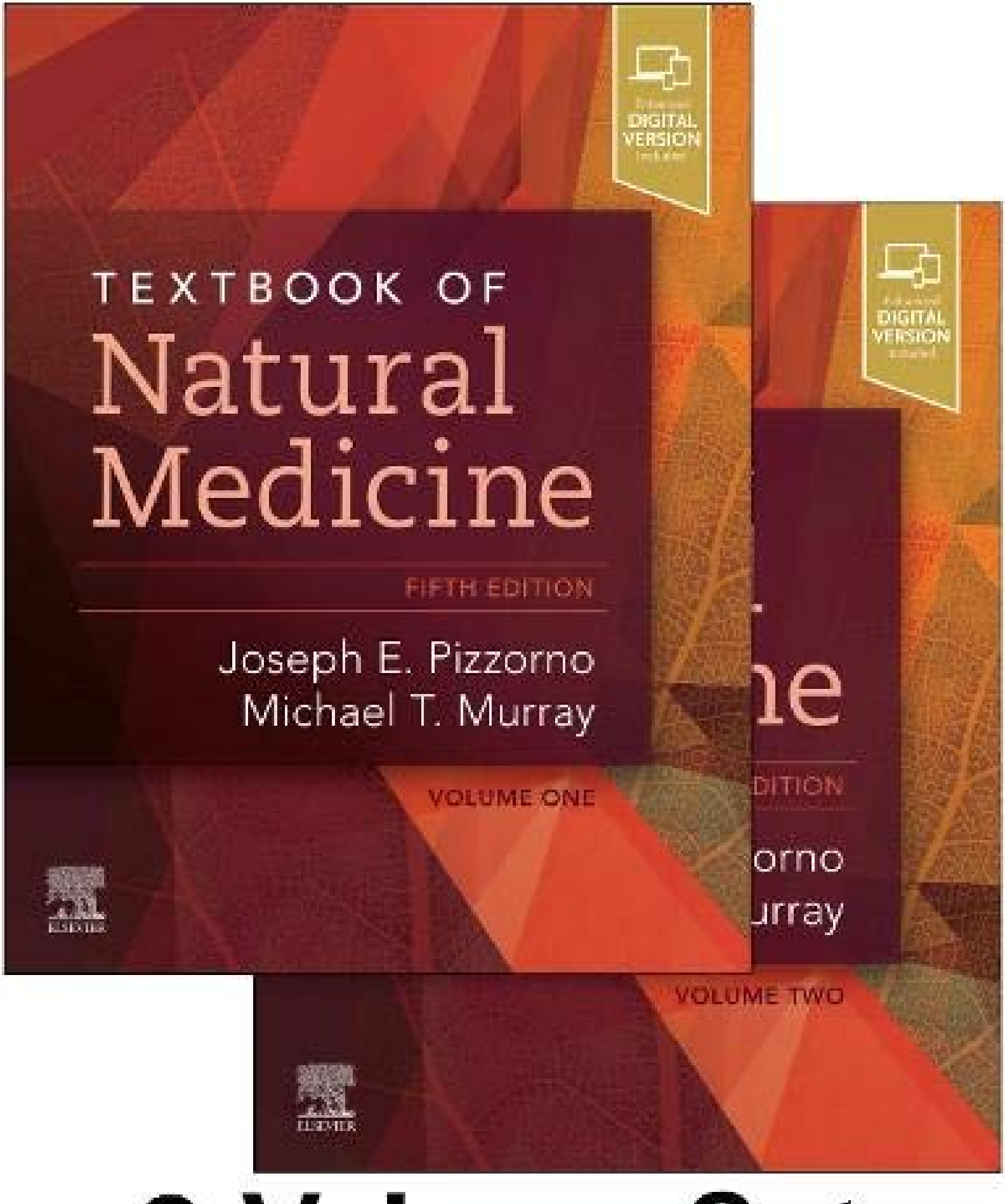

DIGITAL VERSION
TEXTBOOK OF
Natural Medicine
FIFTH EDITION
Joseph E. Pizzorno
Michael T. Murray
VOLUME ONE
ELSEVIER
DIGITAL VERSION
VOLUME TWO
ELSEVIER

2-Volume Set

TEXTBOOK OF Natural Medicine

FIFTH EDITION

EDITED BY

Joseph E. Pizzorno, ND
Editor in Chief, *Integrative Medicine: A Clinician's Journal,* Eagan, Minnesota;
President Emeritus, Bastyr University, Kenmore, Washington;
Chair, Science Board, Bioclinical Naturals, Burnaby, British Columbia

Michael T. Murray, ND
President and CEO, Dr. Murray Natural Living, Inc., Scottsdale, Arizona;
Chief Science Officer, Enzymedica, Venice, Florida

ELSEVIER

Elsevier
3251 Riverport Lane
St. Louis, Missouri 63043

TEXTBOOK OF NATURAL MEDICINE, FIFTH EDITION

ISBN: 978-0-323-52342-4
Volume 1: 978-0-323-52326-4
Volume 2: 978-0-323-52325-7

Notice

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds or experiments described herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made. To the fullest extent of the law, no responsibility is assumed by Elsevier, authors, editors or contributors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Previous editions copyrighted 2013, 2006, 1999, and 1993.

International Standard Book Number: 978-0-323-52342-4

Senior Content Strategist: Linda Woodard
Senior Content Development Specialist: Rebecca Leenhouts
Publishing Services Manager: Julie Eddy
Book Production Specialist: Clay S. Broeker
Design Direction: Margaret Reid

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1

To Dr. John Bastyr and all the natural healers of the past and future who bring the "healing power of nature" to all the people of the world.
Dr. Bastyr, the namesake for Bastyr University, exemplified the ideal physician/healer/teacher we endeavor to become in our professional lives.
We pass on a few of his words of wisdom to all who strive to provide the best of health care and healing: "Always touch your patients—let them know you care," and "Always read at least one research article or learn a new remedy before you retire at night."

CONTRIBUTORS

Kathy Abascal, BS, JD, RH(AHG)
Vashon, Washington

Yaser Abdelhamid, ND, LAc, MS, BS, BA
Licensed Acupuncturist
Center for Integrative and Lifestyle Medicine
Cleveland Clinic
Cleveland, Ohio

Zemphira Alavidze, PhD

Lise Alschuler, ND
Professor of Clinical Medicine Assistant Director, and Fellowship in Integrative Medicine
Program of Integrative Medicine
University of Arizona
Tucson, Arizona

Sidney MacDonald Baker, MD
Independent Retirement Home
Sag Harbor, New York

Stephen Barrie, ND, PhD
Senior Executive
Viome
Bellevue, Washington

David Barry, BS, BAppSci (Hons), DC, ND
Clinical Research Coordinator
Emeritus Research
Camberwell, Victoria, Australia
Senior Lecturer, Naturopathy
Endeavour College of Natural Health
Melbourne, Victoria, Australia

Peter W. Bennett, ND
Clinic Director
Patient Care
Meditrine Naturopathic Medical Clinic
Langley, British Columbia, Canada

Bob G. Blasdel, PhD
Research Director
Vésale Pharma
Noville-sur-Mehaigne, Belgium

Peter B. Bongiorno, ND, MSAc, LAc
Co-Medical Director
Naturopathic Medicine
Inner Source Health
New York, New York

Rachelle S. Bradley, ND
Private Practice
Heartland Naturopathic Clinic
Omaha, Nebraska

Warren M. Brown, ND
Clinical Science Liaison
Medical Affairs
Genova Diagnostics
Asheville, North Carolina

Michael J. Chapman, ND
Medical Education Specialist
Medical Affairs
Genova Diagnostics
Asheville, North Carolina

Alan G. Christianson, NMD
President and Executive
Integrative Health
Scottsdale, Arizona

Anthony J. Cichoke Jr., BS, BS, MA, MA, PhD, DC, DACBN
Portland, Oregon

George W. Cody, JD, MA
Consulting Historian
Edmonds, Washington

Kevin L. Conroy, ND
Owner
Private Practice
Port Angeles Natural Health
Port Angeles, West Virginia

Peter J. D'Adamo, ND, MIFHI
Professor Clinical Sciences
University of Bridgeport College of Naturopathic Medicine
Coder/Developer Opus 23 & SWAMI

Jade Dandy, ND, MSiMR
The Healing Hut Clinic
Eagle, Idaho
National University of Natural Medicine
Portland, Oregon

Patricia M. Devers, DO
Medical Education Specialist
Department of Medical Affairs
Genova Diagnostics, Inc.

Jamie Doughty, BSc, ND
Medical Director
Naturopathic Medicine
Tummy Temple
Olympia, Washington

William Eisner, BSc
Pediatrics/Cardiology
Duke University
Durham, North Carolina

Terry M. Elder, DC
Instructor
Clinical Sciences
National University of Health Sciences
Lombard, Illinois

Geovanni Espinosa, ND, LAc, IFMCP, CNS
Faculty Clinical Assistant Professor
NYU Langone Health, Urology
Educator
Institute for Functional Medicine
New York, New York

Ralph Esposito, ND, LAc
Adjunct Faculty
New York University
New York, New York

Susan Ann Gaylord, PhD
Director, Program on Integrative Medicine
Physical Medicine and Rehabilitation
University of North Carolina (UNC)
Chapel Hill, North Carolina
Associate Professor
Physical Medicine and Rehabilitation
UNC School of Medicine
Chapel Hill, North Carolina

Alan Goldhamer, DC
Director
Residential Health Education Program
TrueNorth Health Center
Santa Rosa, California
Chairman of the Board
Research/Education
TrueNorth Health Foundation
Santa Rosa, California

Andrea Gruszecki, ND
Science Support Specialist
Meridian Valley Laboratory
Tukwilla, Washington

Jason A. Hawrelak, ND, BNat(Hons), PhD
Senior Lecturer in Complementary and Alternative Medicines
College of Health & Medicine
University of Tasmania
Hobart, Tasmania, Australia
Visiting Research Fellow
Australian Research Centre for Complementary and Integrative Medicine
University of Technology Sydney
Sydney, New South Wales, Australia

Bethany Montgomery Hays, MD
Assistant Clinical Professor
Maine Medical Center Dept Ob/Gyn
Tufts University School of Medicine
Portland, Maine

Leah Hechtman, MSci (RHHG), BHSc (Nat), ND
PhD Candidate
Department of Obstetrics, Gynaecology and Neonatology | Faculty of Medicine
University of Sydney
Sydney, New South Wales, Australia
President
National Herbalists Association of Australia
Sydney, New South Wales, Australia
Director and Clinician
The Natural Health and Fertility Centre
Sydney, New South Wales, Australia

Wendy Hodsdon, ND
Adjunct Faculty
Department of Graduate Studies
National University of Natural Medicine
Portland, Oregon
Adjunct Faculty
Maryland University of Integrative Health
Laurel, Maryland

Naomi Hoyle, MD
Eliava Phage Therapy Center
Phage Therapy
Eliava Foundation
Tbilisi, Georgia

Corene Humphreys, ND, BHSc, Dip Med Herb, Dip Hom, QTA
Director
Nutritional Medicine

Mary James, ND
Medical Editor
Naturopathic Doctor News & Review
Scottsdale, Arizona
Expert Panel Member
Women's Health Network
Portland, Maine

Maeba Jonas, MDiv
Ordained Minister
United Church of Christ

Wayne Jonas, MD
Executive Director
Samueli Integrative Health Programs
H&S Ventures
Alexandria, Virginia

Robert Kachko, ND, LAc
Practitioner
Naturopathic Medicine
Inner Source Health
New York, New York
Chief Executive Officer
TribeRx
New York, New York

Joseph Katzinger, ND
Science Director
SaluGenecists
Seattle, Washington

Parris M. Kidd, BSc, PhD
Chief Science Officer and Director of Quality
BrainMD Health
Amen Clinics
Costa Mesa, California

Richard J. Kitaeff, MA, ND, Dip Ac, LAc
Doctor and Clinic Director
New Health Medical Center
Edmonds, Washington
Staff Acupuncturist
Neurology
Northwest Hospital
Seattle, Washington
Clinical Affiliate Faculty
Acupuncture and Oriental Medicine
Bastyr University
Seattle, Washington

Cheryl Kos, ND
Developer
Content
Personalized Medicine Lifestyle Institute
Bainbridge Island, Washington

Thomas A. Kruzel, MT, ND
Rockwood Natural Medicine Clinic
Scottsdale, Arizona

Sarah Kuhl, MD, PhD
Physician
Medicine
VA Northern California
Martinez, California

Elizabeth Kutter, BS, PhD
Faculty Emeritas
Bacteriophage Lab
The Evergreen State College
Olympia, Washington

Michael Alexander Lane, MD
Assistant Professor
Department of Neurology
Oregon Health and Sciences University
Portland, Oregon

Pina LoGiudice, ND, LAc
Co-Owner
Innersource Natural Health and Acupuncture, PC
Huntington, New York

Robert Luby, MD
Executive Director of Medical Education
Medical Education
Institute for Functional Medicine
Federal Way, Washington

Tennille Marx, ND, CFS
Independent Researcher
Kenmore, Washington

Helen (Verhesen) Messier
Founder & Chief Medical Officer
Medical Intelligence Learning Labs, Inc.
San Jose, California

Steven C. Milkis, ND
Owner
Green Lake Natural Medicine
Seattle, Washington

Gaetano Morello, ND
Clinician, Complex Chronic Disease Program
BC Women's Hospital
Vancouver, Canada

Gerard E. Mullin, MD
Associate Professor of Medicine
Gastroenterology and Hepatology
Johns Hopkins School of Medicine
Baltimore, Maryland

Stephen P. Myers, ND, BMed PhD
Professor and Director NatMed Research Unit
Southern Cross University
New South Wales, Australia

Toshia R. Myers, BS, MA, MPhil, PhD
Research Director
Research
TrueNorth Health Foundation
Santa Rosa, California

Tara Nayak, ND
Naturopathic Physician
Philadelphia, Pennsylvania

Mark Harrison Nolting, ND, EAMP
Senior Medical Director
Physical Medicine
TivityHealth
Chandler, Arizona
Medical Director
Edmonds Wellness Clinic
Edmonds, Washington

John Nowicki, ND
Medical Writer, Research Associate
Medical Research Team
Integrative Medicine Advisors, LLC
Seattle, Washington

Brian Orr, BA, BS, ND
Owner
Country Doc: Integrative Medical Specialty
Seattle, Washington

Kristaps Paddock, ND
Medical Director
Charm City Natural Health
Baltimore, Maryland

Cristiana I. Paul, MS Nutrition
Independent Research Consultant
Nutritional Biochemistry Research
Cristiana Paul Consulting
Los Angeles, California

Nicole Pierce, ND
Co-creator
The Vervain Collective
Garden City, Idaho

Lara Pizzorno, MAR, MA, LMT
Senior Medical Writer and Editor
Writing and Editorial Staff
Integrative Medicine Advisors, LLC
Seattle, Washington
Senior Medical Editor
SaluGenecists, Inc.
Seattle, Washington

Terry Arden Pollock, BS, MS
Medical Education Specialist
Medical Affairs
Genova Diagnostics
Asheville, North Carolina

Dirk W. Powell, BS, ND
Adjunct Professor
Naturopathic Medicine
Bastyr University
Kent, Washington

Lahnor Powell, ND, MPH
Medical Education Specialist
Department of Medical Affairs
Genova Diagnostics
Duluth, Georgia

Matt Pratt-Hyatt, PhD
Associate Lab Director
The Great Plains Laboratory, Inc.
Lenexa, Kansas

David Quig, PhD
Vice President
Scientific Support
Doctor's Data, Inc.
St. Charles, Illinois

John C. Reed, MD, MDiv
Founding VP and Fellow of the American Academy of Medical Acupuncture
Fellow of the Osteopathic Cranial Academy
Diplomate of the American Board of Family Medicine
Diplomate of the American Board of Integrative Medicine
Founding Member, American Holistic Medical Association

Ron Reichert, BA, ND
Naturopathic Physician
North Vancouver, Canada

Corey Resnick, ND
President
Integrative Health and Nutrition, Inc.
Lake Oswego, Oregon
Member
Medical Advisory Board
Integrative Therapeutics
Green Bay, Wisconsin

Sally J. Rockwell, PhD, CCN
Deceased

Elaine Roe, MD
Physician, Hall Health Center
University of Washington
Seattle, Washington

Robert A. Ronzio, PhD
Executive Director
Research and Educational Services
Insight Learning Institute
Austin, Texas

Angela Sadlon, ND
All Encompassing Healthcare
Centralia, Washington

Alexander G. Schauss, PhD
Senior Director of Research
Natural and Medicinal Products Research
AIBMR Life Sciences, Inc.
Seattle, Washington
Research Associate
Bio5 Institute
University of Arizona
Tucson, Arizona
Research Associate
Geosciences
University of Arizona
Tucson, Arizona

Michael Scott, ND, MSA
Doctor
Private Practice
UrbanHealthWorks
Boulder, Colorado

Tracey Seipel, FANPA, ABC
Fellow of the Australian Naturopathic Practitioners Association
American Botanical Council
Queensland, Australia

William Shaw, PhD
President
Great Planes Laboratory
Kansas City, Missouri

Ann Shippy, MD
Functional Medicine Physician
Environmental Health Expert
Austin, Texas

Barbara Siminovich-Blok, ND, LAc
Clinical Assistant Professor
Rusk Rehabilitation
New York University Langone Medical Center
New York, New York
Adjunct Professor
Health Sciences
Touro College
New York, New York

Anna Sitkoff, BS, ND
Herbalist
Naturopathic Medicine
Bastyr University
Seattle, Washington

Pamela Snider, ND
Executive and Senior Editor
Foundations of Naturopathic Medicine Project
Foundations of Naturopathic Medicine Institute
Snoqualmie, Washington
Associate Professor
College of Naturopathic Medicine
National University of Natural Medicine
Portland, Oregon
Faculty
School of Naturopathic Medicine
Bastyr University
Kenmore, Washington
Co-Founder
Integrative Health Policy Consortium
Conifer, Washington

Virender Sodhi, MD (Ayurveda), ND
Founder
Ayurvedic Naturopathic Medical Clinic
Bellevue, Washington
Founder and Chief Executive Officer
Ayush Herbs
Redmond, Washington

Nick Soloway, LMT, DC, LAc
Private Practice
Helena, Montana

Lindsey Stuart, MS, CNM
Certified Nurse Midwife
Boulder, Colorado

Cory Szybala, ND
Alumni
Naturopathic Medicine
National University of Natural Medicine
Portland, Oregon

Mollie Parker Szybala, ND, MPH
Doctor
Naturopathic Medicine
Sun Valley Natural Medicine
Ketchum, Idaho

Jade Teta, ND
Owner/Founder/CEO
Metabolic Effect Inc.
Greensboro/Winston-Salem, North Carolina

Keoni Teta, ND
Owner
The Naturopathic Health Clinic of North Carolina
Greensboro/Winston-Salem, North Carolina

Brice Thompson, ND, MS
Postdoctoral Scholar
Department of Pharmaceutics
University of Washington
Seattle, Washington

Sherry Torkos, BSc, Phm, RPh
Pharmacist and Author
Fort Erie, Ontario, Canada

Jessica Tran, ND, MBA
Private Practice
Environmental Medicine
Wellness Integrative Naturopathic Center, Inc.
Irvine, California

Michael Traub, ND, DHANP, FABNO
Medical Director
Dermatology
Lokahi Health Center
Kailua Kona, Hawaii
Clinical Professor of Graduate Medical Education
Postgraduate Education
Bastyr University
Seattle, Washington

Roy Upton, RH
President
American Herbal Pharmacopoeia
Scotts Valley, California

Venessa Wahler, ND
Lead ND
Naturopathic Medicine
Tummy Temple
Seattle, Washington

Edward C. Wallace, ND, DC
Medical Education Specialist
Medical Affairs
Genova Diagnostics
Asheville, North Carolina

Terry Willard, CIH, PhD
Founder
Wild Rose College of Natural Healing
Calgary, Canada

Vijayshree Yadav, MD, MCR, FAAN
Associate Professor
Neurology
Oregon Health & Science University
Portland, Oregon

Eric L. Yarnell, ND, RH(AHG)
Professor
Botanical Medicine
Bastyr University
Kenmore, Washington
Chief Medical Officer
Northwest Naturopathic Urology
Seattle, Washington

Jared Zeff, ND
Naturopathic Physician
Salmon Creek Clinic
Portland, Oregon

Heather Zwickey, PhD
Professor
School of Graduate Studies
National University of Natural Medicine
Portland, Oregon
Adjunct Faculty
Neurology
Oregon Health and Science University
Portland, Oregon
Human Nutrition and Functional Medicine
University of Western States
Portland, Oregon

PREFACE

This fifth edition of the *Textbook of Natural Medicine* (which has now been in publication since 1985) brings several new features and changes to our structure and format. We are especially excited that we are in full color for the first time, including images and figures. These dramatically improve our ability to present, in a more understandable and visually interesting way, the key concepts of and insights into the underlying causes of dysfunction and disease. We are also delighted that with all the new chapters and graphics, Elsevier has moved us back to the two-volume format. To better fit the content into two logical volumes, we changed the order (and some of the titles) of the sections. Syndromes and Special Topics moved to Section V because these fit better in Volume 2 with Section VI, Diseases. Pharmacology of Natural Medicines moved to Section IV because this fits better with Volume 1. As usual, we offer many new chapters, and we think the new chapter on sarcopenia is of particular importance. In addition to new chapters, some chapters have been renamed for better consistency, and some have been moved to sections that we felt were more appropriate. To facilitate utilization, the sections are now color coded, and we have provided alphabetical tabs to help readers in searching for specific diseases. Closely related diseases have been placed in a single chapter—for example, depression, dysthymia, manic phase, and seasonal affective disorder are all located in the chapter on affective disorders chapter—so becoming familiar with these groupings is essential for finding specific diseases. There are now 14 appendices that provide additional resources for the clinician. We worked with authors to make their writing more succinct and eliminate unnecessary content. We also reduced the length of Section VI by removing duplication of content from Section V in the therapeutics portion of the chapters. We hope you will be as pleased with the latest edition as we are.

Due to the substantial increase in pages this edition, to keep down costs we had to move all of the approximately 20,000 references to the online version.

Joseph E. Pizzorno
Michael T. Murray

ACKNOWLEDGMENTS

We would like to thank Inta Ozols, the original commissioning editor, our executive assistant Lavelle Brown (who so effectively organized and managed all the authors and chapters), and the dedicated staff at Elsevier (Kristin Wilhelm, Linda Woodard, Laurie Gower, Becky Leenhouts, Jeff Patterson, Julie Eddy, Clay Broeker, Margaret Reid, Deanna Sorenson, and Allison Kieffer) for their excellent work in making this the best edition ever.

SECTION 1

Philosophy of Natural Medicine

One of the key features of the various schools of natural medicine that differentiates them from conventional medicine is their strong philosophical foundation. The basic philosophical premise of naturopathic medicine, for example, is that there is an inherent healing power in nature and in every human being. We believe that a primary role of the physician is to "remove the blocks to cure" and enhance this innate healing power within his or her patients.

In many ways, this was the most difficult section of the textbook to write because, before this textbook, no comprehensive history of the social, political, and philosophical development of naturopathic medicine had ever been written. Even in the halcyon years of the 1920s and 1930s, the profession was never able to agree upon a concise philosophy. This situation has now changed.

In this section, we provide well-documented chapters detailing the roots of American natural medicine. After a century of maturation, the naturopathic profession has now widely agreed to a comprehensive definition, set of principles, and system of case analysis that provide a systematic guide for the application of these concepts in a clinical setting.

The seven fundamental principles of naturopathic medicine are as follows:

The healing power of nature *(vis medicatrix naturae)*
First, do no harm *(primum non nocere)*
Find the cause *(tolle causam)*
Treat the whole person
Preventive medicine
Wellness
Doctor as teacher

These principles translate into the following questions the practitioner applies when analyzing a case:

- What is the first cause; what is contributing now?
- How is the body trying to heal itself?
- What is the minimum level of intervention needed to facilitate the self-healing process?
- What are the patient's underlying functional weaknesses?
- What education does the patient need to understand why he or she is sick and how to become healthier?
- How does the patient's physical disease relate to his or her psychological and spiritual health?

We have further expanded on the philosophical basis of naturopathic medicine by having these concepts addressed by several authors whose backgrounds allow each of them a unique and, we believe, complementary insight into some of the fundamental questions of the goals of health care. Although the dominant school of medicine has essentially ignored these issues, we believe that the true physician cannot function without a sound philosophical basis to guide his or her actions. Without more than a superficial understanding of health and disease, the physician is more likely to function as a technician, temporarily alleviating symptoms while allowing the real disease to progress past the point of recovery. The huge and increasing burden of chronic disease in all age groups clearly validates the predictions of the founders of naturopathic medicine that primarily treating symptoms, while not addressing causes, results in increased chronic disease.

1

Functional Medicine: A 21st-Century Model of Patient Care and Medical Education

Robert Luby, MD, and *Leo Galland*, AB, MD*

OUTLINE

In this chapter, the basic principles, constructs, and methodology of functional medicine are reviewed. It is not the purpose of this chapter to recapitulate the range and depth of the science underlying functional medicine; books and monographs covering that material in great detail are already available for the interested clinician and for use in health professional schools (see Bibliography at the end of the chapter). The purpose is to describe how functional medicine is organized to deliver personalized systems medicine and is equipped to respond to the challenge of treating complex chronic disease more effectively.

WHAT IS FUNCTIONAL MEDICINE?

Functional medicine encompasses a dynamic approach to assessing, preventing, and treating complex chronic disease. It helps clinicians of all disciplines identify and ameliorate dysfunctions in the physiology and biochemistry of the human body as a primary method of improving patient health. This model of practice emphasizes that chronic disease is almost always preceded by a period of declining function in one or more of the body's physiological organizing systems. Returning patients to health requires reversing (or substantially improving) the specific dysfunctions that contributed to the disease state. Those dysfunctions are, for each of us, the result of lifelong interactions among diet, environment, lifestyle choices, and genetic predispositions. Each patient, therefore, represents a unique, complex, and interwoven set of influences on intrinsic functionality that, over time, set the stage for the development of disease or the maintenance of health. To manage the complexity inherent in this approach, functional medicine has adopted practical models for obtaining and evaluating clinical information that leads to individualized patient-centered therapies.

Historically, the word *functional* was used somewhat pejoratively in medicine. It implied a disability associated with either a geriatric or psychiatric problem. The authors suggest, however, that this is a very limited definition of an extremely useful word. The medical profession has not really produced an efficient method for identifying and assessing changes in basic physiological processes that produce symptoms of increasing duration, intensity, and frequency, although it is known that such alterations in function often represent the first signs of conditions that, at a later stage, become pathophysiologically definable diseases. By broadening the use of *functional* to encompass this view, functional medicine becomes the science and art of detecting and reversing alterations in function that clearly move a patient toward chronic disease over the course of a lifetime.

One way to conceptualize where functional medicine falls in the continuum of health and health care is to examine the functional medicine "tree." In its approach to complex chronic disease, functional medicine encompasses the whole domain represented by the graphic shown in Fig. 1.1, but it first addresses the patient's core clinical imbalances (found in the functional physiological organizing systems); the fundamental lifestyle factors that contribute to chronic disease; and the antecedents, triggers, and mediators that initiate and maintain the disease state. Diagnosis, of course, is part of the functional medicine model, but the emphasis is on understanding and improving the functional core of the human being as the starting point for intervention.

Functional medicine clinicians focus on restoring balance and improved function in the dysregulated systems by strengthening the fundamental physiological processes that underlie them and by adjusting the environmental and lifestyle inputs that nurture or impair them. This approach leads to therapies that focus on restoring health and function, rather than simply controlling signs and symptoms.

PRINCIPLES

Seven basic principles characterize the functional medicine paradigm:

- Acknowledging the biochemical individuality of each human being, based on the concepts of genetic and environmental uniqueness
- Incorporating a patient-centered rather than a disease-centered approach to treatment

*Previous edition contributor

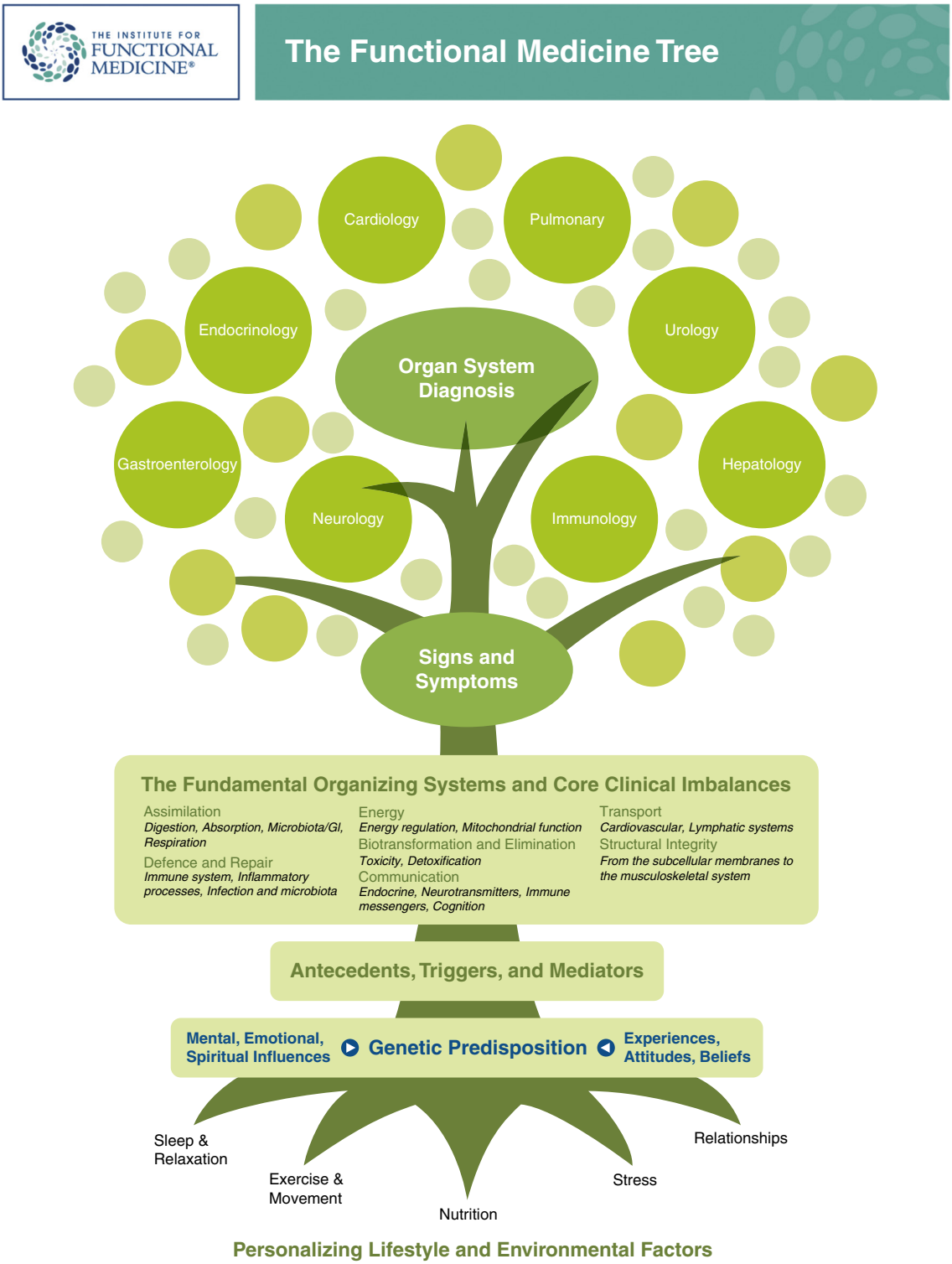


Fig. 1.1 The continuum of health and health care: the functional medicine tree. (Courtesy the Institute for Functional Medicine.)

- Seeking a dynamic balance among the internal and external factors in a patient's body, mind, and spirit
- Addressing the web-like interconnections of internal physiological factors
- Identifying health as a positive vitality—not merely the absence of disease—and emphasizing those factors that encourage a vigorous physiology
- Promoting organ, cellular, and subcellular function as the means of enhancing the health span, not just the life span, of each patient
- Staying abreast of emerging research—a science- and evidence-based approach

LIFESTYLE AND ENVIRONMENTAL FACTORS

The building blocks of life, and the primary influences on them, are found at the base of the functional medicine tree graphic (see Fig. 1.1). When we talk about influencing gene expression, we are interested in the interaction between lifestyle and environment in the broadest sense and any genetic predispositions with which a person may have been born—in a word, the epigenome. (Epigenetics is the study of how environmental factors can affect gene expression without altering the actual DNA sequence and how these changes can be inherited through generations.) Many environmental factors that affect gene expression are (or appear to be) a matter of choice (such as diet and exercise), others are very difficult for the individual patient to alter or escape (air and water quality, toxic exposures), and still others may be the result of unavoidable accidents (trauma, exposure to harmful microorganisms). Some factors that may appear modifiable are heavily influenced by the patient's economic status—if you are poor, for example, it may be impossible to choose more nutritious food, decrease stress in the workplace and at home, or take the time to exercise and rest properly. Existing health status is also a powerful influence on the patient's ability to alter environmental input. If you have chronic pain, exercise may be extremely difficult; if you are depressed, self-activation is a major challenge.

The influence of these lifestyle and environment factors on the human organism is indisputable,[1,2] and they are often powerful agents in the attempt to restore health. Neglecting to address them in favor of merely writing a prescription—whether for pharmaceutical agents, nutraceuticals, or botanicals—means the cause of the underlying dysfunction may itself remain unaddressed and further able to contribute to the genesis of other disease conditions. In general terms, the following factors should be considered when working to reverse dysfunction or disease and restore health:

- Diet (type, quality, and quantity of food; food preparation; calories, fats, proteins, carbohydrates)
- Nutrients (both dietary and supplemental)
- Air and water
- Microorganisms (and the general condition of the soil in which food is grown)
- Physical exercise
- Trauma
- Psychosocial and spiritual factors, such as meaning and purpose, relationships, work, community, economic status, stress, and belief systems
- Xenobiotics
- Radiation

FUNDAMENTAL PHYSIOLOGICAL PROCESSES

There are certain physiological processes that are necessary to life. These are the "upstream" processes that can go awry and create "downstream" dysfunctions that eventually become expressed as disease entities. Functional medicine requires that clinicians consider these in evaluating patients so that interventions can target the most fundamental level possible. These processes are as follows:

1. Communication
   - Intracellular
   - Intercellular
   - Extracellular
2. Bioenergetics/energy transformation
3. Assimilation
4. Structural integrity
5. Biotransformation/elimination
6. Defense and repair
7. Transport/circulation

These fundamental physiological processes are usually taught early in health professions curricula, where they are appropriately presented as the foundation of modern, scientific patient care. Unfortunately, subsequent training in the clinical sciences often fails to fully integrate knowledge of the functional mechanisms of disease with therapeutics and prevention, emphasizing organ system diagnosis instead.[3] Focusing predominantly on organ-system diagnosis without examining the underlying physiology that produced the patient's signs, symptoms, and disease often leads to managing patient care by matching diagnosis to pharmacology. The job of the health care provider then becomes a technical exercise in finding the drug or procedure that best fits the diagnosis (not necessarily the patient or the underlying physiological dysfunction), leading to a significant curtailment of critical thinking pathways: "Medicine, it seems, has little regard for a complete description of how myriad pathways result in any clinical state."[4]

Even more important, pharmacological treatments (and even natural remedies) are often prescribed without careful consideration of their physiological effects across all organ systems, physiological processes, and genetic variations.[5] This was notably exemplified by the cyclooxygenase-2 inhibitor drugs that were so wildly successful on their introduction, only to be subsequently withdrawn or substantially narrowed in use because of collateral damage.[6,7]

CORE CLINICAL IMBALANCES

The functional medicine approach to assessment, both before and after diagnosis, charts a course using different navigational assumptions. Every health condition instigates a quest for information centered on understanding when and how the specific biological system(s) under examination became dysregulated and began manifesting dysfunction and/or disease. Analyzing all the elements of the patient's story, the signs and symptoms, and the laboratory assessment through a matrix focused on functionality requires analytical thinking and a willingness on the part of the clinician to reflect deeply on the underlying biochemistry and physiology. The foundational principles of how the human organism functions—and how its systems communicate and interact—are essential to the process of linking ideas about multifactorial causation with the perceptible effects called disease or dysfunction.

To assist clinicians in this process, functional medicine identified and organized a set of core clinical imbalances that are linked to the fundamental physiological processes (organizing systems). These serve to marry the mechanisms of disease with the manifestations and diagnoses of disease. Many common underlying pathways of disease are reflected in these clinical imbalances. The following list of imbalanced systems and processes is not definitive, but some of the most common examples are provided. We recommend that the organizing systems be considered in the order as shown in the following list:

- Digestion
- Absorption

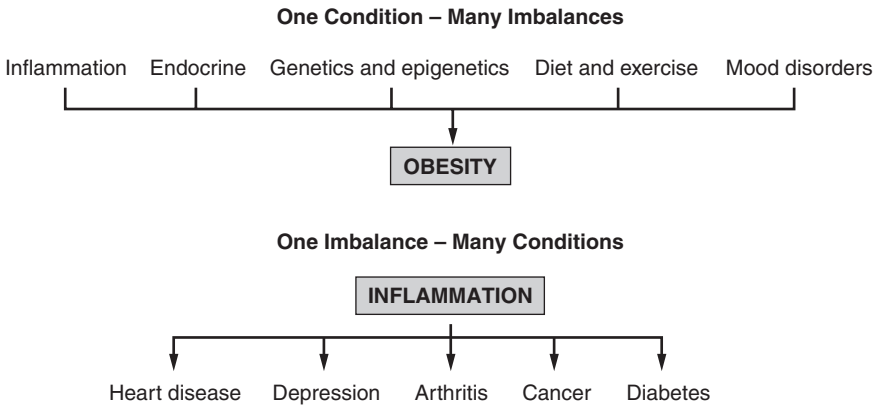


Fig. 1.2 Core clinical imbalances—multiple influences. (Courtesy the Institute for Functional Medicine.)

- Microbiome/gastrointestinal
- Respiration
- Immune system
- Inflammatory processes
- Infection and microbiome
- Energy regulation
- Mitochondrial function
- Toxicity
- Detoxification
- Endocrine
- Neurotransmitter
- Immune messengers
- Cognition
- From the subcellular membranes
- To the musculoskeletal system

Using this construct, it becomes much clearer that one disease and/or condition may have multiple causes (i.e., multiple clinical imbalances), just as one fundamental imbalance may be at the root of many seemingly disparate conditions (Fig. 1.2).

The most important precept to remember about functional medicine is that restoring balance—in the patient's lifestyle and/or environment and in the body's fundamental physiological processes—is the key to restoring health.

ANTECEDENTS, TRIGGERS, AND MEDIATORS[8]

What modern science has taught us about the genesis of disease can be represented by three words: *triggers, mediators,* and *antecedents.* Triggers are discrete entities or events that provoke disease or its symptoms. Microbes are an example. The greatest scientific discovery of the 19th century was the microbial etiology of the major epidemic diseases. Triggers are usually insufficient in and of themselves for disease formation; however, host response is an essential component.

It is, therefore, the functional medicine practitioner's job to know not just the patient's ailments or diagnoses but also the physical and social environment in which illness occurs, the dietary habits of the person (present diet and preillness diet), his or her beliefs about the illness, the effect of illness on social and psychological function, factors that aggravate or ameliorate symptoms, and factors that predispose to illness or facilitate recovery. This information is necessary for establishing a functional medicine treatment plan.

Identifying the biochemical mediators that underlie host responses was the most productive field of biomedical research during the second half of the 20th century. Mediators, as the word implies, do not cause disease. They are intermediaries that contribute to the manifestation and/or continuation of disease. Antecedents are factors that predispose to acute or chronic illness. For a person who is ill, antecedents form the illness diathesis. From the perspective of prevention, they are risk factors. Knowledge of antecedents provides a rational structure for the organization of preventive medicine and public health.

Medical genomics seeks to better understand disease by identifying the phenotypic expression of disease-related genes and their products. The application of genomic science to clinical medicine requires the integration of antecedents (genes and the factors controlling their expression) with mediators (the downstream products of gene activation). Mediators, triggers, and antecedents are not only key biomedical concepts; they are also important psychosocial concepts. In person-centered diagnosis, the mediators, triggers, and antecedents for each person's illness form the focus of the clinical investigation.

Antecedents and the Origins of Illness

Understanding the antecedents of illness helps the physician understand the unique characteristics of each patient as they relate to his or her current health status. Antecedents may be thought of as congenital or developmental. The most important congenital factor is gender: women and men differ sharply in susceptibility to many disorders. The most important developmental factor is age; what ails children is rarely the same as what ails the elderly. Beyond these obvious factors lies a diversity as complex as the genetic differences and separate life experiences that distinguish one person from another.

Triggers and the Provocation of Illness

A trigger is anything that initiates an acute illness or the emergence of symptoms. The distinction between a trigger and a precipitating event is relative, not absolute; the distinction helps organize the patient's story. As a general rule, triggers only provoke illness as long as the person is exposed to them (or for a short while afterward), whereas a precipitating event initiates a change in health status that persists long after the exposure ends.

Common triggers include physical or psychic trauma, microbes, drugs, allergens, foods (or even the act of eating or drinking), environmental toxins, temperature change, stressful life events, adverse social interactions, and powerful memories. For some conditions, the trigger is such an essential part of our concept of the disease that the two cannot be separated; the disease is either named after the trigger (e.g., strep throat) or the absence of the trigger negates the diagnosis (e.g., concussion cannot occur without head trauma). For chronic ailments like asthma, arthritis, or migraine headaches, multiple interacting triggers may be present. All triggers, however, exert their effects through the activation of host-derived mediators. In closed-head trauma, for example, activation of N-methyl-d-aspartic acid receptors, induction of nitric oxide synthase, and liberation of free intraneuronal calcium

BOX 1.1 Common Illness Mediators

Biochemical Hormones
Neurotransmitters
Neuropeptides
Cytokines
Free radicals
Transcription factors

Subatomic
Ions
Electrons
Electrical and magnetic fields

Cognitive/Emotional
Fear of pain or loss
Feelings or personal beliefs about illness
Poor self-esteem, low perceived self-efficacy
Learned helplessness
Lack of relevant health information

Social/Cultural
Reinforcement for staying sick
Behavioral conditioning
Lack of resources because of social isolation or poverty
The nature of the sick role and the doctor–patient relationship

determine the late effects. Intravenous magnesium at the time of trauma attenuates the severity by altering the mediator response.[9,10] Sensitivity to different triggers often varies among persons with similar ailments. A prime task of the functional practitioner is to help patients identify important triggers for their ailments and develop strategies for eliminating them or diminishing their virulence.

Mediators and the Formation of Illness

A mediator is anything that produces or perpetuates symptoms or damages tissues of the body, including certain behaviors. Mediators vary in form and substance. They may be biochemical (e.g., prostanoids and cytokines), ionic (e.g., hydrogen ions), social (e.g., reinforcement for staying ill), psychological (e.g., fear), or cultural (e.g., beliefs about the nature of illness). A list of common mediators is presented in Box 1.1. Illness in any single person usually involves multiple interacting mediators. Biochemical, psychosocial, and cultural mediators interact continuously in the formation of illness.

CONSTRUCTING THE MODEL

Assessment

Combining the principles, lifestyle and environment factors, fundamental physiological processes, antecedents, triggers, mediators, and core clinical imbalances demands a new architecture for gathering and sorting information for clinical practice—in effect, a new heuristic to serve the practice of functional medicine. (Heuristics are rules of thumb—ways of thinking or acting—that develop through experimentation and enable more efficient and effective processing of data.) This new model includes an explicit emphasis on principles and mechanisms that infuse meaning into the diagnosis and deepen the clinician's understanding of the multivalent contributors to physiological dysfunction. Any methodology for constructing a coherent story and an effective therapeutic plan in the context of complex chronic illness must be flexible and adaptive. Like an accordion file that compresses and expands upon demand, the amount and kind of data collected will necessarily change in accordance with the patient's situation and the clinician's time and ability to piece together the underlying threads of dysfunction.

The conventional assessment process involving the chief complaint, history of present illness, and past medical history sections must be expanded (Fig. 1.3) to include a thorough investigation of antecedents, triggers, and mediators and a systematic evaluation of any imbalances within the fundamental organizing systems. Personalized medical care without this expanded investigation falls short.

The Functional Medicine Matrix Model

Distilling the data from the expanded history, physical examination, and laboratory findings into a narrative storyline that includes antecedents, triggers, and mediators can be challenging. Key to developing a thorough narrative is organizing the story using the Functional Medicine Matrix Model form (Fig. 1.4).

The matrix form helps organize and prioritize information and also clarifies the level of present understanding, thus illuminating where further investigation is needed. For example:

- Indicators of inflammation on the matrix might lead the clinician to request tests for specific inflammatory markers (such as highly sensitive C-reactive protein, interleukin levels, and/or homocysteine).
- Essential fatty acid levels, methylation pathway abnormalities, and organic acid metabolites help determine the adequacy of dietary and nutrient intakes.
- Markers of detoxification (glucuronidation and sulfation, cytochrome P450 enzyme heterogeneity) can determine the functional capacity for molecular biotransformation.
- Neurotransmitters and their metabolites (vanilmandelate, homovanillate, 5-hydroxyindoleacetate, quinolinate) and hormone cascades (gonadal and adrenal) have obvious utility in exploring messenger molecule balance.
- Computed tomographic scans, magnetic resonance imaging (MRI), or plain radiographs extend the view of the patient's structural dysfunctions. The use of bone scans, dual-energy x-ray absorptiometry scans, or bone resorption markers[11,12] can be useful in further exploring the web-like interactions of the matrix.
- Newer, useful technologies such as functional MRIs, single-photon emission computed tomography, and positron emission tomographic scans offer a more comprehensive assessment of metabolic function within organ systems.

It is the process of completing a comprehensive history and physical using the expanded functional medicine heuristic and then charting these findings on the matrix that best directs the choice of diagnostic evaluations and successful treatment.

Therapies should be chosen for their potential effect on the most significant imbalances of the particular patient. A completed matrix form facilitates review of common pathways, mechanisms, and mediators of disease and helps clinicians select points of leverage for treatment strategies. However, even with the matrix as an aid to synthesizing and prioritizing information, it can be very useful to consider the effect of each variable at five different levels:

1. Whole-body interventions: Because the human organism is a complex adaptive system, with countless points of access, interventions at one level will affect points of activity in other areas as well. For example, improving the patient's sleep beneficially influences the immune response, melatonin levels, and T-cell lymphocyte levels and helps decrease oxidative stress. Exercise reduces stress, improves insulin sensitivity, and improves detoxification. Reducing stress (and/or improving stress management) reduces cortisol

Chief Complaint (CC)
History of Present Illness (HPI)
Past Medical History (PMH)
– Explore antecedents, triggers, and mediators of CC, HPI, and PMH
Family Medical History
– Genetic predispositions?
Review of Organ Systems (ROS)
Medication and Supplement History
Dietary History
Social, Lifestyle, Exercise History
Physical Examination (PE)
Laboratory and Imaging Evaluations
Explore Core Clinical Imbalances:
Assimilation Imbalances
Digestion
Absorption
Microbiota/GI
Respiration
Defense and Repair Imbalances
Immune system
Inflammatory processes
Infection and microbiota
Energy Imbalances
Energy regulation
Mitochondrial function
Biotransformation and Elimination Imbalances
Toxicity
Detoxification
Communication Imbalances
Endocrine
Neurotransmitter
Immune messengers
Cognition
Structural Integrity Imbalances
From the subcellular membranes to the musculoskeletal system
Initial Assessment:
– Enter data on Matrix form; look for common themes
– Review underlying mechanisms of disease
– Recapitulate patient's story
– Organ system-based diagnosis
– Functional medicine assessment: underlying mechanisms of disease; genetic and environmental influences
Treatment Plan:
– Individualized
– Dietary, lifestyle, environmental
– Nutritional, botanical, psychosocial, energetic, spiritual
– May include pharmaceuticals and/or procedures

Fig. 1.3 Expanding the accordion file: the functional medicine assessment heuristic. (Courtesy the Institute for Functional Medicine.)

levels, improves sleep, improves emotional well-being, and reduces the risk of heart disease. Changing the diet has myriad effects on health, from reducing inflammation to reversing coronary artery disease.

2. Organ-system interventions: These interventions are used more frequently in the acute presentation of illness. Examples include splinting; draining lesions; repairing lacerations; reducing fractures, pneumothoraxes, hernias, or obstructions; or removing a stone to reestablish whole-organ function. There are many interventions that improve organ function. For example, bronchodilators improve air exchange, thereby decreasing hypoxia, reducing oxidative stress, and improving metabolic function and oxygenation in a patient with reactive airway disease.
3. Metabolic or cellular interventions: Cellular health can be addressed by ensuring the adequacy of macronutrients, essential amino acids, vitamins, and cofactor minerals in the diet (or, if necessary, from supplementation). An individual's metabolic enzyme polymorphisms can profoundly affect his or her nutrient requirements. For example, adding conjugated linoleic acid to the diet can alter the peroxisome proliferator–activated receptor system, affect body weight, and modulate the inflammatory response.[13–15] However, in a person who is diabetic or insulin resistant, adding conjugated linoleic acid may induce hyperproinsulinemia, which is detrimental.[16,17] Altering the types and proportions of carbohydrates in the diet may increase insulin sensitivity, reduce insulin secretion, and fundamentally alter metabolism in the insulin-resistant patient. Supporting liver detoxification pathways with supplemental glycine and *N*-acetylcysteine improves the endogenous production of adequate glutathione, an essential antioxidant in the central nervous system and gastrointestinal tract.

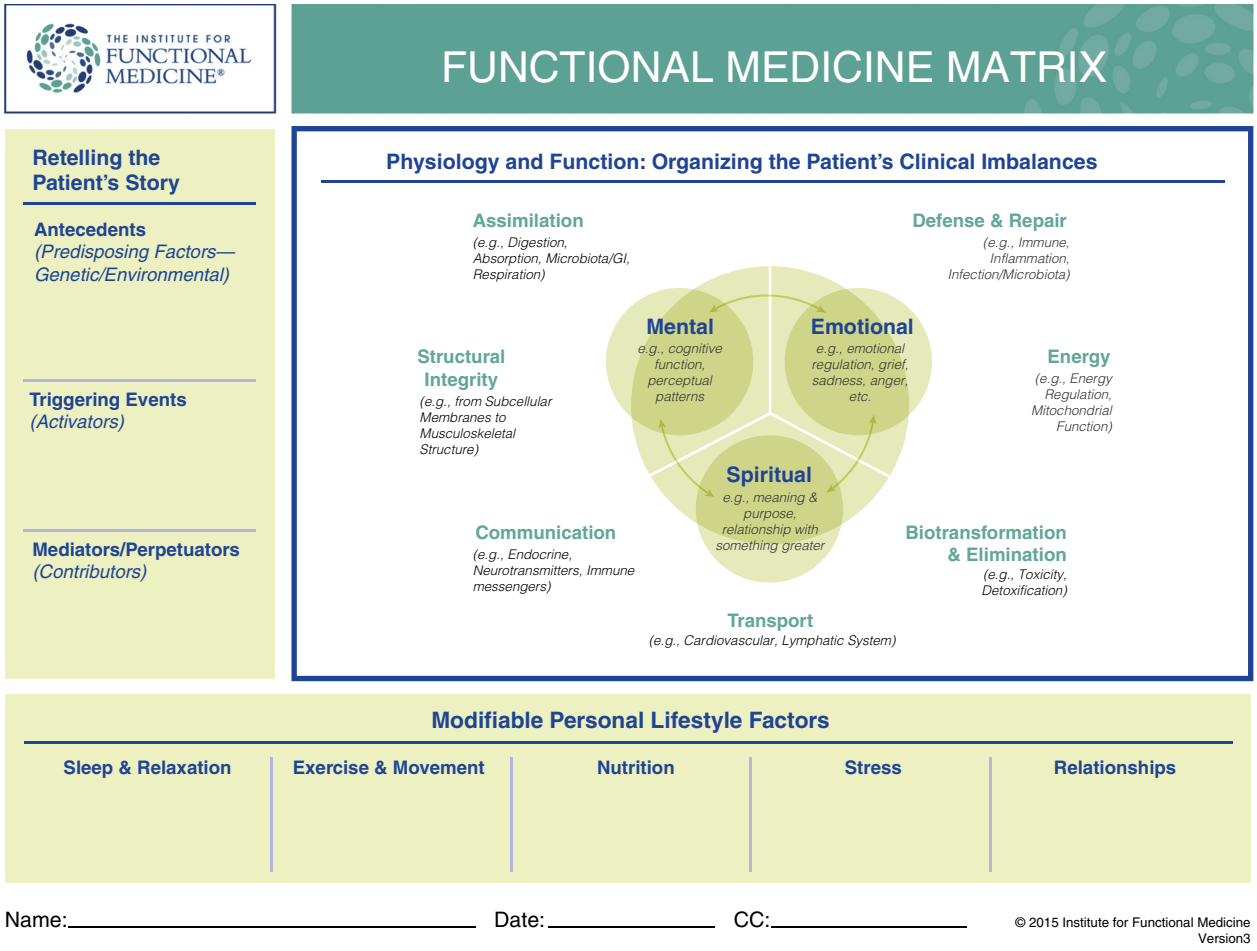


Fig. 1.4 The Functional Medicine Matrix Model. (Courtesy the Institute for Functional Medicine.)

4. Subcellular/mitochondrial interventions: There are many examples of nutrients that support mitochondrial function.[18,19] Inadequate iron intake causes oxidants to leak from mitochondria, damaging mitochondrial function and mitochondrial DNA. Making sure there is sufficient iron helps alleviate this problem. Inadequate zinc intake (found in more than 10% of the U.S. population) causes oxidation and DNA damage in human cells.[19] Ensuring the adequacy of antioxidants and cofactors for the at-risk individual must be considered in each part of the matrix. Carnitine, for example, is required as a carrier for the transport of fatty acids from the cytosol into the mitochondria, improving the efficiency of β-oxidation of fatty acids and resultant adenosine triphosphate production. In patients who have lost significant weight, carnitine undernutrition can result in fatty acids undergoing ω-oxidation, a far less efficient form of metabolism.[20] Patients with low carnitine may also respond to riboflavin supplementation.[20]
5. Subcellular/gene-expression interventions: Many compounds interact at the gene level to alter cellular response, thereby affecting health and healing. Any intervention that alters nuclear factor-κB entering the nucleus, binding to DNA, and activating genes that encode inflammatory modulators, such as interleukin-6 (and thus C-reactive protein), cyclooxygenase-2, interleukin-1, lipoxygenase, inducible nitric oxide synthase, tumor necrosis factor-α, or a number of adhesion molecules, will affect many disease conditions.[21,22] There are many ways to alter the environmental triggers for nuclear factor-κB, including lowering oxidative stress; altering emotional stress; and consuming adequate phytonutrients, antioxidants, alpha-lipoic acid, eicosapentaenoic acid, docosahexaenoic acid, and γ-linoleic acid.[21] Adequate vitamin A allows the appropriate interaction of vitamin A–retinoic acid with more than 370 genes.[23] Vitamin D in its most active form intercalates with a retinol protein and the DNA exon and modulates many aspects of metabolism, including cell division in both healthy and cancerous breast, colon, prostate, and skin tissue.[24] Vitamin D has key roles in controlling inflammation, calcium homeostasis, bone metabolism, cardiovascular and endocrine physiology, and healing.[24]

Experience using this model, along with improved pattern-recognition skills, will often lessen the need for extensive laboratory assessments. However, there will always be certain clinical conundrums that simply cannot be assessed without objective data, and for most patients, there may be an irreducible minimum of laboratory assessments required to accumulate information. For example, in the clinical workup of autism spectrum disorders in children, heavy-metal exposure and toxicity may play an important role. The heavy-metal body burden cannot be sensibly assessed without laboratory studies. In most initial workups, laboratory and imaging technologies can be reserved for those complex cases in which the initial interventions prove insufficient to the task of functional explication. When clinical acumen and educated steps in both assessments and therapeutic trials do not yield expected improvement, laboratory testing often provides rewarding information. This is frequently the context for focused genomic testing.

The Healing Partnership

No discussion of the functional medicine model would be complete without mention of the therapeutic relationship. Partnerships are

formed to achieve an objective. For example, a business partnership forms to engage in commercial transactions for financial gain; a marriage partnership forms to build a caring, supportive, home-centered environment. A healing partnership forms to heal the patient through the integrated application of both the art of medicine (insight driven) and the science of medicine (evidence driven). An effective partnership requires that trust and rapport be established. Patients must feel comfortable telling their stories and revealing intimate information and significant events.

In the 20th century, contemporary medicine, traditionally considered a healing profession, evolved away from the role of healing the sick to that of curing disease through modern science. Research into this transition revealed that healing was traditionally associated with themes of wholeness, narrative, and spirituality. Professionals and patients alike report healing as an intensely personal, subjective experience involving a reconciliation of meaning for an individual and a perception of wholeness. The biomedical model as currently configured no longer encompasses these characteristics.

Contemporary medicine considers the wholeness of healing to be beyond its orthodoxy—the domain of the nonscientific and nonmedical.[25] We disagree. To grasp the profound importance of the healing partnership to the creation of a system of medicine adequate to the demands of the 21st century, an emerging body of relevant research was reviewed.[26–28] As Louise Acheson, MD, MS, associate editor of the *Annals of Family Practice,* articulated insightfully in that journal[29]: "It is challenging to research this ineffable process called healing."

Hsu and colleagues asked focus groups of nurses, physicians, medical assistants, and randomly selected patients to define healing and describe what facilitates or impedes it.[30] The groups arrived at surprisingly convergent definitions: "Healing is a dynamic process of recovering from a trauma or illness by working toward realistic goals, restoring function, and regaining a personal sense of balance and peace." They heard from diverse participants that "healing is a journey" and "relationships are essential to healing."

Research into the role of healing in the medical environment has generated some thoughtful and robust investigations. Scott et al.'s[26] research into the healing relationship found very similar descriptions to those of Hsu et al.[30] The participants in the study[27] articulated aspects of the healing partnership as follows:

1. Valuing and creating a nonjudgmental emotional bond
2. Appreciating power and consciously managing clinician power in ways that would most benefit the patient
3. Abiding and displaying a commitment to caring for patients over time

Three relational outcomes result from these processes: trust, hope, and a sense of being known. Clinician competencies that facilitate these processes are self-confidence, emotional self-management, mindfulness, and knowledge.[27] In this rich soil, the healing partnership flourishes.

The characteristics of a conventional therapeutic encounter are fundamentally different from a healing partnership, and each emerges from specific emphases in training. In the therapeutic encounter, the relationship forms to assess and treat a medical problem using (usually) an organ-system structure, a differential diagnosis process, and a treatment toolbox focused on pharmacology and medical procedures. The therapeutic encounter pares down the information flow between physician and patient to the minimum needed to identify the organ-system domain of most probable dysfunction, followed by a sorting system search (the differential diagnosis heuristic). The purpose of this relationship is to arrive at the most probable diagnosis as quickly as possible and select an intervention based on probable efficacy. The relationship is a left-brain–guided conversation controlled by the clinician and characterized by algorithmic processing and statistical thinking.[31,32]

The functional medicine healing partnership forms with a related but broader purpose: to help the patient heal by identifying the underlying mechanisms and influences that initiated and continue to mediate the patient's illness(es). This type of relationship emphasizes shared responsibility for identifying the causes of the patient's condition and achieving insight about enduring solutions. The healing partnership is critical to the delivery of personalized systems of medicine and to managing the uncertainty (choices under risk) inherent in clinical practice. In the healing partnership, the appropriate utilization and integration of left-brain and right-brain functions are found.

In language, we have the fullest expression of the integration of left- and right-brain function. Language is so complex that the brain has to process it in different ways simultaneously—both denotatively and connotatively. For complexity and nuance to emerge in language, the left brain needs to see the trees, and the right brain helps us see and understand the forest.[33,34]

The starting point for creating a healing partnership is the patient's experience. People, not diseases, can heal. Mindful integration of brain function is at the heart of a healing partnership. Some of the basic steps for establishing a healing partnership include the following:

1. Allowing patients to express, without interruption, their story about why they have come to see you. (Research focused on the therapeutic encounter has repeatedly found that clinicians interrupt the patient's flow of conversation within the first 18 seconds or less, often denying the patient an opportunity to finish.[35]) The manner in which the patient frames the initial concerns often presages later insight into the root causes. Any interruption in this early stage of narrative moves the patient back into left-brain processing and away from insight.[36]
2. After focusing on the chief concerns, encouraging the patient's narrative regarding the present illness(es). Clarifications can be elicited by further open-ended questioning (e.g., "Tell me more about that"; "What else do you think might be going on?"). During this portion of the interview, there is a switching back and forth between right- and left-brain functions.
   - During this conversation, signs and symptoms of the present illness are distributed by the practitioner into the Functional Medicine Matrix Model form as previously described.
   - Analysis of the data thus collected proceeds by assessing probable underlying causes—based on evidence about common underlying mechanisms of disease—and ongoing mediators of the disease.
3. Next, conveying to the patient in the simplest terms possible that to achieve lasting solutions to the problem(s) for which the patient has come seeking help, a few fundamental questions must be asked and answered to understand the problem in the context of the patient's personal life. This framing of the interview process moves the endeavor from a left-brain compilation to a narrative that encourages insight—based on complex pattern recognition—about the root causes of the problem.
4. At this stage, control is shared with the patient: "Without your help, we cannot understand your medical problem in the depth and breadth you deserve." Implementing this shared investigation can be facilitated by certain approaches:
   a. For determining antecedent conditions, the following questions are useful:
      - When was the last time you felt well? When were you free of this problem?
      - What were the circumstances surrounding the appearance of the problem?
      - Have similar problems appeared in family members?

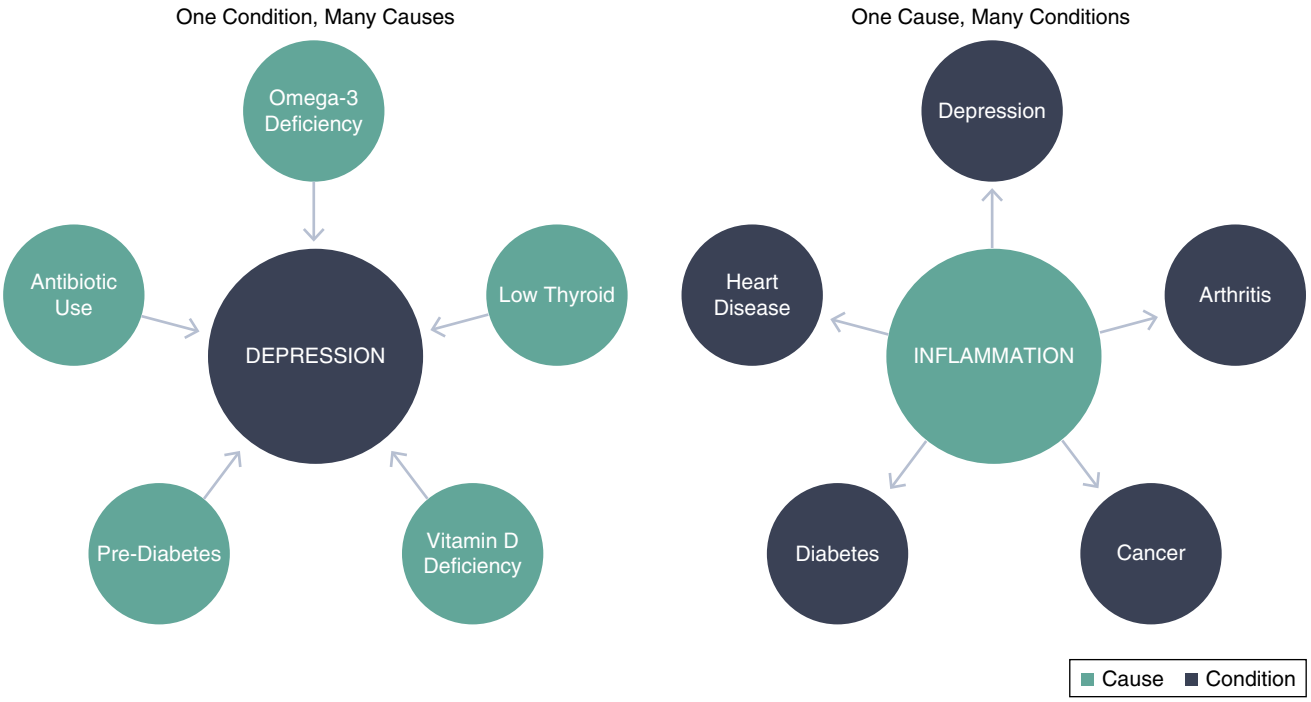


Fig. 1.5 Overview of the functional medicine (FM) model. (Courtesy the Institute for Functional Medicine.)

b. For triggers, the following question is critical:
   - What conditions, activities, or events seemed to initiate the problem? (Microbes and stressful personal events are examples but illustrate quite different categories of triggers. Triggers by themselves are usually insufficient for disease formation, so triggers must be viewed within the context of the antecedent conditions.)

c. Mediators of the problem are influences that help perpetuate it.
   - There can be specific mediators of diseases in the patient's activities, lifestyle, and environment. Many diverse factors can affect the host's response to stressors.
   - Any of the core clinical imbalances, discussed previously and shown on the Functional Medicine Matrix Model, can transform what might have been a temporary change in homeostasis into a chronic allostatic condition.

It helps at this juncture to emphasize again that the following issues are elemental in forming a healing partnership:

- Only the patient can inform the partnership about the conditions that provided the soil from which the problem(s) under examination emerged. The patient literally owns the keys to the joint deliberation that can provide insight into the process of achieving a healing outcome.
- The professional brings experience, wisdom, tools, and techniques and works to create the context for a healing insight to emerge.
- The patient's information, input, mindful pursuit of insight, and engagement become "the horse before the cart." The cart carries the clinician—the person who guides the journey using evidence, experience, and judgment and who contributes the potential for expert insight.

The crux of the healing partnership is an equal investment of focus by both clinician and patient. They work together to identify the right places to apply leverage for change. Patients must commit to engage both their left-brain skills and their right-brain function to inform and guide the exploration to the next steps in assessment, therapy, understanding, and insight. Clinicians must also engage both the left-brain computational skills and the right-brain pattern-recognition functions that, when used together, can generate insight about the patient's story. An overview of the functional medicine model is given in Fig. 1.5.

INTEGRATION OF CARE

Functional medicine explicitly recognizes that no single profession can cover all the viable therapeutic options. Interventions and practitioners will differ by training, licensure, specialty focus, and even by beliefs and ethnic heritage. However, all health care disciplines (and all medical specialties) can—to the degree allowed by their training and licensure and assuming a good background in Western medical science—use a functional medicine approach, including integrating the matrix as a basic template for organizing and coupling knowledge and data. Consequently, functional medicine can provide a common language, a flexible architecture, and a unified model to facilitate integrated and integrative care. Regardless of the discipline in which the clinician has been trained, developing a network of capable, collaborative practitioners with whom to comanage challenging patients and to whom referrals can be made for therapies outside the primary clinician's own expertise will enrich patient care and strengthen the clinician–patient relationship.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Goetzel RZ. Do prevention or treatment services save money? The wrong debate. *Health Aff*. 2009;28(1):37–41.
2. Probst-Hensch NM. Chronic age-related diseases share risk factors: do they share pathophysiological mechanisms and why does that matter? *Swiss Med Wkly*. 2010;140:w13072. Available at http://www.smw.ch/index.php?id=smw-2010-13072. Accessed October 11, 2010.
3. Magid CS. Developing tolerance for ambiguity. *JAMA*. 2001;285(1):88.
4. Rees J. Complex disease and the new clinical sciences. *Science*. 2002;296: 698–701.
5. Radford T. Top scientist warns of "sickness" in US health system. *BMJ*. 2003;326:416. https://doi.org/10.1136/bmj.326.7386.416/b.
6. Vioxx. Lessons for Health Canada and the FDA. *CMAJ*. 2005;172(11):5.
7. Juni P, Nartey L, Reichenbach S, et al. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet*. 2004;364:2021–2029.
8. This section was excerpted and adapted from Galland L. Patient-centered care: antecedents triggers, and mediators. In: *Textbook of Functional Medicine*, Ch. 8.
9. Cernak I, Savic VJ, Kotur J, et al. Characterization of plasma magnesium concentration and oxidative stress following graded traumatic brain injury in humans. *J Neurotrauma*. 2000;17(1):53–68.
10. Vink R, Nimmo AJ, Cernak I. An overview of new and novel pharmacotherapies for use in traumatic brain injury. *Clin Exp Pharmacol Physiol*. 2001;28(11):919–921.
11. Yu SL, Ho LM, Lim BC, Sim ML. Urinary deoxypyridinoline is a useful biochemical bone marker for the management of postmenopausal osteoporosis. *Ann Acad Med Singapore*. 1998;27(4):527–529.
12. Palomba S, Orio F, Colao A, et al. Effect of estrogen replacement plus low-dose alendronate treatment on bone density in surgically postmenopausal women with osteoporosis. *J Clin Endocrinol Metab*. 1002;87(4): 1502–1508.
13. Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, et al. Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARa. *J Lipid Res*. 1999;40:1426–1433.
14. Gaullier JM, Halse J, Hoye K, et al. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *Am J Clin Nutr*. 2004;79:1118–1125.
15. O'Shea M, Bassaganya-Riera J, Mohede IC. Immunomodulatory properties of conjugated linoleic acid. *Am J Clin Nutr*. 2004;79(S): 1199S–1206S.
16. Malloney F, Yeow TP, Mullen A, et al. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 DM. *Am J Clin Nutr*. 2004;80(4):887–895.
17. Riserus U, Vessby B, Arner P, Zethelius B. Supplementation with CLA induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. *Diabetalogia*. 2004;47(6):1016–1019.
18. Ames BN. The metabolic tune-up: metabolic harmony and disease prevention. *J Nutr*. 2003;133:1544S–1548S.
19. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased Km): relevance to genetic disease and polymorphisms. *Am J Clin Nutr*. 2002;75(4):616–658.
20. Bralley JA, Lord RS. *Laboratory Evaluations in Molecular Medicine: Nutrients, Toxicants and Metabolic Controls*. Atlanta: Institute for Advances in Molecular Medicine; 2001.
21. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kB pathway in the treatment of inflammation and cancer. *J Clin Invest*. 2001;107(2):135–142.
22. Tak PP, Firestein GS. NF-kB: a key role in inflammatory disease. *J Clin Invest*. 2001;107(1):7–11.
23. Balmer JE, Blomhoff R. Gene expression regulation by retinoic acid. *J Lipid Res*. 2002;43:1773–1808.
24. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular diseases. *Am J Clin Nutr*. 2004;80(suppl 6). 1678S–1688S.
25. Egnew TR. The meaning of healing: transcending suffering. *Ann Fam Med*. 2005;3(3):255–262.
26. Scott JG, Cohen D, DiCicco-Bloom B, et al. Understanding healing relationships in primary care. *Ann Fam Med*. 2008;6(4):315–322.
27. Miller WL, Crabtree BF, Duffy MB, et al. Research guidelines for assessing the impact of healing relationships in clinical medicine. *Altern Ther Health Med*. 2003;9(suppl 3):A80–A95.
28. Jackson C. Healing ourselves, healing others? first in a series. *Holist Nurs Pract*. 2004;18(2):67–81.
29. Acheson L. Community care, healing, and excellence in research. *Ann Fam Med*. 2008;6:290–291.
30. Hsu C, Phillips WR, Sherman KJ, et al. Healing in primary care: a vision shared by patients, physicians, nurses, and clinical staff. *Ann Fam Med*. 2008;6(4):307–314.
31. Brown M, Brown G, Sharma S. *Evidence-Based to Value-Based Medicine*. Chicago, IL: AMA Press; 2005.
32. Sackett DL, Straus SE, Richardson WS, Rosenberg W, Haynes RB. *Evidence-Based Medicine: How to Practice and Teach EBM* (3rd ed.). New York: Churchill Livingstone.
33. Fiore S, Schooler J. Right hemisphere contributions to creative problem solving: converging evidence for divergent thinking. In: Beeman M, Chiarello C, eds. *Right hemisphere language comprehension: perspectives from cognitive neuroscience*. Philadelphia, PA: Erlbaum Publishing; 1998:255–284.
34. Seger CA, Desmond JE, Glover GH, et al. fMRI evidence for right hemisphere involvement in processing unusual semantic relationships. *Neuropsychology*. 2000;14:361–369.
35. Beckman DB, et al. The effect of physician behavior on the collection of data. *Ann Intern Med*. 1984;101:692–696.
36. Lehrer J. The annals of science: the eureka hunt. *The New Yorker*. 2008:s40–s45.

BIBLIOGRAPHY

Galland L, Lafferty H. *Gastrointestinal Dysregulation: Connections to Chronic Disease. (Functional Medicine Monograph)*. Gig Harbor, WA: The Institute for Functional Medicine; 2008.

Hedaya R, Quinn S. Depression: advancing the paradigm (Functional Medicine Monograph). Gig Harbor, WA: The Institute for Functional Medicine. In: Jones DS, ed. *Textbook of functional medicine*. Gig Harbor, WA: The Institute for Functional Medicine; 2008.

Jones DS, Hofmann L, Quinn S. *21st Century Medicine: a New Model for Medical Education and Practice (White Paper)*. Gig Harbor, WA: The Institute for Functional Medicine; 2009.

Lukaczer D, Jones DS, Lerman RH, et al. *Clinical Nutrition: A Functional Approach*. 2nd ed. Gig Harbor, WA: The Institute for Functional Medicine; 2004.

Vasquez A. *Musculoskeletal Pain: Expanded Clinical Strategies (Functional Medicine Monograph)*. Gig Harbor, WA: The Institute for Functional Medicine; 2008.

2

A Hierarchy of Healing: The Therapeutic Order

A Unifying Theory of Naturopathic Medicine

Stephen P. Myers, ND, BMed PhD, Pamela Snider, ND, Jared Zeff, ND, and *Zora DeGrandpre*, MS, ND*

OUTLINE

A BRIEF HISTORY OF NATUROPATHIC MEDICINE[1]

In 1900 Benedict Lust "invented" naturopathy, as an eclectic practice that combined many natural therapies and therapeutic systems under the umbrella of a comprehensive philosophy and system of practice based on the European nature cure movement that flourished in the 1800s. At the core of this philosophy was the *vis medicatrix naturae* (healing power of nature) and the naturalistic concept of vitalism. As such, naturopathic medicine has deep historical roots and represents a lineage of Western natural medicine that can be traced back to the Roman, Greek, Egyptian, and Mesopotamian cultures and, conceptually, to many traditional and indigenous world medicines.

The modern naturopathic profession originated with Lust, and it grew under his tireless efforts. He crisscrossed the United States, lecturing and lobbying for legislation to license naturopathy, testifying for naturopaths indicted for practicing medicine without a license, and traveling to many events and conferences to help build the profession. He also wrote extensively, including two monthly newspapers (*The Naturopath* and *Herald of Health*) for nearly 40 years, to foster and popularize the profession, and through his efforts, the naturopathic profession grew rapidly.[2–4] By the 1940s, naturopathic medicine had developed a number of 4-year medical schools and had achieved licensure in about one third of the United States, the District of Columbia, four Canadian provinces, and a number of other countries.[3,5]

*Previous edition contributor

The profession went through a period of decline, marked with internal disunity and paralleled by the rise of biomedicine and the promise of wonder drugs. By 1957, there was only one naturopathic college left. By 1975, only eight states still licensed naturopathic physicians, and by 1979, there were only six. A survey conducted in 1980 revealed that there were only about 175 naturopathic practitioners still licensed and practicing in the United States and Canada.[6] In contrast, in 1951, the number was approximately 3000.[7]

The decline of naturopathic medicine after a rapid rise was due to several factors. By the 1930s, a significant tension developed within the profession regarding clinical naturopathic practice based on traditional principles; the development of unified standards; and the role of experimental, reductionist science as an element of professional development.[8,9] Many naturopathic doctors questioned the capacity for the reductionist scientific paradigm to research naturopathic medicine objectively in its full scope.[8,10,11] This tension split the profession of naturopathic physicians from within after the death of Lust in the late 1940s, at a time when the profession was subject to both significant external forces and internal leadership challenges.

This perception created a mistrust of science and research. Science was also frequently used as a bludgeon against naturopathic medicine, and the biases inherent in what became the dominant paradigm of scientific reductionism made a culture of scientific progress in the profession challenging. The discovery of effective antibiotics elevated the standard medical profession to dominant and unquestioned stature by

a culture that turned to mechanistic science as an unquestioned authority. The dawning of the atomic age reinforced a fundamental place for science in a society increasingly dominated by scientific discovery. In this culture, standard medicine, with its growing political and economic strength, was able to force the near elimination of naturopathic medicine through the repeal or "sunsetting" of licensure acts.[2,3,12]

In 1956, as the last early doctor of naturopathy (ND) educational program ended (at the Western States College of Chiropractic), several doctors, including Drs. Ralph Weiss, Charles Stone, W. Martin Bleything, and Frank Spaulding, created the National College of Naturopathic Medicine in Portland, Oregon, to keep the profession alive. However, that school was nearly invisible as the last vestige of a dying profession and rarely attracted as many as 10 new students a year. The profession was considered dead by its historical adversaries.

The culture of America, dominated by standard medicine since the 1940s, however, began to change by the late 1960s. The promise of science and antibiotics was beginning to seem less than perfect. Chronic disease was increasing in prevalence as acute infection was becoming less predominant, and standard medicine had no "penicillin" for chronic diseases. In the late 1970s, scholars in family medicine proposed a biopsychosocial model of care in response to a prevailing perception of a growing crisis in standard medicine.[13] The publication of Engel's "The Need for a New Medical Model" in April 1977 signaled the founding of the field of family medicine based on a holistic philosophy. This shifting culture within standard medicine paralleled a broader social movement in support of alternative health practices and environmental awareness. Elements of the culture were rebelling against plastics and cheap synthetics, seeking more natural solutions. The publication of Rachael Carson's *Silent Spring* in 1962, an indictment of chemical pesticides and environmental damage, marked a turning point in cultural thinking. In *Silent Spring,* Carson challenged the practices of agricultural scientists and the government and called for a change in the way humankind viewed the natural world.[14] New evidence of the dangers of radiation, synthetic pesticides, and herbicides and environmental degradation from industrial pollution was creating a new ethic. Organic farming, natural fibers, and other similar possibilities were starting to capture attention. A few began seeking natural alternatives in medicine. By the late 1960s and early 1970s, enrollments at the National College of Naturopathic Medicine began to reach into the 20s. The 1974 class numbered 23 students. In 1975 the National College enrolled a class of 63 students.[15] The profession was experiencing a resurgence.

In 1978, with a desire to create a college based on science-based natural medicine, Joseph E. Pizzorno, ND, LM, and his colleagues—Les Griffith, ND, LM; Bill Mitchell, ND; and Sheila Quinn—created the John Bastyr College of Naturopathic Medicine in Seattle, Washington. With the creation of Bastyr, named after the eminent naturopathic physician Dr. John Bartholomew Bastyr (1912–1995), the profession entered a new phase. Not only did this new college double the profession's capacity to produce new doctors, but it also firmly placed the profession on the ground of scientific research and validation. "Science-based natural medicine," coined by Dr. Pizzorno, was a major driving force behind the creation and mission of Bastyr. Both Drs. Bastyr and Pizzorno had significant influence and leadership in achieving this focus.

One of Dr. Bastyr's important legacies was to establish a foundation and a model for reconciling the perceived conflict between science and the deeply established healing practices and principles of naturopathic medicine. Kirchfeld and Boyle[4] described his landmark contribution as follows:

> *Although naturopathic colleges in the early 1900s did include basic sciences training, it was not until Dr. John Bastyr (1912–1995) and his firm, efficient and professional leadership that science and research-based training in natural medicine was inspired to reach its fullest potential. Dr. Bastyr, whose vision was one of "naturopathy's empirical successes documented and proven by scientific methods," was himself the prototype of the modern naturopathic doctor, who culls the latest findings from the scientific literature, applies them in ways consistent with naturopathic principles and verifies the results with appropriate studies.*

Bastyr also saw a tremendous expansion in both allopathic and naturopathic medical knowledge, and he played a major role in making sure the best of both were integrated into naturopathic medical education.[4,16]

Bastyr met Lust on two occasions and was closely tied to the nature cure tradition of Kneipp through two influential women: his mother, and his mentor, Dr. Elizabeth Peters, who studied with Father Kneipp. He effortlessly integrated the clinical theories and practices of naturopathy with the latest scientific studies and helped create a new and truly original form of modern primary clinical care within naturopathic medicine. He spent the 20th century preparing the nature cure of the 19th century for entry into the 21st century.[2,16] Today's philosophic debates within the profession are no longer about science. They now tend to center on both sides of the earlier debate and include challenges to the nature cure tradition. A current debate, for instance, is about the role of "green allopathy" within the profession: the tendency to use botanical medicine or nutritional supplements as a simple "green drug" or pharmaceutical replacement therapy. This is in contrast to implementing the full range of healing practices derived from the nature cure tradition and within the framework of the therapeutic order construct to stimulate health restoration as the foundation for reversing disease, alongside, or instead of, botanical medicine or nutritional supplements. Professional consensus appears strong that the full range of naturopathic healing practices must be retained, strengthened, and engaged in the process of education and scientific research and discovery in the 21st century.[17–19]

ORIGINAL PHILOSOPHY AND THEORY

Through the initial 50-year period of professional growth and development (1896–1945), naturopathic medicine had no clear and concise statement of identity. The profession was whatever Lust said it was. He defined "naturopathy" or "nature cure" as both a way of life and a concept of healing that used various natural means of treating human infirmities and disease states. The "natural means" were integrated into naturopathic medicine by Lust and others based on the emerging naturopathic theory of healing and disease etiology. The earliest therapies associated with the term involved a combination of American hygienics and Austro-Germanic nature cure and hydrotherapy. Leaders in this field included Kuhne, Lindlahr, Trall, Kellogg, Holbrook, Tilden, Graham, McFadden, Rikli, Thomson, and others who wrote foundational naturopathic medical treatises or developed naturopathic clinical theory, philosophy, and texts to enhance, agree with, and diverge from Lust's original work.[20–28]

The bulk of professional theory was found in Lust's magazines, *Herald of Health* and *The Naturopath.* These publications displayed the prodigious writings of Lust but did not contain a comprehensive and definitive statement of either philosophy or clinical theory. Lust often stated that all natural therapies fell under the purview of naturopathy. Several texts were considered as somewhat definitive by various aspects of the profession at different times. These texts included Adolph Just's *Return to Nature* (1896), Louis Kuhne's *The New Science of Healing* (1899), and the seven-volume *Natural Therapeutics* by Henry Lindlahr, MD, which was published in the early 1900s. Lindlahr's *Nature Cure* (1913) was considered a seminal work in naturopathic theory, laying

the groundwork for a systematic approach to naturopathic treatment and diagnosis. Lindlahr ultimately presented the most coherent naturopathic theory extant, summarized in his *Catechism of Nature Cure,* which presented a five-part therapeutic progression:

1. "Return to nature"—attending to the basics of diet, dress, exercise, rest, etc.
2. Elementary remedies—water, air, light, electricity
3. Chemical remedies—botanicals, homeopathy, etc.
4. Mechanical remedies—manipulations, massage, etc.
5. Mental/spiritual remedies—prayer, positive thinking, doing good works, etc.[29]

Lindlahr's five-step therapeutic progression follows the *Catechism's* disease causation model: "The primary cause of disease, barring accidental or surgical injury to the human organism and surroundings hostile to human life, is violation of Nature's Laws." The effects of violation of nature's laws on the physical human organism are also the primary causes of disease because they inhibit normal function, lower vitality, and result in tissue destruction:

Primary
- Lowered vitality
- Abnormal composition of blood and lymph
- Accumulation of waste, morbid matter, and poisons in the system

Secondary
- Hereditary/constitutional
- Fevers, inflammation
- Mechanical luxations
- Weakening and loss of reason, will, etc.[29]

In 1948 Spitler wrote *Basic Naturopathy, a Textbook,*[10] and in 1951 Wendel wrote *Standardized Naturopathy.*[11] These texts presented somewhat different approaches; Spitler's text emphasized theory and philosophy, whereas Wendel's text was written, as evidenced by the title, to emphasize the standard naturopathic practices of the day, with an eye toward regulatory practice. In contrast, Kuts-Cheraux's *Naturopathic Materia Medica,* written in 1953, was produced to satisfy a statutory demand by the Arizona legislature but persisted as one of the few extant guides of that era. Practitioners relied on a number of earlier texts, many of which arose from the German hydrotherapy practitioners[30–35] or the eclectic school of medicine (a refinement and expansion of the earlier "Thomsonian" system of medicine)[36–40] and predated the formal American naturopathic profession (1900). However, by the late 1950s, publications diminished. The profession was generally considered on its last gasp, an anachronism of the pre-antibiotic era.

During the process of winning licensure, naturopathic medicine was defined formally by the various licensure statutes, but these definitions were legal and scope-of-practice definitions, often in conflict with each other, reflecting different standards of practice in different jurisdictions. In 1965 the U.S. Department of Labor's *Dictionary of Occupational Titles*[41] presented the most formal and widespread definition. The definition was not without controversy because it reflected one of the internally competing views of the profession, primarily, the nature cure perspective:

> *Diagnoses, treats and cares for patients using a system of practice that bases treatment of physiological function and abnormal conditions on natural laws governing the human body. Utilizes physiological, psychological and mechanical methods such as air, water, light, heat, earth, phytotherapy, food and herbs therapy, psychotherapy, electrotherapy, physiotherapy, minor and orificial therapy, mechanotherapy, naturopathic corrections and manipulations, and natural methods or modalities together with natural medicines, natural processed food and herbs and natural remedies. Excludes major surgery, therapeutic use of x-ray and radium, and the use of drugs, except those assimilable substances containing elements or compounds which are components of body tissues and physiologically compatible to body processes for the maintenance of life.*[41]

This definition did not list drugs or surgery within the scope of modalities available to the profession. It defined the profession by therapeutic modality and was more limited than most of the statutes under which naturopathic physicians practiced,[42] even in 1975, when there were only eight licensing authorities still active.

MODERN NATUROPATHIC CLINICAL THEORY: THE PROCESS OF DEVELOPMENT

> *Medical philosophy comprises the underlying premises on which a health care system is based. Once a system is acknowledged, it is subject to debate. In Naturopathic medicine, the philosophical debates are a valuable, ongoing process which helps the understanding of health and disease evolve in an orderly and truth-revealing fashion.*
>
> ***Randall Bradley, ND***[43]

After the profession's decline in the 1950s and 1960s, a rebirth was experienced, more grounded in medical sciences and fueled by a young generation with few teachers. The profession's roots were neglected out of ignorance, for the most part, along with a youthful arrogance. By the early 1980s, it was apparent that attempts to regenerate the progress made by Lust would require the creation of a unified professional organization and all which that entailed: accreditation for schools, national standards in education and licensure, clinical research, and the articulation of a coherent definition of the profession for legislative purposes, as well as for its own internal development. These accomplishments would be necessary to be able to demonstrate the uniqueness and validity of the profession, guide its educational process, and justify its status as a separate and distinct medical profession.

In 1987 the newly formed (1985) American Association of Naturopathic Physicians (AANP) began this task of developing a unified professional organization under the leadership of James Sensenig, ND (president), and Cathy Rogers, ND (vice president). Four tasks were developed, and committees with specific chairs were delegated. One task was to pursue accreditation of our schools through governmental accreditation bodies, headed by Joe Pizzorno, ND. Another was to create a standard, national licensure examination, independent of the profession, headed by Edwin Smith, ND. A third was to create a peer-reviewed journal that the profession could use to demonstrate its rational basis, headed by Peter D'Adamo, ND. The fourth was a committee to head the creation of a new definition of naturopathic medicine headed by Pamela Snider, ND, and Jared Zeff ND, LAc. The "Select Committee on the Definition of Naturopathic Medicine" succeeded in its 3-year project, which culminated in the unanimous adoption by the AANP's House of Delegates (HOD) of a comprehensive, consensus definition of naturopathic medicine in 1989 at the annual convention held at Rippling River, Oregon.[44–46] The unique aspect of this definition was its basis in definitive principles, rather than therapeutic modalities, as the defining characteristics of the profession. In passing this resolution, the HOD also asserted that the principles would continue to evolve with the progress of knowledge and should be formally reexamined by the profession as needed, perhaps every 5 years.[44–49]

In September 1996 the AANP HOD passed a resolution to review three proposed principles of practice that had been recommended as

additions to the AANP definition of naturopathic medicine originally passed by the HOD in 1989. These three new proposed principles were rejected, and the AANP HOD reconfirmed the 1989 AANP definition unanimously in 2000. The results of a profession-wide survey conducted from 1996 to 1998 on these three new proposed principles demonstrated that although there was lively input, the profession agreed strongly that the original definition was accurate and should remain intact. The HOD recommended that the discussion be moved to the academic community involved in clinical theory, research, and practice for pursuit through scholarly dialogue.[50–54] This formed the basis for further efforts to articulate a clinical theory. AANP members stated in 1987 to 1989 during the definition process: "These principles are the skeleton, the core of naturopathic theory. There will be more growth from this foundation."[46] By 1997, this growth in modern clinical theory was evident.

The first statement of such a theory was published in the AANP's *Journal of Naturopathic Medicine* in 1997 in an article titled "The Process of Healing, a Unifying Theory of Naturopathic Medicine."[55] This article contained three fundamental concepts that were presented as an organizing theory for the many therapeutic systems and modalities used within the profession and were based on the principles articulated in the consensus AANP definition of naturopathic medicine. The first of these was the characterization of disease as a process rather than a pathological entity. The second was the focus on the determinants of health rather than on pathology. The third was the concept of a therapeutic hierarchy.

This article also signaled the emergence of a growing dialogue among physicians, faculty, leaders, and scholars of naturopathic philosophy concerning theory in naturopathic medicine. The hope and dialogue sparked by this article were the natural next step of a profession redefining itself both in the light of today's advances in health care and with respect to the foundations of philosophy at the traditional heart of naturopathic medicine. This dialogue naturally followed the discussions of the definition process and created a vehicle for emerging models and concepts to be built on the bones of the principles. The essence and inherent concepts of traditional naturopathic philosophy were carried in the hearts and minds of a new generation of naturopathic physicians into the 21st century—these modern naturopathic students and naturopathic physicians began to gather to articulate, redefine, and reunify the heart of the medicine.

This new dialogue was formally launched in 1996, when the AANP Convention opened with the plenary session "Towards a Unifying Theory of Naturopathic Medicine," with four naturopathic physicians presenting facets of emerging modern naturopathic theory. The session closed with an open microphone. The impassioned and powerful comments of the naturopathic profession throughout the United States and Canada engaged in the vital process of deepening and clarifying its unifying theory. Dr. Zeff presented "The Process of Healing: The Hierarchy of Therapeutics"; Dr. Mitchell presented "The Physics of Adjacency, Intention, Naturopathic Medicine, and Gaia"; Dr. Sensenig presented "Back to the Future: Reintroducing Vitalism as a New Paradigm"; and Dr. Snider announced the Integration Project, inviting the profession to engage in it by "sharing a beautiful and inspiring anguish—the labor pains of naturopathic theory in the twenty-first century. We know what we have done, and we know there is much more…The foundation is laid. We are ready now for development and integration."[56]

Days later, in September 1996, the Consortium of Naturopathic Medical Colleges (now the American Association of Naturopathic Medical Colleges [AANMC]) formally adopted and launched the Integration Project, an initiative to integrate naturopathic theory and philosophy throughout all divisions of all naturopathic college curricula, from basic sciences to clinical training. A key element of the project engaged the further development and refinement of naturopathic theory. The project was cochaired by Drs. Snider and Zeff from 1996 to 2003. Steering members from all North American naturopathic colleges participated and contributed.[46] Methods included professional and scholarly research, expert teams, symposiums, and training. The result was the fostering of systematic inquiry among academicians, clinicians, and researchers concerning the underlying theory of naturopathic medicine, bringing the fruits of this work and inquiry into the classroom and into scientific discussion.[57]

The Integration Project sustained both formal and informal dialogue since its inception in 1996, which continues today through the Foundations of Naturopathic Medicine Institute. The work has engaged faculty and scholars of naturopathic philosophy in the United States, Canada, the United Kingdom, Australia, and many other countries where naturopathy is established or is professionalizing. It has also engaged institutional leaders and practicing doctors and faculty in all areas of the profession. Why? Naturopathic philosophy is deeply felt as the "commons" of naturopathic medicine: a place where the profession meets—one that is owned by all naturopathic physicians—that reflects, holds, and deepens the heart of naturopathic medicine. The philosophy of naturopathic medicine *is* the foundation and heart of naturopathic medicine and consists of its heritage, knowledge base, concepts, and knowledge codification; its clinical decision making; its integration and initiation of scientific research; and its public policy positions. The philosophy remains valid by evolving with the progress of knowledge, the progress of science, and the progress of the human spirit. It is for this reason medicine is seen as an art and a science. Because naturopathic philosophy engages the intuitively felt mission of nature doctors, it is vital that the profession periodically gathers to renew and revitalize progress regarding its unifying foundations.

The Integration Project sparked a wide range of activities in all six ND colleges at that time, resulting in all-college retreats to share tools, retreats for training of non-ND faculty in naturopathic philosophy, integration of a basic sciences curriculum, expert-team revision of core competencies across departments ranging from nutrition to case management and counseling, development of clinical tools and seminars for clinic faculty, creation of new courses, and the integration of important research questions derived from naturopathic philosophy into research studies and initiatives.[58] The latest effort, the Foundations of Naturopathic Medicine Institute and Project (textbook codification and symposia series; see www.foundationsproject.com) includes its development and presentation of the founding educational module on emunctorology, an essentially naturopathic science, during 2009 and 2010. This is a joint effort of faculty from several of our schools, led by Drs. Thom Kruzel, Rita Bettenberg and Stephen Myers.

North American core competencies for naturopathic philosophy and clinical theory were developed by faculty representing all accredited ND colleges in a landmark AANMC retreat in 2000. The AANMC's Dean's Council formally adopted these competencies in 2000 and recommended that they be integrated throughout curricula in all ND colleges. These national core competencies included the process of healing theory, Lindlahr's model, and the hierarchy of therapeutics (the therapeutic order).[59,60]

Finally, many meetings with scholars and teachers of naturopathic theory and other faculty and leaders—formal and informal—resulted in the further development and refinement of the hierarchy of therapeutics developed by Dr. Zeff in 1997.

Drs. Snider and Zeff and worked closely with each other and then with other naturopathic theory faculty from AANMC colleges in a

BOX 2.1 Working Definition of Naturopathic Nutrition

Consensus Statement from Naturopathic Nutrition Faculty Retreat, Naturopathy and Nutrition Panel and Southern Cross University, June 2003, Preamble

Naturopathic medicine is a distinct system of primary health care—an art, science, philosophy and practice of diagnosis, as well as treatment and prevention of illness. Naturopathic medicine is distinguished by the principles that underlie and determine its practice. These principles include the healing power of nature *(vis medicatrix naturae),* identification and treatment of the causes *(tolle causam),* the promise to first do no harm *(primum non nocere),* doctor as teacher *(docere),* treatment of the whole person, and emphasis on prevention. These principles give rise to a practice that emphasizes the individual and empowers him or her to greater responsibility in personal health care and maintenance.

Definition

Naturopathic nutrition is the practice of nutrition in the context of naturopathic medicine.

Naturopathic nutrition integrates both scientific nutrition and the principles of naturopathic medicine into a distinct approach to nutritional practice.

Core components of naturopathic nutrition are:

Respect for the traditional and empirical naturopathic approach to nutritional knowledge

The value of food as medicine

An understanding that whole foods are greater than the sum of their parts and recognition that they have vitality (properties beyond physiochemical constituents)

Individuals have unique interactions with their nutritional environments

Practice

In the context of the definition, and with respect to the therapeutic order, the practice of naturopathic nutrition may include the appropriate use of the following:

Behavioral and lifestyle counseling

Diet therapy (including health maintenance, therapeutic diets, and dietary modification)

Food selection, preparation, and medicinal cooking

Therapeutic application of foods with specific functions

Traditional approaches to detoxification

Therapeutic fasting strategies

Nutritional supplementation

series of revisions. Drs. Snider and Zeff collaborated in 1998 to develop the hierarchy of therapeutics into the "therapeutic order." The therapeutic order was subsequently explored and refined through a series of faculty retreats and meetings, as well as through experience with students and through student feedback. A key finding of the clinical faculty at Bastyr University was the emphasis on the principle "holism: treat the whole person" and respect for the patient's own unique healing order and his or her values as a context for applying the therapeutic order to clinical decision making.[61] The therapeutic order, or hierarchy of healing, is now incorporated into ND college curricula throughout the United States, Canada, Australia, and New Zealand. For example, an important international outgrowth of the profession's development of theory is the adoption of the unified "Working Definition of Naturopathic Nutrition" in June 2003 by the Australian naturopathic profession (Box 2.1). The 3-year project, fostered by Dr. Stephen Myers, brought together nutrition faculty from naturopathic medicine colleges throughout Australia. The project was cohosted by the Naturopathy and Nutrition Panel, an independent group of naturopaths and nutrition educators whose mission is to foster and support the development of the science, teaching, and practice of naturopathic nutrition, and the School of Natural and Complementary Medicine at Southern Cross University. The definition evolved over two retreats attended by more than 40 faculty members involved in teaching nutrition as part of a naturopathic medicine education. It commenced as a general agreement within the group that there was a real and distinct difference between conventional nutritional concepts and naturopathic nutritional theory. The general agreement was that the distinction between the two had been poorly defined to date and had been the source of dissonance between the naturopathic and science faculty within the colleges. The obvious next step was to define that difference to ensure that nutrition curriculum within naturopathic medicine colleges reflected the core elements of naturopathic nutrition. At the second retreat held in June 2003, the working definition was adopted with a recommendation that it be widely circulated within the naturopathic medicine profession to commence a dialogue aimed at both appropriate revision and broad adoption. This process created a much-needed consensus definition of naturopathic nutrition. This definition is based on the AANP defining principles and incorporates the therapeutic order theory.

The AANP Definition of Naturopathic Medicine position paper was reviewed again in 2010 and reratified unanimously in 2011 by the AANP House of Delegates. "Prescription medications" were added to the single Treatment and Care section and both Naturopathic Practice sections in the 5-page paper (see www.naturopathic.org).

In 2015 the World Naturopathic Federation, founded in 2015, published its first international survey results on naturopathic medicine's core concepts and education in two white papers: *The World Naturopathic Federation Report: Findings From the 1st World Naturopathic Federation Survey* (2015) and *WNF White Paper: Philosophies, and Principles, Theories* (2017). The therapeutic order was reported in the top three (2015) and top five (2017) theory concepts utilized by the profession across the world. The results of the 2015 report stated that the AANP Definition of Naturopathic Medicine position paper and its six principles were widely accepted as written by professionalizing naturopaths in countries responding to the survey, at an average rate of 95%.[62–65]

A THEORY OF NATUROPATHIC MEDICINE

Standard medicine, or biomedicine, has a simple and elegant paradigm. Simply stated, it is "the diagnosis and treatment of disease." In practice, this statement contains several assumptions. One assumption is that illness can be understood in terms of discrete diseases (i.e., human illnesses can be divided into identifiable entities, such as measles or specific forms of cancer, etc.). The next assumption is that "cure" is the elimination of the disease entity. The third assumption is that this is accomplished by the evidence-based application of pharmaceuticals, surgeries, or similar treatments to eliminate, palliate, or suppress the entity and its symptomatic expressions. These are so obvious that they are not commonly considered. They form the background thinking in medical decision making: "identify and treat the disease."

The elegance of this model, and the science behind it, has taken standard medicine to its highest point in history as a reliable vehicle to ease human illness, and its application has saved countless lives. The understanding of the physician, at least about the nature of pathology, has never been as complete as now. However, illness has a near-infinite capacity to baffle the physician. New diseases arise, such as Legionnaire's disease, human immunodeficiency virus/acquired immune deficiency syndrome, and Lyme disease, and shifts occur in disease focus, such as the shift between 1900 and 2000 from acute infection to chronic illness as the predominant cause of death.[66]

Beyond these obvious changes, even with the current depth of understanding, the standard medical world often lacks the ability to effectively understand and cure chronic disease, and treatment tends to become a task of the management of symptoms and the attempt to reduce long-term damage and other consequences rather than actual cure of the illness. So, even representing an apex of human achievement as it does, standard medicine is not without its weaknesses. Its greatest weaknesses include its relatively high cost,[67] its tendency to create iatrogenic disease,[67] and its inability to cure chronic illness as easily as it once cured pneumonia with penicillin or tuberculosis with streptomycin. Compounding the problem is the growing prevalence of antibiotic-resistant infections.[68,69] Part of the reason for the failures within biomedical science is its mechanistic basis. Breaking the body down to its constituent parts has led to a fundamental ignorance of and disrespect for the wholeness of the individual, the natural laws of physiology governing health and healing, and particularly for all things spiritual (the transpersonal domains). Inherent in the dictum—diagnose and treat the disease—is the general neglect of the larger understanding that disease is a process conducted by and within an intelligent organism that is constantly attempting to heal itself, with disease manifestations often being expressions of this self-healing endeavor. As noted by Pizzorno et al.,[70] this intelligent organism strives for optimal function and health. Human beings "are natural organisms, our genomes developed and expressed in the natural world. The patterns and processes inherent in nature are inherent in us. We exist as a part of complex patterns of matter, energy, and spirit. Nature doctors have observed the natural processes of these patterns in health and disease and determined that there is an inherent drive toward health that lives within the patterns and processes of nature."

The uniqueness of naturopathic medicine is not in its therapeutic modalities or the "natural" alternatives to the drugs and surgeries of standard medicine. It is the clinical theory that governs the selection and application of these modalities, captured in the unifying definition adopted in 1989 and expressed more specifically in the continuing articulation of clinical theory. That is, it is the way the naturopathic physician thinks about illness and healing.

The first element of this theory is based on the first defining principle: *vis medicatrix naturae.* It is based on the understanding that disease can be seen as a process and an entity. One can analyze the process of illness and derive some understanding. However, to do this, one needs to examine the assumptions underlying this concept. The governing assumptions of standard medicine are principally that diseases are entities and that drugs and surgery can eliminate these entities from the suffering person. These are not the governing assumptions of naturopathic medicine.

Illness and Healing as Process

Naturopathic medicine can be characterized by a different model than "identify and treat the disease." "The restoration of health" would be a better characterization. Naturopathic physicians adopted the following elegantly brief definition of naturopathic medicine in 1989 in an AANP position paper: "Naturopathic physicians treat disease by restoring health."[45] Immediately, a significant difference is made clear: standard medicine is disease based; naturopathic medicine is health based. Although naturopathic medical students study pathology with the same intensity and depth as standard medical students, as well as its concomitant diagnoses, the naturopathic medical student learns to apply that information in a different context. In standard medicine, pathology and diagnosis are the basis for the discernment of the disease "entity" that afflicts the patient, the first of the two steps of identifying and destroying the entity of affliction. In naturopathic medicine, however, disease is seen much more as a process than as an entity. Rather than viewing the ill patient as experiencing a "disease," the naturopathic physician views the ill person as functioning within a process of disturbance and recovery, in the context of nature and natural systems. Various factors disturb normal health. If the physician can identify these disturbances and moderate them (or at least some of them), the illness and its effects abate. As disturbances are removed, the body can improve in function, and in doing so, health naturally improves. The natural tendency of the body is to maintain itself in as normal a state of health as is possible—this is the basis of homeostatic principles.[71] The role of the physician facilitates this self-healing process.

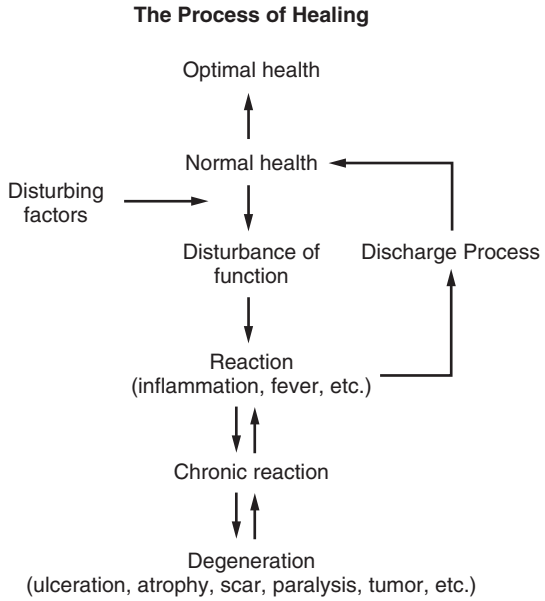


Fig. 2.1 The process of healing. Copyright 1997. (Used by permission. Jared L. Zeff, ND, LAc.)

The obvious first task of the naturopathic physician, therefore, is to determine what is disturbing the health so that these causative elements may be ameliorated. Disease is the process whereby the intelligent body reacts to disturbing elements. It employs such processes as inflammation and fever to help restore its health. In general, one can graph this process simply, as in Fig. 2.1.

The Naturopathic Model in Acute Illness

One can see "illness as process" most easily in the common cold. Within standard medical understanding, the common cold is caused by a virus, from among a family of pathological viruses that can infect a person. The immune system responds, developing appropriate antibodies, which eventually neutralize the virus. There is no "cure" yet discovered, except time. Medications are used to ameliorate the symptomatic experience: aspirin or acetaminophen for fever, decongestants to dry the mucus discharge, and so forth. These measures are not cures; they reduce the symptomatic expression of the "cold" but often lengthen the process. In naturopathic medicine, the cold is seen not as a disease entity but as part of a fundamental process whereby the body restores itself to health.

If the virus were the sole cause of the common cold, then everyone who came into contact with a sufficient dose of the virus would get the cold. Obviously, this does not happen. Susceptibility factors include immune competence, fatigue, vitality, genetics, and other host factors.[72] The virus enters a milieu in which all these factors affect the process. Once the virus enters the system, and if it overcomes resistance factors (Box 2.2), one begins to see disturbance of function, as illustrated in Fig. 2.1. One does not feel quite right. One may

BOX 2.2 Scientific Considerations: The Immune Response and Resistance Factors

Once inside the body, the rhinovirus binds to cellular receptors (primarily the intercellular adhesion molecule-1 [ICAM-1]) or to the low-density lipoprotein (LDL) receptor. The viral particles are then internalized and begin to take over the cellular machinery to produce intact virions.[72,103] At this stage, the body can sometimes mount an adequate defense via cell-mediated immunity to overcome the viral incursion. If we have been previously exposed to the virus, the body's humoral immune response will rapidly produce antibodies to the viral protein, which can also lead to eradication of the microbe. These two immune responses explain why some individuals may develop the full condition, whereas others will shake off the exposure within a few hours. If the viral load overcomes the body's innate defenses, the virus replicates unabated. In the process of replication, the virus not only disrupts the cellular mechanisms but also damages them as well by infecting the surface epithelium and the macrophages[104] and fibroblasts.[105] Naturopathic physicians are interested in the factors that lead to greater immune competence and health restoration through the process of healing and the health practices that support it. French physiologist Claude Bernard (1813–1878) said that the inner terrain or "milieu interieur" was the cause of disease, not the microbes; this concept underpins the naturopathic approach.

BOX 2.3 Scientific Considerations: Consequences of Suppressing the Body's Response

Current research shows that future pathologies may be linked to "suppression" of early rhinovirus infection. These include childhood asthma, adult asthma, and chronic obstructive pulmonary disease (COPD).[106,107] Individuals with asthma are known to have subtle deficiencies in production of type I and type III interferon (IFN),[108,109] indicating that for some asthma patients, early exposure to the rhinovirus predisposes them to asthma, and that the suppression of the normal response may be critical in the future development of asthma. With these effects in mind, the naturopathic physician does not look solely at the virus as a pathogenic entity but also seeks to determine how the patient responds to the virus, thereby determining the most reasonable approach to aiding the patient's natural responses and moderating the patient's long-term health strategies. Suppression of the body's natural responses is avoided. The long-term use of corticosteroids is a prime example of suppression and its consequences.[137,140]

begin to get a sore throat, the first inflammatory reaction, occurring at the point of entry of the virus into the body. The immune factors described may overcome the virus at this point, may be insufficient, or may be suppressed. All of this is mutable to some extent and is affected by host factors, such as nutritional status and fatigue, and can be influenced by taking immune tonics, vitamin C, and other supplements.

To the individual with the condition, the "cold" may proceed into a general state of fatigue and inflammation, possibly fever followed by mucus discharge, cough, and other symptoms, as the body processes and responds to the virus and its effects; eventually, the body overcomes it and eliminates the results.

In the naturopathic model, the cold is not understood so much to be a separate disease entity but a general and fundamental process of disturbance and recovery within the living body. It is a method whereby the body restores itself after a sufficient amount of disturbance accumulates within the system. This is why the cold has no "cure." It is the *cure* for what ails the body. In the naturopathic model of health, it is the support of this "adaptive response"—the restoration of balance that is the central point—through which the process is the "cure" (Box 2.3).

BOX 2.4 Scientific Considerations: The Role of Environment in Chronic Illness

Environmental and lifestyle disturbances are a profound driver in the naturopathic model of health. The scientific evidence is now irrefutable that the national and global burden of chronic disease is highly dependent on modifiable behavioral factors. In a recent study of the causes of death, it was found that tobacco, poor diet and lack of physical activity, alcohol and drug use, toxic agents, and vehicular and firearm incidents were the leading actual causes of death.[76] Other factors included frank malnutrition (as opposed to poor nutrition), unsafe sexual practices, and poor sanitation.[77,78] It has been definitively shown, for example, that diet and lifestyle changes can prevent some forms of diabetes[101,102] and other chronic diseases[142,143] that are leading causes of death in the United States.[76,101,102]

The early naturopathic philosophers and clinicians predicted that the treatment of acute disease by suppressing symptoms (discussed in more depth later in the chapter) would result in more chronic disease. The current disease burden in the Western world certainly confirms this century-old prediction.

The Naturopathic Model in Chronic Illness

Chronic illness arises, in general, when any or all of three factors occur:

1. The disturbing factors persist, such as a chronically improper diet, which continues to burden the body cumulatively, as the digestive processes slowly weaken under the stress of the improper or inadequate diet.
2. The reactive potential is blocked or suppressed, often by drugs, which interfere with the capacity of the body to process and remove its disturbances.
3. The vitality of the system is insufficient, or has become too overwhelmed, to mount a significant and sufficient reaction.

Again, as Lindlahr stated in *Nature Cure,* Chapters 2 and 4, disease is caused by one or more of the following as a result of violating nature's laws of healthy living:

Lowered vitality
Abnormal composition of blood and lymph
Accumulation of morbid matter and poisons

As any of these factors either continue to accumulate and disturb function or reduce the ability of the body to purge the disturbance, the body slides into a chronic, weakened reactive state, with possible episodes of intermittent reaction, and is perceived to be in a persistent chronic illness. Ultimately, as function is sufficiently disturbed, structures or functions are damaged, and chronic inflammation becomes ulceration or scar tissue formation. In terms of the allostatic model, the balance has been disrupted, and there is no more adaptive potential. Atrophy, paralysis, or even tumor formation[73–75] may occur. All of this is the body manifestly doing the best it can for itself in the presence of persistent disturbing factors and with respect to the limitations and range of vitality influenced by the constitution, psycho-emotional/spiritual state, and genotype of the person and his or her surrounding environment (Boxes 2.4 and 2.5).

Reversal of this overwhelmed condition is rarely accomplished by medicating the pathological state. This often results in the control of symptoms but with the persistence of the illness while ideally controlling its more dangerous aspects using higher force interventions, such as pharmaceutical drugs and surgical intervention. Reversal is more likely accomplished by identifying and ameliorating the disturbance and, as necessary, strengthening or supporting the individual response or reactive potential. The first step in this process is to identify and reduce disturbing factors.

BOX 2.5 Scientific Considerations: Chronic Illness and the Adaptive Response

Regarding the responses of an overwhelmed or chronically disturbed organism, it has been argued recently that the anemia of chronic disease is an adaptive biological response rather than a harmful disorder and is associated with a number of chronic states.[92] Citing a number of studies, it was also argued that it was the treatment of the anemia of chronic disease among critically ill patients and those with renal failure and cancer (e.g., breast cancer and head and neck cancers) that was associated with the greater mortality. The U.S. Food and Drug Administration issued a warning against the use of erythropoiesis-stimulating agents in those cancer patients not undergoing chemotherapy or radiation therapy.[143]

States where the normal compensatory mechanisms become overwhelmed or suppressed (reducing the reactive potential of the body) include states of chronic oxidative stress[144] and inflammatory processes.[145,146] It is not, however, solely a matter of an overwhelmed or chronically disturbed organism that is critical to the process of disease progression. Adaptive responses are also of vital importance to the development of chronic disease. Research has shown that these evolutionarily preserved adaptive mechanisms of physical activity, insulin sensitivity, and fat storage are essential in the prevention of chronic disease states.[141,142] In the development of type 2 diabetes, for example, there is increasing evidence that it is the individual's maladaptation to lack of physical activity that appears to lead to decreased insulin sensitivity and increased fat storage, which can then lead to a plethora of chronic diseases, many characterized by states of chronic inflammation[147] and oxidative stress. Continuing basic and clinical studies indicate that many of the processes currently regarded in mainstream medicine as harmful have been evolutionarily retained to provide an adaptive advantage.[148,149] The *Harvard Health Letter* recently published an article describing inflammation as part of the "Unifying Theory of Disease"[150] giving support to the argument that inflammation is crucial in both health and disease and that chronic diseases arise when the inflammatory process occurs without appropriate control. The allostatic model also provides a theoretical basis for naturopathic clinical theory. The allostatic model describes the process of achieving stability (homeostasis) through changes in the homeostatic "set points" or control boundaries.[82–85] Homeostasis, the maintenance of stability in biochemical and physiological processes, is essential for life—and allostasis, the "resetting" of the homeostatic "set points," is essential for the maintenance of homeostasis. As it develops through the various iterations of researchers and clinicians, the model emphasizes the need to look beyond the current linear-reductionist model of disease and toward a more holistic and balanced approach to disease conditions.

The adaptive response of the organism to insult or frank structural damage is a concept that also has support outside naturopathic medicine. For example, Schnaper et al.[151] described a conceptual framework for progressive kidney disease where the initial disease develops through an injury of some nature that provokes a cellular response as an adaptation to the original injury. Where this cellular response is effective, no progressive kidney disease may ensue. If, however, there is a maladaptation, these attempts at self-repair may lead to progressive loss of nephrons and chronic kidney disease.

THE DETERMINANTS OF HEALTH

To reduce the disturbance, one must identify the disturbance. In standard medicine, the first step is to identify the pathology, which is then treated. In naturopathic medicine, one must come to understand what is disturbing the health. To do this, the physician needs to understand what determines health in the first place. The physician can then evaluate the patient in these terms and come to understand what is disturbing the natural state of health. Such a list could be created by any doctor, certainly any naturopathic physician. The authors propose the use of the list in Box 2.6.

Some of these determinants have been discussed—those modifiable behavioral factors such as drug and alcohol use, poor diet or frank malnutrition, lack of physical exercise, environmental and socioeconomic factors, and unsafe sexual practices[76–79] (Box 2.7). Many of these behavioral factors have major psychological and spiritual components, and the effect can be increased stress on both the individual and the family, with all its attendant consequences.[79–81] The naturopathic physician evaluates the patient with these areas in mind, looking for aspects of disturbance, first in the spirit and most generally in diet, digestion, and stress in its various aspects. In this evaluation, the naturopathic physician brings to bear a body of knowledge somewhat unique to naturopathic medicine to evaluate not solely in terms of pathological entity but also in terms of normal function and subclinical functional disturbance (Box 2.8). By locating areas of abnormal function or disturbance, the naturopathic physician acts or recommends ways to ameliorate the disturbance.

As disturbing factors or insults to the system are reduced, the natural tendency of the system is to improve and optimize its function, directing the system back toward normalcy, or homeostasis. In more conventional medical terms, this is one of the fundamental concepts of the allostatic model.[80,82–85] In naturopathic thinking, this is the removal of the obstacles to cure, which allows the emerging action of the *vis medicatrix naturae*, the vital force, the healing power of nature. This is the first step in the hierarchy of healing and what naturopathic physicians may call the overarching model in the clinical theory (the process of healing) of naturopathic medicine: the therapeutic order. This process can be seen in the naturopathic model of healing in Fig. 2.1.

THERAPEUTIC ORDER AND NATUROPATHIC ASSESSMENT

The Assessment Order: Components of a Vitalistic Assessment of Illness, Healing, and Health

> *One thing I have learned in TCM, is that the assessment part implies the treatment, because the treatment is to balance what is imbalanced.*
>
> ***Christy Lee Engel ND, Lac, Bastyr University, 2014***

The assessment order is a set of prioritized components of a vitalistic naturopathic assessment of the patient based on, or dictated by observations of the

- nature,
- locus, or
- center of gravity of the degenerative (disease) process.

This degenerative disease process is evaluated in the context of natural health and healing systems, which interconnect

- mind, body, and spirit;
- the natural, cultural, and socioeconomic environments;
- our heredity, and
- how we live.

These components have been recognized from ancient times through the present.[86] Inclusive of conventional pathological evaluation, the naturopathic assessment order is a guideline to identifying and assessing various types, levels of, and priorities in the underlying causation of degenerative and dysfunctional conditions. The assessment order provides an ordered, nonrigid, dynamic framework (leading to the *therapeutic* order) for gauging the "center of gravity" of the disease process (the most efficient level at which to intervene to engage the patient's healing response). By carefully assessing the status of the patient's health and vitality and identifying components currently contributing to the disease process, the underlying causes become evident. These components

BOX 2.6 Naturopathic Medicine Determinants of Health, Factors That Influence Health

Inborn Determinants
Genetic makeup (genotype)
Intrauterine/congenital factors
  Intrauterine influences: maternal nutrition, health, and lifestyle
  Maternal exposures: drugs, toxins, illnesses, viruses, psycho-emotional
Constitution: determines susceptibility

Disturbances/Disturbing Factors
Illnesses: Patho-biography
Medical interventions (or lack of)
Physical and emotional exposures, stresses and trauma
Toxic and harmful substances
Trauma (physical/emotional)
Toxemia
Addictions
Environmental disturbances, stress: environmental, physical, emotional

How We Live—Hygienic, Lifestyle, Psycho-emotional, Spiritual, Socioeconomic, and Environmental Factors

Spirit
Spiritual life/practice
Self-assessment
Relationship to larger universe (trust, consciousness, compassion)

Exposure to Nature/Environment
Fresh air
Clean water
Natural light
Geography and ecosystem
Exposure to natural systems, wild places, cycles

Diet, Nutrition, and Digestion
Unadulterated food
Optimal nutrition

Rest and Exercise
Rest and sleep
Recreation
Exercise and movement
Breath
Vital force, vital reserve, energy
Structural integrity

Socioeconomic Factors
Loving and being loved
Meaningful work
Culture
Community
Government/public policy
Environment
Income and economic
Health care (quality and access)
Education

From Snider P, Zeff J, Myers S, DeGrandpre Z, et al. Course syllabus: NM5114, Naturopathic Clinical Theory. Seattle, WA: Bastyr University; 1997–2012.

BOX 2.7 Scientific Considerations: Subclinical Inflammation and Chronic Illness

It is becoming increasingly evident that many chronic diseases may have a long subclinical phase, most involving the inflammatory process. As mentioned, a chronic, subclinical inflammatory state has been linked to a number of disorders, including insulin resistance,[152] obesity,[153] vascular disease,[154–157] hypertension,[158] and aging.[159]

BOX 2.8 Scientific Considerations: Determinants of Health Within Public and Community Health Concerns

There exists an increasing consensus that Crohn's disease and ulcerative colitis result from the combined effects of four important factors, none of which is individually sufficient to cause the disease. These four factors are the global changes in the environment, alterations in the microbiome of the intestine, multiple genetic factors, and aberrations or maladaptations in both the innate and adaptive immune systems.[160–163] These four factors, considered to be vital to the development and the increased rates of irritable bowel disease, are quite similar to the determinants of health described in Box 2.6. This serves as a further example of the growing appreciation for the similarities (with important differences) between naturopathic medicine and public and community health.

are assessed for presence, absence, onset, triggers, depth, duration, and modalities and for physiological, psychospiritual, mental, biofield, organ system, and tissue targets. Components to be assessed include (1) determinants of health; (2) vitality; psychospiritual, mental, and energetic availability; and vital force; (3) physiological and energetic systems; (4) structure and musculoskeletal components; (5) the pathology itself, its biochemistry, histology, and pathophysiology; and (6 and 7) the level and strength of specific, targeted, managerial, and higher-force interventions necessary for patient safety and reduction of suffering.

Using the assessment order also engages the power of the patient–physician relationship: *docere*. "If the whole reason for the assessment is to develop a treatment plan [suitable to the patient's safety and health recovery], that is one way to look at it. When we add the *docere* experience between physician and patient, then we add the complexity of the two systems, the intention of the physician, the energetic of the *Vis*—and we have a whole new dynamic in the assessment process, which itself begins to be the treatment process," notes Christy Lee Engel ND, LAc.

The case-taking and evaluation process thus begins the treatment process. It is the foundation of the doctor–patient relationship. Subjective and objective data contribute to both the pathological and the vitalistic assessment. The pathological assessment is viewed as partial although valuable information within the context of the entire vitalistic naturopathic assessment. The naturopathic assessment, in effect, places the disease process, its specific pathophysiology, and its staging within the broader context of the patient's vitality, constitution, etiologic factors (never been well since), and underlying or root causes and leads to the level of intervention suggested by the therapeutic order.

The patient's story or patho-biography (Box 2.9) is an essential and powerful tool for making a complete naturopathic assessment. All information, subjective and objective (S, O), leads to the diagnosis and naturopathic assessment summary using the naturopathic assessment order (A) and to the treatment plan using the naturopathic therapeutic order (P).

The Naturopathic Medicine Assessment Order

Components of a vitalistic assessment of illness, healing, and health

I. Evaluate Conditions for Health—Assess Naturopathic Medicine Determinants of Health

Identify/assess inborn and constitutional factors (innate vitality and susceptibility)—genetics, epigenetics, constitution, elements, and individual perceptions and values.
Identify/assess disturbing factors (obstacles to healing)—behavioral, hygienic, socioeconomic, environmental, psycho-spiritual, and cultural determinants.
Identify/assess health-promoting factors—behavioral, hygienic, socioeconomic, environmental, psycho-spiritual, and cultural determinants.

II. Evaluate *Vis Medicatrix Naturae*: The Healing Power and Processes of Nature

Assess vital force, vitality, energetic/biofield, and stage and status of healing/illness processes.
Assess vital reserve and vitality.
Assess spiritual state.
Assess awareness, energy, and biofield.
Assess vital force, direction, and intensity in healing versus illness process simillimum, signature, dual effect, chronobiology, minimum dose
- suppression, return of old symptoms/retracing
- Hering's rules—direction of symptom progression
- Felt sense by patient of trust, energy, awareness, ability to love

Assess impact of intention, healing practices, and healing interaction on healing response.
Assess healing response—strength, direction, response versus reaction or crisis.

III. Conduct a Functional Assessment of Physiological and Bioenergetic Systems

Assess disturbances in physiological, energetic, and organ and cellular system functions and interrelationships—over-/underactivity, burden, obstruction, disorder, nourishment. Examples include neuroendocrine, digestive, emunctories, psycho-spiritual, and so forth.

IV. Evaluate Structural Obstacles to Health

Assess musculoskeletal and structural integrity.
Assess need for nutrients, movement, and exercise to support musculoskeletal integrity.

V. Conduct Pathological Assessment

Assess symptoms, urgency, suffering, and potential for damage.

VI. Assess Need, Risks, and Benefits of Highest-Force Interventions

Patho-biography, follow-up, physical examination, signs, symptoms, lab imaging

BOX 2.9 The Pathobiography

In spite of the organic roots of our medical genesis, any [physician] must [not] consider... illness purely as a material process of organic alteration. The integration of this illness in its anatomo-clinical aspect in the patient as a person enables us to discover the morbid dynamics underlying the pathological process. The "patho-biographic" case history assumes particular interest as it involves the entire psychic, emotional, affective life of the patient, his cravings, frustrations, achievements, anxiety to succeed, his perspectives. His patho-biographic past is no more than the process of psycho-physical adaptation of the individual to his circumstances and where physiopathological alterations are no more than the objective expression and the ultimate result of such adaptation."

Used with the permission of Dr. Eugenio Candegabe, Journal of the Society of Homoeopaths.

THERAPEUTIC ORDER

The naturopathic medicine therapeutic order is a natural hierarchy of therapeutic intervention based on or dictated by observations of the nature of the healing process from ancient times through the present.[86]

The therapeutic order is a systematic approach to engaging the patient's healing response by working with the order of effective intervention inherent in the healing power and processes of nature. This order is simultaneously linear, holarchical, and recursive and functions as a multilayered, complex system powered by the vital force. It is either limited or increased in its efficiency by the level of the patient's vitality. By removing obstacles to healing, establishing health-promoting factors (giving the body and spirit what it needs), and stimulating the vital force, vitality is increased, igniting the orderly self-healing processes of *vis medicatrix naturae.*

Naturopathic physicians have long recognized (Box 2.10) that (1) the healing process is observable—a natural phenomenon (law of nature) seen consistently in health, healing, and illness (e.g., similar to laws of biology, physics, and regularities of other natural sciences); (2) "Naturopathic medicine recognizes this healing process to be ordered and intelligent" (Snider and Zeff et al., AANP House of Delegates, 1989, 2001, 2011). The principle *vis medicatrix naturae* guides the physician to ignite this ordered process by removing obstacles to healing (disturbing factors) and establishing a healthy internal and external environment (AANP 1989). This is accomplished by establishing individualized global health determinants that "treat disease by restoring health." Less detailed therapeutic orders also exist in traditional Chinese, Tibetan, Ayurvedic, and Unani medicine theories.

The therapeutic order is a natural ordering of the modalities of naturopathic medicine and their application. The concept is somewhat

BOX 2.10 Nature's Healing Order—Lindlahr

Lindlahr referred to the *vis medicatrix naturae* as "the constructive principle in nature." In 1914 he described the healing order this way: "The underlying causes of disease must be removed before we can cure chronic disease and bring about a normal condition of the organism…" the true healer is … the *vis medicatrix nature* which ... endeavors to repair, to heal and to restore all that the physician can do is to remove obstructions and to establish normal conditions within and around the patient, so that the healer within can do his work to the best advantage. . . . Though we cannot heal and give life, we can in many ways assist the healer within. We can teach and explain Nature's Laws, we can remove obstructions and we can make the conditions within and around the patient more favorable for the action of Nature's healing forces."

Lindlahr, H. *Nature Cure*. http://www.fulltextarchive.com/pdfs/Nature-Cure.pdf. Nature Cure. 1913; 460, 532.

BOX 2.11 The Therapeutic Order: Hierarchy of Healing

1. Establish the conditions for health.
   Identify and remove disturbing factors.
   Institute a more healthful regimen.
2. Stimulate the healing power of nature *(vis medicatrix naturae):* the self-healing processes.
3. Address weakened or damaged systems or organs.
   Strengthen the immune system.
   Decrease toxicity.
   Normalize inflammatory function.
   Optimize metabolic function.
   Balance regulatory systems.
   Enhance regeneration.
   Harmonize with your life force.[2]
4. Correct structural integrity.
5. Address pathology: Use specific natural substances, modalities, or interventions.
6. Address pathology: Use specific pharmacological or synthetic substances.
7. Suppress or surgically remove pathology.

The actual therapeutic order may change, depending on the individual patient's needs for safe and effective care. The needs of the patient are primary in determining the appropriate approach to therapy.

Acute and chronic concerns are both addressed using the therapeutic order.[91] Acute concerns are addressed first to avoid further damage, risk, or harm to the patient. The point of entry for assessment and therapy is dependent on each patient's need for effective and safe care, healing, and prevention of suffering or degeneration.[2,91]

From Zeff J, Snider P. Course syllabus: NM5131, Naturopathic clinical theory. Seattle, WA: Bastyr University; 1997–2005.

plastic, in that one must evaluate the unique needs, and even the unique healing requirements, of the specific patient or situation.[87] However, the nature of healing dictates a general approach to treatment. In general, this order is listed in Box 2.11.

An analogy for the therapeutic order in Australian integrative medicine is what is called the "softer option" model of patient care.[88] This model recognizes that, given a choice, the patient will generally choose the softer option, provided that this does not limit a harder option if the softer option fails. By way of example, given a choice between an antibiotic and amputation for a minor cut finger, most people would choose the softer option. Expanding this range of choice to an herbal cream, antiseptic (herbal or nonherbal), and a Band-Aid; an antibiotic; or amputation, we develop a therapeutic order ranging from the softest option (the least force) to the hardest option (the higher-force intervention). The therapeutic order can be seen as a progression of therapeutic interventions that begins with this "softer option."

Acute and Chronic Concerns

As discussed previously, there is an inherent drive toward health that is observable within the patterns and processes of nature. The drive is not perfect. There are times when, unguided, unassisted, or unstopped, the drive goes astray, causing preventable harm or even death in patients; the constructive healing intention[89] becomes destructive pathology. The ND is trained to know, respect, and work with this drive in both acute and chronic illness, using the therapeutic order, and to know when to wait or do nothing, act preventively, assist, amplify, palliate, intervene, manipulate, control, or even suppress using the principle of the least force.[90] Acute and chronic concerns are both addressed and managed using the therapeutic order.[91] Acute concerns are addressed first to avoid further damage, risk, or harm to the patient. The point of entry for assessment and therapy is dependent on each patient's need for effective and safe care, healing, and prevention of suffering and degeneration.[70,91]

Naturopathic physicians avoid suppression of symptoms in acute circumstances unless necessary for patients' well-being and safety. Instead, wherever possible, therapies for acute concerns use the least force (minimizing toxic side effects, suppression of natural functions, and physiological burdens) available to intervene effectively, healing or palliating as needed. The full range of modalities, from nutrition to homeopathy, botanical and physical medicine, hydrotherapy, counseling, prescriptive medication, and surgery, is available to the patient as the naturopathic physician works to apply the least force in providing effective preventive, acute, and chronic care.[91]

Establish the Conditions for Health

Identify and Remove Disturbing Factors

If one understands health to be the natural state and "disturbance" the original culprit, then identifying and reducing disturbance is the obvious first step, unless there is immediate danger to life or limb, in which case acting to reduce suffering and preserve life or limb is paramount. In most chronic disease, neither is immediately threatened. This understanding dictates the primary treatment goal the physician must attend to: the identification and amelioration of those factors disturbing health, especially factors that most disturb health (inappropriate diet, excessive stress, and spiritual disharmony). To understand what disturbs health, one must understand what determines health. The naturopathic physician evaluates a patient with reference to the determinants of health to discover wherein the patient's health is disturbed. In this step, the physician is essentially removing the obstacles to cure and allowing the *vis medicatrix naturae* to do its work.

Among these many possibilities, the most significant are attitude, diet, digestion, psychological and other stressors, and what might be called "spiritual integrity." Humans have a transpersonal dimension and can be seen as spiritual beings. *Spiritual* here is not defined by religion or belief in a deity or deities; it is that component of individuals that gives rise to their inner compass, their "joie de vivre" and their internal meaning of life, their core beliefs, and their values. Perceived in this way, it can be seen that many people in society are experiencing "spiritual crises."[92] Although the general purview of the physician is the body, that instrument cannot be separated from the spirit that animates it. If the spirit is disturbed, the body cannot be fundamentally healthy. Hahnemann, the brilliant and insightful founder of homeopathy, instructed physicians to attend to the spirit.[93] Disturbance in the spirit permeates the body and eventuates physical manifestation. Physicians are responsible

BOX 2.12 Scientific Considerations: Toxemia Today

Using conventional medical terminology, disorders derived from environmental, dietary, and lifestyle factors are termed *idiopathic environmental intolerances, multiple chemical sensitivities,*[98,164–166] *or sometimes oxidative stress disorders.*[167–171] The terminology may be different, but each term describes the same symptomatology. Environmental toxins accumulate, and chronic inflammation increases. These exogenous and endogenous toxins and the lack of exercise stress the system further. The ketogenic diet to control epilepsy may be considered one example of the successful application of diet to control symptoms.[172]

for perceiving such disturbances and addressing them. At colleges of naturopathic medicine in Australia, the United Kingdom, and North America, faculty work with naturopathic medicine students to develop their ability to perceive the spiritual nature of an individual as a foundational skill in addressing the spiritual crises or fundamental needs that have a profound effect on health and well-being. Using this definition, both atheists and agnostics can be seen to have a spiritual aspect. This definition also removes spirituality from religiosity in a way that does not denigrate any individual religious belief, allowing the naturopathic clinician to explore this aspect as part of routine care.

One of the oldest concepts in naturopathic medicine is the concept of toxemia. Toxemia is the generation and accumulation of metabolic wastes and exogenous toxins within the body. These toxins may be the results of maldigestive processes, intermediate metabolites, environmental xenobiotics, and colon bacterial metabolites, for example. These toxins become irritants within the body, resulting in the inflammation of tissues and the ultimate interference with normal biochemical processes.[94] The maldigestive and dysbiotic[95,96] origin of these internally and externally derived toxins is the result of an inappropriate diet, broad-spectrum antibiotics, and the effects of excessive stress on digestion.[97] Eating a diet that cannot be easily digested or that is out of appropriate nutrient balance for the individual results in the creation of metabolic toxins in the intestines.[95–98] Stress, causing the excessive secretion of cortisol and adrenaline, results in the decrease of blood flow to the digestive process, among other effects,[80,82–85] which decreases the efficient functioning of digestion and increases the tendency toward maldigestion, dysbiosis, and toxemia. Physicians can now easily measure the degree of toxemia in various ways (urinary indican or phenol[98]). The older concept of toxemia,[99,100] with scientific advances in its understanding[91,99] (Box 2.12), may now be productively combined with an understanding of the newer concept of allostasis[82–85] and the historical[89,100] and reemerging discussion on the inflammatory component of many, if not most, chronic diseases. Spiritual disharmony, inappropriate diet, digestive disturbance, stress, and toxemia (leading to inflammation) are considered primary causes of chronic illness and must be addressed if healing is to occur. Beyond these, other disturbing factors must be discerned and addressed, whichever pertain to the individual patient.[101,152–154,158,159,165–171]

Institute a Healthier Regimen

As a corollary of the first step, once physicians have determined major contributing factors to illness, they construct a healthier regimen for the patient. Some disturbing factors can be eliminated, like inappropriate dietary elements.[110,111] Others are a matter of different choices or living differently. The basics to consider are appropriate diet, appropriate rest and exercise, stress moderation, a healthy environment, and a sense of spiritual fulfillment.[82,92,141,142,145,146,148]

If this model is correct, these measures alone should result in enhanced health. The problem arises in knowing how to do these things. What is an appropriate diet? This is an area of considerable controversy. Physicians think about diet in many different ways. The goal of dietary improvement is to reduce the symptomatic consequences of the patient's diet and provide optimal nutrition to the patient. The point here, regardless of how this is done, is that it is central and essential for fundamental health improvement. If the diet is not correct, if digestion is not appropriate, if nutrition is not adequate, the patient cannot appropriately function or improve, and the scene is potentially set for chronic inflammatory conditions and the resetting of the adaptive allostatic and homeostatic set points. If the diet and digestion are appropriate, the basis for improvement in other areas is enhanced.

The same is true with these other fundamental elements, to which Lindlahr referred in the first element of his catechism, "return to nature": exercise, rest, dress, and so forth.[29] These have been expanded in the "determinants of health." They create the basis for improvement. What this really means is to change the "terrain," the conditions in which the disease has formed—not only to change but to improve the conditions so that there is less basis for the disease. Hahnemann addresses this on the first page of his *Organon of Medicine*.[93] He identified four tasks for the physician: to understand the true nature of illness, "what is to be cured"; to understand the healing potential of medicines (whether they enhance or suppress function); to understand obstacles to recovery and how to remove them (the determinants of health); and to understand the elements that derange health and how to correct them so that recovery may be permanent.[93] Changing and improving the terrain in which the disease developed is the obvious first step in bringing about improvement. This sets up the basis for the following elements to have the most beneficial effects.

Stimulate the Self-Healing Mechanisms

A certain percentage of patients will improve sufficiently simply by removing disturbing factors and establishing a healthier regimen. Most require more work. Once the patient is prepared, once the terrain is beginning to clear of disturbing factors, then one begins to apply stimulation to the self-healing mechanisms. The basis of this approach is the underlying recognition of the *vis medicatrix naturae,* the tendency of the body to be self-healing, the wisdom and intelligence within the system that constantly tends toward the healthiest expression of function, and the healing "forces" in the natural environment (air, water, light, etc.). The body heals itself. The physician can help create the circumstances to promote this. Then, as necessary, the physician stimulates the system. This also requires that attention be given to the patient's emotional state of mind because the psychological condition of the patient is often of major importance.[113,114]

One of the best ways to do this is through constitutional hydrotherapy, as developed by Otis G. Carroll, ND, early in the past century. This procedure is simple, involving the placement of hot and then cold towels on the trunk and back, in a specific sequence (depending on the patient), usually accompanied by a sine-wave stimulation of the digestive tract. This is a dynamic treatment, simple, inexpensive, and universally applicable. It helps recover digestive function, stimulates toxin elimination, "cleanses the blood," enhances immune function, and has several other effects. It moves the system along toward a healthier state.[115] Exercise often achieves similar results. Many naturopathic modalities can be used to stimulate the overall vital force.

More specific approaches to stimulation, although general in effect, are applied differently to each patient and have a less general effect than those previously mentioned. Homeopathy and acupuncture[116–118] are often the primary methods of such stimulation. They add little to the system; they are not gross chemical treatments. They work with what is there, stimulating a reaction, stimulating function, and correcting disturbed patterns.

Each method helps move the system out of its disturbed state and, with the reduction of encumbrance, helps move it toward health.

Finally, exposure to the patterns, rhythms, and forces of nature is a traditional part of naturopathic medicine and the tradition of nature doctors throughout the world. As previously noted, "We exist as part of complex patterns of matter, energy, and spirit,"[2] and the natural progression of these patterns, and the drive toward health inherent in them, is a natural ally for the physician. Exposure to appropriate rhythms, patterns, and forces of nature strengthens vitality and stimulates the healing power of nature.

Support Weakened or Damaged Systems or Organs

Some systems or functions require more than stimulation to improve. Some organs are weakened or damaged (e.g., adrenal fatigue after prolonged stress), and some systems are blocked or congested (e.g., the hepatic detoxification pathways) and require extra help. This is where naturopathic physicians use their vast natural medicinary. Botanical medicines can affect any system or organ, enhancing its function, improving its circulation, providing specific nutrition, and stimulating repair. Glandular substances can be applied to a similar purpose. Plus, there are a growing number of evidence-based "nutraceuticals"—biological compounds that enhance metabolic pathways and provide specific substances to enhance metabolic function.[119–130]

Naturopathic physicians can also apply specific homeopathic medications, usually in the lower potencies, which act nutritively and can stimulate specific organs or functions. This method, generally referred to as *drainage,* can be used to stimulate detoxification of specific substances from the body in general or of specific organ systems or tissues. Dr. Pizzorno's work in *Total Wellness* and *The Toxin Solution*[131,132]*;* the work of "functional medicine" leader Jeffrey Bland, PhD; and the *Textbook of Functional Medicine* by Jones[90] exemplify the clinical strategies applied at this level of the therapeutic order. These strategies are used to restore optimal function to an entire physiological system (immune, cardiovascular, detoxification, life force, endocrine).[131,132]

One can also use specific exercises to stimulate or enhance organ health. Some systems of yoga and qi gong are organ specific. Specific applications of hydrotherapy and other physiotherapy systems can be applied to enhance the function of organs or tissues.

It has been the clinical experience of many naturopathic physicians that these methods, combined with an appropriate diet and a healthier regimen, along with constitutional hydrotherapy, appropriate homeopathy, and acupuncture, bring most health problems back to normal, without negative consequence, rapidly, efficiently, and permanently.

Address Structural Integrity

Many structural problems result from generalized stress of some kind on internal systems. For example, midback misalignment or discomfort (T1–T12) is often found associated with a history of underlying stress on the digestive organs, the enervation of which originates at those spinal segments. One can manipulate the vertebra back into proper alignment or massage contracted musculature, but until one corrects the underlying functional disturbance, there will be a tendency to repeated structural misalignment. In some circumstances, the singular problem may be simply structural disintegrity. One may have fallen or been hit in some fashion and simply needs the neck manipulated back into proper alignment and the surrounding soft tissue relaxed. There may be no dietary error or other disturbance aside from the original injury, and correction requires only simple manipulation or therapeutic massage. This is an example of the flexibility of the therapeutic order concept. In this case, first-order therapeutics manipulate the cervical spine or relax chronically contracted muscles. Usually, however, the problem of structure is part of the larger problem, and such intervention becomes a fourth-order therapeutic.[70]

Reintegrating structure can occur in many ways, one of which is the method of "bone cracking" known to the ancient Greeks and Chinese and probably all other ancient healing cultures. However, there are nonforce manipulative systems that include many modalities of therapeutic massage. Some systems of exercise are designed to reintegrate and maintain normal structural relationships. Any of these might be appropriate to a specific patient. By approaching the problem in the context of the therapeutic order, one can expect structural corrections to be required only occasionally and for the results to be more or less permanent.

Address Pathology: Use Specific Natural Substances, Modalities, or Interventions

Having gone through the first four steps of this therapeutic hierarchy, most patients improve. The improvement is based on the sound footing of the underlying correction or removal of fundamental causative elements. It is also based on the intrinsic nature of the body to heal itself using the least possible force. Most pathology improves or disappears under these circumstances, but sometimes it is necessary to address pathology. This may be the case because the particular pathology may be threatening to life or limb. Acting on this threat is imperative. It can often be done with naturopathic means, directed specifically against the pathology. Biochemical or genetic individuality also can demand an emphasis at this level of intervention.

One of the major conflicts in naturopathic medicine is that some practitioners find it expedient to diagnose and treat pathology (the standard medical model) rather than pursue a naturopathic model of practice. This approach tends to be less satisfying and less productive of the most elegant outcomes and the long-term continued health of the patient. It also reduces the capacity of the physician to treat, such as in cases where there is no evidence-based treatment for the pathology in question, or where there is no clear diagnosis (i.e., no distinct pathology to treat). This approach is increasingly referred to as "green allopathy." However, the vast body of knowledge that naturopathic education presents in this arena makes such an approach seductive, especially in a culture that more or less expects, supports, reinforces, and pays for a biomedical ("allopathic") approach to diagnosis and treatment.

It is easy to do this. The culture is accustomed to this model and often expects to encounter this in the naturopathic physician's office. In some states, such as Oregon, Washington, and Arizona, where the naturopathic formulary includes most antibiotics and many pharmaceutical drugs, one can practice almost without distinction from a medical doctor. The typical naturopathic formulary is often sufficient to prescribe on a strictly pathological basis.

The problem with this is that it is generally not as effective, especially in the treatment of chronic disease. The value of naturopathic medicine in our culture is not that naturopathic physicians can function almost like medical doctors, with a "natural" formulary instead of drugs. It is that they offer a fundamentally different approach, one based on the restoration of health rather than the treatment of disease.

Given all of this, it still may be useful to directly address the pathological entity or its etiology.[112,133-136] When treating an antibiotic-resistant infection, for example, it may be useful to apply botanical medicines with specific antibiotic properties, along with immune tonics and the more fundamental steps of this therapeutic hierarchy. In difficult cases, such as many cancers, using agents that have specific, pathology-based therapeutics may be an essential element of comprehensive treatment. The naturopathic formulary provides a vast and increasing number of such options. One advantage of such treatment

is that, in general, when applied by a knowledgeable practitioner, it rarely adds more burden or toxicity to the system. Naturopathic pathology-based treatments still follow the dictum "do no harm."

Address Pathology: Use Specific Pharmacological or Synthetic Substances

About 800,000 medical doctors and osteopathic physicians in the United States are trained in the science of pathology-based treatment, using pharmaceuticals and surgery, for example. There are times when such an approach is necessary to preserve life, limb, or function. Although some naturopathic physicians, by training and by statute, may prescribe pharmaceuticals or perform minor office procedures and surgeries, naturopathic physicians may also refer patients in need of such services to appropriate standard medical doctors (MDs) or medically trained osteopaths (DOs). In a growing number of states, NDs can legally provide an expanding range of prescription drugs. Although this is an important tool for the naturopathic primary caregiver, this privilege requires enhanced responsibility for the ND to prescribe those substances only as needed—and to thoroughly rely on applying the least force appropriate to effect recovery and protect patient safety. Both Dr. Lust (at the end of his life) and Dr. Bastyr recognized the need for NDs to have the ability to access, as needed, prescriptive medications and perform minor office procedures to function as primary caregivers. However, both admonished that the philosophy and principles of the medicine guide their judicious use—only as truly needed, based on the least force necessary to restore the patient to health.

Naturopathic physicians are well trained in this regard and respect the necessity and utility of standard medical practice in appropriate situations. Some disagreement exists regarding which situations may be appropriate. The AANP has developed position papers to resolve some of these questions.

In general, although recognizing the necessity of such treatment, most naturopathic physicians also recognize that such treatment often carries consequences that also must be addressed.

Suppress Pathology

Sometimes it is necessary, when there is a risk of harm to the patient's health or tissue or to relieve suffering, to suppress pathology. Medical doctors are especially trained in this art and have powerful and effective tools with which to do this. Unfortunately, suppression, because it does not fundamentally remove or address essential causative factors (e.g., dietary error) often results in the development of other, often deeper disturbance or pathology. Because much pathological expression is the result of the actual self-healing mechanisms (e.g., inflammation), suppressive measures, in general, work in opposition to the *vis medicatrix naturae.* The result of suppression is that the fundamental disturbing factors are still at play within the person, still disrupting function to some extent, whereas the suppression reduces the symptomatic expression and resolution of disturbance. One simple example of this is the overuse of oral corticosteroidal anti-inflammatory and antihistaminic drugs in the treatment of acute asthma. This usually effectively opens the airways. However, prolonged use weakens the patient. If the treatment persists, the patient may become immune compromised or osteoporotic or can develop psychological disorders. These symptoms are part of the long-term effects of steroids.[137] It may, of necessity, maintain breathing, but the long-term cost to the organism can be high.

Suppression, although it may be lifesaving, often has serious consequences. With standard medical methods of care, the cure of chronic illness is often elusive. This is the benefit of the naturopathic approach: by taking a nonsuppressive course of action, based on sound physiological principles, one can often restore health without recourse to the potential damage of suppression. Naturopathic physicians, although recognizing the occasional necessity of suppressive approaches, generally avoid suppression, which is a primary way in which physicians can inflict harm, even with the best of intentions.

THEORY IN NATUROPATHIC MEDICINE

The therapeutic hierarchy is based on the observation of the nature of healing and the inherent order of the healing process. It is part of a unifying theory of naturopathic medicine, an outgrowth of the principles that underlie naturopathic thinking. It provides the physician with instructions that order the many therapeutic modalities used by the practice.

The consensus definition of naturopathic medicine, adopted by the AANP in 1989, is a statement of identity, distinguishing naturopathic medicine from other systems of medical thought. Contained within it is a set of instructions regarding the practice of the medicine. The three concepts discussed here—"disease as process," "the determinants of health," and "the therapeutic order"—are an articulation of these instructions. They are presented as a clinical theory of naturopathic medicine. They have been crystallized, as is the definition, from the observation by nature doctors throughout time and across many traditions of the nature of health, disease, and healing. They provide the physician with instructions. These instructions include a procedure for thinking about human illness in such a way that one can approach its cure in an ordered fashion by understanding its process as an expression of the *vis medicatrix naturae.* It provides the framework for truly evaluating the patient as a whole being: spiritual, mental/emotional, and physical, rather than as a category of pathology. Plus, the theory of naturopathic medicine provides the physician a system for organizing and efficiently integrating the vast therapeutic array provided in naturopathic medicine. Ultimately, it satisfies Hahnemann's observation of the ideal role of medicine, that "the highest ideal of cure is rapid, gentle and permanent restoration of the health … in the shortest, most reliable and most harmless way, upon easily comprehensible principles."[93] The roots of the observations that form this theory are traceable through the mid- and early 20th century, to the traditional theory of the 19th-century European nature cure, and to the roots and theories of traditional world medicines. Hippocrates's writings on the *vis medicatrix naturae* form a foundation that historically underpins the development of this theory.[138,139]

Finally, it is observable across many traditional world medicines that various healing orders are described. Such structures hold implications for public and community health priorities and suggest the reprioritization of healthcare priorities and financing. Implications for public policy and the growing national disease debt invite exploration.

Although this presentation is not comprehensive, the attempt has been made to demonstrate these roots, at least in some of their major articulations. The work presented here is a continuation of this historical process, which ultimately is driven by the true mission of the physician: to ease suffering and to preserve life.

> *What Methods of Cure Are in Conformity with the Constructive Principle in Nature? Those methods which: Establish normal surroundings and natural habits of life in accord with Nature's Laws. Economize vital force. Build up the blood on a natural basis, that is, supply the blood with its natural constituents in right proportions. Promote the elimination of waste matter and poisons without in any way injuring the human body. Arouse the individual in the highest possible degree to the consciousness of personal accountability and the necessity of intelligent personal effort and self-help.*[29]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. See Chapter 5 for a more comprehensive and more fully documented—with some differences—history of naturopathic medicine.
2. Pizzorno J, Snider P. Naturopathic medicine. In: Micozzi MS, ed. *The Fundamentals of Complementary and Alternative Medicine.* New York: Churchill Livingstone; multiple editions.
3. Cody G. The history of naturopathic medicine. In: Pizzorno J, Murray M, eds. *Textbook of Natural Medicine*. New York: Churchill Livingstone; 1999.
4. Kirchfeld F, Boyle W. *The Nature Doctors: Pioneers in Naturopathic Medicine*. Portland, OR: Medicina Biologica; 1994.
5. Schramm A, ed. *Yearbook of the International Society of Naturopathic Physicians and Emerson University Research Council.* Los Angeles: International Society of Naturopathic Physicians; 1945.
6. Tribe W. Personal communication. *National College Professional Survey*. Portland, OR: National College of Naturopathic Medicine; 2008.
7. Wendel P. *Standardized Naturopathy*. Brooklyn, NY: Paul Wendel; 1951.
8. Kirchfeld F, Boyle W. *Nature Doctors*. East Palestine, OH: Buckeye Naturopathic Press; 1994.
9. Freibott G. *Report Submitted to Lanso Cavasos, Secretary of Education*. Washington, DC: U.S. Department of Education; 1990.
10. Spitler HR. *Basic Naturopathy, a Textbook*. New York: American Naturopathic Association; 1948.
11. Wendel P. *Standardized Naturopathy*. Brooklyn, NY: Paul Wendel; 1951.
12. Coulter H. *Divided Legacy: A History of the Schism in Medical Thought*. Washington, DC: Weehawken Book Company; 1973.
13. Engel GL. The need for a new medical model: a challenge for biomedicine. *Science*. 1977;196:129–136.
14. Lear L. Rachel Carson Biography. http://www.wilderness.net/index.cfm?fuse=feature0407. Accessed August 10, 2011.
15. Enrollment records. National College of Naturopathic Medicine. Accessed June 20, 2004.
16. Kirchfeld F, Boyle W. *The Nature Doctors: Pioneers in Naturopathic Medicine*. Portland, OR: Medicina Biologica; 1994.
17. Snider P. The future of naturopathic medical education—primary care integrative natural medicine: the healing power of nature. In: Cronin M, ed. *Best of Naturopathic Medicine: Anthology 1996: Celebrating 100 Years of Naturopathic Medicine*. Tempe, AZ: Southwest College of Naturopathic Medicine Publications; 1996.
18. Snider P. Integration project survey results: report to the AANMC dean's council [Database]; 1999.
19. Standish L, Calabrese C, Snider P, et al. Naturopathic medical research agenda: report to NCCAM: Draft. 2004:5.
20. Tilden JH. *Toxemia Explained: An Antidote to Fear, Frenzy, and the Popular Mad Chasing After So-Called Cures: The True Interpretation of the Cause of Disease, How to Cure Is an Obvious Sequence.* Rev. ed. Denver: FJ Wolf; 1926.
21. Trall R. *The True Healing Art*. New York: Fowler & Wells; 1880.
22. Graham S. *Greatest Health Discovery: Natural Hygiene & Its Evolution Past, Present & Future*. Chicago: Natural Hygiene Press; 1860.
23. Kellogg J. *Rational Hydrotherapy*. 2nd ed. Philadelphia: FA Davis Co; 1903.
24. Kuhne L, Lust B. *Neo-Naturopathy: The New Science of Healing or the Doctrine of the Unity of Diseases*. Butler, NJ; 1917.
25. Kuhne L, Lust B. *The Science of Facial Expression: The New System of Diagnosis, Based on Original Researches and Discoveries*. Butler, NJ; 1917.
26. McFadden B. *MacFadden's Encyclopedia of Physical Culture*. Vol. 5. New York: Physical Culture Publishing; 1920.
27. Rikli A. *Die Grundlerhren der Naturheilkunde einschliesslich "Dia atmospharische Kure," "Es werde Licht" und "Abschiedsworte" [The Fundamental Doctrines of Nature Cure including "the Atmospheric Cure," "Let There Be Light" and "Words of Farewell"]*. 9th ed. Wolfsburg, Germany: G. Rikli; 1911.
28. Tilden JH. *Impaired Health: Its Cause and Cure—A Repudiation of the Conventional Treatment of Disease*. 2nd ed. Denver: Tilden; 1921.
29. Lindlahr H. *Nature Cure: Philosophy and Practice Based on the Unity of Disease and Cure*. Chicago: Nature Cure Publishing; 1913.
30. Kneipp S. *Thus Shalt Thou Live*. Kempten, Bavaria: Koesel; 1889.
31. Kneipp S. *My Water Cure*. UK: Thorsons; 1979 (reprint of 1891 edition).
32. Kneipp S. *My Will*. Kempten, Bavaria: Koesel; 1894.
33. Trall RT. *Hydropathic Encyclopedia: A System of Hydropathy and Hygiene*. New York: Fowlers & Wells; 1851.
34. Rausse JH. *Der Geist der Graffenberger Wasserkur*. Zeitz: Schieferdecker; 1838.
35. Rikli A. *Die Thermodiatetik oder das tagliche thermoelectrische Licht und Luftbad in Verbindung mit naturfemasser Diat als zukunftige Heilmethode*. Vienna: Braumueller; 1869.
36. Thomson S. *A Brief Sketch of the Causes and Treatment of Disease*. Boston: EG House; 1821.
37. Beach W. *A Treatise on Anatomy, Physiology and Health*. New York: W. Beach; 1847.
38. Ellingwood F. *American Materia Medica, Therapeutics, and Pharmacognosy*. Evanston: Ellingwood's Therapeutist; 1919.
39. Felter H. *The Eclectic Materia Medica, Pharmacology, and Therapeutics*. Cincinnati: John K Scudder; 1922.
40. Boyle W. *The Herb Doctors*. East Palestine, OH: Buckeye Naturopathic Press; 1988.
41. *Dictionary of Occupational Titles*. Vol. 1. 3rd ed. Washington, DC: U.S. Department of Labor; 1965.
42. Schram A. Acts and laws. In: *Yearbook of the International Society of Naturopathic Physicians & Emerson University Research Council*. Los Angeles: International Society of Naturopathic Physicians; 1945.
43. Bradley R. Philosophy of naturopathic medicine. In: Pizzorno J, Murray M, eds. *Textbook of Natural Medicine*. New York: Churchill Livingstone; 1985.
44. AANP House of Delegates Resolution. Rippling River, OR; 1989.
45. Select Committee on the Definition of Naturopathic Medicine, Snider P, Zeff J, co-chairs. *Definition of Naturopathic Medicine: AANP Position Paper*. Rippling River, OR: AANP; 1989.
46. Select Committee on the Definition of Naturopathic Medicine. AANP 1987–1989. Report submitted to AANP in 1988, final recommendation submitted to AANP House of Delegates Rippling River, OR; 1989.
47. Snider P, Zeff J. Personal letters and communications, 1987–1989.
48. Zeff J. Convention theme: "what is a naturopathic physician?" *AANP Q News*. 1988;3(1):11.
49. Snider P, Zeff J. Definition of naturopathic medicine: first draft. *AANP Q News*. 1988;3:6–8.
50. North American Association of Naturopathic Medical Colleges Integration Project Survey 1997–1999. Preliminary report. October 26, 1999. Snider P, Zeff J, co-chairs. Mitchell M, Bastyr University Integration Project Student Task Force Chair. Monwai M, database and research assistant.
51. Snider P, Zeff J. Integration Project Report on Survey Data and Proposed Principles of Naturopathic Medicine to the AANMC Dean's Council; 1999.
52. The integration project update 2000: AANP house of delegates principles survey, presented by Mitchell M, IP student task force chair 1997–2000. Comments presented by Snider S, Zeff J, co-chairs integration project 1996–2000. Monwai M, database manager. Saunders F, data analyst.
53. O'Keefe M, Milliman B, Zeff J. Proposed New Principles of Naturopathic Medicine: Wellness, Least Force, Relieve Suffering. Submitted to the AANP House of Delegates; 1996.
54. Resolution introduced in House of Delegates regarding new principles: passed 2000 AANP convention, Seattle, WA. The House of Delegates recommended that the discussion be moved to the academic community involved in clinical theory and practice for development.
55. Zeff J. The process of healing: a unifying theory of naturopathic medicine. *J Naturopath Med*. 1997;1:122–126.
56. Snider P, Zeff J, Sensenig J, et al. Towards a Unifying Theory of Naturopathic Medicine. AANP Plenary Session. Portland, OR: AANP; 1996.
57. Snider P. Integration project: timeline, scope of work, goals, methods. Proposal adopted by CNMC 1996, readopted by AANMC 1997–1998.
58. CNME report from Bastyr University. Standard XI and appendices: curriculum; 1999.
59. AANMC Dean's Council minutes and correspondence; 2000.
60. Snider P, Downey C. Co-chairs. Invitation Letter, Supporting Information, Agenda, Minutes, Tools and Materials. AANMC Integration Project Retreat for Naturopathic Philosophy and Clinical Theory Faculty, Basic Sciences Chairs and Clinic Directors; 2001:20–21.

61. Snider P, Payne S. Making Naturopathic Curriculum more Naturopathic: Agendas, Minutes, 1999-2001. Clinic Faculty Task Force on Integration. Faculty Development Retreat. Bastyr University; 1998.
62. *World Naturopathic Federation Report: Findings From the 1st World Naturopathic Federation Survey*. http://worldnaturopathicfederation.org/wnf-publications/. 2015; 11, 12. Accessed July 16, 2019.
63. *World Naturopathic Federation Report: Findings From the 1st World Naturopathic Federation Survey*. http://worldnaturopathicfederation.org/wnf-publications/. 2015; 12. Accessed July 16, 2019.
64. Findings From the 1st World Naturopathic Federation Survey. http://worldnaturopathicfederation.org/wnf-publications/. 2015; 13. Accessed July 16, 2019.
65. WNF white paper: philosophies, and principles, theories. http://worldnaturopathicfederation.org/wp-content/uploads/2015/12/White-Paper_FINAL.pdf. 2017; 40. Accessed July 16, 2019.
66. Kott A, Fruh D, et al. http://www.fightchronicdisease.org/sites/default/files/docs/2009_PFCDAlmanac_0.pdf. Accessed August 10, 2011.
67. Starfirld B. Is US health really the best in the world? *JAMA*. 2000;284(4):483–495.
68. Goldmann DA, Weinstein RA, Wenzel RP, et al. Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospitals—a challenge to hospital leadership. *JAMA*. 1996;275(3):234–240.
69. Eggleston K, Zhang RF, Zeckhauser RJ. The global challenge of antimicrobial resistance: insights from economic analysis. *Int J Env Res Pub Health*. 2010;7(8):3141–3149.
70. Pizzorno JE, Snider P, Katzinger J. Naturopathic medicine. In: Micozzi MS, ed. *Fundamentals of Complementary and Alternative Medicine.* Philadelphia: Churchill Livingstone; 159–192.
71. Cannon W. Organization for physiological homeostasis. *Physiol Rev*. 1929;9(3):399–431.
72. Gern JE. The ABCs of rhinoviruses, wheezing, and asthma. *J Virol*. 2010;84(15):7418–7426.
73. Gourgiotis S, Kocher HM, Solaini L, et al. Gallbladder cancer. *Am J Surg*. 2008;196(2):252–264.
74. Duong TH, Flowers LC. Vulvo-vaginal cancers: risks, evaluation, prevention and early detection. *Obstet Gynecol Clin North Am*. 2007; 34(4):783–802, x.
75. Bhattacharyya N, Frankenthaler R, Gomolin H, et al. Clinical and pathologic characterization of mucosa-associated lymphoid tissue lymphoma of the head and neck. *Ann Otol Rhinol Laryngol*. 1998;107(9 Pt 1):801–806.
76. Ezzati M, Lopez AD, Rodgers A, et al., and the Comparative Risk Assessment Group. Selected major risk factors and global and regional burden of disease. *Lancet*. 2002;360:1347–1360.
77. McGinnis JM, Foege WH. Actual causes of death in the United States. *JAMA*. 1993;270(18):2207–2212.
78. Ridker PM, Cushman M, Stampfer MJ, et al., Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336(14):973–979.
79. Woolf K, Reese CE, Mason MP, et al. Physical activity is associated with risk factors for chronic disease across adult women's life cycle. *J Am Diet Assoc*. 2008;108(6):948–959.
80. Martinez-Lavin M, Vargas A. Complex adaptive systems allostasis in fibromyalgia. *Rheum Dis Clin North Am*. 2009;35(2):285–298.
81. Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults—the Adverse Childhood Experiences (ACE) study. *Am J Prev Med*. 1998;14(4):245–258.
82. Goldstein DS. Computer models of stress, allostasis, and acute and chronic diseases. *Ann N Y Acad Sci*. 2008;1148:223–231.
83. Romero LM, Dickens MJ, Cyr NE. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Horm Behav*. 2009;55(3):375–389.
84. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338(3):171–179.
85. McEwen BS, Wingfield JC. What is in a name? Integrating homeostasis, allostasis and stress. *Horm Behav*. 2010;57(2):105–111.
86. Hippocrates. The Genuine Works of Hippocrates. Adams F, trans. Baltimore: Williams & Wilkins; 1939.
87. Zeff J, Snyder P. Course Syllabus: NM5171, Naturopathic Clinical Theory. Seattle: Bastyr University; 1997–2005.
88. Myers SP. Personal Communication; 2010.
89. Lindlahr H. *Nature Cure Catechism*. 2nd ed. Chicago: Nature Cure Publishing; 1914.
90. Jones DS, ed. *Textbook of Functional Medicine*. Gig Harbor, WA: The Institute for Functional Medicine; 2005.
91. Huyck A, Lichtenstein B, Broderick K. Personal Communication. Kenmore, WA: Bastyr University Faculty; 2011.
92. Seaward BL. Stress and human spirituality 2000: at the cross roads of physics and metaphysics. *J App Psycho Biofeedback*. 2000;25(4):241–246.
93. Hahnemann S. *Organon of Medicine*. Philadelphia: Boericke & Tafel; 1922.
94. Hirata K, Ikeda S, Honma T, et al. Sepsis and cholestasis: basic findings in the sinusoid and bile canaliculus. *J Hepatobiliary Pancreat Surg*. 2001;8(1):20–26.
95. Vanderploeg R, Panaccione R, Ghosh S, Rioux K. Influences of intestinal bacteria in human inflammatory bowel disease. *Infect Dis Clin North Am*. 2010;24(4):977–993.
96. Farrell RJ, LaMont JT. Microbial factors in inflammatory bowel disease. *Gastroenterol Clin North Am*. 2002;31(1):41–62.
97. Fleming SA, Gutknecht NC. Naturopathy and the primary care practice. *Prim Care*. 2010;37(1):119–136.
98. Lord RS, Bralley JA. Clinical applications of urinary organic acids. Part 2. Dysbiosis markers. *Alt Med Rev*. 2008;13(4):292–306.
99. Orrock P. Naturopathic physical medicine. In: Chaitow L, ed. *Naturopathic Physical Medicine*. St Louis, MO: Elsevier; 2008.
100. Kuhne L, Lust B. *Neo Naturopathy: The New Science of Healing or the Doctrine of Unity of Diseases*. Whitefish, MT: Kessinger Publishing; 2003.
101. Korkina L, Scordo MG, Deeva I, et al. The chemical defensive system in the pathobiology of idiopathic environment-associated diseases. *Curr Drug Metab*. 2009;10(8):914–931.
102. Lord RS, Bralley JA. Clinical applications of urinary organic acids. Part 1: detoxification markers. *Alt Med Rev*. 2008;13(3):205–215.
103. MacDowell AL, Bacharier LB. Infectious triggers of asthma. *Immunol Allergy Clin North Am*. 2005;25(1):45–66.
104. Gern JE. Mechanisms of virus-induced asthma. *J Pediatr*. 2003; 142(suppl 2):S9–S13; discussion S13–4.
105. Ghildyal R, Dagher H, Donninger H, et al. Rhinovirus infects primary human airway fibroblasts and induces a neutrophil chemokine and a permeability factor. *J Med Virol*. 2005;75:608–615.
106. Mallia P, Contoli M, Caramori G, et al. Exacerbations of asthma and chronic obstructive pulmonary disease (COPD): focus on virus induced exacerbations. *Curr Pharm Des*. 2007;13:73–97.
107. Papadopoulos NG, Psarras S. Rhinoviruses in the pathogenesis of asthma. *Curr Allergy Asthma Rep*. 2003;3:137–145.
108. Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005;201:937–947.
109. Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med*. 2006;12:1023–1026.
110. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*. 2001;344(18):1343–1350.
111. Saaristo T, Moilanen L, Korpi-Hyovalti E, et al. Lifestyle intervention for prevention of type 2 diabetes in primary health care one-year follow-up of the Finnish National Diabetes Prevention Program (FIN-D2D). *Diabetes Care*. 2010;33(10):2146–2151.
112. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004;291(10):1238–1246.
113. Glaser R, Kiecolt-Glaser JK. Science and society: stress-induced immune dysfunction: implications for health. *Nature Rev Immuno*. 2005;5(3): 243–251.
114. Zhu J, Kennedy DN, Cao X, et al. Neural transmission of acupuncture signal. In: Xia Y, Cao X, Wu G, Cheng J, eds. *Acupuncture Therapy for Neurological Diseases: A Neurobiological View*. New York: Springer; 2010:81–103.

115. Boyle W, Saine A. *Naturopathic Hydrotherapy*. East Palestine, OH: Buckeye Naturopathic Press; 1988.
116. Lin JG, Chen WL. Acupuncture analgesia: a review of its mechanisms of actions. *Am J Chin Med*. 2008;36(4):635–645.
117. Wang SM, Kain ZN, White P. Acupuncture analgesia: I. The scientific basis. *Anesth Analg*. 2008;106(2):602–610.
118. Hurwitz EL, Aker PD, Adams AH, et al. Manipulation and mobilization of the cervical spine: a systematic review of the literature. *Spine*. 1996;21(15):1746–1759.
119. Eussen S, Klungel O, Garssen J, et al. Support of drug therapy using functional foods and dietary supplements: focus on statin therapy. *Br J Nutr*. 2010;103(9):1260–1277.
120. Rosenbaum CC, O'Mathúna DP, Chavez M, Shields K. Antioxidants and anti-inflammatory dietary supplements for osteoarthritis and rheumatoid arthritis. *Altern Ther Health Med*. 2010;16(2):32–40.
121. Derijk RH, van Leeuwen N, Klok MD, Zitman FG. Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. *Eur J Pharmacol*. 2008;585(2-3):492–501.
122. Nicolson GL. Lipid replacement therapy: a nutraceutical approach for reducing cancer-associated fatigue and the adverse effects of cancer therapy while restoring mitochondrial function. *Can Met Rev*. 2010; 29(3):543–552.
123. Lane JA, Mehra RK, Carrington SD, et al. The food glycome: A source of protection against pathogen colonization in the gastrointestinal tract. *Int J Food Microbio*. 2010;142(1-2):1–13.
124. Iriti M, Faoro F. Grape phytochemicals: a bouquet of old and new nutraceuticals for human health. *Med Hypotheis*. 2006;67(4):833–838.
125. Lucas HJ, Brauch CM, Settas L, et al. Fibromyalgia: new concepts of pathogenesis and treatment. *Int J Immunopath Pharm*. 2006;19(1):5–9.
126. Ferrari CKB. Functional foods, herbs and nutraceuticals: towards biochemical mechanisms of healthy aging. *Biogeront*. 2004;5(5):275–289.
127. Hardy G, Hardy I, Ball PA. Nutraceuticals: a pharmaceutical viewpoint: part II. *Curr Opin Clin Nut Met Care*. 2003;6(6):661–671.
128. Ferrari CKB, Torres EAFS. Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. *Biomed Pharmacother*. 2003;57(5-6):251–260.
129. McVeigh GE, Morgan D, Dixon L. Endothelial-dependent vasodilation in chronic heart failure is improved by dietary fish oil supplementation. *FASEB Meeting on Experimental Biology*: translating the Genome, Date: April 11–15, 2003; San Diego, CA. *Faseb J*. 2003;17(4–5): Abstract No. 769.3.
130. Barrett B, Kiefer D, Rabago D. Assessing the risks and benefits of herbal medicine: an overview of scientific evidence. *Alt Ther Health Med*. 1999;5(4):40–48.
131. Pizzorno J. *Total Wellness*. Rocklin, CA: Prima Publishing; 1996.
132. Pizzorno J. *The Toxin Solution*. San Francisco, CA: HarperOne; 2017.
133. Spelman K, Burns J, Nichols D, et al. Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. *Alt Med Rev*. 2006;11(2):128–150.
134. Boon H, Wong J. Botanical medicine and cancer: a review of the safety and efficacy. *Exp Opin Pharmacother*. 2004;5(12):2485–2501.
135. Halcon L, Milkus K. Staphylococcus aureus and wounds: a review of tea tree oil as a promising antimicrobial. *Am J Inf Cont*. 2004;32(7): 402–408.
136. Dvorkin L, Song KY. Herbs for benign prostatic hyperplasia. *Ann Pharmacother*. 2002;36(9):1443–1452.
137. Lipworth BJ. Systemic adverse effects of inhaled corticosteroid therapy—a systematic review and meta-analysis. *Arch Int Med*. 1999;159(9):941–955.
138. Snider P, Weeks J. Design Principles for Healthcare Renewal. http://www.thecollaboration.org. Accessed June 4, 2004.
139. Weeks J. Integrative medicine industry leadership summit 2001. *Altern Ther Health Med*. 2002;8:S3–S11.
140. Zonana-Nacach A, Barr SG, Magder LS, et al. Damage in systemic lupus erythematosus and its association with corticosteroids. *Arth Rheum*. 2000;43(8):1801–1808.
141. Hanson MA, Gluckman PD. Developmental origins of health and disease: new insights. *Basic Clin Pharmacol Toxicol*. 2008;102(2):90–93.
142. Booth FW, Laye MJ, Lees SJ, et al. Reduced physical activity and risk of chronic disease: the biology behind the consequences. *Eur J Appl Physiol*. 2008;102(4):381–390.
143. Zarychanski R, Houston DS. Anemia of chronic disease: a harmful disorder or an adaptive, beneficial response? *CMAJ*. 2008;179(4): 333–337.
144. Erythropoiesis-Stimulating Agents (ESAs) http://www.fda.gov/Drugs/DrugSafety/DrugSafetyPodcasts/ucm077204.htm. Accessed September 10, 2011.
145. Moylan JS, Reid MB. Oxidative stress, chronic disease, and muscle wasting. *Muscle Nerve*. 2007;35(4):411–429.
146. Green CR, Nicholson LF. Interrupting the inflammatory cycle in chronic diseases–do gap junctions provide the answer? *Cell Biol Int*. 2008;32(12):1578–1583.
147. Subramanian V, Ferrante AW. Obesity, inflammation, and macrophages. *Nestle Nutr Workshop Ser Pediatr Program*. 2009;63:151–159; discussion 159-162, 259-268.
148. Booth FW, Lees SJ. Fundamental questions about genes, inactivity, and chronic diseases. *Physiol Genomics*. 2007;28(2):146–157.
149. Bojalil R. Are we finally taming inflammation? *Crit Care Med*. 2007; 35(4):1215–1216.
150. Inflammation: a unifying theory of disease? Research is showing that chronic inflammation may be the common factor in many diseases. *Harv Health Lett*. 2006;31(6):4–5.
151. Schnaper HW, Hubchak SC, Runyan CE, et al. A conceptual framework for the molecular pathogenesis of progressive kidney disease. *Ped Nephr*. 2010;25(11):2223–2230.
152. Festa A, et al. The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obesity*. 2001;25(10):1407–1415.
153. Faber DR, van der Graaf Y, Westerink J, Visseren FLJ. Increased visceral adipose tissue mass is associated with increased C-reactive protein in patients with manifest vascular diseases. *Atherosclerosis*. 2010;212(1):274–280.
154. Ghanem FA, Movahed A. Inflammation in high blood pressure: a clinician perspective. *J Am Soc Hypertension*. 2007;1(2):113–119.
155. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135–1143.
156. Ridker PM, Buring JE, Shih J, et al. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation*. 1998;98(8):731–733.
157. Rozanski A, Blumenthal JA, Kaplan J. Act of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation*. 1999;99(16):2192–2217.
158. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. *Experimen Gerontol*. 2004;39(5):687–699.
159. Droge W. Free radicals in the physiological control of cell function. *Physio Rev*. 2002;82(1):47–95.
160. Xavier RJ, Podolsky D. Unraveling the pathogenesis of inflammatory bowel disease. *Nature*. 2007;448:427–434.
161. Fiocchi C. Susceptibility genes and overall pathogenesis of inflammatory bowel disease: where do we stand? *J Dig Dis*. 2009;2:26–35.
162. Kaser A, Zeissing S, Blumberg RS. Inflammatory bowel disease. *Ann Rev Immunol*. 2010;28:573–621.
163. Giovane A, Napoli C. Protective effects of food on cardiovascular diseases. In: Sauer H, Shah Ajay M, Laurindo FRM, eds. *Studies on Cardiovascular Disorders*. New York: Humana Press; 2010:455–471.
164. Schirbel A, Fiocchi C. Inflammatory bowel disease: established and evolving considerations on its etiopathogenesis and therapy. *J Dig Dis*. 2010;11(5):266–276.
165. Rossi G, Nucera E, Patriarca G, et al. Multiple chemical sensitivity: current concepts. *Int J Immunopathol Pharmacol*. 2007;20(suppl 1): 5–7.
166. Spencer TR, Schur PM. The challenge of multiple chemical sensitivity. *J Environ Health*. 2008;70(10):24–27.
167. Das-Munshi J, Rubin GJ, Wessely S. Multiple chemical sensitivities: review. *Curr Opin Otolaryngol Head Neck Surg*. 2007;15(4):274–280.
168. Angulo P. Medical Progress-nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346(16):1221–1231.

169. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*. 1999;48(1):1–9.
170. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Bio*. 2007;39(1):44–84.
171. Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004;114(12):1752–1761.
172. Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia*. 2007;48(1):43–58.

FURTHER READINGS

Festa A, et al. Chronic subclinical inflammation as part of the insulin resistance syndrome: the insulin resistance atherosclerosis study (IRAS). *Circulation*. 2000;102(1):42–47.
Kiecolt-Glaser JK, McGuire L, Robles TF, et al. Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. *Ann Rev Psych*. 2002;53:83–107.
Shekelle PG, Adams AH, Chassin MR, et al. Spinal manipulation for low-back pain. *Ann Int Med*. 1992;117(7):590–598.
Woods JA, Vieira VJ, Keylock KT. Exercise, inflammation, and innate immunity. *Immunol Allergy Clin North Am*. 2009;29(2):381–393.
Yetley EA. Multivitamin and multimineral dietary supplements: definitions, characterization, bioavailability, and drug interactions. *Am J Clin Nutr*. 2007;85(1):S269–S276.

3

The History of Naturopathic Medicine: Origins and Overview

George W. Cody, JD, MA, and *Heidi Hascall*, MA*

OUTLINE

Editors' Note: This is the first of two chapters comprehensively presenting the origins and evolution of naturopathic medicine. Quotes from key historical figures are used extensively in both chapters to illustrate how the ideas of naturopathic medicine originated and evolved. One challenge with this approach is that the language use and terminology more than a century ago are somewhat different from the current vocabulary. In addition, these pioneers were limited by the very early stages of the biological sciences because rigorous research into physiology and pathology was just beginning. Thus, from the modern perspective, some ideas may seem quaint, awkward, or "unscientific" based on our current understanding. Nonetheless, the concepts of health and disease they developed, despite the limited biological research, were remarkably insightful and, as well demonstrated by the more than 200 chapters in this textbook, have almost all now been fully validated. In fact, a number of their dire predictions of increased incidence of chronic disease if conventional medicine practices became dominant have now been conclusively demonstrated to have been correct. We urge the reader to focus on the evolution of the concepts and not be distracted by the antiquated terminology.

INTRODUCTION

Naturopathy, as a generally used term, emerged in America from the writings and promotion of Benedict Lust. Naturopathy, or "nature cure," is both a way of life and a concept of healing that employs various natural means of treating human infirmities and disease states. The earliest mechanisms of healing associated with the term, as used by Lust, involved a combination of hygienics and hydropathy (hydrotherapy). The term itself was coined in 1895 by Dr. John Scheel of New York City to describe his method of health care. However, earlier forerunners of these concepts already existed in the history of natural healing, both in America and in the Austro-Germanic European core.

Lust came to this country from Germany in the 1890s as a disciple of Father Sebastian Kneipp, a Dominican priest, and as an emissary dispatched by Father Kneipp to bring hydrotherapy to America. Lust purchased the term *naturopathy* from Scheel in 1902 to describe the eclectic compilation of doctrines of natural healing that he envisioned to be the future of natural medicine. In January 1902, Lust, who had been publishing the *Kneipp Water Cure Monthly* and its German-language counterpart in New York since 1896, changed the name of the journal to *The Naturopath and Herald of Health* and evoked the dawn of a new health care era with the following editorial:

> *Naturopathy is a hybrid word. It is purposely so. No single tongue could distinguish a system whose origin, scope and purpose is universal—broad as the world, deep as love, high as heaven. Naturopathy was not born of a sudden or a happen-so. Its progenitors have for eons been projecting thoughts and ideas and ideals whose culminations are crystallized in the new Therapy. Connaro, doling out his few fixed ounces of food and drink each day in his determined exemplification of Dietotherapy; Priessnitz, agonizing, despised and dejected through the long years of Hydropathy's travail; the Woerishofen priest, laboring lovingly in his little parish home for the thousands who journeyed Germany over for the Kneipp cure; Kuhne, living vicariously and dying a martyr for the sake of Serotherapy; A.T. Still, studying and struggling and enduring for his faith in Osteopathy; Bernarr Macfadden, fired by the will to make Physical Culture popular; Helen Willmans, threading the mazes of Mental Science, and finally emerging triumphant; Orrison Sweet Maraden, throbbing in sympathy with human faults and failures, and longing to realize Success to all mankind—these and hosts of*

* Previous edition contributor

others have brought into being single systems whose focal features are perpetuated in Naturopathy.

Jesus Christ—I say it reverently—knew the possibility of physical immortality. He believed in bodily beauty; He founded Mental Healing; He perfected Spirit-power. And Naturopathy will include ultimately the supreme forces that made the Man of Galilee omnipotent.The scope of Naturopathy is from the first kiss of the new-found lovers to the burying of the centenarian whose birth was the symbol of their perfected one-ness. It includes ideally every life-phase of the id, the embryo, the foetus, the birth, the babe, the child, the youth, the man, the lover, the husband, the father, the patriarch, the soul.

We believe in strong, pure, beautiful bodies thrilling perpetually with the glorious power of radiating health. We want every man, woman and child in this great land to know and embody and feel the truths of right living that mean conscious mastery. We plead for the renouncing of poisons from the coffee, white flour, glucose, lard, and like venom of the American table to patent medicines, tobacco, liquor and the other inevitable recourse of perverted appetite. We long for the time when an eight-hour day may enable every worker to stop existing long enough to live; when the spirit of universal brotherhood shall animate business and society and the church; when every American may have a little cottage of his own, and a bit of ground where he may combine Aerotherapy, Heliotherapy, Geotherapy, Aristophagy and nature's other forces with home and peace and happiness and things forbidden to flat-dwellers; when people may stop doing and thinking and being for others and be for themselves; when true love and divine marriage and pre-natal culture and controlled parenthood may fill this world with germ-gods instead of humanized animals.In a word, Naturopathy stands for the reconciling, harmonizing and unifying of nature, humanity and God.

Fundamentally therapeutic because men need healing; elementally educational because men need teaching; ultimately inspirational because men need empowering, it encompasses the realm of human progress and destiny.

Perhaps a word of appreciation is due Mr. John H. Scheel, who first used the term "Naturopathic" in connection with his Sanitarium "Badekur," and who has courteously allowed us to share the name. It was chosen out of some 150 submitted, as most comprehensive and enduring. All our present plans are looking forward some five or ten or fifty years when Naturopathy shall be the greatest system in the world.

Actually the present development of Naturopathy is pitifully inadequate, and we shall from time to time present plans and ask suggestions for the surpassing achievement of our world-wide purpose. Dietetics, Physical Culture and Hydropathy are the measures upon which Naturopathy is to build; mental culture is the means, and soul-selfhood is the motive.

If the infinite immensity of plan, plea and purpose of this particular magazine and movement were told you, you would simply smile in your condescendingly superior way and straightway forget. Not having learned as yet what a brain and imagination and a will can do, you consider Naturopathy an ordinarily innocuous affair, with a lukewarm purpose back of it, and an ebbing future ahead of it. Such is the character of the average wishy-washy health movement and tumultuous wave of reform.

Your incredulous smile would not discomfit us—we do not importune your belief, or your help, or your money. Wherein we differ from the orthodox self-labeled reformer, who cries for sympathy and cringes for shekels.

We need money most persistently—a million dollars could be used to advantage in a single branch of the work already definitely planned and awaiting materialization; and we need co-operation in a hundred different ways. But these are not the things we expect or deem best. Criticism, fair, full and unsparing is the one thing of value you can give this paper. Let me explain. Change is the keynote of this January issue—in form, title, make-up. If it please you, your subscription and a word to your still-benighted friends is ample appreciation. But if you don't like it, say so. Tell us wherein the paper is inefficient or redundant or ill-advised, how it will more nearly fit into your personal needs, what we can do to make it the broadest, deepest, truest, most inspiring of the mighty host of printed powers. The most salient letter of less than 300 words will be printed in full, and we shall ask to present the writer with a subscription-receipt for life.

By to-morrow you will probably have forgotten this request; by the day after you will have dropped back into your old ways of criminal eating and foolish drinking and sagged standing and congested sitting and narrow thinking and deadly fearing—until the next progress paper of New Thought or Mental Science or Dietetics or Physical Culture prods you into momentary activity.

Between now and December we shall tell you just how to preserve the right attitude, physical and mental, without a single external aid; and how, every moment of every day, to tingle and pulsate and leap with the boundless ecstasy of manhood consciously nearing perfection.

A BRIEF HISTORY OF EARLY AMERICAN MEDICINE WITH AN EMPHASIS ON NATURAL HEALING

To understand the evolutionary history of naturopathic medicine in this country, it is necessary to view the internal development of the profession against the historical, social, and cultural backdrop of American social history.

Medicine in America: 1800–1875

In the America of 1800, although a professional medical class existed, medicine was primarily domestically oriented. An individual who fell ill was commonly nursed by a friend or family member who relied upon William Buchan's *Domestic Medicine* (1769), John Wesley's *Primitive Physic* (1747), or John Gunn's *Domestic Medicine* (1830).[1]

Professional Medicine

Professional medicine transferred from England and Scotland to America in prerevolutionary days. However, 18th- and early-19th-century America considered the concept of creating a small, elite, learned profession to run counter to the political and institutional concepts of early American democracy.[1]

The first medical school in the American colonies opened in 1765 at what was then the College of Philadelphia (later the University of Pennsylvania), and the school was dominated by revolutionary leader and physician Benjamin Rush, a signatory to the Declaration of Independence. The proliferation of medical schools to train the new professional medical class began seriously after the war of 1812. Between 1810 and 1820, new schools were established in Baltimore, Lexington, Cincinnati, and even in rural communities in Vermont and Western New York. Between 1820 and 1850, substantial numbers of schools were established in the western rural states. By 1850, there were 42 medical schools recognized in the United States, although there were only 3 in all of France.

Generally, these schools were started by a group of five to seven local physicians approaching a local college with the idea of establishing a medical school in conjunction with the college's educational facilities. The schools were largely apprenticeship based, and the professors received their remuneration directly from fees paid by the students.

The requirements for a doctor of medicine (MD) degree in late-18th- and early-19th-century America were roughly as follows:

- Knowledge of Latin and natural and experimental philosophy
- Three years of serving an apprenticeship under practicing physicians
- Attending two terms of lectures and passing of attendant examinations
- A thesis

Additionally, graduating students had to be at least 21 years of age.[1]

The rise of any professional class is gradual and marked by difficulties, and varying concepts existed as to the demarcation of a "professional" physician. Contrasts included graduates of medical school versus nongraduates, medical society members versus nonmembers, and licensed physicians versus unlicensed "doctors." Licensing statutes came into existence between 1830 and 1850 but were soon repealed because they were considered "undemocratic" during the apex of Jacksonian democracy. [1]

Thomsonianism

In 1822 the rise in popularity of Samuel Thomson and his publication of *New Guide to Health* helped frustrate the creation of a professional medical class. Thomson's work was a compilation of his personal view of medical theory and American Indian herbal and medical botanical lore. Thomson espoused the belief that disease had one general cause—"cold" influences on the human body—and that disease therefore had one general remedy: "heat." Unlike the followers of Benjamin Rush and the American "heroic" medical tradition who advocated blood-letting, leeching, and the substantial use of mineral-based purgatives such as antimony and mercury, Thomson believed that minerals were sources of "cold" because they came from the ground and that vegetation, which grew toward the sun, represented "heat." [1]

As noted in Griggs's *Green Pharmacy* (the best history of herbal medicine to date), Thomson's theory developed as follows[2]:

> *Instead, he elaborated a theory of his own, of the utmost simplicity: "All diseases … are brought about by a decrease or derangement of the vital fluids by taking cold or the loss of animal warmth … the name of the complaint depends upon what part of the body has become so weak as to be affected. If the lungs, it is consumption, or the pleura, pleurisy; if the limbs, it is rheumatism, or the bowels, colic or cholera morbus … all these different diseases may be removed by a restoration of the vital energy, and removing the obstructions which the disease has generated …"*
>
> *Thus the great object of his treatment was always to raise and restore the body's vital heat: "All … that medicine can do in the expulsion of disorder, is to kindle up the decaying spark, and restore its energy till it glows in all its wonted vigor."*

Thomson's view was that individuals could be self-treating if they had a sincere "guide to health" philosophy and a copy of his book, *New Guide to Health*. The right to sell "family franchises" for the use of the Thomsonian method of healing was the basis of a profound lay movement between 1822 and Thomson's death in 1843. Thomson adamantly believed that no professional medical class should exist and that democratic medicine was best practiced by laypersons within a Thomsonian "family" unit.

By 1839, Thomson claimed to have sold some 100,000 of these family franchises called "friendly botanic societies." Although he professed to have solely the interests of the individual at heart, his system was sold at a profit under the protection of a patent he obtained in 1813.

The Eclectic School of Medicine

Some of the "botanics" (professional Thomsonian doctors) wanted to separate themselves from the lay movement by creating requirements and standards for the practice of Thomsonian medicine. Thomson, however, was adamantly against a medical school founded on his views. Thus it was not until the decade after Thomson's death that independent Thomsonians founded a medical college (in Cincinnati) and began to dominate the Thomsonian movement. These Thomsonian botanics were later absorbed into the medical sectarian movement known as the "eclectic school," which originated with the New Yorker Wooster Beach.

Beach was another of medical history's fascinating characters. From a well-established New England family, he started his medical studies at an early age, apprenticing under an old German herbal doctor, Jacob Tidd. After Tidd died, Beach enrolled in the Barclay Street Medical University in New York. Griggs[2] described the following:

> *Beach's burning ambition was to reform medical practice generally—not to alienate the entire profession by savage attacks from without—and he was convinced that he would be in a stronger position to do so if he were himself a diplomatized doctor. The faculty occasionally listened to criticism from within their own number: against onslaughts of "illiterate quacks" like Samuel Thomson, they simply closed ranks in complacent hostility.*

After opening his own practice in New York, Beach set out to win over fellow members of the New York Medical Society (into which he had been warmly introduced by the screening committee) to his point of view that heroic medicine was inherently dangerous to mankind and should be reduced to the gentler theories of herbal medicine. He was summarily ostracized from the medical society.

To Beach, this was a bitter blow, but he soon founded his own school in New York, calling the clinic and educational facility the "United States Infirmary." However, because of continued pressure from the medical society, he was unable to obtain charter authority to issue legitimate diplomas. He then located a financially ailing but legally chartered school, Worthington College, in Ohio. He opened a full-scale medical college; out of its classrooms, he launched what became known as the *Eclectic School of Medical Theory*. Griggs related the following[2]:

> *Beach had a new name for his practice: While explaining to a friend his notions of combining what was useful in the old practice with what was best in the new, the friend exclaimed, "You are an eclectic!" to which, according to legend, Beach replied, "You have given me the term which I have wanted: I am an eclectic!"*

Cincinnati subsequently became the focal point of the eclectic movement, and the E. M. Institute medical school remained until 1938 (the last eclectic school to exist in America).[3] The concepts of this sect helped form some of the theoretical underpinnings of Lust's naturopathy. Lust himself graduated from the Eclectic Medical College of the City of New York in the first decade of the 1900s.

Despite his criticism of the early allopathic movement (although the followers of Rush were not yet known as *allopaths*, a term reputed to have been coined by Samuel Hahnemann) for their "heroic" tendencies, Thomson's medical theories were "heroic" in their own fashion. Although he did not advocate blood-letting, heavy metal poisoning, and leeching, botanic purgatives—particularly *Lobelia inflata* (Indian tobacco)—were a substantial part of the therapy.

The Hygienic School of Thought

One other forerunner of American naturopathy, also originating as a lay movement, grew into existence at this time. This was the "hygienic" school, which had its genesis in the popular teachings of Sylvester Graham and William Alcott.

Graham began preaching the doctrines of temperance and hygiene in 1830. In 1839 he published *Lectures on the Science of Human Life,*

two hefty volumes that prescribed healthy dietary habits. He emphasized a moderate lifestyle, recommending an antiflesh diet and bran bread as an alternative to bolted or white bread.

Alcott dominated the scene in Boston during this same period and, together with Graham, saw that the American hygienic movement—at least as a lay doctrine—was well established.[4]

Homeopathy

By 1840, the profession of homeopathy had also been transplanted to America from Germany. Homeopathy, the creation of an early German physician, Samuel Hahnemann (1755–1843), had three central doctrines:

- The "law of similars" (that like cures like)
- That the effect of a medication could be heightened by its administration in minute doses (the more diluted the dose, the greater the "dynamic" effect)
- That nearly all diseases were the result of a suppressed itch, or "psora"

The view was that a patient's natural symptom-producing disease would be displaced after homeopathic medication by a similar, but much weaker, artificial disease that the body's immune system could easily overcome.

Originally, most homeopaths in this country were converted orthodox medical men, or "allopaths." The high rate of conversion made this particular medical sect the archenemy of the rising orthodox medical profession. (For a more detailed discussion of homeopathy, see Chapter 39.)

The first homeopathic medical school was founded in 1850 in Cleveland; the last purely homeopathic medical school, based in Philadelphia, survived into the early 1930s. [1]

The Rise and Fall of the Sects

Although these two nonallopathic sects were popular, they never comprised more than one fifth of the professional medical class in America. Homeopathy at its highest point reached roughly 15% and the eclectic school roughly 5%. However, their very existence for many years kept the exclusive recognition desired by the orthodox profession from coming within its grasp. Homeopathy was distasteful to the more conventional medical men not only because it resulted in the conversion of a substantial number of their peers but also because homeopaths generally also made a better income. The rejection of the eclectic school was more fundamental: it had its roots in a lay movement that challenged the validity of a privileged professional medical class.

The existence of three professional medical groups—the orthodox school, the homeopaths, and the eclectics—combined with the Jacksonian view of democracy that prevailed in mid-19th-century America, resulted in the repeal of virtually all medical licensing statutes existing before 1850. However, by the 1870s and 1880s, all three medical groups began to voice support for the restoration of medical licensing.

Views differ as to what caused the homeopathic and eclectic schools to disappear from the medical scene in the 50 years after 1875. One view defined a sect as follows[5]:

> *A sect consists of a number of physicians, together with their professional institutions, who utilize a distinctive set of medically invalid therapies which are rejected by other sects.*

By this definition, the orthodox or allopathic school was just as sectarian as the homeopathic and eclectic schools. Rothstein's view was that these two 19th-century sects disappeared because, beginning in the 1870s, the orthodox school grasped the European idea of "scientific medicine." Based on the research of such men as Pasteur and Koch and the "germ theory," this approach supposedly proved to be the medically proper view of valid therapy and gained public recognition because of its truth.

Another view was that the convergence of the needs of the three sects for professional medical recognition (which began in the 1870s and continued into the early 1900s) and the "progressive era" led to a political alliance in which the majority orthodox school was ultimately dominant by sheer weight of numbers and internal political authority. Starr[1] noted the following:

> *Both the homeopaths and eclectics wanted to share in the legal privileges of the profession. Only afterward did they lose their popularity. When homeopathic and eclectic doctors were shunned and denounced by the regular profession, they thrived, but the more they gained an access to the privileges of regular physicians, the more their numbers declined. The turn of the century was both the point of acceptance and the moment of incipient disintegration.*

In any event, this development was an integral part of the drive toward professional authority and autonomy established during the progressive era (1900–1917). It was acceptable to the homeopaths and the eclectics because they controlled medical schools that continued to teach and maintain their own professional authority and autonomy. However, it was after these professional goals were attained that the lesser schools of medical thought went into rapid decline. [1]

The American Influence

From 1850 to 1900, the medical counterculture continued to establish itself in America. From its lay roots in the teachings of the hygienic movement grew professional medical recognition, albeit a small minority and "irregular" view, that hygiene and hydropathy were the basis of sound medical thought (much like the Thomsonian transition to botanic and eclectic medicine).

Russell Trall

The earliest physician who had a significant effect on the later growth of naturopathy as a philosophic movement was Russell Trall, MD. As noted in James Whorton's *Crusaders for Fitness*,[4] he "passed like a meteor through the American hydropathic and hygienic movement":

> *The exemplar of the physical educator-hydropath was Russell Thatcher Trall. Still another physician who had lost his faith in regular therapy, Trall opened the second water cure establishment in America, in New York City in 1844. Immediately he combined the full Preissnitzian armamentarium of baths with regulation of diet, air, exercise and sleep. He would eventually open and or direct any number of other hydropathic institutions around the country, as well as edit the Water-Cure Journal, the Hydropathic Review, and a temperance journal. He authored several books, including popular sex manuals which perpetuated Graham-like concepts into the 1890s, sold Graham crackers and physiology texts at his New York office, was a charter member (and officer) of the American Vegetarian Society, presided over a short-lived World Health Association, and so on. His crowning accomplishment was the Hygeian Home, a "model Health Institution [which] is beautifully situated on the Delaware River between Trenton and Philadelphia." A drawing presents it as a palatial establishment with expansive grounds for walking and riding, facilities for rowing, sailing, and swimming, and even a grove for open-air "dancing gymnastics." It was the grandest of water cures, and lived beyond the Civil War period, which saw the demise of most hydropathic hospitals. True, Trall had to struggle to keep his head above water during the 1860s, but by the 1870s he had a firm financial footing (being stabilized by tuition fees from the attached Hygeio-therapeutic College). With Trall's death in 1877, however, the hydropathic phase of health reform passed.*

As made evident later in this chapter, this plethora of activity was similar to that engaged in by Benedict Lust between 1896 and his death in 1945, when he worked to establish naturopathy. The Hygeian Home and later "Yungborn" establishments at Butler, New Jersey, and Tangerine, Florida, were similar to European nature cure sanitariums, such as the original Yungborn founded by Adolph Just and the spa/sanitarium facilities of Preissnitz, Kneipp, and Just.

Trall gave a famous address to the Smithsonian Institution in Washington, DC, in 1862, under the sponsorship of the Washington Lecture Association. "The true healing art: or hygienic vs drug medication," a 2.5-hour lecture purported to have received rapt attention, was devoted to Trall's belief in the hygienic system and in hydropathy as the true healing art. The address was reprinted by Fowler and Wells (New York, 1880) with an introduction written by Trall, before his death in 1877.

Trall also founded the first school of natural healing arts in this country to have a 4-year curriculum and the authorization to confer the degree of MD. It was founded in 1852 as a "hydropathic and physiological school" and was chartered by the New York State Legislature in 1857 under the name "New York Hygio-Therapeutic College," with the legislature's authorization to confer the MD degree.

In 1862 Trall went to Europe to attend the International Temperance Convention. He took a prominent part at this meeting of reformers, specifically related to the use of alcohol as a beverage and as a medicine. He eventually published more than 25 books on the subjects of physiology, hydropathy, hygiene, vegetarianism, and temperance, among many others.

The most valuable and enduring of these was his *Hydropathic Encyclopedia,* a volume of nearly 1000 pages that covered the theory and practice of hydropathy and the philosophy and treatment of diseases advanced by older schools of medicine. At the time of his death, according to the December 1877 *Phrenological Journal* cover article featuring a lengthy obituary of Trall, this encyclopedia had sold more than 40,000 copies since its original publication in 1851.

For more than 15 years, Trall was editor of the *Water-Cure Journal* (also published by Fowler and Wells). During this period, the journal went through several name changes, including the *Hygienic Teacher* and *The Herald of Health.* When Lust originally opened the American School of Naturopathy, an English-language version of *Kneipp's Water-cure* (or *Meine Wasser-kurr* in German) being unavailable, he used only the works and writings of Trall as his texts.

Martin Luther Holbrook

By 1871, Trall moved from New York to the Hygeian Home on the Delaware River. His water-cure establishment in New York became the New Hygienic Institute. One of its coproprietors was Martin Luther Holbrook, who later replaced Trall as the editor of *The Herald of Health.* Professor Whorton noted the following[4]:

> *But Holbrook's greatest service to the cause was as an editor. In 1866 he replaced Trall at the head of The Herald of Health, which had descended from the Water-Cure Journal and Herald of Reforms (1845–1861) by the way of the Hygienic Teacher and Water-Cure Journal (1862). Under Holbrook's direction the periodical would pass through two more name changes (Journal of Hygiene Herald of Health, 1893–1897, and Omega, 1898–1900) before merging with Physical Culture.*

Trall and Holbrook both advanced the idea that physicians should teach the maintenance of health rather than simply providing a last resort in times of health crisis. Besides providing a strong editorial voice espousing vegetarianism, the evils of tobacco and drugs, and the value of bathing and exercise, dietetics and nutrition, along with personal hygiene, were strongly advanced by Holbrook and others of the hygienic movement during this era. Whorton described the idea as follows[4]:

> *The orthodox hygienists of the progressive years were equally enthused by the recent progress of nutrition, of course, and exploited it for their own ends, but their utilization of science hardly stopped with dietetics. Medical bacteriology was another area of remarkable discovery, bacteriologists having provided, in the short space of the last quarter of the 19th century, an understanding, at long last, of the nature of infection. This new science's implications for hygienic ideology were profound—when Holbrook locked horns with female fashion, for example, he did not attack the bulky, ground-length skirts still in style with the crude Grahamite objection that the skirt was too heavy. Rather he forced a gasp from his readers with an account of watching a smartly dressed lady unwittingly drag her skirt "over some virulent, revolting looking sputum, which some unfortunate consumptive had expectorated."*

Holbrook expanded on the work of Graham, Alcott, and Trall and, working with an awareness of the European concepts developed by Preissnitz and Kneipp, laid further groundwork for the concepts later advanced by Lust, Lindlahr, and others[4]:

> *For disease to result, the latter had to provide a suitable culture medium, had to be susceptible. As yet, most physicians were still so excited at having discovered the causative agents of infection that they were paying less than adequate notice to the host. Radical hygienists, however, were bent just as far in the other direction. They were inclined to see bacteria as merely impotent organisms that throve only in individuals whose hygienic carelessness had made their body compost heaps. Tuberculosis is contagious, Holbrook acknowledged, but "the degree of vital resistance is the real element of protection. When there is no preparation of the soil by heredity, predisposition or lowered health standard, the individual is amply guarded against the attack." A theory favored by many others was that germs were the effect of disease rather than its cause; tissues corrupted by poor hygiene offered microbes, all harmless, an environment in which they could thrive.*

In addition to introducing the works of Kneipp and his teachings to the American hygienic healthcare movement, Holbrook was a leader of the fight against vivisection and vaccination[4]:

> *Vivisection and vaccination were but two of the practices of medicine criticized in the late 19th century. Therapy also continued to be an object of protest. Although the heroism of standard treatment had declined markedly since mid-century, a prescription was still the reward of any visit to the doctor, and drugless alternatives to healing were appearing in protest. Holbrook published frequent favorable commentaries on the revised water cure system of Germany's Kneipp. A combination of baths, herbal teas, and hardening exercises, the system had some vogue in the 1890's before flowering into naturopathy. Holbrook's journal also gave positive notices to osteopathy and "chiropathy" [chiropractic], commending them for not going to the "drugstore or ransack[ing] creation for remedies nor load[ing] the blood with poison." But though bathing and musculoskeletal manipulation were natural and nonpoisonous, Holbrook preferred to give the body complete responsibility for healing itself. Rest and proper diet were the medicines of this doctor who billed himself as a "hygienic physician" and censured ordinary physicians for being engrossed with disease rather than health.*

The Beginnings of "Scientific Medicine"

While the hygienic movement was making its effect, the orthodox medical profession, in alliance with the homeopaths and eclectics, was making significant advances. The orthodox profession, through the political efforts of the American Medical Association (AMA), first tried to remove sectarian and irregular practitioners by segregating them from the medical profession altogether. It did so by formulating and publishing its first national medical code of ethics in 1847. (In 1846 the orthodox profession formed the AMA to represent its professional views.) The code condemned proprietary patents (even carrying over into a physician's development of surgical or other medical implements, which led to its greatest criticism); encouraged the adoption of uniform rules for payment in geographic areas; condemned the practice of contract work; prohibited advertising and fee-sharing even among specialists and general practitioners; eliminated blacks and women; and, most significantly, prohibited any consultation or contact with irregulars or sectarian practitioners. The code stated the following[6]:

> *No one can be considered as a regular practitioner, or a fit associate in consultation, whose practice is based on an exclusive dogma, to the rejection of the accumulated experience of the profession, and of the aids actually furnished by anatomy, physiology, pathology, and organic chemistry.*

In the late 1870s and into the 1880s the major sects—the orthodox or allopathic school, the homeopaths, and the eclectics—began to find more reason to cooperate to obtain common professional goals. These included the enactment of new licensing laws and the creation of a "respectable" medical educational system. Also at this time, the concept of "scientific medicine" was brought to America. (Although Starr differed from Rothstein regarding the causes of the decline of the homeopathic and eclectic sectarian schools, he noted that Rothstein clearly documented the 19th-century transition of medicine into a recognized professional class composed of both the minority sects and the orthodox school.)

This transition from conflict between the major sects resulted in the erosion of the implementation of the code of ethics; the cooperation among the sects to revive medical licensing standards; the admission of sectarian physicians to regular medical societies; and ultimately, a structural reorganization of the AMA, which occurred between 1875 and 1903.[15]

Once the cooperation among the three medical views began, the medical class dominated by the regular school came fully into power. The homeopathic and eclectic schools of thought met their demise finally because of two significant events: (1) the rapid creation of new medical educational standards between 1900 and 1910, culminating in the publication of the famous "Flexner Report" (1910), and (2) the effective infusion of millions of dollars into selected allopathic medical schools by the newly created capitalistic philanthropic foundations, principally the Carnegie and Rockefeller Foundations.

The Foundations

The effect of the monies from the Carnegie and Rockefeller Foundations was clearly documented[7] and described in detail in Brown's *Rockefeller Medicine Men*.[8] The effect of the monies from these foundations, contributed to medical schools that met the AMA's views on medical education and philosophy, cannot be underestimated.

This process has been well documented.[1,7,9,10] As discussed by Burrows,[10] these educational reforms allowed the AMA to forge a new alliance with state legislators and push quickly for medical licensing designed to reward the educational and medical expertise of the newly orthodox "scientific medicine" and to the exclusion of all others.

Medical Education in Transition

Based on the rising example of scientific medicine and its necessary connection to research, the educational laboratory, and a more thorough scientific education as a preamble to medical practice, Harvard University (under the presidency of Charles Elliott) created a 4-year medical educational program in 1871. The primal modern medical educational curriculum was devised and set in motion more than 20 years later at Johns Hopkins University under the leadership of William Osler and William Welch, using the resources from the original endowment of the hospital and university from the estate of Johns Hopkins.[1]

Other schools followed suit. By the time the AMA set up its Council on Medical Education in 1904, it was made up of five members from the faculties of schools modeled on the Johns Hopkins prototype. This committee set out to visit and rate each of the 160 medical schools then in operation in the country. The ratings used were class A (acceptable), class B (doubtful), and class C (unacceptable).

Eighty-two schools received a class A rating, led by Harvard, Rush (Chicago), Western Reserve, the University of California, and notably, Johns Hopkins. Forty-six schools received a class B rating, and 32 received a class C rating. The class C schools were mostly in rural areas, and many of them were proprietary in nature.

Flexner Report

Subsequent to the AMA ratings, the Council on Medical Education applied to the Carnegie Foundation to commission an independent report to verify its work. Abraham Flexner, a young, energetic, and noted educator, was chosen for this task by the Carnegie Foundation and accompanied by the secretary (Nathan Colwell, MD) of the Council on Medical Education, who participated in all of the committee site visits.

Flexner visited each of the 162 operating U.S. medical schools. The widely publicized *Flexner Report* put the nails in the coffins of all schools with class C ratings and many with class B ratings. Significantly, the educational programs of all but one eclectic school (in Cincinnati) and one homeopathic school (in Philadelphia) were eliminated by 1918.

The eclectic medical schools, in particular, were severely affected by the report. Griggs explained this effect as follows[2]:

> *Of the eight Eclectic schools, the Report declared that none had "anything remotely resembling the laboratory equipment which is claimed in their catalogs." Three of them were under-equipped; the rest "are without exception filthy and almost bare. They have at best grimy little laboratories … a few microscopes, some bottles containing discolored and unlabeled pathological material, in an incubator out of commission, and a horrid dissecting room." The Report found them more culpable than a regular school for these inadequacies: "… the Eclectics are drug-mad; yet, with the exception of the Cincinnati and New York schools, they are not equipped to teach the drugs or drug therapy which constitutes their sole reason for existence."*

The other regular schools that conducted homeopathic or eclectic programs had by that time phased them out in the name of "scientific medicine" (see also Haller[3]).

Pharmaceutical Industry

During this same time, the AMA, through several of its efforts, began a significant alliance with the organized pharmaceutical industry of the United States, shaping it in a manner acceptable to the allopathic profession.[1,9,11]

The New "Sects"

The period from 1890 to 1905 saw the rise of three new medical sects and several other smaller "irregular" schools that replaced those soon to pass from the scene. In Missouri, Andrew Taylor Still, originally trained as an orthodox practitioner, founded the school of medical thought known as "osteopathy" and in 1892 opened the American School of Osteopathy in Kirksville, Missouri. In 1895 Daniel David Palmer, originally a magnetic healer from Davenport, Iowa, performed the first spinal manipulation, which gave rise to the school he termed "chiropractic." He formally published his findings in 1910, after having founded a chiropractic school in Davenport, Iowa. In 1902 Lust founded the American School of Naturopathy in New York.

Although some of the following discussions are devoted to the schools of healing called *osteopathy* and *chiropractic,* only that portion of their histories related to the history of naturopathy is mentioned.[12] (A full study of osteopathic medicine in America may be found in *The D.O.'s* by Gevitz,[13] and a reasonable sketch of chiropractic medicine may be found in Kapling's chapter in *Alternative Medicine.*[12])

As noted by Starr,[1] these new sects, including Christian Science, formulated by Mary Baker Eddy,[14] either rose or fell on their own without ever completely allying with orthodox medicine. Starr theorized that these sects arose late enough that the orthodox profession and its political action arm, the AMA, had no need to ally with them and would rather battle with them publicly. This made these sectarian views separate and distinct from the homeopathic and eclectic schools.

THE FOUNDING OF NATUROPATHIC MEDICINE

Benedict Lust

Lust came to the United States in 1892 at the age of 23. He suffered from a debilitating condition in his late teens while growing up in Michelbach, Baden, Germany, and was sent by his father to undergo the Kneipp cure at Woerishofen. He stayed there from mid-1890 to early 1892; not only was he "cured" of his condition, but he also became a protégé of Kneipp. Dispatched by Kneipp to bring the principles of the Kneipp water cure to America, he emigrated to New York City.

By making contact in New York with other German Americans who were also becoming aware of the Kneipp principles, Lust participated in the founding of the first "Kneipp Society," which was organized in Jersey City, New Jersey, in October 1896.

Lust also attended the first organizational meeting (in mid-October 1896) of the Kneipp Society of Brooklyn. Subsequently, through Lust's organization and contacts, Kneipp Societies were founded in Boston; Chicago; Cleveland; Denver; Cincinnati; Philadelphia; Columbus; Buffalo and Rochester, New York; New Haven, Connecticut; San Francisco; New Mexico; and Mineola on Long Island, New York.

The members of these organizations were provided with copies of the *Kneipp Blatter* and a companion English publication Lust began to put out called *The Kneipp Water Cure Monthly.*

The first "sanatorium" using Kneipp's principles was organized in this country shortly before Lust's arrival. Charles Lauterwasser, an earlier student of Kneipp's who called himself a hydrothic physician and natural scientist, opened the Kneipp and Nature Cure Sanatorium in Newark, New Jersey, in 1891.

In 1895 the Brooklyn Light and Water-Cure Institute was established in Brooklyn, New York, by L. Staden and his wife Carola, both graduates of Lindlahr's Hygienic College in Dresden, Germany. According to their advertising, they specialized in natural healing, Kneipp water treatment, and Kuhne's and Preissnitz's principles (including diet cure, electric light baths [both white and blue], electric vibration massage, Swedish massage and movements, and Thure Brandt massage).

In 1895 Lust opened the Kneipp Water-Cure Institute in New York City, listing himself as the owner and Dr. William Steffens as the residing physician. At the same address (on 59th Street) in October of that year, Lust opened the first "Kneipp store." In the originating November 1896 edition of *The Kneipp Water Cure Monthly* and *Kneipp Blatter,* he advertised his store and sanitarium as personally authorized by Kneipp. In the first part of 1896, just before his organizing of various Kneipp Societies around the New York area, Lust returned to Woerishofen to study further with Kneipp.

Kneipp died in Germany, at Woerishofen, in June 1897. With his passing, Lust was no longer bound strictly to the principles of the Kneipp water cure. He had begun to associate earlier with other German American physicians, principally Dr. Hugo R. Wendel (a German-trained *Naturarzt*), who began, in 1897, to practice in New York and New Jersey as a licensed osteopathic physician. In 1896 Lust entered the Universal Osteopathic College of New York, graduated in 1898, and became licensed as an osteopathic physician. In 1897 Lust became an American citizen.

Once he was licensed to practice as a healthcare physician in his own right, Lust began the transition toward the concept of "naturopathy." Between 1898 and 1902, when he adopted the term *naturopath,* Lust acquired a chiropractic education and changed the name of his Kneipp store to the Health Food Store (the original facility to use that name and concept in this country), specializing in providing organic foods and the materials necessary for drugless cures. He also began the New York School of Massage (listed as established in 1896) and the American School of Chiropractic, all within the same facility—Lust's Kneipp Institute.

Photographs of this facility taken between 1902 and 1907, when the facility moved to another location, show a five-story building listing "Benedict Lust—Naturopath, Publisher, Importer."

He returned to Germany in 1907 to visit with Dr. Baumgarten, Kneipp's medical successor at the Woerishofen facility, which was then run, in cooperation with Baumgarten, by the Reverend Prior Reily, the former secretary to Kneipp and his lay successor at Woerishofen. As directed by Kneipp, Reily had completed, after Kneipp's death, Kneipp's masterwork *Das grosse Kneipp—Buch.* Lust maintained contact with the partnership of Reily and Baumgarten throughout the early part of the 20th century.

In 1902 when he purchased and began using the term *naturopathy* and calling himself a "naturopath," Lust, in addition to his New York School of Massage and American School of Chiropractic, his various publications, and his operation of the Health Food Store, began to operate the American School of Naturopathy, all at the same 59th Street New York address.

By 1907 Lust's enterprises had grown sufficiently large that he moved them to a 55-room building. It housed the Naturopathic Institute, Clinic, and Hospital; the American Schools of Naturopathy and Chiropractic; the now-entitled Original Health Food Store; Lust's publishing enterprises; and the New York School of Massage. The operation remained in this four-story building, roughly twice the size of the original facility, from 1907 to 1915.

From 1912 to 1914, Lust took a "sabbatical" from his operations to further his medical education. By this time, he had founded his large estate-like sanitarium in Butler, New Jersey, known as "Yungborn" after the German sanitarium operation of Adolph Just.

In 1912 he attended the Homeopathic Medical College in New York, which, in 1913, granted him a degree in homeopathic medicine, and in 1914, he received his degree in eclectic medicine. In early 1914 Lust traveled to Florida and obtained an MD's license on the basis of his graduation from the Homeopathic Medical College and the Eclectic Medical College of New York City.

He founded another "Yungborn" sanitarium facility in Tangerine, Florida, and for the rest of his life, while continuing his publications, he engaged in active public lecturing. He also continued to maintain a practice in New York City and operated the sanitariums in Florida and New Jersey. His schools were operated primarily by Hugo R. Wendel.

From 1902, when he began to use the term *naturopathy,* until 1918, Lust replaced the Kneipp Societies with the Naturopathic Society of America. Then, in December 1919, the Naturopathic Society of America was formally dissolved because of its insolvency, and Lust founded the American Naturopathic Association (ANA). Thereafter, 18 states incorporated the association.

In 1918, as part of his effort to replace the Naturopathic Society of America (an operation into which he invested a great deal of his funds and resources in an attempt to organize a naturopathic profession) and replace it with the ANA, Lust published the first *Universal Naturopathic Directory and Buyer's Guide* (a "yearbook of drugless therapy").

Although a completely new version was never actually published, despite Lust's announced intention to make this volume an annual publication, annual supplements were published in either *The Naturopath and Herald of Health* or its companion publication *Nature's Path* (which commenced publication in 1925). The *Naturopath and Herald of Health,* sometimes printed with the two phrases reversed, was published from 1902 to 1927 and from 1934 until after Lust's death in 1945.

This volume documented the merging of the German and American influences that guided Lust in his development of the practice of naturopathy. The voluminous tome, which ran to 1416 pages, was dedicated to:

> *The memory of all those noble pioneers and discoverers who have died in the faith of Naturopathy, and to their courageous successors in the art of drugless healing, all of whom have suffered persecution for saving human lives that medical autocracy could not save, this work is respectfully dedicated by its editor Benedict Lust, N.D., M.D., "The Yungborn," Butler, New Jersey, U.S.A., April 1, 1918.*

Lust's introduction is reprinted here in its entirety to show the purpose of the directory and the status of the profession in the early 1900s:

Introduction

To the Naturopathic Profession, the Professors of Natural Healing in all its branches, the Professors of Scientific Diet, Hydrotherapy, Heliotherapy, Electrotherapy, Neuropathy, Osteopathy, Chiropractic, Naprapathy, Magnetopathy, Phytotherapy, Exercise, Swedish Movements, Curative Gymnastics, Physical and Mental Culture, Balneopathy, and all forms of Drugless Healing, the Faculties of all Drugless Colleges, Institutions, Schools, and all Professors of Hygiene and Sanitation; Manufacturers of Naturopathic Supplies; Publishers of Health Literature, and Natural Healing Societies, GREETINGS: I have the honor to present to your consideration and goodwill, this Volume, No. 1, Year 1918–1919, of the Universal Naturopathic Directory, Year Book of Drugless Healing, and Buyers' Guide.

For twenty-two years past, the need of a directory for Drugless Therapy has been felt. The medical world is in a condition of intense evolution at the present time. It is evolving from the Drugging School of Therapy to the Drugless School. People by the million have lost confidence in the virtues of Allopathy and are turning with joyful confidence to the Professions of Natural Healing until it has been estimated that there are at least forty thousand practitioners of Naturopathic healing in the United States.The motto that IN UNITY THERE IS STRENGTH is the foundation of the present enterprise.

Hitherto, the drugless profession has lacked that prestige in the eyes of the public, which comes from the continuous existence of a big institution, duly organized and wielding the immense authority which is derived no less from organization and history than from the virtues of the principles that are held and practiced by such institutions. The public at large instantaneously respects an institution that is thoroughly organized and has its root earthed in history.

The time has fully arrived when the drugless profession should no longer exist in the form of isolated units, not knowing one another and caring but little for such knowledge. Our profession has been, as it were, as sheep without a shepherd, but the various individuals that constitute this movement so pregnant with benefits to humanity, are now collected for the first time into a Directory and Year-Book of Drugless Healing, which alone will give immense weight and dignity to the standing of the individuals mentioned therein.Not only will the book add to the prestige of the practitioner in the eyes of his patients, but when the scattered members of our profession in every State desire to obtain legislative action on behalf of their profession and themselves, the appeal of such a work as our directory will, in the eyes of legislators, gain for them a much more respectful hearing than could otherwise be obtained.

Now, for the first time, the drugless practitioner finds himself one of a vast army of professional men and women who are employing the most healthful forces of nature to rejuvenate and regenerate the world. But the book itself throws a powerful light upon every phase of drugless healing and annihilates time and distance in investigating WHO IS WHO in the realm of Drugless Therapy.

A most sincere effort has been made to obtain the name and address of every adherent of the Rational School of Medicine who practices his profession within the United States, Canada and the British Isles. It is impossible at this stage of Naturopathic history, which is still largely in the making, to obtain the name and address of every such practitioner. There were some who, even when appealed to, refused to respond to our invitation, not understanding the object of our work. Many of even the most intelligent members have refused to advertise their professional cards in our pages. But we can only attribute these drawbacks to the fact that every new institution that has suddenly dawned upon human intelligence will find that a certain proportion of people who do not understand the nature of the enterprise because the brain cells that would appreciate the benefits that are sought to be conferred upon them, are undeveloped, but a goodly proportion of our Naturopaths have gladly responded to the invitation to advertise their specialty in our columns. These, of course, constitute the brightest and most successful of our practitioners and their examples in this respect should be followed by every practitioner whose card does not appear in this book.We take it for granted that every one of the forty thousand practitioners of Naturopathy is in favor of the enterprise represented by this Directory. This work is a tool of his trade and not to possess this book is a serious handicap in the race for success.

Here will be found an Index of by far the larger number of Naturopaths in the country arranged in Alphabetic, Geographic and Naturopathic sections. Besides this, there is a classified Buyers' Guide that gives immediate information regarding where you can find special supplies, or a certain apparatus, or a certain book or magazine, its name, and where it is published. The list of Institutions with the curriculum of each will be found exceedingly useful.

Natural healing, that has drifted so long, and, by reason of a lack of organization, has been made for so many years the football of official medicine, to be kicked by any one who thought fit to do so, has now arrived at such a pitch of power that it has shaken the old system of bureaucratic medicine to its foundations. The

professors of the irrational theories of life, health and disease, that are looking for victims to be inoculated with dangerous drugs and animalized vaccines and serums, have begun to fear the growth of this young giant of medical healing that demands medical freedom, social justice and equal rights for the new healing system that exists alone for the betterment and uplifting of humanity. I want every Professor of Drugless Therapy to become my friend and co-worker in the great cause to which we are committed, and those whose names are not recorded in this book should send them to me without delay. It will be of far greater interest and value to themselves to have their professional card included amongst those who advertise with us than the few dollars that such advertisement costs.

It will be noted that there are quite a number of Drugless Healers belonging to foreign countries (particularly those of the Western Hemisphere) represented in this Directory. The profession of medicine is not confined to any race, country, clime or religion. It is a universal profession and demands universal recognition. It will be a great honor to the Directory, as well as to the Naturopathic profession at large to have every Naturopathic practitioner, from the Arctic Circle to the furthest limits of Patagonia, represented in the pages of this immense and most helpful work.

I expect that the Directory for the year 1920 will be larger and even more important than the present Directory and that it will contain the names of thousands of practitioners that are not included in the present work.

The publication of this Directory will aid in abolishing whatever evils of sectarianism, narrow-mindedness and lack of loyalty to the cause to which we are devoted, that may exist. That it will promote a fraternal spirit among all exponents of natural healing, and create an increase of their prestige and power to resist the encroachments of official medicine on their constitutional rights of liberty and the pursuit of happiness, by favorably influencing Legislators, Law courts, City Councils and Boards of Health everywhere, is the sincere belief of the editor and publisher.

Having introduced the volume, Lust leads off with his article entitled "The principles, aim, and program of the nature cure system."

Again, this relatively brief article is reproduced here in its entirety so that one can see the merging of influences:

The Principles, Aim, and Program of the Nature Cure System

Since the earliest ages, Medical Science has been of all sciences the most unscientific. Its professors, with few exceptions, have sought to cure disease by the magic of pills and potions and poisons that attacked the ailment with the idea of suppressing the symptoms instead of attacking the real cause of the ailment.

Medical science has always believed in the superstition that the use of chemical substances which are harmful and destructive to human life will prove an efficient substitute for the violation of laws, and in this way encourages the belief that a man may go the limit in self indulgences that weaken and destroy his physical system, and then hope to be absolved from his physical ailments by swallowing a few pills, or submitting to an injection of a serum or vaccine, that are supposed to act as vicarious redeemers of the physical organism and counteract life-long practices that are poisonous and wholly destructive to the patient's well-being.

From the earliest ages to the present time, the priests of medicine have discovered that it is ten times easier to obtain ten dollars from a man by acting upon his superstition, than it is to extract one dollar from him, by appealing to reason and common sense. Having this key to a gold mine within their grasp, we find official medicine indulging at all times in the most blatant, outrageous, freakish and unscientific methods of curing disease, because the methods were in harmony with the medical prestige of the physician.

Away back in pre-historic times, disease was regarded as a demon to be exorcized from its victim, and the medicine man of his tribe belabored the body of his patient with a bag in which rattled bones and feathers, and no doubt in extreme cases the tremendous faith in this process of cure that was engendered in the mind of the patient really cured some ailments for which mental science and not the bag of bones and feathers should be given credit.

Coming down to the middle ages, the Witches' Broth—one ingredient of which was the blood of a child murderer drawn in the dark of the moon—was sworn to, by official medicine, as a remedy for every disease.

In a later period, the "docteur a la mode," between his taking pinches of snuff from a gold snuff box, would order the patient bled as a remedy for what he denominated spirits, vapors, megrims, or miasms.

Following this pseudo-scientific diagnosis and method of cure, came the drugging phase in which symptoms of disease were unmercifully attacked by all kinds of drugs, alkalis, acids and poisons which were supposed, that by suffocating the symptoms of disease, by smothering their destructive energy, to thus enhance the vitality of the individual. All these cures have had their inception, their period of extensive application, and their certain desuetude. The contemporary fashion of healing disease is that of serums, inoculations and vaccines, which, instead of being an improvement on the fake medicines of former ages are of no value in the cure of disease, but on the contrary introduce lesions into the human body of the most distressing and deadly import.

The policy of expediency is at the basis of medical drug healing. It is along the lines of self-indulgence, indifference, ignorance and lack of self-control that drug medicine lives, moves and has its being. The sleeping swineries of mankind are wholly exploited by a system of medical treatment, founded on poisonous and revolting products, whose chemical composition and whose mode of attacking disease, are equally unknown to their originators, and this is called "Scientific medicine."

Like the alchemist of old who circulated the false belief that he could transmute the baser metals into gold, in like manner the vivisector claims that he can coin the agony of animals into cures for human disease. He insists on cursing animals that he may bless mankind with such curses.

To understand how revolting these products are, let us just refer to the vaccine matter which is supposed to be an efficient preventive of smallpox. Who would be fool enough to swallow the putrid pus and corruption scraped from the foulest sores of smallpox that has been implanted in the body of a calf? Even if any one would be fool enough to drink so atrocious a substance, its danger might be neutralized by the digestive juices of the intestinal tract. But it is a far greater danger to the organism when inoculated into the blood and tissues direct, where no digestive substances can possibly neutralize its poison.The natural system for curing disease is based on a return to nature in regulating the diet, breathing, exercising, bathing and the employment of various forces to eliminate the poisonous products in the system, and so raise the vitality of the patient to a proper standard of health.

Official medicine has in all ages simply attacked the symptoms of disease without paying any attention to the causes thereof, but natural healing is concerned far more with removing the causes of disease, than merely curing its symptoms. This is the glory of this new school of medicine that it cures by removing the causes of the ailment, and is the only rational method of practicing medicine.

It begins its cures by avoiding the uses of drugs and hence is styled the system of drugless healing. It came first into vogue in Germany and its most famous exponents in that country were Priessnitz, Schroth, Kuhne, Kneipp, Rickli, Lahmann, Just, Ehret, Engelhardt, and others.

In Sweden, Ling and others developed various systems of mechano-therapy and curative gymnastics.

In America, Palmer invented Chiropractic; McCormick, Ophthalmology. Still originated Osteopathy; Weltmer, suggestive Therapeutics. Lindlahr combined the essentials of various natural methods, while Kellogg, Tilden, Schultz, Trall, Lust, Lahn, Arnold, Struch, Havard, Davis, Jackson, Walters, Deininger, Tyrell, Collins and others, have each of them spent a lifetime in studying and putting into practice the best ideas of drugless healing and have greatly enlarged and enriched the new school of medicine.

Life Maltreated by Allopathy

The prime object of natural healing is to give the principle of life the line of least resistance, that it may enable man to possess the most abundant health.

What Is Life?

The finite mind of man fails to comprehend the nature of this mysterious principle. The philosopher says "Life is the sum of the forces that resist death," but that definition only increases its obscurity. Life is a most precious endowment of protoplasm, of the various combinations of oxygen, hydrogen, carbon and nitrogen, and other purely mineral substances in forming organic tissues. As Othello says, referring to Desdemona's life, which he compares to the light of a candle—

"If I quench thee thou flaming minister,
I can thy former light restore
Should I repent me; but once put out THY light,
I know not whence is that Promethean heat
That can thy light relume."

The spark of life flickers in the sockets of millions and is about to go out. What system of medicine will most surely restore that flickering spark to a steady, burning flame?

Will [it be] the system that employs poisonous vaccines, serums and inoculations, whose medical value has to be supported by the most mendacious statements, and whose practitioners are far more intent on their emoluments and fame, than they are in the practice of humanity?

The Allopathic system, which includes nine tenths of all medical practitioners, is known by its fruits, but it is an appalling fact that infant mortality, insanity, heart disease, arteriosclerosis, cancer, debility, impoverished constitutions, degeneracy, idiocy and inefficiency have enormously increased, particularly during the last twenty-five years, that is, during the regime of inoculations, serums and vaccines.

Naturopathy, on the other hand, so far as it has been developed, and so far as official medicine will allow it to act, leaves no such trail of disease, disaster and death behind it. Natural healing is emancipation from medical superstition, ignorance and tyranny. It is the true Elixir of Life.

The Allopaths have endeavored to cure sick humanity on the basis of the highly erroneous idea that man can change the laws of nature that govern our being, and cure the cause of disease by simply ignoring it. To cure disease by poisoning its symptoms is medical manslaughter.

Dr. Schwenninger of Germany says: "We are suffering under the curse of the past mistakes of our profession. For thousands of years medical doctors have been educating the public into the false belief that poisonous drugs can give health. This belief has become in the public mind such a deep-seated superstition, that those of us who know better and who would like to adopt more sensible, natural methods of cure, can do so only at the peril of losing practice and reputation.

"The average medical man is at his best but a devoted bigot to this vain school-craft, which we call the Medical Art and which alone in this age of science has made no perceptible progress since the days of its earliest teachers. They call it recognized science! Recognized ignorance! The science of to-day is the ignorance of to-morrow. Every year some bold guess lights up as truth to which but the year before the schoolmen of science were as blind as moles."

And Dr. O.W. Holmes, Professor of Anatomy in Harvard University, states: "The disgrace of medicine has been that colossal system of self-deception, in obedience to which mines have been emptied of their cankering minerals, entrails of animals taxed for their impurities, the poison bags of reptiles drained of their venom, and all the inconceivable abominations thus obtained thrust down the throats of human beings, suffering from some fault of organization, nourishment, or vital stimulation."

And these misguided drug doctors are not only not ashamed of their work, but they have induced subservient legislators to pass laws that perpetuate the age-long scandal of allopathic importance, and the degenerative influence of the poisons, and to actually make it a crime on the part of nature doctors to cure a man of his ailment. The brazen effrontery of these medical despots has no limits. They boast of making the State legislators their catspaw in arresting, fining and imprisoning the professors of natural healing for saving human life. Legislators have no right to sit in judgment over the claims of rival schools of healing. They see tens of thousands of sick people go down to their graves by being denied the cures that the employers of nature's forces alone can give them. It is their business to provide for the various schools of medicine a fair field and no favor.

A citizen has an inalienable right to liberty in the pursuit of happiness. Yet the real saviors of mankind are persecuted by the medical oligarchy which is responsible for compulsory vaccination, compulsory medical inspection of public school children, and the demands for State and Federal departments of health, all for the ostensible good of the people, but in reality for the gain of the Medical Trust.

The Naturopaths

The Naturopaths are desirous of freedom for all schools of medicine. They are responsible practitioners who are willing to be examined by an impartial council, appointed by and acting for the State, who will testify to the life and character of every drugless physician before he is entitled to practice medicine. Not one invidious discrimination should be made between the different schools of medicine. The state should see to it that each school should have a full opportunity to do its best for the up-lifting of its citizens.

The Program of Naturopathic Cure

1. ELIMINATION OF EVIL HABITS, or the weeds of life, such as overeating, alcoholic drinks, drugs, the use of tea, coffee and cocoa that contain poisons, meat eating, improper hours of living, waste of vital forces, lowered vitality, sexual and social aberrations, worry, etc.
2. CORRECTIVE HABITS. Correct breathing, correct exercise, right mental attitude. Moderation in the pursuit of health and wealth.
3. NEW PRINCIPLES OF LIVING. Proper fasting, selection of food, hydropathy, light and air baths, mud baths, osteopathy, chiropractic and other forms of mechano-therapy, mineral salts obtained in organic form, electropathy, heliopathy, steam or Turkish baths, sitz baths, etc.

Natural healing is the most desirable factor in the regeneration of the race. It is a return to nature in methods of living and treatment. It makes use of the elementary forces of nature, of chemical selection of foods that will constitute a correct medical dietary. The diet of civilized man is devitalized, is poor in essential organic salts. The fact that foods are cooked in so many ways and are salted, spiced, sweetened and otherwise made attractive to the palate, induces people to over-eat, and over eating does more harm than under feeding. High protein food and lazy habits are the cause of cancer, Bright's disease, rheumatism and the poisons of auto-intoxication.

There is really but one healing force in existence and that is Nature herself, which means the inherent restorative power of the organism to overcome disease. Now the question is, can this power be appropriated and guided more readily by extrinsic or intrinsic methods? That is to say, is it more amenable to combat disease by irritating drugs, vaccines and serums employed by superstitious moderns, or by the bland intrinsic congenial forces of Natural Therapeutics, that are employed by this new school of medicine, that is Naturopathy, which is the only orthodox school of medicine? Are not these natural forces much more orthodox than the artificial resources of the druggist? The practical application of these natural agencies, duly suited to the individual case, are true signs that the art of healing has been elaborated by the aid of absolutely harmless, congenial treatments, under whose ministration the death rate is but five per cent of persons treated as compared with fifty per cent under the present allopathic methods.

The Germanic Influence

The philosophic origins of naturopathy were Germanic. The most significant influences, except those of Russell Trall, the osteopathic concepts of A.T. Still (at this time, strictly the correction of spinal lesions by adjustment), and the chiropractic principles of D. D. Palmer, were originally Germanic. (This was well established in the January 1902 editorial in *Water Cure Monthly*.)

The specific influences on which Lust drew for his work, in order of their chronologic contributions to the system of naturopathy, are the following:

1. Vincent Preissnitz (1799–1851)
2. Johann Schroth (1798–1856)
3. Father Sebastian Kneipp (1821–1897)
4. Arnold Rickli (1823–1926)
5. Louis Kuhne (c. 1823–1907)
6. Henry Lahman (no dates known)
7. F. E. Bilz (1823–1903)
8. Adolph Just (1859–1939).

Also of note were Theodor Hahn and T. Meltzer, who, in the 1860s, were well known for their work in the movement called, in German, *Naturatz* or "naturism."

In photographs accompanying his article "The principles, aim and program of the nature cure system," Lust described each of these thinkers as follows:

1. VINCENT PREISSNITZ, of Graefenberg, Silesia. Founder of Hydropathy. Born October 4, 1799. A pioneer Naturopath, prosecuted by the medical authorities of his day, and convicted of using witchcraft, because he cured his patients by the use of water, air, diet and exercise. He took his patients back to Nature—to the woods, the streams, the open fieldstreated them with Nature's own forces and fed them on natural foods. His fame spread over the whole of Europe, and even to America. His cured patients were numbered by the thousands. The Preissnitz compress or bandage is in the medical literature. Preissnitz is no more, but his spirit lives in every true Naturopath.

2. JOHANN SCHROTH, a layperson, not described in Lust's directory but often talked of in later works and prominently mentioned for his curative theories in Bilz's master work, The Natural Method of Healing. Schroth smashed his right knee in an accident with a horse and it remained stiff in spite of repeated medical treatment. At last, a priest told Schroth that Preissnitz's methods might help, and Schroth decided to give them a try. In order to avoid frequent changing of the packs that were directed by Preissnitz, he placed several packs on top of one another, wrapping the whole portion with a woolen cloth. He left this pack on the injured knee for several hours and produced a moist heat which he theorized to cause the poisonous toxins to dissolve and be swept away. These packs are still used as part of the "Schroth cure" and have reportedly become famous for their blood-cleansing effect. (From an article in the March 1937 Naturopath and Herald of Health by Dr. T.M. Schippel.) As noted by Bilz, the Schroth cure, called by Bilz "the regenerative treatment," was developed for treatment of chronic diseases through the use of an extreme diet following total fasting by withdrawing of all food and drink and then the use of totally dry grain products and the eventual reintroduction of fluids.

3. FATHER SEBASTIAN KNEIPP, of course, is much described and the photos include one of Kneipp lecturing to the multitudes at Wandelhale at Woerishofen, attending Pope Leo XIII in 1893, noting this is the only consultation on health care matters that Kneipp ever consented to outside of Woerishofen, though many famous and aristocratic individuals desired his counsel, and a picture of Kneipp with the Archdukes Joseph and Francis Ferdinand of Austria walking barefoot in new-fallen snow for purposes of hardening the constitution. It was noted that the older Archduke was cured by Kneipp of Bright's disease in 1892, and it noted that the Archduke Joseph, in appreciation of this cure, donated a public park in the town of Woerishofenat a cost of $150,000 florens. The Archduke Francis Ferdinand, the son of Archduke Joseph, was the individual whose murder precipitated World War I. There is a further picture of Kneipp surrounded consultation to numerous patients.

4. ARNOLD RICKLI, founder of the light and light and aircures (atmospheric cure). Dr. Rickli was one of the foremost exponents of natural living and healing. In 1848, he established at Veldes, Krain, Austria, the first institution of light and air cure or as it was called in Europe the "atmospheric cure." In a limited way (rather very late) his ideas have been adopted by the medical profession in America for the cure of consumption. He was an ardent disciple of the vegetarian diet and exemplified the principles of natural living in his own life. The enclosed photo shows him at the age of 97, when he was still active and healthy. He has since passed on, but his work still lives as a testimonial of his untiring efforts. He was the founder and for over 50 years the President of the National Austrian Vegetarian Association.

5. LOUIS KUHNE wrote, in 1891, The New Science of Healing, the greatest work of basic principles in natural healing. In the tradition of Natural Healing and prevention, Kuhne has been described as one who "… advocated sun, steam baths, a vegetarian diet, and whole-wheat bread … in these relatively early days." His renowned work constitutes the only true scientific philosophy for the application of all Drugless Methods. He was the first to give to the world the comprehensible idea of pathology and the first to proclaim the doctrine of the "unity of cure." His book Facial Expression gives the means of diagnosing a patient's pathological condition and determining the amount and location of the systemic encumbrance. He is the founder and first Master of Naturopathy.

6. DR. H. LAHMAN. When the University of Leipzig expelled H. Lahman for his spreading medical sedition among the students, it added a staunch advocate to natural healing. Dr. Lahman finished his medical education in Switzerland and returned to Germany to refute in practice the false ideas of medical science. He later founded the largest Nature Cure institution in the world at Weisser Hirsch, near Dresden, Saxony. He was a strong believer in the "Light and Air" cure and constructed the first appliances for the administration of electric light treatment and baths. He was the author of several books on Diet, Nature Cure and Heliotherapy. As noted in Other Healers, Other Cures: "Heinrich Lahmann came along to stress no salt on foods and no water with meals …" His works on diet are authoritative and his "nutritive salts theory" forms the basis of rational dietetic treatment. This work has but recently come to light in America, and progressive dietitians are forsaking their old, worn-out, high protein, chemical and caloric theories for the "organic salts theory." Carque, Lindlahr, McCann, and other wide awake food scientists have adopted it as a basis for their work. Dr. Lahman was a medical nihilist. He denounced medicine as unscientific and entirely experimental in its practice and lived to prove the saneness of his ideas as evidenced by his thousands of cured patients.

7. PROFESSOR F.E. BILZ. That real physicians are born, not made, is well illustrated in the case of Dr. Bilz, who achieved his first success in healing as a lay practitioner. As a mark of gratitude, a wealthy patient presented him with land and a castle in which to found a Nature Cure anitarium.… The Bilz institution at Dresden-Rdebeul, Germany, became world renowned and was long considered the center of the Nature Cure movement. Professor Bilz is the author of the first Naturopathic encyclopedia, The Natural Method of Healing, which has been translated into a dozen languages, and in German alone has run into 150 editions. He has written many works on Nature Cure and Natural Life, among them being The Future State, in which he predicted the present World War, and advocated a federation of nations as the only logical solution of international problems.

8. ADOLPH JUST, famous author of Return to Nature and founder of original "Yungborn" in Germany.

Both Adolph Just's *Return to Nature* and Louis Kuhne's *The Natural Science of Healing* were translated into English by Lust and released through his publication house.

The Convergence With American Influences

The Universal Naturopathic Directory was truly eclectic in its compilation and composition. Besides the Lust articles, the volume included "How I became acquainted with nature cure" by Henry Lindlahr, MD, ND (which was reproduced in large part in the introduction to volume 1 of Lindlahr[15]); "The nature cure" by Carl Strueh, MD, ND; "Naturopathy" by Harry E. Brook, ND; "The present position of naturopathy and allied therapeutic measures in the British Isles" by Allen Pattreiouex, ND; "Why all drugless methods?" by Per Nelson; and "Efficiency in drugless healing" by Edward Earle Purinton (a reprint of the 1917 publication, referred to earlier, which was composed of a series of articles published in *The Herald of Health and Naturopath* between August 1914 and February 1916).

The volume also contained Louis Kuhne's "Neo-naturopathy (the new science of healing)," in the first publication of the translation by Lust, and articles on electrotherapy, neuropathy, dietology, chiropractic, mechanotherapy, osteopathy, phytotherapy, apyrtropher, physical culture, optometry, hydrotherapy, orthopedics, pathology, natural healing and living, astroscopy, phrenology, and physiology—all of which were specially commissioned for the directory from practitioners and authors considered expert in these subjects.

Also included was a national directory of drugless physicians in alphabetical order, geographically arranged and itemized by profession; biographic notes on American contributors of note; the naturopathic book catalog; a guide to natural healing and natural life books and periodicals; a classified list of medical works; a series of book reviews; a buyer's guide for naturopathic supplies; and, in addition to extensive indexes, a "parting word" by Lust.

The volume contained numerous advertisements for naturopathic schools, sanitariums, and individual practices, and it closed with the following note:

> *This, then, completes Volume 1 of the Naturopathic Directory, Drugless Yearbook and Buyer's Guide for the years 1918 and 1919. Into it, has been placed the conscientious labor of many willing hearts, hands and minds. It is their contribution to the noble cause of natural healing. It will stand as a monument to their endeavors, as well as a memorial to the great souls, the fathers of natural healing, who have passed on.*
>
> *Let this, then, herald a new era—the era wherein man shall recognize the omniscience of Nature, and shall profit through conforming to her laws.*

In the biographic sections, it is apparent that Lust owed a great deal of the feeling of camaraderie in the nature cure movement to some varied American practitioners. The most prominent of these had their biographic sections contained in the 1918 directory. Two of them deserve specific note and attention: Palmer and Still.

Lust met A. T. Still in 1915 in Kirksville, Missouri, shortly before Still's death. From their meetings, Lust noted later in the *Naturopath and Herald of Health* (June 1937) that Still believed that osteopathy by "compromising with medicine is doomed as the school that could have incorporated all the natural and biological healing arts." Lust wanted naturopathy to fill this void.

Lust also had a lengthy acquaintance with B. J. Palmer (the son of D. D. Palmer, the founder of chiropractic), who, following in his father's footsteps, put Davenport, Iowa, and the Palmer Chiropractic College on the map.

Lust also became connected with Henry Lindlahr, MD, ND, of Chicago (as noted in the autobiographical sketch contained in the directory[16] and reprinted in volume 1 of Lindlahr[15]). Lindlahr was a rising businessman in Chicago with all the bad habits of the "gay nineties" era. In his 30s, he became chronically ill. He had gone to the orthodox practitioners of his day and received no relief.

Then he was exposed to Schroth's works, and in following them began to feel somewhat better. Subsequently, he liquidated all his assets and went to a German sanitarium to be cured and to learn nature cure. He returned to Chicago and enrolled in the Homeopathic/Eclectic College of Illinois. In 1903 he opened a sanitarium, which included a residential sanitarium, located in Elmhurst, Illinois; a "transient" clinic (office) on State Street in Chicago; and "Lindlahr's Health Food Store."

Shortly thereafter, he founded the Lindlahr College of Natural Therapeutics, which included hospital internships at the sanitarium. The institution became one of the leading naturopathic colleges of the day. In 1908 he began to publish *Nature Cure Magazine* and began publishing his series of *Philosophy of Natural Therapeutics,* with volume 1 ("Philosophy") in 1918. This was followed by volume 2 ("Practice") in 1919; volume 3 ("Dietetics"; republished with revisions as originally published in 1914); and, in 1923, volume 6 ("Iridiagnosis"). The intended volumes 4 and 5 were in production at the time of Lindlahr's death in 1927. As described in *Other Healers, Other Cures*[17]:

Henry Lindlahr, another American, is remembered for his conviction that disease did not represent an invasion of molecules, but the body's way of healing something. In other words, he viewed symptoms as a positive physiological response—proof that the body is fighting whatever's wrong. Accordingly, a fever is a "healthy" sign and one should be let alone, unless it is dangerously high, of course.

The effect of all these gentlemen on the development of naturopathy in America, under Lust's guidance, was profound.

From these beginnings, the naturopathic movement gathered strength and continued to grow through the 1920s and 1930s, having a major effect on natural healing and natural lifestyles in the United States.

Along the way, Lust was greatly influenced by the writings of John H. Tilden, MD (largely published between 1915 and 1925). Tilden was originally a practicing physician in Denver who became disenchanted with orthodox medicine and began to rely heavily on dietetics and nutrition, formulating his theories of "auto-intoxication" (the effect of fecal matter remaining too long in the digestive process) and "toxemia."

Lust was also greatly influenced by Elmer Lee, MD, who became a practicing naturopath around 1910 and whose movement was called the "hygienic system," following the earlier works of Russell Trall. Lee published *Health Culture* for many years.

In addition to John Tilden, MD, and Elmer Lee, MD, another medical doctor, John Harvey Kellogg, MD, who turned to more nutritionally based natural healing concepts, was greatly respected by Lust. Kellogg was renowned through his connection with the Battle Creek Sanitarium. The sanitarium itself was originally founded in the 1860s as a Seventh-Day Adventist institution designed to perpetuate the Grahamite philosophies of Sylvester Graham and William Alcott. The sanitarium was on the verge of being closed, however, because of economic failure, when, in 1876, Kellogg, a new and more dynamic physician-in-chief, was appointed.

Kellogg, born in 1852, was a "sickly child" who, at the age of 14, ran across the works of Graham and converted to vegetarianism. At the age of 20, he studied for a term at Trall's Hygio-Therapeutic College and then earned a medical degree at New York's Bellevue Medical School. He maintained an affiliation with the regular schools of medicine during his lifetime, due more to his practice of surgery than his beliefs in the area of health care.[4]

Kellogg designated his concepts, which were basically the hygienic system of healthful living, "biologic living." Principally, Kellogg defended vegetarianism, attacked sexual misconduct and the evils of alcohol, and was a prolific writer throughout the late 19th century and early 20th century. He produced a popular periodical, *Good Health,* which continued in existence until 1955. When Kellogg died in 1943 at the age of 91, he had had more than 300,000 patients through the Battle Creek Sanitarium (which he had renamed from the Western Health Reform Institute shortly after his appointment in 1876), including many celebrities, and the "San" became nationally well known.

Kellogg, along with Tilden and Elie Metchnikoff (director of the prestigious Pasteur Institute and winner of the 1908 Nobel Prize for a contribution to immunology), wrote prolifically on the theory of auto-intoxication. Kellogg, in particular, felt that humans, in the process of digesting meat, produced various intestinal self-poisons that contributed to auto-intoxication.

As a result, Kellogg became a near fanatic on the subject of helping humans return to a more healthy, natural state by returning to the naturally designed usage of the colon. He felt that the average modern colon was devitalized by the combination of sedentary living, the custom of sitting rather than squatting to defecate, and the modern civilized habit of ignoring "nature's call" out of an undue concern for politeness. Further, Kellogg concentrated on the fact that the modern diet had insufficient bulk and roughage to stimulate the bowels to proper action.

Kellogg was also extremely interested in hydrotherapy. In the 1890s he established a laboratory at the San to study the clinical applications of hydrotherapy. This led, in 1902, to his writing *Rational Hydrotherapy.* The preface espoused a philosophy of drugless healing that came to be one of the bases of the hydrotherapy school of medical thought in America.

Tilden, as mentioned, was of a similar mind. He must have been to have provided natural healthcare literature with his 200-plus-page dissertation entitled "Constipation," with a chapter devoted to the evils of not responding when nature called.

This belief in the "evils" drawing away from the natural condition of the colon was extremely important to Kellogg's work.[4] Because of Lust's interest, Kellogg's *The New Dietetics* (1921) became one of the bibles of naturopathic literature.[18]

Lust was also influenced by the works of Sidney Weltmer, the father of "suggestive therapeutics." The theory behind Professor Weltmer's work was that whether it was the mind or the body that first lost its grip on health, the two were inseparably related. When the problem originated in the body, the mind nonetheless lost its ability and desire to overcome the disease because the patient "felt sick" and consequently slid further into the diseased state. Alternatively, if the mind first lost its ability and desire to "be healthy" and some physical infirmity followed, the patient was susceptible to being overcome by disease.

Weltmer's work dealt specifically with the psychological process of desiring to be healthy. Lust enthusiastically backed Weltmer's work and had him appear on the programs at various annual conventions of the American Naturopathic Association (which commenced after its founding in 1919).

Lust was also personal friends with and a deep admirer of Bernarr MacFadden.[19] MacFadden was the founder of the "physical culture" school of health and healing, also known as "physcultopathy." This school of healing gave birth across the country to gymnasiums at which exercise programs, designed to allow the individual man or woman to maintain the most perfect state of health and human condition possible, were developed and taught.[4] *Other Healers, Other Cures* described it as follows[15]:

The next Naturopathic star, after Kellogg, was Bernarr MacFadden, the physical culturist who built a magazine-publishing empire (his first magazine was Physical Culture founded in 1898). MacFadden proselytizes for exercise and fresh vegetables, hardly eccentric notions. But his flamboyant efforts to publicize them and his occasional crack-pot ideas (like freezing the unemployed, then thawing them out when the Depression was over) alienated many people. Still, he was his own best advertisement. He fathered nine children by four wives and was parachuting from planes in his 80s. One of MacFadden's admirers was that arch-foe of the medical profession, George Bernard Shaw, the longevous eccentric in his own right.

Lust was also interested in, and helped publicize, "zone therapy," originated by Joe Shelby Riley, DC, a chiropractor based in Washington, DC, and one of the early practitioners of "broad chiropractic." Zone therapy was an early forerunner of acupressure as it related "pressures and manipulations of the fingers and tongue, and percussion on the spinal column, according to the relation of the fingers to certain zones of the body."[17]

Several other American drugless healers contributed to a broad range of "-opathies" that Lust merged into his growing view of naturopathy as the eclectic compilation of methods of natural healing. The

Universal Directory also contained a complete list of osteopaths and chiropractors as drugless healers within the realm of Lust's view of naturopathic theory. His other significant compatriots at the time of the publication of the directory were Carl Stueh, described by Lust as "one of the first medical men in this country who gave up medicine and operation for natural healing"; F. W. Collins, MD, DO, DC, an early graduate of the American School of Naturopathy (1907) who went on to graduate from the New Jersey College of Osteopathy (1909) and the Palmer School of Chiropractic (1912); another "broad chiropractor," Anthony Matijaca, MD, ND, DO, the naturopathic resident expert in electrotherapy and an associate editor of the *Herald and Health Naturopath* (the inverted name of the Lust journal at the time of the directory); and Carl Schultz, ND, DO, MD, president and general manager of the Naturopathic Institute and Sanatorium of California, essentially the second school in the country to pursue the education of physicians under the name of "naturopathy."

In *Inner Hygiene: Constipation and the Pursuit of Health in Modern Society,* Whorton[20] offered his first assessments of the work of Lust as it related to the emergence of naturopathy in the early 20th century:

> *Most of the drugless clan also identified themselves as practitioners of naturopathy, a system of practice that grew out of hydropathy, as well as German water-cure and nature-cure traditions. Organized in the late 1890s under the leadership of German immigrant Benedict Lust, naturopathy sought to cure the full scope of human ills with natural agents (herbs, water, air, sunlight, electricity, massage, and others), agents that supported and stimulated the body's own healing mechanisms.*

In his extensive assessments of Lust's work and writings in *Nature Cures: The History of Alternative Medicine in America,* Whorton[21] attempted to put the philosophic development of naturopathy in a reasonable historical context:

> *However much a dreamer Lust was in some respects, he was an insightful realist in others. He was correct in believing that simply giving nature support as it ran its course was the best one could do with many diseases in his day. He was correct in seeing self-abuse as the source of much physical, and emotional, suffering and attacked it with an ardor that MDs could not bring to the task until nearly a century later. Recent medical lamentations over the evils of smoking, sexual promiscuity, and other risky behaviors adopted in the thoughtless chase after pleasure have nothing on Lust's jeremiads …*
>
> *Lust was right in reprimanding allopaths for focusing so strongly on disease as to lose sight of the importance of promoting health. He was right in appreciating the need to "individualize" the treatment of each patient—and in seeing patient self-responsibility as part of that individualization.*

EARLY-20TH-CENTURY MEDICINE

The Metamorphosis of Orthodox Medicine

Naturopathy's formative years, and in some respects its halcyon days, were from 1900 to 1917. In many jurisdictions, modern licensing laws were not yet in effect, so varied schools of healing were openly practiced. By 1920, however, the American world of medicine had undergone a sharp transition, culminating in four decades of change.

A look at the structure of early medical care in the United States is instructive, even as practiced and dominated by the orthodox school, when noting the changes that occurred between 1875 and 1920.

In 1875 the following was descriptive of American medical practice:

- The practice, even in urban areas, sent the doctor to the patient; the "house call" was the norm.
- There was little modern licensing regulation.
- Hospitals were charitable institutions where persons too poor to otherwise receive health care were usually sent when ill.
- The AMA, although formed in 1846 and generally representative of the professional goals of the regular or orthodox school of medicine, had scarcely begun to make any political inroads at all.
- Medical schools required little or no college education for entrance and were largely apprenticeship based and proprietary in nature, having changed little throughout the century.
- Although some doctors had begun to specialize, to do so was far from the norm. The major recognized specialties were surgery, obstetrics, and gynecology.
- Many different types of doctors existed, and society's reaction to the profession neither recognized specific expertise nor necessarily rewarded professionals in medical practice well.
- Although the orthodox school made up roughly 80% of professional medical practitioners, the homeopaths and the eclectics were visible and respected in their own communities for their abilities and expertise, and much of the public relied on other "irregular" practitioners.

By comparison, in 1920, a total metamorphosis of the medical profession had occurred:

- By 1920, practices had become office oriented and clinic oriented.
- Modern licensing principles had become fully developed, and physicians and surgeons were licensed in all jurisdictions. Most other healthcare providers had some licensing restrictions placed on them, if they were recognized at all.
- Due largely to the introduction into surgery of the practice of antiseptic techniques and aseptic procedures and a correspondent decline in operative mortality, institutional care in the hospital became increasingly accepted. Also, clinical pathology and diagnostic laboratory procedures had become well developed, and the hospital had become a major training and clinical research facility that was generally more acceptable to the patient.
- The AMA was approaching the peak of its political power, having exercised, through its Council on Medical Education and its Council of Pharmacy and Chemistry, major effects on medical schools and the pharmaceutical industry.
- The transition to research- and education-based medical schools, instead of practitioner apprenticeships and proprietary education, had become complete. All recognized medical schools had a 4-year curriculum, with an undergraduate degree or substantial undergraduate study required as a prerequisite. In addition, most schools, in conjunction with most licensing statutes, required a year's internship.
- Specialization was becoming well developed, and the number of specialty groups had increased considerably. This would continue throughout the 1930s and into the early 1940s.
- Professional authority and autonomy had undergone a substantial transition, and the allopathic physician was now recognized as a medical expert.
- By 1922, the last eclectic school was on the verge of closure, and in the early 1930s, the last of the homeopathic schools in the United States was also on the verge of closure. The influence of these sects on orthodox medicine had dwindled to almost nothing. Naturopaths and other alternative healthcare practitioners had adopted the areas of expertise previously considered the territory of homeopaths and eclectics.

The Halcyon Years of Naturopathy

In 1924 Morris Fishbein succeeded George Simmons as editor of the *Journal of the American Medical Association (JAMA).* Fishbein had

joined the editorial staff of *JAMA* under Simmons immediately after his graduation from Chicago's Rush Medical School in 1913. Campion pointed out the following[9]:

> *Over the years, Fishbein not only established himself as the gifted editor of the most widely read medical journal in the United States; he also learned how to extend his editorial position, how to project his opinions nationwide. He became, as the saying went in those years, a "personality." TIME referred to him as "the nation's most ubiquitous, the most widely maligned, and perhaps most influential medico."*

In addition to his development of *JAMA* as an editorial and personal voice, Fishbein also continually railed against "quackery." Lust, among others, including MacFadden, became Fishbein's epitome of quackery. When MacFadden became a wealthy man, after his publishing company included popular magazines like *True Confessions* and *True Detective,* he began campaigning for the 1936 Republican presidential nomination. In response, a physician submitted, under the initials "K.G.," a tongue-in-cheek listing of the cabinet that would exist under MacFadden, including the newly created "Secretary of Aviation" for Lust. Lust was a popular figure by this time who conducted such a busy lecture schedule and practice, alternating between the "Yungborns" in Butler, New Jersey, and Tangerine, Florida, that he had become almost as well known as an airline traveler. Lust devoted a complete editorial in *Nature's Path* to a response.

Although Fishbein had *JAMA* as a personal editorial outlet, Lust had his own publications. Commencing with the *Naturopath and Herald of Health* in 1902 (which changed its name to *Herald of Health and Naturopath* in 1918), Lust continually published this and other monthly journals. In 1919 it became the official journal of the ANA, mailed to all members. Each edition contained the editorial column "Dr. Lust Speaking."

In the early 1920s, the "health fad" movement was reaching its peak in terms of public awareness and interest. As described, somewhat wistfully, in his June 1937 column, Lust announced the approach of the 41st Congress of Natural Healing under his guidance:

> *The progress of our movement could be observed in our wonderful congresses, in 1914 Butler, N.J., 1915 Atlantic City, 1916 in Chicago, 1917 Cleveland, 1918 New York, 1919 Philadelphia, 1920 and 1921 again New York, and 1922 in Washington, D.C., where we had the full support and backing of the Congress of the United States. President Harding received the president and the delegates of our convention and we were the guests of the City of Washington. Through the strenuous efforts of Dr. T.M. Schippel, Hon. Congresswoman Catherine Langley of Kentucky, and eight years of hard work financed and sustained by Dr. Schippel and her powerful friends in Congress, Naturopathy was fully legalized as a healing art in the District of Columbia and the definition was placed on record and the law affirmed and amended by another act which has been fully published over and over again in the official journal of the A.N.A., Naturopath.*
>
> *In 1923 in Chicago, with the help and financing of the great and never-to-be forgotten Dr. Henry Lindlahr, we had a great convention. Not only were all the Naturopaths there but even to an extent our congress was recognized and acknowledged as official and of great importance by the medical people, particularly by the Health Commissioner of Chicago. We held a banquet, and there were discussions covering all platforms of the healing art. It was the first congress in the United States where medicine and Naturopathy in all its branches such as the general old-time Nature Cure, Hydrotherapy and Diet, Osteopathy, Naprapathy, Chiropractic, Neuropathy and Physiotherapy were represented on the same platform. The speakers represented every modern school of healing and the movement at that time was in the direction of an entirely recognized and independent school of healing. There were two camps, official medicine and official Naturopathy, the medical camp having all that is good and bad in medicine and surgery and all the other schools of healing that had sold their birthright and trusted to the allurement of organized medicine, such as Homeopaths, Eclectics, Physio-medics, and the Osteopaths to a large extent. The Osteopaths were always in the wrong camp when they went on mixed boards and Dr. Andrew Taylor Still, the father of Osteopathy, told me in 1915 that by compromising with medicine Osteopathy is doomed as the school that could have incorporated all of the natural and biological healing arts.*
>
> *The year following we had the great congress in Los Angeles which has never been duplicated. We had to meet in two hotels because the crowds ran over 10,000. The glorious banquet will never be forgotten and the congress celebrated and demonstrated that the initial and first intent of the A.N.A. to teach the public Natural Living and Nature Cure was realized. We will never forget the glorious week in Los Angeles where the authorities and the whole city joined us. The success of that congress was largely due to the talent of Dr. Fred Hirsch, the successor to Prof. Arnold Ehret and the noble and generous Naturopaths of the A.N.A. of Cal. There was never a second congress like that.*
>
> *Then we had the great congresses of New York in 1925, Indianapolis 1926, Philadelphia 1927, Minneapolis 1928, Portland, Oregon 1929, New York 1930, Milwaukee 1931, Washington, D.C., 1932, Chicago 1933, Denver 1934, San Diego 1935, and Omaha 1936.*

In 1925 Lust began to try to reach more of the general populace through the lay publication *Nature's Path. The Naturopath* and *Nature's Path* were later merged because the self-supporting advertising and subscription monies were more available by publication to the general populace than to the members of the association (*The Naturopath,* 1902–1927; *Nature's Path,* 1925–1927; merged 1927–1933; separated 1934–1938; *Nature's Path,* 1939–1945).

How large a professional movement Lust inspired during this period of naturopathy's emergence is difficult to gauge. An extensive government survey was not undertaken until 1965. However, as Whorton described in *Nature Cures,*[21] naturopathy had an effect:

> *Those were messages that had enough appeal, evidently, to allow naturopathy to expand steadily through the first decades of the century until by 1923 Lust could estimate that there were nine thousand naturopaths, a "vast army of professional men and women" working on all continents to "rejuvenate and regenerate the world." His figures were undoubtedly inflated. An independent study [the work of the CCMC discussed later] put the number of naturopaths at "possibly 1500," allowing that if the "allied groups" that advocated drugless healing under other names [physiotherapy, sanipractic] were added on the total may reach 2500. Yet whatever their numbers, naturopaths had grown into a force not to be ignored.*

Although Lust's claim of 9000 naturopaths worldwide is impossible to assess, 5000 practitioners may be a reasonable estimate of the reach of his naturopathy in the United States by the late 1920s and into the 1930s. As Whorton[21] reported, the mixer orientation within chiropractic was also becoming a growing presence. This orientation was a philosophy that tended to merge chiropractic and naturopathy in education and practice.[22] Although homeopathy has undergone a small revival in recent years, very few MDs now practice it. It is currently mainly of interest to naturopaths, who earn doctor of naturopathy (ND) degrees, and to a few chiropractors. Naturopaths closely resemble

chiropractors in that they use spinal manipulative therapy and because so-called mixer chiropractors also use naturopathic methods such as heat, cold, hydrotherapy, physiotherapy, dietary supplements, and even some herbal and homeopathic remedies, which is why the traditional, or "straight," chiropractors disparagingly call them "medipractors." Until the middle of the 20th century, a few mixer schools offered both DC and ND degrees, either as alternatives or together after an additional semester of study. Whorton noted a "1930 survey in which some 1,800 chiropractors participated, found, for example, that 1,124 employed hydrotherapy, 1,173 used light therapy, 1,257 provided electrotherapy, and a full 1,352 trusted vibration therapy."[21]

In January 1934 Lust commenced republication of the title *Naturopath and Herald of Health* in addition to *Nature's Path.* Each volume opened with his personal column, different for each publication. Both publications were issued through 1938, when the *Nature's Path* again became the sole publication until Lust's death in 1945.

After the *Universal Directory,* Lust continued to write volumes on naturopathic principles, although he was more of a synthesizer, organizer, lecturer, and essayist than a scientific documenter of naturopathic principles. His most enduring contributions may remain his early translations of Kuhne's and Just's works.

During the 1920s and up until 1937, Lust's brand of "quackery," so labeled by Fishbein, was in its most popular phase. Although the institutional markings of the orthodox school had gained ascendancy, before 1937, it had no real therapeutic success in the treatment of disease outside of the broad advancements in public health.

Lust's naturopathy, together with chiropractic and osteopathy, continued to be on the outside looking in, this lack of therapeutic advancement notwithstanding. Practitioners of all three movements were continually prosecuted for practicing medicine without a license, although they often won their cases by establishing to juries that their practices were (even according to the testimony of medical men) not the same as medicine at all. At the time, orthodox practitioners could offer little or no expectation of cure for many diseases, and the "health food and natural health" movement was generally popular.

During the 1920s Gaylord Hauser, later to become the health food guru of the Hollywood set, came to Lust as a seriously ill young man. Lust, through the application of the nature cure, removed Hauser's afflictions and was rewarded by Hauser's lifelong devotion. His regular columns in *Nature's Path* became widely read among the Hollywood set.

As noted in *Other Healers, Other Cures*[15]:

> *The last big name in Naturopathy was Gaylord Hauser, a Viennese-Born food scientist (as one of his early books identified him) turned to Naturopathy in his later years. He is best remembered for advising the eating of living foods, not dead foods, and for escorting Greta Garbo around. In addition to fresh fruits and vegetables, Hauser's "Wonder Foods" were skimmed milk, brewer's yeast, wheat germ, yogurt, and blackstrap molasses.*

The naturopathic journals of the 1920s and 1930s are instructive. Much of the dietary advice focused on poor eating habits, including the lack of fiber in the diet and an overreliance on red meat as a protein source. More than half a century later in the 1980s, the pronouncements of the orthodox profession, the National Institute of Health, and the National Cancer Institute finally accepted the validity of these early assertions by naturopaths that poor dietary and living habits (particularly smoking) would lead to degenerative diseases, including cancers associated with the lungs, the digestive tract, and the colon.

The December 1928 volume of *Nature's Path* was the first American publication of the works of Herman J. DeWolff, a Dutch epidemiologist who was one of the first individuals to assert, based on studies of the incidence of cancer in the Netherlands, that there was a correlation between exposure to petrochemicals and various types of cancers. He saw a connection between chemical fertilizers and their usage in some soils (principally clay) that led to poisons remaining in vegetables after they had arrived at the market and were purchased for consumption. Again, it was 50 years before orthodox medicine began to accept the wisdom of such concerns.

As Whorton noted in *Nature Cures,* naturopaths were less successful than osteopaths and chiropractors in accomplishing professionalization by the elevation of professional standards, including professional education. This occurred despite the formation of a National Board of Naturopathic Examiners of the ANA in 1940. There was constant internal bickering, which "by the 1940s had taken on a more ominous tone." Although "standards at naturopathic schools were steadily raised from the 1940s on, thanks to both professional idealism and the requirements of state licensing laws," based on "a perusal of the statutes of the dozen states in which naturopaths were licensed in the late 1940s," the divisive trends within naturopathy "would not begin to be reversed until the 1970s."

Whorton[21] observed that there was no misunderstanding where Lust himself stood on the need for professional standards:

> *Obtaining their own licensing statutes was perceived by alternative practitioners as a critical measure for purging incompetence and quackery from their own rank. "Where there is no official recognition and regulation," the founder of naturopathy, Benedict Lust maintained, "you will find the plotters, the thieves, the charlatans … [The] riff-raff opportunists bring the whole art into disrepute." By the time Lust said this, shortly before his death in 1945, frustrating experience had demonstrated that "that is the fate of any science—any profession—which the unjust laws have placed beyond the pale." In following the evolution of alternative medicine over the first third of the twentieth century, it is essential to keep in mind that constant battle of each system to bring itself within the pale.*

The Emerging Dominance of American Medical Association Medicine

In 1937 the status of conventional (allopathic) medicine began to change. The change came with the beginning of the era of "miracle medicine." Lewis Thomas, in his interesting work *The Youngest Science,*[23] compared his education and internship as a physician with his father's life as a physician. His father believed that bedside manner was more important than any actual medication offered by the physician. His father went into general surgery so that he could offer some service to his patients that actually made some change in their condition. Thomas pointed out that the major growth of "scientific medicine" until 1937 advanced diagnosis rather than offering any hope of cure.

This introduction of "miracle medicine," the social effects of World War II on health care, and the death of Lust in 1945 all combined to contribute a precipitous decline for naturopathy and natural healing in the United States. (During the war, the necessity for crisis surgical intervention techniques for battlefront conditions encouraged the use of morphine, sulfa drugs, and penicillin for diseases not previously encountered in civilian life by American combat soldiers. This resulted in the rapid development of higher-technology approaches to medicine and highly visible successes.)

Lust recognized this, and his editorializing became, if anything, even more strident. From the introduction of sulfa drugs in 1937 to the Salk vaccine's release in 1955, the American public became used to annual developments of miracle vaccines and antibiotics.

Lust died in September 1945 at the Yungborn facility in Butler, New Jersey, preparing to attend the 49th Annual Congress of his American ANA. In August 1945, for the official program of that congress held in October 1945 just after his death, he dictated the following remarks:

> *What is the present condition of Naturopathy? What is its future? I can give my opinion in a very few words. For fifty years I have been in the thick of the fight to bring to the American people the Nature Cure. During that period I have had an opportunity to judge what Naturopathy has done, and can accomplish and the type of men and women, past and present, who make up the Naturopathic ranks.*
>
> *Let us take the present situation first. What is Naturopathy accomplishing? The answer to that is: "Everything." Naturopathy holds the key for the prevention, alleviation and cure of every ailment, to man and beast alike. It has never failed in the hands of a competent Naturopath. Whatever the body can "catch"—that same body, with proper handling, can eliminate. And that takes in cancer, tumors, arthritis, cataract and the whole gamut of "incurable medical" disease and ailments. During my years of practice I, personally, have seen every type of human ailment and so-called serious "disease" give way to the simple, proven Naturopathic methods. I make no exception to that statement.*
>
> *Now let us see the type of men and women who are the Naturopaths of today. Many of them are fine, upstanding individuals, believing fully in the effectiveness of their chosen profession—willing to give their all for the sake of alleviating human suffering and ready to fight for their rights to the last ditch. More power to them! But there are others who claim to be Naturopaths who are woeful misfits. Yes, and there are outright fakers and cheats masking as Naturopaths. That is the fate of any science—any profession—which the unjust laws have placed beyond the pale. Where there is no official recognition and regulation, you will find the plotters, the thieves, the charlatans operating on the same basis as the conscientious practitioners. And these riff-raff opportunists bring the whole art into disrepute. Frankly such conditions cannot be remedied until suitable safeguards are erected by law, or by the profession itself, around the practice of Naturopathy. That will come in time.*
>
> *Now let us look at the future. What do we see? The gradual recognition of this true healing art—not only because of the efforts of the present conscientious practitioners but because of the bungling, asinine mistakes of orthodox medicine—Naturopathy's greatest enemy. The fiasco of the sulpha drugs as emphasized disastrously in our armed forces is just one straw in the wind. The murderous Schick test—that deadly "prevention" of diphtheria—is another. All these medical crimes are steadily piling up. They are slowly, but inevitably, creating a public distrust in all things medical. This increasing lack of confidence in the infallibility of Modern Medicine will eventually make itself felt to such an extent that the man on the street will turn upon these self-constituted oppressors and not only demand but force a change. I may not be here to witness this revolution but I believe with all my soul that it is coming. Yes, the future of Naturopathy is indeed bright. It merely requires that each and every true Naturopath carry on—carry on—to the best of his and her abilities. May God bless you all.*

The effects of postwar events on osteopathy and chiropractic were completely different from the effect on naturopathy. In the early days of osteopathy, there was a significant split between the strict drugless system advocated by A. T. Still (osteopathy's originator) and the beliefs of many MDs who converted to osteopathy because of its therapeutic value. The latter group did not want to abandon all of the techniques they had previously learned and all of the drugs they had previously used when those therapy techniques were sometimes effective. Ultimately, most schools of osteopathy, commencing with the school based in Los Angeles, converted to more of an imitation of modern orthodox medicine. These developments led to more of an accommodation between the California osteopaths and the members of the California Medical Association. (This developing cooperation between the California Osteopathic and Medical Association was one of the major issues leading to the downfall, in 1949, of Fishbein's editorial voice in *JAMA*.) Thus osteopathy found a place in professional medicine, at the cost of its drugless healing roots and therapies.[9]

Naturopathy had become an element of chiropractic education and practice at least as early as 1910 with the founding of the Peerless College of Chiropractic and Naturopathy in Portland, Oregon.[22] From this point on, naturopathic education developed in two tracks: schools of naturopathy owned and operated by naturopaths and chiropractic schools that had naturopathic curricula in addition to the core chiropractic programs. These latter schools were a central part of the mixer orientation within chiropractic.[22,24]

Initial assessments of schools of naturopathy occurred in the 1920s and 1930s. These assessments came from those within, or allied with, allopathy and were therefore hardly unbiased, but much of the information in these assessments seemed credible. The progression of education in naturopathy would be expected to have been similar to that of chiropractic, if somewhat smaller in scale. In this regard, Wardwell noted[22]:

> *Wiese and Ferguson[25] identified 392 different chiropractic schools as having existed in the United States. When those for which there is no evidence of more than a year of operation are eliminated, the number is reduced to 188. Most of them probably produced few graduates—the number of schools increased rapidly to their largest between 1910 and 1926, and then contracted, particularly during the depression of the 1930s and World War II.*

The history of schools of naturopathy followed much the same pattern. Whorton[21] noted in *Nature Cures* that this was the case. The operators of these schools seemed, at least on the surface, aware of the kind of criticisms to which proprietary trade and professional schools were subjected: limited facilities, limited resources, and an emphasis on collecting revenue versus providing a full professional education.[22,24] The leading operators of schools of naturopathy sought, at least on paper, to respond to these criticisms. By letter agreement dated October 7, 1922, four of the most identifiable leaders of naturopathy—Benedict Lust, Joe Shelby Riley, F. W. Collins, and Henry Lindlahr—committed to the formation of the Associated Naturopathic Schools and Colleges of America and committed themselves, as "the Presidents of Naturopathic Schools in the United States of America," to specific educational minimums "on and after January 2, 1923": "all matriculants must have a primary school education[26] and all naturopath courses must be composed of 4 years of 6 months each." Additionally, the letter provided that "time allowance or credits may be given to practitioners in the field who desire to take up the naturopathic courses, and to licensed physicians of other methods of healing," with the amount of such credit being left to each school's discretion.

In the summer and fall of 1927 representatives of the AMA's Council on Medical Education and Hospitals conducted inspections—unannounced and incognito—of schools of "chiropody, chiropractic, naturopathy, optometry, osteopathy, physical therapy, as well as a large number of institutions." From these inspections, several reports were generated, including the Council's report on "Schools of Chiropractic and Naturopathy in the United States,"

which appeared first as part of the Council's *Annual Report, 1928,* and later as reprinted in *JAMA*. The report identified 40 schools of chiropractic and 10 schools of naturopathy and detailed the inspections of "some schools of Chiropractic and of Naturopathy": Palmer School of Chiropractic (a straight school); National College of Chiropractic (a mixer school that was reported as having recently purchased and assimilated Lindlahr College of Natural Therapeutics); Los Angeles College of Chiropractic (another leading mixer school); the combined American School of Naturopathy, Inc. and American School of Chiropractic, Inc. (Lust's own New York City schools, although Lust was observed to have been "in Florida" at the time of the inspections); and the Naturopathic College and Hospital of Philadelphia. The reports were predictably negative with regard to facilities, resources, and the clearly proprietary nature of the establishments.

Louis Reed of the Committee on the Costs of Medical Care (CCMC), in discussing "naturopathic schools,"[24] relied heavily on this report from the AMA's Council and observed that "in 1927, according to the American Medical Association, there existed twelve naturopathic colleges with not over 200 students. These figures would probably hold good for the present time." Reed also concluded that there were "a considerable number of miscellaneous drugless healers of a type similar to chiropractors practice in this country" as of 1932 and that "the naturopaths form the largest group of these practitioners.… Of these various cults, only the naturopaths and the sanipractors have any considerable membership. Many of the (other) cults are really part of the naturopathic group."[24]

As to numbers of drugless practitioners, Reed observed that "only the roughest estimate can be made—probably there are about 2500," of which naturopaths "number possibly 1500," and sanipractors—"only the name distinguishes sanipractors from the naturopaths"—numbered some 500 in their Washington state "stronghold." Reed also observed that as of 1932: "A few states—Connecticut, Florida, Oregon, South Carolina, Utah, Washington and the District of Columbia—provide for licensing of naturopaths as limited practitioners.… In addition to those mentioned, certain states (Alabama, Colorado, Illinois, Indiana, Michigan, Ohio, Pennsylvania, New Jersey, and Wyoming) make (other) provision for the licensing of drugless or limited practitioners."[24]

Reed's work for the CCMC, although clearly biased against all of the healing philosophies he identified as "medical cults" (a la Fishbein), principally osteopathy, chiropractic, and naturopathy, was the only work that attempted to survey the presence and effect of these schools of healing in the United States in the 1920s and 1930s.[27] A decade later, in April 1945, another work of this kind appeared in the *Rhode Island Medical Journal.* The article, "Naturopathic Legislation and Education," was written by the Rhode Island Medical Society's executive secretary, John E. Farrell, to set out some of the society's reasons for opposing legislation that would license naturopathy in Rhode Island. The article noted that according to the 1942–1943 Report of the Committee on Education of the ANA, 13 schools of naturopathy in the United States met the criteria of the ANA; the article went on to make a lengthy "Report on Schools" through visits to most of the identified schools.[28] The predictable criticisms of these schools as underfinanced, underresourced, and proprietary in nature appeared once again, although by actual detail of description, National College (Chicago) and Western States (Portland) seemed to be well-established, functioning mixer schools of chiropractic and naturopathy.

The effect on chiropractic of the post–World War II years was somewhat different. Because of educational recognition under the G.I. Bill, the number of chiropractors in the country grew substantially, and their effect on the populace grew accordingly. The sect eventually grew powerful enough in terms of numbers and economic clout that it could pose a legal challenge to the orthodox monopoly of the AMA. However, in the immediate postwar years, the AMA gained tremendous political clout. Combined with the American Legion and the National Board of Realtors,[29] these three groups posed a powerful political triumvirate before the U.S. Congress.

These years, called the years of the "great fear" in Caute's book by that name,[30] were the years during which to be unorthodox was to be "un-American."

Across the country, courts began to take the view that naturopaths were not truly doctors because they espoused doctrines from "the dark ages of medicine" (something American medicine had apparently come out of in 1937) and that drugless healers were intended by law to operate without "drugs" (which became defined as anything a person would ingest or apply externally for any remedial medical purpose). In this regard, the Washington State Supreme Court case of *Kelly v. Carroll*[31] and the Arizona State Supreme Court case of *Kuts-Cheraux v. Wilson* document how significant limitations were placed on naturopaths under the guise of calling them "drugless healers."

In the state of Tennessee, as a reaction to the 1939 publication of the book *Back to Eden* by herbalist Jethro Kloss, court action initiated by the Tennessee State Medical Association led first to the publishers being forbidden to advertise the book for any therapeutic purpose. They were allowed only to acknowledge that it was in stock. Then, after a serious licensing scandal during the war years, the Tennessee State Legislature declared the practice of naturopathy in the state of Tennessee to be a gross misdemeanor, punishable by up to 1 year in jail.

Although it was under considerable public pressure in those years, the ANA undertook some of its most scholarly work, coordinating all the systems of naturopathy under commission. This resulted in the publication of a basic textbook on naturopathy (*Basic Naturopathy,* published in 1948 by the ANA[32]) and a significant work compiling all the known theories of botanical medicine (as commissioned by the ANA's successor after its 1950 name change to the American Naturopathic Physicians and Surgeons Association), the *Naturae Medicina,* published in 1953.[33] Naturopathic medicine began splintering when Lust's ANA was succeeded by six different organizations in the mid-1950s.

The primary organizations among these were the successor to the ANA, which underwent a name change in 1950 to the American Naturopathic Physician and Surgeon's Association and subsequently changed to the American Association of Naturopathic Physicians (AANP) in 1956, and the International Society of Naturopathic Physicians formed under the leadership of M. T. Campenella of Florida shortly after Lust's death, with its American offshoot, the National Association of Naturopathic Physicians.

In the face of the AMA's determination to eliminate chiropractic, and with it, naturopathy—healing philosophies that were linked through the mixer orientation within chiropractic (during the 1930s and through the 1960s the majority camp within a divided chiropractic)—naturopathy went through a period of decline described by Hans Baer (see Bibliography).

Walter Wardwell was a sociology professor who became an early leader in what developed as a subspecialty in the 1950s: medical sociology. His earliest work, starting with his doctoral dissertation (1951) at Harvard, focused on chiropractic as an example of a marginalized health profession (see Bibliography). As early as his doctoral dissertation, Wardwell discussed naturopathy as an adjunct discipline to

chiropractic in the context of the continuing division of chiropractic into mixers and straights. As he later noted[34]:

> *Comparison of the survival of chiropractic with that of osteopathy and naturopathy is a quite different matter which does not involve metaphysical or epistemological differences between them. Furthermore, the overlap in theory between chiropractic, osteopathy and naturopathy is very great. Differences between osteopathic and chiropractic manipulative treatment appear to be more a matter of who applies the technique rather than differences in technique itself. The distinction between chiropractors and naturopaths is even more blurred because they often trained at the same schools and sometimes they studied both fields simultaneously. As recently as 1948, three of the currently accredited chiropractic colleges offered N.D. (Doctor of Naturopathy) and D.C. (Doctor of Chiropractic) degrees.*

In this context he described naturopathy as a school of healing that became extinct as two historical factors converged: the death of Lust in 1945, leaving naturopathy without its "founder," and the mandate in the early 1950s by the major mixers' professional group, the National Chiropractic Association (NCA), that it would no longer accredit chiropractic schools that granted degrees in naturopathy:

> *In the case of naturopathy, chiropractic's victory is nearly complete. Although there may still be up to 2000 naturopaths in practice[35] with naturopaths licensed in a few states, and one small school in Portland, Oregon, still offers naturopathic degrees, none of the schools that formerly offered both chiropractic and naturopathic degrees currently does so. With practically no new recruits entering the profession, naturopathy must disappear.*

By the late 1970s, Wardwell had learned of efforts in the Pacific Northwest to keep naturopathy alive. In his chapter in the *Handbook of Medical Sociology,* Wardwell noted this presence in the Northwest (which had received no mention in the first two editions in 1963 and 1972)[36]:

> *The accrediting of chiropractic colleges is encouraging uniformity, not only in curricula but in scope of practice. Those colleges that formerly offered the Doctor of Naturopathy (N.D.) as well as the D.C. degree have ceased doing so, leaving naturopathy with only one remaining small college in Portland, Oregon.*

In his masterwork, *Chiropractic: History and Evolution of a New Profession* (1992),[22] Wardwell devoted substantial attention to the effect of naturopathy on the mixer orientation within chiropractic and traced naturopathy's final educational decline to the untimely death in 1954 of William A. Budden, DC, ND, the president of the Western States Chiropractic College (WSCC; Portland, Oregon). After Budden's death, the WSCC continued to teach naturopathy until 1958 but dropped its ND degree program in 1956. This was the last resistance to the position of the accrediting committee of the NCA, and no chiropractic ND programs remained. Wardwell observed, though, that the seeds of a naturopathic reemergence had been planted in the Northwest after Budden's death and that naturopathy might survive.

The last ND diplomas were granted at the WSCC in 1958 to students who were enrolled in the ND program at the time of Budden's death. Brinker[37] noted the following:

> *Political pressure from the chiropractic profession had begun in the late 1940s to force chiropractic schools to relinquish programs granting naturopathic degrees. After threatening loss of accreditation, the National Chiropractic Association finally forced Western States College to drop its School of Naturopathy in 1956, and it became exclusively Western States Chiropractic College.*

Efforts to keep naturopathy alive through education and licensure were examined by two reports prepared in 1958, a time when the Utah legislature was reexamining naturopathy's licensure in the aftermath of a case from the Utah Supreme Court that had dealt its practicing NDs a crippling blow. The first was *A Study of the Healing Arts With a Particular Emphasis Upon Naturopathy* (November 1958), prepared as "A Report to the Utah Legislative Council" by legislative council staff. As part of its work, the staff conducted inquiries of and site visits to seven schools accredited by the Utah Naturopathy Examining Board as of August 1957.

Separately, the Bureau of Economic and Business Research[38] of the University of Utah (BEBR) undertook a study focusing on schools that had granted naturopathy degrees and produced *Survey of Naturopathic Schools* ("Prepared for the Utah State Medical Society," December 1958). Preparation of the study was, as noted in the title, undertaken by the university research program at the request of the state medical society, but the preparation of the study was independent, and "no attempt was made by that group to influence the results of the study" (Foreword and Acknowledgements).

The BEBR study, done with the requested cooperation of investigators from five other universities located in various sections of the United States, surveyed all of the schools listed by Utah licensees as schools of graduation or schools attended, using records maintained by the Utah Department of Business Registration.[10] Because the state of naturopathic education in the 1950s is relevant, some observations from this study are worth noting[39]:

> *One of the most important results to emerge from this study is that there are virtually no schools now teaching naturopathy.*

Of the 26 schools investigated during this study, only 9 were still in existence in the fall of 1958. Of these nine, only three are now granting naturopathic degrees, and two others are teaching naturopathy.

Of the three schools granting ND degrees, the study found that one school, Sierra States University in California, began offering a "postgraduate" ND degree after the most highly respected chiropractic program in the country, Los Angeles College of Chiropractic, had discontinued its ND degree program in 1948. National College of Naturopathic Medicine (NCNM), the Oregon school, had—in 1957, its first year of operation—four ND students who were starting at NCNM and 60 enrolled "postgraduate" DCs pursuing ND degrees. The school had been recognized by the Utah examining board but had not yet granted degrees. The third school granting ND degrees as of 1957 to 1958 was the Central States College of Physiatrics in Eaton, Ohio, essentially the one-man operation of H. Riley Spitler, author of *Basic Naturopathy* (published by the ANA in 1948). This school granted a doctor of mechanotherapy (DM) degree, recognized in only Ohio and Alabama by law, or an ND degree to anyone who sought licensure in a state where an ND degree would qualify a graduate for licensure. The course of study for both degrees was the same, and the school had graduated 10 students in the previous 2 years. Its ND degrees were recognized in Utah.

By 1955, the AANP, as it ultimately became known, had recognized only two schools of naturopathic medicine, the Central States College of Physiatrics in Eaton, Ohio, under the leadership of H. Riley Spitler, and Western States College of Chiropractic and Naturopathy located outside Portland, Oregon, under the leadership of W. A. Budden. Budden was a Lindlahr graduate and among the group that took over control of the Lindlahr College after Lindlahr's death in the 1920s. He moved west in 1929 when the northwestern states, including Oregon, became a bastion for naturopaths in this country.

This state of affairs was accurately described by Homola[40] in his book on the history and evolution of chiropractic:

> *As of 1958, only five states (Arizona, Connecticut, Oregon, Virginia and Utah) separately classified and provided licensing provisions for the naturopath. A few states, however, did permit licensing of drugless healers following examination by [a] board. (A good number of states have repealed their laws licensing naturopaths in recent years.) Chiropractic schools that employ the use of physiotherapy teach a course that is very similar to the practice of naturopathy. Likewise, the three or four naturopathic schools still operating today have a curriculum similar to that of many chiropractic colleges. In fact, at least four chiropractic colleges awarded naturopathic degrees along with the chiropractic degree before they came under the jurisdiction of the national Chiropractic Association. With the approval of this organization, the schools were prohibited from issuing naturopathic degrees. This practically amounted to a death-dealing blow to the profession of naturopathy.*[40]

In 1967 the U.S. Department of Health, Education, and Welfare; the Public Health Service; and the National Center for Health Statistics (NCHS) published Public Health Service Publication No. 1758, *State Licensing of Health Occupations.* With the assistance of the Council of State Governments, the NCHS collected data regarding the licensure of health professionals at the state level. "Chapter 8: Naturopaths" recorded the available data for the naturopathic profession as of the mid-1960s. In summary, the NCHS identified five states and the District of Columbia as licensing naturopaths as of 1967: Arizona, Connecticut, Hawaii, Oregon, and Utah. California and Florida were identified as renewing existing licenses but granting no new licenses. The publication reported that by 1965, California had renewed 66 licenses and Florida, 136. Licenses in effect by state were as follows: Arizona (100), Connecticut (47), Hawaii (14), Oregon (148), and Utah (42). No numbers were provided for the District of Columbia. The report stated the following[41]:

> *In addition to Doctors of Naturopathy (ND) there are other limited branches of medicine; these have not been included in the study. In the State of Washington the Drugless Therapeutics Examining Committee functions (for such licensure). The Ohio law states which branches are to be specified on certificates issued by the State Medical Board to limited practitioners. No attempt has been made to collect information on these drugless healers who are few in number.*

Active state practitioners were also numbered (although the reason for the differentiation is not clear), as follows: Arizona (53), Connecticut (29), Hawaii (13), and Oregon (121). Given the existence of approximately 50 practitioners at the time in Washington, and some practicing in Idaho under a decision of the Idaho Supreme Court, there appear to have been perhaps as many as 600 to 700 remaining naturopaths practicing at the end of the 1960s.[42]

According to documentation provided to the federal Department of Health, Education, and Welfare in 1968 by the again-remaining professional association—the National Association of Naturopathic Physicians—only 17 degrees were granted from 1960 to 1968. By 1968, this association had 168 members and estimated that there were perhaps 500 "active" naturopaths in the United States. Congress adopted Medicare in 1965. The legislation covered payment for the services of physicians (essentially MDs and DOs), hospital services, and "other therapeutic services" that would commonly be provided through these conventional means. As Wardwell reported,[22] in 1967, Congress directed the secretary of the Department of Health, Education, and Welfare (HEW), Wilbur Cohen, to study the inclusion services of "additional types of licensed practitioners." The surgeon general and other HEW staff prepared the resulting *Independent Practitioners Under Medicare* using advisory committees only (Wardwell served on the Expert Review Committee for Chiropractic and Naturopathy), which actually had little input. This report documented the ebb tide of naturopathy's "period of decline," as Baer later labeled it.[43] The section of the report *Naturopathy* concluded that as of 1968:

> *Naturopathic theory and practice are not based on the body of basic knowledge related to health, disease, and health care which has been widely accepted by the scientific community. Moreover, irrespective of its theory, the scope and quality of naturopathic education do not prepare the practitioner to make an adequate diagnosis and provide adequate treatment.*

Considering the state of the profession in 1968, these negative assessments were hardly unexpected.

THE MODERN REJUVENATION

After the counterculture years of the late 1960s and feeding of an American disenchantment with organized medicine that began after the miracle-drug era faded, exposing some of orthodox medicine's limitations, alternative medicine began to gain new respect. Naturopathic medicine underwent an era of rejuvenation as a late-1970s consumer interest in more "holistic" medicine began to emerge.

As succinctly described in Cassedy's[44] *Medicine in America: A Short History,* this phenomenon, which was not limited to naturopathic medicine, was consistent with the modern and continuing "search for health beyond orthodox medicine":

> *It should not have been surprising to anyone that certain organized therapeutic sects continue to exist in mid–twentieth century America as successful and conspicuous alternatives to regular medicine. This is not to say that they offer the same threats to the medical establishment or play the same roles as their nineteenth-century counterparts had, as complete therapeutic systems. But they do continue to hold a strong collective appeal for individuals who mistrust or are somehow disenchanted with mainline medicine. They have appealed also to antiauthoritarian sentiments that flourish throughout society. Moreover, as earlier, they satisfy various needs that regular medicine continues to neglect or ignore.*

The same author, in describing the post–World War II decades and the changing fortunes of such healing theories as naturopathic medicine, observed as follows:

> *The period also brought about the renewal or updating of certain previously widely used therapies and considerable experimentation with others, some of them exotic. To an extent this trend represented the rediscovery by trained physicians, nurses, and other regular health professionals of certain values and older styles of therapy. The participation of such professionals proved to be an essential ingredient in the rebirth of several such therapies. However, the major reason for the new successes was the wide-spread active interest and involvement of America's literate lay people in the search for more personal or humane forms of treatment.*

As another author, John Duffy,[45] observed in *From Humors to Medical Science:*

Since health is too closely related to cultural, social, and economic factors to be left exclusively to doctors, American lay people have always engaged in do-it-yourself medicine, resorted to "irregulars and quacks," and supported health movements. As a result of the current fad for physical fitness, our streets are beset by sweat-suited individuals of all ages doggedly jogging their way to health and long life. In addition, stores selling "natural" foods are flourishing, physical fitness salons have become a major business, and anti-smoking and weight-loss clinics and workshops are attracting thousands of individuals bent on leading cleaner and leaner lives. And those for whom physical activity in itself is not enough are seeking physical and mental well-being through faith healing, yoga, and a host of major and minor gurus.

When neither mental effort nor physical exercise can solve medical problems, the sceptics of modern medicine can always turn to the irregulars. A recent estimate places a number of Americans who have relied on an irregular practitioner at some time in their lives at 60 million, and, aided by the high cost of orthodox medicine, irregular medical practice appears to be on the rise.

At the beginning of this period of rejuvenation, the profession's educational institutions had dwindled to one, the National College of Naturopathic Medicine (which had branches in Seattle, Washington, and Portland, Oregon), which was founded after the death of R. A. Budden and the conversion of Western States College to a straight school of chiropractic. Kruger's[15] book *Other Healers, Other Cures* described it as follows in 1974:

Today, Naturopaths in seventeen states are licensed to diagnose, treat, and prescribe for any human disease through the use of air, light, heat, herbs, nutrition, electrotherapy, physiotherapy, manipulations, and minor surgery. At present, one can earn an D.N. [a misnomer, actually—N.D.] degree at the National College of Naturopathic Medicine in Seattle and Emporia, Kansas, [where, by contract, the first 2 years of the 4-year medical education were then taught], or the new North American Naturopathic Institute in North Arlington, New Jersey [there is also a school in Montreal]. The four-year curriculum covers many standard medical courses—anatomy, bacteriology, urology, pathology, physiology, X-ray reading etc.—but also includes botanical medicine, hydrotherapy, electrotherapy, and manipulative technique.

The public, by the late 1970s, was particularly ripe for another rejuvenation of naturopathy's brand of "alternative" health care. As described in Murphy's *Enter the Physician: The Transformation of Domestic Medicine, 1760–1860,* when discussing this cyclical rejuvenation in the mid-20th century[46]:

Contemporary crusaders still stress prevention as the layperson's primary duty, but a growing chorus is calling for every person to assume the newly proactive role in his or her own health care.

What would this entail? There are probably as many answers to this question as there are respondents, but it is striking to note how many of the solutions would have been familiar to our ancestors who lived between 1760 and 1860. One recurring idea, for instance, is that each person knows his or her own constitution history the best and therefore has a duty to communicate that knowledge to medical personnel. Another is a refurbished concept of *vis medicatrix naturae,* the belief that many diseases are self-limiting and therefore do not require much medical intervention—and certainly not the amount or the sort to which contemporary Americans are accustomed. Most significantly, today's analysts are calling on professionals and nonprofessionals to build and nurture a healthcare partnership very much like that envisioned by 19th-century health publicists: a partnership based on mutual respect, clear understanding, and faithful execution. In that scenario, both as it originally evolved and in its updated version, it is the doctor who directs treatment, but crucial to a successful outcome are the informed and responsible actions of the patients, other caregivers, and the patient's family and friends.

In 1978 the John Bastyr College of Naturopathic Medicine was formed in Seattle, Washington, by Joseph E. Pizzorno, ND (founding president), Lester E. Griffith, ND, and William Mitchell, ND (all graduates of the National College of Naturopathic Medicine), and Sheila Quinn, who felt that it was necessary to have more institutions devoted to naturopathic care and the teaching of naturopathic therapeutics. To differentiate Bastyr from the other "irregular"[45] schools, Pizzorno coined the term *science-based natural medicine* and developed the curriculum to implement it. Bastyr's cofounder and first president, Joseph Pizzorno, recognized that "anecdotal and unverified 'cures', particularly when associated with unusual therapies do our cause little good." Consequently, instruction at the school "concentrated more on the scientifically verifiable aspects of natural medicine and less on the relatively anecdotal nature cure aspects."[21]

In *Other Healers, Unorthodox Medicine in America,*[47] a volume written to provide "a scholarly perspective on unorthodox movements and practices that have arisen in the United States" (from the editor's preface), author Martin Kauffman, a modern expert in homeopathy from the Department of History at Westfield State College, detailed Bastyr's homeopathic requirements to graduate:

In 1978, three naturopathic practitioners in Seattle founded the John Bastyr College of Naturopathic Medicine. During the sixth quarter all students at that school are required to take 44 hours of course work in homeopathy, after which they may elect another 66 hours and up to 238 hours of clinical homeopathic instruction. The significance of the naturopathic schools to the resurgence of homeopathy is demonstrated by the fact that "about one third of the graduating class specialized in homeopathic practice, a total of about 50 each year in all."[47]

During the late 1970s, other naturopathic doctors also recognized the need to establish educational institutions for students of naturopathic medicine; subsequent efforts included colleges in Arizona (the Arizona College of Naturopathic Medicine), Oregon (the American College of Naturopathic Medicine), and California (the Pacific College of Naturopathic Medicine). Unfortunately, none of these three survived.

As public demand for natural healing grew in the 1980s and 1990s, the emerging profession continued to grow a breadth and quality of educational opportunity for those seeking accredited doctorate-level programs in naturopathic medicine.

With thriving enrollments at Bastyr and National College, the Council on Naturopathic Medicine was founded in 1978 to establish and oversee educational standards, and today it is recognized by the U.S. Secretary of Education as the national accrediting agency for programs leading to the doctor of naturopathic medicine (ND or NMD) or doctor of naturopathy (ND) degree. To further build on the cornerstone of accredited education and ensure educational quality, in 1986 the Naturopathic Physician Licensing Examination became the first national board examination for graduates; today, graduates must pass a two-part medical examination in biomedical and clinical sciences before they are eligible to use the title "ND." This examination is modeled after the conventional medical board examination for allopathic graduates, the U.S. Medical Licensing Exam, which assigns the "MD" license. This training was described in detail in a report

from 2001 by the University of California San Francisco Center for Health Professions: "Naturopathic physicians are typically trained in a wide array of alternative therapies including herbology, homeopathy, massage, hydrotherapy, physical medicine, behavioral medicine, Traditional Chinese medicine, Ayurvedic medicine, acupuncture, and nutrition therapy, as well as clinical practices such as minor surgery, pharmacology and obstetrics."[48]

With educational standards set, throughout the 1990s and 2000s, a select group of new programs and institutions attained accreditation status with the Council on Naturopathic Medicine: Southwest College of Naturopathic Medicine and Health Sciences, Tempe, Arizona; the College of Naturopathic Medicine at the University of Bridgeport, Connecticut; the Canadian College of Naturopathic Medicine, Toronto, Ontario; and, in 2011, the Boucher Institute of Naturopathic Medicine, British Columbia, Canada. The establishment of multiple geographic locations for this type of education paves a solid future for the profession, providing hundreds of newly graduated naturopathic doctors every year in the United States and Canada.

There are favorable commentaries on the current state of naturopathic medicine. *Other Healers, Unorthodox Medicine in America*[47] is a volume written to provide "a scholarly perspective on unorthodox movements and practices that have arisen in the United States." As described in the *Encyclopedia of Alternative Health Care* by Olsen[49]:

> *While naturopathic medicine is now legal (in several states), many naturopaths practicing in other states are old-timers, practicing under their original "drugless therapy" licenses, issued before laws prohibiting new naturopathic practices went into effect.*

In cooperation with regional associations, the AANP has won licensure and scope-of-practice protection at a steady rate on par with the growth of schools accredited by the Association of Accredited Naturopathic Medical Colleges. As of 2011, 15 U.S. states, the District of Columbia, five Canadian provinces, and the U.S. territories of Puerto Rico and the U.S. Virgin Islands regulate the naturopathic profession (Box 3.1).

Baer's interest in the evolution of chiropractic as a philosophy of healing led him to Wardwell's work and to Wardwell's earlier scholarship, which had been tied to the mixer orientation within chiropractic. Baer took note of his descriptions of naturopathy as a near-extinct philosophy. Predictions of extinction were consistent among the assessments of social scientists in the 1970s and continued into the mid-1980s. Twaddle and Hessler, Rosengren, Whorton, and most notably, Wardwell all discussed naturopathy as a once-observable but marginalized philosophy of health and healing at odds with the conventional medical claims of a scientific medicine (see Bibliography). These social scientists placed naturopathy's demise sometime in the 1950s when chiropractic severed its open naturopathic link by terminating ND programs.

Baer, before Wardwell, took special note of Bastyr and the professionalization represented by its scientific medicine–based curriculum and the publication of a John Bastyr College of Naturopathic Medicine project, *The Textbook of Natural Medicine.*

In his 1992 *Medical Anthropology* article[43] "The Potential Rejuvenation of American Naturopathy as a Consequence of the Holistic Health Movement," Baer detailed his own view of Naturopathy's "three stages of development" noted at the outset of this chapter. Besides relying on material covered in the original chapter of "The History of Naturopathic Medicine," which first appeared in 1985, Baer covered much of the new material regarding the emerging (1900–1930s) and declining (1940–1970s) stages of naturopathy.

Baer particularly broke new ground with his recognition of a "potential rejuvenation" of naturopathy as naturopathic medicine and his recognition that the profession had knowingly or unknowingly adopted a recognized survival strategy as a matter of organizational policy: professionalization. Baer also advanced a theory regarding the "potential rejuvenation" as tied to the emergence in the 1970s of holistic medicine. Holistic medicine, as a philosophy of healing, had a cultural affinity with the eclecticism inherent in naturopathic philosophy. In his 2001 book[50] *Biomedicine and Alternative Healing Systems in America,* Baer updated this view of the status of naturopathic medicine in a chapter entitled "Naturopathy and Acupuncture as Secondary Professionalized Heterodox Medical Systems." With the passage of the additional 10 years, Baer observed[50]:

> *Unlike chiropractic, which no longer poses a serious threat to biomedicine because of its status as a specialty emphasizing spinal manipulation, a rejuvenated naturopathy finds itself in direct competition with biomedicine because both systems claim to provide a comprehensive approach to health care.*
>
> *As osteopathy and chiropractic did earlier, naturopathy … [is] increasingly incorporating the theory and social organization of biomedicine.*
>
> *[N]aturopathy with [its] reductionist philosophy and [its] focus on individual responsibility for healthy living may well undergo further growth in an era of growing health costs.*

BOX 3.1 States/Districts/Provinces That Regulate the Naturopathic Profession (updated 2018)

States
Alaska
Arizona
California
Colorado
Connecticut
Hawaii
Idaho
Kansas
Maine
Massachusetts
Minnesota
Montana
New Hampshire
North Dakota
Oregon
Pennsylvania
Rhode Island
Utah
Vermont
Washington

Districts and Territories
District of Columbia
Puerto Rico
U.S. Virgin Islands

Provinces
Alberta
British Columbia
Manitoba
Nova Scotia
Ontario
Saskatchewan

THE 21ST CENTURY AWAITS

Baer carried his examination of the sociopolitical aspects forward in his 2001 article "The Sociopolitical Status of U.S. Naturopathy at the Dawn of the 21st Century,"[51] which examined the state of naturopathic medicine as it prepared to enter the 21st century. Although "professionalized naturopathy has undergone tremendous growth and legitimization since the late 1970s, nevertheless, it finds itself in a tenuous situation at the dawn of the twenty-first century in that its strength is confined primarily to the Far West and New England; it faces increasing competition from the partially professionalized and lay naturopaths; and it faces the danger of being overshadowed by a powerful biomedical system that is increasingly incorporating aspects of holistic health into its own practice."

He offered no definitive answers to these questions of naturopathic medicine's future, but he also highlighted areas needing further attention by social scientists: continued exploration of the reasons for naturopathy's decline and rejuvenation and continued study of the naturopathic profession in recognition of its state of professionalization.

In closing, Baer observed: "In sum, while changes in the popular ideas about health and healing unleashed the social forces that enabled professional naturopathy to get back on its feet, those same social forces may overwhelm its core claim to being a unique, natural approach to healing."

Whorton expressed the view that in many respects the transition from the marginalized naturopathy to the professionalized naturopathic medicine has now been accomplished.[52] He traced his view of this transformation as part of the larger transformation "from alternative medicine to complementary medicine" on the part of osteopathy, chiropractic, and naturopathy. Whorton described the factors that allowed this transformation even after the death of Lust in 1945: the issue of the "field's lack of a scientific basis" was determined internally when the "died-in-the-wool believers in 'nature cure'" were outlasted by the "liberal practitioners belonging to the so-called western group, naturopaths concentrated in the western states who recognized the validity of mainstream medicine's scientific foundation and sought to incorporate biomedical science into their own system and apply it under the guidelines of naturopathic philosophy."

As Whorton noted, "a key figure among the pseudomedicals was John Bastyr—a practitioner in Seattle since the 1930s, and particularly well-known for his advocacy of natural childbirth." Bastyr, Whorton noted, "recognized the necessity of naturopathy staying abreast of advances in biomedical science and applying those advances 'in ways consistent with naturopathic principles.'"[21] Bastyr was directly involved with the formation and maintenance of the NCNM during the years of naturopathy's decline and lived to see much of "the short history of John Bastyr College [of Naturopathic Medicine] ... the most compelling illustration of the triumphant rebirth of naturopathy as naturopathic medicine."[21]

Bastyr has been called the "father of modern naturopathic medicine" by Pizzorno, ND,[52] the moving spirit behind the professionalization of naturopathic medicine and the founding president of Bastyr University. No individual has carried the practice of NDs in the United States in the way that Lust did, but Bastyr and the others profiled by Kirchfeld and Boyle in *Nature Doctors* kept naturopathy alive during its decline in the 1950s and 1960s so that it could, in time, reemerge.

The movement continues to grow, and thus the effect of natural healing has come full circle. In an era where the statistical number of persons born who are expected to contract cancer, now recognized as a degenerative disease, has increased rather than declined and where the incidence of other degenerative diseases (arthritis, arteriosclerosis, atherosclerosis, etc.) has increased in direct relation to the lengthening of life expectancies produced by improved sanitation and nutrition (although speciously claimed by AMA medicine to be the result of their therapies), the early teachings of Lust, Lindlahr, and others appear to have more validity than ever.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Starr P. *Social Transformation of American Medicine*. New York: Basic Books; 1983.
2. Griggs B. *Green Pharmacy*. London: Jill: Norman, & Hobhouse; 1981.
3. Medical Protestants Haller J. *The Eclectics in American Medicine*. Carbondale, IL: Southern Illinois University Press; 1994.
4. Whorton J. *Crusaders for Fitness*. Princeton, NJ: Princeton Press; 1982.
5. Rothstein W. *American Physicians in the 19th Century*. Baltimore: Johns Hopkins Press; 1972.
6. Haller J. *American Medicine in Transition, 1850–1910*. Urbana, IL: University of Illinois Press; 1981.
7. Rosen G. *The Structure of American Medical Practice*. Philadelphia: University of Pennsylvania; 1983.
8. Brown ER. *Rockefeller Medicine Men*. Berkeley, CA: University of California Press; 1978.
9. Campion F. *AMA & US Health Policy Since 1940*. Chicago: AMA Publishers; 1984.
10. Burrows J. *Organized Medicine in the Progressive Era*. Baltimore: Johns Hopkins Press; 1977.
11. Coulter H. *Divided Legacy*. Vol. 2. Washington, DC: Wehawken Books; 1973.
12. Salmon JW. *Alternative Medicines*. New York: Tavistock; 1984.
13. Gevitz N. *The D.O.'s*. Baltimore: Johns Hopkins Press; 1982.
14. Silberger J. *Mary Baker Eddy*. Boston: Little Brown; 1980.
15. Lindlahr H. *Philosophy of Natural Therapeutics*. Vol. 1. England: Maidstone: Maidstone Osteopathic; 1918.
16. Lust B. *Universal Directory of Naturopathy*. Butler, NJ: Lust; 1918.
17. Kruger H. *Other Healers, Other Cures. A Guide to Alternative Medicine*. New York: Bobbs-Merrill; 1974.
18. Kellogg JH. *New Dietetics*. Battle Creek, MI: Modern Medical Publications; 1923.
19. Ernst R. *Weakness Is a Crime: The Life of Bernarr MacFadden*. Syracuse, NY: Syracuse University Press; 1991.
20. Whorton J. *Inner Hygiene: Constipation & the Pursuit of Health in Modern Society*. New York: Oxford University Press; 2000.
21. Whorton J. *Nature Cures: The History of Alternative Medicine in America*. New York: Oxford University Press; 2002.
22. Wardwell WI. *Chiropractic; History and Evolution of a New Practice*. St. Louis: Mosby; 1992.
23. Thomas L. *The Youngest Science*. Boston: Viking; 1983.
24. Reed L. *The Healing Cults. Publication No. 16 of the Committee on the Costs of Medical Care*. Chicago: University Press; 1932.
25. Wiese G, Ferguson A. How many chiropractic schools? An analysis of institutions that offered the D.C. degree. *Chiropract Hist*. 1988;8(1): 27–36.
26. "Primary education"—circa 1922—was an eighth-grade education, and this educational base would have been the same as that required by chiropractic.
27. The results of Reed's work are also summarized in the CCMC's Publication No. 27, "The Costs of Medical Care" (Falk, Rorem, and Ring [1933], p. 292), as "Naturopaths and Other Drugless Healers."
28. With lengthy discussion of Central States College of Physiatrics (Eaton, Ohio), the Colorado Mineral Health School (Denver), Columbia College of Naturopathy (Kansas City), First National University of Naturopathy (Newark, New Jersey, earlier the United States School), the Metropolitan College (Cleveland), the Nashville College of Drugless Therapy (Tennessee), the National College of Drugless Physicians (part of the National College of Chiropractic, Chicago), the Polytechnic College and Clinic of Natural Therapeutics (Fort Wayne, Indiana), the Southern University of Naturopathy and Physio-Medicine (Miami), the University of Natural Healing Arts (Denver), and the Western States College (Portland, Oregon).
29. Goulden J. *The Best Years*. New York: Atheneum; 1976.
30. Caute D. *The Great Fear*. New York: Simon & Schuster; 1978.
31. The defendant was Otis G. Carroll of Spokane, Washington. He and his brother, Robert V. Carroll, Sr., of Seattle, were longtime associates of Benedict Lust. As members of Lust's American Naturopathic Association, they had advanced naturopathy's presence in Washington State through the Washington State Naturopathic Association.
32. Spitler HR. *Basic Naturopathy*. Des Moines: ANA; 1948.
33. Kuts-Cheraux AW. *Naturae Medicina*. Des Moines: ANPSA; 1953.
34. Wardwell WI. Comparative factors in the survival of chiropractic: a comparative view. *Sociol Symp*. 1978;22:6–17.
35. As to those calling themselves naturopaths, this number was considerably too high, as will become apparent.
36. Wardwell WI. Limited and marginal practitioners. In: Freeman H, Levine S, Reeder LG, eds. *Handbook of Medical Sociology*. Upper Saddle River, NJ: Prentice-Hall; 1979:240–242.
37. Brinker F. The role of botanical medicine in 100 years of American naturopathy. *Herbal Gram*. 1998;42:49–59.
38. Now the National Bureau of Economic Research.
39. Bureau of Economic and Business Research. *Survey of Naturopathic Schools*. Salt Lake City: University of Utah; 1958.
40. Homola S. *Bonesetting, Chiropractic and Cultism*. Panama City, FL: Critique Books; 1963.
41. Cohen W. Naturopathy. In: *Independent Practitioners Under Medicare: A Report to Congress*. Washington, DC: US Department of Health, Education, and Welfare; 1968.
42. The State Licensing of Health Occupations; U.S. Department of Health, Education, and Welfare; and the National Center for Health Statistics Public Health Service Publication No. 1758 (1967) reported: "Naturopaths are specifically licensed in at least five States and the District of Columbia. The absence of a State from this list does not imply that there are no licensed naturopaths. Illinois, for example, could be covered by the medical practice act. Texas and Virginia provide for naturopaths on examining boards but no information is available on licensing practices. Elsewhere licensing powers have been abolished and no new licenses have been issued; for example, in 1965 naturopathic licenses renewed in California numbered 66 and in Florida, 136."
43. Baer HA. The potential rejuvenation of American naturopathy as a consequence of the holistic health movement. *Med Anthropol Q*. 1992;13:369–383.
44. Cassedy JH. *Medicine in America: A Short History*. Baltimore: Johns Hopkins University Press; 1991.
45. Duffy J. *From Humors to Medical Science: A History of American Medicine*. Urbana, IL: University of Illinois Press; 1993:350.
46. Murphy LR. *Enter the Physician: The Transformation of Domestic Medicine, 1760–1860*. Tuscaloosa: University of Alabama Press; 1991.
47. Kaufmann M. Homeopathy in America. In: Gevitz N, ed. *Other Healers: Unorthodox Medicine in America*. Baltimore: Johns Hopkins University Press; 1988:99–123.
48. Hough HJ, Dower C, O'Neil EH. *Profile of a Profession: Naturopathic Practice*. San Francisco: Center for Health Professions; 2001.
49. Olsen KG. *The Encyclopedia of Alternative Health Care*. New York: Pocket Books; 1989.
50. Baer HA. *Biomedicine and Alternative Healing Systems in America*. Madison: University of Wisconsin Press; 2001.
51. Baer HA. The sociopolitical status of U.S. naturopathy at the dawn of the 21st century. *Med Anthropol*. 2001;15(3):329–346.
52. Pizzorno Jr JP, Bastyr J. The father of modern naturopathic medicine. *Integr Med*. 2004;3:28–29.

GENERAL BIBLIOGRAPHY

Baer HA. Organizational rejuvenation of osteopathy. *Soc Sci Med*. 1981;15A:701–711.

Baer HA. A comparative view of a heterodox system: chiropractic in america and britain. *Med Anthropol*. 1984;8:151–168.

Baer HA. The American dominative medical system as a reflection of social values in the larger society. *Soc Sci Med*. 1989;28:1103–1112.

Barrett S, Herbert V. *The Vitamin Pushers: How the Health Food Industry Is Selling America a Bill of Goods*. New York: Prometheus Books; 1994.

Barrett S, Jarvis W. *The Health Robbers: A Close Look at Quackery in America*. New York: Prometheus Books; 1993.

Berlinger H. *A System of Medicine: Philanthropic Foundations in the Flexner Era*. New York: Tavistock Publishers; 1985.

Berman A, Flannery MA. *America's Botanico-Medical Movements: Vox Populi*. Oxford, MS: Pharmaceutical Products Press; 2001.
Bloomfield RJ. Naturopathy in traditional medicine and health care coverage. In: Bannerman RH, Burton J, Wen-Chieh C, eds. *Traditional Medicine and Health Care Coverage*. Geneva: World Health Organization; 1983.
Breiger G. *Medical America in the 19th century*. Baltimore: Johns Hopkins Press; 1972.
Cody G. History of naturopathic medicine. In: Pizzorno J, Murray M, eds. *Textbook of Natural Medicine*. Orlando FL: Churchill Livingstone; 1999:17–40.
Coward R. *The Whole Truth: The Myth of Alternative Health*. London: Faber & Faber; 1989.
Duffy J. *The Healers*. Urbana, IL: University of Illinois Press; 1976.
Engel J. *Doctors and Reformers*. Columbia: University of South Carolina Press; 2001.
Farrell JB. Naturopathic legislation and education. *Rhode Island Med J*. 1945;28:248–263.
Fishbein M. *The Medical Follies*. New York: Boni & Liveright; 1925.
Fishbein M. *The New Medical Follies*. New York: Boni & Liveright; 1925.
Fishbein M. *Quacks and Quackeries of the Healing Cults*. Girard, KS: Haldeman-Julius Publications; 1927.
Fishbein M. *Fads and Quackery in Healing*. New York: Covici, Friede Publishers; 1932.
Flannery MA. *John Uri Lloyd: The Great American Eclectic*. Carbondale: Southern Illinois University Press; 1998.
Goodenough J. *Dr Goodenough's Home Cures & Herbal Remedies*. New York: Crown; 1982.
Gort EH, Coburn D. Naturopathy in Canada: changing relationships to medicine, chiropractic and the state, social science and medicine. *Soc Sci Med*. 1988;26:1061–1072.
Green H. *Fit for America: Health, Fitness, Sport & American Society*. New York: Pantheon Books; 1986.
Griggs B. *Green Pharmacy*. London: Jill, Norman & Hobhouse; 1981.
Haller Jr JS. *Medical Protestants: The Eclectics in American Medicine, 1825–1939*. Carbondale: Southern Illinois University Press; 1994.
Haller Jr JS. *Kindly Medicine: Physio-Medicalism in America, 1836–1911*. Kent, OH: Kent State University Press; 1997.
Haller Jr JS. *A Profile in Alternative Medicine: The Eclectic Medical College of Cincinnati, 1845–1942*. Kent, OH: Kent State University Press; 1999.
Inglis B, West R. *Alternative Health Guide*. New York: Knopf; 1983.
International Society of Naturopathic Physicians Yearbook. Los Angeles: ISNP; 1948.
Kaufmann M. Homeopathy in America. In: Gevitz N, ed. *Other Healers: Unorthodox Medicine in America*. Baltimore: Johns Hopkins University Press; 1988.
Kirchfeld F, Boyle W. *Nature Doctors: Pioneers in Naturopathic Medicine*. Buckeye, OH: Buckeye Naturopathic Press; 1994.
Ludmerer K. *Learning to Heal*. New York: Basic Books; 1985.
Manger LN. *A History of Medicine*. New York: Marcel Dekker; 1992.
Maretzki TW. The "Kur" in West Germany. *Soc Sci Med*. 1987;24:12.
Maretzki TW, Seidler E. Biomedicine and naturopathic healing in West Germany: a historical and ethnomedical view of a stormy relationship. *Cult Med Soc*. 1985;9:383–421.
McKeown T. *The Role of Medicine: Dream, Mirage, or Nemesis?*. London: Nuffield Provincial Hospitals Trust; 1976.
Mills D. *Study of Chiropractors, Osteopaths and Naturopaths in Canada*. Ottawa, Canada: Royal Commission on Health Services; 1966.
Rogers N. *An Alternative Path: The Making and Remaking of Hahnemann Medical College and Hospital of Philadelphia*. New Brunswick, NJ: Rutgers University Press; 1998.
Rosenberg C. *The Care of Strangers: The Rise of America's Hospital System*. New York: Basic Books; 1987.
Rosengren WR. *Sociology of Medicine: Diversity, Conflict and Change*. New York: Harper & Row; 1980.
Roth J. *Health Purifiers and Their Enemies: A Study of the Natural Health Movement in the United States*. New York: Prodist; 1976.
Rothstein W. *American Physicians in the 19th century*. Baltimore: Johns Hopkins Press; 1972.
Serrentino J. *How Natural Remedies Work*. Vancouver, BC: Hartley & Marks; 1991.
Twaddle AC, Hessler RM. *A Sociology of Health*. New York: Macmillan; 1977.
Twaddle AC, Hessler RM. *A Sociology of Health*. Rev ed. New York: Macmillan; 1987.
Utah Legislative Council Staff. A study of the healing arts with particular emphasis upon naturopathy (a report to the legislature). In: Vollmer HM, Mills DL, eds. *Professionalization*. Upper Saddle River, NJ: Prentice-Hall; 1958.
Wardwell WI. *Social Strain and Social Adjustment in the Marginal Role of the Chiropractor (PhD dissertation)*. Boston: Harvard University; 1951.
Wardwell WI. A marginal professional role: the chiropractor. *Social Forces*. 1952;30:339–348.
Wardwell WI. The reduction of strain in a marginal social role. *Am J Sociol*. 1955;61:16–25.
Wardwell WI. Orthodox and unorthodox practitioners: changing relationships and the future status of chiropractors. In: Wallis R, Morley P, eds. *Marginal Medicine*. London: Peter Cohen; 1976.
Wardwell WI. The present and future role of the chiropractor. In: Haldemann S, ed. *Modern Developments in Chiropractic*. New York: Appleton; 1980:25–41.
Wardwell WI. Chiropractors: challengers of medical domination. In: Roth J, ed. *Research in the Sociology of Health Care*. Greenwich, CT: JAI Press; 1982:207–250.
Wardwell WI. Chiropractors. In: Gevitz N, ed. *Other Healers: Unorthodox Medicine in America*. Baltimore: Johns Hopkins University Press; 1988.
Whorton JC. Drugless healing in the 1920s: the therapeutic cult of sanipractic. *Pharm Hist*. 1985;28:14–25.
Wirt A. *Health & Healing*. New York: Houghton Mifflin; 1983.
Wohl S. *Medical Industrial Complex*. New York: Harmony; 1983.

NATUROPATHIC BIBLIOGRAPHY

Abbot JK. *Essentials of Medical Electricity*. Philadelphia: WB Saunders; 1915.
Altman N. *The Chiropractic Alternative: How the Chiropractic Health Care System Can Help Keep You Well*. Los Angeles: JP Tarcher; 1948.
Barber ED. *Osteopathy Complete*. Kansas City: Private; 1896.
Baruch S. *An Epitome of Hydro-Therapy*. Philadelphia: WB Saunders; 1920.
Benjamin H. *Everybody's Guide to Nature Cure*. 7th ed. London: Thorsons; 1981.
Bennet HC. *The Electro-Therapeutic Guide*. Lima, OH: National College of Electro-therapeutics; 1912.
Bilz FE. *The Natural Method of Healing*. New York: Bilz, International News; 1898.
Dejarnette MB. *Technic & Practice of Bloodless Surgery*. Nebraska City, NE: Private; 1939.
Downing CH. *Principles & Practice of Osteopathy*. Kansas City: Williams; 1923.
Filden JH. *Impaired Health (Its Cause & Cure)*. 2nd ed. Denver: Private; 1921.
Finkel H. *Health via Nature*. New York: Barness Printing & Society for Public Health Education; 1925.
Foster AL. *Foster's System of Non-Medicinal Therapy*. Chicago: National Publishing Association; 1919.
Fuller RC. *Alternative Medicine and American Religious Life*. New York: Oxford University Press; 1989.
Goetz EW. *Manual of Osteopathy*. Cincinnati: Nature's Cure; 1909.
Gottsschalk FB. *Practical Electro-Therapeutics. Hammond*. Frank Betz; 1904.
Graham RL. *Hydro-Hygiene*. New York: Thompson-Barlow; 1923.
Inglis B. *Natural Medicine*. London: William Collins; 1979.
Johnson AC. *Principles & Practice of Drugless Therapeutics*. Los Angeles: Chiropractic Education Extension Bureau; 1946.
Just A. *Return to Nature*. Lust B, trans. Butler, NJ: Lust Publications; 1922.
Kellogg JF. *Rational Hydrotherapy*. Battle Creek, MI: Modern Medical Publications; 1901:1902.
Kellogg JH. *New Dietetics*. Battle Creek, MI: Modern Medical Publications; 1923.
King FX. *Rudolf Steiner and Holistic Medicine*. York Beach, MA: Nicolas-Hays; 1987.

Kuhne L. *Neo-Naturopathy (New Science of Healing)* Lust B, trans. Butler, NJ: Lust Publications; 1918.
Lust B. *Universal Directory of Naturopathy*. Butler, NJ: Lust Publications; 1918.
MacFadden B. *Power & Beauty of Superb Womanhood*. NJ: Physical Culture Publications; 1901.
MacFadden B. *Building of Vital Power*. New York: Physical Culture Publications; 1904.
Murray CH. *Practice of Osteopathy*. Elgin, IL: Private; 1906.
Murray MT, Pizzorno JE. *Encyclopedia of Natural Medicine*. Rocklin: CA: Prima; 1998.
Pizzorno JE. *Total Wellness*. Rocklin, CA: Prima; 1996.
Richter JT. *Nature—The Healer*. Los Angeles: Private; 1949.
Spitler HR. *Basic Naturopathy*. Des Moines: ANA; 1948.
Trall RT. *Hydropathic Encyclopedia*. Vols. 1–3. New York: SR Wells; 1880.
Turner RN. *Naturopathic Medicine: Treating the Whole Person*. London: Thorsons; 1984.
Weltmer E. *Practice of Suggestive Therapeutics*. Nevada, MO: Weltmer Institute; 1913.

4

The History of Naturopathic Medicine

A New and Revisionist Perspective: The Lost Years of the 20th Century

George W. Cody, JD, MA

OUTLINE

INTRODUCTION AND AUTHOR'S NOTE

When earlier chapters on natural healing in the United States were researched and written, the available sources were primarily the extensive publications of Benedict Lust and the works of other historians. When other historians had looked extensively at naturopathy—historians such as James Whorton, Hans Baer, or (recently) Susan Cayleff—they also relied on primarily on Benedict Lust.

This has left a historical gap between Lust's death in 1945 and the 1970s when a modern naturopathic medicine emerged and began making its own historical record. But there is a hidden history of natural healing between 1945 and 1975 that this chapter will seek to explore and document—a history that is surprisingly rich and deep.

Discovering and documenting the history of this period had to wait until resources became available for research. Five new resources that became available in the past 10 to 15 years are at the heart of this new chapter. All of these resources have become available—accessible for research—due to modern technology.

The first is the library resources of the modern naturopathic colleges, primarily Bastyr University and what is today the National University of Natural Medicine (NUNM). These resources, though, have largely not been digitized and required old-fashioned "shoe leather" research.

This work is greatly indebted to the "shoe leather" of Eric Blake, ND, of Portland, Oregon (and to Mitchell Stargrove, ND, also of the Portland area, who introduced this author to Eric's work). Eric had spent many hours about 15 years ago researching the stacks of material in the NUNM "rare book" room with an eye to what Benedict Lust either did not tell us or any off-track items that were buried in Lust's publications. This led to many hours of conversation and correspondence between this author and Eric that led down the path of a new historical synthesis.

Second, the John Bastyr archives at Bastyr University then opened up material that Dr. Bastyr had saved from this era that had been in boxes, unexplored. Chief among these was a collection (not complete) of the publications of the Western American Naturopathic Association (ANA)/American Naturopathic Physicians and Surgeons Association/ American Association of Naturopathic Physicians from 1947 to 1954. Dr. Bastyr was a protégé of Dr. Robert V. Carroll Sr., who had brought Bastyr into the world and practice of naturopathy, as these records documented. (Thanks are owed to the staff of the library at Bastyr University, especially Jane Saxton and Linda Tally.)

Third is the archive materials at the W.A. Budden Library of the University of Western States (UWS) in Portland, Oregon. The UWS is the modern successor to Dr. Budden's Western States College where Dr. Budden was president from 1929 to 1954. What was not already digitized and accessible on the UWS website was scanned and made available to this author by the archive librarian, Katie Lockwood. She also deserves a large credit for advancing this historical work product.

Fourth is two resources on the history of the UWS and of chiropractic: the history of the UWS by Lester Lamm, DC, and the history of chiropractic education by Keating, Rehm, and Callender, which includes the detailed minutes of the Council on Education of National Chiropractic Association, within which Dr. Budden participated, operated, and influenced.[1]

Fifth, and finally, a massive amount of newspaper archive material has been digitized and made available. Coupled with search-engine capacities, a completely buried panorama of natural healing history has gone through a supernova process. Armed with these voluminous materials, the questions "Who were the naturopaths?" and "What happened to them?" can at last start to be answered.

Editors' Note: This is the second of two chapters documenting the origins and evolution of naturopathic medicine. Although these were written to stand alone, a full understanding requires reading both chapters.

PART ONE: WHO WERE THE NATUROPATHS?

Natural healing advanced a science-based alternative to the American medical profession of the 20th century; this alternative philosophy started first as naturopathy as promulgated by Benedict Lust, and then its concepts and practice evolved into naturopathic medicine primarily through the work of three early pioneers: Henry Lindlahr, William C. Schulze, and Walter B. Cannon, all medical doctors disenchanted by conventional medicine moving in what they considered the wrong direction. By the early 1950s, natural healing—an alternative to conventional medicine practiced by chiropractors and naturopaths—reached its peak. Practitioners were spread across the United States, and their common philosophy was based on a belief in the *vital force*—the inherent healing power within all of us. The leadership of W.A. Budden and Robert V. Carroll was critical to the professional growth of natural healing from the mid-1930s to the early 1950s. They led a professionalization movement within natural healing that brought these practitioners to the peak that was reached in the post-WWII United States.

IN THE BEGINNING

In the early 20th century there was medicine as practiced by medical doctors ("MDs") and as represented in the United States by the American Medical Association (AMA). The AMA became, over the first 75 years of the 20th century, one of the most powerful political interest groups in US history and became known as "organized medicine."[2]

There were alternatives that emerged early in the 20th century, primarily in the form of chiropractic, naturopathy, and other "drugless' schools of healing as well as in the work of MDs who diverged from the AMA's concept of "scientific medicine." Scientific medicine was based on Louis Pasteur's "germ theory."

Germ Theory and Conventional Medicine

At the turn of the 20th century, as Howard Berliner (1985) has documented, straight germ theory (germ *x* causes disease *y*) became established as the core concept of "scientific medicine" in American allopathic medical education. At the time, the major rivalry in American medicine was between allopaths (about 60% of medical practitioners) and homeopaths (about 30%, the balance being Eclectics and physio-medicalists).

Once the substantial resources of the Rockefeller philanthropies were put behind scientific medical education based on germ theory—with the full consent of the AMA—American medicine became synonymous with the germ theory. This, the Flexner Report (1910), and medical research devoted to finding pharmaceuticals to defeat germs to "cure" disease were all funded by Rockefeller philanthropies and led to what is considered to be the dominant paradigm in American medicine.[3]

As Berliner documents, this was largely the result of a determination to put the Rockefeller philanthropic resources behind this concept of "scientific medicine" not, at the time, any clear clinical superiority on the part of the allopathic philosophy. As of the period in which this took place, largely 1900 to 1920, there was no clinical validation for scientific medicine in terms of the discovery of specific pharmaceuticals of demonstrated efficacy.

Be'champ, Bernard, and the Alternatives to Germ Theory

The concept of an alternative to the germ theory of disease traces to two French contemporaries of Louis Pasteur: Augustine Be'champ and Claude Bernard. Pasteur's work asserting microbes as the cause of disease has been well documented and lauded and does not need to be reproduced here.

Be'champ's theory was, put as simply as possible in the biological sense, that "germs" are always present in our environment and do not "cause" disease. Disease is related, rather, to the physiology of the host, the human (or mammalian) body, not to the germs per se. What we observe in microbiology relative to disease is the resultant by-product of the body's failed attempt to reject a pathogenic microbe, a function that a healthy body's autoimmune system should accomplish.[4]

Bernard's work in physiology was much celebrated in the 19th century. As noted by Charles Gross (1998): "Today the fame of Claude Bernard rests primarily (if not entirely) on his idea that the maintenance of the stability of the internal environment *(milieu interieur)* is a prerequisite for the development of a complex nervous system." But as noted by Gross and others, although Bernard advanced this idea between 1854 and his death in 1874, it "had no impact for over 50 years after its formulation." Why did this "insight that the 'constancy of the internal environment is the condition for the free life' (have) no significance (indeed no meaning) for biologists for more than 50 years?" One major reason was that "Pasteur's new bacteriology and its omnipresent, omnipotent germs, were dominating the biomedical Zeitgeist."[5]

The Early Integrators

In the pre-WWII years, the work of three MDs that diverged from germ-theory orthodoxy became central to the emergence of integrative medicine. These three MDs are Henry Lindlahr, William Charles Schulze, and Walter B. Cannon.

Henry Lindlahr and Integration in Clinical Practice

The standard biography of Lindlahr is that of Kirchfeld and Boyle in *Nature Doctors.*[6] His own therapeutic philosophy is set out in his four-volume work *Natural Therapeutics*, published in 1923 and republished in 1975 as edited by Jocelyn Proby, DO.[7] The four volumes are *Philosophy, Practice, Dietetics,* and *Iridiagnosis.*

Lindlahr started his own school in Chicago in 1914 and built a substantial sanitarium, clinic, and college operation as well.[8] A central tenet of Lindlahr's work, in spite of his medical training, was that the allopathic approach to healing was wrong. There were, he said, "two principal methods of treating disease. One is the combative, the other the preventive… The slogan of modern medical science is, 'Kill the germ and cure the disease'… The combative method fights disease with disease, poison with poison, and germs with germs."[9]

On the other hand, "The preventive method does not wait until disease is fully developed and gained ascendancy in the body, but concentrates its best endeavors on preventing, by hygienic living and natural methods of treatment, the development of disease."[10]

In *Philosophy*, he states: "It is the intent of this volume to warn against the exploitation of destructive combative methods to the neglect of preventive constructive and conservative methods. If these teachings contribute something toward this end they will have fulfilled their mission."

His work is consistent with Walter Cannon's physiological insights, and a decade before Cannon's *Wisdom of the Body* was published, he wrote in *Philosophy:*

> *The diet expert, the hydrotherapist, the physical culturist, the adjuster of the spine, the mental healer and the Christian Scientist, pay little attention to the pathological conditions or the symptoms of disease. Each of these, in accordance with his theory of disease and cure, regulates the diet and habits of living on a natural basis, promotes elimination, teaches correct breathing and wholesome exercise, corrects the mechanical lesions of the body, or establishes the right mental and emotional attitude, and, in so far as he succeeds in doing this, builds health and so diminishes the possibility of disease. The successful doctor of the future will have to fall in line with the procession and do more teaching than prescribing.*[11]

Lindlahr strongly advocated for Be'champ's theories, especially in his *Philosophy* volume. He had discovered Be'champ's work, he said, through the earliest work of Ethel Douglas Hume.[12] Specifically, Lindlahr noted that he had then "made a careful study of [Be'champ's] last work, entitled *The Blood,* in which he summarizes the mycrozymian theory of cell life." From this study, Lindlahr found "a rational, scientific explanation of the origin, growth and life activities of germs and of the normal living cells of vegetable, animal and human bodies."[13] At two points in *Philosophy,* Lindlahr discusses at length the understanding of Be'champ's work that he has gained from his own intense study of Be'champ's writings and how from his own clinical observations and experience, and from Be'champ's work, he comes to advance three primary manifestations of disease. These manifestations of disease are (1) lowered vitality (vitality is the body's strength of positive resistance and recuperative power, the "vital force" of cellular function); (2) abnormal composition of blood and lymph; and (3) accumulation of waste, morbid matter, and poisons in the system.[14]

William Charles Schulze and Integration in Medical Education

Schulze was an MD (of Rush Medical College) who, in 1914, purchased the National School of Chiropractic and broadened its curriculum to include the basic sciences as well as "physiological therapeutics" and mechano-therapy.[15] The name was changed in 1920 to the National College of Chiropractic (NCC). His most enduring influence may have come from acquiring and merging Lindlahr's school after Lindlahr's untimely passing in 1924 and in his mentoring influences on W.A. (Alfred) Budden, DC, ND, of Portland's Western States College and, later on, Joseph Janse, DC, ND, the postwar president of National College (1945–1983).[16]

As Keating and Rehm noted about Schulze:

> *Part of the Schulze legacy is the tradition of broad-scope, "rational chiropractic," or what Palmer called "mixing." As an MD, Schulze had been trained in medical and presumably some minor surgical procedures, but he had apparently committed himself to "drugless healing" early in his career. However, drugless healing, which involved a variety of naturopathic methods, was anathema to the Palmer branch of the profession. The physician-chiropractor would quickly run afoul of the adiagnostic, nontherapeutic, subluxation-only forces in the profession.*[17]

Schulze sought "to promote a professionalism among students and doctors which could rival that of medical competitors," and under Schulze, the NCC introduced laboratory courses in pathology, biochemistry, bacteriology, and toxicology, together with a "strong commitment to diagnostic training," all in response to the adoption in the late 1920s of the Basic Science Law. The NCC adopted the motto "four ways to beat the basic science law": (1) Study basic science. (2) Study basic science. (3) Study basic science. (4) Study basic science. Schulze came under much criticism from the "straights" in chiropractic, and this only increased after he purchased the Lindlahr School of Natural Therapeutics from Henry Lindlahr's estate and transferred "the entire student body and the better part of the faculty" to the NCC.[18] In 1928 this part of the National school was formed into the National College of Drugless Physicians, which was National's ND degree program.

The *National College of Chiropractic Journal* became a voice that extended beyond the NCC itself by the early 1920s, becoming a professional voice as well, challenging B.J. Palmer's "straight" chiropractic philosophy as well as the antagonism of Morris Fishbein, MD, the editor of the *Journal of the American Medical Association (JAMA).* This voice was first found under the NCC secretary, A.J. Forster, MD, DC, and then "under the editorship of William Alfred Budden, DC, ND, an English immigrant and former economics instructor at the University of Alberta who graduated from the NCC in 1923."[19]

From the mid-1920s, Schulze was involved more in the professional activities than in the day-to-day operations of the NCC, and always in the "mixer" camp—first in the American Chiropractic Association (ACA) and then starting in 1930 in the successor National Chiropractic (NCA). Schulze then followed a busy, nationwide speaking schedule in the early 1930s, advocating consistently for the broad-scope professional values of the NCA as taught at the NCC/NCDP. At the 1934 annual meeting of the NCA (May 1934), Schulze—a regular speaker at these annual meetings—noted in his speech that at the time, "harmony among Chiropractors and Drugless practitioners, especially the Naturopaths, was good to look upon."[20]

In 1934 Dr. Schulze joined a convention tour coined "the Northwest Circuit" organized by C.O. Watkins, DC, of Montana that had its speakers speak at NCA-affiliated conventions held in Minnesota, North Dakota, Montana, Washington, British Columbia, Idaho, Utah, Wyoming, and Colorado. The Washington stop was for the meeting of the Northwest Chiropractors Association and was a gathering of 300 attendees from Oregon, Washington, Idaho, Northern California, and British Columbia in September.[21]

The last full year that Dr. Schulze was actively engaged was 1934; he was in ill health for much of 1935 and passed away in September 1936. When he passed away, one of the very many appreciation letters honoring his productive life was sent by Dr. Robert Carrol as the president of the Washington State Naturopathic Association, saying, "The entire drugless profession has lost a friend and teacher."[22]

In 2002 Rehm and Keating noted as Dr. Schulze's great accomplishment, "Schulze created an intellectual environment that would be

rivalled in the middle age of the profession only at Budden's Western States College."[23]

Walter B. Cannon and Integration in Medical Research

Cannon did extensive research in physiology, his area of teaching at Harvard. He concurred with Bernard's concept of the "internal environment" of the human body and coined the term *homeostasis* to describe the body's need to respond physiologically to the external environment to maintain a stable internal environment, which he described as a primary function of the central nervous system.[24] His concept was that through what he called the "wisdom of the body," mammalian forms such as the human body "may be confronted by dangerous conditions in the outer world and by equally dangerous possibilities within the body, and yet continue to live and carry their functions with relatively little disturbance," something that Hippocrates had called *vis medicatrix naturae.*

In a distinctive passage in *The Wisdom of the Body,* Cannon set forth a concept that became central to the postwar chiropractor-naturopaths:

> *The fathers of medicine made use of an expression, "the healing forces of nature," the vis medicatrix naturae. It indicates, of course, recognition of the fact that processes of repair after injury, and of restoration to health after disease, go on quite independent of any treatment that the physician may give …*
>
> *In the first place, the well-trained physician is acquainted with the possibilities and limitations of self-regulation and self-repair in the body. He is instructed in that knowledge and employs it not only for his own intelligent action but also as a means of encouragement for the patient who looks to him for counsel …*
>
> *Again, the physician realizes better than the layman that many of the remarkable capacities of the organism for self-adjustment require time—all of the processes of repair belong in that class—and that they can play an important role in restoring the organism to efficiency only if they are given the chance that time provides …*
>
> *Furthermore the physician realizes that he has at his command therapeutic with which he can support or replace the physiological self-righting or self-protective processes we have been considering …*
>
> *Finally a great service which the physician renders is the bringing of hope and good cheer to his patients. He has seen at work in many cases the restorative processes of the organism … When we are afflicted and our bodily resources seem low, we should think of these powers of protection and healing which are ready to work for the bodily welfare.*[25]

Lindlahr was the greatest *direct* influence on the post-WWII philosophy of natural therapeutics, the central core of "drugless" or "nonmedical" philosophy. Lindlahr's and Cannon's work have a remarkable consistency between them, although Lindlahr was largely influenced by Be'champ and Cannon by Bernard. Together they advanced the work of these two 19th-century French scientists into the 20th century, and in doing so, they advanced a scientific basis for an "alternative" to the germ theory that was at the core of conventional "scientific medicine." Schulze pioneered the education of physicians in a professional "drugless" therapeutics consistent with the theoretical work of Lindlahr and Cannon.

THE EARLY 20TH CENTURY

The shaping of America's modern healthcare landscape began in what historians call the Progressive Era—roughly 1900 through the end of World War II in 1919. At the turn of the 20th century, the AMA had begun the elevation of allopaths over homeopaths and Eclectic physicians—all MDs—and had completed this process by the end of the Progressive Era, aided by the 1910 Flexner Report and the efforts of the Rockefeller and Carnegie Foundations (see previous discussion). By the early 1920s, what had become the AMA's monopoly structure within the medical profession was in place.[26]

Drugless Healing

But as homeopaths and eclectics disappeared, a wide range of practitioners known as "drugless healers" emerged. In this period the licensing structure that became a critical part of the AMA's monopoly structure had not been fully adopted across the United States, and these drugless healers, probably several thousand of them, were to be found in practice. The practitioners of manipulation modalities—including osteopaths and chiropractors, but others as well, such as mechanotherapists and naprapaths—were "drugless." So were myriad others, including practitioners of neuropathy, physcultopathy (physical culture), sanipractic, food science, suggestive therapeutics, and Swedish movement. Some of these drugless healing practitioners were specifically regional; naprapaths were almost exclusively found in Illinois, where D.J. Palmer protégé Simon Oakley had founded his school, and sanipractors were originally exclusive to the state of Washington before advancing their presence into British Columbia in Canada.[27]

Benedict Lust

In 1902 Lust originated his use of the term *naturopathy* and began his development of a theory and philosophy of health and healing "to describe the eclectic compilation of doctrines of natural healing that he envisioned was to be the future of natural medicine." Lust launched his career as the progenitor of naturopathy, adopting that name for his eclectic brand of natural therapeutics and placing the term *naturopath* firmly in the title of his monthly publications, which continued under his status as editor and publisher until his death in 1945.[28] One of the anomalies of Lust's work was that for at least 30 years, there was no firm definition of *naturopathy*; rather, Lust clearly attempted to incorporate all methods of "drugless healing" and "natural therapeutics" into his philosophy of naturopathy. This included the original concept of osteopathy devised by Dr. Still and chiropractic as devised by D.D. Palmer. In Lust's view, these were all pieces of naturopathy, linked together by not being "allopathic medicine."[29]

This "drugless" label could only incorporate Still's osteopathy in its original form, which did not incorporate a *materia medica,* as described, for instance, in Charles Hazzard's *Principles of Osteopathy* (3rd edition, 1899).[30] Where chiropractic was concerned, Lust's naturopathy became clearly allied with the "mixer" philosophy, and both Lust and the mixers were in conflict at the time with the "straights" led by B.J. Palmer.[31]

Naturopathy and Chiropractic

Precisely when chiropractic and naturopathy first became melded into a symbiotic relationship is historically murky. D.D. Palmer did not—at least as historically reported—practice or teach naturopathy or openly associate himself with it. His son, B.J., was adamantly opposed to anything that would dilute the purity of "straight" chiropractic. Benedict Lust, the historical progenitor of naturopathy in America, taught and endorsed chiropractic very early in the 20th century, but it was Solon Langworthy, an early student of D.D.'s, who opened the second identifiable school of chiropractic in 1903 and based its curriculum on mixing nature cure with chiropractic.

Palmer was the originator of chiropractic, of course, but he adopted a kind of "Johnny Appleseed" approach to his spinal manipulation insights, "planting" the concept of chiropractic adjustment more than

anything else. He was traveling constantly after 1902 and granting the right to practice and to educate others in his methods to recipients of his written "diplomas."

It was B.J. Palmer, the son, who adopted a proprietary interest in chiropractic after his graduation from D.D.'s instruction in 1902, much as Lust did in naturopathy. Both chiropractic and naturopathy could best be described as social movements in the field of health and healing in their first two decades of evolution. It was not until the 1920s that others began to work at the professionalization of chiropractic and naturopathy, and many of the most influential of these who became connected with naturopathy were chiropractic "mixers," becoming known in time as the chiropractor-naturopaths or "DC, NDs."

Nature Cure and the Vital Force

Benedict Lust's vision of drugless healing, although it continued to "expand," as noted by Susan Cayleff, was always intended to be consistent with Germanic 19th-century "nature cure." As noted by Henry Lindlahr is his 1915 book *Nature Cure,* the original concepts were credited to Vincent Preissnitz.[32] As noted by Lindlahr, nature cure became "the idea of drugless healing [which] spread over Germany and over the civilized world."[33]

Citing Lindlahr, Susan Cayleff summarized American nature cure's idea of human sickness this way:

> *Henry Lindlahr, MD, a leader in naturopathic philosophy, explained the five specific conditions that caused disease; lowered vitality; abnormal composition of blood and lymph, resulting mainly from wrong eating and drinking; accumulation of waste, producing morbid matter and poison in one's system; mechanical lesions, that is pressure, tension or strain on nerves and nerve centers caused by luxations (dislocations) of bony structures or straining of muscles and ligaments; and discordant or destructive mental and emotional attitude. These conditions more or less remained the core of naturopathy for decades.*[34]

Treatment by means of nature cure theory relied on the body's own drive to maintain health—to achieve what Walter Cannon later called "homeostasis"—by recognition of what was labeled the "vital force." The work of F.E. Bilz, a German MD, was very influential in this regard. Bilz first published his synthesis of German nature cure in Germany in 1898, and in 1901 he published *Natural Method of Healing: A Complete Guide to Health (translated from the latest German edition)* as the English language version of his work.[35]

Bilz noted: "[I]t is known that we cannot heal a disease with the remedy we apply, but that it is the vital force within us which heals, and that we need but aid it, [and] our position becomes a far easier one."[36] This "vital power," the "power of healing," Bilz said, "resides in man himself … divine nature placed it there at the creation of each being.'' Adopting this concept, Lindlahr noted that all healing must "economize vital force" because it is the vital force that "is the Supreme power and intelligence, acting in and through every atom, molecule and cell in the human body which is the true healer, the *vis medicatrix nature* which always endeavors to repair, to heal and to restore …"[37]

GENESIS OF POST–WWI PROFESSIONALISM

To understand the DC, NDs and their professionalization requires going beyond the career of Benedict Lust and the natural living and healing movement that he founded. It also requires more historical background.

The committed professionalization process that followed began in the late 1920s and continued through the first years after WWII. The focus was on moving the educational process and the clinical practice of both naturopathy and chiropractic past the "founder's grip" of Benedict Lust and B.J. Palmer by means of the creation of stable residential colleges and stable state and national professional organizations.

This task was compounded with regard to both of these professions by the committed drive by organized medicine in the United States (primarily in the form of the AMA and its state and local constituencies) toward medical dominance. To respond to the determination of medicine to achieve this dominance, a resistance based on the core values of "Americanism" was required, along with personal resilience and tenacity.

A Short Course in Medical Dominance

Medical dominance is best understood by reference to the book of this same name by Australian sociologist Evan Willis.[38] The subject can be supplemented by a very useful work by another sociologist, Saul Rosenthal, *A Sociology of Chiropractic.*[39]

These sociologists argue that organized medicine has had as a goal since at least 1900 the achievement of medical dominance in domains: achievement of complete control over its own work (autonomy), achievement of complete control over the work of others in health care (dominion), and achievement of complete control over all matters of public policy within the health domain (medical sovereignty).

Willis's argument is sophisticated and extensive; indeed, his discussion of the subject is a book-length treatise. But the short version as it relates to the "exclusion" of "alternative" practices like chiropractic and naturopathy can be summarized. Relying on earlier work by Howard Berliner and others, he demonstrates that medicine's dominance was achieved through the allopathic claiming of the mantle of "science" for its work. This was done through the adoption of the germ theory of disease.[40]

This, in turn, had two advantages, as Willis argues: first, individual clinical skill became less important than extensive schooling within a laboratory and hospital-based system ("clinical skill" versus "clinical science"), and second, health became an individual scientific problem, not a social, environmental or lifestyle problem.

The "Great Trade"

In the United States, this manifested itself in the early 20th century as "the Great Trade" described by Fredric Wolinsky: "[B]y 1925, the AMA had gained a monopoly over the production and licensing of physicians. This included the power to determine what the curriculum should be, how many students should be admitted, which students should be admitted, and how many faculty there should be for each student. Thus 1910 marked a trade of importance between society (as represented by state and federal governments) and the AMA. The trade gave the AMA the exclusive right and sole power to regulate the medical profession. In return, the AMA was to give society the best and most efficient medical care system possible. Society has clearly lived up to its part of the bargain …"[41]

American Exceptionalism

"American exceptionalism" or "Americanism" has been analyzed extensively in the book of the same name by Professor Seymour Martin Lipset, one of the most distinguished US academicians. Professor Lipset gives this synopsis of Americanism: "The American Creed can be described in five terms: liberty, egalitarianism, individualism, populism and *laissez-faire.*"[42]

Medical dominance strikes at each of these five values, all in the name of "scientific medicine." It is based on using the power of the state to enforce the "great trade" as public policy, the antithesis of populism. It is corporatist, not individualist and *laissez-faire.* It creates a

favored class of medical professionals over serving egalitarianism, and by the exercise of the power of the state, it constrains the liberty of the patient as a consumer.

A Time to Build a Profession: The 1930s

The drugless healing concepts of nature cure became, by the 1930s, the philosophical basis for a professional alternative to conventional medicine in the form of the chiropractor-naturopaths, the "DC, NDs." By the mid-1930s, as Susan Cayleff notes, Benedict Lust came to abandon "therapeutic inclusivity" and declared that a clear and fixed professional identity was necessary.[43] For some others who had already formed a professional identity and founded schools and colleges, this moment of self-reckoning came not a moment too soon. It was time to bring all of this into focus as a professional identity once and for all.

The Move Toward Professionalization

By the 1930s, the concept of "drugless healing" began to change, and significantly. The majority within osteopathy was moving to add *materia medica* to osteopathic manipulation and prescribing in line with allopathic thinking and the germ theory.[44] The majority within chiropractic were "mixers," perhaps 70% of chiropractors.[45] And within naturopathy, the amalgamation period of the previous 30 years was giving way to identification as naturopaths and abandonment of other drugless labels.[46]

In the case of osteopathy, the conflict between the originalists and the modernists played out within the American Osteopathic Association (AOA) in the form of battles over "standards" applicable to the osteopathic colleges. Among chiropractors, two professional associations emerged by the early 1930s, the National Chiropractic Association (NCA), which was the association for the "mixers" within chiropractic, and the B.J. Palmer–led Chiropractic Health Bureau, which in 1941 became the International Chiropractic Association—the organization of the "straights."

From the AMA's perspective, the "straights" were the most easily labeled as a "healing cult." The straights stood politically for 18-month schooling directed toward identifying "subluxations" of the spine and resolution by manual spinal adjustment as the treatment for all human ailments. A high school education was considered sufficient as a prerequisite for a "straight" chiropractic education.

The NCA had adopted a 4-year residency education requirement by the end of the 1930s, led by the Metropolitan Chiropractic College, Western States College (WSC or Western States) and the National Chiropractic College. A leader in consistently upgrading educational standards within the NCA was W.A. Budden of Western States.

Within naturopathy, the transition in the 1930s was more complex. As pointed out by Susan Cayleff, by 1935 Benedict Lust moved away from considering all drugless healers part of naturopathy, regardless of how they identified themselves. After three decades, he instead declared that all naturopaths needed to identify as such, encouraging all remaining drugless healers to openly join the naturopathic movement.[47] Kirchfeld and Boyle assert that Lust had gone beyond the establishment and popularization of the American naturopathic movement by the end of the 1920s and "must be credited with four other accomplishments." These were founding the American School of Naturopathy, founding the ANA, his publications, and "the legal status of naturopathy attained as a result of his efforts," which they call "the most tangible of his efforts." They note that "it is difficult to separate the success of Lust's organizations from that of his publications."[48]

By the early 1930s, the success of Lust's efforts must be deemed "qualified."

A 1927 survey by a committee of the AMA of all "schools of chiropractic and naturopathy" found Lust's combined American School of Chiropractic and American School of Naturopathy to be a night school program operating classes for 3 hours each weeknight for a 4-year, 9-months-per-year course. No catalog was published due to expense, but the school was described in Lust's monthly publications. The school as of 1927 and forward was not established to function in the era of the Basic Science Law, and moreover, neither chiropractic nor naturopathy was licensed in New York. In 1935 Lust was found guilty of illegally (under New York law) issuing diplomas awarding a "doctor" degree without a lawful state charter.

It is true that Lust's ongoing influence was from his publications and from his continued popularization of naturopathy through his travels and his speeches. Through this popularization, others were able to obtain legal status for naturopaths in several states, but this was largely accomplished by others in the movement. Lust's own operations were in New York (school and publications), New Jersey (his first Yungborn Sanitarium) and Florida (second Yungborn Sanitarium). Of these states, only Florida granted naturopaths recognized legal status, and this was accomplished by others in the movement.

By the 1930s, an alternate vision formed within the ANS and within some of the NCA schools that offered ND degrees in addition to DC degrees. As Lust's influence declined during WWII and after his passing in 1945, others came forward with a competing vision of the relationship between chiropractic and naturopathy, of naturopathic education, and of nonmedical clinical practice. How this came about is a piece of history that has not been well documented before. The story centers on a few men and women, the most prominent of whom are W.A. (Alfred) Budden of Western States College, with its Schools of Chiropractic and Naturopathy, and Robert V. Carroll of the ANA.

Dr. Budden and Evolution of the Profession in the Pacific Northwest

Budden's Early Career and Arrival in Oregon

W.A. Budden, DC, ND, educated at Schulze's National College and later a Pacific Northwest (NW) transplant, was a leader in the professional development movement of drugless physicians. Over time, Budden acquired several staunch allies in this effort.

The alliance of DCs and NDs in the Pacific NW began through the efforts of Dr. Budden and took root during the remarkable Oregon ballot campaign of 1934. This alliance continued to grow in the aftermath of the ballot fight, as Dr. Budden lived by what he considered to be the lessons of this formative campaign.

This ballot campaign of 1934 was Dr. Budden's brainchild. Budden himself had come west from Chicago to Portland, Oregon, steeped in a "mixer's" amalgam of core chiropractic, physiotherapy, and Lindlahr's natural therapeutics. Budden attended Schulze's National College from 1922 to 1924. Upon graduation as a DC, he joined the faculty and in 1925 succeeded the college's previous dean and school journal editor.[49]

It was at this time that National purchased and absorbed Lindlahr's College of Natural Therapeutics, the premier drugless school of the time. Budden was integrally involved in the integration of the Lindlahr programs into national as the college's ND degree program. As an educator and administrator at National through the 1920s, Budden also authored a textbook for use at National: *Physiotherapy: Technique and Treatment.*[50] In 1929 Budden moved to Portland, and his career as an educator began in earnest.

When Budden arrived in Portland in 1928, he purchased the Pacific Chiropractic College for cash; in 1933 the Pacific College was reincorporated and renamed the Western States College. Western States' core mission was "for the purpose of operating a college that would offer

DC and ND degrees together with training nurses and health technicians and maintain clinics and hospitals."[51]

When he arrived, Budden also networked with both Oregon's chiropractors and naturopaths. Chiropractic had been licensed first in Oregon in 1915; licensure for naturopathy was more recently established, in 1927.[52] He spoke at the Oregon Chiropractic Association's 22nd Annual Meeting in July of 1929, and then in July of 1930, he spoke at the annual meetings of both the Oregon Chiropractic Association and the Oregon Naturopathic Association. These speaking appearances became a tradition carried out many times with each group over the years until his death in 1954.[53]

Oregon adopted a Basic Science Law in 1933.[54] Dr. Budden's initial view was that the Basic Science Law was the medical profession's creation intended specifically to blunt the rise of any competition to MDs, specifically that of DOs, DCs, and NDs.

But Budden's first effort was to try to craft a political response to a political problem. As Budden later related the history of this effort, "October, 1933, saw the formulation of a joint legislative committee to manage the drafting of and the campaign for an amendment to the constitution of Oregon regulating the practice of the healing arts." The Joint Legislative Committee (JLC) was the joint committee of the Oregon Association of Chiropractic Physicians and the Oregon Naturopathic Association, and as Dr. Budden described, it was "composed of an equal number of chiropractors and naturopaths."[55]

The first action of the JLC was to work with legal counsel to draft what became known as the "Healing Arts Amendment," a proposed amendment to the Oregon Constitution by citizen's initiative.[56] As the news report in *The Oregonian,* Portland's and Oregon's largest newspaper, reported, "petitions for this measure contained approximately 47,000 signatures against the 26,667 required by law. The completed petitions were brought to Salem [Oregon's capital] by a caravan of 14 automobiles."[57]

On Election Day, after an exhaustive 6-month effort, the ballot measure went down by a 3-to-1 margin. This attempt to curtail medical dominance in Oregon was, on the surface, not successful. Nonetheless, Dr. Budden, over time, took heart from what he considered to be the "lessons learned" from the campaign.[58] Moreover, he gained some considerable respect for his willingness to engage in politics and the tenacity with which he could wage a political campaign even with limited resources, and this would be to his advantage over the years.[59]

These were lessons that he seemed to take to heart and that animated him for the next 20 years of his career as an educator and a consummate professional. From these "lessons learned," Dr. Budden committed himself to several things. He accepted the basic sciences as a necessary part of a "nonmedical" physician's education, although he continued to argue that professional examinations were best given to candidates by each profession's licensing board. He actually, in time, came to view the Oregon examinations as quite fairly conducted, and his students gained a high passing rate as the curriculum focused on these subjects as part of the core education in both chiropractic and naturopathy.[60]

He continued an extensive public-speaking schedule, regularly appearing through the 1930s, the war years, and in the postwar era before lay audiences like the HEL and the Biochemistry League as well as holding speaking events at Western States and giving weekly radio talks.

He continued at all times to commit his WSC programs to provide a sound and thorough education to his students in both the School of Chiropractic and the School of Naturopathy. He worked tirelessly to improve the standards for all similar colleges and took the lead at every turn in increasing the coursework and prerequisites required.[61]

These alliances and friendships were important to the WSC and to professional development in the Pacific NW. Dr. Budden, and his DC, ND allies, built stability into the education and organization of chiropractic and naturopathy, especially in the western United States and the Pacific NW, but much of their work is forgotten today.

One of these alliances will be discussed here as part of Dr. Budden's efforts to secure strength and stability for both chiropractic and naturopathy in the Pacific NW and at WSC: that with Robert Carroll, DO, ND, of Seattle.

Robert V. Carroll, ND—Leader of NDs in Washington

One of the strongest leaders and professional organizers in his own right was Robert V. Carroll Sr., DO, ND. Dr. Carroll had built the naturopathic profession in Washington. Licensing in Washington was granted under the Drugless Healing Act of 1919. Licenses were actually issued for the practice of sanipractic, a drugless school unique to Washington.[62] But Carroll, although his license said "Sanipractic," was determined to build a distinct identity professionally and allied early with Benedict Lust's ANA.[63]

Robert V. Carroll (Sr.) was also educated in Chicago after WW I, graduating from the American College of Osteopathy and the Lindlahr College of Natural Therapeutics (both Lindlahr schools) in 1923. Carroll finished his studies with Lindlahr and his school faculties just a year before Lindlahr's sudden death from an infection in late March of 1924. Lindlahr's signature is on Carroll's Doctor of Natural Therapeutics diploma,[64] and Carroll spoke often in later years of having been a student of Lindlahr himself.

Carroll moved west to Washington right after graduation, stopping for a year to practice with his brother, O.G., in Spokane before moving on to Seattle.[65] By 1930 he had begun the process of professional organization in Washington, founding the Washington Association of Drugless Physicians and serving as its president for 8 years, with the organization's name changing to the Washington State Naturopathic Association in 1934.[66]

The Beginning of the Budden–Carroll Alliance

The complete origin of Carroll's association with Dr. Budden and his support for Western States is unclear. Both Drs. Carroll and Budden studied in Chicago at about the same time. Dr. Budden made an alliance with the DCs and NDs in Oregon in the early 1930s and as a leader in the drugless professions in Washington. Dr. Carroll had his own connections with the Oregon NDs. Yet still, any connection that they had before the 1934 Ballot Campaign is not yet documented.

But in the immediate aftermath of the campaign, they joined forces, as reported by Benedict Lust. In February 1935, the same month that the *Chiropractic Journal* published Dr. Budden's "Medical Propaganda" article detailing B.J. Palmer's efforts to defeat the Oregon Ballot Campaign, Lust used his "Dr. Lust Speaking" platform in the *Naturopath and Herald of Health*[67] to similarly interfere in professional efforts in the Pacific Northwest (subtitled "Schisms"):

> *Word has come to us of a meeting that was called for Portland, Ore., in December last for the purpose of the "unification and coordination of Naturopathy and Chiropractic." This went out on the letterhead of the Washington Naturopathic Association with headquarters in Seattle and was signed by the president, Dr. Robert V. Carroll.*

Another letter from the Western States College of Portland signed by Dr. A. Budden, calls for a meeting to be held in Seattle on January 12th to put the "finishing touches" on an organization to be known as the International non-Medical Alliance.

Let us say right here and now that we are against any alliance between Naturopaths and Chiropractors. (Naturopath and Herald of Health, February 1935, p. 34)

Lust went on to say that alliances with the chiropractors in California and elsewhere had helped chiropractors and hurt naturopaths and that furthermore, there should only be the ANA to speak for naturopaths and no other associations or groups that purport to represent naturopaths.

"We have no fault to find with Dr. Carroll or Dr. Budden. We are however utterly opposed to the formation of other organizations that would usurp the prerogatives and program of the A.N.A. This organization has stood the test of time."

NATURAL HEALING AT ITS PEAK

In March 1952 Henry J. Schlichting Jr., ND, appeared before the 42nd annual convention of the Oregon Association of Naturopathic Physicians in Portland, Oregon. At the time, Schlichting was the president of the American Naturopathic Physicians and Surgeons Association (ANPSA).[68] Schlichting was quoted as saying that two major national issues facing naturopaths were that alternative schools got no tax support and that naturopaths could not be admitted to tax-supported hospitals. This created "a heavy demand on our profession and the lay public to meet rising … costs."

Schlichting was reported to have told the convention, "Despite these problems the profession 'is making definite progress on a national scale as evidenced by licensing in over 20 states,' [and] insurance companies are recognizing naturopaths 'because they are getting satisfactory results.'"[69]

Although the "licensing in over 20 states" was a generous count, there was no question that at the time Schlichting was speaking, the natural healing alternative was at its peak. Schlichting was from Midlands, Texas, a West Texas city where all major oil companies had a presence in a state where there were almost 500 licensed naturopaths.[70] Specific licensing of naturopathy was in place in 8 states (out of 48); 2 states had naturopaths practicing under drugless licensing, and about a dozen other states had broad, "mixer" licensing of chiropractors. In other states, naturopaths were fairly openly practicing without licensing but consistently pursuing legislation. Natural healers were practicing in about 40 of the 48 states.[71]

What Is the Vital Force?

What bound this natural healing profession together was a belief in the vital force and a resistance to "suppressive drugs," those pharmaceuticals that relieved symptoms without treating the underlying disease state. Between the mid-1930s and the early 1950s—separate from Benedict Lust and his publications—this doctrine of the vital force became central to natural healing in a manner most consistent with Walter Cannon's concept of *homeostasis:*

Yet, we must ever keep in mind that there is no disease to be cured; there are only sick people to be healed…

The physician must support the inherent nature of the patient by whatever means…By supporting the inherent power—the vital force—we re-establish a harmonious functioning of the disordered parts or functions.

It is not the physician that cures, but the indwelling vital force that heals.

Since it is the vital force that heals, we must seek those methods and do for the patient those things which will best support the natural healing powers of the particular person; we must be careful to do nothing that would interfere with that healing force.

If we are not to interfere with the workings of the vital force in its attempt to heal, then we must carry on our practice in conformity with … the laws of nature.

The mere use of a naturopathic method or modality does not mean you are practicing Naturopathy in conformity with its principles and philosophy. If such methods are used as a suppressive treatment, the physician is practicing Allopathic and not Naturopathic medicine.

As naturopathic physicians we must work … in accordance with natural law.

("Editorial" by A.R. Hedges, DC, ND,[72] Journal of the ANA, May 1950)

Credit to Budden and Carroll

The leadership of W. A. Budden and Robert V. Carroll was critical to the professional growth of natural healing from the mid-1930s to the early 1950s. Their alliance had begun formally in late 1934.[73] From 1935 forward, Budden was *the* trailblazer in natural healing education. Robert Carroll became a trailblazer in the professional organization of natural healers, and through a network of common associates, they each supported the work of the other.

Budden accepted the basic sciences as a necessary part of a "nonmedical" physician's education, although he continued to argue that professional examinations were best given to candidates by each profession's licensing board. He actually, in time, came to view the Oregon examinations as quite fairly conducted, and his students gained a high passing rate as the curriculum focused on these subjects as part of the core education in both chiropractic and naturopathy.[74]

Education

He continued at all times to commit his WSC programs to provide a sound and thorough education to his students in both the School of Chiropractic and the School of Naturopathy. He worked tirelessly to improve the standards for all similar colleges and took the lead at every turn in increasing the coursework and prerequisites required.[75]

But it must be understood that Dr. Budden considered chiropractic and naturopathy as complementary, as part of a complete package, and his friends and allies were like-minded; they were "DC, NDs." Emblematic of this view are two events that took place in the mid-1930s: WSC joined in the school alliance known as the "Affiliated Universities of Natural Healing," and Dr. Budden recommitted Western States to a broad natural healing, drugless, progressive curriculum.

The first of these, the affiliation, was the brainchild of Homer G. Beatty, DC, ND, the president of the University of the Natural Healing Arts (UNHA) in Denver, Colorado. The four schools that were advertised in 1935 as being "affiliated" were Western States and the UNHA, joined by the Metropolitan College of Chiropractic and Physiotherapy of Cleveland, Ohio, and the University of the Healing Arts of Hartford, Connecticut. These schools were affiliated in recognizing that the goal of "a regular standard, four years of nine months each, course in Chiropractic and allied subjects is warranted by our profession and offered by the … school members of this affiliation."[76]

The commitment to curriculum was significant, and WSC was a leader in this area, *especially* in naturopathy. Western States was, as of 1933, located at 538 S.E. Alder Street, Portland, and remained at this location until late 1939. The WSC Schedule of Classes and Hours, first printed in May 1933 and in use throughout the college's stay at the S.E. Alder location, set out a curriculum for both the chiropractic and naturopathy programs of 4000 hours of total study over 4 school years, each consisting of 8 months of residential attendance. The school year was September through July of the following year.[77]

The programs had 2750 hours of common study, starting with the basic sciences, and then "upper-class" requirements of 1250 hours specific to each program. Both courses of study had coursework in physiotherapy, electrotherapy, and hydrotherapy, with the major differences between the programs being the chiropractic coursework in clinical neurology versus the naturopathy coursework in herbology and biochemistry.

In actual practice, students enrolled in the chiropractic program and then added the naturopathy program as provided for in the schedule: "After receiving either the D.C. degree or the N.D. degree, the other degree may be secured by an additional 4 months' work; both degrees cannot be awarded within the regular course." The graduating class of July 1937 was typical, with seven DC graduates, four of whom also received the ND degree.[78]

And Dr. Budden was firm in his commitment to a broad natural healing education and a corresponding view of the DC and ND professions. In 1935 he wrote to the *Chiropractic Journal* to object to the idea that "coagulation of tonsils and dehydration of hemorrhoids" were construed as surgery and therefore not defensible as part of chiropractic. He said, "Western States College stands foursquare behind the members of the profession who are engaged in the practice of electrotherapy as a part of chiropractic."[79] As the Schedule of Classes and Hours noted, under Oregon law, chiropractic was "that system of adjusting with the hand or hands the articulation of the bony framework of the human body, and the employment and practice of physiotherapy, electrotherapy and hydrotherapy." As Dr. Budden's letter went on to say: "There is no reason to back down or retreat from the position we have already established." And he did not, either in the classroom, in clinical practice, or in any public forum.

And finally, he made friendships and alliances deeply within both the chiropractic and naturopathic professions of his day. His chiropractic contributions and their effects on the DC profession have been written about elsewhere, but his naturopathic contributions and their effects have not been written about, and so some attention to his efforts in naturopathic education and professionalism will be paid here. He is known as a great chiropractic educator, one of the classic "schoolmen," but he is also remembered "as a great naturopath."[80]

A Profession

When Benedict Lust criticized the alliance formed by Budden and Carroll in late 1934, he was well aware of the Oregon Ballot Campaign and how hard the DC, ND alliance had fought in that 1934 effort. To emphasize the "push" that was necessary in that fight, Budden had addressed the 1934 Oregon Naturopathic Association annual meeting in June on the fight ahead. And through A.R. Hedges, DC, ND, and others in the ND community (which may have included Washington's Carroll) and to boost interest, the ONA had Lust come out from New York for the meeting.[81]

Carroll, by all accounts of the events of the next 15 years of activities within the naturopathic profession, was frustrated by Lust's interference in the political situation in the Pacific NW. What Lust had said struck at the very philosophy of the WSC with its School of Chiropractic and School of Naturopathy. Over the next 15 years, Carroll did three things: he wrested control of the ANA out of the hands of Lust, the "president for life," he made it a much more professional organization, and he backed Dr. Budden and Western States at every turn in the process.[82]

At the 1935 ANA convention in San Diego, Carroll pushed through a new constitution and supporting bylaws modeled on those perfected by the AMA. The state associations would have House of Delegates members based on the membership size of each state. The Board of Directors and Officers would be elected annually, and the Board would conduct much of the business of the ANA, primarily through its Executive Committee. Lust was elected again as president, but the "president-for-life" status was effectively rescinded.[83]

From this point forward, there were more voices within the ANA.

Gradually, Robert Carroll took control of the reins of the naturopathic profession. The final split from the personal grip of Benedict Lust occurred in 1942. The annual convention of the ANA was scheduled for June 1942 in Chicago. The news of this location for the annual meeting had been released at the 1941 annual convention in St. Louis and continually publicized since November of that year.[84] By the spring of 1942, Lust came to realize that he was to be challenged for the presidency of the ANA by a group led by Carroll that was seeking a more committed professional development within naturopathy. This group was largely from states that had licensing laws of some kind in place for naturopaths.[85]

As these naturopaths met in Chicago and elected Frederick Dugdale of Portland, Maine, as their president, Lust and his close associates, Jesse Mercer Gehman of New Jersey and T.M. (Teresa) Schippell of Washington, D.C., hurriedly convened their own meeting in Atlantic City, New Jersey. This meeting of about 70 naturopaths from the eastern United States, almost entirely from unlicensed states, was declared the convention of "the real ANA."[86]

Even though Lust would contest the validity of what he called the "pseudogroup" of "pseudo Naturopaths," until he passed away in the late summer of 1945—and his eastern followers would continue this even longer—it is clear that Dr. Carroll and the Western group acted within the full authority of the constitution and bylaws of the association and were in the "right" in this dispute.

In any event, as Schippell wrote at the time, the western "insurgents" led by Robert Carroll had been working to "attract many outstanding naturopaths to their ranks, (and bring) in many state organizations to their membership" and had many "well-known practitioners."[87] Carroll assumed the presidency of the western ANA at the July 1946 convention and held the office for 3 years, until July 1949.

The Merging of Efforts

Carroll was a friend of Western States as president of the ANA and afterward as the group's past president. In many ways, Western States came to have a favored status among schools that were connected with the teaching of naturopathy where Dr. Carroll and the western ANA were concerned. As the western ANA grew in stature, it began to make three goals clear: to unify all naturopaths in one professional organization (which meant unity with the smaller eastern group left after the death of Benedict Lust in 1945), to advance the goal of a naturopathic profession based in licensed states, and to develop its own clear educational standards. Unity was supposed to take place at the strong and successful Salt Lake City convention in July 1948, during Carroll's presidency. It did not. But professionalism was much advanced by the creation of a strong committee and organizational structure that Carroll ushered in and by the enlistment of quality professionals like Alton C. Johnson, DC, ND, of California—the author of *Principles and Practice of Drugless Therapeutics*—into the western ANA membership.

And the ANA adopted Budden's model of a 4-year, 36-month residence course of study as its educational standard, passing a resolution at its July 1949 convention in Houston, Texas, against recognizing any school that (1) offered any of its instruction by correspondence; (2) offered diplomas rather than a course of instruction; (3) offered to grant multiple degrees for the same course of instruction; (4) granted any advanced standing or transfer credit that was based on study at schools not recognized by the ANA, the American Osteopathic Association, or the National Chiropractic Association.[88]

This was done as some of the last business conducted under Carroll's presidency. In December 1949, the *Journal of the American Naturopathic Association* first published its list of approved schools, listing three schools that would require basic science credits from an outside institution of higher education and Western States as the only school offering residency education in all 4 years of the required curriculum.

Moreover, Carroll strengthened the connections between the Oregon DC, ND community and Western States with the western ANA. Carroll personally quelled unrest among some of the ND community and Western States in the postwar years. He visited a regular meeting of the Oregon Naturopathic Association in December 1947 while ANA president, together with his successor as president of the Washington State Naturopathic Association (Dr. Helena Winters of Kelso, Washington). He then made another visit to the ONA monthly meeting a year later in December 1948. As reported in *Oregon Pioneer,* unrest began in the fall of 1948 within the ND community around Western States that the school was becoming known more as a chiropractic school, or a school of "chiropractic and drugless physicians," outside of the college. Carroll made the purpose of his 1948 visit as ANA president to express his support for Dr. Budden and Western States as Budden saw fit to operate the college.[89]

After leaving the presidency in July 1949, Carroll remained active—somewhat more behind the scenes—in matters of the ANA and the Pacific Northwest. The largest issue for the Western ANA for 1950 was the unfinished business of unification with the remaining naturopaths in the eastern group, and for the year between the annual conventions of 1949 and 1950, this was almost all-consuming. Additionally, legal issues arose in Washington State in 1950 regarding the 1919 Drugless Healing Act under which the naturopaths in that state were licensed. These legal issues threatened to do severe damage to the profession's legal status.[90]

Unification under the Western group's national structure was achieved in St. Louis in 1950, although the amalgamation remained messy until the very end. Then, Dr. Robert V. Carroll, a true giant within the naturopathic profession and a friend of Western States College until the end, passed away suddenly in April 1951.[91]

Firming Up the Curriculum—Chiropractic and Naturopathy

From the time that Dr. Budden opened the "new" or "converted" Western States College in 1934, the curricula in both the School of Chiropractic and the School of Naturopathy showed his imprint.[92] The primary philosophy-of-practice texts were Joy Loban's *Technic and Practice of Chiropractic,* a text authored in 1915 by a leading faculty member from the National College program, and in naturopathy, Otto Juettner's *A Treatise on Medical Practice (the Art and Science of Non-Medical Therapeutic Methods),* a text by a leading Eclectic and physio-medical practitioner. Juettner's work was first published in 1916 and republished by Benedict Lust's New York publishing house.[93]

The other practice texts in use for the 1930s were Goldthwaite's *Body Mechanics and* Marlin's *Manipulative Treatment* for nonspinal adjustive technique, Grieve's *Modern Herbal* for knowledge of herbal remedies, Luke's *Manual of Natural Therapy* for hydrotherapy, Kovac's *Electrotherapy and Light Therapy* for electrotherapy, Sherman's *Chemistry of Food and Nutrition* for dietetics, and Boyd's *Preventive Medicine* for hygiene and public health. The eclectic nature of these texts shows Budden's wide knowledge of the subject areas included in the Western States curricula, but the use of Loban and Juettner's works also reflects the fact that although the professions had grown significantly since WWI, the available scholarship had not.

This began to change at the end of the 1930s with the publication in 1939 of Homer G. Beatty's (of the University of the Natural Healing Arts in Denver) *Anatomical Adjustive Technique* and Alton Johnson's first edition of *The Principles and Practice of Drugless Therapeutics.* Also published in 1939 by the National College of Chiropractic was *Chiropractic Principles and Technic* by Biron, Wells, and Houser of the NCC faculty; this was the first real advancement of the work begun at NCC by Joy Loban in the much earlier days of the profession. And in the late 1930s, John Robinson Verner's *The Logic and Science of Chiropractic* first appeared.

However, the onset of WWII not only slowed the progress of the colleges, but also the rationing of all materials, including paper, made the widespread use of these new works of scholarship problematic. For 1940 to 1941, the 1938 to 1939 WSC catalog simply had the additional date "1940 & 1941" stamped on the cover; no new catalog appears to have been printed until 1944, and no changes were made to the curricula for the duration of wartime.[94]

The 1944 catalog, which was used through 1947, included as new texts added to the curricula those by Beatty (added in anatomical adjustment); Biron, Wells, and Houser (added in palpation); and Johnson (1st edition; added in electrotherapy).[95] Also, *Rational Bacteriology* by chiropractic scholars Verner and Weiant was added to the more standard text by Zimmer in bacteriology.

New Developments in Postwar Scholarship

It was not until the fall term of 1947 that new scholarship began to appear in use at an accelerated pace at Western States. *The Logic and Science of Chiropractic* by Verner (3rd edition, 1946) was added to the curriculum in chiropractic; in naturopathy, Thomas Lake, DC, ND's *Treatment by Neuropathy and the Encyclopedia of Physical and Manual Therapeutics* was added. This latter work was a lengthy treatise published in 1946 on what was more generally known as mechanotherapy by a chiropractor-naturopath of some substantial reputation based in southeastern Washington State.[96]

Dr. Budden on Chiropractic and Naturopathy

The first postwar catalog published was entitled "Bulletin of the Western States College, Announcement of the School of Chiropractic and School of Naturopathy." The college symbol was now a hand holding a torch with the peroration *fiat lux,* or "let there be light."

For this first postwar catalog, Dr. Budden penned his own descriptions of chiropractic and naturopathy, both as to Pacific NW history and as to philosophy. This material became a constant in WSC's postwar catalogs into the mid-1950s.[97]

Prospective students and interested parties were given this introduction to the WSC School of Chiropractic:

> *School of Chiropractic, Founded 1908.*
>
> *The history of Chiropractic is largely the history of its schools. This is particularly true in the Northwest, where the energy and vision of the founder brought forth the D.D. Palmer School of Chiropractic in Portland, Oregon.*

As early as 1908 Dr. Palmer, together with Dr. LaValley, opened the doors of this pioneer institution.

Since that day Portland has been the seat of Chiropractic learning in the Northwest. Always an institute devoted to this purpose has stood in the Rose City. The Western States College is therefore the lineal descendant and beneficiary of all that has gone before. It is carrying on the work of the founder as he would have desired it, in this modern manner.

Chiropractic literature also found its birthplace in Oregon. The original text, "The Chiropractic Adjuster," by D.D. Palmer, was published in Portland."

This was the introduction to the School of Naturopathy:

School of Naturopathy, Founded 1930

The Latins spoke of it as the Vis Medicatrix Naturae—a remedial force or impulse. The Germans called it Natur Heiling, the remedial impulse of Nature, the self-recuperative power of the bodily system, independent of medicine.

It has been described, also, as the capability of living tissue, animal or vegetable, to remedy and remove disease, or to repair the healing power of nature.

United in man with the dynamics of the mind, this matchless force constitutes the basis of naturopathic therapy.

To intelligently enlist it in the fight against disease is the whole art of non-medical healing.

These two short pieces, authored by Dr. Budden, contain his synopses of chiropractic and naturopathy and present the central core of his nonmedical philosophy.

More Curriculum and Postwar Scholarship

By the fall term of 1949, as the Western States Class of 1953 was entering school, the full flowering of postwar scholarship in both chiropractic and naturopathy was available, and Dr. Budden took advantage of the newest works in crafting his curriculum for each program. Through the course of the college terms from fall 1949 to spring 1953 when the class of 1953 was in attendance at WSC, the chiropractic and naturopathy students were educated in two additional new works of nonmedical scholarship: Janse et al., *Chiropractic Principles and Technic,* 2nd edition, and H. Riley Spitler's newly published *Basic Naturopathy*. This latter text was commissioned and published by Carroll's western ANA with the intention of providing a definitive text in naturopathy. The scholarship is thorough and sound, and it holds up well today as "cutting-edge" work in its time.[98]

Budden paired Spitler's text with a classic from the Eclectic–physio-medical school of medical philosophy, Clymer's *Nature's Healing Agents: The Medicines of Nature* (2nd edition, 1926) based on an adaptation of the Thomsonian System. This combination of Verner, Janse, Spitler, and Clymer plus the coursework in dietetics, physical fitness, body mechanics, electrotherapy, physiotherapy, and hydrotherapy demonstrated the clear imprint of Schulze and Lindlahr on Budden's conception of the nonmedical physician.[99]

It cannot be overstated that Budden's students were the broad-scope chiropractors and naturopaths of the postwar era. The WSC students Budden produced kept these professions alive, especially in the Northwest, for the next 30 years, into the mid-1970s.

This postwar era was the zenith of the period when WSC was *the* pacesetting educational and scholarship beacon in chiropractic and naturopathy. This postwar period was the WSC era when WSC was the intellectual environment that would be rivaled in the middle age of the professions perhaps only at National College.[100]

A.R. Hedges, DC, ND, and W. Martin Bleything, DC, ND—First Connections

Another of the friends and allies Dr. Budden first grew close to professionally during the 1930s was A.R. Hedges, DC, ND, of Medford, Oregon. Medford is a smaller city about 275 miles due south of Portland, located just north of the Oregon–California state line. In 1930 when Portland's population was just over 30,000, Medford's population was just over 11,000, and the "greater Medford" population (within a 5-mile radius of the city center) was just under 17,000.[101]

A.R. Hedges had been practicing as a drugless physician in Medford since 1911. His advertisements for his practice with his wife Louisa for their "chiropractic-naturopathic" offices had appeared in the *Medford Mail-Tribune* as early as 1913.[102] Both A.R. and Louisa appeared in Benedict Lust's *Universal Encyclopedia Directory and Buyer's Guide—Year Book of Drugless Therapy for 1918–1919* as active drugless healers in Oregon.

Whether by coincidence or not, Dr. Hedges did not appear on the statewide Oregon scene as a leader in either chiropractic or naturopathy until 1929, just as Dr. Budden was arriving on the scene as well. From here on, the professional arc of these two DC, NDs intersected continually.

At the June 1929 annual meeting of the Oregon Chiropractic Association (OCA), the forerunner of the OACP, Hedges was named by the OCA president to the convention's resolutions committee. This is the first time he appears in any news coverage of OCA affairs. It was at this conference that Dr. Budden gave his first of many addresses to the Oregon profession. Although it is not clear that the two first met each other at this meeting, Dr. Budden drove down to Medford in March 1930 for a Saturday evening meeting of the Southern Oregon Branch of the OCA held at Dr. Hedges's home in Medford. The Southern Oregon Branch was the professional business section for the southwestern region of the state between annual meetings of the OCA.[103]

Budden was in the company of the OCA's president and the secretary of the state Board of Examiners, both doctors from Portland. Plans were being made for the next annual meeting, which was to be held in Medford for the first time. The upcoming legislative session and legislative planning were also discussed.[104] Dr. Budden would attend meetings of the Southern Oregon Branch many more times over the years.

By the next summer, Hedges was named to his first 3-year term on the Oregon Board of Naturopathic Examiners, serving from 1930 to 1933. Over the course of the 1930s, Dr. Budden and Dr. Hedges crossed paths many times during the professional activities of both the OCA/OACP (the progressive "mixer" group of the state) and the naturopaths of Oregon. This was particularly true during the 1934 Ballot Campaign, in which Dr. Hedges actively participated.[105]

It would be during the war and postwar years that the association of Drs. Budden, Carroll, and Hedges would have its greatest import for Western States College. But before moving the story forward into the war years, it is necessary to introduce W. Martin Bleything.

Dr. Wallace Martin Bleything

Dr. Wallace Martin Bleything first appeared on the Oregon scene in 1937 when he was on the speaker's roster for the 1937 annual meeting of the Oregon Association of Chiropractic Physicians, the successor to the Oregon Chiropractic Association. This was a meeting held at WSC that also featured Dr. Carroll as a speaker, and the 1937 WSC commencement was held in conjunction with this event.

The subject of Bleything's speech on this occasion is not known from the news clip available, but at the time, Bleything was working for a research laboratory in Los Angeles. In 1941, just before the United States entered WWII, Bleything appeared as a speaker before an osteopathic postgraduate meeting in Amarillo, Texas, giving a series of talks on endocrinology, a subject for which he was listed as a "nationally-known expert."[106] Just after war broke out, he was awarded his Master of Chiropractic degree from the California Chiropractic College of Oakland, California.[107]

Hedges and Bleything—the War and After

As America went to war, Hedges's professional profile continued to rise as a physician who had been in practice for 30 years. In July 1942 he was elected vice president of the Oregon Association of Chiropractic Physicians, and in December of that year he was named once again to the Oregon Board of Naturopathic Examiners to finish a term (through July 1, 1943) for a member of the Board who had passed away.[108]

In July 1944 he was elected the OACP president at the annual meeting, and he served as president until July 1946. The vice president elected with him in 1944 was another DC, ND, J. W. Sargent, who had worked with Hedges and Budden going back to the 1934 Ballot Campaign if not longer.[109] In fact, there was clearly considerable overlap in the 1930s and 1940s between the OACP and the Oregon Naturopathic Association. In 1946 as his successor as OACP president was being elected, Hedges was reappointed to a full 3-year term on the Oregon Board of Naturopathic Examiners.[110]

In 1949 at the July convention of the ANA in Houston, Hedges was elected second vice president (VP), taking office as Carroll stepped down as president. By November 1949, his hometown Medford newspaper was reporting on his travels across the country in his national position.[111] Hedges was now positioned to protect the interests of WSC with the national ANA as well as Carroll, and he continued to do so.

Hedges served as second VP of the western ANA for 1 year until July 1950; then, as part of the newly unified ANA, he was elected first VP and twice reelected, serving as first VP from 1950 to 1953. At the 1951 annual meeting, the association had gone through a name change to the American Naturopathic Physicians and Surgeons Association. Hedges wrote the editorial in what was now the *Journal of the ANPSA* explaining the long story behind the earlier schism, unification, and the reason for the name change.[112]

He also wrote a series of editorials in the association journal eloquently explaining the difference in core philosophy between the allopaths on one hand and naturopaths and chiropractors on the other; the difference between viewing the physical organism as operating in "conformity with the laws of chemistry" versus the view that the body "is in a vital realm, presided over by a vital force" that maintains life; and the difference between treating symptoms with suppressive means versus "supporting the vital force" to "regain harmonious function" and "to do nothing that would interfere with that healing force."[113]

In July 1953 he was elected president, and he was reelected July 1954, serving until mid-1955. This was all capped off for Dr. Hedges when he appeared before the Oregon Naturopathic Association annual meeting in March 1954 as national president.[114] Throughout these years, Hedges succeeded Carroll in guarding the interests of the Western States program at both the state and national levels. In 1947 the Los Angeles College of Chiropractic dropped its ND degree program. In 1949 the Metropolitan College of Chiropractic closed. Then in 1950, Homer Beatty of Denver's University of the Natural Healing Arts suddenly passed away, and the National College of Chiropractic dropped its ND degree program. Through these events, the other legitimate, 4-year residency programs were lost. As the largest and strongest organization of naturopaths, the ANPSA had a vested interest in the existence and success of WSC.

That brings matters back to Dr. Budden and WSC as the war ended in 1945. Dr. Wallace Martin Bleything, known most often as W. Martin Bleything or Martin Bleything, appears to have been linked primarily to Western States in the postwar era. As the war came to a close, Western States was hanging together but uneasily, as already described. With peace came the wave of returning veterans and a postwar boom of interest fueled by the Servicemen's Readjustment Act of 1944, or the "G.I. Bill." About 2.2 million returning veterans used their G.I. Bill benefits to attend colleges or universities, the classification attained by Western States through the Veteran's Administration, which certified institutions of higher education.[115]

As Lester Lamm described the circumstances of Western States in *Oregon Pioneer:*

> *The shortage of students produced by the Great Depression and made worse by World War II disappeared almost overnight, leaving the college with a completely new challenge: what to do with more applicants than the institution could manage.*
>
> *The G.I. Bill filled college and university classrooms across the nation, and the Western States College was the fortunate beneficiary of escalating applications and enrollment numbers. The explosion of growth was unanticipated and the college was not prepared for the magnitude of the student influx it experienced.*
>
> *The makeup of the student population was also challenging. Students demanded the administration provide them with a higher quality education, in a more appropriate facility.*[116]

The school resolved the facilities space problem in late 1946 by purchasing a building on S. E. Alder in Portland, a former lodge building. As A.E. Homewood described it, "this was far from ideal, or a place of beauty, but did offer the necessary space for expansion."[117] Qualified faculty was another matter. The size of the new student population surpassed the method of Dr. Budden doing a lot of instruction supplemented by local practitioners. Enter, among others, Dr. Bleything.

Bleything had a varied and colorful background, but most relevant to Western States, he had grown up in Portland, then moved to Seattle shortly before World War I. Interrupted by service during the first war, he had studied chemistry and then worked and studied at Grace University Hospital, a homeopathic and drugless physician training program combined with a sanitarium maternity ward run largely by a homeopathic, fully licensed MD. John Bastyr, DC, ND, after whom Bastyr University is named, had interned there, and Dr. Robert Carroll had been on the sanitarium staff, both during the 1930s. Between 1932 and 1942, Bleything had worked at a research laboratory in Los Angeles as a colloidal chemist, as well as graduating from chiropractic college.[118]

Bleything arrived at WSC in 1947 and joined the faculty in first- and second-year basic sciences and in third- and fourth-year practice courses in chiropractic. He got licensed in Oregon as a DC and became a member of the OACP. Within another year, he got licensed as an ND and joined the ONA. By 1951 he had been recruited by A.R. Hedges to take over as editor of the *Journal of the American Naturopathic Association* and had become the lead faculty member in instruction in the naturopathy program at Western States (while still continuing with the basic sciences). Again, Western States had connections deep into the national affairs of naturopathy and was the example naturopaths always turned to when the education of NDs was challenged.[119]

The Western State College Class of 1953

By 1946 the way was now clear for the Western States programs and the chiropractic and naturopathic professions to establish themselves in peacetime. In the fall quarter of 1949, the Western States class of 1953 entered the college. By the time the class of 1953 graduated, the majority had finished with both DC and ND degrees, and the graduating class of the School of Naturopathy was the largest class of ND degree holders ever at WSC. It was also the largest such class anywhere for another 30 years—until the John Bastyr College of Naturopathic Medicine in Seattle graduated its first full class in 1982. The class of 1953 would make its mark in keeping alive both broad-scope chiropractic in Oregon and elsewhere and naturopathy in Oregon, Washington, and the Canadian province of British Columbia for that same 30-year period.

In the immediate postwar period, 1946 to 1952, the AMA—or organized medicine—was consolidating its power within the US healthcare system. Medical dominance was present, but as a threat to alternative practitioners, it was not yet at full throttle. This was, looking back historically, because of the AMA's obsession with the threat that organized medicine called "socialized medicine"—a healthcare system controlled by the federal government.[120]

The AMA and President Truman

Universal health care—a totally government-funded healthcare system—was a pronounced political goal of President Harry Truman. The political and public relations machinery of the AMA was almost entirely directed at this "threat" to the "American Way" for all of President Truman's postwar term in office. While organized medicine was politically preoccupied, chiropractic and naturopathy were able to advance. President Truman left office in January 1953, and by the summer of 1953, the AMA was starting a national campaign to eliminate chiropractors and naturopaths as competitors, but until then, battles were fought on a state-by-state basis.[121]

As previously discussed, the National Chiropractic Association (NCA)—the chiropractic "mixer" group—was directing its accredited colleges to shut down any of their nonchiropractic degree programs, specifically any ND degree programs. After National Chiropractic College complied in 1950, Dr. Budden was the lone holdout in maintaining a naturopathy program. The push in this direction was led by John Nugent, the "Flexner of Chiropractic." In meetings of the education council of the NCA, Dr. Budden was merely resistant; behind closed doors, Dr. Budden and Dr. Nugent had loud, if personally respectful, disagreements about this subject. Dr. Budden was unyielding.[122]

And so the postwar period of 1946 to 1952 was a period of professional development in natural healing and of advances in clinical science and in serious scholarship. But in DC, ND education, Western States was alone after 1950. This was, at the same time, WSC's most fruitful period under Dr. Budden fueled by the G.I. Bill—the only public funding program for DC and ND education until the rise in the 1980s of student loan and Pell Grant funding.

Enter the Class of 1953

By the fall quarter of 1949, the college had settled into a former lodge building at 4535 S. E. 63rd Street. The curriculum was set as well, and the students were expected to attend straight through for three quarters plus summer term in each of the first 3 years of attendance, and two quarters in the fourth year. The schedule started the first week of October and went through the end of July of the following year; the fall, winter, and spring quarters were each 3 months long, and summer term was the month of July. Each regular term was 300 classroom hours of instruction, and summer term was 100 hours, for a total of 1000 hours the first year. This increased to 1160 for the second year, 1260 for the third year, followed by a fall and winter term fourth year of 940 hours. Degree completion for either the chiropractic or naturopathic degree came at the end of the spring quarter the fourth year.[123]

The first 2 years were intense with the basic sciences curriculum (as such programs are today) together with either Introduction to Chiropractic or to Introduction to Naturopathy, followed by Principles of either discipline included in the first 2 years. The students largely lived and breathed this coursework for 4 years, with most of the social activities that the students had being activities like clubs and dances at the college. This class of 1953 was mostly veterans, a bit older, a bit more ready to get to work and on with life, and mostly married. And they were from throughout the country and from British Columbia, Canada. There were eight each from Oregon and Washington; three from California; two each from Missouri, North Dakota, Minnesota, and British Columbia; and one each from Nebraska, Illinois, Michigan, Colorado, Montana, and Ohio. There were five women in the class and two African Americans, one of whom, being from Miami, had come the farthest from home to study at Western States.

Emblematic of the initiative of this class of 1953, class members started a monthly news publication entitled *The Synergist: Western States College Voice of the Student Body,* "published in the interest of UNITY among all interested drugless healing arts."[124] This monthly publication is virtually a journal of this class of 1953, disappearing as a monthly when the class of 1953 graduated and surviving as a bimonthly only for another year or so. But while this class was enrolled at WSC, it was their voice.

Another aspect of the quality of WSC and its student body, and the class of 1953, particularly, was its racial integration. Two members of this class that entered in the fall of 1949 were male African American students who graduated with the class of 1953. One of these students came completely across the country, from Miami, Florida. Jackie Robinson "broke the color barrier" in baseball in 1947. President Truman desegregated the American Armed Forces by Executive Order in 1948. The Warren Court did not strike down the segregation concept in public education until 1954. The enrollment of these two students and their integration into the graduating class of 1953 speaks volumes about the progressiveness of Dr. Budden, of WSC, of the students of the class of 1953, and of the city of Portland in general.

The members of the class participated in two designated "fraternities," Sigma Phi Kappa, a chiropractic fraternity founded in 1912 and chartered at Western States in 1948, and Phi Nu Sigma, a naturopathic fraternity started at Western States modeled on Sigma Phi Kappa. Phi Nu Signa announced in *The Synergist* for February 1953 that it was starting the process of "expanding its sphere of influence by providing for and inviting practicing Naturopathic Doctors to join its ranks to help promote Naturopathy and naturopathic principle and practice." This last item seemed to be a parting gift from the leaders of the class of 1953 to the fraternity and also a way to stay involved themselves as new NDs.

As Reported in *The Synergist*

The initiative of the class of 1953 becomes clear in looking at its own documentation of itself and of the larger WSC student body in *The Synergist.* By the time of the class of 1953 commencement ceremonies in March and July of 1953, *The Synergist* was publishing regular news submitted for publication by both the OACP and the ONA. This provided the student body with a regular source of professional news, and *The Synergist* became a publication subscribed to by the professions as well.

In the postwar era, Western States became a fixture of natural healing under Dr. Budden. *The Synergist* captured this in documenting the professional activities and meetings that took place at the college and the participation of Dr. Budden, the faculty, and student body in these events. As an example, *The Synergist* for May 1952 recapped the annual OANP meeting that had been held at WSC in March 1952, at which the featured keynote speaker had been Henry J. Schlichting Jr., ND, national president of the ANPSA.[125] The same edition of *The Synergist* reported the complete program for the annual meeting of the Oregon Association of Chiropractic Physicians to be held at WSC the first week of June that year.

Dr. Schlichting, it was reported, had given an open speech to WSC students and staff, to physicians, and to the public, as well as a speech to the convention banquet. *The Synergist* reported in the column "Naturo-News" authored by Dr. R.A. Rombaugh of Independence, Oregon, that Dr. Schlichting "lauded the pioneers and early educators and complimented Dr. W.A. Budden, Western States College Director on the fine job in building the College to today's high level of standards." The schools Dr. Schlichting said, were "the life blood of any profession."

Another initiative of the class of 1953 was an annual WSC picnic. The first reported "WSC Annual Picnic" was to be held Sunday, June 15, 1952, but was postponed a week due to inclement weather. But when it was held on June 22, 1952, as reported in *The Synergist* for July 1952, it left all attendees and participants "looking forward to the picnic next year." More impressively, the picnic was attended by both members of the Oregon Association of Naturopathic Physicians and the Washington Association of Naturopathic Physicians, as reported in the *Journal of the American Naturopathic Physicians and Surgeons Association* (vol. 5, no. 2, June 1952).

More Professional Matters for Dr. Budden

Three other events of interest occurred during 1953 that affected the class and were noted in *The Synergist.* First, the Oregon chiropractic scope of practice came under assault again, from the medical profession on one side and the "straight" chiropractic group on the other. A breakfast meeting of the Oregon Joint Legislative Council held Saturday, March 7, 1953, was broadcast on radio and moderated by radio commentator (and future Oregon governor) Tom McCall. The specific subject was pending Senate Bill 134, yet another bill to strip obstetrics and minor surgery from the Oregon chiropractic scope of practice.[126]

Dr. Budden appeared with another physician from the OACP representing the Joint Legislative Committee of chiropractors and naturopaths. The medical view was provided by representatives of the Multnomah County Medical Association, both of them clinical professors at the University of Oregon Medical School, one in OB/GYN and one in general surgery. When asked by McCall about the fact that Palmer College did not teach these subjects as "The Fountainhead" of chiropractic, Budden related some of the history of "straights" and "mixers" and told the audience that one major issue was that the Palmer school did not wish to go to the expense of teaching these subjects even though it had adopted a 4-year curriculum in 1953.

By the following Tuesday, the Oregon Chiropractic Research Association—the Oregon "straight" organization, a group in Oregon about one-fourth the size of the OACP—had issued a strongly worded communique to the radio station that broadcast the discussion and to *The Oregonian* newspaper.

The statement said that the OCRA had nothing to do with the pending legislation and no interest in it, but it took offense at Budden's characterization, noting that Palmer devoted 4485 class hours to "straight" adjustment technique and that Budden "attempted" to teach a laundry list: spinal adjustment, obstetrics, surgery, eye-ear-nose-throat practice, proctology, proctology, removal of tonsils (by electrotherapy), administering anesthetics, use of hypodermics, electrotherapy, hydrotherapy, physiotherapy, "and such" in 4240 class hours.[127]

In the long run, once again, no legislation was passed, altering the Oregon scope of practice.

Some Positive Developments

What did pass at the insistence of Dr. Budden and Western States College, and with the support of both professions, was House Bill 271 (NDs) and 272 (DCs) increasing the educational requirements to high school *plus* 2 years of college credit from an accredited college or university. This was noted in an approving editorial in *The Synergist* for February 1953, penned by Appa Anderson.

Finally, it was noted in *The Synergist* for March 1953 that as president, Dr. A.R. Hedges was bringing the American Naturopathic Physicians and Surgeons Association convention to Portland that summer, in July. The general chairman for the convention was WSC's Professor W. Martin Bleything.[128]

1953 for the Class of '53

The April 1953 issue of *The Synergist* contained a column by the editor of the *O.A.C.P. Journal;* this column had by now become a regular presence in *The Synergist.* The editor was A.C. Johnson, WSC class of 1951. He paid a special tribute to the class of 1953, saying "without reservation, I would like to praise a universally qualified and most ambitious group." He paid special tribute to those "from this group, (that) have become the working force that has published *The Synergist* during these past four years," and closed with: "To the class of '53, and with gratitude to you who have done so much to keep the college, the students, and the profession interested and united, we, the profession, wish you God speed in the gratifying endeavor that awaits you."

And then this remarkable class prepared to graduate and pass into the history of Western States. For some comparison, in June 1949, WSC graduated 36 DC degrees and 16 ND degrees, with 11 graduating with dual degrees. In March 1950, WSC graduated 29 DC degrees and 2 ND degrees. In March 1951, 21 DCs graduated and 4 ND degrees. In March 1952, 31 DCs and 1 ND graduated.

The class was scheduled to graduate in March 1953, and most were graduating as DCs. Because there was interest expressed among class members in receiving dual degrees, a special spring quarter schedule had been arranged, focused exclusively on ND therapeutics. *The Synergist* had reported this development from time to time over the 1952–1953 school year. It was noted in the March 1953 publication: "much of the class will be around after Graduation to take a post-graduate course in Naturopathy, so we really won't be saying our goodbyes for a while" This course for DCs required a full spring quarter of coursework in Cyriax's *Text-book of Orthopaedic Medicine, Volume II* on massage therapy, Mausert's *Herbs for Health,* and Spitler's *Basic Naturopathy* in a course designed by Drs. Budden and Bleything.

The class of 1953 had two commencement ceremonies, one in March and one in late June. In March, 33 DCs and 2 NDs graduated, with 7 DCs also receiving the BTS degree and 1 ND receiving the BTS degree. In July, at a special commencement at the end of the spring quarter, 33 NDs were graduated, and 1 received the BTS degree. Twenty-six of these degrees went to DC graduates from March 1953, three went to March 1952 DC graduates who returned for the spring quarter course, and six ND degrees were received by ND candidates who finished studies in June with the extra quarter's work.

The late-June ND degree ceremony was staged as an evening event on the second night of the ANPSA convention, as reported in the *Journal of the ANPSA* for September 1952 (vol. 6, no. 6). "The

commencement address was delivered by that outstanding authority on nutrition of the University of Missouri, William Albert Albrecht, Ph.D. Dr. Albrecht's address was indeed inspiring, not only to the graduates but to all in attendance." And, as the reporter noted: "It was indeed an inspiration to see this fine young group of naturopathic physicians entering the profession. The profession needs more young naturopathic physicians to assure its future."[129]

Some Stalwarts of 1953

Joseph Boucher, the ND degree graduate who had been student body president for 1952 to 1953, received the William J. Gallagher award as the outstanding graduate of the class of 1953, with Appa Anderson being noted by Dr. Budden in announcing the award as a close second. And then the class of 1953 left Western States to conquer the world. But this class was the fruition of all that Dr. Budden and the chiropractic and naturopathic professions had built at Western States in the face of challenges and opposition.

Dr. Budden died unexpectedly in August 1954, less than a week after returning from attending his last NCA meeting.[130] By the time the class of 1953 finished its first year at Western States, the ND program was the only remaining ND program in the United States.[131] Within 2 years of the death of Dr. Budden, the ND program at Western States was discontinued; courts had restricted the legal scope of practice in Washington and Arizona; and licensing of NDs was lost completely in Texas due to actions by the courts and in South Carolina, Utah, and Florida by legislative action. How this happened is another piece of history still to be told, but a great deal of it curbed broad-scope or "mixer" practices in those states, and a large factor was medical dominance, what the educators and physicians discussed here had battled for 25 years. Things did not improve until at least the 1980s, another part of the story yet to be told.

But that should in no way diminish what was done at Western States College and in *both* its School of Chiropractic and its School of Naturopathy. From the class of 1953, Joseph A. "Joe" Boucher, ND, BTS, became a giant of Canadian naturopathy in his own right,[132] Appa Anderson stayed at Western States for an impressive career of her own in chiropractic radiology,[133] and Professor W. Martin Bleything was one of the founders of today's National University of Natural Medicine, together with one of his fellow faculty members and a former Western States student from the class of 1952.[134] Ralph M. Failor, who—along with his wife Hazel—received a DC degree in March and an ND degree in July, would succeed Dr. Budden as president of WSC. He would in time become a big part of the history of WSC.[135]

What happened was remarkable and needs to be better known.

PART TWO: WHAT HAPPENED TO THEM?

Moving On

There may be no more exemplary story in the development of natural healing among chiropractor-naturopaths than the story of Henry J. Schlichting Jr., of Midland, Texas. Schlichting was trained as a chiropractor in Oklahoma and moved to Texas in 1941, setting himself up as a naturopath. At the time, neither chiropractors nor naturopaths were licensed in Texas. Schlichting became a leader within natural healing professionals, first within Texas and then nationally. He became a trusted ally of both Robert Carroll and of Dr. Budden at Western States College. He and his Texas naturopaths achieved licensed status in 1949. In the early 1950s, all looked bright, and then it all turned dark.

On the evening of July 17, 1953, the commencement for 37 recipients of the degree of doctor of naturopathy took place in the auditorium at Western States College in Portland, Oregon. This special commencement was scheduled as the Tuesday night program for the 1953 annual convention of the American Naturopathic Physicians and Surgeons Association, as the immediate past president of the ANPSA Henry J. Schlichting Jr. of Midland, Texas, was in attendance.[136]

As Schlichting had said a year earlier in a speech at WSC, the prospects for naturopaths seemed good as this WSC class of 1953 graduated, most of them as DC, NDs. But the forces of medical dominance were building, starting back home in Texas, even as Schlichting was attending the convention in Portland. Within 6 years, natural healing in the United States would be much diminished. No career demonstrates this as clearly as that of Schlichting, a remarkable man and physician who, by the end of the 1950s, had been barred by the State of Texas from practicing his chosen profession and who had lost almost everything.

When the class of 1953 graduated from Western States, the leadership of the naturopathic part of the natural healing professions was in Henry Schlichting's hands as the president of the ANA, with A.R. Hedges in line to become Schlichting's successor. There were headwinds, though, just beginning to be felt.

HEADWINDS

Some of these headwinds had been building within the naturopathic movement itself since the 1930s. Benedict Lust criticized Dr. Budden and Dr. Carroll in early 1935 for proposing a continuing alliance based in the Pacific NW between broad-scope chiropractors and naturopaths. The 1935 annual ANA convention a few months later in San Diego was a crossroads event for ND as a profession. Lust opened the convention with a presidential address that seemed to mark a "scofflaw" phase in Lust's career as the head of the naturopathic movement. He told the convention that he had been prosecuted—and persecuted—for issuing diplomas to "doctors" from the American School of Naturopathy without a New York State charter to do so. The State of New York, he charged, was operating as an arm of the AMA's "Medical Trust." Only naturopaths, he told the convention, could decide who deserved to be called naturopaths, not the state or the Medical Trust. Naturopathy needed to be accepted by the public to be legitimate, not by the state.

But for the first time, Robert Carroll brought a countervailing view to an ANA convention. These countervailing visions of the future of naturopathy were the visions that played themselves out over the next 10 years within the ANA. The ANA could not be a movement run by a permanent leadership but needed to truly be a professional organization seeking to advance a natural healing profession. In adopting a new constitution and bylaws proposed by Carroll, the ANA accepted Carroll's vision of the future over Lust's vision from the past.

By 1937 Carroll was comfortable that the ANA was in a good place; now it was time for the profession to accept that fact. At the end of 1937, as the chairman of the Executive Committee of the ANA, Carroll issued "An Appeal to All Naturopaths." He opened by saying that he for some time he had been "alarmed and rather disappointed with the seeming indifference of many of the Drugless Physicians to our National Association." "Progressive Naturopaths," he said, were committed to the goal, through the ANA, that "our profession will take its place as a scientific body of learned naturopaths." With science on its side, progressive naturopaths could, for example, "tell the world just why Sulfanilamide is detrimental to the human body, and in just what kind of cases it is fatal or contraindicated—This must be our objective if we ever hope to merit the respect of the public and our educational institutions." Changes had been made in the ANA in 1935 "to pull our profession out of the adolescent state, which we have seemingly been unable to pass."

As part of the change in ANA structure, the leadership of the ANA also gained some input into submission of ANA professional material in *Naturopath and Herald of Health* in exchange for ANA dues monies

being used to support Lust's costs of publication. Using this position, Carroll had an article by Dr. Budden on the effects of the Basic Science Laws published in *NHH*. The article also appeared in one of the leading chiropractic journals.

Revisiting Basic Science

This article was written in Budden's inimitable style and was written only a couple of years away from the Oregon 1934 ballot fight that marked Budden's initial response to Oregon adopting its Basic Science Law. In a classic Americanism argument, Budden pointed out that a "whole generation of college-bred men and women" would be good national policy as there "should be more and evermore of our youth attending institutes of higher learning, and provisions should be made to make this possible." But there were policies that were intended to work against "Americans who value democracy" and that had the "sinister objective, nothing less than the establishment of exclusive privileges in education."

This was where, Budden argued, the Basic Sciences Laws were directed. They were designed as a test of university-level sciences divorced from the application of the sciences to drugless, nonmedical professions. This was at a time when "the drugless world had developed its own schools and colleges; institutions of learning peculiar to human therapeutics from the non-medical standpoint, well-equipped and staffed by competent teachers."

"Drugless schools," he pointed out, "have no state support and few endowments; they must depend on contributions from alumni and upon tuition." And as "a great national magazine (had) brought to light the unpleasant truth [was] that more people gave allegiance to physiological and drugless methods than to purely medical treatments." If "the drugless schools continued to flourish and to increase in value to the community and the country at large, it soon be too late to attack them. Thus the basic science idea was born."

Budden went on to argue that "it is important to note that in most states, and in the state of Oregon in particular," the sciences called the basic sciences—anatomy, chemistry, physiology, pathology and public health—were already taught and tested on for chiropractic and naturopathy. The purpose of the "extra" examination seemed to Budden to be an attempt to raise a barrier to drugless, nonmedical students of chiropractic and naturopathy with a clear ulterior and undemocratic motive: to keep these practitioners out of the marketplace: "Proponents of the law maintain that by this arrangement, which they contend is fair to all alike, the public is assured of a higher grade of practitioner. The police power of the state should be wielded to protect the public: not one particular group of physicians. The way of safety for the citizen is not in uniformity of thought in the healing arts, but in diversity."

Dr. Budden had come to be recognized by Robert Carroll as one of the leading "schoolmen" in natural healing. Although Budden was very concerned about "a board composed of university lecturers or Deans, men who know nothing of drugless practice and care less, prejudiced even before they occupy positions on the basic science board," he came to believe within less than 10 years of dealing with the Oregon board that the examinations were fairly held—which will be addressed later in this chapter.

A similar set of countervailing visions to those playing out within naturopathy played out within the National Chiropractic Association. In 1939 the influential broad-scope (or "liberal") chiropractor from Montana, C. O. Watkins, DC, argued in an influential article[137] that chiropractors should not favor separate licensing and degree status for naturopathy; rather, chiropractic statutes should be sought legislatively, recognizing that naturopathy was incorporated within a broad conception of "liberal" chiropractic and should be recognized as such.

In the article, Watkins noted that nationwide, there were "16,000 chiropractors, 95 per cent using other than straight Chiropractic" and "2000 naturopaths, many of them holding Chiropractic licenses who could also be considered liberal chiropractors." Furthermore, Watkins noted, the NCA had considered the issue of backing naturopathic legislation and decided on a different policy: "That the NCA oppose any plan that would cause the passage of separate physio-therapy laws or naturopathic laws to cover liberal chiropractors, but rather favor liberalization of Chiropractic legislation where it desirable to legalize liberal practice."

Looking back through the lens of historical hindsight, it seems that these policy differences fostered a diffusion of energies that would have been better spent seeking recognition for natural healing in the most expansive way possible, wherever possible.

And Scandals

Although most developments that have been discussed thus far were positive, in the 1940s, there were scandals involving practitioners identified as "naturopaths" as well. In 1938 a group of liberal chiropractors that identified themselves as chiropractor-naturopaths had formed the American Naturopathic Association of Michigan. Their leaders were charged with bribing members of the Michigan state legislature during the 1939 and 1941 legislative sessions. The charges alleged that bribes had been paid in an effort to get a naturopathic law enacted, and reports of the investigation by an inquiry judge, including the charges brought and the trials and guilty pleas in the case, dominated upper Midwest headlines from late 1944 through the end of 1945.

A much bigger scandal—one that received much wider and more sensational coverage—emerged in Tennessee. By the time the events there had played out, the courts had laid the groundwork for the assault on naturopaths that took place 10 years later in the mid-1950s. Organized naturopathy came to Tennessee in December 1937 when the ANA of Tennessee was chartered. Guy W. Cheatham, DC, ND, had established the Nashville College of Chiropractic and Naturopathy earlier in the 1930s, and the school and the ANA of Tennessee operated in an unlicensed vacuum for several years while Cheatham was active in the NCA efforts on chiropractic education and in ANA national affairs. George A. Floden, DC, ND, of Los Angeles, California, was affiliated with Cheatham's college and lectured there on a regular basis.

Matters in Tennessee changed significantly and abruptly in 1943 when the Speaker of the Tennessee House of Representatives, backed by the Crump political machine based in Shelby County (Memphis), pushed through a naturopathic licensing law over the veto of the state's governor. A 1946 investigation suggested that two naturopaths who later were named to the naturopathic licensing board funneled several thousand dollars to the Speaker (one bookkeeping entry showed $7835 for "1943 legislature"), their golfing buddy, in the form of "friendly" golfing bets.

By 1946 the examining board, which by statute kept its own books and records of licenses issued, had issued 917 Tennessee licenses to "naturopaths" from as far away as California, Alaska, Mexico, Canada, and South Africa. The number could actually have been more than 1000 licenses issued, as the books and records of the examining board "disappeared" from the offices of one of the board's members while the records were under subpoena by state prosecutors. But investigation showed that some diplomas and licenses were sold as a package: $1500 for a diploma and $1500 for a license.

In December 1946, 27 indictments were issued by a grand jury in Nashville. Those charged included Guy Cheatham DC, ND, and his California associate George Floden, DC, ND, along with a California associate of Floden. Two Texas NDs who lectured at Cheatham's Nashville College were charged as well, and in the grand jury's 34-page

indictment, it was alleged that all schools of naturopathy in Tennessee were "diploma mills." Cheatham had, admittedly, issued "naturopathy" diplomas to earlier chiropractic graduates of his college so that they could apply for naturopathy licenses after the 1943 statute went into effect.

In all, 17 defendants submitted *nolo contendere* ("no contest") pleas and were fined between $100 and $1000. The largest fines of $1000 went to two of the three members of the licensing board that the prosecutor said were "to blame for the conspiracy." Charges against Flodden and his California associate were dropped when the State of California denied extradition to Tennessee on the charges.

More importantly in the long run, the 1947 Tennessee legislature repealed the naturopathy act, criminalized any future practice of naturopathy in Tennessee, and invalidated all existing licenses to practice naturopathy in that state as of January 1947. Ten practitioners—all members of the ANA of Tennessee and all "clean" of any taint from the indictments—filed suit, seeking a declaration by the courts that the repealer statute was constitutionally invalid because it deprived them of their valid property interest in their licenses to practice without due process and without any finding that naturopathy itself was a threat to the health and welfare of the citizens of Tennessee.

A chancery court (trial-level) judge agreed, holding that the legislature did not have the constitutional authority to rescind the right to practice a legalized profession and to revoke, *carte blanche,* an entire class of professional licenses from practitioners who were without fault. A request to enjoin *any* enforcement of the statute through prosecutions for practicing medicine without a license was denied as beyond the authority of the court.

But before 1947 was over, the Tennessee Supreme Court had reversed this judgment. The Supreme Court held that the allegations of massive fraud in issuing licenses were serious enough to justify a sweeping response under the state's constitutional police powers, without the necessity to review each license in question. Moreover, the Tennessee court held that it was well within the police power of the state legislature, under the same constitutional police powers, to repeal an entire class of professional licenses—especially in the healthcare domain—*at any time, within its reasonable discretion.* That is, where licensing was concerned, the legislature taketh, and the legislature taketh away.

The plaintiff naturopaths petitioned the US Supreme Court for review of the case and of the Tennessee Supreme Court's holding on these constitutional issues. Review was denied. The table was set for a later broad assault on the licensing of natural healers across multiple states in the 1950s.

And Repercussions

The mischief and the stain originating in Tennessee spread quite quickly as well. In early 1947 as the scope of the Tennessee scandal was emerging and as the Tennessee legislature was repealing the Tennessee licensing law, investigations of licensing of naturopaths began first in Connecticut and then in South Carolina. In Connecticut, the state health commissioner—by state law a position held by an MD—withheld licenses from 28 applicants who had been approved for licensing by the Connecticut Board of Naturopathic Examiners starting in 1942 because none of the applicants had passed state licensing examinations, neither the basic science examination nor the naturopathic licensing examination. All of the applicants had been approved based on a Connecticut healing arts licensing reciprocity statute. All of the applicants sought reciprocity based on licenses issued in South Carolina—a state with no basic science examination.

In 1946 three applicants brought suit in two actions against the health commissioner seeking a court order directing that their licenses be issued immediately (through a *writ of mandamus* or "mandate"). Both applicants had failed the basic science examination more than once and then presented South Carolina licenses to the Connecticut Board, which had approved reciprocity applications. The applicants prevailed first in chancery (trial) court, then in the Connecticut Supreme Court.

The courts—the chancery court in June 1946 and the Connecticut Supreme Court in April 1947—held that the health commissioner served in a ministerial capacity, that is, the health commissioner had no discretion in issuing licenses when the Naturopathic Board had approved the applications. The courts also found that the reciprocity statute allowed the approval without examination, but the Connecticut Supreme Court agreed with the trial court that the health commissioner was trying to protect the public interest. The Connecticut Supreme Court noted further that if evidence showed that the applications were fraudulent or the actions of the Board were taken in bad faith, members of the Board should be removed from office.

Based on the issues developed in the court proceedings, the Connecticut police—which had the responsibility for licensing background checks—began an investigation into the reciprocity applications that lasted 6 months. This led in turn to an overlapping investigation by the state police in South Carolina. A clear pattern was documented: applicants from schools not recognized by South Carolina (which recognized only the National College of Chicago and Metropolitan of Cleveland as of 1947), primarily applicants with diplomas from Lust's American College of New York, had obtained licenses in Tennessee between 1943 and 1946, and then been licensed in South Carolina without examination.

Twenty-four applicants had then applied for licensing in Connecticut, again based on reciprocity. An investigation done in South Carolina by two Connecticut police detectives disclosed that only four applicants had actually spent any time practicing in South Carolina, and no time had been spent in Tennessee. The South Carolina Board had suspended the practice of accepting reciprocity applications from Tennessee in February 1947 when the Tennessee legislature outlawed naturopathy, but in 1947 and 1948, legislative pressure built up on the Board, and ND licensing in the state was under threat of repeal. By 1949 the repeal threat had been survived—for the present.

Much of this was a result of Connecticut adopting a Basic Science Law when only National, Metropolitan, Western States, UNHA of Denver, and Los Angeles College—all chiropractic colleges with ND degree programs—had legitimate 4-year residency programs with a basic sciences curriculum. How had this been worked through by these colleges? By 1944 Dr. Budden's view of the Oregon Basic Sciences Examining Board had changed based on 10 years of experience with the Board and its examinations.

More Basic Science

In the October 1944 issue of *The National Chiropractic Journal,* Budden offered his updated assessment of the Oregon Basic Science Law in the article "Effects of Basic Science Law in Oregon." By this time, Budden noted that "we should like to make it clear … that we—the faculty and myself—have had some ten years of experience with the preparation of students for this test and, as a consequence, we feel that we make speak with some authority." This notwithstanding that "public and candid discussion of the merits and demerits of basic science legislation has been regarded as a species of treason to Chiropractic (and Naturopathy)."

But strictly directed to the Oregon experience, the Basic Science Law in general had shown his students to be ready join "one of the learned profession," passing an examination given by a board of college and university professors chosen by the Board of Higher Education that "conducts its affairs with equity and intelligence (with) examinations

that are fairly held and the papers fairly marked." From all the evidence Budden had observed—and from a success rate of 25 out of 30 students passing the examination the first time (83.33%)—Budden believed "that if a student follows the courses covering the required subjects as they are given in the accredited schools, faithfully and with diligence he will pass the test."

He still noted that the public health part of the examination was based totally on the medical approach to the subject, requiring that the faculty had to teach students both "traditional" public health and the nonmedical alternative thinking. But he felt this was a price to pay in order that a higher level of nonmedical education was achieved, medical propaganda about a low level of nonmedical education was "wiped out," success on the examinations favorably influenced the courts and the legislature, and there was more favorable treatment of the nonmedical professions by the outside the medical domain "all-around."

Finally, Budden noted that "of late efforts have been made to circumvent the law by setting up very dubious and possibly illegal 'reciprocity.'" This, he said, was self-defeating: "There is only one way; to qualify enough candidates to show that the level of education makes two [licensing] examinations for the right to practice the healing arts preposterous."

In this thinking, as in many respects, Dr. Budden was almost 40 years ahead of his time.

Dr. Schlichting

Henry J. (Hank) Schlichting Jr. was born in Fowler, Kansas, in 1915 and was raised—or, as he put it, "reared"—in Weatherford, Oklahoma. By 1938, at age 23, he had graduated from Oklahoma City's Carver Chiropractic College and relocated to Amarillo in the Texas Panhandle, where he joined "Dr. Roy G. Moore's Chiropractic Hospital—Serving the Entire Southwest" as "Assistant Specializing in Dislocations and Fractures." By the fall of 1941, he had relocated to Midland, Texas, and opened his own practice, the Modern Health Clinic. He advertised himself as "Dr. Henry Schlichting, Jr., Naturopathic Physician Specializing in Fractures and Dislocations."[138]

How Schlichting came to call himself a naturopath is somewhat unclear. In the mid-1930s there was no licensing in Oklahoma or Texas for either chiropractors or naturopaths. Carver Chiropractic College in Oklahoma City taught obstetrics, minor surgery, and a broader use of adjustive technique than "straight" chiropractic. Texas was mostly dominated by straights through the influence of the Texas Chiropractic College in San Antonio. Although Schlichting's early training was in chiropractic, and he was first in chiropractic practice in the Amarillo, Texas, area when he settled in Midland in 1941, he allied himself with the naturopaths in Texas and always called himself a naturopath.[139]

Also, by the fall of 1941, he had joined the newly formed ANA of Texas. At the organization's first statewide convention in Dallas—attended by more than 500 initial members—he was elected secretary-treasurer. This quick ascension into the leadership ranks of the naturopaths of Texas led in turn to his connection with the western ANA group and with Robert V. Carroll.[140]

Dr. Schlichting and the Western ANA

The 1942 convention of the ANA in Chicago was boycotted by Benedict Lust, Jesse Mercer Gehmann, T. J. Schippell, and their allies that became the eastern group. They held a "rump" convention of about 75 eastern naturopaths in Atlantic City in anticipation of the Chicago convention electing someone other than Lust as ANA president. In Chicago, Fredric Dugdale of Portland, Maine, was elected president. The president of the ANA of Texas, which since its formation in 1940 had delivered the biggest state representation to the ANA, was H. A. Brown of Canyon, Texas. He was elected first VP of the national ANA under Dugdale (Robert Carroll stayed as chairman of the Board of Directors), and in 1944 Brown was elected national president. When Carroll succeeded Brown as ANA president in 1946, he tapped Schlichting as his secretary.[141]

What made Schlichting stand out to Robert Carroll must be guessed at, but Carroll was a superb leader and organization man, and he recruited many significant naturopaths into the profession and into the western ANA. He recruited John Bastyr and many others in Washington State into naturopathy before moving on to the national stage. Schlichting had much to commend him: he became a strong ally of Harry Brown in the Texas ANA, an organization that grew to more than 400 members, most of whom also joined the national ANA; he was a talented writer and speaker; and he was a strong organization man.

Once he was placed as national secretary by Carroll, Schlichting brought all of these talents to the national ANA. He continued to advance naturopathy in Texas, and he built a busy, thriving practice in Midland. Throughout the 1940s he advertised specializing in fractures and dislocations. Midland was a western Texas oil town, and oil roughneck work was notorious for its physical toll. The Carver techniques emphasized minor surgery and a "structural" approach to chiropractic. This became known in chiropractic as the Carver Technique, and as it evolved, it became one of the roots of naturopathic physical medicine.[142]

More Background on Clinical Practice

Willard Carver "opened the Carver-Denny School of Chiropractic in Oklahoma City in 1906, which in 1908 became the Carver Chiropractic College Carver's philosophy gave equal importance to any anatomically produced 'nerve occlusion,' whether or not related to the vertebral column, while his *structural* approach to biomechanics became more 'holistic' than B.J.'s [Palmer] segmental one-bone-out-of-place approach."[143]

Carver established four chiropractic schools, in New York City; Washington, D.C.; and Denver in addition to Oklahoma City. It was the Denver school that became most significant to natural healing education and clinical technique. As Walter Wardwell described the relevant history: "Homer G. Beatty (1897–1951), who had graduated from Carver's Oklahoma school in 1922, became the dean of the Colorado school in 1923 and its president in 1924, serving until his death in 1951." In 1939 Beatty published *Anatomical Adjustive Technique.* By 1935 the school was reorganized as the nonprofit University of the Natural Healing Arts, which offered three doctoral degrees, D.C., N.D. and D.P.T. [Doctor of Physical Therapy], the last requiring 3 years of study rather than the 4 required for the others."[144]

Homer G. Beatty, DC, ND, is a part of the story of the professionalization of natural healing for several reasons. He was a part of the educational efforts of the National Chiropractic Association from its commencement in the early 1930s. He adopted the 4-year residency educational model for the UNHA DC and ND degree programs in lockstep with Budden at Western States. Although Colorado did not adopt licensure separately for NDs, his ND program at UNHA provided about half of the licensed NDs in the neighboring state of Utah by the mid-1950s. And perhaps most importantly, his book *Anatomical Adjustive Technique,* which described methods of treatment by manual adjustment for the entire anatomy, became a cornerstone for natural physical medicine.

It was this type of clinical technique that was used by Schlichting in his West Texas practice. These clinical techniques were supplemented by the treatment methods illustrated in Alton Johnson's *Principles and Practice of Drugless Therapeutics,* the first edition of which was also published in 1939. Johnson, another DC, ND, covered physiotherapy,

electrotherapy, and hydrotherapy—in addition to adjustive technique—in his book on clinical science for natural healers. Both of these works were integrated by Budden into the postwar curriculum at Western States, and both Beatty and Johnson became members of the western ANA after the war. In Johnson's case, he was recruited by Carroll and Schlichting to attend the 1948 western ANA convention in Salt Lake City and prevailed upon by them to accept the chairmanship of a new ANA committee on physiotherapy. In this position, Johnson wrote or edited a regular physiotherapy column in the journal of the association for several years (while also writing regularly for the journal *The Scientific Chiropractor*), and in the early 1950s he served a 3-year term on the national association's Board of Directors.[145]

These were the clinical techniques at the center of Schlichting's clinical practice during the 1940s when he established his practice and became a leader in the national movement of natural healers. He also became a civic leader in Midland, as a prototypical American joiner of voluntary civic associations: the Lions Club, the Jaycees, the Toastmasters, and various civic improvement efforts. Dr. Schlichting, or "Doc" to the citizens of Midland, practiced and lived very visibly in his adopted hometown, as many members of the western ANA did in the 1940s before natural healing came under assault by the AMA in the 1950s.

Back to the National Scene

As a national officer by 1946, Schlichting found himself in the middle of the dispute over control of the American Naturopathic Association, chartered in Washington, D.C. by Benedict Lust in 1919. After the "pseudo-group insurrection" of 1942, Lust remained embittered and denied the legitimacy of the "western ANA" until his death in August 1945.

But for these 3 years, the nation was at war, and many were diverted from much of civilian life. The westerners largely built a communication network, held annual meetings, and waited out the war. Peacetime would come, and by circumstance, when it did, Benedict Lust had died. Then in peacetime, internecine warfare broke out among the naturopaths, as both Carroll and his western group and the eastern group led by Jesse Mercer Gehmann, T.M. Schippell, and a new face, Paul Wendel, laid claim to the "ANA." Working with Carroll, Schlichting and a few others built up their Western "pseudo-group" while defending their right to being "the real ANA" and while constantly pushing the concept of unifying all naturopaths within one organization.[146]

Looking through the historical record, it seems clear that Robert Carroll had followed a methodical campaign to make the ANA into a true professional organization and that he had done so by amending the ANA constitution, bylaws, and governing structure. The western group had every right to be recognized as the legally constituted ANA organization as WWII ended, and Carroll's ANA represented the broad-scope practitioners from the urban areas of about 20 states, including all of the licensed states. The eastern group that survived Lust, though, continued his dispute of their claim of legitimacy from 1946 to 1950, and the issue was a constant distraction.[147]

Through 1946 and 1947 the western group, for various reasons—including a postwar paper shortage—struggled to produce a monthly publication for its membership. During this time the leadership communicated with members through a series of "Dear Doctor" newsletters that Schlichting sent out from his Midland, Texas, office as "American Naturopathic Association, Inc., Office of the Secretary." In January 1948, at long last the *Journal of the ANA* debuted, and the western ANA became a more established presence on the cultural, political, and professional scene. With much anticipation of a "Unity Convention," the group convened in July 1948 in Salt Lake City, Utah, for its annual meeting. Although the organization had grown in strength and presence—and more than 300 naturopaths attended the meeting from about 20 states—"unity" was not achieved, and Carroll passed the reins of the presidency to Schlichting.[148]

As of the summer of 1948, there were ND degree programs at National College of Chiropractic in Chicago, UNHA in Denver, Western States in Portland, and Metropolitan College in Cleveland, Ohio, that were legitimate 4-year residency colleges, as well as a program at the Los Angeles College of Chiropractic that was in a state of flux, although soon to be disbanded completely by the National Chiropractic Association. Schlichting took the presidency from Carroll at a time when the future for chiropractor-naturopaths looked promising.[149]

A historical note here is in order. Almost all of the naturopaths in the western group were chiropractors who had branched out in classic "mixer" fashion. With the exception of Robert Carroll himself, who had been a direct student of Henry Lindlahr and who had begun to call himself a "naturopath" instead of a drugless healer in the early 1930s, a historical tracking of every ND leader from the postwar era leads back to an early chiropractic college. As just two examples (Schlichting's education has been covered), Harry Riley Spitler (the lead editor of *Basic Naturopathy)* graduated from Ross Chiropractic College in Fort Wayne, Indiana, before WWI, and John Bastyr (after whom today's Bastyr University is named) graduated from the Seattle Chiropractic College in the early 1930s. Spitler was also on the faculty of the Metropolitan College from the mid-1930s until WWII. Also, in several states, "mixers" became identified as naturopaths because chiropractic "straights" took control of chiropractic licensing. Washington, South Carolina, Utah, and Texas were such states.[150]

Dr. Schlichting Becomes President

When Schlichting took over as president of the western ANA, things were at a critical juncture for natural healers, whether they identified as chiropractors or naturopaths both nationally or back home in Texas. Schlichting, while running a busy, growing practice and emerging as a civic leader in Midland, was up to the task of growing the ANA as an organization *and* achieving legal recognition for naturopaths in Texas. In doing so, he followed Robert Carroll's model: practice openly and proudly as an "ND" and "Dr.," be a civic leader, push constantly for recognition for naturopaths and legitimate ND school programs, and do everything possible to unify the profession within the ANA.[151]

As the incoming president in 1948, Schlichting had set the ANA 1949 convention for Houston, Texas, to take place coinciding with the opening of Houston's newest—and in keeping with the mottos of Texas "biggest and best"—luxury hotel. As 200 Texas naturopaths joined with 200 out-of-state naturopaths for the ANA's largest convention, Schlichting was able to announce to the attendees that the Texas legislature had passed a Texas licensing law as part of a legislative "deal," and the governor of Texas was signing off on the legislation. Within 2 years, Texas was the home to more than 400 licensed NDs—the largest licensed state in terms of numbers and the largest source of Western ANA members.[152]

The nature of the "deal" became critical within just a few years. The Texas State Medical Association wanted Texas to adopt a Basic Science Law. The Texas chiropractors wanted a licensing law for "straight" chiropractic. An earlier law had been struck down by the Texas Supreme Court for violating a provision of the Texas Constitution that prohibited giving preference to any "school of medicine." And the growing naturopathic group of 500 and counting wanted recognition. A group of legislators brokered a deal: a Basic Science Law would be adopted

first; then, subject to it, a chiropractic law crafted to withstand challenge would be adopted; and finally, a pending naturopathic bill would be adopted.[153]

The Basic Science Law passed both houses of the Texas legislature handily, the chiropractic law was adopted by slightly tighter margins, and then the naturopathic law passed the state House fairly comfortably. But then, in a harbinger of things to come and with its Basic Science Law in hand, the state medical association tried to kill the deal with a push against the ND bill in the state Senate. The bill, after much delay, passed the state Senate by one vote, 23-22, in July 1949, while the profession was in Houston for its convention.[154]

NATUROPATHS AT THEIR PEAK

The Late 1940s and Professional Growth

Under Schlichting's leadership as national and state leader—and soon as a member of the first naturopathic licensing board in Texas—the profession continued to grow. But as noted earlier, the number of schools started to decline. Los Angeles dropped its program, and Metropolitan closed up in 1949. National dropped its program in 1950 under pressure from the National Chiropractic Association. Suddenly in 1951, Homer G. Beatty, DC, ND, of the UNHA in Denver passed away and with him, in short order, so did another ND program; only Western States under Dr. Budden was left.[155]

In Memoriam—Robert V. Carroll, Sr.

And then suddenly in March 1951, Robert V. Carroll—Schlichting's mentor and the true "father" of the modern naturopathic profession passed away. As Schlichting and the editors of the *Journal of the ANA* memorialized Robert Carroll's life and career[156]

In MEMORIAM

Dr. Robert V. Carroll, Sr., died Friday, May 11th, of internal hemorrhage followed by coronary embolism. He died as he lived, suddenly and dramatically. There had been no prior illness. He went from excellent health to death in twenty-four hours.

The naturopathic field has lost one of its greatest fighters. His was not the fight for individual stature; his was the fight for rightful and legal recognition of Naturopathy. He did not aspire to a statue, a pedestal or plaque exhorting his name in superlatives; his desire was to see naturopathy reach its honored place in the sun and to be a proud member of that profession.

Now, his three score and ten has been completed. His earthly body has been laid away, but his spirit will march on. He has left a high mark on the wall. When we can measure up to it, his dream will have come true.

The world has lost a man, the nation, a citizen, and naturopathy, a leader. But in our hearts each of us knows that the loss cannot be described in words alone. He admired and respected the qualities in others that fired them to opposition. In his heart he had only friends, agreeing friends and disagreeing friends. All will miss him.

In the 1950s Henry Schlichting and the naturopaths faced challenging times that could not be foreseen in 1951. Robert Carroll's leadership and vision would be missed.

1947–1950 and Forward

When Robert V. Carroll passed away unexpectedly in May of 1951, the natural healing profession that he had helped build with 20 years of diligent work was trending upward. When Dr. Carroll passed from the scene, national leadership was then held by Henry J. Schlichting Jr., of Midland, Texas, and by A.R. Hedges of Medford, Oregon. Educational leadership was in the hands of W. A. Budden of Western States College, Portland, Oregon, and Joseph Janse of National College of Chicago, Illinois. Texas had licensed naturopaths and chiropractors in 1949 as part of a legislative "deal" in which the Texas legislature also adopted a Basic Science Law.

In 1950 the Georgia legislature had licensed naturopaths, and the Nevada legislature had also adopted licensing statutes in 1951. In the negative column at the time of Carroll's passing, National College had dropped its formal ND degree program in 1950 under pressure from the National Chiropractic Association's Council on Education, and the governor of the state of Nevada had vetoed the Nevada legislation just after the legislature adjourned. Joe Janse would proudly call himself a DC, ND, well into the 1960s and preside over a broad, "liberal" chiropractic curriculum at National, but the National College decision reduced legitimate, 4-year residency ND education to only Western States.[157]

In Texas, Henry Schlichting—"Doc" to the Midland, Texas, community—sat on a three-member naturopathic board that had processed and accepted more than 400 applications for licensing on a "grandfather" basis that was part of the 1949 legislation. Texas became both the largest state membership base for the western ANA and the largest licensed state in naturopathy. The natural healers seemed to have weathered the Tennessee scandal by 1951 and to have absorbed the lesson that H. Riley Spitler called "Remember Tennessee."[158]

Naturopathy in Connecticut[159] could have suffered much more than it did in the aftermath of the Tennessee scandal, but the Connecticut Supreme Court ruled in April 1947 that the Connecticut State Board of Naturopathic Examiners—not the state commissioner of health, a medical doctor—was the legal decision maker in licensing. As early as 1942 the health commissioner had delayed the issuing of licenses approved by the Board when the approval was under the licensing reciprocity statute and from South Carolina, a state with no Basic Science Law and that approved schools that were not on the Connecticut Board's approved schools list. The commissioner's position was that the reciprocity statue required licensing by a state with licensing requirements comparable to those in effect in Connecticut.

By 1946, when this issue emerged into public awareness, licenses issued in Tennessee—where the diploma mill and licensing fraud scandal was first coming to light—also started appearing in reciprocity applications. By the time two applicants, both with diplomas from Lust's American School in New York (which had lost its state charter in 1935) and with licenses from South Carolina and Tennessee, prevailed in the Connecticut Supreme Court, the legislature had repealed the reciprocity statutes at the urging of the governor, and the state attorney general had begun a review of all existing licenses to determine whether evidence of fraud existed in the application process.

The Board revoked 17 licenses on its own, and the attorney general revoked several more after a trial that focused on the scandal in Tennessee and the loose practices in South Carolina in its own handling of the reciprocity issue. Extensive testimony was introduced by the attorney general's office about the Tennessee licensing scandal from depositions taken in Tennessee, and also about the extent to which South Carolina "rubber stamped" reciprocity application in 1945 and 1946 supported by Tennessee licenses.

In the long run, the Connecticut Board emerged with its licensing authority intact for applicants who graduated from properly approved schools and after successful passage of licensing examinations. The same proved to be true in South Carolina but only after the Board there went through a major legislative scrutiny process and adverse publicity of its own.[160] During the 1947 South Carolina legislative session, the state House of Representatives passed a "concurrent resolution" asking

for an inquiry into "examinations and personal qualifications required of applicants to practice naturopathy in this state and the propriety of granting licenses therefore."

During the 1948 legislative session, the full resolution was addressed between the House and the Senate (which was more favorable to the naturopaths), instructing the state Naturopathic Board to clean up its own house and report to the legislature by the 1949 session. As reported in the press, "The assembly … asked the State Board of Examiners to look into this situation, remedy it if possible."

In February 1949, the Board reported to the legislature "that it had revoked a number of reciprocity licenses, giving the holders a chance to regain them by taking state examinations … Most did and were relicensed … But at least one holder of a license refused to accept the board's order and has brought court action against it." The Board requested stricter licensing laws and stronger disciplinary powers, and the legislature obliged.

After the Scandals

These two states were the most directly affected by the Tennessee scandal. Connecticut was "purged" by the actions of state authorities; South Carolina was purged by aggressive action of the State Naturopathic Board. Licensing in both states survived the Tennessee infection. As a reflection of post-Tennessee reality, the 1949 licensing of naturopaths in Texas included a specific reference in its "grandfather" provisions that provided that licenses issued in the state of Tennessee were not to be considered for any purpose, recognizing that the Tennessee legislature had acted to invalidate any licenses that had been issued.[161]

With a substantial base to work with in Texas, Schlichting decided that the naturopaths should address their own education issues by establishing a legitimate 4-year residency college within the state to be supported primarily by the Texas profession. Lack of "Class A naturopathic colleges," Schlichting wrote in announcing the founding of SCNM to the profession, "is a threat to the perpetuation of our profession." In 1951 the Southern College of Naturopathic Medicine (SCNM) was chartered, and an agreement was made with the newly named Texas Southmost College—a 25-year-old institution previously known as Brownsville Junior College and located in Brownsville, Texas, to serve as the home for SCNM.

The administrative offices for were located in a building on the Texas Southmost campus, and the premed and basic science courses for SCNM students were taken through cross-enrollment at Texas Southmost. The first major event sponsored by SCNM was a 2-week postgraduate seminar cosponsored with the Texas Naturopathic Physicians Association and held on the Texas Southmost campus.[162]

A Change of Identity

The summer of 1951 was eventful for the naturopaths for several reasons. Meeting in Miami Beach, Florida, the western ANA changed its name to the American Naturopathic Physicians and Surgeons Association (ANPSA), reelected Dr. Schlichting as its president, and established its corporate charter and located its headquarters in Des Moines, Iowa. The reasons behind this organizational restructuring were explained in articles by Schlichting and by A.R. Hedges in the September 1951 issue of what was now the *Journal of the ANPSA*. The changes were necessitated, they said, by the last resistance to the newly unified ANA continuing to lay claim to the name American Naturopathic Association. The group felt strong enough to take on a "post-Lustian" identity in representing the natural healing professions.

Robert Carroll would likely have never accepted this one step. He proudly carried the banner of the ANA and felt—correctly—that he was entitled to do so. But Schlichting and Hedges felt that the profession could use the new strength that came with licensed status for naturopaths in Texas, added to the existing licensed states, to support a modern profession, a reinvigorated educational system and a staunch commitment to the *vital force*. Carroll had always preached the value of science in explaining the theory of natural healing and the value of supporting work like Cannon's vision of *homeostasis*. Schlichting preached the value of a professional identity as "family physicians" in a general family practice that included minor surgery and obstetrics.

The new professional organization, he pointed out, was formed to "promote the public health and to perpetuate and advance the science, art and practice of the naturopathic school of medicine; to accomplish such objectives by attaining high standards of naturopathic education and by constantly stimulating and furthering the profession's interest in and knowledge of the diagnosis, treatment and prevention of … disease and ill health." As things trended upward for scientific natural healing in 1951, it was through this vision.[163]

Medical Dominance Arises

But all of this began to change dramatically in 1953, and what seemed so promising at the beginning of the 1950s was totally eroded by the end of the decade. No developments demonstrated this as much as the history of natural healing in Texas over just 10 short years. The force of medical dominance began to rear its head in 1953 at the annual convention of the AMA. A resolution was introduced by the Alabama delegation to the House of Delegates to attack chiropractic and naturopathy at their "weakest point," their school and colleges.

At the behest of AMA leadership, this topic was referred to the AMA educational committee rather than passed on by the House of Delegates. The educational committee felt that the AMA should refrain at the time from weighing in directly on the schools and colleges of other professions for political reasons, but the sentiment of the resolution was taken by AMA leadership as a strong interest by the medical profession in moving politically against these remaining "healing cults." The matter was passed back to the state medical societies to deal with at the state level, with the full support of the national association.[164]

Historian Monte Poen called the AMA "the country's richest and most influential post-World War II lobby." In assessing the powerful effect of the AMA's lobbying, Poen (from his research in the 1970s) noted that "As to the role played by organized medicine, I have become more impressed by the medical community's ability to influence public opinion" in the post-WWII 1940s and 1950s.[165]

At the state level, organized medicine used its ability to influence public opinion to sway the views of mainstream newspapers, government officials, and state legislators. This was important because professional legitimization is established in the United States on a state-by-state basis. This has been true since 1889 when the US Supreme Court decided *Dent v. West Virginia*. The history of this first medical licensing case is the subject of James Mohr's *Licensed to Practice: The Supreme Court Defines the American Medical Profession*.[166]

Dent, the petitioner in the case, was an Eclectic physician at a time in history when there were three schools of medicine: the Regulars (called "allopaths" according to Samuel Hahnemann), the homeopaths (as homeopathy was conceived by Hahnemann), and the Eclectics (which included the physio-medicalists). The Regulars in West Virginia founded the West Virginia Medical Society in 1867 and were the moving force behind the state licensing law adopted by the state legislature in 1882. By adopting legislation that required education at a Regular school, the licensing law barred practitioners from the two other schools of medicine from practicing in West Virginia.

As David Korostyshevsky summarized the key aspects of the situation in his review of Mohr's book in the *Journal of the History of Medicine*[167]:

Grounding his analysis in both legal and medical historiographies, Mohr argues that while the American public supported public health efforts to control epidemic disease, medical licensing was not a popular reform. It was instead "a consciously engineered policy, drafted and passed through the concerted efforts of a specific subset of physicians, the elite Regulars" of the Medical Society of West Virginia (156 of Mohr). Mohr also challenges the interpretation that medical licensing was a response to the growing complexity of scientific medicine. Because scientific medicine did not produce tangible results until the 1930s, the push for medical licensing is a consequence of economic and political factors, not strictly scientific ones. Finally, Mohr shows that the Supreme Court upheld a version of medical licensing that relied on the quality of a physician's education as the only measure of competence.

Because the Regulars were the largest of the three schools of medicine in 19th-century America, the *Dent* decision allowed the Regulars to achieve the elimination of the two other schools on a state-by-state basis, which, by the early 20th century, went a long way toward eliminating the other schools.

THE BEGINNING OF THE END

The Texas Medical Wars

All of this became relevant to events in Texas just as the naturopaths under Schlichting's leadership had begun to achieve success professionally and to create an educational institution that would fill the void left by the NCA decision to require chiropractic schools to abandon the training of naturopaths. As Schlichting transferred the presidency of the ANPSA to Hedges in 1952, he focused even more on his position as secretary of the Texas State Naturopathic Examining Board.[168] After Robert Carroll's unexpected passing, Budden and Hedges further advanced natural healing in Oregon—and tried to secure natural healing throughout the Pacific NW. Schlichting tried to do so in Texas at the same time, hoping to secure the Southwest (Texas in addition to Arizona) for natural healing as well.

The success of the naturopaths in Texas was targeted by the Texas Medical Association and the Texas Medical Board in 1953. The medical campaign against the naturopaths began in a remarkable way. When the 1949 legislature adopted the Texas Naturopathic Act (Article 4950d, Vernon Codified Statutes), its passage was during the term in office of Texas Attorney General Price Daniel (1947–1953). When Schlichting, as secretary of the Texas State Board of Naturopathic Examiners, sought guidance from the attorney general (AG) on the "grandfather clause" of the Naturopathic Act in 1952, that guidance was provided under AG Opinion V-1486, dated July 29, 1952, directed to Schlichting as a state official seeking a necessary interpretation of state law. Such guidance to state officials was—and is—a function of the AG's office under the Texas Constitution and Texas law.

In 1953 Price Daniel—later a US Senator (1953–1957), governor of Texas (1957–1963), and justice of the Texas Supreme Court (1971–1978)—was succeeded as AG by John Ben Shepherd. Shepherd's office received a letter requesting consideration of two questions challenging naturopathic validity under the Texas Constitution from the criminal district attorney of San Antonio, Texas.[169] That district attorney was considering bringing action against naturopaths in Bexar County, Texas, if the Naturopathic Act should be invalid. This action was being requested by the county medical society and state medical association.

In this situation, the Texas AG can—on a discretionary basis—serve a unique function, that of "an alternate Supreme Court."[170] This function is discretionary, but with the matter under consideration by a new AG, briefs were solicited by the AG's office and submitted on the issue. In spite of "three very able briefs" arguing in favor of the enforceability of the Naturopathic Act, G Opinion S-60, dated June 29, 1953, was issued, finding the act to violate the Texas Constitution: "SUMMARY: The Naturopathic Act, Article 4590d. V.C.S. violates the provisions of Art. XVI, Sec. 31 of the Constitution of Texas in that it gives a preference to one segment of the healing arts. To rule otherwise would require a holding that the Act is uncertain and indefinite and thus unconstitutional. *State Ex. Rel. Halsted,* 182 S.W. 2d 479 (Tex. Crim. 1944)."

The complete loss of naturopathy in Texas and the end of the Texas career of Henry J. ("Doc") Schlichting Jr., ND, would not be final for another 5 years. But this was the beginning of the end, and in the next 5 years Georgia, South Carolina, Florida, and Utah would be lost as well. Medical dominance as wielded by organized medicine was under way.

The fallout for naturopaths in Texas after the AG Opinion S-60 issued in July 1953 developed slowly at first. The 1953 session of the Texas legislature had just ended 2 months earlier, so there was no immediate opportunity to seek legislative relief. At the same time, although AG Opinion S-60 had considerable meaning, it was not the same as a decision by the courts. Schlichting and the Texas NDs worked quietly behind the scenes to take stock of the situation and to plan how to proceed. Judging by the material in the *Journal of the ANPSA* and the Texas newspapers, a decision was made by the Texas Naturopathic Physicians Association not to publicize the problems created for the Texas NDs by the attorney general's opinion.

Back to Education: WSC and *Natura Medicina*

Events for naturopaths for the rest of 1953 largely took place outside of Texas. After the very successful ANPSA convention in Portland and the Western States commencement for the ND class of 1953, the news at the end of the year was the 5-years-in-the-making publication of *Natura Medicina.* Within the first year of work on *Basic Naturopathy,* it became apparent to the primary editors—H. Riley Spitler and Pers Nelson of Connecticut—that inclusion of medicinal substances used in naturopathy would need to be reserved for later so that *Basic Naturopathy* would be focused on and confined to theory. President Robert Carroll of the ANA appointed a committee in April of 1947 to prepare a textbook on medicinal substances in use in naturopathy. The committee was formally designated the Natura Medica, Formulary, and Therapeutics Committee. The committee first convened to arrange committee assignments at the 1947 convention in Detroit; A.W. Kuts-Cheraux had been appointed committee chair. Originally the sections for inclusion were vitamins, cell salts, botanicals, and endocrines.[171] Correspondence was sent out by the committee to gather information from "the men in the field," as Kuts-Cheraux reported to the 1948 convention in Salt Lake City. Several complications that emerged from this survey of the profession were outlined by the committee chair in this report: (1) many of the botanicals in use had not been "subjected to the usual chemical analyses, alkaloid and glucoside determination … physiological properties and pharmacological action is very vague"; (2) many favorite botanicals had been identified by practitioners in "homely lay terms"; (3) "many agents endorsed by some practitioners were condemned by others as of no value"; and (4) a major issue that had not been "satisfactorily settled" had been the inclusion of Harrison Act narcotics that had been legalized by the Florida courts for use. Five more years would be required to bring all of the committee's work to its fruition.

The end product was worth the wait in general terms; the book was cutting edge in its contents and scope.[172] In addition to Kuts-Cheraux, there were major contributions to the work by Dr. Herbert Clough on the formulary of selected botanicals and by Dr. Helena Winters of Kelso, Washington, on vitamins and tissue cell salts (with assistance from Dr. H. Riley Spitler, chief author of *Basic Naturopathy*). Reinforced by the entirety of the book's approach to natural medicine was what had become the organizing philosophy of naturopaths in clinical practice: health and healing were emphasized rather than conventional medicine's prevention and treatment of disease, the vital force and homeostasis were a critical core element of the philosophy, and suppressive pharmaceuticals with their side effects were to be avoided.

But it arrived at an unfortunate time, just as the assault on naturopaths and chiropractors by organized medicine was getting under way.

The textbook, for instance, arrived too late to be put into use at Western States during the 1953–1954 school year. Dr. Budden's unexpected death in August 1954 kept the textbook from ever being incorporated into the curriculum. And as events would play out, this cutting-edge work arrived as the naturopathic profession was contracted severely by governmental action in the mid-1950s.

When Dr. Budden passed away, a remarkable force of nature, a remarkable liberal chiropractor, and a great naturopath was lost to the world and to natural healing. The *Journal of the National Chiropractic Association* for September 1954 had "A Tribute" by J.J. (John) Nugent, DC, director of education:

> *Dr. W. A. Budden, director of the Western States College of Chiropractic, Portland, Oregon, died suddenly in Portland on August 1, exactly one week after his return from a meeting of the Council on Education of the National Chiropractic Association.*
>
> *Dr. Budden was one of the pioneer leaders in chiropractic education …*
>
> *As much as any man in our profession, he espoused and introduced high educational standards in our schools.*
>
> *An important and forceful representative of our interests, his authoritative voice was respected and listened to in our legislative halls …*
>
> *To many, Dr. Budden's passing will mean that a great chiropractor, thinker, and educator has passed into history. And it is so! He was one of chiropractic's great …*
>
> *He was a vigorous and indomitable fighter for truth as he saw it for freedom of the individual, and, above all, for intellectual integrity …*
>
> *We will miss him sorely, the … profession has suffered an irreparable loss …*
>
> *Dr. Budden was born a gentleman, and lived and died by that high code.*
>
> *We shall not forget him!*

What was true for chiropractic was true for naturopathy as far as Dr. Budden's career and commitment were concerned. Both professions owe a deep debt to Dr. Budden that *should not* be overlooked and certainly not forgotten.

Events were building to a devastating outcome: the naturopaths were going to lose Western States College as an educational base and Texas as a professional base. When Dr. Budden died in August 1954, Western States itself was at a critical juncture. In the 6 months before Budden passed away, the three issues that would cause such trouble for Western States after his death had begun to appear on the horizon.

WSC and Hard Times

First, the postwar boom fueled by the G.I. Bill was coming to an end. It is difficult to overstate the effect on the chiropractic colleges generally, and Western States specifically, of the G.I. Bill adopted by Congress as the Serviceman's Readjustment Act of 1944. One of the main provisions of the act was the funding of higher education for those who elected this benefit. The funding available covered all of the costs of higher education: tuition, fees, and textbooks. This was true at public and private liberal arts universities and colleges but also true at WSC. By the time the act expired, almost 3 million veterans had attended institutions of higher education nationally, paid for by the federal government.[173]

Second, the new matriculation requirements that Budden had established at WSC and had convinced the Oregon legislature to put into law were taking effect. In an article entitled "The Aspects of Two Years Preprofessional Study as an Entrance Requirement," published in the *Journal of the National Chiropractic Association* in March 1954, Budden noted that "economically, the schools … that follow the two-year plan must expect to experience an, at least temporary, set back in revenue, and it would certainly be unwise … for an institution to venture in [this] direction unless its financial underpinning is of the caliber to absorb the shock through the lean years."

Third, the issue of Dr. Budden continuing the WSC of Naturopathy began to become a serious irritation within the Council on Education (COE) of the NCA. Indeed, there is every indication that only Dr. Budden's personal standing within the NCA and the COE kept the issue from becoming a more persistent matter while he was alive. At the semiannual meeting of the COE held February 11 to 13, 1954, in San Antonio, Texas, Budden raised the subject himself. The minutes for Friday afternoon, February 12, 1954, reflect that "Dr. Budden asked for a frank and open discussion on the Naturopathic issue."[174]

The conversation continued for the rest of the afternoon and again the next morning, as reflected in the minutes. Dr. John Nugent "reminded Dr. Budden that the Western States College was the only remaining school on the accredited list that still conducted a course in Naturopathy." Dr. Budden set out his position on the matter. It was better to "sustain a reputable school of naturopathy" to establish an educational standard that legislatures could look to. Legislation in both Idaho and South Dakota that would have diluted educational standards substantially had been defeated by pointing to Western States as the baseline for naturopathic education. On that note, the afternoon adjournment was taken.

When matters picked up the next morning, "Dr. Budden gave a complete review of the history of naturopathy and asked the Council to give him concise opinions and expression of decision" that he could share with the WSC Board of Directors so that "future policies of Western States College could be determined." Budden was reminded that at the midyear meeting in 1950, both Dr. Janse of National, together with his administrator, and Dr. Budden had been told to discontinue courses in naturopathy. Dr. Budden's Canadian protégé Dr. A.E. Homewood (dean of the Canadian Memorial College of Chiropractic [CMCC], chartered in 1945) "inquired as to the attitude of the Council" if the CMCC initiated courses in naturopathy "to accommodate those Provinces in Canada that sustained naturopathic laws?" Budden also inquired "whether the Council had the right to remove a college from the accredited list if it continued to conduct a naturopathic course?"

WSC and the NCA

The consensus of the council was stated as follows: (1) The Council "would frown on" the CMCC initiating any naturopathic courses, as

a degree or otherwise; (2) Dr. Budden was advised to tell the directors of the Health Resource Foundation as the governors of WSC that "recommended that the course and school of naturopathy as conducted at Western States College be discontinued as soon as obligations and commitments could be fulfilled or terminated"; and (3) the council "does have the right to remove a college from the accredited list if a naturopathic program was operated concurrently with the chiropractic program at an accredited school."

Three weeks later Dr. Budden presided over the 1954 commencement for a class of four ND degree recipients and 26 DC degree recipients, 13 of whom also received the BTS degree.[175] This was the state of matters when Dr. Budden passed away suddenly in August of that year—1954.

Back to Texas

In 1954 Schlichting left most of the national efforts to A.R. Hedges, who had the advantage of working from Oregon and so of having a stable licensing situation around him. The year was spent in Texas getting ready for the 1955 legislative session and weathering a summer of bad news that was beyond the profession's immediate control. Harry Hoxsey was a controversial figure before naturopathy was licensed in Texas in 1949, if not widely known outside the Southwest. After being pursued in the Midwest and charged with practicing medicine without a license for the use of his Hoxsey cancer treatment, Hoxsey opened his Dallas cancer clinic, which grew to a substantial patient volume by the early 1950s.[176]

The Texas Medical Wars

Relevant to naturopathy in Texas, Harry Hoxsey applied for licensing in 1950 under the grandfather provisions of the Texas law, and under the statute, he was deemed qualified to be licensed. First Carroll, and then Schlichting, kept Hoxsey away from the ANA/ANPSA, although Hoxsey had his supporters. But in 1954 when Hoxsey was in the national headlines and consistently reported on in the Dallas newspapers, he was called "the Dallas Naturopath who runs a cancer clinic," and headlines like "Dallas Naturopath Enjoined from Claiming Cancer Cure" appeared. Hoxsey, with the backing of a Pennsylvania state senator, had opened a cancer treatment clinic in the Miners Hospital in Spangler, Pennsylvania. The facility was run by the United Mineworkers Union, and the senator was the hospital's administrator. Convinced of Hoxsey's success in cancer treatment the full backing of the facility was arranged.

But at this point, the federal government moved in to bar the shipment of Hoxsey treatment preparations from Texas to Pennsylvania and the use of the "drugs" in that state. Several months of publicity about the legal dispute joined together "naturopath" and "fake cancer cure" in news coverage. None of this would prove helpful when the Texas legislature went into session in January 1955 and the Texas Naturopathic Physicians Association (TNPA) sought to have legislation passed that would overcome the AG's objection to the naturopathic law adopted in 1949.

The TNPA legislative committee under Schlichting's chairmanship hired a well-placed executive secretary who had been the executive director of the Texas American Legion to pursue a legislative fix to the licensing situation. New legislation was prepared with the assistance of former legislators who were practicing attorneys with an eye to resolving the AG's constitutional concerns. The legislation—House Bill 6—was introduced in the state House of Representatives in January at the start of the legislative session. It was quickly passed out of the House Public Health Committee, catching the medical profession off-guard. Then, just as quickly, members of the House started receiving a "flood of protests [that] began to pile up on legislators' desks." In mid-February, the bill was sent to the State Affairs Committee by a 92-46 vote of the House, where the legislation died.[177]

More Hard Times at WSC

After Dr. Budden's sudden passing, the leadership of the governing board of WSC—the Health Research Foundation (HRF)—fell to Dr. Milton Higgens of Couer d'Alene, Idaho. Dr. Higgens was himself a DC, ND, although Idaho had not licensed naturopaths. Dr. Higgens had been the Idaho representative to the NCA for several years and had been a friend and colleague of Dr. Budden and a director of the HRF for many years as well. Higgens realized in short order that the HRF would have to succeed Dr. Budden in setting WSC policy and that he would need to find someone to take over day-to-day management of WSC as well.[178]

In December 1954, Dr. Ralph Failor, DC, ND, a Portland-based alumnus of WSC (a class of 1953 member) signed on to do this job. As correspondence between Dr. Failor and Dr. Higgens demonstrates that Failor was established at WSC as more of a senior VP; he managed the college day to day on-site but with tightly constrained authority. He wrote full letters to Dr. Higgens once or twice a week and sought and accepted guidance from Dr. Higgens constantly. Dr. Higgens's guidance came in responsive correspondence, also once or twice a week.

The Failor–Higgens correspondence from December 1954 through April 1955, has three constant themes: on a day-to-day, week-to-week basis finances were very tight; the prospects for the future were very bleak; and the chiropractic and naturopathic professions were no help, although they were always "supportive"—lots of "thoughts and prayers."

The professions were particularly frustrating to Dr. Failor. As of April 1955, Dr. Failor had met with the "liberal" DCs of Oregon and Washington as well as the NDs from both states and the NDs from British Columbia. The DCs in Oregon were "supportive" but more concerned that the NCA needed to help the practicing profession more than anything. Legislative matters kept arising, and Dr. Budden was not around to address these anymore. The Washington NDs and "liberal," NCA-connected DCs needed WSC's structural appearance for their purposes, more than the actual institution; both professional groups needed to be able to cite WSC as the "model" for DC and ND education but took the existence of the college for granted.

Nothing highlighted this more than a proposal from the Oregon NDs when Dr. Failor met with their leadership to ask for financial help. As communicated by Dr. Failor to Dr. Higgens: "They asked if we would accept money sufficient to put out a Naturopathic catalogue. I said yes we would. Then, they said, well that is contingent on your [sic] writing the catalogue as we want it written." Meetings with the Washington NDs focused on the NDs submitting legislation that would use the WSC curriculum as an educational model.

Dr. Failor had received contact from both the Florida and South Carolina ND Boards asking for catalogs from WSC to document that an ND educational institution did exist. Dr. Failor wrote to Dwight James, the national executive secretary, to clarify the situation; on behalf the national AANP, Mr. James offered "that the word phytotherapy instead of Herbology, and Naturopathic Medicine instead of non-medical was to be almost a must in the new issue [of the catalogue]."

By the end of the spring term in 1955, Dr. Failor had become frustrated and dismayed by what he saw as WSC's financial status and even more so with what he saw as WSC's financial prognosis. As early as February 1955, Dr. Failor was recommending to the trustees of the Health Resource Foundation that governed WSC that the doors be closed, but the Board refused this assessment.

As Western State's historian Lester Lamm described the situation in the first academic year after Dr. Budden's death:

> *Unfortunately for Dr. Failor, he unknowingly accepted the leadership position at the beginning of one of the most troubling periods in the college's history. The deluge of postwar students that created the boom period dried up overnight, leaving the college to face almost insurmountable financial difficulties. In response to student demands for a better education in better facilities, the college had moved to a new campus in 1947. It had retooled its curriculum, increased its admission requirements, added new faculty and staff, and assumed additional obligations in anticipation of a financially robust future. In short, by 1955, the college was overextended.*

The peak year of G.I. Bill college enrollment nationally was 1947. Across the country. institutions of higher education scrambled to accommodate demand, adding and improving facilitates and faculty. As Lamm noted, WSC was part of this great national response to a newly created marketplace for higher education that became a core piece of the postwar American middle class. This cumulative effect on Western States was borne out by a March 1955 report by Dr. Failor to the HRF Board:

> *At a meeting of the board a month later, Dr. Failor shared a compilation of enrollment data from the previous four years. The trend was beyond alarming; it prognosticated doom.*

1.1.1.1.1.1.1.1 1950–51	*1.1.1.1.1.1.1.2 177*
1.1.1.1.1.1.1.3 1951–52	*1.1.1.1.1.1.1.4 151*
1.1.1.1.1.1.1.5 1952–53	*1.1.1.1.1.1.1.6 125*
1.1.1.1.1.1.1.7 1953–54	*1.1.1.1.1.1.1.8 99*
1.1.1.1.1.1.1.9 Spring 1955	*1.1.1.1.1.1.1.10 68*

> *Only 24 students were projected to enroll in the fall class.*

The declining enrollment through 1953 to 1954 was consistent with the G.I. Bill wave effect, and by the fall of 1954, it was known that the G.I. Bill benefits were scheduled to expire in June 1956 and were not expected to be further extended by the Congress. This meant that students who enrolled after the fall term in 1952 would at some point have to pay their own way. For the entering class of 1954–1955 (the class of 1958), the numbers were further depressed by the new 2-year college attendance prerequisite. Dr. Failor reported that of 49 solid applications for admission in the fall term of 1954, 30 were advised that they did not meet the entrance requirements, and only 19 students could be accepted.

Dr. Failor and WSC's School of Naturopathy

Some of this was undoubtedly foreseen by Dr. Budden, and it seemed like Dr. Budden had been forced to navigate the choppy seas of being a DC, ND "schoolman" for 25 years. What his plan might have been, though, no one seemed to know. The HRF Board was now two members who had worked with Dr. Budden for years plus Dr. Budden's replacement directly recruited by Dr. Nugent of the Council of Education of the NCA. They continually instructed Dr. Failor to stay the course and carry out Dr. Budden's vision. It was hard, though, for Dr. Failor to see the way.

As Dr. Lamm reports:

> *Dr. Failor met with a number of naturopathic trade organizations throughout the Pacific Northwest over the subsequent months to solicit support. Most in the naturopathic community voiced support of the college, but little in the way of direct financial backing ever materialized. What did materialize, however, was the naturopaths' growing resentment for the lack of representation on the HRF board and at the college. The naturopathic community had not gotten over being ignored when they first brought their concerns about equitable representation to Dr. Budden in 1948. Dr. Failor's appeal to the naturopaths for support only widened the rift between the two disciplines.*

Dr. Budden had always been in a position to speak to the naturopaths as one of them. This personal relationship earned over 25 years was gone. Unlike 1948, Robert Carroll was not around to tell the naturopaths to "buck up" and support the only school that they had. In May 1955, matters came to a head at a special meeting of the HRF Board with the naturopaths of WSC, plus members of the naturopathic and chiropractic professions from Oregon, Washington, and British Columbia—those most affected by developments at WSC.

This meeting followed a meeting on the evening of Thursday, May 12, 1955, of Dr. Failor, Dr. Higgens, and Dr. Williamson, the secretary of the HRF Board of Trustees. Also present by invitation were two well-placed DC, NDs, Drs. Ralph Hill and Ross Elliott. The discussion focused on the "paramount" financial issues: that monthly revenue was $4000, whereas expenses were $500; with faculty underpaid, fixed costs deemed "excessive," and "old, delinquent accounts" also deemed excessive. Further discussed was that the HRF trustees were constantly accused of falsifying the financial status (i.e., "crying poor"), carrying out a "silence campaign" (i.e., refusing to provide specifics publicly), and that "Dr. Higgens was trying to close the College."

Also, the various DC and ND professional interests in Oregon and Washington were discussed as they affected WSC. These interests were summarized in the meeting minutes: "Chiropractic: O.A.C.P. Liberals—want controls; Advertisers—want controls; Straights—want no part; Naturopaths: Nature Group—want no part; Medical Group—want shots and medicine included in the Naturopathic Curriculum at College (Not permissible under our Charter.)." In short, the professional groups either wanted much more say where the college was concerned or had no interest in the affairs of the college.

Drs. Higgens and Williamson decided, according to the minutes, to "call a meeting of the Full Board for Saturday, May 14th, 1955." In addition to the HRF Board, it was determined that the professional groups, specifically including the OACP and "officials of the naturopathic Physicians," would be invited to provide "council [sic] and suggestions."

On the afternoon of May 14, the meeting took place. Unfortunately, a reading of the minutes of the meeting can only be summarized as "things went badly."

Naturopathy in the Cross Hairs

Dr. A.R. Hedges—then finishing his third year as the president of the national naturopaths (now named the American Association of Naturopathic Physicians [AANP])—could not attend due to a family illness. This was unfortunate, as from the notes of the meeting, it appeared that Dr. Higgens and Dr. Hedges got along, but most tellingly, it appeared that Dr. Higgens and Dr. Bleything did not—or at least that Dr. Bleything was suspicious of Dr. Higgens's longtime membership in and connections with the NCA and Higgens's friendship with Dr. John Nugent of the NCA.

One of the issues that had the naturopaths most agitated was the attitude of the Council on Education of the NCA. As Dr. Lamm reports:

> *Sensitive information contained in correspondence and conversations between WSC and the Council on Education had been leaked to the naturopaths. Most alarming to the naturopaths was the suggestion that WSC would be denied accreditation from NCE if it didn't discontinue offering the ND degree … A letter from the CE was read to the attendees, which clearly described its position*

regarding the relationship between the two professions. Even though the letter contained no threat from the CE to deny WSC accreditation, it was clear a "divorce" was being encouraged.

The attitude that drove the Council on education—made up of the other NCA "schoolmen"—was summarized by Dr. Higgens as "[the] Association felt that WSC was taking too many Chiropractic students and making Naturopaths of them." Higgens told the naturopaths at the meeting that Dr. Nugent—even though as the director of education for the NCA he reported to the Council, and was therefore "the Messenger"—was "recommending against great pressure that we continue Naturopathy at WSC, is fighting the battle for us in the NCA." And in fairness, Dr. Budden did *not* take chiropractic students and make them into naturopaths; he took students and made them into chiropractic and chiropractic-naturopathic *physicians.* And as Higgens told the meeting, there "is no difference between Chiropractic and naturopathy, in this state [Oregon] where most men have two licenses—most do not know which they are practicing under."

Events over the next year would bear out what Higgens said about Dr. Nugent. Although Dr. Nugent generally did not favor dual-degree programs, he had been a staunch friend and admirer of Dr. Budden as a schoolman and honored Dr. Budden's vision after Budden was gone. The rest of the schoolmen on the NCA Council had tolerated Budden's position while he was alive, on the Council and in their midst. They were not inclined to be tolerant after Dr. Budden could no longer argue the case himself.

Bleything told Higgens at the meeting that if naturopaths were on the Board of the HRF and therefore had more say about the future of WSC, the naturopathic profession would contribute to the college. The overriding concern appearing in the meeting minutes was that the NCA Council would threaten to strip WSC's accreditation unless the naturopathy program was dropped and that because the HRF Board was three chiropractors now, they would abandon the ND program if the threat was made.

Higgens acknowledged that this could materialize as soon as the July NCA meeting. Bleything told the meeting, and Higgens, that if the HRF Board was expanded from three to five and the new seats were given to the naturopaths to fill, the naturopaths would commit financial resources to WSC, as the matter had been put before the Oregon Naturopathic Physicians Association, stating, "If Naturopathic profession gets representation on that Board you will get $10,000 without bowing to Nugent or anybody else." Higgens was openly resistant to expanding the Board, but when the meeting ended at 5:45 that afternoon, the HRF founders group convened to Dr. Failor's office, and by six they announced that they had voted to expand the HRF Board to five members, at least two DCs and two NDs.

Dr. Higgens had become the dominant player in the present and future of WSC since the passing of Dr. Budden. He was dedicated to the continuing existence of Western States as a chiropractic college, accredited by the National Chiropractic Association. From his correspondence with Dr. Failor, it is apparent that although he had his own dedication to the School of Naturopathy, he had no expectations that the naturopathic profession would be of any financial help in the effort to keep the school open. He had learned through Dr. Failor's efforts not to have expectations of the Oregon DCs as well. As an executive director of the NCA, he *did* have expectations of the NCA Council on Education and the NCA generally. Critical to him, as a result, was maintaining NCA accreditation.

On June 4, 1955, the 46th Annual Commencement of the Western States College of Chiropractic and Naturopathy was held. In spite of financial pressures, WSC was still open, and in spite of the critical observations by the Council of the NCA, 22 doctor of chiropractic degrees and 1 doctor of naturopathy were awarded (along with three X-Ray Technician Certificates and two Laboratory Technician Certificates).

The NCA met in early July, and although the Council minutes from that meeting reflect no on-the-record discussion of the WSC naturopathy program, immediately after the Council meeting, the HRF reversed itself and reinstated the three-member Board provision. For whatever reason, no financial commitment to WSC ever materialized from the naturopaths, and as of the 1955–1956 school year, the matter remained unresolved, with the naturopathy program under a cloud.

THE END

Things remained as dire as they had been during the main school year as the summer term was to begin. Dr. Failor had advised Dr. Higgens in correspondence that Dr. Bleything would organize and run the summer term on a no-pay basis. Bleything and others who similarly volunteered taught the summer term, and on September 27, 1955, 13 of the DC graduates from June received their ND degrees, and the lone ND graduate from June—Canadian Robert Fleming—received his DC degree.

But neither the school nor the naturopathy program was out of the woods. Dr. Lamm writes:

By the July 23, 1955, meeting of the HRF board, it was apparent to the trustees that continuing an affiliation with the naturopaths would be problematic if WSC was to achieve accreditation. At the meeting it was said, 'We are privileged to continue naturopathy… [until] we can gracefully work out a solution.' … It was clear by the reaction of the naturopaths that financial support for the college would not be found with them.

How or why this "was clear" based on events between May 19 and July 23 *does not* seem clear. It seems that before the addition of two new members to the Board could be implemented, and presumably based on issues that arose during the NCA meeting in Atlantic City, July 4–8, 1955, the Board withdrew the one condition that the naturopaths put on their financial support for WSC. (The minutes of the Council on Education meeting during this NCA annual convention do not reflect any on-the-record discussion of the naturopathy program. Dr. Failor *was* present and participated in the 3 days of Council meetings, which focused considerable attention to the 2-year college preparation admission requirement.)

How or why the naturopaths let the established WSC naturopathy program be discontinued is lost in the fog of history. It is clear that the naturopaths associated with Western States trusted Dr. Budden and worked well with the Oregon Association of Chiropractic Physicians, the Oregon NCA affiliate. Indeed, as Dr. Higgens observed, the professions in Oregon had substantial overlapping membership. But Dr. Failor, as a DC, ND, asked for help that was not forthcoming: "Dr. Failor met with a number of naturopathic trade organizations throughout the Pacific Northwest over the subsequent months to solicit support. Most in the naturopathic community voiced support of the college, but little in the way of direct financial backing ever materialized."

So the determination of whether to continue the naturopathy program or not was left to the HRF Board and its dealings with the NCA Council, which was hostile to the naturopathy program. Again, in fairness, Dr. Higgens told the Washington NDs in January and again in March that they should dispatch a representative or representatives of their own to the NCA Council to present the naturopathy program as an integral part of Western States and ask that both WSC's ND program *and* its accreditation be maintained as they had been during Dr. Budden's lifetime. But they never did so.

The End, Part 2

Matters came to a head during the midyear NCA Council meeting in Toronto, Canada, February 15–17, 1956, as reflected in the meeting minutes. In September 1955, as the 1955–1956 school year had begun, Dr. Higgens had stepped in to try to at least stabilize WSC's finances:

> *In response to a clearly desperate situation and in an effort to bring about financial stability, Dr. Higgens stepped forward on Sept. 8, 1955, and committed his personal assets by securing a $39,000 mortgage on behalf of the college and took out a $10,000 life insurance policy, naming the college as beneficiary. This was not the first time Dr. Higgens had reached for his checkbook to rescue the college, nor would it be the last. Regardless of the deteriorating situation at the college, and perhaps in spite of it, he was committed to doing everything possible to guarantee survival of WSC.*

If WSC had been Dr. Budden's school for 25 years, it was now firmly Dr. Higgens's school, governed by him and the two fellow chiropractors on the HRF Board. At the meeting in May 1955, Dr. Higgens had been openly questioned about his personal commitment to continuing WSC as a School of Chiropractic *and* Naturopathy. By the time of the February 1956 NCA Council meeting, it seems apparent that Dr. Higgens's commitment was to maintain an NCA-accredited School of Chiropractic, even—or perhaps especially—if that meant dropping the Naturopathy program at WSC. Although Dr. Failor, as the day-to-day manager of WSC in-the-trenches would have welcomed the financial support of the naturopaths and *could* have saved the Naturopathy program if support had been forthcoming, when support *never* materialized, the decision was really that of Dr. Higgens.

The minutes of the February NCA Council meeting tell the story. Dr. Failor had advised that he could not attend and represent WSC "because of pressing responsibilities at the college." And, one assumes, because of the cost of attending the meeting.

The meeting was scheduled for 3 days, Wednesday, February 8, through Friday, February 10, 1956. The "*first* point of consideration placed before the Council from the prepared agenda was the matter of the Western States College of Portland, Oregon, still conducting a course in naturopathy" (emphasis provided). The Chair of the Council (Dr. Thure Peterson) read an "extended statement" prepared by Dr. Nugent and sent to Dr. Higgens "in relation to the matter." The position set out in the statement was to advise Dr. Higgens and the HRF Board that the Council "considers it inopportune for any of the accredited colleges to seek to sustain a naturopathic course."

The Council further "encouraged the board to consider the *necessity* of discontinuing the course as soon as possible, *even at cost and sacrifice of certain advantages that it might represent*" (emphasis provided). Council member Dr. Hendricks noted that it was time for Western States to join Los Angeles and National and "discontinue its affiliations with the naturopathic profession." Failure to do so "would constitute an embarrassment to the Council."

Then Dr. Nugent spoke, and it was apparent that he—regardless of personal feelings on naturopathic programs—felt bound to try to honor the lifelong commitment of his friend and colleague Dr. Budden:

> *Dr. Nugent sought to advise the Council that relinquishing the naturopathic course by Western States College would impose on the college and its board of trustees tremendous economic problems and rob it of certain allegiance … It was the conviction of Dr. Nugent that a decision by Western States College to discontinue the naturopathic course would deal a severe blow to naturopathy and that unless the profession were to organize its own school it might well represent the demise of the profession …With Western States out of the picture of naturopathic education only two schools would be left that issued naturopathic degrees and these were institutions of minor quality and influence; namely the Great lakes College of Mechanotherapy and in Dayton, Ohio, and Spitler College of Naturopathy in Eaton, Ohio.*

These observations by Dr. Nugent came straight from Dr. Budden's long-stated positions expressed during many talks on the subject with Dr. Nugent and others in NCA leadership. In deference to Dr. Budden, Dr. Nugent set this entire argument out for the Council. He then communicated that these may not be the concerns of the current HRF Board governing WSC. Dr. Nugent told the Council that "upon the death of Dr. Budden, he [Dr. Nugent] had been asked by Dr. Higgens to help manage the Western States [College] and to help solve the naturopathic situation … that Dr. Higgens, personally, had invested thousands of dollars in the Western State College [because of his intimate friendship with Dr. Budden], yet he was ready to sacrifice the same if the naturopathic problem could be resolved; that he, Dr. Higgens, was anxious to have the Western States College sever its association with naturopathic education."

After all was considered "it was the general disposition of the Council that patience should be exercised in relation to the circumstances of Western State College," as through "the death of Dr. Budden … the present administration and board of trustees of the college had inherited many compromising and not readily solved problems." Dr. Nugent advised the Council that he was certain that before the Council meetings concluded, "he would obtain word that "a definite decision to eliminate the naturopathic course at Western States had been consummated."

And Naturopathy Is Finished at WSC

And so it was. As the Council convened on the morning of Friday, February 10, the Council was to hear the report of the Council on Educational Standards (Accrediting Committee). The Accrediting Committee proposed that the Council adopt a resolution that Western States "beginning September 1, 1956 … should seek to discontinue students for training in naturopathy" and "terminate its commitment to those naturopathic students enrolled." Dr. Nugent advised that he had spoken several times to Drs. Higgens and Failor since Wednesday morning, and that they understood that this step would be necessary "although it would involve extended financial loss and … the college might not be able to survive." Further that they had announced the day before during an all-school meeting that the decision had been made to discontinue the naturopathic course."

The Council meeting minutes note that the Council had received a copy of "A Statement of Policy on Western States College" that constituted the formal announcement of the decision to all concerned, and that the Council had responded through the Council secretary, Dr. Janse, acknowledging the "courage and integrity" that this decision had required. Within a short period of time, the naturopaths associated with Western States determined to start an independent naturopathy program, initially intended to provide an ND program for WSC's DC graduates who were desirous of an ND degree. In July, three NDs previously associated with WSC, Drs. Bleything, Spaulding, and Stone, chartered an Oregon not-for-profit corporation, the National College of Naturopathic Medicine (NCNM).

But this separation was the kind of "divorce" that left both parties worse off. Dr. Higgens had clearly hoped that the NCA would lend financial assistance to WSC with the "naturopathic issue" resolved. Instead, WSC suffered a severe enrollment decline and financial distress for almost 15 years, with average DC class sizes of 12 to 15 and a total enrollment of 50 to 60. The NCA responded with "hopes and

prayers," restricted accreditation, and suggestions that WSC should be merged with either Los Angeles or National—the two stronger programs with which they had a historical affinity.

Meantime, the NCNM proved to be a difficult proposition, as the ND profession of Oregon, Washington, and British Columbia tried to build a 4-year residency program. It was 15 years before the program—moved to Seattle in terms of actual facilities—began to have decent attendance in the early 1970s.

And all of this had taken its toll on Dr. Failor, DC, ND, as well. As Dr. Lamm reports:

> *Dr. Failor approached the Oregon Association of Chiropractic Physicians (OACP) with his doubts that the college could survive beyond July 1, 1956. The total student population was 73 and a number of disgruntled students announced their plans to transfer to the new naturopathic program starting in the fall. Dr. Higgins appealed to the National Chiropractic Association to support a policy compelling all chiropractic colleges to adopt a two-year, pre-professional college education admissions requirement. The NCA refused to budge.*
>
> *By summer 1956, frustration with lack of support from the NCA, CE, OACP, NDs and DCs; compounded by the student, staff and faculty discontent on campus and a worsening financial situation at the college was more than Dr. Failor was willing to tolerate any longer. He submitted his letter of resignation to the HRF. Even though he had repeatedly recommended closure of the college and left the institution saddened and disappointed, he was effective in instituting stopgap measures that kept the college solvent. Without Dr. Failor's efforts, the college would have closed.*

The educational structure of naturopaths had been severely damaged. And in Texas, the profession began to suffer damage as well.

Back to the Texas Medical Wars

The full story of the 1955 Texas legislative session where the naturopaths were concerned would not be told until the legislature reconvened in 1957, but by now, it had become well known that the AG had determined the old law unconstitutional.

After the legislature recessed without action, the state comptroller refused to pay any further warrants issued by the existing Naturopathic Examining Board given the AG's ruling, effectively putting the Board out of business.[179] In January 1956, using legal counsel retained by the TNPA, the Board filed suit against the comptroller asking the court to uphold the statute and order the comptroller to pay its bills. In May 1956, the district court judge hearing the case ruled in favor of the Naturopathic Examining Board and against the comptroller represented by the AG. The district court upheld the statute as constitutional as to the licensing and registration of naturopaths and directed the payment of warrants issued by the Board.

The comptroller's office filed an appeal, and the matter began working its way through the Texas appellate courts.

In 1957 matters played themselves out badly for the Texas naturopaths in a very public way.[180] The 1957 Texas legislative session began in late January 1957. At the time, the appellate case on the constitutionality of the 1949 statute was pending. Henry Schlichting had been succeeded as president of the TNPA by Howard Harman of San Antonio. The naturopaths were trying in this session to once again get legislation passed that would deal with the AG's opinion. At the end of February, Dr. Harmon went to the Speaker of the Texas House of Representatives with a tape recording on which a member of the Texas House appeared to solicit a bribe to withdraw legislation that would repeal the 1949 law and ban naturopaths from practicing in Texas.

This led to House investigative committee proceedings, a grand jury investigation, an indictment against the legislator, and the legislator's conviction. All of this played out between March 1957 and September 1957, with constant newspaper coverage throughout the state of Texas. It also had two unfortunate effects: few legislators wanted to have anything to do with the naturopaths while this was going on, and first the House investigative committee and then the grand jury dug up the story of the naturopaths' activities during the 1955 legislative session.

What emerged was that the naturopaths had raised a "war chest" of as much as $52,000 for the 1955 session and had spread around among members of the legislature perhaps as much as $37,000 or more to try to get legislation passed. In 2018 dollars, this would be $480,000 dollars raised and $345,000 passed out in legislative "cash gifts." It was rumors of this type of largesse that drew the interest of a young member of the House of Representatives to Harmon as TNPA president while he was in Austin—the state capital—for the 1957 session. The bribe solicited was $5000 total, $3000 for the representative and $2000 to share "with others." In 2018 dollars, this would be $45,000 total, $28,000 for the representative, and $17,000 to "share."

The naturopath caught up in the inquiry into the 1955 legislative activities of the Texas Naturopathic Physicians Association was Henry J. Schlichting Jr.

Texas Medical Wars Continued—1957

While the 1957 Texas legislative fireworks were playing out, the legal process was playing out at the same time.[181] In late January 1957, as the legislative session was getting under way, the Texas Court of Appeals upheld the AG's opinion and reversed the district court. The court held that the 1949 Naturopathic Act was unconstitutional, as the AG's opinion had concluded. The legal effect, as stated by the court, was to completely undo the legislative gains that the naturopaths had been granted under the 1949 "deal" that had given the medical profession a Basic Science Law: "The judgment of the Trial Court is reversed and judgment is here rendered declaring the Naturopathy Act, Art. 4590d V.A.C.S., *void*" (emphasis provided).

In February, Dr. Howard Harmon of the Texas Naturopathic Physicians Association tape-recorded House of Representatives member James Cox soliciting a bribe and took the recording to the Speaker of the House. In March, a legislative investigative committee was convened by the Speaker and—after an emotional "farewell" address to the House—Cox tendered his resignation, which was accepted by the governor. In April, a grand jury was convened, which took testimony about the bribe allegations and also about another matter that had emerged during the legislative hearing: allegations of a "slush fund" that had been used by the TNPA during the 1955 legislative session to "educate" House members with regard to legislation proposed by the TNPA after the AG's opinion had been issued in 1953.

If these assertions about the size of the "slush fund" and the extent of "educational" efforts were true, it had been for naught in 1955. The remedial legislation had been reported out of the House Public Health Committee early in the 1955 session—allegedly as a result of the TNPA "education" efforts—only to die before going any further. When the bill was quickly pushed through the committee process, the medical association took note, and before the matter could come to the House floor for a vote, a "flood of protests" from MDs "began to pile up on legislator's desks," and the bill stalled.

As the House investigation was proceeding in April, the statements of a "mystery witness" were produced by the Texas Department of Public Safety (DPS). The committee abruptly stopped its hearings and referred the matter to the grand jury. In mid-April, the grand jury heard from other NDs appearing before them about the "mystery witness," who was identified as Henry Schlichting Jr. Other NDs told the

press that "Schlichting was the only person who knew how the association's money was spent during past sessions of the legislature." On April 30, 1957, the grand jury heard from Schlichting, who had given a lengthy interview and sworn statement to the Texas DPS the week before.

After his appearance, Schlichting steadfastly gave "no comment" statements to the press, and never did he disclose what he testified to, nor the details of what the TNPA had done with its "slush funds" and "educational" efforts over many years. Another ND who appeared before the grand jury told the press that Schlichting "held his hand on the purse strings since 1943 and still does [in 1957]." But whatever Schlichting disclosed to DPS investigators and the grand jury in March and April of 1957, no indictments were ever issued against anyone accept Representative Cox for soliciting the bribe from Dr. Harmon.

But the entire Harmon–Cox–naturopaths bribery affair had a dampening effect on both the 1957 legislative session and the naturopaths as an interest group desperately needing legislative action. In an extensive two-part investigative report,[182] a reporter for the *El Paso Herald-Post* noted for the "Frantic Fifty-Fifth" legislative session that lobbyists had gone into hibernation when "the naturopaths got cute with their tape recorders" as the legislature began "shaking with a severe case of capital J jitters resulting from the naturopath tape recording in the Representative Cox bribery scandal." Just as significant for the naturopaths was the direct effect on them:

> *You won't find any naturopaths trying to influence legislation during these nervous days. They're now on the inactive list as far as lobbying is concerned—maybe because they figure they've raised their share of turmoil this session, or because they're too busy testifying before the Travis County Grand Jury, whose deliberations are being watched with such big interest by the legislators … Dr. Howard Harmon, the naturopath lobbyist whose tape recording brought about the Cox indictment, hasn't eased the situation any with his forecast that half a dozen lawmakers besides Representative Cox have ample reason to worry.*[183]

In so many ways, these developments could not have come at a worse time for the naturopaths in Texas. On May 1, 1957—the day after Schlichting appeared before the grand jury as the "mystery witness"—the Texas Supreme Court refused to hear the writ of error (appeal) by the Naturopathic Board of Examiners from the court of appeals decision against the 1949 Naturopathy Act. On June 12, 1957, the Texas Supreme Court rejected a request for rehearing of the application for a writ of error, leaving the naturopaths without any further relief at the state-court level. Within 60 days, the naturopaths applied for relief from the US Supreme Court; throughout these various legal steps, the Texas courts stayed enforcement of practicing medicine violations pending the final legal determination of the validity of the 1949 law.

In October, former representative Cox went on trial for bribery, and the newspapers throughout the state had the naturopaths on the front pages again. After the testimony of Dr. Harman and the playing of the recording that Harman had produced, Cox was found guilty as charged. On November 12, 1957 the US Supreme Court declined review of the Texas court decisions on the 1949 naturopathy law.

Texas—The End

On December 3, a massive statewide series of legal actions was taken across 27 Texas counties[184] coordinated by the Texas AG, who vowed to "run the naturopaths out of Texas." In the first "test case" arising out these actions taken in the name of the Texas State Board of Medical Examiners, an injunction was entered on December 9 against Henry Schlichting, enjoining him from continuing to practice naturopathy in the state of Texas without a license to practice medicine. Multiple other injunctions were entered against naturopaths in other counties across the state. For good measure, the AG asked for—and received—an injunction against the continued existence and activities of the Texas Naturopathic Physicians Association.[185]

The naturopaths in Texas—about 450 of them—were in fact out of business.

The case against Schlichting was appealed, and an accelerated review was granted by the Texas Supreme Court. Arguments were heard by the court in January 1958, and a decision was handed down on February 19, 1958. The Texas Supreme Court upheld the injunction against Schlichting. The US Supreme Court later denied review. The Texas career of Dr. Henry J. Schlichting Jr., naturopathic physician, was over.[186]

Schlichting stayed on in Midland, where he had built a solid reputation as a citizen, for another 7 years, practicing as an audiologist working for one of the largest hearing aid companies in the United States. His hearing clinics were regularly advertised, and his appearances at out-of-state gatherings of naturopaths were mentioned in the Midland and Pacific NW newspapers. Then, in 1965, he left his adopted home state of Texas and established a naturopathic practice in Phoenix, Arizona. In 1973 at the age of only 58, he died suddenly at home on a Sunday afternoon. He was taken back "home" to Midland as his resting place.[187]

Three More States Fall: 1955 to 1957

With both WDC and the state of Texas lost to naturopathy, the decline of natural healing accelerated. Licensing was lost in Georgia and in South Carolina, both in 1956. In Utah and Florida—after an extensive review of the profession and its lack of educational institutions—prohibitions were put in place against any further issue of licenses as of 1957. In Florida, after a successful court challenge to the initial action, new legislation was passed in 1959 reinstating the ban on further licenses. In all of these cases, three issues turned the tide against naturopaths: no legitimate school, declining numbers nationally (from no new graduates and 450 licenses gone in Texas), and intense opposition to natural healing by the AMA and its state constituencies.

Naturopaths were first licensed in Florida in 1927.[188] At that time, Benedict Lust had for some years operated his second Yungborn Sanitarium in Tangerine, Florida, in the Tampa Bay region of the state. The legislation adopted defined naturopathy as a "drugless" law, consistent with pre-1939 concepts of that term: "The use and practice of psychological, mechanical and material health sciences to aid in purifying, cleansing and normalizing human tissues for preservation or restoration of health … employs heat, light, water, electricity, psychology, diet, massage and other manipulative methods."

Naturopaths were licensed in South Carolina in 1937 under the leadership of M. S. Dantzler of Spartanburg. South Carolina was a state where the early chiropractic movement was dominated by "straights," and the "mixers" became naturopaths to achieve their own identity before the legislature. The original 1937 law was modeled directly after the Florida statute. ("The use and practice of psychological, mechanical and material health sciences to aid in purifying, cleansing and normalizing human tissues for preservation or restoration of health … employs heat, light, water, electricity, psychology, diet, massage and other manipulative methods").[189]

In 1941—on the eve of WWII—the statute was amended with this addition: "The use and practice of phytotherapy, minor surgery, obstetrics and gynecology, autotherapy and biologicals shall be a part of and included in the practice of naturopathy." The difficulties weathered by naturopaths in South Carolina from 1947 to 1949 after the Tennessee scandal have been discussed. Significantly, these problems were resolved by giving the state Board *more* enforcement powers so that the Board could better police the profession.

The Florida Saga Begins

In Florida, naturopaths in the greater Miami area of the state pushed to have the law interpreted to allow the right to prescribe drugs after the 1939 regulations were put in place at the federal level. Using the courts, this effort was successful in 1947 in convincing the Florida Supreme Court to interpret the reference to "phytotherapy" in the Florida statutes to include all drugs derived in any way from plant origins, including morphine and opium derivatives. In 1949 this created a significant—and predictable—backlash.[190]

In early April 1949, the State Board of Health, through its five-member governing body, adopted a resolution urging legislative action regarding "the licensing and practice of naturopathic physicians," with a finding that: "The board has information that the practice of naturopathy including the licensing of naturopathic physicians, and the treatment of patients of licensed naturopathic physicians is being conducted in such a manner as to be detrimental to the public health of Florida."

The next day, legislation was introduced in the Florida Senate to make the practice of naturopathy unlawful in the state, and the bill was referred to the Senate Committee on Public Health. Pending the hearing by the committee on the bill, the State Health Officer, a medical doctor, picked up the attack on licensed naturopaths, who at the time numbered 239. The concerns expressed were that naturopaths had been "drugless physicians" who opposed drugs as contrary to the natural maintenance of health and natural methods of healing, that naturopaths had no training in the use of *materia medica,* that no other state allowed such practices by naturopaths, and that the state of Tennessee had just determined that much of naturopathic education was a fraud, yet some Tennessee licensees were among the NDs in Florida now using drugs.

On April 19, 1949, the Florida Naturopathic Physicians Association ran a quarter-page ad in the *Miami Herald,* Florida's largest newspaper. The ad was entitled "NATUROPATHS ANSWER THE ATTACKS OF THE STATE BOARD OF HEALTH, An Open Letter to: Wilson T. Sowder, M.D., State Health Officer." In this "open letter," the NDs defended their record of having "used narcotic drugs under Federal Government regulation [for] several years during which they did not abuse or misuse the privilege." The letter further defended the way in which NDs designated their practices; conformed to the laws, including the court decisions on prescription authority and naturopathic educational standards established under the law; and questioned the good faith of an MD as "promoting monopolistic legislation … of the American Medical Association" rather than serving "the people of Florida."

The Senate committee held an all-day hearing the next day and heard a carefully marshaled presentation on these issues, including hearing the testimony of Harry Avery—the Tennessee investigator—as well as a state narcotics investigator and a regional federal narcotics inspector. The naturopaths were represented by legal counsel who was allowed to question the witnesses who appeared. More than 100 NDs attended the session. Although both narcotics inspectors argued against the NDs having prescription authority, both acknowledged under questioning that there had been no reported narcotic violations against Florida naturopaths.

As the hearing closed, the sentiment of the committee seemed to be that although tighter regulation would be in order, the bill outlawing naturopathy was too much. But the record was mixed. Most of the NDs had been licensed before the Basic Science Law had been adopted. Of the 239 licensees only 22—all from National College in Chicago—had passed the BSL examination. Many practitioners had diplomas from Lust's American College of Naturopathy; it was admitted into the record that Lust had been charged in 1934 with operating an unchartered school in New York. As the hearing closed, the committee chair put into the record a stack of 700 telegrams and 50 letters opposing the bill and 5 letters supporting the bill.

The committee voted at about 8 o'clock in the evening to not report the bill out to the Senate by a vote of two "for" and seven "against." One takeaway was important, though: one of the "for" votes was from State Senator Leroy Collins of Tallahassee. In the late 1950s, Collins was to become Florida's governor.[191]

The staff reporter who covered the hearings—indeed, the story of the Board of Health position on naturopaths—also contributed a lengthy four-part series on naturopaths in Florida. The one school represented in Florida among the licensed NDs that was clearly legitimate was the National College of Drugless Physicians—the Lindlahr school—founded in 1908 and part of the National College of Chiropractic. About 40 alumni were practicing in Florida, and "even the critical state medical societies that have commented on naturopathic schools don't charge that this college doesn't offer full four-year training, even though they do complain that it does not offer a four-year course devoted *exclusively* to naturopathy" (emphasis provided).[192]

At the conclusion of the reporting on the legislative matters and the general investigation of naturopaths in Florida, the reporter concluded the series with these observations: "Better regulation, rather than prohibition is probably the answer. If naturopaths were not permitted … drugs; … prominently identified themselves as naturopaths; if stricter examination of schools … [was] required—the public and naturopathy would be benefitted."

The Three-State Region

Geographically Georgia and South Carolina share a long common border, South Carolina to the east and Georgia to the west. The common border cuts a line through a common rural area. The Georgia newspapers covered the reports out of South Carolina through the 1947 to 1949 events, and in 1950, the Georgia naturopaths had general legislative support built up for establishing a licensing board that could "cull the quacks" from the state. The acceptance of this idea in South Carolina was noted, and the Georgia legislature followed suit.

Then in 1955, issues arose in both South Carolina and Florida. Geographically "in the middle," Georgia was affected as well. The South Carolina issues started when the state AG ruled that the law did not allow the naturopaths to prescribe drugs, specifically plant-based narcotics such as opium derivatives. The NDs frankly pushed things too far when they took the AG to court over the issue. Although they won the first rounds of the legal battle, the state medical association used the prospect of NDs claiming prescriptions rights, including to narcotics, to raise the issue of these practices being a threat to public safety.[193]

The prescription of such plant-related drugs had first arisen in Florida in the 1940s, and the courts in Florida had held that the NDs had these prescription rights.

But this issue raised conflict in Florida in 1955 as well through a series of investigative articles in the *Miami Daily News* entitled "Who Are the Naturopaths?" The articles—four in number—raised the same questions being raised in the South Carolina legislature: Where did these naturopaths go to school, and how has it come to pass that they can prescribe drugs and do minor surgery?[194]

In South Carolina and Florida, these questions in 1955 and 1956 provoked no acceptable answers. The National College program had been closed in 1950, the Western States program in early 1956. After the scare in 1949, the Florida NDs went on with business as usual. In fact, when the 1955 *Miami News* investigation was reported in November 1955, the only school pointed to by the Examining Board as approved was Western States. Then the program was discontinued in February 1956.

Leroy Collins was now the governor of Florida, and the *Miami News* investigation called him out by name for his legislative opposition to

naturopaths in Florida. Governor Collins commissioned a report from the State Bureau of Narcotics on the state of naturopathy. When the report documented that things had not seemed to have changed since 1949, he announced that one of his legislative priorities for 1957 was outlawing naturopathy in Florida.[195]

In 1955 South Carolina outlawed naturopathy; in 1956 Georgia outlawed naturopathy; in 1957 Florida outlawed the issuing of any new licenses to practice naturopathy. When the original legislation was struck down by the Florida courts, the 1959 legislature renewed the ban on issuing further licenses. Ultimately, the bans in these three states and Texas were upheld by the courts as within the police powers of state legislatures. In the meantime, things were not going well out West either.

Utah

The Early Days

In Utah the authorization to legally practice naturopathy first appeared in 1925 when the legislature passed legal authority for an examining board for physiotherapy and naturopathy under the Utah Department of Registration.[196] The governor at the time vetoed the measure on the basis that such practices were authorized to be licensed under the state medical board as treatment by a practitioner without the use of medicine or surgery. In October 1934, there were 15 naturopaths registered with the Department of Registration through the authorization of the medical board. Finally in 1937, a separate naturopathic board was authorized by the legislature, and the legislation was signed into law this time around. In 1939 legislation was passed into law raising the educational standards for naturopaths to require 4 years of post–high school education.

In each legislative session after 1939—1941, 1943, and 1945—legislation was proposed but not passed to allow for "practicing as a naturopathic physician and surgeon, including obstetrics." But in 1946, it emerged that the naturopathic examining board had been conducting a supplemental examination for obstetrics and minor surgery in addition to a basic naturopathy examination just as the medical board had done before the examination authority had been transferred. These supplemental licenses were then issued by the Department of Registration to license holders.

Utah Opens Up

This only became publicly known in September 1946, when a *second* AG opinion issued in May 1946, was publicly issued and reported by the Utah newspapers.[197] The story was this: In January 1946, a new department director had asked for an opinion from the AG whether naturopaths could be licensed to practice surgery and obstetrics. The AG's Letter of Opinion answered the question no, as both surgery and obstetrics were the practice of medicine, not naturopathy. In so answering, the opinion noted that there was no such thing as "minor surgery"; under Utah law, surgery was surgery.

In May, a *second* AG opinion letter was sent to the Department of Registration with the recognition that since 1939, naturopaths had been examined in "minor surgery and obstetrics," and upon successful passing of the tests, they had been issued licenses to practice. This opinion noted that the statutes "were not free of ambiguity," and therefore the practice of the Department would be deemed an existing "administrative interpretation" of the law, effective until either the legislature modified the statutes or the courts ruled otherwise. Moreover, the opinion went on to state that although licensed naturopaths could not use or prescribe drugs, those licensed to practice obstetrics and surgery *could* use and prescribe those drugs that "were recognized requirements in obstetrics or minor surgery." The opinion further stated that although naturopaths could not use drugs in general practice, they *could* keep on hand or prescribe drugs for communicable diseases or emergencies (e.g., antidotes for poisons) in practice.

And so, as a matter of statutory interpretation, Utah became a state that allowed very broad practices to naturopaths. The legislature never did adopt any statutory language that modified the statutes interpreted by the AG, and the courts never reviewed the matter; this was the status quo until September 1955. In 1955 the Utah Board of Health—not the Department of Registration—requested a *new* AG opinion on the same statutory issues. At the time, the Department of Registration listed 82 naturopaths, of whom 66 were authorized for obstetrics and minor surgery.[198]

And Shuts Down Again

The 1955 AG opinion reviewed the same licensing statutes as the 1946 opinions and came to a completely different conclusion. Naturopaths could not be licensed to do obstetrics and minor surgery nor prescribe drugs. Under this new opinion, the Department gave notice in November 1955 that it would be canceling all of the licenses issued to naturopaths to practice obstetrics and minor surgery, effective January 1, 1956. The naturopaths appealed to the courts but lost before the Utah Supreme Court in June 1956.[199] In the 1957 legislative session, legislation was passed that would have allowed the continued practice of obstetrics, minor surgery, and the prescription of drugs to "reinstate the status quo." This legislation was vetoed by the governor after the session ended.

Also, legislation was passed to fund and commission a study of the education and practices of naturopaths for the use of the 1959 legislative session. The report was devastating in its assessment of the state of naturopathic education in 1959. Essentially, the only school in existence was the National College of Naturopathic Medicine in Portland, Oregon, which at that time was in its early, rudimentary stage. Based on this assessment, no legislative relief was granted to licensed naturopaths in Utah, who were now firmly reduced to practicing without any obstetrics, minor surgery, or prescription rights.[200]

The 1959 legislature did pass a Basic Science Law. Naturopaths *were* authorized to take the midwifery examination and become licensed midwives as well as NDs but only after taking the BSL examinations. Also, naturopaths could take the obstetrics and minor surgery examinations as given by the medical board (not their own licensing board) but only after passing the BSL examination (this according to another AG opinion requested by the naturopathic board in 1961). And finally, from yet *another* 1961 AG opinion, naturopaths were not licensed to do spinal adjustment because by law, naturopaths were separate from chiropractic. From this point, naturopathy in Utah went into a long decline as existing practitioners retired from practice and new practitioners were scarce.[201]

Washington State Under Siege

In Washington, naturopaths had been in practice under the Washington Drugless Healing Act of 1919 since Robert V. Carroll had founded the Washington State Naturopathic Association in 1934. Before that time, all drugless practitioner licenses listed "Sanipractic," and practitioners either called themselves "drugless physicians" or "Sanipractors." Sanipractors used the initials "SP" for Sanipractic Physician. Licenses continued to be issued for "Sanipractic," but many practitioners demonstrated their alliance with Carroll—and through Carroll's WSNA, their alliance with the national ANA—by using the initials "ND" in practice.

After adverse court decisions in 1947 (drugless healers cannot practice surgery or obstetrics), 1950 (drugless healers are not "doctors" and are not allowed standard malpractice defenses), and 1957 (drugless healers can be prosecuted for practicing medicine without a license for using "Dr." or "physician," conducting physical examinations), the Washington naturopaths were completely unsuccessful in getting any legislative relief over the intense opposition of the medical profession. The NDs obtained 53 signatures on a petition submitted to

the Washington licensing department under the Drugless Healing Act and at least gained the right to be licensed to practice "Naturopathy" in Washington. For about 10 years, this obtained a little legal relief for them from some of the bad case law that applied to "Sanipractors."[202]

A Dismal State of Affairs: The 1960s

This once proud and vigorous natural healing movement that existed from after WWII for about 10 years had gone into such decline—as described here—that the naturopaths' self-reported state as of 1968 showed a professional movement almost gone.[203] In 1968 consideration was given by the US Department of Health and Human Services to the request by the National Association of Naturopathic Physicians to have naturopaths accepted as independent practitioners under Medicare. As part of this process, a lengthy questionnaire was completed and submitted by John W. Noble, DC, ND. Noble was a 1937 graduate of Western States College of both the School of Chiropractic and the School of Naturopathy. As of 1968, Noble was a licensed ND in Oregon, practicing in Portland. He was NANP president and also maintained the administrative offices of the National College of Naturopathic Medicine, which had recently moved its main instructional facilities to Seattle.

As of 1968, he reported that the NANP had 168 members from Washington (26), Idaho (26), Connecticut (24), Oregon (20), California (17), Kansas (16), and New York (7). Of these states, Washington, Idaho, Kansas, and New York were not considered licensed states, so almost half of the NANP membership came from unlicensed jurisdictions. Noble reported on behalf of NCNM that from 1960 to 1967, 16 students had graduated, and as of 1967/1968, 7 were enrolled.

According to the 1965 US government publication *State Licensing of Health Occupations,* there were 351 licenses in effect in Arizona, Connecticut, Hawaii, Oregon, Utah, and the District of Columbia—the states still issuing licenses.[204] Also, 202 licenses were renewed in Florida and California—states that were not issuing new licenses as of 1965. This was a total of 553 active licenses. Together with identifiable practitioners in the unlicensed states, there may have been 700 or so identifiable naturopaths, some practicing in extremely restrictive circumstances.

A Future Resurrection

It would not be until the mid-1970s that this serious decline of natural healing in the United States would begin to be reversed and not until the late 1980s that natural healing would return to an equivalency with the profession that was built between the mid-1930s and the early 1950s by Carroll, Budden, Schlichting, and the others discussed here. That was not, obviously, from lack of commitment or effort on their part.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lamm, Lester. *Oregon Pioneer: The Journey of Chiropractic Education in the Northwest* (Portland, OR, University of Western States); Keating, Joseph C., Jr., Callender, Alana K., and Cleveland, Carl S. III (1998) *A History of Chiropractic Education in North America* (Published by Council on Chiropractic Education, Scottsdale, AZ); 2014.
2. Garceau, O. *The Political life of the American Medical Association.* (Cambridge, MA: Harvard University Press; Truman), D., (1951). *The Governmental Process* (New York: Knopf); The American Medical Association: Power, Purpose and Politics in Organized Medicine. (1954) *The Yale Law Journal*. 1941; 63(7): 937–1022.
3. Berliner H. *A System of Scientific Medicine: Philanthropic Foundations in the Flexner Era*. New York: Tavistock; 1985.
4. Appleton N. *Rethinking Pasteur's Germ Theory*. Berkeley, CA: North Atlantic Books; 2002.
5. Gross CG. Claude Bernard and the constancy of the internal environment. *Neuroscientist*. 1998;4:380–385.
6. Kirchfeld Boyle. *Nature Doctors: Pioneers in Naturopathic Medicine*. East Palestine, OH: Buckeye Press; 1994.
7. Lindlahr HMD, Proby CP, eds. *Philosophy of Natural Therapeutics*. Kent, England: Maidstone Osteopathic Clinic; 1924. Reprint 1975.
8. *Ibid, note 5*.
9. *Ibid, note 6, p. 5*.
10. Ibid, *note* 6, p. 8.
11. *Ibid, note 6, pp. 6-7*.
12. *Ibid, note 6, p. 10*.
13. *Ibid, note 6, p. 10*.
14. *Ibid, note 6, pp. 28-35*.
15. Keating Jr JC, Rehm WS, William C, Schulze MD. D.C. (1870-1936). From mail-order mechano-therapists to scholarship and professionalism among drugless physicians. *Chiropractic Journal of Australia*. 1995;25(3):82–92. Sept.
16. *Ibid.*
17. *Ibid.*
18. *Ibid.*
19. *Ibid.*
20. *Ibid.*
21. Ibid. *Seattle meeting reported in The Seattle Times*; 1934.
22. *Ibid.*
23. *Ibid.* Dr. Budden and Western States College, with its School of Chiropractic and School of Naturopathy, become part of the story in due course.
24. Cannon WB. *The Wisdom of the Body*. 2nd ed. New York: W.W. Norton & Co; 1939.
25. *Ibid, pp*. 240-243.
26. Burrow James G. *Organized Medicine in the Progressive Era: The Move Toward Monopoly*. Baltimore: Johns Hopkins University Press; 1977.
27. Reed Louis S. *The Healing Cults; A Study of Sectarian Medical Practice*. Chicago, IL: Univ. of Chicago Press; 1932.
28. See Cody, George (1985) "History of Naturopathic Medicine," in *Textbook of Natural Medicine*, Pizzorno and Murray, editors, Seattle, WA, Bastyr College Publications (1993) and New York, Churchill Livingstone (1999).
29. See *ibid;* also see the extensive work by Susan Cayleff in Cayleff (2016) *Nature's Path: A History of Naturopathic Healing in America*, Baltimore, Johns Hopkins University Press, and especially pp. 34-50 and 210-217.
30. Charles. Hazzard. *Principles of Osteopathy.* Kirksville, MO: self-published. republished Andesite Press; 1899.
31. See Cayleff, *Nature's Path,* citing several examples.
32. Lindlahr Henry. *Nature Cure; republication by*. USA: ReadaClassic.com (2010); 1913:12–14.
33. *Ibid*, at p. 12.
34. Cayleff, *Nature's Path* at pp. 54-55.
35. Bilz, F. E. *Natural Method of Healing: A Complete Guide to Health (translated from the latest German edition),* Leipzig-London-Paris, F. E. Bilz, publisher, through the International News, Co., New York, in the USA; 1901.
36. *Ibid*, at p. 7.
37. Lindlahr, *Nature Cure*, at pp. 17, 20.
38. Evan Willis. *Medical Dominance*. Sydney, Australia: George Allen & Unwin publishers; 1983.
39. Rosenthal Sau. *Lewiston, Maine*. Edwin Mellen Pressl; 1986.
40. Berliner H. *A System of Scientific Medicine: Philanthropic Foundations in the Flexner Era*. New York: Tavistock; 1985.
41. Wolinsky Fredric D. *The Sociology of Health: Principles, Professions and Issues.* Boston: Little, Brown; 1980:215.
42. Lipset Seymour Martin. *American Exceptionalism; A Double-Edged Sword*. New York: W.W. Norton; 1996.
43. Cayleff, *Nature's Path* at pp. 193, 215.
44. Norman Gevitz. *The DOs: Osteopathic Medicine in America*. 2nd ed. Baltimore, MD: Johns Hopkins University Press; 2004.
45. Wardwell Walter I. *Chiropractic: History and Evolution of a New Profession*. St. Louis, MO: Mosby-Elsevier; 1992.
46. Susan Cayleff. *Nature's Path: A History of Naturopathic Healing in America*. Baltimore, MD: Johns Hopkins University Press; 2016.
47. Cayleff, *Nature's Path*.
48. Kirchfeld Boyle. *Nature Doctors: Pioneers in Naturopathic Medicine*. East Palestine, OH: Buckeye Press; 1994.
49. Lamm Lester. *Oregon Pioneer: The Journey of Chiropractic Education in the Northwest*. Portland, OR: University of Western States; 2014:13–14.
50. *Ibid*.
51. *Ibid*.
52. *Ibid* at pp. 14–15.
53. For an extended review of the concept and application of the Basic Science Law see Gevitz Norman. 'A Coarse Sieve': Basic Science Boards and Medical Licensure in the United States. *Journal of the History of Medicine and Allied Sciences*. 1988;43:36–63.
54. For an extended review of the concept and application of the Basic Science Law, see "A Coarse Sieve: Basic Science Boards and Medical Licensure in the United States," by Norman Gevitz.
55. Budden WA. *Medical Propaganda – Aided by B.J. Palmer, Defeats Health Care Amendment*. The Chiropractic Journal; 1935.
56. For an extended review of the concept and application of the Basic Science Law, see "A Coarse Sieve: Basic Science Boards and Medical Licensure in the United States," by Norman Gevitz.
57. Ibid.
58. The Oregonian. *2 Initiative Bills Will Face Voters*; 1934.
59. Budden WA. *Medical Propaganda – Aided by B.J. Palmer, Defeats Health Care Amendment*. The Chiropractic Journal; 1935.
60. Oregon law required expenditure reporting, and $4819.03 was the officially reported expenditure filed by the "yes" campaign. The "no" campaign spent twice as much, with over $6,000 coming from the Oregon Hospital Association. *The Oregonian,* November 17, 1934; November 18, 1934; November 23, 1934.
61. Lamm, *Oregon Pioneer,* pp. 25-26.
62. Lamm, *Oregon Pioneer*.
63. With regard to Sanipractic, its presence in Washington, and its relationship to naturopathy, see Reed, Louis S. *The Healing Cults; A Study of Sectarian Medical Practice* Chicago, IL, Univ. of Chicago Press; 1932.
64. *The Seattle Times,* Sunday, April 24, notes a wire story Saturday—the day before—from Washington, D.C., stating that Carroll has been announced to speak before the ANA convention that July. This is the first reported connection of Carroll with the national ANA.
65. Carroll's original diplomas are in Bastyr University's John Bastyr archival collections,
66. Kirchfeld and Boyle, *Nature Doctors,* pp. 257–260, covers the relationship between the Carroll brothers.
67. The records of the Washington State Naturopathic Association including the 1930s are in the John Bastyr Archive collection at Bastyr University as maintained and donated by Kenneth Harmon Freeman, ND.
68. Lust and his publications are discussed in detail in both Cayleff, *Nature's Path,* and Kirchfeld and Boyle, *Nature Doctors*.
69. Schlichting succeeded Robert Carroll as president of the western ANA in 1949; in 1951 the association changed its name to the ANPSA.
70. *The Oregonian* newspaper, Portland, Oregon, for March 9, 1952 at page 29.
71. Schlichting gave this number in an interview on the first day of the convention (Thursday); the interview appeared in *The Oregonian* on Friday, March 7, 1952, at p. 45.

72. Licensed states were Connecticut, South Carolina, Georgia, Florida, Texas, Arizona, Utah, and Oregon. Naturopaths were licensed in Washington as drugless healers and in Ohio as "others-mechanotherapy."
73. The life and career of A.R. Hedges, DC, ND, of Medford, Oregon, will be discussed in a future column.
74. See previous column in *IMCJ*.
75. Lamm Lester. *Oregon Pioneer: The Journey of Chiropractic Education in the Northwest*. Portland, OR: University of Western States; 2014:25–26.
76. *Ibid* at p. 6.
77. Keating Joseph Jr C, Callender Alana K, Cleveland III Carl S. *A History of Chiropractic Education in North America*. Scottsdale, AZ: Published by Council on Chiropractic Education; 1998:86–87.
78. The Western States College, School of Chiropractic and School of Naturopathy, Schedule of Classes and Hours was obtained from the library archive collection at the University of Western States.
79. *The Oregonian* newspaper of Portland, Oregon, for Monday, June 28, 1937, had the announcement of the commencement to be held that evening.
80. As quoted in *A History of Chiropractic Education in North America*, at pp. 85-86.
81. As noted in the author's personal correspondence with Gerald Farnsworth, DC, ND, of British Columbia, Canada (June 2015). He was a personal and professional friend and colleague of Joseph Boucher, ND, WSC class of 1953, and the brother of Earl Farnsworth, ND, class of 1955.
82. *The Oregonian*, Monday, June 11, 1934, reports on the meeting from the week before, noting that Harold Hulme of the campaign was reelected secretary-treasurer, that Dr. Budden had passed out diplomas for the postgraduate course as well as speaking on "The Dawn of Medical Freedom," and that Dr. Benedict Lust of New York was the principal banquet speaker.
83. A lot of the story up to 1943 is told—from Dr. Lust's point of view—in the *Naturopath and Herald of Health* (*NHH)* volumes for July 1942, "Dr. Lust Speaking," and January 1943, "The American Naturopathic Association Its Purposes and Objectives." Any other volumes of *NHH* and any other sources will be noted.
84. The constitution and bylaws adopted in 1935 were printed and published in *Naturopath and Herald of Health,* November 1935 and January 1936.
85. *Naturopath and Herald of Health,* November, 1941; January, 1942.
86. *Naturopath and Herald of Health*, July, 1942; August, 1942; January, 1943; Newsletter from the American Naturopathic Association, Office of the Secretary, Midland, Texas, dated March 1, 1947, listing Robert A. Carroll as President; *American Naturopath,* Volume III, No. 4, June 1947; *Herald of Health and Naturopath,* October, 1947; November, 1947 (Published by T.J. Schippell, Wash., D.C.) in the editor's column, "This Month with Dr. Schippell," all have been parsed through to gather this history. This material is in the collected archives at the National University of Natural Health, Portland, Oregon.
87. *Ibid*.
88. *Herald of Health and Naturopath,* September, 1947 (Vol. 52, No. 9). Following Dr. Lust's death in 1945 Dr. Schippell took over publication of this publication in the name of the (eastern) ANA, each monthly cover noting: "Founded in 1896 by Benedict Lust, Father of Naturopathy," with the main editorials being "This Month with Dr. T.M. Schippell" and "Dr. Lust Speaking (reprints)."
89. "Outline of Curriculum for Schools and Colleges Teaching Naturopathy" by the ANA Committee on Education and Council on Schools and Colleges, adopted by the ANA House of delegates at the Annual meeting July, 1948 at Salt Lake City, and reported in *Journal of the ANA, Inc.*, December-January, 1948-1949, pp. 11,21.
90. The first visit is reported in *The Oregonian,* Friday, December 3, 1947, and the second visit is reported in the *Journal of the ANA,* February, 1949, at p. 13; "National News Notes" report on the ONA's convention in December 1948, also attended by a student delegation from Western States of 23 students, and further, in the Newsletter of the Washington State Naturopathic Association for February 1948, a copy of which is the John Bastyr Archive papers at Bastyr University.
91. *Kelly v. O.G. Carroll*, 36 Wn.2d 482, 219 P.2d 79 (1950).
92. *Journal of the ANA,* vol. 4, no. 6, June,1951, at p. 8 has an "In Memoriam" box noting Carroll's passing on Friday, May 11, 1951; Obituary in *Seattle Times*, Sunday, May 13, 1951.
93. The primary tools for this analysis are the catalogs for the programs at WSC maintained in the UWS library archives and the textbooks referred to in these catalogs. The catalogs available will be referenced as the curriculum for each period is discussed.
94. Reference is to the catalogs in use in 1934–1937 and 1938–1939; UWS library archives.
95. UWS library archives.
96. *Ibid*.
97. Lake's *Treatment* is described by Susan Cayleff at p. 38 of *Nature's Path: Naturopathic Healing in America* (2016), Baltimore, Johns Hopkins Press, as presenting osteopathy, chiropractic, and naturopathy as a complementary set of skills necessary to a complete physician.
98. UWS library archives.
99. H. Riley Spitler, DC, ND, OD, of Eaton, Ohio was a pre-WWI graduate of Ross Chiropractic College of Fort Wayne, Indiana. Spitler was truly a renaissance man who deserves his own biography, and later in this series, he will get one. Among other things, he was a medical board examiner in neuropathy in Ohio for over 15 years; a member of the faculty of the Metropolitan Chiropractic College of Cleveland, Ohio, in mechanotherapy; and the originator of the syntonic principle that is still influential in optometry today.
100. UWS library archives.
101. The reference is to Rehm and Keating, as discussed previously. See *IMCJ* for February 2018, vol. 17, no. 1 at p. 20
102. Medford (OR) *Mail-Tribune,* Tuesday, June 10, 1930—front-page story reporting the newest census figures.
103. Medford (OR) *Mail-Tribune,* Sunday, April 4, 1943, references Hedges's 32 years in practice in Medford and observing an annual "Health Week"; Wednesday, February 28, 1962, notes Hedges as receiving only the fourth "Lifetime Membership" in the Oregon Association of Chiropractic Physicians, having been in practice in Medford since 1911; the first of many regular ads for the Hedges practice that has been located in newspaper archives is in the edition for Wednesday, April 9, 1913.
104. *Medford (OR) Mail-Tribune,* Tuesday, March 4, 1930.
105. *Ibid*.
106. Lamm Lester. *Oregon Pioneer: The Journey of Chiropractic Education in the Northwest*. Portland, OR: University of Western States; 2014:22.
107. *Amarillo Globe-Times,* Tuesday, July 15, 1941.
108. *Oakland Tribune,* Thursday, December 18, 1941.
109. *The Oregonian,* Sunday, July 26, 1942, reported the election as VP; Friday, January 1, 1943, reported the Board appointment.
110. The Sunday, June 3, 1934, edition of *The Oregonian* had reported on the 25th Annual Meeting of the ONA, at which Budden spoke on the Ballot Campaign, and Hedges and Sargent and others participated in "a clinic demonstrating naturopathic procedures."
111. *(Salem) Statesman-Journal*, Sunday, June 30, 1946; term effective July 2.
112. *Medford Mail Tribune,* Tuesday, November 29, 1949.
113. See "Editorial' in *Journal of the American Naturopathic Association,* vol. 3, no. 11, November, 1950.
114. See "Editorial". *Journal of the American Naturopathic Association*. June 1950;3(5). May 1950; see also "Editorial" in vol. 3, no. 6,.
115. Sunday, March 7, 1954, *The Oregonian*—meeting announcement for Friday, March 12, 1954.
116. Altschuler Glenn C, Blumin Stuart M. *The GI Bill: A New Deal for Veterans*. New York: Oxford Univ Press; 2009.
117. *Oregon Pioneer*, at p. 23.
118. A.E. Homewood at p. 30.
119. Martin Bleything's background is discussed at length in a newspaper profile when he also graduated from Portland's Lewis and Clark University with a BA in journalism in 1955—at age 65. The information about Grace University comes from materials in the John Bastyr Archives at Bastyr University.
120. *Journal of the ANA,* vol. 4, no. 6, June 1951.
121. There is a great deal of academic scholarship about these events, most extensively in Monte Poen's *Harry S. Truman Versus the Medical Lobby: The Genesis of Medicare* (1979), Columbia, MO, University of Missouri Press. For capsule glimpses of the political power of the AMA, see Truman, David B. (1951; 2nd ed. 1971), New York, Alfred Knopf Publishing,

pp. 170–177, 231–232. For the AMA battle with President Truman, see Hacker, Jacob S. (2002) *The Divided Welfare State: The Battle over Public and Private Social Benefits in the United States*, New York, Cambridge University Press, pp. 222–237. For a discussion of how these issues relate to the elements of medical dominance in U.S. healthcare policy, see Morone, James A. (1990; rev. ed. 1998), *The Democratic Wish: Popular Participation and the Limits of American Government*, revised edition New Haven, CT, Yale University Press

122. The AMA campaign that began in 1953 will be discussed more extensively later in the chapter.
123. Lamm Lester. *Oregon Pioneer: The Journey of Chiropractic Education in the Northwest*. Portland, OR: University of Western States; 2014.
124. This curriculum information comes from archived the college catalogs, officially called "Bulletin of the Western States College." These are archived in the library collection in the W. A. Budden Library at the University of Western States. For this section, these Bulletins have been referenced: Volume No. XXV, June 1949, Annual Catalog Schools of Chiropractic and Naturopathy for 1949–1950; Volume No. XXVI, June 1950; Annual Catalog Schools of Chiropractic and Naturopathy for 1950–1951; Volume No. XXVII, March 1951, Annual Catalog Schools of Chiropractic and Naturopathy for 1951–1952; No. XXVII (2), March 1951; Annual Catalog School of Naturopathy for 1951–1952; No. XXVIII, May 1952, Annual Catalog Schools of Chiropractic and Naturopathy for 1952–1953.
125. *The Synergist* was the monthly "Voice of the Student Body" of the time. As vol. 4, no. 7 was April 1953 as the class of 1953 was graduating, this monthly looks like publication was begun as the class arrived in fall 1949. These sketches of campus life come from the several volumes archived at UWS.
126. See previous references to Dr. Schlichting's speeches on the state of naturopathy, circa 1952.
127. *The Oregonian,* Saturday, March 7, 1953.
128. *The Oregonian,* Wednesday, March 11, 1953.
129. The convention news itself was in *The Oregonian,* Friday, July 17, 1953, with lengthy interview coverage of the convention appearance by soils and food sciences professor from the University of Missouri, Dr. William Albracht.
130. *Ibid*.
131. An obituary in *The Oregonian,* Wednesday, August 18, 1954, notes his death on August 1. The circumstances are described in *Oregon Pioneer*, at p. 27.
132. *Oregon Pioneer,* pp. 33-34.
133. *Nature Doctors,* by Kirchfeld and Boyle at pp. 297–302 covers the career in naturopathy of Joe Boucher.
134. *Oregon Pioneer,* see especially 104–105.
135. *Nature Doctors* p. 310; more discussion of this later in the chapter.
136. *Ibid*.
137. *Journal of the ANPSA,* September, 1952 (volume 6, number 6).
138. The *National Chiropractic Journal,* September, 1939 (Vol. 8, No. 9).
139. *El Paso Herald-Post,* Monday, April 8, 1957; *Amarillo Globe-Times (TX)*, Monday, May 09, 1938; *Amarillo Sunday News and Globe*, Sunday, August 14, 1938; *Midland Reporter-Telegram*, Wednesday, November 26, 1941 and Monday, November 24, 1941 and Monday, January 12, 1942.
140. Various issues of the *Midland Reporter-Telegram* from 1941–1944 carried ads for Dr. Schlichting's practice "emphasizing fractures and dislocations"; the Carver technique is discussed in Wardwell, Walter (1992) *Chiropractic: History and Evolution of a New Profession* (St. Louis, Mosby, Inc.).
141. *Midland Reporter-Telegram,* Monday, November 24, 1941.
142. See previous column re: ANA convention of 1942 and *The Canyon (TX) News,* Thursday, November 2 and Thursday, November 9, 1944, and Thursday, October 25, 1945 re: H. A. Brown, ND.
143. See Note 132.
144. Walter Wardwell. *Chiropractic: History and Evolution of a New Profession*. St. Louis: Mosby, Inc; 1992.
145. Ibid, p. 89.
146. Johnson, Alton Cornelius (1939), *Principles and Practice of Drugless Therapeutics* (Los Angeles, Chiropractic Educational Extension Bureau); see previous column on Western States' postwar curriculum; see *Journal of the ANA* and *ANPSA* 1948–1954, especially *Journal of the ANA* September 1948 (vol. 1, no. 9) at page 18 for Johnson's initial column on physiotherapy and his explanation of being recruited for and acceptance of his position.
147. See previous columns and specifically: *Naturopath and Herald of Health*, July 1942; August 1942; January 1943; Newsletter from the American Naturopathic Association, Office of the Secretary, Midland, Texas, dated March 1, 1947, listing Robert A. Carroll as president; *American Naturopath,* vo. III, no. 4, June 1947; *Herald of Health and Naturopath,* October, 1947; November, 1947 (Published by T. J. Schippell, Washington, D.C.) in the editor's column, "This Month with Dr. Schippell"; all have been parsed through to gather this history. This material is in the collected archives at the National University of Natural Health, Portland, Oregon.
148. *Ibid.* The constitution and bylaws adopted in 1935 were printed and published in *Naturopath and Herald of Health*, November 1935 and January 1936; *Naturopath and Herald of Health*, November 1941; January 1942.
149. See also *Journal of the ANA,* April 1948 (vol. 1, no. 4); August 1948 (vol. 1, no. 8); and September 1948 (vol. 1, no. 9).
150. Wardwell Walter. *Chiropractic: History and Evolution of a New Profession*. St. Louis: Mosby, Inc; 1992; Keating Joseph C Jr, Callender Alana K, Cleveland Carl S III. *A History of Chiropractic Education in North America*. Scottsdale, AZ: Council on Chiropractic Education; 1998.
151. *Fort Wayne (IN) News and Sentinel,* July 23, 1918; Keating et al. (note xiii); John Bastyr's diploma and other information are in archives or on display at Bastyr University; for the story of mixers licensed as NDs in "straight" states such as Utah and South Carolina, see Phillips, Reed B. (2006) *Joseph Janse: The Apostle of Chiropractic Education* (Reed Phillips, publisher).
152. See *El Paso Herald-Post*, Monday, April 8, 1957.
153. *Journal of the ANA* July, 1949 (vol. 2, no. 7).
154. *Journal of the ANA* July, 1949 (vol. 2, no. 7).
155. Keating Joseph Jr C, Callender Alana K, Cleveland Carl III S. *A History of Chiropractic Education in North America* (Scottsdale, AZ. Council on Chiropractic Education; 1998.
156. *Journal of the ANA* June, 1951 (Vol. 4, No.6)—W. Martin Bleything, Editor.
157. Journal of the ANA June, 1951 (Vol. 4, No.6)—W. Martin Bleything, Editor.
158. *Journal of the ANA* June, 1951 (Vol. 4, No.6); *Reno Evening Gazette,* March 6, 7, 15, 1951; *The Atlanta Constitution,* February, 17, 1950; Keating, Joseph C., Jr., Callender, Alana K., and Cleveland, Carl S. III (1998) *A History of Chiropractic Education in North America, (*Scottsdale, AZ, Council on Chiropractic Education).
159. Attorney general's Opinion Letter V-1486, July 29, 1952, to Henry J. Schlichting, ND, Secretary, Texas Board of Naturopathic Examiners; *Journal of the ANA*, September 1948, for Dr. Spitler's remarks on Tennessee.
160. The Connecticut story told here is taken from coverage by *The Hartford Courant* for the following dates: June 6, August 20, 1946; March 20, April 24, May 7 and 29, July 16, 1947; and January 22 and 23, 1948.
161. The South Carolina story is taken from the coverage of the *Charleston (SC) News and Courier* for June 27, 1946; April 24, May 14 and 28, November 15, 1947; and April 11, 1949.
162. AG Opinion Letter V-1486, July 29, 1952, to Henry J. Schlichting, ND, Secretary, Texas Board of Naturopathic Examiners.
163. *Journal of the ANA* August, 1951; *Journal of the ANPSA* October and November, 1951.
164. *Journal of ANPSA*, September, 1951.
165. *Proceedings of the House of Delegates*. American Medical Association Annual Meeting; 1953.
166. Poen Monte M, Harry S. *Truman Versus the Medical Lobby*. Columbia, MO, Preface: University of Missouri Press; 1979.
167. (2013) (The Johns Hopkins University Press, Baltimore).
168. Volume 71, Issue 3, July 1, 2016.
169. AG Opinion Letter V-1486, July 29, 1952, to Henry J. Schlichting, ND, Secretary, Texas Board of Naturopathic Examiners.
170. See AG Opinion Letter S-60, to Austin F. Anderson, Criminal District Attorney, June 29, 1953, "Re: Constitutionality of Article 4590d, V.C.S. relating to the practice of naturopathy."
171. See Sabota, Leo M. and Martin, J. David "The Texas Attorney General-An Alternate State Supreme Court," in Kraemer, Carin and Maxwell (1975) *Understanding Texas Politics* (West Publishing Co., St. Paul, MN).
172. Committee Report to 1948 Convention of the ANA, Salt Lake City, UT, June, 1948, as reported in *Journal of the ANA* for September, 1948.

173. Kuts-Cheraux AW. *Naturae Medicina and Naturopathic Dispensatory. American Naturopathic Physicians & Surgeons Assn., Des Moines, IA*. Chattanooga, TN: Lulu Enterprises, Inc; 1953. Reprint (2007).
174. Altschuler Glenn C, Blumin Stuart M. *The GI Bill: A New Deal for Veterans*. New York: Oxford Univ. Press; 2009.
175. Keating Joseph Jr C, Callender Alana K, Cleveland Carl III S. *A History of Chiropractic Education in North America*. Scottsdale, AZ: Published by Council on Chiropractic Education; 1998.
176. Commencement announcement—UWS Archives.
177. See *Pittsburgh Press,* Sunday, June 27, 1954, for extensive coverage of the Hoxsey cancer treatment events in Pennsylvania in the summer of 1954. Hoxsey had been battling the U.S. government since 1950 in Texas, but it was only in 1953 and 1954 that he began being described as a "naturopath." See *Dallas Morning News,* Tuesday, June 30, 1953; for more on Hoxsey's entire career, which began in his native Iowa in the 1930s, see *The Los Angeles Times,* September 29, 1988, review of the documentary *Hoxsey: Quacks Who Cure Cancer*.
178. *Abilene Reporter-News, February 8, 1955; The Cameron (TX) Herald,* February 17, 1955; *The Corpus Christi (TX) Caller-Times*, February 3, 1955.
179. The history of WSC after Dr. Budden's death through the discontinuance of the School of Naturopathy is taken from *Oregon Pioneer,* pp. 31–35; *A History of Chiropractic Education in North America*, pp. 361–372, especially; and correspondence between Dr. Ralph Failor and Dr. Higgens and associated records from Dr. Failor's tenure found in the UWS library archives.
180. *The Austin (TX) American,* January 25, 1956; March 28, 1956; *Austin (TX) American-Statesman,* May 25, 1956.
181. There were over 1000 news stories statewide in Texas in 1957 reporting the Texas naturopaths' saga. This telling here draws from all of them, but specifically from the coverage by *The Austin (TX) American* for these dates: February 27, March 1, 4, 5, 8, 10, 13, 17, 19, 24, 28, April 3, 8, 12, 21, 24, May 2, 3, 8, 9, 10, 15, 23, 31, July 1, 7, 9, 13, 15, 25, 26, August 1, October 6, 8, 11, 12, 14, 21; and the *Austin (TX) American-Statesman* for February 28, March 4, 7, 12, 15, 20, 28, April 1, 3, 19, 27, May 6, 8, 31, July 1, 3, 8, 13, 25, October 2, 16. Also *The Odessa (TX) American* for April 19, 1957, about the "mystery witness."
182. *Valley Morning Star (Harlingen, TX)*, January 31, 1957 (Associated Press).
183. *El Paso Herald-Post* May 1 and May 3, 1957; C.I. Douglas, Reporter.
184. *Ibid*.
185. *Austin American* December 3, 9, 23, 24, 29; *Austin American-Statesman* 12/ 2.
186. *Ibid*.
187. *Austin American* January, 23, February 20, March 26; *Austin American-Statesman* February 19, 1958.
188. *Big Spring Herald (TX)* January 22, 1961; *Midland Reporter-Telegram (TX)* April 30, 1962; *Arizona Republic* September 7, 1965; *Arizona Republic,* June 5, 1973; *Lubbock Avalanche-Journal*, June 6, 1973.
189. *The Miami Herald* April 19, April 20, 1949.
190. *The State,* Columbia, SC, March 17, 1955.
191. *The Miami Herald* April 19, 20, 1949.
192. *The Miami Herald*, April 20, 1949.
193. Lawrence Thompson, Reporter, *The Miami Herald*.
194. *Charleston News and Courier*, May 1, 1955.
195. *Miami Daily News,* Jane wood, Reporter, November 23, 24, December 1, 2, 1955
196. *Miami Daily News,* April 4, 1957.
197. *Salt Lake Telegram,* March 24, 1925; *Salt City Telegram,* March 5, 1937; *The Salt Lake Tribune,* February 16, 1939; *Salt Lake Telegram,* legislative coverage February of each legislative session, 1941, 1943, 1945.
198. *Salt Lake Tribune*, September 20, 1946.
199. *The Ogden (UT) Standard-Examiner,* September 3, 1955.
200. *The Daily Herald (Provo, Utah),* June 19, 1956; August 9, 1956; December 21, 1956.
201. *The Ogden Standard-Examiner (Ogden, UT)*, March 22, 1957.
202. *Salt Lake City Tribune,* January 29, 1959; *The Ogden Standard-Examiner (Ogden, UT),* March 25, 1959; *The Salt Lake Tribune*, November 2, 1961.
203. *State v. Houck,* 32 Wn. (2d) 681,203 P. (2d) 693 (1949); *Kelly v. Carroll,* 36 Wn.2d 482, 219 P.2d 79 (1950); *State v. Kelsey*, 46 Wn.2d 617, 283 P.2d 982 (1955).
204. Independent Practitioners Under Medicare: A Report to the Congress. U.S. Department of Health, Education and Welfare, William J. Cohen, Secretary, Naturopathy; 1968:126–145.
205. *Ibid* at page 137.

5

Philosophy of Naturopathic Medicine

Rachelle S. Bradley, ND

OUTLINE

INTRODUCTION

This chapter examines the philosophical foundation of naturopathic medicine and its modern applications. Unlike most other health care systems, naturopathy is not identified by any particular therapy or modalities (e.g., conventional medicine, drugs and surgery; chiropractic, spinal manipulation). A wide variety of therapeutic styles and modalities are found within the naturopathic community (Box 5.1). For example, there are still practitioners who adhere to the strict "nature cure" tradition and focus only on diet, "detoxification," lifestyle modification, and hydrotherapy. There are also those who specialize in homeopathy, acupuncture, or natural childbirth. At the other end of the spectrum are naturopathic physicians who use botanical medicines, nutraceuticals, and pharmacology extensively to manipulate the body's biochemistry and physiology. Finally, there is the majority, who practice an eclectic naturopathic practice that includes a little of everything.

Since its inception 130 years ago, naturopathic medicine has been an eclectic system of health care. This characteristic has allowed it to adopt many of the more effective elements of natural and alternative medicine and to adopt conventional medicine's basic and clinical sciences, diagnostics, and pharmacology. Through all of this eclecticism, naturopathic medicine has always identified the Latin expression *vis medicatrix naturae* (the healing power of nature) as its philosophical linchpin.

However, the expression *vis medicatrix naturae,* by itself, does not provide a clear picture of naturopathic medical philosophy or an understanding of the practice of naturopathic medicine in all of its varied forms. With the profession's history of eclecticism, no two practitioners treat any individual patient exactly alike. This situation has its advantages (e.g., individualization of each patient's care, more therapeutic options) but also makes it difficult to perceive the profession's philosophical cohesiveness. Another major disadvantage of this eclecticism is the difficulty in developing consistent practice standards.

To attempt to solve this problem, the modern profession has articulated a general statement of naturopathic principles that expand on *vis medicatrix naturae* (Box 5.2). However, to gain a more in-depth understanding of naturopathic medicine, one must discuss medical philosophy in general.

MEDICAL PHILOSOPHY

The issues fundamental to a discussion of medical philosophy have changed little since naturopathy first appeared as a distinct profession at the end of the 19th century. What has changed is the level of understanding of the biological process and the language of science. Most people who study the early writers on naturopathic medical philosophy quickly get lost in the archaic language and arguments used to justify the theories. This chapter translates these concepts and issues into modern terms.

Vitalism Versus Mechanism

Historically, there have been two main medical philosophies, those of vitalism and mechanism. Their origins can be traced to the Hippocratic writings of ancient Greece. Throughout history, the line separating these two schools of thought has not always been clear, but their philosophical perspectives have generally been in opposition. The conflicting goals and philosophical foundations of these two concepts remain relevant as the modern practices of conventional and alternative physicians come into conflict. As will be seen, the foundations of naturopathic medical philosophy are found in vitalism. However, naturopathy also recognizes the practical value of the mechanistic approach to health care.

Mechanism

Up to the early part of the 20th century, there was considerable debate over the issue of vitalism versus mechanism in the field of biology. The mechanists, or materialists, maintained that the phenomenon of life could be explained exclusively as the product of a complex series of chemical and physical reactions. They denied the possibility that the animate had any special quality that distinguished it from the

BOX 5.1 Naturopathic Modalities

Naturopathic physicians are trained to use a number of diagnostic and treatment techniques. These modalities include the following:

- *Diagnosis.* All the conventional clinical laboratory, physical diagnosis, and imaging (e.g., radiography) techniques, as well as holistic evaluation techniques
- *Counseling.* Lifestyle, nutritional, and psychological
- *Natural medicines.* Nutraceuticals (i.e., all food constituents, constituents of biochemical pathways, etc.), botanical medicine, and homeopathy
- *Physical medicine.* Hydrotherapy, naturopathic manipulative therapy, physiotherapy modalities, exercise therapy, and acupuncture
- *Family practice.* Natural childbirth, minor surgery, natural hormones, biologicals, and pharmaceuticals

BOX 5.2 The Principles of Naturopathic Medicine

The Healing Power of Nature: *Vis Medicatrix Naturae*

Nature acts powerfully through healing mechanisms in the body and mind to maintain and restore health. Naturopathic physicians work to restore and support these inherent healing systems when they have broken down by using methods, medicines, and techniques that are in harmony with natural processes.

First Do No Harm: *Primum Non Nocere*

Naturopathic physicians prefer noninvasive treatments that minimize the risks of harmful side effects. They are trained to know which patients they can treat safely and which ones they must refer to other health care practitioners.

Find the Cause: *Tolle Causam*

Every illness has an underlying cause, often in aspects of the lifestyle, diet, or habits of the individual. A naturopathic physician is trained to find and remove the underlying cause of a disease.

Doctor as Teacher: *Docere*

A principal objective of naturopathic medicine is to educate the patient and emphasize self-responsibility for health. Naturopathic physicians also recognize and employ the therapeutic potential of the doctor–patient relationship.

Treat the Whole Person

Health or disease comes from a complex interaction of physical, emotional, dietary, genetic, environmental, lifestyle, and other factors. Naturopathic physicians treat the whole person, taking all of these factors into account.

Preventive Medicine

The naturopathic approach to health care can prevent minor illnesses from developing into more serious or chronic degenerative diseases. Patients are taught the principles with which to live a healthy life; by following these principles they can prevent major illnesses.

inanimate. It was their contention that the only difference between life and nonlife was the degree of complexity of the system.

Mechanism has several other distinctive characteristics. Its most obvious is that it is reductionistic. *Reductionism* is often used as a synonym for *mechanism.* Mechanistic science is also characterized by an emphasis on linear causality. Without this emphasis on reductionism and linear causality, Western science and medicine would probably have not been so successful. As the 20th century advanced, each new discovery in biological and medical science reinforced the arguments for mechanism, until, by the middle of the century, the biology community had almost exclusively embraced the philosophy of mechanism.

Mechanism is the philosophical foundation of biomedical science and conventional medicine. It is especially visible in the treatment modalities of surgery and most pharmaceuticals. Mechanistic medicine identifies disease and its accompanying signs and symptoms as simply the result of a disruption of normal chemical reactions and physical activities. Such disruptions are caused by the direct interference in these reactions and activities of a "pathogenic agent." (For the purposes of this discussion, the expression "pathogenic agent" refers to any known or unknown etiological agent, influence, or condition; examples are microbial agents, autotoxins, genetic defects, environmental toxins, non–end-product metabolites, and physical and emotional stress and trauma.) A living organism, then, is simply a very complex machine that, as a result of external agents and influences and "wear and tear," breaks down. Because the signs and symptoms of disease are thought to be caused only by these mechanical disruptions and interference with reactions, they are considered to be completely destructive phenomena and are therefore to be eliminated. Disappearance of the signs and symptoms indicates that the pathogenic agent and its resulting disease have been eradicated or, more likely, controlled. The goals of mechanistic medicine tend to be the quick removal of the signs, symptoms, and pathogenic agent.

Mechanistic medicine is being practiced in cases in which the intention of the therapy is to intervene in the perceived mechanism of the disease and/or to relieve the symptoms. Examples would be the use of antihistamines to relieve rhinitis, vitamin B_6 to help carpal tunnel syndrome, emergency care for traumatic injuries, coronary bypass surgery for blocked arteries, and insulin in juvenile-onset diabetes. Mechanism is also being used when an identified pathogenic agent is directly attacked or eliminated; for example, the use of antibiotics or the isolation of a patient from a particular allergen. Clearly, mechanistic medicine can be very effective in achieving its goals. In the presence of modern medical technology, it is easy to see how this philosophy came to dominate biology, medicine, and the attention of the public.

However, the unsolved problems of mechanistic medicine—particularly those of chronic degenerative disease; authoritarianism, which alienates patients from responsibility for their own health; and the rising cost of health care—suggest that there are limits to the mechanistic perspective and explain why vitalism has not disappeared and is in resurgence.

Vitalism

The philosophy of vitalism is based on the concept that life is too well organized to be explained simply as a complex assemblage of chemical and physical reactions (i.e., a living system is more than just the sum of its parts). This is in contrast to the mechanist's contention that "the only difference between life and non-life is the degree of complexity."

Throughout the 19th century, the debate between vitalism and mechanism was carried out mostly by biologists and, in medicine, between the "regular" doctors and those doctors who would now be called alternative. In the medicine of the 19th and early 20th centuries these would have been homeopathic, hydrotherapy, nature cure, and eclectic doctors—all medical doctors with equivalent credentials under the laws of the time. Although the specific terms of "vitalism" and "mechanism" were not necessarily the nomenclature of their debate, the perspectives were the same.

Interestingly, through most of the 19th century this debate within the medical community was distinctively not based on science as we currently think of it. The "regular" doctors of the era, as represented by the American Medical Association, were still strongly influenced by Galen's theory of disease of the four humors with its imaginary anatomy and physiology, bleeding, leeches, mercury, and other horrific treatments. Both the homeopaths and eclectic doctors argued based on

empirical evidence; on the other side, the regular doctors argued based on a dogmatic theory that was more than 1500 years old and unsupported by any evidence. Harris Coulter produced the seminal work on this debate in his three-volume book, *The Divided Legacy*.

The debate between vitalism and mechanism within the field of biology is well documented within the biology journals of the time. This was an era of amazing discoveries about how life functioned. Naturally, this is where the focus of this debate took place for biologists. As the secrets of cellular metabolism were revealed, this debate lurched from one specific argument to the next. The issue was where in the living organism did "God" have direct control. For example, at one point it was argued that the "seat of the soul" was the cell. As the cell was better understood, the place that was the point of God's intervention was postulated to be the nucleus. As research further revealed how the organelles functioned, the vitalistic biologists gave up ground until vitalism as a distinct philosophy in biology was finally abandoned.

The error that doomed the vitalistic-oriented biologists was that they were all reductionistic in the same way as the mechanistic biologists. Reductionistic science seems completely able to learn how life functions from a biochemical and biophysical perspective. Eventually, all of the individual chemical and physical reactions that are found in the processes of life will probably be identified. However, the vitalistic biologists missed the most essential aspect of vitalism: *holism*.

In naturopathy's early years there were few interactions between it and the academic and research worlds. The great authors and practitioners came to naturopathy through "conversion," in other words, most had been cured of some health problem by a natural cure and felt naturopathy and curing the sick was now their calling. There is no evidence that these naturopaths even knew that this debate between vitalism and mechanism was going on in the biology literature. Research in this early era of naturopathy consisted of observing nature and applying these observations to treating patients. This led to a deep appreciation of "nature's" desire for balance and order (what a physiologist would call homeostasis). This holistic perspective, combined with the results of the naturopathic treatments, was the empirical evidence that drove their understanding of health and disease. It was only in the latter half of the 20th century that the field of naturopathic medicine began to converge with the academic and research worlds. Since the 1970s this convergence has moved at breakneck speed, until today there is no longer any real distinction (although this is not evident in some of the politically motivated diatribes against the field of natural medicine). However, by this time the academic and research worlds had long since forgotten about vitalism.

An organism's unique complexity—as demonstrated by its ability to grow and develop, respond to stimuli, reproduce, and repair itself—requires a level of organization and coordination that suggests a distinct quality that is not readily explained by mechanism. This is studied extensively by all medical students in physiology class as the "normal" homeostatic process common to all living organisms. However, the tendency in conventional medical school is to put the concept aside when the student moves on to study pathology and the clinical sciences. Yet up to the point of death, maintaining homeostasis is a prime, if not the primary, driving force in all living organisms. To think that homeostasis is only an important factor in "normal" physiological processes and has no relevance in pathology is to ignore all of the basic sciences. All life is attempting to return to this ideal state whenever injured or ill. The only point in the life cycle that an organism is no longer "trying" to maintain homeostasis is death.

Reductionistic science has done a wonderful job elucidating the functions of the various components of life, but it tends to focus the researcher and the physician on the disease process as an isolated phenomenon rather than the result of a complex reaction of the whole organism to a pathological agent. Fortunately, the debate between the vitalistic and mechanistic perspectives in the modern era focuses on the more relevant and holistic general concepts. Although modern vitalism is inherently holistic in its view, there is no conflict with the findings of biomedical science. What is significant is not the individual biochemical or biophysical reactions, but the fact that they are all coordinated to such a degree as to produce the special activities of a living organism. Because there is no inanimate counterpart to this level of complexity and organization, homeostasis is the most dramatic general argument in favor of vitalism.

A less dramatic argument supporting the vitalistic perspective is the "problem of entropy." Entropy is the tendency of any closed system to find equilibrium, that is, the state of least organization. In other words, systems tend to run down and become less complex over time. In defiance of this universal rule, life, up until the point of death, consistently creates more complex systems out of simple ones. To do this, life actively pursues external matter and energy to incorporate into itself while also selectively eliminating byproducts from its use of this matter and energy.

When the problem of entropy is examined on the molecular level, the same individual chemical processes and elements may be found in both animate and inanimate systems. In the inanimate system, however, there is a constant move toward a state of chemical equilibrium. This type of system cannot maintain an unstable chemical state and always seeks stabilization. Even after the addition of external exciting energy, the system returns to the simplest, least reactive state possible. The animate system is virtually the opposite. It is continuously in a state of dynamic chemical instability, actively seeking energy to maintain this instability and consistently moving to more complex and more organized states (and back again). It is only at the onset of death that an animate system begins to move toward equilibrium, and, of course, then it is no longer animate.

The third general argument in favor of a vitalistic view of life is evolution. For evolution to exist as a force in nature, generations of living organisms have to survive long enough to grow, reproduce, and then evolve. For this survival to take place, the organisms' homeostatic and repair processes must be consistently directed toward maintaining a state of balance with the external environment (i.e., health). Any organism that does not behave biochemically and physiologically in this manner dies and cannot evolve. Thus the phenomenon of evolution, as the action of countless living organisms over eons, multiplies life's antientropic quality and is incompatible with a mechanistic view of living systems.

These easily observable examples of life's "special quality" suggest an "organizing force" that goes beyond what is possible from mere chemistry. This quality that makes life unique should not be mistaken as a metaphysical concept, although an argument for or against such concepts is not intended here. The point is only that vitalism is a medical philosophy based on observable scientific phenomena. Unfortunately, a definitive definition of this quality (in the old literature called the "vital force," defense mechanism, or simply "Nature") will have to wait for vitalistically or holistically oriented researchers. Reductionistic research has not provided much clarification of these special qualities of life—just ask a modern reductionistic biologist to explain how homeostasis works. They can describe what happens on a biochemical and biophysical level, but they cannot describe why it happens.

At this point in the discussion, not many mechanistic practitioners would have reason to be uncomfortable. However, the conflict becomes evident with examination of the premises on which the practice of vitalistic medicine is based. What truly separates vitalism from mechanism and makes it useful as a medical philosophy is its perspective on disease and its associated symptoms.

BOX 5.3 Cure, Suppression, Palliation, and Healing

Cure: A cure occurs when:
(a) a treatment is given to the person;
(b) the signs and symptoms of the disease go away;
(c) the treatment is removed and the signs and symptoms stay away; and
(d) the whole person is healthier and less likely to get sick than before the illness.

This is almost always going to occur only when the whole person was treated, and not just the disease or its symptoms. Palliation and suppression never lead to cure in and of themselves.

Palliation: Palliation occurs when:
(a) a treatment is given for the disease;
(b) the signs and symptoms of the disease go away; but
(c) when the treatment is removed the signs and symptoms return.

The symptoms of the disease are simply being controlled (not cured) as long as the treatment is continued.

It is a classic error of many practitioners and patients to equate palliation with moving toward cure. Palliation is on the opposite end of the spectrum as cure and is closer to suppression. Palliation can be useful but, in and of itself, never leads to cure—other more vitalistic and holistic interventions are necessary and may be as simple as changing to a healthier diet, removing some obstacle to recovery, or reducing stress, or as complex as classical homeopathy or traditional Chinese medicine. When palliation is used over a long enough time, suppression is the natural consequence.

Palliation is the most common result of almost all health care interventions. This is especially true of conventional medicine but also true for much of alternative medicine as well. Unfortunately, both the practitioner and the patient's expectations are frequently satisfied with palliation. This is the most frustrating aspect of modern health care, whether conventional or alternative. Too few people are striving for a cure.

Suppression: Suppression is when:
(a) a treatment is given for a disease;
(b) the signs and symptoms of the disease go away;
(c) the treatment is removed and the signs and symptoms stay away; but
(d) the whole person is less healthy.

Although the symptoms of concern are better, the whole person is worse, which leads to more and worse disease in the future. In conventional medicine suppression is often a goal. Alternative medicine tries for a higher standard, but because palliation is often what happens, suppression can occur here, too.

Suppression frequently occurs because a treatment is given for a symptom or disease rather than the whole person being treated. Suppression may lead later to another more invasive illness.

Healing: Healing is what a living organism (body–mind) does, or attempts to do, for itself. A treatment can only:
(a) control signs and symptoms (palliate or suppress);
(b) support life in a crisis (palliate or suppress);
(c) attack an invading organism such as bacteria or remove a pathological agent such as a toxin or allergen (palliate);
(d) mechanically repair tissues that have been damaged or are malformed (palliate); or
(e) support and/or stimulate the organism's innate healing processes while the body–mind does the work of healing itself (cure).

Curative treatment involves stimulating the whole organism to heal itself. The palliation and suppression of symptoms does not help stimulate self-healing. Palliation tends to create the opposite effect and suppression actually gets in the way of the whole body–mind's efforts to self-heal.

Meaning of Disease

Vitalism maintains that the pathogenic agent does not directly cause most symptoms accompanying disease; rather, they are the result of the organism's intrinsic response or reaction to the agent and the organism's attempt to defend and heal itself. Symptoms, then, are part of a constructive phenomenon that is the best "choice" the organism can make, given the circumstances at any particular time.

Symptoms can be further described as arising from two situations. The first and most common situation is when the symptoms are from what would traditionally be called a "healing reaction"—the organism's concerted and organized attempt to defend and heal itself (i.e., the organism's homeostatic process). These healing reactions produce what can be called "homeostatic symptoms." Examples are fever and inflammation in infections, almost any reaction of the immune system, and many of the symptoms of chronic disease.

This interpretation of symptoms is generally ignored by mechanism. Instead, it views a symptom as the result of a destructive process and focuses on intervening by relieving the symptom or manipulating the pathological mechanism. Mechanistic medicine is therefore most often working contrary to homeostasis and the organism's attempt at healing (this is usually its intent). When this therapeutic approach is effective, vitalists call the result a "suppression" (Box 5.3). This approach to health care is so pervasive that most people, lay and professional alike, still think nothing of suppressing mild fevers with antipyretics. In contrast, vitalism considers these homeostatic symptoms to be the product of a constructive phenomenon and therapeutically stimulates and encourages this directed healing process.

In contrast, vitalism considers these symptoms to be the product of a constructive phenomenon and therapeutically stimulates and encourages this directed healing process. Rather than simply trying to eliminate a pathogenic agent, as mechanistic therapy might, vitalism focuses more on augmenting the organism's resistance to that agent. That is not to say that vitalists object to removing the agent, only that it should be done in the context of simultaneously increasing resistance (in other words, decreasing susceptibility). The importance of this approach becomes evident when one recognizes that disease is only possible when both a pathogenic agent and a susceptibility to that agent are present.

Healing reactions can take several forms. In the first type, an organism's response to a pathogenic agent does not produce symptoms. When the organism is capable of easily defending itself from the agent, no symptoms are perceivable. This is a common homeostatic process and is demonstrated when a potential pathogen, such as β-hemolytic streptococcus, is cultured from a healthy person's throat. However, when the organism is more susceptible or the relative strength of the pathogenic agent is greater, a threshold is reached and symptoms become perceivable. Successful healing reactions of this type include vigorous acute diseases that quickly resolve. The early naturopaths would have called these acute reactions "healing crises." As the susceptibility of the organism increases relative to the strength of the pathogenic agent, there is a greater likelihood that the healing attempt will not be successful. When such a reaction is unsuccessful but vigorous, death may result, unless there is timely application of vitalistic or mechanistic therapy. Examples of this situation are acute bacterial meningitis and cholera.

When the healing attempt is feeble and therefore ineffective, the reaction usually goes into the "chronic disease" stage. Vitalists observe that suppression seems to increase the likelihood that the reaction will be forced to go into such a chronic stage. In this situation the reaction is "smoldering," and most often the organism cannot overcome the

pathogenic agent unassisted. It just "holds its own," and as the organism's general health decreases over the years, the reaction gradually degenerates, producing symptoms that become less homeostatic as it moves to an end-stage pathology. Palliating the symptoms during this phase of the disease contributes to the declining health over time because palliation means that the underlying susceptibility or problem is not being addressed in a curative manner. If the organism can be therapeutically stimulated to produce a more vigorous healing reaction, it can often successfully complete the original healing attempt. This augmented reaction is another example of a naturopathic healing crisis and would also be called an "aggravation" by the vitalists who practice homeopathic medicine.

Intervening mechanistically by relieving symptoms does little to stimulate or encourage the healing response; it usually actually inhibits the healing response. In contrast, vitalistic therapies can be very effective in helping these healing reactions, because the goals of such therapies are precisely the same as those of the organism. Thus it is thought that vitalistic medicine works because, by honoring this process and thereby strengthening the whole organism, it encourages a more effective healing effort. Ideally, the organism is then able to accelerate and complete its reaction against the pathogenic agent, leading to the permanent disappearance of the symptoms as it returns to a state of health.

It would be naive to say that every stage of the healing reaction is positive and in the best interest of the organism or that no symptoms should be palliated. The modern vitalist acknowledges that palliative intervention is sometimes necessary. In contrast, it is important to note that routine mechanistic intervention can encourage its own worst-case scenarios. When mechanistic therapies successfully suppress an organism's chosen healing reaction, a less effective and less desirable response is often produced. Therefore when suppression occurs, it can lead to a more complicated medical situation. Consequently, the very practice of mechanistic medicine tends to reinforce its practitioner's conviction that such intervention is usually necessary. It should be noted, however, that not all mechanistic intervention leads to suppression. It happens less often when the pathogenic agent can be readily eliminated, such as the use of an antibiotic in nonrecurring acute bacterial infections, or when relatively noninvasive therapies are used, such as natural medicines.

The second type of symptom-producing situation occurs when the organism produces symptoms in response to an organic lesion that arises from the direct pathological influence of a pathogenic agent. These could be called "morbid symptoms," examples of which are symptoms from the mass of an invasive tumor, shortness of breath from emphysema, and pain of an injury or myocardial infarction. It should be mentioned that even these symptoms are the result of the organism's overall effort to maintain homeostasis, and homeostatic symptoms are also often present. In addition, a morbid symptom is not necessarily without utility. For instance, pain is valuable as an indication of tissue damage. As can be seen, many, if not most, of these situations involve end-stage disease. Here mechanistic therapies can be very positive when the goals of the therapy do not conflict with those of the organism.

There are instances when invasive mechanistic intervention will probably be required to save "life and limb." These include such conditions as birth and genetic defects, serious traumatic injuries, crisis situations, overwhelming infections, and many malignancies. Unfortunately, mechanistic intervention does not guarantee a successful outcome either. Even in these situations, however, the effectiveness of vitalistic and natural therapy should not be underestimated, and their concurrent use will certainly augment any mechanistic intervention.

The concept of homeostatic and morbid symptoms can be a useful tool to help the understanding of the healing and disease processes, but in many situations it may not be possible to categorize the type of symptoms produced. A rough rule of thumb, however, would be that virtually all symptoms accompanying reversible or functional diseases are homeostatic. In contrast, many of the symptoms associated with traumatic injury and end-stage pathology would be morbid symptoms.

Scientific Medicine

Although mechanism and vitalism represent opposing perspectives, the systems of medicine that represent these philosophies can be successfully tested and examined with the scientific method.[1] That is not to say that the philosophy of vitalism has been unquestionably proven—only that the validity of vitalistic interventions can be scientifically demonstrated. If a therapy can be proven effective, the effectiveness implies the accuracy of the philosophy on which it is based. Unfortunately, very few of the vast resources of the biomedical community have been directed toward investigating vitalistic medicine.

Conventional medicine, as the dominant health care system and a representative of mechanism, has claimed for itself the title "scientific medicine." However, it is inherently no more or less scientific than vitalistic medicine. A system is scientific only when it has met the criteria of the scientific method. This method requires the collection of data through observation and experimentation and the formulation and testing of hypotheses. Nonprejudicial science can effectively study any system, but the researcher must understand the system's particular paradigm. Experiments on a vitalistic therapy based on a reductionistic and mechanistic model are not going to be constructed to show success, or if they do show success, it will be entirely fortuitous.

The criteria of the scientific method can be met by vitalistic medicine, but only when the researchers recognize that it cannot be studied as though it is reductionistic or based on a simplistic model of linear causality. When the experimental model acknowledges the complexity of a living system in a social context (i.e., holism and circular causality/feedback loops), vitalistic medicine proves to be both verifiable and reproducible and, thus, scientific. Unfortunately, because of its current political and economic dominance, conventional medicine is in the position to dictate (through economic and publication control) that research, and therefore the scientific method will be applied primarily to itself. The result is that most conventional practitioners dismiss vitalistic medicine, along with all alternatives, as unscientific. Ironically, most vitalistic physicians also have extensive training in mechanistic and/or conventional medicine. Generally, they are capable of practicing mechanistically and do so to greater or lesser degrees.

NATUROPATHIC PHILOSOPHY

Vis Medicatrix Naturae

Naturopathic physicians assert that all true healing is a result of *vis medicatrix naturae* (the healing power of nature). Unfortunately, some people in the field of alternative medicine (including some naturopathic physicians and students) have mistakenly translocated this concept to the therapy. These practitioners tend to operate as though this "healing power" is an intrinsic property of the natural therapy or medicinal substance itself. In contrast, proponents of vitalism and naturopathic medicine have always understood that the "healing power of nature" is an inherent property of the living organism. *Vis medicatrix naturae* is the living organism's "desire" and ability to heal itself. As mentioned, the homeostatic process best exemplifies this.

Historically, naturopathy is a vitalistic system of medicine. However, over the past 130 years its eclecticism has allowed it to incorporate a

number of therapies that can function mechanistically. What makes these mechanistic therapies acceptable, given naturopathic medicine's vitalistic foundation, is the emphasis on meeting each patient's pragmatic health care needs. So the application of *vis medicatrix naturae* in practice is constantly adjusted depending on the situation at hand.

Ideally, naturopathic practice involves only the use of therapies that support the organism and encourage its intrinsic healing process to work more effectively while avoiding the use of medicines and procedures that interfere with natural functions or have harmful side effects. Natural medicines and therapies are therefore preferred, because when they are used properly and in appropriate circumstances, they are the least harmful, least invasive, and best able to work in harmony with the intrinsic natural healing process. In addition, their constituents have been encountered in nature for millions of years. This long period of exposure has enabled the body to develop metabolic pathways capable of effectively using, processing, and detoxifying these medicines.

The total organism is involved in the healing attempt, so the most effective approach to diagnosis and treatment is to consider the whole person. In addition to physical and laboratory findings, important consideration is given to the patient's attitude, psychological and spiritual state, social circumstances, lifestyle, diet, heredity, and environment. Careful attention to each person's unique individuality and susceptibility to disease is critical to the proper evaluation and treatment of any health problem.

Naturopathic physicians contend that most disease is the direct result of the ignorance and violation of what would be traditionally called "natural living laws." These general lifestyle rules (including diet) are based on the concept that there is an environment (both internal and external) that optimizes the health of an organism. Analysis of the lifestyles of Paleolithic and healthy primitive and modern cultures gives naturopathic physicians and their progenitors many clues as to what a healthy lifestyle should involve.

Throughout most of modern history, biomedical science has focused primarily on researching the sick. Recently it has finally begun to evaluate what constitutes a healthy lifestyle. To no one's surprise, this lifestyle looks like the same one advocated by naturopaths for the past 130 years. A healthy lifestyle could be generalized to include the following:

- Consuming natural unrefined foods
- Getting adequate amounts of exercise and rest
- Living a moderately paced lifestyle
- Having constructive and creative attitudes
- Connecting to other people socially
- Being present to the spiritual aspects of life
- Avoiding toxins and polluted environments
- Maintaining proper elimination

It is also important to control these areas during illness to remove as many unnecessary stresses as possible and to optimize the chances that the organism's healing attempt will be successful. Therefore patient education and responsibility, lifestyle modification, and preventive medicine are fundamental to naturopathic practice.

Although the practice of naturopathic medicine is grounded in *vis medicatrix naturae,* it also recognizes that mechanistic intervention in the disease process is sometimes efficacious and, at times, absolutely necessary. Therefore naturopathic physicians treat patients with a wide variety of vitalistic and mechanistic therapeutic modalities. It is the circumstances and the goal of the therapy that ultimately determines which approaches are used. Naturopathic physicians have a long-standing tradition of integrating the best aspects of traditional, alternative, and conventional medicine in the interest of the patient. As appropriate, patients are referred to other health care practitioners. Whenever possible, every effort is made to use all treatment techniques in a manner that is harmonious with the naturopathic philosophy.

Natural Medicines and Therapies

Traditionally, medicines administered and prescribed by naturopathic physicians have been primarily natural and relatively unprocessed. Four categories of natural medicines can be defined.

The first consists of substances found in nature that have been only minimally processed. Examples include, but are not limited to, foods, clean air and water, and whole herbs. The early "nature cure" practitioners used this category primarily. The second category involves agents extracted or made from naturally occurring products. Although these medicines have undergone processing, their constituents are still in the form found in the original natural substance. These first two types of natural medicinal substances have synergistic constituents that allow their use at lower doses with a resultant broader and safer therapeutic index. Examples of this category are tinctures and other botanical extracts (some of which are standardized on one or more constituents known to be clinically effective), homeopathic medicines, glandular extracts, and other substances of animal origin.

The third category of natural medicines comprises those highly processed medicinal substances that are derived from a natural source. Often everything has been removed from such substances but the identified active ingredient, and they no longer have any synergistic constituents. Examples of these are the many new nutraceuticals made from plant substances, constituents of biochemical pathways, enzymes, amino acids, minerals, vitamins, and other food extracts.

The fourth category that may be considered natural are those manufactured medicines that are presumed to be identical to naturally occurring substances. They have the advantage of being less expensive and are typically available in higher concentrations. Examples of these manufactured natural medicines include bioidentical hormones, synthetic vitamins, and analogues of plant and animal constituents. However, their use is a compromise because:

- It is difficult to determine whether they are the equivalent of the natural product.
- They lack natural synergistic components.
- They may include contaminants from the manufacturing process; these contaminants are often chemically and structurally similar to the desired medicine but generally interfere with the normal pathways rather than enhance them.

Naturopathic physicians also use many natural physical therapies. What makes a therapy natural is that it is derived from a phenomenon of nature and is used to stimulate the body to heal itself. Examples of these phenomena are air, light, heat, electricity, sound, and mechanical force. Some of these natural therapies are mechanical and manual manipulation of the bony and soft tissues (naturopathic manipulative therapy), physiotherapy modalities (e.g., electrotherapy and ultrasound), hydrotherapy, and exercise therapy. Naturopathic physicians also use lifestyle modification, counseling, and suggestive therapeutics. These therapies are all discussed in more detail in other chapters.

Family and Specialty Practice

Naturopathic physicians, like other types of primary care providers, develop practices that meet their personal interests and skills. Although most are engaged in general and family practice, some also specialized in particular therapeutic modalities and/or types of health problems. In all situations, however, the emphasis is still on treating the whole person. The practice of family medicine requires the use of some medicines, techniques, and devices that are not natural but belong among the comprehensive family practice services offered by the naturopathic profession.

In the modern era of naturopathic medicine many states have expanded the scope of practice so that naturopathic physicians now practice much like other primary care practitioners with pharmaceutical prescribing rights. However, naturopathic physicians generally approach the use of pharmaceuticals differently than conventional physicians. They are seen as temporary interventions to be used to support the patient while other, more vitalistic natural therapies are used to help the patient recover his or her health with the ultimate goal of no longer needing the pharmaceutical.

Many naturopaths have also developed advanced expertise in specific natural therapeutic modalities. These practitioners have usually invested in postgraduate training, such as that available through residencies. Three therapeutic specialties that merit mention are natural childbirth, acupuncture, and homeopathy. There is also a growing trend of specializing in organ systems (e.g., gastroenterology) or diseases (e.g., cardiology).

THE PHILOSOPHICAL CONTINUUM

When the various healing systems are examined and placed on a philosophical continuum, mechanism and vitalism are on different ends of the same health care spectrum. Both ends of this health care continuum have their strengths and weaknesses. Mechanistic medicine is effective for trauma, crisis care, end-stage disease, and many acute disorders. However, it is clearly a failure for most chronic disease. Conventional medicine considers most chronic diseases incurable. Vitalistic medicine, in contrast, has its most dramatic successes with chronic disease and is effective with many kinds of acute disease. It is not very effective with trauma and crisis care or with end-stage disease, although it can be a very useful complement to conventional medicine. As can be seen, both ends of the health care spectrum are necessary if every patient's health care needs are to be met.

Although aspects of naturopathic medicine (e.g., constitutional hydrotherapy) and conventional medicine (e.g., chemotherapy) represent the archetypes of vitalism and mechanism, the space between the ends of this spectrum is a gray area within which both naturopathic and conventional physicians operate on a continual basis. Naturopathic physicians integrate vitalistic therapies with mechanistic therapies, but it is not possible for everyone to be experts in everything. The vast majority of naturopathic or conventional physicians cannot learn and competently practice all types of health care. Consequently, to effectively meet society's health care needs, it is necessary to create an integrated/collaborative health care system. Such a system would have both vitalistic and mechanistic practitioners working together in the same clinical settings.

The trends of popular culture and biomedical science that are finally beginning to study alternative medicine suggest that the creation of an integrated health care system is now well under way. However, it takes no great skill for a mechanistic medical doctor to switch from giving a synthetic drug for a disease to giving a natural medicinal substance (both mechanistically oriented interventions) without understanding vitalistic thinking. If naturopathic medicine becomes just another mechanistic system using natural medical substances to treat disease (instead of a system identified with treating the whole person vitalistically), it will lose its unique niche in an integrated health care system. To survive and thrive in this new environment, naturopathic medicine must keep its vitalistic roots. With a thorough grounding in *vis medicatrix naturae,* modern naturopathic medicine will flourish and achieve a leadership position as the dominant health care paradigm shifts to the integrated medicine of the future.

CONCLUSION

The practice of naturopathic medicine can be summarized most simply as helping the body–mind heal itself in the least invasive, most fundamentally curative manner possible. This approach is not tied to any particular therapy or modality, but rather is oriented to a rational blend of vitalistic and mechanistic principles working with the whole person and educating the patient in the ways of health.

As naturopathic knowledge of health and disease grows, new therapies and approaches to health care will be added as they satisfy the principle of *vis medicatrix naturae.* With integration of the larger health care system, naturopathic medicine's place is assured as the profession that truly understands each unique human being's power to heal.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. A thorough review of all health care modalities in use today reveals some that could be considered metaphysical. These include such things as prayer, faith healing, psychic healing, healing touch, touch for health, and medical dowsing. Generally speaking, the actual operator of the therapy must call on God or have some special endogenous skill or "power" that goes beyond intellectual knowledge. This makes these modalities "operator-dependent" and, thus, cannot be validated separately from the practitioner—greatly increasing the difficulty of their scientific verification. Consequently, these modalities are not historically relevant to this discussion of medical philosophy.

FURTHER READING

Baer HA. The potential rejuvenation of American naturopathy as a consequence of the holistic health movement. *Med Anthropol*. 1992;13:369–383.

Coulter HL. *Divided Legacy.* Richmond, CA: North Atlantic Books.

Coulter HL. *Homeopathic Science and Modern Medicine.* Richmond, CA: North Atlantic Books.

Dubos R. *Mirage of Health: Utopias, Progress, and Biological Change.* New York: Harper.

Kirchfeld F, Boyle W. *Nature Doctors: Pioneers in Naturopathic Medicine.* Portland, OR: Medicina Biologica.

Lindlahr H. *Philosophy of Natural Therapeutics.* Maidstone, Kent, UK: Maidstone Osteopathic Clinic.

McKee J. Holistic health and the critique of western medicine. *Soc Sci Med*. 1988;26:775–784.

McKeown T. *The Role of Medicine: Dream, Mirage or Nemesis?* Oxford: Basil Blackwell; 1980.

Payer L. *Medicine and Culture: Varieties of Treatment in the United States, England, West Germany, and France.* New York: Henry Holt and Company.

Schubert-Soldern R. *Mechanism and Vitalism: Philosophical Aspects of Biology*. South Bend, IN: University of Notre Dame Press.

Selys H. *The Stress of Life.* New York: McGraw-Hill.

Sinnott E. *The Bridge of Life: From Matter to Spirit.* New York: Simon and Schuster.

Spitler HR. *Basic Naturopathy: A Textbook.* New York: American Naturopathic Association.

Zeff JL. The process of healing: a unifying theory of naturopathic medicine. *J Nat Med*. 1997;7:122–125.

6

Placebo and the Power to Heal

Peter W. Bennett, ND

OUTLINE

INTRODUCTION

As living organisms, we have evolved with an innate capacity for self-healing. In the clinical setting, naturopathic physicians have always relied on this self-healing capability of the individual. The healing power of the mind and body, the "life force," is a cornerstone of naturopathic philosophy and treatment. Naturopathic physicians describe this with a phrase attributed to Hippocrates: *vis medicatrix naturae*. The "inner" power to heal is one of the great mysteries of medicine, and it behooves us as physicians, regardless of our licensing body, to understand the biology and physiology that surround the processes that guide and control this phenomenon. Following this logic, a central problem to be explored and considered is that placebo response has shown medical science that the mind of a patient has a powerful role in therapy. Although the dictionary definition of mind is described as a person's thoughts and consciousness, centuries of writings from many societies and cultures on philosophy, religion, psychology, cognitive science, neuroscience, and artificial intelligence have struggled to clearly define the complex interaction of personality, emotion, perception, memory, thinking, judgment, and spiritual insight. Adding to the difficulty of the semantics of exploring the healing power of the mind is the difficulty to definitively define the scope of physiology and function of the *mind*.[1] Because the definition and understanding of the human mind challenge a consensus of our collective insight and understanding, it is natural that the function and uses of placebos are an area of myth and misunderstanding rather than an area of wisdom and insight.

As time marches on, medicine is beginning to acknowledge, stimulate, and utilize the subliminal healing capacity of the mind. A review of the placebo literature in *Lancet*[2] concluded that the placebo effect has a complex physiological multisystem dimension and should be encouraged in the clinical situation to optimize health and healing—a perspective reached by this author 30 years ago and published in the first edition of this textbook. In November 2000, 17 health centers and agencies gathered together for 3 days to explore the science of self-healing hidden in the power of placebo. This conference focused on the powerful mind–brain physiology of the placebo effect and its potential for affecting the course of human disease.[3] One of the conclusions of the conference was that the "placebo response" has potential use for medical application and needs further exploration.

Research has shown that the impressions and thoughts in a patient's mind, the attending physician's intention, and the combined effect of their relationship have a measurable effect on the health of the patient. The ability of the patient's mind to affect the process of virtually every disease has been well documented,[4,5] and the internal mechanisms and pathways by which the mind can positively or negatively affect the immune and healing processes has been investigated in the scientific literature of psychoneuroimmunology.[6,7] As the body of knowledge documenting the critical role of the patient's psyche in the therapeutic environment has grown, it has become increasingly important for all schools of medicine to teach the healing potential of the human mind.

Conventional medical thinking has turned its opinion of the placebo effect from that of a 19th-century pejorative to a concept that sums up the complex mind–body interactions affecting the power of

people to heal.[3] Unfortunately, the most modern abuse of the concept of the placebo comes from biased critics of alternative medicine who have chosen to label the beneficial effects of these therapies as merely from the placebo. These critics dismiss the science of natural healing as an imaginary phenomenon and the last resort for quack doctors who have no real medical treatments to offer their patients.[8]

The most interesting aspect of the placebo literature is the exploration of the extent of the potential of the mind to influence human health. The "power of placebo" draws on the innate ability of the body to spontaneously heal itself, a fundamental principle of naturopathic medicine. This point separates the care delivered by naturopathic physicians from the pharmaceutical and surgical approaches of current medical "standard-of-care" procedures. If common medical texts on internal medicine or ambulatory care are examined, the word *healing* is not found in the index. Except for the diagnostic evaluation of "self-limiting diseases" and "spontaneous regression," the ability of the human organism to self-right and repair from a state of acute or chronic disease is not explored in modern medicine except under the designation "placebo response." The placebo response therefore represents all the "unknown" variables that conspire to heal a patient despite pharmaceutical and surgical intervention. Although it seems to be a natural area to develop in clinical and hospital settings, the fundamental separation of mind and body in conventional medical thinking may be slowing down a standardization of care that actively engages the hopes and beliefs of all patients undergoing treatment.

PLACEBO RESPONSE

The placebo response represents the power of the mind, through intention, to effect (1) a change in oneself, (2) a change in those around one, and (3) a change in the environment in which one lives. Intention has been observed to affect machines[9] and remote biological systems.[10] Distantly influenced systems include another person's electrodermal activity, blood pressure, and muscular activity; the spatial orientation of fish; the locomotor activity of small mammals; and the rate of hemolysis of human red blood cells. Prayer, an example of intention, has been extensively studied as a therapeutic healing modality.[11] One study showed a dramatic result in cardiac intensive unit recovery when patients were prayed for by someone at a distant location.[12] Patients in this study were 5 times less likely to require antibiotics, 3 times less likely to experience pulmonary edema, 12 times less likely to require endotracheal intubation, and significantly less likely to experience cardiac mortality.

Our biological systems must conform to the laws of physics. Modern physics has investigated the effect of an observer on the system observed. It has been shown that an electron will acquire a definite axis of measurement in the process of measurement. Bell's theorem supports the idea that our universe consists of particles unified instantly as an indivisible whole; our biological homeostatic systems cannot be analyzed in terms of independent parts. The interconnected nature of our biological systems has been known for thousands of years; the ancient Buddhist concept of "interdependent phenomena" or Pratītyasamutpāda accurately describes this paradigm. The Buddhist concept of interconnectedness and interdependency does not imply a Newtonian billiard-ball effect of a cause or causation, but rather an interdependent "held" state of a plurality of conditions and causes. The idea that a healing response can be generated without genuine causation, such as drug treatment, seems to violate the laws of biological systems. Comparing the healing response generated by a thought or intention might not violate the laws of physics, but it has a difficult place in a medical philosophy of cause and effect driven by pharmacodynamics. Interestingly, humans are not the only organisms to be affected by the placebo response; it has been observed in *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* through cell signaling indicating a phenotypic response to sensory input, which shows that the placebo phenomenon is not ignoring cause and effect—it merely indicates the potential for more subtle self-regulation than that referenced in modern medical standards of care.[13]

Our current medical system is gradually shifting with the developments in modern physics. These modern ideas of biological systems are diametrically opposed to Cartesian paradigms that our internal and external environment consists of separate parts joined by local connections. Medicine must take a "quantum leap" to catch up with the knowledge we possess about our environment through quantum physics. We can see clearly that it is impossible for a doctor to observe a patient without that observation having an effect on the health of the patient.

Pierre Teilhard de Chardin postulated, and Rupert Sheldrake proved, the possibility of a "morphogenetic field" for the subliminal communication to all members of our species.[14] The effect of human thought on other members of society has been described in human society since the beginning of our earliest cultures.

Naturopathic physicians believe that the body has a powerful ability to maintain health and repair to a healthy state after disease by virtue of its inherent power of vitality. This homeostatic healing mechanism has been selected by nature in the same way that the organs that we consider to be vital to our survival have been selected. Healing happens unaided by simply maintaining an environment that does not obstruct the path of cure.

Because the placebo literature documents the philosophical foundations of the naturopathic healthcare model, it is important to review the full scope of this subject. Integrating known placebo initiators in clinical practice is essential for good patient care.

WHY STUDY THE PLACEBO EFFECT?

For hundreds of years, physicians have watched their patients respond to therapies with a wide range of results. Some patients recover fully, whereas others, with apparently identical diseases and therapies, wither and die. Today, a skilled physician can correctly diagnose the condition of a patient by applying the sophisticated techniques of modern medicine. Then, an appropriate therapy, the efficacy of which has been thoroughly proven in research and clinical trials, can be prescribed. Through this process the patient will have received the best care available through current medical technology. However, if the diagnosis, therapy, and therapeutic interaction do not stimulate the hope, faith, and belief of the patient, the chances of success are measurably diminished. In the literature on the placebo effect[15]; psychoneuroimmunology[6]; and psychosomatic,[16] behavioral,[17,18] and psychiatric[19] medicine, it has been repeatedly demonstrated that the beliefs of both the patient and the doctor, and their trust in each other and the process, generate a significant portion of the therapeutic results.[20]

The placebo and its effect are not separate from any aspect of the therapeutic interaction, nor are they "nuisance variables" muddying a clear clinical picture. Rather, they send the physician a strong message: it is a patient's own belief system that mobilizes the inherent healing powers of the mind. By studying the placebo effect, a physician is better able to fully harness this power to trigger internal healing mechanisms. Yet despite the quantity of documentation, the placebo effect remains one of the most misunderstood areas in modern medicine.

The physician should always strive to stimulate self-healing, or the placebo effect, as fully as possible to maximize its potential for healing. Someday physicians will be able to explore the deepest recesses of the unconscious to directly access therapies that assist the body in the

restoration of internal homeostasis. The optimal model for health care is the marriage of appropriate medical technology with the factors that have been shown to generate the placebo effect. This exciting scenario shines on the horizon as the health care of the future.

Because the doctor–patient relationship is such fertile ground for stimulating the healing response,[21–23] it serves a physician well to comprehend the nature of the placebo phenomenon to fully realize this potential for healing.

HISTORY OF PLACEBO

Both the modern physician and primitive medicine men and shamans of the past used ineffective therapies to stimulate healing in their patients. As Shapiro observed, "the true importance of placebo emerges with a review of the history of medical treatment."[24] It was noted that the historical therapies of the medical profession and traditional healers, "purging, puking, poisoning, puncturing, cutting, cupping, blistering, bleeding, leeching, heating, freezing, sweating, and shocking,"[25] worked because of the placebo effect. Although these practices might seem ludicrous in retrospect, all of these therapies were once considered effective. As an embarrassing epilogue, the placebo literature shows that ineffective procedures are just as pervasive in modern medicine as in the jungle hut of the shaman. We must therefore ask ourselves how unfounded medical therapies can survive peer-reviewed literature and centuries of cultural acceptance.

The power of the patient's belief in the potential for a cure has been consistently observed throughout history. Both Galen and Hippocrates recognized the strong effect of the mind on disease and recommended that faith, treatment ritual, and a sound doctor–patient relationship could provide important therapeutic results.[26] Recognition of the power of positive expectation was recorded frequently in the medical literature of the 17th and 18th centuries. It was in the 18th century that the use of placebos was first defined as a "commonplace method of medicine."[27] As the importance of drug therapy grew in the 19th century, the term *placebo* became identified with medicines involving substances that resembled drugs. However, in the 1940s, because of the increase in double-blind research, it became associated with inert substances that were used to replace active medication.

ORIGIN OF THE TERM *PLACEBO*

The original Latin meaning of *placebo* is "I shall please."[28] Although the term had a purely medical application in the first half of the 20th century, its meaning has been subject to various interpretations throughout the past several hundred years.

Before the 1940s, placebos were pharmacologically inactive substances, such as saline and lactose pills, used to satisfy patients that something was being done for them—in other words, the doctor was "pleasing" the patient. The 1940s and 1950s saw an explosion of the use of double-blind experimental procedures to evaluate the growing number of new drugs and medical procedures. Suspicion arose that all medical therapies contained an element of the placebo phenomenon.[29] This new understanding pressed the scientific community to offer new, far broader definitions.

Shapiro[25] offered the classic definition of a placebo:

> *Any therapeutic procedure (or that component of any therapeutic procedure) which is given deliberately to have an effect, or unknowingly has an effect on a patient, symptom, syndrome, or disease, but which is objectively without specific activity for the condition being treated. The therapeutic procedure may be given with or without the conscious knowledge that the procedure is a placebo, may be an active (non-inert) or inactive (inert) procedure, and includes, therefore, all medical procedures no matter how specific—oral and parenteral medications, topical preparations, inhalants, and mechanical, surgical, and psychotherapeutic procedures. The placebo must be differentiated from the placebo effect which may or may not occur and which may be favorable or unfavorable. The placebo effect is defined as the changes produced by placebos. The placebo is also used to describe an adequate control in research.*

A more accurate definition would be the following: The placebo effect is the process of a physician working with the self-healing processes of a patient. The placebo response is healing that results from the patient's own natural survival and homeostatic defense mechanisms.

Modern placebo definitions extend to its nature, properties, and effects. A placebo can be known or unknown, active or inactive, positive or negative in results (placebo effect vs. nocebo effect), and can extend to all forms of diagnostic or therapeutic modalities,[30] as further defined in Box 6.1.

BOX 6.1 Types of Placebos

1. Known placebo: Placebo used in a single-blind experiment. The doctor knows it is a placebo, but the patient does not.
2. Unknown placebo: Double-blind use of placebo. Neither the doctor nor the patient knows that the medication is a placebo.
3. Active placebo: Any substance that has an intrinsic physiological effect that is irrelevant to the ensuing placebo effect. The vasodilating effect of niacin would make it a good active placebo.
4. Inactive placebo: Any substance that is used with medicinal intent but that has no inherent physiological effect. Aside from the glucose effect in a sugar pill (or, to complicate things, an allergic reaction to some component of the supposedly inert substance), it has no physiological effect.
5. Placebo effect: Any changes that occur in a patient as the result of placebo therapy.
6. Nocebo effect: Any changes that occur as a result of placebo therapy that are perceived as negative or counterproductive to the path of cure.

CLINICAL OBSERVATIONS OF "KNOWN" PLACEBO THERAPY

One of the more dramatic examples of the placebo effect reported in the medical literature involved a patient with advanced lymphosarcoma, which Klopfer[31] reported was highly susceptible to the patient's faith in an experimental drug called Krebiozen. When the patient was started on the drug injections, his enthusiasm was so intense that "The tumor masses had melted like snowballs on a hot stove, and in only a few days, they were half their original size!"[31] The injections were continued until the patient was discharged from the hospital and had regained a full and normal life, a complete reversal of his disease and its grim prognosis.

Within 2 months of this recovery, reports that the drug Krebiozen was ineffectual were leaked to the press. Learning of this report, the patient quickly began to revert to his former condition. Suspicious of the patient's relapse, his doctors decided to take advantage of the opportunity to test the dramatic regenerative capabilities of the mind; a single-blind study was performed on the patient using pure placebo. He was told that a new version of Krebiozen had been developed that overcame the difficulties described in the press, and some of the drug was promised to him as soon as it could be procured.

BOX 6.2 Symptoms and Side Effects of Placebo Response

- Anger[206]
- Anorexia[51]
- Behavioral changes[207]
- Depression[62]
- Dermatitis medicamentosa[51]
- Diarrhea[51]
- Drowsiness[62]
- Epigastric pain[51]
- Hallucinations[56]
- Headache[208]
- Lightheadedness[51]
- Palpitation[51]
- Pupillary dilation[36]
- Rash[51]
- Weakness[51]

With much pomp and ceremony, a saline water placebo was injected, increasing the patient's expectations to a fevered pitch. The recovery from his second near-terminal state was even more dramatic than the first. Tumor masses melted, chest fluid vanished, he became ambulatory, and he even went back to flying again. At this time he was certainly the picture of health. The water injections were continued because they worked such wonders. He then remained symptom-free for more than 2 months. At this time, the final American Medical Association announcement appeared in the press—"nationwide tests show Krebiozen to be a worthless drug in the treatment of cancer." Within a few days of this report, the patient was readmitted to the hospital in extremis. His faith was now gone, his last hope had vanished, and he succumbed in less than 2 days.[31]

Other famous placebo case studies are one reported by Cannon[32] on "voodoo death" caused by belief and one reported by Kirkpatrick,[33] who documented the spontaneous regression of lupus erythematosus resulting, in part, from the patient's belief in the removal of a curse.

Other Clinical Observations

Belief sickens, belief kills, belief heals.[34]

Evans[35] and Beecher[36] reviewed, between them, 26 double-blind studies on the efficacy of active analgesic drugs in the treatment of pain. Independently, they concluded that 35% of patients with pain experienced a 50% reduction in their symptoms after placebo medication. These were particularly remarkable results when viewed in the context of Evans's observation that with a standard dose of morphine, only 75% of the patients experienced a 50% reduction in pain. In calculating the efficiency index of placebo analgesia, a method often used to determine the relative efficiency of drugs, placebo was 0.56 as effective as a standard dose of morphine. This prompted Evans to remark, "Thus, on average, placebo is not a third as effective as a standard injection of morphine in reducing severe clinical pain of various kinds but is in fact 56% as effective."[35]

As discussed previously, the placebo phenomenon has been evaluated in a wide variety of clinical settings in addition to pain management (Box 6.2). When a phenomenon such as the placebo effect has been observed to be active in diverse clinical situations, such as surgery, drug therapy, psychotherapy, and biofeedback, and over a range of physical and mental symptoms, the conclusion that it must be a factor in all aspects of medicine is inescapable.

In addition to the variety of positive effects that placebos produce are the *nocebo effects*, perceived as counterproductive to the therapeutic goals. Widely ranging negative side effects to placebos have been reported in the medical literature.[37] These side effects are frequently consistent with those of the medication that patients believe they are getting. For example, the studies that measure the effects of a supposed aspirin usually show nocebo effects of ulcerlike pain.[38] One study showed that suggestion seems to be a primary cause of nocebo reactions, in contrast to the strong conditioning component found in the placebo response.[39]

In homeopathy, aggravations and ameliorations are commonly seen when a placebo is given to fend off a patient's need to take a medication while the homeopathic physician is waiting to see whether a high-potency remedy will effect a cure. Homeopathic doctors report that placebos can cause anxiety and loneliness as well as calmness and immediate relief from insomnia.[40]

PLACEBO MYTHS

An investigation of the understanding of placebos found in the current medical literature revealed the misconceptions that prevail about the nature of placebo therapy and its effectiveness.[40] A study undertaken to examine doctors' and nurses' attitudes about the efficacy and use of placebos showed that both groups underestimated the number of patients who could be helped by placebo.[41] Physicians showed a consistent pattern of placebo use, as follows:

- Placebos were used to prove the patient wrong through the diagnosis of psychogenic symptoms in patients who were thought to be exaggerating, imagining, or faking their symptoms.
- Placebos were used in the treatment of alcoholic, psychotic, and demanding patients who were disliked by the staff of the hospital.
- Placebos were used as a treatment in situations in which standard treatments failed or the patient was getting worse.

These misconceptions regarding the nature of the placebo accounted for its widespread misuse in patients who were perceived as uncooperative or who were suspected of malingering.

Myths about placebos continue to hinder a full understanding of the power inherent in this aspect of health care. The most common myths are discussed here.[42]

Myth 1

"Because placebos tend to be physiologically inert, it is not possible for them to have an effect on physiological homeostasis."

Fact: Research shows that placebos have a wide range of effects (Table 6.1) that are found throughout all aspects of human physiology.

Myth 2

"Placebos are useful only with symptoms that are associated with psychological or psychosomatic complaints. Patients who need a placebo are hypochondriacs with vivid imaginations and need to be palliated with something to please them."

Fact: Placebos have been shown to be effective in the care of all types of patients, with a consistent level of positive results for a wide variety of accurately diagnosed diseases.

Beecher[20] was one of the first to compile a listing of the therapeutic effectiveness of placebo, thereby uncovering the wide range of therapeutic applications that were previously thought to be limited only to pain control. He concluded, "there is too little scientific as well as clinical appreciation of how important unawareness of these placebo effects can be and how devastating to experimental studies as well as to sound clinical judgement lack of attention to them can be."[20]

TABLE 6.1 Physiological Changes Induced by Placebo

Physiological Function	Physiological Changes
Heart	Improved exercise tolerance[190,191]
	Decreased serum lipoproteins[192]
	Improved T waves[57]
	Decreased pulse rate and arterial pressure[59]
Sympathetic stimulation	Decreased tremulousness, sweating, and tachycardia[51]
Claudication	Increased walking distance[193]
Opioid dependence	Addictive drug withdrawal[194]
Postsurgical trauma	Decreased facial swelling[195]
Diabetic blood sugar dyscrasias (NIDDM)	Lowered fasting blood sugar[196,197]
Gastrointestinal secretion and motility	Decreased gastric acid secretion[198]
	Changes in gastric motility[165,199]
	Healing of duodenal ulcers[200]
Hypertension	Lowered blood pressure[201–203]
	Reduced urinary catecholamines[204]
Motor dysfunction	Improved tremor magnitude[205]

NIDDM, non–insulin-dependent diabetes mellitus.

BOX 6.3 Conditions That Have Been Shown to Respond to Placebo

- Angina[54,190,191,209,210]
- Anxiety[51,211,212]
- Arthritis[38,168,213]
- Asthma[214–217]
- Behavioral problems[218]
- Claudication, intermittent[193]
- Common cold[219–222]
- Cough[223]
- Depression[224,225]
- Diabetes (non–insulin-dependent diabetes mellitus)[196,197]
- Drug dependence[51]
- Dysmenorrhea[226]
- Dyspepsia[227]
- Gastric ulcers[228]
- Hayfever[229,230]
- Headaches, temporal and vascular [231–233]
- Hypertension[234,235]
- Labor and postpartum pain[236]
- Premenstrual syndrome[237]
- Ménière's disease[238]
- Nausea of pregnancy[51]
- Pain[106,239]
- Psychoneuroses[52,240]
- Rhinitis[241]
- Sleep disturbances[242]
- Tremor[54]

The large and ever-growing number of studies on placebos and double-blind research (Box 6.3) supports the following assertion made by Beecher[20] 30 years ago:

> *Many "effective" drugs have power only a little greater than that of placebo. To separate out even fairly great true effects above those of placebo is manifestly difficult to impossible on the basis of clinical impression. Many a drug has been extolled on the basis of clinical impression when the only power it had was that of a placebo.*

Myth 3

"The placebo effect is found only with substances that are inert."

Fact: The placebo phenomenon has been observed across a wide spectrum of medical disciplines including surgery,[43] drug therapy,[44] and biofeedback.[45]

Myth 4

"The patient who responds to placebo therapy can be characterized as someone who is of a typical neurotic disposition."[41]

Fact: Although many studies have tried to impute a personality type, disposition,[45,46] or certain epidemiological class[47] to the patient who responds to placebo, this has yet to be well demonstrated because, in the right circumstances, any person can become a placebo reactor.[48,49]

After reviewing the bulk of the research on this subject, Bush[50] and Wolf and Pinsky[51] concluded that the attempts to pigeonhole personalities into a clinical profile ignored the complexity of the human mind. Gliedman et al.[52] similarly reported that age, sex, marital status, social class, and intelligence were unimportant factors in determining a patient's response to placebo. Wolf summarized that attempts to identify placebo reactors need to

> *identify the nature of the symptom being treated, the motivation of the patient and physician, the nature of the test agent, its mode of administration and the life situation of the subject at the time he is tested. The significant point here is not the apparently conflicting findings of investigators with respect to placebo reactors, but rather that in any given situation, responses to a placebo may vary as compared to any other situation and the significance of situations to human subjects cannot be precisely duplicated.*[51]

PHARMACODYNAMICS

The physiological response of the "inert and inactive" placebo extends into the realm of drug pharmacodynamics. Dose–response time curves, cumulative effects (increasing therapeutic efficacy with repeated doses),[53] variable strengths of analgesia based on a patient's drug expectation,[54] drug interactions,[51,55] and carryover effects[46,56] have all been demonstrated. The effects of placebos are so pronounced that some observers have suggested that they can exceed the effects attributable to potent pharmacological agents.[51]

Packaging and Delivery

Several studies found that the effectiveness of placebo therapy depends on the mode of delivery.[57] For example, one study found that green tablets improved anxiety and yellow tablets improved depression,[58] whereas another study found that blue capsules were more sedative and pink capsules were more stimulating.[59] Placebo injections appeared to be more effective than oral administration after oral placebo has failed to relieve the patient's symptoms.[38]

Placebo Interactions

Benson[60] wrote that the patient's belief was also a powerful force in determining the level of relief afforded by the placebo. An increase in patient expectation enhances the physician's ability to elicit a placebo response. Even if patients know that they are receiving placebos, the expectation and relief brought about by the therapeutic interaction

provide positive results.[61] The importance of expectation was further demonstrated by the observation that the greater the stress level of the patient and the greater his or her need for assistance, the greater the effectiveness of placebo.[62] This was seen even in patient responses to psychotropic drugs: D-lysergic acid diethylamide tartrate 25 (LSD-25) could have no effect if the patient was told that the drug was a placebo.[45,63]

Patients, such as war heroes, who had severe injuries but did not have great mental suffering attached to their pain needed less pain medication than persons with similar injuries who had pain that engendered anxiety and connoted disaster.[64]

PLACEBO HEALING MECHANISMS

When animals or humans can react to their own deviations from homeostasis and when these deviations set off restorative processes, therapeutic intervention, including placebo, has an already existing substrate of recovery for exploitation.[17]

A human being has an intrinsic ability to "self-right"—*vis medicatrix naturae* (the healing power of nature). This is the keystone of a philosophy that has been held for thousands of years by naturally oriented physicians (see Chapter 5). The concept of a homeostatic, self-regulating mechanism is central to the understanding of basic concepts of physiology: negative feedback loops control virtually all systems of the body. According to Guyton,[65] "the body is actually a social order of about 75 trillion cells organized into different functional structures.… [E]ach cell benefits from homeostasis and in turn each cell contributes its share toward the maintenance of homeostasis."

The body can maintain health and reestablish a healthy state after disease by virtue of its inherent vitality. This is part of the definition of a homeostatic mechanism; it has been selected by nature in the same way that organs vital to our survival have been selected. The surviving species are those most fitted and best able to cope with dysfunction. Those organisms that can tolerate the greatest stresses and still maintain normal physiology are the hardiest survivors and ensure the species' ability to increase the limits of its adaptation. Therefore, given that an organism is self-maintaining when in an environment that it has been selected for, healing happens unaided through simply maintaining an environment that does not obstruct the path of cure. As Norman Cousins[66] observed, "without any help, the human body is able to prescribe for itself. It does so because of a healing system that is no less real than the circulatory system, the digestive system, the nervous system, or any of the other systems that define human beings and enable them to function."

The Role of Emotions

Starting in the 1970s and early 1980s, review articles began to examine the effect of the mind on the immune system, emphasizing mechanisms and pathways that gave rise to a new field called psychneuroimmunology.[5] Reviews of studies that explored how specific emotions can increase cancer susceptibility,[67,68] examined the effect of emotions and recovery from cancer,[69] investigated the increased incidence of sudden and rapid death during psychological stress,[70] and monitored the changes in immune function during emotional stress[71,72] all confirmed that emotions play a powerful role in the prognosis of a patient. Cannon[32] and Tregear[73] documented dramatic case histories of pioneering anthropologists who witnessed the power of taboos and curses to kill strong, healthy men and women in third-world cultures throughout Africa, South America, and the South Pacific. Tregear[73] wrote, "I have seen a strong young man die the same day he was tauped [tabooed]; the victims die under it as though their strength ran out as water."

The *Vis Medicatrix Naturae*

The healing process described as *vis medicatrix naturae* demonstrates the significant power and potential of the self-generated healing capacity. For a physician, there is no more powerful stimulator of this healing mechanism, the placebo effect, than a strong doctor–patient interaction. Just walking through the door of the physician's office nudges a patient's internal homeostatic mechanisms into seeking higher levels of health, healing, and adaptation. The placebo effect is a result or effect of the patient's seeking the assistance of the doctor's ability to heal and cure. As Benson[60] noted,

> *When we dissected the placebo effect a number of years ago, we found three basic components: One, the belief and expectation of the patient; two, the belief and expectation of the physician; and three, the interaction between the physician and the patient. When these are in concert, the placebo effect is operative.… Perhaps nothing is being transmitted from the healer to the patient, but rather it's the belief the patient has in the healer that's helpful.*

Conscious Control Over Homeostasis

The body has two internal forces to maintain homeostasis: a lower drive and a higher drive. The lower drive is the inherent internal healing mechanism, the vital force, or the primitive life support and repair mechanism that can operate even in a person who is asleep, unconscious, or comatose. The higher drive is the power of the mind and emotions to intervene and affect the course of health and disease by depressing or stimulating internal healing capacities. The effect of this drive can be seen in the clinical observation of patients who move toward spontaneous remission of a life-threatening disease through positive emotional support[15,69] and in patients who fail to express emotions compatible with the body's attempts to survive.[69]

In any disease process, the consciousness of the patient decides the effectiveness of any therapy. It has been suggested that widely ranging nondrug stimuli have the capacity to modulate human functioning.[74] It is emerging in the medical literature that any sensory stimuli or mental activity is able to alter disease progression. This extends to the thoughts and intentions of those connected to the patient. Experiments in remote intention–generated healing and prayer showed that the intention of others was a factor in the homeostatic capabilities of the mind and body. The fact that the homeostatic mechanism can sense and respond to these remote intentions is a reflection of the power of the human mind. Some authors believe that there is a physiological basis for the unlimited possibility of human voluntary control.[75] The conclusion that awareness or "mind," anyone's mind—the patient, the doctor, or someone who is aware of the patient—can alter the patient's physiology is a testament to the "holos" concept in different schools of alternative and complementary medicine. This idea flies so deeply in the face of our mechanistic model of medicine, it forces a complete paradigm shift in the conventional social ethos of medical care.

The ultimate control of psyche over soma demonstrates the priority of the conscious mind over physiological processes such as immunity and pain control.[76] This puts an enormous responsibility on the physician. He or she must take full account of a patient's mental and emotional states when treating chronic or life-threatening disease.

Physiological Mechanisms

Identification of a biochemical mechanism for placebo analgesia has done more to change the image of placebo therapy than any amount of arguing about the importance of beliefs and the mind.[77]

The mechanisms of the placebo response have been suggested to be a mixture of psychological interactions[78] and cognitive states[79] mediating physiological responses.[19] The psychological components

of the patient's placebo effect have been shown to include decreased anxiety and increased relaxation,[54] conditioning,[18] expectation,[23] and well-being generated by the establishment of a sound doctor–patient relationship.[80,81]

Review articles summarized a wide range of receptor-agonist mechanisms driving the neural pathways in different parts of the brain.[82] To date, endorphin, dopamine, cholecystokinin, interleukins, growth hormone, and cortisol have been implicated. The physiological mechanisms of the placebo effect were suggested to include chemicals, catalysts, and enzymes. It is believed that steroids, catecholamines,[15] the autonomic nervous system,[19,83] neuropeptides, and endorphins[84] are also involved. These physiological mechanisms interrelate synergistically and are currently being researched within the rapidly developing field of psychoneuroimmunology,[7] through which the links between depression, affective disorders, emotions, and the immune system and central nervous system (CNS) are being explored. Susceptibility to depression and sensitivity to pain have now been found to be mediated through neurotransmitters such as catecholamines, serotonin, and dopamine.

The current model for explaining the mechanism by which emotions, mood, and psychological stress suppress immune function involves cerebral–hypothalamic and pituitary interaction, which translates stress and anxiety into an autonomic–endocrine response. This response adversely affects the immune function, particularly after chronic stimulation. Stressful stimulation is received in the sensory cortex of the brain and is then referred to the limbic system and the hypothalamus. This interface of higher-brain functions and homeostatic regulating centers provides the communication link between the psyche and soma. According to Rossi,[19] "The hypothalamus is thus the major output pathway of the limbic system. It integrates the sensory-perceptual, emotional, and cognitive function of the mind with the biology of the body."

The nerve centers that control both branches of the autonomic nervous system (both parasympathetic and sympathetic), nerve cells that secrete endocrine-releasing factors, and neural pathways that release hormones directly into the posterior pituitary are in the hypothalamus. The corticosteroids and catecholamines from sympathetic stimulation are key factors in the alteration of disease susceptibility in response to stress. Corticosteroids inhibit the function of both macrophages and lymphocytes, as well as lymphocyte proliferation.[85] Corticosteroids also cause the thymic and lymphoid atrophy noted by Hans Selye in his experiments on stress-induced immune dysfunction.[86]

The autonomic release of catecholamines stimulates receptors on the surface of lymphocytes, thereby increasing their maturation rate. When lymphocytes are in a mature state, their ability to kill bacteria and cancer cells and produce interferon seems to become paralyzed.[87] Thus a population of mature lymphocytes develops, ready to defend the body from infection and inflammation, yet remains paralyzed until the "red alert" signal of sympathetic fight or flight is turned off, signaling the appropriate time to rest and repair.

A number of other peptides, E-type prostaglandins, somatotropin, histamine, insulin, endorphins, antidiuretic hormone, and parathyroid hormone all have receptor sites on lymphocytes and can stimulate the same cyclic adenosine monophosphate–mediated response resulting in lymphocyte maturation and inhibition.[85] A study of the effect of catecholamines on the human immune system showed that when a physiological dose of epinephrine was injected into a healthy volunteer, there was an increase in the number of circulating suppressor T lymphocytes and a decrease in the number of circulating helper T lymphocytes (changes similar to those found in acquired immunodeficiency syndrome [AIDS]).[85]

Neurophysiology of Placebo Response

Medical research has continued to expand the understanding of the placebo healing response, extending the understanding of the complexity of the brain functions that control healing in the body.[88] One review article did an excellent job of summarizing the psychobiological mechanisms involved in the wide array of medical conditions observed in the placebo response literature.[2] Imaging techniques such as positron emission tomography and magnetic resonance imaging (MRI) have literally illuminated the areas of the brain involved in generating the placebo effect.[89] One fascinating development is the indication that the placebo effect may be especially useful in depression, anxiety, substance abuse, and neurodegenerative diseases like Parkinson's disease and Alzheimer's disease. Research has indicated that the conditioning and anticipation of the patient have a potent effect of stimulating specific brain region activity associated with pain modulation and neurohormonal regulation.

Brain Region Activity

Some of the most interesting research on placebos has evolved out of the new MRI technology. This functional MRI (fMRI) can measure blood flow into specific areas of the brain. One study showed that expectation or hope was able to stimulate a certain part of the brain that is activated by pain medications and is associated with pain relief. Placebo analgesia was found to be related to decreased brain activity in pain-sensitive brain regions, including the thalamus, insula, and anterior cingulate cortex. It was also associated with increased activity during anticipation of pain in the prefrontal cortex, providing evidence that placebos alter the experience of pain.[90]

In another study, researchers found that empathy could activate a portion of the brain. They showed that some of the brain regions involved in feeling physical pain became activated when someone empathized with another's pain. Using fMRI, study participants were observed when they experienced a painful stimulus, and the results were compared with those elicited when the participants observed their spouses receiving a similar pain stimulus. The bilateral anterior insula, rostral anterior cingulate cortex, brainstem, and cerebellum were activated when participants received pain and also by a signal that a loved one experienced pain.[91] A group of researchers at the University of California at Los Angeles, using a new technology called quantitative electroencephalography, showed that "effective" placebo treatment induced changes in brain function that were different from those associated with antidepressant medication. Placebo responders (those who showed a response to placebo) showed a significant increase in prefrontal activity starting early in treatment that was not seen in medication responders or in participants who showed no response to medication or placebo. Because a high percentage of antidepressant medication represents the placebo effect, it is important to be able to predict who will be placebo responders.[92]

Placebo and Stress Physiology

The stress "letdown" of a patient in the therapeutic environment is one of the mechanisms that produces the placebo effect. It results from the patient's perception that a transition from a stressful situation to a nonstressful situation has occurred. Mowrer[87] observed that with a decrease in anxiety, there is a concomitant increase in hope, signifying that the period of suffering is over. Certain familiar images and signals, such as white coats, syringes, behavioral procedures, and clinical protocol, create a conditioned response—relief now that help has arrived. Evans and Hoyle[54] similarly observed that "the reduction of fear through the shared expectations that the doctor's medicine will work—even if unknown to the patient it is placebo—mediates powerful therapeutic effects."

The placebo effect in the clinical environment transforms the emotional and mental stress of the patient. These effects, also observed and described by Franz Alexander,[16] Hans Selye,[86] George Solomon,[93] and Walter Cannon,[94] allow the patient to escape the "fight-or-flight" response that can cause and maintain the state of illness.

Physiological and Psychological Stress

Selye[86] demonstrated that physiological stress can have a dramatic effect on the immune and endocrine systems of the body. Laudenslager[95] went on to show that it is not just stress that creates these physiological changes; the perception that stress is "inescapable" is critical to the response. More recently, studies on the effects of psychological stress demonstrated significant changes in immune capability. Maladjustment to "life-change stress" correlated with reduced activity of natural killer cells,[93] decreased T- and B-cell responsivity,[71] and diminished lymphocyte cytotoxicity.[96] For example, Riley[97] observed increased tumor activity in a controlled stress environment and concluded:

> *Emotional, psychosocial, or anxiety-stimulated stress produces increased plasma concentrations of adrenaline, corticosteroids and other hormones through well-known neuroendocrine pathways. A direct consequence of these increased corticoid concentrations is the injury to elements of the immunologic apparatus, which may leave the subject vulnerable to the action of the latent oncogenic viruses, newly transformed cancer cells, or other incipient pathologic processes that are normally held in check by an intact immune system.*

The damage to the immune system by stress, mediated through the hypothalamic–pituitary axis, has been shown to be due to the increase in serum levels of cortisol. In one study, elderly caregivers were shown to have higher cortisol levels and poor antibody response to influenza vaccine.[98] The effect of cortisol on immune and other regulatory functions, such as the regulation of blood sugar, dehydroepiandrosterone (DHEA), insulin, testosterone, and bone resorption, flag it as having highly destructive potential. Anxiety, depression, heart disease, AIDS, and osteoporosis have all been linked with elevated cortisol levels. DHEA, another adrenal hormone, is also modulated by stress physiology, although it seems to have the opposite effect of cortisol. High levels of DHEA seem to protect the body from the damaging effects of elevated cortisol. Ratios of DHEA to cortisol are highly predictive of the individual's ability to tolerate stress.[99]

Current reviews of the literature relating psychological stress and immune dysfunction support the hypothesis that homeostatic immune mechanisms, both humoral and cellular, are significantly impaired by both natural and experimental stress.[5,61,84,100] Hypertension,[101] common colds,[102] coronary artery disease,[103] and myocardial ischemia[104] were linked to adverse stress physiology. Stress even has the ability to increase the permeability of the blood–brain barrier.[105] The implications of stress-related alterations in the blood–brain barrier expose important insights into enigmatic diseases like chronic fatigue syndrome and stress-induced neurological disorders.

Endorphins, Hormones, and Neuropeptides

> *…one rapidly activated psychoneuroendocrine mechanism through which a placebo stimulus may reduce both depression and pain is produced by stimulating the endorphin system.*[18]

Research on endorphins is a relatively new area of study in the field of psychoneuroimmunology. Original research by Levine et al.[106] suggested that the pain relief noted in placebo studies could be explained by the simple mechanism of endorphin-mediated actions. The original emphasis on endorphins and enkephalins was plausible, considering their known modulation of pain and mood functions. This position was further supported by later observations that depression increased chronic clinical pain[107] and that decreased activity in endogenous opioids may be part of the pathophysiology of depression.[108] With the information that placebo can stimulate endorphins, Levine et al.[106] believed that an explanation for the action of placebos had finally been found. Furthermore, research showed that an endorphin-mediated, pain-suppressant placebo effect could be abolished with the use of Naloxone, an opioid antagonist.[109] The same authors went on to further show that endorphin-mediated placebo effects penetrated other physiological systems besides pain management.[110] However, this hypothesis failed to account for the broad spectrum of placebo effects as well as for the fact that the analgesia associated with hypnosis was not affected by an opioid antagonist.[111,112] It is important to note that later literature suggested that Levine et al.[106] were not entirely wrong in implicating the role of endorphins in the placebo mechanism; rather, these researchers were right for the wrong reason.

TABLE 6.2 Effects of Endorphins on the Immune System

Immune System Function	Endorphin Effect(S)
Lymphocyte production	Increased and decreased
Chemotaxis	Increased
T-cell sensitivity to prostaglandin E_2	Increased
Antibody production	Increased and decreased
Complement	Binding of fractions C5B-C9
T-cell proliferation	Modulation of
Natural killer cell function	Modulation of
B-cell differentiation	Modulation of

Endorphins are mainly derived from three precursor proteins (by separate biochemical processes).[113] These opioid peptides are released from central and peripheral areas in response to pain, stress, and emotions and perform many physiological functions, of which analgesia is but one.[114] However, it is becoming evident that the boundaries between the CNS and the immune system are not as clear as once thought. The several known effects of endorphins on immune system function are listed in Table 6.2.[115]

When the functions of neurotransmitters such as endorphins are found to have such an intimate relationship with immune integrity, the paradigm of a body with functions performed independently by its parts—a Newtonian type of thinking—begins to lose credibility. To further blur the already hazy distinction between the CNS and the immune system, research demonstrated that endorphins and peptide hormones, such as adrenocorticotropic hormone, thyroid-stimulating hormone, human chorionic gonadotropin, and luteinizing hormone, are produced by lymphocytes.[115]

It is clear that the demarcation between the CNS and the immune system is impossible to distinguish. The brain and the immune system are the only tissues in the body that have a memory, and the level of communication between the two argues a taxonomy that identifies them as one. Evidence of the innervation of the thymus gland, bone marrow, spleen, and lymph nodes supports the finding that the immune system is subject to efferent CNS information.[115] In addition, studies demonstrating the atrophy of the thymus and lymphatic tissues in the absence of growth hormone,[116] adrenocorticotropic hormone, and increased steroid production by adrenal cells after interferon stimulation indicate that "in the future it will be difficult to distinguish the receptors and signals that are used within and between the neuroendocrine and immune system."[115]

BOX 6.4 Six Principles of Optimizing Placebo Response in Clinical Practice

- *Prima non nocerum:* Prioritize a hierarchy of therapeutic interventions.
- *Tollem causum:* Remove the obstacles.
- Support the therapeutic relationship.
- Enhance positive emotional states.
- Implement therapeutic conditioning or learning.
- Use altered states of consciousness.

CLINICAL APPLICATION

Whether a clinician intends to initiate a placebo effect in a clinical setting or not, the mind of the patient will initiate some subliminal healing effects according to the patient's hope, expectation, conditioning, anxiety reduction, and meaning around the disease and treatment. A recent article in *Lancet*[2] on placebos concluded, "Any ethical assessment of efforts to promote placebo effects in clinical practice first requires knowledge as to the clinical relevance and importance of placebo effects."

A physician with an interest in psychopharmacological treatment, which can be expensive, elaborate, detailed, time consuming, esoteric, and dangerous, usually has considerable knowledge about such treatment. He or she is interested in the symptoms of the patient and the differential response to various drugs and is careful to observe side effects, which may be dangerous. The physician may encourage the patient to call at any time if side effects develop.[26]

The application of the placebo phenomenon in clinical practice should not be a vague attempt to replace the skill of the medically trained physician with obscure "hand waving," incantations, and inert lactose pills. In primary care and specialty clinical practice, the physician's intent should be to optimize patient care by engaging restorative defense mechanisms. To effectively apply current placebo research, the physician must understand several principles (listed in Box 6.4 and discussed here).

Prima Non Nocerum: Prioritize a Treatment Program and Establish a Hierarchy of Care

Prima non nocerum is the Hippocratic injunction dictating that a physician care for the patient so that self-healing mechanisms can engage. This ancient phrase means "Do not disturb the organism's ability to heal itself." The body must be given the full range of possibilities in allowing the power of homeostasis, *vis medicatrix naturae*, to have its optimum capability. "Doing no harm" means that a patient is supplied with the level of medical intervention that is appropriate to his or her ability to maintain life support. The job of the physician is to determine when homeostasis or the defense mechanism has lost the ability to respond to disease.

Acute traumatic swelling and inflammation and shock are examples of the human defense mechanism responding in a way that threatens the health of the organism. It is most interesting that the organism would make choices, as in shock and inflammation, that could kill it. To practice the principle of *prima non nocerum*, a physician must learn when to act and when to let the body heal itself. This is the highest art of medicine; each case and situation is different, and it is up to the physician to interpret the needs of the moment. By implication, the physician who seeks to apply this principle understands the principles of physiology on which human life depends for homeostasis.

Prima non nocerum does not necessarily mean that a physician withholds invasive therapy: it is the physician's responsibility to determine when the body is unable to reestablish homeostasis and therapy is indicated. If an arm must be severed to save the patient's life, there is no violation of *prima non nocerum*.

However, to enhance the principle of *prima non nocerum*, a physician sometimes must withhold therapies and must be content to leave the patient to self-heal. Hippocrates understood the wisdom of letting the body heal on its own, which is implicit in the injunction to "do no harm." The following account of the treatment of Charles II of England is a case in point[117]:

> *A pint of blood was extracted from his right arm and a half pint from his left shoulder, followed by an emetic, two physics, and an enema comprised of fifteen substances; the royal head was shaved and a blister raised; then sneezing powder, more emetics, and bleeding, soothing potions, a plaster of pitch and pigeon dung on his feet, poisons containing ten different substances, chiefly herbs, finally forty drops of extract of human skull and an application of bezoar stone; after which his majesty died.*

When this treatment is compared with modern procedures, such as mammary artery ligation for the relief of angina—a procedure that has no more benefit than sham artery ligation—it appears that physicians continued throughout the centuries to rely on the placebo effect for the care and cure of their patients. Recently, the invasive standard-of-care procedure percutaneous coronary intervention (PCI) of angioplasty and placing coronary stents was shown to be no better than placebo for stable angina. Missing from the medical community discussion on this study was the remarkable fact that some individuals who underwent the sham "placebo" PCI procedure had results that demonstrated that the placebo effect can affect cardiac perfusion.[118] Our medical culture has very little interest in exploring the physiological limits of self-healing.

Because this effect plays such an important role in health care, simple, noninvasive, and effective treatments should be the goal of all therapeutic approaches. Robert Burton[119] wrote in 1628, "an empiric oftentimes, and a silly chirurgeon, doth more strange cures than a rational physician… because the patient puts confidence in him."

The rational physician will also recognize that healing and curing are not necessarily the same. If a patient is helped in any way by the doctor, with or without the use of a placebo, the path of cure has been assisted, although the specific disease may not have responded. Not all patients can be cured, but most patients can be helped.

Tollem Causum: Remove the Cause of Disease

Tollem causum is the principle that seeks to remove the obstacles to cure. The forces "inhibiting the floodgates of health from opening" must be removed for the full force of the patient's beliefs to effect the path of cure. This concept is fundamental to the philosophy of naturopathic medicine, with its strong emphasis on diet, detoxification, and a pattern of living that is consistent and compatible with the context in which humans evolved. Obstacles to cure block the self-healing capacity of the organism. Contamination with heavy metals and xenobiotics (see Chapter 35), focal infections, electromagnetic pollution, scar tissue, genetic metabolic abnormalities, and parenchymal organ damage defeat the best therapeutic intentions and must be addressed.

The patient's habitat is an important aspect of the therapeutic protocol, not only in the diagnosis and care of internal mental and physiological dysfunction but also in determining which environmental factors may be contributing to dysfunction and disease. These factors might include diet, lifestyle, and living environment. It is of the utmost importance to remove a patient from surroundings that are associated with illness or to assist the patient in creating an environment more conducive to health.

Factors that provide conditioning that reinforces the disease process can be associated directly or indirectly with one's environment. For example, if animals are returned to situations where their experimental neuroses were induced, their pathological behavior reactivates.[120] When a patient leaves the offending environment to receive treatment from a physician, the prognosis is correspondingly more favorable.[17] The physician has the added advantage of a patient's heightened expectation during an office visit; a patient's positive associations with the "healing" environment increase his or her receptivity to treatment.[121] If the home or work environment is a source of "disease" and an obstacle to cure, providing an alternative environment may be a most helpful way to remove the obstacles to cure.

Support the Therapeutic Relationship

Confidence should surround all aspects of the therapeutic interaction. The patient must have confidence in the doctor's ability to assist a cure; the doctor must have confidence in the efficacy of his or her therapy[122]; and there must be an understanding or relationship between the doctor and patient that is mutually conducive to respect, trust, and compassion.

The quality of the doctor–patient relationship is paramount. The therapeutic approach to a patient that optimizes the confidence of the patient in the skill of the doctor stimulates the inherent self-regulating healing mechanisms by relaxing the anxiety the patient has about the illness. Anxiety is a well-known immunosuppressant and aggravates the body's defense mechanisms. An optimum therapeutic relationship, when combined with the clinical skill to remove the cause of homeostatic dysfunction, is the height of therapeutic acumen. As Lewith[123] so accurately stated, "The general practitioner may therefore wish to employ all his knowledge, enthusiasm, consultation technique and sympathy, to create the best possible atmosphere in which to elicit a placebo response from the patient."

Current research on factors contributing to the genesis of the placebo effect consistently document the importance of the doctor–patient relationship.[124–126] The healing power of the therapeutic interaction has been demonstrated by the commencement of the placebo effect even before the actual administration of the pill.[127]

The physician facilitates the cultivation of a sound relationship by developing good communication skills. The art of the bedside manner has been recognized throughout history as the primary skill a successful physician needs.[128] The history of medicine is as much a history of the relationship between doctor and patient as the evolution of medical technology and techniques. Through centuries in which doctors were doing more harm than good, little more than the esteem of their clientele sustained the medical profession. But however little real help the doctor had to offer, it was to him that people turned when illness struck.[129]

Bedside manner has been found in clinical studies to entirely alter the course of double-blind studies, and the quality of a therapeutic encounter has been found to facilitate or disrupt the efficacy of a treatment.[130] Listening to the patient,[130] the verbal and nonverbal communication of the physician, the amount of time spent with the patient,[131] patient education,[132] the demeanor of the physician,[133,] and interview skills[131] have been suggested as factors and components of effective physician communication skills. Communication between the doctor and patient is not simply a process of one party talking and the other side listening. Deep communication between both sides is a process of "interbrain" synchronization—literally, the brain waves of two people talking begin to match each other, as described in a research study by researchers at the Basque research center BCBL.[134] This synchronization or lack of it may be an important aspect of interpersonal communication. The examination of placebo dynamics uncovers the complexity, depth, and importance of the doctor–patient relationship. Many factors may be responsible for the varying therapeutic effects of the physician–patient relationship. An open-minded investigation of this relationship brings us, incredibly, to the possibility of brain-to-brain coupling, an interconnected matrix of the mind of the healer and the healed.[135] Learning to "listen" or synchronize with the patient you are communicating with ensures optimum transfer of information, hope, empathy, and a host of essential dynamics between doctor and patient.

Touch is an important form of communication and is sometimes forgotten as a key aspect of the doctor–patient relationship. Highly skilled clinicians with many years of experience, such as the now deceased Dr. John Bastyr (whose remarkable healing abilities inspired the founding of Bastyr University by those privileged to have been his students), frequently impressed upon clinicians the importance of always using diagnostic and therapeutic touch during a patient visit. The doctor's touch can be diagnostic, therapeutic, and, perhaps most important, a means of communicating that he or she is deeply attuned to the problems, needs, and fears of the patient.[133] Touch can heal by increasing tissue mobility and fluid exchange, as in massage, or by relieving pain, as demonstrated by research on healers who use their hands.[136] Touch has also been documented in well-designed double-blind research to extend an unusual healing power that can be transmitted through the hands to plants and animals.[137]

Among other methods of enhancing confidence between the doctor and patient, the setting in which a doctor provides therapy to a patient also determines its effectiveness. The doctor's office setting is very important for optimum and effective treatment: tools and support systems are more accessible, and a heightened patient response results from seeking out the "healing" environment. In a clinical trial with hypertensive patients, placebo alone was not as effective as when it was administered in conjunction with hospitalization. The visit to the physician represents a search for changes that cannot be found through "self-care" or over-the-counter medicines. According to Frank[138]:

> *In short, it appeared that the placebo situation relieved chiefly anxiety and depression, that the degree of relief was unrelated to personality and autonomic measures, and that the patients who responded strongly to a placebo at one time might not at another. In conjunction, these results suggest that the extent of responsiveness to a placebo depends on the interaction of the patient's state at a particular time with certain properties of the situation. The finding that administration of tests and questionnaires seemed to have at least as beneficial an effect as had the pill implies that any interaction between patient and situation that heightens expectations of help may lead to symptom reduction and improvement in mood. The aspects of the situation producing this effect include not only presentation of a symbol of the physician's healing powers (a pill), but any attention and interest shown by professional personnel.*

This phenomenon was also observed in industry and termed the *Hawthorne effect.* As a direct result of the greater attention factory workers received during investigation, the quality of their work improved.[139] In conclusion, the importance of a doctor–patient relationship and the confidence that it engenders shows that all human beings need to share their feelings and experience the therapeutic benefits of touch: the doctor–patient relationship provides an ideal way to meet these fundamental needs.

Enhance Positive Emotional States

> *Love in all its subtleties is nothing more, and nothing less, than … the psychical convergence of the universe upon itself.*
>
> ***—Pierre Teilhard de Chardin, The Phenomenon of Man***

For optimum enhancement of the psychoneuroimmune system, the physician must assist the patient in developing practices that amplify positive emotional states and reduce a negative emotional state. A negative mental state (anxiety, stress, panic, anger, depression, neurotic behavior, self-deprecation, self-destructive feelings and tendencies, and a weak will to live) hinders the ideal functioning of the psychoneuroimmune endocrine axis, disrupting homeostasis. Engle[140] termed this the *giving-up/given-up complex:*

> *Study of the life settings in which patients fall ill reveals that illness is commonly preceded by a period of psychological disturbance, during which the individual feels unable to cope. This has been designated the giving-up/given-up complex and has the following five characteristics: a feeling of giving up, experienced as helplessness or hopelessness; a depreciated image of the self; a sense of loss of gratification from relationships or roles in life; a feeling of disruption of the sense of continuity between past, present, and future; and a reactivation of earlier periods of giving-up. It is proposed that this state reflects the temporary failure of the mental coping mechanisms with a consequent activation of neurally regulated biologic emergency patterns. Changes in body economy so evoked may alter the organism's capability to deal with concurrent pathogenic processes, permitting disease to develop.*

The importance of reducing negative mental states in acute and chronic conditions has been discussed extensively.[70] Acute psychological stress is documented to cause various forms of cardiopulmonary dysfunction and even death.[61]

Chronic mental and emotional strain causes a breakdown of the immune system and can lead to disease. The homeostatic processes become overwhelmed by autoimmune, microbial, or neoplastic invasion. Major writers on the subject of acute and chronic stress emphasize the high priority of managing the physiologically and immunologically destructive effects of the human body's response to stress. Pelletier[141] listed hypertension, arteriosclerosis, migraine headache, cancer, chronic bronchitis, emphysema, asthma, and arthritis as disease processes that are caused or exacerbated by stress physiology. A study researching the relationship between resistance to streptococcal infections in families and stress load in the family found a positive correlation.[142] Another study on the psychosomatic susceptibility to infectious mononucleosis found that two psychosocial factors, high motivation and poor academic performance, significantly increased the risk of "disease" infection.[143] In still another, anticipation of mood and menstrual discomfort were positively correlated and manipulated, thereby supporting the suspicion that expectations act as a determinant of mood.[144]

The conclusion that there is no acute, chronic, or degenerative disease that is not affected by a patient's mental and emotional state must be drawn from the pervasive immunoendocrine effects generated by the mind and emotions. Wolf[145] and Cousins[146] wrote of the power of panic as a factor in myocardial infarction; Marbach et al.[107] described depression as a component in myofascial pain dysfunction; and Shekelle et al.[147] noted, in a 17-year follow-up study, a twofold increase in the incidence of cancer in depressed patients. The clinical scenarios these observers described imply that the placebo effect can control the onset and advance of a disease by shutting down the destructive thoughts, images, and feelings that mediate stress.

Enhancing positive emotions is the corollary of controlling the damaging effects of negative mental and emotional states. Laughter,[23] hope,[148] acceptance,[64] and the reduction of suffering[149] have been shown to speed the course of healing and reduce the level of pain and distress reported by patients. Although pain is sometimes the only language nature can use to adequately communicate to the patient that something is in need of healing, "the relief of suffering and the cure of disease must be seen as twin obligations of a medical profession that is truly dedicated to the sick."[149]

Acceptance has been observed to be a key factor that assists patients in better understanding their pain.[64] Acceptance does not mean complacency in the face of disease but a rational understanding of the situation and the limitations that can sometimes accompany a disease process.

The importance of cultivating hope in a patient also cannot be underestimated.[150] The fact that a patient seeks the help of a physician or "caregiver" already implies a substrate of hope and is a signal that the patient can visualize the potential for recovery. The treatment needs to merely stimulate this willingness to envision a future of health. Hope is an embodiment of the patient's and the doctor's ability to visualize an image of healing and recovery. This process is a recurrent theme in imagery therapy,[151] visualization therapy,[152] therapeutic touch,[153] and psychic healing.[154] Hope is both an active and a passive placebo. The *passive hope placebo* is that which is brought with the patient as the act of seeking help generates a level of unspoken faith in an image or potential for cure. The *active hope placebo* is generated by the physician, who consciously instills a vision or image of cure in the patient as an adjunct to therapy.

Frank[138] performed a double-blind study in which patients were divided into control and induction groups. The induction group was led through a process whereby their hope was strengthened to conform with the expectations of the therapist[138]:

> *It introduces some perceptual clarity into the process of treatment; and to the extent that all our therapists adhered roughly to the insight model of therapy, it helped to bring the patient's expectations in line with what actually occurred in treatment, and also helped him behave in accordance with the therapist's expectations of a good patient.*

The induction group was actually being consciously strengthened to a level of optimal response but was not being led into false expectations.[155]

This type of patient education or active placebo is a necessary and useful tool for framing and directing a positive outlook and prognosis. If a patient can conceive of a state of wellness, then that state of wellness can be achieved. It is the job and domain of the physician to discover those images, emotions, and perceptions that reside in the conscious and subconscious mind of the patient that block the image of a positive state of health. He or she must actively work to control these with the same level of intent as with any presenting gross complaint or physiological dysfunction. Finding these dysfunctional mental substrates and working with the patient to try to change them is fundamental to treating the true cause of disease (see earlier discussion of *tollem causum*).

Research demonstrated the importance of positive and negative thinking in heart disease and cancer, the two areas of disease that cause the highest death rate. Doctors' health care management protocols should reflect this research in the same way that attention to a proper diet is part of a management approach to high serum cholesterol. It is now clearly established, for instance, that even low levels of stress trigger the onset of myocardial ischemia.[156] We also know from the work of Steven Greer[157] and David Spiegel[158] that attitude and emotional exploration are critical to breast cancer survival. Knowing these scientific facts, all doctors must have strategies for helping their patients explore the areas of stress management, group therapy and support groups, and skills in building positive attitudes.

Implement Therapeutic Conditioning or Learning

> *Those who remain at least dimly aware that everything they say or do to a patient conveys a major or minor, positive or negative, and helpful or harmful psychological impact are likely to be more effective physicians.*[159]

Conditioning of the mind has been suggested as a mechanism by which the placebo effect becomes a learned response.[17,19,138] The future of the therapeutic application of placebo will probably hinge primarily on the use of conditioning. A doctor who can understand this will pay close attention to the stimuli of his or her patients and modify these stimuli in a scientific way to help treat immune-related and neurologically related diseases.

Modern psychology acknowledges two models of conditioning or reinforcement of learning behavior, operant and classical. Operant conditioning is a behavior response that theoretically occurs in the presence of some stimulus that is a positive reinforcement; for example, a rat will learn to press a conditioning bar if a food pellet is dispensed as a result. Classical conditioning is a behavior response created by the simultaneous pairing of unconditioned and conditioned stimuli before an evoked response. This is best illustrated by the experiments of Pavlov and his "salivating dog." In Pavlov's experiment with the conditioning of a dog's salivary response to the ringing of a bell, the bell ring is the conditioned stimuli, and the food is the unconditioned stimuli. The salivation is the unconditioned response to the food that becomes the conditioned response. When the dog finally associates the bell ring with the food, the ringing alone causes salivation, the conditioned response.

The principle of classical conditioning has far-reaching implications for the diagnosis and treatment of disease because of the pervasive and permeating implications that conditioning has in all the sensory stimuli of daily existence, in sickness and in health: "Pavlov's teachings, concepts and basic notions afford the real and ultimately scientific basis for the recognition of the potentialities of medical science attacking diseases from both the psychic and somatic sides."[160]

For the purposes of this discussion, one must recognize that classical conditioning happens randomly in our environment and is closely linked to health and healing phenomena. Subconsciously, we note random events and associate them with previous events and observations, independent of an intended learning behavior. Operant conditioning happens in the context of reward, and classical conditioning happens in the context of associated stimuli. There is a much greater predominance and range of associated stimuli for classical conditioning than for operant conditioning for the genesis of the placebo effect. This is because the operant depends on reward, although operant conditioning can happen in the medical model: "Pain-killing drugs that I have taken in the past kill pain; therefore this capsule, which is a painkiller, will kill my pain."

Gliedman et al.[17] noted that drugs that affect the CNS are readily conditioned, whereas drugs that affect the peripheral nervous system and are secretory stimulators (e.g., atropine and pilocarpine) do not result in the establishment of a conditioned response. The primary importance of psychological states to CNS excitation demonstrates that the pivotal loci of command for conditioning reside within the hypothalamus and the limbic system. Therefore a doctor who can induce a state of central excitation in the patient can encourage and condition the patient to make those changes that are deemed necessary for the recovery of health.

The conditioning of a patient to a placebo response is modified by learning stimuli associated with the illness, the stimuli of the doctor and the therapeutic setting, the stimuli of the therapy, previous health, medical therapy, and authority-related experiences.[161] The way that all of these factors interact in the psyche of the patient determines the nature of the placebo response that is achieved.

Satiation obscures the conditioned response, whereas situations of increased stress seem to potentiate the responsiveness of the placebo effect.[64] The placebo effect, conditioning, and learning may therefore be subject to the nature of central excitatory states as well as levels of stress and distress.

The physiological breadth of the placebo response in humans can now be understood in terms of the variety of interactions and effects that drugs, therapeutic procedures, and sensory phenomena of the medical environment have on the psychosomatic matrix of a patient's consciousness. Rossi[19] noted that this complicated web of sensory processing reveals how any facet of therapy "that alters any aspect of the body's sensory, perceptual or physiologic responsiveness on any level can disrupt the more or less fragile state-dependant encoding of symptoms and thereby evoke a 'nonspecific' but real healing effect that we call the placebo response."

The scientific basis of therapeutic applications of psychoneuroimmunology is based on classical conditioning. Ader and Cohen[162] performed research to show that the immune system could be conditioned for therapeutic purposes. They conditioned immunosuppression in rats by injecting them with a conditioned stimulus of cyclophosphamide (a potent immunosuppressing agent) while feeding them a saccharine solution as an unconditioned stimulus.[162] The idea of conditioning for immunomodulation in human patients is therefore a promising therapeutic modality. Applying conditioning techniques for the treatment of systemic lupus erythematosus involving a dosage that normally had minimal results resulted in a delay in the development of the disease.[163]

To fully account for the extent of previous and future conditioning in a patient, the physician must take a complete and exhaustive history to explore the influences of family, work, accidents, emotional predispositions, medical history, and neutral stimuli as contributing factors during the onset of an illness. Lifestyle and emotional, behavioral, or physiological factors might contribute to maintaining the state-dependent learning pattern of disease and dysfunction or give clues to a successful therapeutic intervention. A good example of this is the demonstration by Batterman and Lower[164] of increased analgesic effectiveness based on similar previous therapy. A physician who knows which therapies succeeded and which failed can take advantage of the patient's conditioning and encourage biochemical pathways that the body has learned. Drug or therapeutic interventions are not procedures that can be predicted in the same way that in vivo experimental results can.

The variables involved in human responses to therapy are clearly underestimated in the current rush of research-oriented therapeutic evaluation.[165] Therefore a patient who has been treated by a number of physicians or practitioners for a complaint and has received no results or relief has been conditioned to believe that consultation and treatment by a physician will provide no positive changes. When the patient visits the next practitioner, even if this practitioner can offer a diagnosis and treatment that are correct answers to the long-sought cure, there are very real patient conditioning factors that must still be considered.

Consider the case of a young woman who underwent treatment for breast cancer and the clinical course of the ensuing metastases. Each time she had a positive response to therapy, she experienced a subsequent remanifestation of the cancer. The result of this conditioning was that she came to equate each new course of chemotherapy as a herald of some new manifestation: she "was torn between a desire to live and the fear that allowing hope to emerge again would merely expose her to misery if the treatment failed."[166]

The parameters of conditioning in a clinical setting extend to all aspects of the patient's sensory perceptions. Consciously or unconsciously, the physician provides an environment for patient learning. Lipkin[167] pointed out that every drug, every apparatus, every injection, and every piece of information or advice carries a suggestion of help and hope, regardless of the physiological effects that may accompany it. The physician must realize that patients are taking in all the information about the surroundings, interactions, and therapy and are making associations that can potentially affect the course of their responsiveness to therapy.

Mowrer[86] observed that the "safety signals" of syringes, laboratory coats, and behavioral procedures were all retained in the patient's psyche for future association. A physician can skillfully take advantage of these signals by encouraging and cultivating response generalization or by associating previous therapeutic situations with subsequent treatments by means of unconditioned stimuli, such as office music, odors, and images. Giving patients some sort of unconditioned stimulus that can be taken home allows them to associate with the conditioned response, eliciting the memory of the therapeutic interaction while patients are away from the doctor's office. These unconditioned stimuli or placebos can be given in multiples at one time[81]; changed for more powerful stimuli[168]; and delivered at the end of an induction, suggestion, or imagery procedure. They should not be limited to pills or other apparent medicaments and should extend to sounds, smells, visualizations, and feelings.

It should be remembered that therapeutic conditioning depends on a perceived physiological shift or change in the patient as described in the theory and research of biofeedback.[169] This shift can be experienced as a sense of relaxation, increased warmth or circulation, altered autonomic tone, or a change in some sensory perception. Patients know immediately when there is no change in their disease or dysfunction after they have been given placebo.[170] Therefore some patients need a more active form of therapeutic management that allows for some level of perceived change. Ideally this perception would be a sense of being free from pain or alteration from a state of abnormal physiological function to a state of improved physiological function. Acupuncture, spinal manipulation, drug therapy, physiotherapy, hydrotherapy, and surgery are all therapies that can create an immediate biochemical effect that is perceived by the patient.

The optimum model to apply to the concept of conditioning therapy and the selection of an appropriate therapy or modality was proposed by Greene and Laskin[171] in their evaluation of myofascial pain dysfunction. During an 11-year follow-up study of patients with myofascial pain dysfunction, these researchers concluded that when comparing the effectiveness of a wide variety of reversible and nonreversible (surgical) therapies, conservative and reversible therapies were the most important and appropriate treatment factors for the patient's health and well-being. Focusing on patient communication, educating patients about the reversibility of the condition and the nature of muscle dysfunction as it relates to stress–pain–spasm, developing a therapeutic strategy based on increasing patient awareness and self-management skills, and selecting a flexible treatment strategy were all found to be essential for achieving a good initial response that could lead to long-term wellness. Greene and Laskin[171] believe that the specifics as to which therapy is most indicated are not as important as the need to focus on the nature of presenting musculoskeletal problems and the factors and complexity of the treatment environment.

The routine use of active pharmacological substances reinforces the relationship between conditioned and unconditioned stimuli. However, the routine use of unconditioned stimuli in the absence of a conditioned response weakens the therapeutic efficacy of the practitioner and has been described as "placebo sag."[18] Therefore the learning of a conditioned response from unconditioned stimuli could diminish if the conditioned stimuli fail to produce an adequate or reliable conditioned response. Without the intermittent demonstration of active strength, the placebo effect will get weaker and weaker.

The implications of placebo sag for practitioners of alternative medicine, who try to work with the body's own defense mechanisms without overwhelming medical intervention, are that periodic use of perceptually active therapy is needed to support a patient who is not able to respond or responds too slowly to a gentler therapeutic nudge. In this case the physician must recondition the vital force to open a path to homeostasis.

In a sense, this may be a paradigm of the therapeutic situation, in which changes toward health are induced in the patient by a doctor who is able to cultivate a basic state of arousal, presumably central in nature. This state of arousal causes the patient to become accessible to the doctor's expectations of the patient.[17]

The typical placebo burst, in which a therapy is initially effective after a short period but then wanes, is now understood in terms of the placebo sag from a lack of effective unconditioned stimuli to maintain the conditioned framework.[168] Physicians who lack the ability to extract themselves from a series of unsuccessful therapies risk eventual placebo sag[18]:

> *[T]herapists who primarily use their active strengths (or unconditioned stimuli) paradoxically will get stronger placebo effects than quacks, will enjoy escalating credibility, and will seem as miracle men—when in fact perhaps only half their miracles can be traced to their active ingredients while the other half is a function of the anticipatory (or conditioned) response elicited by their conditioned features.*

Because the visit to a physician is often initiated by the physical pain of the patient, it stands to reason that skillful pain management is a high priority in establishing a therapeutic conditioned response. Pain management by hypnosis, transcutaneous electrical nerve stimulation, therapeutic touch, direct or indirect manipulation, imagery, acupuncture, meditation,[172] and an understanding that aims to elicit the nature of suffering[166] can all be valuable therapeutic adjuncts to establishing a therapeutic environment that conditions the patient for the full potentiation of his or her healing capabilities. (See Chapter 42 for a full discussion of these techniques.)

With the recent development of standardization of, research into, and concentration of the active components of plant medicines, vitamins, and biochemical precursors, naturopathic medicine and other forms of alternative medicine stand on a stronger therapeutic base because of an ever-growing verification of the pharmaceutical and therapeutic armamentarium. These therapeutic modalities are characterized by safe yet physiologically active substances and procedures; therefore they provide some defense against placebo sag.

Use Altered States of Consciousness

Since ancient times, aboriginal humans have recognized the tremendous therapeutic power that lies dormant in the subconscious mind. For thousands of years, shamans and medicine men have used trance states to engage the most subtle aspects of the patient's subconscious to affect factors in disease pathogenesis and prognosis.[173] In modern medicine, it has been documented that shamanistic healing involving altered states can offer dramatic "spontaneous remissions[33];" the mechanisms of this process have been explored in the theory and application of hypnosis.[4,76]

Most currently accepted techniques employed to trigger the subconscious to effect positive changes in somatic or psychic health involve hypnosis. The placebo effect has been linked with hypnosis, or

"low arousal states," which are therefore believed to be critical factors in the evaluation of the mechanisms and perimeters of placebo.[160] A review of the literature documenting the potency of hypnosis and the observed results of placebo clearly demonstrated that these two areas yielded remarkably similar clinical results. The inquiry into hypnosis grew out of the simple intent to validate the effectiveness of the mind in healing processes, whereas most placebo literature grew out of the intent to demonstrate a certain percentage of chance, fluke, spontaneous remission, or psychosomatic illness as a factor to be ruled out in the delivery of intelligent, scientific health care. Using these antiquated definitions of placebo and hypnosis, one is led to believe that hypnosis describes a process of healing based on the skillful guidance of a qualified practitioner and that placebo describes a process based on chance, regardless of the professional circumstances. On closer inspection, the distinction between the two blurs: they appear to be much the same process.

Illness, healing, and health states shift constantly in the homeostatic system, a system that is affected by stimuli received through the different levels of awareness and can be accessed, investigated, and modified by a variety of techniques. These include placebo, hypnosis, and induced altered states of consciousness. Rossi[19] noted that because memory depends on and is limited to the level of awareness in which the memory was acquired, it is "state-bound information":

> *State dependent memory, learning, and behavior phenomena are the missing link in all previous theories of mind body relationships.… The major thrust of these hypotheses is that mind-body information and state-dependent memory, learning and behavior mediated by the limbic-hypothalamic system, are the two fundamental processes of mind-body communication and healing.… The new approach to mind-body healing and therapeutic hypnosis may be conceptualized as processes of accessing and utilizing state-dependent memory, learning and behavior systems that encode symptoms and problems and then reframing them for more integrated levels of adaptation and development.*

Some psychosomatic phenomena are coded into the behavior of an individual through state-induced patterning. Until the patient can access the state in which somatic complaints are induced, possibly through hypnosis or other methods that break the sympathetic dominance of "encoded" shock,[174] the psyche cannot clear them from the soma[19]:

> *A person in a traumatic car accident experiences an intense rush of the alarm reaction hormones. His detailed memories of the accident are intertwined with the complex psychophysiological state associated with these hormones. When he returns to his usual or "normal" psychophysiological states of awareness a few hours or days later, the memories of the accident become fuzzy or, in really severe cases … the victim may be completely amnesic. The memories of the accident have become "state-bound"—that is, they are bound to the precise psychophysiological state evoked by the alarm reaction, together with its associated sensory-perceptual impressions.*

In accessing these psychosomatic state-dependent areas of homeostatic dysfunction, the physician must use techniques that relax the conscious mind and allow access to subconscious content for reframing. The nature of the visit to a physician encourages a patient into more accessible unconscious states, as demonstrated by higher placebo effects when patients present in a hospital setting.[121] These labile states of consciousness are quite natural; humans constantly cycle in and out of different consciousness states.[121] These cycles, or ultradian rhythms, are described as alternating cycles of hemispherical dominance that change every 1½ hours.

When these cycles are interrupted by behavioral stress, psychosomatic behavioral responses such as ulcers, gastritis, asthma attacks, and rashes develop.[175] A change in these rhythms manifests as a period of psychic repose. If an individual is in the midst of performing a task, daydreaming or the perceived need for a rest or coffee break may be the external manifestation of an internally sensed signal of a change in rhythm. This is also a period when one is highly susceptible to hypnotic suggestion. Because these rhythms are very flexible and labile, they can be invoked through hypnosis, or if the physician senses a natural lull indicating a hemispherical switch, a "natural" trance can be induced.

Centuries ago in India, practitioners of hatha yoga observed the effect of mental states on the breathing patterns of an individual. With anger, frustration, and mental instability, the breath reflects a short, arrhythmic pattern that mirrors the disturbed psyche of the person. Conversely, when a person is in a peaceful, relaxed, deep meditative state, the breath is long, rhythmic, and barely perceptible. Their discovery formed the basis for the development of breathing exercises called *pranayama* (literally, regulation or restraint of the vital energy), which aimed to calm the breath so that deep states of meditation and focused concentration could be attained. Current research has affirmed the powerful effect these exercises have on asthma, diabetes, chronic gastrointestinal disorders, and psychosomatic and psychiatric dysfunction.[176] Traditional literature on the ethnomedical effects of training the mind and energy (prana, qi, ki, lung) in India, China, and Tibet consistently remark on the antiaging effects of these training methods. Research suggests that meditation may have a deep antiaging response on human functioning, potentially measurable in the epigenetic aging effect.[177]

Therapeutic exercises that use somatic stimuli to effect changes in the psyche create fertile environments for stimulating the placebo response. A breathing technique used to decrease sympathetic tone or alter nostril predominance for causing shifts in hemispherical activity,[178] an exercise to release fascial muscle tension and thereby effect mood-enhancing blood flow in the brain,[179,180] and a biofeedback treatment that aids in slowing the heart rate and decreasing negative emotional states[169] are all examples of how the psyche can be accessed by the soma. The whole process of eliciting the placebo response involves an attempt to marshal all the reserves and potential for healing through a doctor–patient interaction, engaging both the patient's mind and body to reestablish homeostatic equilibrium. Therapeutic meditation training has shown benefit in modifying pain.[181] Engaging in a formal or traditional method of meditation training may give the patient enhanced insight into the nature of his or her mind and thereby elicit psychological and physical health benefits.[182] Trained meditators exhibit unique abilities of mental functioning.[183] Medical applications of mind-training methods might be better served by relying on methods with deep cultural experience, at the expense of adopting a liberal, nondenominational, nonsectarian method of "relaxation therapy," as it appears not all awareness therapies are created equal. Traditional meditation-training methods offer the possibility of sustained and enhanced awareness of mental states, which may contribute to positive medical outcomes.[184]

Healthcare professionals can use the wisdom of psychosomatic therapies as a central part of their therapeutic protocol. In addition to the specific therapeutic regimen, treatment of the whole patient can be achieved through these harmonious techniques. If physicians could persuade patients to care daily for their emotions, minds, and spirits

the way they care for their hair or teeth, the effectiveness of any prescribed treatment would be greatly enhanced. As a primary therapeutic adjunct and important basis for preventive medicine, this line of treatment is all too often ignored.

ETHICS

There are two forms of "conscious" placebo use by the physician. The use of a placebo as a gentle therapeutic agent by a practitioner is very different from the use of a placebo in a controlled trial in which the possibility of a known therapy is withheld in a treatment group. Some researchers believe that the use of placebos in clinical trials breaches the Declaration of Helsinki, which states that every patient should be assured of the best proven diagnostic and therapeutic method.[185] The ethical problems of delivering health care in a research design in which there is a possibility of a favorable outcome, and half of the group is denied access to this possible favorable outcome, make it a troubling issue.

The ethical use of placebos has also been questioned in an attempt to determine whether a physician should be deceiving patients during the process of healing.[186] Although some writers advocate a restricted use of pure and impure placebos because of their "deceptive" nature,[169] it becomes clear in a brief review of the current literature[6] that any argument for or against the use of placebo assumes the existence of medical procedures that are free of a potential placebo effect. Brody[29] concluded that a placebo can be called the "lie that heals." However, closer examination shows that it is not the lie that does the healing but, rather, the relationship between the patient and doctor that stimulates a natural self-healing mechanism via psychological, symbolic, and biological intervention[29]:

> *For some time, medical science has looked almost exclusively at technical means of diagnosis and treatment; the doctor/patient relationship that forms the setting for their application has been naively viewed as a noncontributory background factor, relegated to the amorphous realm of the "art of medicine," or simply ignored. In this setting, the placebo effect has inevitably been viewed as a nuisance variable, interfering with our ability to elicit "clean data" from clinical trials; and deception in medicine has been seen either as an unimportant side issue or as a tolerated means toward an end. But as the doctor/patient is rediscovered as a worthy focus for medical research and medical education, the placebo effect assumes center stage as one approach to a more sophisticated understanding of this relationship.*

A physician's correct understanding of the nature of placebo therapy has been observed as able to coexist with its inaccurate use and abuse.[41] It has been recommended, however, that (1) a pure placebo should not be prescribed unless the physician has examined the exact indications even more carefully than when prescribing specific therapy, and (2) to avoid missing a disease process that can be easily treated with an empirically proven protocol (e.g., vitamin B_{12}–deficient peripheral neuropathy), the physician should not relax a diagnostic protocol because a patient seems to be responding to a placebo.[186]

The final ethical hurdle of placebo use, or any medical treatment, for that matter, is the abuse of hope in the patient's path of healing. It is one thing to make a harmless recommendation that provides no therapeutic value, but it is another to subject a patient to the known consequences of a dangerous procedure in the pursuit of a dubious outcome. Hope can be abused, leading the patient to experience unreasonable suffering.[187]

CONCLUSION

Health practitioners must be equipped with a better understanding of placebo therapeutics.[10,188] For many years now, the study of placebos has been recommended to doctors and other healthcare professionals. The ideal environment for the dissemination of the therapeutic implications of the doctor–patient relationship is in medical schools as a required part of the curriculum. After finding a pattern of misuse and misunderstanding about the nature and efficacy of placebo, Goodwin et al.[41] recommended that better education might result in more effective placebo use.

In 1938 Houston[128] wrote of the need to reaffirm the art of medicine because he perceived a trend in medicine that invested in a concept of the therapeutic doctor–patient interaction as "undisciplined thought." Houston's remedy for the intellectual bias that viewed medicine as a "tight, fast-set science" was to emphasize the importance of psychobiology in medical schools[129]:

> *One of the most hopeful moves in medical education is teaching to first-year students the elements of psychobiology. A system of belief is implanted best in the young. It would be my suggestion that psychobiology be taught in the premedical years, that the doctor/patient relationship be the beginning of medical studies. A deep insight into this fundamental philosophy is a chief concern of the internist.*

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jensen MP, et al. *Int J Clin Exp Hypn*. 2015;63(1):34–75. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4220267/. Accessed 16 Aug 2019.
2. Finniss DG, Kaptchuk TJ, Miller F, et al. Biological, clinical, and ethical advances of placebo effects. *Lancet*. 2010;375:686–965.
3. Guess H, Kleinman A, Kusek JW, et al. *The Science of the Placebo: Toward an Interdisciplinary Research Agenda*. London: BMJ Books; 2002.
4. Hall H. Hypnosis and the immune system. *Am J Clin Hypertens*. 1982;25:92–103.
5. Rogers M, Dubey D, Reich P. Influence of the psyche and brain on immunity and disease susceptibility. *Psychosom Med*. 1979;41:147–164.
6. Ader R, ed. *Psychoneuroimmunology*. New York: Academic Press; 1981.
7. White L, Tursky B, Schwartz GE, eds. *Placebo: Theory, Research and Mechanisms*. New York: Guilford Press; 1985.
8. Barrett S. Health frauds and quackery. *FDA Consum*. 1977;11:12–17.
9. Thompson R. Numerical analysis and theoretical modeling of causal effects of conscious intention. *Subtle Energies Energy Med J*. 1991;2:47–70.
10. Braud W. Conscious interactions with remote biological systems: anomalous intentionality effects. *Subtle Energies Energy Med J*. 1991;2:1–46.
11. Dossey L. *Healing Words: The Power of Prayer and the Practice of Medicine*. San Francisco: Harper Collins; 10.
12. Byrd R. Positive therapeutic effects of intercessory prayer in a coronary care unit population. *South Med J*. 1988;81:826–829.
13. Harvey S, Beedie CJ. Studying placebo effects in model organisms will help us understand them in humans. *Biol Lett*. 2017;13:20170585.
14. Sheldrake R. *The Presence of the Past: Morphic Resonance and the Habits of Nature*. New York: Times Books; 1988.
15. Benson E. The placebo effect. *JAMA*. 1975;232:1225–1227.
16. Alexander F. *Psychosomatic Medicine: Its Principles and Applications*. New York: Norton; 1987.
17. Gliedman LH, Gantt WH, Teitelbaum HA. Some implications of conditional reflex studies for placebo research. *Am J Psychol*. 1957;113:1103–1107.
18. Wickramasekara I. The placebo as a conditioned response. *Advances*. 1984;1:109–135.
19. Rossi E. *The Psychobiology of Mind-Body Healing*. New York: Norton; 1986.
20. Beecher HK. The powerful placebo. *JAMA*. 1955;159:1602–1606.
21. Everson TC, Cole WH. *Spontaneous Regression of Cancer*. Philadelphia: WB Saunders; 1966.
22. Booth G. Psychological aspects of "spontaneous" regressions of cancer. *J Am Acad Psychoanal*. 1973;1:303–317.
23. Cousins N. Anatomy of an illness (as perceived by the patient). *N Engl J Med*. 1976;295:1458–1463.
24. Shapiro AK. A contribution to a history of the placebo effect. *Behav Sci*. 1960;5:109–135.
25. Shapiro AK. Factors contributing to the placebo effect: their implication for psychotherapy. *Am J Psychother*. 1964;18:73–88.
26. Shapiro AK. Placebogenics and iatroplacebogenics. *Med Times*. 1964;92:1037–1043.
27. Berg AO. The placebo effect reconsidered. *J Fam Pract*. 1983;17:647–650.
28. Webster's II . *New Riverside University Dictionary*. Boston: Riverside Publishing Co; 1984.
29. Brody H. The lie that heals: the ethics of giving placebos. *Ann Intern Med*. 1982;97:112–118.
30. Benson H. The nocebo effect: history and physiology. *Prev Med*. 1997;26:612–615.
31. Klopfer B. Psychological variables in human cancer. *J Proj Tech*. 1957;21:331–340.
32. Cannon WB. "Voodoo" death. *Psychosomatic Med*. 1957;19:182–190.
33. Kirkpatrick RA. Witchcraft and lupus erythematosus. *JAMA*. 1981;245(19):1937.
34. Hahn R. A sociocultural model of illness and healing. In: White L, Tursky B, Schwartz G, eds. *Placebo: Theory, Research and Mechanisms*. New York: Guilford Press; 1985.
35. Evans FJ. Unraveling placebo effects. *Advances*. 1984;1:11–19.
36. Beecher HK. *Measurement of Subjective Responses: Quantitative Effect of Drugs*. New York: Oxford University Press; 1959.
37. Chamsi-Pasha M, Albar M, Chamsi-Pasha H. Minimizing nocebo effect: pragmatic approach. *Avicenna J Med*. 2017;7(4):139–143.
38. Traut EF, Passarellu EW. Placebos in the treatment of rheumatoid arthritis and other rheumatic conditions. *Ann Rheum Dis*. 1957;16:18–22.
39. Colloca L, Sigaudo M, Benedetti F. The role of learning in nocebo and placebo effects. *Pain*. 2008;136:211–218.
40. Elmore D. Personal correspondence, April 1988.
41. Goodwin JS, Goodwin JM, Vogel AV. Knowledge and use of placebos by house officers and nurses. *Ann Intern Med*. 1979;91:106–110.
42. Vogel AV, Goodwin JS, Goodwin JM. The therapeutics of placebo. *Am Fam Physician*. 1980;22:105–109.
43. Beecher HK. Surgery as placebo: a quantitative study of bias. *JAMA*. 1961;176:1102–1107.
44. Wolf S. The pharmacology of placebos. *Pharmacol Rev*. 1959;11:689–704.
45. Linton HB, Langs RJ. Placebo reactions in a study of lysergic acid diethylamide. *Arch Gen Psych*. 1962;6:369–383.
46. Lasagna L, Mosteller F, von Felsinger JM, et al. A study of the placebo response. *Am J Med*. 1954;16:770–779.
47. Moertel CG, Taylor WF, Roth A, et al. Who responds to sugar pills? *Mayo Clin Proc*. 1976;51:96–100.
48. Parkhouse J. Placebo reactor. *Nature*. 1963;199:308.
49. Fisher S. The placebo reactor: thesis, antithesis, synthesis and hypothesis. *Dis Nerv Syst*. 1967;28:510–515.
50. Bush P. The placebo effect. *J Am Pharm Assoc*. 1974;14:671–674.
51. Wolf S, Pinsky RH. Effects of placebo administration and occurrence of toxic reactions. *JAMA*. 1954;155:339–341.
52. Gliedman LH, Nash Jr EH, Imber SD, et al. Reduction of symptoms by pharmacologically inert substances and by short-term psychotherapy. *AMA Arch Neurol Psychiatry*. 1958;79:345–351.
53. Lasagna L, Laties VG, Dohan JL. Further studies on the pharmacology of placebo administration. *J Clin Invest*. 1958;37:533–537.
54. Evans W, Hoyle C. The comparative value of drugs used in the continuous treatment of angina pectoris. *Q J Med*. 1933;2:311–338.
55. Dinnerstein AJ, Halm J. Modification of placebo effects by means of drugs: effects of aspirin and placebo on self-rated moods. *J Abnorm Psychol*. 1970;75:308–314.
56. Lasagna L, von Felsinger JM, Beecher HK. Drug-induced changes in man. I. Observations on healthy subjects, chronically ill patients, and postaddicts. *JAMA*. 1955;157:1006–1020.
57. Shevchuk YM. A medical marvel. *Can Pharm Rev*. 1987:57–600.
58. Schapira K, McClelland HA, Griffiths NR, et al. Study on the effects of tablet color in the treatment of anxiety states. *BMJ*. 1970;1:446–449.
59. Blackwell B, Bloomfield SS, Buncher CR. Demonstration to medical students of placebo responses and non-drug factors. *Lancet*. 1972;1:1279–1282.
60. Benson H. Looking beyond the relaxation response. *Revision*. 1984;7:50–55.
61. Darko DF. A brief tour of psychoneuroimmunology. *Ann Allergy*. 1986;57:233–238.
62. Beecher HK. Evidence for increased effectiveness of placebos with increased stress. *Am J Physiol*. 1956;187:163–169.
63. Reed CF, Witt PN. Factors contributing to unexpected reactions in two human drug-placebo experiments. *Confin Psychiatr*. 1965;8:57–68.
64. Beecher HK. Relationship of significance of wound to pain experienced. *JAMA*. 1956;161:1609–1613.
65. Guyton AC. *Textbook of Medical Physiology*. Philadelphia: WB Saunders; 1981.
66. Foreword Cousins N. In: Locke S, Colligan D, eds. *The Healer Within*. New York: New American Library; 1986.
67. Thomas CB, Duszynski KR, Shaffer JW. Family attitudes reported in youth as potential predictors of cancer. *Psychosom Med*. 1979;1:287–302.
68. Schekelle RB, Raynor Jr WJ, Ostfeld AM, et al. Psychological depression and 17-year risk of death from cancer. *Psychosom Med*. 1981;43:117–125.
69. Dreher H. Cancer and the mind: current concepts in psycho-oncology. *Advances*. 1987;4:27–43.
70. Bartrop RW, Luckhurst E, Lazarus L, et al. Depressed lymphocyte function after bereavement. *Lancet*. 1977;1:834–836.
71. Schleifer SJ, Keller SE, Camerino M, et al. Suppression of lymphocyte stimulation following bereavement. *JAMA*. 1983;250:374–377.

72. Engle GL. Sudden and rapid death during psychological stress: folklore or folk wisdom? *Ann Intern Med*. 1971;74:771–782.
73. Tregear EJ. *Anthr Inst*. 1890;19:100.
74. Kiecolt-Glaser JK, Glaser R. Psychoneuroimmunology: can psychological interventions modulate immunity? *J Consult Clin Psychol*. 1992;60(4):569–575.
75. Gough WC, Shacklett RL. The science of connectiveness. Part 3: the human experience. *Subtle Energies Energy Med J*. 1993;4:187–211.
76. Steggles S, Stam HJ, Fehr R, et al. Hypnosis and cancer: an annotated bibliography 1960–1985. *Am J Clin Hyperten*. 1987;29:281–290.
77. Weil A. *Health and Healing*. Boston: Houghton Mifflin; 1983.
78. Benedetti F, et al. Neurobiological mechanisms of the placebo effect. *J Neurosci*. 2005;25(45):10390–10402.
79. Ashar YK, et al. Brain mechanisms of the placebo effect: an affective appraisal account. *Annu Rev Clin Psychol*. 2017;13:73–98.
80. Freund J, Krupp G, Goodenough D. The doctor patient relationship and the drug effect. *Clin Pharmacol Ther*. 1972;13:172–180.
81. Greene CS, Laskin DM. Long-term evaluation of treatment for myofascial pain dysfunction syndrome. *J Am Dent Assoc*. 1983;107:235–236.
82. Benedetti F. Mechanisms of placebo and placebo-related effects across diseases and treatments. *Annu Rev Pharmacol Toxicol*. 2008;48:33–60.
83. Sternbach RA. The effects of instructional sets on autonomic responsivity. *Psychophysiology*. 1964;62:67–72.
84. Levine JD, Gordon NC, Fields HL. The mechanism of placebo analgesia. *Lancet*. 1978;2:654–657.
85. Borysenko J. Psychoneuroimmunology: behavioral factors and the immune response. *Revision*. 1984;7:56–65.
86. Selye H. The general adaptation syndrome and the diseases of adaptation. *J Clin Endocrinol*. 1946;6:117–230.
87. Mowrer OH. *Learning Theory and Behavior*. New York: Wiley; 1960.
88. Benedetti F, Mayberg HS, Wager TD, et al. Neurobiological mechanisms of the placebo effect. *J Neurosci*. 2005;25(45):10390–10402.
89. Mayberg HS, Silva JA, Brannan SK, et al. The functional neuroanatomy of the placebo effect. *Am J Psychiatry*. 2002;159:728–737.
90. Wager TD, Rilling JK, Smith EE, et al. Placebo-induced changes in FMRI in the anticipation and experience of pain. *Science*. 2004;303:1162–1167.
91. Singer T, Seymour B, O'Doherty J, et al. Empathy for pain involves the affective but not sensory components of pain. *Science*. 2004;303:1157–1162.
92. Leuchter AF, Cook IA, Witte EA, et al. Changes in brain function of depressed subjects during treatment with placebo. *Am J Psychiatry*. 2002;159:122–129.
93. Solomon GF. The emerging field of psychoneuroimmunology, with a special note on AIDS. *Advances*. 1985;2:6–19.
94. Cannon W. Stresses and strains of homeostasis. *Am J Med Sci*. 1935;22:1–18.
95. Laudenslager ML. Coping and immunosuppression: inescapable but not escapable shock suppresses lymphocyte proliferation. *Science*. 1983;221:568–570.
96. Monjan AA, Collector MI. Stress-induced modulation of the immune response. *Science*. 1977;196:307–308.
97. Riley V. Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science*. 1981;212:1100–1109.
98. Vedhara K, Cox NK, Wilcock GK, et al. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet*. 1999;353:627–631.
99. Scott LV, Salahuddin F, Cooney J, et al. Differences in adrenal steroid profile in chronic fatigue syndrome, in depression and in health. *J Affect Disord*. 1999;54:129–137.
100. Locke S, Colligan D. *The Healer Within*. New York: New American Library; 1986.
101. Schnall PL, Pieper C, Schwartz JE, et al. The relationship between "job strain" workplace diastolic blood pressure, and left ventricular mass index: results of a case controlled study. *JAMA*. 1990;263:1929–1935.
102. Bower B. Emotional stress linked to common cold. *Science News*. 1991;140:132.
103. Fackelmann KA. Stress puts squeeze on clogged vessels. *Science News*. 1991;140:309.
104. Mittleman MA, Maclure M. Mental stress during daily life triggers myocardial ischemia. *JAMA*. 1977;277:1558–1559.
105. Vergano D. Stress may weaken the blood brain barrier. *Science News*. 1996;150(24):375.
106. Levine JD, Gordon NC, Fields HL. The mechanism of placebo analgesia. *Lancet*. 1978;2:654–657.
107. Marbach JJ, Dworkin SF. Chronic MPD group therapy and psychodynamics. *J Am Dent Assoc*. 1975;90:827–833.
108. Gold MS, Pottash AC, Sweeny D. Antimanic, antidepressant and antipanic effects of opiates: clinical, neuroanatomical and biochemical evidence. In: Verebey K, ed. *Opioids in Mental Illness: Theories, Clinical Observations, and Treatment Possibilities*. New York: New York Academy of Sciences; 1982.
109. Benedetti F, Arduino C, Amanzio M. Somatotopic activation of opioid systems by target-directed expectations of analgesia. *J Neurosci*. 1999;19:3639–3648.
110. Benedetti F, Amanzio M, Baldi S, et al. Inducing placebo respiratory depressant responses in humans via opioid receptors. *Euro J Neurosci*. 1999;11:625–631.
111. Goldstein A, Grevert P. Placebo analgesia, endorphins and naloxone. *Lancet*. 1978;2(8104-5):1385.
112. Barber J, Mayer D. Evaluation of the efficacy and neural mechanism of a hypnotic analgesia procedure in experimental and clinical dental pain. *Pain*. 1977;4:41–48.
113. Krieger DT. Brain peptides: what, where and why. *Science*. 1983;222:975–985.
114. Heijnen CJ, Ballieux RE. Influence of opioid peptides on the immune system. *Advances*. 1986;3:114–1121.
115. Blalock JE. The immune system as a sensory organ. *J Immunol*. 1984;132:1067–1070.
116. Pierpaoli W, Sorkin E. Hormones and immunologic capacity. I. Effect of heterologous anti-growth hormone (ASTH) antiserum on thymus and peripheral lymphatic tissue in mice. Induction of a wasting syndrome. *J Immunol*. 1968;101:1036–1043.
117. Haggard HW. *Devils, Drugs, and Doctors; the Story of the Science of Healing From the Medicine-Man to Doctor*. New York: Harper & Brothers; 1929.
118. Al-Lamee R, et al. Percutaneous coronary intervention in stable angina (ORBITA): a double-blind, randomised controlled trial. *Lancet*. 2017. https://doi.org/10.1016/S0140-6736(17)32714-9. [Epub ahead of print].
119. Burton R. *The Anatomy of Melancholy*. New York: Empire State Book Company; 1929.
120. Gantt WH. Principles of nervous breakdown-schizokinesis and autokinesis. *Ann N Y Acad Sci*. 1953;56:143–163.
121. Klerman GL. Assessing the influence of the hospital milieu upon the effectiveness of psychiatric drug therapy. *J Nerv Ment Dis*. 1963;137:143–154.
122. Wheatly D. Influence of doctors' and patients' attitudes in the treatment of neurotic illness. *Lancet*. 1967;2:1133–1135.
123. Lewith GT. Every doctor a walking placebo. *Comp Med Res*. 1987;2:10–18.
124. Benson H, Epstein MD, The placebo effect. A neglected asset in the care of patients. *JAMA*. 1975;232:1225–1227.
125. Brody HL. The lie that heals: the ethics of giving placebos. *Ann Intern Med*. 1982;97:112–118.
126. Jensen PS. The doctor-patient relationship: headed for impasse or improvement? *Ann Intern Med*. 1981;95:769–771.
127. Nash EH, Frank JD, Imber SD, et al. Selected effects of inert medication on psychiatric patients. *Am J Psychother*. 1964;18(suppl 1):33–48.
128. Houston WR. The doctor himself as a therapeutic agent. *Ann Intern Med*. 1938;11:1416–1425.
129. Freund J, Krupp G, Goodenough D, et al. The doctor-patient relationship and the drug effect. *Clin Pharmacol Ther*. 1972;13:172–180.
130. Budd MA, Zimmerman ME. The potentiating clinician: combining scientific and linguistic competence. *Advances*. 1986;3:40–45.
131. Bogdonoff MD, Nichols CR, Klein RF, et al. The doctor-patient relationship: a suggested practical and purposeful approach. *JAMA*. 1965;192:45–48.
132. Egbert LD, Battit GE,Welch CE, et al. Reduction of postoperative pain by encouragement and instruction of patients. *N Engl J Med*. 1964;270:825–827.

133. Bruhn JG. The doctor's touch: tactile communication in the doctor-patient relationship. *South Med J*. 1978;71:1469–1473.
134. Pérez A, Carreiras M, Duñabeitia JA. Brain-to-brain entrainment: EEG interbrain synchronization while speaking and listening. *Sci Rep*. 2017;7(1):4190. https://doi.org/10.1038/s41598-017-04464-4.
135. Hasson U, et al. Brain-to-brain coupling: a mechanism for creating and sharing a social world. *Trends Cogn Sci*. 2012;16(2):114–121. https://doi.org/10.1016/j.tics.2011.12.007.
136. Krieger D. *The Therapeutic Touch*. New York: Prentice-Hall; 1979.
137. Grad B. Some biological effects of the "laying on of hands": a review of experiments with animals and plants. *J Am Soc Psychical Res*. 1965;59:95–127.
138. Frank J. The role of hope in psychotherapy. *Int J Psychiatry*. 1968;5:383–395.
139. Roethlisberger FJ, Dickson WJ. *Management and the Worker*. Cambridge, MA: Harvard University Press; 1961.
140. Engel GL. A life setting conducive to illness: the giving-up-given-up complex. *Bull Menninger Clin*. 1968;32:355–365.
141. Pelletier KR. *Mind as Healer, Mind as Slayer: A Holistic Approach to Preventing Stress Disorders*. New York: Delacorte Press/St. Lawrence; 1977.
142. Meyer RJ, Haggerty RJ. Streptococcal infections in families: factors altering individual susceptibility. *Pediatrics*. 1962;29:539–549.
143. Kasl SV. Psychosocial risk factors in the development of infectious mononucleosis. *Psychosom Med*. 1979;41:445–466.
144. Olasov B, Jackson J. Effects of expectancies on women's reports of moods during the menstrual cycle. *Psychosom Med*. 1987;49:65–78.
145. Wolf S. The end of the rope: the role of the brain in cardiac death. *Can Med Assoc J*. 1967;97:1022–1025.
146. Cousins N. *The Healing Heart: Antidotes to Panic and Helplessness*. New York: Norton; 1983.
147. Shekelle RB, Raynor Jr WJ, Ostfeld AM, et al. Psychological depression and 17-year risk of death from cancer. *Psychosom Med*. 1981;43:117–125.
148. Gottschalk LA. Hope and other deterrents to illness. *Am J Psychother*. 1985;39:515–524.
149. Cassel EJ. The nature of suffering and the goals of medicine. *N Engl J Med*. 1982;306:639–645.
150. Bruhn JG. Therapeutic value of hope. *South Med J*. 1984;77:215–219.
151. Achterberg J. *Imagery in Healing: Shamanism and Modern Medicine*. Boston: New Science Library; 1985.
152. Simonton OC. *Getting Well Again: A Step-by-step, Self-Help Guide to Overcoming Cancer for Patients and Their Families*. Los Angeles: JP Tarcher; 1978.
153. Krieger D. *The Therapeutic Touch: How to Use Your Hands to Help or to Heal*. Englewood Cliffs, NJ: Prentice-Hall; 1979.
154. Kunz D, ed. *Spiritual Aspects of the Healing Arts*. Wheaton, IL: Theosophical Publishing; 1985.
155. Frank J. The role of hope in psychotherapy. *Int J Psychiatry*. 1968;5:383–395.
156. Mittleman MA, Maclure M. Mental stress during daily life triggers myocardial ischemia. *JAMA*. 1997;277:1558–1559.
157. Greer S, Moorey S, Baruch JD, et al. Adjuvant psychological therapy for patients with cancer: a prospective randomised trial. *BMJ*. 1992;304:675–680.
158. Spiegel D. Psychosocial aspects of breast cancer treatment. *Semin Oncol*. 1997;24(1 suppl 1):S136–S147.
159. Cornell Conference on Therapy. The use of placebos in therapy. *NY State Med J*. 1946;46:1718–1726.
160. Volgyesi FA. "School for patients," hypnosis therapy and psychoprophylaxis. *BMJ Hypertens*. 1954;5:8–17.
161. Epstein JB. Understanding placebos in dentistry. *J Am Dent Assoc*. 1984;109:71–74.
162. Ader R, Cohen N. Behaviorally conditioned immunosuppression. *Psychosom Med*. 1975;37:333–340.
163. Ader R. Behaviorally Conditioned Modulation of Immunity. In: Guillemin R, Cohn M, Melnechuk T, eds. *Neural Modulation of Immunity: Proceedings of an International Symposium Held Under the Auspices of the Princess Liliane Cardiology Foundation in Brussels, Belgium, October 27 and 28, 1983*. New York: Raven Press; 1985.
164. Batterman RC, Lower WR. Placebo responsiveness—influence of previous therapy. *Curr Ther Res Clin Exp*. 1968;10:136–143.
165. Wolf S. Effects of suggestion and conditioning on the action of chemical agents in human subjects: the pharmacology of placebos. *J Clin Invest*. 1950;29:100–109.
166. Cassel EJ. The nature of suffering and the goals of medicine. *N Engl J Med*. 1982;306:639–645.
167. Lipkin M. Suggestion and healing. *Perspect Biol Med*. 1984;28:121–126.
168. Morrison RA, Woodmansey A, Young AJ. Placebo responses in an arthritis trial. *Ann Rheum Dis*. 1961;20:179–185.
169. Schwartz GE. Biofeedback, self-regulation, and patterning of physiological processes. *Am Sci*. 1975;63:314–324.
170. Modell W, Houde RW. Factors influencing clinical evaluation of drugs. *JAMA*. 1958;167:2190–2199.
171. Greene CS, Laskin DM. Long-term evaluation of treatment of myofascial pain-dysfunction syndrome: a comparative analysis. *J Am Dent Assoc*. 1983;107:235–238.
172. Kabat-Zinn J. An outpatient program in behavioral medicine for chronic pain patients based on the practice of mindfulness meditation: theoretical considerations and preliminary results. *Gen Hosp Psychiatry*. 1982;4:33–47.
173. Halifax J. *Shamanic Voices: A Survey of Visionary Narratives*. New York: Dutton; 1979.
174. Klinghardt DK. Neural therapy. *J Neurol Orthop Med Surg*. 1993;14:109–114.
175. Orr WC, Hoffman HJ, Hegge FW. Ultradian rhythms in extended performance. *Aerosp Med*. 1974;45:995–1000.
176. Goyeche J. Yoga as therapy in psychosomatic medicine. *Psychother Psychosom*. 1979;31:373–381.
177. Chaix R, et al. Epigenetic clock analysis in long-term meditators. *Psychoneuroendocrinology*. 2017;85:210–214. https://doi.org/10.1016/j.psyneuen.2017.08.016. E.
178. Werntz D. *Cerebral Hemispheric Activity and Autonomic Nervous Function [Doctoral Dissertation]*. San Diego: University of California; 1981.
179. Zajonc RB. Emotion and facial efference: a theory reclaimed. *Science*. 1985;228:15–21.
180. Critchley EM. The human face. *BMJ (Clin Res Ed)*. 1985;291:1222–1223.
181. Panta P. The possible role of meditation in myofascial pain syndrome: a new hypothesis. *Indian J Palliat Care*. 2017;23(2):180–187. https://doi.org/10.4103/0973-1075.204239.
182. Karunamuni ND. The five-aggregate model of the mind. *SAGE Open J*. 2015:1–7. https://doi.org/10.1177/2158244015583860.
183. Braboszcz C, et al. Increased gamma brainwave amplitude compared to control in three different meditation traditions. *PLoS One*. 2017;12(1):e0170647. https://doi.org/10.1371/journal.pone.0170647.
184. Adler-Neal AL, Zeidan F. Mindfulness meditation for fibromyalgia: mechanistic and clinical considerations. *Curr Rheumatol Rep*. 2017;19(9):59. https://doi.org/10.1007/s11926.017-0686.0.
185. Rothman KJ, Michaels KB. The continuing unethical use of placebo controls. *N Engl J Med*. 1994;331:394–398.
186. Bok S. The ethics of giving placebos. *Sci Am*. 1974;231:17–23.
187. Mukerjee S. The anatomy of hope: care of the vital organ. *New York Times*. 2004.
188. Leslie A. Ethics and practice of placebo therapy. *Am J Med*. 1954;16:854–862.
189. Kostis JB, Krieger S, Cosgrove N, et al. The mechanism of placebo effect on exercise tolerance in angina pectoris. *Am J Cardiol*. 1982;49:1001.
190. Benson H, McCallie DP. Angina pectoris and the placebo effect. *N Engl J Med*. 1979;300:1424–1429.
191. Rinzler SH, Travell J, Bakst H, et al. Effect of heparin in effort angina. *Am J Med*. 1953;14:438–447.
192. Porter JM, Cutler BS, Lee BY, et al. Pentoxifylline efficacy in the treatment of intermittent claudication: multicenter controlled double-blind trial with objective assessment of chronic occlusive arterial disease patients. *Am Heart J*. 1982;104:66–72.
193. Vinar O. Dependence on a placebo: a case report. *Br J Psychiatry*. 1969;115:1189–1190.

194. Hashish I, Harvey W. Anti-inflammatory effects of ultrasound therapy: evidence for a major placebo effect. *Br J Rheumatol*. 1986;25:77–81.
195. Katz HM, Bissel G. Blood sugar lowering effects of chlorpropamide and tolbutamide. *Diabetes*. 1965;14:650–657.
196. Singer DL, Hurwitz D. Long-term experience with sulfonylureas and placebo. *N Engl J Med*. 1967;277:450–460.
197. Wolf S. Symposium on clinical drug evaluation and human pharmacology. IV. Placebos: problems and pitfalls. *Clin Pharmacol Ther*. 1962;3: 254–257.
198. Abbot FK, Mack M, Wolf S. The action of banthine on the stomach and duodenum of man with observations on the effects of placebos. *Gastroenterology*. 1952;20:249–261.
199. Brogden RN, Carmine AA, Heel RC, et al. Ranitidine: a review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases. *Drugs*. 1982;24:267–303.
200. Grenfell RF, Briggs AH, Holland WC. Antihypertensive drugs evaluated in a controlled double study. *South Med J*. 1963;56:1410–1416.
201. Reader R. Therapeutic trials in mild hypertension. *Med J Aust*. 1984;140:752–754.
202. Gould BA, Mann S, Davies AB, et al. Does placebo lower blood pressure? *Lancet*. 1981;2:1377–1381.
203. Hossmann V, FitzGerald GA, Dollery CT. Influence of hospitalization and placebo therapy on blood pressure and sympathetic function in essential hypertension. *Hypertension*. 1981;3:113–118.
204. Calzetti S, Findley LJ, Gresty MA, et al. Metoprolol and propranolol in essential tremor: a double blind, controlled study. *J Neurol Neurosurg Psychiatry*. 1981;44:814–819.
205. Linton HB, Langs RJ. Placebo reactions in a study of lysergic acid diethylamide (LSD-25). *Arch Gen Psychiatry*. 1962;6:53–67.
206. Cytryn L, Gilbert A. The effectiveness of tranquilizing drugs plus supportive psychotherapy in treating behavior disorders of children: a double-blind study of eighty outpatients. *Am J Orthopsychiatry*. 1960;30:113–129.
207. Keats AS, Beecher HK. Analgesic potency and side action liability in man of heptazone, WIN 1161-2, 6.methyl dihydromorphine, Meptopon, levo-isomethadone and pentobarbital sodium as a further effort to refine methods of evaluation of analgesic drugs. *J Pharmacol Exp Ther*. 1952;105:109–129.
208. Diamond EG, Kittle CF, Crockett JE. Comparison of internal mammary artery ligation and sham operation for angina pectoris. *Am J Cardiol*. 1960;5:483–486.
209. Cobb LA, Thomas GJ, Dillard DH, et al. An evaluation of the internal-mammary-artery ligation by a double-blind technique. *N Engl J Med*. 1959;269:1115–1118.
210. Uhlenhuth EH, Canter A, Neustadt JO, et al. The symptomatic relief of anxiety with meprobamate, phenobarbital, and placebo. *Am J Psychiatry*. 1959;115:905–910.
211. Solomon K, Hart R. Pitfalls and prospects in clinical research on antianxiety drugs: benzodiazepines and placebo—a research review. *J Clin Psychiatry*. 1978;39:823–831.
212. Cederlof S, Jonson G. Intraarticular prednisolone injection for osteoarthritis of the knee: a double blind test with placebo. *Acta Chir Scand*. 1966;132:532–537.
213. Wayne EJ. Placebos. *BMJ*. 1956;2:157.
214. Godfrey S, Konig P. Suppression of exercise-induced asthma by salbutamol, theophylline, atropine, cromolyn, and placebo in a group of asthmatic children. *Pediatrics*. 1975;56:930–934.
215. Luparello T, Lyons HA, Bleecher ER, et al. Influences of suggestion on airway reactivity in asthmatic subjects. *Psychosom Med*. 1968;30:819–825.
216. Godfrey S, Silverman M. Demonstration by placebo response in asthma by means of exercise testing. *J Psychosom Res*. 1973;17:293–297.
217. Molling PA, Lockner AW, Sauls RJ, et al. Committed delinquent boys: the impact of perphenazine and of placebo. *Arch Gen Psychiatry*. 1962;7:70–78.
218. Green FH, Andrews CH, Bain WA, et al. Clinical trials of antihistamine drugs in the prevention and treatment of the common cold. *BMJ*. 1950;2:425–429.
219. Diehl HS, Baker AB, Cowan DW. Cold vaccines: a further evaluation. *JAMA*. 1940;115:593–594.
220. Buck C, Gowdey CW. A clinical trial of a quaternary ammonium antiseptic lozenge in the treatment of the common cold. *Can Med Assoc J*. 1962;86:489–491.
221. Diehl HS. Medicinal treatment of the common cold. *JAMA*. 1933;101: 2042–2050.
222. Hillis BR. The assessment of cough suppressing drugs. *Lancet*. 1952;1: 1230–1235.
223. Malitz S, Kanzler M. Are antidepressants better than placebos? *Am J Psychiatry*. 1971;127:605–611.
224. Morris JB, Beck AT. The efficacy of antidepressant drugs. *Arch Gen Psychiatry*. 1974;30:667–674.
225. Budoff PW. Zomepirac sodium in the treatment of primary dysmenorrhea syndrome. *N Engl J Med*. 1982;307:714–719.
226. Nyren O, Adami HO, Bates S, et al. Absence of therapeutic benefit from antacids or cimetidine in non-ulcer dyspepsia. *N Engl J Med*. 1986;314:339–343.
227. Sturdevant RA, Isenberg JI, Secrist D, et al. Antacid and placebo produced similar pain relief in duodenal ulcer patients. *Gastroenterology*. 1977;72:1–5.
228. Wise PG, Rosenthal RR, Killian P, et al. A controlled study of placebo treatment of hayfever (abstract). *J Allergy Clin Immunol*. 1979;63:216.
229. Baldwin H. Conference on therapy. *Am J Med*. 1954;17:72.
230. Frey GH. The role of placebo response in clinical headache evaluations. *Headache*. 1961;1:31–38.
231. Jellinek EM. Clinical tests on comparative effectiveness of analgesic drugs. *Biometrics Bull*. 1946;2:87–91.
232. Sillaanpaa M. Clonidine prophylaxis of childhood migraine and other vascular headache. *Headache*. 1977;17:28–31.
233. Untreated mild hypertension. A report by the management committee of the australian therapeutic trial in mild hypertension. *Lancet*. 1982;1:185–191.
234. Patel C, Marmot MG, Terry DJ. Controlled trial of biofeedback-aided behavioural methods in reducing mild hypertension. *BMJ (Clin Res Ed)*. 1981;282:2005–2008.
235. Liberman R. An experimental study of the placebo response under three different situations of pain. *J Psychiatr Res*. 1964;33:233–246.
236. Maddocks S, Hahn P, Moller F, et al. A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome. *Am J Obstet Gynecol*. 1986;154:573–581.
237. Thomsen J, Bretlau P, Tos M, et al. Placebo effect in surgery for Meniere's disease: three-year follow-up. *Otolaryngol Head Neck Surg*. 1983;91:183–186.
238. Posner J, Burke CA. The effects of naloxone on opiate and placebo analgesia in healthy volunteers. *Psychopharmacology*. 1985;87:468–472.
239. Barron A, Beckering B, Rudy LH, et al. A double blind study comparing RO4-0403, trifluoperazine and a placebo in chronically ill mental patients. *Am J Psychiatry*. 1961;118:347–348.
240. Schultz JI, Johnson JD, Freedman SO. Double-blind trial comparing flunisolide and placebo for the treatment of perennial rhinitis. *Clin Allergy*. 1978;8:313–320.
241. Straus B, Eisenberg J. Hypnotic effects of an antihistamine—methapyrilene hydrochloride. *Ann Intern Med*. 1955;42:574–582.

7

Positive Mental Attitude

John Nowicki, ND, and *Michael T. Murray, ND*

OUTLINE

INTRODUCTION

A positive mental attitude is one of the foundational elements of good health. This axiom has been contemplated by philosophers and physicians since the time of Plato and Hippocrates. Attitude is reflected by *explanatory style,* a term developed by noted psychologist Martin Seligman to describe a cognitive personality variable that reflects how people habitually explain the causes of life events.[1] Explanatory style was used to describe individual differences in response to negative events during the attributional reformulation of the learned helplessness model of depression developed by Seligman (described in Appendix 12). The Attributional Style Questionnaire developed by Seligman or the Revised Optimism-Pessimism (PSM) scale of the Minnesota Multiphasic Personality Inventory (MMPI) can be used to determine an individual's level of optimism.

In addition to simple conventional wisdom, modern research has also verified the important role that attitude—the collection of habitual thoughts and emotions—plays in determining the length and quality of life. Specifically, studies using various scales to assess attitude, including the PSM scale of the MMPI, have shown that individuals with a pessimistic explanatory style have poorer health, are prone to depression, are more frequent users of medical and mental health care delivery systems, exhibit more cognitive decline and impaired immune function with aging, and have a shorter survival rate compared with optimists.[1–8] One study involved a large cohort of 5566 people who completed a survey at two time points, aged 51 to 56 years at time 1 and aged 63 to 67 years at time 2. This survey included a questionnaire to determine positive psychological well-being by measuring self-acceptance, autonomy, purpose in life, positive relationships with others, environmental mastery, and personal growth. The results showed that people with low positive well-being were 7.16 times more likely to be depressed 10 years later.[9] This research highlighted the fact that although life is full of events that are beyond one's control, people can control their responses to such events. Attitude plays a significant role in determining how people view and respond to the stresses and challenges of life.

EFFECT OF ATTITUDE, PERSONALITY, EMOTIONS ON HEALTH

Longevity

In 1981 the Leisure World Cohort Study undertook a prospective cohort study of nearly 14,000 elderly women and men in a California retirement community to study the relationship between mental attitude and longevity and successful aging.[10] Participants completed a postal survey including seven positively worded items from the Zung self-rating depression scale and were followed to death or December 31, 2016 (a 35-year span), whichever came first. In both men and women, a more negative attitude was associated with significantly higher mortality. The risk of death significantly increased by 2% (women) and 4% (men) for each unit decrease in total attitude score. Overall, the multivariable-adjusted hazard ratio (HR) for death for individuals in the lowest versus the highest quarter of total attitude was 1.24 (1.16–1.32) for women and 1.30 (1.19–1.41) for men. Thus strategies to improve mental outlook may help improve the quantity as well as the quality of life.

Immune Function

The importance of attitude to human health has been examined in the links among the brain, emotions, and the immune system. Research in the field of psychoneuroimmunology indicates that every part of the immune system is connected to the brain in some way, either via a direct nervous tissue connection or through the complex language of chemical messengers and hormones. What scientists are discovering is that every thought, emotion, and experience sends a message to the immune system that either enhances or impairs its ability to function. A simplistic view is that positive emotions, such as joy, happiness, and optimism, tend to boost immune system function, whereas negative emotions, such as depression, sadness, and pessimism, tend to suppress it.

Studies examining immune function in optimists versus pessimists have demonstrated significantly better immune function in the optimists. Specifically, studies have shown that, compared with pessimists, optimists have increased secretory immunoglobulin-A function, natural killer cell activity, and cell-mediated immunity, which is demonstrated by better ratios of helper to suppressor T-cells.[6,11–14]

The immune system is so critical to preventing cancer that if emotions and attitude were risk factors for cancer, one would expect to see an increased risk of cancer in people who have long-standing depression or a pessimistic attitude. Research supports this association; for example, smokers who are depressed have a much greater risk of lung cancer than smokers who are not depressed.[15]

Depression and the harboring of other negative emotions contribute to an increased risk of cancer in several ways. Most research has focused on the effect of depression and other negative emotions on natural killer cells. Considerable scientific evidence has documented the link between a higher risk of cancer and negative emotions, stress, and a low level or low activity of natural killer cells.[16] Negative emotions and stress paralyze many aspects of immune function and literally can cause natural killer cells to burst.[16,17] Furthermore, the prototypical cancer personality—an individual who suppresses anger, avoids conflicts, and has a tendency to have feelings of helplessness—has lower natural killer cell activity than other personality types.[13,14] These studies also indicate that individuals with a personality type that is prone to cancer have an exaggerated response to stress, which compounds the detrimental effects stress has on natural killer cells and the entire immune system.

Depression and stress not only affect the immune system but also appear to hinder the cell's ability to repair damage to DNA. Most carcinogens cause cancer by directly damaging DNA in cells, thereby producing abnormal cells. Some of the most important protective mechanisms against cancer in the cell's nucleus are the enzymes responsible for the repair or destruction of damaged DNA. Several studies have shown that depression and stress alter these DNA repair mechanisms. For example, in one study, lymphocytes from depressed patients demonstrated impairment in the ability to repair cellular DNA damaged by exposure to x-rays.[18,19]

Just as research has identified personality, emotional, and attitude traits that are associated with impaired immune function, the field of psychoneuroimmunology has likewise identified a collection of "immune power" traits that include a positive mental attitude; an effective strategy for dealing with stress; and a capacity to confide traumas, challenges, and feelings to oneself and others.[16,20]

Cardiovascular Health

The cardiovascular system is another system intricately tied to emotions and attitude. The relationship of an optimistic or pessimistic explanatory style with the incidence of coronary heart disease was examined as part of the Veterans Affairs Normative Aging Study, an ongoing cohort study of older men.[8] These men were assessed by the MMPI PSM scale. During an average 10-year follow up, 162 cases of incident coronary heart disease occurred: 71 cases of incident nonfatal myocardial infarction, 31 cases of fatal coronary heart disease, and 60 cases of angina pectoris. Men reporting high levels of optimism had a 45% lower risk for angina pectoris, nonfatal myocardial infarction, and coronary heart disease death than men reporting high levels of pessimism. Interestingly, a clear dose–response relationship was found between levels of optimism and each outcome.

To illustrate how closely the cardiovascular system is linked to attitude, one study showed how measures of optimism and pessimism affected ambulatory blood pressure.[21] Pessimistic adults had higher blood pressure levels than optimistic adults, suggesting that pessimism has broad physiological consequences. Affective well-being (happiness and pleasure) and eudaimonia (sense of autonomy and purposeful engagement with life) have been associated with smaller waist circumference, healthier lipid profiles (e.g., greater high-density lipoprotein cholesterol [HDL-C], lower levels of triglycerides), higher levels of serum antioxidants, and lower levels of inflammatory markers (e.g., C-reactive protein [CRP], fibrinogen).[22]

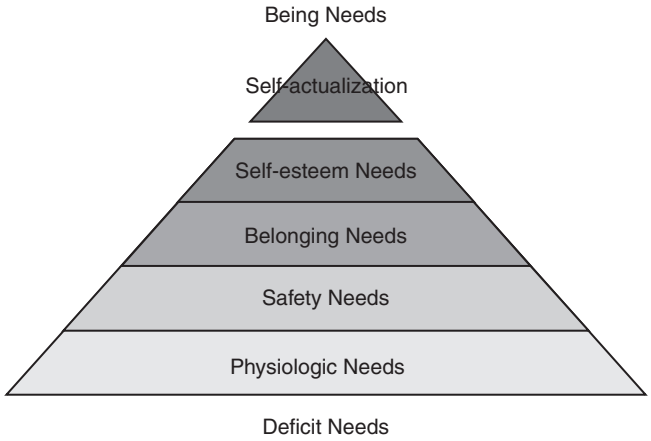


Fig. 7.1 Maslow's hierarchy of needs.

Excessive anger, worrying, and other negative emotions have also been shown to be associated with an increased risk for cardiovascular disease; however, these emotions may simply reflect a pessimistic explanatory style.

SELF-ACTUALIZATION

A physician's role should include facilitating the health of the patient as well as helping the patient achieve *self-actualization,* which is a concept developed by Abraham Maslow, the founding father of humanistic psychology. His work and theories were the result of more than 30 years of intense research on psychologically healthy people. Essentially, Maslow was the first psychologist to study healthy people. He strongly believed that the study of healthy people would create a firm foundation for the theories and values of a new psychotherapy.

Maslow discovered that healthy individuals are motivated toward self-actualization, a process of "ongoing actualization of potentials, capacities, talents, as fulfillment of a mission (or call, fate, destiny, or vocation), as a fuller knowledge of, and acceptance of, the person's own intrinsic nature, as an increasing trend toward unity, integration, or synergy within the person."[23]

Maslow developed a five-step pyramid of human needs in which personality development progresses from one step to the next. The needs of the lower levels must be satisfied before the next level can be achieved. When needs are met, the individual moves toward well-being and health. Fig. 7.1 displays Maslow's hierarchy of needs.

The primary needs that form the base of the pyramid are basic survival or physiological requirements: the satisfaction of hunger, thirst, sexuality, and shelter. The second step consists of safety needs, which are essential for dealing with the world: security, order, and stability. The individual then progresses to the third step, which involves the ability to love and be loved: belonging. The fourth step involves self-esteem and self-respect: approval, recognition, and acceptance. The final step is self-actualization: the use of one's creative potential for self-fulfillment.

In modern life, a person's occupation often correlates with the ability to achieve these needs. Table 7.1 provides an application of Maslow's hierarchy of needs in an occupational environment.

Maslow studied self-actualized people and noted that they had strikingly similar characteristics. Some of Maslow's key findings, in an abbreviated form, include the following:

- Self-actualized people perceive reality more effectively than others and are more comfortable with it. They have an unusual ability to

TABLE 7.1 Practical Application of Maslow's Hierarchy of Needs

Level of Need	General Rewards	Occupational Factors
Self-actualization	Growth	Challenging job
	Achievement	Opportunities for creativity
	Advancement	Achievement in work
	Creativity	Promotion
Self-esteem	Self-respect	Social recognition
	Status	Job title
	Prestige	High status of job
		Feedback from the job itself
Belonging	Love	Work groups or teams
	Friendship	Supervision
	Belongingness	Professional associations
Safety	Security	Health and safety
	Stability	Job security
	Protection	Contract of employment
Physiological	Food	Pay
	Water	Working conditions
	Sleep	
	Sex	

detect the spurious, the fake, and the dishonest in personality. They judge experiences, people, and things correctly and efficiently. They possess an ability to be objective about their own strengths, possibilities, and limitations. This self-awareness enables them to clearly define values, goals, desires, and feelings. They are not frightened by uncertainty.

- Self-actualized people have an acceptance of self, others, and nature. They can accept their own human shortcomings without condemnation. They do not have an absolute lack of guilt, shame, sadness, anxiety, and defensiveness, but they do not experience these feelings to unnecessary or unrealistic degrees. When they do feel guilty or regretful, they do something about it. Generally, they do not feel bad about discrepancies between what is and what ought to be.
- Self-actualized people are relatively spontaneous in their behavior and even more spontaneous in their inner lives, thoughts, and impulses. They are unconventional in their impulses, thoughts, and consciousness. They are rarely nonconformists, but they seldom allow convention to keep them from doing anything they consider important or basic.
- Self-actualized people have a problem-solving orientation toward life instead of a self-orientation. They commonly have a mission in life, some problem outside themselves that enlists much of their energies. In general, this mission is unselfish and is involved with the philosophical and ethical.
- Self-actualized people have a quality of detachment and a need for privacy. Often, it is possible for them to remain above the battle, to be undisturbed by what upsets others. They are self-governing people who find meaning in being active, responsible, self-disciplined, and decisive rather than being pawns or helplessly ruled by others.
- Self-actualized people have a wonderful capacity to appreciate the basic pleasures of life, such as nature, children, music, and sex, again and again. They approach these basic experiences with awe, pleasure, wonder, and even ecstasy.
- Self-actualized people commonly have mystical or "peak" experiences, times of intense emotions in which they transcend the self. During a peak experience, they have feelings of limitless horizons and unlimited power while simultaneously feeling more helpless than ever before. There is a loss of place and time and feelings of great ecstasy, wonder, and awe. The peak experience ends with the conviction that something extremely important and valuable has happened, and thus the person is transformed and strengthened by the experience to some extent.
- Self-actualized people have deep feelings of identification with, sympathy for, and affection for other people despite occasional anger, impatience, or disgust.
- Self-actualized people have deeper and more profound interpersonal relationships than most other adults, but not necessarily deeper than children's. They are capable of more closeness, greater love, more perfect identification, and more erasing of ego boundaries than other people would consider possible. One consequence is that self-actualized people have especially deep ties with relatively few individuals, and their circle of friends is small. They tend to be kind or at least patient with almost everyone, yet they speak realistically and harshly of those who they feel deserve it, especially hypocritical, pretentious, pompous, or self-inflated individuals.
- Self-actualized people are democratic in the deepest possible sense. They are friendly toward everyone, regardless of class, education, political beliefs, race, and color. They believe it is possible to learn something from everyone. They are humble, in the sense of being aware of how little they know in comparison with what could be known and what is known by others.
- Self-actualized people are strongly ethical and moral. However, their notions of right and wrong and good and evil are often unconventional. For example, a self-actualized person would never consider segregation, apartheid, or racism to be morally right, although it may be legal.
- Self-actualized people have a keen, unhostile sense of humor. They do not laugh at jokes that hurt other people or are aimed at others' inferiority. They can make fun of others in general or of themselves when they are foolish or try to be big when they are small. They are inclined toward thoughtful humor that elicits a smile, is intrinsic to the situation, and is spontaneous.
- Self-actualized people are highly imaginative and creative. The creativeness of a self-actualized individual is not of the special talent type, such as Mozart's, but rather is like the naive and universal creativeness of unspoiled children.

CLINICAL APPLICATION OF LEARNED OPTIMISM

The new psychology that Maslow's work referred to may turn out to be "positive clinical psychology."[24] This field of practice was born in 1998 when Martin Seligman chose it as the theme for his term as president of the American Psychological Association.[25] Positive clinical psychology aims to change clinical psychology to have an equally weighted focus on both positive and negative functioning.[26] The approach is based on five key bodies of empirical findings: (1) the absence of positive well-being leads to the development of disorder over time[9]; (2) the absence of positive characteristics predicts disorder above and beyond the presence of negative characteristics[9]; (3) positive characteristics interact with negative life events to predict disorder (so studying only negative life events would produce misleading results)[27]; (4) many aspects of well-being range from extremely negative functioning, through a neutral midpoint, to positive well-being (possibly including happiness to depression and anxiety to relaxation continuums),[28] making it impossible to study exclusively negative or positive well-being; and (5) positive interventions can be as effective as other more commonly used approaches, such as cognitive therapy.[29]

Positive clinical psychology ultimately involves helping patients become optimistic, which, according to Martin Seligman, is our natural tendency.[30] Optimism not only is a necessary step toward achieving optimal health but is also critical to happiness and a higher quality of life.

In many instances, it is not what happens in one's life that determines one's direction; to a large degree, it is the response to those challenges that shapes the quality of life and determines one's level of health. Surprisingly, it is often true that hardship, heartbreak, disappointment, and failure serve as the sparks for joy, ecstasy, compassion, and success. The determining factor is whether these challenges are viewed as stepping-stones or stumbling blocks.

A person's attitude is like his or her physical body: it must be conditioned to be strong and positive. Conditioning an attitude to be positive and optimistic requires adopting specific healthy habits. Four key areas of focus for helping patients develop a positive mental attitude are as follows:

1. Help them become aware of self-talk. Tell them that all people conduct a constant running dialogue in their heads. In time, the things people say to themselves and others percolate down into their subconscious minds. Those inner thoughts, in turn, affect the way people think and feel. Naturally, a steady stream of negative thoughts will have a negative effect on a person's mood, immune system, and quality of life. The cure is to become aware of self-talk and then to consciously work to feed positive self-talk messages to the subconscious mind.
2. Help them ask better questions. The quality of a person's life is equal to the quality of the questions habitually asked. For example, if a person experiences a setback, does he or she think, "Why am I so stupid? Why do bad things always happen to me?" or "Okay, what can be learned from this situation so that it never happens again? What can I do to make the situation better?" Clearly, the latter response is healthier. Regardless of the situation, asking better questions is bound to improve one's attitude. Some examples of questions that can improve attitude and self-esteem when asked regularly include the following:
   "What am I most happy about in my life right now?"
   "What am I most excited about in my life right now?"
   "What am I most grateful about in my life right now?"
   "What am I enjoying most in my life right now?"
   "What am I committed to in my life right now?"
   "Whom do I love? Who loves me?"
   "What must I do today to achieve my long-term goal?"
3. Help them experience gratitude. A large body of recent work has suggested that people who are more grateful have higher levels of well-being and are happier, less depressed, less stressed, and more satisfied with their lives and social relationships.[31,32] Gratitude appears to have one of the strongest links with mental health of any character trait. Helping instill a sense of gratitude has been shown to be a very successful intervention. In one study, participants were randomly assigned to one of six therapeutic interventions designed to improve the participants' overall quality of life.[33] Of these six interventions, it was found that the biggest short-term effects came from a "gratitude visit," where participants wrote and delivered a letter of gratitude to someone in their lives. This simple gesture showed a rise in happiness scores by 10% and a significant fall in depression scores, the results of which lasted up to 1 month after the visit. The act of writing "gratitude journals," in which participants wrote down three things they were grateful for every day, had longer-lasting effects on happiness scores. The greatest benefits with this practice were usually found to occur around 6 months after it began. Similar practices have shown comparable benefits.
4. Help them set positive goals. Learning to set achievable goals is a powerful method for building a positive attitude and raising self-esteem. Achieving goals creates a success cycle: a person feels better about him- or herself, and the better he or she feels, the more likely he or she is to succeed. Some guidelines for helping patients set healthy goals include the following:
   - Be specific. The more clearly the goal is defined, the more likely it will be achieved. For example, if a person wants to lose weight, he or she should define the desired weight and the body fat percentage or measurements to be achieved.
   - State the goal in positive terms and in the present tense; avoid negative words. It's better to say, "I enjoy eating healthy, low-calorie, nutritious foods" than to say, "I will not eat sugar, candy, ice cream, and other fattening foods."
   - Make the goal attainable and realistic. Start out with goals that are easily attainable, like drinking six glasses of water a day or switching from white to whole-grain bread. Initially choosing easily attainable goals creates a success cycle that helps build a positive self-image. Little things add up to make a major difference in the way a person feels about him- or herself.

Counseling is necessary for the severely pessimistic individual. Forms of cognitive therapy appear to be the most useful therapy. Cognitions comprise the whole system of thoughts, beliefs, mental images, and feelings. Cognitive therapy can be as effective as the use of antidepressant drugs in the treatment of moderate depression; in addition, there tends to be a lower risk of relapse—the return of depression—with cognitive therapy.[34] One reason for this is that cognitive therapy teaches people practical skills they can use to combat depression anytime, anywhere, and every day for the rest of their lives. Cognitive therapy avoids the long, drawn-out (and expensive) process of psychoanalysis. It is a practical, solution-oriented psychotherapy that teaches skills a person can apply to improve quality of life.

Mental health specialists trained in cognitive therapy seek to change the way the depressed person consciously thinks about failure, defeat, loss, and helplessness. To do so, they employ five basic tactics that help patients do the following:

- Recognize the automatic negative thoughts that flit through consciousness at the times when they feel the worst.
- Dispute the negative thoughts by focusing on contrary evidence.
- Learn a different explanation to dispute the automatic negative thoughts.
- Avoid rumination (the constant churning of a thought in one's mind) by helping the patient better control his or her thoughts.
- Question depression-causing negative thoughts and beliefs and replace them with empowering, positive thoughts and beliefs.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Peterson C, Seligman M, Valliant G. Pessimistic explanatory style as a risk factor for physical illness: a thirty-five year longitudinal study. *J Pers Soc Psychol*. 1988;55:23–27.
2. Maruta T, Colligan RC, Malinchoc M, Offord KP. Optimism-pessimism assessed in the 1960s and self-reported health status 30 years later. *Mayo Clin Proc*. 2002;77:748–753.
3. Taylor SE, Kemeny ME, Reed GM, et al. Psychological resources, positive illusions, and health. *Am Psychol*. 2000;55:99–109.
4. Schweizer K, Beck-Seyffer A, Schneider R. Cognitive bias of optimism and its influence on psychological well-being. *Psychol Rep*. 1999;84: 627–636.
5. Chang EC, Sanna LJ. Optimism, pessimism, and positive and negative affectivity in middle-aged adults: a test of a cognitive-affective model of psychological adjustment. *Psychol Aging*. 2001;16:524–531.
6. Segerstrom SC. Optimism, goal conflict, and stressor-related immune change. *J Behav Med*. 2001;24:441–467.
7. Maruta T, Colligan RC, Malinchoc M, Offord KP. Optimists vs pessimists: survival rate among medical patients over a 30-year period. *Mayo Clin Proc*. 2000;75:140–143.
8. Kubzansky LD, Sparrow D, Vokonas P, Kawachi I. Is the glass half empty or half full? A prospective study of optimism and coronary heart disease in the normative aging study. *Psychosom Med*. 2001;63:910–916.
9. Wood AM, Joseph S. The absence of positive psychological (eudemonic) well-being as a risk factor for depression: a ten year cohort study. *J Affect Disord*. 2010;122:213–217.
10. Paganini-Hill A, Kawas CH, Corrada MM. Positive mental attitude associated with lower 35-year mortality: the leisure world cohort study. *J Aging Research*. 2018;2126368. PubMed PMID: 30595919.
11. Brennan FX, Charnetski CJ. Explanatory style and immunoglobulin A (IgA). *Integr Physiol Behav Sci*. 2000;35:251–255.
12. Kamen-Siegel L, Rodin J, Seligman ME, Dwyer J. Explanatory style and cell-mediated immunity in elderly men and women. *Health Psychol*. 1991;10:229–235.
13. Imai K, Nakachi K. Personality types, lifestyle, and sensitivity to mental stress in association with NK activity. *Int J Hyg Environ Health*. 2001;204:67–73.
14. Segerstrom SC. Personality and the immune system: models, methods, and mechanisms. *Ann Behav Med*. 2000;22:180–190.
15. Jung W, Irwin M. Reduction of natural killer cytotoxic activity in major depression: interaction between depression and cigarette smoking. *Psychosom Med*. 1999;61:263–270.
16. Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Emotions, morbidity, and mortality: new perspectives from psychoneuroimmunology. *Annu Rev Psychol*. 2002;53:83–107.
17. Maddock C, Pariante CM. How does stress affect you? An overview of stress, immunity, depression and disease. *Epidemiol Psychiatr Soc*. 2001;10:153–162.
18. Kiecolt-Glaser JK, Stephens R, Lipitz P, et al. Distress and DNA repair in human lymphocytes. *J Behav Med*. 1985;8:311–320.
19. Glaser R, Thorn BE, Tarr KL, et al. Effects of stress on methyltransferase synthesis: an important DNA repair enzyme. *Health Psychol*. 1985;4:403–412.
20. Kiecolt-Glaser JK, Glaser R. Psychoneuroimmunology and cancer: fact or fiction? *Eur J Cancer*. 1999;35:1603–1607.
21. Raikkonen K, Matthews KA, Flory JD, et al. Effects of optimism, pessimism, and trait anxiety on ambulatory blood pressure and mood during everyday life. *J Pers Soc Psychol*. 1999;76:104–113.
22. Steptoe A, Demakakos P, de Oliveira C, Wardle J. Distinctive biological correlates of positive psychological well-being in older men and women. *Psychosomatic Medicine*. 2012;74(5):501–508. PubMed PMID: 22511728.
23. Maslow A. *The Farther Reaches of Human Nature*. New York: Viking; 1971.
24. Lambert MJ, Erekson DM. Positive psychology and humanistic tradition. *J Psychother Integration*. 2008;18:222–232.
25. Seligman MEP, Csikszentmihalyi M. Positive psychology: an introduction. *Am Psychol*. 2000;55:5–14.
26. Wood AM, Tarrier N. Positive clinical psychology: a new vision and strategy for integrated research and practice. *Clin Psychol Rev*. 2010;30(7):819–829.
27. Johnson J, Gooding PA, Wood AM, et al. Resilience to suicidal ideation in psychosis: positive self-appraisals buffer the impact of hopelessness. *Behav Res Ther*. 2010;48(9):883–889.
28. Wood AM, Taylor PT, Joseph S. Does the CES-D measure a continuum from depression to happiness? Comparing substantive and artifactual models. *Psychiatry Res*. 2010;177:120–123.
29. Geraghty AWA, Wood AM, Hyland ME. Attrition from self-directed interventions: Investigating the relationship between psychological predictors, intervention content and dropout from a body dissatisfaction intervention. *Social Sci Med*. 2009;71:31–37.
30. Seligman M. *Learned Optimism*. New York: Knopf; 1991.
31. Wood AM, Froh JJ, Geraghty AW. Gratitude and well-being: a review and theoretical integration. *Clin Psychol Rev*. 2010;30(7):890–905.
32. Wood AM, Joseph S, Maltby J. Gratitude predicts psychological well-being above the Big Five facets. *Pers Individ Dif*. 2009;46:443–447.
33. Seligman MEP, Steen TA, Park N, Peterson C. Positive psychology progress: empirical validation of interventions. *Am Psychol*. 2005;60:410–421.
34. Casacalenda N, Perry JC, Looper K. Remission in major depressive disorder: a comparison of pharmacotherapy, psychotherapy, and control conditions. *Am J Psychiatry*. 2002;159:1354–1360.

SECTION 2

Primary and Adjunctive Diagnostic Procedures

During the 20th century, tremendous progress was made in the development of laboratory procedures for the diagnosis of disease. However, this work focused primarily on pathological processes—typically advanced disease; little was done to help the physician recognize physiological abnormalities before they progress to the pathological stage. The problem is further aggravated for doctors of preventive/integrative/functional/natural medicine, who need to evaluate in an objective manner the nutritional status, lifestyle, physiological competency, toxic load, and vitality of their patients. The few available tests that exist tend to be oriented to measuring absolute values rather than functional indices and generally indicate abnormal values only after serious dysfunction develops.

In this section, we have compiled useful assessment methodologies we believe will greatly aid healthcare professionals who want more objective tests in their evaluation of the pathophysiological status of their patients and the causes of dysfunction. These are not meant to replace the standard, pathologically oriented, diagnostic procedures. Rather, we are encouraging the use of methodologies that aid in the early diagnosis of disease susceptibility, quantification of the processes that usually precede clinical disease, and ways to objectively assess foundational causes like functional nutritional deficiencies and toxic load. Where possible, preference is given to tests that measure the uniqueness of the patient's biochemistry rather than abstract absolute values. In keeping with the metabolic and scientific orientation of this textbook, the emphasis has been placed on those procedures that have strong support in the research literature.

Most of these laboratory procedures are on the cutting edge of our understanding of the assessment of the physiological function of metabolically unique individuals. Because it is an emerging field, few experts exist, and many are employed by or associated with the commercial laboratories performing the procedures.

8

Apoptosis in Health and Diseases

Lise Alschuler, ND, and *Aristo Vojdani*, PhD, MSc, CLS*

OUTLINE

INTRODUCTION

Apoptosis is a distinct form of cell death controlled by an internally encoded suicide program. It is believed to occur in the majority of animal cells. It is a distinct event that triggers characteristic morphological and biological changes in the cellular life cycle. It is common during embryogenesis, normal tissue and organ involution, and cytotoxic immunological reactions, and it occurs naturally at the end of the life span of differentiated cells. Apoptosis can also be induced in cells by the application of a number of different agents, including physiological activators, heat shock, bacterial toxins, oncogenes, chemotherapeutic drugs, various toxic chemicals, ultraviolet and γ-radiation, and hypoxia. When apoptosis occurs, the nucleus and cytoplasm of the cell often fragment into membrane-bound apoptotic bodies, which are then phagocytized by neighboring cells. Alternatively, during necrosis, cell death occurs by direct injury to cells, resulting in cellular lysing and the release of cytoplasmic components into the surrounding environment, often inducing an inflammatory response in the tissue. Apoptosis may occur in one cell, leaving surrounding cells unaffected, as opposed to necrosis, which affects multiple cells simultaneously.

A landmark of cellular self-destruction by apoptosis is the activation of nucleases and proteases that degrade the higher-order chromatin structure of the DNA into fragments of 50 to 300 kilobases and subsequently into smaller DNA pieces of about 200 base pairs in length. Activation of proteases, notably aspartate-specific cysteinyl proteases, referred to as caspases, is of primary relevance to apoptosis. Caspase-3 is considered to be the key mediator of apoptosis of mammalian cells, with apoptotic cells characterized by significant caspase 3 activation.[1] Caspase-3 expression may be measured with immunohistochemical staining with caspase 3 antibodies at 1:50 dilution (DAKO, Carpinteria, CA).[2] Using fluorescent-labeled reagents, it is also possible to tag the DNA break and identify the percentage of apoptotic cells with a high degree of accuracy.[3–8] Unfortunately, there is no test for apoptosis that is clinically relevant. Although there are methodological limitations, essentially the interpretation of an apoptotic assay is fraught with unknowns—the significance of any result being dependent on a lack of a reference standard, itself determinant upon the type and number of cells assayed, the duration of apoptosis, and the timing and duration of the test.[9]

Measurable Features of Apoptosis for Research Purposes

One of the most easily measured features of apoptotic cells is the breakup of the genomic DNA by cellular nucleases. These DNA fragments can be extracted from apoptotic cells and result in the appearance of DNA laddering when the DNA is analyzed by agarose gel electrophoresis. The DNA of nonapoptotic cells, which remains largely intact, does not display this laddering on agarose gels during electrophoresis. The large number of DNA fragments appearing in apoptotic cells results in a multitude of 3′-hydroxyl termini of DNA ends. This property can also be used to identify apoptotic cells by labeling the DNA breaks with fluorescent-tagged deoxyuridine triphosphate nucleotides. The enzyme terminal deoxynucleotidyl transferase catalyzes a template-independent addition of deoxyribonucleotide triphosphates to the 3′-hydroxyl ends of double- or single-stranded DNA. A substantial number of these sites are available in apoptotic cells, providing the basis for the single-step fluorescent labeling and flow cytometric method. Nonapoptotic cells do not incorporate significant amounts of the fluorescent-tagged deoxyuridine triphosphate nucleotides due to the lack of exposed 3′-hydroxyl DNA ends.

Apoptosis can also be characterized by changes in cell membrane structure. During apoptosis, the cell membrane's phospholipid asymmetry changes—phosphatidylserine is exposed on the outer membrane, whereas membrane integrity is maintained. Annexin V specifically binds phosphatidylserine, whereas propidium iodide is a DNA-binding fluorochrome. When a cell population is exposed to both reagents, apoptotic cells stain positive for annexin V and negative for propidium iodide; necrotic cells stain positive for both, and live cells stain negative for both.[5]

*Previous edition contributor

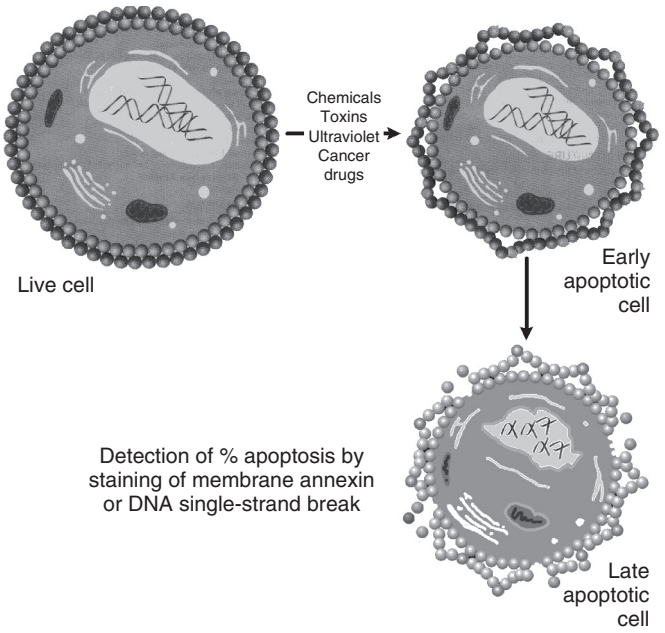


Fig. 8.1 Detection of apoptosis using damaged membrane or DNA single-strand break and flow cytometry.

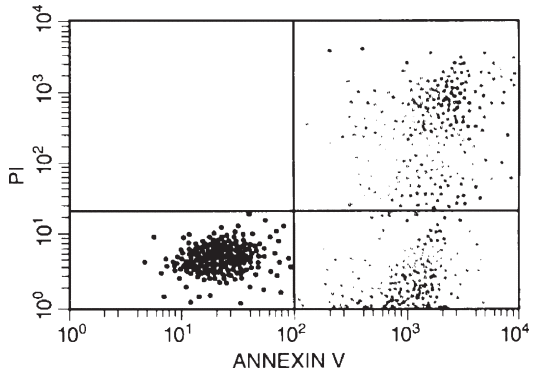


Fig. 8.2 Separation of cells by flow cytometry and detection of apoptotic population.

This process of apoptosis and its analysis by flow cytometry are shown in Figs. 8.1 and 8.2.

Another assessment of apoptosis involves ex vivo cell analysis. Specifically, the expression of active caspase-3 along with the Bcl-2:Bax ratio as markers of apoptosis can be measured. Immunohistochemical staining will reveal the expression of these apoptotic-related proteins, caspase-3 and cleaved caspase-3; the latter is indicative of apoptosis.[10] Bcl-2 is antiapoptotic gene product that exists in ratio to Bax and Bak, which are proapoptotic gene products. This ratio is indicative of the degree of apoptosis, with a decreased Bcl-2:Bax ratio indicative of apoptosis. Cells from Bax(−/−) and Bak(−/−) knockout animals do not respond to apoptosis inducers. In these cells, cytochrome C is not released from the mitochondrial membrane to initiate the caspase cascade.[11] Thus Bax and Bak are critical to apoptosis, and their expression in relation to Bcl-2 is highly correlative to apoptosis.

Different Stages of Apoptosis

The process of apoptosis is divided into three stages:

- Induction
- Sensing or triggering
- Execution

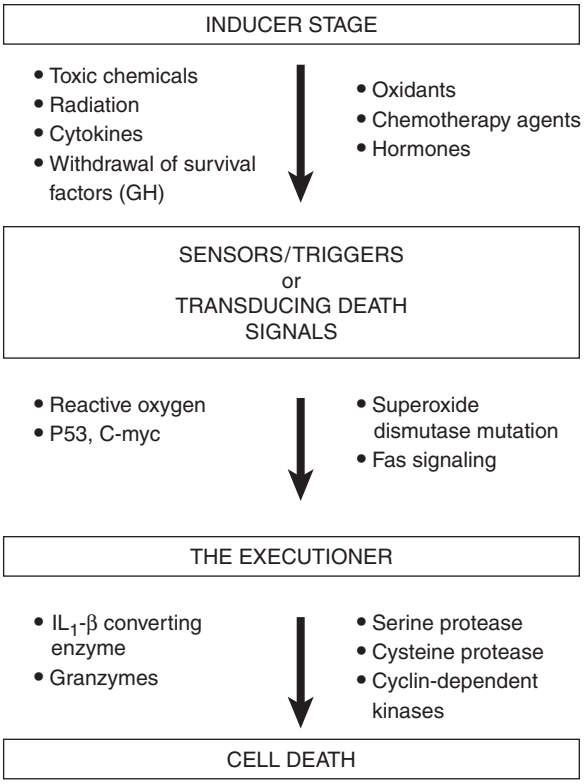


Fig. 8.3 Various stages of "inside-out" cell death or apoptosis.

These stages of apoptosis are depicted in Fig. 8.3. Induction represents the initial events that signal a cell so that apoptosis may begin. This induction phase may be induced by various physical agents, such as toxic chemicals, hypoxia, radiation, chemotherapy agents, hormones, and CD95 or Fas ligation. It has been proposed that the induction stage of apoptosis is prevented by many antioxidants (vitamin C, β-carotene, and vitamin E) and also by various biological response modifiers, including lentinan, thymic hormones, viral antigens, and cytokines.

It is important to note that both extrinsic and intrinsic pathways of apoptosis induction converge in the mitochondria and that the prevention or initiation of apoptosis from this point is the result of cellular redox status.[12] Persistent low to moderate oxidation is consistent with the prevention of apoptosis. Low to moderate oxidative stress within the mitochondrial membrane activates Bcl-2. Bcl-2 maintains the intracellular redox status at a level that is optimal for the cell. Bcl-2 does this by increasing the mitochondrial burn rate of oxygen, thereby increasing electron leakage and oxygen concentration, relative to hydrogen peroxide, in the mitochondrial membrane. This reduces the permeability of the inner mitochondrial membrane and the opening of apoptotic pores. Further, Bcl-2 physically sequesters proapoptotic proteins, Bax and Bak.[13] Bax and Bak normally facilitate the release of cytochrome C from the inner mitochondrial membrane and out into the cytosol, where it initiates the caspase cascade. However, when oxidative stress is acute or high, the inhibitory role of Bcl-2 is altered, and apoptosis can occur. Also, higher levels of reactive oxidative stress generate more hydrogen peroxide within the mitochondria, which, in turn, oxidizes other mitochondrial components, such as cardiolipin, and inner membrane phospholipid. Oxidized cardiolipin moves to the outer mitochondrial membrane and recruits Bax and Bak, thus enabling the release of cytochrome C through Bax- and Bak-controlled pores, finally activating caspases and apoptosis.[14] Also in response to higher oxidative stress, p53 translocates from the cell's nucleus to the mitochondria, where it is de-ubiquitinated and subsequently activates Bax- and Bak-induced apoptosis.[15]

There is, however, a limit to the proapoptotic response to oxidative stress. When oxidative stress is overwhelmingly high and/or prolonged, oxidative damage occurs to cytochrome C and to downstream caspases preventing apoptosis. There are other apoptotic escape mechanisms present in cells as well. Mitochondrial paraoxonase enzymes PON2 and PON3 interact with ubiquinone to reduce oxygen release. This mitigates oxidation of cardiolipin, creating a resistance to apoptosis. In fact, PON2 and PON3 have been established as key factors in the prevention of atherosclerosis because endothelial and macrophage death are the basis of atherogenic plaques.[16]

The induction of apoptosis is entirely dependent on the redox state of the cell and the mitochondrial response to oxidation. Mitochondria can respond to moderate or acute oxidative stress by initiating apoptosis; however, excessive and/or prolonged oxidative stress prevents apoptosis. Glutathione plays a major role in controlling mitochondrial oxidative stress. Reduced glutathione (GSH) donates electrons to reactive oxygen species (ROS), thereby preventing the generation of hydrogen peroxide. In the course of donating the electron, glutathione itself becomes oxidized (GSSG). Oxidized glutathione is then regenerated back to its reduced state through the action of glutathione reductase. On the one hand, the presence of GSH facilitates the destruction of hydrogen peroxide, thereby preventing the release of cytochrome C and delaying apoptosis. This allows the cell to undergo repair, instead of apoptosis, when the oxidative stress is temporary and/or low. On the other hand, by regulating the amount of oxidation, GSH preserves mitochondrial membrane integrity and cytochrome C, ensuring that the capacity for apoptosis in the face of high and/or prolonged oxidative stress is present. If there is a deficiency of reduced glutathione or damage to glutathione reductase (for instance by arsenic trioxide or lead) in the context of high oxidative stress, the cell will be unable to initiate apoptosis.[17] Instead, the cell will be forced to undergo unprogrammed cell death, or necrosis. This is an inflammatory event and will ultimately further contribute to the regional oxidative stress. Thus the induction of apoptosis is intimately connected with the cellular redox potential of cells, itself regulated primarily by glutathione.

The induction stage is followed by a decision on whether or not the cell will undergo apoptosis. The decision to die is under the control of a number of different pathways or cellular sensors that induce the apoptosis signal, which then triggers the central mechanisms. During this stage, enzymes such as interleukin-1β–converting enzymes, serine protease, cysteine protease, granzymes, and cyclin-dependent kinases become activated. Once activated, these enzymes dismantle the cell and trigger the cell-surface changes that cause direct cell recognition and engulfment of the dying cells by phagocytes. These central events are prevented by various antioxidants and biological response modifiers.

Apoptosis Is Induced by Chemicals to Control Malignancy

Many chemicals have the capacity to bind to DNA, form DNA adducts, or cause DNA single-strand breaks, possibly leading to cancer. However, the body is equipped with many factors, enzymes, suppressor genes, and cellular sensors, all with the capacity to prevent the consequences of this DNA damage by activating apoptosis-inducing signals.

The role of apoptosis in regulating tissue growth is readily apparent in the simple equation in which the rate of growth is equal to the difference between the rates of cell proliferation and cell death. Thus tissues expand if the rate of proliferation exceeds the rate of cell death. This is one of the reasons for suggesting that defects in apoptosis may contribute to the transformed state.

An important prediction of the relevance of apoptosis to malignancy is that the rate of apoptosis versus mitosis should influence the behavior of a tumor. Recently, the relationship between the apoptotic and mitotic indexes in a tumor was demonstrated as predictive of outcome: a higher ratio of apoptosis to mitosis within the tumor correlated with a positive prognosis. Further, it was found that this was not simply a function of cell death per se. Tumors with a high incidence of necrosis rather than apoptosis were correlated with a poor prognosis. It therefore follows that treatments or conditions that favor apoptosis should have desirable effects and that defects in the pathways leading to apoptosis are likely to play important roles in the process of oncogenesis.[6,7]

Many reactive chemicals and drugs, such as acetaminophen, diquat, carbon tetrachloride, quinones, cyanide, polyhydroxy polyether, methyl mercury, and organotin, have been implicated in apoptosis (programmed cell death) and necrosis (toxic cell death).[18–25]

Most research on chemical induction of apoptosis is carried out with primary cultures of cell lines (e.g., neurons, thymocytes, carcinoma cells, leukemia cells, neuroblastoma, breast cancer cells, lymphoma); little has been published on the in vivo effects of chemicals on apoptotic cells in animal models and none in humans. Therefore it was of interest to examine the effects of exposure to low levels of benzene, as well as through drinking water concentrations of up to 14 ppb, on the apoptotic cell population, as well as to examine possible changes in the cell cycle progression.[18]

Evidence is sufficient for the carcinogenicity of benzene in humans; therefore there is no safe level of exposure to this chemical or its metabolites. Published case reports, a case series, epidemiological studies, and both cohort and case-control studies have shown statistically significant associations between leukemia and occupational exposure to benzene and benzene-containing solvents.[26,27]

It has been indicated that possibly 800,000 persons are exposed to benzene from coke-oven emissions at levels of less than 0.1 ppm, and 5 million may be exposed to benzene from petroleum refinery emissions at levels of 0.1 to 1 ppm. Since these studies, numerous chemicals have been implicated in apoptosis (or programmed cell death), which arises from damage to DNA. One of the authors, Vojdani, along with collaborators, hypothesized that in individuals with a certain genetic makeup, benzene or its metabolites act as haptens, which may induce programmed cell death. The study involved a group of 60 male and female subjects who were exposed to benzene-contaminated water (at concentrations up to 14 ppm for a period of 3–5 years).[18] For comparison, a control group consisting of 30 healthy males and females with a similar age distribution and without a history of exposure to benzene were recruited. Using flow cytometry, the peripheral blood lymphocytes of both groups were tested for the percentage of apoptotic cell population. When exposed individuals were compared with the control group, statistically significant differences between each mean group were detected (27.5 ± 2.4 and 10 ± 2.6, respectively), indicating an increased rate of apoptosis in 86.6% of exposed individuals ($P < 0.0001$; Mann–Whitney U-test). Flow cytometry analysis of apoptosis in a healthy control and a patient with chronic fatigue syndrome is shown in Fig. 8.4.

It has been demonstrated that benzene induction of apoptosis is caused by a discrete block of the cell-cycle progression. There is a tendency for normal cells to commit "suicide" when deprived of usual growth factors or physical contact with their neighbors due to chemical exposure, which may represent a built-in defense against metastasis. Prompt activation of apoptosis in tumor cells that leave their native tissue presumably eliminates many metastatic cells before they have a chance to proliferate. In cancer, it is tumor cells that neglect to sacrifice themselves or forget to die. Researchers increasingly describe cancer as

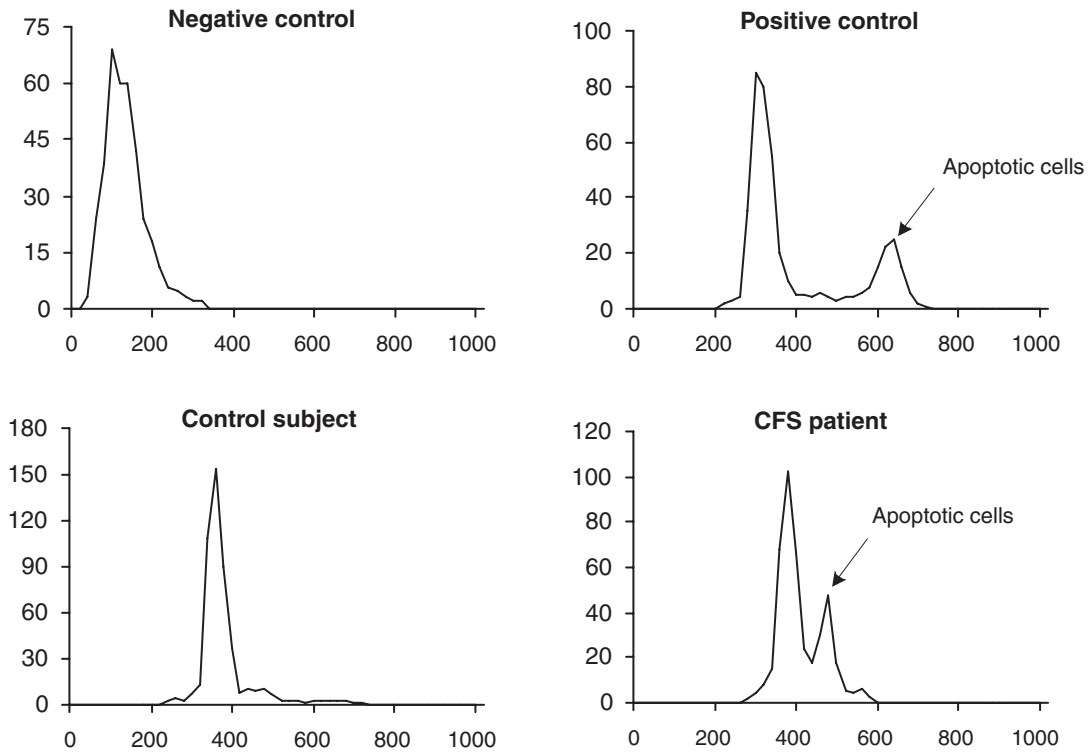


Fig. 8.4 Enhanced apoptotic cell population in benzene-exposed individuals with chronic fatigue syndrome. Flow cytometry analysis of apoptotic cell population in negative control cells (HL-60 leukemic cell line), positive control cells (HL-60 leukemic cells treated with the Apogen camptothecin), control subjects, and benzene-exposed individuals. Peripheral blood leukocytes were isolated, cultured for 12 hours, fixed in paraformaldehyde, labeled with fluorescent-tagged deoxyuridine triphosphate nucleotides, and analyzed for apoptosis by flow cytometry.

a disease involving both excessive proliferation of cells and abandonment of their ability to die. The dysregulation of apoptosis in malignant cells underlies both the initiation and progression of cancer.

Cancer develops after a cell accumulates mutations in several genes that control cell growth and survival. When a mutation seems irreparable, the affected cell usually kills itself rather than risk becoming deranged and potentially dangerous. However, if the cell does not die, it or its progeny may live long enough to accumulate mutations that enable it to divide uncontrollably and metastasize.

In many tumors, genetic damage apparently fails to induce apoptosis because the constituent cells have inactivated the gene that codes for the p53 protein. This protein can lead to activation of the cell's apoptotic machinery when DNA is injured by environmental agents, such as benzene or its metabolites. Therefore it is important to study cell suicide in health and diseases.

CLINICAL APPLICATIONS

Apoptosis in Cancer

The failure of apoptosis in malignant cells in the context of irreparable DNA damage leads to tumor progression. Cancer therapies, namely chemotherapy and radiation, control cancer by inflicting cell damage, which, in turn, triggers apoptosis. Unfortunately, >50% of all human cancers involve a mutation of p53, a central gene in apoptosis. p53 stimulates both the extrinsic death receptor pathway of apoptosis as well as the intrinsic mitochondrial pathway involving a decreased Bcl-2:Bax ratio. Thus it is imperative to find therapies that promote apoptosis independent of p53. Promising therapies in this regard include curcumin[28] derived from *Curcuma longa,* genistein derived from soy,[29] and resveratrol,[30] all of which are under investigative study for this application. Another promising cancer treatment involves the use of recombinant human apoptosis ligands to induce tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). These ligands induce apoptosis via TRAIL, a selective death receptor pathway in a broad range of cancer cell lines, while sparing most normal cell types.[31]

The therapeutic potential for TRAIL-inducing ligands is most promising in combination with cytotoxic chemotherapy agents. Advances in cancer therapy are likely to come in the area of targeted therapies, the majority of which trigger specific receptor-driven pathways that culminate in apoptosis. The centrality of apoptosis induction in cancer cannot be overstated.

Apoptosis in Autoimmune Diseases

In cancer, it is the tumor cells that forget to die; in autoimmunity, immune cells fail to die when they are supposed to. Virtually all tissues harbor apoptotic cells at one time or another. Damaged cells usually commit suicide for the greater good of the body; when this does not occur, disease may develop. Autoimmunity occurs when the antigen receptors on immune cells recognize specific antigens on healthy cells and cause the cells bearing those particular substances to die. Autoimmune disease results from perpetuated immune-mediated tissue destruction and can involve immune cells that are resistant to apoptosis. Under normal conditions, the body allows a certain number of self-reactive lymphocytes to circulate. These cells normally do little harm, but they can become overactive through several processes. For instance, if these reactive lymphocytes recognize some foreign antigen such as microbes on food and haptenic chemicals, then exposure to that antigen causes them to become excited. If, due to molecular

mimicry, these antigens are similar to normal tissues, the activated cells may expand their numbers and attack the healthy tissue, thus causing an autoimmune disease.[3,32,33]

Autoimmune reactions usually are self-limited—they disappear when the antigens that originally set them off are cleared away. In some instances, however, the autoreactive lymphocytes survive longer than they should and continue to induce apoptosis in normal cells. Some evidence in animals and humans has indicated that extended survival of autoreactive cells is implicated in at least two chronic autoimmune syndromes—systemic lupus erythematosus and rheumatoid arthritis. In other words, the lymphocytes undergo too little apoptosis, with the result that normal cells undergo too much.[34,35]

Apoptosis During Viral Infection

Disturbance in the regulation of apoptosis is a component in various diseases. Viral illnesses are among the diseases caused by apoptosis dysregulation. After entering a cell, viruses attempt to shut down the cell's ability to make any proteins except those needed to produce more virus. This act of stalling host protein synthesis is enough to induce many kinds of cells to undergo apoptosis. If the host cell dies, the virus is also eliminated. Therefore certain viruses have evolved ways to inhibit apoptosis in the cells they infect.

Epstein–Barr virus, which causes mononucleosis and has been linked to lymphomas in humans, uses a mechanism that has been seen in other viruses. Epstein–Barr virus produces substances that inhibit apoptosis. Papillomavirus, a major cause of cervical cancer, inactivates p53, a central mediator of apoptosis. Cowpox virus, a relative of which is used as the smallpox vaccine, is another virus that inhibits caspase activation and attendant apoptosis. Investigators interested in antiviral therapy are now exploring ways to block the activity of the antiapoptotic molecules manufactured by viruses.[34]

Apoptosis in Acquired Immunodeficiency Syndrome

Induction of apoptosis by viruses in healthy cells is believed to contribute to the immune deficiency found in patients with acquired immunodeficiency syndrome (AIDS). In these patients, infection with human immunodeficiency virus (HIV) causes T-helper cells to die. As T-helper cells gradually disappear, cytotoxic cells, such as natural killer cells, also perish through apoptosis because they cannot survive without the growth signals produced by T-helper cells. When the number of T cells dwindles, so does the body's ability to fight infections, especially viral and parasitical infections. Researchers have shown that many more helper cells succumb in addition to those that are infected with HIV. It is also highly probable that a large number of the cells die through apoptosis. Apparently, Fas plays a crucial role in this process.

Normally, T cells make functional Fas only after they have been active for a few days and are ready to die. However, helper cells from AIDS patients may display high amounts of functional Fas even before the cells have encountered an antigen. This display of Fas would be expected to cause the cells to undergo apoptosis prematurely whenever they encounter Fas ligand on other cells (such as on T cells already activated against HIV or other microbes). In addition, if the primed cells encounter the antigen recognized by their receptors, they may trigger their own death.

It is also possible that oxygen free radicals trigger the suicide of virus-free T cells. These highly reactive substances are produced by inflammatory cells drawn to infected lymph nodes in patients with HIV. Free radicals can damage DNA and membranes in cells. They will cause necrosis if they do extensive damage, but they can induce apoptosis if the damage is more subtle. In support of the free-radical theory, researchers have found that molecules capable of neutralizing free radicals prevent apoptosis in T cells obtained from patients with AIDS.[34,35]

Therapies with antiapoptotic medication, such as Trolox, a water-soluble analog of vitamin E that prevents oxidative stress, and pyrrolidine dithiocarbamate, a potent inhibitor of nuclear factor-κB, are now the focus of AIDS and autoimmune disease studies.[36,37]

Additionally, protease inhibitors, which are the mainstay of HIV therapy, inhibit apoptosis in immune cells.[38]

The mechanism underlying the apoptosis inhibition is as of yet unknown, but interestingly, supratherapeutic doses of protease inhibitors have an opposite, proapoptotic effect.

Apoptosis in the Heart and Brain

In contrast to cancer, where cells forget to die and insufficient apoptosis occurs, excessive apoptosis accounts for much of the cell death that follows heart attacks and strokes. In the heart, vessel blockage decimates cells that were fully dependent on the vessel. Those cells die by necrosis, partly because they are catastrophically starved of the oxygen and glucose they need to maintain themselves and partly because calcium ions, which are normally pumped out of the cell, rise to toxic levels.

Over the course of a few days, cells surrounding the dead zone, which initially survive because they continue to receive nourishment from other blood vessels, can die as well. Later, however, many cells die by necrosis after being overwhelmed by the destructive free radicals that are released when inflammatory cells swarm into the dead zone to remove necrotic tissue. The less injured cells commit suicide by apoptosis.

If the patient is treated by restoring blood flow, still more cells may die by necrosis or apoptosis because reperfusion leads to a transient increase in the production of free radicals. Similarly, in strokes due to inflammation, the release of such neurotransmitters as glutamate leads to necrosis and apoptosis. Understanding of the factors that lead to the tissue death accompanying heart attack, stroke, and reperfusion has led to new ideas for treatment. Notably, cell death might be limited by drugs and other agents that block free-radical production or inhibit proteases.

Apoptosis also accounts for much of the pathology seen in such diseases as Alzheimer's, Parkinson's, Huntington's, and amyotrophic lateral sclerosis (Lou Gehrig's disease), which are marked by the loss of brain neurons. Elevated apoptosis in these neurological diseases seems to be related to a lack of production of the nerve growth factor and to free-radical damage. It seems likely that a combination of such factors could cause many cells to destroy themselves. Manipulation of this process of cell killing may help in treating these neurological diseases. Studies in animal models imply that long-term delivery of nerve growth factors could protect against programmed cell death in these conditions. Therefore a greater understanding of the mechanisms involved in cell death should greatly enhance those important steps.[32,36,39]

CONCLUSIONS

Apoptosis and cell proliferation play an important role in development, differentiation, homeostasis, and aging.[4–8] The balance established between these two processes depends on various growth and death signals that are influenced by diet, nutrition, lifestyle, and other environmental factors. When the equilibrium between life and death is disrupted by aberrant signals (e.g., low levels of antioxidants in the blood or tissue cells), either tissue growth or atrophy occurs.

Under normal conditions with optimal nutritional factors, tissue homeostasis is sustained by balancing the effects of mitosis and apoptosis. The importance of this balance can clearly be seen when one of these processes becomes predominant (Fig. 8.5). The apoptotic potential within each cell is critical for the health of the host. Apoptosis is

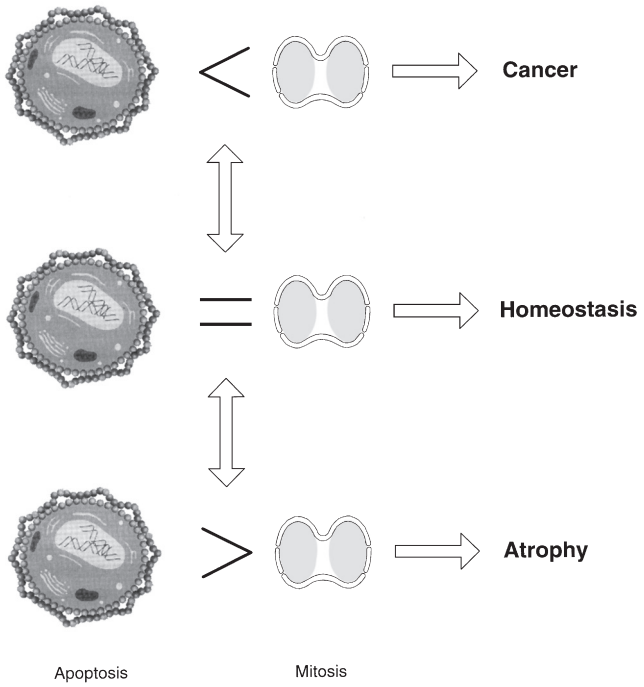


Fig. 8.5 The balance or imbalance between the rate of apoptosis and mitosis determines tissue homeostasis, atrophy, cell proliferation, and the development of cancer.

an elegant response to oxidative stress. This seemingly heroic sacrifice of self for the greater good underpins healthy living. Imbalance of apoptosis regulators, genetic mutations, and viral infections thwarts the healing effect of apoptosis. Finding ways to restore apoptotic and redox balance is critical to health.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Yang Min, Antoine Daniel J, Weemhoff James L, et al. Biomarkers distinguish apoptotic and necrotic cell death during hepatic ischemia-reperfusion injury in mice. *Liver Transpl*. 2014;20(11):1372–1382.
2. Mohamed AK, Magdy M. Caspase 3 role and immunohistochemical expression in assessment of apoptosis as a feature of H1N1 vaccine-caused drug-induced liver injury (DILI). *Electron Physician*. 2017;9(5):4261–4273.
3. Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol*. 1980;68:251–306.
4. White E. Life, death and the pursuit of apoptosis. *Genes Dev*. 1996;10:1–15.
5. Jarvis WD, Kolesnick RN, Fornari FA, et al. Induction of apoptotic DNA damage and cell death by activation of the sphingomyelin pathway. *Proc Natl Acad Sci U S A*. 1994;91:73–77.
6. Green DR, Martin SJ. The killer and the executioner: how apoptosis controls malignancy. *Curr Opin Immunol*. 1995;7:694–703.
7. Arends MJ, McGregor AH, Wyllie AH. Apoptosis is inversely related to necrosis and determines net growth in tumors bearing constitutively expressed myc, ras and HPV oncogenes. *J Pathol*. 1994;144:1045–1057.
8. Marchetti P, Hirsch T, Zamzami M, et al. Mitochondrial permeability triggers lymphocyte apoptosis. *J Immunol*. 1996;157:4830–4836.
9. Renehan AG, Booth C, Potten CS. What is apoptosis, and why is it important? *BMJ*. 2001;322(7301):1536–1538.
10. Amatya JL, Takeshima Y, Shrestha L, et al. Evaluation of apoptosis and immunohistochemical expression of the apoptosis-related proteins in mesothelioma. *Hiroshima J Med Sci*. 2010;59(2):27–33.
11. Kandasamy K, Srinivasula SM, Alnemri ES, et al. Involvement of proapoptotic molecules Bax and Bak in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced mitochondrial disruption and apoptosis: differential regulation of cytochrome C and Smac/DIABLO release. *Cancer Res*. 2003;63(7):1712–1721.
12. Watson WH, Cai J, Jones DP. Diet and apoptosis. *Annu Rev Nutr*. 2000;20:485–505.
13. Krishna S, Low I, Pervaiz S. Regulation of mitochondrial metabolism: yet another facet in the biology of the oncoprotein Bcl-2. *Biochem J*. 2011;435:545–551.
14. Li XX, Tsoi B, Kurihara H, He RR. Cardiolipin and its different properties in mitophagy and apoptosis. *J Histochem Cytochem*. 2015;63(5):301–311.
15. Szczepanek K, Lesnefsky EJ, Larner AC. Multi-tasking: nuclear transcription factors with novel roles in the mitochondria. *Trends Cell Biol*. 2012;22(8):429–437.
16. Witte I, Foerstermann U, Devarajan A, Reddy ST, Horke S. Protectors or traitors: the roles of PON2 and PON3 in atherosclerosis and cancer. *J Lipids*. 2012;2012:342806.
17. Ray A, Chatterjee S, Mukherjee S, Bhattacharya S. Arsenic trioxide induced indirect and direct inhibition of glutathione reductase leads to apoptosis in rat hepatocytes. *Biometals*. 2014;27(3):483–494.
18. Vojdani A, Mordechai E, Brautbar N. Abnormal apoptosis and cell cycle progression in humans exposed to methyl tertiary-butyl ether and benzene contaminating water. *Human Exp Toxicol*. 1997;16:485–494.
19. Walker PR, Smith C, Youdale T, et al. Topoisomerase II-reactive chemotherapeutic drugs induce apoptosis in thymocytes. *Cancer Res*. 1991;51:1078–1085.
20. Brown DB, Sun XM, Cohen GM. Dexamethasone-induced apoptosis involves cleavage of DNA to large fragments prior to internucleosomal fragmentation. *J Biol Chem*. 1993;268:3037–3039.
21. Reynolds ES, Kanz MF, Chicco P, Moslen MT. 1.1-Dichloroethylene: an apoptotic hepatotoxin? *Environ Health Perspect*. 1984;57:313–320.
22. Aw TY, Nicotera P, Manzo L, Orrenius S. Tributyltin stimulates apoptosis in rat thymocytes. *Arch Biochem Biophys*. 1990;283:46–50.
23. Rossi AD, Larsson O, Manzo L, et al. Modification of Ca^{2+} signaling by inorganic mercury in PC12 cells. *FASEB*. 1993;7:1507–1514.
24. Kunimoto M. Methyl mercury induces apoptosis of rat cerebellar neurons in primary culture. *Biochem Biophys Res Commun*. 1994;204:310–317.
25. Vivian B, Rossi AD, Chow SC, Nicotera P. Organotin compounds induce calcium overload and apoptosis in PC12 cells. *Neurotoxicology*. 1995;16:19–25.
26. Ledda-Columbano GM, Coni P, Curto M, et al. Induction of two different modes of cell death, apoptosis and necrosis in rat liver after a single dose of thioacetamide. *Am J Pathol*. 1991;139:1099–1109.
27. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Benzene, Draft Report*. Atlanta, GA: Department of Health and Human Services.
28. Saha A, Kuzuhara T, Echigo N, Fujii A, et al. Apoptosis of human lung cancer cells by curcumin mediated through up-regulation of "growth arrest and DNA damage inducible genes 45 and 153." *Biol Pharm Bull*. 2010;33(8):1291–1299.
29. Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. *Oncogene*. 1999;18:3166–3172.
30. Fulda S, Debatin KM. Sensitization for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by the chemopreventive agent resveratrol. *Cancer Res*. 2004;64(1):337–346.
31. Ashkenazi A, Holland P, Eckhardt SG. Ligand-based targeting of apoptosis in cancer: the potential of recombinant human apoptosis ligand 2/tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). *J Clin Oncol*. 2008;26(21):3621–3630.
32. National Institute of Environmental Health Sciences. *Sixth Annual Report on Carcinogens. Benzene Case No. 71-43-2:35*. Research Triangle Park, NC: National Institute of Environmental Health Sciences
33. Golstein P, Ojcius DM, Ding-E Young J. Cell death mechanisms and the immune system. *Immunol Rev*. 1991;121:29–65.
34. Cohen JJ, Duke RC, Fadok VA, Sellins KS. Apoptosis and programmed cell death in immunity. *Ann Rev Immunol*. 1992;10:267–293.
35. Duke RC, Ojcius DM, Ding-E Young J. Cell suicide in health and disease. *Sci Am*. 1996;275:80–87.
36. Martin SJ, Green DR. Protease activation during apoptosis: death by a thousand cuts. *Cell*. 1995;82:349–352.
37. Forrest VJ, Kang Y, McClain DE, et al. Oxidative stress-induced apoptosis prevented by Trolox. *Free Radic Biol Med*. 1994;16:675–684.
38. Rizza SA, Badley AD. HIV protease inhibitors impact on apoptosis. *Med Chem*. 2008;4(1):75–79.
39. Schreck R, Meier B, Mannel DN, et al. Dithiocarbamates as potent inhibitors of nuclear factor kB activation in intact cells. *J Exp Med*. 1992;175:1181–1194.

9

Bacterial Overgrowth of the Small Intestine Breath Test

Mary James, ND

OUTLINE

INTRODUCTION

Small intestinal bacterial overgrowth (SIBO) is an abnormal colonization within the small bowel by bacteria normally found in the colon, mouth, or pharynx.[1] SIBO is a major contributor to irritable bowel syndrome (IBS) and uncomfortable symptoms such as bloating, abdominal discomfort, and changes in the stool (e.g., diarrhea).[2] It is also a potentially serious disorder that can lead to problems such as malabsorption and weight loss, anemia, malnutrition,[2] increased intestinal permeability,[3] and bone loss.[4] Breath testing for hydrogen (H_2) and methane (CH_4) provides a simple, noninvasive means of detecting SIBO. Once SIBO has been identified, antimicrobials are typically administered to eradicate the bacteria. Concurrent attention to underlying causes is also essential in preventing recurrences.

Conditions Associated With Small Intestinal Bacterial Overgrowth

SIBO, whose overall prevalence is not yet clear, is an overlooked contributing factor in several common disorders.[5] Several studies, for example, have demonstrated the presence of SIBO in patients with IBS.[6,7] In one study, in which 84% of patients with IBS tested positive for SIBO (vs. 20% of healthy controls), successful eradication of SIBO led to a 75% improvement (compared with a 36.7% improvement in those with incomplete eradication and an 11% improvement in participants receiving a placebo).[8] SIBO has also been observed in patients with acne rosacea,[9] Crohn's disease,[10] restless legs syndrome,[11] nonalcoholic fatty liver disease,[12] interstitial cystitis,[13] chronic prostatitis,[14] chronic fatigue syndrome,[15] and fibromyalgia.[16] SIBO may increase intestinal permeability (a.k.a. "leaky gut"),[17] an abnormality that was shown in a small study to resolve in 75% of patients successfully treated for SIBO.[3] Investigators who found leaky gut in 37.5% of patients with fibromyalgia suggested exposure of immune cells to luminal antigens and consequent immune modulation as a likely mechanism for the pain syndrome.[18]

A variety of anatomical and motor disorders of the small bowel can lead to SIBO, including surgical blind loops, diverticula, strictures, adhesions, tumors, fistulas,[19] sclerodermas,[20] intestinal pseudoobstruction,[21] and diabetic enteropathy.[22] Jejunal diverticulosis[5] and Crohn's disease[23] have both been associated with SIBO, particularly in patients with previous intestinal surgery. Because the symptoms of Crohn's disease and SIBO can be similar, symptoms from SIBO can be mistaken for a Crohn's-related acute flare.[10] Although the concentration of bacteria normally increases exponentially toward the distal end of the small intestine,[19] far fewer bacteria inhabit the small intestine than do the colon.[24] A common feature of most of these disorders is stasis of small bowel contents, which allows bacterial concentrations to increasingly resemble those of the large intestine (Box 9.1).[24,25] Although many of the bacteria found in SIBO are beneficial within the colon, these same microorganisms can have deleterious effects within the delicate environment of the small intestine.

Interestingly, many patients with celiac disease whose symptoms persist despite a gluten-free diet have been shown to have SIBO, with improvement only after bacterial eradication.[26] The incidence of SIBO also increases with age.[27] It has been found that 64% of individuals more than 75 years of age with chronic diarrhea have colonic-type flora in their small bowels,[5] and that SIBO is the most common cause of clinically significant malabsorption in elderly persons.[19]

Pathophysiology

Two major factors that control the numbers and types of bacteria within the small bowel are intestinal motility and gastric acid secretion.[19,24] Accordingly, SIBO has been associated with both intestinal stasis and hypochlorhydria.[25] Other factors influencing SIBO include pancreatic enzyme secretion,[28] disaccharidase production by microvilli,[29] ileocecal valve function,[30,84] bile salts, luminal pH, oxidation-reduction potential,[24] and migrating motor complex function.[31]

The migrating motor complex (MMC) is a system of electrical waves that "migrate" throughout the small intestine, serving to propel

BOX 9.1 Causes of Small Intestinal Bacterial Overgrowth

- Achlorhydria, hypochlorhydria, drug-induced hypoacidity
- Chronic constipation
- Stasis resulting from structural changes (e.g., diverticulosis, blind loops, radiation damage, stricture, fistulas, intestinal pseudo-obstruction, adhesions resulting from prior surgery)
- Dysfunctional migrating motor complex
- Chronic pancreatic insufficiency
- Disaccharidase deficiencies (e.g., lactase)
- Dysfunctional ileocecal valve
- Immunodeficiency (especially of secretory immunoglobulin A)
- Diabetes mellitus
- Scleroderma
- Crohn's disease

luminal contents all the way from the stomach to the terminal ileum over a period of 113 to 230 minutes, depending on the individual.[32] The MMC has been referred to as the "intestinal housekeeper." Its influence on motility is independent of the peristalsis that occurs in the large intestine. For instance, whereas colonic peristalsis is stimulated by eating a meal, the MMC is only active in the fasting state.

The MMC consists of four phases. Phase I, which takes place in the stomach, is devoid of contractions. Phase II is composed of low-amplitude, irregular contractions that progress from the stomach to the small intestine. Phase III, initiating in either the stomach or the small intestine, is the most active phase of the MMC; contractions are of high amplitude and travel the length of the small bowel, serving to cleanse it of food from a recent meal. Phase IV represents a brief transition period back to phase I.[31,32] MMC dysfunction has been demonstrated in many individuals with SIBO, especially in Phase III.[33]

For SIBO to produce clinical consequences, an adequate concentration of organisms with particular metabolic properties within specific locations of the small intestine is required. For example, a heavy concentration of strict anaerobes and coliforms in the proximal small intestine is more likely to be associated with malabsorption than a flora consisting of fewer strict anaerobes or coliforms or when strict anaerobes or coliforms are confined to the distal small intestine.[24] For this reason, SIBO may be asymptomatic in some individuals yet produce signs and symptoms in others. Box 9.2 outlines clinical signs and symptoms that should alert the practitioner to consider testing for SIBO.

Signs and Symptoms

The classic SIBO syndrome is characterized by megaloblastic anemia resulting from vitamin B_{12} deficiency and weight loss and diarrhea secondary to fat malabsorption.[24] However, many patients present with nonspecific symptoms 1 to 2 hours after a meal, including bloating, flatulence, and abdominal pain resulting from bacterial fermentation of intraluminal sugars and associated gas production, and constipation-predominant SIBO is also possible (Box 9.3).[15,26]

Via secretory and osmotic processes, diarrhea may occur even in the absence of significant steatorrhea. Unabsorbed fats and bile salts are modified by bacteria in the colon to hydroxylated fats and free bile acids, respectively, which stimulate colonic secretion of water and electrolytes.[24]

Bile salts, essential to fat emulsification and assimilation, must be conjugated with taurine or glycine to function properly. In SIBO, bacteria in the proximal small intestine can deconjugate bile salts to form free bile acids.[24] This can have two major clinical repercussions:

BOX 9.2 When to Consider Breath Testing for Small Intestinal Bacterial Overgrowth

- Gas, bloating, or diarrhea, usually after eating
- Irritable bowel syndrome, either diarrhea or constipation-dependent
- Unexplained weight loss
- Evidence of malabsorption
- Chronic hypochlorhydria or achlorhydria
- Use of acid-blocking medications (especially proton-pump inhibitors)
- Prior intestinal surgery, chronic constipation, or other causes of intestinal stasis
- Intolerance of disaccharides (e.g., lactose)
- Unexplained vitamin B_{12} deficiency, weight loss, or bone loss
- Unexplained nutrient insufficiencies (e.g., calcium, magnesium, fat-soluble vitamins)
- Unexplained "leaky gut"
- Crohn's disease (especially if history of strictures or small bowel resection)
- Restless legs syndrome
- Nonalcoholic fatty liver disease
- Interstitial cystitis

BOX 9.3 Signs and Symptoms of Bacterial Overgrowth

- Gas, bloating, and flatulence
- Diarrhea or constipation
- Abdominal cramping
- Steatorrhea
- Lactose intolerance
- Megaloblastic anemia

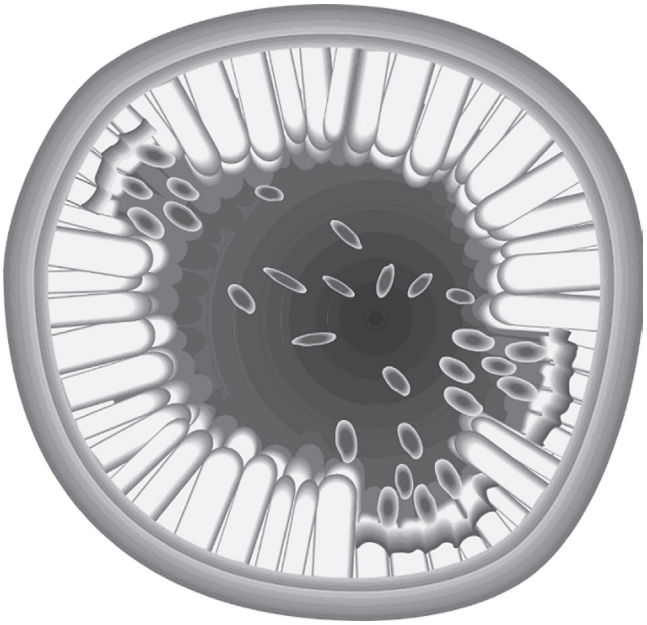


Fig. 9.1 In small intestinal bacterial overgrowth, free bile acids can damage the brush border, resulting in reduced enzyme activity and maldigestion. (Courtesy of Genova Diagnostics, Asheville, NC.)

(1) free bile acids can promote mucosal damage (Fig. 9.1), resulting in reduced brush-border enzyme activities (especially lactase),[34] defects in mucosal uptake of sugars and amino acids, enteric blood loss, and protein-losing enteropathy; or (2) the conjugated bile salt

concentration may fall below the concentration necessary for effective micelle formation, resulting in fat malabsorption, steatorrhea,[24,25] and deficiencies of fat-soluble vitamins.[2]

Fat malabsorption in SIBO can also result from mechanical interference, specifically the formation of a pseudomembrane, thought to represent a maladaptive defense mechanism against the bacterial overgrowth.[35]

Unabsorbed fatty acids can form insoluble soaps with calcium and magnesium, rendering them unavailable.[2] Osteomalacia, night blindness, hypocalcemic tetany,[19] or metabolic bone disease[4] may develop as a consequence of lipid malabsorption in patients with SIBO. Although rare, iron-deficiency anemia may result from blood loss[2] or possibly from an inflammation-induced upregulation of hepcidin, the body's main iron-regulating hormone.[36] SIBO may also lead to vitamin B_{12} deficiency, with megaloblastic anemia and low serum cobalamin levels.[19,24] Although intrinsic factor is not altered by anaerobic bacteria, microbes are capable of detaching vitamin B_{12} from the intrinsic factor, as well as directly using B_{12}.[30] Either mechanism can make the vitamin unavailable. Paradoxically, serum folate values are usually normal or even elevated in SIBO, a result of the bacterial synthesis of the vitamin.[37]

Hypoproteinemia may also occur in SIBO, secondary to protein-losing enteropathy and protein malabsorption.[2,25] In addition, bacteria may metabolize proteins to ammonia and fatty acids, thereby rendering them unavailable to the host.[34]

The composition of bacterial populations contaminating the small bowel is complex and variable.[19] However, the diagnosis of SIBO tends to be oriented less to the identification of specific microorganisms and more to overall bacterial concentrations.[19]

DIAGNOSIS

Endoscopy

Culture of a small bowel aspirate (typically jejunal or duodenal) via endoscopy is a direct method for diagnosing SIBO; abnormally high bacterial counts confirm the diagnosis.[25] Although this technique has been considered the gold standard for diagnosing SIBO, intubation methods are invasive, time-consuming, uncomfortable, and expensive. It also has several shortcomings: (1) because the aspirate is typically taken from only one location, SIBO in the more distal end of the small bowel or concentrated in a large diverticulum or blind loop may be missed[38]; (2) false positives can result from bacterial contamination from the mouth or esophagus[39]; and (3) the traditional threshold of $>10^5$ cfu/mL is not well validated and may only be appropriate for patients with blind loop syndrome as a result of past surgeries (e.g., Billroth II procedure). It is now generally agreed that a lower cutoff of 10^3 cfu/mL is sufficient for a diagnosis of SIBO.[40,41]

Breath Testing

Breath tests were devised as less invasive alternatives to intubation and culture, offering greater patient comfort and convenience. They also offer good sensitivity[42]: a meta-analysis of 12 studies found that lactulose and glucose hydrogen breath testing identified SIBO in 54% and 31% of patients with IBS, respectively, compared with only 4% of the patients via jejunal aspirate and culture.[43]

Breath tests are based on the ability of intestinal microbes to ferment carbohydrates, producing H_2 or CH_4 in the process. A fraction of these gases naturally diffuses from the bowel to the circulation and is excreted with expired air. Because there is no other metabolic source of H_2 and CH_4, pulmonary excretion of these gases is used as a measure of bacterial fermentation during the passage through the bowel.[44]

Breath tests for SIBO commonly employ either lactulose or glucose, a prescribed dose of which is ingested following 1 to 2 days of dietary fiber restriction and a 12-hour fast.[45] In all cases, intestinal bacteria modify the challenge substance, producing an early peak in breath gas values in patients with SIBO. Lactulose is a synthetic, nonabsorbed disaccharide that offers the advantage of traveling the full length of the small intestine. An early H_2 (and/or CH_4) peak is typically followed by a prolonged gas peak representing colonic bacterial activity (approximately 90 minutes into the collection process).[42,46] Glucose, an absorbable monosaccharide, is not suitable for patients with blood sugar disorders such as diabetes, and its rapid absorption reduces the test's sensitivity in the distal ileum.[42] However, its superior diagnostic accuracy in some studies has led to a growing consensus in favor of glucose over lactulose.[47] Differences in methodology between studies may have contributed to the wide range of sensitivities and specificities for lactulose versus glucose (e.g., 31% to 68% and 44% to 100%, respectively, for lactulose; vs. 20% to 93% and 30% to 86%, respectively, for glucose).[41]

During a breath test, breath specimens are collected by exhaling into a special mouthpiece connected to a vacuum-sealed collection tube. A fasting (prechallenge) breath specimen is collected, a specified amount of lactulose or glucose is ingested, and then nine more breath specimens are typically collected at timed intervals every 15 to 20 minutes. Breath levels of H_2 and CH_4 are plotted over time, with earlier rises in breath gas values corresponding to more proximal portions of the small intestine. CO_2 is also measured because insufficient amounts of CO_2 will invalidate results for H_2 and CH_4.

Hydrogen Versus Methane Versus Hydrogen Sulfide

Many studies using carbohydrate challenges have measured only breath H_2. However, 30% to 50% of H_2 producers also produce CH_4,[48] most likely a result of "methanogenic" bacteria, which consume H_2, producing CH_4 in the process.[45] Individuals whose intestines harbor methanogenic bacteria typically produce greater amounts of breath CH_4 during the test, thus being potentially missed on a test measuring only H_2.[49] Because of the lack of consistency and standardization across studies, clinics, and practitioners, a North American group of clinician scientists met for discussion in May 2015.[40] Consensus was reached that CH_4 should be measured along with H_2, especially in cases of constipation or slow transit time.

Some individuals with SIBO also appear to produce hydrogen sulfide (H_2S), a result of intestinal sulfate-reducing bacteria utilizing H_2 equivalents such as acetate and formate. H_2S is not apparent on the standard breath test measuring H_2 and CH_4, and in such a case, the breath gases values may form a flat line. Currently available gas chromatography equipment cannot detect H_2S. However, a preliminary study examining breath H_2S offers promise in diagnosing H_2-negative individuals with SIBO.[50]

Clinical correlations have been noted between various disorders and the production of H_2 versus CH_4. In one study, individuals producing higher amounts of H_2 relative to CH_4 reported significantly increased bloating and cramping after carbohydrate ingestion, whereas individuals producing high CH_4 reported no significant increase in these symptoms.[51] Specific IBS symptoms also vary with breath gas values. For example, CH_4 production has been associated more with constipation-predominant IBS, whereas H_2 production tends to be more associated with diarrhea.[52] SIBO, in general, appears to be more common in diarrhea-predominant IBS than in constipation-predominant IBS.[43]

Interpretation of Breath Testing

Lactulose is normally not fermented until it reaches the bacteria-rich colon. As a result, the typical fasting breath sample contains less than 20 ppm of H_2 or CH_4. An increase in breath gas levels in the later breath specimens (90 and 120 minutes) usually reflects colonic bacterial fermentation and is considered normal. Lack of the expected colonic peak can result from antibiotics or an acidic colonic pH.[53,54] In patients with SIBO, the lactulose is typically fermented in the small intestine, resulting in an early peak in breath gas values.[42]

According to the consensus meeting for SIBO in May 2015, a positive test result (indicating SIBO) is defined by a rise in H_2 of ≥20 ppm above baseline (or a rise in CH_4 of ≥10 ppm above baseline), occurring less than 90 minutes after lactulose or glucose ingestion.[40] A double-peak is not considered necessary for diagnosis. Elevated baseline values occur in up to one-third of patients with SIBO[53] and have been proposed to relate to the fermentation of endogenous brush-border glycoproteins,[55] although future studies are needed to confirm the clinical significance of this finding.[40]

Various factors may interfere with the breath test, resulting in false-negative or false-positive results. Detailed instructions for breath collection help minimize this interference.

False-positive results. The following factors may account for a false-positive result on a breath test:

- Failure to fast for at least 12 hours before the test or to avoid dietary fiber the day before collection can result in excessive "background noise" that contributes to the overall concentration of breath gases.[29]
- Sleeping, smoking, or eating shortly before or during sample collection can increase concentrations of breath gases.[56]
- Fermentation by oropharyngeal flora can lead to early, transient elevations in breath gases after carbohydrate ingestion.[57] As a result, it is recommended that teeth and tongue brushing be performed before specimen collection.

False-negative results. False-positive results on a breath test can be caused by the following factors:

- Diarrhea or the recent administration of antimicrobials can temporarily reduce the concentration of gut bacteria,[58] thus obscuring SIBO. Laxatives and enemas pose a similar risk.[59] Patients are advised to wait at least 1 week after antibiotic therapy before performing the test.
- SIBO confined to the distal ileum may go undetected if the breath gas peak produced in the ileum merges with the breath gases produced by the colonic flora.[42]
- Rapid intestinal transit may cause delayed increases in breath gases, leading to a rise only after the lactulose has already reached the cecum.[60] This is particularly relevant for patients with SIBO who have undergone small bowel resection.

TREATMENT OF SIBO

For a successful clinical outcome, the treatment of SIBO should not only eradicate the bacterial overgrowth but also address symptoms, underlying causes, and complications of SIBO, such as nutrient deficiencies. Although relapses are common, identifying and treating the individual root causes of SIBO can greatly minimize this potential.

Bacterial Eradication

Most patients with clinically significant SIBO host an intestinal flora consisting largely of anaerobes; however, some patients harbor a predominance of gram-negative aerobes, such as *Escherichia coli, Klebsiella,* and *Pseudomonas.*[61] As a result, the most effective antimicrobial agents are those that target both aerobic and anaerobic microorganisms.[19]

Antibiotics

According to a meta-analysis of 10 studies using different antibiotics to treat SIBO, antibiotics were superior to placebo in normalizing H_2 breath tests (51% efficacy for antibiotics compared with 9.8% for placebo).[62] Historically, the first-line antibiotic for SIBO has been tetracycline (250 mg four times daily for 7 days).[19] However, the high prevalence of bacterial resistance to this drug (up to 60% of patients with SIBO)[19] has led to the use of alternative antibiotics. Common alternatives include metronidazole, clindamycin, neomycin, and rifaximin; amoxicillin, ampicillin, chloramphenicol, erythromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole have been used less frequently.[41] The duration of treatment has varied in studies as much as the choice of antibiotic, ranging from 5 days to 1 month.

The minimally absorbed and broad-spectrum antibiotic rifaximin is increasingly recognized for its effectiveness and minimum of side effects.[63] Rifaximin has been widely studied for its use in functional bowel disorders, including SIBO, and was approved by the U.S. Food and Drug Administration (FDA) in 2015 for diarrhea-predominant IBS. In one study, a 7-day course of rifaximin at 400 mg three times daily normalized breath H_2 excretion in 70% of patients with SIBO, whereas tetracycline normalized H_2 excretion in only 27% of patients.[64] In a larger 7-day study, rifaximin at 400 mg three times daily normalized H_2 excretion in 63.4% of patients with SIBO, compared with 43.7% of patients with SIBO taking metronidazole.[65] Longer treatment durations of 10 to 14 days are common.[63]

In cases of excess CH_4 production, the addition of neomycin (e.g., 500 mg twice daily) to rifaximin, ideally for 14 days, has been found to be more effective than rifaximin alone.[66,67] This is because *Methanobrevibacter smithii,* the bacterium considered most responsible for CH_4 production in the gut, is commonly resistant to many antibiotics.

A poor response to antibiotics may indicate mucosal disease, antibiotic resistance, antibiotic-associated diarrhea, or an incorrect diagnosis.[30,68] Recurrence of symptoms after treatment suggests the need for follow-up testing and possible retreatment, as well as a closer examination of underlying causes. Older age, history of surgery such as appendectomy, and chronic use of proton-pump inhibitors increase the likelihood of recurrence.[69] Because prolonged antibiotic therapy significantly raises the risk of diarrhea, *Clostridium difficile* infection, and bacterial resistance,[19] the administration of probiotics is often advised to minimize such side effects.[29] Certain probiotics may also reduce breath H_2 in some patients with SIBO.[70] However, this is an area of ongoing research in SIBO because probiotics have also been observed to exacerbate symptoms in patients.[71]

Herbal Antibiotics

Because conventional antibiotics have shown variable success in eradicating SIBO and can come with side effects (most) or a high price tag (rifaximin), interest has been steadily growing in the use of various botanical agents with antimicrobial activity. Small intestinal fungal overgrowth (SIFO) is also present in some patients with SIBO; thus herbal agents may offer the additional advantage of antifungal activity. Anecdotally, some of the more common antimicrobial and/or antifungal herbs used for SIBO include berberine sulfate or berberine-containing herbs, for example, goldenseal *(Hydrastis canadensis),* barberry *(Berberis vulgaris),* or goldthread *(Coptis chinensis);* oregano *(Origanum vulgare);* allicin (stabilized garlic extract); and neem *(Azadirachta indica).*

In the first formal study examining the efficacy of herbal agents in patients with confirmed SIBO, over a 4-week period, 67 patients received rifaximin and 37 patients received various herbal

combinations (totaling 27 herbs) known to have antibacterial and/or antifungal properties.[72] At follow-up, 46% of the patients in the herbal therapy group had negative lactulose breath tests, compared with 34% of the patients in the rifaximin group.

Antibiotic Alternatives

Peppermint oil, which has been used successfully in patients with IBS, is a volatile oil with antimicrobial properties.[73] Although enteric-coated peppermint oil (dose of 0.2 mL three times a day) dramatically reduced gastrointestinal symptoms and reduced breath H_2 in a patient with SIBO,[74] further research is needed before drawing conclusions about its effectiveness in SIBO.

Addressing the Underlying Causes

Bacterial overgrowth of the small intestine may easily recur if the root causes are not addressed.

Restoration of Gastric Acidity

Because gastric acidity is a critical deterrent to SIBO, restoration of normal stomach pH in patients with hypochlorhydria or achlorhydria is essential. This may include the use of betaine hydrochloride with meals or the discontinuation of antacid medications. A 2017 meta-analysis of 19 studies concluded that the use of proton-pump inhibitors moderately increases the risk of SIBO.[75]

Normalization of Intestinal Motility

As mentioned, intestinal stasis is a major contributing factor to SIBO. When not a result of anatomical or organic causes, reduced motility may be improved with measures such as increased dietary fiber (especially partially hydrolyzed guar gum, which may be safer in SIBO than other forms of fiber[76] and even enhance the effect of rifaximin[77]), water, probiotics, stress management, and exercise.

As stated, impairments in the migrating motor complex (MMC) have been noted in patients with SIBO. The MMC is influenced by both gastrointestinal hormones and the central nervous system. The most active phase of the MMC, Phase III, is induced by serotonin, motilin, and ghrelin. Accordingly, low doses of promotility agents such as serotonin agonists (e.g., tegaserod, prucalopride, or cisapride) and motilin receptor agonists (e.g., azithromycin or erythromycin) are increasingly included in a comprehensive approach to SIBO patients, aimed at preventing relapse[71]; low-dose naltrexone, an opioid antagonist that interacts with the immune system, is also sometimes employed.[78] This is an area of continuing research. In one such study of patients successfully treated for SIBO, tegaserod was shown to dramatically extend symptom-free days posttreatment, and both tegaserod and erythromycin were superior to no prevention at all.[79]

Abdominal/pelvic adhesions (e.g., from prior surgeries) can sometimes restrict motility in the gastrointestinal tract. Anecdotally, such adhesions may be amenable to visceral manipulation.

Dietary Support

An elemental formula supplies daily nutrition in an easy-to-assimilate form and does not contain carbohydrate residues that can feed bacteria. In a study of 124 patients with IBS with SIBO, an elemental diet was found to normalize lactulose breath tests in 80% of patients after 14 days, a statistic not observed with antibiotics; another five patients achieved a negative breath test after following the diet for an additional 6 days, raising the overall success rate to 85%.[80] The precise mechanisms behind the success of an elemental diet are still unclear, although they are postulated to include nutrient deprivation of enteric microbes, stimulation of Phase III of the MMC, and/or stimulation of intestinal immunity.[80]

Diet is increasingly recognized as a critical factor determining success in the treatment of SIBO. Because bacteria thrive on enteric carbohydrates, restricting dietary fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) can theoretically help reduce symptoms and reduce bacterial counts. A systematic review found that a low-FODMAP diet ameliorated IBS symptoms in all FODMAP studies examined.[81] Adequate spacing of meals (e.g., at least 4–5 hours apart) is another important therapeutic consideration, based on the fact that the MMC is only active in the fasting state.

Patients with SIBO may become lactose intolerant as a result of disaccharidase deficiency. This is often ameliorated with bacterial eradication[82]; however, temporary avoidance of all disaccharides—the premise of the "specific carbohydrate diet"—can also help "starve" the excess bacteria and allow healing of the intestinal lining.[83]

Substituting more easily absorbed medium-chain triglycerides for most dietary fat may be helpful in patients with diarrhea and steatorrhea.[19]

Further research is needed into the role of diet in the treatment and especially the maintenance of remission in SIBO. In the meantime, practitioners continue to experiment and refine various approaches.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bouhnik Y, Alain S, Attar A, et al. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am J Gastroenterol*. 1997;94:1327–1331.
2. Bures J, Cyrany J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol*. 2010;16(24):2978–2990.
3. Lauritano EC, Valenza V, Sparano L, et al. Small intestinal bacterial overgrowth and intestinal permeability. *Scand J Gastroenterol*. 2010;45(9):1131–1132.
4. Di Stefano M, Veneto G, Malservisi S, et al. Small intestine bacterial overgrowth and metabolic bone disease. *Dig Dis Sci*. 2001;46:1077–1082.
5. Thomas PD, Forbes A, Green J, et al. Guidelines for the investigation of chronic diarrhoea. *Gut*. 2003;52(suppl 5):v1–v15.
6. Reddymasu SC, Sostarich S, McCallum RW. Small intestinal bacterial overgrowth in irritable bowel syndrome: are there any predictors? *BMC Gastroenterol*. 2010;10:23.
7. Giamarellos-Bourboulis EJ, Pyleris E, Barbatzas C, et al. Small intestinal bacterial overgrowth is associated with irritable bowel syndrome and is independent of proton pump inhibitor usage. *BMC Gastroenterol*. 2016;16(1):67.
8. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. A double-blind, randomized, placebo-controlled study. *Am J Gastroenterol*. 2003;98(2):412–419.
9. Parodi A, Paolino S, Greco A, et al. Small intestinal bacterial overgrowth in rosacea: clinical effectiveness of its eradication. *Clin Gastroenterol Hepatol*. 2008;6(7):759–764.
10. Klaus J, Spaniol U, Adler G, et al. Small intestinal bacterial overgrowth mimicking acute flare as a pitfall in patients with Crohn's disease. *BMC Gastroenterol*. 2009;9:61.
11. Weinstock LB, Walters AS. Restless legs syndrome is associated with irritable bowel syndrome and small intestinal bacterial overgrowth. *Sleep Med*. 2011;12(6):610–613.
12. Fialho A, Fialho A, Thota P, et al. Small intestinal bacterial overgrowth is associated with non-alcoholic fatty liver disease. *J Gastrointestin Liver Dis*. 2016;25(2):159–165.
13. Weinstock LB, Klutke CG, Lin HC. Small intestinal bacterial overgrowth in patients with interstitial cystitis and gastrointestinal symptoms. *Dig Dis Sci*. 2008;53(5):1246–1251.
14. Weinstock LB, Geng B, Brandes SB. Chronic prostatitis and small intestinal bacterial overgrowth: effect of rifaximin. *Can J Urol*. 2011;18(4):5826–5830.
15. Logan AC, Venket Rao A, Irani D. Chronic fatigue syndrome: lactic acid bacteria may be of therapeutic value. *Med Hypotheses*. 2003;60(6):915–923.
16. Pimentel M, Wallace D, Hallegua D, et al. A link between irritable bowel syndrome and fibromyalgia may be related to findings on lactulose breath testing. *Ann Rheum Dis*. 2004;63(4):450–452.
17. Riordan SM, McIver CJ, Thomas DH, et al. Luminal bacteria and small-intestinal permeability. *Scand J Gastroenterol*. 1997;32:556–563.
18. Goebel A, Buhner S, Schedel R, et al. Altered intestinal permeability in patients with primary fibromyalgia and in patients with complex regional pain syndrome. *Rheumatology (Oxford)*. 2008;47(8):1223–1237.
19. Toskes PP. Bacterial overgrowth of the gastrointestinal tract. *Adv Intern Med*. 1993;38:387–407.
20. Rose S, Young MA, Reynolds JC. Gastrointestinal manifestations of scleroderma. *Gastroenterol Clin North Am*. 1998;27:563–594.
21. Camilleri M. Small bowel motility disorders. *Rev Gastroenterol Mex*. 1994;59:120–126.
22. Virally-Monod M, Tielmans D, Kevorkian JP, et al. Chronic diarrhoea and diabetes mellitus: prevalence of small intestinal bacterial overgrowth. *Diabetes Metab*. 1998;24:530–536.
23. Castiglione F, Del Vecchio Blanco G, Rispo A, et al. Orocecal transit time and bacterial overgrowth in patients with Crohn's disease. *J Clin Gastroenterol*. 2000;31:63–66.
24. Anonymous. Small intestinal bacterial overgrowth syndrome. *Gastroenterol*. 1981;80:834–845.
25. Kirsch M. Bacterial overgrowth. *Am J Gastroenterol*. 1990;85:231–237.
26. Tursi A, Brandimarte G, Giorgetti G. High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. *Am J Gastroenterol*. 2003;98:839–843.
27. Riordan SM, McIver CJ, Wakefield D, et al. Small intestinal bacterial overgrowth in the symptomatic elderly. *Am J Gastroenterol*. 1997;92:47–51.
28. Hill M. Normal and pathological microbial flora of the upper gastrointestinal tract. *Scand J Gastroenterol*. 1985;111(suppl):1–6.
29. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. *J Nutr*. 2000;130(suppl):396S–402S.
30. King CE, Toskes PP. Small intestine bacterial overgrowth. *Gastroenterol*. 1979;76:1035–1055.
31. Deloose E, Janssen P, Depoortere I, Tack J. The migrating motor complex: control mechanisms and its role in health and disease. *Nat Rev Gastroenterol Hepatol*. 2012;9(5):271–285.
32. Deloose E, Tack J. Redefining the functional roles of the gastrointestinal migrating motor complex and motilin in small bacterial overgrowth and hunger signaling. *Am J Physiol Gastrointest Liver Physiol*. 2016;310(4):G228–G233.
33. Pimentel M, Soffer EE, Chow EJ, et al. Lower frequency of MMC is found in IBS subjects with abnormal lactulose breath test, suggesting bacterial overgrowth. *Dig Dis Sci*. 2002;47(12):2639–2643.
34. Sherman P, Lichtman S. Small bowel bacterial overgrowth syndrome. *Dig Dis Sci*. 1987;5:157–171.
35. Fagundes-Neto U, De Martini-Costa S, Pedroso MZ, Scaletsky IC. Studies of the small bowel surface by scanning electron microscopy in infants with persistent diarrhea. *Braz J Med Biol Res*. 2000;33:1437–1442.
36. Weinstock LB, Walters AS, Paueksakon P. Restless legs syndrome—theoretical roles of inflammatory and immune mechanisms. *Sleep Med Rev*. 2012;16(4):341–354.
37. Camilo E, Zimmerman J, Mason JB, et al. Folate synthesized by bacteria in the human upper small intestine is assimilated by the host. *Gastroenterol*. 1996;110:991–998.
38. O'Leary C, Quigley EM. Small bowel bacterial overgrowth, celiac disease, and IBS: what are the real associations? *Am J Gastroenterol*. 2003;98:720–722.
39. Sachdev AH, Pimentel M. Gastrointestinal bacterial overgrowth: pathogenesis and clinical significance. *Ther Adv Chronic Dis*. 2013;4(5):223–231.
40. Rezaie A, Buresi M, Lembo A, et al. Hydrogen and methane-based breath testing in gastrointestinal disorders: the North American consensus. *Am J Gastroenterol*. 2017;112(5):775–784.
41. Khoshini R, Dai SC, Lezcano S, Pimentel M. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. *Dig Dis Sci*. 2008;53(6):1443–1454.
42. Rhodes JM, Middleton P, Jewell DP. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. *Scand J Gastroenterol*. 1979;14:333–336.
43. Ford A, Spiegel BM, Talley NJ, Moayyedi P. Small intestinal bacterial overgrowth in irritable bowel syndrome: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2009;7(12):1279–1286.
44. Brummer RJ, Armbrecht U, Bosaeus I, et al. The hydrogen (H_2) breath test: sampling methods and the influence of dietary fibre on fasting level. *Scand J Gastroenterol*. 1985;20:1007–1013.
45. Hamilton LH. In: *Breath Tests and Gastroenterology*. 2nd ed. Milwaukee: QuinTron Instrument Company; 1998.
46. Cloarec D, Bornet F, Gouilloud S, et al. Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. *Gut*. 1990;31:300–304.
47. Gupta A, Chey WD. Breath testing for small intestinal bacterial overgrowth: a means to enrich rifaximin responders in IBS patients? *Am J Gastroenterol*. 2016;111(3):305–306.
48. Rumessen JJ, Nordgaard-Andersen I, Gudmand-Hoyer E. Carbohydrate malabsorption: quantification by methane and hydrogen breath tests. *Scand J Gastroenterol*. 1994;29:826–832.
49. Corazza G, Strocchi A, Sorge M, et al. Prevalence and consistency of low breath H_2 excretion following lactulose ingestion: possible implications for the clinical use of the H_2 breath test. *Dig Dis Sci*. 1993;38:2010–2016.
50. Banik GD, De A, Som S, et al. Hydrogen sulphide in exhaled breath: a potential biomarker for small intestinal bacterial overgrowth in IBS. *J Breath Res*. 2016;10(2):026010.

51. Kajs TM, Fitzgerald JA, Buckner RY, et al. Influence of a methanogenic flora on the breath H_2 and symptom response to ingestion of sorbitol or oat fiber. *Am J Gastroenterol*. 1997;92:89–94.
52. Pimentel M, Mayer AG, Park S, et al. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig Dis Sci*. 2003;48:86–92.
53. Romagnuolo J, Schiller D, Bailey RJ. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol*. 2002;97:1113–1126.
54. Vogelsang H, Ferenci P, Frotz S, et al. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. *Gut*. 1988;29:21–26.
55. Perman JA, Modler S. Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. *Gastroenterol*. 1982;83: 388–393.
56. Solomons N. Evaluation of carbohydrate absorption: the hydrogen breath test in clinical practice. *Clin Nutr J*. 1984;3:71–78.
57. Thompson DG, O'Brien JD, Hardie JM. Influence of the oropharyngeal microflora on the measurement of exhaled breath hydrogen. *Gastroenterol*. 1986;91:853–860.
58. Gilat T, Ben Hur H, Gelman-Malachi E, et al. Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut*. 1978;19:602–605.
59. Solomons NW, Garcia R, Schneider R, et al. H_2 breath tests during diarrhea. *Acta Paediatr Scand*. 1979;68:171–172.
60. Caride VJ, Prokop EK, Troncale FJ, et al. Scintigraphic determination of small intestinal transit time: comparison with the hydrogen breath technique. *Gastroenterol*. 1984;86:714–720.
61. Kocoshis SA, Schletewitz K, Lovelace G, Laine RA. Duodenal bile acids among children: keto derivatives and aerobic small bowel bacterial overgrowth. *J Pediatr Gastroenterol Nutr*. 1987;6:686–696.
62. Shah SC, Day LW, Somsouk M, Sewell JL. Meta-analysis: antibiotic therapy for small intestinal bacterial overgrowth. *Aliment Pharmacol Ther*. 2013;38(8):925–934.
63. Gupta K, Ghuman HS, Handa SV. Review of rifaximin: latest treatment frontier for irritable bowel syndrome mechanism of action and clinical profile. *Clin Med Insights Gastroenterol*. 2017;10:1179552217728905.
64. Di Stefano M, Malservisi S, Veneto G, et al. Rifaximin versus chlortetracycline in the short-term treatment of small intestinal bacterial overgrowth. *Aliment Pharmacol Ther*. 2000;14:551–556.
65. Lauritano EC, Gabrielli M, Scarpellini E, et al. Antibiotic therapy in small intestinal bacterial overgrowth: rifaximin versus metronidazole. *Eur Rev Med Pharmacol Sci*. 2009;13(2):111–116.
66. Pimentel M, Chang C, Chua KS, et al. Antibiotic treatment of constipation-predominant irritable bowel syndrome. *Dig Dis Sci*. 2014;59(6): 1278–1285.
67. Low K, Hwang L, Hua J, et al. A combination of rifaximin and neomycin is most effective in treating irritable bowel syndrome patients with methane on lactulose breath test. *J Clin Gastroenterol*. 2010;44(8):547–550.
68. Bjorneklett A, Hoverstad T, Hovig T. Bacterial overgrowth. *Scand J Gastroenterol*. 1985;109(suppl):123–132.
69. Lauritano EC, Gabrielli M, Scarpellini E, et al. Small intestinal bacterial overgrowth recurrence after antibiotic therapy. *Am J Gastroenterol*. 2008;103(8):2031–2035.
70. Gaon D, Garmendia C, Murrielo NO, et al. Effect of *Lactobacillus* strains (*L. casei* and *L. acidophilus* strains cereal) on bacterial overgrowth-related chronic diarrhea. *Medicina (Brazil)*. 2002;62:159–163.
71. Rezaie A, Pimentel M, Rao SS. How to test and treat small intestinal bacterial overgrowth: an evidence-based approach. *Curr Gastroenterol Rep*. 2016;18(2):8.
72. Chedid V, Dhalla S, Clarke JO, et al. Herbal therapy is equivalent to rifaximin for the treatment of small intestinal bacterial overgrowth. *Glob Adv Health Med*. 2014;3(3):16–24.
73. Shapiro S, Meier A, Guggenheim B. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. *Oral Microbiol Immunol*. 1994;9:202–208.
74. Logan AC, Beaulne TM. The treatment of small intestinal bacterial overgrowth with enteric-coated peppermint oil: a case report. *Alt Med Rev*. 2002;7:410–417.
75. Su T, Lai S, Lee A, et al. Meta-analysis: proton pump inhibitors moderately increase the risk of small intestinal bacterial overgrowth. *J Gastroenterol*. 2017;2. [Epub ahead of print]. https://doi.org/10.1007/s00535-017-1371-9.
76. Quartarone G. Role of PHGG as a dietary fiber: a review article. *Minerva Gastroenterol Dietol*. 2013;59(4):329–340.
77. Furnari M, Parodi A, Gemignani L, et al. Clinical trial: the combination of rifaximin with partially hydrolysed guar gum is more effective than rifaximin alone in eradicating small intestinal bacterial overgrowth. *Aliment Pharmacol Ther*. 2010;32(8):1000–1006.
78. Ploesser J, Weinstock LB, Thomas E. Low dose naltrexone: side effects and efficacy in gastrointestinal disorders. *Int J Pharm Compd*. 2010;14(2):171–173.
79. Pimentel M, Morales W, Lezcano S, et al. Low-dose nocturnal tegaserod or erythromycin delays symptom recurrence after treatment of irritable bowel syndrome based on presumed bacterial overgrowth. *Gastroenterol Hepatol (NY)*. 2009;5(6):435–442.
80. Pimentel M, Constantino T, Kong Y, et al. A 14-day elemental diet is highly effective in normalizing the lactulose breath test. *Dig Dis Sci*. 2004;49(1):73–77.
81. Rao SS, Yu S, Fedewa A. Systematic review: dietary fibre and FODMAP-restricted diet in the management of constipation and irritable bowel syndrome. *Aliment Pharmacol Ther*. 2015;41(12):1256–1270.
82. Nucera C, Lupascu A, Gabrielli M, et al. Sugar intolerance in irritable bowel syndrome: the role of small bowel bacterial overgrowth. *Gastroenterol*. 2004;126(4 suppl 2):A511.
83. Gottschall E. *Breaking the Vicious Cycle*. Baltimore: Kirkton Press; 1994.
84. Chander Roland B, Mullin GE, Passi M, et al. A prospective evaluation of ileocecal valve dysfunction and intestinal motility derangements in small intestinal bacterial overgrowth. *Dig Dis Sci*. 2017;4. [Epub ahead of print]. https://doi.org/10.1007/s10620-017-4726-4.

10

Cell-Signaling Analysis

Lise Alschuler, ND, and *Aristo Vojdani*, PhD, MSc, CLS*

OUTLINE

INTRODUCTION

Signaling pathways in normal cells consist of growth and control messages from the outer surface deep into the nucleus. In the nucleus, the cell-cycle clock collects different messages, which are used to determine when the cell should divide. Cancer cells often proliferate excessively because genetic mutations cause induction of stimulatory pathways and issue too many "go-ahead" signals, or the inhibitory pathways can no longer control the stimulatory pathways.[1]

Impressive evidence has now been gathered with regard to the destination of stimulatory and inhibitory pathways in the cell. These pathways converge on a molecular apparatus in the cell nucleus that is often referred to as the *cell-cycle clock.* The clock is the executive decision maker of the cell; apparently, it runs amok in virtually all types of human cancer. In a normal cell, the clock integrates the mixture of growth-regulating signals received by the cell and decides whether the cell should pass through its life cycle. If the answer is positive, the clock leads the process.

THE CELL CYCLE

A schematic of the classic cell cycle is shown in Fig. 10.1. The cell-cycle compartments are drawn such that their horizontal position reflects their respective DNA content. Cells that contain only one complement of DNA from each parent (2C) are referred to as *diploid cells.* Cells that have duplicated their genome, and thus have 4C amounts of DNA, are called *tetraploid cells.*

The cell cycle is classically divided into the following phases:

- G_0
- G_1
- S
- G_2
- M

The cell-cycle phase of G_1 was historically considered to be a time when diploid (2C) cells had little observable activity. Because this time precedes DNA synthesis, the term *Gap 1 (G_1)* was coined. It is known that there is quite a bit of transcription and protein synthesis during this phase. At a certain point in the cell's life, the DNA synthetic machinery turns on. This phase of the cell's life is labeled *S* for synthesis. As the cell proceeds through this phase, its DNA content increases from 2C to 4C. At the end of S, the cell duplicates its genome and now is in the tetraploid state. After the S phase, the cell again enters a phase that was historically thought to be quiescent. Because this phase is the second gap region, it is referred to as G_2. In the G_2 phase, the cell

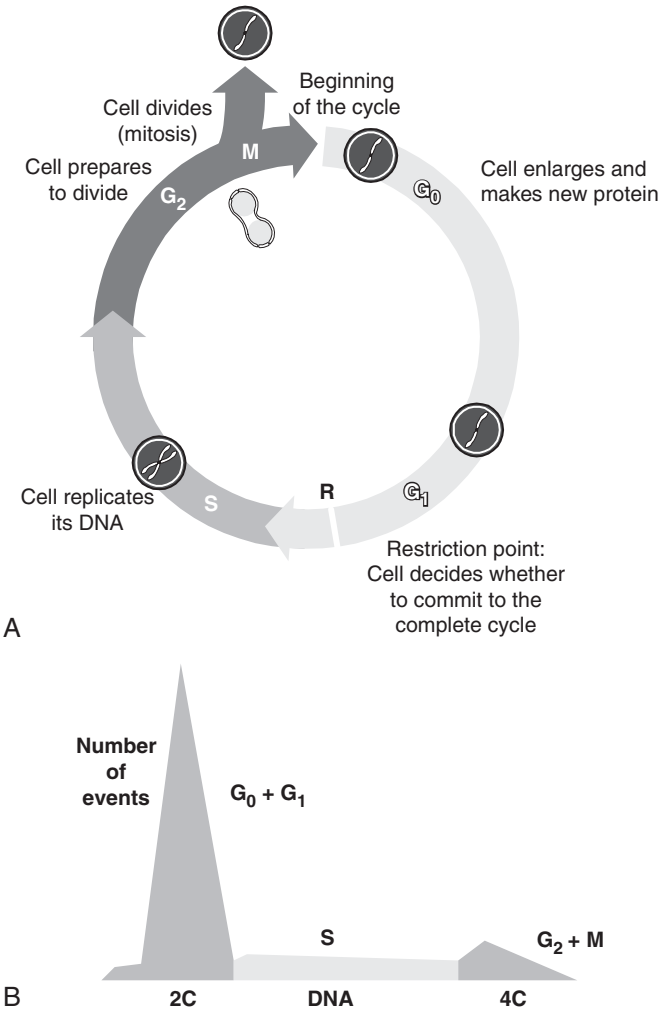


FIG. 10.1 Stages of the cell cycle (G_0, G_1, S, G_2, and M phases) (A) and DNA histogram (B) generated by flow cytometry.

*Previous edition contributor

produces the necessary proteins that play a major role in cytokinesis. After a highly variable amount of time, the cell enters mitosis *(M)*. DNA content remains constant at 4C until the cell actually divides at the end of telophase.

The enlarged parent cell finally reaches the point where it divides in half to produce its two daughters, each of which is endowed with a complete set of chromosomes. The new daughter cells immediately enter G_1 and may go through the full cycle again. Alternatively, they may stop cycling temporarily or permanently.[2–4] Telomeres, condensed chromatin caps on the ends of chromosomes, dictate the ultimate number of cell divisions that can occur. With each cell division, the telomeres shorten, ultimately to the point that destabilizes the chromosomes sufficiently enough to disallow further mitosis. Thus telomeres are considered to be the "mitotic clock." Telomere shortening is linked to aging, sedentarism, chronic stress, and age-related diseases, including cancer, coronary artery disease, and heart failure. Cell-proliferation capacity, the cellular environment, and epigenetic factors affect telomere length and therefore cells' mitotic capacity.

Telomere length can be assessed, and a clinical test is available. The telomere length is reported as a telomere score, which is a calculation of the telomere length derived from nucleated white blood cells. This result is then compared with the average telomere length of a similarly aged sample population. Although the transferability of this information to other bodily tissues is not well characterized, telomere length measured in this way has been correlated to clinical outcomes such as cancer incidence and mortality from cancer.[5] This information can be used to prioritize interventions that increase telomere length, such as dietary interventions, stress reduction, and antioxidant and vitamin therapies.[6]

Another influence on the cell-cycle clock is circadian rhythms, or the circadian clock. The circadian clock is the result of molecular clocks in each cell, circadian physiology, and, ultimately, the suprachiasmatic nuclei in the hypothalamus. The circadian clock regulates the activity and expression of proteins related to cell-cycle checkpoints, and in turn these checkpoints regulate circadian-clock proteins. Every cell in the body has circadian-clock proteins, so-called peripheral oscillators, which exert rhythmic control of mitochondrial morphology, energy metabolism, and cell division.[7] This has significant clinical implications, particularly in the area of cancer treatment. Both the toxicity and efficacy of cytotoxic agents can vary by more than 50% as a function of when they are dosed in experimental models.[8] Although the clinical implications of this have not yet been discovered, the administration of cytotoxic agents in accordance with the circadian-induced activity of the target cells is gaining ground as a reasonable therapeutic approach.

FLOW CYTOMETRY TO ASSESS CELL-CYCLE STATUS

Flow cytometry identifies cells as they "flow" through a detector while being illuminated with intense light. Tissues are generally disaggregated into single-cell suspensions and stained with one or more fluorescent dyes. The cells are forced to flow within a sheath of fluid, eventually being intersected and interrogated by an intense light source, such as a laser beam. As the cell enters the laser beam, it scatters light in all directions. The measurement of light scattered in the forward direction yields information on the particle's size. Scattered light at right angles to the incident light beam provides information on the internal granularity of the cell. If the cell has been stained with one or more fluorescent dyes, a correlated measurement of more than one cellular parameter can be achieved.

The cell cycle is challenging to study because almost any method can cause perturbations to the activity under study. Newer methods to study the cell cycle allow the ability to assess the cell cycle in living cells. One such method involves labeling the subpopulations of living cells in each phase of the cell cycle with fluorescent proteins. Then, using imaging, one can track and quantify cells in specific phases of the cell cycle using these live cell sensors. This method avoids perturbations to the cell cycle from the test and also allows for the assessment of external influences on the cell cycle.[9]

CLINICAL APPLICATION

Patients Exposed to Carcinogenic Chemicals and Patients With Chronic Fatigue Syndrome

To determine whether peripheral blood lymphocytes (PBLs) isolated from individuals with chronic fatigue syndrome (CFS) and chemically exposed patients represent a discrete block in cell-cycle progression, PBLs isolated from patients with CFS and control individuals were cultured, harvested, fixed, stained with propidium iodide, and analyzed by flow cytometry. The nonapoptotic cell population in PBLs isolated from individuals with CFS consisted of cells arrested in the late S and G_2/M boundaries compared with healthy controls. The arrest was characterized by increased S and G_2/M phases of the cell cycle (from 9%–33% and from 4%–21%, respectively) (Table 10.1 and Fig. 10.2) at the expense of G_0/G_1. Such an abnormality in cell-cycle progression indicates abnormal mitotic cell division in patients who have been exposed to chemicals and who have CFS. From these results,

TABLE 10.1 Percentage of Different Phases of Cell Cycle in Healthy Controls and Patients Exposed to Chemicals

Phase	Healthy Controls	Chemically Exposed
G_0/G_1	88.6 ± 1.4	51.7 ± 2.4
S	8.6 ± 1.2	33.2 ± 4.3
G_2/M	3.6 ± 0.82	21.0 ± 2.6

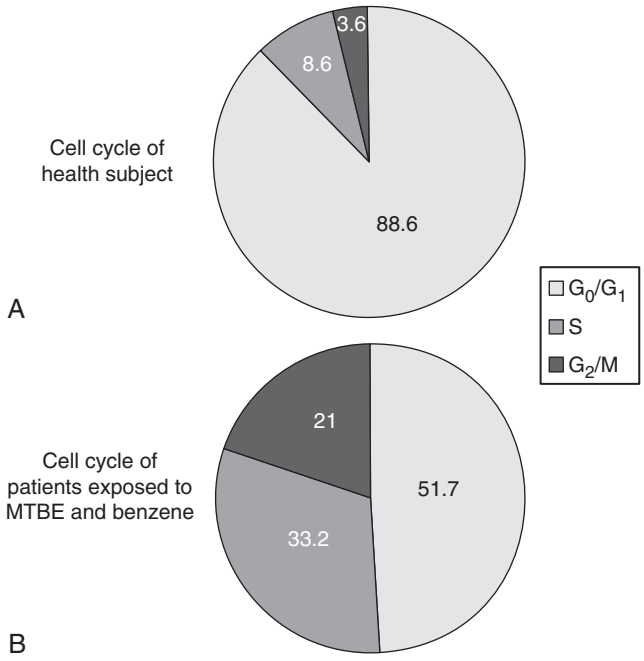


FIG. 10.2 Cell-cycle analysis of peripheral blood lymphocytes from healthy controls (A) and patients exposed to MTBE and benzene (B). Note that in patients' samples, the majority of cells switched from G_0/G_1 to S and G_2/M phases.

it was concluded that the PBLs of patients with chemical exposure and CFS grow inappropriately, not only because the signaling pathways in the cells are perturbed but also because the cell-cycle clock becomes deranged and stimulatory messages become greater than the inhibitory pathways.[10,11] However, to limit cell proliferation and avoid cancer, the human body equips cells with certain backup systems that guard against runaway division. One such backup system present in the lymphocytes of patients with CFS provokes the cell to undergo apoptosis. This programmed cell death occurs if some of the cell's essential components are deregulated or damaged. For example, injury to chromosomal DNA can trigger apoptosis.[1,10,11]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Weinberg RA. How cancer arises. *Sci Am*. 1996;275:62–70.
2. Wheeless LL, Coon JS, Cox C, et al. Precision of DNA flow cytometry in inter-institutional analyses. *Cytometry*. 1991;12:405–412.
3. Wersto RP, Liblit RL, Koss LG. Flow cytometric DNA analysis of human solid tumors: a review of the interpretation of DNA histograms. *Hum Pathol*. 1991;22:1085–1098.
4. Shankey TV, Rabinovitch PS, Bagwell B, et al. Guidelines for implementation of clinical DNA cytometry. *Cytometry*. 1993;14:472–477.
5. Willeit P, Willeit J, Mayr A, et al. Telomere length and risk of incident cancer and cancer mortality. *JAMA*. 2010;304(1):69–75.
6. Xu Q, Parks CG, DeRoo LA, et al. Multivitamin use and telomere length in women. *Am J Clin Nutr*. 2009;89(6):1857–1863.
7. Schmitt K, Grimm A, Dallmann R, et al. Circadian control of DRP1 activity regulates mitochondrial dynamics and bioenergetics. *Cell Metab*. 2018;27(3):657–666.
8. Lévi F, Focan C, Karaboué A, et al. Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. *Adv Drug Deliv Rev*. 2007;59(9–10):1015–1035.
9. Henderson L, Bortone DS, Lim C, Zambon AC. Classic "broken cell" techniques and newer live cell methods for cell cycle assessment. *Am J Physiol Cell Physiol*. 2013;304(10):C927–C938.
10. Vojdani A, Ghoneum M, Choppa PC, et al. Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA. *J Intern Med*. 1997;242:465–478.
11. Vojdani A, Mordechai E, Brautbar N. Abnormal apoptosis and cell cycle progression in humans exposed to methyl tertiary-butyl ether and benzene contaminating water. *Human Exp Toxicol*. 1997;16:485–494.

11

Erythrocyte Sedimentation Rate

Michael T. Murray, ND

OUTLINE

INTRODUCTION

The erythrocyte sedimentation rate (ESR), the rate at which erythrocytes settle out of nonclotted blood in 1 hour, has been one of the most widely performed laboratory tests in the past 75 years. Used primarily to detect occult processes and monitor inflammatory conditions, the ESR test has changed little since 1918 when Fahraeus discovered that the erythrocytes of pregnant women sedimented in plasma more rapidly than they did in nonpregnant women. Since its incorporation into standard laboratory diagnosis, the ESR has been shrouded in medical myths and is often misinterpreted or misused. This chapter provides rational guidelines for its use as a nonspecific measure of inflammatory, infectious, neoplastic, and cardiovascular diseases.[1–4]

ERYTHROCYTE AGGREGATION

Normally, erythrocytes settle quite slowly as the gravitational force of the erythrocyte's mass is counteracted by the buoyant force of the erythrocyte's volume. However, when erythrocytes aggregate, they sediment relatively rapidly because the proportional increase in their total mass exceeds the proportional increase in their volume.

Therefore the major determinant in the sedimentation rate of erythrocytes is erythrocyte aggregation, which usually occurs along a single axis (rouleaux formation). The aggregation of erythrocytes is largely determined by electrostatic forces. Under normal circumstances, erythrocytes have a negative charge and therefore repel each other. However, many plasma proteins are positively charged and neutralize the surface charge of erythrocytes, thereby reducing repulsive forces and promoting aggregation.

The relative contribution of the various "acute-phase" reactant proteins to aggregation is shown in Table 11.1. One protein that has no direct effect on the ESR in physiologic concentrations but is associated with certain inflammatory, degenerative, and neoplastic diseases is C-reactive protein (CRP). Its major function is facilitation of the complement system. Like ESR, the measurement of CRP is used in the monitoring of patients with chronic inflammatory conditions.[1,2] An elevated CRP provides evidence of an inflammatory process despite a normal ESR. Therefore, when used in conjunction with the ESR, it greatly increases the sensitivity in detecting inflammatory and/or infectious processes, especially when variables such as anemia confound the ESR.

The ESR is also elevated in patients with proteinemias (myeloma, macroglobulinemia, cryoglobulinemia, and cold agglutinin disease).[1–4] Disorders of erythrocytes such as various anemias will alter the ESR and may interfere with accurate interpretation.[1–4] Because the ESR is directly proportional to the mass of the erythrocyte and inversely proportional to its surface area, large erythrocytes sediment more rapidly than smaller cells. Therefore in macrocytic anemia, there is an increased ESR, and in microcytic anemia, there is a decreased ESR.

Although the usefulness of ESR determination has decreased as new methods of evaluating disease have been developed, it remains quite helpful in the diagnosis of some diseases, such as temporal arteritis and polymyalgia rheumatica. Perhaps more useful is its ability to monitor these conditions and others, including chronic inflammatory diseases such as rheumatoid arthritis (RA), Hodgkin disease, and other cancers. Although the use of the ESR as a screening test to identify patients who have serious disease is not supported by the literature, it does provide a general gauge of inflammatory processes in the body. It is well accepted that an extreme elevation of the ESR is strongly associated with serious underlying disease, most often infection, collagen vascular disease, or metastatic malignancy. Recently there has been a growing appreciation of the value of the ESR as a marker for atherosclerosis and coronary artery disease.[5,6] In addition, as a sign of chronic low-grade inflammation, it may be helpful as a marker for other conditions as well. For example, in a study of 49,321 Swedish males aged 18 to 20 years, screened for general health and for mental and physical capacity at compulsory conscription examination before military service, there was an inverse correlation between ESR and performance on an IQ test.[7] This result indicated that low-grade inflammation, as indicated by the ESR, was associated with reduced cognitive abilities at ages 18 to 20 years.

PROCEDURES

Various methods for determination of the ESR have been developed. Currently, the Westergren method is recommended by the International Committee for Standardization in Hematology.

TABLE 11.1 Relative Contribution of Acute-Phase Reactant Proteins to Erythrocyte Aggregation

Blood Constituent	Relative Contribution
Fibrinogen	10
β-Globulin	5
α-Globulin	2
Albumin	1

Westergren Method

In the standard Westergren method, the following procedure is used:

1. Dilute venous blood 4:1 with anticoagulant sodium citrate.
2. Put in a 200-mm-long, 2.5-mm-internal-diameter glass tube (Westergren tube).
3. Allow it to stand in a vertical position for 1 hour.
4. At the end of 1 hour, the distance from the meniscus to the top of the column of erythrocytes is recorded as the ESR.

The modified Westergren method uses ethylenediaminetetraacetic acid rather than sodium citrate as an anticoagulant and is more convenient because the same tube of blood can be used for other hematologic studies. The standard and modified methods give identical results.[1–4]

Wintrobe Method

The second most commonly used method is the Wintrobe method. This method is performed with a 100-mm tube (Wintrobe tube) containing oxalate as the anticoagulant. It is more sensitive than the Westergren method in the "normal" to "mildly elevated" range; however, in the more highly elevated ESR, the short tube leads to relatively insensitive readings resulting from packing of cells.[2]

Results

The results of both the Westergren and Wintrobe methods are listed in Box 11.1.

BOX 11.1 Results of Westergren and Wintrobe Methods

- Westergren (normal results)
  - Men: 0 to 10 mm/h
  - Women: 0 to 15 mm/h
  - Children: 0 to 10 mm/h
- Wintrobe (normal results)
  - Men: <6.5 mm/h
  - Women: <16 mm/h

INTERPRETATION

Several factors may result in false-positive or false-negative ESR values; the more significant of these are listed in Box 11.2. In addition, it is important to recognize that in acute disease, the change in sedimentation rate may lag behind the temperature elevation and leukocytosis for 6 to 24 hours, and in unruptured acute appendicitis, the rate may be normal. In convalescence, the increased rate tends to persist longer than the fever or leukocytosis. Box 11.3 lists the most common clinical implications of changes in the ESR. An ESR value exceeding 100 mm/h has a 90% predictive value for serious underlying disease—most often infection, collagen vascular disease, or metastatic tumor.[1]

BOX 11.2 Factors Interfering With the Erythrocyte Sedimentation Rate

False Increase

- Elevated levels of fibrinogen, globulins, and cholesterol
- High room temperature
- Macrocytic anemia
- Menstruation
- Pregnancy
- Running a refrigerated blood sample before it has returned to room temperature
- Tilted erythrocyte sedimentation rate tube
- Certain drugs: dextran, methyldopa, methysergide, oral contraceptive agents, penicillamine, procainamide, theophylline, trifluperidol, vitamin A

False Decrease

- Cachexia
- Clotting of blood sample
- Elevated bile salts
- Elevated phospholipids
- Delay of >2 hours in running the test
- High doses of adrenal steroids
- Hypofibrinogenemia
- Hyperglycemia
- Hyperalbuminemia
- Leukocytosis
- Microcytic anemia
- Newborn
- Certain drugs: adrenocorticotropic hormone, cortisone, ethambutol, quinine, salicylates

Data from references 1 and 4.

Elevated Erythrocyte Sedimentation Rate

Asymptomatic Patients

The presence of an elevated ESR as the only manifestation of a disease process is quite rare. However, when present, it can be highly significant. For example, in one study of 17 patients whose increased ESR was the sole initial clue to disease, two had tuberculosis, one had colon cancer, one had systemic lupus erythematosus, three had ankylosing spondylitis (diagnoses that typically become apparent only after several years of observation), and four men had a persistently elevated ESR several years before a myocardial infarction. The remaining patients developed myeloma, prostate cancer, psoriasis, benign monoclonal gammopathy, and pancreatic cancer.[2]

Although the ESR generally makes a small contribution to disease detection in asymptomatic persons, the presence of an elevated ESR as the only clue to illness in an asymptomatic person indicates the need for a careful diagnostic workup; it may be the first sign of an occult malignancy or a chronic inflammatory disease. The laboratory evaluation for an asymptomatic patient with an elevated ESR should include a complete blood cell count with differentials, blood urea nitrogen and creatinine, alkaline phosphatase measurement, serum protein electrophoresis, urinalysis, guaiac tests of stool, and chest radiograph.[2]

An ESR that exceeds 100 mm/h is definitely associated with infection, malignancy, or connective tissue disease. Rarely does disease remain undiagnosed when the ESR is greatly elevated.[1–4] If an elevated ESR cannot be explained after further clinical evaluation, the ESR should be repeated in 1 month.

BOX 11.3 Clinical Implications of Changes in the Erythrocyte Sedimentation Rate

Increased Rate

- Acute heavy-metal poisoning
- All collagen diseases
- Carcinoma
- Cell or tissue destruction
- Gouty arthritis
- Infections
- Inflammatory diseases
- Leukemia
- Multiple myeloma
- Myocardial infarction
- Nephritis
- Pneumonia
- Rheumatoid arthritis
- Syphilis
- Toxemia

Decreased Rate

- Congestive heart failure
- Polycythemia
- Sickle cell disease

As a Predictor of Coronary Artery Disease

An elevated ESR in white men aged 45 to 64 years was found to be an independent risk factor for coronary heart disease in the National Health and Nutrition Examination Survey-I.[5] The risk was highest when the ESR was greater than 22 mm/h. Subsequent studies and the growing awareness that atherosclerosis is associated with chronic inflammation has given support to the use of the ESR as a prognostic indicator for the risk of coronary artery disease.[6,8] It was hypothesized and later shown that an elevated ESR reflected an elevated blood fibrinogen level (see Chapter 148 for further discussion of fibrinogen). The association of an elevated ESR as a harbinger of coronary artery disease precedes an elevated CRP and can be viewed as an easy-to-use and inexpensive method that indirectly indicates fibrinogen level and aids in the early detection of microinflammation and eventual atherosclerotic burden, even in apparently healthy people.[9,10]

Symptomatic Patients

The ESR is sometimes used to provide confirmation of a disease process when the history and physical findings point toward a specific diagnosis. Although the ESR itself does not diagnose a specific disease, when combined with information gathered in a history and physical, and in conjunction with other laboratory tests, it can be a great help in the formation of a specific diagnosis.

In patients with vague, unsubstantiated illness, the ESR offers limited benefit because of the lack of specificity and the presence of a normal ESR in a wide variety of illnesses. However, the ESR offers great diagnostic benefit when other signs, symptoms, and laboratory findings are present. This is particularly true in malignancies, temporal arteritis and polymyalgia rheumatica, inflammatory arthritis, and suspected infection.

Erythrocyte Sedimentation Rate in Cancer

Malignancy is quite common in symptomatic patients with an elevated ESR. In one study, 70 (8.8%) of 790 clinic patients with an elevated ESR had cancer.[1] However, of the 70 patients with malignancy and an increased ESR, 68 had local signs that led directly to the diagnosis. Thus occult malignancy was present in only 2 of the 790 patients. In addition, the ESR is often normal in patients with cancer, indicating that the ESR should not be relied on as a test to exclude occult malignancy in patients with vague symptoms.

In a prospective follow-up of 300 patients with prostate cancer, an ESR greater than 37 mm/h was associated with a higher incidence of disease progression and death. These findings paralleled other prognostic indicators (e.g., tumor, node, and metastasis staging; grade; performance status; and age) but also indicated that ESR provided additional value in the monitoring of these patients and presumably others with invasive cancer.[3]

Temporal Arteritis and Polymyalgia Rheumatica

Temporal arteritis and polymyalgia rheumatica are related syndromes that can occur together or alone. Both occur in older individuals and are associated with an increased ESR. Symptoms of temporal arteritis include unilateral throbbing headache, scalp sensitivity, visual symptoms, jaw claudication, and localized thickening or loss of pulsation of the temporal artery. Polymyalgia rheumatica is a fast-developing condition characterized by pain and stiffness of the pelvis and/or shoulder girdle, in association with fever, anemia, malaise, and weight loss. It is typically self-limited to 1 to 2 years.

Determination of the ESR is of critical importance in the diagnosis and management of patients with temporal arteritis, a condition that can result in blindness caused by obstruction of the ophthalmic arteries. When the clinical evidence for temporal arteritis is limited, a normal ESR reduces the probability of the disease to less than 1%. When the clinical evidence is strong, it is extremely rare to have a normal ESR. Achievement of a normal ESR by using anti-inflammatory agents, such as cortisone or the natural medicines (e.g., *Curcuma longa,* fish oils, *Ginkgo biloba*), greatly reduces the risk of developing blindness.[1,2]

Inflammatory Arthritis

The ESR is sometimes used to distinguish inflammatory arthritis from other causes of joint symptoms. This is particularly true in the differentiation of RA, which has an elevated ESR, and osteoarthritis, which typically has a normal ESR. Because ESR is not a specific indicator of RA, it is not appropriate to place much value on it as an independent diagnostic predictor of RA. An elevated ESR in patients with joint symptoms simply indicates an active inflammatory process.[1,2]

Suspected Infection

Leukocytosis and fever are better indicators of an acute infectious process than the ESR because the ESR is typically normal during the first stages of infection. However, the ESR is of some value in the differentiation of an intact versus ruptured appendix. Researchers demonstrated that only 2 of 25 patients with nonruptured appendicitis had an ESR of greater than 20 mm/h. In contrast, 67% of patients with ruptured appendixes had an elevated ESR.[1] CRP, however, has emerged as a more accurate measure. The value of CRP for the surgical indication of appendicitis is 4.95 mg/dL.[11] CRP has also been shown to correlate better with joint sepsis than ESR.[11]

Decreased Erythrocyte Sedimentation Rate

The causes of a low ESR (0–1 mm/h) are listed in Boxes 13.2 and 13.3. A low ESR is generally of little significance and may actually indicate a healthy state.[1–4]

Monitoring of Disease Activity

The ESR is well recognized as an aid in monitoring the activity of such inflammatory diseases as temporal arteritis, polymyalgia rheumatica,

and RA.[1–3] In general, improvements in ESR levels generally reflect clinical improvement, and vice versa.

Temporal Arteritis and Polymyalgia Rheumatica

The ESR is the most widely used test for assessing disease activity in patients with temporal arteritis and polymyalgia rheumatica because these conditions have few specific clinical indicators of disease activity. However, both the ESR and clinical status need to be monitored in these patients, and appropriate therapy instituted, if the ESR increases or if there is a worsening in the clinical picture.[1,2,12]

Rheumatoid Arthritis

Although 5% to 10% of patients with RA have a normal ESR, the ESR generally parallels disease activity. Monitoring the patient's ESR therefore provides invaluable feedback on therapeutic effect. An isolated elevated ESR is not useful for prognosis, but sustained extreme elevation of the ESR is associated with a poor prognosis.[1–3] It is important to note that in a meta-analysis of 63 clinical trials or observational studies of RA treatment, the authors concluded that ESR was more sensitive to change than CRP and was the preferred measure of the acute-phase response in RA in clinical practice.[13]

Other Inflammatory Diseases

Other inflammatory diseases, particularly autoimmune diseases such as systemic lupus erythematosus, can be monitored by the ESR in a fashion similar to that for RA.[1-3]

SUMMARY

The ESR is a simple, valuable, and useful laboratory procedure. Although it is a nonspecific indicator of inflammation, an elevated ESR (i.e., greater than 80 mm/h) indicates the presence of significant disease in more than 95% of individuals. The ESR should never be used as the sole diagnostic test. Clinical presentation, comprehensive history, laboratory investigation, and other diagnostic procedures should always be considered when interpreting ESR results. The ESR may be used as a nonspecific gauge of therapeutic efficacy and as a monitoring tool in several inflammatory conditions, including temporal arteritis, polymyalgia rheumatica, RA, certain malignancies, and atherosclerosis.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bray C, Bell LN, Liang H, et al. Erythrocyte sedimentation rate and c-reactive protein measurements and their relevance in clinical medicine. *WMJ*. 2016;115(6):31721.
2. Brigden ML. Clinical utility of the erythrocyte sedimentation rate. *Am Fam Physician*. 1999;60:1443–1450.
3. Bedell SE, Bush BT. Erythrocyte sedimentation rate, from folklore to facts. *Am J Med*. 1985;78:1001–1009.
4. Saadeh C. The erythrocyte sedimentation rate: old and new clinical applications. *South Med J*. 1998;91:220–225.
5. Gillum RF, Mussolino ME, Makuc DM. Erythrocyte sedimentation rate and coronary heart disease: the NHANES I epidemiologic follow-up study. *J Clin Epidemiol*. 1995;48:353–361.
6. Erikssen G, Liestol K, Bjornholt JV, et al. Erythrocyte sedimentation rate: a possible marker of atherosclerosis and a strong predictor of coronary heart disease mortality. *Eur Heart J*. 2000;211:1614–1620.
7. Karlsson H, Ahlborg B, Dalman C. Association between erythrocyte sedimentation rate and IQ in Swedish males aged 18-20. *Brain Behav Immun*. 2010;24(6):868–873.
8. Danesh J, Collins R, Peto R. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. *Eur Heart J*. 2000;21:515–520.
9. Natali A, L'Abbate A, Ferrannini E. Erythrocyte sedimentation rate, coronary atherosclerosis, and cardiac mortality. *Eur Heart J*. 2003;24(7):639–648.
10. Steinvil A, Shapira I, Arbel Y, et al. Determinants of the erythrocyte sedimentation rate in the era of microinflammation: excluding subjects with elevated C-reactive protein levels. *Am J Clin Pathol*. 2008;129(3):486–491.
11. Ernst AA, Weiss SJ, Tracy LA. Usefulness of CRP and ESR in predicting septic joints. *South Med J*. 2010;103(6):522–526.
12. Cantini F, Salvarani C, Olivieri I, et al. Erythrocyte sedimentation rate and C-reactive protein in the evaluation of disease activity and severity in polymyalgia rheumatica: a prospective follow-up study. *Semin Arthritis Rheum*. 2000;30:17–24.
13. Ward MM. Relative sensitivity to change of the erythrocyte sedimentation rate and serum C-reactive protein concentration in rheumatoid arthritis. *J Rheumatol*. 2004;31:884–895.

12

Fantus Test

Dirk W. Powell, BS, ND

OUTLINE

INTRODUCTION

The Fantus test is a simple in-clinic test for measuring urinary chlorides.[1] It is used to estimate dietary sodium chloride intake.[2] For this purpose, it has been shown to give clinically useful results if two factors are taken into consideration[3]:

- The concentration of urinary chloride is proportional to urinary sodium except with increased ingestion of nonsodium chloride salts (e.g., ammonium chloride or potassium chloride).
- Urinary output of sodium chloride may not be a reliable indicator of sodium intake when an individual has a sodium-wasting disorder (Table 12.1).

CLINICAL APPLICATION

The Fantus test was first described by J. B. Fantus in 1936.[4] It was originally used for the detection of salt and water depletion.[5] However, this test is also useful for monitoring excessive sodium chloride intake. Increased sodium chloride ingestion has been implicated as a causal or aggravating factor in many cardiovascular diseases, hypertension, stroke, osteoporosis, and chronic kidney disease.

Since Ambard and Beaujard implicated salt as a major factor in the pathogenesis of hypertension in 1904,[6] conflicting opinions and contradictory data have confused the understanding of the effect of sodium on blood pressure. In 1944 Kempner published a classic article describing the effective treatment of essential hypertension using a salt-restricted "Rice Diet."[7] Many studies have subsequently confirmed the effect of sodium chloride ingestion on blood pressure and the effectiveness of sodium-restricted diets in treating hypertension.[8–13] Evidence suggests that sodium alone does not raise blood pressure, as had been previously thought, but rather that chloride also plays an important role in the elevation of blood pressure when consumed as sodium chloride, or common table salt.[14,15] Sodium chloride restriction has also demonstrated improvements in chronic kidney disease and cardiovascular disease.[16] Another somewhat surprising finding has been that excessive sodium chloride consumption increases system acidity, theorized to be a result of impairment in the kidney's ability to excrete acid waste products of metabolism.[17]

The Fantus test is an indirect test for sodium and actually measures the chloride ion concentration. Ninety percent of the sodium that Americans consume is in the form of sodium chloride.[18] Considering that sodium chloride intake, rather than sodium ion alone, is a significant factor in blood pressure elevation and blood volume expansion, the Fantus test can be more clinically useful than direct tests for urinary sodium when managing hypertension or other disorders affected by sodium chloride.[13,14]

Research shows a strong dose-dependent relationship between excessive salt ingestion and hypertension.[19] Sodium reduction has been found to reduce and prevent heart attack and stroke.[20,21] Blood pressure is observed to begin falling within weeks of reduced salt ingestion.[22] The health advantages of limiting sodium chloride ingestion are apparent considering the preponderance of evidence that supports the health benefits of salt restriction, particularly with cardiovascular disease, hypertension, osteoporosis, and chronic kidney disease. It is estimated that reducing the average daily sodium intake to 2300 mg could reduce cases of high blood pressure by 11,000,000 annually.[23]

However, attempting to restrict sodium chloride ingestion to recommended or therapeutic levels presents two challenges. First, higher levels of sodium chloride are found in most processed and restaurant foods, which account for more than 70% of the sodium consumed.[24] Second, Americans 2 years old or older are conditioned to accept and prefer highly salted food and consume an average of more than 3400 mg of sodium daily.[25] The 2015–2020 Dietary Guidelines for Americans recommend that Americans consume less than 2300 mg of sodium each day as part of a healthy eating pattern.[26]

Because the Fantus test is an inexpensive, simple in-clinic test, it can effectively monitor sodium chloride intake where frequent sampling is useful in the management of salt-restricted diets. Salt restriction can be beneficial with several disorders, including hypertension, congestive heart failure, premenstrual syndrome, headache, osteoporosis, edema, kidney stones, and chronic kidney disorders.

People who are ingesting excessive amounts of salt may be without overt symptoms or signs of disease. It has been observed in animal studies that a high intake of sodium chloride decreases longevity as a factor separate from blood pressure.[27] In another study, salt-induced

TABLE 12.1 Conditions Where Salt Restriction Can De Deleterious

- Adrenocortical insufficiency
- Addison syndrome
- Renal disease with loss of sodium
- Increased sweat or gastrointestinal loss of electrolytes
- Chronic fatigue syndrome

angiotensin elevation was found to adversely affect the myocardium in humans, even when blood pressure remained within normal limits.[28] It has been observed that the adverse effects of excess salt can persist for an extended time after excess salt ingestion has been discontinued.[29,30]

Identifying people who at risk for the harmful effects of excessive salt consumption is an important aspect of preventive medicine. Included in this group are individuals with chronic renal disease, osteoporosis, hypertension or a family history of hypertension, and individuals over the age of 50.[31]

The Fantus test is also useful in monitoring salt intake in clinical conditions where restricted sodium chloride intake can be an aggravating factor or a trigger for serious symptoms, such as in Addison disease, postural hypotension, chronic fatigue syndrome,[32] and electrolyte imbalance (see Table 12.1).

PROCEDURE

The Fantus reagent is prepared so that the number of drops of silver nitrate ($AgNO_3$) used in the titration step can be recorded as grams of sodium per liter of urine (g Na/L urine).

Method

1. Collect a 24-hour urine sample.
2. Measure and record the 24-hour volume.
3. In a test tube, add one drop of 20% potassium chromate (K_2CrO_4) solution to 10 drops of the sample of the 24-hour urine specimen.
4. Titrate (add one drop at a time) with the 2.9% silver nitrate ($AgNO_3$) solution.
5. Record the number of drops needed for the color to turn from yellow to brown as g sodium/L.

Required Reagents

- AgNO3: 2.9% aqueous solution
- K2CrO4: 20% aqueous solution

Results

Fantus test results are measured as NaCl but can be converted to Na (NaCl × 0.39 = Na).

- Typical Western diet: >3000 mg Na/24 h
- Salt-restricted diet: 2300 mg Na/24 h (or less)

INTERPRETATION

The results of a Fantus test generally reflect the salt content of the diet. The typical Western diet contains 3.4 g of sodium in a 24-hour period. On a salt-restricted diet, the goal is typically to reduce 24-hour urine sodium to 2.3 g Na/24 h or less. The minimum 24-hour sodium intake considered safe is 0.5 g Na/24 h for healthy individuals with intact adrenocortical function. Some conditions require higher sodium chloride intake (see Table 12.1).

A random urine can be tested for urine sodium with the Fantus test and can give an approximation of 24-hour sodium ingestion. A sodium intake of 3.4 g Na/day with an intake of 2750 mL of water from both solids and fluids corresponds to the average water turnover for a 70-kg adult. After evaporation from the lungs and other losses, the amount of water excreted by the kidneys is approximately 1500 mL/24 h.[33] A urinary sodium excretion of 3 g in 24 hours would thus produce a urinary sodium concentration of 2 g/L and a urinary sodium chloride concentration of 5.1 g/L (NaCl). A 24-hour urine sample provides a more accurate estimate of 24-hour sodium intake (i.e., 24-hour urine volume × urinary sodium concentration approximates 24-hour sodium intake).

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. DeGowin E, DeGowin RI. New York, NY: Macmillan; 1969:37.
2. Luft FC, Fineberg NS, Sloan RS. Overnight urine collections to estimate sodium intake. *Hypertension*. 1982;(4):494–498.
3. Taylor WH. Use and interpretation of the Fantus estimation of urinary chloride. *Med J*. 1951;2(4740):1125–1128. PMID:14869835.
4. Fantus JB. Fluid postoperatively: statistical study. *JAMA*. 1936;107:14.
5. Marriott H. Water and salt depletion. *Br Med J*. 1947;1:328.
6. Ambard L, Beaujard E. Causes de l'hypertension arteriole. *Arch Gen Med*. 1904;1:520–533.
7. Kempner W. Treatment of kidney disease and hypertensive vascular disease with rice diet. III. *North Carolina Med J*. 1945;6(61–87):117–161.
8. Grohman A, Harrison T, Mason M, et al. Sodium restriction in the diet for hypertension. *JAMA*. 1945;129:533–537.
9. Chapman CB, Gibbons T, Henschel A. The effect of the rice-fruit diet on the composition of the body. *N Engl J Med*. 1950;243:899–905.
10. Dustan HP, Bravo EL, Tarazi RC. Volume-dependent essential and steroid hypertension. *Am J Cardiol*. 1973;31:606–615.
11. Dustan HR, Tarazi RC, Bravo EL. Diuretic and diet treatment of hypertension. *Arch Intern Med*. 1974;133:1007–1013.
12. Parijs J, Joossens JV, Van der Linden L, et al. Moderate sodium restriction and diuretics in the treatment of hypertension. *Am Heart J*. 1973;85:22–34.
13. American Heart Association. *Changes You Can Make to Manage High Blood Pressure*. 2017.
14. Kotchen TA, Kotchen JM. Dietary sodium and blood pressure: interactions with other nutrients. *Am J of Clin Nutr*. 1997;65(suppl 2):708S–711S.
15. Kurtz T, Morris R. Dietary chloride as a determinant of sodium-dependent hypertension. *Science*. 1983;222:1139–1141.
16. Sanghavi S, Vassaldotti JA. Dietary sodium: a therapeutic target in the treatment of hypertension and CKD. *J Ren Nutr*. 2013;23(3):223–227. PMID:23611551.
17. Pizzorno J1, Frassetto LA, Katzinger J. Diet-induced acidosis: is it real and clinically relevant? *Br J Nutr*. 2010;103(8):1185–1194.
18. Institute of Medicine. *Dietary Reference Intakes for Water, Potassium, Sodium Chloride, and Sulfate*. Washington, DC: National Academies Press; 2004.
19. Institute of Medicine. *Dietary Reference Intakes for Water, Potassium, Sodium Chloride, and Sulfate*. Washington, DC: National Academies Press; 2004.
20. Aburto NJ, Das S. *Effect of Reduced Sodium Intake on Blood Pressure, Renal Function, Blood Lipids and Other Potential Adverse Effects*. Geneva, Switzerland: World Health Organization; 2012.
21. Cogswell ME, Mugavero K, Bowman BA, Frieden TR. Dietary sodium and cardiovascular disease risk- measurement matters. *N Engl J Med*. 2016;375:580–586.
22. He FJ, Li J, MacGregor GA. Effect of longer-term modest salt reduction on blood pressure. *Cochrane Database Syst Rev*. 2013;4:CD004937.
23. Palar K, Sturm R. Potential societal savings from reduced sodium consumption in the U.S. adult population. *Am J Health Promot*. 2009;24(1):49–57.
24. Harnack LI, Cogswell ME, Shikany JM, et al. Sources of sodium in U.S. adults from 3 geographic regions. *Circulation*. 2017;135:1775–1783.
25. U.S. Department of Health and Human Services, U.S. Department of Agriculture. What we eat in America. *NHANES*. 2013–2014, Table 37.1017.
26. U.S. Department of Health and Human Services, U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 2015.
27. Meneely GR, Batterbee HD. High sodium-low potassium environment and hypertension. *Am J Cardiol*. 1976;38:768–785.
28. Khairallah P, Sen S, Tarazi R. Angiotensin, protein biosynthesis and cardiovascular hypertrophy (abstr). *Am J Cardiol*. 1976;37:148.
29. Tobian L, Ishii M, Duke M. Relationship of cytoplasmic granules in renal papillary interstitial cells to "post-salt" hypertension. *J Lab Clin Med*. 1969;73:309–319.
30. Dahl LK. Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. *J Exp Med*. 1961;114:231–236.
31. Scribner BH. Salt and hypertension. *JAMA*. 1983;250:388–389.
32. De Lorenzo F, Hargreaves J, Kakkar VV. Pathogenesis and management of delayed orthostatic hypotension in patients with chronic fatigue syndrome. *Clin Auton Res*. 1997;7(4):185–190. PMID: 9292244.
33. Marriott H. Water and salt depletion. *Br Med J*. 1947;1:328.

13

Fatty Acid Profiling

Patricia M. Devers, DO, and *Warren M. Brown, ND*

OUTLINE

INTRODUCTION

Dietary fat intake and its emerging role in chronic disease highlight the need for proper fatty acid measurement and profiling. Many studies have implicated fatty acid imbalances in various conditions,[1–8] including:

- Cardiovascular disease
- Chronic inflammatory conditions
- Neurological and psychiatric disease
- Cancer
- Diabetes
- Polycystic ovary syndrome
- Chronic obstructive pulmonary disease
- Asthma

Unlike other macronutrients, fat is one of the most difficult dietary intake components to quantify. Patients often misreport their fat intake, and food composition tables are frequently incorrect. There is no single biomarker to assess total dietary fat intake.[9] Although the body digests dietary fat into fatty acids, measuring fatty acids cannot validate fat intake because some fatty acids do not come from the diet and are endogenously made. However, there are some biomarkers that reflect essential fatty acid consumption and others that reflect endogenously produced fatty acids. To interpret these measurements, it is important to first understand the definition, physiology, and metabolism of fatty acids.

Fatty acids are simple in structure: a carbon backbone with a carboxyl group (COO) at one end and a methyl group (CH_3) at the other. They function as energy-storage units, cell membrane structural units, and eicosanoid precursors.

Dietary fats are digested into fatty acids, which are then absorbed into the circulation. Three fatty acids can join with glycerol to form triglyceride molecules. Fatty acids are found in serum, cell membranes, and adipocytes.

Although there are many fatty acid dietary sources, such as olive oil and fish, some food sources are more critical than others. The term *essential fatty acid* means these are essential for life and must come from the diet. There are only two essential fatty acids: alpha-linolenic acid (ALA) and linoleic acid (LA). ALA is found in foods such as flax, chia, walnuts, and unhydrogenated soybean oil.[10,11] LA food sources include sunflower seeds, corn oil, and nuts.[12]

Besides essential dietary fatty acids, there are fatty acids that are made endogenously. The body makes them using three processes: synthesis, elongation, and desaturation.

First, fatty acids can be synthesized from acetyl coenzyme A (acetyl-CoA) units made from dietary carbohydrate digestion and metabolism. Insulin's lipogenic activity allows excess glucose to be converted into triglycerides in the liver. Hence, triglycerides and fatty acids can be made through carbohydrate metabolism.[13]

Next, fatty acids can be formed using elongase and desaturase enzymes. Some endogenous fatty acids are formed by adding carbons to dietary fatty acid backbones: they are elongated by elongase enzymes. In desaturation, some of the single bonds in the carbon backbone are converted to double bonds. Desaturation enzymes create different fatty acids[9,14] (Fig. 13.1).

STRUCTURE AND NOMENCLATURE

As mentioned previously, fatty acids are simple in structure: a carbon backbone with a carboxyl group (COO) at one end and a methyl group (CH_4) at the other. The methyl group is labeled omega (ω).

When profiling fatty acids, nomenclature can be complex. In general, they are designated based on the length of the carbon atom backbone, the number of double bonds, and the first double bond's distance from the carbon chain opposite the carboxyl group.[9,15]

The fatty acid backbone usually ranges between 6 and 22 carbons in length, sometimes longer. Because of the variation in the carbon atom backbone members, fatty acids are categorized as short-chain, medium-chain, long-chain, and very-long-chain fatty acids.

Fatty acids are also classified by a double bond's presence or absence, which determines their saturation degree. Saturated fatty acids have no double bonds. Unsaturated fatty acids have one or more double bonds between carbon atoms. Because fatty acids are necessary for forming cell membranes, saturation can play a role in cell membrane fluidity.

Monounsaturated fatty acids (MUFAs), found in olive oil and avocados, have one carbon-carbon double bond, which can occur at

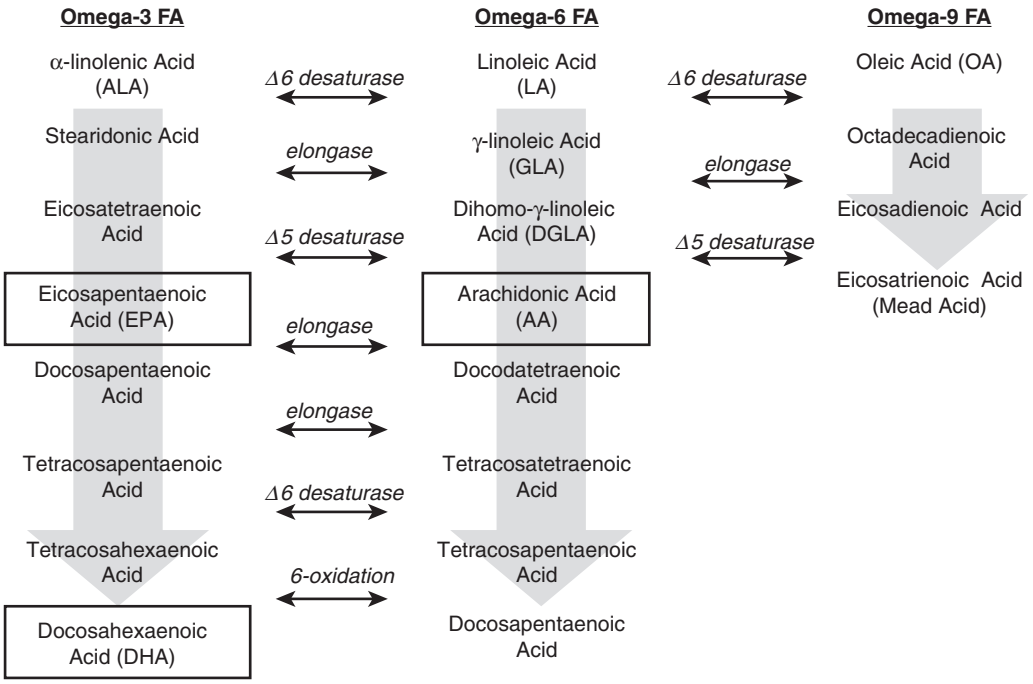


Fig. 13.1 Fatty acid metabolism, elongation and desaturation of essential fatty acids. Chang MI, Puder M, Gura KM. The use of fish oil lipid emulsion in the treatment of intestinal failure associated liver disease (IFALD). *Nutrients*. 2012;4(12):1828 -1850.

different positions within their carbon backbone. The double-bond position designates its name. The most common MUFA is oleic acid, which is found in olive oil. It has a double bond at the ninth carbon and is therefore an omega-9 fatty acid (ω-9).

Polyunsaturated fatty acids (PUFAs), found in foods such as salmon and sunflower seeds, contain more than one double bond. In PUFAs, the first double bond may be found between the third and fourth carbon atom from the ω; these are called omega-3 fatty acids (ω-3). If the first double bond is between the sixth and seventh carbon atoms, then they are called omega-6 fatty acids (ω-6).[15] It is important to note that some laboratories denote *omega* using the letter *n*. For example, omega-3 fatty acids may also be reported as n-3.

If the hydrogen atoms on either side of the double bond are in the same configuration, it is termed a *cis* configuration. Most fatty acids are in the cis configuration. When the hydrogen atoms change configuration, they are considered *trans*. Trans isomers may be induced during industrial processing of unsaturated oil or found in the gastrointestinal (GI) tracts of ruminant animals (cattle, sheep, goats, and deer). Trans fatty acids produced by industrial processing, such as partially hydrogenated vegetable oil, have been shown to cause endothelial dysfunction and may affect cardiovascular risk factors. Ruminant trans fats, as found in dairy products, may be beneficial.[16,17]

In fatty acid profiling, it is important to understand this nomenclature because the abbreviations can vary depending on the laboratory used. For example, stearic acid is a saturated fatty acid with 18 carbons and no double bonds (18:0), whereas oleic acid has 18 carbon bonds and one double bond in the n-9 position (18:1n9). Additionally, eicosapentaenoic acid (EPA) has 20 carbons and multiple double bonds and is represented as 20:5n3. This numerical scheme is the systematic nomenclature commonly used by clinical laboratories. It is also possible to describe fatty acid double bonds in relation to the carbon chain's acidic end, symbolized as delta (Δ). Hence, EPA can also be represented as 20:5 Δ5,8,11,14,17.

MEASUREMENT

Monitoring a patient's fatty acid status offers a way to target dietary therapeutics and alter disease progression. For this reason, usual or long-term dietary intake markers are important. Fatty acids can be measured as free fatty acids in serum, as erythrocyte membrane components, or in adipose tissue.

Adipose tissue fatty acid measurement estimates fatty acid intake ranging from 6 months to 2 years.[18] It is therefore the most ideal reflection of dietary patterns. However, adipose tissue biopsy is not practical. Plasma and erythrocyte assessments are more commonly used because of the ease of specimen collection. Because the life of a red blood cell averages 90 to 120 days, erythrocyte fatty acid assessment is more reflective of long-term status and is therefore preferred to plasma evaluation.[18] For example, omega-3 plasma levels have been shown to be influenced by an acutely high fish oil dose or a short-term dietary increase.[19]

Fatty acid biomarkers are sometimes validated by examining the correlation with measured dietary fat intake. Saturated and monounsaturated fatty acid measurements may not always reflect dietary fat intake because these can be endogenously synthesized from carbohydrates.[20]

Erythrocyte fatty acids are reported as a percentage in the red blood cell (RBC) membrane. When dealing with RBC percentages, one must realize that each fatty acid has an effect on the other percentages. For example, fish oil supplementation (n-3) may increase the overall n-3 percentage, which by default may lower the n-6 percentage.

Plasma measurements are expressed as a percentage or absolute total lipid volume concentration. Again, the percentages are interdependent on the whole. After an omega-3 dose, the peak *concentration* can be observed at 6 hours, whereas the peak *percentage* can be seen at 24 hours. Plasma-based metrics can be sensitive to fasting status and acute intake. In general, RBC-based metrics are more stable over time.[21]

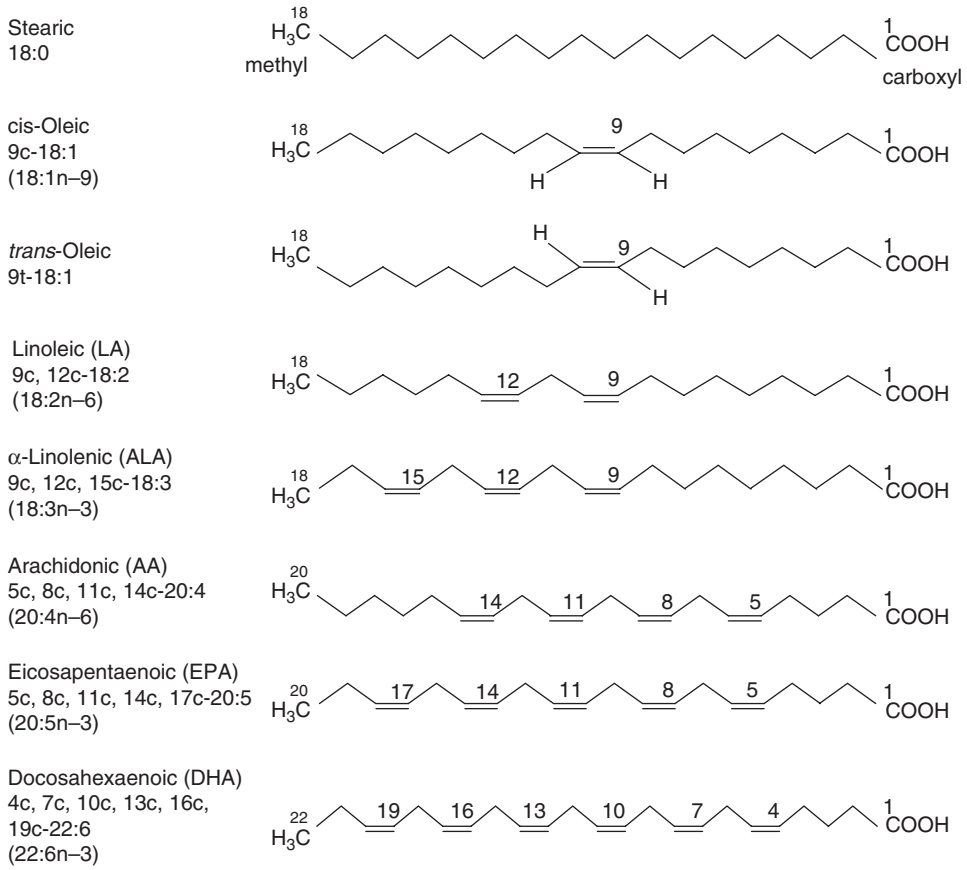


Fig. 13.2 Common dietary fatty acids. Structures of some common dietary fatty acids. Ratnayake W, M, N, Galli C: Fat and Fatty Acid Terminology, Methods of Analysis and Fat Digestion and Metabolism: A Background Review Paper. *Ann Nutr Metab* 2009;55:8–43. https://doi.org/10.1159/000228994

CLINICAL SIGNIFICANCE OF RESULTS

See Fig. 13.2.

Omega-3 (n-3) Polyunsaturated Fatty Acids

Omega-3 fatty acids are positively correlated with healthy aging throughout life and are essential for brain function and cardiovascular health. The average American diet is deficient in n-3 food sources such as oily fish, nuts, flax, and green leafy vegetables. Deficiencies in n-3 fatty acids can result in neurodevelopmental and behavioral disorders, visual changes, skin abnormalities, and heart disease.[22–26]

Many studies show that n-3 fatty acids have significantly positive effects on infant development, cancer, cardiovascular disease, depression, attention deficit hyperactivity disorder, and cognitive decline. These health benefits are mediated through several different mechanisms, including alterations in cell membrane composition and function, anti-inflammatory effects, gene expression, and eicosanoid production.[27]

- **Alpha-linoleic acid (ALA)** is an essential n-3 fatty acid that must be supplied from the diet. From ALA, other important n-3 fatty acids can be endogenously produced by enzymatic elongation and desaturation. ALA is the 18-carbon, 3-double-bond (18:3n3) precursor to make eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), although these are also found in fish oils. ALA dietary sources include green leafy vegetables, walnuts, chia, hemp, and certain plant oils like flaxseed oil and unhydrogenated soybean oil. Several studies on high dietary intake of ALA and higher ALA blood levels show an association with lower fatal coronary artery disease risk.[11] However, there are significant variations in genetics, nutrition, and toxin load that greatly affect an individual's ability to make this multistep conversion.
- **Eicosapentaenoic acid (EPA)** is an omega-3 fatty acid with a 20-carbon chain and five cis-double bonds (20:5n3). It can be enzymatically converted from ALA; however, the efficiency of enzymatic conversion is much lower compared with the absorption from EPA-containing foods. EPA can be obtained by eating oily fish such as cod, mackerel, salmon, and sardines. It is also available in fish oil supplements.

EPA acts as a precursor for prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 eicosanoids. EPA has beneficial effects on multiple atherosclerotic and inflammatory processes, including endothelial function, oxidative stress, foam cell formation, inflammatory cytokine production and release, plaque formation/progression, platelet aggregation, thrombus formation, and plaque rupture. EPA also reduces atherogenic dyslipidemia and has other beneficial effects arising from its inclusion in membrane phospholipids.[28]

- **Docosapentaenoic acid (DPA)** is made by adding carbons to the backbone of EPA using the enzyme delta-5 elongase. It is an intermediary product between EPA and docosahexaenoic acid (DHA) and can retro-convert back to EPA. It is denoted as 22:5n3. Relatively little is known about the potentially distinct DPA health benefits. Although small amounts are found in seafood, it is more often synthesized from EPA.[29]
- **Docosahexaenoic acid (DHA)** is a DPA metabolite with 22 carbons and six double bonds (22:6n3). DHA's endogenous synthesis from its precursor is extremely low, so measured levels largely reflect

seafood consumption or fish oil supplementation. Fish oil supplementation is commonly a combination of purified EPA and DHA.

Cardiovascular disease researchers have studied seafood consumption and fish oil supplementation for both disease prevention and treatment. Fish oil supplementation has been shown to improve serum lipid levels. It prevents cardiac dysrhythmias by increasing cardiac cell membrane fluidity and prevents inflammatory cytokines from binding to their receptors.[27]

In cardiovascular risk assessment, the Omega-3 Index has emerged as an important biomarker in stratifying patients for targeted therapeutics. The Omega-3 Index is the sum of EPA + DHA percentages in RBCs. A target of >8% is optimal, whereas less than 4% denotes increased cardiovascular risk. Fish oil supplementation and dietary changes using fish, flax, chia, and walnuts are often used as therapeutic interventions.

EPA and DHA are also important in fetal brain development. DHA levels positively correlate with the cognitive and retinal development of the fetus.[27] Additionally, there is a role for n-3 in cancer prevention. Diets rich in n-3 (fish oil, flaxseed) and high n-3 erythrocyte concentrations are inversely related to colorectal and breast cancer development, likely due to n-3's anti-inflammatory properties.[27]

Omega-6 (n-6) Polyunsaturated Fatty Acids

Omega-6 fatty acids play a vital role in many physiological functions. They are particularly important for maintaining bone health, regulating metabolism, and stimulating hair and skin growth. In spite of this, n-6 fatty acids are controversial.

The current standard American diet reflects a higher n-6 intake compared with n-3. Dietary sources include vegetable oils and animal fats. Many human evolutionary diets had a 1:1 dietary ratio of n-3:n-6. The fatty acid intake dietary shift toward n-6 sources associated with the cultivation of food and feeding corn to livestock has been implicated in recent increases in disease.[30] The issues surrounding n-6 fatty acids include potentially proinflammatory effects, their increased susceptibility to oxidation, and their competition with the n-3 fatty acids for the enzymatic elongation and desaturation pathways.

Most of the concern regarding n-6 effects revolves around one of its downstream metabolites: arachidonic acid (AA). AA is the primary precursor in the inflammatory cascade. However, AA is not the only n-6 metabolite, and other n-6 fatty acids can be beneficial. For example, dihomo-gamma linoleic acid (DGLA) is a downstream n-6 fatty acid with many anti-inflammatory benefits. Additionally, linoleic acid has shown significant benefits for cardiovascular risks.

- **Linoleic acid (LA)** is an essential n-6 and denoted as 18:2n6. It is the predominant n-6 in the Western diet, obtained mainly from vegetable oils and nuts. Higher LA intake has been shown to reduce low-density lipoprotein cholesterol, promote insulin sensitivity, and reduce hypertension risk. There is a significant inverse relationship between dietary LA intake and coronary artery disease when using LA to replace dietary carbohydrates and/or saturated fats.[12]
- **Gamma linolenic acid (GLA)** is classified as 18:3n6. It can be produced from the essential LA using the enzyme delta-6 desaturase. This is a very slow enzymatic reaction that is further restricted by systemic inflammation, acute and chronic disease, and vitamin and mineral deficiencies such as zinc and cobalt deficiencies.[31] However, GLA can be supplemented using black currant, borage oil, and evening primrose.

GLA levels are important because GLA is the direct precursor to produce dihomo-gamma linolenic acid (DGLA), which is a highly beneficial and anti-inflammatory n-6. GLA supplementation is rapidly metabolized to form DGLA and therefore is a common therapeutic intervention.[32] However, GLA supplementation can also increase the downstream metabolite AA.[8] Supplementing fish oils (EPA/DHA) along with GLA may mitigate this downstream AA conversion because of enzymatic competition for the delta-5-desaturase enzyme. Delta-5-desaturase is responsible for both AA production and EPA metabolism.

- **Dihomo-gamma-linolenic acid (DGLA)**, 20:3n6, has no significant dietary source (very small amounts can be found in some animal products) and is only metabolized from GLA. Research has confirmed that the inability to convert precursor fatty acids to DGLA is associated with many conditions, including diabetes, cancer, and cardiovascular disease.[33]

DGLA exerts anti-inflammatory effects when it is metabolized into eicosanoids and prostaglandins. These two oxidative DGLA metabolites have shown clinical efficacy by suppressing chronic inflammation, lowering blood pressure via vasodilation, inhibiting smooth muscle proliferation associated with atherosclerotic plaque development, arresting cancer cell growth, and aiding in tumor cell differentiation.[33]

- **Arachidonic acid (AA)**, 20:4n6, is a downstream LA metabolite. There are also preformed AA dietary sources, such as animal fats, eggs, poultry, organ meats, and fish. AA is stored in cell membranes and released in response to injury. After AA release, it can be metabolized into eicosanoids through four different pathways: cyclooxygenase, lipoxygenase, cytochrome P450, and oxygen-species–triggered reactions. These pathways yield prostaglandins, isoprostanes, thromboxane, leukotrienes, lipoxins, and epoxyeicosatrienoic acids. Each can act to promote the inflammatory cascade.[34] AA-derived eicosanoids have important roles in immunopathology and have been implicated in inflammation, autoimmunity, allergic diseases, and cancer.[35]

Monounsaturated Fatty Acids

MUFAs are different from other fatty acids because they have only one double bond in their carbon chain. The carbon atom number making up the backbone and the position of this double bond distinguishes one from another and changes their nomenclature. For example, if the double bond is in the seventh position on the carbon backbone, it is known as an omega-7 fatty acid.

The most common dietary MUFA sources are olive oil and nuts. Diets rich in MUFAs have shown beneficial effects on total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. MUFA diets have been shown to reduce LDL oxidative susceptibility, decrease platelet aggregation, increase fibrinolysis, and increase bleeding time.[36] The mechanism involved in MUFAs' health benefits is still being investigated. However, it has been proposed that changes in the composition of very-low-density lipoprotein (VLDL), VLDL enzymes, and VLDL proteins involved in VLDL catabolism decrease plasma triacylglycerol concentrations. Therefore the rates of VLDL production and triacylglycerol clearance may be altered because of dietary fat type and amount.[36]

- **Oleic acid (OA)** has an 18-carbon backbone with one double bond at the ninth carbon. Therefore it is known as an omega-9 fatty acid, 18:1n9. Olive oil is the main source of OA, which is the most represented MUFA in the diet. OA has attracted much attention because of the wide array of literature extolling the "Mediterranean diet," which is rich in olive oil and nuts. Oleic acid has been shown to reduce saturated fatty acids' inflammatory effects on endothelial cells due to a dampening of cytokine activation.[37,38]
- **Nervonic acid (NA)** is an important omega-9 fatty acid, 24:1n9. NA can be found in small amounts in borage and vegetable oils, but mainly it is an oleic acid elongation product.[39] It is abundant in the brain's white matter and necessary for nerve-cell myelin biosynthesis. NA is essential for brain growth and the maintenance of

peripheral nervous tissue enriched with sphingomyelin.[40] Alterations in NA plasma levels have been implicated in mood disorders and demyelinating disorders. Nervonic acid supplementation may also mitigate diabetic neuropathy.[41]

- **Palmitoleic acid (PA)** is a monounsaturated omega-7 fatty acid, 16:1n7. The main PA dietary sources are dairy products and macadamia nuts. However, PA can also be synthesized from triglyceride breakdown or de novo from surplus carbohydrates.

Dairy products are rich in the trans isomer of PA, whereas macadamia nuts contain the cis isomer. In some studies, the trans isomer from dairy has been associated with less inflammation and lower diabetes risk than other trans fats. PA is involved in insulin sensitivity by exerting distinct effects on insulin signaling and glucose uptake.[42]

Macadamia nuts are associated with improving lipid profiles. However, whether the lipid-lowering effects are due to PA specifically, or other oils or nutrients found in these nuts, remains uncertain.[42]

- **Vaccenic acid (VA)** is a monounsaturated omega-7 fatty acid that is also classified as a trans fatty acid (trans-11-18:1n7). It is a positional and geometric isomer of oleic acid. Unlike trans fatty acids produced industrially, VA is naturally occurring. It is formed when saturated fatty acids are bacterially fermented in the GI tracts of ruminant animals (cattle, sheep, and goats). Dairy products (cheese, milk, butter) and meat from these animals contain VA.[43] Although animal and cell studies suggest that VA may be lipid lowering and antiatherogenic, human studies are limited.

Trans Fatty Acids

Trans fatty acids (TFAs) are unsaturated fatty acids with at least one double bond in the trans configuration. There are two primary dietary trans fat sources: naturally occurring TFAs and industrially produced TFAs.

Naturally occurring TFAs are consumed in meats and dairy products from cows, sheep, goats and other ruminant animals. As noted previously, these ruminant trans fats are produced through bacterial metabolism in the animal's GI tract.[44]

But the more common dietary source in the American diet is industrially formed TFAs, as formed in vegetable oil's hydrogenation or partial hydrogenation. The hydrogenation process converts vegetable oils into semisolid fats for use in margarines, commercial cooking, and manufacturing processes. These hydrogenated oils are increasingly used to improve grocery shelf life, increase vegetable oil stability during deep frying, and enhance taste in baked goods. The major TFA sources in the American diet are deep-fried fast foods, bakery products, packaged snack foods, and margarines.[17]

In recent years, TFAs' dietary implications in public health have received increasing attention. The U.S. Food and Drug Administration (FDA) ruled, effective January 1, 2006, that the nutrition labels for all conventional foods and supplements must indicate trans fatty acid content. This was prompted by the evidence that trans fats promote inflammation and increase coronary heart disease risk. Trans fats also increase triglycerides, increase Lp(a) levels, and reduce LDL particle size.[17]

- **Elaidic acid (EA)** is an oleic acid trans isomer (trans-9-18:1). It is the predominant trans fatty acid in the Western diet. EA is found in margarine, partially hydrogenated vegetable oils, and fried foods. EA, like all TFAs, has been extensively studied for its role in increasing cardiovascular risk and adversely affecting lipid profiles. Additionally, EA has recently been shown to enhance metastatic cancer progression.[45]
- **Vaccenic acid (VA)**, as noted previously, is a monounsaturated omega-7 trans fatty acid. It is formed in ruminant animals' GI tracts and consumed in the diet as butter, cheese, and meats from these animals. Although, as with all TFAs, increased VA intake and elevated levels increase risk, there is evidence that VA shows some health benefit in rodent and animal studies, although human studies are limited.[16] VA's metabolic fate has not been extensively studied. It is well absorbed from the diet, but from there, it is either rapidly oxidized or metabolized to other lipids. Data are evolving as to whether VA is preferentially oxidized for energy.[46]

Saturated Fatty Acids

Saturated fatty acids (SFAs) are made up of a carbon chain with no double bonds. Because fatty acids are cell-membrane structural units, this saturated configuration contributes to decreased cell-membrane fluidity. SFAs are not essential nutrients. They are mainly obtained through dietary intake of animal fats. However, the body is capable of synthesizing SFAs from carbohydrates via de novo lipogenesis. The synthesized SFAs are the same FAs found in dietary animal fats. Therefore reducing disease risk using dietary modification should also include carbohydrate reduction.

Most saturated fatty acid studies focus solely on their tendency to alter lipoprotein metabolism and influence cholesterol levels. Additionally, several studies have demonstrated that SFAs may cause adipose tissue inflammation. This inflammatory process involves the signaling of toll-like receptor 4 (TLR4), a sensor that binds lipopolysaccharides. This receptor activation signals nuclear factor-kappa B (NFk-B) production, which triggers the release of inflammatory cytokines like interleukin 1 and 6 (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-α).[47] This inflammatory process is implicated in overall dietary SFA disease risk.

- **Palmitic acid,** or PA (16:0), is a saturated fat naturally found in animal meat and dairy products, as well as in palm and coconut oils. Recent studies reveal evidence that PA excess causes mitochondrial dysfunction mediated by oxidative stress, an effect known as lipotoxicity. Although investigations are ongoing, PA has been linked to an increase in cardiovascular disease risk, cancer risk, and diabetes.[48]
- **Stearic acid** (18:0) is found mainly in meat and dairy products. Stearic acid (SA) increases Lp(a) levels, although the effects are less harmful than those of trans fatty acids.[49] SA is the only saturated fatty acid with a net neutral effect on serum cholesterol ratios.

Although meat is a primary SFA source, not all meat is created equal. Grass-fed beef contains less total fat and tends to contain more conjugated linoleic acid and antioxidants. Grass-fed beef also tends to have much higher cholesterol-neutral stearic acid (18:0) and less cholesterol-elevating SFAs, such as myristic (C14:0) and palmitic (C16:0) FAs.[50]

Lauric acid (12:0) is a medium-/long-chain fatty acid most often found in coconut oil. Lauric acid's metabolic and physiological properties account for many of coconut oil's properties. Lauric acid can be classified as either a medium-chain or long-chain fatty acid. In terms of digestion, however, it behaves more as a long-chain fatty acid because it gets absorbed with chylomicrons. Detailed studies show that most ingested lauric acid is converted directly to energy rather than stored as fat. Most medium-chain fatty acids are absorbed directly into the portal vein and used for energy.[51]

One of coconut oil's advantages is its resistance to oxidation and polymerization, which makes it a stable cooking oil. Because of its high SFA content (92%), coconut oil has always been classified, along with butter, palm oil, and animal fats, as a saturated fat source that should be consumed in low levels. Although the common perception remains that saturated fatty acids are bad, an increasing number of researchers contend that medium-chain fatty acids are less hazardous than hydrogenated vegetable oils, such as soybean and corn oils.[52] Although lauric

acid is a saturated fatty acid, there is no link between lauric acid and high cholesterol.[53] Infrequent to moderate coconut oil use does not seem to raise cholesterol levels.[51,54]

Fatty Acid Ratios

Despite the knowledge that fatty acids are implicated in many chronic diseases, there is a fundamental gap in fatty acid research. No accepted established reference ranges define optimal levels. This results in difficulty interpreting results in relation to disease progression and risk. Therefore ratios between specific fatty acids, separately and in groups, are often used.[55]

- **Omega-6/Omega-3 Ratio:** As touched on previously, Western diets have changed over the past 100 years. A 1:1 balance (n-6:n-3) existed throughout evolutionary history. The current Western diet averages 15:1 to 16.7:1. The shift to n-6 fatty acids correlates with an increase in many chronic conditions, such as cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma, and depression. The proinflammatory effects of preformed AA, and its formation from n-6 precursors, favors increased thromboxane A2, leukotriene B4, IL-1β, IL-6, TNF-α, and C-reactive protein (CRP). By decreasing n-6 dietary intake and balancing this with increases in flax, walnut, chia, fish, and purified fish oils, these inflammatory effects can be mitigated.[55]
- **Arachidonic Acid/Eicosapentaenoic Acid (AA/EPA) Ratio:** EPA, an n-3 fatty acid, metabolically competes with the inflammatory AA to use the same enzyme, delta-5-desaturase, for metabolism. Omega-6 fatty acid dietary intake, specifically animal fats rich in AA, alters essential fatty acid metabolism in favor of inflammation. Adding fish oils, or increasing overall n-3 intake with oily fish, flax, walnut, and chia, shifts delta-5-desaturase activity toward n-3 metabolism and therefore decreases n-6 precursor conversion to AA. Additionally, a decrease in animal fats is helpful.
- **Linoleic Acid/Dihomo-Gamma-Linoleic Acid (LA:DGLA) Ratio:** The n-6 fatty acid DGLA is anti-inflammatory, and the ability to convert precursor n-6 fatty acids to DGLA is paramount because of its ability to alter disease risk and progression.[33] The enzyme responsible for this conversion is delta-6-elongase, which relies heavily on zinc as a cofactor. Delta-6-elongase is quite sensitive to early-stage zinc deficiency. The ratio of LA:DGLA is often used as a zinc status biomarker in humans, in the absence of confounding factors, such as infection or stress. The richest dietary zinc sources include red meats and liver, nuts, seeds, and grains.[56]

Omega-3 Index

The Omega-3 Index was first proposed in 2004 by William S. Harris, PhD, and Clemons von Schacky, MD, as a way of evaluating the risk of death related to coronary artery disease (CAD). It is defined as the RBC percentage sum of EPA + DHA. Estimations show a cardioprotective target to be >8%, and the level associated with increased cardiovascular risk is <4%. The Omega-3 Index allows clinicians to risk-stratify patients and target therapy with purified fish oils or increased dietary intake of n-3–rich foods. Therapeutic interventions are shown to contribute to a reduced risk of CAD-related death.[57–59]

SUMMARY

Fatty acid profiling aids clinicians in disease risk stratification and can direct dietary modification. Patients often misreport fatty acid intake, and food consumption tables can be unreliable. Fatty acid profiling uncovers deficiencies and imbalances that have clinical implications. Dietary and therapeutic targeting may be warranted. Several dietary and supplementation recommendations can be found in Table 13.1, although this list is not exhaustive. Due diligence is recommended regarding supplementation and dietary intake.

TABLE 13.1 Summary of common fatty acid abnormalities with corresponding clinical associations, and treatment considerations.

Biomarker	Abnormality	Clinical Association	Considerations
OMEGA-3			
ALA	Low	Essential FA	Flax, chia, walnut, unhydrogenated soybean oil[10,11]
EPA	Low	Eicosanoid substrate Anti-inflammatory	Oily fish, purified fish oils[28,29]
DPA	Low	Membrane fluidity	EPA, oily fish, fish oil[29]
DHA	Low	Anti-inflammatory	Oily fish, fish oil supplementaion[29]
OMEGA-6			
LA	Low	Essential FA	Sunflower, corn oil, nuts[12]
GLA	Low	Anti-inflammatory, precursor to DGLA	GLA supplementation, evening primrose, borage, black currant. Recommend adding EPA[32]
DGLA	Low	Anti-inflammatory	Add GLA, as above. Recommend adding EPA[32]
Monounsaturated			
OA	Low	Anti-inflammatory, maintains endothelial function	Olive oil[37,60]
NA	Low	Nerve-cell myelin production, elongation product of OA	Olive oil[61]
PA	Low	Anti-inflammatory, serum lipid alteration	Macadamia nuts[42]
VA	High	May contribute to atherogenesis	Decrease beef, dairy, margarine[17,43]
Saturated			
Palmitic	High	Decreased membrane fluidity	Decrease meat, dairy, carbohydrates[47,48]
Stearic	High	Alteration serum lipids	Decrease meat, dairy[49]

TABLE 13.1 Summary of common fatty acid abnormalities with corresponding clinical associations, and treatment considerations—cont'd

Biomarker	Abnormality	Clinical Association	Considerations
Lauric	High	Decreased membrane fluidity	Decrease coconut oil, dairy[51,53,54]
Trans			
Elaidic	High	Proatherogenic	Decrease processed foods, hydrogenated oils, margarine[17,62]
Ratios			
LA/DGLA	High	Enzyme inhibition	Zinc supplementation, or dietary zinc sources: red meats and liver, nuts, seeds, and grains.[56]
AA/EPA	High	Proinflammatory imbalance of FA	Purified fish oil supplementation or fish, Decrease animal fats[63]
ω-6/ω-3	High	Proinflammatory imbalance of FA	Increase dietary fish, walnut, chia, fish oil supplementation Decrease meat, dairy[64]

AA, Arachidonic acid; *ALA,* alpha-linolenic acid; *DGLA,* dihomo-gamma linoleic acid; *DHA,* docosahexaenoic acid; *DPA,* docosapentaenoic acid; *EPA,* eicosapentaenoic acid; *FA,* fatty acid; *GLA,* gamma linolenic acid; *LA,* linoleic acid; *NA,* nervonic acid; *OA,* oleic acid; *PA,* palmitoleic acid; *VA,* vaccenic acid.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Eckel RH, Jakicic JM, Ard JD, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63(25 Pt B):2960–2984.
2. Romano A, Koczwara JB, Gallelli CA, et al. Fats for thoughts: an update on brain fatty acid metabolism. *Int J Biochem Cell Biol*. 2017;84:40–45.
3. Brasky TM, Darke AK, Song X, et al. Plasma phospholipid fatty acids and prostate cancer risk in the SELECT trial. *J Nat Cancer Ins*. 2013;105(15):1132–1141.
4. Liu J, Ma DW. The role of n-3 polyunsaturated fatty acids in the prevention and treatment of breast cancer. *Nutrients*. 2014;6(11):5184–5223.
5. Gan RW, Demoruelle MK, Deane KD, et al. Omega-3 fatty acids are associated with a lower prevalence of autoantibodies in shared epitope-positive subjects at risk for rheumatoid arthritis. *Ann Rheum Dis*. 2017;76(1):147–152.
6. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol*. 2013;75(3):645–662.
7. Muley P, Shah M, Muley A. Omega-3 fatty acids supplementation in children to prevent asthma: is it worthy? A systematic review and meta-analysis. *J Allergy (Cairo)*. 2015;2015:312052.
8. Xu Y, Qian SY. Anti-cancer activities of ω-6 polyunsaturated fatty acids. *Biomed J*. 2014;37(3):112.
9. Arab L. Biomarkers of fat and fatty acid intake. *J Nutr*. 2003;133(3):925S–932S.
10. Harris WS. Alpha-linolenic acid: a gift from the land? *Circulation*. 2005;111(22):2872–2874.
11. Sacks FM, Lichtenstein AH, Wu JH, et al. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. *Circulation*. 2017;136(3):e1–e23.
12. Farvid MS, Ding M, Pan A, et al. Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. *Circulation*. 2014;130(18):1568–1578.
13. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep*. 2001;2(4):282–286.
14. Chang MI, Puder M, Gura KM. The use of fish oil lipid emulsion in the treatment of intestinal failure associated liver disease (IFALD). *Nutrients*. 2012;4(12):1828–1850.
15. Rustan AC, Drevon CA. *Fatty acids: structures and properties*. eLS; 2005.
16. Gebauer SK, Chardigny JM, Jakobsen MU, et al. Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies. *Adv Nutri (Bethesda, Md)*. 2011;2(4):332–354.
17. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *New Engl J Med*. 2006;354(15):1601–1613.
18. Hodson L, Eyles HC, McLachlan KJ, Bell ML, Green TJ, Skeaff CM. Plasma and erythrocyte fatty acids reflect intakes of saturated and n-6 PUFA within a similar time frame. *J Nutri*. 2014;144(1):33–41.
19. Harris WS, Varvel SA, Pottala JV, Warnick GR, McConnell JP. Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: implications for clinical utility. *J Clini Lipidol*. 2013;7(5):433–440.
20. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clini Nutri*. 2007;86(1):74–81.
21. Harris WS, Varvel SA, Pottala JV, Warnick GR, McConnell JP. Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: implications for clinical utility. *J Clini Lipidol*.7(5):433–440.
22. Richardson AJ. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *Int Rev Psychiatry*. 2006;18(2):155–172.
23. Simopoulos AP. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. *Molecular Neurobiol*. 2011;44(2):203–215.
24. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002;106(21):2747–2757.
25. SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res*. 2005;24(1):87–138.
26. McCusker MM, Grant-Kels JM. Healing fats of the skin: the structural and immunologic roles of the ω-6 and ω-3 fatty acids. *Clin Dermatol*. 2010;28(4):440–451.
27. Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. *J Am Diet Assoc*. 2009;109(4):668–679.
28. Borow KM, Nelson JR, Mason RP. Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis. *Atherosclerosis*. 2015;242(1):357–366.
29. Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J Nutr*. 2012;142(3):614s–625s.
30. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)*. 2008;233(6):674–688.
31. Knez M, Stangoulis JCR, Zec M, et al. An initial evaluation of newly proposed biomarker of zinc status in humans - linoleic acid: dihomo-gamma-linolenic acid (LA:DGLA) ratio. *Clin Nutr ESPEN*. 2016;15:85–92.
32. Kapoor R, Huang Y-S. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. *Curr Pharm Biotechnol*. 2006;7(6):531–534.
33. Wang X, Lin H, Gu Y. Multiple roles of dihomo-γ-linolenic acid against proliferation diseases. *Lipids Health Dis*. 2012;11(1):25.
34. Astudillo AM, Balgoma D, Balboa MA, Balsinde J. Dynamics of arachidonic acid mobilization by inflammatory cells. *Biochim Biophys Acta (BBA) Mol Cell Biol L*. 2012;1821(2):249–256.
35. Harizi H, Corcuff J-B, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends Mole Med*. 2008;14(10):461–469.
36. Kris-Etherton PM, Pearson TA, Wan Y, et al. High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am J Clin Nutri*. 1999;70(6):1009–1015.
37. Carluccio MA, Massaro M, Bonfrate C, et al. Oleic acid inhibits endothelial activation. *Arterioscler Thromb Vasc Biol*. 1999;19(2):220–228.
38. Urpi-Sarda M, Casas R, Chiva-Blanch G, et al. Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis. *Pharmacol Res*. 2012;65(6):577–583.
39. Takkunen M, Agren J, Kuusisto J, Laakso M, Uusitupa M, Schwab U. Dietary fat in relation to erythrocyte fatty acid composition in men. *Lipids*. 2013;48(11):1093–1102.
40. Kageyama Y, Kasahara T, Nakamura T, et al. Plasma nervonic acid is a potential biomarker for major depressive disorder: a pilot study. *Int J Neuropsychopharmacol*. 2018;21(3):207–215.
41. Fox TE, Bewley MC, Unrath KA, et al. Circulating sphingolipid biomarkers in models of type 1 diabetes. *J Lipid Res*. 2011;52(3):509–517.
42. Bernstein AM, Roizen MF, Martinez L. Purified palmitoleic acid for the reduction of high-sensitivity C-reactive protein and serum lipids: a double-blinded, randomized, placebo controlled study. *J Clin Lipidol*. 2014;8(6):612–617.
43. Gebauer SK, Destaillats F, Dionisi F, Krauss RM, Baer DJ. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. *Am J Clin Nutri*. 2015;102(6):1339–1346.
44. Remig V, Franklin B, Margolis S, Kostas G, Nece T, Street JC. Trans fats in America: a review of their use, consumption, health implications, and regulation. *J Am Dietetic Ass*. 2010;110(4):585–592.
45. Ohmori H, Fujii K, Kadochi Y, et al. Elaidic Acid, a Trans-Fatty Acid, Enhances the Metastasis of Colorectal Cancer Cells. *Pathobiology: J Immunopathol Mol Cellular Biol*. 2017;84(3):144–151.
46. Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. *Appl Physiol Nutri Metabol*. 2009;34(5):979–991.

47. Ruiz-Núñez B, Dijck-Brouwer DAJ, Muskiet FAJ. The relation of saturated fatty acids with low-grade inflammation and cardiovascular disease. *J Nutri Biochem*. 2016;36:1–20.
48. Mancini A, Imperlini E, Nigro E, et al. Biological and nutritional properties of palm oil and palmitic acid: effects on health. *Molecules (Basel, Switzerland)*. 2015;20(9):17339–17361.
49. Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M. Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein (a), and lipid transfer proteins in healthy subjects. *Am J Clin Nutri*. 1997;65(5):1419–1426.
50. Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutri J*. 2010;9(1):10.
51. Eyres L, Eyres MF, Chisholm A, Brown RC. Coconut oil consumption and cardiovascular risk factors in humans. *Nutri Rev*. 2016;74(4):267–280.
52. Pehowich D, Gomes A, Barnes J. Fatty acid composition and possible health effects of coconut constituents. *West Indian Med J*. 2000;49(2):128–133.
53. Gervajio GC. Fatty acids and derivatives from coconut oil. In: Shahidi F, ed. *Bailey's Industrial Oil and Fat Products*. New York: Wiley; 2005.
54. Pehowich D, Gomes A, Barnes J. Fatty acid composition and possible health effects of coconut constituents. *West Indian Med J*. 2000;49(2):128.
55. Abdelmagid SA, Clarke SE, Nielsen DE, et al. Comprehensive profiling of plasma fatty acid concentrations in young healthy Canadian adults. *PloS one*. 2015;10(2):e0116195.
56. Knez M, Stangoulis JC, Glibetic M, Tako E. The linoleic acid: dihomo-γ-linolenic acid ratio (LA: DGLA)—an emerging biomarker of Zn status. *Nutrients*. 2017;9(8):825.
57. Harris WS. Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as a new risk factor. *Pharmacol Res*. 2007;55(3):217–223.
58. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med*. 2004;39(1):212–220.
59. Baghai TC, Varallo-Bedarida G, Born C, et al. Major depressive disorder is associated with cardiovascular risk factors and low Omega-3 Index. *J Clin Psychiatry*. 2011;72(9):1242.
60. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis*. 2014;13(1):154.
61. Harbige LS, Sharief MK. Polyunsaturated fatty acids in the pathogenesis and treatment of multiple sclerosis. *Br J Nutri*. 2007;98(S1):S46–S53.
62. Sundram K, Ismail A, Hayes KC, Jeyamalar R, Pathmanathan R. Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *J Nutr*. 1997;127(3):514s–520s.
63. Rizzo AM, Montorfano G, Negroni M, et al. A rapid method for determining arachidonic:eicosapentaenoic acid ratios in whole blood lipids: correlation with erythrocyte membrane ratios and validation in a large Italian population of various ages and pathologies. *Lipids Health Dis*. 2010;9(1):7.
64. Simopoulos A. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother*. 2006;60(9):502–507.

14

Food Hypersensitivities

Wendy Hodsdon, ND

OUTLINE

INTRODUCTION

The definitions of terms used for adverse food reactions are variable in the literature, among clinicians, and among the general public. The terms *food hypersensitivity* and *food intolerance* are both used to describe any adverse food reactions. More specific terms, such as *allergies, sensitivities, hypersensitivities,* and *intolerances* are often used interchangeably, although they refer to different types of food reactions. Some more specific nomenclature has been proposed, including *toxic food reactions, IgE-mediated food allergy, non-IgE mediated allergy,* and *nonallergic/nonimmunological diseases.*[1] The National Institute of Allergy and Infectious Diseases defines a food allergy as "an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food." This definition includes all immune-mediated reactions without distinguishing the types of immune responses that cause food reactions but does not consider nonimmunological adverse food reactions.[2,3]

The terms used in this chapter are a compilation of the nomenclature described in the literature.[1,2,4] Food hypersensitivity is the all-encompassing description for nontoxic food reactions. Toxic food reactions include food poisoning due to eating contaminated food from an infectious organism (bacteria, virus, or parasite) or a poison in foods and are not addressed in this chapter. Immunological hypersensitivity includes both food allergies (immunoglobulin E [IgE]-mediated allergy) and food sensitivities (non–IgE-mediated allergy). Food intolerances or nonimmunological hypersensitivities describe reactions due to the lack of an enzyme to digest or process food or food additives or transport disorders.[1,5] Table 14.1 defines the terms along with commonly associated clinical conditions.

The percentage of people with food allergies, sensitivities, and intolerances is objectively unknown because many people do not attribute their symptoms to the food they are eating or attribute symptoms to specific foods but with no objective validation. In 2017 a study looking at food allergies in electronic medical records found that 3.6% of adults in America had verified IgE-mediated food allergies.[6] Yet it is estimated that 30% of individuals alter their diet due to a perceived adverse reaction to food.[7] Adverse reactions to foods (food hypersensitivities) are common, and the prevalence of food allergies is rising.[8]

The emphasis in research has been on the classic food allergies, also known as IgE-mediated food allergies. IgE-mediated allergy reactions affect millions of people, cause significant symptoms that can lead to death, and have high healthcare costs.[8]

Recently, more attention is turning to clinical conditions that are related to food but are not immunologically related. Immunological food allergies do not explain the full range of clinical pathology seen with patients in relation to foods. Food sensitivities are also known as non–IgE-mediated reactions and mixed IgE and non–IgE-mediated reactions. Clinical conditions associated with food sensitivities are diseases such as nonceliac gluten sensitivity, irritable bowel syndrome, eosinophilic esophagitis, food protein–induced enterocolitis syndrome (FPIES), fixed food eruptions, and systemic nickel allergy syndrome.[8] The pathophysiology causing these conditions is being debated in the literature. Sicherer and Sampson in their 2017 review article state, "Despite ongoing investigation, there continues to be little basic understanding of the immunopathogenic mechanisms underlying non-IgE-mediated food allergies."[8] Because the mechanisms are unknown, the descriptions and nomenclature are not clear. This author suggests that *food sensitivities* is the best term to describe non–IgE-mediated allergies, including delayed-onset/chronic and cell-mediated food reactions, that occur from an immunological reaction to foods. Food-specific IgG reactions would be included in the definition of food sensitivities, along with other adverse food reactions with an immunological basis.

The term *food intolerance* is reserved for nonimmunologically mediated reactions to foods from a lack of an enzyme, either a digestive enzyme or a processing enzyme, that causes a reaction to foods. Food intolerances, with this definition, could define 15% to 20% of all adverse food reactions.[3] Many food intolerances are associated with enzyme deficiencies or transportation disorders associated with particular genetic polymorphisms. Examples of food constituents that people are intolerant of include lactose, salicylates, sulfites, sodium glutamate, fructose, histamine, sucrose, tyramine, nickel, preservatives, and more.[3] When these conditions are severe, they are identified at birth and classified according to the metabolic system they affect. Some food intolerances are mild or can be acquired or recognized later in life as people have changes in

TABLE 14.1 Food Hypersensitivity Nomenclature

Adverse Food Reactions = Food Hypersensitivities		
Immune-Mediated Adverse Food Reactions = Immunological Hypersensitivity		Nonimmunological Hypersensitivity
Food Allergy = IgE-Mediated Allergy	**Food Sensitivity =** Non–IgE-Mediated Allergy	**Food Intolerance**
Atopic allergy and non-atopic IgE-mediated allergy	Type IIa, type III, and type IV hypersensitivities	Enzyme deficiencies with subdivisions based on causative agent
Examples include: • Anaphylaxis • Urticaria • Angioedema	Examples include: • Celiac disease • Atopic dermatitis • Eosinophilic esophagitis • Eosinophilic gastroenteritis—food protein induced • Allergic proctocolitis—food protein induced • Enterocolitis syndrome • Oral allergy syndrome	Examples include: • Alcohol intolerance • Lactose intolerance • Salicylate intolerance • Sucrose intolerance • Hereditary fructose • Intolerance • Biogenic amine intolerance • Gliadin intolerance

Modified from Dreborg S. Debates in allergy medicine: food intolerance does not exist. *World Allergy Organ J.* 2015;8:1–6. Bolded terms are proposed by author and/or editor.

dietary habits, digestive enzymes, and liver function or increased chemical exposures (affecting DNA transcription of enzymes and/or microbiome).

This chapter focuses on testing for the immune-mediated reactions (including classical food allergies and food sensitivities) and food intolerances. Various testing methods are used as tools to identify a person's reactive foods. The adverse food reaction test depends on the hypothesized type of food reaction (most often determined by the patient's symptoms) along with the patient's medical history and physical examination. This chapter provides a review of the immunology of adverse food reactions and then looks at the different testing methods.

IMMUNOLOGY OF FOOD ALLERGIES AND FOOD SENSITIVITIES

Most individuals do not have food allergies or sensitivities. Instead, they eat a variety of foods, digest them with varying levels of efficiency, and absorb what they need, and their immune systems develop oral tolerance to the food. Oral tolerance describes how the body learns to not react to an antigen (food) when it is delivered through the oral route.[4] The factors that influence tolerance include the person's genetics, microflora, digestive function, vitamin A levels, lipids, and gut epithelial barrier; the route of exposure (skin versus oral route); and the nature and form of the food when it is consumed.[4]

The mucosal immune system is quite complex, consisting of many parts and functions. Key to food reactions are the regulatory T-cells (Treg cells) in Peyer's patches and mesenteric lymph nodes that produce interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor-β (TGF-β). These cytokines promote B-cell class switching to secretory IgA antibodies (sIgA) and act as a general immunosuppressant for both T and B cell responses.[9] When functioning properly, these allow tolerance to food while still fighting pathogens (bacteria, viruses, parasites, toxins).

Food allergies or IgE-mediated allergy reactions persist in patients with an inappropriate Th2 immune response. Patients with food allergies have food specific CD4+ T cells that express IL-4, IL-5, and IL-13.[9] Research into what causes a breakdown in tolerance and promotes food allergies suggests that adjuvants, alternative routes of sensitization,[9] and loss of intestinal permeability control are all contributing to the increase in food allergies.[10] Adjuvants are substances that stimulate the immune system when given with an antigen. For example, in an illustrative study, mice given cholera toxin (CT) were sensitized to peanut and milk proteins.[11] Allergen-specific IgE antibodies and Th2 cytokines occurred and induced an anaphylaxis response to peanut and milk proteins. The study showed alterations in dendritic cells presenting antigens and in the mesenteric lymph nodes inducing the allergic response.[11] Another hypothesis is that alternative routes of sensitization may be contributing to food allergy reactions. Primary exposure to a food allergen through nonoral routes, such as using creams with peanut oil on the skin, has been implicated in inducing an allergic response.[12] Another paper showed that skin exposure desensitized the allergic response and might help induce a tolerance response in intact skin.[13] Increased intestinal permeability contributes to food allergies through various mechanisms. Efficiency of digestion, transportation of food into the mucosa, mast cell degranulation, and induction of sIgA and cytokines (high levels of IL-4 and IL-9 in the intestines) all contribute to inducing more allergic reactions.[10] The research has focused on understanding food allergy (IgE-mediated allergy) reactions rather than food sensitivity (non-IgE allergy) reactions, although many factors relate to both types of reactions. Genetic background, environmental factors, stress, infection, malnutrition, and microbiota balance all contribute to tolerance and immune-mediated hypersensitivities.[10]

FOOD ALLERGY/HYPERSENSITIVITY/INTOLERANCE TESTING METHODS

A variety of ways to test for adverse food reactions are available, including oral food challenge (OFC), skin-prick testing (SPT), enzyme-linked immunosorbent assay (ELISA) for IgE or IgG antibodies, allergen-specific IgE (sIgE), component-resolved diagnostic tests (CRDs), atopy patch test (APT), breath testing (BT), and controversial energetic testing methods. As immunological methods become more sophisticated, more specific and accurate tests are likely in the future. All methods give different information about adverse food reactions and have specific advantages and disadvantages. Evaluation of adverse food reactions always starts with taking a patient history aimed at identifying the relationship between specific foods and the occurrence of physical symptoms and family predisposition to evaluate possible genetic risk factors. The type of test used depends on the clinical presentation and the results of past tests. Clinical interpretation is required to understand the relevance of the results regardless of the type of testing used.

Oral Food Challenge

The OFC is the most common test used to identify and verify adverse food reactions. The test is extremely versatile and can be used to identify a variety of adverse food reactions, including food allergies, sensitivities, and intolerances. OFCs are indicated to identify the food responsible for acute or chronic symptoms, to expand the diet in patients who have developed tolerance to previously reactive foods, and to identify foods that may have been reactive on other types of testing due to cross-reactivity of antigens.[14]

For patients at risk of severe IgE reaction, OFC for food allergies is performed in an allergist's office or hospital because of the risk of a life-threatening anaphylaxis response.[14,15] A patient with suspected food allergies will eat a gradually increasing amount of food under medical supervision until an age-appropriate dose is reached or symptoms develop. Symptoms often develop in minutes for a food allergy but can appear a couple of hours after the test.[7] This method is recommended for confirming food allergy reactions to reactive foods from SPT and sIgE tests.[14,16]

Food sensitivities and intolerances often have symptoms that are delayed (hours to days after ingesting a food) and are usually not life threatening; therefore OFC can safely be recommended by a variety of clinicians to confirm these types of food reactions.

Method: When testing for food allergies, the OFC can be performed as an open feeding, in which the patient is aware of what he or she is eating; a single-blind OFC, in which the patient is unaware of the food he or she is being fed but the allergist knows what is being tested; or a double-blind, placebo-controlled food challenge (DBPCFC), in which the test food and placebo food are concealed from both the patient and doctor. The DBPCFC is time consuming and difficult to administer and is mostly used in research studies.[15] The challenge food should be eaten in its usual form or the form implicated in the allergic reaction because food processing can significantly affect the allergenic properties of the proteins.[7] OFC is contraindicated when there has been a severe anaphylactic reaction to the test food and when SPT or sIgE exceeds cutoff values, indicating a high probability of severe symptoms with an OFC.

When testing for food sensitivities or intolerances, it is beneficial to eliminate potentially reactive foods from the diet for 8 weeks and then reintroduce foods, watching for symptoms.[2] The precise method will depend on the type of food reaction one is looking for, the clinical presentation of the client, the ability of the person to adhere to a restricted diet, and the preference of the clinician. Methods for testing food sensitivities and intolerances are as follows:

Eliminate potentially reactive foods for 8 weeks:

1. Single suspect food
2. Commonly reactive foods (dairy, eggs, gluten, citrus, nuts/seeds, shellfish, and others)
3. Foods that appear to correlate to physical symptoms
4. Identified reactive foods on food sensitivity test (e.g., food-specific IgG testing)
5. Potentially intolerant foods (e.g., foods containing salicylates or fructose)

Reintroduction in food sensitivities or food intolerances:

1. Encourage the client to keep a journal of symptoms and the timing of reintroduction.
2. Add one food back to the diet by eating 1 serving of the food and waiting 3 days to see if any reactions occur.
3. If no symptoms appear, increase the dose of the food to 2 or 3 servings in a day and observe reactions for the next 3 days.
4. If a reaction occurs, eliminate that food and wait until the symptoms resolve before reintroducing the next food.
5. Continue with the process until all suspected food sensitivities or intolerances have been tested.

Advantages: This method will identify any type of reaction to foods and can be used to confirm a reaction and motivate food elimination compliance, as well as identifying food sensitivities or intolerances. The method is very adaptable and can accommodate a wide variety of people.

Disadvantages: An OFC for a food allergy should only be done with resuscitation equipment available due to possible severe reactions. People can influence the results based on their ideas rather than real reactions when using an open feeding. It is difficult to disguise food for a blinded test. Scoring of symptoms can be useful when evaluating results.[16] When eliminating and reintroducing foods to test for food sensitivities or intolerances, the method can be time consuming and requires the ability to be on a restricted diet for months while keeping good records of symptoms and diet. This method is not appropriate for people with eating disorders or orthorexia.

Clinical Evaluation: Clinicians can consider the severity of the food reaction, the timing and type of symptoms present, and past reactions when developing a specific, individualized protocol. Oral food challenges can be used to confirm associations between foods and symptoms, which can help motivate dietary changes.

Skin-Prick Testing

SPT is the most common test performed by conventional allopathic allergists (MDs) to confirm IgE-mediated allergies, including food allergies and environmental allergies.

Method: SPT is performed by placing a drop of commercially prepared allergen on the skin of the forearm or back and "pricking" or lightly lancing the skin to a depth of 1 mm using a sterile, disposable lancet. After 3 seconds, blot the solution from the skin. A positive and negative control (histamine [10 mg/mL] and physiological glycerine) are used to confirm the reactions. The person is allergic to the allergen when he or she has erythema or swelling greater than that of the positive control in 15 minutes. This method identifies reactions to allergens that produce an IgE-mediated allergy.[17,18] Immunologically, the IgE antibodies that the body has made from past exposure to the allergen are bound to Mast cells that degranulate when the allergen is encountered during the test. A prick + prick (P+P) test is used with fresh foods that have unstable proteins, such as vegetables. This test is done by pricking the fresh food then pricking the patient's skin.[14]

Advantages: SPT is an inexpensive, well-standardized, simple, and low-risk diagnostic test that is more sensitive than ELISA testing for IgE antibodies. There is a high negative predictive value, meaning if there is no reaction on the skin test, there is little possibility of an IgE-mediated allergy assuming the food contains stable proteins in the extracts (such as casein from cow's milk, egg ovomucoid, albumin, and peanut vicilins). When the proteins are not as stable, there is a low predictive value.[14] The test is very accurate for identifying environmental allergies.

Disadvantages: SPT requires that a person be exposed to the allergen before being tested so that the body can produce IgE antibodies. The test can be uncomfortable for patients and is contraindicated in patients with extensive skin disease, those taking antihistamines or other drugs that cannot be discontinued, or those with a recent history of anaphylaxis.[17] The commercial extracts used for SPT contain a mix of major and minor allergenic proteins and can partially degrade during the extraction process. Because these are biological extracts, they may contain cross-reactive allergens.[14] Proteins that degrade with heat have a low predictive value (meaning a negative test does not rule out the possibility of an allergy). With strong clinical indications of food reactions, a P+P test can be used with fresh food.[14] An obvious additional disadvantage is that it primarily detects only IgE-type immunological reactions.

Clinical Evaluation: This test is usually performed by a board-certified allergist. People who report being "tested for food allergies already" have usually had their IgE-mediated food allergies tested through SPT. Intradermal skin testing is a similar, more sensitive test and involves injecting a small amount of the suspected allergen into the skin. This type of testing is more sensitive than SPT but has a higher risk of death, and its value for food allergies is questionable.[17,19]

Radioallergosorbent Testing

Radioallergosorbent testing (RAST) was the first in vitro blood test reported in the literature for identification of IgE antibodies to specific allergens.[20] RAST testing has been replaced by ELISA testing, which is another in vitro test that uses fluorescence instead of radioactivity to perform the test. The basic method of the RAST test has followed different forms as the technology has evolved.

Method: RAST testing is performed using human serum to identify the presence and amount of an antigen-specific IgE antibody present in the serum. In brief, the method involves binding a specific allergen to a solid substrate and adding serum from the person being tested. Antibodies in the serum will bind to the allergen. The sample is rinsed, leaving the bound antibodies behind. A radioactive marker is added to bind to the IgE antibodies bound to the substrate. The radioactive signal can be measured and is proportional to the amount of allergen-specific IgE antibody in the serum. RAST identifies food allergies (IgE-mediated hypersensitivity reactions).[21]

Advantages: This type of testing allows for more convenient identification of allergens in the blood rather than using an SPT. RAST sets the stage for second-generation, allergen-specific antibody tests (ELISA tests) using the same basic method.[22] The newer antibody tests can measure different types of antibodies, use higher-quality allergen extracts, and use different matrix substrates that improve the test accuracy and sensitivity.

Disadvantages: Antibody testing is limiting in that a person needs to be recently exposed to an allergen to have antibodies and a positive result. This type of testing specifically only looks at IgE antibodies and is hampered by cross-reactive proteins and low-quality test agents.[21]

Clinical Evaluation: RAST testing in its original form is not used currently. It has been replaced by quantitative, automated technology, bringing more accuracy and precision to this type of testing.

Enzyme-Linked Immunosorbent Assay

ELISA is a second-generation, allergen-specific antibody test also known as a solid-phase immunoassay. The technology has advanced significantly since RAST was developed in 1961, creating a variety of changes in this type of testing. ELISA test results are reported qualitatively (positive or negative), semiqualitatively (very low, low, moderate, or high reactivity), or qualitatively in the case of some specific tests.

Method: ELISA testing is similar to the RAST test in that an antigen (allergen) is bound to a solid substrate, and serum or whole blood is added to allow for antibodies to bind to the allergen being tested. The sample is rinsed to remove unbound antibodies, and a fluorescent marker is added to bind to the desired antibody. ELISA tests can be used to identify IgE, IgG, IgA, and IgM antibodies depending on the fluorescent marker that is added. sIgE levels are measured routinely to identify food allergies.[8] IgG antibodies could be related to food sensitivities (non-IgE-mediated allergy), although the clinical significance of IgG antibodies to foods is still being debated in the research literature.[23–25] ELISA tests can be performed with whole blood (IgG, IgA), serum (IgE, IgG, IgA, IgM), or saliva (IgA) samples.

Advantages: ELISA testing is versatile, relatively inexpensive, easy to prepare, and readily available, making it a useful screening tool for food reactions.[26] sIgE levels are measured routinely to identify food allergies but should not be used alone to diagnose food allergies.[2] The use of sIgE tests eliminates the risk of systemic reactions that can occur using SPT and OFC,[26] although OFC by a qualified allergist is recommended to confirm food allergies.[16] sIgG testing is generally accepted in the literature for celiac disease, nonceliac gluten sensitivity, and irritable bowel syndrome[24,27] and for patients with unidentified allergic symptoms[25] but is not verified for other symptoms related to food sensitivities.

Disadvantages: A person must have recently eaten the food in question for it to yield a positive result on an sIgG test. More frequent exposure to specific IgG-mediated food sensitivities will induce higher IgG antibody levels in reactive individuals. The amounts of IgG antibodies will decrease as long as the immune system is not reactivated by the food. Cross-reactions between specific food antigens and other agents can cause a positive test reaction even when a person has never eaten the food.

sIgE testing has high false-positive rates, possibly due to cross-reactivity between different similar food proteins.[28] The quality and form of the antigen (food) are difficult to standardize due to natural variability in the extracts and degradation of the testing reagents, which can cause low assay sensitivity.[26] Low assay specificity is found because of the complex mixture of proteins, both allergenic and nonallergenic, in the extracts.[26]

One research study looking at the differences between raw versus processed food allergens showed higher reactivity for processed foods.[29] Most commercially available ELISA tests are made from raw foods, which may mean some processed foods are missed in the testing. Teadorowicz's review article shows how the Maillard reaction increases immunogenicity in food proteins by skewing T-cells to a Th2 response, which most likely affects both allergies and sensitivities.[30] Eggs appear to be a special case, in which cooked eggs (baked) are less reactive than raw or boiled eggs in allergy tests (SPT and IgE ELISAs confirmed with oral food challenge), which highlights the importance of specifying the food antigen in the test being used.[31]

Clinical Evaluation: A positive sIgE test is used with the patient medical history, physical examination, and closely supervised OFC by an allergist to confirm food allergies.[8] Nonconventional medicine practitioners will often use sIgG tests to identify food sensitivities that have not been identified with SPT or sIgE. Confirmation of a food sensitivity also relies on patient history, the physical examination, and the results of sIgG and OFC. The clinical significance of sIgG tests is being debated in the literature, with many articles correctly concluding that sIgG testing does not confirm that a patient has food allergies.[32,33] The research literature is starting to associate positive sIgG tests with specific clinical conditions associated with non–IgE-mediated reactions, such as celiac disease, irritable bowel disease, food protein–induced enterocolitis syndrome, atopic dermatitis, eosinophilic gastrointestinal disorders, and depressive disorders.[24,25,27,34–36] Further research is needed to more fully understand the role of food sensitivities and sIgG testing in clinical pathology.

Specific IgE to Foods

Automated sIgE tests (e.g., ImmunoCAP, Immulite, and HYTEC-288) are automated systems that mimic the ELISA method of IgE antibody detection using traditional extracts containing multiple proteins that are also known as *singleplex extracts.*[22,37,38] Technological advances have allowed quantification of IgE antibodies and specific IgE antibody levels but are not identical between assays and cannot be compared directly.[22,38]

Method: The method is similar to ELISA testing but uses an automated machine and different solid substrates to increase the sensitivity and specificity of the test.

Advantages and Disadvantages: This technology improves on ELISA technology, but it is more expensive and is available only for IgE antibodies. Differences in the composition of the antigens used in the various assays can affect the results of the specificity and comparability of the tests.[22]

Clinical Evaluation: For identifying specific IgE allergens, these methods are less sensitive than skin testing but more sensitive and specific than ELISA testing. This is the most common type of serum testing ordered by a conventional allergist. An OFC is still recommended with automated sIgE tests.[15,18,39]

Component-Resolved Diagnostic Tests or Molecular-Based Diagnosis

CRDs or MBD (ImmunoCAP-ISAC 112) is an IgE antibody technology that uses specific proteins in a food rather than extracts containing a complex combination of food proteins. This allows for identification of which proteins a patient is sensitized to and whether they are dangerous, commonly allergic proteins or relatively harmless proteins.[8,14] This type of testing is sometimes referred to as *multiplex sIgE testing* because it tests for multiple individual allergic proteins.

Method: The microarray biochip is made with purified proteins or recombinant proteins from different common allergens. Common allergens tested include peanut, hazelnut, walnut, soy, rosacea fruits, wheat, and shrimp.[40] Proteins are bound to a microarray biochip, and a small amount of serum or plasma is added. Samples are processed and read with an automated machine to allow protein-specific IgE to be detected.[41]

Advantages and Disadvantages: This technology is being used to identify specific proteins in foods that a person is reacting to. This allows clinicians to correlate clinical reactivity with IgE antibodies to specific proteins in foods or identify if cross-reactivity is occurring with foods carrying homologous proteins.[15,40,42] The utility of CRD has been tested for a variety of commonly allergenic foods and has increased specificity and decreased sensitivity compared with traditional SPT and serum sIgE testing.[37,43,44] The cost of CRD is considered a significant disadvantage for this method.[45]

Clinical Evaluation: Currently, CRD is recommended as an adjunct to SPT and sIgE testing to avoid performing oral food challenges when the risk of severe symptoms is suspected.[15] A more accurate specific immunotherapy (SIT) prescription can be recommended based on the results of CRD for treatment of food allergies.[42,45]

Atopy Patch Test

The APT is most commonly used to identify irritant contact dermatitis and was researched to assess for delayed cell-mediated hypersensitivities to foods (type IV hypersensitivities) but has been shown not to be a useful test for food allergy and is not currently recommended for evaluation of adverse food reactions in the allergy literature.[2,14] APT has also not been shown to be useful in food sensitivities (non–IgE-mediated food allergies) or in children with atopic dermatitis or gastrointestinal reactions to foods, although there may be benefit in more research into these areas.[7] APT may be most useful to identify food intolerance to nickel, incorrectly named systemic nickel allergy syndrome (SNAS). Symptoms include contact dermatitis and gastrointestinal symptoms after eating foods that contain high levels of nickel.[46,47]

Method: APT is performed by using adhesive tape to adhere particular potential allergens to the skin. The patches are left in place for 48 hours, and the skin is examined at 48 and 72 hours for a response. If the skin develops redness or blisters, the person is allergic to the substance.[48]

Advantages: APT is excellent for identifying topical triggers for contact dermatitis. It could be used to identify patients with systemic reactions to nickel from eating nickel-rich foods such as almonds, chickpeas, cocoa, peanuts, and walnuts.[46,47]

Disadvantages: The research examining APT for food sensitivities, atopic eczema, and gastrointestinal diseases has been shown to be unreliable.[2,7] There are not any standardized food reagents for APT, and there is significant variability in interpretation, making it not a useful test for food allergies or sensitivities.[49]

Clinical Evaluation: APT for nickel reactivity may help identify a nickel intolerance.[46,50,51] A controlled, oral food challenge is still necessary to verify SNAS, including a low-nickel diet, which can be challenging to implement.[52]

Breath Testing

BT can be used to determine enzyme deficiencies in some food intolerances and small-intestinal bacterial overgrowth. Many common gastrointestinal disorders with irritable bowel–like symptoms or carbohydrate maldigestion can be identified with BT.

Method: Patients need to prepare for BT by avoiding antibiotics or probiotics for 2 weeks before the test, avoiding prokinetics or laxatives 7 days before the test, and eating only low-fiber meals 24 hours before the test. The patient needs to fast from solids or liquids for 12 hours before the test. A baseline breath sample is taken of hydrogen (or sometimes methane because 20% of patients are not hydrogen producers). The patient is then asked to drink a carbohydrate solution (lactose, fructose, or sorbitol), and breath samples are collected every 20 minutes for the next 2 to 5 hours. Patient symptoms are also recorded. Positive breath tests have increased hydrogen or methane levels.[5,53,54]

Advantages: This diagnostic test is relatively inexpensive, safe, and simple to perform.[54]

Disadvantages: The test can be difficult to prepare for and time consuming to perform. It can take 2 to 5 hours to assess carbohydrate malabsorption. There is variability in how the test is interpreted, and it can be positive for people without clinical symptoms.[54] More research is needed to determine doses for pediatric populations, assess the effect of pre-/probiotics on the test, determine the effect of diet on the test, establish the significance of baseline elevations of hydrogen despite appropriate preparation, and determine optimal time intervals for BT of different substances.[54]

Clinical Evaluation: BT may become a useful diagnostic tool for establishing food intolerances caused by enzyme deficiencies or carbohydrate malabsorption. Although BT is routinely done to diagnose small-intestinal bacterial overgrowth (SIBO), more research will need to be done to establish its use in food intolerances.

Basophil Activation Test

The basophil activation test (BAT) is considered a promising test to identify allergenic foods, but there are challenges in its use outside the research setting. These tests are expensive and require fresh blood and a flow cytometer, which limits their effective use.[8,55,56] OFC can be dangerous in some patients with clinical food allergies. BAT is an in vitro test that is being researched to identify allergies without the need for an OFC after an equivocal SPT or sIgE.[55,57] BAT has the potential to distinguish food allergies from cross-reactivity and to monitor allergic patients on therapy.[56,58] Although this type of testing is not used routinely at this time for allergies, it holds promise for the future.[57,58]

Energetic Methods of Food Sensitivity Testing

Energetic methods of food sensitivity testing, such as Electroacupuncture According to Voll [EAV], Vega testing, Carroll testing, applied kinesiology, electrodermal screening (EDS), and bio-resonance therapy (BRT), are some of the ways alternative medicine practitioners test for adverse food reactions, describing the reaction as energetic and

often not specifying whether the reaction is an allergy, sensitivity, or intolerance. These tests are not validated and most often do not give consistent results.[32,49,59] One study showed that "kinesiology as a diagnostic tool is not more useful than random guessing" after the test was not able to reliably identify wasp venom allergy.[60]

Methods: The methods of these types of tests vary and are generally not elucidated. Most appear to be measuring galvanic skin resistance through an electrical circuit.

Advantages and Disadvantages: Some practitioners have reported good success with these types of devices in identifying food sensitivities and intolerances. Clinical trials using Vega testing and EDS have not shown them to be reliable compared with traditional allergy testing,[59,61] and these tests are not recommended for the identification of food allergies. A review of applied kinesiology literature showed the studies to be unreliable, although many of the studies quoted in the review showed favorable clinical results.[62,63]

Clinical Evaluation: Scientifically, energetic testing methods are unsupported in the literature and should not be used to identify adverse food reactions.

Other Testing Methods

Other testing methods include lymphocyte response testing (ELISA/ACT), cell-size variability testing (ALCAT), provocation-neutralization testing, cytotoxic food allergy testing, and Nambudripad's Allergy Elimination Techniques (NAET), all of which have been used clinically for adverse food reaction testing and treatment. Anecdotal evidence in the form of case studies is positive for NAET[64,65] but could not be found for other testing methods. Systematic, scientific studies either have not been done to verify the clinical relevance of these methods or have shown the tests to be unreliable and clinically questionable.[49,66–68] Review articles suggest that these methods lack scientific rationale, standardization, and reproducibility.[2,14,32,49] Cell-size variability testing had random results with the same sample in a study examining the reproducibility and reliability of this testing method.[69]

CONCLUSIONS

Testing for reactions to foods is being used in clinical practice to confirm or exclude a suspected diagnosis of food allergies, sensitivities, or intolerances and is used as a tool to motivate dietary changes. Selection of the type of diagnostic test and interpretation of the results must be directed by the patient's clinical history, presentation, and the type of suspected adverse food reaction. Clarity around the terms used to describe adverse food reactions will help with the selection and interpretation of testing.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Dreborg S. Debates in allergy medicine: food intolerance does not exist. *World Allergy Organ J*. 2015;8:1–6.
2. Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. *J Allergy Clin Immunol*. 2010;126:1105–1118.
3. The differential diagnosis of food intolerance. *Dtsch Aerzteblatt Online* 2009. https://doi.org/10.3238/arztebl.2009.0359.
4. Brandtzaeg P. Food allergy: separating the science from the mythology. *Nat Rev Gastroenterol Hepatol*. 2010;7:380–400.
5. Berni Canani R, Pezzella V, Amoroso A, et al. Diagnosing and treating intolerance to carbohydrates in children. *Nutrients*. 2016;8:157.
6. Acker WW, Plasek JM, Blumenthal KG, et al. Prevalence of food allergies and intolerances documented in electronic health records. *J. Allergy Clin Immunol*. 2017;140:1587–1591.
7. European Academy of Allergy and Clinical Immunology. EAACI guidelines 2016. In: *A European Declaration on Allergen Immunotherapy*. Zurich, Switzerland: EAACI; 2016.
8. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention and management. *J Allergy Clin Immunol*. 2017. https://doi.org/10.1016/j.jaci.2017.11.003.
9. Steele L, Mayer L, Berin MC. Mucosal immunology of tolerance and allergy in the gastrointestinal tract. *Immunol Res*. 2012;54:75–82.
10. Perrier C, Corthésy B. Gut permeability and food allergies: gut permeability and food allergies. *Clin Exp Allergy*. 2011;41:20–28.
11. Adel-Patient K, Bernard H, Ah-Leung S, et al. Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin. *Allergy*. 2005;60:658–664.
12. Lack G, Fox D, Northstone K, et al. Factors associated with the development of peanut allergy in childhood. *N Engl J Med*. 2003;348:977–985.
13. Dioszeghy V, Mondoulet L, Dhelft V, et al. Epicutaneous immunotherapy results in rapid allergen uptake by dendritic cells through intact skin and downregulates the allergen-specific response in sensitized mice. *J Immunol*. 2011;186:5629–5637.
14. On behalf of the Food Allergy Study Group (ATI) of the Italian Society of Allergy, Asthma and Clinical Immunology (SIAAIC), et al. Guidelines for the use and interpretation of diagnostic methods in adult food allergy. *Clin Mol Allergy*. 2015;13.
15. Kattan JD, Sicherer SH. Optimizing the diagnosis of food allergy. *Immunol Allergy Clin North Am*. 2015;35:61–76.
16. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology–European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol*. 2012;130:1260–1274.
17. Fatteh S, Rekkerth DJ, Hadley JA. Skin prick/puncture testing in North America: a call for standards and consistency. *Allergy Asthma Clin Immunol*. 2014;10:44.
18. Valenta R, Hochwallner H, Linhart B, et al. Food allergies: the basics. *Gastroenterology*. 2015;148:1120–1131.
19. Carr TF, Saltoun CA. Skin testing in allergy. *Allergy Asthma Proc*. 2012;33:S6–S8.
20. Hamilton RG, Franklin Adkinson N. In vitro assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol*. 2004;114:213–225.
21. Gleich GJ, Yunginger JW. The radioallergosorbent test: a method to measure IgE antibodies, IgG blocking antibodies, and the potency of allergy extracts. *Bull N Y Acad Med*. 1981;57:559.
22. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol*. 2010;125:S284–S296.
23. Abrams EM, Sicherer SH. Diagnosis and management of food allergy. *Can Med Assoc J*. 2016;188:1087.
24. Guo H, Jiang T, Wang J, et al. The value of eliminating foods according to food-specific immunoglobulin G antibodies in irritable bowel syndrome with diarrhoea. *J Int Med Res*. 2012;40:204–210.
25. Shakoor Z, Al Faifi A, Al Amro B, et al. Prevalence of IgG-mediated food intolerance among patients with allergic symptoms. *Ann Saudi Med*. 2016;36:386–390.
26. Steckelbroeck S, Ballmer-Weber BK, Vieths S. Potential, pitfalls, and prospects of food allergy diagnostics with recombinant allergens or synthetic sequential epitopes. *J Allergy Clin Immunol*. 2008;121:1323–1330.
27. Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. *Am J Gastroenterol*. 2005;100:1550–1557.
28. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol*. 2016;16:751–765.
29. Vojdani A. Detection of IgE, IgG, IgA and IgM antibodies against raw and processed food antigens. *Nutr Metab*. 2009;6:22.
30. Teodorowicz M, van Neerven J, Savelkoul H. Food processing: the influence of the Maillard reaction on immunogenicity and allergenicity of food proteins. *Nutrients*. 2017;9:835.
31. Calvani M, Arasi S, Bianchi A, et al. Is it possible to make a diagnosis of raw, heated, and baked egg allergy in children using cutoffs? A systematic review. *Pediatr Allergy Immunol*. 2015;26:509–521.
32. Beyer K, Teuber SS. Food allergy diagnostics: scientific and unproven procedures. *Curr Opin Allergy Clin Immunol*. 2005;5:261–266.
33. Hofmaier S, Comberiati P, Matricardi PM. Immunoglobulin G in IgE-mediated allergy and allergen-specific immunotherapy. *Eur Ann Allergy Clin Immunol*. 2014;46:6–11.
34. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology*. 2014;147:602–609.
35. Czaja-Bulsa G, Bulsa M, Gębala A. Food IgG4 antibodies are elevated not only in children with wheat allergy but also in children with gastrointestinal diseases. *BMC Gastroenterol*. 2016;16:39.
36. Karakuła-Juchnowicz H, Szachta P, Opolska A, et al. The role of IgG hypersensitivity in the pathogenesis and therapy of depressive disorders. *Nutr Neurosci*. 2016:1–9. https://doi.org/10.1179/1476830514Y.0000000158.
37. Huss-Marp J, Gutermuth J, Schäffner I, et al. Comparison of molecular and extract-based allergy diagnostics with multiplex and singleplex analysis. *Allergo J Int*. 2015;24:46–53.
38. Park KH, Lee J, Sim DW, et al. Comparison of singleplex specific IgE detection immunoassays: immunocap phadia 250 and immulite 2000 3gallergy. *Ann Lab Med*. 2018;38:23.
39. Manea I, Ailenei E, Deleanu D. Overview of food allergy diagnosis. *Clujul Med*. 2016;89:5.
40. Tuano KS, Davis CM. Utility of component-resolved diagnostics in food allergy. *Curr Allergy Asthma Rep*. 2015;15:32.
41. Shreffler WG. Microarrayed recombinant allergens for diagnostic testing. *J Allergy Clin Immunol*. 2011;127:843–849.
42. Ferreira F, Wolf M, Wallner M. Molecular approach to allergy diagnosis and therapy. *Yonsei Med J*. 2014;55:839.
43. Alessandri C, Zennaro D, Scala E, et al. Ovomucoid (Gal d 1) specific IgE detected by microarray system predict tolerability to boiled hen's egg and an increased risk to progress to multiple environmental allergen sensitisation. *Clin Exp Allergy*. 2012;42:441–450.
44. D'Urbano LE, Pellegrino K, Artesani MC, et al. Performance of a component-based allergen-microarray in the diagnosis of cow's milk and hen's egg allergy: allergen-microarray in the diagnosis of milk and egg allergy. *Clin Exp Allergy*. 2010;40:1561–1570.
45. Larenas-Linnemann D, Luna-Pech JA, Mösges R. Debates in allergy medicine: allergy skin testing cannot be replaced by molecular diagnosis in the near future. *World Allergy Organ J*. 2017;10:32.
46. Da Mata Perez L, França A, Zimmerman J. Systemic nickel allergy syndrome. *World Allergy Organ J*. 2015;8:A89.
47. Di Gioacchino M, Ricciardi L, De Pità O, et al. Nickel oral hyposensitization in patients with systemic nickel allergy syndrome. *Ann Med*. 2014;46:31–37.
48. Dickel H, Geier J, Kreft B, et al. Comparing reliabilities of strip and conventional patch testing: reliability of strip patch test. *Contact Dermatitis*. 2017;76:342–349.
49. Hammond C, Lieberman JA. Unproven diagnostic tests for food allergy. *Immunol Allergy Clin North Am*. 2018;38:153–163.

50. Aslan N, Sezikli M, Erdal E. Nickel sensitivity in patients with gastroesophageal reflux disease. *Cutan Ocul Toxicol*. 2017;36:347–350.
51. Braga M, Quecchia C, Perotta C, et al. Systemic nickel allergy syndrome: nosologic framework and usefulness of diet regimen for diagnosis. *Int J Immunopathol Pharmacol*. 2013;26:707–716.
52. Sharma A. Low nickel diet in dermatology. *Indian J Dermatol*. 2013;58:240.
53. Methodology and indications of H_2-breath testing in gastrointestinal diseases: the Rome consensus conference. *Aliment Pharmacol Ther*. 2009;29: 1–49.
54. Rezaie A, Buresi M, Lembo A, et al. Hydrogen and methane-based breath testing in gastrointestinal disorders: The North American consensus. *Am J Gastroenterol*. 2017;112:775–784.
55. Santos AF, Lack G. Basophil activation test: food challenge in a test tube or specialist research tool? *Clin Transl Allergy*. 2016;6:10.
56. Syed A, Kohli A, Nadeau KC. Food allergy diagnosis and therapy: where are we now? *Immunotherapy*. 2013;5:931–944.
57. Santos AF, Shreffler WG. Road map for the clinical application of the basophil activation test in food allergy. *Clin Exp Allergy*. 2017;47:1115–1124.
58. Sicherer SH, Allen K, Lack G, et al. Critical issues in food allergy: a National Academies consensus report. *Pediatrics*. 2017;140:e20170194.
59. Lewith GT, Kenyon JN, Broomfield J, et al. Is electrodermal testing as effective as skin prick tests for diagnosing allergies? A double blind, randomised block design study. *BMJ*. 2001;322:131–134.
60. Lüdtke R, Kunz B, Seeber N, et al. Test-retest-reliability and validity of the kinesiology muscle test. *Complement Ther Med*. 2001;9:141–145.
61. Semizzi M, Senna G, Crivellaro M, et al. A double-blind, placebo-controlled study on the diagnostic accuracy of an electrodermal test in allergic subjects. *Clin Exp Allergy*. 2002;32:928–932.
62. Cuthbert SC, Goodheart GJ. On the reliability and validity of manual muscle testing: a literature review. *Chiropr Osteopat*. 2007;15:4.
63. Schmitt WH, Leisman G. Correlation of applied kinesiology muscle testing findings with serum immunoglobulin levels for food allergies. *Int J Neurosci*. 1998;96:237–244.
64. Nambudripad R. Alleviation of peanut allergy through Nambudripad's allergy elimination techniques (NAET): a case report. *Glob Adv Health Med*. 2014;3:40–42.
65. Terwee CB. Successful treatment of food allergy with nambudripad's allergy elimination techniques (NAET) in a 3-year old: a case report. *Cases J*. 2008;1:166.
66. Jewett DL, Fein G, Greenberg MH. A double-blind study of symptom provocation to determine food sensitivity. *N Engl J Med*. 1990;323: 429–433.
67. Stein HL. Unreliability of cytotoxic food testing. *West J Med*. 1975;122:491.
68. Teuber SS, Vogt PJ. An unproven technique with potentially fatal outcome: provocation/neutralization in a patient with systemic mastocytosis. *Ann Allergy Asthma Immunol*. 1999;82:61–65.
69. Hodsdon W, Zwickey H. NMJ original research: reproducibility and reliability of two food allergy testing methods. *Nat Med J*. 2010;2:8–13.

15

Genomics, Nutrigenomics, and the Promise of Personalized Medicine

Brian Orr, BA, BS, ND, T. Michael Culp, MA, NMD,* and *Patrick Hanaway*, MD*

OUTLINE

INTRODUCTION

About 3 billion years ago, the experiment known as "life" began on Earth. All living creatures from the five kingdoms are descended from a single common ancestor. We know that all creatures on this planet are related to one another because we all share the same digital "language of life," known as DNA and RNA, made from five simple nucleic acids, connected along a sugar-phosphate chain. If we could meet our primordial progenitor, we might not recognize it as living at all. We suspect it was nothing more than an RNA receptor-catalyst that somehow replicated itself by consuming chemicals in its immediate environment. These simple "ribo-organisms" were inherently unstable, forming and falling apart quite easily. Only over hundreds of millions of years did these organisms gain more stability when single-stranded RNA evolved into double-stranded DNA, and even more stability when the DNA began coding for proteins that would eventually evolve into cellular structure.[1]

Inherent in this narrative is a central fact that is easy to overlook: life began and continues to evolve within specific environments. We all know the classic riddle, "Which came first, the chicken or the egg?" From the perspective of evolutionary biology, this is not much of a riddle because the chicken's ancestors and their eggs preceded the arrival of the chicken by at least 100 million years. However, on closer reflection, the riddle asks a far deeper and more perplexing question, "What is the relationship between the individual and its environment, between the chicken and its egg?" A hospitable environment had to precede the development of life, and no new life can evolve unless there is an environment to support it, but environments change over time, and a species must change to accommodate the new changes in its environment or invariably become extinct, as the vast majority of species that have lived on this planet have done. The central premise of Charles Darwin's grand theory of the origin of species speaks of survival of the fittest, but over time the fittest species is the one that can adapt best to a changing environment. Adaptation is not exclusive to the development of new species; it also plays a critical role in the survival of any individual within a species as well.

The 21st century may well be remembered as the century in which science first truly began to understand the complex interaction between the genetic information inherent in every individual and the environment to which that individual is exposed.

GENES AND ENVIRONMENT—NATURE AND NURTURE

"Environment" is broadly understood in genetics to include everything that is not the genetic information, or genome, itself. Environment includes climate, physical surroundings, exogenous chemicals or toxins, and infectious agents, but it also includes diet, lifestyle, and behavioral factors. Scientists once believed that genes were immutable archives of digital information that simply coded for proteins, which in turn determined the structure and function of an individual's (analog) body. However, it is now irrefutable that gene expression is substantially affected by and sensitive to environmental change. Genes quite literally respond to the environment to which they are exposed.[2] Because genes respond to the environment we subject them to, the environment itself can be proactively manipulated to alter gene expression and, therefore, to change the health state of the individual. This is the central premise of preventive or functional genomics.

In the interactive symphony between genes and environment, the balance between the two may be altered whenever either changes. Because the environment is inherently unpredictable, one effective strategy to increase the chances of survival has been promoting genetic variation within a species because slightly altered individuals may survive environmental change, whereas others may not. It is rather like a strategy for winning a lottery: odds of winning increase linearly with the greater variety of numbers that one can choose. Variety may truly be the spice of life. If populations from a single species diverge into different environments, the selective pressure over time may eventually lead to the creation of separate species.

The human genome is composed of approximately 3 billion nucleotides of genetic code. If you compare your DNA with the next person you happen to meet, you would find that about 1 in every 1000

*Previous edition contributor

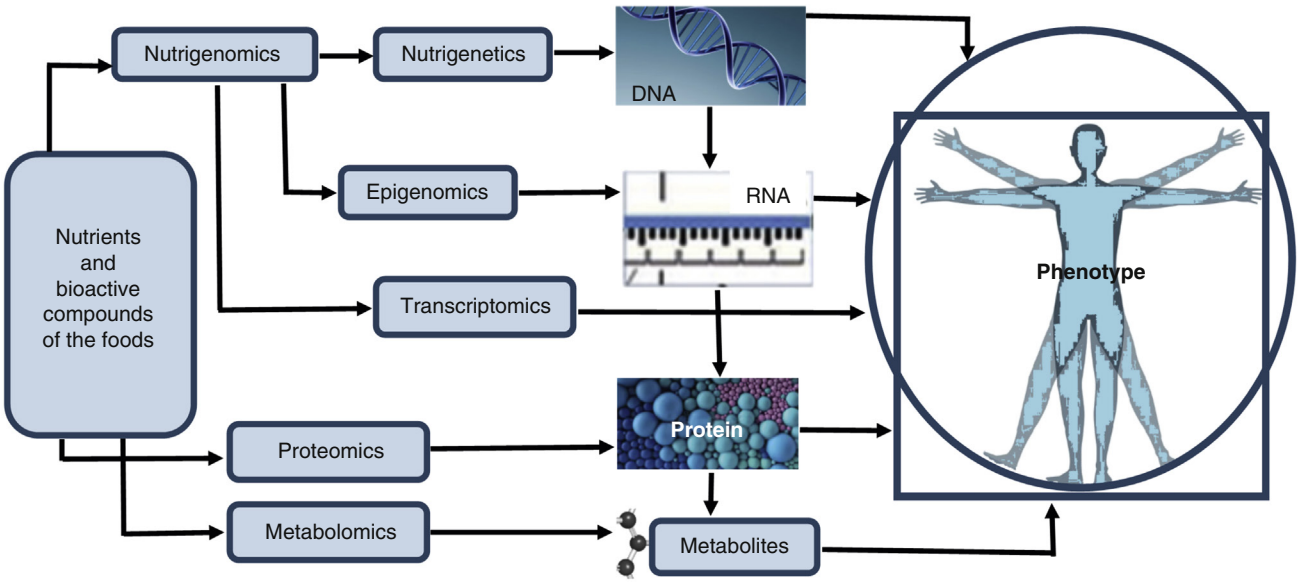


Fig. 15.1 The effects of the nutrients over the genome, proteome, and metabolome. (From Sales NM, Pelegrinin PB, Goersch, MC. Nutrigenomics: definitions and advances of this new science. *J Nutr Metab.* 2014; 202759. PubMed PMID: 24795820.)

nucleotides are different. That means that there are 3 million reasons why the two of you are not the same. These subtle variations of the genetic code are known as polymorphisms (literally, "many shapes," because if the change in the genetic code results in an amino acid substitution, the resulting proteins will have different shapes and slightly altered functions as well). These polymorphisms are largely responsible for our biochemical individuality. Our polymorphisms help make us unique individuals. There are many types of polymorphisms, but by far the most common is the single-nucleotide polymorphism (abbreviated SNP and pronounced "snip"), in which a single nucleotide of the DNA is altered. The sum of all of an individual's polymorphisms significantly affects protein synthesis and physiological function, rendering each individual biologically and biochemically unique.

WHY DO POLYMORPHISMS EXIST?

The theory of natural selection has two central tenets: (1) all organisms compete for limited resources, and (2) organisms with some advantage in acquiring those resources are more likely to survive, to thrive, and to pass on that advantage to their offspring. Polymorphic variations are the ultimate source of these advantages. All polymorphisms that are found to be common in a species must afford some adaptive advantage to survival in some specific environment. Genetic polymorphisms are preserved and become more prevalent in a species when they endow a better chance of survival or of reproduction. The cumulative weight of slight genetic variations over time is the means by which variability within a species arises and by which new species also emerge. However, what may be good for a species and its evolution may not be good for a specific individual because the environmental pressures exerted on a species over millions of years may be very different from the environment encountered in the present.

In individuals, altered polymorphic genes (the legacy of evolution) produce altered proteins, and altered proteins exhibit altered functions. For any given individual, altered protein function may be beneficial, neutral, or harmful, depending on the environment to which he or she is exposed. Prevalent polymorphisms are likely to be beneficial in certain environments but harmful in others. Because we cannot change our genes, the goal of preventive genomics is to alter an individual's environment based on his or her specific genetic variations to optimize his or her genetic potential. Genes themselves cannot be modified, but gene expression can be. One of the common misconceptions of polymorphisms is that they reveal only limitations, but in reality they reveal an individual's potential.

NUTRIGENETICS AND NUTRIGENOMICS

From a clinical perspective, changes in diet are one major way of altering the gene–environment balance and improving an individual's health. Two distinct but interrelated fields of inquiry and knowledge are emerging. Nutrigenetics, also referred to as personalized nutrition, is based on the understanding that our genetic polymorphisms change the way we respond physiologically to specific nutrients. By studying specific polymorphisms and their physiological response to specific nutrients, we can determine a more optimal nutritional regimen for a specific individual based on his or her specific polymorphisms. Nutrigenomics, by contrast, focuses on the effects of specific nutrients (both macro and micro) on the genome and, subsequently, on the body's resulting total pool of proteins (the proteome) and on all its subsequent metabolic activity (the metabolome) as well (Fig. 15.1).[2,3]

Food is, by definition, derived from other living organisms. Inherent in any food is an information code that we read and interpret by eating that food. There is a literal exchange of information that passes between the eaten and the eater. Not surprisingly, central to life is our ability to coordinate our metabolic activities with nutrient availability. Signals from the exterior world (food, toxins, weather, stress, etc.) turn on and turn off specific metabolic processes to improve our chances of survival in an ever-changing world.[4]

The most extreme example of the interaction of food availability and metabolic change is the situation, common in nature, when no food at all is available (i.e., famine). Clive McCay at Cornell University in the 1930s found that by restricting calorie intake in rats by at least 25% from the free-feeding level, he could substantially

increase their average and extreme life expectancies, delay the age of tumor onset, delay cessation of reproductive function, and preserve functional homeostasis or stress response capacity.[5] Since that time, no major investigation has failed to demonstrate significant benefits to health and longevity from calorie restriction. Calorie restriction lowers the level of insulin exposure, which in turn lowers the overall growth-factor exposure, improves age-declining maintenance of mitochondrial function, and helps maintain a long-term favorable balance of the antagonism of insulin to growth hormone.[6] From an evolutionary perspective, this makes intuitive sense. Only when calories are abundant does an individual have the metabolic resources for growth and reproduction. Survival during times of famine necessitates metabolic shifts that conserve resources and promote repair because growth is not an option.[7]

Reducing calorie intake not only lowers overall insulin exposure but is now known to activate the transcription of a family of genes known as sirtuins (SIRTs). The SIRT enzymes appear to have first arisen in primordial eukaryotes, possibly to help them cope with adverse conditions, like famine, and today are found in all plants, yeast, and animals. In response to calorie restriction, SIRT-1 stimulates the production of new mitochondria in skeletal muscle and liver cells, thereby increasing the capacity for metabolic repair and energy production. New mitochondria produce fewer free radicals than the old mitochondria, which leads to less free-radical damage and to delayed onset of metabolic aging. SIRT-1 also has a cascading effect on multiple genes, leading to increased catalase activity, increased free fatty acid oxidation for energy, and reduced inflammation via suppression of the enzyme nuclear factor-κB (NF-κB).[8]

The obvious problem with calorie restriction, however, is that humans like food. Recent studies on the effects of the polyphenol compound resveratrol (initially isolated from grape skins) on obese, sedentary mice suggested that it could mimic the beneficial health effects of calorie restriction even while the mice continued to eat a high-calorie, high-fat diet and did not exercise. Dietary supplementation with resveratrol was found to oppose the effects of the high-calorie diet in 144 of 153 altered biochemical pathways, most of which could be attributed to its activation of the transcription of the enzyme SIRT-1. Resveratrol increased insulin sensitivity, reduced insulin-like growth factor-1 production, increased adenosine monophosphate–activated protein kinase, increased peroxisome proliferator-activated receptor-coactivator-1 (PPAR-1) activity, increased the number of mitochondria, and improved overall motor function.[9] Such research suggests that at some future time we just may be able to have our cake, eat it too, and suffer few of the metabolic consequences of overeating. Nevertheless, the best current nutritional advice for longevity and health remains simply, "eat less." Not only does eating fewer calories lower the insulin load, but it activates the transcription of numerous enzymes that promote mitochondrial regeneration, health, and longevity.

Nutrigenomics helps elucidate the myriad effects of specific nutrients on our genome, as well as helps us rethink and understand the mechanisms by which other nutrients act on our physiology. *Ginkgo biloba* is a commonly prescribed medicinal herb that is known to increase peripheral microcirculation and to contain rather potent antioxidants. The vasodilation allows delivery of these antioxidant compounds to poorly vascularized areas, like the brain. Not surprisingly, ginkgo is commonly used for impaired memory and mental function as we age. Using gene chip assays, researchers examined cellular extracts to look for altered levels of messenger RNA, an accurate measure of gene activity. In vitro studies with human bladder cancer cells that were incubated with ginkgo resulted in suppression of gene transcription by more than 50% in 16 genes and induction of 139 genes by more than 100%. The overall effect of adding ginkgo was to activate genes that code for improved mitochondrial function and antioxidant protection. Subsequent in vitro mouse studies showed activity of more than 12,000 genes and a preferential activation of genes within the brain, with induction of more than 200% of 43 genes in the cortex and 13 genes in the hippocampus, including those genes that promote nerve cell growth, differentiation, regulation, and function, as well as increased mitochondrial activity and antioxidant protection.[10]

This elegant research points to a new understanding of why and how herbal medicines or other specific nutrients can act to change our physiology, but it also helps explain why specific herbs or nutrients act preferentially on specific tissues or organ systems. It is not just *G. biloba* that acts to alter gene transcription. Every medicinal herb, every nutrient, every food, and every pharmaceutical medicine is likely to act in a similar fashion. These compounds do not just have a gross chemical effect on our physiology; they also alter gene expression. Nutrigenomics is forcing us to rethink the ways in which our bodies respond to environmental stimuli in the form of food, herbs, or medicine.

In some areas, nutrigenomics and nutrigenetics can overlap. We propose the use of the term "preventive genomics" to include both nutrigenomics and nutrigenetics because they cannot always be easily separated. To illustrate, PPAR-γ is a nuclear hormone receptor that regulates many cellular functions, such as nutrient metabolism, cell proliferation, and cell differentiation, in response to dietary macronutrients, specifically to carbohydrate and fat intake. PPAR-γ integrates the cellular control of energy, lipid, and glucose homeostasis. Its activation by increased dietary fat or sugar intake is clearly an example of nutrigenomic interaction. However, there is a common polymorphism in the gene that codes for PPAR-γ in which an alanine is substituted for a proline at the 12th amino acid in the protein (the polymorphism is referred to as PPAR-γ P12A). Individuals with the 12A variant display a greater metabolic tolerance to a high-fat diet, leading to a significantly reduced risk of developing insulin resistance, type 2 diabetes, coronary artery disease, and central obesity when consuming a typical Western diet.[11] Individuals with the 12P variant are more sensitive to the ill effects of excessive dietary saturated fat intake, suggesting a clear therapeutic dietary strategy in P-allele carriers to prevent obesity, diabetes, and heart disease.[12]

There are many polymorphisms, like the PPAR-γ proline variation, that exist in a large percentage of the population and appear to increase the risk for certain serious diseases. They beg the question, why do these seemingly harmful polymorphisms exist? It is important to remember that the PPAR-γ proline variation is harmful only in individuals who eat a typical Western diet that is high in calories and saturated fat. It behooves us to remember that in most of nature and for most of human history, too much food to eat was rarely a major risk factor. Quite the opposite was true when food scarcity in winter and famine were common occurrences. In these environments, the ability to extract more nutrition from the same caloric intake would be a distinct advantage for survival. It is only in the past 50 to 100 years in our culture of affluence that these variations have begun to pose significant risks to our health. We call these genes "thrifty genes," a term first coined by D. L. Coleman to explain why the Pima Indians from the desert of the American Southwest were prone to developing obesity and diabetes. Thousands of years of survival in that harsh environment selected for genes that made this group incredibly efficient at retaining calories from food—a distinct adaptive advantage when the food supply was scarce. However, with the 24-hour grocery only a car ride away, their genes are significantly less well adapted to survive.[13] We see similar gene variations that increase inflammation throughout the body. This seems counterproductive until we realize that infectious disease has been a major environmental risk throughout evolution,

and inflammation and immune activation are essentially the same biological process. It is imperative to remember that every polymorphism that exists in humans with significant prevalence confers protection and advantages to survival in some environment. Our task as clinicians is to identify that environment and to recommend it to those patients with that genetic variation.

NATURE VERSUS NURTURE

The reality is that the prevention and cure of complex diseases and syndromes are not to be found exclusively in our genes or our environment but in the interactive symphony between the two. Nature (our genes) provides a plastic template that is largely adaptable to a wide range of environments ("survival of the most adaptable"), and slight variations in those genes can cause altered responses to specific environments (nutrigenetics). In contrast, nurture (our environment) switches genes on and off, largely controlling gene expression (nutrigenomics).

To illustrate this idea of gene–environment interaction, consider the research of Caspi et al.[14] They studied variations in the promoter sequence for the gene coding for monoamine oxidase-A (MAO-A) and found that a promoter polymorphism caused some people to have high-activity MAO-A genes and others to have low-activity genes. Those with high activity MAO-A would deactivate catecholamine neurotransmitters, like dopamine and noradrenaline, more rapidly. They then examined whether these genes played a role in antisocial and violent behavior in men who had been abused as children. Remarkably, they found that men with the high-activity MAO-A gene were virtually immune to the effects of maltreatment as children, seldom if ever becoming violent offenders, whereas men with the low-activity MAO-A gene were much more antisocial and violent, but only if they themselves were abused as children. In other words, for violent behavior to manifest in adulthood, both the low-activity gene (nature) and childhood maltreatment (nurture) needed to be present. If either was missing from the equation, the adult was very likely to be well socialized and nonviolent.

The Centers for Disease Control and Prevention (CDC) published a *Gene-Environment Interaction Fact Sheet* in August 2000[15] that outlines the basic principles of a broad understanding of the causal interaction of genes and environment in human disease. In it, the CDC makes four main points:

1. Virtually all human diseases result from the interaction of genetic susceptibility and modifiable environmental factors.
2. Variations in genetic makeup are associated with almost all disease.
3. Genetic variations do not cause disease but, rather, influence a person's susceptibility to environmental factors.
4. Genetic information can be used to target interventions.

In this brief paper, the CDC essentially outlined the chief tenets of nutrigenetics and of preventive genomics.

Ironically, these ideas are hardly new. In 1909 Archibald Garrod[16] published *Inborn Errors of Metabolism,* in which, after identifying the first human disease that behaved as a true mendelian recessive trait (alkaptonuria), he went further to construct a sweeping hypothesis that altered heredity was "the seat of chemical individuality." "Inborn errors of metabolism," he wrote, "are due to a failure of a step in the metabolic sequence due to loss or malfunction of an enzyme." By examining the subtle end products of metabolism, he continued, we should be able to identify the differences that altered heredity produces in each individual. This is a remarkable insight, given that the words *gene* and *genetic* did not exist in 1909, and it would be roughly 50 years before the structure and true function of DNA were confirmed. Moreover, Garrod's book was published 3 years before the first vitamin was discovered, so he would have had no notion of vitamins as cofactors in enzymatic reactions. He concluded his prophetic work by envisioning the complex interaction between our unique genetic constitution and environmental factors in the exquisitely simple statement "These idiosyncrasies may be summed up in the proverbial saying that one man's meat is another man's poison."

THE CLINICAL UTILITY OF NUTRIGENOMICS AND NUTRIGENETICS

The point of using genetic and genomic information in a clinical setting is to personalize the therapeutic regimen and develop an effective strategy toward true disease prevention. It is a common mistake, however, to think that somehow the new preventive genomic information we can access will make all previous therapies obsolete. This is evident in the mind-set that thinks we can attribute disease risk to polymorphisms without reference to environment (read any genetic newspaper headline). Simply put, genetic information is no more and no less valuable than environmental information. We need them both to make an optimal difference. Furthermore, at this stage in preventive genomic research, there are, at best, only 100 or so polymorphisms about which we have sufficient clinical information to make personalized nutritional recommendations.[17] Although it is useful information, it is insufficient for comprehensive nutritional and therapeutic recommendations.

Carl Sagan once said, "If you want to make an apple pie from scratch, you must first create the universe."[98] Fortunately for making an apple pie, the universe has already been created, and fortunately for making comprehensive nutritional recommendations, natural selection has been active since the beginning of life on this planet. As humans, we and our ancestors have been adapting to environments for the past 3 billion years, if you want to think of all life, or for a mere 600 million years, if you want to think of eukaryotic cells with aerobic respiration. Either way, it is a lot of experimental trial and error that brought us to the present. The proper starting point of nutrigenomics and nutrigenetics is not necessarily genetics but good epidemiology. Good epidemiology can provide the information necessary to determine the best nutrition and lifestyle for the average person. Specific genetic polymorphisms can help health professionals modify these recommendations to meet the specific genetic needs of an individual patient.

Discovering the optimal nutrition and lifestyle for a specific individual will depend on developing a functional matrix that is minimally composed of the following:

1. Good epidemiological dietary and lifestyle data
2. Specific genomic polymorphisms that alter specific macronutrient and micronutrient requirements
3. Functional laboratory assessment of individual function, physiology, and micronutrient status

In terms of Western diets, the Mediterranean diet is one of the best studied, and from an epidemiological perspective, it is arguably the best for disease prevention and optimal health. It has been demonstrated to reduce cholesterol and triglycerides; to prevent atherosclerosis and high blood pressure; and to reduce the risk of senility, stroke, heart disease, insulin resistance, type 2 diabetes, and numerous cancers, including those of the breast, prostate, and colon.[18] Among elderly Europeans, a Mediterranean diet combined with moderate daily activity and no smoking reduces all-cause mortality by 67%, and switching to this healthier diet and lifestyle at age 70 reduced mortality rates by 50%.[19]

Clearly, these results are impressive, but the central tenet of preventive genomics is that these risks can be reduced further with the judicious application of nutrigenetic information gathered from the analysis of individual

polymorphisms. Not all polymorphisms are clinically relevant. Most of our interindividual genetic variation occurs in sections of the DNA that do not actually code for proteins. About 97% of our DNA is either of viral origin, repeats, or simply junk DNA that codes for nothing. To make genomic information clinically useful, polymorphisms must meet four essential criteria; they must be relevant, prevalent, modifiable, and measurable.

1. First, the only polymorphisms of clinical interest in the genome are those that exert an effect on some specific aspect of our biochemistry and physiology (relevant).
2. Second, given our current knowledge of the human genome, only polymorphisms that exist in a substantial portion of a population are likely to be able to be demonstrated in epidemiological and case-controlled studies to be clinically relevant—in essence, we compare polymorphisms that occur in substantial numbers in both groups (prevalent).
3. Third, only polymorphisms whose genetic expression is modifiable via reasonable clinical intervention are clinically useful. Such an intervention may be any modification of environment, including diet, lifestyle, and targeted nutraceuticals or pharmaceuticals. Although genes themselves are not modifiable, the phenotype they generate is modifiable via environmental changes because environment turns genes on and off.
4. Finally, because our genes do not themselves change, we must be able to measure changes in our functional physiology to determine that the environmental changes implemented have been effective in modifying the phenotypic or physiological expression of our unique genes. For this purpose, functional testing must be available and used in conjunction with genomic testing for polymorphisms (measurable).

To illustrate this model of clinical utility, let us consider the example of the detoxification and antioxidant protection afforded by the enzyme glutathione-S-transferase (GST). High levels of toxins and oxidative stress are associated with numerous degenerative diseases and with the aging process itself. Thus an enzyme like GST that protects against such oxidative damage is highly relevant to our health. The GST polymorphisms have been associated with numerous cancers in epidemiological and case-control studies.[20,21] Although there are several isoforms of GST in the body, the μ isoform, *GSTM1,* is the most common in the liver. More than 75% of Caucasians and about 25% of Africans exhibit mutations resulting in complete *GSTM1* deletion.[22] Because other isoforms of GST and other antioxidant pathways exist, it is possible to improve antioxidant protection by maintaining a high reduction potential through exogenous avenues, including dietary antioxidants. Furthermore, men and women who lack the *GSTM1* gene altogether can increase their serum glutathione levels by 16% and 38%, respectively, and their other GST activity by 6% and 8%, respectively, simply by eating four or more servings of brassica vegetables weekly. Interestingly, individuals possessing a *GSTM1* gene showed significantly less benefit.[23] Similar benefits were seen from regular brassica vegetable consumption in reducing colorectal cancer by 53%[24] and lung cancer in smokers by 70%,[25] but again only in individuals lacking the *GSTM1* gene. Thus the increased oxidative stress is attenuated by regular brassica vegetable consumption. Finally, we can validate the clinical efficacy of our intervention strategy by measuring functional levels of oxidative stress through any number of simple laboratory tests, such as urine lipid peroxides, 8-hydroxy-deoxyguanosine, serum glutathione, urine, or plasma cysteine/cysteine ratio, for example. The biological effects of our interventions are measurable.

Personalized Nutrition and Nutrigenetics

Most of the clinically relevant nutrigenomic information available relates to the relationship between polymorphisms with increased chronic disease risk and the dietary therapies and supplements that have been demonstrated to treat the physiological imbalance effectively. This is particularly true of SNPs. In as many as one third of genetic polymorphisms, the corresponding enzyme has a decreased binding affinity for a vitamin or mineral coenzyme, resulting in a lower rate of reaction and altered enzyme function (Fig. 15.2). In a series of review articles, Ames[26] and Ames et al.[27] provided evidence of more than 50 human diseases involving defective enzymes that could be remedied or ameliorated by the administration of higher doses of vitamins or minerals, which at least partially restore enzyme activity. Thus the clinical validity of high-dose nutrient therapy is established but only in genetically susceptible individuals.

CLINICAL APPLICATION

Cardiovascular Disease

Cardiovascular disease accounts for approximately 40% of all deaths in most industrialized countries and is also responsible for significant morbidity and diminished quality of life. Furthermore, using data

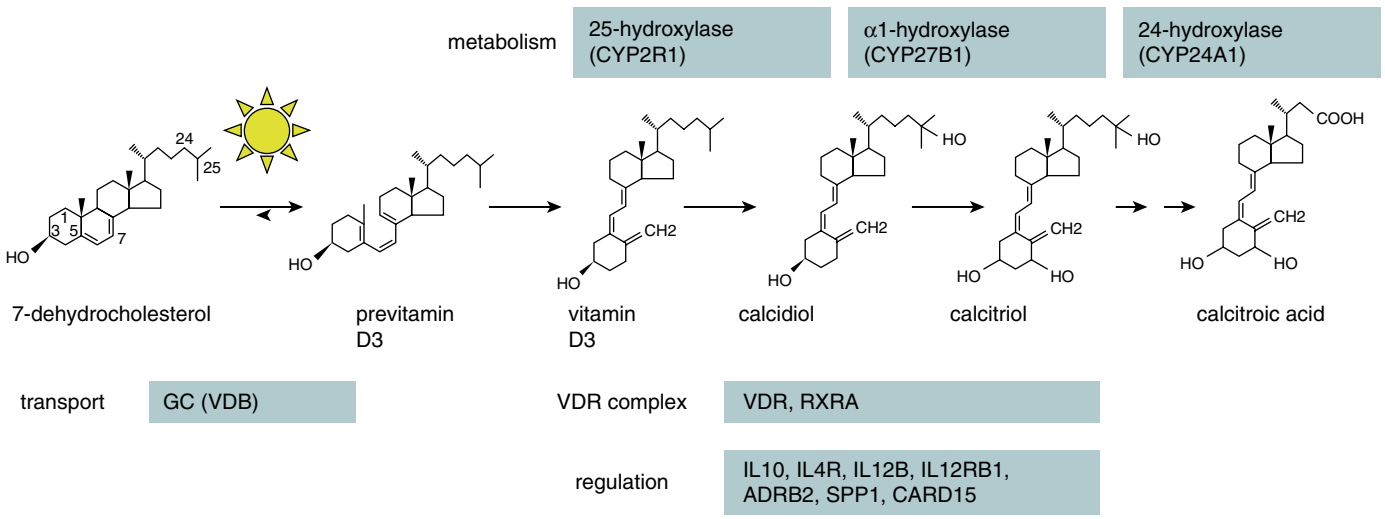


Fig. 15.2 Pathway diagram of genes involved in vitamin D metabolism. (From Wjst M, Altmuller J, Faus-Kessler T, et al. Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signaling pathway. *Resp. Res.* 2006;7:60. PubMed PMID: 16600026.)

from more than 84,000 women who were monitored for 14 years, epidemiology researchers at Harvard University estimated that about 83% of cardiovascular disease could be decreased if everyone ate a reasonably healthy diet, exercised daily, did not smoke, maintained a normal weight, and drank 1 to 2 alcoholic beverages per day.[28] Although these five diet and lifestyle changes seem fairly simple, only 3.1% of the women in the study actually adopted them. Not only is compliance an issue, but epidemiological studies raise deeper philosophical questions because such studies assume that all participants are essentially similar to one another. Are the same therapies equally effective for all individuals? Do all individuals need the same quantities of these diet and lifestyle modifications? Can preventive genomic testing help us individualize our therapeutic protocols?

Let us begin by asking what proportions of carbohydrate, protein, and fat in the diet are the most effective in maintaining normal serum cholesterol levels. Is it a low-fat, low-cholesterol diet as touted by Nathan Pritikin and Dean Ornish? Or perhaps a 40-30-30 (40% carbohydrate, 30% protein, 30% fat), low-calorie "Zone" diet as proposed by Barry Sears? Or a low-carbohydrate, high-protein, high-fat diet such as that made popular by Robert Atkins? Logically they cannot all be the best diet for everyone, yet some people swear by each of them as the answer for an optimal diet.

There is mounting evidence that optimal macronutrient proportions in the diet may be a function of specific polymorphisms. Apolipoprotein-E (Apo-E) is a molecule that mediates the interaction of chylomicron remnants and intermediate-density lipoprotein particles with lipoprotein receptors, including the low-density lipoprotein (LDL) receptor and the chylomicron remnant or Apo-E receptor. It has been suggested that Apo-E allelic variation may account for ≤7% of the variation in total and LDL-cholesterol concentrations in the general population.[29] There are three variations of the Apo-E gene, known as Apo-E2, Apo-E3, and Apo-E4, and these polymorphic variants are important genetic modifiers of serum lipid responses, which consequently may significantly affect an individual's risk for development of coronary artery disease. (Technically, each of these polymorphisms is a haplotype: two individual SNPs at different points in the Apo-E gene that are linked.) Population studies have shown that plasma LDL-cholesterol levels are highest in subjects carrying the Apo-E4 allele, intermediate in those with the Apo-E3 allele, and lowest in those with the Apo-E2 allele.[30] The E3/E3 genotype is the most common in all populations and serves as the benchmark genotype for comparison with any other possible genotype. The Apo-E4 allele is associated with a moderately increased risk of atherosclerosis and coronary artery disease (odds ratio [OR] = 1.53 for men and 1.99 for women in one study).[31] The increase in the risk of all cardiovascular disease in men carrying the Apo-E4 allele is 63% and is 56% for women.[32] Conversely, LDL-cholesterol levels declined with each Apo-E2 allele by 8.8 mg/dL in Hispanics, by 25.6 mg/dL in non-Hispanic white persons, and by 18.1 mg/dL in African Americans.[33]

The association of the Apo-E4 allele with elevated serum cholesterol levels is greatest in populations that consume diets rich in saturated fat and cholesterol compared with other populations. Thus the higher LDL-cholesterol levels observed in people carrying the Apo-E4 allele are manifested primarily in the presence of an atherogenic diet characteristic of Western societies and that the response to dietary saturated fat and cholesterol may differ among individuals with different Apo-E genotypes. Many studies have been conducted to prove this hypothesis.[34] Given that each person inherits two alleles, there are six possible genotypes: E2/E2, E2/E3, E3/E3, E2/E4, E3/E4, and E4/E4. In patients with elevated serum total and LDL-cholesterol, the cholesterol-lowering response to a low-fat, low-cholesterol diet increased as the sum of the allele numbers increased (in other words, response improved in the order of the previously listed six genotypes).[35,36] Conversely, individuals carrying an E4 allele who ate a high-fat diet were much more likely to have elevated serum cholesterol.[37] In contrast, serum triglyceride levels were found to be significantly higher in men, and to a lesser extent in women, who carried an E2 allele. Moreover, the triglyceride levels of individuals with an E2 allele showed a dose-dependent relationship to table sugar consumption, an association not shared with the other genetic variations.[38] Dietary sucrose consumption also increased very low-density lipoprotein (VLDL) cholesterol and triglycerides in Apo-E2 carriers. In contrast, E3/E3 individuals were found to have the lowest triglyceride levels, and E4 carriers were found to have intermediate levels.[39] In another multiethnic study, plasma triglyceride levels were inversely correlated with the number of Apo-E4 alleles (175, 159, and 143 mg/dL with 0, 1, and 2 alleles, respectively).[40] Another study found E4 carriers to have elevations in triglycerides, but only those who consumed alcohol regularly.[41] A study of Turkish men who did not consume alcohol found no elevated triglyceride values in E4 carriers.[42] The most effective dietary therapy to lower triglycerides is restricting carbohydrate, especially sugar, consumption. Because Apo-E2 men are prone to significant triglyceride elevations and their levels are less sensitive to dietary fat intake, a lower-carbohydrate, higher-protein, higher-fat diet may be therapeutically desirable for them.

Individuals with the E3/E3 genotype are moderately affected by dietary fat intake but tend to have lower triglyceride levels than the other genotypes. In one study, individuals with high cholesterol and triglyceride values were treated with a short-term (7-day) very low-calorie juice fast (208 calories/day), and their responses were stratified by Apo-E genotype.[43] Only E3/E3 individuals experienced significant improvement in all parameters, experiencing reductions in LDL cholesterol by 10% and in triglyceride values by 18%. In these individuals, it is tempting to speculate that the low-calorie, moderate 40-30-30 carbohydrate-protein-fat ratio "Zone" diet might be more effective in treating both hypercholesterolemia and hypertriglyceridemia, although no clinical trials to validate this hypothesis have yet been published. Also, in this trial, E2 carriers experienced a 31% drop in LDL levels but a 15% increase in triglyceride levels, lending some additional support for the carbohydrate-sensitive triglyceride hypothesis. E4 carriers had an opposite response to E2 carriers: a dramatic reduction of triglyceride levels by 49% but a rise in LDL levels by 13% while eating only 200 calories/day.

Other dietary interventions that affected cholesterol levels were fiber and alcohol. High intake of soluble fiber (5.7 g/day) reduced LDL cholesterol by 6.6% and 5.6%, respectively, in E3 and E4 allele carriers but had little effect on E2 carriers.[44] Similarly, a study of Korean patients with coronary artery disease found that replacing white rice with whole grains produced the greatest benefit in E3/E3 individuals (12% reduction in triglyceride, 8% reduction in LDL cholesterol, and 8% increase in high-density lipoprotein [HDL] cholesterol levels), as well as moderate benefit in E4 carriers (including a 15% increase in HDL cholesterol values), but there was absolutely no effect on cholesterol levels in E2 carriers.[45] Thus a high-fiber diet may be clinically most useful for E3/E3 individuals, modestly useful for E4 carriers, but not useful for E2 carriers.

The modest consumption (1–2 drinks) of alcohol daily has been shown epidemiologically to be extremely protective against coronary artery disease in the general population. However, Apo-E genotyping reveals that the benefits are not the same for everyone. In a comparison of nondrinkers with drinkers of alcohol, women who were drinkers had lower cholesterol (total and LDL) than women who did not drink, regardless of Apo-E genotype. In men, however, Apo-E2 carriers who were drinkers had lower cholesterol, but Apo-E4 carriers had higher

TABLE 15.1 Lipid-Lowering Therapies Stratified According to Apolipoprotein-E (Apo-E) Genotype

	GENOTYPE[a]		
Therapy	**E2/2 or E2/E3**	**E3/E3**	**E4/E3 or E4/E4**
Diet	Lower carbohydrate	Low calorie, moderate fat	Low fat, no cholesterol Soluble fiber
Alcohol intake	Daily (women and men) (little effect for men)	Daily (women) None (men)	Daily (women)
Exercise	Moderate	Moderate	High-intensity
Pharmaceutical/nutraceutical	Bile sequestrants Statins	Statins	Probucol Statins

[a]The E2/E4 genotype is extremely rare and therefore lacks statistical power in both association studies and prospective trials.

cholesterol, and alcohol consumption had no significant effect for Apo-E3/E3 individuals.[46]

It is worth observing that moderate exercise, although not dietary therapy, was found to be effective in improving serum lipids in E2 and E3 genotypes but not in E4 carriers.[47] In another study, high-intensity physical exercise was most effective in E4 carriers.[48] Thus E2 and E3 carriers may benefit from modest daily exercise, but for an individual with an E4 allele to benefit from exercise, that exercise must be high intensity.

Similarly, knowledge of Apo-E genotype may allow for a more discriminating and more effective use of targeted pharmaceuticals for patients with lipid abnormalities. Statin drugs exhibited a greater cholesterol-lowering effect in Apo-E2 carriers, followed by Apo-E3/E3, with less effectiveness for Apo-E4 carriers. Apo-E2 carriers also showed a better response to gemfibrozil and cholestyramine than the other genotypes. The greatest cholesterol-lowering effects for Apo-E4 carriers came from probucol, a potent antioxidant.[31] However, although statins had a weaker cholesterol-lowering effect in Apo-E4 carriers, statins were most protective in preventing a second heart attack in men with an E4 allele. Statin use reduced the risk of a second heart attack in E4 carriers by 64% compared with only 33% for other genotypes, even though statins reduced LDL cholesterol levels least in Apo-E4 genotypes.[49]

More prospective clinical trials are needed to fully elucidate the optimal therapeutic dietary interventions to protect against coronary artery disease in individuals based on their Apo-E genotype status. However, distinct patterns are beginning to emerge in terms of dietary and lifestyle management for the treatment of coronary artery disease; these are summarized in Table 15.1.

Other variants within the Apo-E gene locus have also been investigated in relation to the association with lipid phenotypes and response to dietary intervention. Evidence suggests that variability in the Apo-E promoter region is associated, independently of the traditional E2, E3, and E4 alleles, with plasma lipid levels,[50] dietary response,[51–53] and cardiovascular disease risk.[54]

Apo-E polymorphisms are the best studied in terms of their association with atherosclerosis and coronary artery disease, but other polymorphisms exert similar effects. Cholesteryl ester transfer protein (CETP) is responsible for the transfer of insoluble cholesteryl esters from HDL to other lipoproteins.[55] The Taq1B polymorphisms of the *CETP* gene result in increased CETP levels, with impaired ability to remove cholesterol from the cells and the bloodstream. The Taq1B polymorphism occurs with lower HDL cholesterol levels and an increased risk of development of atherosclerosis and coronary artery disease.[56,57] In one study, individuals who were homozygous for the Taq1B polymorphism had larger reductions in LDL and VLDL levels through eating a low-fat diet with a high ratio of polyunsaturated to saturated dietary fat.[58] Furthermore, daily moderate alcohol consumption raised HDL levels substantially but only in individuals with the CETP polymorphism.[59,60]

E-selectin (SELE) is a glycoprotein molecule that adheres circulating neutrophils to the endothelial lining of blood vessels. E-selectin is expressed on the surface of endothelial cells after stimulation by inflammatory mediators (mediated by NF-κB). Several polymorphisms in the SELE gene increase adhesion activity of E-selectin, dramatically increasing the risk of atherosclerosis and premature coronary artery disease.[61,62]

The primary therapeutic aim for carriers of a SELE polymorphism is to decrease NF-κB stimulation, in turn reducing SELE expression. Maintaining high antioxidant potential has been shown to decrease NF-κB activation.[63,64] Thus higher levels of dietary antioxidants and possibly supplemental antioxidants may be beneficial. The proinflammatory cytokines tumor necrosis factor-α and interleukin-1 also increase NF-κB activation. Antioxidants, fish oil,[65] and milk thistle (silymarin) supplementation have each been shown to suppress interleukin-1 and tumor necrosis factor-α production directly and would therefore lower NF-κB activation.

The *MLXIPL* gene codes for carbohydrate-responsive element-binding protein (ChREBP). This protein is a transcription factor that binds and activates carbohydrate response element (ChoRE) motifs in the promoter regions of genes responsible for triglyceride synthesis and glycolysis in the liver. Thus ChREBP appears to play a major role in determining fasting triglyceride levels in the blood. Additionally, the *MLXIPL* gene is one of about 26 genes that is deleted in Williams–Beuren syndrome, a neurodevelopmental disorder with several cardiovascular defects.

A SNP in the MLXIPL gene (specifically, the C allele of the G771C polymorphism) shows a strong association with elevated triglyceride levels (OR = 1.29 per copy of the C allele for triglyceride levels >1.7 mmol/L). Median triglyceride levels are 2.07, 1.96 and 1.75 mmol/L for the C/C genotype, C/G genotype, and G/G genotype, respectively.[66]

Conversely, the G allele of the G771C polymorphism is protective against several cardiovascular risk factors.[67] The G allele is associated with lower triglyceride levels (−9.86 mg/dL per G allele; OR for hyperlipidemia = 0.73). Furthermore, stronger adherence to a Mediterranean diet enhances the triglyceride-lowering effect of the G allele (OR = 0.63), and this protection is attenuated when the adherence is low (OR = 0.88). The Mediterranean diet also significantly lowered the risk of cardiovascular pathology in carriers of the G allele more so than in C/C homozygotes on the same diet (hazard ratio = 0.34) and compared with carriers of the G allele who were not on the Mediterranean diet (whose hazard ratio = 0.90).[68]

The *SERPINE1* gene (formerly known as PAI-1) codes for plasminogen activator inhibitor-1. This protein is a member of the serpin family and inhibits both the tissue-type and urokinase-type plasminogen activators. Plasminogen activators are responsible for the conversion of plasminogen to plasmin, which, in turn, degrades fibrin. Thus plasminogen activator inhibitor-1 appears to play a major role in determining the proliferative response to vascular injury by inhibiting the degradation of fibrin and several extracellular matrix proteins by plasmin.

A SNP in the *SERPINE1* gene called the rs6950982 polymorphism is associated with higher levels of several lipids in the blood. People who carry the G allele have higher total cholesterol (A/A: 208, A/G: 210, G/G: 218 mg/dL), higher LDL cholesterol (A/A: 127, A/G: 131, G/G: 136 mg/dL), and higher triglycerides (A/A: 131, A/G: 128, G/G: 154 mg/dL) in the blood. Additionally, men who carry the G allele have higher systolic blood pressure (A/A + A/G: 141.4 mm Hg vs. G/G: 149.8 mm Hg) and diastolic blood pressure (A/A: 81.5, A/G: 82.1, G/G: 85.7 mm Hg). Both men and women with the G/G genotype respond well to the Mediterranean diet for controlling triglyceride levels (~175 mg/dL in the lower-adherence group vs. ~125 mg/dL in the higher-adherence group).[69]

Electrolytes and Hypertension

Hypertension is an independent risk factor for coronary artery disease and for stroke. The therapeutic response to sodium restriction in hypertensive individuals is highly variable. A SNP in the angiotensinogen gene *(AGT)* allows an amino acid substitution in which threonine (T) replaces methionine (M) at amino acid 235. The T allele is associated with increased production of angiotensin, with a tendency for higher blood pressure. There is a stepwise increase of serum AGT from MM to MT to TT genotypes among persons with hypertension[70] and those with normal blood pressure.[71] Although there have been some discrepancies between studies, a meta-analysis of all studies published between 1992 and 1996 showed the 235T allele had a consistent, mild association with hypertension (OR = 1.20), a positive family history of hypertension (OR = 1.42), and more severe hypertension (OR = 1.34).[72] A more recent study (2016) puts the risk of hypertension in carriers of two copies of the 235T allele at an OR of 1.8.[73]

The 235T allele of the *AGT* gene is associated with greater blood pressure decreases than the 235M allele after an intervention to reduce sodium intake (>5 mg/day).[74] Persons with the TT and MT genotypes showed significant systolic blood pressure reductions when consuming mineral salt compared with control subjects ($P < 0.02$ and $P < 0.001$, respectively), but persons with the MM genotype did not ($P < 0.10$). The net adjusted systolic and diastolic blood pressure reduction was –8.6 mm Hg systolic/–3.9 mm Hg diastolic for persons with the TT genotype, –9.0/–5.2 mm Hg for those with the MT genotype, and –5.3/–1.0 mm Hg for the MM genotype.

Aerobic exercise was effective in reducing blood pressure but only in the MM (–3.7 mm Hg) and MT (–3.4 mm Hg) genotypes, and not in the TT (–0.4 mm Hg) individuals, among 477 previously sedentary white Americans.[75] Finally, the use of angiotensin-converting enzyme inhibitors produced more dramatic reductions in blood pressure in 235T allele carriers (TT and MT) than in the MM genotype,[76,77] illustrating the notion that diverse modifications in "environment" (diet, exercise, targeted pharmaceuticals) may produce similar alterations in phenotype (in this case, blood pressure) among genetically susceptible individuals.

Homocysteine and Micronutrients

As mentioned previously, one common physiological effect of SNPs is a decreased binding affinity of a vitamin or mineral coenzyme for the structurally altered enzyme. There is mounting evidence that in most cases, the diminished rate of reaction of that polymorphic enzyme can increase with high-dose cofactor micronutrient supplementation.[78] Perhaps the best-studied example of this model is the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), which is responsible for remethylating homocysteine into methionine, allowing methylation reactions to occur efficiently in the body. When methylation reactions are impaired, plasma homocysteine levels rise (Fig. 15.3). Elevated homocysteine values have been associated with an increased risk of atherosclerosis,[79] risk of early coronary artery disease,[80,81] increased risk of hemostasis and venous thrombosis,[82,83] decreased bone density,[84] neural tube defects, spina bifida,[85,86] Alzheimer's disease, macular degeneration, hearing loss,[87] and acute leukemia in adults.[88] The risk of heart disease increases for heterozygotes as well as for 677T/T homozygotes.[89] Folic acid and vitamins B_2 (as flavin adenine dinucleotide [FAD]), B_6, and B_{12} are all cofactors in the methylation cycle, and a dietary deficiency of any of these vitamins can result in elevated homocysteine levels. However, elevated homocysteine levels can also result from two common polymorphisms in MTHFR: a 677C-T nucleotide substitution and a 1298A-C nucleotide substitution. Someone with a 677T/T genotype has a 50% reduction in MTHFR enzyme activity, whereas a 1298C/C genotype has a 32% reduction. In one study, approximately 28% of patients with elevated homocysteine did not respond to supplementation with folic acid, vitamin B_{12}, and vitamin B_6 because they had the 677T/T genotype

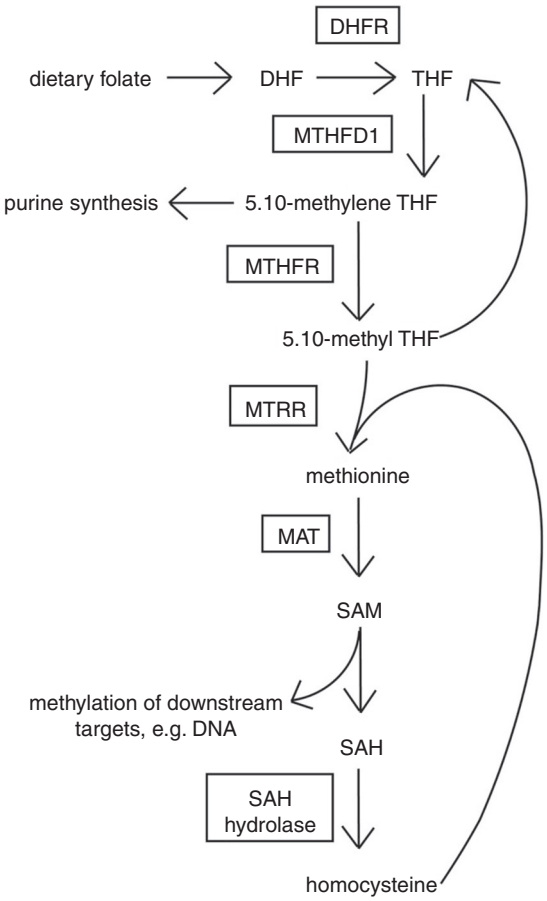


Fig. 15.3 Schematic of the folic acid metabolic cycle. (From Spellicy CJ, Kosten TR, Hamon SC, et al. The MTHFR C677T variant is associated with responsiveness to disulfiram treatment for cocaine dependency. *Front. Psychiatr.* 2013;3:109. PubMed PMID: 233335901.)

and severely impaired MTHFR activity.[90] It is worth noting that the 677C-T polymorphism occurs in the FAD site of MTHFR, suggesting that vitamin B_2 supplementation may be a critical component.[91]

Three rational therapeutic strategies may help resolve elevated homocysteine levels. First is high-dose supplementation of the vitamin cofactors of MTHFR, which relies on the simple logic of the law of mass action in a chemical reaction: higher concentrations of substrates drive the chemical reaction forward. Second, the metabolic products of the MTHFR enzyme, namely, 5-methyltetrahydrofolate, may be supplemented. Third, alternate remethylation pathways may be stimulated by supplementing trimethylglycine or betaine as alternate methyl-group donors. Considering the low cost and high degree of safety associated with each of these options, all three strategies may be employed simultaneously. Regardless, serial plasma homocysteine measurements allow the practitioner to determine whether the therapy employed has been successful.

Although the science of nutrigenomics is still in its infancy, several principles have become clear, with an enormous potential impact for clinical medicine. Nutrients act as dietary signals that alter gene expression (nutrigenomics). In individuals with specific polymorphisms, their specific genetic variation may render that individual more or less sensitive to specific nutrients or environmental stimuli and, therefore, may help determine optimal diet and lifestyle (nutrigenetics). Knowing the effects of these environmental stimuli on gene expression allows us to use this information proactively to alter gene expression and to mitigate risk for developing various forms of chronic disease, like cardiovascular disease. These principles of nutrigenomics and nutrigenetics hold true for macronutrient balance in the diet and for micronutrients, such as vitamins, minerals, and electrolytes. Although our primary focus here has been on nutrient–gene interactions, the same principles hold true for lifestyle changes and targeted pharmaceuticals. Nutritional intervention is merely a more specific application of environmental modification to alter gene expression. What should be clear is that as our knowledge of gene–gene and gene–environment interactions increases, so too will our capacity for delivery of increasingly personalized and primary prevention.

GENOMIC TESTING

From Early Detection to Prevention

We use the term *susceptibility gene* to refer to a polymorphism that may render an individual more susceptible to the development of a chronic disease when exposed to an adverse environment. Most susceptibility genes have a low positive predictive value (the probability that a disease will develop in a person with a positive test result) and a low attributable risk (the proportion of cases of a disease that can be attributed to a susceptibility gene).[92] Therefore some researchers have questioned the clinical utility of susceptibility genetic testing,[93] but such arguments, by applying a single-gene/single-disease model to susceptibility genes, miss the clinical relevance. Susceptibility genes, much like taking a family history, must be seen as important but incomplete contributors in what is invariably a multifactorial risk assessment. In terms of health outcomes, preventive genomic polymorphisms raise risk modestly and are additive in their effects (gene–gene interactions), and their actual phenotype expression is strongly affected by diet, lifestyle, and environment (gene–environment interactions). Rather than negate their clinical utility because of a low positive predictive value and a low attributable risk, these polymorphisms begin to offer a molecular basis for understanding the pathophysiology of complex multifactorial diseases, and an understanding of the environmental factors that affect gene expression begins to evoke effective therapeutic strategies.

Because environment is broadly understood to refer to anything outside the genome itself, therapeutic regimens may be constructed to include any portion of the environment that has been shown to affect gene expression and phenotype. This is truly a holistic approach because effective therapeutic strategies may involve diet, nutritional, and targeted pharmaceutical supplementation; lifestyle and behavioral modification; and the avoidance or elimination of toxins, xenobiotics, and microbes. Intervention at any level of our "environment" may prove clinically beneficial.

Functional medicine is the clinical discipline designed to promote health, to anticipate and prevent disease, or to correct an existing disease by improving physiological function. The underlying assumption is that health and disease lie on the same continuum, and the connecting thread of the continuum is physiological function. Before the manifestation of any frank disease, a progressive loss of homeostasis and increasing dysfunction occur. Clinical intervention in this strategy may begin at the earliest signs of imbalance.[94] The promise of preventive genomics is that the point of effective intervention may begin even earlier, before the beginnings of physiological dysfunction.

Clinical Challenges

Preventive and nutritional genomic testing in clinical practice has its critics, who predominantly argue that there are insufficient numbers of clinical trials to demonstrate the diagnostic and therapeutic efficacy of genomic profiles.[95] However, rather than demonstrating that all pregenomic testing is "premature and scientifically unsound," as suggested by Dr. Muin Khoury of the CDC,[96] such objections merely underscore the perspective shared here. Namely, it is precisely because chronic disease is multifactorial with complex gene–gene and gene–environment interactions that the diagnostic and therapeutic utility of preventive genomic testing must meet the four criteria of being relevant, prevalent, modifiable, and measurable. The last criterion, measurable, is critical at this early stage of preventive genomic testing. We must be able to measure beneficial phenotypic, physiological changes in individuals that result from the therapeutic strategy employed. There will always remain a possibility that other genetic polymorphisms or other environmental influences that we have not yet identified may also affect an individual's disease risk.

A similar cautionary argument should be made in cases in which current genomic knowledge offers conflicting therapeutic advice. A man with an Apo-E4 allele presumably should not drink alcohol, but what if he also has a CETP Taq1B polymorphism for which alcohol has been shown to boost HDL cholesterol levels dramatically? At present, the answer is unknown. Fortunately, because fractionated lipid levels are easily measurable, the effects of moderate, daily alcohol intake on such a specific individual's lipid profile may be easily ascertained. Thus medicine must ultimately be empirical, relying on trial and error until the desired result (e.g., lower serum cholesterol) is achieved.

Bioethical Considerations: Opportunities and Potential for Discrimination

Genetic information, because it represents an unchangeable state, has the potential, at least in theory, to be used in a manner by insurers, employers, and society at large. However, it should be noted that the paradigm for such discrimination views genetic information as representing an individual's immutable limitations, as typified by single-gene diseases (e.g., Tay-Sachs disease, Huntington's disease, sickle cell anemia).

A fundamental cornerstone of preventive genomics is the idea that the phenotypic outcome of any unique genotype is modifiable through environmental change. Preventive genomic testing, rather than revealing an individual's genetic limitations, more accurately reflects an

individual's genetic potential, given the right environment. Genetic polymorphisms have the potential to guide an individual to adopt the appropriate dietary, lifestyle, and environmental changes that can optimize health and longevity.

Now that we know there are irrefutable connections between genes and environment, and we are learning their myriad effects on health and disease, ignorance is no longer ethically neutral. Choosing not to use genomic information in treating patients, now that its health implications are being effectively documented, is ethically untenable. For the first time in the history of medicine, preventive genomic testing allows us to assess individual risk for the development of chronic diseases, to develop comprehensive risk-reduction strategies before imbalances in homeostasis occur, and to institute optimal therapy interventions for patients who are already sick. This new personalized medicine, made possible by the advent of preventive genomics and nutrigenomics, offers practitioners and patients new opportunities for the prevention and treatment of disease and for the promotion of optimal health.

SUMMARY

Preventive genomic testing exploring both nutrigenomics and nutrigenetics is both new and exciting. It affords practitioners both novel and effective avenues to develop personalized therapeutic regimens and to promote optimal disease-prevention strategies. Although what we know is dwarfed by what we do not know, this imbalance does not negate the current therapeutic power that the past 20 years of genomic research has revealed. We agree with the following assertions made by Loktionov[97]:

- There are many examples of effective research on gene–environment interactions.
- There is sufficient evidence to make clinical recommendations on the basis of individualized genomic predisposition.
- There is a need for research on traits that protect from, as well as predispose to, disease.
- Environment modification, especially dietary changes, may be the easiest and most efficient way to influence the risks of many diseases common today.

It is little wonder that Paul Berg, the pioneering researcher in recombinant DNA and genetic engineering and the winner of the Nobel Prize for Chemistry in 1980, once quipped, "At the time, our goal was to focus on the molecular and genetic basis of disease as the starting point for new forms of medicine."[99] However, it is equally true to say that all disease is environmental even when it is also genetic. The symphony between nature and nurture is the very essence of life and health for all creatures who call this Earth their home.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gesteland RF, Atkins JF. *The RNA World*. NY: Cold Spring Harbor Laboratory Press: Cold Spring Harbor; 1993.
2. Ridley M. *Nature via Nurture: Genes, Experience, and What Makes Us Human*. New York: Harper Collins; 2003.
3. Muller M, Kersten S. Nutrigenomics: goals and strategies. *Nat Rev Genet*. 2003;4:315–322.
4. Yun AJ, Lee PY, Doux JD. Are we eating more than we think? Illegitimate signaling and xenohormesis as participants in the pathogenesis of obesity. *Med Hypotheses*. 2006;67:36–40.
5. McCay CM, Crowell MF. Prolonging the life span. *Scientific Monthly*. 1934;39:405–414.
6. Parr T. Insulin exposure and aging theory. *Gerontology*. 1997;43:182–200.
7. Frame LT, Hart RW, Leakey JE. Caloric restriction as a mechanism in mediating resistance to environmental disease. *Environ Health Perspect*. 1998;106(suppl 1):313–324.
8. Elliott PJ, Jirousek M. Sirtuins: novel targets for metabolic disease. *Curr Opin Investig Drugs*. 2008;9:371–378.
9. Baur JA, Pearson KJ, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006;444:337–342.
10. Gohil K. Genomic responses to herbal extracts: lessons from in vitro and in vivo studies with an extract of Ginkgo biloba. *Biochem Pharmacol*. 2002;64:913–917.
11. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPAR gamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet*. 1998;20:284–287.
12. Robitaille J, Després JP, Pérusse L, et al. The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Québec Family Study. *Clin Genet*. 2003;63:109–116.
13. Coleman DL. Diabetes and obesity: thrifty mutants? *Nutr Rev*. 1978;36:129–132.
14. Caspi A, McClay J, Moffitt TE, et al. Role of genotype in the cycle of violence in maltreated children. *Science*. 2002;297:851–854.
15. Centers for Disease Control. The Office of Public Health Genomics. Genomics Fact Sheet. http://www.cdc.gov/genomics/public/eFactSheet/transcript.htm#menu. Accessed 2/21/2012.
16. Garrod AE. *Inborn Errors of Metabolism*. London: H Frowde and Hodder & Stoughton; 1909.
17. Costa V, Casamassimi A, Ciccodicola A. Nutritional genomics era: opportunities toward a genome-tailored nutritional regimen. *J Nutr Biochem*. 2010;21:457–467.
18. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet. *Nutr Rev*. 2006;64:S27–S47.
19. Knoops KT, de Groot LC, et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. *JAMA*. 2004;292:1433–1439.
20. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology*. 2000;61:154–166.
21. Fortes C, Mastroeni S, Botta G, et al. Glutathion S-transferase M1 null genotype, household pesticides exposure and cutaneous melanoma. *Melanoma Res*. 2016;6:625–630.
22. Huang RS, Chen P, Wisel S, et al. Population-specific GSTM1 copy number variation. *Hum Mol Genet*. 2009;2:366–372.
23. Lampe JW, Chen C, Li S, et al. Modulation of human glutathione S-transferases by botanically defined vegetable diets. *Cancer Epidemiol Biomarkers Prev*. 2000;9:787–793.
24. Lin HJ, Probst-Hensch NM, Louie AD, et al. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev*. 1998;7:647–652.
25. Zhao B, Seow A, Lee EJ, et al. Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol Biomarkers Prev*. 2001;10:1063–1067.
26. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. *Am J Clin Nutr*. 2002;75:616–658.
27. Ames BN. Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J Nucleic Acids*. 2010. https://doi.org/10.4061/2010/725071.
28. Stampfer MJ, Hu FB, Manson JE, et al. Primary prevention of coronary heart disease in women through diet and lifestyle. *N Engl J Med*. 2000;343:16–22.
29. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis*. 1988;8:1–21.
30. Schaefer EJ, Lamon-Fava S, Johnson S, et al. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. *Arterioscler Thromb*. 1994;14:1105–1113.
31. Wilson PW, Myers RH, Larson MG, et al. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease: the Framingham Offspring Study. *JAMA*. 1994;272:1666–1671.
32. Lahoz C, Schaefer EJ, Cupples LA, et al. Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. *Atherosclerosis*. 2001;3:529–537.
33. Pablos-Mendez A, Mayeux R, Ngai C, et al. Association of apo E polymorphism with plasma lipid levels in a multiethnic elderly population. *Arterioscler Thromb Vasc Biol*. 1997;17:3534–3541.
34. Corella D, Ordovas JM. Single nucleotide polymorphisms that influence lipid metabolism: interaction with dietary factors. *Annu Rev Nutr*. 2005;25:341–390.
35. Ordovas JM, Lopez-Miranda J, Mata P, et al. Gene-diet interaction in determining plasma lipid response to dietary intervention. *Atherosclerosis*. 1995;118(suppl):S11–S27.
36. Gylling H, Miettinen TA. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. *J Lipid Res*. 1992;33:1361–1371.
37. Tikkanen MJ, Huttunen JK, Ehnholm C, et al. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. *Arteriosclerosis*. 1990;10:285–288.
38. Erkilla AT, Sarkkinen ES, Perez-Jimenez F, et al. APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose. *Am J Clin Nutr*. 2001;73:746–752.
39. Wilson PW, Myers RH, Larson MG, et al. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease: the Framingham Offspring Study. *JAMA*. 1994;272:1666–1671.
40. Pablos-Mendez A, Mayeux R, Ngai C, et al. Association of apo E polymorphism with plasma lipid levels in a multiethnic elderly population. *Arterioscler Thromb Vasc Biol*. 1997;17:3534–3541.
41. Frikke-Schmidt R, Nordestgaard BG, Agerholm-Larsen B, et al. Context-dependent and invariant associations between lipids, lipoproteins, and apolipoproteins and apolipoprotein E genotype. *J Lipid Res*. 2000;41:1812–1822.
42. Mahley RW, Palaoglu KE, Atak Z, et al. Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. *J Lipid Res*. 1995;36:839–859.
43. Lehtimaki T, Frankberg-Lakkala H, Solakivi T, et al. The effect of short-term fasting, apolipoprotein E gene polymorphism, and sex on plasma lipids. *Am J Clin Nutr*. 1997;66:599–605.
44. Wolever TM, Hegele RA, Connelly PW, et al. Long-term effect of soluble-fiber foods on postprandial fat metabolism in dyslipidemic subjects with apo E3 and apo E4 genotypes. *Am J Clin Nutr*. 1997;66:584–590.
45. Jang Y, Kim OY, Park HY, et al. Effect of apolipoprotein E polymorphism on the serum lipid and insulin response to whole grain consumption in coronary artery disease patients. *Nutr Res*. 2001;21:1463–1473.
46. Corella D, Tucker K, Lahoz C, et al. Alcohol drinking determines the effect of the APOE locus on LDL-cholesterol concentrations in men: the Framingham Offspring Study. *Am J Clin Nutr*. 2001;73:736–745.
47. Hagberg JM, Wilund KR, Ferrell RE. APO E gene and gene-environment effects on plasma lipoprotein-lipid levels. *Physiol Genomics*. 2000;4:101–108.
48. Bernstein MS, Costanza MC, James RW, et al. Physical activity may modulate effects of ApoE genotype on lipid profile. *Arterioscler Thromb Vasc Biol*. 2002;22:133–140.
49. Gerdes LU, Gerdes C, Kervinen K, et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survi-

vors of myocardial infarction: a substudy of the Scandinavian Simvastatin Survival Study. *Circulation*. 2000;101:1366–1371.
50. Viiri LE, Loimaala A, Nenonen A, et al. The association of the apolipoprotein E gene promoter polymorphisms and haplotypes with serum lipid and lipoprotein concentrations. *Atherosclerosis*. 2005;179:161–167.
51. Moreno JA, Lopez-Miranda J, Marin C, et al. The influence of the apolipoprotein E gene promoter (-219G/T) polymorphism on postprandial lipoprotein metabolism in young normolipemic males. *J Lipid Res*. 2003;44:2059–2064.
52. Moreno JA, Perez-Jimenez F, Marin C, et al. Apolipoprotein E gene promoter -219G->T polymorphism increases LDL-cholesterol concentrations and susceptibility to oxidation in response to a diet rich in saturated fat. *Am J Clin Nutr*. 2004;80:1404–1409.
53. Moreno JA, Perez-Jimenez F, Marin C, et al. The apolipoprotein E gene promoter (-219G/T) polymorphism determines insulin sensitivity in response to dietary fat in healthy young adults. *J Nutr*. 2005;135:2535–2540.
54. Stengård JH, Frikke-Schmidt R, Tybjaerg-Hansen A, et al. Variation in 5′ promoter region of the APOE gene contributes to predicting ischemic heart disease (IHD) in the population at large: the Copenhagen City Heart Study. *Ann Hum Genet*. 2007;71:762–771.
55. Oliveira HC, Ma L, Milne R, et al. Cholesteryl ester transfer protein activity enhances plasma cholesteryl ester formation: studies in CETP transgenic mice and human genetic CETP deficiency. *Arterioscler Thromb Vasc Biol*. 1997;17:1045–1052.
56. Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis: the Regression Growth Evaluation Statin Study Group. *N Engl J Med*. 1998;338:86–93.
57. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*. 1996;97:2917–2923.
58. Dullaart RP, Hoogenberg K, Riemens SC, et al. Cholesteryl ester transfer protein gene polymorphism is a determinant of HDL cholesterol and of the lipoprotein response to a lipid-lowering diet in type 1 diabetes. *Diabetes*. 1997;46:2082–2087.
59. Fumeron F, Betoulle D, Luc G, et al. Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *J Clin Invest*. 1995;96:1664–1671.
60. Hannuksela ML, Liinamaa MJ, Kesaniemi YA, et al. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis*. 1994;110:35–44.
61. Wenzel K, Felix S, Kleber FX, et al. E-selectin polymorphism and atherosclerosis: an association study. *Hum Mol Genet*. 1994;3:1935–1937.
62. Zheng F, Chevalier JA, Zhang LQ, et al. An HphI polymorphism in the E-selectin gene is associated with premature coronary artery disease. *Clin Genet*. 2001;59:58–64.
63. Bowie G, O'Neill LA. Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. *J Immunol*. 2000;165:7180–7188.
64. Bowie A, O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol*. 2000;59:13–23.
65. Meydani SN, Endres S, Woods MM, et al. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr*. 1991;121:547–555.
66. Pan LA, Chen YC, Huang H, et al. G771C Polymorphism in the MLXIPL gene is associated with a risk of coronary artery disease in the Chinese: a case-control study. *Cardiology*. 2009;114:174–178.
67. Guo S, Zheng F, Qui X, Yang N. ChREBP gene polymorphisms are associated with coronary artery disease in Han population of Hubei province. *Clin Chim Acta*. 2001;412:1854–1860.
68. Ortega-Azorín C, Sorlí JV, Estruch R, et al. Amino acid change in the carbohydrate response element binding protein is associated with lower triglycerides and myocardial infarction incidence depending on level of adherence to the Mediterranean diet in the PREDIMED trial. *Circ Cardiovas Genet*. 2014;1:49–58.
69. Sotos-Prieto M, Guillén M, Portolés O, et al. Association between the rs6950982 polymorphism near the SERPINE1 gene and blood pressure and lipid parameters in a high-cardiovascular-risk population: interaction with Mediterranean diet. *Genes Nutr*. 2013;4:401–409.
70. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 1992;71:169–180.
71. Winkelmann BR, Russ AP, Nauck M, et al. Angiotensinogen M235T polymorphism is associated with plasma angiotensinogen and cardiovascular disease. *Am Heart J*. 1999;137:698–705.
72. Kunz R, Kreutz R, Beige J, et al. Association between the angiotensinogen 235T-variant and essential hypertension in whites: a systematic review and methodological appraisal. *Hypertension*. 1997;30:1331–1337.
73. Bonfim-Silva R, Guimarães LO, Souza Santos J, et al. Case-control association study of polymorphisms in the angiotensinogen and angiotensin-converting enzyme genes and coronary artery disease and systemic artery hypertension in African-Brazilians and Caucasian-Brazilians. *J Genet*. 2016;95:63–69.
74. Hunt SC, Geleijnse JM, Wu LL, et al. Enhanced blood pressure response to mild sodium reduction in subjects with the 235T variant of the angiotensinogen gene. *Am J Hypertens*. 1999;12:460–466.
75. Rankinen T, Gagnon J, Perusse L, et al. AGT M235T and ACE ID polymorphisms and exercise blood pressure in the HERITAGE Family Study. *Am J Physiol Heart Circ Physiol*. 2000;279:H368–H374.
76. Hingorani AD, Jia H, Stevens PA, et al. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens*. 1995;13:1602–1609.
77. Turner ST, Schwartz GL, Chapman AB, et al. Antihypertensive pharmacogenetics: getting the right drug into the right patient. *J Hypertens*. 2001;19:1–11.
78. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. *Am J Clin Nutr*. 2002;75:616–658.
79. Bova I, Chapman J, Sylantiev C, et al. The A677V methylenetetrahydrofolate reductase gene polymorphism and carotid atherosclerosis. *Stroke*. 1999;30:2180–2182.
80. Gallagher PM, Meleady R, Shields DC, et al. Homocysteine and risk of premature coronary heart disease: evidence for a common gene mutation. *Circulation*. 1996;94:2154–2158.
81. Kluijtmans LA, Kastelein JJ, Lindemans J, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation*. 1997;96:2573–2577.
82. Kluijtmans LA, Boers GH, Verbruggen B, et al. Homozygous cystathionine beta-synthase deficiency, combined with factor V Leiden or thermolabile methylenetetrahydrofolate reductase in the risk of venous thrombosis. *Blood*. 1998;91:2015–2018.
83. Gemmati D, Previati M, Serino ML, et al. Low folate levels and thermolabile methylenetetrahydrofolate reductase as primary determinant of mild hyperhomocysteinemia in normal and thromboembolic subjects. *Arterioscler Thromb Vasc Biol*. 1999;19:1761–1767.
84. Miyao M, Morita H, Hosoi T, et al. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. *Calcif Tissue Int*. 2000;66:190–194.
85. Martinez de Villarreal LE, Delgado-Enciso I, Valdéz-Leal R, et al. Folate levels and N(5), N(10)-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. *Arch Med Res*. 2001;32:277–282.
86. Ou CY, Stevenson RE, Brown VK, et al. 5,10-Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. *Am J Med Genet*. 1996;63:610–614.
87. Kim J, Kim H, Roh H, Kwon Y. Causes of hyperhomocysteinemia and its pathological significance. *Arch Pharm Res*. 2018;41:372–383.
88. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci U S A*. 1999;96:12810–12815.

89. Kluijtmans LA, Whitehead AS. Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease; evidence that all three MTHFR C677T genotypes confer different levels of risk. *Eur Heart J*. 2001;22:294–299.
90. Engbersen AM, Franken DG, Boers GH, et al. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet*. 1995;56:142–150.
91. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): relevance to genetic disease and polymorphisms. *Am J Clin Nutr*. 2002;75:616–658.
92. Khoury MJ, Newill CA, Chase GA. Epidemiologic evaluation of screening for risk factors: application to genetic screening. *Am J Public Health*. 1985;75:1204–1208.
93. Holtzman NA, Marteau TM. Will genetics revolutionize medicine? *N Engl J Med*. 2000;343:141–144.
94. Bland JS. The use of complementary medicine for healthy aging. *Altern Ther Health Med*. 1998;4:42–48.
95. Haga SB, Khoury MJ, Burke W. Genomic profiling to promote a healthy lifestyle: not ready for prime time. *Nat Genet*. 2003;34:347–350.
96. Khoury MJ. Genetics and genomics in practice: the continuum from genetic disease to genetic information in health and disease. *Genet Med*. 2003;5:261–268.
97. Loktionov A. Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases (review). *J Nutr Biochem*. 2003;14:426–451.
98. Sagan C. Cosmos Episode 9 The Lives Of The Stars. Fremantle Media Enterprises; 2009.
99. Schechter R. Beckman Center celebrates ten years at the forefront of biomedicine. Stanford University.https://news.stanford.edu/news/1999/april28/beckman-428.html. Published 1999. Accessed March 10, 2020.

16

Hair Mineral Analysis

Nick Soloway, LMT, DC, LAc, and *Steve Austin*, ND*

OUTLINE

INTRODUCTION

Despite many research studies since the original publication of this chapter in 1985, there has been little change in the *diagnostic* value of hair analysis (HA). Outside its accepted use for monitoring of heavy-metal toxicity, at this time HA still has only limited clinical application.

In recent years, several attempts have been made to standardize HA testing techniques,[1,2] but laboratories still do not have an agreement on procedures for handling a hair sample.[3–5,6–8,2,9,10]

Many conditions and diseases have been tested for abnormal HA patterns, and a number have been shown to have patterns differing significantly from the norm, including the following:

- Learning disabilities[11–13]
- Birth defects[14,15]
- Hyperactivity[16–18]
- Down syndrome[19,20]
- Neurosis and psychosis[21]
- Senile dementia[22]
- Autism[23,24,25,26,27]
- Alopecia areata[28]
- Insulin-dependent diabetes mellitus[29,30]
- Cystic fibrosis[31]
- Beta-thalassemia[32]
- Spasticity in children[33]
- Repeated exposure to radiographs[34–36]
- Nasopharyngeal cancer[37,38]
- Aplastic anemia[39]
- Breast cancer[40,41,42]
- Bone mineral density[43,44]
- Type 2 diabetes[45]
- Alzheimer's disease[46,47]
- Hypertension[48,49,50,51]
- Hay fever[52]
- Atopic dermatitis[53]
- Insulin resistance/metabolic syndrome[54,55,56,57,58]
- Fibromyalgia[59]
- Hemodialysis[60]
- Amblyopia[61]
- Multiple sclerosis[62]
- Cancer[63,64,65–68,69,70]
- Bipolar disease[71]
- Schizophrenia[72]
- Parkinson's disease[73]
- In vitro fertilization (IVF) pregnancy[74]
- Preeclampsia[75]

HA has been used successfully to test for drug abuse,[76–82,83] and studies have been performed in several locations examining the validity of using HA to test for drug use before the reinstatement of driving licenses.[84,85] That said, however, drug metabolites are not found in most commercial hair analyses.

HA appears to offer potential as a correlating diagnostic tool in a few of the listed conditions, although the hair mineral patterns should not be used exclusively for diagnosis. For example, the high hair sodium values in infants with cystic fibrosis shows very little overlap with those in controls, and one study demonstrated that children with learning disabilities can be diagnosed with 98% accuracy because of a consistent pattern of high hair values of cadmium, manganese, and chromium in conjunction with low values of lithium and cobalt.[11] The usefulness of HA as a research tool hardly can be questioned. However, significant controversy exists about the use of the method for the clinical diagnosis of diseases other than heavy-metal toxicity and as an indicator of nutritional status. Moreover, multiple studies question the interlaboratory and intralaboratory accuracy of HA.[86,87,88–90,6-8,2,91] The difference between research and clinical use is significant. Whereas it may be of interest in a research setting that patients with various skin conditions have lower mean levels of hair magnesium than controls, the two groups overlap so much that the procedure is diagnostically

* Previous edition contributor

useless.[28] Moreover, even when an altered HA pattern has been associated with a disease, generally there are few investigations as to whether mineral supplementation would affect the clinical condition or even revert the hair mineral pattern to normal. Several studies have looked at the relationship between diet and hair mineral status.[49,92–96,51] with varying conclusions. Some showed some dietary influences on hair mineral status,[93–95,96,97] one showed no correlation,[95] and another showed variable correlation.[97] Not all hair minerals showed a relationship to intake, and many of the studies had limitations because of a limited time of reporting nutrient intake. Hair grows approximately 1.5 cm per month, so the hair sample usually contains mineral content outside of the nutrient reporting time. Growth rate also varies with ethnicity.[98] One study tested hair grown in a 2-year period and found a correlation between low hair Ca, Mg, Sr, and BA and an increased presence of hypertension.[51] Gender has been found to result in significant differences in hair minerals,[99,100] which can complicate the interpretation of test results. "Levels of elements in the hair are not strictly comparable between different areas of the world."[101]

Wide overlap of values in disease and control groups is frequently the case with HA, resulting in excessive false-positive and false-negative results. For example, mentally retarded patients have been found to have hair lead, sodium, and potassium hair values approximately twice those of controls,[19] but the standard deviations are extremely large for some minerals (for sodium, 1644.71 ± 1814.93 versus 744.43 ± 1987[28]; and for potassium, 870.15 ± 1009.19 versus 408.35 ± 689.99), and the large overlap greatly reduces the clinical use of HA. In addition, many of the altered HA patterns associated with the diseases previously listed are as yet unconfirmed.

MINERALS

Calcium and Magnesium

Hair calcium and magnesium values were found to be elevated in patients with fibromyalgia,[102] and another study found decreased levels along with iron and manganese.[59] Higher levels of calcium and magnesium were seen in amblyopic children.[61] Chronic stress in children had an association with elevated Ca/Mg ratios.[103] High hair calcium content has been associated with a reduced risk of coronary heart disease,[104] although higher levels were found in hypertensive, obese patients with insulin resistance.[49] Higher hair calcium was associated with an enhanced augmentation index, a measure of arterial stiffness.[105] Low hair calcium content has been found in the last trimester of pregnancy,[106] and hair calcium content increased in response to supplementation during pregnancy.[107] Low hair magnesium levels have been reported in autistic children, children with attention deficit hyperactivity disorder, patients with various skin disorders, and patients with several types of leukemia, whereas high levels have been reported in conjunction with dyslexia and Prader–Willi syndrome.[18,23,28,108–110] An increase in the Ca/Mg ratio seemed to correlate to increased insulin resistance.[55] In contrast, another study saw a decrease in hair calcium and magnesium in metabolic syndrome.[56] A decrease in the Ca/Mg ratio was seen in Parkinson's disease.[73] The meaning of these associations remains unknown for the most part, and contrasting findings make interpretation and usefulness questionable.

A 2001 study concluded that analysis of hair calcium and phosphorus content was of value as a complementary detection tool in abnormalities of bone metabolism.[111] Elevations in both calcium and magnesium were correlated with a low dietary calcium/magnesium ratio in one study, suggesting that this finding may be indicative of an induced hyperparathyroidism,[112] but that hypothesis remains unproven. Hair calcium and magnesium also vary in response to the hardness and pH of the water in which the hair is usually washed.[104] Water consumption from different sources was reported to change hair calcium and magnesium levels; higher hair levels correlated to an increased risk of kidney stone formation.[113] Supplementation of dietary magnesium has been reported to increase hair magnesium levels in deficient children.[114] Nonetheless, in one study of congenital hypomagnesemia, researchers concluded that hair magnesium level was not a useful tool in monitoring mineral status because the values were higher in affected subjects than in subjects who were not deficient.[115] A 2013 study showed that a high hair calcium level was associated with low calcium intake and low bone mineral density.[44]

There is limited evidence to support the use of hair calcium and magnesium measurements in clinical diagnosis at this time.

Chromium

Hair chromium content is low in insulin-dependent diabetics,[30] although there is much overlap with normal persons. Increased insulin resistance was associated with lower hair chromium,[55,58] Chromium levels decrease with age,[32] but the meaning of this change remains unknown. Hair and tissue chromium levels vary greatly during pregnancy, being very high during the first few months of normal pregnancy and subsequently decreasing.[116,117] Late in pregnancy, hair chromium content typically becomes low,[118] suggesting deficiency.[76] However, high hair chromium value in pregnancy is associated with low-birth-weight infants.[119] In patients with gestational diabetes, hair chromium content is high early in pregnancy but decreases in late pregnancy.[117] Increasing dietary intake of chromium has been linked to increasing hair chromium values,[120] but supplemental chromium does not seem to alter hair levels.[116,121] Chromium was found to be higher in patients with a fixed orthodontic appliance. The range of amounts was 8.94 ± 13.1 μg, although the mean was stated to be above the 90th percentile.[122]

Although normal and deficiency ranges should be more clearly defined, hair chromium content appears to have potential future use in clinical settings. However, current knowledge remains inadequate to help clinicians treat patients on the basis of abnormal hair chromium levels alone.

Copper

Oral contraceptive use is associated with decreased hair copper and increased serum copper.[123] High hair copper levels are associated with being female, lactation, idiopathic scoliosis, and pregnancy in some but not all studies.[124–127] Surprisingly, conditions that affect systemic copper status have been shown not to affect hair levels. Copper deficiency,[128] Wilson disease,[129,130] and cirrhosis[131] do not significantly alter hair copper content. Hair copper levels also vary with geographical location.[132] However, fur and liver copper values have been found to correlate in rats,[131] and one study reported that supplemental copper raises hair copper levels.[133] Hair color also has been found to influence the levels of copper in the hair.[127,134]

At this time, hair copper measurement appears unreliable for clinical application.[124]

Manganese

Levels of hair manganese in mothers of infants with congenital malformations and their offspring were significantly lower in one study,[14] which suggest that maternal hair manganese levels may be used as an indicator of the risk for malformations. Hair manganese was inversely associated with working memory in children[135] but found not to correlate with developmental scores.[136] Both nonsignificantly altered[137] and normal levels of manganese[138] have been

reported in patients with epilepsy.[137] Hair manganese values have been reported to be elevated in people with violent behavior, with varying levels of significance.[138,139] Manganese was lower in fibromyalgia,[59] in children with amblyopia,[61] and in rheumatoid arthritis.[140] Evidence that manganese supplementation affects behavior does not appear to exist at present.

Hair manganese may serve as a useful research tool in the study of altered behavior, but the most promising value of hair manganese may lie in the prediction of congenital malformations.

Selenium

Levels of selenium in well water and hair show good correlation.[141] High hair selenium levels are seen in toxicity[142,143] and hyperlipidemia,[144] and low levels are seen in deficiency.[145] Low hair selenium values have been reported in babies with neural tube defects and their mothers.[15] Selenium appears to be transferred transplacentally as measured by hair analysis.[146] Lower selenium was seen in patients with phenylketonuria,[147] cancer,[63,67,68] bipolar disease,[71] and steatohepatitis.[148] Tissue selenium values reflect short-term variations in intake,[82] and hair levels of the element rise significantly after supplementation.[149,150]

As with chromium, insufficient data are currently available to establish reliable norms for measurement of hair selenium values, and the incidence of false-positive and false-negative results remains unknown. Hair selenium measurement shows promise for clinical use when these problems are resolved, although they haven't been as of this writing.

Sodium and Potassium

It is generally accepted, even by proponents of HA, that hair sodium and potassium do not reflect dietary status. High elevations of hair sodium may be diagnostic in cystic fibrosis[31] but require confirmation. A relatively low Na/K ratio has been reported in celiac disease.[151] Although many HA advocates cite low hair sodium and potassium as indicative of "adrenal exhaustion," the only (preliminary) study exploring this subject reported that hair sodium and potassium do not correlate with adrenal function.[152] There have not been any subsequent studies. Except for cystic fibrosis, hair sodium and potassium appear to hold little promise for clinical use.

Zinc

Hair zinc levels have received more research attention than any other mineral. Low hair zinc has been associated with zinc deficiency, anorexia nervosa, hyperactivity, gender, age, atherosclerosis, beta-thalassemia, vegetarianism, lung cancer, leukemia, celiac disease, epilepsy in males, epilepsy in general, short stature in childhood, poverty, insulin-dependent diabetes mellitus, neural tube defects, Alzheimer's disease,[46] hypertension,[48] atopic dermatitis,[153] breast cancer,[153] schizophrenia,[72] hypertension,[154] prostate cancer,[67] liver cancer,[150] poor growth in children,[155] and during pregnancy.[17,29,32,108,156–174] It also has been reported in neonates if the time between pregnancies is short.[175] High hair zinc was associated with low serum zinc in Kashin–Beck disease.[175]

Because a few of these conditions have been associated with potential zinc deficiencies and supplemental zinc has been shown to increase hair zinc levels,[154] practitioners who use HA often rely on hair zinc as an indicator of zinc status. However, one trial reported that hair zinc levels declined after supplementation.[107,156,157,168,176,177]

Other factors that affect hair zinc levels also interfere with the clinical use of this tool. Shampooing and dyeing affect hair zinc levels,[178] as do the sex of the subject,[178–182] age,[127] and hair growth rate. Malnourished children have shown both low[171,183,184] and high[185] hair zinc.

Poor correlations between hair zinc and height,[187] weight, and zinc consumption also have been reported,[133,179] although one study reported a correlation among hair zinc, weight, and zinc consumption.[186] Another study found that obese people of both sexes had higher hair zinc than those of normal weight and that there was a correlation between the degree of obesity and higher hair zinc levels.[187]

Although low hair zinc levels have been reported in patients with insulin-dependent diabetes mellitus,[176] there is generally considerable overlap between cases and controls. To further complicate the picture, in a study of female descendants of noninsulin-dependent (NIDDM) parents, hair zinc was found to be significantly higher compared with women with no family history of NIDDM.[188]

At this time there is no definitive understanding of the meaning of abnormal hair zinc levels. The hypothesis that high hair zinc levels reflect acute deficiency, whereas low levels indicate chronic deficiency, remains unproven.

Other Minerals

Hair iron was found to correlate positively with serum ferritin[189] and other laboratory markers of iron status,[190] although the clinical relevance of this finding is unclear.[191] Hair lithium has been reported to show a linear response to extradietary sources.[13] Low hair levels of lithium also have been reported in conjunction with heart disease, learning disability, and violent behavior.[13] However, research findings are far from the point at which hair lithium could be used to help clinicians diagnose or treat these conditions.[13] It is suggested that whole-body iodine status can be assessed with HA.[192]

DRUG ABUSE

HA has been used to detect drugs of abuse and their metabolites when urine tests were negative.[76,78] Hair analysis has been used as evidence in a court of law concerning past drug abuse.[77] Although there are concerns about the role of HA in testing for drug abuse,[193] HA does seem to be a valuable tool in drug screening.[79–82,194] As mentioned, drug metabolites and not mineral levels are measured in these trials, and drug metabolites typically are not reported on most commercially available hair analyses.

RATIOS

The experimental documentation for most of the "ideal" ratios that have been published by several HA companies has not been substantiated in the research literature. The Zn/Cu ratio has been reported to be altered in violent patients.[139] This ratio was also elevated in survivors of myocardial infarcts.[195] Ca/Mg ratios were elevated in insulin resistance[55] and stress[102] coronary artery calcification.[196] Strong correlations between several mineral ratios and atherosclerosis also have been reported[197]; however, the clinical significance of these ratios is not known. The Mg/Zn ratios helped separate healthy (<1/1) from epileptic (>1/1) subjects in one study.[165]

DISCUSSION

Hair mineral analysis is conceptually enticing and potentially a valuable clinical tool. However, problems abound with standardization of sampling, handling, and analysis of hair samples.[86,87] Additionally, reference values can differ widely,[198,199,10,200] and many variables affect the results.[201]

BOX 16.1 Factors That Affect Hair Mineral Composition

- Gender[134,99,100,211]
- Age[134,212,213]
- Ethnicity[98]
- Environment[214–216,101,211]
- Hair color[127,134]
- Cold waving
- Bleaching
- Exogenous contamination[104]
- Variations in mineral content within a given sample

This is further aggravated by the reporting of different results on the same sample from different laboratories.[87,202,90,7] The distribution of elements within the lipid and nonlipid portions of hair and the treatment of the sample before testing may explain these variances.[5,203,8] As noted, dietary intakes do not consistently correlate with hair levels, and hair color, sex, age, and other variables appear to significantly affect levels of some minerals. Separate norms for age, gender, and hair color are now being established.[127,204,205,99] However, reviews of the literature attempting to establish reference values continue to show a large variance in mean values, standard deviations, and ranges.[132,206,57,91] Although many laboratories make nutritional recommendations, there is no basis for the nutritional advice suggested by some laboratories. See Box 16-1.

Several studies have concluded a diagnostic value of HA[53,64,105,69,192,70] in specific circumstances. However, to date, there haven't been additional studies validating the results of these studies, which reduces the power of their conclusions.

As a result of the gulf between available information on the one hand and the clinical interest of some practitioners on the other, several articles have appeared decrying the misuses of HA.[86,87,207–209] Hambidge[208] has said, "There is a wide gulf between the limited and mainly tentative justification for their use on an individual basis and the current exploitation of multielement chemical analysis of human hair."

Klevay and associates[210] acknowledge the experimental usefulness of HA but go on to say that "its use in clinical medicine for diagnosis, prognosis, and therapy will remain limited until validation by the standard methods of clinical investigation is achieved." Steindel and Howanitz[209] conclude the following:

> *Physicians and other health care professionals who are considering ordering hair analysis to assess nutritional status or who are basing nutritional counseling or therapy on hair analysis should reconsider this approach unless and until the reliability of hair analysis value is established and evidence becomes available that clinical recommendations based on hair analysis improve patient outcomes.*

Even though these comments are over a decade old, the conclusions are still pertinent. The accumulated results of several hundreds of studies do not warrant the use of HA as an independent diagnostic tool. The widespread use of speculative diagnostic procedures invites condemnation, especially when the burden of these speculations falls on the pocketbook and psyche of the patient. HA is a valid and useful screening tool for toxic metal exposure (lead, mercury, cadmium, arsenic, and selenium) and drugs of abuse. It has the potential of becoming a clinical tool of considerable use in other areas. The current nonstandardization of laboratory procedures is more likely to hinder this development than to help it.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Passwater RA, Cranton EM. *Trace Elements, Hair Analysis and Nutrition*. New Canaan, CT: Keats Publishing; 1983.
2. Chojnacka K. Zielińska A Reference values for hair minerals of Polish students. *Environ Toxicol Pharmacol*. 2010;29(3):314–319.
3. Blaurock-Busch E. Attacking and defending hair mineral analysis. *Townsend Lett Doc Patient*. 2001;251:86–89.
4. Sen J, Das Chaudhuri AB. Brief communication: choice of washing method of hair samples for trace element analysis in environmental studies. *Am J Phys Anthropol*. 2001;115:289–291.
5. Bass DA, et al. Trace element analysis in hair: factors determining accuracy, precision and reliability. *Altern Med Rev*. 2001;6:472–481.
6. Mikulewicz M, Chojnacka K. Reference values of elements in human hair: a systematic review. *Environ Toxicol Pharmacol*. 2013;36(3):1077–1086.
7. Namkoong S, Hong SP. Reliability on intra-laboratory and inter-laboratory data of hair mineral analysis comparing with blood analysis. *Ann Dermatol*. 2013;25(1):67–72.
8. Kempson IM, Skinner WM. A comparison of washing methods for hair mineral analysis: internal versus external effects. *Biol Trace Elem Res*. 2012;150(1-3):10–14.
9. Wołowiec P, Michalak I. Hair analysis in health assessment. *Clin Chim Acta*. 2013;419:139–171.
10. Mikulewicz M, Chojnacka K. Reference values of elements in human hair: a systematic review. *Environ Toxicol Pharmacol*. 2013;36(3):1077–1086.
11. Pihl RO, Parkes M. Hair element content in learning disabled children. *Science*. 1977;198:204.
12. Marlowe M, Moon C. Hair aluminum concentration and nonadaptive classroom behavior. *J Adv Med*. 1988;1:135–142.
13. Schrauzer GN, Shrestha KP, Flores-Arce MF. Lithium in scalp hair of adults, students, and violent criminals. Effects of supplementation and evidence for interactions of lithium with vitamin B12 and with other trace minerals. *Biol Trace Elem Res*. 1992;34:161–176.
14. Saner G, Dagoglu T, Ozden T. Hair manganese concentrations in newborns and their mothers. *Am J Clin Nutr*. 1985;41:1042–1044.
15. Guvenc H, et al. Low levels of selenium in mothers and their newborns in pregnancies with a neural tube defect. *Pediatrics*. 1995;95:879–882.
16. Barlow PJ. A pilot study on the metal levels in the hair of hyperactive children. *Med Hypotheses*. 1983;11:309.
17. Barlow PJ, et al. Trace metal abnormalities in long-stay hyperactive mentally handicapped children and agitated senile dements. *J R Soc Med*. 1986;79:581–583.
18. Kozielec T, Starobrat-Hermelin B. Assessment of magnesium levels in children with attention deficit hyperactivity disorder (ADHD). *Magn Res*. 1997;10:143–148.
19. Marlowe M, et al. Hair mineral content as a predictor of mental retardation. *J Orthomol Psychiatry*. 1983;12:26.
20. Shrestha KP, Carrera AE. Hair trace elements and mental retardation among children. *Arch Environ Health*. 1988;43:396–398.
21. Kracke K. Biochemical bases for behaviour disorders in children. *J Orthomol Psychiatry*. 1982;11:289.
22. Vobecky J, et al. Hair and urine chromium content in 20 hospitalized female psychogeriatric patients and mentally healthy controls. *Nutr Rep Int*. 1980;22:49–55.
23. Marlowe M, et al. Decreased magnesium in the hair of autistic children. *J Orthomol Psychiatry*. 1984;13:117.
24. Blaurock-Busch E, Amin OR. Heavy metals and trace elements in hair and urine of a sample of arab children with autistic spectrum disorder. *Maedica (Buchar)*. 2011;6(4):247–257.
25. Al-Farsi YM, Waly MI. Levels of heavy metals and essential minerals in hair samples of children with autism in Oman: a case-control study. *Biol Trace Elem Res*. 2013;151(2):181–186.
26. Blaurock-Busch E, Amin OR. Toxic metals and essential elements in hair and severity of symptoms among children with autism. *Maedica (Buchar)*. 2012;7(1):38–48.
27. do Nascimento SN, Barth A. Cognitive deficits and ALA-D-inhibition in children exposed to multiple metals. *Environ Res*. 2015;136:387–395.
28. Cotton DW, Porters JE, Spruit D. Magnesium content of the hair in alopecia areata atypica. *Dermatologica*. 1976;152:60–62.
29. Amador M, et al. Hair-zinc concentrations in diabetic children. *Lancet*. 1975;2:1146.
30. Hambidge KM, Rodgerson DO, O'Brien D. Concentration of chromium in the hair of normal children and children with juvenile diabetes mellitus. *Diabetes*. 1968;17:517–519.
31. Kopito L, Elian E, Shwachman H. Sodium, potassium, calcium, and magnesium in hair from neonates with cystic fibrosis and in amniotic fluid from mothers of such children. *Pediatrics*. 1972;49:620–624.
32. Dogru U, Arcasoy A, Cavdar AO. Zinc levels of plasma, erythrocyte, hair and urine in homozygote beta-thalassemia. *Acta Haematol*. 1979;62:41.
33. Man CK, Zheng YH, Mak PK. Hair analysis of spastic children in Hong Kong. *Sci Total Environ*. 1996;191:291–295.
34. Majumdar S, Chatterjee J, Chaudhuri K. Ultrastructural and trace metal studies on radiographers' hair and nails. *Biol Trace Elem Res*. 1999;67:127–136.
35. Chatterjee J, et al. Trace metal levels of X-ray technicians' blood and hair. *Biol Trace Elem Res*. 1994;46:211–227.
36. Man AC, Zheng YH, Mak PK. Structural and trace element changes in scalp hair of radiographers. *Biol Trace Elem Res*. 1998;63:11–18.
37. Leung PL, Huang HM. Following the recovery of naso-pharyngeal cancer patients by trace elements in hair using statistical pattern recognition methods. *Biol Trace Elem Res*. 1998;62:235–253.
38. Leung PL, Huang HM. Analysis of trace elements in the hair of volunteers suffering from nasopharyngeal cancer. *Biol Trace Elem Res*. 1997;57:19–25.
39. Wang J, et al. Contents of trace elements in the hair of aplastic anemia patients and their treatment based on an overall analysis of symptoms and signs. *J Trad Chin Med*. 1994;14:98–100.
40. Joo NS, et al. Hair iron and other minerals' level in breast cancer patients. *Biol Trace Elem Res*. 2009;129(1-3):28–35.
41. Pasha Q, Malik SA. Comparison of trace elements in the scalp hair of malignant and benign breast lesions versus healthy women. *Biol Trace Elem Res*. 2010;134(2):160–173.
42. Benderli Cihan Y, Sözen S. Trace elements and heavy metals in hair of stage III breast cancer patients. *Biol Trace Elem Res*. 2011;144(1-3):360–379.
43. Song CH, et al. Associations of calcium and magnesium in serum and hair with bone mineral density in premenopausal women. *Biol Trace Elem Res*. 2007;118:1–9.
44. Park SJ, Lee SH. Hair calcium concentration is associated with calcium intake and bone mineral density. *Int J Vitam Nutr Res*. 2013;83(3):154–161.
45. Huang H, et al. Hybrid progressive algorith to recognise type II diabetic based on hair mineral element contents. *Conf Proc IEEE Eng Med Biol Soc*. 2005;5:4716–4718.
46. Koç ER, Ilhan A. A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants. *Turk J Med Sci*. 2015;45(5):1034–1039.
47. Koseoglu E, Koseoglu R. Trace metal concentrations in hair and nails from Alzheimer's disease patients: Relations with clinical severity. *J Trace Elem Med Biol*. 2017;39:124–128.
48. Marcinek K, Suliburska J. Evaluation of mineral status in hypertensive patients undergoing pharmacotherapy. *Rocz Panstw Zakl Hig*. 2015;66(1):61–67.
49. Suliburska J, Bogdański P. Dietary intake and serum and hair concentrations of minerals and their relationship with serum lipids and glucose levels in hypertensive and obese patients with insulin resistance. *Biol Trace Elem Res*. 2011;139(2):137–150.
50. Rodenas S, Sánchez-Muniz FJ. Blood pressure of omnivorous and semi-vegetarian postmenopausal women and their relationship with dietary and hair concentrations of essential and toxic metals. *Nutr Hosp*. 2011;26(4):874–883.
51. Wang B, Yan L. Alkaline-earth elements of scalp hair and presence of hypertension in housewives: a perspective of chronic effect. *Chemosphere*. 2017;181:134–141.
52. Yamashiro K, Ogata F. Relationship between hay fever and mineral concentration in the hair, lifestyle or aging. *Yakugaku Zasshi*. 2017;137(8):1035–1040.
53. Yamada T, Saunders T. Cohort study for prevention of atopic dermatitis using hair mineral contents. *J Trace Elem Med Biol*. 2013;27(2):126–131.

54. Park SB, Choi SW, Nam AY. Hair tissue mineral analysis and metabolic syndrome. *Bio Trace Elem Res*. 2009;130(3):218–228.
55. Chung JH, Yum KS. Correlation of hair mineral concentrations with insulin resistance in Korean males. *Biol Trace Elem Res*. 2012;150 (1-3):26–30.
56. Park SB1, Choi SW. Hair tissue mineral analysis and metabolic syndrome. *Biol Trace Elem Res*. 2009;130(3):218–228.
57. Choi WS, Kim SH. Relationships of hair mineral concentrations with insulin resistance in metabolic syndrome. *Biol Trace Elem Res*. 2014;158(3):323–329.
58. Kim HN, Song SW. Concentrations of chromium, selenium, and copper in the hair of viscerally obese adults are associated with insulin resistance. *Biol Trace Elem Res*. 2014;158(2):152–157.
59. Kim YS, Kim KM. Women with fibromyalgia have lower levels of calcium, magnesium, iron and manganese in hair mineral analysis. *J Korean Med Sci*. 2011;26(10):1253–1257.
60. Ochi A, Ishimura E. Trace elements in the hair of hemodialysis patients. *Biol Trace Elem Res*. 2011;143(2):825–834.
61. Zhu Q, Chen J. Mineral elements in the hair of amblyopic children. *Biol Trace Elem Res*. 2011;141(1-3):119–125.
62. Tamburo E, Varrica D. Trace elements in scalp hair samples from patients with relapsing-remitting multiple sclerosis. *PLoS One*. 2015;10(4): e0122142.
63. Czerny B, Krupka K. Screening of trace elements in hair of the female population with different types of cancers in Wielkopolska region of Poland. *Scientific World J*. 2014;2014:953181.
64. Golasik M, Przybyłowicz A. Essential metals profile of the hair and nails of patients with laryngeal cancer. *J Trace Elem Med Biol*. 2015;31:67–73.
65. Qayyum MA, Shah MH. Comparative study of trace elements in blood, scalp hair and nails of prostate cancer patients in relation to healthy donors. *Biol Trace Elem Res*. 2014;162(1-3):46–57.
66. Pasha Q1, Malik SA. Investigation of trace metals in the blood plasma and scalp hair of gastrointestinal cancer patients in comparison with controls. *Clin Chim Acta*. 2010;411(7-8):531–539.
67. Karimi G, Shahar S. Association between trace element and heavy metal levels in hair and nail with prostate cancer. *Asian Pac J Cancer Prev*. 2012;13(9):4249–4253.
68. Kolachi NF, Kazi TG. Investigation of essential trace and toxic elements in biological samples (blood, serum and scalp hair) of liver cirrhotic/cancer female patients before and after mineral supplementation. *Clin Nutr*. 2012;31(6):967–973.
69. Wozniak A, Napierala M. Metal concentrations in hair of patients with various head and neck cancers as a diagnostic aid. *Biometals*. 2016;29(1):81–93.
70. Golasik M, Jawień W. Classification models based on the level of metals in hair and nails of laryngeal cancer patients: diagnosis support or rather speculation? *Metallomics*. 2015;7(3):455–465.
71. Pradeep AS, Naga Raju GJ. Trace elemental distribution in the scalp hair of bipolars using PIXE technique. *Med Hypotheses*. 2014;82(4):470–477.
72. Rahman A, Azad MA. Zinc, manganese, calcium, copper, and cadmium level in scalp hair samples of schizophrenic patients. *Biol Trace Elem Res*. 2009;127(2):102–108.
73. Stefano F, Cinzia N. Hair microelement profile as a prognostic tool in Parkinson's disease. *Toxics*. 2016;4(4).
74. Skalny AV, Tinkov AA. Hair Trace Element and Electrolyte Content in Women with Natural and In Vitro Fertilization-Induced Pregnancy. *Biol Trace Elem Res*. 2017.
75. Maduray K, Moodley J. Elemental analysis of serum and hair from pre-eclamptic South African women. *J Trace Elem Med Biol*. 2017;43:180–186.
76. Graham K, et al. Determination of gestational cocaine exposure by hair analysis. *JAMA*. 1989;262:3328–3330.
77. Strang J, Marsh A, DeSouza N. Hair analysis for drugs of abuse. *Lancet*. 1990;335:740.
78. Gaillard Y, Vayssette F, Pepin G. Compared interest between hair analysis and urinalysis in doping controls. Results for amphetamines, corticosteroids and anabolic steroids in racing cyclists. *Forensic Sci Int*. 2000;107:361–379.
79. Nakahara Y. Hair analysis for abused and therapeutic drugs. *J Chromatogr B Biomed Sci Appl*. 1999;733:161–180.
80. Gaillard Y, Pepin G. Testing hair for pharmaceuticals. *J Chromatogr B Biomed Sci Appl*. 1999;733:231–246.
81. Rivier L. Is there a place for hair analysis in doping controls? *Forensic Sci Int*. 2000;107:309–323.
82. Klien J, Karaskov T, Koren G. Clinical applications of hair testing for drugs of abuse: the Canadian experience. *Forensic Sci Int*. 2000;107:281–288.
83. Kintz P, Villain M, Cirimele V. Hair analysis for drug detection. *Ther Drug Monit*. 2006;28:442–446.
84. Kronstrand R, et al. Hair analysis for drugs in driver's license regranting. A Swedish pilot study. *Forensic Sci Int*. 2010;196:55–58.
85. Polla M, et al. Hait testing is superior to urine to disclose cocaine consumption in driver's license regranting. *Forensic Sci Int*. 2009;189:e41–e43.
86. Barrett S. Commercial hair analysis. Science or scam? *JAMA*. 1985;254:141–145.
87. Seidel S, et al. Assessment of commercial laboratories performing hair mineral analysis. *JAMA*. 2001;285:67–72.
88. Morley N Ford R. Hair-element analysis—still on the fringe. *Child Care Health Dev*. 2002;28(suppl 1):31–34.
89. Shamberger RJ. Validity of hair mineral testing. *Biol Trace Elem Res*. 2002;87(1-3):1–28.
90. Drasch G, Roider G. Assessment of hair mineral analysis commercially offered in Germany. *J Trace Elem Med Biol*. 2002;16(1):27–31.
91. Pozebon D, Scheffler GL. Elemental hair analysis: a review of procedures and applications. *Anal Chim Acta*. 2017;992:1–23.
92. Kim HY, Lee JY. Nutrient intakes and hair mineral contents of young children. *Pediatr Gastroenterol Hepatol Nutr*. 2016;19(2):123–129.
93. Tueller DJ, Eggett DL. A preliminary study of a Peruvian diet using dietary analysis and hair mineral content as indicators. *Biol Trace Elem Res*. 2013;155(2):161–168.
94. Chojnacka K, Zielińska A. The effect of dietary habits on mineral composition of human scalp hair. *Environ Toxicol Pharmacol*. 2010;30(2):188–194.
95. Hong SR, Lee SM. Association between hair mineral and age, BMI and nutrient intakes among Korean female adults. *Nutr Res Pract*. 2009 Fall;3(3):212–219.
96. Oyoo-Okoth E, Admiraal W. Element profiles in hair and nails of children reflect the uptake from food and the environment. *Environ Toxicol Chem*. 2012;31(7):1461–1469.
97. Vanaelst B, Huybrechts I. Mineral concentrations in hair of belgian elementary school girls: reference values and relationship with food consumption frequencies. *Biol Trace Elem Res*. 2012;150(1-3):56–67.
98. Loussouarn G, Lozano I. Diversity in human hair growth, diameter, colour and shape. An in vivo study on young adults from 24 different ethnic groups observed in the five continents. *Eur J Dermatol*. 2016;26(2):144–154.
99. E T, D V. Gender as a key factor in trace metal and metalloid content of human scalp hair. A multi-site study. *Sci Total Environ*. 2016;573:996–1002.
100. Drobyshev EJ, Solovyev ND. Trace element biomonitoring in hair of school children from a polluted area by sector field inductively coupled plasma mass spectrometry. *J Trace Elem Med Biol*. 2017;39:14–20.
101. Tamburo E, Varrica D. Coverage intervals for trace elements in human scalp hair are site specific. *Environ Toxicol Pharmacol*. 2015;39(1):70–76.
102. Ng SY. Hair calcium and magnesium levels in patients with fibromyalgia: a case center study. *J Manipulative Physiol Ther*. 1999;22:586–593.
103. Vanaelst B, Michels N. Cross-sectional relationship between chronic stress and mineral concentrations in hair of elementary school girls. *Biol Trace Elem Res*. 2013;153(1-3):41–49.
104. Noble RE. Uptake of calcium and magnesium by human scalp hair from waters of different geographic locations. *Sci Total Environ*. 1999;239:189–193.
105. Kim OY, Baek SH. Association of increased hair calcium levels and enhanced augmentation index (AIx): a marker of arterial stiffness. *Biol Trace Elem Res*. 2010;138(1-3):90–98.
106. Huang HM, et al. Hair and serum calcium, iron, copper, and zinc levels during normal pregnancy at three trimesters. *Biol Trace Elem Res*. 1999;69:111–120.
107. Leung PL, et al. Hair concentrations of calcium, iron and zinc in pregnant women and effects of supplementation. *Biol Trace Elem Res*. 1999;69:269–282.

108. Sahin G, et al. High prevalence of chronic magnesium deficiency in T cell lymphoblastic leukemia and chronic zinc deficiency in children with acute lymphoblastic leukemia and malignant lymphoma. *Leuk Lymphoma*. 2000;39:555–562.
109. Capel ID, et al. Comparisons of concentrations of some trace, bulk, and toxic metals in the hair of normal and dyslexic children. *Clin Chem*. 1981;27:879–881.
110. Marlowe M, et al. Hair minerals and diet of Prader-Willi syndrome youth. *J Autism Dev Disord*. 1987;17:365–374.
111. Miekeley N, et al. Elemental anomalies in hair as indicators of endocrinologic pathologies and deficiencies in calcium and bone metabolism. *J Trace Elem Med Biol*. 2001;15:46–55.
112. Bland J. Dietary calcium, phosphorus and their relationships to bone formation and parathyroid activity. *J John Bastyr Col Nat Med*. 1979;1:3.
113. Panhwar AH, Kazi TG. Evaluation of calcium and magnesium in scalp hair samples of population consuming different drinking water: risk of kidney stone. *Biol Trace Elem Res*. 2013;156(1-3):67–73.
114. Kozielec T, et al. The influence of magnesium supplementation on magnesium and calcium concentrations in hair of children with magnesium shortage. *Magn Res*. 2001;14:33–38.
115. Guillard O, et al. Congenital hypomagnesemia: alternatives to tissue biopsies for monitoring body magnesium status. *Clin Biochem*. 1992;25:463–465.
116. Hambidge KM, Franklin ML, Jacobs MA. Changes in hair chromium concentrations with increasing distances from hair roots. *Am J Clin Nutr*. 1972;25:380–383.
117. Aharoni A, et al. Hair chromium content of women with gestational diabetes compared with nondiabetic pregnant women. *Am J Clin Nutr*. 1992;55:104–107.
118. Jeejeebhoy KN, et al. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am J Clin Nutr*. 1977;30:531–538.
119. Rowland A. Trace metals leave more than trace effects. *Sci News*. 1984;125:373.
120. Hunt AE, Allen KGD, Smith BA. Effect of chromium supplementation on hair chromium concentration and diabetic status. *Fed Proc*. 1983;24:925.
121. Hambidge KM, Franklin ML, Jacobs MA. Hair chromium concentration. Effects of sample washing and external environment. *Am J Clin Nutr*. 1972;25:384–389.
122. Mikulewicz M, Wołowiec P. Metal ions released from fixed orthodontic appliance affect hair mineral content. *Biol Trace Elem Res*. 2015;163(1-2):11–18.
123. Deeming SB, Weber CW. Hair analysis of trace minerals in human subjects as influenced by age, sex, and contraceptive drugs. *Am J Clin Nutr*. 1978;31:1175.
124. Klevay LM. Hair as a biopsy material. II. Assessment of copper nutriture. *Am J Clin Nutr*. 1970;23:1194–1202.
125. Pratt WB, Phippen WG. Elevated hair copper levels in idiopathic scoliosis. Preliminary observations. *Spine*. 1980;5:230–233.
126. Vir SC, Love AH, Thompson W. Serum and hair concentrations of copper during pregnancy. *Am J Clin Nutr*. 1981;34:2382–2388.
127. Bertazzo A, et al. Determination of copper and zinc levels in human hair: influence of sex, age, and hair pigmentation. *Biol Trace Elem Res*. 1996;52:37–53.
128. Bradfield RB, et al. Hair copper in copper deficiency. *Lancet*. 1980;2:343–344.
129. Mediros D, et al. Trace elements in human hair. *Lancet*. 1982;2:608.
130. Watt F, et al. Analysis of copper and lead in hair using the nuclear microscope; results from normal subjects, and patients with Wilson's disease and lead poisoning. *Analyst*. 1995;120:789–791.
131. Epstein O, et al. Hair copper in primary biliary cirrhosis. *Am J Clin Nutr*. 1980;33:967.
132. Iyengar V, Woittiez J. Trace elements in human clinical specimens: evaluation of literature data to identify reference values. *Clin Chem*. 1988;34:474–481.
133. Jacob RA, Klevay LM, Logan Jr GM. Hair as a biopsy material. V. Hair metal as an index of hepatic metal in rats: copper and zinc. *Am J Clin Nutr*. 1978;31:477–480.
134. Sturaro A, et al. The influence of color, age, and sex on the content of zinc, copper, nickel, manganese, and lead in human hair. *Biol Trace Elem Res*. 1994;40:1–8.
135. Nascimento S, Baierle M. Associations among environmental exposure to manganese, neuropsychological performance, oxidative damage and kidney biomarkers in children. *Environ Res*. 2016;147:32–43.
136. Rink SM, Ardoino G. Associations between hair manganese levels and cognitive, language, and motor development in preschool children from Montevideo, Uruguay. *Arch Environ Occup Health*. 2014;69(1):46–54.
137. Papavasilious PS, et al. Seizure disorders and trace metals: manganese tissue levels in treated epileptics. *Neurology*. 1979;29:1466–1473.
138. Gottschalk LA, et al. Abnormalities in the hair trace elements as indicators of aberrant behavior. *Comp Psychiatry*. 1991;32:229–237.
139. Cromwell PF, et al. Hair mineral analysis: biochemical imbalances and violent criminal behavior. *Psychol Rep*. 1989;64:259–266.
140. Afridi HI, Kazi TG. Association between essential trace and toxic elements in scalp hair samples of smokers rheumatoid arthritis subjects. *Sci Total Environ*. 2011;412–413:93–100.
141. Valentine JL, Kang HK, Spivey GH. Selenium levels in human blood, urine, and hair in response to exposure via drinking water. *Environ Res*. 1978;17:347–355.
142. Yang GQ, et al. Endemic selenium intoxication of humans in China. *Am J Clin Nutr*. 1983;37:872–881.
143. Srivastava AK, et al. Hair selenium as a monitoring tool for occupational exposures in relation to clinical profile. *J Toxicol Environ Health*. 1997;51:437–445.
144. Fülöp P, Seres I. Increased hair selenium concentration in hyperlipidemic patients. *J Cell Mol Med*. 2013;17(3):350–355.
145. Keshan Disease Research Group of the Chinese Academy of Medical Sciences, Beijing. Epidemiological studies on the etiologic relationships of selenium and Keshan disease. *Chin Med J*. 1979;92:477.
146. Momčilović B, Prejac J. High hair selenium mother to fetus transfer after the Brazil nuts consumption. *J Trace Elem Med Biol*. 2016;33:110–113.
147. Jochum F, et al. Effects of a low selenium state in patients with phenylketonuria. *Acta Paediatr*. 1997;86:775–777.
148. Pan D, Huang H. Hair selenium levels in hepatic steatosis patients. *Biol Trace Elem Res*. 2013;152(3):305–309.
149. Gallagher ML, et al. Selenium levels in new growth hair and in whole blood during injection of a selenium supplement for six weeks. *Nutr Res*. 1984;4:577–582.
150. Terada A, et al. Selenium administration to a ten-year-old patient receiving long-term total parenteral nutrition (TPN): changes in selenium concentration in the blood and hair. *J Trace Elem Med Biol*. 1998;10:1–5.
151. Maugh II TH. Hair: a diagnostic tool to complement blood serum and urine. *Science*. 1978;202:1271–1273.
152. Wright JV, Severtson RB. Observations on the interpretations of hair mineral analysis in human medicine. *J Intl Acad Prev Med*. 1982:13–16.
153. Kim JE, Yoo SR. Hair zinc levels and the efficacy of oral zinc supplementation in patients with atopic dermatitis. *Acta Derm Venereol*. 2014;94(5):558–562.
154. Afridi HI, Brabazon D. Evaluation of essential trace and toxic elements in scalp hair samples of smokers and alcohol user hypertensive patients. *Biol Trace Elem Res*. 2011;143(3):1349–1366.
155. Han TH, Lee J. Hair zinc level analysis and correlative micronutrients in children presenting with malnutrition and poor growth. *Pediatr Gastroenterol Hepatol Nutr*. 2016;19(4):259–268.
156. Pekarek RS, et al. Abnormal cellular immune responses during acquired zinc deficiency. *Am J Clin Nutr*. 1979;32:1466–1471.
157. Ward NI. Assessment of zinc status and oral supplementation in anorexia nervosa. *J Nutr Med*. 1990;1:171–177.
158. Gibson RS, et al. Growth in children from the Wosera subdistrict, Papua New Guinea, in relation to energy and protein intakes and zinc status. *Am J Clin Nutr*. 1991;53:782–789.
159. Zachwieja Z, et al. Evaluation of zinc status in children's hair. *Biol Trace Elem Res*. 1995;47:141–145.
160. Bales CW, et al. Zinc, magnesium, copper and protein concentrations in human saliva: age- and sex-related differences. *Am J Clin Nutr*. 1990;51:462–469.
161. Strain WH, Pories WJ. Zinc levels of hair as tools in zinc metabolism. In: Prasad A, ed. *Zinc Metabolism*. Springfield, IL: Charles C Thomas; 1966:363.

162. Freeland-Graves JH, Bodzy PW, Eppright MA. Zinc status of vegetarians. *J Am Diet Assoc*. 1980;77:655–661.
163. Piccinini L, et al. A case-control study on selenium, zinc, and copper in plasma and hair of subjects affected by breast cancer and lung cancer. *Biol Trace Elem Res*. 1996;51:23–30.
164. Varkonyi A, et al. Coeliac disease: always something to discover. *Scand J Gastroenterol Suppl*. 1998;228:122–129.
165. Ashraf W, et al. Utilization of scalp hair for evaluating epilepsy in male and female groups of the Pakistan population. *Sci Total Environ*. 1995;164:69–73.
166. Ilhan A, et al. Serum and hair trace element levels in patients with epilepsy and healthy subjects: does antiepileptic therapy affect the element concentration of hair? *Eur J Neurol*. 1999;6:705–709.
167. Bradfield RB, Hambidge KM. Problems with hair zinc status as an indicator of body zinc status. *Lancet*. 1980;1:363.
168. Anonymous. Zinc supplementation in healthy short children. *Int Clin Nutr Rev*. 1984;4:28.
169. Perrone L, et al. Long-term zinc and iron supplementation in children of short stature: effect of growth and on trace element content in tissues. *J Trace Elem Med Biol*. 1999;13:51–56.
170. Hambidge KM, et al. Zinc nutrition of preschool children in the Denver Head Start program. *Am J Clin Nutr*. 1976;29:734–738.
171. Weber CW, et al. Trace elements in the hair of healthy and malnourished children. *J Trop Pediatr*. 1990;36:230–234.
172. Gibson RS, et al. A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. *Am J Clin Nutr*. 1989;49:1266–1273.
173. Srinivas M, et al. Association between lower hair zinc levels and neural tube defects. *Indian J Pediatr*. 2001;68:519–522.
174. Rathi SS, et al. Zinc levels in women and newborns. *Indian J Pediatr*. 1999;66:681–684.
175. Wang X, Zhang P. Serum and hair zinc levels in patients with endemic osteochondropathy in China: a meta-analysis. *Biol Trace Elem Res*. 2017.
176. Greger JL, Geissler AH. Effects of zinc supplementation on taste acuity of the aged. *Am J Clin Nutr*. 1978;31:633–637.
177. Sandstead HH, et al. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. *Am J Clin Nutr*. 1998;68:470S–475S.
178. Clanet P, et al. Effects of some cosmetics on copper and zinc concentrations in human scalp hair. *Clin Chem*. 1982;28:2450–2451.
179. McKenzie JM. Content of zinc in serum, urine, hair, and toenails of New Zealand adults. *Am J Clin Nutr*. 1979;32:570–579.
180. Heinersdorff N, Taylor TG. Concentration of zinc in the hair of schoolchildren. *Arch Dis Child*. 1979;54:958–960.
181. Smit Vanderkooy PD, Gibson RS. Food consumption patterns of Canadian preschool children in relation to zinc and growth status. *Am J Clin Nutr*. 1987;45:609–616.
182. Meng Z. Age- and sex-related differences in zinc and lead levels in human hair. *Biol Trace Elem Res*. 1998;61:79–87.
183. Weber CW, Pearson PB, Nelson GW. Mineral levels in hair of malnourished children. *Fed Proc*. 1980;39:551.
184. Saner G. Hair trace element concentrations in patients with protein-energy malnutrition. *Nutr Rep Int*. 1985;32:263–268.
185. Erten J, et al. Hair zinc levels in healthy and malnourished children. *Am J Clin Nutr*. 1978;31:1172.
186. Hogan SE. Dietary intake and hair zinc status of persons with severe physical and developmental disabilities. *Nutr Res*. 1996;16:401–412.
187. Taneja SK, Mahajan M, Arya P. Excess bioavailability of zinc may cause obesity in humans. *Experientia*. 1996;52:31–33.
188. Taneja SK, et al. Assessment of copper and zinc status in hair and urine of young women descendants of NIDDM parents. *Biol Trace Elem Res*. 1998;62:255–264.
189. Bisse E, et al. Hair iron content: possible marker to complement monitoring therapy of iron deficiency in patients with chronic inflammatory bowel disease? *Clin Chem*. 1996;42:1270–1274.
190. Sahin C, Pala C. Measurement of hair iron concentration as a marker of body iron content. *Biomed Rep*. 2015 May;3(3):383–387.
191. Beutler E. Hair iron content: possible marker to complement monitoring therapy of iron deficiency in patients with chronic inflammatory bowel disease? *Clin Chem*. 1997;43:1099–1100.
192. Prejac J, Višnjević V. A novel concept to derive iodine status of human populations from frequency distribution properties of a hair iodine concentration. *J Trace Elem Med Biol*. 2014;28(2):205–211.
193. Bailey DN. Drug screening in an unconventional matrix: hair analysis. *JAMA*. 1989;262:3331.
194. Xiang P, Shen M. Review: Drug concentrations in hair and their relevance in drug facilitated crimes. *J Forensic Leg Med*. 2015;36:126–135.
195. Bialkowska M, et al. Hair zinc and copper concentration in survivors of myocardial infarction. *Ann Nutr Metab*. 1987;31:327–332.
196. Park B, Kim MH. High calcium-magnesium ratio in hair is associated with coronary artery calcification in middle-aged and elderly individuals. *Biol Trace Elem Res*. 2017.
197. Fernandez-Britto JE, et al. Coronary atherosclerosis and chemical trace elements in the hair. A canonical correlation study of autopsy subjects, using an arthrometric system and the X-ray fluorescence analysis. *Zentralbl Pathol*. 1993;139:61–65.
198. Miekeley N, Dias Carneiro MT, da Silveira CL. How reliable are human hair reference intervals for trace elements? *Sci Total Environ*. 1998;218:9–17.
199. Druyan ME, et al. Determination of reference ranges for elements in human scalp hair. *Biol Trace Elem Res*. 1998;62:183–197.
200. Llorente Ballesteros MT, Navarro Serrano I. Reference levels of trace elements in hair samples from children and adolescents in Madrid, Spain. *J Trace Elem Med Biol*. 2017;43:113–120.
201. Austin S. Hair analysis: a reappraisal. *NCNM Rev*. 1982;3:21.
202. Spilker BA, Maugh TH. How useful is hair analysis? *J Energy Med*. 1980;1:15–19.
203. Attar KM, Abdel-Aal MA, Debayle P. Distribution of trace elements in the lipid and non lipid matter of hair. *Clin Chem*. 1990;36:477–480.
204. Perrone L, et al. Trace elements in hair of healthy children sampled by age and sex. *Biol Trace Elem Res*. 1996;51:71–76.
205. Senofonte Violante N, Caroli S. Assessment of reference values for elements in human hair of urban schoolboys. *J Trace Elem Med Biol*. 2000;14:6–13.
206. Taylor A. Usefulness of measurements of trace elements in hair. *Ann Clin Biochem*. 1986;23:364–378.
207. Rivlin RS. Misuse of hair analysis for nutritional assessment. *Am J Med*. 1983;75:489–493.
208. Hambidge KM. Hair analyses: worthless for vitamins, limited for minerals. *Am J Clin Nutr*. 1982;36:943–949.
209. Steindel SJ, Howanitz PJ. The uncertainty of hair analysis for trace metals. *JAMA*. 2001;285:83–85.
210. Klevay LM, et al. Hair analysis in clinical and experimental medicine. *Am J Clin Nutr*. 1987;46:233–236.
211. Varrica D, Tamburo E. Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis-Inglesiente (Sardinia, Italy). *Environ Res*. 2014;134:366–374.
212. Sakai T, Wariishi M, Nishiyama K. Changes in trace element concentrations in hair of growing children. *Biol Trace Elem Res*. 2000;77:43–51.
213. Nowak B, Kozlowski H. Heavy metals in human hair and teeth: the correlation with metal concentration in the environment. *Biol Trace Elem Res*. 1998;62:213–228.
214. Tommaseo Ponzetta M, et al. Trace elements in the human scalp hair and soil in Irian Jaya. *Biol Trace Elem Res*. 1998;62:199–212.
215. Batzevich VA. Hair trace element analysis in human ecology studies. *Sci Total Environ*. 1995;164:89–98.
216. Hamilton T, Scweinsberg F. The reliability of conclusions based on hair mineral analysis in individual diagnostic. *Versicherungsmedizin*. 2004;56:136–140.

17

Heidelberg pH Capsule Gastric Analysis

Stephen Barrie, ND, PhD, and *Michael T. Murray, ND*

OUTLINE

INTRODUCTION

Proper digestion is a prerequisite for optimum health, and incomplete or disordered digestion can be a major contributor to the development of many diseases. The problem is not only that ingestion of the best nutritional substances may be of little benefit when breakdown and assimilation are inadequate but also that incompletely digested macromolecules can be inappropriately absorbed into the systemic circulation. This process can lead to various immune complex deposition diseases and is now theorized to be an integral part of the etiology of food allergies.

Adequate gastric hydrogen chloride (HCl) is also necessary for the protection of the gastrointestinal tract from ingested pathogens and for the maintenance of the intestinal microbiome. Healthy gut flora is known to be important for proper immune function, vitamin absorption, and the prevention of opportunistic infections such as *Candida albicans* in the gut.

The Heidelberg gastric analysis technique was developed to measure the hydrogen ion concentration (pH) of the digestive tract and determine the acid secretory ability of the parietal cells. Its use of radiotelemetry allows the gathering of this important information in a convenient and accurate manner.

The Heidelberg pH capsule system had its origin about 50 years ago at Heidelberg University in Germany. In research sponsored by Telefunken, a West German electronics firm, the inventor H. G. Noeller studied gastric acidity in 10,000 people. Since then, about 140 studies, according to a PubMed inquiry, have used the Heidelberg system to investigate various aspects of digestion.[1–7] Physicians and researchers now use this technique for measuring the pH of the digestive system.

PHYSIOLOGY OF DIGESTION IN THE STOMACH

The epithelium of the stomach contains many gastric glands. These tubular glands consist of parietal, chief, and mucous cells. The antral portion of the stomach produces the digestive hormone gastrin, the release of which is stimulated by the following:

- Vagal nerve stimulation
- The physical bulk of the ingested food distending the stomach
- Partially digested proteins

After gastrin is absorbed into the bloodstream, it is carried to the gastric glands, where it stimulates the parietal cells to produce HCl acid and, to a lesser extent, the chief cells to produce digestive enzymes (such as pepsin and intrinsic factor). With adequate stimulation, the parietal cells increase their production of HCl by as much as eightfold.

When the pH of the stomach reaches about 2, the gastrin mechanism becomes blocked, and feedback causes the parietal cells to decrease the production of HCl. This concentration of hydrogen ions (by a factor of 100,000) is an energy-dependent process.

Dietary protein is composed of amino acids held together by peptide linkages. Pepsin A, the major gastric protease, cleaves these in the stomach and is most active at pH values of between 2 and 3. It is inactive at a pH of 5 and above. Consequently, to have any significant digestive effect in the stomach, the gastric juices must be acidic.

Trypsin (a protein-splitting enzyme secreted by the pancreas) completes the process, yielding amino acids and dipeptides. The biochemical messenger that stimulates this pancreatic secretion is the acidic bolus of food moving from the stomach into the duodenum.

PROCEDURE

Equipment

The Heidelberg system consists of the following equipment:

- Radiotelemetry capsule—a hard plastic capsule (about 2 cm long × 0.8 cm in diameter) that contains a miniature radio transmitter, a pH sensing device, and a saline-activated battery
- Waistband antenna—receives the signal from the capsule and relays it to the receiver
- Receiver/recorder—receives and translates the signal. The pH reading is displayed on a meter and recorded by a continuous printer for a permanent record. The receiver also contains a calibration probe used to calibrate each capsule with known pH 1 and 7 solutions.
- Heater block—maintains the calibrating solutions at 37°C

Methods

The test can be conducted in two ways: the tethered capsule repeat challenge and the flow-through method. Each gives different information and has its advantages and disadvantages. For both procedures, the test begins after the patient has fasted (food and liquid) for 8 hours.

The Tethered Capsule Repeat Challenge

In the tethered capsule repeat challenge, the capsule is tethered so that it remains in the stomach while the stomach is challenged by the ingestion of a saturated sodium bicarbonate solution (i.e., baking soda).[8] The challenge solution triggers a rise in stomach pH and a subsequent attempt by the parietal cells to reestablish appropriate acidity. The majority of people have a normal initial pH of between 1 and 2.3. Abnormalities of stomach secretions are usually found only after the stomach is challenged. (A more involved protocol can be found in Wright.[8])

The procedure is as follows:

1. The waistband antenna is fastened around the patient's waist, and the receiver/recorder is turned on and calibrated.
2. The patient swallows the capsule, which is attached to a 1-m-long, thin cotton thread (a small amount of distilled water is allowed). The pH reading typically starts at 7 and falls toward 1. After about 5 minutes, the capsule reaches the bottom of the stomach (which normally displays a pH of between 1 and 2), and the remaining thread is taped to the cheek to prevent movement of the capsule out of the stomach and into the intestine.
3. If the fasting pH is normal, the patient swallows the first challenge of 5 mL of the alkaline solution. Within 30 seconds, the pH normally rises to 7, and the patient is asked to lie down on his or her left side (to keep the stomach contents in as long as possible).
4. If stomach function is normal and acid is secreted sufficiently in response to the alkali challenge, the pH returns to normal (between 1 and 2) within 20 minutes.
5. The challenge is repeated up to four times, as long as the response time is within 20 minutes.

Flow-Through Capsule

In the procedure for the flow-through capsule, the capsule is not tethered to a thread and is allowed to move freely from the stomach into the duodenum and the rest of the small intestine. The proponents of this method claim that this allows measurement of the gastric emptying time and intestinal pH, both of which are important parameters.

INTERPRETATION

Results may be classified as normal, hypochlorhydria, achlorhydria, and hyperchlorhydria.

Normal. The patient successfully reacidifies after four challenges (Fig. 17.1). Curve number 1 shows the capsule entering the digestive tract, number 2 shows the capsule reaching the bottom of the stomach and alkaline challenge occurring, and number 3 shows a pH rise after swallow and subsequent reacidification within 20 minutes.

Hypochlorhydria. The patient requires more than 20 minutes to reacidify (Fig. 17.2). Curve number 1 shows a pH of 1 being reached after 30 minutes. Note that on the third challenge, the pH comes back only to about 4.

Achlorhydria. The patient's stomach shows little acid secretion and is not able to secrete enough acid to bring the pH below 4, even on the first challenge (Fig. 17.3). The pH remains at about 4.2 for almost 2 hours.

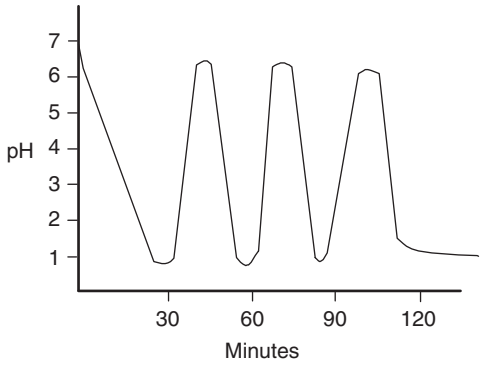


Fig. 17.1 Normal Heidelberg gastrogram.

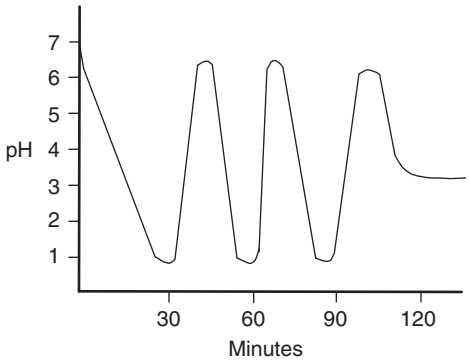


Fig. 17.2 Hypochlorhydric gastrogram.

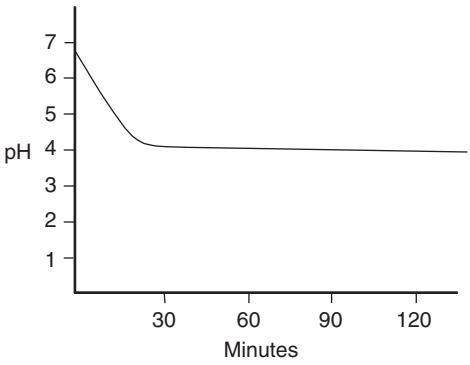


Fig. 17.3 Achlorhydric gastrogram.

Hyperchlorhydria. The gastrogram shows extremely rapid reacidification (within 5 minutes) after each challenge.

Depending on specific curve components, some investigators believe that mucous quantity, fresh or chronic ulcers, and acute gastritis conditions can sometimes be identified.

CLINICAL APPLICATION

Gastritis, indigestion, heartburn, and symptoms of gastroesophageal reflux can be associated with either HCl deficiency or excess. Proper distinction is important for several obvious reasons. Patients with hypo- or achlorhydria incorrectly treated for hyperacidity via acid-blocking drugs may experience symptom relief but also further deterioration of the intestinal structure, function, and intestinal microbiome. Direct measurement of gastric acid secretion through endoscopy, intubation, and aspiration is uncomfortable and unacceptable to many patients. The Heidelberg pH

BOX 17.1 Common Symptoms of Low Gastric Acidity

- Bloating, belching, burning, and flatulence immediately after meals
- A sense of "fullness" after eating
- Indigestion, diarrhea, or constipation
- Multiple food allergies
- Nausea after taking supplements
- Itching around the rectum

Modified from Wright JV. *Healing with nutrition.* Emmaus, PA: Rodale Press; 1985.

BOX 17.2 Common Signs of Low Gastric Acidity

- Weak, peeling, and cracked fingernails
- Dilated capillaries in the cheeks and nose (in nonalcoholics)
- Postadolescent acne
- Iron deficiency
- Chronic intestinal parasites or abnormal flora
- Undigested food in stool
- Chronic candidal infections
- Upper digestive tract gassiness

Modified from Wright JV. *Healing with nutrition.* Emmaus, PA: Rodale Press; 1985.

capsule system offers a convenient and accurate outpatient testing system to clinicians interested in evaluating *functional* gastric acid output.

HCl, pepsin, and intrinsic factor are directly involved in digestion and contribute to the chemical changes in the intestines that assist in the absorption of many nutritional factors. For example, vitamin B_{12} absorption requires intrinsic factor, whereas zinc, calcium, and iron are less efficiently assimilated when gastric acidity is low.[9–11]

INDICATIONS

Many symptoms and signs suggest impaired acid secretory ability, and a number of specific diseases have been found to be associated with achlorhydria and hypochlorhydria (particularly gastric carcinoma and human leukocyte antigen-B_8–related autoimmune diseases). These are listed in Boxes 17.1, 17.2, and 17.3.[12–23] The presence of *Helicobacter pylori* is a major factor in both achlorhydria and hypochlorhydria.[24,25] *H. pylori* induce strong inflammatory responses and a transitory hypochlorhydria, which can progress in ~2% of patients to atrophic gastritis, dysplasia, or gastric adenocarcinoma.

Aging

Numerous studies have shown that acid secretory ability decreases with age. Low stomach acidity has been found in more than half of those older than age 60.[26,27] One study of the elderly found that their tissue

BOX 17.3 Diseases Associated With Low Gastric Acidity

1. Addison's disease
2. Asthma
3. Celiac disease
4. *Dermatitis herpetiformis*
5. Diabetes mellitus
6. Eczema
7. Gallbladder disease
8. Gastric carcinoma
9. Grave's disease
10. Chronic autoimmune disorders
11. Hepatitis
12. Human immunodeficiency virus/acquired immunodeficiency syndrome
13. Chronic hives
14. *Helicobacter pylori* infection
15. Lupus erythematosus
16. Myasthenia gravis
17. Osteoporosis
18. Pernicious anemia
19. Psoriasis
20. Rheumatoid arthritis
21. Rosacea
22. Sjögren's syndrome
23. Thyrotoxicosis
24. Hyperthyroidism and hypothyroidism
25. Vitiligo

Data from references 12 through 24.

nutrient levels could be saturated only through the use of intramuscular supplementation; oral supplementation was ineffective. The authors speculated that this was caused by atrophy of various digestive organs.[28]

CONCLUSION

The Heidelberg pH capsule system is an effective and convenient method to determine gastric acid secretory ability under conditions simulating ingestion of food. The results are extremely valuable in identifying the large number of people who have impaired secretion function. The ramifications of impaired acid secretion are widespread. This technology has become an accepted method of assessing gastric pH in several research settings.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Noeller HG. The use of a radiotransmitter capsule for the measurement of gastric pH. *German Medical Monthly*. 1961;6:3.
2. Noeller HG. Results of examinations of stomach functions with the endo-radio capsule—a new appliance for assisting stomach diagnosis. *Fortschritte der Medizin*. 1962;80:351–363.
3. Steinberg WJ, Mina FA, Pick PG. Heidelberg capsule. In vitro evaluation of a new instrument for measuring intragastric pH. *J Pharm Sci*. 1965;54:772–778.
4. Stavney LS, Hamilton T, Sircus W. Evaluation of the pH-sensitive telemetry capsule in the estimation of gastric secretory capacity. *Am J Dig Dis*. 1966;11:753–760.
5. Dabney R, Yarbrough I, McAlhany JC. Evaluation of the Heidelberg capsule method of tubeless gastric analysis. *Am J Surg*. 1969;117(2):185–192.
6. Andres MR, Bingham JR. Tubeless gastric analysis with a radio-telemetry pill. *CMA*. 1970;102:1087–1089.
7. Mojaverian P, Ferguson RK, Vlasses PH, et al. Estimation of gastric residence time of the Heidelberg capsule in humans. *Gastroenterol*. 1985;89:392–397.
8. Wright J. A proposal for standardized challenge testing of gastric acid secretory capacity using the Heidelberg capsule radiotelemetry system. *J John Bastyr Coll Nat Med*. 1979;1:3–11.
9. Brewer GJ. Effect of intragastric pH on the absorption of oral zinc acetate and zinc oxide in young healthy volunteers. *J Parent Ent Nutr*. 1995;19:393–397.
10. Mahoney AW, Hendricks DG. Role of gastric acid in the utilization of dietary calcium by the rat. *Nutr Metab. 17*74;16:375–382.
11. Jacobs A, Rhodes J. Gastric factors influencing iron absorption in anaemic patients. *Scan J Hematol*. 1967;4:105–110.
12. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med*. 2001;345:784–789.
13. Howitz J, Schwartz M. Vitiligo, achlorhydria, and pernicious anemia. *Lancet*. 1971:1331–1334.
14. Bray GW. The hypochlorhydria of asthma in childhood. *BMJ*. 1930:181–197.
15. Hosking DJ, Moody F, Stewart IM, et al. Vagal impairment of gastric secretion in diabetic autonomic neuropathy. *BMJ*. 1975:588–590.
16. Rabinowitch IM. Achlorhydria and its clinical significance in diabetes mellitus. *Am J Dig Dis*. 1949;18:322–333.
17. Carper WM, Butler TJ, Kilby JO, et al. Gallstones, gastric secretion and flatulent dyspepsia. *Lancet*. 1967:413–415.
18. Rawls WB, Ancona VC. Chronic urticaria associated with hypochlorhydria or achlorhydria. *Rev Gastroent*. 1950;18:267–271.
19. Gianella RA, Broitman SA, Zamcheck N. Influence of gastric acidity on bacterial and parasitic enteric infections. *Ann Intern Med*. 1973;78:271–276.
20. De Witte TJ, Geerdink PJ, Lamers CB. Hypochlorhydria and hypergastrinemia in rheumatoid arthritis. *Ann Rheum Dis*. 1979;38:14–17.
21. Ryle JA, Barber HW. Gastric analysis in acne rosacea. *Lancet*. 1920:1195–1196.
22. Ayres S. Gastric secretion in psoriasis, eczema and dermatitis herpetiformis. *Arch Derm*. 1929;20:854–859.
23. Dotevall G, Walan A. Gastric secretion of acid and intrinsic factor in patients with hyper and hypothyroidism. *Acta Med Scand*. 1969;186:529–533.
24. Smolka AJ, Backert S. How Helicobacter pylori infection controls gastric acid secretion. *J Gastroenterol*. 2012;47:609–618.
25. Adamu MA, Weck MN, Gao L. Incidence of chronic atrophic gastritis: systematic review and meta-analysis of follow-up studies. *Eur J Epidemiol*. 2010;25(7):439–448.
26. Rafsky HA, Weingarten M. A study of the gastric secretory response in the aged. *Gastroenterol*. 1946;8:348–352.
27. Davies D, James TG. An investigation into the gastric secretion of a hundred normal persons over the age of sixty. *Brit J Med*. 1930:1–14.
28. Baker H, Frank O, Jaslow SP. Oral versus intramuscular vitamin supplementation for hypovitaminosis in the elderly. *J Am Geriat Soc*. 1980;48:42–45.

18

Immune Function Assessment

Heather Zwickey, PhD, and *Brice Thompson, ND, MS*

OUTLINE

INTRODUCTION

Immune physiology involves complex interactions between cells and proteins to mediate an effective response to infectious disease. A healthy immune system eliminates pathogenic microbes while simultaneously creating a biodynamic relationship with normal microflora. Because the immune system must also effectively distinguish between dangerous foreign invaders and self-antigens, immune dysfunction can result in infection, autoimmunity, cancer, allergies, and asthma. Healthy immune function is affected by age, gender, sleep, nutrition, environmental toxins, and exercise. Natural therapies such as herbs, meditation, hydrotherapy, and supplements can also enhance and manipulate immune reactions.

A variety of tests can be used to evaluate the immune system. To determine what type of immune assessment to use for each clinical diagnosis, it is important to understand how underlying immune physiology contributes to a clinical picture. Clinicians can assess the qualitative or functional activity of immune cells. Qualitative data may include things like types, number, and activation states of cells. Cells of the immune system express different proteins on their surface. These proteins, called biomarkers, allow identification of different cells as well as their activation state. To assess immunodeficiency and lymphoproliferative diseases, the number of T cells and B cells must be quantified. Analysis of the functional activity of immune cells involves putting the cells into an in vitro assay system; adding antigen or mitogen; and measuring a function such as cytotoxicity, cytokine production, or antibody secretion. The levels of proteins that are produced by lymphocytes and monocytes, such as cytokines and antibodies, can be analyzed as well. Tests of genetic predisposition to disease can be evaluated with genetic tests. An overview of the assays used to measure cells is given in Table 18.1. A description of these tests is provided in Box 18.1.

ANTIBODIES

Blood

Antibody (also called immunoglobulin) is a protein made by B cells in response to an antigen. Each B cell has specificity for one antigen. This specificity is determined by gene rearrangement long before a B cell ever encounters an antigen. The antibody is found both on the surface of B cells and is secreted. B cells are primarily found in the lymph nodes, Peyer's patches of the gut, and the spleen (80%); however, antibody is readily detectable in the blood and other bodily fluids.

Antibodies look like the letter "Y" (Fig. 18.1). Each part of the antibody has a different function: the arms bind specifically to antigen, whereas the base (the Fc region) allows the antibody to bind to receptors. This base region is called the "isotype." Antibodies come in multiple isotypes based on the constant region of their heavy chain. These different isotypes have functional importance. Some isotypes are better than others at triggering the complement cascade. The Fc receptors of other isotypes allow them to activate specific cells, such as mast cells and eosinophils.

Antibodies are essential to measuring immune function because they can act as both an indicator of disease and as a tool to measure the levels of other proteins and cells. Monoclonal antibodies are a powerful tool (see Box 18.2).

Measuring serum antibody is essential in patients who have repeated or severe infections. Low antibody levels in these patients may indicate

TABLE 18.1 Overview of Assays to Measure Specific Immunity

Type of Immunity	Assays
Humoral immunity	Antibody production: ELISA, cytometric bead array
Cellular immunity	Cytokines: ELISA, ELISPOT, cytometric bead array, RIA, intracellular cytokine staining
	Cytotoxicity: 51 chromium release assay
	Proliferation: CFSE, lymphocyte proliferation assay
Genetics	SNPs genetic microarray

CFSE, Carboxyfluorescein succinimidyl ester; *ELISA,* enzyme-linked immunosorbent assay; *ELISPOT,* enzyme-linked immunosorbent spot; *RIA,* radioimmunoassay; *SNP,* single-nucleotide polymorphisms.

BOX 18.1 Testing Methods

ELISA

The enzyme-linked immunosorbent assay (ELISA) is an assay used to measure proteins of the immune system. In ELISA, an antigen is fixed to the surface of a 96-well plate, and then a specific antibody is incubated with the antigen so that it forms an immunocomplex. This antibody is linked to an enzyme that allows the antigen to be converted into a detectable signal, most often a change in color. The more antigen that is present, the darker the color.

ELISPOT

A variation of the ELISA is the enzyme-linked immunosorbent assay (ELISPOT). Peripheral blood mononuclear cells (PBMCs) are plated on a filter-bottom 96-well plate coated with anticytokine antibody. The plate is cultured for 24 to 48 hours to allow cytokine secretion and capture on the plate. Cells are washed off and detector antibody is added, followed by an enzyme substrate. Cytokine-secreting cells are identified as spots of secreted cytokine.

Cytometric Bead Array

A cytometric bead array uses multiplexed beads labeled with capture antibodies for specific analytes, such as cytokines or other serum proteins. Serum is added together with Phycoerythrin (PE)-labeled detector antibody. The antibody-antigen complex is run through the flow cytometer, and software calculates the level of each analyte based on PE fluorescence of each bead population relative to a standard curve.

Intracelluar Cytokine Staining

The intracellular cytokine-staining technique uses the flow cytometer to ensure production of cytokines in short-term stimulated whole blood or PBMCs before the cytokines are secreted from the cells. One advantage of this assay is that multiple cell-surface and intracellular markers can be analyzed in combination using multiparameter flow cytometry.

Chromium-51 Release Assay

To test the ability of CD8 T cells or natural killers (NKs) to kill, target cells are incubated with a radioactive isotope of chromium-51, which can be released when the cell dies. The CD8 T cells or NKs are then placed in serial dilution with the antigen-presenting cells. Antigen is added in the case of the CD8 T cells. When the target cells are killed by the CD8s, the amount of radioactivity or enzyme can be analyzed.

Lymphocyte Proliferation Assay

The proliferation capability of T cells can be measured by isolating PBMCs, separating the T cells, and incubating with tritiated thymidine (^{3}H). A mitogen such as lipopolysaccharide is added to the cell culture. This mitogen may induce cell proliferation. As the cells divide, they incorporate the ^{3}H into the DNA, and analysis of radioactivity can determine how many cell divisions occurred. An ELISA may also be performed to measure cytokine production by the CD4 T cell.

Carboxyfluorescein Succinimidyl Ester

Carboxyfluorescein succinimidyl ester (CFSE) can be used to measure cell division. In this assay, the parent cell is incubated with CFSE, which is incorporated into DNA during cell division. As the cell divides, each of the daughter cells contains half of the CFSE stain. Every subsequent cell division halves the amount of CFSE in the daughter cells. When these cells are run through a flow cytometer, a characteristic pattern is seen (see Fig. 18.2).

an immunodeficiency. Patients with myelomas and lymphoproliferative disorders may have high antibody levels. For example, patients with alcoholic liver disease often have a polyclonal expansion of immunoglobulin-A (IgA), whereas patients with systemic lupus erythematosus (SLE) and Sjögren's syndrome have polyclonal IgG expansion.[1]

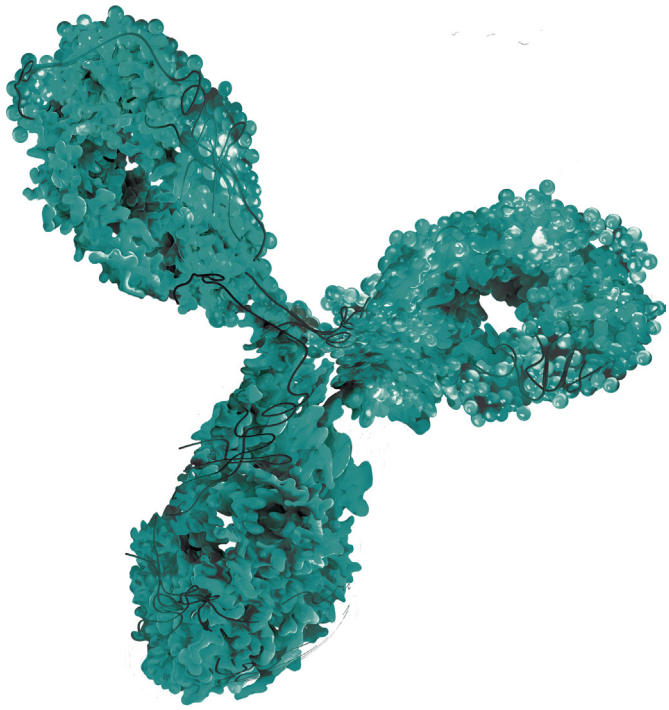


Fig. 18.1 Molecular structure of a human antibody. (mirror-images/iStock.com)

BOX 18.2 Monoclonal Antibodies

One of the primary tools used in many of the assays for immune function is the monoclonal antibody. Monoclonal antibodies are the product of a single B cell. To generate a monoclonal antibody, an antigen is injected into an animal, and the resultant B cells are isolated. These B cells are diluted, and a single B cell is fused with a nonsecreting myeloma cell line to form hybrids. These hybrids then produce the monoclonal antibodies highly specific for the injected antigen. In contrast, when an animal is injected with an antigen, polyclonal antibodies are generated. Many different B cells specific for that antigen release antibody, which is harvested and purified. This collection of antibodies is called polyclonal because, unlike monoclonal antibodies, it has many specificities to the same antigen. Polyclonal and monoclonal antibodies can be tagged with enzymes for fluorescence to detect the original protein antigens for which they are specific.[1]

Many proteins have monoclonal antibodies specific for them, including cell-surface markers, cytokines, and even other antibodies. The quality of the monoclonal antibody can determine the sensitivity and specificity of the test used.

The type of isotype made by B cells can provide clues as to the immunological status of the patient (Table 18.2).

The route and duration of exposure, type of antigen, and genetic background may all affect the isotype of antibody that is produced, as well as the subclass of antibody produced. Upon immediate exposure to a novel infectious organism, the body produces immunoglobulin-M (IgM). After the B cell makes IgM, it class switches to another isotype, as determined by a combination of activation proteins and cytokines. If the patient has been exposed to an infectious agent, the antibody usually will class switch to IgG.

Immunoglobulin-G

IgG is the major antibody found in blood, and it consists of four subclasses, originally described in the 1980s according to their abundance

TABLE 18.2 Antibody Function

Isotype	Serum Levels[a]	Subclasses	Function/Description
IgM	0.5–2.0		Secreted during initial infection; makes large antibody–antigen complexes
IgD	Trace		Function unknown
IgG			Coats microorganisms, making them more likely to be phagocytosed by macrophages, dendritic cells, and neutrophils; neutralizes toxins
	5.0–12	IgG1	Responds to protein antigens
	2.0–6.0	IgG2	Primarily responds to polysaccharide antigens; binds poorly to Fc receptors
	0.5–1.0	IgG3	Responds to protein antigens
	0.1–1.0	IgG4	Response to repeated antigenic exposure; binds poorly to Fc receptors
IgE	Trace		Binds to Fc receptors on mast cells/eosinophils and basophils to elicit allergic response; also involved in response to parasites and worms
IgA			Provides protection at mucosal surfaces
	0.5–3.0	IgA1	Monomeric form found in blood; can trigger inflammation through FcαR1
	0–0.2	IgA2	Dimeric form found in secretions like tears, saliva, and breast milk

Ig, Immunoglobulin.
[a]Serum levels presented in grams per liter.

in serum. IgG1 and IgG2 are present in much higher concentrations than IgG3 and IgG4. All subclasses of IgG are low in pediatric populations. Deficiency in some IgG subclasses results in increased susceptibility to bacterial infections.[2] IgG subtypes vary in their ability to activate complement and Fc-receptor binding. IgG3 has a strong affinity for Fc receptors and has the greatest ability to activate complement. The type of antigen can influence the subclass of antibody response. For example, IgG2 antibodies appear to influence polysaccharide antigens, whereas protein antigens and whole bacteria preferentially elicit IgG1.[3–5]

IgG4 antibodies, found at the lowest concentration in the serum, are generated to repeatedly presented antigens. IgG4-deficient individuals may be more susceptible to pyogenic infections of the respiratory tract. Antibodies against dietary antigens are frequently IgG4. In a community survey of 40 individuals, antifood antibodies to milk, egg, and fish of the IgG4 subclass were found in a significant proportion of a healthy population, indicating that IgG4 antibodies against food antigens cannot serve as markers of atopic disease. Because IgG4 cannot activate complement, it has been hypothesized that it may serve for protective clearance mechanisms and may be a desirable response to dietary antigens.[6] IgG1 and IgG2 have also been found for dietary antigens, but the immunological outcome of these subclasses may be food intolerance that is not caused by immunoglobulin-E (IgE)-mediated hypersensitivity.[7] A larger discussion of the immune response to food can be found in Chapter 14.

Antibodies of each of the IgG subclasses can be detected by a variety of techniques, including radioactive iodine labeling; antigen-coated red cells; immunofluorescence with antigen-coated Sepharose; radioimmunoassay (RIA); and, most commonly, enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (Fig. 18.2).[8]

Immunoglobulin-A

IgA can also be made to infectious agents and occurs in two forms, IgA1 and IgA2. IgA in serum predominantly exists in the monomeric form, IgA1. The ratio of IgA1:IgA2 in serum is about 9:1. IgA found in secretions, termed secretory IgA, occurs as dimers. The ratio of IgA1:IgA2 in secretions varies, but it is approximately 6:4 in saliva.[9] Because IgA is found at mucosal surfaces and lines the mucosal surface of the gut, it is not uncommon to find IgA antibody specific for food antigens. Because IgA is at high levels in secretions, it can be found in saliva in addition to serum, and salivary IgA tests are readily available.[10,11]

Immunoglobulin-E

An immune response to parasites, specifically worms, triggers an IgE response.[12] IgE elicits an immune response by binding to Fc receptors on mast cells, eosinophils, and basophils, causing degranulation and cytokine release. In atopic individuals, IgE is also made to allergens. IgE is at low levels in the blood. Measurement of total serum IgE is useful in patients in whom parasitical infection is suspected, but it is not valuable for measuring allergies. Thus the most common method for allergy testing is the skin-prick test.[13] In this test, a small amount of the suspected allergen is placed on the skin. Then the skin is pricked so that the allergen goes under the skin's surface. If the patient is allergic to the allergen, swelling and redness will appear within 15 to 20 minutes.

Total Antibodies

Measuring total antibody in blood involves protein electrophoresis or ELISA and is valuable. However, quantifying the total amount of antibody specific for an antigen is even more desirable. Antibody titers for common antigens, such as those found in vaccines, can be ordered. Each laboratory has its own reference levels for titers because the sensitivity of the test is related to the specificity and affinity of the reagents used.

Autoantibodies

Specific antibodies, rather than total levels, are also measured for circulating autoantibodies. These antibodies can be detected with immunofluorescence, RIA, and ELISA.[14] Indirect immunofluorescence is a method often used to investigate autoantibody presence, specifically Antinuclear antibody (ANA). This method involves growing liver cells in a petri dish, exposing the cells to patient serum, then washing any excess antibody away. If the serum contains ANA, these antibodies will stay attached to the nuclear region of the liver cells. The plate is then incubated with an antibody that has a fluorescent tag and is specific to human IgG. When examined under a microscope, a nuclear staining pattern can be observed if the patient has ANA (Fig. 18.3). If the patient does not have ANA, a more diffuse staining pattern occurs. This method is the least sensitive of all the antibody tests, yet it is the gold standard in diagnosing several autoimmune conditions, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).[15] Another method of immunofluorescence antibody visualization uses the patient's tissue as opposed to human cells in a petri dish. This method takes patient tissue that is frozen, and sections are cut on a

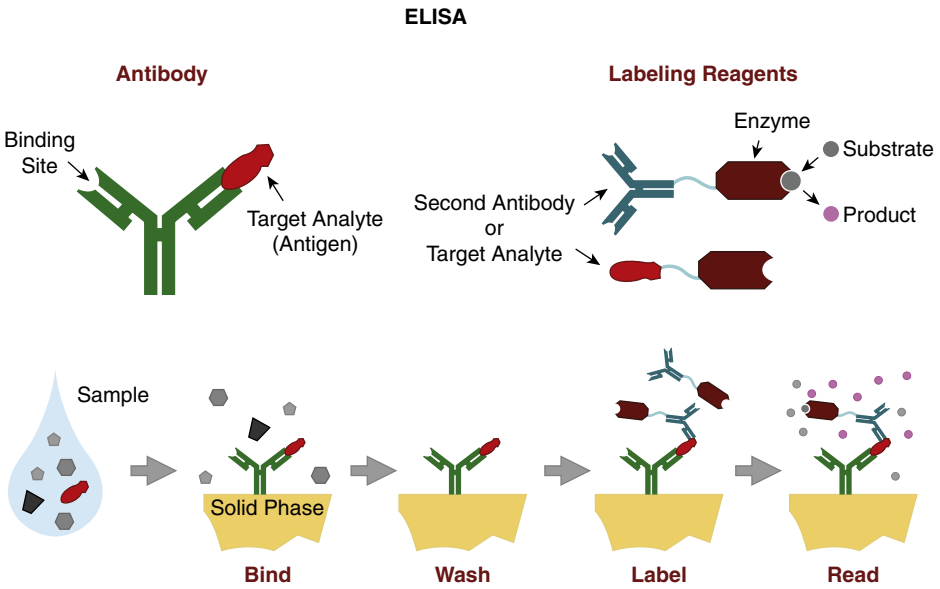


Fig. 18.2 Enzyme-linked immunosorbent assay (ELISA). A capture antibody specific for an antigen in a sample is attached to the bottom of a plate. The sample (blood, saliva, etc.) is added, and excess sample is washed from the plate. A secondary antibody also specific for the antigen is added to the plate. This secondary antibody is labeled with an enzyme. When the substrate for the enzyme is added to the plate, it elicits a color change. The intensity of the color, as measured by spectrophotometry, indicates the amount of antigen that is present.

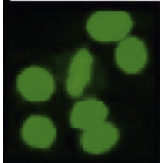
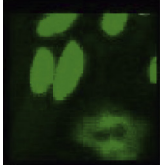
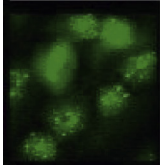
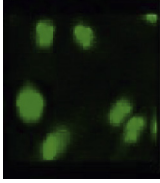
Pattern	Associated Antigens	Clinical Associations
	Double stranded DNA, histones, DNA/histone complexes, others	SLE, drug-induced lupus, and other conditions
	"ENA antigens": Sm, RNP, SS-A/Ro, SS-B/La Nuclear matrix antigens: (hnRNP), others	Autoimmune rheumatic diseases: SLE, MCTD, Sjögren's syndrome, and other conditions
	Centromere proteins A, B, C located at the kinetochore plates	Scleroderma (CREST syndrome) primary biliary cirrhosis
	Fibrillarin, RNA polymerase, NOR-90, Scl-70, Pm/Scl, ribosomal antigens, others	Autoimmune rheumatic diseases: scleroderma, myositis, SLE, and others

Fig. 18.3 Antinuclear antibody (ANA) immunostaining pattern and clinical associations.

cryostat. This tissue is incubated with patient serum, such that autoantibodies in the serum can bind. A fluorescently tagged secondary antibody specific for human immunoglobulin is then used to detect the autoantibodies. Immunofluorescence examination of biopsy specimens of damaged or normal tissue may reveal deposits of immunoglobulins caused by antibodies reacting with an organ or tissue-specific antigens. This approach is especially important in the diagnosis of antiglomerular basement antibody disease and bullous skin disorders but may show false positives when used for SLE. Current recommendations do not support testing for autoantibodies due to their low specificity, particularly ANA testing, unless clinical suspicion for immune involvement is suspected[16] (see Table 18.3 for a listing of common autoantibodies).

TABLE 18.3 Common Autoantibodies

Autoantibody	Clinical Relevance
Antinuclear antibody	Systemic rheumatic diseases
Smooth-muscle antibody	Nonspecific liver damage; chronic hepatitis
Antimitochondrial antibody	Primary biliary cirrhosis
Endomysial antibody	Celiac disease; dermatitis; psoriasis
Antineutrophil cytoplasmic antibody	Vasculitis
Gastric parietal cell antibody	Pernicious anemia
Adrenal antibody	Idiopathic Addison's disease
Pancreatic islet cell antibody	Insulin-dependent diabetes mellitus
Skin antibodies	Pemphigus vulgaris; bullous pemphigoid
Antiacetylcholine receptor	Myasthenia gravis
Antimyelin basic protein	Multiple sclerosis
Double-stranded DNA autoantibody	Systemic lupus erythematosus
Thyroid stimulating hormone antibody	Graves' disease

RIA is a far more sensitive test used for measuring antibodies that are in low concentrations. In RIA, an antibody specific for human antibody is tagged with a radioactive isotope. Because radioactivity is more sensitive than fluorescence, when this antibody is incubated with an autoantibody, the contact detects even low levels of autoantibody.

Organism-Specific Antibodies

Antibody testing can also be used when determining a diagnosis of conditions like infectious mononucleosis due to Epstein–Barr virus (EBV). EBV does not always induce mononucleosis. In fact, only 30% to 40% of those who are infected with EBV will develop mononucleosis.[17] With clinical evidence, serological testing can be performed to confirm the diagnosis. Typically, a monospot is performed. A monospot is a form of heterophilic testing, which means that serum is tested against phylogenetically unrelated species' red blood cells (RBCs), typically horse, ox, or goat. The test is considered positive if the RBCs stick together (agglutination). The antibodies produced early in the infectious process of EBV will cross-react and bind to proteins on the surface of the nonhuman RBCs. If the monospot is positive, a diagnosis can be made. If it is negative, it does not rule out mononucleosis, and further testing can be done at this time.

Testing for IgM and IgG antibodies against the EBV capsid antigen is a more specific test for mononucleosis. The presence of IgM indicates an acute infection. IgG antibodies against EBV nuclear antigen appear 6 to 12 weeks after infection and persist for life. Their presence early in the course of illness effectively rules out EBV infection as a diagnosis. In contrast, IgG antibodies against "EBV early antigen" can signify a recent infection. Early antigen antibodies can be further broken down to anti-D and anti-R antibodies. Anti-D antibodies are consistent with early infection and disappear after resolution.[18]

Measuring antibody titers is a way of assessing vaccination status in individuals who are unsure of their vaccination history or to confirm vaccine protection. Some people are "nonresponders" to vaccines, particularly the hepatitis B vaccine. These individuals do not generate a robust response to a vaccine. Their antibody titers may be below 10 mIU/mL, whereas >10 mIU/mL signifies that an individual is immune. Nonresponders may require further follow-up to determine the etiological mechanism as to why they are nonresponsive to the vaccine.[19] Additionally, vaccine titer testing may be used in patients who wish to demonstrate that they have active immunity to an infectious agent and do not desire to have additional vaccinations. In cases of pregnancy, vaccine titers may be measured on parents and siblings of the newborn to ensure they are immune to the most common childhood diseases. This strategy capitalizes on the concept of "herd immunity" to ensure newborns' protection against preventable diseases.

Urine

When an antibody is made, the antibody light chains, κ and λ, are made in excess. These are present as free forms in serum and urine. Although small amounts of light chains are found in everyone, people with renal damage excrete higher levels of light chains in their urine. Free light chains are associated with malignant plasma dyscrasia and other lymphocyte-related immunoproliferative disorders. Intact immunoglobulin can also be found in urine.[20]

Cerebrospinal Fluid

IgG can be measured in the cerebrospinal fluid (CSF), which is important in the diagnosis of several CNS disease processes, such as multiple sclerosis, neurosyphilis, and subacute sclerosing panencephalitis.[21] A sample of CSF and serum is obtained, and the amount of IgG and albumin is recorded in a ratio of IgG:albumin for both samples. Albumin is not synthesized in the brain, so comparing this IgG:albumin ratio of the CSF to the serum sample provides an indirect indication of how much IgG has been synthesized within the brain.[1]

MEASUREMENT OF COMPLEMENT AND HIGH-SENSITIVITY C-REACTIVE PROTEIN

Complement

Complement is a series of proteins that result in the destruction of microbes. Assays for complement can either be those that recognize individual complement components (e.g., ELISA for C3 or C4) or functional activity of complement, such as lysis. Measurement of C3 and C4 levels is sufficient, except for rare patients with genetic deficiencies, such as hereditary angioedema. Low levels of complement are more significant than high levels. For example, complement levels may be low in patients with glomerulonephritis or SLE.[22,23] However, the complement levels return to normal when patients are in remission.

High-Sensitivity C-Reactive Protein

C-reactive protein (CRP) is involved in the complement pathway (Fig. 18.4). CRP functions by opsonizing bacteria, marking them for destruction by the complement cascade. Because it is readily detectable in the blood, CRP has become an important marker of inflammation. CRP is released early in infection (the first 4–24 hours) during the same window that many other components of inflammation are being activated, such as inflammatory cytokines. CRP levels are an indirect measure of the cytokine interleukin (IL)-6. IL-6 induces production of CRP as part of the initial immune response against an invading pathogen that triggers the rest of the immune response.[24] However, because it is far easier to measure than cytokines or other proteins, high levels of CRP are a more consistent marker of a recent infection or heavy immunological involvement, as is the case in several autoimmune diseases.[25] Laboratories consistently offer a high-sensitivity test for CRP, called hs-CRP. This test is more sensitive than the original test for CRP, allowing the detection of lower levels of CRP. Consistently high levels of hs-CRP may be diagnostic of chronic inflammation and are values commonly used to determine immune involvement or even cardiovascular disease risk.[26]

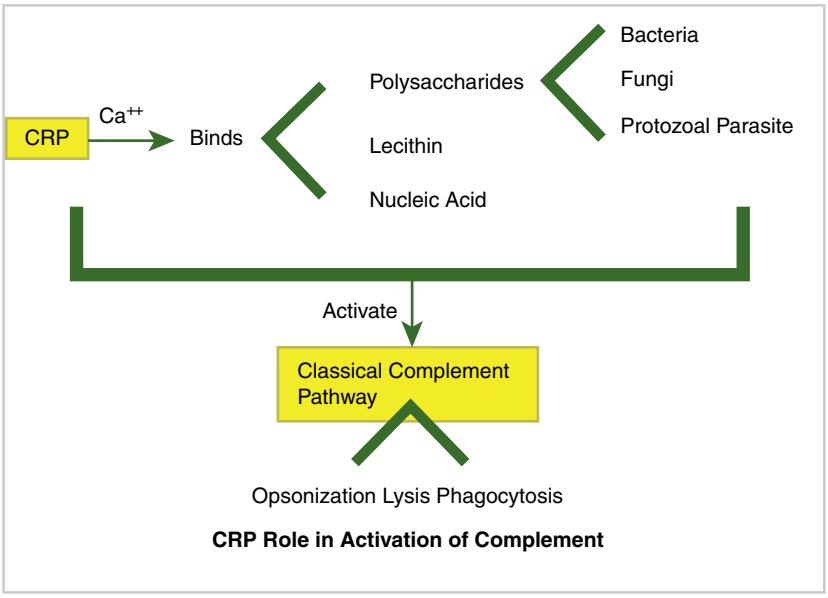


Fig. 18.4 C-reactive protein (CRP) activates the complement pathway.

TABLE 18.4 Regulatory T Cells

Type of Regulatory Cell	Cytokines Made	Action	Development of
Treg cells (CD4+ CD25+ CD152+)[a]	TGF-β, IL-35	Control overall autoimmune state?	Thymus
Type I NKT (cells)	Immunoregulatory		Thymus
(NK cells that express a semi-invariant TCR)	Proinflammatory		
Tr1 cells (CD4+ CD152+)	High IL-10 Moderate TGF-β and IFN-γ No IL-2 or IL-4	Suppress MS; suppress intestinal inflammation; cytolysis	Periphery systemic differentiate from Th0
Th3 cells (CD4+)	High TGF-β Low IL-4 and IL-10 No IFN-γ and IL-2	Oral tolerance	Periphery differentiate in intestinal mucosa
T suppressor cells (CD8+ CD28–)	High IL-10	Suppress DC maturation and function	Periphery

Adapted from Venken et al. 2010[29] and Vignali et al. 2008.[30]
DC, Dendritic cell; *IFN,* interferon; *IL,* interleukin; *MS,* multiple sclerosis; *NK,* natural killer; *NKT,* natural killer T cells; *TCR,* T-cell receptor; *TGF,* tumor growth factor; *Treg,* regulatory T cells.
[a]nTreg (natural Treg) and iTreg (inducible Treg).

MEASUREMENT OF CELLS

Lymphocyte Subpopulations

T Cells

CD4 T cells make cytokines that direct various arms of the immune system. CD8 T cells kill infected cells. The proteins on the surface of T cells that allow them to be identified are listed in Table 18.4. To qualitatively identify T cells and separate them from other cell types, two proteins are used: CD3 and CD4 or CD8. CD3 is a signaling molecule associated with the T cell receptor and is on all T cells. CD4 and CD8 function to increase the interaction between the T cell and the antigen presenting cell. A sample of how T cells appear on a flow cytometry dot plot is in Box 18.3. In healthy individuals, the CD4/CD8 ratio should be approximately 2:1. This ratio may differ in some ethnic groups.[27] CD4/CD8 ratios can be used to evaluate disease progression in patients with human immunodeficiency virus infection.

When a clinician approaches a patient with suspected immunodeficiency, he or she may decide to recommend a functional test. Immunodeficiency could be caused by a cell's inability to proliferate. In this case a lymphocyte proliferation assay or a carboxyfluorescein succinimidyl ester (CFSE) assay would be completed.

BOX 18.3 Sample Flow Cytometry Dot Plot

Flow Cytometry

Flow cytometry is a technique for counting cells and other microscopical particles such as cytokines and proteins. Similar to microscopy, flow cytometry allows the identification of cells based on their morphology and cell-surface proteins; however, flow cytometry evaluates cell populations rather than individual cells. Cells are incubated with monoclonal antibodies specific for certain proteins on their surface. Each antibody is tagged with a different color fluorochrome. The cells are then suspended in fluid and passed through the flow cytometer. Light from lasers in the flow cytometer passes through the cells, allowing detectors on the other side to read the light scatter from the cells based on their granularity and size. The detectors also evaluate the colors of the fluorescence associated with each individual cell. The resulting data are plotted using specialized software.

CD4 T Helper Subsets

CD4 T cells secrete discrete patterns of cytokines. Each pattern is beneficial for different types of immune responses. A single CD4 T cell specific for a single antigen can secrete any pattern of cytokines but will be skewed toward secreting one pattern or another depending on

the initial trigger. CD4 T helper subsets cannot be identified by surface markers. However, because CD4 T helper subsets secrete different patterns of cytokines, intracellular cytokine staining can discriminate between subsets.

The Th0 pattern is secreted by a naïve T cell, that is, one that has not yet seen antigen. It is the cytokine pattern that is designed to simply keep T cells alive. Th0 cells secrete IL-2.

The Th1 pattern is secreted by T cells exposed to bacterial or viral antigens. Overactivity of Th1 T cells has been linked to many autoimmune diseases. Th1 T cells secrete interferon-γ, as well as some other cytokines.

The Th2 pattern is secreted by T cells exposed to worms and parasites but is also involved in allergy and asthma. Th2 T cells secrete IL-4, IL-5, and IL-13.

The Th3 pattern is secreted in response to food. Because the immune response to food should be relatively inert, Th3 cells are considered a type of regulatory T cell (Treg) and are involved in immunological tolerance. Th3 T cells secrete tumor growth factor-β.

The Th9 pattern in T cells is closely linked to Th2, and until recently, they were considered to be the same. Th9 T cells produce higher quantities of IL-9 and do not produce IL-4, in direct contradiction to the cytokines produced by Th2 T cells. Th9 T cells have been implicated in the pathophysiology of asthma and other pulmonic conditions. On the other hand, they may play an important role in cancer surveillance and anticancer activities.[28]

The Th17 pattern is secreted in response to molds and extracellular bacteria. This population of T cells has recently been linked to many autoimmune diseases. Th17 T cells secrete IL-17.

Regulatory T Cells

Tregs play a key role in maintaining tolerance. The loss of Tregs is associated with disease, especially autoimmune disease. Studies of Tregs have been limited largely because of their considerable diversity and lack of unique surface markers; the current state of the science is given in Table 18.4.[29,30] Much of the knowledge about Tregs is from mouse models. Human Tregs have been phenotypically identified as CD4+ CD25+, CD4+ $CD25^{high}$, or CD4+ CD25+ FoxP3+ cells, based on how they stain and present in flow cytometry. CD39 is another protein on the surface of Tregs that may allow reliable identification.[31] Although most CD4 T cells express CD127, Tregs display lower surface expression of this marker.[32] Tregs behave similarly to other regulatory cells, such as Th3 cells found in the gut and Tr1 cells found in the periphery, but Tregs originate in the thymus and produce different cytokines upon stimulation than these other types of regulatory cells. Activation of Th1, Th2, and Tregs can be measured with the expression of CD69.[33]

B Cells

B cells are identified by the surface markers CD19 and CD20 (Table 18.5). In addition to being markers for B cells, CD20 is a marker of non-Hodgkin lymphoma and is a current drug target.[34] Monoclonal antibodies directed at CD19 are therapeutic targets in rheumatoid arthritis.[35] The products of B cells are antibodies and can be measured as described previously.

Monocytes and Neutrophils

Neutrophils and monocytes act early in disease. Tethered to the blood vessel walls, neutrophils are some of the first cells to reach infectious agents. Monocytes can quickly extravasate into tissues, differentiating into macrophages and joining tissue macrophages already present at the site of infection. If monocytes and neutrophils are not functioning properly, severe infections can result. It is rare to find patients with low-functioning monocytes because the consequences can be extreme.

TABLE 18.5 Cell Markers

Antigen	Identity/Function
CD3	Present on all T cells
CD4	Present on T helper cells and some dendritic cells
CD8	Present on cytotoxic T lymphocytes
CD16/CD56	Combination identifies NK cells
CD19/CD20	B-cell markers
Markers of T cell activation	
CD25	IL-2 receptor; serves as a marker for Treg cells
CD28	Activation marker on CD4 and CD8 T cells
CD38	Expressed on T cells after activation; function unknown
CD39	Acts on the nucleotide base responsible for enzymatic cleavage of ATP to AMP; marker for Tregs
CD45RA	Marker of a naïve/resting T cell
CD45RO	Marker of a recently activated T cell
CD69	Early activation marker for T cells, as well as B, NK, monocytes, and neutrophils[24]
CD71	Early activation marker for T cells
CD127	α-Chain of the IL-7 receptor; absence identifies Tregs

AMP, Adenosine monophosphate; *ATP,* adenosine triphosphate; *IL,* interleukin; *NK,* natural killer; *Treg,* regulatory T cells.

When patients have severe staphylococcal or fungal infections, neutrophil numbers should be evaluated, and function should be tested. Neutropenia is more common in neutrophil dysfunction. CD18 is a consistent and reliable marker for neutrophils and can be used as a marker for flow cytometry.[36] In healthy individuals, the neutrophil count normally exceeds 1.5×10^9/L. Mild neutropenia is usually asymptomatic, whereas moderate to severe reductions in numbers are associated with a progressive increase in the risk and severity of infections. Episodes of infection are likely to be life threatening when the neutrophil count falls below 0.5×10^9/L. Neutropenia is a frequent side effect of chemotherapy.[37]

Neutrophil function consists of adhesion, migration, chemotaxis, phagocytosis, and a respiratory burst. Defects in the respiratory burst can cause chronic granulomatosis disease. One simple method to evaluate neutrophil function involves a nitro blue tetrazolium test. In this test, neutrophils ingest and reduce a soluble yellow dye to an intracellular blue crystal. Neutrophils are separated and then stimulated with endotoxin (lipopolysaccharide), and the cells are viewed microscopically.

Neutrophils and monocytes can kill microbes with a respiratory burst. Another test of neutrophil function takes advantage of this activity. Neutrophils or monocytes are incubated with live microbes, such as *Staphylococcus aureus.* After the cells have phagocytosed the organisms, they are washed to remove extracellular organisms. The cells are then lysed, and the lysate is cultured on nutrient agar. Live bacteria will grow on the agar. The number of viable organisms inversely reflects the degree of intracellular killing.

Natural Killer Cells

When cells are infected with a virus or become cancerous, they often express less major histocompatibility complex on their surface. This makes them a target for natural killer (NK) cells. NKs express surface receptors that allow them to recognize the absence of major histocompatibility complex on a target cell and kill it. In addition to this mechanism, NK cells can be identified with CD56.[38] Like CD8 activity, NK function can be measured with a 51-chromium release assay (Box 18.3).

TABLE 18.6 Cytokine Patterns

Immunological Event	Name of Pattern	Cytokines
Inflammation	Proinflammatory	IL-1
		IL-6
		IL-8[a]
		TNF-α
T activation	Th1	IFN-γ
		IL-2
		TNF-α
		GM-CSF
	Th2	IL-4
		IL-5
		IL-10
		IL-13
	Th17	IL-17
T regulation	Th3	TGF-β
		IL-4
		IL-10
	Treg	TGF-β
	Tr1	IL-10
		TGF-β
		IFN-γ

GM-CSF, Granulocyte-macrophage colony-stimulating factor; *IFN,* interferon; *IL,* interleukin; *TGF,* tumor growth factor; *TNF,* tumor necrosis factor; *Treg,* regulatory T cell.
[a]IL-8 is a chemokine.

CYTOKINES

Cytokines are immunomodulatory molecules that play an essential role in cell–cell communication and immune reactions. They are produced by most immune cell types, and many physical symptoms of immune dysfunction are triggered by cytokines. Cytokine expression has been studied extensively in autoimmune disorders, chronic inflammation, and other diseases. Cytokine expression is most accurate when measured locally at the tissue site; however, systemic cytokine levels can be detected in serum and saliva. Cytokines may contribute to disease progression and are thus potential targets for therapeutic intervention.[39,40]

Cytokine levels can be tested with ELISA or cytometric bead array. Because clinical testing for cytokines is relatively new, clinical cytokine testing is not as validated as other forms of testing. Furthermore, cytokine levels are affected by exercise, diet, sleep, age, and gender. Cytokines tend to be secreted in patterns (Table 18.6). Because many natural therapies can modify cytokine expression, skilled clinicians can switch people from one pattern of cytokines to another.

GENETICS

The genetic control of immunity has long been recognized. Many autoimmune diseases map to specific genetic loci. Polymorphisms in the human leukocyte antigen have been linked to insulin-dependent diabetes mellitus, multiple sclerosis, and rheumatoid arthritis. Single-nucleotide polymorphisms (SNPs) in cytokine and cytokine receptor genes are associated with the variability of immune response between individuals to infectious disease.[41] Some clinical laboratories now offer testing for SNPs. SNPs are detected using polymerase chain reaction (PCR).[42] Gene primer pairs for the candidate gene are used to amplify the DNA sequence of the patient's gene. The patient's gene is then sequenced and compared with a standard sequence.

Genetic microarray provides a second test for genetic variability and may allow clinicians to pinpoint genes that are key players in different diseases (Fig. 18.5). In addition to determining patterns of gene expression that contribute to complex disorders, microarray could elucidate gene interactions that lead to clinical symptoms or tell a physician whether a patient is responding to therapy. Microarray involves isolating RNA from tissue, labeling it with fluorescent probe, and then comparing it with a standardized or control tissue by combining the RNA samples and hybridizing them to a chip. The color patterns are read by a computer. The expression of some genes (housekeeping genes) tends to be more stable and can act as a control. There are still several obstacles to overcome with genetic microarray technology, but clinical utility is likely.[43]

CLINICAL SYMPTOMS

It would be an oversight to exclude clinical symptoms as one of the primary ways that the immune system is evaluated. Clinical symptoms usually occur after an immune response is under way, but they are often the first sign to the patient and physician that something may be amiss. Physicians recognized an immune response long before the first immune cells and proteins were discovered. The classic symptoms of inflammation—calor (heat), dolor (pain), rubor (redness), and tumor (swelling)—were originally described and recorded by Aulus (Aurelius) Cornelius, a Roman physician and medical writer who lived from about 30 BC to 45 AD. These hallmarks of inflammation are caused by many complex interactions between cells and cytokines of the immune and nervous system and allow a clinician to make some assumption about immune processes without even drawing blood. For example, calor (heat) refers to fever. If a patient has a fever, a physician may assume that IL-1β is elevated. Table 18.7 illustrates which immune processes underlie the hallmarks of inflammation.

Sickness Behavior

Proinflammatory cytokines act in the brain to induce nonspecific symptoms of infection. In addition to triggering fever, cytokines elicit profound psychological and behavioral changes. Sick individuals experience several symptoms, including malaise, fatigue, weakness, an inability to concentrate, and listlessness. They become sleepy (hypersomnia) and experience depressed activity and a desire to be isolated. These infection-induced changes are referred to as "sickness behavior."[44] Sickness behaviors have a variety of advantages to both the sick individual and the population at large. Malaise, fatigue, and listlessness cause a person to rest and thus conserve energy. Rest further aids the body by decreasing heat loss, whereas shivering increases heat production. Social isolation may help prevent the spread of the infection to others.[45]

Most sickness behaviors are caused by cytokines, and they may help an observant clinician make a diagnosis. For example, fatigue and malaise are most commonly triggered by IL-1β. Interestingly, in some people, tumor necrosis factor-α and IL-6 may cause anger or hostility, and this emotional response leads to the social isolation.[46]

SUMMARY

Choosing appropriate immunological tests requires physiological, economic, and ethical considerations. To choose the right test for the patient, the reliability of the tests, as well as their specificity and sensitivity, must be considered. Assessing the immune system is further complicated by its sensitivity to the environment. Thus running tests in duplicate or triplicate may be necessary to validate results.

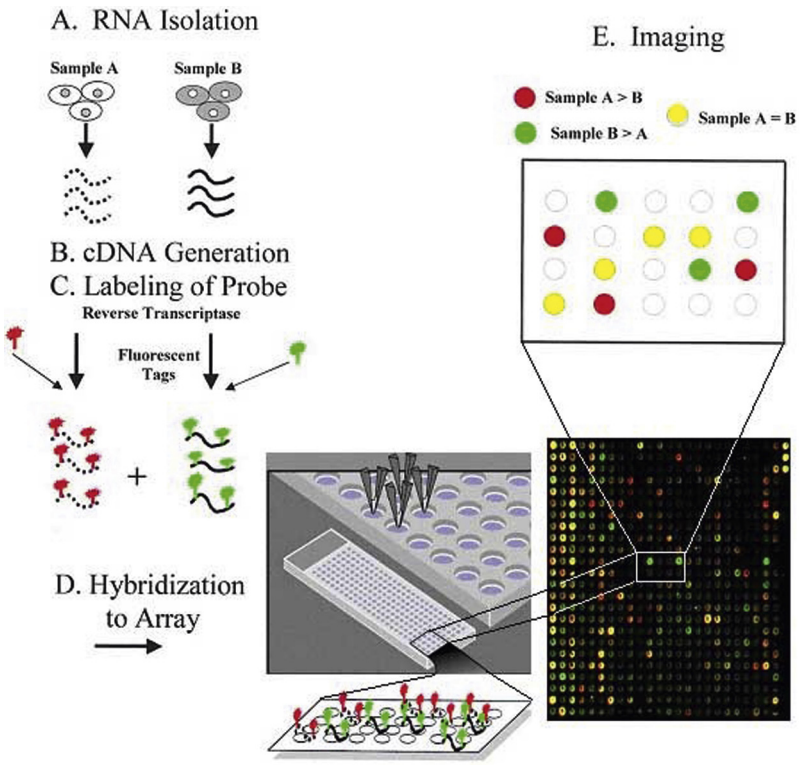


Fig. 18.5 DNA microarray. RNA is prepared from a collection of cells, normal and/or diseased. The RNA is translated into cDNA in vitro. The cDNA from each cell type is labeled with a different color of fluorescent dye. These cDNAs are then added to a chip or bead that has been previously hybridized with DNA probes. A computer analyzes the color of the DNA spots on the chip or bead. Relative difference in color indicates difference in gene expression between the two cell types.

TABLE 18.7 Hallmarks of Inflammation

Latin	English	Cytokines and Cells Involved
Calor	Heat	IL-1β, IL-6 act at the hippocampus, causing the body to increase blood flow, resulting in heat.
Dolor	Pain	TNF-α and IL-1β lead to localized edema that nerve endings sense as pain.
Rubor	Redness	TNF-α and IL-1β cause vasodilation and increased blood flow.
Tumor	Swelling	TNF-α and IL-1β cause increased permeability of the capillaries.

IL, Interleukin; *TNF,* tumor necrosis factor.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Chapel H, Haeney M, Misbah S, et al. *Techniques in Clinical Immunology. Essentials of Clinical Immunology*. Hoboken, NJ: Blackwell Publishing; 2006:306–625.
2. Ferrante A, Rowan-Kelly B, Beard LJ, Maxwell GM. An enzyme-linked immunosorbent assay for the quantitation of human IgG subclasses using monoclonal antibodies. *J Immunol Methods*. 1986;93:207–212.
3. Bird P, et al. ELISA measurement of IgG subclass production in culture supernatants using monoclonal antibodies. *J Immunol Methods*. 1987;104:149–158.
4. Ruths S, Driedijk PC, Weening RS, Out TA. ELISA procedures for the measurement of IgG subclass antibodies to bacterial antigens. *J Immunol Methods*. 1991;140:67–78.
5. Persson MA, Hammarström L, Smith CI. Enzyme-linked immunosorbent assay for subclass distribution of human IgG and IgA antigen-specific antibodies. *J Immunol Methods*. 1985;78:109–121.
6. Husby S, Jensenius JC, Svehag SE. ELISA quantitation of IgG subclass antibodies to dietary antigens. *J Immunol Methods*. 1985;82:321–331.
7. Gocki J, Bartuzi Z. Role of immunoglobulin G antibodies in diagnosis of food allergy. *Adv Dermatol Allergol Dermatol Alergol*. 2016;33:253–256.
8. Kemeny DM, Urbanek R, Richards D, Greenall C. Development of a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for detection of human IgG subclass antibodies. *J Immunol Methods*. 1987;96: 47–56.
9. de Fijter JW, van den Wall Bake AW, Braam CA, van Es LA, Daha MR. Immunoglobulin A subclass measurement in serum and saliva: sensitivity of detection of dimeric IgA2 in ELISA depends on the antibody used. *J Immunol Methods*. 1995;187:221–232.
10. Landry RG. Specific serum IgA titres as realistic indicators of treatment prognoses. *Int Dent J*. 1995;45:267–274.
11. Friedman MG. Radioimmunoassay for the detection of virus-specific IgA antibodies in saliva. *J Immunol Methods*. 1982;54:203–211.
12. Feary J, Britton J, Leonardi-Bee J. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. *Allergy*. 2011;66:569–578.
13. Lieberman JA, Sicherer SH. Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge. *Curr Allergy Asthma Rep*. 2011;11:58–64.
14. Safi MAA. An overview of various labeled assays used in medical laboratory diagnosis. Immune and non-immune assays. *Saudi Med J*. 2010;31:359–368.
15. Lam N-CV, Ghetu MV, Bieniek ML. Systemic lupus erythematosus: primary care approach to diagnosis and management. *Am Fam Physician*. 2016;94:284–294.
16. Yazdany J, et al. Choosing wisely: the American College of Rheumatology's top 5 list of things physicians and patients should question. *Arthritis Care Res*. 2013;65:329–339.
17. Womack J, Jimenez M. Common questions about infectious mononucleosis. *Am Fam Physician*. 2015;91:372–376.
18. Marshall-Andon T, Heinz P. How to use … the Monospot and other heterophile antibody tests. *Arch Dis Child—Educ Pract*. 2017;102:188–193.
19. Zaffina S, et al. Repeated vaccinations do not improve specific immune defenses against Hepatitis B in non-responder health care workers. *Vaccine*. 2014;32:6902–6910.
20. Nakano T, Nagata A. ELISAs for free human immunoglobulin light chains in serum: improvement of assay specificity by using two specific antibodies in a sandwich detection method. *J Immunol Methods*. 2004;293: 183–189.
21. Fredrikson S. Clinical usefulness of cerebrospinal fluid evaluation. *Int MS J*. 2010;17:24–27.
22. Kao AH, et al. Erythrocyte C3d and C4d for monitoring disease activity in systemic lupus erythematosus. *Arthritis Rheum*. 2010;62:837–844.
23. Ponticelli C, Coppo R, Salvadori M. Glomerular diseases and transplantation: similarities in pathogenetic mechanisms and treatment options. *Nephrol Dial Transplant.*. 2011;26:35–41.
24. Nakken B, et al. Biomarkers for rheumatoid arthritis: from molecular processes to diagnostic applications-current concepts and future perspectives. *Immunol Lett*. 2017;189:13–18.
25. Genest J. C-reactive protein: risk factor, biomarker and/or therapeutic target? *Can J Cardiol*. 2010;26(suppl A):41A–44A.
26. Plazak W, et al. Influence of chronic inflammation and autoimmunity on coronary calcifications and myocardial perfusion defects in systemic lupus erythematosus patients. *Inflamm Res*. 2011;60:973.
27. Jiang W, et al. Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. *Clin Diagn Lab Immunol*. 2004;11:811–813.
28. Zhao P, Xiao X, Ghobrial RM, Li XC. IL-9 and Th9 cells: progress and challenges. *Int Immunol*. 2013;25:547–551.
29. Venken K, Hellings N, Liblau R, Stinissen P. Disturbed regulatory T cell homeostasis in multiple sclerosis. *Trends Mol Med*. 2010;16:58–68.
30. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol*. 2008;8:523.
31. Mandapathil M, Lang S, Gorelik E, Whiteside TL. Isolation of functional human regulatory T cells (Treg) from the peripheral blood based on the CD39 expression. *J Immunol Methods*. 2009;346:55–63.
32. Hartigan-O'Connor DJ, Poon C, Sinclair E, McCune JM. Human CD4+ regulatory T cells express lower levels of the IL-7 receptor alpha chain (CD127), allowing consistent identification and sorting of live cells. *J Immunol Methods*. 2007;319:41–52.
33. Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. *Stem Cells Dayt Ohio*. 1994;12:456–465.
34. van Meerten T, Hagenbeek A. CD20-targeted therapy: the next generation of antibodies. *Semin Hematol*. 2010;47:199–210.
35. Tedder TF. CD19: a promising B cell target for rheumatoid arthritis. *Nat Rev Rheumatol*. 2009;5:572–577.
36. Stroncek D. Neutrophil alloantigens. *Transfus Med Rev*. 2002;16:67–75.
37. Lyman GH. A comparison of international guidelines for the prevention of chemotherapy-induced neutropenia. *Curr Opin Hematol*. 2011;18:1–10.
38. Timmons BW, Cieslak T. Human natural killer cell subsets and acute exercise: a brief review. *Exerc Immunol Rev*. 2008;14:8–23.
39. Fox PC, Brennan M, Di Sun P. Cytokine expression in human labial minor salivary gland epithelial cells in health and disease. *Arch Oral Biol*. 1999;44(suppl 1):S49–S52.
40. Suh KI, Kim YK, Kho HS. Salivary levels of IL-1beta, IL-6, IL-8, and TNF-alpha in patients with burning mouth syndrome. *Arch Oral Biol*. 2009;54:797–802.
41. Pankratz VS, Vierkant RA, O'Byrne MM, Ovsyannikova IG, Poland GA. Associations between SNPs in candidate immune-relevant genes and rubella antibody levels: a multigenic assessment. *BMC Immunol*. 2010;11:48.
42. Germer S, Holland MJ, Higuchi R. High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. *Genome Res*. 2000;10:258–266.
43. Kim K, Zakharkin SO, Allison DB. Expectations, validity, and reality in gene expression profiling. *J Clin Epidemiol*. 2010;63:950–959.
44. Kent S, Bluthé RM, Kelley KW, Dantzer R. Sickness behavior as a new target for drug development. *Trends Pharmacol Sci*. 1992;13:24–28.
45. Kelley KW, et al. Cytokine-induced sickness behavior. *Brain Behav Immun*. 2003;17(suppl 1):S112–S118.
46. Eisenberger NI, Inagaki TK, Mashal NM, Irwin MR. Inflammation and social experience: an inflammatory challenge induces feelings of social disconnection in addition to depressed mood. *Brain Behav Immun*. 2010;24:558–563.

19

Intestinal Permeability

Corene Humphreys, ND, BHSc, Dip Med Herb, Dip Hom, QTA

OUTLINE

INTRODUCTION

The intestinal epithelial barrier (IEB) is a dynamic, multicomponent barrier tasked with the important role of keeping out harmful molecules from the external environment while simultaneously allowing the absorption of nutrients and water vital for host survival. Over the past decade, substantial scientific and clinical research has been undertaken in this field of physiology. Alterations in IEB function have now been implicated in the pathogenesis of a number of chronic inflammatory diseases, including intestinal diseases, cardiometabolic disorders, autoimmune diseases, lung disease, neurological conditions, and systemic infectious diseases. This chapter outlines the core components and pathophysiological roles of the IEB and how they relate to specific disease states. It also outlines key diagnostic tests to identify changes in gut permeability, along with evidence-based therapies to restore intestinal barrier homeostasis.

COMPONENTS OF THE INTESTINAL BARRIER

Spanning approximately 300 to 400 m^2, the gastrointestinal barrier represents the largest surface area in the body that separates the external environment from the internal milieu.[1,2] The primary roles of the IEB are to protect the host from luminal pathogenic bacteria, intraluminal toxins, and antigens while simultaneously allowing for the passage of dietary nutrients, electrolytes, and water.[1,3–5] Structures of the IEB comprise four diverse components: microbiological, physical, chemical, and immunological barriers (Fig. 19.1).[1]

The Microbiological Barrier

The gut microbiota is now recognized as an integral component of the intestinal epithelial barrier for a number of reasons: (1) it promotes resistance to the colonization of pathogenic organisms by competing for attachment sites, (2) it provides nutrients, and (3) it releases antimicrobial substances. The microbiota is also involved in the digestion and absorption of luminal nutrients, which in turn supply energy to intestinal epithelial cells.[6–9]

Over 90% of the human microbiota is derived from seven phyla: *Firmicutes* (gram positive), *Bacteroides* (gram negative), *Proteobacteria* (gram negative), *Fusobacteria* (gram negative), *Verrucomicobia* (gram negative), *Cyanobacteria* (gram negative), and *Actinobacteria* (gram positive), with *Firmicutes* and *Bacteroides* as the dominant species.[10–12]

A dysbiosis can be defined as a reduction in microbial diversity and a combination of the loss of beneficial bacteria such as *Bacteroides* strains and butyrate-producing bacteria such as *Firmicutes*[10] and a rise in pathobionts[12] (symbiotic bacteria that become pathogenic under certain conditions), including *Proteobacteria,* which encompasses gram-negative *Escherichia coli.*[13]

Lipopolysaccharides (LPSs) constitute the major lipidic components of the outer wall of gram-negative bacteria. They are vital for the structure and functional integrity of these bacteria, providing a barrier that protects against penetration of bile salts and other antimicrobial agents while allowing the passage of small hydrophobic compounds. For the host, however, persistent migration of LPSs into the circulatory system can have deleterious consequences.[12] LPSs have also been identified in the scientific literature as "endotoxins."[13] Endotoxins bind to receptors, initiating an adaptive immune response and a signaling cascade, leading to activation of proinflammatory genes.[14] Impaired digestive function along with gut-derived microbial toxins trigger both the onset and maintenance of chronic low-grade inflammation.[15] This, in turn, increases intestinal permeability, allowing the translocation of microbiome-derived LPSs into the bloodstream, resulting in a two- to threefold increase in serum LPS concentration, which can reach a threshold termed *metabolic endotoxemia* (ME). ME may

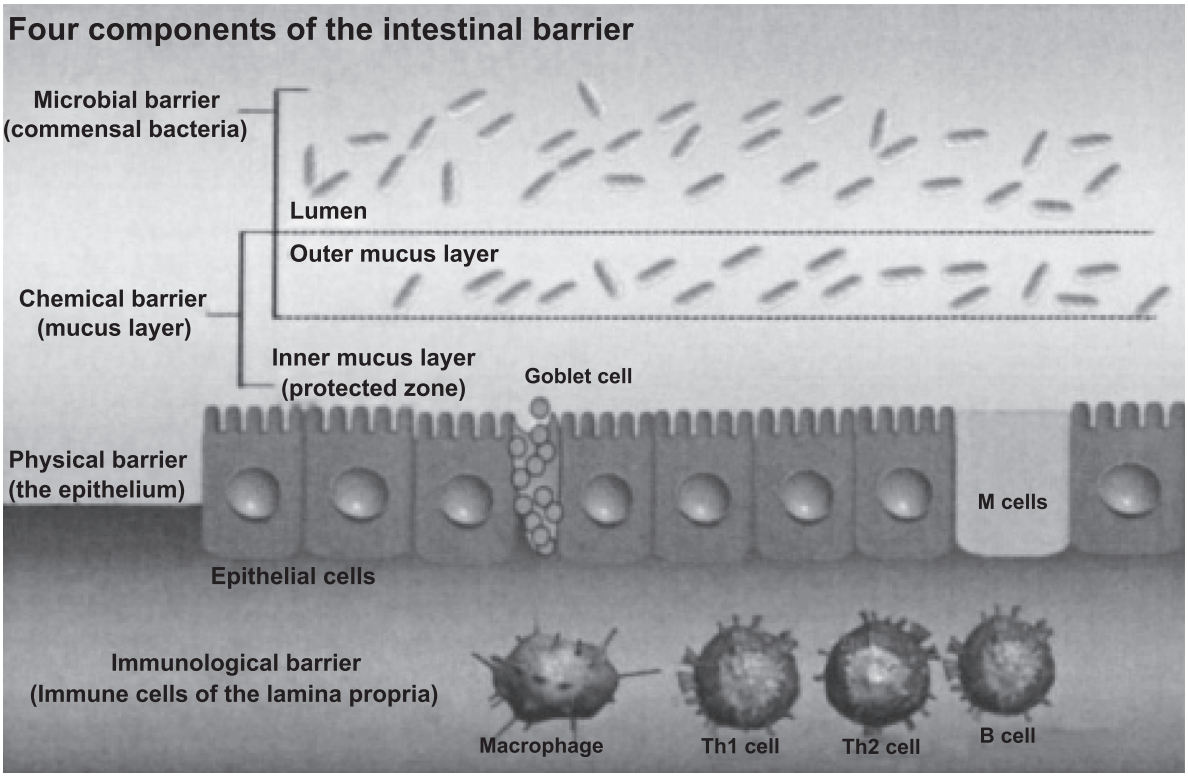


Fig. 19.1 Components of the intestinal barrier. (From Ji J, Qu H, Shu D. Crosstalk between bioactive peptide and intestinal barrier in gut homeostasis. *Curr. Protein Peptide Sci.* 2015;16[7]:604–612.)

trigger toll-like receptor-4 (TLR-4)–mediated inflammatory activation, eliciting chronic low-grade proinflammatory and prooxidative stress. Endotoxemia has been implicated in a number of inflammatory diseases, including sepsis, septic shock, diabetes, and, more recently, obesity-related metabolic disorders. Gut microbiota constitutes the major source of LPSs, and the main portal of entry into the systemic circulation is via direct diffusion through increased intestinal permeability, or from the absorption and incorporation of LPSs into chylomicrons after a lipid-rich meal.[12,16]

Research shows that impaired intestinal barrier function is often associated with alterations in the gut microbiome. When this occurs, instead of maintaining the homeostasis of the immune system, the microbiota can initiate and perpetuate inflammatory responses, thereby triggering or exacerbating intestinal and systemic diseases such as inflammatory bowel disease (IBD) and metabolic syndrome.[11,13,17,18]

The Physical Barrier

The physical barrier comprises the mucus layer, intestinal epithelial cells, and intercellular junctional complexes.[6] The chief role of this component of the IEB is to prevent bacterial adhesion (mucus layer) and regulate transcellular and paracellular diffusion of molecules into the systemic circulation (epithelial cells and tight junctions).[19]

The Mucosal Layer

Immediately below the microbiota and overlying the epithelium is the mucus layer, which functions as both a physical barrier and a biochemical barrier.[5] As the first physical barrier, the mucus layer limits the direct interactions of the intestinal epithelium with microbes, antigens, and toxins while simultaneously allowing nutrient absorption.[17,18,20] The mucosal layer also acts as a lubricant for intestinal motility.[21]

Specialized enterocytes called goblet cells secrete mucus, which is composed of proteins, carbohydrates, lipids, and water.[22] A core component of mucus is large glycoproteins, which belong in the family of secretory-gel–forming mucins. In both the small and large intestine, MUC2 is the primary secretory mucin produced and secreted by goblet cells and forms the main structural unit of the mucus layer.[5,22] As well as acting as the first line of defense against physical and chemical injury from luminal microbial and antigenic contents, secreted mucins serve as a food source for microbes and also provide binding sites for bacteria.[12,19,22,23] At the same time, microbiota and their microbial products are able to modulate mucin synthesis and release through a "crosstalk" feedback mechanism. Thus a symbiotic relationship exists whereby microbiota and mucins work collectively to prevent the invasion of enteric pathogens.[22]

Intestinal Epithelial Cells

Below the mucus layer is the intestinal epithelium, which is organized into a monolayer of invaginations called crypts and villi (finger-like projections) in the small intestine. The bottom of the crypt is composed of stem cells, which differentiate into mature cell lineages as they migrate up toward the villus.[22,24,25]

To date, at least seven different cell lineages have been identified: enterocytes, goblet cells, Paneth cells, microfold cells (M cells), endocrine cells, cup cells, and tuft cells.[6,21] The exact roles of cup and tuft cells have not been fully elucidated.[6] The key physiological roles of the main intestinal epithelial cells are outlined as follows:

Enterocytes comprise approximately 90% of IECs and play an important role in the absorption of nutrients while also serving as a barrier to prevent the translocation of luminal contents into host tissues.[6,19]

Goblet cells secrete mucin and other mucus constituents that form a net-like structure to protect microorganisms from reaching the epithelial surface.[26] They have also been shown to secrete antimicrobial peptides (AMPs) known as trefoil factors, which are involved in intestinal defense and mucosal repair.[1]

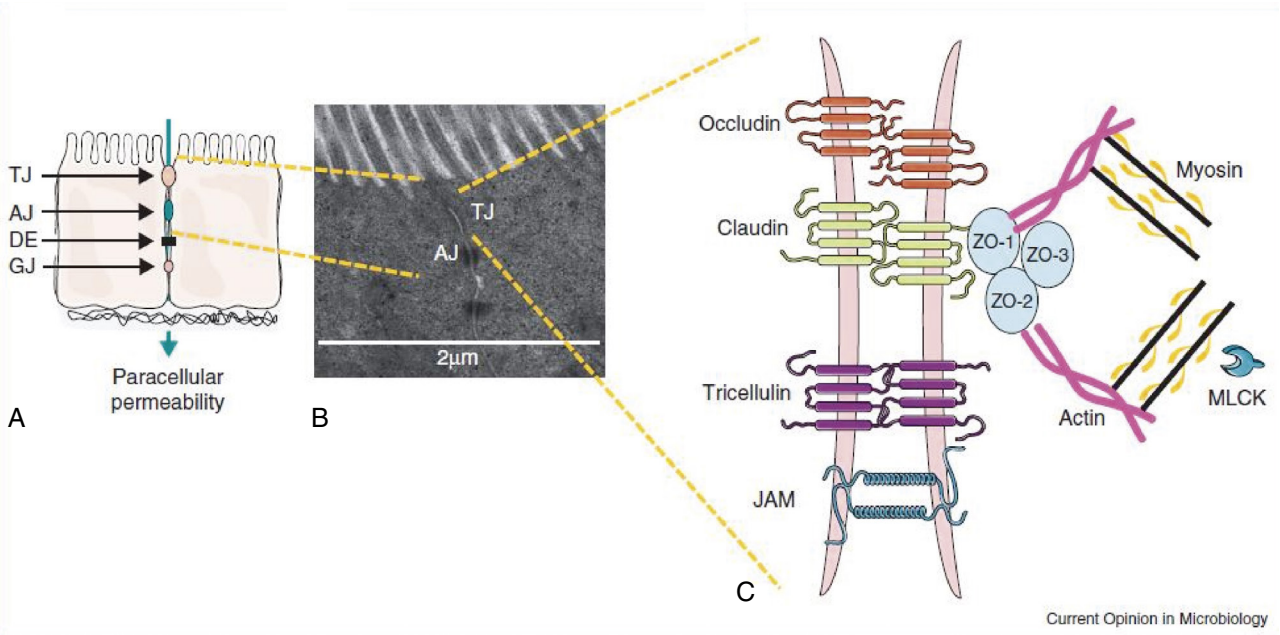


Fig. 19.2 Transmembrane and Intracellular Mechanisms of Paracellular Permeability. (From Barreau F, Hugot JP. Intestinal barrier dysfunction triggered by invasive bacteria. *Curr. Opin. Microbiol.* 2014;17:91-98.)

Paneth cells are composed of large numbers of secretory granules filled with AMPs, and as such, they support and mediate the biochemical barrier function.[5] Antimicrobial substances secreted by Paneth cells include defensins, lysozyme, and regenerating islet-derived protein 3-gamma (RegIIIγ) proteins, all of which help protect against infection from bacteria, fungi, yeasts, and viruses.[1,26,27]

Microfold (M) cells represent the antigenic component of the intestinal epithelial barrier. Located above the Peyer's patches and intestinal lymphoid follicles, M cells are involved in the process of sampling antigenic material, including food antigens, pathogenic bacteria, and their bacterial components, from the lumen and transcytosing to the underlying immune system.[28–31] Commensal bacteria can also be internalized and exported to the gut-associated lymphoid tissue (GALT) via this pathway.[31,32]

Enteroendocrine cells are sensory intestinal epithelial cells that detect and respond to luminal nutrient content by secreting peptide hormones to regulate digestive enzyme secretion, intestinal pH, gastric emptying, satiety, electrolytes, and motility.[33–36] Hormonal mediators secreted by enteroendocrine cells include serotonin, motilin, glucagon-like peptide NPY, and substance P.[34] Enteroendocrine cells comprise less than 1% of the overall intestinal epithelial population, of which serotonin-secreting enterochromaffin cells are the most abundant subtype.[33] Despite their small representation, because they are dispersed throughout the gastrointestinal tract, these cells collectively constitute the largest endocrine system in humans.[35]

Intercellular Junctional Complexes

Intestinal epithelial cells are tightly bound to one another by at least four intercellular junctional complexes, which are vital for the integrity of the epithelial barrier.[37,38] From apical to basal, the intercellular junctions are composed of tight junctions (zonula occludins), adherens junctions (zonula adherens), desmosomes, and gap junctions (Fig. 19.2).[9,19,39,40]

Tight Junctions (Zonula Occludins)

Tight junctions represent the most apical (the side toward the lumen) structure of the intercellular junctional complex and are composed of at least 50 different transmembrane proteins.[9,18,21,37,38] Numerous in vitro and animal studies have identified tight junction disruption as the leading cause of altered intestinal barrier function.[38,39] The primary function of tight junctions is to provide a physical barrier that selectively allows for the passage of water, ions, and small solutes in the space between neighboring cells while limiting access to pathogens, toxins, and xenobiotics.[18,24,41–43]

The four integral transmembrane proteins that make up the tight-junction complex are occludin, the claudin family, tricellulin, and the junctional adhesion molecule (JAM).[39,40] Occludin, tricellulin, and JAM play a regulatory role in tight-junction structure and permeability,[3,20,27,34,44,45] whereas the claudin family is primarily responsible for intestinal barrier function.[26,27,46] Claudins can be differentiated into two main types: barrier enhancing (those that decrease paracellular permeability) and pore forming (claudins that increase paracellular permeability).[20,26,31,46] The specific claudins identified with the sealing function that decreases paracellular permeability include claudin-1, -3, -4, -5, -8, -9, -11, -14, and -19.[18,31,47] Claudins linked to pore formation and enhanced permeability include claudin 2, -7, -10, -12, -15, and -23.[18,48]

Zonula occludins proteins (ZO-1, ZO-2, and ZO-3) are intracellular scaffold proteins that anchor the transmembrane proteins to the cytoskeleton of the cell.[18,27,42,46] Zonulin is recognized as the only physiological regulator of intracellular tight junctions and also plays an important role in the balance between tolerance and immunity.[49,50] Gluten and bacteria (commensals and pathogenic) in the intestinal mucosa have been identified as stimuli that can trigger the release of zonulin.[49,51]

Together, transmembrane and zonulin proteins form a connective network around intestinal epithelial cells via their interaction with the perijunctional actomyosin ring (a dense ring of filamentous actin and myosin that encircles the cell at the region of the adherens junctions and tight junctions).[9,17,18,21,24] Tight-junction functionality is also

regulated in part by myosin light chain (MLC) phosphorylation with the enzyme MLC kinase, which causes actin to contract and open the junctional gap. This results in increased permeability to electrolytes and other small organic molecules.[11,18,20,43]

Historically, the tight-junction complex was thought to be a simple, static paracellular seal. New evidence shows that tight junctions are dynamically and selectively permeable and are able to regulate paracellular flux in both a size- and charge-selective manner.[43,47,51,52] Physiological and pathological factors known to influence tight-junction permeability include dietary peptides, probiotics, enteric pathogens, mucosal immune activation, cytokines, proteases, cellular stress, growth factors, and enteric nervous system signaling.[25,50,52]

Adherens Junctions (Zonula Adherens), Desmosomes, and Gap Junctions

Located immediately below the tight junctions are the adherens junctions, followed by desmosomes and then gap junctions.[24] Adherens junctions and desmosomes form strong adhesive bonds between IECs to hold cells together and protect against mechanical disruption of the epithelial sheet. They are also involved in cell-to-cell communication and are home to proteins that direct epithelial polarization.[12,18,20,24,39,45] Gap junctions are also involved in intracellular communication and help facilitate the exchange of small molecules between intestinal epithelial cells.[9,48,53] Unlike tight junctions, however, these proteins do not regulate paracellular permeability.[12,18,20,24,39,45]

The Biochemical Barrier

Embedded in the mucus layer and far into the gastrointestinal lumen resides the biochemical barrier. The main role of the biochemical barrier is to reduce the load of colonized bacteria and help prevent luminal antigens from having direct contact with host cells.[6] Components that make up the chemical barrier include digestive secretions, AMPs, cytokines, and other inflammatory mediators such as proteases.[26]

Digestive Secretions

The first line of biochemical defense resides within the lumen, where gastric acid, pancreatic juice, and bile participate in the integrity of the intestinal epithelial barrier by altering the pH, thereby creating a bactericidal environment for pathogenic organisms.[19,21] In addition to their antimicrobial role, digestive enzymes degrade dietary proteins, thereby preventing larger immunogenic peptides from reaching the small intestine.[30]

Antimicrobial Peptides

AMPs are secreted by virtually all epithelial cells in the intestine as well as immune cells.[9,18] The chief role of AMPs is to protect the intestinal epithelial barrier against invading pathogens and control the colonization of commensal organisms.[6,12,32,54] AMPs are active against a variety of organisms, including gram-positive and gram-negative bacteria, parasites, protozoa, fungi, and enveloped viruses.[18,54] AMPs destroy bacteria either by enzymatic attack or via nonenzymatic mechanisms that interfere with bacterial membranes via electrostatic interactions.[32] To date, several types of AMPs have been identified, including α-defensins and β-defensins, C-type lectins, cathelicidin, lysozyme, and intestinal alkaline phosphatase.[6] In addition to their role as antimicrobial agents, AMPs also represent a link between the innate and adaptive immune systems.[5,9]

Cytokines

Intestinal epithelial barrier function is also regulated by cytokines, which can have barrier-disrupting or barrier-enhancing effects, depending on the particular cytokine involved (Table 19.1).[3,45] The secretion of proinflammatory cytokines in response to dysbiotic microbiota and other noxious stimuli has been shown to impair gut barrier function and induce the reorganization of a number of tight-junction–associated proteins.[7,34,42]

TABLE 19.1 Effects of Cytokines and Growth Factors on Intestinal Epithelial Barrier Function

Cytokine/Growth Factor	Effects on Intestinal Barrier Function
IFN-γ	↑ paracellular permeability
TNF-α	↑ paracellular and transcellular permeability
IL-1β	↑ paracellular and transcellular permeability
IL-4	↑ paracellular permeability
IL-6	↑ paracellular permeability to cations
IL-10	Restores intestinal barrier defects (neutralizes IFN-γ)
IL-13	↑ paracellular permeability
IL-17	↓ paracellular permeability
TGF-α	Protects against barrier defects (neutralizes free radicals)
TGF-β	↓ paracellular permeability
EGF	Protects against barrier defects (neutralizes free radicals)

EGF, Epidermal growth factor; *IFN-γ,* interferon-gamma; *IL,* interleukin; *TGF-α,* transforming growth factor-alpha; *TGF-β,* transforming growth factor-beta; *TNF-α,* tumor necrosis factor-alpha.

Proteases

Proteases can be present on both the apical and basolateral sides of the intestinal wall. In the gut lumen, they are either produced endogenously (e.g., pancreatic proteases) or from bacteria and food particles. In the lamina propria, proteases are released into the mucosa by macrophages, neutrophils, and mast cells to regulate inflammation.[2] Proteases play a dual role, acting as both degrading enzymes and signaling molecules. In the gastrointestinal mucosa, they are involved in the degradation of the extracellular matrix, mucosal proteins, and bacteria. As signaling molecules, their proteolytic action is capable of altering the structure and function of tight junctions, thereby increasing intestinal permeability.[2,26]

The Immunological Barrier

Residing immediately below the epithelial layer is the lamina propria, which is home to the intestinal immune system.[13] The innate and adaptive immune systems contribute to the immunological barrier, and together, they form the gut-associated lymphoid tissue (GALT), which contains up to 70% of the entire body's immune cells.[19,21] Within the GALT are organized lymphoid follicles, including Peyer's patches, which are primarily located in the distal ileum, and isolated lymph follicles that are mainly found in the colon.[28] The immune cells that make up the innate and adaptive immunity of the gastrointestinal tract include dendritic cells, monocyte/macrophages, neutrophils, mast cells, B cells, and T cells.[28] Collectively, these cells are responsible for antigen sampling and immunological responses to pathogenic microorganisms as well as immune tolerance to commensal flora.[19,21,50]

Secretory immunoglobulin A (sIgA) is the first line of adaptive immune defense and constitutes the major immunoglobulin class in the gastrointestinal tract.[1,18] Immunoglobulin A (IgA) is secreted by

plasma cells (effector B cells) in the lamina propria and is then transcytosed through the intestinal epithelium (with the aid of the polymeric immunoglobulin receptor) and secreted into the lumen as sIgA antibodies.[1,6,13,28] Once in the lumen, sIgA binds to bacteria and viruses, thereby preventing them from attaching to and colonizing epithelial surfaces.[18,26] Another way in which sIgA exerts antimicrobial effects is by entrapment of bacteria in the mucus layer, reducing bacterial virulence factors and phagocytosis of intracellular pathogens as it migrates through the epithelium.[13,55]

LUMINAL TRANSPORT ACROSS THE INTESTINAL EPITHELIUM

Epithelial cells allow for selective permeability by three distinct pathways: the transcellular route, the paracellular route, and the more recently identified unrestricted pathway.[20,39,45]

The **transcellular transport pathway** facilitates the passage of larger molecules (molecular weight [MW]: >600 Da) through the intestinal epithelial cells.[25] Nutrients such as sugars, amino acids, peptides, fatty acids, and vitamins and minerals utilize this pathway.[40,45] Food antigens, bacteria, and bacterial products, including LPSs, primarily use the transcellular route via M cells and other enterocytes such as goblet cells.[12,25,40] These antigens are normally degraded by lysozymes within the intestinal epithelium and enter the lamina propria as small, nonimmunogenic peptides.[17] To date, a number of different transcellular pathways have been described for different luminal compounds based on their size, hydrophobicity, and other chemical traits[12,25,45,56]:

Transcellular uptake—this route is through the plasma membrane of epithelial cells. Small hydrophilic and lipophilic compounds and ions utilize this pathway.

Active transport—nutrients such as sugars, amino acids, and vitamins and minerals migrate through the epithelial barrier using transporters, which requires energy (hence the name "active" transport).

Endocytosis—larger molecules, including proteins, larger peptides, bacteria, and bacterial products, are endocytosed into vesicles and move through the intestinal epithelium via transcytosis and posterior exocytosis.

The paracellular pathway allows for the transport of ions (mostly cations), water, and other inert solutes of low molecular weight (<600 Da) through the spaces between the epithelial cells.[30,34] Unlike the transcellular pathway, the movement of molecules through the paracellular route occurs by diffusion.[57] Currently, three different routes of paracellular flux have been identified, termed the pore, leak, and unrestricted pathways.[39,43] The pore pathway regulates the passage of small-size solutes (approximately 5–10 Å).[20] It is a high-capacity, size-selective, and charge-selective route.[24,52,58] Pore pathway permeability is mainly regulated by subsets of the claudin family of tight-junction proteins.[24,39] Cytokines that enhance the expression of claudin-2 proteins, such as interleukin (IL)-4 and IL-13, upregulate the pore pathway, leading to increased passage of water, sodium, and small cations.[2,20,43,52] The leak pathway allows for the passage of large molecules (up to 125 Å) and is a low-capacity, size-nonselective, and charge-nonselective paracellular route.[20,52,58] Leak pathway permeability appears to be regulated by zonulin, occludin, tricellulin, and MLC kinase.[39,43,58]

The unrestricted pathway this is thought to be a tight-junction–independent pathway that becomes active after direct epithelial damage (i.e., erosions and ulcers). This is a high-capacity, size-nonselective, and charge-nonselective route that allows for the passage of vast quantities of both large (including microbes) and small molecules across the epithelial barrier.[20,39]

Under homeostatic conditions (when the epithelium is intact), luminal flux occurs across both the leak and the pore pathways (Fig. 19.3).[39,52] Pathological insults such as infection, immune-mediated stimuli, and ischemia can compromise the epithelial barrier, leading to an increased flux across the pore pathway, the leak pathway, and the unrestricted pathway.[20] When there is extensive epithelial damage (e.g., necrotizing enterocolitis), the unrestricted pathway becomes the dominant route of transmucosal flux.[20,39]

ASSESSING INTESTINAL BARRIER FUNCTION

The assessment of intestinal barrier function is currently based on two different methods: active and passive measurement. Active tests evaluate trans- and/or paracellular urinary recovery of orally administered probes, whereas passive assessment analyzes the consequences of a disrupted intestinal barrier (i.e., the translocation of luminal compounds and tight-junction proteins into the systemic circulation).[27] Because there is no universal marker to assess all facets of intestinal barrier dysfunction and because each test has varied clinical utility and limitations, the use of more than one test may be necessary for a more comprehensive evaluation of leaky gut.[25,30,59]

Active Assessment of Barrier Function

Active assessment of barrier function loss is predicated on the understanding that orally administered probes of different molecular weights have variable ability to cross the paracellular pathway unless the intestinal barrier has been compromised in some way.[26,27,29] When barrier function loss occurs, the ingested probes are able to cross the intestinal barrier and enter the circulation, and they can be recovered in the urine after renal excretion.[26,27]

Currently, there are a number of different marker probes that can be employed to assess active barrier function. These include the sugar probes lactulose, mannitol, rhamnose, and cellobiose as well as polyethylene glycols (PEG 400, PEG 1500, PEG 4000) and chromium-labeled ethylenediamine tetra-acetic acid (Cr-EDTA).[26,57] For the purposes of this chapter, only the dual-sugar and multisugar tests will be discussed in detail because they are the most commonly used profiles in clinical practice.

Dual-Sugar Test

The lactulose/mannitol dual-sugar test (DST) has been used for more than 50 years and has long been considered the gold standard for the assessment of intestinal permeability.[25,29,30,38,60] The monosaccharide mannitol is a small molecule (MW 182 Da) thought to cross the pore pathway freely, independent of barrier function loss, and thus can be considered a measure of intestinal surface area.[26,27,52,61] The disaccharide lactulose is a larger molecule (MW 342 Da) thought to cross the leak pathway or the unrestricted pathway (after epithelial damage) and is considered a marker of barrier integrity.[4,26,52,61]

DSTs usually evaluate lactulose and mannitol recoveries separately, as well as the lactulose/mannitol (L/M) ratio, for a more complete evaluation of intestinal barrier function.[38]

The ratio of the urinary concentration of lactulose and mannitol represents a measure of the sum of leak pathway permeability and epithelial damage normalized to the surface area.[27,52]

Multisugar Test

A more comprehensive assessment of intestinal permeability can be achieved by the addition of other test probes.[26] This is known as the multisugar test (MST), which involves the simultaneous ingestion of sucrose, lactulose, mannitol, and sucralose to evaluate gastroduodenal, small intestinal, and large intestinal permeability, respectively.[26,27,47]

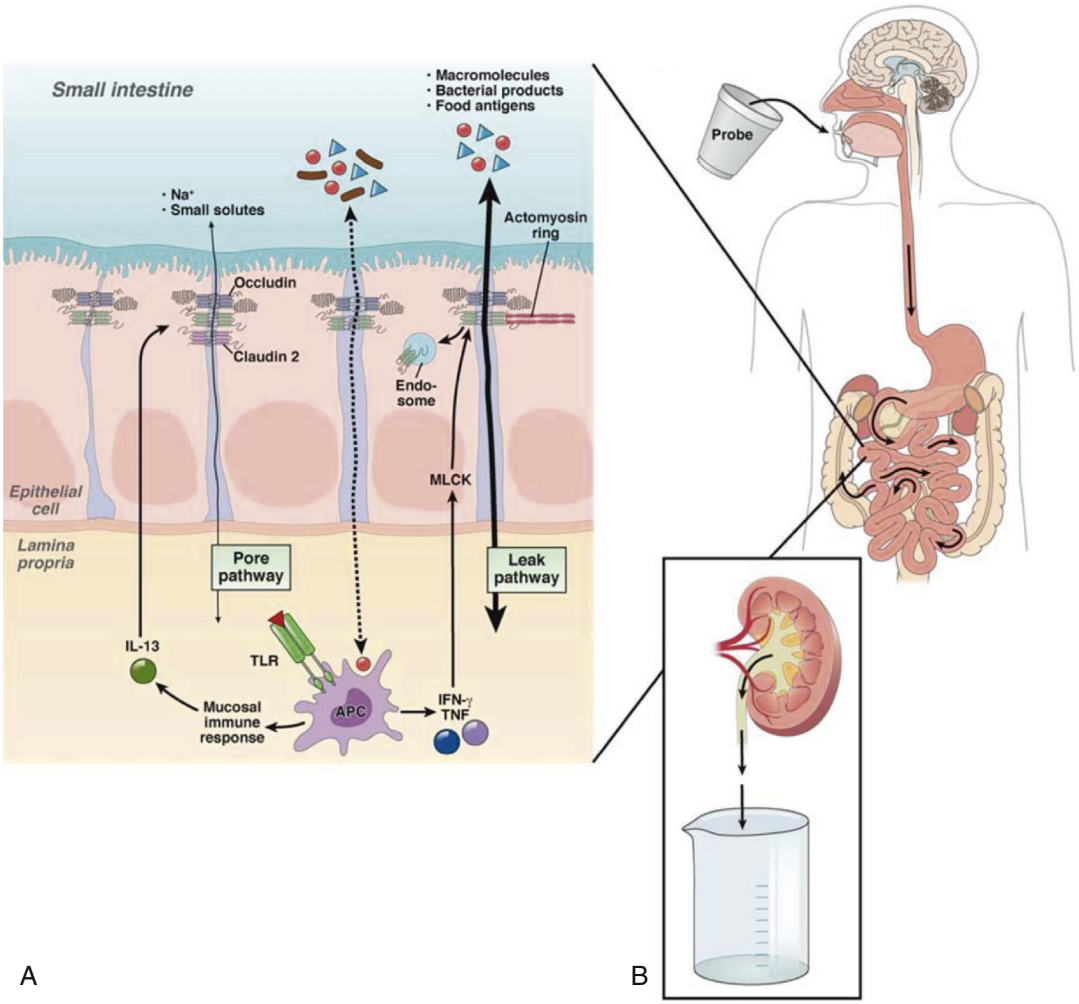


Fig. 19.3 Intestinal permeability pathways and mechanisms of analysis. (From Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? *Clin Gastroenterol Hepatol.* 2013;11[9]:1075–1083.)

The disaccharide sucrose is used for gastroduodenal permeability because this sugar is rapidly hydrolyzed by sucrase, an enzyme secreted in large quantities by enterocytes in the duodenum.[38,47,62] As with the DST, lactulose and either mannitol or rhamnose monosaccharides are used for small intestinal permeability because they are degraded in the cecum by colonic bacteria.[25,27,52] Sucralose is an artificial sweetener with a comparable mass to lactulose (MW 397.64 Da). Because this probe is not metabolized and fermented by colonic bacteria, it can serve as a marker for colonic permeability. Sucralose is thought to be absorbed via the leak paracellular pathway.[25,28,27,61–63]

Factors That Affect the Excretion of Sugar Probes

The excretion of orally ingested sugar probes depends on many factors, which can be categorized as follows[25–25,47,62,64]:

- **Premucosal factors:** proper digestion, gastric dilution and emptying, intestinal dilution, transit time, bacterial degradation, alcohol consumption, smoking, and certain medications (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs]).
- **Mucosal factors:** brush-border hydrolysis, permeation pathways, and blood flow.
- **Postmucosal factors:** endogenous sugar production, metabolism, tissue distribution, renal excretion, and proper urine test collection.

Limitations of the Dual-Sugar and Multisugar Tests

Recently, the efficacy of the DST and MST have been called into question by scientists who claim these profiles are not indicative of the transport of macromolecules, such as bacterial toxins and food antigens, that use the transcellular pathway to enter the lamina propria.[29,30] Research shows that only molecules of 5000 Da or greater are capable of challenging the intestinal immune system and initiating a T-cell–mediated response with downstream cytokine and antibody production.[29]

Probes with higher molecular mass, such as proteins and bacteria, have been put forward as alternative markers to determine whether increased intestinal permeability is sufficient to allow bacterial toxins (e.g., LPSs), food antigens and other macromolecules to permeate the gut wall.[30,38,59]

Passive Assessment of Barrier Function

The passive assessment of barrier function loss is based on the understanding that luminal substances such as bacterial endotoxins, bacterial by-products, and tight-junction barrier proteins only migrate into the systemic circulation after barrier function loss. Quantification of plasma or serum levels of these compounds is therefore considered a reflection of the integrity of the intestinal barrier.[26,27]

Examples of passive tests to measure intestinal permeability include zonulin, endotoxin (LPS), intestinal fatty acid–binding protein (FABP), and endotoxin core antibodies (EndoCAbs). Although they hold the advantage of being snapshot tests that don't require a challenge substance or the time-consuming collection of urine, the substances assayed can be difficult to measure due to technical challenges (e.g., endotoxin assays) or endogenous factors such as hepatic metabolism.[26,27] For example, LPS can be readily measured in the portal vein of animals but is challenging to measure in the peripheral blood of humans and requires careful standardization.[4,26] Additionally, certain luminal compounds (e.g., FABP and EndoCAb) are only able to detect acute intestinal damage and thus are more suited to the hospital environment.[25,26] Currently, one of the most common tests offered by functional diagnostic laboratories to assess passive barrier integrity is zonulin.

Zonulin

Zonulin (MW 47,000 Da) is a biomarker of the intestinal barrier integrity of the small intestine.[37,49,65,66] At this time, it is recognized as the only physiological protein known to reversibly regulate intestinal permeability by disassembling intercellular tight junctions.[37,67,68] The secretion of zonulin is mainly derived from the liver but also other tissue, including enterocytes; adipose tissue; the brain, heart, lungs, kidney, and skin; and immune cells.[37,51,69]

In addition to being a marker of intestinal permeability, zonulin may also play a protective role. Zonulin upregulation may be a secondary response to altered metabolism and inflammation, and through its immune-modulating and anti-inflammatory effects, it may help protect tissue from injury.[37]

Zonulin expression has been shown to be upregulated in a number of metabolic,[37,49,67,68,70–74] inflammatory,[75–77] autoimmune,[38,67,78,79] neurodegenerative,[17,29,50,66] and tumoral diseases.[37,49,65,67] Elevated levels of zonulin have also been identified in children with autism spectrum disorders.[80,81]

Test limitations of zonulin

Because zonulin is secreted by many extraintestinal tissues, serum zonulin levels represent a culmination of zonulin from intestinal secretion and other organs, such as adipose tissue, the liver, the brain, the heart, immune cells, the lungs, the kidneys, and the skin.[37,51,69] This may explain why no correlation has been found between serum and fecal zonulin.[82] To date, clinical data on the use of zonulin in patients with IBD is limited.[51] As such, serum zonulin might best be described as a biomarker of metabolic disorders, such as low-grade inflammation, obesity, and metabolic syndrome, and autoimmune conditions, whereas fecal zonulin may be a more representative analyte to evaluate increased intestinal permeability.[37]

Zonulin has demonstrated high intraday variability, which could be due to how it is metabolized within the body and its circulating half-life.[65,77] Because zonulin is a high-molecular-weight protein, it is capable of eliciting an innate immune-mediated response. As such, when zonulin enters the submucosa and, thereafter, the systemic circulation, it can either be engulfed by macrophages or processed by Kupffer cells in the liver. Thus, depending on the host's innate immune response, zonulin levels in the bloodstream may vary.[65] To date, the half-life of zonulin is not known; rather, it is hypothesized to be very short (minutes to hours) based on the half-lives of other proteins of similar size, such as LPSs.[65,76,83] Due to the high day-to-day variability, some researchers have instead proposed testing for IgA and IgG antibodies against zonulin, occludin, and other tight-junction proteins, which are considered more stable markers of tight-junction permeability.[65]

FACTORS AFFECTING INTESTINAL PERMEABILITY

A number of factors have been shown to interfere with the integrity of intestinal barrier function, including enteric infections, a high-fat diet, gluten, food allergens, and drugs such as NSAIDs and proton-pump inhibitors (Fig. 19.4).[4]

Enteric Infections

Virtually all enteric pathogens disrupt the tight-junction barrier (Table 19.2), although the mechanisms by which they do so can vary from organism to organism.[45] Other mechanisms by which enteric pathogens increase paracellular and transcellular permeability include the upregulation of proinflammatory cytokines and immune cell infiltration.[7,40]

Protozoal infections are also associated with damage to the intestinal wall, specifically those caused by *Giardia intestinalis*,[46,84] *Entamoeba histolytica*,[22,46,85] and *Blastocystis hominis*.[86] Protozoa conventionally classified as nonpathogenic (e.g., *Entamoeba coli*) do not appear to alter intestinal permeability.[86]

High-Fat Diet

The Western diet is characterized by a high intake of dietary fats (particularly saturated fatty acids) and sugar (especially fructose) and a low intake of fiber. The long-term effects of an energy-rich, nutrient-poor diet are associated with a number of metabolic disorders that have their origins in altered microbiota and/or increased intestinal permeability with enhanced endotoxin in the portal vein.[26] Consumption of a high-fat diet leads to upregulation of the transcellular pathway (via chylomicron–LPS cotransport), which occurs independent of altered paracellular permeability.[87,88]

Gluten/Gliadin

As previously mentioned, gluten and the gluten component gliadin, along with bacteria, have been identified as the major stimuli that can trigger the release of zonulin.[51,89] It has been postulated that zonulin-driven opening of the tight junctions may be a host defense mechanism to flush out microorganisms and upregulate the innate immune system to protect against colonization of the small intestine.[37,49]

Food Allergies and Intolerance

For food allergies to manifest, intraluminal antigens must first penetrate the intestinal epithelial barrier before gaining access to mast cells in the subepithelial compartment and triggering hypersensitivity reactions.[90] Under normal conditions, most dietary proteins (~90%) are digested by gastric and pancreatic proteases and brush-border enzymes and transformed into peptides and amino acids before being absorbed by enterocytes via the transcellular pathway.[30,90] Once inside the enterocytes, any remaining intact proteins are ordinarily broken down inside lysosomal compartments, thereby preventing undigested proteins from entering the lamina propria.[17,91,92] A small amount (~10%) of intact proteins or partially digested peptides will cross the intestinal wall via the paracellular route. This pathway is regulated by intercellular tight junctions and, once translocated, results in antigen-specific immune responses that support immune tolerance to nonself antigens.[17,89]

Prolonged upregulation of paracellular permeability allows large amounts of nonself antigens to enter the lamina propria, which initiates an allergic reaction characterized by mast-cell recruitment and IgE-mediated antibody production. Inflammatory mediators such as cytokines and proteases also involved in the immune response set up a self-perpetuating situation leading to further gut barrier damage and increased entry of allergens into the circulation.[4,57,93]

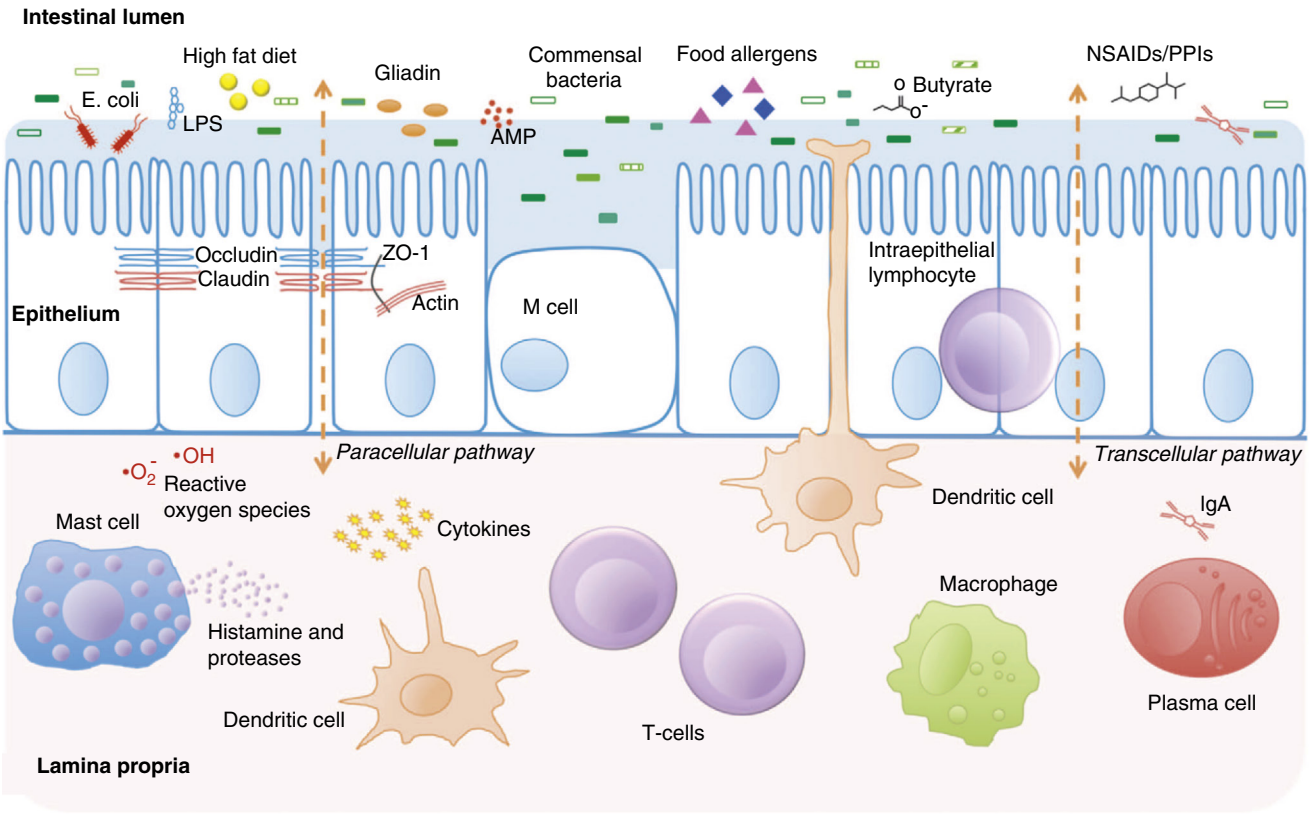


Fig. 19.4 Factors associated with impaired intestinal permeability. (From Konig J, Wells J, Cani PD, et al. Human Intestinal barrier function in health and disease. *Clin Transl Gastroenterol*. 2016;7[10]:e196.)

TABLE 19.2 Enteric Pathogens Associated With Altered Tight-Junction Regulation[4,34,40,45]

Campylobacter jejuni
Clostridium difficile
Clostridium perfringens
Enterohemorrhagic *Escherichia coli*
Enteropathogenic *E. coli*
Pseudomonas aeruginosa
Pseudomonas fluorescens
Salmonella typhimurium
Shigella dysenteriae
Shigella flexneri
Vibrio cholerae
Yersinia enterocolitica
Dengue virus
Norovirus
Reovirus
Rotavirus

Nonsteroidal Anti-Inflammatory Drugs and Proton-Pump Inhibitors

Altered gut barrier function is estimated to occur in 60% to 80% of patients taking NSAID medication.[4] Even short-term NSAID therapy has been shown to cause changes in intestinal permeability similar to those seen in patients with active IBD.[63,94] Ingestion of NSAIDs initially damages the enterocytes and increases gut permeability. The damaged mucosa is then exposed to endogenous substances such as bile acids, bacterial degradation products, and hydrolytic and proteolytic enzymes, with subsequent immune activation and inflammation.[4,95] Both cyclooxygenase (COX)-1 and COX-2 inhibitors have been implicated in small bowel damage.[95]

Proton-pump inhibitors (PPIs) are another commonly prescribed drug, often coadministered with NSAIDs. Their deleterious effects on intestinal barrier function primarily target the stomach and colon.[4] In addition to decreasing gastric production of HCl, these medications inhibit contractile activity, which interferes with the tight-junction complex via the intracellular actin–myosin cytoskeleton. Research suggests they may also affect paracellular permeability through phosphate-mediated dephosphorylation of tight-junction proteins.[4]

Exposure to Xenobiotic Pollutants

More than 25 tons of food will be processed over the course of an individual's life span, representing the largest load of antigens and xenobiotics confronting the human body. Infection, inflammatory cytokines, nutrient transporter activation, and noxious environmental toxins all alter intestinal permeability. Persistent organic pollutants (POPs) bioaccumulate in human and animal tissue and biomagnify in food chains, thus increasing their concentration and toxicity in the environment. Pollutants that are highly fat soluble are readily reabsorbed via passive diffusion in both the intestines and proximal tubules. These lipophilic compounds can also be actively transported with bile salts back into the bloodstream. POPs damage DNA and mitochondria, stimulate the expression of tumor necrosis factor (TNF)-α, and increase inflammatory cytokines, all of which contribute to increased

intestinal permeability. Alcohol ingestion also increases intestinal permeability to endotoxins and macromolecules, allowing increased toxic and antigenic effects.[96]

Cholinergic signaling is also involved in the regulation of the barrier function. Fluoridated drinking water can cause gastrointestinal distress as fluoride is converted to hydrogen fluoride in the stomach. Chronic gastritis with associated histological changes, including scanty microvilli, surface abrasions, and desquamated epithelium, as well as IBD, have been observed in individuals after consuming fluoride for long periods of time.[97] These have been shown to result from the effect of fluoride on the cholinergic pathway.

Olmesartan, an angiotensin II receptor blocker, has been associated with the development of a sprue-like enteropathy characterized by severe diarrhea and histopathological changes in the intestine.[98,99] A study from the Mayo Clinic first reported the association between olmesartan and enteropathy.[100] Twenty-two patients presented with severe diarrhea and weight loss and biopsies consistent with celiac sprue. However, serological testing for celiac disease was negative, and no patient responded to a gluten-free diet. After cessation of the medication, clinical symptoms resolved quickly, and the histological changes disappeared.

Many studies have shown that disruption of tight junctions leads to a rise in epithelial permeability. For example, methotrexate induces ZO-1 dephosphorylation, which in turn is associated with a change in the protein's localization in epithelial cells in the small intestine; this change may contribute to leakage of the intestinal barrier.[101] Chlorpyrifos, an organophosphate insecticide, has been linked to microbial dysbiosis and modification of the function of the enteric nervous system and its associated cholinergic signaling, which alters the epithelial barrier function and changes tight-junction protein expression.[102]

GASTROINTESTINAL AND SYSTEMIC DISEASES ASSOCIATED WITH ALTERED INTESTINAL BARRIER FUNCTION

Alterations in gut barrier function have been implicated in the pathogenesis of a number of chronic inflammatory diseases, including intestinal diseases, cardiometabolic disorders, autoimmune diseases, lung conditions, neurological disorders, and systemic infectious diseases (Table 19.3).[4,17,39,62] There is much debate among the medical and scientific communities as to whether disrupted intestinal barrier function is an initiating factor or epiphenomenon of inflammatory diseases.[17,45,47,48] To date, the gastrointestinal and systemic diseases with the strongest evidence of altered intestinal barrier function as an initiating and/or developing factor include celiac disease, IBD, irritable bowel syndrome, and type 1 diabetes.[45]

Numerous in vitro, animal, and ex vivo studies indicate that altered intestinal permeability can lead to the development of chronic inflammatory diseases by influencing the mucosal immune system, but only in genetically susceptible hosts with initial exposure to an environmental trigger (i.e., microbial or viral infection, dietary proteins, and toxic chemicals).[6,17,29] Fasano and colleagues have identified a common chain of events triggering the development of autoimmune diseases such as celiac disease and type 1 diabetes. The initiating step is the genetic susceptibility of the host's immune system to identify and misinterpret an environmental antigen from the small intestinal tract. The second step involves the host being exposed to the antigen, and the final step involves the antigen being presented to the mucosal immune system following paracellular passage and entry into the submucosa. Delivery of the antigen to the submucosa triggers a multiorgan process that leads to the development of an autoimmune response.[49]

TABLE 19.3 Gastrointestinal and Extraintestinal Conditions Associated With Intestinal Permeability[4,17,23,45,48]

Gastrointestinal Conditions	Extraintestinal Conditions
Alcoholic liver disease	Acute lung injury
Celiac disease	Asthma
Enteric infections and infestations	Atherosclerosis
Food allergies and intolerance	Atopic allergies
Hepatic encephalopathy	Autism spectrum disorders
Inflammatory bowel disease	Coronary artery disease
Irritable bowel syndrome (IBS)	Environmental enteropathy
Liver cirrhosis	Food allergies
Liver fibrosis	Graft-versus-host disease
Necrotizing enterocolitis	HIV infection
Nonalcoholic steatohepatitis	Hypertension
Nonceliac gluten sensitivity	Insulin resistance
Pancreatitis	Mediterranean fever
Small intestinal bowel overgrowth	Metabolic syndrome
Steatosis	Multiorgan failure syndrome
	Multiple sclerosis
	Neuropsychiatric illnesses
	Obesity
	Osteoporosis
	Parkinson's disease
	Rheumatoid arthritis
	Septicemia
	Type 1 diabetes
	Type 2 diabetes

THERAPEUTIC CONSIDERATIONS

Nutrition has been shown to have a pronounced effect on the microbiota, intestinal barrier function, and the passage of endotoxins into the circulation.[87] Therefore encouraging patients to make positive dietary changes should be part of a holistic treatment plan to restore intestinal barrier function.[26] Consuming a more traditional diet that is rich in fruits and vegetables, whole grains, and foods with prebiotic properties while minimizing processed and refined foods, total fat, and total calories can help reduce circulating endotoxins.[26,87] Such recommendations are supported by research that demonstrated a 71% rise in plasma endotoxins with a Western-style diet and a 31% reduction in circulating endotoxins following the consumption of a more traditional diet.[96]

Nutraceutical Therapies

Given that increased intestinal permeability may well be a consequence of the disease, versus the initiating event, researchers recommend treating the underlying disease(s).[39] For example, IBD is associated with significant increases in proinflammatory cytokines, which are known to increase both paracellular and transcellular permeability.[45] Treating IBD with anti-TNF antibodies or other recognized therapies that address the underlying immune activation has been shown to significantly reduce intestinal permeability.[39]

Natural agents with the greatest evidence for their use in protecting the intestinal epithelium include probiotics and the amino acid glutamine. Other nutraceutical agents with clinical and in vitro evidence to support intestinal barrier function are also described in the following sections.

Probiotics

Probiotics are thought to protect the intestinal mucosa and epithelial barrier through several different mechanisms[6,11,97]:

1. By enhancing the expression and secretion of mucin and β-defensins, thereby preventing the proliferation and migration of commensal and pathogenic organisms across the mucus layer
2. By increasing the production and secretion of sIgA to protect bacteria and their antigens from epithelial colonization
3. By altering gene expression and/or microbiota composition and increasing numbers of commensal bacteria
4. By producing antimicrobial factors (e.g., bacteriocins) that kill or inhibit pathogenic bacteria
5. By enhancing the production of tight-junction transmembrane proteins

Positive peer-reviewed clinical trials evaluating probiotic intervention with intestinal permeability as a primary endpoint are summarized in Table 19.4.

Glutamine

Glutamine is considered a conditionally essential amino acid that serves as a preferential fuel for rapidly dividing mucosal cells such as enterocytes, colonocytes, and immune cells.[45,103,104] It plays a vital role in the maintenance of mucosal growth and in its structure and function, particularly in times of metabolic stress where demand outweighs supply. For example, catabolic states such as burns, sepsis, and recovery from major surgery are associated with low glutamine status and accompanying epithelial atrophy and increased intestinal permeability.[47,103,105] Glutamine also protects against chemotherapy-induced alterations in gut permeability.[106,107] Supplementation with glutamine has been shown to restore intestinal membrane permeability and protect against TNF-α–induced bacterial translocation after intestinal injury.[47,104] In vitro studies suggest glutamine helps restore paracellular permeability by regulating the expression of the tight-junction proteins zonulin and occludin.[56,108]

Given its role in intestinal metabolism and immunity, glutamine was thought to be a promising therapeutic agent for patients with IBD. However, research in this cohort is sparse and conflicting, with studies suggesting it may actually exacerbate symptoms.[103,109,110] Supplementation with glutamine may enhance T-cell function, which can lead to the upregulation of proinflammatory cytokines and aggravate Crohn's disease.[110] Glutamine may also indirectly increase nitric oxide synthesis via its metabolism from citrulline to arginine, with resultant inflammation and tissue injury in patients with ulcerative colitis and Crohn's disease.[111] Some researchers have postulated that discrepant findings may be a result of the timing and duration of glutamine supplementation. It is thought that glutamine intervention should be administered before or at the onset of injury and, failing that, for a longer duration than most studies have allowed.[56] Dosing may also be a critical factor because studies that have yielded positive outcomes for patients with IBS with diarrhea (IBS-D)[112] and hospitalized,[113] postoperative,[114,115] chemotherapy,[106,107] and radiotherapy patients[116] have supplemented with 20 to 30 grams per day (intravenously or orally) for 5 to 30 days.

Miscellaneous Nutraceuticals

Other nutrients with preliminary clinical evidence for supporting intestinal barrier function include bovine colostrum, lactoferrin, and zinc. Bovine colostrum may have potential as a natural agent to protect against NSAID-induced alterations in mucosal integrity by downregulating proinflammatory cytokines.[117–120] Recombinant lactoferrin has also been shown to reduce NSAID-mediated small intestinal permeability following a short-term indomethacin challenge.[121] High-dose zinc (110 mg three times a day for 8 weeks) was reported to reduce intestinal permeability in patients with Crohn's disease in remission.[122]

Natural agents with experimental evidence for supporting intestinal barrier regulation include polyphenols, vitamins A and D, polyunsaturated fatty acids (PUFAs), and butyrate.

To date, a number of polyphenols have been investigated, including quercetin, kaempferol, myricetin, genistein, catechin, and curcumin. The main mechanisms by which polyphenols help restore intestinal permeability are through enhancing tight-junction assembly and neutralizing proinflammatory cytokines (in particular, TNF-α, IL-1β, and IFN-γ).[26,45,46,56]

Vitamin A and D receptors are expressed in a number of organs, including the intestinal epithelium, where they play a role in gene transcription and regulation.[45] Both of these fat-soluble vitamins have been shown to support gut barrier integrity and improve immune function.[56,123] Vitamin A helps regulate the growth and differentiation of intestinal epithelial cells. In animal models and in vitro studies, a deficiency of vitamin A is associated with altered commensal bacteria, reduced villous height, and dysregulated tight-junction protein assembly.[26,124] Vitamin D is thought to protect the intestinal barrier by inducing the expression of tight-junction proteins (specifically ZO-1 and claudin).[6] Low levels of vitamin D are associated with reduced mucosal barrier integrity and increased susceptibility to mucosal damage, which may in turn increase the risk of IBD.[125] In other experimental studies, vitamin D deficiency demonstrated alterations in the microbiome and intestinal epithelial barrier dysfunction.[123]

Animal studies suggest polyunsaturated omega-3 fatty acids can improve villous height and intestinal barrier function and reduce LPS-induced intestinal damage.[46] In vitro research shows that PUFAs support paracellular permeability by modulating occludin and zonulin tight-junction proteins.[126]

Butyrate is a short-chain fatty acid (SCFA) produced by the anaerobic microbial fermentation of oligosaccharides that serves as an important metabolic fuel for colonic bacteria.[28] In vitro studies have demonstrated that butyric acid supports intestinal barrier function by regulating the assembly of the tight-junction proteins zonulin and occludin.[127] Based on animal research, butyrate improves tight-junction integrity by improving transepithelial resistance. It has also been shown to prevent the release of TNF-induced IL-8 secretion, suggesting a therapeutic role for butyrate in inflammatory diseases of the colon.[46,128]

Pharmaceutical Medications for Altered Intestinal Permeability

Larazotide Acetate

The most promising pharmaceutical drug candidate for the treatment of altered intestinal barrier function is larazotide acetate. Also known as AT-1001, larazotide is a zonulin antagonist that protects against gliadin-induced alterations in paracellular permeability in patients with celiac disease.[39] Phase I and Phase II clinical trials have demonstrated an excellent safety and tolerability profile.[126] Although all trials demonstrated a reduction in tissue transglutaminase and intestinal and extraintestinal symptoms after a gluten challenge, improvements in the lactulose/mannitol ratio were reported only in the inpatient setting.[129–132] Phase III clinical trials are currently underway, and its use for other chronic inflammatory diseases associated with zonulin dysregulation is also being explored.[17,49]

Disodium Cromoglycate

The pharmacological agent disodium cromoglycate is a recognized mast-cell stabilizer that has been used for the treatment of IgE-mediated gut permeability.[133,134] More recently, it has demonstrated

TABLE 19.4 Probiotic Intervention Studies Assessing Intestinal Barrier Function

Probiotic(s)	Study Design	Indication(s)	Dose	Outcomes
Lactobacillus rhamnosus GG[144]	Randomized, double-blind, placebo-controlled trial	Atopic dermatitis and food allergies in infants ($n = 27$) Eczema in nursing mothers ($n = 10$)	5×10^8 CFU/g infant formula for one month 2×10^{10} CFU twice daily for one month (mothers)	Alleviated intestinal inflammation and promoted endogenous barrier mechanisms by reducing TNF-α and IL-4
Lactobacillus rhamnosus 19070–2, *Lactobacillus reuteri* DSM 1224[145]	Randomized, double-blind, placebo-controlled crossover trial	Atopic dermatitis (moderate to severe) in children aged 1–13 years ($n = 41$)	2×10^{10} CFU twice daily for 6 weeks	Improved gut barrier (reduced lactulose/mannitol ratio)
Lactobacillus acidophilus-11 *Lactobacillus plantarum* 1258 *Bifidobacterium longum*-88[146]	Randomized, double-blind, placebo-controlled trial	Colorectal carcinoma patients undergoing colectomy aged 25–75 years ($n = 150$)	2.6×10^{14} CFU/day for 6 days preoperatively and 10 days postoperatively	Reduced the rate of postoperative infection and septicemia Reduced serum zonulin concentrations
Lactobacillus acidophilus-11 *Lactobacillus plantarum* 1258 *Bifidobacterium longum*-88[147]	Randomized, double-blind, placebo-controlled trial	Colorectal liver metastases in adults aged 25–75 years ($n = 150$)	2.6×10^{14} CFU/day for 6 days preoperatively and 10 days postoperatively	Reduced the rate of postoperative infection and septicemia Reduced serum zonulin concentrations
Saccharomyces boulardii-17[148]	Randomized, single-blind, placebo-controlled trial	Crohn's disease in adults aged 19–54 ($n = 34$)	200 mg lyophilized *Saccharomyces boulardii* (4×10^8 CFU) every 8 hours for 3 months	Improved intestinal permeability Prevented the activation of NF-κB and downstream proinflammatory cytokines
Lactobacillus rhamnosus GG[149]	Randomized, double-blind, placebo-controlled trial	Gastroenteritis in infants and children aged 9–21 months ($n = 124$)	1×10^{10} CFU/day for 4 weeks	Improved intestinal permeability Increased IgG antibody levels
Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium longum[150]	Randomized, single-blind, placebo-controlled trial	Diarrhea-predominant irritable bowel syndrome (IBS-D) in adults aged 32–57 ($n = 30$)	200g Fermented milk containing *Streptococcus thermophilus* 1×10^8 CFU, *Lactobacillus bulgaricus* 1×10^7 CFU, *Lactobacillus acidophilus* 1×10^7 CFU, *Bifidobacterium longum* 1×10^7 CFU twice daily for 4 weeks	Decreased small bowel permeability and improved mean global IBS scores
Lactobacillus rhamnosus GG[151]	Randomized, single-blind, placebo-controlled trial	IBS or functional abdominal pain in children aged 5–14 years ($n = 136$)	3×10^9 CFU twice daily for 8 weeks	Improved intestinal permeability Reduced frequency and severity of abdominal pain
Lactobacillus rhamnosus GG[152]	Randomized, double-blind, placebo-controlled trial	NSAID-induced alterations in gastric permeability in adult males aged 18–38 ($n = 16$)	2.4×10^9 CFU/day for 5 days	Protected the integrity of the gastric mucosal barrier against indomethacin therapy
Bifidobacterium animalis ssp. lactis 420 (B420) and/or Litesse Ultra polydextrose (LU)[153]	Randomized, double-blind, placebo-controlled trial	Overweight and obese adults aged 18–65 ($n = 225$)	1×10^{10} CFU/day for 6 months	B420 and B420 + LU reduced waist circumference and food intake Reduced blood zonulin and hs-CRP correlated with reduced trunk fat mass
Bifidobacterium lactis + Preterm infant formula (Prenan Nestle)[154]	Prospective, randomized case-control study	Preterm infants 27–36 weeks' gestation ($n = 75$)	2×10^7 CFU/g infant formula for 30 days	Decreased intestinal permeability and increased head growth
Lactobacillus acidophilus W22 *Lactobacillus casei* W56 *Lactobacillus paracasei* W20, *Lactobacillus plantarum* W62 *Lactococcus lactis* W19 *Bifidobacterium bifidum* W23 *Bifidobacterium lactis* W51 (Ecologic 825)[155]	Controlled clinical trial	Patients with ulcerative colitis with active pouchitis aged 32–71 years ($n = 16$)	2.5×10^9 CFU twice daily for 8 weeks	Probiotics restored the mucosal barrier to *Escherichia coli* Bacterial diversity correlated with barrier function

CFU, colony-forming unit; *hs-CRP,* high-sensitivity C-reactive protein; *IBS,* irritable bowel syndrome; *IgG,* immunoglobulin G; *IL-4,* interleukin-4; *NF-κB,* nuclear factor-kappa B; *NSAID,* nonsteroidal anti-inflammatory drug; *TNF-α,* tumor necrosis factor-alpha.

promising results in reducing diarrhea-predominate IBS symptoms[135] and functional dyspepsia,[136] although the exact mechanisms by which it improves barrier function have yet to be determined.

A number of pharmaceutical drugs used for the treatment of gastrointestinal diseases are also capable of modifying intestinal permeability. These include steroids, aminosalicylates, anti-TNF agents, and mucosal protectors.[21]

Corticosteroids

Corticosteroids have long been used to induce remission in Crohn's disease. Prednisolone therapy has been shown to reduce intestinal permeability by approximately 50% in patients with Crohn's disease (evidenced by improvements in the lactulose/mannitol ratio).[137] Other clinical research has demonstrated a decrease in permeability in children and adolescents with active Crohn's disease and ulcerative colitis (assessed with the lactulose/rhamnose ratio).[138] The ability of corticosteroids to inhibit the expression of TNF-α and nuclear factor (NF)-κB represent the underlying mechanisms by which these drugs protect against damage to the intestinal barrier.[139]

Aminosalicylates

5-aminosalicylic acid derivatives are often employed for the treatment of uncomplicated, mild to moderate IBD. In vitro studies show that these medications (particularly mesalamine) support the reestablishment of the mucosal barrier by enhancing epithelial restitution and proliferation.[140,141]

Anti-TNF agents

Anti-TNF agents such as infliximab are a more recent class of drugs used to treat IBD. As their name suggests, they work by inhibiting the TNF pathway, thereby reducing inflammation and restoring mucosal integrity.[39,142]

Mucosal Protectors

Mucosal protectors have a long-standing history of use in the treatment of peptic disease. Drugs such as sucralfate and bismuth help protect epithelial cells from gastric acid and pepsin.[143] Gelatin tannate works by forming a protective mucoadhesive layer and reducing the intestinal inflammation and bacterial fermentation associated with infectious diarrhea.[21]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ji J, Qu H, Shu D. Crosstalk between bioactive peptide and intestinal barrier in gut homeostasis. *Curr Protein Peptide Sci*. 2015;16(7):604–612.
2. Van Spaendonk H, Ceuleers H, Witters L, et al. Regulation of intestinal permeability: the role of proteases. *World J Gastroenterol*. 2017;23(12):2106–2123.
3. Lee SH. Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intest Res*. 2015;13(1):11–18.
4. Konig J, Wells J, Cani PD, et al. Human intestinal barrier function in health and disease. *Clin Transl Gastroenterol*. 2016;7(10):e196.
5. Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World J Gastroenterol*. 2014;20(5):1165–1179.
6. Mu Q, Kirby J, Reilly CM, Luo XM. Leaky gut as a danger signal for autoimmune diseases. *Front Immunol*. 2017;8:598.
7. Castoldi A, Favero de Aguiar C, Moraes-Vieira PM, Olsen Saraiva Camara N. They must hold tight: junction proteins, microbiota and immunity in intestinal mucosa. *Curr Protein Peptide Sci*. 2015;16(7):655–671.
8. Zaiss MM, Harris NL. Interactions between the intestinal microbiome and helminth parasites. *Parasite Immunol*. 2016;38(1):5–11.
9. Wells JM, Brummer RJ, Derrien M, et al. Homeostasis of the gut barrier and potential biomarkers. *Am J Physiol*. 2017;312(3):G171–G193.
10. Lau E, Carvalho D, Pina-Vaz C, Barbosa JA, Freitas P. Beyond gut microbiota: understanding obesity and type 2 diabetes. *Hormones (Athens, Greece)*. 2015;14(3):358–369.
11. Caricilli AM, Castoldi A, Camara NO. Intestinal barrier: a gentlemen's agreement between microbiota and immunity. *World J Gastrointest Pathophysiol*. 2014;5(1):18–32.
12. Guerville M, Boudry G. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. *Am J Physiol*. 2016;311(1):G1–G15.
13. van der Meulen TA, Harmsen H, Bootsma H, Spijkervet F, Kroese F, Vissink A. The microbiome-systemic diseases connection. *Oral Dis*. 2016;22(8):719–734.
14. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature*. 2000;406(6797):782–787.
15. Laugerette F1, Vors C, Peretti N, et al. Complex links between dietary lipids, endogenous endotoxins and metabolic inflammation. *Biochimie*. 2011;93(1):39–45.
16. Jayashree B, Bibin YS, Prabhu D, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem*. 2014;388(1–2):203–210.
17. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers*. 2016;4(4):e1251384.
18. Sanchez de Medina F, Romero-Calvo I, Mascaraque C, Martinez-Augustin O. Intestinal inflammation and mucosal barrier function. *Inflamm Bowel Dis*. 2014;20(12):2394–2404.
19. Viggiano D, Ianiro G, Vanella G, et al. Gut barrier in health and disease: focus on childhood. *Eur Rev Med Pharmacol Sci*. 2015;19(6):1077–1085.
20. France MM, Turner JR. The mucosal barrier at a glance. *J Cell Sci*. 2017;130(2):307–314.
21. Scaldaferri F, Pizzoferrato M, Gerardi V, Lopetuso L, Gasbarrini A. The gut barrier: new acquisitions and therapeutic approaches. *J Clin Gastroenterol*. 2012;46(suppl l):S12–S17.
22. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep*. 2010;12(5):319–330.
23. Nagpal R, Kumar M, Yadav AK, et al. Gut microbiota in health and disease: an overview focused on metabolic inflammation. *Beneficial Microbes*. 2016;7(2):181–194.
24. Neunlist M, Van Landeghem L, Mahe MM, Derkinderen P, des Varannes SB, Rolli-Derkinderen M. The digestive neuronal-glial-epithelial unit: a new actor in gut health and disease. *Nat Rev Gastroenterol Hepatol*. 2013;10(2):90–100.
25. Galipeau HJ, Verdu EF. The complex task of measuring intestinal permeability in basic and clinical science. *Neurogastroenterol Motil*. 2016;28(7):957–965.
26. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol*. 2014;14:189.
27. Grootjans J, Thuijls G, Verdam F, Derikx JP, Lenaerts K, Buurman WA. Non-invasive assessment of barrier integrity and function of the human gut. *World J Gastrointest Surg*. 2010;2(3):61–69.
28. Brandtzaeg P. Gate-keeper function of the intestinal epithelium. *Beneficial Microbes*. 2013;4(1):67–82.
29. Vojdani A. For the assessment of intestinal permeability, size matters. *Altern Ther Health Med*. 2013;19(1):12–24.
30. Menard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. *Mucosal Immunol*. 2010;3(3):247–259.
31. Markov AG, Aschenbach JR, Amasheh S. The epithelial barrier and beyond: claudins as amplifiers of physiological organ functions. *IUBMB Life*. 2017;69(5):290–296.
32. Brandl K, Schnabl B. Is intestinal inflammation linking dysbiosis to gut barrier dysfunction during liver disease? *Expert Rev Gastroenterol Hepatol*. 2015;9(8):1069–1076.
33. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int J Exp Pathol*. 2011;92(4):219–231.
34. Snoek SA, Verstege MI, Boeckxstaens GE, van den Wijngaard RM, de Jonge WJ. The enteric nervous system as a regulator of intestinal epithelial barrier function in health and disease. *Expert Rev Gastroenterol Hepatol*. 2010;4(5):637–651.
35. Worthington JJ, Reimann F, Gribble FM. Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. *Mucosal Immunol*. 2018;11(1):3–20.
36. Gribble FM, Reimann F. Signalling in the gut endocrine axis. *Physiol Behav*. 2017;176:183–188.
37. Ohlsson B, Orho-Melander M, Nilsson PM. Higher levels of serum zonulin may rather be associated with increased risk of obesity and hyperlipidemia, than with gastrointestinal symptoms or disease manifestations. *Int J Mol Sci*. 2017;18(3).
38. Salles Teixeira TF, Boroni Moreira AP, Silva Souza NC, Frias R, Gouveia Peluzio Mdo C. Intestinal permeability measurements: general aspects and possible pitfalls. *Nutricion Hospitalaria*. 2014;29(2):269–281.
39. Odenwald MA, Turner JR. The intestinal epithelial barrier: a therapeutic target? *Nat Rev Gastroenterol Hepatol*. 2017;14(1):9–21.
40. Barreau F, Hugot JP. Intestinal barrier dysfunction triggered by invasive bacteria. *Curr Opin Microbiol*. 2014;17:91–98.
41. Yu YB, Li YQ. Enteric glial cells and their role in the intestinal epithelial barrier. *World J Gastroenterol*. 2014;20(32):11273–11280.
42. Ahmad R, Sorrell MF, Batra SK, Dhawan P, Singh AB. Gut permeability and mucosal inflammation: bad, good or context dependent. *Mucosal Immunol*. 2017;10(2):307–317.
43. Liang GH, Weber CR. Molecular aspects of tight junction barrier function. *Curr Opin Pharmacol*. 2014;19:84–89.
44. Krug SM, Schulzke JD, Fromm M. Tight junction, selective permeability, and related diseases. *Semin Cell Dev Biol*. 2014;36:166–176.
45. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci*. 2013;70(4):631–659.
46. Lu Z, Ding L, Lu Q, Chen YH. Claudins in intestines: distribution and functional significance in health and diseases. *Tissue Barriers*. 2013;1(3):e24978.
47. Camilleri M, Madsen K, Spiller R, Greenwood-Van Meerveld B, Verne GN. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol Motil*. 2012;24(6):503–512.
48. Quigley EM. Leaky gut—concept or clinical entity? *Curr Opin Gastroenterol*. 2016;32(2):74–79.
49. Fasano A. Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications. *Clin Gastroenterol Hepatol*. 2012;10(10):1096–1100.

50. Campbell AW. The gut, intestinal permeability, and autoimmunity. *Altern Ther Health Med*. 2015;21(1):6–7.
51. Vanuytsel T, Vermeire S, Cleynen I. The role of haptoglobin and its related protein, zonulin, in inflammatory bowel disease. *Tissue Barriers*. 2013;1(5):e27321.
52. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? *Clin Gastroenterol Hepatol*. 2013;11(9):1075–1083.
53. Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr*. 2011;141(5):769–776.
54. Kopp ZA, Jain U, Van Limbergen J, Stadnyk AW. Do antimicrobial peptides and complement collaborate in the intestinal mucosa? *Front Immunol*. 2015;6:17.
55. Alexopoulou A, Agiasotelli D, Vasilieva LE, Dourakis SP. Bacterial translocation markers in liver cirrhosis. *Ann Gastroenterol*. 2017;30(5):486–497.
56. Farre R, Vicario M. Abnormal barrier function in gastrointestinal disorders. *Handb Exp Pharmacol*. 2017;239:193–217.
57. Mishra A, Makharia GK. Techniques of functional and motility test: how to perform and interpret intestinal permeability. *J Neurogastroenterol Motil*. 2012;18(4):443–447.
58. Turner JR, Buschmann MM, Romero-Calvo I, Sailer A, Shen L. The role of molecular remodeling in differential regulation of tight junction permeability. *Semin Cell Dev Biol*. 2014;36:204–212.
59. Wang L, Llorente C, Hartmann P, Yang AM, Chen P, Schnabl B. Methods to determine intestinal permeability and bacterial translocation during liver disease. *J Immunol Methods*. 2015;421:44–53.
60. Johnston SD, Smye M, Watson RG, McMillan SA, Trimble ER, Love AH. Lactulose-mannitol intestinal permeability test: a useful screening test for adult coeliac disease. *Ann Clin Biochem*. 2000;37(Pt 4):512–519.
61. Addobbati R, Pascolo L, Di Toro N, Sebastiani GB, Martellossi S, Not T. Influence of urine volume on the assessment of intestinal permeability in affected children by multiple sugar probes. *Clin Chem Lab Med*. 2014;52(2):227–235.
62. Shaikh M, Rajan K, Forsyth CB, Voigt RM, Keshavarzian A. Simultaneous gas-chromatographic urinary measurement of sugar probes to assess intestinal permeability: use of time course analysis to optimize its use to assess regional gut permeability. *Clin Chim Acta; Int J Clin Chem*. 2015;442:24–32.
63. van Wijck K, Verlinden TJ, van Eijk HM, et al. Novel multi-sugar assay for site-specific gastrointestinal permeability analysis: a randomized controlled crossover trial. *Clin Nutr*. 2013;32(2):245–251.
64. Bjarnason I. Intestinal permeability. *Gut*. 1994;35(suppl 1):S18–S22.
65. Vojdani A, Vojdani E, Kharrazian D. Fluctuation of zonulin levels in blood vs stability of antibodies. *World J Gastroenterol*. 2017;23(31):5669–5679.
66. Schwiertz A, Spiegel J, Dillmann U, et al. Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. *Parkinsonism Relat Disord*. 2018.
67. Zhang D, Zhang L, Zheng Y, Yue F, Russell RD, Zeng Y. Circulating zonulin levels in newly diagnosed Chinese type 2 diabetes patients. *Diabetes Res Clin Pract*. 2014;106(2):312–318.
68. Zhang D, Zhang L, Yue F, Zheng Y, Russell R. Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation. *Eur J Endocrinol/Eur Fed Endocrine Soc*. 2015;172(1):29–36.
69. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci*. 2000;113(Pt 24):4435–4440.
70. Hendy OM, Elsabaawy MM, Aref MM, Khalaf FM, Oda AMA, El Shazly HM. Evaluation of circulating zonulin as a potential marker in the pathogenesis of nonalcoholic fatty liver disease. *APMIS*. 2017;125(7):607–613.
71. Pacifico L, Bonci E, Marandola L, Romaggioli S, Bascetta S, Chiesa C. Increased circulating zonulin in children with biopsy-proven nonalcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(45):17107–17114.
72. Mokkala K, Tertti K, Ronnemaa T, Vahlberg T, Laitinen K. Evaluation of serum zonulin for use as an early predictor for gestational diabetes. *Nutr Diabetes*. 2017;7(3):e253.
73. Zak-Golab A, Kocelak P, Aptekorz M, et al. Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. *Int J Endocrinol*. 2013;2013:674106.
74. Kume T, Acar S, Tuhan H, et al. The relationship between serum zonulin level and clinical and laboratory parameters of childhood obesity. *J Clin Res Pediatr Endocrinol*. 2017;9(1):31–38.
75. Qi Y, Goel R, Kim S, et al. Intestinal permeability biomarker zonulin is elevated in healthy aging. *J Am Med Dir Assoc*. 2017;18(9):810.e811–810, e814.
76. Klaus DA, Motal MC, Burger-Klepp U, et al. Increased plasma zonulin in patients with sepsis. *Biochemia Medica*. 2013;23(1):107–111.
77. Sheen YH, Jee HM, Kim DH, et al. Serum zonulin is associated with presence and severity of atopic dermatitis in children, independent of total IgE and eosinophil. *Clin Exp Allergy*. 2018.
78. Duerksen DR, Wilhelm-Boyles C, Veitch R, Kryszak D, Parry DM. A comparison of antibody testing, permeability testing, and zonulin levels with small-bowel biopsy in celiac disease patients on a gluten-free diet. *Dig Dis Sci*. 2010;55(4):1026–1031.
79. Malickova K, Francova I, Lukas M, et al. Fecal zonulin is elevated in Crohn's disease and in cigarette smokers. *Pract Lab Med*. 2017;9:39–44.
80. Esnafoglu E, Cirrik S, Ayyildiz SN, et al. Increased serum zonulin levels as an intestinal permeability marker in autistic subjects. *J Pediatr*. 2017;188:240–244.
81. Fasano A, Hill I. Serum zonulin, gut permeability, and the pathogenesis of autism spectrum disorders: cause, effect, or an epiphenomenon? *J Pediatr*. 2017;188:15–17.
82. Ohlsson B, Roth B, Larsson E, Hoglund P. Calprotectin in serum and zonulin in serum and feces are elevated after introduction of a diet with lower carbohydrate content and higher fiber, fat and protein contents. *Biomed Rep*. 2017;6(4):411–422.
83. Yao Z, Mates JM, Cheplowitz AM, et al. Blood-borne lipopolysaccharide is rapidly eliminated by liver sinusoidal endothelial cells via high-density lipoprotein. *J Immunol*. 2016;197(6):2390–2399.
84. Buret AG, Mitchell K, Muench DG, Scott KG. Giardia lamblia disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitol*. 2002;125(Pt 1):11–19.
85. Cereijido M, Contreras RG, Flores-Benitez D, et al. New diseases derived or associated with the tight junction. *Arch Med Res*. 2007;38(5):465–478.
86. Dagci H, Ustun S, Taner MS, Ersoz G, Karacasu F, Budak S. Protozoon infections and intestinal permeability. *Acta Tropica*. 2002;81(1):1–5.
87. Brown BI. Nutritional management of metabolic endotoxemia: a clinical review. *Altern Ther Health Med*. 2017;23(4):42–54.
88. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: is it real and is it relevant? *Biochimie*. 2016;124:11–20.
89. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev*. 2011;91(1):151–175.
90. Laitinen K, Isolauri E. Management of food allergy: vitamins, fatty acids or probiotics? *Eur J Gastroenterol Hepatol*. 2005;17(12):1305–1311.
91. Yu LC. The epithelial gatekeeper against food allergy. *Pediatr Neonatol*. 2009;50(6):247–254.
92. Heyman M. Gut barrier dysfunction in food allergy. *Eur J Gastroenterol Hepatol*. 2005;17(12):1279–1285.
93. Fasano A. Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. *Am J Pathol*. 2008;173(5):1243–1252.
94. Aabakken L. Small-bowel side-effects of non-steroidal anti-inflammatory drugs. *Eur J Gastroenterol Hepatol*. 1999;11(4):383–388.
95. Bjarnason I, Takeuchi K. Intestinal permeability in the pathogenesis of NSAID-induced enteropathy. *J Gastroenterol*. 2009;44(suppl 19):23–29.
96. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology*. 2012;142(5):1100–1101.e1102.
97. Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol*. 2010;298(6):G807–819.

98. Campos Ruiz A, Urtasun Arlegui L, Marra-Lopez Valenciano C. Sprue-like enteropathy linked to olmesartan. *Rev Esp Enferm Dig*. 2016;108(5):292–293.
99. Ianiro G, Bibbo S, Montalto M, Ricci R, Gasbarrini A, Cammarota G. Systematic review: Sprue-like enteropathy associated with olmesartan. *Aliment Pharmacol Ther*. 2014;40(1):16–23.
100. Rubio-Tapia A, Herman ML, Ludvigsson JF, et al. Severe spruelike enteropathy associated with olmesartan. *Mayo Clin Proc*. 2012;87(8):732–738.
101. Hamada K, Shitara Y, Sekine S, Horie T. Zonula occludens-1 alterations and enhanced intestinal permeability in methotrexate-treated rats. *Cancer Chemother Pharmacol*. 2010;66(6):1031–1038.
102. Requile M, Gonzalez-Alvarez DO, Delanaud S, et al. Use of a combination of in vitro models to investigate the impact of chlorpyrifos and inulin on the intestinal microbiota and the permeability of the intestinal mucosa. *Environ Sci Pollut Res Int*. 2018;25(23):22529–22540.
103. Akobeng AK, Miller V, Stanton J, Elbadri AM, Thomas AG. Double-blind randomized controlled trial of glutamine-enriched polymeric diet in the treatment of active Crohn's disease. *Aliment Pharmacol Ther*. 2000;30(1):78–84.
104. Clark EC, Patel SD, Chadwick PR, Warhurst G, Curry A, Carlson GL. Glutamine deprivation facilitates tumour necrosis factor induced bacterial translocation in Caco-2 cells by depletion of enterocyte fuel substrate. *Gut*. 2003;52(2):224–230.
105. Tang ZF, Ling YB, Lin N, Hao Z, Xu RY. Glutamine and recombinant human growth hormone protect intestinal barrier function following portal hypertension surgery. *World J Gastroenterol*. 2007;13(15):2223–2228.
106. Li Y, Ping X, Yu B, Liu F, Ni X, Li J. Clinical trial: prophylactic intravenous alanyl-glutamine reduces the severity of gastrointestinal toxicity induced by chemotherapy—a randomized crossover study. *Aliment Pharmacol Ther*. 2009;30(5):452–458.
107. Li Y, Yu Z, Liu F, Tan L, Wu B, Li J. Oral glutamine ameliorates chemotherapy-induced changes of intestinal permeability and does not interfere with the antitumor effect of chemotherapy in patients with breast cancer: a prospective randomized trial. *Tumori*. 2006;92(5):396–401.
108. Beutheu S, Ghouzali I, Galas L, Dechelotte P, Coeffier M. Glutamine and arginine improve permeability and tight junction protein expression in methotrexate-treated Caco-2 cells. *Clin Nutr (Edinburgh, Scotland)*. 2013;32(5):863–869.
109. Den Hond E, Hiele M, Peeters M, Ghoos Y, Rutgeerts P. Effect of long-term oral glutamine supplements on small intestinal permeability in patients with Crohn's disease. *JPEN*. 1999;23(1):7–11.
110. Akobeng AK, Miller V, Thomas AG, Richmond K. Glutamine supplementation and intestinal permeability in Crohn's disease. *JPEN*. 2000;24(3):196.
111. Rachmilewitz D, Stamler JS, Bachwich D, Karmeli F, Ackerman Z, Podolsky DK. Enhanced colonic nitric oxide generation and nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Gut*. 1995;36(5):718–723.
112. Basra S, Verne GN, Zhou QQ. Randomized placebo-controlled trial of glutamine for the treatment of diarrhea-predominant IBS. *Gastroenterology*. 2013;144(5):S160–S160.
113. Conejero R, Bonet A, Grau T, et al. Effect of a glutamine-enriched enteral diet on intestinal permeability and infectious morbidity at 28 days in critically ill patients with systemic inflammatory response syndrome: a randomized, single-blind, prospective, multicenter study. *Nutr*. 2002;18(9):716–721.
114. Quan ZF, Yang C, Li N, Li JS. Effect of glutamine on change in early postoperative intestinal permeability and its relation to systemic inflammatory response. *World J Gastroenterol*. 2004;10(13):1992–1994.
115. Jian ZM, Cao JD, Zhu XG, et al. The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: a randomized, double-blind, controlled study of 120 patients. *JPEN*. 1999;23(suppl 5):S62–S66.
116. Yoshida S, Matsui M, Shirouzu Y, Fujita H, Yamana H, Shirouzu K. Effects of glutamine supplements and radiochemotherapy on systemic immune and gut barrier function in patients with advanced esophageal cancer. *Ann Surg*. 1998;227(4):485–491.
117. Playford RJ, MacDonald CE, Calnan DP, et al. Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. *Clin Sci (Lond)*. 2001;100(6):627–633.
118. Choi HS, Jung KH, Lee SC, et al. Bovine colostrum prevents bacterial translocation in an intestinal ischemia/reperfusion-injured rat model. *J Med Food*. 2009;12(1):37–46.
119. Playford RJ, Floyd DN, Macdonald CE, et al. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. *Gut*. 1999;44(5):653–658.
120. Playford RJ, Macdonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *Am J Clin Nutr*. 2000;72(1):5–14.
121. Troost FJ, Saris WH, Brummer RJ. Recombinant human lactoferrin ingestion attenuates indomethacin-induced enteropathy in vivo in healthy volunteers. *Eur J Clin Nutr*. 2003;57(12):1579–1585.
122. Sturniolo GC, Di Leo V, Ferronato A, D'Odorico A, D'Inca R. Zinc supplementation tightens "leaky gut" in Crohn's disease. *Inflamm Bowel Dis*. 2001;7(2):94–98.
123. Assa A, Vong L, Pinnell LJ, Avitzur N, Johnson-Henry KC, Sherman PM. Vitamin D deficiency promotes epithelial barrier dysfunction and intestinal inflammation. *J Infect Dis*. 2014;210(8):1296–1305.
124. Osanai M, Nishikiori N, Murata M, Chiba H, Kojima T, Sawada N. Cellular retinoic acid bioavailability determines epithelial integrity: role of retinoic acid receptor alpha agonists in colitis. *Mol Pharmacol*. 2007;71(1):250–258.
125. Kong J, Zhang Z, Musch MW, et al. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol*. 2008;294(1):G208–G216.
126. Beguin P, Errachid A, Larondelle Y, Schneider YJ. Effect of polyunsaturated fatty acids on tight junctions in a model of the human intestinal epithelium under normal and inflammatory conditions. *Am J Physiol*. 2013;4(6):923–931.
127. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr*. 2009;139(9):1619–1625.
128. Venkatraman A, Ramakrishna BS, Pulimood AB, Patra S, Murthy S. Increased permeability in dextran sulphate colitis in rats: time course of development and effect of butyrate. *Scand J Gastroenterol*. 2000;35(10):1053–1059.
129. Khaleghi S, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: focus on larazotide acetate. *Therap Adv Gastroenterol*. 2016;9(1):37–49.
130. Kelly CP, Green PH, Murray JA, et al. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study. *Aliment Pharmacol Ther*. 2013;37(2):252–262.

131. Leffler DA, Kelly CP, Abdallah HZ, et al. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. *Am J Gastroenterol*. 2012;107(10):1554–1562.
132. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. *Aliment Pharmacol Ther*. 2007;26(5):757–766.
133. Andre F, Andre C, Feknous M, Colin L, Cavagna S. Digestive permeability to different-sized molecules and to sodium cromoglycate in food allergy. *Allergy Proc*. 1991;12(5):293–298.
134. Falth-Magnusson K, Kjellman NI, Magnusson KE, Sundqvist T. Intestinal permeability in healthy and allergic children before and after sodium-cromoglycate treatment assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000). *Clin Allergy*. 1984;14(3):277–286.
135. Lobo B, Pigrau M, Martinez C, et al. Clinical benefit and intestinal mucosal transcriptome modulation after long-term mast cell stabilization with oral disodium cromoglycate in diarrhea-predominant irritable bowel syndrome (IBS-D) patients. *Gastroenterology*. 2015;148. S494–S494.
136. Friesen CA, Sandridge L, Andre L, Roberts CC, Abdel-Rahman SM. Mucosal eosinophilia and response to H1/H2 antagonist and cromolyn therapy in pediatric dyspepsia. *Clin Pediatr*. 2006;45(2):143–147.
137. Wild GE, Waschke KA, Bitton A, Thomson AB. The mechanisms of prednisone inhibition of inflammation in Crohn's disease involve changes in intestinal permeability, mucosal TNFalpha production and nuclear factor kappa B expression. *Aliment Pharmacol Ther*. 2003;18(3):309–317.
138. Miki K, Moore DJ, Butler RN, Southcott E, Couper RT, Davidson GP. The sugar permeability test reflects disease activity in children and adolescents with inflammatory bowel disease. *J Pediatr*. 1998;133(6):750–754.
139. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)*. 1998;94(6):557–572.
140. Baumgart DC, Vierziger K, Sturm A, Wiedenmann B, Dignass AU. Mesalamine promotes intestinal epithelial wound healing in vitro through a TGF-beta-independent mechanism. *Scand J Gastroenterol*. 2005;40(8):958–964.
141. Di Paolo MC, Merrett MN, Crotty B, Jewell DP. 5-Aminosalicylic acid inhibits the impaired epithelial barrier function induced by gamma interferon. *Gut*. 1996;38(1):115–119.
142. Suenaert P, Bulteel V, Lemmens L, et al. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn's disease. *Am J Gastroenterol*. 2002;97(8):2000–2004.
143. Copeman M, Matuz J, Leonard AJ, Pearson JP, Dettmar PW, Allen A. The gastroduodenal mucus barrier and its role in protection against luminal pepsins: the effect of 16,16 dimethyl prostaglandin E2, carbopol-polyacrylate, sucralfate and bismuth subsalicylate. *J Gastroenterol Hepatol*. 1994;9(suppl 1):S55–S59.
144. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol*. 1997;99(2):179–185.
145. Rosenfeldt V, Benfeldt E, Valerius NH, Paerregaard A, Michaelsen KF. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr*. 2004;145(5):612–616.
146. Liu ZH, Huang MJ, Zhang XW, et al. The effects of perioperative probiotic treatment on serum zonulin concentration and subsequent postoperative infectious complications after colorectal cancer surgery: a double-center and double-blind randomized clinical trial. *Am J Clin Nutr*. 2013;97(1):117–126.
147. Liu Z, Li C, Huang M, et al. Positive regulatory effects of perioperative probiotic treatment on postoperative liver complications after colorectal liver metastases surgery: a double-center and double-blind randomized clinical trial. *BMC Gastroenterol*. 2015;15:34.
148. Garcia Vilela E, De Lourdes De Abreu Ferrari M, Oswaldo Da Gama Torres H, et al. Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Crohn's disease in remission. *Scand J Gastroenterol*. 2008;43(7):842–848.
149. Sindhu KN, Sowmyanarayanan TV, Paul A, et al. Immune response and intestinal permeability in children with acute gastroenteritis treated with *Lactobacillus rhamnosus* GG: a randomized, double-blind, placebo-controlled trial. *Clin Infect Dis*. 2014;58(8):1107–1115.
150. Zeng J, Li YQ, Zuo XL, Zhen YB, Yang J, Liu CH. Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther*. 2008;28(8):994–1002.
151. Francavilla R, Miniello V, Magista AM, et al. A randomized controlled trial of Lactobacillus GG in children with functional abdominal pain. *Pediatr*. 2010;126(6):e1445–e1452.
152. Gotteland M, Cruchet S, Verbeke S. Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indomethacin in humans. *Aliment Pharmacol Ther*. 2001;15(1):11–17.
153. Stenman LK, Lehtinen MJ, Meland N, et al. Probiotic with or without fiber controls body fat mass, associated with serum zonulin, in overweight and obese adults—randomized controlled trial. *EBioMed*. 2016;13:190–200.
154. Stratiki Z, Costalos C, Sevastiadou S, et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. *Early Hum Dev*. 2007;83(9):575–579.
155. Persborn M, Gerritsen J, Wallon C, Carlsson A, Akkermans LM, Soderholm JD. The effects of probiotics on barrier function and mucosal pouch microbiota during maintenance treatment for severe pouchitis in patients with ulcerative colitis. *Aliment Pharmacol Ther*. 2013;38(7):772–783.

20

Laboratory Tests for the Determination of Vitamin Status

Joseph E. Pizzorno, ND

OUTLINE

INTRODUCTION

Laboratory assessment of vitamin status is a significant challenge for the clinician. Although measurement of blood and serum levels is easily available, their clinical value is limited to the detection of severe deficiencies. Detection of functional deficiencies requires far more sophistication. A number of diverse methods have been used by researchers and clinicians to try to answer the question, "Does this patient need more of a specific vitamin?" A number of strategies are available to the clinician, three of which will be mentioned here.

The traditional method is to measure levels in specific tissues such as blood, serum, red cells, and white cells. The advantage of this strategy is that establishing population standards is straightforward. However, a key disadvantage of this strategy is that it is absolute (i.e., it is a measure of level, but not function). As can be seen in Fig. 20.1, although a person may have adequate blood levels of a nutrition, indications of inadequacy such as homocysteine will often indicate a functional deficiency even though the blood levels are supposedly adequate.

As our understanding of the genomics of nutrition has evolved, we have now added another more nuanced question: "Does this patient need the activated version of a specific vitamin?" Several chapters on nutrition in this textbook present research showing that a surprisingly large portion of the population does not convert the dietary version of one or more nutrients into one or more of its activated forms. For example, see Chapter 125 Vitamin A for a discussion of the research showing that a surprising 25% to 35% of the general population poorly converts beta-carotene to vitamin A.

A second strategy then is to measure molecules that may increase or decrease when inadequate functional levels of a nutrient is available. For example, inadequate activated folate or vitamin B_6 will result in elevated levels of homocysteine. Chapter 210, Porphyrias provides numerous examples of changes in urinary excretion of multiple molecules that indicate nutritional inadequacy.

A third strategy is to take some of the patient's blood and then use some measure of cellular function and determine the amount of each nutrient needed to optimize function. This is an intriguing idea in need of substantial clinical validation.

ASSESSMENT OF WATER-SOLUBLE VITAMIN STATUS

See Table 20.1 for laboratory tests and optimal ranges for common vitamins.[1–5]

Ascorbic Acid (Vitamin C)

Assessment of vitamin C is particularly difficult because ascorbate readily oxidizes in assay samples. In addition, serum levels reflect recent dietary uptake rather than actual tissue levels. Research in an animal model of vitamin C deficiency (the *Gulo* mouse) clearly demonstrated that a dietary intake that does not lead to serum saturation of vitamin C results in tissue deficits.[6] Serum saturation of vitamin C was required to achieve tissue concentrations similar to wild-type animals, which can synthesize ascorbate. In humans, maximum serum saturation from oral dosing was predicted to be roughly one sixtieth of that achieved with intravenous administration, highlighting the inability of serum levels to predict optimal physiological function.[7,8] Leukocyte levels are not as susceptible to dietary intake but are also readily affected by infection, hypoglycemia, and many common prescription and over-the-counter drugs. Additionally, the uptake of ascorbate into leukocytes significantly underestimates the relative uptake of ascorbate into muscle tissue (which contains roughly two thirds of total body ascorbate), indicating that muscle may be a more labile pool for vitamin C, and more prone to deficiency.[9] The popular lingual ascorbate test does not appear to be reliable because it does not correlate well with leukocyte or serum levels.[10] The loading test, if carefully controlled, is probably most accurate, although good standard ranges have yet to

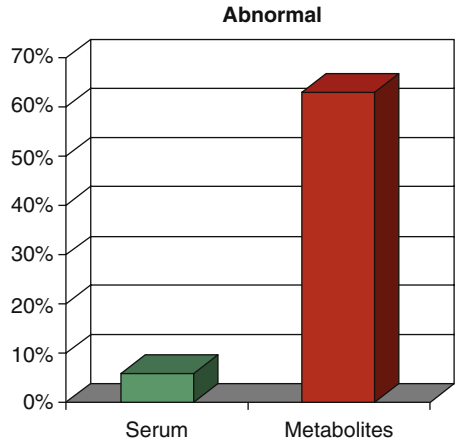


Fig. 20.1 Predicting deficiency by blood level versus metabolite level. (Modified from Joosten E, van den Berg A, Riezler R, et al. Metabolic evidence that deficiencies of vitamin-B12 [cobalamin], folate, and B-6 occur commonly in elderly people. *Am J Clin Nutr.* 1992;58[4]:468–476.)

be determined. Discovery of ascorbate-dependent enzymes involved in cell signaling pathways and epigenetic modulation offer the possibility for more functional analysis in the future, although unfortunately no definitive analysis is currently available.[11] Lastly, polymorphisms in vitamin C transport genes may also be relevant when assessing status; for example, variants in vitamin C transport genes have been associated with serum vitamin C concentrations and modify the strength of the correlation between dietary vitamin C and serum ascorbic acid.[12] Some of these polymorphisms have also been linked to a variety of disease risks, including coronary heart disease, inflammatory bowel disease (IBD), and cancer.[13,14]

Biotin

Biotin is a vitamin B complex especially affected by oral antibiotics. Food is a poor source of this vitamin, making humans more dependent on gut flora sources. Elevation of 3-hydroxyisovalerate (3-HIA) resulting from deficiency of the biotin-dependent enzyme appears a useful measure. Additionally, two studies in which healthy volunteers were intentionally made biotin deficient suggested that elevated urinary levels of 3-HIA-carnitine might be a sensitive indicator, even for a marginal deficiency. This offers greater accuracy and is less prone to laboratory error than the traditional urinary 3-HIA.[15,16] Plasma levels of 3-HIA-carnitine may also prove to be a sensitive marker and reduce the dependency on renal function for an accurate determination, an important consideration during pregnancy.[17]

Folate

Serum folate is too greatly affected by recent consumption to be clinically useful; however, elevated serum folate levels (>45.3 nmol/L) in the presence of a vitamin B_{12} deficiency may reflect a functional folate deficiency known as a "methyl trap" (i.e., a build-up of 5-MTHF that cannot be converted to tetrahydrofolate, because methionine synthesis is impaired).[18] Homocysteine levels may be elevated because of deficiency of vitamins B_6 and/or B_{12} and folate. Erythrocyte folate is more accurate and considered a more reliable indicator of tissue status; it is not influenced by recent dietary intake and represents a 4-month average. Evaluation of other B vitamin status, particularly B_{12}, may be necessary to rule out a folate deficiency. Although the presence of neutrophil hypersegmentation has been used to identify folate deficiency, this also occurs with B_{12} and iron deficiency, making it a very nonspecific marker.[19] Recent advances in mass spectrometry allow for measurement of folate species, including 5-MTHF, THF,

TABLE 20.1 Laboratory Tests and Optimal Ranges for Common Vitamins

Nutrient	Test	Acceptable Level
Water-Soluble		
Ascorbic acid	Serum	>0.3 mg/dL
	Leukocyte	30 mcg/10^8 WBCs
	Load test	0.3–2.0 mg/h in control
		24–49 mg/h after 500 mg
Biotin	3-hydroxyisovalerate	<20 mcg/mg creatinine (overnight urine)
Folate	Erythrocyte folate	>160–650 ng/mL (~350 nmol/L) Note: cut-off recommended for pregnant women is >906 nmol/L
	Serum homocysteine	<10 μmol/L
Niacin	Urinary *N*-methylnicotinamide	>1.6 mg/g creatinine
	2-pyridone 5-carboxamide (2-PYR)	>1.6 mg/g creatinine
	RBC NAD/NADP	>1.3
Pantothenic acid	Urinary pantothenic acid	>1 mg/day
Pyridoxine	Serum level	5.0–50.0 ug/L
	Plasma pyridoxal 5-phosphate	>30 nmol/L
	Urinary 4-PA	>3.0 mol/d
	Serum homocysteine	<9.0 μmol/L
Riboflavin	EGRAC	<1.3
Thiamine	RBC transketolase	<15% increase
	Whole blood thiamine (HPLC)	>16 ng/mL
Vitamin B_{12}	Serum B_{12}	>150 pg/mL
	Urinary methylmalonic acid	<5 mcg/mg creatinine
	Serum methylmalonic acid	<0.45 μmol/L
	Serum homocysteine	<9.0 μmol/L
	Holotranscobalamin	>30 pmol/L
Fat-Soluble		
Vitamin A	Plasma retinol:	15–60 mcg/dL:
	0–5 mo	>20
	6 mo–17 y	>30
	Adult	>20
Vitamin D	25 (OH) vitamin D	40–80 ng/mL
Vitamin E	Plasma α-tocopherol	>16.2 μmol/L
	α-tocopherol:cholestrol	>5.2 μmol/L
Vitamin K	% serum uncarboxylated osteocalcin	<20 ? (optimal not yet determined)

Data from Tierney LM, McPhee SJ, Papdakis MA. *Current Medical Diagnosis and Treatment*. Stamford, CT: Appleton & Lange; Rubenstein E, Federman DD. *Scientific American Medicine*. New York: Scientific American; Werbach MR. *Textbook of Nutritional Medicine*. Tarzana, CA: Third Line Press; Brally J, Lord RS. *Laboratory Evaluations in Molecular Medicine*. Norcross, GA: Institute for Advances in Molecular Medicine; Homocysteine Lowering Trialists Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ*. 1998;316:894–898.
ALT, Alanine aminotransferase; *AST*, aspartate aminotransferase; *EGOT*, erythrocyte glutamic oxaloacetic transaminase; *EGPT*, erythrocyte glutamic pyruvic transaminase: *FAD*, flavin adenine dinucleotide; H_2O_2, hydrogen peroxide; *HPLC*, high-performance liquid chromatography; *NAD*, nicotinamide adenine dinucleotide; *NADP*, nicotinamide adenine dinucleotide phosphate; *PA*, pyridoxic acid; *RBC*, red blood cell; *WBC*, white blood cell.

5,10-methyleneTHF, methenylTHF/10-formylTHF, and formylTHF, though this test is not widely available.[20]

Niacin

Although measurement of nicotinic acid in the blood is not very reliable, measurement of its metabolites provides a clinically useful function assessment. Several metabolite tests are now available, including urinary levels of 2-pyridone 5-carboxymide and *n*-methylnicotinamide.[21,22]

Pantothenic Acid

Serum pantothenic acid does not correspond well with dietary intake, although erythrocyte levels are more closely correlated.[23] Measurement of urinary excretion of pantothenic acid appears reliable.

Pyridoxine

Several procedures are available for assessing vitamin B_6 status. Unfortunately, substantial agreement on the best methodology has not been established, because variations in phenotypes significantly alter the results of functional and loading tests. The active form of pyridoxine (pyridoxal 5-phosphate [P5P]) is involved in some 60 enzymes, so deficient activity of these enzymes can be measured as a functional assessment of pyridoxine. Plasma levels of P5P appear to be a better functional indicator than erythrocyte levels, at least in patients with rheumatoid arthritis (RA).[24] Plasma P5P levels show an inverse relationship with C-reactive protein, with low levels observed in a number of inflammatory conditions, including RA, IBD, stroke, and diabetes and, at least in RA and IBD, P5P levels are inversely related to disease severity. It has been suggested that this is caused by a tissue-specific mobilization of P5P to the site of inflammation.[25] Plasma levels below 30 nmol/L (considered borderline deficient) have independently been associated with an increased risk for coronary artery disease, with a particularly high risk when combined with high-sensitivity C-reactive protein.[26,27] High plasma P5P levels have also been associated with a 34% reduced risk for all cancers, according to a recent meta-analysis of nearly 2 million participants.[28] Although suitable for most circumstances, plasma P5P does not appear to be reliable during pregnancy or the acute phase of myocardial infarction, and alternatives should be used. Additionally, it can also be affected by albumin concentration, alkaline phosphatase activity, and alcohol consumption, and decreases after glucose or carbohydrate intake. Urinary 4-pyridoxic acid (PA) is a useful marker of recent intake only, whereas erythrocyte aminotransferase and erythrocyte alanine aminotransferase activation by pyridoxal phosphate may be a better indicator of long-term status.[29] Elevated homocysteine may also be a sign of deficiency, at least in some populations.[30] Plasma 4-PA has also been suggested as a marker, as it is less influenced by inflammation, though it is significantly influenced by renal function. Ratios of various metabolites, such as the PAr index (PA/[P5P+ plasma pyridoxal]) and the ratio of 3-hydroxykynurenine to xanthurenic acid appear to mitigate the effect of some of the many confounders for vitamin B6 status.[31]

Riboflavin

The most common measure of riboflavin is red blood cell glutathione reductase activity. The enzyme is stimulated in vitro by the addition of flavin adenine dinucleotide, and expressed as erythrocyte glutathione reductase activation coefficient (EGRAC). An EGRAC greater than 1.30 is typically used as a cut-off (with higher values indicative of suboptimal status), although one recent study suggested this threshold for deficiency might need to be raised. This same study also demonstrated that riboflavin supplementation caused an increase in hemoglobin status among women with the lowest riboflavin intakes, despite no change in iron intake or absorption.[32] EGRAC is not reliable in individuals with glucose-6-phosphate dehydrogenase deficiency. In this population, pyridoxamine phosphate oxidase may be more appropriate.[33] Blood riboflavin levels are not reliable because of technical difficulties in measurement. Lastly, homocysteine levels may be relevant for those with genetic variation in the methylenetetrahydrofolate reductase gene. Individuals with the TT genotype of the C677 T polymorphism may have increased riboflavin needs.[34]

Thiamine

The most common measure of thiamine is erythrocyte transketolase activity. The enzyme is stimulated in vitro by the addition of thiamine pyrophosphate. Elevation in activity greater than 15% indicates a functional deficiency. The test is not reliable in patients with diabetes mellitus, pernicious anemia, or a significant negative nitrogen balance. For example, growing evidence has indicated that a tissue deficiency of thiamine exists in patients with diabetes, and may increase the risk for vascular and neurological complications. Despite markedly reduced plasma levels of thiamine among individuals with type 1 and type 2 diabetes, erythrocyte transketolase activity remained normal, largely because of an upregulation of red blood cell thiamine transporter levels.[35,36] Advances in high-performance liquid chromatography technology now allow for direct measurement of either whole blood or erythrocyte thiamine, which may be a valuable alternative.[37,38] Both serum and plasma thiamine levels have poor sensitivity and specificity for a thiamine deficiency. Urinary thiamine excretion less than 40 mcg or less than 27 mcg/g creatinine also suggests a deficiency.[39]

Additionally, measurement of amino acids and their ketoacid analogs that are excreted in thiamine deficiency is being used. (See Brally and Lord[4] for a more complete discussion.)

Vitamin B_{12}

Serum levels of vitamin B_{12} are of some value, although they do not track cerebrospinal fluid levels very well, and only a small portion (20%–30%) of total cobalamin levels are bound to transcobalamin and taken up via receptor-mediated cellular uptake. Additionally, at typical cut-off levels, serum B_{12} may miss as many as 45% of B_{12} deficient patients if used in isolation.[40] Erythrocyte cell size is also not reliable, because neurological signs and symptoms can precede macrocytosis by 6 to 12 months.

Serum levels of holotranscobalamin may be the optimal first-line diagnostic procedure, at least among elderly patients. It has been suggested to be one of the earliest markers of negative B_{12} balance, and its diagnostic accuracy is not affected by renal insufficiency, a problem with total cobalamin levels and methylmalonic acid.[41,42] Using a cut-off of 30 and 60 pmol/L holotranscobalamin, a sensitivity of 65% and 90% and a specificity of 90% and 55%, respectively, were shown against a methylmalonic acid standard.[43]

Measurement of either urinary or serum methylmalonic acid is considered fairly sensitive and specific in those with healthy renal function, especially when expressed as a ratio with the urinary creatinine measurement.[44,45] Elevated homocysteine concentrations may indicate deficiency, but it is not specific to B_{12}.

ASSESSMENT OF FAT-SOLUBLE VITAMIN STATUS

Vitamin A

Although liver biopsy is the most accurate method of vitamin A assessment, other less invasive and less expensive methodologies are more appropriate. As with most other nutrients, serum levels of vitamin A fall significantly only after tissue reserves have been depleted, and are subject to numerous laboratory challenges as well as other factors (e.g.,

infection, protein status). Serum retinol binding protein is sometimes used as an alternative to serum retinol because it avoids many of these complications, although it is also susceptible to artificial decreases caused by inflammation or protein malnutrition.[46] Optimal cut-offs have not been clearly established for retinol binding protein, although levels greater than 0.825 μmol/L have been suggested for children and greater than 1.05 μmol/L for adults.[47,48] The dark adaptation test detects early deficiency of this nutrient, and provides a useful alternative (see Chapter 27). The retinol isotope dilution test, using deuterium- or (13) C-labeled retinol, has been cited as the most sensitive indirect marker of vitamin A status, useful for assessment of both deficiency and excess, but is not widely available.[49,50] Lastly, given the variation in the ability to convert β-carotene to retinol, the use of plasma carotene levels is probably not a useful marker of vitamin A status.[51]

Vitamin D

The widespread deficiency of vitamin D has gained a much greater degree of recognition, with most recommendations based on 25(OH) vitamin D levels. Most functional indicators, such as levels of parathyroid hormone, point to a level of 25(OH) vitamin D greater than or equal to 80 nmol/L as being sufficient, although some evidence has suggested higher levels might be optimal, particularly for cancer prevention.[52-54] Prostate cancer may be the outlier among cancers, as a recent meta-analysis of 21 studies found an increase in risk with increasing 25(OH) vitamin D levels.[55] However, a 2018 meta-analysis found that higher 25(OH) levels were associated with reduced mortality among prostate cancer patients, similarly to most other cancers.[56,57] Lastly, the measurement of free 25(OH) vitamin D may have its own clinical utility, such as vitamin D assessment during pregnancy, as it measures the functional activity and is less dependent on changes in vitamin D-binding protein levels. Only recently have reference ranges been suggested.[58,59]

Vitamin E

Despite dietary intake of γ-tocopherol being tenfold greater than α-tocopherol, the preference of the α-tocopherol transfer protein for α-tocopherol results in much higher serum levels, which is why the latter is often used as a biomarker. Emerging evidence suggests that despite the ease in measurement, and the widespread supplementation with α-tocopherol, mixed tocopherols (such as the γ form) are likely of greater physiological importance.[60,61] Unfortunately, optimal levels of other biomarkers have not yet been established, although one review reported a γ-tocopherol levels range between 2 and 5 μmol/L.[62] Somewhat concerning, data from nearly 6000 adults in the NHANES study found signs of more advanced cellular aging (measured by telomere length) in individuals with higher γ-tocopherol levels (α-tocopherol was not associated).[63] It is also important to adjust vitamin E levels to lipid levels, such as triglycerides or cholesterol, with the latter more commonly used.

Adipose and platelet levels have been suggested to be better biomarkers, as have cells with low-density lipoprotein receptors, such as mononuclear leukocytes and buccal mucosal cells.[64]

Vitamin K

In recent years the importance of vitamin K for physiological functions other than blood coagulation has been recognized, including functions related to both bone and vascular disease. For this reason, the traditional methods of assessment, such as prothrombin and clotting assays, may not be sufficient markers of vitamin K status, at least in regard to these other functions.[65] Better functional markers may be serum uncarboxylated osteocalcin (ucOC) levels, or perhaps a serum carboxylated OC to serum total OC ratio. One review suggested that a percent of ucOC greater than 20 is probably optimal, although genetic variations may modify this to some degree.[66-68] Another functional marker is the level of inactive matrix Gla-protein (MGP), referred to as dephospho-uncarboxylated MGP (dp-ucMGP), or sometimes as a ratio indicating the proportion of MGP that is carboxylated. This marker has been associated with arterial stiffness among diabetics and a risk factor for cardiovascular disease.[69,70] However, it is not clear that any of the carboxylated proteins reflect more than short-term vitamin K status. Additionally, although they may accurately assess the activity of menaquinones, it is not clear that they assess the different activity of phylloquinone.[71,72]

CONCLUSION

As described here, many procedures are now available for the assessment of absolute and functional vitamin status. Although research continues in this important area, the reader is advised to carefully study the discussion of urinary organic acids profiling (see Chapter 29). Using metabolic products excreted in the urine now allows the clinician far greater specificity in recognizing dysfunctional enzyme systems, whether they are a result of genetic deviations or nutritional deficiencies.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Tierney LM, McPhee SJ, Papdakis MA. *Current Medical Diagnosis and Treatment.* Stamford, CT: Appleton & Lange.
2. Rubenstein E, Federman DD. *Scientific American Medicine.* New York: Scientific American.
3. Werbach MR. *Textbook of Nutritional Medicine.* Tarzana, CA: Third Line Press.
4. Brally J, Lord RS. *Laboratory Evaluations in Molecular Medicine.* Norcross, GA: Institute for Advances in Molecular Medicine.
5. Homocysteine Lowering Trialists Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ*. 1998;316:894–898.
6. Vissers MC, Bozonet SM, Pearson JF, et al. Dietary ascorbate intake affects steady state tissue concentrations in vitamin C-deficient mice: tissue deficiency after suboptimal intake and superior bioavailability from a food source (kiwifruit). *Am J Clin Nutr*. 2011;93(2):292–301.
7. Padayatty SJ, Sun H, Wang Y. Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med*. 2004;140(7):533–537.
8. Cullen JJ. Ascorbate induces autophagy in pancreatic cancer. *Autophagy*. 2010;6(3):421–422.
9. Carr AC, Bozonet SM, Pullar JM, et al. Human skeletal muscle ascorbate is highly responsive to changes in vitamin C intake and plasma concentrations. *Am J Clin Nutr*. 2013;97(4):800–807. PMID: 23446899.
10. Leggott PJ, Robertson PB, Rothman DL, et al. Response of lingual ascorbic acid test and salivary ascorbate levels to changes in ascorbic acid intake. *J Dent Res*. 1986;65(2):131–134. PMID: 3455968.
11. Pagé EL, Chan DA, Giaccia AJ, et al. Hypoxia-inducible factor-1alpha stabilization in nonhypoxic conditions: role of oxidation and intracellular ascorbate depletion. *Mol Biol Cell*. 2008;19(1):86–94.
12. Cahill LE, El-Sohemy A. Vitamin C transporter gene polymorphisms, dietary vitamin C and serum ascorbic acid. *J Nutrigenet Nutrigenomics*. 2009;2(6):292–301. PMID: 20588054.
13. Shaghaghi MA, Kloss O, Eck P. Genetic variation in human vitamin C transporter genes in common complex diseases. *Adv Nutr*. 2016;7(2):287–298. PMID: 26980812.
14. Padayatty SJ, Levine M. Vitamin C: the known and the unknown and Goldilocks. *Oral Dis*. 2016;22(6):463–493. PMID: 26808119.
15. Horvath TD, Stratton SL, Bogusiewicz A, et al. Quantitative measurement of urinary excretion of 3-hydroxyisovaleryl carnitine by LC-MS/MS as an indicator of biotin status in humans. *Anal Chem*. 2010;82(22):9543–9548.
16. Stratton SL, Horvath TD, Bogusiewicz A, et al. Urinary excretion of 3-hydroxyisovaleryl carnitine is an early and sensitive indicator of marginal biotin deficiency in humans. *J Nutr*. 2011;141(3):353–358.
17. Stratton SL, Horvath TD, Bogusiewicz A, et al. Plasma concentration of 3-hydroxyisovaleryl carnitine is an early and sensitive indicator of marginal biotin deficiency in humans. *Am J Clin Nutr*. 2010;92(6):1399–1405.
18. Sobczyńska-Malefora A, Harrington DJ. Laboratory assessment of folate (vitamin B9) status. *J Clin Pathol*. 2018;71(11):949–956. PMID: 30228213.
19. Sipahi T, Tavil B, Unver Y. Neutrophil hypersegmentation in children with iron deficiency anemia. *Pediatr Hematol Oncol*. 2002;19(4):235–238.
20. Blackmore S, Pfeiffer CM, Lee A, et al. Isotope dilution-LC-MS/MS reference method assessment of serum folate assay accuracy and proficiency testing consensus mean. *Clin Chem*. 2011;57(7):986–994. PMID: 21561849.
21. Seal AJ, Creeke PI, Dibari F, et al. Low and deficient niacin status and pellagra are endemic in postwar Angola. *Am J Clin Nutr*. 2007;85(1):218–224.
22. Fu CS, Swendseid ME, Jacob RA, et al. Biochemical markers for assessment of niacin status in young men: levels of erythrocyte niacin coenzymes and plasma tryptophan. *J Nutr*. 1989;119(12):1949–1955.
23. Eissenstat BR, Wyse BW. Hansen RG. Pantothenic acid status of adolescents. *Am J Clin Nutr*. 1986;44(6):931–937.
24. Chiang EP, Bagley PJ, Roubenoff R, et al. Plasma pyridoxal 5'-phosphate concentration is correlated with functional vitamin B-6 indices in patients with rheumatoid arthritis and marginal vitamin B-6 status. *J Nutr*. 2003;133(4):1056–1059.
25. Ueland PM, Ulvik A, Rios-Avila L, et al. Direct and functional biomarkers of vitamin B6 status. *Annu Rev Nutr*. 2015;35:33–70. PMID: 25974692.
26. Lin PT, Cheng CH, Liaw YP, et al. Low pyridoxal 5'-phosphate is associated with increased risk of coronary artery disease. *Nutrition*. 2006;22(11–12):1146–1151.
27. Cheng CH, Lin PT, Liaw YP, et al. Plasma pyridoxal 5'-phosphate and high-sensitivity C-reactive protein are independently associated with an increased risk of coronary artery disease. *Nutrition*. 2008;24(3):239–244.
28. Mocellin S, Briarava M, Pilati P. Vitamin B6 and cancer risk: a Field Synopsis and meta-analysis. *J Natl Cancer Inst*. 2017;109(3):1–9. PMID: 28376200.
29. Hansen CM, Shultz TD, Kwak HK, et al. Assessment of vitamin B-6 status in young women consuming a controlled diet containing four levels of vitamin B-6 provides an estimated average requirement and recommended dietary allowance. *J Nutr*. 2001;131(6):1777–1786.
30. Woolf K, Manore MM. Elevated plasma homocysteine and low vitamin B-6 status in nonsupplementing older women with rheumatoid arthritis. *J Am Diet Assoc*. 2008;108(3):443–453; discussion 454.
31. Ueland PM, Ulvik A, Rios-Avila L, et al. Direct and functional biomarkers of vitamin B6 status. *Annu Rev Nutr*. 2015;35:33–70. PMID: 25974692.
32. Powers HJ, Hill MH, Mushtaq S, et al. Correcting a marginal riboflavin deficiency improves hematologic status in young women in the United Kingdom (RIBOFEM). *Am J Clin Nutr*. 2011;93(6):1274–1284.
33. Mushtaq S, Su H, Hill MH, et al. Erythrocyte pyridoxamine phosphate oxidase activity: a potential biomarker of riboflavin status? *Am J Clin Nutr*. 2009;90(5):1151–1159.
34. Hoey L, McNulty H, Strain JJ. Studies of biomarker responses to intervention with riboflavin: a systematic review. *Am J Clin Nutr*. 2009;89(6):1960S–1980S.
35. Rabbani N, Thornalley PJ. Emerging role of thiamine therapy for prevention and treatment of early stage diabetic nephropathy. *Diabetes Obes Metab*. 2011;13(7):577–583.
36. Thornalley PJ, Babaei-Jadidi R, Al Ali H, et al. High prevalence of low plasma thiamine concentration in diabetes linked to a marker of vascular disease. *Diabetologia*. 2007;50(10):2164–2170.
37. Lima LF, Leite HP, Taddei JA. Low blood thiamine concentrations in children upon admission to the intensive care unit: risk factors and prognostic significance. *Am J Clin Nutr*. 2011;93(1):57–61.
38. Talwar D, Davidson H, Cooney J, et al. Vitamin B(1) status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: comparison with erythrocyte transketolase activation assay. *Clin Chem*. 2000;46(5):704–710.
39. Frank LL. Thiamin in clinical practice. *JPEN J Parenter Enteral Nutr*. 2015;39(5):503–520. PMID: 25564426.
40. Harrington DJ. Laboratory assessment of vitamin B12 status. *J Clin Pathol*. 2017;70(2):168–173.
41. Valente E, Scott JM, Ueland PM, et al. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B12 status in the elderly. *Clin Chem*. 2011;57(6):856–863.
42. Bamonti F, Moscato GA, Novembrino C, et al. Determination of serum holotranscobalamin concentrations with the AxSYM active B(12) assay: cut-off point evaluation in the clinical laboratory. *Clin Chem Lab Med*. 2010;48(2):249–253.
43. Harrington DJ. Laboratory assessment of vitamin B12 status. *J Clin Pathol*. 2017;70(2):168–173.
44. Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. *Clin Chem*. 2007;53(5):963–970.
45. Vogiatzoglou A, Oulhaj A, Smith AD. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. *Clin Chem*. 2009;55(12):2198–2206.
46. de Pee S, Dary O. Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. *J Nutr*. 2002;132(suppl 9):2895S–2901S.
47. Gorstein JL, Dary O, Pongtorn, et al. Feasibility of using retinol-binding protein from capillary blood specimens to estimate serum retinol concentrations and the prevalence of vitamin A deficiency in low-resource settings. *Public Health Nutr*. 2008;11(5):513–520.
48. Baeten JM, Richardson BA, Bankson DD, et al. Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infec-

tion, protein malnutrition, and the acute phase response. *Am J Clin Nutr*. 2004;79(2):218–225.
49. Gannon BM, Tanumihardjo SA. Comparisons among equations used for retinol isotope dilution in the assessment of total body stores and total liver reserves. *J Nutr*. 2015;145(5):847–854. PMID: 25809683.
50. Valentine AR1, Davis CR, Tanumihardjo SA. Vitamin A isotope dilution predicts liver stores in line with long-term vitamin A intake above the current Recommended Dietary Allowance for young adult women. *Am J Clin Nutr*. 2013;98(5):1192–1199. PMID: 24047915.
51. Leung WC, Hessel S, Méplan C, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. *FASEB J*. 2009;23(4):1041–1053.
52. Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmun Rev*. 2010;9(11):709–715.
53. Binkley N, Ramamurthy R, Krueger D. Low vitamin D status: definition, prevalence, consequences, and correction. *Endocrinol Metab Clin North Am*. 2010;39(2):287–301.
54. Garland CF, French CB, Baggerly LL, et al. Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. *Anticancer Res*. 2011;31(2):607–611.
55. Xu Y, Shao X, Yao Y, et al. Positive association between circulating 25-hydroxyvitamin D levels and prostate cancer risk: new findings from an updated meta-analysis. *J Cancer Res Clin Oncol*. 2014;140(9):1465–1477.
56. Song ZY, Yao Q, Zhuo Z, et al. Circulating vitamin D level and mortality in prostate cancer patients: a dose-response meta-analysis. *Endocr Connect*. 2018;7(12):R294–R303. PMID: 30352424.
57. Mondul AM, Weinstein SJ, Layne TM, et al. Vitamin D and cancer risk and mortality: state of the science, gaps, and challenges. *Epidemiol Rev*. 2017;39(1):28–48. PMID: 28486651.
58. Tsuprykov O, Buse C, Skoblo R, et al. Reference intervals for measured and calculated free 25-hydroxyvitamin D in normal pregnancy. *J Steroid Biochem Mol Biol*. 2018;181:80–87. PMID: 29567112.
59. Tsuprykov O, Chen X, Hocher CF, et al. Why should we measure free 25(OH) vitamin D? *J Steroid Biochem Mol Biol*. 2018;180:87–104. PMID: 29217467.
60. Usoro OB, Mousa SA. Vitamin E forms in Alzheimer's disease: a review of controversial and clinical experiences. *Crit Rev Food Sci Nutr*. 2010;50(5):414–419.
61. Devaraj S, Leonard S, Traber MG, et al. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med*. 2008;44(6):1203–1208.
62. Traber MG. Vitamin E regulatory mechanisms. *Annu Rev Nutr*. 2007;27:347–362.
63. Tucker LA. Alpha- and Gamma-Tocopherol and telomere length in 5768 us men and women: a nhanes study. *Nutrients*. 2017;9(6). pii: E601. PMID: 28629117.
64. Morrissey PA, Sheehy PJ. Optimal nutrition: vitamin E. *Proc Nutr Soc*. 1999;58(2):459–468.
65. Cranenburg EC, Schurgers LJ, Vermeer C, et al. The coagulation vitamin that became omnipotent. *Thromb Haemost*. 2007;98(1):120–125.
66. Iwamoto J, Sato Y, Takeda T, et al. High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: a review of the literature. *Nutr Res*. 2009;29(4):221–228.
67. McKeown NM, Jacques PF, Gundberg CM. Dietary and nondietary determinants of vitamin K biochemical measures in men and women. *J Nutr*. 2002;132(6):1329–1334.
68. Nimptsch K, Nieters A, Hailer S, et al. The association between dietary vitamin K intake and serum undercarboxylated osteocalcin is modulated by vitamin K epoxide reductase genotype. *Br J Nutr*. 2009;101(12):1812–1820.
69. van den Heuvel EG, van Schoor NM, Lips P, et al. Circulating uncarboxylated matrix Gla protein, a marker of vitamin K status, as a risk factor of cardiovascular disease. *Maturitas*. 2014;77(2):137–141.
70. Sardana M, Vasim I, Varakantam S, et al. Inactive matrix Gla-protein and arterial stiffness in type 2 diabetes mellitus. *Am J Hypertens*. 2017;30(2):196–201. PMID: 27927630.
71. Fusaro M, Gallieni M, Rizzo MA, et al. Vitamin K plasma levels determination in human health. *Clin Chem Lab Med*. 2017;55(6):789–799.
72. Shea MK, Booth SL. Concepts and Controversies in Evaluating vitamin K status in population-based studies. *Nutrients*. 2016;8(1). pii: E8. PMID: 26729160.

21

Lactose Intolerance Testing

Brian Orr, BA, BS, ND, Tina Kaczor, ND, FABNO,* and *Jeff Baker*, ND, DHANP, CCH*

OUTLINE

INTRODUCTION

Lactose malabsorption results from the inability to properly digest lactose, the disaccharide found in mammalian milk. Lactase is an enzyme in the brush border (microvilli) of the small intestine responsible for cleaving lactose into absorbable monosaccharides. Lactase enzyme deficiency leads to lactose malabsorption because the gut is unable to absorb the larger disaccharide. When symptoms such as diarrhea, bloating, flatulence, or abdominal discomfort result from this malabsorption, a diagnosis of lactose intolerance is given. Lactose intolerance affects an estimated 25% to 36% of Americans and 67% to 75% of adults worldwide.[1–3] As shown in Table 21.1, the condition occurs in people of all ethnic backgrounds, with a near 100% prevalence observed in Asians. Within the United States, prevalence parallels country of origin, with the highest rates in African Americans, Hispanic Americans, Asian Americans, and Native Americans.[4]

TABLE 21.1 Prevalence of Lactose Intolerance by Ethnic Group

Group	Prevalence (%)
African blacks	97–100
Asians	90–100
North American blacks	70–75
Hispanics	70–80
Persons of Mediterranean descent	60–90
Persons of Jewish descent	60–80
North American whites	7–15
Northern Europeans	1–5

The generalized nature of abdominal symptoms of lactose intolerance can obscure proper diagnosis,[5] resulting in symptom management through medical intervention. However, when properly diagnosed, lactose intolerance may be treated with patient education, lactase supplementation, and dietary modifications,[6,7] eliminating the need for medical interventions. In patients experiencing symptoms, a nutritional history and objective measures of lactose malabsorption can lead to the diagnosis of lactose intolerance. Objective measures for lactose malabsorption include stool pH testing, lactose tolerance testing, and hydrogen/methane breath testing. In addition, genomic tests are also available to assess the presence or absence of polymorphisms linked to lactase enzyme deficiency.[3]

Controversy surrounds the subject of lactose intolerance stemming from the dramatic decrease in dairy consumption that can result from its diagnosis. Numerous studies showed a correlation between decreased dairy consumption and a rise in calcium-dependent conditions, such as osteoporosis, heart disease, and colon cancer.[8–13] Studies also indicated that food-based calcium was a better source of this nutrient than supplements. Because dairy foods are the richest and best-absorbed dietary sources of calcium, a careful diagnosis should be made before these foods are eliminated from the patient's diet. Dietary limitation of calcium-rich foods should be done only in the context of objectively proven lactose intolerance. There is significant variability in the severity of symptoms,[14] and many patients with lactose malabsorption are able to consume moderate amounts of lactose without symptoms,[15] so both the diagnosis and treatment should be pursued under professional guidance.[16]

PATHOPHYSIOLOGY

Lactose is a disaccharide that can be hydrolyzed by the lactase enzyme lactase-phlorizin hydrolase (LPH) into glucose and galactose, which are actively absorbed in the small intestine (Fig. 21.1). When LPH activity is decreased in the brush border (microvilli) of the distal duodenum and proximal jejunum, then lactose malabsorption results. The presence of lactose malabsorption does not always result in lactose intolerance. Only when the amount of lactose ingested exceeds

*Previous edition contributor

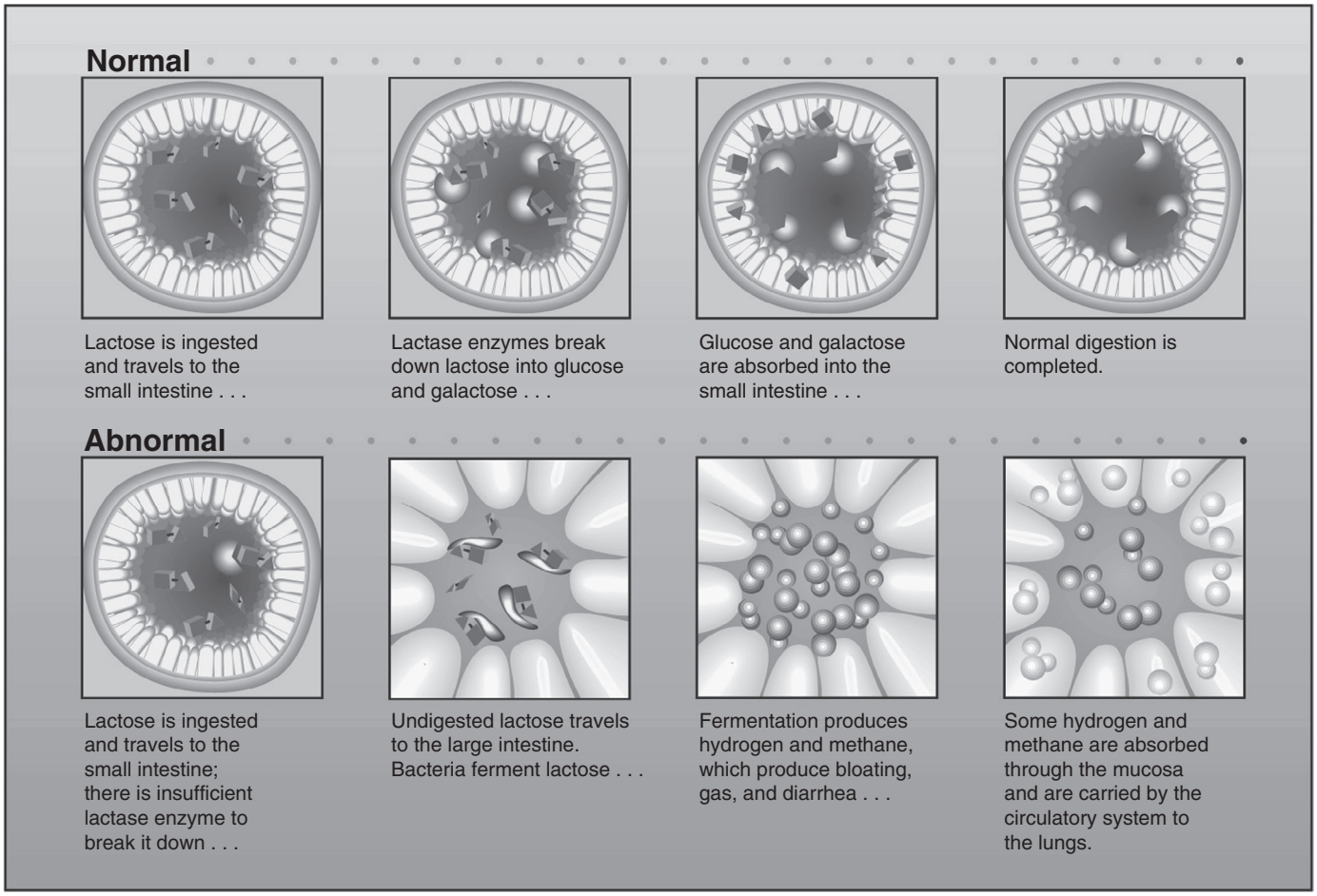


Fig. 21.1 Lactose digestion.

digestive capacity does lactose persist in the lumen, ultimately reaching the colon, where it may lead to osmotic retention of fluid and bacterial fermentation, thus causing the symptoms characteristic of lactose intolerance (see Fig. 21.1).[17] Therefore symptoms of lactose intolerance often require several contributing factors in addition to LPH deficiency, including (1) relatively high lactose load, (2) gastric emptying rate,[18,19] (3) rapid small bowel transit, (4) heightened visceral sensitivity,[20] (5) colonic water absorption capacity,[19] and/or (6) altered bowel flora.[21]

ADULT-TYPE (PRIMARY) LACTOSE DEFICIENCY

The majority of the world's population has a normal decrease in LPH synthesis after weaning. Two genes appear responsible for the control of LPH synthesis. These include the *LCT* gene that encodes the lactase enzyme itself and the *MCM6* gene that encodes two regulatory regions that activate the promoter that transcribes the *LCT* gene.[22] The most common genotype of the *MCM6* gene confers the phenotype of primary lactase deficiency (lactase nonpersistence) by downregulating *LCT* promotion upon weaning.[22,23] Genetically, nucleotides in the *MCM6* gene appear to be primarily responsible for this developmentally programmed loss of transcription of the *LCT* gene.[22,23] However, single-nucleotide polymorphisms (SNPs) in the *MCM6* gene can confer the phenotype of lactase persistence by extending the transcription of the *LCT* gene into adulthood.[22,24,25]

It is theorized that maintaining the ability to digest lactose into adulthood (lactase persistence) is the result of a relatively new evolutionary genetic mutation that occurred after the domestication of farm animals.[2,26] Preservation of lactase synthesis has been associated with several distinct SNPs on the *MCM6* gene that include the following: *–13910C>T, –13915T>G, –14010G>C, –13937G>A, –13907G>C,* and *–13913T>C.* Respective to these SNPs, the variant alleles associated with lactase persistence are *T, T, C, G, G,* and *T.*[3,22,25,27–30] Although the presence of any these SNPs suggests lactase persistence, their absence does not equate to lactase nonpersistence because there appear to be other SNPs that have not yet been determined.[31]

ACQUIRED (SECONDARY) LACTASE DEFICIENCY

Because LPH is located in the brush border (microvilli) of gut mucosal cells, LPH deficiency may be secondary to diseases that damage these cells. Lactose intolerance has been observed as a secondary feature in celiac disease,[32] tropical sprue, acute gastroenteritis, chemotherapy-induced mucositis,[33] cystic fibrosis, alcoholism,[34] pelvic radiation therapy,[35] and Crohn's disease.[36] In secondary lactase deficiency, treating the underlying condition and resultant restoration of mucosal integrity often restores lactase activity.[37]

CONGENITAL LACTASE DEFICIENCY

Congenital lactase deficiency is a rare inborn error of metabolism characterized by very low or absent lactase activity in the intestinal microvilli at birth. Unlike lactase nonpersistence mutations, which affect upstream enhancer regions of *LCT,* mutations in the *LCT* gene itself appear to be responsible for congenital lactase deficiency.[38] Clinical symptoms include severe diarrhea, dehydration, and malnutrition and

BOX 21.1 Symptoms of Lactose Intolerance

Indigestion
Bloating
Flatulence
Nausea
Diarrhea
Failure to thrive
Abdominal cramps

often appear during the first week with the consumption of lactose. Separately, preterm infants may exhibit symptoms of lactase deficiency if born before 34 weeks' gestation, a condition called developmental lactase deficiency.[39] As expected, preterm infants can gain function of the lactase enzyme with time.

LACTOSE INTOLERANCE VERSUS DAIRY ALLERGY

Lactose intolerance and dairy allergy are separate pathologies. Lactose intolerance results from the maldigestion of dairy carbohydrate (lactose), whereas dairy allergy is an immune response to dairy proteins (e.g., casein, lactalbumin, whey). Dairy allergy may involve reactions (e.g., systemic anaphylaxis) that affect the gastrointestinal tract, skin, respiratory tract, or multiple systems. These immediate reactions are often mediated by immunoglobulin-E (IgE) and can cause severe morbidity and even death; however, in such cases, dietary elimination of dairy products is associated with good prognosis.[40] The prevalence of IgE-mediated allergic reaction to dairy protein in the general population is estimated at 1% to 3%, being highest in infants and lowest in adults. However, the prevalence of IgG-mediated allergic reactions may be higher.

DIAGNOSIS OF LACTOSE INTOLERANCE

Clinical suspicion of lactose intolerance should be raised when foods containing milk or milk products produce symptoms of gas, bloating, cramping, or diarrhea (Box 21.1). A short course of dietary manipulation, with careful removal of milk-containing products, can strengthen the case for lactose intolerance as a diagnosis. Stool acidity testing, oral lactose tolerance testing, breath tests for hydrogen and methane, and/or genomic evaluation for *LCT* haplotypes should be used to confirm the diagnosis. Because the presence of lactase deficiency does not always result in lactose intolerance, genomic testing should never be used as a sole determinant in diagnosis. Breath testing provides the most reliable, noninvasive means of determining lactose malabsorption but must be used in the context of symptomology to make the diagnosis of lactose intolerance. In contrast, a positive breath test coupled with the absence of *LCT* haplotyping indicating primary lactase deficiency suggests a secondary causation, the diagnosis of which should be pursued.

There have been conflicting results regarding the presence of lactose intolerance in patients with irritable bowel syndrome (IBS). By definition, the diagnosis of IBS is a functional diagnosis that is made when all organic causes of symptoms have been ruled out. However, studies have shown that patients with IBS have a high rate of lactose intolerance, with the resolution of IBS symptoms upon lactose limitation or removal.[41–43] Rana et al.[44] found that patients with diarrhea-predominant IBS had a higher incidence of lactose intolerance (82%) than patients who had either spastic-type IBS or features of both IBS types. For this reason, lactose intolerance should always be investigated in cases of IBS, a condition that affects up to 20% of Americans.[45]

BOX 21.2 Sources of Lactose

Obvious Sources
Milk (whole, skim, dry powdered, evaporated)
Cheeses
Butter, many margarines
Goat's milk
Half-and-half cream
Ice cream and many sherbets
Yogurt

Hidden Sources
Artificial sweeteners containing lactose
Breads, biscuits and crackers, doughnuts made with milk
Breading on fried foods
Breakfast and baby cereals containing milk solids
Buttered or creamed foods (soups and vegetables)
Cake and pudding mixes, many frostings
Candies with milk chocolate
Cookies made with milk
Hot dogs, luncheon meats, sausage, hash, processed and canned meats
Mayonnaise and salad dressings made with milk
Nondairy creamers (except for Coffee Rich)

A number of studies have suggested that transient lactose intolerance is also associated with infantile colic.[46,47] For susceptible children with lactose maldigestion, only 12 g of lactose (~1 cup of milk) daily has been shown to be associated with increased abdominal pain.[48]

Nutritional History

A detailed history of the patient's average consumption of lactose-containing food should be obtained. Often, patients do not consider yogurt, ice cream, chocolate milk, and milk ingested with cereal as important sources of lactose. They also may not be aware that lactose is added to many nondairy products to provide texture, flavor, and browning and to absorb flavors, aromas, and food colors (Box 21.2). In addition, because of its excellent binding ability, lactose is contained in many drugs and over-the-counter products (Box 21.3). Identifying all sources of lactose is necessary to (1) identify the potential relationship between gastrointestinal symptoms and lactose consumption and (2) develop an effective lactose-free diet, if necessary.

Empirical Testing (Trial Elimination of Milk Products From the Diet)

If the patient experiences symptoms after consuming food products containing lactose, the temporary exclusion of all lactose-containing products from the diet as a preliminary diagnostic procedure may be helpful. However, the diagnosis of lactose intolerance should not be based solely on the elimination of milk products. This subjective test may be misleading if hidden sources of lactose (see Box 21.3) are not removed or if unrelated symptoms coincidently abate during this period.[49]

Stool Testing

The fermentation of undigested disaccharides of any kind within the colon results in the overproduction of fatty acids by colonic bacteria, which lowers the pH of the stool. Normal stool pH is between 6.0 and 7.0 in young children and adults. Although infants may normally have a lower pH due to a high-lactose diet, a pH of less than 5.3 suggests maldigestion. Although pH testing can be used to raise the clinical suspicion of malabsorption, further workup is warranted to make a diagnosis of lactose intolerance.

BOX 21.3 Prescription and Over-the-Counter Drugs Containing Lactose

Prescription Drugs

Ativan
Bumex
Calan
Coumadin tablets
Erythromycin
Glucotrol
Lasix
Lotronex
Mevacor
Premarin
Prilosec
Propecia
Reglan
Synthroid
Vasotec
Xanax

Over-the-Counter Drugs

Actifed tablets
Allbee C-800 Plus iron tablets
Benadryl tablets
Chlor-Trimeton Allergy tablets
Ferro-Sequels
Imodium A-D caplets
Marezine tablets
Pepcid AC chewable
Slow FE iron tablets
Sudafed Plus tablets
Unifed Chewable tablets

Breath Testing

Breath testing is the method of choice for diagnosing lactose maldigestion.[50] It is sensitive and specific,[51] simple to perform, noninvasive, and inexpensive.[49] Breath testing is based on the ability of intestinal microbes to ferment carbohydrates, in this case lactose, producing hydrogen or methane in the process. A fraction of these gases naturally diffuses from the bowel to the circulation and is eliminated via the lungs. Because there is no other metabolic production of hydrogen and methane, pulmonary excretion of these gases may be used as an indirect measure of lactose maldigestion, indicating lactase deficiency.[52]

The benefits of breath testing are as follows:

- Its results correlate strongly with the symptoms of lactose intolerance.[53]
- It can be done at home by the patient or in the physician's office.[54]
- The lower challenge dose of lactose causes significantly fewer side effects than the large doses used in blood and urine galactose tests.[55]
- The breath hydrogen/methane test is the standard in pediatric cases in which other tests would be difficult to perform.[56–58]

Historically, breath testing measured hydrogen only. However, Tormo et al.[59] showed that methane is produced instead of hydrogen in some patients with lactose malabsorption. They concluded that measuring both gases was necessary for accurate diagnosis of lactose maldigestion. Other researchers suggested that although methane was produced predominantly in some cases, hydrogen production correlated more strongly with symptoms; therefore hydrogen testing alone might be sufficient for the diagnosis of lactose intolerance.[60,61] Breath testing using a radiolabeled carbon molecule (^{13}C) in the lactose structure showed poor correlation with lactose intolerance, as evidenced by poor $^{13}CO_2$ output by the lungs.[53]

Procedure

After an overnight fast, a baseline breath sample is collected 30 minutes after rising. The patient then ingests a challenge dose of lactose (up to a maximum of 25 grams), and breath samples are collected 1, 2, and 3 hours after ingestion of the challenge dose.[62,63]

Interpretation

If lactose maldigestion is present, breath levels of hydrogen or methane will rise within 1 to 2 hours after ingesting the lactose challenge. As little as 2 g of carbohydrate reaching the colon produces a detectable increase in breath hydrogen.[64]

Hydrogen and Methane Responses

The normal breath hydrogen level in a healthy, fasting patient is less than 10 ppm. Patients with lactose malabsorption show an increase in breath hydrogen concentration of 20 ppm or more during the test.[65,66]

The normal breath methane level in a fasting patient is 0 to 7 ppm. An increase of at least 12 ppm of methane alone during the test is considered positive for lactose malabsorption, regardless of the hydrogen response.[67–69]

If both breath hydrogen and methane rise after a lactose challenge, the two responses are added to estimate the degree of malabsorption. The increases in breath hydrogen and methane levels together must be 20 ppm or more to suggest lactose malabsorption.[69] The extent of elevation relates to the degree of malabsorption.

False-Positive Results

False-positive results occur rarely and are usually a consequence of the following interfering factors:

- *Fiber intake.* Fiber should be avoided 24 hours before the test. Ingesting fiber in food or in supplements increases fermentation and hydrogen production.[70–72]
- *Exposure to tobacco smoke.* Tobacco smoke increases hydrogen levels and should be avoided immediately before and during testing.[73]
- *Sleeping.* Sleeping between breath sample collections may increase both hydrogen and methane levels.[74]

False-Negative Results

Breath hydrogen/methane testing has a false-negative rate of approximately 5%; the false-negative rate is 10% if only hydrogen is measured. False-negative results can occur because of the following factors:

- Use of lactase supplements.[55]
- Use of antibiotics before the test. Antibiotics decrease the bacteria that ferment lactose.[75]
- Use of laxatives or enemas before the test. These decrease hydrogen and methane responses in patients with lactose malabsorption and reduce fermentation in the colon.[76]
- Severe diarrhea or hyperacidic colon contents. Hyperacidity inhibits the production of hydrogen and promotes the production of methane by colonic bacteria.[77,78]

High Baseline Levels

An elevated baseline level of breath hydrogen indicates that one or more interfering factors are present. Testing must be repeated to obtain reliable results. A baseline breath hydrogen level of greater than 10 ppm can be due to the following:

- Improper fasting
- Consumption of high-fiber foods the day before testing
- Performance of test immediately after awakening[67]

A baseline breath hydrogen level of more than 20 ppm can be due to the following:

- Possible small intestine bacterial overgrowth.[79] Elevated fasting levels of hydrogen occur in up to one third of patients with small intestinal bacterial overgrowth[80] and may be caused by the fermentation of endogenous brush-border glycoproteins.[81]
- Small intestine bacterial overgrowth may elevate baseline methane readings as well.[67,79,81,82]

Genomic Testing

Genomic testing requires a single blood sample and avoids the diet restrictions, lengthy collection regimen, and potential for abdominal symptoms that are associated with using an oral lactose challenge. The *LCT* gene on chromosome 2 encodes for the enzyme LPH, and the wild type is associated with lactase nonpersistence. Lactase persistence is associated with two polymorphisms on the enhancer region upstream from *LCT,* C/T_{13910} and G/A_{22018}. Heterozygotes are considered to have lactase persistence but with intermediate lactase activity. Homozygotes T/T_{13910} and G/G_{22018} have the lactase persistence genotype, whereas C/C and G/G homozygotes carry the wild-type nonpersistence genotype. Genomic testing, although useful, only establishes a lactase enzyme deficiency, the presence of which does not always lead to lactose intolerance symptoms. Heterozygotes, although considered carriers of lactase persistence genotyping, can have bouts of impaired lactase activity due to stress or infection, rendering intermittent bouts of lactose intolerance.[83] Therefore testing for lactose malabsorption through an objective measure of lactose malabsorption, such as hydrogen breath testing, is still warranted.[84]

Tissue Sampling

An endoscopic biopsy of the jejunum can assess the presence of lactase as well as other disaccharidases, although this test is rarely used due to the expense and discomfort involved. Morphologically, enterocytes would not show abnormality in cases of primary lactase deficiency but may show blunting of microvilli or inflammatory changes associated with secondary causes of lactose intolerance.

Blood Testing

Lactose tolerance testing (LTT) was formerly used but has been replaced by more sensitive breath testing, as previously described. LTT required a large dose of glucose challenge, up to 50 g, which often led to abdominal symptoms that are avoided with the lower doses used in breath testing. If the rise in serum glucose after challenge was less than 26 mg/dL during the ensuing 2 hours, then lactose maldigestion was presumed. Another similar test uses radiolabeled lactose,[18] C-lactose. Although it has been shown to have the ability to differentiate between those who can digest lactose and those with maldigestion, this method has not been evaluated for its ability to identify symptoms directly related to lactose intolerance,[79] and it is not available clinically.

OTHER TYPES OF SUGAR INTOLERANCE

The inability to properly digest other types of saccharides can produce symptoms similar to those caused by lactose intolerance. For this reason, it may be necessary to investigate other types of sugar intolerance, especially in patients whose clinical test results do not support a diagnosis of lactose intolerance. Commonly ingested sugars, such as fructose, sucrose, and maltose, should be considered if sugar intolerance is suspected in the presence of normal breath and genetic testing. (See Chapter 9, Bacterial Overgrowth of the Small Intestine Breath Test, for a more in-depth discussion of carbohydrate intolerance testing.)

SUMMARY

The majority of the world's population is lactase deficient. However, lactose intolerance may not develop in all of these individuals. A comprehensive evaluation that incorporates diagnostic testing, the patient's nutritional history, and the relationship between diet and gastrointestinal symptoms is necessary for an accurate diagnosis. Proper diagnosis of lactose intolerance allows for dietary modifications that may allow a limited amount of dairy products in some lactose-intolerant individuals. Exclusion of lactose-containing foods altogether should be done only for those requiring strict avoidance, with careful attention to the replacement of lost nutrients, such as calcium. Breath testing of hydrogen/methane production after a lactose challenge is the diagnostic method of choice.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Srinivasan R, Minocha A. When to suspect lactose intolerance: symptomatic, ethnic, and laboratory clues. *Postgrad Med*. 1998;104:109–111, 115–116, 122–123.
2. Storhaug CL, Fosse SK, Fadnes LT. Country, regional, and global estimates for lactose malabsorption in adults: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2017;10:738–746.
3. Mattar R, Ferraz de Campos Mazo D, Carrilho FJ. Lactose intolerance: diagnosis, genetic, and clinical factors. *Clin Exp Gastroenterol*. 2012;5:113–121.
4. National Digestive Diseases Information Clearinghouse. A service of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). *Lactose Intolerance.* Last updated Feb 24. https://www.niddk.nih.gov/health-information/digestive-diseases/lactose-intolerance/definition-facts. Accessed 11/28/2010.
5. Vernia P, Di Camillo M, Marinaro V. Lactose malabsorption, irritable bowel syndrome and self-reported milk intolerance. *Dig Liver Dis*. 2001;33:234–239.
6. Rusynyk RA, Still CD. Lactose intolerance. *J Am Osteopath Assoc*. 2001;101:S10–S12.
7. Felicilda-Reynaldo RF, Kenneally M. Digestive enzyme replacement therapy: pancreatic enzymes and lactase. *Medsurg Nurs*. 2016;25:182–185.
8. Park YK, Yetley EA, Calvo MS. *Calcium intake levels in the United States*: issues and considerations. http://www.fao.org/DOCREP/W7336T/w7336t06.htm. Accessed 8/30/2011.
9. Lipkin M, Newmark H. Calcium and the prevention of colon cancer. *J Cell Biochem*. 1995;22(suppl):65–73.
10. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. dash collaborative research group. *N Engl J Med*. 1997;336:1117–1124.
11. Griffith LE, Guyatt GH, Cook RJ, et al. The influence of dietary and nondietary calcium supplementation on blood pressure: an updated metaanalysis of randomized controlled trials. *Am J Hypertens*. 1999;12:84–92.
12. Lupton JR. Dairy products and colon cancer: mechanisms of the protective effect. *Am J Clin Nutr*. 1997;66:1065–1066.
13. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium polyp prevention study group. *N Engl J Med*. 1999;340:101–107.
14. Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice—myths and realities. *Aliment Pharmacol Ther*. 2008;27:93–103.
15. Shaukat A, Levitt MD, Taylor BC, et al. Systematic review: effective management strategies for lactose intolerance. *Ann Intern Med*. 2010;152:797–803.
16. Aurisicchio LN, Pitchumoni CS. Lactose intolerance: recognizing the link between diet and discomfort. *Postgrad Med*. 1994;95:113–116, 119–120.
17. Berkow R, Fletcher AJ, eds. *The Merck Manual*. Rahway, NJ: Merck; 1992:822–830.
18. Yamada T, Alpers DH. *Textbook of Gastroenterology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2003.
19. Suchy FJ, Brannon PM, Carpenter TO, et al. National Institutes of Health consensus development conference: lactose intolerance and health. *Ann Intern Med*. 2010;152:792–796.
20. Di Stefano M, Miceli E, Mazzocchi S, et al. Visceral hypersensitivity and intolerance symptoms in lactose malabsorption. *Neurogastroenterol Motil*. 2007;19:887–895.
21. Almeida JA, Kim R, Stoita A, et al. Lactose malabsorption in the elderly: role of small intestinal bacterial overgrowth. *Scand J Gastroenterol*. 2008;43:146–154.
22. Enattah NS, Sahi T, Savilahti E, et al. Identification of a variant associated with adult-type hypolactasia. *Nat Genet*. 2002;30:233–237.
23. Ingram C, Mulcare C, Itan Y, et al. Lactose digestion and the evolutionary genetics of lactase persistence. *Human Genetics*. 2009;124:579–591.
24. Rasinperä H, Savilahti E, Enattah NS, et al. A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut*. 2004;53:1571–1576.
25. Troelsen JT. Adult-type hypolactasia and regulation of lactase expression. *Biochim Biophys Acta*. 2005;1723:19–32.
26. Enattah NS, Sahi T, Savilahti E, et al. Identification of a variant associated with adult-type hypolactasia. *Nat Genet*. 2002;30:233–237.
27. Tishkoff SA, Reed FA, Ranciaro A, et al. Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet*. 2007;39:31–40.
28. Torniaien S, Parker MI, Homberg V, et al. Screening of variants for lactase persistence/non-persistence in populations from South Africa and Ghana. *BMC Genet*. 2009;10:1471–2156.
29. Piepoli A, Schirru E, Mastrorilli A, et al. Genotyping of the lactase-phlorizin hydrolase c/t-13910 polymorphisms by means of a new rapid denaturing high-performance liquid chromatography-based assay in healthy subjects and colorectal cancer patients. *J Biomol Screen*. 2007;12:733–739.
30. Ingram CJ, Elamin MF, Mulcare CA, et al. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Hum Genet*. 2007;120:779–788.
31. Ingram C, Raga T, Tarekegn A, et al. Multiple rare variants as a cause of a common phenotype: several different lactase persistence associated alleles in a single ethnic group. *J Molecular Evolution*. 2009;69:579–588.
32. Ojetti V, Nucera Ga, Migneco A, et al. High prevalence of celiac disease in patients with lactose intolerance. *Digestion*. 2005;71:106–110.
33. Osterlund P, Ruotsalainen T, Peuhkuri K, et al. Lactose intolerance associated with adjuvant 5-fluorouracil-based chemotherapy for colorectal cancer. *Clin Gastroenterol Hepatol*. 2004;2:696–703.
34. Keshavarzian A, Iber FL, Dangleis MD, Cornish R. Intestinal-transit and lactose intolerance in chronic alcoholics. *Am J Clin Nutr*. 1986;44:70–76.
35. Henriksson R, Franzen L, Sandstrom K, et al. Effects of active addition of bacterial cultures in fermented milk to patients with chronic bowel discomfort following irradiation. *Support Care Cancer*. 1995;3:81–83.
36. von Tirpitz C, Kohn C, Steinkamp M, et al. Lactose intolerance in active Crohn's disease: clinical value of duodenal lactase analysis. *J Clin Gastroenterol*. 2002;34:49–53.
37. Kanabar D, Randhawa M, Clayton P. Improvement of symptoms in infant colic following reduction of lactose load with lactase. *J Hum Nutr Diet*. 2001;14:359–363.
38. Torniainen S, Freddara R, Routi T, et al. Four novel mutations in the lactase gene (LCT) underlying congenital lactase deficiency (CLD). *BMC Gastroenterol*. 2009;9:8.
39. Heyman MB. Nutrition. Lactose intolerance in infants, children, and adolescents. *Pediatrics*. 2006;118:1279–1286.
40. Bahna SL. Cow's milk allergy versus cow milk intolerance. *Ann Allergy Asthma Immunol*. 2002;89:56–60.
41. Bernardessilva C, Pereira A, Defatimaalvesdamota G, et al. Lactase persistence/non-persistence variants, C/T_13910 and G/A_22018, as a diagnostic tool for lactose intolerance in IBS patients. *Clin Chimica Acta*. 2007;386:7–11.
42. Gremse DA, Nguyenduc GH, et al. Irritable bowel syndrome and lactose maldigestion in recurrent abdominal pain in childhood. *South Med J*. 1999;92:778–781.
43. Vernia P, Ricciardi MR, Frandina C, et al. Lactose malabsorption and irritable bowel syndrome: effect of a long-term lactose-free diet. *Ital J Gastroenterol*. 1995;27:117–121.
44. Rana SV, Mandal AK, Kochhar R, et al. Lactose intolerance in different types of irritable bowel syndrome in north Indians. *Trop Gastroenterol*. 2001;22:202–204.
45. Zaman A. Irritable bowel syndrome. *Clin Cornerstone*. 2002;4:22–33.
46. Moore DJ, Robb TA, Davidson GP. Breath hydrogen response to milk containing lactose in colicky and noncolicky infants. *J Pediatr*. 1988;113:979–984.
47. Gremse DA, Greer AS, Vacik J. Abdominal pain associated with lactose ingestion in children with lactose intolerance. *Clin Pediatr*. 2003;42:341–345.
48. Murphy MS, Sood M, Johnson T. Use of the lactose H_2 breath test to monitor mucosal healing in coeliac disease. *Acta Paediatr*. 2002;91:141–144.
49. Montes RG, Perman JA. Lactose intolerance. Pinpointing the source of nonspecific gastrointestinal symptoms. *Postgrad Med*. 1991;89:175–178, 181–184.
50. Moore BJ. Dairy foods: are they politically correct? *Nutr Today*. 2003;38:82–90.
51. Newcomer AD, McGill DB, Thomas PJ. Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med*. 1975;293:1232–1236.

52. Brummer RJ, Armbrecht U, Bosaeus I, et al. The hydrogen (H_2) breath test. Sampling methods and the influence of dietary fibre on fasting level. *Scand J Gastroenterol*. 1985;20:1007–1013.
53. Zhong Y, Yin W, Huang C. Study on the expired gas of subjects with lactose intolerance by using H_2/$13CO_2$ breath test [in Chinese]. *Wei Sheng Yan Jiu*. 2002;31:180–183.
54. Metz G, Jenkins DJ, Peters TJ, et al. Breath hydrogen as a diagnostic method for hypolactasia. *Lancet*. 1975;1:155–157.
55. Lerch MM, Rieband HC, Feldberg W. Concordance of indirect methods for the detection of lactose malabsorption in diabetic and nondiabetic subjects. *Digestion*. 1991;48:81–88.
56. Barillas-Mury C, Solomons NW. Test-retest reproducibility of hydrogen breath test for lactose maldigestion in preschool children. *J Pediatr Gastroenterol Nutr*. 1987;6:281–285.
57. Barillas-Mury C, Solomons NW. Variance in fasting breath hydrogen concentrations in Guatemalan preschool children. *J Pediatr Gastroenterol Nutr*. 1987;6:109–113.
58. Solomons NW, Barillas C. The cut-off criterion for a positive hydrogen breath test in children: a reappraisal. *J Pediatr Gastroenterol Nutr*. 1986;5:920–925.
59. Tormo R, Bertaccini A, Conde M, et al. Methane and hydrogen exhalation in normal children and in lactose malabsorption. *Early Hum Dev*. 2001;65(suppl):S165–S172.
60. Vernia P, Camillo MD, Marinaro V. Effect of predominant methanogenic flora on the outcome of lactose breath test in irritable bowel syndrome patients. *Eur J Clin Nutr*. 2003;57:1116–1119.
61. Montes RG, Saavedra JM, Perman JA. Relationship between methane production and breath hydrogen excretion in lactose-malabsorbing individuals. *Dig Dis Sci*. 1993;38:445–448.
62. Bond JH, Levitt MD. Quantitative measurement of lactose absorption. *Gastroenterology*. 1976;70:1058–1062.
63. Robb TA, Davidson GP. Two-hour lactose breath hydrogen test. *J Pediatr Gastroenterol Nutr*. 1987;6:481–482.
64. Levitt MD. Production and excretion of hydrogen gas in man. *N Engl J Med*. 1969;281:122–127.
65. Newcomer AD. Screening tests for carbohydrate malabsorption. *J Pediatr Gastroenterol Nutr*. 1984;3:6–8.
66. Jain NK, Patel VP, Pitchumoni CS. Efficacy of activated charcoal (AC) in reducing intestinal gas: a double blind clinical trial. *Am J Gastroenterol*. 1986;81:532–535.
67. Hamilton LH. *Breath Tests and Gastroenterology*. Milwaukee: QuinTron Instruments; 1998.
68. Cloarec D, Bornet F, Gouilloud S, et al. Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. *Gut*. 1990;31:300–304.
69. Fritz M, Siebert G, Kasper H. Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture. *Palatini Br J Nutr*. 1985;54:389–400.
70. Behall KM, Scholfield DJ, van der Sluijs AM. Breath hydrogen and methane expiration in men and women after oat extract consumption. *J Nutr*. 1998;128:79–84.
71. Behall KM, Howe JC. Breath-hydrogen production and amylose content of the diet. *Am J Clin Nutr*. 1997;65:1783–1789.
72. Kondo T, Nakae Y. Breath hydrogen and methane excretion produced by commercial beverages containing dietary fiber. *J Gastroenterol*. 1996;31:654–658.
73. Rosenthal A, Solomons NW. Time-course of cigarette smoke contamination of clinical hydrogen breath-analysis tests. *Clin Chem*. 1983;29:1980–1981.
74. Solomons N. Evaluation of carbohydrate absorption: the hydrogen breath test in clinical practice. *Clin Nutr J*. 1984;3:71–78.
75. Gilat T, Ben Hur H, Gelman-Malachi E, et al. Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut*. 1978;19:602–605.
76. Solomons NW, Garcia R, Schneider R, et al. H_2 breath tests during diarrhea. *Acta Paediatr Scand*. 1979;68:171–172.
77. Vogelsang H, Ferenci P, Frotz S, et al. Acidic colonic microclimate—possible reason for false negative hydrogen breath tests. *Gut*. 1988;29:21–26.
78. Perman JA, Modler S, Olson AC. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora: studies in vivo and in vitro. *J Clin Invest*. 1981;67:643–650.
79. Kerlin P, Wong L. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology*. 1988;95:982–988.
80. Romagnuolo J, Schiller D, Bailey RJ. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. *Am J Gastroenterol*. 2002;97:1113–1126.
81. Perman JA, Modler S. Glycoproteins as substrates for production of hydrogen and methane by colonic bacterial flora. *Gastroenterology*. 1982;83:388–393.
82. Kerlin P, Wong L. Lactose tolerance despite hypolactasia in adult celiac disease. *J Gastroenterol Hepatol*. 1987;2:233–237.
83. Matthews SB, Waud JP, Roberts AG. Systemic lactose intolerance: a new perspective on an old problem. *Postgraduate Med J*. 2005;81:167–173.
84. Waud JP, Matthews SB, Campbell AK. Measurement of breath hydrogen and methane, together with lactase genotype, defines the current best practice for investigation of lactose sensitivity. *Ann Clin Biochem*. 2008;45:50–58.

22

Metal Toxicity: Assessment of Exposure and Retention

David Quig, PhD

OUTLINE

INTRODUCTION

It is imperative that clinicians understand and apply in practice the distinction between metal toxicity, as clearly defined by standards of care, and the *toxic effects of metals* that may be associated with the net retention of toxic elements; it is not just a matter of semantics. The incidence of high-level exposure to toxic metals and acute poisoning/toxicity is rare and is most commonly associated with occupational sources. Associated symptoms are well defined and accepted. A plethora of published research has clearly defined many of the specific biochemical mechanisms by which even much lower levels of metals elicit a vast array of adverse effects that can culminate in neurotoxicity, nephrotoxicity, cardiovascular and pulmonary disease, cancer, teratogenicity, and dysregulation of immune function. Despite knowledge of the adverse effects of metals on the most basic biochemical processes that may affect human health, the fact that environmental exposure with cumulative retention of "subthreshold" levels of toxic elements may warrant clinical intervention is not generally accepted by the dominant medical community. However, with respect to lead, mercury, and cadmium, a consultant to the National Institute of Environmental Health Sciences stated that the margin between the levels of exposure for people in industrialized nations and the levels of exposure that are currently recognized as producing the lowest adverse effect levels is small.[1,2]

The established laboratory tests accepted for a diagnosis of metal toxicity (e.g., blood lead) do not provide an estimate of the actual levels of metals that have accumulated in the body. The purpose of this chapter is to provide an overview of the various laboratory tests for the (1) objective assessment of exposure to toxic metals and (2) estimation of net retention of toxic metals. It is beyond the scope of this chapter to discuss the sources and symptoms associated with net retention of the most commonly encountered metals. (See Crinnion and Pizzorno, *Clinical Environmental Medicine,* Elsevier, 2018, for a comprehensive discussion of toxic metals.) Thorough reviews of these topics have been published elsewhere.[3–7]

The most commonly encountered toxic metals (mercury, lead, cadmium, and arsenic) are natural constituents of the earth's crust, but their increasing abundance in air, water, and surface soil results primarily from industrialization and energy production (pollution). Consequently, the environment today has become contaminated to the point that everyone, regardless of occupation, is at higher risk for at least long-term, low-level exposure to toxic metals. However, consistent with the basic principles of toxicology, confirmation of exposure is by no means valid documentation of clinically significant retention. In reality, the toxic effects of metals for an *individual* are exhibited when the level of retention exceeds physiological tolerance, and net retention (body burden) is determined by the relative rates of toxic metal assimilation and excretion.

Two important concepts that have been largely overlooked with respect to metal toxicity may explain the discrepancies of opinion expressed by practitioners of conventional medicine and preventive medicine. The first is the potential for the combined effects of multiple toxic metals, which can have additive, but also synergistic, adverse physiological effects (and, of course, toxic chemical exposure aggravates the problem),[8,10] even in children with environmental exposures.[9] This concept should be further extended to include consideration of the potential combined effects of organ toxicants and toxic metals, the total toxic load. The broad heading of "toxic chemical entities" includes not only naturally occurring and synthetic exogenous compounds but also noxious endogenous compounds derived from a severely disrupted dysbiotic or poorly functioning gastrointestinal (GI) metabolome. An additional factor is the remarkable individual variability in susceptibility or tolerance to toxic elements. Established precedence for the phenomena has been provided by such observations as the rapid contact allergic response that is elicited by mercury in a small percentage of a population.[11] Individual variability in susceptibility is determined in part by genetic polymorphisms, nutritional status, and the total toxic load. The factors of multiple toxicants and individual variability impede simple interpretation of any laboratory test result for an individual patient.

No single definitive test can be used to diagnose the toxic effects of excessive retention of toxic elements; any test result must be interpreted in conjunction with a thorough review of a patient's physical findings,

exposure history, and symptoms. However, the symptoms associated with toxic metal retention appear to be diverse and rather nondescript, and they may not be fully expressed until later in life. Clear examples of such latency of symptom expression have been provided for lead and hypertension[12] and cardiovascular mortality.[13] Therefore to address the needs of clinicians who focus on preventive medicine as opposed to crisis management, the following review of laboratory tests emphasizes testing that has greater sensitivity with respect to the detection of the bioaccumulation of toxic elements. Emphasis has been placed on the distinction between testing that is most appropriate for assessment of exposure versus net retention.

ASSESSMENT OF TOXIC METAL EXPOSURE

Hair Elemental Analysis

When performed properly, hair elemental analysis can serve as a qualitative screening test for exposure to toxic metals, but it is not a reliable method for the diagnosis of metal toxicity or net retention. Hair is an excretory tissue that can provide a cumulative record of bioavailable trace elements in the body, and the hair content of mercury, arsenic, lead, and thallium has been used as evidence for the cause of death.[14] Once metals are incorporated into growing hair, there is no back exchange into the body; the concentration of metals in hair is usually far greater than that in blood or urine. The length of the hair specimen analyzed dictates the duration of time during which exposure occurred, and segmental analysis of hair can be used forensically to estimate the temporal course of exposure. A study of the lead and mercury content of hair from a long-deceased president of the United States was performed at the Armed Forces Institute of Pathology in Washington, DC, exemplifying the potential utility of hair analysis for exposure to toxic metals.[15] Detection of toxic metals in hair actually predates that in blood and urine.[16] A growing number of peer-reviewed publications support the value of elemental analysis of hair specimens for the detection of exposure to toxins, and some national laboratories have been performing hair elemental analysis for mercury, lead, and arsenic for years. Arsenic in both hair and urine confirmed arsenic exposure from an arsenical pesticide in an individual with peripheral neuropathy and macrocytosis.[17] Hair levels of lead, manganese, cadmium, and other toxic metals have been correlated with psychological conditions and deviant or violent behaviors.[18] Lead, cadmium, and mercury levels in children's hair have been inversely correlated with childhood intelligence. Hair analysis has been used to identify historical as opposed to current exposure to lead.[20] School children with relatively high levels of lead in their hair had slower reaction times and less flexibility in changing their focus of attention than children with relatively low concentrations of lead in their hair.[21] More recently, hair levels of lead and barium were found to be higher in rural populations in relation to their proximity to oil fields (contaminated water) in the Thar Jath oil fields in South Sudan.[19] The Agency for Toxic Substances and Disease Registry (ATSDRss), the U.S. Environmental Protection Agency, and the National Academy of Sciences recognize the scientific validity of hair mercury levels as an indicator of maternal and fetal exposure to methylmercury. In a cognitive performance study of children in the Faroe Islands, there were detectable effects on brain function in the children whose mothers had elevated levels of hair mercury.[22] A history of fish consumption and mercury in hair samples are considered the best indicators of human exposure to methylmercury.[23] Fish consumption among Scandinavian[25] and Tyrrhenian men,[26] Amazonian children,[27] and people from the Minamata Bay area[15] and the San Francisco Bay area[24] was positively correlated with hair and blood mercury levels. Note that hair elemental analysis definitely provides useful information about exposure to methylmercury (fish consumption); however, it is not nearly as useful for disclosing information about exposure to inorganic mercury as derived from dental amalgams.[15] The concentration of methylmercury in hair is about 300 times higher than that in blood.[28] In sharp contrast, about 75% of total hair arsenic is present in the inorganic form.[29]

Although an increasing number of peer-reviewed published studies support the clinical utility of elemental analysis of hair for the assessment of exposure to specific toxic metal forms, some considerations prevent its acceptance by governmental agencies in the United States. In June 2001, the Agency for Toxic Substances and Disease Registry (ATSDR) convened a panel of scientists with some expertise in hair analysis or risk assessment to explore "the state of the science of hair analysis."[30] Overall, the discussion was objective and focused on the existing scientific data. In a summary statement from the meeting, it was concluded that "in general, hair analysis results can provide limited qualitative insights into environmental exposures and rarely can answer questions about potential health effects."[27]

The primary concerns raised by the group pertained to uncertainties about the quantitative relationship among the actual "internal dose," the rate of incorporation into hair, and the current lack of well-established data to enable one to predict potential health effects for a given concentration of a specific metal in hair. Such criticisms are irrelevant to those who understand the limitations of hair elemental analysis.

Overinterpretation of the results of elemental analysis of hair is a serious concern shared by science-based laboratories and astute clinicians as well as the ATSDR. It should be kept in mind that the ATSDR has been interested in the use of hair analysis as an initial screening tool for inexpensive and noninvasive monitoring of populations in the vicinity of known sites of contamination. The goal of the ATSDR to be able to use hair elemental analysis as confirmation of toxicity is quite different from the use of the test in preventive or environmental medicine to provide an initial indication of exposure.

The consensus report by the ATSDR is consistent with the aforementioned statement that hair analysis can provide some qualitative information about exposure to toxic metals but does not provide a basis for the diagnosis of metal toxicity. As such, hair analysis may be helpful to clinicians as a step toward identifying potential health problems that may be associated with toxic metal exposures before overt symptoms are expressed. Further testing should be performed before treatment options are considered. The clinician should be wary of laboratories that perform hair analysis as a vehicle to sell nutritional supplements and should be aware of interlaboratory variation.[31] Clinicians are encouraged to use only laboratories that can validate their certification or accreditation and incorporate state-of-the-art methodologies for washing, digesting, and analyzing hair specimens.[32] Appropriate quality control characteristics and the validation of the establishment of reference ranges, accuracy, precision, and reliability of state-of-the-art hair analysis have been described.[33-35]

Blood Analysis: Toxic Metal Exposure

For the most commonly encountered toxic metals, the current standard for diagnosis of metal toxicity is abnormally high concentrations in whole blood or urine (e.g., thallium, arsenic). However, blood analysis for toxic metals is a better indicator of exposure than toxicity in most cases. Distribution of metals, such as lead, in the body has been long recognized as initially dependent on the rate of delivery via the blood to various tissues and organs.[36] Subsequent redistribution then depends on the relative affinities of tissues for the metals and toxicokinetics that can vary markedly among individuals. Tissue affinities for metals are determined in large part by the high relative intracellular concentrations of reduced glutathione and metallothionein.[37]

Furthermore, blood levels can fluctuate considerably with intermittent exposure and assimilation. Thus as stated by the ATSDR and the Centers for Disease Control and Prevention, the concentration of lead in the blood reflects mainly the exposure history of the previous few months and does not necessarily reflect the larger burden and much slower elimination kinetics of lead in bone.[10]

Examining kinetic models of metal metabolism shows that the blood compartment has the shortest half-life. Metals leave blood by excretion (urine, bile, and sweat) and transfer to tissues. The retention by tissues, such as bone, kidneys, and brain, accounts for the much longer biological half-lives of most toxic metals in the body. This simple concept has been clearly demonstrated in numerous studies and in the *Physician's Desk Reference.*[29] Adult and pediatric patients who were diagnosed with lead toxicity on the basis of elevated blood lead values exhibited marked reductions in blood lead levels after chelation therapy with Chemet (DMSA).

However, 2 weeks after cessation of chelation, blood lead levels rebounded to between 60% and 85% of pretreatment levels (the rebound effect has been associated with all pharmacological chelators). The relationship between blood lead levels and the quantity of lead excreted in urine after calcium disodium edetate (Ca-EDT) DMSA chelation is nonlinear, in that arithmetic increases in blood lead are associated with exponential increases in urine lead excretion.[1,39]

Under extreme conditions of grossly excessive retention of metals (long-term occupational exposure), the equilibrium between tissue stores and blood can result in blood metal levels that are at or above the established threshold values for the diagnosis of metal toxicity but still do not indicate the extent of total body metal retention. The currently established standard of blood lead levels for the assessment of lead toxicity in children is disturbing because no minimum response levels have been established for lead because a threshold has yet to be defined for the most sensitive effect of lead neurotoxicity.[45]

Interestingly, separation of blood into the plasma versus red blood cell components can provide valuable information to the clinician about the primary sources of exposure to mercury. Approximately 95% of methylmercury, most commonly derived from contaminated fish, partitions into red blood cells,[46,47] whereas about 90% of inorganic mercury (amalgams, occupational exposure) is found in the plasma compartment bound to albumin, cysteine, and nonspecific proteins.[32]

Because the first step in successful detoxification is to remove the source of exposure, documentation of the primary source of exposure to mercury can be instrumental for efficient detoxification. Blood arsenic levels, albeit with a very short half-life (approximately 6 hours), reflect exposure to inorganic arsenic but not dietary organic arsenic (shellfish), which is rapidly excreted in the urine.[48,92] The blood levels of metals derived from orthopedic metal implants have come to the forefront, especially with respect to metal-on-metal (M/M) total hip arthroplasty. All patients with such M/M prostheses will have elevated levels of cobalt and chromium.[40,41] The wear-related release of the metal debris has been causally associated with not only local tissue damage[42] but also remote adverse effects on a wide array of physiological/biochemical processes and functions.[43,44]

Urinalysis of Toxic Elements Exposure: Unprovoked

In general, urinalysis for toxic metals does not provide a scientifically valid basis for the diagnosis of metal toxicity. However, in some cases it provides an indication of very recent or ongoing exposure. Such is not necessarily the case for lead because urinary lead is generally not a useful biomarker to estimate low-level exposure. However, elevated urinary lead-chelate complexes resulting from the EDTA Ca-EDTA or DMSA mobilization test provide a good means to estimate net retention of lead.

A different scenario exists for organic arsenic and inorganic and methylmercury. The most commonly accepted biomarker for exposure to inorganic mercury is the urinary level of inorganic mercury.[49] However, the World Health Organization (WHO) standard for occupational exposure is very high (50 mcg/g creatinine).[50] This high standard has been challenged because neurological impairment has been reported for occupationally exposed subjects[51,52] whose urinary mercury levels were well below the WHO standard. Evidence that urinary mercury levels are indicative of exposure to implanted mercury amalgams has also been published.[53] In a study of more than 1000 Vietnam-era veterans reported by the National Institute of Dental Research, a highly statistically significant correlation was detected between the level of amalgam exposure and urinary mercury levels. Several other studies have reported an association between amalgam exposure and urinary mercury levels.[54,56-58] Elevated levels of urinary arsenic have been detected in workers during periods of occupational exposure, including copper smelting, spraying of insecticides or herbicides, and application of wood treatments.[61]

Arsenic can be markedly and transiently elevated in individuals within 48 hours after consumption of shellfish that contain high levels of relatively nontoxic species of organic arsenic (e.g., arsenobetaine, arsenocholine).[62] Urinary mercury is frequently elevated in consumers who regularly consume fish.[15,63] Therefore analysis of an unprovoked urine specimen is highly recommended to avoid alarmism and misinterpretation of the results of a urinary metals provocation test. Patients should be instructed to abstain from the consumption of fish and shellfish for about a week before a chelation challenge is performed. Elevated urinary values of arsenic and mercury associated with the specific dietary and occupational conditions reflect recent or ongoing high-level exposure but are not necessarily reflective of the body burden of the specific elements. Although blood metal levels reflect transient transport in the body, urinary levels qualitatively reflect excretion of an unknown fraction of the total body pools of assimilated metals.

Urinalysis: Biomarkers of Renal Cadmium Toxicity

Toxic metals such as cadmium, mercury, and lead are known to be nephrotoxic at high levels of assimilation. Cadmium is of particular concern because of its exceedingly long residence time in the kidneys.[64] Therefore in addition to urine levels of cadmium, urinary biomarkers of renal damage should be assessed for documentation of cadmium toxicity. Early markers of cadmium-induced renal damage include proteinuria, glucosuria, aminoaciduria, hypercalciuria, and polyuria.[65] Sensitive urinary biomarkers for more advanced cadmium-induced renal tubular damage include elevated levels of the low-molecular-weight protein β_2-microglobulin, retinal-binding protein, *N*-acetyl-β-D-glucosaminidase (NAG), and Cystatin C (especially in patients where serum creatinine may be misleading (e.g., very obese, elderly, or malnourished patients).[66] Abnormal urinary levels of NAG have also been reported in association with markedly high urinary mercury levels (35 mcg/g creatinine) in chlor-alkali workers with long-term exposure to inorganic mercury.[67] It is noteworthy that the urinary NAG levels were correlated with urinary mercury and integrated dose of exposure but not with concurrently measured blood mercury levels.[53] Thus it appears that assessment of urinary biomarkers of renal damage may be useful in the diagnosis of toxicity in cases of very high exposure to cadmium and perhaps mercury. However, negative findings for the renal biomarkers do not exclude the possibility of related nephrotoxicity retention that is most commonly encountered in general clinical practice.

ASSESSMENT OF RETENTION: URINALYSIS, PROVOCATION TESTS

The best currently available method to estimate the level of retention of toxic metals in the body is urinalysis for toxic metals after the administration of chelating agents.[55] In the 1970s, the value of a standardized calcium-disodium EDTA (Ca-Na_2-EDTA) provocative test was recognized as sensitive to determine the mobile and potentially toxic body lead stores and to assess response to chelation therapy in pediatric patients with high blood lead levels.[68–70] Subsequently, Markowitz and Rosen[61] described the results of a comparable, yet more convenient test that would permit use in a greater number of qualifying patients.

The concept and value of using metal complexing agents to estimate the net retention of readily accessible toxic elements has gained some acceptance, but it is not accepted by the American College of Medical Toxicology.[59]

That position is in direct conflict with a renowned nephrologist who has stated that "the best measure for assessing the total accumulation of lead in the body is the calcium disodium EDTA lead mobilization test."[60] Several other pharmaceutical agents are widely used for this purpose. The most commonly used pharmaceutical agents are Ca-Na_2-EDTA, Dimaval-(RS)-2,3-dimercapto-propropane-1 sulfonate (DMPS), and mes-2,3-dimercaptosuccinic acid (DMSA). Nonpharmaceutical compounds that have also been used as possible provocation agents include N-acetyl-cysteine (N-AC) and potassium-citrate (K-citrate).[72] The latter compounds are not chelators by definition and have not been studied extensively or compared with the efficacy of the well-established Ca-EDTA lead mobilization test.

EDTA

Ca-Na_2-EDTA is the only form of EDTA approved for lead decorporation by the U.S. Food and Drug Administration (FDA). It has been used for decades as the provocation agent and therapeutic agent of choice for patients with high blood lead levels. The true chelator is also well known to be effective for increasing the urinary excretion of antimony, gadolinium, iron, cobalt, trivalent chromium, copper, nickel, cadmium, and manganese.[73] Ca-EDTA also has a high affinity for zinc and, if not used properly, can cause zinc depletion. Clinicians are strongly encouraged to assess glomerular function before use (Ca-EDTA mobilized metals are excreted in the urine). Before attempting to embark on the safe and effective use of Ca-EDTA, clinicians should attend specific training courses. Although the "slow-push" Ca-Na_2-EDTA protocol introduced in the United States appears to be effective for increasing the urinary excretion of lead,[74] it has not been formally evaluated for safety. Ca-EDTA is not an effective chelator of mercury as are the dithiol metal complexing agents discussed next.

DMPS

DMPS appears to be the most productive agent for the mobilization of mercury, as determined in vitro[75] and in a comparative study during the Iraqi mercury crisis, in which people were acutely poisoned after consumption of grains contaminated with a methylmercury-containing fungicide.[76] More relevant to the typically encountered clinical situation, a DMPS provocation study was conducted with volunteer college students.[77] Subjects with and without amalgam fillings were given 300 mg DMPS (orally), and all urine was collected over the subsequent 9 hours. DMPS raised mean urinary mercury for the nonamalgam group from 0.27 to 5.1 mcg and that of the amalgam group from 0.7 to 17.2 mcg over the 9-hour period. A highly significant positive correlation was detected between the amount of mercury excreted after the DMPS challenge and amalgam surface area.

An additional study supports the value of provocation testing for people occupationally exposed to mercury vapors. A comparison of urinary mercury levels was made before and after oral administration of a 300-mg dose of DMPS to dental technicians, dentists, and nonoccupationally exposed controls.[46] Unprovoked urine mercury levels were comparable for dental technicians and dentists and were about five times higher than those in controls. Compared with preprovocation values, DMPS-induced urinary mercury (micrograms per 6 hours) increased by a factor of 87, 49, and 34 for the technicians, dentists, and controls, respectively. Post-DMPS urinary mercury levels were 16 and 6 times higher than in controls for the technicians and dentists, respectively. The group's mean urinary mercury for the technicians after DMPS was 424 ± 85 mcg/6 h. The baseline urinary coproporphyrin level, which is an established biomarker of mercury-induced disruption of heme biosynthesis, was significantly correlated with urinary mercury levels after DMPS but was not correlated with baseline urinary mercury levels. The researchers concluded that post-DMPS urinary mercury levels were better indicators of exposure and retention than unprovoked urinary mercury levels.

The pharmacokinetics of DMPS have been well defined,[78,79] and the efficacy of DMPS for detoxification of mercury,[80] arsenic,[81] and lead (pediatric)[82] has been documented. Although DMPS is not approved by the FDA, it is registered in Germany with the German Drug Regulatory Authorities and is available in the oral form without a prescription.[68] DMPS is no longer available to clinicians in the US. DMPS is associated with a very low incidence of serious side effects,[68] and its safety in general was evident from the observations made during the course of extensive IV administration (250 mg every 4 hours for 12 consecutive days) in a young woman who had severe arsenic toxicity.[71] The most commonly reported side effects associated with DMPS are nausea, weakness, vertigo, chills, fever, cutaneous reactions/itching, erythema multiforme, and elevations of transaminases.[69,71,72] An extensive review of the German literature about the pharmacokinetics, affinities for various metals, and side effects of DMPS is available.[72]

The recommended IV dose of DMPS is 3 to 5 mg/kg (not to exceed 250 mg to avoid hypotension).

A detailed description of an extensively used oral DMPS provocation protocol has been presented,[68] as well as an IV DMPS provocation protocol.[6] To establish the basal urinary rate of metal excretion, the patient is instructed to fast overnight and collect a first-morning urine specimen. In the morning, with an empty stomach and after the bladder is emptied, the patient is given about 300 mg DMPS (5 to 10 mg/kg) orally or 3 to 5 mg/kg intravenously. All urine is collected for the subsequent 6 hours.[68] A light meal (no seafood or fish) may be consumed about 2 hours after oral ingestion, and fluid consumption is encouraged. The specific laboratory instructions should be followed for shipping the specimen. Metals mobilized by DMPS are excreted primarily by the kidney and to a much lesser extent by the liver (biliary/fecal).[68]

Equilibrium and stability constants (in vitro) for various DMPS–metal complexes have been presented.[83] In the clinical setting, DMPS is effective for the mobilization and excretion of bismuth, mercury (organic and inorganic), copper, lead, arsenic, antimony, nickel, tin, tungsten, and gold but does not affect thallium, aluminum, or uranium excretion. In the majority of adult patients, mercury is the predominant metal excreted after DMPS, and elevation of copper is normal (about five times higher than the preprovoked urine level). As mercury levels decline during detoxification therapy, it is common to see increased urinary levels of other metals, such as lead and tin, with subsequent challenges. The shifting pattern of the metal species excreted is based on a

combination of affinities of DMPS for the different metals as well as on mass competition for metal-binding sites. There are no well-established guidelines for the interpretation of the results of the DMPS challenge test. Therefore conclusions about toxicity cannot be made from the DMPS test results alone. Consideration has to be given to the overall medical examination, medical and exposure history, and presenting symptoms. If a decision is made to proceed with some form of detoxification therapy, the initial challenge result can serve as a reference point against which subsequent challenge results can be compared with to evaluate the efficacy of treatment. The levels of other toxic metals excreted should always be considered, and one should note that DMPS does not provide direct information as to the level of mercury present in the central nervous system. It is beyond the scope of this chapter to discuss protocols for metal detoxification, but it is emphasized that if a pharmaceutical metal complexing agent is to be used, the glomerular filtration rate must be assessed before initiation and periodically during the therapy.

Some clinicians use transdermal DMPS for both diagnosis and treatment. However, one study found no measurable presence in blood or urinary DMPS after a standard dose.[84]

DMSA

Another dithiol metal-complexing agent, DMSA, is also widely used for provocation testing, as well as detoxification therapy for lead, mercury species, and other sulfhydryl reactive metals (e.g., arsenic, antimony). Several studies demonstrated the effectiveness of DMSA to increase the urinary excretion of lead[85–87] and mercury and decrease the blood levels of these metals.[30,88–90]

DMSA was approved by the FDA for lead detoxification in children with lead poisoning and is an agent of choice for lead detoxification in children and adults. DMSA appears to be considered by some to be the "new chelator" for lead. Only about 20% of orally administered DMSA is systemically available after a single dose.[29] DMSA, when used in conjunction with Ca-Na_2-EDTA, increases cumulative urinary lead excretion and ameliorates the Ca-EDTA–mediated redistribution of lead to soft tissues.[81,91] DMSA is restricted to the extracellular compartment and does not have direct access to the elements retained in the cells or interstitial fluid, and it does not appear to cross a healthy blood–brain barrier. In rodent models, however, DMSA has been demonstrated to be effective in decreasing the levels of lead and mercury in the brain.[77,82,83,85] Animal studies also indicated greater efficacy of DMSA for lead detoxification when concomitantly administered with antioxidants.[78,79] This is true for all bona fide metal-complexing and metal-chelating agents. N-acetyl cysteine alone appears to enhance lead excretion to a much lesser degree than DMSA, but in contrast, the potential effects of α-lipoic acid alone are equivocal. To date, no reliable studies have been published to indicate that lipoic acid is an effective metal-binding agent that has a net effect on metal excretion in humans, and the potential for lipoic acid–induced mercury redistribution, particularly to the brain, is a significant consideration.[86]

DMSA is generally well tolerated with common but mild side effects, including GI bloating and/or gas, occasional loose stools, and skin rash. Assessment of liver enzyme levels is recommended before and periodically during extended administration. Compared with DMPS and Ca-EDTA, DMSA has minimal effects on essential elements such as copper and zinc. DMSA is excreted almost exclusively as a mixed disulfide with two molecules of cysteine.[87] Thorough reviews of the pharmacokinetics and clinical use of DMSA have been presented.[6,68,69,88] It should be noted that there is a tremendous difference between the DMSA protocol described for acute lead poisoning[76] and that commonly used for chronic lead retention.

Various protocols for DMSA provocation testing have been suggested.[6,89] However, a convenient and productive provocation protocol has been described that entails giving a single dose of DMSA orally (30 mg/kg, not more than 2 g) on an empty stomach and bladder followed by collection of all urine for the subsequent 6 hours.[62] The peak rate of excretion of metals occurs after about 3 hours. The protocol is well tolerated, enhances compliance, and significantly reduces exposure to the compound. Patients should be advised in advance that their urine will transiently have a foul, sulfurous odor and that transient GI inconvenience may occur.

Nonpharmaceutical Agents

Nonpharmaceutical compounds, such as *N*-acetyl-L-cysteine (*N*-AC) and potassium citrate (K-citrate), have been tested for efficacy as provocation agents for mercury in one published study.[62] Urinary mercury levels, expressed as micrograms per liter, were compared before and after a single oral dose of *N*-AC (30 mg/kg), K-citrate (5 g in 200 mL water), DMSA (30 mg/kg), or DMPS (Unithiol, 250 mg in 5 mL water). Basal urinary mercury levels (about 5 ug/L) were comparable for all of the treatment groups, each of which contained 16 to 65 multisymptomatic subjects. All subjects either had dental amalgams or had recently undergone removal of amalgams. Urine was collected for 3 hours after the challenge compounds were given, except that urine was collected for only 2 hours after the DMPS. The different collection time for the DMPS prohibits valid comparisons of the effects of the other agents. The high bolus doses of *N*-AC and K-citrate significantly increased urinary mercury, by 131% and 83%, respectively, compared with basal values. Under these conditions of different collection times, DMSA and DMPS increased mercury excretion by 163% and 135%, respectively. No mention was made of the urine volumes associated with the different test groups, and, clearly, the data would have been easier to interpret had the results been standardized per gram urine creatinine.

Grossly misleading values for urinary metals can be associated with the expression of excreted metals per unit volume because urinary output can vary considerably. No other data are currently available to permit further evaluation of the value of the nonpharmaceutical compounds as provocation agents. These two compounds also should be tested in animal models to determine whether they are associated with significant redistribution of metals among various tissues because neither is a true chelator, and their stability constants are relatively low. Currently, no definitive studies are available to assess the utility of parentally administered reduced glutathione as a direct metal-complexing agent. A final note about other routes of administration of various authentic and potential metal detoxification agents, particularly with respect to provocation testing, is that the author has not been able to find any published research addressing the systemic bioavailability or efficacy of any agent that is given via a transdermal delivery system.

FECAL METALS ANALYSIS

Several toxic metals, including mercury, lead, thallium, and cadmium, are naturally excreted primarily or partially in bile. Therefore under certain conditions, analysis of fecal metals, without provocation, may provide at least qualitative information about the rate of biliary excretion of assimilated metals. However, contaminated foods present a significant source of exposure to metals, and metals that have not been assimilated by the gastrointestinal tract can contribute overwhelmingly to the total amount of metals measured in a fecal specimen. In addition to dietary contamination, fecal mercury is very much influenced by the amount of mercury that is present in the mouth in the form of mercury amalgams.[90] Fecal mercury concentrations, expressed per gram of dry weight, are roughly an order of magnitude higher in people who have an average of six to eight medium-sized amalgams than in individuals who are amalgam-free.[90] Day-to-day variability in fecal mercury levels in amalgam bearers is remarkably small. Fecal mercury levels are highly correlated with the number of

amalgams (Fig. 22.1). Further, it has been demonstrated that fecal mercury levels decline significantly after extraction of amalgams.[91] Therefore levels of fecal metals generally are more a representation of exposure to metals than an indication of total body retention. Current research efforts are focusing on the identification of metal-complexing agents or phytonutrients that increase the biliary/fecal excretion of metals. Fecal metals have been analyzed in autistic children ($n = 54$), and, on average, metal levels were significantly higher compared with those in age-matched, neurotypical controls ($n = 83$).[83] The reason for the higher levels in association with autism is not known, and pica is a possible issue that may contribute to higher levels of exposure in patients with autism.

CONCLUSION

Long-term, low-level exposure to environmental toxins is a growing global problem, and evidence is accumulating to link the bioaccumulation of toxic metals in humans to subtle and overt long-term toxic effects, poor health, and substantially increased disease risk. Increasing numbers of patients dissatisfied with the care provided by clinicians who rely only on methods for the assessment of acute metal poisoning are seeking out clinicians who are aware of the value of tools that are yet to be accepted for the assessment of subacute metal toxicity. Analyses of metals in hair, blood, and urine all have advantages and disadvantages, and no single currently available laboratory test can unequivocally permit a valid diagnosis of "subclinical" metal toxicity. The results of the various tests discussed in this chapter, along with a complete medical examination, exposure history, and other findings, can be used to design a comprehensive therapeutic detoxification program. Table 22.1 provides an overview of the value of the various tests for assessing a patient's potential problem with toxic elements. To close, it is emphasized that there is a need for better chelating agents that have much greater volumes of distribution and the ability to *safely* cross a normal blood–brain barrier and directly remove neurotoxic elements from the central nervous system. Perhaps research toward finding a cure for Parkinson's disease or premature dementia will provide such agents.

REFERENCES

See www.expertconsult.com for a complete list of references.

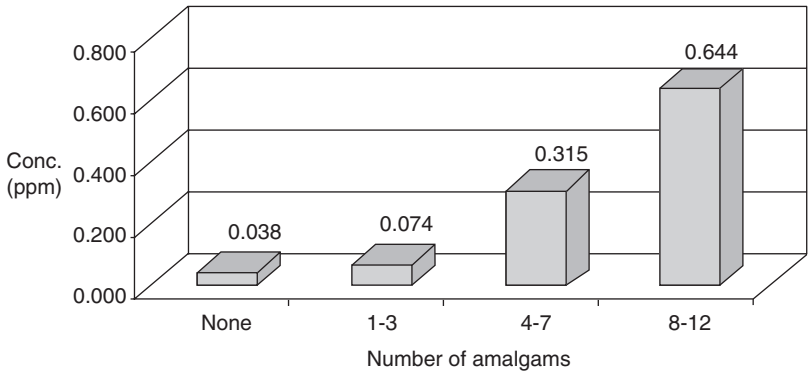


Fig. 22.1 Fecal mercury levels versus the number of dental amalgams. Fecal mercury (microgram per kilogram dry weight) was plotted against number of amalgams for 200 subjects. (Data from Bass DA, Urek K, Quig D. Measurement of mercury in feces. Poster presented at American Association of Clinical Chemistry Conference, New Orleans, July 1999.)

TABLE 22.1 Summary of the Potential Clinical Value of Hair Analysis and Urinalysis

		URINE (RETENTION)			
Metal/Metalloid	**Hair (exposure)**	**No Provocation**	**DMSA**	**DMPS**	**Ca-Na_2-EDTA**
Aluminum	Fair	Poor	Poor	Poor	Fair, best with deferoxamine
Antimony	Good	Poor	Good	Good	Good
Arsenic	Inorganic, Good	Poor	Good	Excellent	Poor
Cadmium	Fair	Poor	Fair	Fair	Good with IV GSH
Inorganic mercury	Poor to fair	Poor	Excellent	Excellent	Poor
Iron	Poor	Poor	Poor	Poor	Good, best with deferoxamine
Lead	Good	Poor	Good	Fair	Excellent
Nickel	Poor	Poor	Fair	Fair	Good
Organic mercury	Excellent	Poor	Excellent	Excellent	Poor
Tin	Poor	Poor	Good	Good	Good
Tungsten	Good	Poor	Fair	Fair	Poor
Uranium	Good	Poor	Poor	Poor	Poor

Summary of the potential value of hair analysis (exposure) and the most commonly used provocation agents used in conjunction with urinalysis for the detection of retention of specific metals. The qualitative guidelines are based on the author's perception of affinities (in vivo) derived from the examination of thousands of test results and stability constants as determined under highly defined conditions in vitro. The information provided does not include the potential use of adjunctive agents and/or protocols and does not cover all known metal complexing agents.
Ca-Na_2-EDTA, calcium-disodium ethylenediaminetetraacetic acid; *DMPS,* Dimaval-(RS)-2,3-dimercapto-propane-1 sulfonate; *DMSA,* meso-2,3-dimercaptosuccinic acid; *GSH,* glutathione; *IV,* intravenous.

REFERENCES

1. Goyer R. Chelation of toxic metals: current interests. *Env Health Persp*. 1995;103:988–989.
2. Goyer RA, Cherian MG, Jones MM, et al. Role of chelating agents for prevention, intervention, and treatment of exposures to toxic metals. *Env Health Persp*. 1995;11:1048–1341.
3. Patrick L. Mercury toxicity and antioxidants. Part I. Role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity. *Altern Med Rev*. 2002;7:456–471.
4. Quig D. Cysteine metabolism and metal toxicity. *Altern Med Rev*. 1998;3:262–270.
5. Patrick L. Toxic metals and antioxidants. Part II. Arsenic and cadmium toxicity. *Altern Med Rev*. 2003;8:106–128.
6. Crinnion WJ. Environmental medicine. Part III. Long-term effects of chronic mercury exposure. *Altern Med Rev*. 2000;5:209–223.
7. Grandjean P. International perspectives of lead exposure and lead toxicity. *Neurol Toxicol*. 1993;14:9–14.
8. Schubert J, Riley EJ, Tyler SA. Combined toxic effects in toxicology, rapid systematic testing procedure: cadmium, mercury and lead. *J Toxicol Environ Med*. 1978;4:763–776.
9. Burbure C, Buchet JP, Leroyer A, et al. Renal and neurological effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. *Env Hlth Persp*. 2006;114:584–590.
10. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for lead. Update. https://www.atsdr.cdc.gov/toxprofiles/index.asp. Accessed July 6, 2018.
11. Finne K, Goransson K, Winkler L. Oral lichen planus and contact allergy to mercury. *Int J Oral Surg*. 1982;11:236–239.
12. Malm O, Branches FJ, Akagi H, et al. Mercury and methylmercury in fish and human hair from the Tapajos River basin. *Brazil Sci Total Environ*. 1995;175:141–150.
13. Salonen JT, Seppanen K, Nyssonen K, et al. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. *Circulation*. 1995;91:646–655.
14. Holsbeek L, Das HK, Joiris CR. Mercury in human hair and relation to fish consumption in Bangladesh. *Sci Total Environ*. 1996;186:181–188.
15. Harada M, Nakanishi J, Kunuma S. The present mercury contents of scalp hair and clinical symptoms in inhabitants of the Minamata area. *Environ Res*. 1998;77:160–164.
16. Phelps RW, Clarkson TW, Kershaw TG, et al. Interrelationships of blood and hair mercury concentrations in a North American population exposed to methylmercury. *Arch Environ Health*. 1980;35:161–168.
17. Yamato N. Concentration and chemical species of arsenic in human urine and hair. *Bull Environ Contam Toxicol*. 1988;40:633–640.
18. Eastern Research Group. *Hair Analysis Panel Discussion: Exploring the State of the Science*. Lexington, MA: Agency for Toxic Substances and Disease Registry; 2001.
19. Pragst F, Stieglitz K, Quig D, et al. High concentrations of lead and barium in hair of the rural population caused by water pollution in the Thar Jath oilfields in South Sudan. *J Forensic Sci International*. 2017. https://doi.org/10.1016/j.foresciint.2016.12.022.
20. Agency for Toxic Substances and Disease Registry (ATSDR). Document 03-0300: hair sampling testing-scientific. PM65 http://www.atsdr.cdc.gov/hair_analysis. Accessed June 3, 2003.
21. Siedel S, Kreutzer R, Smith D, et al. Assessment of commercial laboratories performing hair mineral analysis. *JAMA*. 2001;285:67–72.
22. Steindel SJ, Howanitz PJ. The uncertainty of hair analysis for trace metals. *JAMA*. 2001;285:83–85.
23. Puchyr RF, Bass DA, Gajewski R. Preparation of hair for measurement of elements by inductively coupled mass spectrometry (ICP-MS). *Biol Trace Elem Res*. 1998;62:167–182.
24. Hightower JM, Moore D. Mercury levels in high-end consumers of fish. *Env Hlth Persp*. 2003;111:604–608.
25. Bass DA, Hickok D, Quig D, et al. Trace element analysis in hair: factors determining accuracy, precision, and reliability. *Altern Med Rev*. 2001;6:472–481.
26. Druyan ME, Bass DA, Puchyr R, et al. Determination of reference ranges for elements in human scalp hair. *Biol Trace Elem Res*. 1998;62:183–197.
27. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Lead*. Atlanta, GA: Public Health Service, U.S. Department of Health and Human Services; 1993.
28. Sanders BM, Goering PL, Jenkins KJ. The role of general and metal-specific cellular responses in protection and repair of metal-induced damage: stress proteins and metallothioneins. In: Chang LW, ed. *Toxicology of Metals*. New York: CRC Press; 1996:165–187.
29. *Physicians' Desk Reference*. 54th ed. Montvale, NJ: Medical Economics; 2000.
30. Hoet P, Buchet J-P, Decerf L, et al. Clinical evaluation of a lead mobilization test using the chelating agent dimercaptosuccinic acid. *Clin Chem*. 2006;52:88–96.
31. Gehardsson L, Skerfving F. Concepts on biological markers and biomonitoring for metal toxicity. In: Chang L, ed. *Toxicology of Metals*. New York: CRC Press; 1996:81–110.
32. Ishihari N. Inorganic and organic mercury in blood, urine and hair in low level mercury vapor exposure. *Int Arch Occup Environ Health*. 1978;40:249–253.
33. Foa V, Colombi A, Maroni M, et al. The speciation and chemical forms of arsenic in the biological monitoring of exposure to inorganic arsenic. *Sci Total Environ*. 1984;34:241–259.
34. Iffland R. Arsenic. In: Seiler HG, Sigel A, Sigel H, eds. *Handbook on Metals in Clinical and Analytical Chemistry*. New York: Dekker; 1994:237–245.
35. Clarkson TW. Principles of risk assessment. *Adv Dent Res*. 1992;6:22–27.
36. World Health Organization (WHO). *Inorganic Mercury*. *Environmental Health Criteria 118. World Health Organization*. Geneva: International Program on Chemical Safety; 1991.
37. Echeverria D, Heyer NJ, Martin MD, et al. Behavioral effects of low-level exposure to Hgo among dentists. *Neurotoxicol Teratol*. 1995;17:161–168.
38. Langworth S, Almkvist O, Soderman E, et al. Effects of occupational exposure to mercury vapour on the central nervous system. *Br J Indust Med*. 1992;49:545–555.
39. Gilbert SG, Weiss B. A rationale for lowering blood lead action level from 10 to 2 ug/dL. *Neurotoxicol*. 006;27:693–701.
40. Sampson B, Hart A. Clinical usefulness of blood metal measurements to assess the failure of metal-on-metal hip implants. *Ann Clin Biochem*. 2012;49:118–131.
41. Jantzen C, et al. Chromium and cobalt ion concentrations in blood and serum following various types of metal-on-metal hip arthroplasties. *Acta Orhtopaedica*. 2013;84:229–236.
42. Sansone V, et al. The effects on bone cells of metal ions released from orthopaedic implants—a review. *Clin Cases Min Bone Metab*. 2013;10:34–40.
43. Mayo Clinic. Cobalt, chromium, molybdenum, titanium, molybdenum, serum—clinical interpretive. https://www.mayomedicallaboratories.com/testcatalog/Clinical+and+Interpretive/89367. Accessed September 22, 2017.
44. Tower S. Arthroprosthetic cobaltism: neurological and cardiac manifestations in two patients with metal-on-metal arthroplasty: a case report. *J Bone Joint Surg*. 2010;92:1–5.
45. Centers for Disease Control and Prevention. It is now clear from a plethora of published research that there is no safe blood level of lead in children. https://www.cdc.gov/nech/lead/. Accessed January 1, 24, 2018.
46. Gonzalez-Ramirez D, Maiorino RM, Zuniga-Charles M. Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans. II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterey, Mexico. *J Pharmacol Exper Ther*. 1995;272:264–274.
47. Schweinsberg F. Risk estimation of mercury intake from different sources. *Toxicol Lett*. 1994;72:345–351.
48. Begerow J, Zander D, Freier I, et al. Long-term mercury excretion in urine after removal of amalgam fillings. *Int Arch Occup Environ Health*. 1994;66:209–212.
49. Buchet JP, Pauwels J Lauwerys R. Assessment of exposure to inorganic arsenic following ingestion of marine organisms. *Environ Res*. 1994;66:44–51.

50. National Research Council. *Toxicological Effects of Methylmercury*. Washington, DC: National Academies Press; 2000.
51. Lauwerys R, Bernard A, Roels HA, et al. Cadmium exposure markers as predictors of nephrotoxic effects. *Clin Chem*. 1994;40:1391–1394.
52. Herber RFM. Cadmium. In: Seiler HG, Sigel A, Sigel H, eds. *Handbook on Metals in Clinical and Analytical Chemistry*. New York: Dekker; 1994:202.
53. Verschoor M, Herber R, Van Hemmen J, et al. Renal function of workers with low-level cadmium exposure. *Scand J Work Environ Health*. 1987;13:232–238.
54. Barregard L, Hultberg B, Schutz A, et al. Enzymuria in workers exposed to inorganic mercury. *Int Occup Environ Health*. 1988;61:65–69.
55. Inigues JL, Leverger G, Dolfus C. Lead mobilization test in children with lead poisoning: validation of a 5-hour EDTA calcium disodium provocation test. *Arch Ped Adol Med*. 1995;149:338–340.
56. Centers for Disease Control. Preventing lead poisoning in young children. *J Pediatr*. 1978;93:709–714.
57. Chisolm Jr JJ. Chelation therapy in children with subclinical plumbism. *J Pediatr*. 1974;53:441–449.
58. Chisolm Jr JJ. The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood. *J Pediatr*. 1968;73:1–15.
59. Charlton N, Wallace KL. Post-chelator challenge urinary metal testing: position statement. http://www.acmt.net/cig/page.cig? Accessed August 22, 2009.
60. Kathuria A. Lead nephropathy. http://emedicine.medscape.com/article/242605-overview. Accessed November 7, 2011.
61. Markowitz ME, Rosen JF. Assessment of lead stores in children: validation of an 8-hour CaNa2EDTA provocative test. *J Pediatr*. 1984;104:337–341.
62. Hibberd AR, Howard MA, Hunnisett AG. Mercury from dental amalgam fillings: studies on oral chelating agents for assessing and reducing mercury burdens in humans. *J Nutr Environ Med*. 1998;8:219–231.
63. Rozema TC. Protocols for chelation therapy. *J Adv Med*. 1997;10:3–100.
64. Quig DW. Systematic and functional assessment of nutritional status and exposure to toxins. Paper presented at Orthomolecular Health and Medicine Conference, San Francisco; 2002.
65. Keith RL, Setiarahardojo I, Fernando Q, et al. Utilization of renal slices to evaluate the efficiency of chelating agents for removing mercury from the kidney. *Toxicology*. 1997;116:67–75.
66. Clarkson TW, Magos L, Cox C, et al. Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak. *J Pharmacol Exp Therap*. 1981;218:74–83.
67. Aposhian HV, Bruce DC, Alter W, et al. Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: correlation with dental amalgams. *FASEB J*. 1992;6:2472–2476.
68. Aposhian HV, Maiorino RM, Gonzalez-Ramirez D, et al. Mobilization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology*. 1995;97:23–38.
69. Aposhian HV. DMSA and DMPS: water soluble antidotes for heavy metal poisoning. *Ann Rev Toxicol*. 1983;23:193–215.
70. Campbell JR, Clarkson TW, Omar MD. The therapeutic use of 2,3-dimercaptopropane-1-sulfonate in two cases of inorganic mercury poisoning. *JAMA*. 1986;256:3127–3130.
71. Wax PM, Thorton CA. Recovery from severe arsenic-induced peripheral neuropathy with 2,3-dimercapto-1-propanesulfonic acid. *Clin Toxicol*. 2000;38:777–780.
72. Dimaval (DMPS). *Scientific Product Monograph*. 7th ed. Berlin: Heyl; 2008.
73. Dimival (DMPS). *Summary of Scientific Literature*. Houston: Heyltex Corp.; 1991.
74. Graziano JH, Siris ES, LoIacono NJ, et al. 2,3-dimercaptosuccinic acid as an antidote for lead intoxication. *Clin Pharmacol Ther*. 1985;37:431–438.
75. Grandjean P, Jacobsen IA, Jorgensen PJ. Chronic lead poisoning treated with dimercaptosuccinic acid. *Pharmacol Toxicol*. 1991;68:266–269.
76. Roels HA, Boeckx M, Ceulemans E. Urinary excretion of mercury after occupational exposure to mercury vapour and influence of the chelating agent meso-2,3-dimercaptosuccinic acid (DMSA). *Br J Ind Med*. 1991;48:247–253.
77. Smith D, Bayer L, Strupp B. Efficiency of succimer chelation for reducing brain lead levels in a rodent model. *Environ Res*. 1998;78:168–176.
78. Pande M, Mehta A, Pant BP, et al. Combined administration of a chelating agent and an antioxidant in the prevention and treatment of acute lead intoxication. *Environ Toxicol Pharmacol*. 2001;9:173–184.
79. Pande M, Flora SJS. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimer in rats. *Toxicology*. 2002;177:187–196.
80. Lee B, Schwartz BS, Stewart W, et al. Provocation chelation with DMSA and EDTA: evidence for differential access to lead storage sites. *Occup Environ Med*. 1995;52:13–19.
81. Besunder JB, Super DM, Anderson RL. Comparison of dimercaptosuccinic acid and calcium disodium ethylenediaminetetraacetic acid versus dimercaptopropanol and ethylenediaminetetraacetic acid in children with lead poisoning. *J Pediatr*. 1997;130:966–971.
82. Aaseth J. Treatment of mercury and lead poisonings with dimercaptosuccinic acid and sodium dimercaptopropane sulfate. *Analyst*. 1995;120:853–854.
83. Jones MM, Singh PK, Kostial K, et al. Comparative in vivo lead mobilization with meso- and rac-2,3-dimercapto-succinic acids in Albino Wistar rats. *Pharmacol Toxicol*. 1997;186:182–186.
84. Cohen JP, Ruha AM, Curry SC, et al. Plasma and urine dimercaptopropanesulfonate concentrations after dermal application of transdermal DMPS (TD-DMPS). *J Med Toxicol*. 2013;9(1):9–15. PMID: 23143832.
85. Gong Z, Evans HL. Effects of chelation with meso-dimercaptosuccinic acid (DMSA) before and after the appearance of lead-induced neurotoxicity in the rat. *Toxicol Appl Pharmacol*. 1997;144:205–214.
86. Gregus Z, Stein AF, Varga F, et al. Effect of lipoic acid on biliary excretion of glutathione and metals. *Toxicol Appl Pharmacol*. 1992;114:88–96.
87. Maiorino RM, Aposhian MM, Xu ZF, et al. Determination and metabolism of thiol chelating agents. XV. The meso-2,3-dimercaptosuccinic acid-cysteine (1:2) mixed disulfide, a major urinary metabolite of DMSA in the human, increases the urinary excretion of lead in the rat. *J Pharmacol Exp Therap*. 1993;267:1221–1226.
88. Miller A. Dimercaptosuccinic acid (DMSA), a non-toxic, water-soluble treatment for heavy metal toxicity. *Altern Med Rev*. 1998;3:199–207.
89. Frumkin H, Manning CC, Williams PL, et al. Diagnostic chelation challenge with DMSA: a biomarker of long-term mercury exposure? *Environ Health Persp*. 2001;109:167–171.
90. Bass DA, Urek K, Quig D. Measurement of mercury in feces. Poster presented at American Association of Clinical Chemistry Conference, July 25-29. New Orleans; 1999.
91. Bjorkman L, Sandborg-Englund G, Ekstrand J. Mercury in saliva and feces after removal of amalgam fillings. *Toxicol Appl Pharmacol*. 1997;144:156–162.
92. Apostoli P, Bartoli D, Alessio L, et al. Biological monitoring of occupational exposure to inorganic arsenic. *Occup Environ Med*. 1999;56:825–832.

23

Mineral Status Evaluation

Andrea Gruszecki, ND

OUTLINE

INTRODUCTION

Minerals are inorganic elements found in the earth's crust; they are found in soil and water in varying proportions. The therapeutic effect of minerals has been recognized for more than 2000 years in Asian medicine[1] and Western civilization. As early as the 2nd century BC, salt was mixed in vinegar with other spices and prescribed for various health conditions.[2] The Native American *Dine*' (Navajo) people traditionally used juniper ash in recipes to maintain their health; juniper ash is now recognized as a source of calcium.[3] In Europe, calcium was first recognized as an essential element (for fowl) in 1790 by Dr. George Fordyce, and iodine was first recognized as a component of thyroxine by chemist Eugen Baumann in 1896.[4] Scientific studies assessing the bioavailability of minerals in humans first appeared in the literature in the 1960s,[5,6,7] and over the ensuing years, it became clear that minerals play an important role in the biochemistry of the human body.[8,9]

The human body may contain over 60 elements; currently, 15 of these elements are considered essential. Although up to 25 elements may be considered functional in human physiology, the question of which elements are essential for human life and health remains unresolved for some of the micromineral ("trace") elements.[10] Either a deficiency or an excess of physiologically active minerals may have deleterious effects on multiple enzyme systems, nerve tissues, and organs, including the brain, heart, thyroid, liver, kidneys, and skin. Reliable assessments of mineral element status are required to evaluate and monitor a patient's mineral status.[11] Analytical considerations for assessment may include specimen selection, collection equipment, preanalytical variables, and methodology.[12]

SPECIMEN SELECTION

The majority of nutritional elements are best evaluated in blood or urine.[13] Hair may be useful in the determination of some mineral element deficiencies.[14] (See Chapter 16, Hair Mineral Analysis, for a critical review of the research.)

Blood

If blood is selected as the specimen type, further consideration should be given to which compartment of blood (serum, cell, or both) is required for accurate evaluation. For example, the cations sodium, potassium, calcium, and chloride are important extracellular components of the blood's serum compartment. Clinically significant electrolyte abnormalities are common with poor oral intake, age over 65, alcoholism, diuretic use, and/or recent history of electrolyte abnormality. These clinically present with symptoms such as vomiting, chronic hypertension, recent seizure, and/or muscle weakness.[15] Urinary electrolytes are more commonly analyzed in hospital settings and may provide insight regarding volume status, hyponatremia, acute kidney injury, metabolic alkalosis, hypokalemia, and urine anion gap (net charge).[16] The use of older diuretic medications may also disrupt electrolyte balance and influence the selection of specimens.[17] Recent dietary intake may rapidly alter serum levels of other minerals or cause wide day-to-day variation in serum or urine levels. Nutrient elements that serve as cofactors in proteins and enzymes, such as magnesium[18] and zinc,[19] may be primarily intracellular and may be best assessed from within blood cells (erythrocytes). Whole blood and intracellular assessments provide a longer window of exposure than serum samples

because the elements within blood cells represent 60 to 120 days of nutrient element exposure. Both compartments may be assessed with a whole-blood specimen, which may be preferred if an excess of a nutrient element, such as manganese, iron, or copper, is suspected.[13]

Urine

Urine may be the preferred specimen for nutrients such as iodine.[11] Urinary assessments require normal renal function for accuracy. Animal studies indicate that excess mineral consumption is excreted in the urine to maintain homeostasis, and excretion may rapidly decrease to reflect an insufficient intake of all minerals.[20]

Hair

Hair may accurately reflect a nutrient element deficiency, and some hair mineral ratios have been associated with medical disorders.[21] However, the research has been inconsistent. Newer studies are confirming relationships between hair and blood for some nutrient elements and disproving others.[22] Other studies are exploring the relationship between hair element concentrations and specific disease states.[23] The interpretation of hair results may be confounded by the ease with which hair can be contaminated by external sources of exposure. With that proviso, hair analysis can accurately reflect exposure to, and absorption of, a limited number of elements (e.g., chromium) or deficiencies of others (e.g., copper). Research is ongoing to define the physiologic mechanisms that incorporate nutrient elements into hair tissues. Currently, for many mineral elements, a quantitative measurement from a hair sample may not represent a quantitative measurement of an ingested dose of mineral from the diet and supplements.[24] In addition, external contamination may mask a deficiency or artificially increase the amount of mineral element found in a hair sample because external contaminants bind to the cysteine residues of the hair.[25] The most appropriate use of hair analysis appears to be in the assessment of toxic metal exposure. The utility of hair analysis remains highly controversial; it remains an "unproven practice" according to the American Medical Association, yet it is approved by the Centers for Disease Control and Prevention for the assessment of methylmercury and other toxic metals.[26]

COLLECTION EQUIPMENT

Clinicians may employ whole-blood or urine analysis in the evaluation of mineral status because these fluids are the simplest and most economical to collect and transport.[12,14] Tests of whole blood require no centrifugation and, like urine, require no special treatment other than collection, refrigeration, and shipping in approved containers. If samples of cellular blood components are collected, they must be centrifuged immediately per the laboratory's instructions. If not centrifuged in a timely manner, cells may disintegrate, falsely increasing the amount of nutrient elements in the serum or plasma of the sample and falsely decreasing the amount of nutrient element available in the surviving cells. Appropriate, mineral-free containers provided by the laboratory must be used with most element collections to prevent possible leaching of elements from collection devices or shipping containers. Hair analysis has the benefit of convenience and low cost. Hair samples are noninvasive, are stable at room temperature, and require no onsite processing to improve sample quality.

PREANALYTICAL VARIABLES

Preanalytical variables in the specimen-collection process occur before the receipt of a specimen by the laboratory.[12,27,28] Careful attention to collection, preparation, and shipping may decrease preanalytical errors that may affect laboratory results. The timing of specimen collections may be important because some results may be affected by a recent intake of meals or medicines. Variables such as age, gender, ethnicity, time of day (diurnal rhythms), season, tobacco use, or the presence of comorbid conditions may alter analyte levels and must be borne in mind by the interpreting physician. Alterations in renal function may be particularly problematic for the collection of urine specimens. Liver disease or inflammation may alter the levels of acute-phase and metallothionein proteins that bind to nutrient elements and metals.[29] Renal wasting disorders may increase urine levels and decrease blood levels of creatinine, minerals, and other analytes. Renal clearance disorders may decrease urine levels of creatinine, minerals, and other analytes.[30] Medications, such as diuretics, may alter renal function and mineral levels; all medications may be reviewed with the patient's pharmacist before interpreting results.

METHODOLOGY

The analytical method used by a laboratory to assess nutrient elements and other metals must be sensitive, specific, accurate, precise, and timely.[12] The detection limit of the methodology is important for the analysis of trace or "ultratrace" microminerals. Spectrometry methodologies, such as inductively coupled plasma-mass spectrometry or inductively coupled plasma-optical emission spectrometry, are considered state of the art. Reputable laboratories are Clinical Laboratory Improvement Amendments (CLIA) certified, use highly trained personnel, and choose to participate in third-party proficiency testing.[31] Proficiency testing ensures the reproducibility and reliability of the laboratory's internal quality control programs (IQCPs) and ensures reliable, reproducible results.[32] Some of the quality controls that may be used in each batch of samples processed include the use of reagent blanks, the replication of analyses to ensure precision, and the use of certified reference samples with known amounts of the metals of interest to ensure accuracy, specificity, and sensitivity. Reference values for some minerals have been standardized in the literature; for other elements, laboratories must establish their own reference values.

MINERALS AND DISEASE

Several minerals are required to maintain the necessary functions of life.[12,11,33] Minerals may be considered essential if a deficiency (lack) of the mineral results in significant disease or death. Nonessential minerals may affect physiologic function and have health benefits, but they have not yet been proven essential. Macrominerals are required in daily amounts of milligrams to grams; microminerals are required in daily amounts of micrograms to milligrams. Some minerals, such as electrolytes, dissociate into charged ions in the body fluids (electrolytes) and may be important in the maintenance of membrane potentials needed for muscle contractions and nerve conduction. Both macro- and microminerals are necessary cofactors in metabolic functions, and either an excess or a deficiency may be detrimental to health.

Homeostatic regulation maintains the balance of nutrient elements in the body. Regulation mechanisms may include intestinal assimilation, protein chaperones for blood transportation, storage in body tissues, and excretion mechanisms (Fig. 23.1).

Homeostatic mechanisms may be disrupted by genetics, poor diet, substances that block assimilation, toxins that displace minerals or compete for receptor sites on enzymes and cells, diseases of malabsorption, liver disease, or renal disorders. Either over- or undernutrition may be problematic for mineral homeostasis.[34] High levels of meat consumption in Western diets may excessively raise iron levels, and consumption of processed foods may result in a dietary deficiency

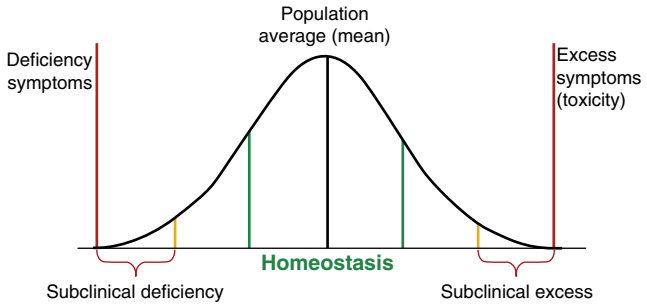


Fig. 23.1 The level of a mineral element in a tissue sample is determined by mineral availability and individual variance in assimilation, transport, and cellular uptake capacity.[12,37]

of multiple minerals.[35,36] Inherited mutations that disrupt mineral homeostasis are rare, but important, disruptors of patient health. The most common disorders include the iron-storage disorder hemochromatosis, the copper disorders Wilson disease and Menkes syndrome, the zinc-deficiency disorder acrodermatitis enteropathica, and sulfite oxidase deficiency (lack of molybdenum cofactor). The effects of inherited variation in the general population on mineral status is an important new area of research.[37]

Levels of various nutrient elements have been associated with disease states and may be considered clinical biomarkers.[8] Cirrhosis may lower levels of serum selenium,[38] calcium,[39] magnesium,[40] and zinc.[41] Emphysema or certain cancers may increase serum copper levels, and both copper and manganese levels may increase during congestive heart failure, infection, or psychoses.[42] Heart tissue levels of selenium, iron, copper, zinc, and phosphorus have been associated with ejection fraction and cardiac index.[43] In men infected with human immunodeficiency virus (HIV), helper T-type 4 cells appear closely correlated with serum magnesium concentration.[44] Although other associations have been observed between trace minerals and breast cancer,[45] gastrointestinal malignancy,[46] and malignant ascites,[47] there have been null studies for these conditions as well. It is important to remember that observed associations between certain disorders may be variable and based on patient status, ethnicity, or the stage of the disease when studied; both may confound the results of such studies.

The ratios between some trace elements have been associated with specific disorders. Levels of magnesium and copper, and their ratios, may be predictive for lower limb ischemia, atherosclerosis obliterans, and risk of aortic aneurysm.[48] The relative concentrations of copper, zinc, and selenium in whole blood and thyroid tissue may present in specific patterns for various thyroid disorders and thyroid cancer. Serum copper and copper/zinc ratios were shown to be increased in breast cancer but not in benign breast diseases.[49] In another study, serum copper-to-zinc ratios were shown to be of diagnostic and prognostic value in head, face, and neck cancer, with alterations in copper, zinc, and the copper-to-zinc ratio related to the stage of the disease.[50] Clinicians specializing in the treatment of certain disorders may wish to review the literature for associations between mineral status and the disorder of interest.

ESSENTIAL MACROMINERALS

Calcium

Calcium is the most common cation in the body and the primary component of tooth and bone.[51] Normal calcium levels are necessary for muscle contraction, enzyme function, protein stabilization, neuron firing, hormone release,[52] and blood coagulation.[53] Calcium may also act as a second messenger for some cell-signaling pathways. Calcium reserves in the body are maintained through multiple mechanisms, which include parathyroid hormone, dietary calcium intake, assimilation of calcium from the digestive tract, and renal calcium excretion. Disruption of these calcium homeostasis mechanisms may result in either hypercalcemia or hypocalcemia.[54,55]

The parathyroid gland monitors the blood calcium level and responds to low calcium concentrations by releasing parathyroid hormone (PTH). PTH increases the level of 1,25-dihydroxyvitamin D (calcitriol) and stimulates bone resorption to increase blood calcium levels. Serum calcium is so closely regulated by the parathyroid gland that its use as an indicator of calcium balance is not reliable when considered in isolation. Calcium is also regulated as it is transported across cell membranes; calcium status affects the function of the endoplasmic reticulum, the mitochondria,[56] and the sarcoplasmic reticulum of muscle cells. Measurement of physiologically active ionized calcium may be more useful in the independent evaluation of calcium status.

Hypercalcemia is defined as a total serum calcium concentration of greater than 10.4 mg/dL or an ionized serum calcium of greater than 5.2 mg/dL.[55,57] Hypercalcemia may occur because of excessive bone resorption (immobilization), hyperparathyroidism, vitamin D toxicity, and the presence of cancer or granulomatous disorders. Hypercalcemia occurs when the kidney's capacity to excrete calcium is exceeded. Mild hypercalcemia may be asymptomatic. More severe hypercalcemia may result in anorexia, nausea, vomiting, constipation, abdominal pain, fatigue, polyuria, arrhythmia, and hypertension.[58] Severe hypercalcemia may progress to symptoms of confusion, delirium, and coma. Hypercalcemia may induce renal insufficiency, nephrogenic diabetes insipidus, kidney stones, or vascular and soft tissue calcifications.

Hypocalcemia is defined as a total serum calcium concentration of less than 8.8 mg/dL or an ionized calcium level of less than 4.7 mg/dL with normal plasma proteins.[55,59] Hypocalcemia may occur because of hypoparathyroidism or pseudohypoparathyroidism, vitamin D deficiency or dependency, magnesium deficiency, acute pancreatitis, hyperphosphatemia, or renal tubular disease. Renal tubular disease may increase calcium excretion into the urine or decrease the conversion of precursors into active 1,25-dihydroxyvitamin D. During pregnancy, serum calcium and albumin levels may decrease. Medications, such as anticonvulsants, rifampin, furosemide, or bisphosphonates, may contribute to hypocalcemia. Steroid medications may decrease the gastrointestinal absorption of calcium. Hypocalcemia is typically asymptomatic but may present with dry and scaly skin, brittle nails, coarse hair, muscle cramps (legs or back), swelling of the optic nerve (papilledema), and cardiac arrhythmia or neuropsychiatric symptoms such as depression, cognitive decline, or psychosis due to diffuse encephalopathy.[58,59] More severe hypocalcemia may progress to neuromuscular irritability (spasms, tetany) and sensory parasthesias. Hypocalcemia may be associated with liver disease, nephrotic syndrome, congestive heart failure, or malnutrition.

Calcium may be evaluated in serum, whole blood, urine, or hair samples.[54,55] Serum calcium is reported as total calcium (approximately 40% protein-bound and 60% ionized calcium ions). High serum protein (albumin) levels may falsely elevate and low serum protein levels may decrease serum calcium levels. A whole-blood analysis of calcium will assess calcium present in serum, the cell membranes, and intracellularly. Urinary calcium levels will reflect gastrointestinal assimilation, bone turnover, and renal filtration. Up to 300 mg/24 hours may be excreted by subjects on unrestricted diets. Hair calcium may reflect nutritional status, and the hair calcium-to-magnesium ratio has been associated with levels of coronary artery calcification in older subjects.[60,61] Hair mineral deposition may also be altered by the presence of comorbid disorders.[62] However, hair contamination may occur because of calcium-rich "hard" water or hair products (perms, dyes, bleaches).[63]

Calcium (Ca) may be considered in relation to phosphorus (P), and a Ca:P ratio of 1.3:1 has been suggested for the maintenance of bone density.[64] Reduced calcium and increased phosphorus intake is common in Western diets and results in a low Ca:P ratio. Food sources of calcium include dairy products, canned fish with bones (e.g., salmon, sardines), green leafy vegetables, nuts, and seeds. Assessment of dietary intake of calcium is confounded by multiple factors that affect absorption, such as the quantity of fiber and other natural chelators in the diet,[65] gastric acidity, the ratio of dietary calcium to phosphorus and magnesium, gut transit time, and other factors.

Chloride

Because chloride is a halogen, most do not consider it a mineral, but technically it is classified as such. Chloride is the most common anion in the body and is primarily found in the extracellular blood compartment (serum or plasma).[66,67] Chloride, with sodium, potassium, and bicarbonate, regulates water distribution, osmotic pressure, pH, and ion balance in the extracellular compartment of the blood. Chloride is necessary for the production of hydrochloric acid in the stomach and is also essential in cellular pump functions. Chloride levels are regulated by renal excretion into urine, and chloride may also be excreted in sweat.

Chloride is typically evaluated as part of an electrolyte (serum elements) panel; levels of chloride (Cl) and sodium (Na) indicate the amount of salt (NaCl) in the blood. Symptoms of an electrolyte imbalance may include chronic vomiting, diarrhea, weakness, or respiratory distress. Chloride and other electrolytes may become imbalanced during episodes of metabolic or respiratory acidosis or alkalosis. Renal disorders may also disrupt electrolyte balances.[68] Dehydration, diuretic medications, and high doses of either antacids or baking soda may lower chloride levels. Glucocorticoid or mineralocorticoid medications may alter electrolyte balance and increase urine output.[69] Chloride ions are obtained through the diet and normally pass easily through the intestinal barrier.

Chloride may be evaluated in serum and urine, and urinary chloride is a necessary component of a full electrolyte assessment or metabolic panel.[66,67] Chloride sweat testing may be used in the diagnosis of cystic fibrosis.[70] Urine chloride analysis, and often the analysis of urine sodium as well, may be important when considering alkalosis or acidosis or when assessing high or low serum chloride levels. Depending on the patient's condition, serum and urine chloride levels may differ. Urinary chloride may help determine whether chloride loss is related to salt loss or due to an excess of adrenal hormones (cortisol or aldosterone), which may alter electrolyte excretion. Increased serum chloride (hyperchloremia) may occur because of dehydration, Cushing disease, or kidney disease (renal clearance disorders). Acid–base dysregulation resulting in metabolic acidosis or respiratory alkalosis may also increase serum chloride.[71] Low serum chloride (hypochloremia) may occur because of congestive heart failure, chronic vomiting, or Addison disease. Lung diseases, such as emphysema, may result in chronic respiratory acidosis and metabolic alkalosis, lowering serum chloride. Decreased urinary chloride may occur because of Cushing disease, primary aldosteronism, congestive heart failure, gastrointestinal malabsorption, or diarrhea. Increased urinary chloride may occur because of dehydration, Addison disease, high salt intake, or insufficient calorie intake. If both urinary chloride and sodium are elevated in a patient on a salt-restricted diet, the patient may not be diet-compliant. See Chapter 12, Fantus Test, for the use of urinary chloride as a measure of salt consumption.

Magnesium

Magnesium is a cation found primarily in the intracellular (35%–45%) compartment of the blood.[55,72,73] Up to 55% of magnesium is found in bone, and this reservoir may be mobilized for the maintenance of serum levels. Serum magnesium levels may affect nerve conduction, muscle contraction, and cardiovascular functions. Transportation of magnesium across the cell membrane is regulated by dedicated transporters. Magnesium is a cofactor for over 300 intracellular enzymes that participate in energy production, oxidative phosphorylation, glycolysis, cell replication, nucleotide (DNA) metabolism, and protein synthesis. Approximately 80% of a cell's magnesium is bound to ATP, which helps make the ATP bioactive.[74]

Hypermagnesemia (serum concentration >2.6 mg/dL) is uncommon without magnesium supplementation (or ingested as laxatives or antacids).[75] Other causes may include renal failure, hyperparathyroidism, dehydration, early diabetic acidosis, Addison disease, rhabdomyolysis (muscle breakdown), or a history of familial hypocalciuric hypercalcemia. Increased serum magnesium and copper levels may be seen with seizure disorders. High magnesium concentrations may decrease serum calcium levels. Medications that may increase magnesium levels include aspirin, thyroid medication, potassium-sparing diuretics, lithium carbonate, and antibiotics (review medications with a pharmacist).[74] Magnesium intoxication may occur if magnesium is supplemented and renal failure is present. Deep tendon reflexes are lost as magnesium levels increase above 5 mg/dL. As levels rise higher, magnesium depresses the nervous system; symptoms of magnesium intoxication may include hyporeflexia, hypotension, lethargy, disorientation, respiratory depression, and cardiac arrhythmia leading to cardiac arrest (>15 mg/dL).

Hypomagnesemia occurs because of inadequate magnesium intake, malabsorption, or increased excretion from the kidney or gastrointestinal tract.[76] Low magnesium concentrations may be associated with poor diet, alcoholism, inflammatory bowel disorders, poorly controlled diabetes, chronic diarrhea, recent hospitalization or surgery, or toxemia of pregnancy.[77] Magnesium blood levels are also lower in the second and third trimesters of a normal pregnancy. Medications such as proton-pump inhibitors, antacids, diuretics, digoxin, insulin, laxatives, phenytoin, glucocorticoids, steroid hormones, hormone antagonists, some antibiotics, antihistamines, and antivirals may decrease magnesium levels (review medications with a pharmacist).[74] High doses of calcium, vitamin D, or caffeine may further exacerbate magnesium losses. Low magnesium concentrations may result in symptoms of neuromuscular excitability (muscle cramps, spasms) or cardiac arrhythmia. Good dietary sources include green leafy vegetables, nuts, soybeans, and cocoa mass. Hard water may contain magnesium salts and contribute to magnesium intake.

Serum magnesium may be the most commonly used but least accurate assessment of magnesium status.[55] The serum magnesium concentration may be influenced by recent dietary intake and other factors; it constitutes only about 1% to 3% of total body magnesium and does not reflect magnesium levels in cells.[77] The binding of magnesium to serum proteins is subject to many uncontrollable variables. Serum magnesium levels as low as 1.2 mEq/L have been measured in patients with normal total body magnesium.[78] Measurement of magnesium status presents some difficulties; currently, the magnesium retention (load or tolerance) test may be the most accurate, although cumbersome, method of assessment in adults.[79] The retention test requires a parenteral infusion of magnesium with subsequent urine collection to evaluate urinary magnesium.[80]

Other methods of assessing magnesium include ionized magnesium concentrations (in blood, plasma, or serum), urinary magnesium, and erythrocyte concentrations.[55] Erythrocyte concentrations of magnesium are not subject to the same transient fluctuations as serum and may be more reliable indicators of status than serum levels.[81] The analysis of white blood cell (WBC) magnesium content has also been

explored. The concentration of magnesium in leukocytes has been inversely associated with the risk of tachyarrhythmias. WBC assessments of magnesium may not be accurate in all patients; inherited variation in the *MAGT1* magnesium transporter gene[82] may reduce the amount of magnesium in WBCs and result in a form of immunodeficiency. The best test to evaluate total body magnesium sufficiency may well be whole-blood analysis because this will assess total magnesium in serum, intracellularly, and in the cell membranes of both red blood cells (RBCs) and WBCs. Ionized magnesium and total magnesium appear to provide similar information.[83] Increased urinary excretion in individuals with type 1 diabetes has been shown to decrease erythrocyte levels, without significant hypomagnesemia.[84] A 24-hour urinary magnesium may be useful to reflect dietary changes in magnesium intake.[85] Healthy kidneys restrict the excretion of magnesium when concentrations or intakes are low and permit excretion when serum levels are restored. The current scientific literature remains divided in regard to hair Mg levels reflecting intracellular levels. External Mg contamination of hair may result from recent hair treatment, hair color, or hard water exposure, confounding hair element evaluations.

Phosphorus

The element phosphorus is a necessary component in cell membranes, DNA, and RNA.[55,86] Phosphorus is primarily used in bone mineralization (85%), adenosine triphosphate (ATP) metabolism, pH homeostasis, and cell signaling. Phosphorus is bound to oxygen in all living systems and is found in the body as phosphate (PO_4^{3-}). Phosphorus, in the form of phosphoryl groups (PO_3), is attached to and removed from molecules during cell signaling. Phosphorus homeostasis is regulated by the parathyroid gland and vitamin D.

Hyperphosphatemia may occur because of renal insufficiency (GFR < 30 mL/min), hypoparathyroidism, pseudohypoparathyroidism (hormone resistance), or granulomatous disease (immunodeficiency).[86,87] Less commonly, hyperphosphatemia may occur because of diabetic ketoacidosis, rhabdomyolysis, tumor lysis syndrome (cancer therapy), and excessive exercise or occasionally because of the excessive use of oral or rectal (enema) phosphate salts. The condition is asymptomatic but may present with symptoms if hypocalcemia is also present (dry and scaly skin, brittle nails, coarse hair, muscle cramps [legs or back], swelling of the optic nerve [papilledema], cardiac arrhythmia, or neuropsychiatric symptoms). Elevated serum phosphate has been associated with an increased risk of cardiovascular disease, heart failure, and kidney disease. Chronic hyperphosphatemia may induce the precipitation of calcium into vascular and soft tissues; the calcifications may appear on imaging studies.

The kidney normally compensates for low phosphorus intake. Hypophosphatemia occurs when serum phosphate is less than 2.5 mg/dL.[86,88] Symptoms of hypophosphatemia may include anorexia, anemia, muscle weakness, bone pain, increased susceptibility to infections, peripheral parasthesias, loss of coordination, or respiratory distress. In children, chronic hypophosphatemia may present as rickets; in adults, it may present as osteomalacia. Causes of hypophosphatemia include alcoholism, respiratory alkalosis, the recovery phase of diabetic ketoacidosis, hyperparathyroidism, Cushing syndrome, hypothyroidism, vitamin D deficiency, malabsorption syndromes, renal wasting, severe anorexia nervosa, or the presence of other electrolyte imbalances (hypomagnesemia or hypokalemia). The excessive use of theophylline (respiratory inhalers) or antacids and the chronic use of diuretic medications may also deplete phosphate levels.

Phosphate is measured in serum, plasma, or urine.[55] Serum or plasma phosphate results may be compromised if specimens are hemolyzed, have a high fat content, or have high bilirubin concentrations. With normal renal function, urinary phosphorus reflects dietary status. Hair phosphorus has not been documented to reflect dietary status or biochemical phosphorus status.

Potassium

Potassium is the primary intracellular cation.[89–91] Intracellular potassium concentrations may be 30 times higher than serum or plasma levels. The high intracellular levels are maintained by adenosine triphosphate (ATP)-dependent cell membrane transporters, which exchange sodium for potassium. The sodium–potassium "pumps" are essential for the maintenance of the ionic gradients (membrane potentials) needed for muscle contractions and nerve conduction. Potassium diffuses out of cells with the concentration gradient if the pump's activity is inhibited. The kidney is slow to adapt to changes in potassium concentrations in the blood.

Hyperkalemia is defined as a serum potassium concentration of greater than 5.5 mEq/L.[89,90,92] Symptoms of hyperkalemia may include peripheral tingling or paresthesias; muscle weakness, which rarely progresses to flaccid paralysis; and cardiac arrhythmia, which may progress to ventricular fibrillation or asystole. Hyperkalemia may be asymptomatic until arrhythmias occur. The causes may include increased potassium intake (supplements), kidney injury or chronic renal disease that decrease excretion, hypoaldosteronism, Addison disease (with sodium depletion), congenital adrenal hyperplasia (salt wasting), metabolic acidosis, and dehydration. The ingestion of large amounts of fruit or fruit juices may increase potassium levels. Medications that may contribute to hyperkalemia include potassium-sparing diuretics, angiotensin-converting enzyme (ACE) inhibitors, nonsteroidal anti-inflammatory agents (NSAIDs), heparin, digitalis, α- and β-blocker antihypertensives, and angiotensin receptor blockers. "Pseudohyperkalemia" may occur if comorbid platelet, erythrocyte, or mixed-type blood disorders are present and may result in preanalytical platelet activation or hemolysis.[93] It can also occur from excessive, long-term consumption of glycyrrhizin from licorice root. Plasma potassium may be more accurate in such patients.

Hypokalemia is defined as a serum potassium concentration of less than 3.5 mEq/L.[89,90,94] Hypokalemia may be most apparent in erythrocytes; potassium is primarily an intracellular electrolyte.[95] Symptoms of potassium loss may begin with fatigue or muscle weakness and cramping and progress to tetany, rhabdomyolysis (muscle breakdown), respiratory distress, muscle and intestinal paralysis (bloating, constipation, pain), and cardiac arrhythmias. Chronic hypokalemia may impair the kidney's ability to concentrate urine and result in polyuria (excessive urination) and polydipsia (excessive thirst). Hypokalemia is usually caused by decreased potassium intake or increased potassium losses from the gastrointestinal tract or the kidney. Gastrointestinal losses may be precipitated by severe or chronic vomiting or diarrhea, laxative abuse, bowel diversion surgery, villous adenoma of the colon (rare), or the ingestion of bentonite clay (binds potassium). Renal losses may occur because of Cushing syndrome, hyperaldosteronism, congenital renal hyperplasia, acquired renal tubular dysfunction, or inherited renal-wasting disorders (Bartter, Gitelman, Liddle, or Fanconi syndrome). Hypomagnesemia may induce increased renal excretion of potassium, as may excessive intake of glycyrrhizin, a component of black licorice candies and licorice root herbal supplements. A potassium shift from the intracellular to the extracellular compartment may occur because of hyperthyroidism or the rare, inherited condition familial periodic paralysis. Medications that may contribute to hypokalemia include β-adrenergic agonists, decongestants, bronchodilators, diuretics, mineralocorticoids, glucocorticoids, antibiotics, caffeine, sodium polystyrene sulfonate, and labor-suppressing medications. Fruits, vegetables, seeds, and nuts are good dietary sources of potassium. Potassium supplements may be required by some individuals, but their use must be carefully monitored.

Potassium concentrations may be evaluated in serum, plasma, erythrocytes, whole blood, or urine.[89] Hair potassium has not currently been associated with either dietary intake or nutrient status. Reference values are different for plasma and serum samples. Platelets rupture during coagulation, and the increased potassium is found in the serum, so the serum reference values are higher. The patient's platelet count may have an effect on the potassium level in serum samples as well; high platelet counts (thrombocythemia, thrombocytosis) may result in pseudohyperkalemia. Blood samples must be processed quickly and correctly to prevent preanalytical errors that may occur. Cooling of a sample before separation may falsely increase serum or plasma potassium levels. Leaving unseparated samples at room temperature for long periods before processing may falsely lower serum or plasma potassium levels. Delayed transport, rough handling, or failure to release the arm tourniquet before blood collection may cause tissue damage and falsely elevate potassium samples as erythrocytes break open. In addition, serum or plasma concentrations may not reflect intracellular losses during early potassium deficiency. The renal tubular response is slow to adapt to potassium depletion and may take a week or more to adjust ion exchanges and conserve potassium.

Erythrocyte (RBC) potassium concentrations may be a more accurate indicator of the potassium content in other types of cells than serum or plasma.[95] Although RBCs do not have nuclei, the sodium–potassium membrane pump that maintains the proper influx and efflux of these ions is intact and may respond quickly to restore intracellular levels when potassium becomes available. The rapid restoration of intracellular levels is essential for the continued firing of neurons and for cardiac contractility. Reduced intracellular levels have been associated with changes in the cardiac recovery phase (electrocardiograms).[96] Evidence indicates that obesity may decrease the efficiency of the sodium–potassium pumps.[97] The whole-blood potassium concentration may be almost as accurate as the RBC potassium level because 98% of potassium is intracellular.[98]

Urinary potassium may be used to confirm and diagnose abnormal blood potassium levels.[89] Urinary renal excretion of potassium is also used to estimate daily potassium intakes. A 24-hour collection is the preferred reference method, although collections of shorter duration (7 p.m.–7 a.m.) may also be acceptable.[99] Within the reference values, the highest tertile (third) of potassium excretion has been associated with a decreased risk of hypertension,[100] cardiovascular events, and mortality.[101] Urinary potassium concentrations may decrease because of low aldosterone levels or the use of medications such as NSAIDs, beta blockers, or pharmaceutical lithium. Urinary potassium levels may increase because of renal disease or rhabdomyolysis (muscle breakdown).

Sodium

Sodium is the primary cation in the extracellular fluid.[89,102] The sodium–potassium ATPase pumps maintain the intracellular potassium and extracellular sodium concentration gradients and establish the membrane potentials necessary for neuron firing, cardiac function, and muscle contraction. Sodium is evaluated as part of an electrolyte (serum elements) panel; levels of sodium (Na) and chloride (Cl) indicate the amount of salt (NaCl) in the blood.[103] Symptoms of an electrolyte imbalance may include chronic vomiting, diarrhea, weakness, or respiratory distress. Electrolytes are commonly monitored in patients with hypertension, heart failure, and liver or kidney disease.

Sodium is absorbed by the gastrointestinal system, and the kidneys excrete all but 1 to 2 meEq/L of the approximately 200 meEq/L of sodium chloride ingested daily. Sodium, with chloride, potassium, and bicarbonate, regulates water distribution, osmotic pressure, pH, and ion balance in the extracellular compartment of the blood. Disruption of the body's fluid volume or fluid-compartment equilibrium may occur if there is a loss, gain, or inappropriate retention of either sodium or water. The regulation of extracellular water and sodium status occurs in the renal tubules of the kidneys. The extracellular fluid volume includes the blood volume, and signaling systems that regulate blood pressure, including the renin–angiotensin–aldosterone system, antidiuretic hormone, and renal dopamine, may alter sodium and water regulation in the kidney.

Hypernatremia is a hyperosmolar condition, defined as serum sodium of greater than 145 mEq/L, and occurs when water loss is greater than water intake.[102,104] Hypernatremia is uncommon with normal kidney function but may occur because of dehydration, Cushing syndrome, diabetes insipidus, adrenal tumors (secrete deoxycorticosterone), congenital adrenal hyperplasia, osmotic diarrhea, hypothalamic disorders, or an impaired thirst mechanism.[105] Hypernatremia may be more common in the aged, those with impaired mental status, and infants because these individuals may have difficulty rehydrating unassisted. Hypernatremia commonly presents with thirst and proceeds to neurologic symptoms (confusion, agitation, dry mucous membranes, decreased urination, muscle spasms, parasthesias, tremors, ataxia, seizures, coma) as water shifts from cells into the extracellular compartment. Medications such as anabolic steroids, diuretics, glucocorticoids, laxatives, cough medications, and oral contraceptives may increase sodium levels (review medications with a pharmacist). Hypernatremia resulting from salt ingestion rarely occurs with normal renal function. The treatment of hypernatremia depends on the water (fluid) status of the patient. Hypernatremia may be hypovolemic (extracellular water loss greater than sodium loss), euvolemic (increased sodium but normal extracellular water levels), or hypervolemic (sodium levels increase and extracellular water increases). Urine sodium and osmolality may be used with serum assessments to determine whether the kidney is the cause.

Hyponatremia is defined as a serum sodium level of less than 136 mEq/L and indicates an excess of extracellular water volume compared with sodium concentration.[89,102,106] Low serum sodium levels may be caused by a water–sodium imbalance or by the presence of excess glucose or lipids in the blood ("pseudohyponatremia"). Causes of sodium loss may include diarrhea or vomiting, renal disease or injury, mineralocorticoid deficiency, Addison disease, hypothyroidism, syndrome of inappropriate antidiuretic hormone secretion (SIADH), cirrhosis, heart failure, pancreatitis, rhabdomyolysis, physical or emotional distress (increased vasopressin), small bowel obstruction, or increased fluid intake. Endurance or extreme sports athletes may ingest large quantities of water during events and experience acute hyponatremia if they do not replace salt as well. Symptoms of hyponatremia include nausea, weakness, confusion, and lethargy and may progress to ocular palsy, confusion, and coma if the sodium loss becomes severe. Medications that may contribute to hyponatremia include thiazide diuretics, barbiturates, carbamazepine, chlorpropamide, clofibrate, opioids, tolbutamide, ACE inhibitors, tricyclic antidepressants, NSAIDs, and vincristine. Dietary sources primarily include table salt, sea vegetables (e.g., kelp, seaweed), dairy products, and spinach.

Hyponatremia may occur during isosmotic, hyperosmotic, or hypoosmotic conditions.[89,106,107] Isosmotic hyponatremia is actually pseudohyponatreima; plasma sodium is decreased, but osmolality, glucose, and urea levels are normal. The phenomenon is exclusive to samples assessed using flame emission spectrophotometry or ion-selective electrodes and only occurs in samples from patients with hyperproteinemia or severe hyperlipidemia. Hyperosmotic hyponatremia occurs most commonly because of severe hyperglycemia as the water and sodium shift compartments to compensate for the excess glucose. Sodium levels will decrease about 1.6 mEq/L for every 100 mg/dL increase in glucose.

Hypoosmotic hyponatremia occurs because of a greater loss of sodium (depletional) compared with water or because of an increase in the extracellular water volume (dilutional).

Depletional hyponatremia presents clinically with orthostatic hypotension, tachycardia, and decreased skin turgor ("tenting"). Low urine sodium during depletional hyponatremia indicates an appropriate renal response; the loss is likely from the gastrointestinal tract or the skin (excessive sweating). High urine sodium indicates renal wasting of sodium, metabolic alkalosis, or the use of thiazide diuretics. Dilutional hyponatremia occurs because of water retention and presents clinically as weight gain or edema. This may occur because of renal disease or nephrotic syndrome, congestive heart failure, or hepatic cirrhosis. If hypoosmotic hyponatremia presents with normal extracellular water volume, likely causes include SIADH and deliberate excess water intake.

Sodium is most commonly measured in blood or urine, although it may also be measured in sweat, feces, and gastrointestinal fluids.[89] Whole blood, serum, or heparinized plasma may all be used to assess sodium concentrations, although no information on the status of the extracellular compartment will be available from whole-blood analysis. Urinary sodium is always evaluated as a component of sodium blood level evaluations. Urinary sodium may be increased because of an increased blood sodium level, Addison's disease, diuretic use, or renal wasting of sodium. Higher urinary sodium levels have been associated with an increased risk of cardiovascular events and mortality.[108] Urinary sodium levels may be low because blood levels are low; because urinary excretion of sodium is compromised (nephrotic syndrome); or because of dehydration, congestive heart failure, or liver disease.

ESSENTIAL MICROMINERALS

Copper

Copper is an essential component of copper-incorporating oxidase enzymes and other metalloproteins.[12,109,110] Copper-dependent enzymes are necessary for energy production (cytochrome c oxidase), collagen cross-linking, hemoglobin synthesis, norepinephrine synthesis, histamine catabolism (diamine oxidase), melanin synthesis, and antioxidant status (superoxide dismutase). Two thirds (about 60 mg) of total body copper is sequestered in bone or muscle tissues. Free copper ions may interact with cellular components to generate free radicals. Special "chaperone" proteins keep intracellular copper bound to prevent this cellular damage by free copper ions; however, inherited variations in chaperone proteins may compromise their ability to bind copper.[111] Copper absorbed from the gastrointestinal tract is bound to albumin and transported to the liver. Ninety percent of the copper exported from the liver is bound to ceruloplasmin; urinary excretion is limited to ionic, not bound, copper. Both copper excesses and deficiencies may be inherited or acquired. Inherited conditions are the result of genetic mutations and are usually X-linked (maternal inheritance). Acquired conditions may be the result of environmental exposures or supplementation choices.

Excess copper in the body may have toxic effects.[12,109,110,112] High levels of intracellular copper may inhibit protein synthesis or gene expression. Copper excess may be acquired or the result of inheritance. True acquired toxicity (poisoning) is rare but may occur because of contamination (storage of acidic foods or beverages in copper containers, contaminated water, or cooking in corroded copper or with brass utensils). Symptoms after ingestion may include nausea, vomiting, and diarrhea. Excess copper is primarily excreted in the bile, and liver disorders that decrease bile excretion may increase copper levels. Subclinical acquired copper excess may require a liver biopsy with visualization of Mallory hyaline bodies for confirmation. Rarely, large amounts of copper may be accidentally absorbed through the skin or ingested (gram quantities). Symptoms may then progress to hepatotoxicity, hemolytic anemia, and anuria (renal failure) and may result in death if not promptly treated. High copper levels may be seen with rheumatoid arthritis and some types of cancers. Serum copper and magnesium may be increased with seizure disorders.[113] The use of carbamazepine or phenobarbital may increase copper blood levels (review medications with a pharmacist). Individuals with Wilson disease, a genetic disorder of impaired copper transportation, may be at risk for toxic effects at copper intake levels considered normal for the average population and may present with low ceruloplasmin; Kayser–Fleischer rings in the cornea; and hepatic, neurologic, psychiatric, or reproductive disorders.[114]

Clinical hypocupremia is rarely noted because of dietary deficiency.[12,109,110,112] Acquired copper deficiencies may occur because of childhood protein deficiency (kwashiorkor), persistent infant diarrhea (cow's milk–based diet), malabsorption syndromes, gastric surgery, or excessive zinc supplementation (>50 mg daily). Copper deficiency may delay normal development or result in symptoms of neutropenia, osteopenia, myelopathy, neuropathy, optic neuritis, and iron-resistant hypochromic anemia. Levels of serum copper and ceruloplasmin may, or may not, be low. Copper deficiency may be found in association with premature birth, cystic fibrosis, or aceruloplasminemia (iron deposition). Menkes syndrome is an X-linked mutation in males that results in low ceruloplasmin and low copper levels in serum, the liver, and copper-dependent proteins. Severe symptoms (intellectual disability, gastrointestinal symptoms, hypopigmentation, skeletal changes, hair texture changes, arterial ruptures) occur in affected individuals, and the diagnosis is usually made in early childhood. Individuals with less severe disease may have partial enzyme activity and a milder form of the disease (occipital horn syndrome). Subcutaneous injections may restore peripheral copper levels, but passage through the blood–brain barrier remains limited. Dietary copper sources include beans, eggs, fish, fresh fruits, liver, milk, mushrooms, nuts, oysters, peas, poultry, and whole grains.

Copper may be evaluated in serum, plasma, whole blood, erythrocytes, urine, stool, and hair.[11,12] Hair may provide an estimate of nutritional copper status, and hair copper levels may increase or decrease in certain disease states.[115–117] However, hair cannot discern the presence or absence of inherited copper metabolic disorders, and hair may be easily contaminated by copper-based products used in pools, hot tubs, or plumbing. Fecal copper levels reflect excess dietary copper from bile excretion and unassimilated dietary copper in the stool. Urinary copper reflects the excretion of ionic copper and may be used in conjunction with blood levels to assess the presence of inherited copper metabolic disorders. Accurate urinary copper assessments require normal renal function. Urinary copper may be very high if patients with Wilson disease are treated with chelators during urine collection. Wilson disease is diagnosed when serum ceruloplasmin is low but urinary copper excretion is high.[114] Menkes disease is likely if blood, urine, and hepatic copper levels are low. The most frequently used assessments of copper status are serum or plasma copper and ceruloplasmin.[11] If excess copper intake or exposure is suspected, then whole blood (total copper) and ceruloplasmin may be the best choice because the sample includes both intracellular and extracellular copper. Erythrocyte copper may have some utility in certain patient populations, such as patients with Alzheimer's disease, where elevated RBC copper has been found in Alzheimer's subjects but not in controls.[118]

Iodine

The element iodine, ingested as the compound iodide, is essential for the synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3).[19,119] Metabolic activity (ATP production, protein

synthesis, enzyme activity, development of skeleton and nervous system, etc.) is regulated by thyroid activity. Thyroid function is regulated by thyroid-stimulating hormone (TSH) from the pituitary gland, which increases the uptake of iodine by the gland. Approximately 80 μg of iodine is used to synthesize thyroid hormones daily. A healthy thyroid gland concentrates about 75% of total body iodine (15–20 mg). Iodide intake varies based on location and the amount of iodide available in the soil. Landlocked populations and those at higher altitudes, far from the ocean, may be more at risk of iodine deficiency. The accumulation of radioactive iodine (^{131}I) in the body may occur after accidental emissions from nuclear power plants and increase the risk of developing thyroid cancer; the uptake of ^{131}I is less likely with adequate iodine status.

Iodine excess may occur if doses of greater than 1.1 mg daily are chronically ingested by iodine-sufficient adults.[120,121] Exceeding this dose may increase the risk of iodine-induced dysfunction in susceptible populations, such as individuals with iodine deficiency, preexisting thyroid disease, the elderly, neonates, or during pregnancy (prenatal exposure). Although there are biological mechanisms in place to control thyroid hormone synthesis during iodine excess,[122] the iodine exposure may also increase TSH and the risk of goiter and hypothyroidism in some susceptible individuals and in neonates.[123] Excess iodine may also increase the risk of thyroiditis and thyroid papillary cancer. The accidental ingestion of more than 50 mg of iodine daily for 2 months by 43 older adults occurred during a study, and the effects were reviewed in a separate study.[124] Of the 43 subjects taking the high iodide dose, 10 subjects developed hypothyroidism (elevated TSH) while taking the iodide; all but two had returned to normal TSH levels 1 month after discontinuing the iodide supplement. Three subjects developed hyperthyroidism (low TSH) while taking the iodide dose, which persisted 1 month after discontinuation. High levels of iodine exposure may occur if iodinated radiographic contrasts or medications such as amiodarone are used (review medications with pharmacist). Iodine excess may result in symptoms of increased salivation, gastrointestinal irritation, acne-like skin lesions, and a brassy taste in the mouth. Acute iodine poisoning may include symptoms of a burning feeling in the mouth, throat, and stomach; fever; nausea, vomiting, and diarrhea; weak pulse; and cyanosis. Symptoms may progress to coma. High doses of iodine may be contraindicated with antithyroid medications, ACE inhibitors, and potassium-sparing diuretics and during pregnancy. Large doses of potassium iodide may decrease the efficacy of the anticoagulant coumarin.

Iodine deficiency may result in various disorders.[119–121] If iodine intake is less than 100 μg per day, TSH levels will rise. During deficiency, the thyroid gland is stimulated by increasing levels of TSH and increases in size because of overstimulation by TSH (colloid goiter). Most individuals remain euthyroid, but if iodine deficiency is severe, hypothyroidism may result. Iodine deficiency in adults may impair cognitive function and is the most common cause of goitrous hypothyroidism. Perinatal iodine restriction may have permanent developmental effects on cognition and may result in lower IQ levels. During pregnancy, iodine requirements roughly double. Maternal deficiency may increase the risk of attention deficit and hyperactivity. Severe maternal deficiency (development of goiter) causes cretinism (cognitive impairment, deafness, motor impairments, poor growth, developmental delay), miscarriage, or stillbirth. Food sources of iodine include sea vegetables, seafood, dairy, eggs, and grains; all food sources are highly variable in iodine content due to local conditions and production practices. Dietary iodide is easily assimilated by the gastrointestinal tract, but consumption of goitrogenic (soy, cassava, cruciferous vegetables, some beans, millet, and sweet potato varieties) foods may prevent uptake. Iodized table salt may be an acceptable source of iodide; however, restaurants and processed food manufacturers rarely use iodized salt in food production. Adequate selenium, iron, and vitamin A are required to support iodine use by the thyroid gland. Tobacco use may impair iodine uptake by the thyroid gland. The presence of intestinal parasites, such as *Ascaris lumbricoides,* hookworm, or *Entamoeba histolytica,* may prevent iodide absorption by the gastrointestinal tract.[125] Many environmental toxins, especially metals, impair iodine absorption and utilization (see Chapter 183, Hypothyroidism).

Urinary iodine is the international standard for the evaluation of iodine status because more than 90% of dietary iodine is excreted in the urine within 24 to 48 hours of ingestion.[11] Although often used as a surrogate,[126] the assessment of serum thyroid biomarkers has limited utility in the evaluation of iodine status.[127,128] Whereas some studies show no association between TSH, T4, and urine iodine levels, other studies indicate that serum T4 may be a conditionally useful biomarker in children and adults. Interventional studies indicate that serum TSH levels may be reflective of iodine status in newborns but are not a sensitive indicator of iodine status in developing children or adults, although serum thyroglobulin may be used in school-aged children. Similar studies indicate that serum TSH may be a useful indicator of iodine status in pregnant or nursing women. Serum T4 is not considered accurate for pregnant or nursing women. For serum T4 to be an accurate surrogate for iodine, there must be a moderate baseline T4 status.

Iodine intake over the past few days is best reflected in urinary iodine assessments. The World Health Organization defines iodine deficiency as median urinary iodine concentrations of less than 100 μg/L (< 150 μg/L during pregnancy).[126] Urinary iodine excretion of greater than 300 μg (> 500 μg during pregnancy) is considered excessive. Urinary iodine of less than 20 μg/L is considered severe deficiency. Multiple 24-hour urine collections are recommended to estimate iodine intake in individual subjects. It has been suggested, in a non–peer-reviewed trade magazine, that whole-body sufficiency of iodine may be assessed in urine using an "iodine loading test." The interpretation of the results of this test presupposes specific receptor/storage sites that take up and store iodine/iodide.[129] When body storage of iodine/iodide is optimal, the percentage excretion of an oral loading dose of iodine/iodide excreted in urine is maximal; some authors purport that body stores are optimal when excretion is 90% or more.

Iron

Iron is distributed into virtually all compartments in the body.[130] Iron is used by peroxidase, cytochrome, and other enzymes, such as cystathionine-β-synthase (CBS) and enzymes of the tricarboxylic acid cycle. Iron is found in nonenyzme proteins such as hemoglobin, myoglobin, and mitochondrial iron–sulfur clusters[131]; storage proteins such as ferritin or hemosiderin; and in the transport protein apo-transferrin. Strict conservation of iron stores in the body, even when iron is in excess, results in the loss of approximately only 1% of total body iron daily. Regulation of iron intake occurs in the gastrointestinal tract, where iron assimilation may be modulated by hepcidin so that about 1 milligram of iron is absorbed daily.[132] Both iron excess (overload) and iron deficiency may impair homeostasis[133]; the abnormal distribution of iron during various disease processes may contribute to their symptomology and presentation.

Iron overload may be acquired or hereditary.[132,134] Acquired iron overload (hemosiderosis) may occur because of excessive iron therapy, hemodialysis, multiple blood transfusions (for sideroblastic anemia, hemolytic anemia, pyruvate kinase deficiency, and thalassemia major), acute overdose, chronic liver disease, or alcoholism. Individuals with β-thalassemia intermedia may develop iron overload because of increased intestinal absorption. An acute iron overdose damages organs and intestines, resulting in vomiting and diarrhea.

TABLE 23.1 Anemia of Inflammatory Response[a,130,141,143]

Disorder	Serum Iron	TIBC	UIBC	Transferrin	Transferrin % saturation	Ferritin	Hemoglobin
Iron deficiency	Low	High	Low	High	Low	Low	Low
B_{12} deficiency	High	Low	High	Low	High	High	Low
Anemia of chronic disease	Normal or Low	Normal or Low	Normal or High	Normal or Low	Normal or Low	Normal or High	Low
Hemochromatosis	High	Low	High	Low	High	High	Normal
Thalassemia	High	Low	High	Low	High	High	Low
Porphyria cutanea tarda	High	Low	High	Low	High	High	Normal

TIBC, Total iron-binding capacity; *UIBC,* unsaturated iron-binding capacity
[a]Adapted from the Iron Disorders Institute. http://www.irondisorders.org. Accessed November 1, 2017.

Hereditary hemochromatosis occurs primarily in Caucasians of Northern European descent and is the result of mutations in the *HFE* gene. The disease is characterized by iron accumulation in the liver and other organs (C282Y homozygous or C282Y heterozygous together with a heterozygous H63D or S55C).[135] Early symptoms include fatigue and lethargy; characteristic late symptoms include bronzing of the skin (palms), cirrhosis, diabetes, and increased risk of hepatocellular carcinoma and neurodegenerative diseases.[132,133,136] Juvenile hereditary hemochromatosis, from mutations in the *HEF2* or *HAMP* genes, results in symptoms that present before 30 years of age and that include hypogonadotropic hypogonadism, cardiomyopathy, arthropathy, and liver fibrosis or cirrhosis.[137] Other mutations result in African iron overload (also known as Bantu siderosis) and ferroprotein deficiency, iron-storage diseases that deposit iron primarily into macrophages. Inherited disorders such as aceruloplasminemia, hypotransferrinemia, Friedreich ataxia, and porphyria cutanea tarda may also result in iron overload.

Iron deficiency is one of the most common mineral deficiencies worldwide,[130,132,134] although isolated iron deficiency is uncommon in the United States.[133] Iron deficiency is most common in children, reproductive-aged women, and the elderly. Deficiency may be secondary to poor diet, obesity, chronic kidney disease, gastrointestinal malabsorption, gastrointestinal tumors,[138] gastrointestinal parasites, gastric bypass surgery, or blood loss. Blood losses may result from comorbid disease, blood donations, or extreme endurance training. Iron depletion occurs in gradual stages, and different biomarkers are useful at different stages. Iron-deficiency anemia may result in symptoms such as fatigue, pallor, headaches, dizziness, impaired cognitive function, behavior or motor difficulties (developing children), poor immune responses, decline in exercise performance, tachycardia, dyspnea, and difficulty regulating temperature (iodine deficiency or comorbid hypothyroidism). Symptoms may progress to nail spooning, brittle nails, loss of taste, angular cheilosis (skin lesions at the corners of the mouth), atrophic glossitis ("bald," sore, tongue),[139] or pica. Co-occurring deficiencies in vitamin A, copper, or zinc may further impair iron metabolism. Dietary sources include beans, dark green leafy vegetables, dates and figs, dried fruits, egg yolk, fish, molasses, nuts, organ meats, red meat, shellfish, and whole and enriched grains. Iron deficiency may increase manganese absorption by the gastrointestinal tract.

A variety of tests and methods may be used to assess iron status,[130,132,134] and a variety of physiologic conditions must be considered when interpreting iron results (see Table 23.1).[130,140] Serum iron, total iron-binding capacity (TIBC), serum transferrin, serum ferritin, hematocrit, hemoglobin, soluble transferrin receptor, and zinc protoporphyrin may all play a role in diagnosing iron disorders. The use of various commercial methodologies to measure some of these analytes requires each laboratory to establish its own reference values for those tests. Although the majority of iron is sequestered inside the erythrocytes as heme iron, and iron supplementation improves RBC hemoglobin content (during pregnancy), erythrocyte iron is not commonly measured in conventional laboratories.[142] Iron test results may be affected by diurnal variation (test in morning), stage of menstrual cycle, use of iron supplements or intake of a high-iron diet, oral contraceptives (raise iron values), hepatitis, and the presence of acute or chronic inflammation. Methotrexate may increase iron test results. Testosterone, high-dose aspirin, metformin, and adrenocorticotropic hormone may decrease iron test results.

Serum iron evaluates the level of iron in circulation.[140] A low serum iron with a high transferrin or TIBC may be an indication of iron deficiency.[130,143] During chronic diseases, serum iron, transferrin, and TIBC are all decreased. Serum iron increases, and unsaturated iron-binding capacity decreases during iron overload. High levels of stress or sleep deprivation may transiently decrease serum iron levels.

TIBC is assessed by saturating a blood sample with iron and comparing the saturated sample (transferrin saturation) with the serum iron results to obtain the TIBC and the transferrin percent saturation:

$$\text{Transferrin \% saturation} = \frac{\text{serum} \times 100}{\text{TIBC}}$$

Normally, about 30% of transferrin is bound to iron. Transferrin levels will decrease because of liver disease, nephrotic syndrome, low protein intake, or iron overload, and the transferrin saturation will increase. TIBC levels will increase during iron deficiency, and the transferrin saturation will decrease. Plasma iron levels decline in stage 2 iron deficiency, and TIBC rises.

Serum transferrin represents the unsaturated iron-binding capacity (UIBC) and may be used instead of the more expensive transferrin saturation test:

$$\text{TIBC} = \text{UIBC} + \text{serum iron}$$

Transferrin levels may decrease because of nephrotic syndrome or other renal-wasting disorders.[144] Hemoglobin levels usually remain in range during stage 2 iron deficiency, whereas serum transferrin receptor concentrations increase.

Ferritin is the primary intracellular iron-storage protein, and serum ferritin estimates the amount of iron stored in the body.[130] Serum ferritin decreases during the first stage of iron deficiency as iron stores are depleted and bone marrow iron decreases. Low levels of ferritin indicate iron deficiency; however, high levels may occur not only with iron-storage disorders but with a variety of acute and chronic diseases or with the presence of malignancy.

Hemoglobin is found within erythrocytes, and the hematocrit value roughly estimates the number of RBCs.[144] Hemoglobin and

hematocrit values are assessed during a complete blood count. In the third stage, iron-deficiency anemia (IDA), hemoglobin and hematocrit levels decrease, and microcytic hypochromic anemia occurs. An elevated hematocrit may indicate polycythemia, dehydration, pulmonary disease, congenital heart disease, renal tumor, tobacco use, or high-altitude living. Hematocrit results may also be altered by recent blood transfusion or donation.

The soluble transferrin receptor (sTfR) is used when ferritin results may be compromised by a preexisting or concurrent inflammatory disorder (ferritin is an acute-phase reactant during inflammation).[145,146] The sTfR assessment is not affected by the level of inflammation and may be used to discern whether anemia is caused by iron deficiency or chronic disease. sTfR rises with the onset of iron-deficient erythropoiesis and continues to rise as iron deficiency progresses to anemia. sTfR will also elevate because of hemolytic anemias, myelodysplastic syndromes, and the use of erythropoietic stimulation medications. Importantly, sTfR does not rise during anemia of chronic disease.

Zinc protoporphyrin (ZPP) is a heme-precursor protein.[147] When there is insufficient iron to synthesize heme, or if an exposure to the toxic element lead occurs, ZPP levels rise. ZPP is measured in the free erythrocyte protoporphyrin test, and a ZPP:heme ratio may be included in the results. A change in the ZPP:heme ratio may be the first indication of insufficient iron reserves in pediatric populations, and the ratio will shift before symptoms of iron deficiency appear. ZPP may also be elevated due to comorbid infection, inflammatory disorders, anemia of chronic disease, or inherited porphyria. The results must be interpreted with the patient's symptoms and history in mind.

Iron levels may occasionally be evaluated in urine or hair. Urinary iron excretion is variable and is not considered to reflect tissue iron stores; however, increased urinary iron excretion may be an indicator of exposure to the toxic element lead.[148] Increased iron excretion may be a causative factor in intractable IDA and may be evaluated in such cases.[149] The iron levels in hair have not been correlated with more conventional blood estimates of iron status; however, research continues in this area. Recent studies have correlated low hair iron (and other elements) with growth retardation[62] and demonstrated a positive association between hair iron levels and levels of behavioral traits (novelty seeking, extraversion) and physical activity.[150]

Manganese

Manganese is an essential cofactor for enzymes in multiple biochemical pathways, including antioxidant protection (mitochondrial superoxide dismutase), glucose synthesis (pyruvate carboxylase), the urea cycle (arginase), and connective tissue and cartilage synthesis (glycosyltransferases).[12,151] Although essential, manganese at high levels may have toxic effects in the nervous system. Manganese and iron may compete for uptake in the gastrointestinal system. Manganese uptake increases during iron deficiency and decreases during a high-iron meal. Manganese is transported in the body bound to albumin or transferrin and excreted from the body primarily through the bile.

Manganese levels of greater than 5.4 µg/L serum or greater than 20 µg/L whole blood indicate manganese exposure or retention.[12,152] High levels of manganese exposure may occur because of occupational inhalation of manganese dust; inhalation of methylcyclopentadienyl manganese tricarbonyl (MMT) gasoline additive; or ingestion of manganese in supplements, food, or water.[151] Exposed infants and children may not excrete manganese efficiently until their hepatic systems are fully developed, and adults with liver disease may have cholestasis and increased manganese retention. Symptoms of hypermanganesemia may contribute to hepatic encephalitis and resemble the neurologic symptoms of Wilson disease or Parkinson's disease; however, manganism is unresponsive to L-dopa therapy.[152–154] In rare individuals, a mutation in the SLC30A10 zinc/manganese cellular transporter may result in a syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia,[155] which may run in families.

Human manganese deficiency has been demonstrated in small experiments or during chronic parenteral nutrition.[12,151,152] Symptoms in children may include growth delays and skeletal abnormalities. Symptoms in adults may include low cholesterol; skin rash or lesions; abnormal glucose tolerance; and increased blood calcium, phosphorus, and alkaline phosphatase. Human studies indicate that low manganese levels may increase the risk of seizures[156] and may be associated with Alzheimer's disease and risk of cognitive impairment.[157] Dietary sources include dark green leafy vegetables, dried fruits, dried legumes, nuts, and whole grains.

Manganese levels may be assessed in serum, whole blood, erythrocytes, urine, and hair.[12,13,158,159] For serum collections, a plastic cannula should be used to prevent contamination from steel needles, and specimens should be discarded and redrawn if hemolysis occurs during sample collection or processing. Serum manganese levels represent approximately 30% of total body manganese and may vary based on recent dietary intake. Serum manganese may also require an evaluation of lymphocyte manganese superoxide dismutase activity to accurately assess nutritional deficiency. Whole-blood levels may be used to monitor manganese excess and may provide a more accurate assessment of recent dietary intake over the life span of the RBC (60–120 days).[160] Erythrocyte manganese represents about 60% of total manganese and may provide further diagnostic insight for specific conditions such as prolidase deficiency (imidodipeptiduria),[161] which presents with increased erythrocyte manganese and normal serum levels. Urinary manganese is considered to have a short half-life postexposure and may better reflect recent dietary and environmental exposures rather than body nutritional status. Hair manganese has been evaluated in multiple studies,[162–164] but the possibility of external contamination must be eliminated before the interpretation of results.[165]

Molybdenum

Molybdenum is an essential cofactor for enzymes involved in sulfur metabolism (e.g., sulfite oxidase), uric acid synthesis (xanthine oxidase), and detoxification (aldehyde oxidase, mitochondrial amidoxime reducing component).[12,166,167] The element must be complexed into a pterin protein to act as a cofactor in biochemical reactions,[168] and inherited deficiency of the cofactor results in microcephaly, developmental delay, and neurologic symptoms within a week of birth. Affected individuals have increased levels of urinary sulfite, S-sulfocysteine, xanthine, and hypoxanthine and decreased uric acid in their blood.[169] Molybdenum is assimilated in the gastrointestinal tract as molybdate (MoO^{2-}_4); once assimilated, more than 80% of the molybdate binds to erythrocyte proteins. Excess dietary molybdenum is excreted by the kidneys, and experimental ingestion of up to 1.5 mg daily for 24 days produced no adverse effects in young men.[170]

Dietary molybdenum excess in humans is not well documented. Ingestion of 10 to 15 mg of molybdenum in the daily diet occurs in some isolated Armenian populations and has been correlated with increased blood levels of uric acid. Increased uric acid, arthralgias, and ceruloplasmin levels have been reported with occupational molybdenum exposures.[12]

Reported dietary deficiency is rare but has been documented when molybdenum-deficient parental nutrition has been administered to patients. Chronic molybdenum deficiency causes sulfite toxicity.[171] Deficiency symptoms included tachycardia, tachypnea, headache, nausea, vomiting, and progression to coma. Animal studies indicate that tungsten exposures may increase the excretion of molybdenum, even when molybdenum is deficient in the diet.[172] Dietary sources of molybdenum include beans, peas, red meats, and whole grains.

Molybdenum was once considered difficult to measure, but inductively coupled plasma mass spectrometry has sufficient sensitivity to measure molybdenum in biological samples, such as whole blood, erythrocytes, serum, plasma, or urine.[12] The average value for whole blood is 1 μg/L; for plasma or serum, the average is 0.5 μg/L. Urinary molybdenum ranges, on average, from 40 to 60 μg/L daily and varies with dietary intakes. Urinary molybdenum has been compared with femoral neck and lumbar bone mineral densities, and an inverse relationship has been found: the higher the urinary molybdenum, the lower the bone mineral density.[173]

Selenium

Selenium is an essential component of selenoproteins vital for various metabolic and antioxidant functions.[12,174,175] Selenoproteins all contain selenocysteine, which is now considered the 21st amino acid. Glutathione peroxidase (antioxidant), iodothyronine deiodinase (thyroid hormone), thioredoxin reductase (intracellular redox status), and selenophosphate synthase (selenoprotein synthesis) all require selenocysteine in their structures, as do the antioxidants selenoprotein P and selenoprotein W. The human synthesis pathway has only recently been defined and may be affected by inherited variations in the pathway enzymes,[176] with clinically significant results. Animal studies indicate that selenium may have synergistic antioxidant effects with vitamin E.[177]

Dietary selenium is primarily selenomethionine. Both organic and inorganic selenium may be absorbed by the gastrointestinal system, although the absorption of some inorganic selenium may require the presence of glutathione.[178] Intakes in the United States and Canada are generally considered sufficient. Selenium sufficiency may reflect dependence on local food production; in some areas of the world, selenium-deficient soils and dependence on locally grown foods may predispose human populations to selenium deficiency; in other areas, high selenium levels may be found. Excess selenium is normally excreted by the kidneys.

Toxic levels of selenium exposure may occur because of supplementation, industrial exposure, or high levels in local soil or water, which may bioaccumulate into food plants and animals.[12,174,175] Chronic exposure to high amounts of selenium in food, water, or soil may increase the risk of amyotrophic lateral sclerosis.[179] Inorganic selenates may be more toxic than organic selenium compounds and may cause symptoms at lower levels of exposure.[180] Early signs and symptoms of selenosis (chronic exposure) include a "garlic" odor on the breath, brittle nails, and hair loss and progress to fatigue, irritability, gastrointestinal symptoms, mottled teeth, skin rashes, and neurologic disorders. Toxic effects have been associated with selenium intakes of 850 μg daily. The tolerable upper intake level for adults is 400 μg daily from all sources, but newer evidence indicates that daily intake in excess of 250 to 300 μg daily may increase the risk for type 2 diabetes, dermatitis, and hair loss.

Selenium deficiency may occur because of low levels in local soil and water, because of malabsorption syndromes, or because of inherited variations in selenocysteine insertion protein (SECISBP2 tRNA), which impairs thyroid hormone synthesis and antioxidant status and decreases plasma selenium levels.[176] Systemic selenoprotein deficiency results in photosensitivity and hearing loss; the effects of partially active pathways are unknown at present.

Symptoms of severe deficiency may include muscle weakness, muscle wasting, and cardiomyopathy. Severe deficiency may also decrease male fertility.[12,174,175] Severe dietary selenium deficiency during child development may result in multifactorial Keshan (cardiomyopathy) or Kashin–Beck (joint deformities, dwarfism) disease; these conditions are seen primarily in areas of China with selenium-deficient soil. The risk of deficiency may be increased because of bariatric surgery, malabsorption syndromes, renal dialysis, and specialized medical diets (phenylketonuria, homocystinuria, maple syrup urine disease). Selenium levels may be lower in HIV+ individuals in developing countries or due to local selenium-deficient soils. Marginal selenium deficiency may impair cognitive function,[181] thyroid function, and immune or inflammatory responses. Deficiency may increase the risk of cardiovascular disease, reproductive disorders, or mood disorders. Poor immune responses in selenium-deficient individuals may increase viral virulence and the severity of viral diseases. The use of cisplatin or other medications may decrease selenium levels (review all medications with a pharmacist). Dietary sources of selenium include Brazil nuts,[182] fish, seafood, meat, brown rice, and sunflower seeds.

Selenium status may be evaluated by whole blood, serum, plasma, urine, and hair.[12] The types of selenium ingested may sequester in different compartments in the blood; organic selenium increases plasma and urine levels, whereas inorganic selenite does not.[183] Whole-blood analysis measures selenium in both the intracellular and extracellular compartments and may be the best assessment if selenium excess is suspected. Serum and plasma are the most commonly assessed, despite the fact that serum levels may decrease during acute-phase activation or chronic inflammation.[184] Lower levels of serum selenium have been associated with an increased risk of anemia in adults over 65 years old.[185] Erythrocyte levels remain stable, even as serum or plasma levels decrease, and may provide a better assessment of recent dietary intakes over the life span of the RBC (60–120 days).[186] Urinary selenium levels reflect recent dietary and environmental exposures.[187] After the injection of 2,3 dimercaptopropane-1-sulfonate (DMPS), less urinary selenium is excreted by subjects with amalgams, which indicates that selenium may bind to mercury.[188] Hair selenium has been correlated with plasma levels in Polish subjects.[189] Altered hair selenium levels have been demonstrated in subjects with nonalcoholic fatty liver disease[190] and hyperlipidemia,[191] compared with controls. Hair is easily contaminated from external sources, and selenium from antidandruff shampoos may greatly increase hair selenium levels.[192]

Zinc

Zinc is an essential cofactor and catalyst in approximately 300 enzymes.[12,193,194] Zinc, cysteine, and histidine combine to form folded "zinc finger" compounds used in cellular metabolism as a structural stabilizer in proteins and membranes or as a regulator of gene expression. Adequate zinc is required for normal growth and development. Zinc is also used during the conversion of vitamin A precursors, cell signaling, hormone synthesis, and nerve transmission. Blood plasma contains less than 1% of total body zinc, and 80% of plasma zinc is bound to albumin. Zinc in erythrocytes is present as the enzyme carbonic anhydrase; RBC levels are about 10 times greater than plasma levels. Zinc assimilation in the gastrointestinal tract is variable based on the availability of zinc in the diet; zinc absorption may range from 20% to 50% of the zinc in a sufficient diet and may increase to 100% absorption of the zinc in a deficient diet. Zinc uptake may be inhibited if large amounts of supplemental iron are taken simultaneously or due to inherited variations in the *ZIP4* (SLC39A4) gene.[195] Excess zinc is excreted into the gut and incorporated into stool or excreted by the kidneys.

Zinc, in excess, has toxic effects.[12,194,196] Excessive supplementation, accidental exposure from galvanized food or beverage containers, or industrial exposure to zinc-oxide fumes may all result in toxic levels of zinc. Symptoms of toxic levels of zinc ingestion may include headaches, abdominal pain, vomiting, diarrhea, and loss of appetite. Symptoms of inhalation (metal fume fever) may include fever, sweating, shortness of breath, nausea, fatigue, and myalgias 4 to 12 hours

postexposure; symptoms then resolve after 12 to 24 hours in a zinc-free environment. Chronically high levels of zinc exposure may decrease copper levels and result in nerve damage. Chronic high doses of zinc (100–150 mg daily) block gastrointestinal copper assimilation and result in low blood copper levels. Signs and symptoms include erythrocyte microcytosis (small RBCs), neutropenia (low WBCs), poor immune responses, and an increased risk of urinary lithiasis (kidney stones).[197] Higher zinc doses of 140 to 450 mg daily may alter iron metabolism and reduce levels of high-density lipoproteins. Local applications of zinc in the nasal passages may damage olfactory nerves; intranasal zinc sprays and gels are no longer recommended because the loss of smell may be permanent.

Marginal zinc deficiency may be prevalent in developing countries or areas dependent on cereal grain-based diets high in phytate and fiber.[193] In the United States and Canada, zinc deficiency may occur because of inadequate intake, increased excretion, or increased requirement.[12,194] Zinc deficiency may occur because of poor diet, gastrointestinal malabsorption, chronic diarrhea, cystic fibrosis, liver disorders, alcoholism, diabetes mellitus, chronic kidney disease, sickle cell disease, HIV+ status, physical trauma, pregnancy, and lactation. Severe zinc deficiency may cause symptoms of intrauterine growth restriction, impaired development, short stature,[198] loss of appetite, immune dysfunction, weight loss, delayed wound healing, taste abnormalities, poor concentration, and fatigue. Symptoms may progress to night blindness, hair loss, diarrhea, delayed sexual development, male hypogonadism, impotence, and eye lesions or skin lesions.

The very young and very old may have an increased risk of zinc deficiency.[12,194,199,200] Inherited variations in the metal-binding protein metallothionein 2A (MT2A-5 A/G) may result in GG phenotypes having lower zinc and, perhaps, higher copper and lead levels than the wild-type AA phenotype.[201] Inherited variations in the *ZIP4* zinc transporter (SLC39A4) may result in severe zinc deficiency (acrodermatitis enteropathica) with classic indications of low (<30 µg/dL) serum zinc, periacral and periorificial dermatitis, alopecia, and diarrhea.[199] Inherited variations may be suspected if zinc is persistently low (< 50 µg/dL), with symptoms of poor growth, anorexia, and mild nonspecific dermatitis that improve with zinc supplementation. Severe zinc deficiency in infants may occasionally occur because of inherited variations in zinc transporter 2 (SLC30A2) specific to the mammary gland, which results in low levels of zinc in breast milk but normal maternal zinc levels. The zinc content of breast milk decreases after the first 6 months; dietary zinc is required to meet infant needs at approximately 6 months postpartum.

Approximately 40% of individuals over 60 years old may be zinc deficient if no supplemental zinc is used; even with supplementation over 20% may still be zinc deficient due to comorbid conditions or gastrointestinal dysfunctions.[12,200] Strict vegetarians or vegans on primarily grain-based diets may require 50% more dietary zinc because of the decreased availability of zinc in the diet.[193] High intakes of supplemental iron or calcium may impair zinc uptake; patients may be monitored for zinc status during supplementation. Zinc excretion may increase because of muscle breakdown and excretion of ketone bodies during extended fasting or the use of extremely low-calorie or ketogenic diets. Medication use may alter zinc uptake. Tetracycline, quinolone antibiotics, or bisphosphonates lose efficacy and reduce zinc assimilation if taken together (consult with a pharmacist for dosing regimens). Anticonvulsants (sodium valproate, etc.), thiazide diuretics, and ethambutol may all increase zinc excretion. Medications such as penicillamine, used to treat Wilson disease, or diethylenetriamine penta-acetate (DTPA), used for iron overload, may severely deplete zinc. Review all medications with a pharmacist if zinc–medication interactions are suspected. Dietary sources of zinc include oysters, crab, meats, beans, chickpeas, and cashews. Soaking beans, grains, and seeds before cooking and the use of leavened grain products increase bioavailable zinc from plant-based foods.[193]

Although zinc may be measured in whole blood, serum, plasma, erythrocytes, urine, and hair, nutritional zinc status is difficult to discern because homeostatic mechanisms maintain plasma zinc until the deficiency is severe.[12,202] Plasma zinc is the most commonly used assessment; however, plasma zinc is considered insensitive to dietary intakes, has a diurnal shift (higher in the morning), and must be interpreted in conjunction with serum albumin levels, urinary zinc, and an assessment of acute-phase reactants (zinc levels decrease; copper levels increase).[203] Plasma is preferred over serum because zinc levels may be 5% to 15% higher in serum samples. A repeated fasting plasma zinc of less than 70 µg/dL is suspicious for marginal deficiency, and a level of less than 30 µg/dL indicates deficiency. Whole blood and erythrocytes may also be used and may have different associations with health and disease.[204–206] Because the majority of zinc is intracellular and bound to enzymes, whole-blood levels may provide the best overall blood assessment of zinc status. Urinary zinc may be used in conjunction with serum zinc levels to determine whether kidney function is a contributing cause for abnormal serum/plasma results. Increased urinary zinc excretion may deplete serum zinc levels. Renal failure may decrease urinary zinc and increase serum/plasma levels. Urinary zinc levels may also reflect dietary intake, but they are not sensitive enough to be used as a screening test for mild or moderate deficiency.[202] Hair samples may indicate zinc deficiency but may be easily contaminated by external sources such as hair products, which may artificially elevate hair zinc levels.

POTENTIALLY ESSENTIAL MINERALS

Bromine

Bromine is an unstable element found in nature as bromide. Although in high amounts bromine or bromide exposures may have toxic effects, increasing evidence indicates that lower levels of bromide in the human body may be beneficial, and perhaps essential, for human health.[10,207–209] Brominated proteins may play a role in neurologic signaling, immunity, collagen synthesis, and rapid eye movement (REM) sleep.[210]

High levels of bromine or bromide exposure may be associated with negative effects. Bromide is found in food, water, and seawater. Industrial exposure to bromine gas may occur; bromine may be used in pool or hot tub cleaners, pesticides, and fire retardants.[211] Brominated vegetable oils may be used in food preparation or added to soft drinks.[212] Bromide may be found in veterinary medications and occasionally in human pharmaceuticals (Germany, Japan).[213] Pyridostigmine bromide was distributed and may have been taken by some U.S. troops during the first Persian Gulf war as a nerve agent antidote.[214] Bromide levels may be higher in areas of radioactive fallout.[215] Signs of acute inhalation exposure may include dyspnea, cough, headache, dizziness, and irritation of mucous membranes. Acute ingestion of a large amount of bromine may result in gastrointestinal symptoms (nausea, vomiting, etc.). Chronic, excessive ingestion of brominated vegetable oils may result in dermatologic or neurologic symptoms. Higher plasma bromine levels have been associated with increased levels of TSH.[216]

Low levels of bromide intake may alter acetylcholinesterase signaling,[207] sleep quality,[209] collagen cross-linking,[208] and the function of G protein-coupled receptor 7 (GPR7) in the nervous system.[217] The antimicrobial function of eosinophil peroxidase in WBCs is inhibited by low bromine levels.[10] Bromine levels in serum and erythrocytes are decreased by peritoneal dialysis.[218] Dietary sources of bromide include fish, grains, and nuts.

Bromine has been measured in blood,[219] serum,[220] and urine.[221] In whole blood, typical bromide levels may range from 0.3 to 1.2 mg/dL. Whole blood levels of greater than 1.2 mg/dL have been associated with altered electroencephalogram readings. Serum levels of greater than 1250 µg/mL are considered toxic. Urinary bromide may be used to monitor industrial exposures to 1-bromopropane or other bromine/bromide compounds.

Chromium

Chromium (as a component of "glucose tolerance factor" or chromodulin) was proposed as an essential element more than 50 years ago after patients on long-term parenteral nutrition developed glucose intolerance, ataxia, peripheral neuropathy, and weight loss, which improved with the addition of trivalent chromium.[12,222–224] Chromium may be biologically active, yet multiple studies have failed to confirm the presence of biological chromium-binding compounds in humans. Studies have also failed to confirm the claims that chromium improves body composition or muscle mass.[225,226] Although recent studies in Asian populations have correlated lower plasma chromium levels with metabolic syndrome and type 2 diabetes,[227] the majority of randomized controlled trials do not demonstrate evidence of prevention or treatment of glucose intolerance or type 2 diabetes at the usual supplement dose of 200 µg daily. For most glucose-intolerant subjects, higher doses may be required to achieve benefit, but safety studies to support the use of higher doses are lacking. There is some evidence that increasing chromium intake for mildly glucose-intolerant subjects on low-chromium diets may be beneficial,[228] and one study using a biotin-chromium supplement with obese subjects with type 2 diabetes reported improvements in glucose response and biomarkers for cardiovascular disease and atherogenesis.[229] Less than 3% of dietary chromium is absorbed in the gut, although vitamin C, niacin, NSAIDs, aspirin, beta blockers, corticosteroids, or insulin may increase absorption (animal studies). The supplement chromium picolinate may have greater bioavailability, passing directly through the jejunum and being incorporated into cells. Dietary trivalent chromium is bound to transferrin or albumin in the plasma, and excess is excreted in the urine.

The toxic effects of chromium vary based on whether the exposure is trivalent or hexavalent chromium. Trivalent chromium (Cr^{III}) is considered nontoxic at normal dietary intake and supplementation levels; however, hexavalent chromium (Cr^{VI}) is considered toxic in any amount. Chromium may be ingested, inhaled (industrial exposures), or absorbed through the skin.[230,231] Ingestion of hexavalent chromium may result in inflammatory changes in small intestine, pancreas, and liver tissues (animals) and may increase the risk of gastrointestinal and lung cancers. Respiratory exposure may result in bronchial asthma, respiratory tract polyps, nasal and septal ulcerations, or nasopharyngeal irritation and inflammation and may increase the risk of lung cancer. Topical exposure to very high levels of hexavalent chromium may result in allergic dermatitis. Hexavalent chromium is a component of joint-replacement prosthetics and may be found in drinking water supplies as a contaminant.[232] Trivalent chromium is found in supplements and dietary chromium. Although few symptoms are associated with trivalent chromium exposure, human studies indicate that long-term chromium picolinate supplementation ranging from 600 to 2400 µg/day may result in kidney or liver damage in some individuals. Patients with comorbid renal or liver disorders may be at particular risk.

True dietary deficiency has not been documented for chromium. The most likely cause of low chromium levels is increased urinary excretion from poor diet, renal disorders, or intense athletic training.[233] Diets high in simple carbohydrates may increase chromium excretion. Trivalent chromium may be obtained from whole grains, brans, green beans, broccoli, nuts, and egg yolks.

Chromium may be measured in serum, plasma,[227] erythrocytes, whole blood, and urine by laboratories using element-free collection devices and inductively coupled plasma mass spectrometry (ICP-MS) technology.[12] Serum values are considered normal at 0.1 to 0.2 µg/L. Recent evidence indicates that RBC chromium levels primarily reflect exposure to toxic hexavalent chromium (Cr VI) because trivalent chromium does not enter erythrocytes.[234] Whole blood provides an assessment of both compartments and, therefore, total chromium exposure. Urinary chromium levels may not reflect dietary intake because renal excretion may be altered by exercise and high-carbohydrate diets.[233]

Fluorine

Fluorine, as the metal compound fluoride, is found naturally in water sources at varying levels.[235] Fluoride is not considered essential because deficiency does not result in overt death or disease; however, lack of fluoride increases the risk of dental caries and gum disease, which may contribute to cardiovascular or chronic inflammatory disease. Fluoride is absorbed by the gastrointestinal system and moves quickly from the circulation into the bones and teeth, where 95% of the body's fluoride is found. Approximately 90% of excess fluoride is excreted in the urine. Fluoride is deliberately added to water supplies in the United States and some areas of Canada.[12,236] Internationally, fluoride-poor areas may supplement salt or milk with fluoride instead. In the body, fluoride is incorporated into hydroxyapatite, which, as fluoroapatite, strengthens bones and tooth enamel.[10] Although fluoride may increase the density of osteoporotic bone, it has not been shown to alter the underlying structural defects in the bone trabecular pattern that increase the risk of fracture.[237]

Excess fluoride exposure may occur industrially, from high levels of fluoride found naturally in springs or groundwater (>4 mg/L), or from the overuse of fluoride-containing products such as powdered infant formula, tea (*Camellia sinensis*), fluoride supplements, or fluoridated dental products.[237] Young children may be at risk if they swallow fluoridated toothpaste.[12] Medications, such as anesthetics, antibiotics, antiinflammatories, and cancer treatments, may contain fluoride. ^{18}F-fluorodeoxyglucose is used as an imaging agent for positron emission tomography (PET) scans.[10] Low-salt diets may decrease the element's excretion in the urine and increase fluoride retention. Symptoms of acute fluoride poisoning may include gastrointestinal symptoms and progress to excessive salivation, excessive lacrimation, sweating, and muscle weakness. Chronic ingestion of higher levels of fluoride may contribute to the risk of hypothyroidism.[238] A recent meta-analysis indicates that in utero exposures may contribute to lower IQ levels at fluoride exposure levels considered safe for adults.[239] The most obvious sign of chronic excess fluoride ingestion is fluorosis, the development of chalky, white patches on erupting teeth, which may stain yellow or brown. Continued exposure (plasma fluoride > 4 micromoles/L) may weaken or pit tooth enamel and progress in adults to osteosclerosis (excessive bone hardening), spinal exostosis (new abnormal bone growth), and genu valgum (knock-knee).[240] Symptoms of skeletal fluorosis may include joint pain and stiffness and may progress to calcification of ligaments, immobility, muscle wasting, and spinal cord compression. The use of the antifungal voriconazole may result in symptoms of fluorosis.[241]

Low levels of fluoride are associated with an increased risk of dental caries.[12,237] Dietary sources include bone-in marine fish (sardines, etc.), tea, grape juice, and crab.

Fluoride may be measured in plasma or urine.[12] Individuals drinking U.S. fluoridated water may have plasma fluoride levels of 0.1 to 0.4 mmol/L and urinary fluoride levels of 0.2 to 3.2 mg/L.[242] Drinking water samples may also be assessed for fluoride concentration with laboratory analysis. For urine and water samples, a special fluoride-specific

electrode is usually used to directly determine concentration. Tissue (blood) samples require special processing to remove the fluoride from the organic material before measurement.

Lithium

The element lithium is found sporadically in natural drinking water sources and is not considered an essential element, although it may have biological effects at low doses.[10] Lithium is absorbed by the gastrointestinal tract and excreted from the kidneys much like sodium.[243]

Lithium has been shown to upregulate the expression of glutathione S-transferase[244] and the antiapoptosis genes *BCL2* and *IRS2* in vitro. Lithium also down-regulates the proapoptosis genes Bcl2-associated agonist of cell death *(BAD)* and Bcl-2 homologous antagonist/killer *(BAK1)*.[10] Lithium has also been shown in vitro to inhibit the expression of glycogen synthase kinase-3β, an enzyme involved in Alzheimer's disease pathogenesis. Lithium exposure may alter thyroid function; urinary lithium levels have been inversely associated with plasma concentrations of T4 and positively associated with TSH in women,[245] and plasma levels of lithium carbonate have been associated with TSH levels in children.[246]

High levels of lithium carbonate are used as a pharmaceutical in the treatment of mania and bipolar disorder; responses to lithium therapy may depend in part on the patient's ethnicity and inheritance of glutamate decarboxylase-like protein 1 *(GADL1)*.[10] *GADL1* variants rs17026688, rs17026651, and IVS8 + 48*del*G may predict lithium response in patients of Han Chinese descent.[247] Because of its chemical similarities to sodium, renal lithium conservation may be increased in dehydrated patients.[243] Toxicity information is based on studies of lithium carbonate. High levels of lithium carbonate may result in lethargy, dizziness, weakness, poor coordination, slurring speech, gastrointestinal symptoms, confusion, or restlessness.[248] Chronic exposure to high levels of lithium carbonate may impair renal and thyroid function. Blood levels greater than 2.5 mmol/L may result in tremors, muscle rigidity, hyperreflexia, seizures, and renal failure. Prenatal exposure to lithium carbonate during the first trimester may result in the development of cardiac malformations.[249] The use of medications such as acetazolamide, ACE inhibitors, angiotensin II receptor antagonists, antacids, caffeine, calcium channel blockers, NSAIDs, theophylline, or psychiatric medications such as haloperidol, methyldopa, and selective serotonin reuptake inhibitors may alter the metabolism of lithium carbonate.[250]

Symptoms of deficiency may affect the central nervous system, and behavioral disorders have been associated with low lithium levels.[10] Population studies have associated lower levels of lithium exposure (2.0–5.0 μg/L) in drinking water with a higher risk of dementia, vascular dementia, and Alzheimer's disease compared with populations with more than 10 μg/L in drinking water.[251] No association has been found in population studies for the incidence of bipolar disorder and lithium levels in drinking water.[252] Low hair lithium levels have been associated with heart disease, learning disabilities, and violent behavior.[253]

Lithium levels may be evaluated in serum,[243] erythrocytes,[254] urine,[255] or hair.[253] Serum is most commonly used to assess levels of lithium carbonate and may correlate with RBC levels.[256] Urinary lithium values were found to range from 11.0 to 50.5 μg/L in a Japanese population. Hair lithium levels averaged 0.063 μg/g in a study of U.S. adults, and hair levels may correlate with lithium supplementation.

Vanadium

Vanadium is not currently considered an essential nutrient cofactor for any human enzyme, although it is essential for some rodents (rats) and other mammals.[257] Because of this, animal studies using this element may not translate directly into human populations. Vanadium may compete with phosphate-binding sites in human enzymes.[258] Increasing evidence suggests vanadium may have effects on cell metabolism and immune responses, particularly humoral B cell responses,[259] and sodium–potassium ATPase.[260] The oxidation state of vanadium may determine whether it is found in the cell or the plasma. Vanadium^{IV} binds to transferrin in the blood and is primarily found in the plasma. Intracellular vanadium is found primarily in the kidneys, spleen, bones, and liver, and is typically found as vanadium^{IV} or vanadium^{V}. Small human studies indicate that vanadium may result in minor improvements in glucose metabolism, glycogen synthesis, and HbA1c levels in subjects with type 2 diabetes; however, vanadium may also increase triglyceride levels, and large, well-controlled studies are needed to confirm findings and establish dosing.[261,262] Assimilation of vanadium by the gastrointestinal tract is poor (3%–20%). Vanadium is primarily excreted through the kidneys.

High levels of vanadium exposure, either ingested or inhaled, have toxic effects.[263–265] Toxic vanadium compounds may be encountered in industrial settings and released into the environment as industrial waste (coal emissions, fly ash, fuel oils, jet exhaust).[266] Several organic vanadium compounds have been tested as treatment agents for diabetes and cancer. Inhalation of vanadium compounds may result in cough, sputum, exertional dyspnea, wheezing, headache, nausea, palpitations, nosebleed, abnormal breath sounds (wheezes, rales, rhonchi), eye irritation, and throat inflammation. Ingestion of vanadium compounds may result in headache, nausea, and diarrhea. Chronic high levels of exposure may progress to distal tremors (hands), diarrhea, blood cell abnormalities, hair and nail changes, green-hued tongue, hypertension, and enlarged liver. Industrial exposures to vanadium compounds have been associated with altered response times, cognition, and mood in Chinese factory workers.[267]

Although vanadium deficiency has not been studied in humans, low levels of vanadium have been associated with several chronic diseases. A study of Chinese industrial workers associated low levels of vanadium with an increased risk of atherosclerosis (higher cholesterol, lower high-density lipoprotein C [HDL-C], poor apoB:apoA1 ratios); the association was strongest in male subjects. Another study found an inverse correlation between new-onset type 2 diabetes in Chinese individuals and plasma vanadium levels.[268] The subjects with the lowest levels of vanadium had the highest risk of diabetes. Food sources of vanadium include mushrooms, shellfish, peppers, parsley, dill weed, grain, and grain products.

Vanadium levels may be evaluated in whole blood, serum, urine, or hair.[265] Serum or whole blood is preferred because very little vanadium is found in RBCs. The average serum concentration due to dietary vanadium exposures is less than or equal to 2 μg/L. The average urinary vanadium level from normal background exposures (based on study controls) was found to be 2.7 μg/L. Women in the highest third of urinary vanadium concentration were shown to have a lower risk of breast cancer (64%–40%) compared with those in the lowest third.[269] Hair levels of vanadium have not been correlated with ingestion in studies of children exposed to elevated vanadium in drinking water.

CONCLUSION

Newer technologies and methodologies continue to improve the detection of mineral elements. Testing for mineral excess or deficiency may be an important step in the diagnosis of patients and may be necessary to confirm the effectiveness of supplementation or dietary changes.[270,271] It is important to remember that a laboratory result is only as good as the laboratory performing the assay. Reputable laboratories will have quality assurance and quality control procedures in place[32,272] and will participate in third-party proficiency testing when available.

Proper collection and analytic procedures must be followed to ensure accurate results. For mineral element analysis, such procedures may include the preanalytical preparation of hair samples.[273] For blood samples, element-free collection equipment is essential to prevent contamination and false high results. Careful preparation of separated blood components is also required to ensure the accuracy of results. Urinary samples may occasionally be contaminated by personal hygiene products, and hair may be easily contaminated by minerals in bathing water or by elements in hair products.[274] The interpretation of results must always include the consideration of the patient's history and physical condition because, in the end, it is the patient who is treated, not the laboratory results.[273]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Foster S, Yue C. *Herbal Emissaries: Bringing Chinese Herbs to the West*. Randolph, VT: Healing Arts Press; 1992.
2. Gratzer W. *Terrors of the Table: The Curious History of Nutrition*. Oxford: Oxford University Press; 2006.
3. Morales L. *To Get Calcium, Navajos Burn Juniper Branches to Eat the Ash*. Northern Public Radio Morning Edition. Broadcast August 21, 2017 http://www.npr.org/sections/thesalt/2017/08/21/544191316/to-get-calcium-navajos-burn-juniper-branches-to-eat-the-ash. Accessed September 22, 2017.
4. Carpenter KJ. A short history of nutritional science: part 2 (1885–1912). *J Nutr*. 2003;133(4):975–984.
5. Lowman JT, Krivit W. New in vivo tracer method with the use of nonradioactive isotopes and activation analysis. *J Lab Clin Med*. 1963;61: 1042–1048.
6. McPherson GD. Stable isotopes of calcium as tracers in studies of mineral metabolism. *Acta Orthop Scand*. 1965;78:1–86.
7. Donaldson GWK, Johnson PF, Tothill P. Red cell survival time in man as measured by 50Cr and activation analysis. *BMJ*. 1968;2: 585–587.
8. Bringquist RF, Demay MB, Kronenberg HM. Hormones and disorders of mineral metabolism. In: *Williams Textbook of Endocrinology*. 11th ed. Philadelphia: WB Saunders; 2007:1203–1268.
9. Rosen CJ, Compston JE, Lian JB. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Hoboken, NJ: Wiley; 2013.
10. Chellan P, Sadler P. The elements of life and medicines. *Philos Trans Series A Math Phys Eng Sci*. 2015;373(2037).
11. Hambidge M. Biomarkers of trace mineral intake and status. *J Nutri*. 2003;133(suppl 3):948S–955S.
12. Shenkin A, Baines M, Fell G, Lyon TDG. *Chapter 30 Vitamins and Trace Elements. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
13. American Association for Clinical Chemistry. *Trace minerals*; 2013. https://labtestsonline.org/understanding/analytes/trace-minerals/tab/test. Accessed September 21, 2017.
14. Milne D. *Laboratory Assessment of Trace Element and Mineral Status. Clinical Nutrition of the Essential Trace Elements and Minerals*. Totowa, NJ: Humana Press; 2000:69–90.
15. Grover C. *Electrolytes*. WebMD LLC; 2012. http://www.medscape.com/viewarticle/769279. Accessed September 21, 2017.
16. Reddi AS. Fluid, interpretation of urine electrolytes and osmolality. In: *Electrolyte and Acid-Base Disorders Clinical Evaluation and Management*. Cham, Switzerland: Springer; 2014.
17. Liamis G, Rodenburg EM, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Electrolyte disorders in community subjects: prevalence and risk factors. *Am J Med*. 2013;126(3):256–263 (ISSN: 1555-7162).
18. Guo H, Lee J, Guo M, Lu Y, Tang F, et al. Status of intracellular and extracellular magnesium concentration in patients with cardiac syndrome X. *Acta Cardiologica*. 2005;60(3):259–263.
19. Mierzecki A, Strecker D, Radomska K. A pilot study on zinc levels in patients with rheumatoid arthritis. *Biol Trace Element Res*. 2011;143(2):854–862.
20. Hartmann H, Bandt C, Glatzel P. Influence of changing oral mineral supply on kidney functions including renal fractional excretion of calcium, magnesium and phosphate in cows. *Berl Munch Tierarztl Wochenschr* vol: 114 (7-8) pp: 267-272.
21. Park B, Kim M, Cha C, Lee Y, Kim K. High calcium–magnesium ratio in hair is associated with coronary artery calcification in middle-aged and elderly individuals. *Biol Trace Element Res*. 2017;179(1):52–58.
22. Rodrigues J, Batista B. Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. *Sci Total Environ*. 2008;405:370–376.
23. Olabanji I, Oluyemi E, Fatoye F, Ngila J. Elemental composition of blood and hair of mentally-ill patients using ICP-OES techniques. *Int J Biol Chem Sci*. 2011;5(2):663–679.
24. Kempson I, Lombi E. Hair analysis as a biomonitor for toxicology, disease and health status. *Chem Soc Rev*. 2011;40(7):3915–3940.
25. Chojnacka K, Saeid A, Michalak I, Mikulewicz M. Effects of local industry on heavy metals content in human hair. *Pol J Environ Stud*. 2012;21(6):1563–1570.
26. Centers for Disease Control. *Health Care Providers Analysis of Hair Samples PDF*. Agency for Toxic Substances and Disease Registry; 2003. https://www.atsdr.cdc.gov/HAC/hair_analysis/03-0330HairSampleTesting-Scientific.pdf. Accessed September 21, 2017.
27. Rana S. No preanalytical errors in laboratory testing: a beneficial aspect for patients. *Indian J Clin Biochem IJCB*. 2012;27(4):319–321.
28. Simundic A, Lippi G. Preanalytical phase—a continuous challenge for laboratory professionals. *Biochem Med*. 2012;22(2):145–149.
29. Bremner I, Beattie JH. Metallothionein and the trace minerals. *Annual Rev Nutri*. 1990;10:63–83.
30. Malkina A. Chronic kidney disease. *Merck Manual Professional Version*. http://www.merckmanuals.com/professional/genitourinary-disorders/chronic-kidney-disease/chronic-kidney-disease. Accessed September 22, 2017.
31. The Joint Commission. *Laboratory proficiency testing*; 2017. https://www.jointcommission.org/accreditation/lab_proficiency_testing.aspx. Accessed September 26, 2017.
32. Theodorsson E. Quality assurance in clinical chemistry: a touch of statistics and a lot of common sense. *J Med Biochem*. 2016;35(2):103–112.
33. Johnson L. *Overview of minerals*. Merck Manual Professional Version; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/overview-of-minerals. Accessed September 19, 2017.
34. Tzioumis E, Adair L. Childhood dual burden of under- and overnutrition in low- and middle-income countries: a critical review. *Food Nutri Bull*. 2014;35(2):230–243.
35. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. *BMC Med*. 2012;10(1):119.
36. Massey L. Dietary animal and plant protein and human bone health: a whole foods approach. *J Nutri*. 2003;133(3):862S–865S.
37. Stover P. Influence of human genetic variation on nutritional requirements. *Am J Clin Nutri*. 2006;83(2):436S–442S.
38. Burk RF, Early DS, Hill KE, et al. Plasma selenium in patients with cirrhosis. *Hepatology*. 2003;27:794–798.
39. Whelton MJ, Kehayoglou AK, Agnew JE, et al. Calcium absorption in parenchymatous and biliary liver disease. *Gut*. 1971;12:978–983.
40. Lim P, Jacob E. Magnesium deficiency in liver cirrhosis. *Q J Med*. 1972;41:291–300.
41. Ozsoylu S, Akgun N. Defective neutrophils motility. *Am J Dis Child*. 1985;139:10.
42. Sullivan JF, Blotcky AJ, Jetton MM, et al. Serum levels of selenium, calcium, copper, magnesium, manganese and zinc in various human diseases. *J Nutr*. 1979;109:1432–1437.
43. Oster O, Dahm M, Oelert H. Element concentrations (selenium, copper, zinc, iron, magnesium, potassium, phosphorous) in heart tissue of patients with coronary heart disease correlated with physiological parameters of the heart. *Eur Heart J*. 1993;14:770–774.
44. Beck KW, Schramel P, Hedl A, et al. Serum trace element levels in HIV-infected subjects. *Biol Trace Elem Res*. 1990;25:89–96.
45. Kuo HW, Chen SF, Wu CC, et al. Serum and tissue trace elements in patients with breast cancer in Taiwan. *Biol Trace Elem Res*. 2002;89: 1–11.
46. Goyal M, Kalwar A, Vyas R, Bhati A. A study of serum zinc, selenium and copper levels in carcinoma of esophagus patients. *Ind J Clin Biochem*. 2006;21(1):208–210.
47. Celik HA, Aydin HH, Ozsaran A, et al. Trace elements analysis of ascitic fluid in benign and malignant diseases. *Clin Biochem*. 2002;35:477–481.
48. Iskra M, Majewski W, Piorunska-Stolzmann M. Modifications of magnesium and copper concentrations in serum and arterial wall of patients with vascular diseases related to ageing, atherosclerosis and aortic aneurysm. *Mag Res*. 2002;15(3-4):279–285.
49. Kucharzewski M, Braziewicz J, Majewska U, et al. Copper, zinc, and selenium in whole blood and thyroid tissue of people with various thyroid diseases. *Biol Trace Elem Res*. 2003;93:9–18.

50. Gupta SK, Shukla VK, Vaidya MP, et al. Serum trace elements and Cu/Zn ratio in breast cancer patients. *J Surg Oncol*. 1991;46:178–181.
51. Higdon J, Weaver C. *Calcium*. Linus Pauling Institute, Oregon State University; 2017. http://lpi.oregonstate.edu/mic/minerals/calcium. Accessed September 26, 2017.
52. Komolov I, Fedotov V, Gudoshnikov V, Fazekas I, Rappay G. Calcium modifies the effects of thyroliberin and somatostatin on hormone release from cell cultures of the rat anterior pituitary. *Endokrinologie*. 1982;80(3):318–324.
53. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Ind J Anaesthesia*. 2014;58(5):515–523.
54. Lewis III JL. *Overview of disorders of calcium concentration*. Merck Manual Professional Version; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/overview-of-disorders-of-calcium-concentration. Accessed September 26, 2017.
55. Shenkin A, Baines M, Fell G, Lyon TDG. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
56. Lewi Brookes P, Yoon Y, Robotham J, Anders M, Sheu S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol*. 2004;287(4):C817–C833.
57. Lewis III JL. *Hypercalcemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypercalcemia. Accessed September 26, 2017.
58. National Institutes of Health. *Calcium fact sheet for health professionals*; 2016. https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/. Accessed September 28, 2017.
59. Lewis III JL. *Hypocalcemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypocalcemia. Accessed September 26, 2017.
60. Jeruszka-Bielak M, Brzozowska A. Relationship between nutritional habits and hair calcium levels in young women. *Biol Trace Element Res*. 2011;144(1–3):63–76.
61. Park B, Kim M, Cha C, Lee Y, Kim K. High calcium–magnesium ratio in hair is associated with coronary artery calcification in middle-aged and elderly individuals. *Biol Trace Element Res*. 2017;179(1):52–58.
62. Ozmen H, Akarsu S, Polat F, Cukurovali A. The levels of calcium and magnesium, and of selected trace elements, in whole blood and scalp hair of children with growth retardation. *Iranian J Pediatri*. 2013;23(2):125–130.
63. Bass DA, Hickok D, Quig D, Urek K. Trace element analysis in hair: factors determining accuracy, precision, and reliability. *Altern Med Rev*. 2001;6(5):472–481.
64. Pereira D, Lima R, de Lima R, Gonçalves M, de Morais L, et al. Association between obesity and calcium:phosphorus ratio in the habitual diets of adults in a city of northeastern Brazil: an epidemiological study. *Nutri J*. 2013;12(1):90.
65. Al Hasan SM, Hassan M, Saha S, Islam M, Billah M, et al. Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: a cross-sectional study. *BMC Nutri*. 2016;2(1):24.
66. Shenkin A, Baines M, Fell G, Lyon TDG. Electrolytes and blood gases. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
67. American Association for Clinical Chemistry. *Chloride*; 2016. https://labtestsonline.org/understanding/analytes/chloride/tab/test. Accessed September 27, 2017.
68. Mahaladar A. Acid base and fluid electrolyte disturbances in chronic kidney disease. *Clin Queries Nephrol*. 2012;1(4):295–299.
69. Thunhorst R, Beltz T, Johnson A. Glucocorticoids increase salt appetite by promoting water and sodium excretion. *Am J Physiol Regul Integr Comp Physiol*. 2007;293(3):R1444–R1451.
70. Mishra A, Greaves R, Massie J. The relevance of sweat testing for the diagnosis of cystic fibrosis in the genomic era. *Clin Biochem Rev*. 2005;26(4):135–153.
71. Lewis III JL. *Acid-base disorders*. Merck Manual Professional Version; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/acid-base-regulation-and-disorders/acid-base-disorders#v987379. Accessed September 27, 2017.
72. Higdon J, Volpe S. *Magnesium*. Linus Pauling Institute, Oregon State University; 2014. http://lpi.oregonstate.edu/mic/minerals/magnesium. Accessed September 27, 2017.
73. American Association for Clinical Chemistry. Magnesium. https://labtestsonline.org/understanding/analytes/magnesium/tab/test; 2013. Accessed September 28, 2017.
74. Schwalfenberg G, Genuis S. The importance of magnesium in clinical healthcare. *Scientifica*. 2017;(2017):1–14.
75. Lewis III JL. *Hypermagnesemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypermagnesemia. Accessed September 28, 2017.
76. Lewis III JL. *Hypomagnesemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypomagnesemia. Accessed September 28, 2017.
77. Seelig M. *Magnesium Deficiency in the Pathogenesis of Disease*. New York: Plenum Medical Books; 1980.
78. Kisters K, Tepel M, Spieker C, et al. Decreased membrane Mg2+ concentrations in a subgroup of hypertensives: membrane model for the pathogenesis of primary hypertension. *Am J Hypertens*. 1998;11:1390–1393.
79. Holm C, Jepsen J, Sjøgaard G, Hessov I. A magnesium load test in the diagnosis of magnesium deficiency. *Hum Nutri Clin Nutri*. 1987;41(4):301–306.
80. National Institutes of Health. *Magnesium fact sheet for professionals*; 2016. https://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/. Accessed September 28, 2017.
81. Abraham A, Rosenman D, Meshulam Z, Zion M, Eylath U. Serum, lymphocyte, and erythrocyte potassium, magnesium, and calcium concentrations and their relation to tachyarrhythmias in patients with acute myocardial infarction. *Am J Med*. 1986;81(6):983–988.
82. Genetics Home Reference. *MAGT1 gene*. U.S. National Library of Medicine; 2014. https://ghr.nlm.nih.gov/gene/MAGT1#. Accessed September 27, 2017.
83. Standley C, Whitty JE, Mason BA, Cotton DB. Serum ionized magnesium levels in normal and preeclamptic gestation. *Obstetr Gynecol*. 1997;89(1):24–27.
84. Gürlek A, Bayraktar M, Özaltin N. Intracellular magnesium depletion relates to increased urinary magnesium loss in type I diabetes. *Hormone Metabol Res*. 1998;30(02):99–102.
85. Joosten M, Gansevoort R, Bakker S. Assessing magnesium by 24-h urinary excretion. *Am J Clin Nutri*. 2015;101(1):240–241.
86. Higdon J, Calvo M. *Phosphorus*. Linus Pauling Institute, Oregon State University; 2014. http://lpi.oregonstate.edu/mic/minerals/phosphorus. Accessed September 29, 2017.
87. Lewis III JL. *Hyperphosphatemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hyperphosphatemia. Accessed September 29, 2017.
88. Lewis III JL. *Hypophosphatemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypophosphatemia. Accessed October 2, 2017.
89. Shenkin A, Baines M, Fell G, Lyon TDG. Physiology and disorders of water, electrolyte, and acid-base metabolism. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
90. Higdon J, Lin P. *Potassium*. Linus Pauling Institute, Oregon State University; 2010. http://lpi.oregonstate.edu/mic/minerals/potassium. Accessed October 2, 2017.
91. American Association for Clinical Chemistry. *Potassium*; 2016. https://labtestsonline.org/understanding/analytes/potassium/tab/test. Accessed October 2, 2017.
92. Lewis III JL. *Hyperkalemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hyperkalemia. Accessed October 2, 2017.
93. Sevastos N, Theodossiades G, Archimandritis A. Pseudohyperkalemia in serum: a new insight into an old phenomenon. *Clin Med Res*. 2008;6(1):30–32.

94. Lewis III JL. *Hypokalemia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypokalemia. Accessed October 2, 2017.
95. Bahemuka M, Hodkinson H. Red-blood-cell potassium as a practical index of potassium status in elderly patients. *Age Ageing*. 1976;5(1):24–30.
96. Barbagallo Sangiorgi G, Costanza G, Di Sciacca A, Frada' G, Cupidi G, et al. Serum potassium levels, red-blood-cell potassium and alterations of the repolarization phase of electrocardiography in old subjects. *Age Ageing*. 1984;13(5):309–312.
97. De Luise M, Blackburn G, Flier J. Reduced activity of the red-cell sodium-potassium pump in human obesity. *N Engl J Med*. 1980;303(18):1017–1022.
98. Lans H, Stein I, Meyeb K. The relation of serum potassium to erythrocyte potassium in normal subjects and patients with potassium deficiency. *Am J Med Sci*. 1952;223:65–74.
99. Mill J, Silva A, Baldo M, Molina M, Rodrigues S. Correlation between sodium and potassium excretion in 24- and 12-h urine samples. *Brazil J Med Biol Res*. 2012;45(9):799–805.
100. Kieneker L, Gansevoort R, Mukamal K, de Boer R, Navis G, et al. Urinary potassium excretion and risk of developing hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension*. 2014;64(4):769–776.
101. O'Donnell M, Mente A, Rangarajan S, McQueen M, Wang X, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. *N Engl J Med*. 2014;371(7):612–623.
102. Higdon J, Pruess H. *Sodium (chloride)*. Linus Pauling Institute, Oregon State University; 2016. http://lpi.oregonstate.edu/mic/minerals/sodium. Accessed October 4, 2017.
103. American Association for Clinical Chemistry. *Sodium*; 2016. https://labtestsonline.org/understanding/analytes/sodium/tab/test. Accessed October 4, 2017.
104. Lewis III JL. *Hypernatremia. Merck Manual Professional Version*; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hypernatremia. Accessed October 4, 2017.
105. Andersson B, Rundgren M. Thirst and Its Disorders. *Annual Rev Med*. 1982;33(1):231–239.
106. Lewis III JL. *Hyponatremia*. Merck Manual Professional Version; 2016. http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hyponatremia. Accessed October 4, 2017.
107. American Association for Clinical Chemistry. *Osmolality*. 2016. https://labtestsonline.org/understanding/analytes/osmolality/tab/test. Accessed October 4, 2017.
108. O'Donnell M, Mente A, Rangarajan S, McQueen M, Wang X, et al. Urinary Sodium and potassium excretion, mortality, and cardiovascular events. *N Engl J Med*. 2014;371(7):612–623.
109. American Association for Clinical Chemistry. *Copper*; 2015. https://labtestsonline.org/understanding/analytes/copper/tab/test. Accessed October 10, 2017.
110. Higdon J, Prohaska J. *Copper*. Linus Pauling Institute, Oregon State University; 2014. http://lpi.oregonstate.edu/mic/minerals/copper. Accessed October 10, 2017.
111. Devi SRB, Aloysius DM, Sulochana KN. Copper transporters and chaperones: their function on angiogenesis and cellular signalling. *J Biosci*. 2016;41(3):487–496.
112. Johnson LE. *Copper. Merck Manual of Medicine Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/copper. Accessed October 10, 2017.
113. Prasad R, Singh A, Das BK, Upadhyay RS, Singh TB, Mishra OP. Cerebrospinal fluid and serum zinc, copper, magnesium and calcium levels in children with idiopathic seizure. *J Clin Diag Res*. 2009;3(6):1841–1846.
114. Johnson LE. *Wilson disease (Wilson's disease; inherited copper toxicity)Merck Manual of Medicine Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/wilson-disease. Accessed October 10, 2017.
115. Koç E, Ilhan A, Aytürk Zübeyde, Acar B, Gürler M, et al. A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants. *Tur J Med Sci*. 2015;45(5):1034–1039.
116. Gürgöze M, Ölçücü A, Aygün A, Taskin E, Kiliç M. Serum and hair levels of zinc, selenium, iron, and copper in children with iron-deficiency anemia. *Biol Trace Element Res*. 2006;111(1–3):23–30.
117. González-Reimers E, Alemán-Valls M, Barroso-Guerrero F, Santolaria-Fernández F, López-Lirola L, et al. Hair zinc and copper in chronic alcoholics. *Biol Trace Element Res*. 2002;85(3):269–275.
118. Vaz F, Fermino B, Haskel M, Wouk J, de Freitas G, et al. The relationship between copper, iron, and selenium levels and Alzheimer disease. *Biol Trace Element Res*. 2017;1–7.
119. Higdon J, Pearce E. *Iodine*. Linus Pauling Institute, Oregon State University; 2015. http://lpi.oregonstate.edu/mic/minerals/iodine. Accessed October 11, 2017.
120. Johnson L. *Iodine*. Merck Manual Professional Version; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/iodine. Accessed October 11, 2017.
121. National Institutes of Health. *Iodine fact sheet for health professionals*; 2011. https://ods.od.nih.gov/factsheets/Iodine-HealthProfessional/. Accessed October 11, 2017.
122. Bürgi H. Iodine excess. *Best Pract Res Clin Endocrinol Metab*. 2010;24(1):107–115.
123. Connelly K, Boston B, Pearce E, Sesser D, Snyder D, et al. Congenital hypothyroidism caused by excess prenatal maternal iodine ingestion. *J Pediatri*. 2012;161(4):760–762.
124. Thomson C, Campbell J, Miller J, Skeaff S. Minimal impact of excess iodate intake on thyroid hormones and selenium status in older New Zealanders. *Eur J Endocrinol*. 2011;165(5):745–752.
125. Furnée C, West C, van der Haar F, Hautvast J. Effect of intestinal parasite treatment on the efficacy of oral iodized oil for correcting iodine deficiency in schoolchildren. *Am J Clin Nutri*. 1997;66(6):1422–1427.
126. World Health Organization, UNICEF, ICCIDD. 4.2 Urinary iodine. In: *Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination: A Guide for Programme Managers*. 3rd ed. Geneva: World Health Organization; 2007: 32–34.
127. Ristic-Medic D, Piskackova Z, Hooper L, Ruprich J, Casgrain A, et al. Methods of assessment of iodine status in humans: a systematic review. *Am J Clin Nutri*. 2009;89(6):2052S–2069S.
128. Soldin O, Tractenberg R, Pezzullo J. Do thyroxine and thyroid-stimulating hormone levels reflect urinary iodine concentrations? *Ther Drug Monitoring*. 2005;27(2):178–185.
129. Abraham GE. The safe and effective implementation of orthoiodosupplementation in medical practice. *Original Internist*. 2004;11:17–36.
130. Shenkin A, Baines M, Fell G, Lyon TDG. Hemoglobin, iron and bilirubin. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
131. Fontecave M. Iron-sulfur clusters: ever-expanding roles. *Nat Chem Biol*. 2006;2(4):171–174.
132. Higdon J, Wessling-Resnick M. *Iron*. Linus Pauling Institute, Oregon State University; 2016. http://lpi.oregonstate.edu/mic/minerals/iron. Accessed October 13, 2017.
133. National Institutes of Health. *Iron dietary supplement fact sheet*; 2016. https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/. Accessed October 13, 2017.
134. Johnson L. *Iron. Merck Manual Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/iron. Accessed October 13, 2017.
135. American Association for Clinical Chemistry. *More details on the HFE gene*; 2013. https://labtestsonline.org/understanding/conditions/hfe-gene/. Accessed October 16, 2017.
136. Ekanayake D, Roddick C, Powell L. Recent advances in hemochromatosis: a 2015 update. *Hepatol Int*. 2015;9(2):174–182.
137. Goldberg YP. Juvenile hereditary hemochromatosis. GeneReviews® [Internet]; 2011. https://www.ncbi.nlm.nih.gov/books/NBK1170/. Accessed October 13, 2017.
138. Niv E, Elis A, Zissin R, Naftali T, Novis B, et al. Iron deficiency anemia in patients without gastrointestinal symptoms—a prospective study. *Fam Pract*. 2004;22(1):58–61.

139. Fletcher J, Mather J, Lewis M, Whiting G. Mouth lesions in iron-deficient anemia: relationship to Candida albicans in saliva and to impairment of lymphocyte transformation. *J Infect Dis*. 1975;131(1):44–50.
140. American Association for Clinical Chemistry. *Iron tests*; 2013. https://labtestsonline.org/understanding/analytes/iron/tab/test. Accessed October 16, 2017.
141. American Association for Clinical Chemistry. *Hematocrit*. 2015. https://labtestsonline.org/understanding/analytes/hematocrit/tab/test/. Accessed October 16, 2017.
142. Schoorl M, Schoorl M, Van der Gaag D, Bartels P. Effects of iron supplementation on red blood cell hemoglobin content in pregnancy. *Hematol Rep*. 2012;4(4).
143. American Association for Clinical Chemistry. *TIBC, UIBC, and transferrin*; 2013. https://labtestsonline.org/understanding/analytes/tibc/tab/test/. Accessed October 16, 2017.
144. Rifkind D, Kravetz H, Knight V, Schade A. Urinary excretion of iron-binding protein in the nephrotic syndrome. *N Engl J Med*. 1961;265(3):115–118.
145. Skikne B. Serum transferrin receptor. *Am J Hematol*. 2008;83(11):872–875.
146. American Association for Clinical Chemistry. *Soluble transferrin receptor*; 2015. https://labtestsonline.org/understanding/analytes/soluble-transferrin-receptor/tab/test/. Accessed October 17, 2017.
147. American Association for Clinical Chemistry. *Zinc protoporphyrin*; 2014. https://labtestsonline.org/understanding/analytes/zpp/tab/test. Accessed October 17, 2017.
148. Orlando E, Raffi G, Alessandri M. Urinary concentration of iron and lead in a group of workers exposed to the risk of lead poisoning. *Med Lavoro*. 1965;56(11):732–737. ref.22.
149. Kimura I, Yamana M, Nishishita A, Sugiyama M, Miyata A. Urinary iron excretion test in iron deficiency anemia. *Acta Med Okayama*. 1980;34(1):37–41.
150. Takeuchi H, Taki Y, Sekiguchi A, Nouchi R, Kotozaki Y, et al. Association of hair iron levels with creativity and psychological variables related to creativity. *Front Hum Neurosci*. 2013;7:875.
151. Higdon J, Aschner M. *Manganese*. Linus Pauling Institute, Oregon State University; 2010. http://lpi.oregonstate.edu/mic/minerals/manganese. Accessed October 17, 2017.
152. Johnson L. *Manganese. Merck Manual Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/manganese. Accessed October 17, 2017.
153. Cannon J, Greenamyre J. The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicol Sci Off J Soc Toxicol*. 2011;124(2):225–250.
154. Guilarte T. Manganese and Parkinson's disease: a critical review and new findings. *Environ Health Perspect*. 2010;118(8):1071–1080.
155. Tuschl K, Clayton P, Gospe S, Gulab S, Ibrahim S, et al. Syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia caused by mutations in SLC30A10, a manganese transporter in man. *Am J Hum Gen*. 2012;90(3):457–466.
156. Gonzalez-Reyes RE, Gutierrez-Alvarez AM, Moreno CB. Manganese and epilepsy: a systematic review of the literature. *Brain Res Rev*. 2007;53(2):332–336.
157. Du K, Liu M, Pan Y, Zhong X, Wei M. Association of serum manganese levels with Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. *Nutrients*. 2017;9(3):231.
158. Hatano S, Nishi Y, Usui T. Erythrocyte manganese concentration in healthy Japanese children, adults, and the elderly, and in cord blood. *Am J Clin Nutri*. 1983;37(3):457–460.
159. Laohaudomchok W, Lin X, Herrick R, Fang S, Cavallari J, et al. Toenail, blood, and urine as biomarkers of manganese exposure. *J Occup Environ Med*. 2011;53(5):506–510.
160. Franco R. Measurement of red cell lifespan and aging. *Transfus Med Hemother: Off Org Deutschen Gesellschaft Transfus Immunhamatol*. 2012;39(5):302–307.
161. Lombeck I, Wendel U, Versieck J, van Ballenberghe L, Bremer H, et al. Increased manganese content and reduced arginase activity in erythrocytes of a patient with prolidase deficiency (iminodipeptiduria). *Eur J Pediatr*. 1986;144(6):571–573.
162. Kilic E, Saraymen R, Demiroglu A, Ok E. Chromium and manganese levels in the scalp hair of normals and patients with breast cancer. *Biol Trace Element Res*. 2004;102(1–3):019–026.
163. Molina-Villalba I, Lacasaña M, Rodríguez-Barranco M, Hernández A, Gonzalez-Alzaga B, et al. Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. *Chemosphere*. 2015;124:83–91.
164. Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. *Environ Health Perspect*. 2007;115(1):122–127.
165. Ashraf W, Jaffar M, Mohammad D. Trace metal contamination study on scalp hair of occupationally exposed workers. *Bull Environ Contam Toxicol*. 1994;53(4).
166. Higdon J, Mendel R. *Molybdenum*. Linus Pauling Institute, Oregon State University; 2010. http://lpi.oregonstate.edu/mic/minerals/molybdenum. Accessed October 18, 2017.
167. Klein J, Busch J, Potting C, Baker M, Langer T, et al. The mitochondrial amidoxime-reducing component (mARC1) is a novel signal-anchored protein of the outer mitochondrial membrane. *J Biol Chem*. 2012;287(51):42795–42803.
168. Mendel R. The molybdenum cofactor. *J Biol Chem*. 2013;288(19):13165–13172.
169. Genetics Home Reference. *molybdenum cofactor deficiency*. U.S. National Library of Medicine; 2017. https://ghr.nlm.nih.gov/condition/molybdenum-cofactor-deficiency#genes. Accessed October 18, 2017.
170. Turnlund J, Keyes W, Peiffer G. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. *Am J Clin Nutri*. 1995;62(4):790–796.
171. Johnson L. *Molybdenum. Merck Manual Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/molybdenum. Accessed October 18, 2017.
172. Yoshida M, Nakagawa M, Hosomi R, Nishiyama T, Fukunaga K. Low molybdenum state induced by tungsten as a model of molybdenum deficiency in rats. *Biol Trace Element Res*. 2015;165(1):75–80.
173. Lewis R, Johns L, Meeker J. Exploratory analysis of the potential relationship between urinary molybdenum and bone mineral density among adult men and women from NHANES 2007–2010. *Chemosphere*. 2016;164:677–682.
174. Higdon J, Tsuji P. *Selenium*. Linus Pauling Institute, Oregon State University; 2015. http://lpi.oregonstate.edu/mic/minerals/selenium. Accessed October 20, 2017.
175. National Institutes of Health. *Selenium dietary supplement fact sheet*; 2016. https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/. Accessed October 20, 2017.
176. Schoenmakers E, Carlson B, Agostini M, Moran C, Rajanayagam O, et al. Mutation in human selenocysteine transfer RNA selectively disrupts selenoprotein synthesis. *J Clin Investig*. 2016;126(3):992–996.
177. Schwenke D, Behr S. Quantification in vivo of increased LDL content and rate of LDL degradation in normal rabbit aorta occurring at sites susceptible to early atherosclerotic lesions. *Cir Res*. 1988;62(4):699–710.
178. Gammelgaard B, Rasmussen L, Gabel-Jensen C, Steffansen B. Estimating intestinal absorption of inorganic and organic selenium compounds by in vitro flux and biotransformation studies in caco-2 cells and ICP-MS detection. *Biol Trace Element Res*. 2012;145(2):248–256.
179. Sanz Alaejos M, Díaz Romero C. Urinary selenium concentrations. *Clin Chem*. 1993;39(10):2040–2052.
180. Vinceti M, Filippini T, Cilloni S, Bargellini A, Vergoni A, et al. Health risk assessment of environmental selenium: emerging evidence and challenges (Review). *Mol Med Rep*. 2017;15(5):3323–3335.
181. Pillai R, Uyehara-Lock J, Bellinger F. Selenium and selenoprotein function in brain disorders. *IUBMB Life*. 2014;66(4):229–239.
182. Thomson C, Chisholm A, McLachlan S, Campbell J. Brazil nuts: an effective way to improve selenium status. *Am J Clin Nutri*. 2008;87(2):379–384.
183. Burk R, Norsworthy B, Hill K, Motley A, Byrne D. Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. *Cancer Epidemiol Biomarker Prev*. 2006;15(4):804–810.

184. Stefanowicz F, Talwar D, O'Reilly D, Dickinson N, Atkinson J, et al. Erythrocyte selenium concentration as a marker of selenium status. *Clin Nutri*. 2013;32(5):837–842.
185. Semba R, Ricks M, Ferrucci L, Xue Q, Guralnik J, et al. Low serum selenium is associated with anemia among older adults in the United States. *Eur J Clin Nutri*. 2009;63(1):93–99.
186. Lane H, Warren D, Taylor B, Stool E. Blood selenium and glutathione peroxidase levels and dietary selenium of free-living and institutionalized elderly subjects. *Proceedings of the Society for Experimental Biology and Medicine*. Vol. 173(1). New York: Society for Experimental Biology and Medicine; 1983:87–95.
187. Hawkes W, Richter B, Alkan Z, Souza E, Derricote M, et al. Response of Selenium status indicators to supplementation of healthy North American men with high-selenium yeast. *Biol Trace Element Res*. 2008;122(2):107–121.
188. Høl P, Vamnes J, Gjerdet N, Eide R, Isrenn R. Dental amalgam affects urinary selenium excretion. *Biol Trace Element Res*. 2002;85(2):137–147.
189. Hác E, Krechniak J, Szyszko M. Selenium levels in human plasma and hair in northern Poland. *Biol Trace Element Res*. 2002;85(3):277–285.
190. Pan D, Huang H. Hair selenium levels in hepatic steatosis patients. *Biol Trace Element Res*. 2013;152(3):305–309.
191. Fülöp P, Seres I, Jenei Z, Juhász I, Paragh G. Increased hair selenium concentration in hyperlipidemic patients. *J Cellul Molecul Med*. 2013;17(3):350–355.
192. van Cutsem J, van Gerven F, Fransen J, Schrooten P, Janssen P. The in vitro antifungal activity of ketoconazole, zinc pyrithione, and selenium sulfide against Pityrosporum and their efficacy as a shampoo in the treatment of experimental pityrosporosis in guinea pigs. *J Am Acad Dermatol*. 1990;22(6):993–998.
193. Higdon J, Ho E. *Zinc*. Linus Pauling Institute, Oregon State University; 2015. http://lpi.oregonstate.edu/mic/minerals/zinc. Accessed October 23, 2017.
194. National Institutes of Health. *Zinc dietary supplement fact sheet*; 2016. https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/. Accessed October 23, 2017.
195. Engelken J, Carnero-Montoro E, Pybus M, Andrews G, Lalueza-Fox C, et al. Extreme population differences in the human zinc transporter ZIP4 (SLC39A4) are explained by positive selection in sub-Saharan Africa. *PLoS Gen*. 2014;10(2):e1004128. https://doi.org/10.1371/journal.pgen.1004128.
196. Johnson L. *Zinc. Merck Manual Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/zinc. Accessed October 23, 2017.
197. Johnson A, Munoz A, Gottlieb J, Jarrard D. High dose zinc increases hospital admissions due to genitourinary complications. *J Urol*. 2007;177(2):639–643.
198. Jumaan R. Serum copper, zinc and copper/zinc ratio and their relationship to age and growth status in Yemeni adolescent girls. *Sultan Qaboos Univ Med J*. 2008;8(3):291–299.
199. Krebs N. Update on zinc deficiency and excess in clinical pediatric practice. *Annal Nutri Metabol*. 2013;62(suppl. 1):19–29.
200. Ma J, Betts N. Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII). *J Nutri*. 2000;130(11):2838–2843.
201. Kayaaltı Z, Aliyev V, Söylemezoğlu T. The potential effect of metallothionein 2A−5 A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. *Toxicol Appl Pharmacol*. 2011;256(1):1–7.
202. Lowe N, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. *Am J Clin Nutri*. 2009;89(6):2040S–2051S.
203. Gruys E, Toussaint M, Niewold T, Koopmans S. Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci*. 2005;6B(11):1045–1056.
204. Zhang L, Lu L, Pan Y, Ding C, Xu D, et al. Baseline blood levels of manganese, lead, cadmium, copper, and zinc in residents of Beijing suburb. *Environ Res*. 2015;140:10–17.
205. Schultze B, Lind P, Larsson A, Lind L. Whole blood and serum concentrations of metals in a Swedish population-based sample. *Scand J Clin Laboratory Investig*. 2014;74(2):143–148.
206. Buxaderas S, Farré-Rovira R. Whole blood and serum zinc levels in relation to sex and age. *Rev Espanola Fisiol*. 1985;41(4):463–470.
207. Yamane M, Abe A, Yanagisawa I. Anticholinesterase action of a bromine compound isolated from human cerebrospinal fluid. *J Neurochem*. 1984;42(6):1650–1654.
208. McCall A, Cummings C, Bhave G, Vanacore R, Page-McCaw A, et al. Bromine is an essential trace element for assembly of collagen IV scaffolds in tissue development and architecture. *Cell*. 2014;157(6):1380–1392.
209. Canavese C, De Costanzi E, Stratta P, Sabbioni E. A role for bromine deficiency in sleep disturbances of long-term dialysis patients. *Am J Kid Dis*. 2006;48(6):1018–1019.
210. Torii S, Mitsumori K, Inubushi S, Yanagisawa I. The REM sleep-inducing action of a naturally occurring organic bromine compound in the encéphale isolé cat. *Psychopharmacologia*. 1973;29(1):65–75.
211. van Leeuwen F, Hanemaaijer R, Loeber J. The effect of sodium bromide on thyroid function. In: *The Target Organ and the Toxic Process*. Berlin, Heidelberg: Springer; 1988:93–97.
212. Zeratsky K. Should I be worried that my favorite soda contains brominated vegetable oil? *Mayo Clinic Healthy Lifestyle Nutrition and Healthy Eating*; 2017. https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/expert-answers/bvo/faq-20058236. Accessed October 25, 2017.
213. Drugs.com. *Potassium bromide*; 2017. https://www.drugs.com/international/potassium-bromide.html. Accessed October 25, 2017.
214. U.S. Department of Veteran's Affairs. Pyridostigmine bromide and Gulf War veterans. https://www.publichealth.va.gov/exposures/gulfwar/sources/pyridostigmine-bromide.asp. Accessed October 25, 2017.
215. Wielopolski L, Adams WH, Heotis PM. Blood bromine levels in a Pacific atoll population. *Environ Res*. 1986;41(1):91–98.
216. Allain P, Berre S, Krari N, Laine P, Barbot N, et al. Bromine and thyroid hormone activity. *J Clin Pathol*. 1993;46(5):456–458.
217. Fujii R, Yoshida H, Fukusumi S, Habata Y, Hosoya M, Kawamata Y, Yano T, Hinuma S, et al. Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. *J Biol Chem*. 2002;277(37):34010–34016.
218. Wallaeys B, Cornelis R, Lameire N. The Trace Elements Br, Co, Cr, Cs, Cu, Fe, Mn, Rb, Se and Zn in serum, packed cells, and dialysate of COPD patients. *Front Peritoneal Dial*. 1986:478–481.
219. Mayo Medical Laboratory. *Bromide, blood*; 2015. https://www.mayomedicallaboratories.com/test-notifications/attachment.php?id=40801. Accessed October 25, 2017.
220. Shenkin A, Baines M, Fell G, Lyon TDG. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
221. Hanley KW, Petersen M, Curwin BD, Sanderson WT. Urinary bromide and breathing zone concentrations of 1-bromopropane from workers exposed to flexible foam spray adhesives. *Annals Occup Hygiene*. 2006;50(6):599–607.
222. Johnson L. *Chromium. Merck Manual Professional Version*; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/chromium. Accessed October 26, 2017.
223. Vincent JB. Chromium: is it essential, pharmacologically relevant, or toxic? In: *Interrelations Between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences*. Vol 13. Dordrecht: Springer; 2013.
224. Higdon J, Vincent J. *Chromium*. Linus Pauling Institute; 2014. http://lpi.oregonstate.edu/mic/minerals/chromium. Accessed October 26, 2017.
225. Kobla H, Volpe S. Chromium, exercise, and body composition. *Crit Rev Food Sci Nutri*. 2000;40(4):291–308.
226. Tian H, Guo X, Wang X, He Z, Sun R, et al. Chromium picolinate supplementation for overweight or obese adults. *Cochrane Database Syst Rev*. 2013;(11):CD010063.
227. Chen S, Jin X, Shan Z, Li S, Yin J, et al. Inverse association of plasma chromium levels with newly diagnosed type 2 diabetes: a case-control study. *Nutrients*. 2017 Mar;9(3):294.
228. Anderson R, Polansky M, Bryden N, Canary J. Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutri*. 1991;54(5):909–916.
229. Geohas J, Daly A, Juturu V, Finch M, Komorowski J. Chromium picolinate and biotin combination reduces atherogenic index of plasma in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Am J Med Sci*. 2007;333(3):145–153.

230. Agency for Toxic Substances & Disease Registry. *Toxicological profile for chromium*; 2015. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=62&tid=17. Accessed October 26, 2017.
231. O'Flaherty E, Kerger B, Hays S, Paustenbach D. A physiologically based model for the ingestion of chromium (III) and chromium (VI) by humans. *Toxicol Sci Off J Soc Toxicol*. 2001;60(2):196–213.
232. Linos A, Petralias A, Christophi CA, Christoforidou E, Kouroutou P, et al. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece—An ecological study. *Environ Health*. 2011;10(1):50.
233. Lukaski HC. Magnesium, zinc, and chromium nutriture and physical activity. *Am J Clin Nutr*. 2000;72(2 suppl):585S–593S.
234. Devoy J, Géhin A, Müller S, Melczer M, Remy A, et al. Evaluation of chromium in red blood cells as an indicator of exposure to hexavalent chromium: an in vitro study. *Toxicol Letters*. 2016;255:63–70.
235. Division of Oral Health, National Center for Chronic Disease Prevention and Health Promotion. *Water fluoridation basics*. Centers for Disease Control and Prevention; 2016. https://www.cdc.gov/fluoridation/basics/index.htm. Accessed October 27, 2017.
236. Rabb-Waytowich D. Water fluoridation in Canada: past and present. *J Canad Dent Ass*. 2009. http://www.cda-adc.ca/jcda/vol-75/issue-6/451.html. Accessed October 27, 2017.
237. Higdon J, Warren J. *Fluoride*. Linus Pauling Institute; 2014. http://lpi.oregonstate.edu/mic/minerals/fluoride. Accessed October 27, 2017.
238. Peckham S, Lowery D, Spencer S. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. *J Epidemiol Commun Health*. 2015;69(7):619–624.
239. Choi A, Sun G, Zhang Y, Grandjean P. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. *Environ Health Perspect*. 2012;120(10):1362–1368.
240. Johnson L. *Fluorine*. Merck Manual Professional Version; 2017. http://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/fluorine. Accessed October 27, 2017.
241. Skiles J, Imel E, Christenson J, Bell J, Hulbert M. Fluorosis because of prolonged voriconazole therapy in a teenager with acute myelogenous leukemia. *J Clin Oncol Off J Am Soc Clin Oncol*. 2011;29(32):e779–e782.
242. Cardos VES, Whitford GH, Aoyama H, et al. Daily variations in human plasma fluoride concentrations. *J Fluorine Chem*. 2008;129:1193–1119.
243. Shenkin A, Baines M, Fell G, Lyon TDG. Therapeutic drugs and their management. In: Burtis CA, Ashwood ER, Bruns DE, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Philadelphia, PA: Elsevier; 2006.
244. Shao L, Cui J, Young L, Wang J. The effect of mood stabilizer lithium on expression and activity of glutathione s-transferase isoenzymes. *Neuroscience*. 2008;151(2):518–524.
245. Broberg K, Concha G, Engström K, Lindvall M, Grandér M, et al. Lithium in drinking water and thyroid function. *Environ Health Perspect*. 2011;119(6):827–830.
246. Findling R, Robb A, McNamara N, Pavuluri M, Kafantaris V, et al. Lithium in the acute treatment of bipolar I disorder: a double-blind, placebo-controlled study. *Pediatrics*. 2015;136(5):885–894.
247. Chen C, Lee C, Lee M, Ouyang W, Chen C, et al. Variant GADL1 and response to lithium therapy in bipolar I disorder. *N Engl J Med*. 2014;370(2):119–128.
248. American Association for Clinical Chemistry. *Lithium*; 2017. https://labtestsonline.org/understanding/analytes/lithium/tab/test. Accessed October 30, 2017.
249. Patorno E, Huybrechts K, Bateman B, Cohen J, Desai R, et al. Lithium use in pregnancy and the risk of cardiac malformations. *N Engl J Med*. 2017;376(23):2245–2254.
250. U.S. National Library of Medicine. *Lithium*; 2017. https://medlineplus.gov/druginfo/meds/a681039.html. Accessed October 30, 2017.
251. Kessing L, Gerds T, Knudsen N, Jørgensen L, Kristiansen S, et al. Association of lithium in drinking water with the incidence of dementia. *JAMA Psychiatry*. Oct 1;74(10):1005–1010. https://doi.org/10.1001/jamapsychiatry.2017.2362.
252. Kessing L, Gerds T, Knudsen N, Jørgensen L, Kristiansen S, et al. Lithium in drinking water and the incidence of bipolar disorder: a nation-wide population-based study. *Bipolar Disord*. 2017. https://doi.org/10.1111/bdi.12524.
253. Schrauzer G, Shrestha K, Flores-Arce M. Lithium in scalp hair of adults, students, and violent criminals. *Biol Trace Element Res*. 1992;34(2):161–176.
254. Summerton A, Harvey N, Forrest A. New direct method for measuring red cell lithium. *J Clin Pathol*. 1989;42(4):435–437.
255. Usuda K, Kono K, Dote T, Watanabe M, Shimizu H, et al. An overview of boron, lithium, and strontium in human health and profiles of these elements in urine of Japanese. *Environ Health Prevent Med*. 2007;12(6):231–237.
256. Hisayasu G, Cohen J, Nelson R. Determination of plasma and erythrocyte lithium concentrations by atomic absorption spectrophotometry. *Clin Chem*. 1977;23(1):41–45.
257. Harland B, Harden-Williams B. Is vanadium of human nutritional importance yet? *J Am Dietetic Ass*. 1994;94(8):891–894.
258. Seargeant L, Stinson R. Inhibition of human alkaline phosphatases by vanadate. *Biochem J*. 2979;181(1).
259. Tsave O, Petanidis S, Kioseoglou E, Yavropoulou M, Yovos J, et al. Role of vanadium in cellular and molecular immunology: association with immune-related inflammation and pharmacotoxicology mechanisms. *Oxid Med Cell Longev*. 2016;2016:1–10.
260. Anke M. Vanadium. An element both essential and toxic to plants, animals and humans? *Anales Real Acad Nacional Farm*. 2005;70(4):961–999.
261. Cohen N, Halberstam M, Shlimovich P, Chang C, Shamoon H, et al. Oral vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with non-insulin-dependent diabetes mellitus. *J Clin Investig*. 1995;95(6):2501–2509.
262. Cusi K, Cukier S, DeFronzo R, Torres M, Puchulu F, et al. Vanadyl sulfate improves hepatic and muscle insulin sensitivity in type 2 diabetes. *J Clin Endocrinol Metabol*. 2001;86(3):1410–1417.
263. National Institute of Environmental Health Sciences. *Chemical information review document for oral exposure to tetravalent and pentavalent vanadium compounds*; 2008. https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/niehs_vanadium_compounds_508.pdf. Accessed October 30, 2017.
264. Agency for Toxic Substances & Disease Registry. *Toxicological profile for vanadium*; 2015. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=276&tid=50. Accessed October 31, 2017.
265. Agency for Toxic Substances & Disease Registry. *Public health statement for vanadium*; 2012. https://www.atsdr.cdc.gov/PHS/PHS.asp?id=274&tid=50. Accessed October 31, 2017.
266. Boyle Karleen. *Evaluating particulate emissions from jet engines: analysis of chemical and physical characteristics and potential impacts on coastal environments and human health.* Transportation Research Record 1517. Los Angeles: University of California, Los Angeles; 1996.
267. Li H, Zhou D, Zhang Q, Feng C, Zheng W, et al. Vanadium exposure-induced neurobehavioral alterations among Chinese workers. *NeuroToxicology*. 2013;36:49–54.
268. Wang X, Sun T, Liu J, Shan Z, Jin Y, et al. Inverse association of plasma vanadium levels with newly diagnosed type 2 diabetes in a Chinese population. *Am J Epidemiol*. 2014;180(4):378–384.
269. Tang L, Su Y, He J, Chen W, Su F, et al. Urinary Titanium and vanadium and breast cancer: a case-control study. *Nutri Cancer*. 2012;64(3):368–376.
270. Jacobs DG, Jacobs DO, Kudsk KA, Moore FA, Oswanski MF, Poole GV, Sacks GS, et al. Practice management guidelines for nutritional support of the trauma patient. *J Trauma Acute Care Surg*. 2004;57(3):660–679.
271. Burritt M, Anderson CE. Laboratory assessment of nutritional status. *Hum Pathol*. 1984;15(2):130–133.
272. World Health Organization. *In vitro diagnostics and laboratory technology quality assurance*; 2017. http://www.who.int/diagnostics_laboratory/quality/en/. Accessed October 31, 2017.
273. Bharadwaj S, Ginoya S, Tandon P, Gohel T, Guirguis J, et al. Malnutrition: laboratory markers vs nutritional assessment. *Gastroenterol Rep*. 2016;4(4):272–280.
274. Druyan ME, Bass D, Puchyr R, Urek K, Quig D, Harmon E, Marquardt W. Determination of reference ranges for elements in human scalp hair. *Biol Trace Element Res*. 1998;62:183–197.

24

Mold Exposure Assessment

Ann Shippy, MD

OUTLINE

INTRODUCTION

Chronic exposure to environmental toxins, including mycotoxins and microbial volatile organic compounds (mVOCs) from mold, is an important emerging area of medicine. Despite recent advances in the technology to test for mold and mycotoxins, there is much more that needs to be known to provide the best care. Identifying patients affected by mold is challenging because there are no standards of care for testing or treatment. Many physicians are not even aware of the effects of mycotoxins, and medical organization statements have not incorporated the most recent research and testing capabilities in their position statements and recommendations. How many years of research and legal battles did it take to identify smoking tobacco as a health hazard and then to change public policy? Most people now realize that breathing in smoke and air pollution is detrimental to their health. At this time, most people and healthcare providers do not realize the harmful effect of the inhalation or ingestion of mold toxins from buildings that are commonly water damaged and many food products. It is likely that, one day, we will look back at mold exposures in food and buildings in the same way we look at the danger of tobacco use now—it seems so obvious.

DEFINITIONS

Mycotoxins: Mycotoxins are fungal secondary metabolites that bioaccumulate, leading to carryover and concentration into animal fluids, organs, and tissues. As a consequence, mycotoxin determination in biological samples from humans and animals has been reported worldwide. Because most mycotoxins show toxic effects at low concentrations and considering the extremely low levels present in biological samples, the application of reliable detection methods is required.[1]

mVOCs: mVOCs are chemicals with low molecular weights, high vapor pressure, and low water solubility produced by fungi and bacteria during metabolism. These chemical characteristics allow mVOCs to easily evaporate into the air or "off-gas," and they may have an odor. Volatile organic compounds (VOCs) can be produced through industrial or biological processes. In the industrial setting, VOCs are commonly used or are created as by-products in the manufacture of paints, pharmaceuticals, refrigerants, petroleum fuels, dry cleaners, furniture, household cleaners, and other common products.

CHALLENGES

1. There has been little research on the effects of chronic exposure to low levels of mycotoxins and mVOCs in humans. This means much of the clinical application has to be extrapolated from primarily cell and animal studies and a few human studies.
2. The technology to detect mycotoxins and mVOCs in humans or the environment is in its infancy. Thus there are no defined, well-accepted protocols. There is considerable noise and confusion over the best practices, although there has been moderate progress in the past decade in developing technology to detect mycotoxins in food, the environment, and humans.
3. There is a lack of research on how to treat patients affected by toxic mold most effectively and the best ways to monitor their progress.
4. There is a lack of research to identify the safe levels of mycotoxins and mVOCs accounting for the synergy of other environmental toxins or the genetics and nutritional status of the individual.
5. The U.S. government and other governments and organizations, such as the World Health Organization (WHO), have not yet updated their policies based on progress with the testing technology that has been added in the past decade to improve the detection of mycotoxins and correlate them to human illness. Much more research needs to be funded.
6. Mycotoxin-induced illness is likely a significant health-damaging epidemic based on the high prevalence of water-damaged buildings and the extensive presence of mycotoxins in the food supply.
7. Addressing this epidemic may be an enormous financial burden because many buildings such as residences, schools, businesses, and governments that affect the health of their inhabitants are in need of extensive remediation for acceptable or optimal human health.

8. The governmental regulations of mycotoxins in food vary greatly by country. Global standards are needed that account for the synergy of mycotoxins. This will also have a significant financial impact as even more batches of crops and products are deemed inedible.

Symptoms to Consider in Mycotoxin Illness from Contaminated Food or Inhalation

At the time most government policies and medical organization statements were published, there was no testing available to correlate mycotoxins present in humans and the environment with human disease, and thus such policies are outdated. Most of the research on mycotoxins is done on animals or other organisms. Because controlling mold in animal feed is a challenging, ongoing worldwide issue, there is extensive animal research. Standard medical education teaches clinicians to consider mold only as an allergy or asthma issue or in the differential diagnosis for an infectious disease in immunocompromised patients, not as a toxicity issue. Certainly, mold infection is a recognized cause of morbidity and mortality in transplant patients on immunosuppression, patients on chemotherapy, and those with autoimmunity or immunodeficiency.[2] Few health care providers are aware of the toxic effects of mold on their patients. As more health care providers gain experience in recognizing mold-related illness, emerging research is showing promise for identifying mold toxin exposure as an underlying contributor to many illnesses.

Research estimates that up to 50% of residential and work environments in North America have had water damage[3] and that mold exposure should be considered in all patients with any chronic respiratory condition. Of particular significance is the finding that in adult-onset asthma, two thirds of cases are caused by exposure to water-damaged buildings. The relationship of indoor mold exposure from water-damaged buildings to other health conditions has less research available. Mold exposure has been linked to neurotoxicity, autoimmunity, immunotoxicity, and cytotoxicity. The carcinogenic effects of food-borne mold contamination are well established.

Research supports that environmental toxins drive disease in the general population, not just those who are genetically or nutritionally the most susceptible. Mycotoxins and mVOCs are joining the categories of pesticides, metals, solvents, and persistent organic pollutants in affecting health and longevity.

Positions of National and International Organizations on Mold and Nonrespiratory Conditions

World Health Organization

- "Microbial growth may result in greater numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucans and volatile organic compounds in indoor air. The causative agents of adverse health effects have not been identified conclusively, but **an excess level of any of these agents in the indoor environment is a potential health hazard."**[3]
- Mycotoxins, or fungal toxins, are low-relative-molecular-mass biomolecules produced by fungi, some of which are toxic to animals and human beings. Mycotoxins are known to interfere with RNA synthesis and may cause DNA damage. Some fungal species may produce various mycotoxins, depending on the substrate. In the case of *Penicillium,* one such compound is penicillin, a strong antibiotic. Several mycotoxins (e.g., aflatoxin from *Aspergillus flavus* and *Aspergillus parasiticus*) are potent carcinogens. Many mycotoxins are immunotoxic, but the trichothecene mycotoxins are immunostimulating at low doses.[4] Numerous mycotoxins have been classified by their distinct chemical structures and reactive functional groups, including primary and secondary amines, hydroxyl or phenolic groups, lactams, carboxylic acids, and amides. The mycotoxins that have perhaps received the most attention are the trichothecenes, produced by *Stachybotrys chartarum.* Bloom[5] showed that several mycotoxins produced by *S. chartarum* and *Aspergillus versicolor* (i.e., macrocyclic trichothecenes, trichodermin, sterigmatocystin, and satratoxin G) could be present in most samples of materials and settled dust from buildings with current or past damage from dampness or water damage. Charpin-Kadouch[6] compared the levels of macrocyclic trichothecenes in samples from 15 flooded dwellings known to be contaminated with *S. chartarum* or *Chaetomium* and a group of nine dwellings without visible mold. The level of macrocyclic trichothecenes was significantly higher in floor dust from the moldy houses than from the reference dwellings; the levels in wall samples from moldy houses were also higher.

Centers for Disease Control and Prevention[7]

- A link between other adverse health effects, such as acute idiopathic pulmonary hemorrhage among infants, memory loss, or lethargy, and molds, including the mold *S. chartarum (Stachybotrys atra),* has not been proven. Further studies are needed to find out what causes acute idiopathic hemorrhage and other adverse health effects.
- Standards for judging what is an acceptable, tolerable, or normal quantity of mold have not been established.
- In summary, *S. chartarum (S. atra)* and other molds may cause health symptoms that are nonspecific. At present, there is no test that proves an association between *S. chartarum (S. atra)* and particular health symptoms. Individuals with persistent symptoms should see their physician. However, if *S. chartarum (S. atra)* or other molds are found in a building, prudent practice recommends that they be removed.

U.S. Environmental Protection Agency

The U.S. Environmental Protection Agency (EPA) website has extensive resources to address identifying, preventing, and remediating water damage and mold, plus guidelines for schools. However, at the time of this writing, the medical content has not been updated since 2004.

Water-Damaged Buildings

Water-damaged buildings expose their occupants to a diverse range of toxins, with many physiologically damaging effects. Chemical, microbial, and physical processes that break down building materials produce toxins. Tables 24.1 and 24.2 list the metabolite toxins released, organisms involved, physiological effects induced, and disease associations. Tables 24.3 and 24.4 shows that bacterial growth also releases toxins in water-damaged buildings.

The strongest correlations to mold exposure and symptoms are neurological and immunological, but such exposure can affect all systems of the body. Food-borne mycotoxins have been shown to cause cancer, impaired child growth, neural tube defects, immunotoxicity, gastroenteritis, and renal disease.[8] Studies comparing mycotoxins with pesticides show that mycotoxins can be more toxic than pesticides.[9] Even more concerning is the research showing that fungal metabolites have synergistic genotoxic and other harmful effects.[10]

mVOCs are low-molecular-weight compounds and include numerous alcohols, esters, ethers, ketones, aldehydes, terpenoids, thiols, and their derivatives. They diffuse into the air and enter the body through the lungs and skin. mVOCs are even more toxic than the chemicals traditionally thought of as being industrial toxins. A study comparing fungal VOCs showed a greater toxic effect than formaldehyde, xylene, benzene, and toluene.[11] I-octen-3-ol studies predict it to be more toxic to human embryonic stems cells than toluene.[12] Increased levels of biomarkers such as myeloperoxidase, lysozyme, and eosinophil cationic protein are related to symptoms of headache, nausea, and mucosal irritation as inflammation increases from exposures.

Neurotoxicity is clearly associated with mold exposure and other toxin exposures. One study evaluated neurobehavioral and pulmonary

TABLE 24.1 Toxic Metabolites Produced by Bacteria Isolated From Water-Damaged Materials and Indoor Air

Metabolite	Organisms	Physiological Effects	Disease
Valinomycin	*Streptomyces griseus*	Mitochondrial poison	Unknown
Leptomycin B	*Streptomyces species*	Inhibition of inducible nitric oxide synthetase	Unknown
Toxic peptide	*Bacillus amyloliquefaciens*	Depolarized transmembrane. Decreased ATP and NADH cell death	Unknown
Mitochondrial toxin	*Bacillus pumilus*	Disruption of mitochondrial membrane	Unknown
Mitochondrial toxin	*Nocardiopsis species*	Disruption of mitochondrial membrane	Unknown
Cytostatic compounds	*Coculture of S. chartarum and S. californicus*	Cytotoxic compounds that are just as toxic as doxorubicin and AMD	Unknown

AMD, actinomycin D; *ATP*, adenosine triphosphate; *NADH*, nicotinamide adenine dinucleotide.
Adapted from Thrasher JD, Crawley S. The biocontaminants and complexity of damp indoor spaces: more than what meets the eyes. *Toxicol Ind Health*. 2009;(9-10):583-615

TABLE 24.2 Mycotoxins Produced by Toxic Molds

Metabolite	Organisms	Physiological Effects	Disease
Gliotoxin	*Aspergillus fumigatus, terres, flavus, niger; Trichoderma virens; Penicillium* spp.; *Candia albicans*	Immune toxicity, immunosuppression, neurotoxicity	Invasive aspergillosis
Aflatoxin B1, kojic acid, aspergillic acid, nitropropionic acid	*Aspergillus flavus*	Liver pathology and cancer; immune toxicity; neurotoxicity	Carcinogenesis
Fumigaclavines, fumitoxins, fumitermorgens, verruculogen, gliotoxin	*A. fumigatus*	Lung disease; neurotoxicity; tremors; immune toxicity	Aspergillosis
Ochratoxin A		Immunosuppression	Balkan endemic nephropathy (BEN)
Urinary tract tumors	*A. niger*		BEN
Aspergillosis	*Penicillium verrucsom*	Lung disease	
Ochratoxin A	*Aspergillus ochraceus*	Nephropathology	Urinary tract damage
Penicillic acid, xanthomegnin, viomellein, vioxanthin			Tumors
Sterigmatocystin, 5-methoxy-sterigmatocystin	*Aspergillus versicolor*	Liver pathology and cancer	Carcinogenesis
Chaetomiums	*Chaetomium globosum*	Cytotoxicity	Unknown
Chaetoglobosum A and C		Cell division	Unknown
Griseofulvin	*Memnoniella echinata*	Carcinogenesis?	Unknown
Dechlorogrseofulvins			Reproductive toxin
Trichodermin			Hypersensitivity?
Trichodermo		Protein synthesis inhibition	
Mycophenolic acid	*Penicillium brevicompactum*	Cytotoxic; mutagen	Unknown
Botryodiploidin	*Penicillium expansum*	Immune toxicity; cytotoxic	Unknown
Patulin, citrinin, chaetoglobosin, roquefortine C			Tremors
Verrucosidins	*Penicillium plonicium*	Cytotoxicity	Tremors
Penicillic acid, nephrotoxic glycopeptides			Nephropathology
Trichothecenes	*Trichoderma* spp.	Trichothecene toxicity	Unknown
Trichodermol, trichodermin, gliotoxin, viridin		Immunotoxicity	Immune impairment
Fumonisins	*Fusarium verticillioides (aka moniliforme)*	Neural tube defects in animals and humans	Central nervous system (CNS) birth defects
Spirocyclic	*Stachybotrys chartarum*	Respiratory bleeding	Pulmonary bleeding
Drimanes, roridin		Protein synthesis inhibition	
Satratoxins (F, G, H)		Neurotoxicity	
Hydroxyroridin E		Cytotoxicity	
Verrucarin J, trichodermin, dolabellanes, altrones B and C, stachybotrylactams		Immune toxicity	

Table 24.2 demonstrates that mycotoxins disrupt mitochondrial function; misbalance nitric oxide synthesis; and create inflammatory mediators, neurotoxicity, cytotoxicity, immune suppression, carcinogenesis, and mutagenesis. This long list can cause patients' symptoms to be diverse and complex.
Adapted from Campbell AW, Thrasher JD, Gray MR, Vojdani A. Mold and mycotoxins: effects on the neurological and immune systems in humans. *Adv Appl Microbiol*. 2004;55:375–406.

TABLE 24.3 Symptoms Caused by Mold Toxicity/Water-Damaged Buildings

Symptom	% in Exposed Population	% in Controls	*p*-Value
Memory problems	5.1	3.3	0.0002
Spaciness	4.8	3.2	0.0007
Excessive fatigue	5.8	4.3	0.0001
Coughing	4.6	3.2	0.001
Slurred speech	4.5	3.1	0.002
Weak voice	4.1	2.8	0.003
Watery eyes	4.6	3.4	0.004
Lightheadedness	4.4	3.2	0.006
Dizziness	4.3	3.1	0.005
Weakness	4.2	3.0	0.008
Headache	5.2	4.1	0.005
Throat discomfort	4.5	3.4	0.008
Sinus discomfort	4.7	3.6	0.01
Coordination problems	4.0	2.9	0.01
Nasal symptoms	5.1	4.1	0.02
Bloating	4.2	3.2	0.02
Visual changes	3.9	2.9	0.02
Rash	3.9	2.9	0.02

Adapted from Campbell AW, Thrasher JD, Gray MR, Vojdani A. Mold and mycotoxins: effects on the neurological and immune systems in humans. *Adv Appl Microbiol.* 2004;55:375–406; Pizzorno J, Shippy A. Is mold toxicity really a problem for our patients? part 2-nonrespiratory conditions. *Integr Med (Encinitas).* 2016 Jun;15:8-14.

impairment via comparison among three groups: (1) those exposed to indoor molds, (2) those exposed to chemicals, and (3) "unexposed" community referents.[13] Both the mold- and chemical-exposed groups had similar findings of decreased balance, longer reaction times, increased blink reflex latency, increased color discrimination errors, decreased visual field, and reduced grip strength; measures of cognitive and memory performance were abnormal.

Another study of individuals exposed to mold in their homes found multiple neurological deficits in 70% and abnormalities in T and B cells in more than 80% of the patients.[14] A study of individuals working in a well-documented water-damaged school building compared with "unexposed" controls found statistically significant loss of visual contrast sensitivity (VCS), a sensitive measure of neurodysfunction, as well as increased respiratory problems.[15]

Exposure to mold/damp buildings increases the production of multiple inflammatory molecules and alters immune function mediators. The immune systems of those working in damp buildings react to exposure with a 2- to 1000-fold increased production of a wide variety of these inflammatory/immune mediators.[16] Animal studies clearly show mold-induced immunotoxicity as well.

In some cases, multiple sclerosis could be a mold toxin disease from gliotoxin produced by various species of *Aspergillus* and *Candida.* Gliotoxin suppresses immune function, increases the permeability of the blood–brain barrier, and is highly neurotoxic.[17]

In a study on chronic fatigue syndrome (CFS), a high correlation was found between the presence of mycotoxins in the patient's urine and having a diagnosis of CFS. Of participants, 93% had one mycotoxin present, and 30% had two or more mycotoxins present.[18]

Importantly, as the incidence of autoimmune disorders increases, cases of autoimmune diseases and related symptoms have been reported among the occupants of damp buildings.[19] When seeing patients with autoimmune diagnosis, it is important to consider mold exposure as an underlying trigger that is potentially treatable.

ASSESSMENT OF MOLD EXPOSURE

IgG or IgE Allergy Serum Testing

IgG or IgE allergy serum testing is an older method, but is sometimes helpful in raising the suspicion of current mold exposure. Some practitioners also use it to see if IgE and/or IgG levels are reduced with treatment, to evaluate the effectiveness of remediation/avoidance of exposure, and to consider immunotherapy. It is not conclusive, but levels of antibodies can correlate with the types and levels of mold the patient is being exposed to.

Mycotoxin Urinary Levels

The latest acceptable technology tests urine samples. Results are not conclusive if negative because all mycotoxins cannot yet be tested for. See the advantages and disadvantages of the two available technologies in Table 24.5.

Inflammatory Markers and Hormones

Inflammatory markers and hormones can be indicators for toxic exposures but are not conclusive if positive or negative. They can be helpful in monitoring a patient's progress. These markers are part of the Shoemaker protocol.

Proteomics

Proteomic has promise in the assessment of mold exposure, but currently this approach is used only in research and is expensive.

Genetics

Genetic testing can be helpful to establish individual treatment programs. Consider human leukocyte antigen (HLA) type, methylation, P450, *COMT*, glutathione, *NAT*, *VDR*, and mitochondrial and histamine single-nucleotide polymorphisms (SNPs). Toxins affect individuals in different ways depending on their genetics, synergistic toxins present, and nutritional status. Early genetic testing can help identify those most susceptible to mold toxins and other environmental toxins.

Visual Contrast Sensitivity Testing

Visual contrast sensitivity (VCS) measures the ability to see details at low contrast levels. Although it is useful for early detection of neurodegeneration, it does not differentiate the cause. Biotoxins reduce available oxygen to the optic nerves as a result of reduced blood flow, which can affect the ability to detect the "edge" between light and dark and lead to a reduction in night vision and increased light sensitivity.

This is an easily available and inexpensive test. However, it is a nonspecific test of neurological function (i.e., not diagnostic for mycotoxin illness). A positive test may reveal a 92% chance that a patient has biotoxin illness, but again, it does not determine which toxin—it could be mold toxins or an environmental neurotoxin, such as mercury. Positive tests usually indicate mold exposure but can also be attributed to Lyme disease or other biotoxins.

Testing needs to be conducted at a distance of 18 inches from the screen and should be done in the daytime.

False positives can occur with cataracts or other vision abnormalities

Laboratory Testing Technology

Testing for the mycotoxin load present in humans via sampling tissue and body fluids is still very limited. There are likely hundreds of mycotoxins, possibly even an order of magnitude more, but only a few can be commercially tested for in human samples or the environment, except in research laboratories.

Qualitative polymerase chain reaction (QPCR) can now be used to detect and quantify the presence of 45 molds in human tissue and the environment.

TABLE 24.4 Health Hazard Evaluation Report HETA 2005-0135-3116, Alcee Fortier Senior High School New Orleans, Louisiana, September 2010, Department of Health and Human Services, Centers for Disease Control and Prevention, Workplace Safety and Health, National Institute for Occupational Safety and Health

	AFSHS	WHHS	Prevalence Ratio	*p*-Value
Lower Respiratory				
Cough	35 (43)	11 (10)	4.16 (2.26, 7.68)	<0.01
Wheezing or whistling in chest	19 (23)	2 (2)	12.13 (2.91, 50.62)	<0.01
Chest tightness	22 (27)	0	+inf (7.69, +inf)	<0.01
Unusual shortness of breath	19 (24)	4 (4)	6.22 (2.20, 17.56)	<0.01
Upper Respiratory				
Sinus problems	27 (33)	14 (13)	2.44 (1.37, 4.35)	<0.01
Dry or irritated eyes	16 (20)	12 (11)	1.72 (0.86, 3.44)	0.12
Nosebleeds	3 (4)	1 (1)	3.70 (0.53, 47.02)	0.33
Sore or dry throat	21 (24)	13 (13)	1.95 (1.04, 3.67)	0.03
Frequent sneezing	17 (20)	4 (4)	5.23 (1.83, 14.96)	<0.01
Stuffy nose	25 (29)	10 (10)	3.09 (1.57, 6.07)	<0.01
Runny nose	22 (25)	7 (7)	3.87 (1.73, 8.62)	<0.01
Constitutional				
Fever or sweats	14 (16)	4 (4)	4.10 (1.40, 12.01)	<0.01
Aching all over	12 (14)	4 (4)	3.71 (1.24, 11.08)	0.01
Unusual tiredness or fatigue	25 (31)	18 (17)	1.78 (1.04, 3.03)	0.03
Headache	30 (35)	21 (20)	1.74 (1.08, 2.81)	0.02
Neurobehavioral				
Difficulty concentrating	15 (18)	4 (4)	4.63 (1.60, 13.44)	<0.01
Confusion or disorientation	8 (10)	2 (2)	5.05 (1.25, 29.56)	0.02
Trouble remembering things	15 (17)	5 (5)	3.59 (1.36, 9.47)	<0.01
Irritability	19 (22)	15 (14)	1.51 (0.82, 2.80)	0.18
Depression	6 (7)	2 (2)	3.74 (0.87, 20.82)	0.14
Change in sleep patterns	16 (19)	4 (4)	4.99 (1.73, 14.37)	<0.01
Rash, dermatitis, or eczema (on face, neck, arms, or hands)	12 (14)	4 (4)	3.70 (1.24, 11.06)	0.01

Enzyme-Linked Immunosorbent Assay

An enzyme-linked immunosorbent assay (ELISA) is currently available through Real Time Labs (RTL).

- What it tests for: 16 of the most common mycotoxins, especially for *Macrocyclic trichothecenes* (most toxic trichothecenes), for which the laboratory has a U.S. patent
  - Ochratoxin A
  - Aflatoxins (B1, B2, G1, G2)
  - Trichothecenes (satratoxin G, satratoxin H, isosatratoxin F, rodirin A, rodirin E, rodirin H, rodirin L-2, verrucarin A, verrucarin J)
  - Gliotoxin
  - Chaetoglobosin A
- What these mycotoxins correspond to regarding mold presence:
  - Aflatoxin = *Aspergillus flavus*
  - Ochratoxin A = *Aspergillus ochraceus, Aspergillus niger, Penicillium verrucosum*
  - Gliotoxin = *Aspergillus fumigatus,* possibly
  - Macrocyclic trichothecenes = *S. chartarum*
  - Simple trichothecenes = *Fusarium* species
  - Chaetoglobosin = *Chaetomium*
- What it misses: More than 50,000 different species of mold are not test for; only 200 are currently documented to cause serious health risks to humans or animals.
- Methods: College of American Pathologists (CAP) certified, Clinical Laboratory Improvement Amendments (CLIA) accredited; ELISA (antibodies prepared against mycotoxins); very sensitive test (can detect traces of mycotoxins in the ng/mL [= ppb])
  - Urine-based (blood test not validated in blood components, per RTL): turnaround is 10 days after receiving the sample.
  - Options are available to test for mycotoxins in the sputum, bronchoalveolar lavage (BAL), nasal wash, and tissue sample (requires consulting with medical team at RTL to discuss preparation).
  - Is there a test for food-based mycotoxins? Per RTL, it is "unlikely" that aflatoxin- or ochratoxin-positive urine correlates to food-based mycotoxins.
- Troubleshooting: If the test result is negative but the patient is very sick, it can indicate that the individual has poor detoxification and isn't able to remove/liberate mycotoxins from the body; a positive test does *not* differentiate current exposure from previous exposure.
- Other tests: RTL can test clothing and other things for mycotoxins and also performs Environmental Relative Moldiness index (ERMI) and mycotoxin testing from environmental samples; it can also test sinuses for mold (molecular fungal DNA panel).

Mass Spectrometry

Mass spectrometry testing is currently available through Great Plains Labs.

- What it tests for: Seven different mycotoxins from four species of mold, with more on the way
  - Aflatoxin M—main metabolite of aflatoxin B1 (from *Aspergillus*)

TABLE 24.5 Comparison of ELISA and Mass Spectrometry for Mycotoxin Testing

ELISA		MASS SPECTROSCOPY	
Advantages	**Disadvantages**	**Advantages**	**Disadvantage**
Well characterized, well understood Trusted Straightforward to troubleshoot Good sensitivity and selectivity with broad dynamic-range antibody against specific class of analytes	Must have a specific antibody (mono- or polyclonal in nature) Limited range of analytes to be detected	Well characterized, used in drug testing, wide range of analytes can be tested High sensitivity and precision for identification of analytes	Narrow dynamic range of analytes, must have parent compound for comparison to specimen Selectivity of analyte is not as good and as easy to optimize Heavy training of technologist required High cost of equipment and maintenance
Instruments are flexible and scalable, can be manual process or automated	Assays are multistep; lot-to-lot variation in the antibody product must be carefully controlled	Sample volumes are low	
	Reagent usage and antibody cost can be high	Day-to-day reagent costs are low	
Equipment is low cost	Time to complete assay is 1–3 hours Technologist must be well trained on pipetting and washing techniques	Samples can be run and analyzed in minutes	

  - Ochratoxin A (produced from *Aspergillus* and *Penicillium* families)
  - Sterigmatocystin—closely related to aflatoxin (produced from *Aspergillus, Penicillium,* and *Bipolaris*)
  - Roridin E—a macrocyclic trichothecene (from *Fusarium, Myrothecium,* and *Stachybotrys*)
  - Verrucarin A—a macrocyclic trichothecene mycotoxin (from *Stachybotrys, Fusarium,* and *Myrothecium*)
  - Enniatin B1—categorized as cyclohexadepsipeptides (from *Fusarium*)
  - Zearalenone—produced by *Fusarium*
- Methods: Advanced liquid chromatography mass spectrometry (MS/MS); very sensitive (can identify parts per trillion [ppt; vs. ppb in ELISA testing])
  - Urine-based sample
  - History: Released in 2017

TREATMENT

Unfortunately, there are no widely accepted protocols and very limited data on treatment efficacy. Treating environmental illness, including mold, requires a comprehensive approach.

1. Avoidance

The primary pillar of treatment is to minimize additional toxin exposure. Once an individual is symptomatic, he or she may become sicker, or not improve, if he or she is exposed to any other environmental toxins. Make sure that the patient eliminates all of the toxin exposures that he or she can. Guide the patient to avoid new cars, new paint, flame retardants, plastic, pumping gas, cosmetics, pesticide, and preservatives and other contaminants in food and water. Exposure to all toxins must be minimized as much as possible. The first step is to identify the source of the mold. Consider home, work, school, car, farming (silos, hay, and animal feed), and food. It is also possible that the patient is colonized/infected with mold that is producing the toxins. Even the brain has been shown to harbor mold, contributing to Alzheimer's disease.[20]

2. Enhance Detoxification Pathways

Enhancing detoxification pathways, optimizing the gastrointestinal tract, and supporting mitochondrial function are critical for most patients to fully recover. Although there is limited research directly related to mold, treatment options to consider include the following:

- N-acetylcysteine (NAC): NAC reduces the oxidative damage and inhibits the apoptosis induced by *Fusarium* toxins in porcine kidney cells. It is effective through multiple pathways.[21–23]
- Binders: Clay, charcoal, modified citrus pectin, cholestyramine/Welchol[24,25]
- Glycine
- Sauna—infrared if possible[26]
- Hyperbaric: Hyperbaric oxygen treatment improved attention deficit disorder and slowed reaction time in mold-exposed subjects in 10 sessions.[27]
- Liposomal glutathione[28]
- Hormonal support as indicated, including thyroid, estrogen, dehydroepiandrosterone (DHEA), testosterone, and melatonin[29,30]
- Curcumin[31]
- Vitamin E[29,32]
- Quercetin, selenium, glucomannan, nucleotides, antimicrobial peptides, probiotics, polyunsaturated fatty acids, oligosaccharides, and plant extracts: These products inhibit trichothecene-induced oxidative stress by (1) inhibiting reactive oxygen species (ROS) generation and induced DNA damage and lipid peroxidation, (2) increasing antioxidant enzyme activity, (3) blocking the mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB signaling pathways, (4) inhibiting caspase activity and apoptosis, (5) protecting mitochondria, and (6) regulating anti-inflammatory actions.[33]

3. Comprehensive, Integrated Care

Integration of genetic SNPs, proteomics, exposomics, and other markers of the cellular function of the immune, detoxification, mitochondrial, and methylation systems may help identify those most affected, as well as identify potential treatment options. In addition, assessing potential nutritional deficiencies and levels of other environmental toxins may help identify those with increased individual susceptibility. Given the complex nature of the interaction between the human genome and environmental toxins, the science of bioinformatics will be necessary to more fully understand the full severity and magnitude of mold exposure and its effects on human health.

CONCLUSION

Mold metabolites have a harmful impact on human health. It is imperative that more research be done to further understand the effects of mold on human health, identify the best ways to test patients and indoor environments, and develop optimal treatment approaches for patients, especially given the common occurrence of water damage in buildings and the presence of mycotoxins in food.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Escrivá L, Font G, Manyes L, Berrada H. Studies on the presence of mycotoxins in biological samples: an overview. *Toxins (Basel)*. 2017;9(8):E251. https://doi.org/10.3390/toxins9080251.
2. Webb BJ, Ferraro JP, Rea S, et al. Epidemiology and clinical features of invasive fungal infection in a US health care network. *Open Forum Infectious Diseases*. 2018;5(8):ofy187. https://doi.org/10.1093/ofid/ofy187.
3. *Who Guidelines for Indoor Air Quality: Dampness and Mould*. World Health Organization; 2009. https://www.who.int/airpollution/guidelines/dampness-mould/en/. Accessed 21 January 2020.
4. Halstensen AS, Nordby KC, Eduard W, Klemsdal SS. Real-time PCR detection of toxigenic Fusarium in airborne and settled grain dust and associations with trichothecene mycotoxins. *J Environ Monit*. 2006;8(12):1235–1241.
5. Bloom E, Bal K, Nyman E, et al. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by Stachybotrys and Aspergillus spp. in indoor environments. *Appl Environ Microbiol*. 2007;73(13):4211–4217.
6. Charpin-Kadouch C, Maurel G, Felipo R, et al. Mycotoxin identification in moldy dwellings. *J Appl Toxicol*. 2006;26(6):475–479.
7. Centers for Disease Control and Prevention. Mold. Web site. https://www.cdc.gov/mold/default.htm. Accessed 21 January 2020.
8. Wu F, Groopman JD, Pestka JJ. Public health impacts of foodborne mycotoxins. *Annu Rev Food Sci Technol*. 2014;5:351–372.
9. Paterson RR, Lima N. Toxicology of mycotoxins. *EXS*. 2010;100:31–63.
10. Interference with mutagenic aflatoxin B1-induced checkpoints through antagonistic action of ochratoxin A in intestinal cancer cells: a molecular explanation on potential risk of crosstalk between carcinogens. *Oncotarget*. 2016. https://doi.org/10.18632/oncotarget.8914. [Epub ahead of print].
11. Inamdar AA, Zaman T, Morath SU. et al. Drosophila melanogaster as a model to characterize fungal volatile organic compounds. *Environ Toxicol*. 2014;29(7):829–836.
12. Inamdar AA, Moore JC, Cohen RI, Bennett JW. A model to evaluate the cytotoxicity of the fungal volatile organic compound 1-octen-3-ol in human embryonic stem cells. *Mycopathologia*. 2012;173:13–20.
13. Kilburn KH. Neurobehavioral and pulmonary impairment in 105 adults with indoor exposure to molds compared to 100 exposed to chemicals. *Toxicol Ind Health*. 2009;25(9-10):681–692.
14. Rea WJ, Didriksen N, Simon TR, et al. Effects of toxic exposure to molds and mycotoxins in building-related illnesses. *Arch Environ Health*. 2003;58(7):399–405.
15. Thomas G, Burton NC, Mueller C, et al. Comparison of work-related symptoms and visual contrast sensitivity between employees at a severely water-damaged school and a school without significant water damage. *Am J Ind Med*. 2012;55(9):844–854.
16. Rosenblum Lichtenstein JH, Hsu YH, Gavin IM, et al. Environmental mold and mycotoxin exposures elicit specific cytokine and chemokine responses. *PLoS One*. 2015;10(5):e0126926.
17. Purzycki CB, Shain DH. Fungal toxins and multiple sclerosis: a compelling connection. *Brain Res Bull*. 2010;82(1-2):4–6.
18. Brewer JH, Thrasher JD, Straus DC, et al. Detection of mycotoxins in patients with chronic fatigue syndrome. *Toxins (Basel)*. 2013;5(4):605–617.
19. Myllykangas-Luosujärvi R, Seuri M, Husman T, et al. A cluster of inflammatory rheumatic diseases in a moisture-damaged office. *Clin Exp Rheumatol*. 2002;20(6):833–836.
20. Pisa D, Alonso R, Rábano A. Different brain regions are infected with fungi in Alzheimer's disease. *Sci Rep*. 2015;5:15015. https://doi.org/10.1038/srep15015.
21. Zhang W, Zhang S, Zhang M, et al. Individual and combined effects of Fusarium toxins on apoptosis in PK15 cells and the protective role of N-acetylcysteine. *Food Chem Toxicol*. 2018;111:27–43.
22. Venkataramana M, Chandra Nayaka S, Anand T, et al. Zearalenone induced toxicity in SHSY-5Y cells: the role of oxidative stress evidenced by N-acetyl cysteine. *Food Chem Toxicol*. 2014;65:335–342.
23. Arbillaga L, Azqueta A, Ezpeleta O, et al. Oxidative DNA damage induced by Ochratoxin A in the HK-2 human kidney cell line: evidence of the relationship with cytotoxicity. *Mutagenesis*. 2007;22(1):35–42.
24. De Mil T, Devreese M, De Baere S, et al. Characterization of 27 mycotoxin binders and the relation with in vitro zearalenone adsorption at a single concentration. *Toxins (Basel)*. 2015;7(1):21–33.
25. Hope J. A review of the mechanism of injury and treatment approaches for illness resulting from exposure to water-damaged buildings, mold, and mycotoxins. *The Scientific World Journal*. 2013:2013. https://doi.org/10.1155/2013/767482.
26. Rea WJ. A large case-series of successful treatment of patients exposed to mold and mycotoxin. *Clin Ther*. 2018;40(6):889–893.
27. Ezra N, Dang K, Heuser G. Improvement of attention span and reaction time with hyperbaric oxygen treatment in patients with toxic injury due to mold exposure. *Eur J Clin Microbiol Infect Dis*. 2011;30(1):1–6.
28. Wang X, Jin C, Zhong Y, et al. Glutathione reduction of patulin-evoked cytotoxicity in HEK293 cells by the prevention of oxidative damage and the mitochondrial apoptotic pathway. *J Agric Food Chem*. 2018;66(29):7775–7785.
29. Akinrinmade FJ, Akinrinde AS, Amid A. Changes in serum cytokine levels, hepatic and intestinal morphology in aflatoxin B1-induced injury: modulatory roles of melatonin and flavonoid-rich fractions from Chromolena odorata. *Mycotoxin Res*. 2016;32(2):53–60.
30. Lan M, Han J, Pan M-H, et al. Melatonin protects against defects induced by DEoxynivalenol. *J Pineal Res*. 2018;65(1):e12477. https://doi.org/10.1111/jpi.12477.
31. Ruan D, Wang WC, Lin CX, et al. Effects of curcumin on performance, antioxidation, intestinal barrier and mitochondrial function in ducks fed corn contaminated with ochratoxin A. *Animal*. 2019;13(1):42–52.
32. Yılmaz S, Kaya E, Comakli S. Vitamin E (α tocopherol) attenuates toxicity and oxidative stress induced by aflatoxin in rats. *Adv Clin Exp Med*. 2017;26(6):907–917.
33. Wu Q, Wang X, Nepovimova E, et al. Antioxidant agents against trichothecenes: new hints for oxidative stress treatment. *Oncotarget*. 2017;8(66):110708–110726.

RESOURCES

https://www.cdc.gov/mold/
https://www.epa.gov/mold
https://www.nachi.org/documents/world-health-organization-guidelines-for-indoor-air-quality-dampness-and-mold.pdf
https://www.food.gov.uk/business-guidance/mycotoxins
http://www.fao.org/food/food-safety-quality/a-z-index/mycotoxins/en/
Milićević, D. R., Skrinjar, M., & Baltić, T. (2010). Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. Toxins, 2(4), 572–592. https://doi.org/10.3390/toxins2040572.

25

Nonmetallic Toxic Chemical Assessment

William Shaw, PhD

OUTLINE

INTRODUCTION

Nonmetal toxic chemicals cause diseases of virtually every organ in the human body and in virtually all animals, including fish, amphibians, reptiles, birds, and mammals. In addition, such chemicals may alter the microbiome in the soil and water and in the gastrointestinal tracts of animals. Such toxic chemicals come from multiple sources, including the air, water, food, pharmaceuticals, cosmetics, cleaning agents, and prescription and over-the-counter medications. Roughly 13,000 chemicals are used in cosmetics, of which only 10% have been properly evaluated for safety.

Doris Rapp, a physician and pioneer in the field of environmental medicine, reported that in 1998, more than 80,000 different chemicals with a total weight of 1.2 billion pounds were used in the United States.[1] With a population of about 300 million people at the time, that calculates to 4 pounds of chemicals for every person in the nation. Furthermore, less than 10% of the chemicals used have been even partially evaluated for safety, and in the majority of those evaluated, there was no assessment of safety for women and children. Dr. Rapps books *Is This Your Childs World?*[2] and *Our Toxic World*[1] are excellent, comprehensive works on the clinical effects of both metal and nonmetal toxic chemicals.

Furthermore, the vast majority of people today are not exposed to a single chemical but to a wide variety of chemicals. In such cases, the cumulative effects of multiple chemical exposures over long periods of time are much greater than just the additive effects of such chemicals. The Environmental Working Group's study commissioned five laboratories to examine the umbilical cord blood of 10 babies of African American, Hispanic, and Asian heritage and found more than 200 chemicals in each newborn.[3]

Results from the widest-ranging European survey of human toxic contamination show that 76 persistent, bioaccumulative, and toxic industrial chemicals were present in the blood of those tested.[4] The World Wildlife Fund (WWF) and the Co-operative Bank collected and analyzed the blood of 47 people from all over Europe. These included 39 Members of the European Parliament (MEPs),[4] observers from accession countries, 1 former MEP, and 3 WWF staff, representing 17 countries in Europe.[4] Blood samples were analyzed for an unprecedented 101 chemicals from five groups: organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), brominated flame retardants, phthalates, and perfluorinated compounds (PFCs). A total of 76 different chemicals from the 101 looked for were found in the blood of those tested.

The highest number of chemicals found in one person was 54, and the median number of chemicals detected was 41. At least 13 of the same chemicals were found in every single person tested, including chemicals banned in Europe over 20 years ago as well as chemicals in widespread use today, such as phthalates and PFCs. This huge toxic burden is more fully covered in Chapter 35, Environmental Medicine, and comprehensively in *Clinical Environmental Medicine* (Crinnion and Pizzorno, Elsevier, 2018).

NONMETALLIC TOXIC CHEMICALS, METALLIC TOXIC CHEMICALS, AND TOXIC METALS

The reason for differentiating metal and nonmetal toxic chemicals is that these two groups are analyzed in blood and urine by different technologies. The toxic metals were primarily tested first by atomic absorption and later by induction-coupled plasma mass spectrometry (ICP-MS). The nonmetal toxic chemicals have been tested effectively with gas chromatography, followed by gas chromatography combined with mass spectrometry (GCMS), and then with liquid chromatography combined with mass spectrometry (LC/MS). The main advantage of ICP-MS for toxic metals is that a large number of metals (50 or more) can be tested simultaneously on a single sample, such as blood, urine, stool, or hair, without the use of chromatography. Testing of nonmetal toxic chemicals typically requires much more complex sample extraction and almost always complex chromatography. These differences in the analytical testing of metal and nonmetal toxic chemicals have made the testing of metal toxic chemicals faster and cheaper than the testing of nonmetal toxic chemicals, resulting in much greater research and knowledge regarding metal toxic chemicals compared with nonmetal toxic chemicals. The ease of analytical testing has also led to much more regulation on contamination with toxic metals compared with nonmetal toxic chemicals.

Until very recently, screening tests for a wide variety of toxic chemicals were only available by the clinician ordering a few tests at a time from large reference laboratories. Because most individuals had no idea what chemicals they were exposed to, it was prohibitively expensive to order a large number of chemical tests. Recently, however, tests for a large variety of toxic chemicals have become available.

COMMON TOXIC CHEMICALS AND DISEASE

Some of the most common diseases associated with toxic chemical exposure include diabetes; impaired fertility; developmental disorders, such as attention deficit disorder and autism; psychiatric disorders; autoimmune diseases; neurotoxicity, including psychiatric disorders; and cancer.[1] Because many toxic chemicals affect the function of mitochondria, fatigue and exercise intolerance may be common in toxic exposures.[5] The marked decline in sperm counts and testosterone in males over the past few decades poses an existential threat to the human race and even to all life on Earth.[1] Excessive toxic chemical exposure should be ruled out as a cause of virtually any chronic disease. Some have discounted this toxic load with remarks such as: Oh well, why should I worry about toxic chemical exposure if everyone has it?

Although many people frequently have no detectable amount of specific toxic chemicals, every single person tested in the Great Plains Laboratory (GPL) had some detectable chemicals in their urine samples. The most important factor for toxic chemical tests is *not* whether there are detectable amounts, but if there are *high* amounts compared with a normal population or if their level exceeds the threshold found to be associated with increased disease risk.

Very high concentrations of toxic chemicals, such as those above the 95th percentiles of the entire population and especially values that are multiples of the 95th percentiles, are usually associated with severe health impairment. Those with multiple exposures are at even greater risk. Some of the highest toxic chemical values found at GPL have been in young children with autism. People who think they have healthy lifestyles and who consume organic food are often shocked at the amounts of chemicals found in their urine samples. For example, one of these individuals found her urine perchlorate value was more than 50 times the 95th percentile value for perchlorate. Organic farms frequently have nonorganic farms or golf courses as neighbors. When the golf course or nonorganic farm is sprayed, the chemicals used can travel many miles on a windy day or even evaporate and become a part of the next rainfall. One of the best sources of reference ranges is those from the US Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES).[6] (Parenthetically, this NHANES group was the author's first employer after earning his PhD degree in biochemistry.)

The NHANES study is one of the best data sources for toxic chemical data because it selects the population to be evaluated using highly sophisticated statistical techniques that evaluate virtually all people of both sexes in the United States of every age, race, and geographical region. The survey is unique in that it combines interviews and physical examinations of the subjects tested. The survey uses top-notch analytical methods, employing mass spectrometry in most cases. The statistical analysis of the data is also top notch, and the number of subjects is quite large, ranging from 1000 to 2000. Fig. 25.1 shows an example of the NHANES data for dimethylphosphate, a major metabolite of many different organophosphate pesticides. The NHANES reports have similar data for hundreds of environmental chemicals. Urine data are always reported with creatinine corrections, allowing for comparisons of data from individuals with differing fluid intakes. The statistical analyses of the data are performed by the National Center for Health Statistics (NCHS). Some of the most important toxic chemicals are those used in agriculture as pesticides, primarily as insecticides and herbicides that kill weeds, which are considered to be any plants that grow where humans do not want them growing.

Toxic chemicals may affect every organ in the body, but some of the toxic chemicals of greatest concern are those that affect the brain and nervous system, those that affect reproduction and fertility by mutagenesis or other mechanisms, and those that affect the development of children. In addition to chemicals that harm the human body directly, some chemicals may cause harm by altering the microbiome of the soil and the intestinal tracts of both humans and animals.

INDICATIONS FOR TOXIC CHEMICAL TESTING

Toxic chemical testing should be used whenever the patient has an occupational exposure to chemicals used in the workplace, such as the following:

- Pesticide applicators or agricultural workers exposed to glyphosate, organophosphate pesticides, 2,4-dichlorophenoxyacetic acid (2,4-D), or pyrethroid pesticides
- Workers in plastics factories using phthalates or vinyl chloride
- Workers in any type of industrial factories in which volatile solvents such as benzene, xylene, butadiene, propylene oxide, ethylene oxide, acrylonitrile, bromopropane, and others are regularly used
- Patients who have been treated with intravenous solutions from plastic bags or administered through plastic intravenous tubing, and may currently be contaminated with phthalates or vinyl chloride
- Workers in furniture, upholstery, and drapery industries using a variety of flame retardants, many of which are highly toxic
- Individuals who commonly employ paints, stains, or varnishes in their employment (including individuals using these chemicals occasionally, such as repainting the inside of the house, who may sometimes have symptoms such as severe fatigue)
- Individuals involved in dry cleaning or cleaning of industrial or military equipment
- Firefighters, police officers, and other rescue workers exposed to a wide variety of chemicals in general disasters such as the 9/11 terrorist attacks or other large fires
- Pilots, flight attendants, and even passengers because they can be exposed to jet engine exhaust and/or chemicals used to disinfect conduits that are circulated through the air system of the plane
- Physicians, nurses, other medical personnel, and restaurant workers who frequently wash their hands using toxic antibacterial hand soaps containing hexachlorophene, para-chloro-metaxylenol (PCMX), triclosan, and triclocarban
- Workers like police officers or car and airplane mechanics who may be exposed to vehicle exhaust fumes

The list of conditions for which toxic chemicals should be tested for people who do not obviously have occupation exposures includes the following:

- Patients with any illness that is life-threatening or severely debilitating, such as cancer, multiple sclerosis, autism, attention deficit disorder, Alzheimer's disease, and many others
- Patients with chronic illnesses that are not severely debilitating or life-threatening but are significantly reducing quality of life, such as arthritis, fatigue, and others
- Patients (both men and women) with impaired fertility
- Children with birth defects or failure to reach developmental milestones
- Women and men who want to reduce their chances of having a baby with a significant disease
- Any individual who wants to optimize his or her health and fitness

A list of toxic chemicals that are commonly found in human urine samples is given in Table 25.1. Note that some of the chemicals tested are common metabolites of more than one parent toxic chemical.

An entire book (such as *Clinical Environmental Medicine*) is needed to cover even most of the chemicals in Table 25.1 and the other chemicals[7-39] that are usually tested in the blood. A few summaries of the use and toxicity of some of the most commonly used major toxic chemicals follow.

Urinary *para*-Nitrophenol (1999-2010)

Metabolite of Ethyl Parathion, Methyl Parathion, and Nitrobenzene

Geometric mean and selected percentiles of urine concentrations (in µg/L) for the U.S. population from the National Health and Nutrition Examination Survey.

	Survey years	Geometric mean (95% conf. interval)	Selected percentiles (95% confidence interval) 50th	75th	90th	95th	Sample size
Total	99-00	*	< LOD	< LOD	**2.50** (1.40-4.50)	**5.00** (2.90-11.0)	1989
	01-02	*	< LOD	**1.33** (1.20-1.46)	**2.69** (2.39-3.01)	**3.72** (3.46-4.15)	2975
	07-08	**.673** (.595-.761)	**.740** (.660-.830)	**1.49** (1.32-1.66)	**2.77** (2.19-3.45)	**4.50** (3.50-5.42)	2564
	09-10	**.454** (.407-.506)	**.510** (.440-.580)	**1.09** (1.00-1.19)	**2.18** (1.99-2.34)	**3.14** (2.85-3.55)	2744
Age group							
6-11 years	99-00	*	< LOD	**.940** (<LOD-2.40)	**2.67** (1.70-3.80)	**4.30** (2.70-6.40)	479
	01-02	*	**.790** (<LOD-.910)	**1.49** (1.36-1.61)	**2.89** (2.22-3.58)	**4.10** (3.01-4.74)	565
	07-08	**.803** (.678-.952)	**.890** (.760-1.01)	**1.66** (1.26-1.99)	**2.85** (2.10-3.94)	**4.37** (2.91-6.75)	383
	09-10	**.506** (.426-.601)	**.600** (.500-.720)	**1.20** (.980-1.43)	**2.21** (1.74-2.73)	**2.85** (2.28-3.81)	386
12-19 years	99-00	*	< LOD	< LOD	**3.40** (1.60-5.70)	**5.70** (2.60-19.0)	680
	01-02	*	**.730** (<LOD-.910)	**1.45** (1.32-1.61)	**2.66** (2.15-3.11)	**3.34** (3.11-4.01)	813
	07-08	**.769** (.614-.962)	**.850** (.680-1.02)	**1.49** (1.28-1.74)	**2.79** (1.94-3.45)	**3.47** (2.97-4.48)	387
	09-10	**.430** (.375-.493)	**.520** (.460-.590)	**.950** (.870-1.09)	**1.84** (1.43-2.03)	**2.37** (1.84-2.98)	401
20-59 years	99-00	*	< LOD	< LOD	**2.30** (1.20-5.70)	**4.50** (2.30-16.0)	830
	01-02	*	< LOD	**1.28** (1.09-1.47)	**2.69** (2.32-3.10)	**3.72** (3.37-4.24)	1099
	07-08	**.658** (.574-.754)	**.720** (.640-.840)	**1.49** (1.31-1.65)	**2.77** (2.10-3.70)	**4.68** (3.37-5.56)	1173
	09-10	**.452** (.400-.511)	**.510** (.420-.590)	**1.12** (1.00-1.24)	**2.16** (1.91-2.39)	**3.27** (2.84-3.58)	1308
60 years and older	01-02	*	< LOD	**1.29** (1.07-1.49)	**2.66** (2.11-3.39)	**4.01** (3.17-7.19)	498
	07-08	**.607** (.512-.720)	**.610** (.550-.710)	**1.41** (1.14-1.76)	**2.81** (2.19-3.90)	**4.70** (2.90-6.91)	621
	09-10	**.453** (.386-.530)	**.460** (.380-.580)	**1.06** (.970-1.33)	**2.42** (1.87-3.00)	**3.65** (3.00-4.36)	649
Gender							
Males	99-00	*	< LOD	< LOD	**2.50** (1.40-4.50)	**4.50** (2.50-14.0)	971
	01-02	*	**.760** (.450-.880)	**1.49** (1.32-1.63)	**3.01** (2.66-3.33)	**4.13** (3.61-4.92)	1395
	07-08	**.782** (.690-.887)	**.850** (.740-.980)	**1.59** (1.43-1.73)	**2.85** (2.19-3.53)	**4.52** (3.47-5.01)	1282
	09-10	**.524** (.472-.581)	**.590** (.490-.670)	**1.30** (1.14-1.42)	**2.29** (2.07-2.54)	**3.29** (2.90-3.73)	1342
Females	99-00	*	< LOD	< LOD	**2.50** (1.30-5.70)	**5.70** (2.90-9.50)	1018
	01-02	*	< LOD	**1.18** (1.01-1.37)	**2.29** (1.95-2.69)	**3.52** (3.16-3.77)	1580
	07-08	**.582** (.510-.664)	**.640** (.550-.720)	**1.32** (1.10-1.59)	**2.72** (2.16-3.35)	**4.37** (3.09-5.64)	1282
	09-10	**.396** (.352-.446)	**.440** (.380-.510)	**.960** (.860-1.06)	**2.01** (1.70-2.26)	**3.07** (2.62-3.55)	1402
Race/ethnicity							
Mexican Americans	99-00	*	< LOD	**1.70** (<LOD-3.50)	**5.80** (2.60-24.0)	**22.0** (3.60-36.0)	695
	01-02	*	**.680** (<LOD-.840)	**1.33** (1.08-1.58)	**2.61** (1.91-3.41)	**3.64** (2.70-5.73)	744
	07-08	**.624** (.542-.720)	**.700** (.560-.810)	**1.37** (1.16-1.52)	**2.58** (2.03-3.33)	**4.46** (2.79-6.91)	494
	09-10	**.484** (.392-.599)	**.560** (.440-.710)	**1.30** (1.03-1.46)	**2.21** (1.78-2.46)	**3.07** (2.39-3.73)	602
Non-Hispanic blacks	99-00	*	< LOD	**1.20** (<LOD-2.60)	**2.90** (1.70-6.00)	**4.80** (2.50-9.20)	518
	01-02	*	**.850** (<LOD-1.10)	**1.76** (1.36-2.15)	**3.13** (2.47-4.26)	**4.92** (3.75-6.36)	752
	07-08	**.826** (.716-.952)	**.860** (.760-1.01)	**1.71** (1.45-1.92)	**3.15** (2.56-3.90)	**4.72** (3.91-5.68)	568
	09-10	**.505** (.381-.670)	**.570** (.400-.820)	**1.30** (1.01-1.50)	**2.19** (1.80-2.63)	**3.49** (2.57-4.28)	504
Non-Hispanic whites	99-00	*	< LOD	< LOD	**2.10** (<LOD-6.33)	**4.20** (2.10-11.0)	603
	01-02	*	< LOD	**1.29** (1.14-1.42)	**2.70** (2.38-3.10)	**3.71** (3.38-4.00)	1259
	07-08	**.623** (.531-.730)	**.690** (.610-.790)	**1.36** (1.19-1.59)	**2.51** (1.89-3.08)	**3.63** (2.82-5.48)	1075
	09-10	**.440** (.388-.499)	**.490** (.410-.580)	**1.03** (.930-1.12)	**2.18** (1.89-2.48)	**3.14** (2.67-3.62)	1197

Limit of detection (LOD, see Data Analysis section) for Survey years 99-00, 01-02, 07-08, and 09-10 are 0.8, 0.1, 0.1, and 0.1 respectively.
< LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.
* Not calculated: proportion of results below limit of detection was too high to provide a valid result.

Biomonitoring Summary: http://www.cdc.gov/biomonitoring/Nitrobenzene_BiomonitoringSummary.html

Fig. 25.1 Representative table from the Centers for Disease Control and Prevention (CDC) National Health and Examination Survey (NHANES) publication *Fourth National Report on Human Exposure to Environmental Chemicals* (February 2015, p. 128). The survey covers a number of years so that the reader or researcher can determine trends in the exposure of the population. Thus the values for the 75th, 90th, and 99th percentile appear to be steadily increasing over the last 10 years (1999-2008) of the survey, indicating greater toxic exposure to the population. This trend is apparent in all age groups. (From Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals. February 2015. https://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf.)

TABLE 25.1 Common Metabolites of Toxic Chemicals Tested in Urine

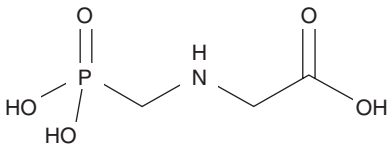
Parent Toxic Chemical	Metabolite Commonly Tested in Urine	Brief Summary of Toxicity
Xylene[32]	2-Methylhippuric acid (2MHA) 3-Methylhippuric acid (3MHA) 4-Methylhippuric acid (4MHA)	These are metabolites of xylenes, solvents found in paints, lacquers, cleaning agents, pesticides, and gasoline. Exposure to xylenes generates methylhippuric acid isomers. Avoid/reduce exposure to these substances.
Benzene[33]	N-Acetyl phenyl cysteine (NAP)	NAP is a metabolite of benzene. Benzene is a solvent that is widespread in the environment. It is found in cigarette smoke and gasoline and is a by-product of all types of combustion, including motor vehicle exhaust. Treatment consists of removing sources of exposure.
Styrene/ethylbenzene[32]	Phenylglyoxylic acid (PGO)	Exposure to environmental or workplace styrene may increase phenylglyoxylic and mandelic acid. Reduce exposure by eliminating the use of plastic and Styrofoam containers for cooking, reheating, eating, or drinking. Elimination of styrene can be accelerated by supplementing with glutathione and N-acetyl cysteine (NAC).
MTBE/ETBE[34]	2-Hydroxyisobutyric acid (2HIB)	2-HIB is formed endogenously as a product of branched-chain amino acid degradation and ketogenesis. This compound is also the major metabolite of gasoline octane enhancers such as MTBE and ETBE. Elevated levels indicate environmental exposure. Use of purified water is useful if local water is contaminated.
Diethylphthalates[35]	Monoethyl phthalate (MEP)	MEP from diethyl phthalate is the most abundant phthalate metabolite found in urine. Diethyl phthalate is used in plastic products. Elevated values indicate exposure from various possible sources. Elimination of phthalates may be accelerated by sauna treatment.
Organophosphates[36]	Dimethylphosphate (DMP) Diethylphosphate (DEP)	DMP and DEP are major metabolites of 147 organophosphate pesticides. Reduce exposure by eating organic foods and avoiding the use of pesticides in your home or garden. Living near agricultural areas or golf courses and areas regularly sprayed with pesticides will increase exposure. Elimination of organophosphates can be accelerated by sauna treatment.
Pyrethroids[37]	3-Phenoxybenzoic acid (3PBA)	3-PBA is a metabolite of six different pyrethroid insecticides. Elimination can be accelerated by sauna treatment.
2,4-Dichlorophenoxyacetic acid (2,4-D)[38]	Parent measured	2,4-D was an ingredient in Agent Orange and is most commonly used in agriculture of genetically modified foods and as a weed killer for lawns. Reduce exposure by eating organic foods and avoiding the use of pesticides in your home or garden.
Mitochondrial dysfunction marker	Tiglylglycine (TG)[39]	TG is a marker for mitochondrial dysfunction. Mutations of mitochondrial DNA may result from exposure to toxic chemicals, infections, inflammation, and nutritional deficiencies.
Acrylamide[79]	N-acetyl-S-(2-carbamoylethyl)-cysteine (NAE)	NAE is a metabolite of acrylamide, which is detoxified through a two-step process. First, acrylamide is metabolized by the cytochrome P450s. Second, it is conjugated to glutathione to make it more water soluble. Acrylamide is used in many industrial processes, such as plastics, food packaging, cosmetics, nail polish, dyes, and treatment of drinking water. High levels of acrylamide can elevate a patient's risk of cancer and cause neurological damage. Supplementation with glutathione can assist in the elimination of this compound.
Diphenyl phosphate[13-16]	Diphenyl phosphate	This is a metabolite of the organophosphate flame retardant triphenyl phosphate (TPHP), which is used in plastics, electronic equipment, nail polish, and resins. TPHP can cause endocrine disruption. Studies have also linked TPHP to reproductive and developmental problems.
Perchlorate[17-19]	Perchlorate	Perchlorate is used in the production of rocket fuel, missiles, fireworks, flares, explosives, fertilizers, and bleach. Studies show that perchlorate is often found to contaminate water supplies and food sources. It can disrupt the thyroid's ability to produce hormones. The US Environmental Protection Agency has also labeled perchlorate a likely human carcinogen. Patients who are high in perchlorate can use a reverse-osmosis water treatment system to remove perchlorate.
1,3 Butadiene[20-23]	N-Acetyl (3,4-dihydroxybutyl) cysteine (NABD)	NABD is a metabolite of 1,3 butadiene, which is evidence of exposure to synthetic rubber, such as tires. 1,3 butadiene is a known carcinogen and has been linked to increased risk of cardiovascular disease. Individuals who come into contact with rubber, such as car tires, could absorb 1,3 butadiene through the skin.
Propylene oxide[24-25]	N-Acetyl (2,hydroxypropl) cysteine (NAHP)	NAHP is a metabolite of propylene oxide, which is used in the production of plastics and as a fumigant. It is also used in the preparation of lubricants, surfactants, and oil demulsifiers and as a food additive, an herbicide, a microbicide, an insecticide, a fungicide, and a miticide. Propylene oxide is a probable human carcinogen.
1-Bromopropane[26-28]	N-Acetyl (propyl) cysteine (NAPR)	NAPR is a metabolite of 1-bromopropane. Chronic exposure can lead to decreased cognitive function and impairment of the central nervous system. Acute exposure can lead to headaches.

TABLE 25.1 Common Metabolites of Toxic Chemicals Tested in Urine—cont'd

Parent Toxic Chemical	Metabolite Commonly Tested in Urine	Brief Summary of Toxicity
Ethylene oxide[29-31] Vinyl chloride Halopropane	2-Hydroxyethyl mercapturic acid (HEMA)	HEMA is a metabolite of ethylene oxide, which is used in the production of agrochemicals, detergents, pharmaceuticals, and personal care products. Chronic exposure to ethylene oxide has been determined to be mutagenic to humans. HEMA is also a metabolite of vinyl chloride and halopropane, which are used in many commercial chemical processes, such as foam gluing, dry cleaning, and in the production of solvents. Supplementation with glutathione should assist in the detoxification process of these chemicals.
Acrylonitrile[10-12]	N-Acetyl (2-cyanoethyl) cysteine (NACE)	NACE is a metabolite of acrylonitrile, which is used in the production of acrylic fibers, resins, and rubber. Acrylonitrile is metabolized by the cytochrome P450s and then conjugated to glutathione. Supplementation with glutathione should assist in the detoxification of acrylonitrile.

THE MOST COMMON TOXIC CHEMICALS

Glyphosate



Glyphosate is one of the most prevalent chemicals used in agriculture. Some countries use glyphosate in greater amounts than all other agricultural chemicals combined. Monsanto brought it to market in 1974 under the trade name **Roundup**, and Monsanto's last commercially relevant US patent expired in 2000.[40] Many companies now produce the herbicide. By 2016 there was a 100-fold increase from the late 1970s in the frequency of application and volume of glyphosate-based herbicides applied, with further increases expected in the future, partly in response to the global emergence and spread of glyphosate-resistant weeds.[40]

The amount of glyphosate used as a weed killer or herbicide proliferated with the development of genetically modified foods that were genetically modified to be resistant to the plant-killing action of glyphosate. The availability of genetically modified food plants that were patented by Monsanto was a major factor in the tremendous increase in glyphosate use. Corn and soy seeds were the first crops to be marketed as Roundup Ready. Current glyphosate-resistant crops include soy, corn, canola, alfalfa, sugar beets, and cotton, with wheat still under development. In 2015, 89% of corn, 94% of soybeans, and 89% of cotton produced in the United States were genetically modified to be glyphosate-tolerant.[40]

In addition to the use of glyphosate on genetically modified crops, glyphosate is also applied to many nongenetically modified crops as a desiccant in the process called preharvest **crop desiccation**, referring to the application of an herbicide to a crop shortly before harvest. As a systemic herbicide, glyphosate is not a true desiccant because it can take weeks rather than days for the crop to die back after application.[41] Nongenetically modified foods with which glyphosate has been used as a desiccant include wheat, barley, oats, sunflowers, potatoes, lentils, garbanzo beans, and many others.[42] In addition, glyphosate is also used as a desiccant for nonfood crops such as cotton and tobacco.[43] The shikimic acid pathway of weeds and common nongenetically modified (non-GMO) food plants produces aromatic amino acids needed for plant growth; glyphosate inhibits a key enzyme in this pathway, leading to the death of the weed or non-GMO plant. The DNA of GMO corn, soy, and other food plants is modified so that it is relatively resistant to glyphosate. The makers of glyphosate claim that the toxicity of glyphosate is negligible because humans and other mammals lack the shikimic acid pathway, but research to disprove the toxic effects of glyphosate on many other biological processes is missing.

The human safety of glyphosate has been seriously questioned by a broad group of 14 health experts who are predominantly professors in institutions of environmental health or medical schools or are other environmental professionals.[44]

Starting in the mid-1990s, a chronic kidney disease of unknown etiology (CKDu) was discovered among the rice paddy farmers in the North Central Province of Sri Lanka.[45] Individuals with this kidney disease have substantially higher amounts of glyphosate in their urine than individuals without kidney disease. The government of Sri Lanka subsequently banned the use of glyphosate in that country.

In the following two decades, the disease spread rapidly to other farming areas. The age-standardized prevalence of the disease is estimated at 15%,[45] affecting a total population of 400,000 patients, with an estimated death toll of approximately 20,000 in the period[45] The unique feature of CKDu is that its etiology does not include commonly known risk factors for CKD, such as diabetes mellitus, hypertension, or glomerular nephritis.[45]

A similar incidence of deaths due to CKDu in sugar cane workers has been reported in Central America.[45] In those cases, it is suspected that toxic chemicals such as arsenic may form chelation complexes with glyphosate that prolong the persistence of glyphosate in the soil and water and that may also lead to contamination of humans who drink water containing such metal chelates. Such chelates of glyphosate prolong the life of glyphosate in the environment to as long as 22 years.

Although glyphosate was designed to kill weeds, it also kills susceptible bacteria that have biochemical pathways similar to plants and weeds. Shehata et al.[46] found that glyphosate exposure in poultry can lead to a marked increase in pathogenic bacteria, such as *Salmonella* and *Clostridia* species, that were resistant to glyphosate in the stool samples of the poultry and to a significant decrease in beneficial flora, such as *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis,* and *Lactobacillus* species, which are susceptible to glyphosate.

In addition, ingestion of the herbicide could be a significant predisposing factor that has been associated with an increase in diseases mediated by *Clostridium botulinum*[47,48] in cattle, and perhaps even in the farmers exposed to infected cattle.[49] *C. botulinum* is one of three species of *Clostridia* bacteria that produce large quantities of the precursors of 3-[3-hydroxyphenyl]-3- hydroxypropionic acid (HPHPA), a phenolic compound.[50] This compound inhibits the enzyme dopamine–β-hydroxylase that converts dopamine to norepinephrine in neurons in

the brain and in the sympathetic nervous system.[50] Excessive amounts of the dopamine metabolite homovanillic acid have been found to be prevalent in urine samples from children with autism.[45]

Shaw reported[45] on a case study of triplets exposed to high amounts of glyphosate due to excessive ingestion of GMO corn tortillas. Two of the children had autism, and the third had a suspected seizure disorder. All of the children had high biochemical markers indicating mitochondrial damage. All three children had markedly elevated urinary glyphosate, with their mean baseline value—34.4 µg/g creatinine—being 25.5 times the median value of 1.35 µg/g creatinine and 24.1 times the mean value of 1.43 µg/g creatinine of the study's internal reference range. The glyphosate value in the urine of one of the retested triplets decreased dramatically (94% decrease) after switching to an organic food diet, and symptoms of autism declined considerably after organic diet implementation.

The female of the triplets did not have elevated phenolic compounds on either organic acid test but did have a significantly elevated value of the metabolite tiglylglycine, a marker for mitochondria complex 1 dysfunction or mutations in the mitochondrial electron transport chain.[45] In addition, the triplets mother also had significantly elevated tiglylglycine.

All of the triplets had elevated succinic acid in their urine, an indicator of mitochondrial dysfunction (see Chapter 29, Urinary Organic Acids),[45] on at least one of the two organic acids tests performed on these patients. Both males had elevated succinic acid on one of the two samples, whereas the female had elevated values on both organic acid tests.

Succinic acid is metabolized by the enzyme succinic dehydrogenase, which is significant in that it is both a Krebs cycle enzyme and a component—complex 2—of the mitochondrial electron transport chain, making this metabolite a marker of mitochondrial complex 2 dysfunction. Succinic acid has been found to be a sensitive marker for a variety of other toxic chemicals as well, including both metal and nonmetal toxic chemicals.

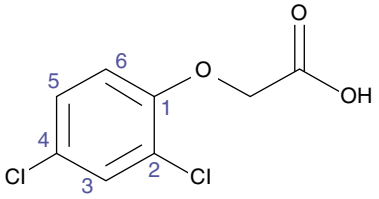
In addition to elevated markers associated with mitochondria damage, the high quantity of phenolic compounds produced by *Clostridia* bacteria in the two male members of the triplets is consistent with the previous reports that glyphosate can alter the intestinal flora of exposed animals and humans. Both males with the elevated *Clostridia* markers had depressed conversion of dopamine to norepinephrine, **leading to overproduction of dopamine, a feature prevalent in autism**.[45] The pH of certain compartments of the neurons is a critical factor in maintaining neurotransmitters in the biochemical form that is most stable and least toxic.

The synaptic vesicles that store dopamine and norepinephrine maintain an acidic pH of around 5.2, a pH that maintains the optimum stability of these neurotransmitters. These vesicles also contain dopamine-beta-hydroxylase that converts dopamine to norepinephrine. If this enzyme is blocked by *Clostridia* metabolites, dopamine accumulates in the synaptic vesicles to the point that excessive dopamine leaks out of the synaptic vesicles and enters the cytoplasm, which has a pH of 7.4, a pH at which dopamine is highly unstable and is converted to a number of highly unstable and toxic metabolites called dopamine quinones and aminochromes.[45] Paradoxically, some of these toxic metabolites become even more toxic when they react with glutathione. Dopamine toxic metabolites covalently bind to the mitochondrial proteins and the neuronal structural proteins actin and a/b-tubulin of the cytoskeleton, inducing autophagy, the formation of clusters of condensed chromatin, mitochondrial DNA damage, oxidative damage, impaired mitochondrial energy production, and finally cell death.[45]

In addition to autism and kidney disease associations, the International Agency for Research on Cancer of the World Health Organization (WHO) published a summary of its monograph on glyphosate and classified it as probably carcinogenic in humans (category 2A) based on epidemiological, animal, and invitro studies.[51] Seralini etal.[52] reported that female rats exposed to glyphosate or to glyphosate in a commercial product called Roundup, in which other chemicals are present, developed mammary or pituitary tumors at a rate higher than controls. Treated male rats developed increased rates of liver necrosis and kidney abnormalities compared with controls. The findings of abnormal kidneys in the exposed rats are consistent with the kidney disease described in Asia that is associated with thousands of deaths related to high glyphosate in the urine. A meta-analysis published in 2014 identified an increased risk of non-Hodgkins lymphoma in workers exposed to glyphosate formulations.[53]

One of the highest values for urine glyphosate recorded at The Great Plains Laboratory (GPL) was in a man who only ate organic food but who used chewing tobacco regularly. Tobacco that is dried appears to be saturated with glyphosate.[43]

2, 4-Dichlorophenoxyacetic Acid (2, 4-D)



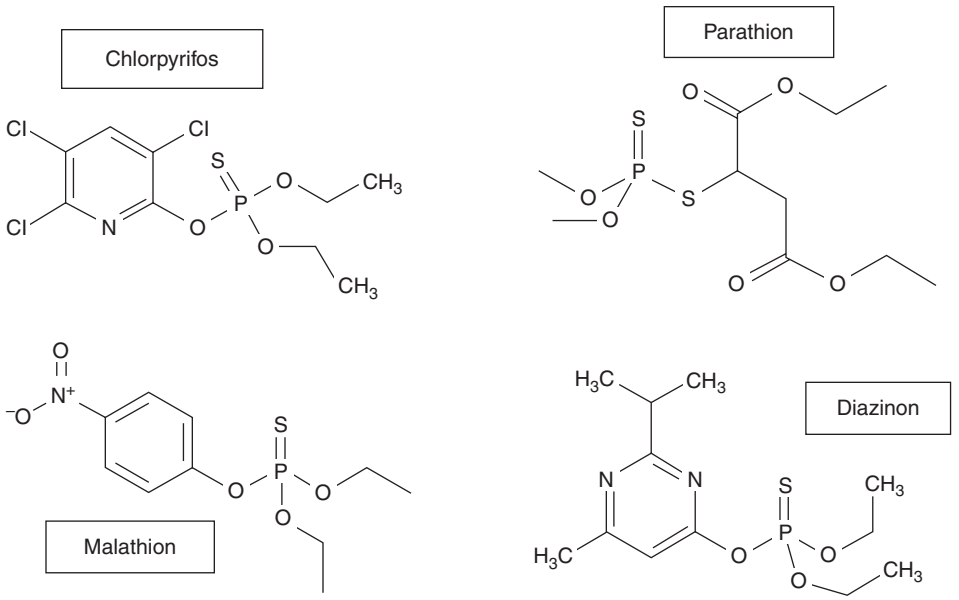
2, 4-D is a major herbicide intended to kill unwanted vegetation such as broadleaf weeds and woody plants. It is used in agriculture and on residential properties. People can be exposed to herbicides by breathing them in the air, by skin contact from residential use or living near application sites, and by eating contaminated food and drinking contaminated water.[54] Neuritis, weakness, nausea, abdominal pain, headache, dizziness, peripheral neuropathy, stupor, seizures, brain damage, and impaired reflexes have been associated with dermal or oral exposure.[55] 2, 4-D is a known endocrine disruptor and can block hormone distribution and cause glandular breakdown. It is linked to immune system damage, birth defects, and reproductive issues, possibly due to its frequent contamination with dioxins.[55] Small amounts of 2, 4-D may be found in many urine samples because of widespread environmental contamination. 2,4-D was a major component of a chemical mixture called Agent Orange used by the US military during the Vietnam War to increase the visibility for warplanes by destroying plant undergrowth and crops.[54]

Some of the toxicity of Agent Orange has been attributed to contaminating molecules called dioxins that arose during manufacturing. 2, 4-D may not be as contaminated with dioxins as in the past.[56] Although the presence of dioxins in 2, 4-D has been banned, they have recently still been detected. A July 2013 Four Corners investigation found elevated levels of dioxins in a generic version of 2, 4-D, one of Australias most widely used herbicides. One scientist said the product tested by Four Corners, which was imported from China, had one of the highest dioxin readings for 2, 4-D in the past 10 to 20 years, and could pose potential health risks.[57]

Recently, genetically modified food plants resistant to both glyphosate and 2, 4-D have been introduced into agriculture, using herbicides containing both glyphosate and 2, 4-D.[58]

The highest value at GPL was that of a child with autism with a urine value 28 times the 95th percentile. The child had a urine succinic acid (a marker for mitochondrial complex 2 abnormality) more than 10 times the upper limit of normal, a value more abnormal than individuals with proven mitochondrial DNA deletions. The tiglylglycine marker was in the normal range, indicating that the effects of the 2, 4-D were primarily on complex 2 versus complex 1 of the mitochondrial respiratory chain. The child was treated by sauna therapy for about an hour almost daily for 3 months, along with an organic food diet, after which a repeat test found no detectable 2, 4-D and a considerable improvement in autistic symptoms. The source of 2, 4-D was unable to be identified.

Organophosphates



Organophosphates are one of the most toxic groups of substances used throughout the world. Four common organophosphate structures are illustrated in the accompanying graphic. There are nearly 3 million poisonings per year, resulting in 200,000 deaths.[59] They are often used as biochemical weapons and terrorist agents but are most commonly used in pesticide formulations.[59] Children exposed to organophosphates have more than twice the risk of developing pervasive developmental disorder (PDD), an autism spectrum disorder, and children born to mothers living within 500 m of fields where organochlorine pesticides were used were more than six times more likely to develop autism than children whose mothers did not live near such fields.[60] Maternal organophosphate exposure has been associated with various adverse outcomes, including having shorter pregnancies and children with impaired reflexes.[60]

Approximately 340 million kilograms of pesticide active ingredient is used agriculturally in the United States annually, and 85% of US households store at least one pesticide for home use.[61] These insecticides kill insects (and mammals such as humans) by the inhibition of the enzyme acetyl-cholinesterase and other enzymes in which serine is part of the enzyme active site, such as dipeptidyl peptidase IV. When acetylcholine breakdown is inhibited, overstimulation can lead to constant nerve transmission or overstimulation of neurons or muscles, resulting in excessive salivation, abnormal behavior, diarrhea, urinary incontinence, vomiting, tremors, muscle paralysis, and even death.[62] High exposure levels have been associated with attention deficit, memory impairment, and pervasive developmental disorders.[60] Exposure has also been linked to violent behavior, depression, and suicide and may have played a role in the onset of Gulf War syndrome.[63-65] If levels are high, toxicity can be measured by decreased cholinesterase or pseudocholinesterase activity in plasma.[62] Acute toxicity is treated with atropine and/or pralidoxime.[66] Organophosphate exposure can be reduced by eating organic foods, avoiding the use of pesticides in the house or garden, avoiding residence near agricultural areas or golf courses, and staying indoors if insecticides are being sprayed. Lice shampoo, pet flea collars, and flea spray are also major sources of organophosphates. Remove sources of exposure if possible. A total of 147 different organophosphate pesticides can be detected by their metabolites DEP and DMP[67] (Table 25.1). Patients with values for organophosphates over the 95th percentile of the reference range with which GPL consulted included those with autism, Aspergers syndrome (a form of mild autism), severe uncontrolled temper tantrums, amyotrophic lateral sclerosis (ALS), vascular dementia, Parkinson's disease, and Huntingtons syndrome. One of the children with autism with high urine organophosphates was from the Miami, Florida, area, in which organophosphates had been widely sprayed to control mosquitoes implicated in the spread of the Zika virus. Initial results from GPL suggest organophosphates are the most common toxic chemical group in which the urine values of patients commonly exceed the 95th percentile.

TREATMENT OF NONMETAL TOXIC CHEMICAL EXPOSURE

The first and most important step in all chemical exposures is to identify the source of the toxic chemical exposure and remove the patient from the exposure. This may be simple if the patient is industrially exposed in a chemical plant or is a pest exterminator who is exposed to pesticides every day. Chemicals in cosmetics, such as phthalates, triphenylphosphates, and solvents, may be absorbed through the skin. Many individuals are exposed to chemicals through their ingestion of food and water, so switching to an organic diet is highly effective in removing pesticides and herbicides from the body. It is important to understand the difference in the terms *organic* and *non-GMO*. Organic foods are foods that have not received treatment with synthetic pesticides, herbicides, or other restricted chemicals. Non-GMO labels on foods mean the foods are not from genetically modified plants, but the food could still be highly contaminated with toxic chemicals used as pesticides and herbicides. In the case of the triplets with autism discussed previously, there was a 94% reduction of glyphosate in less than 2 months by switching to an organic diet.

Use of supplements that combine with various metabolites of toxic chemicals is also useful to accelerate the detoxification of large numbers of toxic chemicals. Glutathione and glutathione precursors such as n-acetylcysteine frequently combine with oxidation products of Phase I detoxification enzymes to form less toxic Phase II detoxification conjugates. Liposomal glutathione, taken orally, appears to be more effective than plain glutathione because of better absorption from the gastrointestinal tract. Although glutathione may be given

intravenously as well, oral administration is often cheaper and more convenient than intravenous infusions for long detoxification protocols. Glycine, an amino acid, may also be used orally to increase the excretion of oxidized metabolites of benzene and xylene as glycine conjugates.

Sauna treatment is another common treatment used for detoxification. Mercola and Crinnion have outlined a comprehensive sauna treatment for detoxification, with some protocols using flush niacin (not niacinamide or time-release niacin).[68,69]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rapp D. *Our Toxic World: A Wakeup Call*. Buffalo, NY: Environmental Medical Research Foundation; 2004.
2. Rapp D. *Is This Your Child's World?*. New York: Bantam Books; 1996.
3. Tests find more than 200 chemicals in newborn umbilical cord blood. https://www.scientificamerican.com/article/newborn-babies-chemicals-exposure-bpa/. Accessed 1/28/2018.
4. European parliamentarians contaminated by 76 chemicals. http://wwf.panda.org/wwf_news/?12622/European-parliamentarians-contaminated-by-76-chemicals. Accessed 1/30/2018.
5. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Mol Psychiatry*. 2012;17(4):389–401.
6. Fourth National Report on Human Exposure to Environmental Chemicals February 2015:128. https://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf. Accessed August 27, 2019.
7. Feroe AG, Attanasio R, Scinicariello F. Acrolein metabolites, diabetes and insulin resistance. *Environ Res*. 2016;148:1–6. https://doi.org/10.1016/j.envres.2016.03.015.
8. Semla M, Goc Z, Martiniakova M, Omelka R, Formicki G. Acrylamide: a common food toxin related to physiological functions and health. *Physiol Res*. 2017;66:205–217.
9. Duale N, etal. Biomarkers of human exposure to acrylamide and relation to polymorphisms in metabolizing genes. *Toxicol Sci*. 2009;108:90–99. https://doi.org/10.1093/toxsci/kfn269.
10. Jakubowski M, Linhart I, Pielas G, Kopecky J. 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. *Br J Ind Med*. 1987;44:834–840.
11. Acrylonitrile. Report on carcinogens: carcinogen profiles. 2011;12:2830.
12. Woutersen RA. Toxicologic profile of acrylonitrile. *Scand J Work Environ Health*. 1998;24(suppl 2):5–9.
13. Stapleton HM, Misenheimer J, Hoffman K, Webster TF. Flame retardant associations between children's handwipes and house dust. *Chemosphere*. 2014;116:54–60. https://doi.org/10.1016/j.chemosphere.2013.12.100.
14. Cooper EM, Covaci A, van Nuijs AL, Webster TF, Stapleton HM. Analysis of the flame retardant metabolites bis(1,3-dichloro-2-propyl) phosphate (BDCPP) and diphenyl phosphate (DPP) in urine using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem*. 2011;401:2123–2132. https://doi.org/10.1007/s00216-011-5294-7.
15. Li X, Rose SE, Montine KS, Keene CD, Montine TJ. Antagonism of neuronal prostaglandin E(2) receptor subtype 1 mitigates amyloid beta neurotoxicity invitro. *J Neuroimmune Pharmacol*. 2013;8:87–93. https://doi.org/10.1007/s11481-012-9380-1.
16. Dishaw LV, Macaulay LJ, Roberts SC, Stapleton HM. Exposures, mechanisms, and impacts of endocrine-active flame retardants. *Curr Opin Pharmacol*. 2014;19:125–133. https://doi.org/10.1016/j.coph.2014.09.018.
17. Steinmaus CM. Perchlorate in water Supplies: sources, exposures, and health effects. *Curr Environ Health Rep*. 2016;3:136–143. https://doi.org/10.1007/s40572-016-0087-y.
18. Leung AM, Pearce EN, Braverman LE. Environmental perchlorate exposure: potential adverse thyroid effects. *Curr Opin Endocrinol Diabetes Obes*. 2014;21:372–376. https://doi.org/10.1097/med.0000000000000090.
19. Lewandowski TA, Peterson MK, Charnley G. Iodine supplementation and drinking-water perchlorate mitigation. *Food Chem Toxicol*. 2015;80:261–270. https://doi.org/10.1016/j.fct.2015.03.014.
20. Jain RB. Levels of selected urinary metabolites of volatile organic compounds among children aged 6-11 years. *Environ Res*. 2015;142:461–470. https://doi.org/10.1016/j.envres.2015.07.023.
21. Symanski E, etal. Air toxics and early childhood acute lymphocytic leukemia in Texas, a population based case control study. *Environ Health*. 2016;15:70. https://doi.org/10.1186/s12940-016-0154-8.
22. St Helen G, etal. Intake of toxic and carcinogenic volatile organic compounds from secondhand smoke in motor vehicles. *Cancer Epidemiol. Biomarkers Prev*. 2014;23:2774–2782. https://doi.org/10.1158/1055-9965.epi-14-0548.
23. von Ehrenstein OS, Aralis H, Cockburn M, Ritz B. In utero exposure to toxic air pollutants and risk of childhood autism. *Epidemiology (Cambridge, Mass.)*. 2014;25:851–858. https://doi.org/10.1097/ede.0000000000000150.
24. Garcia E, Hurley S, Nelson DO, Hertz A, Reynolds P. Hazardous air pollutants and breast cancer risk in California teachers: a cohort study. *Environ Health*. 2015;14:14. https://doi.org/10.1186/1476-069x-14-14.
25. Propylene oxide. *Rep Carcinog*. 2011;12:367–369.
26. Hanley KW, Petersen MR, Cheever KL, Luo L. N-acetyl-S-(n-propyl)-l-cysteine in urine from workers exposed to 1-bromopropane in foam cushion spray adhesives. *Ann Occup Hyg*. 2009;53:759–769. https://doi.org/10.1093/annhyg/mep051.
27. Report on carcinogens monograph on 1-bromopropane. Report on carcinogens. *Monograph on*. 2013:1–168.
28. Boyle EB, etal. Assessment of exposure to VOCs among pregnant women in the national children's study. *Int J Environ Res Public Health*. 2016;13:376. https://doi.org/10.3390/ijerph13040376.
29. Kloth S, Baur X, Goen T, Budnik LT. Accidental exposure to gas emissions from transit goods treated for pest control. *Environ Health*. 2014;13:110. https://doi.org/10.1186/1476-069x-13-110.
30. Konduracka E, Krzemieniecki K, Gajos G. Relationship between everyday use cosmetics and female breast cancer. *Pol Arch Intern Med*. 2014;124:264–269.
31. Yuan JM, etal. Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. *Carcinogenesis*. 2012;33:804–809. https://doi.org/10.1093/carcin/bgs026.
32. Marchese S, Curini R, Gentili A. Daniela Perret and Lucia Mainero Rocca Simultaneous determination of the urinary metabolites of benzene, toluene, xylene and styrene using high-performance liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*. 2004;18:265–272.
33. Protano C, Andreoli R, Manini P, Vitali M. Urinary trans, trans-muconic acid and S-phenylmercapturic acid (N-acetylphenycysteine) are indicative of exposure to urban benzene pollution during childhood. *Sci Total Environ*. 2012;435436:115–123. https://doi.org/10.1016/j.scitotenv. 2012.07.004. Epub 2012 Jul 28.
34. Alexander A, Rosner E, Dekant W. Biotransformation and Kinetics of excretion of Ethyl tert-Butyl ether in rats and humans. *Toxicol Sci*. 2000;53:194–201.
35. Mark Kao L, Ruoff B, Nancy Bower, Aoki T, Smart C, Mannens G. Pharmacokinetics, metabolism and excretion of 14C-monoethyl phthalate (MEP) and 14C-diethyl phthalate (DEP) after single oral and IV administration in the juvenile dog. *Xenobiotica*. 2011;42(4):389–397. https://doi.org/10.3109/00498254.2011.627478.
36. CDC Biomonitoring Summary Organophosphorus Insecticides: Dialkyl Phosphate Metabolites. https://www.cdc.gov/biomonitoring/OP-DPM_BiomonitoringSummary.html. Accessed August 27, 2019.
37. Oulhote Y, Maryse F. Bouchard urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian Children. *Environ Health Perspect*. 2013;121:11–12.
38. Hughes DL, Ritter DJ, Wilson RD. Determination of 2,4-dichlorophenoxyacetic acid (2,4-D) in human urine with mass selective detection. *J Environ Sci Health B*. 2001 Nov;36(6):755–764.
39. Bennett M, Powell J, Daniel J, etal. Tiglylglycine excreted in urine in metabolism and the respiratory chain measured by stable isotope dilution GC-MS. *Clin Chem*. 1994;40(10):1879–1883.
40. Glyphosate. https://en.wikipedia.org/wiki/Glyphosate. Accessed 5/3/2018.
41. Brian Hall (August 12, 2014). Direct Harvest: Do you need a desiccant or a pre-harvest glyphosate? Field Crop News.
42. CrOp Desiccation. https://en.wikipedia.org/wiki/Crop_desiccation#cite_note-15. Accessed August 27, 2019.
43. 90% Of All Tobacco Is Full of Pesticides, Herbicides, and GMOs By Robert Harrington Posted On June 16, 2015. http://naturalsociety.com/90-of-all-tobacco-is-gmo-full-of-pesticides-and- herbicides/. Accessed 1/30/2018.
44. Myers JP, Antoniou MN, Blumberg B, etal. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ Health*. 2016;15(19):13. https://doi.org/10.1186/s12940- 016-0117-.

45. Shaw W. Elevated urinary glyphosate and *Clostridia* metabolites with altered dopamine metabolism in triplets with two of the three having an autistic spectrum disorder and the other a suspected seizure disorder: a case study with potential implications to autism. *Integr Med*. 2017;16:50–57.
46. Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota invitro. *Curr Microbiol*. 2013;66(4):350–358.
47. Krüger M, Shehata AA, Schrödl W, Rodloff A. Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*. *Anaerobe*. 2013;20:74–78. https://doi.org/10.1016/j.anaerobe.2013.01.005. Epub 2013 Feb 6.
48. Rulff R, Schrdl W, Basiouni S, Neuhaus J, Krger M Is downer cow syndrome related to chronic botulism? *Pol J Vet Sci.* 2015;18(4):759765. doi: 10.1515/pjvs-2015-0098. The presence of a significantly higher C. *botulinum* ABE and CD antibody levels in DCS cows than in the healthy animals suggests that chronic C. *botulinum* toxico-infection could be a predisposing factor for DCS.
49. Rodloff AC, Kruger M. Chronic *Clostridium botulinum* infections in farmers. *Anaerobe*. 2012;18(2):226–228.
50. Shaw W. *Clostridia* bacteria in the gastrointestinal tract as a major cause of depression and other neuropsychiatric disorders. Role of *Clostridia* metabolites 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) and 4-cresol in the alteration of dopamine and norepinephrine metabolism. In: Greenblatt J, Kelly B, eds. *Integrative Psychiatry for Depression: Redefining Models for Assessment, Treatment, and Prevention of Mood Disorders*. New York: Taylor and Francis; 2016:31–48.
51. Guyton KZ, Loomis D, Grosse Y, etal. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol*. 2015;16(5):490–491.
52. Séralini GE, Clair E, Mesnage R, etal. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environ Sci Eur*. 2014;26:14.
53. Schinasi L, Leon ME. Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*. 2014;11(4):4449–4527.
54. 2,4-Dichlorophenoxyacetic acid. https://en.wikipedia.org/wiki/2,4-Dichlorophenoxyacetic_acid#cite_note-31. Accessed August 27, 2019.
55. 2,4-D updated by Steven Gilbert (Jun 09, 2014). http://www.toxipedia.org/display/toxipedia/2%2C4-D. Accessed June 9, 2014.
56. 2,3,7,8-Tetrachlorodibenzodioxin. https://en.wikipedia.org/wiki/2,3,7,8-Tetrachlorodibenzodioxin. Accessed August 27, 2019.
57. Four Corners By Janine Cohen (July 22, 2013). Four Corners Investigation Finds Dangerous Dioxins in Widely Used Herbicide 24D. Abc.net.au. Accessed May 3, 2014.
58. Resources & Rewards. Enlist.com. http://www.enlist.com/en/resources-and-rewards.html. Published 2020. Accessed February 29, 2020.
59. Organophosphate poisoning https://en.wikipedia.org/wiki/Organophosphate_poisoning. Accessed August 27, 2019.
60. Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environ Health Perspect*. 2007;115:1482–1489. https://doi.org/10.1289/ehp.10168.
61. Bradman A, Eskenazi B, Barr DB, etal. Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. *McKone Environ Health Perspect*. 2005;113:1802–1807.
62. Rosane Maria Salvi, Diogo Lara R, Eduardo Ghisolfi S, Luis Portela V, Renato Dias D, Diogo Souza O. Neuropsychiatric evaluation in subjects chronically exposed to organophosphate pesticides. *Toxicol Sci*. 2003;72:267–271.
63. Phillips KF, Laxmikant S. Repeated low-dose organophosphate DFP exposure leads to the development of depression and cognitive impairment in a rat model of Gulf War Illness Deshpande. *Neurotoxicology*. 2016;52:127–133.
64. Chronic Neurological Sequelae to Organophosphate Pesticide Poisoning Kyle Steenland, Barbara Jenkins, Richard Ames G, Michael O'Malley, David Chrislip, John Russo, Steenland etal. *Am J Public Health*. 1994;84:5.
65. Davies R, Ahmed G, Freer T. Psychiatric aspects of chronic exposure to organophosphates: diagnosis and management. *Adv Psychiatr Treat*. 2000;6:356–361.
66. Nurulain SM. Different approaches to acute organophosphorus poison treatment. *J Pak Med Assoc*. 2012;62(7):712–717.
67. National Research Council. *Pesticides in the Diets of Infants and Children*. Washington, DC: National Academies Press; 1993.
68. Niacin, Exercise, and SaunaA Simple and Effective Detox Program That Can Significantly Improve Your Health May 04, 2014 Joseph Mercola https://articles.mercola.com/sites/articles/archive/2014/05/04/detoxification-program.aspx. Accessed August 27, 2019.
69. Crinnion WJ. Sauna as a valuable clinical tool for cardiovascular, autoimmune, toxicant- induced and other chronic health problems. *Altern Med Rev*. 2011;16(3):215–225.

26

Oral Manifestations of Nutritional Status

Michael T. Murray, ND

OUTLINE

INTRODUCTION

The structures and lining of the oral cavity offer valuable and easily accessible information on the nutritional status of an individual. Lesions may indicate a nutrient deficiency or may be manifestations of gastrointestinal or other disease.[1] Because of the very rapid cell turnover of the oral mucosa, these lesions often may precede other manifestations of nutrient deficiency or systemic disease. Some typical lesions are mucosal ulceration, cheilosis, gingivitis, and glossitis. The majority of people in the United States are deficient in one or more nutrients, so signs of nutritional deficiency are common.

THE HEALTHY MOUTH

The ventral surface of the healthy tongue is covered with a smooth, pink mucous membrane and lymphoid follicles. On the dorsal surface, the filiform, fungiform, and circumvallate papillae (which contain the organs of taste) produce a rough, grayish-red appearance. The thick epithelial tufts of the filiform papillae give the tongue its characteristic grayish-white coating, whereas the globular, pale red fungiform papillae give it a speckled pink appearance. Furrows are not characteristic of the healthy tongue.

The buccal mucosa has a grayish-red color and may be crossed by fine grayish ridges where it touches the closed teeth.

Healthy gums have a light-reddish appearance and cover the roots of the teeth completely.

The assessment of oral health, obviously, must include the health and status of the teeth. Patients should be asked about their dental history and encouraged to have regular dental checkups. Here are some key nutritional considerations for the examination of the teeth[2]:

- Having fewer than 20 teeth as an adult is associated with a significantly reduced capacity to eat nutritious foods, such as salads, raw fruit and vegetables, nuts, and whole-grain products. The same applies even to denture wearers.
- Bulimia may manifest as erosive tooth wear.
- Osteoporosis may manifest initially as changes in jaw structure and/or loose teeth.
- The presence of mercury amalgams may be associated with systemic disease, especially neurological conditions.

ABNORMALITIES OF THE ORAL MEMBRANES

Table 26.1 summarizes the typical oral manifestations associated with a particular nutrient deficiency, and Table 26.2 summarizes common disorders associated with oral manifestations.[1–12]

In general, ulceration should be considered a nonspecific expression of a disease state. A search for the etiology will usually result in a specific therapy. Aphthous stomatitis is a common example of a mucosal ulceration and is discussed in detail in Chapter 147.

Similarly, cheilosis is a common expression of an acquired nutrient deficiency. Gingivitis is associated with the classic signs of scurvy, but other nutrients have now been shown to play a role in gingival health; this subject is discussed in Chapter 208. Glossitis is associated with numerous vitamin deficiency states, each with a characteristic appearance.

Like glossitis, intraoral burning represents a nonspecific expression of a possible nutrient deficiency or systemic disease.[3–5] Possible causes in addition to those listed in Table 26.2 are as follows: xerostomia; dentures; deficiencies of iron, vitamin B_{12}, folic acid, vitamin B_6, and protein; steatorrhea; antibiotic use; changes in mucosal innervation; and anxiety states.

In people who do not wear dentures, nutritional disorders are the most common causative factors.[2]

Leukoplakia is any white lesion of the oral cavity that cannot be removed by rubbing the mucosal surface. Although lesions are usually only a sign of chronic irritation, 2% to 6% represent either dysplasia or early invasive squamous cell carcinoma.[4]

Erythroplakia is similar to leukoplakia, except that it has a definite erythematous component. This is a far more serious sign, with 90% of such lesions representing dysplasia or carcinoma.

TABLE 26.1 Oral Signs of Nutrient Deficiency

Nutrient	Signs of Oral Deficiency
Vitamins	
Biotin	Geographical tongue, atrophy of lingual papillae
Folic acid	Gingivitis, glossitis with atrophy or hypertrophy of filiform papillae, cheilosis
Niacin	Intraoral burning, canker sores, halitosis, glossitis, tongue swollen with red tip and sides, swollen red fungiform papillae, filiform papillae becoming inflamed and losing their epithelial tufts (giving the characteristic slick red appearance)
Pyridoxine	Intraoral burning, glossitis, mucosal ulcerations and erosions, cheilosis
Vitamin B_{12}	Intraoral burning, mucosal ulcerations and erosions, painful glossitis with a beefy red or fiery appearance, eventually resulting in an atrophic (smooth and shiny) tongue
Riboflavin	Soreness and intraoral burning, cheilosis, angular stomatitis, glossitis with a magenta tongue
Vitamin C	Sore and bleeding gums, deep blue-red color to gums, loose teeth, follicular hyperkeratosis
Vitamin D	Intraoral burning
Vitamin E	Glossitis
Minerals	
Calcium	Periodontal disease, tooth decay
Iron	Cheilosis, atrophic glossitis, gingivitis, candidiasis, intraoral burning or pain, mucosal ulcerations and erosions, pallor
Zinc	Cheilosis, atrophic glossitis, gingivitis, candidiasis, intraoral burning or pain, mucosal ulcerations and erosions, pallor

Data from Pflipsen M, Zenchenko Y. Nutrition for oral health and oral manifestations of poor nutrition and unhealthy habits. *Gen Dent*. 2017;65(6):36–43. Budtz-Jorgensen E, Chung JP, Rapin CH. Nutrition and oral health. *Best Pract Res Clin Gastroenterol*. 2001;15:885–896. Basker RM, Sturdee DW, Davenport JC. Patients with burning mouths. *Br Dent J*. 1978;145:9–16. Maragou P, Ivanyi L. Serum zinc levels in patients with burning mouth syndrome. *Oral Surg Oral Med Oral Pathol*. 1991;71:447–450. Ship JA, Grushka M, Lipton JA, et al. Burning mouth syndrome: an update. *J Am Dental Assoc*. 1995;126:843–853. Werbach MR. *Nutritional Influences on Disease*. Tarzana, CA: Third Line Press; 1993. Shepherd A. The impact of oral health on nutritional status. *Nurs Stand*. 2002;16:37–38. Hornick B. Diet and nutrition: implications for oral health. *J Dent Hyg*. 2002;76:67–78. Enwonwu CO, Sanders C. Nutrition: impact on oral and systemic health. *Compend Contin Educ Dent*. 2001;22:12–18. Rugg-Gunn AJ. Nutrition, diet and oral health. *J R Coll Surg Edinb*. 2001;46:320–328. Mojon P, Budtz-Jorgensen E, Rapin CH. Relationship between oral health and nutrition in very old people. *Age Ageing*. 1999;28:463–468. Walls AW. Oral health and nutrition. *Age Ageing*. 1999;28:419–420.

TABLE 26.2 Common Disorders Associated With Oral Manifestations

Oral Manifestation	Disorder(s)
Cheilosis	Crohn's disease, acrodermatitis enteropathica, alcoholism, celiac disease, malabsorption syndrome
Gingivitis	Crohn's disease, anorexia nervosa, celiac disease, scurvy
Erythroplakia	Dysplasia or carcinoma
Glossitis	Crohn's disease, diabetes, alcoholism, celiac disease, malabsorption syndrome, pernicious anemia, iron-deficiency anemia, amyloidosis, carcinoid syndrome, cigarette smoking, anemia
Intraoral burning	Menopause, diabetes mellitus, esophageal reflux, Sjögren's syndrome
Leukoplakia	Chronic irritation, dysplasia, early invasive squamous-cell carcinoma
Ulcerations, erosions	Crohn's disease, ulcerative colitis, celiac erosions disease, corticosteroid use, acrodermatitis enteropathica, anorexia nervosa, pernicious anemia, iron-deficient anemia, mercury poisoning, nicotine withdrawal

Data from Budtz-Jorgensen E, Chung JP, Rapin CH. Nutrition and oral health. *Best Pract Res Clin Gastroenterol*. 2001;15:885–896. Basker RM, Sturdee DW, Davenport JC. Patients with burning mouths. *Br Dent J*. 1978;145:9–16. Maragou P, Ivanyi L. Serum zinc levels in patients with burning mouth syndrome. *Oral Surg Oral Med Oral Pathol*. 1991;71:447–450. Ship JA, Grushka M, Lipton JA, et al. Burning mouth syndrome: an update. *J Am Dental Assoc*. 1995;126:843–853. Werbach MR. *Nutritional Influences on Disease*. Tarzana, CA: Third Line Press; 1993. Shepherd A. The impact of oral health on nutritional status. *Nurs Stand*. 2002;16:37–38. Hornick B. Diet and nutrition: implications for oral health. *J Dent Hyg*. 2002;76:67–78. Enwonwu CO, Sanders C. Nutrition: impact on oral and systemic health. *Compend Contin Educ Dent*. 2001;22:12–18. Rugg-Gunn AJ. Nutrition, diet and oral health. *J R Coll Surg Edinb*. 2001;46:320–328. Mojon P, Budtz-Jorgensen E, Rapin CH. Relationship between oral health and nutrition in very old people. *Age Ageing*. 1999;28:463–468. Walls AW. Oral health and nutrition. *Age Ageing*. 1999;28:419–420.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Pflipsen M, Zenchenko Y. Nutrition for oral health and oral manifestations of poor nutrition and unhealthy habits. *Gen Dent*. 2017;65(6):36–43.
2. Budtz-Jorgensen E, Chung JP, Rapin CH. Nutrition and oral health. *Best Pract Res Clin Gastroenterol*. 2001;15:885–896.
3. Basker RM, Sturdee DW, Davenport JC. Patients with burning mouths. *Br Dent J*. 1978;145:9–16.
4. Maragou P, Ivanyi L. Serum zinc levels in patients with burning mouth syndrome. *Oral Surg Oral Med Oral Pathol*. 1991;71:447–450.
5. Ship JA, Grushka M, Lipton JA, et al. Burning mouth syndrome: an update. *J Am Dental Assoc*. 1995;126:843–853.
6. Werbach MR. *Nutritional Influences on Disease*. Tarzana, CA: Third Line Press; 1993.
7. Shepherd A. The impact of oral health on nutritional status. *Nurs Stand*. 2002;16:37–38.
8. Hornick B. Diet and nutrition: implications for oral health. *J Dent Hyg*. 2002;76:67–78.
9. Enwonwu CO, Sanders C. Nutrition: impact on oral and systemic health. *Compend Contin Educ Dent*. 2001;22:12–18.
10. Rugg-Gunn AJ. Nutrition, diet and oral health. *J R Coll Surg Edinb*. 2001;46:320–328.
11. Mojon P, Budtz-Jorgensen E, Rapin CH. Relationship between oral health and nutrition in very old people. *Age Ageing*. 1999;28:463–468.
12. Walls AW. Oral health and nutrition. *Age Ageing*. 1999;28:419–420.

Rapid Dark Adaptation Test

Dirk W. Powell, BS, ND

OUTLINE

INTRODUCTION

The earliest sign of functional vitamin A deficiency is a decrease in dark adaptation, or poor night vision. Serum retinol levels are not predictive of subclinical states. However, classic dark adaptation testing is a cumbersome and time-consuming process (usually taking 45 minutes or longer).

A rapid test (6 minutes) was described by Thornton[1] and evaluated by Vinton and Russell.[2] This rapid dark adaptation test (RDAT) has significant clinical utility. The basis for the test is the measurement of the time of the so-called Purkinje shift. This term refers to the shifting of peak retinal wavelength sensitivity from the red toward the blue end of the visual spectrum during the transition from day (cone-mediated) vision to night (rod-mediated) vision. When color vision is nonfunctional, this shift causes the intensity, not the color, of blue to appear brighter than red under dim lighting.

CLINICAL APPLICATION

The RDAT is a clinical screening tool appropriate for use in adults and in children and could complement biochemical determination of vitamin A and/or zinc in clinical settings.[3] Although various dark adaptation tests have the advantage of being an in vivo test, and therefore directly relevant to function, they are somewhat less specific. Table 27.1 lists conditions that may give abnormal dark adaptation results despite normal serum levels of vitamin A.

PROCEDURE

Method

1. The procedure is explained to the subject.
2. The subject's vision is light-adapted by fixation on a standard x-ray viewing box for 1 minute at a distance of 0.5 meters. The x-ray viewing box is then turned off (the darkroom light remains on).
3. The subject is given all 18 disks mixed in random order, and a stopwatch is started.
4. The subject separates the white and then the blue disks as fast as possible. Under these controlled lighting conditions, the subject will not be able to recognize the colors, because the cones cannot distinguish color with the limited light available. The ability to separate the disks by brightness therefore depends on the rods. Any disk mistakenly separated by the subject is returned to the original pile until 100% accuracy of sorting is achieved, at which point the stopwatch is stopped and the time is recorded as the result.
5. The first test performed by a subject should be redone to allow for learning and standardization.

Required Equipment

- Lightproof room
- A standard darkroom light fixture fitted with a 7.5-W bulb and a neutral-density filter (allowing 1% transmittance). The bottom of the fixture is suspended 1.2 m above the work surface so that the target brightness on the work area is approximately 0.0068 candela/m^2.
- Munsell color disks with matte finish: five white disks (N9.5/-), six blue disks (5PB5/10), and seven red disks (5R5/10) (available from Munsell Color and Macbeth Division, Baltimore, MD)
- A nonreflective work surface
- A stopwatch
- A standard x-ray viewing box

TABLE 27.1 Conditions CAUSING Abnormal RDAT Results in Subjects With Normal Levels of Vitamin A

- Zinc deficiency
- Cataract
- Retinitis pigmentosa
- Diabetic retinopathy
- Macular degeneration[4,5]
- Severe errors of refraction
- Miosis caused by pharmaceutical agents
- Tinted corrective lenses

RESULTS

Normals[2]:

- 20 to 39 years old: 3.03 ± 1.00 minutes
- 40 to 60 years old: 4.41 ± 0.83 minutes

Vitamin A deficient:

- 7.63 ± 1.79 minutes

INTERPRETATION

The RDAT resultant time depends largely on the individual setting. However, the difference in the RDAT time between normal and vitamin A–deficient individuals is significant, and therefore standardization is easily achieved. The normal values depend on the age of the subject, with older subjects having an increased RDAT time.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Thornton SP. A rapid test for dark adaptation. *Ann Ophthalmol*. 1977;9:731–734.
2. Vinton NE, Russell RM. Evaluation of a rapid test of dark adaptation. *Am J Clin Nutr*. 1981;34:1961–1966.
3. Solomons NW, Russell RM, Vinton E, Guerrero AM, Mejia L. Application of a rapid dark adaptation test in children. *J Pediatr Gastroenterol Nutr*. 1982;1(4):571–574. PMID:7186069.
4. Jackson GR, Edwards JG. A short-duration dark adaptation protocol for assessment of age-related maculopathy. *J Ocul Biol Dis Infor*. 2008;1(1):7–11. PMID: 20072631.
5. Jackson GR, Scott IU, Kim IK, Quillen DA, Iannaccone A, Edwards JG. Diagnostic sensitivity and specificity of dark adaptometry for detection of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2014;10(3):1427–1431. PMID: 24550363.

28

Biomarkers for Stool Analysis

Joseph Katzinger, ND

OUTLINE

INTRODUCTION

Gastrointestinal (GI) dysfunction is a remarkably common experience in the United States.[1] The popular media are saturated with advertisements promising to soothe a wide variety of complaints, including (but by no means limited to) constipation, indigestion, reflux, bloating, and cramping. Those seeking relief beyond that offered on their local pharmacy shelves are substantial in number: GI-related complaints account for a significant percentage of visits made to primary care providers' offices.[2] As a group, many GI diseases carry significant morbidity and mortality—and many are currently poorly treated or in some cases completely untreated.[3] Yet the burden of GI diseases goes beyond individual physical costs: one survey estimated that over $40 billion is dedicated to addressing GI-related issues annually.[4] This is likely to be an underestimate, however, as a 2015 analysis estimated the hospitalization cost alone due to just GI hemorrhages in the United States at $5 billion annually.[5] Given the substantive toll that GI-related illnesses place on patients and the healthcare system—and given that current treatment methods to address them may be less than effective—it must be asked: What options do clinicians have to fully support their patients?[6]

Laboratory diagnostics that assess the stool for insight into the state of health of the gut have existed for decades, yet the evolution of technology is such that more sensitive and specific tools now exist for easy clinical application. Biomarkers for stool analysis measure key physiological aspects of the gut: digestion/absorption (D), immunology/inflammation/infectious processes (I), and gut microflora balance (G)—or DIG. This physiological assessment of DIG mirrors functional status and provides a critical, noninvasive way to evaluate and clinically approach the patient with GI complaints (Fig. 28.1). Assessed together, stool analysis biomarkers provide an effective means of capturing underlying physiological disruption that manifests as disease—eliminating the need for costly diagnostic testing where unnecessary; highlighting those individuals specifically at risk for organic disease who require immediate specialty referral; and identifying the source of gut dysfunction to apply simple, cost-effective treatments.

OVERVIEW OF THE GASTROINTESTINAL TRACT

A popular adage expounds, "You are what you eat." Over the course of a lifetime, it is estimated that a well-nourished adult will consume between 25 and 50 tons of food.[7] Yet given that these macronutrients—fats, carbohydrates, and proteins—must ultimately be broken down, taken up, and assimilated, perhaps the more appropriate maxim is this: "You are what you digest and absorb."

Once taken in, dietary foodstuffs undergo a series of complex digestive processes in this tube within a tube. The human gut has a massive absorptive surface area to capture macronutrients and micronutrients critical to optimal physiological function, yet the impacts of its processes are increasingly understood to go beyond traditionally defined roles of digestion and absorption. The mucosal surface of the GI tract is the primary interface with our external environment, and the interaction of gut commensal microflora with this mucosal surface is critical for differentiating self from nonself—a phenomenon known as "oral tolerance." In addition, more than 70% of the body's immune tissue sits within the gut and is both closely interconnected to—and continuously interacting with—the largest concentration of nervous tissue outside of the central nervous system (CNS). These immunomodulatory and neuroendocrine functions give credence to clinical concern that gut dysfunction may be a root cause not only at the level

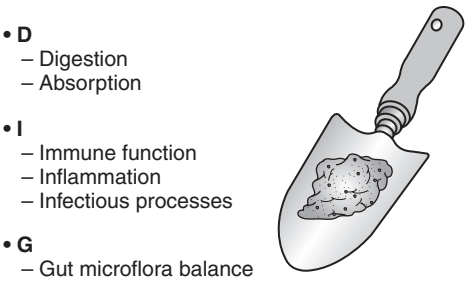


Fig. 28.1 Key physiological and functional processes of the gastrointestinal tract: DIG.

of local digestive issues like constipation, gastroesophageal reflux disease, and irritable bowel syndrome (IBS) but also for many chronic, systemic illnesses like inflammatory bowel disease (IBD), Hashimoto thyroiditis, systemic lupus erythematosus (SLE), rheumatoid arthritis, and migraine.

The following sections provide a broad-based overview of digestive physiology, followed by an in-depth look at key aspects of the gut utilizing the DIG framework—digestion/absorption (D), immunology/inflammation/infectious processes (I), and gut microflora balance (G)—and the biomarkers that provide insight into their function and/or dysfunction.

DIGESTIVE PHYSIOLOGY 101

Brain

As anyone watching a late-night food commercial may attest to, the process of digestion begins long before any food enters the mouth or stomach. The cephalic phase of digestion begins when anticipatory, visual, gustatory, and/or olfactory stimuli activate central nervous system (CNS) centers and result in parasympathetic stimulation of gastric activity.[8]

Mouth

Homogenization of ingested food first occurs in the mouth via the grinding and mixing action of the teeth. Salivary glands provide hydration to form a bolus and protect the pharyngeal and esophageal mucosa, primarily with secretory immunoglobulin-A (IgA) antibodies. The process of starch and fat hydrolysis is initiated with the release of lingual lipase, salivary amylase, and ptyalin.[9,10]

Esophagus

As the food bolus is swallowed, the epiglottis closes, providing a one-way valve that allows the food to pass into the GI tract and not the respiratory tract. Food is then transported down the esophagus in peristaltic waves past the lower esophageal sphincter and into the stomach.

Stomach

The stomach produces churning action and initiates protein and lipid hydrolysis. Peptides, amino acids, and fatty acids released in this process synchronize the release of pancreatic juice and bile into the small intestine.[9] About 2 L of gastric juice is produced each day, containing several important components.[11] Hydrochloric acid (secreted by the parietal cells) activates pepsinogen to convert to pepsin, which renders some minerals (e.g., calcium and iron) more absorbable. It also creates an essentially sterile environment to prevent bacterial overgrowth. Mucus forms an acid- and pepsin-resistant coating for the stomach lining. Gastric lipase secreted by gastric mucosa begins to hydrolyze triglycerols, producing 1,2-diacylglycerols and fatty acids. The optimal pH of gastric lipase is about 4, but the enzyme is active up to pH 6 or 6.5.[12] Several GI hormones, including gastrin, somatostatin, and ghrelin, are also produced by cells within the stomach.[13]

Liver and Biliary System

The liver is the largest gland in the body and performs an extraordinary number of tasks that affect every system of the body. Although the liver has critical vascular and metabolic functions, its secretory and excretory functions—especially as related to the synthesis and secretion of bile acids—are those that most directly affect digestion.

Because the by-products of lipid digestion have limited ability to dissolve in water, they have difficulty reaching the epithelial surface of the small intestine, which is covered by the unstirred water layer. To be more effectively absorbed, they must form aggregates (micelles) with bile acids (which act as biological detergents) that can penetrate the unstirred water layer. Without bile acids, only a small number of lipid molecules permeate through the water layer to be taken up by the brush-border membrane of the enterocytes. Finally, fat-soluble antioxidants (vitamins A, D, E, and K, as well as lutein and lycopene) also require bile acids for optimal absorption and utilization.

Pancreas

The pancreas plays a vital role in supporting the ongoing breakdown of the semifluid mass of partially digested food that flows from the stomach to the small intestine (chyme). Pancreatic duct cells secrete bicarbonate, which is critical for rapidly neutralizing gastric acid to preclude damage to the duodenal mucosa. In addition, pancreatic exocrine function results in the secretion of proteases, amylases, and lipases that further break down macronutrients (proteins, carbohydrates, and lipids) so that their components are readily absorbed across the intestinal mucosa.

Primary pancreatic exocrine insufficiency results from organic diseases of the pancreas (e.g., chronic pancreatitis, cystic fibrosis, severe acute necrotizing pancreatitis, pancreatic cancer). Secondary pancreatic exocrine insufficiency results from extrapancreatic diseases such as celiac disease and Crohn's disease, GI and pancreatic surgical resection, as well as any condition that results in a compromise or blunting of the small intestinal brush border. These include inflammatory processes, infectious agents, food allergies, and dysbiosis and/or small bowel bacterial overgrowth. Pancreatic exocrine insufficiency has now been recognized to result from both type 1 diabetes (26%–57%) and type 2 diabetes (20%–36%).[14] Whether of primary or secondary etiology, any compromise of pancreatic exocrine function can result in significant malnutrition-related morbidity and mortality.[15]

Small Intestine

Most digestion and assimilation occur in the small intestine. Digestion is mediated chiefly by pancreatic enzymes, including proteolytic enzymes, nucleolytic enzymes, lipases, α-amylase, and phospholipase A_2, which hydrolyze macromolecules to oligomers, dimers, or monomers.[9] Pancreatic enzymes are most active in the neutral pH range,[11,16] and bicarbonate begins the process of neutralizing stomach acid. Pancreatic proteases are secreted into the duodenum as inactive precursors, trypsinogen, chymotrypsinogen, and proelastase, and are activated by brush-border enzymes, such as enterokinase, to form trypsin, chymotrypsin, and elastase, respectively.[11]

The digestive and absorption capacities of the small intestine are greatly enhanced by circular folds and fingerlike projections of the intestinal mucosa called microvilli. This region, dubbed the "brush border," increases the luminal surface area of the intestinal wall by about 600 times, creating a total absorptive surface of approximately 200 to 250 m,[2] essentially the size of a tennis court.[11,16,17]

Even after prolonged contact with pancreatic enzymes, a substantial portion of ingested carbohydrates and amino acids depend on hydrolytic enzymes (disaccharidases such as maltase, sucrase, lactase, enterokinase, and peptidases) produced by the brush-border membrane to complete their digestion.[11]

For this reason, damage to the intestinal brush border—resulting from inflammation, trauma, parasitical infection, bacterial overgrowth, long-term nonsteroidal anti-inflammatory drug use, and other factors—may interfere significantly with the proper absorption of nutrients. Such damage also can disrupt the intercellular tight junctions that are vital to the protective-barrier function of the gut mucosal layer, increasing the intestinal permeability of potentially harmful macromolecules, such as bacteria and toxins, into the systemic circulation. This state of increased intestinal permeability, sometimes referred to as "leaky gut," has been cited as a potential contributory or causative factor in gluten enteropathy, Crohn's disease, food allergy, various arthritides and autoimmune diseases, chemotherapy complications, and a variety of other clinical conditions.[18–22]

Recent work has implicated zonulin, the human analog to an endotoxin produced by *Vibrio cholera,* as the master regulator of intercellular tight junctions. The two known triggers of zonulin release, which in turn lead to tight junction disassembly, are bacterial toxins and gliadin.[23]

Large Intestine

A primary role of the large intestine is to absorb water, about 1 L/day. The large intestine also provides an environment for microbial fermentation of soluble fiber, starch, and undigested carbohydrates. Soluble fibers are more readily fermented than insoluble fibers.[24]

Nondigestible carbohydrates (e.g., fiber), polysaccharides, and oligosaccharides contain chemical bonds that make them incapable of complete digestion by pancreatic or brush-border enzymes. As they move through the digestive tract, these undigested compounds play an important role in intestinal transport mechanisms. Once in the colon, they are cleaved and modified by enzymes produced by resident colonic bacteria to form short-chain fatty acids (SCFAs) and various gases, such as methane, hydrogen, and carbon dioxide.[12]

ASSESSING GASTROINTESTINAL HEALTH: DIG

As noted previously, a clinical approach to evaluating the functional state of gut health can be one that is based on the primary physiological roles of the gut. This approach is captured by the mnemonic DIG: digestion/absorption (D), immunology/inflammation/infectious processes (I), and gut microflora balance (G).

DIGESTION/ABSORPTION

Digestive Dysfunction

The first step in optimizing GI health is optimizing digestive function. Because numerous organs are involved in the digestive process, maldigestion can occur with malfunction at any level of digestive physiology, resulting in both local and systemic signs and symptoms. Maldigestion may result from an inadequate cephalic phase, leading to inadequate secretion of salivary amylases and proteases; insufficient mastication; hypochlorhydria; pancreatic insufficiency; bile insufficiency; small intestinal villous atrophy; and brush-border enzyme destruction.

As an example, hypochlorhydria has been hypothesized to be the root cause of a number of clinical conditions, including reduced mineral and amino acid absorption, depression, and other syndromes.[25,26]

In addition, interest has been expressed in the medical literature about the connection between the robust use of pharmaceuticals promoting gastric acid blockade and chronic clinical complaints. Research indicates that small bowel bacterial overgrowth and associated disorders, such as blunting of brush-border enzyme activity and decreased pancreatic exocrine function, may be an iatrogenic consequence of overprescribing of proton-pump inhibitors.[27–29] Given that long-term use of drugs that markedly reduce gastric acid secretion may negatively affect dietary and nutraceutical calcium absorption, concern has also been raised about their potential to increase the risk of fracture in individuals with osteoporosis, although the greatest potential for this may be in patients already at risk for loss of bone mineral density.[30–33]

A 2019 meta-analysis, which included 32 studies and over 2 million individuals, found an increase in risk for any-site, hip, and spine fractures associated with the use of proton-pump inhibitors, which was strengthened by the duration of use, suggesting causality. Importantly, this increase in fracture risk was not associated with a decrease in bone mineral density.[34]

Maldigestion associated with pancreatic exocrine insufficiency results in inadequate delivery of enzymes to the small intestine and can lead to the inadequate breakdown of fats, carbohydrates, or protein. The net effect is poor nutrition and an unhealthy environment for the flora of the large intestine. Significant decreases in exocrine pancreatic secretion have been associated with the aging process[35] and, clinically, are associated with osteoporosis[36,37] and diabetes.[38–40]

Absorptive Dysfunction

Malabsorption can be characterized by abnormal fecal excretion of fat (steatorrhea); abdominal pain; and variable malabsorption of fats, fat-soluble vitamins, other vitamins, proteins, carbohydrates, minerals, and water. Common causes include the following:

- Maldigestion
- Hypochlorhydria
- Small bowel bacterial overgrowth
- Deficient bile production, resulting in inadequate solubility of fatty acids[12]
- Chronic inflammation of the small intestine
- Rapid transit, which does not allow adequate time for absorption

Malabsorption often increases with age[41]: atrophic gastritis is estimated to affect 10% to 30% of individuals more than 50 years old and often leads to inadequate absorption of key nutrients, such as vitamin B_{12}.[42] To date, deficiencies in vitamin B_{12}, vitamin C, vitamin D, magnesium, calcium, and iron have all been associated with hypochlorhydria.[43,44]

General malabsorption syndromes are associated with many disorders of the intestinal tract, including celiac disease, gluten enteropathy, IBD, infectious processes such as giardiasis and cryptosporidiosis, lactose intolerance, and eosinophilic gastroenteritis. Because amino acids, carbohydrates, vitamins, and minerals are absorbed through different processes, individuals with malabsorption are at risk for the development of a wide range of nutrient deficiencies.

Biomarkers for Digestion/Absorption

Pancreatic Elastase 1

Pancreatic elastase 1 (PE1) is a proteolytic enzyme secreted exclusively by the human pancreas, and as such, it reflects overall pancreatic exocrine function.[45] It is an extremely stable, reliable, and specific marker[46] and correlates well with gold-standard, stimulated pancreatic function tests.[47] PE1 is concentrated fivefold to sixfold higher in feces than when it enters the duodenum, reflecting the overall stability of this biomarker in the GI tract.[48] It is not degraded during intestinal transit and is generally not affected by increases or decreases in intestinal transit time,[49] although its sensitivity may be diminished in cases of liquid diarrhea.[50] Unlike other pancreatic markers, such as chymotrypsin,

PE1 results are not affected by pancreatic enzyme replacement therapy,[46,47] a feature that makes it a valuable marker for monitoring and adjusting exogenous enzyme replacement.[46]

PE1 appears to have less sensitivity when diagnosing mild exocrine pancreatic insufficiency and may have limited use in differentiating pancreatic from nonpancreatic steatorrhea or diarrhea.[51] Previous analyses suggest a sensitivity of 54% to 65%, 75%, and 95% to 100% for mild, moderate, and severe insufficiency, respectively, with an overall specificity of 79%. It should be noted that other indirect assessments of pancreatic function, such as the 72-hour fecal fat test, have very poor sensitivity for mild/moderate insufficiency.[52]

Although research typically indicates levels of PE1 less than 200 mcg/g to be consistent with normal pancreatic exocrine function, healthy individuals produce on average 500 mcg/g of PE1. PE1 levels greater than 200 mcg/g and less than 500 mcg/g suggest a deviation from optimal pancreatic function, values between 100 and 200 mcg/g suggest moderate pancreatic insufficiency, and values less than 100 mcg/g are considered to be consistent with severe pancreatic insufficiency.[53,54] Enzymatic support of pancreatic insufficiency is warranted, and the strength of supplementation—typically based on the level of lipase units—should be modulated based on the degree of pancreatic insufficiency indicated by the PE1 level (Table 28.1).

Reduced PE1 has been reported clinically in patients with type 1 and type 2 diabetes,[55,56] chronic pancreatitis,[57,58] osteoporosis,[37] cystic fibrosis,[59] trauma states,[55] and intestinal malabsorption states associated with mucosal atrophy such as celiac disease.[60,61]

Putrefactive Short-Chain Fatty Acids

Valerate, isovalerate, and isobutyrate are produced exclusively by bacterial fermentation of proteinaceous material (polypeptides and amino acids). These SCFAs are putrefactive, and their presence suggests underlying maldigestion and/or malabsorption from dysfunctional states, such as hypochlorhydria or exocrine pancreatic insufficiency,[62] or bacterial overgrowth in the small intestine. Other causes are GI disease (resulting from the fermentation of blood or mucosal cells delivered to the colon)[63] and rapid transit time (resulting from inadequate time for digestion and absorption of peptides and amino acids).[64]

Increased levels of SCFAs, including acetate, propionate, butyrate, and valerate, have all been associated with increased adiposity and obesity, although decreased SCFA levels have been associated with colorectal cancer.[65,66] A 2019 systematic review and meta-analysis found that levels among those with IBS differed from healthy controls, but they were not consistently elevated. Those with constipation-predominant IBS had lower butyrate and propionate levels, whereas those with diarrhea-predominant IBS had elevated levels of butyrate only.[67]

Fecal Fats

Fecal fats include triglycerides, long-chain fatty acids, cholesterol, and phospholipids, and are derived primarily from the dietary ingestion of fat. Elevated levels of fecal fats in the stool suggest fat malabsorption (steatorrhea), which can occur as a result of maldigestion and/or impaired uptake of fatty acids. In addition, any condition that results in impairment of lipase activity and bile acid production and release may result in fat malabsorption. These include pancreatic insufficiency, cholestasis, short bowel syndrome, and celiac disease.[68]

TABLE 28.1 Pancreatic Elastase 1 Levels, Degree of Pancreatic Insufficiency, and Enzyme Supplementation

>500 mcg/g	Optimal
351–500 mcg/g	Normal, but not optimal
201–350 mcg/g	Mild insufficiency
101–200 mcg/g	Moderate insufficiency
<100 mcg/g	Severe insufficiency

IMMUNOLOGY/INFLAMMATION/INFECTIOUS PROCESSES

As noted previously, more than 70% of the body's immune tissue sits within the GI tract.[69] This immune tissue has a complex and dynamic relationship with the commensal microflora that colonizes its surface—the primary interface with an external environment rich in both supportive dietary antigens as well as pathogenic agents. The gut commensal microflora exists in this macronutrient-dense intestinal environment and is a significant contributor to the process of digestion, building of essential nutrients, and holding nonbeneficial bacterial counts in check. How is it, then, that host immune function is modulated to allow for the acceptance of self (as well as supportive commensal organisms and dietary antigens), even as it is vigilantly surveying for and combating against pathogenic microorganisms?

The answer appears to lie with an exquisitely balanced, ongoing inflammatory response by the intestinal immune system: it is capable of mounting strong inflammatory responses against pathogenic agents while concomitantly providing inhibitory signals that preclude responses against commensal bacteria.[70,71] Any alteration of this equilibrium, for example, as a result of gut microflora imbalance, genetic predisposition, or immune dysregulation, will result in an upregulation of the gut inflammatory response. Diagnostically, this may be reflected by the release of the biomarker calprotectin from neutrophils.[72] Clinically, the breakdown of this intestinal immune balance has been implicated in the development of diseases marked by significant inflammation, such as IBD,[73–75] and has been postulated to play a role in the development of extraintestinal allergic and autoimmune disorders.[76] For example, dysbiosis appears to be a hallmark of rheumatoid arthritis, and the effectiveness of recently developed biopharmaceuticals, such as etanercept, may in part be due to a shift in the microbiota composition.[77] Alterations in microbiota populations have also been reported for conditions not recognized as having an autoimmune component, for example, hypertension, and atherosclerotic cardiovascular disease has been associated with microbial shifts.[78,79] Similarly, changes to gut microbiota populations may drive a variety of pathways that contribute to diabetes and obesity (Fig. 28.2). Microflora imbalance has also been reported in patients with IBS.[80]

Biomarkers for Immune Function, Inflammation, and Infectious Processes

Eosinophil Protein X

Eosinophil protein X (EPX), also known as eosinophil-derived neurotoxin (EDN), is a basic cellular protein with potent cytotoxic and neurotoxic properties and is a marker of eosinophil activation and degranulation. Mobilization of eosinophils in the gut appears to occur in response to immune-mediated inflammatory responses.[81,82]

Clinically, fecal elevations of EPX have been described in IBD, IgE-mediated food allergy, parasitical infections, and collagenous colitis.[83–93] For example, in a small study, fecal EPX levels were shown to out-predict both skin-prick tests and atopy patch tests for diagnosing cow's milk allergy in toddlers.[94]

Calprotectin

Calprotectin belongs to a group of calcium-binding neutrophil-derived proteins. Because neutrophils are typically mobilized in response to increased mucosal permeability, cell or tissue damage, and infectious processes, elevations in calprotectin may be seen in the presence of inflammatory, neoplastic, and/or infectious disease processes.[95,96]

Clinical application of this biomarker has been directed toward discerning the best approach for the patient with functional abdominal symptomology. Specifically, calprotectin has been evaluated (in conjunction with Rome criteria) as a diagnostic biomarker to distinguish between IBS and IBD.[97–99] Surveys indicated that up to 12% of primary care visits comprised IBS-related complaints, and such visits accounted for 28% of referrals to gastroenterologists.[100,101] Because symptoms often overlap, early IBD may be inappropriately labeled as IBS. At least one study reported that more than 25% of patients with Crohn's disease had been misdiagnosed as having IBS in the prodromal stages of their disease.[102] Attempts to define organic disease in patients with IBS inevitably results in the application of more invasive diagnostic imaging procedures and escalated healthcare costs.[6]

Calprotectin has been found to correlate closely with IBD activity[103] and provides a highly sensitive, specific, and noninvasive alternative for assessing inflammatory activity in IBD.[99,104] Fecal calprotectin levels have been shown to correlate significantly with histological and endoscopic assessments of disease activity in ulcerative colitis[105] and with indium-111–labeled white blood cells (the gold standard for assessing gut inflammation) in patients with Crohn's disease.[106] In one report, more than 95% of patients with IBD had elevated fecal calprotectin levels.[107]

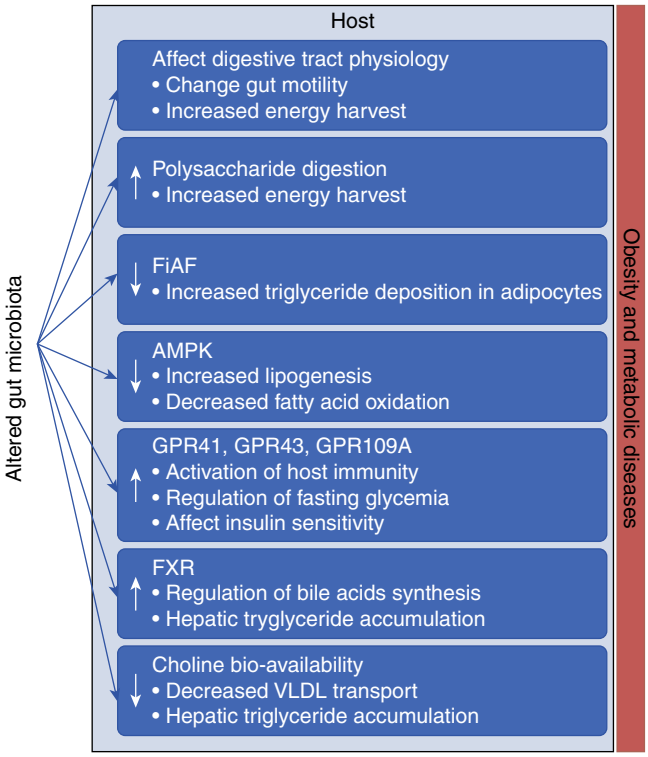


Fig. 28.2 Metabolic and immune interactions between gut microbes and the host in obesity and the metabolic syndrome. (From Boulangé CL, Neves AL, Chilloux J, et al. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med.* 2016 Apr 20;8[1]:42. PMID: 27098727. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

Calprotectin has been used to predict relapse in patients with established IBD and to evaluate the effectiveness of therapeutic regimens as well.[83,108,109] In 2017 a systematic review was published in *Inflammatory Bowel Diseases,* which found that two consecutively elevated calprotectin values were associated with 53% to 83% likelihood of relapse within the next 2 to 3 months among asymptomatic patients with IBD.[110]

Because the severity of inflammation is indicated by varying levels of calprotectin, the treatment options and urgency of diagnostic intervention vary as well (Table 28.2).

Infectious Agents

Helicobacter pylori. *Helicobacter pylori* remains a prevalent worldwide infection, with infection rates in middle-aged adults reaching more than 80% in areas of low socioeconomic status and 20% to 50% in most industrialized countries.[111] *H. pylori* continues to be a causal link for the development of peptic ulcer disease (an estimated 10% to 20% lifetime risk in infected individuals),[112] as well as a variety of other GI concerns ranging in severity from dyspepsia to gastric malignancy.[113,114]

Several diagnostic options exist in assessing possible *H. pylori* infection. Although histological confirmation remains the gold standard, noninvasive measures such as urea breath testing, *H. pylori* stool antigen (HpSA) testing, and *H. pylori* polymerase chain reaction (PCR) are currently available. A 2018 Cochrane review found that at a 90% specificity, HpSA testing has a sensitivity of 83%, less accurate than urea breath testing for the diagnosis of infection.[115]

HpSA diagnostics have been approved by the US Food and Drug Administration for the detection of *H. pylori* pre- and posteradication therapy. This biomarker indicates the presence of *H. pylori* antigens shed directly into the stool and provides increased ease of clinical use as well as diagnostic performance characteristics comparable to urea breath testing in both adult and pediatric populations.[116,117] It has been shown to accurately indicate treatment failure at 7 days after completion of eradication therapy (compared with 4 weeks for urea breath testing),[117–119] an important consideration for directing additional clinical intervention in patients who remain symptomatic or develop recurrent symptoms posttherapy.

Although PCR testing for *H. pylori* is currently offered as a noninvasive diagnostic alternative to endoscopy and biopsy as well, concerns have been raised about the high degree of false-positive results with this methodology.[120,121]

Clostridium difficile. *Clostridium difficile* is an anaerobic, spore-forming, gram-positive bacterium that can be part of the normal intestinal flora. Although typically considered an opportunistic

TABLE 28.2 Calprotectin Levels, Inflammation Severity, and Clinical Intervention

<50 mcg/g	No inflammation
50–120 mcg/g	Mild inflammation
• Evaluate for dysbiosis, allergies, and infection.	
120–250 mcg/g	Moderate inflammation
• Evaluate aggressively for source of inflammation.	
>250 mcg/g	Severe inflammation
• Refer for diagnostic imaging (i.e., colonoscopy).	
• Begin treatment in patients with inflammatory bowel disease (IBD). Therapy should persist until biomarker indicates that inflammation has resolved and mucosal healing has occurred.	

infection occurring after antibiotic therapy, the epidemiology of *C. difficile* infection has shifted significantly. Its incidence has increased dramatically worldwide in the past decade, and new at-risk populations include individuals with community-acquired infection and no previous exposure to antibiotics, children, and pregnant women.[122]

C. difficile produces two toxins, A and B, which are its main virulence factors, and human infection can take many forms. These range from asymptomatic carrier states[123] and recurrent mild-to-moderate IBS-like manifestations to severe symptoms indistinguishable from colitis (cramping, diarrhea, urgency, mucus, and blood) that may result in a misdiagnosis of IBD.[124]

Parasitical Agents

Research suggests that parasitical organisms may also act as etiological agents of gut inflammation and dysfunction, including abdominal pain, diarrhea, and constipation.[125] One study found *Blastocystis hominis* to be present four times more frequently in patients with IBS compared with control subjects. In addition, symptoms resolved in 80% of subjects with IBS when *B. hominis* was treated.[126]

Other parasitical agents identified by stool analysis that have been isolated from patients with clinical symptomology include *Dientamoeba fragilis, Entamoeba* spp., *Endolimax nana,* and *Giardia lamblia.*[127–129] A review by an American diagnostic laboratory routinely surveying stool for parasitical agents found that 23.5% of nearly 14,000 clinical samples meeting *International Classification of Diseases,* 9th edition, criteria for IBS tested positive for the presence of at least one parasite.[130] *B. hominis* was the most commonly identified organism (12.5% of positive samples), followed by *D. fragilis* (3.8%), *Entamoeba* spp. (3.4%), *E. nana* (2.2%), and *G. lamblia* (0.7%). Similar results have been reported by laboratories outside of the United States as well.[131]

In 2017 a systematic review of 45 studies found that following infectious enteritis, more than 10% of individuals develop IBS, a more than fourfold increase in risk compared with those without infectious enteritis.[132]

GUT MICROFLORA BALANCE

At birth, the human GI tract is sterile. Bacterial colonization begins within days of delivery and evolves continuously over the first 1 to 2 years of life.[133] Early gut microflora development is affected by a variety of factors: location and type of birthing, breastfeeding versus formula-feeding, and antibiotic use in infancy and childhood.[134–137] *Bifidobacteria* are typically the first organisms to populate the newborn gut, and research suggests that any event that results in inadequate gut colonization during early life may lead to an increased risk of infectious, allergic, and autoimmune disorders later in life.[138] Once established, the bacterial composition of the gut tends to remain relatively constant over the lifetime of an adult,[139] although environmental (pharmaceuticals, toxins, infectious agents), lifestyle (stress), and dietary (glycemic load, fiber content, essential fatty acid composition, macronutrient/micronutrient composition, and pH balance) factors may induce shifts in commensal flora balance.[140,141]

Bacteria are present at every level of the GI tract and can be found in increasing density as one progresses from the upper gut (esophagus and stomach, 10^3 to 10^5 organisms/mL luminal content) to the lower gut (large intestine, 10^{10} to 10^{12} organisms/mL luminal content; feces, 10^9 to 10^{11} organisms/mL luminal content). In addition, the human gut hosts a massive diversity of microflora species—estimates range from 2000 to 35,000 bacterial species in the human gut; because many of these species are not cultivatable, it is difficult to derive a definitive estimate.[142,143] A recent project, the Metagenomics of the Human Intestinal Tract (MetaHit), cataloged nearly 10 million genes in the human gut microbiome.[144] Anaerobic species comprise the vast majority of the microbiome in healthy individuals; these include obligate anaerobes such as *Bacteroides, Bifidobacterium, Lactobacillus,* and *Clostridium,* as well as facultative anaerobes such as *Escherichia coli, Citrobacter, Enterobacter, Proteus,* and *Klebsiella.*[145] At counts of greater than 100 trillion bacteria colonizing the GI tract, these nonhuman cells vastly outnumber the total number of human cells in the body. With a metabolic capacity greater than the liver—and accounting for roughly 3 lb of body mass—this dynamic population of organisms could be considered a hidden organ in its own right.[146]

As noted previously, the bacteria that inhabit the human GI tract play a significant role in metabolic processes in the body. These include fermentation, SCFA and undigested dietary fiber metabolism, primary bile acid deconjugation, vitamin synthesis, and energy production. In addition, they are key immunomodulators, exert trophic effects on intestinal epithelia, and are a critical defense against pathogens—not only by competing for nutrients, space, and adherence intraluminally but also by producing bactericidal substances.[147] For this reason, alterations in gut microbial balance (dysbiosis) may result in health-related consequences at both the intestinal and extraintestinal levels.[80,148–150]

In dysbiosis, organisms of typically low intrinsic virulence, including bacteria, yeasts, and protozoa, may grow or migrate beyond their normal limitations. This can result in intensified microbial competition for nutrients, damage to the gut mucosal layer, and alterations of host immune function[151] and, as a consequence, may increase the risk for nutritional deficiencies, inflammation, autoimmune disorders, and neoplasia.[147,152–156]

Given the potential for disease that results from altered host–flora dynamics, restoration of balance as a means of restoring health has been a fundamental tenet of natural medicine and an increasing area of research in traditional medical spheres. However, the question should be asked: What defines optimal gut ecology?

Analysis of stool samples has been historically used to provide insight into the microbial composition of the gut. Specifically, levels of growth on plated culture media can be quantified and reflect the density of bacteria in the distal colon. Studies comparing electron microscopical evaluation of the microflora with fecal culture suggest that 50% to 80% of the total microscopic composition is recovered with stool culture methodologies. Limitations presented with the utilization of culture techniques include selectivity of growth based on media used and challenges posed by recovering strict anaerobes in a standard culture environment. Despite these limitations, the use of molecular probes suggests that there is good agreement on the degree of biodiversity when fecal culture is compared with 16S rDNA sequence analysis. In addition, biomarkers that reflect the metabolic activity of the GI microflora provide insight into the optimal functioning of the microbiome.[157]

With the advent of the Human Microbiome Project, increased focus has been placed on the use of molecular probe technology to characterize the microbial populations of the human body. A surprising 80% of the bacteria populating the human gut, previously unknown and thought to be unculturable, was revealed with these probe technologies.[158] What has emerged in recent years is the field of "culturomics," a revolution in bacterial culture technology that has permitted the isolation of hundreds of new bacterial species using multiple culture conditions and prolonged incubation times, combined with matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry and 16S rRNA sequencing. There are a number of

Fig. 28.3 All 2776 species isolated at least once from humans using culture. Using the online tool Wordle (http://www.wordle.net), the size of the name of each species is proportional to the number of times it occurs in the database. In this image, only the species name was taken into consideration, excluding the genus. (From Bilen M, Dufour JC, Lagier JC, et al. The contribution of culturomics to the repertoire of isolated human bacterial and archaeal species. *Microbiome*. 2018;May 24;6[1]:94. PMID: 29793532. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

techniques that have advanced culture technology, including the use of diffusion chambers (the use of membranes that allow nutrients to enter but confine bacterial cells); this alone increased the number of cultured colonies 300-fold.[159,160] Fig. 28.3 shows all 2776 species isolated from humans at least once using culture, an incredible and very recent increase in the number of culturable species.

Given ongoing evidence linking the GI microflora, health, and disease, these research initiatives have sought to identify a core gut microbiome. A limited number of common species have been demonstrated in the human intestinal microbiota to date, indicating the promise of applying molecular probe techniques in defining a beneficial bacterial composition. Given that research consistently demonstrates significant interindividual diversity and high intraindividual specificity, variability of microflora composition between individuals appears to be the norm, especially with aging.[161,162] In addition, although molecular probes have the capability of identifying the vast pool of species inhabiting the human gut, questions have been raised about the clinical relevance of such identification, as was noted previously with *H. pylori* PCR versus stool antigen testing. This is of particular concern when parasitical and/or pathogenic organisms are identified because probe technology does not currently have the capacity to differentiate between the DNA of living organisms (active infection) and DNA fragments indicative of past infectious processes or incidental exposure (the so-called "viability bias"). Additionally, metagenomic studies are limited by the extraction bias; that is, the diversity of the bacterial revealed is a function of the extraction protocol.[163] It seems likely that advanced culture methods combined with genomic analyses may provide the best overall picture of gut microbial diversity.

Ultimately, characterization of the microflora is undertaken to correct imbalances that may be contributing to acute or chronic states of illness. Supportive therapies such as prebiotics and probiotics (see Chapters 104 and 105 on prebiotics and probiotics) are standard clinical tools for optimizing gut health, and supplementation has been shown to be of benefit in an array of medical conditions, including atopic dermatitis,[145,164] acute infectious and antibiotic-associated diarrhea,[165–167] cancer prevention,[154] IBS,[168,169] prevention of necrotizing enterocolitis,[170] prevention and treatment of Pouchitis,[171] and ulcerative colitis.[172–175]

Although evidence exists that demonstrates clinical benefit, there is little consensus regarding what constitutes the most optimal therapeutic strain(s), concentrations, or timeline of treatment. With the emergence of molecular technology, there may be a point in the near future that allows individual targeting of probiotic therapies. Until that time, phenotypic markers that provide insight into the health of the microbiome—biomarkers reflecting digestion/absorption (D), immunology/inflammation/infectious processes (I), and gut microflora balance (G)—may be utilized to identify and monitor patients needing support.

BIOMARKERS FOR GASTROINTESTINAL MICROFLORA BALANCE AND METABOLIC FUNCTION

Beneficial Bacteria

Bifidobacterium, Lactobacillus, and nonpathogenic *E. coli* are the predominant strains of beneficial flora,[135] with the obligate anaerobic *Bifidobacteria* constituting about one fourth of the microbial flora found in adults.[176] In the distal colon, *Bifidobacteria* outnumber *Lactobacilli* by 1000:1.[177] Quantification of *Bifidobacteria* via stool culture typically demonstrates recovery in the 3+ or 4+ ranges in a healthy microbial milieu. The facultative aerobe *Lactobacilli* and nonpathogenic *E. coli* are both typically recovered in the 1+ or 2+ ranges.[178]

Adequate amounts of *Bifidobacterium, Lactobacillus,* and nonpathogenic *E. coli* are essential for the maintenance of optimal digestive functioning, and as such, these species are utilized in culture diagnostics as indicators of gut microflora health. Recovery of their numbers in less-than-optimal ranges suggests alterations in gut microbial balance and indicates a clinical need to ascertain the underlying etiology of the disruption and, ultimately, to assess the need for therapeutic support. The composition and distribution of the GI flora are affected by a variety of factors, as noted previously, including dietary intake, infectious agents, pharmaceuticals, and age. Stress also exerts a profound impact on gut flora and physiology, including increasing intestinal permeability, increasing bacterial adherence, and decreasing intraluminal levels of *Lactobacilli.*[141]

Restoration of balance and improvement of clinical symptoms may be achieved by probiotic supplementation. As indicated in the peer-reviewed literature, increasing levels of probiotic support may be required with increasing levels of disease state severity (Fig. 28.4).[179]

Additional Bacteria

Bacterial cultures are capable of identifying any number of additional organisms present in the individual human gut. These organisms may or may not exert a pathogenic effect depending on the state of balance or imbalance of the gut microbial milieu in toto. Putting it another way: sometimes these agents are the source of gut dysfunction, and sometimes their presence is merely a reflection of underlying gut dysfunction.

Overgrowth of additional bacteria may cause clinical and subclinical malabsorption and increase bowel permeability to large molecules. Abnormalities of the immune or mechanical barriers can lead to enhanced uptake of inflammatory luminal macromolecules and pathogenic bacteria. Bacterial antigens are capable of inducing antibodies, which cross-react with host antibodies, forming systemic immune complexes.[180,181] This process has been implicated in the etiology of chronic gut inflammation, as well as SLE and other connective tissue diseases.[182,183]

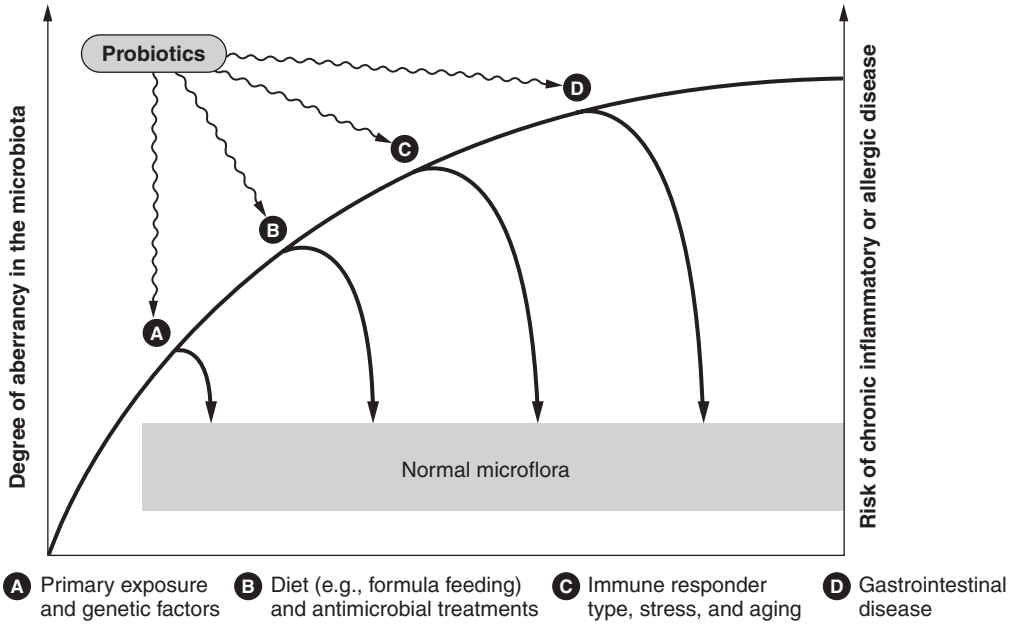


Fig. 28.4 Probiotic recommendations by disease. (From Isolauri E, Kirijavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut.* 2002;50:iii54–iii59. http://gut.bmj.com/content/50/suppl_3/iii54.full.pdf. Used with permission from BMJ Publishing Ltd. Rights were not granted to include this figure in electronic media. Please refer to the printed book. 2012.)

Bacterial species such as *Klebsiella* and *Proteus* are not routinely surveyed as components of standard microbiological assessments, but both have demonstrated antigenic cross-reactivity to human leukocyte antigens (HLAs). *Klebsiella* has been associated with ankylosing spondylitis when cross-reactivity occurs with the HLA-B27 antigen. Similarly, *Proteus mirabilis* has been associated with rheumatoid arthritis and is known to cross-react with the HLA-DR4 antigen.[184,185]

Mycology

The GI tract in healthy individuals normally harbors small amounts of yeast. However, colonic yeast infections have attracted attention and controversy as a possible cause of chronic complex illnesses.[186] Investigators have hypothesized that *Candida albicans* may be involved in the etiology of allergic disorders such as scleroderma and asthma,[152,187] chronic fatigue,[188] chemical sensitivity,[186] and IBD.[189] For example, a recent analysis of 235 individuals with IBD found that the fungal microbiota is also shifted compared with healthy controls, including a reduced proportion of *Saccharomyces cerevisiae* and an increased proportion of *C. albicans.*[190]

Biomarkers for Microflora Metabolic Activity

As mentioned previously, biomarkers that reflect the metabolic activity of the GI microflora can provide an adjunctive assessment of the microbial status of the gut. Diagnostic assays utilized include those that evaluate SCFAs, β-glucuronidase, and bile acid metabolism.[165]

Beneficial Short-Chain Fatty Acids

SCFAs are produced by the anaerobic bacterial fermentation of primarily nonabsorbed dietary fiber.[191] The rate and amount of SCFA production depends on the composition and density of microflora present in the colon, the substrate source, and GI transit time.[192]

There are three primary beneficial SCFAs—acetate, n-butyrate, and propionate—yet most clinical and research emphasis has been placed on n-butyrate. Butyrate plays a major role in the physiology of the colonic mucosa and serves as the major energy source for the colonocyte.[193] In addition, research suggests that it may modulate inflammation in IBD via regulation of proinflammatory cytokines[194,195] and may play a role in the prevention of colon cancer.[196]

Beta-Glucuronidase

Beta-glucuronidase is an inducible enzyme elaborated by anaerobic *E. coli, Peptostreptococcus, Bacteroides,* and *Clostridium.*[197,198] By uncoupling glucuronides (xenobiotics and endogenous compounds detoxified via the glucuronidation pathway), this enzyme can deconjugate potential toxins, enhancing the formation of local carcinogens in the bowel[199] and promoting the enterohepatic recirculation of toxins, hormones,[200] and various drugs[201] in the body. For this reason, excess amounts of the enzyme may promote a higher risk of colon cancer. Both the incidence of colon cancer and levels of β-glucuronidase tend to be higher in individuals consuming Western diets (high in saturated fat, low in fiber) than in individuals consuming diets high in fiber and low in saturated fat.[202]

However, an adequate amount of β-glucuronidase activity is probably important to maintain normal enterohepatic recirculation of endogenous compounds, such as vitamin D,[203] thyroid hormone,[204] and estrogen.[205]

Bile Acids

The relationship between dietary fat and increased risk of colon cancer is believed to hinge on the excess production of bile acids and the bacterial conversion of conjugated primary bile acids to potentially dangerous unconjugated secondary bile acids.[206] Studies have found that a higher ratio of secondary bile acids, specifically, an elevated ratio of lithocholic acid to deoxycholic acid (LCA/DCA ratio), is associated with higher susceptibility to polyps and colorectal cancer.[207] Increased secondary bile acid excretion and a rise in the LCA/DCA ratio have also been observed in patients with gallstones.[208] An increase in secondary bile acids and a decrease in primary bile acids have recently been observed among patients with heart failure, with a tentative link to overall survival.[209]

BOX 28.1 Biomarkers for Stool Analysis: Clinical Vignettes

A 21-year-old woman presents with a history of intermittent recurrent abdominal cramping, bloating and/or gas, and occasional diarrhea over the past 2 years. She has been evaluated by her college health clinic on numerous occasions, has been told that her symptoms were primarily "stress related," and has been advised to cut back on her coffee intake during the day. She has recently been evaluated for complaints of fatigue and has been diagnosed with hypothyroidism (including elevated thyroid antibodies), for which she is now being treated. While home during a break, the patient was asked to submit a stool analysis by her primary care doctor, who was concerned that some underlying etiology had not been determined. Results were significant for the following:

Pancreatic elastase = 150 (normal level, more than 200)

- At this level, the pancreatic elastase clinically indicates moderate pancreatic insufficiency and the need for pancreatic enzyme support. Evaluation of the underlying cause of pancreatic insufficiency is warranted.

Calprotectin = 22 (normal level, less than 50)

- A normal calprotectin level indicates no inflammation due to neutrophilic activity in the gastrointestinal tract.

Eosinophil protein X = 13.1 (normal level, less than 7.0)

- An elevated eosinophil protein X may be associated clinically with celiac disease, parasitical infections, and/or immunoglobulin-E–mediated food allergies.

Microscopy = no evidence of parasite infection or altered gut flora

Given that celiac disease can be consistent with the patient's clinical symptoms and may be suggested on stool analysis by an elevated eosinophil protein X and compromised pancreatic exocrine function (low pancreatic elastase), the doctor decided to evaluate the patient for celiac disease. Blood serologies (positive tissue transglutaminase immunoglobulin-A and positive antiendomysial antibody) confirmed the diagnosis of celiac disease. The patient was placed on a gluten-free diet and given pancreatic digestive enzyme support. At follow-up, the patient reported that her symptoms had markedly improved—appearing only during times of dietary indiscretion.

BOX 28.2 Biomarkers for Stool Analysis: Clinical Vignette

A 42-year-old woman presents with a 3-year history of irregular bowel movements and intermittent lower abdominal pain, for which she has seen her primary care provider many times. She also has been diagnosed with depression and is currently using a tricyclic antidepressant, which she notes does not make a significant difference in her abdominal pain. She has used fiber, antidiarrheals, probiotics, and diet changes in the past to no avail. Upon evaluation, her new primary physician chooses to use stool analysis to evaluate the root cause of her illness. While waiting for the stool test to return, empiric use of probiotics at 10 billion colony-forming units per day has made no difference. The following results were noted:

Pancreatic elastase = 482 (normal level, more than 200)

- At this level, the pancreatic elastase clinically indicates normal pancreatic function and no need for pancreatic enzyme support.

Calprotectin = 286 (normal level, less than 50)

- This level of elevation of calprotectin indicates severe gastrointestinal tract inflammation and warrants referral to a specialist for further investigation (i.e., colonoscopy).

Microscopy = no evidence of parasitical infection or altered gut flora

The patient is referred for colonoscopy for inflammatory changes in the gastrointestinal tract. She is diagnosed with ulcerative colitis and placed on steroids and 5-aminosalicylic acid. For this patient, stool analysis allowed for early detection and appropriate referral to a gastroenterologist. Subsequent evaluations of gastrointestinal inflammation with the calprotectin biomarker can be used to monitor mucosal healing and determine whether repair is sufficient and/or complete or to monitor for relapse in patients with inflammatory bowel disease (IBD).

Bacterial overgrowth markedly increases the concentration of unconjugated bile acids, and this mechanism may play an important role in the pathophysiology of gut mucosal injury.[210] Probiotic treatment with *L. reuteri* has been demonstrated to lower the bioavailable concentration of toxic bile acids.[206]

SUMMARY

The philosophical framework that describes GI health to be the foundation of optimal health is not a new one. The *Sushruta Samhita,* an ancient Ayurvedic text, alludes to this in the following passage: "A person whose basic emotional and physical tendencies are in balance, whose digestive power is balanced, whose bodily tissues, elimination functions and activities are in balance, and whose mind, sense and soul are filled with vitality, that person is said to be healthy." If we hold this tenet to be true, then tools that allow us to diagnostically illuminate the imbalances that create symptoms and disease states in our patients—and ultimately lead to interventions to support them—are clinically important. Biomarkers for stool analysis provide a useful approach to identify the underlying pathologies that present as gut dysfunction, thus eliminating the need for costly diagnostic testing where unnecessary; highlighting those individuals specifically at risk for severe disease who do require significant diagnostic intervention; and identifying the source of gut dysfunction to apply simple, cost-effective treatments (Boxes 28.1 and 28.2).

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jones R, Murfin D. Gastrointestinal problems in the community and in general practice. In: Jones R, ed. *Gastrointestinal Problems in General Practice*. Oxford: Oxford University Press; 1993.
2. Shaheen NJ. The burden of gastrointestinal and liver diseases in GI epidemiology. In: Talley NJ, Locke RG, Saito Y, eds. *GI Epidemiology*. Malden: MA Blackwell Publishing; 2007:8–16.
3. Shaheen NJ, Hansen RA, Morgan DR, et al. The burden of gastrointestinal and liver diseases, 2006. *Am J Gastroenterol*. 2006;101:2128–2138.
4. Sandler RS, Everhart JE, Donowitz M, et al. The burden of selected digestive disease in the United States. *Gastroenterol*. 2002;122:1500.
5. Peery AF, Crockett SD, Barritt AS, et al. Burden of gastrointestinal, liver, and pancreatic diseases in the United States. *Gastroenterology*. 2015;149(7):1731–1741.e3. PMID: 2632713.
6. Drossman DA, Morris CB, Schneck S, et al. International survey of patients with IBS: symptom features and their severity, health status, treatments, and risk taking to achieve clinical benefit. *J Clin Gastroenterol*. 2009;43:541–550.
7. Brantzaeg P. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci*. 2002;964:13–45.
8. Power ML, Schulkin J. Anticipatory physiological regulation in feeding biology: cephalic phase responses. *Appetite*. 2008;50:194–206.
9. Hopfer U. Digestion and absorption of nutritional constituents. In: Devlin TM, ed. *Textbook of Biochemistry with Clinical Correlations.* New York: Wiley-Liss; 1081–1113.
10. Valdez IH, Fox PC. Interactions of the salivary and gastrointestinal systems. I. The role of saliva in digestion. *Dig Dis Sci*. 1991;9:125–132.
11. Johnson LR. In: *Gastrointestinal Physiology*. St Louis: Mosby; 2002.
12. Tso P, Crissinger K. Digestion and absorption of lipids. In: Stipanuk MH, ed. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: WB Saunders; 2000:125–140.
13. Hunt RH, Camilleri M, Crowe SE, et al. The stomach in health and disease. *Gut*. 2015;64(10):1650–1668. PMID: 26342014.
14. Singh VK, Haupt ME, Geller DE, et al. Less common etiologies of exocrine pancreatic insufficiency. *World J Gastroenterol*. 2017;23(39):7059–7076. PMID: 29093615.
15. Dominguez-Munoz JE. Pancreatic enzyme therapy for pancreatic exocrine insufficiency. *Curr Gastroenterol Rep*. 2007;9:116–122.
16. Tso P, Crissinger K. Overview of digestion and absorption. In: Stipanuk MH, ed. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: WB Saunders; 2000:75–90.
17. Guyton AC, Hall JE. *Textbook of Medical Physiology*. Philadelphia: WB Saunders; 1996.
18. Smecuol E, Sugai E, Niveloni S, et al. *Gastrointestinal Permeability in Dermatitis Herpetiformis and Increasing Severity of Enteropathy (abstract #2012)*. Atlanta, GA: Presented at Digestive Disease Week and the 102nd Annual Meeting of the American Gastroenterological Association; 2001:20–23.
19. Tepper RE, Simon D, Brandt LJ, et al. Intestinal permeability in patients infected with the human immunodeficiency virus. *Am J Gastroenterol*. 1994;89:878–882.
20. O'Dwyer ST, Michie HR, Ziegler TR, et al. A single dose of endotoxin increases intestinal permeability in healthy humans. *Arch Surg*. 1988;123:1459–1464.
21. Rooney PJ, Jenkins RT, Buchanan WW. A short review of the relationship between intestinal permeability and inflammatory joint disease. *Clin Exp Rheumatol*. 1990;8:75–83.
22. DeWitt RC, Kudsk KA. The gut's role in metabolism, mucosal barrier function, and gut immunology. *Infect Dis Clin North Am*. 1999;13:465–481.
23. Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers*. 2016;4(4):e1251384. PMID: 28123927.
24. Lupton JR, Turner ND. Dietary fiber. In: Stipanuk MH, ed. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: WB Saunders; 2000:143–154.
25. Cater 2nd RE. The clinical importance of hypochlorhydria (consequence of chronic Helicobacter infection): its possible etiological role in mineral and amino acid malabsorption, depression, and syndromes. *Med Hypotheses*. 1992;39:375–383.
26. Kassarjian Z, Russell RM. Hypochlorhydria: a factor in nutrition. *Annu Rev Nutr*. 1989;9:271–285.
27. Bures J, Cyrany J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol*. 2010;16:2978–2990.
28. Lombardo L, Foti M, Ruggia O, et al. Increased incidence of small intestinal bacterial overgrowth during proton pump inhibitor therapy. *Clin Gastroenterol Hepatol*. 2010;8:504–508.
29. Spiegel BM, Chey WD, Chang L. Bacterial overgrowth and irritable bowel syndrome: unifying hypothesis or a spurious consequence of proton pump inhibitors? *Am J Gastroenterol*. 2008;103:2972–2976.
30. Sipponen R, Harkonen M. Hypochlorhydric stomach: a risk condition for calcium malabsorption and osteoporosis? *Scand J Gastroenterol*. 2010;45:133–138.
31. Corley DA, Kubo A, Zhao W, et al. Proton pump inhibitors and histamine-2 receptor antagonists are associated with hip fractures among at-risk patients. *Gastroenterol*. 2010;139:93–101.
32. Gray SL, LaCroix AZ, Larson J, et al. Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women: results from the women's health initiative. *Arch Intern Med*. 2010;170:765–771.
33. Targownik LE, Lix LM, Leung S, et al. Proton-pump inhibitor use is not associated with osteoporosis or accelerated bone mineral density loss. *Gastroenterol*. 2010;138:896–904.
34. Liu J, Li X, Fan L, et al. Proton pump inhibitors therapy and risk of bone diseases: An update meta-analysis. *Life Sci*. 2019;218:213–223. PMID: 30605646.
35. Vellas B, Balas D, Albarede JL. Effects of aging process on digestive functions. *Compr Ther*. 1991;17:46–52.
36. Teichmann J, Lange U, Hardt PD, et al. Pancreatic elastase 1 in patients with osteoporosis (abstract). Presented at Kongress der Deutschen Gesellschaft fur Innere Medizin, Wiesbaden, Germany, April 22, 2001.
37. Mann ST, Mann V, Stracke H, et al. Fecal elastase 1 and vitamin D3 in patients with osteoporotic bone fractures. *Eur J Med Res*. 2008;13:68–72. 25.
38. Hardt PD, Krauss A, Bretz L, et al. Pancreatic exocrine function in patients with type 1 and type 2 diabetes mellitus. *Acta Diabetol*. 2000;37:105–110.
39. Hardt PD, Krauss A, Bretz L, et al. Pancreatic exocrine function in patients with type 1 and type 2 diabetes mellitus. *Acta Diabetol*. 2000;37(3):105–110.
40. Maire F, Le Baleur Y, Rebours V, et al. Outcome of patients with type 1 or 2 autoimmune pancreatitis. *Am J Gastroenterol*. 2011;106(1):151–156.
41. Russell RM. Changes in gastrointestinal function attributed to aging. *Am J Clin Nutr*. 1992;55(6 suppl 1):1203S–1207S.
42. Shane B. Folic acid, vitamin B12, and vitamin B6. In: Stipanuk MH, ed. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: WB Saunders; 2000:483–518.
43. Cavalcoli F, Zilli A, Conte D, et al. Micronutrient deficiencies in patients with chronic atrophic autoimmune gastritis: A review. *World J Gastroenterol*. 2017;23(4):563–572. PMID: 28216963.
44. EJ1 Hoorn, van der Hoek J, de Man RA, et al. A case series of proton pump inhibitor-induced hypomagnesemia. *Am J Kidney Dis*. 2010;56(1):112–116. PMID: 20189276.
45. Dominici R, Franzini C. Fecal elastase-1 as a test for pancreatic function: a review. *Clin Chem Lab Med*. 2002;40:325–332.
46. Gullo L, Ventrucci M, Tomassetti P, et al. Fecal elastase 1 determination in chronic pancreatitis. *Dig Dis Sci*. 1999;44:210–213.
47. Cade A, Walters MP, McGinley N, et al. Evaluation of fecal pancreatic elastase-1 as a measure of pancreatic exocrine function in children with cystic fibrosis. *Pediatr Pulmonol*. 2000;29:172–176.
48. Sziegoleit A, Linder D. Studies on the sterol-binding capacity of human pancreatic elastase-1. *Gastroenterol*. 1991;100:768–774.
49. Dominguez-Munoz JE, Hieronymus C, Sauerbruch T, et al. Fecal elastase test: evaluation of a new noninvasive pancreatic function test. *Am J Gastroenterol*. 1995;90:1834–1837.

50. Brydon WG, Kingstone K, Ghosh S. Limitations of faecal elastase-1 and chymotrypsin as tests of exocrine pancreatic disease in adults. *Ann Clin Biochem*. 2004;41:78–81.
51. Lankisch PG. Now that fecal elastase is available in the United States, should clinicians start using it? *Curr Gastroenterol Rep*. 2004;6:126–131.
52. Domínguez-Muñoz JE, D Hardt P, Lerch MM, et al. Potential for screening for pancreatic exocrine insufficiency using the fecal elastase-1 test. *Dig Dis Sci*. 2017;62(5):1119–1130. PMID: 28315028.
53. Maconi G, Dominici R, Molteni M, et al. Prevalence of pancreatic insufficiency in inflammatory bowel diseases. Assessment by fecal elastase-1. *Dig Dis Sci*. 2008;53:262–270.
54. Senkal M, Ceylan B, Deska T, et al. Exocrine pancreas disfunction in severely traumatised patients and early enteral nutrition. *Ulus Travma Acil Cerrahi Derg*. 2008;14:34–39.
55. Nunes AC, Pontes JM, Rosa A, et al. Screening for pancreatic exocrine insufficiency in patients with diabetes mellitus. *Am J Gastroenterol*. 2003;98:2672–2675.
56. Hardt PD, Hauenschild A, Nalop J, et al. High prevalence of exocrine pancreatic insufficiency in diabetes mellitus. A multicenter study screening fecal elastase 1 concentrations in 1,021 diabetic patients. *Pancreatol*. 2003;3:395–402.
57. Girish BN, Rajesh G, Vaidyanathan K, et al. Fecal elastase1 and acid steatocrit estimation in chronic pancreatitis. *Indian J Gastroenterol*. 2009;28:201–205.
58. Mann ST, Stracke H, Lange U, et al. Vitamin D3 in patients with various grades of chronic pancreatitis, according to morphological and functional criteria of the pancreas. *Dig Dis Sci*. 2003;48:533–538.
59. Walkowiak J, Lisowska A, Przyslawski J, et al. Faecal elastase-1 test is superior to faecal lipase test in the assessment of exocrine pancreatic function in cystic fibrosis. *Acta Paediatr*. 2004;93:1042–1045.
60. Evans KE, et al. Pancreatic insufficiency in adult celiac disease: do patients require long-term enzyme supplementation? *Dig Dis Sci*. 2010;55:2999–3004.
61. Nousia-Arvanitakis S, Fotoulaki M, Tendzidou K, et al. Subclinical exocrine pancreatic dysfunction resulting from decreased cholecystokinin secretion in the presence of intestinal villous atrophy. *J Pediatr Gastroenterol Nutr*. 2006;43:307–312.
62. Nakamura T, Tabeke K, Terada A, et al. Short-chain carboxylic acid in the feces of patients with pancreatic insufficiency. *J Acta Gastroenterol Bleg*. 1993;56:326–331.
63. Rasmussen HS, Holtug K, Mortensen PB. Degradation of amino acids to short-chain fatty acids in humans. An in vitro study. *Scand J Gastroenterol*. 1988;23:178–182.
64. Oufir LE, Barry JL, Flourie B, et al. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. *Eur J Clin Nutr*. 2000;54:603–609.
65. Fernandes J, Su W, Rahat-Rozenbloom S, et al. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutr Diabetes*. 2014;4:e121. PMID: 24979150.
66. Chen HM, Yu YN, Wang JL, et al. Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. *Am J Clin Nutr*. 2013;97(5):1044–1052. PMID: 23553152.
67. Sun Q, Jia Q, Song L, Duan L. Alterations in fecal short-chain fatty acids in patients with irritable bowel syndrome: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2019;98(7):e14513. PMID: 30762787.
68. Hammer HF. Pancreatic exocrine insufficiency: diagnostic evaluation and replacement therapy with pancreatic enzymes. *Dig Dis*. 2010;28:339–343.
69. Salminen S, Bouley C, Boutron-Ruyault MC, et al. Functional food science and gastrointestinal physiology and function. *Br J Nutr*. 1998;80:S147–S171.
70. Honda K, Takeda K. Regulatory mechanisms of immune responses to intestinal bacteria. *Mucosal Immunol*. 2009;2:187–196.
71. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature*. 2000;406:782–787.
72. Mahida YR. Epithelial cell responses. *Best Pract Res Clin Gastro*. 2004;18:241–253.
73. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest*. 2007;117:514–521.
74. Shannahan F. Host-flora interactions in inflammatory bowel disease. *Inflamm Bowel Dis*. 2004;10:S16–S24.
75. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol*. 2003;3:521–533.
76. Belkaid Y, Liesenfeld O, Maizels RM. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: induction and control of regulatory T cells in the gastrointestinal tract: consequences for local and peripheral immune responses. *Clin Exp Immunol*. 2010;160:35–41.
77. Picchianti-Diamanti A, Panebianco C, Salemi S, et al. Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. *Int J Mol Sci*. 2018;19(10):E2938. PMID: 30261687.
78. Jie Z, Xia H, Zhong SL, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017;8(1):845. PMID: 29018189.
79. Yan Q, Gu Y, Li X, et al. Alterations of the gut microbiome in hypertension. *Front Cell Infect Microbiol*. 2017;7:381. PMID: 28884091.
80. Nobaek S, Johansson ML, Molin G, et al. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol*. 2000;95:1231–1238.
81. Carlson M, Raab Y, Peterson C, et al. Increased intraluminal release of eosinophil granule proteins EPO, ECP, EPX, and cytokines in ulcerative colitis and proctitis in segmental perfusion. *Am J Gastroenterol*. 1999;94:1876–1883.
82. Rothenberg ME, Mishra A, Brandt EB, et al. Gastrointestinal eosinophils. *Immunol Rev*. 2001;179:139–155.
83. Wagner M, Peterson CG, Ridefelt P, et al. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J Gastroenterol*. 2008;14:5584–5589.
84. Peterson CG, Eklund E, Taha Y, et al. A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease. *Am J Gastroenterol*. 2002;97:1755–1762.
85. Saitoh O, Kojima K, Sugi K, et al. Fecal eosinophil granule–derived proteins reflect disease activity in inflammatory bowel disease. *Am J Gastroenterol*. 1999;94:3513–3520.
86. van Odijk J, Peterson CG, Ahlstedt S, et al. Measurements of eosinophil activation before and after food challenges in adults with food hypersensitivity. *Int Arch Allergy Immunol*. 2006;140:334–341.
87. Pucci N, November E, Cammarata MG, et al. Scoring atopic dermatitis in infants and young children: distinctive features of the SCO-RAD index. *Allergy*. 2005;60:113–116.
88. Magnusson J, Gellerstedt M, Ahlstedt S, et al. A kinetic study in adults with food hypersensitivity assessed as eosinophil activation in fecal samples. *Clin Exp Allergy*. 2003;33:1052–1059.
89. Majamaa H, Laine S, Miettinen A. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. *Clin Exp Allergy*. 1999;29:1502–1506.
90. Koot BG, ten Kate FJ, Juffrie M, et al. Does Giardia lamblia cause villous atrophy in children? a retrospective cohort study of the histological abnormalities in giardiasis. *J Pediatr Gastroenterol Nutr*. 2009;49:304–308.
91. Reimert CM, Tukahebwa EM, Kabatereine NB, et al. Assessment of Schistosoma mansoni induced intestinal inflammation by means of eosinophil cationic protein, eosinophil protein X and myeloperoxidase before and after treatment with praziquantel. *Acta Trop*. 2008;105:253–259.
92. Lettesjo H, Hansson T, Peterson C, et al. Detection of inflammatory markers in stools from patients with irritable bowel syndrome and collagenous colitis. *Scand J Gastroenterol*. 2006;41:54–59.
93. Bischoff SC, Grabowsky J, Manns MP. Quantification of inflammatory mediators in stool samples of patients with inflammatory bowel disorders and controls. *Dig Dis Sci*. 1997;42:394–403.
94. Kalach N, Kapel N, Waligora-Dupriet AJ, et al. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin Chem Lab Med*. 2013;51(2):351–361. PMID: 23087088.

95. von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol*. 2007;102:803–813.
96. Tibble J, Sigthorsson G, Foster R, et al. Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma. *Gut*. 2001;49:402–408.
97. Sprakes MB, Hamlin PJ, Ford AC. Utility of fecal calprotectin in differentiating active inflammatory bowel disease from coexistent irritable bowel syndrome. *Am J Gastroenterol*. 2011;106:166–167.
98. Langhorst J, Elsenbruch S, Koelzer J, et al. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol*. 2008;103:162–169.
99. Tibble JA, Sigthorsson G, Foster R, et al. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterol*. 2002;123:450–460.
100. Mitchell CM, Drossman DA. Survey of the AGA membership relating to patients with functional gastrointestinal disorders. *Gastroenterol*. 1987;92:1282–1284.
101. Thompson WG, Heaton KW, Smyth GT, et al. Irritable bowel syndrome in general practice: prevalence, characteristics and referral. *Gut*. 2000;46:78–82.
102. Pimentel M, Tabibzadeh S, Kirit-Kiriak V, et al. Prolonged prodrome of gastrointestinal symptoms is associated with Crohn's disease but not ulcerative colitis. *Gastroenterol*. 1999;116:G3453 (AGA abstracts).
103. Bunn SK, Bisset WM, Main MJ, et al. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2001;32:171–177.
104. Wassell J, Dolwani S, Metzner M, et al. Faecal calprotectin: a new marker for Crohn's disease? *Ann Clin Biochem*. 2004;41:230–232.
105. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion*. 1997;58:176–180.
106. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 1999;34:50–54.
107. Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. *World J Gastroenterol*. 2001;7:460–465.
108. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. *BMC Res Notes*. 2009;2:221.
109. Xiang JY, Ouyang Q, Li GD. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. *World J Gastroenterol*. 2008;14:53–57.
110. Heida A1, Park KT, van Rheenen PF. Clinical utility of fecal calprotectin monitoring in asymptomatic patients with inflammatory bowel disease: a systematic review and practical guide. *Inflamm Bowel Dis*. 2017;23(6):894–902. PMID: 28511198.
111. Feldman RA. Epidemiologic observations and open questions about disease and infection caused by *Helicobacter pylori*. In: Achtman M, Suerbaum S, eds. *Helicobacter Pylori: Molecular and Cellular Biology*. Wymondham, United Kingdom: Horizon Scientific Press; 2001:29–51.
112. Duggan A. *Helicobacter pylori:* when is treatment now indicated? *Int Med J*. 2002;32:465–469.
113. Chey WD, Wong BC. Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am J Gastroenterol*. 2007;102:1808–1825.
114. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med*. 2001;345:784–789.
115. Best LM, Takwoingi Y, Siddique S, et al. Non-invasive diagnostic tests for *Helicobacter pylori* infection. *Cochrane Database Syst Rev*. 2018;3:CD012080. PMID: 29543326.
116. De Korwin JD. Advantages and limitations of diagnostic methods for *H. pylori* infection. *Gastroenterol Clin Biol*. 2003;27:380–390.
117. Vaira D, Vakil N, Menegatti M, et al. The stool antigen test for detection of *Helicobacter pylori* after eradication therapy. *Ann Intern Med*. 2002;19(136):280–287.
118. Bravo LE, Realpe JL, Campo C, et al. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for *Helicobacter pylori* infection. *Am J Gastroenterol*. 1999;94:2380–2383.
119. Vakil N, Rhew D, Soll A, Ofman JJ. The cost-effectiveness of diagnostic testing strategies for *Helicobacter pylori*. *Am J Gastroenterol*. 2000;95:1691–1698.
120. Sugimoto M, Wu JY, Abudayyeh S, et al. Unreliability of results of PCR detection of *Helicobacter pylori* in clinical or environmental samples. *J Clin Microbiol*. 2009;47:738–742.
121. Zambon CF, Basso D, Navaglia F, et al. Non-invasive diagnosis of *Helicobacter pylori* infection: simplified 13C-urea breath test, stool antigen testing, or DNA PCR in human feces in a clinical laboratory setting? *Clin Biochem*. 2004;37:261–267.
122. Ananthakrishnan AN. *Clostridium difficile* infection: epidemiology, risk factors and management. *Nat Rev Gastroenterol Hepatol*. 2011;8:17–26.
123. Barbut F, Petit J-C. Epidemiology of *Clostridium difficile*-associated infections. *Clin Microbiol Infect*. 2001;7:405–410.
124. Yassin SF, Young-Fadok TM, Zein NN, et al. *Clostridium difficile*-associated diarrhea and colitis. *Mayo Clin Proc*. 2001;76:725–730.
125. Stark D, van Hal S, Marriott D, et al. Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. *Int J Parasitol*. 2007;37:11–20.
126. Yakoob J, Jafri W, Jafri N, et al. Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. *Am J Trop Med Hyg*. 2004;70:383–385.
127. Yakoob J, Jafri W, Beg MA, et al. Blastocystis hominis and Dientamoeba fragilis in patients fulfilling irritable bowel syndrome criteria. *Parasitol Res*. 2010;107:679–684.
128. Tungtrongchitr A, Manatsathit S, Kositchaiwat C, et al. *Blastocystis hominis* infection in irritable bowel syndrome patients. *Southeast Asian J Trop Med Public Health*. 2004;35:705–710.
129. Garcia LS, Shimizu RY, Bernard CN. Detection of *Giardia lamblia, Entamoeba histolytica/Entamoeba dispar,* and *Cryptosporidium parvum* antigens in human fecal specimens using the triage parasite panel enzyme immunoassay. *J Clin Microbiol*. 2000;38:3337–3340.
130. *Personal Communication with Dr. Patrick Hanaway, Chief Medical Officer*. Genova Diagnostics; 2009.
131. Lagacé-Wiens PR, VanCaeseele PG, Koschik C. Dientamoeba fragilis: an emerging role in intestinal disease. *CMAJ*. 2006;175:468–469.
132. Klem F, Wadhwa A, Prokop LJ, et al. Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: a systematic review and meta-analysis. *Gastroenterology*. 2017;152(5):1042–1054.e1. PMID: 28069350.
133. Kligler B, Hanaway P, Cohrssen A. Probiotics in children. *Pediatr Clin North Am*. 2007;54:949–967.
134. Fallani M, Young D, Scott J, et al. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J Pediatr Gastroenterol Nutr*. 2010;51:77–84.
135. Isolauri E, Kirjavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut*. 2002;50(S3):III54–III59.
136. Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiotia in early infancy. *Pediatrics*. 2006;118:511–521.
137. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Peds Gastro Nutr*. 2000;30:60–67.
138. Sherman PM, Cabana M, Gibson GR, et al. Potential roles and clinical utility of prebiotics in newborns, infants, and children: proceedings from a global prebiotic summit meeting, New York City, June 27-28, 2008. *J Pediatr*. 2009;155:S61–S70.
139. Zoetendal EG, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol*. 1998;64:3854–3859.

140. Cordain L, Eaton SB, Sebastian A, et al. Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr*. 2005;81:341–354.
141. Hart A, Kamm MA. Review article: mechanisms of initiation and perpetuation of gut inflammation by stress. *Aliment Pharmacol Ther*. 2002;16:2017–2028.
142. Hugon P, Dufour J-C, Colson P, et al. A comprehensive repertoire of prokaryotic species identified in human beings. *Lancet Infect Dis*. 2015;15:1211–1219. https://doi.org/10.1016/S1473-3099(15)00293-5 PMID: 26311042.
143. Frank DN, Pace NR. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol*. 2008;24(1):4–10. PMID: 18043225.
144. Li J1, Jia H2, Cai X2, Zhong H, et al. An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol*. 2014;32(8):834–841. PMID: 24997786.
145. Ramakrishna BS. The normal bacterial flora of the human intestine and its regulation. *J Clin Gastroenterol*. 2007;41:S2–S6.
146. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep*. 2006;7:688–693.
147. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet*. 2003;361:512–519.
148. Jia W, Li H, Zhao L, et al. Gut microbiota: a potential new territory for drug targeting. *Nat Rev Drug Discov*. 2008;7:123–129.
149. Frank DN, St Amand AL, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A*. 2007;104:13780–13785.
150. Guarner F. Enteric flora in health and disease. *Digestion*. 2006;73(suppl 1):5–12.
151. Bland JS, Costarella L, Levin B, et al. *Clinical Nutrition: A Functional Approach*. Gig Harbor, WA: Institute for Functional Medicine; 1999.
152. Palma-Carlos AG, Palma-Carlos ML, Costa AC. *Candida* and allergy. *Allergy Immunol*. 2002;34:322–324.
153. Rook GA. The changing microbial environment and chronic inflammatory disorders. *Allergy Asthma Clin Immunol*. 2008;4:117–124.
154. McGarr SE, Ridlon JM, Hylemon PB. Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. *J Clin Gastroenterol*. 2005;39:98–109.
155. Rook GA, Adams V, Hunt J, et al. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. *Springer Semin Immunopathol*. 2004;25:237–255.
156. Brandtzaeg PE. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci*. 2002;964:13–45.
157. Tannock GW. Molecular assessment of intestinal microflora. *Am J Clin Nutr*. 2001;73(suppl 2):410S–414S.
158. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science*. 2005;308(5728):1635–1638. PMID: 15831718.
159. Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science*. 2002;296(5570):1127–1129. PMID: 12004133.
160. Lagier JC, Dubourg G, Million M, et al. Culturing the human microbiota and culturomics. *Nat Rev Microbiol*. 2018:540–550. 29937540.
161. Tap J, Mondot S, Levenez F, et al. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol*. 2009;11:2574–2584.
162. Claesson MJ, Cusack S, O'Sullivan O, et al. Microbes and Health Sackler Colloquium: Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci U S A*. 2011;108(supp1):4586–4591.
163. Brooks JP, Edwards DJ, Harwich Jr MD, et al. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. *BMC Microbiol*. 2015;15:66. 25880246.
164. Kalliomäki M, Salminen S, Poussa T, et al. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361:1869–1871.
165. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol*. 2006;101:812–822.
166. Allen SJ, Okoko B, Martinez E, et al. Probiotics for treating infectious diarrhoea. *Cochrane Database Syst Rev*. 2004;2:CD003048.
167. Van Niel CW, Feudtner C, Garrison MM, et al. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics*. 2002;109:678–684.
168. Brenner DM, Moeller MJ, Chey WD, et al. The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Am J Gastroenterol*. 2009;104:1033–1049.
169. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterol*. 2005;128:541–551.
170. Guthmann F, Kluthe C, Bührer C. Probiotics for prevention of necrotising enterocolitis: an updated meta-analysis. *Klin Padiatr*. 2010;222:284–290.
171. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut*. 2004;53:108–114.
172. Zigra PI, Maipa VE, Alamanos YP. Probiotics and remission of ulcerative colitis: a systematic review. *Neth J Med*. 2007;65:411–418.
173. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*. 2005;54:242–249.
174. Tursi A, Brandimarte G, Giorgetti GM, et al. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med Sci Monit*. 2004;10:PI126–PI131.
175. Kato K, Mizuno S, Umesaki Y, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther*. 2004;20:1133–1141.
176. Modler HW, McKellar MC, Yaguchi M. Bifidobacteria and bifidogenic factors. *Can Inst Food Sci Tech J*. 1990;23:29–41.
177. Hart AL, Stagg AJ, Frame M, et al. The role of the gut flora in health and disease, and its modification as therapy. *Aliment Pharmacol Ther*. 2002;16:1383–1393.
178. Tannock GW. The normal microflora: new concepts in health promotion. *Microbiol Sci*. 1988;5:4–8.
179. Verna EC, Lucak S. Use of probiotics in gastrointestinal disorders: what to recommend? *Therap Adv Gastroenterol*. 2010;3:307–319.
180. Peters TJ, Bjarnson I. Uses and abuses of intestinal permeability measurements. *Can J Gastroenterol*. 1988;2:127–132.
181. Madara JL, Nash S, Moore R, et al. Structure and function of the intestinal epithelial barrier in health and disease. *Monogr Pathol*. 1990;31:306–324.
182. Apperloo-Renkema HZ, Bootsma H, Mulder BI, et al. Host-microflora interaction in systemic lupus erythematosus (SLE): circulating antibodies to the indigenous bacteria of the intestinal tract. *Epidemiol Infect*. 1995;114:133–141.
183. Romanov VA, Shilkina NP, Gul'neva MI, et al. Microflora in patients with systemic connective tissue diseases. *Vestn Ross Akad Med Nauk*. 2008;4:10–14.
184. Pöllänen R, Sillat T, Pajarinen J, et al. Microbial antigens mediate HLA-B27 diseases via TLRs. *J Autoimmun*. 2009;32:172–177.
185. Ebringer A, Rashid T, Wilson C. Rheumatoid arthritis, *Proteus,* anti-CCP antibodies and karl popper. *Autoimmun Rev*. 2010;9:216–223.
186. Birdsall TC. Gastrointestinal candidiasis: fact or fiction? *Altern Med Rev*. 1997;2:346–354.
187. Rose S, Young MA, Reynolds JC. Gastrointestinal manifestations of scleroderma. *Gastroenterol Clin North Am*. 1998;27:563–594.
188. Cater RE. II. Chronic intestinal candidiasis as a possible etiological factor in the chronic fatigue syndrome. *Med Hypotheses*. 1995;44:507–515.
189. Trojanowska D, Zwolinska-Wcislo M, Tokarczyk M, et al. The role of *Candida* in inflammatory bowel disease. Estimation of transmission of *C. albicans* fungi in gastrointestinal tract based on genetic affinity between strains. *Med Sci Monit*. 2010;16:CR451–CR457.
190. Sokol H, Leducq V, Aschard H, et al. Fungal microbiota dysbiosis in IBD. *Gut*. 2017;66(6):1039–1048. PMID: 26843508.
191. Clausen MR, Bonnen H, Mortensen PB. Colonic fermentation of dietary fibre to short chain fatty acids in patients with adenomatous polyps and colonic cancer. *Gut*. 1991;32:923–928.
192. Wong JM, de Souza R, Kendall CW, et al. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol*. 2006;40:235–243.

193. Thibault R, Blachier F, Darcy-Vrillon B, et al. Butyrate utilization by the colonic mucosa in inflammatory bowel diseases: a transport deficiency. *Inflamm Bowel Dis*. 2010;16:684–695.
194. Weng M, Walker WA, Sanderson IR. Butyrate regulates the expression of pathogen-triggered IL-8 in intestinal epithelia. *Pediatr Res*. 2007;62:542–546.
195. Tedelind S, Westberg F, Kjerrulf M, et al. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World J Gastroenterol*. 2007;13:2826–2832.
196. Tang Y, Chen Y, Jiang H, et al. The role of short-chain fatty acids in orchestrating two types of programmed cell death in colon cancer. *Autophagy*. 2011;7:235–237.
197. Benno Y, Mitsuoka T. Impact of *Bifidobacterium longum* on human fecal microflora. *Microbiol Immunol*. 1992;36:683–694.
198. Cole CB, Fuller R, Mallet AK, et al. The influence of the host on expression of intestinal microbial enzyme activities involved in metabolism of foreign compounds. *J Appl Bacteriol*. 1985;59:549–553.
199. Kim DH, Jin YH. Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. *Arch Pharm Res*. 2001;24:564–567.
200. Adlercreutz H, Martin F, Pulkkinen M, et al. Intestinal metabolism of estrogens. *J Clin Endocrinol Metab*. 1976;43:497–505.
201. Kuhn JG. Pharmacology of irinotecan. *Oncology*. 1998;12(8 suppl 6):39–42.
202. Reddy BS, Wynder EL. Large-bowel carcinogenesis: fecal constituents of populations with diverse incidence rates of colon cancer. *J Natl Cancer Inst*. 1973;50:1437–1442.
203. Kumar R, Nagubandi S, Mattox VR, et al. Enterohepatic physiology of 1,25-dihydroxyvitamin D3. *J Clin Invest*. 1980;65:277–284.
204. Hazenberg MP, de Herder WW, Visser TJ. Hydrolysis of iodothyronine conjugates by intestinal bacteria. *FEMS Microbiol Rev*. 1988;4:9–16.
205. Gorbach SL. Estrogens, breast cancer, and intestinal flora. *Rev Infect Dis*. 1984;6(suppl 1):S85–S90.
206. De Boever P, Wouters R, Verschaeve L, et al. Protective effect of the bile salt hydrolase-active *Lactobacillus reuteri* against bile salt cytotoxicity. *Appl Microbiol Biotechnol*. 2000;53:709–714.
207. Imray CH, Radley S, Davis A, et al. Faecal unconjugated bile acids in patients with colorectal cancer or polyps. *Gut*. 1992;33:1239–1245.
208. Mamianetti A, Garrido D, Carducci CN, et al. Fecal bile acid excretion profile in gallstone patients. *Medicina*. 1999;59:269–273.
209. Mayerhofer CCK, Ueland T, Broch K, et al. Increased secondary/primary bile acid ratio in chronic heart failure. *J Card Fail*. 2017;23(9):666–671. PMID: 28688889.
210. Theisen J, Nehra D, Citron D, et al. Suppression of gastric acid secretion in patients with gastroesophageal reflux disease results in gastric bacterial overgrowth and deconjugation of bile acids. *J Gastrointest Surg*. 2000;4:50–54.

29

Organic Acid Profiling

Michael J. Chapman, ND, Edward C. Wallace, ND, DC, and *Terry Arden Pollock, BS, MS*

OUTLINE

INTRODUCTION

In 1909 Dr. Archibald Garrod stood before the Royal College of Physicians in London and presented his concept of "inborn error of metabolism." He used his clinical expertise in childhood illness, combined with insight from his mentorship in genetics, to make the argument for biochemical individuality.[1] Garrod believed organic acidurias were an example of genetic inheritance that could be linked to biochemical individuality and illness.[2] His concept was extremely controversial at the time because Mendelian genetics had generated major opposition.[3]

More than 100 years later, a multitude of inborn errors have been characterized through advances in molecular genomics.[2] Although urine organic acid profiling remains a valuable diagnostic tool for screening genetic disorders (as in the 1900s),[4] a new utility for organic acid profiling has emerged. Enzyme dysfunction (such as seen in inborn errors) leads to abnormal organic acids in the urine. Profiling these acids has been shown to provide insight into altered enzyme function. This chapter discusses the multitude of factors that lead to enzyme dysfunction and demonstrates that organic acid profiling is not limited to only inborn errors. Instead, organic acids are clinically relevant to uncover any factor driving enzyme dysfunction, whether *genetic, nutritional,* or *environmental* in origin.

The human genome project has taken Dr. Garrod's concept of genetic individuality to a new level. This genomic revolution has led to extensive research into the subtle differences in genes, particularly through single-nucleotide polymorphisms (SNPs). We are learning more each day about how person-to-person genetic variation predisposes to enzymatic dysfunction.

Furthermore, enzyme function can be influenced by environmental factors such as nutritional inadequacies, dysbiosis, toxic exposure, mitochondrial dysfunction, hormone imbalance, inflammation, and chronic disease.[5–7] For example, vitamin B_{12} deficiency leads to the excretion of methylmalonic acid in the urine, mimicking the inborn error of metabolism known as methylmalonic aciduria.[8] The ability of the environment to influence enzyme production and activity is referred to as epigenetics.

With a new understanding of genetic individuality and epigenomics in mind, this chapter demonstrates how organic acid profiling can assist the clinician in assessing both endogenous and exogenous factors that limit metabolic pathways and shows that organic acid testing ultimately leads to opportunities for therapeutic intervention. Organic acid profiling aids the clinician in designing personalized and targeted therapeutic interventions by providing a direct analysis of metabolic pathways.

LABORATORY ORGANIC ACID PROFILES

Organic acids are compounds produced through normal metabolic processes. They are commonly measured by first-morning void urinalysis and are often categorized into groups based on their clinical relevance. Common organic acid categories include the following:

- Organic acids produced by gut microbiota
- Organic acids of mitochondrial dysfunction
- Organic acids related to neurotransmitter metabolism
- Organic acids related to vitamin-dependent pathways
- Organic acids from toxic exposures and detoxification pathways

Organic acid interpretation requires clinical insight into the array of factors that can precipitate abnormal findings. The human metabolism is an incredibly complex and dynamic system, and clinicians often place emphasis on both individual biomarkers and abnormal biomarker patterns that may provide clinical insight. The following sections review these main categories of organic acids and discuss the clinical utility of measuring these biomarkers and metabolic pathways. Although more than 300 organic acids have been reported in the literature, this chapter focuses only on those most frequently presented on laboratory profiles. Note that urine testing should be avoided in patients with renal compromise because it can lead to false-positive urine findings.

Organic Acids Produced by Gut Microbiota

The gastrointestinal microbiome plays an important role in health and disease progression. One role of the gut flora is to digest and ferment components from the food, such as fibers, fatty acids, phenols, and many other compounds.[9] When gut bacteria act on these compounds, they create by-products, such as B vitamins. Several organic acids are also produced by gut bacterial fermentation and are systemically absorbed by the body. Bacterially derived compounds (also referred to as bacterial metabolites) can have significant beneficial or harmful activity in the body. These bacterial metabolites can be detected

through urine organic acid testing, and they have been studied in a variety of conditions.

Clinically profiling bacterial metabolites can assist in the detection of unhealthy gut bacteria alterations, commonly called dysbiosis. Antibiotic use has been correlated with decreased excretion of bacterial organic acids.[10,11] Conversely, excessive bacterial growth in the gastrointestinal (GI) tract (such as in small intestinal bacterial overgrowth [SIBO]; see Chapter 9) may lead to a predictable global increase in bacterial metabolite production.[12] Evaluating bacterially derived organic acids can, therefore, provide insight into the relative abundance of GI microbiota and their metabolic activity.

Other factors, such as dietary habits, can strongly influence the urine levels of these organic acids. Accordingly, individual test results require careful interpretation and clinical correlation, including an assessment of the dietary factors present at the time of testing.

Malabsorption Markers (Tryptophan and Phenylalanine Metabolites)

Protein malabsorption can lead to excessive bacterial production of particular organic acids. Hartnup disease is a well-researched example of this phenomenon. In this condition, amino acid absorption (particularly tryptophan) is impaired in the intestine and leads to excessive bacterial fermentation of tryptophan to indole metabolites (indole-3-acetate).[13] Therefore Hartnup disease serves as a model for assessing bacterial fermentation products as markers of amino acid malabsorption.

Indoleacetic acid (IAA), or indole-3-acetate, is produced by gut bacterial fermentation of the amino acid tryptophan (as is seen in Hartnup disease).[14] Urinary IAA may also function as a biomarker for abnormal tryptophan metabolism, which is a characteristic feature in neurocognitive disorders.[15] IAA is considered to be systemically inflammatory, and the literature has correlated excessive IAA production with chronic diseases such as cardiovascular disease.[16] Indoleacetic aciduria has also been found in autistic patients, which is intriguing, given that autism is commonly associated with both dysbiosis and disordered tryptophan metabolism.[17]

IAA was previously thought to be primarily derived from the bacteria *Clostridia bartletti* or similar *Clostridia* species[18]; however, common gut microbes such as *Escherichia coli* and *Saccharomyces* spp. have demonstrated the ability to synthesize IAA.[19,20] Therefore IAA ultimately may be shown to be another way that gut bacteria can produce systemically-active compounds that can disrupt metabolic pathways.

Indican is another indole compound commonly correlated with protein malabsorption and has been used to assess dysbiosis and overgrowth.[21–23]

Phenylacetic acid and 4-hydroxyphenylacetic acid are also produced by gut bacterial fermentation of amino acids, similar to IAA.[11,18] Clinicians have used them as malabsorption markers alongside IAA; however, other dietary factors contribute to their production (Table 29.1). Therefore these markers should also be thought of as phenolic metabolites, which are discussed in the next section.

Dysbiosis Markers (Phenolic Metabolites): DHPPA3, 3-HPA, 4-HPA, PAA, HPHPA, Benzoic Acid, Hippuric Acid, D-Lactate

There are numerous organic acids that originate, at least in part, from gut microbiota. These acids have been used clinically as an indirect assessment of dysbiosis. Many of these bacterial metabolites originate from the fermentation of dietary phenols and flavonoids. Therefore the greater the bacterial numbers/activity in the GI tract, the higher the levels of bacterial metabolites found in the urine.

Diets high in polyphenols can be reflected as elevated urine levels in organic acid testing (Table 29.2). Specifically, bacteria easily metabolize dietary substrates such as flavonoids, catechins, and caffeic acids into these organic acids.[24] This understanding leads to an alternate clinical utility for the phenolic metabolites. In the absence of dysbiosis, levels of phenolic metabolites may serve as a marker for the intake of healthy, antioxidant-rich foods.

Clinically, urinary phenylacetic acid has been shown to inversely correlate to depressive symptoms.[25–27] There are similar findings for both **3-hydroxyphenylacetic acid** (3-HPAA) and **4-hydroxyphenylacetic acid** (4-HPAA).[7,28] All of these microbial by-products may exhibit COX-2 inhibition and free-radical–scavenging properties, which may reduce colon cancer risk. This lends further support to the utility of these markers as an indication of antioxidant consumption.[29–31] When these biomarkers are not suspected to be elevated due to dietary influence, abnormalities may be due to imbalances in gut flora which often lead clinicians to assess GI function and treat where appropriate.[7]

Bacterial metabolism of polyphenols results in the excretion of **benzoic acid** and **hippuric acid**. Urinary benzoic acid can also originate from foods containing the preservative sodium benzoate. Hippuric acid is formed when sodium benzoate is conjugated with glycine.[32] Elevated levels of urinary hippuric acid have been associated with several conditions that may be linked to dysbiosis.[33,34] For example, elevated urinary hippurate was associated with an increase in blood pressure, which is due to the direct effect of gut-microbial products on blood pressure. However in other studies low hippuric acid excretion has also been attributed to dysbiosis, which supports its use as a biomarker for gut microbial alterations.[35]

3,4-Dihydroxyphenylpropionic acid (DHPPA) is a fermentation by-product of dietary phenols that are produced by several bacteria (including some *Clostridia* spp.).[36–40] DHPPA and other bacterial metabolites were previously thought to identify the presence of specific dysbiotic bacteria. However, as with IAA, ongoing research has shown that there are more bacteria capable of synthesizing these metabolites than previously thought.

Similarly, 3-(3'-hydroxyphenyl)-3-hydroxypropionic acid (**HPHPA**) has been associated with *Clostridia* spp. and autism in a few small studies.[41] However, elevated urinary levels of this metabolite have also been found after the consumption of tea, grapes, and citrus foods (particularly orange juice).[42–45] In the absence of high dietary intake of phenol-rich foods, production of HPHPA may be due to alterations in gut bacteria and always warrant follow-up stool testing to confirm a clinical suspicion of dysbiosis or infection.

Urinary excretion of **D-lactate** has been used as a marker for carbohydrate malabsorption and small bowel dysbiosis.[46] There was initial debate around the origin of this metabolite. Early research suggested it was solely produced from gut bacteria (particularly *Lactobacillus* spp.).[47] The most prevalent GI condition associated with D-lactic acidosis is short bowel syndrome.[48,49] However, research now shows that D-lactate can also originate from human metabolism.[50] Elevated urinary excretion has been found in multiple disease conditions, including diabetes, inflammatory bowel disease (IBD), infection, and trauma, and may be relevant in chronic fatigue syndrome.[50] Therefore D-lactate may be a better indicator of metabolic disturbance from chronic disease progression than gut dysbiosis.

Yeast and Fungal Markers (Arabinose, Arabinitol, Citramalic Acid, Tartaric Acid)

Candidiasis is a controversial topic among integrative, functional, and natural medicine practitioners. Despite being a frequently used term, the diagnosis of "*Candida* overgrowth" or "systemic *Candida*" is still a topic of debate, except in invasive candidiasis, a fungal sepsis. That being said, *gastrointestinal yeast* is a relatively common finding that ultimately may or may not reflect dysbiosis and/or infection.

Clinicians have used organic acid findings (as an alternative to stool testing) in an effort to detect the presence of gastrointestinal yeast. **D-arabinitol** is a sugar alcohol that is produced specifically by

TABLE 29.1 Organic Acids and Common Metabolic Associations

Analyte	Metabolic Pathway	Clinical Associations
Indoleacetic acid	Gut bacterial metabolism	Chronic inflammation, neurocognitive disorders, cardiovascular disease
Indican	Gut bacterial metabolism	Protein malabsorption/intake
Phenylacetic acid	Gut bacterial metabolism	Polyphenol intake
3-Hydroxyphenylacetic acid	Gut bacterial metabolism	Polyphenol intake, COX-2 inhibition, reduced colon cancer risk
4-Hydroxyphenylacetic acid	Gut bacterial metabolism	Polyphenol intake, COX-2 inhibition, reduced colon cancer risk
Benzoic acid	Gut bacterial metabolism	Polyphenol intake, sodium benzoate intake
Hippuric acid	Gut bacterial metabolism	Polyphenol intake, sodium benzoate intake
3,4-DHPPA	Gut bacterial metabolism	Polyphenol intake
HPHPA	Gut bacterial metabolism	Flavanoid intake
D-lactate	Bacterial or human by-product	Small bowel dysbiosis, diabetes, IBD, infection, chronic fatigue syndrome
D-arabinitol	Yeast product	Systemic invasive candidiasis
Arabinose	Fruit sugar	Fruit intake
Citramalic acid	Fruit sugar	Fruit intake
Tartaric acid	Phenolic food compound	Polyphenol intake, food additive/preservative
Adipic acid	Fatty acid metabolism	Insulin resistance/diabetes, fasting, ketogenic diet
Suberic acid	Fatty acid metabolism	Insulin resistance/diabetes, fasting, ketogenic diet
Ethylmalonic acid	Fatty acid metabolism	Insulin resistance/diabetes fasting, ketogenic diet
Pyruvic acid	Glycolysis	High carbohydrate intake
Lactic acid	Anaerobic metabolism	Hypoxic states: anemia, sleep apnea, recent exercise
β-hydroxybutyric acid	Ketone production	Insulin resistance/diabetes, fasting, ketogenic diet
β-hydroxymethylglutaric acid	HMG pathway	Ketosis, statin therapy (theoretically)
Citric acid	Citric acid cycle	Diabetes/metabolic syndrome, depression, hypertension, fibromyalgia, cancer, IBD, ASD, mitochondrial dysfunction
Cis-aconitic acid	Citric acid cycle	Mitochondrial dysfunction
Isocitric acid	Citric acid cycle	Mitochondrial dysfunction
α-Ketoglutaric acid	Citric acid cycle	Mitochondrial dysfunction
Succinic acid	Citric acid cycle	Mitochondrial dysfunction
Malic acid	Citric acid cycle	Mitochondrial dysfunction
Vanilmandelic acid	Epi-/norepinephrine metabolism	Neuroendocrine tumors, psychological stress, PTSD
3-Methoxy-4-hydroxyphenylglycol	Epi-/norepinephrine metabolism	Mood disorder (low), behavioral disorders (low)
Homovanillic acid	Dopamine metabolism	Fatigue, sleep apnea, mood disorder (low), neuroblastoma
5-Hydroxyindoleacetic acid	Serotonin metabolism	SSRI/5-HTP supplementation, neuroendocrine tumors, chronic migraine, inflammation, metabolic syndrome, cardiovascular disease, IBS-D
Kynurenic acid	Tryptophan metabolism	Mood disorder, chronic inflammation, insulin resistance
Quinolinic acid	Tryptophan metabolism	Mood disorder, chronic inflammation, insulin resistance
α-Ketoisovaleric acid	Amino acid metabolism	Muscle catabolism, BCAA supplementation, insulin resistance, B-vitamin need
α-Ketoisocaproic acid	Amino acid metabolism	Muscle catabolism, BCAA supplementation, insulin resistance, B-vitamin need
α-Keto-β-methylvaleric acid	Amino acid metabolism	Muscle catabolism, BCAA supplementation, insulin resistance, B-vitamin need
Formiminoglutamic acid	Amino acid metabolism	Alcohol consumption, OCP use, nitrous oxide exposure, folate/vitamin B_{12} Need
Methylmalonic acid	Amino acid metabolism	Vitamin B_{12} deficiency, inborn error
Xanthurenic acid	Tryptophan metabolism	Inflammation, vitamin B_6 need
3-Hydroxyisovaleric acid	Amino acid metabolism	Biotin need
3-Hydroxypropionic acid	Amino acid metabolism	Small bowel dysbiosis
Glutaric acid	Amino acid metabolism	Inborn error
Isovalerylglycine	Amino acid metabolism	Inborn error
α-Ketophenylacetic acid	Styrene exposure	Oxidative damage
α-Hydroxyisobutyric acid	MTBE exposure	Oxidative damage
2-Methylhippuric acid	Xylene exposure	Oxidative damage
Glucaric acid	Phase I and II detox	Oxidative stress, antioxidant intake
P-hydroxyphenyllactic acid	Gut bacterial metabolism	Unknown
Orotic acid	Urea cycle	Alcoholism, allopurinol use, inborn error
Pyroglutamic acid	Glutathione pathway	Poor amino acid status, poor glycine intake
α-Hydroxybutyric acid	Glutathione pathway	Insulin resistance, smoking, alcohol intake

ASD, Autism spectrum disorder; *BCAA,* branch-chain amino acid; *3,4-DHPPA,* 3,4-dihydroxyphenylpropionic acid; *HMG,* β-hydroxymethylglutaric acid; *HPHPA,* 3-(3′-hydroxyphenyl)-3-hydroxypropionic acid; *IBD,* inflammatory bowel disease; *IBS-D,* irritable bowel syndrome, diarrhea predominant; *MTBE,* methyl tert-butyl ether; *OCP,* oral contraceptive pill; *PTSD,* posttraumatic stress disorder; *SSRI,* selective serotonin reuptake inhibitor.

TABLE 29.2 Common Dietary Sources of Gut Bacterial Metabolites

Urinary Metabolite	Common Dietary Sources
Indoleacetic acid, Indican	High tryptophan intake, green/black tea[61]
Phenylacetic acid	Wine/grapes[62]
3,4-Dihydroxyphenopropionic acid	Whole grains, chocolate, coffee, green/black tea, olives/olive oil, citrus fruits (animal studies)[36,38,40,63–69]
3-Hydroxyphenylacetic acid and 4-hydroxyphenlyacetic acid	Wine/grapes,[62,65] cranberries,[66] green/black tea,[40] berries,[67,68] orange juice,[69] grape seed extract[70]
Benzoic acid/hippuric acid	Orange juice[71], elderberry,[72] huckleberry,[73] food preservative,[74] berries,[75] other flavanoids[76]
Arabinose	Widely distributed, grains, commercial sweeteners[77]
Citramalic acid	Apples,[54,55] cranberries,[56] sugar beets[57]
Tartaric acid	Wine/grapes,[78] chocolate,[79] food additive/preservative

Candida spp., and high urinary levels have been found in individuals with systemic invasive candidiasis.[51,52] However, it is not known if urinary D-arabinitol levels correlate to GI *Candida* spp.

Conversely, **arabinose** is not made by *Candida* but, rather, is a pentose sugar that can be fermented by *Candida* into D-arabinitol. **Arabinose, citramalic acid**, and **tartaric acid** were reported to be elevated in one case study of children with autistic features.[53] However, there is no research, to date, correlating these markers to clinical candidiasis. Because these organic acids are likely dietary in origin (particularly fruit),[54–58] abnormal markers warrant follow-up stool testing to confirm a suspicion of gastrointestinal candidiasis.59 As these markers may come from fruit intake, and given that many clinicians anecdotally monitor fruit intake when suspecting GI candidiasis, the former organic acids can aid in the management of GI yeast; however, this hypothesis has not been fully evaluated in the literature.

Looking ahead, the field of metabolomic profiling has been analyzing organic acid patterns in multiple disease populations. As more becomes known, it may eventually lead to an expanded application of bacterial metabolite profiling. If we know which organic acids are of bacterial origin and what "organic acid footprint" is associated with a disease, we can then begin to unravel the role that our gut flora plays in the development or prevention of that specific disease.[60] At this time we work with non-specific indicators of the "bacterial organic acid footprint" to demonstrate the presence or absence of dysbiosis.

Organic Acids of Mitochondrial Dysfunction

Clinicians use these organic acids as indicators of mitochondrial function, and abnormalities can provide therapeutic opportunities to support cellular and metabolic energy.

Fatty Acid Oxidation (Adipic Acid, Suberic Acid, and Ethylmalonic Acid)

In the mitochondria, fatty acids are converted into fuel sources through a process called beta-oxidation. Beta-oxidation is a critical step in meeting cellular energy demands. The first step in beta-oxidation is the conversion of fatty acids into acetyl coenzyme A (acetyl-CoA), which is the fuel used by the citric acid cycle to generate adenosine triphosphate (ATP). Fatty acid conversion is dependent on carnitine because these fatty acids must be transported across the mitochondrial membrane via the "carnitine shuttle."[80]

When beta-oxidation is impaired, fats are increasingly metabolized by an alternate pathway (omega-oxidation), resulting in elevated levels of specific dicarboxylic acids, such as **adipic**, **suberic**, and **ethylmalonic acids**.[81,82] Impaired beta-oxidation can occur due to carnitine deficiency or dysfunction of the enzymes involved and may lead to an accumulation of these dicarboxylic acids.[83,84] These dicarboxylic acids are then excreted in the urine. Elevated adipic, suberic, or ethylmalonic acid excretion is also characteristic of ketosis, such as in insulin resistance/diabetes, fasting, or low carbohydrate intake.[84]

Beyond metabolic ketosis, free fatty acid accumulation is a significant independent clinical finding because these acids lead to mitochondrial dysfunction by injuring the cell membrane and increasing free-radical production.[80,85] Therefore monitoring these acids provides insight into mitochondrial impairment. Adipic acid excretion has been found to be elevated in chronic alcohol use in males, suggesting another source of mitochondrial dysfunction.[86] However, clinicians should be aware that a high intake of medium-chain triglycerides (MCT oil, coconut, etc.) could also lead to isolated elevations of fatty acid metabolites.[87]

The "carnitine shuttle" is critically important to beta-oxidation. Therefore carnitine supplementation may help attenuate mitochondrial dysfunction and improve ATP synthesis.[80,88] Vitamin B_2 and magnesium availability also play an important role in optimal beta-oxidation, making them viable intervention options for elevated urinary fatty acids (such as in glutaric aciduria).[89–91]

Mitochondrial dysfunction has been implicated as a characteristic in innumerable chronic diseases. Therefore organic acid profiling can be a method to uncover subclinical disruptions of beta-oxidation and is a valuable tool for investigating mitochondrial dysfunction in the clinical setting.[92] Nutritional intervention, including, but not limited to, B vitamins, antioxidants, and carnitine, may be helpful when laboratory signs of mitochondrial dysfunction are found.[93]

Glycolysis (Pyruvic Acid and Lactic Acid)

Carbohydrates are another source of the cellular fuel acetyl-CoA. Carbohydrates typically contain glucose as a component, which is converted into **pyruvic acid** by a large enzyme called the pyruvate dehydrogenase complex (PDC). PDC requires multiple cofactors derived from vitamins B_1, B_2, B_3, and B_5. Thiamine (vitamin B_1) appears to be particularly important to the PDC enzyme.[94] In cases of an increased rate of glycolysis, vitamin B_1 supplementation was able to decrease lactic acid and pyruvic acid.[95] Pyruvic acid is also metabolized by another enzyme named pyruvate carboxylase, which requires biotin as a cofactor. When elevated pyruvic acid is paired with elevated 3-hydroxyisovaleric acid (discussed later), clinicians may evaluate biotin needs.

Conversely, elevated lactic acid and pyruvic acid in the urine can be caused by several inborn errors, such as pyruvate carboxylase deficiency.[96,97] Elevated urinary **lactic acid** may also originate from impaired glycemic control, such as is seen in insulin resistance and alcohol dependence.[98–100] Furthermore, lactic acid increases during hypoxic states and has been found to be elevated in sleep apnea.[101] Lastly, lactic acid is produced during strenuous exercise and has been used as a biomarker for exercise exertion.[102]

Citric Acid Cycle Metabolites

The citric acid cycle, or tricarboxylic acid cycle (TCA cycle), is a group of biochemical interactions that occur in every living cell's mitochondria. This cycle of reactions provides the necessary molecules for oxidative phosphorylation and is the biggest source for producing ATP, the main cellular energy source (Fig. 29.1).[103,104] Each step of the citric acid cycle is dependent on nutrient cofactors to assist in catalyzing each chemical reaction.

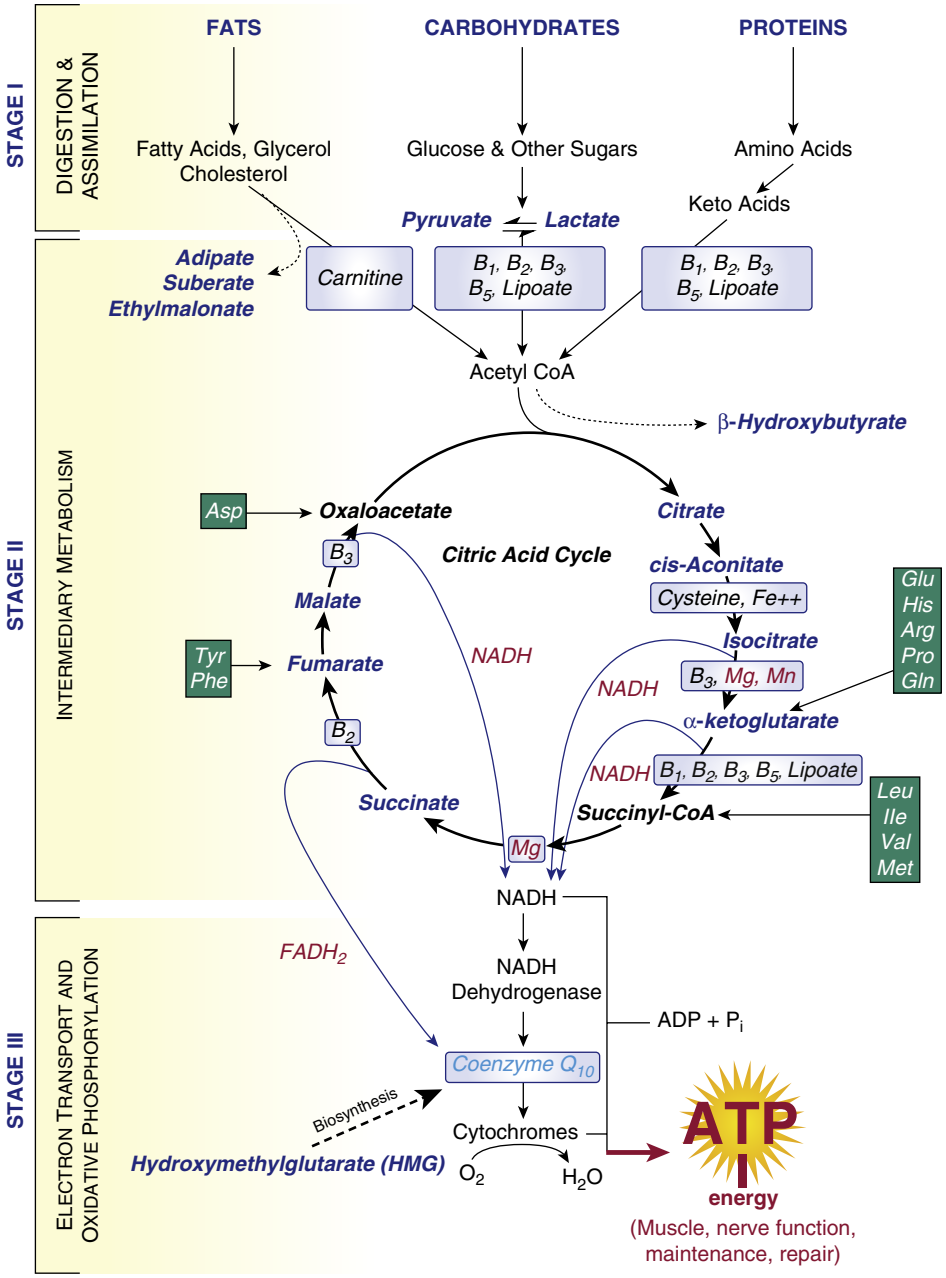


Fig. 29.1 Cellular energy pathways and commonly profiled organic acids.

β-hydroxybutyric acid (BHBA) is a ketone body. Ketones are generated in the liver when glucose metabolism is unable to meet energy demands. Heightened ketone production (ketosis) occurs during states such as prolonged fasting, exercise, and metabolic disease.[105] Elevations of β-hydroxybutyric acid can be used as an early risk indicator for diabetes, predicting impaired glucose tolerance and worsening glycemic control.[106–108] Low carbohydrate intake and ketogenic diets are likely to contribute to BHBA levels.

β-hydroxymethylglutaric acid (HMG) is another keto acid made from the molecule HMG-CoA and is a precursor to cholesterol and coenzyme Q_{10} synthesis. Inhibition of HMG-CoA (through statin therapy) may lead to higher circulating levels of HMG, showing up as elevated urinary HMG. Several inborn errors are known to produce 3-hydroxy-3-methylglutaric aciduria, with varying degrees of onset and clinical manifestations, ranging from neurodevelopmental disorders to cardiomyopathy.[109]

Citric acid cycle intermediates are tricarboxylic acids that make up the backbone of the TCA cycle. The predominance of literature surrounding abnormal citric acid cycle organic acid excretion is in the field of inborn errors of metabolism. However, advancing metabolomics research has begun to investigate metabolic "footprints" of various chronic diseases.[110–113] For example, altered urinary citric acid excretion has been demonstrated in metabolomics studies of diabetes/metabolic disease,[114–116] major depressive disorder,[28] hypertension,[117] fibromyalgia,[118] cancer,[119,120] and immune-mediated inflammatory disease (e.g., IBD).[121] Excretion of citric acid intermediates may also be abnormal in autism.[5,122,123] Furthermore, alterations in TCA cycle mechanics, leading to abnormal excretion of intermediates, has been associated with mitochondrial dysfunction,[124,125] linking them to conditions such as neurocognitive disease, diabetes, cancer, mood disorders, cardiovascular disease, and chronic fatigue syndrome.[92]

Many nutrient cofactors, particularly B vitamins and essential minerals, have demonstrated the capacity to protect mitochondrial function and are critical to the citric acid cycle.[94,126] Abnormal TCA cycle intermediates may reflect dysfunction of the TCA enzymes due to nutritional status, vitamin deficiency, toxicity, genetic polymorphism, or disease condition.[5,6,127–134] Abnormal urinary excretion of these acids may provide a window into various clinical conditions, as well as a potential therapeutic target to correct mitochondrial dysfunction.[103]

Genomics research may lead us to a greater understanding of how genetic individuality plays a role in the subclinical disruption of citric acid cycle mechanics. This information generates a novel utility for interpreting organic acids in the urine.[127] For example, homozygous enzyme defects of the citric acid cycle have been extensively studied. Recent studies suggest heterozygous defects may be tumorigenic.[131] If nutritional supplementation can attenuate these enzymatic weaknesses, whether they are genetic or acquired, clinical analysis of these metabolites may have vast clinical applications.[132] Attenuating subtle metabolic disruptions with adequate vitamin cofactor supplementation may significantly aid in chronic disease prevention or management.

Organic Acids of Neurotransmitter Metabolism

Neurotransmitter metabolites are an important group of organic acids. These compounds are breakdown products of neurotransmitter metabolism and can offer insight into neurotransmitter synthesis and degradation. Altered neurotransmitter metabolism can be due to enzyme impairment from chronic disease states, stress, genetic susceptibility, environmental toxins, and/or nutritional deficiencies.

Vanilmandelic acid (VMA) and **3-methoxy-4-hydroxyphenylglycol** (MHPG) originate from the breakdown of catecholamines, such as epinephrine and norepinephrine (Fig. 29.2). Catecholamines are integrally related to the stress response, both in the brain and peripherally. Therefore catecholamine metabolites have been used to assess sympathetic activity.[133] Much of the research on these markers has been focused on detecting neuroendocrine tumors and paragangliomas (particularly with VMA).[134–136] Elevated urinary VMA correlates to other physiological stress conditions, such as during competition, posttraumatic stress disorder (PTSD), and daily psycho-emotional stress.[137–140]

Low levels of catecholamine metabolites, particularly MHPG, have been found in mood and behavioral disorders.[141,142] Researchers have used this organic acid as an index for depression.[143] It is also interesting to note that because catecholamines are increased in response to exercise, MHPG has been explored as a method to evaluate the effectiveness of exercise as a therapeutic intervention in patients with affective disorders.[143,144]

Lower urinary MHPG has also been found in patients with attention deficit hyperactivity disorder (ADHD).[145,146] Disruption of catecholamine neurotransmission is a central hypothesis in ADHD, in both animal and human models.[147] Atomoxetine (a selective norepinephrine reuptake inhibitor) has efficacy in ADHD and may block norepinephrine in the central nervous system (CNS), allowing its breakdown and excretion.[148] Therefore urinary MHPG may be useful as a biomarker for serotonin–norepinephrine reuptake inhibitor (SNRI) therapy.[148,149]

Homovanillic acid (HVA) originates from dopamine. Similar to vanilmandelic acid, urinary HVA is elevated in neural crest tumors, such as neuroblastoma.[135,150] Dopamine is involved in learning and behavior modulation, and elevated urinary HVA has also been used as a dopamine turnover biomarker, seen in autism and other neurobehavioral disorders.[151] Dopamine also plays a role in the circadian rhythm, and elevated HVA correlates to dopamine-induced tiredness. For example, patients with obstructive sleep apnea present with varying degrees of sleepiness, and patients with apnea presenting with sleepiness and fatigue have much higher dopamine metabolite levels compared with patients with apnea with less sleepiness.[152] Conversely, low HVA levels may reflect low dopamine turnover in mood disorders, whereby medications that increase HVA correlate to therapeutic responsiveness.[153]

5-hydroxyindoleacetic acid (5-HIAA) is a metabolic by-product of serotonin.[154] Urinary 5-HIAA has been used as a predictive and prognostic biomarker for neuroendocrine tumors.[155–158] However, because it is a breakdown product of serotonin, patients taking over-the-counter 5-HTP, those taking psychiatric medications, or those who consume tryptophan-rich foods may anecdotally have high 5-HIAA levels in their urine.[158–160]

Aside from these factors, elevated 5-HIAA has been used as a marker of excess serotonin turnover in the periphery, which is associated with several clinical conditions. For example, higher levels have been associated with serotonin-induced infertility in men.[161] Similar risk associations have been found in chronic migraine, inflammation, metabolic syndrome, and cardiovascular disease.[162–164] Low urinary 5-HIAA levels has been found in some patient populations. One hypothesis of this association is that the need for niacin (vitamin B_3) shunts tryptophan away from serotonin production and into the kynurenine pathway which leads to NAD synthesis. Clinically, one may consider additional niacin support to correct this metabolic disturbance.[165]

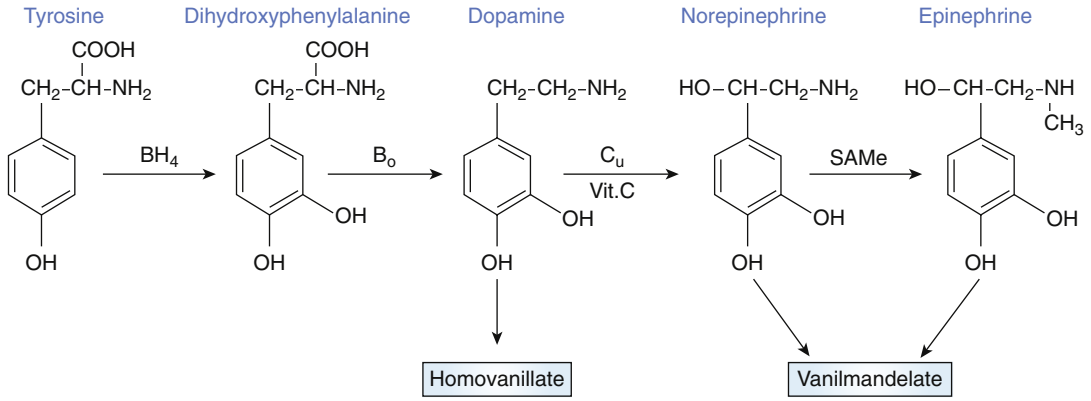


Fig. 29.2 Catecholamine metabolism and urinary metabolites.

The overwhelming majority of circulating serotonin is produced in the GI tract, not the central nervous system.[166] Therefore urinary 5-HIAA is primarily a reflection of gut-derived serotonin.[167,168] In the GI tract, alterations of serotonin metabolism have been associated with IBS. Higher urinary 5-HIAA was found in constipation-predominant irritable bowel syndrome (IBS),[169] whereas lower levels have been found in diarrhea-predominant IBS.[170] In a recent IBS study, 5-HTP supplementation led to increased turnover to 5-HIAA compared with controls (suggesting dysfunctional serotonin metabolism); however, supplementation ultimately improved intestinal barrier function.[171]

Tryptophan metabolism produces urinary **kynurenic acid** and **quinolinic acid**. Tryptophan is the amino acid precursor of serotonin. However, tryptophan is primarily metabolized in a different pathway, known as the *kynurenine pathway*. This alternate pathway is upregulated in response to inflammation and stress, which may cause deficient serotonin production.[172] Therefore urinary kynurenic acid and quinolinic acid are relevant in mood disorders and chronic inflammation.[173] Kynurenic acid can become further metabolized into quinolinic acid, which is an inflammatory and neurotoxic metabolite associated with depression.[174] Some laboratories calculate the ratio of kynurenic acid to quinolinic acid, whereby a low kyurenic:quinolinic ratio is suggestive of disturbed kynurenine metabolism leading to excessive quinolinic acid production (seen in mood disorders).[175] These metabolites also play a role in the development of insulin resistance and diabetes, which are common findings in depressed patients.[173]

The kynurenine pathway is particularly sensitive to vitamin B_6 deficiency, which has been shown to increase urinary kynurenic acid and xanthurenic acid (discussed in the next section).[176,177] Altered tryptophan metabolism and vitamin B_6 deficiency are suspected complications of the use of the oral contraceptive pill (OCP).[177,178] Vitamin B_6 supplementation has also been used to attenuate these alterations (at least partly); however, OCP use may disrupt tryptophan metabolism through other unknown mechanisms. Also, as previously mentioned, a major end-product of the kynurenine pathway is NAD, usually supplied by vitamin B_3. Therefore, clinicians may look at elevated kynurenine metabolites as an indication for needs for vitamin B_3.[165]

Organic Acids Related to Vitamin-Dependent Pathways

Some organic acids are used as specific markers to assess functional levels of vitamin cofactors. By examining pathways that are strongly influenced by nutrient availability, clinicians can home in on potential nutrient insufficiencies that can be targeted therapeutically.

Branch-Chain Amino Acid Intermediates (α-Keto Acids)

In the body, amino acids can be broken down to provide metabolites used in energy production. To do so, branch-chain amino acids (leucine, isoleucine, and valine) must be transported into the mitochondria via the carnitine shuttle. They are then converted into α-keto acids: **α-ketoisovaleric**, **α-ketoisocaproic**, and **α-keto-β-methylvaleric acids**.

After entering the mitochondria, α-keto acids are further metabolized by an enzyme complex known as the branch-chain α-keto acid dehydrogenase complex (BCKDC). BCKDC is the same enzyme responsible for the inborn metabolism error maple syrup urine disease. This enzyme complex requires multiple cofactors derived from vitamins B_1, B_2, B_3, and B_5 and lipoic acid. Because the breakdown of α-keto acids is dependent on BCKDC, deficiencies in the enzyme's cofactors may lead to increased α-keto acids in the systemic circulation and the urine (Fig. 29.3).[179]

Optimally functioning branch-chain amino acid (BCAA) metabolism is important in a variety of clinical conditions. As a group, BCAAs participate in protein synthesis, stimulate insulin release, spare lean muscle during weight loss or aging, and promote wound healing.[180] Elevated plasma BCAAs are associated with insulin resistance, which may be a result of decreased BCAA catabolism for energy production.[181] This metabolic disturbance could be compounded if nutrient deficiencies (such as vitamins B_1, B_2, B_3, and B_5) limit the activity of BCKDC.[179]

Therapeutically, B-vitamin support may be indicated with elevated urinary α-keto acids levels. In one study, supplementation demonstrated the ability to reduce α-keto acid excretion in healthy individuals, suggesting that elevated urinary α-keto acids is an indicator of the need for B vitamins.[182] Also, high BCAA turnover (such as in strenuous exercise, catabolic states, or BCAA supplementation) may contribute to the excretion of α-keto acids. The ability of B vitamins to attenuate these clinical states has not been fully researched.

Folate and B_{12} Markers: Formiminoglutamic Acid and Methylmalonic Acid

Formiminoglutamic acid (FIGlu) is the intermediate metabolite in the pathway that converts histidine to glutamic acid.[183] FIGlu metabolism depends on tetrahydrofolate (BH_4), a folate derivative, and accumulates with inadequate available folate. FIGlu accumulation is seen most dramatically in the inborn error formiminoglutamic aciduria. However, urinary FIGlu measurements have also been used as an indirect assessment of folate deficiency, dating back to the 1950s.[184] Initially called the "FIGlu test," patients with folate deficiency were shown to produce higher urinary FIGlu when given a large dose of L-histidine.[184]

In the literature, urinary FIGlu testing continues to be a marker for folate deficiency.[185,186] Compared with known folate-deficient patients, one study demonstrated that FIGlu has a high degree of specificity for

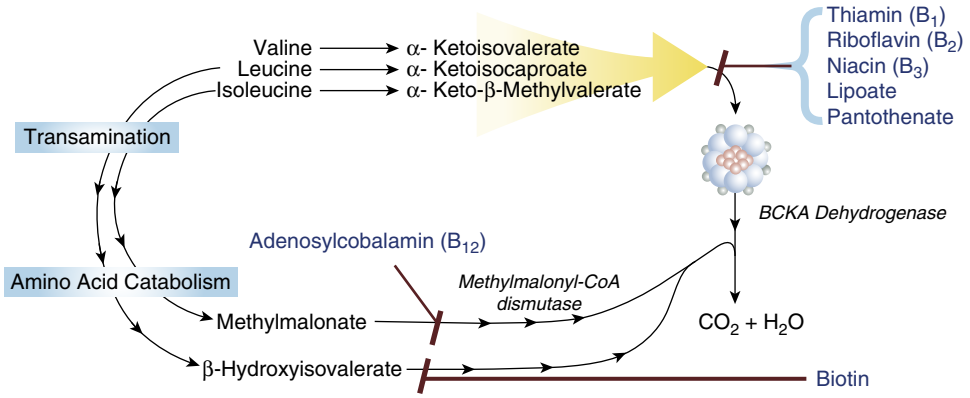


Fig. 29.3 Amino acid metabolism and B-vitamin cofactors.

detecting folate deficiency.[187] Multiple clinical conditions are associated with high urinary FIGlu, such as alcohol consumption, pregnancy, and birth control use.[188,189] Whether this is due to true folate depletion or another unknown mechanism is still debated.

Aside from folate evaluation, formiminoglutamic acid may reflect vitamin B_{12} status because folate recycling requires B_{12} as a cofactor. Vitamin B_{12} replacement decreased FIGlu levels in one study.[190] Both folate and vitamin B_{12} are critically important to methylation, which may support the utility of urinary FIGlu as a marker for disordered methylation capacity, as is seen in alcoholism.[191] Lending further clinical evidence, nitrous oxide (NO) is a known inhibitor of the enzyme that recycles folate (using B_{12}), and exposure to NO transiently increased urinary FIGlu, mimicking B_{12} deficiency.[192]

Methylmalonic acid (MMA) is an intermediate of methionine conversion to succinic acid for energy use. This pathway is vitamin B_{12} dependent, and MMA accumulation is indicative of the inborn error methylmalonic acidemia.[193] This inborn error can arise from variants in multiple genes, and it can present itself at any time in life, with varying degrees of severity.[194] Also, subtle genetic variants (SNPs) that can predispose individuals to B_{12} deficiency have been discovered.[195]

Because MMA conversion requires vitamin B_{12}, elevated levels of MMA are indicative of B_{12} deficiency.[196–199] Methylmalonic acid, as a functional biomarker, is considered a more sensitive index of B_{12} status compared with serum B_{12}.[200–204] Urinary MMA correlates with serum MMA, making the simple urine test a useful screening tool for B_{12} deficiency in at-risk populations, such as the elderly or patients with GI dysfunction.[196,202] Vitamin B_{12} therapy lowers MMA, and monitoring this metabolite may help prevent the consequences of B_{12} deficiency, such as cognitive decline and neuropathy.[204–206]

Other B-Vitamin Markers: Xanthurenic Acid, 3-Hydroxypropionic Acid, 3-Hydroxyisovaleric Acid, Glutaric Acid, Isovalerylglycine

Xanthurenic acid is a product of the tryptophan-kynurenine pathway discussed previously. Disturbances in this pathway have been evaluated in a variety of clinical conditions, including immune suppression, cancer progression, and immune/inflammatory reactions (such as in IBD).[207] The entire pathway is heavily dependent on vitamin B_6 availability; therefore xanthurenic acid has been studied as a biomarker for B_6 status.[208–210] Functional B_6 deficiency can be a consequence of dysglycemia, caused by inflammatory upregulation of the kynurenine pathway, ultimately leading to elevated levels of xanthurenic acid.[211] Also previously mentioned, kynurenine metabolites may become elevated when there are needs for vitamin B_3, which may lead clinicians to consider vitamin B_3 supplementation to address tryptophan-kynurenine abnormalities.[165]

Biotin, also referred to as vitamin B_7, is an essential vitamin cofactor in several enzymatic pathways. Certain clinical circumstances, such as pregnancy, vegetarianism, and anticonvulsant therapy, have been demonstrated to induce marginal biotin deficiency.[212,213] The organic acid **3-hydroxyisovaleric acid** is a functional marker for biotin status and can predict early biotin deficiency before serum biotin is affected.[213–216] **3-hydroxypropionic acid** is another biotin deficiency marker that has been studied; however, it does not appear to have the same diagnostic sensitivity.[217]

Glutaric acid and **isovalerylglycine** are two acids that are extremely elevated in inborn errors of acyl-CoA dehydrogenase. There are multiple forms of acyl-CoA dehydrogenase deficiencies, and most of them are diagnosed in neonates.[218] However, milder forms of this rare mitochondrial disorder exist, can have adult-onset presentation, and are responsive to riboflavin (vitamin B_2) and carnitine therapy.[218,219] Another interesting study demonstrated that elevated urinary levels of isovalerylglycine were found in anorexia nervosa patients. This is believed to be due to the effect of poor thyroid conversion of vitamin B_2 into active FAD, which is normalized in some patients after a refeeding program. Therefore vitamin B_2 need may be demonstrated by this urinary marker. Other causes of mitochondrial dysfunction may have a similar effect on beta-oxidation causing abnormalities of these markers.

Organic Acids From Toxic Exposures and Detoxification Pathways

Some organic acids relate to toxic exposure and detoxification capacity. These discrete biomarkers have increasing clinical utility in today's world (see Chapter 30, Urinary Porphyrins, and Chapter 35, Environmental Medicine). The following subsections provide a few examples of markers that help identify current exposures and the potential need for detoxification support.

Toxic Exposure Metabolites: α-Ketophenylacetic Acid, α-Hydroxyisobutyric Acid, 2-Methylhippurate

α-Ketophenylacetic acid (more commonly known as phenylglyoxylic acid) is a breakdown product of the petrochemical styrene. Styrene is a plastic ingredient found in numerous products, including insulation, fiberglass, carpet backing, pipes, and car and boat parts.[220] The most common source of styrene exposure is plastic food containers. Phenylglyoxylic acid (PGA) accounts for the majority of styrene detoxification in the body and serves as an exposure biomarker.[221] PGA can also originate from benzene, toluene, ethylbenzene, and xylene exposure.[222–224] Therefore high levels of urinary PGA warrant an investigation of any petrochemical contact because these chemicals are suspected to cause DNA damage.[221,224]

α-Hydroxyisobutyric acid is the major urinary metabolite of an industrial solvent called methyl tert-butyl ether (MTBE). MTBE was used as an additive to gasoline until the mid-2000s, when it was gradually phased out. However, considerable underground leakage has led to widespread groundwater and soil contamination.[225] Concerns about the tumorigenicity of chronic exposure to MTBE has been debated in the literature for a long time and played a significant role in removing MTBE from gasoline. However, it is still used in other industrial settings. Interestingly, MTBE is a powerful solvent, and injection of MTBE into the gallbladder has been a successful intervention to dissolve gallstones.[226,227]

Another organic acid commonly found in the urine is **2-methylhippurate**, which is a xylene-breakdown metabolite. Humans are exposed to xylene most commonly through volatile organic compounds (VOCs) from paints and varnish.[228] 2-methylhippuratue has also been found to be elevated in individuals exposed to pesticides.[229]

Detoxification Pathway Markers: Glucaric Acid, P-Hydroxyphenyllactic Acid, Orotic Acid, Pyroglutamic Acid, α-Hydroxybutyric Acid

Glucaric acid (or glucarate) is frequently used as an indirect marker for Phase I detoxification.[230–232] Many environmental chemicals, including pesticides and xenobiotics, are detoxified through Phase I enzymes. Therefore urinary glucaric acid is a valuable biomarker for exposure, as well as exposure-induced upregulation of detoxification enzymes.[233,234] Glucaric salts (such as calcium-D-glucarate), along with antioxidant-rich foods, are often used therapeutically to support detoxification. Therefore it is important to know that these therapies provide sources of glucaric acid that could influence urinary results.[235,236]

Traditionally, urine screening for elevated **orotic acid** was used to identify the inborn error orotic aciduria and as a means of evaluating other hyperammonemias.[237] Orotic acid has also been interpreted,

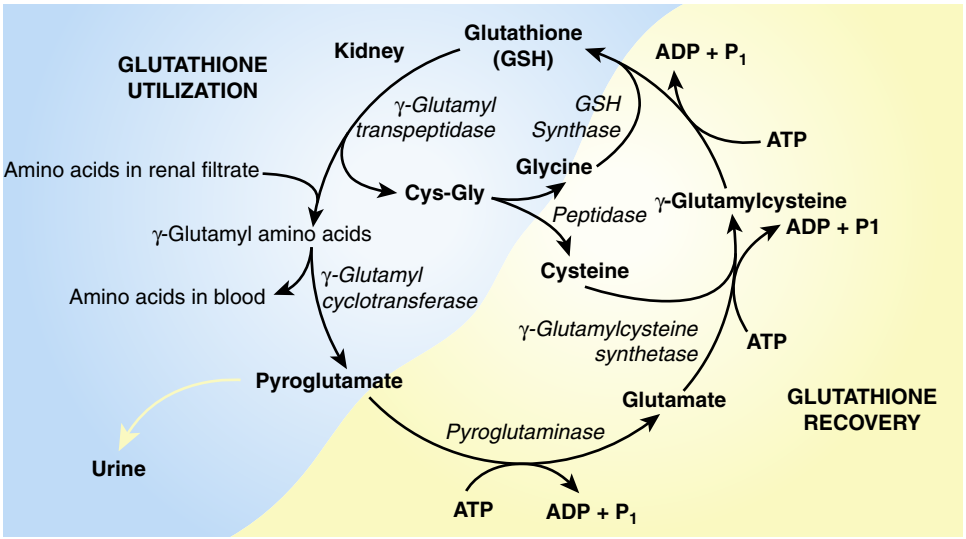


Fig. 29.4 The gamma-glutamyl cycle.

anecdotally, as a reflection of arginine status and urea cycle dysfunction; however, routine use of this biomarker to assess subclinical nutrient deficiency or hepatotoxicity is not well established in the literature.[238,239] Also, urinary orotic acid may arise from inhibition of other biochemical pathways. For example, the pyrimidine pathway used in nucleotide synthesis is inhibited by drug therapies such as allopurinol. This inhibition results in elevated urinary orotic acid.[240] Similar to orotic acid, urinary **sulfate** has also been used by clinicians to assess detoxification capacity. However, urinary sulfate has been shown to be heavily influenced by dietary habits, and therefore it is a relatively nonspecific biomarker.[241]

Pyroglutamic acid (also known as 5-oxoproline) is a metabolite of the gamma-glutamyl cycle. The gamma-glutamyl cycle is important because it assists in glutathione production and recycling (Fig. 29.4).[242] Glutathione is a powerful antioxidant, primarily responsible for cellular detoxification and redox balance.[242]

Glutathione is a tripeptide, consisting of three amino acids: glutamate, cysteine, and glycine. Glutathione synthesis requires sufficient availability of these amino acids, and deficiency of any of the three amino acids can affect glutathione production.[242] Elevated pyroglutamic acid may reflect nutritional needs for the amino acid glutathione precursors, particular glycine.[243,244] In individuals consuming low-protein/vegetarian diets, elevated pyroglutamic acid levels were significantly reduced when protein was supplemented.[245] To lend further support to pyroglutamic acid as a marker for glycine need, glycine supplementation has demonstrated the capacity to return high pyroglutamic acid excretion to normal and increase glutathione levels.[246]

Whereas the gamma-glutamyl cycle is responsible for recycling glutathione, it is the transsulfuration pathway that produces glutathione. The biomarker **α-hydroxybutyrate** (2-hydroxybuturic acid [2-HB]) is hypothesized to be a marker for upregulation of the transsulfuration pathway.[238] However, the majority of the literature demonstrates that 2-HB has a stronger correlation with dysglycemia. Some articles have suggested that it is one of the most significant biomarkers available for early determination of insulin resistance and metabolic disturbance.[247,248] Metabolic stress creates an imbalance in NADH/NAD ratios, which leads directly to the production of 2-HB.[249,250] 2-HB levels may be affected by smoking, alcohol consumption, exercise, and dietary habits. Thus 2-HB could prove to be a biomarker for unhealthy lifestyle habits.[251]

SUMMARY

Organic acid analysis is a powerful clinical tool. Originating as a way to diagnose inborn errors of metabolism, it now has been adopted into the evolving field of metabolomics, genomics, and personalized medicine. Clinicians aiming to target individual metabolic weaknesses use organic acid analysis as a backbone assessment. Numerous examples have been discussed in the medical profession of the dramatic positive clinical outcomes that have resulted from targeted treatments based on organic acid abnormalities.

Looking to the future, many more organic acids will likely be evaluated and added to current organic acid profiles. Organic acid profiling will continue to expand our understanding of the multitude of factors that precipitate individual susceptibility to chronic diseases and, therefore, guide the therapies that can help prevent them. Organic acid analysis provides insight into genetic metabolic weaknesses, environmental insults, and diet and lifestyle factors that limit optimal biochemical function. These abnormalities are likely at the root of innumerable chronic diseases, and correcting these abnormalities could have profound application in overall disease regression or prevention. Tomorrow's medicine will be an integration of Dr. Garrod's genetic individuality concept with functional profiling of biochemical pathways, ultimately making organic acid profiling critically important to clinicians focused on personalized medical care.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Scriver CR. Garrod's Croonian Lectures (1908) and the charter 'Inborn Errors of Metabolism': albinism, alkaptonuria, cystinuria, and pentosuria at age 100 in 2008. *J Inherit Metab Dis*. 2008;31(5):580–598.
2. Martins AM. Inborn errors of metabolism: a clinical overview. *Sao Paulo Med J*. 1999;117:251–265.
3. Weatherall DJ. The centenary of Garrod's Croonian lectures. *Clin Med(London, England)*. 2008;8(3):309–311.
4. Tanaka K, Hine DG, West-Dull A, Lynn TB. Gas-chromatographic method of analysis for urinary organic acids. I. Retention indices of 155 metabolically important compounds. *Clin Chem*. 1980;26(13):1839–1846.
5. Tsoukalas D, Alegakis A, Fragkiadaki P, et al. Application of metabolomics: focus on the quantification of organic acids in healthy adults. *Int J Mol Med*. 2017;40(1):112–120.
6. Astarita G, Langridge J. An emerging role for metabolomics in nutrition science. *J Nutrigenet Nutrigenomics*. 2013;6(4–5):181–200.
7. Zheng P, Wang Y, Chen L, et al. Identification and validation of urinary metabolite biomarkers for major depressive disorder. *Mol Cell Proteomics: MCP*. 2013;12(1):207–214.
8. van Wijngaarden JP, Dhonukshe-Rutten RAM, Brouwer-Brolsma EM, et al. Vitamin B12 intake and related biomarkers: associations in a Dutch Elderly population. *J Nut Health Aging*. 2017;21(10):1268–1276.
9. Shortt C, Hasselwander O, Meynier A, et al. Systematic review of the effects of the intestinal microbiota on selected nutrients and non-nutrients. *Eur J Nutr*. 2017.
10. Hryhorczuk LM, Novak EA, Gershon S. Gut flora and urinary phenylacetic acid. *Science (New York, NY)*. 1984;226(4677):996.
11. Mora Brugues J, Gonzalez Sastre F. Influence of intestinal flora on the elimination of phenylacetic acid in urine. *Clin Chem*. 1986;32(1 Pt 1):223.
12. Grace E, Shaw C, Whelan K, Andreyev HJN. Review article: small intestinal bacterial overgrowth—prevalence, clinical features, current and developing diagnostic tests, and treatment. *Aliment Pharmacol Ther*. 2013;38(7):674–688.
13. Wong PW, Lambert AM, Pillai PM, Jones PM. Observations on nicotinic acid therapy in Hartnup disease. *Arch Dis Child*. 1967;42(226):642–646.
14. Blaut M, Clavel T. Metabolic diversity of the intestinal microbiota: implications for health and disease. *J Nutr*. 2007;137(3):751S–755S.
15. Karu N, McKercher C, Nichols DS, et al. Tryptophan metabolism, its relation to inflammation and stress markers and association with psychological and cognitive functioning: Tasmanian Chronic Kidney Disease pilot study. *BMC Nephrol*. 2016;17(1):171.
16. Sallée M, Dou L, Cerini C, Poitevin S, Brunet P, Burtey S. The Aryl hydrocarbon receptor-activating effect of uremic toxins from tryptophan metabolism: a new concept to understand cardiovascular complications of chronic kidney disease. *Toxins*. 2014;6(3):934–949.
17. Gevi F, Zolla L, Gabriele S, Persico AM. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. *Molecular Autism*. 2016;7:47.
18. Russell WR, Duncan SH, Scobbie L, et al. Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. *Mol Nutr Food Res*. 2013;57(3):523–535.
19. Rao RP, Hunter A, Kashpur O, Normanly J. Aberrant synthesis of indole-3-acetic acid in Saccharomyces cerevisiae triggers morphogenic transition, a virulence trait of pathogenic fungi. *Genetics*. 2010;185(1):211–220.
20. Evenepoel P, Meijers BKI, Bammens BRM, Verbeke K. Uremic toxins originating from colonic microbial metabolism. *Kidney Int*. 76:S12-S19.
21. Aarbakke J, Schjonsby H. Value of urinary simple phenol and indican determinations in the diagnosis of the stagnant loop syndrome. *Scand J Gastroenterol*. 1976;11(4):409–414.
22. King CE, Toskes PP. Small intestine bacterial overgrowth. *Gastroenterology*. 1979;76(5):1035–1055.
23. Novis BH, Bank S, Marks IN, Redmond AO. The value of estimating urinary indican. *S Afr Med J*. 1971;45(41):1167–1170.
24. Del Rio D, Stalmach A, Calani L, Crozier A. Bioavailability of coffee chlorogenic acids and green tea flavan-3-ols. *Nutrients*. 2010;2(8):820.
25. Sabelli H, Fawcett J, Gusovsky F, Edwards J, Jeffriess H, Javaid J. Phenylacetic acid as an indicator in bipolar affective disorders. *J Clin Psychopharmacol*. 1983;3(4):268–270.
26. Sabelli HC, Fawcett J, Gusovsky F, et al. Clinical studies on the phenylethylamine hypothesis of affective disorder: urine and blood phenylacetic acid and phenylalanine dietary supplements. *J Clin Psychiat*. 1986;47(2):66–70.
27. Sabelli HC, Javaid JI, Fawcett J, Kravitz HM, Wynn P. Urinary phenylacetic acid in panic disorder with and without depression. *Acta Psychiatr Scand*. 1990;82(1):14–16.
28. Zheng P, Chen JJ, Zhou CJ, et al. Identification of sex-specific urinary biomarkers for major depressive disorder by combined application of NMR- and GC-MS-based metabonomics. *Transl Psychiatry*. 2016;6(11):e955.
29. Amic A, Markovic Z, Markovic JMD, Jeremic S, Lucic B, Amic D. Free radical scavenging and COX-2 inhibition by simple colon metabolites of polyphenols: a theoretical approach. *Computl Biol Chem*. 2016;65:45–53.
30. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(1):230S–242S.
31. Selma MV, Espín JC, Tomás-Barberán FA. Interaction between phenolics and gut microbiota: role in human health. *J Agricult Food Chem*. 2009;57(15):6485–6501.
32. Badenhorst CP, Erasmus E, van der Sluis R, Nortje C, van Dijk AA. A new perspective on the importance of glycine conjugation in the metabolism of aromatic acids. *Drug Metabol Rev*. 2014;46(3):343–361.
33. Lees HJ, Swann JR, Wilson ID, Nicholson JK, Holmes E. Hippurate: the natural history of a mammalian-microbial cometabolite. *J Proteome Res*. 2013;12(4):1527–1546.
34. Loo RL, Zou X, Appel LJ, Nicholson JK, Holmes E. Characterization of metabolic responses to healthy diets and association with blood pressure: application to the Optimal Macronutrient Intake Trial for Heart Health (OmniHeart), a randomized controlled study. *Am J Clin Nutr*. 2018;107(3):323–334.
35. Williams HR, Cox IJ, Walker DG, et al. Differences in gut microbial metabolism are responsible for reduced hippurate synthesis in Crohn's disease. *BMC Gastroenterology*. 2010;10:108.
36. Hanske L, Loh G, Sczesny S, Blaut M, Braune A. The bioavailability of apigenin-7-glucoside is influenced by human intestinal microbiota in rats. *J Nutr*. 2009;139(6):1095–1102.
37. Pereira-Caro G, Fernandez-Quiros B, Ludwig IA, Pradas I, Crozier A, Moreno-Rojas JM. Catabolism of citrus flavanones by the probiotics Bifidobacterium longum and Lactobacillus rhamnosus. *Eur J Nutr*. 2016.
38. Rios LY, Gonthier M-P, Rémésy C, et al. Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am J Clin Nutr*. 2003;77(4):912–918.
39. Ludwig IA, Paz de Pena M, Concepcion C, Alan C. Catabolism of coffee chlorogenic acids by human colonic microbiota. *BioFactors (Oxford, England)*. 2013;39(6):623–632.
40. Henning SM, Wang P, Abgaryan N, et al. Phenolic acid concentrations in plasma and urine from men consuming green or black tea and potential chemopreventive properties for colon cancer. *Mol Nutr Food Res*. 2013;57(3):483–493.
41. Xiong X, Liu D, Wang Y, Zeng T, Peng Y. Urinary 3-(3-hydroxyphenyl)-3-hydroxypropionic acid, 3-hydroxyphenylacetic acid, and 3-hydroxyhippuric acid are elevated in children with autism spectrum disorders. *BioMed Res Int*. 2016;2016:9485412.
42. van Dorsten FA, Grun CH, van Velzen EJ, Jacobs DM, Draijer R, van Duynhoven JP. The metabolic fate of red wine and grape juice polyphenols in humans assessed by metabolomics. *Mol Nutr Food Res*. 2010;54(7):897–908.
43. Roowi S, Stalmach A, Mullen W, Lean MEJ, Edwards CA, Crozier A. Green tea flavan-3-ols: colonic degradation and urinary excretion of catabolites by humans. *J Agric Food Chem*. 2010;58(2):1296–1304.
44. Pereira-Caro G, Borges G, van der Hooft J, et al. Orange juice (poly)phenols are highly bioavailable in humans. *Am J Clin Nutr*. 2014;100(5):1378–1384.

45. Roowi S, Mullen W, Edwards CA, Crozier A. Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans. *Mol Nutr Food Res*. 2009;53(S1):S68–S75.
46. Wallis A, Ball M, McKechnie S, Butt H, Lewis DP, Bruck D. Examining clinical similarities between myalgic encephalomyelitis/chronic fatigue syndrome and d-lactic acidosis: a systematic review. *J Trans Med*. 2017;15:129.
47. Thurn JR, Pierpont GL, Ludvigsen CW, Eckfeldt JH. D-lactate encephalopathy. *Am J Med*. 1985;79(6):717–721.
48. Adeva-Andany M, Lopez-Ojen M, Funcasta-Calderon R, et al. Comprehensive review on lactate metabolism in human health. *Mitochondrion*. 2014;17:76–100.
49. Kowlgi NG, Chhabra L. D-Lactic acidosis: an underrecognized complication of short bowel syndrome. *Gastroenterol Res Pract*. 2015;2015:8.
50. Scheijen JLJM, Hanssen NMJ, van de Waarenburg MPH, Jonkers DMAE, Stehouwer CDA, Schalkwijk CG. L(+) and D(−) lactate are increased in plasma and urine samples of type 2 diabetes as measured by a simultaneous quantification of L(+) and D(−) lactate by reversed-phase liquid chromatography tandem mass spectrometry. *Exp Diabetes Res*. 2012;2012:234812.
51. Bernard EM, Wong B, Armstrong D. Stereoisomeric configuration of arabinitol in serum, urine, and tissues in invasive candidiasis. *J Infect Dis*. 1985;151(4):711–715.
52. Yeo SF, Wong B. Current status of nonculture methods for diagnosis of invasive fungal infections. *Clin Microbiol Rev*. 2002;15(3):465–484.
53. Shaw W, Kassen E, Chaves E. Increased urinary excretion of analogs of Krebs cycle metabolites and arabinose in two brothers with autistic features. *Clin Chem*. 1995;41(8 Pt 1):1094–1104.
54. Sugimoto N, Forsline P, Beaudry R. Volatile profiles of members of the USDA Geneva Malus Core Collection: utility in evaluation of a hypothesized biosynthetic pathway for esters derived from 2-methylbutanoate and 2-methylbutan-1-ol. *J Agric Food Chem*. 2015;63(7):2106–2116.
55. Hulme AC. The isolation of l-citramalic acid from the peel of the apple fruit. *Biochimica et Biophysica Acta*. 1954;14(1):36–43.
56. Liu H, Garrett TJ, Su Z, Khoo C, Gu L. UHPLC-Q-Orbitrap-HRMS-based global metabolomics reveal metabolome modifications in plasma of young women after cranberry juice consumption. *J Nutr Biochem*. 2017;45:67–76.
57. Khorassani R, Hettwer U, Ratzinger A, Steingrobe B, Karlovsky P, Claassen N. Citramalic acid and salicylic acid in sugar beet root exudates solubilize soil phosphorus. *BMC Plant Biol*. 2011;11:121.
58. Marconi O, Floridi S, Montanari L. Organic acids profile in tomato juice by HPLC with UV detection. *J Food Quality*. 2007;30(2):253–266.
59. Akins RS, Angkustsiri K, Hansen RL. Complementary and alternative medicine in autism: an evidence-based approach to negotiating safe and efficacious interventions with families. *Neurotherapeutics*. 2010;7(3):307–319.
60. Rojo D, Mendez-Garcia C, Raczkowska BA, et al. Exploring the human microbiome from multiple perspectives: factors altering its composition and function. *FEMS Microbiol Rev*. 2017;41(4):453–478.
61. van der Hooft JJ, de Vos RC, Mihaleva V, et al. Structural elucidation and quantification of phenolic conjugates present in human urine after tea intake. *Anal Chem*. 2012;84(16):7263–7271.
62. Jacobs DM, Fuhrmann JC, van Dorsten FA, et al. Impact of short-term intake of red wine and grape polyphenol extract on the human metabolome. *J Agric Food Chem*. 2012;60(12):3078–3085.
63. Gonthier M-P, Verny M-A, Besson C, Rémésy C, Scalbert A. Chlorogenic Acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J Nutr*. 2003;133(6):1853–1859.
64. Booth AN, Jones FT. Metabolic fate of hesperidin, eriodictyol, homoeriodictyol, and diosmin. *J Biol Chem*. 1958;230(2):661–668.
65. Zamora-Ros R, Achaintre D, Rothwell JA, et al. Urinary excretions of 34 dietary polyphenols and their associations with lifestyle factors in the EPIC cohort study. *Scientific Rep*. 2016;6:26905.
66. Feliciano RP, Boeres A, Massacessi L, et al. Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols. *Arch Biochem Biophys*. 2016;599:31–41.
67. Gill CI, McDougall GJ, Glidewell S, et al. Profiling of phenols in human fecal water after raspberry supplementation. *J Agric Food Chem*. 2010;58(19):10389–10395.
68. Koli R, Erlund I, Jula A, Marniemi J, Mattila P, Alfthan G. Bioavailability of various polyphenols from a diet containing moderate amounts of berries. *J Agric Food Chem*. 2010;58(7):3927–3932.
69. Roowi S, Mullen W, Edwards CA, Crozier A. Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans. *Mol Nutr Food Res*. 2009;53(suppl 1):S68–S75.
70. Ward NC, Croft KD, Puddey IB, Hodgson JM. Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic acid, an important metabolite of proanthocyanidins in humans. *J Agric Food Chem*. 2004;52(17):5545–5549.
71. Pereira-Caro G, Ludwig IA, Polyviou T, et al. Identification of plasma and urinary metabolites and catabolites derived from orange juice (poly)phenols: analysis by high-performance liquid chromatography-high-resolution mass spectrometry. *J Agric Food Chem*. 2016;64(28):5724–5735.
72. de Ferrars RM, Cassidy A, Curtis P, Kay CD. Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. *Mol Nutr Food Res*. 2014;58(3):490–502.
73. Heinrich J, Valentova K, Vacek J, et al. Metabolic profiling of phenolic acids and oxidative stress markers after consumption of Lonicera caerulea L. fruit. *J Agric Food Chem*. 2013;61(19):4526–4532.
74. Leth T, Christensen T, Larsen IK. Estimated intake of benzoic and sorbic acids in Denmark. *Food Additives & Contaminants Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*. 2010;27(6):783–792.
75. Williamson G, Clifford MN. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br J Nutr*. 2010;104(suppl 3):S48–S66.
76. Loke WM, Jenner AM, Proudfoot JM, et al. A metabolite profiling approach to identify biomarkers of flavonoid intake in humans. *J Nutr*. 2009;139(12):2309–2314.
77. Krog-Mikkelsen I, Hels O, Tetens I, Holst JJ, Andersen JR, Bukhave K. The effects of L-arabinose on intestinal sucrase activity: dose-response studies in vitro and in humans. *Am J Clin Nutr*. 2011;94(2):472–478.
78. Regueiro J, Vallverdú-Queralt A, Simal-Gándara J, Estruch R, Lamuela-Raventós RM. Urinary tartaric acid as a potential biomarker for the dietary assessment of moderate wine consumption: a randomised controlled trial. *Br J Nutr*. 2014;111(9):1680–1685.
79. Lawson AM, Chalmers RA, Watts RW. Urinary organic acids in man. I. Normal patterns. *Clin Chem*. 1976;22(8):1283–1287.
80. Sharma S, Black SM. Carnitine homeostasis, mitochondrial function, and cardiovascular disease. *Drug Discov Today Dis Mech*. 2009;6(1–4):e31–e39.
81. Gregersen N, Ingerslev J. The excretion of C6-C10-dicarboxylic acids in the urine of newborn infants during starvation. Evidence for omega-oxidation of fatty acids in the newborn. *Acta Paediatr Scand*. 1979;68(5):677–681.
82. Rinaldo P, Welch RD, Previs SF, et al. Ethylmalonic/adipic aciduria: effects of oral medium-chain triglycerides, carnitine, and glycine on urinary excretion of organic acids, acylcarnitines, and acylglycines. *Pediatr Res*. 1991;30(3):216–221.
83. Semba RD, Trehan I, Li X, et al. Environmental enteric dysfunction is associated with carnitine deficiency and altered fatty acid oxidation. *E Bio Med*. 2017;17:57–66.
84. Villarreal-Pérez JZ, Villarreal-Martínez JZ, Lavalle-González FJ, et al. Plasma and urine metabolic profiles are reflective of altered beta-oxidation in non-diabetic obese subjects and patients with type 2 diabetes mellitus. *Diabetol Metabol Syndr*. 2014;6:129.
85. Chang B, Nishikawa M, Nishiguchi S, Inoue M. L-carnitine inhibits hepatocarcinogenesis via protection of mitochondria. *Int J Cancer*. 2005;113(5):719–729.
86. Ma X, Baraona E, Goozner BG, Lieber CS. Gender differences in medium-chain dicarboxylic aciduria in alcoholic men and women. *Am J Med*. 1999;106(1):70–75.
87. Tserng KY, Griffin RL, Kerr DS. Distinction of dicarboxylic aciduria due to medium-chain triglyceride feeding from that due to abnormal fatty acid oxidation and fasting in children. *Metabolism*. 1996;45(2):162–167.

88. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. *Pharmacol Rev*. 2002;54(1):101–127.
89. Kaluzna-Czaplinska J, Socha E, Rynkowski J. B vitamin supplementation reduces excretion of urinary dicarboxylic acids in autistic children. *Nutr Res (New York, NY)*. 2011;31(7):497–502.
90. Nagao M, Tanaka K. FAD-dependent regulation of transcription, translation, post-translational processing, and post-processing stability of various mitochondrial acyl-CoA dehydrogenases and of electron transfer flavoprotein and the site of holoenzyme formation. *J Biol Chem*. 1992;267(25):17925–17932.
91. Olsen Rikke K, Koňaříková E, Giancaspero Teresa A, et al. Riboflavin-responsive and non-responsive mutations in FAD synthase cause multiple acyl-CoA Dehydrogenase and combined respiratory-chain deficiency. *Am J Hum Gene*. 2016;98(6):1130–1145.
92. Pieczenik SR, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. *Exp Mol Pathol*. 2007;83(1):84–92.
93. Fenech M. Vitamins associated with brain aging, mild cognitive impairment, and Alzheimer disease: biomarkers, epidemiological and experimental evidence, plausible mechanisms, and knowledge gaps. *Advan Nutr (Bethesda, Md)*. 2017;8(6):958–970.
94. Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. *Chem-Biol Interact*. 2006;163(1–2):94–112.
95. Falder S, Silla R, Phillips M, et al. Thiamine supplementation increases serum thiamine and reduces pyruvate and lactate levels in burn patients. *Burns*. 2010;36(2):261–269.
96. Bhandary S, Aguan K. Pyruvate dehydrogenase complex deficiency and its relationship with epilepsy frequency—an overview. *Epilepsy Res*. 2015;116:40–52.
97. Maesaka H, Komiya K, Misugi K, Tada K. Hyperalaninemia hyperpyruvicemia and lactic acidosis due to pyruvate carboxylase deficiency of the liver; treatment with thiamine and lipoic acid. *Eur J Pediatr*. 1976;122(2):159–168.
98. Schlecht I, Gronwald W, Behrens G, et al. Visceral adipose tissue but not subcutaneous adipose tissue is associated with urine and serum metabolites. *PloS One*. 2017;12(4):e0175133.
99. Friedrich N, Skaaby T, Pietzner M, et al. Identification of urine metabolites associated with 5-year changes in biomarkers of glucose homoeostasis. *Diabetes Metabol*. 2017.
100. Mostafa H, Amin AM, Teh CH, Murugaiyah V, Arif NH, Ibrahim B. Metabolic phenotyping of urine for discriminating alcohol-dependent from social drinkers and alcohol-naive subjects. *Drug Alcohol Depend*. 2016;169:80–84.
101. Hira HS, Shukla A, Kaur A, Kapoor S. Serum uric acid and lactate levels among patients with obstructive sleep apnea syndrome: which is a better marker of hypoxemia? *Ann Saudi Med*. 2012;32(1):37–42.
102. Nikolaidis S, Kosmidis I, Sougioultzis M, Kabasakalis A, Mougios V. Diurnal variation and reliability of the urine lactate concentration after maximal exercise. *Chronobiol Int*. 2018;35(1):24–34.
103. Nicolson GL. Mitochondrial dysfunction and chronic disease: treatment with natural supplements. *Altern Ther Health Med*. 2014;20(suppl 1):18–25.
104. Akram M. Citric acid cycle and role of its intermediates in metabolism. *Cell Biochem Biophys*. 2014;68(3):475–478.
105. Newman JC, Verdin E. β-hydroxybutyrate: much more than a metabolite. *Diabetes Res Clin Pract*. 2014;106(2):173–181.
106. Trico D, Prinsen H, Giannini C, et al. Elevated alpha-hydroxybutyrate and branched-chain amino acid levels predict deterioration of glycemic control in adolescents. *J Clin Endocrinol Metabol*. 2017;102(7):2473–2481.
107. Mahendran Y, Vangipurapu J, Cederberg H, et al. Association of ketone body levels with hyperglycemia and type 2 diabetes in 9,398 Finnish men. *Diabetes*. 2013;62(10):3618–3626.
108. Cobb J, Eckhart A, Perichon R, et al. A novel test for IGT utilizing metabolite markers of glucose tolerance. *J Diabetes Sci Technol*. 2015;9(1):69–76.
109. Wortmann SB, Kluijtmans LA, Engelke UFH, Wevers RA, Morava E. The 3-methylglutaconic acidurias: what's new? *J Inher Metabol Dis*. 2012;35(1):13–22.
110. Puchades-Carrasco L, Pineda-Lucena A. Metabolomics applications in precision medicine: an oncological perspective. *Curr Top Med Chem*. 2017;17(24):2740–2751.
111. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome Biol*. 2017;18(1):83.
112. Basak T, Varshney S, Akhtar S, Sengupta S. Understanding different facets of cardiovascular diseases based on model systems to human studies: a proteomic and metabolomic perspective. *J Proteomics*. 2015;127(Pt A):50–60.
113. Kałużna-Czaplińska J. Noninvasive urinary organic acids test to assess biochemical and nutritional individuality in autistic children. *Clin Biochem*. 2011;44(8):686–691.
114. Zuppi C, Messana I, Tapanainen P, et al. Proton nuclear magnetic resonance spectral profiles of urine from children and adolescents with type 1 diabetes. *Clin Chem*. 2002;48(4):660–662.
115. van Doorn M, Vogels J, Tas A, et al. Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in Type 2 diabetes mellitus patients and healthy volunteers. *Br J Clin Pharmacol*. 2007;63(5):562–574.
116. Waldram A, Holmes E, Wang Y, et al. Top-down systems biology modeling of host metabotype-microbiome associations in obese rodents. *J Proteome Res*. 2009;8(5):2361–2375.
117. Martin-Lorenzo M, Martinez PJ, Baldan-Martin M, et al. Citric acid metabolism in resistant hypertension: underlying mechanisms and metabolic prediction of treatment response. *Hypertension (Dallas, Tex: 1979)*. 2017;70(5):1049–1056.
118. Malatji BG, Meyer H, Mason S, et al. A diagnostic biomarker profile for fibromyalgia syndrome based on an NMR metabolomics study of selected patients and controls. *BMC Neurology*. 2017;17(1):88.
119. Zhang T, Wu X, Ke C, et al. Identification of potential biomarkers for ovarian cancer by urinary metabolomic profiling. *J Proteome Res*. 2013;12(1):505–512.
120. Liang Q, Wang C, Li B. Metabolomic analysis using liquid chromatography/mass spectrometry for gastric cancer. *Appl Biochem Biotechnol*. 2015;176(8):2170–2184.
121. Alonso A, Julia A, Vinaixa M, et al. Urine metabolome profiling of immune-mediated inflammatory diseases. *BMC Med*. 2016;14(1):133.
122. Kaluzna-Czaplinska J. Noninvasive urinary organic acids test to assess biochemical and nutritional individuality in autistic children. *Clin Biochem*. 2011;44(8–9):686–691.
123. Noto A, Fanos V, Barberini L, et al. The urinary metabolomics profile of an Italian autistic children population and their unaffected siblings. *J Maternal-Fetal Neon Med*. 2014;27(suppl 2):46–52.
124. Dimmock DP, Lawlor MW. Presentation and diagnostic evaluation of mitochondrial disease. *Pediatr Clin N Am*. 2017;64(1):161–171.
125. Haas RH, Parikh S, Falk MJ, et al. The in-depth evaluation of suspected mitochondrial disease: the Mitochondrial Medicine Society's Committee on Diagnosis. *Mol Gene Metabol*. 2008;94(1):16–37.
126. Wojtczak L, Slyshenkov VS. Protection by pantothenic acid against apoptosis and cell damage by oxygen free radicals—the role of glutathione. *BioFactors (Oxford, England)*. 2003;17(1–4):61–73.
127. Anderson NM, Mucka P, Kern JG, Feng H. The emerging role and targetability of the TCA cycle in cancer metabolism. *Protein Cell*. 2017.
128. Cardaci S, Ciriolo MR. TCA Cycle defects and cancer: when metabolism tunes redox state. *Int J Cell Biol*. 2012;2012:161837.
129. Rojczyk-Golebiewska E, Kucharzewski M. Influence of chosen metals on the citric acid cycle. *Pol Merkur Lekarski*. 2013;34(201):175–178.
130. Strydom CRC. *The Effect of Selected Metals on the central Metabolic Pathways in Biology: A Review*. Water SA; 2006.
131. Cardaci S, Ciriolo MR. TCA Cycle defects and cancer: when metabolism tunes redox state. *Int J Cell Biol*. 2012;2012:9.
132. Penes NO, Weber B, Paun SD. Role of genetic polymorphism in nutritional supplementation therapy in personalized medicine. *Rom J Morphol Embryol*. 2017;58(1):53–58.
133. Oeltmann T, Carson R, Shannon JR, Ketch T, Robertson D. Assessment of O-methylated catecholamine levels in plasma and urine for diagnosis of

autonomic disorders. *Auton Neurosci: Basic & Clinical*. 2004;116(1–2):1–10.
134. Tohmola N, Itkonen O, Turpeinen U, Joenvaara S, Renkonen R, Hamalainen E. Preanalytical validation and reference values for a mass spectrometric assay of serum vanillylmandelic acid for screening of catecholamine secreting neuroendocrine tumors. *Clin Chim Acta*. 2015;446:206–212.
135. Barco S, Gennai I, Reggiardo G, et al. Urinary homovanillic and vanillylmandelic acid in the diagnosis of neuroblastoma: report from the Italian Cooperative Group for Neuroblastoma. *Clin Biochem*. 2014;47(9):848–852.
136. Monteleone M, Naccarato A, Sindona G, Tagarelli A. A reliable and simple method for the assay of neuroendocrine tumor markers in human urine by solid-phase microextraction-gas chromatography-triple quadrupole mass spectrometry. *Anal Chim Acta*. 2013;759:66–73.
137. Helin P, Kuoppasalmi K, Laakso J, Harkonen M. Human urinary biogenic amines and some physiological responses during situation stress. *Int J Psychophysiol*. 1988;6(2):125–132.
138. Fukuda M, Hata A, Niwa S, et al. Plasma vanillylmandelic acid level as an index of psychological stress response in normal subjects. *Psychiatr Res*. 1996;63(1):7–16.
139. Brantley PJ, Dietz LS, McKnight GT, Jones GN, Tulley R. Convergence between the daily stress Inventory and endocrine measures of stress. *J Consult Clin Psychol*. 1988;56(4):549–551.
140. Dikanovic M, Kadojic D, Demarin V, et al. The effect of stress hormones on cerebral hemodynamics in patients with chronic posttraumatic stress disorder. *Acta Clinica Croatica*. 2009;48(4):405–411.
141. Chappell JC, Eisenhofer G, Owens MJ, et al. Effects of duloxetine on norepinephrine and serotonin transporter activity in healthy subjects. *J Clin Psychopharmacol*. 2014;34(1):9–16.
142. Vincent S, Bieck PR, Garland EM, et al. Clinical assessment of norepinephrine transporter blockade through biochemical and pharmacological profiles. *Circulation*. 2004;109(25):3202–3207.
143. Dabidy Roshan V, Pourasghar M, Mohammadian Z. The efficacy of intermittent walking in water on the rate of MHPG sulfate and the severity of depression. *Iran J Psychiat Behav Sci*. 2011;5(2):26–31.
144. Goode DJ, Dekirmenjian H, Meltzer HY, Maas JW. Relation of exercise to MHPG excretion in normal subjects. *Arch General Psychiat*. 1973;29(3):391–396.
145. Shekim WO, Javaid J, Davis JM, Bylund DB. Urinary MHPG and HVA excretion in boys with attention deficit disorder and hyperactivity treated with d-amphetamine. *Biol Psychiat*. 1983;18(6):707–714.
146. Scassellati C, Bonvicini C, Faraone SV, Gennarelli M. Biomarkers and attention-deficit/hyperactivity disorder: a systematic review and meta-analyses. *J Am Acad Child Adolesc Psychiatr*. 2012;51(10):1003–1019. e1020.
147. Russell VA, Sagvolden T, Johansen EB. Animal models of attention-deficit hyperactivity disorder. *Behav Brain Func*. 2005;1(1):9.
148. Montoya A, Escobar R, Garcia-Polavieja MJ, et al. Changes of urine dihydroxyphenylglycol to norepinephrine ratio in children with attention-deficit hyperactivity disorder (ADHD) treated with atomoxetine. *J Child Neurol*. 2011;26(1):31–36.
149. Hopkins SC, Sunkaraneni S, Skende E, et al. Pharmacokinetics and exposure-response relationships of dasotraline in the treatment of attention-deficit/hyperactivity disorder in adults. *Clin Drug Invest*. 2016;36(2):137–146.
150. Nishi M, Miyake H, Takeda T, Takasugi N, Hanai J, Kawai T. Urinary vanillylmandelic acid and homovanillic acid levels in randomly-sampled urine for the mass screening of neuroblastoma. *Jpn J Clin Oncol*. 1990;20(3):268–270.
151. Kaluzna-Czaplinska J, Socha E, Rynkowski J. Determination of homovanillic acid and vanillylmandelic acid in urine of autistic children by gas chromatography/mass spectrometry. *Med Sci Monit*. 2010;16(9):Cr445–450.
152. Paik MJ, Kim DK, Nguyen DT, et al. Correlation of daytime sleepiness with urine metabolites in patients with obstructive sleep apnea. *Sleep Breathing*. 2014;18(3):517–523.
153. Santagostino G, Cucchi ML, Frattini P, et al. The influence of alprazolam on the monoaminergic neurotransmitter systems in dysthymic patients. Relationship to clinical response. *Pharmacopsychiat*. 1998;31(4):131–136.
154. Tellez MR, Mamikunian G, O'Dorisio TM, Vinik AI, Woltering EA. A single fasting plasma 5-HIAA value correlates with 24-hour urinary 5-HIAA values and other biomarkers in midgut neuroendocrine tumors (NETs). *Pancreas*. 2013;42(3):405–410.
155. Ardill JE, Armstrong L, Smye M, Doherty R, McCance DR, Johnston BT. Neuroendocrine tumours of the small bowel: interpretation of raised circulating chromogranin A, urinary 5 hydroxy indole acetic acid and circulating neurokinin A. *QJM*. 2016;109(2):111–115.
156. Chan DL, Clarke SJ, Diakos CI, et al. Prognostic and predictive biomarkers in neuroendocrine tumours. *Crit Rev Oncol/Hematol*. 2017;113:268–282.
157. Adaway JE, Dobson R, Walsh J, et al. Serum and plasma 5-hydroxyindoleacetic acid as an alternative to 24-h urine 5-hydroxyindoleacetic acid measurement. *Ann Clin Biochem*. 2016;53(Pt 5):554–560.
158. Joy T, Walsh G, Tokmakejian S, Van Uum SH. Increase of urinary 5-hydroxyindoleacetic acid excretion but not serum chromogranin A following over-the-counter 5-hydroxytryptophan intake. *Can J Gastroenterol*. 2008;22(1):49–53.
159. Corcuff JB, Chardon L, El Hajji Ridah I, Brossaud J. Urinary sampling for 5HIAA and metanephrines determination: revisiting the recommendations. *Endocrine Connections*. 2017;6(6):R87–r98.
160. Feldman JM, Lee EM. Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. *Am J Clin Nutr*. 1985;42(4):639–643.
161. Ortiz Á, Espino J, Bejarano I, et al. The correlation between urinary 5-hydroxyindoleacetic acid and sperm quality in infertile men and rotating shift workers. *Reprod Biol Endocrinol: RB&E*. 2010;8. 138-138.
162. Gasparini CF, Smith RA, Griffiths LR. Genetic and biochemical changes of the serotonergic system in migraine pathobiology. *J Headache Pain*. 2017;18(1):20.
163. Afarideh M, Behdadnia A, Noshad S, et al. Association of peripheral 5-hydroxyindole-3-acetic acid, a serotonin derivative, with metabolic syndrome and low-grade inflammation. *Endocr Pract*. 2015;21(7):711–718.
164. Fukui M, Tanaka M, Toda H, et al. High plasma 5-hydroxyindole-3-acetic acid concentrations in subjects with metabolic syndrome. *Diabetes Care*. 2012;35(1):163–167.
165. Payne IR, Walsh EM, Whittenburg EJR. Relationship of dietary tryptophan and niacin to tryptophan metabolism in schizophrenics and nonschizophrenics. *Am J Clin Nutr*. 1974;27(6):565–571.
166. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes*. 2013;20(1):14–21.
167. Bousser M-G, Elghozi J-L, Laude D, Soisson T. Urinary 5-HIAA in migraine: evidence of lowered excretion in young adult females. *Cephalalgia*. 1986;6(4):205–209.
168. Erspamer V, Testini A. Observations on the release and turnover rate of 5-hydroxytryptamine in the gastrointestinal tract. *J Pharm Pharmacol*. 1959;11(1):618–623.
169. Yazar A, Buyukafpar K, Polat G, et al. The urinary 5-hydroxyindole acetic acid and plasma nitric oxide levels in irritable bowel syndrome: a preliminary study. *Scottish Med J*. 2005;50(1):27–29.
170. Moskwa A, Chojnacki J, Wisiewska-Jarosinska M, et al. Determination of homovanillic acid and vanillylmandelic acid in urine of autistic children by gas chromatography/mass spectrometry. *Pol Merkur Lekarski*. 2007;22(131):366–368.
171. Keszthelyi D, Troost FJ, Jonkers DM, et al. Serotonergic reinforcement of intestinal barrier function is impaired in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2014;40(4):392–402.
172. Jeon SW, Kim YK. Inflammation-induced depression: its pathophysiology and therapeutic implications. *J Neuroimmunol*. 2017;313:92–98.
173. Oxenkrug G. Serotonin-kynurenine hypothesis of depression: historical overview and recent developments. *Curr Drug Targets*. 2013;14(5):514–521.
174. Zheng P, Chen JJ, Huang T, et al. A novel urinary metabolite signature for diagnosing major depressive disorder. *J Proteome Res*. 2013;12(12):5904–5911.

175. Savitz J, Drevets WC, Wurfel BE, et al. Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder. *Brain Behavior Immunity*. 2015;46:55–59.
176. Bender DA, Njagi EN, Danielian PS. Tryptophan metabolism in vitamin B6-deficient mice. *Br J Nutr*. 1990;63(1):27–36.
177. Rios-Avila L, Coats B, Chi Y-Y, et al. Metabolite profile analysis reveals association of vitamin B-6 with metabolites related to one-carbon metabolism and tryptophan catabolism but not with biomarkers of inflammation in oral contraceptive users and reveals the effects of oral contraceptives on these processes. *J Nutr*. 2015;145(1):87–95.
178. Donald EA, Bossé TR. The vitamin B6 requirement in oral contraceptive users II. Assessment by tryptophan metabolites, vitamin B6, and pyridoxic acid levels in urine. *Am J Clin Nutr*. 1979;32(5):1024–1032.
179. Shibata K, Nakata C, Fukuwatari T. High-performance liquid chromatographic method for profiling 2-oxo acids in urine and its application in evaluating vitamin status in rats. *Biosci Biotechnol Biochem*. 2016;80(2):304–312.
180. Harris RA, Joshi M, Jeoung NH, Obayashi M. Overview of the molecular and biochemical basis of branched-chain amino acid catabolism. *J Nutr*. 2005;135(suppl 6):1527s–1530s.
181. Adams SH. Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. *Adv Nutr*. 2011;2(6):445–456.
182. Shibata K, Sakamoto M. Urinary branched-chain 2-oxo acids as a biomarker for function of B-group vitamins in humans. *J Nutr Sci Vitaminol*. 2016;62(4):220–228.
183. Cooperman JM, Lopez R. The role of histidine in the anemia of folate deficiency. *Exp Biol Med (Maywood, NJ)*. 2002;227(11):998–1000.
184. Arakawa T, Ohara K, Fujii M, Hirata K, Takahashi Y. Serum folate activity and urinary formiminoglutamic acid following histidine load in infants and children. *Tohoku J Exp Med*. 1965;87(3):199–204.
185. Labadarios D, Dickerson JW, Parke DV, Lucas EG, Obuwa GH. The effects of chronic drug administration on hepatic enzyme induction and folate metabolism. *Br J Clin Pharmacol*. 1978;5(2):167–173.
186. Friedman S, Shachter A, Eckerling B, Eichhorn F, Rutenberg A. Fromino-glutamic acid (Figlu) excretion and abortion. *Panminerva Medica*. 1977;19(4):271–274.
187. Marin GH, Tentoni J, Cicchetti G. Megaloblastic anemia: rapid and economical study. *Sangre*. 1997;42(3):235–238.
188. Rosenauerova-Ostra A, Hilgertova J, Sonka J. Urinary formiminoglutamate in man. Normal values related to sex and age. Effects of low calorie intake and alcohol consumption. *Clin Chim Acta*. 1976;73(1):39–43.
189. Shojania AM. Oral contraceptives: effect of folate and vitamin B12 metabolism. *Can Med Assoc J*. 1982;126(3):244–247.
190. Hansen OP, Drivsholm A, Hippe E, Quadros E, Linnell JC. Interrelationships between Vitamin B12 and folic acid in myelomatosis: cobalamin coenzyme and tetrahydrofolic acid function. *Scand J Haematol*. 1978;20(4):360–370.
191. Li K, Wahlqvist ML, Li D. Nutrition, one-carbon metabolism and neural tube defects: a review. *Nutrients*. 2016;8(11).
192. Armstrong P, Rae PW, Gray WM, Spence AA. Nitrous oxide and formiminoglutamic acid: excretion in surgical patients and anaesthetists. *Br J Anaesth*. 1991;66(2):163–169.
193. Chen M, Zhuang J, Yang J, Wang D, Yang Q. Atypical hemolytic uremic syndrome induced by CblC subtype of methylmalonic academia: a case report and literature review. *Medicine*. 2017;96(43):e8284.
194. Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. *J Inherit Metab Dis*. 2012;35(1):91–102.
195. Miller JW, Ramos MI, Garrod MG, Flynn MA, Green R. Transcobalamin II 775G>C polymorphism and indices of vitamin B12 status in healthy older adults. *Blood*. 2002;100(2):718–720.
196. Kwok T, Cheng G, Lai WK, Poon P, Woo J, Pang CP. Use of fasting urinary methylmalonic acid to screen for metabolic vitamin B12 deficiency in older persons. *Nutrition (Burbank, Los Angeles County, Calif)*. 2004;20(9):764–768.
197. Gultepe M, Ozcan O, Avsar K, Cetin M, Ozdemir AS, Gok M. Urine methylmalonic acid measurements for the assessment of cobalamin deficiency related to neuropsychiatric disorders. *Clin Biochem*. 2003;36(4):275–282.
198. Wiersinga WJ, de Rooij SE, Huijmans JG, Fischer C, Hoekstra JB. Diagnosis of vitamin B12 deficiency revised. *Nederlands Tijdschrift Voor Geneeskunde*. 2005;149(50):2789–2794.
199. Dhillon VS, Zabaras D, Almond T, Cavuoto P, James-Martin G, Fenech M. Whey protein isolate improves vitamin B12 and folate status in elderly Australians with subclinical deficiency of vitamin B12. *Mol Nutr Food Res*. 2017;61(5).
200. Harrington DJ. Laboratory assessment of vitamin B12 status. *J Clin Pathol*. 2017;70(2):168–173.
201. Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. *Clin Chem Lab Med*. 2003;41(11):1478–1488.
202. Ward MG, Kariyawasam VC, Mogan SB, et al. Prevalence and risk factors for functional vitamin B12 deficiency in patients with Crohn's disease. *Inflamm Bowel Dis*. 2015;21(12):2839–2847.
203. Hin H, Clarke R, Sherliker P, et al. Clinical relevance of low serum vitamin B12 concentrations in older people: the Banbury B12 study. *Age Ageing*. 2006;35(4):416–422.
204. Tangney CC, Tang Y, Evans DA, Morris MC. Biochemical indicators of vitamin B12 and folate insufficiency and cognitive decline. *Neurology*. 2009;72(4):361–367.
205. Favrat B, Vaucher P, Herzig L, et al. Oral vitamin B12 for patients suspected of subtle cobalamin deficiency: a multicentre pragmatic randomised controlled trial. *BMC Family Practice*. 2011;12:2–2.
206. Sun AL, Ni YH, Li XB, et al. Urinary methylmalonic acid as an indicator of early vitamin B12 deficiency and its role in polyneuropathy in type 2 diabetes. *J Diabetes Res*. 2014;2014:921616.
207. Ciorba MA. Kynurenine pathway metabolites: relevant to vitamin B-6 deficiency and beyond. *Am J Clin Nutr*. 2013;98(4):863–864.
208. Chen CF, Liu TZ, Lan WH, et al. Novel spectrophotometric method for the quantitation of urinary xanthurenic acid and its application in identifying individuals with hyperhomocysteinemia associated with Vitamin B(6) deficiency. *Bio Med Res Int*. 2013;2013:678476.
209. Rios-Avila L, Nijhout HF, Reed MC, Sitren HS, Gregory JF. A mathematical model of tryptophan metabolism via the kynurenine pathway provides insights into the effects of vitamin B-6 deficiency, tryptophan loading, and induction of tryptophan 2,3-dioxygenase on tryptophan metabolites. *J Nutr*. 2013;143(9):1509–1519.
210. Ulvik A, Theofylaktopoulou D, Midttun O, Nygard O, Eussen SJ, Ueland PM. Substrate product ratios of enzymes in the kynurenine pathway measured in plasma as indicators of functional vitamin B-6 status. *Am J Clin Nutr*. 2013;98(4):934–940.
211. Oxenkrug GF. Increased plasma levels of xanthurenic and kynurenic acids in type 2 diabetes. *Mol Neurobiol*. 2015;52(2):805–810.
212. Perry CA, West AA, Gayle A, et al. Pregnancy and lactation alter biomarkers of biotin metabolism in women consuming a controlled diet. *J Nutr*. 2014;144(12):1977–1984.
213. Mock DM, Henrich CL, Carnell N, Mock NI. Indicators of marginal biotin deficiency and repletion in humans: validation of 3-hydroxyisovaleric acid excretion and a leucine challenge. *Am J Clin Nutr*. 2002;76(5):1061–1068.
214. Mock NI, Malik MI, Stumbo PJ, Bishop WP, Mock DM. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased biotin status in experimental biotin deficiency. *Am J Clin Nutr*. 1997;65(4):951–958.
215. Mock DM, Stratton SL, Horvath TD, et al. Urinary excretion of 3-hydroxyisovaleric acid and 3-hydroxyisovaleryl carnitine increases in response to a leucine challenge in marginally biotin-deficient humans. *J Nutr*. 2011;141(11):1925–1930.
216. Stratton SL, Horvath TD, Bogusiewicz A, et al. Urinary excretion of 3-hydroxyisovaleryl carnitine is an early and sensitive indicator of marginal biotin deficiency in humans. *J Nutr*. 2011;141(3):353–358.
217. Mock DM, Henrich-Shell CL, Carnell N, Stumbo P, Mock NI. 3-Hydroxypropionic acid and methylcitric acid are not reliable indicators of marginal biotin deficiency in humans. *J Nutr*. 2004;134(2):317–320.
218. Pollard LM, Williams NR, Espinoza L, et al. Diagnosis, treatment, and long-term outcomes of late-onset (type III) multiple acyl-CoA dehydrogenase deficiency. *J Child Neurol*. 2010;25(8):954–960.

219. Behin A, Acquaviva-Bourdain C, Souvannanorath S, et al. Multiple acyl-CoA dehydrogenase deficiency (MADD) as a cause of late-onset treatable metabolic disease. *Revue Neurologique*. 2016;172(3):231–241.
220. Pleban FT, Oketope O, Shrestha L. Occupational styrene exposure on auditory function among adults: a systematic review of selected workers. *Safety and Health at Work*. 2017;8(4):329–336.
221. Rueff J, Teixeira JP, Santos LS, Gaspar JF. Genetic effects and biotoxicity monitoring of occupational styrene exposure. *Clin Chim Acta*. 2009;399(1–2):8–23.
222. Hajizadeh Y, Teiri H, Nazmara S, Parseh I. Environmental and biological monitoring of exposures to VOCs in a petrochemical complex in Iran. *Environ Sci Pollut Res Int*. 2018;25(7):6656–6667.
223. Suh JH, Lee HY, Kim U, et al. Simultaneous determination of benzene, toluene, ethylbenzene, and xylene metabolites in human urine using electromembrane extraction combined with liquid chromatography and tandem mass spectrometry. *J Sep Sci*. 2015;38(24):4276–4285.
224. Costa C, Costa S, Silva S, et al. DNA damage and susceptibility assessment in industrial workers exposed to styrene. *J Toxicol Environ Health A*. 2012;75(13–15):735–746.
225. Amberg A, Rosner E, Dekant W. Toxicokinetics of methyl tert-butyl ether and its metabolites in humans after oral exposure. *Toxicol Sci*. 2001;61(1):62–67.
226. Cheng JS, Lai KH, Lo GH, Ng WW, Tam TN, Huang SM. In vitro dissolution of cholesterol and brown pigmented gallstones: a comparison of MTBE, DMSO and BA-EDTA. *Zhonghua Yi Xue Za Zhi (Taipei)*. 2000;63(9):667–672.
227. Hofmann AF, Amelsberg A, Esch O, et al. Successful topical dissolution of cholesterol gallbladder stones using ethyl propionate. *Dig Dis Sci*. 1997;42(6):1274–1282.
228. Boyle EB, Viet SM, Wright DJ, et al. Assessment of exposure to VOCs among pregnant women in the National Children's Study. *Int J Environ Res Pub Health*. 2016;13(4):376.
229. Choi CY, Cho N, Park SY, Park S, Gil HW, Hong SY. Urine methyl hippuric acid levels in acute pesticide poisoning: estimation of ingested xylene volume and association with clinical outcome parameters. *J Korean Med Sci*. 2017;32(12):2051–2057.
230. Morimoto S, Koda T, Suidzu T, et al. Significance of urinary glucaric acid measurement and its application to paclitaxel therapy. *Cancer Chemotherapy*. 2009;36(11):1857–1861.
231. Hasegawa K, Shimidzu K, Morimoto S, et al. Improvement of urinary d-glucaric acid assay and its application. *Yakugaku Zasshi*. 2008;128(1):135–140.
232. Hunter J, Maxwell JD, Carrella M, Stewart DA, Williams R. Urinary D-glucaric-acid excretion as a test for hepatic enzyme induction in man. *Lancet (London, England)*. 1971;1(7699):572–575.
233. Edwards JW, Priestly BG. Effect of occupational exposure to aldrin on urinary D-glucaric acid, plasma dieldrin, and lymphocyte sister chromatid exchange. *Int Arch Occup Environ Health*. 1994;66(4):229–234.
234. Hogue CJ, Brewster MA. The potential of exposure biomarkers in epidemiologic studies of reproductive health. *Environ Health Perspect*. 1991;90:261–269.
235. Zółtaszek R, Hanausek M, Kiliańska ZM, Walaszek Z. The biological role of D-glucaric acid and its derivatives: potential use in medicine. *Postepy Hig Med Dosw (Online)*. 2008;62:451–462. http://europepmc.org/abstract/MED/18772850. Accessed 2008/09/04/.
236. Hanausek M, Walaszek Z, Slaga TJ. Detoxifying cancer causing agents to prevent cancer. *Integr Cancer Ther*. 2003;2(2):139–144.
237. Salerno C, Crifo C. Diagnostic value of urinary orotic acid levels: applicable separation methods. *J Chromatogr B, Analyt Technol Biomed Life Sci*. 2002;781(1–2):57–71.
238. Lord RS, Bralley JA. Clinical applications of urinary organic acids. Part I: detoxification markers. *Altern Med Rev*. 2008;13(3):205–215.
239. Dash SK, Chauhan M, Varma V, Sharma R, Kansal S, Chawla R. Hyperammonemic coma in a post-partum patient with undiagnosed urea cycle defect. *Indian J Crit Care Med*. 2013;17(2):107–110.
240. Brosnan ME, Brosnan JT. Orotic acid excretion and arginine metabolism. *J Nutr*. 2007;137(6 suppl 2):1656s–1661s.
241. van den Born JC, Frenay AR, Bakker SJ, et al. High urinary sulfate concentration is associated with reduced risk of renal disease progression in type 2 diabetes. *Nitric Oxide*. 2016;55–56:18–24.
242. Liu Y, Hyde AS, Simpson MA, Barycki JJ. Emerging regulatory paradigms in glutathione metabolism. *Adv Cancer Res*. 2014;122:69–101.
243. Friesen RW, Novak EM, Hasman D, Innis SM. Relationship of dimethylglycine, choline, and betaine with oxoproline in plasma of pregnant women and their newborn infants. *J Nutr*. 2007;137(12):2641–2646.
244. Metges CC, Yu YM, Cai W, et al. Oxoproline kinetics and oxoproline urinary excretion during glycine- or sulfur amino acid-free diets in humans. *Am J Physiol Endocrinol Metabol*. 2000;278(5):E868–E876.
245. Jackson AA, Persaud C, Meakins TS, Bundy R. Urinary excretion of 5-L-oxoproline (pyroglutamic acid) is increased in normal adults consuming vegetarian or low protein diets. *J Nutr*. 1996;126(11):2813–2822.
246. Persaud C, Forrester T, Jackson AA. Urinary excretion of 5-L-oxoproline (pyroglutamic acid) is increased during recovery from severe childhood malnutrition and responds to supplemental glycine. *J Nutr*. 1996;126(11):2823–2830.
247. Gall WE, Beebe K, Lawton KA, et al. alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. *PloS One*. 2010;5(5):e10883.
248. Cobb J, Eckhart A, Motsinger-Reif A, Carr B, Groop L, Ferrannini E. alpha-Hydroxybutyric acid is a selective metabolite biomarker of impaired glucose tolerance. *Diabetes Care*. 2016;39(6):988–995.
249. Ferrannini E, Natali A, Camastra S, et al. Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. *Diabetes*. 2013;62(5):1730–1737.
250. Landaas S, Pettersen JE. Clinical conditions associated with urinary excretion of 2-hydroxybutyric acid. *Scand J Clin Lab Invest*. 1975;35(3):259–266.
251. Imaki M, Kawabata K, Yoshida Y, Nakamura T, Tanada S. Evaluation of the effects of various factors on the serum alpha hydroxybutyrate dehydrogenase activity in young females. *App Hum Sci*. 1995;14(6):297–302.

30

Urinary Porphyrins for the Detection of Heavy Metal and Toxic Chemical Exposure

Michael J. Chapman, ND

OUTLINE

INTRODUCTION

Today's world is one of unprecedented toxicity. The planet's increasing industrialization has led to the release of metals and the synthesis of new-to-nature chemicals that are designed to be difficult for biological systems to break down, which has resulted in substantial soil, water, food, and air contamination. Throughout this modernization, there have been instances of human exposures that have caused dramatic and immediate health consequences. However, little emphasis has been placed on how chronic low-level exposure contributes to chronic disease. Now, more than ever, there is a need to bring greater focus on the role of environmental exposures and to address the growing toxic burden of the entire population.[1]

One of the earliest discoveries relating to the role toxic metals play in disrupting homeostasis is the mechanism by which lead poisoning alters heme synthesis.[2–5] When heme synthesis is interrupted, heme metabolites, known as porphyrins, build up in tissues and are subsequently excreted in the urine at higher levels.[6] With this new understanding, the ability to measure porphyrins came into clinical practice as a tool that helps clinicians assess the contribution of toxic exposure to the disease process.

DESCRIPTION

Porphyrin urinary excretion, also referred to as porphyrinuria, has been an area of clinical investigation since the 1800s. Porphyrinuria was originally discovered as an inborn error of metabolism and led to the clinical characterization of congenital porphyria.[7] Eventually, porphyrins were uncovered as precursors of heme metabolism, and what was termed porphyrinuria is the result of a disruption in heme production. Since that time, the heme pathway has been extensively studied, along with the enzymes responsible for heme synthesis. As additional enzyme pathway defects became evident, the symptomatic presentation of each inherited metabolic error became collectively known as *porphyrias*.[8]

Urine porphyrin measurement has been an important tool for the clinical diagnosis of porphyrias, and its usage has largely remained relegated to this purpose. However, urine porphyrin quantitative assessment, not caused by an inborn error of metabolism, remains an area of intrigue in both the scientific literature and in clinical practice. Any factor that alters the functionality of one or more of the enzymes involved in heme metabolism may result in altered porphyrin excretion. This has been studied in metabolic dysfunction, drug responses, and toxic exposures.[9] Because the nature of clinical porphyrias is well established, the majority of this chapter's content is dedicated to the use of urinary porphyrin assessment to detect minor disturbances of heme metabolism.

HEME BIOSYNTHESIS

Heme is an indispensable protein in the body. It serves as the body's substrate for essential protein production, such as hemoglobin for oxygen exchange, and for the Phase I detoxification enzymes and the cytochromes (a, b, c, and P450) used in electron transport and detoxification. The heme-synthesis pathway involves eight enzymatic steps (Fig. 30.1). Flaws in any of these eight enzymes can ultimately impair heme synthesis, leading to increased heme precursors called porphyrinogens. These porphyrinogens are then quickly oxidized to the porphyrin form as they are removed from the body.[6]

However, in severe enzyme deficiency, porphyrins can accumulate in the body, leading to potentially life-threatening health complications as seen in acute and chronic porphyrias (Table 30.1).[8] Most of the enzyme defects that lead to porphyria are inherited conditions. Porphyrias have historically been clinically classified in multiple ways, either based on clinical presentation or by the nature of the inherited defect.[6,8] The urinary analysis of these porphyrins has been a great tool to aid in the diagnosis of porphyria.

In addition to aiding in the diagnosis of congenital and acquired porphyrias, urinary measurement has also been studied in cases of more subtle heme-synthesis errors. These include cases of toxic exposures, oxidative and metabolic stress, and specific clinical conditions such as autism.[6,9] By studying these cases, attempts have been made to characterize particular patterns of porphyrinuria to gain greater insight into the causes and consequences of disrupted heme biosynthesis.

PORPHYRINURIA IN ENVIRONMENTAL EXPOSURE

Lead exposure is one of the most famous and well-known porphyrinuria inducers. Lead affects three different enzymes in the heme pathway and can produce characteristic elevations in urinary porphyrins, which have been well described in the literature.[4] The typical pattern of porphyrin excretion seen in lead poisoning is an increase in δ-aminolevulinic acid (ALA) and coproporphyrin. This occurs as a result of inhibition of the heme-synthesis enzymes δ-aminolevulinate dehydratase and coproporphyrinogen oxidase, respectively.[2,4,6] Because of the consistency of this finding, it has been suggested that urinary

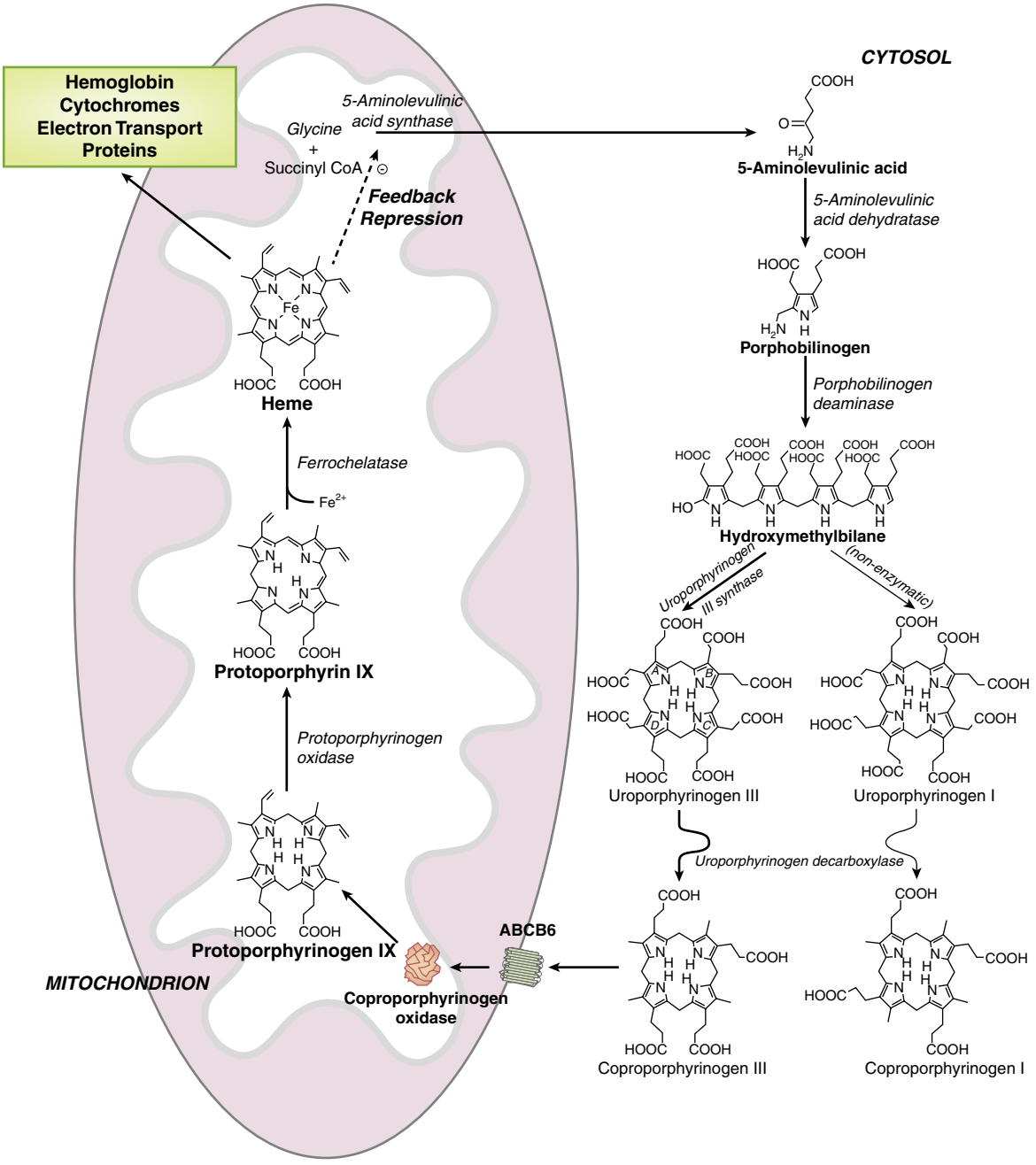


Fig. 30.1 Biosynthesis of heme.

TABLE 30.1 Types of Porphyrias

Porphyria	Enzyme Deficiency	Urine Findings[8]
Acute intermittent porphyria	Porphobilinogen deaminase	ALA, PBG, uroporphyrins
Hereditary coproporphyria	Coproporphyrinogen oxidase	ALA, PBG, coproporphyrins
Variegate porphyria	Protoporphyrinogen oxidase	ALA, PBG, coproporphyrins
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Uroporphyrins, heptaporphyrins
Hepatoerythropoietic porphyria		
Protoporphyria	Fellochelatase	None
ALA dehydratase porphyria	ALA dehydratase	ALA, coproporphyrins
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Uroporphyrins, heptaporphyrins

ALA, δ-aminolevulinic acid; *PGB,* porphobilinogen.

TABLE 30.2 Excretion Patterns in Common Environmental Exposures

Common Environmental Trigger	Common Urine Findings[a]
Lead	ALA, coproporphyrin III[4]
Mercury	Coproporphyrins, pentacarboxylporphyrin, precoproporphyrin
Arsenic	Uroporphyrins, coproporphyrins[28]
Alcohol	ALA, total porphyrins[32]
Numerous organic chemicals[b]	Uroporphyrins, coproporphyrins, total porphyrins[6]

ALA, δ-aminolevulinic acid.

Note: Certain environmental exposures have demonstrated particular patterns of porphyrin excretion. However, when interpreting urinary porphyrin testing, due diligence should be applied to rule out any inherited porphyria if suspected. Toxic metal analysis can aid in the diagnosis of metal toxicity.

[a]These patterns have been demonstrated in the literature and in clinical practice; however, it should be stated that these are general guidelines. Positive findings warrant further investigation into toxic exposure. Also, other factors (e.g., medications, liver disease, malignancy, and other chronic conditions) can contribute to altered porphyrin excretion.

[b]Porphyrin excretion patterns have been studied in a limited number of organic chemical exposures. A comprehensive workup is recommended when assessing any form of environmental toxic exposure.

porphyrin analysis may be considered as part of monitoring lead exposure in combination with blood lead levels.[10,11]

More recently, the use of urinary porphyrin profiles has been researched with respect to mercury exposure and toxicity. The bulk of this scientific exploration is attributed to Dr. James Wood's work at the Department of Environmental Health at the University of Washington.[12–19] In multiple animal and human studies, a pattern of porphyrin excretion was detected as a result of mercury exposure (Table 30.2). A statistically significant increase was seen in the excretion of three discrete porphyrins: coproporphyrin, pentacarboxylporphyrin, and an atypical porphyrin they referred to as precoproporphyrin.[12,13,15,19,20] This atypical porphyrin would later be renamed keto-isocoproporphyrin.[21,22] This pattern correlated with the dose and timing of mercury exposure. These three porphyrins were also elevated in dentists routinely exposed to mercury vapors, specifically in dentists with higher urinary mercury excretion.[19] Interestingly, in another study involving dentists, these three porphyrins were also significantly decreased after chelation therapy.[13] One study of dentists found that porphyrin excretion was better correlated with clinical symptoms associated with mercury toxicity than urine mercury levels.[23] Ultimately, a few published articles claimed that this porphyrin pattern was a useful biomarker for assessing the total body burden of mercury.[24–26] Additionally, these researchers suggested that porphyrin profiling may be more reliable than urine mercury testing for detecting subclinical mercury exposure correlating with the toxic neurological effects.[20,24]

Altered urinary porphyrin excretion has been documented in other environmental toxic exposures, such as arsenic,[27–31] cadmium, organic chemicals,[6] and alcohol,[32] and in combinations of multiple toxic exposures.[33] Many other toxicants may play a role in porphyrinuria and need further investigation. Although urinary porphyrin excretion appears to consistently correlate with toxic metal exposure, the degree to which an individual's detoxification capacity plays a role in susceptibility to toxins is still under investigation.[17,34] It may ultimately be determined that both increased environmental exposure and reduced detoxification contribute to the degree of heme-pathway disruption.

CLINICAL APPLICATIONS OF PORPHYRINURIA

Porphyrin profiles have been used clinically in assessing certain patient populations. The most recent intrigue regarding porphyrin assessment is in autism. Investigation into risk factors that might contribute to the rise in autism over recent years led to concern about the role mercury exposure plays in autism pathogenesis.[35–38] As more research began to implicate mercury exposure as a plausible factor in the development of autism spectrum disorders (ASDs), porphyrin testing was used as a noninvasive and independent method for assessing exposure.[39] Indeed, several studies of autism patients showed significant porphyrin excretion compared with controls, often corresponding to the porphyrin patterns characteristic of mercury exposure.[40] These results were replicated across multiple continents.[41,42] Clinically, porphyrin profiles are still used in this patient population to assess toxic metal body burden. However, reduced detoxification capacity and increased oxidative stress have also been suspected in autism.[43–45] Therefore the multifactorial nature of childhood neurobehavioral disorders should be strongly emphasized.[46] Reduction in porphyrin excretion after chelation therapy, along with emerging evidence surrounding mercury, ASD, and heme disturbances, makes porphyrin profiling an important clinical tool.[14,29,47]

Aside from managing autism and toxic exposures, the association between oxidative stress and porphyrin excretion is clinically significant. Knowing the amount of oxidative stressors the average patient encounters daily, clinicians must consider the role these exposures play in other chronic health complaints. With the growing understanding of genetic individuality and variation, porphyrin excretion may provide a window into genetic susceptibility to oxidative stress.[17] Further studies are needed to evaluate these roles in various patient populations, such as neurological disorders,[48] malignancy,[49] and mood disorders, to name a few.

CONCLUSION

Urine porphyrin profiling can be a noninvasive and valuable tool for chronic disease assessment and management. There is compelling evidence that environmental exposure, whether severe or nascent, has the potential to be a major contributing factor in the pathogenesis of chronic illness. Specific toxicants may also demonstrate unique patterns of porphyrin excretion because eof specific heme-synthesis enzyme abnormalities. Often, the difficulty in clinically assessing toxicity is the degree to which these toxins are stored in tissues rather than in the circulation, making it difficult to assess the total body burden. Because heme synthesis takes place in specific tissues (predominantly bone, kidney, and the liver), tissue toxins may affect heme synthesis, leading to porphyrinuria. It should be noted that many common medications can affect the heme pathway. Further studies are warranted to elucidate the triggers leading to altered urine porphyrin levels. Therefore clinical correlation and discretion are important when interpreting any given test result.

Finally, care and expertise should be applied in the management of environmental exposure and toxicity. The primary approach is the identification and removal of ongoing exposures. In addition to the physical examination, a thorough exposure history, combined with a well-designed environmental questionnaire, can help identify toxic triggers. Second, intake of antioxidant-rich nutrients and vitamins helps increase detoxification and elimination. Last, ensuring optimal detoxification via gastrointestinal elimination, appropriate skin care, and sweat depuration therapy goes a long way toward toxin removal.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev*. 2005;14(8):1847–1850.
2. Lichtman HC, Feldman F. In vitro pyrrole and porphyrin synthesis in lead poisoning and iron deficiency. *J Clin Invest*. 1963;42(6):830–839.
3. Oskarsson A, Fowler BA. Effects of lead on the heme biosynthetic pathway in rat kidney. *Exp Mol Pathol*. 1985;43(3):409–417.
4. Fujita H, Nishitani C, Ogawa K. Lead, chemical porphyria, and heme as a biological mediator. *Tohoku J Exp Med*. 2002;196(2):53–64.
5. Piomelli S, Seaman C, Kapoor S. Lead-induced abnormalities of porphyrin metabolism the relationship with iron deficiency. *Ann N Y Acad Sci*. 1987;514(1):278–288.
6. Daniell WE, Stockbridge HL, Labbe RF, et al. Environmental chemical exposures and disturbances of heme synthesis. *Environ Health Perspec*. 1997;105(suppl 1):37–53.
7. Goldberg A, McColl KE, Moore M, Rimington C. *Disorders of Porphyrin Metabolism*. Springer Science & Business Media; 2013.
8. Puy H, Gouya L, Deybach J-C. Porphyrias. *The Lancet*. 2010;375(9718):924–937.
9. Doss MO. Porphyrinurias and occupational disease. *Ann N Y Acad Sci*. 1987;514:204–218.
10. Klotz K, Goen T. Human biomonitoring of lead exposure. *Met Ions Life Sci*. 2017;17.
11. Wang Q, Ye LX, Zhao HH, Chen JW, Zhou YK. Benchmark dose approach for low-level lead induced haematogenesis inhibition and associations of childhood intelligences with ALAD activity and ALA levels. *Sci Total Environ*. 2011;409(10):1806–1810.
12. Woods JS. Porphyrin metabolism as indicator of metal exposure and toxicity. In: Goyer RA, Cherian MG, eds. *Toxicology of Metals: Biochemical Aspects*. Berlin, Heidelberg: Springer Berlin Heidelberg; 1995:19–52.
13. Woods JS. Altered porphyrin metabolism as a biomarker of mercury exposure and toxicity. *Can J Physiol Pharmacol*. 1996;74(2):210–215.
14. Woods JS, Armel SE, Fulton DI, et al. Urinary porphyrin excretion in neurotypical and autistic children. *Environ Health Perspect*. 2010;118(10):1450–1457.
15. Woods JS, Bowers MA, Davis HA. Urinary porphyrin profiles as biomarkers of trace metal exposure and toxicity: studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicol Appl Pharmacol*. 1991;110(3):464–476.
16. Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM. The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. *Toxicol Appl Pharmacol*. 2005;206(2):113–120.
17. Woods JS, Heyer NJ, Russo JE, Martin MD, Farin FM. Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: summary findings from the Casa Pia Children's Amalgam clinical trial. *Neurotoxicol*. 2014;44:288–302.
18. Woods JS, Martin MD, Leroux BG, et al. Urinary porphyrin excretion in normal children and adolescents. *Clin Chim Acta*. 2009;405(1-2):104–109.
19. Woods JS, Martin MD, Naleway CA, Echeverria D. Urinary porphyrin profiles as a biomarker of mercury exposure: studies on dentists with occupational exposure to mercury vapor. *J Toxicol Environ Health*. 1993;40(2-3):235–246.
20. Pingree SD, Simmonds PL, Rummel KT, Woods JS. Quantitative evaluation of urinary porphyrins as a measure of kidney mercury content and mercury body burden during prolonged methylmercury exposure in rats. *Toxicol Sci*. 2001;61(2):234–240.
21. dos Santos APM, Mateus ML, Carvalho CML, Batoréu MCC. Biomarkers of exposure and effect as indicators of the interference of selenomethionine on methylmercury toxicity. *Toxicol Lett*. 2007;169(2):121–128.
22. Heyer NJ, Bittner AC, Echeverria D, Woods JS. A cascade analysis of the interaction of mercury and coproporphyrinogen oxidase (CPOX) polymorphism on the heme biosynthetic pathway and porphyrin production. *Toxicol Lett*. 2006;161(2):159–166.
23. Echeverria D, Heyer NJ, Martin MD, Naleway CA, Woods JS, Bittner AC. Behavioral effects of low-level exposure to elemental Hg among dentists. *Neurotoxicol Teratol*. 1995;17(2):161–168.
24. Fowler BA. Porphyrinurias induced by mercury and other metals. *Toxicol Sci*. 2001;61(2):197–198.
25. Kern JK, Geier DA, Ayzac F, Adams JB, Mehta JA, Geier MR. Toxicity biomarkers among US children compared to a similar cohort in France: a blinded study measuring urinary porphyrins. *Environ Toxicol Chem*. 2011;93(1-2):396–405.
26. Geier DA, Geier MR. A prospective assessment of porphyrins in autistic disorders: a potential marker for heavy metal exposure. *Neurotox Res*. 2006;10(1):57–64.
27. Ng JC, Wang JP, Zheng B, et al. Urinary porphyrins as biomarkers for arsenic exposure among susceptible populations in Guizhou province, China. *Toxicol Appl Pharmacol*. 2005;206(2):176–184.
28. Marchiset-Ferlay N, Savanovitch C, Sauvant-Rochat M-P. What is the best biomarker to assess arsenic exposure via drinking water? *Environ int*. 2012;39(1):150–171.
29. Heyer NJ, Echeverria D, Woods JS. Disordered porphyrin metabolism: a potential biological marker for autism risk assessment. *Autism Res*. 2012;5(2):84–92.
30. Wang JP, Qi L, Zheng B, Liu F, Moore MR, Ng JC. Porphyrins as early biomarkers for arsenic exposure in animals and humans. *Cell Mol Biol (Noisy-le-Grand, France)*. 2002;48(8):835–843.
31. Hernandez-Zavala A, Del Razo LM, Garcia-Vargas GG, et al. Altered activity of heme biosynthesis pathway enzymes in individuals chronically exposed to arsenic in Mexico. *Arch Toxicol*. 1999;73(2):90–95.
32. Doss MO, Kuhnel A, Gross U. Alcohol and porphyrin metabolism. *Alcohol Alcohol*. 2000;35(2):109–125.
33. Fowler BA, Mahaffey KR. Interactions among lead, cadmium, and arsenic in relation to porphyrin excretion patterns. *Environ Health Perspect*. 1978;25:87–90.
34. Li T, Woods JS. Cloning, expression, and biochemical properties of CPOX4, a genetic variant of coproporphyrinogen oxidase that affects susceptibility to mercury toxicity in humans. *Toxicolo Sci*. 2009;109(2):228–236.
35. Geier DA, Geier MR. A two-phased population epidemiological study of the safety of thimerosal-containing vaccines: a follow-up analysis. *Med Sci Monit*. 2005;11(4):160–170.
36. Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuro endocrinology letters*. 2005;26(5):439–446.
37. Palmer RF, Blanchard S, Stein Z, Mandell D, Miller C. Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas. *Health & place*. 2006;12(2):203–209.
38. Bradstreet JJ. A case-control study of mercury burden in children with autistic spectrum disorders. *J Coll Physicians Surg*. 2003;8(3):76–79.
39. Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R. Porphyrinuria in childhood autistic disorder: implications for environmental toxicity. *Toxicol Appl Pharmacol*. 2006;214(2):99–108.
40. Geier DA, Geier MR. A prospective study of mercury toxicity biomarkers in autistic spectrum disorders. *J Toxicol Environ Health*. 2007;70(20):1723–1730.
41. Austin DW, Shandley K. An investigation of porphyrinuria in Australian children with autism. *J Toxicol Environ Health Sci Part A*. 2008;71(20):1349–1351.
42. Khaled EM, Meguid NA, Bjorklund G, et al. Altered urinary porphyrins and mercury exposure as biomarkers for autism severity in Egyptian children with autism spectrum disorder. *Metab Brain Dis*. 2016;31(6):1419–1426.
43. James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J clin nut*. 2004;80(6):1611–1617.
44. James SJ, Melnyk S, Jernigan S, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141b(8):947–956.

45. Youn S-I, Jin S-H, Kim S-H, Lim S. Porphyrinuria in Korean children with autism: correlation with oxidative stress. *J Toxicol Environ Health*. 2010;73(10):701–710.
46. Bradstreet JJ, Smith S, Baral M, Rossignol DA. Biomarker-guided interventions of clinically relevant conditions associated with autism spectrum disorders and attention deficit hyperactivity disorder. *Altern Med Revc*. 2010;15(1):15–32.
47. Geier DA, Kern JK, Homme KG, Geier MR. Abnormal brain connectivity spectrum disorders following thimerosal administration: a prospective longitudinal case–control assessment of medical records in the vaccine safety datalink. *Dose-Response*. 2017;15(1).
48. Geier DA, Pretorius HT, Richards NM, Geier MR. A quantitative evaluation of brain dysfunction and body-burden of toxic metals. *Med Sci Monit*. 2012;18(7):CR425–CR431.
49. Inoue K, Ota U, Ishizuka M, et al. Porphyrins as urinary biomarkers for bladder cancer after 5-aminolevulinic acid (ALA) administration: the potential of photodynamic screening for tumors. *Photodiagnosis Photodyn Ther*. 2013;10(4):484–489.

31

Urine Indican Test (Obermeyer Test)

Dirk W. Powell, BS, ND

OUTLINE

INTRODUCTION

An increased level of urinary indican indicates bacterial dysbiosis or small intestine bacterial overgrowth (SIBO). Indole is produced when tryptophan, an essential amino acid, is converted to *2,3-benzopyrrole* by intestinal bacterial cleavage of the tryptophan side chain. The indole produced in the small intestine is mostly excreted in feces, but some is also absorbed into the systemic circulation.

Indole that is absorbed from the small intestine is converted to indican (*1H-indol-3-ol hydrogen sulfate ester*) in the liver. Indican is conjugated with potassium sulfate or glucuronic acid. After reentering the general circulation, indican is then excreted by the kidneys, and the concentration of indican can be measured in the urine (Fig. 31.1).

CLINICAL APPLICATION

Because most of the endogenous indoles have a side chain that prevents cleavage and are metabolized to skatole, the production of indicans (indoxyl potassium sulfate and indoxyl glucuronate) reflects bacterial activity in the gastrointestinal tract, particularly in the small intestine. Table 31.1 lists conditions where increased levels of indican are found.[1-4] Elevated levels of indican are considered an indicator of the overgrowth of anaerobic bacteria in the small intestine, impaired protein digestion, or excess protein (tryptophan) ingestion.

Procedure

The detection of indican depends on its oxidation to indigo blue by Obermeyer reagent and then the concentration of the indigo blue in a layer of chloroform for measurement.

Method

1. Place 5 mL of fresh urine in a test tube.
2. Add 5 mL of Obermeyer reagent.
3. Mix.
4. Add 2 mL of chloroform and invert several times.
5. Allow the chloroform to settle and observe.
6. The results are then rated by the color present in the chloroform layer.

The Obermeyer test requires the use of a hood to exhaust toxic fumes and caution to avoid skin contact with Obermeyer reagent.

Reagents

- Obermeyer reagent: dissolve 0.8 g ferric chloride in 100 mL concentrated hydrochloric acid (*caution:* caustic).
- Chloroform (*caution:* volatile and toxic; keep tightly capped)

Results

Most tests result in varying depths of blue, as shown in Table 31.2. However, some patients have as-yet-unidentified molecules that can modify the colors produced by this methodology. In Table 31.2, column 1 lists the ranking, column 2 shows the typical blue color, and column 3 lists color variants.

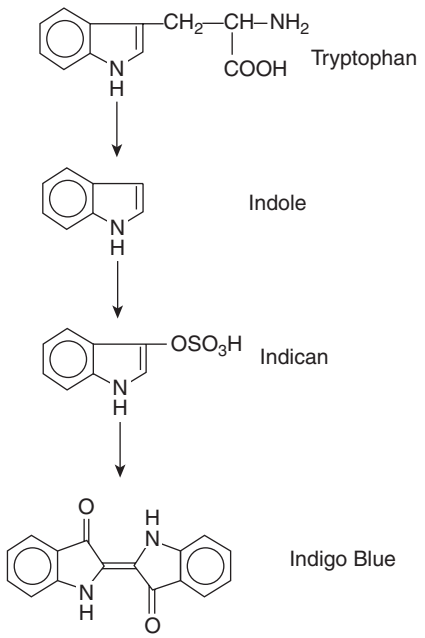


Fig. 31.1 Conversion of tryptophan to indican and indigo blue.

TABLE 31.1 Conditions Associated With Elevated Levels of Indican[1–4]

Hypochlorhydria (parietal cell dysfunction, antacids)
Pancreatic insufficiency (deficient trypsin, chymotrypsin, pepsin)
Excess protein intake
Increased GI transit time (gastroparesis, inefficient peristalsis)
Gastric ulcer or cancer
Inflammatory bowel disease
Celiac disease
Biliary and intestinal obstruction
Small intestine bacterial overgrowth
Gastrectomy or bariatric surgery
Jejunal diverticulosis
Scleroderma
Hartnup disease

GI, Gastrointestinal.

TABLE 31.2 Interpretation of Obermeyer Test Results

Toxic Level	Color	Color Variants
0		Urine color
1		Light blue or light green
2		Blue, green, or golden brown
3		Violet, indigo, or dark brown
4		Jet black

- Urine color: 0 (negative or normal)
- Light blue or light green: +1
- Blue, green, or golden brown: +2
- Violet, indigo, or dark brown: +3
- Jet black: +4

INTERPRETATION

A positive result can be associated with the disorders listed in Table 31.1, hypochlorhydria, pancreatic insufficiency, SIBO, an excessively high protein intake. The latter indication can be controlled by the institution of a diet with moderate protein (less than 60 g daily) for 2 days before testing.

CONCLUSION

The Obermeyer test is an in-office diagnostic test that can be used to identify gastrointestinal (GI) dysfunction or dysbiosis. A positive test may indicate the need for additional testing for gastrointestinal pathogens, SIBO, or altered GI function. The Obermeyer test for urine indican can be a clinically useful tool for identifying underlying causes of GI-related disorders and for optimizing GI health.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Davidson I, Henry JB. *Todd-Sanford Clinical Diagnosis and Management by Laboratory Methods*. 15th ed. Philadelphia: WB Saunders; 1974:773–775.
2. Greenberger N, Saegh S, Ruppert RD. Urine indican excretion in malabsorptive disorders. *Gastroenterol*. 1968;55:204–211.
3. Curzon G, Walsh J. Value of measuring urinary indican excretion. *Gut*. 1966;7:711.
4. Asatoor AM, Craske J, London DR, et al. Indole production in Hartnup disease. *Lancet*. 1963;1:126–128.

SECTION 3

Therapeutic Modalities and Practice Specialties

This section presents a historical, scientific, and clinical review of the schools of thought and modalities of natural medicine by its various names. We have compiled the work of experts in their fields into what we hope the reader will find a concise, yet useful, description of these practices, modalities, and specialties. Because of the clinically oriented and unconventional nature of these disciplines, the scientific evaluation of their theories and efficacy has been limited in the past. Happily, published research in natural medicine has increased dramatically since *A Textbook of Natural Medicine* was first published in 1985.

Although this textbook is strongly oriented to the scientific method and the use of the peer-reviewed research for documentation of the efficacy of a therapy, the widespread clinical use and long history of patient satisfaction demand that they be given far more clinical attention. Although the mechanisms of action of several have yet to be elicited, their research foundation continues to strengthen.

32

Acupuncture

Mark Harrison Nolting, ND, EAMP, and *John C. Reed, MD, MDiv*

OUTLINE

INTRODUCTION

This chapter presents acupuncture at a time when the practice has been increasingly recognizable and is a popular therapeutic practice around the world. Although research into the efficacy of acupuncture abounds, quality research studies that are acceptable to many in conventional medicine seem elusive. Professional skepticism of the practice of acupuncture is easy to find online. We intend for this chapter to inform and encourage the discussion of acupuncture within the current state of practice, promote understanding and consideration of the history of acupuncture, and explore the variety of practice options. Importantly, this chapter also serves as a presentation of updated research information, providing the basis for what we consider to be the availability of good evidence of efficacy.

Acupuncture needling is one of the most visual and widely practiced traditional medicine therapies in the world today.[1] Public acceptance and utilization of acupuncture as a profession and a therapeutic modality steadily increased across the globe between 1980 and 2018. In the United States, where the growth of acupuncture has been most impressive during these years, we have seen the numbers of licensed nonphysician practitioners and physician practitioners expand rapidly. In Washington State, for example, the number of acupuncture licensees jumped by 227% during the 10 years from 1997 to 2007, the most of any of the complementary and alternative (CAM) medicine practices, including chiropractic. The same report showed the growth of chiropractic licenses was only 14%, whereas that of physicians was 34%. Naturopathic physician licenses also increased considerably during the same period, by 134%.[2]

PROFESSIONAL ORGANIZATIONS

The following U.S.-based organizations were formed to guide, support, and monitor all aspects of acupuncture practice in the United States, from credentialing and professional practice to education and research:

- National Certification Commission for Acupuncture and Oriental Medicine (NCCAOM; founded in 1982): the nonprofit professional certifying agency for acupuncture and oriental medicine practice[3]
- American Association of Acupuncture and Oriental Medicine (AAAOM; founded in 1981): the largest acupuncturist professional trade organization[4]
- Council of Colleges of Acupuncture and Oriental Medicine (CCAOM; founded in 1982): the 501(c)(6) voluntary membership association for 57 acupuncture schools and programs in the United States[5]
- Accreditation Commission for Acupuncture and Oriental Medicine (ACAOM; 1989): the national organization recognized by the U.S. Department of Education to accredit acupuncture and Oriental medicine (AOM) schools and programs in the United States[6]
- American Board of Chiropractic Acupuncture (ABCA; founded in 2005): the nonprofit organization that is the sole chiropractic acupuncture credentialing organization recognized by the American Chiropractic Association[7]
- American Academy of Medical Acupuncture (AAMA; founded in 1987): the largest physician acupuncturist (MD, DO) professional trade organization[8]
- American Board of Medical Acupuncture (ABMA; founded in 2000): the nonprofit credentialing organization setting national standards in training and examination for diplomates in medical acupuncture[9]
- Society of Acupuncture Research (SAR; founded in 1993): an interprofessional organization of researchers and clinicians dedicated to improving the quality and increasing the awareness of research in acupuncture, herbal therapy, and other modalities of Oriental medicine[10]

BOX 32.1 Education

Acupuncture Training	Physician Training	Other
Licensed acupuncturist (LAc standard): • 3–4 years professional, post-bachelor's standard • 1500–2000 hours acupuncture specific • Advanced professional doctorate programs (PD and DAOM/Doctorate of Acupuncture and Oriental Medicine)	MD, DO, ND, DC: • 300 hours or less, acupuncture specific • 100 hours for chiropractic add-on	Physical therapist, nurse: • 40–50 hours of "dry needling" National Acupuncture Detox Association detoxification technicians: • 100 hours or less

TABLE 32.1 China TCM Statistics 2017

TCM professionals, total	613,000
TCM doctors	482,000
TCM interns	14,000
TCM pharmacy professionals	117,000
Acupuncture hospitals	6
TCM doctors per 10,000 population	2.69 (0.69–0.83, United States)
Chinese investment in TCM	1.7 billion yuan

TCM, Traditional Chinese medicine.

- National Acupuncture Detoxification Association (NADA; founded in 1985): a not-for-profit 501(c)(3) training and advocacy organization that encourages community wellness through the use of a standardized 5-point ear acupuncture protocol initially developed to assist in substance abuse withdrawal[11]
- American Society of Acupuncturists (ASA; founded in 2017): an umbrella organization that grew out of the informal Council of State [Acupuncture] Associations. This 501(c)(6) not-for-profit organization has 24 state associations with a total current membership of 4000. It seeks to provide resources for the promotion of professional acupuncture practitioners in their respective state practice situations.[12]

As a result of the ongoing work of these organizations, the following milestones had been achieved as of 2018[3,5,11]:

- Licensure: 47 states plus Washington, DC
- Licensed acupuncturists: estimated 25,000 to 30,000
- CCAOM schools and branches: 57
- NCCAOM diplomates since 1982: 22,000
- NADA-trained providers in 23 states

There are several types of providers who receive training in acupuncture,[3–9,11,12] as noted in Box 32.1.

On occasion, professionals such as medical doctors, naturopathic physicians, and chiropractors undergo extensive training in acupuncture, but it is more common to receive specific professional training composed of far fewer hours.

For perspective and comparative purposes, Table 32.1 provides traditional Chinese medicine (TCM) statistics from mainland China for the years 2007 and 2008. Acupuncture is considered a key component of the practice of "modern" TCM, along with herbal medicine, massage, nutrition, qigong, and health promotion.[13–19]

The World Health Organization stated in 2001 that "Chinese medicine, particularly acupuncture, is the most widely used traditional medicine. It is practiced in every region of the world."[20] Although this statement has not been revised as of 2018, there is every reason to believe that this remains true.

TERMINOLOGY

When it comes to discussing acupuncture in the context of its integration into Western health care systems, we should recognize that term *acupuncture* is ambiguous, and its meaning can differ based on its context of use. For clarification, the common different meanings of *acupuncture* are as follows:

1. Acupuncture is a physical medicine procedure involving the insertion of filiform needles into the body at specific points to effect therapeutic functional changes in nerve, connective tissue, muscular, and vascular structures and internal organ systems. Procedures that elicit these therapeutic responses through point stimulation using heat (moxibustion), pressure, laser light frequencies, sound frequencies, and electrical currents in the milli- to microampere range are best termed "acupuncture-related techniques," which can be more clearly described by adding clarifying terms, such as *electroacupuncture, sonopuncture, acupressure,* and so forth.[21]
2. Acupuncture is a multicomponent health care practice: The "practice of acupuncture" involves history taking, physical examination, diagnosis, patient education, and acupuncture procedure planning regarding point selection, stimulation intensity, and session frequency. In the practice of acupuncture, a practitioner typically incorporates other traditional or modern physical medicine interventions, such as heating of acupoints (traditional moxibustion), suction cupping, bleeding, soft tissue scraping, manual tissue and joint manipulation, electrostimulation, laser stimulation, and therapeutic exercise. The practice of acupuncture can be relevant to patient outcome goals, including goals in acute care, chronic illness care, and preventive or wellness care. The present-day theories and methods of the practice of acupuncture grew out of the health care traditions from China, Japan, Korea, and Vietnam and the dissemination of this practice through the West since the 17th century. Recent acupuncture basic science research is illuminating the biomedical correlations to aspects of acupuncture methods and practice that were previously only understood via translations of the metaphors used in the ancient Chinese acupuncture texts.[22]
3. The term *acupuncture* is used in many cases to refer to the distinct entry-level health profession that specializes in the practice of acupuncture and related techniques according to a licensed or registered scope of practice delimited by U.S. state laws. The designation of these "professional acupuncturists" varies, with the most familiar terms being licensed acupuncturist (LAc), certified acupuncturist (CA), East Asian medicine provider (EAMP), Oriental medical doctor (OMD), and doctor of acupuncture and oriental medicine (DAOM). Although the initial licensing authorities were concerned with the safety and competency of practitioners inserting needles into patients, most professional acupuncturists in the United States have been trained in East Asian traditional diagnostic and therapeutic models that are broader in scope than the traditional application of needle-based physical medicine treatments. This profession has worked to progressively expanded the legal scope of practice to reflect the practice of a comprehensive natural health care system.

In the state of California, for example, acupuncture licensure was expanded to reflect this trend within the professional acupuncturist community. "The theory and practice of acupuncture is based

on Asian medicine (also known as traditional, classical Chinese or Oriental medicine), a comprehensive natural health care system that has been in use in Asian countries for thousands of years to preserve health and diagnose, treat, and prevent illness."[23]

The practice of the California licensed acupuncture profession, according to Business and Professions Code Section 4937(b) of California, now legally includes the following: "to perform or prescribe the use of oriental massage, acupressure, breathing techniques, exercise, or nutrition, including the incorporation of drugless substances and herbs as dietary supplements to promote health."[24]

In this process of expanding the scope of practice beyond the physical medicine practice of acupuncture, the profession in Washington State redefined itself in legislation as *East Asian medical practitioners (EAMPs)*.

This expansion of the scope of professional acupuncturists beyond the clinical practice of a physical medicine–based needling procedure into a broad-based "natural medicine" scope involving herbal treatments, manual therapies, and lifestyle practices is likely a result of two factors: first, the perceived need among the public for more personalized, "natural," and gentler health services than those of typical biomedicine; and second, the creation of TCM as an organized diagnostic and treatment practice style in the modern Peoples Republic and the resulting export of TCM as the principal type of diagnosis and treatment approach taught in U.S. acupuncture programs and colleges. The TCM style is most compatible with the use of herbal treatments for internal medicine problems and with the use of needling as an adjunct therapy. For clarity, there are many styles of acupuncture practice. In Japan, for example, traditional Japanese medicine includes the acupuncture practitioners who use needles, moxa, and other tools to deal with the physical medicine aspects of health and illness and includes the 10,000+ regular medical doctors who use Kampo medicines (standardized herbal formulae) to complement pharmaceutical drugs and diet and lifestyle intervention for the care of internal medicine health problems.[25]

In November 1997, the National Institutes of Health (NIH) convened a consensus development conference on the subject of acupuncture. After discussion among a board of experts, the following working definition was included in the NIH Consensus Statement:

> *Acupuncture describes a family of procedures involving stimulation of anatomical locations on the skin by a variety of techniques. There are a variety of approaches to diagnosis and treatment in American acupuncture, which incorporates medical traditions from China, Japan, Korea, and other countries. The most studied mechanism of stimulation of acupuncture points employs penetration of the skin by thin, solid, metallic needles, which are manipulated manually or by electrical stimulation.*[26]

The NIH conference has had a lasting positive effect on the growth of acupuncture in the United States. The definitions and recommendations for further research have sparked wider acceptance within the medical profession and promoted the consensus that acupuncture as a procedure and as a mode of practice is ready to be incorporated into the education, research, and practice schemes of Western medicine. Because of the increasing basic and clinical research in acupuncture, the NIH conference documents kept the NIH definition of the practice of American acupuncture within definition levels 1 and 2 in the previous list. It did not include the diagnostic and therapeutic practices of TCM herbal internal medicine as part of the necessary model of "acupuncture practice."

We describe acupuncture and related physical medicine techniques as a standalone health care practice and also discuss the types of licensed providers who currently offer it within their scope of practice, including the LAc providers of East Asian natural medicine, the medical and osteopathic physicians, the chiropractic and naturopathic physicians, the physical therapists, and the drug detox technicians. All of these providers have been contributing to the dissemination of acupuncture throughout the health care system and to its accessibility to the general public. This chapter describes the various styles of acupuncture practice and the evidence-informed clinical applications of acupuncture so that readers will be better informed about effective clinical referrals based on patients' needs and desires.

CLINICAL APPLICATIONS

The clinical applications of acupuncture can be divided into three areas: biomechanical medicine, internal medicine, and mind–body medicine.

Biomechanical medicine refers to approaches that improve or restore the structure and functions of the muscles, nerves, connective tissue, ligaments, and bones that support pain-free movement and resilience to mechanical stress. Examples include low back pain, joint strains, radiculopathies, and sports injuries.

Internal medicine refers to disorders of internal organ systems and the neuroendocrine regulation of the body; examples are migraine headaches, diabetes, constipation, and infertility.

Mind–body medicine refers to psychological and emotional disorders ranging from stress reactions to anxiety, depression, cognitive dysfunction, and interpersonal and social adjustment disorders.

THEORY AND PRACTICE STYLES

Acupuncture is the Western name for a technique involving stimulation of particular anatomically mapped locations or palpable tender zones of the body by heat applications, mechanical stimulation, or the insertion of fine needles. Classical approaches to the theory and organization of this health care practice date back as many as 4000 to 5000 years. For contemporary practitioners, their practice styles of acupuncture are based on an understanding of several fundamental concepts, as discussed in the following subsections.

Yin/Yang Balance

Yin/yang balance is the central theory in the practice of acupuncture. It refers to relative biophysiological balance and can be clinically observed in neurological signaling or in cellular and organ metabolism as activation versus inhibition, in tissues as inflammation versus inflammation control, in thermoregulation as heating versus cooling, in autonomic activity as sympathetic versus parasympathetic tone, and in vascular tone as vasodilation versus vasoconstriction.

Qi, Blood, and Fluid

Qi, blood, and fluid can be clinically observed in the vascular system as oxygenated versus deoxygenated blood flow, as venous drainage versus venous congestion, in lymphatic flow as determined by tissue tension and by intravascular versus extravascular fluid exchange, and by physicochemical phase shifts in biological fluids between liquid, solid, and gel (traditionally translated as "phlegm") states.

Five Phases

The five phases (or five elements or five movements) are clinically observable patterns of genetically expressed physiological tendencies and body morphotypes, which resonate with personality strengths and weaknesses and with environmental and dietary preferences and susceptibilities. This approach can be clinically useful in determining which acu-stimulation targets could rebalance specific physiology or psychological factors that are promoting the yin/yang or qi, blood,

and fluid imbalances. This treatment approach varies from the TCM approach by being a more indirect or nonlinear approach to achieving healthier homeostasis. Five-element acupuncture is a refined form of constitutional acupuncture. Practitioners of this style will identify the deeper and lifelong biopsychosocial patterns underlying their patients' ailments. They attempt an artful rebalancing of the interwoven patterns of dysfunction using the special channel acupoints that classically have inherent nourishing and controlling relationships among the five-phase physiology of that particular person.

Zang Fu Organ Functional Metabolism Qualities

The Zang Fu are the Chinese "organs" and related physiological functions. There are five solid "Zang" yin organs (liver, heart, spleen/pancreas, kidneys, and lungs) and five hollow "Fu" yang organs (gallbladder, small intestines, stomach, large intestine, and urinary bladder). In addition, there are two "organs" that represent distributed physiological functions that the ancient Chinese associated with a related body anatomy. The *San Jiao* or "triple energizer" is a yang organ that roughly corresponds to the overall restorative and functional activities of all the organs in the upper, middle, and lower body cavities. In traditional teaching, it is considered to be a Fu organ with the function of transporting water. In modern thinking, it seems to correspond to the physiological function of the mitochondria, whose activities provide the metabolic energy of the internal organs. The paired yin "organ" is the *Xin-Bao,* or "heart protector," which has been poorly translated in English as "pericardium."

Depending solely on the Western name without a thorough understanding of the traditional Chinese meaning leads to much confusion clinically. In clinical practice, the heart-protector function often corresponds to the regulation of sympathetic tone. In practice, the Chinese term for urinary bladder function seems to correspond to the embryological and functional aspects associated with the entire cloacal region in comparative anatomy. The liver in the Chinese sense is much more a functional system with many body-wide interactions. Among other things, the Chinese *Gan* (liver) stores blood and qi and is responsible for the smooth transmission of qi and blood throughout the body. It also has a chief role in emotions; an unbalanced *Gan* (liver) results in anger, high blood pressure, and a general state of agitation. In the TCM diagnostic approach, the Zang Fu and yin/yang principles lead to poetic explanatory diagnoses. Clinical symptoms such as dizziness, vertigo, insomnia, dry throat, and lumbago could be ascribed to a deficiency of liver and kidney yin. Cold limbs, lumbago, diarrhea, and scanty urine could be ascribed to a deficiency of spleen and kidney yang.

Six-Channel Acupuncture *(Jing Luo)*

Six-channel acupuncture *(Jing Luo)* is explained in the classical text *Sang Han Lung*. This approach facilitates interactions among organs, tissues, nerves, and blood vessels via electromechanical needle stimulation of the semiconductive connective tissue web that permeates the body but can be accessed by major acupoints.

Neuroanatomical Acupuncture

Neuroanatomical acupuncture is a style of practice inclusive of recent biomedical studies in animals of electroacupuncture effects on the central and peripheral nervous system[27] and anecdotal clinical experience with myofascial "trigger-point" mechanisms.[24,26,28,29-37]

The treatment of tender knots in muscles by intramuscular stimulation is a "non–channel-based acupuncture. It was termed "Ashi" or "ouch point" acupuncture by the ancients and was probably the first form of acupuncture practiced in the West from the time of the Dutch physician Willem Ten Rhyne, who visited Japan sometime in the early 17th century.[38] Dr. Franklin Bache (Benjamin Franklin's great-grandson) described his clinical trials utilizing acupuncture on prisoners with various conditions in an 1825 article in the *North American Medical and Surgical Journal.* The descriptions of his empirical needling intervention suggest he knew nothing about classical point, channel, or related theories.[39]

This form of acupuncture was essentially rediscovered by Dr. Janet Travell, who, along with Dr. David Simon, published texts for the treatment of myofascial pain with trigger-point therapy. They discovered that the stimulation of these palpable points with hydremic needles was effective even if they did not inject local anesthetics. Over the intervening several decades, this has led to the promotion of a simplified "dry-needling" style of acupuncture among U.S. physical therapists and some chiropractic physicians. Conversely, basic research done in modern China with electroacupuncture has contributed to the broader neuroanatomical style of acupuncture by demonstrating that different frequencies of electronic stimulation via acupoints and myotome and sclerotome regions can boost specific types of endorphins and enkephalins, achieving a level of pain relief not seen with simple trigger-point releases or channel-based manipulation with needles and moxibustion.[27]

Modern research has found a high degree of overlap between the mapped trigger points of Travell and Simons and the classical acupoints that are related to the anatomy of the connective tissue major and auxiliary channels[37]:

Microsytem acupuncture
Ear (auricular) acupuncture
Korean hand acupuncture
Scalp acupuncture: Chinese, YNSA (Yamamoto New Scalp Acupuncture)

SCOPE OF PRACTICE

Similar to the challenges in universal acceptance of an exact definition of the term *acupuncture,* the clinical practice of acupuncture also varies across the world and in different U.S. jurisdictions. As noted, there were acupuncture practice acts in 47 U.S. states and the District of Columbia as of early 2018. Although there is a core of therapeutic acceptance from state to state, there are wide variations in expanded scopes. Although most states use the designation "LAc," a few have begun to use other credentials, such as "doctor of acupuncture" in Rhode Island, "doctor of Oriental medicine" in a few other states, and "East Asian medicine practitioner" in Washington State.[40]

New Mexico is a good example of perhaps the broadest acupuncture scope in the United States. In New Mexico, the practice of "Oriental medicine" includes homeopathy, prescriptive drug authority (with expanded practice certification), laser therapy, injection, and intravenous (IV) therapy within its doctor of Oriental medicine (DOM) designation.[41]

Although California law has in many ways set the pace for the profession through the years, with a scope that now defines acupuncturists as primary health care professionals with a practice inclusive of Asian massage, acupuncture, herbal medicine, exercises, breathing techniques, and dietary supplements, among others, it is the Washington State law that forged entirely new ground for acupuncturists in the United States.

The stage was set for the renaming and overhaul of the state acupuncture law for a number of reasons, including the strong desire by a Korean Washington State legislator to rid the Western world of the term *Oriental,* an upcoming sunset review, and several confusing and deficient parts of current acupuncture law. What resulted was the insertion of the term *Asian* in place of *Oriental* in the Washington State acupuncture regulations. "East Asian medicine means a health care service utilizing East Asian medicine diagnosis and treatment to

promote health and treat organic or functional disorders and includes the following: Acupuncture (or lancets), electrical, mechanical, magnetic devices, moxibustion, acupressure, cupping, dermal friction technique, infrared, sonopuncture, laserpuncture, point injection therapy (aquapuncture), dietary advice (including recommendation and sale of herbs, vitamins, minerals), breathing, relaxation and East Asian exercise, Qi Gong, East Asian massage and Tui na, and superficial heat and cold therapies."[42]

Acupuncturists in the state of Washington are now licensed under the EAMP credential.[43]

SAFETY

Acupuncture therapy is generally regarded as a safe and relatively pain-free procedure. There are many forms and styles of acupuncture therapy practiced; some have a reputation for potentially being more painful at times, but generally the procedure in the United States is accompanied by very little reporting of adverse effects. The treatment provided by most licensed acupuncturists is painless. Millions of treatments have been performed by licensed acupuncturists in the United States who have taken "clean needle" training as part of their basic acupuncture training. For the past 15 years, it has been the standard of care in acupuncture practice that single-use disposable needles are used in all treatments.

Recently, there has been renewed discussion about the safety of acupuncture, sparked by a *British Medical Journal (BMJ)* editorial piece that put a cautionary spin on the view that acupuncture needling is entirely safe or at least has virtually no side effects.

The article, "Acupuncture Transmitted Infections," although highlighting the skin infection risk of acupuncture needling, also identified only "more than 50 cases" to date (2010) worldwide. Nevertheless, the authors' main opinion stated that these types of infections were underreported.[44] An older report also published in the *BMJ* by Vincent seems appropriately titled: "The Safety of Acupuncture; Acupuncture Is Safe in the Hands of Competent Practitioners." Vincent stated, "The conclusion that acupuncture is a very safe intervention in the hands of a competent practitioner seems justified on the evidence available."[45] A large review study published in 2008 focused on acupuncture safety and efficacy in pediatrics. The authors "found evidence of some efficacy and low risk associated with acupuncture in pediatrics." They stated some caution as well, noting "the safety of acupuncture is a serious concern, particularly in pediatrics. Because acupuncture's mechanism is not known, the use of needles in children becomes questionable." They also stated, "nevertheless, it seems acupuncture is a safe complementary/alternative medicine modality for pediatric patients on the basis of the data we reviewed."[46]

The NCCAOM and the AAAOM jointly authored a response to the *BMJ* editorial, stating that "the article neglected to mention that the incident of infections is drastically reduced when a consumer seeks a qualified practitioner who has met the rigorous standards of the NCCAOM certification which includes passing of the Council of Colleges of Acupuncture and Oriental Medicine (CCAOM) clean needle technique course, a prerequisite to becoming an NCCAOM certified practitioner."[47] They stated in another article that "while acupuncture is a highly effective and valuable form of healthcare, it is not an entirely risk-free medical procedure, and should be administered by practitioners with the proper training who follow accepted guidelines of practice."[48]

Acupuncture has a low relative risk. The NIH Consensus Statement on Acupuncture published in 1998 found that the incidence of adverse effects is substantially lower than that of many drugs or other accepted procedures for the same conditions.[49] Systematic reviews and surveys have clarified that acupuncture is safe when performed by appropriately trained practitioners, with infrequent minor side effects, such as feeling relaxed, elated, tired, or having sensation or itching at the point of insertion. Rare, serious complications, such as infection or pneumothorax, are directly related to insufficient training. Safe use of acupuncture has also been established in pediatrics and for women who are pregnant.[49]

EVIDENCE-BASED AND EVIDENCE-INFORMED ACUPUNCTURE

Acupuncture practice has steadily increased across the world since the 1970s. Although it was once viewed as a strictly Asian practice, acupuncture is now practiced by a wide variety of medical and nonmedical practitioners. Each type of health care discipline has at least some of its members practicing acupuncture. Of course, the popularity and spread of the practice do not mean acupuncture has become mainstream or effective. Where is the evidence proving or disapproving the practice? Skeptics abound within established medical practice. At the same time, there is an increasing body of acupuncture research to consider, review, and evaluate. According to the Society for Acupuncture Research, "every month, an average of 100 acupuncture-related articles are published in more than 50 journals."[50] Although this may not seem impressive in the greater context of evidence-based medicine as a whole, it does represent a great increase over the years in research focused on the field.

A defining moment in acupuncture research in the West came with the conclusion of the NIH Consensus Conference in 1997. For the first time, a Western panel of medical experts gathered to review, critique, and set guidelines for the use of acupuncture in the medical field in the United States. Before this conference, research in acupuncture was acknowledged by some Western doctors but largely dismissed. The view of Asian studies and of studies from other countries in general did not make an effect on the U.S. radar. The conference helped focus the attention, collect what research had been completed to date, and concluded that there was merit in the use of acupuncture for certain conditions and, most importantly, that there was a real need and demand that acupuncture be better studied and used. Acupuncture was finally "on the map" in the West.[51]

Progress was made in the 10 years after the NIH conference, as was detailed by the 2007 Society for Acupuncture Research (SAR) International Symposium.[52] The symposium detailed a number of important research conclusions, highlighting growing evidence for the effectiveness of acupuncture treatment for chronic pain while detailing the issues concerning sham acupuncture in trials and a number of basic science trials demonstrating the physiological effects of needling.

Conventional medical practice has grown within a system of great scrutiny and self-analysis. A massive research culture spins off studies by the thousands. The double-blinded, placebo-controlled trial has become the hallmark of the "appropriately conducted research trial." Acupuncture evolved in a completely different culture, that of China, where, ironically, as we were "discovering" the Chinese traditional system of acupuncture, not so many years ago, the Chinese began a wholesale discovery of traditional Western medicine. Acupuncture and all that is TCM have been extensively researched in China. The quality and breadth of acupuncture research in China and Asia in general have increased greatly since the first edition of this textbook.

Large research academies, such as the China Academy of Traditional Chinese Medicine in Beijing, and numerous universities and schools of TCM have greatly improved the research culture in China since the 1970s. Although there is considerable emphasis on herbal research, there is still quite a lot of research examining the other areas of TCM, including acupuncture.[53-56]

In 2014, the Veterans Administration (VA) published an evidence map of acupuncture.[57,66-81] This document is a broad overview of the evidence base for acupuncture and is based on 183 systemic reviews from 2005 through 2013. The map estimated the research volume and effectiveness of acupuncture on the basis of the published reviews. According to the document, assessments to evaluate the value of acupuncture need to take into account multiple factors, including the following:

- Clinical efficiency
- Risk and benefits compared with standard treatment
- Costs and cost-effectiveness
- Patient satisfaction
- Patient preferences for a particular diagnosis

The evidence map of acupuncture for pain summarizes the clinical indication of pain. The bubble plot summarizes the results of 59 systematic reviews for 21 distinct indications relevant to the outcome of pain.

Evidence of a positive effect with high confidence was found for dysmenorrhea, cancer pain, and labor pain. The wellness-relevant indications and outcomes bubble plot summarize 43 systematic reviews and 3 large randomized controlled trials (RCTs) with 20 distinct indications. Evidence of a potential positive effect with high confidence was found for insomnia, smoking cessation, and restless leg syndrome. Results for the mental health bubble map represented 17 systematic reviews with summary effects for 9 clinical indications. There was a potential positive effect with high confidence for depression and schizophrenia. Medium confidence was found for the treatment of anxiety and posttraumatic stress disorder (PTSD).[57]

An excellent source of current research studies regarding integrative medicine including acupuncture is the Academic Consortium for Integrative Medicine and Health. The organization's website notes the following: "The Consortium was founded in 1999 by eight academic health centers including Duke University, Harvard University, Stanford University, University of California, San Francisco, University of Arizona, University of Maryland, University of Massachusetts, and the University of Minnesota. Now with 72 institutional members, the Consortium continues to grow and represents thousands of scientists, educators, clinicians and other health professionals who share an interest in the field of Integrative Medicine and Health."[58]

One of the topic areas within the Consortium is "moving beyond medications" to an area of collected information on evidence-based nonpharmacological pain approaches. Available for review is a white paper on strategies for comprehensive pain care. Also included in this section are evidence summaries for treating pain, as follows:

- Acupuncture in the management of cancer pain
- Acupuncture therapy for chronic pain
- Acupuncture therapy for postoperative pain with opioid sparing
- Acupuncture therapy acute pain (not perioperative)[58]

One of the studies highlighted included a review of several studies performed in emergency departments (EDs) in the United States between 2014 and 2016 and provided evidence of the effective use of acupuncture for acute nonperioperative pain.

A randomized study involved 300 ED patients who received either IV morphine or acupuncture. Acupuncture proved better than morphine in pain relief by 14%. Acupuncture also provided pain relief in a shorter time frame, by 6 to 18 minutes. There were also far fewer side effects, only 4 compared with 86 in the morphine group.[59]

In another summarized study, this one of feasibility and acceptability, 182 ED patients who received only acupuncture had a decrease in pain comparable to that of patients who had received analgesics. In addition, the acupuncture-only group had a reduction in anxiety levels.[60] The 2017 Clinical Guidelines of the American College of Physicians for acute, subacute, and chronic low back pain recommend that patients should select nonpharmacologic treatment with superficial heat, massage, acupuncture, or spinal manipulation.[59]

In multiple systematic reviews with meta-analyses, acupuncture was found to be effective in reducing postsurgical pain compared with sham acupuncture, controls, and usual care, with a reduction in opioid need (21% opioid reduction at 8 hours, 23% at 24 hours, and 29% at 72 hours postsurgery) and with lowered incidence of opioid-related side effects such as nausea, dizziness, sedation, pruritus, and urinary retention.[60-65]

SUMMARY

By most accounts, acupuncture has been around for approximately 5000 years. The basic practice of placing needles (made of metal, stone, bone, wood, or other materials) into select points on the body to effect change, be it pain relief or the calming of anxiety, has not really changed much. As we have briefly touched on in this chapter, the history of acupuncture is rich. Standards of practice vary widely, and critical areas of study have yet to be adequately delineated. Yet here we are in 2018 with a much larger and richer database of study—from RCTs to therapeutic outcomes—at our fingertips to discuss than we had in 1985 when this text was first published.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. National Center for Complementary and Integrative Medicine (NCCIH). http://nccih.nih.gov/health/acupuncture. Accessed 03/30/2018.
2. Washington State Department of Health, Health Systems Quality Assurance. *Annual Report, Health Professions Discipline and Regulatory Activities*. 2008. http://doh.wa.gov/hsqa/documents/2008UDA. Accessed 10/25/10.
3. National Certification Commission for Acupuncture and Oriental Medicine (NCCAOM). http://nccaom.org. Accessed 03/30/2018.
4. American Association of Acupuncture and Oriental Medicine (AAAOM). http://aaaomonline.org. Accessed 03/30/2018.
5. Council of Colleges of Acupuncture and Oriental Medicine (CCAOM). http://ccaom.org. Accessed 03/30/2018.
6. Accreditation Commission for Acupuncture and Oriental Medicine (ACAOM). http://www.acaom.org. Accessed 03/30/2018.
7. American Board of Chiropractic Acupuncture (ABCA). https://www.americanboardofchiropracticacupuncture.org. Accessed 06/16/2018.
8. American Academy of Medical Acupuncture (AAMA). https://www.medicalacupuncture.org. Accessed 06/16/2018.
9. American Board of Medical Acupuncture (ABMA). https://www.dabma.org. Accessed 06/16/2018.
10. Society of Acupuncture Research (SARS). https://www.acupunctureresearch.org. Accessed 06/16/2018.
11. National Acupuncture Detox Association (NADA) https://www.acudetox.com/index.php/about-nada. Accessed 06/17/2018.
12. American Society of Acupuncture (ASA). https://www.asacu.org. Accessed 06/17/2018.
13. The American Acupuncturist. *Terry Courtney: Sherman Cohn on Historical Perspectives Regarding the Formalization of the US Chinese Medicine Profession.* American Association of Acupuncture and Oriental Medicine (AAAOM). Fall 2010. http://aaaomonline.org. Accessed 10/25/10.
14. *Clinician's and Educators' Desk Reference on the Licensed Complementary and Alternative Healthcare Professions.* Seattle, Washington: Academic Consortium for Complementary and Alternative Health Care (ACCAHC). http://accahc.org. Accessed 10/30/10.
15. Council of Colleges of Acupuncture and Oriental Medicine. *Know Your Acupuncturist.* http://ccaom.org. Accessed 10/26/10.
16. Volker S. *Currents of Tradition in Chinese Medicine, 1626-2006*. Seattle, WA: Eastland Press Inc; 2007.
17. *2010 Census and Estimated range of Licensed Acupuncturists in the USA*; 2010. American Census results. http://2010census.gov. Accessed 10/30/10.
18. China Statistical Year Book of Chinese Medicine, issued by the State Administration of TCM in China; 2008.
19. Report on Advances in TCM and Chinese Herbal Materia Medica, issued by the China Academy of Science and Technology and the China Association of Chinese Medicine in 2009.
20. World Health Organization. *Traditional Medicine.* Fact Sheet No. 134. http://who.int/medi-acentre/factsheets/fs134/en. Accessed 10/25/10.
21. Tao L. Acupuncture: what underlies needle administration? Evidence-based complementary and alternative medicine. *eCAM*. 2009;6:185–193.
22. Washington East Asian Medicine Association. http://weama.info. Accessed 3/30/2018.
23. *A Consumer's Guide to Acupuncture and Asian Medicine,* California Department of Consumer's Affairs. http://nhcsp.com. Accessed 03/30/2018.
24. California Acupuncture Board. www.acupuncture.ca.gov. Accessed 03/30/2018.
25. Kaptchuk TJ. East Asian medicine (letter to the editor). *Ann Intern Med*. 2000;137:703.
26. Acupuncture. Consensus Development Conference Statement. National Institutes of Health. Available online at the NIH National Consensus Program website. http://consensus.nih.gov. Accessed 09/13/11.
27. Han, Jishan, Craig, William. *Personal communication*.
28. Lewith GT. *The History of Acupuncture in China. Acupuncture, its Place in Western Medical Science*. Suffolk, UK: Green Press; 1983.
29. *Webster's Encyclopedic Unabridged Dictionary of the English Language.* New York: Gramercy Books; 1996.
30. Yang I-Yen. *Personal communication*; 1997.
31. In: Zhufan X, Xiaokai H, eds. *Dictionary of traditional Chinese medicine*. Hong Kong: The Commercial Press; 1984:342.
32. Veith I. *The Yellow Emperor's Classic of Internal Medicine*. Baltimore: Williams & Wilkins; 1966.
33. Maoshing N. In: *The Yellow Emperor's classic of medicine: a new translation of the Neijing Suwen with commentary*. Boston: Shambhala.
34. Porkert M, Hempen CH. *Chinese Academy of TCM, classical acupuncture—the standard textbook.* Dinkelscherben, Germany: Phainon Edititrus & Media GmbH; 1995.
35. Wei-kang F. In: *The story of Chinese acupuncture and moxibustion*. Peking: Foreign Languages Press; 1975.
36. Hoizey D, Hoizey M. *A history of Chinese medicine*. Vancouver, BC: UBC Press; 1993.
37. Simons David G. *Travell Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual: Two Volume Set: Second Edition/Volume 1 and First Edition/Volume 2* published November 1st. LWW; 1998.
38. State Administration of Traditional Chinese Medicine. *Advanced Textbook on Traditional Chinese Medicine and Pharmacology*. Vol. 1. Beijing: New World Press; 1995.
39. Devitt M, Bache Franklin. In: *A Pioneer of American Acupuncture.* Acupuncture Fall News 2010, The American Acupuncturist. http://aaaomonline.org. Accessed 10/30/10.
40. National Certification Commission for Acupuncture and Oriental Medicine (NCCAOM). http://nccaom.org. Accessed 3/30/2018.
41. New Mexico acupuncture regulations. http://nmcpr.state.nm.us/nmac. Accessed 3/13/11.
42. California acupuncture practice law. http://acupuncture.ca.gov. Accessed 3/30/2018.
43. Washington State Department of Health RCW 18.06.010. http://doh.wa.gov. Accessed 3/30/2018.
44. Woo CY, Lin AW, Lau KP. Acupuncture transmitted infections. *BMJ*. 2010;340:c1268.
45. Vincent C. The safety of acupuncture. *BMJ*. 2001;323:467–468.
46. Jindal V, Gee A, Mansky PJ. Safety and efficacy of acupuncture in children: a review of the evidence. *J Pediatr Hematol Oncol*. 2008;30(6):431–442.
47. *Response to British Medical Journal Editorial Regarding Unsafe Practice of Acupuncture.* http://ncaaom.org. Accessed 3/14/11.
48. *The safety of Acupuncture and Oriental Medicine.* http://ncaaom.org. Accessed 03/30/2018.
49. *NIH Consensus Statement*. vol. 15; 1997:3–5. Number.
50. Acupunctureresearch.org, assessed online 06/05/2018.
51. *Acupuncture*. National Center for Complementary and Alternative Medicine; 2002. Publication No. D003.
52. Society for Acupuncture Research. http://acupunctureresearch.org. Accessed 03/30/2018.
53. Aetna Clinical Policy Bulletin. *Acupuncture.* Number: 0135. Copyright 2001-2010 Aetna Inc. http://aetna.com/cpd/medical/data/100_199/0135. Accessed 03/30/2018.
54. Natural Standard. http://naturalstandard.com/index-abstract.asp?create-abstract=/monograph5/alternativemodalities/acupuncture.asp. Accessed 10/27/10.
55. Witt CM, Jena S, Brinkhaus B, et al. Acupuncture for patients with chronic neck pain. *Pain*. 2006;125:98–106.
56. Wei-Yu F, et al. Traditional Chinese medical journals currently published in mainland China. *J Altern Complement Med*. 2008;14(5):595–609. https://doi.org/10.1089/acm.2008.0009.
57. Hempel S, Taylor SL, Solloway M, et al. *Evidence Map of Acupuncture* VAESP Project #05-226. ; 2013.
58. Accessed online imconsortium.org 07/30/2018.
59. Grissa MH, Baccouche H, Boubaker H, et al. Acupuncture vs intravenous morphine in the management of acute pain in the ED. *Am J Emerg Med*. 2016;34(11):2112–2116.
60. Reinstein AS, Erickson LO, Griffin KH, et al. Acceptability, adaptation, and clinical outcomes of acupuncture provided in the emergency department: a retrospective pilot study. *Pain Med*. 2016.

61. Arya Nielsen, PhD Consortium Pain Task Force Evidence Group. Evidence-Based Nonpharmacologic Strategies for Comprehensive Pain Care The Consortium Pain Task Force White Paper © www.nonpharmpaincare.org. Accessed 06/16/2019.
62. Sun Y, Gan TJ, Dubose JW, Habib AS. Acupuncture and related techniques for postoperative pain: a systemic review of randomized controlled trials. *Br J Anaesth*. 2008;101:151–160.
63. Unschuld PU. *Nan-Ching: the Classic of Difficult Issues*. Berkeley: University of California Press; 1986.
64. Unschuld PU. *Medicine in China: A History of Ideas*. Berkeley: University of California Press; 1985.
65. Unschuld PU, ed. In: *Approaches to traditional Chinese medical literature. Proceedings of an International Symposium on Translation Methodologies and Terminologies*. Dordrecht, The Netherlands: Kluwer; 1989:517.
66. Zheng CH, Zhang MM, Huang GY, Wang W. The role of acupuncture in assisted reproductive technology. *Evid Based Complement Alternat Med*. 2012;2012:543924.
67. Haake M, Muller HH, Schade-Brittinger C, et al. German Acupuncture Trials (GERAC) for chronic low back pain: randomized, multicenter, blinded, parallel-group trial with 3 groups. *Arch Intern Med*. 2007;167(17):1892–1898.
68. Lund I, Lundeberg T. Are minimal, superficial or sham acupuncture procedures acceptable as inert placebo controls? *Acupunct Med Mar*. 2006;24(1):13–15.
69. Zhang H, Bian Z, Lin Z. Are acupoints specific for diseases? A systematic review of the randomized controlled trials with sham acupuncture controls. *Chin Med*. 2010;5:1. Evidence Map of Acupuncture Evidence-based Synthesis Program 459 CONTENTS 34.
70. Linde K, Niemann K, Schneider A, Meissner K. How large are the nonspecific effects of acupuncture? A meta-analysis of randomized controlled trials (Provisional abstract). *BMC Medicine*. 2010;75(1). http://onlinelibrary.wiley.com/o/cochrane/cldare/articles/DARE12011001754/frame.html. Accessed 06/16/2019.
71. Moroz A, Freed B, Tiedemann L, Bang H, Howell M, Park JJ. Blinding measured: a systematic review of randomized controlled trials of acupuncture. *Evid Based Complement Alternat Med*. 2013;2013:708251.
72. White A, Foster N, Cummings M, Barlas P. The effectiveness of acupuncture for osteoarthritis of the knee: a systematic review. *Acupunct Med*. 2006;24:40–48.
73. Shi GX, Yang XM, Liu CZ, Wang LP. Factors contributing to therapeutic effects evaluated in acupuncture clinical trials. *Trials*. 2012;13:42.
74. MacPherson H, Hammerschlag R. Acupuncture and the emerging evidence base: contrived contr oversy and rational debate. *J Acupunct Meridian Stud*. Aug 2012;5(4):141–147.
75. Liu YQ, Ma LX, Xing JM, et al. Does Traditional Chinese Medicine pattern affect acupoint specific effect? Analysis of data from a multicenter, randomized, controlled trial for primary dysmenorrhea. *J Altern Complement Med. Jan* 2012;19(1):43–49.
76. He L, Zhou MK, Zhou D, Li N, Wu B. Acupuncture for Bell's palsy: a systematic review. *Chinese Journal of Evidence-Based Medicine*. 2005;5(2):106–109, 129.
77. Yeung WF, Chung KF, Poon MM, et al. Prescription of Chinese herbal medicine and selection of acupoints in pattern-based traditional Chinese medicine treatment for insomnia: a systematic review. *Evidence-Based Complementary and Alternative Medicine*. 2012. Article Number 902578.
78. Zheng H, Chen M, Wu X, Li Y, Liang FR. Manage migraine with acupuncture: a review of acupuncture protocols in randomized controlled trials. *Am J Chin Med*. 2010;38(4):639–650.
79. Prady SL, Burch J, Crouch S, Macpherson H. Controlling practitioner-patient relationships in acupuncture trials: a systematic review and meta-regression. *Acupunct Med. Mar*. 2013;15.
80. Kim SY, Lee H, Chae Y, Park HJ. A systematic review of cost-effectiveness analyses alongside randomised controlled trials of acupuncture. *Acupunct Med*. Dec 2012;30(4):273–285.
81. Li Y, Liu LA, Zhao L, et al. [Acupuncture and moxibustion for peripheral facial palsy at different stages: multi-central large-sample randomized controlled trial]. *Zhongguo Zhen Jiu*. 2011;31(4):289–293.

33

Ayurveda: The Science of Life and Mother of the Healing Arts

Virender Sodhi, MD (Ayurveda), ND

OUTLINE

INTRODUCTION

Ayurveda is one of the most ancient systems of medicine known today. The origins of this science of life (*Ayu,* "life," and *Veda,* "knowledge"), although difficult to pinpoint, have been placed by scholars of ancient Indian Ayurvedic literature somewhere around 6000 BC.[1]

Ayurveda is a holistic science of health and a balance in lifestyle. Disease is seen as an imbalance, and its treatment involves diverse strategies to restore optimal function and balance. Using dietary alterations, yoga, and exercise, along with elaborate surgical techniques and complex, integrated herbal formulas, the Ayurvedic physician treats the whole person, removing disease completely by ending the imbalance that created it.

HISTORY

In ancient India, it was custom for a teacher's instruction to be recorded by his students, who would eventually repeat the same information orally to their own disciples. Thus according to the different interpretations given by various disciples of Ayurveda, a number of treatises were written. Although specific instructions differed, the basic principles remained the same.

Ayurvedic teachings were orally transmitted for thousands of years and then written down in melodious Sanskrit poetry. The contents of a number of Sanskrit verses, or *shlokas,* although written many centuries ago, still sound a note of familiarity in today's scientific environment. Ayurveda, in its first recorded form (*vedas:* the world's oldest literature), is specifically called *Atharveda.*

The Development of Ayurvedic Medicine

Hindu legend holds that after seeing the suffering of human beings, Lord Brahma, the god of creation, elaborated ways to ease that suffering to Daksha, who, in turn, taught them to the Ashwin twins. Fig. 33.1 presents the chronology of Ayurveda's development.

Dhanvantari and Bhardwaj separately developed the surgical and medical aspects of Ayurveda around the 9th century BC. Their students recorded these principles in great detail in compendia that are called *Samhitas.*

The *Sushruta Samhita,* one of the most widely accepted Ayurvedic texts, emphasizes the surgical aspects of therapy. Its author, Sushruta, is considered the father of surgery (particularly of plastic and reconstructive surgery). The medical teachings of Charak were a synthesis of earlier work. His material has become a classic text of the nonsurgical medical wisdom of Ayurveda. Successive generations have modified his work, the *Samhita.*

The Major Schools and Specialties

School of Physicians *(Atreya Sampradaya)*

Charak wrote a complete text on Ayurvedic medicine in which he revised the work of Agnivesh. Charak's text described the *Tridosh* physiology (*Vat, Pit,* and *Kaph*), seven *Dhatus* (tissues), and three *Malas* (excretions). His text covered the pathophysiology and treatment of diseases, human constitution *(Prakriti),* classifications and preparations of drugs, diet, "right conduct," medical ethics, and many other aspects of medicine.

School of Surgeons *(Dhanvantari Sampradaya)*

Sushruta wrote the first comprehensive works on surgery. These were later revised by Nagarjuna in the 2nd century AD. The major subjects in his texts were the following:

- Injections
- Preoperative care

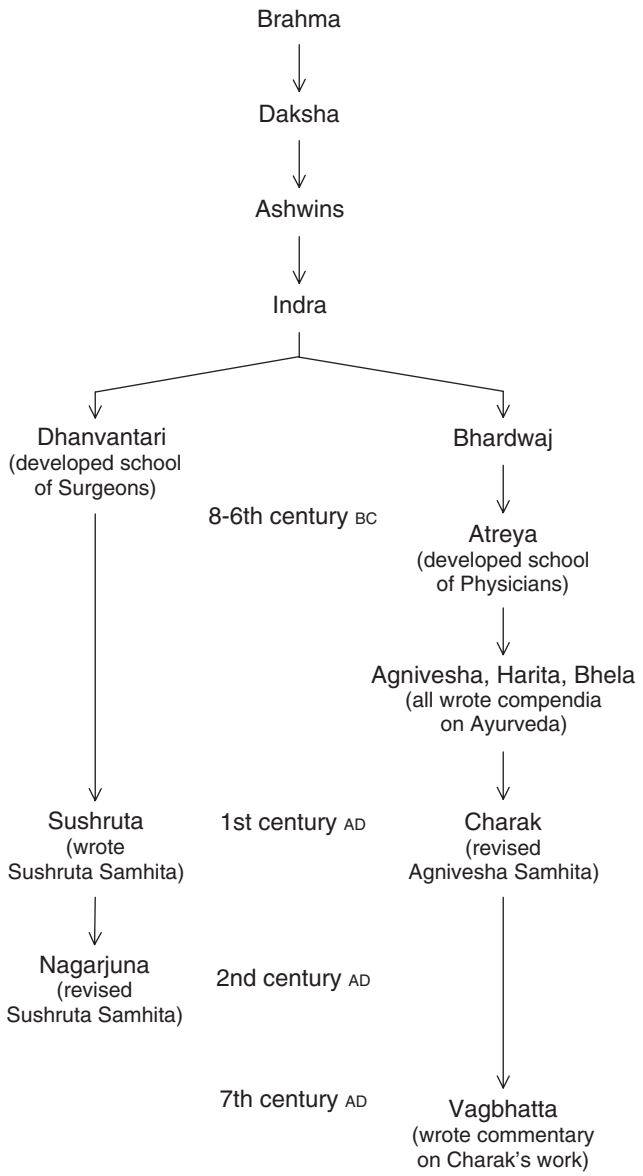


Fig. 33.1 The chronology of Ayurveda.

- Postoperative care
- Suturing
- Asepsis
- Sterilization
- Operation theaters
- Hospitals

Sushruta described 141 types of instruments and listed 40 types of surgeries and surgical techniques for treating cataracts, hemorrhoids, hernias, and bone problems, for cosmetic and plastic purposes, and for the removal of kidney stones and gallstones.

BRANCHES OF AYURVEDA

Ayurveda encompasses eight specialties or branches. They comprise a system developed to prevent and cure disease and also to achieve and maintain excellent health. Table 33.1 lists the branches.

Philosophy

Ayurvedic philosophy is based on the Samkhya philosophy of creation. It has influenced major strains of philosophy in both Eastern and Western civilizations. The word *Samkhya* is derived from the Sanskrit words *Sat* ("truth") and *Khya* ("to know"). The Rishi Kapila (*Rishi* means "realized beings" or "seers of truth") realized the Samkhya philosophy of creation. They perceived the following:

- The close relationship between humans and the universe: humans are a microcosm, a universe within themselves, whereas the external environment is the macrocosm.
- The source of all existence is cosmic consciousness, manifest as male (*Shiva* or *Purusha*) and female (*Shakti* or *Prakriti*) energy.

Purusha is formless, colorless, and "beyond" attribute. *Prakriti* has form, color, awareness, and choice. *Prakriti* creates all the forms of the universe and has three attributes *(Gunas): Satva* (essence), *Rajas* (movement), and *Tamas* (inertia). It is also represented by *Brahma* (the god of creation), *Vishnu* (the god of protection), and *Shiva* (the god of destruction), which together comprise a cycle active in this universe.

In *Prakriti,* the three attributes are in balance. Whenever this balance is disturbed, the attributes interact to bring about the evolution of the universe, yielding the cosmic vibration of *Aum.* The cosmic intellect (the *Mahad*) manifests itself as ego *(Ahamkar),* which, through the help of *Satva,* manifests the five senses and five motor organs, which together constitute the "organic universe." Ego further manifests into the five basic elements (space, air, fire, water, and earth), which, under the influence of *Tamas,* create the "inorganic universe."

Satva is a creative potential *(Brahma), Rajas* is a kinetic protective force *(Vishnu),* and *Tamas* is a potentially destructive force *(Shiva).* These three—*Brahma, Vishnu,* and *Shiva*—are constantly operating in the universe.

TABLE 33.1 Specialties in Ayurveda

Ayurvedic Name	English Name
Shalya Tantra	General surgery
Shalkya	Ophthalmology and otorhinolaryngology
Kaya Chikitsa	Medicine
Bhutvidya	Psychiatry
Kumar-Bhritya	Pediatrics, obstetrics, and gynecology
Agada Tantra	Toxicology and jurisprudence
Rasayana	Geriatrics
Vajikaran	Fertility and sterility

Five Basic Elements and the Universe (Panchbhuta Philosophy)

The Rishis perceived that consciousness consists of five basic elements: ether (space), air, fire, water, and earth. At the beginning of the world, consciousness was without form, existing as the subtle vibration of the cosmic "soundless" sound *Aum.*

Within these vibrations appeared the element ether. Ether started to move, creating air. The movement of ether also produced friction and, through friction, generated heat, then fire. From the heat of the fire, ethereal elements dissolved and liquefied into water. Water then solidified to form molecules of earth. Thus all matter was born from the five elements. These five elements exist in energy forms.

Five Elements and the Senses

The five elements also connect with the five senses: ether—hearing, air—touch, fire—vision, water—taste, and earth—smell, and they are present in certain physiological functions. Expressing the functions of the sensory organs are five actions (Table 33.2). In this manner, the

TABLE 33.2 The Five Elements and the Senses

Element	Sense	Organ	Action	Vehicle of Action
Ether	Hearing	Ear	Speech	Mouth
Air	Touch	Skin	Holding, giving, receiving	Hand
Fire	Vision	Eye	Walking	Feet
Water	Taste	Tongue	Procreation	Genitals
Earth	Smell	Nose	Excretion	Anus

BOX 33.1 Properties of *Dosha*

Vat	*Pit*	*Kaph*
Dry	Oily	Oily
Light	Light	Heavy
Cold	Hot	Cold
Rough	Liquid	Slimy
Subtle	Penetrating	Soft
Mobile	Mobile	Static
Clear		Dense
Dispersing		Slow
Smells sour		

elements are directly related to humans' abilities both to perceive the external environment in which they live and to respond to it:

- Ether is the medium through which sound travels. The ear is the organ of hearing, expressing its action through the organ of speech, which creates meaningful sound.
- Air is related to skin and the sense of touch. Its organ of action is the hand, which is especially sensitive. The hand performs the actions of holding, giving, and receiving.
- Fire produces light, heat, and color and is thus related to vision and direction. Its organ is the eye.
- Water relates to the organ of taste. The tongue is also related to the action of the genitals, the penis and clitoris. In Ayurveda, the penis and clitoris are called the lower tongues. By controlling the upper tongue, one naturally controls the lower tongue.
- The earth element relates to the sense of smell, and the nose is its organ.

Physiology

The five elements manifest within the body as the Tridosha (*Dosha* means "protective" or, when out of balance, "disease producing"). The Tridosha are the three humors, or basic principles, described earlier—*Vat, Pit,* and *Kaph.*

From the bodily combination of ether and air comes the bodily air principle, *Vat Dosha.* Likewise, fire and water combine as *Pit Dosha,* or fire principle, and earth and water produce the *Kaph Dosha,* or water principle.

These three control all biological, psychological, and physiopathologic functions of the body, mind, and consciousness. They produce natural urges and individual tastes in food, flavor, and temperature. They govern the maintenance and destruction of bodily tissue and the elimination of waste products. They also are responsible for psychological phenomena, including the emotions of fear, anger, greed, and the highest order of emotions: understanding, compassion, and love.

Properties of *Dosha*

Vat, Pit, and *Kaph* control all human biological, psychological, and physiopathologic functions and have subtle properties, as shown in Box 33.1.

The *Doshas* increase by similar properties and are diminished by opposite properties. For example, *Vat* is dry, light, and cold, so any food, medicine, lifestyle, or behavior that increases these qualities increases *Vat* within the body. Conversely, oily, heavy, or hot factors decrease *Vat.*

Functions of *Tridosha*

Each humor has a specific action. *Vat* is the principle of movement and may be called the bodily air principle, as opposed to the environmental air principle. *Vat* is a subtle energy governing all biological movement—breathing, blinking, muscle and joint movement, heartbeat, and all nerve and membrane contractions and expansions. In addition, it controls the psychological functions governing the emotions of fear and anxiety. *Vat* also controls pain, tremors, and spasms. Broadly speaking, the whole nervous system can be labeled as *Vat* function. The large intestine, pelvic cavity, bones, skin, ears, and thighs are the places of *Vat*. Any excess of *Vat* accumulates in these areas.

Pit, or bodily fire, governs digestion, absorption, assimilation, nutrition, temperature, skin color, luster of the eye, intelligence, and understanding. It arouses anger, hate, and jealousy. The small intestine, stomach, blood, sweat glands, fat, eyes, and skin are the places of *Pit;* all metabolism is governed by *Pit.*

Kaph, biological water, is the cement of the body, providing for physical structure. It is responsible for body resistance and biological strength. It lubricates the joints; provides moisture to skin; and promotes wound healing, strength, vigor, and stability. It supports memory, gives energy to the heart and lungs, and maintains immunity. *Kaph* is present within the throat, chest, head, sinuses, nose, mouth, stomach, joints, cytoplasm, plasma, and liquid secretions. Psychologically, *Kaph* governs attachment, greed, long-standing envy, calmness, forgiveness, and love.

A balance of the *Dosha* is necessary for optimal health. Together, they govern all metabolic activities: anabolism *(Kaph),* catabolism *(Vat),* and metabolism *(Pit).*

Individual Psychosomatic Constitutions—*Prakriti*

Prakriti, a Sanskrit word composed of *pra* ("before") and *akriti* ("creativity"), denotes the constitution of each individual as determined at conception. At the time of fertilization, permutations of *Vat, Pit,* and *Kaph* determine the constitution of the new individual, with maleness or femaleness dominating other traits. These basic traits are also shaped by other important factors, such as diet, lifestyle, behavior, emotions, and seasons.

As illustrated in Box 33.2, up to seven different constitutions can exist depending on the permutation and combination of *Vat, Pit,* and *Kaph. Prakriti* is genetically determined; the basic constitution, the combination of the three humors, remains unchanged throughout an individual's lifetime. The combination can, however, respond to environmental changes.

Life is considered a sacred path in Ayurveda, a ceaseless interaction between the internal *(Tridosha)* environment and the external environment, or the sum of cosmic forces. To counteract external change, an individual may create a balance in the internal forces by altering diet, lifestyle, and behavior. The characteristics of the corresponding psychosomatic constitutions are listed in Table 33.3.

Mental Constitutions

On the mental and astral planes, three *Gunas* (attributes of female energy or *Prakriti*) correspond to the three humors that make up the physical constitution. In the Ayurvedic system, the *Gunas* are *Satva, Rajas,* and *Tamas*. They provide the basis for the distinctions in human temperament and individual differences in psychological and moral dispositions. These attributes are further subdivided, but that is beyond the scope of this chapter.

Satva

The *Satva* type of mind expresses essence, understanding, purity, clarity, compassion, and love. People of Satvic psyche (*Satva* temperament) have healthy bodies and pure behavior and consciousness. They believe in the existence of God, are religious, and are often holy persons.

Rajas

The *Raja* type of mind operates on the sensual level. Such persons are interested in business, prosperity, power, prestige, and position. They enjoy wealth, are generally extroverted, and are politically minded.

BOX 33.2 The Seven Types of Constitution

- *Vat*
- *Pit*
- *Kaph*
- *Vat-Pit*
- *Pit-Kaph*
- *Kaph-Vat*
- *Vat-Pit-Kaph*

Tamas

The *Tamas* type is distinguished by its ignorance, inertia, heaviness, and dullness. Tamasic people are lazy, selfish, and destructive by nature. They show little respect to others. All their activities are egocentric. The Satvic person attains self-realization without much effort, whereas those of Rajasic or Tamasic mind have difficulty. These three subtle mental energies are responsible for behavior patterns that may be altered and improved through practice and spiritual discipline, such as yoga and meditation.

HEALTH AND DISEASE

Health is defined in Ayurveda as the soundness of body *(Shrira)*, mind *(Manas)*, and soul *(Atma)*. Each part of this tripod of life should receive equal attention to ensure that the individual achieves sound health. Ayurvedic medicine stresses that psychic influences strongly affect the body in health and disease, a fact that must also be taken into account in modern therapeutics.

Modern science takes pride in its understanding of physiology but, in doing so, has emphasized fragmentation, isolation, and disunity. Instead of wholeness and interaction, this modern view accepts only physical objects as causes of disease, whereas these objects are merely the agents of disease, able to cause specific symptoms but only in a susceptible host. Disease is the result of a disruption of the spontaneous flow of nature's intelligence within physiology. When people violate nature's laws and cannot adequately rid themselves of the results of this action, they acquire diseases.

Ayurveda conceives the body as being composed of three principal divisions: three *Doshas* (humors), seven *Dhatus* (tissues), and three *Malas* (excretions).

The three *Doshas* regulate cell functions. *Pit* gives energy and is responsible for cellular, enzymatic, and metabolic functions; *Kaph*

TABLE 33.3 Psychosomatic Constitutions *(Prakriti)*

Characteristic	*Vat*	*Pit*	*Kaph*
Body frame	Tall or small, thin, ill-nourished, hard, dry, cold	Medium, many moles, well nourished, pimples, patches of freckles, pigment, tender appearance	Stout, well-nourished, big, oily, greasy, cold, beautiful
Skin	Dry, cracked, tough, broken, brownish, black	Soft, thin, yellow, red, pink	Greasy, soft, yellow, white
Hair on head	Brown, scanty, coarse, curved, wavy, wrinkled	Pinkish, yellow, moderate, soft, baldness, premature graying	Black, plentiful, firm, wavy, curved
Head	Small size	Moderate in size	Big and steady
Eyes	Small, dry, thin, muddy brown, unsteady gaze, not pleasant looking	Thin, yellow, pink coppery, quickly become inflamed, pleasant looking	Big, white, pink, pleasant looking, thick, fixed, greasy
Body weight	Less	Moderate	Heavy
Action of body	Quick and unsteady walk, quick actions and movements	Moderate, intelligent actions	Steady walk, slow and dignified
Eliminations	Less, constipated	Copious, watery	Moderate, solid
Strength	Less, tires quickly	Moderate	Strong, hard worker, can withstand strains
Body odor	No smell, less sweating	Heavy sweating, bad smell	Less sweating, pleasant smell
Voice	Feeble, broken, hidden, hoarse, unpleasant	High pitch	Pleasant, deep bass voice, good tone
Speech	Quick, very talkative	Moderate	Slow, definite
Sleep	Less sleep, wakeful at night	Moderate	Sleeps a good deal
Dreams	Moving in sky, action	Cold, flowers, sun, fire, lightning, red, frightful dreams	Ponds, lakes, flowers, swans, beautiful sights
Sex	Less sex capacity, less semen/menses, few children	Less sex capacity, few children	Great sex capacity
Life expectancy	Short	Moderate	Long-lived
Reaction to disease	Quick to get diseases, usually nervous disorders	Moderate, usually inflammations	Good resistance, usually cold and phlegm type
Reaction to drugs	Minimum doses required	Intolerance to drugs	Slow, tolerates high doses

helps in synthesizing blocks of cells; and *Vat* controls the other two. A balance of these *Doshas,* good-quality tissues (the seven *Dhatus*), and a certain character of excretions are essential for maintaining health.

As explained earlier, an individual is born with a particular *Dosha* predominating in his or her constitution *(Prakriti).* This predominant *Dosha,* quite apart from genetic, age, environment, and dietary factors, may make an individual susceptible to a certain disease. For example, *Pit Prakriti* individuals are more prone to develop a disease syndrome with symptoms similar to a peptic ulcer. This is a result of hyperactivity of the *Pit Dosha,* which regulates enzymatic activity. Hyperpepsinogenemic individuals are more susceptible to duodenal ulcer formation.[2]

Ayurveda teaches that the origin of most diseases is found either in an exogenous or endogenous *Dosha* imbalance or in an inherent or acquired weakness of the tissues. Therefore, the successful treatment or prevention of disease consists of normalizing cellular functions by correcting any *Dosha* imbalance or improving inherent tissue vitality. For example, in the treatment of cancer, the use of agents cytotoxic to the cancer cells is important. However, equally important is potentiation of the immune system and stimulation of the body's own healing mechanisms.

MODES OF THERAPY

Once a diagnosis is made, Ayurveda offers various modes of therapy. The treatment is chosen according to patient constitution and the disease process. Modalities include dietary alterations, botanical medicines, minerals, animal products, exercise, yoga, meditation, counseling, and surgery.

Diet

Ayurveda places great emphasis on diet, for both its direct effect on the individual's physiological state and its influence on the action of medicines. Proper assimilation of dietary constituents is essential for the maintenance of good health. Improper assimilation results in the formation of intermediary products of digestion that have toxic properties and are therefore treated as foreign by the body. Such toxic products are called *Ama* (this leads to the concepts of immune and autoimmune disorders).

Arthritic diseases such as rheumatoid arthritis are attributed to an accumulation of *Ama.* Could these toxic intermediates—macromolecules absorbed transmucosally from the intestine (in nutritionally insignificant amounts)—provoke strong immune reactions? The human gut has a complex system to control the continuous onslaught of antigenic substances derived from food, microorganisms, and toxins.[3] It has been suggested that absorption of such compounds could underlie the pathogenesis of disease in the gut and in distant sites such as the liver and spleen.[4]

Ayurveda stresses prevention of the formation and accumulation of *Ama* through appropriate diet and the use of therapies to improve digestion. It also considers various dietary factors that trigger or eliminate certain diseases.[5] That is why Ayurveda places emphasis on diet according to the patient's psychosomatic constitution *(Prakriti),* time of day *(Dincharya),* and season *(Ritucharya).* For example, a person with the *Pit* psychosomatic constitution should not eat foods that are hot, pungent, or spicy at noon in the summer because this tends to increase diseases of inflammation.

Ayurveda prescribes specific diets for several psychiatric disorders. Recent research supports this approach. Brain levels of the neurotransmitters 5-hydroxytryptamine, catecholamine, and acetylcholine have been found to be influenced by dietary constituents. Consequently, it has been suggested that normal brain functions and mental disease can be altered by diet.[6] Recent exciting developments include the successful treatment of mental depression with neurotransmitter precursors.[7,8]

Ayurveda also prescribes certain diets *(Pathya)* during drug therapies because dietary constituents are believed to influence drug action.

BOX 33.3 Host- and Drug-Related Factors

Drug Factors	
Prakriti	Constitution of the drug
Guna	Properties
Prabhava	Activity (potency)
Desh	Place
Ritu	Season
Grahan	Storage
Nihit	Transport
Sanskar	Refinement
Matra	Dose
Sanyog	Combination
Adhishthan	Ability to reach site of action
Host Factors	
Prakriti	Constitution of host
Vayam	Age
Vikriti	Pathological condition
Sar	System strength
Satamya	Tolerability
Satva	Psychological state
Ahar Shakti	Digestive capacity
Vyayam Shakti	Exercise tolerance
Balam	Strength of host

Individualization of Medicinal Therapy

Medicinal therapy is highly individualized in Ayurveda.[9] The choice and dose of medicine are influenced not only by disease but by the individual's constitution and the environmental conditions likely to affect that individual's *Doshas.*

For example, *Piper rotudum* (black pepper) and *Zingiber officinale* (ginger), which increase *Pit* (increasing stomach acid and pepsin secretion), are used cautiously in individuals with a *Pit* constitution. Another example is in the treatment of the patient with hypertension. Ayurveda prescribes *Terminalia chebula* for the treatment of hypertensive patients who have *Vat Prakriti,* whereas for the patient with *Pit Prakriti,* one uses *Terminalia arjuna.*

In the *Vimanastrana,* Charak presented an interesting discussion of host- and drug-related factors that help in the determination of the drug and dosage (Box 33.3).

Ayurveda also emphasizes proper timing for the administration of medicines. Considering that chronopharmacology (the study of the timing of drug administration in relation to physiological function) has only recently been developed as a branch of modern therapeutics, it seems remarkable that such astute observations about the timing of medicaments were made so many centuries ago.

Pharmacy in Ayurveda

In Ayurveda, pharmacy is highly developed. Almost 70 books contain more than 8000 recipes for the preparation of different medicines, most of which are derived from minerals and plants. Many formulations are available, including simple distillates *(Arka),* decoctions *(Kwatha),* tinctures *(Avleha),* powders *(Churna),* pills (*Vati, Goti,* and

Modak), fermented products *(Asva),* and medicated oils (*Taila* and *Ghrita*). The oil preparations are particularly useful because they help target the sites of action.

Detailed descriptions of the methods are recommended to ensure a medicine is suitable for human use.[10] One pharmaceutic technique, *Samskara* ("refinement"), is known as *Shudhi* ("purification"), a process that eliminates the toxicity of some minerals and plants. Another practice is the administration of drugs in combination *(Samyoga)* to reduce toxicity and increase efficacy.

RESEARCH

Considerable modern research has proven the efficacy of Ayurvedic herbal preparations, and research has now moved to elucidating their mechanisms and sites of action.[11] Recent studies demonstrated probable genomic bases for metabolic differences as predicted by *Prakriti*. This genetic variant may provide newer approaches to pharmacogenomics. Extensive studies on *Prakriti* subtypes and genome mapping, especially of other important drug-metabolizing enzyme polymorphisms (e.g., CYP2C19, CYP2D6, CYP2C9, CYP3A4, TPMT), would be useful to understand a possible *Prakriti* pharmacogenomics relationship to its correlating genotype, *Prakriti,* and drug metabolism.[12]

Curcumin, the active principle of *Curcuma longa,* has been found to exert a powerful anti-inflammatory effect by blocking many inflammatory pathways, reversing insulin resistance, and reversing cancer by repairing defective genes.[13] Further, it has been shown to selectively inhibit platelet prostaglandin production while sparing vascular endothelial prostaglandin synthesis.

Many plant preparations have been used to strengthen general host resistance. *Rasayna, Jeevaniya,* and *Balya* increase tissue resistance to disease, a concept similar to "prohost therapy" as put forward by Hadden.[14] Prohost therapy claims to augment cellular responses and, consequently, ameliorate disease states.[15]

A paper published in *Phytotherapy Research* in May 2011 found that aqueous extract of *Withania somnifera* root had an ability to inhibit the formation of mature amyloid-β fibrils in vitro. Amyloid plaques in the brain cause Alzheimer's disease. This is an exciting discovery and may help many people prevent Alzheimer's disease.[16] Perhaps most exciting, however, is the current research demonstrating the efficacy of Ayurvedic herbal preparations in conditions for which modern medicine has had limited or no success. For example, animal studies showed that *Withania somnifera (Ashwagandha)* can reverse the immunosuppression of cyclophosphamide, azathioprine, and prednisolone, and a 50% alcoholic extract of *Phyllanthus emblica* protects the liver from paracetamol.[17,18] Recent human studies found that patients with acne vulgaris demonstrated substantial improvement, with no significant side effects, after using *Sunder vati* compared with placebo. Interestingly, the study found three other Ayurvedic formulas ineffective. Patients with osteoarthritis experienced substantial, highly significant improvement in pain and disability with no significant side effects (however, the radiological findings did not improve).[19]

AYURVEDIC AND MODERN MEDICINE

In evaluating Ayurvedic medicine by modern standards, one encounters a number of difficulties. First, there is a wide variation in the quantity of pharmacologically active substances in plants. In addition, many findings are more subjective than objective. The modern scientist has difficulty recognizing subjective experiences because no reliable methodology has been developed to measure and reproduce such experiences. Yet totally objective experience, which completely disregards the subjective, may be wrong and even dangerous.

There is no such thing as complete objectivity. What people claim as such is merely agreement among many minds. Subjective experience is limited. Senses vary in ability, and reality for one is unreality for another. When using the tools of quantitative, technical science to describe biological and living systems phenomena, one soon encounters limits. Artificial distinctions must be imposed to reduce variables and interpret nonlinear events intelligibly.

Science has often confused the map for the actual reality. Take the example of color vision in bees. The eye of a bee is more sensitive to blue, violet, and ultraviolet light, whereas receptors in the human eye more readily detect red, green, and blue wavelengths. Thus bees are nearly blind to red light, and humans are quite blind to ultraviolet light. These differing characteristics result in members of each species forming a completely different perception of the same object. Color, rather than being a part of the "reality" of a perceived object, is an expression of sensory apparatus, determined by each species' or individual's unique pattern of interneural connections. Even more interesting is the fact that, within the same species, perception may vary considerably.

Ayurvedic philosophy may strike the contemporary reader as unnecessarily complex for the conceptual territory it addresses. However, it is actually quite succinct and relevant to modern life. Its precepts have influenced many systems of healing, including naturopathic medicine, through several paths of its root traditions. It emerged alongside systems known to early Persians, Greeks, and the Chinese. Modern medicine is itself a distillation of these same rich traditions. When one compares Ayurvedic concepts with those of preindustrial Europe, much similarity becomes apparent.

Ayurgenomics

Understanding Ayurvedic Insights Toward Individualized Genomic Medicine

Individualized medicine is the core essence of Ayurveda. The *Tridosha* system in Ayurveda is used to guide the individualization of treatments. The Ayurvedic physicians observe the patient's body type, mental activity, emotional tendencies, and physiological functions to make an assessment of the unique combination of *Tridosha,* called *Prakriti.* The *Prakriti* of the individual is like the expression of the individual's genome today.

A unique, integrative "Ayurgenomics" medicine approach can be used to reinforce the essence of Ayurveda with the modern science of genomics. Ayurveda follows an approach that has noteworthy parallels with modern-day personalized genomic medicine advances in the understanding and management of health and disease. Every individual is born with a specific proportion of *Tridosha,* which is partially determined genetically but also affected by the environment during fetal development. In Ayurvedic medicine, diseases are believed to originate based on the imbalances in *Doshas,* and the primary goal of Ayurveda is to make sure that the *Doshas* maintain their homeostatic state.

The individuation explained by *Dosha* balance is a precursor to the modern genomic individuation. Even when the modern technology did not exist, ancient Vedic sages used the power of observation to determine the constitution of an individual. The Vedic sages gave complete attention to the patient by interviewing the patient and examining the patient. Old Ayurvedic texts give an immense amount of details about symptoms, disease progression, and physical examination, and these details were translated into the language of *Tridosha.* This knowledge informs the practitioner about the genomic individuality (i.e., *Prakriti*) of the person. In the contemporary world, the *Prakriti* or constitution of the individual is the sum expression of the individual's genome. This individual genomic signature is expressed by the

individual's symptoms, likes and dislikes, mental and emotional tendencies, and modern physiological measures.

Recently, the field of Ayurgenomics has emerged to test the validation of the works of traditional Ayurvedic physicians. In one study, researchers performed genetic testing of individuals with three unique constitutions: *Vat, Pit,* or *Kaph.*[20] The genetic study was matched with the constitution, and genetic patterns were observed. The *Vat* individuals had more active transport components. The *Pit* individuals had greater activity and responsiveness of blood components such as various blood cells, platelets, and immune cells. The *Kaph* individuals had a larger share of lipid components, such as cholesterol, triglycerides, and structural proteins. These results clearly demonstrated the qualities of *Doshas* seen in Ayurvedic texts: *Vat*—movement, *Pit*—metabolism, *Kaph*—structure and stability.

In another investigation, the constitutional expression of individuals from distinct climates was tested.[21] The scientists looked at the *EGLN1* gene, an oxygen sensor that activates other genes in the body to adapt to low oxygen concentrations. It was hypothesized that expression of the *EGLN1* gene would be greater in high-altitude populations. Because this gene is linked to adapting and oxygen metabolism, it was also thought that this population might have more *Pit* individuals. They found both hypotheses to be true: individuals in higher altitudes had greater expression of the *EGLN1* gene, and there was a higher number of *Pit* individuals in this population.

Another Ayurgenomics research study looked at rheumatoid arthritis.[22] Rheumatoid arthritis involves inflammation and joint pain. Hence, there is an increased activation of inflammatory pathways. According to Ayurveda, the fundamental characteristics between *Vat*-dominant and *Pit*-dominant individuals suffering from rheumatoid arthritis are unique. The study reaffirms constitutional-based individuality. The *Vat* individuals had greater expression of inflammatory markers, interleukin-1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), whereas the *Pit* individuals had greater expression of oxidative stress markers SOD3 and PON1. *Kaph* individuals did not demonstrate either inflammatory or oxidative pathways. Overall, Ayurgenomics established some underlying mechanisms of the destructive process of rheumatoid arthritis based on *Prakriti.*

The idea of *Prakriti* in Ayurveda and its correlation with genomics was observed more than a decade ago. Some studies did find the relationship between *Prakriti* classification with genetic information and the link of single-nucleotide polymorphisms (SNPs) in *HLA-DRB1, CYP2C19, EGLN1,* genes related to inflammatory and oxidative stress, cardiovascular disease markers for various blood cells, DNA methylation alterations, and risk factors for cardiovascular and inflammatory diseases. Perhaps these studies have demonstrated the association of specific genes with the phenotype of a particular *Prakriti,* but the first attempt to organize the *Prakritis* using genome-wide SNP markers and find a scientific basis for *Prakriti* classification was made in 2015.[23]

The investigators performed genome-wide SNP analysis of 262 male individuals belonging to three different *Prakritis.* They discovered that 52 SNPs were significantly different between *Prakritis.* The scientists also analyzed 297 Indian population samples with known ancestry. They found out that the *PGM1* gene correlates with the phenotype of *Pit.* We know that in Ayurveda, characteristics of Pitta include digestion, metabolism, and energy production. Interestingly, the *PGM1* gene is in the center of many metabolic pathways, such as glycolysis and gluconeogenesis. Research shows that the function of the gene directly correlates with the role of *Pit* in metabolism as described in ancient Ayurvedic literature, *Charak Samhita*. This provides evidence that the phenotypic classification of Ayurveda has a genetic basis, and its *Prakriti*-based practice complements personalized medicine.

SUMMARY

Only some glimpses of Ayurveda are presented here. This ancient system of medicine, developed over centuries, has a consistent and logical framework. Ayurveda gives detailed instructions for the preservation of health and the treatment of disease. The ancient system faced a setback when modern medicine subjected all knowledge to experimental and statistical verification, which, although useful, is limited by the tools available and the perceptions underlying the questions asked. Furthermore, modern medicine finds human bodies to have no variability within studies, creating the inability to study a pattern in differences in body types. Now that considerable knowledge of cellular physiology has accumulated and more sensitive modern biomedical research tools exist, we may be able to evaluate the concepts of Ayurveda more effectively. Ayurgenomics is already being practiced in Ayurvedic clinics all over the globe, filling in gaps present in modern medical science and creating a platform for integrative medicine.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gordon BL. *Medicine Throughout Antiquity*. Philadelphia: FA Davis; 1949.
2. Rotter JI, Sones JQ, Samloff IM, et al. Duodenal-ulcer disease associated with elevated serum pepsinogen I: an inherited autosomal dominant disorder. *N Engl J Med*. 1979;300:63–66.
3. Walker WA. Antigen handling by the gut. *Arch Dis Child*. 1978;53:527–531.
4. Tagesson C, Sjodahl R, Thoren B. Passage of molecules through the wall of the gastrointestinal tract. *Scand J Gastroent*. 1978;13:519–524.
5. Gulabkunverba S. *Charak Sutra 26, Charak Samhita*. Jamnagar, India: Ayurvedic Society. 1949;124–141.
6. Ferstorm JD. Dietary precursors and brain neurotransmitter formation. *Annu Rev Med*. 1981;32:413–425.
7. Copen A, Shaw DM, Farrell MB. Potentiation of the antidepressive effect of a monoamine oxidase inhibitor by tryptophan. *Lancet*. 1963;1(7272):79–81.
8. Gelenberg AJ, Wojcik JD, Growden JH, et al. Tyrosine for the treatment of depression. *Am J Psychiat*. 1980;137:622–623.
9. Sasdri RD, Gulabkunverba S. *Charak Sutra 8*. Jamnagar, India: Ayurvedic Society. 1949;105.
10. Budar Peth YJB. *Sharangdhar Samhita. Part II*. Pune, India: YJ Dixit; 1908.
11. Satyavati GV, Raina MK, Sharma M. *Medicinal Plants of India*. Vol. 1 and 2. New Delhi: Indian Council of Medical Research; 1976.
12. Yogita G, Kalpana J, Bhushan P. Traditional medicine to modern pharmacogenomics: Ayurveda Prakriti type and CYP2C19 gene polymorphism associated with the metabolic variability. *Evid Based Complement Alternat Med*. 2011;1–5. Article ID 249528 https://doi.org/10.1093/ecam/nep2062009.
13. Bharat B. Aggarwal targeting, inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. *Annu Rev Nutr*. 2010;30:14.1–14.27.
14. Hadden JW. Immunomodulators in the immunotherapy of cancer and other diseases. *Trends Pharm Sci*. 1982;3:191–194.
15. Srivastava R, Puri V, Srimal RC, et al. Effect of curcumin on platelet aggregation and vascular prostaglandin synthesis. *A rzneimittelforschung (Drug Research)*. 1986;36:715–717.
16. Kumar S, Harris RJ, Seal CJ, et al. An aqueous extract of *Withania somnifera* root inhibits amyloid β fibril formation in vitro. *Phytother Res*. 2011. https://doi.org/10.1002/ptr.3512.
17. Ziauddin M, Phansalkar N, Patki P, et al. Studies on the immunomodulatory effects of ashwagandha. *J Ethnopharmacol*. 1996;50:69–76.
18. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on *Phyllanthus emblica* linn. and quercetin. *Indian J Exp Biol*. 1995;33:261–268.
19. Paranjpe P, Kulkarni PH. Comparative efficacy of four Ayurvedic formulations in the treatment of acne vulgaris: a double-blind randomised placebo-controlled clinical evaluation. *J Ethnopharmacol*. 1995;49:127–132.
20. Prasher B, et al. Whole genome expression and biochemical correlates of extreme constitutional types defined in ayurveda. *J Transl Med*. 2008;6(48):1–12.
21. Aggarwal S, et al. EGLN1 involvement in high altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. *Proc Natl Acad Sc U S A*. 2010. Oct 18. (2010).
22. Juyal RC, et al. Potential of ayurgenomics approach in complex trait research: leads from a pilot study on rheumatoid arthritis. *Plos ONE*. 2012;7(9):e45752.
23. Govindaraj P, Nizamuddin S, Sharath A, et al. Genome-wide analysis correlates ayurveda prakriti. *Scientific Reports*. 2015;5(1).

FURTHER READING

Budwar Peth YJB. *Sharangdhai Samhita, Part 1* Pune, India: Dixit; 1908.

Dahanukar SA, Date SG, Karainchikar SM. Cytoprotective effect of *Terminalia chebula* and *Asparagus racemosa* on gastric mucosa. *Indian Drugs*. 1983;20:442–495.

Dahanukar SA, Karandikar SM. Evaluation of antiallergic activity of *Piper longum*. *Indian Drugs*. 1984;21:377–383.

Gulabkunverba S, Samhita Charak, Shamita Sushruta. *Chaunkamba Orientale*. India: Ayurvedic Society: Varanasi. Jamnagar. 1980.

Kerup PVN. In: Bannerman RH, Burton J, Wen-Chieh C, eds. *Traditional Medicine and Health Care Coverage*. Geneva: World Health Organization; 1983:50–58.

Kulkarni RR, Patki PS, Jog VP, et al. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. *J Ethnopharmacol*. 1991;33:91–95.

Lad V. *Ayurveda, The Science of Self Healing*. Santa Fe, NM: Lotus Press; 1984.

Lad V. Ayurveda. A holistic medical approach. *Probe*. 1987;26:293–297.

McIntosh RP. The importance of timing in hormone and drug delivery. *Trends Pharm Sci*. 1984;5:492–501.

Meerson FA. *Adaptation, Stress and Prophylaxis*. New York: Springer-Verlag; 1984.

Rege NN, Dahanukar SA, Karandikar SM. Hepatoprotective effect of *Tinospora cordifolia* against carbon tetrachloride induced liver damage. *Indian Drugs*. 1984;21:544–580.

Singhal GD, Tripathi SN, Chaturvedi GN. *Fundamental and Plastic Surgery Considerations in Ancient Indian Surgery*. Varanasi, India: Singhal Publications; 1981.

Srikantamurthy KR. *Clinical Methods in Ayurveda*. Varanasi, India: Chaukhambha Orientalia; 1996.

Thakkar C. *Ayurveda, the Indian Art and Science of Medicine*. New York: Noble Offset Printers; 1990.

34

Botanical Medicine—A Modern Perspective

Michael T. Murray, ND

OUTLINE

INTRODUCTION

The term *herb* refers to a plant used for medicinal purposes. Are herbs effective medicinal agents, or is their use merely a reflection of folklore, outdated theories, and myth? To the uninformed, herbs are generally thought of as ineffective medicines used before the advent of more effective synthetic drugs. To others, herbs are simply sources of compounds to isolate and then market as drugs. However, to some, herbs and crude plant extracts are effective medicines to be respected and appreciated.

For many people of the world, it is still the case that herbal medicines are the only therapeutic agents available. In the late 1990s, the World Health Organization estimated that about 80% of the world's population relies on herbs for primary healthcare needs.[1] This widespread use of herbal medicines is not restricted to developing countries; it has been estimated that 70% of all medical doctors in France and Germany regularly prescribe herbal preparations. And there is no question that herbal medicine is also flourishing in the United States.

Although herbal medicine has existed since the dawn of time, knowledge of how plants affect human physiology remains largely unexplored. Many individuals formulate their view of herbal medicine based on opinion, philosophy, and ideology. This chapter seeks to facilitate an informed view of herbal medicine. The past and future of herbal medicines are discussed. We believe that the continued evolution of the tradition of herbal medicine can only be accomplished within the context of continued scientific investigation.

Throughout the world, but especially in Europe, the United States, Canada, Australia, and Japan, a tremendous renaissance in the use and appreciation of herbal medicine occurred in the latter part of the 20th century. For example, in the United States, the sale of herbal products skyrocketed from $200 million in 1988 to more than $3.5 billion in 1997 before steadily growing to more than $8 billion in sales in 2017.[2]

The rebirth of herbal medicine, especially in developed countries, is largely based on a renewed interest by the public and scientific researchers. During the past 30 to 40 years, there has been a huge increase in scientific information concerning plants, crude plant extracts, and various substances from plants as medicinal agents. For example, a PubMed search of the term "herbal medicine" yielded more than 38,000 hits in 2018. Combine the term with "clinical trial" and the number is more than 2600. There had been more than 350 clinical trials with *Ginkgo biloba* alone as of 2018.

THE ROLE OF HERBS IN MODERN PHARMACY

Plants still play a major role in modern pharmacy. For the past 70 years, about 25% of all prescription drugs in the United States and other developed countries have contained active constituents obtained from plants. Digoxin, codeine, colchicine, morphine, vincristine, and yohimbine are some popular examples (see Table 34.1 for more). Many over-the-counter (OTC) preparations are also composed of plant compounds. Pharmacognosy, the study of natural drugs and their constituents, plays a major role in current drug development. Unfortunately, the standard path of the approval of a drug is a process that typically takes 10 to 18 years at a total cost somewhere between $350 million and $5 billion.

Because a plant cannot be patented, plants are screened for biological activity, and then the so-called "active" constituents (compounds) are isolated and typically chemically modified to produce unique substances. If the compound is powerful enough, the drug company begins the process to procure U.S. Food and Drug Administration (FDA) approval. Of 520 new drugs approved by the FDA or comparable entities in developed countries, 30 came directly from natural product sources, and another 173 were either semisynthetic from a natural source or modeled after a naturally occurring compound.[1]

Because of the expense and lack of patent protection, few clinical evaluations were done before 1980 on whole plants or crude plant extracts as medicinal agents per se. A key factor in contributing to more research into herbal medicines after this time was the development

in Europe of regulatory policies and practices that made it economically feasible for companies to do research. For example, in Germany, beginning in 1978, regulations were instituted that allowed herbal products to be marketed with drug claims if they are proven to be safe and effective through a special monograph system.[3] A special commission (Commission E) authored the initial series of 400 monographs on herbal products similar to the over-the-counter (OTC) monographs in the United States. Whether the herbal product was made available by prescription or OTC was based on its application and safety of use based on its monograph. If a manufacturer of an herbal product meets the quality requirements of the monograph or produces additional evidence of safety and effectiveness, which can include data from existing literature, anecdotal information from practicing physicians, and limited clinical studies, it is viewed as safe and effective. Herbal products sold in pharmacies are also reimbursed by insurance if they are prescribed by a physician.

In contrast, in the United States, extracts identical to those approved in Germany and other countries as drugs are available as "dietary supplements," and manufacturers are prohibited from making any therapeutic claims for their products. No medicinal claims are allowed for most herbal products in the United States because the FDA requires the same standard of absolute proof as required for new drugs. For decades, the FDA has rejected the idea of establishing an independent "expert advisory panel" for the development of monographs similar to Germany's Commission E monographs, as well as other ideas to create a suitable framework for the marketing of herbal products in the United States.

The monograph system in Germany allows companies to market their products according to the guidelines of Commission E. With the ability to make appropriate claims, many companies have achieved success with their products, enabling them to fund the necessary research to gain greater acceptance within mainstream, conventional medicine. The use of St. John's wort extract in the treatment of depression is a perfect case in point to illustrate how Commission E monographs led to significant documentation of the efficacy of plants with a long history of folk use for depression.

When the Commission E monograph for St. John's wort came out in 1984, it identified the constituent hypericin as the active constituent and permitted the medicinal use of the herb (in average doses of 2 to 4 g of herb, or 0.2 to1 mg total hypericin) for depression, anxiety, or nervous excitement.

Originally, it was thought that hypericin acted as an inhibitor of the enzyme monoamine oxidase, thereby resulting in the increase of central nervous system monoamines such as serotonin and dopamine. However, it was later shown that St. John's wort does not inhibit monoamine oxidase in vivo.[4] The antidepressant activities appear to be related more to serotonin-reuptake inhibition as occurs with the drugs Prozac, Paxil, and Zoloft; modulation of neuroendocrine function; downregulation of β-adrenergic receptors; and upregulation of serotonin receptors in the brain areas that are implicated in depression.[4,5] In addition, it appears that although hypericin is an important marker, other compounds such as flavonoids are also thought to play a major role in the pharmacology of St. John's wort. The key point here is that the further understanding and documentation of the clinical effectiveness of St. John's wort extract was largely the direct result of a commercial incentive created by the existence of Commission E.[6]

Science Fueled the "Herbal Renaissance"

Improvements in analytic techniques and modern pharmacology gave researchers the tools and understanding necessary to better evaluate herbal medicines. Improvements in plant cultivation techniques and the quality of herbal extracts (quality control and standardization) have also led to the development of some effective plant medicines. These advances have created a renaissance in the appreciation and use of herbal medicine. It seems that science and medicine have finally advanced to a level where nature can be appreciated rather than discounted. The scientific investigation of plant medicines is replacing some of the mystery and romance of herbalism with a greater understanding of the ways in which herbs work. Thirty years ago, it was impossible to determine exactly how herbs promoted their healing effects because analytic science had not advanced to a sufficient level of sophistication. This point is well illustrated by the fact that the main mechanism of action responsible for aspirin's anti-inflammatory effect was not understood until the early 1970s, and its mechanism of action for pain relief has yet to be fully understood.

Because the mechanism of therapeutic action of a particular herb or its constituents could not be fully elicited, many effective plant medicines were erroneously labeled as possessing no pharmacological activity. Now, researchers equipped with greater understanding and more sophisticated technology are rediscovering the wonder of plants as medicinal agents. Much of the increased understanding is, interestingly, a result of synthetic drug research.

For example, one of the modern classes of drugs is calcium channel blockers. These agents block the entry of calcium into smooth muscle cells, thereby inhibiting contraction and promoting muscular relaxation. Calcium-channel-blocking drugs are currently being used in the treatment of high blood pressure, angina, asthma, and other conditions associated with smooth muscle contraction. They represent a highly evolved stage of modern drug pharmacy. After calcium channel drugs became better understood, it was discovered that many herbs contain components that possess calcium-channel-blocking activity. In most cases, the historical use of these herbs corresponded to their calcium-channel-blocking activity.

In addition to possessing currently understood pharmacological activity, many herbs possess pharmacological actions that are not consistent with modern pharmacological understanding. For example, an herb often appears to affect homeostatic control mechanisms to aid normalization of many bodily processes. When there is a hyperstate, the herb has a lowering effect, and when there is a hypostate, it has a heightening effect. This action is totally baffling to orthodox pharmacologists but not to experienced herbalists, who have used terms such as *alterative, amphoteric, adaptogen,* or *tonic* to describe this effect.

The Advantages of Herbal Medicines

In general, herbal preparations are thought to have three major advantages: lower cost, fewer side effects, and medicinal effects that tend to normalize physiological function. When used most effectively, the mechanism of action of an herb often corrects the underlying cause of a disorder. In contrast, synthetic drugs are often designed to alleviate the symptom or effect without addressing the underlying cause. They generally act as specific agonists or inhibitors of enzymes or receptor sites. Interestingly, research has often shown that for many plants, the whole plant or crude extract is much more effective than isolated constituents. In many instances, multiple components produce multiple pharmacological actions. This is a key concept because, in general, most drugs poison a specific enzyme. In contrast, herbal medicines, with their multiple constituents, work broadly, modulating multiple enzyme systems.

Herbal medicine will certainly play a major role in future medicine. As modern medicine gains more knowledge and understanding about health and disease, it is adopting therapies that are more natural and less toxic. Lifestyle modification, stress reduction, exercise, meditation, dietary changes, and many other traditional naturopathic therapies are becoming much more popular in standard medical circles. This illustrates the paradigm shift that is occurring in medicine.

With the continuing advancement in science and technology, there has been a great improvement in the quality of herbal medicines available and in the understanding of their optimal clinical use. Improvements in cultivation techniques coupled with improvements in quality control and standardization of potency will continue to increase the effectiveness of herbal medicines.

THE STUDY OF HERBAL MEDICINE

The study of herbal medicine spans the breadth of pharmacology, the study of the history, source, physical and chemical properties, mechanisms of action, absorption, distribution, biotransformation, excretion, and therapeutic uses of "drugs." In many respects, the pharmacological investigation of herbal medicine is just beginning. This textbook is replete with examples of herbs whose historical use is being justified by new investigations into their pharmacology.

The History of Herbal Medicine

The history of the use of plants as medicines is full of interesting stories and fascinating facts. The evolution that occurred in herbal medicine over the centuries is only beginning to be recognized as more natural medicines gain acceptance. Interestingly, this acceptance is largely a result of increased scientific investigation.

A trend exists toward using natural substances, including compounds found in the human body, such as interferon, interleukin, insulin, and human growth hormone, as well as foods, food components, herbs, and herbal compounds. More and more researchers are discovering the tremendous healing properties of these natural compounds and their advantages over synthetic medicines and surgery in the treatment of many health conditions. Through these scientific investigations, a trend toward natural medicine is emerging. To better appreciate this evolutionary trend, this section presents some of the historical aspects of herbal medicine. Much of the following discussion is derived from Barbara Griggs's *Green Pharmacy: A History of Herbal Medicine.*[7]

In the Beginning

Plants have been used as medicines since the dawn of animal life. The initial use of plants as medicines by humans is thought to have been a result of "instinctive" dowsing. Animals in the wild still provide evidence that this phenomenon occurs. Animals, with a few notable exceptions, eat plants that heal them and avoid plants that do them harm. Presumably humans also possessed this instinct at one time.

As civilizations developed, medicine men and women were responsible for transmitting the information on herbs to their successors. Before the advent of written language, this information was handed down by verbal and experiential means.

Besides instinctive dowsing, it was commonly believed that plants had been signed by the "creator" with some visible or other clue that would indicate its therapeutic use. This concept is commonly referred to as the "doctrine of signatures." Common examples of this doctrine are the following:

- *Panax ginseng* (ginseng): its roots bear a strong resemblance to a human figure, and its general use is as a tonic.
- *Caulophyllum thalictroides* (blue cohosh): its branches are arranged like limbs in spasm, indicating its usefulness in the treatment of muscular spasm.
- *Sanguinaria canadensis* (bloodroot): its roots and sap are a beautiful blood color, corresponding to its traditional use as a "blood purifier."
- *Lobelia inflata* (lobelia): its flowers are shaped like a stomach, corresponding to its emetic qualities.
- *Hydrastis canadensis* (goldenseal): its yellow-green root signifies its use in jaundice as well as infectious processes.

All of these uses have been confirmed by recent research.

Materia Medicas

With the development of written language, materia medicas (books containing prescribing information on herbs) became the vehicle of passing information about the medicinal use of herbs to future herbalists. Materia medicas were recorded in ancient China, Babylon, Egypt, India, Greece, and other parts of the world. From these materia medicas, it is quite obvious that herbal medicines were highly respected therapies in ancient times.

Galen's Influence

No system, rules, or classification of Western herbal materia medicas existed until the first century AD, when Galen, the Greek physician who founded experimental physiology, established his system of rules and classification. Galen's classification was based on Hippocratic medicine (i.e., balance of the four humors: blood, yellow and black bile, and phlegm) and a profound belief in a beneficent nature. Although his system is considered seriously flawed in the light of modern medical knowledge, Galen is historically considered to be the founder of scientific herbalism.

Galen evaluated and classified each plant according to its relation to Hippocratic medical theory. Although based initially in Hippocratic principles, Galen constructed his own elaborate, and rigid, system of medicine. Galen's work also signified the beginning of a clear division between the professional physician and the traditional healer. Because only the well educated could understand Galen's system, and even with the best schooling it remained a mystery to many, all challenges to the professional physician were effectively squelched by dogma.

Galen's system dominated European medical thinking for 1500 years. Perhaps if the Roman Empire had continued to flourish, others would have surfaced to develop alternative theories. Instead, Galenical medicine reigned unchallenged throughout the Middle Ages. By the 19th century, the "professional" physician, confident in his supposedly superior knowledge, took Galenical philosophy to an extreme probably never imagined by Galen, by the adoption of bloodletting, purging, and administering exotic medicines. This was in direct contrast to the traditional healer's patient use of traditional herbs and tremendous faith in the healing power of nature.

The Black Plague and Syphilis

Although Galenical medicine dominated the Middle Ages, herbal medicine was still deeply entrenched in European culture. The Black Plague of 1348 may have been the beginning of a change in medical thought because conventional medicine was totally useless. As nearly one third of Europeans died during this plague, the public began to lose faith in Galenical medicine. Nearly 150 years later, another blow was dealt to Galenical medicine, when syphilis became the major medical problem. Unlike the Black Death, patients with syphilis tended to survive longer, giving physicians more time to experiment with treatments. At this time, perhaps the greatest hoax in the history of medicine began. Mercury became the standard medical treatment for syphilis despite the fact that even Galen thought mercury too poisonous to use.

Syphilis did, however, open the door for the use of some new herbs from the Americas. A French physician, Nicholas Monardes, published a comprehensive account of sarsaparilla and several other "new" drugs in the treatment of syphilis in 1574. Many Europeans at the time believed that syphilis had come to Europe from the West Indies with Columbus's sailors, and because there was a general belief that whatever disease was native to a country might be cured by the medicinal

herbs growing in that region, it was only natural for sarsaparilla to become a popular remedy. Because the standard treatment for syphilis was the use of mercury, which often resulted in greater morbidity than did syphilis, sarsaparilla was a welcome alternative. Despite initial excitement, Monardes's sarsaparilla cure eventually lost favor, probably due to other components in the cure; specifically, patients were confined to a warm room for 30 days and for the following 40 days were required to abstain from both wine and sexual intercourse.

Although the public popularity of sarsaparilla waned, it continued to be used in the treatment of syphilis. During military operations in Portugal in 1812, a British Inspector General of Hospitals noted that the Portuguese soldiers suffering from syphilis who used sarsaparilla recovered much faster and more completely than their British counterparts who were treated with mercury.

Sarsaparilla was also used by the Chinese in the treatment of syphilis. Later clinical observations in China would demonstrate, through the use of blood tests, that sarsaparilla is effective in about 90% of cases of acute syphilis and 50% of cases of chronic syphilis.

Although sarsaparilla was clearly more beneficial than mercury in the treatment of syphilis, mercury was the standard medical treatment of choice for more than four and a half centuries. Some historians have stated that "the use of mercury in the treatment of syphilis may have been the most colossal hoax ever perpetrated" in the history of medicine. Mercury represented a new kind of medicine, one formulated and prepared in a laboratory using the new techniques of chemistry. It helped prepare the way for future synthetic and mineral drugs at the expense of herbal medicines.

Challenges to Galenical Medicine

The 1500s also saw a strong challenge to Galenical medicine from within the traditional circles. Specifically, Paracelsus, an alchemist who believed strongly in the doctrine of signatures, was responsible for founding modern pharmaceutical medicine. Paracelsus is probably most remembered for the development of laudanum (tincture of opium). After Paracelsus, Galenical preparations and treatments fell greatly out of favor.

In public circles, herbal medicine was regaining some respect as well. In the early 1600s, Culpepper, an English pharmacist, published his book *The English Physician.* Instead of requiring patients to purchase expensive exotic or imported drugs, Culpepper recommended the herbs his clients and readers had growing in their own backyards. Although Culpepper's herbal philosophy is based on astrological rationalizations, it reinforced a strong English tradition of domestic herbal medicine. This came at a time when professional physicians were beginning to become contemptuous of herbal medicine.

Meanwhile, in the Americas during the 1600s and 1700s, herbs used traditionally by Native Americans were becoming quite popular, especially in the treatment of malaria and scurvy. Herbal medicine continued to gain even greater respect in the late 1700s, as exemplified by English physician Withering's classic description of digitalis. However, mercury, bleeding, and purging were still the "standard" medical treatments, epitomized by George Washington's death from complications incurred during treatment of a sore throat (i.e., he was bled to death).

The Thomsonian and Eclectic Movements

During the early 1800s, standard medicine may have been ready to reconsider traditional herbal remedies, but then came the Thomsonian movement. Samuel Thomson (1769 to 1843) patented a system of herbal medicine that, in 1839, claimed more than 3 million faithful followers. Although Thomson brought back to medicine the vitalistic Hippocratic idea of *vis medicatrix naturae* and gained widespread public support for the use of herbal medicine, the Thomsonian movement was probably detrimental to medical reform.

Thomsonians became locked in prejudice and dogma and insisted that all medical knowledge was complete and could be found in Thomson's works. These and other claims roused scorn, indignation, rage, and resentment in the average North American doctor. Frequently based on purging through the use of herbal emetics, Thomson's treatments were often as harsh as the standard treatments of the times (for further discussion, see Chapter 4).

During the 1800s, the eclectic movement attempted to bridge the gaps among standard medical thought, Thomsonianism, and traditional herbal medicines. Rather than attack the existing medical system, the eclectic movement sought to bring about reform by educating physicians about the use of herbal medicines. Several eclectic medical colleges were established, and for a while it appeared that the eclectic movement was making headway in its attempt to reform the medical system from within.

The movement eventually failed, however. Several factors were probably responsible for this: a split in the ranks, which diluted the movement; harsh measures like mercury, calomel, and bloodletting were finally discarded by the conventional professional physician, due to a decrease in infectious disease as a result of improved sanitation and hygiene; and perhaps most important, the failure to establish and sustain quality medical schools.

The Flexner report on medical education in 1910 spelled doom for the eclectics; by 1920, seven of the eight schools that had existed before the report closed, with the last school closing in 1938. Meanwhile, the standard medical schools, aided by the Rockefeller Foundation, flourished, promoting the growth of the modern pharmaceutical industry and the current near-monopoly of the medical profession.

The Growth of the Pharmaceutical Industry

Because a plant cannot be patented, little research was done before the latter part of the 20th century. Instead, pharmaceutical firms and researchers screened plants for biological activity, and then the so-called active constituents were isolated and chemically modified to produce unique, patentable compounds. Much to the dismay of the researchers was the discovery that in many instances, the isolated constituents were less biologically active than the crude herb. Because the crude herb provided no economic reward to the American pharmaceutical firm, the crude herb or extract never reached the marketplace. In contrast, European policies on herbal medicines made it economically feasible for companies to research and develop phytopharmaceuticals. The policies in the United States contributed to the tremendous growth of the pharmaceutical industry and loss of appreciation and respect for herbal medicine.

The herbal industry further compounded the problem by failing to provide quality herbal products or take advantage of technological advances that allowed for the standardization of chemical constituents. The herb that best exemplifies the failure to adopt standardization techniques is digitalis. One batch of crude digitalis might have a low level of active constituents, making the crude herb ineffective, whereas the next batch might be unusually high in active constituents, resulting in toxicity or even death when standard amounts are used. The lack of standardization made it easier for U.S. pharmaceutical firms to rationalize their economic need to isolate, purify, and chemically modify the active constituents of digitalis so they could market these compounds as drugs. The problem with using the pure active constituent is that the safe dosage range is smaller: digitalis toxicity and death have increased dramatically as a result of purification. Toxicity was less of a factor when using the crude herb because overconsumption of potentially toxic doses resulted in vomiting or diarrhea, thus avoiding the severe heart disturbance and death that now occur with pure digitalis cardiac glycoside drugs.

Fortunately, several European and Asian pharmaceutical firms began specializing in phytopharmaceuticals in the latter half of the 20th century. These companies have played a prominent role in researching, developing, and promoting herbal medicines.

Research is demonstrating that crude extracts often have greater therapeutic benefit than the isolated "active" constituent. This occurrence has been long known in other parts of the world, but in this country, isolated plant drugs are still thought of as having the greatest therapeutic effect. This myth is gradually being eroded as our knowledge of herbal medicines increases.

If current standardization techniques had been available earlier in this century, it is possible that many current prescription drugs would be herbal extracts instead of isolated and modified active constituents or synthetic chemicals.

IMPORTANT NORTH AMERICAN HERBAL MEDICINE ORGANIZATIONS

The American Botanical Council

One of the critical entities in promoting the responsible use of botanical medicine in the United States has been the American Botanical Council (ABC), an independent, nonprofit research and education organization dedicated to providing accurate and reliable information on botanical medicine for consumers, health care practitioners, researchers, educators, industry, and the media. Founded by Mark Blumenthal in 1988, the ABC has a robust website and publishes *HerbalGram,* a peer-reviewed quarterly journal, along with providing other useful publications and services. For example, Blumenthal and the ABC were largely responsible for translating the entire Commission E monographs into English, and the complete expanded version is now provided on the ABC website.[8] ABC is also the publisher of four books, including *The ABC Clinical Guide to Herbs,* a continuing education and reference book, which contains extensive monographs on the safety and efficacy of 30 popular herbs.

In addition, the ABC provides proactive, science-based information about herbal medicine to the media through its Media Education program. The ABC also provides an internship program for students of pharmacy and dietetics, as well as other training and certification programs. For more information, go to http://www.herbalgram.org.

American Herbal Pharmacopoeia

The American Herbal Pharmacopoeia (AHP) was founded in 1995 by herbalist Roy Upton, with unrestricted funding initially provided by Planetary Herbals and now by many organizations. The AHP produces critically reviewed and scientifically validated monographs that provide an internationally recognized standard for herbal medicine identification, adulteration recognition, extraction, and quality control. These standards help ensure the identity, purity, and quality of botanical raw materials and manufactured products. Each monograph also presents a complete and critical review of the traditional and scientific literature regarding the efficacy and safety of herbal medicines. More information can be found at http://www.herbal-ahp.org.

FINAL COMMENTS

Although the future looks extremely promising for herbal medicine, its growth and continued success will ultimately be determined by the following:

- Continued scientific investigation and clinical research
- Adoption of recognized standards of quality by manufacturers
- Governmental regulations that allow meaningful therapeutic and health claims

TABLE 34.1 Classic Examples of Drugs From Plants With a Correlation to Their Traditional Use

Drug	Clinical Use	Botanical Source
Atropine	Anticholinergic	*Atropa belladonna*
Caffeine	Central nervous system stimulant	*Cola nitida*
Camphor	Rubefacient	*Cinnamomum camphora*
Cocaine	Local anesthetic	*Erythroxylon coca*
Codeine	Analgesic/antitussive	*Papaver somniferum*
Colchicine	Antigout	*Colchicum autumnale*
Digitoxin	Cardiotonic	*Digitalis purpurea*
Digoxin	Cardiotonic	*Digitalis lanata*
Emetine	Amebicide/emetic	*Cephaelis ipecacuanha*
Ephedrine	Sympathomimetic	*Ephedra sinica*
Gossypol	Male contraceptive	*Gossypium* spp.
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Kawain	Tranquilizer	*Piper methysticum*
Methoxsalen	Psoriasis/vitiligo	*Ammi majus*
Morphine	Analgesic	*Papaver somniferum*
Noscapine	Antitussive	*Papaver somniferum*
Physostigmine	Cholinesterase inhibitor	*Physostigma venenosum*
Pilocarpine	Parasympathomimetic	*Pilocarpus jaborandi*
Podophyllotoxin	Topical wart remedy	*Podophyllum peltatum*
Quabain	Cardiotonic	*Strophanthus gratus*
Quinine	Antimalarial	*Cinchona ledgeriana*
Reserpine	Antihypertensive	*Rauwolfia serpentine*
Scopolamine	Sedative	*Datura metel*
Sennosides	Laxative	*Cassia* spp.
Theophylline	Bronchodilator	*Camellia sinensis*
Tubocurarine	Muscle relaxant	*Chondrodendron tomentosum*
Yohimbine	Male erectile dysfunction	*Pausinystalia yohimbe*

Data from De Smet, PA. The role of plant-derived drugs and herbal medicines in healthcare. *Drugs.* 1997;54:801–840.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. De Smet PA. The role of plant-derived drugs and herbal medicines in healthcare. *Drugs*. 1997;54:801–840.
2. American Botanical Council. Retail Sales of Herbal Supplements in US Increase by 8.5% in 2017, Topping $8 Billion. http://cms.herbalgram.org/press/2018/PRMarketReport9_11_2018.html. Accessed 6 Aug 2019.
3. Keller K. Legal requirements for the use of phytopharmaceutical drugs in the Federal Republic of Germany. *J Ethnopharmacol*. 1991;32:225–229.
4. Thiede HM, Walper A. Inhibition of MAO and COMT by hypericum extracts and hypericin. *J Geriatr Psychiatry Neurol*. 1994;7:S54–S56.
5. Perovic S, Muller WEG. Pharmacological profile of hypericum extract. Effect of serotonin uptake by postsynaptic receptors. *Arzneim Forsch*. 1995;45:1145–1148.
6. Butterweck V. Mechanism of action of St. John's wort in depression: what is known? *CNS Drugs*. 2003;17:539–562.
7. Griggs B. *Green Pharmacy. A History of Herbal Medicine*. London: Robert Hale; 1981.
8. American Botanical Council. The Commission E monographs. http://cms.herbalgram.org/commissione/index.html. Accessed 6 Aug 2019.

35

Environmental Medicine

Joseph E. Pizzorno, ND, and *Walter Crinnion*, BS, ND*

OUTLINE

ENVIRONMENTAL TOXIC LOAD

The 20th century, with its promise of "Better Living Through Chemistry," resulted in a host of chemical toxicant-related illnesses (referred to here as "environmental illnesses"). We are experiencing the relatively new medical diagnoses of sick (closed) building syndrome[1] and multiple chemical sensitivity,[2] both of which are known to be related to overexposure to environmental contaminants. The rates of asthma, allergy, autism spectrum disorder, and attention deficit hyperactivity disorder, as well as childhood brain tumors, obesity, and diabetes, are all skyrocketing in concert with the level of environmental pollution. Indeed, most of the primary chronic, noninfective health problems plaguing our modern world have been linked to the growing environmental burden found in all people. Included in this list are the most common cardiovascular, respiratory, neurological, immunological, and endocrine problems.

This chapter summarizes key aspects of environmental medicine every clinician should know. Clinicians wanting to delve into this important area more deeply will find this topic extensively addressed in *Clinical Environmental Medicine* (Crinnion and Pizzorno, Elsevier, St. Louis, MO, 2018).

The Toxic Burden

The main principle of environmental medicine (EM) is "total load."[3] Many patients want to point to the one chemical exposure predating their illness as *the* compound that made them ill. This approach coincides with the traditional approach of toxicology that focuses on a single toxicant, ignoring the fact that all people have multiple toxicants present. In most cases, the symptom-initiating exposure was merely the one that tipped the individual's total load "over the edge," leading to a health crisis. EM takes into account the possible synergism of the total load of all toxicants and toxins as a causative factor in illness.[4–7]

The U.S. Centers for Disease Control and Prevention (CDC) has been conducting ongoing laboratory assessment of samples gathered in the National Health and Nutrition Examination Survey (NHANES) trial to quantify the total load of xenobiotic compounds present in the average U.S. resident.[8] The overall purpose of this work is to "provide unique exposure information to scientists, physicians, and health officials to help prevent exposure to some environmental chemicals."

The goals of this survey include the following:

- To determine which chemicals are found in the U.S. population and at what concentrations
- For chemicals with known toxicity levels (as defined by the field of toxicology), to determine the prevalence of people with levels that exceed the safe limit (e.g., a blood lead level that is ≥10 mcg/dL)
- To establish reference values that can be used by physicians and scientists to determine whether a person or group has an unusually high exposure—especially helpful to identify population groups that merit further assessment of exposure sources or health effects

Before the CDC's publication of these reports, standard laboratory reference values for these compounds reflected industrial workplace standards. The new CDC values give us, instead, a population-based reference range, allowing identification of those with higher-than-normal burdens of these toxic compounds. To date, samples of urine, blood, and serum collected from the NHANES participants have been assessed for the presence of 246 environmental chemicals or their metabolites. Of these 246 compounds, 120 were ubiquitous enough to have mean values assigned (Table 35.1).

Exposure to these persistent and nonpersistent compounds begins during the fetal stage because they all appear to easily cross the placental barrier.[9] To this maternally transferred burden is added the daily exposures to xenobiotics in the air, food, water, and personal care products. Cord blood samples taken at birth offer an accurate reading of which xenobiotics the fetus is being exposed to. The Environmental Working Group (EWG) tested the cord blood of 10 babies born in US hospitals for the presence of 413 toxic compounds.[10] Of those, 287 were detected in the samples, with an average of 200 of these compounds present for each infant. Table 35.2 shows the compounds that were looked for in the cord blood samples and how many were found.

A number of compounds, including pharmaceutical agents, illegal drugs, heavy metals, and pesticides, have been found in meconium

*Previous edition contributor

TABLE 35.1 Ubiquitously Found Toxicants in U.S. NHANES Participants[8]

Compound	No. Tested	No. Found
Acrylamides	2	2
Benzophenone-3 (sunscreen)	2	1
Bisphenol A	1	1
Chlorinated pesticides	16	5
Cotinine, nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (smoking)	2	2
Dichlorophenol	3	3
Dioxins/furans	17	2
Herbicides	23	2
Metals	26	16
Methyl tert-butyl ether (MTBE)	1	1
Nitrate	1	1
Organophosphate pesticide metabolites	16	4
Parabens	3	3
Perchlorate	1	1
Perfluorinated compounds	12	7
Phthalates	16	12
Polybrominated diphenyl ethers	11	5
Polychlorinated biphenyls (PCBs)	40	33
Polycyclic aromatic hydrocarbons (exhaust)	10	10
Pyrethroid pesticides	5	1
Solvents	28	6
Thiocyanate	1	1
Trihalomethanes (chlorination by-products)	4	2
Triclosan (disinfectant)	1	1

TABLE 35.2 Xenobiotics Found in the Cord Blood of 10 Children Born in U.S. Hospitals[10]

Compound	No. Tested	No. Found	Pollutant Source
Mercury	1	1	Seafood, dental amalgams
Polycyclic aromatic hydrocarbons (PAHs)	18	9	Combustion by-product (from tailpipes and cigarettes)
Polybrominated dioxins and furans (PBDDs/Fs)	12	7	Contaminants in brominated flame retardants
Polychlorinated dioxins and furans (PCDDs/Fs)	17	11	By-products of plastic production (PVC), industrial bleaching, and incineration
Perfluorinated chemicals (PFCs)	12	9	Teflon, Scotchgard, fabric and carpet protectors, food wrap coatings
Chlorinated pesticides	28	21	Maternal transfer, sardines, farmed salmon
Polybrominated diphenyl ethers (PBDEs)	46	32	Flame retardants; high in house dust and farmed salmon
Polychlorinated naphthalenes	70	50	Wood preservatives, varnishes
Polychlorinated biphenyls (PCBs)	209	147	High in farmed salmon and sardines

samples.[11] Included in the meconium toxicant assays were organophosphate pesticide (OP) metabolites that were not looked for in the EWG cord blood study. This is quite a load for each child to start out with, especially because the liver, brain, and endocrine and immune systems are all still developing, and many of these compounds are toxic to those tissues.

Table 35.3 shows a typical report from the CDC. As can be seen, the body load of this common metabolite of neurotoxic OPs has been increasing relentlessly in the general population.[12]

Other Factors Affecting Clinical Impact of Total Load

EM physicians also consider many other factors besides toxicant levels as part of the total load on an individual. Many of these factors will negatively affect the ability of the body to clear these toxicants from the blood or the tissue or to protect itself from the damage caused by these compounds. Such factors include the following:

- **Genetics.** Polymorphisms in liver Phase I and Phase II biotransformation enzymes can reduce the ability of the body to metabolize xenobiotic toxicants.[13]
- **Nutrient deficiencies.** Deficiencies of nutrients needed for biotransformation (Phases I and II), excretion (Phase III), and protection against toxicant damage can result in reduced clearance and increased oxidative damage.[14]
- **Dietary choices.** The amounts and ratios of proteins, fats, and carbohydrates can either enhance or inhibit the clearance of toxicants from the bloodstream.[15]
- **Emotional/mental/spiritual stressors.** Stresses from family, jobs, society, relationships, and other sources can directly affect health, toxin clearance, and clinical reaction to environmental toxicants.[16]
- **Lifestyle.** Daily choices can have a direct bearing on pollutant exposures and how well the body responds to its toxic burden. This includes the amount of sleep, exercise, and leisure time one gets.
- **Overall health and wellness.**
- **Infections, disease, organ function.** The health of organs, tissues, and cells has a profound influence on the ability to clear toxicants efficiently[17] and to resist toxic damage. The presence of pathogenic organisms that release their own toxins can be a decisive contributor to the total load.
- **Microbiome.** Especially if endotoxicity is present.
- **Electromagnetic field (EMF) exposure.**

Sources of Environmental Toxins

- Maternal-fetal transfer
- Air, indoor and outdoor
- Food
- Personal care products
- Water

In the previous section, the EWG newborn study[10] highlighted the fact that mothers unwittingly pass a host of toxic compounds to their children during gestation. After birth, the toxic exposures primarily come from indoor air (especially in one's residence) and the food that is consumed. The U.S. Environmental Protection Agency did a number of total exposure assessment methodology studies in the 1980s,

TABLE 35.3 Urinary Dimethylphosphate (DMP) (1999–2008): Metabolite of Several Organophosphorus Insecticides, Geometrical Mean and Selected Percentiles of Urine Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey

Categories	Survey Years	Geometrical mean (95% confidence interval [CI])	50th Percentile (95% CI)	75th Percentile (95% CI)	90th Percentile (95% CI)	95th Percentile (95% CI)	Sample Size
Total	99–00	a	.740 (<LOD-1.40)	2.90 (2.10–4.00)	7.90 (6.20–8.90)	14.0 (10.0–19.0)	1949
	01–02	a	<LOD	3.32 (2.90–3.74)	8.40 (7.11–9.23)	13.4 (11.4–14.9)	3017
	03–04	a	<LOD	4.15 (3.39–4.94)	9.54 (7.46–11.9)	15.1 (12.8–18.2)	2494
	05–06	a	<LOD	5.21 (4.15–6.56)	17.2 (13.8–20.5)	29.6 (24.3–35.2)	2635
	07–08	a	<LOD	7.91 (6.64–9.07)	20.8 (18.2–24.4)	35.6 (30.3–39.4)	2593
Age group							
6–11 years	99–00	1.58 (1.15–2.18)	1.10 (.580–2.20)	4.40 (2.80–6.80)	10.0 (7.80–21.0)	22.0 (15.0–33.0)	471
	01–02	a	.970 (<LOD-2.00)	5.04 (3.31–7.66)	12.2 (9.10–15.1)	18.3 (12.6–41.7)	576
	03–04	a	<LOD	4.53 (3.34–5.96)	11.0 (5.62–17.9)	16.2 (7.46–28.3)	310
	05–06	a	<LOD	9.92 (3.24–20.0)	27.1 (16.5–46.6)	46.6 (27.1–63.0)	350
	07–08	a	<LOD	12.5 (7.22–16.4)	29.7 (20.3–39.1)	43.3 (29.7–62.7)	385
12–19 years	99–00	a	.670 (<LOD-1.80)	3.80 (2.50–4.90)	9.90 (6.20–18.0)	22.0 (13.0–29.0)	664
	01–02	a	.670 (<LOD-1.31)	4.27 (3.41–5.35)	9.27 (7.80–12.3)	14.7 (11.8–21.3)	822
	03–04	a	1.20 (<LOD-2.27)	4.61 (3.47–6.72)	10.9 (7.90–15.0)	20.9 (12.5–26.8)	717
	05–06	a	<LOD	7.32 (1.35–12.4)	22.6 (16.1–36.1)	44.0 (27.6–54.0)	718
	07–08	a	<LOD	10.0 (7.22–14.5)	25.5 (16.8–35.6)	36.2 (25.5–49.6)	391
20–59 years	99–00	a	.680 (<LOD-1.30)	2.70 (1.80–3.70)	6.60 (5.70–8.10)	9.70 (8.80–14.0)	814
	01–02	a	<LOD	2.95 (2.35–3.41)	6.95 (5.80–8.82)	11.5 (9.66–13.7)	1121
	03–04	a	<LOD	3.75 (2.84–4.88)	8.52 (6.86–10.7)	14.1 (10.8–17.5)	938
	05–06	a	<LOD	4.09 (2.45–6.03)	13.8 (9.82–19.8)	24.6 (19.8–32.8)	1092
	07–08	a	<LOD	6.07 (4.26–7.69)	18.0 (13.6–22.6)	30.3 (23.2–37.9)	1180
60 years and older	01–02	a	.700 (<LOD-1.50)	3.68 (2.91–4.56)	8.93 (6.79–10.7)	14.4 (9.60–19.1)	498
	03–04	a	2.04 (<LOD-2.63)	4.60 (3.83–5.15)	11.3 (7.68–13.4)	16.3(12.4–19.4)	529
	05–06	a	<LOD	6.75 (4.75–9.32)	16.8 (11.7–22.1)	26.3 (18.9–38.8)	475
	07–08	a	<LOD	10.8 (8.64–13.0)	24.9 (19.2–32.4)	40.5 (32.5–47.4)	637
Gender							
Males	99–00	a	.670 (<LOD-1.30)	2.90 (2.20–4.00)	7.90 (6.00–9.30)	18.0 (10.0–24.0)	952
	01–02	a	<LOD	3.49 (2.90–4.15)	8.40 (6.95–10.3)	12.8 (11.3–14.7)	1420
	03–04	a	<LOD	4.03 (3.30–4.91)	8.49 (6.82–11.9)	15.1 (11.3–20.0)	1221
	05–06	a	<LOD	4.10 (2.37–6.40)	15.9 (12.4–20.9)	28.1 (22.3–36.7)	1246
	07–08	a	<LOD	7.75 (6.50–9.63)	20.8 (17.6–26.6)	36.1 (27.0–47.4)	1295
Females	99–00	a	.790 (<LOD-1.60)	2.90 (2.00–4.20)	7.80 (5.70–9.00)	11.0 (9.00–18.0)	997
	01–02	a	<LOD	3.06 (2.63–3.63)	8.38 (6.56–9.63)	13.7 (10.9–17.6)	1597
	03–04	a	1.08 (<LOD-1.92)	4.29 (3.31–5.28)	10.4 (7.64–12.9)	14.8 (13.3–18.0)	1273
	05–06	a	<LOD	6.40 (5.04–7.21)	18.7 (14.6–20.8)	30.9 (24.6–41.1)	1389
	07–08	a	<LOD	7.91 (6.18–9.36)	20.6 (16.5–25.5)	34.2 (26.6–41.5)	1298

LOD, Limit of detection; *<LOD,* less than the limit of detection, which may vary for some chemicals by year and by individual sample.
The LOD for survey years 1999–2000, 2001–2002, 2003–2004, 2005–2006, and 2007–2008 was 0.58, 0.5, 0.5, 0.47, and 0.47, respectively.
[a]Not calculated: proportion of results below LOD was too high to provide a valid result.

which showed that indoor air typically contained higher levels of environmental chemicals than outdoor air (even in towns with multiple chemical plants). The majority of compounds found in the indoor air were solvents from smoking, dry-cleaned clothes, home furnishings, and home cleaning agents. Pesticide use in the home and garden is also a contributing factor to the burden of pollutants in indoor air and dust.[18,19]

Food is the other route through which the majority of our xenobiotic load is delivered. The U.S. Food and Drug Administration (FDA) has an ongoing Total Diet Study that measures a set list of foods for a variety of environmental contaminants, including heavy metals and plasticizer compounds.[20] The U.S. Department of Agriculture (USDA) has also been doing an ongoing measurement of pesticide residues on the most commonly consumed fruits and vegetables.[21] The data published in this USDA report form the basis for the "dirty dozen" list of the most toxic fruits and vegetables, which are published on the web by the EWG.[22] This listing gives the consumer information on how to avoid produce containing high levels of agricultural chemicals.

High levels of glyphosate residues are found in all Roundup Ready soybeans, whereas no residue was found in organically raised soybeans.[23] All soy protein isolates from genetically modified (GM) soy contained residues of glyphosate and its metabolite in levels up to 2.7 ug/g.[24] Fortunately, no residues were found in soy milk or soybean oil, nor were any found in corn oil, cow's milk, whole-milk powder, or breast milk. However, glyphosate residues have been found in wheat.[25]

High-molecular-weight phthalates (plasticizers) contaminate food based on the length of time food is stored in plastic wrapping, the fat content of the food, and whether or not heat is involved. Microwaving foods in plastic wrap increases phthalate migration.[26] Levels of plasticizers are high in store-wrapped meat, poultry, fish, and cheese, with cheese having the highest level of plasticizers. This is most likely due to the fat content of each of these foods. Contrary to an ongoing Internet myth, freezing food in plastic containers does not increase plastic levels in the food. Plastic levels in unheated plastic-wrapped foods only increased in those foods after they were heated.[27] Exposure to low-molecular-weight phthalates, however, comes primarily from fragrances and other personal care products.[28] On average, the consumption of one canned food item daily increased urinary bisphenol A (BPA) by 24%, and consuming two items increased urinary BPA by 54%.[29] However, not all canned foods increased urinary BPA to the same degree. Consumption of one canned soup increased urinary levels by 229%, canned pasta by 70%, and canned fruits and vegetables by 41%. Consuming one can of soup daily for 5 days has been shown to increase urinary BPA levels by more than 1000%.[30] Data from NHANES 2005–2006 revealed that drinking soda pop and consuming school lunches and meals prepared outside of the home were the most significant sources of BPA.[31]

Butter has been used as a sampling agent to assess the regional and global distribution of polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs) around the globe. When butter was sampled for these compounds, the highest levels of PCBs were found in butter from Europe and North America.[32] For individuals living in North America, the greatest source of PCB exposure came from butter and fish.[33] Sardines have the highest concentration of the highly toxic PCBs 138, 153, and 180, followed closely by farmed salmon and then hamburger.[34]

In the 2004–2005 FDA Total Diet Study, 100% of the salmon samples were found to contain PCBs and dichlorodiphenyldichloroethylene (DDE). Currently 50% of world fish consumption comes from aquaculture,[35] and two thirds of all salmon consumed in North America is farmed.[36] Farmed salmon is estimated to be responsible for 97% of dietary POP exposure.[37] POP levels were assessed in 700+ salmon (totaling approximately 2 metric tons of farmed and wild salmon) from around the globe.[38] Fourteen persistent chlorinated compounds were found in significantly higher levels in farmed salmon than in wild salmon. The only compound that did not reach statistical significance was lindane, which was still higher in farmed versus wild salmon. The four compounds with the greatest differences were PCBs, dioxins, Toxaphene, and dieldrin. The PCB content in farmed salmon averaged 42.5 ng/g, whereas the wild Alaskan salmon averaged only 3.2 ng/g. Fish samples from farms off the coast of Washington State and Chile had lower PCB concentrations than salmon farmed by Scotland and the Faroe Islands. POP levels in the fish samples paralleled the POP levels in the fish pellets used in those areas.[39] Another study revealed the average PCB content in farm-raised Atlantic Salmon to be 28 to 38 ng/g, whereas total PCB content in wild Alaskan salmon averaged 2.8 to 13.7 ng/g.[40] The PCBs found in Atlantic salmon, dominated by highly toxic PCBs 138, 153, and 180, are different from those in Alaskan salmon, which have fewer chlorine molecules, have greater water solubility, and are far less toxic to humans. Some changes have been made to the fish pellets that are fed to farmed salmon, resulting in a decrease in some of the POPs present in the fish.[41] Unfortunately, the levels of PCBs 135, 153, and 180 have remained about the same. Farmed shrimp and tilapia, along with cod, are relatively free of the POPs found in farmed salmon, providing less toxic fish alternatives.

Lead and arsenic are common water contaminants that vary by geographical area as well as the age of the water pipes and the type of disinfection used.

Many areas, including much of the United States, have high levels of inorganic arsenic in the groundwater (Fig. 35.1).[42]

Other commonly found water pollutants are the gasoline additive methyl tert-butyl ether (MTBE),[43,44] hexavalent chromium,[45,46] perchlorate (rocket fuel),[47] perfluorocarbons (in areas where industry is using perfluorinated compounds [PFCs]),[48,49] and trichloroethylene (TCE),[50] along with fluoride,[51] trihalomethanes,[52] pesticides, solvents,[53] other industrial chemicals, and pharmaceuticals.[54–56] In contrast, commonly used personal care products contain low-molecular-weight phthalates[57] and parabens.[58]

ADVERSE HEALTH EFFECTS

Numerous studies have been published on the adverse health effects of individual xenobiotic pollutants. Although the areas of impact cover all the systems in the body, this chapter focuses on the documented immunotoxic, neurotoxic, and endocrinotoxic effects of these chemicals.

Immunotoxicity

Of the three major systems affected by xenobiotic burden (immune, neurological, and endocrine), the signs and symptoms of immunotoxicity are very often the first to occur in a patient's history. The classic picture of immunotoxicity is reduced cell-mediated immunity leading to chronic infections (reduced Th1 function), allergies and asthma (increased Th2 function), chemical sensitivity, and autoimmunity (overactive Th2 or Th17 activity).[59] The first manifestation of immunotoxicity often seen in a case history is the development of asthma and allergies. Multiple environmental toxicants are associated with increased incidences of allergic reactivity to the environment and to foods (Table 35.4). The chemicals that lead the way in causing atopic reactions include both OPs and chlorinated pesticides, solvents, and combustion by-products, including tobacco smoke and diesel exhaust.

Cell-Mediated Immune Dysfunction

Unfortunately, many of the xenobiotics that have been ubiquitously found are very potent suppressors of cell-mediated immune

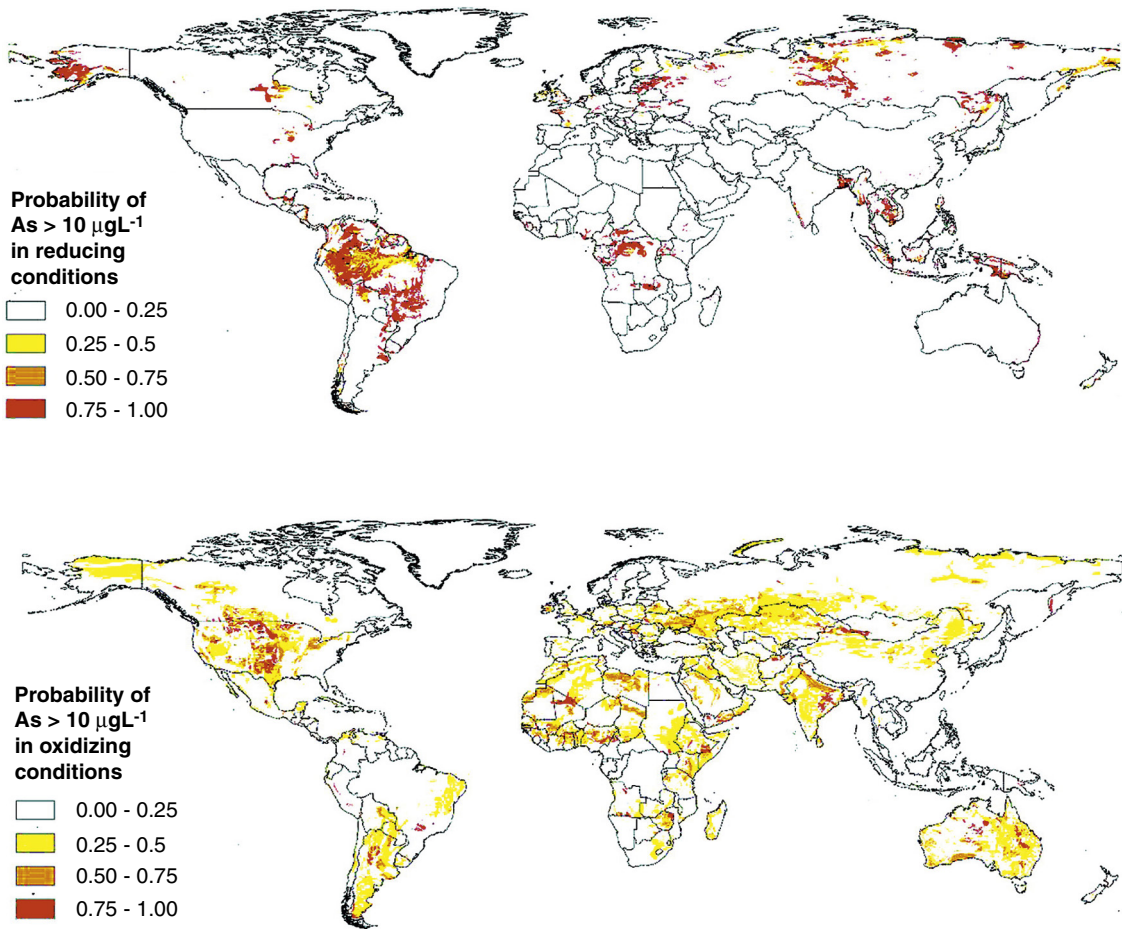


Fig. 35.1 Global groundwater arsenic concentrations. (From Amini M, Abbaspour KC, Berg M, et al. Statistical modeling of global geogenic arsenic contamination in groundwater. *Environ Sci Technol*. 2008;42(10):3669–3675. PubMed PMID: 18546706.)

TABLE 35.4 Common Environmental Pollutants Associated with Low Th1 and High Th2

Diesel exhaust particles (DEPs)[60,61]
Polycyclic aromatic hydrocarbons[62]
Particulate matter $(PM)_{2.5}$[63,64]
Lead[65,66]
Mercury (thimerosal and methylmercury)[67,68]
Tributyl tin[69]
Organophosphate pesticides[70]
Dichlorodiphenyltrichloroethane (DDT)/dichlorodiphenyldichloroethylene (DDE)[71]
Broadleaf chlorophenoxy herbicides[72]
Trichloroethylene in drinking water[73]
Polychlorinated biphenyl (PCB) 118[74]
Benzene[75]
Bisphenol A[76]
Bisphenol A (prenatal exposure)[77]
Phthalates[78]
Dioxin[79]
Toluene diisocyanate (from indoor building materials)[80]
Trimellitic anhydride (from indoor building materials)[39]
Perfluorooctanesulfonate (PFOS)[81]
Gas stove in the home[82]
Maternal agricultural work

responses. DDE (the main metabolite of dichlorodiphenyltrichloroethane [DDT]) causes apoptosis of peripheral blood mononuclear cells, resulting in fewer macrophages roaming the body to phagocytize invading pathogens and trigger an immune response.[83,84] Mercury, another commonly found xenobiotic, also increases apoptosis of both monocytes and lymphocytes and reduces the phagocytic ability of the monocytes. It has been demonstrated that workers occupationally exposed to mercury vapor exhibited a diminished capacity to produce both tumor necrosis factor-α (TNF-α) and interleukin-1.[85]

The chemicals produced by combustion, polyaromatic hydrocarbons (PAHs), have been shown to have similar depressing effects on the immune system, including decreased T-cell–dependent antibody response, decreased splenic activity, diminished T-cell effector functions, and suppression of T-cell cytotoxic induction, and they lower the activity of natural killer (NK) cells.[86] PAH exposure results in impaired systemic immunity and increased risk of allergy in humans.[87] The OPs, which are not as biologically persistent as chlorinated pesticides, are also toxic to the immune system. They have been found to cause decreased percentages of CD4 and CD5 cells, increased numbers and percentages of CD26 cells, increased incidence of atopy and antibiotic sensitivity, and high rates of autoimmunity. This elevation in autoimmunity is reflected by high levels of antibodies to smooth muscle, parietal cells, brush border, thyroid, and myelin and elevated antinuclear antibody (ANA).[88]

Allergy and Asthma

Traffic-related air pollutants increase the risk of 1-year-old children becoming allergic to both foods and airborne allergens.[89] As children

continue to be exposed to vehicular exhaust, their risk of developing food allergies by age 4 increases by 230%, far higher than their risk of having pollen allergies by that age (83% increase).[90] Multiple studies have demonstrated a clear association between vehicular exhaust and asthma in both children and adults.[91–93] Children living in areas with denser traffic were 250% more likely to present to a local emergency department with asthma.[94] The spikes of asthmatic symptoms in children rise with daily spikes of vehicular pollution and can persist for up to 2 days after a spike.[95] When exposure to high traffic was combined with mycotoxin (mold contamination) exposure in the home, the risk for asthma went from a 75% increase to 5.85-fold increase.[96]

Diesel vehicles, which are most closely associated with these immunological effects, comprised 70% of all new vehicle sales in France and 50% in the rest of Europe in 2010.[97] In the United States, the sale of diesel vehicles increased by almost 40% in 2010 alone. Diesel exhaust particles (DEPs) interfere with immune system regulation by suppressing nuclear factor kappa B (NF-κB), reducing NF-κB cytokine production.[98] DEPs reduce levels of the antiviral cytokine interferon-gamma (IFN-γ) and IFN-γ mRNA more potently than either dexamethasone or cyclosporine A.[99] DEP also dramatically diminishes NK cell function in human volunteers.[100,101] This reduction of Th1 function, resulting in diminished cell-mediated immunity, is the likely cause for DEP exposure also increasing the rates of respiratory infections.[102–105]

DEP exposure in animals results in increased allergen-specific Th2/TH17 cells in the lungs, which enhances allergic reactivity. Not surprisingly, when mice were exposed to egg albumin *after* DEP exposure, they produced more immunoglobulin E (IgE) to ovalbumin than mice that were not exposed to DEP.[106] In atopic individuals with null GSTT1 genotypes, DEP exposure resulted in even higher interleukin (IL)-5 production.[107] Other studies have confirmed the increase in Th2 cytokine production after DEP exposure and have shown that this exposure leads to increased reactivity to ragweed,[108] cedar pollen,[109] birch pollen,[110] and egg protein.[111] Simultaneous exposure to DEP and allergens leads to a more rapid development of allergic reactivity to those allergens than would occur without DEP presence.[112]

Autoimmunity

In the past 30 years, the incidence and prevalence of autoimmune diseases have been steadily rising.[113] Currently, rheumatic, endocrine, gastrointestinal, and neurological autoimmune diseases are increasing annually at a rate of 7.1%, 6.3%, 6.2%, and 3.7%, respectively. Comparing old rates with current rates, the greatest jumps are found in celiac disease, diabetes 1, and myasthenia gravis. These increases in autoimmunity have been linked to common environmental pollutants. The notion of chemically induced autoimmune states is, of course, not new because many chemicals are known to induce the onset of systemic lupus erythematosus. Some chemicals, like formaldehyde and other volatile organic compounds, may induce tissue-specific autoimmune reactions by acting as haptens.[114] These low-molecular-weight molecules bind to various tissues in the body, making a new antigenic combination. But the elevation of Th2 and Th17 cytokine pictures secondary to pollutant exposure is the clearest pathway for these autoimmune problems. Autoimmunity highlighted by connective tissue destruction is associated with an increased Th17 immune response. Published research has already shown that particulate matter, diesel exhaust, BPA, trichloroethylene, cigarette smoke, PCBs, and paraquat have been linked to conditions of increased Th17. Most of those have also been clearly linked to increased rates of autoimmune disorders. Although the findings have not yet been associated with increasing Th17 levels, women in the NHANES 1999–2004 study with the highest versus lowest levels of hair and blood mercury were either 410% or 232% more likely to have elevated ANA levels, respectively.[115] Individuals with higher levels of mercury exposure are also more likely to have elevated ANA levels.[116,117] In the 2003–2004 NHANES, those persons with the highest levels of PCBs were over four times more likely to have elevated ANA levels than those with a lower PCB burden.[118]

Twelve persons who were residentially exposed to the chlorpyrifos were found to have elevated antibody levels between 1 and 4.5 years after the exposure occurred.[119] The most common autoimmune antibodies found were anti–smooth muscle, causing autoimmune hepatitis (odds ratio [OR] 3.99), and anti–parietal cell, leading to diminished hydrochloric acid production (OR 9.7), along with antithyroid and antimyelin antibodies. Animals exposed simultaneously to mercury and trichloroethylene developed autoimmune hepatitis, whereas those exposed to only one of those two compounds for 8 weeks failed to show this pathology.[120] Four groups of patients with long-term formaldehyde exposure (mobile home dwellers, office workers in closed buildings, persons who had been removed from their formaldehyde exposure for 1 year, and persons with occupational exposure) demonstrated similar autoantibody production.[121] Mobile home dwellers had a 144-fold higher risk of having anti–parietal cell antibodies than nonmobile home dwellers, whereas office workers were 40.5-fold more likely to have anti–parietal cell antibodies. Levels of anti–smooth muscle, antimitochondrial, and anti–brush border were also higher in those with formaldehyde exposure.

Elevated levels of particulate matter $(PM)_{2.5}$-containing traffic-related air pollution have been associated with circulating levels of anti-dsDNA, a classic marker for systemic lupus erythematosus (SLE).[122] Persons exposed to higher levels of $PM_{2.5}$ in Quebec were more likely to have SLE or one of the other autoimmune connective tissue disorders (Sjogren's syndrome, scleroderma, polymyositis, or dermatomyositis).[123] PM_{10}, NO_2, and CO have all been shown to be risk factors for juvenile-onset SLE, with each increase of 13.4 ug/m^3 in PM_{10} increasing disease risk by 34%.[124]

Chemical Sensitivity

Chemical sensitivity (CS), also termed multiple chemical sensitivity (MCS), idiopathic environmental intolerance (IEI),[125] and toxicant-induced loss of tolerance (TILT),[126,127] is a modern medical phenomenon that is now present in up to 30% of the population.[128] Individuals with CS experience adverse physical, mental, or emotional symptoms after being exposed to ambient levels of common environmental chemicals. Individuals with chemical sensitivity often show the same cytokine imbalances already discussed in this section.[129] However, CS may not be a manifestation of only immunotoxicity—there is also evidence of limbic kindling or neural sensitization in persons with this problem.[130,131]

Neurotoxicity

The neurological system is also a frequent target for xenobiotic compounds. Some patients will present primarily with neurotoxicity symptoms, whereas others may exhibit immunotoxicity signs and symptoms first. The most common neurotoxicity symptoms include reduced cognitive functioning (often referred to by the patient as "brain fog" or "crooked brain"), headache, memory problems, and mood disorders. Tremors, balance problems, and anxiety can also be present (Box 35.1).

The nervous system is a unique target for toxic agents in several ways:

1. The adult neuron does not divide, and therefore, replacement of lost neurons is not possible. Nerve cells killed by toxins cannot regenerate.
2. The blood–brain barrier does not block nonpolar substances or items that are actively transported.

BOX 35.1 Neurotoxicity Presentations

- Organophosphates
  - Poor cognition
  - Poor attention
  - Depression
  - Fatigue
  - Headache
  - Tremors
  - Paresthesia
  - Slower reaction time
- Chronic Low-Level Chlorinated Pesticides
  - Poor cognition
  - Short-term memory loss
  - Balance problems
  - Depression
  - Fatigue
- Solvents
  - Poor cognition
  - Short-term memory loss
  - Depression
  - Irritability
  - Fatigue
  - Headache

3. Because the normal function of the nervous system requires the action of a complex, integrated network, damage to even a small portion of the nervous system sometimes can result in marked effects on function.
4. Neurons are dependent on glucose and oxygen, and some cell bodies exist at borderline levels of oxygen. If high energy demands are placed on the system and the delivery of oxygen is reduced, then cell death may occur.
5. Because of the high lipid content (myelin), there is an accumulation and storage of lipophilic xenobiotics.
6. Neurons have high surface areas and, therefore, increased exposure to toxic compounds.
7. Neuroinflammation is fairly easy to start and very difficult to alleviate.

Unfortunately, a great many of the common toxic xenobiotics in our bodies are potent neurotoxins. All of the major classes of pesticides kill pests by virtue of their neurotoxic actions. Chlorinated pesticides and pyrethroids disrupt the ion flow along the axon, whereas OPs (developed in Germany between the First and Second World Wars for use in warfare[132]) and carbamates are potent acetylcholinesterase inhibitors (resulting in excessive acetylcholine levels in the synaptic clefts). Solvents, some of which were originally used as anesthetics, dampen the propagation and transmission of electrical impulses along the nerve axons. All of these agents produce various forms of toxic encephalopathy (acute or chronic, selective, or diffuse toxic encephalopathies). Many environmentally ill patients present to their physicians with chief complaints that fit this diagnostic category.

Other common pollutants are indirect neurotoxins and affect the neurological system adversely by causing neuroinflammation, resulting in cognitive decline, mood imbalances, pain disorders, and chronic neurological diseases.

Pesticide Neurotoxicity

Organophosphates. The average clinician will rarely see an individual with acute organophosphate poisoning. Instead, they will see persons presenting with depression, headache, fatigue, cognitive issues, and other problems that could tend to be attributed to other issues of aging. The following OP worker studies highlight these problems.

Greenhouse workers who were exposed to OPs exhibited higher incidences of depression, headache, tremors, and paresthesias.[133] Polish female greenhouse workers exposed to OPs exhibited longer reaction time and reduced motor steadiness than unexposed workers. They also reported increased tension, depression, and fatigue more often than controls.[134] Dutch farmers and gardeners who used OPs frequently had a much higher risk of developing mild cognitive dysfunction than others.[135] Farmers repeatedly exposed to OPs from sheep dip showed much greater vulnerability to psychiatric disorders than controls (quarry workers). They also performed worse than controls on cognitive testing that assessed attention span and how fast they processed information.[136] None of the persons in any of these studies fit the definition of acute OP poisoning as set by the field of toxicology, including no evidence of low acetylcholinesterase levels.

A study of persons previously poisoned by OPs revealed that OP-induced neurotoxicity can persist after the incident, manifesting as problems with memory, abstraction, intellectual functioning, mood, and motor reflexes. They also had greater distress and complaints of disability.[137]

Chlorinated pesticides. The use of chlorinated pesticides was almost completely banned in the United States by the 1980s, and thus the symptomatic picture of acute organochlorine poisoning is rarely seen. Instead, low-level organochlorine neurotoxicity presents with generalized neurological dysfunction very similar to what is found with OPs.

Early controlled trials of airborne exposure to low levels of DDT revealed that exposed subjects would experience neurological symptoms, including dimming of vision, a drawing sensation at the base of the nose or behind the eyes, a sense of fullness deep inside the skull, headache, slowness of thought, inability to concentrate, and short-term memory loss.[138] Various muscle symptoms also occurred, including weakness, fatigue, dysphagia, and ataxia.

A study of retired malaria control workers and non-DDT-exposed controls revealed that the exposed group had significantly poorer performance on cognitive, sensory, and motor testing that persisted long after exposure ended.[139] They did particularly poorly on the cognitive testing (verbal attention, visuomotor speed, and sequencing) and reported significantly more psychiatric symptoms than controls reported. Additionally, children who were exposed to "background, low-level concentrations" of DDT while in utero revealed decreases of up to 7.86 (standard error, 3.21) points in the verbal scale and 10.86 (standard error, 4.33) points in the memory scale compared with children whose concentrations were <0.05 ng/mL.[140]

The neurological effects of chlordane exposure were studied in a group of persons who lived in an apartment complex where chlordane was used. Seven years after the application occurred, residents and former residents of the complex were assessed with neuropsychiatric testing.[141] Significant changes were found in the exposed persons, including reduced reaction time; balance dysfunction (shown by increased sway speed); and reductions in cognitive function, perceptual motor speed, and immediate and delayed verbal recall. They also had worse scores for mood, including increased tension, depression, anger, and fatigue.

Solvents. Chronic toxic encephalopathy (CTE) from solvent exposure will often gradually improve in 50% of cases with the elimination of solvent exposure (Box 35.2). However, 50% will not improve from mere avoidance in the time frame of 6 to 42 months after initial assessment, far longer than the clearance of solvents would suggest. It is interesting to note that in one study, persons on

BOX 35.2 Chronic Toxic Encephalopathy: Designations of Solvent-Induced Neurotoxicity

1. Affective syndrome: neuropsychiatric symptoms, no signs of impairment, reversible
2. Mild chronic toxic encephalopathy: neuropsychiatric symptoms, proven impairments, uncertain reversibility
3. Severe chronic toxic encephalopathy: more severe neuropsychiatric symptoms, more pronounced impairments, usually irreversible

Data from World Health Organization meeting on Organic Solvents, Copenhagen, Denmark, 1985.

antidepressants were nearly four times more likely to have persistent solvent-induced CTE than those not on the medications.[142] Although the study failed to list which antidepressants were used, many of the commonly used antidepressant medications are known inhibitors of the CYP system in the liver, thereby prolonging the half-life of xenobiotics. Workers with a genetic polymorphism affecting glutathione conjugation also had a dramatically increased risk for the development of CTE.[143]

White-collar employees with low-level toluene exposure have exhibited significant deficiencies in continuous performances tests and tests measuring processing speed and memory.[144] Additionally, shipyard painters were found to have significantly higher scores for neurotic behaviors than controls.[145] They were also found to have significantly greater problems with short-term memory, concentration, fatigue, dizziness, and insomnia and noted more trouble with a feeling of pressure in the chest and perspiration without work. One of the questions most frequently answered affirmatively by the group (in significantly higher levels than controls) was, "Do you often have to go back to check things that you have done, such as turned off the stove, locked the door, etc.?"[146]

Female workers exposed to toluene showed significantly more problems with manual dexterity, visual scanning, and verbal memory.[147] The authors of this study noted that the workers who exhibited these changes on neurobehavioral testing showed absolutely no clinical signs of toxicity!

Heavy Metal Neurotoxicity

Lead. Lead is a well-recognized neurotoxin that causes multiple problems in exposed children, including reduced IQ scores[148] and attention and behavioral problems.[149] Parents of children with lead burdens have reported that their children experienced more somatic complaints and delinquent, aggressive, internalizing, and externalizing behavior.[150] Their teachers have reported that the children had more problems with anxious/depressed behavior; social problems; attention problems; and delinquent, aggressive, internalizing, and externalizing behavior. Such findings contributed to the removal of lead from gasoline and paint in the United States in 1978.

When persons who were exposed to lead in childhood were studied 20 years later, neurological deficits were still found. In this group, significant adverse central and peripheral neurological effects were present. Peripheral nerve function was altered, as were measures of coordination, reaction time, dexterity, learning, and mood.[151] When bone lead burden was measured in older individuals, none of whom had industrial exposures to lead, a clear association was correlated with mental function. As the total bone lead (measured by fluoroscopy of the tibia) increased, cognitive function decreased in a group of older individuals from Baltimore.[152] The cumulative lifetime lead burden has been associated with increased risk for the development of parkinsonism.[153,154]

Even though blood lead levels (BLLs) have been steadily dropping in the past few decades, these lower lead levels are still associated with neurological deficits in children, leading some to propose that there is not a safe BLL. Approximately 7.4 IQ points were lost in children with a BLL of <10 ug/dL versus 4.6 IQ points in those with a per-unit increase in BLL of >10 ug/dL.[155] The IQ losses in children with a BLL of <10 ug/dL were confirmed in a subsequent study of 6-year-old children[156] and in children in seven international population studies.[157] IQ loss with BLLs above 1.71 ug/dL has also been found in a study of Italian adolescents. In this study, a doubling of the BLL equated to a 2.4-pt reduction in IQ. The researchers stated that for each 0.19-ug/dL increase in BLL, 1 IQ point was lost.[158] In a North Carolina study, BLLs as low as 2 ug/dL were associated with poorer performance in the classroom for school-aged children.[159]

Mercury-Induced Neurotoxicity. Mercury in both organic and elemental forms is neurotoxic, and methylmercury (organic) is the most well-known neurotoxin. The widespread pollution of Minamata Bay, Japan, by methylmercury in the 1950s provided researchers with a clear picture of methylmercury poisoning through daily fish consumption. Known as Minamata disease, the neurotoxic signs included ataxia, speech impairment, constriction of visual fields, hypoesthesia, dysarthria, hearing impairment, and sensory disturbances. These neurological problems persisted and were found in other areas of Japan as the mercury contamination spread through fish consumption.[160] Follow-up studies in the Minamata area almost 40 years after the spill and almost 30 years since a fishing ban was enacted for the area showed the persistence of mercury neurotoxicity. Male residents in fishing villages in the area in 1995 reported significantly higher prevalence than town-resident controls for the following complaints: stiffness, dysesthesia, hand tremor, dizziness, loss of pain sensation, cramping, atrophy of the upper arm musculature, arthralgia, insomnia, and lumbago. Female residents of the fishing villages had significantly higher incidents of complaints of leg tremor, tinnitus, loss of touch sensation, leg muscular atrophy, and muscular weakness.[161]

Fish-eating Amazonian children with hair mercury levels above 10 mcg/g showed deficits in neuropsychological tests for motor function, attention, and visuospatial performance that correlated with their hair mercury concentrations.[162] Patients in an internal medicine practice in San Francisco who consumed large fish regularly and had mercury blood levels above 5 mcg/L were most likely to present with fatigue, hair loss, trouble thinking, memory loss, muscle aches, headaches, and a metallic taste in the mouth.[163]

The majority of individuals who had their amalgam fillings removed noted psychological improvements, including a reduction in anger outbursts, depression, irritability, and fatigue.[164]

Indirect Neurotoxicity—Neuroinflammation

All of the environmental pollutants have demonstrated powerful prooxidant activity; in addition, they deplete the levels of reduced glutathione in the brain tissue and inhibit the function of the antioxidant enzymes. Neurons and their supporting glial cells are directly affected by this oxidative stress and diminished antioxidant protective ability. Oxidative stress activates the glial cells, leading to increased production and release of the proinflammatory chemicals interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), IFN-γ, and TNF-α.

The resulting neuroinflammation is a key component in the pathobiology of headaches and chronic pain,[165] cognitive decline,[166,167] mood disorders,[168] traumatic brain injury,[169] and neurodegenerative diseases such as Alzheimer's disease,[170] attention deficit hyperactivity disorder (ADHD),[171] amyotrophic lateral sclerosis (ALS),[172] autism,[173]

multiple sclerosis,[174] bipolarity,[175] seizures,[176] and parkinsonism.[177] Neuroinflammation has been shown to be present in parkinsonism and appears to be responsible for all the non–dopamine-depletion-related manifestations.[178]

Neuroinflammation is triggered by traumatic brain injury,[179] endotoxicity (circulating levels of lipopolysaccharides from gram-negative bacterial cell walls),[180] elevated blood sugar (glycation end products),[181] stress,[182] and a host of environmental toxicants such as air pollutants,[183] heavy metals,[184] and OPs.[185] Vehicular exhaust produces particulate matter in a range of sizes, all of which carry a variety of polycyclic aromatic hydrocarbons.

Endocrine Toxicity

The most common endocrine diagnoses associated with xenobiotic burden include the following:

1. Infertility (both male and female)
2. Low testosterone in males less than 55 years of age
3. Hypothyroidism (especially autoimmune)
4. Adult-onset diabetes
5. Obesity

Infertility

Herbicides and organophosphate pesticides. Ontario women who used pesticides were 3.8-fold more likely to be infertile.[186] Five compounds reduced females' fecundity rates to between 24% and 49%: dicamba (0.51), glyphosate (0.61), 2,4-D (0.71), organophosphates (0.75), and thiocarbamates (0.76). Spanish greenhouse sprayers exposed to OPs had higher rates of spontaneous abortion rates, along with higher rates of depression and headaches.[187] Although these health problems were clearly evident, no significant decrease in erythrocyte acetylcholinesterase was found. This reproduced the findings of other studies showing apparent adverse neurological organophosphate health effects without markers of acute OP toxicity. A study in California farming counties showed a clear association with pesticide spraying and fetal death due to congenital anomalies.[188] In this study, when an OP or carbamate pesticide was sprayed in one of 8 adjacent square miles of women's residences during the third to eighth week of pregnancy, the OR of fetal death was 1.4. When the spraying occurred within 1 square mile of a residence, the OR increased to 2.2.

Chlorinated pesticides and polychlorinated biphenyls. Hexachlorocyclohexane (HCH) serum levels were significantly associated with miscarriage history and uterine fibroid presence among patients in a German reproductive clinic.[189] Higher HCH and PCB levels were also noted more frequently in women with antithyroid and antinuclear antibodies. PCB exposure and elevated antithyroid antibodies have also been reported elsewhere.[190] PCB serum levels were significantly associated with endometriosis, and increasing DDT levels with reduced conception. Pentachlorophenol (PCP) levels were also found to be associated with increased miscarriage rates and levels of ANA.

A noncontrolled study in Canada looked for the presence of certain xenobiotics in the serum and follicular fluid from infertile females and seminal plasma from their male partners.[191] These 18 couples all attended an in vitro fertilization program in hopes of becoming parents. The researchers found DDE, mirex, HCH, trichlorobenzene, and three different PCBs in more than 50% of all follicular fluid samples. Four different PCBs, DDE, and endosulfan were also found in over 50% of all serum samples. DDE was the most frequently found contaminant, had the highest residue, and was negatively associated with fertilization. Of the couples tested, those who failed to achieve pregnancy with in vitro fertilization methods generally had higher toxic levels than successful couples. It is unfortunate that these authors did not get a matched control group of fertile couples to compare the toxic burden.

In the same Canadian study, women with higher DDE levels had greater trouble conceiving. This finding was also noted in the previously mentioned German study and was recently redocumented as a generational effect as well in a group of women from Oakland, California.[192] In this study, serum levels of DDT/DDE were measured from maternal samples at the time of birth. The time it took their daughters to conceive was then followed for 28 to 31 years. Differing generational effects were found for both DDT and DDE. Maternal levels of DDT were associated with reduced fecundity (32% less) in their daughters, whereas DDE levels were associated with increased fecundity (16% greater).

Solvents. Reduced fecundity has also been shown in female toluene workers (fecundity rate [FR] 0.47) and in laboratory workers exposed to organic solvents (FR 0.79).[193,194] Wives of men who worked around organic solvents also showed decreased fecundity (FR 0.36).[195] Occupational exposure to organic solvents is associated with both longer time to pregnancy and increased risk for spontaneous abortion once conception occurs.[196] Chinese female chemical plant workers exposed to organic solvents experienced a very high risk for spontaneous abortions (OR 2.9).[197] Of the myriad of petrochemicals they were exposed to, benzene (OR 2.5), gasoline (OR 1.8), and hydrogen sulfide (OR 2.3) all showed independent significant association with miscarriages. Solvent-exposed pharmaceutical factory workers also showed increased spontaneous abortion risk from multiple solvent exposures that increased along with the number of solvents they were exposed to.[198]

Air pollutants. Smoking more than 10 cigarettes daily has been associated with reduced fertility in women (FR 0.66), with the OR for infertility being 1.64.[199] Because tobacco smoke contains high benzene and styrene levels, along with heavy metals and polycyclic aromatic hydrocarbons, it is difficult to say which xenobiotic would have the greatest effect on fertility. Female smokers also experience higher rates of ectopic pregnancies and spontaneous abortions,[200] along with greater rates of stillbirths (OR 2.0) and infant mortality (OR 1.8).[201] Mothers who quit smoking in the first trimester showed the same rates of stillbirth and infant mortality as nonsmoking women. When Ireland banned smoking in the workplace, rates of preterm births dropped by 25%.[202]

Women living closest to busy roadways have slightly higher rates of infertility than those living further away.[203,204] Brief exposure to elevated levels of PM_{10} has been shown to increase the rates of miscarriage by up to 2.6 times.[205] Mothers exposed to higher ambient air pollution were found to have significantly higher levels of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in the white blood cells of their cord blood.[206] The PAH-DNA adducts were also significantly higher in infertile men than in fertile men.

Hypothyroidism

PCBs have been closely studied for their possible adverse effects on thyroid hormonal status and function. Both PCBs and thyroxine are made of two connected benzene rings with attached halogens, with PCBs having chlorine molecules and thyroxine having iodine. Interestingly, the common human contaminant BPA is also structurally similar, with two bromine molecules on each of the rings. PCBs have demonstrated in vitro activity in binding transthyretin, a transport protein mechanism for thyroxine.[207] POPs also appear to adversely affect the thyroid hormone–metabolizing enzymes (uridine-diphosphate-glucuronyltransferases, iodothyronine deiodinases, and sulfotransferases) found in the liver and brain.[208]

Elevated levels of both antiperoxidase and antithyroglobulin were found in workers in a PCB manufacturing plant.[209] This is reflective of the earlier noted study,[188] in which PCBs and HCH were frequently found in infertile German women with antithyroid and antinuclear antibodies. Surprisingly, no difference was found in the levels of T4 and thyroid-stimulating hormone (TSH) in these 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) workers versus the control group.

PCB serum levels of consumers of fish from the Baltic Sea were found to have a significant inverse correlation between PCB levels and TT3, along with a nonstatistically significant inverse correlation with TT4.[210] German children showed a significant positive correlation between PCB serum levels and elevation of TSH,[211] and they also exhibited a significant inverse correlation between serum PCB levels and FT4, as one would expect with increasing TSH levels.

Chlorinated pesticides have also been associated with hypothyroidism. A study of Indian women looked for any association between DDT and dieldrin in women with elevated TSH and depleted T4. DDT was found to be higher in all of the hypothyroid women, although not significantly, but dieldrin was significantly associated with low T4 levels.[212]

Adult-Onset Diabetes

The incidence of adult-onset diabetes has risen in the past decade at rates much higher than previously recorded.[213] Although type 2 diabetes mellitus (T2DM) has long been associated with a variety of lifestyle choices, new information is powerfully pointing to a number of common pollutants as major risk factors.[214] Inorganic arsenic, naturally present in groundwater throughout the world, has been clearly linked to the development of T2DM, whereas arsenic from food-based sources has not.[215,216] The presence of persistent organic pollutants has also been linked to the development of diabetes. The NHANES participants with the lowest levels of PCB 153 in their blood were 2.5 times more likely to develop diabetes than persons without PCB 153, whereas those individuals in the highest quartiles of this toxicant were 6.8 times more likely to have this disorder.[217] The termiticides transnonachlor and oxychlordane each increased the risk of T2DM from 2.7-fold, for those with the lowest blood levels of these chlordane residues, to 11.8- and 6.5-fold, respectively, for those with the highest blood levels. When the sums of all six bioaccumulative toxins were combined, the odds of developing diabetes went from a low of fourteenfold to a high of 37.7-fold for those with the greatest fat-soluble burden. Evidence of the increased risk for T2DM from a variety of POPs continues to mount.[218,219]

Not all persons are living in areas with higher groundwater arsenic, and exposure to POPs is not as common as it was several decades ago. But all persons are exposed to various levels of air pollution, which is strongly associated with the development of both obesity and diabetes. Ambient nitrogen dioxide has been positively linked to reduced B-cell function and reduced insulin sensitivity in Latino youth living in Los Angeles, in whom it was also found to contribute to increased weight.[220] Additionally, ambient levels of $PM_{2.5}$ have been positively associated with the incidence of T2DM.[221,222]

Urinary phthalate and BPA levels have also been associated with increased diabetes risk, although not all BPA studies demonstrate a significant risk.[223–226] Urinary phthalate levels are also positively associated with increased weight and waist circumference.[227,228]

ASSESSMENT

History

A comprehensive history is the cornerstone of detecting xenobiotic-induced health problems. When taking a history, attention should be paid to both occupational and nonoccupational chemical exposures. Occupational exposures can be the ones the client is most aware of and should always be followed up with a request for the Material Safety Data Sheet on each chemical from the employer. Nonoccupational exposures are harder to document, but in a family practice they are often found to be the main culprit. Specific questions should be asked about the history of residences: where the patient lived, how new the structure is, age of carpeting, time of remodel, type of heating, use of indoor or outdoor pesticides, attached garage, and so forth. The presence of environmentally induced illnesses will often become very clear when the clinician compares the chronological history of all the client's health complaints with the client's residential and occupational exposures.

However, because of the ubiquitous nature of the chemicals in question, a history may not always give a clear picture of which chemical caused the illness in question.

Laboratory Tests

Laboratory tests may be helpful in cases where the history is not. A standard complete blood count and general blood chemistry test is usually nonremarkable in cases of chronic low-level exposures (although Crinnion often finds leukopenia present in his population of toxic patients). In his patient population, the author (Crinnion) has rarely found elevated levels of liver enzymes, even when solvents and pesticides are present in the serum. This has also been reported in the literature with persons occupationally exposed to solvents.[229]

Serum tests for the most commonly found PCBs, chlorinated pesticides, and aromatic solvents are currently available. Urine assays for the metabolites of organophosphate pesticides, glyphosate (from Roundup), low- and high-molecular-weight phthalates, and other common chemical pollutants are also currently available to the clinician. The publication of the CDC's national reports provides national reference values for many of these compounds that the clinician can use when interpreting the laboratory reports. For the biologically persistent PCBs and chlorinated pesticides, the CDC gives levels both in parts per billion and as lipid-adjusted (nanogram per gram lipid) values. These fat-soluble toxins are released from adipose tissue along with triglycerides and cholesterol.[230] Previously published research actually found that quantifying the fat-soluble xenobiotics according to the amount of lipids in the blood corresponded well with the concentration of xenobiotics present in adipose biopsies of the same person.[231] These findings are the reason why the CDC presented lipid-adjusted levels for these specific POPs. Lipid-adjusted values offer a glimpse of the POP adipose burden and allow the clinician an opportunity to monitor the effectiveness of depuration treatments.

Current OP exposure can be identified by the presence of OP metabolites in the urine. Similar testing can be done for phthalate (plasticizers) metabolites, BPA, and parabens. All of these compounds are nonpersistent, and their presence indicates regular, ongoing exposure. These tests can be highly useful in helping people attain a diet and lifestyle that are free from several serious toxic families.

Heavy metals can be measured in the hair, blood, urine, and stool. Blood levels are mostly indicative of current exposure levels and are still considered by many physicians to be the only valid method of heavy-metal assessment. Unfortunately, the blood reference values for heavy metals are typically based on industrial medicine standards for toxicity in the workplace. Fortunately, the CDC national reports again provide the national normal ranges for most of the heavy metals in blood and in urine (given as microgram per liter and microgram per gram creatinine). A nonchallenged random or first-morning urine test will provide information on total body cadmium levels and current exposures to mercury, lead, and arsenic.[232,233]

An estimation of the body burden of mercury and lead can be derived from a metal-mobilization test (MMT). MMTs use urine metal assessment before and after the administration of a metal-mobilizing agent. The total amount of metal in the pre- and postchallenge urine samples needs to be calculated as part of this method (ug/sample). Intravenous calcium edetate (CaEDTA) and oral 2,3-dimercaptosuccinic acid (DMSA) have both been used for lead-mobilization testing.[234–238] DMPS has been used for mercury-mobilization testing, and DMSA can also be used.[239]

TREATMENT

Effective treatment for chemically induced illnesses begins with avoidance of further exposures, which is the major step for all nonpersistent pollutants. Supplementation to support xenobiotic elimination and antioxidant protection is always beneficial. For persistent compounds, depuration techniques are necessary to reduce their body burden.

Avoidance of Further Chemical Exposure

This step is critical, although it is often the most difficult step in terms of compliance. In cases of multiple chemical sensitivities, simply having a chemically safe dwelling can bring improvement.[240] Compounds to be avoided include solvents, paints, exhaust fumes, perfumes, hair sprays, new furniture, carpeting, cabinetry, plastics, and gas or oil heat, among others. Because the home is the environment that is most in one's control, the home must be made a "safe oasis." If there are building materials that are off-gassing chemicals and these materials cannot be removed, they should be sealed. There are a number of good books available to help with these issues.[241]

The single greatest source of solvent exposure in the home comes from smoking inside, which also provides combustion by-products, cancer-causing chemicals, and heavy metals. After smoking, the next greatest sources are carpeting, new cabinetry, and new paint. Cleaning supplies, including tile cleaners, perfumes, air fresheners, and the presence of dry-cleaned clothing, complete the rest of the top indoor polluters. Exposure to all of these can be reduced with simple lifestyle choices, such as the following:

- Do not wear shoes indoors.
- Replace furnace filters every 6 weeks with high-quality pleated filters (rated minimum efficiency reporting value [MERV] 7–9).
- Air out dry-cleaned clothing in the garage or car trunk for a week before bringing it into the house.
- Do not smoke indoors.
- Consider replacing carpet with tile or stone flooring.
- Use nonscented laundry detergent and fabric softener.
- Consider getting an air purifier that has both charcoal and high-efficiency particulate air (HEPA) filters (e.g., IQAir and Blue Air). Make sure to get one with enough cubic feet of air purified each minute to clear the air in the bedroom at least once every 30 minutes.

In addition to avoiding environmental chemicals, any foods that cause adverse reactions should be avoided (see Chapter 14 on food hypersensitivities). Organic foods should be chosen wherever possible, as well as purified water. Avoiding the "dirty dozen" fruits and vegetables (www.foodnews.org), along with all farmed salmon, high-mercury fish, and nonorganic dairy products, will significantly reduce toxin exposures through the diet.

Dietary Support

The best macrodiet choice (in addition to organic foods) is to consume a high-protein, low-carbohydrate, and low-fat diet. Such a diet helps Phase I biotransformation significantly.

Protein

The metabolism of toxic chemicals and drugs has been shown to be impaired by protein deprivation. Increased toxicity of chemical compounds and drugs has been reported with protein deficiency. Protein deficiency decreases the activity of liver mixed-function oxidase (MFO) systems, which increases the half-life of numerous toxic chemicals and drugs and potentiates drug action and toxicity. The quantity and quality of protein in the diet alters both Phase I and Phase II reactions in drug metabolism (a gelatin diet induces very low MFO activity). The toxicity of organochlorinated compounds (OCCs), acetylcholinesterase inhibitors, herbicides, and fungicides have all been shown to be increased severalfold by protein deficiency. Reduced clearance of antipyrine and increased half-life of antipyrine have been found in Asian vegetarians with low dietary protein intake. This was not found in white vegetarians with adequate protein intake.

Although protein deficiency clearly reduces the ability of the body to adequately metabolize chemicals,[242] the opposite also appears to be true. Isocalorically increasing the ratio of dietary protein to carbohydrate ratio in well-nourished volunteers was shown to enhance the clearance of antipyrine and theophylline. Although it is not clear if the effect of the protein was due to the amino acid content alone, it is known that methionine and cysteine deficiency led to a reduction of intestinal and hepatic Phase I enzyme activity. Hepatic Phase I activity can also be suppressed by folic acid and choline deficiency. Low methionine intake also affects selenium metabolism by making less selenium available for glutathione-peroxidase biosynthesis. High sugar intake is also known to reduce the clearance of certain chemicals from the liver.

Fiber

Rice bran fiber (RBF), which can be found in a diet high in brown rice or using RBF as a fiber supplement, has been shown to have a high binding ability for PCBs and other toxins, including the combustion by-product benzo(a)pyrene, in a laboratory setting.[243] When measured against other fibers, RBF demonstrated the ability to dramatically reduce the reabsorption (termed "hepatic recycling") of PCBs from the intestines in animals.[244] Hepatic recycling occurs after the liver dumps some of these toxins into the intestines with bile, but when these compounds make it to the intestine, they are reabsorbed into the bloodstream and sent back to the liver—a process facilitated by low fiber and deconjugating bacteria in the gut. This recycling pattern is the main reason why such a minute amount of these toxicants actually makes it out of the body. Although RBF helps break this recycling and gets more toxic xenobiotics to leave, wheat bran showed absolutely no benefit in this regard.[245] A study using either spinach fiber or RBF in PCB-exposed animals showed that RBF increased fecal PCB excretion by 6.6 times and spinach fiber by 4.1 times.[246] In another animal study, 10% RBF in the diet increased the excretion of toxic furans by 4.5 times that of placebo.[247] A study of PCB-burdened patients who consumed 7 to 10 g of a fermented RBF product (which is available as a food item in Japan) three times daily (after each meal) for a year found that they had twice the amount of dioxin excretion as controls.[248] While bran fiber has shown many benefits, as much of rice is contaminated with arsenic. The prescriber must ensure the recommended product is not contaminated.

Chlorophyll, Seaweed, Chlorella

Chlorophyll has long been thought of as a blood purifier, and recent studies have documented its effectiveness at helping clear POPs, but not toxic metals, from the body. As previously mentioned, both

chlorophyll-containing spinach fiber and matcha green tea increase the excretion of POPs. The seaweed nori, which also contains chlorophyll, was tested in rats to measure its power in helping clear dioxins. Rats fed a diet of 10% nori had an increase in fecal excretion of two different dioxins at levels 5.5 and 6.0 times more than the control group.[249] Chlorella, long a popular "detoxification" agent, was also tried with dioxin-contaminated rats. The group of rats given chlorella had increased dioxin excretion that varied from 30% to over 300% higher than the control group.[250] Postulating that it was the chlorophyll content of the chlorella that was the active ingredient, this group of researchers then set out to measure that hypothesis.

The effectiveness of chlorophyll alone on the excretion of dioxins and furans from rats was quickly established. The higher the content of chlorophyll in the diet, the greater the excretion of these persistent fat-soluble toxins in the feces. The ranges of chlorophyll went from a low of 0.1% to a high of 0.5% in the diet. A diet with 0.1% chlorophyll is roughly equivalent to consuming 10% of your diet as spinach or 20% as seaweed. In the 0.1% group, the fecal excretion of the various toxins ranged from 40% to 80% greater than that of the control group. At the end of the study, all of the animals that were given chlorophyll had a lower total body burden of these persistent toxins than their counterparts.[250]

Similar results were found with chlorophyll-containing vegetables. When POP-burdened rats were fed a diet with or without 10% dioxin and furan, elimination occurred in proportion to the chlorophyll content of the vegetables.[251] The vegetables with the smallest increase in fecal dioxin excretion (60% to 300% increase) were Chinese cabbage, broccoli, onion, Welsh onion, cabbage, and celery. Kale, Chinese chive, Shungiku, Chingensai, green lettuce, and sweet peppers increased dioxin excretion by 330% to 480%. Topping the charts with an increased dioxin excretion of 760% to 1160% were Komatsuna, mitsuba, spinach, and perilla.

Nutritional Supplementation

In cases of toxic overload, supplementation can be tricky because some persons with severe chemical reactivity can react to many of the nutrients that are normally needed. In his four-volume treatise *Chemical Sensitivity,* Rea stated that his chemically burdened patients were typically deficient in magnesium, selenium, and vitamin B_6 (whether or not they were taking oral supplements of these).[3,4] Supplementation with high levels of antioxidants is typically recommended because all of these xenobiotics have been shown to cause oxidative damage.

Depuration (Cleansing)

The removal of impurities from the body is known as depuration. Colonic irrigations and sauna have been used for depuration in addition to the use of RBF and chlorophyll.

The earliest articles on the use of saunas for depuration came from clinics associated with the Church of Scientology, using the "Hubbard Purification Rundown." Designed by L. Ron Hubbard, the protocol uses exercise, high-temperature saunas, increasing doses of niacin, and electrolyte replacement.[252] This protocol has been used to reduce levels of PCBs, polybrominated biphenyls (PBBs), and hexachlorobenzene.[253] More recently, Genuis et al. have published studies on the effectiveness of sweating for depuration. A small group of patients with chronic illness along with an equal number of healthy controls provided serum, urine, and sweat samples. Far-infrared (FIR) saunas were used by 10, standard Finnish steam saunas by 7, and exercise alone by 3. Both BPA and phthalates were documented in the sweat and urine from individuals in the study who used either FIR or Finnish saunas.[254,255] BPA and di(2-ethylhexyl)phthalate (DEHP) were both found to be present in the sweat of individuals who did not have these toxicants present in their serum, indicating that both have some degree of storage in the subcutaneous fat pads. Mono-(2-ethylhexyl) phthalate (MEHP) was found in the sweat at concentrations double that in the urine. Five polybrominated diphenyl ether (PBDE) flame retardants found in the serum of these individuals were also mobilized into their sweat with sauna use.[256] As with both BPA and phthalates, some PBDE cogeners were present in the sweat while not being present in the serum.

A comprehensive naturopathic protocol of diet, supplements, hydrotherapy, colonic irrigations, and sauna for treating environmentally poisoned individuals was developed by the author (Crinnion) and has been in use for more than 20 years. An outcome study on persons using this protocol for a variety of chemically induced ills revealed it to be surprisingly effective. Of all the various problems treated with the depuration protocol, 83% of the participants rated their results as good or great. The two conditions in which 100% of the participants reported great results were asthma ($n = 3$), and addiction recovery ($n = 1$). There were several problem (chief complaint) categories in which 100% of the participants rated their results as moderate/good or great. Those categories were autoimmune, dermatological, gastrointestinal, and/or liver. The categories with the next highest ratings of moderate/good and great were fatigue, with 92% improvement; allergies, with 85% improvement; and chemical sensitivities, with 84% improvement.[257]

The Crinnion Depuration Protocol

The Crinnion Depuration Protocol consists of the following components:

1. **Daily exercise.** Exercise usually consists of using an exercycle, rebounder, or brisk walking to begin lipolysis and diaphoresis.
2. **Thermal chambers.** Up to three 60-minute "sauna" sessions, with temperatures at a range of 120°F to 135°F, followed by 10- to 15-minute cool-down periods. Glass-bottled spring water was given along with electrolyte replacement. Although the Hubbard clinics use higher temperatures, it was observed that lower temperatures reduced the frequency of adverse effects and increased the release of chemicals in the sweat.
3. **Constitutional hydrotherapy.** This uses alternating hot and cold towels with sine-wave stimulation as done by O. G. Carrol, ND, and Harold Dick, ND.[258] This therapy has been used for decades to stimulate the body's own self-healing activity. We found that it also increased the amount of toxin-laden bile dumped from the liver into the intestines. Also assisting the choleretic and cholagogue action on the liver is an herbal capsule taken daily consisting of *Chelidonium, Chionanthus, Taraxacum, Arctium lappa, Silybum* mar., and *Urtica dioca.*
4. **Colonic irrigation.** Gravity-fed machines are used to gently introduce triple-filtered water into the large intestines, providing an avenue for toxic bile to rapidly leave the body. Individuals will routinely have "liver dumps" of bile that range in color from yellow to red, with occasional gray or brown. The color of bile normally ranges from green to yellow to orange to red, depending on the amount of time of exposure to bacterial action in the bowel. However, in this situation, we believe that it is dumped from the liver and rapidly passed through the small intestines, similar to what is seen in "gastric dumping" syndrome. We are therefore unable to account for the differences in the color of this effluent. In some patients with heavy agricultural exposure, we have seen higher amounts of fluorescent yellow bile. Hence, the color of the bile may be more attributable to the chemical compounds in the bile than the bacterial action upon the bile. We have documented that chlorinated pesticides are present in the effluent, which we refer to as "bile dumps."
5. **Constitutional homeopathy.** This has been used primarily as a stand-alone treatment, with the use of any other supplemental and treatments prohibited. However, we have found it to be a valuable component of this protocol and completely

compatible with all the other treatment methods involved. It appears most beneficial in those individuals who need a boost for their vital force to get them moving toward healing and for those who are stuck in emotional issues that they have not been willing to look at.

6. **Body therapies (massage, Shiatsu, craniosacral, visceral, chiropractic).** These are done as needed for the individual to treat specific musculoskeletal problems and to assist in mobilizing toxins that are stored in the tissues.
7. **Counseling.** Mental and emotional toxins are as big of a problem as physical and/or chemical toxicants. When people are exposed to powerful emotional toxins (abuse, etc.) that they have no outlet for handling, they end up "stuffing" the emotional toxins. When this emotional stuffing occurs, any physical toxins that they are exposed to at that time are also stuffed (stored) rather than being eliminated. Because of this, when they start to mobilize the physical toxins, the old emotional issues will come back into consciousness. When individuals choose to again suppress the emotional issues (rather than facing them), their physical cleansing will also stop. This is evidenced by the observations that they will stop sweating in the thermal chambers, stop heating the cold towels in the hydrotherapy, and stop having good liver releases in the colonic therapy. When the emotional "toxins" are faced and released, then these cleansing parameters are returned to former levels. Assisting all of the individuals going through this depuration protocol with their emotional issues helps them cleanse physically.

This protocol is run on a daily basis that usually lasts between 4 and 8 weeks (five sessions weekly). This protocol begins the depuration process, which then must be continued once the individual returns home. Most individuals will need to continue regular cleansing (use of home saunas, hydrotherapy, and colon therapy) for at least 12 months after completing their intensive in-office cleansing. When testing for serum levels of chlorinated pesticides, PCBs, and solvents is repeated along with immune parameters after 12 months, improvements are routinely seen. In addition to symptomatic relief, improvements in the laboratory reports (decrease in serum xenobiotics and reduction in autoantibodies, etc.) are clearly evident.

SUMMARY

Environmental chemicals and toxic metals are ubiquitous in our environment and in all humans at varying levels. Damage by these xenobiotics can be found in virtually every cell, tissue, and organ system in the human body. The most susceptible systems appear to be the immune, neurological, and hormonal systems. Many of the chronic health problems caused by toxic xenobiotics in these systems often present as classic degenerations that are considered to be the result of aging. Classic immunotoxicity manifestations include the development of allergies and asthma, chemical reactivity, chronic infections, and autoimmunity. Neurotoxicity typically presents with decreased cognition, headache, fatigue, short-term memory loss, and mood disorders. Endocrine toxicity can result in hypothyroidism, obesity, infertility, and diabetes.

Exposure to many of the ubiquitous xenobiotic toxicants can be easily eliminated by simple lifestyle choices (Box 33.3), although the burden of fat-stored pollutants can be reduced by depuration techniques that often can be handled through dietary changes. The diagnosis of xenobiotic-induced damage can be elucidated by a comprehensive history, along with the presence of pollutant-specific biomarkers.

BOX 35.3 Treatment

Avoiding toxic exposures is mostly in one's control.

Diet and Home Air:

1. Avoid the 12 most toxic fruits and vegetables (peaches, apples, bell peppers, celery, blueberries, kale, nectarines, strawberries, cherries, pears, imported grapes, spinach, lettuce, potatoes); use organic varieties of these instead.
2. Freely eat all of the 15 least toxic fruits and vegetables (onions, avocado, sweet corn, pineapples, mango, asparagus, sweet peas, kiwi fruit, cabbage, eggplant, cantaloupe, watermelon, grapefruit, sweet potato, honeydew melon).
3. Do not eat any farmed or Atlantic salmon.
4. Freely eat Alaskan salmon (available fresh only from June until October; canned and frozen Alaskan salmon available year-round).
   a. Alaskan/Pacific salmon will always be labeled as King (Chinook), Red (Sockeye), or Silver (Coho) Salmon. If that distinction is not given, it is farmed salmon being passed off as Alaskan.
5. Avoid the fish with the highest mercury content (shark, swordfish, king mackerel, tuna, orange roughy, marlin, Chilean bass, lobster, halibut, snapper).
6. Freely eat the fish with the lowest mercury content (clam, ocean perch, Alaskan salmon, tilapia, flounder, sole, catfish).
7. Avoid all sugar—sugar reduces the ability of the liver to clear toxic compounds out of the bloodstream.
8. Begin to reduce the toxicity of the air inside the home:
   a. Don't wear shoes indoors.
   b. Replace the furnace filters every 6 weeks with high-quality pleated filters (rated minimum efficiency reporting value [MERV] 7–9).
   c. Air out dry-cleaned clothing in the garage or car trunk for a week before bringing it into the house.
   d. Do not smoke indoors.
   e. Consider replacing the carpet with tile or stone flooring.
   f. Use nonscented laundry detergent and fabric softener.
   g. Consider getting an air purifier—the best are IQAir and Blue Air. Make sure you get one with enough cubic feet of air purified each minute to clear the air in your bedroom at least once every 30 minutes.
   h. Follow the directions for step-by-step reduction of indoor air pollution sources in *Clean, Green and Lean* (New York: Wiley).

Dietary Things to Do:

1. Consume broccoli and other members of the *Brassica* family daily.
2. Consume green tea daily.
3. Increase intake of green foods (green leafy vegetables) daily.
4. Consume brown rice daily, or take a rice-fiber supplement.

Basic Supplementation:

1. Multivitamin/multimineral—take a quality product daily.
2. Vitamin C—begin with 3000 mg/day; for cases of high toxicity, a total of at least 9000 mg/day may be needed (unless this causes diarrhea).
3. N-acetyl cysteine—1500 to 1800 mg/day. This nutrient helps increase the amount of glutathione in the body.
4. Magnesium citrate—1 capsule (140 mg) up to three times daily.
5. A good probiotic—1/day.
6. Rice bran fiber—1 tablespoon in water or juice after each meal.
7. Whey protein powder—2 scoops daily in water or organic juice—high-quality whey protein will help boost glutathione levels and increase the ability of the liver to clear toxins out of the blood.

Cleansing:

1. Begin doing colonic irrigations. This is the best way to reduce the total toxic burden. It works best to do five colonics in the first 2 weeks, which will typically reduce the circulating toxic load enough for both the patient and the clinician to notice the difference.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jansz J. Sick building syndrome. In: Quah SR, ed. *International Encyclopedia of Public Health*. 2nd ed. New York: Academic Press; 2017. https://doi.org/10.1016/B978-0-12-803678-5.00407-0.
2. Genuis SJ. Chemical sensitivity: pathophysiology or pathopsychology? *Clin Ther*. 2013;35(5):572–577. PubMed PMID: 23642291.
3. Rea WJ. *Chemical Sensitivity*. Vol 1. Boca Raton: CRC Press; 1992:17–21.
4. Rea WJ. *Chemical Sensitivity*. Vol 1–2. Boca Raton: Lewis; 1992.
5. Pellacani C, Tagliaferri S, Caglieri A, et al. Synergistic interactions between PBDEs and PCBs in human neuroblastoma cells. *Environ Toxicol*. 2014;29(4):418–427. PubMed PMID: 22434561.
6. Axelrad JC, Howard CV, McLean WG. Interactions between pesticides and components of pesticide formulations in an in vitro neurotoxicity test. *Toxicol*. 2002;173(3):259–268. PubMed PMID: 11960678.
7. Gardner Jr HS, Brennan LM, Toussaint MW, et al. Environmental complex mixture toxicity assessment. *Environ Health Perspect*. 1998;106(suppl 6):1299–1305. PubMed PMID: 9860885.
8. Centers for Disease Control and Prevention. Exposure report. http://www.cdc.gov/exposurereport/. Accessed April 13, 2017.
9. Myllynen P, Pasanen M, Pelkonen O. Human placenta: a human organ for developmental toxicology research and biomonitoring. *Placenta*. 2005;26:361–371. PubMed PMID: 15850640.
10. Environmental Working Group. Body burden: the pollution in newborns. http://www.ewg.org/research/body-burden-pollution-newborns. Accessed April 13, 2017.
11. Barr DB, Bishop A, Needham LL. Concentrations of xenobiotic chemicals in the maternal-fetal unit. *Reprod Toxicol*. 2007;23:260–266. PubMed PMID: 17386996.
12. Centers for Disease Control and Prevention. *fourth national report on human exposure to environmental chemicals*; 2018. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Mar2018.pdf. Accessed July 9, 2018.
13. Knudsen LE, Norppa H, Gamborg MO, et al. Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. *Cancer Epidemiol Biomarkers Prev*. 1999;8(4 Pt 1):303–310. PubMed PMID: 10207633.
14. Zheltova AA, Kharitonova MV, Iezhitsa IN, Spasov AA. Magnesium deficiency and oxidative stress: an update. *Biomed (Taipei)*. 2016;6(4):20. Epub 2016 Nov 17. PubMed PMID: 27854048.
15. Chen HW, Tsai CW, Yang JJ, et al. The combined effects of garlic oil and fish oil on the hepatic antioxidant and drug-metabolizing enzymes of rats. *Br J Nutr*. 2003;89(2):189–200. PubMed PMID: 12575903.
16. Cooney CM. Stress-pollution interactions: an emerging issue in children's health research. *Environ Health Perspect*. 2011;119(10):a430–a435. PubMed PMID: 22069778.
17. Blouin RA, Farrell GC, Ioannides C, Renton K, Watlington CO. Impact of diseases on detoxication. *J Biochem Mol Toxicol*. 1999;13(3,4):215–218. PubMed PMID: 10098907.
18. Lioy PJ, Wallace L, Pellizzari E. Indoor/outdoor, and personal monitor and breath analysis relationships for selected volatile organic compounds measured at three homes during New Jersey TEAM-1987. *J Expo Anal Environ Epidemiol*. 1991;1(1):45–61. PubMed PMID: 1824311.
19. Lemley AT, Hedge A, Obendorf SK, Hong S, Kim J, Muss TM, Varner CJ. Selected pesticide residues in house dust from farmers' homes in central New York State, USA. *Bull Environ Contam Toxicol*. 2002;69(2):155–163. PubMed PMID: 12107690.
20. U.S. Food and Drug Administration. Total Diet Study. https://www.fda.gov/Food/FoodScienceResearch/TotalDietStudy/default.htm. Accessed December, 2017.
21. Agricultural Marketing Service, U.S. Department of Agriculture. Pesticide data program. https://www.ams.usda.gov/datasets/pdp. Accessed December, 2017.
22. Environmental Working Group. Shopper's guide to pesticide in produce. https://www.ewg.org/foodnews/#.WimHeGWzt0k. Accessed December, 2017.
23. Bøhn T, Cuhra M, Traavik T, et al. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. *Food Chem*. 2014;153:207–215. PubMed PMID: 24491722.
24. Ehling S, Reddy TM. Analysis of glyphosate and aminomethylphosphonic acid in nutritional ingredients and milk by derivatization with fluorenylmethyloxycarbonyl chloride and liquid chromatography-mass spectrometry. *J Agric Food Chem*. 2015;63(48):10562–10568. PubMed PMID: 26568409.
25. Granby K, Johannesen S, Vahl M. Analysis of glyphosate residues in cereals using liquid chromatography-mass spectrometry (LC-MS/MS). *Food Addit Contam*. 2003;20(8):692–698. PubMed PMID: 13129785.
26. Castle L, Nichol J, Gilbert J. Migration of polyisobutylene from polyethylene/polyisobutylene films into foods during domestic and microwave oven use. *Food Addit Contam*. 1992;9(4):315–330. PubMed PMID: 1493881.
27. Page BD, Lacroix GM. The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985–1989: a survey. *Food Addit Contam*. 1995;12(1):129–151. PubMed PMID: 7758627.
28. Duty SM, Ackerman RM, Calafat AM, Hauser R. Personal care product use predicts urinary concentrations of some phthalate monoesters. *Environ Health Perspect*. 2005;113(11):1530–1535. PubMed PMID: 16263507.
29. Hartle JC, Navas-Acien A, Lawrence RS. The consumption of canned food and beverages and urinary bisphenol a concentrations in NHANES 2003–2008. *Environ Res*. 2016;150:375–382. PubMed PMID: 27362993.
30. Carwile JL, Ye X, Zhou X, et al. Canned soup consumption and urinary bisphenol A: a randomized crossover trial. *JAMA*. 2011;306(20):2218–2220. PubMed PMID: 22110104.
31. Lakind JS, Naiman DQ. Daily intake of bisphenol A and potential sources of exposure: 2005–2006 national health and nutrition examination survey. *J Expo Sci Environ Epidemiol*. 2011;21(3):272–279. PubMed PMID: 20237498.
32. Kalantzi OI, Alcock RE, Johnston PA, et al. The global distribution of PCBs and organochlorine pesticides in butter. *Environ Sci Technol*. 2001;35(6):1013–1018. PubMed PMID: 11347908.
33. Newsome WH, Davies DJ, Sun WF. Residues of polychlorinated biphenyls (PCB) in fatty foods of the Canadian diet. *Food Addit Contam*. 1998;15(1):19–29. PubMed PMID: 9534869.
34. Schecter A, Colacino J, Haffner D, et al. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. *Environ Health Perspect*. 2010;118(6):796–802. PubMed PMID: 20146964.
35. National Oceanic and Atmospheric Administration. Aquaculture. http://www.nmfs.noaa.gov/aquaculture/faqs/faq_aq_101.html#4howmuch. Accessed April 29, 2017.
36. Knapp G, Roheim CA, Anderson JL. The great salmon run. http://www.iser.uaa.alaska.edu/Publications/2007_01-GreatSalmonRun.pdf. Accessed April 29, 2017.
37. van Leeuwen SP, van Velzen MJ, Swart CP, et al. Halogenated contaminants in farmed salmon, trout, tilapia, pangasius, and shrimp. *Environ Sci Technol*. 2009;43(11):4009–4015. PubMed PMID: 19569323.
38. Hites RA, Foran JA, Carpenter DO, et al. Global assessment of organic contaminant in farmed salmon. *Science*. 2004;303:226–229. PubMed PMID: 14716013.
39. Carlson DL, Hites RA. Polychlorinated biphenyls in salmon and salmon feed: global differences and bioaccumulation. *Environ Sci Technol*. 2005;39(19):7389–7395. PubMed PMID: 16245806.
40. Ikonomou MG, Higgs DA, Gibbs M, et al. Flesh quality of market-size farmed and wild British Columbia salmon. *Environ Sci Technol*. 2007;41(2):437–443. PubMed PMID: 17310704.
41. Nøstbakken OJ, Hove HT, Duinker A, et al. Contaminant levels in Norwegian farmed Atlantic salmon *(Salmo salar)* in the 13-year period from 1999 to 2011. *Environ Int*. 2015;74:274–280. PubMed PMID: 25454244.
42. Eawag. Risk maps. http://www.eawag.ch/en/research/humanwelfare/drinkingwater/wrq/risk-maps/. Accessed November 2017.
43. Connor JA, Kamath R, Walker KL, McHugh TE. Review of quantitative surveys of the length and stability of MTBE, TBA, and benzene plumes in groundwater at UST sites. *Ground Water*. 2015;53(2):195–206. PubMed PMID: 25040137.

44. Ayotte JD, Mrazik BR, Argue DM, McGarry FJ. Occurrence of methyl tert-butyl ether (MTBE) in public and private wells, Rockingham County, New Hampshire. https://pubs.usgs.gov/fs/2004/3119/. Accessed November, 2017.
45. Andrews D, Walker B. "Erin Brockovich" carcinogen in tap water of more than 200 million Americans. https://www.ewg.org/research/chromium-six-found-in-us-tap-water#ref6. Accessed November, 2017.
46. Tziritis E, Kelepertzis E, Korres G, et al. Hexavalent chromium contamination in groundwaters of Thiva Basin, central Greece. *Bull Environ Contam Toxicol*. 2012;89(5):1073–1077. PubMed PMID: 22996651.
47. Lau FK, deCastro BR, Mills-Herring L, et al. Urinary perchlorate as a measure of dietary and drinking water exposure in a representative sample of the United States population 2001-2008. *J Expo Sci Environ Epidemiol*. 2013;23(2):207–214. PubMed PMID: 23188482.
48. Post GB, Cohn PD, Cooper KR. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. *Environ Res*. 2012;116:93–117. PubMed PMID: 22560884.
49. Hu XC, Andrews DQ, Lindstrom AB, et al. Detection of poly- and perfluoroalkyl substances (PFASs) in U.S. drinking water linked to industrial sites, military fire training areas, and wastewater treatment plants. *Environ Sci Technol Lett*. 2016;3(10):344–350. PubMed PMID: 27752509.
50. Ruckart PZ, Bove FJ, Shanley 3rd E, Maslia M. Evaluation of contaminated drinking water and male breast cancer at Marine Corps Base Camp Lejeune, North Carolina: a case control study. *Environ Health*. 2015;14:74. PubMed PMID: 26376727.
51. Centers for Disease Control and Prevention. Community water fluoridation. https://www.cdc.gov/fluoridation/statistics/2012stats.htm. Accessed November, 2017.
52. Colman J, Rice GE, Wright JM, et al. Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action. *Toxicol Appl Pharmacol*. 2011;254(2):100–126. PubMed PMID: 21296098.
53. Moran MJ, Zogorski JS, Squillace PJ. Chlorinated solvents in groundwater of the United States. *Environ Sci Technol*. 2007;41(1):74–81. PubMed PMID: 17265929.
54. Glassmeyer ST, Furlong ET, Kolpin DW, et al. Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States. *Sci Total Environ*. 2017;581–582: 909–922. PubMed PMID: 28024752.
55. Yang YY, Zhao JL, Liu YS, et al. Pharmaceuticals and personal care products (PPCPs) and artificial sweeteners (ASs) in surface and ground waters and their application as indication of wastewater contamination. *Sci Total Environ*. 2017. pii:S0048–9697(17)32959-5. PubMed PMID: 29089128.
56. Furlong ET, Batt AL, Glassmeyer ST, et al. Nationwide reconnaissance of contaminants of emerging concern in source and treated drinking waters of the United States: Pharmaceuticals. *Sci Total Environ*. 2017;579:1629–1642. PubMed PMID: 28040194.
57. Nassan FL, Coull BA, Gaskins AJ, et al. Personal care product use in men and urinary concentrations of select phthalate metabolites and parabens: results from the Environment And Reproductive Health (EARTH) study. *Environ Health Perspect*. 2017;125(8):087012. PubMed PMID: 28886595.
58. Guo Y, Wang L, Kannan K. Phthalates and parabens in personal care products from China: concentrations and human exposure. *Arch Environ Contam Toxicol*. 2014;66(1):113–119. PubMed PMID: 23880707.
59. Rea WJ. *Chemical Sensitivities*. Vol 1. Boca Raton: CRC Press; 1992.
60. Ohtani T, Nakagawa S, Kurosawa M, et al. Cellular basis of the role of diesel exhaust particles in inducing Th2-dominant response. *J Immunol*. 2005;174(4):2412–2419. PubMed PMID: 15699178.
61. Chang Y, Sénéchal S, de Nadai P, et al. Diesel exhaust exposure favors TH2 cell recruitment in nonatopic subjects by differentially regulating chemokine production. *J Allergy Clin Immunol*. 2006;118(2):354–360. PubMed PMID: 16890758.
62. Yanagisawa R, Koike E, Win-Shwe TT, et al. Low-dose benzo[a] pyrene aggravates allergic airway inflammation in mice. *J Appl Toxicol*. 2016;36(11):1496–1504. PubMed PMID: 26918773.
63. Zhao C, Liao J, Chu W, et al. Involvement of TLR2 and TLR4 and Th1/Th2 shift in inflammatory responses induced by fine ambient particulate matter in mice. *Inhal Toxicol*. 2012;24(13):918–927. Erratum in: *Inhal Toxicol*. 2013;25(3):178. PubMed PMID: 23121301.
64. Dobreva ZG, Kostadinova GS, Popov BN, et al. Proinflammatory and anti-inflammatory cytokines in adolescents from Southeast Bulgarian cities with different levels of air pollution. *Toxicol Ind Health*. 2015;31(12):1210–1217. PubMed PMID: 23771874.
65. Iavicoli I, Marinaccio A, Castellino N, Carelli G. Altered cytokine production in mice exposed to lead acetate. *Int J Immunopathol Pharmacol*. 2004;17(suppl 2):97–102. PubMed PMID: 15345199.
66. Heo Y, Lee WT, Lawrence DA. In vivo the environmental pollutants lead and mercury induce oligoclonal T cell responses skewed toward type-2 reactivities. *Cell Immunol*. 1997;179(2):185–195. PubMed PMID: 9268502.
67. Agrawal A, Kaushal P, Agrawal S, et al. Thimerosal induces TH2 responses via influencing cytokine secretion by human dendritic cells. *J Leukoc Biol*. 2007;81(2):474–482. PubMed PMID: 17079650.
68. de Vos G, Abotaga S, Liao Z, et al. Selective effect of mercury on Th2-type cytokine production in humans. *Immunopharmacol Immunotoxicol*. 2007;29(3–4):537–548. PubMed PMID: 18075863.
69. Kato T, Tada-Oikawa S, Wang L, et al. Endocrine disruptors found in food contaminants enhance allergic sensitization through an oxidative stress that promotes the development of allergic airway inflammation. *Toxicol Appl Pharmacol*. 2013;273(1):10–18. PubMed PMID: 24035973.
70. Fukuyama T, Tajima Y, Ueda H, et al. Prior exposure to immunosuppressive organophosphorus or organochlorine compounds aggravates the T(H)1- and T(H)2-type allergy caused by topical sensitization to 2,4-dinitrochlorobenzene and trimellitic anhydride. *J Immunotoxicol*. 2011;8(2):170–182. PubMed PMID: 21534883.
71. Daniel V, Huber W, Bauer K, et al. Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4. *Arch Environ Health*. 2002;57(6):541–547. PubMed PMID: 12696651.
72. Kim HA, Kim EM, Park YC, et al. Immunotoxicological effects of Agent Orange exposure to the Vietnam War Korean veterans. *Ind Health*. 2003;41(3):158–166. PubMed PMID: 12916745.
73. Seo M, Yamagiwa T, Kobayashi R, et al. Augmentation of antigen-stimulated allergic responses by a small amount of trichloroethylene ingestion from drinking water. *Regul Toxicol Pharmacol*. 2008;52(2):140–146. PubMed PMID: 18721841.
74. Gaspar-Ramírez O, Pérez-Vázquez FJ, Pruneda-Álvarez LG, et al. Effect of polychlorinated biphenyls 118 and 153 on Th1/Th2 cells differentiation. *Immunopharmacol Immunotoxicol*. 2012;34(4):627–632. PubMed PMID: 22233178.
75. Gillis B, Gavin IM, Arbieva Z, et al. Identification of human cell responses to benzene and benzene metabolites. *Genomics*. 2007;90(3):324–333. PubMed PMID: 17572062.
76. Yan H, Takamoto M, Sugane K. Exposure to bisphenol A prenatally or in adulthood promotes T(H)2 cytokine production associated with reduction of CD4CD25 regulatory T cells. *Environ Health Perspect*. 2008;116(4):514–519. PubMed PMID: 18414636.
77. Yoshino S, Yamaki K, Li X, Sai T, et al. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunol*. 2004;112(3):489–495. PubMed PMID: 15196218.
78. Bornehag CG, Nanberg E. Phthalate exposure and asthma in children. *Int J Androl*. 2010;33(2):333–345. PubMed PMID: 20059582.
79. Fujimaki H, Nohara K, Kobayashi T, et al. Effect of a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin on immune function in male NC/Nga mice. *Toxicol Sci*. 2002;66(1):117–124. PubMed PMID: 11861978.
80. Fukuyama T, Ueda H, Hayashi K, et al. Detection of low-level environmental chemical allergy by a long-term sensitization method. *Toxicol Lett*. 2008;180(1):1–8. PubMed PMID: 18571882.
81. Zheng L, Dong GH, Zhang YH, et al. Type 1 and Type 2 cytokines imbalance in adult male C57BL/6 mice following a 7-day oral exposure to perfluorooctanesulfonate (PFOS). *J Immunotoxicol*. 2011;8(1):30–38. PubMed PMID: 21299352.
82. Duramad P, Harley K, Lipsett M, et al. Early environmental exposures and intracellular Th1/Th2 cytokine profiles in 24-month-old children living in an agricultural area. *Environ Health Perspect*. 2006;114(12): 1916–1922. PubMed PMID: 17185285.

83. Pérez-Maldonado IN, Díaz-Barriga F, de la Fuente H, et al. DDT induces apoptosis in human mononuclear cells in vitro and is associated with increased apoptosis in exposed children. *Environ Res*. 2004;94(1):38–46. PubMed PMID: 14643285.
84. Pérez-Maldonado IN, Athanasiadou M, Yáñez L, et al. DDE-induced apoptosis in children exposed to the DDT metabolite. *Sci Total Environ*. 2006;370(2–3):343–351. PubMed PMID: 16904735.
85. Langworth S, Elinder CG, Sundqvist KG. Minor effects of low exposure to inorganic mercury on the human immune system. *Scand J Work Environ Health*. 1993;19(6):405–413. PubMed PMID: 8153593.
86. Haschek WM, Rousseaux CG. *Haschek and Rousseaux's Handbook of Toxicologic Pathology.* Maryland Heights, MO: Academic Press, 2013.
87. Hew KM, Walker AI, Kohli A, et al. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. *Clin Exp Allergy*. 2015;45(1):238–248. PubMed PMID: 25048800.
88. Thrasher JD, Madison R, Broughton A. Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. *Arch Environ Health*. 1993;48(2):89–93. PubMed PMID: 7682805.
89. Sbihi H, Allen RW, Becker A, et al. Perinatal exposure to traffic-related air pollution and atopy at 1 year of age in a multi-center Canadian birth cohort study. *Environ Health Perspect*. 2015;123(9):902–908. PubMed PMID: 25826816.
90. Gruzieva O, Bellander T, Eneroth K, et al. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J Allergy Clin Immunol*. 2012;129(1):240–246. PubMed PMID: 22104609.
91. Middleton N, Yiallouros P, Nicolaou N, et al. Residential exposure to motor vehicle emissions and the risk of wheezing among 7-8 year-old schoolchildren: a city-wide cross-sectional study in Nicosia, Cyprus. *Environ Health*. 2010;9:28. PubMed PMID: 20565827.
92. Gehring U, Wijga AH, Brauer M, et al. Traffic-related air pollution and the development of asthma and allergies during the first 8 years of life. *Am J Respir Crit Care Med*. 2010;181(6):596–603. PubMed PMID: 19965811.
93. Brauer M, Hoek G, Smit HA, et al. Air pollution and development of asthma, allergy and infections in a birth cohort. *Eur Respir J*. 2007;29(5):879–888. PubMed PMID: 17251230.
94. Pereira G, De Vos AJ, Cook A. Residential traffic exposure and children's emergency department presentation for asthma: a spatial study. *Int J Health Geogr*. 2009;8:63. PubMed PMID: 19930672.
95. Spira-Cohen A, Chen LC, Kendall M, et al. Personal exposures to traffic-related air pollution and acute respiratory health among Bronx schoolchildren with asthma. *Environ Health Perspect*. 2011;119(4):559–565. Erratum in: *Environ Health Perspect*. 2011;119(4):564. PubMed PMID: 21216722.
96. Ryan PH, Bernstein DI, Lockey J, et al. Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years. *Am J Respir Crit Care Med*. 2009;180(11):1068–1075. PubMed PMID: 19745206.
97. Autoblog Sitemap. Autoblog. http://autos.yahoo.com/green_center-article_110/. Accessed January 2, 2012.
98. Sarkar S, Song Y, Sarkar S, Kipen HM, et al. Suppression of the NF-κB pathway by diesel exhaust particles impairs human antimycobacterial immunity. *J Immunol*. 2012;188(6):2778–2793. PubMed PMID: 22345648.
99. Ohtani T, Nakagawa S, Kurosawa M, et al. Cellular basis of the role of diesel exhaust particles in inducing Th-2 dominant response. *J Immunol*. 2005;174(4):2412–2419. PubMed PMID: 15699178.
100. Pawlak EA, Noah TL, Zhou H, et al. Diesel exposure suppresses natural killer cell function and resolution of eosinophil inflammation: a randomized controlled trial of exposure in allergic rhinitics. *Part Fibre Toxicol*. 2016;13(1):24. PubMed PMID: 27154411.
101. Müller L, Chehrazi CV, Henderson MW, et al. Diesel exhaust particles modify natural killer cell function and cytokine release. *Part Fibre Toxicol*. 2013;10:16. PubMed PMID: 23618096.
102. Castranova V, Ma JY, Yang HM, et al. Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. *Environ Health Perspect*. 2001;109(suppl 4):609–612. PubMed PMID: 11544172.
103. Yin XJ, Dong CC, Ma JY, et al. Sustained effect of inhaled diesel exhaust particles on T-lymphocyte-mediated immune responses against *Listeria monocytogenes*. *Toxicol Sci*. 2005;88(1):73–81. PubMed PMID: 16107554.
104. Takano H, Yanagisawa R, Inoue K. Components of diesel exhaust particles diversely enhance a variety of respiratory diseases related to infection or allergy: extracted organic chemicals and the residual particles after extraction differently affect respiratory diseases. *J Clin Biochem Nutr*. 2007;40(2):101–107. PubMed PMID: 18188411.
105. Yang HM, Antonini JM, Barger MW, et al. Diesel exhaust particles suppress macrophage function and slow the pulmonary clearance of *Listeria monocytogenes* in rats. *Environ Health Perspect*. 2001;109(5):515–521. PubMed PMID: 11401764.
106. Takafuji S, Suzuki S, Koizumi K, et al. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. *J Allergy Clin Immunol*. 1987;79:639–645. PubMed PMID: 2435776.
107. Carlsten C, Blomberg A, Pui M, et al. Diesel exhaust augments allergen-induced lower airway inflammation in allergic individuals: a controlled human exposure study. *Thorax* 2016;71(1):35–44. Erratum in: *Thorax*. 2016;71(4):385. PubMed PMID: 26574583.
108. Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J Immunol*. 1997;158(5):2406–2413. PubMed PMID: 9036991.
109. Maejima K, Tamura K, Nakajima T, et al. Effects of the inhalation of diesel exhaust, Kanto loam dust, or diesel exhaust without particles on immune responses in mice exposed to Japanese cedar *(Cryptomeria japonica)* pollen. *Inhal Toxicol*. 2001;13(11):1047–1063. PubMed PMID: 11696873.
110. Lubitz S, Schober W, Pusch G, et al. Polycyclic aromatic hydrocarbons from diesel emissions exert proallergic effects in birch pollen allergic individuals through enhanced mediator release from basophils. *Environ Toxicol*. 2010;25(2):188–197. PubMed PMID: 19382185.
111. Nilsen A, Hagemann R, Eide I. The adjuvant activity of diesel exhaust particles and carbon black on systemic IgE production to ovalbumin in mice after intranasal instillation. *Toxicol*. 1997;124(3):225–232. PubMed PMID: 9482124.
112. Nel AE, Diaz-Sanchez D, Ng D, et al. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. *J Allergy Clin Immunol*. 1998;102(4 Pt 1):539–554. PubMed PMID: 9802360.
113. Lerner A, Jeremias P, Matthias T. The world incidence and prevalence of autoimmune disease is increasing. *Int J Celiac Dis*. 2015;3(4):151–155.
114. Vojdani A, Ghoneum M, Brautbar N. Immune alteration associated with exposure to toxic chemicals. *Toxicol Ind Health*. 1992;8(5):239–254. PubMed PMID: 1455435.
115. Somers EC, Ganser MA, Warren JS, et al. Mercury exposure and antinuclear antibodies among females of reproductive age in the United States: NHANES. *Environ Health Perspect*. 2015;123(8):792–798. PubMed PMID: 25665152.
116. Silva IA, Nyland JF, Gorman A, et al. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. *Environ Health*. 2004;3(1):11. PubMed PMID: 15522122.
117. Gardner RM, Nyland JF, Silva IA, et al. Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. *Environ Res*. 2010;110(4):345–354. PubMed PMID: 20176347.
118. Gallagher CM, McElroy AE, Smith DM, et al. Polychlorinated biphenyls, mercury, and antinuclear antibody positivity, NHANES 2003-2004. *Int J Hyg Environ Health*. 2013;216(6):721–727. PubMed PMID: 23419585.
119. Thrasher JD, Madison R, Broughton A. Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. *Arch Environ Med*. 1993;48(2):89–93.
120. Gilbert KM, Rowley B, Gomez-Acevedo H, Blossom SJ. Coexposure to mercury increases immunotoxicity of trichloroethylene. *Toxicol Sci*. 2011;119(2):281–292. PubMed PMID: 21084432.

121. Thrasher JD, Broughton A, Madison R. Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. *Arch Environ Health*. 1990;45(4):217–223.
122. Bernatsky S, Fournier M, Pineau CA, et al. Associations between ambient fine particulate levels and disease activity in patients with systemic lupus erythematosus (SLE). *Environ Health Perspect*. 2011;119(1):45–49. PubMed PMID: 20870568.
123. Bernatsky S, Smargiassi A, Barnabe C, et al. Fine particulate air pollution and systemic autoimmune rheumatic disease in two Canadian provinces. *Environ Res*. 2016;146:85–91. PubMed PMID: 26724462.
124. Fernandes EC, Silva CA, Braga AL, et al. Exposure to air pollutants and disease activity in juvenile-onset systemic lupus erythematosus patients. *Arthritis Care Res (Hoboken)*. 2015;67(11):1609–1614. PubMed PMID: 25892357.
125. Idiopathic environmental intolerances. American Academy of Allergy, Asthma and Immunology (AAAAI) Board of Directors. *J Allergy Clin Immunol*. 1999;103(1 Pt 1):36–40. PubMed PMID: 9893182.
126. Miller CS. Are we on the threshold of a new theory of disease? Toxicant-induced loss of tolerance and its relationship to addiction and abdiction. *Toxicol Ind Health*. 1999;15(3-4):284–294. PubMed PMID: 10416280.
127. Miller C, Ashford N, Doty R, et al. Empirical approaches for the investigations of toxicant-induced loss of tolerance. *Environ Health Perspect*. 1997;105(suppl 2):515–519. PubMed PMID: 9167989.
128. Caress SM, Steinemann AC. Prevalence of fragrance sensitivity in the American population. *J Environ Health*. 2009;71(7):46–50. PubMed PMID: 19326669.
129. Heuser G, Wojdani A, Heuser S. *Diagnostic markers of multiple chemical sensitivity. Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council*. Washington, DC: National Academy Press; 1992.
130. Bell IR. White paper: Neuropsychiatric aspect so sensitivity to low-level chemicals: a neural sensitization model. *Toxicol Ind Health*. 1994;10(4-5):277–312. PubMed PMID: 7778100.
131. Bell IR, Hardin EE, Baldwin CM, Schwartz GE. Increased limbic system symptomatology and sensitizability of young adults with chemical and noise sensitivities. *Environ Res*. 1995;70(2):84–97. PubMed PMID: 8674484.
132. Chambers JE, Levi PE, eds. *Organophosphates: Chemistry, Fate, and Effects*. San Diego, CA: Academic Press; 1992.
133. Parrón T, Hernández AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. *Hum Exp Toxicol*. 1996;15(12):957–963. PubMed PMID: 8981099.
134. Bazylewicz-Walczak B, Majczakowa W, Szymczak M. Behavioral effects of occupational exposure to organophosphorus pesticides in female greenhouse planting workers. *Neurotoxicol*. 1999;20(5):819–826. PubMed PMID: 10591517.
135. Bosma H, van Boxtel MP, Ponds RW, et al. Pesticide exposure and risk of mild cognitive dysfunction. *Lancet*. 2000;356(9233):912–913. PubMed PMID: 11036900.
136. Stephens R, Spurgeon A, Calvert IA, et al. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet*. 1995;345(8958):1135–1139. PubMed PMID: 7723544.
137. Savage EP, Keefe TJ, Mounce LM, et al. Chronic neurological sequelae of acute organophosphate pesticide poisoning. *Arch Environ Health*. 1988;43(1):38–45. PubMed PMID: 3355242.
138. Kailin EW, Hastings A. Cerebral disturbances from small amounts of DDT. A controlled study. *Med Ann Dist Columbia*. 1966;35(10):519–524. PubMed PMID: 5223191.
139. van Wendel de Joode B, Wesseling C, Kromhout H, et al. Chronic nervous-system effects of long-term occupational exposure to DDT. *Lancet*. 2001;357(9261):1014–1016. PubMed PMID: 11293598.
140. Ribas-Fitó N, Torrent M, Carrizo D, et al. In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers. *Am J Epidemiol*. 2006;164(10):955–962. PubMed PMID: 16968864.
141. Kilburn KH, Thornton JC. Protracted neurotoxicity from chlordane sprayed to kill termites. *Environ Health Perspect*. 1995;103(7-8):690–694. PubMed PMID: 7588480.
142. Dryson EW, Ogden JA. Organic solvent induced chronic toxic encephalopathy: extent of recovery, and associated factors, following cessation of exposure. *Neurotoxicol*. 2000;21(5):659–665. PubMed PMID: 11130269.
143. Bruning T, Lammert M, Kempkes M, et al. Influence of polymorphism of GSTM1 and GSTT1 for risk of renal cell cancer in workers with long-term high occupational exposures to trichloroethene. *Arch Toxicol*. 1997;71:596–599. PubMed PMID: 9285043.
144. Tsai SY, Chen JD, Chao WY, Wang JD. Neurobehavioral effects of occupational exposure to low-level organic solvents among Taiwanese workers in paint factories. *Environ Res*. 1997;73(1-2):146–155. PubMed PMID: 9311540.
145. Chen R, Dick F, Semple S, et al. Exposure to organic solvents and personality. *Occup Environ Med*. 2001;58(1):14–18. PubMed PMID: 11119629.
146. Chen R, Dick F, Seaton A. Health effects of solvent exposure among dockyard painters: mortality and neuropsychological symptoms. *Occup Environ Med*. 1999;56(6):383–387. PubMed PMID: 10474533.
147. Foo SC, Jeyaratnam J, Koh D. Chronic neurobehavioural effects of toluene. *Br J Ind Med*. 1990;47(7):480–484. PubMed PMID: 2383518.
148. Needleman HL, Gatsonis CA. Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. *JAMA*. 1990;263(5):673–678. PubMed PMID: 2136923.
149. Tuthill RW. Hair lead levels related to children's classroom attention-deficit behavior. *Arch Environ Health*. 1996;51(3):214–220. PubMed PMID: 8687242.
150. Needleman HL, Riess JA, Tobin MJ, et al. Bone lead levels and delinquent behavior. *JAMA*. 1996;275(5):363–369. PubMed PMID: 8569015.
151. Stokes L, Letz R, Gerr F, et al. Neurotoxicity in young adults 20 years after childhood exposure to lead: the Bunker Hill experience. *Occup Environ Med*. 1998;55(8):507–516. PubMed PMID: 9849536.
152. Bandeen-Roche K, Glass TA, Bolla KI, et al. Cumulative lead dose and cognitive function in older adults. *Epidemiol*. 2009;20(6):831–839. PubMed PMID: 19752734.
153. Coon S, Stark A, Peterson E, et al. Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect*. 2006;114(12):1872–1876. PubMed PMID: 17185278.
154. Weuve J, Press DZ, Grodstein F, et al. Cumulative exposure to lead and cognition in persons with Parkinson's disease. *Mov Disord*. 2013;28(2):176–182. PubMed PMID: 23143985.
155. Canfield RL, Henderson Jr CR, Cory-Slechta DA, et al. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med*. 2003;348(16):1517–1526. PubMed PMID: 12700371.
156. Jusko TA, Henderson CR, Lanphear BP, et al. Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age. *Environ Health Perspect*. 2008;116(2):243–248. PubMed PMID: 18288325.
157. Lanphear BP, Hornung R, Khoury J, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect*. 2005;113(7):894–899. PubMed PMID: 16002379.
158. Lucchini RG, Zoni S, Guazzetti S, et al. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. *Environ Res*. 2012;118:65–71. PubMed PMID: 22925625.
159. Miranda ML, Kim D, Galeano MA, et al. The relationship between early childhood blood lead levels and performance on end-of-grade tests. *Environ Health Perspect*. 2007;115(8):1242–1247. PubMed PMID: 17687454.
160. Ninomiya T, Ohmori H, Hashimoto K, et al. Expansion of methylmercury poisoning outside of Minamata: an epidemiological study on chronic methylmercury poisoning outside of Minamata. *Environ Res*. 1995 Jul;70(1):47–50. PubMed PMID: 8603658.
161. Fukuda Y, Ushijima K, Kitano T, et al. An analysis of subjective complaints in a population living in a methylmercury-polluted area. *Environ Res*. 1999;81(2):100–107. PubMed PMID: 10433841.
162. Grandjean P, White RF, Nielsen A, et al. Methylmercury neurotoxicity in Amazonian children downstream from gold mining. *Environ Health Perspect*. 1999;107(7):587–591. PubMed PMID: 10379006.
163. Hightower JM, Moore D. Mercury levels in high-end consumers of fish. *Environ Health Perspect*. 2003;111(4):604–608. 12676623.

164. Siblerud RL. The relationship between mercury from dental amalgam and mental health. *Am J Psychother*. 1989;43(4):575–587. PubMed PMID: 2618948.
165. Gu Y, Yang DK, Spinas E, et al. Role of TNF in mast cell neuroinflammation and pain. *J Biol Regul Homeost Agents*. 2015;29(4):787–791. PubMed PMID: 26753638.
166. Reis PA, Alexandre PC, D'Avila JC, et al. Statins prevent cognitive impairment after sepsis by reverting neuroinflammation, and microcirculatory/endothelial dysfunction. *Brain Behav Immun*. 2016. pii: S0889–1591(16)30496-2. PubMed PMID: 27833044.
167. Bilbo SD, Smith SH, Schwarz JM. A lifespan approach to neuroinflammatory and cognitive disorders: a critical role for glia. *J Neuroimmune Pharmacol*. 2012;7(1):24–41. PubMed PMID: 21822589.
168. Leonard BE. Impact of inflammation on neurotransmitter changes in major depression: an insight into the action of antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:261–267. PubMed PMID: 24189118.
169. Cherry JD, Tripodis Y, Alvarez VE, et al. Microglial neuroinflammation contributes to tau accumulation in chronic traumatic encephalopathy. *Acta Neuropathol Commun*. 2016;4(1):112. PubMed PMID: 27793189.
170. Fuster-Matanzo A, Llorens-Martín M, Hernández F, Avila J. Role of neuroinflammation in adult neurogenesis and Alzheimer disease: therapeutic approaches. *Mediators Inflamm*. 2013;2013:260925. PubMed PMID: 23690659.
171. Hariri M, Djazayery A, Djalali M, et al. Effect of n-3 supplementation on hyperactivity, oxidative stress and inflammatory mediators in children with attention-deficit-hyperactivity disorder. *Malays J Nutr*. 2012;18(3):329–335. PubMed PMID: 24568073.
172. Hooten KG, Beers DR, Zhao W, Appel SH. Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. *Neurotherapeutics*. 2015;12(2):364–375. PubMed PMID: 25567201.
173. Di Marco B, Bonaccorso CM, Aloisi E, et al. Neuro-inflammatory mechanisms in developmental disorders associated with intellectual disability and autism spectrum disorder: a neuroimmune perspective. *CNS Neurol Disord Drug Targets*. 2016;15(4):448–463. PubMed PMID: 26996174.
174. Nguyen LT, Ramanathan M, Weinstock-Guttman B, et al. Sex differences in in vitro pro-inflammatory cytokine production from peripheral blood of multiple sclerosis patients. *J Neurol Sci*. 2003;209(1-2):93–99. PubMed PMID: 12686409.
175. Pantović-Stefanović M, Petronijević N, Dunjić-Kostić B, et al. sVCAM-1, sICAM-1, TNF-α, and IL-6 levels in bipolar disorder type I: acute, longitudinal, and therapeutic implications. *World J Biol Psychiatry*. 2016:1–34. PubMed PMID: 27841086.
176. de Vries EE, van den Munckhof B, Braun KP, et al. Inflammatory mediators in human epilepsy: a systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2016;63:177–190. PubMed PMID: 26877106.
177. Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. *Neurobiol Dis*. 2010;37(3):510–518. PubMed PMID: 19913097.
178. Machado V, Zöller T, Attaai A, Spittau B. Microglia-mediated neuroinflammation and neurotrophic factor-induced protection in the MPTP mouse model of Parkinson's disease—lessons from transgenic mice. *Int J Mol Sci*. 2016;17(2). PubMed PMID: 26821015.
179. Mietto BS, Mostacada K, Martinez AM. Neurotrauma and inflammation: CNS and PNS responses. *Mediators Inflamm*. 2015;2015:251204. PubMed PMID: 25918475.
180. Shi Z, Ren H, Huang Z, et al. Fish oil prevents lipopolysaccharide-induced depressive-like behavior by inhibiting neuroinflammation. *Mol Neurobiol*. 2016. [Epub ahead of print]. PubMed PMID: 27815837.
181. Pugazhenthi S, Qin L, Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta*. 2016. pii: S0925–4439(16)30097–7. PubMed PMID: 27156888.
182. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun*. 2007;7:901–912. PubMed PMID: 17475444.
183. Levesque S, Surace MJ, McDonald J, Block ML. Air pollution & the brain: Subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. *J Neuroinflammation*. 2011;8:105. PubMed PMID: 21864400.
184. Monnet-Tschudi F, Zurich MG, Boschat C, Corbaz A, Honegger P. Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev Environ Health*. 2006;21(2):105–117. PubMed PMID: 16898674.
185. Banks CN, Lein PJ. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. *Neurotoxicol*. 2012;33(3):575–584. Review. PubMed PMID: 22342984.
186. Curtis KM, Savitz DA, Weinberg CR, Arbuckle TE. The effect of pesticide exposure on time to pregnancy. Epidemiol. 1999;10(2):112–117. Erratum in: *Epidemiol* 1999;10(4):470. PubMed PMID: 10069244.
187. Parrón T, Hernández AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. *Hum Exp Toxicol*. 1996;15(12):957–963. PubMed PMID: 8981099.
188. Bell EM, Hertz-Picciotto I, Beaumont JJ. A case-control study of pesticides and fetal death due to congenital anomalies. *Epidemiol*. 2001;12(2):148–156. Erratum in: *Epidemiol*. 2001;12(5):596. PubMed PMID: 11246574.
189. Gerhard I, Monga B, Krähe J, Runnebaum B. Chlorinated hydrocarbons in infertile women. *Environ Res*. 1999;80(4):299–310. PubMed PMID: 10330303.
190. Langer P, Tajtáková M, Fodor G, et al. Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls. *Eur J Endocrinol*. 1998;139(4):402–409. PubMed PMID: 9820616.
191. Younglai EV, Foster WG, Hughes EG, et al. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch Environ Contam Toxicol*. 2002;43(1):121–126. PubMed PMID: 12045882.
192. Cohn BA, Cirillo PM, Wolff MS, et al. DDT and DDE exposure in mothers and time to pregnancy in daughters. *Lancet*. 2003;361(9376):2205–2206. Erratum in: *Lancet*. 2003;362(9394):1504. PubMed PMID: 12842376.
193. Plenge-Bönig A, Karmaus W. Exposure to toluene in the printing industry is associated with subfecundity in women but not in men. *Occup Environ Med*. 1999;56(7):443–448. PubMed PMID: 10472314.
194. Wennborg H, Bodin L, Vainio H, Axelsson G. Solvent use and time to pregnancy among female personnel in biomedical laboratories in Sweden. *Occup Environ Med*. 2001;58(4):225–231. PubMed PMID: 11245738.
195. Sallmén M, Lindbohm ML, Anttila A, et al. Time to pregnancy among the wives of men exposed to organic solvents. *Occup Environ Med*. 1998;55(1):24–30. PubMed PMID: 9536159.
196. Attarchi MS, Ashouri M, Labbafinejad Y, Mohammadi S. Assessment of time to pregnancy and spontaneous abortion status following occupational exposure to organic solvents mixture. *Int Arch Occup Environ Health*. 2012;85(3):295–303. PubMed PMID: 21681482.
197. Xu X, Cho SI, Sammel M, et al. Association of petrochemical exposure with spontaneous abortion. *Occup Environ Med*. 1998;55(1):31–36. PubMed PMID: 9536160.
198. Taskinen H, Lindbohm ML, Hemminki K. Spontaneous abortions among women working in the pharmaceutical industry. *Br J Ind Med*. 1986;43(3):199–205. Erratum in: *Br J Ind Med* 1986;43(6):432. PubMed PMID: 3947584.
199. Axmon A, Rylander L, Stromberg U, Hagmar L. Time to pregnancy and infertility among women with a high intake of fish contaminated with persistent organochlorine compounds. *Scand J Word Environ Health*. 2000;26(3):199–206. PubMed PMID: 10901111.
200. Burguet A, Agnani G. [Smoking, fertility and very preterm birth]. *Gynecol Obstet Biol Reprod (Paris)*. 2003;32(1 suppl): 1S9–16.
201. Wisborg K, Kesmodel U, Henrikson TB, et al. Exposure to tobacco smoke in utero and the risk of stillbirth and death in the first year of life. *Am J Epidemiol*. 2001;154:322–327. PubMed PMID: 11495855.
202. Kabir Z, Clarke V, Conroy R, et al. Low birthweight and preterm birth rates 1 year before and after the Irish workplace smoking ban. *BJOG*. 2009;116(13):1782–1787. PubMed PMID: 19832830.
203. Mahalingaiah S, Hart JE, Laden F, et al. Adult air pollution exposure and risk of infertility in the Nurses' Health Study II. *Hum Reprod*. 2016;31(3):638–647. PubMed PMID: 26724803.

204. Nieuwenhuijsen MJ, Basagaña X, Dadvand P, et al. Air pollution and human fertility rates. *Environ Int*. 2014;70:9–14. PubMed PMID: 24879367.
205. Perin PM, Maluf M, Czeresnia CE, et al. Effects of exposure to high levels of particulate air pollution during the follicular phase of the conception cycle on pregnancy outcome in couples undergoing in vitro fertilization and embryo transfer. *Fertil Steril*. 2010;93(1):301–303. PubMed PMID: 19631320.
206. Perera FP, Jedrychowski W, Rauh V, Whyatt RM. Molecular epidemiological research on the effects of environmental pollutants on the fetus. *Environ Health Perspect*. 1999;107(suppl 3):451–460. PubMed PMID: 10346993.
207. Lans MC, Spiertz C, Brouwer A, Koeman JH. Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *Eur J Pharmacol*. 1994;270(2-3):129–136. PubMed PMID: 8039542.
208. Brouwer A, Morse DC, Lans MC, et al. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol Ind Health*. 1998;14(1–2):59–84. PubMed PMID: 9460170.
209. Langer P, Tajtáková M, Fodor G, et al. Increased thyroid volume and prevalence of thyroid disorders in an area heavily polluted by polychlorinated biphenyls. *Eur J Endocrinol*. 1998;139(4):402–409. PubMed PMID: 9820616.
210. Hagmar L, Rylander L, Dyremark E, et al. Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. *Int Arch Occup Environ Health*. 2001;74(3):184–188. PubMed PMID: 11355292.
211. Osius N, Karmaus W, Kruse H, Witten J. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ Health Perspect*. 1999;107(10):843–849. PubMed PMID: 10504153.
212. Rathore M, Bhatnagar P, Mathur D, Saxena GN. Burden of organochlorine pesticides in blood and its effect on thyroid hormones in women. *Sci Total Environ*. 2002;295(1-3):207–215. PubMed PMID: 12186288.
213. Centers for Disease Control and Prevention. Long term trends in diabetes. https://www.cdc.gov/diabetes/statistics/slides/long_term_trends.pdf. Accessed December, 2017.
214. Song Y, Chou EL, Baecker A, et al. Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related metabolic traits: a systematic review and meta-analysis. *J Diabetes*. 2016;8(4):516–532. PubMed PMID: 26119400.
215. Grau-Pérez M, Kuo CC, Spratlen M, et al. The association of arsenic exposure and metabolism with type 1 and type 2 diabetes in youth: the search case-control study. *Diabetes Care*. 2017;40(1):46–53. PubMed PMID: 27810988.
216. Lampron-Goulet É, Gagnon F, Langlois MF. Association between consumption of private well water contaminated by low levels of arsenic and dysglycemia in a rural region of Quebec, Canada. *Environ Res*. 2017;159:232–238. PubMed PMID: 28818805.
217. Lee DH, Lee IK, Song K, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the national health and examination survey 1999-2002. *Diabetes Care*. 2006;29(7):1638–1644. PubMed PMID: 16801591.
218. Lee YM, Ha CM, Kim SA, et al. Low-dose persistent organic pollutants impair insulin secretory function of pancreatic β-cells: human and in vitro evidence. *Diabetes*. 2017;66(10):2669–2680. PubMed PMID: 28720696.
219. Grice BA, Nelson RG, Williams DE, et al. Associations between persistent organic pollutants, type 2 diabetes, diabetic nephropathy and mortality. *Occup Environ Med*. 2017;74(7):521–527. PubMed PMID: 28438788.
220. Alderete TL, Habre R, Toledo-Corral CM, et al. Longitudinal associations between ambient air pollution with insulin sensitivity, β-cell function, and adiposity in Los Angeles Latino children. *Diabetes*. 2017;66(7):1789–1796. PubMed PMID: 28137791.
221. Hansen AB, Ravnskjær L, Loft S, et al. Long-term exposure to fine particulate matter and incidence of diabetes in the Danish nurse cohort. *Environ Int*. 2016;91:243–250. PubMed PMID: 26989812.
222. Coogan PF, White LF, Yu J, et al. PM2.5 and diabetes and hypertension incidence in the black women's health study. *Epidemiol*. 2016;27(2):202–210. PubMed PMID: 26595125.
223. Sun Q, Cornelis MC, Townsend MK, et al. Association of urinary concentrations of bisphenol A and phthalate metabolites with risk of type 2 diabetes: a prospective investigation in the Nurses' Health Study (NHS) and NHSII cohorts. *Environ Health Perspect*. 2014;122(6):616–623. PubMed PMID: 24633239.
224. Silver MK, O'Neill MS, Sowers MR, Park SK. Urinary bisphenol A and type-2 diabetes in U.S. adults: data from NHANES 2003-2008. *PLoS One*. 2011;6(10):e26868. PubMed PMID: 22046388.
225. Piecha R, Š Svačina, Malý M, et al. Urine levels of phthalate metabolites and bisphenol A in relation to main metabolic syndrome components: dyslipidemia, hypertension and type 2 diabetes. a pilot study. *Cent Eur J Public Health*. 2016;24(4):297–301. PubMed PMID: 28095285.
226. Sabanayagam C, Teppala S, Shankar A. Relationship between urinary bisphenol A levels and prediabetes among subjects free of diabetes. *Acta Diabetol*. 2013;50(4):625–631. PubMed PMID: 23636267.
227. Kim JH, Park H, Lee J, et al. Association of diethylhexyl phthalate with obesity-related markers and body mass change from birth to 3 months of age. *J Epidemiol Community Health*. 2016;70(5):466–472. PubMed PMID: 26834143.
228. Hatch EE, Nelson JW, Qureshi MM, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. *Environ Health*. 2008;7:27. PubMed PMID: 18522739.
229. Lundberg I, Håkansson M. Normal serum activities of liver enzymes in Swedish paint industry workers with heavy exposure to organic solvents. *Br J Ind Med*. 1985;42(9):596–600. PubMed PMID: 2864077.
230. Norén K, Weistrand C, Karpe F. Distribution of PCB congeners, DDE, hexachlorobenzene, and methylsulfonyl metabolites of PCB and DDE among various fractions of human blood plasma. *Arch Environ Contam Toxicol*. 1999;37(3):408–414. PubMed PMID: 10473799.
231. Phillips DL, Pirkle JL, Burse VW, et al. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol*. 1989;18(4):495–500. PubMed PMID: 2505694.
232. Crinnion WJ. The benefits of pre- and post-challenge urine heavy metal testing: Part 1. *Altern Med Rev*. 2009;14(1):3–8. PubMed PMID: 19364190.
233. Crinnion WJ. The benefit of pre- and post-challenge urine heavy metal testing: part 2. *Altern Med Rev*. 2009;14(2):103–108. PubMed PMID: 19594221.
234. Lin JL, Lin-Tan DT, Hsu KH, Yu CC. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. *N Engl J Med*. 2003;348(4):277–286. PubMed PMID: 12540640.
235. Chen KH, Lin JL, Lin-Tan DT, et al. Effect of chelation therapy on progressive diabetic nephropathy in patients with type 2 diabetes and high-normal body lead burdens. *Am J Kidney Dis*. 2012;60(4):530–538. PubMed PMID: 22721929.
236. Markowitz ME, Rosen JF. Need for the lead mobilization test in children with lead poisoning. *J Pediatr*. 1991;119(2):305–310. PubMed PMID: 1907320.
237. Hoet P, Buchet JP, Decerf L, et al. Clinical evaluation of a lead mobilization test using the chelating agent dimercaptosuccinic acid. *Clin Chem*. 2006;52(1):88–96. PubMed PMID: 16239340.
238. Khan DA, Qayyum S, Saleem S, Khan FA. Evaluation of lead body burden in occupational workers by lead mobilization test. *J Pak Med Assoc*. 2009;59(6):350–354. PubMed PMID: 19534366.
239. Gonzalez-Ramirez D, Maiorino RM, Zuniga-Charles M, et al. Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, Mexico. *J Pharmacol Exp Ther*. 1995;272(1):264–274. PubMed PMID: 7815341.
240. Gibson PR, Elms AN, Ruding LA. Perceived treatment efficacy for conventional and alternative therapies reported by persons with multiple chemical sensitivity. *Environ Health Perspect*. 2003;111(12):1498–1504. PubMed PMID: 12948890.
241. Good books on this subject are *Less Toxic Living* by Carolyn P. Gorman, available from Environmental Health Center, Dallas, Texas ([214]368-4132); *Success in the Clean Bedroom* by Natalie Golos, Pinnacle Publishers, Rochester, New York, 1992. *Your Home, Your Health, Your Well-Being* by David Rousseau, published by Hartley and Marks, Vancouver, British Columbia, Canada, 1988. *The Toxin Solution* by Joseph Pizzorno, HarperOne, San Francisco, California, 2017.

242. Meydani M. Dietary effects on detoxification processes. In: Hathcock J, ed. *Nutritional Toxicology*. Vol. II. Maryland Heights, MO: Academic Press; 1987.
243. Sera N, Morita K, Nagasoe M, et al. Binding effect of polychlorinated compounds and environmental carcinogens on rice bran fiber. *J Nutr Biochem*. 2005;16(1):50–58. PubMed PMID: 15629241.
244. Kimura Y, Nagata Y, Buddington RK. Some dietary fibers increase elimination of orally administered polychlorinated biphenyls but not that of retinol in mice. *J Nutr*. 2004;134(1):135–142. PubMed PMID: 14704306.
245. De Vos S, De Schrijver R. Polychlorinated biphenyl distribution and faecal excretion in rats fed wheat bran. *Chemosphere*. 2005;61(3):374–382. PubMed PMID: 16182854.
246. Morita K, Hamamura K, Iida T. [Binding of PCB by several types of dietary fiber in vivo and in vitro]. *Fukuoka Igaku Zasshi*. 1995;86(5): 212–217. PubMed PMID: 7628811.
247. Morita K, Hirakawa H, Matsueda T, et al. [Stimulating effect of dietary fiber on fecal excretion of polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) in rats]. *Fukuoka Igaku Zasshi*. 1993;84(5):273–281. PubMed PMID: 8392484.
248. Nagayama J, Takasuga T, Tsuji H, et al. Active elimination of causative PCDFs/DDs congeners of Yusho by one year intake of FBRA in Japanese people. *Fukuoka Igaku Zasshi*. 2003;94(5):118–125. PubMed PMID: 12872712.
249. Morita K, Tobiishi K. Increasing effect of nori on the fecal excretion of dioxin by rats. *Biosci Biotechnol Biochem*. 2002;66(11):2306–2313. PubMed PMID: 12506965.
250. Morita K, Ogata M, Hasegawa T. Chlorophyll derived from *Chlorella* inhibits dioxin absorption from the gastrointestinal tract and accelerates dioxin excretion in rats. *Environ Health Perspect*. 2001;109(3):289–294. PubMed PMID: 11333191.
251. Morita K, Matsueda T, Iida T. [Effect of green vegetable on digestive tract absorption of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in rats]. *Fukuoka Igaku Zasshi*. 1999;90(5):171–183. PubMed PMID: 10396873.
252. Schnare DW, Denk G, Shields M, et al. Evaluation of a detoxification treatment for fat stored xenobiotics. *Med Hypothesis*. 1982;9:265–282.
253. Tretjak Z, Shields M, Beckmann SL. PCB reduction and clinical improvement by detoxification: an unexploited approach? *Hum Exp Toxicol*. 1990;9(4):235–244. PubMed PMID: 2143911.
254. Genuis SJ, Beesoon S, Birkholz D, Lobo RA. Human excretion of bisphenol A: blood, urine, and sweat (BUS) study. *J Environ Public Health*. 2012;2012:185731. PubMed PMID: 22253637.
255. Genuis SJ, Beesoon S, Lobo RA, Birkholz D. Human elimination of phthalate compounds: blood, urine, and sweat (BUS) study. *Sci World J*. 2012;2012:615068. PubMed PMID: 23213291.
256. Genuis SK, Birkholz D, Genuis SJ. Human excretion of polybrominated diphenyl ether flame retardants: blood, urine, and sweat study. *Biomed Res Int*. 2017;2017:3676089. PubMed PMID: 28373979.
257. Crinnion WJ. Results of a decade of naturopathic treatment for environmental illnesses. *J Naturopathic Med*. 1997;17:21–27.
258. Boyle W, Saine A. *Lectures in Naturopathic Hydrotherapy*. OH: Buckeye Press; 1988.

36

The Exercise Prescription

Keoni Teta, ND, Jade Teta, ND, and *Ralph Esposito, ND, LAc*

OUTLINE

INTRODUCTION

Movement has been inextricably linked to health, well-being, and survival throughout human existence. It served as the means to procure food and was the key tool in protecting ourselves in times of danger. The ability to fight, flee, migrate, and forage was at the very core of life. Today, movement is no longer required for survival, and food is easily available with little need to expend energy. Yet the ancient connection between movement and metabolism remains permanently imprinted into our physiological function. Combating the diseases of the modern day is impossible without restoring movement as a permanent way of life.

Because a full discussion of movement and exercise physiology is beyond the scope of this chapter, this chapter will instead focus on movement as medicine for the most common diseases, with a special focus on the overlapping mechanisms that account for exercise's beneficial effect. A strong emphasis is given to newer understandings of exercise physiology and metabolism and how this information relates to the practice of medicine and the historical human condition.

The obesity epidemic, by most estimates, is the greatest health challenge of this century. It already represents one of the largest modifiable risk factors for increased morbidity and mortality, with close to 66% of the U.S. population being overweight or obese.[1–3] Because of the huge challenges obesity represents and the powerful role exercise can play, this chapter is framed in the context of weight management. Other related health issues, such as exercise approaches for inflammation, cardiovascular disease, cancer, mood, sexual dysfunction, and the brain, are also discussed.

EXERCISE IN HISTORICAL CONTEXT

Human movement patterns have always necessitated multiple parameters of fitness. Understanding these historical movement patterns provides a guiding context in which to view exercise. Although research in exercise is essential to understand, without a guiding context, it can be difficult to provide an appropriate exercise prescription. Viewing movement through the lens of human evolution, we believe, provides the frame of reference necessary to understand how to use movement as medicine.

Longer-duration, lower-intensity exercise made up the majority of early human activity.[4–6] Walking was the chief means of movement for millions of years. Early humans walked most of the day and did this every day. Their survival was dependent on it. Research suggests that early *Homo sapiens* foraging on the plains of Africa covered a range of over 10 miles daily, hauling weapons, game, and tools.[4–7] Analysis of modern-day hunter–gatherers confirms this degree of walking activity.

In addition to this lower-intensity activity, survival also depended on higher-intensity, shorter-duration activity. Sprinting away from danger or after wild game and lifting and hauling were essential. Both women and men engaged in these more anaerobic bouts of exercise. Although in most cultures women did not participate in the hunt, they did lift and haul wood, help construct shelters, and carry babies. The average baby was carried almost 1000 miles in the first 2 years of life in more traditional cultures.[5] The more intense activities of hunting, hauling, and foraging were done in what is termed the "paleolithic rhythm," where more intense activities were spaced out over 2 to 4 nonconsecutive days per week.[5,7]

Moderate-intensity exercise, much like aerobic exercise today, was also a part of life. However, this exercise did not take the shape of going for a jog or leisure run. Aerobic exercise was mostly in the form of sporadic running after food and dancing, which occupied an important place in the culture of ancient peoples and still does in modern-day hunter–gatherer societies. These dances can last from one to a few hours and take place several times during the week.[5–7]

The movement patterns of our historical ancestors were a necessary part of life and were fully integrated into the lifestyle. This movement was what could be best described as "cross-training," with elements of lifting, jumping, running, walking, throwing, climbing, hauling, hiking, or whatever movement pattern was required to procure food, stay protected from the elements, and defend territory. Our species is adapted from an environment where food was not guaranteed and a nomadic hunter–gatherer way of living dominated. This understanding is essential for the appropriate prescription of exercise. Hunter–gatherer societies in the past and present have been analyzed through a number of different scientific tools, which show that they do not experience the same degenerative and chronic diseases of modern, Westernized peoples.[4,8,9]

After taking into account body weight, the modern-day human energy expenditure is estimated to be only 38% of that of our hunter–gatherer ancestors.[5] It has been shown that for modern-day humans to approximate the level of activity of hunter–gatherers, they would have to walk 12 miles/day in addition to other activity. [5] Policy leaders on appropriate levels of human activity, such as the American College of Sports Medicine, recommend movement patterns estimated to be 44% less than that of our prehistoric ancestors and modern-day hunter–gatherers.[5]

Several studies have shown that total energy consumption from exercise may be the primary health-promoting property of movement.[5,10,11] Given the drastically different environment in which humans now reside, we are left with a serious dilemma. How do we get people to do an activity not required for their survival, that takes time, and is not an enjoyable activity for many? It is useful to remember that the number-one reason given for not participating in exercise is time.[12] These facts, and the historical frame of reference provided by evolutionary movement patterns, create a problem. Exercise prescriptions must deliver acceptable caloric expenditure, should mimic the cross-training attributes of our ancestors, but must also be time conscious and "doable" for the average population.

A NEW PERSPECTIVE ON MOVEMENT

It is easy to look at the data presented and quickly move to a "more is better" approach to exercise. However, movement quality is an important consideration before simply instructing more exercise. Quality in this segment is discussed in terms of more functional and efficient movement. In other words, movement that provides the perfect balance of range of motion and stability leads to more efficient animation of the body. The body is a chain of parts that are completely integrated. Individual exercises, although viewed in isolation by us, are actually complex and highly entrained patterns of neurological connections translated into muscular action. These "movement patterns" are the building blocks on which all exercise is constructed. Without a strong foundation, the benefits of exercise can sometimes become a liability, increasing the risk of injury, pain, and dysfunction.

It is both irresponsible and shortsighted to focus exercise prescriptions solely on delivering greater metabolic stimulus if there is not a strong and appropriate base of mobility and stability through basic movement patterns. In other words, doing more activity may not be beneficial if it simply reinforces poor movement mechanics. We must keep in mind that our ancestors were able to move more because they also moved better.

Human development and survival from infancy to adulthood required mastery of movement. The neurological and muscular systems are built as a unit and cannot be separated. Movement should not be thought of in terms of isolated anatomy but rather as an integrated system that moves in patterns. A squat, for example, is not simply a matter of contracting certain muscles that move joints. It is instead a coded and rehearsed pattern of neurological firing translated into physical movement.

Gray Cook, physical therapist and the foremost expert on functional movement, is the author of two of the most foundational pieces of work in movement science. In his books *Athletic Body in Balance: Optimal Movement Skills* and *Conditioning for Performance* and *Movement: Functional Movement Systems,* Cook described movement science as analogous to the relationship between computer software and hardware.[13,14] The muscles and joints act as the hardware, but the neuromuscular firing pattern is the software. Software obviously is what controls and informs the computer, and so it is with the nervous system controlling the muscle. To address movement, he rightly pointed out that we need to work movement patterns and not simply isolate parts.

The brain does not view exercise as individual muscles working. Instead, the brain has a code or a pattern for each movement. This pattern consists of thousands of different neurons firing in very specific ways to elicit a coordinated action, resulting in a movement. Each movement is to the brain like a computer software program; movement science calls this a motor program. It is these motor programs that need to be attended to in terms of movement quality.

Although exercise science has developed core principles and protocols to be followed, movement has not developed such protocols. Up until very recently, there has been no standardized understanding of what constitutes normal functional movement. This gap has now been filled by the work of Gray Cook and his colleagues. They have put together a system of screening and analyzing movement so that it can be objectively measured, tracked, and corrected. If we are to use movement as medicine and stay true to the natural medicine axiom of "first, do no harm," we must establish a standard of practice in movement medicine.

STANDARDS OF MOVEMENT

The Functional Movement Screen and Selective Functional Movement Assessment

The Functional Movement Screen, or FMS, as it is popularly known, is "a ranking and grading system that documents movement patterns that are key to normal function." By acting as a screen for movement, the FMS pinpoints movement dysfunctions that may predispose to injury and/or inefficiency.

The FMS has a scoring system that allows for a hierarchical ranking of the most limiting patterns or asymmetries unique to an individual. This in turn provides direction to clinicians as to what corrective strategies should be implemented and when. The FMS score can then be used to systematically address underlying movement dysfunctions and restore a base of movement from which conditioning can be built.

The FMS is complimented by the Selective Functional Movement Assessment (SFMA). The SFMA is the diagnostic system of functional movement. For the clinician concerned with movement, the two work hand-in-hand. The FMS is both the precursor to and the follow-up from the SFMA. The FMS looks at seven key movement patterns, including a squat, lunge, and hurdle step. Its purpose is to ascertain a baseline of mobility and stability and to determine whether movement

is pain-free. The SFMA is the clinician's tool for the diagnostic determination of when pain is present. Together, the screen and the assessment provide a movement system that allows tailored movement plans that can find, address, and track changes in movement from pain and dysfunction to recovery and optimal movement.

The FMS is a screening tool for the clinician; it is not diagnostic but rather prognostic. The SFMA is diagnostic and helps the clinician pinpoint the issue and further prioritize treatment. Once a patient has been discharged from treatment and no longer has pain with movement, movement quality must still be measured. This is where again the FMS is used.

Several recent studies showed the FMS to be a reliable and repeatable screening tool for movement quality, but they also proved its ability to predict injury in several high-population groups. Minick et al.[15] showed that the FMS demonstrated substantial interrater reliability by both novice and expert users. This demonstrates an important quality of any clinical tool, that of repeatability and objective evaluation in the hands of different users.

When the FMS was put to the test in very active population groups, its usefulness became clear. American football players were analyzed in a 2009 study in the *Scandinavian Journal of Medicine, Science and Sports;* they demonstrated reliable improvements in FMS scores from a standardized intervention based on the screening examination and established an FMS score cutoff predictive of increased injuries.[16]

Firefighters benefitted from an FMS-guided functional exercise program.[16]The FMS-guided intervention resulted in a 62% reduction in time off of work because of injuries and reduced injuries by 42% during a 12-month period.

Movement pattern performance is an important precursor to increased exercise participation and is an important addition to the exercise world. The FMS and SFMA are new and essential tools for the use of exercise as medicine. They provide a much-needed "standard operating procedure" for movement quality. Gray Cook and colleagues have a clinical website (http://www.functionalmovement.com) that teaches and instructs on movement systems and provides valuable clinical tools for clinicians.

New Insights on Exercise for Weight Loss

Once movement quality is attended to, exercise quality needs to be addressed. The current approaches of exercise for weight loss and health are still centered on the low-intensity, "aerobic-zone," calorie-burning paradigms. Recent understanding is redefining this narrow approach to health and fitness. A caloric-focused model of metabolism may not be the most useful way to view exercise. If we look at track athletes, both elite marathoners and sprinters have very low percentages of body fat. Sprinters, however, have less body fat and higher amounts of muscle mass, yet they exercise in a very different way.[17–19] Sprinters engage in short bursts of anaerobic effort lasting seconds, whereas marathoners run in an "aerobic zone" for hours and use large amounts of caloric energy. If the aerobic model of exercise truly is the best way to gain fitness and fat loss, why is there a discrepancy between these two groups of athletes? In analyzing recent data, we can see that anaerobic contributions to energy expenditure now need to be considered in exercise prescription.[20–22]

There is a common misunderstanding of fat-burning percentages in exercise. Many still perceive exercise done in the aerobic zone, usually defined as between 65% and 80% of maximum heart rate (MHR), as burning the most fat per unit time. This is not the case. Lower exercise intensities burn a higher proportion of fat compared with sugar, relatively speaking. However, exercise beyond the aerobic training zone burns more total energy and fat because it is a higher intensity.

Suppose two people exercise for 30 minutes. Person A jogs at an intensity of 60% MHR, whereas person B jogs at an intensity of 60% MHR and then, every few minutes, sprints for a short period, reaching over 90% MHR before returning to a lower intensity. Assume person A burned 200 calories, 60% coming from fat and 40% from sugar. Person A therefore used 120 total units of fat and 80 units of sugar (200 calories × 0.60 = 120 fat calories). Person B exercised at a higher intensity, burning a lower percentage of fat. Assume Person B used 50% fat and 50% sugar but burned 100 more calories because of the higher workload. Person B therefore burned 150 units of fat and 150 units of sugar (300 × 0.50 = 150 fat calories). When the energy burned is totaled, person B burns more energy (300 calories) and more total fat (150 units compared with 120 units) than person A, despite using a lower percentage of the energy as fat. This example shows that higher-intensity exercise of the same duration surpasses lower-intensity exercise in fat calorie use. There are further metabolic consequences created through higher-intensity exercise that make its application in exercise prescription for fitness and fat loss more intriguing.

Does Aerobic Exercise Work for Weight Loss?

A 2009 review by Melanson et al.[23] looked at the effect exercise had on metabolic stimulation. The study primarily looked at moderate-intensity aerobic exercises like jogging, biking, or swimming while including a small sample of anaerobic exercise studies. It showed that aerobic exercise of moderate intensity did not provide a metabolic advantage aside from the calories burned during activity. A previous meta-analysis done over a 25-year period came to a similar conclusion.[24] This study analyzed the data from over 400 studies comparing the effects of diet alone, aerobic exercise alone, or diet plus aerobic exercise on weight loss. The results showed that aerobic exercise did not provide a significant advantage to weight loss over diet by itself. Although aerobic exercise has been shown to be a reliable tool in the maintenance of weight loss,[22,23] these studies suggest it may not be enough to elicit significant fat-loss effects.[24–27]

At the same time, new lines of research show potentially promising effects from more anaerobic modalities. High-intensity interval training (HIIT) is a method of alternating highly anaerobic activity with more relaxed aerobic movement. This method allows the benefits of intense exertion under more tolerable conditions. Rest is required to sustain intense activity for any length of time. Weight training is another anaerobic-centered activity. These more anaerobic-dominated exercise approaches have unique benefits that may complement the calorie-burning effects of aerobic exercise.

Aerobic Versus Anaerobic Exercise

In very simple terms, aerobic metabolism takes place in the mitochondria and requires the use of oxygen. Anaerobic metabolism proceeds through a different pathway and requires neither the involvement of the electron transport chain nor oxygen. It is well known that as exercise intensity increases, anaerobic metabolism dominates; unfortunately, the exact anaerobic contribution to energy production is exceedingly difficult to measure. The standard way to approximate calorie expenditure and substrate utilization during exercise is through the measure of respiratory gases. The ratio of carbon dioxide expelled to oxygen consumed can give a predictable evaluation of not only energy use but also fuel utilization—glucose versus fat.

However, this method is only valid at lower exercise intensities. At higher intensities, the relationship is less clear. To help address this error, researchers also measure excess postexercise oxygen consumption (EPOC). This is a measure of the recovery energy expenditure after exercise, and it has been thought to consist of anaerobic contributions to exercise as well. There is

some argument as to how meaningful this EPOC effect can be. Many researchers claim the effect does not last long, only several hours, and amounts to, at best, 15% of total calories burned.[28–30] However, these approximations come largely from studies with lower exercise intensities involving standard aerobic exercise protocols.

Studies using highly anaerobic protocols including cardiovascular interval protocols and weight training showed a much different picture. In 2001 Schuenke et al.[31] showed that circuit resistance training, using heavy weights and short rest periods lasting only 31 minutes, were able to generate an EPOC that persisted for 48 hours. The results showed that metabolism 24 and 48 hours after the exercise session was increased by 21% and 19%, respectively. The researchers pointed out that for a typical 180-lb individual, "this equates to 773 calories expended post exercise" while resting and as a result of the workout. This is far from insignificant and greatly exceeds the 15% many researchers quote for EPOC. Similar findings were shown in women using a similar resistance-training protocol. In women, the elevation in metabolic rate lasted 16 hours.[32–34] The same findings were seen with HIIT protocols, with significant EPOC values lasting up to 24 hours.[33–35]

Exercise Burn and "After-Burn"

Dr. Christopher Scott of the University of Southern Maine published extensively in this area and is the author of one of the authoritative textbooks in this field, *A Primer for Exercise and Nutritional Sciences: Thermodynamics, Bioenergetics, and Metabolism*.[36] In his works, Dr. Scott points out that EPOC does not fully explain anaerobic energy use and that the anaerobic contributions to exercise might be even greater than originally thought, especially where lactic acid production is concerned. Dr. Scott emphasized that to fully account for calories burned during exercise, three components must be measured: (1) calories burned aerobically during exercise, (2) calories burned aerobically after exercise (EPOC), and (3) anaerobic calories burned from exercise.[37–41] EPOC and the anaerobic lactic acid measurements for exercise should be considered separately according to Dr. Scott.

A 2005 study by Dr. Scott demonstrated the potential ramifications of anaerobic exercise. This study compared a 3.5-minute aerobic exercise challenge with three work-equivalent 15-second sprints.[42] Calorie use during the exercise bout was calculated to be 29 kcal for the aerobic exercise and approximately 4 kcal for the sprinting. However, when the EPOC contribution was added to the two exercise bouts, energy use rose to 36 kcal for the aerobic bout and 39 kcal for the sprint exercise. Finally, when the purely anaerobic contribution was added to the calorie totals, the numbers for the anaerobic sprint exercise rose significantly. The final tally was 39 kcal for the aerobic exercise compared with 65 kcal for the sprint exercise. By adding both EPOC and the anaerobic contribution to the original calorie total, the sprint exercise was shown to far surpass the aerobic exercise in calories burned. This is interesting when one considers the aerobic exercise session took over four times longer to complete (210 vs. 45 seconds). Without including both the EPOC and anaerobic energy use, a full 94% of the calories used during the sprinting would go uncounted.

In studies published in 2006 and 2009 in the *Journal of Strength and Conditioning Research,* the same researchers quantified anaerobic energy use during weight lifting.[37,41] Using the method of measuring and quantifying all three components of calorie burn (aerobic metabolism during exercise, EPOC, and anaerobic contributions), these studies showed that weight-training exercise burned 70% more calories than originally thought.

Hormonal Versus Caloric Exercise

To understand the full ramifications of this new exercise science, the discussion must move to hormonal metabolism. Hormones as we describe them in this chapter refer to all signaling molecules in the body, including steroid hormones, muscle-derived cytokines (myokines), and other signaling molecules. Hormonal messengers directly influence the fat-burning stimulus during exercise and may be responsible for part of the EPOC after-burn as well.

Brief bouts of anaerobic exercise appear to adjust hormones for greater caloric burn during and after exercise.[43] This increased energy use is partly explained by EPOC. This is a measure of how much oxygen the body consumes in the hours and days after a workout. An example of EPOC in the acute sense is climbing a long flight of stairs. While walking up the stairs, breathing is labored, but respiration becomes most difficult after reaching the top. The body does this to recover the "debt" of oxygen used during activity. The EPOC created by climbing a flight of steps is an example of the much larger metabolic effect created from intense movement.

Exercise of sufficient intensity elevates stress hormones like adrenaline, noradrenaline, and cortisol. Together these hormones ensure the switch to glucose metabolism, which historically supplied the energy to fight or flee. As exercise intensity is elevated further, anaerobic contributions to metabolism increase. This produces lactate (lactic acid), which, contrary to common belief, is not a waste product, but a physiological buffer and signaling molecule.[44–46] As lactate rises, it is correlated with, and some studies suggest actually induces, the anabolic steroids testosterone and human growth hormone (HGH).[47–49] This "hormonal soup," catecholamines along with cortisol, HGH, and testosterone, acts synergistically to increase postexercise fat loss and lean muscle tissue production, thus creating a more fit and functional physiology.

Aerobic and anaerobic exercise have different hormonal effects. Hormones do not work in isolation, and like people, they behave differently depending on the social environment. Interestingly, the hormone cortisol, often seen as a fat-storing hormone because of its insulin-desensitizing effect, behaves differently when combined with growth hormone and testosterone.[46,50,51] When cortisol is "socializing" with testosterone and growth hormone, its catabolic action on muscle is blocked, fat storing at the belly is attenuated, and the three may synergistically enhance fat burning.[51–54] Attempting to blunt the cortisol response to high-intensity exercise may be counterproductive for fat burning and not necessary in the context of growth hormones.[55–58]

Long-duration, lower-intensity cardiovascular exercise behaves differently in regard to cortisol. There are also key changes in hunger hormones that are different between lower-intensity aerobic exercises compared with higher-intensity anaerobic workouts. With aerobic-zone exercise, there is a danger of cortisol increasing unopposed by the growth-promoting hormones.[50] This, along with a lowering of leptin and an increase in ghrelin, can lead to compensatory eating with specific cravings for fatty, sugary, and salty foods.[59–65] Higher-intensity, shorter-duration activity has the opposite effect on ghrelin, with a more balanced ratio of catabolic versus anabolic hormones.[62,63] This may explain why standard aerobic prescriptions have not been shown to be as effective for optimal body composition.[23,24,66–69]

Aerobic Zone Versus Interval Training

A 2001 study compared standard aerobic-zone training and anaerobic-interval exercise in women.[70] The anaerobic-interval group exercised for 2 minutes at a highly intense 97% of MHR. They then rested by doing 3 minutes of low-intensity activity. The more aerobic group performed moderately intense activity at close to 70% of MHR. The researchers made sure that each group burned 300 calories. Despite

exercising longer and burning the same amount of calories, the aerobic group lost less body fat at the end of the study compared with the interval group. In addition, fitness in the interval group was substantially greater than in the aerobic group.

A similar study published in the same journal in 1996 showed that an anaerobic-interval group burned significantly more fat than their aerobically trained counterparts.[71] Not only did the interval group burn greater amounts of fat during exercise, but they also exhibited increased fat-burning effects that persisted for 24 hours after the exercise had stopped. The interval group was able to accomplish this with an exercise session that was a full 15 minutes shorter than the aerobic group.

A 1994 study[35] tracked two groups of people, one group doing aerobic training for a period of 20 weeks, whereas a second group was followed for 15 weeks and engaged in HIIT. The researchers wanted to see how each program would affect body composition. The aerobic group burned 48% more calories than the interval group (120.4 vs. 57.9 MJ) during exercise. The interval group, however, enjoyed a ninefold greater loss in subcutaneous fat. At the conclusion of the study, muscle biopsy analysis showed that resting levels of 3-hydroxyacyl coenzyme A dehydrogenase, a marker of fatty acid oxidation, were significantly elevated in the interval group but not in the aerobic group.[35]

A 2008 study looked at intense intermittent exercise compared with steady-state aerobics.[72] Forty-five healthy women between the ages of 18 and 30 were recruited for the study, divided into three groups, and studied for 15 weeks. One group did HIIT, where they sprinted on a bike for 8 seconds followed by a 12-second rest. This was repeated for 20 minutes. Another group did moderately intense peddling that was sustained for 40 minutes. The final group did no exercise. At the end of the 15 weeks, the high-intensity interval group lost 2.5 lb of fat, whereas the aerobic group actually gained 0.6 lb of fat. A measure of the fat-related hormones leptin and insulin were also positively affected in the HIIT group compared with the steady-state group. This was accomplished with a workout that was half as long (20 vs. 40 minutes) as the steady-state's group.

Resistance Training Studies

Research hints that resistance training may also provide unique benefits for fat loss and fitness. Recent analyses showed that the clear distinctions once set for aerobic exercise and resistance training are no longer delineated so clearly. Circuit training routines have been shown to provide an aerobic stimulus great enough for cardiovascular benefit while providing strength-training benefits.[73,74] Resistance training may also have great applicability for not just fitness and fat loss but also diseases of insulin resistance.[75,76] Weight training, compared with aerobic exercise, greatly attenuates the natural loss of muscle mass that occurs with aging and dieting[77] and improves body composition and self-esteem better than aerobic exercise modalities.[78–80]

Resistance training may also have a much greater effect on EPOC. Two studies already discussed[32] showed significant metabolic elevations in men lasting up to 48 hours and in women lasting up to 16 hours. These workouts used exercise regimens that were more intense than most studied resistance-training programs, but they were also shorter. Combining the benefits of resistance training with cardiovascular exercise seems to have the most benefit.[81] This type of training is called concurrent exercise and involves resistance-training workouts that are followed immediately by aerobic exercise or vice versa. Studies showed that these approaches afforded the benefits of both aerobic and resistance-training workouts.[82–85] This "cross-training" approach falls in line with historical movement patterns and saves time.

Concurrent exercise approaches follow two patterns, serial concurrent exercise (SCE) and integrated concurrent exercise (ICE). In SCE, the two modalities (aerobic and resistance training) are done one right after the other, whereas in the integrated format, the two modalities are alternated; a weight-training movement is done, followed by a cardiovascular "burst" of exercise. Based on studies, it appears the benefits of this type of approach can be amplified further using the ICE approach. In a series of three studies in 2008, all published in the *Journal of Strength and Conditioning Research,* Davis et al.[86–88] showed the potential benefit of ICE protocols. An ICE workout consisted of brief 60-second bursts of "cardio-acceleration" placed between traditional weight-training sets. This protocol was able to dramatically amplify multiple fitness parameters over and above the same workout volume done in an SCE format.[87] Fat loss in the ICE group was close to 10 times greater than in the SCE group over an 11-week period. This constituted a time commitment of just over 4 h/week. Because the work was equivalent in both groups, it was the combination of exercises that made the difference. This same workout protocol showed the ability to improve cardiorespiratory and cardiovascular parameters even in well-trained athletes.[86] It also decreased the delayed-onset muscle soreness, the feeling of soreness lasting 24 to 48 hours after the workout, in the ICE group.[88] Similar protocols showed the same promising results in body composition change and cardiovascular benefits.[89,90]

Safety of Interval Exercise

HIIT, and intense weight training, can be used effectively and safely when combined with the appropriate use of heart rate (HR) monitoring, perceived exertion rate (PER), and the use of intervals: periods of exertion followed by rest. Studies show that this type of activity is manageable in several illness models, including chronic obstructive pulmonary disease,[91,92] post–coronary artery bypass patients,[93] congestive heart failure,[93] and heart transplantation patients.[94] This type of anaerobic stimulus more realistically mimics real-world challenge and allows for self-paced exercise that is safe, tolerable, and more beneficial for many heart and lung patients.[91–98] Cardiac patients also may have less risk with this type of activity because it has more favorable effects on ST-segment changes and heart rate variability (HRV).[96–98]

Monitoring HR is useful for any health care provider prescribing exercise. It is important to understand that HR equations are merely estimates based on age; there is much variability. HR equations, in general, underestimate HRs in the very fit. The old HR equation of 220 − age is an inferior HR equation that underestimates HR in the old and overestimates in the young.[99] Newer equations allow for better predictive value. Based on current understanding, women and men should use separate equations for predicted HR percentages. For men, the MHR equation should be 208 − age (0.7).[99] For women, this equation should be 206 − age (0.88).[100] It is also useful to know the equation for translating percent of MHR to percent of oxygen uptake (VO_2), and vice versa. That equation is %MHR = (0.64) %VO_2 + 37.

Because multiple drugs and disease states can interfere with HR, exertion ratings used alone or combined with HR are a more useful clinical tool. In exercise research, a 16-point exertion scale called the Borg scale is used. It is a cumbersome tool to use and teach in clinical practice. A simple 1 to 10, with 10 being maximum exertion and 1 being at rest, is a far more useful tool clinically. It is easy to teach and implement. Traditional aerobic exercise scores between 6 and 8 on the 1 to 10 scale. HIIT will reach between 8 and 10 on the work phase and between 1 and 4 on the rest phase. In clinical practice, this is a useful aid in working with exercise intensity. Combining HR percent monitoring with PERs and heart rate recovery (HRR) allows for tight control of workout safety.

Two other useful clinical tools for health care practitioners are the "talk test" and HRR. Exertion and respiration are closely linked. The ability of a person to talk during exercise is a direct indication

of whether they have crossed into the anaerobic zone.[101] For the unfit, this usually occurs around 55% of VO_2 max, and for the very fit, it occurs around 85% VO_2 max. This corresponds to 72% MHR and 91% MHR, respectively, leaving the average healthy exerciser right around 80% of MHR for the anaerobic threshold, at which point the ability to talk will be compromised. Correlating this "talk test" with an individual's HR allows practitioners to closely monitor exercise. HRR is a measure of how fast the heart recovers from exertion and is an indication of sympathetic and parasympathetic tone. A healthy heart should recover at least 25 beats/min or more within 1 minute after exertion. A heart that recovers 10 or fewer beats in 1 minute is a concern that should constitute a referral to a cardiologist.[102]

Muscle–Body Messengers and Inflammation

If we were able to see, in real-time, the molecular interactions occurring within muscles and fat tissue, we would see a unique interaction of muscle communication via myokines. Every time the body moves, muscles release signaling molecules that communicate to the rest of the body. The endocrine properties of muscle, like fat, have been confirmed.[103–105] We are now learning that myokines, cytokines derived specifically from muscle, give instructions to the body about how to function and adapt and may very well hold the key to controlling chronic inflammation.

Interleukin-6: The Exercise Factor

Of the numerous myokines, interleukin-6 (IL-6) is one of the most important in understanding the inflammatory response. IL-6 is generally known as a proinflammatory cytokine and a member of the inflammatory triad: tumor necrosis factor-alpha (TNF-α), IL-1, and IL-6. When all three of these molecules are released in the bloodstream, we find a proinflammatory effect, typically found with infection and sepsis. However, when released during exercise, IL-6 can have protective effects.[106,107] Skeletal muscle contraction induces increases in IL-6 while keeping TNF-alpha and IL-1 levels at bay via IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors (sTNFRs).[108–110] IL-1ra inhibits interleukin-1-beta (IL-1β), a major proinflammatory cytokine, but also induces IL-10, which is not only anti-inflammatory itself but sets off a cascade stimulating other anti-inflammatory cytokines while concurrently inhibiting proinflammatory cytokines such as TNF-α. [106,109,110]

The IL-6 effect implicates exercise as a first-line defense against inflammation and thus suggests the benefits of resistance training in chronic illness and inflammatory conditions such as rheumatoid arthritis, heart disease, diabetes, and cancer.[111–115] Increasing exercise intensity, full-body muscle contraction, and muscle glycogen depletion are the major exercise elements enhancing IL-6 release from muscle.[104,116–118] These factors together can induce an increase of plasma IL-6 that is 20- to 100-fold over resting levels.[104,118] At these levels, IL-6 begins to exert influence over the body, relaying messages about the metabolic needs of the muscle. In this way, IL-6 acts more like a hormone than a cytokine by sending communications from muscle to adipose tissue, immune cells, and the liver instructing the body to burn fat via adenosine monophosphate kinase (AMPK) [116,119] and hormone-sensitive lipase (LPL),[113] control glucose regulation by improving insulin sensitivity,[120] and inhibit the production of the proinflammatory cytokines.[121,122]

Interleukin-15: The Arnold Cytokine

A potent antiobesity and anti-inflammatory myokine, IL-15 has gained the title the "Arnold cytokine" after the famous bodybuilder Arnold Schwarzenegger. This myokine is a potent anabolic factor[123] that is upregulated in trained skeletal muscle, particularly type 2 muscle fibers, which are most activated in resistance training over endurance exercise.[124,125] Furthermore, IL-15 decreases fat deposition, reduces white adipose tissue, lowers visceral adiposity, and possibly may inhibit TNF-α, thus promoting an anti-inflammatory benefit. These effects are desirable because muscle mass is frequently lost along with fat in aerobic-centered exercise programs.

We do know that an accumulation of adipose tissue, particularly visceral adipose tissue, induces chronic systemic inflammation via macrophage infiltration, thus leading to many downstream effects, such as insulin resistance, cardiovascular disease, diabetes, and neurodegenerative disease. IL-15, like IL-6, crosses over, having hormone-like action affecting the body systemically, thus explaining the important role these myokines play in the anti-inflammatory benefits of exercise.[126]

Lactate

Lactate has been correlated with several positive benefits to exercise. It is now believed that lactate is directly related to some of these beneficial changes through hormone-like action. As exercise intensity escalates, there is a large surge in catecholamine production. This occurs to supply the body with much-needed blood sugar to fuel intense exercise. As the exercise intensity increases, aerobic physiology becomes "maxed out," forcing the body to become more anaerobic. This switch, believed in the past to be caused by an oxygen deficit, is currently understood to indicate mitochondrial shuttle saturation. When this occurs, pyruvate and hydrogen ions begin to accumulate in the cytosol. It is the accumulation of hydrogen ions that lowers the pH of the cell, causing fatigue and the familiar muscle burn of intense exercise. In this situation, lactate is quickly formed. Lactate can then be recycled back to glucose via gluconeogenesis, be burned directly by conversion back to pyruvate and entry into the Krebs cycle, and/or engage cellular machinery as a signaling molecule.

The major signal sent by lactate during exercise seems to be an "adaptation signal." Analysis of this action shows that lactate has two major functions: first, it increases cellular events, leading to mitochondrial generation,[127,128] and second, it stimulates the release of growth-promoting hormones, including HGH and testosterone.[46,129–131]

The major action on cellular signaling seems to be the upregulation of MCT-1.[128] MCT-1 is an embedded protein in the mitochondrial membrane that speeds the formation of pyruvate from lactate, thus making existing mitochondria more efficient. In addition, research by Hashimoto et al.[127] in 2007 elucidated a complex mechanism whereby lactate interacts directly with key genes involved in mitochondrial biogenesis. Taken together, this opens a whole new understanding for improving metabolic efficiency. By harnessing the power of lactate, mitochondrial levels can be increased and enhanced, resulting in many beneficial effects on total physiology.

The idea of lactate as a hormone is a novel concept. However, it has been made clear through several studies over the past few years that lactate is a key signaling molecule in exercise metabolism. Interestingly, a lactate receptor called GPR81 was recently isolated in rats, confirming it does act like a hormone.[132] Other studies have shown that lactate can directly stimulate the release of testosterone, progesterone, and HGH.[44,129,133–135] This lends further credibility to lactate's role in helping the body adapt and grow.[136]

Testosterone and Human Growth Hormone

Testosterone and HGH, in particular, are key players in human metabolism, especially as they pertain to physique development, combating obesity, and antiaging effects. Testosterone and HGH, in addition to

other effects, increase lean muscle tissue and decrease fat mass. Both of these anabolic hormones are more pronounced with anaerobic exercise.

Exercise Approaches to Inflammation

IL-6's release from muscle cells is not a nervous system phenomenon and is not based on muscle injury. It seems the impetus for IL-6 release is mechanical.[105,118] In other words, just the act of movement is all that is required. However, there are ways to amplify IL-6 production during exercise. The science of exercise metabolism now goes far beyond simple calories. The ability to harness the far-ranging hormonal and cytokine effects of exercise can be accomplished through the use of short-duration, high-intensity exercise techniques used in athletic populations for decades. Although the term *high intensity* has the tendency to cause reservation, these tools and techniques can be adapted for use in even the least fit and most populations with inflammation.[32,112,137]

Before discussing the techniques in this approach to exercise, it is important to define why short, intense exercise may be more useful than traditional approaches. The damage associated with chronic inflammation is compounded by a lack of offsetting growth factors. The body produces these growth factors in response to intense exercise. Testosterone and especially growth hormone are known to be factors linked closely with intensity. The word *intense* as used here means exercise that is glycogen depleting—that is, it significantly reduces the body's muscle and liver sugar stores. Only two types of exercise are likely to produce these effects, long-duration exercise lasting hours or short, intense, sprint-type exercise. There are obvious constraints to prescribing hour-long exercise sessions because lack of time is the number-one reason cited for lack of exercise participation, making short, intense exercise more realistic. In addition, the overall hormonal response to long-duration exercise is counterproductive because it raises cortisol levels above the body's ability to compensate with growth promoters.[51–57,138]

This type of exercise also makes sense because it creates a hormonal environment that produces sustained fat burning and muscle growth.[32,58,70] The amount of glycogen reduction is directly correlated with IL-6 release, and high-intensity exercise is shown to increase IL-6 and catecholamines together.[105,109,117,118] Catecholamines have their own independent effect in lowering TNF-α and IL-1, synergistically enhancing IL-6. Combining these known effects with techniques that can deliver the same benefit in less time presents the opportunity to supply these anti-inflammatory effects in short time periods.[109,122,139]

EXERCISE PROTOCOLS

Holistic Fitness Prescription

Based on the research reviewed in this chapter of the new understandings emerging from exercise research, it seems a cross-training approach in line with our historic movement patterns would provide the most benefit. This approach would include walking, aerobic-zone exercise, resistance training, and cardiovascular interval training. However, realizing exercise prescription needs to be feasible and time consciousness necessitates combining elements to generate the most health benefit.

Putting this information in the historical context, it would appear that daily walking should be done as much as feasible. Walking should be viewed as a necessity rather than exercise. Based on historical information, walking constituted the predominant activity of our ancestors. Walking not only burns calories but also has restorative and relaxing effects. Because walking and eating are generally incompatible, it may be a suitable replacement for eating behavior. Walking may best be done in the evening and for hormonal benefit; walking in a natural setting may be more beneficial than walking in more urban settings.[140] Studies have shown positive effects on lowering cortisol.[141]

In addition to walking, aerobic-zone training should be instituted as well. Based on most data, an aerobic program lasting between 20 and 60 minutes 3 to 5 days/week seems optimal. Based on new information, aerobic exercise may be best for weight maintenance and not the most optimal for attaining weight loss. Given the new data on HIIT, the time spent doing aerobic exercise may be able to be cut in half. A HIIT program done 3 times/week for 20 to 40 minutes could save considerable time[72] and provide the same cardiovascular benefit and perhaps better weight-loss results without the compensatory food behaviors.[59–65]

Recommendations capitalizing on the concept of non–exercise-associated thermogenesis (NEAT)[142] and burst training[140-143] may have special applicability in today's time-starved world. NEAT involves accumulating as much non–exercise-related activity as possible and includes everything from washing the dishes to fidgeting with a pencil, to nervously bouncing a leg up and down and even nonsitting time. Burst training is a concept popularized by Mark Smith, PhD, and involves accumulating several 1-minute anaerobic bouts of activity throughout the day. Several interesting studies showed this approach was able to generate clinically meaningful weight-loss results with only minutes of exercise per day.[142,143] Instituting behavioral changes that involve parking further away, doing a burst of activity by running up the steps, standing rather than sitting, and even fidgeting when standing or sitting still can all have a significant effect on total energy expenditure.[144]

Weight-training programs should also be a center point of health, fitness, and fat loss.[145] Resistance exercise excels at maintaining muscle mass and helps attenuate rebound weight gain.[146] Three full-body workouts per week, using moderately heavy weights in the 8 to 12 repetition range for 3 to 5 sets appears optimal. Again, because time is an increasingly limiting factor, integrating resistance training with interval training into one workout 3 to 5 times/week for 30 to 60 minutes can save considerable time yet still yield significant caloric expenditure and fat loss.

Rest-Based Training Method for Exercise Prescription

Rest and movement are often seen as opposites, but they are actually complementary and dependent on each other. Exercisers told to run as fast as possible for 10 minutes will necessarily regulate intensity to complete the task. If they were instead told to run as fast as possible for 10 seconds, the intensity could be dramatically elevated. True high-intensity exercise is impossible to achieve without rest. Quality rest leads to quality work, and vice versa.

HIIT and intense weight lifting have always coupled work with rest. One issue with these workouts is the rigid structure. The work/rest ratios "force" individuals of varying fitness levels to work at mandated levels. This works to lower intensity, inducing the same pacing effect seen in traditional aerobic exercise. These types of workouts are often too intense for many and can create psychological resistance to participating.

Rest-based training (RBT) uses rest, autonomy, and time manipulation to optimize intensity for all fitness levels. It combines the latest in exercise science and motivational psychology. RBT enjoys the same physiological benefits of intense interval exercise and weight training but with key psychological benefits.

RBT differs in the application of rest. Although interval training and weight training have clearly defined work and rest ratios, RBT leaves the exerciser in charge of the duration of rest. The language employed in this type of training is "push until you can't, rest until you can." This shift in paradigm acts as reverse psychology for exercisers.

The motivational psychology of exercise is an important consideration regarding exercise consistency, frequency, and intensity. The primary goal or purpose of interval training is to maximize work effort across all work bouts and employ the shortest recovery time possible to maximize the training stimulus. Contrary to popular belief, research has shown that exercisers who have autonomy over their workout parameters will often work harder and are able to self-regulate to an optimal work/rest ratio for their physiology.[146,147]

There are four key attributes in our RBT system. All are geared toward maximizing work effort in a safe and scalable way. The key tenets of rest-based exercise are represented by the acronym REST.

Rest Based

Pushing to the point of rest is actually the goal of a rest-based workout. By putting the focus on rest as opposed to work, RBT not only automatically increases the quality of work but also makes exercise psychologically easier.[146–148] When exercisers know they have permission to rest, they may voluntarily work harder without even being consciously aware they are doing so.

Interestingly, animal research showed that intermittent exercise is inherent and may be an evolutionary adaptation to maximize distances covered per unit time.[147] Animals naturally engage in sporadic work/rest ratios during movement and self-regulate exercise to optimize both performance and recovery. Research showed that humans have the same capability.[146]

Extrinsic Focus

A major inhibitor of intensity is exercisers' focus on intrinsic sensations such as breathlessness, burning, and other uncomfortable feelings.[146,148] RBT uses strategies that focus participants away from these intrinsic sensations to more extrinsic factors. Workout parameters change quickly, monotony is minimized, circuits are used, exercise timing is limited, and different movement strategies are incorporated in the same workout. All of this is designed almost as a distraction technique so that the exercisers focus more on what they are doing versus what they are feeling. This helps them work harder and therefore rest more often.

Self-Determined

In psychology research, self-determination theory posits that when people are given control and choice over their options, internal motivation automatically increases.[149–151] With RBT, there is structure in the workout, but the exerciser is left in complete control over how hard to work, when to rest, and for how long and even has flexibility regarding exercise choice and modifications. These factors not only serve to increase the quality of work within a session[146] but can also improve exercise adherence from session to session.[146,149–153]

Time Conscious

Given that time and intensity are so closely linked, harder workouts by necessity must be shorter. RBT workouts can be as short as a 1-minute burst repeated multiple times throughout the day to as long as 40 minutes of continuous exercise employing start-and-stop working and resting. Workouts lasting over 40 minutes suffer in intensity and may have negative hormonal consequences.[62,63,72]

Work/rest ratios employed by traditional interval and weight-lifting workouts work for some but can be imperfect for most. By focusing on rest in a workout and allowing exercisers control over when they rest and for how long, optimal intensity for results can be achieved in a safe, scalable way. A 96-year-old grandfather would be able to use the same workout approach to deliver optimal intensity for him, whereas a 24-year-old elite athlete could use the concept to deliver an optimal intensity as well. Rest-based exercise can be seen as a new functional model for fitness and fat loss.

CLINICAL APPLICATIONS

Exercise and the Heart

Aerobic-zone exercise is a well-known preventive and treatment strategy for cardiovascular disease. Virtually all health care providers are aware of the aerobic exercise prescription advocated by most policy-making organizations as "20 to 60 minutes of aerobic exercise done at an intensity of 65% to 80% of MHR on all or most days."[156] Given that these recommendations are well established and universally accepted, we will focus our attention here on new research related to the heart.

Since the publication of the book *Aerobics* by Kenneth Cooper in 1968, participation in activities such as jogging, biking, swimming, marathons, and triathlon training has steadily risen. Newer research suggests, however, that this approach can be taken too far and that exercise for health, fitness, and fat loss should not be so one-dimensional. Dr. Cooper himself has conceded "In that book I said, 'The more exercise, the better.' But by 1982, after too many telephone calls from distraught widows whose 55-year-old, overweight smoker husbands had died jogging, I realized I had made a mistake. … Exercise wasn't a panacea that could fix everything, it had risks.… There may be a point of diminishing returns."[157] To improve our return on exercise investment, there must be adequate rest between sessions. Resting may also include active resting such as walking, restorative yoga, meditation, and tai qi. However, sleep is certainly the most important aspect of restoration, with 8 to 10 hours being necessary to reap the most from exercise. Of note is that the older the age, the more rest that may be needed between exercise sessions.

The risks of excessive aerobic exercise are now better understood. Ultra-endurance exercise, such as marathon running and triathlons, can have a negative effect on heart function. Intense, long-duration aerobic activity dramatically elevates oxidative stress in the heart, alters left and right ventricle function, and causes damage in otherwise normal hearts.[158–160] This is not to say aerobic exercise is dangerous but rather to highlight the potential downside of an aerobically dominated exercise prescription.

Research studying ultramarathon runners has found that these athletes experience significant detrimental changes in their immunological, hematological, and biochemical parameters. Ultra-endurance training in cold conditions may significantly impair immune function via changes in neutrophils, immature neutrophils, and monocytes, suggesting an increased risk of infection.[154] Furthermore, cardiovascular illness and renal dysfunction are evident with increases in blood viscosity,[155] postexercise anemia, increased creatine kinase (CK) and brain natriuretic peptide (BNP), and increased copeptin.[156,157]

On the other hand, there is some research suggesting that at least at the cellular level, certain biochemical processes are put into play with ultra-endurance athletes that suggest such exercise may impart longevity. One of the ways this can be assessed is via telomere length and cellular senescence. Aerobic exercise prolongs and prevents shortening of leukocyte telomeres and promotes telomere homeostasis by the action of human telomerase reverse transcriptase (TERT), the shelterin complex (or telosome), and miRNA.[158,159] Cellular senescence appears to be slowed in endurance athletes compared with their sedentary counterparts, with reductions in DNA damage, inflammatory markers, and induction of telomerase, which is associated with longer telomere length and longevity.[160]

Obesity may actually be a better target for exercise therapy because it is so closely related to heart disease. Any exercise approach that helps lower body fat can be viewed as a heart-healthy plan. Because

so many view exercise as a time-consuming chore and because lack of time is the number-one reason cited for lack of participation in exercise, interval-training exercise prescriptions may be better suited to heart protection. High-intensity interval training, as evident in several studies, provides both the cardiovascular and aerobic benefits of endurance and moderate-intensity training while providing the benefits of weight-resistance exercise. Improvements in insulin sensitivity, VO_2 max, and cardiac function[161] and increased muscular irisin are seen with high-intensity training over moderate intensity.[162] Yet a key limiting factor in exercise is actually physically exercising, but compared with moderate continuous exercise, high-intensity exercise with short bursts of action is more enjoyable and improves patient compliance.[163,164]

Interval Training and the Heart and Vascular System

The theory behind interval training is that the heart, like all muscles, needs to be challenged to repair and grow stronger. Theoretically, interval training allows the advantage of a harder challenge with less risk because each high-intensity bout is followed by recovery. If done correctly, the heart is forced to alternate between sympathetic stimulation and parasympathetic recovery. This, it is argued, is a more functional way to exercise because it trains HRR and heart stimulation. Because many cardiac events come from sudden unexpected anaerobic challenges, like shoveling the first winter snow, running through an airport to catch a plane, or walking up a large flight of steps, HIIT is believed to help the body prepare for these challenges by teaching the heart to work and recover quickly.

HRV and spontaneous cardiac baroreflex provide functional measures of cardiac parasympathetic activity. A study in April 2005 showed the effect of interval training on both.[96] Eleven healthy elderly men, mean age 74 years, underwent an intensive 14-week interval training program involving nine 1-minute bouts of exercise at 85% MHR followed by 4 minutes of recovery at 65% MHR. Aerobic capacity increased 18.6%, whereas HRV showed a significant parasympathetic shift at night from pre- to posttraining. The cardiac baroreflex response also improved, with 10 of the 11 participants showing a favorable response.

Benefits of HIIT may be good for peripheral vascular disease and cardiovascular disease. A 2006 study reported an observational study on the effect of HIIT on intermittent claudication.[165] A total of 47 patients were included in the study. Patients were asked to walk on a treadmill to maximal claudication pain six times in each exercise session with 3 minutes of rest in between. Once a patient could walk continuously for 6 minutes without reaching maximal pain, the speed and/or grade was increased. A rehabilitation score was calculated as the product of speed and grade achieved by the participants. Results showed that HIIT led to clinical improvement in symptoms and a higher tolerable workload by patients. No adverse effects were seen from the treatment, suggesting those with peripheral arterial disease could both tolerate and benefit from HIIT.

In 2007, Wisløff et al.[166] shed some light on the cardiovascular effects of HIIT versus traditional aerobic training for heart failure patients. Twenty-seven patients with stable postinfarction heart failure were randomized to either aerobic exercise (70% MHR) or HIIT (95% MHR for 4 minutes separated by 3 minutes at 50%–70% MHR) 3 times/week for 12 weeks or to a control group. The control group was told to follow the exercise advice of their family doctor and met for 47 minutes of walking at 70% MHR every third week. All groups did exercise on a treadmill. The major finding was that HIIT was superior to traditional aerobic training with regard to reversal of left ventricular (LV) remodeling, aerobic capacity, endothelial function, and quality of life. With respect to LV remodeling, in the HIIT group, the LV diastolic and systolic diameters decreased by 12% and 15%, and estimated LV end-diastolic and end-systolic volumes dropped by 18% and 25%, respectively. Prohormone BNP, a marker of hypertrophy and severity of heart failure, declined by 40% in the HIIT group. There was no change in the traditional aerobic group or control group in LV remodeling, pointing to a rather profound effect of HIIT on traditional aerobic exercise in this patient population.

As mentioned, HIIT has been shown to be safe and well tolerated in chronic obstructive pulmonary disease,[91,92] postbypass patients,[93] congestive heart failure,[93] and even heart transplantation patients.[94] As discussed, HIIT may realistically mimic real-world challenges. It can also be tailored into a self-paced format that is safe, tolerable, and beneficial for many cardiovascular conditions.[95–97] It was shown in one study to have more favorable effects on ST-segment changes than traditional aerobic exercise, in addition to the positive effects on HRV.[96–98,100]

Obviously, physicians prescribing exercise for high-risk patients will not want to completely abandon traditional aerobic exercise in favor of HIIT. Traditional aerobic exercise is well established to both treat and prevent cardiovascular diseases. More studies need to be done on HIIT, but it appears it may have some unique cardiovascular benefits. There are some considerations when prescribing this form of exercise. Work/rest ratios should be larger for the more fit and smaller for the less fit or more frail. A beginning exercise protocol for a younger fit person wanting to optimize cardiac prevention would be a work/rest ratio of 1:2. More frail and less fit clients will want to start with a work/rest ratio of 1:4 or greater. The RBT approach highlighted previously may provide increased benefit and safety when using intervals.

Exercise and Bone

When prescribing exercise for bone, there are several important considerations: the mechanostat theory, the concept of osteogenic shear forces, and most importantly, knowing that bone density does not necessarily equal bone strength. A strong bone is both dense and flexible, whereas a weak bone has only one or neither of those qualities.[167]

The mechanostat theory states that bone has set points of minimum effective strain (MES) that determine whether or not bone will be gained or lost.[168–170] If forces on bone increase more than what the bone is accustomed to (i.e., relative MES), mineral flux into bone increases. This, along with compensatory structural changes, helps bone become competent enough to deal with increased functional demand. Likewise, if demands on bone decrease and fall below the MES threshold, bone metabolism changes result in weaker bone.[170] The latter scenario often prevails in age and illness and can happen very quickly. During periods of immobilization, bone mineral density can decline as fast as 1% per month.[171,172]

To understand the concept of osteogenic shear forces, one must have some idea of the bone's anatomy. Cortical bone contains lacunae (hollowed-out areas) that are connected via canals (canaliculi). These lacunae house osteocytes that extend dendritic arms through the canaliculi to communicate with other osteocytes. Osteocytes act as mechanoreceptors. The osteocyte and its dendritic arms are bathed in fluid. The movement of this fluid is believed to be "sensed" by the osteocytes. The degree of intensity of this fluid movement dictates the degree of bone-building stimulation. Research hints that exercise that creates large "shear forces," such as jumping, creates the most potent stimuli for bone growth. Another key factor in this physiology is that small jostling forces have a much weaker effect, and continuous exercise may work against bone building by causing desensitization of osteocytes. Optimal bone-building exercise therefore is sporadic in nature, alternating work and rest, and creates large strain and shear forces. Multidirectional jumps and hops seem to be most ideal.

Measuring bone density alone does not provide an adequate measure of bone strength.[167] To determine the full effect of exercise, all bone parameters should be assessed. To properly ascertain bone strength, cortical thickness and bone diameter are equally important compared with bone mass. Cortical bone, the outer portion of bone, is responsible for 80% of bone strength. Thicker bones are also stronger bones. By measuring density, cortical bone, and thickness, we get a much more accurate picture of how much force a bone can handle. Effective exercise should address all these areas. In addition, bone-building exercise should be aimed at developing functional parameters that prevent a fall in the first place.[17,173] This means that exercise modalities that build strength, flexibility, balance, and reaction speed should not be ignored.

Exercise and Cancer

Yoga therapy has been shown to predict survivability in breast cancer,[174] is correlated with better outcomes in cancers of the head and neck,[175] and allays anxiety- and stress-related dysfunction in cancer treatment.[176,177] The research on yoga and its use in cancer is gaining ground. A report by Brauer et al.[178] in 2010 looked at the leading cancer treatment hospitals in America. This study showed that 56% of these centers incorporated yoga into their clinic offerings.

The wasting of cancer, cachexia, is a difficult condition to treat, with few therapeutic options. Resistance training has several unique mechanisms that seem to address several of the underlying mechanisms of cachexia.[179] Resistance exercise is able to increase phosphorylation of mammalian target of rapamycin (mTOR) substrates and p70S6 k.[180] This suggests it has the potential to induce mTOR, a major contributor to decreased muscle synthesis.[181] Resistance training is also a powerful modulator of adenosine triphosphate–dependent ubiquitin-proteasome system (UPS) activity by way of inflammatory cytokine modulation. Two review articles in 2007 by Pajak et al.[182] and Al Majid et al.[183] showed several complicated mechanisms of how resistance exercise works. Resistance exercise can release IL-6, which is anti-inflammatory when released by muscle,[85] and IL-15, which is strongly anabolic. Muscle-derived IL-6 suppresses TNF-α and IL-1. This action strongly decreases the overactivity of adenosine triphosphate–dependent UPS, which may be the most important component of cancer cachexia. IL-15 also will increase the type II muscle fibers that seem to be selectively lost in cachexia.

Exercise and Brain Neuroplasticity

Conditions of the brain that cause, or have the potential to cause, dementia-like illnesses are at epidemic proportions, with some experts suggesting that by 2050, upwards of 130 million people worldwide will have dementia.[184] With recent studies showing the strong connection and mechanism by which blood glucose and insulin resistance worsen neurocognitive health,[185] it would also be legitimate to suggest exercise to be a protective tool against neurocognitive disorders; the research is surely in agreement.

If there is one medication that outperforms any other drug in improving outcomes in Parkinson's disease (PD), Alzheimer's disease (AD), stroke recovery, depression, anxiety, attention deficit hyperactive disorder (ADHD), age-related cognitive decline, and multiple sclerosis, then exercise is it.

The mechanism by which exercise exhibits these significant benefits is via induction of neuroplasticity, neurogenesis, and neurotrophy. The ability of the brain to reorganize, create new neural pathways, induce neuronal activity, and enhance the growth and survival of neuronal tissue is influenced by exercise.

Neurotrophic factors such as BDNF, IGF-1, and VEGF play an intricate role as neuroprotective agents, with BDNF seeming to be the most potent.[186] Exercise induces the production of BDNF, which freely crosses the blood–brain barrier (BBB),[187] resulting in hippocampal neurogenesis, dendritic complexity, and synaptic plasticity.[188,189] One mechanism through which environmental toxins induce dementia is by impairing the production of BDNF. Increased BDNF levels may be responsible for enhancing hippocampal volume and improving cognitive function, whereas decreased levels of BDNF, along with IGF-1, are associated with Alzheimer's disease.[190] It is suspected that the benefits of BDNF are derived from its ability to induce hippocampal and cortical neuronal growth and mitochondrial biogenesis and reduce neuroinflammation.[191]

Vascular endothelial growth factor (VEGF), induced by exercise, is known for improving vascular circulation, and its role in inducing hippocampal neurogenesis is evident in humans.[192] Additionally, IGF-1, which is positively correlated with exercise intensity, is released into the blood postexercise and can, like BDNF, bypass the BBB and affect neuroprotective actions in the brain.[193] IGF-1 works in tandem with BDNF to improve neuroplasticity, and furthermore, it provides protection against oxidative damage and neuro-excitotoxicity.[194,195]

These mechanistic pathways explain the ability of exercise to attenuate cognitive decline. These mechanisms then translate to actual outcomes in human trials. Physical activity and exercise have been shown to improve learning capacity[196] in adults and offspring[197]; enhance recovery from traumatic brain injuries[198]; improve gait and function in PD; and improve memory,[199] language ability, attention, and other cognitive parameters in AD.[200,201]

Exercise and physical activity not only spare brain volume, which slowly declines with age, but also increase gray and white matter in various areas of the brain in older adults.[202] The volume of gray and white matter in the prefrontal cortex (involved with working memory and executive functions) and temporal lobe (involved with long-term memory) has been shown to be preserved with physical activity, along with attenuation of hippocampal volume, which is a predictor of time to progression in AD.[203] Increases in gray matter were shown in as early as 6 months and lasting up to 13 years, suggesting exercise has a prolonged benefit on neuroplasticity.[204] Furthermore, increased gray matter not only reduces the risk of cognitive impairment but improves cognitive function and prevents dementia in the elderly.[204,205]

Overall, exercise, whether it is aerobic or resistance training, is beneficial for neuroplasticity and cognitive health. However, most benefits have been observed with high-intensity training, exceeding 30 minutes daily and done for more than 6 months.[206] Including resistance exercise not only provides neurological benefits but also tackles many of the comorbidities of aging as discussed in this chapter, such as frailty, sarcopenia, and cardiometabolic disease.

Exercise for the Brain and Mood

Exercise increases cerebral blood flow and metabolism and therefore affects both mood and cognitive function. Exercise has been shown to have many positive benefits on mood, especially in the realm of depression, anxiety, and self-esteem.[207] In one depression study, 50 adults classified with depressed mood on the Profile of Mood States-Short Form Depression Scale (POMS) were studied over 10 weeks.[208] The treatment consisted of moderate-intensity aerobic exercise for 30 minutes three times per week. At the end of the study, 62% of the exercise group was classified as normal on the POMS compared with 29% in the control group. Another study in the elderly showed comparable results.[209] Several mood parameters were assessed, including both depression and anxiety. The study group had decreased scores for depression and anxiety and showed significant improvement in quality-of-life scores.

Two other studies on depression showed exercise as a potential primary therapy in major depression and among patients refractory to

drug therapy. A study published in 2001 showed that a small group of subjects with major depression who underwent a daily 30-minute walking program had a clinically relevant and statistically significant benefit from exercise in as little as 12 days.[210] The program was a moderate-intensity interval-walking program alternating from "somewhat hard" to half that pace on the rest phase. Even subjects refractive to drug therapy were able to improve with the exercise program. Considering the well-known response delay of 2 to 4 weeks with the use of medications, this study showed aerobic exercise as a more immediate fix and a smart integrative approach with drug treatment.

Direct comparisons of exercise versus drug therapy (sertraline) for major depression showed exercise as a powerful mood enhancer.[211] Subjects performed aerobic exercise on a treadmill three times per week and were followed over a 12-month period with respect to Zoloft therapy, exercise and Zoloft therapy, and exercise alone. The exercise-alone group had significantly better outcomes in terms of treatment effect, remission, and relapse than either the Zoloft group or the Zoloft group plus exercise. Although it is not completely understood, the mechanism by which exercise improves depression and mood-related disorders is multifactorial but does show similar mechanisms to antidepressant medications, but it also surpasses the palliative effects of medications and improves neurocognitive markers.[212] Exercise has been shown to inhibit GSK-3-beta, improve hippocampal neurogenesis, increase brain-derived neurotrophic factor (BDNF), and increase regulation of mTOR.[213,214]

Exercise and Anxiety

Exercise has favorable effects on anxiety. Past studies have shown exercise to be an anxiolytic in symptoms induced by caffeine and carbon dioxide,[215,216] but these compounds did not result in panic. One study showed that direct treatment of patients with panic disorder with aerobic exercise led to significant improvement compared with placebo.[217] The study was over a 10-week period, and the maximum effect was seen toward the end of the study. This points to exercise as both a preventative and alternative treatment strategy for anxiety. The acute effects of exercise may even be able to allay panic attacks. A recent study used exercise as a treatment against induced panic attacks.[218] Cholecystokinin tetrapeptide (CCK-4) is a reliable inducer of panic attacks in humans and mimics the mechanisms associated with anxiety-induced panic attacks. Fifteen subjects were studied in a crossover design. Exercise for 30 minutes at 70% of maximum oxygen consumption was compared against no exercise. The next day, subjects received a bolus of CCK-4 and were monitored for signs and symptoms of panic attack. Previous exercise the day before produced half as many panic attacks, 6 compared with 12. This appears to indicate exercise as a modifier of anxiety-induced panic attacks.

Exercise and Memory

Exercise also affects memory and has shown benefit in multiple brain parameters.[219] Even weight lifting can improve brain function in the elderly.[220] Exercise is known to increase neurotransmission and myelin synthesis. Taken together, this can increase both the speed and quality of mental activity. Studies have shown that exercise positively affects attention deficit disorder/ADHD, dementia, AD, and other mental disorders.[192–195] Recent studies also showed that exercise has the potential to create new neurons.[221,222]

SUMMARY

This chapter illustrated the vast potential of exercise to both prevent and treat multiple medical issues, from obesity to mental and emotional dysfunction. There are many aspects of exercise and health that were not covered or were only discussed briefly. However, the key concepts covered should allow smart manipulation of exercise parameters to fit many conditions. It is our hope that understanding the new science of exercise, and the historical construct in which movement evolved, will allow health practitioners to move beyond one-dimensional exercise models focused solely on aerobic exercise to a more holistic and functional model of prescriptive movement.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA*. 2002;288(14):1723–1727.
2. Wang Y, Beydoun MA. The obesity epidemic in the United States—gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. *Epidemiol Rev*. 2007;29(1):6–28. https://doi.org/10.1093/epirev/mxm007.
3. Smith KB, Smith MS. Obesity Statistics. *Prim Care Clin Off Pract*. 2016;43(1):121–135. https://doi.org/10.1016/j.pop.2015.10.001.
4. Cordain L, Gotshall RW, Eaton SB. Evolutionary aspects of exercise. *World Rev Nutr Diet*. 1997;81:49–60.
5. Cordain L, Gotshall RW, Eaton SB, Eaton SB. Physical activity, energy expenditure and fitness: an evolutionary perspective. *Int J Sports Med*. 1998;19(5):328–335. https://doi.org/10.1055/s-2007-971926.
6. Lee RB. *The !Kung San: Men, Women and Work in a Foraging Society*. 1st ed. London: Cambridge University Press; 1979.
7. Sahlins MD, Lee R, Devore I. Notes on the original affluent society. In: Adline, ed. *Man the Hunter*. Chicago: Aldine; 1968:85–89.
8. Cordain L, Friel J. The Paleolithic Athlete: The Original Cross-Trainer. In: Rodale, ed. *The Paleo Diet for Athletes: The Ancient Nutritional Formula for Peak Athletic Performance*. PA: Emmaus; 2012.
9. Eaton SB, Konner M, Shostak M. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. *Am J Med*. 1988;84(4):739–749.
10. Unick JL, Otto AD, Goodpaster BH, Helsel DL, Pellegrini CA, Jakicic JM. Acute effect of walking on energy intake in overweight/obese women. *Appetite*. 2010;55(3):413–419. https://doi.org/10.1016/j.appet.2010.07.012.
11. King JA, Wasse LK, Broom DR, Stensel DJ. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med Sci Sport Exerc*. 2010;42(3):485–492. https://doi.org/10.1249/MSS.0b013e3181ba10c4.
12. Burton NW, Turrell G. Occupation, hours worked, and leisure-time physical activity. *Prev Med (Baltim)*. 2000;31(6):673–681. https://doi.org/10.1006/pmed.2000.0763.
13. Cook G. *Athletic Body In Balance: Optimal Movement Skills and Conditioning for Performance*. 1st ed. Champaign, Ill: Human Kinetics Publishers; 2003.
14. Cook G. *Movement: Functional Movement Systems: Screening, Assessment, Corrective Strategies*. 1st ed. Aptos, CA: Lotus Pub; 2011.
15. Minick KI, Kiesel KB, Burton L, Taylor A, Plisky P, Butler RJ. Interrater reliability of the functional movement screen. *J Strength Cond Res*. 2010;24(2):479–486. https://doi.org/10.1519/JSC.0b013e3181c09c04.
16. Kiesel K, Plisky P, Butler R. Functional movement test scores improve following a standardized off-season intervention program in professional football players. *Scand J Med Sci Sports*. 2011;21(2):287–292. https://doi.org/10.1111/j.1600-0838.2009.01038.x.
17. Carbuhn AF, Fernandez TE, Bragg AF, Green JS, Crouse SF. Sport and training influence bone and body composition in women collegiate athletes. *J strength Cond Res*. 2010;24(7):1710–1717. https://doi.org/10.1519/JSC.0b013e3181d09eb3.
18. Spenst LF, Martin AD, Drinkwater DT. Muscle mass of competitive male athletes. *J Sports Sci*. 1993;11(1):3–8. https://doi.org/10.1080/02640419308729956.
19. Barnard RJ, Grimditch GK, Wilmore JH. Physiological characteristics of sprint and endurance masters runners. *Med Sci Sports*. 1979;11(2):167–171.
20. Jabbour G, Iancu H-D, Paulin A. Effects of high-intensity training on anaerobic and aerobic contributions to total energy release during repeated supramaximal exercise in obese adults. *Sport Med - open*. 2015;1(1):36. https://doi.org/10.1186/s40798-015-0035-7.
21. Bertuzzi R, Kiss MAPDM, Damasceno M, Oliveira RSF, Lima-Silva AE. Association between anaerobic components of the maximal accumulated oxygen deficit and 30-second Wingate test. *Brazilian J Med Biol Res = Rev Bras Pesqui medicas e Biol*. 2015;48(3):261–266. https://doi.org/10.1590/1414-431X20144043.
22. Jabbour G, Iancu H-D, Mauriège P, Joanisse DR, Martin LJ. High-intensity interval training improves performance in young and older individuals by increasing mechanical efficiency. *Physiol Rep*. 2017;5(7):e13232. https://doi.org/10.14814/phy2.13232.
23. Melanson EL, MacLean PS, Hill JO. Exercise improves fat metabolism in muscle but does not increase 24-h fat oxidation. *Exerc Sport Sci Rev*. 2009;37(2):93–101. https://doi.org/10.1097/JES.0b013e31819c2f0b.
24. Miller WC, Koceja DM, Hamilton EJ. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int J Obes Relat Metab Disord*. 1997;21(10):941–947.
25. Rosenkilde M, Auerbach P, Reichkendler MH, Ploug T, Stallknecht BM, Sjödin A. Body fat loss and compensatory mechanisms in response to different doses of aerobic exercise—a randomized controlled trial in overweight sedentary males. *Am J Physiol Regul Integr Comp Physiol*. 2012;303(6):R571–R579. https://doi.org/10.1152/ajpregu.00141.2012.
26. Rosenkilde M, Reichkendler MH, Auerbach P, et al. Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: a randomized controlled trial. *J Appl Physiol*. 2013;115(11):1599–1609. https://doi.org/10.1152/japplphysiol.00680.2013.
27. Schubert MM, Palumbo E, Seay RF, Spain KK, Clarke HE. Energy compensation after sprint- and high-intensity interval training. In: Fisher G, ed. *PLoS One*. 2017;12(12):e0189590. https://doi.org/10.1371/journal.pone.0189590.
28. Kravitz L. Calorie burning: it's time to think "outside the box". *IDEA Fit J*. 2009;6:32–38.
29. LeCheminant JD, Jacobsen DJ, Bailey BW, et al. Effects of long-term aerobic exercise on EPOC. *Int J Sports Med*. 2008;29(1):53–58. https://doi.org/10.1055/s-2007-965111.
30. LaForgia J, Withers RT, Gore CJ. Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *J Sports Sci*. 2006;24(12):1247–1264. https://doi.org/10.1080/02640410600552064.
31. Schuenke MD, Mikat RP, McBride JM. Effect of an acute period of resistance exercise on excess post-exercise oxygen consumption: implications for body mass management. *Eur J Appl Physiol*. 2002;86(5):411–417. https://doi.org/10.1007/s00421-001-0568-y.
32. Osterberg KL, Melby CL. Effect of acute resistance exercise on postexercise oxygen consumption and resting metabolic rate in young women. *Int J Sport Nutr Exerc Metab*. 2000;10(1):71–81.
33. Tucker WJ, Angadi SS, Gaesser GA. Excess postexercise oxygen consumption after high-intensity and sprint interval exercise, and continuous steady-state exercise. *J strength Cond Res*. 2016;30(11):3090–3097. https://doi.org/10.1519/JSC.0000000000001399.
34. Williams CB, Zelt JGE, Castellani LN, et al. Changes in mechanisms proposed to mediate fat loss following an acute bout of high-intensity interval and endurance exercise. *Appl Physiol Nutr Metab*. 2013;38(12):1236–1244. https://doi.org/10.1139/apnm-2013-0101.
35. Tremblay A, Simoneau JA, Bouchard C. Impact of exercise intensity on body fatness and skeletal muscle metabolism. *Metabolism*. 1994;43(7):814–818.
36. Scott CB. *A Primer for the Exercise and Nutrition Sciences: Thermodynamics, Bioenergetics, Metabolism*. New York: Humana Press; 2008.
37. Scott CB. Estimating energy expenditure for brief bouts of exercise with acute recovery. *Appl Physiol Nutr Metab*. 2006;31(2):144–149. https://doi.org/10.1139/h05-013.
38. Scott CB. Contribution of blood lactate to the energy expenditure of weight training. *J strength Cond Res*. 2006;20(2):404–411. https://doi.org/10.1519/R-17495.1.
39. Scott CB. Contribution of anaerobic energy expenditure to whole body thermogenesis. *Nutr Metab (Lond)*. 2005;2(1):14. https://doi.org/10.1186/1743-7075-2-14.
40. Scott CB, Kemp RB. Direct and indirect calorimetry of lactate oxidation: implications for whole-body energy expenditure. *J Sports Sci*. 2005;23(1):15–19. https://doi.org/10.1080/02640410410001716760.
41. Scott CB, Croteau A, Ravlo T. Energy expenditure before, during, and after the bench press. *J Strength Cond Res*. 2009;23(2):611–618. https://doi.org/10.1519/JSC.0b013e31818c2845.
42. Scott C. Misconceptions about Aerobic and Anaerobic energy expenditure. *J Int Soc Sports Nutr*. 2005;2(2):32. https://doi.org/10.1186/1550-2783-2-2-32.
43. Bell GJ, Syrotuik D, Martin TP, Burnham R, Quinney HA. Effect of concurrent strength and endurance training on skeletal muscle

properties and hormone concentrations in humans. *Eur J Appl Physiol*. 2000;81(5):418–427. https://doi.org/10.1007/s004210050063.
44. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol*. 2004;558(Pt 1):5–30. https://doi.org/10.1113/jphysiol.2003.058701.
45. Chwalbínska-Moneta J, Krysztofiak F, Ziemba A, Nazar K, Kaciuba-Uściłko H. Threshold increases in plasma growth hormone in relation to plasma catecholamine and blood lactate concentrations during progressive exercise in endurance-trained athletes. *Eur J Appl Physiol Occup Physiol*. 1996;73(1-2):117–120.
46. Godfrey RJ, Madgwick Z, Whyte GP. The exercise-induced growth hormone response in athletes. *Sports Med*. 2003;33(8):599–613.
47. Turner MJ, Howley ET, Tanaka H, Ashraf M, Bassett DR, Keefer DJ. Effect of graded epinephrine infusion on blood lactate response to exercise. *J Appl Physiol*. 1995;79(4):1206–1211. https://doi.org/10.1152/jappl.1995.79.4.1206.
48. Takahashi H, Irizawa M, Komura T, Kikuchi K, Ebisu Y, Nakayama H. Relationship among blood lactate and plasma catecholamine levels during exercise in acute hypoxia. *Appl Human Sci*. 1995;14(1):49–53.
49. Kaiser P, Tesch P. Effects of acute β-adrenergic blockade on blood and muscle lactate concentration during submaximal exercise. *Int J Sports Med*. 1983;04(04):275–277. https://doi.org/10.1055/s-2008-1026049.
50. Jacks DE, Sowash J, Anning J, McGloughlin T, Andres F. Effect of exercise at three exercise intensities on salivary cortisol. *J strength Cond Res*. 2002;16(2):286–289.
51. Ottosson M, Lönnroth P, Björntorp P, Edén S. Effects of cortisol and growth hormone on lipolysis in human adipose tissue. *J Clin Endocrinol Metab*. 2000;85(2):799–803. https://doi.org/10.1210/jcem.85.2.6358.
52. Crawford BAL, Liu PY, Kean MT, Bleasel JF, Handelsman DJ. Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. *J Clin Endocrinol Metab*. 2003;88(7):3167–3176. https://doi.org/10.1210/jc.2002-021827.
53. Björntorp P. Hormonal control of regional fat distribution. *Hum Reprod*. 1997;12(suppl 1):21–25.
54. McCarty MF. Modulation of adipocyte lipoprotein lipase expression as a strategy for preventing or treating visceral obesity. *Med Hypotheses*. 2001;57(2):192–200. https://doi.org/10.1054/mehy.2001.1317.
55. Ottosson M, Vikman-Adolfsson K, Enerbäck S, Elander A, Björntorp P, Edén S. Growth hormone inhibits lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab*. 1995;80(3):936–941. https://doi.org/10.1210/jcem.80.3.7883853.
56. Samra JS, Clark ML, Humphreys SM, MacDonald IA, Bannister PA, Frayn KN. Effects of Physiological Hypercortisolemia on the Regulation of Lipolysis in Subcutaneous Adipose Tissue 1. *J Clin Endocrinol Metab*. 1998;83(2):626–631. https://doi.org/10.1210/jcem.83.2.4547.
57. Djurhuus CB, Gravholt CH, Nielsen S, Pedersen SB, Møller N, Schmitz O. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. *Am J Physiol Metab*. 2004;286(3):E488–E494. https://doi.org/10.1152/ajpendo.00199.2003.
58. Kraemer WJ, Gordon SE, Fleck SJ, et al. Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med*. 1991;12(2):228–235. https://doi.org/10.1055/s-2007-1024673.
59. Church TS, Martin CK, Thompson AM, Earnest CP, Mikus CR, Blair SN. Changes in weight, waist circumference and compensatory responses with different doses of exercise among sedentary, overweight postmenopausal women. In: Sorensen TIA, ed. *PLoS One*. 2009;4(2):e4515. https://doi.org/10.1371/journal.pone.0004515.
60. Karamouzis I, Karamouzis M, Vrabas IS, et al. The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. *Clin Chem Lab Med*. 2002;40(2):132–136. https://doi.org/10.1515/CCLM.2002.023.
61. Broom DR, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am J Physiol Regul Integr Comp Physiol*. 2009;296(1):R29–R35. https://doi.org/10.1152/ajpregu.90706.2008.
62. Erdmann J, Tahbaz R, Lippl F, Wagenpfeil S, Schusdziarra V. Plasma ghrelin levels during exercise — Effects of intensity and duration. *Regul Pept*. 2007;143(1-3):127–135. https://doi.org/10.1016/j.regpep.2007.05.002.
63. Bozinovski NC, Bellissimo N, Thomas SG, Pencharz PB, Goode RC, Anderson GH. The effect of duration of exercise at the ventilation threshold on subjective appetite and short-term food intake in 9 to 14 year old boys and girls. *Int J Behav Nutr Phys Act*. 2009;6(1):66. https://doi.org/10.1186/1479-5868-6-66.
64. McMurray RG, Hackney AC. Interactions of metabolic hormones, adipose tissue and exercise. *Sports Med*. 2005;35(5):393–412.
65. Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology*. 2001;26(1):37–49.
66. Sjödin AM, Forslund AH, Westerterp KR, Andersson AB, Forslund JM, Hambraeus LM. The influence of physical activity on BMR. *Med Sci Sports Exerc*. 1996;28(1):85–91.
67. Kraemer WJ, Volek JS, Clark KL, et al. Influence of exercise training on physiological and performance changes with weight loss in men. *Med Sci Sports Exerc*. 1999;31(9):1320–1329.
68. Wilmore JH, Després JP, Stanforth PR, et al. Alterations in body weight and composition consequent to 20 wk of endurance training: the HERITAGE Family Study. *Am J Clin Nutr*. 1999;70(3):346–352.
69. Kraemer WJ, Volek JS, Clark KL, et al. Physiological adaptations to a weight-loss dietary regimen and exercise programs in women. *J Appl Physiol*. 1997;83(1):270–279. https://doi.org/10.1152/jappl.1997.83.1.270.
70. King J, Panton L, Broeder C, Browder K, Quindry J, Rhea L. A comparison of high intensity vs. low intensity exercise on body composition in overweight women. *Med. Sci. Sports Exerc*. 2001;33(5). https://doi.org/10.1097/00005768-200105001-01285.
71. Treuth MS, Hunter GR, Williams M. Effects of exercise intensity on 24-h energy expenditure and substrate oxidation. *Med Sci Sports Exerc*. 1996;28(9):1138–1143.
72. Trapp EG, Chisholm DJ, Freund J, Boutcher SH. The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *Int J Obes (Lond)*. 2008;32(4):684–691. https://doi.org/10.1038/sj.ijo.0803781.
73. Meka N, Katragadda S, Cherian B, Arora RR. Review: endurance exercise and resistance training in cardiovascular disease. *Ther Adv Cardiovasc Dis*. 2008;2(2):115–121. https://doi.org/10.1177/1753944708089701.
74. Pierson LM, Herbert WG, Norton HJ, et al. Effects of combined aerobic and resistance training versus aerobic training alone in cardiac rehabilitation. *J Cardiopulm Rehabil*. 21(2):101–110.
75. Tokmakidis SP, Zois CE, Volaklis KA, Kotsa K, Touvra A-M. The effects of a combined strength and aerobic exercise program on glucose control and insulin action in women with type 2 diabetes. *Eur J Appl Physiol*. 2004;92(4-5):437–442. https://doi.org/10.1007/s00421-004-1174-6.
76. Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D. Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. *Diabetes Res Clin Pract*. 2002;56(2):115–123.
77. Bryner RW, Ullrich IH, Sauers J, et al. Effects of resistance vs. aerobic training combined with an 800 calorie liquid diet on lean body mass and resting metabolic rate. *J Am Coll Nutr*. 1999;18(2):115–121.
78. Velez A, Golem DL, Arent SM. The impact of a 12-week resistance training program on strength, body composition, and self-concept of Hispanic adolescents. *J strength Cond Res*. 2010;24(4):1065–1073. https://doi.org/10.1519/JSC.0b013e3181cc230a.
79. Dionigi RA, Cannon J. Older adults' perceived changes in physical self-worth associated with resistance training. *Res Q Exerc Sport*. 2009;80(2):269–280. https://doi.org/10.1080/02701367.2009.10599562.
80. Reel JJ, Greenleaf C, Baker WK, et al. Relations of Body Concerns and Exercise Behavior: a meta-analysis. *Psychol Rep*. 2007;101(3):927–942. https://doi.org/10.2466/pr0.101.3.927-942.
81. Chicco AJ. Exercise training in prevention and rehabilitation: which training mode is best? *Minerva Cardioangiol*. 2008;56(5):557–570.
82. Bouchla A, Karatzanos E, Dimopoulos S, et al. The addition of strength training to aerobic interval training: effects on muscle strength and body composition in CHF patients. *J Cardiopulm Rehabil Prev*. 2011;31(1):47–51. https://doi.org/10.1097/HCR.0b013e3181e174d7.

83. Voet NB, van der Kooi EL, Riphagen II , Lindeman E, van Engelen BG, Geurts AC. Strength training and aerobic exercise training for muscle disease. In: Voet NB, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd; 2010:CD003907. https://doi.org/10.1002/14651858.CD003907.pub3.
84. Schjerve IE, Tyldum GA, Tjønna AE, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. *Clin Sci (Lond)*. 2008;115(9):283–293. https://doi.org/10.1042/CS20070332.
85. Teta J, Teta K. Anti-inflammatory effects of high-intensity exercise. Townsend letter. Published http://www.townsendletter.com/Feb-March2009/exercise0209.htm; 2009. Accessed February 23, 2018.
86. Davis WJ, Wood DT, Andrews RG, Elkind LM, Davis WB. Concurrent training enhances athletes' cardiovascular and cardiorespiratory measures. *J strength Cond Res*. 2008;22(5):1503–1514. https://doi.org/10.1519/JSC.0b013e3181739f9f.
87. Davis WJ, Wood DT, Andrews RG, Elkind LM, Davis WB. Concurrent training enhances athletes' strength, muscle endurance, and other measures. *J strength Cond Res*. 2008;22(5):1487–1502. https://doi.org/10.1519/JSC.0b013e3181739f08.
88. Davis WJ, Wood DT, Andrews RG, Elkind LM, Davis WB. Elimination of Delayed-Onset Muscle Soreness by Pre-resistance Cardioacceleration before Each Set. *J Strength Cond Res*. 2008;22(1):212–225. https://doi.org/10.1519/JSC.0b013e31815f93a1.
89. Paoli A, Pacelli F, Bargossi AM, et al. Effects of three distinct protocols of fitness training on body composition, strength and blood lactate. *J Sports Med Phys Fitness*. 2010;50(1):43–51.
90. Monteiro AG, Alveno DA, Prado M, et al. Acute physiological responses to different circuit training protocols. *J Sports Med Phys Fitness*. 2008;48(4):438–442.
91. Kaelin M, Swank A, Barnard KL, Adams KJ, Beach P, Newman J. Physical fitness and quality of life outcomes in a pulmonary rehabilitation program utilizing symptom limited interval training and resistance training. *J Exerc Physiol Online*. 2001;4:30–37.
92. Butcher SJ, Jones RL. The impact of exercise training intensity on change in physiological function in patients with chronic obstructive pulmonary disease. *Sports Med*. 2006;36(4):307–325.
93. Meyer K, Samek L, Schwaibold M, et al. Interval training in patients with severe chronic heart failure: analysis and recommendations for exercise procedures. *Med Sci Sports Exerc*. 1997;29(3):306–312.
94. Pokan R, Von Duvillard SP, Ludwig J, et al. Effect of high-volume and -intensity endurance training in heart transplant recipients. *Med Sci Sports Exerc*. 2004;36(12):2011–2016.
95. Tanasescu M, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Exercise type and intensity in relation to coronary heart disease in men. *JAMA*. 2000;288(16):1994.
96. Pichot V, Roche F, Denis C, et al. Interval training in elderly men increases both heart rate variability and baroreflex activity. *Clin Auton Res*. 2005;15(2):107–115. https://doi.org/10.1007/s10286-005-0251-1.
97. Ehsani AA, Biello DR, Schultz J, Sobel BE, Holloszy JO. Improvement of left ventricular contractile function by exercise training in patients with coronary artery disease. *Circulation*. 1986;74(2):350–358.
98. Warburton DER, McKenzie DC, Haykowsky MJ, et al. Effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease. *Am J Cardiol*. 2005;95(9):1080–1084. https://doi.org/10.1016/j.amjcard.2004.12.063.
99. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. *J Am Coll Cardiol*. 2001;37(1):153–156.
100. Gulati M, Shaw LJ, Thisted RA, Black HR, Bairey Merz CN, Arnsdorf MF. Heart rate response to exercise stress testing in asymptomatic women: the st. James women take heart project. *Circulation*. 2010;122(2):130–137. https://doi.org/10.1161/CIRCULATIONAHA.110.939249.
101. Foster C, Porcari JP, Anderson J, et al. The talk test as a marker of exercise training intensity. *J Cardiopulm Rehabil Prev*. 2008;28(1):24–30. https://doi.org/10.1097/01.HCR.0000311504.41775.78. quiz 31–32.
102. Wang L, Su SW, Celler BG. Time constant of heart rate recovery after low level exercise as a useful measure of cardiovascular fitness. *Conf Proc. Annu Int Conf IEEE Eng Med Biol Soc IEEE Eng Med Biol Soc Annu Conf*. 2006;1:1799–1802. https://doi.org/10.1109/IEMBS.2006.259568.
103. Pedersen BK, Febbraio M. Muscle-derived interleukin-6—a possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav Immun*. 2005;19(5):371–376. https://doi.org/10.1016/j.bbi.2005.04.008.
104. Ostrowski K, Schjerling P, Pedersen BK. Physical activity and plasma interleukin-6 in humans - effect of intensity of exercise. *Eur J Appl Physiol*. 2000;83(6):512–515. https://doi.org/10.1007/s004210000312.
105. Pedersen BK, Ostrowski K, Rohde T, Bruunsgaard H. The cytokine response to strenuous exercise. *Can J Physiol Pharmacol*. 1998;76(5):505–511.
106. Petersen EW, Carey AL, Sacchetti M, et al. Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am J Physiol Metab*. 2005;288(1):E155–E162. https://doi.org/10.1152/ajpendo.00257.2004.
107. Tomas E, Kelly M, Xiang X, et al. Metabolic and hormonal interactions between muscle and adipose tissue. *Proc Nutr Soc*. 2004;63(02):381–385. https://doi.org/10.1079/PNS2004356.
108. Pedersen BK, Steensberg A, Fischer C, et al. Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil*. 2003;24(2-3):113–119.
109. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab*. 2003;285(2):E433–E437. https://doi.org/10.1152/ajpendo.00074.2003.
110. Trujillo ME, Lee M-J, Sullivan S, et al. Tumor necrosis factor alpha and glucocorticoid synergistically increase leptin production in human adipose tissue: role for p38 mitogen-activated protein kinase. *J Clin Endocrinol Metab*. 2006;91(4):1484–1490. https://doi.org/10.1210/jc.2005-1901.
111. Carey AL, Bruce CR, Sacchetti M, et al. Interleukin-6 and tumor necrosis factor-? are not increased in patients with Type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. *Diabetologia*. 2004;47(6):1029–1037. https://doi.org/10.1007/s00125-004-1403-x.
112. Häkkinen A, Pakarinen A, Hannonen P, et al. Effects of prolonged combined strength and endurance training on physical fitness, body composition and serum hormones in women with rheumatoid arthritis and in healthy controls. *Clin Exp Rheumatol*. 23(4):505–512.
113. Watt MJ, Carey AL, Wolsk-Petersen E, Kraemer FB, Pedersen BK, Febbraio MA. Hormone-sensitive lipase is reduced in the adipose tissue of patients with type 2 diabetes mellitus: influence of IL-6 infusion. *Diabetologia*. 2005;48(1):105–112. https://doi.org/10.1007/s00125-004-1598-x.
114. Wallenius V, Wallenius K, Ahrén B, et al. Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med*. 2002;8(1):75–79. https://doi.org/10.1038/nm0102-75.
115. Kubaszek A, Pihlajamäki J, Komarovski V, et al. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes*. 2003;52(7):1872–1876.
116. MacDonald C, Wojtaszewski JFP, Pedersen BK, Kiens B, Richter EA. Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity. *J Appl Physiol*. 2003;95(6):2273–2277. https://doi.org/10.1152/japplphysiol.00242.2003.
117. Steensberg A, Febbraio MA, Osada T, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol*. 2001;537(Pt 2):633–639.
118. Peake JM, Suzuki K, Hordern M, Wilson G, Nosaka K, Coombes JS. Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur J Appl Physiol*. 2005;95(5-6):514–521. https://doi.org/10.1007/s00421-005-0035-2.
119. Kelly M, Keller C, Avilucea PR, et al. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun*. 2004;320(2):449–454. https://doi.org/10.1016/j.bbrc.2004.05.188.
120. Knudsen JG, Gudiksen A, Bertholdt L, et al. Skeletal muscle IL-6 regulates muscle substrate utilization and adipose tissue metabolism during recovery from an acute bout of exercise. In: Philp A, ed. *PLoS One*. 2017;12(12):e0189301. https://doi.org/10.1371/journal.pone.0189301.
121. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insu-

lin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes*. 2005;54(10):2939–2945.
122. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol*. 2005;98(4):1154–1162. https://doi.org/10.1152/japplphysiol.00164.2004.
123. Furmanczyk PS, Quinn LS. Interleukin-15 increases myosin accretion in human skeletal myogenic cultures. *Cell Biol Int*. 2003;27(10):845–851.
124. Nielsen AR, Mounier R, Plomgaard P, et al. Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. *J Physiol*. 2007;584(Pt 1):305–312. https://doi.org/10.1113/jphysiol.2007.139618.
125. Riechman SE, Balasekaran G, Roth SM, Ferrell RE. Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses. *J Appl Physiol*. 2004;97(6):2214–2219. https://doi.org/10.1152/japplphysiol.00491.2004.
126. Pedersen BK, Åkerström TCA, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol*. 2007;103(3):1093–1098. https://doi.org/10.1152/japplphysiol.00080.2007.
127. Hashimoto T, Hussien R, Oommen S, Gohil K, Brooks GA. Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *FASEB J*. 2007;21(10):2602–2612. https://doi.org/10.1096/fj.07-8174com.
128. Hashimoto T, Brooks GA. Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med Sci Sport Exerc*. 2008;40(3):486–494. https://doi.org/10.1249/MSS.0b013e31815fcb04.
129. Luger A, Watschinger B, Deuster P, Svoboda T, Clodi M, Chrousos GP. Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. *Neuroendocrinology*. 1992;56(1):112–117. https://doi.org/10.1159/000126912.
130. Lin H, Wang SW, Wang RY, Wang PS. Stimulatory effect of lactate on testosterone production by rat Leydig cells. *J Cell Biochem*. 83(1): 147–154.
131. Chen C-C, Chen C-W, Lin P-H, et al. Interactive effect of corticosterone and lactate on regulation of testosterone production in rat leydig cells.
J Cell Physiol. 2017;232(8):2135–2144. https://doi.org/10.1002/jcp.25700.
132. Liu C, Wu J, Zhu J, et al. Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. *J Biol Chem*. 2009;284(5):2811–2822. https://doi.org/10.1074/jbc.M806409200.
133. Godfrey RJ, Whyte GP, Buckley J, Quinlivan R. The role of lactate in the exercise-induced human growth hormone response: evidence from McArdle disease. *Br J Sports Med*. 2009;43(7):521–525. https://doi.org/10.1136/bjsm.2007.041970.
134. Lu SS, Lau CP, Tung YF, et al. Lactate stimulates progesterone secretion via an increase in cAMP production in exercised female rats. *Am J Physiol*. 1996;271(5 Pt 1):E910–E915. https://doi.org/10.1152/ajpendo.1996.271.5.E910.
135. Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Integr Comp Physiol*. 2004;287(3):R502–R516. https://doi.org/10.1152/ajpregu.00114.2004.
136. Novelle MG, Contreras C, Romero-Picó A, López M, Diéguez C. Irisin, two years later. *Int J Endocrinol*. 2013;2013:746281. https://doi.org/10.1155/2013/746281.
137. Abramson JL, Vaccarino V. Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. *Arch Intern Med*. 2002;162(11):1286–1292.
138. Djurhuus CB, Gravholt CH, Nielsen S, et al. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. *Am J Physiol Endocrinol Metab*. 2002;283(1):E172–E177. https://doi.org/10.1152/ajpendo.00544.2001.
139. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. *J Leukoc Biol*. 2005;78(4):819–835. https://doi.org/10.1189/jlb.0505247.
140. Burgomaster KA, Hughes SC, Heigenhauser GJF, Bradwell SN, Gibala MJ. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol*. 2005;98(6):1985–1990. https://doi.org/10.1152/japplphysiol.01095.2004.
141. Park BJ, Tsunetsugu Y, Kasetani T, Kagawa T, Miyazaki Y. The physiological effects of Shinrin-yoku (taking in the forest atmosphere or forest bathing): evidence from field experiments in 24 forests across Japan. *Environ Health Prev Med*. 2010;15(1):18–26. https://doi.org/10.1007/s12199-009-0086-9.
142. Smith MJ. Sprint interval training- "it's a HIIT!". http://www.strength-coach.com/Sprint_Interval_Training[1].pdf. Accessed November 1, 2012.
143. Jakicic JM, Wing RR, Butler BA, Robertson RJ. Prescribing exercise in multiple short bouts versus one continuous bout: effects on adherence, cardiorespiratory fitness, and weight loss in overweight women. *Int J Obes Relat Metab Disord*. 1995;19(12):893–901.
144. Kotz CM, Levine JA. Role of nonexercise activity thermogenesis (NEAT) in obesity. *Minn Med*. 2005;88(9):54–57.
145. Hunter GR, Byrne NM, Sirikul B, et al. Resistance training conserves fat-free mass and resting energy expenditure following weight loss. *Obesity (Silver Spring)*. 2008;16(5):1045–1051. https://doi.org/10.1038/oby.2008.38.
146. Edwards AM, Bentley MB, Mann ME, Seaholme TS. Self-pacing in interval training: a teleoanticipatory approach. *Psychophysiology*. 2011;48(1):136–141. https://doi.org/10.1111/j.1469-8986.2010.01034.x.
147. Ekkekakis P. Let them roam free? Physiological and psychological evidence for the potential of self-selected exercise intensity in public health. *Sports Med*. 2009;39(10):857–888. https://doi.org/10.2165/11315210-000000000-00000.
148. Rose EA, Parfitt G. Exercise experience influences affective and motivational outcomes of prescribed and self-selected intensity exercise. *Scand J Med Sci Sports*. 2012;22(2):265–277. https://doi.org/10.1111/j.1600-0838.2010.01161.x.
149. Deci E, Vansteenkiste M. Self-determination theory and basic need satisfaction: understanding human development in positive psychology. *Ric di Psicol*. 2004;27:23–40.
150. Markland D, Ryan RM, Tobin VJ, Rollnick S. Motivational Interviewing and Self–Determination Theory. *J Soc Clin Psychol*. 2005;24(6):811–831. https://doi.org/10.1521/jscp.2005.24.6.811.
151. Ryan RM, Deci EL. Self-determination theory and the facilitation of intrinsic motivation, social development, and well-being. *Am Psychol*. 2000;55(1):68–78.
152. Williams DM. Exercise, affect, and adherence: an integrated model and a case for self-paced exercise. *J Sport Exerc Psychol*. 2008;30(5):471–496.
153. Duncan LR, Hall CR, Wilson PM, Jenny O. Exercise motivation: a cross-sectional analysis examining its relationships with frequency, intensity, and duration of exercise. *Int J Behav Nutr Phys Act*. 2010;7:7. https://doi.org/10.1186/1479-5868-7-7.
154. Žákovská A, Knechtle B, Chlíbková D, Miličková M, Rosemann T, Nikolaidis PT. The Effect of a 100-km Ultra-Marathon under Freezing conditions on selected immunological and hematological parameters. *Front Physiol*. 2017;8:638. https://doi.org/10.3389/fphys.2017.00638.
155. Liu C-H, Tseng Y-F, Lai J-I, et al. The changes of red blood cell viscoelasticity and sports anemia in male 24-hr ultra-marathoners. *J Chinese Med Assoc*. 2017. https://doi.org/10.1016/j.jcma.2017.09.011.
156. Christensen DL, Espino D, Infante-Ramírez R, et al. Transient cardiac dysfunction but elevated cardiac and kidney biomarkers 24 h following an ultra-distance running event in Mexican Tarahumara. *Extrem Physiol Med*. 2017;6(1):3. https://doi.org/10.1186/s13728-017-0057-5.
157. Yoon JH, Park Y, Ahn J, Shin KA, Kim YJ. Changes in the markers of cardiac damage in men following long-distance and ultra-long-distance running races. *J Sports Med Phys Fitness*. 2016;56(3):295–301.
158. Laye MJ, Solomon TPJ, Karstoft K, Pedersen KK, Nielsen SD, Pedersen BK. Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol*. 2012;112(5):773–781. https://doi.org/10.1152/japplphysiol.00997.2011.
159. Chilton WL, Marques FZ, West J, et al. Acute Exercise Leads to Regulation of Telomere-Associated Genes and MicroRNA Expression in Immune

Cells. In: Saretzki G, ed. *PLoS One*. 2014;9(4):e92088. https://doi.org/10.1371/journal.pone.0092088.
160. Song Z, Von Figura G, Liu Y, et al. Lifestyle impacts on the aging-associated expression of biomarkers of DNA damage and telomere dysfunction in human blood. *Aging Cell*. 2010;9(4):607–615. https://doi.org/10.1111/j.1474-9726.2010.00583.x.
161. Hwang C-L, Yoo J-K, Kim H-K, et al. Novel all-extremity high-intensity interval training improves aerobic fitness, cardiac function and insulin resistance in healthy older adults. *Exp Gerontol*. 2016;82:112–119. https://doi.org/10.1016/j.exger.2016.06.009.
162. Archundia-Herrera C, Macias-Cervantes M, Ruiz-Muñoz B, Vargas-Ortiz K, Kornhauser C, Perez-Vazquez V. Muscle irisin response to aerobic vs HIIT in overweight female adolescents. *Diabetol Metab Syndr*. 2017;9:101. https://doi.org/10.1186/s13098-017-0302-5.
163. Thum JS, Parsons G, Whittle T, Astorino TA. High-intensity interval training elicits higher enjoyment than moderate intensity continuous exercise. *PLoS One*. 2017;12(1):e0166299. https://doi.org/10.1371/journal.pone.0166299.
164. Vella CA, Taylor K, Drummer D. High-intensity interval and moderate-intensity continuous training elicit similar enjoyment and adherence levels in overweight and obese adults. *Eur J Sport Sci*. 2017;17(9):1203–1211. https://doi.org/10.1080/17461391.2017.1359679.
165. Adams J, Ogola G, Stafford P, Koutras P, Hartman J. High-intensity interval training for intermittent claudication in a vascular rehabilitation program. *J Vasc Nurs*. 2006;24(2):46–49. https://doi.org/10.1016/j.jvn.2006.03.002.
166. Wisløff U, Støylen A, Loennechen JP, et al. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation*. 2007;115(24):3086–3094. https://doi.org/10.1161/CIRCULATIONAHA.106.675041.
167. Watts NB. Fundamentals and pitfalls of bone densitometry using dual-energy X-ray absorptiometry (DXA). *Osteoporos Int*. 2004;15(11):847–854. https://doi.org/10.1007/s00198-004-1681-7.
168. Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. *Bone Miner*. 1987;2(2):73–85.
169. Skerry TM. One mechanostat or many? Modifications of the site-specific response of bone to mechanical loading by nature and nurture. *J Musculoskelet Neuronal Interact*. 6(2):122–127.
170. Lanyon LE. Using functional loading to influence bone mass and architecture: objectives, mechanisms, and relationship with estrogen of the mechanically adaptive process in bone. *Bone*. 1996;18(suppl 1):37S–43S.
171. Shackelford LC, LeBlanc AD, Driscoll TB, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. *J Appl Physiol*. 2004;97(1):119–129. https://doi.org/10.1152/japplphysiol.00741.2003.
172. LeBlanc A, Schneider V, Shackelford L, et al. Bone mineral and lean tissue loss after long duration space flight. *J Musculoskelet Neuronal Interact*. 2000;1(2):157–160.
173. Frimel TN, Sinacore DR, Villareal DT. Exercise attenuates the weight-loss-induced reduction in muscle mass in frail obese older adults. *Med Sci Sports Exerc*. 2008;40(7):1213–1219. https://doi.org/10.1249/MSS.0b013e31816a85ce.
174. Danhauer SC, Mihalko SL, Russell GB, et al. Restorative yoga for women with breast cancer: findings from a randomized pilot study. *Psychooncology*. 2009;18(4):360–368. https://doi.org/10.1002/pon.1503.
175. Silver HJ, de Campos Graf Guimaraes C, Pedruzzi P, et al. Predictors of functional decline in locally advanced head and neck cancer patients from south Brazil. *Head Neck*. 2010;32(9):1217–1225. https://doi.org/10.1002/hed.21322.
176. Vadiraja HS, Raghavendra RM, Nagarathna R, et al. Effects of a yoga program on cortisol rhythm and mood states in early breast cancer patients undergoing adjuvant radiotherapy: a randomized controlled trial. *Integr Cancer Ther*. 2009;8(1):37–46. https://doi.org/10.1177/1534735409331456.
177. Rao MR, Raghuram N, Nagendra HR, et al. Anxiolytic effects of a yoga program in early breast cancer patients undergoing conventional treatment: a randomized controlled trial. *Complement Ther Med*. 2009;17(1):1–8. https://doi.org/10.1016/j.ctim.2008.05.005.
178. Brauer JA, El Sehamy A, Metz JM, Mao JJ. Complementary and alternative medicine and supportive care at leading cancer centers: a systematic analysis of websites. *J Altern Complement Med*. 2010;16(2):183–186. https://doi.org/10.1089/acm.2009.0354.
179. de Lima C, Alves LE, Iagher F, et al. Anaerobic exercise reduces tumor growth, cancer cachexia and increases macrophage and lymphocyte response in Walker 256 tumor-bearing rats. *Eur J Appl Physiol*. 2008;104(6):957–964. https://doi.org/10.1007/s00421-008-0849-9.
180. Baar K. Training for endurance and strength: lessons from cell signaling. *Med Sci Sports Exerc*. 2006;38(11):1939–1944. https://doi.org/10.1249/01.mss.0000233799.62153.19.
181. Song Z, Moore DR, Hodson N, et al. Resistance exercise initiates mechanistic target of rapamycin (mTOR) translocation and protein complex co-localisation in human skeletal muscle. *Sci Rep*. 2017;7(1):5028. https://doi.org/10.1038/s41598-017-05483-x.
182. Pajak B, Orzechowska S, Pijet B, et al. Crossroads of cytokine signaling—the chase to stop muscle cachexia. *J Physiol Pharmacol*. 2008;59(suppl 9):251–264.
183. Al-Majid S, Waters H. The biological mechanisms of cancer-related skeletal muscle wasting: the role of progressive resistance exercise. *Biol Res Nurs*. 2008;10(1):7–20. https://doi.org/10.1177/1099800408317345.
184. Prince M, Wimo A, Guerchet M, Ali G, Y.T. W, Prina M. *World Alzheimer Report 2015. The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Costs and Trends*. London; 2015.
185. Leszek J, Trypka E, Tarasov V, Ashraf G, Aliev G. Type 3 diabetes mellitus: a novel implication of alzheimers disease. *Curr Top Med Chem*. 2017;17(12):1331–1335. https://doi.org/10.2174/1568026617666170103163403.
186. Marosi K, Mattson MP. BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol Metab*. 2014;25(2):89–98. https://doi.org/10.1016/j.tem.2013.10.006.
187. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology*. 1998;37(12):1553–1561.
188. Eadie BD, Redila VA, Christie BR. Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J Comp Neurol*. 2005;486(1):39–47. https://doi.org/10.1002/cne.20493.
189. van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. *J Neurosci*. 2005;25(38):8680–8685. https://doi.org/10.1523/JNEUROSCI.1731-05.2005.
190. Laske C, Stellos K, Hoffmann N, et al. Higher BDNF serum levels predict slower cognitive decline in Alzheimer's disease patients. *Int J Neuropsychopharmacol*. 2011;14(03):399–404. https://doi.org/10.1017/S1461145710001008.
191. Mattson MP, Moehl K, Ghena N, Schmaedick M, Cheng A. Intermittent metabolic switching, neuroplasticity and brain health. *Nat Rev Neurosci*. 2018;19(2):63–80. https://doi.org/10.1038/nrn.2017.156.
192. Fabel K, Fabel K, Tam B, et al. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *Eur J Neurosci*. 2003;18(10):2803–2812.
193. Carro E, Nuñez A, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J Neurosci*. 2000;20(8):2926–2933.
194. Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *J Neurosci*. 2001;21(15):5678–5684.
195. Arwert LI, Deijen JB, Drent ML. The relation between insulin-like growth factor I levels and cognition in healthy elderly: a meta-analysis. *Growth Horm IGF Res*. 2005;15(6):416–422. https://doi.org/10.1016/j.ghir.2005.09.001.
196. Winter B, Breitenstein C, Mooren FC, et al. High impact running improves learning. *Neurobiol Learn Mem*. 2007;87(4):597–609. https://doi.org/10.1016/j.nlm.2006.11.003.
197. Robinson AM, Bucci DJ. Physical exercise during pregnancy improves object recognition memory in adult offspring. *Neuroscience*. 2014;256:53–60. https://doi.org/10.1016/j.neuroscience.2013.10.012.
198. Chin LM, Keyser RE, Dsurney J, Chan L. Improved cognitive performance following aerobic exercise training in people with traumatic

brain injury. *Arch Phys Med Rehabil*. 2015;96(4):754–759. https://doi.org/10.1016/j.apmr.2014.11.009.
199. Wise EK, Hoffman JM, Powell JM, Bombardier CH, Bell KR. Benefits of exercise maintenance after traumatic brain injury. *Arch Phys Med Rehabil*. 2012;93(8):1319–1323. https://doi.org/10.1016/j.apmr.2012.05.009.
200. Lautenschlager NT, Cox KL, Flicker L, et al. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease. *JAMA*. 2008;300(9):1027. https://doi.org/10.1001/jama.300.9.1027.
201. Jean L, M-È Bergeron, Thivierge S, Simard M. Cognitive intervention programs for individuals with mild cognitive impairment: systematic review of the literature. *Am J Geriatr Psychiatry*. 2010;18(4):281–296. https://doi.org/10.1097/JGP.0b013e3181c37ce9.
202. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A*. 2011;108(7):3017–3022. https://doi.org/10.1073/pnas.1015950108.
203. Erickson KI, Prakash RS, Voss MW, et al. Aerobic Fitness is Associated With Hippocampal Volume in Elderly Humans. *Hippocampus*. 2009;19(10):1030. https://doi.org/10.1002/HIPO.20547.
204. Erickson KI, Raji CA, Lopez OL, et al. Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study. *Neurology*. 2010;75(16):1415–1422. https://doi.org/10.1212/WNL.0b013e3181f88359.
205. Rovio S, Spulber G, Nieminen LJ, et al. The effect of midlife physical activity on structural brain changes in the elderly. *Neurobiol Aging*. 2010;31(11):1927–1936. https://doi.org/10.1016/j.neurobiolaging.2008.10.007.
206. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci*. 2003;14(2):125–130. https://doi.org/10.1111/1467-9280.t01-1-01430.
207. Salmon P. Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. *Clin Psychol Rev*. 2001;21(1):33–61.
208. Annesi JJ. Changes in depressed mood associated with 10 weeks of moderate cardiovascular exercise in formerly sedentary adults. *Psychol Rep*. 2005;96(3 Pt 1):855–862. https://doi.org/10.2466/pr0.96.3.855-862.
209. Antunes HKM, Stella SG, Santos RF, Bueno OFA, de Mello MT. Depression, anxiety and quality of life scores in seniors after an endurance exercise program. *Rev Bras Psiquiatr*. 2005;27(4):266–271. doi:/S1516-44462005000400003.
210. Dimeo F, Bauer M, Varahram I, Proest G, Halter U. Benefits from aerobic exercise in patients with major depression: a pilot study. *Br J Sports Med*. 2001;35(2):114–117.
211. Babyak M, Blumenthal JA, Herman S, et al. Exercise treatment for major depression: maintenance of therapeutic benefit at 10 months. *Psychosom Med*. 62(5):633–638.
212. Lee H-J, Baek S-S. Role of exercise on molecular mechanisms in the regulation of antidepressant effects. *J Exerc Rehabil*. 2017;13(6):617–620. https://doi.org/10.12965/jer.1735188.594.
213. Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behav Brain Res*. 2001;120(1):87–95.
214. Cotman CW, Engesser-Cesar C. Exercise enhances and protects brain function. *Exerc Sport Sci Rev*. 2002;30(2):75–79.
215. Youngstedt SD, O'Connor PJ, Crabbe JB, Dishman RK. Acute exercise reduces caffeine-induced anxiogenesis. *Med Sci Sports Exerc*. 1998;30(5):740–745.
216. Esquivel G, Schruers K, Kuipers H, Griez E. The effects of acute exercise and high lactate levels on 35% CO2 challenge in healthy volunteers. *Acta Psychiatr Scand*. 2002;106(5):394–397.
217. Ströhle A, Feller C, Onken M, Godemann F, Heinz A, Dimeo F. The acute antipanic activity of aerobic exercise. *Am J Psychiatry*. 2005;162(12):2376–2378. https://doi.org/10.1176/appi.ajp.162.12.2376.
218. Broocks A, Bandelow B, Pekrun G, et al. Comparison of aerobic exercise, clomipramine, and placebo in the treatment of panic disorder. *Am J Psychiatry*. 1998;155(5):603–609. https://doi.org/10.1176/ajp.155.5.603.
219. Mirochnic S, Wolf S, Staufenbiel M, Kempermann G. Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. *Hippocampus*. 2009;19(10):1008–1018. https://doi.org/10.1002/hipo.20560.
220. Cassilhas RC, Viana VAR, Grassmann V, et al. The Impact of Resistance Exercise on the Cognitive Function of the Elderly. *Med Sci Sport Exerc*. 2007;39(8):1401–1407. https://doi.org/10.1249/mss.0b013e318060111f.
221. Hillman CH, Erickson KI, Kramer AF. Be smart, exercise your heart: exercise effects on brain and cognition. *Nat Rev Neurosci*. 2008;9(1):58–65. https://doi.org/10.1038/nrn2298.
222. Marks BL, Katz LM, Smith JK. Exercise and the Aging Mind: Buffing the baby boomer's body and brain. *Phys Sportsmed*. 2009;37(1):119–125. https://doi.org/10.3810/PSM.2009.04.1692.

37

Fasting

Toshia R. Myers, BS, MA, MPhil, PhD, and *Alan Goldhamer, DC*

OUTLINE

INTRODUCTION

Fasting is broadly defined as the voluntary practice of partially or completely abstaining from caloric foods and beverages.[1] Fasting methods differ based on the amount and type of calories consumed daily, time period for daily caloric consumption, fasting duration, as well as rationale.[1-6] Therapeutic prolonged water-only fasting is the protracted (i.e., ≥2 days) consumption of only water for purposes of health promotion.[1,2,7] Most humans have sufficient nutrient reserves to safely undergo a prolonged water-only fast (herein called "fast") for 40 days[8-10] depending on body mass index, fat and muscle percentages, activity levels, and state of health. Human survival during fasting is supported by the ability to enter ketosis and utilize ketone bodies as an alternate energy source for the brain and other organs.[11] Ketone bodies potentially modulate some of the molecular and cellular adaptations observed during nutrient deprivation.[12] Preliminary evidence correlating beneficial clinical outcomes with fasting[13-17] supports the continued research and clinical application of this method.

This chapter reviews various aspects of therapeutic fasting in humans including historical context, physiological responses, clinical research, and clinical application. Caloric restriction and intermittent fasting in humans and other organisms are reviewed in depth elsewhere.[3,4,6,18]

HISTORICAL CONTEXT

Evolutionary biologists speculate that *Homo sapiens* evolved with unpredictable access to food resources, which likely became more unpredictable after the advent of agriculture.[19] Several evolutionary theories attempt to explain how periodic food scarcity shaped our fat storage capacity,[20] but there are fewer theories on how it contributed to our efficient utilization of ketone bodies as an alternate fuel. There is also limited understanding of how periodic food scarcity influenced our long-standing therapeutic, spiritual, and sociological relationship with fasting.[1] Historical texts indicate that, for millennia, people of nearly all cultures and religions have practiced various fasting methods.[21-23] Over the past 200 years, therapeutic fasting has gone in and out of fashion with both allopathic and alternative health practitioners. Therapeutic fasting has once again emerged as a potential tool to treat the pandemic of degenerative diseases largely caused by the overconsumption of highly processed foods.[1-4] The type of therapeutic fasting currently practiced is based on the tenets of Natural Hygiene, which was founded by Isaac Jennings, MD (1788–1874)[24] and later popularized by Herbert M. Shelton, ND (1895–1985).[25-32]

PHYSIOLOGY

Humans gradually transition from the fed state through the fasted state, ultimately terminating in starvation. This transition is regulated by metabolic, endocrine, and neuronal adaptations that ensure whole-body energy requirements are continuously met[8,11] and appear to modulate molecular and cellular mechanisms associated with retardation of aging processes, at least in studied organisms.[3,4,12,33,34] Early reports on human fasting physiology[9,35-37] as well as research by Cahill and colleagues[11,38-86] contributed substantially to our knowledge of

TABLE 37.1 Metabolic Phases of Fasting

Phase	Predominant Metabolic State	Duration (Post Final Caloric Intake)	Primary Tissue/ Organ Involvement	Main Features
I	Glycolysis	Approximately 4–6 hours	Blood Liver	• Food is digested • Glucose, amino acids, and TAG absorbed into the blood • Glucose converted to glycogen via glycogenesis for storage in the liver
II	Glycogenolysis	Approximately 6–24 hours	Liver	• Decreased blood glucose levels stimulates glucagon secretion • Glucagon stimulates glycogenolysis of hepatic glycogen reserves to glucose • Glycogenolysis supplies 75% of glucose requirements, remaining 25% from gluconeogenesis
III	Gluconeogenesis	Approximately 24–48 hours	Liver	• Gluconeogenesis meets the majority of glucose requirements • Blood glucose levels decline but are still utilized by all tissue except liver
IV	Gluconeogenesis > Ketogenesis	Approximately 48 hours until 5–7 days	Kidneys Fatty tissue Liver	• Renal gluconeogenesis increases • Lipolysis of TAGs into glycerol and fatty acids are converted to glucose and ketone bodies, respectively • Ketone bodies in urine steadily increase
V	Ketogenesis > Gluconeogenesis	Approximately 5–7 days until starvation	Fatty tissue Liver	• Energy requirements primarily met through fat metabolism • Starvation begins when essential protein is catabolized to meet energy requirements

TAG, Triacylglycerol.

human physiological responses to fasting. Metabolic processes were described in five phases based on the transition through the postabsorptive state and near-steady state of prolonged fasting (Table 37.1).[11] Throughout this transition, metabolic states are often concurrent and have variable rates depending on an individual's age, sex, and nutrient reserves.[87] It should be noted that there appear to be marked differences between the metabolism of lean and obese individuals.[73,74,88,89] For example, lean individuals have increased concentrations of ketone bodies and an increased percentage of energy derived from protein oxidation while fasting.[73]

Metabolic Phase I (0–4/6 Hours)

Phase I begins immediately after caloric ingestion, when carbohydrates, proteins, and fats are digested into sugars (primarily glucose), amino acids (single or peptides), and fatty acids (triacylglycerol, TAG), respectively.[11] All macronutrients are absorbed into the blood, transported to the liver for processing and/or storage, and transported back to the blood to maintain physiological processes. Glucose and amino acids are absorbed directly into the bloodstream through the small intestine. TAGs are broken down into glycerols and free fatty acids, which form micelles that enter enterocytes where they are reconverted to TAGs and packaged in chylomicrons. Chylomicrons are absorbed into the lymphoid system through the small intestine and then released into the bloodstream. In this phase, high blood glucose levels stimulate the pancreatic β cells to secrete insulin above basal levels. Insulin regulates the uptake and metabolism of glucose, amino acids, and fatty acids. At this phase, all tissues preferentially utilize glucose as fuel, except the liver and heart, which prefer α-ketoacids and fatty acids, respectively. Glucose is transported into cells where it is catabolized through glycolysis. During glycolysis, glucose is converted to pyruvate that is oxidized to acetyl-coenzyme A (CoA) that enters the tricarboxylic (TCA) cycle, ultimately producing adenosine tri-phosphate (ATP). Increased glycolysis in the liver results in an excess of acetyl-CoA that is used to synthesize TAGs, which are packaged into very-low-density lipoproteins for storage and secretion when blood glucose is low. Insulin also regulates the uptake of glucose by adipocytes, where it is converted through glycolysis to glycerol 3-phosphate and acetyl-CoA, which are then synthesized into TAG. High levels of blood glucose enable hepatocytes and muscle cells to convert glucose to glycogen—the storage form of glucose—through glycogenesis. The liver and muscles store approximately 100 and 300 grams of glycogen, respectively.[8]

Metabolic Stage Phase II (4–6 hours)

Phase II begins approximately 4 to 6 hours after final caloric intake.[11] Decreased blood glucose levels stimulate pancreatic α cells to secrete glucagon above basal levels. Glucagon is an insulin antagonist that stimulates glycogenolysis of hepatic glycogen to glucose. Hepatic glycogen reserves are depleted within approximately 24 hours of fasting. Glycogenolysis also converts muscle glycogen to glucose 6-phosphate. However, muscle cells lack the enzyme glucose 6-phosphatase that is required to release glucose; therefore muscle glycogen is not used to meet whole-body glucose requirements. During this early fasted phase, hepatic glycogenolysis provides about 75% and gluconeogenesis accounts for the other 25% of daily glucose requirements of all tissue except the liver.[8]

Metabolic Phase III (1–2 days)

Phase III lasts from approximately 24 to 48 hours after final caloric intake.[11] During this time, gluconeogenesis is the primary metabolic pathway supplying daily glucose requirements.[38-40,90] Gluconeogenesis, primarily in the liver, produces glucose from the noncarbohydrate carbon substrates glycerol, lactate, and amino acids.[8,91] TAG hydrolysis forms glycerol and fatty acids in adipose tissue. Glycerol is converted to dihydroxyacetone phosphate, which is used to produce glucose in the liver, after which it is exported to extrahepatic tissue. In skeletal muscle, when rates of glycolysis exceed the TCA cycle, excess lactate is produced and transported to the liver where it is converted to pyruvate and then to glucose through the Cori cycle. Glucose is then transported back to muscle cells or used to meet whole-body glucose requirements.[85] Amino acids are primarily used to make protein, but when glucose is

TABLE 37.2 Energy Reserves in Typical 70 kg Human[186-190]

	GLUCOSE/GLYCOGEN		PROTEIN		TRIGLYCERIDE	
Tissue (Weight)	g	kcal	g	kcal	g	kcal
Blood (10 kg)	15	60	100	400	5	45
Liver (1 kg)	100	400	100	400	50	450
Intestines (1 kg)	0	0	100	400	0	0
Brain (1.4 kg)	2	8	40	160	0	0
Muscle (30 kg)	300	1200	4000	16,000	600	5400
Adipose (15 kg)	200	80	300	1200	12,000	108,000
Skin, lung, spleen (4 kg)	13	52	240	960	40	360
Total (62.4 kg)	450	1800	4880	19,520	12,695	114,255

kg, kilogram; *g*, gram; *kcal*, kilocalorie.
Data from Elkeles RS, Tavil AS. *Biochemical Aspects of Human Disease*. Boston: Blackwell Scientific; 1983.

low, the breakdown of digestive and glycolytic enzymes, skeletal muscle, and other connective tissue provides amino acids for gluconeogenesis. In skeletal muscle, ammonium is produced as a by-product of protein catabolism, but it is unable to be converted to urea for removal through urine, as in the liver. Excess ammonium results in transamination of surplus pyruvate to ultimately form alanine. The glucose-alanine or Cahill cycle transports the glucogenic amino acid, alanine, from skeletal muscle to the liver to produce glucose that can then be used by extrahepatic tissue. Although all amino acids, with the exception of lysine and leucine, are glucogenic, alanine and glutamine are the predominate amino acids used in gluconeogenesis in the liver and kidneys, respectively.[92,93] Eventually, the renal cortex synthesizes more glucose through gluconeogenesis than does the liver.[64] During this phase, blood glucose begins to decline, but glucose is still utilized by all tissue except the liver.[8,91] In the fasted state, blood glucose levels routinely drop down to 40 mg/dL or lower.[11,90,94,95]

Metabolic Phase IV (2–5/7 days)

Phase IV begins approximately 48 hours after final caloric intake and lasts until approximately day 5 to 7.[11] At this phase, renal gluconeogenesis becomes progressively more important in the maintenance of blood glucose levels. Additionally, reduced blood glucose and increased glucagon levels induce adipocytes to increase lipolysis of TAGs into fatty acids and glycerol. Glycerol is converted to glucose through gluconeogenesis as described previously. Fatty acids bound to albumin are transported to the liver, muscle, and other tissues. Fatty acids in the liver are broken down by β oxidation to form acetyl-CoA. When acetyl-CoA exceeds the capacity of the TCA cycle because of reduced oxaloacetate availability, it is used to synthesize the ketone bodies acetoacetic acid (AcAc), acetone, and β-hydroxybutyric acid (βOHB) through ketogenesis.[8] The liver is unable to utilize ketone bodies for fuel, which results in large quantities of ketones, primarily AcAc and βOHB, to be secreted into the bloodstream. Within the mitochondria of extrahepatic tissue, βOHB is further oxidized to AcAc that is then transported to the TCA cycle. Increased ketones are typically identified through urinalysis by day three of the fasted state.[90] Except for red blood cells, the renal medulla, and the liver, all tissues, including the brain, is able to utilize ketone bodies for energy. By the end of Phase IV, the brain's energy requirements are met primarily by ketone bodies.[8,11]

Metabolic Phase V (Until Nutrient Depletion Begins)

Phase V begins when rates of ketogenesis exceed gluconeogenesis and continues until starvation begins.[11] The length of this phase depends on an individual's body mass index, fat and muscle percentages, physical activity levels, and state of health. Studies on respiratory quotient and urinary nitrogen have demonstrated that adipose TAG stores meet the majority of whole-body energy requirements during prolonged fasting.[35,49,51,56,85] Meeting energy requirements through fat metabolism decreases dependency on gluconeogenesis, thus sparing protein.[86,96] The brain begins utilizing ketone bodies, primarily βOHB, after approximately 4 days. This adaption is essential because brain glycogen content is very low (0.1%). The brain (40 g/day) and other tissues (40 g/day) still have an obligatory need for approximately 80 g/day of glucose, which is met through gluconeogenesis.[8,97] Starvation begins when essential proteins are catabolized to meet energy requirements.[8] Based on average nutrient reserves, a 70-kg human can fast for 2 to 3 months before entering starvation (Tables 37.2 and 37.3).[8,35,49,51,97,185-189]

TABLE 37.3 Utilization of Energy Reserves

Energy Source	Reserve[a]
Glucose	1 hour
Digestion	4–8 hours
Glycogen	12 hours
Amino acid	48 hours
Protein	3 weeks[b] 24 weeks (obligate use)
Triglycerides	8 weeks

[a]Based on 100% utilization in a 70-kg human.
[b]If protein were the only fuel used for gluconeogenesis.

PHYSIOLOGICAL EFFECTS

Although data in mammalian model organisms suggest that intermittent fasting has various effects on the neuroendocrine system, such as increased synaptic plasticity and parasympathetic tone,[98] data on neuronal and endocrine adaptations during prolonged water-only fasting in humans are lacking. Preliminary research has shown that during prolonged fasting human growth hormone, reverse T3, adrenaline, noradrenaline, dehydroepiandrosterone (DHEA), sex hormone-binding globulin, and cortisol increase,[94,95,99-102] whereas thyroid-stimulating hormone, T3, luteinizing hormone, follicle-stimulating hormone, and testosterone decrease.[94,95,99-101] Specific details on fluctuations over the course of a prolonged fast; differences between individuals based on age, sex, and nutrient reserves; and the downstream effects of these changes remain to be elucidated.

Weight decreases in response to caloric deprivation. During prolonged fasting, weight loss averages 0.9 kg/day during the first week and decreases to 0.3 kg/day by the third week.[90] Initial rapid weight loss

is primarily caused by water and sodium diuresis.[90,103] Other changes include decreased pulse rate[9,35] and blood pressure (BP),[9,35,37,104] as well as a transient small increase and then a slow drop in the basal metabolic rate by about 1% per day until stabilizing at about 75% of normal.[104] Electrocardiography often demonstrates cardiac adaptations, including sinus bradycardia, decreased QRS complex and T-wave amplitude, elongation of the QT interval, and shifts to the right of the QRS and T-wave axes, which normalize upon refeeding.[9,90,104,105] Physiological responses typically return to prefast levels upon caloric consumption.

CLINICAL RESEARCH

Research, primarily conducted in model organisms, has uncovered several potentially health-promoting cellular and molecular responses to nutrient deprivation, such as hormone modulation, reduced oxidative stress, and increased autophagy.[3,4] Additional research is needed to conclusively determine whether fasting produces similar mechanistic responses or how these responses might affect clinical outcomes in humans. A century of fasting literature[1,106] and limited clinical evidence[15,17,107-110] suggest that the method has beneficial health outcomes, but the substantial amount of data is largely inconclusive as a result of methodological limitations. For example, there are essentially no randomized controlled trials (RCTs) on the efficacy of fasting in the treatment of any disease.

There is an unsubstantiated perception that therapeutic fasting is unsafe.[5] The misconception is associated with a period during which an extreme form of water-only fasting was used to treat obesity.[111,112] During this period, there were several deaths[111,113-115] reported out of approximately 1000 documented fasting cases.[112,116-123] These deaths could likely have been prevented had unintentionally harmful fasting practices, such as arbitrarily long fasting lengths, uninformed electrolyte monitoring and refeeding practices, and fasting patients with contraindications,[114,120,124-129] not been used. Until recently, there were no peer-reviewed assessments of adverse events during fasting. A recent retrospective study describes the adverse events (AEs; classified according to the Common Terminology Criteria for Adverse Events and MedDRA terminology) that occurred during prolonged water-only fasting visits (2–40 days; n = 768) at a medically supervised fasting center.[7] The study found that the highest-grade AEs experienced during the majority visits (72%; n = 555) were mild to moderate in nature and are known to commonly occur during fasting (e.g., nausea, back pain, headache, and presyncope). There were two serious adverse events (grade 4 hyponatremia on fasting day 7 and grade 3 dehydration on fasting day 3), which resolved without further complication, and there were zero deaths. Overall, the data suggest that the method is safe, when conducted under medical supervision using the protocol described.

The following is a description of literature published on the effects of therapeutic fasting during which only water or, in some cases of early research, acaloric liquids and/or vitamin/mineral supplementation was administered. Unfortunately, in many early publications, the fasting method was not adequately described, and these studies are not included here.

CASE REPORTS

Case reports describe novel, informative clinical cases of 1 to 3 patients. They can inform clinicians and patients and guide the course of clinical research. In addition to the reports presented here, there are numerous case reports describing physiology and on the use of fasting to treat obesity.

Appendicitis

A 46-year-old male presented with abdominal pain that was progressively worsening and tenderness with an accompanying mass in the lower right quadrant. He had a normal white blood cell count and slightly elevated erythrocyte sedimentation rate. A retroperitoneal sonogram confirmed an enlarged appendix. The patient opted to undergo a medically supervised, water-only fast for 7 days with 4 days of refeeding rather than surgically remove his appendix. He fasted and refed for 7 and 4 days, respectively. There were no serious complications, and upon termination he had reduced abdominal swelling, no pain or fever, and a normal white blood count. He remained symptom-free at the 3-month, 1-year, and 2-year follow-up visits.[107]

Follicular Lymphoma

A 42-year-old woman with stage IIIa, low-grade follicular lymphoma was reported in *BMJ Case Reports*. After a 21-day water-only fast followed by 10 days of supervised refeeding, her enlarged lymph nodes were no longer palpable and computerized tomography (CT) scans confirmed the size reduction. She did not undergo standard cancer treatment, has maintained a healthy lifestyle, and was symptom-free at the 6-month follow-up visit.[15] At the 3-year follow-up visit, the patient remained symptom-free as indicated by CT/PET scans.

Chronic Posttraumatic Headache

A 52-year-old woman with a 16-year history of chronic posttraumatic headache presented with a constant headache that was described as "dull and achy" with a pain level of 6 to 8/10 that did not improve with standard pharmaceutical medications. She underwent two 40-day medically supervised, water-only fasts with a 6-month intervening period of an exclusively plant-foods diet. At the end of the second fast, she was free of headache symptoms with the exception of an occasional headache that lasted less than 10 minutes with a pain level 1/10. Her body mass index (BMI) reduced from 33.1 kg/m^2 to 18.8 kg/m^2. There were no serious complications, and her serological values remained normal with the exception of slightly increased liver enzymes, which resolved upon refeeding. At the 5-year follow-up visit, she was still symptom-free and had maintained a normal BMI.[110]

CLINICAL STUDIES

Studies on the clinical effects of fasting have been primarily observational in nature. Many studies lack the necessary sample size and controls to conclusively determine outcomes. RCTs are needed to draw conclusions about this method.

Cardiovascular Disease

Early studies suggest that fasting reduces serum triglycerides,[130] BP,[9,35,90,131] and symptoms of congestive heart failure.[125] More recently, medically supervised, water-only fasting was shown to reduce borderline and high hypertension.[14,109] In 68 consecutive patients with borderline high BP, an average of 13 days of water-only fasting reduced systolic BP by an average of 20 mm Hg, with 82% of patients achieving systolic BP below 120 mm Hg.[109] In 174 consecutive patients with high BP, an average of 10 days of water-only fasting resulted in more than 90% of patients becoming normotensive. In patients with systolic BP greater than 180 mm Hg, the average reduction in systolic BP exceeded 60 mm Hg.[108] Preliminary data also suggest that treatment of hypertension with a 14-day medically supervised, water-only fast could reduce combined medical and drug costs by almost $2700 per year per patient.[132]

Cancer and Chemotherapy

Preliminary research suggests that water-only fasting for approximately 2 to 3 days before and/or after chemotherapy ameliorated

commonly reported chemotherapy side effects.[17,133] There is currently a randomized trial being conducted on the effects of 72 hours of water-only fasting in conjunction with chemotherapy.[133]

Diabetes Mellitus

Reports, as early as 1912, suggest that prolonged water-only fasting improves diabetes.[9,134,135] In obese diabetic patients, prolonged water-only fasting substantially improved most parameters of insulin function independent of weight loss.[136]

Epilepsy

Therapeutic fasting has been used since the early 1900s to treat seizures.[135,137] Ketosis, initiated by fasting, decreased the duration, severity, and number of seizures.[138]

Autoimmune Disorders

Several reports suggest that fasting has a beneficial effect on autoimmune disorders, such as chronic urticaria.[139,140] It was found that fasting shortened the early stages of acute glomerulonephritis (reduced glomerular filtration rate, high BP, and edema) and improved prognosis.[141] Rheumatoid arthritis (RA) appears to respond particularly well to fasting. Studies have shown that fasting in arthritis patients results in decreased erythrocyte sedimentation rate (ESR), arthralgia, pain, stiffness, and need for medication.[142-149] Consistent with those findings, a study of 43 patients with definite or classic RA found that a water-only fast of 7 days significantly improved grip strength, pain, swelling of proximal interphalangeal joints, ESR, and functional activity.[150]

Obesity

Therapeutic fasting as a treatment for obesity was popularized in the 1960s.[81,111-114,117-119,121,122,125,131,151-153] In general, fasting results in an initial weight loss of approximately 0.9 kg/day with a gradual decrease to 0.3 kg per day over 30 days.[154] The initial weight lost is primarily that of water, glycogen, and sodium. A study monitoring 121 obese patients for approximately 7 years after fasting an average of 2 months found that after 2 to 3 years, 50% of patients returned to their prefast weights, and that by the end of the study, 90% weighed the same as before their fasts.[122]

CLINICAL APPLICATION

Although rigorous clinical research is lacking, there is substantial clinical anecdotal evidence supporting the use of medically supervised, therapeutic fasting as a safe and effective treatment for a variety of diseases. TrueNorth Health Center (TNHC), the only center in the United States that trains and certifies medical practitioners in water-only fasting,[155] has conducted more than 20,000 medically supervised fasts since 1984. The vast majority of these patients benefited from fasting; less than 1% experienced a serious adverse event and no patients died.[7] Clinicians at TNHC have observed improvement in diseases ranging from lupus to hyperhidrosis to follicular lymphoma. Additional therapeutic fasting centers now exist in the United States, Canada, England, and Australia. Like TNHC, these centers follow the standards of care and principles of ethics established by the International Association of Hygienic Physicians.[156]

Therapeutic fasting conducted under medical supervision at an inpatient facility minimizes adverse events that can arise during fasting because clinical staff can monitor symptoms, order and analyze necessary clinical laboratory tests and procedures, approve adjunctive therapies, appropriately terminate the fast, and supervise postfast recuperation. In most cases, fasting is therapeutically superior to a restricted diet because (1) hunger almost totally disappears,[9,131] (2) ketosis occurs more quickly and efficiently,[9,131] (3) famine edema does not occur,[9] (4) sodium diuresis is more pronounced,[112] (5) weight loss is greater and is typically from fat loss rather than protein catabolism, (6) healing time is shorter, and (7) patient strength may be greater.[31] Restricted diets of vegetable broth or fruit and vegetable juices do not initiate fasting metabolic processes because they contain carbohydrates, protein, and/or fat. Nonetheless, restricted diets are often useful before and after fasting, for patients in whom a healing crisis (i.e., where chronic conditions/symptoms become acute) develops during a fast, and when a fast is contraindicated.[30]

TABLE 37.4 Clinical Stages of Fasting

Stages	Duration	Main Features
I Early fasting	Up to 7 days	• Transient detox symptoms • Preoccupation with eating
II Balanced fasting	Weeks to months	• Acute healing crisis • Health gradually returns
III Starvation	Begins when body increases protein catabolism and can ultimately terminate in death	• Discontinue fasting before this phase • Risk of terminal tissue damage

Therapeutic fasting can be described in three clinical stages (Table 37.4). It should be noted that the clinical stages of fasting do not directly correspond with the metabolic phases described previously. This is because rates of metabolic change occur at a faster rate than patients typically transition through clinical symptoms. For example, clinical stage I can last up to 7 days, at which time metabolism has already progressed into Phase IV or possibly Phase V. There is also a lack of data on how clinical symptoms directly correlate with the metabolic changes that occur while fasting.

Stage I, or early fasting, lasts up to 7 days during which patients can present with common detoxification symptoms of malaise, headaches, and muscle aches that are typically transitory. Patients often express concern for their health and a preoccupation with eating, but any desire for food is likely psychological and has little to do with serious physiological need. Stage II, or balanced fasting, is the most clinically significant fasting stage and can last for weeks to months. This stage typically begins after the patient has entered into a near-steady state of ketosis and adjusted from their dietary habits before fasting. Patients often experience one or more "healing crises" and/or go through less significant detoxification reactions. Extending a fast beyond a notable healing crisis/detoxification reaction rather than stopping midcourse may result in more beneficial health outcomes. During this time, health should gradually return; if not, the patient should be given a thorough medical evaluation. Stage III, also called starvation, occurs when the body increases protein catabolism and can potentially damage essential tissue and, ultimately, terminate in death. Fasting should be discontinued before this stage begins. Predicting optimal fast length is difficult because it is based on many factors, including protein, fat, and electrolyte reserves, individual metabolism, mental health, financial limitations, work and family obligations, severity of disease, age, and sex. Overall, "[the] doctor will look for good practical recovery where the patient is symptom free and signs of regeneration are present."[157] "Fasting to completion" (i.e., exhaustion of nutrient reserves) is no longer practiced nor is it necessary, as consecutive fasting with intervening refeeding appears to be safer and as effective.[157]

Guidelines

The use of a whole plant-foods diet processed without added sugar, oil, and salt before and after fasting is beneficial for reducing symptom

severity during fasting and avoiding complications during refeeding. This diet also promotes prefast bowel movements, which should occur at least daily before fasting, as well as postfast bowel movements, which should quickly develop and pass without complication. It is necessary to adopt a health-promoting lifestyle, including a whole-plant-foods diet, postfast to maximize and maintain any benefits obtained while fasting.

Consumption of 64 to 96 oz/day of pure water (distilled, filtered, or reverse osmosis) is recommended,[31,158] but upwards of 160 oz is commonly ingested without affecting serum sodium levels. Increased water intake appears to reduce detoxification reactions, but excess consumption can cause electrolyte imbalances that are clinically significant and require refeeding. Physiologically, the body is able to modulate "available water" through reduced obligatory water excretion (caused by lower excretion of urea, the major osmotic solute) and by accessing water released from fat catabolism.[85] Upon refeeding there is a sudden shift from a low level of insulin and ketosis to a high level of insulin and glycolysis. As the plasma insulin rises, potassium, phosphate, and magnesium are driven intracellularly and sodium extracellularly, which dilutes the circulating concentrations.[159,160] Sodium restriction during refeeding should be emphasized to not precipitate dilution, edema, or acute heart failure.[78]

In addition to maintaining optimal hydration, rest is essential during fasting. Patients may nap throughout the day. It is also common to experience reduced sleep at night, possibly because of decreased daily activity and increased daytime rest. Brief walks or light stretching are permissible. Rigorous exercise while fasting is discouraged because fuel conservation is necessary to maximize healing and avoid unnecessary gluconeogenesis.[31,158] Even moderate activity can double caloric utilization.[59] In serious chronic disease, an excess of activity has been suspected as cause of death during fasting.[161] Sunlight is also important for general health during fasting, and patients should try to obtain 10 to 20 min/day. However, dehydration resulting from sun exposure can promote orthostatic hypotension and subsequent injury from falls. An increase in heart rate by 10 to 15 beats per minute may indicate excessive sun exposure.

LABORATORY VALUES

Assessment of a fasting patient's progress is not based on a single sign or symptom but on the total clinical picture. Therefore vital signs, including blood pressure and pulse, should be checked daily. Laboratory tests such as a complete blood count and serum chemistry panel and urinalysis are performed weekly and other tests are performed as necessary. Laboratory values during fasting are typically unique to the individual and disease process, but some general observations have been made.[121,154]

Complete Blood Count

Complete blood counts usually show no significant change. Low hemoglobin and hematocrit values[10,162] require that hemolysis or hemorrhage are ruled out, whereas elevations in hematocrit, hemoglobin, and red blood cell counts usually indicate reduced hydration.[78,163] White blood cell (WBC) counts are usually unchanged or decrease slightly with fasting. However, WBCs may increase if infection is present or if WBC counts are low before fasting.

Serum Electrolytes

Serum electrolyte levels are not good indicators of tissue stores, but they are considered the most important blood values during fasting because any significant change necessitates immediate clinical management. All electrolytes decrease over the course of a long fast as mobilized stores are lost, but stores appear to be redistributed even when distilled water is used during prolonged fasts. Serum calcium and chloride concentrations are usually stable but can decrease, especially if vomiting or diarrhea is present. There is a tendency for serum potassium concentrations to decrease, although they can also increase, and values less than 3 mmol/L or higher than 6 mmol/L often require the fast to be terminated. Similarly, serum sodium concentrations can decrease and values less than 130 mmol/L require immediate attention. The total body store of potassium is approximately 55 mEq/kg of body weight, of which 98% is intracellular.[164,165] The total body store of sodium is estimated at 50 mEq/kg, of which approximately 70% is exchangeable.[166,167] The typical daily dietary intake of potassium is 3 to 5 g and sodium is 3 to 7 g. During early fasting, each day the body loses 1.6 to 1.8 g (40–45 mEq) of potassium and 3.5 to 5.8 g (150–250 mEq) of sodium, and these values eventually drop to 0.4 to 0.6 g (10–15 mEq) and 0.02 to 0.35 g (1–15 mEq), respectively.

Liver Enzymes

During medically supervised fasting, the enzymes serum glutamic oxaloacetic transaminase (SGOT/AST) and serum glutamic pyruvic transaminase (SGPT/ALT) are used as biomarkers to assess liver function. These values typically remain in the normal range. However, the values may increase considerably if liver disease is present and may rise even if liver disease is not present. This is usually not a cause for concern as values typically return to normal postfast. For example, at the end of a 40-day fast, an obese patient with a history of chronic pharmaceutical use had a substantial increase in both SGOT and SGPT values. At the beginning of the patient's second fast (6 months later), the values of both enzymes had normalized and had increased only slightly at the end of the second 40-day fast. The enzyme values were at the low end of the reference range at 3-month and 5-year follow-up visits.[16]

Other Blood Values

Triglyceride, cholesterol, and uric acid levels usually rise during fasting,[168,169] indicating mobilization of tissue stores. Postfast values often show a decrease from prefast values,[168,170] but lipid panels may not normalize until 4 to 6 weeks postfast. Serum protein and pancreatic lipase and amylase values usually decline with fasting. A rise in blood urea nitrogen (BUN) value may occur, but a decrease has also been reported.[9,10] Serum creatinine levels can increase,[170] remain stable,[171] or decrease. In cases of increased levels, prompt retesting and/or fast termination are required. In cases of increased levels, prompt retesting and/or fast termination are required. Blood glucose values drop in most patients.[134,172] In some patients, values below 40 mg/dL have been observed and are not typically a cause for concern in the absence of additional signs of hypoglycemia. If the blood glucose value is low before fasting, it may rise after fasting. ESR and C-reactive protein usually drop after fasting, although they may rise during the fast.[148,149]

Urinalysis

Urinalysis is conducted weekly, but it might be difficult to interpret during fasting because the body discards considerable waste via the kidneys. It is not uncommon to see various types of casts, red blood cells, WBC, bilirubin (+1 to +2), protein (trace, +2), and ketones (+4) and, if liver disease is present, urobilinogen elevation. Trace leukocytes and blood are common incidental findings, particularly in women. Specific gravity is commonly elevated (possibly to 1.035), a finding that may reflect inadequate hydration.

Adjunctive Care

Dietary Supplements

During prolonged fasting, macro- and micronutrient imbalance is rare. Protein catabolism and vitamin and mineral excretion decreases as the fast progresses, and typically by day 10 is low enough to maintain homeostasis. Fast termination is preferred to supplementation. Problems such

as nausea and indigestion have been reported when vitamin and mineral supplements were taken during fasting.[122,171] In a report describing vitamin deficiency during fasting, the actual fasting protocol was not described; in addition, the patient's physical activity was not restricted and oral medication for intercurrent illness was maintained during fasting.[122]

Enemas

Enemas are generally not administered or necessary if the fasting patient has daily, healthy bowel movements before fasting begins. To help prevent constipation, a raw and cooked plant-foods diet free from any additives, animal products, or refined carbohydrates for at least 2 days will help promote bowel movement prefast and prevent postfast constipation. If bowel movements do not start early during refeeding, then precautionary methods to avoid fecal compaction should be considered, including stewed prunes, enema, and/or colon hydrotherapy.

Hydrotherapy

Constitutional hydrotherapy and sitz baths have been implemented with fasting. Strong treatments, both in frequency and/or temperature interval size, should be limited to early fasting.

Intravenous Therapy

Intravenous administration requires much care and is best avoided entirely, except for emergent conditions. Saline should be avoided as a result of plasma expansion and edema, which has precipitated acute heart failure. Glucose, in contrast, should be accompanied by vitamin B_1 and B_6 coadministration to avoid acute thiamine deficiency and lactic acidosis.[173-175]

Pharmaceuticals

Pharmaceutical use is contraindicated during prolonged fasting. The primary concern regarding fasting medicated patients is the potentiation of drug action during the fasted state, altered urinary/hepatic metabolism, and known drug side effects and adverse events. Appropriately removing pharmaceuticals allows for ease of clinical assessment while ensuring patient safety. Successful fasts have been administered while maintaining some hormonal medications including insulin, thyroid, and reproductive hormones, often at reduced dosage.

CONTRAINDICATIONS

Contraindications to fasting are few, and each case must be judged individually (Table 37.5). For example, an inexperienced practitioner may assume that emaciated patients should not fast, but in cases of extreme emaciation a short fast (1–3 days) or a series of such short fasts with longer periods of proper intervening feeding may be beneficial.[31] With regard to fasting contraindications Alec Burton stated:

> *I have found few health problems which are absolute contraindications to fasting. In my experience, if the need is evident, the only genuine contraindication is fear…. As for the other conditions often mentioned, e.g. kidney disease, heart impairment, [tuberculosis], etc., they merely require extreme caution, because of the limits imposed by pathology, but they are not inexorable contraindications.*[157]

Relative contraindications to fasting include severe anemia, porphyria, cachexia, anorexia, severe liver or kidney disease, medium-chain acyl-CoA dehydrogenase deficiency, advanced cerebral vascular insufficiency, higher-grade cardiac arrhythmias, certain cancers or psychological disorders, and active gastric ulcer disease.[176] Although fasting is contraindicated in severe renal insufficiency, patients with 65% renal function often normalize as a result of fasting and dietary management. Additionally, fasting pregnant women and children is controversial. Short, medically supervised fasts may be appropriate for pregnant women and children on an individual basis, but long fasts are typically strongly contraindicated and precaution is indicated. Doctors (e.g., Shelton, Benesh, Sidwha, and Burton) with considerable experience fasting pregnant women during all three trimesters have found no adverse effects with fasts of a few days to 2 to 3 weeks, but there are insufficient data to conclude if the practice is safe. Fasting during lactation is not generally advised because milk flow is halted by fasting and is difficult to resume.[31]

SIDE EFFECTS

Medically supervised, prolonged water-only fasting as a therapeutic procedure is generally safe.[7] Side effects of fasting are rarely serious, with the exception of rare electrolyte imbalance, but fasting may uncover disease and reveal weaknesses that were previously subclinical.[117,177] Discomfort during fasting may be due to withdrawal from stimulants, hypoglycemia, acidosis, elimination of wastes, and enhancement of repair. Patients may experience headaches, insomnia, nausea, back pain, dyspepsia, fatigue, skin irritations, presyncope, coated tongue, body odor, aching limbs, palpitations, mucus discharge, and visual and hearing disturbances. Hair growth is usually arrested, and skin may become dry and scaly. Most signs and symptoms are usually brief in duration. In certain cases, complications occur that may necessitate breaking the fast prematurely. Examples of such conditions are shown in Table 37.5.

Fasting also elevates serum uric acid values and uric acid excretion, and if fluid intake is insufficient, gout or renal stones may be precipitated.[178] A few reports have also discussed the development of Wernicke encephalopathy during prolonged fasting,[174,175] but this rarely occurs during therapeutic fasting. Therefore it is difficult to determine whether the condition is related to methodology. Furthermore, the incidence of death at fasting institutions is low, and there is no evidence in the scientific literature to suggest that fasting or starvation can be considered a cause of death. Death during fasting indicates that the remedial efforts of the body have been overpowered by the pathological process. This situation occurs in serious disease, whether the patient is eating or fasting. In examining the fallacy of attributing the cause of death to fasting, Stewart and Fleming wrote, "Fasting short of emaciation is not hazardous; if death results, reasons other than those of the fast should be considered before concluding that all supervised fasts should be discouraged."[179]

CONCLUSION

Prolonged water-only fasting conducted under medical supervision is increasingly recognized as a safe and effective therapy for a number of diseases, but the practice is not for everyone and postfast lifestyle modifications are necessary to maintain any health benefits obtained from fasting. Preliminary research indicates that there is at least some degree of overlap between the physiological responses induced by caloric restriction, intermittent fasting, prolonged fasting, and exercise, such as increased autophagy and insulin sensitivity.[3,180-184] Additional research is necessary to determine the extent to which these methods induce similar physiological responses in humans and if the responses result in clinical health outcomes. Nonetheless, used alone or in combination, these natural therapies could help reduce the overwhelming rates of chronic diseases that humans are experiencing globally. As it is more beneficial and cost effective to maintain rather than repair health, it will be important to determine the effect of these therapies on health span over the course of a life.

TABLE 37.5 Fasting Contraindications, Common Adverse Events, and Serious Complications

Contraindications	Common Adverse Events[a]	Complications[b]
• Severe anemia	• Headaches	• Sudden drop in BP
• Porphyria, cachexia, anorexia	• Insomnia	• Delirium
• Severe liver or kidney disease	• Nausea	• Prolonged hypothermia
• Medium-chain acyl-CoA dehydrogenase deficiency	• Back pain/aching limbs	• Rapid/slow/feeble/irregular pulse
• Advanced cerebral vascular insufficiency	• Dyspepsia	• Extreme weakness
• Higher-grade cardiac arrhythmias	• Fatigue	• Dyspnea
• Certain cancers	• Skin irritations	• Vomiting/diarrhea causing dehydration
• Pregnant women and children	• Presyncope	• Gastrointestinal tract bleeding
	• Coated tongue	• Hepatic decompensation
	• Body odor	• Renal insufficiency
	• Aching limbs	• Cardiac arrhythmias
	• Mucus discharge	• Severe electrolyte imbalance

[a]Common adverse events are typically mild to moderate in nature.
[b]Less common, potentially serious complications that typically require premature termination of fast.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Fredericks R. *Fasting: An Exceptional Human Experience*. All Things Published Well; 2013.
2. Furhman J. *Fasting and Eating for Health: A Medical Doctor's Program for Conquering Disease*. St. Martin's Griffin; 1995.
3. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. *Cell Metab*. 2014;19:181–192. https://doi.org/10.1016/j.cmet.2013.12.008.
4. Longo VD, Panda S. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab*. 2016;23:1048–1059. https://doi.org/10.1016/j.cmet.2016.06.001.
5. Wilhelmi de Toledo F, et al. Fasting therapy - an expert panel update of the 2002 consensus guidelines. *Forsch Komplementmed*. 2013;20:434–443. https://doi.org/10.1159/000357602.
6. Patterson RE, et al. Intermittent fasting and human metabolic health. *J Acad Nutr Diet*. 2015;115:1203–1212. https://doi.org/10.1016/j.jand.2015.02.018.
7. Finnell JS, Saul BC, Goldhamer AC, Myers TR. Is fasting safe? A chart review of adverse events during medically-supervised, water-only fasting. *BMC Comp Altern Med*. 2018;18(1).
8. Berg JM, TJ, Stryer L. *Biochemistry*. 5th ed. WH Freeman; 2002.
9. Keys A, Brozek J, Henschel A, Mickelsen O, Longstreet-Taylor H. *The Biology of Human Starvation*. Vols. 1 and 2. University of Minnesota Press; 1950.
10. Lawlor T, Wells DG. Metabolic hazards of fasting. *Am J Clin Nutr*. 1969;22:1142–1149.
11. Cahill Jr GF. Fuel metabolism in starvation. *Annu Rev Nutr*. 2006;26:1–22. https://doi.org/10.1146/annurev.nutr.26.061505.111258.
12. Puchalska P, Crawford PA. Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. *Cell Metab*. 2017;25:262–284. https://doi.org/10.1016/j.cmet.2016.12.022.
13. Goldhamer AC, Gershfeld N, Goldman DM, Myers TR. Challenging case in clinical practice: long-term relief from chronic posttraumatic headache after water-only fasting and an exclusively plant foods diet. *Alternat Complement Ther*. 2017;23:129–131. https://doi.org/10.1089/act.2017.29117.acg.
14. Goldhamer A, Lisle D, Parpia B, Anderson SV, Campbell TC. Medically supervised water-only fasting in the treatment of hypertension. *J Manipulative Physiol Ther*. 2001;24:335–339. https://doi.org/10.1067/mmt.2001.115263.
15. Goldhamer AC, Klaper M, Foorohar A, Myers TR. Water-only fasting and an exclusively plant foods diet in the management of stage IIIa, low-grade follicular lymphoma. *BMJ Case Rep*. 2015;2015. https://doi.org/10.1136/bcr-2015-211582.
16. Goldhamer AC, et al. Medically supervised water-only fasting in the treatment of borderline hypertension. *J Altern Complement Med*. 2002;8:643–650. https://doi.org/10.1089/107555302320825165.
17. Safdie FM, et al. Fasting and cancer treatment in humans: a case series report. *Aging (Albany NY)*. 2009;1:988–1007. https://doi.org/10.18632/aging.100114.
18. Patterson RE, Sears DD. Metabolic effects of intermittent fasting. *Annu Rev Nutr*. 2017;37:371–393. https://doi.org/10.1146/annurev-nutr-071816-064634.
19. Young VR, Scrimshaw NS. The physiology of starvation. *Sci Am*. 1971;225:14–21.
20. Genne-Bacon EA. Thinking evolutionarily about obesity. *Yale J Biol Med*. 2014;87:99–112.
21. Arbesmann R. Fasting and prophecy in pagan and Christian antiquity. *Traditio*. 1951;7:1–71.
22. MacDermont V. *The Cult of the Seer in the Ancient Middle East: A Contribution to Current Research on Hallucinations Drawn from Coptic and Other Texts*. University of California Press; 1971.
23. Muhammad A. *The Religion of Islam: A Comprehensive Discussion of the Sources, Principles and Practices of Islam*. The Ahmadiyya Anjuman Isha'at Islam; 1936.
24. Burfield-Hazzard L. *The Pioneers of Therapeutic Fasting in America*. Kessinger Publishing; 2005.
25. Graham S, Trall RT, Shelton HM. *The Greatest Health Discovery*. Natural Hygiene Press; 1972.
26. Shelton HM. *The Hygienic System*. Vol. 3. Dr. Shelton's Health School; 1934.
27. Shelton HM. *The Hygienic System*. Vol. 3. Dr. Shelton's Health School; 1950.
28. Shelton HM. *Rubies in the Sand*. Dr. Shelton's Health School; 1961.
29. Shelton HM. *Natural Hygiene: Man's Pristine Way of Life*. Dr. Shelton's Health School; 1968.
30. Shelton HM. *Fasting for Renewal of Life*. Natl Health Assoc; 1974.
31. Shelton HM. *The Science and Fine Art of Fasting*. 5th ed. Natural Hygiene Press; 1978.
32. Shelton HM. *Fasting Can Save Your Life.* 2nd ed. 3rd printing ed. Natural Hygiene Press; 1981.
33. Newman JC, Verdin E. Ketone bodies as signaling metabolites. *Trends Endocrinol Metab*. 2014;25:42–52. https://doi.org/10.1016/j.tem.2013.09.002.
34. Cotter DG, Schugar RC, Crawford PA. Ketone body metabolism and cardiovascular disease. *Am J Physiol Heart Circ Physiol*. 2013;304:H1060–H1076. https://doi.org/10.1152/ajpheart.00646.2012.
35. Benedict FG. *A Study of Prolonged Fasting*. Carnegie Institute; 1915.
36. Penny F. Notes on a thirty days' fast. *Br Med J*. 1909;1:1414–1416.
37. Morgulis S. *Fasting and Undernutrition: A Biological and Sociological Study of Inanition*. EP Dutton & Company; 1923.
38. Aoki TT, Muller WA, Brennan MF, Cahill Jr GF. Effect of glucagon on amino acid and nitrogen metabolism in fasting man. *Metabolism*. 1974;23:805–814.
39. Aoki TT, Muller WA, Cahill Jr GF. Hormonal regulation of glutamine metabolism in fasting man. *Adv Enzyme Regul*. 1972;10:145–151.
40. Aoki TT, Toews CJ, Rossini AA, Ruderman NB, Cahill Jr GF. Glucogenic substrate levels in fasting man. *Adv Enzyme Regul*. 1975;13:329–336.
41. Cahill Jr G, Felig P, Owen O, Wahren J. Metabolic adaptation to prolonged starvation in man. *Nord Med*. 1970;83:89.
42. Cahill Jr GF. Starvation in man. *N Engl J Med*. 1970;28:668–675. https://doi.org/10.1056/NEJM197003192821209.
43. Cahill Jr GF. Starvation in man. *Clin Endocrinol Metab*. 1976;5:397–415.
44. Cahill Jr GF. President's address. Starvation. *Trans Am Clin Climatol Assoc*. 1983;94:1–21.
45. Cahill Jr GF. Survival in starvation. *Am J Clin Nutr*. 1998;68:1–2.
46. Cahill Jr GF, Aoki TT. Starvation and body nitrogen. *Trans Am Clin Climatol Assoc*. 1971;82:43–51.
47. Cahill Jr GF, et al. Hormone-fuel interrelationships during fasting. *J Clin Invest*. 1966;45:1751–1769. https://doi.org/10.1172/JCI105481.
48. Cahill Jr GF, Marliss EB, Aoki TT. Fat and nitrogen metabolism in fasting man. *Horm Metab Res*. 1970;2(suppl 2):181–185.
49. Cahill Jr GF, Owen OE. Starvation and survival. *Trans Am Clin Climatol Assoc*. 1968;79:13–20.
50. Cahill Jr GF, Owen OE. Body fuels and starvation. *Int Psychiatry Clin*. 1970;7:25–36.
51. Cahill Jr GJ, Owen OE, Morgan AP. The consumption of fuels during prolonged starvation. *Adv Enzyme Regul*. 1968;6:143–150.
52. Felig P, Marliss E, Cahill Jr GF. Are plasma amino acid levels elevated in obesity? *N Engl J Med*. 1970;282:166.
53. Felig P, Marliss E, Owen OE, Cahill Jr GF. Blood glucose and gluconeogenesis in fasting man. *Arch Intern Med*. 1969;123:293–298.
54. Felig P, Marliss E, Owen OE, Cahill Jr GF. Role of substrate in the regulation of hepatic gluconeogenesis in fasting man. *Adv Enzyme Regul*. 1969;7:41–46.
55. Felig P, Marliss EB, Cahill Jr GF. Metabolic response to human growth hormone during prolonged starvation. *J Clin Invest*. 1971;50:411–421. https://doi.org/10.1172/JCI106508.
56. Felig P, Owen OE, Morgan AP, Cahill Jr GF. Utilization of metabolic fuels in obese subjects. *Am J Clin Nutr*. 1968;21:1429–1433.
57. Felig P, Owen OE, Wahren J, Cahill Jr GF. Amino acid metabolism during prolonged starvation. *J Clin Invest*. 1969;48:584–594. https://doi.org/10.1172/JCI106017.
58. Felig P, Pozefsky T, Marliss E, Cahill Jr GF. Alanine: key role in gluconeogenesis. *Science*. 1970;167:1003–1004.

59. Cahill, GF Jr. Physiology of acute starvation in man. *Ecol Food Nutri.* 1978;6:221–230. https://doi.org/10.1080/03670244.1978.9990501.
60. Kamm DE, Cahill Jr GF. Effect of acid-base status on renal and hepatic gluconeogenesis in diabetes and fasting. *Am J Physiol.* 1969;216:1207–1212.
61. Marliss E, Aoki TT, Felig P, Pozefsky T, Cahill Jr GF. Hormones and substrates in the regulation of gluconeogenesis in fasting man. *Adv Enzyme Regul.* 1970;8:3–11.
62. Marliss EB, Aoki TT, Unger RH, Soeldner JS, Cahill Jr GF. Glucagon levels and metabolic effects in fasting man. *J Clin Invest.* 1970;49:2256–2270. https://doi.org/10.1172/JCI106445.
63. Muller WA, Aoki TT, Cahill Jr GF. Effect of alanine and glycine on glucagon secretion in postabsorptive and fasting obese man. *J Clin Endocrinol Metab.* 1975;40:418–425. https://doi.org/10.1210/jcem-40-3-418.
64. Owen OE, Felig P, Morgan AP, Wahren J, Cahill Jr GF. Liver and kidney metabolism during prolonged starvation. *J Clin Invest.* 1969;48:574–583. https://doi.org/10.1172/JCI106016.
65. Owen OE, et al. Brain metabolism during fasting. *J Clin Invest.* 1967;46:1589–1595. https://doi.org/10.1172/JCI105650.
66. Afolabi PR, et al. The effect of total starvation and very low energy diet in lean men on kinetics of whole body protein and five hepatic secretory proteins. *Am J Physiol Endocrinol Metab.* 2007;293:E1580–E1589. https://doi.org/10.1152/ajpendo.00169.2007.
67. Elia M, Crozier C, Neale G. Mineral metabolism during short-term starvation in man. *Clin Chim Acta.* 1984;139:37–45.
68. Elia M, Farrell R, Ilic V, Smith R, Williamson DH. The removal of infused leucine after injury, starvation and other conditions in man. *Clin Sci (Lond).* 1980;59:275–283.
69. Elia M, Goren A, Behrens R, Barber RW, Neale G. Effect of total starvation and very low calorie diets on intestinal permeability in man. *Clin Sci (Lond).* 1987;73:205–210.
70. Elia M, Lammert O, Zed C, Neale G. Energy metabolism during exercise in normal subjects undergoing total starvation. *Hum Nutr Clin Nutr.* 1984;38:355–362.
71. Elia M, Martin S, Price C, Hallworth MJ, Neale G. Effect of starvation and elective surgery on hand dynamometry and circulating concentration of various proteins. *Clin Nutr.* 1984;2:173–179.
72. Elia M, Parkinson S. Protein economy during human starvation. *Eur J Clin Nutr.* 1989;43:139–143.
73. Elia M, Stubbs RJ, Henry CJ. Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing total starvation. *Obes Res.* 1999;7:597–604.
74. Elia M, Wood S, Khan K, Pullicino E. Ketone body metabolism in lean male adults during short-term starvation, with particular reference to forearm muscle metabolism. *Clin Sci (Lond).* 1990;78:579–584.
75. Elia M, Zed C, Neale G, Livesey G. The energy cost of triglyceride-fatty acid recycling in nonobese subjects after an overnight fast and four days of starvation. *Metabolism.* 1987;36:251–255.
76. Eliasen MM, et al. Adaptive cellular mechanisms in response to glutamine-starvation. *Front Biosci.* 2006;11:3199–3211.
77. Jackson JM, et al. Macro- and micronutrient losses and nutritional status resulting from 44 days of total fasting in a non-obese man. *Nutrition.* 2006;22:889–897. https://doi.org/10.1016/j.nut.2006.06.001.
78. Korbonits M, Blaine D, Elia M, Powell-Tuck J. Metabolic and hormonal changes during the refeeding period of prolonged fasting. *Eur J Endocrinol.* 2007;157:157–166. https://doi.org/10.1530/EJE-06-0740.
79. Valenta LJ, Elias AN. Modified fasting in treatment of obesity. Effects on serum lipids, electrolytes, liver enzymes, and blood pressure. *Postgrad Med.* 1986;79:263–267.
80. DeFronzo RA, Soman V, Sherwin RS, Hendler R, Felig P. Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. *J Clin Invest.* 1978;62:204–213. https://doi.org/10.1172/JCI109108.
81. Felig P, Cunningham J, Levitt M, Hendler R, Nadel E. Energy expenditure in obesity in fasting and postprandial state. *Am J Physiol.* 1983;244:E45–E51.
82. Felig P, Kim YJ, Lynch V, Hendler R. Amino acid metabolism during starvation in human pregnancy. *J Clin Invest.* 1972;51:1195–1202. https://doi.org/10.1172/JCI106913.
83. Felig P, Lynch V. Starvation in human pregnancy: hypoglycemia, hypoinsulinemia, and hyperketonemia. *Science.* 1970;170:990–992.
84. Fisher M, Sherwin RS, Hendler R, Felig P. Kinetics of glucagon in man: effects of starvation. *Proc Natl Acad Sci U S A.* 1976;73:1735–1739.
85. Saudek CD, Felig P. The metabolic events of starvation. *Am J Med.* 1976;60:117–126.
86. Sherwin RS, Hendler RG, Felig P. Effect of ketone infusions on amino acid and nitrogen metabolism in man. *J Clin Invest.* 1975;55:1382–1390. https://doi.org/10.1172/JCI108057.
87. Varlamov O, Bethea CL, Roberts Jr CT. Sex-specific differences in lipid and glucose metabolism. *Front Endocrinol (Lausanne).* 2014;5:241. https://doi.org/10.3389/fendo.2014.00241.
88. Horowitz JF, Coppack SW, Klein S. Whole-body and adipose tissue glucose metabolism in response to short-term fasting in lean and obese women. *Am J Clin Nutr.* 2001;73:517–522.
89. Horowitz JF, et al. Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol.* 1999;276:E278–E284.
90. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. *West J Med.* 1982;137:379–399.
91. Alberts B, JA, Lewis J, et al. *Molecular Biology of the Cell.* 4th ed. Garland Science; 2002.
92. Stumvoll M, Perriello G, Meyer C, Gerich J. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. *Kidney Int.* 1999;55:778–792. https://doi.org/10.1046/j.1523-1755.1999.055003778.x.
93. Stumvoll M, Meyer C, Kreider M, Perriello G, Gerich J. Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. *Metabolism.* 1998;47:1227–1232.
94. Byerley LO, Heber D. Metabolic effects of triiodothyronine replacement during fasting in obese subjects. *J Clin Endocrinol Metab.* 1996;81:968–976. https://doi.org/10.1210/jcem.81.3.8772559.
95. Webber J, Macdonald IA. The cardiovascular, metabolic and hormonal changes accompanying acute starvation in men and women. *Br J Nutr.* 1994;71:437–447.
96. Sherwin RS. The effect of ketone bodies and dietary carbohydrate intake on protein metabolism. *Acta Chir Scand Suppl.* 1981;507:30–40.
97. Reinmuth OM, Kogure K, Scheinberg P, Shimojyo S. Total cerebral blood flow and metabolism in human brain stem disease. *Neurology.* 1968;18:280–281.
98. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. *Ageing Res Rev.* 2017;39:46–58. https://doi.org/10.1016/j.arr.2016.10.005.
99. Scriba PC, et al. Effects of obesity, total fasting and re-alimentation on L-thyroxine (T4), 3,5,3'-L-triiodothyronine (T3), 3,3',5'-L-triiodothyronine (rT3), thyroxine binding globulin (TBG), cortisol, thyrotrophin, cortisol binding globulin (CBG), transferrin, alpha 2-haptoglobin and complement C'3 in serum. *Acta Endocrinol (Copenh).* 1979;91:629–643.
100. Snel M, et al. Food cues do not modulate the neuroendocrine response to a prolonged fast in healthy men. *Neuroendocrinology.* 2012;96:285–293. https://doi.org/10.1159/000336500.
101. Henson LC, Heber D. Whole body protein breakdown rates and hormonal adaptation in fasted obese subjects. *J Clin Endocrinol Metab.* 1983;57:316–319. https://doi.org/10.1210/jcem-57-2-316.
102. Ho KY, et al. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *J Clin Invest.* 1988;81:968–975. https://doi.org/10.1172/JCI113450.
103. Weinsier RL. Fasting—a review with emphasis on the electrolytes. *Am J Med.* 1971;50:233–240.
104. Consolazio CF, et al. Metabolic aspects of acute starvation in normal humans: performance and cardiovascular evaluation. *Am J Clin Nutr.* 1967;20:684–693.
105. Theorell T, Kjellberg J, Palmblad J. Electrocardiographic changes during total energy deprivation (fasting). *Acta Med Scand.* 1978;203:13–19.
106. Lignot J, LeMaho Y. *Comparative Physiology of Fasting, Starvation, and Food Limitation.* Spinger-Verlag; 2012.
107. Gershfeld N, Sultana P, Goldhamer A. A case of nonpharmacologic conservative management of suspected uncomplicated subacute appendicitis in an adult male. *J Altern Complement Med.* 2011;17:275–277. https://doi.org/10.1089/acm.2010.0253.

108. Goldhamer A, Lisle D, Parpia B, Anderson SV, Campbell TC. Medically supervised water-only fasting in the treatment of hypertension. *J Manipulative Physiol Ther*. 2001;24:335–339. https://doi.org/10.1067/mmt.2001.115263.
109. Goldhamer AC, et al. Medically supervised water-only fasting in the treatment of borderline hypertension. *J Alternat Complement Med (New York, N.Y.)*. 2002;8:643–650. https://doi.org/10.1089/107555302320825165.
110. Goldamer AC, Gershfeld N, Goldman DM, Myers TR. Challenging case in clinical practice: long-term relief from chronic posttraumatic headache after water-only fasting and an exclusively plant-food diet. *Complement Alternat Ther*. 2017;23:129–131.
111. Spencer IO. Death during therapeutic starvation for obesity. *Lancet (London, England)*. 1968;1:1288–1290.
112. Bloom WL. Fasting as an introduction to the treatment of obesity. *Metabol Clin Experiment*. 1959;8:214–220.
113. Garnett ES, Barnard DL, Ford J, Goodbody RA, Woodehouse MA. Gross fragmentation of cardiac myofibrils after therapeutic starvation for obesity. *Lancet*. 1969;293:914–916. https://doi.org/10.1016/S0140-6736(69)92546-X.
114. Cubberley PT, Polster SA, Schulman CL. Lactic acidosis and death after the treatment of obesity by fasting. *N Engl J Med*. 1965;272:628–630. https://doi.org/10.1056/NEJM196503252721208.
115. Norbury FB. Contraindications to long-term fasting. *JAMA*. 1964;188.88–88.
116. Schless GL, Duncan GG. The beneficial effect of intermittent total fasts on the glucose tolerance in obese diabetic patients. *Metabolism*. 1966;15:98–102.
117. Duncan GG, Hunscher MA, Cristofori FC, Duncan TG, Schless GL. Intermittent total fasts and obesity; indications, results and preventable hazards. *Postgrad Med*. 1965;38:523–535.
118. Duncan GG, Duncan TG, Schless GL, Cristofori FC. Some hazards of total fasts in the control of obesity and diabetes and their prevention. *Trans Am Clin Climatol Assoc*. 1964;76:135–141.
119. Duncan GG. Intermittent fasts in the correction and control of intractable obesity. *Trans Am Clin Climatol Assoc*. 1962;74:121–129.
120. Runcie J, Thomson TJ. Prolonged starvation—a dangerous procedure. *Br Med J*. 1970;3:432–435.
121. Thomson TJ, Runcie J, Miller V. Treatment of obesity by total fasting for up to 249 days. *Lancet (London, England)*. 1966;2:992–996.
122. Drenick EJ, Swendseid ME, Blahd WH, Tuttle SG. Prolonged starvation as treatment for severe obesity. *JAMA*. 1964;187:100–105. https://doi.org/10.1001/jama.1964.03060150024006.
123. Stewart WK, Fleming LW. Features of a successful therapeutic fast of 382 days' duration. *Postgraduate Med J*. 1973;49:203–209.
124. Garnett ES, Barnard DL, Ford J, Goodbody RA, Woodehouse MA. Gross fragmentation of cardiac myofibrils after therapeutic starvation for obesity. *Lancet*. 1969;1:914–916.
125. Gilliland IC. Total fasting in the treatment of obesity. *Postgrad Med J*. 1968;44:58–61.
126. Rose M, Greene RM. Cardiovascular complications during prolonged starvation. *West J Med*. 1979;130:170–177.
127. Scobie IN, Durward WF, MacCuish AC. Proximal myopathy after prolonged total therapeutic starvation. *Br Med J*. 1980;280:1212–1213.
128. Scobie IN, MacCuish AC, Kesson CM, McNeil IR. Neutropenia during allopurinol treatment in total therapeutic starvation. *Br Med J*. 1980;280:1163.
129. Spencer IO. Death during therapeutic starvation for obesity. *Lancet*. 1968;1:1288–1290.
130. Vessby B, Selinus I, Lithell H. Serum lipoprotein and lipoprotein lipase in overweight, type II diabetics during and after supplemented fasting. *Arteriosclerosis*. 1985;5:93–100.
131. Duncan GG. Intermittent fasts in the correction and control of intractable obesity. *Trans Am Clin Climatol Ass*. 1963;74:121–129.
132. Goldhamer AC. Initial cost of care results in medically supervised water-only fasting for treating high blood pressure and diabetes. *J Altern Complement Med*. 2002;8:696–697. https://doi.org/10.1089/107555302260511694.
133. Dorff TB, et al. Safety and feasibility of fasting in combination with platinum-based chemotherapy. *BMC Cancer*. 2016;16:360. https://doi.org/10.1186/s12885-016-2370-6.
134. Allen FM. *Studies Concerning Glycosuria and Diabetes*. Harvard University Press; 1913.
135. Guelpa G, Arnold FS. *Auto-intoxication and Dis-Intoxication*. Rebman; 1912.
136. McCarty MF. Maturity-onset diabetes mellitus—toward a physiological appropriate management. *Med Hypotheses*. 1981;7:1265–1285.
137. Guelpa G, Marie A. La lutte contre l'epilepsie par la desintoxication et par la reeducation alimentarie. *Rev Ther Med Chirurgicale*. 1911;78:8–13.
138. Conklin HW. The cause and treatment of epilepsy. *Am J Osteo*. 1922;22:11–14.
139. Okamoto O, Murakami I, Itami S, Takayasu S. Fasting diet therapy for chronic urticaria: report of a case. *J Dermatol*. 1992;19:428–431.
140. Fuhrman J, Sarter B, Calabro DJ. Brief case reports of medically supervised, water-only fasting associated with remission of autoimmune disease. *Altern Ther Health Med*. 2002;8(112):110–111.
141. Brod J, Pavkova L, Fencl V, Hejl Z, Kratkova E. Influence of fasting on the immunological reactions and course of acute glomerulonephritis. *Lancet (London, England)*. 1958;1:760–763.
142. Kjeldsen-Kragh J, et al. Controlled trial of fasting and one-year vegetarian diet in rheumatoid arthritis. *Lancet*. 1991;338:899–902.
143. Kjeldsen-Kragh J, et al. Changes in laboratory variables in rheumatoid arthritis patients during a trial of fasting and one-year vegetarian diet. *Scand J Rheumatol*. 1995;24:85–93.
144. Panush RS. Controversial arthritis remedies. *Bull Rheumatic Dis*. 1984;34:1–10.
145. Schmidt S, et al. Uncontrolled clinical study of the efficacy of ambulant fasting in patients with osteoarthritis. *Forsch Komplementmed*. 2010;17:87–94. https://doi.org/10.1159/000285479.
146. Sköldstam L, Larsson L, Lindström FD. Effect of fasting and lactovegetarian diet on rheumatoid arthritis. *Scand J Rheumatol*. 1979;8:249–255.
147. Sköldstam L, Lindström FD, Lindblom B. Impaired conA suppressor cell activity in patients with rheumatoid arthritis shows normalization during fasting. *Scand J Rheumatol*. 1983;12:369–373.
148. Sundqvist T, et al. Influence of fasting on intestinal permeability and disease activity in patients with rheumatoid arthritis. *Scand J Rheumatol*. 1982;11:33–38.
149. Udén AM, Trang L, Venizelos N, Palmblad J. Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. *Ann Rheum Dis*. 1983;42:45–51.
150. Kroker GF, RM S, Marshall R, et al. Fasting and rheumatoid arthritis: a multicenter study. *Clin Ecol*. 1984;2:137–144.
151. Duncan GG, Jenson WK, Fraser RI, Cristofori FC. Correction and control of intractable obesity. Practicable application of intermittent periods of total fasting. *JAMA*. 1962;181:309–312.
152. Folin O, Denis W. On starvation and obesity, with special reference to acidosis. *J Biol Chem*. 1915;21:183–192.
153. Harrison M, Harden R. The long-term value of fasting in the treatment of obesity. *Lancet*. 1966;288:1340–1342. https://doi.org/10.1016/S0140-6736(66)92085-X.
154. Kerndt PR, Naughton JL, Driscoll CE, Loxterkamp DA. Fasting: the history, pathophysiology and complications. *West J Med*. 1982;137:379–399.
155. Center TH. 2017. http://www.healthpromoting.com/. Accessed 13 Sept 2019.
156. Homepage. International Association of Healthcare Practitioners. 2017. *https://www.iahp.com/*. Accessed 13 Sept 2019.
157. Burton A. Fasting too long. *J Health Sci*. 1979;2:144–146.
158. Carrington H. *Fasting for Health and Long Life*. Health Research; 1963.
159. Mehanna H, Nankivell PC, Moledina J, Travis J. Refeeding syndrome—awareness, prevention and management. *Head Neck Oncol*. 2009;1:4. https://doi.org/10.1186/1758-3284-1-4. Accessed 13 Sept 2019.
160. Mehanna HM, Moledina J, Travis J. Refeeding syndrome: what it is, and how to prevent and treat it. *BMJ*. 2008;336:1495–1498. https://doi.org/10.1136/bmj.a301.

161. Kahan A, Porter AMW. Death during therapeutic starvation. *Lancet*. 1968;291:1378–1379.
162. Rooth G, Carlström S. Therapeutic fasting. *Acta Med Scand*. 1970;187:455–463.
163. Palmblad J, et al. Acute energy deprivation in man: effect on serum immunoglobulins antibody response, complement factors 3 and 4, acute phase reactants and interferon-producing capacity of blood lymphocytes. *Clin Experiment Immunol*. 1977;30:50–55.
164. Walker HK, Hall WD, Hurst JW. *Clinical Methods*. 3rd ed. Butterworths; 1990.
165. Patrick J. Assessment of body potassium stores. *Kidney Int*. 1977;11:476–490.
166. Brennan BL, Yasumura S, Letteri JM, Cohn SH. Total body electrolyte composition and distribution of body water in uremia. *Kidney Int*. 1980;17:364–371.
167. Ellis KJ, Vaswani A, Zanzi I, Cohn SH. Total body sodium and chlorine in normal adults. *Metabolism*. 1976;25:645–654.
168. Valenta LJ, Elias AN. Modified fasting in treatment of obesity. Effects on serum lipids, electrolytes, liver enzymes, and blood pressure. *Postgraduate Med*. 1986;79:263–267.
169. Ende N. Starvation studies with special reference to cholesterol. *Am J Clin Nutr*. 1962;11:270–280.
170. Vessby B, Boberg M, Karlström B, Lithell H, Werner I. Improved metabolic control after supplemented fasting in overweight type II diabetic patients. *Acta Med Scand*. 1984;216:67–74. https://doi.org/10.1111/j.0954-6820.1984.tb03773.x.
171. Rapoport A, From GLA, Husdan H. Metabolic studies in prolonged fasting: I. Inorganic metabolism and kidney function. *Metabolism*. 1965;14:31–46. https://doi.org/10.1016/0026-0495(65)90079-X.
172. Lithell H, et al. A fasting and vegetarian diet treatment trial on chronic inflammatory disorders. *Acta Dermato-Venereologica*. 1983;63:397–403.
173. From the Centers for Disease Control and Prevention. Lactic acidosis traced to thiamine deficiency related to nationwide shortage of multivitamins for total parenteral nutrition—United States, 1997. *JAMA*. 1997;278:109–111.
174. Devathasen G. Wernicke's encephalopathy in prolonged fasting. *Lancet*. 1982;2:1108–1109.
175. Falzi G, Ronchi E. Wernicke's lethal encephalopathy in voluntary, total, prolonged fasting. *Forensic Sci Int*. 1990;47:17–20.
176. Fuhrman J. *Fasting and Eating for Health: A Medical Doctor's Program for Conquering Disease by Joel Fuhrman*. St. Martin's Griffin; 1758.
177. Duncan GG, Duncan TG, Schless GL, Cristofori FC. Contraindications and therapeutic results of fasting in obese patients. *Annals New York Acad Sci*. 1965;131:632–636.
178. Drenick EJ. Hyperuricemia, acute gout, renal insufficiency and urate nephrolithiasis due to starvation. *Arthritis Rheumatism*. 1965;8:988–997. https://doi.org/10.1002/art.1780080509.
179. Stewart WK, Fleming LW. Fragmentation of cardiac myofibrils after therapeutic starvation. *Lancet (London, England)*. 1969;1:1154.
180. Alirezaei M, et al. Short-term fasting induces profound neuronal autophagy. *Autophagy*. 2010;6:702–710.
181. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. *Cell*. 2014;159:1263–1276. https://doi.org/10.1016/j.cell.2014.11.006.
182. He C, Sumpter Jr R, Levine B. Exercise induces autophagy in peripheral tissues and in the brain. *Autophagy*. 2012;8:1548–1551. https://doi.org/10.4161/auto.21327.
183. Kaur J, Debnath J. Autophagy at the crossroads of catabolism and anabolism. *Nat Rev Mol Cell Biol*. 2015;16:461–472. https://doi.org/10.1038/nrm4024.
184. Ntsapi C, Loos B. Caloric restriction and the precision-control of autophagy: a strategy for delaying neurodegenerative disease progression. *Exp Gerontol*. 2016;83:97–111. https://doi.org/10.1016/j.exger.2016.07.014.
185. Elkeles RS, Tavil AS. *Biochemical Aspects of Human Disease*. Blackwell Scientific; 1983.
186. Montgomery R, Dryer RL, Conway TW, Spector AA. *Biochemistry: A Case-Oriented Approach*. 6th ed. CV Mosby; 1996.
187. Olson RE, et al. Nutrition Foundation, Inc; 1984.
188. Shils ME. *Modern Nutrition in Health and Disease*. 9th ed. Lea & Febiger; 1998.
189. White A, Handler P, Smith EL. *Principles of Biochemistry*. 6th ed. McGraw-Hill; 1978.

38

Glandular Therapy

Michael T. Murray, ND

OUTLINE

INTRODUCTION

For almost as long as historical records have been kept, glandular therapy has been an important form of medicine. The basic concept underlying the medicinal use of glandular substances from animals is that "like heals like." For example, if the liver needs support or a patient is suffering from liver disease, then he or she may benefit from eating beef liver. Modern glandular therapy, however, primarily involves the use of concentrated glandular extracts.

A gland is defined as a secretory organ. The internal secretory organs of the body are called endocrine glands. These ductless glands secrete hormones directly into the bloodstream. The glands known to have endocrine function include the pineal, pituitary, thyroid, parathyroid, thymus, adrenal, pancreas, and gonads (testes or ovaries). Although not technically glands, it is common to refer to other organs of the body as glandulars when they are used in glandular therapy. For example, tissue extracts of heart, spleen, prostate, uterus, brain, and other tissues are often used in glandular or organotherapy.

Research has shown that certain glandular preparations and hormones are quite effective when taken orally. A number of glandular preparations are effective orally because of active hormone or enzyme content (e.g., thyroid, adrenal cortex, and pancreatin preparations). A good deal of literature supports pharmaceutical-grade liver, aorta, and thymus extracts, and some support exists for pituitary, spleen, orchic (testes), and ovarian extracts as well. However, despite this scientific support, many still question the effectiveness of glandular products in human health.

A key challenge to the use of glandulars is the lack of widely accepted standards for extraction and quantification. Each manufacturer of a glandular product claims its method of extraction is the most ideal. However, the majority of these contentions are based on theoretic or philosophical grounds, not on research or clinical results. No quality control procedures or standards are enforced in the glandular industry. It is left up to the individual company to adopt quality control and good manufacturing procedures. Nonetheless, many glandular preparations available in the U.S. marketplace appear to be effective.

METHODS OF MANUFACTURE OF GLANDULAR PREPARATIONS

It is critical that properly processed glandular material be used because the biologically active materials, such as enzymes, soluble proteins, natural lipid factors, vitamins, minerals, and hormone precursors, are destroyed or eliminated if the product is not prepared properly.

Most glandular products are derived from beef (bovine) sources, the exception being pancreatic extracts, which are most often derived from pork (porcine) sources. The four most widely known methods of processing are the azeotropic method, salt precipitation, freeze-drying, and predigestion.

The Azeotropic Method

The azeotropic method begins by quick freezing the material at well below 0°F, then washing the material with a solvent (e.g., ethylene dichloride) to remove the fatty tissue. The solvent is then distilled off, and the material is dried and ground into a powder so that it can be placed in tablets or capsules. Although the azeotropic method eliminates the problem of fat-stored toxins like pesticides and healthy metals, unfortunately, it also removes fat-soluble hormones, enzymes, essential fatty acids, and other potentially beneficial materials; in addition, traces of the solvent still remain.

The Salt Precipitation Method

The salt precipitation method involves the maceration of fresh glandular material in salt and water. Because the salt increases the density of the water-soluble material, when the mixture is centrifuged, the lighter fat-soluble material can be separated out. The material is then dried and powdered. The benefit with the salt

precipitation method is that no toxic solvents are used to separate the fatty material. The downside is the increased salt content of the product.

Freeze-Drying

The freeze-drying process involves quickly freezing the glandular material at temperatures of −40°F to −60°F and then placing the material into a vacuum chamber, which removes the water by direct vaporization from its frozen state (hence the term *freeze-drying*). The benefit of freeze-drying is that the product contains a higher concentration of unaltered protein, enzymes, and all of the fat-soluble components. Because the fat is not removed, it is critical that the glands are derived from livestock that have grazed on open ranges not sprayed with pesticides or herbicides. The animals must also be free from antibiotics, synthetic hormones, and infection.

Predigestion

The predigestion method employs plant and animal enzymes or some other method to partially digest or hydrolyze the glandular material. The partially digested material is then passed through a series of filtrations to separate out fat-soluble and large molecules. The purified material is then freeze-dried. This method of extraction is best for glandulars (e.g., liver and thymus) where the polypeptide and other water-soluble fractions are desired.

SPONGIFORM ENCEPHALOPATHY

Bovine spongiform encephalopathy (BSE), also known as mad cow disease, is a transmissible, slowly progressive, degenerative, fatal disease affecting the central nervous system of adult cattle. The transmissible agent in BSE is a modified form of a normal cell surface component known as a prion protein. Unlike infectious organisms, prions are resistant to common treatments, such as heat and digestive secretions. Eating the meat of an animal with BSE may lead to a disease similar to BSE in humans called variant Creutzfeldt–Jakob disease (CJD).

BSE was first reported among cattle in the United Kingdom in 1986 and has been a major concern since then. The outbreak in the United Kingdom may have started from the feeding of scrapie-contaminated sheep meat-and-bone meal to cattle. Scrapie is a disease of sheep that is related to BSE in cattle. The evidence is strong that the outbreak in cattle was amplified in the United Kingdom by feeding rendered bovine meat-and-bone meal to young calves.

BSE has been reported in cattle throughout Europe. There has been a single case in Canada and in the United States from a cow imported from Canada. Wild game in the United States, such as deer and elk, have been affected by a similar disease known as chronic wasting disease. The reason American cattle have been spared may be because of the active surveillance and import measures taken by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA).

The USDA has restricted the importation of live ruminants, such as cows and sheep, and food products from these animals from BSE countries since 1989 and from all European countries since 1997. In addition, the FDA prohibits the use of most mammalian protein in the manufacture of animal feeds given to ruminants because this kind of feeding practice is believed to have initiated and amplified the outbreak of BSE in the United Kingdom.

To reduce the risk of ingesting beef with BSE, it is critical to use products manufactured under the following guidelines:

- The June 2000 European Commission Decision regulating the use of material presenting risks for BSE
- A "Certification of Suitability" by the European Directorate of Quality of Medicines to ensure that the highest-quality, pharmaceutic-grade material is being used
- The FDA's guidelines for sourcing and processing of bovine material

The incidence of all prion diseases (Kuru, CJD, Gerstmann–Sträussler–Scheinker syndrome, and fatal familial insomnia) worldwide is about 1 to 2 per million people per year.[1]

EVIDENCE FOR INTACT PROTEIN ABSORPTION

There is now considerable evidence that large macromolecules pass intact from the normal human gut into the bloodstream. In some instances, the body appears to recognize which molecules it needs to absorb intact and which molecules it needs to break down into smaller units. This phenomenon may help explain the effectiveness of glandular therapy.

Numerous whole proteins have been shown in human and animal studies to be absorbed intact into the bloodstream after oral administration.[2–8] These include human albumin and lactalbumin, bovine albumin, ovalbumin, lactoglobulin, ferritin (molecular weight 500,000), chymotrypsinogen, elastase, and other large molecules.

Furthermore, proteins and polypeptides, as well as various hormones that are absorbed intact from the gut, have been shown to exert effects in target tissues. For example, in addition to thyroxine or thyroid hormone and cortisone, several peptide hormones are known to be biologically active when administered orally, including luteinizing hormone–releasing factor and thyrotropin-releasing hormone.[9,10] Even insulin has been shown to be absorbed orally under certain circumstances (e.g., in the presence of protease inhibitors or hypertonic solutions in the intestines).[11,12]

These data indicate that at least some of the larger molecules in glandular products are absorbed intact to induce physiological effects, particularly polypeptides, which exert hormone or hormone-like action.

CLINICAL APPLICATIONS

An adequate body of research now exists to support the use of orally administered glandular extracts. The following is a brief discussion of several glandular preparations and their use. Table 38.1 lists the primary conditions responding to glandular therapy.

Adrenal Extracts

Oral adrenal extracts have been used in medicine since at least 1931.[13] Adrenal extracts may be made from the whole adrenal gland or just from the adrenal cortex. Whole adrenal extracts (usually in combination with essential nutrients for the adrenal gland) are most often used in cases of low adrenal function, presenting as fatigue, inability to cope with stress, and reduced resistance. Because extracts made from the adrenal cortex contain small amounts of corticosteroids, they are typically used as a "natural" cortisone in severe cases of allergy and inflammation (e.g., asthma, eczema, psoriasis, rheumatoid arthritis).

Dosage

The dosage of adrenal extract depends on the quality and potency of the product. The best measure of an effective dose for a preparation may be the level of stimulation (irritability, restlessness, and insomnia) the patient experiences. When prescribing adrenal extracts, start at one third of the recommended dosage on the label and slowly increase the dosage every 2 days until a stimulatory effect is noted. Once this effect is noticed, reduce the dosage to a level just below the level that produces stimulation.

TABLE 38.1 Therapeutic Uses of Glandular Extracts

Extract	Clinical applications
Adrenal extracts	Chronic fatigue
	Asthma
	Eczema
	Psoriasis
	Rheumatoid arthritis
Aortic glycosaminoglycans	Cerebral and peripheral arterial insufficiency
	Venous insufficiency and varicose veins
	Hemorrhoids
	Vascular retinopathies, including macular degeneration
	Postsurgical edema
Liver extracts	Chronic hepatitis
	Chronic liver disease
Pancreatic extracts	Pancreatic insufficiency
	Cystic fibrosis
	Inflammatory and autoimmune diseases such as rheumatoid arthritis, scleroderma, athletic injuries, and tendinitis
	Cancer
	Infections
Spleen extracts	After splenectomy
	Immune potentiation
	Infection
	Cancer
	Celiac disease
	Dermatitis herpetiformis
	Ulcerative colitis
	Rheumatoid arthritis
	Glomerulonephritis
	Systemic lupus erythematosus
	Vasculitis
	Low white blood cell counts
	Thrombocytopenia
Thymus extracts	Recurrent and chronic viral infections, such as chronic fatigue syndrome, respiratory infections, AIDS, acute hepatitis B infection
	Cancer patients with immune depression from chemotherapy or radiation
	Asthma, hay fever, eczema, and food allergies
	Autoimmune disorders, such as rheumatoid arthritis, lupus erythematosus, and scleroderma
Thyroid extracts	Hypothyroidism

AIDS, acquired immunodeficiency syndrome.

Aortic Glycosaminoglycans

A mixture of highly purified bovine-derived glycosaminoglycans (GAGs) is naturally present in the aorta, including dermatan sulfate, heparan sulfate, hyaluronic acid, and chondroitin sulfate; related hexosaminoglycans have been shown to protect and promote normal artery and vein function. More than 50 clinical studies have shown an orally administered complex of aortic GAGs to be effective in a number of vascular disorders, including the following:

- Cerebral and peripheral arterial insufficiency
- Venous insufficiency and varicose veins
- Hemorrhoids
- Vascular retinopathies, including macular degeneration postsurgical edema[14–23]

Significant improvements in both symptoms and blood flow have been noted.

In addition, aortic GAGs have many important effects that interfere with the progression of atherosclerosis, including prevention of damage to the surface of the artery, formation of damaging blood clots, migration of smooth muscle cells into the intima, formation of fat and cholesterol deposits, and lowering total cholesterol levels while raising high-density lipoprotein cholesterol.[24–28]

Dosage

The dosage of the mixture of highly purified bovine-derived GAGs naturally present in the aorta is 100 mg/day. Similar, but not nearly as impressive, results in the treatment of atherosclerosis have been noted with chondroitin sulfate at a daily dose of 3 g (1 g, three times daily).[29]

Liver Extracts

Beef (bovine) liver extracts and concentrates are a rich natural source of many vitamins and minerals, including iron. Liver extracts can contain as much as 3 to 4 mg of heme iron per gram. In addition to its use as a source of iron and other nutrients, hydrolyzed liver extracts have been used to treat chronic liver diseases since 1896. Numerous scientific investigations into the therapeutic efficacy of liver extracts demonstrated that these extracts improved fat utilization, promoted tissue regeneration, and prevented damage to the liver.[30–33] In short, clinical studies demonstrated that oral administration of hydrolyzed liver extracts can be quite effective in improving liver function.

For example, in one double-blind study, 556 patients with chronic hepatitis were given either 70 mg of liver hydrolysate or a placebo three times daily.[33] After 3 months of treatment, the group that received the liver extract had far lower serum liver enzyme levels. Because the level of liver enzymes in the blood reflects damage to the liver, it can be concluded that liver extract is effective in chronic hepatitis via an ability to improve the function of damaged liver cells, and to prevent further damage to the liver.

Dosage

The dosage is entirely dependent on the concentration, method of preparation, and quality of the liver extract. The highest-quality products are aqueous hydrolyzed extracts because they have the fat-soluble components removed and typically contain more than 20 times the nutritional content of raw liver, including 3 to 4 mg of heme iron per gram.

Contraindication

Liver extracts should not be used in patients experiencing an iron-storage disorder, such as hemochromatosis.

Pancreatic Extracts

Pancreatic enzymes are most often employed in the treatment of pancreatic insufficiency. Pancreatic insufficiency is characterized by impaired digestion, malabsorption, nutrient deficiencies, and abdominal discomfort. Pancreatic enzymes are also used by physicians in the treatment of the following:

- Cystic fibrosis
- Inflammatory and autoimmune diseases like rheumatoid arthritis, scleroderma, athletic injuries, and tendinitis
- Cancer
- Infections

For a full discussion of pancreatic enzymes, see Chapter 111.

Dosage

Full-strength products are preferred to lower-potency pancreatin products because lower-potency products are often diluted with salt, lactose, or galactose to achieve the desired strength (e.g., 4× or 1×). The dosage recommendation for a 10× U.S. Pharmacopoeia (USP) pancreatic enzyme product is 500 to 1000 mg three times a day immediately before meals when used as a digestive aid and at least 20 minutes before meals or on an empty stomach when anti-inflammatory effects are desired.

Pancreatic extracts are generally well tolerated and are not associated with any significant side effects.

Spleen Extracts

As early as the 1930s, orally administered bovine spleen extracts were shown to possess some physiological action in increasing white blood cell counts in patients with extreme deficiencies of white blood cells and being of some benefit in patients with malaria and typhoid fever.[34–36]

Like thymus extracts, pharmaceutical-grade bovine spleen extracts are currently quite popular in Germany for the treatment of infectious conditions and as immune-enhancing agents in cancer. Spleen tissue extracts may be of benefit in enhancing general immune function because many potent immune system–enhancing compounds secreted by the spleen are low-molecular-weight peptides. For example, the potent immunostimulants tuftsin and splenopentin are composed of only four and five amino acids, respectively.

Both tuftsin and splenopentin have been shown to exert profound immune-enhancing activity. Tuftsin stimulates macrophages that have taken up residence in specific tissues like the liver, spleen, and lymph nodes. Tuftsin also helps mobilize other white blood cells to fight against infection and cancer. A deficiency of tuftsin has been associated with signs and symptoms of frequent infections.[37]

Splenopentin, like tuftsin, has also demonstrated significant immune-enhancing effects. Its effects are primarily directed toward enhancing the immune system's response to regulating compounds such as colony-stimulating factors.[38] Colony-stimulating factors, such as interleukin-3, and granulocyte/macrophage colony–stimulating factors stimulate the production of white blood cells. Splenopentin is probably the factor responsible for the results noted in clinical studies during the 1930s in which spleen extracts were used in the treatment of depressed white blood cell counts.

Splenopentin has also been shown to enhance the activity of natural killer cells.[39] Natural killer cells destroy cells that have become cancerous or infected with viruses and are the body's first line of defense against cancer.

In addition to tuftsin and splenopentin, hydrolyzed (predigested) spleen extracts concentrated for peptides demonstrated impressive immune restorative properties in mice.[40] In one study, mice were exposed to radiation to significantly damage the immune system. Mice treated with the spleen extract recovered within 6 to 8 weeks. In contrast, those treated with a placebo recovered after 10 weeks at the earliest.

The primary clinical uses of spleen extracts are after removal of the spleen, and the conditions associated with low spleen function (hyposplenia) are shown in Table 38.2.

Spleen extracts may also be useful in the treatment of low white blood cell counts, bacterial infections, and as an adjunct in cancer therapy. Individuals who have had splenectomies or who have low tuftsin levels or autoimmune conditions linked to low activity of the reticuloendothelial system should use spleen extracts.

Because the spleen is difficult to repair, severe spleen trauma usually requires splenectomy to stop the severe hemorrhage. The spleen is also removed in the medical treatment of certain diseases, such as idiopathic thrombocytic purpura and to determine the extent of Hodgkin disease.

TABLE 38.2 Conditions Associated With Low Spleen Function

- Celiac disease
- Dermatitis herpetiformis
- Ulcerative colitis
- Rheumatoid arthritis
- Glomerulonephritis
- Systemic lupus erythematosus
- Vasculitis
- Thrombocytopenia

Removal of the spleen is associated with an increased risk for infection. This increased risk of infection makes children and adults particularly susceptible to pneumococcal pneumonia. About 2.5% of patients who have their spleen removed die of pneumococcal pneumonia within 5 years of splenectomy. It is often recommended that a child who has undergone a splenectomy receive a pneumococcal vaccine and receive long-term antibiotic treatment. Use of spleen extracts, especially those rich in tuftsin, may be a natural alternative.

Spleen extracts should probably be viewed as a necessary medicine for people who have had their spleen removed. If the thyroid, adrenals, or ovaries are removed, most patients would be prescribed the corresponding hormone. It only makes sense that if the spleen is removed, the body should be supplied with necessary spleen substances like tuftsin and splenopentin.

The increased risk of infection is attributed primarily to a deficiency of tuftsin.[41,42] Tuftsin is produced only in the spleen; without the spleen, there simply is no tuftsin in the circulation. Without tuftsin, the body is without one of its key stimulators of the immune system. Individuals without spleens need an outside source of tuftsin, like spleen extracts.

Dosage

Clinically, hydrolyzed (predigested) products concentrated for tuftsin and splenopentin content are preferable to crude preparations. The daily dose should provide 50 mg tuftsin and splenopentin or roughly 1.5 g of total spleen peptides.

No side effects or adverse effects have been reported with the use of oral spleen preparations.

Polyerga

Before the development of manufacturing techniques for human insulin to be used in the treatment of diabetes, the source was insulin isolated from pig pancreas. In Germany, after World War II, there was a shortage of pigs and subsequently a shortage of insulin. A German researcher, Walter Kuhlmey, MD, PhD, subsequently sought to produce insulin from other animal organs, most notably the spleen. In the process, this led to the serendipitous discovery of Polyerga. Kuhlmey found that the pig spleen extract not only had some insulin-like activity but that it also increased the general sense of well-being and gave people more energy. He named his new product Polyerga (in Latin, *poly* means "multiple," and *erga* means "power").

Initially, Polyerga had a general use. In 1951 an event changed Polyerga's clinical use. Julia Meir was a patient of oncologist Heinrich Pophanken, MD. She had an advanced cancer of the pancreas. She was considered incurable, and her death was expected shortly. In an effort to ease her pain, lift her fatigue, and stimulate a sense of well-being in Julia, Pophanken gave her Polyerga (via injection). Surprisingly, the

dying patient began to recover. Pophanken gave Julia three to six intramuscular injections of Polyerga per week during the first 2 months and then just two injections a week thereafter. In 1954 Julia died of causes unrelated to her cancer. When the autopsy was conducted, the egg-sized tumor in her pancreas had disappeared entirely. This single case history led to the focus of research on Polyerga as a cancer therapy.

Cancer Therapy

Polyerga has been evaluated in several clinical studies in cancer patients. In one of the largest trials, 158 breast cancer patients were divided into two groups. The women in the Polyerga group received injections of Polyerga three times per week, whereas the women in the control group received a placebo injection. In the Polyerga group, the percentage of white blood cells, various parameters of immune function, body weight, and general sense of well-being all improved significantly compared with the control group.[43]

In a double-blind, placebo-controlled study in patients with head and neck cancers, Polyerga was shown to support patients who received the chemotherapy drugs 5-FU and cisplatin.[44] Polyerga was shown to stabilize the levels of lymphocytes. Typically, patients receiving 5-FU, cisplatin, and other chemotherapy drugs experienced a reduction in lymphocytes. This effect was noted in the placebo group but not in the patients receiving Polyerga. In addition, the Polyerga group reported less fatigue and higher energy levels than the placebo group, and there was no weight loss. Prevention of weight loss is a major goal in support of the cancer patient because significant weight loss reduces the tolerance to anticancer drugs, reduces the functioning of the immune system, and is considered to be a major cause of death in the cancer patient.

In another study of 248 cancer patients, giving them Polyerga orally for 4 months improved appetite, reduced pain, increased energy and activity levels, and improved their general sense of well-being.[45] Furthermore, a reduction of the extent of the illness, along with a clear improvement of health, was recorded by observing physicians. The best results were obtained in breast cancer patients and patients experiencing colon and other carcinomas, whereas lung cancer patients and patients with metastases did not seem to respond as well to Polyerga.

Other studies have shown Polyerga to produce similar benefits, as well as enhance survival time and reduce metastasis (spreading) in patients with cancers of the colon, stomach, and lungs.[46] From the totality of existing clinical studies, animal studies, and test tube (in vitro) studies, it can be concluded that Polyerga does the following:

- Exerts significant immune-enhancing effects
- Prevents some side effects of chemotherapy, especially the detrimental effects on the immune system
- Enhances the effectiveness of conventional chemotherapy and radiation treatment
- Prevents metastasis
- Enhances the general sense of well-being, improves energy levels, and prevents detrimental weight loss in cancer patients
- Enhances both the quality of life and survival time in cancer patients

The Mechanism of Action

Many effects of Polyerga may be the result of boosting the output of γ-interferon.[47] This important chemical acts as the communication link between the macrophages and lymphocytes. Reduction in the level of γ-interferon results in significantly impaired immune function. Low γ-interferon levels are common in cancer patients. By increasing γ-interferon levels, Polyerga creates a cascading of effects that lead to enhanced immune function in cancer patients. Polyerga increases the activation of natural killer cells.[48–51]

Other Uses

Polyerga may be helpful any time immune support is required, such as in chronic viral infections, including hepatitis C, human immunodeficiency virus (HIV), and Epstein–Barr syndrome. In a study of 10 patients with chronic hepatitis B, Polyerga administration (intramuscularly twice weekly and orally three tablets daily for 24 weeks) led to complete resolution in 3 of the 10 patients and lowering of liver enzymes in individuals with lower viral loads.[52] Polyerga may also be quite helpful to people who have had their spleens removed.

Dosage

Most studies with Polyerga used injectable preparations. Based on dose-effect studies in animals, as well as human clinical data, an effective oral dosage was determined. Each Polyerga tablet contains 100 mg of polypeptides. The optimum dosage recommendation is one to two tablets three times per day. For patients up to 140 lb body weight, the dosage is one tablet three times per day. For each additional 40 lb body weight, add a tablet (e.g., a 220-lb person would take five tablets daily in divided dosages). There is no toxicity with Polyerga, so there is no concern about overdosage.[53] However, taking more than the recommended dose does not necessarily produce better results. For best results, Polyerga should be ingested on an empty stomach before meals.

Thymus Extracts

A substantial amount of clinical research supports the effectiveness of orally administered thymus extracts. Specifically, numerous clinical trials have shown that oral administration of predigested calf thymus extract, rich in thymus-derived polypeptides, is effective in doing the following:

- Preventing recurrent respiratory infections in children
- Correcting the T-cell defects in HIV infections (acquired immunodeficiency syndrome [AIDS])
- Treating acute hepatitis B infections
- Restoring the number of peripheral leukocytes in cancer patients with chemotherapy-induced depression of white blood cell counts
- Treating asthma, hay fever, and food allergies in children[54,55]

The effectiveness of the thymus extract in these conditions is reflective of broad-spectrum immune system enhancement presumably mediated by improved thymus gland activity. This effect fits in nicely with one of the basic concepts of glandular therapy (i.e., that the oral ingestion of glandular material of a certain animal gland strengthens the corresponding human gland). The result is a broad general effect indicative of improved glandular function. Interestingly, thymus extracts have been shown to normalize the ratio of T-helper cells to suppressor cells, whether the ratio is low, as in AIDS, chronic infections, and cancer, or high, as in allergies, migraine headaches, and autoimmune diseases like rheumatoid arthritis.

Chronic Viral Infections

Recurrent or chronic infections, including chronic fatigue syndrome and chronic postviral syndrome, are characterized by a depressed immune system. This is a difficult condition to treat because of the repetitive cycle of a compromised immune system leading to infection, which leads to damage to the immune system, further weakening resistance to viral infection. Thymus extracts may provide the answer to chronic infections by restoring healthy immune function.

The ability of thymus extracts to treat and then reduce the number of recurrent infections has been studied in groups of children with a history of recurrent respiratory tract infections. Controlled clinical studies revealed not only that orally administered thymus extracts were able to effectively eliminate infection, but also that treatment

over the course of a year significantly reduced the number of respiratory infections and significantly improved numerous immune parameters.[56]

One of the most difficult viral infections for the body to eliminate is type B viral hepatitis. Thymus extracts have been shown to be effective in several clinical studies in both acute and chronic cases. In these studies, a therapeutic effect was noted by accelerated decreases of liver enzymes (transaminases), elimination of the virus, and a higher rate of seroconversion to anti-HBe, signifying clinical remission.[57,58]

The most extreme example of a chronic viral infection is AIDS. Although thymus extracts have not been shown to reverse this difficult disease, studies have shown an ability to improve several immune parameters, including an ability to increase the T-helper cells, a critical goal in AIDS treatment.[59]

Cancer

The primary application of thymus extracts in cancer has been to counteract the immune-suppressing effects of radiation and chemotherapy. The net effect of thymus extract administration is to prevent the tremendous depression of white blood cell levels and activity that result from chemotherapy or radiation.[55,60]

Allergies

In patients with allergies, levels of the allergic immunoglobulin-E antibody and eosinophils are typically elevated, whereas levels of suppressor T cells are typically depressed. These abnormalities are clear indications of altered immune function.

The oral administration of thymus extracts has been shown in double-blind clinical studies to improve the symptoms and course of hay fever, allergic rhinitis, asthma, eczema, and food allergies.[55,61–63] Presumably, this clinical improvement is the result of the restoration of proper immune function because levels of immunoglobulin-E and eosinophils have been shown to be reduced, whereas the ratio of helper to suppressor T cells is improved.

Interestingly, in several clinical studies, children receiving thymus extracts during food allergy elimination diets were often able to tolerate foods that had previously been allergenic and symptom producing.[62,63]

Autoimmune Disorders

Autoimmune disorders, such as rheumatoid arthritis, are characterized by autoimmunity. Central to this immune dysfunction is a high ratio of T-helper to suppressor cells. A high ratio of T-helper to suppressor cells results in increased antibody formation. The higher the ratio, the higher the number of antibodies being produced to damage body structures. In one clinical study, rheumatoid arthritis patients with a ratio of T-helper to suppressor cells of 3.3 achieved normal ratios (1.02–2.46) after 3 months of therapy with a thymus extract.[55]

Although the use of a thymus extract may not result in substantial clinical improvement, it appears to be useful in restoring proper immune function in autoimmune diseases, including rheumatoid arthritis, lupus, and scleroderma.

Dosage

From a practical view, products concentrated and standardized for polypeptide content are preferable to crude preparations. The daily dose should be equivalent to 120 mg pure polypeptides with molecular weights less than 10,000, or roughly 750 mg of the crude polypeptide fraction.

No side effects or adverse effects have been reported with the use of thymus preparations.

Thyroid Extracts

Desiccated natural thyroid is available by prescription according to USP guidelines. Preparations are derived from porcine thyroid glands. Many naturopathic physicians prefer natural thyroid to isolated synthetic T_4 because it contains both thyroxine (T_4) and tri-iodothyronine (T_3). Typical levels of thyroid hormone contained per grain in USP thyroid are 38 mcg of T_4 and 9 mcg of T_3.

The thyroid extracts sold as "nutritional supplements" are required by the FDA to be thyroxine-free. However, it is nearly impossible to remove all the hormone from the gland. These nutritional thyroid preparations can be considered milder forms of desiccated natural thyroid.

The primary use of thyroid preparations is in the medical treatment of hypothyroidism. In all but its mildest forms, treatment involves the use of desiccated thyroid or synthetic thyroid hormone. For more discussion, see Chapter 209.

Dosage

Dosage is determined by clinical evaluation, including blood measurements of thyroid hormones (see Chapter 209 for directions).

SUMMARY

From the scientific data that currently exist, there is enough evidence to support the use of orally administered glandular extracts. For the best results, physicians should choose glandular products made by reputable companies that employ established methods of manufacture to produce extracts of known concentration.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Chen C, Dong XP. Epidemiological characteristics of human prion diseases. *Infect Dis Poverty*. 2016;5:47.
2. Gardner ML. Gastrointestinal absorption of intact proteins. *Annu Rev Nutr*. 1988;8:329–350.
3. Gardner ML. Intestinal assimilation of intact peptides and proteins from the diet—a neglected field? *Biol Rev*. 1984;59:289–331.
4. Udall JN, Walker WA. The physiologic and pathologic basis for the transport of macromolecules across the intestinal tract. *J Pediatr Gastroenterol Nutr*. 1982;1:295–301.
5. Kleine MW, Stauder GM, Beese EW. The intestinal absorption of orally administered hydrolytic enzymes and their effects in the treatment of acute herpes zoster as compared with those of oral acyclovir therapy. *Phytomedicine*. 1995;2:7–15.
6. Hemmings WA, Williams EW. Transport of large breakdown products of dietary protein through the gut wall. *Gut*. 1978;19:715–723.
7. Ambrus JL, Lassman HB, De Marchi JJ. Absorption of exogenous and endogenous proteolytic enzymes. *Clin Pharmacol Ther*. 1967;8:362–368.
8. Kabacoff BB. Absorption of chymotrypsin from the intestinal tract. *Nature*. 1963;199:815–817.
9. Ormiston BJ. Clinical effects of TRH and TSH after IV and oral administration in normal volunteers and patients with thyroid disease. In: Hal R, Werner I, Holgate H, eds. *Frontiers Of Hormone Research*. Vol. 1. Basel, Switzerland: Karger; 1972:45–52.
10. Amoss MS, Rivier J, Guillemin R. Release of gonadotrophins by oral administration of synthetic LRF or tripeptide fragment of LRF. *J Clin Endocrinol Metab*. 1972;35:175–177.
11. Seifert J. Mucosal permeation of macromolecules and particles. *Angiology*. 1966;17:505–513.
12. Laskowski M, Haessler HA, Miech RP, et al. Effect of trypsin inhibitor on passage of insulin across the intestinal barrier. *Science*. 1958;127:1115–1116.
13. Britton SW, Silvette H. Further experiments on cortico-adrenal extract. Its efficacy by mouth. *Science*. 1931;74:440–441.
14. Abate G, Berenga A, Caione F, et al. Controlled multicenter study on the therapeutic effectiveness of mesoglycan in patients with cerebrovascular disease. *Minerva Med*. 1991;82:101–105.
15. Mansi D, Sinsi L, De Michele G, et al. Open trial of mesoglycan in the treatment of cerebrovascular ischemic disease. *Acta Neurol (Napoli)*. 1988;10:108–112.
16. Laurora G, Cesarone SR, De Sanctis MT, et al. Delayed arteriosclerosis progression in high risk subjects treated with mesoglycan. Evaluation of intima-media thickness. *J Cardiovasc Surg*. 1993;34:313–318.
17. Vecchio F, Zanchin G, Maggioni F, et al. Mesoglycan in treatment of patients with cerebral ischemia. Effects on hemorheologic and hematochemical parameters. *Acta Neurol (Napoli)*. 1993;15:449–456.
18. De Donato G, Sangiuolo P. Instrumental evaluation of the effects of mesoglycan in venous disease patients. Prospective randomized double-blind study. In Italian. *Minerva Med*. 1986;77:1927–1931.
19. Oddone G, Fiscella GF, De Franceschi T. Assessment of the effects of oral mesoglycan sulphate in patients with chronic venous pathology of the lower extremities. *Gazz Med Ital*. 1987;146:111–114.
20. Prandoni P, Cattelan AM, Carta M. Long-term sequelae of deep venous thrombosis of the legs. Experience with mesoglycan. In Italian. *Ann Ital Med Int*. 1989;4:378–385.
21. Sangrigoli V, Carra G, Lazzara N, et al. Mesoglycan in acute and chronic venous insufficiency of the legs. In Italian. *Clin Ter*. 1989;129:207–209.
22. Petruzzellis V, Velon A. Therapeutic action of oral mesoglycan in the pharmacologic treatment of the varicose syndrome and its complications. In Italian. *Minerva Med*. 1985;76:543–548.
23. Saggioro A, Chiozzini G, Pallini P, et al. Treatment of hemorrhoidal crisis with mesoglycan sulfate. In Italian. *Minerva Dietol Gastroenterol*. 1985;31:311–315.
24. Stevens RL, Colombo M, Gonzales JJ, et al. The glycosaminoglycans of the human artery and their changes in atherosclerosis. *J Clin Invest*. 1976;58:470–481.
25. Tammi M, Seppala PO, Lehtonen A, et al. Connective tissue components in normal and atherosclerotic human coronary arteries. *Atherosclerosis*. 1978;29:191–194.
26. Day CE, Powell JR, Levy RS. Sulfated polysaccharide inhibition of aortic uptake of low density lipoproteins. *Artery*. 1975;1:126–137.
27. Postiglione A, De Simone B, Rubba P, et al. Effect of oral mesoglycan-sulphate on plasma lipoprotein concentration and on lipoprotein concentration in primary hyperlipidemia. *Pharmacol Res Commun*. 1984;16:1–8.
28. Saba P, Galeone F, Giuntoli F, et al. Hypolipidemic effect of mesoglycan in hyperlipidemic patients. *Curr Ther Res*. 1986;40:761–768.
29. Nakazawa K, Murata K. The therapeutic effect of chondroitin polysulphate in elderly atherosclerotic patients. *J Int Med Res*. 1978;6:217–225.
30. Nagai K. A study of the excretory mechanism of the liver—effect of liver hydrolysate on BSP excretion. *Jpn J Gastroenterol*. 1970;67:633–638.
31. Ohbayashi A, Akioka T, Tasaki H. A study of effects of liver hydrolysate on hepatic circulation. *J Therapy*. 1972;54:1582–1585.
32. Sanbe K, Murata T, Fujisawa K, et al. Treatment of liver disease—with particular reference to liver hydrolysates. *Jpn J Clin Exp Med*. 1973;50:2665–2676.
33. Fujisawa K, Suzuki H, Yamamoto S, et al. Therapeutic effects of liver hydrolysate preparation on chronic hepatitis—a double blind, controlled study. *Asian Med J*. 1984;26:497–526.
34. Minter MM. Agranulocytic angina: treatment of a case with fetal calf spleen. *Texas State J Med*. 1933;2:338–343.
35. Gray GA. The treatment of agranulocytic angina with fetal calf spleen. *Texas State J Med*. 1933;29:366–369.
36. Greer AE. Use of fetal spleen in agranulocytosis. Preliminary report. *Texas State J Med*. 1932;28:338–343.
37. Fridkin M, Najjar VA. Tuftsin: its chemistry, biology, and clinical potential. *Crit Rev Biochem Mol Biol*. 1989;24:1–40.
38. Diezel W, Weber HA, Maciejewski J, et al. The effect of splenopentin (DA SP-5) on in vitro myelopoiesis and on AZT-induced bone marrow toxicity. *Int J Immunopharmacol*. 1993;15:269–273.
39. Rastogi A, Singh VK, Biswas S, et al. Augmentation of human natural killer cells by splenopentin analogs. *FEBS Lett*. 1993;317:93–95.
40. Volk HD, Eckert R, Diamantstein T, et al. Immunorestitutive action of hydrolysates and ultrafiltrates of bovine spleen. *Arzneimittelforschung*. 1991;41:1281–1285.
41. He SW. Effect of splenectomy on phagocytic function of leukocytes. *Zhonghua Wai Ke Za Zhi*. 1989;27:354–356, 381.
42. Spirer Z, Zakuth V, Diamant S, et al. Decreased tuftsin concentrations in patients who have undergone splenectomy. *BMJ*. 1977;2:1574–1576.
43. Berressem P, Frech S, Hartleb M. Additional therapy with Polyerga improve immune reactivity and quality of life in breast cancer patients during rehabilitation. *Tumor Diagnos Ther*. 1995;16:45–48.
44. Borghardt J, Rosien B, Gortelmeyer R, et al. Effects of a spleen peptide preparation as supportive therapy in inoperable head and neck cancer patients. *Arzneimittelforschung*. 2000;50:178–184.
45. Maar K. Improvement of the general condition of tumor patients. *Erfahrungsheilkunde*. 1998;47:60–64.
46. Klose G, Mertens J. Long term results of post-operative treatment of carcinoma of the stomach with Polyerga. *Therapiewoche*. 1977;27:5359–5361.
47. Baier JE, Neumann HA, Taufighi-Chirazi T, et al. Thymopentin, factor AF2, and Polyerga improve impaired mitogen induced interferon-g release of peripheral blood mononuclear cells derived from tumor patients. *Tumor Diagnos Ther*. 1994;15:21–26.
48. Zarkovic N, Hartleb M, Zarkovic K, et al. Spleen peptides (Polyerga) inhibit development of artificial lung metastases of murine mammary carcinoma and increase efficiency of chemotherapy in mice. *Cancer Biother Radiopharm*. 1998;13:25–32.
49. Jurin M, Zarkovic N, Ilic Z, et al. Porcine splenic peptides (Polyerga) decrease the number of experimental lung metastases in mice. *Clin Exp Metastasis*. 1996;14:55–60.
50. de Ojeda G, Diez-Orejas R, Portoles P, et al. Polyerga, a biological response modifier enhancing T-lymphocyte-dependent responses. *Res Exp Med (Berl)*. 1994;194:261–267.
51. Klingmüller M. Spleen peptides activate natural killer cells. *Erfahrungsheilkunde*. 1999;12:756–759.

52. Vassilev M, Antonov K, Theocharov P, et al. Effects of low molecular weight glycoproteins in chronic hepatitis B. *Hepatogastroenterology*. 1996;43:882–886.
53. Hartleb M, Leuschner J. Toxicological profile of a low molecular weight spleen peptide formulation used in supportive cancer therapy. *Arzneimittelforschung*. 1997;47:1047–1051.
54. Cazzola P, Mazzanti P, Bossi G. In vivo modulating effect of a calf thymus acid lysate on human T lymphocyte subsets and CD4+/ CD8+ ratio in the course of different diseases. *Curr Ther Res*. 1987;42:1011–1017.
55. Kouttab NM, Prada M, Cazzola P. Thymomodulin: biological properties and clinical applications. *Med Oncol Tumor Pharmacother*. 1989;6:5–9.
56. Fiocchi A, Borella E, Riva E, et al. A double-blind clinical trial for the evaluation of the therapeutic effectiveness of a calf thymus derivative (Thymomodulin) in children with recurrent respiratory infections. *Thymus*. 1986;8:331–339.
57. Galli M, Crocchiolo P, Negri C, et al. Attempt to treat acute type B hepatitis with an orally administered thymic extract (Thymomodulin). Preliminary results. *Drugs Exp Clin Res*. 1985;11:665–669.
58. Bortolotti F, Cadrobbi P, Criverllaro C, et al. Effect of an orally administered thymic derivative, Thymodulin, in chronic type B hepatitis in children. *Curr Ther Res*. 1988;43:67–72.
59. Valesini G, Barnaba V, Benvenuto R, et al. A calf thymus lysate improves clinical symptoms and T-cell defects in the early stages of HIV infection. Second report. *Eur J Cancer Clin Oncol*. 1987;23:1915–1919.
60. Kang SD, Lee BH, Yang JH, et al. The effects of calf-thymus extract on recovery of bone marrow function in anticancer chemotherapy. *N Med J (Korea)*. 1985;28:11–15.
61. Marzari R, Mazzanti P, Cazzola P, et al. Perennial allergic rhinitis. Prophylaxis of acute episodes using thymomodulin. In Italian. *Minerva Med*. 1987;78:1675–1681.
62. Genova R, Guerra A. Thymomodulin in management of food allergy in children. *Int J Tissue React*. 1986;8:239–242.
63. Cavagni G, Piscopo C, Rigoli E, et al. Food allergy in children: an attempt to improve the effects of the elimination diet with an immunomodulating agent (thymomodulin). A double-blind clinical trial. *Immunopharmacol Immunotoxicol*. 1989;11:131–142.

39

Homeopathy

Andrew Lange, ND,* and *Susan Ann Gaylord, PhD*

OUTLINE

INTRODUCTION

Homeopathy is a highly systematized method of medical therapeutics and clinical evaluation. The term *homeopathy* is derived from the Greek words *homeos*, meaning "similar," and *pathos*, meaning "suffering." The medicines used in this system of therapeutics are chosen according to the Law of Similars (the concept of like curing like), a fundamental homeopathic principle based on the observed relationship between a medicine's ability to produce a specific constellation of signs and symptoms in a healthy individual and the same medicine's ability to cure a sick patient with similar signs and symptoms. This principle was first recognized by Hippocrates, who noticed that herbs given in low doses tended to cure the same symptoms they produced when given in toxic doses.

Homeopathic medicines are derived from a wide variety of plant, mineral, and chemical substances. They are prepared according to standards of the *U.S. Homeopathic Pharmacopoeia*, a revised version of which has been approved by the U.S. Food and Drug Administration and U.S. Congress.

HISTORY

The homeopathic school of medicine was founded by a German physician, Samuel Hahnemann. He had already gained a reputation in chemistry and medicine, having formulated a soluble form of mercury and developed a safer method for its use and having written a number of works on pharmacology, hygiene and public health, industrial toxicology, and psychiatry. His treatise on arsenic poisoning (1786) is still considered authoritative. A prolific writer, Hahnemann collected, compiled, revised, and edited the existing pharmacological knowledge. The work was well received by the medical profession of the time. Hahnemann was one of the most learned men of his generation in medicine, chemistry, and pharmacology, making his later criticisms of medicine all the more significant.[1]

Disillusioned with the theories and practice of 18th-century medicine, Hahnemann retired from practice in 1782 and spent the next 14 years earning a meager living doing chemical research; writing; and translating English, French, Italian, and Latin works. He wrote of his time of practice:

> *It was painful for me to grope in the dark, guided only by books in the treatment of the sick. To prescribe according to this or that (fanciful) view of the nature of diseases, substances that only owed to mere opinion their place in the materia medica; I had conscientious scruples about treating unknown morbid states in my suffering fellow creatures with these unknown medicines which, being powerful substances, may, if they were not exactly suitable (and how could the physician know whether they were suitable or not, seeing that their peculiar special actions were not yet elucidated) easily change life into death, or produce new affections and chronic ailments, which are often more difficult to remove than the original disease.*

In his struggle to determine a reliable basis for therapeutics, he was distressed by his inability to provide medical care for the acute illnesses of even his own growing family. In 1790 during his translation of William Cullen's (a Scottish physician) *Materia Medica,* he added a footnote disagreeing with Cullen's conclusions that the basis of cinchona bark's effectiveness was its bitter and astringent qualities. *Cinchona officinalis* (Peruvian bark), from which the drug quinine is derived, was known to be clinically effective in malaria and intermittent fevers (then called ague). He argued that there were several drugs in common usage that, in smaller doses, had greater bitter and

*Previous edition contributor

astringent qualities yet had no specific action on fevers. As an experiment, Hahnemann took four drachms of cinchona twice daily and soon developed the paroxysmal symptoms characteristic of intermittent fevers.

This duplication of symptoms was a revelation to him and ultimately resulted in his formulation of the concept of determining the properties of a medicine by studying its effects on healthy humans.

Although homeopathy offers a profoundly deep and unified evaluation in the treatment of chronic diseases (see the section "Follow-Up and Case Evaluation"), it gained most of its early reputation in the treatment of acute and epidemic diseases. An uproar was caused in Cincinnati in 1849 when two immigrant German homeopaths, treating cholera with camphor and other homeopathically prescribed remedies, published statistics in newspapers indicating that only 35 of their 1116 treated cases had died. During the 19th century, 33% to 50% of patients with cholera who were given standard medical care died. In the 1879 epidemic of yellow fever, New Orleans homeopaths treated 1945 cases with a mortality rate of 5.6%, whereas standard medical doctors were losing 16% of patients. These and similar statistics had a profound effect on Congress and public opinion.[2]

Over time, homeopaths established their own network of treatment facilities. By 1892, in the United States, they controlled 110 hospitals, 145 dispensaries, 62 orphan asylums and retirement homes, more than 30 nursing homes and sanatoria, and 16 insane asylums.

Constantine Hering established the first homeopathic medical school in the United States in 1835. It later moved from its original site in Allentown, Pennsylvania, to Philadelphia, where it remains today as an orthodox medical school: the Hahnemann Medical College and Hospital. Hering's promotion of homeopathy and development of the materia medica were equaled only by Hahnemann himself. Hering's 10-volume work, *The Guiding Symptoms of Our Materia Medica,* remains a definitive work on the clinical verifications of the homeopathic approach. Unfortunately, of the many medicines introduced by Hering, only nitroglycerine remains in orthodox medical practice as a tribute to his medical genius.

Throughout the world, homeopathy has maintained a consistent tradition. Frederick Harvey Foster Quinn introduced it to England in the 1840s. It has since become a postgraduate medical specialty, recognized by the Department of Health by virtue of an Act of Parliament. Homeopathic hospitals and outpatient clinics are part of England's national health system. Homeopaths have been engaged as personal physicians to the royal family for the past four generations.

Homeopathy is widely practiced in Europe, India, Argentina, and Mexico and is experiencing a renaissance in the United States.

PHILOSOPHY

Provings

Hahnemann defined his method of testing medicines on healthy people as "provings." He expanded his investigations to include a wide range of substances, using his family, friends, and associates as experimental subjects.

Historically, Hahnemann was not the first to use this methodology. In 1760 Anton Stoerck reported testing stramonium *(Datura)* by rubbing it on the skin, inhaling the vapors of the freshly crushed leaves, and finally, ingesting the fresh extract. He theorized that if stramonium disturbs the senses and produces mental derangements in healthy people, it might be administered to maniacs for the purpose of restoring the senses by effecting a change of ideas.

The medical literature contains examples of inadvertent provings: in 1983 a study in the *New England Journal of Medicine* reported that pyridoxine (vitamin B_6), which is used in the treatment of some types of peripheral neuropathy, was also capable of producing neuropathies when given in large doses.[3] In 1796 Hahnemann published, in *Hufeland's Journal,* the fruit of his investigations in the article "Essay on a New Principle for Ascertaining the Curative Power of Drugs, with a Few Glances at Those Hitherto Employed."

Like Treating Like

Hahnemann also recognized the tendency of a natural disease to have a "homeopathic effect" (i.e., a preventive or therapeutic effect) on other diseases with similar symptomatology. Although he ascribed this to the stimulation of the organism to eradicate the disease, he felt the deliberate induction of a disease to be difficult, uncertain, and dangerous.[4] This concept has many parallels in modern medical science. Descriptions of viral interference under natural conditions were described in 1937 by G. Findlely and F. MacCallum, who found that monkeys infected with the Rift Valley fever virus were protected from the more fatal yellow fever virus. They adopted the term "virus interference" and believed that when one virus infects a group of cells, a second virus is somehow excluded.[5] This eventually led to the discovery of interferon in 1957 by Alick Isaacs and Jean Lindenmann. These methods of inducing self-regulation are critical in enhancing the body's ability to recognize and resolve illness.

In 1799 Hahnemann gained increased professional acceptance of his ideas by the successful application of *Atropa belladonna* (deadly nightshade) in the prevention and treatment of scarlet fever (which had at that time reached epidemic proportions). In 1860 it was recommended as the treatment of choice in the National Dispensatory, which stated: "As long as persons are under the influence of belladonna the liability to contract scarlatina is very much diminished."[6]

The *Organon of Medicine*

In 1810 Hahnemann published his *Organon of Medicine,* a book that, through six editions, formed the foundation and definition of the homeopathic practice of medicine. It contains the philosophy, observations, and clinical applications of homeopathy, as well as citations from the historical and current literature of the time. Hahnemann challenged the reductionistic and mechanistic practices of his time, stating that the nature of disease is dynamic and cannot be defined by isolating processes, grasping for an explanation. He further asserted that the cause of disease could not be known and that the categorization of disease states and attempts to manipulate physiology were insufficient because they did not address the integrity and complexity of the organization of the organism as a whole.

He described this organization as dynamic, meaning "in accordance with the animating principle of life," which is the underlying energetic pattern to which matter conforms.

Disease is therefore addressed descriptively in the context of the whole patient, with the patient's unique symptoms being indicative of that individual's vital response to the condition. For any given disease there may be a long list of remedies that have been clinically effective, but it is the individualization and differentiation among medicines, based on the patient's unique indications, that leads to a successful homeopathic prescription.

Vitalism

Disease, in the homeopathic model, is thought to arise from inherent or developed weaknesses in the patient's defense mechanisms, creating a susceptibility to "morbific influences" (e.g., toxic factors in the environment, bacteria, psychological stresses). This viewpoint is considered "vitalistic" (see Chapter 2, Hierarchy of Healing, and Chapter 5, Philosophy of Naturopathic Medicine, for further discussion), and although it does not deny a corporeal reality, it considers pathology to be but a singular focus in a complex net of interactions.

William Boyd, in *A Textbook of Pathology,* discussed the limitations of the causal approach to disease currently in vogue in medicine when he stated[7]:

> *We must admit, however unwillingly, that we seldom or never really know the cause of anything. Many a beautiful idea has been slain by ugly fact. We merely note a constant association with one thing always following another. We say that the tubercle bacillus is the cause of tuberculosis. That is merely another way of saying that the bacillus is associated with a constant type of lesion; it is no explanation of how the lesions are produced by the bacillus. Nor does it explain why some persons and animals are susceptible to the infection while others are immune.*

Vitalism can be better understood in the context of Hahnemann's time, when theories of the causation of disease and its treatment abounded, such as Galen's doctrine that the secondary quality of a medicine (i.e., its action on the disease) could be determined from its primary qualities, such as its taste or smell; the evaluation of medicines by the study of their interactions when mixed with human blood in a jar; iatrochemistry, which had been reduced from the Paracelsian application of spagyric tinctures or oils of metals to dangerous toxic doses; the classification of drugs according to the Dioscoridian approach, which was based on the physiological action (e.g., diuresis, diaphoresis) and chemical composition; and the "doctrine of signatures," which held that the outer form and color of a plant revealed its inner archetypal action.[8,9]

Although some studies of the effects of medicinal agents were done with animals, Hahnemann observed that they had different effects on humans; pigs could safely eat *Nux vomica* in quantities that would immediately kill humans. Dogs could eat *Aconitum napellus,* a deadly poison to humans, without injury. He also rejected the method of testing drugs by studying their effects on the sick as haphazard and unreliable, particularly because the results being sought were often only symptomatic relief rather than eradication of the disease state.

Hahnemann defined the application of medicines whose purpose was to alter physiology or act as an antagonist to disease as the practice of "allopathy" (*allo,* meaning "contrary" in Greek). The current dominant medical system is heavily influenced by the causal and allopathic paradigms. This results in the diagnosis being the focal point of practice, without which appropriate therapy cannot be instituted. The pharmacological approach is usually limited to the end results of disease rather than the origins of pathogenesis. Subsequent problems are classified as unwanted side effects because only the primary action of the pharmaceutical agent is used for treating a specific disease state. By focusing on only the primary effects of a drug, a diverse remaining range of physiological, as well as psychological effects, is ignored.

In the homeopathic model, the side effects are an important part of the agent's action and the body's response to it; by ignoring them, a drug's range of usefulness is greatly limited, whereas its toxicity is increased. Hahnemann's empiric investigations not only led to new applications of medicines but also provided a method for integrating the physical, mental, and emotional effects of a drug. This allowed the treatment of the totality of a patient's symptoms as a dynamic pattern of interaction.

Vitalists stress the teleological behavior of organisms (i.e., the goal directedness and design in biological phenomena). Disease is therefore regarded as a positive expression of the organism's self-regulatory process in response to environmental or other stresses. Disease is not accidental but is rather the effort of the organism to ward off deeper or more internal disorganization. It is the natural wisdom of the body, the *vis medicatrix naturae,* or, using current scientific terminology, the tendency of the body to maintain homeostasis. Medical intervention often acts in conflict with these vital intracellular and extracellular regulatory functions.

Karl Menninger, in 1948, commented on this medical dilemma[10]:

> *I believe that clinicians have come to think more and more in terms of a disturbance in the total economics of the personality, a temporary overwhelming of the efforts of the organism to maintain a continuous internal and external adaptation to continuously changing relationships, threats, pressures, instinctive needs and reality demands … It is the imbalance, the organismic disequilibrium, which is the real pathology, and when that imbalance reaches a degree or duration that threatens the comfort or survival of the individual, it may correctly be denoted disease.*

Homeopathy is a method of specific induction of nonspecific resistance that stimulates the body's inherent defense and self-regulatory mechanisms, rather than taking over a function of the body, initiating dependency on the medicine itself.

THE CLINICAL APPLICATION OF HOMEOPATHIC PRINCIPLES

The homeopathic clinical and therapeutic process consists of three interrelated processes: case taking, evaluation, and prescribing. The process is comprehensive and engages the observations of the patient as well as those of the doctor. Hahnemann described the process in paragraphs 84 to 103 of the *Organon* and stressed the importance of distinguishing between chronic and acute, or self-limiting, disease.

The Homeopathic Interview

The initial history of complaints is elicited from the patient with as little interruption as possible (as long as the patient does not digress unduly) so that the patient's train of thought is not disrupted or directed along lines imposed by the physician's biases. According to Hahnemann:

> *The physician elicits further particulars about each of the patient's statements without ever putting words in his mouth, or asking a question that can be answered only by yes or no, which induces the patient to affirm something untrue or half true or else deny something really there to avoid discomfort or out of desire to please, thereby giving a wrong picture of the disease, which would lead to the wrong treatment.*

An entire review of symptoms is recorded in descriptive detail, taking into consideration all modalities that affect a symptom. Hahnemann emphasized the general symptoms (i.e., those affecting the entire organism) as the leading indications for the remedy. These key symptoms include mental and emotional effects, the metabolism and its reactions to environmental stimuli, sleep positions, food cravings and aversions, thirst, body type, and all manifestations of unconscious and autonomic regulation.

Unique characteristic symptoms, particularly those regarded as "strange, rare, and peculiar," are important considerations in the selection of the remedy. These might be the expression of a paradoxical or unusual relationship, such as pain ameliorated by pressure or the sensation of the legs being made of wood or glass. The association of the start of a disease or symptom complex with an environmental or emotional event can be key and emphasizes the importance of an accurate and extensive interview.

Hahnemann emphasized the importance of taking a comprehensive case, particularly in chronic disease[11]:

> *In chronic diseases in women one should pay particular attention to such things as pregnancy, infertility, sexual desire, confinement, miscarriages, nursing, vaginal discharges, and the condition of the*

monthly flow, especially noting whether it recurs at intervals that are too short or too long, how many days it lasts, whether or not it is interrupted, the quantity, how dark with color, any leukorrhea before or after the flow. If there is leukorrhea, what it is like, what symptoms accompany it, what is its quantity, under what conditions does it appear, what brings it on?

Because the patient's symptoms are the expressions of the body's attempts to heal itself, symptomatic treatment (i.e., many allopathic therapies) can impair the physician's ability to obtain vital information and complicate the taking of the case. This problem has also been recognized by some medical authors, such as Boyd, who stated: "We recognize that the pattern of disease has changed out of recognition during the past 30 to 40 years owing to modern drugs, particularly the antibiotics."[7]

Follow-Up and Case Evaluation

Considering the vitalistic and holistic perspective of the homeopathic approach, a clear definition of cure is necessary to establish the treatment goal. Mere palliation or suppression of symptoms at the cost of the overall vitality and function of the individual is considered negligent by the homeopathic practitioner. For example, if a patient's skin disease is treated and appears to resolve but is followed by asthma, fatigue, and confusion, the treatment is evaluated as having been suppressive. If, upon proper treatment, the more serious lung and systemic disruptions are alleviated and the previous skin lesions return, the patient is considered as progressing toward a cure. When further appropriate therapy results in final alleviation of the skin disease, without any undue stress to the patient, it is then considered a true cure.

This evaluative procedure is part of Hering's Law of Cure, an observation of the principles of curative responses that can be applied to any healing process, regardless of the school of thought. In true healing, according to this set of observations, symptoms follow these patterns:

- From above, down the body to the extremities
- From within to without (often in the form of discharges and other eliminative processes)
- From the most important organs (e.g., the central nervous system) to the least important organs (typically, the skin)
- In reverse order of their appearance (i.e., the chronologically most recent being replaced by those of the earlier stages of the disease and, in some instances, earlier in the patient's life)

Homeopathy holds that the disease first affects the vital force and is manifested initially by a change in the patient's well-being, long before any objective changes can be observed. Illness is usually first recognized when the patient becomes aware of the early manifestations of the disease.

Disease and cure must also be considered in the context of the belief system and culture of the patient. Much of what we call disease arises from the individual's inability to find meaning and purpose. Many forms of healing are capable of enabling the person to integrate into the fabric of daily life and of providing ways to help the person address personal needs for fulfillment.

In his study of disease, Hahnemann noted that there were inherited predispositions to disease, which he related to the improper treatment, and therefore suppression, of skin eruptions and venereal disease. He called these predispositions *miasmas* and, in 1828, published his findings in *Chronic Diseases: Their Nature and Homeopathic Cure.* He observed that many people, despite apparently healthy lifestyles, develop degenerative diseases. These often become established in childhood and continue to plague the person throughout life, despite medical treatment. He described three miasmas: psora, which represents a fundamental flaw in the human ability to eradicate disease related to the suppression of skin disease; syphilis; and sycosis, which is caused by the suppression of the fig wart, or what is now known as human papillomavirus. Hahnemann described the chronic effects of bacterial and viral diseases in his explanation of miasmas. In his discussion of viral diseases such as smallpox and other epidemic diseases, Hahnemann's descriptions of the nature of viruses and their treatment predate their discovery by 50 years. He was a contemporary of Edward Jenner and supported Jenner's use of smallpox vaccination.

More recently, George Vithoulkas, a contemporary homeopathic author and teacher, defined health on three levels: mental, emotional, and physical. The mind should be capable of functioning with clarity, rationality, coherence, and logical sequence. It should be capable of engaging in creative service for the good of others, as well as for the good of oneself, demonstrating freedom from selfishness and possessiveness. On the emotional level, there should be a state of serenity free from excessive passion, a state that should not be confused with a lack of emotional response generated as protection against emotional vulnerability. Finally, on the physical level, there should be freedom from pain. The healing person should experience a subjective sense of well-being and a progressive increase in vitality.[12]

Prescription

Because homeopathy is oriented toward the administration of a single medicine at a time, careful prescribing is important. It is through the application of single medicines that homeopathic physicians have been able to record clinical verification of the provings and amass an impressive body of literature.

Combination homeopathic medicines have been introduced as specific remedies for diseases and therefore have not represented homeopathic methods, although many studies support their efficacy.

The process of selecting the correct remedy involves both careful study of the patient's symptomatology and medical history and matching these with the appropriate remedy. This requires a sound understanding of the homeopathic *materia medica* (see the section "The Study of the *Materia Medica*").

The symptoms of the homeopathic *materia medica* are indexed in repertories that have evolved in both reference books and computer analysis programs.

Homeopathic Pharmacy and Potency Selection

This leads to a discussion of what has remained the greatest mystery of homeopathic medicine (and the source of considerable ridicule and misunderstanding): the use of "potentized" substances.

As Hahnemann began his research, he found that when treating patients according to the Law of Similars, there was an initial aggravation of the symptoms, the "healing crisis," when using the high dosages typical of that era. He empirically tried using progressive dilutions of the medicines, beginning with tinctures from plants and triturations with milk sugar for metals and salts. He made the dilutions serially by mixing 1 drop of the tincture with 100 drops of alcohol, which were then "succussed" (shaken by pounding against a resilient surface) vigorously. He found that, with increasing dilution, the severity of the aggravation lessened while the patient continued to improve, often with deeper and more enduring results. He called these diluted remedies "potentized." As an analytic chemist, he was aware of Avogadro's theories (they were contemporaries), but he persisted in evaluating dilutions beyond the point where chemical activity could be detected.

This challenge to the present understanding of the therapeutic mechanism has been addressed by recent workers who have suggested that the therapeutic properties of the remedy lie in the energetic impression they make on the diluting vehicle (typically alcohol and

water or lactose). Various techniques have been used to determine whether there is a physical difference between the potentized dilution and the unmodified vehicle. These studies have used ultraviolet spectroscopy, conductivity measurements, infrared spectroscopy, surface tension measurements, Raman Laser spectroscopy, nuclear magnetic resonance, and other methods. Much of this work has shown regular peaks and troughs in activity with progressive dilutions, and Heintz claimed that the peaks corresponded to the maximum effects found in the biological studies he reported (see the section "Basic Research").[13]

Mechanism of Action

To date, there is no conclusive understanding of the mechanism of action of the potentizing process. However, this has not inhibited the use of potencies, which have been diluted by a factor of 100 up to 100,000 times ($10^{100,000}$). At this time, most explanations for the mechanism of homeopathic high potencies are provisional (e.g., the postulate that the remedies act in resonance with the magnetic fields of the body, or that the physiochemical properties of water can be modified by a solute and remain so even in the absence of the solute).[14]

This has not affected clinical practice or the demonstration of efficacy in clinical trials any more than the use of aspirin did, despite the fact that the discovery of its mechanism of action through modulation of prostaglandins did not occur until the 1980s. There are many forces whose nature can only be recognized by their results (e.g., gravity). These observations of relationships, confirmations of experience, are the basis of an empiric system. Medicine remains an art in the field of science.

Interestingly, a group operating in the Hematology Department of the School of Pharmacy in Bordeaux, France, tested the effect of both common aspirin and homeopathic preparations on the vascular walls of rats. Aspirin at high concentrations (100 mg/kg) induced a decrease in platelet aggregation (amplitude and speed) as well as a decrease in the area of the thrombi (arterial and venous) and the number of emboli (arterials and venous).

Aspirin at ultralow doses (9, 15, 30 CH) induced an increase in platelet aggregation (amplitude and speed) as well as an increase in the area of thrombi (arterial and venous) and the number of emboli (arterial and venous). The antiaggregation and antithrombotic action of aspirin at high concentrations (100 mg/kg) was inhibited by the concomitant injection of aspirin 15 CH.[15]

This confirmed Hahnemann's observations of the primary and secondary effects of medicines mentioned in the *Organon.*[11] Bellavite described these effects as "biologically active compounds (which) may cause inverse or paradoxical effects on a complex homeostatic system when either the doses of the compound, or the methods of preparation and of administering, or the sensitivity of the target system are changed."[16,17]

Research into both the pharmacological effects of homeopathic preparations and the paradoxical effects of orthodox drugs that confirm the Law of Similars' underlying homeopathic prescribing is a growing body of literature. Certain pharmacological substances, when tested in high dilutions, act on the same biological systems.[18–24]

The reaction to the high dilutions can also be the opposite of that to a drug at low dilutions (e.g., proinflammatory agents can be anti-inflammatory at high dilutions).[25–28]

Paradoxical effects of medicines are the basis of the Arndt–Schultz law in pharmacology and hormesis. The Arndt–Schultz law states that weak stimuli slightly accelerate vital activity, medium-strong stimuli raise it, strong ones suppress it, and strong ones arrest it.[29]

Southam and Erlich[30] reported the stimulatory effect of an antifungal agent when used at low doses and proposed the term "hormesis." Hormesis is defined as "the stimulatory effect of subinhibitory concentrations of any toxic substance on any organism."[31] Hormesis is considered a nonspecific phenomenon that increases the resistance and growth of the treated organism. It exists in all living organisms. This "action–reaction" model shows the efficacy of the "vital activity" in fighting the poison in a nonspecific way, although specific defense molecules are also synthetized.[32–37] A modern and important pathological model showed that a single dose of an antitumoral immunosuppressive substance (cisplatin) induced increased lymphokine-activated killer activity.[38] Wagner and colleagues demonstrated that low doses of cytostatic agents stimulated human granulocyte and lymphocyte growth.[39]

The goals and methods of homeopathic pharmacy have their roots in earlier Paracelsian and spagyric medical systems. The challenge remains to define homeopathic empiric science in the context of a modern science. It may be that homeopathy presents a challenge to pharmaceutical science itself that will bring forth new models for pharmacology. The more central challenge is for homeopathy to discover how it can apply its own critical methods to develop a more effective healthcare service.

The assumption that we can find substances in nature that can alter disease underlies the history of medicine and pharmacology, yet healing remains a mystery. Further studies are necessary to confirm and develop the understanding of the mechanisms and validity of homeopathic medicines.

Determination of Potency

In terms of clinical practice, general guidelines have evolved for the determination of potency. In the sixth edition of the *Organon,* Hahnemann recommends ascending the scale of potencies gradually. In paragraph 248, he suggests that the medicinal solution be "succussed anew with use." In chronic cases, the patient is directed to take one teaspoonful daily or every other day, and in acute diseases, as frequently as needed. If the solution is used up before the problem alleviates, the next higher dilution is used (if still indicated by the symptom pattern).[40]

The higher potencies, whose use largely developed in the United States, are repeated much less frequently and are generally reserved for the experienced practitioner. The more potentized the remedy, the closer it must meet the Law of Similars (i.e., the accuracy of the prescription must be high for a curative effect). Lower potencies are often repeated daily, depending on the condition being treated.

Several ranges of potencies include the decimal scale, which uses a 1:10 dilution; the centesimal scale, which is diluted 1:100; and the LM potencies introduced in the sixth edition of the *Organon,* using daily doses of 1:50,000 dilutions.

It is important to note that the sixth edition was unavailable until 1924, 76 years after Hahnemann's death. The predominant clinical application of homeopathic potencies had developed using an ascending scale. A single dose was used until its action had ceased, when the same potency would be repeated. When that potency seemed to no longer demonstrate an enduring effect, a higher potency was used. There have been regular arguments between low- and high-potency prescribers as to the most effective method.

The Study of the *Materia Medica*

Constantine Hering once stated:

> *A mere acquaintance with the principal symptoms cannot be called studying the materia medica, although we make it the basis of our study. The study of materia medica must be regarded and dealt with in exactly the same manner as that of other natural sciences.*

To give a perspective on the way in which homeopathic physicians organize the proving symptoms into clinical pictures, we draw from an essay on *Sepia* by E. B. Nash[41]:

This is another of our wonderful remedies of which the dominant school knows nothing, except what they have learned from us. Its chief sphere of action seems to be in the abdomen and pelvis, especially in women. No remedy produces stronger symptoms here. We quote from different but equally good observers.

Sensation of bearing down in the pelvic region, with dragging pains from the sacrum; or feeling of bearing down of all pelvic organs. (Hahnemann)

Labor-like pains accompanied with the feeling as though she must cross her legs and "sit close" to keep something from coming out through the vagina. (Guernsey)

Pain in uterus, bearing down, comes from back to abdomen, causing oppression of breathing; crosses limbs to prevent protrusion of parts. (Hering)

Prolapse of the uterus, of the vagina, with pressure as if everything would protrude. (Lippe)

Experience has shown its value in cases of ulceration and congestion of the os and cervix uteri. Its use supersedes all local applications. (Dunham)

No higher authority than the united testimony of these five of our best observers could be brought to show the action of Sepia *upon the pelvic organs.*

Now when we come to examine the provings in Allen's Encyclopedia, *we find that these symptoms were mainly produced by Hahnemann and his provers, and Hahnemann advocated proving remedies in the 30th, and some of them were produced by the 200th, especially those most strongly verified by black-faced type.*

We confess that we cannot understand how so many question the value of potencies for proving or curing. Sepia, *like* Sulphur, *affects the general circulation in a very marked manner. Flashes of heat with perspiration and faintness is almost as characteristic of this remedy as of* Sulphur. *But there are, with* Sepia, *more apt to be associated with them the pelvic symptoms already given, and they are also more apt to occur in conjunction with the climacteric. Indeed, these flashes often seem with* Sepia *to start in the pelvic organs and from thence to spread over the body.*

But this irregularity of circulation extends as far as that of Sulphur. *The hands and feet are hot alternately, that is, if the feet are hot, the hands are cold, and* vice versa. *There is not so much sensation of burning with* Sepia *as with* Sulphur, *but there is actual heat, and the venous congestion, which seems to be the real state of the organs where the pressive bearing down et cetera is felt, is also accompanied with much throbbing and beating.*

This local congestion to the pelvic organs is not simply sensational. There are actual displacements in consequence of it, and the long continued congestion results in inflammations, ulcerations, leukorrheas and even malignancies or cancerous organizations. Induration with a painful **sense** *of stiffness in the uterine region is characteristic.*

This pelvic congestion also affects the rectum in a marked degree. The rectum prolapses, there is a sensation of fullness, or of a foreign substance as of a ball or weight, and oozing of moisture from the rectum. Indeed, the rectal and anal symptoms are almost as strong as the uterine and vaginal. It is impossible to enumerate all the symptoms connected with the circulatory disturbances of Sepia *in such a work as this, only a general study of the Materia Medica can do it.*

The urinary organs come in for their share of symptoms. The same pressure and fullness consequent upon the portal congestion reaches here. We will now proceed to give what we have found to be particularly valuable symptoms under the various organs in this region. "Pressure on bladder and frequent micturation with tension in lower abdomen." "Sediment in the urine like clay; as if clay burnt on the bottom of the vessel; urine very offensive (Indium), *can't endure to have it in the room, it is reddish or may be bloody." This is found mostly in women. With children there is one peculiar symptom which has often been verified. "The child always wets the bed during its first sleep."*

Upon the male organs I have found it particularly useful in chronic infection. There is not much discharge, but a few drops, perhaps, which glue up the orifice of the urethra in the morning; but it is so persistent and the usual remedies will not "dry it up." In my early practice I used to use a weak injection of Sulphate of Zinc, *but it used to annoy me that I could not use it without resorting to local measures.* Sepia *does it in the majority of cases and* Kali iodatum *will do it in the rest. I have, where there was a thick discharge of long standing and the smarting and burning on urination continued, several times finished the case with* Capsicum.

As a rule, this long continued slight, passive gleety discharge is a result of weakness of the male genitals, as is shown by a flaccidity of the organs and frequent seminal emissions. The emissions are thin and watery. Sepia *covers all of this and often sets all to rights in a short time.*

The mind symptoms of Sepia *are like* Pulsatilla, *in that she is sad and cries frequently without knowing the reason why. So if in a tearful mind with uterine disturbances* Pulsatilla *should fail you, the next remedy to be studied is Sepia. But there is another condition of mind not found under* Pulsatilla *or any other remedy in the same degree, and that is, that, notwithstanding there is no sign of dementia from actual brain lesion, the patient, contrary to her usual habit, becomes indifferent to her occupation, her house work, her family or their comfort, even to those whom she loves the best. This is a very peculiar symptom and a genuine keynote for the exhibition of* Sepia…

I once cured a very obstinate case of entero-colitis (so-called cholera infantum), after the complete failure of two eminent allopaths, with Sepia, *the leading symptom being, always worse after taking milk. Oozing of moisture from the anus finds its remedy here sometimes, but oftener in* Antimonium crudum. *The* Sepia *patient is very weak. A short walk fatigues her very much. She faints easily from extremes of cold and eat, after getting wet, from riding in a carriage, while kneeling at church, and on other trifling occasions. This fainting, or sense of sinking faintness, may be found in pregnancy, child bed, or during lactation; or, again, it may come on after hard work, such as "laundry work;" so it has come to be called the "washer woman's" remedy.*

As can be seen by this excerpt, the indications for a remedy are complex, requiring study and understanding.

RESEARCH IN HOMEOPATHY

Homeopathy arose from empiric observations and operates from empiric clinical evidence and phenomenologically descriptive fields.

Samuel Hahnemann was the first to use the methodology of provings, to gather information about the reactions of healthy subjects to the ingestion of minute amounts of substances. Both clinical trials and laboratory research, although relatively sparse and often inconclusive, do point to efficacy and activity beyond placebo. Homeopathy has faced challenges in the scientific as well as political arena because its mechanism of action is still unknown. For example, quoting from a recent review, "according to current pharmacological theory it would appear impossible that homeopathic therapy could have any effect over placebo."[42] However, as the authors go on to say, "The available hypotheses for a possible mechanism of action, however, do not claim that homeopathic remedies act through pharmacological but through biophysical pathways and all include the idea of some form of information transfer."[42] Despite adversaries who claim that research has determined that homeopathy is nothing more than placebo, homeopathy researchers, using increasingly sophisticated research designs, continue to produce intriguing evidence regarding the effects and efficacy of homeopathic substances. This evidence, which emerged from several types of research, including provings, clinical trials, and laboratory studies, may soon converge to build a promising case for homeopathy's effect.

Evidence From a Modern Proving Study

Although traditionally provings, also called *pathogenetic trials*, first involved material doses of substances, Hahnemann himself, as well as later provers, conducted proving studies using potencies beyond Avogadro's number. One recent proving study provided convincing evidence that ultramolecular doses of homeopathic substances can produce symptoms different from placebo.[43] This double-blind, placebo-controlled experimental study randomized 25 medical doctors into three groups who received 30-CH potentized doses of either *Natrum muriaticum* or *Arsenicum album* or an identical placebo. All substances were beyond Avogadro's number in terms of dilution. The main outcome parameter was the number of remedy-specific symptoms per group. The actual remedies themselves were chosen randomly from a list of 20 already-proven remedies that are frequently used in homeopathic practice; this list was not known to subjects or the study team, other than the study director and pharmacist; the two selected remedies were not known to anyone on the study team, including the study director. Participants were advised to ingest five remedy globules on day 1, then 2×5 on day 2, or until symptoms appeared, and then write their symptoms in a diary over 4 days. These symptoms were compiled and reviewed by an independent homeopathic expert, blinded to group assignment, who analyzed each symptom via a computer program for its typicality, or not, for one of the two remedies tested. The results showed that symptoms typical for the respective remedy were significantly more likely to occur in both the *Natrum muriaticum* or *Arsenicum album* groups, whereas nonspecific symptoms were more frequent in the placebo group ($P = 0.001$). Typical symptoms reported by subjects ingesting *Arsenicum album* included "strange restlessness"; "increased desire to swallow"; "lack of desire to urinate"; and "gushing diarrhea." Typical symptoms reported by subjects ingesting *Natrum muriaticum* were "difficulty concentrating"; "slight vertigo when seated"; "tearing pain in scalp"; "twitching in right eye"; and "cramping like labor in abdomen."[43] This impressive finding showed that nonmaterial doses of substances produced specific and distinctive effects—characteristic of the remedy—in subjects who were blinded to which remedy they were ingesting.

Human Clinical Trials

Although the previously described study provides evidence for an effect of ultramolecular homeopathic substances on the human organism, what is of primary interest to humans, and particularly clinicians, is whether such effects are therapeutic. In one early classic study, Gibson et al.[44] published a double-blind clinical trial of homeopathic treatment in rheumatoid arthritis. The 3-month study was elegantly designed in that the prescribing was individualized to the patient's symptoms and was controlled, on a double-blind basis, by giving half the patients the correct remedy and the rest a placebo. All patients continued to use conventional, nonsteroidal anti-inflammatory drugs, and the treated group showed significant improvement in subjective pain, articular index, stiffness, and grip strength. Other published studies have demonstrated the efficacy of homeopathic treatment for the treatment of headache, bruising, cancer-related symptoms, attention deficit hyperactivity disorder in children, asthma, upper respiratory tract infections, otitis media, arthritis, allergies, male infertility, influenza, cardiac insufficiency, herpes, osteoarthritis, acquired immunodeficiency syndrome, and chronic fatigue syndrome.[45–82]

Meta-Analyses and Systematic Reviews of Human Clinical Trials

Hundreds of human clinical trials, of varying quality, with both positive and negative results, have now been carried out. Meta-analyses and systematic reviews have been helpful in identifying promising treatments and areas where more research is needed, thus moving the field forward.

In 1991 one of the first meta-analyses of homeopathic clinical trials, by Kleijnen et al.,[83] was published in the *British Medical Journal,* with a total of 105 controlled trials. The quality of trials was described as poor for two thirds of those reviewed. Positive results for homeopathic treatment were found in 81 trials, leading the authors to state, "The evidence in this review would probably be sufficient for establishing homeopathy as a regular treatment for certain indications."

In 1997 Linde et al.[84] published another meta-analysis of 119 trials that met the inclusion criteria; 89 had adequate data for meta-analysis, and two sets of trials were used to assess reproducibility. The combined odds ratio for the 89 studies was 2.45 in favor of homeopathy, with an odds ratio of 1.78 for the 26 good-quality studies. The authors concluded, "The results of our meta-analysis are not compatible with the hypothesis that the clinical effects of homeopathy are completely due to placebo." In 2014 Mathie et al.[85] published a rigorous, focused systematic review and meta-analysis of randomized controlled trials of individualized homeopathic treatment (typically involving a long interview between the practitioner and the patient) versus placebo, finding 32 eligible randomized controlled trials (RCTs) covering 24 medical conditions, of which 22 had extractable data available for meta-analysis. Although the evidence was of uneven quality and the authors were cautious in their conclusions, their findings were that medicines prescribed in individualized homeopathy may have small, specific treatment effects. In 2017 Mathie et al.[86] published a second rigorous systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of nonindividualized homeopathic treatment and concluded that although the quality of the evidence was low, a meta-analysis of all extractable data indicated a small, statistically significant effect of nonindividualized homeopathic treatment. Their major conclusion was—as in previous systematic reviews in homeopathy—that higher-quality RCT research, with a lower risk of bias, is needed to be able to determine homeopathy's efficacy for conditions or symptoms decisively.

In some instances, accumulating evidence has made it feasible to carry out systematic reviews to evaluate homeopathy's use for the treatment of specific conditions. For example, in a review of homeopathic treatment of people with human immunodeficiency virus or acquired immune deficiency syndrome, five controlled clinical trials

were identified, with evidence for improved CD4 and CD8 counts and specific physical, immunological, neurological, metabolic, and quality-of-life benefits. The author concluded that homeopathy might play a useful role as an adjunctive therapy in this condition.[81] Pilkington et al.[82] examined the use of homeopathy for anxiety and anxiety disorders, concluding that homeopathy might be beneficial. One particularly fruitful and positive area of research, for which there are several positive trials, is in the use of homeopathy for the treatment of respiratory allergies.[74]

Human Effectiveness Studies

Effectiveness research involves the evaluation of a treatment's usefulness in more natural or real-world settings. For example, during World War II, isopathic preparations were given prophylactically, and homeopathic therapies were used in mustard gas burns. A subsequent statistical analysis found that these treatments yielded significant results compared with placebos. The remedies used were mustard gas, *Rhus toxicodendron,* and kali bichromium.[13]

More recently, Witt et al.[87] evaluated health-status changes of chronically ill patients after 8 years of homeopathic treatment. This prospective, multicenter cohort study involved 103 homeopathic primary care practices in Germany and Switzerland; in total, 3709 patients were studied, with the most frequent diagnoses being allergic rhinitis, headache in adults, and atopic dermatitis and multiple recurrent infections in children. Disease severity decreased significantly over this time, with considerable improvement in physical and mental quality of life. Another prospective multicenter observational study of 129 patients treated homeopathically by 48 physicians for low-back pain found sustained physical and quality-of-life improvements, along with decreased use of conventional healthcare services and pharmacological therapies.[88] A similar prospective multicenter observational study evaluated symptom changes in 212 adult patients with chronic migraines who were treated with individualized homeopathy by 68 physicians. Migraine severity showed marked improvement at 3 and 24 months, with improved quality of life and decreased use of conventional health services.[89]

Animal Studies: Field and Laboratory Research

In a now-classic study, *Caulophyllum* (in the 30th centesimal potency) was given to 10 sows to test its efficacy in the control of stillbirths. The results showed a statistically significant drop in the number of stillbirths and led to a larger, uncontrolled study in a whole herd. After 4 months of therapy, piglet mortality dropped from 20% to 2.6%.[90]

Cloudhury obtained dramatic results from injecting mice intraperitoneally with kali phosphoricum, calcarea phosphorica, or ferrum phosphorica (in the 30th decimal potency) 12 days after implantation of fibrosarcoma. Of the 77 treated mice, 52% were cured and survived more than 1 year, whereas all of the 77 controls died within 10 to 15 days.[91]

Scofield, in his review article, discussed numerous experiments with humans, animals, and plants using isopathic treatment for poisoning and experimental liver damage and various in vitro studies.[13]

A number of studies have been conducted to investigate the ability for homeopathic preparations to affect either the elimination or consequences of toxic substances.

There is good evidence that homeopathy may be effective in assisting in the elimination and treatment of heavy metals and other toxins. Studies of arsenic,[92,93] bismuth,[18,19,94] lead,[95] mercury,[96,97] carbon tetrachloride,[20,25] alpha-amanitine (from the mushroom *Amanita phalloides*),[26] and carcinogens such as 2-acetylaminofluorene and phenobarbital[98] have been published.

The use of homeopathic dilutions of hormones and immunomodulators has shown potential. Immunostimulatory effects of high dilutions of thymic hormones and interferons were demonstrated in mice by Bastide's group.[26,99–102] Other studies demonstrated that extremely small amounts of antigens were specific for immunomodulation.[103–107] In one study, half of a group of mice were given preparations of reticuloendothelial tissue from *Francisella tularensis*–infected mice in six different serial agitated dilutions (SADs), three of which were beyond Avogadro's number in terms of molecular content. The other half of the group of mice were given control diluents. All mice were then challenged with lethal doses of *F. tularensis* and evaluated for time to death and total number of deaths per group. The SAD preparations resulted in increased mean times to death (18.6 vs. 13.7 days) and decreased mortality (53% vs. 75%). The protective effect was not related to the level of dilution of the substance.[107]

Magnani et al. used a well-designed mouse model to examine the anxiolytic-like activity of several potencies of ultradiluted *Gelsemium sempervirens,* compared with the drug buspirone and placebo. *Gelsemium*-treated mice spent more time in a lighted compartment (a measure of reduced anxiety), similar to the effects of buspirone and significantly greater than the placebo group. The authors concluded that *Gelsemium* acted on the emotional reactivity of mice, with anxiolytic-like effects even at ultrahigh dilutions.[108]

These and other such dramatic results have tremendous implications in pharmacy, immunology, and clinical health care that demand continued research.

Basic Research

Clinical and experimental data obtained in studies about the effect of homeopathic preparations in inflammatory conditions present a considerable degree of reproducibility.[109–113]

The inability of Ovelgonne et al.[114] and Hirst et al.[115] to replicate their study published in the journal *Nature,* using the human basophil degranulation test (HBDT) to establish the ability for high dilutions to trigger the degranulation of anti–immunoglobulin-E, caused considerable distraction from other more credible research.

Brown and Ennis[116] used different methods to demonstrate the efficacy of high dilutions of histamine to inhibit the activation of basophils using HBDT. Instead of measuring degranulation provoked by ultramolecular dilutions of anti–immunoglobulin-E, as Ovelgonne et al. and Hirst et al. did, they examined the inhibition of activation of basophils by ultramolecular dilutions of histamine.

The experiments used ultramolecular dilutions of histamine (15c to 19c), prepared with vortexing (instead of succussion). The main experiment, performed by all the laboratories, was based on inhibition of basophil activation as measured by degranulation. Flow cytometry experiments at three laboratories showed compatible results, with inhibition of activation as high as 43%. Nearly all experiments showed statistically significant inhibition of basophil activation.[117–120]

Experimental study of homeopathy in allergology[121] effects have been reported in vivo[122–124] as well as in vitro.[125–129]

The physical properties of homeopathic preparations are gaining considerable understanding via research. Studies demonstrated that the physicochemical properties of extremely diluted solutions (EDSs) are different from those of pure, untreated water, notwithstanding the identical chemical composition of the two liquids.[130–132] The same conclusions were inferred by Lo.[133,134] Rey[135] showed that the structure of hydrogen bonds in pure water was different from that of an EDS obtained by an iterative procedure of successive dilutions and succussions and was not identical as expected. Recent studies on the physicochemical properties of water provided evidence that the most studied liquid by far, water, still exhibits unexpected properties.[136–142] Lobyshev et al.[143] showed that low concentrations and electromagnetic fields can produce large-scale realignments of its structure,

which can be either reversible or irreversible. One can deduce from these studies that water and aqueous solutions are complex systems, capable of auto-organization as a consequence of small perturbations of various kinds.

The question of whether water can maintain "memory" of solutes in EDS is best understood by understanding the physical characteristics of water and its ability to form stable clusters and crystals. This aspect of physics is not widely studied but is well documented.[130–133,136,141,144]

Sukal et al.[145] pointed out that ethanol molecules, via which potentized homeopathic substances are prepared and stored, are thought to promote or preserve water structures in the potentized substance. These researchers examined the Fourier transform infrared spectra of various ultradiluted homeopathic substances—*Nux vomica* 30c, *Lycopodium* 30c, *Santonin,* 30c, *Cina* 30c, *Cina* 206c, and *Cina* 1006c—as well as their diluent media, 90% ethanol and ethanol 30c. The potencies differed from each other, and from their diluent media, in the number of oxygen–hydrogen bending vibrational bands, as well as their wave number, shape, and the half-width of the bands. This study illustrated that the medicated sucrose globules used in homeopathic practice can retain specific spectral properties and can be differentiated from each other by Fourier transform infrared spectra with regard to the oxygen–hydrogen bending vibrational band.[145]

Such meticulously designed and executed studies are bringing us ever closer to understanding the mechanisms by which homeopathy may work.

SUMMARY

Homeopathy plays an important role in the context of modern naturopathic medicine. Hahnemann emphasized the importance of lifestyle in the treatment of the patient. One of his primary dictums was to first remove the obstacles to cure, as he said:

> *While taking a case of chronic disease one should examine and weigh the particular conditions of the patient's day to day activities, living habits, diet, domestic situation, and so on. One should ascertain whether there is anything in them which may cause or sustain the disease and remove it to help the cure.*

Unfortunately, homeopathy is also an extremely challenging system to master, requiring a considerable understanding of both case taking and *materia medica* as well as extensive consultation time with the patient. It has therefore often been discarded, even by those aware of its efficacy. Although attempts have been made to reduce it to simpler systems (e.g., allergy desensitizations, vaccinations, Schuessler's cell salts, and isopathic preparations from diseased tissues and heavy metals), they are not considered strictly homeopathic unless prescribed according to their effects upon healthy people or the confirmed observations of cured symptoms.

The greatest challenge to homeopathy is not how it fits into the current scientific paradigm but how it fits into health care. The same biases against homeopathy have been used against other nonpharmacological approaches to medicine, including psychology and nutritional medicine.

The wide acceptance in the homeopathic community of unaccredited educational programs has favored the spread of nonclinically trained homeopaths. Naturopathic medical schools in the United States have remained one of the few resources for clinical training in homeopathy. Homeopathic medical schools in other countries have maintained homeopathy in the context of medical and clinical training.

There have been many individualized styles of prescribing introduced into homeopathy that have created breaches between different practitioners. In addition, many new medicines are being introduced without adequate clinical verification. Unfortunately, there are few forums for critical discourse within the homeopathic community.

The divisions between different schools of homeopathic practice are attempts to parlay new ideas against an orthodox view that solely seeks to maintain the integrity of the principles developed by Hahnemann.

One basic argument by some of these schools has been that because a homeopathic prescription should not be based on a specific disease, extensive training in current medical sciences should not be primary. However, this ignores an essential part of Hahnemann's instructions. In the third paragraph of the *Organon,* he stated, "The physician should distinctly understand…what is curable in diseases in general, and in each case in particular; that is, the recognition of disease."

Correct diagnosis is primary in understanding the context and pathogenesis of a disease. Thus it would not be possible to understand the relation of much of drug proving symptoms, their clinical application, and the evaluation of efficacy or subsequent case management without understanding the nature of disease. Homeopathy is proven in the context of good health care. It is a system of therapeutics, not a replacement for common standards of diagnosis and preventive care.

Homeopathy is representative of a principle found throughout nature, and its role in bringing forth concepts of resonance, constitution, and holism are shared throughout fields of science and healing. Homeopathy represents an integrated, holistic system of natural therapeutics. Its capacity for addressing psychosomatic disease and acute pathology as a dynamic process is unique. It has remained a coherent system, with extensive clinical verification, for more than two centuries. Homeopathy is an economical and effective method that has been established as an integral part of the medical system in many countries. With the resurgence of interest in natural medicine, this discipline will undoubtedly be more widely used.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Coulter HL. *Divided Legacy: A History of the Schism in Medical Thought*. Vol. 2. Washington, DC: Wehawken; 1973:310.
2. Coulter HL. *Divided Legacy: A History of the Schism in Medical Thought*. Vol. 2. Washington, DC: Wehawken; 1973:305.
3. Schaumburg H, Kaplan J, Windebank A, et al. Sensory neuropathy from pyridoxine abuse. *N Engl J Med*. 1983;309:445–448.
4. Hahnemann S. *Organon of Medicine. [Reprint.]*. Los Angeles: JP Tarcher; 1980:42–50.
5. Isaacs A. Interferon. *Sci Am*. 1961;204:51–57.
6. Coulter HL. *Homeopathic Influences in 19th Century Allopathic Therapeutics*. Washington, DC: American Institute of Homeopathy; 1973.
7. Boyd WA. *Textbook of Pathology*. 8th ed. Philadelphia: Lea & Febinger; 1970.
8. Riddle JM. *Dioscorides on Pharmacy and Medicine*. Austin, TX: University of Texas Press; 1985.
9. Pagel W. *Paracelsus: An Introduction to Philosophical Medicine in the Era of the Renaissance*. New York: Karger; 1982.
10. Menninger K. Changing concepts of disease. *Ann Intern Med*. 1948;29:318–325.
11. Hahnemann S. *Organon of Medicine. [Reprint.]*. Los Angeles: JP Tarcher; 1980:94.
12. Vithoulkas G. *The Science of Homeopathy*. New York: Grove Press; 1979.
13. Scofield AM. Experimental research in homeopathy—a critical review. *Br Hom J*. 1984;73:161–180. 211–226.
14. Bellavite P, Signorini A. *Homeopathy: A Frontier in Medical Science*. Berkeley, CA: North Atlantic Books; 1995.
15. The biological action of dilutions of aspirin. Professor Doutremepuich (University of Bordeaux, France). http://www.boiron.com/en/htm/04-politique/fondamentale.htm. Accessed September 30, 2004.
16. Bellavite P, Andrioli G, Lussignoli S, et al. A scientific reappraisal of the "principle of similarity." *Med Hypotheses*. 1997;49:203–212.
17. Teixeira M. Homeopathic use of modern medicines: utilization of the curative rebound effect. *Med Hypotheses*. 2003;60:276–283.
18. Lapp C, Wurmser L, Ney J. Mobilisation de l'arsenic fixé chez le cobaye, sous l'action de doses infinitesimales d'arseniate de sodium. *Therapie*. 1955;10:625–638.
19. Lapp C, Wurmser L, Ney J. Infinitesimal doses of sodium arseniate causing mobilization of fixed arsenic in guinea pigs. *Therapie*. 1955;10:625–638.
20. Bildet J, Aubin M, Baronnet S, et al. Resistance de la cellele hepatique du rat après une intoxication infinitesimale au tetrachlorule de carbone. *Homeopath Fr*. 1984;72:175–181.
21. Aubin M. Elements de pharmacology homeopathique. *Homeopath Fr*. 1984;72:231–235.
22. Bastide M, Doucet-Jaboeuf M, Daurat V. Activity and chronopharmacology of very low doses of physiological immune inducers. *Immunol Today*. 1985;6:234–235.
23. Doutremepuich C, de Seze O, Anne MC, et al. Platelet aggregation on whole blood after administration of ultra low dosage acetylsalicylic acid in healthy volunteers. *Thromb Res*. 1987;47:373–377.
24. Bonamin LV, Martinho KS, Nina AL, et al. Very high dilutions of dexamethasone inhibit its pharmacological effects in vivo. *Br Homeopath J*. 2001;90:198–203.
25. Guere JM, Saurel J, Aubin M, et al. Étude de l´action de différentes dilutions de phosphorus sur l´hépatite toxique du rat. *Homeopathie Fr*. 1984;72:199–204.
26. Guillemain J, Douylliez C, Bastide M, et al. Pharmacologie de l'infinitesimal. *Homeopathie*. 1987;4:35–46.
27. Bastide M. *Signals and Images: Selected Papers from the 7th and 8th GIRI Meeting*. Dordrecht, Netherlands: Kluwer Academic Publishers; 1997.
28. Poitevin B. Review of experimental studies in allergy. *Br Homeopath J*. 1998;87. 89–89.
29. Leeser O. Support of homeopathy by the Arndt-Schulz law. *Hippokrates*. 1953;24:417–421.
30. Southam CM, Erlich J. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology*. 1948;33:515–524.
31. Oberbaum M, Cambar J. Hormesis; dose dependent reverse effects of low and very low doses. In: Endler PC, Schulte J, eds. *Ultra High Dilution: Physiology and Physics*. Dordrecht, Netherlands: Kluwer Academic Publisher; 1994:5–19.
32. Bastide M, Lagache A. *Is similia principle related to the Arndt-Schultz Law and hormesis? Paper presented at the 53 Congress of the IHML International Homeopathic Medical League*. Amsterdam, Netherlands; 1998.
33. Stebbing AR. Growth hormesis: a by-product of control. *Health Phys*. 1987;52:543–547.
34. Stebbing AR. Hormesis: the stimulation of growth by low levels of inhibitors. *Sci Total Environ*. 1982;22:213–234.
35. Halm RP. *Actualité et Modernité de la Recherche Expérimentale en Homéopathie*. Montpellier, France: Thèse Doct. Pharm, Univ.; 1993.
36. Van Wijk R, Wiegant FAC. *Cultured Mammalian Cells in Homeopathy Research—the Similia Principle in Self Recovery*. Utrecht, Netherlands: University Utrecht Publisher; 1994.
37. Van Wijk R, Ooms H, Wiegant FAC, et al. A molecular basis for understanding the benefits from subharmful doses of toxicants; an experimental approach to the concepts of hormesis and the homeopathic similia law. *Environ Manage Health*. 1994;5:13–25.
38. Arinaga S, Adachi M, Karimine N, et al. Enhanced induction of lymphokine-activated killer activity following a single dose of cisplatin in cancer patients. *Int J Immunopharmacol*. 1994;16:519–524.
39. Wagner H, Kreher B, Jurcic K. In vitro stimulation of human granulocytes and lymphocytes by pico- and femtogram quantities of cytostatic agents. *Arzneimittelforschung*. 1988;38:273–275.
40. Hahnemann S. *Organon of Medicine. [Reprint.]*. Los Angeles: JP Tarcher; 1980:248.
41. Nash EB. *Leaders in Homoeopathic Therapeutics [Reprint.]*. 6th ed. New Delhi, India: Jain; 1982:200–206.
42. McCarney RW, Linde K, Lasserson TJ. Homeopathy for chronic asthma. *Cochrane Database Syst Rev*. 2004;1:CD000353.
43. Mollinger H, Schneider R, Walach H. Homeopathic pathogenetic trials produce specific symptoms different from placebo. *Forsch Komplementmed*. 2009;16:105–110.
44. Gibson RG, Gibson SL, MacNeill AD, et al. Homeopathic therapy in rheumatoid arthritis: evaluation by double-blind clinical therapeutic trial. *Br J Clin Pharmacol*. 1980;9:453–459.
45. Walach H, Jonas WB, Ives J, et al. Research on homeopathy: state of the art. *JAltern Complementary Med*. 2005;11:813–829.
46. Seeley BM, Denton AB, Ahn MS, et al. Effect of homeopathic Arnica montana on bruising in face-lifts. *Arch Facial Plastic Surg*. 2006;8:54–59.
47. Weatherley-Jones E, Nicholl JP, Thomas KJ, et al. A randomised, controlled, triple-blind trial of the efficacy of homeopathic treatment for chronic fatigue syndrome. *J Psychosom Res*. 2004;56:189–197.
48. Brigo B, Serpelloni G. Homeopathic treatment of migraines. *Berlin J Res Homeopathy*. 1991;1:98–106.
49. Muscari-Tomaioli G, Allegri F, Miali E, et al. Observational study of quality of life in patients with headache, receiving homeopathic treatment. *Br Homeopath J*. 2001;90:189–197.
50. Walach H, Haeusler W, Lowes T, et al. Classical homeopathic treatment of chronic headaches. *Cephalalgia*. 1997;17:119–126.
51. Ernst E. Homeopathic prophylaxis of headaches and migraine? A systematic review. *J Pain Symptom Manage*. 1999;18:353–357.
52. Walach H, Lowes T, Mussbach D, et al. The long-term effects of homeopathic treatment of chronic headaches: one year follow-up and single case time series analysis. *Br Homeopath J*. 2001;90:63–72.
53. Frei H, Thurneysen A. Homeopathy in acute otitis media in children: treatment effect or spontaneous resolution? *Br Homeopath J*. 2001;90:180–182.
54. Harrison H, Fixsen A, Vickers A. A randomized comparison of homoeopathic and standard care for the treatment of glue ear in children. *Complement Ther Med*. 1999;7:132–135.
55. Jacobs J, Springer DA, Crothers D. Homeopathic treatment of acute otitis media in children: a preliminary randomized placebo-controlled trial. *Pediatr Infect Dis J*. 2001;20:177–183.
56. Lamont J. Homoeopathic treatment of attention deficit hyperactivity disorder. A controlled study. *Br Homeopath J*. 1997;86:196–200.

57. Frei H, Thurneysen A. Treatment for hyperactive children: homeopathy and methylphenidate compared in a family setting. *Br Homeopath J*. 2001;90:183–188.
58. Gerhar I, Wallis E. Individualized homeopathic therapy for male infertility. *Homeopathy*. 2002;91:133–144.
59. Ferley JP, Zmirou D, D'Adhemar D, et al. A controlled evaluation of a homoeopathic preparation in the treatment of influenza-like syndrome. *Br J Clin Pharmacol*. 1989;27:329–335.
60. Schroder D, Weiser M, Klein P. Efficacy of a homeopathic Crataegus preparation compared with usual therapy for mild (NYHA II) cardiac insufficiency: results of an observational cohort study. *Eur J Heart Fail*. 2003;5:319–326.
61. Jenaer M, Henry MF, Garcia A, et al. Evaluation of 2LHERP in preventing recurrences of genital herpes. Institute International 3IDI. *Br Homeopath J*. 2000;89:174–177.
62. Long L, Ernst E. Homeopathic remedies for the treatment of osteoarthritis: a systematic review. *Br Homeopath J*. 2001;90:37–43.
63. Strosser W, Weiser M. Patients with gonarthrosis gaining back mobility: homeopathic in a double blind comparison. *Biol Med*. 2000;29:295–299.
64. Jonas WB, Linde K, Ramirez G. Homeopathy and rheumatic disease. *Rheum Dis Clin North Am*. 2000;26:117–123.
65. Rastogi DP, Singh VP, Singh V, et al. Evaluation of homeopathic therapy in 129 asymptomatic HIV carriers. *Br Homeopath J*. 1993;82:4–8.
66. Rastogi DP, Singh V, Dey SK, et al. A clinical profile of HIV carriers. A 3 year study (abstract). Proceedings of the 2nd International Conference on AIDS in Asia and Pacific, November 1992.
67. Rastogi DP, Singh V, Dey SK, et al. Homoeopathic drugs as immunomodulators (abstract). Proceedings of the 10th International Conference on AIDS=STD, August 1995.
68. Rastogi DP, Singh VP, Singh V. Evaluation of immunorestorative role of homoeopathic medicines in HIV/AIDS (abstract). Proceedings of the 3rd International Conference on AIDS in Asia and Pacific, September 1994.
69. Danninger T, Gallenberger K, Kraeling J. Immunologic changes in healthy probands and HIV infected patients after oral administration of *Staphylococcus aureus* 12c: a pilot study. *Br Homeopath J*. 2000;89:106–115.
70. Wiesenauer M, Ludtke R. The treatment of pollinosis with galphimia glauca D4—a randomized placebo-controlled double-blind clinical trial. *Phytomedicine*. 1995;2:3–6.
71. Kahn J. Homeopathic remedy relieves allergic asthma symptoms. *Medical Tribune*. 1995;5:11.
72. Matusiewicz R, Wasniewuki J, Sterna Bazanska A, et al. Behandlung des chronischen Asthma bronchiale mit einem homoopathischen Komplexmittel. *Erfahrungsheilkunde*. 1999;48:367–374.
73. Matusiewicz R. The homeopathic treatment of corticosteroid-dependent asthma: a double-blind, placebo-controlled study. *Biomed Ther*. 1997;15:117–122.
74. Ullman D, Frass M. A review of homeopathic research in the treatment of respiratory allergies. *Altern Med Rev*. 2010;15:48–56.
75. Poitevin B. Review of experimental studies in allergy. *Br Homeopath J*. 1998;87:89–99.
76. Reilly DT, Taylor MA, McSharry C, et al. Is homoeopathy a placebo response? Controlled trial of homoeopathic potency, with pollen in hayfever as model. *Lancet*. 1986;2:881–886.
77. Taylor MA, Reilly D, Llewellyn-Jones RH, et al. Randomised controlled trial of homoeopathy versus placebo in perennial allergic rhinitis with overview of four trial series. *BMJ*. 2000;321:471–476.
78. Weiser M, Clasen BPE. Controlled double-blind study of a homeopathic sinusitis medication. *Biol Ther*. 1995;13:4–11.
79. de Lange de Klerk ES, Blommers J, Kuik DJ, et al. Effect of homoeopathic medicines on daily burden of symptoms in children with recurrent upper respiratory tract infections. *BMJ*. 1994;309:1329–1332.
80. Taylor MA, Reilly D, Llewellyn-Jones RH, et al. Randomised controlled trial of homoeopathy versus placebo in perennial allergic rhinitis with overview of four trial series. *BMJ*. 2000;321:471–476. Erratum in *BMJ* 2000;321:733.
81. Ullman D. Controlled clinical trials evaluating the homeopathic treatment of people with human immunodeficiency virus or acquired immune deficiency syndrome. *J Altern Complementary Med*. 2003;9:133–141.
82. Pilkington K, Kirkwood G, Rampes H, et al. Homeopathy for anxiety and anxiety disorders: a systematic review of the literature. *Homeopathy*. 2006;95:151–162.
83. Kleijnen J, Knipschild P, ter Riet G. Clinical trials of homeopathy. *BMJ*. 1991;302:316–323.
84. Linde K, Clausius N, Ramirez G, et al. Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. *Lancet*. 1997;350:834–843.
85. Mathie RT, Lloyd SM, Legg LA, et al. Randomized placebo-controlled trials of individualized homeopathic treatment: systematic review and meta-analysis. *Systematic Reviews*. 2014;3:142–157.
86. Mathie RT, Ramparsad N, Legg LA, et al. Randomised, double-blind, placebo-controlled trials of non-individualised homeopathic treatment: systematic review and meta-analysis. *Systematic Reviews*. 2017;3:142–157.
87. Witt CM, Ludtke R, Mengler N, et al. How healthy are chronically ill patients after eight years of homeopathic treatment? Results from a long term observational study. *BMC Public Health*. 2008;8:413–421.
88. Witt CM, Ludtke R, Baur R, et al. Homeopathic treatment of patients with chronic low back pain. A prospective observational study with 2 years' follow up. *Clin J Pain*. 2009;25:334–338.
89. Witt CM, Ludtke R, Willich SN. Homeopathic treatment of patients with migraine: a prospective observational study with a 2-year follow-up period. *J Altern Complementary Med*. 2010;16:347–355.
90. Day CE. Control of stillbirths in pigs using homeopathy. *Vet Rec*. 1984;114:216.
91. Choudhury H. Cure of cancer in experimental mice with certain biochemic salts. *Br Homeopath J*. 1980;69:168–170.
92. Cazin JC, Cazin M, Gaborit JL, et al. A study of the effect of decimal and centesimal dilutions of arsenic on the retention and mobilization of arsenic in the rat. *Hum Toxicol*. 1987;6:315–320.
93. Banerjee P, Bhattacharyya SS, Pathak S, et al. Evidences of protective potentials of microdoses of ultra-high diluted arsenic trioxide in mice receiving repeated injections of arsenic trioxide. *ECAM*. 2009;1–10.
94. Cazin JC, Cazin M, Chaoui A, et al. Influence of several physical factors on the activity of ultra low doses. In: Doutremepuich C, ed. *Ultra Low Doses*. London: Taylor & Francis; 1991:69–80.
95. Fisher P, House I, Belon P, et al. The influence of the homoeopathic remedy plumbum metallicum on the excretion kinetics of lead in rats. *Hum Toxicol*. 1987;6:321–324.
96. Cambar J, Desmouliere A, Cal JC, et al. Mise en évidence de l'effet protecteur de dilutions homéopathiques de Mercurius corrosivus vis-à-vis de la mortalité au chlorure mercurique chez la souris. *Ann Homéopath Fr*. 1983;5:6–12.
97. Guillemain J, Cal JC, Desmouliere A, et al. Effet protecteur de dilutions homéopathiques de metaux néphrotoxiques vis-à-vis d'une intoxication mercurielle. *Cahiers Biothérapie*. 1984;81(suppl):27–34.
98. De Gerlache J, Lans M. Modulation of experimental rat liver carcinogenesis by ultra low doses of the carcinogens. In: Doutremepuich C, ed. *Ultra Low Doses*. London: Taylor & Francis; 1991:17–26.
99. Doucet-Jaboeuf M, Guillemain G, Piechaczyk M, et al. Evaluation de la dose limite d'activité du facteur thymique serique. *CR Acad Sci Paris*. 1982;295:283–286.
100. Doucet-Jaboeuf M, Pelegrin A, Cot MC, et al. Seasonal variations in the humoral immune response in mice following administration of thymic hormones. In *Annual Review of Chronopharmacoly*. Vol 1. Oxford: Pergamon Press; 1984.
101. Bastide M, Daurat V, Doucet-Jaboeuf M, et al. Immunomodulator activity of very low doses of thymulin in mice. *Int J Immunother*. 1987;3:191–200.
102. Daurat V, Dorfman P, Bastide M. Immunomodulatory activity of low doses of interferon alpha, beta in mice. *Biomed Pharmacother*. 1988;42:197–206.
103. Weisman Z, Topper R, Oberbaum M, et al. Immunomodulation of specific immune response to KLH by high dilution of antigen (abstract 19). Proceedings of the 5th GIRI Meeting, Paris, November 20–30, 1991.

104. Bentwich Z, Weisman Z, Topper R, et al. Specific immune response to high dilutions of KLH; transfer of immunological information. In: Bornoroni C, ed. *Omeomed 92*. Bologna: Editrice Compositori; 1993:9–14.
105. Larue F, Cal JC, Guilleman J, et al. Influence du facteur dilution sur l'effet protecteur de Mercurius Corrosivus vis-á-vis de la toxicite induite par le chorure mercurique chez la souris. *Homeopathie Fr*. 1985;73:375–380.
106. Vischniac I. Influence des doses infinite simales de plomb sur l'evolution de l'intoxication au plomb chez l'animal. *Homeopathie Fr*. 1965;53:21–33.
107. Jonas WB, Dillner DK. Protection of mice from tularemia infection with ultra-low, serial agitated dilutions prepared from Francisella tularensis-infected tissue. *J Sci Exploration*. 2000;14:35–52.
108. Magnani P, Conforti A, Zanolin E, et al. Dose-effect study of Gelsemium sempervirens in high dilutions on anxiety-related responses in mice. *Psychopharmacol*. 2010;210:533–545.
109. Davenas E, Poitevin B, Benveniste J. Effect on mouse peritoneal macrophages of orally administered very high dilutions of Sillica. *Eur J Pharmacol*. 1987;135:313–319.
110. Sainte-Laudy J, Belon P. Analysis of immunosuppressive activity of serial dilutions of histamine on human basophil activation by flow cytometry. *Inflamm Res*. 1996;45(suppl 1):s33–s34.
111. Bildet J, Guyot M, Bonnini F, et al. [The effect of dilutions of Apis mellifica and Apium virus on ultraviolet light-induced erythema in the guinea pig.]. *Ann Pharmacol Fr*. 1989;47:24–32. [French].
112. Oberbaum M, Weisman Z, Kakinkovich A, et al. Healing chronic wounds performed on mouse ears using silica (Sio2) as a homeopathic remedy. In: Bastide M, ed. *Signals and Images*. Dordrecht, Netherlands: Kluwer Academic Publishers; 1997:191–199.
113. Belon P, Cumps J, Ennis M, et al. Inhibition of human basophil degranulation by successive histamine dilutions: results of a European multi-centre trial. *Inflamm Res*. 1999;48(suppl 1):S17–S18.
114. Ovelgonne JH, Bol AW, Hop WC, et al. Mechanical agitation of very dilute antiserum against IgE has no effect on basophil staining properties. *Experientia*. 1992;48:504–508.
115. Hirst SJ, Hayes NA, Burridge J, et al. Human basophil degranulation is not triggered by very dilute antiserum against IgE. *Nature*. 1993;366:525–527.
116. Brown V, Ennis M. Flow-cytometric analysis of basophil activation: inhibition by histamine at conventional and homeopathic concentrations. *Inflamm Res*. 2001;50:S47–S48.
117. Belon P, Cumps J, Ennis M, et al. Inhibition of human basophil degranulation by successive histamine dilutions: results of a European multi-centre trial. *Inflamm Res*. 1999;48:S17–S18.
118. Belon P, Cumps J, Ennis M, et al. Histamine dilutions modulate basophil activity. *Inflamm Res*. 2004;53:181–188.
119. Fisher P. A landmark for basic research in homeopathy. *Homeopathy*. 2004;93:162–163.
120. Sainte-Laudy Dr . (France), Professor Mannaionni (University of Florence, Italy), Professor Ennis (Queen's University, Belfast). The effect of dilutions of antigens and various biologic factors on the activity of basophil leukocytes. http://www.boiron.com/en/htm/04-politique/fondamentale_03.htm. Accessed October 8, 2004.
121. Poitevin B. Experimental study of homoeopathy in allergology. *Br Homeopath J*. 1998;87:154–164.
122. Linde K, Jonas WB, Melchart D, et al. Critical review and meta-analysis of serial agitated dilutions in experimental toxicology. *Hum Exp Toxicol*. 1994;13:481–492.
123. Endler PC, Pongratz W, Kastberger G, et al. The effect of highly diluted agitated thyroxine on the climbing activity of frogs. *Vet Hum Toxicol*. 1994;36:56–59.
124. Youbicier-Simo BJ, Boudard F, Mekaouche M, et al. Effects of embryonic bursectomy and in ovo administration of highly diluted bursin on adrenocorticotropic and immune responses of chickens. *Int J Immunother*. 1993;9:169–180.
125. Boyd WE, Brit M. Biochemical and biological evidence of the activity of high potencies. *Br Homeopath J*. 1954;44:7–44.
126. Davenas E, Beauvais F, Amara J, et al. Human basophil degranulation triggered by very dilute antiserum against IgE. *Nature*. 1988;333:816–818.
127. Sainte-Laudy J, Belon P. Analysis of immunosuppressive activity of serial dilutions of histamine on human basophil activation by flow cytometry. *Inflamm Res*. 1996;45(suppl 1):S33–S34.
128. Jonas W, Lin Y, Tortella F. Neuroprotection from glutamate toxicity with ultra-low dose glutamate. *Neuroreport*. 2001;12:335–339.
129. Vickers AJ. Independent replication of pre-clinical research in homeopathy: a systematic review. *Forsch Komplementarmed*. 1999;6:311–320.
130. Elia V, Niccoli M. Thermodynamics of extremely diluted solutions. *Ann N Y Acad Sci*. 1999;879:241–248.
131. Elia V, Niccoli M. New physico-chemical properties of water induced by mechanical treatments. *J Therm Anal Calorim*. 2000;61:527–537.
132. Elia V, Niccoli M. New physico-chemical properties of extremely diluted aqueous solutions. *J Therm Anal Calorim*. 2004;75:815–836.
133. Lo SY. Anomalous state of ice. *Mod Phys Lett B*. 1996;10:909–919.
134. Lo SY, Lo A, Chong LW, et al. Physical properties of water with IE structures. *Mod Phys Lett B*. 1996;10:921–930.
135. Rey L. Thermoluminescence of ultra-high dilutions of lithium chloride and sodium chloride. *Physica A*. 2003;323:67–74.
136. Mishima O, Stanley HE. Decompression-induced melting of ice IV and the liquid-liquid transition in water. *Nature*. 1998;392:164–168.
137. Wourtersen S, Emmerichs U, Bakker HJ. Femtosecond mid-IR pump-probe spectroscopy of liquid water: evidence for a two-component structure. *Science*. 1997;278:658–660.
138. Robinson GW, Cho CH, Gellene GI. Refractive index mysteries of water. *J Phys Chem B*. 2000;104:7179–7182.
139. Woutersen S, Bakker HJ. Resonant intermolecular transfer of vibrational energy in liquid water. *Nature*. 1999;402:507–509.
140. Gregory JK, Clary DC, Liu K, et al. The water dipole moment in water clusters. *Science*. 1997;275:814–817.
141. Ropp J, Lawrence C, Farrar TC, et al. Rotational motion in liquid water is anisotropic: a nuclear magnetic resonance and molecular dynamics study. *J Am Chem Soc*. 2001;123:8047–8052.
142. Errington JR, Debenedetti PG. Relationship between structural order and the anomalies of liquid water. *Nature*. 2001;409:318–321.
143. Lobyshev VI, Shikhlinskaya RE, Ryzhikov BD. Experimental evidence for intrinsic luminescence of water. *J Mol Liquids*. 1999;82:73–81.
144. Elia V, Baiano S, Duro I, et al. Permanent physico-chemical properties of extremely diluted aqueous solutions of homeopathic medicines. *Homeopathy*. 2004;93:144–150.
145. Sukul NC, Ghosh S, Sukul A, et al. Variation in Fourier transform infrared spectra of some homeopathic potencies and their diluent media. *J Altern Complementary Med*. 2005;11:807–812.

FURTHER READING

The research section of this chapter is deeply indebted to the works of Peter Fisher, editor of the *British Homeopathic Journal;* Madeleine Bastide; Paolo Bellavite; and Andrea Signorini. The clinical training the first author received from Alan Sutherland and Marion Belle Rood has continued to be the foundation of sound practice and the inspiration to sustain study in the philosophy of nature.

Books

Bastide M. *Signals and Images*. Dordrecht, Netherlands: Kluwer Academic Publishers; 1997.

Bellavite P, Signorini A. *Homeopathy, a Frontier in Medical Science*. Berkeley, CA: North Atlantic Books; 1995.

Boerke W. *Pocket Manual of Materia Medica With Repertory*. New Delhi, India: Jain; 1936.

Clark J. *Dictionary of Practical Materia Medica*. Vols. 1–3. Essex, England: Health Sciences; 1900.

Edzard E, Hahn EG. *Homeopathy, a Critical Appraisal*. Oxford: Butterworth Heinemann; 1998.

Hahnemann S. *Chronic Diseases: Their Specific Nature and Homeopathic Treatment*. New York: Wm Radde; 1845.

Hahnemann S. *Organon of Medicine. [Reprint.]*. Los Angeles: JP Tharcher; 1982.

Kent JT. *Materia Medica*. Philadelphia: P Blakiston; 1900.

Lange A. *Getting at the Root: Treating the Deepest Source of Disease*. Berkeley, CA: North Atlantic Books; 2002.
Nash EB. *Leaders in Homeopathic Therapeutics*. New Delhi, India: Jain; 1898.
Roberts HA. *The Principles and Art of Cure by Homeopathy*. Essex, England: Health Sciences; 1942.

Journals

Homeopathy, formerly the *British Homeopathic Journal*; the official journal of the Faculty of Homeopathy, London
Homeopathic Links—International Journal for Classical Homeopathy
Simillimum, journal of the Homeopathic Academy of Naturopathic Physicians
The American Homeopath, journal of the North American Society of Homeopaths
The Homeopath, journal of the Society of Homeopaths (UK)

Web Resources

Ad Hom. the Academic Departments of Homeopathy at Glasgow Homeopathic Hospital. http://homeopathyusa.org. Website.
British Homeopathic Library (a library and information service dedicated to the research and practice of homeopathy). http://dspace.dial.pipex.com/hom-inform/index.shtml. Website.
HOMINT Documentation Information System VSM. Alkmaar (Netherlands)/DHU, Karlsruhe (Germany). Contains 35,000 references, major articles, homeopathic journals, literature-search on request. vsm@gn.apc.org. Arjo Bol, information specialist VSM/Susanne Rehm, DHU. Email.
Homeopathic Educational Resources Database. Compiled by Dr. Russell Malcolm of the Glasgow Homeopathic Hospital. ghl@gn.apc.org. Email.

40

Hydrotherapy

Jamie Doughty, BSc, ND, and *Venessa Wahler, ND*

OUTLINE

A BRIEF HISTORY OF NATUROPATHIC MEDICINE INTRODUCTION

Hydrotherapy is a form of physical medicine using the therapeutic application of water in a variety of ways, both internally and externally, on the body. Topical applications of cold or hot water packs, compresses, baths, pools, steams, sweats, showers, enemas, and colonics are all forms of hydrotherapy. Profound, multisystem physiological effects result from the thermodynamic property of water and its effects on an organism's circulation and capability to remove waste and eliminate. Hydrotherapy techniques applied to a patient are a form of stress to the cells that influences their metabolic function, regulates their environment, and provides an opportunity for the body's natural healing processes to take place.

Hydrotherapy has been used by human civilizations for thousands of years and has grown to have literature demonstrating scientific evidence-based applications and uses worldwide today.

Naturopathic physicians are trained in harnessing the curative power of nature through hydrotherapy techniques to treat a myriad of chronic diseases by supporting the innate ability of the human body to heal. Naturopathic medicine follows the principle of "first do no harm," which analyzes the risks versus benefits of any therapeutic intervention. Hydrotherapy is a simple nature cure—a gentle, effective, and curative modality for modern culture. In the words of Dr. Simon Baruch (1840–1921): "No remedy in the entire *materia medica* demands as clear judgment and as much knowledge of the patient's condition as does the application of water."

In North America there are seven different accredited naturopathic educational institutions that all teach and practice the therapeutic application of water through many lecture hours and much clinical experience.[1] The working hypothesis of naturopathic hydrotherapy is that the state of being free from illness and injury is proportional to the normal flow of healthy blood, lymph, and qi.

HISTORY

Water has been associated with medicine and healing since the beginning of recorded history. As an ancient method of treatment, hydrotherapy has been used for thousands of years by people from all over the world and is documented in the writings of many civilizations. In the minds of primitive peoples, water had both supernatural and spiritual power. Religion and water were closely associated for centuries.

Ancient Hydrotherapy

Dating as far back as 10,000 BC, Native Americans used sweat lodges for healing purposes, and the waters of Bath, England, were first used by Paleolithic hunters. The waters of Baden-Baden in Germany have been used for thousands of years, and Ayurvedic steam treatments have been used in India since 5000 BC. Ruins of an ancient bath were discovered in Pakistan, dating back as far as 4500 BC. On the island of Crete, travelers and visitors had access to public baths and running water and would bathe before visiting the king at the palace of Knossos in 2000 BC. The benefits of colon hydrotherapy were recorded as early as 1500 BC, as described in an ancient Egyptian medical document, the "Ebers Papyrus."

Hippocrates, referred to as the "father of medicine," began using water extensively around 400 BC for healing purposes. He prescribed the use of baths in the treatment of both acute and chronic disease, and his writings regarding baths contain some of the earliest dictums on the therapeutic uses of water.[2] Records reveal that water was used in the treatment of rheumatism, fever, inflammation, and other illnesses. Greeks introduced water treatments to the Roman Empire, and by 50 BC, bathing became popular throughout Rome. Roman physicians Galen and Celsus wrote of preventing disease with warm and cold

baths, and by 300 AD, there were approximately 900 baths operating in the city of Rome. When the Roman Empire declined, the use of baths for hygienic, social, and medical purposes also declined.

In the Middle Ages, spanning from 500 to 1500 AD, public bathing was condemned by Christian authorities, and eventually, bathing was discouraged and considered indulgent by the Catholic Church. Although healing methods using water were no longer used in Europe, the knowledge of hydrotherapy was kept alive through monks in monasteries who dedicated themselves to replicating Greek and Roman texts by hand. Islamic countries became the main storage place of classic Western scientific thought. Sentos (public bathhouses) emerged and became popular in Japan, and saunas were invented in Finland. Around that same time, banyas were introduced in Russia. In 1242 AD, hot springs were discovered and developed in Switzerland.

Modern Hydrotherapy

In the 16th century, the Renaissance brought forth a change in medicine, and bathing once again gained popularity for health purposes. Sir John Floyer, an English physician, wrote about "curing madness by cold baths" in his *History of Cold Bathing* published in 1702. His book influenced Johann S. Hahn, who established the principles of modern hydrotherapy in Germany. More than 200 springs were operating in Europe, and private bathrooms and showers were used. During the Scientific Revolution, methods became available to analyze minerals and their effects on the body. In 1810 the first bathroom, which included a sink, toilet, and bathtub, was constructed in a private house in the United States.

The hydrotherapy movement traces its origins to two prominent hydrotherapists: Vincent Priessnitz of Austria and Father Sebastian Kneipp of Bavaria. Priessnitz had no formal training but was inspired to heal his own broken ribs using cold compresses. When he was young, he recalled a man who healed cattle by the external application of cold water. He eventually went on to open his first sanatorium in 1829 in Graefenberg, successfully treating more than 40,000 people with little loss of life. He was also persecuted by medical authorities and convicted of witchcraft because he taught people to heal themselves with the use of water, exercise, and diet.

Priessnitz published *The Cold Water Cure* in 1843, and two establishments later opened in New York City based on his principles. In 1851 Dr. T. L. Nichols and his wife, Mary Grove, opened the American Hydropathic Institute in New York. In 1853 the New York Hydropathic School was established by two medical doctors, Dr. Joel Shew and his associate Dr. Russell Trall.

Father Sebastian Kneipp, initially rejected from the priesthood because he had diabetes mellitus, went on to cure himself with water treatments and diet. After his cure, he was admitted to the priesthood and went on to write *My Water Cure,* published in 1886. In 1891 Father Kneipp treated Benedict Lust, curing him of his medical problems. Lust would later bring Kneipp's teachings to America, influencing Dr. John Harvey Kellogg, an important figure in the modern history of hydrotherapy. Lust eventually played a pivotal role in forming the foundation of naturopathic medicine.

Although the popularity of water treatments began to decline in the late 19th century, Ellen G. White, health reformer and founder of Seventh-Day Adventism, was inspired to open the Western Health Reform Institute, focusing on hydrotherapy, diet, rest, and other natural remedies. One conventional doctor, Simon Baruch, studied hydrotherapy in Europe and brought his teachings to the United States. He spent his life trying to establish hydrotherapy as a science-based treatment, as opposed to the nonscientific form, and taught at Columbia University.

In 1880 Dr. John Harvey Kellogg became the medical director of Battle Creek Sanitarium (formerly Western Health Reform Institute), which became the leading center for natural healing in North America and Europe. He wrote extensively on health, and his book *Rational Hydrotherapy* was published in 1901. In 1917 Kellogg wrote a scientific article praising the effects of colon hydrotherapy in treating the large bowel.[3]

Henry Lindlahr, a real estate tycoon in the United States, after being told he had diabetes mellitus and that no treatments were available, went to Europe to be treated by Father Kneipp. Once he was healed by Kneipp's recommendations of hydrotherapy and proper diet, Lindlahr returned to the United States, received medical training, and established a sanitarium in Chicago in 1906. He wrote *Nature Cure,*[4] the definitive guide to the philosophy and practice of nature cure medicine.

Otis G. Carroll, a modern forefather of naturopathic medicine, studied with Lindlahr and Alex Ledoux, another student of Sebastian Kneipp. Dr. Carroll developed the Constitutional Hydrotherapy treatment in 1923 in Spokane, Washington, which combines compresses with electrical stimulation. Carroll's work was continued by Harold Dick, another naturopath who entered into practice in 1955. In 1990 his work was then followed by his daughter Letitia Dick-Kronenberg, ND. The Windrose Clinic has now provided expert hydrotherapy for more than 50 years. In 2012 her book *The Ultimate Text in Constitutional Hydrotherapy: A 100 Year Tradition of Clinical Practice*[5] was published.

In 1956 Dr. John Bastyr established the National College of Naturopathic Medicine. Hydrotherapy is still taught in naturopathic medical institutions and practiced in their associated teaching clinics as a highly effective, fundamental naturopathic modality. In the late 1980s, *Lectures in Naturopathic Hydrotherapy*[6] was written by Dr. Wade Boyle, ND, and Dr. Andre Saine, ND.

PHYSIOLOGICAL EFFECTS OF WATER

> *No case of chronic disease, no matter how inveterate, unless it has reached an absolutely hopeless stage, should be abandoned as incurable without giving hydrotherapy, with the accompanying regimen of diet and exercise, active or passive, an intelligent and persevering trial.*
>
> ***—Dr. John Harvey Kellogg, 1852–1943***

Water is a universal substance. The gross composition of the human body is water, responding powerfully to its application both internally and externally. Water is the universal solvent in the body; all nutrients, wastes, gases, hormones, and toxins are carried throughout the body in this aqueous base. Water is distributed between the two major fluid compartments, intracellularly and extracellularly, which together constitute the total body water ranging from 55% to 75%, depending on age and gender. Pure water has a neutral pH of 7; it is neither acidic nor alkaline. This neutrality won't alter the pH of the body or of any substances added to the water. Water takes many shapes that can be used in a therapeutic manner to affect physiology. Examples of the various forms of water include liquid, ice, and steam vapor.[7]

Water affects human body systems in various ways depending on the method of administration and the circumstances of the individual. The physiological effects of hydrotherapy are classified as thermal, mechanical, and chemical. Thermal effects are produced by the application of water at temperatures above or below body temperature, with the magnitude of variation from body temperature predicting therapeutic outcomes. Mechanical effects are produced by the effect of water acting on the body surface in the form of sprays, douches, frictions, immersions, whirlpools, and so forth. Chemical effects are produced when water is taken orally, by nasal/sinus irrigation, through vaginal douching, or via colon irrigation.

The effects of the application of water can be generalized or local, depending on the application and administration. Water can be administered internally or externally to the human system. Water has the ability

TABLE 40.1 Definitions and Patient Perceptions of Water Temperature[58]

Water Temperature	Patient Experience
Very cold 32°F–52°F	Painfully cold
Cold 55°F–65°F	Tolerable but uncomfortable
Cool 66°F–80°F	Produces goose bumps
Tepid 81°F–92°F	Slightly below skin temperature
Warm/neutral 93°F–97°F	Comfortable
Hot 98°F–104°F	Tolerable but skin turns red
Very hot 105°F–110°F	Tolerable for short periods
Painfully hot 111°F–124°F	Intolerable
Dangerously hot 125°F	Injuries likely

to modify the physiology of a living body system through temperature effects. With variations of temperature, human body systems need to adapt. In an attempt to accommodate itself (the body) to a change in circumstance, water can change tissues to support the vital processes. The physiological effects of water will vary depending on the individual person's age, biological sex, and physiological condition. (Table 40.1)

Basic Properties of Water

Water has the ability to absorb and radiate large quantities of heat; it is this high specific heat that is the standard by which other specific heats are measured.[8]

- It takes 1 calorie to cool 1 g of water 1°C.
- It takes 80 calories to turn 1 g of water at 0°C into ice (therefore melting ice absorbs 80 times as much heat as does liquid water).
- It takes 540 calories to turn 1 g of water at 100°C into steam; condensing steam gives off 540 times as much heat as does liquid water.

The fluidity of water allows for large areas of application to a single body at one time. A topical application of water will elicit an effect through the skin, which will affect the nervous system, circulation, and finally the tissues. Water is also an excellent conductor of heat by rapidly carrying heat to and away from the body—it is faster than air by 25×.[9]

The modification of water temperature is the most commonly used mode of therapeutic application. The rate of cold or hot temperature exchange depends on the difference in temperature between the two objects, the conducting properties of the objects, the length of time the process is allowed to continue, and the area of the body through which the temperature flows (i.e., skin, fat, or muscle tissue).

Water temperature is described as varying from cold to hot.

Hydrotherapy can have a profound healing effect on the body by the support of healthy blood flow, lymph, and chi. Water can be used to increase circulation and decrease congestion of the blood and lymph, thereby affecting the quantity of these fluids. Water will also affect the quality of blood through the organs of elimination. These emunctories include the skin, liver, kidneys, bowels, and lungs. The quality of blood can be altered through water to affect the organs of digestion and elimination—the stomach, pancreas, small intestine, and colon. For example, increasing blood flow through the application of water can ensure that the body digests and assimilates more nutrients to help build up the quality of the blood.

We can manipulate the blood and body fluids through various applications of hot and cold.

Definitions of the key methods affecting blood and lymph flow are as follows:

1. Direct: local effects of hot and cold applications to a tissue depth of less than 1 cm because thermal receptors are superficial. Direct effects are effective for treating cellulitis in an extremity, mastitis, or tooth infections.
2. Revulsive: alternating hot and cold applications. The hot application magnifies the effect of the cold that follows it. When done in alternating sequences, the effects are additive. Revulsive treatments are very powerful to decongest tissues and produce analgesic effects. Examples include alternating sitz baths and alternating baths to the extremities.
3. Retrostasis: cold application for driving fluid from one area of the body to another.
4. Derivation: hot application for drawing fluid from one area of the body to another. In the presence of congestion in the circulation of an ill patient, a derivative treatment draws proportionally more blood and lymph from the congested parts, whereas in a healthy patient with normal circulation, a derivative treatment draws blood equally from all other parts.
5. Collateral circulation: the fluid dynamics of retrostasis and derivation apply to a restricted area where blood-flow modifications in a superficial artery can change the circulation of a deep artery from the same trunk. For example, a cold application to the start of a superficial branch of an artery will constrict, decreasing blood flow, while the deep branch would dilate to increase blood flow. The converse is also true; a hot application to the start of a superficial branch of an artery will dilate, increasing blood flow, while the deep branch will constrict to decrease blood flow.
6. Arterial trunk reflex: cold or hot applications exerted on an arterial trunk will influence the smaller vessels fed by that trunk. For example, a hot application to an arterial trunk will dilate the trunk and its distal arteries branching off.
7. Spinal cord reflex: symmetrically paired structures of the body are reflexively connected from their vital center to the corresponding area of skin (i.e., extremities).

Principles of Hot and Cold Temperatures

The thermal effect of water is the most commonly used principle of therapeutic hydrotherapy. In general, hot water relaxes and sedates, whereas cold water stimulates, invigorates, and tonifies. Nonetheless, very hot water can stimulate and be destructive, whereas prolonged cold water can be depressive and destructive.[10]

The duration of the application of water is the best predictor of the previously described effects of temperature on human body systems. Short applications will have a stimulating effect, whether it is hot or cold, and long applications will have a depressive effect, whether it is hot or cold. A long application will have an initial vasoconstrictive effect on circulation followed by a secondary vasodilation effect. The definition of a long application of water is one where the temperature doesn't change significantly over time, such as during a shower or bath.

The sequence of effects of hot and cold applications include initial vasoconstriction for both temperatures followed by secondary vasodilation.[11] The final sequence of a cold application ends with vasoconstriction, but with a hot application, we see venostasis. Short cold applications produce a substantial increase in oxygen absorption; carbon dioxide excretion; an increase in nitrogen absorption and excretion; an increase in tissue tone, muscle tone, and vascular tone; and an increase in peripheral white blood cell and red blood cell counts while decreasing blood glucose. Of note, short cold applications have the greatest potential for the stimulation of tissue metabolism.

Hot applications, whether short or long, will somewhat increase oxygen absorption and carbon dioxide excretion, but they will decrease tissue tone and decrease peripheral red and white blood cell counts while increasing blood glucose. Remember, the time of application and the temperature of the water determine the therapeutic and physiological effect on blood flow and can be manipulated using various time and temperature methods.

Principles of Circulation of Blood by Hot and Cold Temperatures

Hydrotherapy is based on the premise that healing is proportional to normal blood flow. When we use alternating hot and cold applications of water, it creates a pumping action in the peripheral heart. The peripheral heart includes skeletal muscle, arteriolar smooth muscle, and capillary dilation or constriction that is different from the central heart. The human body's closed circulatory system determines that it is impossible to have an effect on one part of the circulation without having effects on another part of the system. Again, the temperature of the water affects the skin, which reflexes to the nervous system, the circulatory system, and all the way down to the level of the tissues.

There are several important treatment variables when using hydrotherapy. The first is temperature; the greater the difference between the temperature of the application and the temperature of the human body, the greater the intensity of the treatment will be.

In alternative or revulsive treatments, the greater the contrast in temperature between the hot and cold, the greater the intensity of the treatment.

The duration and frequency of the timing of the application are other variables to manipulate. When the time of the application is extreme, the duration is inversely proportional to the overall intensity of the treatment. For example, short treatments of hot or cold are stimulating to the circulation, and long treatments, whether hot or cold, are depressive to the system.

If the temperature is moderate or tepid, the intensity of the treatment is going to be directly proportional to the duration of the timing of application. The more frequent the application is made, the more intense is the overall effect. Also of note is the time of the day when the application is used and whether it is ideal for a patient's vitality of constitution to enhance the therapeutic effect.

The location of the site of application is another important variable. With derivation treatments, the greater the treatment area, the greater is the intensity of the treatment. With retrostatic treatments, the smaller the treatment area, the more intense is the effect. With alternating-temperature treatments, the greater the size difference between hot and cold, the greater is the intensity of their combined effect. Of note, remember that a cold application should be smaller than a hot application when using alternating treatments.

Incorporating pressure and friction to the treatment area will increase the patient's ability to tolerate an extreme temperature because they increase the intensity of the treatment due to the greater force of water against the body. For example, friction on the skin's surface will allow a patient to tolerate cold without shivering and also draw blood to the surface, which will help increase core heat loss.

In the case of compresses and fomentations, the ability of water vapor to penetrate the material used in covering the patient is inversely proportional to the heating or cooling effects of the compress. The amount of dampness and wetness in the application is also proportionally related to the effect of the temperature on the body.

Fever and Antipyretic Treatments

Heat may be applied to the body in various ways, including hot packs, fomentations, steam, baths, and showers. All hot applications produce physiological responses that are attempts by the body to eliminate and dissipate excess heat to prevent a damaging rise in local and systemic temperatures. The effects produced by hot applications depend on the mode, temperature, duration, and the condition of the patient.

Water at 98°F or above is generally perceived as hot, and water higher than 104°F is considered very hot. At 120°F, an immersion bath becomes unendurable, although small areas of the body, such as the hand, may be conditioned to endure a temperature of 10°F to 15° higher for short periods. The mucous membranes, unlike the skin, may endure temperatures as high as 135°F, which accounts for our ability to drink very hot liquids or benefit from steam inhalation treatment. Although exposure to the high temperatures of hot tubs and saunas has become quite popular in recent years, Kneipp, Priessnitz, and Kellogg all believed that repeated and prolonged use could weaken the individual unless counteracted by frequent cold applications, such as showers or ablutions.

TABLE 40.2 Effect of Treatment Variables on Metabolism

- A short hot application of water will increase circulation and increase metabolism.
- A long hot application of water will decrease circulation and increase metabolism.
- A short cold application of water will increase circulation and increase metabolism.
- A long cold application of water will decrease circulation and decrease metabolism.

Local hot applications result in vasodilation, with a resulting increase in capillary blood flow, oxygen delivery to the tissues, local metabolic activity, and migration of lymphocytes through vessel walls and into the local tissues. Local perspiration is increased, and muscle relaxation occurs through the inhibition of muscle spindles, which decreases tone. Intense moist heat applied for a long period of time (several minutes) has a depth of penetration not exceeding about 3.4 cm. The increased blood flow through the area carries away heat conducted into the tissues from a hot application and limits the depth of penetration. (This "radiator-like" effect can be overcome by more intense applications of heat, and deep tissue destruction can occur.)

General hot applications over half of the body or greater can have dramatic effects on the cardiovascular system. The inability to dissipate heat from a large volume of tissues will result in an increase in body temperature, metabolism, and oxygen consumption. The heart rate increases 10 beats for every 1°F rise in body temperature, whereas blood pressure and cardiac output decrease. The respiratory rate increases 5 to 6 breaths per minute for every 1°F rise in body temperature, whereas the depth of respiration decreases. Hyperventilation may occur and result in respiratory alkalosis. Van't Hoff's law of temperature states that for every temperature rise of 18°F, the velocity of chemical and metabolic reactions in the body is increased 2 to 3 times. Slight increases in temperature can have a profound effect on cellular metabolism and systemic physiology, cell membrane permeability, oxidation, and metabolic rate. (Table 40.2)

Fever is defined as an increase in the regulated body temperature resulting from an elevation of the thermoregulatory set point for the body in the hypothalamus. This is often caused by infectious diseases. Fever is produced when an exogenous pyrogen is phagocytized by a macrophage, which results in the release of interleukin-1, which travels in the blood like a hormone to the hypothalamus. Prostaglandin E2 is produced in the hypothalamus to increase the body's temperature set point. At this point, the normal body temperature is perceived as being too low, so the body shifts into heat-conservation mode by initiating shivering, the vasoconstriction of surface blood vessels, and the contraction of arrector pili muscles in the skin. Interleukin-1 also induces fatigue and sleep to save energy while promoting the production of proteins in the liver that stimulate the immune system. In skeletal muscle tissue, we see liposomal activity that causes protein breakdown for the purpose of adding more amino acids to the blood for tissue repair and energy. This

will be experienced by the patient as myalgias. Gut motility is decreased with a fever above 99.5°F, causing anorexia to depress the appetite to free up energy for the healing process. It is important to fast the patient during fever because clinical experience shows that a fever will rarely go above 104°F in adults when they are fasting. Fasting during fever should be avoided with pregnant, nursing, or diabetic patients.

The purpose of fever in the body is to increase the production of white blood cells and to move those white blood cells into the circulation faster. White blood cell motility and productivity rates are increased with fever. The production of interferon is enhanced, and antibody production increases up to 20 times. During fever, blood concentrations of iron and zinc are reduced to inhibit bacterial growth.

Types of Fever

Types of fevers include the following:

1. Adaptive increases in temperature produced with exercise, by hormones (cortisol, norepinephrine/epinephrine), or in states of dehydration or heat exhaustion
2. Pyrogenic fevers (infectious) produced from bacterial toxins and foreign proteins
3. Artificially induced fever using thermo-hydrotherapy to deliberately elevate body temperature

All fevers can cause the following symptoms in patients:

- Nervous system symptoms, including insomnia, headache, or delirium
- Chilliness with goose bumps
- Shallow, rapid breathing
- Malaise or backaches
- Excessive thirst
- Shallow, rapid breathing
- Increased heart rate and cardiac output
- Anorexia, foul breath, coated tongue
- Hot, dry skin or cold, clammy skin
- Constipation or diarrhea
- Scanty, highly concentrated and colored urine

The following are measurements of normal body temperature: oral, 98.6°F; rectal, 99.6°F to 100°F; and axillary, 97.8°F. The ideal range for fever is 102°F to 103°F; this is optimal for fighting infection and is most effective. At temperatures between 104°F and 107°F, dehydration becomes a concern, and above 107°F, dangerous effects can occur, such as loss of consciousness and irreversible protein denaturation.

The degree of temperature elevation in a fever is determined by the severity of the pathogen and the vitality of the patient. We see this often when children run much higher fevers than adults or in chronically ill patients and the elderly, who can sometimes run only a slight fever if at all.

The best use of hydrotherapy in treating fever is to help the body maintain an optimal temperature between 102°F and 104°F. By using water, we can keep the patient well hydrated and can lower a dangerously high fever or raise a suboptimally low fever. When a patient is in the ideal range for fever and is sweating, the patient needs to be managed by keeping him or her well hydrated, warm, and comfortable.

Dehydration can cause the body temperature to stay elevated longer and will add to the risks for dangerous side effects from fever, such as seizures. The application or internal use of cool water is helpful for a high fever, and warm water externally or internally through teas to help promote sweating is helpful in a low fever or chilly patient. High fevers can result in fluid loss and electrolyte imbalances. Fluid-electrolyte replacement and rehydration formulas should be added to the water (e.g., vegetable broth, salt, diluted juice, or a rehydration drink). Applications of heat during fever are contraindicated in peripheral vascular disease, diabetes mellitus, brain injuries, and stroke.

Effects of Water Temperature on Fever

The effects of water temperature on fever are as follows:

- Short cold applications of temperature result in increased heat loss and increased heat production and, therefore, no net change in body temperature.
- Long cold applications of temperature result in increased heat loss and decreased heat production and, therefore, a decrease in net body temperature unless the patient is shivering.
- Tepid applications increase heat loss and cause no change in production and, therefore, result in a decrease in net body temperature.
- Short hot applications between 3 and 5 minutes greatly decrease heat loss and greatly increase heat production and, therefore, result in a great increase in net body temperature.
- Long hot applications of temperature greatly decrease heat loss and greatly increase heat production, resulting in a substantial net increase in body temperature.
- Very short hot applications of less than 3 minutes will result in a great increase in heat loss as blood is drawn to the surface to improve heat loss in the core of the body, with a slight increase in heat production, therefore resulting in a decrease in net body temperature.

In summary, the only temperature applications that offer a net loss of body temperature are the long cold, tepid, and very short hot types. Tepid and neutral baths are very effective in their ability to carry heat away from the body by conduction without increasing the body's production of heat either by the application of heat or by resulting in the patient shivering. Tepid baths range from 81°F to 92°F, and neutral baths are defined as 93°F to 96°F. Tepid and neutral water affusions involve the pouring of water onto the patient to increase the friction and effect of the treatment. A tepid sponge bath is a gentle and effective treatment for fever reduction, especially in children. The patient loses heat by conduction and evaporation, and the sponging provides gentle friction to bring more blood to the surface.

Be aware of the coasting factor, which dictates that oral body temperature readings will take some time to catch up to the core body temperature. It is recommended to stop the hydrotherapy treatment before the patient reaches the desired oral temperature by 1°F to 2°F.

GENERAL GUIDELINES FOR HYDROTHERAPY

For the Health Care Provider

1. To ensure safe and effective outcomes, it is important to take a full case history for each patient and rule out contraindications to the hydrotherapy treatment. Determine the physical, mental, and emotional status of each individual. Children, the elderly, those with major health problems, those with generally low resistance, obese individuals, and those with severe physical limitations may have a weaker response to hydrotherapy treatments and should be more closely observed.
2. Thoroughly explain each treatment before beginning. Include in your discussion the length of the session, the sequence of events, and any other pertinent details, such as the rationale for the treatment and temperatures to expect. Ensure that the patient understands the specifics of each therapy and that the patient is comfortable with all aspects of the treatment.
3. Check for and record objective findings at the onset of the therapy session. This should include taking the patient's temperature, pulse, and respiration rate.
4. Follow standard precautions that are designed to protect the patient and health care provider.

5. Stay with the client throughout the session or have an emergency signal such as a bell or alert system. Check in periodically with the client. Rerecord the patient's pulse, respiration rate, and temperature, and monitor other physiological markers such as blood sugar as needed.
6. Provide a clean treatment room equipped with all necessary supplies. Encourage hydration and provide clean drinking water. Ensure adequate temperature control in each treatment room.
7. Monitor the patient closely for side effects of the treatment. The client should not become chilled to the point of shivering. If the patient gets cold, consider initiating warming techniques such as friction rubs, offering warm drinks, or applying additional blankets or dressings while continuing with treatments. Oppositely, if the patient becomes overheated (depending on the therapy), initiate cooling techniques such as applying towels dipped in ice water and wrung out or applying ice packs where indicated. If the patient becomes dizzy or lightheaded, assess blood sugar, offer hydration, and encourage rest. Other side effects may include nervousness, heart palpitations, nausea, and aches and pains. In most cases, symptoms are self-limiting and respond to simple interventions and rest.
8. Check the temperature of both hot and cold applications before applying them to a patient's skin. Use an appropriate thermometer when using water in a treatment.
9. Record final pulse, temperature, respiratory rate, and any other pertinent physical examination findings. Adequate documentation of the therapy session will guide ongoing treatments, may prompt future research, and will continue to substantiate hydrotherapy as an effective therapeutic modality.
10. Inform your patient of any posttreatment precautions and recommendations. Some reactions may take place several hours to several days after treatment, so ensure that patients have a way to follow up with you if needed.

For the Patient or Individual

Before Treatment

1. Plan your hydrotherapy session at a time of day when you feel vital and unhurried.
2. Avoid eating 1 to 2 hours before your treatment time. Avoid alcohol and recreational drug use. Stay adequately hydrated.
3. Report all medications and supplements to your health care provider. If performing hydrotherapy at home or in a health facility such as a gym, make sure you check with your doctor first.

During Treatment

1. Report any reactions to your practitioner and stop therapy immediately if adverse effects occur.
2. Remain hydrated throughout the hydrotherapy session.
3. Take measures to warm up or cool down if you become too chilled or overheated, respectively.
4. Mindful breathing exercises may help balance the nervous system and optimize outcomes.
5. Follow safety rules and regulations of the health care facility (e.g., obey time restrictions for saunas and treatment pools).

After Treatment

1. Avoid drafts and excessive heat or cold after hydrotherapy treatments.
2. Be mindful of any side effects of your treatment, and report them to your health care provider.
3. Stay hydrated and follow dietary recommendations or restrictions specific to the hydrotherapy treatment.

General Cautions and Contraindications

Hydrotherapy is generally very safe, but, as with most medical therapies, there are cautions to consider and contraindications to be aware of. When caution is taken in hydrotherapy, it does not mean that the therapy should be avoided; it simply guides us to consider more closely the specific needs of the individual before, during, and after the therapeutic treatment. A contraindication is defined by any special symptom or circumstance that renders the use of a remedy or the carrying out of a procedure inadvisable, usually because of risk.[12]

Hydrotherapy treatments may produce unexpected or undesired effects. When this occurs, it's important to carefully to assess the individual's response to the treatment and ensure that the therapy has been properly administered. If an aggravation from treatment does occur, the therapist will need to review the intensity of the treatment and the length of treatment and reassess the appropriateness for the individual. Modifications can be made if additional treatments are indicated.

Most side effects of hydrotherapy are self-limiting, and the individual will often respond well to rest and simple interventions. Some of these effects include shivering, headache, dizziness or lightheadedness, hyperventilation, heart palpitations, skin irritation, nausea, insomnia, and minor aches and pains. Shivering will occur if the client is allowed to get too cold and therapy is not followed by appropriate warming techniques. Headaches may present secondary to dehydration; as a reaction to the water temperature, blood sugar dips, or blood pressure spikes; or as a result of a detoxification reaction. Detoxification reactions are possible within hours or may occur up to 1 or 2 days after treatment.

Dizziness or lightheadedness experienced during a hydrotherapy treatment may occur as a result of dehydration, decreased blood sugar, and/or decreased blood pressure levels. The client should be given adequate hydration and may be encouraged to lie down and get back up slowly once symptoms have subsided. Keep a source of sugar on hand in the case of hypoglycemia. Hyperventilation can develop from nervousness. A reduction in feelings of tension, as well as increased pain thresholds to hot and cold, can be achieved by having the patient perform deep- and slow-breathing techniques.[13]

Heart palpitations may accompany dizziness and result from elevated body temperature. Other factors affecting heart rate include emotions, body size and position, and medications that are shown to affect pulse rate. The heart rate is normally between 60 beats per minute (bpm) and 100 bpm, although a pulse rate as low as 40 bpm can be seen in very physically fit individuals.

Pregnancy

When used with caution and professional guidance, hydrotherapy can be supportive for women in pregnancy and during the labor process. Although warm-water therapies can promote relaxation and offer relief of minor aches and pains, prolonged exposures to heat sources such as hot tubs, hot baths, saunas, and steam rooms are contraindicated. Hot applications over the abdomen should be avoided. The American College of Nurse-Midwives (ACNM) affirms that "Warm water immersion hydrotherapy during labor provides comfort, supports relaxation, and is a safe and effective nonpharmacological pain relief strategy that promotes physiological childbirth."[14] When evidence-based clinical guidelines are followed, research demonstrates that the use of hydrotherapy for pain relief during labor does not increase risks for healthy women or newborns.[14]

The Organization of Teratology Information Services (OTIS) states that in pregnancy, a body temperature over 101°F can be of concern, especially if it lasts for a long period of time.[15] Hot baths would be a safer way to relax than would a hot tub. In a bath, the upper body remains

out of the water, and the water in the bath cools over time, reducing the risk of overheating. Although hot tubs are not recommended in pregnancy, there are steps to take to reduce risk. These include monitoring body temperature to avoid overheating, limiting time in a hot tub to 10 minutes or less, and reprogramming the hot tub to maintain a lower temperature.[16] The American College of Obstetricians and Gynecologists recommends that pregnant women never let their core body temperature rise above 102.2°F.[16] Neutral baths and Epsom salt hand and foot baths in the later stages of pregnancy have traditionally been used for symptoms of local edema.

According to the UK Teratology Information Service (UKTIS), there are limited data on most pregnancy outcomes after maternal exposure to external heat sources such as saunas, hot tubs, and hot baths, and there are no data relating specifically to steam room exposure.[17] An evidence-based assessment of the potential risks of these treatments in pregnancy is difficult due to the limitations of testing on pregnant females.

Hypertension and Cardiovascular Disease

Hydrotherapy treatments should be used with caution in individuals with cardiovascular disease, and there are several contraindications to consider. These individuals may be taking medications that modify how the body will respond to heat and cold and should be closely monitored. Heat should not be applied over artificial devices such as pacemakers or implantable defibrillators.

Elevated temperatures can cause increased demands on the circulatory system, including significant changes in local blood flow and changes in the size of blood vessels, heart rate, the volume of blood pumped from the heart, and blood pressure. High-temperature treatments such as sauna therapy, hot baths, and steam baths could be potentially dangerous in those with hypertension due to a temporary increase in blood pressure, and thus they should be avoided. Additionally, high-temperature treatments can ultimately reduce blood pressure, leading to fainting or dizziness in a person with low blood pressure.

The heart may not be strong enough for very high-temperature treatments in those with congestive heart failure (CHF), and these treatments should be avoided. In contrast, thermal vasodilation after warm-water bathing and low-temperature sauna bathing at 60°C for 15 minutes has been shown to improve cardiac function in those with CHF.[18]

Local hydrotherapy treatments are used with caution in those with varicose veins, and extreme hot and cold treatments should be avoided. Local hot and cold treatments should be avoided in those with phlebitis. When dealing with a vasoconstriction disorder, such as Raynaud's syndrome, local cold applications are contraindicated. Local hot applications are contraindicated in those with arteriosclerosis, a condition involving thickening, hardening, and loss of elasticity in the walls of the blood vessels.

In patients with coronary risk factors, there may be a risk of precipitating myocardial infarction (MI), or heart attack, when alternating heat exposure during sauna therapy is followed by rapid cooling during a cold-water bath.[19] Interestingly, animal studies have revealed that sauna therapy may serve as a noninvasive therapy for patients with MI because it attenuates cardiac remodeling after MI by improving coronary vascularity in the noninfarcted myocardium.[20]

Cancer

It is known that there are more than 100 types of cancer and that cancer can form almost anywhere in the human body. Individuals who are undergoing cancer treatment should have the approval of their physician before initiating hydrotherapy treatments. Local and whole-body hydrotherapy treatments may be useful for those diagnosed with cancer for reasons other than cancer treatment, such as for pain or relaxation purposes. However, because hydrotherapy treatments at the very least can have effects on the skin and place demands on the circulatory system, it is imperative to treat the whole person and assess where contraindications to treatment may arise.

Daily, brief cold-water stress over many months may enhance antitumor immunity and improve nonlymphoid cancer survival rate.[21] A hypothesis suggests that sudden ice-cold-water immersion can increase blood–brain barrier permeability, thereby increasing the mortality of neurovirulent infections. This could lead to immunotherapy development for some nonlymphoid cancers, including those caused by viral infections.[22]

Hyperthermic treatment of tumors can be traced back to the time of the ancient Greeks, and modern medicine has used hyperthermia as an adjunct cancer treatment in various settings. For example, research has demonstrated that hyperthermia, when added to chemotherapy and/or radiation, may positively affect treatment outcomes for patients with pancreatic cancer.[23] Research has also shown that hyperthermia can have a synergistic effect on the cell-killing effect of chemotherapy drugs and radiation.[24] Because of this, hyperthermia treatments should not be indiscriminately used in those receiving chemotherapy and/or radiation. A study on preventing alopecia caused by chemotherapy revealed that the use of a cold pillow compress could reduce hair follicle inhibition or damage caused by chemotherapeutic agents.[25]

Colon hydrotherapy is an option for colonoscopy preparation for colon cancer screening. A study showed that the quality of colon cleansing, overall tolerance, comfort, and convenience were significantly better for colon hydrotherapy compared with alternative preparations.[26] Colon hydrotherapy is contraindicated in those with cancer of the rectum or colon.

Diabetes

An estimated 30 million people of all ages—or 9.4% of the U.S. population—had diabetes in 2015, reaching a high of 25% among those aged 65 years or older.[27] Because of its prevalence, it's important to understand when hydrotherapy can safely be used for those with diabetes. Type 1 and type 2 diabetes mellitus are both associated with reduced ability to maintain core temperature during thermal stress. This has been related to impairments in the body's ability to dissipate heat, mediated via increases in skin blood flow and sweating during heat stress, as well as a reduced capacity to increase metabolic heat production and to decrease skin blood flow during cold stress.[28] Generally, warm- and neutral-temperature treatments are safer, and recommendations for hydrotherapy treatment should be approved by the patient's physician.

Acute complications of diabetes occur as a result of blood sugar levels that become either too high or too low. Chronic complications of the disease often include nerve damage and tissue damage from chronically elevated blood sugar levels. Cardiovascular disease can develop slowly in those with diabetes, particularly atherosclerosis, so high-temperature treatments such as hot saunas, hot baths, and local hot applications are contraindicated. When diabetes affects the smaller blood vessels of the legs and feet, these vessels may not dilate normally in response to heat. Heat applied to these areas leads to increased metabolism in the heated tissues, which would require more oxygen and other nutrients. When blood vessels cannot supply enough oxygen and other nutrients to the treated tissues, it can result in tissue death.

Another factor to consider is diabetic neuropathy, a nerve condition that causes, among other symptoms, a limited ability to sense pressure and temperature in the extremities. In this situation, the individual may not be able to discern if a hot application is burning the skin or if a cold application is freezing the skin. Therefore hot and cold applications are contraindicated.

Less-extreme-temperature treatments, such as alternating warm (102°F) and cold (55°F) footbaths, used under medical supervision, may help increase circulation and offer treatment for diabetic ulcers. Immersion in Dead Sea water at a temperature of only 95°F for 20 minutes was shown to produce significant reductions in blood glucose in those with type 2 diabetes.[29]

HYDROTHERAPY TECHNIQUES

The range of hydrotherapy treatments is vast, and the ways in which water is applied to the body to initiate a therapeutic response are only limited by the imagination of the therapist. Deciding on which treatment to use requires a thorough understanding of the principles of hydrotherapy, the health status of the patient, and the effects of hot and cold on the body.

Compresses

A compress is essentially the application of water at any temperature through a cloth medium or other compress material, wrung out and applied to the body. In the early 20th century, J. H. Kellogg described 12 types of compresses. They were classified according to their temperature, as (1) very cold; (2) cool or cooling; (3) warm or neutral; (4) hot; (5) very hot; (6) alternate (alternating very hot and very cold); (7) revulsive (short cold followed by prolonged very hot); (8) hot and cold (simultaneously); (9) hot and cold pack; (10) heating or stimulating (applying cold, protected by an additional material and prolonged until warmed by the body); (11) proximal compress; and (12) the irrigating compress.[30] While appreciating that each type of compress has its unique features, four basic types of compresses will be discussed: hot and cold compresses, warming compresses, and alternating hot and cold compresses.

A single compress is the use of only wet material applied to the body. A double compress, in comparison, consists of an initial wet layer covered by a water-resistant—or dry—material covering, such as wool. This double layering acts to prevent cooling by evaporation or radiation. When applied to specific areas of the body, compresses can be referred to by the area being treated (e.g., a throat compress or eye compress).

Cold Compresses and Packs

When a cloth is wrung from cold or ice water and applied to a particular area of the body, it is considered a cold compress. A cold pack, on the other hand, is made from crushed ice or produced commercially as gel packs that are cooled in a freezer. A cold pack requires almost no preparation and allows for more aggressive tissue cooling compared with a compress. Cold packs remain cold for a longer duration than a cold compress; therefore cold compresses are renewed frequently to achieve the desired cold effect. Additionally, cold packs applied for long periods have the potential to cause tissue damage, so treatment time should be limited to 15 to 20 minutes, and the skin should be occasionally monitored. Refer to Table 40.3 for a list of indications and contraindications for local cold applications.

The temperature and type of cold compress will depend on the area of the body being treated as well as the health status of the patient. Cold packs are often applied to larger, flat surfaces, such as the back, due to the limited ability to wrap them around irregularly shaped surfaces. Cold compresses are used more flexibly, can be applied over smaller or delicate surfaces such as the eyes or forehead, and are more suitable for clients who cannot tolerate very cold applications.

To elicit a more specific effect from a cold compress, a practitioner may choose to add certain chemical solutions to the compress, such as Epsom salts, essential oils, and a plethora of herbs. When herbs are used, they are steeped in water to produce a tea, and the compress material is then dipped into it, wrung out, and applied to the body. Adding essential oils to the water is much quicker than preparing herbs and requires an understanding of the effects of the oils on the skin.

TABLE 40.3 Indications and Contraindications for Local Cold Application

Indications for Local Cold Application	Contraindications for Local Cold Application
Muscle strains and joint sprains	Cold client or if the client has an aversion or sensitivity to cold
Support for an overheated individual	Impaired sensation or numbness in a given area
To reduce blood flow to an area of congestion	After eccentric exercises (resistance training)[59]
Acute and chronic low back pain	Poor circulation
To decrease local tissue metabolism via vasoconstriction	Peripheral vascular disease
To decrease muscle spasms	Open wounds
	Malignancy
	Acute asthma, sinusitis, and pleurisy
	Prolonged exposure with lymphedema
	Implanted medical device such as a cardiac pacemaker
	Uncontrolled hypertension
	Caution with children, the elderly, and those with a debilitating condition

Hot Compresses and Fomentations

A hot compress is the local application of moist heat to an area of the body to elicit a rise in local tissue temperature, improve local circulation, support muscle relaxation, and relieve musculoskeletal pain. A hot compress usually consists of a folded cloth dipped in hot water, wrung out, and applied to a surface of the body. Depending on the size of the area being treated, a washcloth, hand towel, or large towel may be used. A fomentation is a particular type of hot compress that delivers moist heat at a higher temperature for a longer period of time. It typically consists of many layers of folded towels, flannel, or other thick material that can absorb water and retain heat. A fomentation is often wrapped with a layer of wool felt to effectively hold heat and then by additional layers of towels to protect the patient's skin.

Similar to modifying a cold compress, a practitioner may choose to add certain substances to a hot compress to elicit a more specific effect. For example, a Thai herbal compress, consisting of Plai or Cassumunar ginger, turmeric, and camphor, may be used for osteoarthritis and muscle pain and can also be used as a treatment of choice to induce lactation.[31] Ground mustard plasters have long been used to warm muscle tissues and treat chronic aches and pains. Because mustard plasters are generally very hot, treatment time is often limited to 15 minutes, and the underlying skin should be checked frequently to avoid irritation or blistering. Castor oil packs can be used to increase local circulation of blood and lymph, relieve muscle and joint pain, soften scar tissue, and relax smooth muscle. Castor oil packs have been shown to effectively control symptoms of constipation.[32] Refer to Table 40.4 for a list of contraindications for local hot applications.

Warming Compresses

A warming compress consists of a cold compress covered by a layer of dry material—typically flannel or wool—that is worn for prolonged

TABLE 40.4 Indications and Contraindications for Local Hot Application

Indications for Local Hot Application	Contraindications for Local Hot Application
Poor local circulation	Sensitivity to heat or aversion to heat
To increase blood flow to an area	Impaired sensation or numbness in a given area
Menstrual cramping	Congestive heart failure
Nervous tension	Malignancy
Muscle soreness and tightness	Open wounds
Stiff and painful joints	Implanted medical device such as a cardiac pacemaker
Chilled client or chilled local area	Swelling and inflammation
To assist in reducing edema and removing waste products from an area of injury	Patients with diabetes: avoid local hot applications to legs and feet
Improve range of motion	Peripheral vascular disease
	Caution with children, the elderly, and those with a debilitating condition
	Pregnancy: avoid hot packs over the abdomen

periods until eventually warmed by the body. The top layer of dry material acts to prevent heat loss by evaporation and allows for the accumulation of heat. Also referred to as a cold double compress, a warming compress is used to improve local circulation, relax muscles, and relieve musculoskeletal pain. There is also a derivative effect that occurs when blood flow is increased as a result of warming the compress.

A popular example of a warming compress is the wet sock treatment. This treatment consists of dipping cotton socks in cold water, wringing them out completely, and applying them to previously warmed feet. Once the feet have been soaked in warm water for 5 to 10 minutes—or warmed by other means—the cold socks are applied. Cold socks can be placed in a freezer for several minutes to increase the intensity of the treatment if there are no contraindications to doing so. A pair of dry wool socks is then applied to cover the cold socks and left overnight to be warmed by the patient. The socks are often dry by morning. The wet sock treatment is commonly used for sinus congestion, upper respiratory infections, bronchitis, cough or sore throat, headaches, and ear infection.

The temperature of the initial cold application will depend on the treatment location and the health status of the individual. The same basic guidelines for cold compresses apply for warming compresses. Because a colder application can elicit a stronger secondary reaction to the cold, caution is taken with weak or debilitated individuals who are unable to generate a strong secondary response.

Alternating Hot and Cold Compresses

Alternating hot and cold compresses—or local contrast treatments—are one of the most efficient ways to improve blood flow through an area of the body. This procedure is a succession of compresses of hot and cold that combine the effects of both the hot and cold compress. The hot compress is applied for up to 3 to 5 minutes, alternating with a cold compress for 30 to 90 seconds. As a general rule, the duration of a hot application should be greater than that of a cold application and cover a slightly larger area. Additionally, the procedure should always begin with the hot compress and end with the cold compress. It is also repeated three or more times.

The heat applied initially during an alternating compress treatment causes dilation of the cutaneous vasculature. A subsequent cold application will cause vasoconstriction followed by vasodilation, allowing for marked improvements in blood flow to the treated site. This can be particularly helpful to stimulate healing and reduce swelling in a joint after injury or surgery. By increasing blood flow, contrast compresses can help relieve pain. For example, alternating compresses were shown to improve foot functionality scores in individuals with heel pain.[33]

Baths

Baths are full or partial immersions of the body into various temperatures of water to produce a wide range of therapeutic effects. With whole-body immersions, the body—except for the head—is immersed in water, which can be performed in a standard bathtub. In a partial-body immersion, only a particular part of the body is immersed in water using a variety of basic containers, such as a bowl, a bucket, or standard bathtub. Additives can be used, such as Epsom salt, sea salt, baking soda, oatmeal, mustard powder, and herbs, to elicit a more specific effect from the bath.

Hot Baths

Depending on the tolerance of the individual as well as the condition being treated, hot baths will generally range in temperature from 100°F to 106°F and are administered for 20 to 60 minutes or longer. Hot baths are used for many types of musculoskeletal pain and to stimulate the immune system, induce sweating, and cleanse the body, among other purposes. They encourage muscle relaxation, enhance circulation, and are efficient at raising core temperature. Hyperthermia treatments are useful for managing infections and can be used as adjunct cancer support. When treatments are limited to a brief period of only a few minutes, peripheral vasodilation may promote heat loss and thereby reduce fever.

The duration of a local hot immersion is typically 15 to 20 minutes and will depend on the condition being treated, as well as the tolerance of the individual. One type of local hot immersion, the hot foot bath, has been shown to relieve fatigue and insomnia in patients undergoing chemotherapy.[34] It can be used as an effective intervention for the management of sleep disturbances after traumatic brain injury.[35]

When performed at home, whole-body hot immersions should be limited to 30 minutes and kept to a temperature of 104°F. An individual should have assistance when performing hyperthermia treatments in a home setting. When performed in a clinical setting, more aggressive treatment can be administered under close supervision. The water temperature should be kept to 106°F, and the duration of treatment should be limited to 60 minutes.

Contraindications to whole-body hot immersions include seizure disorders, pregnancy, cardiovascular disorders, diabetes, loss of sensation, intolerance to heat, multiple sclerosis, and drug or alcohol ingestion. Prolonged hot immersions should be avoided in children, the elderly, weak or anemic individuals, those with severe organic disease, and those with a tendency to hemorrhage.

General precautions should be followed when performing hyperthermia treatments. Patients should wait at least an hour after eating a meal before receiving treatment to avoid symptoms such as nausea and vomiting. Likewise, if a client has gone too long without food, symptoms of hypoglycemia may arise. The individual should be adequately hydrated before, during, and after treatment. Additionally, because hot-water immersions may induce hypotension, the individual could become lightheaded and lose his or her footing. Clients should be instructed to rise slowly from the bath with assistance close by. Headaches may result from hyperthermia treatments. Applying cold compresses to the head and face early and throughout the treatment can prevent this. Hyperventilation sometimes occurs with hot-water

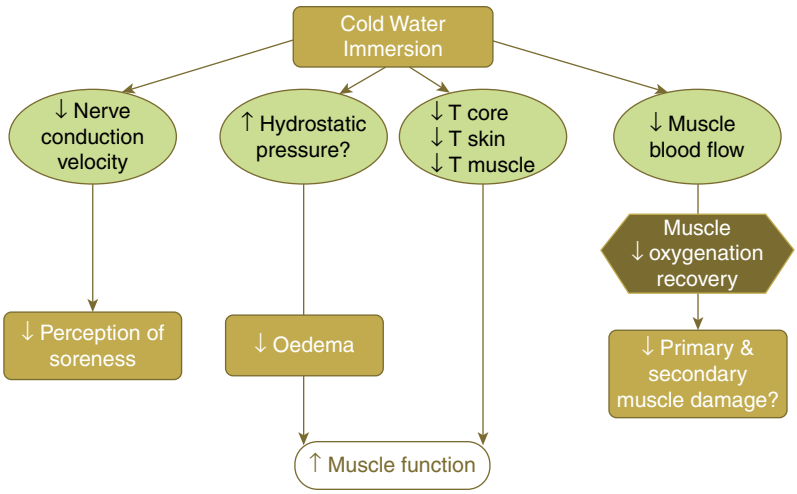


Fig. 40.1 Physiological effects of cold-water immersion. *T,* temperature. (From McGorm H, Roberts LA, Coombes JS, Peake JM. Cold water immersion. *Aspetar Sports Med J.* 2015;4[1].)

immersions and, although rare, may lead to early tetany from respiratory alkalosis. This can be prevented by monitoring the individual's respiratory rate and offering breath coaching as needed.

Neutral Baths

The neutral bath is a head-out, whole-body immersion bath given at a temperature close to that of the body and exerting as little thermal effects as possible. A minor variation in temperature by only a few degrees could result in an altered therapeutic response. Neutral baths are taken for a duration of 15 minutes to 4 hours, although warm water will need to be added after a period of about 20 minutes to maintain the appropriate temperature.

Neutral baths have been shown to lower heart rate, blood pressure, and cortisol levels while preserving metabolic rate and body temperature and increasing urine output.[36] Neutral baths have a calming effect on the nervous system and are indicated for those with insomnia, anxiety, nervousness, and pain. They may be a useful aid in detoxification treatments due to their effects on urine output, as well as an adjunct treatment for peripheral edema. Caution should be taken with those with cardiovascular disease and kidney disease, and the duration of treatment should be limited or treatment avoided in those with eczema. The air in the room should be kept warm, and care should be taken so that the individual does not become chilled after treatment.

Cold and Contrast Baths

A cold, full-body immersion bath is used at a temperature below 60°F and is often limited to a duration of only a few minutes. It generally follows a hot, full-body immersion to reverse the vasodilation of skin after a heat treatment. A meta-analysis concluded that individuals with exercise-induced hyperthermia were cooled twice as fast by cold-water immersion as by passive recovery.[37] Such an invigorating treatment should only be attempted by those in robust health. Cold baths are contraindicated in the very young and the very old; weak and debilitated patients; those who are chilled or have an aversion to cold; and those with kidney disease, cardiovascular disease, and hyperthyroidism.

A local cold immersion bath can be used therapeutically to reduce musculoskeletal pain or swelling in a particular area of the body, such as the arm, hand, or foot. Compared with the effects of using an ice pack or ice massage, a local cold-water immersion is most indicated for inducing therapeutic effects associated with the reduction of motor nerve conduction (Fig. 40.1).[38]

Contrast baths combine both hot- and cold-water immersions and are effective for stimulating blood flow to the skin and decreasing congestion in the body. Similar to other contrast therapies, treatment begins with hot and ends with cold. For local immersions, 3 to 5 minutes of hot is followed by 30 to 90 seconds of cold. Full-body immersion often requires at least 10 minutes of hot, followed by 30 to 90 seconds of cold, and is most appropriate for those in robust health. Local contrast immersions require only a few small buckets and access to hot and cold water, allowing for both home and clinical use. Full contrast immersions are typically available in a spa or clinical setting because the process necessitates two large tubs to accommodate a hot and cold immersion. Contrast water therapy can also be performed easily at home in the shower. Ending a daily hot shower with a full-body cold rinse is tonifying to the circulatory and immune systems. In a long-term prospective study, the frequency and intensity of common colds were reduced after volunteers were regularly treated with contrasting hot and cold showers over a 6-month period.[39]

Sitz Bath

A sitz bath is a partial immersion bath of the pelvic region that has long been used to treat afflictions of the abdomen, pelvis, perineum, and low back. It is carried out in a specifically designed tub—older models resemble a chair-shaped bathtub and are made of porcelain; newer models are designed to sit atop the toilet or are advanced enough to contain their own water heater. The sitz bath can be effectively taken in a standard bathtub or even a plastic tub large enough to immerse the pelvic region. A sitz bath may be taken hot, cold, neutral, or with contrasting hot and cold, and preparations such as Epsom salt, herbs, and essential oils can be added.

Hot sitz baths are analgesic and stimulate pelvic circulation. They have been used to help relieve low back pain, dysmenorrhea, spastic constipation, cramps of the uterus or ureters, ovarian or testicular pain, sciatica, and after cystoscopy or hemorrhoidectomy. After sphincterotomy for anal fissure, the sitz bath provided significant relief of anal burning and increased patient satisfaction score.[40] The hot sitz bath relieves urinary retention in patients after hemorrhoidectomy, with spontaneous micturition more likely at higher water temperatures.[41] Hot sitz baths can be used to raise core temperature as well as to create a fluid-shifting, or derivative, effect. Hot sitz baths significantly reduced urethral stricture after transurethral resection of the prostate.[42] The hot sitz bath has a duration of

3 to 10 minutes and is followed by a cool sponging or spray to the area. A hot footbath may be taken concomitantly, ending with cold water or spray to the feet. Cold compresses may be applied to the forehead or back of the neck during treatment. Hot applications to the pelvis are contraindicated during menses, in pregnancy, and in cases of acute inflammation.

A neutral sitz bath is typically taken for a longer duration of 15 minutes to 2 hours. Warm water may be added to maintain temperature as needed. It has a calming feature, appropriate for acute inflammation, and may offer relief of pruritus. A hot footbath is optional with a neutral sitz bath, and a blanket placed over the patient's upper body may be used to prevent chilling.

A cold sitz bath is used primarily for its tonifying effects—increasing the tone of the smooth muscle of the bladder, uterus, and colon. It offers support for atonic constipation, incontinence, metrorrhagia, subinvolution of the uterus, and enuresis. Cold sitz baths significantly reduce edema[43] and pain after an episiotomy,[44] and aromatherapy could offer perineal wound healing in the postpartum period.[45] The duration of a cold sitz bath is typically 30 seconds to 8 minutes. The patient should be adequately covered to avoid chilling. The cold sitz bath often follows the hot sitz bath. In this case the level of the hot water should be located at least 1 inch above the level of the cold water, which helps prevent chilling.

Contrast sitz baths are a combination of hot and cold sitz baths. The duration of the hot sitz bath is commonly 3 minutes, followed by 30 seconds in the cold sitz bath. The procedure is repeated three times and always ends with cold. Contrast sitz baths are useful for increasing pelvic circulation and smooth muscle tone in the pelvic region. They are indicated for chronic pelvic inflammatory disease, chronic prostatitis, postpartum care, atonic constipation, anal fissures, and after surgical procedures such as hemorrhoidectomy.

Cold Friction Rubs

A cold friction rub is a whole-body friction treatment performed with a cold, wet coarse bath mitt or washcloth and applied in sequential order to the body. It may be used to enhance the effects of another cold treatment, after a heating treatment to prevent the client from becoming chilled, or as a stand-alone treatment to stimulate blood flow to the skin and as a general tonic. Cold friction rubs are especially supportive for immobile or bedridden clients who are not receiving circulatory stimulation through exercise.[46]

The client lies supine, undressed to his or her level of comfort and draped, except for the area being treated. The extent of water saturation of the mitt depends on the cooling effect desired and the health status of the client. The area of the body is treated until the skin becomes reddened. Each area of the body is treated, dried, and draped to retain the heat produced by the body.

This treatment is contraindicated for those who are chilled; have a sensitivity to cold; or have infected, damaged, sunburned, or sensitive skin.

Constitutional Hydrotherapy

Developed in the early 20th century by Dr. O. G. Carroll, the constitutional hydrotherapy method incorporates the use of alternating hot and cold compresses, along with electrical stimulation via sine-wave therapy. Also acting as a warming compress, this therapy is useful for a vast array of conditions; because of its gentle nature, it has very few contraindications when used under the care of a trained naturopathic physician or qualified hydrotherapist.

The therapy begins with the application of two hot towels applied to the patient's torso, extending from the collarbone to the hips. The hot towels are then covered by a wool blanket and left in place for 5 minutes. If the patient is chilly, he or she may need more blankets. At the 5-minute mark, another fresh hot wet towel is applied quickly to the patient's torso, followed immediately by a cold wet towel. The cold towel is left in place for 10 minutes while sine-wave pads are applied to the patient's lower thoracic region and abdomen. The cold towel is checked to ensure that at least the middle portion of the towel is warmed. The patient then turns over, and the sequence is repeated again (two wet hot towels to the back, covered by wool for 5 minutes, followed by a wet cold towel, covered by wool for 10 minutes or until warmed). Dr. Letitia Dick-Kronenberg, ND, is careful to point out that a patient is not to be wrapped with a sheet before applying the wool blanket.[47] She explains that the sheet chills a patient and that the wool blankets are simply applied on top of the towels.

When the hot compress is initially applied, it acts to dilate the superficial vasculature, increasing blood flow to the surface of the body. Once the cold towel is applied, it causes vasoconstriction, shunting the blood back to vital organs. The cold compress covered by wool is warmed by the recirculation of blood back to the surface of the body. The addition of sine-wave therapy allows for a much quicker treatment than a traditional warming compress treatment. The constitutional hydrotherapy treatment takes approximately an hour and always ends with the cold compress.

Constitutional hydrotherapy is used to boost the immune system and bring balance to body functions. A pilot study completed in 2008 at Bastyr University suggests that constitutional hydrotherapy can be safely administered to HIV+ adults and revealed a statistically significant increase in energy as well as increased physical functioning and quality of life.[48] Research by Kate Wiggin, ND, at the National College of Naturopathic Medicine showed a posttreatment increase in leukocyte circulation that remained elevated for 2 hours.[49] Research conducted by Mark Carney, ND, and Bryan McConnell, ND, demonstrated a host of changes in the body after constitutional hydrotherapy treatments. These include a decrease in body fat, increased vitality, decreased pain, and decreased cholesterol levels, to name a few.[50]

Wet Sheet Pack

The wet sheet pack procedure involves wrapping the undressed patient in a cold wet sheet and then covering the patient with dry blankets to insulate, control body temperature, and regulate moisture evaporation. It is recommended to use cold water between 15°C and 20°C for soaking the sheet, and the wet sheet pack must come into close contact with all areas of skin when applied. Dry blankets must be snugly wrapped at the neck and shoulders down to the feet to prevent any airflow and eliminate patient chilling.

The physiological effects of a wet sheet pack are determined by the duration of the treatment and the number of dry covering blankets to control evaporation. There are several stages of heat generation during the wet sheet pack. The first temperature effect is the cooling stage, lasting 5 to 15 minutes as body heat is removed by evaporation. The cooling evaporation stage is helpful for reducing fevers. In the cooling stage, the patient's body is attempting to warm the sheet back to body temperature; it is complete when the patient no longer perceives the wet sheet as cold. The degree of wetness in the sheet can be tailored for individual patient needs; weak and frail patients will require a well-wrung-out sheet, whereas vital patients can benefit from the toning effect of added sheet wetness.

The second neutral stage begins when the sheet reaches or slightly exceeds the temperature of the skin. Here, the property of derivation is acting upon cerebral blood flow by lessening the amount of blood in the brain, which induces a sedative-like stage and tends to induce sleep. The neutral stage can last from 15 minutes to an hour, depending on the vitality of the patient and how many dry covering blankets are used in the wrap. The more dry coverings, the sooner the completion of this stage.

The third stage is the heating and sweating stage. Here, the sheet pack has been warmed, and the rise in skin temperature causes superficial blood vessels to dilate and draw blood away from congested organs. The heating stage can last from 15 minutes to an hour.

Finally, as the patient's body temperature increases, the patient begins to perspire and eliminate toxins through various detoxification processes. Toxins such as those from alcohol, tobacco, coffee, and processed and refined foods can be removed via the diaphoretic process. Febrile patients will reach the elimination phase much sooner than the weak or frail, and all patients will require plenty of fluids (water, electrolytes, teas, etc.) to maintain adequate hydration. The detoxification phase can last up to an hour and should be promptly discontinued if the patient gets chilled or uncomfortable. Upon completion of the wet sheet pack, the patient's skin should be frictioned quickly with a dry towel before the patient redresses.

The wet sheet wrapping sequence and dry blanket applications are illustrated in detail in *Lectures in Naturopathic Hydrotherapy* by Boyle and Saine.[5]

Indications for the wet sheet pack include the following: fevers, insomnia, mania, delirium, restlessness, nervous exhaustion, hypopepsia, detoxification from substances, gout, bronchitis, common colds, influenza, jaundice, measles, scarlet fever, constipation, hepatic congestion, and splenic congestion. There are very few contraindications, but caution should be taken in patients with diabetes, poor circulation, or skin eruptions and in very weak or ill patients.

Colon Hydrotherapy

Colonic hydrotherapy is a healing tradition that has been used by nearly every culture across the earth. The ancient healing tradition of using water to cleanse the colon has been used in India for more than 5000 years in the Ayurvedic medicine methods. Worldwide, many prescriptions for enemas were found on Babylonian and Assyrian tablets. The earliest record of using an appliance to administer an enema is from in the late 14th century. These early methods used tubes or cannulas made of bone, reed, or metal attached to a bladder or sleeve made from silk cloths, animal skin, or an ox bladder. Enemas are documented to have been used by Chinese, Hindu, Greek, Roman, Sumerian, and African cultures.

Modern colonic equipment was introduced into the United States by Kellogg, Dierker, and Vattenborg in the early 1900s. These early machines used large amounts of water to flush the colon. Current equipment uses a disposable plastic speculum and tubing to avoid and omit any danger for cross-contamination between patients. Water pressure, flow, and temperature can be manipulated to maximize therapeutic goals and patient comfort.

Effects of Colonic Hydrotherapy

The effects of colonic hydrotherapy include the following:

1. Removal of solid fecal matter from the colon. Solid fecal material can cause distension of the colon and contribute to prolapse. Hardened fecal material interferes with motility and results in constipation. Decreased physical stress by removal of such material will allow the colon to maintain optimal anatomical position, function, and tone. Pathogenic bacteria and parasites can be removed, thereby relieving the imbalance of putrefactive organisms that contribute to dysbiosis. Also, undigested foodstuffs in the colon will result in overactive fermentation, putrefaction, and rancidification of macronutrients, which encourages the growth of pathogenic bacteria and decreases the growth of healthy bacteria.[51]
2. The removal and dilution of toxins during colonic hydrotherapy allows for a decrease in the overall toxic load on the body through the enterohepatic recirculation. Elimination of toxic metabolites serves to prevent colon cancer and helps increase the growth of beneficial health-promoting bacteria. The removal of toxic compounds produced by colonic bacteria decreases the risk of colon cancer formation due to the dilution and removal of such compounds.
3. Cellular detoxification is augmented with colonic irrigation. Cells will readily release their waste products in a hypotonic solution of water. Cells also become oxygenated through the colon wall by dissolved O_2. Several forms of oxygen can be added to the water, such as ozone or H_2O_2.
4. The water temperature will affect the muscular function of the colon. Warm water between 98°F and 102°F will relax the muscles and decrease spasm. Cool water between 90°F and 95°F will stimulate the muscles to contract to improve tone. This is very helpful with atonic constipation. Retraining the muscles of the colon will address constipation and produce normal daily formed bowel movements.

Indications for Colon Hydrotherapy

Many systemic diseases can be relieved by the removal of toxic material from the colon. Headaches, premenstrual syndrome (PMS), arthritis, dermatitis/eczema, and neurocirculatory disorders are all aggravated by bowel toxemia.[51] The symptoms of PMS are associated with high levels of unconjugated estrogen, which are hepatotoxic. Colon irrigation detoxifies the liver and allows for normal liver function through lavage of toxins via the enterohepatic circulation. Detoxifying the colon takes the burden of detoxifying the entire system off the liver. A liver overburdened by diseases such as cirrhosis, hepatitis, or fatty infiltration; chronic exposure to toxins from alcohol, drugs, solvents, and heavy metals; or chronic exposure to endotoxin is unable to handle the full load, and toxins therefore spill over into the systemic circulation. When toxins enter the bloodstream, they travel to other organ systems, causing tissue damage and destruction usually in the weakest organ system first. This theory to explain disease was coined "autotoxemia" by Dr. John Henry Tilden (1851–1940) in the book *Toxemia Explained,* first published in 1926.

When the liver is overburdened from exogenous or endogenously produced toxins, we see the skin become burdened to eliminate the toxins, and conditions such as acne, eczema, and psoriasis result.

Many autoimmune conditions, such as rheumatoid arthritis (RA), multiple sclerosis (MS), and ankylosing spondylitis (AS), have an overgrowth of pathogenic bacteria in susceptible individuals. Gut bacteria cross-react with genetic markers on the cells of patients with RA, MS, or AS, which can trigger the onset of symptoms. Hashimoto's thyroiditis, Reiter's syndrome, and iritis have also been linked to cross-reactivity with pathogenic gut bacteria.[52]

Colon hydrotherapy eliminates bile from the enterohepatic circulation. Bile removes toxins; therefore overall detoxification is supported. Bile is also acidic in nature, and its removal promotes alkalinity in the tissues, which is health promoting. Colon hydrotherapy can also increase the rate of bile synthesis and secretion by 20%.

Bowel tonification through colon hydrotherapy will address chronic constipation, atonic colon, spastic colon, irritable bowel syndrome (IBS), and neurogenic colon from plegic traumas.

Colon irrigation is effective during fasting protocols, during drug and alcohol detoxification, and in preparation for a colonoscopy procedure.

Contraindications for Colon Hydrotherapy

Contraindications include acute ulcerative colitis, acute Crohn's disease, acute abdomen/appendicitis, colorectal cancer, congenital megacolon (Hirschsprung's disease), acute diverticulitis, and neurogenic

constipation from diabetes. Due to the increasing levels of fluids during colon irrigation, other contraindications include hypokalemia, congestive heart failure (CHF), kidney disease/renal failure, active hemorrhage in the gastrointestinal (GI) tract, and uncontrolled hypertension (HTN). The use of colonic irrigation is avoided in acute stroke; during pregnancy or nursing; and in patients with an aneurysm, bowel perforation, severe anemia, acute abdominal hernia, or recent colorectal surgery.

It is advised to take precautions when using colon hydrotherapy in the deficient, fragile, elderly, or very young patient. Patients with diverticulosis, fecal impaction, long-term steroid use, symptomatic painful fissures or hemorrhoids, hepatitis, and AIDS can be directed to use enemas at home for the treatment of these conditions as a safeguard against aggravation of the condition.

Additives for Implantation

Herbal solutions are common additives for rectal use during colon hydrotherapy. Chlorophyll or wheatgrass will provide nutritive, anti-inflammatory, antioxidant, and soothing effects to help heal the cells of the colon. Chlorophyll provides an alkaline environment and provides electrolytes. Solutions with sodium, potassium, and magnesium will support electrolyte balance, healthy hydration, and smooth muscle contraction (peristalsis).

Retaining coffee implants for 10 to 15 minutes during colon hydrotherapy promotes bile elimination via gallbladder emptying. Organic coffee with higher amounts of caffeine and palmitic acid are used in the Gerson and the Kelley programs to address cancer and chronic disease. In addition, theophylline and theobromine (two other chemicals in coffee) dilate blood vessels and counter inflammation of the gut. Palmitates enhance the enzyme system responsible for the removal of toxic free radicals from the serum, and the fluid of the colonic then stimulates the visceral nervous system to promote peristalsis and the transit of diluted toxic bile from the duodenum and out the rectum.

All the blood in the body passes through the liver every 3 minutes; therefore "colon irrigation represents a form of dialysis of blood across the gut wall."[53]

It has been clinically proven that the compound berberine from goldenseal and Oregon grape provides full-spectrum antibiotic benefits in the treatment of infections of the mucous membranes and parasites while also helping decrease inflammation of the gallbladder and liver. A tinctured berberine implant will have direct action on *Staphylococcus* spp., *Streptomyces* spp., *Chlamydia* spp., *Pseudomonas* spp., *Escherichia coli, Giardia lamblia,* and *Candida albicans*.

Garlic has also been proven to provide broad-spectrum antimicrobial effects against many bacteria, viruses, parasites, and fungi. Garlic is also effective against roundworms and hookworms.

Inflammatory conditions of the colon can benefit from demulcent herbal implants, such as slippery elm, marshmallow root, licorice root, comfrey, and plantain, to help heal and soothe the mucous membranes.

Astringent herbal implants will help dry, tone, and constrict mucous membranes through various phytochemicals, of note for their rich tannin content. Goldenseal, yarrow, bayberry, burdock, myrrh, and yellow dock are used to alleviate various digestive disorders.

Antispasmodic herbs are useful to support normal, rhythmic colon peristalsis; chamomile, catnip, and peppermint will benefit spastic constipation.

Rectal oil infusions of sesame, flax, or olive oil will aid in the removal of hard, retained stool or impacted feces, as well as support dry mucous membranes resulting from many pharmaceutical medications.

Practical Procedural Techniques

Before colon irrigation, it is important to have the patient fully empty the bladder. Then the patient is directed to lie in the lateral decubitus position, facing away from the colon irrigation equipment for speculum insertion. Single-use sterile specula and flexible tubing come in various sizes (adult and small child) and shapes (one or two pieces); most adult patients will require a single-piece speculum. Patients with hemorrhoids or degrees of uterine or bladder prolapse will require a two-piece speculum for comfort of insertion and to minimize tissue prolapse interference during releases. The insertion of the speculum should not hurt if it is properly lubricated with organic coconut oil or sterile lubricating jelly. A slow, steady insertion with deep, full, open-mouth breathing will help relax the internal and external anal sphincters. Once the speculum has been inserted, most patients will benefit from assistance turning on their back for the remainder of the therapy. A supported supine position will allow the therapist to assess the placement of abdominal organs/viscera and the level of congestion, bloating, or tightness of the tissues. A pillow should be behind the head to prevent any neck strain, and a bolster should be placed under the knees to relax the abdominal muscles and hip flexors. Always assess the patient's abdomen with palpation to establish a baseline and identify any areas of tenderness, gas, hardness, or fascial restrictions before beginning the water flow.

The average time for a colon hydrotherapy session is 45 minutes to 1 hour from start to finish. There are several options for water flow into the patient; gravity fills are very gentle and easily tolerable because they derive pressure from the force of gravity. Water is flowing from a height above the level of the patient to allow for 1 psi of natural water pressure.

Pressurized fills have an upper limit for the colon of 2 psi; this safeguard is built into current technology and equipment. Pressured fills can benefit patients who require a more vigorous treatment or when gas or stool clogs the speculum. Most patients release best with gravity fill cycles and pressurized release cycles. The goal is to maximize the efficacy of the water to enter the colon without overwhelming the patient. Most fills will take about 1 to 3 minutes or around 3 to 4 quarts of water to stimulate releases. The client could experience abdominal pressure or resistance as the water is pushing past gas, fecal material, or flexures of the colon; deep breathing and tension relaxation will help should that occur. When the release valve is opened, abdominal massage release techniques and nervous system support will optimize the expulsion of fecal and waste water.

Some patients may benefit from side-lying during the entire colon hydrotherapy session. Side-lying will assist the release of trapped gas and is also helpful in obese patients with tissue requiring more support to allow for the speculum to stay inserted and the tubing to be unobstructed.

If a patient is experiencing water leaking during the fill and release cycles, this is usually due to poor rectal tone or a degree of sigmoid prolapse; side lying and Kegels with cooler water fills will help support the seal between the speculum and rectum.

Treatment Analysis and Expectations

Possible patient reactions during colon hydrotherapy include nausea, abdominal cramping, dizziness, and hot flashes or chills. These reactions are all considered normal and can be moderated by coached breathing, frequent verbal check-ins, pelvic tilting adjustment, and diaphragm or chakra releases. Autonomic system discharges can occur and present with flushing of the skin, sweating, clamminess, muscle fasciculation, or emotional releases. The use of essential oils on the soles of the feet, over the liver, on the chest, or for inhalation will assist in mitigating nervousness and supporting emotional balancing.

The equipment observation tube allows the physician to interpret the fecal material released. Fresh, well-formed stool is normally yellow to light brown in color. Darker brown or black stool indicates prolonged retention or excessive bile secretion. Small, round, hard balls of stool are seen in all cases of constipation. Black, tarry stool can indicate the presence of blood from the upper bowel or ingestion of iron supplements or bismuth. A greenish color can be seen during gallbladder flushes when the bile is flowing and being released quickly; gallstones are often seen. Pale gray stool indicates impaired bile excretion, often found with liver or gallbladder disease. Bright red blood is observed with acute hemorrhoid or colon cancer, whereas pinkish-red waste and water is seen after the consumption of beets. Mucus-coated stool indicates inflammatory conditions such as inflammatory bowel disease (IBD), parasitical infections or dysbiosis, stress, or food sensitivities. Greasy stools and tubing indicate improper fat digestion, whereas foam in the tube indicates excessive fermentation, such as what is seen with bacterial imbalances or overgrowth.

Sauna

A Finnish proverb reads: "The sauna is Finland's medication and a poor [person's] apothecary."[54] The word *sauna* often indicates a bathhouse or spa in many parts of the world. Saunas have been an integral part of Finnish tradition for 2000 years, spreading throughout all of Europe and North America. Early saunas were made from natural materials such as wood, stone, clay, skins, and cloth. The heat source of these saunas was rocks that had been placed in a fire or water that was heated by fire.

With the advent of modern technology came new ways of constructing heat therapy besides a wood-burning fire. Other types of saunas include those heated by gas-burning stoves, electric light bulbs, electric heaters, smoke, steam heat, and infrared energy.

Conventional saunas can be divided into two types: dry heat or steam applied to the entire body inside a wood-paneled cabinet, room, or chamber. Dry-heat saunas and steam rooms will raise the core body temperature, induce sweating, and increase the heart rate. Their effects on the respiratory tract differ; breathing steam room air provides a 100% humidity level, which soothes the mucous membranes of the upper nasal passages, throat, and sinuses and lower respiratory tree, whereas dry-heat saunas are between 10% and 20% humidity.

Saunas will increase epithelial circulation from 5% to 10%, becoming 50% to 70% of the cardiac output, whereas blood flow to internal organs decreases.[55] The increase in skin circulation promotes the healing of wounds, minimizes scar tissue, and aids the recovery of muscle tissue from trauma or exercise. The increased circulatory effect, along with the opening of epithelial cells, will provide a way for toxins to be removed from the body system. Toxins removed by saunas include solvents, pesticides, insecticides, heavy metals, phenols, formaldehyde, bisphenol A (BPA), polychlorinated biphenyls (PCB), and volatile organic compounds (VOCs). Naturopathic physicians should incorporate the use of saunas into any detoxification or weight-loss program.

Saunas can decrease rheumatic pains and increase joint mobility in patients with arthritis, and they can provide relief in chronic pain syndromes such as fibromyalgia. Saunas will also relax spastic or stiff muscles.

The hemodynamic effect of sauna bathing has been documented to support the lowering of systolic and diastolic blood pressure, improved cardiac ejection in congestive heart failure, and enhanced pulmonary function to alleviate asthmatic conditions. Cardiac output increases by 60% to 70% in relation to the increase in heart rate, whereas cardiac stroke volume does not change.[56] Contraindications to sauna therapy include unstable angina pectoris, recent myocardial infarction (MI), and severe aortic stenosis.[57]

A modern technology called infrared sauna was invented in Japan in the mid-1960s. Infrared saunas raise the body temperature directly via radiant heat rather than heating an entire room. The use of far-infrared rays invigorates cells, promoting cellular metabolism and renewal. Far-infrared radiation saunas transmit a specific bandwidth of between 5 to 15 microns to human body cells via a process called cellular resonance. This absorption supports the release of waste products and fat-soluble toxins from the body's tissues (Fig. 40.2).

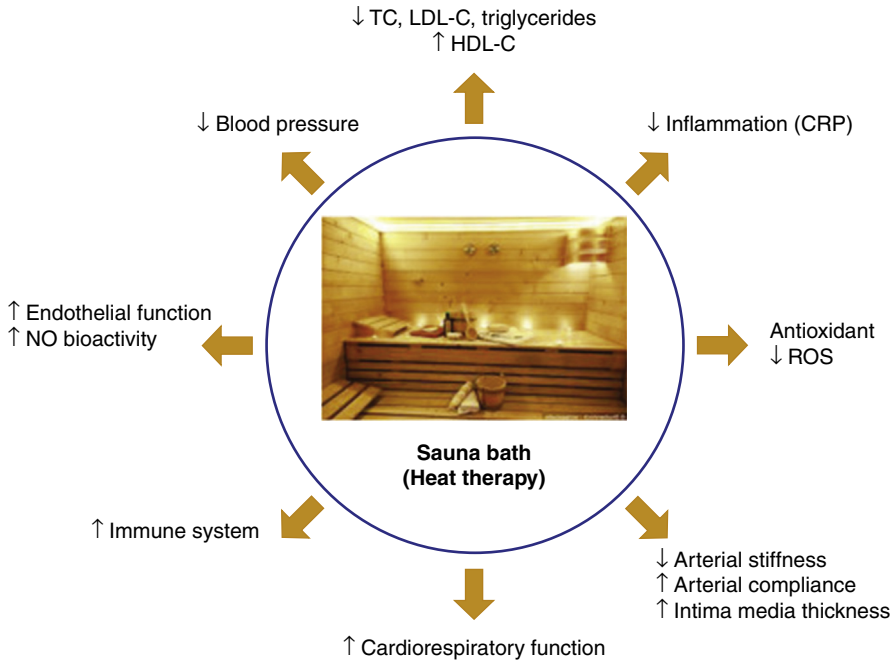


Fig. 40.2 Pleiotropic effects (proposed mechanistic pathways) of Finnish sauna baths. *CRP,* C-reactive protein; *HDL-C,* high-density lipoprotein cholesterol; *LDL-C,* low-density lipoprotein cholesterol; *NO,* nitric oxide; *ROS,* reactive oxygen species; *TC,* total cholesterol. (From Laukkanen JA, Laukkanen T, Kunutsor SK. Cardiovascular and other health benefits of sauna bathing: a review of the evidence. *Mayo Clin Proc.* 2018;93[8]:1111–1121.)

Local Steam Inhalation

When water is heated and converted to steam, the vapor consists of minute water droplets in the air that can be inhaled to warm and relax the respiratory tract. Steam inhalation will liquefy mucus secretions, making them easier to expectorate from the sinuses and lung passages. Spasmodic cough, bronchitis, and asthma can be relieved through the relaxation of the bronchial tree and increased circulation. Steam inhalation is contraindicated with CHF or any severe compromise to the cardiovascular system due to the potential for breathing difficulty with steam.

SUMMARY

This chapter provides an overview of the history of hydrotherapy and its foundational role in naturopathic medicine today. The information provided here serves to alert and inform both the physician and patient of the benefits provided by hydrotherapy, any cautions or contraindications, and the supplies and equipment needed to apply it in a safe manner. Understanding the physiological effects of water and following the general guidelines for hydrotherapy techniques allow for the stimulation of the body's vital force and immune system to facilitate the natural healing process. Literature demonstrating scientific, evidence-based applications of water continues to shed light on this long-standing, versatile, and powerful healing modality.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Association of Accredited Naturopathic Medical Colleges. 6 accredited naturopathic programs across 7 North American campuses. https://aanmc.org/naturopathic-schools/. Accessed August 13, 2019.
2. Hippocrates. Hippocratic writings. In: *The Great Books*. Chicago: William Benton; 1952.
3. Kellogg JH. Should the colon be sacrificed or may it be reformed? *JAMA LXVIII*. 1917;30:26.
4. Lindlahr H. *Nature Cure*. 20th ed. Holicong, PA: Wildside Press; 1922.
5. Boyle W, Saine A. *Lectures in Naturopathic Hydrotherapy*. Sandy, OR: Eclectic Medical Publications; 1988.
6. Dick-Kronenberg L. *The Ultimate Text in Constitutional Hydrotherapy: A 100 Year Tradition of Clinical Practice*. Spokane, WA: The Carroll Institute of Natural Healing; 2012.
7. Baruch S. *The Principles and Practice of Hydrotherapy: A Guide to the Application of Water in Disease for Students and Practitioners of Medicine*. New York: William Wood & Co; 1899:1–3.
8. U.S. Geological Survey. Heat capacity. https://water.usgs.gov/edu/heat-capacity.html. Accessed August 13, 2019.
9. Bowen IS. The ratio of heat losses by conduction and by evaporation from any water surface. *Phys Rev*. 1926;27.
10. Alexiou S. The effect of water temperature on the human body and the swimming effort. *Biol Exerc*. 2014;10(2). https://www.biologyofexercise.com/images/issues/1022.pdf. Accessed August 13, 2019.
11. Smith CJ, Johnson JM. Responses to hyperthermia. Optimizing heat dissipation by convection and evaporation: neural control of skin blood flow and sweating in humans. *Auton Neurosci*. 2016;196:25–36.
12. Contraindication. *Farlex Partner Medical Dictionary*. https://medical-dictionary.thefreedictionary.com/contraindication. Accessed February 5, 2018.
13. Busch V, Magerl W, Kern U, et al. The effect of deep and slow breathing on pain perception, autonomic activity, and mood processing—an experimental study. *Pain Med*. 2012;13(2):215–228.
14. American College of Nurse-Midwives. *Hydrotherapy During Labor and Birth*; 2014. Accessed http://www.midwife.org. Accessed January 16, 2018.
15. *MotherToBaby. Hyperthermia Fact Sheet*. 2017. https://mothertobaby.org/fact-sheets/hyperthermia-pregnancy/. Accessed January 16, 2018.
16. American Pregnancy Association. *Risks and Safety Concerns of Using Hot Tubs While Pregnant*; 2017. http://www.americanpregnancy.org. Accessed January 15, 2018.
17. UK Teratology Information Service. *Use of Saunas and Steam Rooms in Pregnancy*; 2016. http://www.uktis.org. Accessed January 15, 2018.
18. Iiyama J, Matsushita K, Tanaka N, et al. Effects of single low-temperature sauna bathing in patients with severe motor and intellectual disabilities. *Int J Biometeorol*. 2008;52:431–437.
19. Imai Y, Nobuoka S, Nagashima J, et al. Acute myocardial infarction induced by alternating exposure to heat in a sauna and rapid cooling in cold water. *Cardiology*. 1998;90:299–301.
20. Sobajima M, Nozawa T, Shida T, et al. Repeated sauna therapy attenuates ventricular remodeling after myocardial infarction in rats by increasing coronary vascularity of noninfarcted myocardium. *Am J Physiol Heart Circ Physiol*. 2011;301:548–554.
21. Mooventhan A, Nivethitha L. Scientific evidence-based effects of hydrotherapy on various systems of the body. *N Am J Med Sci*. 2014;5:199–209.
22. Shevchuk NA, Radoja S. Possible stimulation of anti-tumor immunity using repeated cold stress: a hypothesis. *Infect Agent Cancer*. 2007;2:20.
23. Van der Horst A, Versteijne E, Besselink MGH, et al. The clinical benefit of hyperthermia in pancreatic cancer: a systematic review. *Int J Hyperthermia*. 2017:1–11.
24. Hildebrandt B, Wust P, Ahlers O, et al. The cellular and molecular basis of hyperthermia. *Crit Rev Oncol Hematol*. 2002;43(1):33–56.
25. Li LC, Wang M, Ning XC. Study on preventing alopecia caused by chemotherapy with cold pillow compresses. *Chin J Nurs*. 1995;30:643–645.
26. Fiorito, JJ, Culpepper-Morgan, JA, Estabrook SG, et al. *Hydrotherapy Compared with PEG-ES Lavage and Aqueous Sodium Phosphate as Bowel Preparation for Elective Colonoscopy: a Prospective, Randomized, Single Blinded Trial. The American College of Gastroenterology Annual Scientific Meeting Abstract*. Bethesda, MD: American College of Gastroenterology; 2006.
27. Centers for Disease Control and Prevention. *U.S. Dept. of Health and Human Services. National Diabetes Statistics Report, 2017*. Atlanta, GA: Centers for Disease Control and Prevention; 2017.
28. Kenny Glen P, Sigal Ronald J, McGinn R. Body temperature regulation in diabetes. *Temperature (Austin)*. 2016;3(1):119–145.
29. Mizrahi E, Liberty I, Tsedek I, et al. The influence of single immersion in dead sea water on glucose, insulin, cortisol and c-peptide levels in type 2 diabetes mellitus patients. *Harefuah*. 2011;150(8):646–649, 688, 689.
30. Kellogg JH. *Rational Hydrotherapy: A Manual of the Physiological and Therapeutic Effects of Hydriatic Procedures, and the Technique of Their Application in the Treatment of Disease*. Battle Creek, MI: Modern Medicine; 1901:772.
31. Dhippayom T, Kongkaew C, Chaiyakunapruk N, et al. Clinical effects of Thai herbal compress: a systematic review and meta-analysis. *Evid-Based Complement Alternat Med*. 2015:1–14.
32. Arslan GG, Eser I. An examination of the effect of castor oil packs on constipation in the elderly. *Complement Ther Clin Pract*. 2011;17(1):58–62.
33. Arankalle D, Wardle J, Nair PM. Alternate hot and cold application in the management of heel pain: a pilot study. *The Foot*. 2016;29:25–28.
34. Yang HL, Chen XP, Lee KC, et al. The effects of warm-water footbath on relieving fatigue and insomnia of the gynecologic cancer patients on chemotherapy. *Cancer Nurs*. 2010;33(6):454–460.
35. Chiu HY, Lin EY, Chiu HT, et al. A feasibility randomized controlled crossover trial of home-based warm footbath to improve sleep in the chronic phase of traumatic brain injury. *J Neurosci Nurs*. 2017;49(6):380–385.
36. Šrámek P, Šimečková M, Janský L, et al. Human physiological responses to immersion into water of different temperatures. *Eur J Appl Physiol*. 2000;81(5):436–442.
37. Nye EA, Edler JR, Eberman LE, et al. Optimizing cold-water immersion for exercise-induced hyperthermia: an evidence-based paper. *J Athl Train*. 2016;51(6):500–501.
38. Herrera E, Sandoval MC, Camargo DM, et al. Motor and sensory nerve conduction are affected differently by ice pack, ice massage, and cold-water immersion. *Phys Ther*. 2010;90(4):581–591.
39. Ernst E, Wirz P, Pecho I. Prevention of common colds by hydrotherapy. *Physiotherapy*. 1990;76:207–210.
40. Gupta PJ. Effects of warm water sitz bath on symptoms in post-anal sphincterotomy in chronic anal fissure—a randomized and controlled study. *World J Surg*. 2007;31(7):1480–1484.
41. Shafik A. Role of warm water bath in inducing micturition in postoperative urinary retention after anorectal operations. *Urol Int*. 1993;50(4):213–217.
42. Park SU, Lee SH, Chung YG, et al. Warm sitz bath: are there benefits after transurethral resection of the prostate? *Korean J Urol*. 2010;51(11):763–766.
43. LaFoy J, Geden EA. Postepisiotomy pain: warm versus cold sitz bath. *J Obstet Gynecol Neonatal Nurs*. 1989;18(5):399–403.
44. Ramler D, Roberts J. A comparison of cold and warm sitz baths for relief of postpartum perineal pain. *J Obstet Gynecol Neonatal Nurs*. 1986;15(6):471–474.
45. Hur MH, Han SH. Clinical trial of aromatherapy on postpartum mother's perineal healing. *Taehan Kanho Hakhoe Chi*. 2004;34(1):53–62.
46. Sinclair M. *Modern Hydrotherapy for the Massage Therapist*. Lippincott Williams & Wilkins; 2007:192.
47. Dick-Kronenberg L. *The Ultimate Text in Constitutional Hydrotherapy: A 100 Year Tradition of Clinical Practice*. Spokane, WA: The Carroll Institute of Natural Healing; 2007:27.
48. Bradley R, Pillsbury C, Huyck A, et al. Clinical pilot of constitutional hydrotherapy in HIV+ adults. Alternative therapies in health and medicine. abstracts from the 2009 North American Research Conference on Complementary & Integrative Medicine. *Explore*. 2009;15:3.
49. Wiggin K, Gravett M, Zwickey H. Effects of constitutional hydrotherapy on peripheral leukocytes. In: *Proceedings of the AANP Convention*. 2004; Seattle, WA.
50. Chaitow L. *Naturopathic Physical Medicine: Theory and Practice for Manual Therapists and Naturopaths*. London: Churchill Livingstone; 2008:532.
51. Bastedo W. Colonic irrigations. *JAMA*. 1932;27.

52. Alvarez WC. Origin of the so-called autointoxication symptoms. *JAMA*. 1919;72:8–13.
53. *Healing Newsletter*, May–June 1986; 13.
54. Virtanen J. *The Finnish Sauna: Peace of Mind, Body and Soul*. New York: Continental Publishing House; 1998.
55. Vuori I. Sauna bather's circulation. *Ann Clin Res*. 1988;20:249–256.
56. Eisalo A, Katila M, Kihlberg J, Takkunen J. Determination of cardiac output from blood pressure measurements and by the dye dilution method. *Ann Med Intern Fenn*. 1995;45:195–201.
57. Keast ML, Adamo KBJ. The Finnish sauna bath and its use in patients with cardiovascular disease. *Cardiopulm Rehabil*. 2000;20(4):225–230.
58. Croze S, Duclaux R, Russek M. Constancy of heat pain characteristics to changes in skin and body temperature. *Brain Res*. 1977;131:367–372.
59. Tseng CY, Lee JP, Tsai YS, et al. Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage. *J Strength Cond Res*. 2013;27(5):1354–1361.

41

Manipulation

Terry M. Elder, DC, and *Robert M. Martinez*, DC, ND*

OUTLINE

THERAPEUTIC KEYS

Manipulation is a passive manual maneuver that introduces movement beyond the passive range of motion (ROM) through the elastic barrier but does not exceed the anatomical barrier.[1] Mobilizations are passive stretches with or without oscillations over which the patient can exert control.[2] Naturopathic, chiropractic, and osteopathic professions use adjusting techniques that range in force from a nearly imperceptible force to high-velocity thrusts causing joint cavitation (popping noise). It should be known that the terms *adjustment* and *manipulation* are synonymous.

As a prudent starting point, the initial evaluation should determine whether manipulation of the patient is appropriate, seeking out "red flags" that contraindicate manipulation, such as fracture, infection, neoplasm, progressive neurological deficit, cord pressure, or cauda equina syndrome.[3,4]

For an extensive list of contraindications and precautions concerning manipulation, see Table 4.1, page 93, in *Chiropractic Technique, Principles & Procedures,* 3rd edition, by Bergman and Peterson.

- A correct differential diagnosis is crucial to the selection of patients, and the functional assessment is crucial to the selection of appropriate manual medicine techniques.[5]
- If gross signs of inflammation are present in a joint (heat, swelling, redness, and pain), and repeated movement to end range (ER) worsens the signs, manipulation in that direction would most likely aggravate the condition.
- When bringing the joint complex to tension, if the pain peripheralizes, it is a relative contraindication to manipulation.
- The practitioner should be alerted that an adverse outcome is likely from manipulation when repetitive motion with progressive force in the direction of the manipulation peripheralizes pain and reduces function.
- Relaxation techniques (heat, muscle work, and calming environment) are helpful to patients complaining of anxiety, muscle tension, stiffness, and aching before manipulation.[6]
- "The goal of manipulation is to restore maximal pain-free movement of the musculoskeletal system and postural balance."[7]
- Do not treat muscle spasm as a primary condition. Muscle spasm is almost always a response of the body to a noxious stimulus. Find the cause and treat it.[8]
- Trigger points are myofascial irritations that are frequently caused by underlying joint fixations. Manipulation performed improperly at hypermobile segments can irritate a trigger point and can precipitate a muscle spasm later that day.[9]

HISTORY

Manipulations are depicted in prehistoric cave drawings and Chinese statues, circa 2700 BC,[10] but Hippocrates is credited with the earliest recorded written physician's prescription of manipulative treatment methods, which changed little until the 6th century. He advocated the key principles of judicious use of force, direction of thrust, and proper levering of joints.[11]

During the Dark Ages, priests provided medical treatment at their monasteries. Kessler stated: "Friar Moultan, of the order of St. Augustine, wrote *The Complete Bonesetter*. The text, which was revised by John Turner in 1656, suggests that manipulation was practiced in medical settings throughout the Middle Ages and Renaissance."[12]

Three main concepts developed during the 1700s still have a major influence on manipulation today. The first held that "vertebral luxation" (bone out of place) was responsible for spinal deformity. The second, the mainstream medical opinion, maintained that "caries of the spine" caused spinal deformity, which was treated with bloodletting and rest, while condemning extension and manipulation as both useless and dangerous and citing concerns about the potentially

*Previous edition contributor

disastrous effects of manipulating tuberculous, neoplastic, rheumatic, or fractured joints. The third held that muscles were the main cause of problems, and treatment should be complete rest or active exercise, as the case warranted.[11]

More recently, questions of vertebral disc herniation, precipitation of cerebral vascular accidents, controversial issues of the cost-effectiveness and efficacy of manipulative treatment, and the lack of a differential diagnosis by many nonallopathic manipulators have become the cause célèbre.

It is interesting to note the following:

- Hippocrates railed against the abuse of manipulative therapy by physicians and others of his time.
- Physicians of the late 1700s assailed one another's methods of treatment (e.g., in *The Lancet,* December 16, 1826, the banner on page 347 appropriately read, "THE YELLOW JOURNAL").
- Surgeons held "bonesetters" in great contempt "when they condescend[ed] to speak at all of bonesetters and their works."[10]
- Bonesetters held their secrets and passed them from father to son.
- Financial competition was noted early in the literature: "It is known to most practitioners of surgery, and has been made known to many to their great cost and loss, that a large portion of the cases of impaired mobility or usefulness of limbs after injury fall into the hands of a class of men called 'bonesetters.'"[13]
- Although there has been a great deal of animosity, and claims of superiority made by the various practitioners of manipulative treatment even to this day, "specific conclusions cannot be derived from the scientific literature for or against either the efficacy of spinal manipulative therapy or the pathophysiologic foundations from which it is derived."[14]

SCHOOLS OF THOUGHT IN MANIPULATION

Bonesetters of England

The bonesetters of England generally held that a bone was out of place and had a "feel" for what was wrong. Hutton described the information gained from a bonesetter as "bring[ing] some spoils out of the camp of the Philistines."[13]

Chiropractic

D.D. Palmer, the "founder" of chiropractic, rediscovered the principle of "lost nerve tone" in a revelation from a deceased friend, Dr. Atkinson, and reestablished this method of healing. D.D. Palmer and his son B.J. Palmer, the "developer" of chiropractic, added one of the most colorful pages to the history of manipulative treatment.

D.D. performed the first chiropractic manipulation on a deaf janitor whose hearing had been lost when he stooped over and felt something give in his back. D.D. reasoned that if the deafness occurred from something slipping out, restoring the vertebra to its correct position should cure the condition: "With this new objective in view, a half-hour's talk persuaded Mr. Lillard to allow me to replace it and his hearing was restored."[15]

Although there are multiple schools of thought in chiropractic, each with its own strengths and weaknesses, the literature supports the need to combine therapeutic exercise with manipulation. *Rehabilitation of the Spine, A Practitioner's Manual,* 2nd ed., edited by Craig Liebenson,[4] is considered a landmark publication by many in the field and addresses this topic at length.

Naturopathic

Almost all naturopathic techniques result from the blending of thoughts of the other schools of medicine. This is appropriate when one realizes there is little new in manipulation, only refining and relabeling. It is also appropriate that naturopathy does not lay claim to originating a school of thought on manipulation but uses this method of treatment when indicated, not exclusively but as part of a therapeutic regimen.

Several naturopathic schools in the past were associated with the chiropractic and eclectic schools of medicine. The genesis of the naturopathic profession is well documented in Chapters 3 and 4, History of Naturopathic Medicine.

Osteopathic

Andrew Still left the practice of allopathic medicine and started a school of osteopathy in Kirksville, Missouri. It is highly probable that the first chiropractor, D.D. Palmer, went to this school and learned some of the techniques, but it is not well documented. The famed "equal but separate" movement of the osteopaths led to a 1921 resolution, submitted at the American Osteopathic Association convention, that allowed the entrance of chiropractors with advanced standing into their schools. Andrew Still, before his death, saw the defection of his osteopathic profession into the ranks of medical orthodoxy.[16–18] Interestingly, manipulation is now only an elective segment in some American osteopathic schools, whereas in England, where osteopathy is not part of the medical establishment, manipulation is still the mainstay of osteopathic practice.

The resurgence of interest in manual medicine has been brought to the forefront by the Osteopathic College at Michigan State University. This school teaches manual medicine to physical therapists, doctors of medicine, and doctors of osteopathy. Greenman's[1] *Principles of Manual Medicine* adds a useful text to the field of manual medicine.

Allopathic

James Cyriax, James Mennell, and John Mennell were brilliant physicians who worked to reintegrate manipulation into medical practice. They wrote valuable texts on manipulative therapy, although they did not totally agree on the effects they achieved with manipulation. Both James and John Mennell held to the correction of lost joint play and denied the effect on the intervertebral disc,[19] whereas Cyriax claimed reduction of a protruding disc.[8]

"Controversy and contention" best describe the higher levels of the respective schools of medical thought. The impression one gets in reading through the literature is intolerance of others' ideas expressed in ad hominem attacks. The mistake "lay" manipulators and "nonphysicians" make is not ineffectiveness but their willingness to seek training outside the fraternal order of the "medical" brotherhood; to address the public directly rather than communicating exclusively within the order; and, worst of all, to openly compete, economically and politically, against the fraternal order.[11,13]

Donald B. Tower, in the chairman's summary at the National Institute of Neurological and Communicative Diseases and Stroke conference in 1975,[20] noted a physician who received little credit for his early contribution to the field: J. Evans Riadore, a London physician who wrote a treatise on the irritation of spinal nerves in 1843. He attributed many diseases to this condition, stating: "If any organ is deficiently supplied with nervous energy of blood, its functions immediately, and sooner or later its structure, become deranged." This was a viewpoint subsequently echoed by osteopaths and chiropractors.[20]

The fifth edition of *Spinal Manipulation* by Bourdillion et al.,[21] the first authors of which were medical manipulators, has been largely reworked from previous editions and heavily influenced by osteopathic methods.

Awareness of manual medicine has been fostered by Calliet, McNabb, Maigne, Maitland, Kaltenborn, Williams, Jirout, Lewit, Janda, Bogduk, McKenzie, and many others.

Physical Therapy

James Cryiax influenced Robin McKenzie, a New Zealand physical therapist who subsequently developed a systematic approach called mechanical diagnosis and treatment (MDT) of musculoskeletal conditions. It is a process of evaluation and treatment of musculoskeletal disorders based on a mechanical history and the patient's symptomatic and mechanical response to movement, positions, and loading.[4] McKenzie's approach is often prejudicially dismissed as "extension exercises for the low back." He proposed a new tack in the approach to back pain that was found to be highly effective in populations with acute and chronic back pain, as well as low in cost.[22] MDT includes McKenzie's observations that most conditions are self-resolving and that focus should be on prevention and recovery of function by teaching patients proper posture and self-management. After years of studying, performing, and researching manipulation, he felt that only 20% of patients needed manipulative therapy, and 80% could self-treat using ER loading strategies learned from books or practitioners.[23]

IS MANUAL MEDICINE THE RIGHT TREATMENT?

Patients with somatic pain caused by psychosocial factors often seek a physical cause and treatment for their pain. If the presenting complaints do not seem to follow a mechanical pattern and the pain diagram is nonanatomic, consider the possibility of psychosocial factors. Psychosocial workplace factors associated with risk of spinal injury include job dissatisfaction, stressful working conditions as perceived by the employee, employer practices reported as being unfair, poor coping skills, lack of recognition at work, low supervisor support, a high frequency of job problems, and negative beliefs of or attitudes toward the consequences of having "low back trouble."[24,25] A major factor in identifying the incidence of future pain is the patient's perception of being disabled.[26] If the patient's history and records include previous evaluations by multiple providers with conflicting and confusing reports from the patient, caution is advised in approaching the management of the patient. If in doubt, seek a consultation with an astute colleague, physiatrist, or appropriate specialist.

Is It Safe to Move?

A clinician must answer the question, "Is it safe to 'move the patient' using conservative therapies, exercise, mobilizations, and manipulations?" Red flags have been shown to have many false positives, resulting in unnecessary additional diagnostic testing. Recent studies showed less than 1% of patients presenting to general practitioners with conditions that warranted further diagnostic evaluation. Red flags are a source of unnecessary medical interventions. When the medical history and examination indicate a serious disease, neurological compromise, or progressive neurological deficit, further evaluation or consultation is warranted. Although the question of safety is usually answered by the provider's clinical training, a general outline of triage is presented as a starting point to help determine whether further evaluation is indicated before initiating or continuing care.[27]

Signs of Neoplasm

The combination of age over 50 years, a history of cancer, unexplained weight loss of more than 10 kg (22 pounds) within 6 months, failure to improve after 1 month of conservative care, and an elevated erythrocyte sedimentation rate (ESR) have a sensitivity of 100% in identifying patients with a neoplasm. Night pain, especially pain that prevents the patient from getting sleep or "drives them from the bed" also generates a high degree of suspicion.[28,29]

Signs of Infection/Inflammation/Illness

Heat, swelling, pain, redness, and loss of function are the cardinal signs of inflammation. Acute inflammation lasts from 1 to 3 days, and chronic inflammation usually is considered from 7 days to 7 weeks but may last up to 12 months. The stages between acute and chronic inflammation overlap, and a continuum exists.[30] The clinical picture may be confused by chronic pain.

Laboratory tests may show signs such as an elevated C-reactive protein, ESR, or other tests for inflammatory arthritides. Signs of infection may be as simple as a low-grade fever, chills, a shift to the left in the white blood cell count, signs of a worsening chronic illness, or frank presentation of an acute illness.[31] Clinical signs are pain that is constant 24 hours a day, described as aching, throbbing, or burning and aggravated by movement, with no position that gives relief.

Manipulation is contraindicated over an area of acute inflammation, but acute pain may be the result of mechanical pain. This confusion can usually be resolved during the palpatory process. During palpation, an acutely inflamed joint will exhibit deep pain upon pressure and movement. This deep pain will persist long after palpation is concluded. Palpation of a manipulable joint restriction will also cause pain, but only as long as the palpation pressure is applied. Treatment of the tissues should be guided by the level of inflammation and the response of the tissue to mechanical testing, according to MDT methodology, to determine whether the patient should rest or pursue specific therapeutic movements. Observe the posttreatment results to see if signs of inflammation worsen, indicating incorrect application or inappropriate therapy.

Signs of Neurological Disorder or Progressive Neurological Deficit

A loss of sensation, decreased or absent deep tendon reflex, loss of muscle strength, and positive nerve root tension are signs of nerve root involvement. If only these neurological findings are present and do not become progressively worse, appropriate conservative care may be effective.[32] Dizziness, drop attacks, diplopia, dysarthria, dysphagia, numbness, nystagmus, and nausea are more concerning signs of central nervous system involvement. Loss of bladder or bowel control or saddle anesthesia requires urgent evaluation with magnetic resonance imaging of the lumbar spine. If progressive neurological deficit of weakness, loss of sensation, and loss of function, or the previously mentioned signs and symptoms, are present, a consultation with a competent colleague, physiatrist, or neurosurgeon is indicated.

Signs of Fracture

A fracture typically has an obvious traumatic cause when the patient is young or middle-aged. It should be noted that even in the young and middle-aged, something as simple as chronic bronchitis with severe cough can result in rib fracture; therefore diagnostic caution is always warranted. In older patients, pathological fractures are a concern, especially if there is a history of prolonged or repeated steroid use. Be wary of occult fracture after motor vehicle accidents or athletic injuries and nontraumatic fractures in the elderly. The red flags suggesting vertebral fracture are age more than 50 years, female sex, major trauma, pain and tenderness, and a distracting painful injury. In particular, look above and below the site of injury, because the force of trauma may be transmitted and cause a fracture some distance from the site of impact.[33] In a pelvic fracture, there is always a fracture in two places in the ring.[34]

Is the Best Treatment Movement or Rest?

Pain can be confusing and lead one to conclude that inflammation is present. If pain is constant aching, throbbing, or burning, it suggests

that the pain has a chemical cause, and it is inflammation. When the pain goes away, even for 30 minutes in a day, it suggests that the pain is due to mechanical pressure on tissue. To illustrate this point, bend your finger backward until you feel strain and hold it. At first you may only feel discomfort, but after an extended period of time, it will become painful. Next, try bending the finger backward beyond strain until it hurts. The pain you feel is due to abnormal stresses on a normal tissue; there is nothing wrong with your finger! You do not need drugs, modalities, or manual therapy. Simply stop overstraining the soft tissues. Robin McKenzie used this seemingly simple example to teach the consequences of sustained ER loading as a common cause of pain—mechanical pain rather than pain from inflammation.[23]

If the pain is caused by inflammation, it is best to rest until the inflammation has been reduced. If the pain is mechanical, it is often best to move, but how far should one move, and in which direction?

Which Direction to Move?

Common approaches to determine which direction to move the patient are orthopedic tests, selective tissue tension tests, and McKenzie's MDT. Each can inform the practitioner of which tissues are involved and help formulate an appropriate treatment program.

Orthopedic Tests

Orthopedic tests are designed to stress the damaged tissue and reproduce the pain of the primary complaint. The examiner is not looking merely for pain to be reported as a result of the maneuver but, rather, pain that is specific for the test and reproduces the pain of the primary complaint. Therefore the following should be remembered:

- The examiner can make the patient worse by forcing tests or performing them incorrectly.
- The least stressful tests should be done first. If the first test causes severe pain, most of the other motions will be painful afterward and confuse the findings.
- The test by definition has a positive response, which correlates to a specific condition that must be produced when the test is performed for the test to be positive (e.g., Lindner's test and Soto Hall's test are performed in the same way, but the findings to report a positive test are different).
- The test must reproduce the pain of the primary complaint and be positive by the test's definition.
- Tests are centered in allopathic medicine to diagnose pathology, fracture, moderate to severe sprain/strain, and dislocation. Although the patient feels pain from the movements of these tests, these are not true positive tests just because the patient reports that the movement hurts.

When multiple orthopedic tests are indiscriminately performed on one visit, without an understanding of the mechanism of action of the stress these maneuvers put on the tissues, the unwitting examiner merely subjects the patient to the trauma of a series of painful maneuvers that only serve to confuse the practitioner and aggravate the patient's condition. Detailed texts that cover this area include those by Magee[35] or Evans[36]; such texts are helpful and should be referred to for additional information. It is even more helpful to attend programs in which experienced practitioners teach skills and knowledge to inexperienced practitioners.

Selective Tissue Tension Tests

This section provides an introduction to the differential diagnosis of soft tissue injuries by means of active and passive movements using the selective tissue tension tests of James Cryiax[8]; this topic is covered in depth by Kessler[12] and Magee.[35]

Active movements. First, active ROMs are performed to determine voluntary ROM and patient status. Measuring active ROMs cannot differentiate whether the loss of function is due to pain, weakness, stiffness, and/or lesion, but it is helpful to determine patient tolerance to motion and how guarded the patient is when moving.

Resisted isometric tests. Next, isometric muscle tests are performed to test the contractile tissue (muscle and tendons) with the joint in neutral to avoid involving the noncontractile tissues. The following results may be noted:

- Normal—painless and full strength
- Minor tear—pain with full strength
- Moderate tear—pain with little strength
- Neurological deficit—no pain and little strength

Passive movements. When the joint is put through passive motion, it reaches an end point, which has an "end-feel" that helps determine the status of the soft tissue around the joint. The end-feel of a joint may be one of the following:

- Normal—the amount of motion and feel of the tissues are appropriate for age.
- Abnormal—normal end-feels that are not normal for the joints and ROMs that are being tested (e.g., the feeling of bone on bone is the normal end-feel at the elbow when extended but is abnormal when it occurs at the ER of knee extension).
- Pathological—these end-feels are present only in joints that have undergone pathological changes.

Normal end-feels

Capsular end-feel. This is a firm, "leathery" feeling, felt, for example, when the normal shoulder is at full external rotation. When felt in conjunction with a capsular pattern of restriction, and in the absence of significant inflammation or effusion, it indicates capsular fibrosis.

Bony end-feel. This feels abrupt, as when moving the normal elbow into full extension. When accompanying a restriction of movement, it may suggest hypertrophic bony changes, such as those that occur with degenerative joint disease, or possible malunion of bony segments after healing of a fracture.

Soft tissue approximation end-feel. This is a soft end-feel, as when fully flexing the normal elbow or knee. It may accompany joint restriction in the presence of significant muscular hypertrophy.

Muscular end-feel. This more rubbery feel resembles what is felt at the extremes of straight-leg raising (SLR) from tension on the hamstrings. It is less abrupt than a capsular end-feel.

Abnormal end-feels. The tissue involved is determined by the response to the passive ROMs and by moving the joint to ER and into closed pack position.

Muscle or tendon. Active and passive movements are limited or painful, or both. Pain is caused by active contraction or passive stretching of the inflamed or torn muscle–tendon unit.

Ligaments. Both active and passive movements are limited or painful, or both, in the same direction due to stretching of the inflamed or torn ligament.

Capsular pattern. Only joints that are controlled by muscles have a capsular pattern. Pain is caused by both active and passive motions, and the limitation of motion is in a specific proportion that is listed in tables in Kessler's text.[12]

Pathological joint end-feels. Pathological joint end-feels indicate a disease process; they are limited and are never normal:

- Empty—bursitis, space-occupying lesion, neoplasm
- Boggy—joint effusion
- Spasm—guarding from inflammation
- Internal derangement—loose body/torn meniscus

McKenzie's Mechanical Diagnosis and Therapy

MDT is an invaluable system for practitioners who perform manual therapy. Rather than a technique, it is a method of diagnosis determined by response to repeated movement and pressure to joints and soft tissue that, once mastered, allows rapid assessment and selection of patients for treatment. It also identifies those not likely to respond to manual therapy so that the provider's treatment program can be altered, or the patient can quickly receive an appropriate referral. Resources available are classes from the McKenzie Institute on MDT, McKenzie's textbooks,[37,38] and a chapter in Liebenson's text.[4] Only a few of the many helpful concepts from the McKenzie method are covered in the following discussion. Keep in mind that McKenzie asserted that only 20% of patients needed manipulative therapy, and prevention is key to success in his model.

Centralization and Peripheralization

A cervical spine problem can refer pain to the shoulder blade, arm, forearm, and hand. A low back problem can refer pain to the sacroiliac joint (SIJ), buttock, thigh, leg, and foot. When the referred pain from the spine is brought closer to the midline in response to movements, position, or load, and remains reduced, it is called "centralization."[22] When the pain moves out from the spine in response to movements, position, or load, it is called "peripheralization." When a movement produces centralization, it is a motion to pursue for treatment. When a movement causes peripheralization, it is a motion to avoid because it will likely worsen the condition. If no movement or position centralizes the pain, it is a poorer prognosis for the response to the treatment method being used. Although this phenomenon is typically associated with vertebral disc problems, it can be observed in many musculoskeletal problems.

End-Range Pain and Pain During Motion

End-range pain (ERP) is felt only when the joint is moved to ER. It is a sign of deranged or adaptively shortened soft tissues around the joint complex. The importance is that this is an indication that stretching and mobilizing techniques should be used before manipulation. If the joint is not mobilized to ER repeatedly, the manipulation may cause aggravation of the complaint. When the joint is fully stretched, after a progression of forces described in the "Performing Manipulation" section, the practitioner can determine whether and in which direction the mobilization or manipulation is indicated.

Pain during motion (PDM) is pain that is felt during joint movement, as well as at the ER. The joint ROM may be restricted as well. PDM is a sign of soft tissue derangement, often thought of as a vertebral disc problem. Movements or positions that increase the PDM and cause increased restriction of movement after the movement is performed should be avoided. When a mobilization or manipulation increases PDM or restriction, it is important to find a motion or position that will reduce the PDM and restore the motion as soon as possible to reduce the irritation of the tissues. Application of ice, analgesic, and/or anti-inflammatory medications may be indicated as well.

PRETREATMENT ASSESSMENT

Functional Assessment

Often the focus is on injury, pathology, and disease, but the majority of musculoskeletal pain is from pathomechanics due to postural and repetitive strain, disuse, deconditioning, fatigue, and nutritional factors that must be addressed if treatment is to be successful. Once the portion of the patient's condition that is a musculoskeletal problem has been established, the mechanical fault should be determined. Manipulative treatment without correction of the mechanical fault results in prolonged treatment and recurrence. Detecting this is sometimes easy, whereas at other times it is a mystery that takes careful investigation, as discussed by McKenzie,[39] Greenman,[1] Lewit,[5] and Liebenson.[4]

Observe the posture of the patient. If he or she is tilted and antalgic to the side and/or forward, due to an episode of neck or back pain, it indicates a large space-occupying mass, which is most often the disc, but it could be other soft tissue or pathology. Proceed slowly and carefully, avoiding any movements that cause peripheralization, increase PDM, or increase the obstruction of motion.

Establish the baseline of the location of the complaint and its response to motion, and then repeat motions to ER. In the cervical spine, test flexion, extension, rotation, and lateral bending. In the lumbar spine, test flexion, extension, and lateral translation. The testing procedures are described in detail, with photos, in Liebenson's text.[4]

Note the ROM. If there are complaints at or below the shoulder blade or buttock, it is important to test reflexes, muscle strength, sensation, and nerve root tension signs to assess if the nerve root is affected. Additional tests can be added as indicated.

Cervical Spine

The cervical spine presents a diagnostic challenge (Fig. 41.1). Although the mechanism of the tests in the lumbar spine is similar, the area is much more delicate and thus requires more careful application.

There is considerable discussion of the topic of vertebrobasilar stroke (VBS) after manipulation of the cervical spine. Tests done in the office have not proved effective, but the history should alert the practitioner to the possibility of VBS insult, and manipulation is

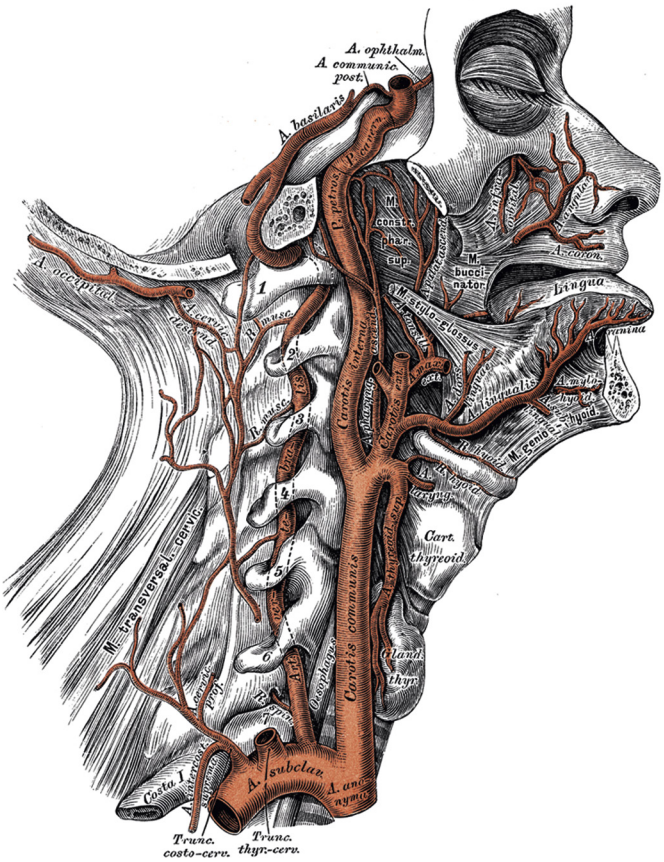


Fig. 41.1 Cervical spine. (From ilbusca/iStock.com.)

contraindicated. The presence of the signs listed in the section on Signs of Neurological Disorder is an important indicator that mobilization or manipulation should not be used. The most important risk factors identified by Terrett[40] are dizziness, unsteadiness, giddiness, vertigo, and sudden severe pain in the side of the head or neck, which is different from any pain the patient has had before. If the practitioner elects to follow the pattern of the progression of force, the occurrence of these symptoms in the early mobilizations contraindicates progressing the force and manipulation. However, VBS has occurred during an examination simply by having the patient turning his or her head. The incidence is small, but missing this clinical presentation is devastating, although not clearly caused by the evaluation or manipulative treatment.[41]

If there is compression of the nerve root or cervical instability, the patient may present holding the head, lifting it cephalically to decompress the spine and root, which is a clear indication to stabilize and transport immediately. Any such presentation is best handled by emergency department staff because even passive ROM assessment may cause permanent injury.

If there is irritation of the brachial plexus or a nerve root, the patient may support the arm in abduction and flexion, often resting the hand on the top of his or her head. This is a sign to proceed cautiously. Any movements that cause peripheralization must be avoided, and movements that produce centralization should be pursued. In cases where there are no deficits and all signs and symptoms are relieved by elevation of the arm, this may be the only treatment needed. If overt signs of nerve root compression are present, mobilization and manipulation must be performed with caution.

Cervical Distraction

If distraction of the head from the shoulders causes aggravation of the patient's complaints at 12 lb of traction (the average weight of the head), stabilization and relaxation techniques are indicated. If distraction of the head from the shoulders causes relief of the radiating pain at 12 lb of traction, slowly increase the traction to 25 lb. If this relieves the complaint of neck pain, it is an indication that mobilization and traction are indicated therapies. If it aggravates the complaint, it is an indication of inflammation or instability and suggests caution in using mobilization or traction.

Cervical compression that causes radiating pain to the arms is a sign that indicates nerve root irritation from the disc and is treated by symptom response that centralizes the arm complaint.

Maximum Cervical Rotation, Lateral Bending, and Compression

If maximum cervical rotation, lateral bending, and compression cause radiating arm pain, it is a sign of nerve root compression, and only a skilled practitioner should attempt manipulation in that direction.

Spurling's Maneuver

When combined cervical rotation, lateral bending, and extension with overpressure (Spurling's maneuver) aggravates or reproduces the complaint or causes radiating pain to the midback or arm, this indicates nerve root irritation, and manipulation in that direction places the patient at risk for aggravation of this condition. If the pain is local, it is a sign of facet irritation that often responds well to manipulation. The practitioner can test the response with repeat mobilization to the ER; if only ERP is produced, then increased force can judiciously be used.

After determining that the lesion is not a "hard" orthopedic or neurological lesion, differential tissue tension tests are used to determine the involved tissue. Appropriate repeated movements to ER and skilled mobilization and/or manipulations are identified by centralization of the symptoms and improved function.

Signs of Upper Limb Tension

Butler[42] described, in detail, the identification of signs of cervical root tension using upper limb tension tests developed by Elvy in 1979. The tests are useful to help guide treatment.

Lumbar Spine and Sacroiliac Joint

During supine SLR, the lumbar nerve roots begin to develop tension at about 35 degrees. If SLR causes leg pain below 30 degrees, it is likely due to stretching of the lumbar nerve root in the presence of a space-occupying lesion or significant hip pathology. SLR above 35 degrees causes increasing ipsilateral nerve root tension and ipsilateral hamstring tightness, and it may expose lumbar dysfunction or derangement as the straight leg moves toward ER. Once the painful ER is found, one lowers the limb to the pain-free range and then performs internal rotation and dorsiflexion of the foot and great toe (Fig. 41.2). If this maneuver causes a return of the leg pain, there is likely tension on the lumbar nerve roots. Because the SLR maneuver stresses multiple tissues, the test can be misleading, and it can be somewhat difficult to determine what a positive finding is until the practitioner's skills develop, but the positive result should be described to help clarify the findings.

Assessing Radiating Leg Symptoms

Following the principle of "Do no harm," one should test reflexes and sensation first, then proceed to active tests such as the Valsalva maneuver to test for increased intrathecal pressure. If radiating pain results from the Valsalva maneuver, it is a sign of a space-occupying lesion. Next, the practitioner should do SLR, adding dorsiflexion of the foot with extension of the great toe to determine whether the root tension reproduces or aggravates the radiating leg symptoms. Assessment with MDT protocols is recommended and is superior to standard orthopedic protocols for functional assessment. Commonly used tests are compression tests (Milgram's bilateral leg raising and Lindner's test [forcefully flexing the trunk while the patient is supine]); these force the disc posteriorly (if herniated) and cause increased intraabdominal pressure, resulting in increased intrathecal pressure (Valsalva effect).

Some additional ideas for finding the area of a lesion in the lumbar spine are as follows:

- Support Adams (the belt test)—With the patient standing, the doctor has him or her bend forward and notes the level of pain. The doctor then secures the patient's pelvis by hugging the anterior

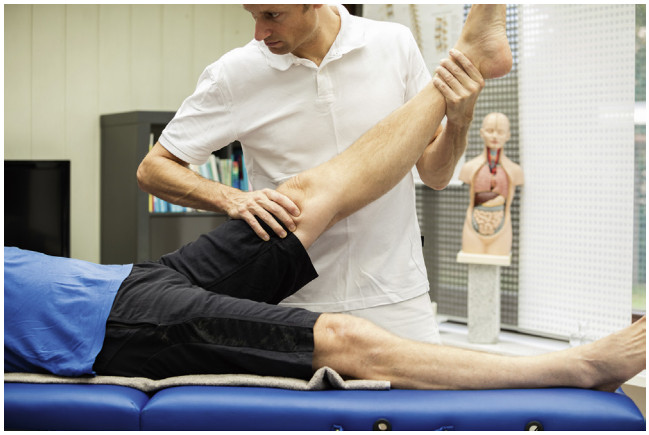


Fig. 41.2 Supine straight-leg raise (Lasegue) test. Jan-Otto/iStock.com.)

superior iliac spines with the arms, pressing the patient's sacrum into the doctor's hip. If the patient's pain is decreased, it indicates the lesion is in the pelvis (probably the SIJ or hip); if pain increases, it is in the lumbar spine.

- Patrick's test—Flexion, abduction, and external rotation of the hip are blocked; this causes pain over the inguinal fossa and into the thigh when a hip lesion is present.

This is only an introduction to one common problem that may present with many variations. A careful study of the mechanism of action of the tests used can confirm, rather than confuse, a diagnostic impression.

Assessing the Sacroiliac Joint

The SIJ is the "dumping ground" for pain from the lumbar spine, just as the shoulder blade is the "dumping ground" for pain from the cervical spine. The key to determining whether the problem is in the SIJ is to rule out all lumbar problems first. Second, Laslett[43] determined that when two or more of the following SIJ tests produce concordant pain, it is highly likely that the SIJ is the pain generator:

1. Gapping test—The patient is supine. The examiner crosses his or her arms, placing the heels of the hands on the anterior superior iliac spine, forcefully pressing laterally and down into the table to "gap" the SIJ, attempting to stretch the anterior SIJ ligaments.
2. Compression test—The patient is lying on his or her side with the painful SIJ up. The pressure is directed to the opposite iliac crest, attempting to compress the anterior SIJ and stretch the posterior SIJ ligaments.
3. Posterior shear or "thigh thrust"—The patient is supine. The practitioner's hand, a small block, or sandbag is placed under the patient's sacrum. The hip is flexed to 90 degrees (perpendicular to the floor), the knee is maximally flexed, and a thrust is applied down the shaft of the femur. One should avoid adduction of the hip, as this will cause pain in normal patients.
4. Pelvic torsion (Gaenslen's test)—The patient is supine at the edge of the table with one thigh extended over the edge of the table. On the other side, the hip and knee are flexed to the chest. Overpressure is applied to the extended thigh to accentuate the posterior rotation of the opposite side. This should be performed on both sides.
5. Sacral thrust—With the patient lying prone, the examiner thrusts down on the sacrum.
6. Cranial shear—With the patient prone, cranial pressure is applied to the apex of the sacrum with the examiner's hands, whereas the painful side ankle is placed between the examiner's knees and is tractioned caudally.

Techniques for correction of these fixations are described by Maigne,[44] Bourdillion,[21] and Maitland.[2]

TREATMENT CONCEPTS

Notes on the Art of Manipulation

Manipulation is an art. Assuming a proper differential diagnosis, one acquires a "feel" that allows recognition of the differences among the types of joint fixations and knowledge of when to manipulate.

When a movement causes pain, it should not be performed unless the diagnosis of the patient's condition is sure and the motion will cause no harm.[44]

Joint Motion

Joint motion descriptions can become elaborate, or one may simply say that there are six degrees of freedom of motion (plus long-axis extension): flexion, extension, left and right rotation, and left and right lateral bending. Not all joints have all six degrees of motion. The normal degrees and ROMs of joints are listed in anatomy and kinesiology texts (Fig. 41.3). If a degree of motion is lost or blocked, the type of fixation and which form of manual medicine is indicated should be determined.

Barrier Concepts

Joint motion is described from neutral to an ER that is ultimately limited by the anatomical barrier, which, if exceeded, causes tissue trauma. The extent of the active ROM can be increased by passive motion to a point at which all of the tissues around the joint have been brought to tension, called the elastic barrier.[1] Beyond the elastic barrier is a small ROM referred to as the paraphysiological space.[45] It is within the paraphysiological space that joint cavitation, the "popping" sound, occurs. When joints and soft tissues are dysfunctional, alterations of ROM may occur both within the ROM and at the end of it. If one focuses only on working in the paraphysiological space, one severely limits the effects that can be made on dysfunction.

Before using manipulation, think about the joint end-feel (the way the joint feels at the end of its ROM) and determine which pattern is present:

- Edematous and boggy end-feel—indicates joint effusion and possibly inflammation. Muscle spasms should not be treated as a primary condition because they are almost always the body's response to a noxious stimulus or pathophysiological fault. The best treatment for muscle spasm generally is to find the origin of the noxious stimulus or pathophysiological fault, which can be joint dysfunction at a distal site, nutritional deficiency of magnesium orP-5-P, and so forth (see Chapter 47, Soft Tissue Manipulation).
- Springy and taut end-feel—ligamentous fixations (as felt in the normal knee in lateral bending stress tests and drawer tests). Manipulation works well on these types of fixations. Surging

Joint		Normal Range of Motion (Degrees)
Shoulder	Flexion	0-180
	Extension	0-60
	Abduction	0-180
	Internal rotation	0-70
	External rotation	0-90
Elbow	Flexion	0-150
Forearm	Pronation	0-80
	Supination	0-80
Wrist	Flexion	0-80
	Extension	0-70
Hip	Flexion	0-120
	Extension	0-30
	Abduction	0-45
	Adduction	0-30
	Internal rotation	0-45
	External rotation	0-45
Knee	Flexion	0-135
Ankle	Dorsiflexion	0-20
	Plantar flexion	0-50

Fig. 41.3 Ranges of motion of various joints. (From American Academy of Orthopedic Surgeons (AAOS) as reported in Appendix B of Reese NB, Bandy WD. *Joint Range of Motion and Muscle Length Testing.* 2nd ed. St. Louis: Saunders; 2010.)

sinusoidal and interferential current, ultrasound, and moist hot packs facilitate the treatment. One can follow with cryotherapy in the subacute stages.

- Bone-on-bone end-feel—usually indicative of degenerative joint disease (as felt in the normal elbow in extension). If there is fine crepitus, mild degenerative joint disease (DJD) may be present; coarse crepitus indicates moderate DJD, and joint creaking may be advanced DJD. Diathermy, contrast baths, and other naturopathic therapies indicated in osteoarthritis are helpful.

Preparation for the Manipulation

The room should be at a comfortable ambient temperature, and the physician should wear no watches or jewelry that might catch the patient's hair or skin.

Performing Manipulation

Manipulation of a joint is performed to correct joint fixation. Once the area to be manipulated has been appropriately assessed:

1. Test the direction of manipulative thrust with repeat mobilization to ER to ascertain that the pain does not peripheralize, the movement does not increase PDM, and the movement does not result in increased obstruction to motion from mobilization, using a progression of force to determine the effect of the direction and force applied.

This is a logical stepwise progression of the force generated first by the patient, then by the practitioner. The advantages are increased patient comfort, ability to "test the waters," and decreased risk of harm from the procedure. The steps are as follows:

Patient-generated force—the patient does all the motion actively.

Patient-generated overpressure—the patient uses a strap, fulcrum, or other device to increase the movement to ER.

Practitioner-generated force—the practitioner mobilizes the joint to ER repeatedly.

Practitioner uses manipulative thrust if positive responses occur that include centralization, reduction of antalgic postures, increased ROM, and decreased PDM. It is a relative contraindication to continuing to step 4 if there is no positive response in steps 1 to 3.

2. Stabilize the area to be manipulated: A point of stabilization is created by one hand and the physician's body weight while the other hand performs the manipulation. Minor corrections of the position of the stabilized part or hand, or both, are frequently interpreted by an observer as twisting or wrenching motions. Twisting and wrenching are difficult to control and may injure the patient.
3. Bring the joint to tension, removing the periarticular tissue slack. Mobilization with tissue slack not taken up is safe, but manipulation when tissue slack is present invites injury.
4. Thrust only when the patient is relaxed and the fixation is felt.

The manipulative thrust can be described according to the following:

- Direction of line of thrust—through the joint space, parallel to joint surfaces, or tangential to the point of fixation.
- Velocity—slow for mobilizations. High velocity can correct joint fixations, and the speed must be faster than the patient's reaction time, or a strain injury will result if the patient's muscular resistance occurs at the time of the manipulative thrust.
- Amplitude—governed by the quality of the health of the tissue, the quality of the fixation, and the location of the condition on the spectrum of the inflammation response.

After the elastic barrier has been stretched, repeated manipulation in the same direction is complicated because the end-feel tension normally felt before a manipulation is reduced or absent for at least 20 minutes. The same phenomenon occurs when one "pops" knuckles. The risk of injury is much greater, and changing to mobilization or active muscular relaxation techniques is recommended rather than repeated manipulation.

Reassess

The condition of the joint fixation should be reassessed after each treatment to determine whether the therapy has been successful.

A single manipulation rarely corrects a problem completely. The use of a joint-scanning technique shows where the problems are that need further evaluation. The goal is to alleviate acute conditions or, if unsuccessful, to turn them into subacute conditions and resolve subacute and chronic conditions before they cause a chronic fatigue response, joint fixation, degeneration, or chronic myositis.

Troubleshooting Technique

Common problems blocking successful manipulation can be overcome by the following:

- Learning joint play analysis on the peripheral joints and practicing manipulations of those joints before attempting to manipulate the spinal joints.
- Practicing the manipulative procedure with repeated light oscillatory movements while modifying the direction to feel the changes in joint tension, thus gaining experience, confidence, and knowledge without inflicting injury.
- Being confident of the procedure; otherwise, the patient may sense the physician's hesitation and guard, preventing the manipulation and increasing the risk of a poor outcome.
- Feeling the fixation of the joint as it is brought to tension; otherwise, the manipulation traumatizes the tissues unnecessarily or does not gain the desired result.
- Learning the arthrology of the joint to be manipulated so as not to put the joint in a "locked" or jammed position that will cause the force of the manipulation to be dispersed to the surrounding tissues and joints.

Personal instruction is invaluable, but unless the rationales of joint mechanics, pathomechanics, and pathophysiology are the basis of the teacher's approach, it can be confusing and lead to erroneous conclusions. The Further Reading list includes texts by Bourdillion, Greenman, Liebenson, and Maitland; these are good sources for learning the basics of the art.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Greenman P. *Principles of Manual Medicine*. Baltimore: Williams & Wilkins; 1996.
2. Maitland GD. *Peripheral Manipulation*. 3rd ed. London: Butterworth-Heinemann; 1991.
3. Bigos S, Bowyer O, Braen G, et al. *Acute Low Back Problems in Adults*. Rockville, MD: US Department of Health and Human Services, Public Health Service, Agency for Health Care Policy and Research; 1994.
4. Liebenson C. *Rehabilitation of the Spine: A Practitioner's Manual*. 2nd ed. Baltimore: Lippincott Williams & Wilkins; 2007.
5. Lewit K. *Manipulative Therapy in Rehabilitation of the Locomotor System*. Boston: Butterworth-Heinemann; 1985.
6. Arnell P, Beattie S. Heat and cold in the treatment of hypertonicity. *J Can Phys Assoc*. 1972;24:61–67.
7. Dvorak J, Dvorak V, Schneider W. *Manual Medicine*. Heidelberg, Germany: Springer Verlag; 1985.
8. Cryiax J. *Textbook of Orthopaedic Medicine*. London: Bailliére Tindall; 1982.
9. Travell JG, Simons DG. *Myofascial Pain and Dysfunction: The Trigger Point Manual*. Baltimore: Williams & Wilkins; 1983.
10. Schafer R. *Chiropractic Health Care: A Conservative Approach to Health Restoration, Maintenance, and Disease Resistance*. Des Moines: Foundation of Chiropractic Education and Research; 1976.
11. Lomax E. Manipulative therapy. In: Buerger AA, Tobis JS, eds. *Approaches to the Validation of Manipulation Therapy*. Springfield, IL: CC Thomas; 1977.
12. Kessler RM. *Management of Common Musculoskeletal Disorders: Physical Therapy Principles and Methods*. Philadelphia: Harper & Row; 1976.
13. Hood WP. On the so-called "bone-setting," its nature and results. *Lancet*. 1871;1(304–310):344–349.
14. Goldstein M, ed. *The Research Status of Spinal Manipulative Therapy: A Workshop Held at the National Institutes of Health, February 2–4, 1975*. Bethesda, MD: U.S. Department of Health, Education, Welfare, Public Health Service, National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke; 1975.
15. Palmer DD. *The Science, Art and Philosophy of Chiropractic*. Portland, OR: Portland Printing House; 1910.
16. Gibbons R. The evolution of chiropractic: medical and social protest in America. Notes on the survival years and after. In: Haldeman S, ed. *Modern Developments in the Principles and Practice of Chiropractic*. New York: Appleton-Century-Crofts; 1980:16–19.
17. Northup G. History and development of osteopathic concepts: osteopathic terminology. In: Goldstein M, ed. *The Research Status of Spinal Manipulative Therapy: A Workshop Held at the National Institutes of Health, February 2–4, 1975*. Bethesda, MD: U.S. Department of Health, Education, Welfare, Public Health Service, National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke; 1975:43–51.
18. Wardwell WI. Discussion: the impact of spinal manipulative therapy on the health care system. In: Goldstein M, ed. *The Research Status of Spinal Manipulative Therapy: A Workshop Held at the National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke, February 2–4, 1975*. Bethesda, MD: U.S. Department of Health, Education, Welfare, Public Health Service, National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke; 1975:20–21.
19. Zhon DA, Mennell JM. *Musculoskeletal Pain: Diagnosis and Physical Treatment*. Boston: Little, Brown and Company; 1976.
20. Tower DB. Chairman summary: evolution and development of the concepts of manipulative therapy. In: Goldstein M, ed. *The Research Status of Spinal Manipulative Therapy: A Workshop Held at the National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke, February 2–4, 1975*. Bethesda, MD: US Department of Health, Education, Welfare, Public Health Service, National Institutes of Health, National Institute of Neurological and Communicative Disorders and Stroke; 1975:59.
21. Bourdillion JF, Day EA, Bookhout MR. *Spinal Manipulation*. Boston: Butterworth-Heinemann; 1992.
22. McKenzie R. *The Lumbar Spine*. Waikanae. New Zealand: Spinal Publications; 1981.
23. McKenzie R, Kubey C. *Seven Steps to a Pain-Free Life: How to Rapidly Relieve Back and Neck Pain Using the McKenzie Method*. New York: Plume; 2000.
24. Krause N. Psychosocial job factors, physical workload, and incidence of work-related spinal injury: a 5-year prospective study of urban transit operators. *Spine*. 1998;23:2507–2516.
25. Feuerstein M, Berkowitz SM, Huang GD. Predictors of occupational low back disability: implications for secondary prevention. *J Occup Environ Med*. 1999;41:1024–1031.
26. Estlander AM, Takala EP, Viikari-Juntura E. Do psychological factors predict changes in musculoskeletal pain? A prospective, two-year follow-up study of a working population. *J Occup Environ Med*. 1998;40:445–453.
27. Williams CM, Maher CG, Hancock MJ, et al. Low back pain and best practice care: a survey of general practice physicians. *Arch Intern Med*. 2010;170:271–277.
28. Deyo RA. Diagnostic evaluation of LBP: reaching a specific diagnosis is often impossible. *Arch Intern Med*. 2002;162:1444–1447.
29. Henschke N, Maher GC, Refshauge KM. Screening for malignancy in low back pain patients: a systematic review. *Eur Spine J*. 2007;16:1673–1679.
30. Robbins SL, Cotran RS. *Pathologic Basis of Disease*. Philadelphia: WB Saunders; 1979.
31. Wasson J, et al. *The Common Symptom Guide: A Guide to the Evaluation of 100 Common Adult and Pediatric Symptoms*. New York: McGraw-Hill; 1984.
32. Beneliyahu DJ. Chiropractic management and manipulative therapy for MRI documented cervical disk herniation. *J Manipulative Physiol Ther*. 1994;17:177–185.
33. Waddell G. An approach to backache. *Br J Hosp Med*. 1982;28(187):190–191. 193–194.
34. Henschke N, Maher GC, Refshauge KM. A systematic review identifies five "red flags" to screen for vertebral fracture in patients with low back pain. *J Clin Epidemiol*. 2008;61:110–118.
35. Magee DJ. *Orthopedic Physical Assessment*. Philadelphia: WB Saunders; 1992.
36. Evans RC. *Illustrated Essentials in Orthopedic Physical Assessment*. St. Louis: Mosby; 1994.
37. McKenzie R. *The Lumbar Spine*. Waikanae, New Zealand: Spinal Publications; 1997.
38. McKenzie R. *The Cervical and Thoracic Spine*. Waikanae, New Zealand: Spinal Publications; 1990.
39. McKenzie R. *Treat Your Own Back*. Waikanae, New Zealand: Spinal Publications; 1997.
40. Terrett AGJ. *Vertebrobasilar Stroke Following Manipulation*. Des Moines, IA: National Chiropractic Mutual Insurance Company.
41. Cassidy JD, Boyle E, Côté P, et al. Risk of vertebrobasilar stroke and chiropractic care. *Spine*. 2008;33:S176–S183.
42. Butler D. *Mobilization of the Nervous System*. New York: Churchill Livingstone; 1991.
43. Laslett M, Williams M. The reliability of selected pain provocation tests for sacroiliac joint pathology. *Spine*. 1994;19:1243–1249.
44. Sandoz R. Some physical mechanisms and effects of spinal adjustments. *Ann Swiss Chirop Assoc*. 1976;6:91–141.
45. Stamford B. Giving injuries the cold treatment. http://www.physsportsmed.com/issues/1996/03_96/cold.htm#avoid. Accessed December 29, 2003.

FURTHER READING

Books

Bourdillion JF, Day EA, Bookhout MR. *Spinal Manipulation*. Boston: Butterworth-Heinemann; 1992.

A good text that covers the basics. It is exceeded in many areas by Greenman's text.

Dagenais S, Haldeman S. *Evidence-Based Management of Low Back Pain*. St. Louis: Elsevier Mosby; 2007.

The North American Spine Society's up-to-date, clinically oriented, evidence-based medicine text. It details and references the description, theory, efficacy and harms of various treatment interventions for low back pain that are readily transferable to many musculoskeletal conditions.

Greenman P. *Principles of Manual Medicine*. Baltimore: Williams & Wilkins; 1996.

A great text that covers the material in a clear, understandable, and usable format.

Haldeman S. *Principles and Practice of Chiropractic*. 2nd ed. New York: Appleton-Century-Crofts; 1992.

The second edition has major changes and new contributors and is an excellent source of information on history, research, diagnosis, and treatment of all phases of manipulation, with special attention to the spine.

Liebenson C. *Rehabilitation of the Spine: A Practitioner's Manual*. 2nd ed. Baltimore: Lippincott Williams & Wilkins; 2007.

Liebenson brought together many fine contributors who address the cutting-edge methods of manual medicine. It covers much of what is needed in clinical practice. Very useful and readable. The second edition is a major update and a gold mine of information.

Maitland GD. *Vertebral Manipulation*. 4th ed. London: Butterworth-Heinemann; 1977.

A physical therapist's approach, with emphasis on patient selection, pretreatment assessment, assessment during treatment, assessment after treatment, and therapeutic approach for each area of the spine.

Maitland GD. *Peripheral Manipulation*. 3rd ed. London: Butterworth-Heinemann; 1991.

The same approach as the previous book, but for the extremities. The author suggests learning the extremities before attempting to learn spinal manipulation. After all, a joint is a joint.

Journals

Henschke N, Maher CG, Refshauge KM, et al. Prevalence of and screening for serious spinal pathology in patients presenting to primary care settings with acute low back pain arthritis. *Rheumatism*. 2009;60:3072–3080.

An in-depth look at the prevalence of red flags in primary care practice and how some red flags are positive for patients without serious pathology.

42

Nonpharmacological Control of Pain

Richard J. Kitaeff, MA, ND, Dip Ac, LAc

OUTLINE

INTRODUCTION

Pain in its myriad forms is one of the most common symptoms for which patients seek relief. Acute pain is an unpleasant experience primarily associated with tissue injury, and the protective response patients have to pain provides the clinician with valuable diagnostic information.

The reaction to pain is highly subjective and, as a function of higher centers, is extremely variable. It is influenced by many factors, depending on the individual patient and his or her situation. When pain becomes chronic, the multifactorial influences (e.g., anxiety, depression, social, cultural, and economic factors, secondary gain) play an even larger role.

When treating a patient for pain, the clinician must first determine the primary cause, the pathogenesis, and the secondary or contributing factors. The relief of pain may then be achieved by removal of the primary cause (e.g., cure of an infection), neutralization of the effect of the stimulus (e.g., emollients for an ulcer), relief of discomfort (e.g., biofeedback), suppression of the disease process (e.g., antiinflammatory agents), and dulling or obliteration of the sense of pain (e.g., analgesics or acupuncture).[1]

Although the medical profession has chosen to emphasize the pharmacological methods of pain control, many nonpharmacological options are available. Their applicability and efficacy are documented here. (Although this chapter liberally utilizes pain control in childbirth, the examples and concepts can be generalized to any situation involving acute and/or chronic pain.)

THE EXPERIENCE OF PAIN

A Psychological Model

Pain is generally acknowledged to be a complex physiological/psychological phenomenon. It involves motivational and emotional components and conceptual interpretation, which may or may not have their basis in actual nociception. Verbal reports of pain and associated behavioral responses are controlled, at least in part, by psychological, cultural, and situational factors.

For acute pain, such as that of childbirth, in which the painful experience can be directly related to nociceptive input, a multiprocess feedback model can be considered. However, one must keep in mind the complexity of the psychological processes intervening between the sensory event and the observable response, ranging from the physiological to the social aspects of personality. These include elements of the following:

- Information processing
- Performance ability
- Attention
- Memory
- Expectancy
- Attitudes and beliefs
- Secondary gain
- Self-concept
- Designated sick roles

In the psychological model, the brain infers information from bodily signs and integrates it with existing personal and situational variables to direct behavior. When consideration must also be given to the interactions with interested observers, such as physicians, family members, and birth attendants, who influence the interpretation with their own experiences and attitudes about pain, the complexity becomes even greater.

According to this model, which does not differ in essence from a general model of stress, a primary appraisal of the personal danger or threat posed by the painful stressor is followed by a secondary appraisal of one's ability to cope, based on emotional feedback, and contributions of situational and sociocultural response factors. On this basis, a woman in labor could choose to regard pain as "positive," "functional," or "creative"; "pain with a purpose"; or alternatively, "part of a process involving injury."[2] In the course of a pain management program carried out with 84 patients with low back pain, those who more strongly endorsed "organic" concepts about the nature and treatment of pain reported higher levels of disability, whereas reductions in reported "organic" pain beliefs improved

reported disability, and endorsement of "psychological" concepts about the nature and treatment of pain was not associated with disability.[3] Several studies indicate that "catastrophizing" predicts pain or is associated with lower pain tolerance.[4,5] This conceptualization of painful stress suggests that intervention could be successful at several levels: cognitive patterning, physiological arousal associated with emotional stress, and control of environmental stimuli. Examples of appropriate strategies are cognitive coping skills such as restructuring and utilization of preparatory information and attention shifts; muscular relaxation, physical or electrical stimulation, and biofeedback techniques; and structuring of the environment in a way conducive to effective coping (such as by making it nonthreatening and comfortable).[6]

Neuropsychological Mechanisms of Pain

According to research on the mechanisms of pain, pain can be treated not only by anesthetic blocks, surgical intervention, and the like but also by influencing the motivational-affective and cognitive factors.[7] The traditional specificity theory of pain, first enunciated by Descartes in the 17th century, holds that pain messages are conducted from specific pain receptors at the periphery through discrete pathways to pain centers in the brain. However, there are individual differences in pain responses, pain is not consistently stopped by cutting or blocking of the "pain pathway," and it is now known that nonpainful types of stimulation activate the A-delta and C fibers (see next paragraph) that are associated with pain. Therefore later modifications of pain theory took into account the patterning of nerve impulses over time to reflect differences in the degree and intensity of stimuli and the summation of signals from an extended area.[8]

One model of pain is the gate-control theory, which Melzack and Wall[9] formulated in 1965. Based on neurological data and a categorization of the words used to describe pain, this theory conceptualizes the pain experience as having sensory-discriminative, motivational-affective, and cognitive-evaluative components or modalities, corresponding to different patterns of nerve impulses. Neurologically, a specialized cluster of nerve cells in the substantia gelatinosa of the spinal column is thought to operate like a valve or gate, controlling nerve signals before they evoke the perception of, and response to, pain. Besides this monitoring of sensory data in the central nervous system, gating is also influenced by the relative amount of activity in large-diameter (A-beta) and small-diameter (A-delta and C) nerve fibers. The large fibers tend to inhibit transmission, or close the gate, preventing pain, and the small fibers tend to facilitate transmission, or open the gate, resulting in pain. The fact that large fibers are activated by pressure, touch, massage, and vibration suggests a mechanism for such pain control techniques as acupressure, acupuncture, and transcutaneous electrical nerve stimulation (TENS). Such stimulation apparently closes the spinal gate via the large-fiber system. Melzack and Casey[7] expanded this theory by proposing the possibility of a higher-level gate, in the reticular or limbic structures of the brain, that probably mediates the drive to escape from unpleasant stimuli. At central nervous system (CNS) levels, the biochemical mechanisms of gate control may involve the endorphins, natural morphinelike substances that have been implicated in the pain-controlling effects produced by acupuncture.[10]

Although the pain circuit generally is considered to include the thalamus, the somatosensory cortex, the anterior cingulate cortex (ACC), the amygdala, the hippocampus, and the prefrontal cortex, recent imaging studies have focused on supraspinal CNS areas associated with the experience of pain: the prefrontal cortex, anterior cingulate cortex, sensory cortex, and insula. The prefrontal cortex (often considered the "executive brain") is thought to encode cognitive aspects of acute and chronic pain, including coping ability and a general inhibitory effect on the severity of pain. The anterior cingulate cortex, part of the limbic system, relates to the affective/emotional or fear and suffering component of pain. The insula can be considered the sensory component of the limbic system. Imaging studies have recently found that different chronic pain conditions appear to involve pain condition–specific patterns of cortical activity. For example, postherpetic neuralgia and osteoarthritis of the knee appear to be represented primarily in the insula, whereas back pain appears to be associated with activity in the prefrontal cortex. Peripheral structures of the nervous system play a role mostly in long-term effects or in chronic pain. Nociceptive input from the periphery can sensitize cortical areas involved in processing, for example, in conditions such as fibromyalgia or chronic low back pain. In such chronic conditions, decreased gray matter in the prefrontal cortex may be associated with a decreased ability to inhibit the experience of pain.[11]

Neuroimaging studies have also shown the right ACC to be activated with unilateral pain. Pain memory, a learned pattern of brain activity, is involved in the central sensitization model of chronic pain, known as "kindling": repeated exposure to painful stimuli leads to increased sensitivity to later noxious stimuli.[12]

The implication of these observations of the brain areas and processes involved in the perception and processing of pain is that a "top-down" psychological process of cognition operated by the neocortex can inhibit lower brain functions (such as emotions) of the subcortical limbic system, as in cognitive learning strategies and techniques such as eye movement desensitization and reprocessing (EMDR).[12]

Toxins that Increase Pain Sensitivity

Contemporary Western diets contain acid precursors more than base precursors, yielding a daily systemic net acid load of varying amounts. For example, when salt (NaCl) is added to the diet, it becomes a condiment, not a requirement, and when consumed acutely in large quantities, NaCl can be noxious. High protein intake and phosphate-rich soft drinks also contribute to an increased load of dietary acid. A blood pH constantly at the lower end of the normal range has been termed *latent acidosis*. It has been suggested that latent chronic acidosis might contribute to an increase in pain symptoms resulting from swelling of connective tissue because eof changed acid–base homeostasis.[13]

Inorganic arsenic exposure exacerbates the degree of inflammatory pain (i.e., enhances pain perception) and could worsen the pathological state of painful inflammatory diseases.[14] Lipopolysaccharides (LPSs), also known as endotoxins, from the cell walls of gram-negative bacteria have been shown to increase sensitivity to visceral and somatic pain and increase musculoskeletal pain sensitivity correlating with both changes in IL-6 and negative mood.[15]

Pain in Childbirth

A psychological/social learning approach to pain emphasizes control of motivation, expectation, focus of attention, and stress and feelings of anxiety, depression, and helplessness. Factors specifically operative in labor pain involve these in addition to social support and the physiological factors of hunger, rest, and muscular tension.[16] All of these factors can contribute to the interpretation of pain being placed on the nociceptive message provided by uterine contractions. The influence of motivation on labor pain was effectively demonstrated in a prospective study of maternal attitudes toward pregnancy in 8000 American women. One of the factors found to be strongly related to maternal attitude toward having a baby was the need for analgesics in labor.[17]

Cultural conditioning may also be fundamental to the labeling of childbirth as painful. Throughout most of the world, analgesics are not required for labor; in fact, a Japanese anesthesiologist suggests that the idea of "painless delivery" is a strange one to his culture.[18] American women, on the other hand, "live through a largely self-fulfilling prophecy of birth as a painful, terrifying ordeal, and/or as a medical, drugged process over which they have no control."[16] This idea relates to body fantasies of injury, brought about in a hospital environment where distress is an expected response to the expulsive reflex.[2]

PAIN CONTROL

Moderating Variables and Psychological Techniques

Psychological Strategies

The psychological strategies recommended for control of labor pain, many of them part of prepared childbirth programs, generally aim to provide control, communication, relaxation, attention focus, and support in addition to physical counterstimuli. Considerable psychological research supports the use of these strategies in the development of pain tolerance.

The significance of various characteristics of an individual's psychological profile has been studied by evaluating the effects on pain perception of such parameters as the following:

- Introversion–extroversion[19–21]
- Augmenters–reducers[22]
- Field dependence[23,24]
- Repression–sensitization[25–27]

For example, on the repression–sensitization axis, repressors may be characterized as people who avoid having to cope with pain. Sensitizers, however, have an obsessive need to cope; they like to be informed in advance about the situation and to have control over it. The superior initial tolerance exhibited by repressors in response to heat and pressure stimuli disappears in repeated trials, showing that the sensitizers' predilection for challenge enables them to endure long-term pain better.

The importance of individual difference variables is also illustrated by the observation that one third of patients undergoing surgical operations do not request pain-killing medication.[28] This common ability to suppress pain indicates that not all surgical patients consider themselves passive victims. In fact, during the postoperative period, pain persists longer for those who accept medication.

Cognitive Strategies

The impetus for devising cognitive strategies to promote tolerance of pain has been particularly supported by investigations showing that pain tolerance increases with greater predictability and perception of control.[29–33] Similarly, preparatory communications and information received before the onset of experimental or surgical pain consistently decrease the subjects' perception of pain.[34–36] Animal studies have demonstrated higher rates of instrumental responses when painful shocks are signaled than when they are unsignaled.[37] Kanfer and Seidner[38] found that subjects who could advance slides of travel pictures at their own rate tolerated iced-water immersion of the hand longer than yoked subjects whose slides were changed by the experimenter.

When surgical patients were given a sense of control by being provided with preparatory information about postoperative discomforts and operative care, in combination with training in rehearsal of realistic, positive aspects of the surgical experience, they showed a significant reduction in postoperative anxiety (as indicated by nurses' observations), requests for sedatives, and length of hospital stay.[39] Furthermore, preparation for second periodontal procedures by auditory and visual messages classified as "control enhancement" was associated with a reduction of pain after a second operation.[40] Subjects who could cognitively redefine a threat of electric shocks as interesting new physiological sensations also reduced stress to a greater extent than subjects not provided with this coping strategy.[41]

A typical cognitive behavioral procedure utilizes "stress inoculation," beginning with an educational phase (in which the client is given a conceptual framework for understanding the nature of his or her stressful reactions), followed by rehearsal of behavioral and cognitive coping skills, based on a set of coping self-statements generated by the client in collaboration with the therapist. Such cognitive-behavioral techniques, sometimes in combination with electromyography (EMG) biofeedback control, have been found successful in the treatment of chronic low back pain.[42–44] Also, cognitive-behavioral strategies have been effective in alleviating the pain of irritable bowel syndrome,[45] temporomandibular joint syndrome,[46,47] cancer,[48] migraine headaches,[49] rheumatic conditions,[50–52] fibromyalgia,[53] and complex regional pain syndrome.[54] Low back pain has been effectively controlled utilizing cognitive-behavioral or social learning strategies addressing fear-avoidance beliefs[55] or catastrophizing,[56b] whereas voxel-based morphometry (VBM) has revealed structural changes in regions of the brain with higher levels of pain intensity and negative cognitions in cases of low back pain.[57] Older adults given pain-coping skills training (PCST) were able to control the pain of osteoarthritis of the knee.[58]

This emphasis on conceptualization, preparatory information, and cognitive transformation seems to have been incorporated into the Read method of natural childbirth, which replaces fear with knowledge about birth.[17] Sheila Kitzinger,[2] in her method of prepared childbirth, similarly emphasizes the necessity of "acquiring knowledge and understanding of what labor involves, the terminology used by obstetricians and midwives, and information about what happens in hospitals."

A study by Stevens and Heide[59] conducted at the University of Wisconsin used iced water to test perception and endurance of pain in subjects who had been taught methods used in childbirth education classes. The control subjects for this training and an additional control group were offered only distraction during the tests. Those who had been taught the techniques reported only about half the pain of that reported by control subjects and endured it 2.5 times longer. The prepared childbirth strategies improved with practice, were effective for pain lasting longer than most contractions in labor, and were more effective than distraction techniques.[59] However, this last finding introduces some confusion because some prepared childbirth methods include either distraction techniques or some other deliberate disposition of one's attention.

Attention Focusing

Distraction or focused attention, mostly utilizing the rhythms of the breath, is essential to the Lamaze method, the most popular prepared childbirth program in America, and is important in the Bradley and other methods. Sheila Kitzinger[2] describes the controlled attention focusing as:

> *concentration on what is happening, one's response to it as a task, and visualization of what is being achieved by the work of the uterus during contractions. The focus may be on the fantasy of the contractions as a shape provided by actual objects (furniture, architectural details, flowers, a painting) in the room, or a combination of these factors.*

Stevens and Heide[59] found that attention focusing functions effectively as analgesia for labor pain. Such strategies are strongly supported by much psychological research. Hospitalized children with chronic

illnesses who were taught distraction techniques were able to reduce measures of distress before and during medical procedures such as intramuscular and intravenous injections.[60] In a study of patients with burn pain, sensory focusing techniques were more successful than distraction techniques in controlling pain, and both were more successful than standard care.[61] The focus may be on a competing response, as in a study by Kanfer and Goldfoot[62] showing that when attention was directed to self-presented external slides, individuals were able to increase their tolerance of the pain of cold water. Focus on a competing response is also shown in the use of hypnosis as an analgesic and in the meditative states of Raj yogis, who pinpoint attention on the tip of the nose or a point on the back of the skull and then do not react physiologically to cold water, bright lights, or sudden sounds.[63,64] Other adepts in unusual feats of pain tolerance, such as having spikes stuck through the skin, either maintain an unfocused attitude, without evaluation, or pinpoint attention totally on the pain but without evaluation.[65] In such cases the attitude of detachment from the pain can be reflected by an undisturbed electroencephalography (EEG) pattern of alpha or beta waves throughout the performance of the feat.

Internet-Based Cognitive-Behavioral Therapy

Numerous recent studies have supported the effective use of the Internet and smartphones to implement cognitive learning strategies for pain management. Based on a meta-analytic review of 23 randomized controlled trials of guided Internet-based cognitive-behavioral therapy (ICBT), improvement was shown in general psychological outcomes, disease-related physical outcomes, and disease-related effect on daily life.[66] Specifically, chronic pain has been reduced through smartphone and Internet applications employing online journals that facilitate improved communication between patient and clinician. Feedback and coaching were provided to patients via Twitter postings and blogs, and online resources and blogs provided patient education.[67] In a randomized controlled trial, an 8-week Internet-based exercise program significantly improved pain and global health status for 81 breast cancer survivors.[68] A guided Internet-delivered self-help cognitive-behavioral therapy program was successful in treating 69 adolescents with chronic pain.[69] A smartphone-based intervention with diaries and personalized therapist feedback based on CBT principles, in addition to access to a website for self-management, reduced pain scores for 140 women with widespread chronic pain.[70] Internet-based telemonitoring of fibromyalgia patients in a 24-week multicomponent intervention influenced key health outcomes.[71]

Virtual Reality for Pain Management

A review of six studies showed that the distraction provided by the virtual reality (VR) technique reduced acute and chronic pain in adults and children in conditions such as headaches or fibromyalgia, sometimes combining VR with modalities such as biofeedback and cognitive-behavioral therapy. At the same time, VR actually seemed to produce neurophysiological changes.[72] Pain was reduced significantly for 50 medical inpatients viewing a three-dimensional (3D) VR experience using the Samsung Gear Oculus VR headset.[73] Thirty patients with various chronic pain conditions utilized a 5-minute head-mounted VR display to reduce their pain rating on a visual analog scale by 33%.[74] Twelve patients with chronic intractable phantom limb pain using machine learning, augmented reality, and VR showed statistically and clinically significant improvement in all metrics of their phantom limb pain.[75]

Relaxation Training

Relaxation training, another essential method of pain control, is found in all childbirth training programs. A considerable body of literature supports its importance in pain control because a state of lowered autonomic arousal is incompatible with anxiety. Although progressive muscular relaxation, systematic desensitization, and autogenic training are all well-established physiological approaches to muscular relaxation, meditation traditions provide quicker methods to achieve what Benson[76] has called the "relaxation response." One of the simplest meditation practices—maintaining a focal awareness of the flow of breath—is taught by Rahima Baldwin[11] in *Special Delivery* and is identical to the ancient practice of vipassana, or insight meditation.

Hypnosis

Hypnosis or autohypnosis is another method used to induce deep relaxation for pain control. It incorporates many of the therapeutic elements already referred to—focused attention, positive expectation, and a supportive or permissive attitude—in making suggestions that alleviate anxiety. Thus its success in pain management may be viewed from a cognitive-behavioral perspective.[77] In one technique, "glove anesthesia" is induced in one hand, and the "numb, heavy wooden feeling" so produced is transferred to the other hand, the face, and eventually to the abdomen to "relieve the discomfort" of uterine contractions (the word *pain* is never used because its use would be countersuggestive).[78] Pain modulation in "highly hypnotizable" subjects has been confirmed through brain measurement of somatosensory event–related potentials (SERPs) to noxious stimuli, with the highest amplitudes for these subjects recorded at frontal and temporal scalp sites.[79]

Control of Environmental Stress

Kitzinger[2] cites animal research to show how environmental stress can interfere with the physiological processes of labor and delivery. Education for childbirth therefore promotes verbal and nonverbal support from the husband, obstetrician, midwife, or anyone else who is part of the birthing environment. Touch relaxation and coaching techniques combine the essential elements of relaxation, massage counterstimulus, and the direct supportive communication of a partner.[11] Several studies agree that comfort in labor is also enhanced by a more vertical position, such as the squatting posture that is adopted in many other cultures.[80–82]

Counterstimulus Methods: Massage, Acupuncture, Transcutaneous Electrical Nerve Stimulation

The hand reflexology method of grasping combs during labor to activate points on the fingertips and balls of the hand that relate to uterine functioning is one example of counterstimulus strategy.[11] Foot reflexology, acupressure, acupuncture, and TENS might also share a common autonomic nervous mode of operation.

Transcutaneous Electrical Nerve Stimulation

The use of TENS to control pain during delivery has been evaluated by several studies. The method used in a Swedish study,[83] which was subsequently replicated in Germany and Britain, was originally developed in the United States by Shealy and Maurer[84] for the control of acute and chronic pain. In a controlled study of experimentally induced cold-pressor pain, the effect of electrical stimulation with TENS electrodes at two traditional acupoints in 20 subjects had an analgesic effect with statistical significance comparable to that of morphine, and the combined effect of TENS with morphine was stronger than TENS alone.[85] A series of controlled randomized double-blind studies on carpal tunnel syndrome pain found that the combination of low-level laser and microcurrent TENS on distant and local points significantly decreased McGill Pain Questionnaire score, sensory and motor latencies, and Phalen and Tinel signs compared with sham treatment.[86]

A British study investigating the relative hypoalgesic effects of different TENS parameters upon experimentally induced mechanical pain found that low-frequency, high-intensity, extrasegmental stimulation (i.e., over an acupuncture point rather than over a nerve distribution) produced a rapid-onset hypoalgesic effect, which increased during the stimulation period and was sustained for 30 minutes after stimulation.[87]

In TENS, the electrodes are generally placed over the painful area to stimulate the cutaneous nerves in that area. For use in labor, four electrodes are placed on either side of the midline of the spine to stimulate the posterior primary rami of the spinal segments (T11–L1 and S2–S4) receiving the painful stimuli during labor. It is interesting to note that these are the loci of acupuncture points (BL-20, BL-27, and BL-28) that are traditionally thought to reflect female reproductive function.

The selection of this area for stimulation is based on Bonica's account of the neurological mechanism of delivery pain.[88] During the first stage, pain receptors are assumed to be activated by contractions of the uterus and dilation of the cervix. The evoked impulses are mediated in afferents that run in the hypogastric nerves and reach the spinal cord via the dorsal roots T10 to L1. The pain is referred to large areas of the abdomen and back. During the second stage, pain is also caused by distention and stretching of the delivery canal, the pelvic floor, the vulva, and the perineum. The pain is localized, and the impulses reach the spinal cord mainly via the pudendal nerves and the dorsal roots S2 to S4. The pain during the first stage is characterized as an ache, considered to be mediated in small-diameter C fibers. During the second stage, the pain has the more localized intensive nature usually identified with the delta afferent fibers.[88,89]

In the typical application of this technique for control of pain during labor, low-intensity stimulation is given continuously, and a high-intensity stimulation can be initiated by the parturient herself whenever pain increases. Stimulation via the thoracic electrodes is maintained throughout the delivery at an amplitude that is maximal for a pleasant sensation, whereas sacral stimulation is added from the later part of the first stage. Table 42.1 summarizes the uniformly good results that have been reported.

Those patients who complained of backache have especially appreciated it. An Austrian study compared the analgesic effects of TENS, pethidine, and placebos on labor pain in 30 parturient women during the first stage of labor. No significant difference was found between the placebo, unspecific TENS, and control groups in the increase in pain during the test period. Patients who had received pethidine and those who had been given TENS experienced considerable relief of pain.[90] It is curious that apart from a passing reference by Shealy and Maurer[84] to its use in labor, no research on the obstetrical application of TENS appeared for many years in any of the U.S. literature. A 1996 review of 30 studies on TENS stimulation of acupuncture points in labor substantiated the conclusions of earlier research.[91]

TABLE 42.1 The Results of the Use of Transcutaneous Electrical Nerve Stimulation for Pain Control in Labor

Study	No. of patients	RESULTS (%)		
		Good	Moderate	None
Augustinsson et al.[83]	147	44	44	12
Andersson et al.[94]	27	48	37	15
Kanfer and Goldfoot[62]	35	20	62	18
Stewart[93]	67	31	56	13
Kubista et al.[138]	102	55	24	21
Bundsen et al.[139]	347	47	42	11

In view of the relatively good results and lack of complications, the consensus of all the preceding studies is that the TENS method is recommended as a primary pain-relieving measure, to which conventional methods can be added as needed. Robson[92] comments that TENS is noninvasive and is believed to be safe for both mother and baby. It is easy to apply and can be operated throughout labor by the doctor, midwife, father, or mother. Augustinsson et al.[83] were most impressed by the lack of complications because the conventional methods, including analgesic and sedative drugs, nitrous oxide inhalation, epidural anesthesia, and local blockades, all possess a varying level of potential risk.[83] Another advantage is that TENS, because it does not give complete analgesia, does not eliminate pain as a diagnostic tool; it can be interrupted whenever needed for clinical evaluation. More important, perhaps, from the point of view of the woman in labor is the fact that her consciousness is not altered to the point of excluding her own active participation in, and experience of, the delivery.

Both Stewart[93] and Augustinsson et al.[83] reported TENS alone to be inadequate for analgesia in the second stage of labor. The second group of researchers regards this difference as possible support for the assumption that C fiber–mediated pain is more amenable to blocking by electrical stimulation than A fiber–mediated pain. Stewart[93] mentions simply that many women did not wish to use the stimulator at that stage because it proved a distraction from their efforts to bear down. In this connection, it is interesting to note that "those who were well prepared and keen on natural childbirth were not always the most enthusiastic and, in fact, two of the early failures were patients who had been to relaxation classes."[93] Robson[92] explains that TENS could distract some patients from their breathing or other focus of attention learned in childbirth preparation classes.

A related issue in the TENS literature is introduced by the comment of Andersson et al.[94] that there was a correlation between the level of hypnotizability and that of pain relief in their subjects. Such a correlation may, of course, imply only susceptibility to any type of therapeutic effect. Neumark et al.[90] tested this effect by including a placebo group that was given no current through the electrodes; these researchers found that the result for the placebo group was not different from that of TENS applied nonspecifically (i.e., incorrectly) but was significantly different from the effect of TENS placed over the relevant nerve distribution and from that of pethidine. Robson,[92] although making no attempt to assess a patient's level of susceptibility to hypnosis, switched off the TENS machine for at least two contractions. All patients asked for it to be switched on again, indicating that the technique was providing pain relief. Augustinsson et al.[83] consider the suggestive effect, if it occurs, to be of minor significance because several investigators have found the pain-reducing effect of TENS to be achieved through demonstrable neurophysiologic mechanisms. Stewart[93] points out that the greater personal contact between patient and attendant essential to the use of this method may introduce an element of suggestibility or distraction that affects the pain experience.

Acupuncture

Thousands of studies have investigated the efficacy and mechanisms of acupuncture analgesia for acute and chronic pain, in surgical operations, and in childbirth. In a review article of 24 studies, Lewith and Machin[95] found that the typical clinical trial showed a 70% efficacy of acupuncture compared with placebo treatment. Reichmanis and Becker[96] found similar results in a review of 17 studies of acupuncture analgesia in experimentally induced pain. A systematic review and meta-analysis concluded that acupuncture relieved pain within 30 minutes of treatment, compared with sham acupuncture and

analgesic injection.[97] In a randomized controlled trial, electroacupuncture administered together with general anesthesia for 64 patients receiving surgical operations in a Malaysian hospital significantly reduced pain ratings and total morphine requirements.[98] On the basis of a thorough review of the clinical and experimental research on acupuncture pain control, Stux and Pomerantz[99] concluded that acupuncture analgesia helps from 55% to 85% of patients with chronic pain, comparing favorably with the effects of potent pain medication (such as morphine, which is 70% effective), and clearly distinct from the placebo effect, which helps 30% to 35%.[99] At the same time, somatosensory EEG–evoked potential studies have provided objective evidence of the analgesic effect of acupuncture.[100–102]

Recent randomized and double-blinded controlled studies have demonstrated the clinical effectiveness of acupuncture in treating chronic lateral epcondylitis[103] and chronic neck pain.[104] In the neck pain study, stimulation at distant points of the neck-related meridians were more effective than sham acupuncture points and "dry-needle" injections of local myofascial trigger points, reducing motion-related pain by one third after a single treatment. A single-blind randomized controlled trial showed that electroacupuncture (EA) is superior to manual acupuncture in treating patients with tennis elbow.[105] A review article on the treatment of fibromyalgia pain for patients at a hospital in Brazil showed improvement with traditional acupuncture, measured on subjective scales and number of tender points.[106] Preoperative EA has led to a reduction in the intraoperative and postoperative requirement for analgesic medications (alfentanil and morphine) in patients receiving gynecological lower abdomen surgery.[107] In fact, surgery that requires general anesthesia in Western countries is routinely performed in Chinese hospitals with the combination of acupuncture and local anesthesia for pain control, providing a considerable decrease in risk for surgical patients. When acupuncture was combined with Western (pharmacological) medicine in the treatment of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), the combined treatment approach was more effective on measures of pain and other symptoms than either acupuncture or Western medicine alone.[108] A randomized trial treating 50 cases of osteoarthritis of the knee, using warm-needling moxibustion, another traditional method of stimulating acupuncture points, was found to significantly relieve pain, improve function and muscle balance, and strengthen extensor and flexor muscle power.[109]

Hyodo and Gega[18] of the Osaka Medical College have reviewed the literature (summarized in Table 42.2) on acupuncture anesthesia and analgesia in normal delivery and found mixed results. For example, Wallis et al.[110] reported that although 19 of their 21 volunteer parturients considered acupuncture unsuccessful in providing analgesia for labor, one third indicated that they would choose acupuncture analgesia in labor again. Some researchers criticize the technique as being inconsistent, unpredictable, incomplete, and time-consuming and interfering with movement and electronic monitoring.

In their own study, Hyodo and Gega[18] tested 32 patients, equally divided between primiparas and multiparas. Low-frequency electrical current was introduced through needles at LI-4, ST-36, and SP-6, a standard therapeutic repertory for sedation of the reproductive organs. The results were assessed in terms of relief noted by the patient (subjective scale) and through the obstetrician's observation (objective scale); among the primiparas, 62.5% found good or excellent effect on the subjective scale, and 62.6% found good or excellent effect on the objective scale; among the multiparas, 93.8% had subjective relief, and 93.7% had objective relief. Overall, 90% of the patients experienced relief of pain within 20 minutes of initiation of acupuncture anesthesia. These researchers noted the considerable disparity in reports of the effectiveness of acupuncture from Japan and the United States, explaining it as a novelty effect. It is natural that in Japan, where no analgesic methods are normally used, the scoring in favor of acupuncture would be high compared with that in the United States.

TABLE 42.2 Results of Acupuncture Analgesia in the Control of Labor Pain

Study	No. of Patients	RESULTS (%) Good	Poor or None
Hyodo and Gega[18]			
Primiparas	16	62.5	37.5
Multiparas	16	93.7	7.3
Ito[140]	80	85	15
Wallis et al.[110]		9–33	67–91
Abouleish and Depp[112]		80.5	19.5

Hyodo and Gega[18] concluded that acupuncture analgesia is useful for delivery, especially because of its safety, even though its results are more erratic and less potent than those of conventional anesthetic techniques. More recently, a randomized controlled trial investigating acupuncture treatment as a complement or an alternative to conventional analgesia for labor in a Swedish hospital found that acupuncture significantly reduced the need for epidural analgesia, and parturients receiving acupuncture achieved a better extent of relaxation than the control group.[111]

A considerable amount of research has focused on determining a mechanism for acupuncture analgesia. A 1995 review of studies on acupuncture effects in pain and disease pointed out that, like exercise, acupuncture produces rhythmic discharges in nerve fibers and causes the release of endogenous opioids and oxytocin. Furthermore, "experimental and clinical evidence suggests that acupuncture may affect the sympathetic system via mechanisms of the hypothalamic and brain-stem levels."[112] Animal studies continue to demonstrate that acupuncture analgesia is mediated in the central and peripheral nervous systems by opioid peptides.[113–115] The cortex and hippocampus appear to participate in the modulation of chronic pain, and the analgesic action of EA seems to operate along this pathway.[114] A study carried out on dogs seems to verify the traditional theory of points of tonification and sedation, through differential production of sympathomimetic and parasympathomimetic effects on the cardiovascular system upon stimulation of different points. Carlsson,[116] a Swedish researcher, concludes that a mechanism for therapeutic acupuncture must include peripheral events that release neuropeptides; spinal mechanisms such as gate control; and supraspinal mechanisms through the descending pain inhibitory system, the sympathetic nervous system, and the hypothalamic–pituitary–adrenal (HPA) axis. He cautions that much of animal and human experimental acupuncture research shows only short-term hypoalgesia, that almost all such experimental research has been performed with EA rather than the more gentle manual style of therapeutic acupuncture, and that pain threshold elevation in human experimental research does not necessarily predict the clinical outcome.

In a study of labor induction and inhibition by EA, Tsuei et al.[117] utilized SP-6 and SP-4 points, which are located in the territory of the L4 dermatome. The spleen meridian, to which these loci belong, runs across the dermatomes of L4, L5, L2, and L1, then upward from T12 to T5. Because the sympathetic nerve controlling the uterus through the pelvic plexus receives preganglionic fibers from T5 to L4, Tsuei et al.[117] concluded that it is highly possible that stimulation of the electropermeable loci within this area may alter the physiological function of the uterus. The LI-4 points of the upper extremities, often added to the spleen meridian points in the acupunctural control of labor pain,

perhaps represent the central approach to the autonomic nervous system because these loci control pain to the head and neck. It should be noted, however, that Motoyama,[118] who has attempted to verify the traditional subtle anatomy of meridian pathways through tests of electrocutaneous resistance at meridian points, claims that these effects cannot be adequately explained in terms of the conventional sympathetic dermatomes but instead imply an alternative bioelectric transmission system.

The discovery of the Head McKenzie sensory zones has shown the possible mediation of the invisible meridians and points of traditional Eastern medicine between internal organs and corresponding skin areas. Nakatani[119] was able to detect the electropermeable line as an apparent viscerocutaneous autonomic nerve reflex when organic diseases were involved. Hyodo[120] has explained acupuncture stimulation as the transmission of impulses centrally from the reactive electropermeable loci, via a sympathetic afferent fiber, and noted that the autonomic nerve in the viscera is stimulated to response by the reverse of the McKenzie theory. An exciting recent development in acupuncture research has been the finding by functional neuroimagery of neuronal correlates of acupuncture stimulation in the human brain. In a controlled study of EA stimulation of GB-34 on the left leg, real EA elicited significantly higher activation over the hypothalamus and primary somatosensory motor cortex than mock and minimal EA, showing that the hypothalamic–limbic system was significantly modulated by EA at acupoints.[121]

Other Methods

The scope of this chapter does not permit a detailed discussion of other promising methods of pain control, but mention can be made of massage, biofeedback, and nutritional and botanical agents for the treatment of pain. A literature search led to the conclusion that massage, particularly acupressure, is effective for low back pain, especially when it is combined with exercises and education, with beneficial effects lasting at least 1 year after the end of treatment.[122] A vast literature is available on biofeedback training for pain. A meta-analysis of 21 controlled studies of biofeedback for chronic low back pain found improvements in pain intensity and other associated measures, both short term and long term.[123] BrightHearts is a biofeedback-assisted relaxation device for children, an iPad app paired with a wireless heart monitor. The application displays a digital geometrical design that responds to changes in heart rate. By slowing the heart rate, children could be taught to achieve relaxation and manage procedural pain and anxiety.[124] Another article suggesting a future direction in pain medicine describes the use of "off-the-shelf," low-cost, and low-bandwidth telemedicine equipment to deliver clinical biofeedback treatment when the patient and provider are in two different locations.[125] In a randomized double-blind trial of 30 patients with chronic maxillofacial pain, a significant reduction in pain scores and an improvement in the tolerance of experimentally induced dental pain were achieved with administration of 3 g daily of the amino acid tryptophan and a high-carbohydrate, low-fat, low-protein diet in comparison with placebo.[126] Among botanical agents for pain, corydalis rhizome or tuber (yanhusuo) is well known in the Chinese materia medica for its analgesic effect, containing combined alkaloids found to be 40% as effective as morphine. Acting probably through inhibition of the reticular activating system, corydaline was shown in one clinical study to decrease or relieve pain in 32 of 44 patients with dysmenorrhea.[127] Casperome, an extract of *Boswellia serrata,* was found effective in pain reduction in several randomized clinical studies of common inflammatory conditions, and the plasma levels of inflammatory markers were also reduced.[128] Based on an extensive literature review, specific traditional medicinal plants and isolated compounds originating from India have been reported as effective for the management of neuropathic pain.[129] Statistically significant improvement in pain resistance and suppression of edema were found in animals treated with UP 1306, a standardized blend of extracts from *Acacia catechu* and *Morus alba,* which was determined through assays to operate as a COX-2 inhibitor, comparable to a 200-mg/kg dose of ibuprofen.[130]

A current controversy revolves around the issue of whether there is objective evidence to support the widespread anecdotal testimonials for the success of cannabis products in controlling pain. An overview of systematic reviews on the efficacy of cannabis-based medicines in neuropathic pain and painful spasms in multiple sclerosis, in addition to the pain of rheumatic diseases and cancer, concluded inconsistent findings.[131] At the same time, another report found inhaled (smoked or vaporized) cannabis consistently effective in reducing noncancer pain, whereas oral cannabinoids seemed to improve the chronic pain of cancer but not acute postoperative pain, chronic abdominal pain, or rheumatoid pain.[132] Yet another study found limited evidence for the benefit of THC/CBD spray in the treatment of neuropathic pain and inadequate evidence for any benefit in the treatment of cancer or rheumatoid pain.[133]

CONCLUSION

This chapter has presented many of the current nonpharmacological strategies for the control of pain. Because the mechanism of pain perception has been shown to involve both physiological and psychological components, the optimal treatment might combine psychological factors, such as preparatory information, attention focus, relaxation, and supportive communication, with the physical stimuli of TENS or acupuncture. In fact, such a multidisciplinary approach to patients with chronic back pain was evaluated after a 4-week program that included back schooling, psychological intervention, and treatment by acupuncture, chiropractic, the Alexander technique, and a pain specialist. Significant improvement was maintained for a period of 6 months.[134]

Dental researchers of the Pediatric Pain Program of the University of California at Los Angeles School of Medicine searched several databases for reports of randomized controlled clinical trials of complementary and alternative medical modalities used to treat chronic facial pain. Three acupuncture trials, eight biofeedback trials, and three relaxation trials met the researchers' inclusion criteria, suggesting that these modalities were comparable to conventional treatment such as an intraoral appliance.[135]

Increasingly, the multidisciplinary pain management team, incorporating a variety of nonpharmacological treatment modalities, is being considered "the optimal method for delivery of comprehensive treatment to patients in pain."[136] The incorporation of "alternative" forms of pain management, including acupuncture, relaxation techniques, hypnosis, biofeedback, and guided imagery, is acknowledged by the Children's Hospital of the Medical College of Wisconsin to complement pharmacological management of children's pain.[137] The selection, balance, and application of these treatment components should be based on consideration of an individual's coping styles. Such a treatment program could be developed to provide more consistently effective analgesia than the individual components can provide separately. Relieving the pain of childbirth, for example, without diminishing or distorting the full consciousness of the experience for the mother, would be consistent with the goals of the contemporary physician of natural medicine.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Krupp MA, Chatton MJ. *Current Medical Diagnosis and Treatment*. Los Altos, CA: Lange Medical; 1984:1–5.
2. Kitzinger S. Pain in childbirth. *J Med Ethics*. 1978;4:119–121.
3. Walsh DA, Radcliffe JC. Pain beliefs and perceived physical disability of patients with chronic low back pain. *Pain*. 2002;97:23–31.
4. Haythronthwaite JA, Lawrence JW, Fauerbach JA. Brief cognitive interventions for burn pain. *Ann Behav Med*. 2001;23:42–49.
5. Piira T, Taplin JE, Goodenough B, von Baeyer CL. Cognitive-behavioural predictors of children's tolerance of laboratory-induced pain: implications for clinical assessment and future direction. *Behav Res Ther*. 2002;40:571–584.
6. Kitaeff R. *Cognitive Strategies for Control of Painful Stress*. Unpublished manuscript; 1979.
7. Melzack R, Casey KC. Sensory, motivational and central control of pain. In: Kenshalo DR, ed. *The Skin Senses: Proceedings*. Springfield, IL: Charles C Thomas; 1968:423–443.
8. Feurerstein M, Skjei E. *Mastering Pain*. New York: Bantam; 1979:17–21.
9. Melzack R, Wall PD. Pain mechanisms: a new theory. *Science*. 1965;150:971–979.
10. Cheng RS, Pomeranz B. Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms: endorphin and non-endorphin systems. *Life Sci*. 1979;25:1957–1962.
11. Jensen Mark P. A neuropsych model of pain: research and clinical implications. *J Pain*. 2010:2–12.
12. Grant M. Neuropsychology of chronic pain. *Neuropsychotherapist*. 2015;24:15–17.
13. Vormann J, Worlitschek M, Goedecke T, Silver B. Supplementation with alkaline minerals reduces symptoms in patients with chronic low back pain. *J Trace Elem Med Biol*. 2001;15(2-3):179–183. PubMed PMID: 11787986.
14. Aguirre-Banuelos P, Escudero-Lourdes C, Sanchez-Pena LC, Del Razo LM, Perez-Urizar J. Inorganic arsenic exposure affects pain behavior and inflammatory response in rat. *Toxicol Appl Pharmacol*. 2008;229(3):374–385. PubMed PMID: 18329681.
15. Wegner A, Elsenbruch S, Maluck J, et al. Inflammation-induced hyperalgesia: effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia. *Brain Behav Immun*. 2014;41:46–54. PubMed PMID: 24814500.
16. Baldwin R. *Special Delivery: the Complete Guide to Informed Birth*. Millbrae, CA: Les Femmes; 1979.
17. Laukaran VH, van den Berg BJ. The relationship of maternal attitude of pregnancy outcomes and obstetric complications: a cohort study of unwanted pregnancy. *Am J Obstet Gynecol*. 1980;136:374–379.
18. Hyodo M, Gega O. Use of acupuncture anesthesia for normal delivery. *Am J Chin Med*. 1977;5:63–69.
19. Davidson PO, McDougall CE. The generality of pain tolerance. *J Psychosom Res*. 1969;13:83–89.
20. Eysenck S. Personality and pain assessment in childbirth of married and unmarried mothers. *J Mental Sci*. 1961;107:417–429.
21. Levine F, Tursky B, Nichols D. Tolerance for pain, extroversion and neuroticism: failure to replicate results. *Percept Mot Skills*. 1966;23:847–850.
22. Morgan AH, Lezard F, Prytulak S, Hilgard ER. Augmenters, reducers, and their reaction to cold-pressor pain in waking and suggested hypnotic analgesia. *J Pers Soc Psychol*. 1970;16:5–11.
23. Mumford JM, Newton AV, Ley P. Personality, pain perception and pain tolerance. *Br J Psychol*. 1973;64:105–107.
24. Sweeney DR, Fine BJ. Pain reactivity and field dependence. *Percept Mot Skills*. 1965;21:757–758.
25. Andrew J. Coping style, stress-relevant learning and recovery from surgery. *Diss Abstr*. 1968;28:1182–1183.
26. Davidson PO, Bobey MJ. Repressor-sensitizer differences on repeated exposure to pain. *Percept Mot Skills*. 1970;31:711–714.
27. Cohen F, Lazarus R. Active coping processes, coping dispositions, and recovery from surgery. *Psychosom Med*. 1973;35:375–389.
28. Chapman CR. *Lecture*. University of Washington; 1979.
29. Bowers K. The effects of UCS temporal uncertainty on heart rate and pain. *Psychophysiology*. 1971;8:382–389.
30. Bandler RJ, Madaras GR, Bem DJ. Self-observation as a source of pain perception. *J Pers Soc Psychol*. 1968;9:205–209.
31. Geer JH, Davison GC, Gatchel RI. Reduction of stress in humans through non-veridical perceived control of aversive stimulation. *J Pers Soc Psychol*. 1970;16:731–738.
32. Pervin L. The need to predict and control under conditions of threat. *J Pers Soc Psychol*. 1963;31:570–585.
33. Staub E, Tursky B, Schwartz GE. Self-control and predictability: their effects on reactions to aversive stimulation. *J Pers Soc Psychol*. 1971;18:157–162.
34. Johnson JE. Effects of accurate expectations about sensations on the sensory and distress components of pain. *J Pers Soc Psychol*. 1973;27:261–275.
35. Neufeld RW, Davidson PO. The effects of vicarious and cognitive rehearsal on pain tolerance. *J Psychosom Res*. 1971;15:329–335.
36. Staub E, Kellett DS. Increasing pain tolerance by information about aversive stimuli. *J Pers Soc Psychol*. 1972;21:198–203.
37. Seligman M, Maier S, Solomon R. Unpredictable and uncontrollable aversive events. In: Brush F, ed. *Aversive Conditioning and Learning*. New York: Academic Press; 1969.
38. Kanfer FH, Seidner ML. Self-control: factors enhancing and tolerance of noxious stimulation. *J Pers Soc Psychol*. 1973;25:381–389.
39. Langer E, Janis I, Wolfer J. *Effects of Cognitive Device and Preparatory Information on Psychological Stress in Surgical Patients*. Unpublished manuscript; 1973.
40. Croog SH, Baume RM, Nalbandian J. Pain response after psychological preparation for repeated periodontal surgery. *J Am Dent Assoc*. 1994;125:1353–1360.
41. Holmes DS, Houston BK. Effectiveness of situation redefinition and affective isolation in coping with stress. *J Pers Soc Psychol*. 1974;29:212–218.
42. Newton-John TR, Spence SH, Schotte D. Cognitive-behavioural therapy versus EMG biofeedback in the treatment of chronic low back pain. *Behav Res Ther*. 1995;33:691–697.
43. Vlaeyen JW, Haazen IW, Schuerman JA, et al. Behavioural rehabilitation of chronic low back pain: comparison of an operant treatment, an operant-cognitive treatment and an operant-respondent treatment. *Br J Clin Psychol*. 1995;34:95–118.
44. Turner JA, Jensen MP. Efficacy of cognitive therapy for chronic low back pain. *Pain*. 1993;52:169–177.
45. van Dulmen AM, Fennis JF, Bleijenberg G. Cognitive-behavioral group therapy for irritable bowel syndrome, effects and long-term follow-up. *Psychosom Med*. 1996;58:508–514.
46. Dworkin SF. Behavioral and educational modalities. *Oral Surg Oral Med Pathol Oral Radiol Endod*. 1997;83:128–133.
47. Gardea MA, Gatchel RJ, Mishra KD. Long-term efficacy of biobehavioral treatment of temporomandibular disorders. *J Behav Med*. 2001;24:341–359.
48. Arathuzik D. Effects of cognitive-behavioral strategies on pain in cancer patients. *Cancer Nurs*. 1994;17:207–214.
49. Osterhaus SO, Passchier J, van der Helm-Hylkema H, et al. Effects of behavioral psychophysiological treatment on school children with migraine in a nonclinical setting: predictors and process variables. *J Pediatr Psychol*. 1993;18:697–715.
50. Basler HD. Group treatment for pain and discomfort. *Patient Educ Couns*. 1993;20:167–175.
51. Sinclair VG, Wallston KA. Predictors of improvement in a cognitive-behavioral intervention for women with rheumatoid arthritis. *Ann Behav Med*. 2001;23:291–297.
52. Astin JA, Beckner W, Soekin K, et al. Psychological interventions for rheumatoid arthritis: a meta-analysis of randomized controlled trials. *Arthritis Rheum*. 2002;47:291–302.
53. Williams DA, Cary MA, Groner KH, et al. Improving physical functional status in patients with fibromyalgia: a brief cognitive behavioral intervention. *J Rheumatol*. 2002;29:1280–1286.
54. Lee BH, Scharff L, Sethna NF, et al. Physical therapy and cognitive-behavioral treatment for complex regional pain syndromes. *J Pediatr*. 2002;141:135–140.

55. Baez S, Hoch MC, Hoch JM. Evaluation of cognitive behavioral interventions and pschoeducation implemented by rehabilitation specialists to treat fear-avoidance beliefs in patients with low back pain: a systematic review. *Arch Phys Med Rehabil*. 2017.
56. Mansell G, Storheim K, Leihting T, Werner EZ, Grotle M. Identification of indirect effects in a cognitive patient education (COPE) intervention for low back pain. *Phys Ther*. 2017;97(12):1138–1146.
57. Chehedi O, Rusu AC, Konietzny K, et al. Brain structural alterations associated with dysfunctional cognitive control of pain in patients with lower back pain. *Eur J Pain*. 2017.
58. Nagasawa Y, Oka K, Shhibata A. The effects of pain coping skills training among older outpatients with osteoarthritis. *Nihon Rohen Igakkai Zasshi*. 2017;54(4):546–554.
59. Stevens RJ, Heide F. Analgesic characteristics of prepared childbirth techniques: attention focusing and systematic relaxation. *J Psychosom Res*. 1977;21:429–438.
60. Dahlquist LM, Busby SM, Slifer KJ, et al. Distraction for children of different ages who undergo repeated needle sticks. *J Pediatr Oncol Nurs*. 2002;19:22–34.
61. Haythronthwaite JA, Lawrence JW, Fauerbach JA. Brief cognitive interventions for burn pain. *Ann Behav Med*. 2001;23:42–49.
62. Kanfer FH, Goldfoot DA. Self-control and tolerance of noxious stimulation. *Psychol Rep*. 1966;18:79–85.
63. Evans MB, Paul GL. Effects of hypnotically suggested analgesia on physiological and subjective responses to cold stress. *J Consult Clin Psychol*. 1970;35:362–371.
64. Anand BK, Chhina ES, Singh B. Some aspects of electroencephalographic studies in yogis. *EEG Clin Neurophysiol*. 1961;13:452–456.
65. Pelletier K, Peper E. The chutzpah factor in altered states of consciousness. *J Humanis Psych*. 1977;17:63–73.
66. Van Beugen S, Fenwerda M, Hoeve D, et al. Internet-based cognitive behavioral therapy for patients with chronic somatic conditions: a meta-analytic review. *J Med Internet Res*. 2014;16(3).
67. Eckard C, Asbury C, Bolduc B, et al. *J Pain Manag Med*. 2016;2(3).
68. Galiano-Castillio W, Cantarevo-Villanueva J, Fernandez-Lao C, et al. Telehealth system: a randomized controlled trial evaluating the impact of an internet-based exercise intervention on quality of life, pain, muscle strength and fatigue in breast cancer survivors. *Cancer*. 2016;122(20):3166–3174.
69. Voerman JS, Remene S, Westendorp T, et al. Effects of a guided internet-delivered self-help intervention for adolescents with chronic pain. *J Pain*. 2015;16(11):1115–1126.
70. Kristjansdottir OB, Fors EA, Eide E, et al. A smartphone-based intervention with diaries and therapist-feedback to reduce catastrophizing and increase functioning in women with chronic widespread pain: randomized controlled trial. *J Med Internet Res*. 2013;15(1).
71. Wilson M, roll JM, Corbett C, Barbosa-Leiker C. Empowering patients with persistent pain using an Internet-based self-management program. *Pain Manag Nurs*. 2015;1b(4):503–514.
72. Gupta A, Scott K, Dukewich M. Innovative technology: using virtual reality in the treatment of pain: does it reduce pain via distraction, or is there more to it? *Pain Med*. 2017.
73. Tashjian VC, Mosaleqhi S, Howard AB, et al. Virtual reality for management of pain in hospitalized patients: results of a controlled trial. *JMIR Ment Health*. 2017;4(1).
74. Jones T, Moore T, Choo J. The impact of virtual reality on chronic pain. *PLOS One*. 2016;11(12).
75. Ortiz-Catalan, Gudmundsdottir RA, Kristoffersen MB, et al. Phantom motor execution facilitated by machine learning and augmented reality as treatment for phantom limb pain. *Lancet*. 2016;338(10062):2885–2894.
76. Benson H. *The Relaxation Response*. New York: Avon; 1976.
77. Chaves JF. Recent advances in the application of hypnosis to pain management. *Am J Clin Hypn*. 1994;37:117–129.
78. Kroger WS. *Clinical and Experimental Hypnosis in Medicine, Dentistry, and Psychology*. Philadelphia: Lippincott; 1963:197–198.
79. De Pascalis V, Magurano MR, Bellusci A, Chen AC. Somatosensory event-related potential and autonomic activity to varying pain reduction cognitive strategies in hypnosis. *Clin Neurophysiol*. 2001;112:1475–1485.
80. Dunn PM. Obstetric delivery today: for better or for worse? *Lancet*. 1976;1:790–793.
81. Flynn A, Kelly J. Continuous fetal monitoring in the ambulant patient in labour. *Br Med J*. 1976;2:842–843.
82. Liu YC. Effects of an upright position during labor. *Am J Nurs*. 1974;74:2202–2205.
83. Augustinsson LE, Bohlin P, Bundsen P, et al. Pain relief during delivery by transcutaneous electrical nerve stimulation. *Pain*. 1977;4:59–65.
84. Shealy CN, Maurer D. Transcutaneous nerve stimulation for control of pain: a preliminary technical note. *Surg Neurol*. 1974;2:45–57.
85. Yuan CS, Attele AS, Dey L, et al. Transcutaneous electrical acupoint stimulation potentiates analgesic effect of morphine. *J Clin Pharmacol*. 2002;42:899–903.
86. Naeser MA, Hahn KA, Lieberman BE, Branco KF. Carpal tunnel syndrome pain treated with low-level laser and microamperes transcutaneous electric nerve stimulation: a controlled study. *Arch Phys Med Rehabil*. 2002;83:978–988.
87. Chesterton LS, Barlas P, Foster NE, et al. Sensory stimulation (TENS): effect of parameter manipulation on mechanical pain thresholds in healthy human subjects. *Pain*. 2002;99:253–262.
88. Bonica JJ. *Fundamental Considerations. Principles and Practice of Obstetric Analgesia and Anesthesia*. Vol 1. Philadelphia: FA Davis; 1967.
89. Bonica JJ. The nature of pain in parturition. *Clin Obstet Gynecol*. 1975;2:499–516.
90. Neumark J, Pauser G, Scherzer W. Pain relief in childbirth: an analysis of the analgesic effects of transcutaneous nerve stimulation (TNS), pethidine and placebos. *Prakt Anaesth*. 1978;13:13–20.
91. Kemp T. The use of transcutaneous electrical nerve stimulation on acupuncture points in labour. *Midwives*. 1996;109:318–320.
92. Robson JE. Transcutaneous nerve stimulation for pain relief in labour. *Anaesthesia*. 1979;34:357–360.
93. Stewart P. Transcutaneous nerve stimulation as a method of analgesia in labour. *Anaesthesia*. 1979;34:361–364.
94. Andersson SA, Block E, Holmgren E. Lagfrekvent transkutan elektrisk stimulering for smartlindring vid forlassning. *Lakartidningen*. 1976;73:2421–2423.
95. Lewith GT, Machin D. On the evaluation of the clinical effects of acupuncture. *Pain*. 1983;16:111–127.
96. Reichmanis M, Becker RO. Relief of experimentally-induced pain by stimulation at acupuncture loci: a review. *Comp Med East West*. 1977;5:281–288.
97. Xiang A, Cheng K, Shen X, Xu P, Liu S. The immediate analgesic effect of acupuncture for pain: a systematic review and meta-analysis. *Evid Based Complement Alternat Med*. 2017:3837194.
98. Praveena Seevaunamtum S, Bhojwani K, Abdulla N. Intraoperative electroacupuncture reduces postoperative pain, analgesic requirements and prevents postoperative nausea and vomiting in gynaecological surgery: a randomized controlled trial. *Anesth Pain Med*. 2016;6(6).
99. Stux G, Pomeranz B. *Basics of Acupuncture*. New York: Springer-Verlag; 1988.
100. Chapman CR, Colpitts YM, Benedetti C, Kitaeff R, et al. Evoked potential assessment of acupunctural analgesia: attempted reversal with naloxone. *Pain*. 1980;9:183–197.
101. Kumar A, Tandon OP, Bhattarcharya A, et al. Somatosensory evoked potential changes following electro-acupuncture therapy in chronic pain patients. *Anaesthesia*. 1995;50:411–414.
102. Xu X, Shibasaki H, Shindo K. Effects of acupuncture on somatosensory evoked potentials: a review. *J Clin Neurophysiol*. 1993;10:370–377.
103. Fink M, Wolkenstein E, Luennemann M, et al. Chronic epicondylitis: effects of real and sham acupuncture treatment: a randomized controlled patient- and examiner-blinded long-term trial. *Forsch Komplementarmed Klass Naturheilkd*. 2002;9:210–215.
104. Irnich D, Behrens N, Gleditsch JM, et al. Immediate effects of dry needling and acupuncture at distant points in chronic neck pain: results of a randomized, double-blind, sham-controlled crossover trial. *Pain*. 2002;99:83–89.
105. Tsui P, Leung MC. Comparison of the effectiveness between manual acupuncture and electro-acupuncture on patients with tennis elbow. *Acupunct Electrother Res*. 2002;27:107–117.

106. Targino RA, Imamura M, Kaziyama HH, et al. Pain treatment with acupuncture for patients with fibromyalgia. *Curr Pain Headache Rep*. 2002;6:379–383.
107. Sim CK, Xu PC, Pua HL, et al. Effects of electroacupuncture on intraoperative and postoperative analgesic requirement. *Acupunct Med*. 2002;20:56–65.
108. Chen G, Xiang J, Ouyang L, et al. Acupuncture combined with western medicine for CP/CPPS: a randomized controlled trial. *Zhongguo Zhen Jiu*. 2016;36(12):1247–1251.
109. Wang X, Hou M, Wang H, Ji F. Warm-needling moxibustion for knee osteoarthritis: a randomized controlled trial. *Zhongguo Zhen Ji*. 2017;37(5):457–462.
110. Wallis L, Shnider SM, Palahniuk RJ, Spivey HT. An evaluation of acupuncture analgesia in obstetrics. *Anesthesiology*. 1974;41:596–601.
111. Abouleish E, Depp R. Acupuncture in obstetrics. *Anesth Analg*. 1975;54:82–88.
112. Ramnero A, Hanson U, Kihlgren M. Acupuncture treatment during labour: a randomized, controlled trial. *Br J Obstet Gynecol*. 2002;109:637–644.
113. Wu GC, Zhu J, Cao X. Involvement of opioid peptides of the preoptic area during electroacupuncture analgesia. *Acupunct Electrother Res*. 1995;20:1–6.
114. Zhu L, Li C, Ji C, Li W. The role of OLS in peripheral acupuncture analgesia in arthritic rats. *Zhen Ci Yan Jiu [Acupunct Res]*. 1993;18:214–218.
115. Zhou L, Jiang JW, Wu GC, Cao XD. Changes of endogenous opioid peptides content in RPGL during acupuncture analgesia. *Sheng Li Xue Bao*. 1993;45:36–43.
116. Carlsson C. Acupuncture mechanisms for clinically relevant long-term effects: reconsideration and a hypothesis. *Acupunct Med*. 2002;20:82–99.
117. Tsuei JJ, Lai Y, Sharma SD. The influence of acupuncture stimulation during pregnancy. *Obstet Gynecol*. 1977;50:448–479.
118. Motoyama H. *How to Measure and Diagnose the Functions of Meridians and Corresponding Internal Organs*. Tokyo: Institute for Religious Psychology; 1976.
119. Nakatani Y. *A guide for Application of Ryodoraku Autonomous Nerve Regulatory Therapy*. Tokyo: Japanese Society of Ryodoraku Autonomic Nervous System; 1972.
120. Hyodo M. *New Management of Pain*. Tokyo: Chiyugai Igakushiya; 1970.
121. Wu MT, Sheen JM, Chuang KH, et al. Neuronal specificity of acupuncture response: a fMRI study with electroacupuncture. *Neuroimage*. 2002;16:1028–1037.
122. Furlan AD, Brosseau L, Imamura M, Irvin E. Massage for low back pain: a systematic review within the framework of the cochrane collaboration back review group. *Spine*. 2002;27:1896–1910.
123. Sielski R, Rief W, Glombiewski JA. Efficacy of biofeedback in chronic back pain: a meta-analysis. *Int J Behav Med*. 2017;24(1):25–41.
124. Morrow AM, Burton KLO, Watanabe MM, Cloyd BH, Klut GA. Developing BrightHearts: a pediatric biofeedback mediated relaxation app to manage procedural pain and anxiety. *Pain Pract*. 2017.
125. Earles J, Folen RA, James LC. Biofeedback using telemedicine: clinical applications and case illustrations. *Behav Med*. 2001;27:77–82.
126. Seltzer S, Dewart D, Pollack RL, Jackson E. The effect of dietary tryptophan on chronic maxillofacial pain and experimental pain tolerance. *J Psychiatr Res*. 1982;83(17):181–186.
127. Bensky D, Gamble A. *Chinese Herbal Medicine Materia Medica*. Seattle: Eastland Press; 1986:389.
128. Riva A, Allegrini P, Franceschi F, et al. A novel boswellic acids delivery form (Casperome) in the management of musculoskeletal disorders: a review. *Eur Rev Med Pharmac Sci*. 2017;21(22):5258–5263.
129. Singh H, Bhushan S, Arova R, Singh Buttar H, Arova S, Singh B. Alternative treatment strategies for neuropathic pain: role of Indian medicinal plants and compounds of plant origin – a review. *Biomed pharmacother*. 2017;92:634–650.
130. Yimam M, Lee YC, Jiao P, et al. UP 1306, a botanical composition with analgesic and anti-inflammatory effect. *Pharmacognosy Res*. 2016;8(3):186–192.
131. Hauser W, Petzke F, Fitzcharles MA. Efficacy, tolerability and safety of cannabis-based medicines for chronic pain management – an overview of systematic reviews. *Eur J Pain*. 2017.
132. Romero-Sandoval EA, Kolano AL, Alvarado-Vazquez PA. Cannabil and cannabinoids for chronic pain. *Curr Rheumatol Rep*. 2017;19(11):69.
133. Hauser W, Fitzcharles MA, Radbruch L, Petzke F. Cannabinoids in pain management and palliative medicine. *Dtsch Arztebl Int*. 2017;114(38):627–634.
134. Elkayam O, Ben Itzhak S, et al. Multidisciplinary approach to chronic back pain: prognostic elements of the outcome. *Clin Exp Rheumatol*. 1996;14:281–288.
135. Myers CD, White BA, Heft MW. A review of complementary and alternative medicine use for treating chronic facial pain. *J Am Dental Asso*. 2002;133:1189–1196.
136. Golden BA. A multidisciplinary approach to nonpharmacologic pain management. *J Am Osteopath Assoc*. 2002;102:S1–S5.
137. Rusy LM, Weisman SJ. Complementary therapies for acute pediatric pain management. *Pediatr Clin North Am*. 2000;47:589–599.
138. Kubista E, Kucera H, Riss P. The effect of transcutaneous nerve stimulation on labor pain. *Geburtshilfe Frauenheilkd*. 1978;38:1079–1084.
139. Bundsen P, Carlsson CA, Forssman L, Tyreman NO. Pain relief during delivery by transcutaneous electrical nerve stimulation. *Prakt Anaesth*. 1978;13:20–28.
140. Ito T. Painless labor with acupuncture anesthesia. *Japan J Anesth*. 1974;23:10–16.

43

Nontransfusion Significance of ABO and ABO-Associated Polymorphisms

Peter J. D'Adamo, ND, MIFHI, Barbara Siminovich-Blok, ND, LAc, and *Tara Nayak, ND*

OUTLINE

INTRODUCTION

> *"Concepts without percepts are empty; percepts without concepts are blind."*
>
> ***Immanuel Kant (1724–1804)***

Since Landsteiner first described the ABO typing system in 1900, the preeminent clinical role of this red blood cell (RBC) antigen–serum antibody system has been the prediction of reactions involving transfused blood and, to a lesser degree, transplanted organs. Despite this exceedingly important role, however, no rational individual should conclude that the proper exchange of blood or other tissues constitutes their primary biological role.

It can be argued that the overwhelming and revolutionizing influence of the ABO system on the history and practice of transfusion medicine and the general reluctance of allopathic medicine to find a workable niche for the nonreducible have had the effect of precluding interest in the nontransfusion significance of ABO polymorphism and, attendant to that, of a lack of any meaningful medical applications.

This chapter examines the biological significance of the ABO blood grouping and the ABH secretor system, with special attention to the effects of ABO expression as a "tipping point" for a variety of physiological and pathological manifestations that may be of interest to the practitioner of natural medicine.

BACKGROUND

Although a comprehensive review of the glycobiology of the ABO system is beyond the scope of this work, the brief overview given here should allow the reader to understand the basic mechanics and nomenclature of the system.

All humans can be typed for ABO blood group. There are four basic blood types: A, B, AB, and O. The system is composed of two antigens and two antibodies. The specific combination of these four components determines that individual's type. Table 43.1 shows the possible permutations of antigens and antibodies with the corresponding ABO types.

ABH ANTIGENS

The ABO blood group antigens are not primary gene products but, instead, enzymatic reaction products catalyzed by enzymes called glycosyltransferases. As depicted in Fig. 43.1, they are synthesized from an oligosaccharide intermediate, "H substance," which is produced by the monosaccharide fucose. Group A or B activity is produced by the addition of a single sugar on the nonreducing end of the H chain.

TABLE 43.1 Possible Combinations of Antibodies and Antigens in the ABO Blood Grouping System

Abo Blood Type	A Antigen	B Antigen	Anti-A Antibody	Anti-B Antibody
A	Yes	No	No	Yes (IgM)
B	No	Yes	Yes (IgM)	No
O	No	No	Yes (IgM, IgG)	Yes (IgM, IgG)
AB	Yes	Yes	No	No

Ig, Immunoglobulin.

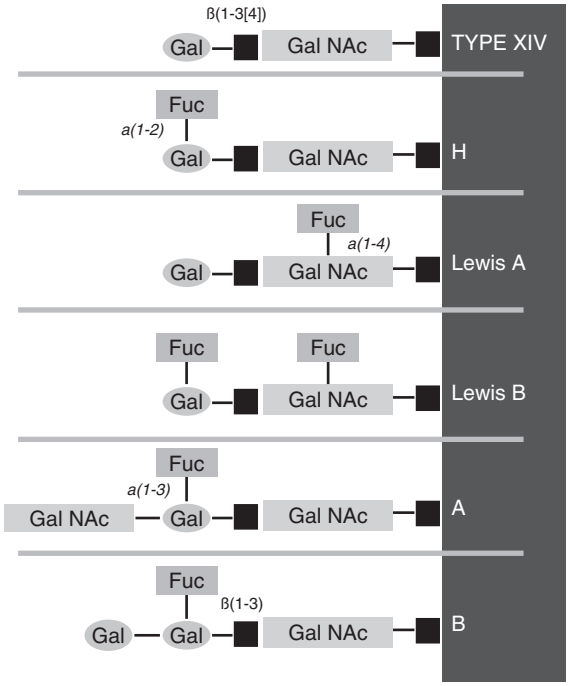


Fig. 43.1 Structures of the nonreducing end of the ABH and Lewis blood group antigen determinants.

Adding the glycoprotein *N*-acetylgalactosamine (GalNAc) to the end of the chain results in blood group A antigenicity. The terminal carbohydrate and the B group antigen is the monosaccharide galactose. There is no true O antigen: the terminal carbohydrate of O (H) antigen is the monosaccharide fucose. We identify the blood types as "ABO" but typically refer to the actual antigen system as "ABH."

ABH substances manifest quite early in life processes.[1] They are detected in the cell membranes and secretions of human embryos at about 5 weeks' gestational age in the epithelium and virtually all vascular endothelium. In fetal organs' endothelial cells, the expression of ABH antigen is ubiquitously upregulated. This is suggestive evidence that blood group antigens serve as early immunomorphological markers of the endothelial differentiation of mesenchymal cells, specifying the location of future blood vessels.[2]

All embryonic epithelia contain the ABH antigen, except those of the nervous system, adrenal glands, and liver. The antigens subsequently disappear from epithelia in an orderly and predictable manner as evidence of morphological differentiation appears. By about the end of the third intrauterine month, the adult pattern of distribution is achieved.

The embryonic expression of ABH antigens may be an important aspect in the pathogenesis of certain diseases: a scrutiny of 2557 medical records for children with type 1 diabetes and controls showed ABO blood group incompatibility in close to 90% of children with diabetes,[3] indicating that maternal–fetal ABO incompatibility may have acted to inhibit normal pancreatic islet formation in the fetus.

The expression of ABH antigens in the adult is tightly regulated, and their reappearance in adult tissue—normally devoid of them—is virtually always a sign of disease. Inappropriate ABH expression is one of the prime manifestations of the aberrant glycosylation state that is a hallmark of malignancy. Instances have been observed indicating that aberrant ABH expression may be paralleled in entirely different organ systems. A link has been demonstrated between ABH antigen expression in normal and neoplastic colonic epithelia and consequent alterations of ABH expression in the thyroid.[4]

ABH Secretors and Nonsecretors

By 1930 it had been shown that some people do and others do not secrete antigens corresponding to their ABO blood group into their saliva. Persons with these substances in saliva (secretors) have more ABH substances in their tissues than those lacking the substance in their saliva (nonsecretors). The ability to secrete behaved as a simple Mendelian function dominant to nonsecretion. Persons with blood groups A, B, and AB who are secretors secrete the antigens corresponding to their blood groups. Group H persons secrete the H substance, as do all other secretors, to a somewhat lesser extent.

ABH substances are secreted by mucous glands in many organs, including the upper respiratory tract, the gastrointestinal tract from the esophagus through the colon, and the uterine cervix. ABH secretor status is a major conditioner of the gut mucosa. ABH secretors have greater quantities of free ABH antigens in the makeup of their intestinal secretions, which has significant effects on bacterial and lectin adherence to the gut microvilli. The secretor gene regulates the synthesis of blood group substances in superficial glands of the gastric and small intestine mucosa. Large amounts of ABH material are found in all secretors,[5–8] characterized by a uniform distribution of blood type antigens in the gastric pits. ABH expression is independent of secretor status: glands situated deep in the mucosa of the pylorus and small intestine (Brunner glands) and gastric parietal glands both produce A and B substances without regard to secretor status.[9]

For a more detailed discussion of the metabolic consequences of ABH secretor status, the reader should refer to the author's article specifically on the subject.[10]

Brush-Border Hydrolases

ABO blood group determines much of the enzyme activity in the tissue (brush border) of the intestine. At least six intestinal hydrolases have ABO blood group antigenic determinants directly related to ABO blood group. The expression of ABH antigens secreted by intestinal glycoproteins is under the control of the secretor gene; therefore these antigens are not detected in the hydrolases of nonsecretor subjects.[11]

Intestinal Alkaline Phosphatase Activity

Intestinal alkaline phosphatase (IAP) is involved with both the breakdown of dietary cholesterol and the absorption of calcium. The activity of IAP and serum alkaline phosphatase (SAP) is strongly correlated with ABH secretor phenotypes. It has been estimated that the SAP activity of nonsecretors is only about 20% of the activity in the secretor groups.[11–15]

ABO polymorphism is linked to the levels and persistence of IAP.[16] Numerous studies have associated group O individuals with the highest alkaline phosphatase activity and group A with the lowest.[17] In addition, one study implied that the group A antigen itself might inactivate IAP.[18]

Bacterial Flora

The role of the ABO blood group in determining the bacteria making up a healthy gastrointestinal ecosystem is particularly strong in ABH secretors. Because ABH secretor status and ABO blood group dictate the presence and specificity of A, B, and H blood group antigens in human gut mucin glycoproteins, their status can influence the populations of bacteria capable of taking up local residence. This occurs because some of the bacteria in the digestive tract are actually capable of producing enzymes that allow them to degrade the terminal sugar of the ABH blood type antigens for a constant food supply.[19] Bacteria capable of degrading blood group B antigen can detach the terminal α-D-galactose. Group A–degrading bacteria can detach the GalNAc. These bacteria

have a competitive advantage and can thrive in the environment created by the preconditioning of ABH secretions. Although comparatively small populations of bacteria produce blood group–degrading enzymes (estimated populations are 10^8 bacteria per gram), the quantity of these bacteria are several orders of magnitude greater in different blood types, and they are much more stable residents. For example, B-degrading bacteria have a population density that is about 50,000-fold greater in blood group B secretors than in other subjects. Similar bacterial specificity and enzyme activity are found in other blood types.[20]

Evidence suggests that ABH nonsecretors have lower levels of immunoglobulin-G (Ig-G)[21,22] and secretory IgA concentrations than secretors.[23,24] ABH nonsecretors appear to have a higher prevalence of a variety of autoimmune diseases, including ankylosing spondylitis, reactive arthritis, psoriatic arthropathy, Sjögren's syndrome, multiple sclerosis, and Graves' disease.[22,25–27]

Studies to determine the patterns associated with the gut microbiome as assessed via fecal sample are still being conducted to determine ABO and ABH secretor status influence. To date, studies have shown that being an ABH secretor is associated with lower diversity index, whereas specific ABO blood groups are associated with allowing strain-specific bacterial expansion based on the bacteria's ability to degrade sugars.[28] For example, the bacteria *Lachnospiraceae* possess beta-galactosidase activity and are therefore higher in blood group B and O secretors due to the presence of D galactose residues on their antigen's terminal chain.[28] Following this thought, as we identify microbial metabolic processes, we can expect to uncover that those strains with preferential utilization of *N*-acetylgalactosamine will have better chances of colonizing the gut of a blood group A secretor.

Blood Group as Self-Declaration and Adhesion Molecules

In the larger world of glycoproteins, ABH antigens are characterized as O-linked glycans (glycosylation at serine or threonine residues by GalNAc). Many proteins in nature carry these dense and complex arrays of covalently attached sugar chains. Their biological roles are particularly important in the construction of complex multicellular organisms and organs.

Glycans are typically on the cellular outer surfaces and outside of secreted macromolecules; therefore they can mediate interactions between organisms. They are also involved in cell-to-cell and cell-to-matrix interactions that are crucial to the development and function of complex multicellular organisms.

Glycans can have a significant effect on fungal, viral, and bacterial pathogenicity.[29] ABH antigens are ubiquitous in nature, found abundantly in foodstuffs (where they are thought to play a role in the induction of opposing blood group antibodies early in life) and in a host of microorganisms.

A 1995 study showed that of 833 fungi harvested from 1977 to 1994, 422 extracts (47.8%) produced agglutination of human red blood cells (RBCs), equally distributed against type O, A, and B cells. The fungal agglutinins, in this case, are desirous of attaching and infecting seeds or other microbes that possess some "ABO blood type" activity of their own.[30]

ABH antigens appear in secretions by 8 to 9 weeks of age, first in the salivary glands and stomach, then throughout the gastrointestinal and vaginal tracts. The ABH variation of blood group antigen expression on vaginal epithelial cells and mucus has a significant role in susceptibility to urinary tract infections in women.[31]

Infertility and Spontaneous Abortion

ABO blood type incompatibility may be a critical factor in infertility. ABO-incompatible mating couples (a type A male fertilizing a type O female) are a common occurrence in miscarriages, especially very early in the gestational term. One study of 288 miscarriages showed that there was an excess of blood type A and type B in otherwise normal fetuses. The researchers concluded that the ABO incompatibility between mother and fetus was likely a cause of early miscarriages, but almost exclusively in chromosomally normal fetuses.[32–35]

In a study of 102 infertile couples, Solish[36] found that 87% were blood type incompatible. This researcher suggested that the infertility was due to the presence of antibodies in the secretions of the mother's genital tract or incompatible sperm from the father. In another study, a total of 589 compatible mating couples were compared with 432 incompatible mating couples. The mean number of living children presented a significant difference. There was a 21% deficiency of type A children in the two groups. Similarly, there was a 16% deficiency of type B children in the two groups. It appears that a 31.9% rate of miscarriage is associated with incompatible matings, compared with 17.15% in compatible matings. This finding has led some researchers to theorize that ABO incompatibility results in "cervical hostility" between the man's blood type antigens, which are present in his sperm, and the woman's opposing antibodies, present in her cervical mucus.[37]

Besides infertility, habitual abortion is also related to ABH secretor status as shown in 2010. The secretor status of 66 couples with recurrent spontaneous abortion (RSA) was obtained and compared with that of couples with successful term pregnancies. It was found that a secretor phenotype of couples with RSA, especially of the husband, could facilitate "reproductive success" and that couples in which both parents were secretors, the RSA probability was extremely diminished.[38] A previous study showed the interaction between ABO blood groups and adenosine deaminase genetic polymorphism during intrauterine life, providing results that could not be explained by strictly conventional immunologic mechanisms. The authors concluded that cell-to-cell interactions involving ABO antigens might have an important role at implantation and that adenosine deaminase, through control of local adenosine concentration, could modulate these interactions, influencing the probability of successful implantation.[39] A third and most recent study comparing secretor status compatibility between mother and child suggested an intrauterine selection against Se^- (nonsecretor) of the embryo carried by an Se^+ (secretor) mother. The selection was dependent on factors influencing the maternal environment.[40]

To measure women's fertility below age 45, follicle-stimulating hormone (FSH) levels are measured. Women with an FSH level greater than 10 are considered to have diminished ovarian reserve. In an ongoing study at Yale University and the Albert Einstein College of Medicine, it was observed that women with type A or AB blood were significantly less likely to have an FSH greater than 10 than were women with type O or B blood. They concluded that type O blood might affect fertility.[41]

Other Immune Correlates

In 1991 D'Adamo reported that individuals of group O blood reporting previous urticaria or anaphylaxis showed high residual titers of anti-A isoagglutinins. A score value was assigned to each agglutination reaction. Individuals with these disorders showed remarkably high titration scores compared with controls of the same blood group, up to three times higher. A mild increase was noted for group O subjects with severe eczema or asthma, but total scores in these subjects were only marginally greater than those of controls. A striking association was shown for group O women experiencing endometriosis.[42]

ABO and Secretor Blood Group Genetics

ABO System

In 1910 Epstein and Ottenberg suggested that the ABO blood group system could be inherited.[43] The determination of ABO status is the result of two codominant alleles and one recessive allele found on chromosome 9q band 34.

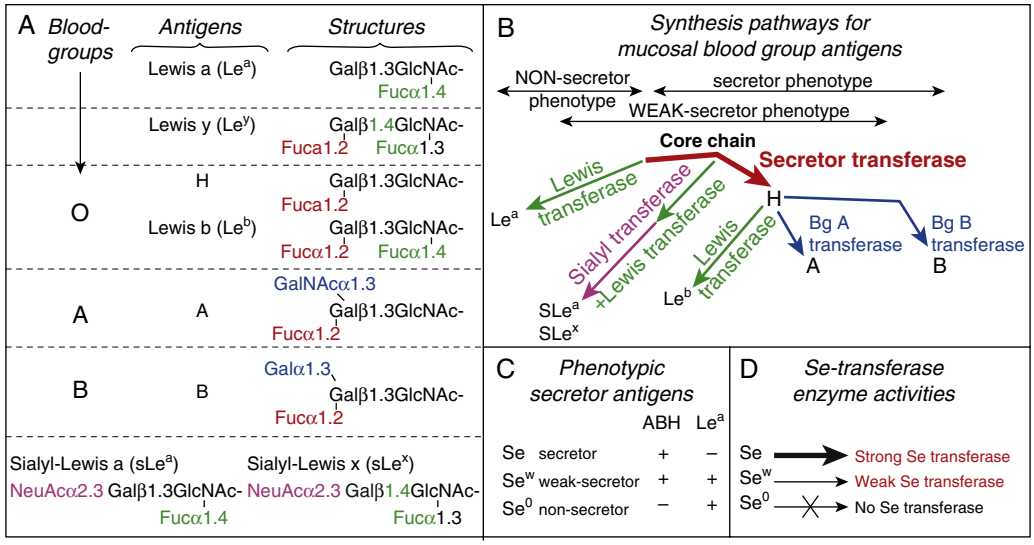


Fig. 43.2 Fucosylated and sialylated blood group (bg) antigens and associated secretor phenotypes. (A) The α1.2-fucosylated *(in red)* H and Le^b antigens define bg O. Bg A and B antigens present additional GalNAc or Gal residues *(blue),* respectively. Le^y and Le^b are both difucosylated. sLea and sLex are sialylated Lewis antigens *(in pink).* (B) Synthesis pathways for bg antigens with corresponding Se phenotypes: Le^a is found in Se^0 individuals, whereas Se^w individuals carry a mix of mucosal Le^a and Le^b. Lea is formed when the Se-transferase is inactive or weak because Le^a is a "dead end" and is not extended further. During inflammation and infection, sialyl-transferases are expressed, and carbohydrate core chains become sialylated in competition with Se-fucosyltransferase. (C) The presence of ABH and Le^a antigens in salivary, milk, and gastrointestinal tract secretions identifies individuals of Se, Se^0, or Se^w phenotype. (D) In Se^w subjects, α1.2 fucosylation is hampered by an enzymatically weak Se-transferase, whereas Se^0 individuals lack Se-transferase activity. (From Linden S, Mahdavi J, Semino-Mora C, Olsen C, Carlstedt I, Boren T, Dubois A. Role of ABO secretor status in mucosal innate immunity and *H. pylori* infection. *PLoS Pathogens.* 2008;4[1]:e2. PubMed PMID: 18179282. https://openi.nlm.nih.gov/detailedresult.php?img=PMC2174967_ppat.0040002.g001&query=ABO+blood+grouping&req=4&npos=202. Accessed October 21, 2018.)

A and B blood group genes are dominant over the O blood group, and the A and B group genes are themselves codominant. The ABH antigens are not primary and direct gene products; they are the reaction products catalyzed by glycosyltransferases type of enzymes.

ABO genes consist of at least seven exons, and the coding sequence in the seven coding exons spans more than 18 kb of the genomic DNA. The single-nucleotide deletion found in most (but not all) of the O alleles that is responsible for the loss of the activity of the enzyme is located in exon 6.[44]

ABH Secretor System

The secretor gene (*FUT2* at 19q13.3) codes for the activity of glycosyltransferases needed to assemble (secrete) free ABH antigen in body fluids like saliva, semen, vaginal fluid goblet cells, and mucous gland cells. This is accomplished in concert with the gene for group O or H *(FUT1).* Secretor status is determined by two alleles on *FUT2, Se* (dominant) and *se* (recessive), with approximately 80% of the population typing as secretors (*SeSe* or *Sese*).

Lewis Blood Groups and Their Association With the ABH Secretor System

The ABH secretor system is a major determinant of the Lewis (Le) blood grouping system (Fig. 43.2). This is due to the fact that, in addition to ABH, *FUT1* and *FUT2* provide the glycans necessary for conversion of Le antigens as well.

Two broad categories of Le blood type exist. These are the "Lewis positive" (either Le^{a+b-} or Le^{a-b+}) and "Lewis negative" (Le^{a-b-}) phenotypes. Depending on race, between 1% and 8% of the population is Lewis negative.

In Lewis-positive phenotypes, Le^a is formed initially, and in the case of nonsecretors (lacking the *Se* gene, *FUT2*), Le^a glycan is attached to the RBCs, and they type as Le^a. In the case of secretors, the *Se* gene activates the *H* gene, which causes an additional sugar to be added to Le^a, converting it to Le^b (see Fig. 43.1).

Among Le-positive individuals, ABH secretors are always Le^{a-b+} because they convert all their Le^a antigen into Le^b. Conversely, among Lewis-positive people, ABH nonsecretors are always Le^{a+b-} because they lack the *FUT2*-dependent glycosyltransferase to accomplish this conversion.

Thus it is often possible (and quite handy) to use the Lewis groups to infer ABH secretor status because Lewis typing is fairly quick to perform and easy to master compared with the traditionally used salivary inhibition test. However, the use of Lewis typing to infer ABH secretor status works only in those individuals who are Lewis-positive (about 9 of every 10 patients). Lewis-negative individuals can either be secretors or nonsecretors. Lewis-negative patients carry important metabolic consequences of their own, worthy of much attention.[10] Table 43.2 shows Lewis blood types and their relationship to ABH secretor and/or nonsecretor status.

Sialylated forms of several Lewis variations (sialyl Lewis A, sialyl Lewis X) are oligosaccharide ligands now considered crucial to the initial adhesion of white blood cells to a site of injury mediated by E-selectins. Large quantities of sialyl Lewis X have also been found on the surfaces of certain tumor and cancer cells, and one of its variants (sialyl 6-sulfo Lewis X) appears to be involved in routine homing processes involving a variety of chemokines.

Individuals of blood group O phenotype run an approximate 1.5- to 2-fold higher risk for development of peptic ulcer disease,[45]

TABLE 43.2 Lewis (Le) Blood Types and Their Relationship to ABH Secretor and/or Nonsecretor Status

Lewis Type	Category	Abh Secretor Status
Le^{a+b-} Le^a antigen but not Le^b	Lewis positive	ABH nonsecretor
Le^{a-b+} Le^b antigen but not Le^a	Lewis positive	ABH secretor
Le^{a-b-} Neither Le^a nor Le^b	Lewis negative	Lewis outcome cannot determine ABH secretor status

although there is no direct correlation between ABO blood group phenotypes and the prevalence of *Helicobacter pylori* infection. However, in addition to H type 1, the Le^b antigen is also a binding receptor for *H. pylori,* and in this capacity, it can best be described as a "virulence-promoting factor." For virulent strains, Le^b antigen binding activity targets the microbes to the epithelial cell surfaces and potentiates the effect of secretion of virulence factors, such as the vacuolating cytotoxin and/or neutrophil activating–recruiting factors.[46]

Linkage and Pleiotropism

Linked genes occur on the same chromosome and are inherited as a single unit. Gene linkage analysis shows several genes linked to the ABO locus. For example, there are strong indications that a gene regulating dopamine β-hydroxylase activity is linked to the ABO blood group locus.[47] Dopamine β-hydroxylase is a key enzyme in the conversion of dopamine to norepinephrine.

This linkage may help explain the continued significance of ABO group as a discreet and significant genetic marker for a variety of affective disorders, including type A behavior in men subsequent to myocardial infarction[48] and bipolar depression,[49,50] each of which has been associated with blood group O. The ABO locus shows putative linkage with platelet monoamine oxidase activity,[51] reduced levels of which have been noted in group O healthy men.[52]

Additional evidence implies that there is a linkage between the ABO gene and the gene that regulates the activity of the enzyme argininosuccinate synthetase, which recycles arginine from citrulline in the production of nitric oxide.[53] A letter to the editor in the journal *Lancet* reported differences between ABO groups in their responsiveness to inhaled nitric oxide therapy: types with a B antigen (B and AB) had less success with this therapy.[54]

Elevated factor VIII (FVIII) levels contribute to venous thrombotic risk. FVIII levels are determined to a large extent by levels of von Willebrand factor (vWF), a protein that protects FVIII against proteolysis.[55] ABO polymorphism is one of the best-characterized genetic modifiers of plasma FVIII; it accounts for approximately 30% of the total genetic effect.[56] Subjects with blood group non-O have higher vWF and FVIII levels than individuals with blood group O.[57]

Digit ratio, a marker of assessing the levels of prenatal exposure to androgens, has yielded mixed results[58,59] as a predictor of androgen stimulation in individuals with polycystic ovarian syndrome. However, preliminary evidence suggests that genes contributing to the expression of 2D:4D reside in the vicinity of the gene loci (chromosomal locations: 9q34.2 and 1p36.11) of the blood groups or that there may be pleiotropic effects on digit ratio as a result of the blood group genes. Associations using digit ratios may require incorporation of the ABO blood group in their interpretation.[60]

Additional Physiological Correlations

In addition to the previously described variations in IAP and brush-border hydrolase activity, several additional circumstances where ABO polymorphism exerts a significant influence on physiology have been reported.

Gastric Acidity

Because the prevalence of both pernicious anemia and gastric cancer is higher in individuals of blood group A and that of duodenal ulcer is higher in those of group O, a hypothesis relating blood group effects on acid secretion was inevitable.[61] Early work confirmed that acid output tended to be greater in group O than in group A subjects.[62,63]

Gastrin and Pepsinogen

In one study, serum pepsinogen A (pepsinogen I) levels were studied in relation to ABO blood group, age, and gender in 700 healthy blood donors. Serum pepsinogen A levels were higher in males than in females and rose with increasing age. Blood group O individuals showed higher serum pepsinogen A levels than blood group A individuals.[64] There is also evidence that the type A antigen in gastric juice binds to pepsin and possibly inactivates it.[65] A study using serum pepsinogen levels as a marker for gastric atrophy showed a high association with blood groups A and B.[66] However, possibly owing to the polygenic nature of pepsinogen activity, one study failed to find any significant difference in pepsinogen levels between ABO groups.[67]

Another study looking at ABO polymorphism and serum gastrin concentration after stimulation by a glycine drink could find no correlation with ABO blood group.[68] However, the study had a simple preprandial and postprandial methodology. In a different study, the concentrations of gastrin were measured in the blood of 121 fasting healthy Greek volunteers of both sexes and different ABO blood types, between ages 20 and 70 years. The testing took place immediately after a test meal was eaten by subjects who had fasted for 8 hours; the measurement was repeated at 10 and 40 minutes post-meal. The researchers found that gastrin levels took 40 minutes to increase after the meal in the blood type A and B subjects but that a significant increase appeared 10 minutes after the meal in the blood type O subjects.[69]

Cholesterol

Although several studies on highly select populations have yielded conflicting results,[70,71] the general consensus is that blood group A individuals have a significantly higher basal cholesterol level than those in other blood groups. The relationship between ABO blood phenotype and total serum cholesterol level was examined in a specific Japanese population. The results showed that cholesterol levels were very significantly elevated in the blood type A group compared with non-A.[72]

ABO blood group and coronary risk factor levels were measured in a nationwide sample of more than 6000 black and white adolescents aged 12 to 17 years. Blood group A was associated with significantly higher serum total cholesterol levels in white females independent of all other risk factors, in white males independent of age and weight, and in Southern black females independent of age and weight.[73] A separate study (the Bogalusa Heart Study) looked at 656 white and 371 black adolescents and found the same results with regard to total cholesterol; even higher levels of low-density lipoprotein cholesterol were found in type A adolescents than in other blood types.[74]

Stress

Several studies have identified differences between ABO group and possible chemical responses to stress. Interestingly, individuals of blood group A appeared to have a lower incidence of "type A personality."[75]

One study evaluated the influence of blood type A versus O coupled with a mirror-drawing stressor on very-low-density lipoprotein toxicity-preventing activity (TxPA). Plasma cortisol levels showed significant ABO variation. Exposure to the stressor significantly decreased TxPA and increased cortisol for the total group of 25 older men. The stress response patterns of the 15 blood type A men were different from those of the 10 type O subjects. The blood type A group had higher initial levels of TxPA and cortisol as well as quicker stress recovery rates than the type O group.[76] Another study showed that blood group A individuals responded to a stressful situation (venipuncture) with higher levels of cortisol and, possibly, of adrenaline.[77]

Rheology

For purposes of this discussion, *rheology* is used to describe the dynamics between blood clotting (moving toward a solid state) and blood thinning (moving toward a liquid state). It might be tempting to substitute the word *viscosity* for *rheology* when talking about blood types and clotting. Viscosity, however, does not cover the "dynamics" of how, when, and why blood can change texture; it only distinguishes one texture state from another.

There is evidence that the rheology of blood may play a role in a variety of chronic anxiety states. Compared with normal subjects, chronic depressive and schizoid patients had very significant differences in their blood rheology and in the ability of their RBCs to aggregate. When patients with schizoid anxiety were compared with those with depressive anxiety, their ratio of albumin to globulin was increased. When patients were divided according to their ABO blood groups, significant differences were found in their albumin:fibrinogen ratio and their blood viscosity. This was particularly true for women who had blood type A and who had depressive anxiety; their blood tended to be substantially "thicker" and to have higher amounts of serum proteins in it than women with similar depression who had blood type O.[78]

Associations between the ABO phenotype and variations in blood rheology have also been reported in high blood pressure[79]; stress[80]; diabetes[81]; heart attack, cancer, and thyroid disease[82]; kidney failure[83]; and malignant melanoma.[84]

Soluble Endothelial Cell Markers

Selectins are cell–cell adhesion molecules that are involved in leukocyte–endothelial cell adhesive interaction, which is required for extravasation at target tissue sites. Three types of selectins have been discovered: L-selectins are generally expressed on almost all leukocytes, E-selectins are inducible on vascular endothelium upon stimulation with cytokines, and P-selectins are found on activated platelets.

The role of thrombomodulin as a C-type lectin is less known. It has a domain that interferes with neutrophil adhesion to endothelial cells. Elevated levels of E-selectin ($P < 0.001$) and thrombomodulin ($P < 0.001$) are linked with blood type A individuals.[85]

PATHOLOGICAL PROCESS AND ABO BLOOD GROUPS

Mental Disease

There are several reports exploring the relationship of ABO groups, ABH and Lewis secretor type, and mental disease such as obsessional illness,[86] bipolar disorder,[87,88] depression,[89] and schizophrenia.[88] A significant part of that research was done in the 1970s and 1980s and does not seem to have been updated or explored much further. Most of the articles reviewed and not discussed here were not conclusive about blood group associations.

Cardiovascular Disease

Stroke

A European study compared 50 patients with stroke. The standard expected frequency of ABO blood types in the surrounding population showed that the frequency of the blood group A in the patients with stroke was 120% greater than would normally be expected. The percentage of blood type B was even higher (159% of expected rate of occurrence). Patients with blood type O were only 85% as likely to experience stroke.[90]

A 1979 study of 220 patients with stroke looked at the viscosity of their blood a few hours after the stroke event. About 80% of the patients had blood cells that easily aggregated. What was especially interesting was the discovery by the researchers that the clotting of blood in patients with A and B blood types was mostly due to fibrinogen, whereas in blood types O and AB, it was caused by other clotting factors.[91]

Individuals with blood types A and AB have a generalized tendency toward problems associated with blood clotting, whereas problems in those with blood types B and O appear to be linked to excessive bleeding and poor clotting. This observation was verified in several studies, the largest being performed in 1460 patients with "stroke" and reported in the *Lancet.* In 329 cases, the cause of death was certified as cerebral thrombosis (brain clot). In the thrombosis cases, there was an excess of patients of blood types A and AB and a deficiency of those with blood types O and B. In the 482 "strokes" that were due to cranial bleeding, the reverse was true: there was a significantly higher proportion of patients with blood types O and B than of those with blood types A and AB.[92]

A 2009 United Kingdom systematic review tried to assess whether the effects of non-O status on thrombosis risk were of the magnitude predicted by its effect on vWF/FVIII levels (Fig. 43.3). They confirmed the historical impression of linkage between some vascular disorders and non-O blood group status. They concluded that the odds ratios were similar to those predicted by the effect of ABO(H) on vWF levels on thrombosis. They proposed further work to understand and refine the effect of reducing O(H) antigen expression and therefore a more widespread adoption of ABO(H) typing.[93]

Peripheral Artery Disease and Venous Thromboembolism

Peripheral artery disease (PAD) occurs in approximately 12% of the adult population. The prevalence of PAD increases with age: almost 20% of people older than 70 years have the disease. E-selectin and thrombomodulin levels are always elevated in intermittent claudication, a disorder almost always found with PAD and carrying a distinct association with blood type A.[94]

In 125 patients experiencing venous thrombosis in a Brazilian population, a higher-than-expected proportion of blood group A and a lower-than-expected proportion of blood group O were observed among the patients.[95] This is consistent with the previously discussed influence of ABO on a third soluble endothelial product, vWF, and its role in thromboembolism. Another study in 2007 confirmed that non-O blood type was independently associated with risk of venous thromboembolism and added to the risk associated with FV Leiden (hypercoagulability).[96] Even in pregnancy, a nested case-control study within a cohort of 71,729 women who gave birth to 126,783 children in Denmark showed that blood groups A and AB might be associated with increased risk estimates for venous thromboembolism in pregnancy and the puerperium.[97]

Heart Disease

There is a clear-cut association with having A and AB phenotypes and an increased risk for heart disease. This association has been reported continuously in the scientific literature over the past 50 years. Individuals who have blood type A have higher rates of heart attack in all age groups, both genders, and all ethnic and national groups.

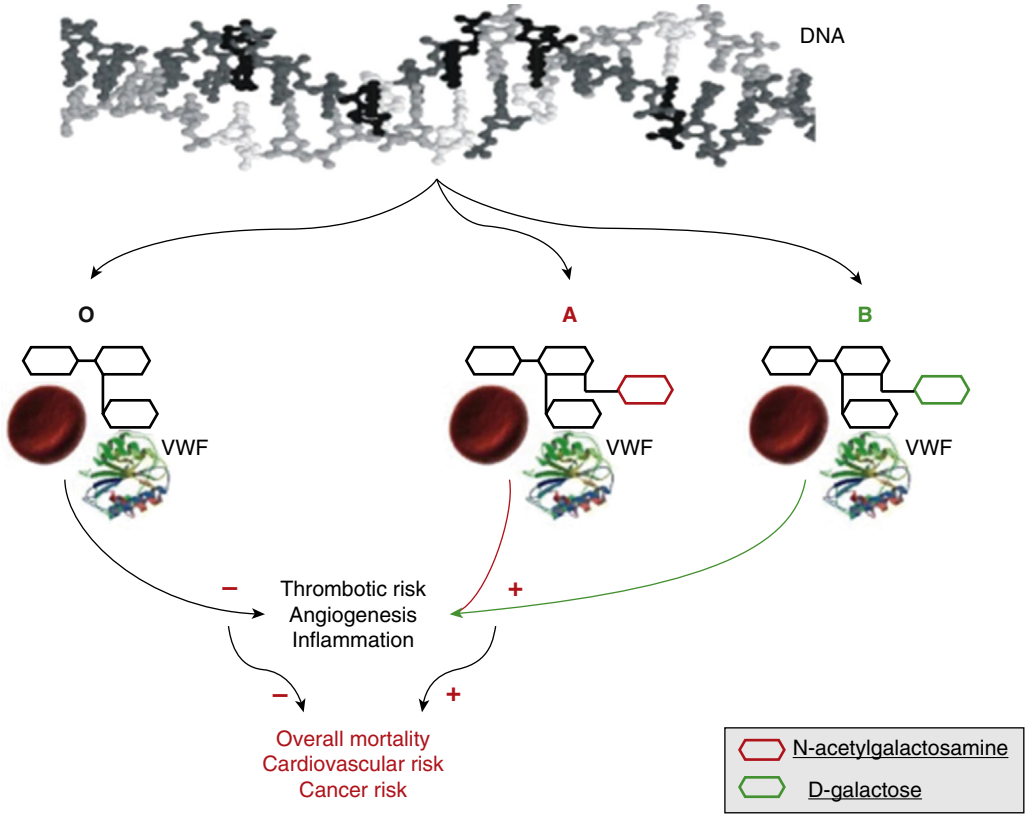


Fig. 43.3 The intriguing relationship between ABO blood group system, von Willebrand factor (vWF), and cardiovascular disease. (From Franchini M, Lippi G. The intriguing relationship between the ABO blood group, cardiovascular disease, and cancer. *BMC Med.* 2015;13:7. PubMed PMID: 25592962. https://openi.nlm.nih.gov/detailedresult.php?img=PMC4295232_12916_2014_250_Fig1_HTML&query=ABO+blood+grouping&req=4&npos=156. Accessed October 21, 2018.)

In 1962 the Framingham Heart Study typed the blood of the surviving 4125 members of the original study group of 5209 people first examined from 1948 to 1951. The most striking observance was the lower rates of nonfatal heart disease in men aged 39 to 72 years with blood type O than in those with blood type A.[98] A 1994 Polish study on patients who underwent coronary bypass surgery with highly advanced arteriosclerosis of the coronary arteries found a significantly higher number of cases with group AB and a lower number of those with group O.[99] A 1981 German study of 13,175 patients showed a prevalence of blood type A in all types of heart disease examined.[100]

In a study of 191 coronary artery bypass candidates, investigators paradoxically found an excess of type O over type A subjects. After examining the data more closely, they concluded that the tendency of type A subjects for more ready development of blood clots ("thrombotic proneness") led to a poorer prognosis. In essence, the blood type A subjects were missing from the study because they had already died in greater numbers, leaving a disproportionate number of type O subjects among the long-term survivors.[101] In a study of male survivors of heart disease, researchers found that there were fewer patients who were type A and younger than 55 years than would have been otherwise expected.[102]

A 1975 Italian study of 746 patients with high blood pressure, 3258 with congenital heart disease, and 4503 with a history of heart attack found a significant lack of patients with type O blood and a significant excess of blood type A patients in the group with myocardial infarction. The study also showed an excess of blood type A patients with high blood pressure and a lack of patients who were blood type B.[103] A 1983 study of 255 women originally investigating the effects of smoking on the rates of heart attack also found several other factors significantly associated with heart attacks in this group, including hypertension, angina pectoris, family history, diabetes mellitus, and blood type A.[104]

Platt et al.[105] examined blood type and heart attacks in two different age groups. The patients were divided into two groups: 65 years or older and younger than 65 years. The predominance of blood type A in patients with cardiac infarction was "highly significant" in both age groups ($P < 0.005$). This study was unique in that it excluded other risk factors, such as smoking, high blood pressure, diabetes, and high cholesterol levels. When the researchers looked specifically at the older group, the predominance of blood group A in those with cardiac infarction was even higher ($P < 0.001$). The researchers concluded, "Our investigation strongly suggests the existence of a genetic factor associated with blood group A and independent of the other risk factors, which is also responsible for a greater incidence of cardiac infarction."[105]

An 8-year study of 7662 British men found that blood type A was linked to the incidence of ischemic heart disease as well as higher total serum cholesterol concentrations.[106] A 2015 study confirmed this association and additionally linked any blood group that is not type O with increased mortality due to cardiovascular disease.[107]

Cancer

> *"Some cancers contain an A-like substance even when they occur in persons who are not A or AB. These observations suggest that in the tissues, both normal and neoplastic, of all persons, there are blood group A-like antigens present at a biochemical level, which are usually inaccessible to the immune system." Mourant (Blood Groups and Disease, 1977)*

Tumor development is usually associated with changes in cell-surface carbohydrates. Cell glycosylation depends on the expression and function of various glycosyltransferases and glycosidases. These enzymes modify the carbohydrate core structure or the terminal carbohydrate structures, resulting in the incomplete synthesis or modification of existing carbohydrates. Considerable research demonstrates that malignant transformation is associated with various and complex alterations in the glycosylation process.

These and other changes provide a selective advantage for tumor cells during their progression to more invasive and metastatic forms. Some of the more studied glycoprotein modifications include the following[108]:

- Increase in *N*-glycation and O-glycation and fucosylation processes
- Increase in mucins
- Alteration in galectin levels and specificities
- Increase in cell-surface sialylation
- Increase in chitinase-like proteins and proteoglycans
- Increase in glycosphingolipids

The blood group–related carbohydrate structures Le(x), sialyl-Le(x), ABH, and Le(y) are examples of terminal carbohydrate structures that are related to tumor prognosis. These structures are of increasing interest because they may function as adhesion molecules or motility factors.[109]

Thomsen-Friedenreich Antigen (Pancarcinoma-Associated Antigen)

Many malignant cells develop a tumor marker called the Thomsen-Friedenreich (T) antigen (TFA). Tn and sialyl Tn (STn) appear to result from somatic mutations in the *Cosmc (C1GALT1C1)*. Diverse neoplastic lesions, including colon cancer and melanoma-derived cell lines, express both Tn and STn antigen due to loss-of-function mutations in *Cosmc*.[110] This antigen is encrypted in normal healthy cells, much like a rock is covered over by water at high tide. T antigen becomes "unsuppressed" only as a cell moves toward malignancy, much like the example of the covered rock, which is uncovered as the tide moves out.

It has been estimated that T antigen (or its precursor, Tn antigen) is expressed in about 90% of all cancers, earning it the appellation "pancarcinoma-associated antigen."[111] As a general rule, an orderly expression of T antigens on a cancer cell usually indicates a relatively favorable outlook. However, a prevalence of Tn antigens on a cancer cell usually denotes a highly aggressive, metastatic cancer, irrespective of the form of cancer or organ involved.[112]

Tn antigen is rarely found in healthy tissue; most people produce TFA agglutinins antibodies to it, probably in response to cross-induction by the gut flora. ABO blood group appears to influence the amount and activity of these antibodies against T and Tn antigens. Although derived from the MN blood type antigen, Tn antigen shows structural homology to the A antigen because they share terminal GalNAc. Because antibodies against Tn antigen cross-react with A glycolipids, Tn antigen was concluded to be an A-like antigen in a broad sense. Blood group A patients with gastric cancer have the greatest and most uniform suppression of TFA agglutinins levels, irrespective of age, cancer stage, or tumor morphology.

Inadvertent Elaboration of Blood Group Antigens

Deletion, reduction, or inappropriate expression of blood group A or B antigen in tumors of blood type A or B individuals is clearly correlated with the level of malignancy and metastatic potential.[113] The most significant variations are summarized in Table 43.3.

A and B blood group antigens are present on carcinoma cells at the early stages of carcinogenesis and tend to disappear at later stages.

TABLE 43.3 Variation in Blood Group Antigen (BGA) Expression in Normal and Neoplastic Tissues

Tissue, Organ	Normal Expression of BGAS	Expression of BGAS in Malignancy	Reference
Colon[a]	Present	Absent	157
Bladder[b]	Absent	Present	158
Prostate	Present	Absent	159
Liver[c]	Absent	Present	160
Squamous	Present	Absent	161
Endometrium[d]	Absent	Present	162
Stomach	Present	Absent	163
Thyroid[a]	Absent	Present	164
Esophagus	Present	Absent	165

[a]Inversely correlated.
[b]BGAs better than all other tumor markers.
[c]BGAs effective at predicting hepatitis transformation to malignancy.
[d]Vast majority of BGAs secreted are H antigen.

TABLE 43.4 Correlation of CA19-9 and DU-PAN-9 Expression in Colorectal Cancer With Lewis (Le) Type

Lewis Phenotype	Ca19–9 Expression[a]	Du-Pan-9 Expression
Le^{a+b-}	Highest levels	Lower levels
Le^{a-b+}	High levels	Lower levels
Le^{a-b-}	Zero to very low levels	Highest levels

[a]Individuals having homozygous inactive Se alleles *(sese)* and homozygous active Le alleles (*LeLe*) exhibited the highest mean CA19-9 value. All of the Lewis-negative individuals (*lele* genotype) had complete absence of CA19-9, irrespective of the Se genotype.[10]

The blood group A antigen may render malignant cells resistant to apoptosis.[114]

Lewis blood groups can modulate the expression of several tumor-associated antigens, including DU-PAN9 and CA19-9. Some researchers have suggested that taking into account Lewis secretor status to establish reference ranges for the tumor antigen measurements might actually be a way to improve their clinical utility. This relationship is shown in Table 43.4.

The association of blood groups with cancer was first demonstrated by Aird et al.[115] in 1953, pointing to group A with gastric carcinoma. One hypothetical mechanism is that the carcinoma cells produce an antigen immunologically related to blood group A, which may have a protective effect, particularly in blood group O individuals, by prevention of the growth and spread of the tumor.[116] It appears that the progression of stomach cells to stomach cancer involves a necessary mutation at the ABO gene, the result of which is the production of A antigen, even if this is not the person's blood type. This "A-like" antigen may not be the true A antigen but, rather, what one researcher called "tumor-associated, A cross-reacting antigens occurring in a wide variety of human adenocarcinomas of hosts belonging to all ABO blood groups."[117] There is also a B-like antigen noted in colon or rectal carcinoma and other malignant processes.[112] More data need to be collected about different malignant processes. A 2009 study in Italy that retrieved tumor data on 15,359 patients was able to show that exocrine pancreatic malignancy

was associated with the ABO blood group, but no other probabilistically significant correlations were made, or correlations were made but discarded, about other malignancy associations.[118]

Epidermal Growth Factor

The epidermal growth factor receptor (EGF-R) bears an antigenic determinant that is closely related to the human blood group A carbohydrate structure. A higher number of high-affinity EGF binding sites was observed in donors with blood group A1 erythrocytes than in donors with blood groups O and B.[119]

That the blood group A antigen can also bind to EGF-Rs is now well documented, so it is not unlikely that free A antigen in individuals with blood groups A and AB (especially if they are secretors) can find their way onto these excess EGF-Rs and act to stimulate cell growth. As with vWF and FVIII, excessive activation of the EGF-R results in cancer cells that become more mobile and better able to develop new and additional blood supplies (angiogenesis).[120]

One reason that low stomach acid is linked to type A blood may have to do with the role of EGF discussed in the previous section on saliva, "ABH Secretors and Nonsecretors." As described, the EGF-R has a high affinity for the type A antigen, and although the major action of EGF is to stimulate repair of the digestive lining, it has also been shown to decrease acid production in the stomach.

Pancreatic Cancer

The state of the art in the field of pancreatic cancer research continues to prove the relationship of malignancy to ABO blood group. A clinical trial for pancreatic ductal adenocarcinoma that involved 3000 people from the Han population showed that higher risk, higher state of malignancy, and less survival after surgery was associated with non-O blood groups.[121] Analyzing ABO genotype alleles with a genome-wide association study (PanScan) identified significant associations at the ABO gene locus with risk of pancreatic cancer. An increase in risk was noted with the addition of each non-O allele. Compared with OO genotype, AA presented a higher risk than AO in the 3000 patients analyzed.[122]

To help identify what aspect of the O allele is involved in lowering the pancreatic cancer risk in 2009, 558,542 single-nucleotide polymorphisms (SNPs) were genotyped in 1900 cancer patients and an equal number of control patients. A link was found between a locus on 9q34 and pancreatic cancer marked by the SNP rs505922. This SNP maps to the first intron of the ABO blood group gene, corroborating the epidemiological fact that people with blood group O may have a lower risk of pancreatic cancer than those with groups A or B.[123]

The enzyme glycosyltransferase is associated with increased pancreatic cancer risk impact rather than actions of other nearby genes on chromosome 9q34. Glycosyltransferase activity is greater for the A1 versus A2 variant. In a 2010 cancer cohort study, an increased risk in participants with A1, but not A2, alleles was observed. The same study showed that the protective effect of the O allele would be equivalent for O01 and O02 variants. Secretor status was not an effect modifier.[124]

Having a non-O ABO blood group and colonization by *H. pylori* are risk factors for pancreatic cancer. In a study at the Yale School of Medicine in 2010, an association between pancreatic cancer risk and CagA-negative *H. pylori* seropositivity was found among individuals with non-O blood type but not among those with O blood type, proving that there is an association between pancreatic cancer and *H. pylori* colonization, particularly for individuals with non-O blood types.[125]

Breast and Other Gynecologic Cancers

The following articles represent a sampler of the advances in understanding relationships between ABH antigens, secretor status, and the incidence and prognosis of different gynecologic-related cancers. In 2009 a correlation between breast cancer in Greek women and ABO blood group was published. One hundred sixty-six female patients with breast cancer were examined. They revealed that blood group A is more often associated with ductal breast cancer (49.6%), in contrast to the other blood groups, and particularly to blood group AB (3.6%). The relative risk of metastasis in Rh (−) patients was 4.2 times higher than that in Rh (+) patients. Consequentially, blood group A, and particularly A (−), has the worst prognosis of all.[126]

Breast cancer researchers have given the aberrant glycosylation moiety T/Tn antigen the moniker "ligand-like complex" (LLC). By virtue of altered antigenicity, LLC allows for both metastatic egress from the regional lymph nodes and detachment from the extracellular matrix and thus is associated with cancers of poor prognosis.[127] We believe that LLC may well be the "A-like," pancarcinoma cross-reacting antigen. Observation of the GalNac-binding lectin from the Roman snail, *Helix pomatia* agglutinin, appears to identify this oligosaccharide[128]; there have been separate reports that "Springer's vaccine" (human group O RBC membrane–derived T/Tn antigen containing traces of phosphoglycolipid A hyperantigen) has had significant effect as an immune modulator in breast carcinoma of even advanced stage.[129]

A study of epithelial ovarian cancer incidence and its association to ABO blood groups and risk was performed in 2010 at Harvard and analyzed data from 49,153 women in the Nurses' Health Study. Compared with women with blood group O, women with blood group AB or B had a nonsignificant 38% increase in ovarian cancer incidence, whereas blood group A was not associated with risk. Combining blood groups AB and B, they observed a statistically significant positive association with presence versus absence of the B antigen for the serous invasive subtype and overall. In this large, prospective cohort of women, the presence of the B antigen was positively associated with ovarian cancer incidence, whereas blood group A was not associated with the same risk.[130]

Another interesting, yet not definitive, association is presented between invasive squamous cell carcinoma (SCC) of the vulva and ABO blood groups. The distribution of ABO blood group for 33 women diagnosed with invasive SCC of the vulva was determined. ABO blood group was also recorded for 100 female patients (controls) who underwent a gynecologic procedure for a nonneoplastic process during the same period. A trend was identified for women with invasive SCC of the vulva to have blood group type A, but this did not determine a definitive association between blood group type A, or any other blood group, and vulvar SCC.[131]

Other Cancers

In two large independent populations, non-O blood group was associated with a decreased risk of skin cancer. The association was statistically significant for nonmelanoma skin cancer. Additional studies are needed to confirm these associations and to define the mechanisms by which ABO blood type or closely linked genetic variants may influence skin cancer risk.[132]

In addition to original studies on gastric carcinoma, a 2016 study associated blood group A with an increased risk of cancer of the pharynx and esophageal adenocarcinoma.[133] The ABO blood group is not only associated with incidence but also prognosis. Research showed that blood group A was a negative prognostic factor in gastric carcinoma.[134] Another 2017 study showed that of gastric cancer patients with a positive postoperative CEA cancer marker, those with blood group AB were more likely to have a better survival rate.[135]

TABLE 43.5 Mechanisms of ABO Influence on Infectious Disease[10]

Class	Mechanism	Description	Example
Selectivity	Adhesion kinetics	Adhesion or lectin specificities of the infectious agent based on particular ABH glycosylation	*Candida albicans* (group O)
	Humoral dynamics	Inadequate isoagglutinin production or activity	*Neisseria gonorrheae* (group B)
	Molecular mimicry	Infectious agent is antigenically similar to host's ABO group	*Giardia lamblia* (group A)
Response variability	Host response	Variation in severity of disease through unique biological response (examples: inappropriate inflammatory response; rosette formation)	Cholera (group O) Dengue fever (group B) Malaria (group A)
	Substrain susceptibility	Different ABO groups often show variations in susceptibility between individual bacterial, fungal, or parasitic species or viral strains.	Influenza (all groups) Malaria (group O vs. group A)

In colorectal cancer, there is no specific association with blood group type, but there is a significant difference between the Rh(+) and Rh(−) groups. Further studies on blood group antigens are needed to elucidate the relationship between these antigens and colorectal cancer.[136]

Infection

To a great extent, infectious diseases, especially the worldwide epidemic diseases, have selective effects. This is demonstrated, *inter alia,* in the different "selection values" in the ABO blood group system. During the eons before antimicrobial intervention, selection variability via ABO polymorphism was a preeminent natural survival mechanism.

The previous section appears to imply a selection disadvantage for group A individuals, and it has been argued that under present-day civilized living conditions, blood type O carriers have a preservation advantage over blood group A carriers.[137] This may be the result of the deletion of the selection factor "infectious disease," which may nevertheless regain importance if environmental changes occur.[138]

Historically, some of the most catastrophic epidemic and endemic diseases are ABO selective and, in many instances, demonstrate ABO variation in morbidity, mortality, or microcharacteristics such as substrain preferentiality and level of inflammatory response. They include cholera (O), smallpox (A), malaria (A), and influenza (variable subsets depending on strain).

The influence of ABO polymorphism on infectious disease appears to stem from a multitude of unique factors. These are encapsulated in Table 43.5. A morbidity and mortality variation among the ABO and secretor groups is presented in Table 43.6. A special examination of polymorphic differences in uropathic infectious disease is presented in Table 43.7.

An interesting study done in Sudan about placental malaria showed that women of eastern Sudan are at risk for placental malaria infection irrespective of their age or parity but that women with blood group O were at higher risk of past placental malaria infection.[139] A higher seroprevalence of IgG *H. pylori*–specific antibodies was observed in gastric ulcer patients (90%) compared with the control group (60%). A significant increase of phenotypes O, A2, and Le^b in *H. pylori*–infected patients was observed. The expression of these antigens had progressive alterations in areas of ulcerous lesions and intestinal metaplasia; therefore ABH and Lewis blood group antigens appear to be a good indicator for cellular alterations in the gastric epithelium.[140]

For a more detailed examination of particular infectious scenarios, the reader is referred to the author's comprehensive survey.[141]

Periodontal Disease and Oral Malignancy

A recent study segregated populations (1220 subjects aged between 20 and 55) into three groups according to Ramfjord's periodontal disease index: healthy, gingivitis, and periodontitis. Blood types were confirmed for each of the participants. Effectively, blood group A showed a significantly higher percentage in the gingivitis group, and blood group O showed a higher percentage in the periodontitis group. The blood group AB showed the least percentage of periodontal disease. The distribution of Rh factor in all groups showed a significantly higher distribution of Rh-positive.[142]

In most human carcinomas, including oral carcinoma, a significant event is decreased expression of histo–blood-group antigens A and B. A relative down-regulation of the glycosyltransferase that is involved in the biosynthesis of A and B antigens is seen in oral carcinomas in association with tumor development. As reviewed previously, the events leading to loss of A transferase activity are related, in some instances, to the loss of heterozygosity involving chromosome 9q34, which is the locus for the ABO gene, and in other cases, to hypermethylation of the ABO gene promoter. Altered blood group antigens in malignant oral tissues may indicate increased cell migration. Some studies showed that normal migrating oral epithelial cells, like malignant cells, show lack of expression of A/B antigens and, therefore, lack of ABH antigens to key receptors controlling adhesion and motility, such as integrins, cadherins, and CD-44.[143]

BLOOD GROUPS AND DIETARY LECTINS

ABO polymorphism is one of the prime determinants of the glycosylation variability of gut mucin. As such, it affords insight into the actions of a class of carbohydrate-binding dietary proteins called lectins, which are increasingly a subject of interest for nutritional researchers.

Historical Perspective

Stillmark at the University of Dorpat in Estonia first identified lectins in 1888. While investigating the toxic effects on the blood of castor bean extract *(Ricinus communis),* he noticed that the RBCs were being agglutinated. He isolated the material responsible for the agglutination and called it ricin.

In 1945 William Boyd of the Boston University School of Medicine discovered that lectins can be blood group specific, being able to agglutinate the RBCs of one type but not those of another. He discovered that lima bean lectin would agglutinate RBCs of human blood type A but not those of O or B. The seeds of *Lotus tetragonolobus* can agglutinate group O specifically, and *Bandeiraea simplicifolia* is specific to group B. The specificity of lectins is so sharply defined that they can differentiate among blood subgroups. *Dolichos biflorens* lectin reacts more vigorously with blood group A1 than with A2.

The word *lectin* was proposed by William Boyd in 1954 to describe a class of blood type–specific agglutinins found in certain plants. The word is allegorical, to a degree, because it derives from a Latin word meaning "to choose."

Actions of Lectins

The surface epithelium of the gut is extensively glycosylated,[144] mainly because most membrane proteins, including hormone and growth

TABLE 43.6 Influence of ABO Polymorphism on Susceptibility to Various Infectious Agents[10]

Strain	Susceptible Phenotype	Comments
Amoebic dysentery	O, A	• Blood groups B and AB have a degree of resistance against developing severe or acute dysentery, especially the amoebic forms.
Candida carriage	O, NS	• *Candida* carriage was associated with blood group O ($P < 0.001$) and, independently, with nonsecretion of blood group antigens ($P < 0.01$). • *Candida albicans* extracellular polymeric material contains a mannoprotein adhesion with a lectin-like affinity for H (type 2) blood group antigen. • There was a significantly higher number of nonsecretors (48.9%) among 174 patients with either oral or vaginal *Candida* infections compared with the proportion of nonsecretors in the local population (26.6%). Nonsecretor saliva actually seemed to enhance *Candida* attachment.
Cholera	O, AB	• Blood group O individuals have a greater risk of infection with cholera and develop the most severe and life-threatening forms of this illness. This has been documented in several studies. • In contrast, type ABs appear to have the highest degree of protection from cholera infections. • Type O had more diarrhea-like stools per day than persons of other blood groups and were more likely to report vomiting and muscle cramps.
Coccidioidomycosis	B	• Blood group B individuals are more prone to disseminated disease after exposure.
Dengue fever	B	• According to researchers, blood group B was strongly associated with the severe form of dengue fever known as dengue hemorrhagic fever.
Dermatophytosis	A	• The fungus *Trichophyton rubrum,* isolated from 54.5% of the patients tested, was more frequent in individuals belonging to blood group A.
Escherichia coli	Variable subsets	• It appears that many forms of *E. coli* capable of causing diarrhea are immunologically "B-like." This results in a substantially higher number of cases of diarrhea among individuals of blood group B and AB people. • However, when it comes to the overall severity of infection with *E. coli,* types B and AB are not alone; type Os also are more likely to get a severe form of diarrhea.
Giardia	A	• Blood group A is more susceptible to giardiasis, especially the asymptomatic form, whereas blood group B is less susceptible to giardiasis.
Helicobacter pylori	O, nonsecretor	• *H. pylori* variants produce a variety of blood group antigens, including A, Lewis (a), and a variety of type 1 H like antigens (O). • Group O would be a moderate risk factor for infection by *H. pylori,* with more severe cases in men. • Group O has a more pronounced inflammatory reaction to *H. pylori.* Group O cells released significantly more IL-6 and TNF in response to *H. pylori* infection. • The Lewis^{a+b-} nonsecretor phenotype and blood group O are relevant genetic markers of peptic ulcer. • The Lewis^{a+b-} nonsecretor phenotype and blood group A were all positively associated with esophageal adenocarcinoma, with concurrent *H. pylori* infection.
Hookworm	O	• A 1972 Egyptian study correlated type O with higher incidence of hookworm and strongyloidiasis.
Influenza	Variable subsets	• Blood group A: Generate a quick and substantial antibody response against influenza type A(H1N1) and especially A(H3N2). The antibody response against influenza B is not quite as dramatic. • Blood group AB: Relatively poor ability to generate high antibody levels against any of the influenza viruses. • Blood group B: Reasonable, but not great ability to generate an antibody response against influenza A (H1N1). Slowest (3–5 months) and weakest ability to generate antibodies against influenza A (H3N2 "Hong Kong") of any blood group. Against influenza B virus, blood group B has a significant advantage and responds differently from either blood group A or O. The blood group B immune response happens much earlier and persists longer. • Blood group O: Moderate ability to generate antibody response against influenza A (H1N1) and A (H3N2) viruses. Antibody response against influenza B is not as dramatic as blood group B.
Malaria	A, AB	• The evidence suggests that blood group A individuals might have a higher predisposition to infection with the *Plasmodium vivax* species, whereas blood group B individuals tend toward higher infection rates with *P. falciparum.* • Malaria-infected RBCs sometimes bind to uninfected RBCs to form clumps, called rosettes. The rosettes can obstruct flow in small blood vessels and lead to tissue damage and severe malaria disease. The tendency for malaria to be worse among As and ABs is due primarily to a greater degree of rosette formation by RBCs with these antigens. • Von Willebrand factor, always elevated in type A, also enhances rosette formation.
Neisseria gonorrheae	B	• The relation of infection with *N. gonorrheae* to the blood groups A, B, AB, and O was examined in 584 women attending a prenatal clinic. The occurrence of gonorrhea was significantly higher in black patients with blood group B than in those with blood groups A, AB, or O. Depending on the ABO blood group, gonorrhea may affect the titers of isohemagglutinins compared with those of uninfected controls. • The isohemagglutinin titers in group O patients were significantly increased ($P < 0.001$) against erythrocytes A, B, and AB. In group A patients, only the titer against AB erythrocytes was significantly increased. • In group B patients, the titer against AB erythrocytes was significantly lower ($P < 0.001$) compared with that in sera of healthy persons.

TABLE 43.6 Influence of ABO Polymorphism on Susceptibility to Various Infectious Agents[10]—cont'd

Strain	Susceptible Phenotype	Comments
Norwalk virus (NV)	O	• It appears that group O RBCs are most easily bound by NV versus group B RBCs that are apparently little bound, if at all. Individuals with an O phenotype were more likely to be infected with NV. • The preferred binding sites are apparently the H type 2 antigen that functions as the viral receptor on human type O RBCs. • The Lewis B antigen (found in secretors) is also a binding site.
Schistosomiasis	A	• Group A tends to be more susceptible to infection, tends to get more intense symptoms after infection, and is much more likely to have damage to organs (e.g., the liver) after infection.
Shigellosis	B, AB	• A strong association between blood group B (and AB to a slightly lesser degree) and shigellosis exists.
Smallpox	A	• Group A has higher mortality from smallpox infection. • Group A individuals also have more reactions from smallpox vaccination. • The leukocytes of peripheral blood of group A individuals showed a poorer binding capacity with respect to the smallpox vaccine virus. • Blood group A also exhibited a high rate of chromosomal aberration after vaccination, resulting to some extent from increased proliferative ability of the cells.
Staphylococcus aureus	A	• Blood group A is much more likely to be a chronic carrier of *S. aureus.* This is partly due to blood group A individuals having a decreased ability to mount an aggressive antibody (or immune) response against this organism.
Streptococcus (group B)	B	• A blood group connection with neonatal group B streptococci infection exists for blood group B. Maternal blood group B is associated with about a doubling of risk for infection among their infants.
Strongyloidiasis	O	• A 1972 Egyptian study correlated type O with a higher incidence of hookworm and strongyloidiasis.
Tuberculosis	O	• Group O blood has a much higher rate of infection with tuberculosis (this is particularly true in individuals of European descent). • Tuberculosis runs a much more aggressive and detrimental course in blood group O, whereas type A is afforded the highest degree of protection. • Typically, during the first 2 years of infection with bacillary tuberculosis, there is a significant excess of infection among individuals with blood groups O and AB.

IL-6, Interleukin-6; *RBCs,* red blood cells; *TNF,* tumor necrosis factor.

TABLE 43.7 Influence of ABO Polymorphism on Susceptibility to Various Uropathic Infectious Agents[10]

Blood Group	Uropathogenic Strains
A	• *Staphylococcus saprophyticus*
B	• *Klebsiella pneumoniae* • *Proteus* spp. • *Pseudomonas* spp.
AB	• *Klebsiella pneumonia* • *Proteus* spp. • *Pseudomonas* spp. • *Staphylococcus saprophyticus*
Nonsecretor	• Uropathogenic *Escherichia coli*

As a general rule, blood group B is most plagued by chronic or recurrent urinary tract infections. Type AB is next on the susceptibility list, followed by type A. Type Os are the most protected. Nonsecretors are much more prone to repeated and severe urinary tract infections.

BOX 43.1 Factors Influencing Glycosylation in the Intestines, Hence Activity of Dietary Lectins

Animal species
Blood group specificity
Age
Particular area of the small intestine
Position along the villi
State of cell maturation
Diet
Bacterial status
Sickness or pathology

Data from Pusztai A, Bardocz S. Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications. *Trends Glycosci Glycotechnol.* 1996;8:149–165.

factor receptors, transport proteins, and brush-border enzymes, are glycosylated before being embedded in the brush-border membrane. Membrane lipids and gangliosides are also glycosylated, and all secreted mucins are carbohydrate-rich glycoproteins. Thus the scope of potential lectin–carbohydrate interactions is quite wide. However, not all lectins react with the epithelium, and even those that do react vary in their ability to recognize and bind to specific types of carbohydrate receptors. Because lectin reactions are quite specific, it is imperative that the correct carbohydrate structures be present on the surface of the gut mucosa. Factors that are known to influence lectin activity are summarized in Box 43.1.

Lectins can have a variety of biological effects, including induction of mitosis in lymphocytes, cellular agglutination via cross-linking of membrane sugars, preferential agglutination of malignant cells, precipitation of polysaccharides and glycoproteins, and activation of complement pathways. The variety of reported effects resulting from the ingestion of dietary lectins is summarized in Table 43.8.

Most lectins in the U.S. diet are resistant to breakdown during gut passage, so they are bound and endocytosed by epithelial cells. Endocytosed lectins act as powerful exogenous growth factors for the

TABLE 43.8 Reported Effects of Dietary Lectins

Action	Description
Induction of interleukins (ILs)	Dietary lectins are known to induce interleukins IL-4 and IL-13. Because lectins can enter the circulation after oral uptake, they might play a role in inducing the so-called early IL-4 required to switch the immune response toward a helper T-cell type 2 response and type I allergy.[166]
Induction of autoimmunity	The interaction of dietary lectins with enterocytes and lymphocytes may facilitate the translocation of both dietary and gut-derived pathogenic antigens to peripheral tissues, which in turn causes persistent peripheral antigenic stimulation. In genetically susceptible individuals, this antigenic stimulation may ultimately result in the expression of autoimmune disease.[167]
Interference with protein digestion	Aminopeptidase activity (the enzyme that breaks down polypeptides into amino acids) is inhibited by several dietary lectins.[168]
Interaction with the brush-border membrane	Lectins, which bind avidly to the brush-border membrane, are potent hyperplastic growth factors for the gut.[169] Binding of lectins to the epithelium is obligatory for growth stimulation, and their growth factor activity is determined mainly by the strength and intensity of their binding.[170]
Antinutrient effects	Rats fed a diet composed principally of raw navy bean flour were smaller and had 50% less ability to absorb glucose and utilize dietary protein than a control group that was fed navy beans in which the lectin had been inactivated.[171]
Enhancement of gut permeability	In one study, rats fed on diets containing kidney beans showed greater intestinal permeability to serum proteins that had been injected into the bloodstream. After challenge with kidney bean proteins, the protein injected into the bloodstream was detected in both the lumen (open space) and the walls of the small intestine. The researchers suggested that dietary lectins may, at least in part, be responsible for the loss of serum proteins and may contribute to other food intolerance secondary to the loss of gut integrity.[172]
Activation of gut hormones	Cholecystokinin is induced by several dietary lectins.[173]
Hormone and growth factor mimicry	Because surface membrane receptors of cells are glycosylated, lectins are good mimics of the effects of endogenous growth factors, hormones, and cytokines in all types of cells.[145] Lectins can mimic the effect of natural ligands and induce similar physiological reactions. It is also possible that bound lectin induces conformational changes in the receptor and/or physically blocks the active site of the receptor, thereby attenuating or completely abolishing the physiological effect of the natural ligand.
Mucotractive effects	Many lectins stimulate the production of mucus. This is possibly a protective function or an allergic response and was for a time thought to be an action of lectins that promised some therapeutic benefit that could be applied in patients with cystic fibrosis.[146]
Mitogenic effects	Several foodstuffs and herbal medicines are known to contain lectins that are capable of inducing T- or B-cell blastogenesis.

small intestine, and they can induce dramatic shifts in its bacterial flora and interfere with its hormone secretion. In addition, lectins that are transported across the gut wall into the systemic circulation can modulate the body's hormone balance, metabolism, and health. In contrast to dietary proteins, lectins resist degradation in the small intestine and are also resistant to breakdown by most gut bacteria. Thus most lectins survive, at least in part, the passage through the digestive tract in an immunologically and functionally intact form.[145]

Although lectin binding is most commonly studied in the small intestine, similar binding can occur throughout the entire digestive tract, from the stomach to the distal colon. However, surface glycosylation varies in the different functional parts of the gut, so lectin binding is not uniform in the digestive tract. Binding of lectins and their endocytosis by enterocytes is more extensive in the colon, where bacterial counts are high. Endocytosis in the small intestine becomes appreciable only in the presence of large numbers of commensal bacteria.[146]

How prevalent are lectins in the diet? A superficial survey done in the early 1980s implied that they are quite extensively distributed in our food supply.[147] In this study, the edible parts of 29 of 88 foods tested, including common salad ingredients, fresh fruits, roasted nuts, and processed cereals, were found to possess significant lectin-like activity as assessed with blood agglutination and bacterial agglutination assays.

Lectin content is a prime area of manipulation for the production of transgenic foods,[148] so they will probably continue to occupy the attention of nutritionally oriented physicians well into the future.

Role of ABO Blood Groups

Because ABO blood group is a prime determinant of glycosylation, it is not surprising that a substantial number of food lectins show ABO specificity. However, equally important but not typically recognized is the author's observation that ABO status appears to influence variability in secondary glycosides, which are not integral to the ABO type of the hosts. These secondary glycosides appear to provide additional conditioning of the mucin manufactured by the particular ABO type, which can explain the apparent effects of panhemagglutinins and other non-ABO specific lectins on one particular ABO type over another. These effects are listed in Table 43.9.

The benefit of using the ABO groups as a predictive device in narrowing down potential food reactions in sensitive subjects lies in its low cost to the patient and the clinician's ability to discern interactions that are not easily testable via standard food allergy isolation methods. A useful resource for clinicians seeking more information on lectins is *Lectser,* the world's most complete lectin characterization database, available at the principal website.[149]

CLINICAL APPLICATIONS OF ABO POLYMORPHISM

The "Blood Type Diet"

The advantage of a low-lectin diet can be exemplified by the best-known application of the ABO polymorphisms, the Blood Type Diet (BTD), popularized by publication in 1997 of the author's *New York Times* bestseller *Eat Right 4 Your Type.*

Eat Right 4 Your Type was the first "diet book" that looked at foods as medicine tailored to the individual rather than simply as part of a generic single-purpose mechanism (i.e., weight loss, cholesterol control). Like the very antigens that it uses for its determination, BTD is "nonreducing," meaning that the food value system is essentially stateless: a food that might cause difficulties in one ABO phenotype may well possess therapeutic value in another, a concept that harkens back to the observation of the Roman philosopher Lucretius: "What is food to one man may be fierce poison to others."

TABLE 43.9 Primary and Secondary Mucin Glycosides Known to Be Associated With ABO Polymorphism

Blood Group	Primary Glycoside	Secondary Glycoside(S)
A	*N*-Acetylgalactosamine	Mannose[174] Glycophorin A[175] Galactosyl-A glycolipids[176] "Band 3"[177]
B	Galactose	N-Neuraminic acid[178] Glycophorin B[175]
O	Fucose	*N*-Acetylglucosamine[179–181]

The BTD inventories a variety of parameters, including the physiological and pathological distinctions between the blood groups as reported in the literature, lectin and agglutinin characterizations (both from the author and from others), and variations in isoagglutinin titer and reactivity with regard to food challenge (both in vitro and in vivo). It also provides specific recommendations regarding foods that should be considered beneficial and should be emphasized and others that may be problematic and might better be avoided.

One advantage conferred by the theory is the notion that certain individuals may be better suited to Paleolithic or vegetarian diets. Despite certain locutions and circumlocutions offered by proponents of one system or the other, most clinicians have ample experience with the simple fact that certain patients make better vegetarians or carnivores than others. Conversely, the ability to identify particular individuals at risk of an adverse reaction to high-protein and/or low-carbohydrate diets can lead to actions aimed at the prevention of such consequences.

For example, high-protein diets are known to upregulate soluble endothelial adhesion factors,[150] whereas a soy-based, lipid-lowering diet is known to decrease endothelial adhesion factors, including vWF, E-selectin, and the intercellular adhesion molecule.[151–153] Because individuals with blood group A have been reported to have increased levels of all three endothelial adhesion factors and, perhaps consequently, a higher incidence of cardiovascular disease, a modified Asian and/or Mediterranean plant-based diet would appear ideal for this phenotype.

Conversely, in 1998, we examined serum cholesterol levels in a small number of group O subjects after a minimum of 1 month of the BTD recommended for that phenotype.[154] Group O individuals following the "Type O Diet" (higher protein, lower carbohydrate) showed a consistent drop in serum cholesterol ($n = 14$; average 237.3 mg/dL before diet, 200.2 mg/dL with diet); in subjects with elevated serum triglyceride values, the results were more pronounced ($n = 6$; average 387.1 mg/dL before diet, 168.8 mg/dL with diet).

In 2009 D'Adamo reported that sequential breath hydrogen levels (BHL) after lactulose administration varied significantly by ABO blood group and secretor status. Breath hydrogen excretion was significantly higher in group A subjects ($n = 35$) versus group O ($n = 31$). Secretor status appeared to influence location and temporality of bacterial overgrowth and BHL, with secretors having significantly higher levels on earlier blows (B1: $P = 0.027$) and nonsecretors having significantly elevated BHLs on later blows (B3: $P = 0.014$, B4: $P = 0.013$). Adoption of blood group–specific dietary recommendations significantly reduced mean BHLs ($n = 40$; $P = 0.007$).[155]

The BTD has produced an extensive library of works, at various levels of information density. *Eat Right 4 Your Type* has been translated into more than 60 languages, and well over 6 million individuals follow the system.[156] Self-reported outcomes collected via the Internet appear to indicate wide consumer satisfaction with the system: in more than 7000 reports, the basic ABO-based system generated an 85% level of satisfaction. Most interestingly, this percentage was essentially unchanged across the individual blood groups. In other words, 85% of the blood type A respondents reported satisfaction with a predominantly plant-based diet, whereas the same percentage of type O individuals reported satisfaction with a high-protein, Paleo-type diet. What this portends for the future of reductionist, one-size-fits-all diet systems remains to be seen.[157]

Materia Medica

ABO polymorphism and its clinical expression have led to new or rediscovered applications and contraindications for several traditional naturopathic treatment modalities. The most significant are summarized in Table 43.10.

SUMMARY

It is our hope that this abbreviated survey provides sufficient information to persuade the reader that ABO and ABO-related polymorphisms, in both genotypic and phenotypic expression, exert biological significance far beyond the surface of an erythrocyte and that this expression is worth factoring into the everyday algorithms of a nutritional practice. Perhaps the advent and acceptance of the newly emerging science of "nutrigenomics" might provide the conceptual framework needed to allow the nontransfusion significance of ABO and secretor polymorphism to parse into meaningful scientific dialogue. At that point, natural medicine could be considered to have the rational beginnings to delivering its long-awaited promise, "Treating the patient, not the disease."

TABLE 43.10 Modalities Known to Exert Effects at Least Partially Preferential to ABO Group

Agent/Species	Common Names	Actions	Notes	References
Fucus vesiculosus	Bladderwrack kelp	Antiadhesive Candicidal	Best suited for O, nonsecretors	182–184
Baptisia tinctoralis	Wild indigo	Induces anti-Tn or cross-reacting antibodies	Best suited for A, AB	185, 186
Urtica dioica rhizome	Stinging nettle root	"Superantigen" lectin T-cell mitogen (CD4+ and CD8+); Candicidal	Not useful in B or AB	87, 188
Helix pomatia agglutinin	Roman or escargot snail	Contains Tn-, LLC-, and "A-like"–specific lectins	Best suited for A, AB	27, 128
1.1.1.1.1.1 Agaricus bisporus	Domestic mushroom	Lectin stimulates insulin release by pancreatic islets. Lectin binds Tn antigen; stimulates differentiation of undifferentiated colon cancer cells; inhibits proliferation of epithelial cell lines.	Best suited for A	89, 190
Marrubium spp.	Horehound	Contains Tn-specific lectins	Best suited for A, AB	91
Salvia spp.	Sage	Contains Tn-specific lectins; attenuates soluble endothelial adhesion factors	Best suited for A, AB	91, 192
Vicia faba	Fava bean	VFA was found to stimulate an undifferentiated colon cancer cell line to differentiate into gland-like structures. The adhesion molecule epCAM is involved in this process. Dietary or therapeutic VFA may slow the progression of colon cancer.	Best suited for A; can be used by O, B	93
Griffonia (Bandeiraea simplicifolia)	African legume from which 5-HTP is extracted	*Griffonia* contains five isolectins, at least three of which are blood group agglutinating. GSA B4 is a serologically Lewis b(Le^b)–active binding lectin claimed to be blood group B specific as well. The "A"-rich lectin preferentially agglutinates blood group A, and the "B"-rich lectin preferentially agglutinates blood group B cells and is specific for alpha-galactose residues.	Not useful in A, AB, B or secretors	94–196
Artocarpus integrifolia	Jackfruit seeds	Contains T- and Tn-specific lectin (jacalin)	Best suited for A, AB; can be used by O, B	197

epCAM, Epithelial cell adhesion molecule; *GSA, Griffonia simplicifolia* agglutinin; *LLC,* ligand-like complex; *VFA, Vicia faba* agglutinin.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Szulman AE. The histological distribution of the blood group substances in man as disclosed by immunofluorescence. III. The A, B, and H antigens in embryos and fetuses from 18 mm in length. *J Exp Med*. 1964;119:503–516.
2. Sarafian V, Dimova P, Georgiev I, et al. ABH blood group antigen significance as markers of endothelial differentiation of mesenchymal cells. *Folia Med (Plovdiv)*. 1997;39:5–9.
3. Dahlquist G, Kallen B. Maternal-child blood group incompatibility and other perinatal events increase the risk for early-onset type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*. 1992;35:671–675.
4. Vowden P, Lowe AD, Lennox ES, et al. Thyroid blood group isoantigen expression: a parallel with ABH isoantigen expression in the distal colon. *Br J Cancer*. 1986;53:721–725.
5. Glynn LE, Holborow EJ, Johnson GD. Distribution of blood group substances in human gastric and duodenal mucosa. *Lancet*. 1957;273:1083–1088.
6. Glynn LE, Holborow EJ. Distribution of blood-group substances in human tissue. *Br Med Bull*. 1959;15:150–153.
7. Holborow EJ, Brown PC, Glynn LE, et al. Distribution of blood group A antigen in human tissues. *Br J Exp Pathol*. 1960;41:430–437.
8. Szulman AE. Histological distribution of blood group substances A and B in man. *J Exp Med*. 1960;111:785–800.
9. Kogure T, Furukawa K. Enzymatic conversion of human group O red cells into group B active cells by alpha-d-galactosyltransferases of sera and salivas from group B and its variant types. *J Immunogenet*. 1976;3:147–154.
10. D'Adamo PJ, Kelly GS. Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. *Altern Med Rev*. 2001;6:390–405.
11. Matsushita M, Irino T, Stigbrand T, et al. Changes in intestinal alkaline phosphatase isoforms in healthy subjects bearing the blood group secretor and non-secretor. *Clin Chim Acta*. 1998;277:13–24.
12. Domar U, Hirano K, Stigbrand T. Serum levels of human alkaline phosphatase isozymes in relation to blood groups. *Clin Chim Acta*. 1991;203:305–313.
13. Mehta NJ, Rege DV, Kulkami MB. Total serum alkaline phosphatase (SAP) and serum cholesterol in relation to secretor status and blood groups in myocardial infarction patients. *Indian Heart J*. 1989;41:82–85.
14. Tibi L, Collier A, Patrick AW, et al. Plasma alkaline phosphatase isoenzymes in diabetes mellitus. *Clin Chim Acta*. 1988;177:147–155.
15. Agbedana EO, Yeldu MH. Serum total, heat and urea stable alkaline phosphatase activities in relation to ABO blood groups and secretor phenotypes. *Afr J Med Sci*. 1996;25:327–329.
16. Walker BA, Eze LC, Tweedie MC, et al. The influence of ABO blood groups, secretor status and fat ingestion on serum alkaline phosphatase. *Clin Chim Acta*. 1971;35:433–444.
17. Stolbach LL, Krant MJ, Fishman WH. Intestinal alkaline phosphatase in chylous effusion: role of ABO blood group and secretor status. *Enzymologia*. 1972;42:431–438.
18. Bayer PM, Hotschek H, Knoth E. Intestinal alkaline phosphatase and the ABO blood group system—a new aspect. *Clin Chim Acta*. 1980;108:81–87.
19. Hoskins LC, Boulding ET. Degradation of blood group antigens in human colon ecosystems. II. A gene interaction in man that affects the fecal population density of certain enteric bacteria. *J Clin Invest*. 1976;57:74–82.
20. Hoskins LC, Boulding ET. Degradation of blood group antigens in human colon ecosystems. I. In vitro production of ABH blood group-degrading enzymes by enteric bacteria. *J Clin Invest*. 1976;57:63–73.
21. Al-Agidi SK, Shukri SM. Association between immunoglobulin levels and known genetic markers in an Iraqi population. *Ann Hum Biol*. 1982;9:565–569.
22. Shinebaum R. ABO blood group and secretor status in the spondyloarthropathies. *FEMS Microbiol Immunol*. 1989;1:389–395.
23. Grundbacher FJ. Immunoglobulins, secretor status, and the incidence of rheumatic fever and rheumatic heart disease. *Hum Hered*. 1972;22:399–404.
24. Grundbacher FJ. Genetic aspects of selective immunoglobulin A deficiency. *J Med Genet*. 1972;9:344–347.
25. Pal A, Hill M, Wordsworth P, et al. Secretor status and ankylosing spondylitis. *J Rheumatol*. 1998;25:318–319.
26. Manthorpe R, Staub Nielsen L, et al. Lewis blood type frequency in patients with primary Sjögren's syndrome. A prospective study including analyses for A1A2BO, secretor, MNSs, P, Duffy, Kell, Lutheran and rhesus blood groups. *Scand J Rheumatol*. 1985;14:159–162.
27. Toft AD, Blackwell CC, Saadi AT, et al. Secretor status and infection in patients with Graves' disease. *Autoimmunity*. 1990;7:279–289.
28. Gampa A, Engen P, Green S, Mutlu E. Associations between fecal microbiota composition and blood group antigens. *Gastroenterology*. 2017;152(5). https://doi.org/10.1016/s0016-5085(17)33433-9.
29. Tosh FD, Douglas LJ. Characterization of a fucoside-binding adhesin of *Candida albicans*. *Infect Immun*. 1992;60:4734–4739.
30. Furukawa K, Ying R, Nakajima T, et al. Hemagglutinins in fungus extracts and their blood group specificity. *Exp Clin Immunogenet*. 1995;12:223–231.
31. Navas EL, Venegas MF, Duncan JL, et al. Blood group antigen expression on vaginal and buccal epithelial cells and mucus in secretor and nonsecretor women. *J Urol*. 1993;149:1492–1498.
32. Godzisz J. Synthesis of natural allohemagglutinins of the ABO system in healthy children aged 3 months to 3 years. *Rev Fr Transfus Immunohematol*. 1979;22:399–412. French.
33. Grundbacher FJ. Genetics of anti-A and anti-B levels. *Transfusion*. 1976;16:48–55.
34. Adewuyi JO, Gwanzura C, Mvere D. Characteristics of anti-A and anti-B in black Zimbabweans. *Vox Sang*. 1994;67:307–309.
35. Lauritsen JG, Grunnet N, Jensen OM. Materno-fetal ABO incompatibility as a cause of spontaneous abortion. *Clin Genet*. 1975;7:308–316.
36. Solish GI. Distribution of ABO isohaemagglutinins among fertile and infertile women. *J Reprod Fertil*. 1969;18:459–474.
37. Cantuaria AA. Blood group incompatibility and cervical hostility in relation to sterility. *Obstet Gynecol*. 1978;51:193–197.
38. Gloria-Bottini F, Nicotra M, Ianniello F, et al. ABH secretor status and reproductive success in couples with primary recurrent spontaneous abortion. *J Obstet Gynaecol*. 2011;37:32–36.
39. Lucarini N, Nicotra M, Gloria-Bottini F, et al. Interaction between ABO blood groups and ADA genetic polymorphism during intrauterine life. A comparative analysis of couples with habitual abortion and normal puerperae delivering a live-born infant. *Hum Genet*. 1995;96:527–531.
40. Gloria-Bottini F, Magrini A, Cozzoli E, et al. ABH secretor genetic polymorphism: evidence of intrauterine selection. *Eur J Obstet Gynecol Reprod Biol*. 2011;154(1):20–23.
41. Locke, Tim. Blood type O linked fertility problems. *Web MD*. http://www.webmd.com/infertility-and-reproduction/news/20101025/blood-type-o-linked-fertility-problems. Accessed November 10, 2011.
42. D'Adamo PJ, Zampieron ER. Does ABO "bias" in natural immunity imply an innate difference in T-cell response? *J Naturopath Med*. 1991;2:11–17.
43. Greenwalt TJ. A short history of transfusion medicine. *Transfusion*. 1997;37:550–563.
44. Yamamoto F. Blood group antigen gene mutation database. *The Burnham Institute*. http://www.bioc.aecom.yu.edu/bgmut/ abo.htm. Accessed March 27, 2004.
45. Aird I, Bentall HH, Mehigan JA, et al. The blood groups in relation to peptic ulceration and carcinoma of colon, rectum, breast, and bronchus. *BMJ*. 1954;4883:315–321.
46. Rad R, Gerhard M, Lang R, et al. The *Helicobacter pylori* blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. *J Immunol*. 2002;168:63033–63041.
47. Wilson AF, Elston RC, Siervogel RM, et al. Linkage of a gene regulating dopamine-beta-hydroxylase activity and the ABO blood group locus. *Am J Hum Genet*. 1988;42:160–166.
48. Neumann JK, Chi DS, Arbogast BW, et al. Relationship between blood groups and behavior patterns in men who have had myocardial infarction. *South Med J*. 1991;84:214–218.
49. Rinieris PM, Stefanis CN, Lykouras EP, et al. Affective disorders and ABO blood types. *Acta Psychiatr Scand*. 1979;60:272–278.

50. Rafaelsen OJ, Shapiro RW. Psychopharmacological studies in genetically determined subgroups of psychiatric patients. *Prog Neuropsychopharmacol*. 1979;3:147–154.
51. Goldin LR, Gershon ES, Lake CR, et al. Segregation and linkage studies of plasma dopamine-beta-hydroxylase (DBH), erythrocyte catechol-O-methyltransferase (COMT), and platelet monoamine oxidase (MAO): possible linkage between the ABO locus and a gene controlling DBH activity. *Am J Hum Genet*. 1982:250–252.
52. Arato M, Bagdy G, Rihmer Z, et al. Reduced platelet MAO activity in healthy male students with blood group O. *Acta Psychiatr Scand*. 1983;67:130–134.
53. Northrup H, Lathrop M, Lu SY, et al. Multilocus linkage analysis with the human argininosuccinate synthetase gene. *Genomics*. 1989;5:442–444.
54. McFadzean J, Tasker RC, Petros AJ. Nitric oxide ABO blood group difference in children (letter). *Lancet*. 1999;353:1414–1415.
55. Nichols WC, Ginsburg D. Von Willebrand disease. *Medicine*. 1997;76:1–20.
56. Nitu-Whalley IC, Lee CA, Griffioen A, et al. Type 1 von Willebrand disease—a clinical retrospective study of the diagnosis, the influence of the ABO blood group and the role of the bleeding history. *Br J Haematol*. 2000;108:259–264.
57. De Visser MC, Sandkuijl LA, Lensen RP, et al. Linkage analysis of factor VIII and von Willebrand factor loci as quantitative trait loci. *J Thromb Haemost*. 2003;1:1771–1776.
58. Cattrall FR, Vollenhoven BJ, Weston GC. Anatomical evidence for in utero androgen exposure in women with polycystic ovary syndrome. *Fertil Steril*. 2005;84:1689–1692.
59. Lujan ME, Bloski TG, Chizen DR, et al. Digit ratios do not serve as anatomical evidence of prenatal androgen exposure in clinical phenotypes of polycystic ovary syndrome. *Hum Reprod*. 2010;25:204–211.
60. Voracek M. Digit ratio (2D:4D), ABO blood group, and the Rhesus factor. *Percept Mot Skills*. 2008;107:737–746.
61. Blood-groups and the intestine [Editorial]. *Lancet*. 1966;2(7475):1232–1233.
62. Sievers ML. Hereditary aspects of gastric secretory function; race and ABO blood groups in relationship to acid and pepsin production. *Am J Med*. 1959;27:246–255.
63. Koster KH, Sindrup E, Seele V. ABO blood-groups and gastric acidity. *Lancet*. 1955;269:52–55.
64. Pals G, Defize J, Pronk JC, et al. Relations between serum pepsinogen levels, pepsinogen phenotypes, ABO blood groups, age and sex in blood donors. *Ann Hum Biol*. 1985;12:403–411.
65. Hristova G. Removal of blood substance "A" from pepsin by gel filtration. *Z Immunitatsforsch Allerg Klin Immunol*. 1968;135:2146–2150.
66. Shibata A, Hamajima N, Ikehara Y, et al. ABO blood type, Lewis and Secretor genotypes, and chronic atrophic gastritis: a cross-sectional study in Japan. *Gastric Cancer*. 2003;6:8–16.
67. Axelsson CK, Szecsi PB, Nielsen MD. Group I pepsinogens in serum. I. Normal range, age and sex relation, diurnal rhythm, seasonal variation, and distribution in ABO blood groups. *Scand J Gastroenterol*. 1984;19:1114–1118.
68. Mazacca G, Budillon F, De Marco M, et al. ABO blood groups and serum gastrin. *Acta Hepatogastroenterol (Stuttg)*. 1975;22:44–46.
69. Melissinos K, Alegakis G, Archimandritis AJ, et al. Serum gastrin concentrations in healthy people of the various ABO blood groups. *Acta Hepatogastroenterol (Stuttg)*. 1978;25:482–486.
70. Contiero E, Chinello GE, Folin M. Serum lipids and lipoproteins associations with ABO blood groups. *Anthropol Anz*. 1994;52:221–230.
71. Tarjan Z, Tonelli M, Duba J, et al. Correlation between ABO and Rh blood groups, serum cholesterol and ischemic heart disease in patients undergoing coronarography. *Orv Hetil*. 1995;136:767–769. Hungarian.
72. Wong FL, Kodama K, Sasaki H, et al. Longitudinal study of the association between ABO phenotype and total serum cholesterol level in a Japanese cohort. *Genet Epidemiol*. 1992;9:405–418.
73. Gillum RF. Blood groups, serum cholesterol, serum uric acid, blood pressure, and obesity in adolescents. *J Natl Med Assoc*. 1991;83:682–688.
74. Fox MH, Webber LS, Thurmon TF, et al. ABO blood group associations with cardiovascular risk factor variables. II. Blood pressure, obesity, and their anthropometric covariables. *Bogalusa Heart Study Hum Biol*. 1986;58:549–584.
75. Mao X, Xu M, Mu S, et al. Study on relationship between human ABO blood groups and type A behavior pattern. *Hua Xi Yi e Da Xue Xue Bao*. 1991;22:93–96. Chinese.
76. Neumann JK, Arbogast BW, Chi DS, et al. Effects of stress and blood type on cortisol and VLDL toxicity preventing activity. *Psychosom Med*. 1992;54:612–619.
77. Locong AH, Roberge AG. Cortisol and catecholamines response to venisection by humans with different blood groups. *Clin Biochem*. 1985;18:67–69.
78. Dintenfass L, Zador I. Blood rheology in patients with depressive and schizoid anxiety. *Biorheology*. 1976;13:33–36.
79. Dintenfass L, Bauer GE. Dynamic blood coagulation and viscosity and degradation of artificial thrombi in patients with hypertension. *Cardiovasc Res*. 1970;4:50–60.
80. Dintenfass L, Zador I. Effect of stress and anxiety on thrombus formation and blood viscosity factors. *Bibl Haematol*. 1975;41:133–139.
81. Dintenfass L, Davis E. Genetic and ethnic influences on blood viscosity and capillaries in diabetes mellitus. *Microvasc Res*. 1977;14:161–172.
82. Dintenfass L, Forbes CD. Effect of fibrinogen on aggregation of red cells and on apparent viscosity of artificial thrombi in haemophilia, myocardial infarction, thyroid disease, cancer and control systems: effect of ABO blood groups. *Microvasc Res*. 1975;9:107–118.
83. Dintenfass L, Steward JH. Formation, consistency and degradation of artificial thrombi in severe renal failure: effect of ABO blood groups. *Thromb Diath Haemorrh*. 1968;20:267–284.
84. Dintenfass L. Some aspects of haemorrheology of metastasis in malignant melanoma. *Haematologia (Budap)*. 1977;11:301–307.
85. Blann AD, Daly RJ, Amiral J. The influence of age, gender and ABO blood group on soluble endothelial cell markers and adhesion molecules. *Br J Haematol*. 1996;92:498–500.
86. McKeon JP, McColl D. ABO blood groups in obsessional illness—state and trait. *Acta Psychiatr Scand*. 1982;65:74–78.
87. Ertas M, Vahip S, Tuğlular I, et al. Lewis and ABO-Rh blood group systems in patients with bipolar affective disorder. *Encephale*. 1990;16:203–204.
88. Mendlewicz J, Massart-Guiot T, Wilmotte J, et al. Blood groups in manic-depressive illness and schizophrenia. *Dis Nerv Syst*. 1974;35:39–41.
89. Shapiro RW, Rafaelsen OJ, Ryder LP, et al. ABO blood groups in unipolar and bipolar manic-depressive patients. *Am J Psychiatry*. 1977;134:197–200.
90. Sostaric V, Bozicevic D, Brinar V, et al. Hereditary antigen characteristics of blood in ischemic cerebrovascular accident. *Neurol Croat*. 1991;40:3–11.
91. Ionescu DA, Ghitescu M, Marcu I, et al. Erythrocyte rheology in acute cerebral thrombosis: effects of ABO blood groups. *Blut*. 1979;39:351–357.
92. Ionescu DA, Marcu I, Bicescu E. Cerebral thrombosis, cerebral haemorrhage, and ABO blood-groups. *Lancet*. 1976;1:278–280.
93. Wu O, Bayoumi N, Vickers MA, et al. ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. *J Thromb Haemost*. 2008;6:62–69.
94. Horby J, Gyrtrup HJ, Grande P, et al. Relation of serum lipoproteins and lipids to the ABO blood groups in patients with intermittent claudication. *J Cardiovasc Surg (Torino)*. 1989;30:533–537.
95. Robinson WM, Roisenberg I. Venous thromboembolism and ABO blood groups in a Brazilian population. *Hum Genet*. 1980;55:129–131.
96. Ohira T, Cushman M, Tsai MY, et al. ABO blood group, other risk factors and incidence of venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). *J Thromb Haemost*. 2007;5:1455–1461.
97. Larsen TB, Johnsen SP, Gislum M, et al. ABO blood groups and risk of venous thromboembolism during pregnancy and the puerperium. A population-based, nested case-control study. *J Thromb Haemost*. 2005;3:300–304.
98. Havlik RJ, Feinleib M, Garrison RJ, et al. Blood-groups and coronary heart-disease. *Lancet*. 1969;2:269–270.
99. Slipko Z, Latuchowska B, Wojtkowska E. Body structure and ABO and Rh blood groups in patients with advanced coronary heart disease after aorto-coronary by-pass surgery. *Pol Arch Med Wewn*. 1994;91:55–60. Polish.

100. Meshalkin EN, Okuneva GN, Vlasov IuA, Vel'tmander NN. ABO and Rh blood groups in cardiovascular pathology. *Kardiologiia*. 1981;21:46–50. [In Russian.].
101. Erikssen J, Thaulow E, Stormorken H, et al. ABO blood groups and coronary heart disease (CHD): a study in subjects with severe and latent CHD. *Thromb Haemost*. 1980;43:137–140.
102. Allan TM. ABO blood groups, age and work in ischaemic heart disease. *Atherosclerosis*. 1975;21:459–461.
103. Galeazzi L, Gualandri V. ABO blood-group phenotypes and pathogenesis of cardiovascular diseases. Congenital, rheumatic and coronaric heart disease and arterial hypertension (author's transl). *G Ital Cardiol*. 1975;5:744–751. Italian.
104. Rosenberg L, Miller DR, Kaufman DW, et al. Myocardial infarction in women under 50 years of age. *JAMA*. 1983;250:2801–2806.
105. Platt D, Muhlberg W, Kiehl L, et al. ABO blood group system, age, sex, risk factors and cardiac infarction. *Arch Gerontol Geriatr*. 1985;4: 241–249.
106. Whincup PH, Cook DG, Phillips AN, et al. ABO blood group and ischaemic heart disease in British men. *BMJ*. 1990;300:1679–1682.
107. Etemadi A, Kamangar F, Islami F, Poustchi H, Pourshams A, Brennan P, et al. Mortality and cancer in relation to ABO blood group phenotypes in the Golestan Cohort Study. *BMC Med*. 2015;13(1). https://doi.org/10.1186/s12916-014-0237-8.
108. D'adamo P. Lectins and Glycoconjugates in Oncology. *Fundamentals of Generative Medicine*. Wilton, CT: Drum Hill Publishing; 2010.
109. Dabelsteen E. Cell surface carbohydrates as prognostic markers in human carcinomas. *J Pathol*. 1996;179:358–369.
110. Ju T, Lanneau GS, Gautam T, et al. Human tumor antigens Tn and sialyl Tn arise from mutations in *Cosmc*. *Cancer Res*. 2008;68:1636–1646.
111. Springer GF. T and Tn pancarcinoma markers: autoantigenic adhesion molecules in pathogenesis, prebiopsy carcinoma-detection, and long-term breast carcinoma immunotherapy. *Crit Rev Oncog*. 1995;6:57–85.
112. Kurtenkov O, Klaamas K, Miljukhina L. The lower level of natural anti-Thomsen-Friedenreich antigen (TFA) agglutinins in sera of patients with gastric cancer related to ABO(H) blood-group phenotype. *Int J Cancer*. 1995;60:781–785.
113. Ichikawa D, Handa K, Hakomori S. Histo-blood group A/B antigen deletion/reduction vs. continuous expression in human tumor cells as correlated with their malignancy. *Int J Cancer*. 1998;76. 284–249.
114. Marionneau S, Le Moullac-Vaidye B, Le Pendu J. Expression of histo-blood group A antigen increases resistance to apoptosis and facilitates escape from immune control of rat colon carcinoma cells. *Glycobiology*. 2002;12:851–856.
115. Aird I, Bentall HH, Roberts JA. A relationship between cancer of stomach and the ABO blood groups. *BMJ*. 1953;1:799–801.
116. Beckman L, Angqvist KA. On the mechanism behind the association between ABO blood groups and gastric carcinoma. *Hum Hered*. 1987;37:140–143.
117. Hirohashi S, Clausen H, Yamada T, et al. Blood group A cross-reacting epitope defined by monoclonal antibodies NCC-LU-35 and -81 expressed in cancer of blood group O or B individuals: its identification as Tn antigen. *Proc Natl Acad Sci U S A*. 1985;82:7039–7043.
118. Iodice S, Maisonneuve P, Botteri E, et al. ABO blood group and cancer. *Eur J Cancer*. 2010;46:3345–3350. [In Italian.].
119. Defize LH, Arndt-Jovin DJ, Jovin TM, et al. A431 cell variants lacking the blood group A antigen display increased high affinity epidermal growth factor-receptor number, protein-tyrosine kinase activity, and receptor turnover. *J Cell Biol*. 1988;107:939–949.
120. Engelmann B, Schumacher U, Haen E. Epidermal growth factor binding sites on human erythrocytes in donors with different ABO blood groups. *Am J Hematol*. 1992;39:239–241.
121. Ben Q, Wang K, Yuan Y, et al. Pancreatic cancer incidence and outcome in relation to ABO blood groups among Han Chinese patients: a case-control study. *Int J Cancer*. 2011;128:1179–1186.
122. Wolpin BM, Kraft P, Gross M, et al. Pancreatic cancer risk and ABO blood group alleles: results from the Pancreatic Cancer Cohort Consortium. *Cancer Res*. 2010;70:1015–1023.
123. Amundadottir L, Kraft P, Stolzenberg-Solomon RZ, et al. Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat Genet*. 2009;41:986–990.
124. Wolpin BM, Kraft PL, Xu M, et al. Variant ABO blood group alleles, secretor status and risk of pancreatic cancer: results from the Pancreatic Cancer Cohort Consortium. *Cancer Epidemiol Biomarkers Prev*. 2010;19:3140–3149.
125. Risch HA, Yu H, Lu L. ABO blood group, *Helicobacter pylori* seropositivity, and risk of pancreatic cancer: a case-control study. *J Natl Cancer Inst*. 2010;102:502–505.
126. Stamatakos M, Kontzoglou K, Safioleas P, et al. Breast cancer incidence in Greek women in relation to ABO blood groups and Rh factor. *Int Semin Surg Oncol*. 2009;18:14.
127. Brooks SA, Leathem AJ. Prediction of lymph node involvement in breast cancer by detection of altered glycosylation in the primary tumour. *Lancet*. 1991;338:71–74.
128. Brooks SA, Hall DM, Buley I. GalNAc glycoprotein expression by breast cell lines, primary breast cancer and normal breast epithelial membrane. *Br J Cancer*. 2001;85:1014–1022.
129. Springer GF. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. *J Mol Med*. 1997;75:594–602.
130. Gates MA, Wolpin BM, Cramer DW, et al. ABO blood group and incidence of epithelial ovarian cancer. *Int J Cancer*. 2011;128:482–486.
131. Redman R, Massoll NA, Wilkinson EJ. Association between invasive squamous cell carcinoma of the vulva and ABO blood group. *J Low Genit Tract Dis*. 2005;9:89–92.
132. Xie J, Qureshi AA, Li Y, et al. ABO blood group and incidence of skin cancer. *PLoS One*. 2010;5:e11972.
133. Vasan SK, Hwang J, Rostgaard K, et al. ABO blood group and risk of cancer: a register-based cohort study of 1.6 million blood donors. *Cancer Epidemiol*. 2016;44:40–43. https://doi.org/10.1016/j.canep.2016.06.005.
134. Xiao S, Feng F, Sun L, et al. Blood type AB predicts promising prognosis in gastric cancer patients with positive preoperative serum CEA. *Banday. Medicine*. 2017;96(47):e8496. https://doi.org/10.1097/MD.0000000000008496.
135. Ye-Qiong X, Ting-Wang J, et al. Prognostic value of ABO blood group in gastric cancer. *J Surg Res*. 2016;201:188–195.
136. Slater G, Itzkowitz S, Azar S, et al. Clinicopathologic correlations of ABO and Rhesus blood type in colorectal cancer. *Dis Colon Rectum*. 1993;36:5–7.
137. Jorgensen G. ABO blood groups in physicians more than 75 years old: on the hypothesis concerning "little more fitness of blood group O. *MMW Munch Med Wochenschr*. 1974;116:649–652. German.
138. Jorgensen G. Human genetics and infectious diseases (author's transl). *MMW Munch Med Wochenschr*. 1981;123:1447–1452. [In German.].
139. Adam I, Babiker S, Mohmmed AA, et al. ABO blood group system and placental malaria in an area of unstable malaria transmission in eastern Sudan. *Malar J*. 2007;6:110.
140. Martins LC, de Oliveira Corvelo TC, Oti HT, et al. ABH and Lewis antigen distributions in blood, saliva and gastric mucosa and *H pylori* infection in gastric ulcer patients. *World J Gastroenterol*. 2006;12: 1120–1124.
141. D'Adamo P. *Eat Right 4 Your Type: The Complete Blood Type Encyclopedia: The A-Z Reference Guide for the Blood Type Connection to Symptoms, Disease, Conditions, Vitamins, Supplements, Herbs, and Food*. New York: Riverhead Books; 2002.
142. Koregol AC, Raghavendra M, Nainegali S, et al. ABO blood groups and rhesus factor: an exploring link to periodontal diseases. *Indian J Dent Res*. 2010;21:364–368.
143. Dabelsteen E, Gao S. ABO blood-group antigens in oral cancer. *J Dentistry Res*. 2005;84:21–28.
144. Pusztai A, Bardocz S. Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications. *Trends Glycosci Glycotechnol*. 1996;8:149–165.
145. Pusztai A. Dietary lectins are metabolic signals for the gut and modulate immune and hormone functions. *Eur J Clin Nutr*. 1993;47:691–699.

146. Freed DJ. Dietary lectins. In: Brostoff J, Challacombe S, eds. *Food Allergy and Intolerance*. Philadelphia: Baillière Tindall Publishers.
147. Bardocz S, Grant G, Ewen SW, et al. Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. *Gut*. 1995;3:353–360.
148. Nachbar MS, Oppenheim JD. Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature. *Am J Clin Nutr*. 1980;33:2338–2345.
149. D'Adamo P. *Eat right 4 your type*. LECster 2.3. http://www.dadamo.com/lecster2/lecster.cgi. Accessed November 15, 2010.
150. Fleming RM. The effect of high-protein diets on coronary blood flow. *Angiology*. 2000;51:817–826.
151. Blann AD, Jackson P, Bath PM, et al. von Willebrand factor, a possible indicator of endothelial cell damage, decreases during long-term compliance with a lipid-lowering diet. *J Intern Med*. 1995;237:557–561.
152. Yildirir A, Tokgozoglu SL, Oduncu T, et al. Soy protein diet significantly improves endothelial function and lipid parameters. *Clin Cardiol*. 2001;24:711–716.
153. Fuentes F, Lopez-Miranda J, Sanchez E, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med*. 2001;134:1115–1119.
154. D'Adamo P. Unpublished results. 1998.
155. D'Adamo PJ. Serum breath hydrogen levels after lactulose administration vary by ABO blood group and secretor status and improve upon adoption of blood group specific dietary recommendations. In: *Proceedings of the Institute for Human Individuality Conference* (June 5-7, 2009). Dolce Conference Center, Norwalk Connecticut. Wilton, CT: Institute for Human Individuality, 54–60.
156. More information, including an extensive knowledge base, is available at the author's primary internet portal. http://www.dadamo.com.
157. *Eat Right for Your Type*. http://www.dadamo.com/survey. Accessed November 12, 2010.
158. Cooper HS, Marshall C, Ruggiero F, et al. Hyperplastic polyps of the colon and rectum. An immunohistochemical study with monoclonal antibodies against blood groups antigens (sialosyl-Lea, Leb, Lex, Ley, A, B, H). *Lab Invest*. 1987;57:421–428.
159. Saegusa M. Isoantigens ABH in bladder tumors as an indicator of malignant potential: a combined study with CEA, BMG, Leu-M1 and H-Ag. *Hinyokika Kiyo*. 1989;35:1311–1321. Japanese.
160. Abel PD, Marsh C, Henderson D, et al. Detection of blood group antigens in frozen sections of prostatic epithelium. *Br J Urol*. 1987;59:430–435.
161. Lin XS. The expression and significance of blood group antigens (BGA) A, B, H, Le(a) and Le(b) in hepatocellular carcinoma and chronic hepatitis. *Zhonghua Bing Li Xue Za Zhi*. 1992;21:24–26. [Chinese/summary translation in English.].
162. Robey-Cafferty S, El Naggar AK, Sahin AA, et al. Prognostic factors in esophageal squamous carcinoma. A study of histologic features, blood group expression, and DNA ploidy. *Am J Clin Pathol*. 1991;95:844–849.
163. Inoue M, Sasagawa T, Saito J, et al. Expression of blood group antigens A, B, H, Lewis-a, and Lewis-b in fetal, normal, and malignant tissues of the uterine endometrium. *Cancer*. 1987;60:2985–2993.
164. Matias-Guiu X, Giux M. ABO (H) blood group antigen expression in gastric mucosa. *Pathol Res Pract*. 1988;183:476–480.
165. Larena A, Vierbuchen M, Fischer R. Blood group antigen expression in malignant tumors of the thyroid: a parallel between medullary and non-medullary carcinomas. *Langenbecks Arch Chir*. 1995;380:269–272.
166. Haas H, Falcone FH, Schramm G, et al. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. *Eur J Immunol*. 1999;29:918–927.
167. Cordain L, Toohey L, Smith MJ, et al. Modulation of immune function by dietary lectins in rheumatoid arthritis. *Br J Nutr*. 2000;83:207–217.
168. Erickson RH, Kim J, Sleisenger MH, et al. Effect of lectins on the activity of brush border membrane-bound enzymes of rat small intestine. *J Pediatr Gastroenterol Nutr*. 1985;4:984–991.
169. Pusztai A. *Plant Lectins*. Cambridge: Cambridge University Press; 1991.
170. Pusztai A, Ewen SWB, Grant G, et al. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion*. 1990;46:308–316.
171. Weinman MD, Allan CH, Trier JS, et al. Repair of microvilli in the rat small intestine after damage with lectins contained in the red kidney bean. *Gastroenterol*. 1989;97:1193–1204.
172. Greer F, Pusztai A. Toxicity of kidney bean *(Phaseolus vulgaris)* in rats: changes in intestinal permeability. *Digestion*. 1985;32:42–46.
173. Jordinson M, Playford RJ, Calam J. Effects of a panel of dietary lectins on cholecystokinin release in rats. *Am J Physiol*. 1997;273:G946–G950.
174. Ewen SW, Pusztai A. Health risks of genetically modified foods. *Lancet*. 1999;354:684.
175. Czirok E, Marton A, Csik M, et al. Virulence factors of *Escherichia coli*. I. Mannose resistant haemagglutinating capacity is associated with serogroup but not with site of infection. *Acta Microbiol Hung*. 1984;31:187–195.
176. Podbielska M, Krotkiewski H. Identification of blood group A and B antigens in human glycophorin. *Arch Immunol Ther Exp (Warsz)*. 2000;48:211–221.
177. Clausen H, Stroud M, Parker J, et al. Monoclonal antibodies directed to the blood group A associated structure, galactosyl-A: specificity and relation to the Thomsen-Friedenreich antigen. *Mol Immunol*. 1988;2:199–204.
178. Slomiany BL, Murty VL, Slomiany A. Structural features of carbohydrate chains in human salivary mucins. *Int J Biochem*. 1993;25:259–265.
179. Kochetkov NK, Derevitskaya VA, Arbatsky NP. The structure of pentasaccharides and hexasaccharides from blood group substance H. *Eur J Biochem*. 1976;67:129–136.
180. Johansson I, Bratt P, Hay DI, et al. Adhesion of *Candida albicans, but not Candida krusei,* to salivary statherin and mimicking host molecules. *Oral Microbiol Immunol*. 2000;15:112–118.
181. Schulte BA, Poon KC, Rao KP, et al. Lectin histochemistry of complex carbohydrates in human cervix. *Histochem J*. 1985;17:627–654.
182. Ferreiros CM, Criado MT. Purification and partial characterization of a *Fucus vesiculosus* agglutinin. *Rev Esp Fisiol*. 1983;39:51–59.
183. Roberts DD, Ginsburg V. Sulfated glycolipids and cell adhesion. *Arch Biochem Biophys*. 1988;267:405–415.
184. Criado MT, Ferreiros CM. Selective interaction of a *Fucus vesiculosus* lectin-like mucopolysaccharide with several *Candida* species. *Ann Microbiol (Paris)*. 1983;134A:149–154.
185. Boericke W. *Pocket Materia Medica*. San Francisco; 1927.
186. Jenkin CR, Jackson GD. Artificial antigens and immunity to infectious disease. *Prog Immunobiol Stand*. 1970;4:493–504.
187. Galelli A, Truffa-Bachi P. *Urtica dioica* agglutinin: a superantigenic lectin from stinging nettle rhizome. *J Immunol*. 1993;151:1821–1831.
188. D'Adamo P. Unpublished observations. 1987–1994.
189. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). *J Endocrinol*. 1998;157:259–266.
190. Yu LG, Fernig DG, White MR, et al. Edible mushroom *(Agaricus bisporus)* lectin, which reversibly inhibits epithelial cell proliferation, blocks nuclear localization sequence-dependent nuclear protein import. *J Biol Chem*. 1999;274:4890–4899.
191. Bird GW, Wingham J. More Tn-specific lectins from seeds of the genus *Labiatae: Hyptis* sp. *Chan, Salvia lyrata* and *Marrubium velutinum. Clin Lab Haematol*. 1982;4:403–404.
192. Ren de C, Du GH, Zhang JT. Inhibitory effect of the water-soluble extract of *Salvia miltiorrhiza* on neutrophil-endothelial adhesion. *Jpn J Pharmacol*. 2002;90:276–280.
193. Jordinson M, El-Hariry I, Calnan D, et al. *Vicia faba* agglutinin, the lectin present in broad beans, stimulates differentiation of undifferentiated colon cancer cells. *Gut*. 1999;44:709–714.
194. Khan F, Khan RH, Sherwani A, et al. Lectins as markers for blood grouping. *Med Sci Monit*. 2002;8:RA293–RA300.
195. Ito N, Nishi K, Kawahara S, et al. Difference in the ability of blood group-specific lectins and monoclonal antibodies to recognize the ABH antigens in human tissues. *Histochem J*. 1990;22:604–614.
196. Vandonselaar M, Delbaere LT, Spohr U, et al. Crystallization of the lectin IV of *Griffonia simplicifolia* and its complexes with the Lewis b and Y human blood group determinants. *J Biol Chem*. 1987;262:10848–10849.
197. Wu AM, Wu JH, Lin LH, et al. Binding profile of *Artocarpus integrifolia* agglutinin (Jacalin). *Life Sci*. 2003;72:2285–2302.

44

Nutritional Medicine

Michael T. Murray, ND

OUTLINE

Let your food be your medicine and let your medicine be your food.
Hippocrates

INTRODUCTION

Nutritional medicine, as described in this textbook, consists of the use of diet and nutritional supplementation as therapeutic modalities. The foundation of nutritional medicine is a health-promoting diet that focuses on the consumption of whole, natural foods grown organically. Nutritional supplementation—the use of vitamins, minerals, and other food factors to support good health as well as to prevent or treat illness—is an important component of nutritional medicine. However, dietary supplements are used as complementary agents, not as sole primary medicines. It is important to emphasize that diet is always primary, and supplementation secondary, within nutritional medicine.

One of the key concepts in nutritional medicine is to supply the necessary nutrients to allow the body to maintain health. The concept of "biochemical individuality" was coined by nutritional biochemist Roger Williams in the 1970s to recognize the wide range in the nutritional needs of humans. These observations also provided the basis for "orthomolecular medicine" as envisioned by two-time Nobel laureate Linus Pauling.

In addition to serving as necessary factors in normal physiology, many nutrients appear to exert pharmacological effects. The advantage of using nutrients at pharmacological dosages is that they are more recognizable and better metabolized by the body, as evidenced by a broader therapeutic index. Even so, the use of nutrients as pharmacological agents is closely akin to drug therapy. That being the case, it is imperative that they be used and monitored appropriately.

EVOLUTIONARY ASPECTS IN HUMAN NUTRITION

Although the human gastrointestinal tract is capable of digesting both animal and plant foods, several physical characteristics indicate that *Homo sapiens* evolved to digest primarily plant foods. Specifically, human teeth are composed of 20 molars, which are perfect for crushing and grinding plant foods, along with eight front incisors, which are well suited for biting into fruits and vegetables. Only the front four canine teeth are designed for meat eating. Human jaws swing both vertically to tear and laterally to crush, but carnivores' jaws swing only vertically. Additional evidence that supports the human evolutionary preference for plant foods is the long length of the human intestinal tract. Carnivores typically have a short bowel, and herbivores have a bowel length proportionally comparable to that of humans. Thus the human bowel length favors plant foods.[1]

A Look at Our Closest Wild Relatives

To answer the question, "What should humans eat?" many researchers look to other primates, such as chimpanzees, monkeys, and gorillas. Nonhuman wild primates are also omnivores or, as often described, herbivores and opportunistic carnivores. They eat mainly fruits and vegetables but may also eat small animals, lizards, and eggs if given the opportunity. Only 1% and 2%, respectively, of the total calories consumed by gorillas and orangutans are animal foods. The remainder of their diet is from plant foods. Because humans are between the weights of the gorilla and orangutan, it has been suggested that humans are designed to eat around 1.5% of their diet as animal foods.[2] Most Americans derive well over 50% of their calories from animal foods.

Although most primates eat a considerable amount of fruit, it is critical to point out that the cultivated fruit in American supermarkets is far different from the nutrient-dense wild fruits these animals rely on. Wild fruits have slightly higher protein contents and a higher content of certain essential vitamins and minerals, but cultivated fruits tend to be higher in sugars. Cultivated fruits are therefore very tasty to humans, but because they have a higher sugar composition and also lack the fibrous pulp and multiple seeds found in wild fruit that slow down the digestion and absorption of sugars, cultivated fruits raise blood sugar levels much more quickly than their wild counterparts.

TABLE 44.1 Estimated Mineral Intakes of Wild Monkeys and Humans

Mineral	Total Daily Intake of 7-Kg Adult Monkey (Mg)	Recommended Daily Allowance for 70-Kg Human (Mg)
Calcium	4571	800
Phosphorus	728	800
Potassium	6419	1600–2000
Sodium	182	500
Magnesium	1323	350
Iron	38.5	10
Manganese	18.2	2.0–5.0
Copper	2.8	1.5–3.0

Wild primates fill up not only on fruit but also on other highly nutritious plant foods. As a result, wild primates weighing one tenth as much as a typical human ingest nearly 10 times the level of vitamin C and much higher amounts of many other vitamins and minerals. Other differences in the wild primate diet are also important to point out, such as a higher ratio of α-linolenic acid, the essential omega-3 fatty acid, to linoleic acid, the essential omega-6 fatty acid[2] (Table 44.1).

Humans Versus Primates

Determining what foods are best suited for humans may not be as simple as looking at the diet of wild primates. There are some structural and physiological differences between humans and apes. A larger, more metabolically active brain is one of the key differences. It has been theorized by some evolutionists that a shift in dietary intake of fats was the likely stimulus for the brain growth in ancient humans. The shift itself was likely the result of limited food availability forcing early humans to collect shellfish and hunt grazing mammals such as antelope and gazelle in addition to gathering plant foods. Archeological data support this association—the brains of humans started to grow and become more developed at about the same time as evidence shows an increase of shellfish consumption and the presence of animal bones being butchered with stone tools at early villages. Data also show that the early humans who lived near water sources and ate seafood experienced the biggest brain change. An increased intake of the omega-3 fatty acid docosahexaenoic acid (DHA) found primarily in fish and seafood, but also in wild game, was perhaps the largest dietary contributor to brain growth.

The thought is that a higher DHA intake led to bigger brains in humans. And, with a bigger brain, early humans were able to engage in more complex social behavior, which led to improved foraging and hunting tactics, which in turn led to even higher-quality food intake, fostering additional brain evolution. In contrast, inland prehistoric humans did not have sufficient access to DHA, and the Neanderthals chose to focus on low-DHA-content meat from larger animals. As a result, both got stuck with limited brain capacity and died off.

The importance of DHA to brain function relates to its role in the composition of brain-cell membranes, and as a result, it influences the following:

- The fluidity of brain cell membranes
- Neurotransmitter synthesis
- Neurotransmitter binding
- Signal transmission
- The activity of key enzymes that break down neurotransmitters like serotonin, epinephrine, dopamine, and norepinephrine

Although improved dietary quality alone cannot fully explain why human brains grew, it appears to have played a critical role. A large part of the brain is made up of omega-3 fatty acids. In fact, 60% of the fats in the brain are omega-3 fatty acids, with DHA being the main type. A higher intake of DHA during pregnancy and early childhood was especially important to human evolution. DHA is critical for healthy brain development both in the womb and in early childhood. About 75% of brain cells are in place before birth, and the other 25% are in place by the age of 1 year—making DHA an essential nutrient for both pregnant mothers and young children. DHA is so important for early brain development that it is now automatically added to baby milk formula, and pregnant women should also strive to get sufficient levels by regularly eating fish or taking fish oil supplements.

The proper function of the brain and nerves also requires the monounsaturated fat oleic acid, the main component of olive oil as well as the chief oil in almonds, pecans, macadamias, peanuts, and avocados. Myelin, the protective sheath that covers communicating neurons, is composed of 30% protein and 70% fat, with the key fat being oleic acid. Again, the key point is that the right fat has critical effects in the human brain.

Hunter–Gatherer Diets

Data from anthropologists looking at hunter–gatherer cultures provide much insight as to what humans evolved to eat; however, it is very important to point out that these groups were not entirely free to determine their diets. Instead, their diets were molded by what was available to them. For example, the diet of the Inuit Eskimos is far different from that of the Australian aborigines. It may not be appropriate to answer the question, "What should humans eat?" simply by looking at these studies. Nonetheless, it is important to point out that regardless of whether hunter–gatherer communities relied on animal or plant foods, the rate of diseases of civilization, such as heart disease and cancer, is extremely low in such communities.[3]

It should also be pointed out that the meat that our ancestors consumed was much different from the meat found in supermarkets today. Domesticated animals have always had higher fat levels than their wild counterparts, but the desire for tender meat has led to the breeding of cattle that produce meat with a fat content of 25% to 30% or more, compared with less than 4% for free-living animals and wild game. In addition, the type of fat is considerably different. Domestic beef contains primarily saturated fats and virtually undetectable amounts of omega-3 fatty acids. In contrast, the fat of wild animals contains more than five times more polyunsaturated fat per gram and has a good amount of beneficial omega-3 fatty acids (approximately 4%).[4]

Considerable evidence indicates that a high intake of red or processed meat increases the risk of mortality. For example, in a cohort study of half a million people aged 50 to 71 years at baseline, men and women in the highest versus lowest quintile of red and processed meat intake had elevated risks for overall mortality.[5]

In another prospective cohort study, subjects were followed from 1980 (women) or 1986 (men) until 2006. Low-carbohydrate diets, either animal-based (emphasizing animal sources of fat and protein) or vegetable-based (emphasizing vegetable sources of fat and protein), were computed from several validated food-frequency questionnaires assessed during follow-up.[6] A low-carbohydrate diet based on animal sources was associated with higher all-cause mortality in both men and women, whereas a vegetable-based, low-carbohydrate diet was associated with lower all-cause and cardiovascular disease mortality rates.

The Importance of a Plant-Based Diet

The evidence supporting diet's role in chronic degenerative diseases is substantial and compelling. There are two basic facts linking the

diet–disease connection: (1) a diet rich in plant foods (i.e., whole grains, legumes, fruits, and vegetables) is protective against many diseases that are extremely common in so-called Western society, and (2) a diet providing a low intake of plant foods is a causative factor in the development of these diseases and provides conditions under which other causative factors are more active. Early naturopathic pioneers were strong proponents of plant-based diets—one of the health movements that helped define the profession was the vegetarian Grahamists. The founding leaders of the profession, Henry Lindlahr, MD, and Benedict Lust, ND, both advocated whole-foods vegetarian diets.

The Pioneering Work of Burkitt and Trowell

Much of the link between diet and chronic disease originated from the work of two medical pioneers, Denis Burkitt, MD, and Hugh Trowell, MD, authors of *Western Diseases: Their Emergence and Prevention*, first published in 1981.[7] Although now extremely well recognized, their work is actually a continuation of the landmark work of Weston A. Price, a dentist and author of *Nutrition and Physical Degeneration*. In the early 1900s, Dr. Price traveled the world, observing changes in teeth and palate (orthodontic) structure as various cultures discarded traditional dietary practices in favor of a more "civilized" diet. Price was able to follow individuals as well as cultures over 20 to 40 years and carefully documented the onset of degenerative diseases as their diets changed. On the basis of extensive studies examining the rate of diseases in various populations (epidemiological data) and his own observations of primitive cultures, Price formulated the following sequence of events:

First stage. In cultures consuming a traditional diet consisting of whole, unprocessed foods, the rate of chronic diseases like heart disease, diabetes, and cancer is quite low.

Second stage. Commencing with eating a more "Western" diet, there is a sharp rise in the number of individuals with obesity and diabetes.

Third stage. As more and more people abandon their traditional diet, conditions that were once quite rare become extremely common. Examples are constipation, hemorrhoids, varicose veins, and appendicitis.

Fourth stage. Finally, with full westernization of the diet, other chronic degenerative or potentially lethal diseases, such as heart disease, cancer, osteoarthritis, rheumatoid arthritis, and gout, become extremely common.

Since Burkitt and Trowell's pioneering research, a virtual landslide of data has continually verified the role of the Western diet as the key factor in nearly every chronic disease, especially obesity and diabetes. Box 44.1 lists diseases with convincing links to a diet low in plant foods. Many of these now-common diseases were extremely rare before the 20th century.

TRENDS IN U.S. FOOD CONSUMPTION

During the 20th century, food consumption patterns changed dramatically (Table 44.2). Total dietary fat intake rose from 32% of the calories in 1909 to 43% by the end of the century. Overall carbohydrate intake dropped from 57% to 46%, and protein intake remained stable at about 11%.

Compounding these detrimental changes are the individual food choices accounting for the changes. The biggest changes include significant rises in the consumption of meat, fats and oils, and sugars and sweeteners in conjunction with the decreased consumption of noncitrus fruits, vegetables, and whole-grain products. The greatest change in the past 100 years of human nutrition is the switch from a diet with a high level of complex carbohydrates, as found naturally occurring in grains and vegetables, to a tremendous and dramatic increase in the

BOX 44.1 Diseases Highly Associated With a Low-Fiber Diet

Metabolic
Obesity, gout, diabetes, kidney stones, gallstones

Cardiovascular
High blood pressure, strokes, heart disease, varicose veins, deep vein thrombosis, pulmonary embolism

Colonic
Constipation, appendicitis, diverticulitis, diverticulosis, hemorrhoids, colon cancer, irritable bowel syndrome, ulcerative colitis, Crohn's disease

Other
Dental caries, autoimmune disorders, pernicious anemia, multiple sclerosis, thyrotoxicosis, psoriasis, acne

TABLE 44.2 Trends in Quantities of Foods Consumed per Capita (Pounds per Year)

Foods	1909	1967	1985	1999
Meat, Poultry, and Fish				
Beef	54	81	73	66
Pork	62	61	62	50
Poultry	18	46	70	68
Fish	12	15	19	15
Total	146	203	224	199
Eggs (each)	37	40	32	32
Dairy Products				
Whole milk	223	232	122	112
Low-fat milk	64	44	112	101
Cheese	5	15	26	30
Other	47	159	190	210
Total	339	450	450	453
Fats and Oils				
Butter	18	6	5	5
Margarine	1	10	11	8
Shortening	8	16	23	22
Lard and tallow	12	5	46	—
Salad and cooking oil	2	16	25	29
Total	41	53	68	70
Fruits				
Citrus	17	60	72	79
Noncitrus				
Fresh	154	73	87	115
Processed	8	35	34	37
Total	179	168	193	231
Vegetables				
Tomatoes	46	36	38	55
Dark green and yellow	34	25	31	39
Other				
Fresh	136	87	96	126
Processed	8	35	34	39
Total	224	183	199	259

Continued

TABLE 44.2 Trends in Quantities of Foods Consumed per Capita (Pounds per Year)—cont'd

Foods	1909	1967	1985	1999
Potatoes, White				
Fresh	182	67	55	49
Processed	0	19	28	91
Total	182	86	83	140
Dry beans, peas, nuts, and soybeans	16	16	18	22
Grain Products				
Wheat products	216	116	122	150
Corn	56	15	17	28
Other grains	19	13	26	24
Total	291	144	165	202
Sugar and Sweeteners				
Refined sugar	77	100	63	68
Syrups and other sweeteners	14	22	90	91
Total	91	122	153	159

Modified from U.S. Department of Agriculture. *Food Review* 2000;23:8–15.

number of calories consumed from simple sugars. Currently, more than half of the carbohydrates being consumed are in the form of sugars (sucrose, corn syrup, etc.) being added to foods as sweetening agents. High consumption of refined sugars is linked to many chronic diseases, including obesity, diabetes, heart disease, and cancer.

THE GOVERNMENT AND NUTRITION EDUCATION

Throughout the years, various governmental organizations have published dietary guidelines, but the recommendations of the U.S. Department of Agriculture (USDA) have become the most widely known. In 1956 the USDA published *Food for Fitness—A Daily Food Guide.* This became popularly known as the Basic Four Food Groups. The Basic Four comprised the following:

1. The Milk Group: milk, cheese, ice cream, and other milk-based foods
2. The Meat Group: meat, fish, poultry, eggs, with dried legumes and nuts as alternatives
3. The Fruit and Vegetable Group
4. The Breads and Cereals Group

One of the major problems with the Basic Four Food Groups model was that it graphically suggested that the food groups were equal in health value. The result was overconsumption of animal products, dietary fat, and refined carbohydrates and insufficient consumption of fiber-rich foods like fruits, vegetables, and legumes. This in turn resulted in diets being responsible for many premature deaths, chronic diseases, and increased healthcare costs.

As the Basic Four Food Groups became outdated, various other governmental and medical organizations developed guidelines of their own, designed to reduce the risk of either a specific chronic degenerative disease, such as cancer or heart disease, or of all chronic diseases.

To create a new model in nutrition education, the USDA first published the "Eating Right Pyramid" in 1992. It received harsh criticisms from numerous experts and other organizations. One big question that should be asked is, "Is it appropriate to have the USDA making these recommendations?" After all, the USDA serves two somewhat

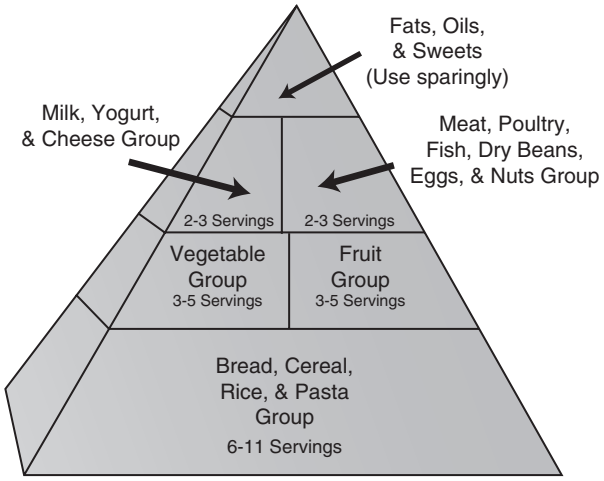


Fig. 44.1 U.S. Department of Agriculture Food Pyramid.

conflicting roles: (1) it represents the food industry, and (2) it oversees educating consumers about nutrition. Many people believe that the pyramid was weighted more toward dairy products, red meat, and grains because of influence from the dairy, beef, and grain farming and processing industries. In other words, the pyramid was designed not to improve the health of Americans but, rather, to promote the USDA agenda of supporting multinational agrifoods giants (Fig. 44.1).

One of the main criticisms of the Eating Right Pyramid is that it does not stress strongly enough the importance of quality food choices. For example, the bottom of the pyramid represents the foods that the USDA thinks should make up the bulk of a healthy diet: the Bread, Cereal, Rice, and Pasta Group. Eating 6 to 11 servings a day from this group is supposedly the path to a healthier life. In this way, the pyramid sets a person up for insulin resistance, obesity, and adult-onset diabetes if he or she consistently chooses refined rather than whole-grain products in this important category. This is one example of how the Eating Right Pyramid does not take into consideration how quickly blood glucose levels rise after eating a certain type of food—an effect referred to as the food's glycemic index (GI). The GI is a numerical scale used to indicate how fast and how high a particular food raises blood glucose (blood sugar) levels. There are two versions of the GI, one based on a standard of comparison with glucose as 100, and the other based on white bread. Foods are tested against the results of the selected standard. Foods with a lower GI create a slower rise in blood sugar, whereas foods with a higher GI create a faster rise in blood sugar.

One of the major problems with the Eating Right Pyramid is that the GIs of some of the foods that the pyramid is directing Americans to eat more of, such as breads, cereals, rice, and pasta, can greatly stress blood sugar control, especially if derived from refined grains, and are now being linked to an increased risk for obesity, diabetes, and cancer. As a result, the goal of the Eating Right Pyramid was to improve the health of Americans and, hopefully, slow down the growing trend toward obesity and diet-related disease, but because of poor individual food choices within the categories, the pyramid has only worsened the problem.

On June 2, 2011 the USDA unveiled a new food icon, MyPlate, to replace the food pyramid (see Fig. 44.2). This simplified illustration is designed to help Americans make healthier food choices. MyPlate is the first step in a multiyear effort to raise awareness and educate consumers about eating more healthfully. The initial launch came with some simple recommendations:

- Balance calories.
- Enjoy your food, but eat less.
- Avoid oversized portions.

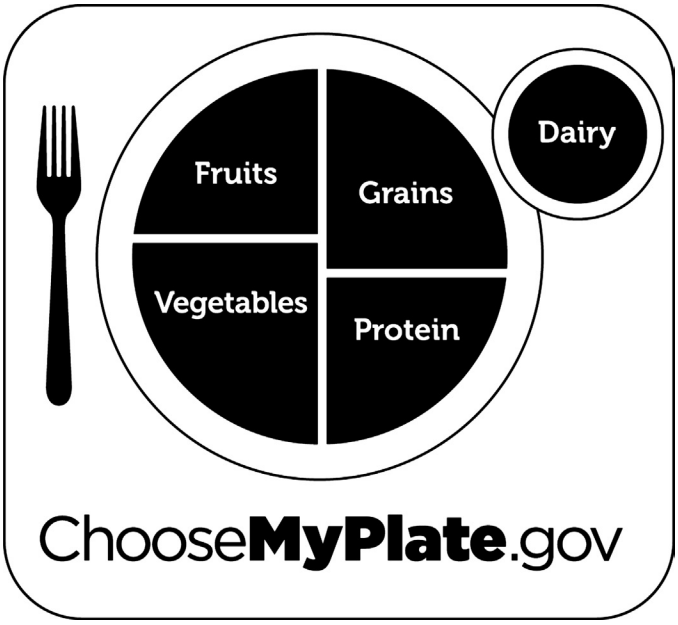


Fig. 44.2 U.S. Department of Agriculture MyPlate icon.

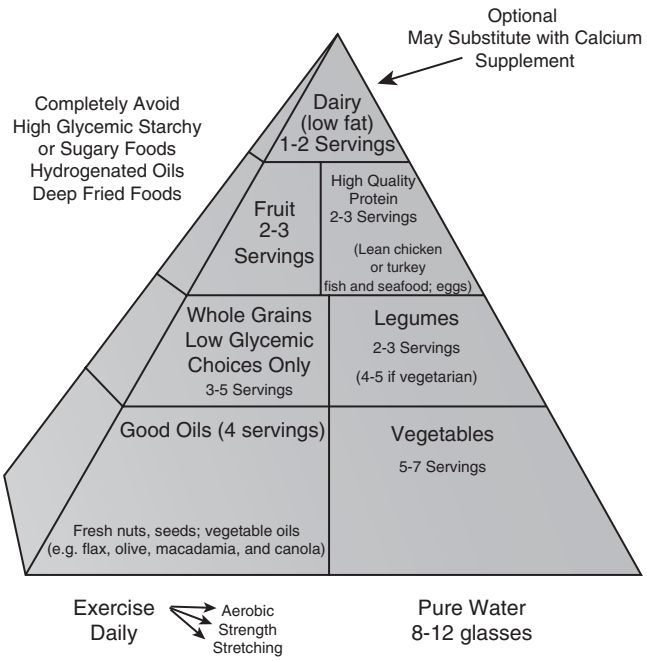


Fig. 44.3 The Optimal Health Food Pyramid.

Foods to Increase

- Make half your plate fruits and vegetables.
- Make at least half your grains whole grains.
- Switch to fat-free or low-fat (1%) milk.

Foods to Reduce

- Compare sodium in foods like soup, bread, and frozen meals and choose the foods with lower numbers.
- Drink water instead of sugary drinks.

However, this new campaign does not appear to be any more successful than prior efforts.

THE OPTIMAL HEALTH FOOD PYRAMID

Based on existing evidence, we have created the Optimal Health Food Pyramid (Fig. 44.3). The major difference from the USDA pyramid is that the Optimal Health Food Pyramid incorporates the best of two of the most healthful diets ever studied—the traditional Mediterranean diet (see later discussion) and the traditional Asian diet. In addition, the Optimal Health Food Pyramid more clearly defines what the healthy components within the categories are and stresses the importance of vegetable oils and regular fish consumption as part of a healthful diet. Appendix 9 provides a patient handout that clearly defines the components of the Optimal Health Food Pyramid.

The Optimal Health Food Diet is based on the following nine principles:

1. Eat a "rainbow" assortment of fruits and vegetables.
2. Reduce exposure to foods contaminated with pesticides and metals.
3. Eat to support blood sugar control.
4. Do not overconsume animal foods.
5. Eat the right types of fats.
6. Keep salt intake low and potassium intake high.
7. Avoid food additives.
8. Take measures to reduce foodborne illness.
9. Drink sufficient amounts of water each day.

1. Eat a "Rainbow" Assortment of Fruits and Vegetables

A diet rich in fruits and vegetables is the best bet for preventing virtually every chronic disease. That fact has been established time and again in scientific studies on large numbers of people. The evidence in support of this recommendation is so strong that it has been endorsed by U.S. government health agencies and by virtually every major medical organization, including the American Cancer Society. "Rainbow" simply means that selecting colorful foods—red, orange, yellow, green, blue, and purple—provides the body the full spectrum of pigments with powerful antioxidant effects as well as the nutrients it needs for optimal function and protection against disease (Box 44.2).

Fruits and vegetables are so important in the battle against cancer that some experts have said, and we believe, that cancer is a result of a "maladaptation" over time to a reduced level of intake of fruits and vegetables. As a study published in the medical journal *Cancer Causes and Control* put it in 1991, "Vegetables and fruit contain the anticarcinogenic cocktail to which we are adapted. We abandon it at our peril."[8] A vast number of substances found in fruits and vegetables are known to protect against cancer. Some experts refer to these as "chemopreventers," but they are better known to many as phytochemicals (Table 44.3). Phytochemicals include pigments such as carotenes, chlorophyll, and flavonoids; dietary fiber; enzymes; vitamin-like compounds; and other minor dietary constituents. Although they work in harmony with antioxidants like vitamin C, vitamin E, and selenium, phytochemicals exert considerably greater protection against cancer than these simple nutrients. Among the most important groups of phytochemicals are pigments such as chlorophyll, carotenes, and flavonoids.

2. Reduce Exposure to Pesticides, Metals, and Food Additives

In the United States more than 1.2 billion pounds of pesticides and herbicides are sprayed on or added to food crops each year. That is roughly 5 lb of pesticides for each man, woman, and child. There is a growing body of evidence demonstrating the correlation between exposure to pesticides and the development of a wide spectrum of pathologies, ranging from obesity to type 2 diabetes, low sperm counts, neurodegenerative diseases, and, of course, cancer. One of the closest links is the plight of the American farmer. The lifestyle of farmers is generally healthy: compared with city dwellers, farmers have access to

BOX 44.2 The "Rainbow" Assortment of Fruits and Vegetables

Red
Apples (red)
Bell peppers (red)
Cherries
Cranberries
Grapefruit
Grapes (red)
Plums (red)
Radishes
Raspberries
Strawberries
Tomatoes
Watermelon

Dark Green
Artichoke
Asparagus
Bell peppers (green)
Broccoli
Brussels sprouts
Chard
Collard greens
Cucumber
Grapes (green)
Green beans
Honeydew melons
Kale
Leeks
Lettuce (dark green types)
Mustard greens
Peas
Spinach
Turnip greens

Yellow and Light Green
Apples (green or yellow)
Avocado
Banana
Bell peppers (yellow)
Bok choy
Cabbage
Cauliflower
Celery
Fennel
Kiwi fruit
Lemons
Lettuce (light green types)
Limes
Onions
Pears (green or yellow)
Pineapple
Squash (yellow)
Zucchini (yellow)

Orange
Apricots
Bell peppers (orange)
Butternut squash
Cantaloupe
Carrots
Mangoes
Oranges
Papaya
Pumpkin
Sweet potatoes (Yams)

Purple
Beets
Blackberries
Blueberries
Cabbage (purple)
Cherries
Currants
Eggplant
Grapes (purple)
Onions (red)
Pears (red)
Plums (purple)
Radishes

lots of fresh food; they breathe clean air, work hard, and have a lower rate of cigarette smoking and alcohol use. Yet studies show that farmers have a higher risk of lymphomas; leukemias; and cancers of the stomach, prostate, brain, and skin.[9] Exposure to pesticides is the primary reason for this occurrence.

Perhaps the most problematic pesticides are the family of halogenated hydrocarbons, such as 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene (DDE), polychlorinated biphenyl (PCB), pentachlorophenol (PCP), dieldrin, and chlordane. These chemicals persist almost indefinitely in the environment. A similar pesticide, dichlorodiphenyltrichloroethane (DDT), has been banned for nearly 30 years, yet it can still be found in soil and root vegetables such as carrots and potatoes. The human body also has a tough time detoxifying and eliminating these compounds. Instead, they end up being stored in fat cells. Moreover, inside the body, these chemicals can act like the hormone estrogen. They are thus suspected as a major cause of the growing epidemic of estrogen-related health problems, including breast cancer.[10] Some evidence also suggests that these chemicals raise the risk of lymphomas, leukemia, and pancreatic cancer as well as play a role in low sperm counts and reduced fertility in men.[11]

Avoiding pesticides is especially important in children of preschool age. Children are at greater risk for two reasons: they eat more food relative to body mass, and they consume more foods higher in pesticide residues—such as juices, fresh fruits, and vegetables. A recent University of Washington study[12] that analyzed levels of breakdown products of organophosphorus pesticides (a class of insecticides that disrupt the nervous system) in the urine of 39 urban and suburban children aged 2 to 4 years found that concentrations of pesticide metabolites were six times lower in the children who ate organic fruits and vegetables than in those who ate conventional produce.

After conducting an analysis of USDA pesticide residue data for all pesticides for 1999 and 2000, the Consumers Union[13] warned parents of small children to limit or avoid conventionally grown foods known to have high pesticide residues, such as cantaloupes, green beans (canned or frozen), pears, strawberries, tomatoes (Mexican grown), and winter squash. The University of Washington study added apples to this list.

Recommendations for Patients to Avoid Pesticides and Metals in the Diet

Patients can avoid consuming pesticides in their foods by following these recommended practices:

1. Do not overconsume foods that have a tendency to concentrate pesticides, such as animal fat, meat, eggs, cheese, and milk.
2. Buy organic produce, which is grown without the aid of synthetic pesticides and fertilizers. Although less than 3% of the total produce in the United States is grown without pesticides, organic produce is widely available.
3. Develop a good relationship with your local grocery store produce manager. Explain your desire to reduce your exposure to pesticides and waxes. Ask what measures the store takes to ensure that pesticide residues are within approved limits. Ask where the store obtains its produce; make sure the store is aware that foreign produce is much more likely to contain excessive levels of pesticides as well as pesticides that have been banned in the United States.
4. Decrease exposure to arsenic. If eating chickens, ensure they were not treated with arsenic compounds. If eating rice, be sure it was not grown with water contaminated with arsenic.
5. Try to buy local produce in season.
6. Peeling off the skin or removing the outer layer of leaves of some produce may be all you need to do to reduce pesticide levels. The downside is that many of the nutritional benefits of fruits and vegetables are concentrated in the skin and outer layers. An alternative measure is to remove surface pesticide residues, waxes, fungicides, and fertilizers by soaking the item in a mild solution of additive-free soap such as Ivory or pure castile soap. All-natural, biodegradable cleansers are also available at most health food stores. To use, spray the food with the cleanser, gently scrub, and rinse.

3. Eat to Support Blood Sugar Control

Refined sugars, white flour products, and other sources of simple sugars are quickly absorbed into the bloodstream, causing a rapid rise in blood sugar. In response, the body boosts secretion of insulin by the pancreas. High-sugar, "junk-food" diets definitely lead to poor blood sugar regulation, obesity, and ultimately, type 2 diabetes and heart disease.[14–16] The stress on the body that they cause, however, including secretion of too much insulin, can promote the growth of cancer and increase the risk of heart disease as well.

As already discussed, the GI of a food refers to how quickly blood sugar levels will rise after it is eaten. However, the GI does not tell how much of that carbohydrate is in a typical serving of a particular food,

TABLE 44.3 Examples of Anticancer Phytochemicals

Phytochemical	Action(s)	Sources
Carotenes	Antioxidants Enhance immune functions	Dark-colored vegetables, such as carrots, squash, spinach, kale, tomatoes, yams (sweet potatoes); fruits such as cantaloupe, apricots, citrus fruits
Coumarin	Antitumor properties Enhances immune functions Stimulates antioxidant mechanisms	Carrots, celery, fennel, beets, citrus fruits
Dithiolthiones, glucosinolates, and thiocyanates	Block cancer-causing compounds from damaging cells Enhance detoxification	Cabbage-family vegetables—broccoli, brussels sprouts, kale, etc.
Flavonoids	Antioxidants Direct antitumor effects Immune-enhancing properties	Fruits, particularly darker fruits, such as berries, cherries, citrus fruits; also tomatoes, peppers, greens
Isoflavonoids	Block estrogen receptors	Soy and other legumes
Lignans	Antioxidants Modulate hormone receptors	Flaxseed and flaxseed oil; whole grains, nuts, seeds
Limonoids	Enhance detoxification Block carcinogens	Citrus fruits, celery
Polyphenols	Antioxidants Block carcinogen formation Modulate hormone receptors	Green tea, chocolate, red wine
Sterols	Block production of carcinogens Modulate hormone receptors	Soy, nuts, seeds

Modified from Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc* 1996;96:1027–1039.

so another tool is needed. The glycemic load (GL) is a relatively new way to assess the effect of carbohydrate consumption that takes the GI into account but gives a fuller picture of the effect that a food has on blood sugar levels than GI alone. A GL of 20 or more is high, a GL of 11 to 19 inclusive is medium, and a GL of 10 or less is low. For example, beets have a high GI but a low GL. Although the carbohydrate in beet root has a high GI, the amount of carbohydrate is low, so a typical serving of cooked beet root has a relatively low GI (about 5). Thus, as long as one eats a reasonable portion of a low-GL food, the effect on blood sugar is acceptable, even if the food has a high GI. For example, an individual with diabetes can enjoy some watermelon (GI 72) as long as he or she keeps the serving size reasonable; the GL for 120 g watermelon is only 4.

In essence, foods that are mostly water (e.g., apple, watermelon), fiber (e.g., beet root, carrot), or air (e.g., popcorn) will not cause a steep rise in blood sugar even if their GIs are high as long as portion sizes are moderate. To help design a healthy diet, we provide a list of the GI, fiber content, and GL of common foods in Appendix 7.

4. Reduce Intake of Meat and Other Animal Foods

Study after study seems to indicate that the higher the intake of meat and other animal products, the higher is the risk of heart disease and cancer, especially the major cancers like those of the colon, breast, prostate, and lung, whereas a diet focusing on plant foods exerts the opposite effect.[17,18]

There are many reasons for this association. Meat lacks the antioxidants and phytochemicals that protect from cancer. At the same time, it contains lots of saturated fat and other potentially carcinogenic compounds—including pesticide residues, heterocyclic amines, and polycyclic aromatic hydrocarbons, which form when meat is grilled, fried, or broiled. The more well done the meat, the higher is the level of amines.

Some proponents of a diet high in meats claim that people should eat the way their "caveman" ancestors did. That argument does not really hold up. As already discussed, the meat of the wild animals that early humans consumed had a fat content of less than 4%. The demand for tender meat has led to the breeding of cattle whose meat contains 25% to 30% or more fat. Domestic beef contains primarily saturated fats and virtually no beneficial omega-3 fatty acids (discussed later), whereas the fat of wild animals contains more than five times the polyunsaturated fat per gram and has substantial amounts (about 4%) of omega-3 fatty acids.

Range-fed animals also contain 10 times as much conjugated linoleic acid (CLA) as grain-fed animals. CLA is a slightly altered form of the essential fatty acid linoleic acid. It occurs naturally in meat and dairy products. CLA was discovered in 1978, when researchers at the University of Wisconsin were looking for cancer-causing compounds that result from cooking. Instead, they found CLA, which appears to be an anticancer compound. Preliminary studies show that CLA might reduce the risk of heart disease and cancer.[19]

Particularly harmful to human health are cured or smoked meats, such as ham, hot dogs, bacon, and jerky, that contain sodium nitrate and/or sodium nitrites—compounds that keep the food from spoiling but that dramatically raise the risk of cancer. These chemicals react with amino acids in foods in the stomach to form highly carcinogenic compounds known as nitrosamines.

Research in adults makes a convincing argument to avoid these foods. Even more compelling is the evidence linking consumption of nitrates to a significantly increased risk of the major childhood cancers (leukemias, lymphomas, and brain cancers), as follows:

- Children who eat 12 hot dogs per month have nearly 10 times the risk of leukemia compared with children who do not eat hot dogs.[20]
- Children who eat hot dogs once a week double their chances of brain tumors; eating hot dogs twice a week triples the risk.[20]

- Pregnant women who eat two servings per day of any cured meat have more than double the risk of bearing children who have brain cancer.[21]
- Children who eat the most ham, bacon, and sausage have three times the risk of lymphoma.[20]
- Children who eat ground meat once a week have twice the risk of acute lymphocytic leukemia compared with those who eat none; eating two or more hamburgers weekly triples the risk.[20]

Fortunately, vegetarian alternatives to these standard components of the American diet are now widely available, and many of them taste quite good. Consumers can find soy hot dogs, soy sausage, soy bacon, and even soy pastrami at their local health food stores as well as in many mainstream grocery stores.

5. Eat the Right Type of Fats

There is no room for debate: a diet high in fat, particularly saturated fat and cholesterol, has been linked to numerous health issues, including cancer. However, just as important as the amount of fat is the type of fat consumed. The goal is to decrease total fat intake (especially intake of saturated fats, trans fatty acids, and omega-6 fats) while increasing the intake of omega-3 fatty acids and monounsaturated fatty acids.

What makes a fat "bad" or "good" has a lot to do with the function of fats in the body's cellular membranes. Membranes are made mostly of fatty acids. What determines the type of fatty acid present in the cell membrane is the type of fat consumed. A diet composed mostly of saturated fat, animal fatty acids, and trans fatty acids (from margarine, shortening, and other sources of hydrogenated vegetable oils) and high in cholesterol results in membranes that are much less fluid in nature than a diet with optimal levels of unsaturated fatty acids. Modern pathology clearly indicates that an alteration in cell membrane function is the central factor in the development of virtually every disease. As it relates to diabetes, abnormal cell membrane structure due to eating the wrong types of fats leads to impaired action of insulin.

Without a healthy membrane, cells lose their ability to hold water, vital nutrients, and electrolytes. They also lose their ability to communicate with other cells and to be controlled by regulating hormones, including insulin. Without the right type of fats in cell membranes, cells simply do not function properly. Considerable evidence indicates that cell membrane dysfunction is a critical factor in the development of many diseases.

The type of dietary fat profile that is linked to many diseases is an abundance of saturated fat and trans fatty acids (hydrogenated vegetable oils) along with a relative insufficiency of monounsaturated and omega-3 fatty acids. One of the key reasons appears to be that because dietary fat determines cell membrane composition, such a dietary pattern leads to reduced membrane fluidity, which in turn causes reduced insulin binding to receptors on cellular membranes and/or reduced insulin action. Particularly harmful to cell membrane function are margarine, vegetable oil shortening, and other foods containing trans fatty acids and partially hydrogenated oils. These "unnatural" forms of fatty acids interfere with the body's ability to use important essential fatty acids and are now linked to an increased risk for heart disease, diabetes, and cancer. Just the opposite effect has been shown for diets high in monounsaturated fats and omega-3 fatty acids.

One diet that appears to be representative of a way of eating that provides an optimal intake of the right types of fat is the traditional Mediterranean diet—a term with a specific meaning. It reflects food patterns typical of some Mediterranean regions in the early 1960s, such as Crete, parts of Greece, and southern Italy. The traditional Mediterranean diet has shown tremendous benefit in fighting heart disease and cancer as well as diabetes and rheumatoid arthritis.[22] It has the following characteristics:

- Olive oil is the principal source of fat.
- It centers on an abundance of plant food (fruit, vegetables, breads, pasta, potatoes, beans, nuts, and seeds).
- Foods are minimally processed, and there is a focus on seasonally fresh and locally grown foods.
- Fresh fruit is the typical daily dessert; sweets containing concentrated sugars or honey are consumed a few times per week at the most.
- Dairy products (principally cheese and yogurt) are consumed daily in low to moderate amounts.
- Fish is consumed on a regular basis.
- Poultry and eggs are consumed in moderate amounts (1–4 times weekly) or not at all.
- Red meat is consumed in low amounts.
- Wine is consumed in low to moderate amounts, normally with meals.

Olive oil consists not only of the monounsaturated fatty acid oleic acid; it also contains several antioxidant agents that may also account for some of its health benefits. Olive oil is particularly valued for its protection against heart disease. It lowers harmful low-density lipoprotein (LDL) cholesterol and increases the level of protective high-density lipoprotein cholesterol. It also helps circulating LDL cholesterol from becoming damaged by free radicals[23,24] and has been proven to contribute to better control of the elevated blood triglycerides that are so common in diabetes. However, it is not only olive oil that produces these benefits—frequent nut consumption is also quite beneficial to health. The Prevention With the Mediterranean Diet (PREDIMED) study is recognized worldwide as a landmark study that marked a turning point in the dietary prevention of chronic diseases because it was not an epidemiological study but a randomized trial. This study showed that when the Mediterranean diet was supplemented with the free provision of either extra virgin olive oil or mixed tree nuts, this intervention reduced the risk of having a cardiovascular event by 30% after 5 years of intervention.[25]

6. Keep Salt Intake Low and Potassium Intake High

The electrolytes—potassium, sodium, chloride, and magnesium—are mineral salts that can conduct electricity when dissolved in water. For optimal health, it is important to consume these nutrients in the proper balance. Too much sodium in the diet from salt (sodium chloride) can disrupt this balance. Many people know that a high-sodium, low-potassium diet can cause high blood pressure and that doing the opposite can lower blood pressure,[26,27] but not as many are aware that the former diet also raises the risk of cancer.[28]

In modern Western society, only 5% of sodium intake comes from the natural ingredients in food. Prepared foods contribute 45% of our sodium intake; 45% is added in cooking, and another 5% is added as a condiment.

Patients can reduce their salt intake by following these tips:

1. Take the salt shaker off the table.
2. Omit added salt from recipes and food preparation.
3. If you absolutely must have the taste of salt, try the salt substitutes such as NoSalt and Nu-Salt. These products are made with potassium chloride and taste very similar to sodium chloride.
4. Learn to enjoy the flavors of unsalted foods.
5. Try flavoring foods with herbs, spices, and lemon juice.
6. Read food labels carefully to determine the amounts of sodium. Learn to recognize ingredients that contain sodium: salt, soy sauce, salt brine, and any ingredient with "sodium" in its name (such as monosodium glutamate) or "baking soda" (sodium bicarbonate).
7. In reading labels and menus, look for words that signal high sodium content, such as smoked, barbecued, pickled, broth, soy sauce,

teriyaki, Creole sauce, marinated, cocktail sauce, tomato base, Parmesan, and mustard sauce.

8. Do not eat canned vegetables or soups, which are often extremely high in sodium.
9. Choose low-salt (reduced-sodium) products when available.

Most Americans have a potassium:sodium (K:Na) ratio of less than 1:2. In other words, they ingest twice as much sodium as potassium. However, experts believe that the optimal dietary potassium:sodium ratio is greater than 5:1—10 times higher than the average intake. However, even this may not be optimal. A natural diet rich in fruits and vegetables can easily produce much higher K:Na ratios because most fruits and vegetables have a K:Na ratio of at least 50:1. The average K:Na ratios for several common fresh fruits and vegetables are as follows:

- Carrots: 75:1
- Potatoes: 110:1
- Apples: 90:1
- Bananas: 440:1
- Oranges: 260:1

7. Avoid Food Additives

Food additives are used to prevent spoiling or to enhance flavor; they include such substances as preservatives, artificial colors, artificial flavorings, and acidifiers. Although the government has banned many synthetic food additives, it should not be assumed that all the additives currently used in the U.S. food supply are safe. Many synthetic food additives that are being linked to such diseases as depression, asthma or other allergy, hyperactivity or learning disabilities in children, and migraine headaches remain in use.[29–32]

The U.S. Food and Drug Administration has approved the use of more than 2800 different food additives. It is estimated that the per-capita daily consumption of these food additives is approximately 13 to 15 g. This amount is astounding and leads to many questions. Which food additives are safe? Which should be avoided? An extremist might argue that no food additive is safe. However, many food additives fulfill important functions in the modern food supply. Many compounds approved as additives are natural in origin and possess health-promoting properties, whereas others are synthetic compounds with known cancer-causing effects. Obviously, the most sensible approach is to focus on whole, natural foods and avoid foods that are highly processed.

An illustration of the problem with food additives is one of the most widely used synthetic food colors: Food, Drug, and Cosmetic Act yellow dye no. 5, or tartrazine. Tartrazine is added to almost every packaged food as well as to many drugs, including some antihistamines, antibiotics, steroids, and sedatives. In the United States, the average daily per-capita consumption of certified dyes is 15 mg, of which 85% is tartrazine; among children, consumption is usually much higher.

Although the overall rate of allergic reactions to tartrazine is quite low in the general population, allergic reactions to tartrazine are extremely common (20%–50%) in individuals sensitive to aspirin as well as other allergic individuals. Like aspirin, tartrazine is a known inducer of asthma, hives, and other allergic conditions, particularly in children. In addition, tartrazine, as well as benzoate and aspirin, increases the production of a compound that raises the number of mast cells in the body. Mast cells are involved in producing histamine and other allergic compounds. A person with more mast cells in the body is typically more prone to allergies. For example, an examination of patients with hives shows that more than 95% have a higher-than-normal number of mast cells.

In studies using provocation tests to determine sensitivity to tartrazine and other food additives in patients with hives, results have ranged from 5% to 46%. Diets eliminating tartrazine as well as other food additives in sensitive individuals have, in many cases, been shown to be of great benefit in patients with hives and other allergic conditions, such as asthma and eczema.

8. Take Measures to Reduce Foodborne Illness

Foodborne illness is caused by the consumption of contaminated foods or beverages. Although the food supply in the United States is one of the safest in the world, the Centers for Disease Control and Prevention estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5000 Americans die each year from foodborne illness.[33] The microbe or toxin enters the body through the gastrointestinal tract and often causes the first symptoms there, so nausea, vomiting, abdominal cramps, and diarrhea are common symptoms in many foodborne diseases. Most cases of foodborne illness are mild, but serious diarrheal disease or other complications may occur.

More than 250 different organisms have been documented as being capable of causing foodborne illness.[34] Most of these cases are infections by a variety of bacteria, viruses, and parasites, but poisonings can also occur as a result of ingestion of harmful toxins or chemicals from organisms that have contaminated the food; for example, botulism occurs when the bacterium *Clostridium botulinum* grows and produces a powerful paralytic toxin in foods. The botulism toxin can produce illness even if the bacteria are no longer present.

Most of the common causes of foodborne infections are microorganisms frequently present in the intestinal tracts of healthy animals. Meat and poultry can become contaminated during slaughter by contact with small amounts of intestinal contents, and fresh fruits and vegetables can be contaminated if they are washed or irrigated with water that is contaminated with animal manure or human sewage.

The most common causes of foodborne infections are the bacteria *Campylobacter, Salmonella,* and *Escherichia coli* O157:H7 and a group of viruses called caliciviruses, also known as the Norwalk and Norwalk-like viruses. Undercooked meat and poultry, raw eggs, unpasteurized milk, and raw shellfish are the most common sources of these organisms.

The foremost measure to reduce the risk of foodborne illness is to cook meat, poultry, and eggs thoroughly. Using a thermometer to measure the internal temperature of meat is a good way to be sure that it is cooked sufficiently to kill bacteria. For example, ground beef should be cooked to an internal temperature of 160°F, poultry should reach a temperature of 185°F, and an egg should be cooked until the yolk is firm.

One must also take care to avoid contaminating foods by making sure to wash hands, utensils, and cutting boards after they have been in contact with raw meat or poultry and before they touch another food. Cooked meat should be served on a clean platter, rather than back on one that held the raw meat. To prepare produce, one should wash fresh fruits and vegetables in running tap water. A soft-bristle brush with a little mild soap can be used. Greens can be soaked in cold water as many times as needed to get them clean.

If further urging is needed, remind the patient that most of the increase in human longevity is due to public health's dramatic success in reducing foodborne infections.

9. Drink Sufficient Amounts of Water Each Day

Water is essential for life. The average amount of water in the human body is about 10 gallons. The recommendation to drink at least 48 oz of water per day to replace the water that is lost through urination, sweat, and breathing is valid. Even mild dehydration impairs physiological and performance responses.[35] Many nutrients dissolve in water so that they can be absorbed more easily in the digestive tract. Similarly, many metabolic processes need to occur in water. Water is a component of

blood and thus is important for transporting chemicals and nutrients to cells and tissues. Each cell is constantly bathed in a watery fluid. Water also carries waste materials from cells to the kidneys for filtering and elimination. Water absorbs and transports heat. For example, the heat produced by muscle cells during exercise is carried by water in the blood to the surface, helping maintain the right temperature balance. The skin cells also release water as perspiration, which helps maintain body temperature.

Several factors are thought to increase the likelihood of chronic mild dehydration: a faulty thirst "alarm" in the brain; dissatisfaction with the taste of water; regular exercise that increases the amount of water lost through sweat; living in a hot, dry climate; and consumption of the natural diuretics caffeine and alcohol.

There is currently a great concern over the U.S. water supply. It is becoming increasingly difficult to find pure water. Most of the water supply is full of chemicals, including not only chlorine and fluoride, which are routinely added, but also a wide range of toxic organic compounds and chemicals, such as PCBs, pesticide residues, and nitrates, and heavy metals such as lead, mercury, and cadmium. It is estimated that lead alone may contaminate the water of more than 40 million Americans. Patients should be encouraged to determine the safety of their tap or well water by contacting their local water companies; most cities have quality assurance programs that perform routine analyses. Patients can simply ask for the most recent analysis.

SUMMARY

The dietary guidelines and principles that are detailed in this chapter represent our answer to the hotly debated question, "What is the best diet?" After a review of several popular diet in detail as well as thousands of scientific articles on the role of diet in human health, our offering here is based on the evolutionary understanding of what constitutes the optimal diet. The bottom line for a health-promoting diet is to reduce the intake of potentially harmful substances—foods laden with empty calories, additives, and artificial sweeteners—and replace them with natural foods, preferably organically grown.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ryde D. What should humans eat? *Practitioner*. 1985;232:415–418.
2. Milton K. Nutritional characteristics of wild primate food: do the diets of our closest living relatives have lessons for us? *Nutrition*. 1999;15:488–498.
3. Cordain L, Eaton SB, Miller JB, et al. The paradoxical nature of hunter-gatherer diets: meat-based, yet non-atherogenic. *Eur J Clin Nutr*. 2002;56(suppl 1):S42–S52.
4. Eaton SB, Eaton 3rd SB. Paleolithic vs. modern diets—selected pathophysiological implications. *Eur J Nutr*. 2000;39:67–70.
5. Sinha R, Cross AJ, Graubard BI, et al. Meat intake and mortality: a prospective study of over half a million people. *Arch Intern Med*. 2009;169:562–571.
6. Fung TT, van Dam RM, Hankinson SE, et al. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. *Ann Intern Med*. 2010;153:289–298.
7. Trowell H, Burkitt D. *Western Diseases: Their Emergence and Prevention*. Cambridge, MA: Harvard University Press; 1981.
8. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. *Cancer Causes Control*. 1991;2:427–442.
9. Gangemi S, Miozzi E, Teodoro M. Occupational exposure to pesticides as a possible risk factor for the development of chronic diseases in humans (Review). *Mol Med Rep*. 2016;14(5):4475–4488.
10. Aronson KJ, Miller AB, Woolcott CG, et al. Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2000;9:55–63.
11. Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. *Sci Total Environ*. 2017;575:525–535.
12. Lu C, Knutson DE, Fisker-Andersen J, et al. Biological monitoring survey of organophosphorus pesticide exposure among preschool children in the Seattle metropolitan area. *Environ Health Perspect*. 2001;109:299–303.
13. Consumers Union. *Do You Know What You're Eating? An Analysis of U.S. Government Data of Pesticide Residues in Foods*. Washington, DC: Consumers Union; 1999.
14. Jenkins DJ, Kendall CW, Augustin LS, et al. Glycemic index: overview of implications in health and disease. *Am J Clin Nutr*. 2002;76:S266–S2673.
15. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. *Am J Clin Nutr*. 2002;76:S274–S280.
16. Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *Am J Clin Nutr*. 2000;71:1455–1461.
17. Godfray HCJ, Aveyard P, Garnett T, et al. Meat consumption, health, and the environment. *Science*. 2018;(6399):361.
18. Yip CSC, Lam W, Fielding R. A summary of meat intakes and health burdens. *Eur J Clin Nutr*. 2018;72(1):18–29.
19. Whigham LD, Cook ME, Atkinson RL. Conjugated linoleic acid: implications for human health. *Pharmacol Res*. 2000;42:503–510.
20. Blot WJ, Henderson BE, Boice Jr JD. Childhood cancer in relation to cured meat intake: review of the epidemiological evidence. *Nutr Cancer*. 1999;34:111–118.
21. Preston-Martin S, Pogoda JM, Mueller BA, et al. Maternal consumption of cured meats and vitamins in relation to pediatric brain tumors. *Cancer Epidemiol Biomarkers Prev*. 1996;5:599–605.
22. Galbete C, Schwingshackl L, Schwedhelm C, et al. Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses. *Eur J Epidemiol*. 2018;33(10):909–931.
23. Alarcon de la Lastra C, Barranco MD, Motilva V, et al. Mediterranean diet and health: biological importance of olive oil. *Curr Pharm Des*. 2001;7:933–950.
24. Visioli F, Franco M, Toledo E, et al. Olive oil and prevention of chronic diseases: Summary of an international conference. *Nutr Metab Cardiovasc Dis*. 2018;28(7):649–656.
25. Guasch-Ferré M, Salas-Salvadó J, Ros E, et al. The PREDIMED trial, Mediterranean diet and health outcomes: how strong is the evidence? *Nutr Metab Cardiovasc Dis*. 2017;27:624–632.
26. Whelton PK, He J. Potassium in preventing and treating high blood pressure. *Semin Nephrol*. 1999;19:494–499.
27. Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. DASH–Sodium Collaborative Research Group. *N Engl J Med*. 2001;344:3–10.
28. Jansson B. Potassium, sodium, and cancer: a review. *J Env Pathol Toxicol Oncol*. 1996;15:65–73.
29. Boris M, Mandel FS. Foods and additives are common causes of the attention deficit hyperactive disorder in children. *Ann Allergy*. 1994;72:462–468.
30. Lessof MH. Reactions to food additives. *Clin Exp Allergy*. 1995;25(suppl 1):27–28.
31. Groten JP, Butler W, Feron VJ, et al. An analysis of the possibility for health implications of joint actions and interactions between food additives. *Regul Toxicol Pharmacol*. 2000;31:77–91.
32. Simon RA. Adverse reactions to food additives. *Curr Allergy Asthma Rep*. 2003;3:62–66.
33. Lasky T. Foodborne illness—old problem, new relevance. *Epidemiology*. 2002;13:593–598.
34. Tauxe RV. Emerging foodborne pathogens. *Int J Food Microbiol*. 2002;78:31–41.
35. Kleiner SM. Water: an essential but overlooked nutrient. *J Am Diet Assoc*. 1999;99:200–206.

45

Peat Therapeutics and Balneotherapy

Mark Groven, ND,* and *Joseph E. Pizzorno, ND*

OUTLINE

INTRODUCTION

Nature has provided many gifts of healing. One of the most outstanding and exciting is peat. The use of organic peat and its constituents is ancient, yet people with pain, injury, and dermatologic, rheumatic, and other conditions are benefiting from modern peat therapy today. This pleasant and safe healing discovery has brought relief and cure for many.[1–18]

Balneology is the study of the art and science of bathing. Balneotherapy is the use of natural thermal mineral waters, additive baths, peloids, and other natural substances, as well as various atmospheric or environmental elements singly or in combination, for the prevention and treatment of disease. The aim of balneotherapy is to change regulatory and reactive functions, leading to improvement of capacity, adaptation, and self-healing.[19]

Peloid refers to the pulp of a substance that is applied to the body. It may be in pack form or bath, either local or whole body. The concentration of peloidal solutions can vary, and peloid solutions should be applied to the skin in a specific manner for a specific condition to optimize results. Common peloids are peat pulp, lake or sea muds, and plant substances.

For many conditions, balneotherapy works synergistically with peloid therapy, and the percutaneous absorption of their constituents along with the physiological and psychological effects provides an excellent therapy for people who can no longer tolerate oral or injectable pharmaceuticals and have chronic degenerative diseases. Life is stressful, and our society is aging. We would be wise to utilize the positive benefits of balneotherapy in the conventional treatment of pain and illness as well as in health maintenance and prevention of disease. The purpose of this chapter is to describe the general concept of balneotherapy, with an emphasis on the therapeutic application of peat. There certainly is a distinction between the application of peat and the application of other muds, such as lake mud or clay. The characteristics of the specific peat mud constituents being used are vitally important, as is the manner of their application.

* Previous edition contributor

BALNEOLOGY

History

Therapeutic bathing is an ancient art and probably the oldest of medical procedures. Hippocrates wrote about the application of therapeutic bath in 400 BC and how it soothed pain in the side, improved respiration, soothed the joints and skin, was diuretic, and removed heaviness of the head. It was suited for those who benefited, but it could be unsuitable if applied in the wrong way. It enjoyed tremendous popularity until about 75 years ago when, along with other natural

techniques, it fell out of favor as conventional medicine produced its modern successes. Since then, the large corpus of empirical wisdom has been expanded on, and much scientific evidence has contributed to the advancement of balneology as a science. Many institutions are teaching hydrotherapy and balneological techniques, and many spas have wonderful programs for people to use. It is not hard to imagine that the reason this art survived and improved is because it can better people's health.

Balneotherapy's modern-day roots lie predominantly in European spas, which have some of the longest continuously running histories of all medical institutions. Millions of patients flock to clinics throughout Europe and the world each year for treatment in hydrology departments under the supervision of physicians and their staffs. Such clinics provide a variety of balneotherapeutic techniques. *Spa therapy* is a term used for the combination of balneotherapy and other techniques usually delivered at a resort setting. The effects of spa therapy are influenced positively by the pleasure of being in a beautiful setting with the stresses of home and work removed. Medical spas are often in an area where the earth's elements are present. The pristine air, the ambient temperature, the humidity or amount of light, nourishing food, and exercise can effect a change in the spirit, mind, and body.[19]

Balneotherapy in Combination With Other Therapies

Many therapies work well together, such as phototherapy and mud therapy in the treatment of psoriasis. Mud pack therapy, peloidotherapy, massage, soft and osseous tissue manipulation, iontophoresis, phonophoresis, and exercise work together.[20] The combination of buoyancy and heat in the water makes sense, for example, in underwater traction bath and massage, which have been shown to reduce the levels of analgesic consumption in patients.[18] In my own experiences with patients, hydrotherapy, with and without mud in combination with other treatments, such as naturopathic manipulation, can work like nothing else to help people heal and stay healthy. A multitude of conditions can be treated with balneotherapeutic methods—pain, injury, and dermatologic and rheumatic conditions rank high.[2,19,21,22] Under the right conditions and with the right application, balneotherapy stimulates healing and speeds recovery.[23]

Physiological Effects

Balneotherapeutics induce direct and indirect actions on the body. The direct actions of balneotherapy take into consideration the physical actions of water on the body, such as hydrostatic pressure, buoyancy, viscosity, and frictional resistance, as well as thermal effects and the chemical and pharmacological effects of the percutaneous absorption of the substance being used.[24] Such substances are found in hot spring waters of various types, such as carbon dioxide, hydrogen sulfide, chloride, sulfate, iron, acid, and radon. Mineral waters contain cations, such as sodium, potassium, calcium, and magnesium, and anions, such as SO_4^-, Cl^-, and HCO_3^-. The concentrations of these ions are usually significantly high (1 g/L). In spring water, levels of nitrogen compounds, such as nitrate, nitrogen dioxide, and nitrogen oxide, are very low, and the water is free of or low in bacteria.[17] Peat muds, plant preparations, and mineral-containing muds are also used. In Europe, peat baths and peloids are traditional. These applications are used in combination with exercise, aquatics, steam bath, sauna therapy, climatotherapy, physical therapy, and pharmacotherapy, among others, with important consideration being given during treatment to the chronobiological and circadian-rhythm phases of the body.[1,14,20,21,25–27]

The indirect actions of balneotherapy arise from the repeated application of therapeutic stimulation, such as climatic exposure to the elements, training effects of exercises, and social and psychological effects arising from changes in the environment. These elements act as a complex stimulation in a nonspecific manner of the physiological function of the organism's central nervous system, autonomic nervous system, endocrine system, immune system, and so on. The result of these stimulations is a reactive response by the body, leading to activation and improvement of capacity, adaptation, and self-healing potential. In other words, balneotherapy has a normalizing effect on the body's systems and rhythms.

Circadian Rhythms

Biorhythms are important in the expression of many conditions.[28,29] Sunlight, mealtimes, and seasonal changes are external cues that, together with internal cues such as blood pressure and respiration, affect the hypothalamus or master clock. This part of the brain then signals hormones, enzymes, and other substances to facilitate healing, produce cells, or cause pain and symptoms. It has been shown that different times of day affect the amount of muscle torque potential, body temperature, and clock-gene messenger RNA (mRNA) expression.[30] A cold or hot foot bath can induce a rise in the temperature of the oral mucosa of up to 1°C, but the extent of the increase in the oral temperature depends on the body's reaction to this stimulus, which is influenced by the body's own internal clock.[1]

Conditions such as arthritis have pain with varying cycles.[31] It is thought that some of these cycles are in synchrony with the moon and the sun. Heart attacks, asthma, and rheumatoid arthritis joint pain are early-morning diseases, so medication and treatment can be tailored for different times of day when it is significantly more helpful and less wasteful when not needed. Serum adrenocorticotropic hormone, prolactin, luteinizing hormones, and immune parameters such as plasma levels of soluble p75 tumor necrosis factor and tumor necrosis factor-α (TNF-α) vary in 24-hour rhythms in the body.[23] These may be affected through balneotherapy, as may melatonin production and expression.[24] Melatonin affects the organization and expression of biorhythms and is easily susceptible to oxidative stress. Symptom remission in some conditions coincides with a normalization of circadian rhythms promoted by balneotherapeutic treatment.[32–33] In a European study, the combination of carbon dioxide bath and mud bath was shown to downregulate the systolic pressure in hypertension while having a balancing effect on biological clocks.[34] A study on fibromyalgia patients, in whom altered reactivity of the hypothalamic–pituitary–adrenal axis was observed, supported the theory that mud pack therapy works in synergy with antidepressant treatment to decrease pain and improve depression. Balneotherapy can promote the body's response systems, such as the stress response system, to achieve homeostasis.[35]

PEAT THERAPEUTICS

History

The ancients used peat extract baths, and the antiseptic properties of peat mud were recognized in World War I when it was applied directly to wounds to prevent infection.[36] Natives used mud externally and internally. Peat has been used as a medicinal preparation in baths and peloid packs extensively in Europe for the past 200 years. This unique substance contains many chemical constituents that can interact with organic and inorganic compounds.[37] The scientific basis for the physical, chemical, and pharmacological effects of peat baths has long been known, and such baths have been used extensively in balneotherapeutic applications in Europe and other parts of the world to treat rheumatic diseases, gynecological disorders, osteoarthritis, lumbago, sciatica, skin diseases, trauma and its sequelae, and many other ailments and afflictions. The many substances in peat offer a vast possibility of medicinal cure applications.[38–48]

It is important to consider the region and origin of the peat being used for medicinal purposes. Low moor peat has been shown to contain higher concentrations of nitrous substances, which are thought to contain a higher content of biologically active substances than the high moor peats or peats taken from a shallower depth. It is not just high nitrous content that makes a certain peat more medically useful but also the quality, type, and amount of the biologically active substances it contains that are the determining factors in its medicinal effect. In Germany, these types of peats are now a national resource.

Skin Response

The skin is a reflex, metabolic, immune, and excretory organ. It affects the autonomic, immune, and circulatory systems and participates in the biosynthesis of not only vitamin D but also acetylcholine, histamine, and serotonin.[2,4,10,15] Significantly higher concentrations of minerals and medicaments can be attained in the epidermis with baths than with systemic flooding via the vascular system. There is percutaneous uptake of many substances by the skin but not in large amounts for most natural substances. Permeation into the dermis does occur especially for humic acids, and uptake beyond the stratum corneum is exemplified by measurable urinary excretion rates.[49,50]

The primary effects of bath components take place within the skin. For instance, hydrogen sulfide acts as a trap for oxygen radicals, functioning to reduce inflammation. It is thought that the action comes from the effect of sulfur on the Langerhans cells, which play a role in immune presentation and inflammation modulation. In this way, skin responses can act as transmitter-activating helper functions. Sulfur-containing peat baths demonstrate a pain-reducing and healing effect on rheumatic and degenerative diseases. One reason may be the reduction in the activity of Langerhans cells in producing cytokines that results from the combination of the components within peat and thermal radiation.[2,7,15,19,22,50,64]

Physiological Effects

Peat has a structure containing micropores, which accounts for its sponge-like water-carrying capacity and its ability to maintain either hot or cold temperatures. When applied, peat produces a gradient rise or fall in temperature, which is especially desirable in a therapeutic bath. A peat bath formulation influences neuromuscular, endocrine, and pulmonary functions, as well as kidney hemodynamics, depending on the consistency and volume of the partial or full bath.[4,10] Peat has well-documented effects, such as tissue dilatation and increases in stroke volume, metabolism, and immunologic stimulation. A peat bath may be preferable to a water bath if one considers the gradient rise and fall of temperature, increased buoyancy, and prevention of heat loss during a bath and the possible positive chemical and pharmacological effects of the constituents of peat.[47,49–52]

Those who have experienced a therapeutic bath can appreciate the feelings of exhilaration and deep relaxation induced by a bath that contains an additive such as peat. The response is affected by the constituents in the water, the temperature of the bath, and the time of day the stimulus is given.[52]

The patient's genetics and physical capacity are also important. A peat bath enhances circulation significantly longer than a water bath.[53] Microcirculatory vasodilation in the skin has been shown to increase even without hyperthermia. The peripheral and deeper arteries, such as the intrauterine vessels, have shown prolonged increased flow after a peat bath. The effects of peat constituents can occur without heat, but heat increases the effect on the body.

The antirheumatic activity of thermal muds has a precise pharmacological character. Therapy can be prescribed according to the characteristics of the mud for specific conditions.[47,49] For example, specific muds work better with phototherapy for psoriasis and atopic dermatitis. The length of time that the peat mud has undergone humification and maturation lends unique characteristics. Maturation of peat muds increases their thermoinsulating, hydration, and, importantly, biochemical characteristics. The sulfoglycolipid content of mature mud differentiates a natural remedy from a specific application with a precise pharmacological character. These sulfoglycolipids are absorbed through the skin and stimulate an antirheumatic effect.[54] Several peat substances are able to permeate the skin.[50] Their absorption and action have been documented by the comparisons of placebo, water baths, and peat baths using Doppler ultrasound measurement. One study that measured circulation in the uterine artery after bath therapy showed that only the peat bath achieved the physiological effect of prolonged vasodilatation and circulation. This effect lasted for several hours after the treatment. It is thought that absorption of peat substances takes place through the hair follicles and apocrine glands via diffusion and partial pinocytosis.[53,55] The fractions of peat components that penetrate the skin include the humic acid fractions: fulvic, ulmic, and volvic. The excitatory effect of humic acid fractions such as fulvic acid affect the reactivity of the α2 and D2 receptors of smooth muscle cells.[51]

The functions of peat in medicinal applications are antimicrobial, antiviral, anti-inflammatory, and antineoplastic, to name a few.[52] Many biochemical effects have been demonstrated in humans and animals. The anti-inflammatory effect of peat mud has been attributed to a sulfoglycolipid associated with a decrease in serum interleukin-1 (IL-1) in patients with arthritis.[56] The effects of mud applications include elevation of protein synthesis, reduction of arachidonic acid, and inhibition of inflammatory mediators such as leukotrienes (LTB4), prostaglandins (PGE-2), and thromboxane. Biological activity is ascribed to peat ingredients, such as sulfur compounds, magnesium, manganese, iron, and humic acids.[6,7,10,11,16,19,24] Mud pack therapy decreases the proinflammation factors IL-1 and TNF-α and the radical-mediated peroxidations nitric oxide and myeloperoxidase.[57] It also increases serum levels of insulin-like growth factor 1, which is cartilage protective. Humic substances spread widely in nature, and when found mainly in highly degraded peat, they have been shown to have a proliferative effect on certain leukocytes. Water-soluble oxihumate, given orally or dermally, increases the proliferative response in mononuclear leukocytes as well as the production and expression of IL-2.[2,36,58]

Hyperthermia

The thermal properties of peat mud applications have been shown to be much greater than those of water baths because of the former's dynamic viscosity, decreased convective cooling, and protective effect on the skin with hot applications.[2] Whole-body, extracorporeal, and local infrared applications of hyperthermia have uses in cancer therapy. Hyperthermic effects include changes in heat shock proteins (HSPs) and upregulation of heart antioxidant defense proteins such as manganese superoxide dismutase.[54] Plasma B-endorphins also rise in response to hot water bathing and may be responsible for the euphoric feeling the bath may bring.[24,59] In a study of patients with cancer, hyperthermia was shown to create the same endorphin rise both after sauna bath and in whole-body infrared hyperthermia.

Whole-body hyperthermia (WBH) stimulates an increase in T cells, such as monocytes, and absolute numbers of white blood cells.[59] Heat increases granulocyte mobility, phagocytic and bactericidal properties, and enzymatic activity.[1] There is an increase in homing response to different tissues of lymphocytes, which contributes to antitumor activity. Hyperthermia may increase lymphocyte migration into inflamed tissue or lymphoid tissues such as lymph nodes and Peyer patches; this effect may help generate the cellular immune response. TNF-α and IL-6

are regulated by the stimulus of hyperthermia.[60–62] HSPs produced by hyperthermia can provide protection against the muscle damage that occurs through a pathological increase in intracellular calcium or uncoupling of the mitochondrial respiratory chain. Hyperthermia provides protection against typical damage from reperfusion after ischemia or with excessive exercise damage. Calcium homeostasis, energy loss, increased free radical–mediated reactions, and activation of apoptosis pathways are affected. I use a thermal application of the partial or full peat-additive bath for the treatment of back pain, musculoskeletal disorders, skin problems, viral illnesses, and more. The use of heat in the right amount is crucial to treatment efficacy.[54,62,63]

CLINICAL APPLICATIONS

Care must be exercised in the selection of patients for any type of thermal therapy. It is important to allow each patient time to adapt by starting with lower-temperature and shorter-duration treatments first. Patients with neurodegenerative diseases like multiple sclerosis (MS) and conditions such as diabetes are not good candidates for WBH. In MS, the excitatory effect on nerves from heat leads to muscle cramping, and in diabetes, heat may lead to an ultimate drop in blood sugar and lightheadedness or loss of consciousness. Peat has many beneficial applications, however, as described here.

Discopathy

A series of treatments using a peat bath first, followed by manual traction and then a peat pack over the affected area of the spine, has worked marvelously for many of my patients with discopathy. The first thing that is helped is the pain; then there is an improvement in structural integrity and function over time. It is necessary to perform these combination treatments three times a week for 3 to 12 weeks if there is significant discopathy.

Myopathy

Muscle pain is easily treated with a thermal peat bath or peat pack. The increase in circulation is very helpful to muscles and can be valuable in assisting with osseous manipulation once the muscles are more relaxed.

Scoliosis

A peat bath followed by traction of the spine can be very helpful in patients with scoliosis who have back pain. Applying a peat pack over the area of spinal pain can also be useful.

Arthritis

Peat treatments have shown efficacy for both osteoarthritis and rheumatoid arthritis. Matrix metalloproteinases (MMP-1 or stromelysin-1) are significantly reduced in mud bath patients with osteoarthritis.[64] One needs to be careful with acute rheumatoid arthritis because treatment may initially aggravate symptoms. Generally, in osteoarthritis, these treatments have been shown in both the literature and my experience to be very beneficial. I have observed significant decreases in swelling and pain with one treatment multiple times in osteoarthritis of the knee and other areas. Generally, a series of either combination bath and pack or single bath or packs is given. Treatments are done every day or two. Emerging research is documenting subjective and objective improvement: serum concentrations of IL-1β, TNF-α, IL-8, IL-6, and TGF-β, as well as eHsp72, were markedly decreased in 21 patients with osteoarthritis of the knee.[65]

A 2018 study of 33 patients with hand osteoarthritis found a statistically significant benefit of peloid therapy compared with a control group that used only hand exercises.[66]

Another recent study, this one of 75 patients suffering chronic lateral epicondylitis, compared peloid with lateral epicondylitis band and found a statistically significant improvement in subjective measures.[67]

A particularly interesting study followed more than 100 patients suffering bilateral knee osteoarthritis for a year after completion of a single cycle of treatment. They found statistically significant benefit for all time periods measured.[68]

Headache

Chronic headaches can respond to peat bath or to peat packs, especially on the neck or over the cervical spine. One must be careful not to overheat the medulla with the cervical pack treatment; thus mud applications are usually applied below the second cervical vertebra.

Hamstring Strain

One of the best applications of bath followed by mud pack is in a hamstring muscle strain. In my experience, athletes even of professional stature have been able to "get back into the game" faster and with more function than they would have with any other type of treatment.

Ankle Sprain

Partial peat bath immersion of the foot and leg for ankle sprain is very effective. One can see in older sprains the immediate reduction of ecchymosis after the treatment. Patients can perform home treatments in a plastic wastepaper basket on a daily basis for three to six treatments. This approach is inexpensive and wonderfully effective.

Hypertension

Ambulatory blood pressure has been shown to be affected positively by balneotherapy. After a series of treatments, blood pressure at rest and during standardized levels of ergonomic exercise tends to decrease, as does nocturnal blood pressure. The effect is sustained sometimes permanently.[34] I do not necessarily recommend this treatment for people with hypertension because people with hypertension have more cardiovascular problems that must be considered before they undergo thermal therapy. Generally, people with mild hypertension tolerate the bath better than people with mild hypotension.

Dermatologic Problems

Psoriasis and atopic dermatitis are the dermatologic conditions more commonly treated with peat bath. I, however, have had very good success in eczema and dermatitis with both peat bath and cream application. There are multiple possibilities with dermal applications.

Scleroderma

A peat bath weekly for 6 to 12 treatments is often a good method of treating scleroderma. Treatment should start with a tolerable temperature and increase slowly over the series of baths so that the patient can adapt.

Human Papillomavirus

The antiviral effects of topical peat application have been demonstrated on several viruses, including the human papillomavirus. Remission and prevention of implantation of the virus have been described. This is a measure that prevents cancer. The antiviral and antineoplastic effects are thought to be associated with the ability of peat constituents, such as humate, to bind on lectin-binding junctions, thereby blocking viral entry into cells.[39,53]

Herpes Virus

Topical treatment of herpes virus skin disease with humic and fulvic fractions of peat has been effective. I have had significant success with

zoster outbreaks. Most important, the topical application of specific peat formulation creams that include humic acids has been very helpful to some of my patients.

Infertility

A study on infertility due to immature follicle maturation syndrome demonstrated good results with peat therapy in comparison with pharmacotherapy. In the peat therapy group, the rate of pregnancy was very good, along with a practically nonexistent spontaneous abortion rate, whereas in the pharmacotherapy group, the rate of spontaneous abortions was very high.[32]

ANKYLOSING SPONDYLITIS

In the treatment of ankylosing spondylitis and spondylitis associated with inflammatory bowel disease, mud therapy showed a decrease in the level of C-reactive protein and an elevation of hemoglobin with a series of treatments. This coincided with a significant reduction in pain and an improvement in function.[35,69,70] Peat bath and pack and just the bath are options. If a patient is not a candidate for thermal bath, the packs over the spine can be very useful.

Hematoma

Organic peat, with its intense vasodilating, anti-inflammatory effects and interactions of ions and mineral properties, enhances the reduction of hematomas. Hematomas treated with thermal peat application have been reported to resolve 50% faster, with no hemosiderin residue, than those treated with only heat applications, which often leaves residues.[71] One can see bruising decrease with pack application immediately after the treatment and an enhancement in the absorption of the hematoma in significantly less time with bath and pack.

Immune Stimulation

Peat baths in combination with hyperthermia demonstrate leukocyte elevation. The immune-stimulating effects of peat baths seen clinically correspond to hematologic changes after baths.[59] Some effects on immune function are due to heat, and others are due to the constituents in peat. A favorable effect is seen on peripheral blood lymphocytes in atherosclerotic disease after hydrogen/sulfide bath, independent of heat.[63] Oxihumate has shown antiviral activity plus immunostimulatory effect on mononuclear lymphocytes while also having very low or no toxicity, showing promise for the treatment of immunocompromised patients.[36] Immune stimulation or regulation may also be due to increases or changes in immunocyte numbers and function and the balancing of chronobiological rhythms.[45,50,71,73,74,78-82] Oral oxihumate, a potassium salt of oxihumic acid, may show some activity in blocking human immunodeficiency virus type 1 infection of MT-2 cells and may be helpful in the topical treatment of herpes virus–induced skin diseases.[36]

There are also internal uses for constituents of peat. Interestingly, electrophoresis of peat mud showed benefits in patients with duodenal ulcer, secondary to hormone shifts and normalization of collagen metabolism in the duodenal mucosa.[15] The internal use of peat fractions, such as humic acid, also increased the proliferation of some T cells.[36]

Fibromyalgia

A recent uncontrolled study evaluated 30 days of daily mud bathing at 40°C for 10 min. In addition to subjective reports of clinical improvement, objective measures showed a reduced C-reactive protein level and increased telomere length in leukocytes.[72]

CLINICAL PROCEDURES

The following procedures should be applied with care and forethought as to diagnosis and the skillful administration of the treatment. These procedures are stimulations to the body, and the thermal effects should not be taken lightly. Patients must be thoroughly screened for contraindications to treatment before undergoing full-body immersion hyperthermia. The very young and very old should, in general, have very gentle hot or cold stimulation, if any at all. Generally, up to three thermal baths are given per week if they are hot. Thermal therapy can be depleting for some patients, so consideration of the patient's vitality is primary.

Hyperthermic Medicinal Peat Bath

The indications for and contraindications to hyperthermic medicinal peat bath are given in Box 45.1.

Materials

- Peat bath material—I use a commercially available peat bath formula containing peat extract, sulfur, wintergreen, and pine oil.
- Tub with water thermometer and safety features like handrails and nonslip floor mats
- Room with table for perspiration time
- Gown or loose-fitting bathing suit
- Two sheets
- Two wool blankets
- Two large towels (one for patient to dry off after treatment and one for head wrap)
- Basin with iced water and a hand towel for cooling patient's face
- Digital thermometer or otothermometer for patient monitoring (no glass mercury thermometers). A microcomputer-based data-acquisition device that records electrocardiogram (ECG) data and body and ambient temperatures during bathing can be used; this is preferred over individual readings of pulse and oral temperature during the bath because the ECG shows heart function and the clinician is freed from the task of doing the monitoring. This device can be a real-time recorder for the bath.[73]
- Exhaust fan or room air filter
- Footstool for entering and leaving tub

The design of the bath area should take into consideration getting patients in and out of the tub and then as directly as possible to a treatment table.

Procedure

1. Before a patient begins treatment, cardiovascular risk and any other conditions that do not respond to or are aggravated by thermal therapy should be ruled out. Once the patient has been classified as to risk factors for thermal therapy, the clinician should ask about previous experience with heat in sauna, steam baths, or other types of infrared heating of the tissues. Much depends on the patient's positive health perspective of the self and of the pain or disease process and the patient's expectations of the therapeutic bath or application.
2. Make sure the tub is clean and without a ring. Check log book on last treatment and cleaning. If any evidence of an unclean tank is seen, it must be cleaned and disinfected before use: wearing rubber gloves, use a soft scouring sponge to scrub the tank with disinfectant soap, followed by a rinse of hot water. Then spray the surface with 10% bleach solution, and wait 10 minutes before rinsing with very hot water. Alternatively, a nonbleach product that is antimicrobial can be used; there are many choices for practitioners to choose from on the market.

BOX 45.1 Indications and Contraindications for Hyperthermic Medicinal Peat Bath

Indications

Acne
Arthritis pain
Back pain
Benign prostatic hypertrophy
Bursitis
Carpal tunnel syndrome
Dermatitis
Eczema
Fibromyalgia
Flu
Fractures
Acute gouty toe
Chronic gout
Gynecological disorders
Headaches
Hematomas
Hives
Insomnia
Lumbalgia
Metabolic disorders
Muscle tension
Neurological disorders
Obesity
Orthopedic disorders
Osteoarthritis
Postoperative rehabilitation
Premenstrual syndrome
Prostatitis
Psoriasis
Rashes
Rheumatoid arthritis
Sciatica
Scleroderma
Skin care
Sprains/strains
Stress relief
Trauma
Viral infections

Contraindications

Acute hypertension
Breastfeeding
Cardiac deficiency
Diabetes
Multiple sclerosis
Neurodegenerative disease
Open wounds
Preexisting high fever
Pregnancy
Pulmonary deficiency
Respiratory insufficiency
Systemic lupus erythematosus

3. Fill the tub to 10 inches from the top with water at a temperature of 104°F to 113°F (40°C–45°C).
4. The starting temperature and possible duration of treatment are determined by the condition.
5. The straight water bath should not exceed 110°F. With peat additive, the temperature should not exceed 113°F.
6. Add peat to the bath.
7. Close monitoring during treatment, by means of periodic recording of the patient's pulse, oral temperature, duration of treatment, and tank temperature, is necessary. A quick spike in pulse above the initial pulse within the first minute or minutes is a contraindication to treatment. Any adverse reaction, such as tingling of the fingers and toes, nausea, headache, lightheadedness, or vertigo, should be evaluated closely and treatment terminated. Some patients may be able to tolerate only a low temperature and short duration for the first treatment. For a patient having a series of treatments, the first treatment is of shorter duration and lower temperature to see how the patient responds. The ability to tolerate treatments should improve as patients acclimate through their series of treatments.
8. The patient should enter extremely still water slowly. It will not feel as hot if the water is still.
9. Have the patient remain still as he or she becomes fully immersed, to help decrease the sensation of intense heat.
10. To treat the pelvis, use a sitz bath rather than a full bath to concentrate the effects of the treatment. A full bath after the sitz bath may be useful.
11. The water will cool as time passes, although the peat material will help maintain the temperature. If the starting temperature was 105°F, hot water may have to be added.
12. Bath duration is 8 to 20 minutes and should not exceed 20 minutes because of the additive pharmacological effects and the tendency for hyperthermia to produce increased metabolism and mobilization of chemicals within the body.
13. If the patient becomes fatigued or distressed, the patient should exit the bath to be wrapped in the waiting sheet and wool blankets; do not wait longer to have the patient leave the bath.
14. The patient must have help exiting the tub, from two people who provide lifting support from under the arms on either side. This is a time to be very careful. The clinician should assist the patient by placing an arm under the patient's arm but having the patient use his or her own ability to walk and get out of the tub. If the clinician tries to lift the patient rather than provide necessary support, the patient's tendency is to put all his or her weight on the clinician, which is not the goal. After thermal therapy, patients are usually fine to walk, but there is a chance of lightheadedness and, in rare cases, vertigo. Patients may need assistance.
15. Encourage the patient to concentrate on walking on his or her own.
16. Have the patient lie down on a fresh sheet and wrap the patient in both sheets and two or three wool blankets. Cover the head with a towel.
17. Continue to monitor pulse and oral temperature for the duration of the 20-minute perspiration time.
18. Rinse a facecloth in cold water and apply for 10 seconds or longer to the patient's face every 1 or 2 minutes during both bath and perspiration times. This is extremely important during the bath.
19. Encourage the patient to relax and help the patient focus on pleasant matters during the bath. The psychological component of the bath is huge. Having patients think and talk about whatever excites and pleases them about their lives or their future during the difficult part of the bath and the treatment will help them tolerate the heat and reframe their disease processes.
20. After the patient has been wrapped from head to foot in sheet and wool blankets (it is not necessary to have blankets under the patient because the table is a good insulator that prevents the blankets from getting wet), allow the patient to go through the hydrotherapy reaction of rise and fall in temperature, pulse, and diaphoresis three times. Then remove the patient from the sheets and allow

him or her to return to normal activities. This cyclic reaction is seen with peat baths. With water baths, the patient may or may not sweat after the bath. With peat baths, perspiration is helpful because there is increased absorption or skin effect if perspiration is allowed to continue after the bath.

21. Have the patient rest and replace electrolytes after treatment. The patient should not do a lot of exercise for 12 to 24 hours after a full bath, especially patients with back problems. The patient should promote good posture in the treated area during this time.
22. During the perspiration time, manual traction can be applied to the spine. This is done by grasping the ankles of the supine patient and pulling for 30 to 45 seconds with enough traction that the patient almost slides on the table. Indications for manual traction are disc problems, scoliosis, and impingement; it is a nice addition to any peat bath for any condition because it feels good to the patient.
23. Advise the patient not to shower with soap for up to 12 hours after the peat bath because absorption rates continue after the bath if peat additives have been used.
24. Patients should dry thoroughly and remain covered, warm, and out of drafts for 3 hours after treatment. This precaution can easily be overlooked and cause aggravation if cold stimulation is allowed to happen. More than one reminder to the patient is necessary because people naturally are ready to cool down after a thermal bath.
25. Clean the tank and room thoroughly after use. Log out times of bath and tank cleaning.

Medicinal Peat Peloid

The indications for and contraindications to this procedure are given in Box 45.2. Care should be taken to avoid burns when performing this application. It is important for the patient to have a strong feeling of warmth without a hot, burning sensation. For a typical application, this heat sensation should last for 20 to 30 minutes and may take a few minutes to start once the mud has been applied. Start timing from the point that the patient first feels warmth. The practitioner should use his or her own touch and sense of heat during the application.

Materials

- Peat poultice material—I use Healing Botanical's professional-use poultice formula containing peat pulp, peat extract, sulfur, wintergreen, and pine oil. Hot water should be added to this dry material 3 minutes before application, and the solution should be mixed so it becomes very slightly supersaturated. The point at which it just becomes shiny and has the consistency of mixed cake batter is perfect. In 1 to 3 minutes, it will be time to apply it to the area being treated. It will still be warm for application because hot water was used.
- Three large towels
- Small towel
- Facecloth
- Small blanket to cover hydrocollator
- Two small stainless-steel basins
- One small paper cup
- Hydrocollator (alternatively, hot water bottle can be used)

Procedure

This is a thermal peat pack meant to be applied for 30 minutes. The procedure is as follows:

1. Make a square layer of peat material about 0.25 inches thick, 2 inches bilaterally over the spine, and 6 to 8 inches long over the spine. If the spinal area is not being treated, the area to be treated is covered. The area of application should be as flat and level as possible.

BOX 45.2 Indications and Contraindications for Medicinal Peat Peloid

Indications

Acne
Arthritis pain
Back pain
Bursitis
Carpal tunnel syndrome
Eczema
Fibromyalgia
Fractures
Acute gouty toe
Chronic gout
Headaches
Hematomas
Hives
Lumbalgia
Molluscum contagiosum
Muscle tension
Orthopedic disorders
Osteoarthritis
Postoperative rehabilitation
Premenstrual syndrome
Prostatitis
Psoriasis
Rashes
Rheumatoid arthritis
Sciatica
Skin care
Sprains
Strains
Stress relief
Trauma

Contraindications

Open wounds
Pregnancy
Very thin, fragile skin
Heat-insensitive skin
Allergies to any of the peloid materials

2. Cover the peat directly with a single-layer warm, wet facecloth. So as to remember the borders of the peloid exactly, ridge the facecloth around the margins of the peat.
3. Border the wet facecloth–covered peloid with a rolled bath towel, making a quarter turn at the corners while folding the towel to match the margin of the peat.
4. Apply one layer of towel over the facecloth and peat material. Depending on the size and mass of the hydrocollator and its temperature, this layer may not be necessary.
5. Put a fresh hydrocollator pack directly over the towel. The hydrocollator should be heated at a gentle boil for 1 hour before use. Do not allow any exposed skin to come in contact with the hydrocollator.
6. Cover the hydrocollator pack with a towel or small blanket to insulate it and prevent heat loss.
7. Have a cup of cold water ready to pour on the wet facecloth–covered peloid if it gets too hot. In a good treatment, the peloid pack should get hot enough to require two to three dousings of water or more.

8. As soon as the patient says that the pack is getting too hot, lift up the hydrocollator pack and towel and pour the water directly over the facecloth–covered peloid until cool. Then replace the hydrocollator and coverings.
9. Never leave the patient unattended with the hydrocollator on the mud!
10. Treatment time is approximately 25 to 30 minutes.
11. To remove the peat from the skin after treatment, slide a small basin along the skin under the peat, scraping the peat into the bowl. Wipe the area with a full facecloth wetted with warm water in a gentle twisting motion back and forth to remove peat residue from the skin.
12. Cover the treated area after treatment to maintain warmth for 3 hours. The patient's clothing is fine, but a wool blanket would do a better job.

Partial-Immersion Medicinal Peat Bath

The indications for and contraindications to this procedure are given in Box 45.3.

Materials

- Deep-well basin; a tall plastic wastebasket size works well for the leg.
- Medicinal peat bath
- Water thermometer
- Small towel

Procedure

1. Fill basin to three-quarters full with 108°F to 114°F water.
2. Add peat to the bath.
3. Have the patient immerse the wrist, ankle, or elbow slowly into the water. Try to immerse the forearm and leg if treating the hand or foot.
4. Keep the body part immersed for 25 minutes.
5. After the treatment, cover the area with a wool sock or clothing and keep covered for 3 hours after treatment.
6. Peat material is often sent home with the patient to do home treatments.
7. Clean up the basin by washing with antimicrobial soap. Then disinfect with 10% bleach solution, and rinse after 10 minutes.

BOX 45.3 Indications and Contraindications of Partial-Immersion Medicinal Peat Bath

Indications
Arthritis pain
Bursitis
Carpal tunnel syndrome
Eczema
Fibromyalgia
Fractures
Acute gouty toe
Chronic gout
Hematomas
Orthopedic disorders
Osteoarthritis
Plantar fascitis
Postoperative rehabilitation
Psoriasis
Rashes
Rheumatoid arthritis
Skin care
Sprains
Strains
Tendinitis
Tenosynovitis
Trauma

Contraindications
Open wounds
Pregnancy
Heat-insensitive area

Combination Full Bath and Peat Pack

Materials

The same materials are needed as described for the two preceding applications.

Procedure

This treatment is used for many conditions. I particularly like it for treatment of vertebral disc injury or degenerative joint conditions. This procedure is also very good for injuries such as hamstring strain. It focuses the balneological effects in the area to which the peloid pack is applied. Performing the peat bath first and then applying the peat pack is the ultimate treatment for those who can tolerate the bath. After the patient steps out of the bath, he or she walks to the table and lies face-up for the regular traction sequence. After three tractions, it is appropriate to turn the patient face-down and apply the peat pack over the spine or area being treated.

ADVERSE REACTIONS

A study out of Russia evaluated peloid therapy in 128 women presenting with bacterial vaginosis and normal prolactin level and 58 women with bacterial vaginosis and concomitant hyperprolactinemia. Although peloid therapy was significantly effective in women suffering vaginosis and normal prolactin levels, the women with elevated prolactin levels clearly suffered worse clinical outcomes and an aggravation of their hormone dysfunction.[74]

SUMMARY

As a physician using balneotherapy for 15 years, I have achieved excellent results in a large percentage of patients who undergo peat therapy. This therapy often helps other therapies work better. Balneotherapy can be primary or adjunctive. The potential effect on the body should not be taken lightly.

I have observed excellent results for the following conditions:

- Arthritis
- Tenosynovitis
- Strains and sprains
- Discopathy
- Plantar fascitis
- Low back pain, including sciatica
- Scoliosis
- Fractures
- Gout
- Muscle pain
- Dermatologic conditions such as eczema

The combination of medical sophistication in diagnosis and application of various balneological methods provides an excellent tool for physicians to treat in a natural way to the great benefit of their patients.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Sukenik S, Flusser D, Abu-Shakra M. The role of spa therapy in various rheumatic diseases. *Rheum Dis Clin North Am*. 1999;25:883–897.
2. Matz H, Orion E, Wolf R. Balneotherapy in dermatology. *Dermatol Ther*. 2003;16:132–140.
3. Braverman DL, Ericksen JJ, Shah RV, et al. Interventions in chronic pain management. New frontiers in pain management: complementary techniques. *Arch Phys Med Rehabil*. 2003;84:S47–S48.
4. Dietrich J. Endocrinological changes after peat therapy. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
5. Tishler M, Yaron M, Brostovski Y. Effect of spa therapy in Tiberias on patients with ankylosing spondylitis. *Clin Rheumatol*. 1995;14:21–25.
6. Guillemin F, Constant F, Collin JF, et al. Short and long-term effect of spa therapy in chronic low back pain. *Br J Rheumatol*. 1994;33(2):148–151.
7. Ponikowska I, Przemyslaw A, Nowobilski KV, et al. The clinical principals of balneology and physical medicine. *Massage Ther J*. 2003;Winter:90–103.
8. Storojenko N, Tupikova V, Kibzun V, et al. *Treatment of Patients Affected by Burns with Hydrosulphur Mineral Water Irrigation at Health Resorts of Sochi (Russia). Presented at 2nd Symposium, Sulfur in Health Resort Medicine*. Bad Nenndorf: Germany; 1994.
9. Faizullin ZZ. *Rehabilitation of Patient with Chronic Persistent Hepatitis by means of Sulphide-Chloride-Sodium-Baths at the Sanatorium Stage. Presented at 2nd Symposium, Sulfur in Health Resort Medicine*. Bad Nenndorf: Germany; 1994.
10. Sukenik S, Buskila D, Neumann L, et al. *Sulphur Bath and Mud Pack Treatment for Rheumatoid Arthritis at the Dead Sea Area*. Beer-Sheva, Israel: Ben-Gurion University of the Negev; 1989.
11. Agishi Y, Ohtsuka Y, eds. *Recent Progress in Medical Balneology and Climatology*. Hokkaido, Japan: Hokkaido University; 1995.
12. Callies R, Kaiser G. Leukocyte evaluation of RA for efficacy of a peat cure. *Physiotherapy*. 1978;30:19–26.
13. Bellometti S, Cecchettin M, Galzigna L. Mud pack therapy in osteoarthrosis: changes in serum levels of chondrocyte markers. *Clin Chim Acta*. 1997;268:101–106.
14. Agishi Y, Ohtsuka Y, Watanabe I, et al. *Effects of Therapeutic Elements on Physiological Functions in Man and Balneotherapy Recent Progress in Medical Balneology and Climatology*. Hokkaido, Japan: Hokkaido University; 1995.
15. Korepanov AM, Zhukova MA, Chernysheva NG, et al. SMC-electrophoresis of peat mud in the treatment of patients with duodenal ulcer in outpatient care unit. *Vopr Kurotol Fizioter Lech Fiz Kult*. 2003;5:22–25.
16. Konrad K, Tatrai T, Hunka A, et al. Controlled trial of balneotherapy in treatment of low back pain. *Ann Rheum Dis*. 1992;51:820–822.
17. Praetzel HG, Aigner UM, Wemert D, et al. Therapeutic effects of sulfur-peat baths on patients with rheumatic muscle pain. *Phys Rehab Kur Med*. 1992;2:92–97.
18. Scheffel KZ, Praetzel HG. Analgesic effects of humic acid bath. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
19. Bender T, Karagulle Z, Balint P, et al. Hydrotherapy, balneotherapy, and spa treatment in pain management. *Rheumatol Int Clini Exp Invest*. 2004. http://springerlink.metapress.com/media/ H82CAPLN2KWW4UP J4P/ Contributions/7/3. Accessed 25 Aug 2019.
20. Magyarosy KL, Resch KH, Krause W, et al. Electromyographic research for the efficacy of function of peat packs on musculature of the back. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
21. Cutolo M, Seriolo B, Craviotto C, et al. Circadian rhythms in RA. *Ann Rheum Dis*. 2003;62:593–596.
22. Pollmann L, Hildebrandt G. *Temperature Changes of the Oral Mucosa Induced by Footbaths: Preventive Effect Against Common Cold Infections*. Marburg, Germany: Institute fur Arbeitsphysiologie und Rehabilitationsforschung der Universitat Marburg; 1993.
23. Gauthier A, Davenne D, Martin A, et al. Time of day effects on isometric and isokinetic torque developed during elbow flexion in humans. *Eur J Appl Physiol*. 2001;84:249–252.
24. Levitskii EF, Abdoulkina NG, Zaitsev AA, et al. The optimization of the duration of the sanitarium-health resort treatment of patients with neurological manifestations of spinal osteochondrosis (I). *Vopr Kurortol Fizioter Lech Fiz Kult*. 1996;(5):26–28.
25. Kasianova IM. Hydrotherapy in the treatment of obesity. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994:131–133.
26. Bellometti S, Cecchettin M, Lalli L, et al. Mud pack treatment increases serum antioxidant defenses in osteoarthrosic patients. *Biomed Pharmacother*. 1996;50:37.
27. Praetzel HG, Aigner UM, Weinert D, et al. *The Analgesic Effects of Sulfur peat Baths for Non-Articular Rheumatic Afflictions. Presented at 2nd Symposium, Sulfur in Health Resort Medicine*. Bad Nenndorf: Germany; 1994.
28. Beer M, Pratzel HG. Indications for gynecological balneotherapy. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
29. Kuhn G, Rohwer J, Buhring M. *Balneotherapy for Progressive Systemic Sclerosis. Presented at 2nd Symposium, Sulfur in Health Resort Medicine*. Bad Nenndorf: Germany; 1994.
30. Sukenik S, Neumann L, Buskila D, et al. Dead Sea bath salts for the treatment of rheumatoid arthritis. *Clin Exp Rheumatol*. 1990;8:353–357.
31. Kotwica S, Split W, Róg-Malinowski M, et al. Spa treatment of sciatic pains at Swieradow. *Neurol Neurochir Pol*. 1976;10:715–722.
32. Siderov VD, Mamiliaeva DR. The current aspects of pelotherapy of patients with rheumatoid arthritis. *Arthritis Rheum*. 1994;37:1132–1137.
33. Beer AM, Grozeva A, Sagorchev P, et al. Comparative study of the thermal properties of mud and peat solutions applied in clinical practice. *Biomed Tech (Berl)*. 2003;48:301–305.
34. Odabasi E, Gul H, Macit E, et al. Lipophilic components of different therapeutic mud species. *J Altern Complement Med*. 2007;13:1115–1118.
35. Konrad K, Tatral T, Vereckel E, et al. Controlled trial of balneotherapy in treatment of low back pain. *Ann Rheum Dis*. 1992;51:820–822.
36. Praetzel HG. Balneologically activated skin functions and their clinical evidence. *J Jpn Assoc Phys Med Balneol Climatol*. 1993;57:11–13.
37. Falch B. Balneophototherapy for atopic dermatitis: new scientific findings in dermatology. *Schweiz Z Ganzheits Medizin*. 2002;14:60–63.
38. Beer AM, Junginger HE, Lukanov J, et al. Evaluation of the permeation of peat substances through human skin in vitro. *Int J Pharm*. 2003;253:169–175.
39. Beer AM, Sagorchev P, Lukanov J. Isolation of biologically active fractions from the water soluble components of fulvic and ulmic acids from peat. *Phytomedicine*. 2002;9:659–666.
40. Kubota K, Kurabayashi H, Tamura K, et al. A transient rise in plasma B-endorphin after a traditional 47°C hot-spring bath in Kusatsu-Spa, Japan. *Life Sci*. 1992;51:1877–1880.
41. Goecke C. Efficacy of peat therapy: health resort medicine. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
42. Maglara AA, Vasilaki A, Jackson MJ, et al. Damage to developing mouse skeletal muscle myotubes in culture: protective effect of heat shock proteins. *J Physiol*. 2003;253:837–846.
43. Solovieva VP, Sotnikova EP, Naumova GV, et al. Biologically active peat preparations and their possible applications in medicine. In: *International Peat Society. Proceedings of the 6th International Peat Congress.* Duluth, MN: International Peat Society; 1980.
44. Tolomio C, Ceschi-Berrini C, Moschin E, et al. Colonization by diatoms and antirheumatic activity of thermal mud. *Cell Biochem Funct*. 1999;17:29–33.
45. Bellometti S, Poletto M, Gregotti C, et al. Mud bath therapy influences nitric oxide, myeloperoxidase and glutathione peroxidase serum levels in arthritic patients. *Int J Clin Pharmacol Res*. 2000;20:69–80.
46. Britschka ZM, Teodoro WR, Velosa AP, et al. The efficacy of Brazilian black mud treatment in chronic experimental arthritis. *Rheumatol Int*. 2007;28:39–45.
47. Zellner M, Hergovics N, Roth E, et al. Human monocyte stimulation by experimental whole body hyperthermia. *Wien Klin Wochenschr*. 2002;114:102–107.

48. Rhind SG, Gannon GA, Shek PN, et al. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. *J Appl Physiol*. 1999;87:1178–1185.
49. Atanackovic D, Pollock K, Corovic A, et al. Effects of 41.8°C whole body hyperthermia (WBH) on T cell homing molecules in patients with advanced malignancies (abstract 3475). *Proc Am Soc Clin Oncol*. 2003;22:864.
50. Carroll NM, Elaraj DM, Puhlmann M, et al. Alterations in tumor necrosis factor-induced endothelial cell procoagulant activity by hyperthermia. *Int J Cancer*. 2004;111:457–462.
51. Haveman J, Van Der Zee J, Wondergem J, et al. Effects of hyperthermia on the peripheral nervous system: a review. *Int J Hyperthermia*. 2004;20:371–391.
52. Bellomiti S, Richelmi P, Tassoni T, et al. Production of matrix metalloproteinases and their inhibitors in osteoarthritic patients undergoing mud bath therapy. *Int J Clin Pharm Res*. 2005;25:77–94.
53. Pizzoferrato A, Garzia I, Cenni E, et al. Beta-endorphin and stress hormones in patients affected by osteoarthritis undergoing thermal mud therapy. *Minerva Med*. 2000;91:239–245.
54. Ortega E, Gálvez I, Hinchado MD, et al. Anti-inflammatory effect as a mechanism of effectiveness underlying the clinical benefits of pelotherapy in osteoarthritis patients: regulation of the altered inflammatory and stress feedback response. *Int J Biometeorol*. 2017;61(10):1777–1785. PMID: 28456879.
55. Given PH, Spackman W, Imbalzano JR, et al. Physiochemical characteristics and levels of microbial activity in some Florida peat swamps. *Int J Coal Geol*. 1983;3:77–99.
56. Kasapoğlu Aksoy M, Altan L, Eröksüz R, Metin Ökmen B. The efficacy of peloid therapy in management of hand osteoarthritis: a pilot study. *Int J Biometeorol*. 2017;61(12):2149–2152. PMID: 28779304.
57. Ökmen BM, Eröksüz R, Altan L, Aksoy MK. Efficacy of peloid therapy in patients with chronic lateral epicondylitis: a randomized, controlled, single blind study. *Int J Biometeorol*. 2017;61(11):1965–1972. PMID: 28620677.
58. Fioravanti A, Bacaro G, Giannitti C, et al. One-year follow-up of mud-bath therapy in patients with bilateral knee osteoarthritis: a randomized, single-blind controlled trial. *Int J Biometeorol*. 2015;59(9):1333–1343. PMID: 25516113.
59. Cozzi F, Podswiadek M, Cardinale G, et al. Mud-bath treatment in spondylitis associated with inflammatory bowel disease—a pilot randomised clinical trial. *Joint Bone Spine*. 2007;74:436–439.
60. Olivera AP, Schirmer MH, Olivera VM. Treatment of hematomas with peat used in balneotherapy. *J Med Esthetics*. 1997;3:1–3.
61. Maeda T, Kudo Y, Horiuchi T, Makino N. Clinical and anti-aging effect of mud-bathing therapy for patients with fibromyalgia. *Mol Cell Biochem*. 2018;444(1-2):87–92. PMID: 29214470.
62. Yoshihiro U, Yoshiharu Y, Caldwell WM, et al. A microcomputer-based data acquisition system for ECG, body and ambient temperatures measurement during bathing. *International ISA Biomed Sci Instrum Symposium*. 2000;36.
63. Boldyreva OA. The influence of balneo- and peloid therapy on the characteristics of the hormonal regulation in the women presenting with bacterial vaginosis. *Vopr Kurortol Fizioter Lech Fiz Kult*. 2015;92(3):22–26. PMID: 26285330.
64. Elkayam O, Wigler I, Tishler M, et al. Effect of spa therapy in Tiberias on patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol*. 1991;18:1799–1803.
65. Artmann C, Pratzel HG. *Influence on the Immune System by Sulfur Water Bath. Presented at Symposium, Sulfur in Health Resort Medicine*. Bad Nenndorf: Germany; 1994.
66. Peter ACR. Proteins of rheumatic afflictions. *Physiotherapy*. 1987;39:331–335.
67. Ohtsuka Y, Yabunaka N, Watanabe I, et al. Platelet antioxidative defense system is modified by balneotherapy and bathing temperature. In: *New Frontiers in Health Resort Medicine, No. 059-04*. Hokkaido, Japan: Noboribetsu Branch Hospital, Hokkaido University School of Medicine; 1996.
68. Ortyl W, Turowski G, Zubel M. The influence of balneotherapy on T-cell populations and direct lymphocytotoxicity in patient with vascular disorders of lower limbs. *Mater Med Pol*. 1991;23(3):175–180.
69. Bellometti S, Galzigna L. Serum levels of a prostaglandin and a leukotriene after thermal mud pack therapy. *J Invest Med*. 1998;46:140–145.
70. Weislaw O, Turowski G, Turowski ZM. The influence of balneotherapy on T-cell populations and direct lymphocytotoxicity in patients with vascular disorders of lower limbs. In: *Health Resort Medicine: 32nd World Congress of the International Society of Medical Hydrology (and Climatology)*. Bad Worishofen: Germany; 1994.
71. Praetzel HG. Forty Years of Medical Balneology and Climatology: Director Medical Institute of Balneology and Climatology. Munich, Germany: Ludwig Maxmillions University;1990.
72. Poensin D, Carpenter PH, Fechoz C, et al. Effects of mud pack treatment on skin microcirculation. *Joint Bone Spine*. 2003;70:367–370.
73. Schmidt KL. Sulfur water. In: *Compendium of Balneology and Cure Medicine*. Darmstadt: Steinkopff; 1989.
74. Basili S, Martini F, Ferroni P, et al. Effects of mud-pack treatment on plasma cytokine and soluble adhesion molecule levels in healthy volunteers. *Clin Chim Acta*. 2001;314:209–214.

46

Rotation Diet:
A Diagnostic and Therapeutic Tool

Sally J. Rockwell, PhD, CCN

OUTLINE

INTRODUCTION

Food allergy and/or intolerance is a common component of many chronic diseases (see Chapter 14). However, it is not readily recognized as problematic. Conventional laboratory diagnosis is not geared toward testing food allergies, and most allergists dismiss the concept of delayed hypersensitivity reactions to foods (see Chapter 14). A time-honored, effective approach to both the diagnosis and treatment of food sensitivities is an elimination–rotation diet. This approach has the advantage of being low cost, and the techniques must be followed assiduously to ensure clinical efficacy. Box 46.1 lists the typical indications for an elimination diet.

THE ELIMINATION DIET

The first step in the elimination diet is to remove from the diet (1) all of the most common allergenic foods—that is, wheat and other glutinous grains, dairy products, eggs, corn, soy and tofu, peanuts, citrus fruits, yeast, and refined sugars—and (2) other often problematic substances, such as highly processed foods, chemicals, additives, preservatives, artificial colorings, flavorings, caffeine (coffee, tea, cola drinks, chocolate), and alcohol.

The object is to avoid the intake of all suspect foods and substances for at least 5 days, or long enough to clear all traces of those foods from the digestive tract. The omitted foods are then reintroduced into the diet one at a time. Keeping an accurate food and symptom diary enables the offending foods to be identified and eliminated; depending on the severity of the initial test response, the foods can be carefully reintroduced into the diet within 3 to 6 months.

Although some authorities recommend eliminating only one food at a time, clinicians in this area have found that multiple allergies are the rule, not the exception. Eliminating only one allergen may not clear symptoms well enough to notice an improvement. The advantages of eliminating all major allergens in the beginning are rapid clearing, minimal adverse reactions, and accurate test results. Also, when symptoms clear rapidly, the patient becomes inspired and eager to continue with the testing.

As Dr. Doris Rapp[1] explained:

> *If there are several tacks in the bottom of a shoe, the whole foot hurts; removing only one tack will make little or no difference. But remove all the tacks, let the foot heal, then add back one tack at a time and the source of pain is isolated and easily identified. If only one tack (or food) is removed, no significant difference will be noted.*

Variations of the elimination diet range from the most stringent plan, involving elimination of all the major allergens, refined foods, and toxic substances, to a more lenient approach, which eliminates only wheat, dairy, and refined sugars. Consideration of the patient's lifestyle, age, weight, general health, food preferences, attitude, and family system determines the approach. For children and pregnant or lactating women, the amount of calories or carbohydrates should not be restricted; one should simply omit the major allergens.

Variations of the Elimination Diet

As can be seen in Table 46.1, all elimination and/or challenge diets are variations of the following four "Rs" procedure:

1. Remove suspect foods for at least 5 days.
2. Reintroduce and/or test by challenge ingestion.
3. Record reactions.
4. Rotate foods, avoiding those shown to cause adverse reactions.

Water Fasting

Water fasting should be implemented only under the close guidance of an experienced health professional. A lifetime of accumulated toxins may be released in a short time, creating unwarranted discomfort and complications that may overwhelm the patient and interfere with testing (see Chapter 37 for a complete description of fasting methodologies).

The Dilute Juice Fast

Less severe than water fasting, and more acceptable to patients, is the dilute juice fast. Diluted juices provide a modest amount of calories, stabilizes blood glucose levels, and decreases the adverse detoxification–withdrawal reactions that may occur during the elimination of all common allergens. The basic procedure is to use three parts of distilled or

BOX 46.1 Indications for the Use of the Elimination Diet

- Documented or suspected food allergies and/or intolerances
- Chronic complaints that have not subsided with treatment
- Patients who have symptoms and whose tests results are all "normal"
- Patients in whom mental health problems are suspected because of lack of significant progress
- Children and teens who are labeled as having attention deficit disorder or as being hyperactive, those who are autistic, and those who have behavior problems

TABLE 46.1 Elimination Diet Variants

Type	Protocol
Water fast	Water-only fast for 5 days; reintroduction of foods
Dilute juice fast	Diluted fruit juice fast for 5 days; reintroduction of omitted foods
Fruit, melon, and vegetable plan	Only fruits, melons, and vegetables for 5 days; reintroduction of omitted foods
Caveman (caveperson) plan	Proteins, nuts, seeds, legumes, fruits, and vegetables for 5 days; reintroduction of omitted foods Only natural, unprocessed foods are eaten

mineral water to one part of fresh, sugar-free juice. The diluted juices are sipped throughout the day. Ideally, a different juice is used each day: celery, carrot, papaya, cranberry, berry, apple, pineapple, and other fruits or vegetables that have not been consumed on a daily basis. Citrus fruits should be avoided. Low-allergen protein supplements, such as Ultra Clear, MEDIPRO Protein Powder, pure free-form amino acids, N Foods, and Vivonex, may be added after the third day. In addition, liberal amounts of pure water should be consumed throughout the day to dilute and flush out toxic substances.

Note: This approach works for most patients with *Candida* yeast infections because the juice is so dilute that it rarely creates a problem. However, commercially prepared juices (especially tomato and citrus juice) often contain molds, which can be problematic.

Fruit and/or Vegetable Plan

In the fruit and/or vegetable plan, the patient consumes unlimited amounts of clean, fresh fruit and melons and raw, steamed, or baked vegetables throughout the day. If *Candida* overgrowth is severe, or if weight loss is desired, vegetables should be emphasized and fruits limited.

The Caveman (Caveperson) Plan

The caveperson plan consists of the following basic foods that the caveperson consumed, after the major allergenic foods have been eliminated: vegetables, fruits, berries, honey, nuts, seeds, beans, peas, sprouts, roots, gourds, poultry, fish, seafood, and wild game.

Procedure

Beginning the Elimination Diet

An ideal elimination diet begins as follows:

- 2 days of diluted juices
- Fruits and vegetables for the next 2 days
- The caveperson diet for 2 days or until symptoms clear

Any one, or any combination, of the previously described elimination plans will clear the patient of allergenic substances. If after 5 days the patient's symptoms have not diminished (or disappeared), continue the diet for another 5 days. If symptoms are not clear in 10 days, begin to rotate foods because the patient may be reacting to a food or substance he or she is consuming every day. (Unsuspected environmental allergens may be contributing to the symptoms also; see "Helpful Hints and Suggestions" later in this chapter.)

Testing by Challenge Ingestion

One suspect food is introduced every other day. The object is to give the patient sufficient calories, build a reliable list of safe foods, and delay unpleasant reactions for as long as possible. The challenge should begin with rarely ingested foods that are least likely to cause adverse reactions. Foods that are known to cause severe reactions should not be tested with challenge.

Suggested testing sequence. Introduce foods back into the diet in the following order whenever possible: vegetables, fruits, melons, beans, nuts and seeds, yeasts, dairy products, and finally, grains. When dairy is added, goat products should be tested first: plain yogurt, cheeses, and then milk. Follow the same process with cow's milk. Grains are best tested in the following order (gluten-containing grains are in italics): quinoa, amaranth, buckwheat, wild rice, brown rice, millet, *barley, spelt,* kamut, teff, oats, *rye,* corn, and lastly, *wheat*. Dietary supplements should also be tested one at a time.

Food and symptom diary. The patient records, chronologically in a notebook, all foods, liquids, supplements, moods, symptoms, and reactions. The foods eaten throughout the day should be noted in either pencil or blue or black ink. Symptoms are highlighted or circled in colored ink so that those adverse reactions stand out clearly. In 2 to 3 weeks, a repetitive pattern of symptoms will be clearly visible, allowing for identification and elimination of the problematic foods or food combinations that cause symptoms.

In the beginning, a patient may not be able to describe in exact words how he or she feels, so a numbering system may be useful for noting general moods throughout the day. Numbers go from 1 (poor) to 10 (excellent); feeling sort of medium, neither poor nor excellent, would be recorded as a 5. In general, depending on the patient's health and motivation, foods causing a mild reaction should be avoided for 3 months, and those causing a severe reaction are best avoided for at least 6 months before being reintroduced.

Testing children. Play a game with children to make testing fun. Have them draw a picture of how they feel, or write their names before and after testing a substance. Note all before-and-after differences in the drawings or writings.

THE DIVERSIFIED ROTATION DIET

The basic concept of the diversified rotation diet is to achieve the following:

- Eliminate all major allergenic substances.
- Eat the remaining foods once every 4 days.
- Allow 2 to 4 days between food families.

Rotation can be simplified by using a template—the "master chart"—with foods correctly arranged according to their botanical family classifications. The spacing of the foods and food families is organized into four columns, one for each day, so the patient simply chooses foods from the appropriate column each day. A color-coding system further simplifies the process of choosing the correct foods.

See Appendix 11 for an easy-to-follow template. The chart provides 4 days between specific foods and 2 days between food families. A second chart (Master Chart II) may be needed for severely sensitive individuals. It provides 4 days between specific foods and 4 days between food families. If necessary, it can be easily adapted to a 7-day rotation.

Using the Master Charts

The clinician uses a pencil to cross out the major allergens, plus any known or suspected problem foods, in each of the four columns. The patient may eat the remaining foods from the appropriate column throughout the day. The foods that are not crossed out are allowed in the diet.

Arrows on the chart (↔) indicate a food that is essentially the only food of a family and that is not cross-reactive with other foods. Therefore it is not restricted to any one particular day and can be moved to another column if additional food choices are needed.

Patients who will be rotating foods for any length of time will welcome the benefits of using the color-coding system. A color is assigned to each day. Each column represents a day: day 1, green, is column 1; day 2, yellow, column 2; day 3, blue, column 3; and day 4, red, column 4. After day 4, the patient returns to day 1 and repeats the process. Food containers are labeled and color-coded to coordinate with the color of the day; for example, green labels, twist-ties, or rubber bands are placed on all containers of foods for day 1. The color-coding system enables the entire family to see at a glance which foods the patient is permitted to eat on each day.

Modifications for Vegans

The master charts can be used for vegans. Simply cross out all foods of animal origin. Legumes (beans and peas) are generally an important part of the vegan diet; thus all legumes should be tested, one at a time, beginning with the least allergenic, rarely eaten beans and proceeding to soy and tofu, with peanuts tested last. Depending on the allergic response to legumes, the patient may be able to tolerate legumes daily, as long as he or she eats a different legume each day. In other words, although the 4-days-between-foods rule continues to be honored, the 1-day-between-food-families rule may be ignored for less-sensitive patients.

Food Preparation

For testing purposes, fresh, organic foods should be used whenever possible. Otherwise, frozen or dehydrated foods are a better choice than canned goods. Most vegetables are best eaten raw, steamed, or baked. Fish, poultry, and meat are best poached, steamed, sautéed, baked, or simmered in a slow cooker (e.g., crock-pot). Soups and stews are fine. Use only sea salt for seasoning; all spices and flavorings must be avoided unless they are individually tested.

Blood glucose levels may need to be stabilized with small, frequent meals throughout the day. Have the patient prepare four mixtures of "trail mix"—nuts and dried fruits—one for each day (refer to the master chart for specific food choices). A supply of healthy snacks must be maintained everywhere: home, school, office, and automobile.

Helpful Hints and Suggestions

Control of Withdrawal Symptoms and Allergenic Reactions

Vitamin C buffered with calcium, potassium, and/or magnesium is suggested as the daily source of vitamin C for allergenic individuals. These substances help balance an acidic body pH and are valuable for neutralizing unpleasant allergic reactions. Stabilization of pH eases the symptoms of withdrawal from allergenic foods and the cravings that occur when the patient is breaking an addiction, whether it is to sugar, wheat, coffee, cigarettes, alcohol, or a drug.

For daily supplementation, begin with 1/8 teaspoon of buffered vitamin C powder (or one 500 mg capsule) in 1/2 cup of water three times a day (between meals and at bedtime). Gradually increase the dosage by an additional 1/8 teaspoon (1 capsule) per dose every 2 days until the patient reaches bowel tolerance—loose stool, gas, or diarrhea—at which point the dosage is reduced. As the patient improves, the vitamin C requirement will likely lessen, and the dosage can be reduced again.

Note: Buffered vitamin C should not be taken with meals because it may neutralize stomach acid, which is commonly deficient in patients with food allergy and/or intolerance (see Chapter 14).

For temporary relief of symptoms or to neutralize an adverse reaction, the patient should mix and drink 1/2 teaspoon of buffered vitamin C in 1/2 cup of water or dilute juice. Available commercial products include Klaire Labs Bicarbs (see http://www.klairelabs.com), Cardiovascular Lab's Tri-Salts (see http://www.cardiovascularlabs.com), and Alka Seltzer gold label (not the blue label, see http://www.alkaseltzer.com); if none of these products is available, 1/2 teaspoon of baking soda in 1/2 cup of water can be useful.

Environmental Allergies

If the patient does not respond to the diet in a timely fashion, and all other possibilities have been ruled out, the clinician should suspect environmental and/or chemical sensitivities. The first step in this instance is to eliminate the use of all scented toiletries, room sprays, and cleaning solutions in the home and workplace.

Ensuring Adequate Protein Intake

The most allergenic foods tend to be those highest in protein; thus care must be taken to ensure that the patient is consuming adequate amounts of protein. Patients with multiple food allergies may need to be referred to an experienced nutritionist to develop a balanced diet.

Patience

The clinician must be especially supportive and patient with individuals who have received only long-term conventional treatment. You may be the first physician to discuss the rewards of listening to their bodies and explain how symptoms are the body's way of communicating. Learning to pay attention to what they eat and drink and the possible adverse symptoms is a vital part of getting well again.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCE

1. Rapp D. *The Impossible Child: In School, At Home*. Buffalo, NY: Practical Allergy Research Foundation; 1986.

47

Soft Tissue Manipulation: Diagnostic and Therapeutic Potential

Terry M. Elder, DC, Leon Chaitow, ND, DO,* and *Tim Irving*, DC, MS, LMT*

OUTLINE

INTRODUCTION

The term *soft tissue* is a ubiquitous and, at times, vague term. Every profession that deals with the mobility, stability, and dynamic function of the neuromusculoskeletal system must embrace diagnostic and therapeutic soft tissue procedures. Medical and therapeutic systems such as those used by naturopaths, chiropractors, osteopaths, allopaths, physical therapists, massage therapists, and body-centered psychotherapists all have their own procedures for identifying and treating a variety of soft tissue dysfunction. Despite their differences, terms used, or the theories advocated by each profession, they all agree to some degree that soft tissue dysfunction can cause and/or indicate disease, injury, aberrant reflex activity, and aberrant movement patterns, the results of which can have minor or profound effects on the health of the individual. In addition, most professionals who diagnose and treat soft tissue dysfunction seem to refer to a common phenomenon: distinct, palpable, usually sensitive areas of soft tissue aberrations or lesions that are either directly or reflexively related to local or organ dysfunction and/or disease.[1,2]

The thoughts, ideas, and theories that are foundational to physical medicine are evolving; so, too, are the thoughts, ideas, and theories foundational to soft tissue diagnostics and therapeutics. One clinically relevant and interesting note is that many systems of health care delivery are moving to more encompassing and holistic ideas about the interrelation of many cells, tissues, organs, and systems to obtain and maintain optimal health and wellness. Functional or lifestyle medicine practitioners look at the various systems of the body in a hierarchical fashion. Treating systems "upstream" or higher up on a hierarchical list of systems allows the practitioner to optimize the health of the individual without chasing down symptoms.[3] Movement therapists and doctors who help improve and stabilize movement are looking at movement patterns instead of single or even dual-planar movement. The idea that movement only transmits from muscle origin to insertion is long gone, clinically incomplete, and inferior to the recent, more functional theories.[4–6] Soft tissue diagnostic and therapeutic procedures are currently evolving; this evolution is occurring as a result of clinical treatment and trials and other empirical methods.

Although the working definition of soft tissue has always included muscles, ligaments, tendons, fascia, and more, each individual tissue was looked at and often treated as a separate entity, not as a functional unit. There has been a recent push to look at functional groups of soft tissues. Ideas like myofascial "chains," "trains," and units are taking the soft tissue world by storm and proving to be clinically superior to some of the incomplete, older, more reductionist ideas.[7–9] Tension relationships are now ideas that permeate soft tissue diagnosis and therapies. Muscle coordination and perception are looked at as functions of these relationships rather than the work of a group of muscles and their respective myoneural units.[7–11]

Soft tissue therapy may be used to accomplish the following:

- Normalize soft tissue dysfunction in preparation for joint manipulation[12–14]
- Restore postural or functional integrity; this has been one of the primary uses of soft tissue therapies for ages, and now many are thinking about this more in terms of restoring healthy tension relationships throughout all of the integrated soft tissue structures of the body[7–9,15]
- Function as part of a comprehensive approach to health care in which tension relationships and subsequent reflex activity, manifested in soft tissues, are used diagnostically and therapeutically[16]

Of final note, professions that classically have not viewed soft tissue as the primary avenue for their treatment benefits have been evolving their paradigms. Acupuncturists specifically have been viewing soft tissue, more specifically fascia, as part of their diagnostic and treatment foundation. Various authors in the acupuncture world have noted that acupuncture points and connective tissue and soft tissue planes have a very intimate relationship and, at times, are indistinguishable.[17–19]

*Previous edition contributor

HEALTH IMPLICATIONS OF SOFT TISSUE MANIPULATION: THE BIG PICTURE

For every therapeutic intervention, we must first determine how best to use the technique and/or intervention. For soft tissue manipulation, there are localized responses that will be addressed later in this chapter; this section is devoted to how soft tissue manipulation affects overall health.

Immune System

Clinically, many who practice soft tissue manipulation have noted various immune responses from patients and clients. The evidence for these effects has been elucidated, mostly through studies of massage therapy. Because the immune system receives messages and is controlled by the nervous and endocrine systems, the effects of soft tissue manipulation regarding the immune system are neuroendocrine in nature.[20]

Emotional

Soft tissue manipulation has been shown to help the emotional status of individuals experiencing depression, eating disorders, excessive anger, rage, rejection, and fear. Using soft tissue manipulation as an adjunct therapy when treating individuals with emotional distress can have widespread systemic effects that may prove to be necessary.[21–26]

Endocrine

Inappropriate amounts of hormones will cause a breakdown in this important system of control. Most notable, especially with regard to soft tissue manipulation, is excess cortisol. Soft tissue manipulation has been found to decrease inappropriately elevated levels of cortisol. The effects of chronically high cortisol levels occur at the hypothalamic–pituitary–axis level and can disrupt and create aberrations in neuroendocrine function. In addition, soft tissue manipulation has also been shown to raise low levels of dopamine and serotonin; this effect has implications in treating addictions, eating disorders, depression, and more.[27–31]

What this author is referring to in this subsection is the effect that soft tissue manipulation has on our ability to maintain homeostasis or homeodynamics after the introduction of a stressful stimulus. According to Selye and proponents of the General Adaptation Syndrome line of thought, our physical, biochemical, and emotional beings can withstand a certain amount of stress.[32] Everyone has a different threshold, and soft tissue manipulation allows the practitioner the opportunity to remove layers of stress so that we can adapt and thrive.

DEFINITION: FUNCTIONAL SOFT TISSUE

As noted in the introduction, thoughts, ideas, and theories surrounding soft tissue diagnosis and manipulation are evolving. The phrase "functional soft tissue" has been used by this author and others. The idea that muscles, bones, tendons, fascia, and ligaments are separate entities is an idea that, although true in a histological sense, is not the whole truth in a functional sense. A more clinically useful idea is that all of these structures transmit tension. Some of them contract, others resist tension, others give way to tension; the bottom line is they all respond and transmit tension. What many consider the apex of the soft tissue evolution is that soft tissue pathologies exist when the transmission of this tension is altered. As a result, various authors have developed ideas that link all connective and soft tissues together functionally. Myers developed the "Anatomy Trains" idea, and Stecco developed ideas that link soft tissues together functionally through fascial motor units, centers of coordination, centers of perception, and centers of fusion. Ida Rolf was on the cutting edge of these ideas and looked at the physical body as a transmitter of tension.[7–9,15]

Taking the idea of functional soft tissues one step further, the fitness and performance professions are evolving closely with the clinical soft tissue manipulation professions to develop very integrative and useful movement therapies. These movement therapies bridge the gap between more passive soft tissue manipulative techniques and exercise. Many effective soft tissue manipulative techniques include active movement and mobilization on the patient's and/or client's part. These therapeutic procedures, combined with more passive soft tissue interventions, are proving to be more effective than passive techniques alone.[33]

SOFT TISSUE PATHOLOGIES

Many of the focal soft tissue lesions and dysfunctions listed can be classified as myofascial points; some are more descriptive, whereas others are the terminology used when describing a diagnosis. This author feels it necessary to note that this is not a comprehensive list of potential soft tissue points and/or lesions, but that most, if not all, soft tissue lesions will fall into these categories:

- Tender points
- Trigger points
- Fascial densifications
- Strains/sprains
- Fibrosis
- Adhesions
- Chapman's reflex points

Researchers and clinicians have used different terminology to describe similar phenomena, resulting in confusion of what is essentially an uncomplicated pattern. In the musculature and connective tissues of the body, often in the regions of the origins and insertions of the muscles, soft tissue points are often commonly found at motor points and in the muscle belly. The commonality, despite differing opinions on diagnostic criteria and the manner in which they are assessed, is palpable, sensitive areas of altered structure resulting from injury, anoxia, irritation, stress, aberrant reflex activity, and/or aberrant movement patterns. Another commonality to these points is their size, which ranges from 0.5 to 1.0 cm across, and their feel, which is described either as harder or firmer than surrounding normal tissue or as having an "edematous," "boggy," "fibrous," or "stringy" feel.[34]

It is often noted that these localized areas of altered structure and function occur in bands of stressed fibers, both fascial and muscular. In all cases, such localized areas are, to a greater or lesser degree, sensitive or tender out of proportion to the amount of pressure exerted.[35] All these points are potential "trigger points," but only those that, upon pressure, are noted to refer pain or other symptoms to a distant (target) area and that are recognizable as "familiar" to the individual are classified as such points.[36,37]

Methods of Identification

Despite all of the advances in imaging, the most widely used clinical procedure to identify soft tissue pathology is a combination of movement, special (orthopedic) tests, and skilled palpation. The diagnostic use of movement patterns will be discussed in the next section; they, too, can be used to locate focal soft tissue lesions and points.[16,38–40]

The definitions of the previously mentioned soft tissue pathologies can be and are debated. Often, clinicians name a focal soft tissue lesion a "trigger point" when many more of us would call it a "tender point." Likewise, fascial densifications can be tender points, trigger-point adhesions, and/or fibrosis. Then there are reflex points that can elucidate functional and pathological problems in and around the viscera; Chapman's points are examples of such points.

There are a variety of methods by which soft tissue changes may be located through palpation. They include the following:

- Traditional massage methods—mainly palpatory awareness during various massage strokes like effleurage, petrissage, tapotement, and vibration.
- Specific palpation techniques—such as those developed by massage, naturopathic, chiropractic, physical therapy, and osteopathic schools. The common thread is the development of fine motor skills and palpatory awareness, the foundation of which is a solid knowledge of structural and functional anatomy.
- Neuromuscular technique—a method of combined assessment and therapy developed in the 1930s by Stanley Lief, an American-trained naturopathic and chiropractic physician working in Europe.
- Skin distraction, pinching, and gliding techniques

All of these, as well as other methods of palpation, may be used to identify areas of local soft tissue dysfunction that may be either sources or results of reflex activity or other local adaptive responses. All of these techniques are used to assess and notice areas of tone, texture, and temperature abnormalities as well as developmental and structural asymmetry.

The analysis of the available information present in localized areas of the soft tissues requires consideration of a variety of classifications and systems. It is necessary to examine some of the systems that have described the same tissue changes in different ways, to compare the similarities and differences in the descriptions of points (discrete, usually sensitive areas of altered structure and function in the soft tissue) and the diagnostic and therapeutic significance ascribed to them.

Aberrant Movement Patterns

As noted in the introduction, soft tissue diagnostics and therapeutics are evolving into a more functional paradigm; with this evolution comes a journey into the idea that movement patterns will help clinicians elucidate soft tissue dysfunction and focal lesions.[4–9,11,36]

Methods of Identification

The simplest description of detecting and diagnosing movement patterns is that they are performed through observation and patient movements (active and passive). All of the techniques for this type of assessment have this in common; which movements and how they should be performed are debatable.

Simple, single planar movement patterns have been assessed by many.[6,11] These are often easier to assess, but their relevance has been questioned over the past 10 years. Multiplanar movement patterns have been purportedly better, more accurate, and more clinically relevant in the assessment of soft tissue and other neuromusculoskeletal structures.[4–9,36] There is no definitive proof as to which strategy and which motions are the most clinically relevant. Clinicians have begun to use patterns described as "primitive" by Gray Cook and Kyle Kiesel.[4,5] The aforementioned clinicians and authors designed a succinct and concise assessment of seven movements. These movements range from deep squats to toe touches, lunges, push-ups, and active straight leg raises. The assessment of these motions has been given a point system: 0 means there is pain with the movement pattern; 1 is difficulty performing it even after some compensatory correction; 2 is difficulty performing it without said compensation but having the ability to perform it with said compensation; and 3 is full ability and stability while performing each pattern. The relevance of each pattern is speculative and debatable. Gray[36] designed a complex assessment of movement patterns through triplanar movements. Although the theory behind his assessments is similar to Cook's and others, his procedures are unique. This is not a concise and neatly packaged technique, but many clinicians are seeing great results with their patients using Gray's theories and applications.

TABLE 47.1 General Soft Tissue Manipulative Technique

Technique	Description
Articulation	Repetitive passive movements employing leverage through variable ranges of the arc
Effleurage	Superficial drainage technique derived from massage therapy
Inhibition/ischemic compression	Describes an objective rather than a method; consists of pressure applied for lengthy periods, slowly applied and slowly released, using thumb contact as a rule
Kneading	Deep or superficial rhythmical pressure, usually applied by thenar or hypothenar eminence
Positional release methods	Approaches that, instead of acting directly on restricted or shortened structures, aim to position them in a state of "ease" by moving away from restriction barriers, allowing a spontaneous normalization to occur, involving neural (muscle spindle) resetting and circulatory enhancement These methods include what is known as strain/counterstrain as well as much craniosacral work
Rhythmic traction	Repetitive attempts to separate articulations to stretch interarticular and periarticular structures
Springing	Repetitive, usually slowly applied, pressure of a gradual nature, often used diagnostically
Stretching	Short and long amplitude attempts at separation of muscular attachments and stretching of ligaments, fascia, and membranes
Vibration	Rapid oscillatory pressure or movement

Soft Tissue Manipulation Techniques

Therapeutic efforts and techniques may be directed toward the diagnosis and treatment of the mechanical aspects of dysfunction (trauma, strain, aberrant movement patterns, etc.) and toward the use of the available information from such reflex areas in a more wide-ranging, holistic approach to the health of the patient.[37]

General, rhythmic techniques are often employed on the soft tissues to relieve local dysfunction and/or to prepare for subsequent adjustment of osseous structure. There are common threads to each technique; many use rocking and other rhythmic movements, compression and ischemic compression, and a variety of contacts (hands, digital pressure, elbows, and various devices). In all of the available soft tissue techniques, the objectives are the improvement of circulation and drainage; release of contracture, fibrosis, and/or adhesion; greater range of movement; decreased pain and improved movement; and stability. Most soft tissue methods can be applied in a stimulatory as well as a relaxing or inhibitory manner, but care should be taken to prevent stimulation from becoming an irritation. Table 47.1 presents a survey of various soft tissue techniques and a brief description of each.

The Potential of Soft Tissue Manipulation

The professions that use manual medicine are experiencing a resurgence in the tradition of "hands-on" assessment, treatments, and therapies. The musculoskeletal system is both the greatest energy consumer and the largest organ of sensory input in the body. This primary machinery of life has long been unappreciated in

therapeutic terms. The development of methods such as strain/counterstrain, muscle energy technique, fascial manipulation, trigger-point therapy, and neuromuscular technique and research, as well as other reflex systems, ensures that the diagnostic and therapeutic potential of the soft tissues is increasingly being recognized and used.

Korr, the premier osteopathic researcher of the second half of the 20th century, summarized another vital implication of soft tissue dysfunction—interference with axonal transport mechanisms—as follows:

> *Any factor that causes derangement of transport mechanism in the axon or that chronically alters the quality or quantity of the axonally transported substances could cause the trophic influences to become detrimental. This alteration in turn would produce aberrations of structure, function and metabolism, thereby contributing to dysfunction and disease. Almost certainly to be included among these harmful factors are the deformation of nerves and roots, such as compression, stretching, angulation and torsion that are known to occur all too commonly in the human being and that are likely to disturb the interaxonal transport mechanisms, intraneural microcirculation and the blood-nerve barrier. Neural structures are especially vulnerable in their passage over highly mobile joints, through bony canals, intervertebral foramina, fascial layers and tonically contracted muscles. Many of these biomechanically induced deformations are of course subject to manipulative amelioration and correction.*[41]

SUMMARY

This survey touched on some of the many ways in which soft tissue dysfunction may impinge upon the economy of the body as a whole. Soft tissue manipulation is an important diagnostic and treatment modality and should be considered an integral part of the practice of any physician or practitioner whose intent is to care for the whole person. Although a great variety of assessment, diagnostic, and treatment procedures exist, there are common threads that transcend their differences.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lewit K. *Manipulative Therapy in Rehabilitation of the Motor System*. Boston, MA: Butterworths; 1985.
2. Baldry P. *Acupuncture, Trigger Points and Musculoskeletal Pain*. London: Churchill Livingstone; 1993.
3. Jones DS, ed. *Textbook of Functional Medicine*. Gig Harbor, WA: The Institute for Functional Medicine; 2005.
4. Cook G. *Athletic Body in Balance*. Champaign, IL: Human Kinetics; 2003.
5. Cook G, Burton L, Kiesel K, et al. *Movement: Functional Movement Systems: Screening, Assessment, Corrective Strategies*. Aptos, CA: On Target Publications; 2010.
6. Sahrmann S. *Movement System Impairment Syndromes of the Extremities, Cervical and Thoracic Spines*. St. Louis: Elsevier; 2010:568.
7. Stecco L. *Fascial Manipulation for Musculoskeletal Pain*. Padova, Italy: Piccin; 2004.
8. Stecco C, Stecco L. *Fascial Manipulation: Practical Part*. Padova, Italy: Piccin; 2009.
9. Myers TW. *Anatomy Trains: Myofascial Meridians for Manual and Movement Therapists*. 2nd ed. New York: Churchill Livingstone; 2008.
10. Liebenson C. *Rehabilitation of the Spine*. Baltimore: Lippincott Williams & Wilkins; 2006.
11. Janda V. Introduction to functional pathology of the motor system. Proceedings of VII Commonwealth and International Conference on Sport. *Physiother Sport*. 1982;3:39–41.
12. Hartman L. *Handbook of Osteopathic Technique*. London: Hutchinson; 1985.
13. DiGiovanna E, ed. *An Osteopathic Approach to Diagnosis and Treatment*. Philadelphia: Lippincott; 1991.
14. Greenman P. *Principles of Manual Medicine*. Baltimore: Williams & Wilkins; 1996.
15. Rolf I. *Rolfing: The Integration of Human Structures*. New York: Perennial Library, Harper and Row; 1977.
16. Chaitow L. *Neuromuscular Technique*. Rochester, VT: Thorsons; 1980.
17. Langevin HM, Churchill DL, Fox JR, et al. Evidence of connective tissue involvement in acupuncture. *FASEB J*. 2002;16:872–874.
18. Langevin HM, Yandow JA. Relationship of acupuncture points and meridians to connective tissue planes. *Anatom Rec (New Anat)*. 2002;269:257–265.
19. Ma YT, Ma M, Cho ZH. *Biomedical Acupuncture for Pain Management: An Integrative Approach*. New York: Churchill Livingstone; 2005.
20. Hernandez-Reif M, Ironson G, Field T, et al. Breast cancer patients have improved immune and neuroendocrine functions following massage therapy. *J Psychosom Res*. 2004;57:45–52.
21. Field T, Grizzle N, Scafidi F, et al. Massage and relaxation therapies' effects on depressed adolescent mothers. *Adolescence*. 1996;31:903–911.
22. Field T, Schanberg S, Kuhn C, et al. Bulimic adolescents benefit from massage therapy. *Adolescence*. 1998;33:555–563.
23. Underdown A, Barlow J, Chung V, et al. Massage intervention for promoting mental and physical health in infants under six months. *Cochrane Database Syst Rev*. 2006;4:CD002892.
24. Cannon W. *Bodily Changes in Pain, Hunger, Fear and Rage: An Account of Recent Research into the Function of Emotional Excitement*. New York: Appleton; 1915.
25. Cannon W. *The Wisdom of the Body*. New York: Norton; 1932.
26. Waersted M, Eken T, Westgaard RH. Psychogenic motor unit activity: a possible muscle injury mechanism studied in a healthy subject. *J Musculoskeletal Pain*. 1993;1:185–190.
27. Du Ruisseau P, Taché Y, Selye H, et al. Effects of chronic stress on pituitary hormone release induced by combined hemi-extirpation of the thyroid, adrenal and ovary in rats. *Neuroendocrinol*. 1977;24:169–182.
28. Field T, Hernandez-Reif M, Diego M, et al. Cortisol decreases and serotonin and dopamine increase following massage therapy. *Int J Neurosci*. 2005;115:1397–1413.
29. Nerbass FB, Feltrim MI, Souza SA, et al. Effects of massage therapy on sleep quality after coronary artery bypass graft surgery. *Clinics (Sao Paulo)*. 2010;65:1105–1110.
30. Smith MJ, Selye H. Stress: reducing the negative effects of stress. *Am J Nurs*. 1979;79:1953–1955.
31. Selye H. Stress and holistic medicine. *Fam Community Health*. 1980;3:85–88.
32. Selye H. The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology and Metabolism*. 1946;6:117–230.
33. Petersen T, Larsen K, Nordsteen J. The McKenzie method compared with manipulation when used adjunctive to information and advice in low back pain patients presenting with centralization or peripheralization. A randomized controlled trial. *Spine*. Published online February 27, 2011.
34. Travell J, Simons D. *Myofascial Pain and Dysfunction: The Trigger Point Manual*. Vol. 1 and 2. Baltimore, MD: Lippincott Williams & Wilkins; 2006.
35. Mennell JM. The therapeutic use of cold. *J Am Osteopath Assoc*. 1975;74:1146–1158.
36. Gray G. Gray Institute—Applied Functional Science. http://www.grayinstitute.com. Accessed March 10, 2011.
37. Jones L. *Strain and Counterstrain*. Colorado Springs, CO: American Academy of Osteopathy; 1981.
38. Mitchell Jr FL. The training and measurement of sensory literacy in relation to osteopathic structural and palpatory diagnosis. *J Am Osteopath Assoc*. 1976;75:874–884.
39. Ebner M. *Connective Tissue Massage: Theory and Therapeutic Application*. Edinburgh: E&S Livingstone; 1962.
40. Dvorak J, Dvorak V. *Manual Medicine: Diagnostics*. New York: Thieme-Stratton; 1984.
41. Korr IM. The spinal cord as organizer of disease processes. IV. Axonal transport and neurotrophic function in relation to somatic dysfunction. *J Am Osteopath Assoc*. 1981;80:451–459.

48

Spirituality, Religion, and Healing

Wayne Jonas, MD, and *Maeba Jonas, MDiv*

OUTLINE

INTRODUCTION—WHY SPIRITUALITY, RELIGION, AND HEALTH?

Spirituality plays a key role in the creation of healing, health, and well-being. This chapter describes what spirituality is; how the research on spirituality and health is evolving; how to appropriately address spirituality and provide information on resources; and processes that can help the provider attend to the spiritual dimension of patients' care and, in doing so, provide whole-person care.

When faced with serious or chronic illness, most people ask the question, "Why?" Physicians and healthcare providers are trained to answer this question in physical terms and will usually launch into explanations involving chemistry, molecules, genetic predispositions, environmental exposures, and so forth. However, for many seeking the full answer to healing, this is not enough. Although patients may be grateful for these explanations, there is another meaning of the question that they may also seek to answer. That question may be expressed like this: "Why did this happen to me? What does this mean for my life? What will happen to me if I die?" These are not medical questions. They are spiritual questions. When asked, 70% to 80% of patients with a serious or chronic disease or those in the hospital will say they would like to discuss spiritual issues, to pray with their provider, or to see a professional chaplain.[1,2] There is a desire by patients to address spiritual dimensions of their lives in health care.

Fortunately, many hospitals respond to this by providing professional spiritual assistance in the form of chaplains and spiritual counselors. Unfortunately, most medical providers in the hospital (and in the clinic) never ask their patients if they need or want spiritual services. Holistic, person-centered care requires this gap be filled. To care for the whole person means that we attend to the mental and spiritual dimension of the individual's being. The roots of the very word *healing* attest to this fact. Derived from the ancient German *hael,* we also get the words *whole* and *holy,* illustrating the unity of these concepts.[3] But this is not just a linguistic or conceptual unity; it is an actual unity with practical implications for health care. Fig. 48.1 illustrates what a whole-person model looks like. The one that I (WJ) use has four dimensions, constructed for simplicity and utility in clinical practice: (1) the external dimension of the body, (2) the behavioral dimension of lifestyle, (3) the social and emotional dimension of the psyche, and (4) the mental and spiritual dimension of the soul. Healthcare professionals are usually trained to attend to one or the other of these dimensions; however, regardless of one's training or focus in health care, care for the whole person requires that we acknowledge the existence and unity of these dimensions and help provide spiritual assistance, whatever our discipline or specialty.

DEFINITIONS—RELIGION, SPIRITUALITY, HEALTH, HEALING

The words *spirit, spiritual,* and *spirituality* have several but mostly consistent meanings:

Spirituality: The quality or condition of being spiritual.

Spiritual: Of, pertaining to, or affecting the spirit or soul, especially from a religious aspect.

Spirit: (1) The animating principle in humans and animals. (2) The immaterial part of a corporeal being.[4]

Many have heard the phrase "spiritual but not religious," but what is the difference between the two? In a general sense, religion refers to a system of beliefs, practices, and traditions agreed upon by a community or institution, often having to do with a connection to a higher power or a higher self. Spirituality is a set of beliefs, ethics, moral convictions, or sense of connectedness practiced by an individual. In other words, religion refers

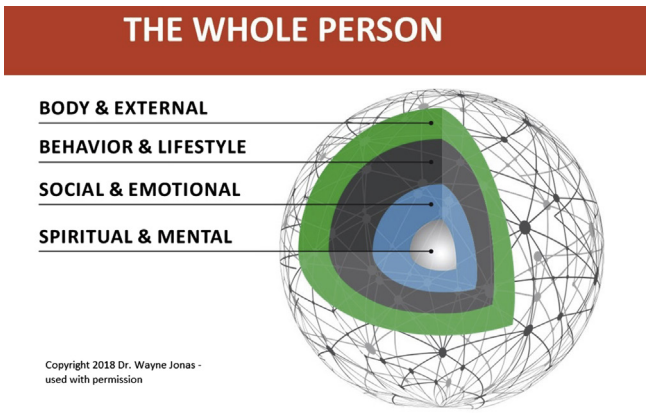


Fig. 48.1 Schematic of a whole person in four dimensions—external, behavior, social/emotional, mental/spiritual.

to spiritual traditions (e.g., Christianity, Judaism, Islam, Buddhism, etc.) and the practices that arise from the cultures and communities surrounding these traditions. In contrast, spirituality is a much more amorphous term that has been used increasingly in many societies by people who may be uncomfortable with the "institutions" of organized religions but still seek a sense of wholeness, connection, or simply peace in their lives.

Spirituality, then, depends on a variety of beliefs concerning the soul, theology, ritual, culture, and so forth. Within specific traditions, there are differing definitions. This might include "being saved" (Evangelical Christian), "submission" (Islam), or "nonattachment" (Buddhism). To favor the idea that one of these concepts is the "truth" is to endorse certain spiritual viewpoints while dismissing others. Given the world's spiritual diversity, each view must be interpreted within the context of its a particular tradition. These traditions share the fundamental goal of giving meaning to the experience and concept of spirit, but each has unique perspectives and values. This creates challenges for the study of spirituality and health, especially in multicultural societies. In the West, the default response has been to either follow Christian theology or to make definitions that are often secular and less specific to any one religious tradition. These definitions are more "transcendental" in scope and seek to make all humans and human behavior spiritual rather than allowing for specificity in religious expression based on the individual patient. These definitions tend to make the meaning of spirituality "fuzzy" or even "vague and contradictory."[5,6]

This also conflates spiritual, religious, and psychological factors, reducing the validity of the terms—such as when a psychological factor like optimism is used as a "spiritual" metric. This can confound results and become tautological. If, however, the meaning of research terms varies from common use, the results may lack ecological validity. These challenges can be seen in the common complaint that very spiritual people are somehow "better" than others or that one must be spiritual to be ethical or compassionate. Broad definitions that universalize spirituality can be especially damaging in pluralistic settings, such as in science and multicultural contexts. To remedy this, we recommend distinguishing between religion and spirituality.[7] For some, all spiritual beliefs and practices are completely within the framework of their religion. For 20% to 30% of Americans, spirituality is largely outside religion.[8,9] A Pew survey found that 24% of Americans say they go to the religious services of at least one faith other than their own. Spiritual and religious pluralism is the norm for many places in the world, especially the United States.[10,11] Although Christianly still dominates the American scene, there are other growing and powerful influences shaping American religious heterodoxy, such as the spiritual traditions of Asia, principally Buddhism and Hinduism (especially meditation and yoga), Islam, and nonreligious spiritual-like experiences.

WHY RELIGION AND SPIRITUALITY ARE IMPORTANT IN HEALTH CARE

It is likely that among our Neolithic ancestors there was little distinction between spirituality and physical healing. Most tribal societies involve shamans in healing, even of the physical body. Most major healing traditions also postulate and have a close connection between the spiritual and physical bodies in their treatment models.[12] The separation between spirituality and health care is largely a modern, Western phenomenon.

Although the advent of the scientific method has created breakthroughs in evidence-based health care, the consequence for conventional medicine is a lack of emphasis on context in any given treatment. The research on "complementary and integrative medicine" and "optimal healing environments" has demonstrated the need for better attention to whole-person health care that has only recently started to return to the healthcare system.[13,14]

In contrast, systems of Eastern medicine, such as Ayurveda or the chakra system in India, acupuncture or tai chi in China, and meditation or singing bowls in Tibet (to name a few), regularly incorporate priests, shamans, or spiritual healers together with prescribed treatments and rituals to address not only the symptoms but also the root of the disease itself.[15] Just as a psychologist might ask probing questions to understand the cause of one's mental distress, in many parts of the world, disease may not only be a physical problem but a result of spiritual misalignment or dis-ease.

Within a Christo-centric framework, science in the West has sought to distance itself from such a model, for fear that assigning spiritual values to a physical problem may result in the outdated and even dangerous conflation of placing responsibility for illness on the patient or the patient's "sins." This, of course, can lead to ostracization or even maltreatment from a medical perspective, which we indeed must try to avoid. However, what if one's religious or spiritual beliefs do not include the concept of sin? In a Buddhist or Hindu context, one's disease might have much more to do with *karma,* a concept that assigns no more responsibility to an individual than the act of being born! Of course, once one has a disease (much like karma), it is possible to do something to treat it, which must occur by addressing not just the symptoms of the illness but also the whole person, and that is where spirituality and ritual become important.

More and more, the defining "spiritual" experiences of people in the West stem from adopting the religious practices of other traditions, such as yoga from Hinduism or meditation or mindfulness, which largely came to the United States from the Buddhist tradition. Because Buddhism is a nontheistic religion (that is, it does necessitate a belief in God), spiritual Westerners find it particularly appealing, and mindfulness has found large applications as a primarily nonreligious spiritual practice. It is important to remember, however, that these now secularized practices have spiritual and even religious roots.[16] Therefore what "spiritual" means to any one patient can vary widely and has great implications for health care. For example, one might identify as Jewish or Christian but may not be able to readily assume the rituals, practices, or even belief system based on these identities alone.

Patients want greater attention to spirituality when they are in the hospital. A 2003 survey of more than 1,700,000 patients, involving 33% of all US hospitals, showed that patient satisfaction with the emotional and spiritual aspects of care had some of the lowest ratings and was an area in great need of improvement.[17] The Joint Commission on Hospital Accreditation requires all hospitals to have a process and place to assess the "spiritual health" of all patients. This is usually done by taking some sort of "spiritual history"[18,19] (discussed later in the chapter).

Prayer is a common practice in the United States and is often directed toward health concerns. A national survey in 2004 revealed that 35% of respondents used prayer for health concerns; 75% of these prayed for wellness, and 22% prayed for the healing of specific medical conditions. Of those praying for specific medical conditions, 69% found prayer very helpful. Factors independently associated with the increased use of prayer included age 33 to 53 years (odds ratio [OR], 1.6), age more than or equal to 54 years (OR, 1.5), female sex (OR, 1.4), and education beyond high school (OR, 1.5). Patients with depression, chronic headaches, back and/or neck pain, digestive problems, and allergies were also associated with more frequent prayer. Only 11% of respondents described using or discussing prayer with their physicians.

Most people in the United States believe that spirituality can powerfully affect human health and want physicians to include faith as part of a comprehensive doctor–patient interview[20]:

- A Gallup poll published in 1990 found that 94% of patients admitted to hospitals stated they believed that spiritual health is as important as physical health, 77% believed that physicians should consider their spiritual needs as part of their medical care, and 37% wanted their physician to discuss their religious beliefs more.[21]

- A telephone survey conducted in 2002 found that 80% of respondents believed God acts through physicians to cure illness; 40% believed God's will was the most important factor in recovery; and 69% said they would want to speak to someone about spiritual concerns if seriously ill, although only 3% would initiate a conversation about spiritual factors with a physician.[22]

Even outside of a faith tradition, nonreligious spirituality is used in finding meaning, whole-person healing, and the cultivation of caring patient–provider relationships.

It seems clear, then, that spirituality, in its various manifestations, such as religion, prayer, rituals, meditation, meaning and purpose, exceptional experiences, and behavior, is a core component of holistic health care. An individual is not only a body but also a spiritual being. Caring for the whole person, then, requires that we ask about and work with the spiritual dimension of health care. But what does this mean in daily practice? How do we address spirituality in routine health care? When is it most needed, and who should provide such care?

RESEARCH ON RELIGIOUS AND SPIRITUAL PRACTICES AND HEALTH

The scientific research on religion, spirituality, and health is a growing field. There are measures for spiritual belief, practice, and experience, as well as research linking spirituality and health.[23,24] However, the subjective nature of spirituality and the inability to randomize patients to most spiritual practices require correlative and mixed-methods approaches for their evaluation, including systematic qualitative research. The following is a brief summary of research on spirituality, religion, prayer, and the effects of intention and selected "biofield" practices common in spiritual practices.

The Health Effect of Religious and Spiritual Practices

There is considerable research showing that participation in religious and spiritual activities, including churchgoing, Bible study, prayer groups, rituals (both community and private), or other behavior oriented to a "higher being" or "God," correlates with health outcomes. Summaries of these studies have been done by a number of scholars, such as Harold Koenig, Larry Dossey, Daniel Benor, Stephan Schwartz, Marilyn Schlitz, Wayne Jonas, and others. My colleagues and I examined this research extensively and published a critical appraisal in the book *Healing, Intention and Energy Medicine: Science, Methodology and Clinical Implications,* from which a summary is provided for this chapter.[25] Nearly 200 studies examining the health effects of religious and spiritual behaviors on various conditions and outcomes have been published. Most of this research shows that religious practices are associated with beneficial effects on health. This includes reduced mortality, improved physical health and quality of life, and reduced mental illness and drug abuse. More than 75% of these studies report statically significant effects on those associations. Few studies report (or even measure) the adverse effects associated with religious practices. The vast majority of this research is epidemiological. This blunts the certainty of these associations because multiple confounding factors besides religious behavior, such as changes in diet, lifestyle and behavior, social connections, and risk and resilience factors, are hard to control. These confounding factors cannot be randomly distributed, but they can be approximated with control comparisons and analyzed.

Intercessory and Healing Prayer

There are a few studies of prayer—defined as healing intention or appeals toward a "higher being or force." Most of these studies examine if health outcomes changed compared with no or less prayer. A review was conducted by John Astin, who focused on randomized studies of distant healing and prayer for clinical conditions. Astin's review included a total of 13 randomized studies on prayer involving 2328 patients. Of these, six (46%) reported statistically significant effects of prayer on at least one outcome and a mean effect size across trials of 0.30, which is considered small in most clinical research. The research had an average quality score of 45% of the maximum using a standard quality score for controlled studies.[26] No effects at this level of evidence were reported for the healing of warts or for alcoholic relapse in a small trial.[27] Four more randomized, controlled trials were published after this review. One was positive, and three reported no significant effects from distant prayer.

"Bioenergy" Healing Research

What is often called "energy healing" involves the intentional direction of spiritual "energy" or a hypothesized "biofield" toward a patient, with the goal of improved health. A review of clinical studies of energy healing was conducted by Sara Warber, MD; Gaia Kile, MSN, NP; and Brenda Gillespie, PhD, of the University of Michigan. The review focused on randomized controlled trials of clinical conditions treated with these energy methods. Nineteen randomized controlled trials involving 1122 patients were included. All but one were on Therapeutic Touch—a semiformalized process used by nurses and others in which a practitioner holds his or her hand over the patient. Eleven (58%) of the studies reported statistically significant effects, with a mean effect size of 0.60—a moderate effect. Positive effects were found for the alleviation of pain in burn patients and reduced anxiety in institutionalized elderly patients. There have been more than 50 studies published on laboratory models of energy research involving mainly cellular and animal models. Although much of the findings are reported as positive, an overview shows no studies that were independently replicated at this time.

Qigong

Qigong is an ancient energy healing method developed in China that claims to accumulate and direct this energy, which is called "qi." Although a vast amount of Chinese literature exists, it is difficult to evaluate because it is mostly in Chinese. We examined both clinical and laboratory studies of "external qi" (qi projected from a practitioner). The evaluation of clinical studies also captures qigong practice, or "internal qi" (qi cultivated by meditation and exercises). In the latter, we focused on blood pressure because this area has the largest amount of evaluable literature. A review was conducted by Michael Mayer, PhD, OMD. Thirty-three controlled studies (out of 72 reports) on the effects of qigong on hypertension were reviewed. All studies reported positive effects, but only five of the studies were randomized. However, the quality of the research was poor (quality score of 13% of maximum). No high-quality replications were done. One study reported adverse effects.

A review of the laboratory (cell and animal) studies of qigong was done by Juliann Kiang, PhD, and Ping Y. Lu. Kiang and Lu sorted through studies from multiple databases (including those in Chinese). Fifty-eight studies (out of 130 reports) had enough detail to review. Studies reported positive effects on a variety of outcomes, including cancer growth and cellular and enzyme changes and immune modulation, in both animals and cell culture. However, the quality of the research was poor (quality score of 20% of maximum), and there were no independent, high-quality replications. Significant publication bias is likely in both the clinical and laboratory studies because positive studies are published much more often than studies showing no effects.[28,29]

Direct Effects of Intention

There are many studies, including high-quality, independent replications, examining the effects of intention on biological and physical parameters. One set of studies examines if a person can affect the conductivity of the skin of another person using mental intention. Another body of research involves examining whether people can alter chance events (e.g., the throw of a die or an electronic random event generator [REG]) by intention. In both these sets of research, there is a clear and highly statistically significant effect of intention on the outcome. Intention clearly has direct effects on a number of parameters. However, these effects are small (often less than 5% difference from controls). Although these studies have proven that intention does have effects, how these studies relate to prayer and other spiritual practices is unclear.

The Effect of Healing in a Clinical Setting

What is the effect of having a spiritual healer as part of medical practice? Two studies have been described by Tim Harlow, MBchB, DCH, and one study was described by Harald Walach, PhD. Initial observations indicated benefits from introducing healers into a clinic where controlled assessments of healing effect were performed. A healer was brought into the conventional practice without compromising conventional medical care or altering the healer's methods. The healer resulted in an improvement in the symptoms and well-being of some (but not all) of the chronically ill patients and changed the perceptions and behavior of doctors, other providers, and patients about spiritual healing. Physicians took more time to listen, and patients communicated more often about the complementary therapies they were using. In a study by Harald Walach, PhD, 12 chronically ill patients offered distant healing had improved quality of life compared with those not treated by healers. This effect was significant, both statistically and clinically. When including these additional quality criteria, the evidence level is B because no high-quality meta-analysis or independent replication has been done.

Advancing Research on Spirituality and Health

There is clearly a need for further research on the relationship between religion, spirituality, and health. But advancing the field is complex. Not only do concepts and definitions in spirituality and healing vary widely, but the models of research used for more tangible constructs often do not provide a good fit with the subtle aspects of spirituality, such as meaning, transcendence, the sacred, prayer, holiness, and so forth. Although measures of spiritual experiences and religious practice have been developed, these may not capture the meaning of faith for many people. Dr. Chris Feudtner, a palliative care pediatrician at the Children's Hospital of Philadelphia, has designed a thoughtful model of research allowing fluid "mediation between domains," both the concrete and fluid concepts in spirituality in health.[30]

In summary, there is evidence to suggest that mind and matter interact in a way that is consistent with the assumptions of distant healing. Mental intention has effects on nonliving random systems (e.g., random number generators) and may have effects on living systems. Although conclusive evidence that these mental interactions result in the healing of specific illnesses is lacking, further quality research should be pursued. For further information, there are a number of references providing details on any part of this analysis.[31]

WHO PROVIDES SPIRITUAL CARE?

With the separation of the spiritual and physical in modern medicine and the decline of formal religions in the West, the role of spiritual care has been distributed across a wide variety of groups. Although spiritual healing, prayer groups, meditation, and other faith-based practices continue to be widespread, modern professional spiritual care has been largely consolidated in clinical pastoral care providers. Before 1906 and what was called the Emmanuel Movement, psychotherapy was part of the domain of both ministers and physicians. However, after the emergence of psychotherapy as a formal discipline, the practice of psychotherapy was formalized under the medical domain, and chaplains wanting to do psychological counseling were required to get special training and certification in that skill.[32] During this time, formal hospital chaplaincy and clinical pastoral education (CPE) emerged as the professional certification for ministers. Those with CPE training are now hired by hospitals specifically to provide spiritual care services. They largely deal with providing spiritual support and care in areas such as depression and pain, dealing with anxiety and anger, coping with the effects of treatments, and dealing with loss and death.[33]

Although ministers have denominational training, CPE provides skills for dealing with multidenominational, multifaith, and even nonreligious beliefs and contexts. Care of hospital staff to assist in coping with stress, burnout, and traumatic experiences is also a frequent role of hospital chaplains.

Chaplains also have a role in assisting with ethical decisions in health care. Challenging treatment decisions and withdrawal of treatment in seriously ill patients and differences in opinions between family members or between medical professionals and family members all may require spiritual discernment and assistance from chaplains. However, medical personnel often do not know how or when to use chaplains in these decisions. Whereas 88% of healthcare providers say that chaplains should be on hospital ethical committees, for example, only 22% to 32% of those committees actually appoint chaplains.[34] Recently, organized educational courses have emerged to help healthcare professionals learn how to better deal with religious and spiritual issues and how to work more closely with chaplains in health care.

Those wanting to learn how to work more closely on these issues would be wise to take such training. One of the most fully developed is the yearly course conducted by Dr. Christina Puchalski, founder of the George Washington Institute of Spirituality and Health (GWish), which provides a comprehensive introduction to team-based spiritual and healthcare integration.[35]

Faith communities have often been integral to the provision of healthcare, from Catholic and Jewish hospital systems to community-based multidenominational groups seeking to address poverty, housing, food insecurity, safety, and other social determinants of health. Recently, the National Academy of Medicine convened a workshop to explore novel faith–health community collaborations addressing population health delivery. The workshop (1) provided an overview of faith-based assets in communities and their relationship to population health and the work of health improvement, (2) highlighted areas where faith-based health assets are using evidence to inform their work and demonstrating effectiveness in improving health outcomes, (3) provided examples of effective partnerships involving faith-based health assets, and (4) shared lessons learned from working with faith-based assets that could contribute toward principles for engagement of healthcare organizations and public health agencies. Here, a prominent body is giving space, time, and thought on how the gap between medical care and faith care could be better filled.[36] Those wanting to affect public health and the "upstream" determinants of health and disease could do so by getting more involved in these community–healthcare collaborations.

TOOLS FOR CARING FOR THE WHOLE PERSON IN DAILY PRACTICE

How can providers and others who deliver their services address the spiritual aspects of patient care without some of the infrastructure

TABLE 48.1[a] Questions for an Integrative Health Visit Using the HOPE Note

Dimension	Questions	Description
Spiritual and mental	***Why do you want to be healthy?*** ***What is most important for you in your life?***	This addresses a person's **inner life:** • Desires, beliefs, and needs • Why they get up in the morning • Their purpose in life **"What matters"** rather than "what's the matter"
Behavioral and lifestyle	***How is your diet?*** *—Listen to the global assessment; count the F&V.* ***How is your sleep?*** *—Listen to the global assessment; determine pattern—rested?* ***How is your activity level?*** *—Assess level and intensity of movement.* ***How is your stress?*** *—What do you do for stress management?*	• Lifestyle and behavior can affect up to 60%–70% of chronic illnesses and are key to reversing chronic disease and improving function. • Behavior change must be connected to what is meaningful for the person, or it cannot be sustained. ***Does the client believe that what he or she does is helping?***
Social and emotional	***How is your social support?*** *—Inquire about family, friends, frequency, fullness.* ***How was your childhood?*** *—Explain that ACE can influence healing*	• Loneliness is epidemic; is a risk factor for disease and death; and influences recovery, resilience, and readmission. • So often, the reason and process for healing have to do with relationships. • Men—spouse; women—friends, family; church; community; work colleagues • The depth (quality) of the relationships is more important than the number (quantity).
External environment	***What is your home like?*** *—Do you have a place and time for yourself?* ***How is your work environment?*** *—Gauge the level of stress, control, and privacy.* ***Do you get out in nature?*** *—Most people underestimate its importance.*	Community, worksite, school, and home environments shape the following: • What a person is able to do • What happens to a person • How safe or stressed a person feels • How well a person flourishes and functions • How long a person lives

ACE, adverse childhood experiences; *F&V*, fruits and vegetables.
[a]Copyright 2018 Dr. Wayne Jonas. Used with permission.

and teams described previously? How can they bring in the mental and spiritual aspects of a patient's life without going beyond their professional domain of providing medical care? One approach is to conduct an "integrative health" visit. Integrative health (IH) is the coordinated intersection of conventional care, complementary medicine, and self-care. The goal of IH is to move from the former to the latter by providing the dialogue and support to help a patient to become engaged in self-care in a rational and evidence-based way. The IH visit starts by asking the patient to describe "what matters" to him or her in life and reflect on what he or she most lives for. What gives the patient meaning, purpose, and joy in life? It is by connecting "what matters" to "what's the matter" that patients both begin and sustain their healing journey. I do this with two tools that get at this very "existential" (shall we say "spiritual") dimension of a person.

The first tool is the Personal Health Inventory (PHI). The PHI is a simple two-page questionnaire given to patients before a visit. It asks them to state what is most important to them in their lives and then to rate (on a scale of 1 to 5) where they are and where they are ready to change in three other domains that determine health: behavioral, social, and emotional and environmental. The PHI sets the stage for a dialogue during the integrative visit to discuss all the domains of healing of a person. Fig. 48.1 shows the elements of the PHI.

The second tool is used during the visit. It is called the HOPE Note. HOPE stands for Healing-Oriented Practices and Environments, and the tool is designed to elicit more details of the patient's personal determinants of health and start a Personal Health Plan (PHP). HOPE asks questions about lifestyle (diet, activity, sleep, stress management); the social and emotional dimension (social support, loneliness); the mental and spiritual dimension (mental attitude; goals and joys in life); and the external environment (exposure to beauty, art, and nature). The discussion seeks to link the mental and spiritual dimension to the other healing pathways of a person. Table 48.1 shows the questions of the HOPE Note.[37]

This approach allows a holistic assessment of the individual in an everyday office visit. From this, the patient's needs and wants can be taken into account and merged into the patient's own personal health plan. From that point, support in the patient's healing journey is provided to actualize the plan. This provides truly patient-centered care. Such care requires a team that includes expertise in medicine, social determinants, lifestyle and behavior change, and spiritual care. It also includes the patient and family members as part of the team.

CONCLUSIONS

The mental and spiritual dimension is a core part of all human beings. As such, any healthcare system or provider that seeks to care for the whole person needs to address this dimension. We know that patients need and want to address spiritual issues when they are ill. We know that there is evidence for the reality and effectiveness of spiritual practices on health outcomes. There are professionals and groups trained and prepared to assist in helping patients with religious and spiritual needs. Tools and training are available to bring the mental and spiritual dimension into the practitioner's daily encounters with patients. Thus it is important for all physicians and other healthcare providers to address the core dimension of spirituality in their practice.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. MacLean CD, Susi B, Phifer N, et al. Patient preference for physician discussion and practice of spirituality: results from a multicenter patient survey. *J Gen Int Med*. 2003;18(1):38–43.
2. Balboni TA, Vanderwerker LC, Block SD, et al. Religiousness and spiritual support among advanced cancer patients and associations with end-of-life treatment preferences and quality of life. *J Clin Oncol Off J Am Soc Clin Oncol*. 2007;25(5):555.
3. Heal (v.). Available at: https://www.etymonline.com/word/heal. Accessed date: 4 December 2018.
4. Brown L. *The New Shorter Oxford English Dictionary on Historical Principles*. Oxford, UK: Clarendon Press; 1993:2989–2990.
5. Zinnbauer BJ, Paragament KI, Cole B, et al. Religion and spirituality: unfuzzying the fuzzy. *J Sci Study Relig*. 1998;36:549–564.
6. Egbert N, Mickley J, Coeling H. A review and application of social scientific measures of religiosity and spirituality: assessing a missing component in health communication research. *Health Commun*. 2004;16(1):7–27.
7. Larson DB, Swyers JP. *Scientific Research on Spirituality and Health: A Consensus Report*. Rockville, MD: National Institute for Healthcare Research; 1998.
8. Kurs K. Are you religious or are you spiritual? voices of a new America. *Spiritual Health Int*. 2001;4:28–31.
9. Gallup Organization. *Gallup Poll Topics: A-Z; Religion*. Washington, DC: Gallup; 2001. Available at: www.gallup.com/poll/indicators/indreligion4.asp. Accessed date: 4 January 2010.
10. Schmidt LE. *Restless Souls: The Making of American Spirituality*. New York: Harper Collins Publisher; 2005.
11. Fuller RC. *Spiritual but Not Religious: Understanding Unchurched America*. New York: Oxford University Press; 2001.
12. Krippner S. Common aspects of traditional healing systems across cultures. *Essentials of Complementary and Alternative Medicine*. 1999:181–199.
13. Jonas WB, Levin JS, eds. *Essentials of Complementary and Alternative Medicine*. Philadelphia: Lippincott Williams & Wilkins; 1999.
14. Sakallaris BR, MacAllister L, Voss M, Smith K, Jonas WB. Optimal healing environments. *Glob Adv Health Med*. 2015;4(3):40–45. https://doi.org/10.7453/gahmj.2015.043.
15. Turner ELB. *Among the Healers: Stories of Spiritual and Ritual Healing Around the World*. Westport, Conn.: Praeger Publishers; 2006.
16. Hauser B. *Yoga Traveling: Bodily Practice in Transcultural Perspective*. Heidelberg: Springer International Publishing; 2013.
17. Clark PA, Drain M, Malone MP. Addressing patients' emotional and spiritual needs. *Joint Comm J Qual Safety*. 2003;29:659–670.
18. *A template for spiritual assessment: a review of the JCAHO requirements and guidelines for implementation*. http://goliath.ecnext.com/coms2/gi_0199-6058670/A-template-for spiritual assessment.html. Accessed date: 5 July 2010.
19. *Association of professional chaplains. Evaluating your spiritual assessment process*. http://www.professionalchaplains.org/uploadedFiles/pdf/JCAHO-evaluating-your-spiritual-assessment-process.pdf. Accessed date: 21 March 2011.
20. King DE, Bushwick B. Beliefs and attitudes of hospital inpatients about faith healing and prayer. *J Fam Pract*. 1994;39:349–352.
21. Princeton Religion Research Center, Gallup G. *Religion in America, Approaching the Year 2000*. Prentice Religion Research Center; 1990.
22. Mansfield CJ, Mitchell J, King DE. The doctor as God's mechanic? Beliefs in the Southeastern United States. *Social Sci Med*. 2002;54(3):399–409.
23. Koenig HG, McCullough M, Larson DB. *Handbook of Religion and Health*. New York: Oxford University Press; 2001.
24. Hufford DJ. *An Analysis of the Field of Spirituality, Religion and Health*. Bryn Mawr, PA: Metanexus Institute; 2005.
25. Jonas WB, Crawford CC, eds. *Healing, Intention and Energy Medicine: Science, Methodology and Clinical Implications*. London: Churchill Livingston; 2003.
26. Astin JA, Harkness E, Ernst E. The efficacy of "distant healing": a systematic review of randomized trials. *Annals Int Med*. 2000;132(11):903–910.
27. Crawford CC, Sparber AG, Jonas WB. A systematic review of the quality of research on hands-on and distance healing: clinical and laboratory studies. *Altern Ther Health Med*. 2003;9(3):A96–A104.
28. Jonas WB, Crawford CC, eds. *Healing, Intention and Energy Medicine: Science, Methodology and Clinical Implications*. London: Churchill Livingston; 2003.
29. Benor DJ. *Spiritual Healing: Scientific Validation of a Healing Revolution*. vol. 1. Wholistic Healing Publications; 2001.
30. Feudtner C, Haney J, Dimmers MA. Spiritual care needs of hospitalized children and their families: a national survey of pastoral care providers' perceptions. *Pediatrics*. 2003;111(1):e67–e72.
31. Jonas WB, Crawford CC, eds. *Healing, Intention and Energy Medicine: Science, Methodology and Clinical Implications*. London: Churchill Livingston; 2003.
32. Caplan E. *Mind Games: American Culture and the Birth of Psychotherapy*. vol. 9. Univ of California Press; 1998.
33. Galek K, Flannelly K, Koenig H, Fogg S. Referrals to chaplains: the role of religion and spirituality in healthcare settings. *Ment Health Relig Cult*. 2007;10(4):363–377.
34. Carey LB, Robinson P, Cohen J. Organ procurement and health care chaplaincy in Australia. *J Relig Health*. 2011;50(3):743–759.
35. *GW Institute for Spirituality and Health*. 2018. https://smhs.gwu.edu/gwish/. Accessed date: 8 October 2019.
36. Faith-Health Collaboration to Improve Community and Population Health. *Proceedings of a Workshop*. Washington, DC: National Academy of Medicine, Roundtable on Population Health; 2018. http://nationalacademies.org/hmd/Activities/PublicHealth/PopulationHealthImprovementRT/2018-MAR-22.aspx. Accessed date: 8 October 2019.
37. The HOPE Note Toolkit. *Samueli Integrative Health Programs*. Alexandria, VA: H&S Ventures; 2018. http://drwaynejonas.com/resources/hope-note/. Accessed date: 8 October 2019.

49

Unani Medicine

Yaser Abdelhamid, ND, LAc, MS, BS, BA

OUTLINE

INTRODUCTION

Unani Medicine: Greco-Arabic Healing and the Islamic Genius

The Arabic term *unānī* derives from the word *Ionian,* which is a general adjective signifying "things-Greek." The linguistic translations and transliterations of this word took place between the Greek, Arab, and Persian civilizations. The particular usage of this term, however, has more often been a descriptive allusion to the composite system of medicine born of the Arab world's inheritance—within the larger framework of Islamic civilization—of the medical tradition of ancient Greece.

The literary sources of Unani medicine (al-tibb al-yunānī, or sometimes referred to simply as "Tibb," or "Hikmat" in Pakistan and Afghanistan) were Arabic translations of ancient Greek, Roman, Egyptian, Arabic, Persian, Indian, and Chinese medical texts. However, there is no question that the initial, primary, and most honored textual sources were of Greek origin. The Arabs immersed themselves in the medical knowledge and wisdom contained in the writings of Hippocrates (Buqarāt) and Galen (Jālinūs),[1] as well as Plato (Aflātūn), Aristotle (Aristatīl), Dioscorides (Dīsqūrīdis), and Empedocles (Abrāqlīdis).

It is well recognized by contemporary historians that the ancient Greek—and to some extent Roman—intellectual heritage of the West would have been almost totally lost were it not for the integrating and synthesizing Arab genius within the Islamic world.[2] In this regard, one must marvel at the Arabs' level of care and precision in "first collecting, then translating, then augmenting, and finally codifying the classical Greco-Roman heritage that Europe had lost."[3] However, "so many people in the West wrongly believe that Islam acted simply as a bridge over which ideas of Antiquity passed to medieval Europe. Nothing could be further from the truth, for no idea, theory, or doctrine entered the citadel of Islamic thought unless it became first Muslimized and integrated into the total world-view of Islam."[4]

Unani Medicine: Comparisons and Contrasts With Other Health Care Systems

Apart from Unani medicine's introduction onto the stage of world history as a legitimate synthesis—in time and space—of primarily Greek and Islamic civilizational influences, there is yet another dimension to Unani medicine that is often overlooked and of great significance. Along with the medical traditions of other civilizations of the past, like the Ayurveda of Hindu India or traditional Chinese medicine, Unani medicine continues to be preserved and persevere—especially in the face of modern cultural trends in many nations that have moved toward changing the old guard of ancient medicine for the shiny hopes and promises of a new system of powerful drugs, "healing steel," and high-tech diagnostic tools and machinery. Unani medicine is still practiced today as an organic, living, breathing, whole system of health care helping millions of people around the world (especially in the Indian subcontinent, Pakistan, Afghanistan, Persia [Iran], China, Indonesia, Malaysia, Bangladesh, Sri Lanka, South Africa, and certain sectors of the Middle East [such as Saudi Arabia, Kuwait, and Dubai]).

Furthermore, Unani medicine historically had the opportunity, capacity, and satisfaction of embracing the numerous folk medicines that existed within the borderlands of the Islamic world, from the traditional use of herbal remedies in ancient Palestine to the folkloric ore-based therapeutics of the people living on the high plateaus of the Himalayan Hindu-Kush mountains.

Unani medicine also shares medical theories, philosophies, cultural identities, and spiritual insights regarding human health and disease with ancient systems of medicine, such as Hindu India's Ayurveda and traditional Chinese medicine, because its knowledge and wisdom hail from the traditional Eastern "Orient" while contrasting tremendously with the single system of contemporary medicine that one could say belongs to the world of the modern Western "Occident."[5] This is what led Hakim Chishti (an American researcher and practitioner of the science and art

of Unani medicine) to present a very thoughtfully indexed comparison between all of these healing systems (as found on http://www.unani.com), whose general points are outlined in Table 49.1.

HISTORY

Contributions and Influences From Antiquity

There were a variety of strands of medicine that came together at the crossroads of Islamic history that proved to be of tremendous significance for the birth of Islamic medicine in general and for Unani medicine in particular.[6]

First, there was the synthesis of the ancient sciences (including medicine) of the Egyptian and Mesopotamian civilizations in Greek life and thought, centered in Alexandria. Only in the light of recent archeological and anthropological discoveries have we discovered the high level of scientific sophistication that was present in these two kingdoms. The medical knowledge attributed to these earlier civilizations became forged in the fire of the Greek intellect into an even more systematic model of diagnostics and therapeutics.

Second, the main centers of Greco-Hellenistic learning were pushed farther East as a result of a decline of intellectual energies due to shifting political and economic realities constantly confronting and fracturing Western Christendom. This encouraged two other civilizations to take up the reins of the transmission of essential elements of Greek knowledge: Persian and Harrānian (i.e., Sabaean). The Sabaeans were independent heirs to the Babylonian and Greek scientific estates through their reliance on the wisdom contained in Hermeticism and neo-Pythagoreanism. Also, it was in the city of Jundishapur that the Persians vivified the sciences coming out of India and China.

In summary, the "scientific knowledge that originated in India, China, and the Hellenistic world was sought out by Arab and Muslim scholars and then translated, refined, synthesized, and augmented at different centers of learning, starting at Jundishapur in Persia around the 6th century—even before the coming of Islam—and then moving

TABLE 49.1 Comparing the Three Great Traditional Healing Systems With Modern Western Medicine[8]

	Unani	Ayurveda	Chinese	Modern Western
Origins	Persia; circa 980 AD	India; circa 2000 BC	China; circa 2700 BC	Europe; United States; late 19th century
Primary dynamic elements	Rūh (spirit force)	Prana (life breath)	Chi (life energy)	Brain and heart
Disease correlates	Humors	Tridoshas	Yin–yang; chi	Named pathology
Disease causes	Imbalance of humoral temperament	Ama is the "harbinger of misery"; the cause of disease	Systemic imbalances; no overriding emphasis on one	Bacteria; virus; fungus; parasite; metabolic disturbances; trauma
Basis of diagnosis	Humors: blood, phlegm, yellow bile, black bile	Tridosha: Vata, Pitta, Kapha	Four diagnoses of TCM	Based on patient's history, physical examination, laboratory testing
Diagnostic models	Restore balance to humors and organ systems	Concept of Shiva-Shakti; balance in the tridosha or three humors system	Achieve balance of yin (passive) and yang (active) physiological functions	Specifically named pathology
Chief diagnostic modality	Differential; mizāj or temperament assessed for each of four humors	Differential; states of consciousness aligned with each of the three humors	Differential, questioning, observation, palpation, and listening; Zang Fu organ syndromes	Differential; named disease
Diagnostic tests	Observation, lifestyle, pulse, urine, stool, and palpation	Tongue, pulse, urine, and palpation	Tongue, pulse, and palpation	Urinalysis, x-rays, and standard blood tests; sampling organ tissues; diagnostic x-rays; angiography (Note: in the United States, as a whole, about 8.5 billion laboratory diagnostic tests were done in 1996.)
Pulse diagnosis	Reveals humoral imbalance in organ system. Taken with three fingers at radial pulse of wrist; more than 1,000 potential factors evaluated in seconds	Correlates pulse to the tridosha or three humors Taken by the index finger of the physician; qualities of pulse are described in terms of several animals	Direct manifestation of the circulatory energy of the body; classical five phase pulse correspondences Taken on the wrist; about 40% reliable as a sole diagnostic method by most TCM practitioners	Speed: fast pulse to slow pulse
Elements of nature	Four: fire, air, water, and earth	Five: fire, earth, water, air, and ether	Five: fire, earth, metal, water, and wood	22 basic elements of chemistry
Main dietary influences	Nonalcoholic; regular fasting; nonporcine	Vegetarian	Rice and vegetables	High refined sugars; alcohol; fats; drugs
Patient participation and will	Empower patient to make changes in diet and lifestyle	High objectivity	Personal determination	Not significant

TABLE 49.1 Comparing the Three Great Traditional Healing Systems With Modern Western Medicine[a]—cont'd

	Unani	Ayurveda	Chinese	Modern Western
Deity of system	Abrahamic monotheism; primarily God (Allah) of Islam	Polytheistic; Panentheistic; Nondualistic Advaita Vedanta (Brahma Siguna, Brahma Nirguna); God of Hinduism	Nontheistic; Confucianism; Taoism (Tao); Buddhism (Amida Buddha, Avalokiteshvara)	Secular atheism; agnosticism; modern evolutionary nihilism
Primary treatment modalities	Diet; herbs; fasting; cupping; purgation; baths; attars (essential oils or medicinal scented perfumes)	Pancha Karma (detoxification);[b] herbs; diet; emetic therapies	Acupuncture, herbs; cupping; moxibustion; diet	Chemotherapy; radiation therapy; pharmaceutical drugs; surgery; rehabilitative physical therapy
Primary treatment objective	Mizān: restore to balance; provoke "the healing crisis"	Clear the entire GI tract; regulate the bowels; improve digestion	Tonification of energy	Symptom suppression; kill germs and bacteria; palliative end-of-life management
Some instruments used	Glass cups	Glass cups	Glass cups; acupuncture needles	Ophthalmoscope, laryngoscope, and x-ray; sphygmomanometer; electrocardiogram; chemical tests of body fluids and tissues
Side effects	Overdose of herbal substances; very rare	Overdose of herbal substances; very rare	Potential for acute symptoms from improper needle techniques; overdose of herbal substances; all very rare	106,000 die annually from improper medications; severe and frequent drug reactions;[c] very common
Cancer rates (WHO rates out of 93 countries)	6th lowest	6th lowest	30th lowest	93rd lowest (worst of all)
Annual per capita health care expenses	$9.45 Source: World Bank	$9.45 Source: World Bank	$3.96 Source: World Bank	$1,301.00 Source: World Bank
Common medicines used	Senna pods, black seed, cumin, ginger	Amla; guggul; Bibhitaki; Triphala	Ginseng, codonopsis, dang quai, astragalus	Antibiotics, antidepressants, corticosteroids, analgesics
Chief complaints	Nonregulation of practitioners; lack of clinics	Nonregulation of practitioners; lack of clinics	Obtuse language	Adverse reactions; patient dissatisfaction; skyrocketing medical costs
Direction of development	Training practitioners in powers of observation; building schools; sources for formulations	Training practitioners; building schools; develop formulations	Integration with Western hospital medicine	Higher costs; more complex diagnostics; genetic medicine; shaped by new healthcare reform laws
Typical U.S. $ cost of treatment	$15–$200	$150–$200	$45–$300	$200–$4,000

GI, gastrointestinal; *TCM,* traditional Chinese medicine; *WHO,* World Health Organization.

[a]Modified from the American Institute of Unani. Comparison of healing systems. http://www.unani.com/comparison.htm. Accessed April 23, 2012.

[b]From Vasant Lad. An Introduction to Panchakarma. http://www.ayurveda.com/pdf/pk_intro.pdf.

[c]"A 1998 report estimated that 106,000 Americans die each year as a result of adverse reactions to prescription medications. This figure represents three times the number of people killed by automobiles and is the fourth leading cause of death in the United States. Only heart disease, cancer, and stroke kill more Americans than adverse reactions to drugs. This staggering figure does not include drugs administered in error or those taken as a suicide gesture. If medication errors were included in this statistic, the death toll would probably be as high as 140,000 deaths per year. As a result of 39 separate studies nationwide, it was found that 3.2 out of every 1000 hospitalized patients die each year as the result of adverse reactions to prescription drugs in each and every hospital in this country. Of the 106,000 people killed each year by an adverse reaction to a prescription drug, 43,000, or 41%, were initially admitted to the hospital because of the adverse drug reaction. The other 59%, or 63,000 patients, were hospitalized for some other cause but developed a fatal reaction to a prescription drug received while hospitalized." (Montague P, National Writers Union, UAW Local 1981/AFL-CIO. Another kind of drug problem. *Rachel's Environment & Health Weekly* 1999;632. http://www.drsuzy.com/summaryInterest.html.)

to Baghdad,[7,8] Cairo, and finally Toledo and Cordoba, from whence this knowledge spread to Western Europe."[3]

The Torchbearers of Islamic Medicine

With the advent of the Islamic spiritual tradition, one must mention the intellectual power and genius found in the physicians of Islam. For if history did not give birth to these giants in the field of medicine, one could not really speak in the present tense at all of contemporary Unani medicine, let alone its subtle historical influences on the character of modern conventional medicine.

Ibn Ishāq (Johannitus Onan), 809–873 AD

Hunayn ibn Ishāq al-ʿIbādi was "the greatest of all translators of this period … [and] an outstanding physician of his day. [He] often translated texts from Greek into Syriac. At other times he would translate directly from Greek into Arabic."[6] He was "reputed to have been paid

for his manuscripts by an equal weight of gold. He and his team of translators rendered the entire body of Greek medical texts, including all the works of Galen, Oribasius, Paul of Aegin, Hippocrates, and the *Materia Medica* of Dioscorides, into Arabic by the end of the ninth century. These translations established the foundations of a uniquely Arab medicine."[3]

Al-Tabari, 810–855 AD

Ali ibn Rabbān al-Tabari was the teacher of the great Rhazes. He was the "author of the first major work of Islamic medicine … entitled *Kitāb Firdaws al-Hikma* ("The Book of the Paradise of Wisdom"). In 360 chapters, he summarized the various branches of medicine, devoting the last discourse, which consisted of 36 chapters alone, to a study of Indian medicine (Ayurveda). The work, the first large compendium of its kind in Islam, is of particular value in the fields of pathology, pharmacology, and diet, and clearly displays the synthetic nature of this new school of medicine, now coming into being."[6]

Al-Rāzi (Rhazes), 841–926 AD

Abu Bakr Muhammad ibn Zakariyya al-Rāzi was born in the town of Rayy in Persia, near what is present-day Tehran. He was reputed to be Islamic medicine's greatest clinician. His works include *al-Kitāb al-Mansūri* (*The Book of Mansūr* or the Latin *Liber Almansoris,* in which Rhazes delineated principles of medical theory, diet, pharmacology, dermatology, oral hygiene, epidemiology, toxicology, and even climatology and its effects on the human body), *al-Judari wa al-Hasbah* (*Smallpox and Measles,* which was the first treatise ever written on the subject), and his magnum opus *al-Kitāb al-Hāwī* (*The Comprehensive Work* or the *Liber Continens* of later Latin translators; this monumental collection of 25 volumes contained all the medical knowledge of the age, including the master's own observation and experience; Fig. 49.1).[3]

Fig. 49.1 This memorialized stained-glass window of Al-Razi (Rhazes), the great Persian physician, is found in Princeton University Chapel. (The Revd Dr A. K. M. Adam, https://www.flickr.com/photos/akma/2150287950/)

Ibn Sīna (Avicenna), 980–1037 AD

Abu 'Ali al-Husayn ibn 'abd Allāh ibn Sīna was born in the city of Bukhāra in what is today Uzbekistan. He was the preeminent physician of his time, earning the epithet of "Prince of Physicians." He began studying medicine at the precocious age of 13, became a physician at 16 years of age, attended to kings and princes at 18 years of age, and was appointed as court physician to the ruler of a Persian province at 20 years of age. His literary corpus includes *Kitāb as-Shifā'* (*The Book of Healing,* which was primarily a text of encyclopedic proportions on medicine and philosophy) and *al-Qanūn fi 'l-Tibb* (*The Canon of Medicine,* a one-million-word text summarizing the entire Hippocratic and Galenic traditions and describing the Syro-Arab and Indo-Persian medical practices of his time). *The Canon* quickly was, for several hundreds of years, the standard medical textbook of the Islamic, medieval Christian, and later Indo-Pakistani worlds. Avicenna's *Canon of Medicine* was "a five-volume compendium of Greek and Islamic healing that became one of the principal textbooks in European universities centuries later."[9]

It is from this remarkable polymath that Unani medicine draws its breath of life. Avicenna is perceived by some to be the "father of Unani medicine," and his *Canon* its gospel (Fig. 49.2).[3]

Post-Avicennan Medicine

Egypt and Syria: Ibn Nafīs

Ibn Nafīs was born in Damascus and died in Cairo in the 12th century AD. Ibn Nafīs has only recently gained fame as the rightful discoverer of pulmonary circulation, which was mistakenly thought to have been identified in the 16th century by Michael Servetus. There have been several studies in recent years that incontestably demonstrated that Ibn Nafīs discovered the lesser circulation of the blood before Servetus. See A. O. Soubani's "The Discovery of the Pulmonary Circulation Revisited," in which this medical doctor and researcher

Fig. 49.2 A portrait of Avicenna. It is from this remarkable polymath that Unani medicine draws its breath of life. Thus some historians perceive Avicenna to be the "father of Unani medicine" and his *Canon* its gospel.[4] (Chouchou2017 [CC BY-SA (https:///creativecommons.org/licenses/by-sa/4.0)].)

pooled together and summarized a host of earlier literature regarding this hallmark of medical subjects.[6,10]

Spain and Morocco: Al-Zahrāwī (Albucasis) and Ibn Rushd (Averroes)

Abu al-Qāsim al-Zahrāwī (976–1013 AD) is considered to be the greatest surgical figure in Islamic medicine. He was also original in this medical arena in inventing and manufacturing the requisite surgical instrumentation that did not exist, or existed previously in very crude form only, to conduct operations as painlessly and effectively as possible. Some of these instruments of surgery have not changed significantly in design for more than 1000 years. A more comprehensive sketch of the significance of Albucasis in the field of surgery can be found in Kasule.[11]

Abu al-Walīd Muhammad ibn Ahmad ibn Muhammad ibn Rushd[12] (1126–1198 AD) was born in Cordoba, where he trained in law and philosophy, although he made his living as a physician. He wrote his *Kitāb al-Kulliyyāt* (the Latin translator's Colliget, *The Book of General Principles*) in which he covered the whole field of medicine in abridged form (Fig. 49.3).

The Eastern Lands of Islam, Persia, and India: Al-Jurjāni, Nurbakhski, and Dara Shukūh

Isma'īl Sharaf al-Dīn al-Jurjāni followed Avicenna by one generation, producing the most important medical encyclopedia in the Persian language, the *Zakhira-i Khwarizmshahi (Treasury Dedicated to the King of Khwarazm)*. The size, as well as the style of the work, places it between Avicenna's *Canon* and Rhazes's *Continens*.

Muhammad Husayni Nurbakhski was a physician of 15th-century Safavid, Persia, who wrote a large medical work entitled *The Quintessence of Experience*. He was the first person to identify and treat several common diseases, including whooping cough.

The 16th and 17th centuries were the major centuries in which Islamic medicine spread into India and came to be known as al-Tibb al-Yunānī (Unani medicine). One of the major patrons of the medical arts in India during this time was Dara Shukūh, a Mogul prince who was also a Sufi and a student of the Vedanta. ʿAyn al-Mulk al-Shirāzī was responsible, at least in large part, for composing *The Medicine of Dara Shukūh,* which is the last great medical encyclopedia written in the world of Islam.

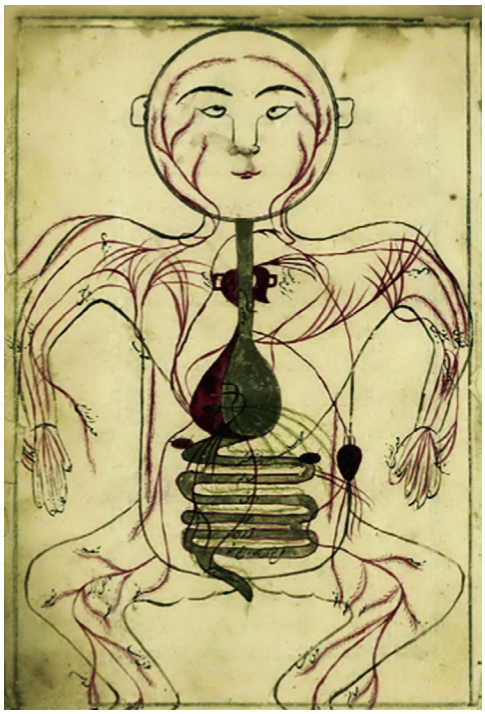


Fig. 49.3 Ibn Naffīs made his discovery of the structure of pulmonary circulation (the "lesser circulation") three centuries before its rediscovery in Europe by Michael Servetus (d. 1553) and Realdo Colombo (d. 1559), both of whom historians mistakenly thought for centuries to have been the original discoverers. (Reproduced with kind permission of Tabriz Central Library.)

METAPHYSICAL AND PHILOSOPHICAL FOUNDATIONS

Unani medicine's philosophical underpinnings are tied to the Islamic spiritual tradition. The true and original meaning of the word *philosophy* as *philo-sophia* ("love of wisdom") has only accidental affinities to what is termed philosophy in the modern lexicon today. Traditional philosophy as *philo-sophia* was, and still is, tied to a spiritual vision of humanity and the cosmos.

Epistemology and Ontology

Epistemology and ontology deal directly with the nature of knowledge—what something "is," how it is obtained, and its methods of use—and the study of existence or "being," respectively. Because Unani medicine is rooted in the Islamic tradition, it is from this spiritual source where one must search for the answers to the questions regarding its theories on knowledge and existence.

Within the Islamic world, as well as other traditional religious worlds, knowledge has a definite hierarchical structure.[13-15] This hierarchy maintains the essential links that exist between Heaven and Earth, between God and humans. First, there exists an acknowledgment of a presiding Divine Knowledge (i.e., God as wise and all-knowing). Following this is the knowledge found in Revelation (i.e., a perfect dispensation from Heaven that enlightens the human intellect).[16] As this revelatory knowledge sparks the heart's depths, it necessarily begins to involve the mind–intellect in its quest for knowledge of this earthly domain (ʻilm al-dunya), this physical world.

Ontologically, the "being-ness" of humanity is intimately connected with the Being of God. God is seen as the Supreme Being Alone, without whom nothing can rightfully claim existence for itself. Thus God's Being is termed Wājib al-Wujūd ("Necessary Being"). In this light, humanity—and everything else in the world of forms—is seen as "borrowing" its existence from the indispensable nature of Divine Being.

Cosmology and the Human Microcosm

The basic cosmologic premise in Islam, as well as in other traditions such as Hinduism, Christianity, and Judaism, is that the Absolute Truth of Divinity (al-Haqq) manifests Itself in the world of forms and within the human heart in a process called the "arc of descent."[17] There is in response to this arc of descent of the Divine a reciprocal "arc of ascent" of the human spirit.[18] These adwār ("arcs" or "cycles") of descent and ascent relate directly to the interdependence of all things on all levels of existence. Everything in creation is perceived as acknowledging, in one form or another, the all-pervading Divine Presence. From the traditional view, the human being is seen as the most concentrated theophany (i.e., a locus of Divine Presence in the world of forms), especially as it concerns the qualities of an active intellect and free will. According to the Hermetic dictum, "As above, so too below," the human being acts as a mirror to the Divine. Furthermore, the Sufis (the mystics of Islam) have a popular saying that addresses this reality on the cosmic scale: al-insān qawn saghīr wa 'l-qawn insān kabīr ("The human is a small universe, and the universe is a large human").

The guiding principle of the dynamic interplay between the Divine Order and the rest of creation is called Tawhīd ("the principle of Divine Unity"). All of the traditional sciences in Islam agree that there exists a dependence of creation on the Divine, an interdependence between all forms in the cosmos, and an intradependence of all elements within each specific form. There is an obvious analogy to the profound knowledge found in that sacred oriental symbol of the Tao's tai chi ("Supreme Principle of Unity") with the complementing and harmonizing forces of yin and yang. This is also known in Islamic cosmologic language as al-wahdah fi 'l-kathrah wa 'l-kathrah fi 'l-wahdah ("the one in the many and the many in the one").

Unani medicine bases its medical theories of diagnosis and treatment on this specific understanding of the intradependent relationships that exist between the four humors as it concerns the subtle (latīf) aspects of human physiology and spiritual psychology (Fig. 49.4).[19]

Teleology and Spiritual Correspondences

The teleological component of Unani medicine's philosophy entails extracting "meaning" and "purpose" in relation to the realities of creation and the human experience. To what end is human life? What is the goal of all of creation? Is there a plan or purpose that governs the created order and all of its particular elements?

The Qur'ān explicitly states that inna li 'Llāhi wa inna ilayhi rāji'ūn ("Verily we belong to God, and to Him is our return"). The human race hurls itself forward, in time and space, toward an inevitable reunion with its Creator. It is this understanding of the nature of things that permits traditional hakīms (the "doctors" of the Unani medical tradition) to find solace in the fact that every patient is ultimately a patient at the doorsteps of the Divine Doctor.

The notion of forcing "heroic" measures upon the sacred human frame is problematic to the philosophical principles upon which Unani medicine is based. This is not to say that surgery and trauma care are perceived in a negative light in Unani medicine. Rather, the question is really one of proportions. The traditional Tabīb ("man of medicine," or physician) always has an eye to the Divine within the human temple. This is really a cultivated virtue that the hakīms practice as part of their art to remain true to the doctrine that "God is everywhere present"; that in keeping with the wisdom of Plato, it is the "eye of the soul" that witnesses "tō Agathōn" ("the Supreme Good"). Therefore any decision made by a hakīm must include an analysis of the risk-to-benefit ratio within the larger ethical framework of the Divine's presence in human life. As a prayerful supplication of ʿAli, the son-in-law of the Prophet Muhammad and the spiritual pole of Shiite Islam, says: Yā man Ismuka dawā', wa dhikruka shifā' ("Oh Ye whose Name is a sacred medicine, and in whom remembrance of Thee [dhikruka] is a healing balm …").

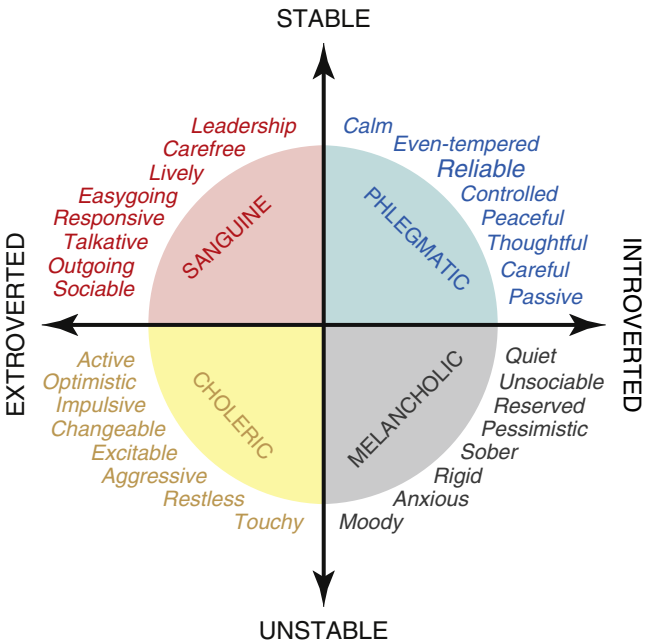


Fig. 49.4 The four humors in psychological profile. (Nada Pop-Jordanova, Olivera Sarakinova, Silvana Markovska-Simoska, Sofija Loleska, Anxiety and Personality Characteristics In Children Undergoing Dental Interventions, Contributions. Sec. Med. Sci., XXXIV 3, 2013. UDC: 616.89-008.441.1-02:616.31-053.2.)

UNANI MEDICAL THEORY IN PRINCIPLE

Vis Medicatrix Naturae

It is only with the "eye of the heart" (ayn al-qalb) that the doctors of Unani medicine are able to gain proper intuition of vision concerning the state of the jism al-latīf (the "subtle body") of the patient in whom is found the flowing, dynamic streams of the humors. It is at the level of the subtle body that the hakīm desires to enliven the self-healing power, the vital force, or the *vis medicatrix naturae* to affect a change in the patient's being in the direction of health and wellness. It is this particular "organizing principle" (al-quwwa al-mudabbirah), as a component of the hidden physiology of the subtle body, that affects a harmonizing reaction in the face of "disease" or illness. One may perceive the vis as being the thread that holds the fabric of the human body, mind, and soul together in close conjunction with the spirit. This is the general medical theory of the "healing power of nature" that Unani medicine posits as a guiding principle, on which is built more complex specific understandings of human health and disease.

A Personal Thought on the Invisible Vis or Quwwa

We encounter many opportunities in life that allow us to witness obvious demonstrations of the *vis medicatrix naturae*. An everyday example of the obvious workings of the vis is a paper cut. Many of us have had this experience and have considered it a very irritating situation. However, if we look carefully at what is actually happening, we cannot but assume that the "natural healing instinct" in the human body, when undergoing injury or trauma, is to mobilize mechanisms that will allow for self-preservation and homeostasis, which would be disorder and chaos. Why should the body even mobilize these mechanisms to begin with? We know to a great degree the science behind these mechanisms, but what we do not know is why these mechanisms should initiate such a nonentropic response. This is still a mystery.

As simple as a paper cut is, it is usually followed by a natural biochemical and physiological inclination toward wound healing (homeostatic repair). We usually never see a paper cut to a finger progressively develop into an open wound, fester with pus and infection, turn gangrenous or necrotic, and die, necessitating amputation of the finger; this is simply not the natural, instinctual response of the human body. In actuality, what we do see happen is the influence of this organizing principle—call it what you may: qi, prana, vis, quwwa, ki, and so forth—leading the organism's biochemistry toward a healing homeostasis, not a death-inducing "diseased" disturbance.

There is yet another common example that relies heavily on the signaling of this "power" or "quwwa" for healing, and that is surgery. How many times have we heard what surgeons say after surgery? It usually goes something like "Well, it's now up to the patient to heal"

or "I've done all I can; the rest is up to the patient" or "I look forward to seeing you for your follow-up to see how you're healing." What do statements like this really mean? I believe that what is actually being invoked is a subconscious acknowledgment of and reliance upon the vis. However, because the language of modern conventional medicine does not give this ever-present healing power a name, it is vicariously mentioned under ambiguous terms, phrases, and allusions. All other traditional systems of medicine in the world have a name for this "elephant in the room" as a distinct reality.

The Doctrine of the Seven Naturals

There are hosts of medical systems around the world with their own understanding of what constitutes the primary and basic functional components that cause health to reign or disease to take root, progress, and finally manifest in constellations of signs and symptoms of illness. In Chinese medicine, we have physiological concepts such as qi, blood, yin, and yang. In conventional medicine, physiology is dominated by the key concepts of organic issues relating to organ tissue morphology (anatomy) and fluid dynamics (medical biochemistry; Fig. 49.5).

In Unani medicine, these concepts fall under the strict heading of umūr al-tabīʿiyyah ("principles of natural physiology"), comprising the "Seven Naturals" that are the pillars and determinants of health. These are as follows (Fig. 49.6):

Elements (arkān)
Temperaments (mizāj)
Humors (akhlāt)
Organs (aʿdā')
Forces, drives, faculties, or powers (quwwāt)
Actions or functions (afʿāl)
Pneuma or spirit substance (rūh)

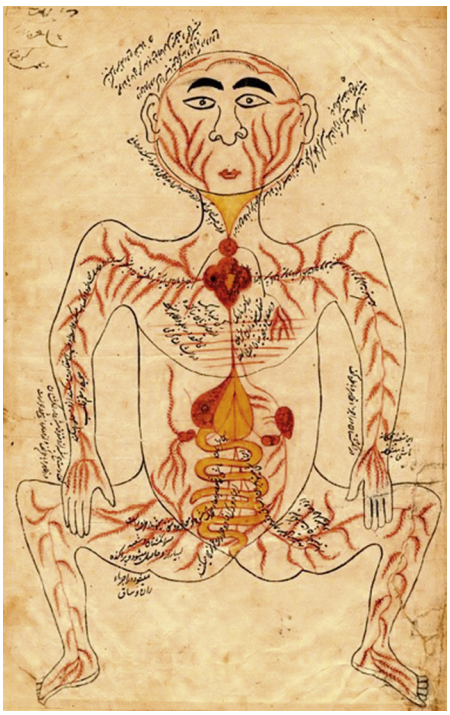


Fig. 49.5 Mansūr ibn Ilyās's 14th-century work on anatomy contained illustrated chapters on five systems of the body: bones, nerves, muscles, veins, and arteries. The page shown depicts the arteries with the internal organs shown in watercolors.

Elements, Qualities, Properties, and States

The elements are the simple building blocks of matter in nature, including the human body. They are nondivisible, but they do have qualities that are both active and passive that determine the states of matter. They are as follows:

Fire (nār), which is hot and dry (hār and yābis): determines the energetic state.
Air (hawā'), which is hot and wet (hār and ratab): determines the gaseous state.
Water (mā'), which is cold and wet (bārid and ratab): determines the liquid state.
Earth (ardh), which is cold and dry (bārid and yābis): determines the solid state.

These elements do not mean literally clods of dirt, buckets of water, and so forth. "Likewise, the burning fire that we see is not the element fire, which is really the potentiality of fire within the substance."[20]

Temperaments

The interplay between the qualities of the elements leads ultimately to a uniform configuration of a temperament. "Each [Temperament] is named after a certain Humor, and is characterized by the predominance of that Humor and its associated basic qualities."[21] The classical temperaments correlate well to today's psychological constitutional types: choleric, melancholic, phlegmatic, and sanguine. Imbalances of temperaments cause disharmony, especially on the mental and emotional fronts. The following is a concise, but thorough medical depiction of the temperaments (Table 49.2).

Humors

There is no doubt that the principle of the humors (Akhlāt) originated with the "father of medicine," Hippocrates, describing it in his book entitled *Tabīʿat al-Insān.*[22] He observed upon examination of blood

Fig. 49.6 A metaphysical vision of the Islamic cosmos: metacosm, macrocosm, microcosm. This depiction shows the seven planetary spheres dominated by seven prophetic presences (in time and space), surrounded by the lunar and zodiacal influences and encompassed by the angelic sphere signifying the Divine as the Immanent Sacred Presence and the Transcendent Beyond Being. (From Celestial map, signs of the Zodiac and lunar mansions in the Zubdat-al Tawarikh, dedicated to the Ottoman Sultan Murad III in 1583.)

TABLE 49.2 The Four Different Temperaments

SANGUINE TEMPERAMENT

Humor	Blood
Basic qualities	Hot and wet
Face	Oval or acorn-shaped face and head. Delicate, well-formed mouth and lips. Beautiful almond-shaped eyes, often brown. An elegant, swanlike neck.
Physique	In youth, balanced, neither too fat nor too thin. Moderate frame and build. Elegant, statuesque form, with ample, luxuriant flesh. Joints well-formed; bones, tendons, veins not prominent. Can put on weight past 40 years, mostly around hips, thighs, buttocks.
Hair	Thick, luxuriant, wavy. Abundant facial and body hair in men.
Skin	Pink, rosy, blushing complexion. Soft, creamy smooth, luxurious feel. Pleasantly warm to the touch.
Appetite	Quite hearty, often greater than digestive capacity. A predilection for rich gourmet foods.
Digestion	Good to moderate; balanced. Can be overwhelmed by excessive food.
Metabolism	Moderate, balanced. Bowel tone can be a bit lax.
Predispositions	Metabolic excesses of the blood. Uremia, gout, diabetes, high cholesterol. Intestinal sluggishness, putrefaction. Congested, sluggish liver and pancreas. Congested blood, bleeding disorders. Respiratory catarrh, congestion, asthma. Urinary conditions, genitourinary disorders. Excessive menstruation in women. Skin conditions, hypersensitivity, capillary congestion.
Urine	Tends to be rich or bright yellow and thick.
Stool	Well-formed, neither too hard nor too soft.
Sweat	Balanced, moderate.
Sleep	Moderate, balanced, sound. Can be some snoring.
Dreams	Usually pleasant, of a charming, amusing, romantic nature. Travel, enjoyment, games, distractions.
Mind	Faculty of judgment well developed. A synthetic intellect that likes to see the whole picture. An optimistic, positive mental outlook. Rather conventional and conformist; good social skills.
Personality	Exuberant, enthusiastic, outgoing. Optimistic, confident, poised, graceful. Expansive, generous. Romantically inclined; loves beauty, esthetics, arts. Sensual, indulgent nature. Sociable, gregarious, light-hearted, cheerful.

CHOLERIC TEMPERAMENT

Humor	Yellow Bile
Basic qualities	Hot and dry
Face	Broad jaw. Sharp nose, high cheekbones. Sharp, angular facial features. Reddish face common. Sharp, fiery, brilliant, penetrating eyes and head.
Physique	Compact, lean, wiry. Good muscle tone and definition. Prominent veins and tendons. Broad chest common. An active, sportive type. Weight gain usually in chest, arms, belly, upper body.
Hair	Often curly. Can also be thin, fine. Balding common in men. Blonde or reddish hair common.
Skin	Ruddy or reddish color if heat predominates; sallow or bright yellow if bile predominates. Rough and dry, quite warm.
Appetite	Sharp and quick. Soon overcome by ravenous hunger. Fond of meat, fried foods, salty or spicy foods, alcohol, intense or stimulating taste sensations.
Digestion	Sharp and quick. Tendency toward gastritis, hyperacidity, acid reflux. When balanced and healthy, can have a "cast iron stomach," able to digest anything.
Metabolism	Strong, fast, active; catabolic dominant. Strong innate heat of metabolism. Liver and bile metabolism can be problematic. Digestive secretions strong, bowel transit time short. Adrenals, sympathetic nervous system dominant. Strong inflammatory reactions.
Predispositions	Fevers, infections, inflammation. Hives, rashes, urticaria. Fatty liver, bilious conditions. Hyperacidity, acid reflux, inflammatory and ulcerative conditions of middle GI tract. Headaches, migraines, irritability. Eyestrain, red sore eyes. Purulent conditions. High cholesterol, cardiovascular disorders. Gingivitis. Bleeding disorders from excess heat, choler in the blood. Hypertension, stress.
Urine	Tends to be scanty, dark, thin. Can be hot or burning.
Stool	Tends toward diarrhea, loose stools. Can have a yellowish color, foul odor.
Sweat	Profuse, especially in summer, or with vigorous physical activity. Strong body odor. Sensitive to hot weather, suffers greatly in summer.
Sleep	Often fitful, restless, disturbed, especially with stress, indigestion. Often tends to wake up early or in the middle of the night.
Dreams	Often of a military or violent nature. Dreams of fire, red things common. Fight or flight, confrontation.
Mind	Bold, daring, original, imaginative, visionary. Ideation faculty well-developed. Brilliant intellect; sharp, penetrating insight. The idea man who prefers to leave the details to others.
Personality	Prone to anger, impatience, irritability; short temper. Bold, courageous, audacious, confrontational, contentious. Dramatic, bombastic manner; high powered personality. The rugged individualist and pioneer; thrives on challenge. The fearless leader. Seeks exhilaration, intense experiences. Driven, "Type A" personality. Prone to extremism, fanaticism.

TABLE 49.2 The Four Different Temperaments—cont'd

MELANCHOLIC TEMPERAMENT

Humor	Black Bile
Basic qualities	Cold and dry
Face	Square or rectangular head and face. Prominent cheekbones, sunken hollow cheeks common. Small, beady eyes. Teeth can be prominent, crooked, or loose. Thin lips.
Physique	Tends to be thin, lean. Knobby, prominent bones and joints common. Prominent veins, sinews, tendons. Muscle tone good, but tends to be stiff, tight. Rib cage long and narrow, with ribs often prominent. Can gain weight in later years, mainly around midriff.
Hair	Color dark, brunette. Thick and straight. Facial and body hair in men tends to be sparse.
Skin	A dull yellow or darkish, swarthy complexion. Feels coarse, dry, leathery, cool. Calluses common.
Appetite	Variable to poor. Varies, fluctuates according to mental or emotional state.
Digestion	Variable to poor; irregular. Digestion also varies according to mental or emotional state. Colic, gas, distension, bloating common.
Metabolism	Often slow. Can also be variable, erratic. Prone to dehydration. Nervous system consumes many nutrients, minerals. GI function variable, erratic; digestive secretions tend to be deficient. Blood tends to be thick. Nutritional deficiencies can cause a craving for sweets, starches. Thyroid tends to be challenged, stressed.
Predispositions	Anorexia, poor appetite. Nervous, colicky digestive disorders. Constipation. Spleen disorders. Nutritional and mineral deficiencies, anemia. Blood sugar problems, hypoglycemia. Wasting, emaciation, dehydration. Poor circulation and immunity. Arthritis, rheumatism, neuromuscular disorders. Nervous and spasmodic afflictions. Dizziness, vertigo, ringing in ears. Nervousness, depression, anxiety, mood swings.
Urine	Tends to be clear and thin.
Stool	Can either be hard, dry, compact; or irregular, porous, club-shaped. Constipation, irritable bowel common.
Sweat	Generally scanty. Can be subtle, thin, furtive, indicating poor immunity. Nervous stress can increase sweating.
Sleep	Difficulty falling asleep, insomnia. Stress, overwork, staying up late aggravates insomnia. Generally a light sleeper.
Dreams	Generally dark, moody, somber, disturbing. Themes of grief or loss common.
Mind	An analytical intellect; detail oriented. Efficient, realistic, pragmatic. Reflective, studious, philosophical. Retentive faculty of memory well-developed. Thinking can be too rigid, dogmatic. A prudent, cautious, pessimistic mental outlook.
Personality	Practical, pragmatic, realistic. Efficient, reliable, dependable. A reflective, stoic, philosophical bent. Can be nervous, high strung. Frugal, austere; can be too attached to material possessions. Serious, averse to gambling and risk taking. Can be moody, depressed, withdrawn. Can easily get stuck in a rut. Excessive attachment to status quo.

PHLEGMATIC TEMPERAMENT

Humor	Phlegm
Basic qualities	Cold and wet
Face	Round face; full cheeks, often dimpled. Soft, rounded features. Double chin, pug nose common. Large, moist eyes. Thick eyelids and eyelashes.
Physique	Heavy frame, stout, with flesh ample and well-developed. Often pudgy, plump, or overweight; obesity common. Joints dimpled, not prominent. Veins not prominent, but can be bluish and visible. Lax muscle tone common. Feet and ankles often puffy, swollen. Women tend to have large breasts. Weight gain especially in lower body.
Hair	Light-colored, blondish hair common. Light facial and body hair in men.
Skin	Pale, pallid complexion; very fair. Soft, delicate, cool, moist skin. Cool, clammy perspiration common, especially in hands and feet.
Appetite	Slow but steady. Craves sweets, dairy products, starchy glutinous foods.
Digestion	Slow but steady to sluggish. Gastric or digestive atony common. Sleepiness, drowsiness after meals common.
Metabolism	Cold, wet, and slow. Conserves energy, favors anabolic metabolism. Congestion, poor circulation, especially in veins and lymphatics. Kidneys slow, hypofunctioning, inefficient. Adrenals and thyroid tend toward hypofunction; basal metabolism low. Metabolic water drowning out metabolic fire.
Predispositions	Phlegm congestion. Water retention, edema. Lymphatic congestion, obstruction. Poor venous circulation. Gastric atony, slow digestion. Hypothyroid, myxedema. Adrenal hypofunction. Weight gain, obesity. Frequent colds and flu. Chronic respiratory conditions, congestion. Swollen legs, ankles, feet. Cellulite. Poor tone of skin, muscles, and fascia.
Urine	Tends to be clear or pale and thick. Tends to be scanty in volume, with excess fluid accumulation in the body.
Stool	Well-formed, but tends to be slightly loose, soft. Bowels tend to be sluggish.
Sweat	Cool, clammy sweat common, especially on hands and feet. Sweating can be easy and profuse, especially with kidney hypofunction. Sensitive to cold weather; suffers greatly in winter.
Sleep	Very deep and sound. Tends toward excessive sleep, somnolence. Snoring common; can be loud or excessive.
Dreams	Generally very languid, placid. Water and aquatic themes common.
Mind	Tends to be dull, foggy, slow. Slow to learn, but once learned, excellent and long retention. Patient, devoted, faithful. Faculty of empathy well-developed. Sentimental, subjective thinking. A calm, good-natured, benevolent mental outlook.
Personality	Good natured, benevolent, kind. Nurturing, compassionate, sympathetic, charitable. Great faith, patience, devotion; tends to be religious, spiritual. Sensitive, sentimental, emotional, empathetic. Passive, slow, sluggish; averse to exertion or exercise. Calm, relaxed, takes life easy. Excessive sluggishness can lead to depression.

GI, gastrointestinal.

From Osborn D. The four temperaments. http://www.greekmedicine.net/. Accessed April 23, 2012. Used with permission.

that the red portion of fresh blood is the blood humor (Dam; corresponding to the sanguinous temperament), the white material mixed with blood is the phlegm humor (Balgham; related to the phlegmatic temperament), the yellow-colored froth on top is the yellow bile humor (Safra; corresponding to the choleric temperament), and the heavy part that sediments down is the black bile humor (Sauda; related to the melancholic temperament).[20]

It is important to realize that the humor associated with the blood and called "sanguine" is not identical with the blood drawn by phlebotomy. Nor is the phlegm the same physical phlegm produced by the lungs. Cameron Gruner, MD, called them "quasi-material," or what some have termed "semi-gaseous vapors."[20]

Similarly, qi is beyond physical or corporeal form. Qi and the Seven Naturals are really and truly best understood as a "formless substance," often referred to in the language of philosophy as a Platonic "form" or "idea."[23] Ultimately, it is understood that true "health is a harmony of [these] humors."[20]

Organs

The organ component in the doctrine of the Seven Naturals is actually very straightforward. There are four primary organs that the rest of the organs support and whose work they may enlist. These are as follows:
Heart: the seat of vital power and heat
Brain: the seat of sensations and movements
Liver: the seat of all vegetative, nutritive, and eliminative powers
Gonads: the seat of generative capacity for reproduction of the species

The medical traditions of antiquity all agree that when considering an approach that is centered on health, wellness, and prevention, the two most important organs to focus emphasis on are the liver and the colon. Modern medicine focuses its emphasis on the heart and the brain, for good reason. "Miracles" occur every day in emergency rooms, operating rooms, trauma care facilities, burn units, first-line responder ambulance services, life flight transportation services, etc. "In fact, emergency medicine is one of the most legitimate and impressive achievements of [modern] orthodox medicine."[20]

Forces, Energies, Faculties, Drives, or Powers

The groundwork energies that animate the Seven Naturals are termed forces or faculties (quwwāt). These forces are the legitimate activating principles for the functioning of the humors. Without the quwwāt, there would be no accomplishment of organ function or of anything else. There are primarily three faculties that give rise to a variety of functions. These faculties are:

Natural (as in "from the world of nature") or vegetative faculty (quwwah tabīʿiyyah): governs the nutritive power of the liver and reproductive powers of the generative organs.

Vital or animal faculty (quwwah haywāniyyah): responsible for preserving the integrity of the vital force.

Psychic or soul faculty (quwwah nafsāniyyah): controls the brain and the rational faculty. "It is of significance to note that in Arabic as in many other languages the words for breath (nafas) and soul (nafs) are related. Therein lies a profound cosmological principle which is also related to the invocation of the Name of God (dhikr) as the central technique of Sufism for spiritual realization."[4]

Actions or Functions

Functions or actions (afʿāl) are the manifesting activities of the previously mentioned humoral and organ powers. The functions ultimately are the by-products of the will or power of the humors and organs. For example, the heart's function is to beat; the stomach's function is to receive food and drink and prepare them for further digestive processing; the brain's function is to allow for reception of stimuli, with higher functioning allowing for the expression of reason and thought.

Pneumata or Spirits

The spirit (Rūh) is the otherworldly vehicle for the transmission of that divine spark of life force, a heavenly command that "descends upon this mixture of the humors and which is the subtle body standing intermediate between the physical body comprised of the humors and the force of life which comes from the world above. It is worth drawing attention to the similarity between the words rūh (spirit) and rīh (the wind or air) in Arabic."[4]

> *The spirit or rūh in its medical sense is, according to Muslim physiologists, and following Galen, of three kinds:*
> *Vital Spirit: hot and dry; has its center in the left ventricle of the heart; preserves life; causes the body to grow, move, and reproduce; and travels within the arteries.*
> *Psychic Spirit: cold and wet, has its center in the brain, causes sensation and movement, and moves within the nerves.*
> *Natural Spirit: hot and wet; has its center in the liver; is concerned with the reception of food, growth, and reproduction; and travels within the veins.*[4]

Thus there is an association between physical health and spirituality. "According to some of Avicenna's writings, and in keeping with the Hermetic Tradition, Avicenna sees these natural laws as embodying a component of Divine creative perfection, which to him explains the tendency inherent in natural systems to direct themselves toward a point of … equilibrium."[24]

Another aim toward understanding the physiology and pathology of the doctrine of the Seven Naturals involves answering the question "to what end?" The answer to this has to do with achieving a healthy constitution for every patient by meeting the goals of the six essential causes (of health; al-asbāb al-sittah al-dharūriyyah)[22]:

- Air
- Food and drink
- Body movement and repose
- Mental movement and repose
- Sleeping habits
- Retention and evacuation

It is these basic causal pillars that ultimately guide the hakīm toward satisfying the criteria for health as set forth by Avicenna himself in his *Canon of Medicine,* which are as follows[25]:

- The complexion of an individual is pleasing, with shades and color that are normal to the individual's respective biological environment.
- Body build is medium, neither too lean nor too heavy.
- Hair is not too profuse nor scanty.
- The feel of the body is balanced in respect to heat, cold, moisture, and dryness.
- Sleep and wakefulness are moderate.
- Movements are free and easy.
- Intellectual functions and memory are good.
- Habits and behavior are balanced between timidity and assertiveness, anger and calm, leniency, humor, pride, and humility.
- Growth and repair are rapid, whereas deterioration is slow.
- Dreams are interesting and pleasing.
- Food is enjoyable.
- Food is digested and assimilated normally.
- Excretory functions are regular.

Techniques of Inquiry and Assessment

Pulse

Unani medicine pulse diagnosis as a science takes tremendous practice and requires sensitivity to the characteristics of 10 physical criteria of the radial pulse[20]:
Quality of expansion (length, width, depth)

Quality of impact (strong, weak, moderate)
Duration of cycle (fast, slow, moderate)
Duration of pause (successive, different, moderate)
Between beats (full, empty, moderate)
Compressibility (hard, soft, moderate)
Pulse perspiration (full, empty, moderate)
Regularity
Order and disorder (ordered, irregular, irregularly disordered)
Rhythm (similar, different, out of rhythm)

Avicenna's pulse diagnosis was so profound that he even "recognized 'physiological psychology' in treating illnesses involving emotions. From the clinical perspective Ibn Sina developed a system for associating changes in the pulse rate with inner feelings which has been viewed as predating the word association test of Carl Jung. He is said to have treated a seriously ill patient by feeling the patient's pulse and reciting aloud to him the names of provinces, districts, towns, streets, and people. By noticing how the patient's pulse quickened when names were mentioned, Ibn Sina deduced that the patient was in love with a girl whose home Ibn Sina was able to locate by the digital [pulse] examination. The man took Ibn Sina's advice, married the girl, and recovered from his illness" (Fig. 49.7).[26]

Uroscopy, the "Urine Wheel," and the "Alvine Discharge" (Fig. 49.8)

Unani medicine demonstrates sophistication in realizing that there is much legitimate insight and wisdom to be gained through the macroscopic study of urine (often termed *uroscopy*) and stool. These tissues of the body reveal indices of the body's internal metabolic terrain and activity. For example, "urine wheels" were used by hakīms to forward assumptions about diagnosis or prognosis, treating urine essentially as a "liquid window through which physicians felt they could view the body's inner workings."[27]

"Laboratory medicine began 6000 years ago with the analysis of human urine, which was called uroscopy until the 17th century and today is termed urinalysis. From ancient times until the Victorian era, urine was used as the primary diagnostic tool. Physicians spoke of urine as a 'divine fluid', or a window into the body. Babylonian and Egyptian physicians began the art of uroscopy. Uroscopy, from the word 'uroscopia,' means 'scientific examination of urine.' The word is derived from the Greek 'ouron' meaning 'urine' and 'skopeo', meaning to 'behold, contemplate, examine, inspect.'"[27]

Building on the uroscopy and medicine of Galen, "Theophilus Protospatharius, a seventh century physician, wrote De Urinis. This manuscript from Byzantium was the first publication exclusively on the subject of urine."[27] It was later used in the system of Unani medicine and translated for use down through the European Middle Ages.

In Avicenna's system of urine inspection, "important points to be considered in using urine in diagnosis are color, density, turbidity,

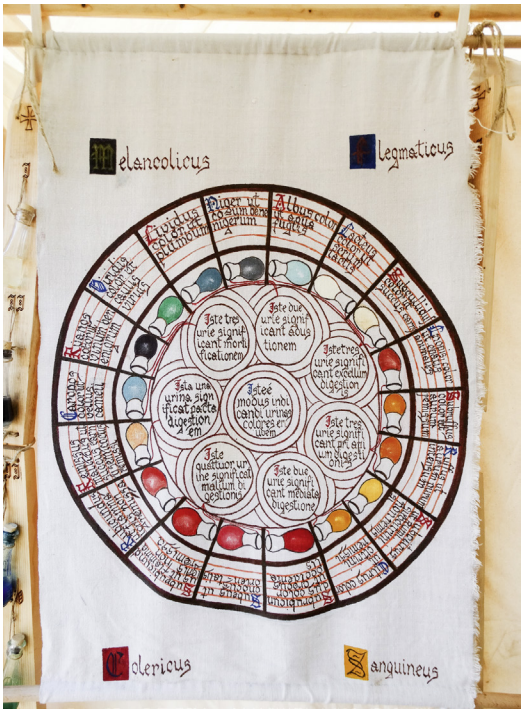


Fig. 49.8 Medieval urine wheel. Labeled in Latin, this 15th-century diagram shows some of the possible colors of urine (outer edge of circle) to help doctors diagnose urine-related disease. Artwork from *Fasciculus Medicinae,* a collection of medieval medical manuscripts published in Europe that date from 1491. (shoricelu/iStock.com.)

Fig. 49.7 Avicenna (Ibn Sīna) Commemorative Medal. UNESCO issued a commemorative medal in 1980 to mark the 1000th anniversary of Ibn Sīna's birth. One face of the medal depicts a scene showing Avicenna surrounded by his disciples (inspired by a miniature in a 17th-century Turkish manuscript), and on the reverse is a phrase by Avicenna in Arabic and Latin: "Cooperate for the well-being of the body and the survival of the human species." (Jaeger, S. (2020). Iran Art Medals - (Artmedals.net) [online] Artmedals.net. Available at: http://www.artmedals.net/iranmedals.html. [Accessed 27 Feb. 2020].)

sediment, quantity, froth, and odor."[25] Of course, one can quickly see allusions in these criteria to even modern macroscopic urinalysis. However, even collection techniques were considered. "Ismail of Jurjani, an 11th century physician, recommended collecting the full amount (of urine) over 24 hours in a large clean vessel and keeping it out of the sun or heat, which could alter color. Ismail also recognized that food and aging altered urine, and required a good night's sleep and empty stomach before collection. He wrote about this in what became the most comprehensive instructional book on urine collection and examination"[27] of his time.

Stool analysis and the patient's history of bowel movements were also tools used by hakīms that allowed for the responsible investigation into the possible causes of illness. Stool has "certain characteristics vis-à-vis color, odor, volume, and consistency," [22] whereas bowel movement history includes "time, duration, and frequency of defecation."[22]

Just to give a brief example, differing stool colors approximate differing illnesses and imbalances of humors. Hakim Moinuddin Chishti summarized this category of investigation by what stool color signifies from an Unani medicine point of view in his book's section called "The Alvine Discharge"[20]:

- White stool: an obstruction of the passages carrying bile; is seen with jaundice.
- Red stool: reveals the crisis point of a disease.
- Black stool: means high oxidation; maturing of a disease caused by imbalance of the black bile humor; bad sign; presence of blood in small or great quantities, which is a sign of diseases of the liver, intestinal ulcer, or rupture of internal vessels.
- Green stool: indicates diminished internal heat.
- Yellow stool: sign of the imbalance of the yellow bile humor (at the onset of illness); is a sign that the body is eliminating harmful substances (at the resolution of an illness).
- Multicolored, pus-filled, sticky stool: very grave sign of the degeneration of internal organs.[20]

UNANI MEDICAL THEORY IN PRACTICE

The Practice Framework

Because of the obvious difficulties that confronted personalized physician-to-patient travel and care, Avicenna drew up plans on the centralizing and standardizing of medical operations for the masses in hospitals. "These hospitals kept records of all their patients and their medical care, something done for the first time in medical history."[26]

"From the tremendous impetus of advancement of medicine supplied by Avicenna, the Arabs took the huddled masses of sick and established them in sleek and elegant hospitals. Their hospitals were immense structures with courtyards and had features such as lecture halls, libraries,[26,28] mosques and chapels (they treated people of all religious beliefs), charity wards, kitchens, and dispensaries. All patients were attended by qualified male and female nurses. The mood at the magnificent Mansūr Hospital of Cairo is reflected in the following account of the amenities arranged for the benefit of all patients:

'Day and night, fifty reciters intoned the Qur'ān aloud. At nightfall, musicians played soft melodies to induce drowsiness in the patients. Professional storytellers entertained the sick with their tales. When the patients left the hospital, they were given enough money so that they would not have to resume work immediately.'"[20]

Furthermore, even before Avicenna's lifetime and progressive influence, "there was a separate hospital in Damascus for lepers, while, in Europe, even six centuries later, lepers were condemned and burned to death by royal decree."[26] The Bimaristans (hospital systems) and smaller "mobile hospitals," which could possibly be regarded as the first ambulances in the world, dedicated to serving under times of civil or community distress, urgency, or emergency,[26] were complex in structure and function.

Therapeutic Modalities

The bridge of therapies, in time and space, between Unani medicine and North American holistic and naturopathic medicine had much to do with the cross-pollinating of research, scholarship, practice, and politics of Europe's (especially old Germany's) "back to nature" movements, which made the trans-Atlantic shift and became rooted in the medical movements known as Eclectic Medicine,[29] Thomsonian Medicine, the American Medical School of Vitalism (which was philosophically opposed to scientific materialism[30]), and the Nature Cure,[31] wherein, like Unani medicine, "the patient is kept close to nature during illness."[22]

Examples of this North American connection with Unani medicine are found in the lives of personalities such as Nicholas Culpeper,[32] Benedict Lust, Henry Lindlahr, or William Kellogg, along with their applied therapies. "By the time of the early eighteenth century, the Tibb system was the basis of virtually all medicine in the civilized world, having been translated and formed as the basis of the work of such men as Father Sebastian Kneipp (1821–1897) and Samuel Hahnemann (1755–1843), the founder of homeopathy, who is reputed to have known Arabic and read Avicenna's works."[20] In this regard, it is important to remember that historically (Fig. 49.9):

> *The practical approaches of the Greeks and Romans were the antecedents of the 19th century nature cure pioneers. They were the only forms of medicine available, and, to this extent, naturopathic and orthodox medicine had what Saks describes as "a relatively undifferentiated past." Indeed, both schools of medicine claim Hippocrates as their progenitor. There is no doubt that Hippocrates set many of the principles we hold dear as naturopathic physicians; in particular, the concept of the healing power of nature (… vis medicatrix naturae)… Hippocrates advocated dietary adjustments,*

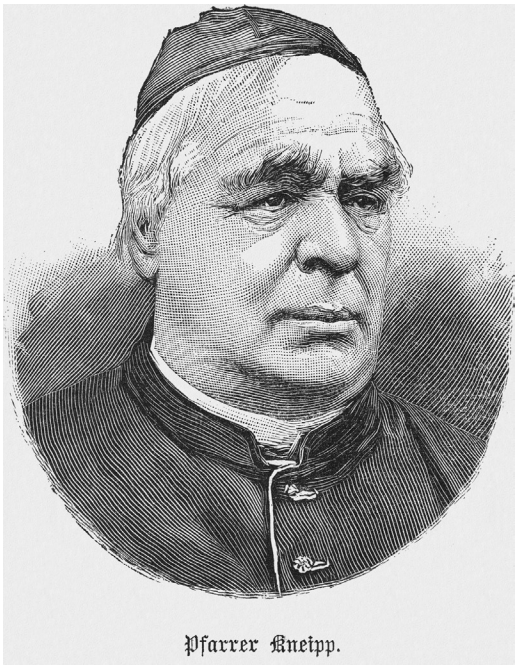


Fig. 49.9 Father Sebastian Kneipp, pioneer of modern hydrotherapy, is shown giving a bath treatment to a patient. (ZU_09/iStock.com.)

fasting, physical therapy, rest, and herbal medicines, but even he and his followers were not above using chemical drugs.[33]

For those who perceive that there is no doubt of Hippocrates's influence on setting the practical and theoretical standards for contemporary naturopathic medicine, then it must hold true that by direct historical relevance—and all that has been stated previously in this chapter regarding this—naturopathic medicine is without question an offspring of the life and thought of the "prince of physicians," Avicenna. It was Avicenna, with his singular intellect, who synthesized and expanded upon all who came before him in his *Canon* and other works and who handed down humoral medicine, along with its practical therapeutics, to generations of physicians after him.

In the late 1950s, a small group of BCNO (British College of Naturopathy, now formally recognized as the British College of Osteopathic Medicine) graduates, medical herbalists, and homeopaths formed the Research Society for Naturopathy to explore a wider range of treatment modalities. They ran seminars on such topics as humoral therapy…[33]

Fig. 49.10 This depiction of the birth of Caesar may be the first illustration of a cesarean section found in the written works of the most famous Muslim chronicler, Al-Biruni (973–1051 ad), as illustrated in the manuscript of al-Bīrūnī, Al-Athār al-Bāqiyah 'an al-Qurūn al-Khāliyah, MS 161, Edinburgh University.

Food-Based or Dietary Therapies

The objective of dietary therapy (ʿilāj bil ghida) is to properly regulate a patient's appetite, in terms of quality or quantity, to maintain the balance of the humors and strengthen the vital force. This is accomplished by prescribing patients food-based protocols, such as restriction, fasting[25,34] (which is an effective, natural process of detoxification and healing[25]), liquid diets, excess eating, semisolid diet, bland diets, and so on.[22] Attention should be paid to the seasons and the requirements of the body in relationship to the environment as to what foods to recommend, depending on the "degree of effectiveness of types of cereals, of leguminous plants, vegetables, root crops, fruits, types of meat, types of milk, of honey and sweet-meats, of water, of drinks, and [edible] aromatic herbs."[8]

Ultimately, "food is one of the most potent and yet safe instruments of establishing balance in a disturbed organism."[25] This is in line with the Hippocratic proclamation, "Let food be your medicine and medicine be your food," as well as conforming to the first principle of therapy, which stipulates "first do no harm." "It is not accidental that the Andalusian physician Abū Marwān ibn Zuhr in the 12th century wrote the first scientific work on diet ever composed, the *Kitāb al-Aghdiyah* ("The Book of Diet")."[4]

Pharmacy, *Materia Medica,* and the Black Seed

Historically, and as far as pharmacology and the *materia medica* is concerned, among the Arab inheritors of Greek knowledge and wisdom, "it is sufficient to say that Dioscorides was indisputably the greatest authority, that his work was many times translated and elaborated"[8] by scholars such as al-Biruni, Ibn-Bajjah (Avempace), and al-Idrisi (the famous geographer whose maps purportedly influenced the maritime travels and adventures of Christopher Columbus; Fig. 49.10).[35]

The predominant therapeutic modality in Unani medicine is without doubt ʿilāj bil dawā' (pharmacotherapy). Raw *materia medica* resources for this therapy came from the plant (90%), mineral (5%), or animal (5%) kingdoms. As Khan noted, "in treatment the principle that each human being is a reflection of all creation is followed by using a whole range of substances."[25]

Some examples of Unani medicine phytotherapy include the conditions and their respective remedies shown in Table 49.3.

The spiritual lore and medicine of the black seed. There is no doubt that the most well-known herb used in Unani medicine, and revered by the cultures of the Middle East and Asia, is the black seed (al-habba al-sauda, or al-habba al-barakah ["the black seed" or "the seed of grace"]). It also goes by the scientific name *Nigella sativa* (Fig. 49.11).[36]

TABLE 49.3 Examples of Unani Medicine Phytotherapy

Condition	Remedy
Fever	Lemon juice with pinch of salt
Constipation	Rose petals with milk
Diarrhea	Amla *(Emblica officinalis)*
Conjunctivitis	Aloe and opium paste
Toothache	Basil leaves paste
Baldness	*Tamarix articulata* in mustard oil
Eczema	Ghee (clarified butter)
Influenza	Long pepper *(Piper longum)*
Asthma	Turmeric powder in milk
Insomnia	Fried cumin seed powder with ripe banana pulp
Menstrual absence	Carrot and black sesame seed cold decoction
Diabetes	*Coccinia, Evolvulus, Eugenia,* and poppy seeds
Obesity	Guggul gum resin *(Commiphora mukul)* powder

Data from Jamal A, and Qadeer HA. *Unani: The science of Graeco-Arabic medicine*. New Delhi, India: Lustre, 1998:25.

The Prophet of Islam specifically mentioned the black seed as he exhorted members of his community to "Use the black seed, which is a healing for all diseases except al-Sām, and al-Sām is Death."[37] With such a pronouncement, one may infer that the prophet was the prototypic hakīm. This observation is important to note because more than 1.6 billion people in the world follow, as closely as possible, the life and thought of the prophet of Islam.

Thus the prophet's statement regarding the black seed elevates and ennobles this plant beyond its investigated scientific benefits.

However, it was posited that "advancements in the methods of analytical chemistry, physiology, pharmacology, and microbiology, etc. have led to the discovery of many active principles of the *N. sativa* [seed] such as nigellicine, nigellidine, nigellimine-N-oxide, thymoquinone, dithymoquinone, thymohydroquinone, nigellone, thymol, arvacrol, oxy-coumarin, 6-methoxycoumarin, and 7-hydroxy-coumarin, alpha-hedrin, steryl-glucoside as well as rich amounts of

Fig. 49.11 The black seed, also known as *Nigella sativa.* (Madeleine Steinbach/Shutterstock.)

Fig. 49.12 Çemberlitaş Hamami, Istanbul, Turkey. Revered Ottoman architect Mimar Sinan built Çemberlitaş Hamami in 1584. It is one of the busiest and most popular due to the separate male and female bathing sections, quality massages, and a location near the Grand Bazaar. (master2/iStock.com.)

flavonoids, tannins, essential fatty acids, essential amino acids, ascorbic acid, iron, and calcium.

"The black seed also has a number of pharmacological effects of profound therapeutic value such as analgesic, anti-inflammatory, anti-histaminic, anti-allergic, anti-oxidant, anti-cancer, immune stimulation, anti-asthmatic, anti-hypertensive, hypoglycemic, anti-bacterial, anti-fungal, anti-viral, and anti-parasitic effects."[37,38]

Environmental Regimens

There are primarily three regimental therapies[22] to take advantage of as part of Unani's movement and manual medicine approach (Fig. 49.12):

- Activity (riyādhāt), which usually means some form of voluntary movement or exercise with the purpose of achieving the following:
  - Organs that perform their functions efficiently
  - Better absorption and assimilation of food and nutrition
  - Clearing of pores in the skin
  - Removal of waste products through the lungs
  - Strengthening the physique
- Massage (tadlīk), which is physical pressure and movement given by another through gentle or aggressive touching, moving, or rubbing of muscles and soft tissues for the purpose of achieving the following:
  - Bracing the body
  - Relaxation response
  - Decreased body fat
  - Enhanced circulation
  - Good organ function
- Thermarium baths (hammāmāt), which are found around the world. These baths sometimes contain mineral constituents (e.g., sulfur) that leech out of the terrestrial basin holding these waters; the benefits of these hot baths include the following:
  - Releasing waste products and impurities through the skin
  - Reducing the viscosity of the humors
  - Reducing obesity

Of these regimental therapies, it is without a doubt the thermo-hydrotherapeutic hammām (the thermarium) that many people may be acquainted with. The hammām is probably more familiar to Western ears as the "Turkish Bath." Worldwide today, there are many medical and recreational resort facilities that incorporate traditional baths of this nature in cavernous, underground, or open-air communal systems.

What follows is an average description of a hammām experience[39]:

> *Traditionally the hammām is made up of a number of chambers with domed white roofs. The "istirāha" is the "rest salon," where visitors are received and un-robed. The inner sanctum of the hammām is usually set down from the istirāha, with further descents into the various steam rooms. The walls of the steam rooms are customarily made from hajar habash (a black stone) which holds heat for a long time. Once you have achieved a good sweat [taʿrīk], you then proceed on to another warm room where the hammāmi greets you.*
>
> *The hammāmi is the person who cleans or exfoliates your body and gives you a [vigorous] massage. One person commented, "I was pummeled, stretched, twisted like a pretzel, and scrubbed with a floor-like brush for about an hour." However, you do emerge glowing and gleaming with your metabolism raring and your fat cells pummeled and detoxified. Finally, you are returned to one of the outer chambers, wrapped up in a robe, and led to a cubicle to rest and relax and enjoy mint tea or juice.*[39]

History records that knowledge of the "medical benefits of the hammām dates back to 200 B.C. when Hippocrates stated, 'Give me the power to create a fever and I shall cure any disease.' Essentially, the steam bath induces a hyperthermia, which raises the body temperature above normal. Hyperthermia stimulates the immune system by increasing production of antibodies and interferon (an antiviral protein with cancer fighting capabilities). A good healthy sweat can help relieve pain and stiffness of joints and muscles, treat respiratory problems like sinusitis, and ward off symptoms of colds or flu. It is also excellent for detoxification, and especially in conjunction with massage it can reduce cellulite by releasing toxins from fat cells. Also, increased blood flow stimulates metabolism and rejuvenates the skin."[39]

In the West, "hydrotherapy enjoyed a great resurgence in 18th and 19th century Austria and Germany, and was a key part of Father Sebastian Kneipp's system of natural therapeutics, which went on to become the basis for Naturopathy. For example, in many European countries that have hot springs or spa resorts, [hydrotherapy] treatments are a recognized subspecialty of medicine."[40]

Surgery, Cauterization, and Bloodletting

In Manfred Ullmann's *Islamic Surveys II* and Seyyed Hossein Nasr's *Islamic Science: An Illustrated Study,* there are illustrated plates demonstrating a cesarean section[8] from an ancient Arabic manuscript; a plate[8] illustrating the diagrams for surgical instrumentation ("Numerous surgical instruments especially various types of scalpels were developed, some quite elaborate and combining as elsewhere utility and beauty, and most have survived relatively unchanged over the ages with only small local variations"[4]) developed and used by

Abu al-Qāsim al-Zahrāwī (known to the West as Albucasis), who was one of the greatest surgeons of antiquity that "took surgery [jarh] out of the hands of barbers and put it into the realm of scientific medicine"[8]; and images of surgical interventions involving hemorrhoidal correction, bone-setting, reductions of dislocations of joints, dental procedures ("Muslim physicians would even make false teeth from the bones of animals[4]), and so forth.[4] "Surgery was also used by the Arabs to correct cataracts."[41]

Many hakīms accept the fact that today, it is probably best to triage the surgical option for patients to surgeons who have been educated and trained in modern medical schools. The same would hold true for cauterization (kay; healing through heating or burning of tissue), which today is accomplished with the help of lasers, bloodletting (fasād; releasing of venous blood to purge toxic or disturbed humoral physiology), and leeching (taʿlīk; the use of leeches to evacuate "corrupt" blood).[42]

Other therapies, such as cupping (hajamāt) and cathartic colonic purgatives (ishāl), are often used hand in hand as supportive or adjunct therapies to surgery as well.

Pneumatology, Wellness, and the "Infallible Remedy"

Hakīms were often part of guilds, craft orders, or philosophical schools of thought that were supported by influential political or religious leaders of their time. Also, these guilds were mostly attached to one of the many mystical brotherhoods of Islam, known as Sufi Tarīqahs (which were spiritually or esoterically oriented "Paths" or "Initiatic Orders" leading to God, realization, and enlightenment for the aspirant).[43]

A hakīm (male doctor) or hakīma (female doctor) was usually invested in practicing more than just his or her medical science but also turning his or her mind, soul, heart, and spirit toward heaven in pursuit of the perfection of the virtues. The very word *hakīm* has as its etymological basis the linguistic root reference in Arabic to someone's attainment of spiritual *hikmah* (wisdom, sagacity, or saintliness) through the practice of the virtues. These virtues are such things as beauty of character; wisdom; respect and compassion for all of creation; charity through self-sacrifice and altruism; and most important of all, constant "remembrance" (dhikr) of God as Divine All-Possibility.

In this light, there is a natural concern and goal on the part of the hakīm for improving the lot of patients in regard to their mental and emotional well-being, not just caring and tending to the health of the physical body.

Unani medicine doctors have always known that wellness is a state of wholeness, a wholeness that cannot be learned through an academic investigation of health and disease. True wellness is a state of total satisfaction and contentment, a state of understanding that everything is as it should be (otherwise, metaphysically speaking, God would not be God, and we would not be we).

Hakīms teach that wellness can be approached through various life lessons, experiential methods, and psychospiritual disciplines. Lao Tzu explained that "at the center of your being you have the answer; you know who you are, and you know what you want."[42] The hakīm will often prescribe a variety of treatments with guidance ("huda")—when tending to the soul of a person—by precipitating in the patient a profound desire to be a more "balanced, emotionally stable, and successful individual who is able to make better decisions and realize better achievements in life."[26] This is accomplished through the practice of pneumatology (traditional psychospiritual counseling and healing), which includes some of the following subjects:

Prayers (personal, supplicatory, or intercessory)[44]

Meditation (fikr), with breath and sacred sound[45]

Contemplation or intellection (tasawwur bil ʿaql)[46]

Dhikr (remembrance through repetition of a sacred formula or Divine Name): This would be very similar to the "Jesus Prayer" or the "Ave Maria" within Orthodox and Western Christianity, as well as the tradition of Japa Yoga within Hinduism.

Recitation of holy scripture: "The Qur'an in its totality, and certain sections and verses in particular, is curative of serious conditions not amenable to other forms of treatment. The methods of using the Qur'an are many: Taʿwīdh ("seeking refuge from harm or evil" with God), Daʿwah ["supplication for help from God"], and Ruqya [reciting sacred verse to a sick person]."[25]

Solitude in nature: We should remember the contemporary resurgence of the direct healing power of nature in the movement of eco-psychology and all that it has to offer.

Visualization[47]

Music therapy: "In addition to baths, drugs, kind and benevolent treatment given to the mentally ill, music therapy and occupational therapy were also employed. These therapies were highly developed. Special choirs and live music bands played daily to entertain the patients by singing, music, and other light-hearted performances."[26]

Forgiveness for self and others[48]

Elimination of unhealthy and disturbed attitudes, thoughts, and behaviors, such as "arrogance, pride, self-deception, lack of concentration, giddiness, frivolousness, irreverence, degrading others, forgetfulness, fear of failure, hypocrisy, excessive emotionality, severe anger, and being inconsiderate."[45,49]

Philosophical counseling[50,51]

Making pilgrimages to local and international holy sites or cities such as Jerusalem, the Kaʿba, Benares, Angkor Wat, the shrine of our Lady of Guadalupe, Lourdes, Mt. Shasta, Mt. Fuji, and so forth[52]

The "Infallible Remedy"

The "Infallible Remedy" is the recitation, in the original sacred Arabic language, of the first chapter of the Noble Qur'ān 41 times for 40 days during the interval between the obligatory canonical dawn prayer and the optional prayer immediately after it. "The Prophet said: 'In Sūrat al-Fātihah there is a balm for all ailments.' He went on to provide the specific instructions for utilizing this most treasured remedy… The accumulated experience of the Sufis confirms that the reading and reciting of Sūrat al-Fātihah with true faith and sincere conviction, cures all maladies, whether spiritual or worldly, external or internal… The Companions [of the Prophet] used to read it for treatment of diseases, both physical and mental."[45] If, for whatever reason, one cannot accomplish this level of intensity or precision in the recitation of the opening chapter of the Holy Qur'ān as methodically instituted and practiced by the Prophet of Islam himself, then "simply recite 11 times: Bismi Llāh ir-Rahmān ir-Rahīm ['In the Name of God, the Most Merciful, the Most Compassionate.']."[45]

The opening chapter of the Qur'an is in Arabic, translated and transliterated. This allows those who do not read Arabic to simply pronounce the words and phrases and thus benefit from the sacred sonorous quality of the recitation as it surrounds and penetrates the human body and its immediate environment with theurgic energy and influence from Above (Fig. 49.13).

Clinical Quick Notes

This section discusses a few condition-specific protocols that are true examples of the diagnostic and therapeutic paradigms of Unani medicine. These cases and the pursuant tables illustrate the clinical mind-set and logic of a hakīm or hakīma in the traditional mode of prescriptive authority.[20,45]

Fig. 49.13 The opening chapter of the Holy Qur'an: Arabic–English translation and transliteration. **1:1 Bismillāhi r-raḥmāni r-raḥīm** In the name of Allah, the Most Gracious, the Ever Merciful. **1:2 Al ḥamdu lillāhi rabbi l-ʿālamīn** All praises to Allah, Lord/Cherisher/Sustainer of the Universe. **1:3 Ar raḥmāni r-raḥīm** The Most Gracious, the Most Merciful. **1:4 Māliki yawmi d-dīn** Sovereign of the Day of Judgment. **1:5 Iyyāka naʿbudu wa iyyāka nastaʿīn** You alone we worship, and You alone we ask for help. **1:6 Ihdinā ṣ-ṣirāṭ al-mustaqīm** Guide us to the true path; **1:7 Ṣirāṭ al-laḍhīna anʿamta ʿalayhim ġhayril maġhḍūbi ʿalayhim walāḍ ḍāllīn** The path of those upon whom You have bestowed your favor, not of those who have earned Your anger, nor of those who go astray. (volkankovancisoy/iStock.com.)

BOX 49.1 The "Opening Chapter" of the Holy Qur'an Written in Arabic

1:1
Bismillāhi r-raḥmāni r-raḥīm
In the Name of Allah, the Most Merciful, the Most Compassionate
1:2
Al ḥamdu lillāhi rabbi l-ʿālamīn
Praise Be to Allah, Lord of All Worlds
1:3
Ar raḥmāni r-raḥīm
The Most Merciful, the Most Compassionate
1:4
Māliki yawmi d-dīn
King of the Day of Judgment
1:5
Iyyāka naʿbudu wa iyyāka nastaʿīn
Thee Alone Do We Worship, and in Thee Alone Do We Trust
1:6
Ihdinā ṣ-ṣirāṭ al-mustaqīm
Guide us to the straight path;
1:7
Ṣirāṭ al-laḍīna anʿamta ʿalayhim ġayril maġḍūbi ʿalayhim walāḍ ḍāllīn
The path of those upon whom you have bestowed your favor, not of those who have earned your
anger, nor of those who go astray.

Bismi 'Llāh Al-Rahmān Al-Rahīm
Translation: "In the Name of God, the Most Merciful, the Most Compassionate." This statement, recognizing the sacred and all-embracing nature of the Divine, traditionally begins any and all literature in the Islamic world. It is an affirmation of the long-held spiritual doctrine that the Divine is everywhere and always present.

Lethargy (Chronic Fatigue)

"Its cause is the prevalence of moisture in the brain, brought on either by phlegm or by blood. Use an enema. Make the person smell vinegar and feed him only light, easily digested foods."[20]

Melancholy (Sadness, Low Mood, or Depression)

"It is caused by an imbalance of the phlegm humor. Use an enema at waking and at bedtime. Eat only soft foods. Sexual intercourse has a remarkable effect in removing melancholy."[20]

Tinnitus (Ringing in the Ears)

"Detoxification of the system should be accomplished first, along with relaxation techniques. However, it has been determined that those who suffer from a constant ringing in the ears continue to 'hear' the sound even if the auditory nerve is severed."[20]

Nosebleed

"A nosebleed can be controlled by cupping the back of the head. If blood is coming out of the right nostril only, also apply cupping to the area of the liver. Apply cupping over the spleen if there is blood coming out of the left nostril only."[20]

Asthma

"If the cause is phlegm, then the remedy is to first eliminate all foods that produce mucus, such as milk, eggs, cheese, fruits, and all sugars of any kind. Syrup of hyssop with warm water three times a day is recommended after a bout with asthma. Rub the chest with linseed oil and beeswax … If the cause of the asthma is too much internal heat affecting the lungs, then the remedy pursued should be with cold drinks and a cucumber pomade on the chest… Gargling with milk and cinnamon with honey added is also recommended."[20]

Coughing

Black Bile. A blackish or dark greenish substance comes out when coughing. Give boiled wheat bran with sugar or honey.

Cough Powder. "This remedy relieves general irritation of the throat and lungs. Use 1/8th teaspoon (in all) of each of the following; skunk cabbage, horehound, African cayenne, bayberry bark, valerian root, and gentian; mix with three ounces molasses, and take 1 teaspoon with hot tea."[20]

Unani Medicinal Herbs Used to Treat Diabetes

Plant species and preparations of Unani medicinal herbs used to treat diabetes, as well as additional uses, are listed in Table 49.4.

Unani Medicinal Herbs Used to Treat Skin Diseases

Plant species and preparations of Unani medicinal herbs used to treat skin diseases, as well as additional uses, are listed in Table 49.5.

SUMMARY

The hakīms of ancient times were persons of tremendous insight and wisdom, both in theory and in practice; inheritors of ancient Greek knowledge; and players on the stage of world history who ultimately allowed for the birth of many aspects of modern medicine. A very brief

TABLE 49.4 Unani Medicinal Herbs Used to Treat Diabetes

Plant Species	Preparation	Additional Uses
Astragalus macrocarpus DC	Leaf decoction	Heart disease
Ceratonia siliqua L.	Leaf decoction	Herpes and lip sores
Cichorium pumilum Jacq.	Foliage decoction	Bacterial infection, poisoning, and rheumatism
Cupressus sempervirens L.	Fruit decoction	Antiseptic and nervous system
Eryngium creticum Lam.	Foliage decoction	Liver diseases, poisoning, anemia, and infertility problems
Juglans regia L.	Leaf and flower decoction	Asthma and sexual weakness
Lupinus varius Gaertn	Soaked seeds	Kidney stones
Mercurialis annua L.	Leaf decoction	Cancer and skin diseases
Morus nigra L.	Leaf, stem, and fruit decoction	Teeth or gum inflammation and cholesterol
Paronychia argentea Lam.	Leaf and flower decoction	Stones in kidney and heart diseases
Pinus halepensis Mill.	Leaf and seed decoction	Sexual weakness
Prosopis farcta Sol. Ex Russell	Foliage decoction	Menstrual cramps and kidney stones
Quercus calliprinos Decne	Fruit and bark decoction	Cancer, bed wetting, and ulcer
Salvia fruticosa Mill.	Foliage infusion	Stomachache, intestinal gas, and inflammation
Sarcopoterium spinosum L.	Leaf, seed, and root decoction	Intestinal pain, kidney diseases, and ulcer
Smilax aspera L.	Fruit and root decoction	Poisoning
Teucrium polium L.	Foliage decoction	Kidney stones, liver diseases, and stomach and intestinal inflammation
Trigonella foenum-graecum L.	Seed decoction	Sexual weakness and stomach and intestinal pain

Data from Azaizeh H, Saad B, Khalil K, Said O. The state of the art of traditional Arab herbal medicine in the eastern region of the Mediterranean: a review. *Evid Based Complement Alternat Med*. 2006;3:229–235.

TABLE 49.5 Unani Medicinal Herbs Used to Treat Skin Diseases

Plant Species	Preparation	Additional Uses
Alcea setosa Boiss.	Leaf, flower, and root decoction	Stomach and intestinal pain, inflammation, and asthma
Ammi visnaga L.	Flower and seed decoction	Kidney inflammation and respiratory system (asthma)
Asphodelus microcarpus Salzm. and Viv.	Bulb and root juice	Ectoderm parasites and jaundice
Cyclamen persicum Mill.	Leaf and bulb decoction	Ear infections
Eruca sativa Miller	Seed oil	Sexual weakness and hair loss
Ficus sycomorus L.	Stem milky sap	Coughing, digestive system, and anemia
Glaucium corniculatum L.	Poultice of macerated roots	Cholesterol and acne
Inula viscosa L. Ait. Inula	Foliage macerated in oil	Muscle relaxation and infertility
Lavandula officinalis Chaix and Kitt	Leaf, flower, and seed infusion	Urinary system, asthma, and nerve system
Lycium europaeum L.	Root decoction	High blood pressure and diabetes
Malva nicaeensis All.	Whole-plant decoction	Coughing and wounds
Myrtus communis L.	Leaf infusion	Stomach or intestinal pain and inflammation
Paronychia harmala L.	Seed infusion in olive oil	Wounds and lice
Sanguisorba minor Scop.	Whole-plant decoction	Ulcer, burns, and wounds
Saponaria mesogitana Boiss.	Root decoction	Liver diseases, stones in kidneys, and joint inflammation
Scolymus maculatus L.	Stem decoction	Intestine and kidney inflammation
Solanum nigrum L.	Foliage decoction	Wounds and sunburn
Tamarix aphylla L. H. Karst.	Leaf decoction	Eye inflammation and fever
Thymelaea hirsuta L. Endl.	Foliage paste	Couching and respiratory system
Viola odorata L.	Foliage decoction	Respiratory system, stomach and intestinal inflammation

Data from Azaizeh H, Saad B, Khalil K, Said O. The state of the art of traditional Arab herbal medicine in the eastern region of the Mediterranean: a review. *Evid Based Complement Alternat Med*. 2006;3:229-235.

and pointed summary follows as an example of what is meant, in the best sense of the phrase, by the statement that "knowledge is power."

- Avicenna was the first to describe meningitis so accurately, and in such detail, that the description has scarcely been added to after 1000 years.
- Avicenna was the first to describe intubation (a surgical procedure to facilitate breathing that Western physicians began using only at the end of the 18th century).
- The use of plaster of Paris for fractures by the Arabs was standard practice; it was "rediscovered" in the West in 1852.
- Surgery was used by the Arabs to correct cataracts.
- Ibn Al Nafis discovered pulmonary circulation.
- A strict system of licensing for medical practitioners was introduced in Baghdad in 931 AD, which included taking the Hippocratic Oath and specific periods of training for doctors.

- There was a system for the inspection of drugs and pharmaceuticals—the equivalent of the U.S. Food and Drug Administration—in Baghdad 1000 years ago.
- The European system of medicine was based on the Arabic system, and even as recently as the early 19th century, students at the Sorbonne had to read the *Canon of Avicenna* as a condition for graduating.
- Unani-Tibbi hospitals were, from the beginning, free to all without discrimination on the basis of religion, sex, ethnicity, or social status.
- Unani-Tibbi hospitals allocated different wards for each classification of disease.
- Hospitals had unlimited water supplies and bathing facilities.
- Before the advent of the printing press, there were extensive handwritten libraries in Baghdad (80,000 volumes), Cordova (600,000 volumes), Cairo (2 million volumes), and Tripoli (3 million volumes).
- All Unani-Tibbi hospitals kept patient records.
- A hospital was established for lepers. As many as six centuries later in Europe, they were still burning lepers to death by royal decree.
- In 830 AD, nurses were brought from Sudan to work in the Qayrawan hospital in Tunisia.
- A system of fountain-cooled air was devised for the comfort of patients with fever.
- Avicenna described the contamination of the body by "foreign bodies" before infection, and Ibn Khatima also described how "minute bodies" entered the body and caused disease—well in advance of Pasteur's discovery of microbes.
- Al Razi was the first to describe smallpox and measles. He was accurate to such a degree that nothing has been added since then.
- Avicenna described tuberculosis as being a communicable disease.
- Avicenna devised the concept of anesthetics. The Arabs developed a "soporific sponge" (impregnated with aromatics and narcotics and held under the patient's nose), which preceded modern anesthesia.
- The Arab surgeon Al Zahrawi was the first to describe hemophilia.
- Al Zahrawi was also the first surgeon in history to use cotton, which is an Arabic word, as a surgical dressing for the control of hemorrhage.
- Avicenna accurately described the surgical treatment of cancer, saying that the excision had to be radical and remove all diseased tissue, including amputation and the removal of veins running in the direction of the tumor. He also recommended cautery of the area if needed.
- Avicenna, Al Razi, and others formed a medical association for the purpose of holding conferences so that the latest developments and advancements in the field of medicine could be debated and passed on to others.[53]

So it seems ironic that "what began as an advanced medical system that set world standards has now come to be regarded as a system of folk medicine. This decline coincided with the decline of the Islamic Empire and the dissolution of the Caliphate, as these were directly responsible for the direction and impetus of Islamic scientific scholars in all fields."[41]

The decline of this ancient system of medicine also proves, if proof be needed, that "the achievements of the Unani-Tibbi practitioners of today bear little resemblance to those of their illustrious predecessors, and some of those claiming to practice traditional medicine are woefully ill-equipped"[41] to do so. Most individuals passing themselves off as Unani practitioners today would be labeled by most knowledgeable persons as folk healers or country herbalists, but not hakīms of the Avicennan type.

Today, it is in the Indo-Pakistani subcontinent or, more generally speaking, the sub-Himalayan regions of the Orient, that Unani medicine continues to thrive as a living school of medicine, competing with three other systems of health care: Ayurveda, traditional Chinese medicine, and modern Western medicine. Although current, modern "Western antipathies to things-Arabic and Muslim"[54,55] seem to have stalled the advance of Unani medicine into the stadium of complementary and alternative therapies in the West, it is slowly and gradually gaining ground as an optional healthcare system for many patients.

In India, the government has set up a Central Council for Research in Unani Medicine, which also has a licensing system for traditional practitioners.[41] India has 18 colleges of Unani medicine, about 100 Unani hospitals, and nearly 1400 beds for inpatients. In addition, there are nearly 900 dispensaries of Unani medicines and about 30,000 registered practitioners. In India alone, the number of persons serviced by Unani medicine is estimated to be at least 23 million from more than 220 villages.[56] Unfortunately, the details of the overall medical situation in Pakistan and other countries are not as well known or are simply not available.[57]

One commentator wrote, "Since both Ayurveda and Greek Medicine are constitutionally based, it's not surprising that improving basic constitutional strength, resilience and resistance to disease should be a very important therapeutic objective in both systems. The methods and modalities employed are also quite similar: diet is first and foremost, followed by simple herbal remedies, lifestyle modification, massage and bodywork, hygienic purification treatments and exercise/gymnastics/yoga. Being humor-based, both systems consider self-poisoning, or auto-intoxication with toxic metabolic residues and superfluous morbid humors to be the primary cause of all disease and pathology, for which they prescribe various hygienic purification treatments and regimes. The Ayurvedic word for toxins, "ama" means, "crude" or "raw"—residues from faulty or incomplete pepsis that haven't been properly integrated into the body and its functioning, and therefore impede it. The word ama has its equivalent in the word 'crudities,' a common term for toxins in Greek Medicine."[58]

In the humble opinion of this author, there is no better time than the present for discovering the beauty, mystery, and power of Unani medicine. This gift from the East—along with Ayurveda and traditional Chinese medicine—will reveal itself to serious seekers as a very real healthcare option offering natural, safe, and effective approaches to healing.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. For an introduction to the significance of Galen from a thoroughly contemporary Western historical and clinical paradigm, see the *Journal of the American Herbalists Guild* 2002;3(1).
2. Venzmer G. *Five Thousand Years of Medicine*. New York, NY: Taplinger Publishing Company
3. Tschanz DW. The arab roots of european medicine. *Aramco World*. 1997;48:20.
4. Nasr SH. *Islamic Science: an Illustrated Study*. Kent, UK:Westerham Press
5. Please see Professor Edward Said's thoroughly engaging study on this topic in his popular book *Orientalism* (Vintage Books, New York, 1979). Furthermore, it should go without saying that *Orient* and *Occident* are terms that transcend time (although one can generalize and say that the world of antiquity was primarily governed by the laws of tradition and the dictates of heaven) and space (although one can summarize the fact that most of what is called the Western world is governed by the laws of secularism dominated by the will of man). As to a logical, brilliant, and intellectually sound study of an understanding of this dichotomy and all that it means, please read S. H. Nasr's *Knowledge and the Sacred* and Professor Harry Oldmeadow's *Traditionalism: Religion in the Light of the Perennial Philosophy*.
6. Nasr SH. *Science and Civilization in Islam and Islamic Science: an Illustrated Study*. Cambridge, UK: The Islamic Texts Society
7. It was in Baghdad, Iraq, that Caliph Ma'mun founded the very famous "Bayt al-Hikma [literally 'House of Wisdom'], a sort of academy, in which translators found a convenient working place equipped with the necessary working facilities" to conduct the highest quality and quantity of manuscript translations, which was demanded of them by the caliph.
8. Ullmann M. Islamic Surveys II: Islamic Medicine. Edinburgh, UK: Edinburgh University Press
9. *Arabic Science, the Language*. http://fatarana.wordpress.com/2008/12/25/arabic-science-the-language. Accessed 1/3/2012.
10. Soubani AO. *The discovery of the pulmonary circulation revisited*. http://www.dankalia.com/science/0020226.htm (1995). Accessed 1/14/2012.
11. Kasule OH. Sr. *Surgery in Islam: a historical and current reappraisal.* http://omarkasule-01.tripod.com/id289.html. Accessed 1/14/2012.
12. In: (eds: Alphen JV, Aris A.) *Oriental Medicine*. Boston, MA: Shambhala Press; 48.
13. Professor Osman Bakar has compiled two wonderful studies on the topic of philosophy (including its epistemological and ontological dimensions) and its relationship to the traditional Islamic sciences in his books, entitled *The History and Philosophy of Islamic Science* and *Classification of Knowledge in Islam*.
14. Bakar O. *Tawhid and Science: Essays on The History and Philosophy of Islamic Science*. Kuala Lumpur, Penang: Secretariat for Islamic Philosophy and Science.
15. Bakar O. *Classification of Knowledge in Islam: a Study in Islamic Philosophies of Science*. Cambridge, UK: Islamic Texts Society.
16. The word *intellect* here is used in its original sense—not as a "fallen word" cut off from its true etymologic source as perceived by many of us in the modern world, but as a word signifying the knowing faculty within the human being that knows immediately and without recourse to analytic or discursive thought, a faculty that is constantly in touch with God. It is precisely this faculty of intellectual intuition that the profound mystic of the German Rhine, Meister Eckhart, described as being the "divine spark" within the human heart that is the seat of all true knowledge and wisdom.
17. There is a famous Qur'ānic verse (41:53) that addresses this claim, wherein the "signs" of God's authorship of creation are known most evidently as *fi 'l-āfāq wa fī anfusihim* ("upon the horizon [of creation] and within their [human] souls").
18. Bakhtiar L. *Sufi: Expressions of the Mystic Quest*. New York, NY: Thames and Hudson.
19. For a scholarly treatment of this subject, see Seyyed Hossein Nasr's *An Introduction to Islamic Cosmological Doctrines* (Albany, NY: State University of New York Press; 1993).
20. Chishti HGM. *The Traditional Healer's Handbook: A Classic Guide to the Medicine of Avicenna*. Rochester, VT: Healing Arts Press.
21. Osborn D. *The four temperaments*. http://www.greekmedicine.net/b_p/Four_Temperaments.html. Accessed 1/2/2012.
22. Ahmad J, Qadeer HA. *Unani: the Science of Graeco-Arabic Medicine*. New Delhi, India: Lustre Press.
23. An excellent discussion concerning the notions of "physical" or "corporeal," Platonic "forms" or "ideas," and "energy" or "quantum" entities within the context of many traditional cultural and spiritual knowledge frameworks can be found in *The Quantum Enigma* and other works by Wolfgang Smith, physicist and Christian scholastic philosopher.
24. *Avicenna's Canon of Medicine*. http://www.traditionalmedicine.net.au/canonavi.htm. Accessed 1/3/2012.
25. Khan MS. *Islamic Medicine*. London, UK: Routledge and Kegan Paul.
26. In: (ed. Athar S.) *Islamic Perspectives in Medicine: a Survey of Islamic Medicine*. Indianapolis, IN: American Trust Publications; 55–56.
27. Armstrong JA. Urinalysis in western culture: a brief history. *Kidney Int*. 2007;71:384.
28. "The library of the Tulum Hospital, which was founded in Cairo in 872 AD (1100 years ago) had 100,000 books. Physicians had their own extensive personal book collections, at a time when printing was unknown and book editing was done by skilled and specialized scribes putting in long hours of manual labor. Mustansiriyya University in Baghdad contained 80,000 volumes; the library of Cordova 600,000 volumes; that of Cairo 2 million, and that of Tripoli 3 million books" (see Athar S. *Islamic Perspectives in Medicine: a Survey of Islamic Medicine.* Indianapolis, IN: American Trust Publications; 55–56.).
29. *Eclectic Medicine*. http://en.wikipedia.org/wiki/Eclectic_medicine. Accessed 1/3/2012.
30. Johnson PE. *The unraveling of scientific materialism*. http://www.arn.org/ftissues/ft9711/articles/johnson.html. Accessed 1/3/2012.
31. Boyle W, Kirchfeld F. *Nature Doctors: Pioneers in Naturopathic Medicine*. Portland, OR:NCNM Press.
32. "Culpeper was an herbalist who followed the classical medical precepts of Hippocrates and Galen. A medical populist, Culpeper's mission was to put medicine and natural healing back into the hands of the people… Culpeper's best known and loved work is his herbal, called *A Complete Herbal* [which has been studied and used by many contemporary naturopathic physicians for academic and clinical practice purposes], which has never really gone out of print since it was first published… *A Complete Herbal* is Culpeper's treatise and alphabetical catalog of the medicinal plants of his native England, including their astrological correspondences and indications." See http://www.greekmedicine.net/whos_who/Nicholas_Culpeper.html.
33. Snider P, Turner RN. Nature cure in europe: the transatlantic journey from pragmatism to principles. *Naturopath Doctor News Rev*. 2010;6.
34. "Biologically, fasting is an effective, natural process of detoxification and healing" (see Khan MS. *Islamic Medicine.* London, UK: Routledge and Kegan Paul.).
35. *More about Al-Idrisi*. http://www.clarklabs.org/about/More-About-Al-Idrisi.cfm. Accessed 1/3/2012.
36. Natural Medicines Comprehensive Database. *Black seed*. http://faculty.ksu.edu.sa/18856/Medicinal%20Plants/Black%20seed.pdf. Accessed 1/3/2012.
37. Randhawa MA. Department of Pharmacology, College of Medicine, King Faisal University, Dammam, Ksa. *J Ayub Med Coll Abbottabad*. 2008;20(1).
38. The scientific references for all of the most recent scientific literature for the black seed's pharmacological profile can be found on the second page at http://www.ayubmed.edu.pk/JAMC/Past/20-2/Editorial.pdf or the article by Dr. Mohammad Akram Randhawa.
39. *What is a Hammam*. http://www.completemorocco.com/morocco-an-introduction/what-is-a-hammam/. Accessed 1/14/2012.
40. Osborn D. *The water cure*. http://www.greekmedicine.net/therapies/The_Water_Cure.html. Accessed 1/3/2012.
41. Fundukian LJ. *Gale Encyclopedia of Alternative Medicine*. 3rd ed. Detroit, MI: Gale, a part of Cengage Learning, Inc.
42. Personal correspondence from Dr. Battata, MD. Dayton, OH: Department of Pathology, Wright State University School of Medicine.

43. For more information on the topic of traditional Sufism, please see works by Martin Lings, such as *What Is Sufism?;* Seyyed Hossein Nasr's *Sufi Essays;* Titus Burckhardt's *An Introduction to Sufism;* William Stoddart's *Sufism;* and one of the most profound esoteric expositions on Sufism available to the general public, which is Frithjof Schuon's *Sufism: Veil and Quintessence*.
44. *Index of 101 Dua'a*. http://www.duas.org/index.htm. Accessed 1/3/2012.
45. Chishti HM. *The Book of Sufi Healing*. Rochester, VT: Inner Traditions International.
46. Ali SM. *The gist of Sufism; the heart of the matter*. http://muslimcanada.org/tazkiya.pdf. Accessed 1/14/2012.
47. Kumar M. *Creative visualization; how to create whatever you want in your life*! http://www.leggeattrazione.altervista.org/visualization.pdf. Accessed 1/14/2012.
48. For an incredible assessment of the various humoral personality types in regard to personality traits, please visit the following website for further information: http://www.intraspec.ca.
49. Sometimes physiologic fever is coincident with such disturbances, but according to the Prophet Muhammad (peace be upon him) this type of fever "constitutes a deep spiritual cleansing causing 'the sins to fall off like leaves being shed from a tree.' These fevers burn off impurities on the soul level."
50. "In ancient and pre-modern times, people saw the practical value of philosophy as a guide for living. . . But then, in the early 1980's, German philosopher Gerd Achenbach decided to revive the old and time-honored practice and offered his services as a philosophical counselor for those seeking to solve their problems philosophically. In 1982, Achenbach founded the Society for Philosophical Praxis to establish philosophical counseling as a profession. Praxis is a Greek word meaning the practical application of a branch of learning—in this case philosophy—to solve life's problems. In classical Greek astrology and counseling, Praxis also refers to one's career, life path, or calling. Helping clients find their career or life path is a specialty of philosophical counseling." For further information about this technique of counseling, see Osborn.
51. Osborn D. *Philosophical counseling*. http://www.greekmedicine.net/therapies/Philosophical_Counseling.html. Accessed 1/3/2012.
52. Greenfield SM. *Pilgrimage, therapy, and the relationship between healing and imagination*. http://www4.uwm.edu/clacs/resources/pubs/pdf/greenfield82.pdf. Accessed 1/14/2012.
53. From Fundukian LJ. *Gale Encyclopedia of Alternative Medicine*. 3rd ed. Detroit, MI: Gale, a part of Cengage Learning, Inc; 2008. Reproduced with permission. http://www.cengage.com/permissions.
54. This was part of a statement made by Dr. Paul Bergner in an email correspondence to an inquirer regarding the issue of Unani medicine.
55. Bergner P. Four humors / 1995 | Henriette's Herbal Homepage. 2020. Henriettes-herb.com. Available at: http://www.henriettes-herb.com/archives/best/1995/four-humors.html. Accessed 6/2/2020.
56. Siddiqui MK. *Unani medicine in India*. http://www.crism.net/news/pdf/unani_medicine_overview_dr_khalid_siddiqui.pdf. Accessed 1/3/2012.
57. Dharmananda S. *Unani medicine with reference to Hamdard of Pakistan and India*. http://www.itmonline.org/arts/unani.htm. Accessed 1/3/2012.
58. Osborn D. *Greek medicine and Ayurveda*. http://www.greekmedicine.net/b_p/Greek_Medicine_and_Ayurveda.html. Accessed 1/3/2012.

FURTHER READING

Books

Abdul HH. *Avicenna's Tract on Cardiac Drugs and Essays on Arab Cardiotherapy*. Karachi, Pakistan: Hamdard Foundation Press; 1983.

trans Adams F. *The Genuine Works of Hippocrates*. Baltimore, MD: Williams & Wilkins Company; 1946.

Ajram K. *The Miracle of Islamic Science*. Cedar Rapids, IA: Knowledge House Publishers; 1992.

Alam MT, Saeed A, Usmanghani K. *Indusyunic Medicine: Traditional Medicine of Herbal, Animal, and Mineral Origin in Pakistan*. Karachi, Pakistan: University of Karachi; 1997.

Arberry AJ, trans. *The Spiritual Physick of Rhazes*. London, UK: John Murray; 1950.

Bakar O. *Classification of Knowledge in Islam*. Cambridge, UK: The Islamic Texts Society; 1998.

Bakar O. *The History and Philosophy of Islamic Science*. Cambridge, UK: The Islamic Texts Society; 1999.

Beshore G. *Science in Early Islamic Cultures*. Danbury, CT: Franklin Watts; 1998.

George LS. *The Golden Age of Islam*. New York, NY: Benchmark Books; 1998.

Jackson R. *Doctors and Diseases in the Roman Empire*. Norman, OK: University of Oklahoma Press; 1988.

Janabi TH. *Clinging to a Myth.* Indianapolis: American Trust Publications; 1990.

Johnstone P, trans. *Medicine of the Prophet*. Cambridge, UK: The Islamic Texts Society; 1998.

Krueger HC. *Avicenna's Poem on Medicine*. Springfield, IL: Charles C. Thomas; 1963.

Nasr SH. *Knowledge and the Sacred*. Albany, NY: State University of New York Press; 1989.

Nasr SH. *An Introduction to Islamic Cosmological Doctrines*. Albany, NY: State University of New York Press; 1993.

Nasr SH. *Religion and the Order of Nature*. New York, NY: Oxford University Press; 1996.

Needleman J. *Sin and Scientism*. San Francisco, CA: Robert Briggs Associates; 1985.

Rahman F. *Health and Medicine in the Islamic Tradition*. New York, NY: The Crossroad Publishing Company; 1989.

Scarborough J. *Roman Medicine*. Ithaca, NY: Cornell University Press; 1969.

Ullmann M. *Islamic Surveys II: Islamic Medicine*. Edinburgh: Edinburgh University Press; 1978.

Journals

Adams A. Reviving our heritage: traditional Islamic medicine. *Q-News.* 2001:35–37.

Bakar O. The common philosophical foundations of traditional medicines. *Sophia*. 1994;6:1–4.

Blackhirst R. The alchemy of traditional foods. *Sacred Web*. 1999;4:47–56.

Darmani NA. Avicenna: the prince of physicians and a giant in pharmacology. *J Islamic Med Assoc N Am*. 1995;26:78–81.

French R. Foretelling the future: Arabic astrology and English medicine in the late twelfth century. *Isis*. 1996;87:453–480.

Smith W. Esoterism and cosmology: from Ptolemy to Dante and Cusanus. *Sophia*. 2002;8:130–152.

Vohora SB. Is the human body a microcosm? *Studies History Med*. 1981:61–86.

Internet

A Brief Description of the Philosophical Foundations of Greco-Arabic Medicine. http://www.traditionalmedicine.net.au/chapter2.htm. Accessed 1/3/2012.

Arab Medical Schools During the 12th and 13th Centuries. http://www.levity.com/alchemy/islam06.html. Accessed 1/3/2012.

Azmi HAA. Concept of matter in Unani Medicine. http://www.ignca.nic.in/ps_04018.htm. Accessed 1/3/2012.

Ead HA. Averroes as a Physician. Accessed 1/3/2012. http://www.levity.com/alchemy/islam21.html.

Ead HA. ed: Arabic (or Islamic) Influence on the Historical Development of Medicine. http://www.levity.com/alchemy/islam19.html. Accessed 1/3/2012.

International Institute of Islamic Medicine. http://www.iiim.org/islamed3.html. Accessed 1/3/2012.

What did the Islamic world contribute to medicine? http://www.youtube.com/watch?v=99xgXC5uxiI&feature=related. Accessed 1/3/2012.

SECTION IV

Pharmacology of Natural Medicines

Careful review of the scientific literature reveals a considerable amount of research documenting the clinical efficacy, pharmacological activity, and toxicology of numerous "natural" medicines. An important purpose of this section is the critical evaluation, from a scientific standpoint, of the historical and clinical use of natural medicines, especially botanicals. Although plants have been used as medicines since antiquity, appreciation of them as effective medicinal agents was greatly diminished until the later part of the 20th century. It is hoped that this section will further revive the appreciation for and the safe and effective use of botanical medicines.

Some plant constituents are discussed as separate entities, and for several botanicals, these constituents may be the most appropriate therapeutic substances. However, we believe that the whole herb should be used whenever possible. Many studies have demonstrated that, in many instances, the physiological and pharmacological effects of a particular plant constituent are diminished when it is given as an isolated component rather than in its naturally occurring environment. Furthermore, besides synergistic and enhancing effects, there appear to be factors in most plants that prevent many of the adverse effects of isolated plant constituents. In understanding the pharmacology, pharmacognosy, and historical use of plants, the student of natural medicine is once again inspired by the miracle of nature as well as the intuition and empiricism that ancient herbalists possessed.

We hope that this section will help better inform our readers of the impressive uses, and potential dangers, of these natural medicines. We also want to stress that botanical medicine should not be used simply as a substitute for the drugs commonly used to treat disease. The true physician of natural medicine will use them as part of the treatment of the whole person and in the context of addressing the causes of the disease and promoting health, not simply treating symptoms.

Finally, considering the unfortunate, continuing trend of forever-increasing use of prescription drugs—and in too many instances polypharmacy with unpredictable results (especially in the elderly)—the astute physician of natural medicines needs to be expert in the potential for interaction between natural and conventional medicines. These interactions can take many forms, such as enhancing or inhibiting: a drug's rate of absorption, the intended pharmaceutical effects, the rate of detoxification, and so forth. Far more research is now available on drug–herb–nutrient interactions than when the first edition came out in 1985. Almost every natural medicine covered in this textbook now has a robust drug interactions subsection.

Glossary of Some Terms Used in Section IV

Abortifacient: a substance that induces abortion.

Acrid: a pungent, biting taste that causes irritation.

Adaptogen: a substance that is safe, increases resistance to stress, and has a balancing effect on body functions.

Adjuvant: a substance that enhances the effect of the medicinal agent or increases the antigenicity of a cancer cell.

Alkaloids: naturally occurring amines arising from heterocyclic and often complex structures that display pharmacological activity. Their common names typically end in *-ine*. They are usually classified according to the chemical structure of their main structures: phenylalkylamines (e.g., ephedrine), pyridine (e.g., nicotine), tropine (e.g., atropine, cocaine), quinoline (e.g., quinine), isoquinoline (e.g., papaverine), phenanthrene (e.g., morphine), purine (e.g., caffeine), imidazole (e.g., pilocarpine), and indole (e.g., physostigmine, yohimbine).

Alterative: a substance that has a balancing effect on a particular body function.

Analgesic: a substance that reduces the sensation of pain.

Androgens: hormones that stimulate male characteristics.

Anthelminthic: a substance that causes the elimination of intestinal worms.

Anthocyanidin: a particular class of flavonoids that gives plants, fruits, and flowers colors ranging from red to blue.

Antidote: a substance that neutralizes or counteracts the effects of a poison.

Aphrodisiac: a substance that increases sexual desire.

Astringent: an agent that causes the contraction of tissue.

Balm: a soothing or healing medicine applied to the skin.

Carminative: a substance that promotes the elimination of intestinal gas.

Carotenes: fat-soluble plant pigments, some of which can be converted into vitamin A.

Cathartic: a substance that stimulates the movement of the bowels; more powerful than a laxative.

Cholagogue: a compound that stimulates the contraction of the gallbladder.

Choleretic: a compound that promotes the flow of bile.

Cholestasis: the stagnation of bile within the liver.

Cholinergic: mimic the action of acetylcholine affecting the parasympathetic portion of the autonomic nervous system and the release of acetylcholine.

Coenzyme: a necessary nonprotein component of an enzyme, usually a vitamin or mineral.

Compress: a pad of linen applied under pressure to an area of skin and held in place; may include an herbal extract.

Decoctions: aqueous extracts prepared by boiling the botanical material with water for a specified period, followed by straining or filtering.

Demulcent: a substance soothing to irritated mucous membranes.

Emulsify: to disperse large fat globules into smaller uniformly distributed particles that can remain in suspension in water.

Enteric coated: a way of coating a tablet or capsule to ensure that it does not dissolve in the stomach so it can reach the intestinal tract.

Enzyme: an organic catalyst that facilitates chemical reactions.

Essential oils: also known as volatile oils, ethereal oils, or essences. They are usually complex mixtures of a wide variety of organic compounds (e.g., alcohols, ketones, phenols, acids, ethers, esters, aldehydes, oxides) that evaporate when exposed to air. They generally represent the odoriferous principles of plants.

Extracts: concentrated forms of natural products obtained by treating crude materials containing these substances with a solvent and then removing the solvent completely or partially from the preparation. The most commonly used extracts are fluid extracts, solid extracts, powdered extracts, tinctures, and native extracts.

Flavonoid: a generic term for a group of flavone-containing compounds that are found widely in nature. They include many of the compounds that account for plant pigments (anthocyanins, anthoxanthins, apigenins, flavones, flavonols, bioflavonols, etc.). These plant pigments exert a wide variety of physiological effects in the human body.

Fluid extracts: these extracts are typically hydroalcoholic solutions with a strength of one part solvent to one part herb. The alcohol content varies with each product. They are, in essence, concentrated tinctures, constructed to represent 1 grain of the crude drug to 1 minim of fluid extract.

Glycosides: sugar-containing compounds composed of a glycone (sugar component) and an aglycone (non–sugar-containing component) that can be cleaved through hydrolysis. The glycone portion may be glucose, rhamnose, xylose, fructose, arabinose, or any other sugar. The aglycone portion can be any kind of organic compound (e.g., sterols, triterpenes, anthraquinones, hydroquinones, tannins, carotenoids, anthocyanidins).

Infusion: tea produced by steeping a botanical in hot water.

Laxative: a substance that promotes the evacuation of the bowels.

LD_{50}: the dosage that will kill 50% of the animals taking the substance.

Lipotropic: promoting the flow of lipids to and from the liver.

Menstruum: solvents used for extraction (e.g., water, alcohol, acetone).

Metalloenzyme: an enzyme that contains a metal linked to its protein structure.

Native extracts: high-potency extracts prepared via concentration under reduced pressure at low temperatures until all solvent is removed.

Oleoresins: primarily mixtures of resins and volatile oils. They either occur naturally or are made by extracting the oily and resinous materials from botanicals with organic solvents (e.g., hexane, acetone, ether, alcohol). The solvent is then removed under vacuum, leaving behind a viscous, semisolid extract that is the oleoresin. Examples of prepared oleoresins are paprika, ginger, and capsicum.

Powdered extract: a solid extract that has been dried to a powder.

Putrefaction: the process of breaking down protein compounds by decay or rotting.

Recommended dietary allowance (RDA): the estimated amount of a nutrient considered to support health.

Resins: complex oxidative products of terpenes that occur naturally as plant exudates or are prepared by alcohol extraction of botanicals that contain resinous principles.

Saponins: nonnitrogenous glycosides, typically with sterol or a triterpene as the aglycone, that possess the common property of foaming, or making suds, when strongly agitated in aqueous solution.

Solid extracts: thin to thick, viscous liquids or semisolids prepared from native extracts by adjusting the latter to the specific strength with suitable diluents. Typically, these extracts are 4:1, that is, one part extract is equivalent to, or derived from, four parts of crude herb.

Standardized extracts: an herbal extract standardized to a defined concentration of a specific compound, usually the one considered most clinically active.

Strength: the potency of an extract or the strength of a botanical extract, which is generally expressed in two ways. If they contain known active principles, their strength is commonly expressed in terms of their content of active principles. Otherwise, their strength is expressed in terms of their concentration of the crude drug. Thus a strength of 4:1 means one part of extract is equivalent to, or derived from, four parts of crude drug. A strength of 1:5 represents one part of extract comparable to 0.2 parts of the crude drug. This ratio method of expressing drug strength does not accurately measure potency because there may be wide variation between manufacturers.

Tinctures: alcoholic or hydroalcoholic solutions usually containing the active principles of botanicals in low concentrations. They are usually prepared by maceration or percolation or by dilution of their corresponding fluid or native extracts. The strengths of tinctures are typically 1:10 or 1:5. Alcohol content varies.

Tonic: a substance that exerts a gentle strengthening effect on the body.

50

Allium cepa (Onion)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Allium cepa (family: *Amaryllidaceous* or *Liliaceous*)
Common name: onion

GENERAL DESCRIPTION

Numerous forms and varieties of onion exist because this perennial or biennial herb is cultivated worldwide. The part used is the fleshy bulb. Common varieties include white globe, yellow globe, and red globe (Fig. 50.1A and B).

Onions range in size, color, and taste depending on their variety. The two general types of large, globe-shaped onions are classified as either spring/summer or storage onions. The former class includes those grown in warm weather climates and mild or sweet in flavor. Included in this group are the Walla Walla, Vidalia, and Maui Sweet onion. Storage onions are grown in colder weather climates and, after harvesting, are dried for several months to attain dry, crisp skins. Storage onions generally have a more pungent flavor and are usually named by their color: white, yellow, or red. Spanish onions fall into this classification. In addition to these large onions, there are smaller varieties, such as the pearl onion.

CHEMICAL COMPOSITION

Onions, like garlic, contain various organic sulfur compounds:

- S-methylcysteine sulfoxide
- Trans-S-(1-propenyl) cysteine sulfoxide
- S-propylcysteine sulfoxide
- Dipropyl disulfide

Onions also contain the enzyme alliinase, which is released when the onion is cut or crushed, causing conversion of trans-S-(1-propenyl) cysteine sulfoxide to the so-called lacrimatory factor (propanethial S-oxide). Other constituents include the following:

- Flavonoids (primarily quercetin)
- Phenolic acids (e.g., caffeic, sinapic, and p-coumaric)
- Sterols
- Saponins
- Pectin
- Volatile oils

HISTORY AND FOLK USE

Although not as valued as a medicinal agent compared with garlic, onion has been used almost as widely. Like garlic *(Allium sativum),* onion has been used as an antispasmodic, carminative, diuretic, expectorant, stomachic, anthelmintic, and anti-infective agent. Externally, it has been used as a rubefacient and poultice, giving relief for skin diseases and insect bites.[1]

A. cepa originated in the central part of Asia, from Iran to Pakistan, and northward into the southern part of Russia. Onions have been revered throughout time not only for their culinary use but also for their therapeutic properties. As early as the 6th century, onions were used as medicine in India. They were popular with the ancient Greeks and Romans and were often dressed with extra seasonings because many people did not find them spicy enough. It was their pungency that made onions popular among poor people throughout the world. Onions were an indispensable vegetable in the cuisines of many European countries during the Middle Ages and later even served as a classic healthy breakfast food. Christopher Columbus brought onions to the West Indies, and their cultivation spread throughout the Western hemisphere. China, India, the United States, Russia, and Spain are among the leading producers of onions.

World onion production has increased dramatically, with current production around 44 million tons per year, making onions the second most important horticultural crop after tomatoes. Because of their storage characteristics and durability for shipping, onions have always been traded more widely than most vegetables. Onions are versatile, often used in many dishes, and accepted by almost all traditions and cultures.[2]

Fig. 50.1 (A) *Allium cepa* bulb. (B) *A. cepa* onion (Yarnell). (A, from coldsnowstorm/iStock.com.)

PHARMACOLOGY

Onions and garlic, due to their similar constituents, have many of the same pharmacological effects. However, significant differences make one more advantageous than the other in certain conditions.

One of the key nutritional qualities of onions is their high content of quercetin. To determine uptake as well as in vivo antioxidant effects of quercetin from onions, six healthy, nonobese, normocholesterolemic female volunteers participated in a randomized two-phase crossover supplementation trial to compare the antioxidant effects associated with (1) a meal of fried onions, and (2) a meal of fried onions and fresh cherry tomatoes.[3] Plasma flavonoids, lymphocyte DNA damage, plasma ascorbic acid, tocopherols and carotenoids, urinary malondialdehyde, and 8-hydroxy-2-deoxyguanosine were determined to assess flavonoid absorption and antioxidant efficacy. The results indicated that the flavonoid glucosides (quercetin-3-glucoside and isorhamnetin-4-glucoside) were significantly elevated in plasma after ingestion of the onion-only meal, and the increases were associated with increased resistance of lymphocyte DNA to DNA strand breakage. A significant decrease in the level of urinary 8-hydroxy-2-deoxyguanosine was evident 4 hours after ingestion of the onion meal. After the combined tomato and onion meal, only quercetin was detected in plasma. Endogenous base oxidation was decreased, but resistance to strand breakage was unchanged. No significant change in the excretion of urinary malondialdehyde occurred after either meal. The conclusions from the study were that both meals—onions and onions with tomatoes—led to transient decreases in biomarkers of oxidative stress, although the biomarkers affected differed. It is possible that the differences in patterns of response reflect the different uptakes of flavonoids, but the underlying mechanism is not yet understood. Onion has a combination of fructans, dietary flavonoids, and organosulphur compounds with functional benefits against diseases. Some of these benefits are a reduction of blood fibrinogen concentration, the enhancement of blood's fibrinolytic activity, antioxidant activity, lipid peroxidation inhibition, a reduction of serum cholesterol, a reduction of respiratory and skin infections, and a decreased risk of diabetes.[4]

Antimicrobial Activity

Although onions exhibit antibacterial, antifungal, and anthelmintic activity, it is not nearly as potent as that of garlic. This suggests that garlic may be better indicated in cases of infection,[5] but onion can usually be consumed in larger quantities than garlic, which may increase the concentration of antimicrobial constituents in vivo to approximate those of garlic.

Cardiovascular Effects

Like garlic, onions and onion extracts were shown to decrease blood lipid levels, increase fibrinolysis, decrease platelet aggregation, and lower blood pressure in several clinical studies.[6,7] Onion oil, compared with garlic oil, was a stronger inhibitor of the enzymes cyclooxygenase and lipoxygenase, which mediate eicosanoid metabolism (prostaglandins, thromboxanes, and leukotrienes).[8] This suggests that onions would also have a greater effect on inhibition of platelet aggregation and other events mediated by eicosanoids. In addition, extracts of onion have been shown to inhibit platelet aggregation via inhibition of diphosphate, epinephrine, arachidonic acid, adenosine, and collagen-induced platelet aggregation and inhibiting the action of platelet-activating factor.[9] Garlic and onion consumption was associated with lower levels of cholesterol and triglycerides, as well as an increase in fibrinolytic activity[10] (see Chapter 51 for details). Because the quantity of onion consumed in the study cited was so much larger than that of garlic (600 g of onion per week compared with 50 g of garlic), an argument could be made that onion consumption was the major determinant.

Onion consumption may also reduce the risk of cardiovascular disease due to its quercetin content. Epidemiological data suggest that those who consume a diet rich in quercetin-containing foods might have a reduced risk of cardiovascular disease. In a double-blind, randomized, cross-over study, subjects ingested an onion soup containing either a high or a low amount of quercetin. Plasma quercetin concentrations and platelet aggregation and signaling were assessed after soup ingestion. The high-quercetin soup contained 69 mg total quercetin compared with the low-quercetin soup containing 5 mg total quercetin. Plasma quercetin concentrations were significantly higher after

the ingestion of the high-quercetin soup than after the ingestion of the low-quercetin soup, and the high-quercetin soup also influenced some aspects of platelet aggregation and signaling.[11]

Diabetes

Onions were shown to have significant oral hypoglycemic action, comparable to that of the prescription oral hypoglycemic agents tolbutamide and phenformin.[12,13] The active hypoglycemic principle in onions is believed to be allyl propyl disulfide, although other constituents, such as quercetin and anthocyanidin, may play a significant role as well. Experimental and clinical evidence suggests that allyl propyl disulfide lowers glucose by competing with insulin (also a disulfide) for degradation sites, thereby increasing the half-life of insulin. Other mechanisms, such as increased hepatic metabolism of glucose or increased insulin secretion, have been proposed.

In one assessment of the hypoglycemic activity of onion consumption in patients with type 1 and type 2 diabetes, the ingestion of 100 g of onion caused a considerable reduction in fasting blood glucose levels by about 89 mg/dL in relation to insulin (145 mg/dL) in patients with type 1 diabetes, and it reduced fasting blood glucose levels by 40 mg/dL compared with glibenclamide (81 mg/dL) in patients with type 2 diabetes, as measured 4 hours later.[14] The same dose of onion produced a significant reduction in induced hyperglycemia (glucose tolerance test) by about 120 mg/dL compared with water (77 mg/dL) and insulin (153 mg/dL) in patients with type 1 diabetes, and it considerably reduced the glucose tolerance test by 159 mg/dL in relation to water (55 mg/dL) and glibenclamide (114 mg/dL) in patients with type 2 diabetes, as measured after 4 hours.

Antiasthmatic Action

Onions have historically been used as antiasthmatic agents. Their action in asthma, as well as in other conditions associated with increased lipoxygenase derivatives (leukotrienes), such as psoriasis and atopic dermatitis, appears to be greater than that of garlic. The net effect is similar to that of cortisol, which inhibits all eicosanoid metabolism via inhibition of phospholipase. Inhibition of leukotriene formation and onion's quercetin and isothiocyanate content are likely the primary factors responsible for onion's antiasthmatic effects. These effects have been confirmed in experimental studies.[15,16]

Antitumor Effects

Various forms of cancer are associated with an imbalance between the activities of two enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC). Histone deacetylase inhibitors (HDACIs) regulate the activity of HDACs and are being used in cancer treatment. Onions have been reported to show potential HDAC-inhibitory activity, exhibiting antitumor effects through the activation of cell-cycle arrest, induction of apoptosis and autophagy, angiogenesis inhibition, and mitotic cell death in cancer cells.[17]

An onion extract was found to be cytotoxic to tumor cells in vitro and to arrest tumor growth when tumor cells were implanted in rats.[18] The onion extract was shown to be nontoxic, with a dose as high as 40 times that of the cytotoxic dose for the tumor cells causing no adverse effects on the host. Another species, *Allium ascalonicum* (shallots), was shown to exhibit significant antileukemic activity in mice.[19] In addition, sulfides found in onions were shown to be effective agents for controlling tumors in both in vitro and in vivo models, and the antitumor effects are likely caused by reversing the antitumor immune system.[20]

One human study evaluated onion consumption and stomach cancer in more than 120,000 men and women between 55 and 69 years of age.[21] After a 3.3-year follow-up, 139 stomach cancers were diagnosed. The researchers found a strong inverse association between onion consumption and stomach cancer incidence but no association with the use of leeks or garlic.

Hair Tonic Effects

Topical application of onions has been shown to help alopecia areata, a patchy and nonscarring hair-loss condition. Any hair-bearing surface may be involved in alopecia areata, and different modalities of treatment have been used to induce hair regrowth. One study compared the effectiveness of topical crude onion juice with tap water in the treatment of patchy alopecia areata.[22] The patients were divided into two groups. The group treated with onion juice consisted of 23 patients (16 males and 7 females) ranging in age from 5 to 42 years old (mean, 22.7 years), and the control group consisted of 15 patients (8 males and 7 females) ranging in age from 3 to 35 years old (mean, 18.3 years). The two groups were advised to apply the treatment twice daily for 2 months. Regrowth of terminal coarse hairs started after 2 weeks of treatment with crude onion juice. At 4 weeks, hair regrowth was seen in 17 patients (73.9%). At 6 weeks, hair regrowth was observed in a total of 20 patients (86.9%) and was significantly higher among males (93.7%) compared with females (71.4%). In contrast, with tap water, hair regrowth was apparent in only two patients at 8 weeks of treatment, with no sex differences. These results indicate that crude onion juice can be an effective topical therapy for patchy alopecia areata.

CLINICAL APPLICATION AND DOSAGE

This chapter highlights the medicinal value of onions, particularly in cardiovascular disease, diabetes mellitus, and inflammatory conditions. Onions can be eaten liberally as part of a nutritious diet. Therapeutic dosages in the various forms are typically equal to 50 to 150 g/day of raw onion.

TOXICOLOGY

Virtually no reports of toxicity have been documented. However, people with heartburn may note an aggravation of symptoms. One study evaluated symptoms of acid reflux in 16 normal subjects and 16 patients with heartburn.[23] Subjects were studied with an esophageal pH probe for 2 hours after eating a plain hamburger and a glass of ice water, and then on another day after an identical meal plus a slice of raw onion. In the patients without acid reflux, ingestion of onions did not increase any of the variables measured (number of reflux episodes, pH less than 4, time of pH less than 4, heartburn episodes, and belches). In contrast, subjects with heartburn experienced a significant increase in all variables. Although the authors of the study concluded that onions could be a potent and long-lasting reflexogenic agent in patients with heartburn, an alternative explanation might be that onions simply improved digestive acid secretion, making the symptoms of reflux more noticeable.

DRUG INTERACTIONS

There are no confirmed drug interactions with onion consumption. Theoretically, because onion consumption may improve blood sugar control, patients with type 2 diabetes taking oral hypoglycemic drugs need to monitor glucose levels because dosage levels of the medication might need to be adjusted.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Vahora SB, Rizwan M, Khan JA. Medicinal uses of common Indian vegetables. *Planta Med*. 1973;23:381–393.
2. Griffiths G, Trueman L, Crowther T, et al. Onions—a global benefit to health. *Phytother Res*. 2002;16:603–615.
3. Boyle S, Dobson V, Duthie S, et al. Absorption and DNA protective effects of flavonoid glycosides from an onion meal. *Eur J Nutr*. 2000;39:213. https://doi.org/10.1007/s003940070014.
4. Bisen PS, Emerald M. Nutritional and therapeutic potential of garlic and onion (*Allium* sp.). *Curr Nutrition Food Sci*. 2016;12(3):190–199. https://doi.org/10.2174/1573401312666160608121954.
5. Elnima EI, Ahmed SA, Mekkawi AG, et al. The antimicrobial activity of garlic and onion extracts. *Pharmazie*. 1983;38:747–748.
6. Louria DB, McAnally JF, Lasser N, et al. Onion extract in treatment of hypertension and hyperlipidemia: a preliminary communication. *Curr Ther Res*. 1985;37:127–131.
7. Mittal MM, Mittal S, Sarin JC, et al. Effects of feeding onion on fibrinolysis, serum cholesterol, platelet aggregation and adhesion. *Indian J Med Sci*. 1974;28:144–148.
8. Norwell DY, Tarr RS. Garlic, vampires, and CHD. *Osteopath Ann*. 1983;11:546–549.
9. Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with hypolipidemic, hemostatic, fibrinolytic, and anticoagulant effects (part 1). *Asian J Pharm Sci Techn*. 2015;5(4):271–284.
10. Sainani GS, Desai DB, Gohre NH, et al. Effect of dietary garlic and onion on serum lipid profile in Jain community. *Indian J Med Res*. 1979;69:776–780.
11. Hubbard GP, Wolffram S, de Vos R, et al. Ingestion of onion soup high in quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in man: a pilot study. *Br J Nutr*. 2006;96:482–488.
12. Bever BO, Zahnd GR. Plants with oral hypoglycemic action. *Q J Crude Drug Res*. 1979;17:139–196.
13. Sharma KK, Gupta RK, Gupta S, et al. Antihyperglycemic effect of onion—effect on fasting blood sugar and induced hyperglycemia in man. *Indian J Med Res*. 1977;65:422–429.
14. Taj Eldin IM, Ahmed EM, Elwahab HM. A preliminary study of the clinical hypoglycemic effects of *Allium cepa* (red onion) in type 1 and type 2 diabetic patients. *Environ Health Insights*. 2010;14:71–77.
15. Dorsch W, Adam O, Weber J, et al. Antiasthmatic effects of onion extracts—detection of benzyl- and other isothiocyanates (mustard oils) as antiasthmatic compounds of plant origin. *Eur J Pharmacol*. 1984;107:17–24.
16. Dorsch W, Weber J. Prevention of allergen-induced bronchial obstruction in sensitized Guinea pigs by crude alcoholic onion extract. *Agents Actions*. 1984;14:626–630.
17. Singh AK, Bishayee A, Pandey AK. Targeting histone deacetylases with natural and synthetic agents: an emerging anticancer strategy. *Nutr*. 2018;10(6). pii: E371. PubMed PMID: 29882797.
18. Nepkar DP, Chander R, Bandekar JR, et al. Cytotoxic effect of onion extract on mouse fibrosarcoma 180 A cells. *Indian J Exp Biol*. 1981;19:598–600.
19. Caldes G, Prescott B. A potential antileukemic substance present in *Allium ascalonicum*. *Planta Med*. 1973;23:99–100.
20. Nohara T, Fujiwara Y, El-Aasr M, et al. Antitumor allium sulfides. *Chem Pharm Bull*. 2017;65(3):209–217. PubMed PMID: 28250342.
21. Sharquie KE, Al-Obaidi HK. Onion juice (*Allium cepa* L.), a new topical treatment for alopecia areata. *J Dermatol*. 2002;29:343–346.
22. Dorant E, van den Brandt PA, Goldbohm RA, et al. Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterol*. 1996;110:12–20.
23. Allen ML, Mellow MH, Robinson MG, et al. The effect of raw onions on acid reflux and reflux symptoms. *Am J Gastroenterol*. 1990;85:377–380.

51

Allium sativum (Garlic)

Michael T. Murray, ND, and John Nowicki, ND

OUTLINE

Allium sativum (family: *Amaryllidaceous* or *Liliaceous*)
Common names: garlic, allium

GENERAL DESCRIPTION

Garlic, a member of the lily family, is a perennial plant that is cultivated worldwide (Fig. 51.1). The garlic bulb is composed of individual cloves enclosed in a white skin. It is the bulb, either fresh or dehydrated, that is used as a spice or medicinal herb.

CHEMICAL COMPOSITION

Garlic contains 0.1% to 0.36% of a volatile oil composed of sulfur-containing compounds, including:

- Allicin
- Diallyl disulfide
- Diallyl trisulfide

Garlic oil is obtained by steamed distillation of the crushed fresh bulbs.[1] These volatile compounds are generally considered responsible for most of the pharmacological properties of garlic. Other constituents of garlic include the following[1,2]:

- Alliin (S-allyl-l-cysteine sulfoxide)
- S-methyl-l-cysteine sulfoxide
- Protein (16.8%, dry weight basis)
- High concentrations of trace minerals (particularly selenium)
- Vitamins
- Glucosinolates
- Enzymes (alliinase, peroxidase, and myrosinase)

Allicin is mainly responsible for garlic's pungent odor (Fig. 51.2). It is formed by the action of the enzyme alliinase on the compound alliin. The essential oil of garlic yields approximately 60% of its weight in allicin after exposure to alliinase. The enzyme is inactivated by heat, which accounts for the fact that cooked garlic produces neither as strong an odor as raw garlic nor nearly as powerful physiological effects.[1]

HISTORY AND FOLK USE

Garlic has been used throughout history for the treatment of a wide variety of conditions. Its usage predates written history. Sanskrit records document the use of garlic remedies approximately 5000 years ago, whereas the Chinese have been using it for at least 3000 years. *The Codex Ebers,* an Egyptian medical papyrus dating to about 1550 BC, mentions garlic as an effective remedy for various ailments, including hypertension, headache, bites, worms, and tumors. Hippocrates, Aristotle, and Pliny cited numerous therapeutic uses for garlic. In general, garlic has been used throughout the world to treat coughs, toothache, earache, dandruff, hypertension, atherosclerosis, hysteria, diarrhea, dysentery, diphtheria, vaginitis, and many other conditions.[1–3]

Stories, verse, and folklore (e.g., its alleged ability to ward off vampires) give historical documentation to garlic's power. Sir John Harrington, in writing *The Englishman's Doctor* in 1609, summarized garlic's virtues and faults[3]:

Garlic then have power to save from death

Bear with it though it maketh unsavory breath,

And scorn not garlic like some that think

It only maketh men wink and drink and stink.

In 1721, during a widespread plague in Marseilles, France, four condemned criminals were recruited to bury the dead. The gravediggers proved to be immune to the disease. Their secret was a concoction they drank consisting of macerated garlic in wine. This became known as vinaigre des quatre voleurs ("four thieves" vinegar), and it is still available in France today.

Garlic's antibiotic activity was noted by Pasteur in 1858. Garlic was used by Albert Schweitzer in Africa to treat amebic dysentery and as an antiseptic in the prevention of gangrene during World Wars I and II.

PHARMACOLOGY

Although garlic has a wide range of well-documented effects, its most important clinical uses are in the areas of infection, cancer prevention, and cardiovascular disease.

Antimicrobial Activity

Garlic has broad-spectrum antimicrobial activity against many genera of bacteria, viruses, worms, and fungi.[4–7] These findings support the historical use of garlic in the treatment of various infectious conditions.

Antibacterial Activity

Dating back to 1944, studies demonstrated that both garlic juice and allicin inhibit the growth of *Staphylococcus, Streptococcus, Bacillus, Brucella,* and *Vibrio* species at low concentrations.[7–9] In more recent studies, using serial dilution and filter paper disk techniques, fresh and vacuum-dried powdered garlic preparations were found to be effective antibiotic agents against many bacteria, as listed in Box 51.1.[4–7,10,11] In these studies, the antimicrobial effects of garlic were compared with commonly used antibiotics, including penicillin, streptomycin, chloramphenicol, erythromycin, and tetracyclines. Besides confirming garlic's well-known antibacterial effects, the studies demonstrated its efficacy in inhibiting the growth of bacteria that had become resistant to one or more of these antibiotics.[7]

Fig. 51.1 *Allium sativum.* (From https://www.istockphoto.com/photo/fresh-garlic-in-the-wicker-basket-gm474939904-64984191.)

Garlic administration has also been shown to significantly reduce the number of coliforms and anaerobes in the feces.[12] One clinical application of garlic's antibacterial activity may be in the treatment of *Helicobacter pylori.* A clinical investigation indicated that garlic intake for long durations (years) was associated with a significantly lower average antibody titer. This suggests an indirect inhibitory effect on the reproduction of *H. pylori* and possibly prevention of more serious peptic ulcer diseases.[13]

Antifungal Activity

Garlic demonstrated significant antifungal activity in many in vitro and in vivo studies.[4,14–19] From a clinical perspective, inhibition of *Candida albicans* has the most significance because both animal and in vitro studies showed garlic to be more potent than nystatin, gentian violet, and six other reputed antifungal agents.[4,15–17] Although allicin and the volatile oil fraction are clearly the most potent active anti-*Candida* components,[20,21] aqueous garlic extracts have been shown in vivo to be effective, even at a dilution of 1:100, against the common tinea corporis, capitis, and cruris fungal skin infections.[15]

One study compared the effects of garlic tablets (1500 mg/d × 7 days) and fluconazole (150 mg/d × 7 days) on *Candida* vaginitis in 100 married women (aged 18–44 years).[22] The results of microscopical evaluation and vaginal discharge culture showed significant differences before and after intervention in both groups, with the overall symptoms of the disease (i.e., redness of the vulva and vagina, discharge, pustulopapular lesions, and abnormal cervix) improving by about 60% in the garlic tablet group and 71.2% in the fluconazole group. In another study at a major Chinese hospital, garlic therapy alone was used effectively in the treatment of cryptococcal meningitis, one of the most serious fungal infections imaginable.[18]

Anthelmintic Effects

Garlic extracts have anthelmintic activity against common intestinal parasites, including *Ascaris lumbricoides* (roundworm) and hookworms.[12,23]

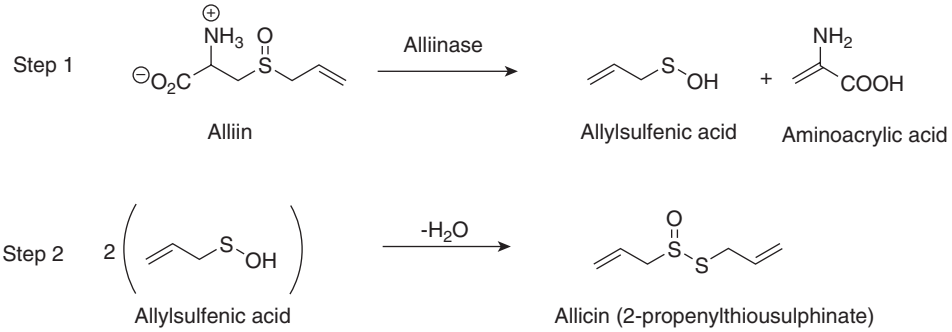


Fig. 51.2 Chemical structure of allicin and mechanism of formation from alliin by the enzyme alliinase. (From Wallock-Richards D, Doherty CJ, Doherty L, Clarke DJ, Place M, Govan JR, Campopiano DJ. Garlic revisited: antimicrobial activity of allicin-containing garlic extracts against Burkholderia cepacian complex. *PLoS One.* 2014;9[12]:e112726.)

BOX 51.1 Clinically Relevant Microbes Inhibited by Garlic

- Bacteria
- Alpha- and β-hemolytic Streptococcus
- *Citrobacter* spp.
- *Escherichia coli*
- *Helicobacter pylori*
- *Klebsiella pneumoniae*
- Mycobacteria
- *Proteus vulgaris*
- *Salmonella enteritidis*
- *Staphylococcus aureus*
- Fungi
- *Candida albicans*
- *Cryptococcus neoformans*
- Helminths
- *Ascaris lumbricoides*
- Hookworms
- Viruses
- Herpes simplex types 1 and 2
- Human rhinovirus type 2
- Parainfluenza virus type 3
- Vaccinia virus
- Vesicular stomatitis virus

Data from Adetumbi MA, Lau BH. *Allium sativum* (garlic)—a natural antibiotic. *Med Hypotheses.* 1983;12:227–237; Koch HP. Garlicin—fact or fiction? *Phytother Res.* 1993;7:278–280; Hughes BG, Lawson L. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* L. (elephant garlic), and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. *Phytother Res.* 1991;5:154–158; Harris JC, Cottrell SL, Plummer S, et al. Antimicrobial properties of *Allium sativum* (garlic). *Appl Microbiol Biotechnol.* 2001;57:282–286; Sharma VD, Sethi MS, Kumar A, et al. Antibacterial property of *Allium sativum* Linn: in vivo and in vitro studies. *Indian J Exp Biol.* 1977;15:466–468; Elnima EI, Ahmed SA, Mekkawi AG, et al. The antimicrobial activity of garlic and onion extracts. *Pharmazie.* 1983;38:747–748.

Antiviral Effects

Garlic's antiviral effects have been demonstrated by its protection of mice from infection with intranasally inoculated influenza virus and by its enhancement of neutralizing antibody production when given with influenza vaccine.[24]

The in vitro virus-killing effects of fresh garlic, allicin, and other sulfur components of garlic were determined against herpes simplex types 1 and 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2. The order for virucidal activity was as follows: ajoene > allicin > allyl methyl thiosulfinate > methyl allyl thiosulfinate.

Ajoene was found in oil macerates of garlic but not in fresh garlic extracts. No antiviral activity was found for alliin, deoxyalliin, diallyl disulfide, or diallyl trisulfide. Fresh garlic extract was virucidal against all viruses tested. The virucidal activity of commercial products was found to be dependent on their preparation processes. Those producing the highest level of allicin and other thiosulfinates had the best virucidal activity.[25] The antiviral activity of an allicin-containing garlic supplement was investigated in 146 subjects randomized to receive a placebo or an allicin-containing garlic supplement, one capsule daily, over a 12-week period.[26] The garlic-treatment group had significantly fewer colds than the placebo group (24 vs. 65 patients). The placebo group, in contrast, recorded significantly more days challenged virally (366 vs. 111 patients) and a significantly longer duration of symptoms (5.01 vs. 1.52 days). This study indicates that allicin-containing garlic supplements can prevent the common cold virus.

Immune-Enhancing Effects

Extensive research has shown that garlic has many immune-potentiating properties, most of which are thought to be due to volatile factors composed of sulfur-containing compounds: allicin, diallyl disulfide, diallyl trisulfide, and others. For example, in vitro studies with allicin showed that it stimulated enhanced cell-mediated cytotoxicity in human peripheral mononuclear cells. In animal models, multiple administration of allicin elicited marked antitumor effects via immunostimulatory mechanisms.[27] Fresh garlic, commercial products containing allicin, and aged garlic preparations have all shown these immune-enhancing properties. Garlic has been shown to enhance the pathogen-attacking activity of T cells, neutrophils, and macrophages, which increase the secretion of interleukin and natural killer (NK) cell activity.[28–31] The increase in killer cell activity was a remarkable 140% in those who ate the equivalent of two bulbs a day and 156% in those who consumed 1800 mg of odorless, aged garlic.

Healthy human participants (n = 120), between 21 and 50 years of age, were recruited for a randomized, double-blind, placebo-controlled parallel-intervention study to consume 2.56 g aged garlic extract (AGE)/d or placebo supplements for 90 days during the cold and flu season.[32] After 45 days of AGE consumption, γδ-T and NK cells proliferated better and were more activated than cells from the placebo group. After 90 days, although the number of illnesses was not significantly different, the AGE group showed reduced cold and flu severity, with a reduction in the number of symptoms, the number of days participants functioned suboptimally, and the number of work/school days missed.

Anticancer Effects

The famous Greek physician Hippocrates prescribed eating garlic as a treatment for cancer. Animal research and some human studies suggest this may have been well-founded advice. It must be kept in mind that much of garlic's anticancer effect is likely an indirect effect via its effect on the immune system. Nonetheless, several garlic components have displayed significant anticancer effects, including enhancing Phase II metabolizing enzymes, antioxidant properties, inhibition of the formation of nitrosamines, direct tumor growth inhibition, and the ability to induce apoptosis.[33–51] Human studies showing garlic's anticancer effects are largely based on epidemiological studies.[33–37] These studies typically show an inverse relationship between cancer rates and garlic consumption. Human studies have also shown that garlic inhibits the formation of nitrosamines (powerful cancer-causing compounds formed during digestion).[47,48]

Cardiovascular Effects

Garlic appears to be an important protective factor against heart disease and strokes via its ability to affect the process of atherosclerosis at many steps (Fig. 51.3). Because there is substantial clinical information on garlic's beneficial effects on the cardiovascular system, the pharmacology is discussed later in the "Clinical Applications" section.

Other Effects

Anti-inflammatory Effects

Garlic extract has demonstrated significant anti-inflammatory activity in experimental models of inflammation.[2,12] This activity is probably a result of garlic's inhibition of the formation of inflammatory compounds. A randomized, double-blind, placebo-controlled,

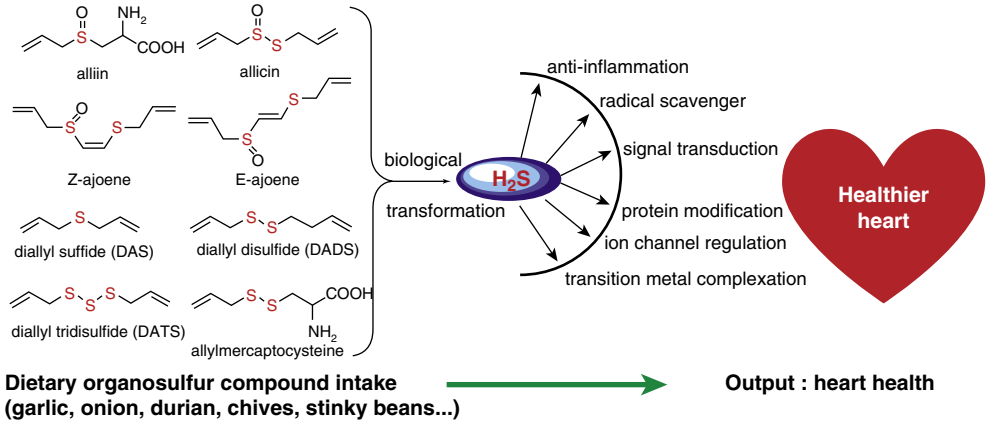


Fig. 51.3 Hydrogen sulfide as the common denominator for bioactivity of dietary organosulfur compounds. (From Tocmo R, Liang D, Lin Y, Huang D. Chemical and biochemical mechanisms underlying the cardioprotective roles of dietary organopolysulfides. *Front Nutr.* 2015;2:1.)

parallel-design trial was conducted to assess the anti-inflammatory and analgesic effects of a garlic supplement on serum resistin and tumor necrosis factor-alpha (TNF-α) concentrations and on pain severity in overweight or obese women with knee osteoarthritis (OA).[52] Eighty postmenopausal overweight or obese women (25 ≤ body mass index [BMI] ≤ 40 kg/m2, age 50–75 years) with mild to moderate knee OA were randomly divided into two groups to receive twice-daily garlic tablets (total: 1000 mg) or placebo for 12 weeks. At week 12, although serum TNF-α levels did not change significantly within or between the two groups, resistin concentrations were significantly decreased in the garlic group (6.41 ± 2.40–5.56 ± 2.16 ng/mL; $P = 0.008$). After supplementation, pain scores were also significantly lower in the garlic group compared with the placebo group (5.3 ± 2.3 vs. 6.2 ± 2.5; $P = 0.043$).

Hypoglycemic Action

Garlic and onions have often been used in the treatment of diabetes. Allicin has been shown to have significant hypoglycemic action. This effect is thought to be due to increased hepatic metabolism, increased release of insulin, and the insulin-sparing effect.[53] The latter mechanism appears to be the major factor because allicin and other sulfhydryl compounds in garlic and onions compete with insulin (also a disulfide protein) for insulin-inactivating compounds, which results in an increase in free insulin.

Interestingly, when allicin was administered to groups of rats fed a high-fructose diet, the control group that was fed a diet enriched by fructose alone continued to gain weight, whereas the groups fed allicin did not.[54] This study indicates that garlic may have some practical use in weight control.

Raw crushed garlic has been shown to have beneficial effects in individuals with metabolic syndrome and may be used as an accompanying remedy for prevention and treatment. In one study, 40 patients with metabolic syndrome underwent treatment with 100 mg/kg body weight of raw crushed garlic 2 times per day added to a standard diet for 4 weeks.[55] Results showed that raw crushed garlic significantly reduced the components of metabolic syndrome, including waist circumference, systolic and diastolic blood pressure, triglycerides, and fasting blood glucose, and significantly increased serum high-density lipoprotein cholesterol.

Miscellaneous Effects

Garlic possesses diuretic, diaphoretic, emmenagogue, and expectorant action.[1,10] A randomized, double-blind, placebo-controlled study found that fermented garlic extract improved serum of gamma-glutamyl transferase (GGT) and alanine transaminase (ALT) levels in adults with mild hepatic dysfunction.[56] It is also a carminative, antispasmodic, and digestant, making it useful in cases of flatulence, nausea, vomiting, colic, and indigestion.[12,57]

COMMERCIAL PREPARATIONS

The modern use of garlic primarily features the use of commercial preparations designed to offer the benefits of garlic without the odor. The marketplace is swamped with garlic products, and each manufacturer claims its product is the best. However, there are vital considerations when choosing a garlic product to prescribe. First, if the primary goal is to lower cholesterol or blood pressure, as well as exert immune-enhancing or antimicrobial effect, it is important to ensure that the product provides a sufficient level of allicin. Because allicin is not actually in the product at significant levels, manufacturers often refer to the allicin potential or allicin yield. These terms signify the amount of allicin produced when allinase is activated in the garlic tablet or powder.

The next issue is not so simple to tell from a label. It involves the quality and character of the enteric coating of the tablet. For the allicin to be liberated within the intestinal tract, the tablet must not only be resistant to the stomach's acid, but it must disintegrate rapidly when it reaches the small intestine. According to research conducted by the renowned garlic experts Lawson et al.,[58] when 24 brands of enteric-coated garlic were analyzed for tablet dissolution using an approved method (U.S. Pharmacopoeia dissolution method 724A), only one brand released the amount of allicin claimed on the label. The second-best brand released only 44% of its label claim, and 75% of the brands released less than 10% of their label claim. Failure to deliver an effective dosage of allicin most assuredly does not lower cholesterol or blood pressure.

Why so many garlic products fail to deliver allicin is basically due to two major problems. First, many of the garlic products contained little alliinase activity. Alliin was found to be plentiful, but because the activity of alliinase was low, the level of allicin formed was also low. Next, many tablets contained excipients (e.g., binders and fillers) that actually inhibit alliinase activity. The alliinase activity in 63% of the brands was less than 10% of the expected activity. The inability to release an effective dose of allicin would explain why so many studies on garlic supplements fail to show benefits in lowering cholesterol or blood pressure.[59] For example, studies done

on one particular garlic supplement before 1993 were mostly positive. The results from these positive studies were the main reason garlic supplements have been allowed to refer to cholesterol-lowering activity in Germany and the United States. However, most studies published since 1995 have failed to show a consistent effect in lowering cholesterol.[60–62]

Although the authors of the negative studies on garlic believe that the underlying reason for the results was a better-designed study, a more likely explanation is that they were due to a poorer-quality tablet. Specifically, research conducted by Lawson showed that tablets manufactured before 1993 were twice as resistant to disintegration in acid as tablets manufactured after 1993 and that the older tablets released three times the amount of allicin than the more recently manufactured tablets.[58] Examination of package labels shows several changes in tablet excipients between the pre-1993 and post-1993 tablets. Again, these excipients are believed to block allinase activity.

Importantly, most studies that show a positive effect of garlic and garlic preparations in reducing cholesterol and blood pressure are those that use products that deliver a sufficient dosage of allicin, other garlic components such as S-allylcysteine, and garlic extracts (e.g., AGE) that protect against atherosclerosis via additional mechanisms, including protection against low-density lipoprotein (LDL) oxidation and improvement of endothelial cell function.

Kyolic, the AGE manufactured by the Wakunaga Wakunaga Pharmaceutical Co., Ltd. (Osaka, Japan), is a highly standardized AGE produced by extraction and aging of organic fresh garlic, at room temperature, for 20 months. The process increases antioxidant levels and converts allicin to mostly stable water-soluble organosulfur compounds, such as S-allylmercaptocysteine and S-allylcysteine. These compounds have high bioavailability and considerable antioxidant effects.[63,64] AGE has minimal cholesterol-lowering and antimicrobial effects, but based on good clinical data, it does have antiatherosclerotic, antiaging, and anticancer effects.

The antioxidant effects of AGE have been shown to prevent LDL oxidation[65] and improve endothelium-dependent vasodilation via increased nitric oxide production and decreased output of inflammatory cytokines. In a double-blind, placebo-controlled, crossover study in 15 men with angiographically proven coronary artery disease, AGE supplementation for 2 weeks significantly improved brachial artery flow–mediated endothelium-dependent dilation by 44%.[66] In a double-blind study of AGE in normal, healthy individuals, dosages between 2.4 and 7.2 g/day were shown to produce a dose-dependent selective inhibition on platelet aggregation and adhesion.[67] In a 1-year study, AGE was shown to produce a modest reduction in the calcium score of 7.5, determined by electron beam tomography, whereas the placebo group demonstrated an average increase in calcium scores of 22.2.[68,69]

Epidemiological and animal studies suggest AGE and its organosulfur constituents, such as S-allylcysteine and S-allylmercaptocysteine, have anticarcinogenic effects. Several clinical studies validated an anticancer effect for AGE. In one double-blinded study in patients with colorectal polyps using high-AGE (AGE 2.4 mL/day) and low-AGE (AGE 0.16 mL/day) doses, in 37 patients chosen as efficacy evaluated subjects, 47.4% (9/19 patients) in the high-AGE and 66.7% (12/18 patients) in the low-AGE group had at least one new adenoma for the first and second interval (0–12 months after intake). The decrease rate of at least one adenoma was 50.0% (7/14 patients) in the high-AGE group for the second interval (6–12 months after intake), whereas there was no decrease in subjects in the low-AGE group.[70] The difference from baseline for total size of adenomas increased in the low-AGE group, whereas an increase in the high-AGE group was suppressed for the second interval. The difference from baseline for the total size of adenomas in subjects who had adenomas at baseline increased in the low-AGE group and decreased in the high-AGE group for the second interval. The results of this study suggest the possibility of preventive and therapeutic effects of AGE on colorectal adenomas, although it would be necessary to investigate these results in larger-scale and longer-term trials.

In a double-blind study in patients with advanced cancer, the primary end point used was a quality of life (QOL) questionnaire based on the Functional Assessment of Cancer Therapy. The sub–end points were changes in NK cell activity and salivary cortisol levels before and after administering AGE. The group consisted of 42 patients with liver cancer (84%), 7 patients with pancreatic cancer (14%), and 1 patient with colon cancer (2%). Drug compliance was relatively good in both the AGE and placebo groups. Although no difference was observed in QOL, both the number of NK cells and NK cell activity increased significantly in the AGE group.[71]

CLINICAL APPLICATIONS

Although garlic has long been used in infectious conditions, a use supported by its antimicrobial and immune-enhancing properties, the primary clinical use of garlic has focused on its role in cardiovascular disease. Specifically, garlic is recommended primarily for its ability to lower cholesterol and blood pressure in the attempt to reduce the risk of dying prematurely from a heart attack or stroke.

In addition to the use of garlic preparations, garlic consumption as a food should be encouraged in patients with high cholesterol levels, high blood pressure, and diabetes.

Cholesterol-Lowering Activity

One of the major areas of focus in garlic's ability to offer significant protection against heart disease and strokes has been the evaluation of its ability to lower blood cholesterol levels.[1,72–76] Unfortunately, the results are inconsistent. Most, but not all, of the double-blind, placebo-controlled studies in patients with initial cholesterol levels greater than 200 given a commercial garlic preparation, providing a daily dose of at least 10 mg alliin or a total allicin potential of 4000 mcg, showed an ability to lower total serum cholesterol levels by about 10% to 12%. In addition, LDL cholesterol decreased by about 15%, high-density lipoprotein (HDL) cholesterol levels usually increased by about 10%, and triglyceride levels typically dropped by 15%,[1,58,59,76–83] whereas most trials not using products that delivered this dosage of allicin failed to produce a lipid-lowering effect.[58–62] However, based on some clinical trials, even using a well-defined garlic preparation is not sufficient assurance that positive results will be seen.[84–87]

One study worth mentioning evaluated the effect of raw garlic and two commonly used garlic supplements against a placebo on cholesterol concentrations in adults with moderate hypercholesterolemia. In the trial, 192 adults with low-density lipoprotein cholesterol (LDL-C) concentrations of 130 to 190 mg/dL were randomly assigned to one of the following four treatment arms: raw garlic, powdered garlic supplement, AGE supplement, or placebo. Garlic product doses equivalent to four average-sized garlic cloves a day were given 6 days/week for 6 months. None of the forms of garlic used, including raw garlic, had statistically or clinically significant effects on LDL-C or other plasma lipid concentrations in adults with moderate hypercholesterolemia.[87]

At present, it can be concluded that the cholesterol-lowering effects of supplemental garlic preparations on cholesterol levels are extremely variable and modest at best. Nonetheless, the combination of lowering

LDL and raising HDL can greatly improve the HDL/LDL ratio, a significant goal in the prevention of heart disease and strokes. Garlic preparations exert several other beneficial effects in preventing heart disease and strokes (discussed under "Platelet Aggregation Inhibition") and have also been shown to improve blood sugar control in diabetics with hyperlipidemia.[88]

In addition to taking a garlic supplement, individuals with high cholesterol levels should eat more garlic and onions because increased dietary intake of garlic and onions can also lower cholesterol levels.[72–75,89] In a 1979 population study, researchers studied three populations of vegetarians in the Jain community in India who consumed differing amounts of garlic and onions.[90,91] Numerous favorable effects on blood lipids, as shown in Table 51.1, were observed in the group that consumed the largest amount. Blood fibrinogen (discussed in "Fibrinolytic Activity") levels were highest in the group eating no onions or garlic. The study was quite significant because the subjects had nearly identical diets, except in garlic and onion ingestion.

Hypertension

Garlic has demonstrated hypotensive action in both experimental animal models and humans with hypertension.[72–75,92–96] A meta-analysis of published and unpublished randomized controlled trials of garlic preparations was conducted to determine the effect of garlic on blood pressure relative to placebo.[92] Eight trials (seven double-blind, one single-blind) were identified as meeting analytic criteria. A total of 415 subjects were included in the analysis. All trials used a dried garlic powder standardized to contain 1.3% alliin at a dosage of 600 to 900 mg/day (corresponding to 7.8 and 11.7 mg of alliin or the equivalent of approximately 1.8–2.7 g of fresh garlic daily). The meta-analysis concluded that garlic preparations designed to yield allicin lowered systolic and diastolic blood pressures over a 1- to 3-month period. The typical drop from pooled data was 11 mm Hg in the systolic and 5 mm Hg in the diastolic. This degree of blood pressure reduction in hypertensives can be quite significant. If the blood pressure–lowering effects of garlic can be maintained, the risk of stroke may be reduced by an estimated 30% to 40% and the risk of heart attack by 20% to 25%.

Platelet Aggregation Inhibition

Excessive platelet aggregation is strongly linked to atherosclerosis, heart disease, and strokes. Garlic preparations standardized for alliin content, as well as garlic oil and AGE, have all demonstrated significant inhibition of platelet aggregation.[67,72–75,97,98] In one study, 120 patients with increased platelet aggregation were given either 900 mg/day of a dried garlic preparation containing 1.3% alliin or a placebo for 4 weeks.[97] In the garlic group, spontaneous platelet aggregation disappeared, the microcirculation of the skin increased by 47.6%, plasma viscosity decreased by 3.2%, diastolic blood pressure dropped from an average of 74 to 67 mm Hg, and fasting blood glucose concentration dropped from an average of 90 to 79 mg/dL.

TABLE 51.1 Effects of Garlic and Onion Consumption on Serum Lipids Under Carefully Matched Diets

Garlic/Onion	Cholesterol	Triglyceride
None	208 mg/dL	109 mg/dL
10/200 g/wk	172 mg/dL	75 mg/dL
50/600 g/wk	159 mg/dL	52 mg/dL

Fibrinolytic Activity

Epidemiological studies have suggested that excessive fibrinogen formation is a major primary risk factor for cardiovascular disease.[99] Garlic preparations standardized for alliin content and garlic oil as well as both fried and raw garlic have been shown to significantly increase serum fibrinolytic activity in humans.[100,101] This increase occurs within the first 6 hours after ingestion and continues for up to 12 hours.

Prevention of Low-Density Lipoprotein Oxidation and Other Vascular Effects

Growing evidence indicates that LDL oxidation plays a significant role in the development of atherosclerosis. Accordingly, substances that prevent oxidation of LDL may slow down atherosclerosis. Garlic is known to exert antioxidant activity and has recently been shown to exert significant effects on preventing LDL oxidation.[65,66,102–105] In one study, healthy human volunteers given 600 mg/day of a garlic preparation providing 7.8 mg alliin for 2 weeks had a 34% lower susceptibility to lipoprotein oxidation compared with controls.[102] These results are significant given the short amount of time in which they were produced, coupled with the importance of reducing lipoprotein oxidation.

A placebo-controlled, double-blind trial involved 23 subjects with coronary artery disease who had at least 75% blockage in one to three major coronary arteries. Two and 4 hours after the subjects ingested a single 300-mg dose of garlic powder, the atherogenicity of their sera was markedly decreased, less cholesterol had accumulated, and there were lower levels of oxidized LDL in human aortic smooth muscle cells cultured with patients' sera after treatment compared with those cultured with sera obtained before administration of garlic. After 3 weeks of therapy at 300 mg, three times daily, blood serum atherogenicity was decreased twofold compared with initial levels.[106]

Because AGE has also shown an ability to prevent LDL oxidation,[104] it is likely that S-allylcysteine and other nonallicin components of garlic play a major role in the protection against LDL oxidation.

Studies have also shown garlic or garlic components to exert positive effects on endothelial function, vascular reactivity, and peripheral blood flow.[107–109] For example, a randomized, double-blind, placebo-controlled trial demonstrated that supplementation with garlic extract favorably modifies endothelial biomarkers, such as high-sensitivity C-reactive protein (hsCRP) and total antioxidant status, associated with cardiovascular risk.[110]

DOSAGE

On the basis of extensive clinical research, a commercial garlic product should provide a daily dose equal to at least 4000 mg of fresh garlic. This dosage translates to at least 10 mg alliin or a total allicin potential of 4000 μg. Alternatively, AGE at a dosage of 2.4 to 7.2 g/day can be used.

TOXICITY

For the vast majority of individuals, garlic is nontoxic at the dosages commonly used. For some, however, it can irritate the digestive tract and cause heartburn. Others are apparently unable to effectively detoxify allicin and other sulfur-containing components. Prolonged feeding of large amounts of raw garlic to rats resulted in anemia, weight loss, and failure to grow.[111] Although the exact toxicity of garlic has yet to be definitively determined, side effects are rare at the recommended dosage. Garlic is thought to be safe during pregnancy and breastfeeding. Two studies showed that babies liked breast milk better from mothers who ate garlic.[112,113]

DRUG INTERACTIONS

Generally, garlic supplements should not be used in patients taking anticoagulant drugs (e.g., Coumadin) or in those scheduled for elective surgery because of garlic's effects on platelet aggregation. The exception to this warning is AGE because it appears safe for use in patients on warfarin therapy.[114]

Although garlic components have shown an ability to affect cytochrome P450 enzymes, they do not appear to interfere with drug metabolism.[115,116]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Koch H, Lawson L. *Garlic: The Science and Therapeutic Application of Allium Sativum L. and Related Species*. Baltimore: Williams & Wilkins; 1996.
2. Raj KP, Parmar RM. Garlic—condiment and medicine. *Indian Drugs*. 1977;15:205–210.
3. Block E. The chemistry of garlic and onions. *Sci Am*. 1985;252:114–119.
4. Adetumbi MA, Lau BH. *Allium sativum* (garlic)—a natural antibiotic. *Med Hypotheses*. 1983;12:227–237.
5. Koch HP. Garlicin—fact or fiction? *Phytother Res*. 1993;7:278–280.
6. Hughes BG, Lawson L. Antimicrobial effects of *Allium sativum* L. (garlic), *Allium ampeloprasum* L. (elephant garlic), and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. *Phytother Res*. 1991;5:154–158.
7. Harris JC, Cottrell SL, Plummer S, et al. Antimicrobial properties of *Allium sativum* (garlic). *Appl Microbiol Biotechnol*. 2001;57:282–286.
8. Huddleston IF, DuFrain J, Barrons K, et al. Antibacterial substances in plants. *J Am Vet Med Assoc*. 1944;105:394–397.
9. Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J Am Chem Soc*. 1944;66:1950–1951.
10. Sharma VD, Sethi MS, Kumar A, et al. Antibacterial property of *Allium sativum* Linn: in vivo and in vitro studies. *Indian J Exp Biol*. 1977;15:466–468.
11. Elnima EI, Ahmed SA, Mekkawi AG, et al. The antimicrobial activity of garlic and onion extracts. *Pharmazie*. 1983;38:747–748.
12. Vohora SB, Rizwan M, Khan JA. Medicinal uses of common Indian vegetables. *Planta Med*. 1973;23:381–393.
13. Salih BA, Abasiyanik FM. Does regular garlic intake affect the prevalence of Helicobacter pylori in asymptomatic subjects? *Saudi Med J*. 2003;24:842–845.
14. Amer M, Taha M, Tosson Z. The effect of aqueous garlic extract on the growth of dermatophytes. *Int J Dermatol*. 1980;19:285–287.
15. Venugopal PV, Venugopal TV. Antidermatophytic activity of garlic (*Allium sativum*) in vitro. *Int J Dermatol*. 1995;34:278–279.
16. Sandhu DK, Warraich MK, Singh S. Sensitivity of yeasts isolated from cases of vaginitis to aqueous extracts of garlic. *Mykosen*. 1980;23:691–698.
17. Prasad G, Sharma VD. Efficacy of garlic (*Allium sativum*) treatment against experimental candidiasis in chicks. *Br Vet J*. 1980;136:448–451.
18. Anonymous. Garlic in cryptococcal meningitis: a preliminary report of 21 cases. *Chin Med J*. 1980;93:123–126.
19. Fromtling RA, Bulmer GS. In vitro effect of aqueous extract of garlic (*Allium sativum*) on the growth and viability of Cryptococcus neoformans. *Mycologia*. 1978;70:397–405.
20. Lemar KM, Turner MP, Lloyd D. Garlic (*Allium sativum*) as an anti-Candida agent: a comparison of the efficacy of fresh garlic and freeze-dried extracts. *J Appl Microbiol*. 2002;93:398–405.
21. Kim JW, Kim YS, Kyung KH. Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts. *J Food Prot*. 2004;67:499–504.
22. Ebrahimy F, Dolatian M, Moatar F, Majd HA. Comparison of the therapeutic effects of Garcin (®) and fluconazole on *Candida vaginitis*. *Singapore Med J*. 2015;56(10):567–572. 26512149.
23. Bastidas GJ. Effect of ingested garlic on *Necator americanus* and *Ancylostoma caninum*. *Am J Trop Med Hyg*. 1969;18:920–923.
24. Nagai K. Experimental studies on the preventive effect of garlic extract against infection with influenza virus. *Jpn J Infect Dis*. 1973;47:321–325.
25. Weber ND, Andersen DO, North JA, et al. In vitro virucidal effects of *Allium sativum* (garlic) extract and compounds. *Planta Med*. 1992;58:417–423.
26. Josling P. Preventing the common cold with a garlic supplement: a double-blind, placebo-controlled survey. *Adv Ther*. 2001;18:189–193.
27. Patya M, Zahalka MA, Vanichkin A, et al. Allicin stimulates lymphocytes and elicits an antitumor effect: a possible role of p21ras. *Int Immunol*. 2004;16:275–281.
28. Morioka N, Sze LL, Morton DL, et al. A protein fraction from aged garlic extract enhances cytotoxicity and proliferation of human lymphocytes mediated by interleukin-2 and concanavalin A. *Cancer Immunol Immunother*. 1993;37:316–322.
29. Lau BH, Yamasaki T, Gridley DS. Garlic compounds modulate macrophage and T-lymphocyte functions. *Mol Biother*. 1991;3:103–107.
30. Kandil OM, Abdullah TH, Elkadi A. Garlic and the immune system in humans. Its effect on natural killer cells. *Fed Proc*. 1987;46:441.
31. Hirao Y, Sumioka I, Nakagami S, et al. Activation of immunoresponder cells by the protein fraction from aged garlic extract. *Phytother Res*. 1987;1:161–164.
32. Percival SS. Aged garlic extract modifies human immunity. *J Nutri*. 2016;146(2):433S–436S. 26764332.
33. Lau BH, Tadi P, Tosk J. *Allium sativum* (garlic) and cancer prevention. *Nutr Res*. 1990;10:937–948.
34. Dorant E, van den Brandt PA, Goldbohm RA, et al. Garlic and its significance for the prevention of cancer in humans: a critical review. *Br J Cancer*. 1993;67:424–429.
35. Dausch JG, Nixon DW. Garlic: a review of its relationship to malignant disease. *Prev Med*. 1990;19:346–361.
36. Thomson M, Ali M. Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. *Curr Cancer Drug Targets*. 2003;3:67–81.
37. Khanum F, Anilakumar KR, Viswanathan KR. Anticarcinogenic properties of garlic: a review. *Crit Rev Food Sci Nutr*. 2004;44:479–488.
38. Choy YM, Kwok TT, Fund KP, et al. Effect of garlic, Chinese medicinal drugs and amino acids on growth of Erlich ascites tumor cells in mice. *Am J Chin Med*. 1983;11:69–73.
39. Lin X, Liu J, Milner J. Dietary garlic powder suppresses in vivo formation of DNA adducts induced by N-nitroso compounds in liver and mammary tissues. *FASEB J*. 1992;6:A1392.
40. Nagabhushan M, Line D, Polverini PJ, et al. Anticarcinogenic action of diallyl sulfide in hamster buccal pouch and forestomach. *Cancer Lett*. 1992;66:207–216.
41. Meng CL, Shyu KW. Inhibition of experimental carcinogenesis by painting with garlic extract. *Nutr Cancer*. 1990;14:207–217.
42. Niukian K, Schwartz J, Shklar G. Effects of onion extract on the development of hamster buccal pouch carcinomas as expressed in tumor burden. *Nutr Cancer*. 1987;9:171–176.
43. Wargovich MJ. Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. *Carcinogenesis*. 1987;3:487–489.
44. Belman S. Onion and garlic oils inhibit tumor promotion. *Carcinogenesis*. 1983;4:1063–1065.
45. Weisberger AS, Pensky J. Tumor inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). *Cancer Res*. 1958;18:1301–1308.
46. Kroening F. Garlic as an inhibitor for spontaneous tumors in mice. *Acta Unio Int Contra Cancrum*. 1964;20:855–856.
47. Mei X, Lin X, Liu JZ, et al. The blocking effect of garlic on the formation of N-nitrosoproline in the human body. *Acta Nutrimenta Sinica*. 1989;11:144–145.
48. Xing M, Wang ML, Xu HX, et al. Garlic and gastric cancer—the effect of garlic on nitrite and nitrate in gastric juice. *Acta Nutrimenta Sinica*. 1982;4:53–55.
49. Sundaresan S, Subramanian P. S-allylcysteine inhibits circulatory lipid peroxidation and promotes antioxidants in N-nitrosodiethylamine-induced carcinogenesis. *Pol J Pharmacol*. 2003;55:37–42.
50. Ledezma E, Apitz-Castro R, Cardier J. Apoptotic and anti-adhesion effect of ajoene, a garlic derived compound, on the murine melanoma B16F10 cells: possible role of caspase-3 and the alpha(4)beta(1) integrin. *Cancer Lett*. 2004;206:35–41.
51. Munday R, Munday CM. Induction of phase II enzymes by aliphatic sulfides derived from garlic and onions: an overview. *Methods Enzymol*. 2004;382:449–456.
52. Dehghani S, Alipoor E, Salimzadeh A, et al. The effect of a garlic supplement on the pro-inflammatory adipocytokines, resistin and tumor necrosis factor-alpha, and on pain severity, in overweight or obese women with knee osteoarthritis. *Phytomedicine*. 2018;48:70–75. 30195882.
53. Bever BO, Zahnd GR. Plants with oral hypoglycemic action. *Q J Crude Drug Res*. 1979;17:139–196.

54. Elkayam A, Mirelman D, Peleg E, et al. The effects of allicin on weight in fructose-induced hyperinsulinemic, hyperlipidemic, hypertensive rats. *Am J Hypertens*. 2003;16:1053–1056.
55. Choudhary PR, Jani RD, Sharma MS. Effect of raw crushed garlic (*Allium sativum L.*) on components of metabolic syndrome. *J Dietary Suppl*. 2018;15(4):499–506. 28956671.
56. Kim HN, Kang SG, Roh YK, Choi MK, Song SW. Efficacy and safety of fermented garlic extract on hepatic function in adults with elevated serum gamma-glutamyl transpeptidase levels: a double-blind, randomized, placebo-controlled trial. *Eur J Nutri*. 2017;56(5):1993–2002. 27743130.
57. Barowsky H, Boyd LJ. The use of garlic (Allistan) in gastrointestinal disturbances. *Rev Gastroenterol*. 1944;11:22–26.
58. Lawson LD, Wang ZJ, Papadimitriou D. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. *Planta Medica*. 2001;67:13–18.
59. Lawson LD, Wang ZJ. Tablet quality: a major problem in clinical trials with garlic supplements. *Forsch Komplementarmed Klass Naturheilkd*. 2000;7:45.
60. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nutr J*. 2002;1:4. Published online 2002 November 19 https://doi.org/10.1186/1475-2891-1-4.
61. Alder R, Lookinland S, Berry JA, et al. A systematic review of the effectiveness of garlic as an anti-hyperlipidemic agent. *J Am Acad Nurse Pract*. 2003;15:120–129.
62. Stevinson C, Pittler MH, Ernst E. Garlic for treating hypercholesterolemia: a meta-analysis of randomized clinical trials. *Ann Intern Med*. 2000;133:420–429.
63. Park JH, Park YK, Park E. Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. *Plant Foods Hum Nutr*. 2009;64:244–249.
64. Kodera Y, Suzuki A, Imada O, et al. Physical, chemical, and biological properties of s-allylcysteine, an amino acid derived from garlic. *J Agric Food Chem*. 2002;50:622–632.
65. Huang CN, Horng JS, Yin MC. Antioxidative and antiglycative effects of six organosulfur compounds in low-density lipoprotein and plasma. *J Agric Food Chem*. 2004;52:3674–3678.
66. Durak I, Kavutcu M, Aytac B, et al. Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J Nutr Biochem*. 2004;15:373–377.
67. Steiner M, Li W. Aged garlic extract, a modulator of cardiovascular risk factors: a dose-finding study on the effects of AGE on platelet functions. *J Nutr*. 2001;131:S980–S984.
68. Williams MJ, Sutherland WH, McCormick MP, et al. Aged garlic extract improves endothelial function in men with coronary artery disease. *Phytother Res*. 2005;19:314–319.
69. Budoff M. Aged garlic extract retards progression of coronary artery calcification. *J Nutr*. 2006;136(suppl 3):S741–S744.
70. Tanaka S, Haruma K, Yoshihara M, et al. Aged garlic extract has potential suppressive effect on colorectal adenomas in humans. *J Nutr*. 2006;136(suppl 3):S821–S826.
71. Ishikawa H, Saeki T, Otani T, et al. Aged garlic extract prevents a decline of NK cell number and activity in patients with advanced cancer. *J Nutr*. 2006;136(suppl 3):S816–S820.
72. Ali M, Thomson M. Consumption of a garlic clove a day could be beneficial in preventing thrombosis. *Prostaglandins Leukot Essent Fatty Acids*. 1995;53:211–212.
73. Lau BH, Adetumbi MA, Sanchez A. *Allium sativum* (garlic) and atherosclerosis. A review. *Nutr Res*. 1983;3:119–128.
74. Kendler BS. Garlic (*Allium sativum*) and onion (*Allium cepa*): a review of their relationship to cardiovascular disease. *Prev Med*. 1987;16:670–685.
75. Ernst E. Cardiovascular effects of garlic (*Allium sativum*): a review. *Pharmatherapeutica*. 1987;5:83–89.
76. Kleijnen J, Knipschild P, ter Riet G. Garlic, onions and cardiovascular risk factors. A review of the evidence from human experiments with emphasis on commercially available preparations. *Br J Clin Pharmacol*. 1989;28:535–544.
77. Warshafsky S, Kamer RS, Sivak SL. Effect of garlic on total serum cholesterol. A meta-analysis. *Ann Intern Med*. 1993;119:599–605.
78. Jain AK, Vargas R, Gotzkowsky S, et al. Can garlic reduce levels of serum lipids? A controlled clinical study. *Am J Med*. 1993;94:632–635.
79. Rotzsch W, Richter V, Rassoul F, et al. Postprandial lipemia under treatment with *Allium sativum*. Controlled double-blind study of subjects with reduced HDL2-cholesterol. *Arzneimittelforschung*. 1992;42:1223–1227. [German].
80. Mader FH. Treatment of hyperlipidaemia with garlic-powder tablets. Evidence from the German Association of General Practitioners' multicentric placebo-controlled double-blind study. *Arzneimittelforschung*. 1990;40:1111–1116.
81. Chernyad'eva IF, Shil'nikova SV, Rogoza AN, et al. Dynamics of interrelationships between the content of lipoprotein particles, fibrinogen, and leukocyte count in the plasma from patients with coronary heart disease treated with Kwai. *Bull Exp Biol Med*. 2003;135:436–439.
82. Sobenin IA, Andrianova IV, Demidova ON, et al. Lipid-lowering effects of time-released garlic powder tablets in double-blinded placebo-controlled randomized study. *J Atheroscler Thromb*. 2008;15:334–338.
83. Ashraf R, Aamir K, Shaikh AR, et al. Effects of garlic on dyslipidemia in patients with type 2 diabetes mellitus. *J Ayub Med Coll Abbottabad*. 2005;17:60–64.
84. Van Doorn MB, Espirito Santo SM, et al. Effect of garlic powder on C-reactive protein and plasma lipids in overweight and smoking subjects. *Am J Clin Nutr*. 2006;84:1324–1329.
85. Peleg A, Hershcovici T, Lipa R, et al. Effect of garlic on lipid profile and psychopathologic parameters in people with mild to moderate hypercholesterolemia. *Isr Med Assoc J*. 2003;5:637–640.
86. Turner B, Mølgaard C, Marckmann P. Effect of garlic (*Allium sativum*) powder tablets on serum lipids, blood pressure and arterial stiffness in normo-lipidaemic volunteers: a randomised, double-blind, placebo-controlled trial. *Br J Nutr*. 2004;92:701–706.
87. Gardner CD, Lawson LD, Block E, et al. Effect of raw garlic vs commercial garlic supplements on plasma lipid concentrations in adults with moderate hypercholesterolemia: a randomized clinical trial. *Arch Intern Med*. 2007;167:346–353.
88. Sobenin IA, Nedosugova LV, Filatova LV, et al. Metabolic effects of time-released garlic powder tablets in type 2 diabetes mellitus: the results of double-blinded placebo-controlled study. *Acta Diabetol*. 2008;45:1–6.
89. Bordia A. Effect of garlic on blood lipids in patients with coronary heart disease. *Am J Clin Nutr*. 1981;34:2100–2103.
90. Sainani GS, Desai DB, Gorhe NH, et al. Effect of dietary garlic and onion on serum lipid profile in Jain community. *Indian J Med Res*. 1979;69:776–780.
91. Sainani GS, Desai DB, Gorhe NH, et al. Dietary garlic, onion and some coagulation parameters in Jain community. *J Assoc Physicians India*. 1979;27:707–712.
92. Silagy CA, Neil HA. A meta-analysis of the effect of garlic on blood pressure. *J Hypertens*. 1994;12:463–468.
93. Petkov V. Plants and hypotensive, antiatheromatous and coronarodilatating action. *Am J Chin Med*. 1979;7:197–236.
94. Foushee DB, Ruffin J, Banerjee U. Garlic as a natural agent for the treatment of hypertension: a preliminary report. *Cytobios*. 1982;34:145–152.
95. Al-Qattan KK, Khan I, Alnaqeeb MA, et al. Mechanism of garlic (*Allium sativum*) induced reduction of hypertension in 2K-1C rats: a possible mediation of Na/H exchanger isoform-1. *Prostaglandins Leukot Essent Fatty Acids*. 2003;69:217–222.
96. Sharifi AM, Darabi R, Akbarloo N. Investigation of antihypertensive mechanism of garlic in 2K1C hypertensive rat. *J Ethnopharmacol*. 2003;86:219–224.
97. Kiesewetter H, Jung F, Pindur G, et al. Effect of garlic on thrombocyte aggregation, microcirculation, and other risk factors. *Int J Clin Pharmacol Ther Toxicol*. 1991;29:151–155.
98. Rahman K, Billington D. Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. *J Nutr*. 2000;130:2662–2665.
99. Ernst E. Fibrinogen: an important risk factor for atherothrombotic diseases. *Ann Med*. 1994;26:15–22.
100. Chutani SK, Bordia A. The effect of fried versus raw garlic on fibrinolytic activity in man. *Atherosclerosis*. 1981;38:417–421.

101. Legnani C, Frascaro M, Guazzaloca G, et al. Effects of dried garlic preparation on fibrinolysis and platelet aggregation in healthy subjects. *Arzneimittelforschung*. 1993;43:119–122.
102. Phelps S, Harris WS. Garlic supplementation and lipoprotein oxidation susceptibility. *Lipids*. 1993;28:475–477.
103. Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. *Phytother Res*. 2003;17:97–106.
104. Dillon SA, Burmi RS, Lowe GM, et al. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein. *Life Sci*. 2003;72:1583–1594.
105. Avci A, Atli T, Ergüder IB, et al. Effects of garlic consumption on plasma and erythrocyte antioxidant parameters in elderly subjects. *Gerontology*. 2008;54:173–176.
106. Orekhov AN, Tertov VV, Sobenin IA, et al. Garlic powder tablets reduce atherogenicity of low density lipoprotein: a placebo-controlled double-blind study. *Nutr Metab Cardiovasc Dis*. 1996;6:21–31.
107. Ganado P, Sanz M, Padilla E, et al. An in vitro study of different extracts and fractions of *Allium sativum* (garlic): vascular reactivity. *J Pharmacol Sci*. 2004;94:434–442.
108. Anim-Nyame N, Sooranna SR, Johnson MR, et al. Garlic supplementation increases peripheral blood flow: a role for interleukin-6? *J Nutr Biochem*. 2004;15:30–36.
109. Baluchnejadmojarad T, Roghani M. Endothelium-dependent and -independent effect of aqueous extract of garlic on vascular reactivity on diabetic rats. *Fitoterapia*. 2003;74:630–637.
110. Szulinska M, Kregielska-Narozna M, Swiatek J, et al. Garlic extract favorably modifies markers of endothelial function in obese patients—randomized double blind placebo-controlled nutritional intervention. *Biomed Pharmacother*. 2018;102:792–797. 29604599.
111. Nakagawa S, Masamoto K, Sumiyoshi H, et al. Effect of raw and extracted-aged garlic juice on growth of young rats and their organs after peroral administration (author's trans). *J Toxicol Sci*. 1980;5:91–112. [Japanese].
112. Mennella JA, Beauchamp GK. Maternal diet alters the sensory qualities of human milk and the nursling's behavior. *Pediatrics*. 1991;88:737–744.
113. Mennella JA, Beauchamp GK. The effects of repeated exposure to garlic-flavored milk on the nursling's behavior. *Pediatr Res*. 1993;34:805–808.
114. Macan H, Uykimpang R, Alconcel M, et al. Aged garlic extract may be safe for patients on warfarin therapy. *J Nutr*. 2006;136(suppl 3):S793–S795.
115. Gallicano K, Foster B, Choudhri S. Effect of short-term administration of garlic supplements on single-dose ritonavir pharmacokinetics in healthy volunteers. *Br J Clin Pharmacol*. 2003;55:199–202.
116. Markowitz JS, Devane CL, Chavin KD, et al. Effects of garlic (*Allium sativum L.*) supplementation on cytochrome P450 2D6 and 3A4 activity in healthy volunteers. *Clin Pharmacol Ther*. 2003;74:170–177.

52

Aloe vera (Cape Aloe)

Michael T. Murray, ND

OUTLINE

Aloe vera (family: Liliaceous)
Common name: cape aloe

GENERAL DESCRIPTION

More than 300 species of aloe plants exist, but the most popular medicinal variety is currently *Aloe vera.* The nomenclature of *A. vera* is somewhat confusing, because the plant has been known by various names, most notably *A. barbadensis* and *A. vulgari.* The geographical origination of the plant is unclear. Historical records indicate that it may have originated from Egypt or the Middle East. Aloe has been introduced and naturalized throughout most of the tropics and warmer regions of the world, including the Caribbean, southern United States, Mexico, Latin America, the Middle East, India, and Asia.[1]

A. vera is a perennial plant with yellow flowers and tough, fleshy triangular or spear-like leaves arising in a rosette configuration. The leaves are up to 20 inches long and 5 inches across at the base, tapering to a point. There may be as many as 30 leaves per plant. The margins of the leaf are characterized by saw-like teeth. Inside, the meaty leaf is filled with gel that arises from a clear, central mucilaginous pulp. Mature aloe measures 1.5 to 4 feet long and has a base of 3 inches or greater in diameter (Fig. 52.1).

The leaf is composed of three distinct layers: an outer layer of tough tissue; a corrugated lining just beneath the outer layer; and the major portion of the leaf, the inner layer consisting of parenchymal cells containing large vacuoles of a semisolid, gelatinous, transparent gel. The bitter latex of the corrugated layer protects the plants from predators. Should an animal bite the leaf, the sap causes irritation. The dried latex (juice) derived from the corrugated layer is the source of the laxative properties of aloe. The parenchymal tissue or gel is the portion of the aloe used in other applications.

Aloe vera Terminology

- *A. vera gel*—naturally occurring, undiluted parenchymal tissue obtained from the decorticated leaves of *A. vera*
- *A. vera concentrate*—*A. vera* gel from which the water has been removed
- *A. vera juice*—an ingestible product containing a minimum of 50% *A. vera* gel
- *A. vera latex*—the bitter yellow liquid derived from the pericyclic tubules of the rind of *A. vera,* the primary constituent of which is aloin.

CHEMICAL COMPOSITION

The main feature of all aloe plant portions is a high water content. Aloe gel contains 99% water, whereas the skin and fillet fractions contain 90% and 98% water, respectively. *A. vera* contains numerous compounds possessing biological activity. Although many botanical medicines have substantial geographical variation in content, commercial aloe is quite consistent. One study found that the composition of the major compounds was remarkably invariable, with aloeresin A, aloesin, and aloin (both epimers A and B) contributing between 70% and 97% of total dry weight in a ratio of approximately 4:3:2, respectively. Minor compounds were less evenly distributed, with aloinoside

Fig. 52.1 Aloe vera habit (Yarnell).

A and aloinoside B found in higher concentrations in Western countries. The aloin content of the exudate varied, but there were no distinct geographical discontinuities.[2]

Anthraquinones

In 1851 the cathartic action of aloe was discovered to be caused by aloin, a lemon yellow powder formed from drying of the bitter latex (Fig. 52.2). From this material several anthracenes have been isolated, the major anthraquinone being barbaloin. Barbaloin and aloin are often referred to synonymously. Although aloe contains other anthraquinone derivatives, including the anthracene known as aloe-emodin, barbaloin is considered the most potent cathartic. As a whole, most of the anthraquinone compounds in aloe are water-soluble glycosides easily separated from the water-insoluble resinous material. However, rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is a lipophilic anthraquinone in *Aloe* sp. that has been shown to exert many pharmacological effects beyond any laxative effect, including hepatoprotective, nephroprotective, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities.[3]

Saccharides

Recent research on *A. vera* has focused on the glycoprotein, mucopolysaccharide, and polysaccharide constituents.[4,5] Aloe contains the polysaccharides galactose, xylose, arabinose, and acetylated mannose or acemannan. The latter has received considerable research attention and has shown significant antiviral and immunopotentiating effects, as well as osteogenic, anti-inflammatory, and antibacterial activity. Acemannan is a water-soluble, long-chain polydispersoid β(1,4)-linked mannan polymer interspersed with *O*-acetyl groups found mainly in aloe gel.[6]

Prostanoids

Several prostanoid compounds have been discovered in *A. vera* extracts.[7] The conversion of essential fatty acids to prostanoids by the enzyme cyclooxygenase in a plant such as *A. vera* is rare. The major unsaturated fatty acid in the plant is γ-linolenic acid (C18:3), which can be converted to icosatrienoic acid, the precursor to prostaglandins of the 1 series. The 1 series prostaglandins are known to exert more favorable effects on inflammation, allergy, platelet aggregation, and wound healing. The presence of γ-linoleic acid or prostaglandins, or both, in a stable medium, along with inhibitors of thromboxane synthesis, may be another important chemical characteristic of aloe responsible for its anti-inflammatory and wound-healing effects.

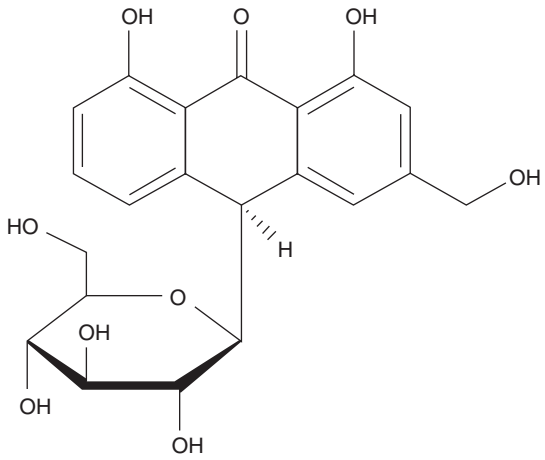


Fig. 52.2 Aloin.

BOX 52.1 Chemical Composition of Aloe Vera

Anthraquinones: Aloin, barbaloin, isobarbaloin, anthranol, aloetic acid, anthracene, ester of cinnamic acid, aloe-emodin, emodin, chrysophanic acid, ethereal oil, resistannol

Saccharides: Cellulose, glucose, mannose, L-rhamnose, aldopentose

Prostanoids: Gamma-linolenic acid

Enzymes: Oxidase, amylase, catalase, lipase, alkaline phosphatase

Amino acids: Lysine, threonine, valine, methionine, leucine, isoleucine, phenylalanine

Vitamins: Vitamins B_1, B_2, B_6, C, and E; folic acid; choline; β-carotene

Minerals: Calcium, sodium, manganese, magnesium, zinc, copper, chromium

Miscellaneous: Cholesterol, triglycerides, steroids, uric acid, lignins, β-sitosterol, gibberellin, salicylic acid

Data from Shelton RM. *Int J Dermatol* 1991;30:679-683.

Superoxide Dismutase

Extracts from the parenchymatous leaf gel and the rind of aloe (*A. barbadensis* Miller) have been shown to contain seven electrophoretically identifiable superoxide dismutases (SODs). Two of these seven are mangano SODs, whereas the other five activities are cupro-zinc SODs.[8]

Other Constituents

Other biological active compounds found in *A. vera* include the following:

- A serine carboxypeptidase
- Salicylates
- Minerals
- Vitamins
- Sterols
- Amino acids

Box 52.1 provides a partial listing of the remarkably diverse range of compounds isolated from *A. vera.*

HISTORY AND FOLK USE

A. vera has a storied history of use. Mesopotamian clay tablets dated 1750 BC indicate that *A. vera* was used for medicinal purposes. Egyptian

records from 550 BC also mentioned aloe for infections of the skin. The ancient Greeks were also aware of aloe's medicinal effects, because both Pliny (23–79 AD) and Dioscorides (first century AD) wrote of aloe's ability to treat wounds and heal infections of the skin. *A. vera* is still widely used in many traditional systems of medicine. In India, for example, in addition to external applications, aloe (whole leaves, the exudate, and the fresh gel) is used as a cathartic, stomachic, and anthelmintic. *A. vera* has been adopted into their materia medicas by many cultures of the world.[1]

In the United States the history of aloe can be traced as far back as the *United States Pharmacopoeia* of 1820, where a number of aloe preparations were described. Most of these preparations were designed to take advantage of aloe's laxative effects. By the early 1900s, more than 27 different aloe preparations were in popular use. In 1920 aloe began being cultivated for pharmaceutic use.

A major development in the modern use of aloe occurred in 1935 when a group of physicians successfully used the fresh juice to treat a patient with facial burns caused by radiographs.[9] The relief offered by aloe in the topical treatment of burns, minor irritations, skin ulcers, and other skin disorders is a major reason why companies supplying dermatological and cosmetic products have incorporated aloe into many of their formulations.

Although more and more of aloe's medicinal effects are being confirmed, it is still predominantly administered without direct medical supervision. Therefore the history and folk use of aloe are continuing to evolve.

PHARMACOLOGY

The pharmacology of aloe is surprisingly diverse. Its laxative, immune potentiation, antimicrobial, and wound-healing activities help explain its wide-ranging folk and clinical applications.

Gastrointestinal Effects

Laxative Effects

Although physicians have prescribed the whole aloe leaf as a cathartic for more than 2000 years, it was not until 1851 that the active principal aloin was discovered.[1] In small doses, aloin acts as a tonic to the digestive system, giving tone to the intestinal muscle. At higher doses, it becomes a strong purgative. Its actions are most obvious on the large intestine, where it increases colonic secretions and peristaltic contractions. In combination with strychnine and belladonna, aloin became one of the most popular laxatives for chronic constipation for many years. Because aloin often causes painful contractions, other anthraquinone laxatives like cascara and senna are now much more popular.[10,11]

A substantial amount of research activity continues in an effort to understand the laxative effects of aloe. Research using the rat large intestine showed that the increase in water content of the large intestine induced by barbaloin preceded the stimulation of peristalsis, attended by diarrhea. Therefore it was suggested that the increase in water content was a more important factor than the stimulation of peristalsis in the diarrhea induced by barbaloin.[12] Further studies by the same researchers suggested that aloe-emodin-9-anthrone (AE-anthrone) produced from barbaloin in the rat large intestine might be the actual chemical mediator of this effect. AE-anthrone not only caused an increase in the intestinal water content but also stimulated mucus secretion.[13]

Bowel Detoxification

In 1985 Bland[14] reported the effect of orally consumed *A. vera* juice on urinary indican, gastrointestinal pH, stool culture, and stool specific gravity in a semicontrolled study of 10 (5 men and 5 women) healthy human subjects.[14] Urinary indican (see Chapter 30) is used as an indicator of the degree to which either dietary protein is malabsorbed or intestinal bacteria are engaged in putrefactive processes. After a full week of drinking 6 oz. of *A. vera* juice three times daily, urinary indican levels decreased one full unit. This suggests that regular *A. vera* juice consumption can lead to improved protein digestion and assimilation or reduced bacterial putrefaction, or both.

TABLE 52.1 Antimicrobial Effects of *Aloe Vera* Extract in Cream Base Compared With Silver Sulfadiazine in Agar (AgSD) Well (6-mm) Diffusion

Organism	Aloe Vera	AgSD
Gram-negative		
Escherichia coli	16	12
Enterobacter cloacae	14	12
Klebsiella pneumoniae	14	6
Pseudomonas aeruginosa	17	12
Gram-positive		
Staphylococcus aureus	18	12
Streptococcus pyogenes	16	12
Streptococcus agalactiae	16	12
Streptococcus faecalis	6	11
Bacillus subtilis	19	14

Data from Robson MC, Heggers JP, Hagstrom WJ. J Burn Care Rehabil 1982;3:157-162. Inhibition zones measured in millimeters.

Inhibition of Gastric Acid Secretion

With Heidelberg gastric analysis, *A. vera* juice was shown to increase gastric pH by an average of 1.88 U. This supports the findings of other researchers that *A. vera* gel can inhibit the secretion of hydrochloric acid. The Heidelberg test also demonstrated that *A. vera* juice can slow down gastric emptying, possibly leading to improved digestion.

Six of the 10 subjects showed marked alterations in stool cultures after the week-long study. This implies that *A. vera* juice may exert some bacteriostatic or fungistatic activity. In the four subjects with positive cultures for yeast, there was a reduction in the number of yeast colonies.

Stool specific gravity was reduced after the week of drinking *A. vera* juice. This implies improved water retention, yet none of the subjects complained of diarrhea or loose stools.

IMMUNE-ENHANCING AND ANTIMICROBIAL ACTIVITY

Antibacterial and Antifungal Activity

Aloe demonstrated activity against many common bacteria and fungi in several studies. In a detailed review of these studies, the researchers assayed the antimicrobial properties of an *A. vera* extract and reviewed the work of others.[15-17] Both mean inhibitory and mean lethal concentrations were determined and compared with silver sulfadiazine (SSD), a potent antiseptic used in the treatment of extensive burns. As shown in Table 52.1,[18] the antimicrobial effects of *A. vera* compare quite favorably with those of SSD. A 60% *A. vera* extract was found to be bactericidal against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Citrobacter* spp., *Enterobacter cloacae, Streptococcus pyogenes,* and *S. agalactiae.* Concentrations of 70% aloe were bactericidal for *Staphylococcus aureus,* 80% for *Escherichia coli,* and 90% for *S. faecalis* and *Candida albicans.* Organisms inhibited in other studies

include *Mycobacterium tuberculosis, Trichophyton* spp., and *Bacillus subtilis.*[2,4,5] The antimicrobial activity against common skin pathogens of *A. vera* gel in a cream base was shown to be slightly better than SSD in agar well diffusion studies.[18]

Antiviral Effects

Acemannan (acetylated mannose) in injectable form has been approved for veterinary use in fibrosarcomas and feline leukemia. Its action in feline leukemia is quite impressive. Feline leukemia, like AIDS, is caused by a retrovirus (feline leukemia virus). The virus is so lethal that once cats develop clinical symptoms, they are usually euthanized. Typically more than 70% of cats die within 8 weeks of the onset of clinical signs. In a study of 44 cats with clinically confirmed feline leukemia, acemannan was injected (2 mg/kg) weekly for 6 weeks and the cats were reexamined 6 weeks after termination of treatment.[19] At the end of the 12-week study, 71% of the cats were alive and in good health.

Acemannan demonstrated significant antiviral activity against several viruses, including feline AIDS, human immunodeficiency virus type 1 (HIV-1), influenza virus, and measles virus.[20-22]

Immune Enhancement

Acemannan is a potent immunostimulant.[18,23-25] Among the effects noted for acemannan are the enhancement of macrophage release of interleukin (IL)-1-α, cytokines, tumor necrosis factor, and nitric oxide release, as well as phagocytosis and nonspecific cytotoxicity. Acemannan also enhances T-cell function and interferon production, although these actions may also be caused by enhanced macrophage function. Macrophage production of cytokines IL-6 and tumor necrosis factor-α were dependent on the dose of acemannan provided. These effects can be substantial. For example, in one study, acemannan was shown to enhance the macrophage respiratory burst (twofold increase above the media controls), phagocytosis (45% compared with 25% in controls), and killing of *C. albicans* (38% killing of *C. albicans* compared with 0%–5% killing in controls).[26]

Hematopoietic Effects

Several complex carbohydrates have been found to significantly stimulate hematopoiesis. CARN 750, a polydispersed β-(1,4)-linked acetylated mannan isolated from the *A. vera* plant, has been shown to have hemato-augmenting properties. Subcutaneous injections of 1 mg/mouse of CARN 750 optimally increased hematopoietic progenitors, measured as IL-3, supported colony-forming units, culture (CFU-C) and high proliferative potential colony-forming cell (HPP-CFC) assays in the spleen. Providing 2 mg/animal of CARN 750 optimally increased bone marrow cellularity, frequency, and absolute number of HPP-CFCs and CFU-Cs. The hematopoietic activity of CARN 750 increased with the frequency of administration. The greatest increase in activity occurred in mice myelosuppressed with radiation.[27]

Anti-inflammatory Activity

A. vera has been shown to exert a number of anti-inflammatory actions, including blocking of the generation of inflammatory mediators like thromboxanes and bradykinin, reducing neutrophil infiltration during inflammation, and reducing edema. Several compounds in aloe are responsible for these actions. The most important are glycoproteins, which inhibit and actually break down bradykinin, a major mediator of pain and inflammation; various anthraquinones; and salicylates. These anti-inflammatory substances may be of significance in both topical and oral applications.

One comprehensive study evaluated the effects of aqueous, chloroform, and ethanol extracts of *A. vera* gel on carrageenan-induced edema in the rat paw and neutrophil migration into the peritoneal cavity stimulated by carrageenan. The capacity of the aqueous extract to inhibit cyclo-oxygenase activity was also evaluated. The aqueous and chloroform extracts decreased the edema induced in the hind paw and the number of neutrophils migrating into the peritoneal cavity, whereas the ethanol extract only decreased the number of neutrophils. The aqueous extract was also found to inhibit prostaglandin E_2 production from arachidonic acid, demonstrating an inhibitory action on cyclooxygenase. The aqueous extract contained anthraglycosides, reductor sugars, and cardiotonic glycosides, whereas the ethanol extract contained saponins, carbohydrates, naphthoquinone, sterols, triterpenoids, and anthraquinones. The chloroform extract contained sterols and anthraquinones.[28]

Another useful aspect of aloe is its ability to inhibit lipid peroxidation and scavenge free radicals. One study measured the activity of seven anthraquinones and four anthrones against nonenzymatic and enzymatic lipid peroxidation in vitro and their ability to scavenge free radicals. Using rat hepatocytes exposed to strong oxidizing agents, dithranol and anthrone provided the strongest inhibition of nonenzymatic peroxidation. Rhein anthrone and aloe-emodin showed the highest inhibitory activity against peroxidation of linoleic acid catalyzed by lipoxygenase. Anthrone, dithranol, and rhein anthrone were the most effective free radical scavengers.[29]

Alcohol Detoxification

Oral administration of aloin (300 mg/kg) given 12 hours before administration of alcohol (3 g/kg) significantly decreases the blood alcohol area under the curve by a remarkable 40%. This suggests an increase in the rate of blood alcohol elimination from the body of 45% to 50%. Analysis of hepatic triglyceride levels revealed that both ethanol and aloin given alone significantly increased the triglyceride levels in a comparable manner. However, the level obtained by the combined treatment of aloin and ethanol was not statistically different from that produced by either treatment alone. The levels of serum L-aspartate:2-oxoglutarate aminotransferase and L-alanine:2-oxoglutarate aminotransferase activities were not increased by acute alcohol intoxication, aloin alone, or the combined treatment of alcohol and aloin.[30]

Wound Healing

The topical effects of *A. vera* appear to be caused by a combination of enhancement of wound healing along with anti-inflammatory, moisturizing, emollient, and antimicrobial actions.[31] *A. vera* contains a number of compounds necessary for wound healing, including vitamin C, vitamin E, and zinc. Unlike many other anti-inflammatory substances, *A. vera* has been shown to stimulate fibroblast and connective tissue formation, thereby promoting wound repair. Finally, aloe appears to stimulate the epidermal growth and repair process, including increasing the manufacture of supportive glycosaminoglycans, presumably as a result of its polysaccharides.[32] Mannose-6-phosphate, the major sugar in the *A. vera* gel, may be its most active growth substance.[33]

Another interesting effect of aloe in wound healing is its ability to counteract the wound-healing suppression effects of cortisone. In one study, *A. vera* at doses of 100 and 300 mg/kg daily for 4 days blocked the wound-healing suppression of hydrocortisone acetate up to 100% using the wound tensile strength assay. The authors suggested this response was because of the growth factors present in *A. vera* masking the wound-healing inhibitors.[34]

CLINICAL APPLICATIONS

Skin Health, Wound Healing, Burns, Frostbite, etc.

Despite growing consumer awareness of *A. vera*'s soothing effects on burns and wound healing during the past 50 years, few human studies

have been conducted.[35] Virtually all of the studies support the topical use of *A. vera* gel, especially in minor burns or skin inflammation. There is also some support for use in more severe tissue damage.

Although limited, the human research has been promising. For example, one study found *A. vera* gel quite successful in three patients with chronic leg ulcers of 5, 7, and 15 years' duration.[36] The gel was applied to the ulcers on gauze bandages. Rapid reduction in ulcer size was noted in all three subjects, and complete resolution occurred in two. Encouraging results were also reported for acne and seborrhea.

In a study of 27 patients with a partial-thickness burn wound, treatment with *A. vera* gel was compared with Vaseline gauze. The average time of healing in the aloe gel area was a statistically significant and dramatic 1 week shorter: 11.9 days compared with 18.2 days for the Vaseline gauze–treated wound. Histological evaluation showed early epithelialization in the *A. vera* gel–treated area.[37]

In another double-blind study, the efficacy of *A. vera* cream for partial-thickness burn wounds was studied and compared with that of SSD. Thirty patients with similar types of second-degree burns at two sites on different parts of the body were included in this study. Each patient had one burn treated with topical SSD and one treated with aloe cream, randomly. The rate of reepithelialization and healing of the partial thickness burns was significantly faster in the site treated with aloe than in the site treated with SSD (15.9 vs 18.73 days, respectively; $P < 0.0001$).[38]

Another study compared the therapeutic effects of systemic pentoxifylline with topical *A. vera* cream in the treatment of frostbite. The frostbitten ears of 10 New Zealand white rabbits were assigned to one of four treatment groups: untreated controls, those treated with *A. vera* cream, those treated with pentoxifylline, and those treated with *A. vera* cream and pentoxifylline. The control group had a 6% tissue survival. Tissue survival was notably improved with pentoxifylline (20%), was better with *A. vera* cream (24%), and was best with the combination therapy (30%).[39]

Aloe may also prove useful in more severe tissue injuries, such as those seen in necrotizing fasciitis. Necrotizing fasciitis usually manifests as a low-grade cellulitis that quickly deteriorates to a limb- and life-threatening soft tissue infection. Immediate surgical débridement is essential, followed by aggressive wound management. An interesting report described excellent results in two cases. Case 1 was a 72-year-old female who, upon presenting to the emergency department with a "sore bottom," was diagnosed with five problems:

- Anal-rectal abscess
- Fournier's gangrene
- Ulcerative colitis
- Chronic blood loss/anemia
- Protein caloric malnutrition

After débridement, her anal-rectal wound extended from the labia to the left buttock. Care was multidisciplinary and included applying a water-based aloe gel and saline-soaked gauze twice a day. After 45 days, the wound exhibited a pink base with granulation tissue and contraction of the wound edges.

Case 2 was a 48-year-old male with seroma of the left leg secondary to a crush injury. Within 3 days he developed deep vein thrombosis in that leg, as well as two large seroma cavities on either side of the thigh. Care included packing with aloe gel and saline-soaked sponges. Two weeks after admission, the anterior wound was covered with a split-thickness skin graft, whereas partial closure of the lateral cavity was attempted unsuccessfully with retention sutures. After 5 weeks, healing was complete for the anterior wound and 95% complete for the posterior wound.[40]

A double-blind study also showed that patients with pressure sores responded equally to an acemannan hydrogel wound dressing or a moist saline gauze wound dressing.[41]

Several studies have also shown that topical aloe preparations can accelerate healing from surgical wounds. In one of the more recent studies, 90 women who had undergone cesarean delivery were randomly divided into two groups. In one group, the wound was dressed with aloe vera gel, whereas simple dressing was used in the control group. Wound healing was assessed 24 hours and 8 days after the cesarean operation using a standard scale. A significant difference was observed between the two groups with respect to the wound healing score 24 hours after the operation, with 45 women in the aloe vera group and 35 in the control group obtaining a zero score.[42]

A topical cream containing 0.5% *Aloe vera* juice powder showed benefits posthemorrhoidectomy. Application of the cream on the surgical site was shown to be effective in reducing postoperative pain both on resting and during defecation, healing time, and analgesic requirements in the patients compared with the placebo group.[43]

Oral ingestion of aloe preparations may also help promote wound healing. In particular, sterol-rich extracts. Sterolic fractions of aloe stimulate collagen and hyaluronic acid production in human dermal fibroblasts. In a 12-week, randomized, double-blind, placebo-controlled study an oral aloe sterol supplement was shown to promote very positive effects on skin elasticity, hydration, and the collagen scores in 64 healthy women (age range 30–59 years; average 44.3 years). The improved collagen score was based on the measured collagen content in the dermis by ultrasound.[44]

Psoriasis

Several studies have shown topical treatment with aloe preparations may help psoriasis. One double-blind, placebo-controlled study evaluating the clinical efficacy and tolerability of topical *A. vera* extract 0.5% in a hydrophilic cream obtained impressive results. Sixty patients (36 males and 24 females) aged 18 to 50 years (mean 25.6 years) with slight to moderate chronic plaque–type psoriasis and psoriasis area, and severity index (PASI) scores between 4.8 and 16.7 (mean 9.3) were enrolled and randomized to two groups. The mean duration of the disease before enrollment was 8.5 years (range 1–21 years). Patients self-administered trial medication topically at home three times daily for 5 consecutive days/week (maximum 4 weeks active treatment). Patients were examined on a weekly basis, and those showing a progressive reduction of lesions, desquamation followed by decreased erythema, infiltration, and lowered PASI score were considered healed. The study was scheduled for 16 weeks with 12 months of follow-up on a monthly basis. The treatment was well tolerated by all the patients, with no adverse drug-related symptoms and no dropouts. By the end of the study, the *A. vera* extract cream had cured 25 of 30 patients (83.3%) compared with the placebo cure rate of only 2 of 30 patients (6.6%), resulting in significant clearing of the psoriatic plaques (328 of 396, or 82.8%) versus placebo (28 of 366, or 7.7%), and a decreased PASI score to a mean of 2.2.[45]

In another study, the efficacy of topical *A. vera* was compared with 0.1% triamcinolone acetonide (TA) in mild to moderate plaque psoriasis. After 8 weeks of treatment, the mean PASI score decreased from 11.6 to 3.9 in the *A. vera* group and from 10.9 to 4.3 in the TA group. The mean Dermatology Life Quality Index scores decreased from 8.6 to 2.5 in the *A. vera* group and from 8.1 to 2.3 in the TA group. These results indicated that both treatments had similar efficacy in improving the quality of life of patients with mild to moderate psoriasis.[46]

Radiation Burns

Research into the topical applications of *A. vera* gel began in the 1930s for the treatment of radiation burns. During the 1930s, radiographs were used therapeutically for cancer, eczema, and other skin complaints, and as a depilatory agent. In 1935 Collins and Collins[9] reported

the success of *A. vera* gel in a single case, a woman with a patch of severe x-ray dermatitis on her forehead. The woman had tried various medical treatments for 8 months, only to have her condition worsen. The Collinses were going to perform a skin graft, but as a temporary measure applied a preparation of fresh whole *A. vera* leaves to reduce the itching. The result was that "Twenty-four hours later she reported that the sensation of itching and burning had entirely subsided," and by 5 weeks "there was complete regeneration of the skin of the forehead and scalp, new hair growth, complete restoration of sensation, and absence of scar." Five months after treatment was started, there was complete healing. The following case reports, although not as positive as this initial study, clearly indicated that *A. vera* was effective in some cases.

Until the 1940s most of the studies on aloe were reported case histories.[4] To substantiate these case studies, animal studies began to appear in the literature. Rowe and colleagues[35] performed several studies in rats with radiation-induced ulcers and determined that fresh aloe pulp was effective, whereas dried aloe powder was not.[4,34]

In 1953 Lushbaugh and Hale,[36] working for the US Atomic Energy Commission, produced one of the most convincing studies of the efficacy of *A. vera* gel. Twenty albino rats were exposed to β-radiation, and different treatments were used on quadrants of the affected area of each animal. The treatments used were fresh *A. vera* leaf, a commercial *A. vera* ointment, application of a dry gauze bandage, and an untreated control. Both fresh *A. vera* and the *A. vera* ointment produced clear improvements. At the end of 2 months, the *A. vera*–treated areas were completely healed, whereas the other two areas had not yet healed at the end of 4 months.

A large placebo-controlled, double-blind study cast doubt on the efficacy of aloe for severe radiation burns. Three Phase III randomized trials were reported in this study. The first one was double-blind, used a placebo gel, and involved 194 women receiving breast or chest wall irradiation. The second trial randomized 108 such patients to *A. vera* gel versus no treatment. Skin dermatitis was scored weekly during both trials both by patients and by health care providers. Skin dermatitis scores were virtually identical on both treatment arms during both trials.[47] The aim of the study was to see if topical *A. vera* gel would be beneficial in reducing the identified skin side effects of radiation therapy, including erythema, pain, itching, dry desquamation, and moist desquamation compared with aqueous cream. The secondary aim was to assess the effect of other factors known to predict severity of radiation skin reaction (i.e., breast size, smoking habit, and one or more drainages of lymphocele after surgery) on other skin side effects. The third study involved more than 225 patients with breast cancer after lumpectomy or partial mastectomy who required a course of radiation therapy using tangential fields. Like the other two studies, *A. vera* gel did not significantly reduce radiation-induced skin side effects.[48]

These surprising results might be explained by another study that compared the efficacy of commercially available gels with an acemannan-rich extract from aloe leaves in the treatment of irradiated mice. Male C3H mice received graded single doses of γ-radiation ranging from 30 to 47.5 Gy to the right leg. In most experiments, the gel was applied daily, beginning immediately after irradiation. To determine the timing of application for the best effect, gel was applied beginning on days −7, 0, or +7 relative to the day of irradiation (day 0) and continuing for 1, 2, 3, 4, or 5 weeks. The right inner thigh of each mouse was scored on a scale of 0 to 3.5 for severity of radiation reaction from the seventh to thirty-fifth day after irradiation. Dose–response curves were obtained by plotting the percentage of mice that reached or exceeded a given peak skin reaction as a function of dose. The researchers found that although the acemannan-rich extract gel was highly effective, the commercially available gel showed no improvement over the control. They also found that the aloe gel had to be applied immediately after irradiation and continued for at least 2 weeks. There was no effect if the aloe gel was applied only before irradiation or beginning 1 week after irradiation. Clearly, the quality and concentration of aloe constituents are crucial if clinical results are to be obtained.[53] In addition, the physical form of the preparation (e.g., gel vs. cream) may be important, as a study using a well-defined aloe extract in a cream showed no benefit in protecting against radiation injury to the skin in women being treated for breast cancer.[49]

One area where *A. vera* gel has shown some benefit is in acute radiation-induced proctitis (ARP), a common side effect that affects up to 50% of patients receiving radiotherapy. In a double-blind placebo-controlled trial, 20 consecutive patients with ARP after external-beam radiation therapy of pelvic malignancies were randomized to receive either *A. vera* 3% or placebo ointment, 1 g twice daily for 4 weeks. These patients presented with at least two of the following symptoms: rectal bleeding, abdominal/rectal pain, diarrhea, or fecal urgency. A symptom index and lifestyle effect was calculated by the addition of the scores from a questionnaire. Results showed a significant ($P < 0.05$) improvement in the symptom index for diarrhea, fecal urgency, clinical presentation total, and lifestyle effect. Hemorrhage and abdominal/rectal pain did not improve significantly. The odds ratios for advantage of *A. vera* over placebo for "clinical presentation total" was 3.97.

Gastric Ulcers, Ulcerative Colitis, and Rectal Disorders

Internal use of *A. vera* gel to treat peptic ulcers was first studied in 1963.[50] Twelve patients with x-ray–confirmed duodenal ulcers were given 1 tablespoon of an emulsion of *A. vera* gel in mineral oil once daily. At the end of 1 year, all patients demonstrated complete recovery and no recurrence. On the basis of experimental evidence, the following factors were considered responsible for the effectiveness:

- *A. vera* gel inactivates pepsin in a reversible fashion. When the stomach is devoid of food, pepsin is inhibited by *A. vera* gel; however, in the presence of food, pepsin is released and allowed to digest the food.
- The gel inhibits the release of hydrochloric acid via interference with histamine binding to the parietal cells.
- *A. vera* gel is an extremely good demulcent that heals and prevents aggravating irritants from reaching the sensitive ulcer.

A. vera gel has also been shown to useful for ulcerative colitis. In the double-blind study, 44 patients were randomly given oral *A. vera* gel or placebo, 100 mL twice daily for 4 weeks. Clinical remission, improvement, and response occurred in 9 (30%), 11 (37%), and 14 (47%), respectively, of 30 patients given aloe vera, compared with 1 (7%), 1 (7%), and 2 (14%), respectively, of 14 patients taking placebo. The Simple Clinical Colitis Activity Index and histological scores decreased significantly during treatment with *A. vera* ($P = 0.01$ and $P = 0.03$, respectively), but not with placebo.[51]

A topical cream containing 0.5% *A. vera* juice powder showed benefits in the treatment of chronic anal fissures.[52] The aloe cream was applied by the patients to the wound site three times per day for 6 weeks. There were statistically significant differences ($P < 0.0001$) in chronic anal fissure pain, hemorrhaging upon defection, and wound healing before and at the end of the first week of treatment with the aloe cream compared with the control group. This aloe preparation was also shown to be great benefit after hemorrhoidectomy (mentioned previously).

Oral Mucosal Disorders

The topical benefits of aloe preparations extend to the oral mucosa. According to a systematic review, aloe preparations have been

evaluated in 15 clinical trials ranging in size from 20 patients to 110 patients with clinically diagnosed oral mucosal lesions. Most of the studies showed statistically significant clinical improvement in such conditions as oral lichen planus, submucous fibrosis, burning mouth syndrome, radiation-induced mucositis, candida-associated denture stomatitis, xerostomic patients, and recurrent aphthous stomatitis.[53]

Oral submucosa fibrosis is a chronic, complex, premalignant condition linked most often to betel nut chewing. Given *A. vera's* anti-inflammatory, wound-healing, antioxidant, and antineoplastic activities, it would appear to be an effective intervention. In the most recent study, the clinical response to *A. vera* juice or gel was comparable to that of intralesional injections of hydrocortisone and hyaluronidase combined with oral antioxidant supplementation.[54]

In the treatment of lichen planus, results from five double-blind clinical trials indicate that aloe vera is more efficient than placebo and has a comparable effect to triamcinolone acetonide.[53,55] In the most recent study, 40 patients with lichen planus were randomly divided into two equal groups. Group A patients received aloe vera gel, whereas group B patients received triamcinolone acetonide. When clinical signs and symptoms were observed after 8 weeks of therapy, it was determined that aloe vera gel was more effective than triamcinolone acetonide in the treatment of oral lichen planus.[56]

Acquired Immunodeficiency Syndrome

Although acemannan has demonstrated some direct antiviral activity against HIV-1 by inhibiting glycosylation of viral glycoproteins, its main promise in treating AIDS and HIV may be to enhance the action of azidothymidine (AZT), one of the antiviral drugs used in the treatment of AIDS. In vitro studies have shown that acemannan combined with suboptimal noncytotoxic concentrations of AZT or acyclovir acts synergistically to inhibit the replication of HIV and herpes simplex type 1 (HSV-1).[22] On the basis of these studies, as well as preliminary human studies, researchers believe that the use of acemannan may reduce the amount of AZT required by as much as 90%. In addition to AZT's cost, its use is often associated with severe side effects, including anemia and granulocytopenia resulting from bone marrow suppression.

Preliminary clinical studies have suggested that acemannan and *A. vera* may be beneficial when administered orally in HIV-positive individuals. For example, in one study, 14 HIV patients prescribed oral acemannan (800 mg/day) demonstrated significant increases in circulating monocytes/macrophages. In particular, there were significant increases in the number of large circulating monocytes, indicating improvement in phagocytizing, processing, and presenting cells in the blood.[57] In a study of 15 AIDS patients receiving an oral dose of acemannan (800 mg/day), the average scores of Modified Walter Reed (MWR) Clinical Evaluation scoring, absolute T-4, absolute T-8, and p24 core antigen levels all improved in those surviving (Table 52.2) at the end of 900 days. Two patients died of AIDS, and another committed suicide. From this study and others, it has been suggested that prognostic criteria to determine the most responsive patients are those with an absolute T-4 count greater than 150/mm^3 and p24 levels less than 300.

TABLE 52.2 Acemannan in the Treatment of Acquired Immunodeficiency Syndrome

Test	Pretreatment	After 900 Days
Modified Walter Reed Clinical Evaluation	65	2
Absolute T-4	322/mm^3	324/mm^3
Absolute T-8	469/mm^3	660/mm^3
p24 Core antigen	5/15	4/12

Data from McDaniel HR, Carpenter RH, Kemp M, et al. *Antiviral Res* 1990;13(suppl 1):117.

Unfortunately, a follow-up study did not reproduce these early, promising results. A comprehensive study assessed the safety and efficacy of acemannan as an adjunctive to antiretroviral therapy among 63 male patients (mean age, 39 years) with advanced HIV disease receiving zidovudine (ZDV) or didanosine (ddI).[58] The randomized, double-blind, placebo-controlled trial provided a large dose of acemannan (400 mg orally four times daily). Eligible patients had CD4 counts of 50 to 300/μL twice within 1 month of study entry and had received 26 months of antiretroviral treatment (ZDV or ddI) at a stable dose for the month before entry. CD4 counts were made every 4 weeks for 48 weeks. p24 antigen was measured at entry and every 12 weeks thereafter. Sequential quantitative lymphocyte cultures for HIV and ZDV pharmacokinetics were performed in a subset of patients.

The mean baseline CD4 counts were 165 and 147/μL in the placebo and acemannan groups, respectively; 90% of the patients were receiving ZDV at entry. Six patients in the acemannan group and five in the placebo group developed AIDS-defining illnesses. There was no statistically significant difference between the groups at 48 weeks with regard to the absolute change or rate of decline of the CD4 count. Among ZDV-treated patients, the median rates of CD4 change (ACD4) in the initial 16 weeks were −121 and −120 cells/year in the placebo and acemannan groups, respectively; ACD4 decline from week 16 to 48 was 0 and −61 cells/year in the acemannan and placebo groups, respectively ($P = 0.11$). No statistical difference between groups occurred with regard to adverse events, p24 antigen, quantitative virology, or pharmacokinetics. Twenty-four patients, 11 receiving placebo and 13 receiving acemannan, discontinued study therapy prematurely (none as a result of serious adverse reactions). The decreased, but not statistically significant, rate of loss of CD4 cells in the acemannan group from weeks 16 to 48 provides a possible ray of hope that long-term use, such as reported previously, may be of value, and should be investigated.

Diabetes

A. vera also exhibits a hypoglycemic effect in both normal and alloxan-induced diabetic mice.[59] Research began in humans with a small study of five patients with type 2 diabetes ingested half a teaspoonful of aloe four times daily for 14 weeks. Fasting blood sugar in each patient fell from a mean of 273 to 151 mg/dL with no change in body weight. The authors concluded that aloe lowered blood glucose levels by an unknown mechanism.[60] A more recent and larger study (49 men and 23 women) provided more support for the efficacy of aloe in combination with glibenclamide in diabetes. Although there was no response to glibenclamide alone, the combination was effective.[61] The patients were provided with 1 tablespoon of aloe gel and 5 mg of glibenclamide twice daily, with 5 mg twice daily of glibenclamide serving as the control. After 2 weeks, fasting blood sugar decreased significantly in the treated group, and by day 42 had decreased from an average of 289 mg/dL to a remarkable 148 mg/dL. Although the drop in serum cholesterol was insignificant, serum triglycerides decreased from 223 mg/dL to (again remarkable) 128 mg/dL by day 42.

In a systematic review of five randomized controlled trials with *A. vera* preparations involving 415 participants with prediabetes or early stage type 2 diabetes, results showed that *A. vera* supplementation significantly reduced the concentrations of fasting blood glucose, glycosylated hemoglobin A1c (Hb A_{1c}), total cholesterol, and low-density lipoprotein-cholesterol at the same time increasing serum high-density lipoprotein-cholesterol levels. Only one adverse event was reported

(but not described) in all of these studies combined. The evidence showed that *A. vera* can effectively reduce the levels of FBG, HbA1c, triglyceride, TC, and LDL-C, and increase the levels of HDL-C in these patients. The proposed mechanism is improved insulin sensitivity by activating AMP-activated muscle protein kinase.[62]

Asthma

Oral administration of an extract of *A. vera* for 6 months was shown to produce good results in the treatment of asthma in some individuals of various ages.[63] The exception was the fact that the *A. vera* extract was not effective in patients dependent on corticosteroids. The mechanism of action is thought to be via restoration of protective mechanisms, followed by augmentation of the immune system.

The extract used in the study was produced from the supernatant of fresh leaves stored in the dark for 7 days at 4°C. The dosage was 5 mL of a 20% solution of the aloe extract in saline twice daily for 24 weeks. Eleven of 27 patients (40%) without corticosteroid dependence reported significant improvement at the study's conclusion.

Studies indicate that subjecting the leaves to dark and cold results in an increase in the polysaccharide fraction. One gram of the crude extract obtained from leaves stored in the cold and dark produced 400 mg of neutral polysaccharide, compared with only 30 mg produced from leaves not subjected to cold or dark.

Antioxidant Support

Daily ingestion of an *A. vera* gel extract for 14 days was shown to increase plasma total antioxidant capacity (TAC) in healthy volunteers without any clinical side effects.[63]

Cancer Prevention

Aloe preparations and components have been shown to exert antigenotoxic and chemopreventive effects in a time-course and dose-dependent manner.[64,65] Aloe preparations have also shown efficacy in treatment of spontaneous neoplasms in dogs and cats.[66] These studies suggest *A. vera* may also play a role in humans. One clinical study sought to evaluate whether the concomitant administration of aloe could enhance the therapeutic results of melatonin in patients with advanced solid tumors for whom no effective standard anticancer therapies were available. The study included 50 patients with lung cancer, gastrointestinal tract tumors, breast cancer, or brain glioblastoma who were treated with melatonin alone (20 mg/day orally in the dark period) or melatonin plus *A. vera* tincture (1 mL twice daily). A partial response was achieved in two of 24 patients treated with melatonin plus aloe and in none of the patients treated with melatonin alone. Stable disease was achieved in 12 of 24 and in seven of 26 patients treated with melatonin plus aloe or melatonin alone, respectively. Therefore the percentage of nonprogressing patients was significantly higher in the group treated with melatonin plus aloe than in the melatonin group (14 of 24 vs. 7 of 26; $P < 0.05$). The percentage of 1-year survival was also significantly higher in patients treated with melatonin plus aloe (9 of 24 vs. 4 of 26; $P < 0.05$).[67]

Contraception

An interesting new application of aloe is as a spermicide. Twenty samples of fresh ejaculate from healthy human volunteers between 20 and 30 years of age were treated in vitro with a 1% concentration of zinc acetate combined with lyophilized *A. barbadensis* (at concentrations of 7.5%–10%). The combination of zinc acetate with lyophilized *A. barbadensis* was shown to possess powerful spermicidal and antiviral effects. This was attributed to their concentration of minerals (boron, barium, calcium, chromium, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc), which were toxic to the sperm tail, causing instant immobilization. Studies with rabbit vaginal epithelium showed no irritation. This is important because nonoxynol-9, the active spermicidal ingredient used in vaginal contraception for more than 30 years, appears to cause cell membrane damage in vaginal and cervical epithelium and may possibly have teratogenic effects.[68]

DOSAGE

A. vera gel can be applied liberally for topical applications. A wide range of products are available on the market; however, simple pure *A. vera* gel is sufficient.

A. vera juice can be consumed orally as a beverage or tonic. Although detailed information is currently lacking as to the optimal dose for these types of products, it is recommended that no more than 1 quart be consumed daily.

The dose of acemannan being used in HIV/AIDS patients is 800 to 1600 mg/day. This corresponds to a dose of approximately 0.5 to 1 L/day for most *A. vera* juice products. However, there may be great variation in the amount of acemannan in various products.

TOXICOLOGY

Although rare, hypersensitivity reactions manifesting as generalized, nummular, eczematous, and papular dermatitis have been reported as a result of topically applied *A. vera* preparations. In general, topical aloe preparations are not useful for treating deep vertical wounds such as those produced during laparoscopy.[69]

Oral aloe preparations are extremely well tolerated. There are a few reports of aloe preparations inducing elevations in hepatic enzymes and even acute liver injury.[70] Thus it may be appropriate to perform annual assessment of liver function enzymes. There are also a few case reports of aloe ingestion leading to thyroid dysfunction (reduced serum triiodothyronine).

DRUG INTERACTIONS

Like other stimulant laxatives, excessive use of aloe dried juice/latex may interact with several drugs. Large doses of aloe may increase the risk of toxicity of antiarrhythmic drugs. Theoretically, potential for adverse effects exists when aloe dried juice/latex is used excessively along with herbs containing cardiac glycosides (e.g., black hellebore, Canadian hemp roots, digitalis leaf, pheasant's eye plant, pleurisy root, squill bulb leaf scales, *Strophanthus* seeds) or with cardiac glycoside drugs (e.g., digoxin). Overuse of aloe dried juice/latex can exacerbate the potassium loss caused by corticosteroids, diuretics, horsetail, and licorice root.

As a result of the hypoglycemic effects of internally consumed *A. vera* gel, people using glyburide drugs to control blood sugar (e.g., Diabeta, Micronase, Glynase) should monitor blood glucose levels closely.

Aloe dried juice/latex causes shorter gastrointestinal transit time, so it may reduce the absorption of some drugs.

Concomitant use of aloe with other stimulant laxative herbs might increase the risk of potassium depletion. In addition to senna leaves and pods and cascara bark, the two herbs most widely used for this purpose, stimulant laxative herbs also include wild cucumber fruit *(Ecbolium elaterium)*, blue flag rhizome, alder buckthorn, European buckthorn, butternut bark, castor oil, colocynth fruit pulp, gamboge bark exudate, jalap root, black root, manna bark exudate, podophyllum root, rhubarb root, and yellow dock root.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Haller Jr JS. A drug for all seasons, medical and pharmacological history of aloe. *Bull N Y Acad Sci*. 1990;66:647–659.
2. van Wyk BE, van Rheede, van Oudtshoorn MC, et al. Geographical variation in the major compounds of Aloe ferox leaf exudate. *Planta Med*. 1995;61:250–253.
3. Zhou YX, Xia W, Yue W, et al. Rhein: a review of pharmacological activities. *Evid Based Complement Alternat Med*. 2015;2015:578107.
4. Cock IE. The genus aloe: phytochemistry and therapeutic uses including treatments for gastrointestinal conditions and chronic inflammation. *Prog Drug Res*. 2015;70:179–235.
5. Akaberi M, Sobhani Z, Javadi B, Sahebkar A, Emami SA. Therapeutic effects of Aloe spp. in traditional and modern medicine: a review. *Biomed Pharmacother*. 2016;84:759–772.
6. Sierra-García GD, Castro-Ríos R, González-Horta A, Lara-Arias J, Chávez-Montes A. Acemannan, an extracted polysaccharide from Aloe vera: a literature review. *Nat Prod Commun*. 2014;9(8):1217–1221.
7. Afzal M, Ali M, Hassan RAH, et al. Identification of some prostanoids in Aloe vera extracts. *Planta Med*. 1991;57:38–40.
8. Sabeh F, Wright T, Norton SJ. Isozymes of superoxide dismutase from Aloe vera. *Enzyme Protein*. 1996;49:212–221.
9. Collins CE, Collins C. Roentgen dermatitis treated with fresh whole leaf Aloe vera. *Am J Roentenol*. 1935;33:396–397.
10. Godding EW. Therapeutics of laxative agents with special reference to anthraquinones. *Pharmacol*. 1976;14(suppl 1):78–101.
11. Anton R, Haag-Berrurier MH. Therapeutic use of natural anthraquinones for other than laxative actions. *Pharmacol*. 1976;20(suppl 1):104–112.
12. Ishii Y, Tanizawa H, Takino Y. Studies of aloe. IV. Mechanism of cathartic effect. *Biol Pharm Bull*. 1994;17:495–497.
13. Ishii Y, Tanizawa H, Takino Y. Studies of aloe. V. Mechanism of cathartic effect. *Biol Pharm Bull*. 1994;17:651–653.
14. Bland J. Effect of orally-consumed Aloe vera juice on human gastrointestinal function. *Preventive Veterinary Med*. 1985;14:152–154.
15. Fly LB, Keim I. Tests of Aloe vera for antibiotic activity. *Econ Bot*. 1963;17:46–48.
16. Lorenzetti LJ, Salisburg R, Beal J, et al. Bacteriostatic property of Aloe vera. *J Pharm Sci*. 1964;53:1287.
17. Heggers JP, Pineless GR, Robson MC. Dermaide Aloe/Aloe vera gel: comparison of the antimicrobial effects. *J Am Med Technol*. 1979;41: 293–294.
18. Robson MC, Heggers JP, Hagstrom WJ. Myth, magic, witchcraft, or fact? Aloe vera revisited. *J Burn Care Rehabil*. 1982;3:157–162.
19. Sheets MA, Unger BA, Giggleman GF, et al. Studies of the effect of acemannan on retrovirus infections: clinical stabilization of feline leukemia virus-infected cats. *Mol Biother*. 1991;3:41–45.
20. Kemp MC, Kahlon JB, Chinnah AD, et al. In-vitro evaluation of the antiviral effects of acemannan on the replication and pathogenesis of HIV-1 and other enveloped viruses: modification of the processing of glycoprotein precursors. *Antiviral Res*. 1990;13(suppl 1):83.
21. Kahlon JB, Kemp MC, Carpenter RH, et al. Inhibition of AIDS virus replication by acemannan in vitro. *Mol Biother*. 1991;3:127–135.
22. Kahlon JB, Kemp MC, Yawei N, et al. In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. *Mol Biother*. 1991;3:214–223.
23. 't Hart LA, Nibbering PH, van den Barselaar MT, et al. Effects of low molecular constituents from Aloe vera gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. *Int J Immunopharmacol*. 1990;12:427–434.
24. Womble D, Helderman JH. Enhancement of allo-responsiveness of human lymphocytes by acemannan (Carrisyn). *Int J Immunopharmacol*. 1988;10:967–974.
25. Zhang L, Tizard IR. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. *Immunopharmacol*. 1996;35:119–128.
26. Stuart RW, Lefkowitz DL, Lincoln JA, et al. Upregulation of phagocytosis and candidicidal activity of macrophages exposed to the immunostimulant acemannan. *Int J Immunopharmacol*. 1997;19:75–82.
27. Egger SF, Brown GS, Kelsey LS, et al. Studies on optimal dose and administration schedule of a hematopoietic stimulatory beta-(1,4)-linked mannan. *Int J Immunopharmacol*. 1996;18:113–126.
28. Vazquez B, Avila G, Segura D, et al. Anti-inflammatory activity of extracts from Aloe vera gel. *J Ethnopharmacol*. 1996;55:69–75.
29. Malterud KE, Farbrot TL, Huse AE, et al. Antioxidant and radical scavenging effects of anthraquinones and anthrones. *Pharmacol*. 1993;47(suppl 1):77–85.
30. Chung JH, Cheong JC, Lee JY, et al. Acceleration of the alcohol oxidation rate in rats with aloin, a quinone derivative of Aloe. *Biochem Pharmacol*. 1996;52:1461–1468.
31. Hashemi SA, Madani SA, Abediankenari S. The review on properties of aloe vera in healing of cutaneous wounds. *Biomed Res Int*. 2015;2015:714216.
32. Chithra P, Sajithlal GB, Chandrakasan G. Influence of Aloe vera on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *J Ethnopharmacol*. 1998;59:179–186.
33. Davis RH, Donato JJ, Hartman GM, et al. Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. *J Am Podiatr Med Assoc*. 1994;84:77–81.
34. Davis RH, DiDonato JJ, Johnson RW, et al. Aloe vera, hydrocortisone, and sterol influence on wound tensile strength and anti-inflammation. *J Am Podiatr Med Assoc*. 1994;84:614–621.
35. Dat AD, Poon F, Pham KB, Doust J. Aloe vera for treating acute and chronic wounds. *Cochrane Database Syst Rev*. 2012;15(2):CD008762.
36. Zawahry M, Hegazy MR, Helal M. Use of aloe in treating leg ulcers and dermatoses. *Int J Dermatol*. 1973;12:68–73.
37. Visuthikosol V, Chowchuen B, Sukwanarat Y, et al. Effect of Aloe vera gel to healing of burn wound. A clinical and histologic study. *J Med Assoc Thai*. 1995;78:403–409.
38. Khorasani G, Hosseinimehr SJ, Azadbakht M, et al. Aloe versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. *Surg Today*. 2009;39:587–591.
39. Miller MB, Koltai PJ. Treatment of experimental frostbite with pentoxifylline and aloe vera cream. *Arch Otolaryngol Head Neck Surg*. 1995;121:678–680.
40. Ardire L. Necrotizing fasciitis: case study of a nursing dilemma. *Ostomy Wound Manage*. 1997;43(30–34):38–40. 36.
41. Thomas DR, Goode PS, LaMaster K, et al. Acemannan hydrogel dressing versus saline dressing for pressure ulcers. A randomized, controlled trial. *Adv Wound Care*. 1998;11:273–276.
42. Molazem Z, Mohseni F1, Younesi M, Keshavarzi S. Aloe vera gel and cesarean wound healing; a randomized controlled clinical trial. *Glob J Health Sci*. 2014;7(1):203–209.
43. Eshghi F, Hosseinimehr SJ, Rahmani N, et al. Effects of Aloe vera cream on posthemorrhoidectomy pain and wound healing: results of a randomized, blind, placebo-control study. *J Altern Complement Med*. 2010;16(6):647–650.
44. Tanaka M, Yamamoto Y, Misawa E, et al. Effects of aloe sterol supplementation on skin elasticity, hydration, and collagen score: a 12-week double-blind, randomized, controlled trial. *Skin Pharmacol Physiol*. 2016;29(6):309–317.
45. Syed TA, Ahmad SA, Holt AH, et al. Management of psoriasis with Aloe vera extract in a hydrophilic cream: a placebo-controlled, double-blind study. *Trop Med Int Health*. 1996;1:505–509.
46. Choonhakarn C, Busaracome P, Sripanidkulchai B, et al. A prospective, randomized clinical trial comparing topical aloe vera with 0.1% triamcinolone acetonide in mild to moderate plaque psoriasis. *J Eur Acad Dermatol Venereol*. 2010;24:168–172.
47. Williams MS, Burk M, Loprinzi CL, et al. Phase III double-blind evaluation of an Aloe vera gel as a prophylactic agent for radiation-induced skin toxicity. *Int J Radiat Oncol Biol Phys*. 1996;36:345–349.
48. Heggie S, Bryant GP, Tripcony L, et al. A phase III study on the efficacy of topical aloe vera gel on irradiated breast tissue. *Cancer Nurs*. 2002;25: 442–451.
49. Hoopfer D, Holloway C, Gabos Z, et al. Three-Arm Randomized Phase III Trial: quality aloe and placebo cream versus powder as skin treatment during breast cancer radiation therapy. *Clin Breast Cancer*. 2015;15(3):181–190.

50. Blitz JJ, Smith JW, Gerard JR. Aloe vera gel in peptic ulcer therapy: preliminary report. *J Am Osteopath Assoc*. 1963;62:731–735.
51. Langmead L, Feakins RM, Goldthorpe S, et al. Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. *Aliment Pharmacol Ther*. 2004;19:739–747.
52. Rahmani N, Khademloo M, Vosoughi K, Assadpour S. Effects of Aloe vera cream on chronic anal fissure pain, wound healing and hemorrhaging upon defection: a prospective double blind clinical trial. *Eur Rev Med Pharmacol Sci*. 2014;18(7):1078–1084.
53. Nair GR, Naidu GS, Jain S, et al. Clinical effectiveness of aloe vera in the management of oral mucosal diseases- a systematic review. *J Clin Diagn Res*. 2016;10(8):ZE01–ZE07.
54. Anuradha A, Patil B, Asha VR. Evaluation of efficacy of aloe vera in the treatment of oral submucous fibrosis - a clinical study. *J Oral Pathol Med*. 2017;46(1):50–55.68. Nair GR.
55. Ali S, Wahbi W. The efficacy of aloe vera in management of oral lichen planus: a systematic review and meta-analysis. *Oral Dis*. 2017;23(7):913–918.
56. Reddy RL, Reddy RS, Ramesh T, et al. Randomized trial of aloe vera gel vs triamcinolone acetonide ointment in the treatment of oral lichen planus. *Quintessence Int*. 2012;43(9):793–800.
57. McDaniel HR, Combs C, McDaniel R, et al. An increase in circulating monocyte/macrophages (MM) is induced by oral acemannan (ACE-M) in HIV-1 patients. *Am J Clin Pathol*. 1990;94:516–517.
58. Montaner JS, Gill J, Singer J, et al. Double-blind placebo-controlled pilot trial of acemannan in advanced human immunodeficiency virus disease. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996;12:153–157.
59. Ajabnoor MA. Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol*. 1990;28:215–220.
60. Gnhannam N, Kingston M, Al-Meshaal IA, et al. The antidiabetic activity of Aloes. *Horm Res*. 1986;24:288–294.
61. Bunyapraphatsara N, Yongchaiyudha A, Rungpitarang S, et al. Antidiabetic activity of Aloe vera juice II. Clinical trial in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine*. 1996;3:245–248.
62. Shida T, Yagi A, Nishirnura H, et al. Effect of Aloe extract on peripheral phagocytosis in adult bronchial asthma. *Planta Medica*. 1985;51:273–275.
63. Prueksrisakul T, Chantarangsu S, Thunyakitpisal P. Effect of daily drinking of Aloe vera gel extract on plasma total antioxidant capacity and oral pathogenic bacteria in healthy volunteer: a short-term study. *J Complement Integr Med*. 2015;12(2):159–164.
64. Kim HS, Lee BM. Inhibition of benzo[a]pyrene-DNA adduct formation by Aloe barbadensis Miller. *Carcinogenesis*. 1997;18:771–776.
65. Peng SY, Norman J, Curtin G, et al. Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator, acemannan. *Mol Biother*. 1991;3:79–87.
66. Harris C, Pierce K, King G, et al. Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms. *Mol Biother*. 1991;3:207–213.
67. Lissoni P, Giani L, Zerbini S, et al. Biotherapy with the pineal immunomodulating hormone melatonin versus melatonin plus aloe vera in untreatable advanced solid neoplasms. *Nat Immun*. 1998;16:27–33.
68. Fahim MS, Wang M. Zinc acetate and lyophilized Aloe barbadensis as vaginal contraceptive. *Contraception*. 1996;53:231–236.
69. Schmidt JM, Greenspoon JS. Aloe vera dermal wound gel is associated with a delay in wound healing. *Obstet Gynecol*. 1991;78:115–117.
70. Parlati L, Voican CS, Perlemuter K, Perlemuter G. Aloe vera-induced acute liver injury: a case report and literature review. *Clin Res Hepatol Gastroenterol*. 2017;41(4):e39–e42.

Angelica Species

Michael T. Murray, ND,* and *John Nowicki, ND*

OUTLINE

Angelica sinensis or polymorpha (family: *Umbelliferae* or *Apiaceae*)
Common names: Chinese angelica, tang-kuei (dong quai)
Angelica acutiloba (family: *Umbelliferae* or *Apiaceae*)
Common name: Japanese angelica
Angelica archangelica (family: *Umbelliferae* or *Apiaceae*)
Common name: European angelica
Angelica atropurpurea (family: *Umbelliferae* or *Apiaceae*)
Common name: American angelica
Angelica silvestris (family: *Umbelliferae* or *Apiaceae*)
Common name: wild angelica

GENERAL DESCRIPTION

Angelica spp. are biennial or perennial plants with hollow fluted stems that rise to a height of 3 to 7 feet. The umbels, or clusters, of greenish-white flowers bloom from May to August (Fig. 53.1AC). The plants are found in damp mountain ravines and meadows, on riverbanks, and in coastal areas; angelica is also a widely cultivated species. In Asia it is grown primarily for its medicinal properties, whereas in the United States and Europe it is cultivated for use as a flavoring agent in most major categories of food products, including alcohol (e.g., bitters, liqueurs, vermouths) and nonalcoholic beverages (e.g., ice cream, candy, gelatins, and puddings). In all species, the roots and rhizomes are the most extensively used portions of the plant.

Angelica sinensis and *Angelica acutiloba*

In Asia the authentic and original angelica species used for medicinal purposes is *A. sinensis* (dong quai), native to China. Although at least nine other angelica species are used in China, dong quai is by far the most highly regarded. For several thousand years, dong quai has been cultivated for medicinal use in the treatment of a variety of disorders. Several hundred years ago, when the supply of Chinese angelica was scarce, the Japanese began to cultivate *A. acutiloba,* an angelica species indigenous to Japan, as a substitute.[1] The two species appear to have similar therapeutic effects despite the following cultural opinions: in China, the Japanese angelica is thought to have no therapeutic value; whereas in Japan, Chinese angelica is thought to have no effect. Experimentally, both species exhibit similar therapeutic effects.

Angelica archangelica and *Angelica atropurpurea*

Historical usage suggests that European angelica *(A. archangelica)* and American angelica *(A. atropurpurea)* have properties different from the Asian species. However, this difference has not been evaluated by chemical analysis.

CHEMICAL COMPOSITION

Angelica sinensis and *Angelica acutiloba*

Although it has been assumed that Chinese and Japanese angelica are similarly composed of various coumarins and flavonoids that are responsible for their medicinal actions, a recent analysis showed that *A. sinensis* contained approximately tenfold higher levels of the key components ferulic acid and Z-ligustilide compared with roots of *A. acutiloba.*[2]

Many medicinal compounds are believed to be present in the essential oil, including the following[3]:

- 3*n*-Butylphthalide
- Cadinene
- Carvacrol
- n-Dodecanal
- Isosafrole

*Previous edition contributor

A

Fig. 53.1 (A) *Angelica* species. (B) *Angelica archangelica* inflorescence. (C) *Angelica dahurica* flower fruit. (A, from JPC-PROD/iStock.com. B and C, from Eric L Yarnell urologynd@gmail.com Also contributor.)

- Linoleic acid
- Palmitic acid
- Safrole
- Sequiterpene
- n-Tetradecanoyl

Angelica archangelica

A. archangelica is also rich in coumarins and is particularly phototoxic. Coumarins including osthole, angelicin, osthenol, umbelliferone, archangelicine, bergapten, and ostruthol are found in significant concentrations, with osthole composing nearly 0.2% of the root. The root is also a good source of flavonoids, including archan-gelenone and caffeic acids. The root contains 0.3% to 1% volatile oil, composed mainly of β-phellandrene, α-pinene, borneol, limonene, and four macrocyclic lactones.[3,4]

HISTORY AND FOLK USE

Angelica sinensis and *Angelica acutiloba*

In Asia, angelica's reputation is perhaps second only to ginseng. Predominantly regarded as a female remedy, angelica has been used to treat dysmenorrhea, amenorrhea, metrorrhagia, and menopausal symptoms, and to facilitate a healthy pregnancy and easy delivery. Angelica is also used in the treatment of abdominal pain, anemia, injuries, arthritis, migraine headache, and many other conditions.[3,5]

Angelica archangelica

One of the most highly praised herbs in old herbal texts, *A. archangelica* was used in all Northern European countries as a protection against contagion, for purifying the blood, and for curing every conceivable malady; it was considered a sovereign remedy for poisons, agues, and all infections. According to one legend, *A. archangelica* was revealed in a dream as a cure for the plague. One explanation for the name is related to its blooming near May 8, the feast day of Michael the Archangel.

A. archangelica has been used for a wide variety of conditions, including flatulent dyspepsia, pleurisy, respiratory catarrh, and bronchitis. The plant was believed to possess carminative, spasmolytic, diaphoretic, expectorant, and diuretic activity.[6] A prospective, open-label trial of Feru-guard, a combination of ferulic acid and an extract of *A. archangelica*, led to significantly reduced subscale scores on the Neuropsychiatric Inventory, indicating that it may be effective for treating the behavioral and psychological symptoms of dementia.[7]

Angelica atropurpurea

American angelica's therapeutic use mirrors that of European angelica. It is most commonly used for heartburn and flatulent colic.[8]

PHARMACOLOGY

The pharmacology of *Angelica* spp. primarily relates to high coumarin content. However, unlike other scientific investigations of botanical medicines, most research on *Angelica* spp. has been done on plant extracts, rather than isolated constituents. The overwhelming majority of studies have been done on the Asian species. Some of the pharmacological activities demonstrated include the following:

- Phytoestrogen activity
- Cardiovascular effects
- Smooth muscle–relaxing effects
- Antioxidant effects
- Analgesic activity
- Antiallergic, immunomodulating, and direct antitumor activity
- Antimicrobial activity

Phytoestrogen Effects

Phytoestrogens (i.e., plant estrogenic substances) are components of many medicinal herbs with historical use in conditions that are now treated by synthetic estrogens. Chinese and Japanese angelica contain weakly active phytoestrogens, much lower in activity than other phytoestrogens, and approximately no more than 1:400 are active as estrogen.[9] This helps explain why angelica was used in conditions of both excessive and deficient estrogen. Phytoestrogens demonstrate an adaptogenic effect by competing with estrogen for binding sites. When estrogen levels are low, they can exert some weak estrogenic activity; when estrogen levels are high, they reduce overall estrogenic activity by occupying estrogen receptor sites. This alterative action of phytoestrogens is probably the basis of the plant's use in amenorrhea and menopause.

Japanese angelica has demonstrated uterine tonic activity, causing an initial increase in uterine contraction followed by relaxation.[10,11] In addition, administration of Japanese angelica to mice resulted in an increase in uterine weight, an increase in the DNA content of the uterus and liver, and an increase in glucose use by the liver and uterus.[1,10] Because of these and other effects, angelica has been referred to as a uterine tonic.

Administration of a standardized ethanol extract in ovariectomized rats exhibited stimulation of the uterine histoarchitecture, a significant cornification in the vaginal epithelium, and a reduction of serum luteinizing hormone concentration, showing the estrogenic nature of the extract. Furthermore, the administration of the extract in intact female rats provoked a significant modification of the vaginal smear in 67% of treated rats.[3,12]

Cardiovascular Effects

Angelica possesses significant hypotensive action largely due to its vasodilator activity.[1,10] Dihydropyranocoumarins and dihydrofuranocoumarins from *Umbelliferous* plants have been shown to possess significant coronary vasodilatory, spasmolytic, and cyclic adenosine monophosphate phosphodiesterase inhibitory properties.[13] The mechanism of action appears to be a result of calcium-channel antagonism. Agents that interact with calcium channels (calcium channel blockers) are involved in the treatment of wide-ranging conditions, including hypertension and angina. Umbelliferous plants, such as angelica, may offer similar effects. Angelica provides additional cardiovascular effects, including negative inotropic and antiarrhythmic action.[1]

Smooth Muscle–Relaxing Activity

Calcium-channel–blocking compounds are also capable of relaxing the smooth muscles of visceral organs. Essential oil of angelica demonstrated relaxing action on the smooth muscles of the intestines and uterus, whereas the water extract produced an initial contraction and then prolonged relaxation.[1,10,11] This confirms its historical use in the treatment of intestinal spasm and uterine cramps. Its action on other smooth muscles could explain its hypotensive action (vascular smooth muscle) and historical use in asthma (bronchial smooth muscle).

Analgesic Activity

Both Chinese and Japanese angelica have demonstrated pain-relieving and mild tranquilizing effects in experimental studies in animals.[1,10,14,15] Angelica's analgesic action was 1.7 times that of aspirin in one study.[15] Several dimetric phthalides, including angesinenolides, exhibit inhibitory activity against COX-2.[16] Its analgesic activity, combined with its smooth muscle–relaxing activity, supports its historical use in such conditions as uterine cramps, trauma, headaches, and arthritis.

Antiallergic, Immunomodulating, and Direct Antitumor Activity

Angelica has a long history of use by Chinese and Japanese herbalists in the prevention and treatment of allergic symptoms in individuals who are sensitive to various substances (e.g., pollen, dust, animal dander, food).[1,17] Its action is related to its ability to inhibit the production of immunoglobulin-E in a selective manner. Because immunoglobulin-E levels in patients with atopic conditions are typically 3 to 10 times greater than the upper limit of normal, angelica may offer some benefit by reducing these elevated antibodies.

Coumarin compounds have demonstrated immune-enhancing activity in both healthy and cancer patients.[18,19] Coumarins have been shown to stimulate macrophages and increase phagocytosis, offering significant protection against metastasis and growth of tumor cells.[18] Upon coumarin administration, macrophages are activated and thus capable of entering the tumor, where a specific destruction of tumor cells may occur.[18,19]

Coumarin compounds of angelica and the polysaccharides of the water extract of Japanese angelica have demonstrated immune-modulating and antitumor activity. They have been shown to possess mitogenic activity to B lymphocytes, interferon-producing activity, antitumor activity, and activation of both classic and alternative complement pathways.[20–24] Chinese angelica has been shown to increase murine interleukin-2 production, stimulate the reticuloendothelial system, and increase the production of tumor necrosis factor.[25–27] The antitumor effects of angelica appear to result from the polysaccharide components because these compounds have been shown to exert antitumor effects on experimental tumor models in vivo and inhibitory effects on invasion and metastasis of cancer cells in vitro.[28–30] The time-effect relation of cytokine response also suggests that macrophages and natural killer cells involved in nonspecific immunity were primarily activated, and helper T cells were secondarily affected by angelica polysaccharides.[31] The effects by coumarins, polysaccharides, and extracts of *Angelica* spp. would seem to support the historical use of angelica as an adjunct in cancer therapy.

Kidney Effects

A 24-week, prospective, multicenter, and randomized controlled trial was performed on 158 patients with primary glomerulonephritis.[32] Compared with patients taking losartan, there was a significant continual decrease of proteinuria in the *A. sinensis* group throughout the 24-week time frame of the study, and from week 16 to week 24, there was a significant reduction in proteinuria.

Antimicrobial Activity

Extracts of Chinese angelica have been shown to possess antibacterial activity against both gram-negative and gram-positive bacteria, whereas extracts of Japanese angelica exhibited no antibacterial action.[9] The inconsistency may be secondary to different essential oil concentrations of the extracts used in the studies. The oil of *A. archangelica* has also exhibited significant antifungal and anthelmintic properties but virtually no antibacterial activity.[4,33,34] Because other herbs have much greater antimicrobial activity, *Angelica* spp. would be considered a less-than-optimum antimicrobial agent.

CLINICAL APPLICATION

Angelica spp. have been used worldwide to treat multiple conditions. At present, *A. archangelica* and *A. atropurpurea* are most indicated as expectorants, antispasmodics, and carminatives in the treatment of respiratory ailments, gas, and abdominal spasm. Chinese angelica (*A. sinensis* or *polymorpha*) and Japanese angelica *(A. acutiloba)* appear most useful in the treatment of disorders of menstruation, menopause

(especially hot flashes), atopic conditions, smooth muscle spasm (e.g., uterine cramps, migraines, abdominal spasm), and possibly as an immunostimulatory adjunct in cancer therapy.

Menopause

By far the most popular use of *Angelica* spp. has been the use of *A. sinensis* in the treatment of menopausal complaints. Although a double-blind, placebo-controlled study in women showed no significant benefit, the preparation used (a dried aqueous extract) was clearly lacking some of the important volatile compounds, although it was standardized for ferulic acid content.[35] A study conducted in China showed that a combination of *A. sinensis, Paeonia lactiflora, Ligusticum monnieri, Atractylodes chinensis, Sclerotium poriae,* and *Alisma orientalis* was effective in roughly 70% of women experiencing menopausal symptoms.[36] In an in vitro study with human bone, the aqueous extract of *A. sinensis* was found to directly stimulate proliferation, alkaline phosphatase activity, protein secretion, and type I collagen synthesis in a dose-dependent manner.[37] Also, in a double-blind study, the combination of 100 mg angelica, 60 mg soy isoflavones, and 50 mg of black cohosh extract significantly reduced menstrual migraines.[38] An herbal product, EstroG-100, containing a combination of standardized extracts of *Cynanchum wilfordii, Phlomis umbrosa,* and *Angelica gigas,* was tested in a randomized, double-blind, placebo-controlled trial on 64 pre-, peri-, and postmenopausal women.[39] The constituting symptoms of vasomotor, paresthesia, insomnia, nervousness, melancholia, vertigo, fatigue, rheumatic pain, and vaginal dryness were significantly improved in the EstroG-100 group, without weight gain or any serious side effects.

The estrogenic aspect of dong quai has been called into question for women with estrogen-dependent breast cancer. In vitro studies have shown that the aqueous extract of dong quai stimulated the growth of breast cancer cells, resulting in the caution against its use in herbal preparations for the treatment of peri- or postmenopausal symptoms, especially in women with breast cancer, pending further study.[40]

DOSAGE

- Dried root or rhizome: 1 to 2 g orally, or by infusion, three times a day
- Tincture (1:5): 1.0 to 2 mL, three times a day
- Fluid extract (1:1): 0.5 to 2 mL, three times a day

TOXICOLOGY

Angelica is generally considered to be of low toxicity. However, it contains many substances that may induce photosensitivity. This should be kept in mind when using any *Umbelliferous* plant. This photoreactive activity can be used therapeutically in the treatment of vitiligo and psoriasis.

Use of *Angelica* spp., particularly *A. sinensis,* in men may lead to gynecomastia.[41]

DRUG INTERACTIONS

Angelica species may interfere with anticoagulants. Although animal studies showed no significant variation in the pharmacokinetic parameters of warfarin after dong quai treatment for either single-dose administration or steady-state concentrations of warfarin, there was a single case report of dong quai possibly being responsible for raising the international normalized ratio in women on long-term coumadin therapy. Therefore, in patients on anticoagulant therapy, caution is advised, and monitoring is necessary.[42]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Hikino H. Recent research on Oriental medicinal plants. *Econ Med Plant Res*. 1985;1:53–85.
2. Zhao KJ, Dong TT, Tu PF, et al. Molecular genetic and chemical assessment of radix *Angelica* (Danggui) in China. *J Agric Food Chem*. 2003;51:2576–2583.
3. Duke JA. *Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1985.
4. Leung AY. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. New York: Wiley; 1980.
5. Duke JA, Ayensu ES. *Medicinal Plants of China*. Algonac, MI: Reference Publications; 1985.
6. Grieve MA. *Modern Herbal*. New York: Dover Publications; 1971.
7. Kimura T, Hayashida H, Murata M, Takamatsu J. Effect of ferulic acid and *Angelica archangelica* extract on behavioral and psychological symptoms of dementia in frontotemporal lobar degeneration and dementia with Lewy bodies. *Geriatr Gerontol Inter*. 2011;11(3):309–314. PubMed PMID: 21272180.
8. Lust J. *The Herb Book*. New York: Bantam Books; 1974.
9. Liu J, Burdette JE, Xu H, etal. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem*. 2001;49:2472–2479.
10. Yoshiro K. The physiological actions of tang-kuei and conidium. *Bull Oriental Healing Arts Inst USA*. 1985;10:269–278.
11. Harada M, Suzuki M, Ozaki Y. Effect of Japanese angelica root and peony root on uterine contraction in the rabbit in situ. *J Pharmacobiodyn*. 1984;7:304–311.
12. Circosta C, Pasquale RD, Palumbo DR, etal. Estrogenic activity of standardized extract of *Angelica sinensis*. *Phytother Res*. 2006;20:665–669.
13. Thastrup O, Fjalland B, Lemmich J. Coronary vasodilatory, spasmolytic and cAMP-phosphodiesterase inhibitory properties of dihydropyranocoumarins and dihydrofuranocoumarins. *Acta Pharmacol Toxicol (Copenh)*. 1983;52:246–253.
14. Tanaka S, Ikeshiro Y, Tabata M, etal. Anti-nociceptive substances from the roots of Angelica acutiloba. *Arzneimittelforschung*. 1977;27:2039–2045.
15. Tanaka S, Kano Y, Tabata M, et al. [Effects of Toki *(Angelica acutiloba Kitawaga)* extracts on writhing and capillary permeability in mice (analgesic and anti-inflammatory effects). *Yakugaku Zasshi*. 1971;91:1098–1104 ([in Japanese]).
16. Ly JL, Zhang LB, Guo LM. Phthalide dimers from *Angelica sinensis* and their COX-2 inhibition activity. *Fitoterapia*. 2018; pii: S0367-326X(18):30822–308230. https://doi.org/10.1016/j.fitote.2018.06.016. PubMed PMID: 29935258.
17. Sung CP, Baker AP, Holden DA, et al. Effect of extracts of *Angelica polymorpha* on reaginic antibody production. *J Nat Prod*. 1982;45:398–406.
18. Casley-Smith . JR. The actions of benzopyrenes on the blood-tissue-lymph system. *Folia Angiol*. 1976;24:7–22.
19. Berkarda B, Bouffard-Eyuboglu H, Derman U. The effect of coumarin derivatives on the immunological system of man. *Agents Actions*. 1983;13:50–52.
20. Ohno N, Matsumoto S, Suzuki I, etal. Biochemical and physicochemical characterization of a mitogen obtained from an oriental crude drug, Tohki *(Angelica actiloba Kitagawa)*. *J Pharmacobiodyn*. 1983;6:903–912.
21. Yamada H, Kiyohara H, Cyong JC, etal. Studies on polysaccharides from *Angelica acutiloba*. Part 1. Fractionation and biological properties of polysaccharides. *Planta Med*. 1984;50:163–167.
22. Yamada H, Kiyohara H, Cyong JC, et al. Studies on polysaccharides from *Angelica acutiloba*-IV. Characterization of an anti-complementary arabinogalactan from the roots of *Angelica acutiloba Kitagawa*. *Mol Immunol*. 1985;22:295–304.
23. Kumazawa Y, Mizunoe K, Otsuka Y. Immunostimulating polysaccharide separated from hot water extract of *Angelica acutiloba Kitagawa* (Yamato Tohki). *Immunology*. 1982;47:75–83.
24. Lu S, Huang H, Wu S. The regulation effects of *Angelica sinensis* on the erythrocytic immune function and IL-2 in mice. *Zhong Yao Cai*. 1997;20:301–303 ([in Chinese]).
25. Weng XC, Zhang P, Gong SS, et al. Effect of immuno-modulating agents on murine IL-2 production. *Immunol Invest*. 1987;16:79–86.
26. Haranaka K, Satomi N, Sakurai A, et al. Antitumor activities and tumor necrosis factor producibility of traditional Chinese medicines and crude drugs. *Cancer Immunol Immunother*. 1985;20:1–5.
27. Shan JJ, Wang Y, Wang SC, et al. Effect of *Angelica sinensis* polysaccharides on lymphocyte proliferation and induction of IFN-gamma. *Yao Xue Xue Bao*. 2002;37:497–500 ([in Chinese]).
28. Shang P, Qian AR, Yang TH, et al. Experimental study of anti-tumor effects of polysaccharides from *Angelica sinensis*. *World J Gastroenterol*. 2003;9:1963–1967.
29. Zheng M, Wang YP. [Experimental study on effect of *Angelica* polysaccharide in inhibitory proliferation and inducing differentiation of K562 cells. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2002;22:54–57 ([in Chinese]).
30. Sun Y, Tang J, Gu X, et al. Water-soluble polysaccharides from *Angelica sinensis* (Oliv.) Diels: preparation, characterization and bioactivity. *Int J Biol Macromol*. 2005;36:283–289.
31. Yang T, Jia M, Meng J, et al. Immunomodulatory activity of polysaccharide isolated from *Angelica sinensis*. *Int J Biol Macromol*. 2006;39:179–184.
32. Shen P, Yang X, He L. Effect of astragali and Angelica particle on proteinuria in Chinese patients with primary glomerulonephritis. *J Trad Chinese Med*. 2016;36(3):299–306. PubMed PMID: 27468543.
33. Rhee JK, Woo KJ, Baek BK, et al. Screening of the wormicidal Chinese raw drugs on *Clonorchis sinensis*. *Am J Chin Med*. 1981;9:277–284.
34. Opdyke DL. Angelica root oil. *Food Cosmet Toxicol*. 1975;13:713–714.
35. Hirata JD, Swiersz LM, Zell B, et al. Does dong quai have estrogenic effects in postmenopausal women? A double-blind, placebo-controlled trial. *Fertil Steril*. 1997;68:981–986.
36. Chang HM, But PPH. *Pharmacology and Applications of Chinese Materia Medica*. Singapore: World Scientific; 1987:489–505.
37. Yang Q, Populo SM, Zhang J, et al. Effect of *Angelica sinensis* on the proliferation of human bone cells. *Clin Chim Acta*. 2002;324:89–97.
38. Burke BE, Olson RD, Cusack BJ. Randomized, controlled trial of phytoestrogen in the prophylactic treatment of menstrual migraine. *Biomed Pharmacother*. 2002;56:283–288.
39. Chang A, Kwak BY, Yi K, Kim JS. The effect of herbal extract (EstroG-100) on pre-, peri-, and post-menopausal women: a randomized double blind, placebo-controlled study. *Phytother Res*. 2012;26(4):510–516. PubMed PMID: 21887807.
40. Lau CB, Ho TC, Chan TW, et al. Use of dong quai *(Angelica sinensis)* to treat peri- or postmenopausal symptoms in women with breast cancer: is it appropriate? *Menopause*. 2005;12:734–740.
41. Goh SY, Loh KC. Gynaecomastia and the herbal tonic dong quai. *Singapore Med J*. 2001;42:115–116.
42. Page 2nd RL, Lawrence JD. Potentiation of warfarin by dong quai. *Pharmacotherapy*. 1999;19:870–876.

Artemisia absinthium (Wormwood)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Artemisia absinthium (family: Asteraceae)
Common names: wormwood

GENERAL DESCRIPTION

Artemisia absinthium generally occurs as an aromatic, semiwoody, perennial subshrub reaching up to 1 m tall, particularly in its native Mediterranean habitat. The leaves and flowers are used as medicine (Fig. 54.1A and B).

CHEMICAL COMPOSITION

The aerial parts of wormwood contain two components considered most important to its medicinal activity: 0.15% to 0.4% monoterpenoids and 0.2% to 1.5% sesquiterpene lactones.[1] The monoterpenoids α- and β-thujone are perhaps the most notorious constituents, given their potential neurotoxicity. Alpha-thujone is believed to be much more toxic and is present as 0% to 60% of the volatile oil (mean 5.8%) compared with 0% to 69.7% of β-thujone (mean 12.5%).[2,3] Chemotypes of wormwood depend on growing environment and genetics, and only certain ones contain thujones—others have transsabinyl acetate, cis-epoxyocimen, or chrysanthenyl acetate as primary compounds instead.[4] Major sesquiterpene lactones present are absinthin, artabsin, matricin, and anabsinthin (Figs. 54.2–54.5).

HISTORY AND FOLK USE

Wormwood was used historically as a digestive bitter in traditional herbal systems of various societies around its native Mediterranean habitat. The Ebers papyrus, an Egyptian medical treatise and one of the oldest extant pieces of writing (circa 1550 BC), mentions wormwood. Pliny the Elder discussed the use of wormwood to expel parasites, as reflected in the common name of the plant, in the 1st century AD.

The medicinal aspects of most of the history of wormwood are often overshadowed by development in the late 18th century of absinthe. The standard account of the rise of absinthe, a liqueur derived in part from wormwood, begins with French expatriate Pierre Ordinaire, who allegedly developed the first absinthe recipe in 1792 in Switzerland.[5] Absinthe schnapps, absinthe-infused ale (known as "purl"), absinthe-infused wine, and other absinthe-containing beverages had been in use since Dioscorides' time and probably before. Absinthe was apparently made by infusing wormwood, garden angelica root, anise fruit, and marjoram herb in ethanol; distilling them; and adding other flavorings, including volatile oils.[5] The resulting product was bright green and intensely bitter, formed a white precipitate with water (known as louche), and had a high alcohol content (50%–80%).[6]

Henri Dubied and his son-in-law Henri Louis Pernod purchased Ordinaire's formula and began producing large quantities of absinthe at the Pernod Fils distillery.[6] One client for their product was the French military, which invaded Algeria from 1844 to 1847 and issued absinthe regularly to the troops to help prevent and treat dysentery. These troops apparently returned to France with a taste for absinthe and increased the popularity of the beverage.

Although originally most popular with people in the lower classes, absinthe caught on among the intelligentsia in the 1860s and 1870s, a time of cultural ferment, particularly in Paris. Parisians would gather in cafes to sip absinthe, often diluted by pouring cold water over a sugar cube held in a perforated spoon into a glass containing a shot of absinthe.[7] Numerous famous artists of the time drank absinthe regularly, including Vincent van Gogh, whose insanity has been blamed (without proof) on his absinthe habit.

The growing popularity of absinthe, combined with a fire at the Pernod Fils distillery in 1901, led to a surge of absinthe-like products on the market. Unfortunately, these imitation products were often made as cheaply as possible by simply mixing volatile oils with alcohol, adding various coloring agents to give the appropriate green hue (including

Fig. 54.1 Wormwood.

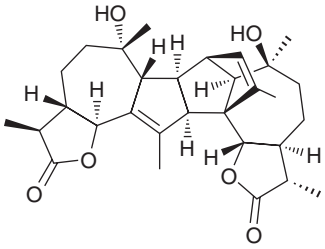


Fig. 54.2 Absinthin. (From https://en.wikipedia.org/wiki/Absinthin. Accessed October 2, 2018.)

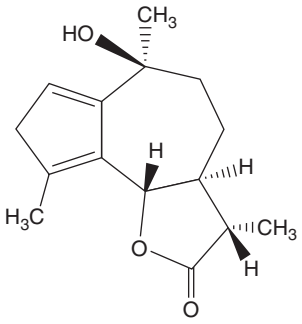


Fig. 54.3 Artabsin. (From http://kanaya.naist.jp/knapsack_jsp/information.jsp?word=C00000173. Accessed October 2, 2018.)

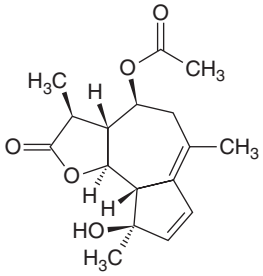


Fig. 54.4 Matricin. (From https://en.wikipedia.org/wiki/Matricin. Accessed October 2, 2019.)

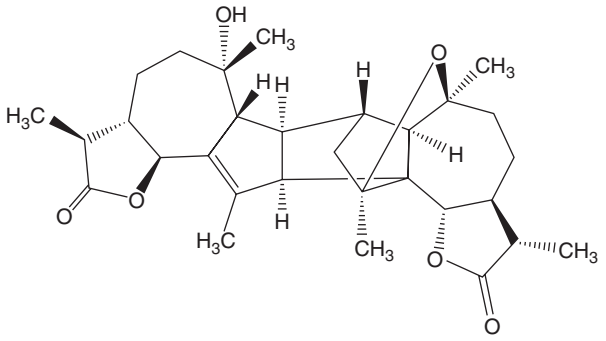


Fig. 54.5 Anabsinthin. (From http://kanaya.naist.jp/knapsack_jsp/information.jsp?mode=r&word=C00020969&key=0. Accessed October 2, 2018.)

copper sulfate), and adding antimony chloride to create the appearance of the white precipitate formed in true absinthe.[5] At the same time, a new phenomenon termed absinthism was becoming widely known. This syndrome was a result of chronic absinthe overindulgence and included bursts of violent aggressiveness followed by prolonged depression, trembling, hallucinations, seizures, and death.[7] However, the presence of adulterated, low-quality, high-ethanol absinthe products on the market and the inability to distinguish the effects of ethanol abuse from those of other compounds in the mixture made it difficult to be certain exactly what was causing absinthism. The levels of the purported neurotoxins thujones in true absinthe were 2 to 4 mg per drink, far below the 10 mg/kg levels needed to induce neurological damage in numerous animal experiments.[8] In the early 20th century, absinthe was banned in most of Europe and the United States. Absinthe, usually with limits on thujones content, is now legal again in many places, including Europe and the United States.

The Eclectics regarded wormwood as a digestive tonic useful in atonic dyspepsia, as a potential treatment for roundworms, and for topical application to sprains and bruises.[9] The dangers of absinthism were well known to these turn-of-the-century American natural physicians, but they did not seem clear on the various problematic elements in the history of absinthe discussed previously.

PHARMACOLOGY

The study of wormwood has focused on four major actions: digestive effects, antiparasitic effects, endocrine effects, and effects on the central nervous system. Growing evidence suggests it is also a potent tumor necrosis factor-α inhibitor. Various reported effects of isolated α- and β-thujone may also be relevant to wormwood's effects.

Digestive Effects

In a controlled clinical trial, wormwood tincture and thujones-free wormwood tincture were both shown to significantly increase bile and pancreatic enzyme secretion in humans, compared with water.[10] The levels of secretion were assessed intraduodenally. These effects seem to be common to many bitter herbs that contain sesquiterpene lactones. Wormwood extract had a modest antidiarrheal effect in one rat model unrelated to infection.[11]

Hepatic Effects

Pretreatment of mice with 500 mg/kg of wormwood extract decreased the 100% lethality of 1 g/kg acetaminophen to 20%.[12] The toxicity of carbon tetrachloride was also reduced by pretreatment of rats with wormwood. Administering 500 mg/kg wormwood extract to rats 6 hours after toxic but sublethal doses of acetaminophen (but not

carbon tetrachloride) significantly reduced hepatic damage. An aqueous extract of wormwood protected mice against carbon tetrachloride- and immune-mediated liver damage, partly by reducing oxidation and partly by inhibiting tumor necrosis factor (TNF)-α and interleukin-1.[13]

Antiparasitic Effects

Wormwood does not appear to contain the same active constituents as sweet Annie *(Artemesia annua),* notably artemisinin, yet aqueous extracts of wormwood showed antimalarial effects in vitro.[14] In mice, oral administration of extracts using 95% ethanol of wormwood was shown to have a potent schizonticidal effect against *Plasmodium berghei,* although not as potent as chloroquine.[15,16] Activity of wormwood against multidrug-resistant *P. falciparum* has been demonstrated in vitro.[16]

Crude extracts of wormwood have been reported to inhibit other organisms in vitro or in animals, including *Trypanosoma brucei, Trichinella spiralis, Leismania amazonensis, Hymenolepsis nana, Naegleria fowleri,* and *Toxocara cati.*[17–23] Crude extract of wormwood was ineffective at treating infection with the nematode *Haemonchus contortus* in gerbils, although infusions and tinctures were effective at treating this infection in sheep and calves.[24,25] Apparently, research in India has shown an effect for wormwood against *Entamoeba histolytica,* but details of this publication could not be obtained.[26]

Endocrine Effects

Wormwood has repeatedly been shown to be antidiabetic and hypoglycemic in multiple animal models of diabetes.[27–30] Contrary to the picture of thujones as largely harmful, a mixture of α- (70%) and β-thujone (20%) 5 mg/kg/day normalized lipid levels in diabetic rats, with no reported adverse effects.[31] A mixture of green tea and wormwood has been shown to both enhance glycemic control and lower elevated lipid levels.

Neurological Effects

Structural and biosynthetic similarities have long been noted among α- and β-thujone and tetrahydrocannabinol from *Cannabis sativa* (marijuana).[32] Although thujones weakly bind cannabinoid receptors in animals and human receptors in vitro, they do not appear to activate them.[33] Wormwood oil also lacked significant cannabimimetic activity in this assay. Thujones' purported hallucinogenic and epileptogenic effects have been disproven except at extremely high concentrations, and "absinthism" has been largely proven to be a result of alcoholism.[34,35] An 80% ethanol extract of wormwood was a strong muscarinic and nicotinic cholinergic receptor agonist in vitro.[36] Methanol extracts of wormwood at concentrations of 100 to 200 mg/kg significantly reduced memory loss and motor incoordination after middle cerebral artery occlusion in rats.[37]

CLINICAL APPLICATIONS

Wormwood was officially approved by the German Commission E for the treatment of patients with dyspepsia, loss of appetite, and biliary dyskinesia.[38] Human research showing that wormwood could increase digestive secretions supports the use of wormwood for loss of appetite, although much more specific work is necessary for definitive proof of efficacy.[10]

Two preliminary open trials have found wormwood teas effective for reducing symptoms and serum transaminase levels in patients with chronic hepatitis B.[39,40] Neither found adverse effects of using up to 6 g per day for 4 weeks. One randomized, single-blind trial found that wormwood was not more effective than placebo for patients with nonalcoholic fatty liver disease.[41]

A randomized, double-blind clinical trial of Crohn's disease found that wormwood extract, standardized to 0.2% to 0.38% absinthin and 0.25% to 0.52% total volatile oil (without thujone), in a base of rose petal, cardamom, and mastic gum or ginger (500 mg three times per day), was more effective than placebo at preventing relapse at 20 weeks.[42] All patients were tapered off corticosteroids after 2 weeks on the medication. At a dose of 750 mg three times per day, this same extract has been shown to lower TNF-α levels in patients with Crohn's disease over 6 weeks.[43]

In an uncontrolled trial, the same extract mentioned earlier, at a dose of 600 mg three times per day in 10 patients with immunoglobulin-A nephropathy not responding to immunosuppressive medications, was effective at decreasing proteinuria and blood pressure over 6 months.[44] Glomerular filtration rate remained stable, and there were no adverse effects.

Wormwood ointment, liniment, and piroxicam were compared in a double-blind, randomized noninferiority trial in adults with knee osteoarthritis.[45] After application of the assigned medication three times daily for 28 days, all groups had improved significantly compared with baseline; there was no significant difference between the groups on outcome measures (assessment of pain, stiffness, and physical function). All treatments were very safe.

In a small clinical trial, 32 Chinese adults with type 2 diabetes were treated with either *Gymnema sylvestre* (gurmar), *Citrullus colocynthis* (bitter apple), wormwood, or placebo capsules for 30 days, all at a dose of 500 mg twice per day.[46] Blinding and randomization were not described. All three herbs significantly decreased fasting blood glucose compared with placebo, with no significant changes in lipid levels.

DOSAGE

Wormwood is primarily used in three forms—tea, tincture, and capsule. Volatile oil should not be used. A typical dose of tea is 1 g (1–2 tsp) dried leaf and flower per cup of water, steeped for 10 to 15 minutes.[47] The tea should be covered during steeping to retain as many volatile constituents as possible. A cup of tea is sipped before meals three times daily. Thujones are water insoluble, so aqueous extracts of the plant are generally low in thujones. A typical dose of tincture is 0.5 to 1 mL three times daily mixed with 2 to 4 oz water, sipped before meals. A typical capsule dose is 2 to 3 g/day in divided doses.[48] Daily thujone intake should be <0.1 mg/kg/day.[49]

TOXICITY

Although much toxicity is ascribed to wormwood, it now appears that this is largely the result of poor research methodology in the early 20th century and improperly conflating thujones, wormwood volatile oil, and absinthe.[34] Any toxicity attributed to the use of various forms of the beverage absinthe must not be confused with the use of teas or tinctures in medicinal doses. Alpha-thujone is considered a neurotoxin and convulsant compound, although these effects are only seen at supratherapeutic doses.[2,50] Beta-thujone, present in much higher levels in wormwood, is generally considered much less toxic.[51] In mice, α- and β-thujone both antagonized γ-aminobutyric acid-A receptors and induced seizures.[52] Alpha-thujone was a much stronger convulsant than β-thujone in this study. This study confirmed previous findings that thujone is rapidly metabolized by the liver and removed from the body in animals.[53]

A 31-year-old man who drank 10 mL of wormwood volatile oil, mistakenly believing it was identical to absinthe, became agitated, belligerent, and incoherent and developed tonic-clonic seizures.[54] Haloperidol improved his mental function, but acute renal failure and rhabdomyolysis were discovered, becoming worse a day later.

Seventeen days after discharge from the hospital, he had recovered completely. More recently, another case was reported of a 53-year-old woman who drank an unspecified amount of wormwood tea and developed rhabdomyolysis and acute renal failure; she recovered completely.[55] A 31-year-old woman who drank wormwood tea was reported to develop disorientation, amnesia, and fever but recovered completely.[55]

These effects have never been documented to occur in patients being treated with reasonable doses of medicinal extracts of or crude wormwood. In one naturopathic medical practice, nine patients over a 3-year period were identified who had been treated with a bitters formula including 11% tincture of wormwood, generally as a treatment for dyspepsia or malabsorption.[56] Administered at a dose of 1 teaspoon (5 mL) three times daily, patients would have been exposed to approximately 0.5 mL wormwood tincture three times daily for weeks to months. For the eight patients with available laboratory data, there was no evidence of renal, hepatic, or other damage. One patient showed improvement in serum liver enzyme levels while taking the bitters formula. None of the patients developed seizures or other signs or symptoms of neurotoxicity. Although retrospective, this study did support the notion that wormwood in this form and at this dose is safe for use in adults as a digestive aid.

Thujones stimulate 5-aminolevulinate synthase in vitro, leading to large increases in porphyrin levels.[57] Although it seems likely that porphyrogenic levels of thujones could be achieved by drinking large volumes of absinthe or volatile oil, levels of intake of tincture, tea, or capsules of wormwood are far lower and unlikely to lead to such high levels. Porphyrogenic effects have not been clearly documented to be induced by wormwood in humans, although thujones and thujone-containing herbs such as wormwood should be avoided by people with porphyria until more information is available.

DRUG INTERACTIONS

No confirmed drug interactions exist for wormwood. It should be avoided in combination with chronic ethanol abuse due to historic reports pertaining to absinthe. Given the low doses of tinctures used, this should not represent any health threat related to ethanol ingestion. Wormwood could theoretically have synergistic porphyrogenic effects with other drugs that can induce porphyric attacks, including barbiturates, hydantoins, and carbamazepine.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bisset NG, Wichtl M. *Herbal Drugs and Phytopharmaceuticals*. Boca Raton, FL: CRC Press; 1994:45–48.
2. Lachenmeier DW, Nathan-Maister D. Systematic misinformation about thujone in pre-ban absinthe. *Deutsche Lebensmittel-Rundschau*. 2007;103:255–263.
3. Ariño A, Arberas I, Renobales G, et al. Essential oil of *Artemisia absinthium L.* From the Spanish Pyrenees. *J Essent Oil Res*. 1999;11:182–184.
4. Nguyen HT, Németh ZE. Sources of variability of wormwood *(Artemisia absinthium L.)* essential oil. *J Appl Res Med Aromatic Plants*. 2016;3:143–150.
5. Vogt DD. Absinthium: a nineteenth-century drug of abuse. *J Ethnopharmacol*. 1981;4:337–342.
6. Vogt DD, Montagne M. Absinthe: behind the emerald mask. *Int J Addict*. 1982;17:1015–1029.
7. Haines JD. Absinthe—return of the green fairy. *J Okla State Med Assoc*. 1998;91:406–407.
8. Tisserand R, Young R. *Essential Oil Safety: A Guide for Health Care Professionals*. 2nd ed. Edinburgh: Churchill Livingstone; 2014.
9. Felter HW. *Eclectic Materia Medica, Pharmacology and Therapeutics*. Sandy, OR: Eclectic Medical Publications; 1998.
10. Baumann IC, Glatzel H, Muth HW. Studies on the effects of wormwood *(Artemisia absinthium L.)* on bile and pancreatic juice secretion in man. *Z Allgemeinmed*. 1975;51:784–791.
11. Calzada F, Arista R, Pérez H. Effect of plants used in Mexico to treat gastrointestinal disorders on charcoal-gum acacia-induced hyperperistalsis in rats. *J Ethnopharmacol*. 2010;128:49–51.
12. Gilani AH, Janbaz KH. Preventive and curative effects of *Artemisia absinthium* on acetaminophen and CCl4-induced hepatotoxicity. *Gen Pharmacol*. 1995;26:309–315.
13. Amat N, Upur H, Blazekovic B. In vivo hepatoprotective activity of the aqueous extract of *Artemisia absinthium L.* against chemically and immunologically induced liver injuries in mice. *J Ethnopharmacol*. 2010;131:478–484.
14. Hernandez H, Mendiola J, Torres D, et al. Effect of aqueous extracts of *Artemisia* on the in vitro culture of *Plasmodium falciparum*. *Fitoterapia*. 1990;61:540–541.
15. Zafar MM, Hamdard ME, Hameed A. Screening of *Artemisia absinthium* for antimalarial effects on *Plasmodium berghei* in mice: a preliminary report. *J Ethnopharmacol*. 1990;30:223–226.
16. Ramazani A, Sardari S, Zakeri S, et al. In vitro antiplasmodial and phytochemical study of five *Artemisia* species from Iran and in vivo activity of two species. *Parasitol Res*. 2010;107:593–599.
17. Nibret E, Wink M. Volatile components of four Ethiopian *Artemisia* species extracts and their in vitro antitrypanosomal and cytotoxic activities. *Phytomedicine*. 2010;17:369–374.
18. Caner A, Döskaya M, Degirmenci A, et al. Comparison of the effects of *Artemisia vulgaris* and *Artemisia absinthium* growing in western Anatolia against trichinellosis *(Trichinella spiralis)* in rats. *Exp Parasitol*. 2008;119:173–179.
19. Tariq KA, Chishti MZ, Ahmad F, Shawl AS. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. *Vet Parasitol*. 2009;160:83–88.
20. Tamargo B, Monzote L, Piñón A, et al. In vitro and in vivo evaluation of essential oil from *Artemisia absinthium L* formulated in nanocochelates against cutaneous leishmaniasis. *Medicines (Basel)*. 2017;4(2):E38.
21. Beshay EVN. Therapeutic efficacy of *Artemisia absinthium* against *Hymenolepis nana*: in vitro and in vivo studies in comparison with the anthelmintic praziquantel. *J Helminthol*. 2018;92(3):298–308.
22. Mendiola J, Bosa M, Perez N, et al. Extracts of *Artemisia abrotanum* and *Artemisia absinthium* inhibit growth of *Naegleria fowleri* in vitro. *Trans R Soc Trop Med Hyg*. 1991;85:78–79.
23. Yıldız K, Başalan M, Duru O, Gökpınar S. Antiparasitic efficiency of *Artemisia absinthium* on *Toxocara cati* in naturally infected cats. *Turkiye Parazitol Derg*. 2011;35(1):10–14.
24. Squires JM, Ferreira JF, Lindsay DS, et al. Effects of artemisinin and *Artemisia* extracts on *Haemonchus contortus* in gerbils (Meriones unguiculatus). *Vet Parasitol*. 2011;175:103–108.
25. Alam S, Mishra A, Sarkar TK, et al. Elucidation of in vitro and in vivo anthelminthic efficacy of *Artemisia absinthium* in sheep and calf model. *Ecol Environ Conservation*. 2016;22(suppl):S255–S259.
26. Tahir M, Khan AB, Siddiqui MMH. Effect of *Artemisia absinthium Linn* in acute intestinal amebiasis. *Presented at Conference of Pharmacology and Symposium on Herbal Drugs.* New Delhi, India: March 15, 1991.
27. Daradka HM, Abas M, Mohammad M, Jaffar M. Antidiabetic effect of *Artemisia absinthium* extracts on alloxan-induced diabetic rats. *Comp Clin Pathol*. 2014;23(6):1733–1742.
28. Goud BJ. Hypoglycemic activity of a methanol extract of *Artemisia absinthium* leaves in experimental rats. *Int J Adv Pharm Res*. 2011;2(7):307–312.
29. Tawfeuk HZ. Hypoglycemic effect of *Artemisia absinthium* and green tea (Camellia sinensis) mixture on alloxan-induced diabetic rats. *J Agroalimentary Processes Technologies*. 2016;22(4):253–262.
30. Goud BJ, Swamy BKC. Antidiabetic activity of *Artemisia absinthium* versus metformin in stz induced diabetic rat. *Int J Adv Res Engineering Appl Sci*. 2016;5(11):1–12.
31. Al-Haj Baddar NW, Aburjai TA, Taha MO, Disi AM. Thujone corrects cholesterol and triglyceride profiles in diabetic rat model. *Nat Prod Res*. 2011;25(12):1180–1184.
32. del Castillo J, Anderson M, Rubottom GM. Marijuana, absinthe and the central nervous system. *Nature*. 1975;253:365–366.
33. Meschler JP, Howlett AC. Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. *Pharmacol Biochem Behav*. 1999;62:473–480.
34. Padosch SA, Lachenmeier DW, Kröner LU. Absinthism: a fictitious 19th century syndrome with present impact. *Substan Abuse Treat Prevent Policy*. 2006;1:14.
35. Lachenmeier DW. Thujone-attributable effects of absinthe are only an urban legend—toxicology uncovers alcohol as real cause of absinthism. *Med Monatsschr Pharm*. 2008;31(3):101–106.
36. Wake G, Court J, Pickering A, et al. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. *J Ethnopharmacol*. 2000;69:105–114.
37. Bora KS, Sharma A. Neuroprotective effect of *Artemisia absinthium L.* on focal ischemia and reperfusion-induced cerebral injury. *J Ethnopharmacol*. 2010;129:403–409.
38. Blumenthal M, Busse WR, Goldberg A, et al. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*. Austin, TX: American Botanical Council; 1988:232–233.
39. Anwar M, Hakeem MH, Siddiqui MM, Tajuddin. Clinical efficacy of *Artemisia absinthium Linn* in viral hepatitis with special reference to ejection fraction of heart. *Hamdard Medicus*. 1998;41(3):93–96.
40. Nikhat S. Clinical efficacy of afsantin (*Artemisia absinthium Linn)* in management of waram-e-kabid (hepatitis). *Hamdard Medicus*. 2007;7(3):103–108.
41. Ahmed NZ, Siddiqui MA. Safety and efficacy of Afsanteen *(Artemisia absinthium L.)* in fatty liver: a randomized single blind controlled study. *Int J Adv Pharm Med Bioallied Sci*. 2015;3(2):106–112.
42. Omer B, Krebs S, Omer H, et al. Steroid-sparing effect of wormwood *(Artemisia absinthium)* in Crohn's disease: a double-blind placebo-controlled study. *Phytomedicine*. 2007;14:87–95.
43. Krebs S, Omer TN, Omer B. Wormwood *(Artemisia absinthium)* suppresses tumour necrosis factor alpha and accelerates healing in patients with Crohn's disease—a controlled clinical trial. *Phytomedicine*. 2010;17:305–309.
44. Krebs S, Omer B, Omer TN, et al. Wormwood *(Artemisia absinthium)* for poorly responsive early-stage IgA nephropathy: a pilot uncontrolled trial. *Am J Kidney Dis*. 2010;56:1095–1099.
45. Basiri Z, Zeraati F, Esna-Ashari F, et al. Topical effects of *Artemisia absinthium* ointment and liniment in comparison with piroxicam gel in patients with knee joint osteoarthritis: a randomized double-blind controlled trial. *Iranian J Med Sci*. 2017;42(6):524–531.
46. Li Y, Zheng M, Zhai X, et al. Effect of *Gymnema sylvestre, Citrullus colocynthis* and *Artemisia absinthium* on blood glucose and lipid profile in diabetic human. *Acta Pol Pharm*. 2015;72:981–985.
47. Windholz M, Budavari S, Blumetti RF, et al. *The Merck Index*. Rahway, NJ: Merck & Co; 1983.

48. Duke JA. *Handbook of Medicinal Herbs*. 2nd ed. Boca Raton, FL: CRC Press; 2002.
49. Lachenmeier DW, Walch SG. The choice of thujone as drug for diabetes. *Nat Prod Res*. 2011;25(20):1890–1892.
50. Summary of data for chemical selection α-thujone 546-80-5. National Toxicology Program. https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/a_thujone.pdf. Accessed October 13, 2011.
51. Arnold WN. Vincent van Gogh and the thujone connection. *JAMA*. 1988;260:3042–3044.
52. Höld KM, Sirisoma NS, Ikeda T, et al. Alpha-thujone (the active component of absinthe): gamma-aminobutyric acid type A receptor modulation and metabolic detoxification. *Proc Natl Acad Sci U S A*. 2000;97:3826–3831.
53. Höld KM, Sirisoma NS, Casida JE. Detoxification of alpha- and beta-thujones (the active ingredients of absinthe): site specificity and species differences in cytochrome p450 oxidation in vitro and in vivo. *Chem Res Toxicol*. 2001;14:589–595.
54. Weisbord SD, Soule JB, Kimmel PL. Poison on line—acute renal failure caused by oil of wormwood purchased through the Internet. *N Engl J Med*. 1997;337:825–827.
55. Altunkaynak Y, Demir N, Ertem DH, et al. Neuropsychiatric symptoms due to *Artemisia absinthium* poisoning. *Turk J Neurol*. 2017;23:84–85.
56. Heron S, Yarnell E. Retrospective analysis of the safety of bitter herbs with an emphasis on *Artemisia absinthium L* (wormwood). *J Naturopathic Med*. 2000;9:32–39.
57. Bonkovsky HL, Cable EE, Cable JW, et al. Porphyrogenic properties of the terpenes camphor, pinene, and thujone. *Biochem Pharmacol*. 1992;43:2359–2368.

55

Artemisia annua (Sweet Wormwood)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

GENERAL DESCRIPTION

The genus Artemisia is named after the Greek goddess Artemis, who apparently used a plant in the genus and gave her name to it.

CHEMICAL COMPOSITION

Sweet wormwood contains several sesquiterpene trioxane lactones, most notably artemisinin (also known as qinghaosu) but also deoxyartemisinin, artemisinic acid, and arteannuin-B.[1] These compounds give the plant a distinctive bitter taste. A peroxide bridge in artemisinin is vital in the antimalarial effects of the compound.[2] Artemisinin is rapidly and readily reduced to dihydroartemisinin in the body. Dihydroartemisinin appears to be the active metabolite of artemisinin and all synthetic artemisinin derivatives developed to date.[3]

Sweet wormwood also contains several flavonoids.[1] In vitro research suggests these flavonoids potentiate the antimalarial activity of the sesquiterpene lactones.[4]

Three semisynthetic derivatives of artemisinin are widely available for use: artemether, artesunate, and arteether.[3] Artemether is a lipophilic β-epimer of dihydroartemisinin and is administered intramuscularly. Artesunate is a hydrophilic hemisuccinate compound administered orally or by injection. Arteether is an ethyl ether derivative of dihydroartemisinin and is lipophilic.

HISTORY AND FOLK USE

The herb, known to the ancient Chinese as qing hao, was recommended for treatment of fever as early as 341 AD, as written in the handbook for prescription for emergencies, *Zhou Hou Bei Ji Fang* by Ge Heng.[3] On the basis of extant written records, qing hao had been prescribed in traditional Chinese medicine for other problems for at least 500 years beforehand.

Qinghaosu, known as artemisinin in the West, was isolated and identified in qing hao in the 1970s in China.[5] It was thoroughly investigated and determined to have excellent antimalarial activity.

PHARMACOLOGY

Antimalarial Effects

Dihydroartemisinin preferentially accumulates in erythrocytes, and malaria-infected erythrocytes accumulate 300 times as much as uninfected erythrocytes.[6] Because active artemisinin compounds all appear to be converted to dihydroartemisinin in vitro and then act as mentioned previously, all these compounds ultimately end up in erythrocytes.

Artemisinin and related molecules appear to act in part by the generation of free radicals, which then damage the membranes of several parasitical organisms.[7] This leads to the rapid death of all stages of malaria parasites.[8] Because the presence of an oxidizing peroxide bridge appears essential to the activity of artemisinin compounds, the oxidative hypothesis is further supported. The importance of iron to this process is unclear. Some investigators suggest that iron chelators such as desferrioxamine increase the activity of artemisinin in vitro.[9]

Artemisinin compounds are synergistic with many other, but not all, antimalarial drugs. Low doses of mefloquine and artemisinin showed definite synergism in vitro against *Plasmodium falciparum,* whereas higher doses showed additive effects.[10] Mefloquine, chloroquine, primaquine, and tetracycline were all potentiated by artemisinin in mice with malaria; the effects of pyrimethamine, cycloguanil, and sulphonamide antibiotics were all decreased.[11]

Antiparasitic Effects

Besides killing *Plasmodium* spp., artemisinin compounds have shown activity against *Schistosoma* spp. and *Clonorchis sinensis.* Numerous animal studies show that artemether and artesunate can prevent and cure infections by *Schistosoma japonicum, Schistosoma mansoni,* and

Schistosoma haematobium.[12–15] It is not clear if artemisinin or dihydroartemisinin is effective. However, artemisinin and the various available semisynthetic variants were all effective at treating *Clonorchis* infections in rats, with no sign of toxicity.[16]

Antineoplastic Effects

Mounting evidence suggests that artemisinin compounds have potent anticancer activity. Artemisinin and quercetagetin 6,7,3′,4′-tetramethyl ether, a flavonoid found in sweet wormwood, both showed activity against several tumor types in vitro.[17] Dihydroartemisinin and artesunate have also been investigated and found to inhibit cancer cells significantly in vitro.[18,19] The presence of ferrous iron appears to be essential for this activity, and sufficient quantities of iron may be necessary to prevent cancer cells from becoming resistant to artemisinin compounds.[18,20]

A combination of ferrous sulfate and dihydroartemisinin proved effective at limiting the growth of implanted fibrosarcomas in rats.[21] The combination did not appear to cause adverse effects. Ferrous sulfate or dihydroartemisinin administered alone was not effective in this model.

CLINICAL APPLICATIONS

Malaria

A meta-analysis confirmed that artemisinin, dihydroartemisinin, artemether, artesunate, and arteether are potent, effective, and safe in patients with uncomplicated malaria compared with other antimalarial agents.[22] In cases of severe or cerebral malaria, these agents appeared to be at least as effective as quinine.[23] No artemisinin compound has been shown to work better than any other, although there is a relative dearth of studies examining this question. It should be noted that most trials have been conducted in Southeast Asia, an area with a high incidence of multidrug-resistant malaria. Profligate use of artemisinin and derivatives by itself (instead of coupled with other drugs and measures as should be) is associated with rising resistance to these agents (Fig. 55.1).[24] Tú Yōuyōu won the Nobel prize for medicine and physiology for her part in the discovery of artemisinin for malaria—the first Chinese woman to win any Nobel prize, and she did so without a postgraduate degree, without ever working outside of China, and without belonging to any scientific academies.

Rising artemisinin resistance, which occurs for a wide range of reasons including widespread sales of counterfeit drug, use of single artemisinin instead of combination therapy, and the expense of the drug, has led to a new approach to malaria. This approach uses whole sweet wormwood and not artemisinin, and as will be shown, has shown it is more effective.

Based on several prior small trials,[25,26] a large double-blind trial was conducted, involving 957 patients aged 5 years and older with malaria in the Democratic Republic of the Congo.[26a] Of these, 943 completed the trial. Participants were randomized to artesunate 4 mg/kg and amodiaquine 10 mg/kg (ASAQ) once daily for three days, an infusion of sweet wormwood providing at most 8.5 mg artemisinin per day, or an infusion of *Artemisia afra* (African wormwood), which had minimal artemisinin. Both infusions were made by adding 5 g of stem and leaf of the respective herbs to 1 L of boiled water and letting it infuse for 10 minutes. It was then strained and drunk in three equal portions throughout the day for 7 days. The dose was the same for children and adults. All patients therefore received some active treatment. They simultaneously received either a placebo tablet (in the herbal infusion groups) or a placebo infusion (made using just 0.2 g of herb, in those taking active ASAQ).

On every clinical measure that matters, sweet and African wormwood were significantly superior to artesunate-amodiaquine. Cure (defined as absence of parasitemia after 28 days) was seen in 89% of African and 96% of sweet wormwood patients, significantly superior compared to just 34% of those receiving ASAQ. Fever resolution occurred within 24 hours with either herb, compared to the significantly longer 48 hours for ASAQ. After 14 days, no patient taking sweet or African wormwood had detectable gametocytes in their blood, while 2% of the drug group did not have clearance. Adverse effects occurred in significantly fewer patients taking wormwood than those taking drugs (5% vs. 43% respectively).

This study calls into question the entire idea of approaching infectious diseases using single molecules. A significant shift of focus of research on malaria treatment and prevention should now go towards complex medicines as are found in herbs. It is particularly notable that the dramatic reduction in gametocytes could open a completely new approach to preventing malaria, as this is the form of the parasite that gets into mosquitos, completing the plasmodial life cycle.

Schistosomiasis

Several double-blind trials conducted in China, the Ivory Coast, and Egypt proved that artemether is effective in reducing the risk of developing schistosomiasis compared with placebo.[27–29] Similar to the work with malaria cited above, a large double-blind trial has assessed the efficacy of sweet and African wormwood in 800 patients with schistosomiasis in the Democratic Republic of the Congo.[29a] Of these, 780 completed the trial. Patients were randomized to either take praziquantel 60 mg/kg daily for 3 days or the same dose of the herbal infusions described in the malaria section. All subjects taking praziquantel also took low-dose herb infusions (0.2 g/L) as a placebo, or if they were taking the herbal infusions at full strength, a placebo tablet. The teas were again administered for 7 days.

All subjects in both herb groups had 100% elimination of eggs in their stools by day 14; this was significantly faster than by day 21 in the praziquantel group. Egg counts dropped by 91% in both herb groups, significantly better than the 67% reduction achieved by the drug. Melena and serum eosinophil levels declined equally well in all groups; hemoglobin levels rose significantly better in the praziquantel and African wormwood groups compared to those taking sweet wormwood. Vomiting, abdominal pain, and headache were common (16% -27% incidence) in the praziquantel group while none of the wormwood groups suffered any significant adverse effects. These herbs should now be considered the standard of care for schistosomiasis, as they are clearly superior in every way to praziquantel based on this very large, rigorous trial.

Cancer

An early case study in a 72-year-old Indian man found that artesunate intramuscularly (IM) 60 mg daily for 15 days followed by artesunate 50 mg once daily by mouth for 6 weeks reduced the size of a laryngeal squamous cell carcinoma by 70% and resolved symptoms.[30] Several other case studies appeared in 2002, including patients with lymphoma, lung, breast, and skin cancer, suggesting that artemisinin and/or artesunate resulted in dramatic improvement.[31] Two cases of metastatic uveal melanoma progressing under conventional chemotherapy had disease stabilization, and one was alive for over 4 years at the time of publication, when artesunate was added to their programs.[32] A case series of 15 patients with prostate cancer (5 postprostatectomy) found that 7 had improvements in their prostate-specific antigen (PSA) levels; all were still alive and without metastasis with an average 9-month follow-up.[33]

The only known double-blind, randomized trial involved 20 patients with resected colorectal cancer treated with oral artesunate

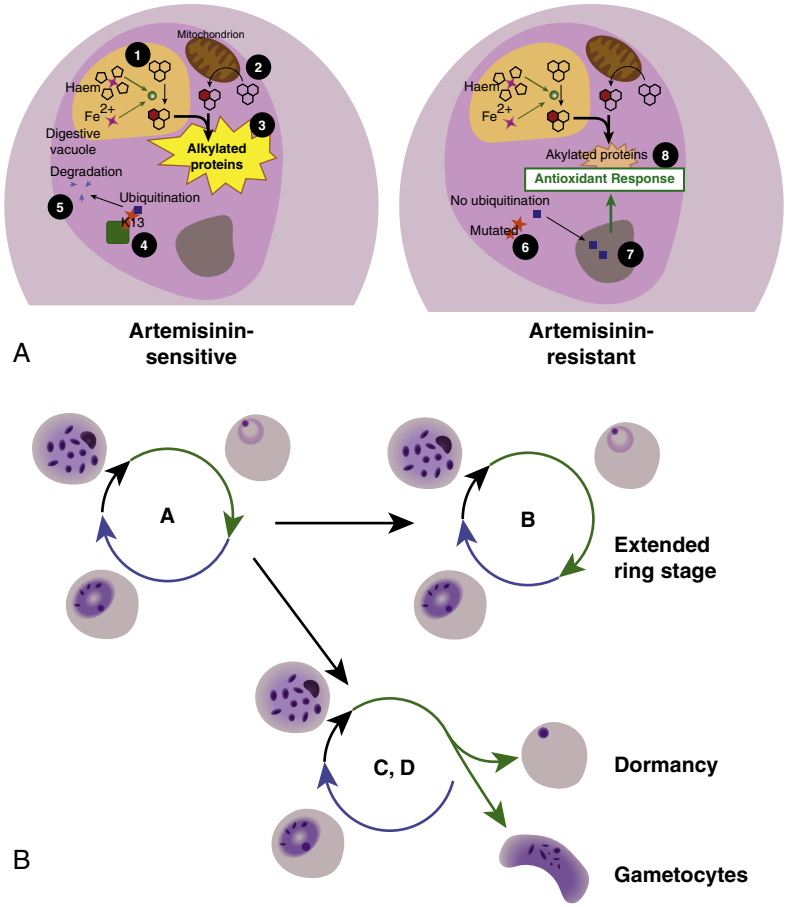


Fig. 55.1 Proposed mechanisms of artemisinin resistance. (A) Relevant biochemical pathways. In ring-stage parasites, artemisinin is primarily activated by heme produced in the process of hemoglobin digestion *(1)* although heme biosynthesis in mitochondria may also contribute *(2)*. Activated artemisinins alkylate nearby proteins in an indiscriminate manner, leading to cell death *(3)*. In artemisinin-sensitive parasites, a transcriptional factor with the potential to upregulate protein turnover and oxidative damage responses is bound via the K13 adaptor *(4)*, leading to its ubiquitination and proteolysis *(5)*. K13 mutation disrupts this binding *(6)*, allowing the factor to enter the nucleus *(7)*, with upregulation of a range of transcriptional responses that can mitigate the downstream consequences of artemisinins *(8)*. (B) Proposed phenotypes associated with artemisinin resistance. The overall length and proportion of time spent in each stage appear relatively fixed in a given strain *(A)*. By extending their ring stage *(B)*, parasites increase the period of reduced vulnerability to artemisinins. An alternative is to increase the proportion of parasites entering dormancy *(C)*, a natural phenomenon observed in all parasite strains that allows escape from relatively short-duration artemisinin exposures in patient treatments. Finally, increasing the proportion of parasites that differentiate into gametocytes *(D)* at a given timepoint could improve the chances of transmission before treatment is administered. (From Woodrow CJ, White NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. *FEMS Microbiol. Rev.* 2017;41[1]:34–48.)

200 mg daily or placebo for 14 days before surgery and found a significantly reduced risk of cancer recurrence in those treated with artesunate.[34] More randomized controlled trials are certainly warranted to determine the optimal dosing and efficacy of artemisinin and artesunate for cancer patients.

ARTHRITIS

In a controlled trial (blinding was not described), 159 patients with rheumatoid arthritis were randomized to take leflunomide and methotrexate alone or coupled with 30 g of sweet wormwood extract daily for 48 weeks.[35] All measures of symptomatic relief and objective measures of inflammation, including C-reactive protein and citrullinated protein antibody levels, were improved significantly more in the sweet wormwood group compared with controls. Corticosteroids were able to be used less in the sweet wormwood group compared with controls, and there were fewer adverse effects as well.

A supercritical carbon dioxide extract of sweet wormwood was tested in 42 patients with knee or hip osteoarthritis.[36] This double-blind, randomized trial compared 75 mg or 150 mg twice daily of the extract with placebo over 12 weeks. Only the low-dose extract group had significant improvements in symptoms, particularly pain, compared with baseline. Between-group comparisons were unfortunately not provided, raising concerns that neither dose of the extract may have been superior to placebo. In an open-label extension of this trial, 28 patients completed an additional 6 months of treatment, and it was found that the benefits seen in the double-blind portion were maintained, with no significant adverse effects.[37]

DOSAGE

The historical dosage of sweet wormwood tea is 5 g/1 L water in three to four divided doses, although doses of up to 30 g daily have also been used.[20,35] The dose of a 1:5 tincture of dried leaf is difficult to extrapolate because a higher ethanol concentration (60%–70%) could be used, which would greatly improve the ability of the solvent to draw artemisinin into the final product. A rough estimate would be 1 to 3 mL three times a day for a healthy adult. The usual dose of isolated artemisinin as a prescription drug for uncomplicated malaria is 500 to 1000 mg/day for 5 days.[20,38] Food does not appear to significantly affect the absorption of artemisinin.[39] Artemisinin and sweet wormwood should not be used for more than 5 to 7 days continuously because they stop being absorbed and stop working at that point.

TOXICITY

The only common adverse effects from the use of sweet wormwood are nausea, loss of appetite, and dizziness.[38] Vomiting is rare. Neurotoxic effects have been observed in experimental animals treated with artemisinin derivatives and in a small handful of humans.[40] These effects have not been observed during the use of whole sweet wormwood or its extracts. Sweet wormwood should be avoided in pregnancy and lactation until more data are available regarding its safety in these situations. Artemisinin derivatives have frequently been used safely in children, particularly when administered by suppository.[41] A single case of encephalopathy has been reported in one breast cancer patient being treated with artemisinin.[42]

DRUG INTERACTIONS

Artemisinin compounds generally show more rapid parasite clearance and fever reduction when combined with mefloquine than either agent given by itself, according to a meta-analysis of clinical trials.[18] Mefloquine's neurological toxicity does not appear to be altered by combination with artemisinin compounds, although the tendency to induce severe vomiting may be slightly lessened.

Artemisinin compounds can be combined safely with doxycycline and tetracycline, according to the results of two clinical trials, although at least one of these trials suggested the combination was less effective than artemether combined with mefloquine.[43,44] Another double-blind trial found that the combination of artesunate and tetracycline was equally effective and much safer than quinine and tetracycline.[45]

Artemisinin may interfere slightly with sulfadoxine-pyrimethamine. In one trial, artemisinin by itself led to insignificantly more rapid parasite clearance than artemisinin combined with sulfadoxine-pyrimethamine.[46] Fever relief was achieved equally rapidly, and adverse effects were absent in both groups. Further research is necessary to determine for certain if there is any negative effect from combining these two agents.

Grapefruit juice initially increases the bioavailability of oral artemether, although it does not stop the inevitable decline in bioavailability of this compound that occurs with repeated dosing over a few days.[47]

Single case studies suggest that artesunate may increase the risk of hepatotoxicity in cancer patients being treated with the drug temozolomide.[48]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Zheng GQ. Cytotoxic terpenoids and flavonoids from Artemisia annua. *Planta Med*. 1994;60:54–57.
2. China Cooperative Research Group. Chemical studies on qinghaosu (artemisinin). On qinghaosu and its derivatives as antimalarials. *J Tradit Chin Med*. 1982;2:3–8.
3. Hien TT, White NJ. Qinghaosu. *Lancet*. 1993;341:603–608.
4. Elford BC, Roberts MF, Phillipson JD, et al. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. *Trans R Soc Trop Med Hyg*. 1987;81:434–436.
5. Coordinating Group for Research on the Structure of Qinghaosu. A new type of sesquiterpene lactone-qinghaosu. *Kexue Tongbao*. 1977;22:142.
6. Gu HM, Warhurst DC, Peters W. Uptake of 3H-dihydroartemisinine by erythrocytes infected with Plasmodium falciparum in vitro. *Trans R Soc Trop Med Hyg*. 1984;78:265–270.
7. Krungkrai SR, Yuthavong Y. The antimalarial action on *Plasmodium falciparum* of qinghaosu and artesunate in combination with agents which modulate oxidant stress. *Trans R Soc Trop Med Hyg*. 1987;81:710–714.
8. Meshnick SR. Artemisinin antimalarials: mechanisms of action and resistance. *Med Trop (Mars)*. 1998;58:13–17.
9. Sibmooh N, Pipitaporn B, Wilairatana P, et al. Effect of artemisinin on lipid peroxidation and fluidity of the erythrocyte membrane in malaria. *Biol Pharm Bull*. 2000;23:1275–1280.
10. Bwijo B, Alin MH, Abbas N, et al. Efficacy of artemisinin and mefloquine combinations against *Plasmodium falciparum*. In vitro simulation of in vivo pharmacokinetics. *Trop Med Int Health*. 1997;2:461–467.
11. Chawira AN, Warhurst DC, Robinson BL, et al. The effect of combinations of qinghaosu (artemisinin) with standard antimalarial drugs in the suppressive treatment of malaria in mice. *Trans R Soc Trop Med Hyg*. 1987;81:554–558.
12. Le WJ, You JQ, Yang YQ, et al. Studies on the efficacy of artemether in experimental schistosomiasis. *Yao Xue Bao*. 1982;17:187–193. [Chinese].
13. Le WJ, You JQ, Mei JY. Chemotherapeutic effect of artesunate in experimental schistosomiasis. *Yao Xue Bao*. 1983;18:619–621. [Chinese].
14. Xiao S, Tanner M, N'Goran EK, et al. Recent investigations of artemether, a novel agent for the prevention of schistosomiasis japonica, mansoni and haematobia. *Acta Trop*. 2002;82:175–181.
15. Yang Y, Xiao S, Tanner M, et al. Histopathological changes in juvenile *Schistosoma haematobium* harboured in hamsters treated with artemether. *Acta Trop*. 2001;79:135–141.
16. Chen RX, Qu ZQ, Zeng MA, et al. Effect of quinhaosu and its derivatives on Clonorchis sinensis in rats. *Chin Pharm Bull*. 1983;18:410–411.
17. Zheng GQ. Cytotoxic terpenoids and flavonoids from *Artemisia annua*. *Planta Med*. 1994;60:54–57.
18. Singh NP, Lai H. Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. *Life Sci*. 2001;70:49–56.
19. Efferth T, Dunstan H, Sauerbrey A, et al. The anti-malarial artesunate is also active against cancer. *Int J Oncol*. 2001;18:767–773.
20. Sadava D, Phillips T, Lin C, et al. Transferrin overcomes drug resistance to artemisinin in human small-cell lung carcinoma cells. *Cancer Lett*. 2002;179:151–156.
21. Moore JC, Lai H, Li JR, et al. Oral administration of dihydro-artemisinin and ferrous sulfate retarded implanted fibrosarcoma growth in the rat. *Cancer Lett*. 1995;98:83–87.
22. McIntosh H.M.; Olliaro P. Artemisinin derivatives for treating uncomplicated malaria (Cochrane Review). In: *The Cochrane Library,* Issue 3. Update Software: Oxford, UK.
23. McIntosh HM, Olliaro P. Artemisinin derivatives for treating severe malaria (Cochrane Review). In: *The Cochrane Library,* Issue 3. Oxford, UK: Update Software.
24. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 2009;361:455–467.
25. Mueller MS, Karhagomba IB, Hirt HM, et al. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. *J Ethnopharmacol*. 2000;73:487–493.
26. Price RN, Nosten F, Luxemburger C, et al. Effects of artemisinin derivatives on malaria transmissibility. Randomized controlled trial. *Lancet*. 1996;347:1654–1658.

26a. Munyangi J, Cornet-Vernet L, Idumbo M, et al. *Artemisia annua* and *Artemisia afra* tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial. *Phytomedicine*. 2019;57:49–56.

27. Xiao SH, Booth M, Tanner M. The prophylactic effects of artemether against Schistosoma japonicum infections. *Parasitol Today*. 2000;16:122–126.
28. Utzinger J, N'Goran EK, N'Dri A, et al. Oral artemether for prevention of Schistosoma mansoni infection: randomised controlled trial. *Lancet*. 2000;355:1320–1325.
29. Elmorshedy H, Tanner M, Bergquist RN, et al. Prophylactic effect of artemether on human *Schistosomiasis mansoni* among Egyptian children: a randomized controlled trial. *Acta Trop*. 2016;158:52–58.

29a. Munyangi J, Cornet-Vernet L, Idumbo M, et al. Effect of *Artemisia annua* and *Artemisia afra* tea infusions on schistosomiasis in a large clinical trial. *Phytomedicine*. 2018;51:233–240.

30. Singh NP, Verma KB. Case report of a laryngeal squamous cell carcinoma treated with artesunate. *Arch Oncol*. 2002;10:279–280.
31. Rowen RJ. Artemisinin: from malaria to cancer treatment. *Townsend Lett Doc Patient*. 2002:86–88.
32. Berger TG, Dieckmann D, Efferth T, et al. Artesunate in the treatment of metastatic uveal melanoma—first experiences. *Oncol Rep*. 2005;14:1599–1603.
33. Yarnell E. Preliminary case series of artemisinin for prostate cancer in a naturopathic practice. *J Restor Med*. 2015;4:24–32.
34. Krishna S, Ganapathi S, Ster IC, et al. A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer. *EBioMedicine*. 2015;2:82–90.
35. Yang M, Guo MY, Luo Y, et al. Effect of *Artemisia annua* extract on treating active rheumatoid arthritis: a randomized controlled trial. *Chin J Integr Med*. 2017;23:496–503.
36. Stebbings S, Beattie E, McNamara D, Hunt S. A pilot randomized, placebo-controlled clinical trial to investigate the efficacy and safety of an extract of *Artemisia annua* administered over 12 weeks, for managing pain, stiffness, and functional limitation associated with osteoarthritis of the hip and knee. *Clin Rheumatol*. 2016;35:1829–1836.
37. Hunt S, Stebbings S, McNamara D. An open-label six-month extension study to investigate the safety and efficacy of an extract of *Artemisia annua* for managing pain, stiffness and functional limitation associated with osteoarthritis of the hip and knee. *N Z Med J*. 2016;129:97–102.
38. Balint GA. Artemisinin and its derivatives: an important new class of antimalarial agents. *Pharmacol Ther*. 2001;90:261–265.
39. Dien TK, de Vries PJ, Khanh NX, et al. Effect of food intake on pharmacokinetics of oral artemisinin in healthy Vietnamese subjects. *Antimicrob Agents Chemother*. 1997;41:1069–1072.
40. Miller LG, Panosian CB. Ataxia and slurred speech after artesunate treatment for falciparum malaria. *N Engl J Med*. 1997;336:1328.
41. Krishna S, Planche T, Agbenyega T, et al. Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanaian children with moderate malaria. *Antimicrob Agents Chemother*. 2001;45:509–516.
42. Panossian LA, Garga NI, Pelletier D. Toxic brainstem encephalopathy after artemisinin treatment for breast cancer. *Ann Neurol*. 2005;58:812–813.
43. Looareesuwan S, Viravan C, Vanijanonta S, et al. Randomized trial of mefloquine-doxycycline, and artesunate-doxycycline for treatment of acute uncomplicated falciparum malaria. *Am J Trop Med Hyg*. 1994;50:784–789.
44. Than M. *Unpublished data obtained from the investigator,* Defence Services General Hospital, Myanmar. Cited in McIntosh HM, Olliaro P. Artemisinin derivatives for treating uncomplicated malaria (Cochrane Review) In: The Cochrane Library, Issue 3. Update Software: Oxford, UK.
45. Duarte EC, Fontes CJ, Gyorkos TW, et al. Randomized controlled trial of artesunate plus tetracycline versus standard treatment (quinine plus tetracycline) for uncomplicated *Plasmodium falciparum* malaria in Brazil. *Am J Trop Med Hyg*. 1996;54:197–202.
46. Tran TH, Arnold K, Nguyen TH, et al. Single dose artemisinin-mefloquine treatment for acute uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg*. 1994;88:688–691.
47. van Agtmael MA, Gupta V, van der Graaf CA, et al. The effect of grapefruit juice on the time-dependent decline of artemether plasma levels in healthy subjects. *Clin Pharmacol Ther*. 1999;66:408–414.
48. Efferth T, Schöttler U, Krishna S, et al. Hepatotoxicity by combination treatment of temozolomide, artesunate and Chinese herbs in a glioblastoma multiforme patient: case report review of the literature. *Arch Toxicol*. 2017;91:1833–1846.

Bee Products—Pollen, Propolis, and Royal Jelly

Michael T. Murray, ND

OUTLINE

GENERAL DESCRIPTION

Historically, one of the most valued groups of natural medicines is that of bee pollen, propolis, and royal jelly.

Bee pollen comes from the male germ cell of flowering plants. As the honeybee travels from flower to flower, it fertilizes the female germ cell. Honeybee pollination enables the reproduction of more than 80% of the world's grains, fruits, vegetables, and legumes. The pollen is collected and brought to the hive, where the bees add enzymes and nectar to the pollen.

Propolis is the resinous substance collected by bees from the leaf buds and barks of trees, especially poplar and conifer trees. The bees use the propolis, along with beeswax, to construct the hive. Propolis has antimicrobial activities that help the hive block out viruses, bacteria, and other organisms.

Royal jelly is a thick, milky substance produced by worker bees to feed the queen bee. The worker bees mix honey and bee pollen with enzymes in the glands of their throats to produce royal jelly. Royal jelly is believed to be a useful nutritional supplement because of the queen bee's superior size, strength, stamina, and longevity compared with other bees.

CHEMICAL COMPOSITION

Bee pollen is often referred to as "nature's most perfect food" because it is a complete protein (typically containing 10%–35% total protein), meaning it contains all eight essential amino acids, and it also provides B vitamins, vitamin C, carotenes, minerals, DNA, RNA, numerous flavonoid molecules, and plant hormones.

Propolis and royal jelly have similar nutritional qualities to pollen but considerably higher levels of different biologically active compounds.[1,2] Royal jelly contains approximately 12% protein, 5% to 6% lipids, and 12% to 15% carbohydrates.

HISTORY AND FOLK USE

The use of bee products for medicinal purposes is as old as beekeeping itself. Chinese texts more than 2000 years old include many mentions of bee products. Hippocrates also wrote about them. Honey was so valued during Roman times that it was often used instead of gold to pay taxes.

Of the bee products, propolis was the most valued as a medicinal agent. Hippocrates prescribed propolis to help heal sores, and external and internal ulcers. Propolis-making bees were also depicted on vases from ancient Egypt, where the sign of the bee was often interwoven with the titles of the kings and used as the motif on ornaments presented as rewards for valor. The ancient Egyptians looked upon bees and their propolis as the source of eternal health and life. In the 17th century, propolis was a major ingredient of healing ointments in the European pharmacopoeia.

PHARMACOLOGY AND CLINICAL APPLICATIONS

The health benefits of bee products are much heralded but insufficiently researched. Some overlap exists in the clinical uses of pollen, propolis, and royal jelly. The following discussion notes the principal uses for each of these bee products, as shown in Box .62.1. This list is likely to grow with continued research.

Bee Pollen

Little research has been done on bee pollen, probably because the financial rewards to justify such an investment are lacking. The research that does exist is limited but impressive. For example, studies in animals show that pollen can promote growth and development, protect against free radical and oxidative damage, and protect against the effects of harmful radiation and toxic exposure to chemical solvents.[3–5] A pollen extract has also been shown to produce significant improvement in menopausal symptoms (headache, urinary incontinence, vaginal dryness, decreased vitality) in double-blind studies.[6] The improvements were achieved although the pollen extract produces no estrogenic effect, an important consideration for women who cannot take estrogens of any kind.[7]

BOX 56.1 Clinical Applications for Bee Products

Bee Pollen
- Allergies
- Antioxidant support
- Energy enhancement
- Menopausal symptoms
- Support for chemotherapy and radiation therapy

Propolis
- Common cold
- Gastrointestinal infections
- Immune enhancement
- Topical anti-inflammatory
- Upper respiratory tract infections
- Vaginitis

Royal Jelly
- Elevated cholesterol levels
- Energy enhancement

Propolis

The primary use of propolis has been in immune system enhancement and infections. Propolis has inherent antimicrobial activity that protects the hive block from viruses, bacteria, and other organisms. Propolis has shown considerable antimicrobial activity in in vitro studies.[8–10] Propolis also stimulates the immune system, according to preliminary human studies.[11,12] In vitro and animal studies have also shown that propolis exerts some antioxidant, liver-protecting, anti-inflammatory, and anticancer properties.[13–17]

A key use of propolis is protecting against and shortening the duration of the common cold. A preliminary human study reported that propolis extract (PE) reduced upper respiratory infections in children.[11] In a double-blind study of 50 patients with the common cold, the group taking PE became symptom-free far more quickly than the placebo group.[18]

Another possible application of propolis is in the treatment of inflammatory bowel diseases like Crohn disease and ulcerative colitis. An anecdotal article described an interesting case of ulcerative colitis that responded to propolis therapy.[19] The author suggested that the antimicrobial and anti-inflammatory properties of propolis might be of value in the treatment of inflammatory bowel diseases.

The antimicrobial properties of propolis may also help protect against parasitical infections in the gastrointestinal tract. One preliminary study of children and adults with giardiasis showed a 52% rate of successful parasite elimination in children and a 60% rate in adults in those given PE (amount not stated).[20] However, these results are not as impressive as those achieved with conventional drugs used against giardiasis, so propolis should typically not be used alone for this condition.

Propolis may also have an effect on vaginal infections. In one study, 97 vaginal yeast strains were evaluated for susceptibility to nystatin and PEs. All the yeasts tested were inhibited by low concentrations of PE (maximum of 393.19 mcg/mL of the total flavonoid content), including an isolate resistant to nystatin, regardless of the clinical conditions of the women and the species of yeast isolated.[21]

Propolis has exerted many positive effects on the oral cavity in clinical studies. This includes its use as a mouthwash, showing benefits in reducing dental plaque[22] and gingivitis,[23] healing oral wounds,[24] improving periodontal pocket depths in type 2 diabetics,[25] and reducing the severity and promoting the resolution of oral mucositis associated with chemotherapy.[26] In the study on oral mucositis, on day 7 of the study, there were significant differences in the incidence rates of oral wounds, mucositis, and oral cavity erythema between the propolis group and the placebo group, with 65% of the propolis group having complete healing.

Propolis has also been shown to exert some benefit in type 2 diabetes in terms of improvement in blood sugar control, periodontal health, antioxidant activity, and healing of foot ulcers (topical use). However, the degree of absolute improvement in any of these areas is not sufficient on its own. For example, in one double-blind study, a dosage of 900 mg per day of propolis showed statistically significant advantage over the placebo, but after 12 weeks, the fasting blood sugar only dropped from an average of 152 to 134 mg/dL, and the glycosylated hemoglobin A1C only dropped from 8.2% to 7.4%.[27] Neither of these improvements reached targeted goals in type 2 diabetes. Similar results were seen in another study using a dosage of 400 mg per day of a propolis product.[25] Nonetheless, propolis appears a useful adjunctive therapy for patients with problems with blood sugar control.

Finally, one of the key components of propolis, caffeic acid phenethyl ester, has been shown to exert selective estrogen-receptor modulation.[28] This action, along with inhibition of receptor activator nuclear factor-κB ligand (RANKL)-activated signaling, implied that it might be an effective agent against osteoporosis. RANKL is essential for osteoclast differentiation, activation, and survival. Low concentrations of caffeic acid phenethyl ester (<1 mcM) dose-dependently inhibited RANKL-induced osteoclastogenesis in cell and bone marrow macrophage cultures, as well as decreasing the capacity of human osteoclasts to resorb bone.[29]

Royal Jelly

Some research on royal jelly found a cholesterol-lowering effect. Specifically, 11 human studies have been published, 8 of which were double blind.[30,31] Of the double-blind studies, only three used an oral preparation. An injectable form was used in the other studies. The results of a detailed analysis of the double-blind studies indicated that with oral preparations, despite shortcomings in the design of the studies and lack of standardization with commercial preparations used, royal jelly decreased total cholesterol levels by about 14% in patients with moderate to severe elevations in blood cholesterol levels (initial values ranging from 210–325 mg/dL). Better results may be achieved when using higher-quality royal jelly products.

DOSAGE

- Bee pollen: usually 1 to 3 tablespoons a day
- Propolis: 100 to 500 mg three times a day
- Royal jelly: 50 to 250 mg of royal jelly one to two times a day

TOXICITY

Allergic reactions are the most common adverse reactions. If there is a known allergy to conifer and poplar trees, the use of bee products should be avoided. Allergic reactions can range from mild (e.g., mild gastrointestinal upset) to severe (e.g., asthma, anaphylaxis [shock], intestinal bleeding, and even death in people who are extremely allergic to bee products).[32]

DRUG INTERACTIONS

No drug interactions have been reported.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem Toxicol*. 1998;36:347–363.
2. Hove SR, Dimick PS, Benton AW. Composition of freshly harvested and commercial royal jelly. *J Apic Res*. 1985;24:52–61.
3. Qian B, Zang X, Liu X. Effects of bee pollen on lipid peroxides and immune response in aging and malnourished mice. *Zhongguo Zhong Yao Za Zhi*. 1990;15:301–303. 319: [Chinese].
4. Xie Y, Wan B, Li W. Effect of bee pollen on maternal nutrition and fetal growth. *Hua Xi Yi Ke Da Xue Bao*. 1994;25:434–437. [Chinese].
5. Ceglecka M, Wojcicki J, Gonet B, et al. Effect of pollen extracts on prolonged poisoning of rats with organic solvents. *Phytother Res*. 1991;5:245–249.
6. Szanto E, Gruber D, Sator M, et al. Placebo-controlled study of Melbrosia in treatment of climacteric symptoms. *Wien Med Wochenschr*. 1994;144:130–133. [German].
7. Einer-Jensen N, Zhao J, Andersen KP, et al. Cimicifuga and Melbrosia lack oestrogenic effects in mice and rats. *Maturitas*. 1996;25:149–153.
8. Tosi B, Donini A, Romagnoli C, et al. Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. *Phytother Res*. 1996;10:335–336.
9. Dobrowolski JW, Vohora SB, Sharma K, et al. Antibacterial, antifungal, antiamebic, antiinflammatory and antipyretic studies on propolis bee products. *J Ethnopharmacol*. 1991;35:77–82.
10. Tichy J, Novak J. Detection of antimicrobials in bee products with activity against viridans streptococci. *J Altern Complement Med*. 2000;6:383–389.
11. Bratter C, Tregel M, Liebenthal C, et al. Prophylactic effectiveness of propolis for immunostimulation: a clinical pilot study. *Forsch Komplementarmed*. 1999;6:256–260. [German].
12. Crisan I, Zaharia CN, Popovici F, et al. Natural propolis extract NIVCRISOL in the treatment of acute and chronic rhinopharyngitis in children. *Rom J Virol*. 1995;46:115–133.
13. Pascual C, Gonzalez R, Torricella RG. Scavenging action of propolis extract against oxygen radicals. *J Ethnopharmacol*. 1994;41:9–13.
14. Lin SC, Lin YH, Chen CF, et al. The hepatoprotective and therapeutic effects of propolis ethanol extract on chronic alcohol-induced liver injuries. *Am J Chin Med*. 1997;25:325–332.
15. Khayyal MT, El-Ghazaly MA, El-Khatib AS. Mechanisms involved in the antiinflammatory effect of propolis extract. *Drugs Exp Clin Res*. 1993;19:197–203.
16. Mirzoeva OK, Calder PC. The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostaglandins Leukot Essent Fatty Acids*. 1996;55:441–449.
17. Choi YH, Lee WY, Nam SY, et al. Apoptosis induced by propolis in human hepatocellular carcinoma cell line. *Int J Mol Med*. 1999;4:29–32.
18. Szmeja Z, Kulczynski B, Sosnowski Z, et al. Therapeutic value of flavonoids in rhinovirus infections. *Otolaryngol Pol*. 1989;43:180–184. [Polish].
19. Golan R. *Propolis. Townsend Letter for Doctors*. 2001;215:66–68.
20. Miyares C, Hollands I, Castaneda C, et al. Clinical trial with a preparation based on propolis "propolisina" in human giardiasis. *Acta Gastroenterol Latinoam*. 1988;18:195–201. [Spanish].
21. Dalben-Dota KF, Faria MG, Bruschi ML, et al. Antifungal activity of propolis extract against yeasts isolated from vaginal exudates. *J Altern Complement Med*. 2010;16:285–290.
22. Santiago KB, Piana GM, Conti BJ, et al. Microbiological control and antibacterial action of a propolis-containing mouthwash and control of dental plaque in humans. *Nat Prod Res*. 2018 Jun;32(12):1441–1445.
23. Bretz WA, Paulino N, Nör JE, Moreira A. The effectiveness of propolis on gingivitis: a randomized controlled trial. *J Altern Complement Med*. 2014;20(12):943–948.
24. Dodwad V, Kukreja BJ. Propolis mouthwash: a new beginning. *J Indian Soc Periodontol*. 2011;15:121–125.
25. El-Sharkawy HM, Anees MM, Van Dyke TE. Propolis improves periodontal status and glycemic control in patients with type 2 diabetes mellitus and chronic periodontitis: a randomized clinical trial. *J Periodontol*. 2016;87(12):1418–1426.
26. AkhavanKarbassi MH, Yazdi MF, Ahadian H, SadrAbad MJ. Randomized double-blind placebo-controlled trial of propolis for oral mucositis in patients receiving chemotherapy for head and neck cancer. *Asian Pac J Cancer Prev*. 2016;17(7):3611–3614.
27. Samadi N, Mozaffari-Khosravi H, Rahmanian M, Askarishahi M. Effects of bee propolis supplementation on glycemic control, lipid profile and insulin resistance indices in patients with type 2 diabetes: a randomized, double-blind clinical trial. *J Integr Med*. 2017;15(2):124–134.
28. Jung BI, Kim MS, Kim HA, et al. Caffeic acid phenethyl ester, a component of beehive propolis, is a novel selective estrogen receptor modulator. *Phytother Res*. 2010;24(2):295–300.
29. Ang ES, Pavlos NJ, Chai LY, et al. Caffeic acid phenethyl ester, an active component of honeybee propolis attenuates osteoclastogenesis and bone resorption via the suppression of RANKL-induced NF-kappaB and NFAT activity. *J Cell Physiol*. 2009;221:642–649.
30. Vittek J. Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia*. 1995;51:927–935.
31. Guo H, Saiga A, Sato M, et al. Royal jelly supplementation improves lipoprotein metabolism in humans. *J Nutr Sci Vitaminol (Tokyo)*. 2007;53:345–348.
32. Greenberger PA, Flais MJ. Bee pollen-induced anaphylactic reaction in an unknowingly sensitized subject. *Ann Allergy Asthma Immunol*. 2001;86:239–242.

Beta-Carotene and Other Carotenoids

Michael T. Murray, ND, and *Bob Capelli**

OUTLINE

INTRODUCTION

The carotenoids represent the most widespread group of naturally occurring pigments in nature. They are a highly colored (red and yellow) group of fat-soluble compounds, composed of hydrocarbons (carotenes) and their oxygenated derivatives (oxycarotenoids or xanthophylls). The basic carotenoid structure consists of eight isoprenoid units with a series of conjugated double bonds. All photosynthetic organisms, whether bacteria or plants, contain carotenoid pigments. These compounds not only function as auxiliary pigments in photosynthesis but also play a crucial role in protecting the organism or plant against photosensitization by its own chlorophyll.

More than 600 carotenoids have been characterized, but only about 30 to 50 are believed to have vitamin A activity, and some 20 are found in human plasma and tissues. The biological activity of a carotenoid has historically been considered synonymous with its corresponding vitamin A activity. Beta-carotene has been termed the most active of the carotenoids because of its higher provitamin A activity (Fig. 57.1). However, recent research suggests that this function of carotenoids has been overemphasized because they have been found to exhibit many other important physiological activities. For a carotenoid to have vitamin A activity, it must have an unaltered β-ionone ring with an attached polyene side chain containing 11 carbon atoms. In contrast, apocarotenoids are compounds that have been shortened by the removal of at least one end of the molecule beyond a designated location (e.g., β-Apo-8′-carotenal has been cleaved at the 8′ carbon). Apocarotenes and xanthophylls have reduced or no vitamin A activity, but many have been shown to have significant antioxidant activity, physiological benefits, and the ability to produce meaningful clinical effects.[1–3] Also, approximately 25% of Caucasians do not effectively convert β-carotene to vitamin A.

*Previous edition contributor

DIETARY SOURCES

Among the richest sources of carotenes are green leafy vegetables. The carotenoids in green plants are found in the chloroplasts with chlorophyll, usually in complexes with a protein or lipid. Beta-carotene is the predominant form in most green leaves. In general, the greater the intensity of the green color, the greater the concentration of β-carotene. Orange-colored fruits and vegetables (e.g., carrots, apricots, mangoes, yams, squash) typically have higher concentrations of provitamin A carotenoids, the provitamin A content again paralleling the intensity of the color. Yellow vegetables have higher concentrations of xanthophylls and hence lower provitamin A activity. In orange and yellow fruits and vegetables, β-carotene concentrations are high, but other provitamin A carotenoids typically predominate. Red and purple vegetables and fruits, such as tomatoes, red cabbage, berries, and plums, contain large portions of non–vitamin A–active pigments, including flavonoids. Legumes, grains, and seeds are also significant sources of carotenoids. Carotenoids are also found

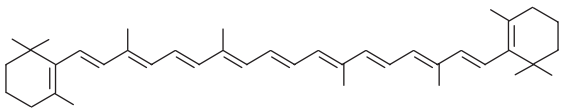


Fig. 57.1 Beta-carotene.

TABLE 57.1 Provitamin A Carotenoids and Food Sources

Carotenoid	Vitamin A Activity (%)	Food Sources
Beta-carotene	100	Green plants, carrots, sweet potatoes, squash, spinach, apricots, green peppers
Alpha-carotene	50–54	Green plants, carrots, squash, corn, watermelons, green peppers, potatoes, apples, peaches
Gamma-carotene	42–50	Carrots, sweet potatoes, corn, tomatoes, watermelons, apricots
Beta-zeacarotene	20–40	Corn, tomatoes, yeast, cherries
Cryptoxanthin	50–60	Corn, green peppers, persimmons, papayas, lemons, oranges prunes, apples, apricots, paprika, poultry
Beta-apo-8′-carotenal	72	Citrus fruit, green plants
Beta-apo-12′-carotenal	120	Alfalfa meal

TABLE 57.2 Non–Provitamin A Carotenoids and Food Sources

Carotene	Vitamin A Activity (%)	Food Sources
Lycopene	0	Tomatoes, carrots, green peppers, apricots, pink grapefruit
Zeaxanthin	0	Spinach, paprika, corn, fruits
Lutein	0	Green plants, corn, potatoes, spinach, carrots, tomatoes, fruits
Canthaxanthin	0	Mushrooms, trout, crustaceans
Crocetin	0	Saffron
Capsanthin	0	Red peppers, paprika
Astaxanthin	0	Salmon, trout, lobster, shrimp, krill

in various animal foods such as salmon and other fish, egg yolks, shellfish, milk, and poultry. Carotenoids are also frequently added to foods as colorants. Table 57.1 lists carotenoids with provitamin A activity found in common food sources, whereas Table 57.2 lists some important carotenoids that have no vitamin A activity. The structures of two of these, lycopene and zeaxanthin, are illustrated in Figs. 57.2 and 57.3.

According to a detailed analysis of the levels of carotenoids in 120 fruits and vegetables, lycopene is found in few.[4] Table 57.3 lists the foods that contained lycopene. These values indicate that lycopene levels are retained in food processing.

Astaxanthin is a vibrantly deep red pigment found predominantly in marine life. When microalgae rich in astaxanthin are consumed by salmon, lobster, shrimp, krill, and other sea life, the intense red pigmentation results in these animals having red or pink flesh or outer shells. Salmon is the richest source, with different species having different levels. Here are the levels per 6-ounce serving:

Wild Sockeye salmon 6.4 mg
Wild Coho salmon 3.6 mg
Farmed Atlantic salmon 1.6 mg
Pink salmon 1.2 mg

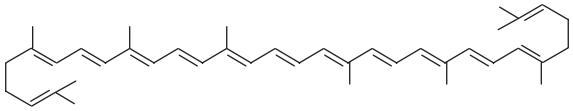


Fig. 57.2 Lycopene.

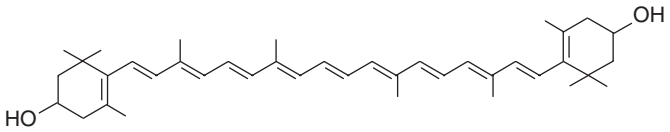


Fig. 57.3 Zeaxanthin.

TABLE 57.3 Lycopene Content of Common Foods

Food	Lycopene (mg/100 g)
Apricot, canned	0.06
Apricot, dried	0.8
Grapefruit (pink and raw)	3.4
Guava juice	3.3
Tomato, raw	3.1
Tomato juice, canned	8.6
Tomato paste, canned	6.5
Tomato sauce, canned	6.3
Watermelon, raw	4.1

METABOLISM

Absorption

Various factors are known to influence the absorption efficacy of vitamin A and carotenoids. Although retinol does not require bile acids to assist absorption, carotenoids do. Other factors that affect vitamin A and carotenoid absorption include the following:

- The presence of fat, protein, and antioxidants in the food
- The presence of bile and a normal complement of pancreatic enzymes in the intestinal lumen
- The integrity of the mucosal cells

The absorption efficiency of dietary vitamin A is usually quite high (80%–90%), with only a slight reduction in efficiency at high doses. In contrast, β-carotene's absorption efficiency is much lower (40%–60%), and it decreases rapidly with increasing dosage. Carotene supplements are better absorbed than the carotenes from foods.[5] Supplying only β-carotene, whether natural or synthetic, may exert a detrimental effect on the absorption of other carotenes. For example, β-carotene supplementation has been shown to inhibit the absorption of lutein.[6] Studies in humans have shown that with β-carotene supplementation, the absorption of lutein can drop by more than 50%.[7] Interestingly, the absorption of lutein from vegetables is five times greater than that of β-carotene.[8]

Transformation in the Intestinal Mucosa

As stated earlier, of the more than 600 carotenoids that have been reasonably well characterized, only about 30 to 50 are believed to have provitamin A activity. However, carotenoids provide the majority of dietary vitamin A for those who have the genetics for conversion. Provitamin A carotene conversion to vitamin A depends on diverse factors:

- Genetics
- Protein status
- Thyroid hormones
- Zinc
- Vitamin C

TABLE 57.4 Distribution of Carotenoids in Some Human Tissues (milligrams per kilogram)

Tissue	Carotenoids	Beta-Carotene
Adrenal	20.1 ± 11.9	10.8 ± 5.5
Liver	8.3 ± 21.3	—
Testis	5 ± 7.7	4.7 ± 2
Fat	3.9 ± 6	1.3 ± 1.1
Pancreas	2.3 ± 1.2	1.1 ± 1
Spleen	1.6 ± 2.2	1.2 ± 0.5
Lung	0.6 ± 1.0	—
Thyroid	0.6 ± 0.4	—

The conversion diminishes as carotene intake increases and when serum retinol levels are adequate. Beta-carotene and other provitamin A carotenes were originally believed to be cleaved by carotene dioxygenase at the 15,15' double bond, which would yield two molecules of all-trans retinal. However, it has been established that the dioxygenase enzyme nonspecifically attacks any one of the double bonds of the β-carotene, resulting in the formation of a corresponding apo-β-carotenal or retinal.[9] The apocarotenal formed can either be degraded to retinal or absorbed. The retinal formed is then converted to retinol by retinaldehyde reductase.

Uncleaved provitamin A carotenoids, apocarotenoids, and non–provitamin A carotenoids, like retinol, are transported in the chylomicra.

Genetics of Conversion of Carotenoids to Vitamin A

Evolving research is showing that many humans are not very effective converting beta-carotene into vitamin A. The enzyme 15,15′-monooxygenase (BCMO1) responsible for converting beta-carotene to vitamin A has six common single-nucleotide polymorphisms (SNPs) that result in the production of a slow to almost completely ineffective BCMO1.[10–12]

Transport, Storage, and Excretion

No specific carrier protein exists in the plasma for carotenoids. These compounds are typically transported in human plasma in association with the plasma lipoproteins, particularly by low-density lipoprotein (LDL). As a consequence, patients with high serum cholesterol or LDL levels tend to have high serum carotene levels. The concentrations found in the plasma usually reflect the dietary concentration, with β-carotene typically comprising only 20% to 25% of the total serum carotene level.[13]

Lycopene is the most predominant carotenoid in human plasma, accounting for more than 50% of the carotenoids in human serum. Owing to its lipophilic nature, lycopene is found to concentrate in the LDL and very-low-density lipoprotein fractions and not in the high-density lipoprotein (HDL) fractions of the serum cholesterol. Interestingly, although trans-lycopene constitutes the predominant isomer in food sources, in human plasma, 50% of the total lycopene has been found as cis isomers. Whether this is due to in vivo isomerization or preferential absorption of cis-lycopene is still unclear. Little is known about in vivo metabolism of lycopene.

Carotenes may be stored in adipose tissue, the liver, other organs (the adrenals, testes, and ovaries have the highest concentrations), and the skin (Table 57.4). Deposition in the skin results in carotenodermia. This is a benign (and probably beneficial) state. Carotenodermia not directly attributable to dietary intake or supplementation, however, may indicate a deficiency in a necessary conversion factor (e.g., zinc, thyroid hormone, vitamin C, or protein).

Astaxanthin behaves differently than other carotenoids due to its unique structural and chemical properties.[14] Astaxanthin possesses six qualitative features that are important to distinguish:

- It can span the entire depth of the cell membrane to protect both the external and internal cell membranes.
- It crosses the blood–brain and blood–retinal barriers to provide its protective properties to the eyes and brain.
- It is more easily incorporated into many cells, including skin, muscle, and nerves.
- It does not become a prooxidant and increase free radical proliferation.

PHYSIOLOGICAL ROLES AND PHARMACOLOGICAL ACTIVITY

Antioxidant Activity

In general, carotenes exert significant antioxidant activity, whereas the antioxidant activity of vitamin A is relatively minor.[1] However, its antioxidant actions are specific in that they are involved primarily in scavenging singlet molecular oxygen and, to a much lesser extent, peroxyl radicals. The efficacy of the various carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule, determining their lowest triplet energy level. The most efficient singlet oxygen-quenching carotene is the open-ring carotenoid lycopene,[15] whereas even more potent is the xanthophyll astaxanthin.[16]

The antioxidant activity of carotenes is thought to be responsible for their anticancer effects. Because aging is associated with free radical damage, a hypothesis developed that carotenes may protect against aging as well. Evidence seems to support this hypothesis. It appears that tissue carotenoid content is the most significant factor in determining the maximal life-span potential (MLSP) of mammalian species ($r = 0.835$ for 12 mammalian species, and for primates alone, $r = 0.939$).[17] For example, the human MLSP of approximately 90 years correlates with a serum carotene level of 50 to 300 mg/dL, whereas other primates, such as the rhesus monkey, have an MLSP of approximately 34 years, correlating with a serum carotene level of 6 to 12 mg/dL.

Although β-carotene has received most of the attention, many carotenoids that have either low or no vitamin A activity exert much greater protection compared with β-carotene. For example, β-carotene generates vitamin A much more efficiently than α-carotene, but α-carotene is approximately 38% stronger as an antioxidant and 10 times more effective in suppressing liver, skin, and lung cancer in animals compared with β-carotene. Even more powerful are lycopene, lutein, and astaxanthin. Studies have shown astaxanthin to exhibit the highest overall antioxidant activity.[16] In singlet oxygen quenching, astaxanthin is 11 times stronger than beta-carotene. In total free radical elimination, natural astaxanthin is:

- 14 times stronger than vitamin E
- 18 times stronger than proanthocyanins
- 21 times stronger than synthetic astaxanthin
- 54 times stronger than beta-carotene

The antioxidant effects of carotenoids are believed to be a key mechanism of action for their role as a protective factor against in aging-related, chronic degenerative diseases such as atherosclerosis, heart disease, strokes, cancer, macular degeneration, and neurodegenerative diseases. Numerous epidemiological studies have shown the association with an increased intake or higher blood levels of various carotenoids and lower incidence of these diseases and aging-related disorders.

Anti-inflammatory Activity

In addition to their antioxidant effects, many carotenoids also act directly and indirectly on many aspects of inflammation that include cellular signaling cascades, such as nuclear factor κB (NF-κB), mitogen-activated protein kinase (MAPK), and nuclear factor erythroid 2–related factor 2 (Nrf2).[18] In particular, astaxanthin has shown significant anti-inflammatory effects.[19] These effects were quite evident in a double-blind, placebo-controlled study in people with rheumatoid arthritis. Subjects took either astaxanthin (12 mg per day) or placebo for 8 weeks. Results showed a steady trend toward improvement in both pain levels and daily satisfaction from the beginning of the study to a midway point after 4 weeks, and then increasing improvement during the last 4 weeks of the study. By the end of 8 weeks, the pain scores had dropped by 35% in the group taking astaxanthin, and the satisfaction scores improved by 40%.[20] These effects were also studied in soccer players.[21] In a double-blind study, 40 male soccer players were randomly assigned to astaxanthin (4 mg daily) and placebo groups. After 90 days, results showed a number of benefits with astaxanthin, including a rise of salivary secretory IgA levels, a decrease in prooxidant-antioxidant balance, a decrease in muscle enzymes levels, and a significant blunting of the systemic inflammatory response as noted by C-reactive protein.

Immune System

Carotenoids have demonstrated significant effects in enhancing immune function.[22] Some of these effects are probably related to an ability to prevent stress-induced thymic involution, as well as promote thymus growth and function and increase interferon's stimulatory action on the immune system.[23] Interferon is a powerful immune-enhancing compound that plays a central role in protection against viral infections.

Reproduction

Beta-carotene reportedly has a specific effect in fertility distinct from its role as a precursor to vitamin A. In bovine nutritional studies, cows fed β-carotene–deficient diets exhibited delayed ovulation and an increase in the number of follicular and luteal cysts.[24,25] The corpus luteum has the highest concentration of β-carotene of any organ measured.[26] The carotene cleavage activity changes with the ovulation cycle, with the highest activity occurring during the midovulation stage. It has been speculated that a proper ratio of carotene to retinol must be maintained to ensure proper corpus luteum function.

Because the corpus luteum produces progesterone, inadequate corpus luteum function could have significant deleterious effects. Inadequate corpus luteum secretory function is one of the characteristic features of infertile or irregular menstrual cycles, or both.[27] Furthermore, reduced corpus luteum function and an increased estrogen-to-progesterone ratio has been implicated in various clinical conditions, including ovarian cysts, premenstrual tension syndrome, fibrocystic breast disease, and breast cancer.[28] Because supplemental β-carotene given to cows significantly improves corpus luteum function in cows and reduces the incidence of ovarian cysts (42% in control group vs. 3% in the β-carotene group), it may have similar effects in humans.

Astaxanthin has also shown benefit in improving reproductive function in animals. Human research has focused exclusively on male fertility and its effects on sperm to date. A double-blind, placebo-controlled, randomized study was done in 30 couples who had been unsuccessfully trying to conceive for more than 12 months. In each couple, the men were diagnosed as being infertile according to World Health Organization (WHO) guidelines, whereas the women showed no signs of infertility. The men in the treatment group were administered 16 mg per day of natural astaxanthin along with other antioxidants, and the men in the control group received placebos. At the end of the study, significant differences between the two groups included the following:

- Sperm linear velocity increased in the astaxanthin group.
- Reactive oxygen species decreased in the astaxanthin group.
- Of the astaxanthin-treated couples, 54.5% got pregnant, versus 10.5% of the placebo couples.

The researchers commented that the results in this trial were better than those in similar trials in which other antioxidants were used. "Functional improvement may be related to the reduction of reactive oxygen species resulting in enhancement of linear velocity and reduction of DNA damage."[29]

Photoprotection

Photo-oxidative processes play a role in the pathology of light-exposed tissues, including the eyes and skin. Age-related macular degeneration affects the macula lutea of the retina, the area of maximal visual acuity. It is a major cause of irreversible blindness among the elderly in the United States. Macular pigments protect against the photo-oxidative processes, which may be related to the antioxidant activities of the macular carotenoids or their light-filtering effects. Lutein and zeaxanthin are responsible for the coloration of the macula lutea. It is important to point out that neither lycopene, α-carotene, or β-carotene is found in this tissue. Lutein supplementation has proven particularly beneficial in offering protection against macular degeneration, as well as improving visual acuity in the early stages of the disease.[30]

In regard to protecting the skin against oxidative damage, although clinical applications have focused on β-carotene (discussed later in the chapter), there is evidence indicating that lycopene and astaxanthin may be better suited for this application.[31,32] For example, in a double-blind trial, lycopene completely inhibited UVA1- and UVA/B-induced upregulation of genes in the skin that are indicators of oxidative stress, photodermatoses, and skin aging.[31]

COMMERCIAL FORMS

Six primary sources of carotenes are on the market:

- Synthetic all-trans β-carotene
- Beta- and α-carotene from the algae *Dunaliella*
- Mixed carotenes from palm oil
- Lutein
- Lycopene
- Astaxanthin

Of the three sources of β-carotene, mixed carotenes from palm oil carotenes seem to be the best form (Table 57.5). Palm oil carotenes appear to give much better antioxidant protection compared with either synthetic or algal derived beta-carotene. The carotene complex of palm oil closely mirrors the pattern in high-carotene foods. In particular, whereas the synthetic version only provides the trans configuration of β-carotene, natural carotene sources like palm oil provide β-carotene in both a trans and cis configuration:

- 60% β-carotene (both trans and cis isomers)
- 34% α-carotene
- 3% γ-carotene
- 3% lycopene

Palm oil carotenes are absorbed about 4 to 10 times better than synthetic all-trans β-carotenes.[32–35] Carotenes from *Dunaliella* have also been shown to be well absorbed.[36] The widespread health concerns over the use of "tropical oils" like palm and coconut do not apply to carotene products extracted from palm oil because the fat content is minimal. In addition, the real problem with palm oil occurs when it is processed (i.e., partially hydrogenated).

TABLE 57.5 Antioxidant Potential of Different Carotene Products (Per 25,000 IU of Vitamin A Activity)

Source	Carotenoid	Quenching Rate	% In source	mg/25,000 IU	Antioxidant Potential
Antioxidant potential	Alpha-carotene	1.9	33	7.36	2.6
	Beta-carotene	1.4	63	14.04	3.66
	Gamma-carotene	2.5	2.5	0.56	0.26
	Lycopene	3.1	0.1	0.02	0.01
	TOTAL				6.53
Algal	Alpha-carotene	1.9	4	0.61	0.22
	Beta-carotene	1.4	96	14.69	3.83
	TOTAL				4.05
Synthetic	Beta-carotene	1.4	100	14.97	3.9
	TOTAL				3.9

Lutein is derived primarily from marigold flowers, whereas lycopene is derived from tomato extracts. Astaxanthin is most often derived from *Haematococcus pluvialis,* an astaxanthin-rich type of algae. Although astaxanthin is found in certain fish oil supplements such as salmon, herring roe, or krill oil, the amounts in these sources are much lower than those provided from extracts of *H. pluvialis.* For example, the level of astaxanthin naturally occurring in a capsule of salmon or krill oil is in the range of 100 mcg (0.1 mg). That amount is not much compared with the 4 to 12 mg per capsule found in most astaxanthin supplements derived from *H. pluvialis.* There are other sources of astaxanthin on the market, but these forms are produced from either chemical synthesis or from genetically modified yeast (*Xanthophyllomyces dendrorhous,* formerly *Phaffia rhodozyma*). These synthetic forms are approved as feed additives and are often fed to salmon in fish farms to give them red flesh, but the synthetic forms differ in stereochemistry and esterification. The synthetic form is also 20 times less effective as an antioxidant, so it is unlikely to have the same benefits as natural astaxanthin.[37]

CLINICAL APPLICATIONS

The primary clinical applications of carotenoids are as follows:

- Prevention of cancer
- Prevention of cardiovascular disease
- Prevention of cognitive decline and dementia
- Macular degeneration and visual processing (see Chapter 195, Macular Degeneration)
- Immune enhancement
- Vaginal candidiasis
- Photosensitivity disorders

Although there is great overlap, it is important to understand that each carotenoid that may exert greater benefit in specific clinical indications.

Prevention of Cancer

Most epidemiological studies have demonstrated a strong inverse correlation between dietary beta-carotene intake and various cancers, especially those involving epithelial tissues (e.g., lung, skin, uterine cervix, gastrointestinal tract, and breast cancer).[38–42] The epidemiological association is much stronger for beta-carotene than for vitamin A. This may reflect beta-carotene's superior antioxidant, immune-potentiating, and anticarcinogenic activity.

Here is an important consideration. Although most of the epidemiological studies employed dietary questionnaires to determine intake, when blood levels were used instead, the results were even better for carotene intake reducing cancer risk. In a systematic review and meta-analysis of prospective studies of dietary intake and blood concentrations of carotenoids and breast cancer risk, beta-carotene intake as determined by dietary questionnaire was associated with a reduced breast cancer risk with a relative risk (RR) of 0.95. In contrast, the summary RR for blood concentrations of 50 µg beta-carotene/dL was 0.82. Interestingly, only beta-carotene was associated with a reduction in RR of the six dietary carotenes assessed in this analysis (alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene, lutein/zeaxanthin, and total carotenoids).

Although there is no argument that a diet high in carotenes protects against cancer, the big question is: "Can β-carotene supplementation reduce the risk of cancer?" The answer appears to be that synthetic β-carotene supplementation does not. Three highly publicized reports on cancer prevention trials featuring synthetic all-*trans* β-carotene in high-risk groups produced negative results. Taking a close look at each of these studies is important to help put things into perspective.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group

This study's population was 29,000 men in Finland who smoked and drank alcohol.[43] The men were given β-carotene (20 mg/day) or vitamin E, or both. The results of this study indicated an 18% increase in lung cancer in the β-carotene group. This result was not totally unexpected, as studies in primates demonstrated that when animals were fed alcohol and β-carotene, they experienced an increase in liver damage as a result of oxidative damage.[44] Other researchers have pointed out that β-carotene is susceptible to oxidative damage.[45] The protection against the oxidative damage of β-carotene is the presence of other antioxidant nutrients.[46] The absence of these protective nutrients could result in the formation of cancer-causing compounds, stressing the importance of relying on foods and broader-spectrum nutritional antioxidant support. Adding support to this statement is the fact that the group receiving both β-carotene and vitamin E did not show an increase in cancer. In the group not receiving β-carotene supplements, there was a strong protective effect of high dietary β-carotene and blood carotene levels against lung cancer. Altogether, these data strongly suggest that the protection offered by β-carotene is only apparent when other important antioxidant nutrients are provided and may not be provided by the synthetic forms.

Carotene and Retinol Efficacy Trial

The second trial reporting on the role of β-carotene in a high-risk group is the Carotene and Retinol Efficacy Trial (CARET).[47] This study comprised more than 18,000 U.S. male and female smokers and

asbestos workers. It was halted 21 months prematurely in January 1996 after 4 years of intervention indicated that β-carotene supplementation (30 mg/day) increased lung cancer by 28% and overall deaths by 17%. Although this appears dramatic, a closer look at the numbers and percentages puts them into proper perspective. Among active smokers, the risk of lung cancer during the CARET study was 5 per 1000. The 28% increase found with β-carotene supplementation increased this number to roughly 6 per 1000.[47]

Interestingly, once again in the group not taking β-carotene, the lowest rate of cancer was found among individuals with the highest blood β-carotene levels, and in former smokers, β-carotene supplementation actually reduced cancer risk by 20%.

The Physician's Health Study

The Physician's Health Study was composed of 22,071 U.S. male physicians who took either 50 mg of β-carotene or a placebo every other day for 12 years. Results demonstrated no significant effect—positive or negative—on cancer or cardiovascular disease, even in the group (11%) that smoked.[48]

General Comments on the "Negative" Studies

Although scientific research is clear that diets high in antioxidants are protective against many cancers, the data are not as solid with antioxidant supplements. Three main points should be kept in mind when discussing research with antioxidants:

- The antioxidant system of the body relies on a complex interplay of many different dietary antioxidants.
- Taking any single antioxidant nutrient is not enough. Total protection requires a strategic, comprehensive dietary and supplement program.
- Although dietary supplements are important, they cannot replace the importance of consuming a diet rich in antioxidants.

A shortcoming of many dietary studies is that researchers often focus on the effects of just one factor. In a way, this is like judging an entire symphony by listening to a single trombone. Such research has its value, but it is not complete and often raises more questions than it answers.

Another issue is that not all antioxidants are created equal. When it comes to quenching free radicals, each may have a somewhat different (and usually narrow) range of activity. For example, β-carotene is an effective quencher of a free radical known as singlet oxygen but is virtually powerless against the dozens of other types of free radicals. As a result, it has a narrow range of benefit and is susceptible to being damaged itself and forming a free radical without additional antioxidant support. Most antioxidants require some sort of "partner" antioxidant that allow them to work more efficiently. Additionally, research has shown that β-carotene itself can become damaged if used alone (i.e., without its partner antioxidants vitamin C, vitamin E, and selenium). For example, although studies showed that synthetic β-carotene supplements given alone actually increased the risk of cancer in smokers, when β-carotene was given along with vitamin E and selenium, it reduced cancer deaths by a significant 13%.[43] Damaged β-carotene is extremely toxic to the liver, the lining of the arteries, and the lungs. This fact alone may explain some of the disappointing results from recent β-carotene studies.

The results of these three studies indicate that synthetic β-carotene supplementation may have adverse effects in high-risk groups for cancer and cardiovascular disease. These studies do not invalidate the hundreds of studies showing the preventive effect of a diet rich in carotenes and nutritional antioxidants against cancer and cardiovascular disease. These results seem to indicate the need for a diet high in carotenes, and if carotene supplementation is desired, people should not smoke, natural forms should be used, and the β-carotene needs to be protected against the formation of toxic derivatives by taking extra vitamins C and E and selenium. That said, even studies with β-carotene supplementation that also used additional vitamins C and E failed to show a protective effect against either cancer or cardiovascular disease.[49,50]

Other Prospective Cancer Prevention Studies

In addition to these three highly publicized studies, several prospective and double-blind studies have shown promising results. In particular, β-carotene supplementation is especially effective in the treatment of early cancerous lesions of the oral cavity and esophagus.[51,52] Although β-carotene has been shown to exert these benefits on its own (in dosages ranging from 15–180 mg/day), one of the most positive studies showing a reduction in cancer risk with supplemental β-carotene to date is one that featured a broader supplement program. The Linxian Cancer Chemoprevention Study was a prospective study of 30,000 rural Chinese adults. In one substudy, subjects received one of four supplement programs:

- Retinol and zinc
- Riboflavin and niacin
- Vitamin C and molybdenum
- Beta-carotene, vitamin E, and selenium (dosages one to three times greater than the U.S. recommended daily allowance)

The latter group demonstrated a 13% decrease in cancer deaths and a reduction of 9% in overall deaths.[53,54] These results again support the notion that a combination of antioxidants is superior to high levels of any single antioxidant.

Lycopene in Prostate Cancer

In one of the first detailed studies of lycopene protection against cancer, Harvard researchers discovered that men who consumed the highest levels of lycopene (6.5 mg/day) in their diet showed a 21% decreased risk of prostate cancer compared with those eating the lowest levels.[55] Men who ate two or more servings of tomato sauce each week were 23% less likely to develop prostate cancer during the 22 years of the study than men who ate less than one serving of tomato sauce each month. In addition to a protective effect, lycopene may exert a therapeutic effect as well. In a study of patients with existing prostate cancer, lycopene supplementation (15 mg/day) was shown to slow tumor growth, shrink the tumor, and lower the level of prostate-specific antigen (PSA), a marker of cancer activity, by 18%.[56]

In another study, the efficacy of lycopene (4 mg/day) plus orchidectomy was compared with orchidectomy alone in the management of advanced prostate cancer.[57] Fifty-four patients with histologically confirmed metastatic prostatic cancer and a performance status of 0 to 2 (WHO) were entered into the trial. At 6 months there was a significant reduction in PSA level in both treatments, but it was more marked in the lycopene group (mean 9.1 and 26.4 ng/mL). After 2 years, these changes were more consistent in the lycopene group (mean 3.01 and 9.02 ng/mL). Eleven (40%) patients in the orchidectomy-alone group and 21 (78%) in the lycopene group had a complete PSA response, with a partial response in 9 (33%) and 4 (15%) and progression in 7 (25%) and 2 (7%), respectively. Bone scans showed that in the orchidectomy arm, only 4 (15%) patients had a complete response versus 8 (30%) in the lycopene group, with a partial response in 19 (70%) and 17 (63%) and progression in 4 (15%) and 2 (7%), respectively. Of the 54 patients who entered the trial, 19 (35%) died: 12 (22%) in the orchidectomy group and 7 (13%) in the lycopene group. Researchers concluded that adding lycopene to orchidectomy produced a more reliable and consistent decrease in serum PSA level; it not only shrunk the primary tumor but also diminished the secondary tumors, providing better relief from bone pain and lower urinary tract symptoms

and improving survival compared with orchidectomy alone. Lycopene appears to be most useful in the early stages of prostate cancer because no clinically relevant benefits were shown for patients with advanced stages of the disease in one clinical trial. Lycopene also does not appear to be effective for androgen-independent prostate cancer.[58] Lycopene does, however, appear to be helpful in benign prostate hyperplasia (BPH). In one double-blind, placebo-controlled trial involving 40 patients with histologically proven BPH free of prostate cancer were randomized to receive either lycopene at a dose of 15 mg/day or placebo for 6 months.[59] The primary end point of the study was the inhibition or reduction of increased serum PSA levels. Symptoms of the disease, as assessed via the International Prostate Symptom Score questionnaire, were improved in both groups, with a significantly greater effect in men taking lycopene supplements.

In a 6-month repeat-biopsy randomized trial among men with high-grade prostatic intraepithelial neoplasia (HGPIN), 58 participants consumed a placebo or lycopene (30 mg/day). Pre- and posttreatment biopsies were immunostained and digitally scored. Pathologists blindly reviewed each biopsy to score histologic features. There were no meaningful differences between the groups in PSA, IGF-1, or IGF-binding protein 3 concentrations, nor any significant differences in expression of MCM-2 or p27 in epithelial nuclei or prevalence of cancer. However, more extensive atrophy and less extensive HGPIN was more common in the lycopene group.[57] Despite large differences in serum lycopene after intervention, no treatment effects were apparent on either the serum or benign tissue end points. Larger studies are warranted to determine whether changes observed in the extent of HGPIN and focal atrophy can be replicated.

Prevention of Cardiovascular Disease

Just as in the case of cancer prevention, although a high intake of carotene-rich foods appears to be protective, the same may not be true for supplementation with synthetic β-carotene.[1–3,60,61] Double-blind trials wherein people were supplemented with β-carotene alone or placebo have not found benefit for synthetic β-carotene supplementation.[61] Three of four trials reported a higher risk of cardiovascular disease in the β-carotene groups compared with those receiving placebo.

Again, a major issue with much research on carotenes in cardiovascular disease protection has been the focus on β-carotene. It may not be the most important marker of protection. A large clinical study evaluating the relationship between carotene status and heart attack (acute myocardial infarction) found that lycopene, but not β-carotene, was protective.[62] Lycopene, lutein, and astaxanthin exert greater antioxidant activity compared with β-carotene in general but specifically against LDL oxidation.[63–65]

Beta-carotene is likely of less importance compared with many other carotenes, especially lycopene, astaxanthin, and lutein, because it does not get incorporated into LDL effectively, although it may help protect the endothelium. These other carotenoids exert more significant effects. For example, lutein is especially important in protecting against LDL oxidation. On the basis of analysis of the different subtypes of LDL, it has been found that lycopene, β-carotene, and cryptoxanthin were mainly located in the larger, less dense LDL particles, whereas astaxanthin, lutein, and zeaxanthin were found preferentially in the smaller, denser LDL particles.[63,66] Because the smaller, denser LDL subtype is most easily oxidized, astaxanthin, lutein, and zeaxanthin are particularly important in protecting against damage to LDL cholesterol.

Despite the focus on protecting LDL cholesterol from damage, it appears that the protective effect of carotenoids against the development of atherosclerosis does not occur early in the progression (i.e., they exert little effect on protecting against oxidative damage to LDL cholesterol) but, rather, later on by some undetermined mechanism. This conclusion is based on studies indicating that carotenoid-enriched diets are associated with less atherosclerotic plaque formation.

Prevention of Cognitive Decline and Dementia

Astaxanthin exerts a number of key protective mechanisms against age-related decline in cognitive function. Because astaxanthin can cross the blood–brain barrier, and because it is a strong and high-quality antioxidant and has anti-inflammatory actions as well, it is logical that it should help prevent neurologic impairment associated with aging because these conditions are directly related to oxidation and inflammation in the brain. Astaxanthin also promotes neurogenesis and plasticity, factors that decline with aging. Neurogenesis is now widely accepted to occur throughout adulthood, primarily in the hippocampus, the key area of the brain essential for learning and memory.[67]

This ability to directly affect neurogenesis and plasticity in the hippocampus is an emerging explanation for some of the observed effects with astaxanthin supplementation. Effects include not only delaying or ameliorating the cognitive impairment associated with normal aging but also improving cognitive function as well. For example, in one double-blind, placebo-controlled clinical trial, 96 healthy middle-aged or elderly subjects were randomly to take either placebo, 6 mg of astaxanthin per day, or 12 mg of astaxanthin per day for 12 weeks. Improvements in the CogHealth battery score were found in the 12-mg group after 12 weeks. Improvements were noted earlier in the Groton Maze Learning Test in both the 6-mg and 12-mg groups.[68]

Immune Enhancement

Carotenes demonstrated a number of immune-enhancing effects in recent studies.[27] However, these effects were demonstrated as far back as 1931, when it was found that a diet rich in carotenes, as determined by blood carotene levels, was inversely related to the number of school days missed by children.[69] Originally, it was thought that the immune-enhancing properties of carotenes were due to their conversion to vitamin A. Now it is known that carotenes exert many immune system–enhancing effects independent of any vitamin A activity.

In one study, the relationship of plasma concentrations of six major carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) with the incidence and severity of acute respiratory infections was determined.[70] The incidence rate ratio of acute respiratory infections at high β-carotene status was 0.71 (95% confidence interval [CI] 0.54–0.92) compared with the group with a low β-carotene concentration. Interestingly, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin were not related to the incidence or severity of infections, indicating that β-carotene may exert the most significant immune-enhancing effects.

One of the most impressive studies with supplemental β-carotene was conducted on normal human volunteers.[71] Results demonstrated that oral β-carotene (180 mg/day, approximately 300,000 international units [IU]) significantly increased the frequency of OKT4+ (helper/inducer T cells) by approximately 30% after 7 days and the frequency of OKT3+ (all T cells) after 14 days.[47] Because T4+ lymphocytes play a critical role in determining host immune status, this study indicates that oral β-carotene may be effective in increasing the immunologic competence of the host in conditions characterized by a selective diminution of the T4 subset of T cells, such as the acquired immunodeficiency syndrome and cancer.

However, rather than supplementing the diet with synthetic β-carotene, it may be more advantageous to use natural carotene sources or to increase the intake of carotene-rich foods. In another study, 126 healthy college students were randomly assigned to one of the following groups:

- Group A, the control group
- Group B, a group that used a 15-mg (25,000 IU) β-carotene supplement daily

- Group C, a group that consumed approximately 15 mg β-carotene per day from carrots

Better results (i.e., increase in white blood cell number and function) were achieved in the group eating the carrots versus those taking the carotene supplement.[72]

Astaxanthin also demonstrates significant immune-enhancing effects. In one double-blind clinical trial in healthy, young women averaging just over 20 years old, the women were separated into three different groups: the control group took a placebo, and the two treatment groups took either 2 mg of astaxanthin per day or 8 mg per day over 8 weeks. Results showed that at either dosage, astaxanthin[19]:

- Increases the total number of antibody-producing B cells
- Amplifies the cytotoxic activity of natural killer cells
- Leads to increased numbers of T cells
- Stimulates lymphocyte (white blood cell) counts
- Significantly increases delayed-type hypersensitivity response
- Dramatically decreases DNA damage
- Reduces C-reactive protein (CRP), the key marker for systemic inflammation

Vaginal Candidiasis

It is a well-established fact that women are more susceptible to vaginal candidiasis when the immune system is depressed, which may be due to low carotene levels. Beta-carotene levels were determined in exfoliated vaginal cells in 22 women with vaginal candidiasis and compared with vaginal cells from 20 controls. The β-carotene level per 1 million cells in the women with vaginal candidiasis was 1.46 ng compared with 8.99 ng in the control group (i.e., one-sixth that of normal).[73]

These results, coupled with β-carotene's known effects on enhancing the immune system, suggest that a low tissue level of β-carotene is associated with vaginal candidiasis, and a high dietary or supplemental intake of β-carotene may be protective against vaginal candidiasis.

Photosensitivity Disorders

Beta-carotene has become the treatment of choice for photosensitivity disorders. It is most effective in the treatment of erythropoietic protoporphyria (EPP).[74] Its effectiveness in other photosensitivity disorders such as polymorphous light eruption, solar urticaria, and discoid lupus erythematosus is significant but not as great.[75–79] Beta-carotene also has a small but significant effect in increasing the exposure at which manifestations of sunburn begin, thus allowing some subjects the opportunity to stay in the sun long enough to get a "tan" for the first time.[79]

Patients with EPP are characterized by elevated levels of porphyrins in blood, feces, and skin and by sensitivity to visible light. This sensitivity manifests after exposure to sunlight as a burning sensation followed by swelling and redness. Topical sunscreens are of no value. The photosensitivity is due to excitation of the porphyrin molecule by ultraviolet radiation, resulting in the production of free radicals that are deleterious to the skin. Direct cell damage results in the release of chemical mediators, which in turn damage other cells, resulting in the manifestations of itching, burning, redness, and swelling.

In EPP, it appears that carotene levels must be maintained in the blood at 600 to 800 mg/dL for optimum effects and that the protective effect is not usually observed until after 4 to 6 weeks of therapy. The actions of β-carotene and other carotenes in human tissue are similar to their action in plant cells (i.e., they function as a cellular screen against sunlight-induced free radical damage).

DOSAGE

Beta-carotene: A daily dosage of 25,000 IU (15 mg of β-carotene) appears to be reasonable for general health. Again, for the best clinical effect, it appears that natural mixed forms of carotene should be used in conjunction with a broad range of other natural antioxidants (see Chapter 108). In the treatment of EPP, the dosage is based on maintaining blood carotene levels between 600 and 800 mg/dL.

- Lycopene: 15 to 30 mg daily.
- Lutein/zeaxanthin: 10 mg of lutein and 2 mg zeaxanthin
- Astaxanthin: 4 to 12 mg daily.

TOXICITY

Supplementing the diet with β-carotene has not been shown to result in any significant toxicity despite its use in high doses in the treatment of numerous photosensitivity disorders (see discussion on "Photosensitivity Disorders"). Occasionally patients complain of loose stools, which usually clears spontaneously and does not necessitate stopping treatment. Elevated carotene levels in the blood do not lead to vitamin A toxicity, nor do they lead to any other significant disturbance besides yellowing of the skin (carotenodermia).

The ingestion of large amounts of carrots or carrot juice (0.45–1 kg/day of fresh carrots for several years) has, however, been shown to cause neutropenia, as well as menstrual disorders.[80,81] Although the blood carotene levels of these patients did reach levels (221–1007 mg/dL) similar to those of patients taking high doses of β-carotene (typically 800 mg/dL), the disturbances are due to some other factor in carrots because neither of these effects nor any others have been observed in subjects consuming high doses of pure β-carotene (e.g., 300,000–600,000 IU/day [180–360 mg β-carotene], which is equivalent to 4–8 lb of raw carrots) over long periods of time.[82–85] Doses up to 1000 mg/kg have been given to rats and rabbits for long periods of time, with no signs of embryotoxicity, toxicity, tumorigenicity, or interference in reproductive functions.[86]

There are no significant side effects or concerns with recommended levels of lycopene, lutein, or astaxanthin.

DRUG INTERACTIONS

One study showed that β-carotene in combination with selenium, vitamin C, and vitamin E appeared to decrease the effectiveness of the combination of simvastatin (Zocor) and niacin. The researchers proposed that this inhibition of hydroxymethylglutaryl coenzyme-A reductase inhibitors could reduce the effectiveness of similar drugs, such as atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), and pravastatin (Pravachol).[87]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ribeiro D, Freitas M, Silva AMS, Carvalho F, Fernandes E. Antioxidant and pro-oxidant activities of carotenoids and their oxidation products. *Food Chem Toxicol*. 2018;120:681–699.
2. Eggersdorfer M, Wyss A. Carotenoids in human nutrition and health. *Arch Biochem Biophys*. 2018;652:18–26.
3. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. Carotenoids: biochemistry, pharmacology and treatment. *Br J Pharmacol*. 2017;174(11):1290–1324.
4. Mangels AR, Holden JM, Beecher GR, et al. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J Am Diet Assoc*. 1993;93:284–296.
5. Brown ED, Micozzi MS, Craft NE, et al. Plasma carotenoids in normal men after a single ingestion of vegetables or purified beta-carotene. *Am J Clin Nutr*. 1989;49:1258–1265.
6. Nierenberg DW, Dain BJ, Mott LA, et al. Effects of 4 y of oral supplementation with beta-carotene on serum concentrations of retinol, tocopherol, and five carotenoids. *Am J Clin Nutr*. 1997;66:315–319.
7. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and beta-carotene when administered to human adults in separate or combined oral doses. *Am J Clin Nutr*. 1995;62:604–610.
8. Van het Hof KH, Brouwer IA, West CE, et al. Bioavailability of lutein from vegetables is 5 times higher than that of beta-carotene. *Am J Clin Nutr*. 1999;70:261–268.
9. Ganguly J, Sastry PS. Mechanism of conversion of beta-carotene into vitamin A—central cleavage versus random cleavage. *World Rev Nutr Diet*. 1985;45:198–220.
10. Leung WC, Hessel S, Méplan C, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. *FASEB J*. 2009;23(4):1041–1053. PMID: 19103647.
11. Lietz G, Oxley A, Leung W, et al. Single nucleotide polymorphisms upstream from the β-carotene 15,15'-monoxygenase gene influence provitamin A conversion efficiency in female volunteers. *J Nutr*. 2012;142(1):161S–165S. https://doi.org/10.3945/jn.111.140756. Epub 2011 Nov 23. PMID: 22113863.
12. Scott KJ, Rodriquez-Amaya D. Pro-vitamin A carotenoid conversion factors: retinol equivalents—fact or fiction? *Food Chemistry*. 2000;69:127–127.
13. Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *J Natl Cancer Inst*. 1984;73:1439–1444.
14. Fakhri S, Abbaszadeh F, Dargahi L, Jorjani M. Astaxanthin: a mechanistic review on its biological activities and health benefits. *Pharmacol Res*. 2018;136:1–20.
15. Hedayati N, Naeini MB, Nezami A, et al. Protective effect of lycopene against chemical and natural toxins: a review. *Biofactors*. 2018. https://doi.org/10.1002/biof.1458.
16. Nishida Y, Yamashita E, Miki W. Comparison of astaxanthin's singlet oxygen quenching activity with common fat and water soluble antioxidants. *Carotenoid Science*. 2007;11:16–20.
17. Cutler RG. Carotenoids and retinol: their possible importance in determining longevity of primate species. *Proc Natl Acad Sci U S A*. 1984;81:7627–7631.
18. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress—implications of cellular signaling pathways and relation to chronic disease prevention. *Nutr Res*. 2014;34(11):907–929.
19. Park J, Chyun J, Kim Y, Line L, Chew B. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutri Metabol*. 2010;7:18.
20. Nir Y, Spiller G. BioAstin helps relieve pain and improves performance in patients with rheumatoid arthritis. *J Am Coll Nutri*. 2002;21(5).
21. Baralic I, Andjelkovic M, Djordjevic B, Dikic N, Radivojevic N, Suzin-Zivkovic V, et al. Effect of astaxanthin supplementation on salivary IgA, oxidative stress, and inflammation in young soccer players. *Evid Based Complement Alternat Med*. 2015;2015:1–9. https://doi.org/10.1155/2015/783761.
22. Hughes DA. Dietary carotenoids and human immune function. *Nutrition*. 2001;17(10):823–827.
23. Bendich A. Beta-carotene and the immune response. *Proc Nutr Soc*. 1991;50:263–274.
24. Folman Y, Rosenberg M, Ascarelli I, et al. The effect of dietary and climatic factors on fertility, and on plasma progesterone and oestradiol-17 beta levels in dairy cows. *J Steroid Biochem*. 1983;19:863–868.
25. Anonymous. Metabolism of beta-carotene by the bovine corpus luteum. *Nutr Rev*. 1983;41:357–358.
26. Lotthammer KH. Importance of beta-carotene for the fertility of dairy cattle. *Feedstuffs*. 1979;51:16–19.
27. O'Fallon JV, Chew BP. The subcellular distribution of beta-carotene in bovine corpus luteum. *Proc Soc Exp Biol Med*. 1984;177:406–411.
28. Sherman BM, Korenman SG. Inadequate corpus luteum function: a pathophysiological interpretation of human breast cancer epidemiology. *Cancer*. 1974;33:1306–1312.
29. Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant "astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian J Androl*. 2005;7(3):257–262.
30. Buscemi S, Corleo D, Di Pace F, et al. The effect of lutein on eye and extra-eye health. *Nutrients*. 2018;10(9):pii:E1321. https://doi.org/10.3390/nu10091321.
31. Grether-Beck S, Marini A, Jaenicke T, Stahl W, Krutmann J. Molecular evidence that oral supplementation with lycopene or lutein protects human skin against ultraviolet radiation: results from a double-blinded, placebo-controlled, crossover study. *Br J Dermatol*. 2017;176(5):1231–1240.
32. Tominaga K, Hongo N, Karato M, Yamashita E. Cosmetic benefits of astaxanthin on human subjects. *Acta Biochim Pol*. 2012;59(1):43–47.
33. Ben-Amotz A, Mokady S, Edelstein S, et al. Bioavailability of a natural isomer mixture as compared with synthetic all-trans-beta-carotene in rats and chicks. *J Nutr*. 1989;119:1013–1019.
34. Mokady S, Avron M, Ben-Amotz A. Accumulation in chick livers of 9-cis versus all-trans beta-carotene. *J Nutr*. 1990;120:889–892.
35. Carughi A, Hooper FG. Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture. *Am J Clin Nutr*. 1994;59:896–899.
36. Zhang J, Wang CR, Xue AN, et al. Effects of red palm oil on serum lipids and plasma carotenoids level in Chinese male adults. *Biomed Environ Sci*. 2003;16:348–354.
37. Morinobu T, Tamai H, Murata T, et al. Changes in beta-carotene levels by long-term administration of natural beta-carotene derived from Dunaliella bardawil in humans. *J Nutr Sci Vitaminol*. 1994;40:421–430.
38. Rao AR, Sarada R, Shylaja MD, Ravishankar GA. Evaluation of hepatoprotective and antioxidant activity of astaxanthin and astaxanthin esters from microalga—*Haematococcus pluvialis*. *J Food Sci Technol*. 2015;52(10):6703–6710.
39. Ziegler RG. A review of the epidemiologic evidence that carotenoids reduce the risk of cancer. *J Nutr*. 1989;119:116–122.
40. Panic N, Nedovic D, Pastorino R, Boccia S, Leoncini E. Carotenoid intake from natural sources and colorectal cancer: a systematic review and meta-analysis of epidemiological studies. *Eur J Cancer Prev*. 2017;26(1):27–37. Review.
41. Yu N, Su X, Wang Z, Dai B, Kang J. Association of dietary vitamin A and β-carotene intake with the risk of lung cancer: a meta-analysis of 19 publications. *Nutrients*. 2015;7(11):9309–9324.
42. Leoncini E, Nedovic D, Panic N, et al. Carotenoid intake from natural sources and head and neck cancer: a systematic review and meta-analysis of epidemiological studies. *Cancer Epidemiol Biomarkers Prev*. 2015;24(7):1003–1011.
43. Aune D, Chan DS, Vieira AR, et al. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr*. 2012;96(2):356–373.
44. The Alpha-Tocopherol; Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med*. 1994;330:1029–1035.

45. Leo MA, Kim C, Lowe N, et al. Interaction of ethanol with beta-carotene: delayed blood clearance and enhanced hepatotoxicity. *Hepatology*. 1992;15:883–891.
46. Krinsky NI. The biological properties of carotenoids. *Pure Appl Chem*. 1994;66:1003–1010.
47. Krinsky NI. Antioxidant functions of carotenoids. *Free Radic Biol Med*. 1989;7:617–635.
48. Omenn G, Goodman G, Thornquist M, et al. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer Res*. 1994;54:2038s–2043s.
49. Rowe PM. Beta-carotene takes a collective beating. *Lancet*. 1996;347:249.
50. Lin J, Cook NR, Albert C, et al. Vitamins C and E and beta carotene supplementation and cancer risk: a randomized controlled trial. *J Natl Cancer Inst*. 2009;101:14–23.
51. Cook NR, Albert CM, Gaziano JM, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. *Arch Intern Med*. 2007;167:1610–1618.
52. Garewal H, Shamdas GJ. Intervention trials with beta-carotene in precancerous conditions of the upper aerodigestive tract. In: Bendich A, Butterworth CE, eds. *Micronutrients in Health and in Disease Prevention*. New York: Marcel Dekker; 1991:127–140.
53. Toma S, Benso S, Albanese E, et al. Treatment of oral leukoplakia with beta-carotene. *Oncology*. 1992;49:77–81.
54. Blot WJ, Li JY, Taylor PR, et al. The Linxian trials: mortality rates by vitamin-mineral intervention group. *Am J Clin Nutr*. 1995;62: S1424–S1426.
55. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst*. 1993;85:1483–1492.
56. Gann PH, Ma J, Giovannucci E, et al. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res*. 1999;59:1225–1230.
57. Kucuk O, Sarkar FH, Sakr W, et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev*. 2001;10:861–868.
58. Ansari MS, Gupta NP. A comparison of lycopene and orchidectomy vs orchidectomy alone in the management of advanced prostate cancer. *BJU Int*. 2003;92:375–378.
59. Jatoi A, Burch P, Hillman D, et al. A tomato-based, lycopene-containing intervention for androgen-independent prostate cancer: results of a Phase II study from the North Central Cancer Treatment Group. *Urology*. 2007;69:289–294.
60. Gann PH, Deaton RJ, Rueter EE, et al. A phase II randomized trial of lycopene-rich tomato extract among men with high-grade prostatic intraepithelial neoplasia. *Nutr Cancer*. 2015;67(7):1104–1112.
61. Street DA, Comstock GW, Salkeld RM, et al. Serum antioxidants and myocardial infarction. Are low levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? *Circulation*. 1994;90:1154–1161.
62. Tavani A, La Vecchia C. Beta-carotene and risk of coronary heart disease. A review of observational and intervention studies. *Biomed Pharmacother*. 1999;53(9):409–416.
63. Agarwal S, Rao AV. Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. *Lipids*. 1998;33:981–984.
64. Lowe GM, Bilton RF, Davies IG, et al. Carotenoid composition and antioxidant potential in subfractions of human low-density lipoprotein. *Ann Clin Biochem*. 1999;36:323–332.
65. Cheng HM, Koutsidis G, Lodge JK, et al. Tomato and lycopene supplementation and cardiovascular risk factors: a systematic review and meta-analysis. *Atherosclerosis*. 2017;257:100–108.
66. Fassett RG, Coombes JS. Astaxanthin, oxidative stress, inflammation and cardiovascular disease. *Future Cardiol*. 2009;5(4):333–342. https://doi.org/10.2217/fca.09.19.
67. Iwamoto T, Hosoda K, Hirano R, et al. Inhibition of low-density lipoprotein oxidation by astaxanthin. *J Atherosclerosis Thrombosis*. 2000;7(4):216–222.
68. Grimmig B, Kim SH, Nash K, et al. Neuroprotective mechanisms of astaxanthin: a potential therapeutic role in preserving cognitive function in age and neurodegeneration. *Geroscience*. 2017;39(1):19–32.
69. Katagiri M, Satoh A, Tsuji S, Shirasawa T. Effects of astaxanthin-rich *Haematococcus pluvialis* extract on cognitive function: a randomized, double-blind, placebo-controlled study. *J Clini Biochemist Nutri*. 2012;51(2):102–107.
70. Clausen SW. Carotenemia and resistance to infection. *Trans Am Pediatr Soc*. 1931;43:27–30.
71. van der Horst-Graat JM, Kok FJ, Schouten EG. Plasma carotenoid concentrations in relation to acute respiratory infections in elderly people. *Br J Nutr*. 2004;92:113–118.
72. Alexander M, Newmark H, Miller RG. Oral beta-carotene can increase the number of OKT4+ cells in human blood. *Immunol Lett*. 1985;9:221–224.
73. Brevard PB. Beta-carotene affects white blood cells in human peripheral blood. *Nutr Rep Int*. 1989;40:139–150.
74. Mikhail MS, Palan PR, Basu J, et al. Decreased beta-carotene levels in exfoliated vaginal epithelial cells in women with vaginal candidiasis. *Am J Reprod Immunol*. 1994;32:221–225.
75. Mathews-Roth MM. Carotenoids in erythropoietic protoporphyria and other photosensitivity diseases. *Ann N Y Acad Sci*. 1993;691:127–138.
76. Swanbeck G, Wennersten G. Treatment of polymorphous light eruptions with beta-carotene. *Acta Derm Venereol*. 1972;52:462–466.
77. Newbold PC. Beta-carotene in the treatment of discoid lupus erythematosus. *Br J Dermatol*. 1976;95:100–101.
78. Fusaro RM, Johnson JA. Hereditary polymorphic light eruption in American Indians—photoprotection and prevention of streptococcal pyoderma and glomerulonephritis. *JAMA*. 1980;244:1456–1459.
79. Mathews-Roth MM, Pathak MA, Parrish J, et al. A clinical trial of the effects of oral beta-carotene on the responses of human skin to solar radiation. *J Invest Dermatol*. 1972;59:349–353.
80. Shoenfeld Y, Shaklai M, Ben-Baruch N, et al. Neutropenia induced by hypercarotenemia. *Lancet*. 1982;1:1245.
81. Kemmann E, Pasquale SA, Skaf R. Amenorrhea associated with carotenemia. *JAMA*. 1983;249:926–929.
82. Mathews-Roth MM. Neutropenia and beta-carotene. *Lancet*. 1982;2:222.
83. Stampfer MJ, Willett W, Hennekens CH. Carotene, carrots, and white blood cells. *Lancet*. 1982;2:615.
84. Mathews-Roth MM, Abraham AA, Gabuzda TG. Beta-carotene content of certain organs from two patients receiving high doses of beta-carotene. *Clin Chem*. 1976;22:922–924.
85. Mathews-Roth MM. Amenorrhea associated with carotenemia. *JAMA*. 1983;250:731.
86. Poh-Fitzpatrick MB, Barbera LG. Absence of crystalline retinopathy after long-term therapy with beta-carotene. *J Am Acad Dermatol*. 1984;11:111–113.
87. Heywood R, Palmer AK, Gregson RL, et al. The toxicity of beta-carotene. *Toxicology*. 1985;36:91–100.

Boron[94]

Joseph E. Pizzorno, ND

OUTLINE

INTRODUCTION

Boron is a ubiquitous constituent of the environment, typically occurring in nature as inorganic borates with minerals replacing the hydrogen ions seen in Fig. 58.1. In human tissues, boron is found as the borate anion and boric acid, predominantly the latter form, with a concentration primarily reflecting dietary intake.[1,2] In trace amounts, boron is essential for the growth of many plants, and although it has yet to be recognized as an essential nutrient for humans, growing data from animal and human studies continue to suggest that boron is important for many life processes. These include embryogenesis; bone growth and maintenance; immune function; regulation of inflammation; psychomotor skill; mineral metabolism; brain function and performance; and the prevention of osteoporosis, osteoarthritis, and prostate, lung, and cervical cancer.[3] It also has been shown to affect the metabolism or action of many biological compounds, including glucose, S-adenosylmethionine, amino acids, triglycerides, macrominerals, and a number of hormones, including vitamin D, insulin, testosterone, and estrogen.[4,5]

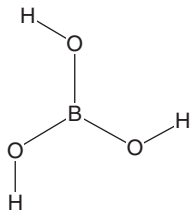


Fig. 58.1 Boric acid.

Sources

Because boron in plants depends on the availability of boron in the soil, the same food crop can vary greatly in boron content depending on where and how it is grown. In general, soils exposed to high degrees of precipitation have decreased levels of boron.[6] Heavy rainfall leaches the highly soluble boric acid from the soil, whereas in more arid regions, evaporation of groundwater results in higher concentrations of boron solutes. Food processing results in additional loss of boron, and the use of high-phosphate and high-potash fertilizers reduces its uptake by plants.[7,8] Foods of plant origin, such as leafy vegetables, noncitrus fruits (e.g., apples), nuts, legumes (peanut butter), avocados, and sea vegetables are considered the best sources of boron.[1,9] Wine has also been shown to contribute appreciable amounts of boron to the diet, and water intake may also be a significant source of total intake.[10] A diet containing an abundance of these items would provide 2 to 6 mg/day of boron.[9,11]

Daily intake of boron depends on several variables. The concentration of boron in water varies considerably according to the geographical source. In some areas, boron in drinking water and water-based beverages may account for most of the total dietary boron intake. Individual food preference greatly influences the daily intake of boron. Fruits, vegetables, tubers, and legumes have higher concentrations of boron than do cereal grains or animal tissues. For adults and seniors, though, the largest source of boron turns out to be instant regular coffee.[12] Given the poor dietary intake of produce in the United States, the top two boron contributors to adult boron intake, coffee and milk, are actually deficient in this nutrient (Table 58.1)[13] compared with other,

TABLE 58.1 Top Contributors of Dietary Boron in the American Diet

Food	Boron (mg/mL)
Coffee, from ground beans	0.029
Milk, whole	0.018
Apples, raw	0.36
Beans, refried	0.4
Potatoes, from French fries	0.11
Orange juice	0.072
Peanut butter	1.145
Wine	0.61
Apple juice	0.18
Cola	0.013

Data from Rainey CJ, Nyquist LA, Christensen RE, et al. Daily boron intake from the American diet. *J Am Diet Assoc.* 1999;99:335–340.

more nutritious foods. Nevertheless, these make up 12% of the total boron intake by virtue of the volume consumed.[13] Boron has also been determined to be a notable contaminant or major ingredient of many personal care products, and it (boric acid) is occasionally used as a food preservative.[14]

In Sydney, Australia, 32 subjects aged 20 to 53 years old were assessed over a 7-day period for their dietary intake of boron. The average boron intake in male and female subjects was found to be 2.28 ± 1.3 and 2.16 ± 1.1 mg/day, respectively.[10] The boron content of selected Australian foods has been found to correlate with values in Finnish and U.S. Food and Drug Administration tables and is presented in Table 58.2 (note, however, the large variations between countries and even regions of a country).[10] Regional differences are certainly important. An analysis of nearly 300 foods that are commonly consumed in Korea found considerable discrepancies with values reported elsewhere. For example, the boron content in Korean apples was found to be one-twelfth that found in German apples. The foods found to be richest in boron (in micrograms/100 g) included buckwheat flour (828), soybeans (1642), mung beans (818), almonds (917), cocoa (2026), coffee powder (approximately 1530), and oolong and green tea powder (1274 and 1316, respectively).[15]

TABLE 58.2 Concentration of Boron in Selected Australian[a] Foods

Food	Boron (mg/100 g)
Almonds	2.82
Apples (red)	0.32
Apricots (dried)	2.11
Avocados	2.06
Bananas	0.16
Beans (red kidney)	1.4
Bran (wheat)	0.32
Brazil nuts	1.72
Broccoli	0.31
Carrots	0.3
Cashews (raw)	1.15
Celery	0.5
Chickpeas	0.71
Dates	1.08
Grapes (red)	0.5
Hazelnuts	2.77
Honey	0.5
Lentils	0.74
Olives	0.35
Onions	0.2
Oranges	0.25
Peaches	0.52
Peanut butter	1.92
Pears	0.32
Potatoes	0.18
Prunes	1.18
Raisins	4.51
Walnuts	1.63
Wine (Shiraz Cabernet)	0.86

[a]Note that there is a large variation between countries.

METABOLISM

Chemical Properties

Elemental boron was first isolated in 1808. It is the first member (atomic number 5) of the metalloid or semiconductor family of elements, including silicon and germanium, and is the only nonmetal of the group IIIA elements. Like carbon, boron has a tendency to form double bonds and macromolecules.[16] Boron, as boric acid, acts as a Lewis acid, accepting hydroxyl (OH^-) ions and leaving an excess of protons.[17] Because boron complexes with organic compounds containing hydroxyl groups, it interacts with sugars and polysaccharides, adenosine-5-phosphate, pyridoxine, riboflavin, dehydroascorbic acid, and pyridine nucleotides.[18] Given boron's affinity for hydroxyl groups, it is possible that it interacts with glycoproteins and glycolipids found in cellular membranes. Animal studies have supported a role for boron in maintaining cellular membrane structure and function, with boron deprivation causing pathological changes in both zebrafish and frogs.[19] Dietary magnesium and essential fatty acids, both of which are involved in cell membrane function, also influence the response to boron deprivation, lending further support for a role for boron. For example, in an animal-based study, boron deprivation was found to alter animal behavior in ways that were modifiable by varying the composition of dietary fatty acids, and vice versa, possibly by altering the fluidity of cellular membranes.[20]

Biochemistry

Boron in food, sodium borate, and boric acid are well absorbed from the digestive tract.[21] However, homeostatic mechanisms for maintaining serum levels of boron exist, with urinary levels mirroring intake.[8] A boron transporter has been identified (NaBC1), and dietary supplementation of boron has been shown to alter its genetic expression.[4,22] Compounds of boron are also absorbed through damaged skin and mucous membranes; however, they do not readily penetrate intact skin.[23]

No accumulation of boron has been observed in soft tissues of animals fed long-term low doses of boron; however, in acute poisoning incidents, the amount of boric acid in brain and liver tissue has been reported to be as high as 2000 ppm. Within a few days of consumption of large amounts of boron, levels in blood and most soft tissues quickly reach a plateau.[24] Tissue homeostasis is maintained by the rapid elimination of excess boron, primarily in the urine, with bile, sweat, and breath also contributing as routes of elimination.[18]

Evidence suggests that supplemental boron does accumulate in bone; however, cessation of exposure to dietary boron results in a rapid drop in bone boron levels. The half-life of boric acid in animals is estimated at about 1 day.[24]

Biological Functions

Boron contributes to living systems by acting indirectly as a proton donor and exerting an influence on cell membrane structure and function.[25] Although the absolute essentiality of boron for plants as well as two animal species (zebrafish and frogs) is well documented, studies to date have not shown it to be unequivocally essential for other species or humans. However, boron supplementation has been shown to affect certain aspects of animal physiological function. In general, supplemental dietary boron has its most marked effects when the diet is deficient in known nutrients.[26]

Recent animal-based data suggest boron may in some way affect the utilization or production of S-adenosylmethionine, which in turn influences homocysteine metabolism, as well as other molecules involved in cellular signaling and differentiation. Rats deprived of boron had increases in plasma cysteine and homocysteine as well as decreases in hepatic S-adenosylmethionine and the related compounds S-adenosylhomocysteine and spermidine. It is possible that many of boron's biological effects are mediated through this mechanism because S-adenosylmethionine is involved in many methylation reactions, including DNA methylation, as well as methylation of other neurotransmitters, phospholipids, and signaling molecules, and is also used frequently as an enzyme substrate.[27]

Evidence suggests that boron might also have an effect on decreasing fasting serum glucose concentrations in postmenopausal women.[28]

Embryo Maturation

Research has shown that boron is an important player in the early stages of life.[3] Boron deficiency negatively affects reproductive ability, as well as embryo development, in both the African clawed frog *(Xenopus laevis)* and the zebrafish. Experimentation with *Xenopus* noted that dietary boron deprivation elicited necrotic egg increases combined with a high frequency of abnormal gastrulation, causing adverse effects during gametogenesis, gamete maturation, embryonic development, and larval maturation.[19] In the zebrafish model, 45% of boron-deprived embryos died during the early postfertilization period. Conversely, only 2% of boron-supplemented embryos died. Other studies with rats and mice corroborated that low boron status might affect reproduction in mammals, although the conclusions from these studies were not as clear.

Life Span

Boron in an animal model has been shown to affect life span, although the process is undefined. Extremes in dietary boron, both a deficiency and an excess, appear to affect the median life span of *Drosophila.* Adding excess during the adult stage decreased life span by as much as 69%, whereas supplementing the diet with low levels of boron increased life span by 9.5%.[29] No research exists for other species.

Brain Function and Performance

Although limited data exist for boron's influence on brain function, existing research is "among the most supportive in demonstrating that boron is a beneficial bioactive element for humans."[30] Brain electrophysiology and cognitive performance were assessed in response to dietary manipulation of boron (approximately 0.25 vs. approximately 3.25 mg boron/2000 kcal/day) in three studies with healthy older men and women. A low boron intake was shown to result in a decrease in the proportion of power in the α-band and an increase in the proportion of power in the δ-band, effects similar to those induced by heavy metals and nonspecific malnutrition, as well as mental drowsiness and reduced alertness. Other changes in left–right symmetry and brain-wave coherence were noted in various sites, indicating an influence on brain function. When contrasted with the high boron intake, low dietary boron resulted in significantly poorer performance ($P < 0.05$) on tasks emphasizing manual dexterity, eye–hand coordination, attention, perception, encoding, and short- and long-term memory. Collectively, the data from these studies indicate that boron may play a role in human brain function, alertness, and cognitive performance.[31]

Interestingly, as mentioned previously, essential fatty acid composition may interact with boron status to influence brain function. A number of biomarkers for low activity were observed in boron-deficient rats when given safflower oil, but fish oil negated these deficits.[20]

Hematologic

Boron supplementation to human subjects who had previously followed a dietary regimen deficient in boron increased blood hemoglobin concentrations, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. It lowered hematocrit, red blood cell count, and platelet count.[32] As discussed in the "Toxicology" section, boron may inhibit the enzyme δ-aminolevulinic acid dehydratase.

Hepatoprotection

Studies of fulminate hepatic failure in Wistar rat models showed that boron pretreatment significantly reduced lipid peroxidation, as well as decreased serum liver enzymes, in these animals. Boron pretreatment also increased the peroxide-metabolizing enzymes' levels of glutathione peroxidase and catalase, as well as glutathione itself.[33,34]

Mineral Metabolism

Boron also affects mineral metabolism as well as related hormones in human subjects. In the first nutritional study with humans involving boron,[23] postmenopausal women first were fed a diet that provided 0.25 mg boron/2000 kcal for 119 days and then were fed the same diet with a boron supplement of 3 mg boron/day for 48 days. The boron supplementation reduced the urinary excretion of calcium and magnesium and elevated the serum concentrations of 17 β-estradiol and testosterone.[9]

In a study designed to determine the effects of boron supplementation on blood and urinary minerals in athletic subjects on Western diets, findings suggested that boron supplementation modestly affected mineral status.[35]

DEFICIENCY SIGNS AND SYMPTOMS

Information on boron deficiency is limited, especially in humans. It is thought that insufficient intake of boron becomes obvious only when the body is stressed in a manner that enhances the need for it. When the diets of animals and humans are manipulated to cause functional deficiencies in nutrients such as calcium, magnesium, vitamin D, essential fatty acids, and methionine, a large number of responses to dietary boron occur.[36] Evidence suggests that more than 21 days on a boron-deficient diet is required to demonstrate detectable effects in humans.[37] The variables that are changed due to a boron-deficient diet abruptly improve about 8 days after boron supplementation is introduced.[9] Evidence indicates that hemodialysis results in an excessive decrease in serum boron compared with controls.[38] Although blood urea is by no means pathognomonic for a boron deficiency, it has been found to be slightly elevated during boron depletion.[39]

NUTRIENT INTERACTIONS

Vitamin D

Considerable evidence indicates that dietary boron alleviates the perturbations in mineral metabolism that are characteristic of vitamin D_3 deficiency.[40] In one study, chicks fed a diet inadequate in vitamin D exhibited decreased food consumption and plasma calcium concentrations and increased plasma concentrations of glucose, β-hydroxybutyrate, triglycerides, triiodothyronine, cholesterol, and alkaline phosphatase activity after 26 days. Supplemental boron returned plasma glucose and triglycerides to concentrations exhibited by chicks fed a diet adequate in vitamin D.[41]

In rachitic chicks, boron elevated the numbers of osteoclasts and alleviated distortion of the marrow sprouts of the proximal tibial epiphysial plate, a distortion characteristic of vitamin D_3 deficiency.[40,42] Higher apparent balance values of calcium, magnesium, and phosphorus have been observed for rats fed a vitamin D–deprived diet if the diet was supplemented with boron.[26]

After supplementation with 3.25 mg boron daily, plasma levels of vitamin D_2 increased in men older than age 45 and postmenopausal women on low-magnesium and low-copper diets.[43] In a very small human study ($n = 8$), healthy male volunteers were given 10 mg boron per day for 1 week, resulting in a nonsignificant 7% rise in vitamin D levels.[5] Inhibition of 24-hydroxylase, the enzyme that catabolizes vitamin D, has been speculated to be the possible mechanism of action, although this has not been demonstrated.[44]

Calcium

Boron supplementation may have a favorable effect on calcium metabolism. A boron supplement of 3 mg/day affected several indexes of mineral metabolism of seven women consuming a low-magnesium diet and five women consuming a diet adequate in magnesium; the women had consumed a conventional diet supplying about 0.25 mg boron per day for 119 days. Boron supplementation modestly reduced the urinary excretion of calcium when dietary magnesium was low.[9]

In men older than age 45 and postmenopausal women, changes caused by boron supplementation included an increased concentration of ionized plasma and total calcium, as well as reduced serum calcitonin concentration and urinary excretion of calcium.[43] A 1993 study demonstrated that a low-boron diet elevated urinary calcium excretion. The high level of calcium excretion was maintained throughout the 6-week study; however, it remained elevated even after boron supplementation began.[45]

Animal studies suggest that boron deprivation does not markedly affect the concentration of calcium (and phosphorus) in bone, but rather, it does affect the concentration of other nutrients associated with osteoblast and osteoclast function, such as magnesium, potassium, copper, and zinc.[30]

Copper

Supplemental boron acts to increase serum levels of both copper and copper-dependent enzymes in humans. Boron supplementation (3 mg/day) to five men older than age 45, four postmenopausal women, and five postmenopausal women on estrogen therapy who had been fed a low-boron diet (0.23 mg/2000 kcal) for 63 days resulted in higher erythrocyte superoxide dismutase, serum enzymatic ceruloplasmin, and plasma copper.[43] In a subsequent study, these same variables were again found to be higher during boron repletion than while subjects were fed a diet low in boron.[46]

Magnesium

When magnesium deprivation is severe enough to cause typical signs of deficiency, a significant interaction between boron and magnesium is found.[16] A combined deficiency of boron and magnesium causes detrimental changes in the bones of animals. Supplemental boron elevates plasma magnesium concentrations and enhances growth.[42]

Boron supplementation resulted in increased serum magnesium concentrations in human female subjects.[24] Boron supplementation increased red blood cell magnesium concentrations.[47] It has been shown that serum magnesium concentrations are greater in sedentary females whose diets are supplemented with boron than in exercising female athletes who are supplemented with boron.[48] This finding, although unexplained to date, may indicate an increased loss of boron through urine and perspiration during exercise.

Phosphorous

Supplemental boron seemed to lower serum phosphorus concentrations in female subjects 20 to 27 years old.[48] However, exercise training diminished these changes,[24] again possibly indicating increased losses or an increased need for boron as a result of exercise. A low-magnesium status, along with supplementation of boron, may depress the urinary excretion of phosphorus. This does not occur in women with an adequate magnesium intake.[9]

Methionine and Arginine

In experimental animals, a beneficial effect is consistently observed after boron supplementation when the diet contains marginal methionine and excessive arginine. Among the signs exhibited by rats fed on a diet marginal in methionine and magnesium are depressed growth and bone magnesium concentration and elevated spleen weight/body weight and kidney weight/body weight ratios. Findings indicate that the severity of these symptoms is alleviated with boron supplementation.[49]

Essential Fatty Acids

Several studies have emerged that suggest some interaction between essential fatty acids and boron. As previously mentioned, boron deprivation was shown to decrease the activity of rats and alter their brain composition, yet supplementation with fish oil (vs. safflower oil) minimized these effects.[20] Similarly, boron deprivation in rats was shown to impair both bone trabecular microarchitecture and cortical bone strength. Interestingly, the use of fish oil instead of safflower oil was also shown to benefit both of these structural markers through a distinct mechanism, yet the benefit of fish oil was limited only to the group receiving adequate boron. In other words, a boron deficiency prevented the normal benefit of fish oil to bone.[50] Lastly, as mentioned previously, boron deprivation was shown to decrease S-adenosylmethionine levels in rats. Feeding them corn oil instead of fish oil had similar effects, although they were likely mediated by a different mechanism.[50]

HORMONE INTERACTIONS

In rats, supplemental dietary boron substantially depressed plasma insulin, plasma pyruvate concentrations, and creatine kinase activity and increased plasma thyroxine (T4) concentrations. Boron supplementation also decreased plasma aspartate transaminase activity.[51] In animal experiments, boron supplementation offset the elevation in plasma alkaline phosphatase caused by vitamin D deficiency.[39] Boron supplementation in rats has also been shown to reduce plasma insulin levels, likely by increasing insulin sensitivity.[52]

An increase in the dietary intake of boron from 0.25 to 3.25 mg/day has been reported to increase plasma 17 β-estradiol by more than 50% and to more than double plasma testosterone levels in postmenopausal women. The elevation seemed more marked when dietary magnesium was low.[9] In a subsequent study of healthy men, boron supplementation resulted in an increase in the concentrations of both plasma

estrogen and testosterone; however, not all published trials support these observations.[10]

Ten male bodybuilders, 20 to 26 years old, were given a 2.5-mg boron supplement for 7 weeks, whereas nine male bodybuilders, 21 to 27 years old, were given a placebo. Because both groups demonstrated significant increases in total testosterone ($P < 0.01$), lean body mass ($P < 0.01$), one-repetition maximum squat ($P < 0.001$), and bench pressing ($P < 0.01$), the authors concluded that the gains were a result of 7 weeks of bodybuilding, not of boron supplementation.[53] However, in a small study of male volunteers, 10 mg supplemental boron per day was associated with an increase in plasma free testosterone and a decrease in mean plasma estradiol levels. A decrease in the inflammatory markers C-reactive protein, tumor necrosis factor-α, and sex hormone–binding globulin was also seen.[5]

One researcher hypothesized that boron might be required for the synthesis of steroid hormones, as well as vitamin D. Because the biosynthesis of steroids such as vitamin D, testosterone, and 17 β-estradiol involves one or more hydroxylation steps, and because of boron's ability as a Lewis acid to complex with hydroxyl groups, boron may assist the addition of hydroxyl groups to the steroid structures.[9] It has also been suggested that boron may act in an unspecified manner to protect hormones from rapid inactivation.[9] The current hypothesis suggests that boron can inhibit a range of microsomal enzymes that insert hydroxyl groups near existing hydroxyls in steroids. These enzymes include those that catabolize 25-hydroxyvitamin D.[54]

Table 58.3 lists boron's effect on selected hormones in either animals or humans. Some of these interactions have only been demonstrated in animal models, whereas others have not been demonstrated unequivocally to date in all age and gender segments of the human population.

CLINICAL APPLICATIONS

Osteoporosis

A considerable body of evidence has shown that both the compositional and functional properties of bone are affected by boron status.[55] In experimental animals, histological findings suggest that supplemental boron enhances maturation of the growth plate.[39] Boron has also been found at the highest concentrations in growing and calcifying areas of long bones.[9] In two human studies, boron deprivation caused changes in indexes associated with calcium metabolism in a manner that could be construed as being detrimental to bone formation and maintenance; these changes were enhanced by a diet low in magnesium.[9,39] The author concluded that boron and magnesium are apparently necessary for optimal calcium metabolism and are thus necessary to prevent the excessive bone loss that often occurs in postmenopausal women and older men.[39]

At the cellular level, in vitro studies have shown that boron may play a role in the regulation of proteins in the morphogenesis of bone, including BMP-4, -6 and -7, as well as the mRNA expression of several extracellular matrix proteins.[56] This is consistent with animal-based studies that suggest boron deprivation causes deficits in bone modeling and remodeling by inhibiting bone formation.[57,58]

Osteoarthritis, Rheumatoid Arthritis, and Regulation of Inflammation

A dietary boron deficiency may be a contributing factor in some cases of arthritis.[59] In areas of the world where boron intake is routinely 1 mg/day or less, the estimated incidence of arthritis ranges from 20% to 70%, whereas in areas where boron intake ranges from 3 to 10 mg/day, the estimated incidence of arthritis ranges from 0% to 10%.[59]

Analytic evidence indicates that persons with arthritis have lower boron concentrations in femur heads, bones, and synovial fluid compared with persons without this disorder.[7]

In 1961 the first anecdotal evidence suggesting that boron may be beneficial for osteoarthritis was presented when one patient had reduced swelling and stiffness and remained symptom-free for 1 year after supplementation with 3 mg of elemental boron twice daily for 3 weeks. A human study also offered evidence that boron supplementation might be beneficial in the treatment of this condition. In a double-blind, placebo-controlled trial of 20 subjects with osteoarthritis, 50% of subjects receiving a daily supplement containing 6 mg of boron noted a subjective improvement in their condition. Only 10% of those receiving placebo improved during the same time interval. Greater improvement was noted in the condition of all joints ($P < 0.01$), as well as less pain on movement ($P < 0.001$), in subjects receiving the boron supplementation.[60]

Clinical observations indicate that children with juvenile arthritis (Still disease) improve with boron supplementation (6–9 mg/day) in 2 to 3 weeks, whereas adults with osteoarthritis may require 2 to 4 months of supplementation before benefits are detected. Persons with rheumatoid arthritis may experience an aggravation of symptoms for 1 to 3 weeks but generally notice improvement within 4 weeks of beginning boron supplementation.[7]

Boron also has been implicated as a potential regulator of inflammatory and immune responses, possibly explaining its benefit for arthritic conditions. For example, boric acid has been shown to inhibit lipopolysaccharide-induced tumor necrosis factor-α formation.[61] Other animal-based studies support an anti-inflammatory role for boron.[62,63]

Prostate, Lung, Breast, and Cervical Cancer

Given that boron may act as a possible modifier of the hormones testosterone and estrogen, some studies have investigated its possible role in prostate cancer. One controlled case study compared the boron intake of 95 prostate cancer cases with that of 8720 male controls. After controlling for age, race, education, smoking, body mass index, dietary caloric intake, and alcohol consumption, this epidemiological screening found the risk of prostate cancer to be inversely proportional to dietary intake of boron in a dose-responsive manner.[64] A more recent study compared the prevalence of prostate cancer among those with high exposure to boron to control populations, and although no significant difference in prevalence was found, they did find a lower prevalence of elevated prostate-specific antigens (PSAs). The authors concluded that boron may interfere with processes related

TABLE 58.3 Boron's Observed Effect on Selected Hormones

Hormone	Increases	Decreases
Alkaline phosphatase		X
Aspartate transaminase[a]		X
Calcitonin		X
Cholecalciferol		X
Creatine kinase[a]	X	
17 Beta-estradiol[b]	X	
Insulin[a]		X
Superoxide dismutase		X
Testosterone[b]	X	
Thyroxine[a]		X

[a]These interactions have only been demonstrated in animal models.
[b]These interactions have not been demonstrated unequivocally to date in all age and gender segments of the human population.

to hyperplasia and carcinogenesis.[65] This is consistent with previous research that purported that boron may act as an inhibitor of PSA. It is thought that uninhibited PSA cleaves insulin-like growth factor binding protein-3, providing increased local levels of insulin-like growth factor 1, which then leads to tumor growth. Most epidemiological studies support a preventative effect of higher dietary boron, although not all do (the only exception used a different database for the boron content of foods).[66–68]

Some in vitro research observed that boric acid inhibits the proliferation of both the hormone-dependent and hormone-independent human prostate cancer cell lines in a dose-dependent manner.[69] In one study, three groups of 10 mice were implanted subcutaneously with human prostate adenocarcinoma cells. Two of the murine groups were given boric acid solutions (at 1.7 or 9 mg/kg of boron per day) by gavage, whereas the control group received only water. After 8 weeks, tumor sizes in the boric acid groups decreased by 38% and 25%, respectively, and serum PSA levels decreased by 88.6% and 86.4%, respectively, compared with the control group. Additionally, significantly less mitotic figures were seen in the boric acid groups. Immunohistochemical studies demonstrated significantly less expression of insulin-like growth factor 1 levels, although circulating levels were unchanged among the groups.[70]

Multiple mechanisms in addition to PSA inhibition have been suggested for boron's chemopreventative properties, including inhibition of stored calcium release from ryanodine receptor agonist sites and induction of apoptosis.[71,72]

This may explain its reported benefit for other cancers as well, for in addition to prostate cancer, diets with greater amounts of boron have been associated with reduced risks of cervical cancer (cytopathologic findings) and lung cancer among women.[73] A dose-dependent risk for lung cancer was seen among women, with those consuming the lowest amounts of boron having a nearly twofold greater risk compared with those in the highest quintile. Additionally, a diet low in boron heightened the risk for lung cancer among those using hormone replacement therapy.[74]

A double-blind, placebo-controlled study of 47 women with invasive ductal carcinoma treated with radiotherapy found that a boron-based gel statistically significantly relieved the pain and discomfort of radiation-induced dermatitis.[75]

Multiple cell-culture, animal, and pilot human studies have found benefit directly or as an adjunct in inhibition of tumor-induced angiogenesis, induction of cancer-cell apoptosis, treatment of multiple myeloma and non-Hodgkin lymphoma, and decreasing the adverse effects of chemotherapy.[76]

For these many reasons, boron-containing compounds are increasingly being used for cancer care, based on a variety of mechanisms.[77] Further human research is needed to determine the full clinical benefits of this interesting element in cancer prevention and treatment.

Wound Healing

Boron has been shown to significantly improve wound healing in humans. Application of a 3% boric acid solution to deep wounds reduced time in intensive care by two thirds.[78] In vitro research showed that a boric-acid solution improved wound healing through action on the extracellular matrix.[79] These beneficial effects are apparently due to the promotion of the activity of elastase, trypsin-like enzymes, collagenase, and alkaline phosphatase.

Dysmenorrhea

In a triple-blind randomized clinical trial study of 113 university students, 10 mg/day of boron from 2 days before the menstrual flow until its third day resulted in significant reduction in severity and duration of pain. The study followed the women for only two cycles, which is unfortunate because the improvement increased by cycle, suggesting that further benefit above the 20% found in the second cycle may be possible.[80]

DOSAGE

The optimal dose of boron for the prevention of osteoporosis and proper physiological function appears to be between 1 and 6 mg/day. It is best to obtain boron by means of a diet abundant in fruits, vegetables, legumes, and nuts, but persons whose diets are limited may need a supplement containing 1 to 3 mg of elemental boron. In patients with either osteoarthritis or rheumatoid arthritis, a trial period of 2 to 4 months with a dose of 3 mg of boron two to three times daily seems to be indicated.

TOXICOLOGY

Although boron is potentially toxic to all organisms and, as boric acid and borax, has been used as a pesticide and food preservative, higher animals usually do not accumulate boron because of their ability to rapidly excrete it.[17] Authenticated cases of poisoning in humans have been few and have primarily been the result of accidental ingestion of insecticides and household products containing borates or use of large amounts of boric acid in the treatment of burns.[81]

Improper use of boric acid–containing antiseptics is still one of the most common causes of toxic accidents in newborns and infants. Because boric acid is readily absorbed through damaged skin, it should not be applied topically to extensive wounds.[21]

In animals, long-term low-level boron exposure has been shown to cause reduced growth, cutaneous disorders, and suppression of male reproductive system function.[82] Studies indicated that male rodents developed testicular atrophy with dietary exposure to boric acid above 4500 ppm and had decreased sperm motility at all exposure levels above 1000 ppm.[83]

Humans given 100 mg of boron intravenously or 270 mg of boric acid orally reported no discomfort and showed no obvious signs of toxicity.[84,85] Drinking water with high boron concentrations in Turkey has not been shown to cause untoward effects in humans exposed over multiple generations.[86] Airborne exposures to boron oxide and its hydration product, boric acid, have been reported to cause respiratory and eye irritation.[87] A fatal outcome was reported after ingestion of 1 g of boric acid by a child; however, adults have survived acute intakes of nearly 300 g.[88] The Food and Nutrition Board of the Institute of Medicine has established a Tolerable Upper Intake Level for boron of 20 mg/day for adults over 18 years of age and as low as 3 mg for children between 1 and 3 years of age.[89] One author suggested that plasma or serum concentrations greater than 300 ng/mL indicate an intake in excess of what is needed to prevent deficiency, whereas levels more than 1 mcg/mL indicate toxicity.[50]

Boron may also have the potential for toxicity during pregnancy. A study in a population of newborns exposed to normal environmental boron levels through pregnancy found a negative relationship between whole blood δ-aminolevulinic acid dehydratase activity and placental boron levels. This is certainly concerning given the neurotoxic effects caused by lead's inhibition of this same enzyme.[90]

Common signs and symptoms of acute boron toxicity (largely based on animal data) include nausea, as well as vomiting and diarrhea that are blue–green in color.[8,88] Other symptoms seen with acute exposure are abdominal pain, an erythematous rash involving both the skin and mucous membranes, stimulation or depression of the central nervous system, convulsions, hyperpyrexia, renal tubular damage, abnormal

liver function, and jaundice.[21] Increased urinary riboflavin excretion has also been reported subsequent to acute boric acid ingestion.[91] Symptoms of long-term intoxication include anorexia, gastrointestinal disturbances, debility, confusion, dermatitis, menstrual disorders, anemia, convulsions, and alopecia.[21] In three cases of work-related exposure, alopecia signs were reversed when boric acid exposure was reduced or eliminated.[92] Because it is excreted primarily in urine, caution should be used for those with impaired renal function.

Because of its ability to increase the excretion of boron, in cases of toxicity, *N*-acetylcysteine is the preferred intervention.[93]

DRUG INTERACTIONS

Boron supplementation may increase serum magnesium, testosterone, and estrogen levels. However, no adverse clinical effects have been reported.

SUMMARY

Although the skeletal response to boron is modified by other nutritional variables, such as calcium, magnesium, vitamin D, methionine, essential fatty acid intake, and arginine, considerable evidence indicates that both the compositional and functional properties of bone are affected by boron status at least somewhat independently of other variables.

Findings suggest that boron is an important nutrient not only for mineral metabolism but also for varied aspects of optimal health in humans, including influences on lipid membrane integrity and cellular signaling pathways. Although all published trials are not in agreement on the effect of boron supplementation on levels of 17 β-estradiol and testosterone, evidence strongly suggests that boron deficiency results in decreased levels in postmenopausal women, whereas supplementation tends to normalize levels in these same women. Boron's effect on sex hormones in other segments of the population is still equivocal, although recent evidence suggests it may increase free testosterone levels at least in men. Topically it has shown clinical benefit for treating some forms of vaginitis (see Chapter 224, Vaginitis, Vulvovaginitis and Vulvodynia). As the mechanisms for its action are more thoroughly understood, which now include influencing S-adenosylmethionine metabolism and regulation of osteogenesis and cellular signaling pathways, therapy with boron may become more targeted and specific.

On the basis of available information, boron appears to offer benefits in the prevention of osteoporosis and arthritis, as well as cancer prevention. It is also a safe and potentially effective mineral to consider in any treatment regimen for both rheumatoid and osteoarthritis, as well as several cancers.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Naghii MR, Samman S. The role of boron in nutrition and metabolism. *Prog Food Nutr Sci*. 1993;17:331–349.
2. Woods WG. An introduction to boron: history, sources, uses, and chemistry. *Env Health Perspect*. 1994;102(suppl 7):5–11.
3. Nielsen FH. The emergence of boron as nutritionally important throughout the life cycle. *Nutrition*. 2000;16:512–514.
4. Liao SF, Monegue JS, Lindemann MD, et al. Dietary supplementation of boron differentially alters expression of borate transporter (NaBCl) mRNA by jejunum and kidney of growing pigs. *Biol Trace Elem Res*. 2011;143(2):901–912. [Epub ahead of print].
5. Naghii MR, Mofid M, Asgari AR. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. *J Trace Elem Med Biol*. 2011;25:54–58.
6. Houng KH. The physiology of boron and molybdenum in plants. In: Okajima H, Uritani I, Houng HK, eds. *The Significance of Minor Elements on Plant Physiology. Taipei: ASPAC*. Food & Fertilizer Technology Center; 1975:61–66.
7. Newnham RE. The role of boron in human nutrition. *J Appl Nutr*. 1994;46:81–85.
8. McBride J. Banishing brittle bones with boron? *Agric Res*. 1987;35(10): 12–13. Nov/Dec.
9. Nielsen FH, Hunt CD, Mullen LM, et al. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J*. 1987;1:394–397.
10. Naghii MR, Wall PM, Samman S. The boron content of selected foods and the estimation of its daily intake among free-living subjects. *J Am Col Nutr*. 1996;15:614–619.
11. McBride J. Banishing brittle bones with boron? *Agric Res*. 1987:12–13.
12. Hunt CD, Meacham SL. Aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc: concentrations in common Western foods and estimated daily intakes by infants; toddlers; and male and female adolescents, adults, and seniors in the United States. *J Am Diet Assoc*. 2001;101:1058–1060.
13. Rainey CJ, Nyquist LA, Christensen RE, et al. Daily boron intake from the American diet. *J Am Diet Assoc*. 1999;99:335–340.
14. Hunt CD, Shuler TR, Mullen LM. Concentration of boron and other elements in human foods and personal-care products. *J Am Diet Assoc*. 1991;91:558–568.
15. Choi MK, Jun YS. Analysis of boron content in frequently consumed foods in Korea. *Biol Trace Elem Res*. 2008;126:13–26.
16. Nielsen FH. Boron—an overlooked element of potential nutritional importance. *Nutr Today*. 1988;23:4–77.
17. Loomis WD, Durst RW. Chemistry and biology of boron. *Biofactors*. 1992;3:229–239.
18. Zittle CA. Reaction of borate with substances of biological interest. *Adv Enzymol Relat Subj Biochem*. 1951;12:493–527.
19. Fort DJ, Rogers RL, McLaughlin DW, et al. Impact of boron deficiency on Xenopus laevis. A summary of biological effects and potential biochemical roles. *Biol Trace Elem Res*. 2002;90:117–142.
20. Nielsen FH, Penland JG. Boron deprivation alters rat behaviour and brain mineral composition differently when fish oil instead of safflower oil is the diet fat source. *Nutr Neurosci*. 2006;9:105–112.
21. Nielsen FH. Ultratrace minerals: boron. In: Shils ME, Young VR, eds. *Modern Nutrition in Health and Disease*. Philadelphia: Lea & Febiger; 1988:281–283.
22. Park M, Li Q, Shcheynikov N, et al. NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. *Mol Cell*. 2004;16:331–341.
23. n: Reynolds JE, ed. *Martindale: The Extra Pharmacopoeia*. London: Royal Pharmaceutical Society;1680.
24. Moseman RF. Chemical disposition of boron in animals and humans. *Environ Health Perspect*. 1994;102:113–117.
25. Barr RD, Barton SA, Schull WJ. Boron levels in man: preliminary evidence of genetic regulation and some implications for human biology. *Med Hypotheses*. 1996;46:286–289.
26. Dupre JN, Keenan MJ, Hegsted M, et al. Effects of dietary boron in rats fed a vitamin D-deficient diet. *Environ Health Perspect*. 1994;102:55–58.
27. Nielsen FH. Boron deprivation decreases liver S-adenosylmethionine and spermidine and increases plasma homocysteine and cysteine in rats. *J Trace Elem Med Biol*. 2009;23:204–213.
28. Hunt CD. Biochemical effects of physiological amounts of dietary boron. *J Trace Elem Exp Med*. 1996;9:185–213.
29. Massie HR, Whitney SJ, Aiello VR, et al. Changes in boron concentration during development and ageing of Drosophila and effect of dietary boron on life span. *Mech Ageing Dev*. 1990;53:1–7.
30. Nielsen FH. Is boron nutritionally relevant? *Nutr Rev*. 2008;66:183–191.
31. Penland JG. Dietary boron, brain function, and cognitive performance. *Environ Health Perspect*. 1994;102(suppl 7):65–72.
32. Nielsen FH, Mullen LM, Nielsen EJ. Dietary boron affects blood cell counts and hemoglobin concentrations in humans. *J Trace Elem Exp Med*. 1991;4:211–223.
33. Ali S, Pawa S, Abdulla M. Fulminant hepatic failure and its protection by boron. *J Hepatol*. 2003;38(suppl 2):190.
34. Pawa S, Ali S. Boron ameliorates fulminant hepatic failure by counteracting the changes associated with the oxidative stress. *Chem Biol Interact*. 2006;160:89–98.
35. Meacham SL, Taper LJ, Volpe SL. Effect of boron supplementation on blood and urinary calcium, magnesium, and phosphorus, and urinary boron in athletic and sedentary women. *Am J Clin Nutr*. 1995;61:341–345.
36. Nielsen FH. New essential trace elements for the life sciences. *Biol Trace Elem Res*. 1990;26–27:599–611.
37. Nielsen FH. Facts and fallacies about boron. *Nutr Today*. 1992;27:6–12.
38. Usuda K, Kono K, Iguchi K, et al. Hemodialysis effect on serum boron level in the patients with long term hemodialysis. *Sci Total Environ*. 1996;191:283–290.
39. Nielsen FH. Studies on the relationship between boron and magnesium which possibly affects the formation and maintenance of bones. *Magnes Trace Elem*. 1990;9:61–69.
40. Hunt CD. The biochemical effects of physiologic amounts of dietary boron in animal nutrition models. *Environ Health Perspect*. 1994;102(suppl 7):35–43.
41. Hunt CD, Herbel JL, Idso JP. Dietary boron modifies the effects of vitamin D3 nutrition on indices of energy substrate utilization and mineral metabolism in the chick. *J Bone Miner Res*. 1994;9:171–182.
42. Hunt CD. Dietary boron modified the effects of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. *Biol Trace Elem Res*. 1989;22:201–220.
43. Nielsen FH, Shuler TR, Gallagher SK. Effects of boron depletion and repletion on blood indicators of calcium status in humans fed a magnesium-low diet. *J Trace Elem Exp Med*. 1990;3:45–54.
44. Miljkovic D, Miljkovic N, McCarty MF. Up-regulatory impact of boron on vitamin D function – does it reflect inhibition of 24-hydroxylase? *Med Hypotheses*. 2004;63:1054–1056.
45. Beattie JH, Peace HS. The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women. *Br J Nutr*. 1993;69:871–884.
46. Nielsen FH. Biochemical and physiologic consequences of boron deprivation in humans. *Environ Health Perspect*. 1994;102(suppl 7):59–63.
47. Hunt CD, Herbel JL, Nielsen FH. Metabolic responses of postmenopausal women to supplemental dietary boron and aluminum during usual and low magnesium intake: boron, calcium, and magnesium absorption and retention and blood mineral concentrations. *Am J Clin Nutr*. 1997;65:803–813.
48. Meacham SL, Taper LJ, Volpe SL. Effects of boron supplementation on bone mineral density and dietary, blood, and urinary calcium, phosphorus, magnesium, and boron in female athletes. *Environ Health Perspect*. 1994;102:79–82.
49. Nielsen FH, Shuler TR, Zimmerman TJ, et al. Magnesium and methionine deprivation affect the response of rats to boron deprivation. *Biol Trace Elem Res*. 1988;17:91–107.
50. Nielsen FH, Stoecker BJ. Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone. *J Trace Elem Med Biol*. 2009;23:195–203.

51. Hunt CD, Herbel JL. Boron affects energy metabolism in the streptozotocin-injected, vitamin D3-deprived rat. *Magnes Trace Elem*. 1992;10:374–386.
52. Bakken NA, Hunt CD. Dietary boron decreases peak pancreatic in situ insulin release in chicks and plasma insulin concentrations in rats regardless of vitamin D or magnesium status. *J Nutr*. 2003;133:3577–3583.
53. Ferrando AA, Green NR. The effect of boron supplementation on lean body mass, plasma testosterone levels, and strength in male bodybuilders. *Int J Sport Nutr*. 1993;3:140–149.
54. Miljkovic D, Miljkovic N, McCarty MF. Up-regulatory impact of boron on vitamin D function—does it reflect inhibition of 24-hydroxylase? *Med Hypotheses*. 2004;63:1054–1056.
55. McCoy H, Kenney MA, Montgomery C, et al. Relation of boron to the composition and mechanical properties of bone. *Environ Health Perspect*. 1994;102:49–53.
56. Hakki SS, Bozkurt BS, Hakki EE. Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). *J Trace Elem Med Biol*. 2010;24:243–250.
57. Gorustovich AA, Steimetz T, Nielsen FH, et al. A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet. *Arch Oral Biol*. 2008;53:677–682.
58. Gorustovich AA, Steimetz T, Nielsen FH, et al. Histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet. *Anat Rec (Hoboken)*. 2008;291:441–447.
59. Newnham RE. Agricultural practices affect arthritis. *Nutr Health*. 1991;7:89–100.
60. Travers RL, Rennie GC, Newnham RE. Boron and arthritis: the result of a double-blind pilot study. *J Nutr Med*. 1990;1:127–132.
61. Cao J, Jiang L, Zhang X, et al. Boric acid inhibits LPS-induced TNF-alpha formation through a thiol-dependent mechanism in THP-1 cells. *J Trace Elem Med Biol*. 2008;22:189–195.
62. Bourgeois AC, Scott ME, Sabally K, et al. Low dietary boron reduces parasite (nematoda) survival and alters cytokine profiles but the infection modifies liver minerals in mice. *J Nutr*. 2007;137:2080–2086.
63. Armstrong TA, Spears JW. Effect of boron supplementation of pig diets on the production of tumor necrosis factor-alpha and interferon-gamma. *J Anim Sci*. 2003;81:2552–2561.
64. Cui Y, Winton MI, Zhang ZF, et al. Dietary boron intake and prostate cancer risk. *Oncol Rep*. 2004;11:887–892.
65. Müezzinoğlu T, Korkmaz M, Neşe N, et al. Prevalence of prostate cancer in high boron-exposed population: a community-based study. *Biol Trace Elem Res*. 2011;144(1-3):49–57.
66. Barranco WT, Hudak PF, Eckhert CD. Evaluation of ecological and in vitro effects of boron on prostate cancer risk (United States). *Cancer Causes Control*. 2007;18:71–77.
67. Cui Y, Winton MI, Zhang ZF, et al. Dietary boron intake and prostate cancer risk. *Oncol Rep*. 2004;11:887–892.
68. Gonzalez A, Peters U, Lampe JW, et al. Boron intake and prostate cancer risk. *Cancer Causes Control*. 2007;18:1131–1140.
69. Barranco WT, Eckhert CD. Boric acid inhibits human prostate cancer cell proliferation. *Cancer Lett*. 2004;216:21–29.
70. Gallardo-Williams MT, Chapin RE, King PE, et al. Boron supplementation inhibits the growth and local expression of IGF-1 in human prostate adenocarcinoma (LNCaP) tumors in nude mice. *Toxicol Pathol*. 2004;32:73–78.
71. Henderson K, Stella SL, Kobylewski S, et al. Receptor activated Ca(2+) release is inhibited by boric acid in prostate cancer cells. *PLoS One*. 2009;4:e6009.
72. Scorei IR. Calcium fructoborate: plant-based dietary boron as potential medicine for cancer therapy. *Front Biosci (Schol Ed)*. 2011;3:205–215.
73. Korkmaz M, Uzgören E, Bakirdere S, et al. Effects of dietary boron on cervical cytopathology and on micronucleus frequency in exfoliated buccal cells. *Environ Toxicol*. 2007;22:17–25.
74. Mahabir S, Spitz MR, Barrera SL, et al. Dietary boron and hormone replacement therapy as risk factors for lung cancer in women. *Am J Epidemiol*. 2008;167:1070–1080.
75. Aysan E, Idiz UO, Elmas L, et al. Effects of boron-based gel on radiation-induced dermatitis in breast cancer: a double-blind, placebo-controlled trial. *J Invest Surg*. 2017;30(3):187–192.
76. Pizzorno L. Nothing Boring About Boron. *IMCJ*. 2015;14(4):35–48.
77. Scorei RI, Popa Jr R. Boron-containing compounds as preventive and chemotherapeutic agents for cancer. *Anticancer Agents Med Chem*. 2010;10:346–351.
78. Blech MF, Martin C, Borrelly J, Hartemann P. Treatment of deep wounds with loss of tissue: value of a 3 percent boric acid solution [in French]. *Presse Med*. 1990;19(22):1050–1052.
79. Benderdour M, Van Bui T, Hess K, Dicko A, Belleville F, Dousset B. Effects of boron derivatives on extracellular matrix formation. *J Trace Elem Med Biol*. 2000;14(3):168–173.
80. Nikkhah S, Dolatian M, Naghii MR, et al. Effects of boron supplementation on the severity and duration of pain in primary dysmenorrhea. *Complement Ther Clin Pract*. 2015;21(2):79–83.
81. Locatelli C, Minoia C, Tonini M, et al. Human toxicology of boron with special reference to boric acid poisoning. *G Ital Med Lav*. 1987;9:141–146.
82. Minoia C, Gregotti C, Di Nucci A, et al. Toxicology and health impact of environmental exposure to boron. A review. *G Ital Med Lav*. 1987;9:119–124.
83. Chapin RE, Ku WW. The reproductive toxicity of boric acid. *Environ Health Perspect*. 1994;102(suppl 7):87–91.
84. Jansen JA, Anderson J, Schou JS. Boric acid single dose pharmacokinetics after intravenous administration to man. *Arch Toxicol*. 1984;55:64–67.
85. Jansen JA, Schou JS, Aggerbeck B. Gastro-intestinal absorption and in vitro release of boric acid from water-emulsifying ointments. *Food Chem Toxicol*. 1984;22:49–53.
86. Sayli BS, Tuccar E, Elhan AH. An assessment of fertility in boron-exposed Turkish subpopulations. *Reprod Toxicol*. 1998;12:297–304.
87. Garabrant DH, Bernstein L, Peters JM, et al. Respiratory and eye irritation from boron oxide and boric acid dusts. *J Occup Med*. 1984;26:584–586.
88. Von Burg R. Boron, boric acid, and boron oxide. *J Appl Toxicol*. 1992;12:149–152.
89. Food and Nutrition Board, Institute of Medicine, National Academies. Dietary reference intakes (DRIs): tolerable upper intake levels, elements https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summary-tables.t8/?report=objectonly. (Accessed 12/23/2018).
90. Huel G, Yazbeck C, Burnel D, et al. Environmental boron exposure and activity of delta-aminolevulinic acid dehydratase (ALA-D) in a newborn population. *Toxicol Sci*. 2004;80:304–309.
91. Pinto J, Huang YP, McConnell RJ, et al. Increased urinary riboflavin excretion resulting from boric acid ingestion. *J Lab Clin Med*. 1978;92:126–134.
92. Beckett WS, Oskvig R, Gaynor ME, et al. Association of reversible alopecia with occupational topical exposure to common borax-containing solutions. *J Am Acad Dermatol*. 2001;44:599–602.
93. Banner Jr W, Koch M, Capin DM, et al. Experimental chelation therapy in chromium, lead, and boron intoxication with N-acetylcysteine and other compounds. *Toxicol Appl Pharmacol*. 1986;83:142–147.
94. The original version of this chapter in a previous edition was reprinted with permission from *Alternative Medicine Review* 1997;2:48–56.

59

Bromelain[107]

Anthony J. Cichoke Jr., BS, BS, MA, MA, PhD, DC, DACBN

OUTLINE

Proteolytic enzyme of *Ananas comosus* (family: Bromeliaceae)
Synonyms: bromelin, plant protease concentrate

INTRODUCTION

Bromelain is a proteolytic (protein-lysing) enzyme found in the stem and the fruit of the pineapple plant *(Ananas comosus)*. Other proteolytic enzymes found in pineapple include ananain and comosain,[1] and there are several nonproteolytic enzymes, such as amylase, peroxidase, and acid phosphatase.[2]

Most bromelain on the market is stem bromelain (which itself is composed of perhaps up to six different types of enzymes, including bromelain A and B).[3] Bromelain is widely used in food processing to prepare precooked cereals, produce protein hydrolysates, chill-proof beer, and tenderize meat; in cosmetics; in diagnostic laboratories; in pharmaceuticals; and in over-the-counter nutritional supplements. Best known as a digestive aid, bromelain supplements, when used systemically, are also used to treat the degenerative effects of aging (including osteoarthritis),[4] several types of cancer,[5–11] circulatory disorders,[5,12–18] dysmenorrhea,[19] immune disorders,[20,21] infections,[22–25] inflammatory diseases and conditions,[26–39] and respiratory tract diseases.[42–45] Topical applications include cosmetics and skin debridement formulations.

Bromelain is a sulfur-containing enzyme, with an optimum pH activity between 3.0 and 8.0.[46] Bromelain has endoproteolytic and catalytic activity and predominantly splits peptides, amides, and ester bonds involving lysine, alanine, tyrosine, glycine, and other basic amino acids.[47]

The extraction of bromelain is usually accomplished by fractionated ultrafiltration and subsequent lyophilization of the juice pressed from the stem of ripe pineapples (frequently from *Ananas comosus* of the cayenne variety). However, cores, leaves, and other waste products remaining after pineapple processing also yield a certain amount of the enzyme.[48]

Bromelain activity within the stem of the pineapple can vary widely depending on the age of the plant, as well as on the storage duration and conditions. A Thai study found that 3-year-old peeled pineapple stems contained substantially more activity than second- and first-year stems as measured in casein-digesting units (CDUs)—48.76% compared with 32.0% and 19.3%, respectively.[49] In addition, storage duration before considerable loss of activity occurred was limited to 3 days at room temperature or 2 weeks in a room cooled to 5°C (41°F).[49]

BROMELAIN SUPPLEMENTS

Hundreds of medical articles on bromelain's therapeutic applications have been published since it was introduced as a medicinal agent in 1957.[50,51] Initially, many studies employed Ananase (Rorer; Washington, PA), an enterically coated bromelain tablet. Since then, studies have included bromelain in virtually every available form including tablets, chewable tablets, capsules, powder, and ointment. In addition, many studies use bromelain in combination with other enzymes or with various vitamins, minerals, and herbs, for wider application.

Bromelain activity is measured in "units," and there are several types of units currently in use. Most bromelain supplements sold in the United States are measured in gelatin-dissolving units (GDUs), although milk-clotting units (MCUs), Fédération Internationale du Pharmaceutiques units (FIPs), and CDUs are also used. However, these measurement methods are neither interchangeable nor comparable. In addition, the activity of any bromelain supplement might be expressed per tablet or capsule or per gram, making comparison between different supplements difficult.

BROMELAIN AS A DIGESTIVE AID

Bromelain has a long history of use as a digestive aid. By the time Columbus and his men arrived in America, natives of Central and

South America were already using bromelain-rich pineapple juice to cure bellyaches and improve digestion. As a proteolytic enzyme, bromelain aids in the digestion of protein foods. Because of bromelain's wide pH activity, it is effective on substrates in the acidic stomach as well as the alkaline small intestine. To aid in digestion, bromelain supplements should be taken with, or just before, a meal. Enzyme supplements are also effective against numerous diseases and conditions when taken between meals in a method referred to as systemic enzyme therapy.

SYSTEMIC ENZYME THERAPY

Bromelain—when taken systemically—can help slow many of the degenerative effects of aging[4,51]; inhibit cancer growth and metastasis[5–10]; prevent the aggregation of blood platelets[5,12–18] and thereby help prevent heart attacks, strokes, and other cardiovascular conditions; improve digestive problems[51–53]; treat dysmenorrhea[19,54]; improve the symptoms of immune conditions[38]; fight infections[22–24,26,55]; effectively speed up healing from inflammatory diseases and conditions[26–39]; fight sinusitis and other respiratory tract diseases[44,45]; and improve antibiotic absorption.[54–56]

To be effective systemically, many enzymes are enterically coated to prevent them from breaking down in the acidic stomach. Enzymes with wide pH, such as bromelain, can also be taken between meals. In this way, they are able to move through the digestive system and into the small intestine, pass through the brush border into the circulatory system, and be used by organs and cells in the body. To be most effective, systemically, it is essential to take enzymes on an empty stomach, approximately 1.5 hours before or after a meal (when used for digestive therapy, enzymes should be ingested approximately 30 minutes before meals). See the "Dosage" section of this chapter.

In the past, it was widely assumed that large protein molecules—such as enzymes—could not survive the digestive process and be reabsorbed intact. However, numerous studies have demonstrated that bromelain—and other proteolytic enzymes—can be absorbed by the small intestine through a number of routes, pass into the bloodstream, and ultimately circulate throughout the body.[57–59] Oral administration is effective, as is parenteral administration.[26,59–61]

Research on dogs indicated that levels of bromelain (after oral administration) peaked at 10 hours and were still detectable after 48 hours, whereas intravenous infusion peaked in 50 minutes and was detectable for 5 hours.[60]

A randomized, controlled, double-blind study on humans demonstrated that the highest plasma concentration of bromelain was achieved at approximately 48 hours.[57,58] A study of 19 men given 3 grams of oral bromelain daily indicated that the enzyme had a plasma half-life of 6 to 9 hours. Researchers estimated that within the 3- to 50-hour period, an average of 10.8 mcg of bromelain was present in plasma.[57] In both animals and humans, studies indicated that up to 40% of the absorbed orally administered bromelain was absorbed intact.[59–61] For an in-depth discussion of enzyme absorption, please refer to Chapter 100 on pancreatic enzymes.

CLINICAL APPLICATIONS

When used in systemic enzyme therapy, bromelain exerts a wide variety of therapeutic effects[26,50,51]:

- Fights many of the disorders associated with aging (e.g., digestive problems,[51] osteoarthritis,[4] and cancer[5–10])
- Assists digestion[51–53]
- Prevents and treats cancer[5–10,62]
- Inhibits blood platelet aggregation[5,12–18]
- Reduces pain[17,63–65]
- Relaxes smooth muscles[12]
- Stimulates the immune system[20,21]
- Fights inflammation[26–33]
- Reduces swelling[28,63,64]
- Improves wound healing[29]
- Relieves sinusitis[44,45]
- Debrides burns and wounds[66–68]
- Improves antibiotic absorption[54–56]

Aging

Unfortunately, the process of aging is accompanied by an increasingly wide variety of health problems, including chronic disorders, cancer, cardiovascular disease, osteoporosis, and arthritis. Further, the number of Americans over 65 is increasing every year. According to the US Census Bureau, over 20% of the American population will be age 65 or older by the year 2050. That figure was only 12% in 2008.[69]

Most commonly, the body loses resiliency as it ages and various degenerative diseases manifest. This is accompanied by a decline in hormone secretion,[70,71] immune system function,[71,72] and enzyme production,[73,74] thus reducing the body's effectiveness at fighting off foreign invaders. The symptoms of aging could be, at least partially, the consequence of these enzymatic reductions. For example, the graying of hair has been attributed to a lack of tyrosinase or a loss of its activity level.

Unlike the known serious side effects of most prescription drugs, bromelain has been shown to effectively fight many of the disorders associated with aging, including digestive problems,[49] osteoarthritis,[4] and cancer,[5–10] but without the serious and long-term side effects of many drugs.

Bromelain is also widely incorporated into lotions, creams, scrubs, facial masks, and other skin care products, where it is believed to improve the skin's appearance by removing dead skin cells.

Cancer

The standard of care for treating cancer has long included surgery, chemotherapy, and radiation, although hormone therapy, stem cell transplantation, and biological therapy (which includes immunotherapy) are increasingly being used. Treatment with bromelain is one form of biological therapy showing considerable promise in the treatment of cancer by preventing its growth,[5] delaying or preventing its metastasis,[6–9] and in some cases, exhibiting a cytotoxic effect on cancer cells.[9,10] In addition, in vitro studies show that bromelain can enhance the therapeutic value of several chemotherapeutics[8,62,75] and can alleviate some of the side effects of hormone therapy in patients with breast cancer.[76]

Skin cancer is the most common form of cancer in the United States.[77] To determine whether bromelain could prevent skin cancer, researchers gave hairless mice 20 mg of bromelain per kilogram of body weight per day for 1 year and then subjected them to ultraviolet light for 15 minutes three times per week for the same period[11] (it is generally agreed that exposure to ultraviolet rays increases an individual's risk of skin cancer). After 1 year, only 40% of the bromelain group developed skin cancer (as opposed to 100% of the control group). In addition, it took the bromelain group twice as long to develop lesions. Another study, conducted over a 6-month period, found that mice receiving 80 mg of bromelain per kilogram of body weight did not develop any abrasions after 2 months.[5]

Bromelain also appears effective at reducing cancer metastasis. An in vitro study on bromelain and glioma (primary brain tumor) cells

found that bromelain significantly and reversibly reduced the migration, adhesion, and invasion of glioma cells.[6] In this study, bromelain did not appear to have a cytotoxic effect, even after a 3-month treatment period.

An in vitro study on four different gastrointestinal cancer cell lines found that treatment with bromelain significantly inhibited their spread.[7] Another study determined that a combination of bromelain and N-acetylcysteine (NAC) was significantly more effective at inhibiting the growth and spread of cancer cells than either bromelain or NAC alone.[8]

A study on human cholangiocarcinoma (CC) cell lines found that bromelain treatment reduced cancer cell viability and inhibited the ability of those cells to migrate and invade.[9] Treatment with bromelain also induced apoptosis, an important function of most, if not all, cancer therapies.[78]

Particularly encouraging are animal studies suggesting antimetastatic properties and inhibition of platelet aggregation associated with metastasis, as well as inhibition of invasiveness and growth of tumor cells. For example, an in vivo study on mice found that bromelain significantly increased the animals' survival time in five of the six tumor lines evaluated.[79] Researchers transplanted the tumor cell lines (leukemia P-388, sarcoma S-37, Ehrlich ascites tumor cells [EAT], Lewis lung carcinoma cells [LLC], M-B16F10 [MB-F10] melanoma, and ADC-755 mammary adenocarcinoma cells) into the mice and 24 hours later administered bromelain either intraperitoneally, intramuscularly, or subcutaneously (depending on tumor type). The life expectancy of the mice treated with bromelain increased compared with the untreated mice or those treated with the control (5-fluorouracil) in all cases except those transplanted with MB-F10 melanoma (the researchers considered bromelain's effect on this tumor cell line to be a nonsignificant increase in survival).

Pharmacological and preclinical studies indicate that bromelain acts as an immunomodulator by inducing the production of distinct cytokines (such as interleukin[IL]-1β, IL-6, and IL-8, as well as tumor necrosis factor-γ), and by raising the impaired immunocytotoxicity of monocytes against tumor cells.[10,26,80–83] These findings were partially confirmed in a study involving 16 mammary tumor patients.[80] In that study, bromelain had a significant effect on monocytes, natural killer cells, and lymphocytes. In those patients who responded to bromelain treatment, monocyte cytotoxicity increased from 7.8% to 54% in b-macrophage-activated killer cells (bMAK-cells) and 16% to 47% in macrophage-activated killer cells (MAK-cells). Another study on breast cancer cell line MCF-7 found that treatment with bromelain significantly decreased the viability of the MCF-7 cells.[84] The researchers found that bromelain's strength was similar to that of taxol.

Bromelain appears to work synergistically with chemotherapeutics and improves their therapeutic efficacy.[62,75] Researchers conducted an in vitro study on gastrointestinal cancer and found that bromelain in combination with N-acetylcysteine potentiated the effect of numerous chemotherapeutics.[75]

An in vitro study on breast cancer cell line MDA-MB-231 found that bromelain in combination with cisplatin induced apoptosis to a higher degree than either bromelain or cisplatin when used as a single agent.[62] Most encouraging was the determination that the addition of bromelain significantly enhanced the activity of cisplatin, allowing for a reduction in the necessary dose of cisplatin, thereby potentially reducing its sometimes-serious side effects.

In addition to its effect on cancer cell growth and metastasis, bromelain may relieve some of the adverse side effects of hormone therapy in the treatment of breast cancer.[76,85] Bromelain in combination with papain, sodium selenite, and *Lens culinaris* lectin was given to 680 women receiving hormone therapy as an adjuvant treatment for breast cancer.[77] After 4 weeks of therapy, those suffering from adverse effects of the hormone therapy (which often include arthralgia and mucosal dryness) experienced a significant reduction in the severity of their symptoms.

A number of mechanisms may be responsible for bromelain's effect on cancer. Some researchers believe that bromelain's proteolytic activity may be responsible for its effect on tumor cells,[6,84] whereas others believe its effectiveness may be due to its other components.[10,26,80]

Circulatory Disorders

Research shows that bromelain is a potent inhibitor of platelet aggregation, both in vivo and in vitro.[5,12–18] Platelet aggregation is a major factor in atherogenesis and can result in heart attack, stroke, acute thrombophlebitis, transient ischemic attack, nightly leg cramps, edema, deep venous thrombosis, ecchymosis, and cellulitis. Bromelain's action could be due to its plasmin-increasing effects (plasmin is a proteolytic enzyme produced by the body whose job is to dissolve fibrin, a protein involved in blood clot formation).[86] Further, research demonstrates that bromelain (in conjunction with potassium and magnesium) can be effective in treating angina pectoris.[18,87]

An ex vivo study on rats found that bromelain offers cardioprotective activity.[88] The rats received an intraperitoneal injection of bromelain twice daily for 15 days before their hearts were removed and ischemia was induced in the heart. Researchers concluded that the bromelain treatment reduced the size of the infarct, limited injury to the myocardium, and thereby, enhanced cardiac function.

Researchers employed bromelain as an adjunct to analgesics in a double-blind study involving 73 patients with acute thrombophlebitis.[17] All symptoms of inflammation, including pain, swelling, tenderness, redness, disability, and elevated skin temperature, decreased. The common daily dose of bromelain (in this study and others) was 60 to 160 mg of 1200 MCU bromelain. Other researchers believe that doses of 400 to 800 mg are necessary in treating patients with thrombophlebitis (and many other conditions as well).[18]

Digestive Disorders

As mentioned previously, when taken with or just before meals, bromelain supplements can regulate digestion. As a proteolytic enzyme, bromelain improves the digestion of protein-containing food. When taken systemically, bromelain is effective in treating pancreatic insufficiency (and is a proven substitute for trypsin or pepsin in cases of pancreatic insufficiency and postpancreatectomy).[51] Researchers in one study found that nearly 22% of subjects over the age of 60 years had pancreatic exocrine insufficiency.[74] Double-blind studies determined that bromelain is particularly effective at treating pancreatic insufficiency when combined with ox bile and pancreatin.[52,53]

Dysmenorrhea

Bromelain in combination with papain is an effective treatment for dysmenorrhea.[19] Bromelain decreases the spasms of a contracted cervix in tested patients, so it is believed to be a smooth muscle relaxant. As-yet-unidentified substances in bromelain may be more beneficial in this regard than the bromelain protease; when isolated and purified, the protease failed to produce a similar effect. Bromelain increases levels of prostaglandin-E (PGE_1)-like compounds while decreasing prostaglandins of the 2-series (e.g., PGE_2 and PGF_{2a}), which is hypothesized to be the basis for bromelain's muscle-relaxing ability.[50]

Endometriosis is a frequent cause of dysmenorrhea and occurs when endometrial tissue grows outside of the uterus. Researchers conducted in vitro and in vivo tests to assess the effect of a combination of N-acetyl cysteine, alpha-lipoic acid, and bromelain on endometriosis.[54] The study results showed that the combination not only

resulted in the developed of fewer and smaller cysts but also had an anti-inflammatory effect by inhibiting the activation of vascular cell adhesion protein 1 (VCAM-1).

Immune Disorders

Bromelain appears to have immunomodulatory effects on immune and autoimmune disorders.[20,21] Understanding and treating immune disorders is becoming increasingly important because these disorders include many chronic conditions. (Please see the "Respiratory Tract Diseases" section of this chapter for a discussion of bromelain and respiratory conditions, including asthma.)

Two of the most devastating immune disorders are immune complex hypersensitivities (e.g., rheumatoid arthritis and glomerulonephritis) and autoimmune disorders (e.g., systemic lupus erythematosus). Immune complex hypersensitivities occur when antigens and antibodies bind, forming a cluster that can lodge in tissues or circulate throughout the body, leading to inflammation and tissue damage. Circulating immune complexes seem to be a major component of numerous immune disorders. Various factors inhibit cellular immunity, but the most important elements seem to be these "blocking factors" (i.e., immune complexes) that cause immune suppression. Autoimmune disorders occur when the body's immune system mistakenly attacks healthy cells. There are dozens of such conditions, including type 1 diabetes mellitus, multiple sclerosis, myasthenia gravis, and systemic lupus erythematosus. Crohn's disease and ulcerative colitis are two types of autoimmune disease, collectively referred to as inflammatory bowel disease (IBD). Researchers conducted an in vitro study to measure the effect of bromelain on the secretion of proinflammatory cytokines in IBD.[38] They determined that bromelain treatment significantly decreased the secretion of cytokines (including granulocyte colony-stimulating factor [G-CSF], interferon gamma [IFN-γ], and tumor necrosis factor [TNF]) and chemokines (including macrophage inflammatory protein-1ß [MIP-1ß]). According to the researchers, the secretion of these proinflammatory substances was due to bromelain's proteolytic activity.

Systemic enzyme therapy can stimulate the body's own defenses, accelerate the inflammatory process, and break down pathogenic immune complexes from the tissues, bringing them into the bloodstream for elimination. It is important to note that because enzyme use liberates immune complexes from the tissues, increasing their presence in the bloodstream, symptoms may actually increase in severity; however, this is only temporary. When used in sufficient quantities, the enzymes can then degrade the immune complexes in the bloodstream (sometimes within a few hours), and the disease symptoms should subside.

Infections

Infections caused by bacteria, viruses, fungi, and parasites can frequently be the most challenging conditions to treat. Bromelain has proven effective in fighting numerous infections, ranging from *Escherichia coli* to bronchitis (see the "Respiratory Tract Diseases" section later in this chapter).

The O157:H7 strain of *E. coli* is a particularly severe and potentially life-threatening bacterial infection that causes nausea, vomiting, and bloody diarrhea in humans and can lead to kidney failure and death. The *E. coli* enterotoxins gain a foothold by adhering to receptors on the intestinal mucosa. Preventing the attachment could also prevent the resulting diarrhea. Researchers administered a single dose of enterically coated protease granules (containing bromelain) to rabbits and then inoculated the animals with different strains of *E. coli*.[22] Of those rabbits given the enzyme mixture and strain H10407, only one animal died, and none of the remaining rabbits developed diarrhea. In the group not inoculated with the enzyme mixture, 87% either developed severe diarrhea or died.[22]

Comparable results were obtained with other bacterial infections. For example, similar studies with bromelain have been conducted on piglets inoculated with K88 positive *E. coli,*[24] and an in vitro study on rabbit ileum found that bromelain was also 51% effective against cholera toxin.[23]

Antibiotics are often the drug of choice to treat bacterial infections. Some studies indicate that bromelain may be as effective as antibiotics in treating numerous infectious processes, including bronchitis, perirectal abscess, pyelonephritis, and cutaneous staphylococcus infection.[26,55] In addition if, and when, treatment with antibiotics becomes necessary, studies show that bromelain can increase serum levels of several antibiotics (including tetracycline, amoxicillin, and penicillin) in different body fluids (including blood, cerebral spinal fluid, mucus, sputum, and urine) and tissues (including appendix, gallbladder, ovary, epithelium, and uterus).[54–56]

Bromelain also shows effectiveness against antibiotic-resistant bacteria, including strains of *Staphylococcus aureus*.[89] *S. aureus* is a gram-positive bacterium responsible for a number of infections, including skin infections (staph infections). Drug-resistant strains of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA), have rendered the infection particularly difficult to treat. Researchers evaluated the effect of bromelain and other enzymes on drug-resistant *S. aureus* biofilms and found that, after a 2-hour treatment with bromelain, the *S. aureus* biomass had been reduced by up to 98%.[89] The researchers concluded that treatment with enzymes may be a beneficial therapy against infections caused by drug-resistant bacteria.

Tuberculosis (TB) is another bacterial infection that appears to respond to bromelain treatment.[25] In an in vitro study, researchers evaluated stem bromelain's effect on murine and human macrophages infected with *Mycobacterium tuberculosis,* the bacterium responsible for TB.[25] Study results show that bromelain treatment reduced the production of foamy macrophages and inhibited the bacteria's viability. Foamy macrophages are produced as part of the inflammatory response and may play an important role in the progression of TB.[90]

Bromelain may also play a role in preventing periodontal disease.[91] An in vitro study found that a bromelain solution restricted the growth of several pathogens, including *Streptococcus mutans, Enterococcus fecalis, Aggregatibacter actinomycetemcomitans,* and *Porphyromonas gingivalis,* implicated in the formation of periodontal disease.[91] The researchers hypothesized that bromelain may have prevented the pathogens from adhering to periodontal tissues.

Inflammatory Diseases and Conditions

The ability of bromelain to decrease inflammation has been documented in various clinical studies and experimental models.[26–39] This anti-inflammatory action is beneficial because the inflammatory process is involved in numerous conditions, including injuries, infections, respiratory tract diseases, and arthritis (many of these conditions are covered separately within this chapter). In addition, numerous studies have noted bromelain's ability to reduce pain, swelling, and bruising and improve the rate of healing after various surgical procedures.[26–30,92] Unlike the majority of nonsteroidal anti-inflammatory drugs (NSAIDs), there seems to be no indication that bromelain promotes bleeding; however, because bromelain inhibits platelet aggregation, it is logical to assume that it *might* increase the risk of bleeding, so care should be exercised (see the section on drug interactions in this chapter).

Some of the earliest studies on bromelain involved athletic injuries. A 1960 study of boxers highlighted bromelain's rehabilitative effects.[29]

Within 4 days of initiating bromelain treatment, 58 of 74 boxers experienced complete resolution of all signs of bruising. Complete clearance of bruising occurred in 8 to 10 days in the remaining 16 boxers receiving bromelain. At the end of 4 days, only 10 boxers in the control group demonstrated complete clearance of bruising, whereas clearance in the remaining 62 in the control group required 7 to 14 days. It is important to note that bromelain's effect on pain reduction is probably not due to any direct analgesic effect but rather to its ability to reduce tissue edema and inflammation.

Fifty-nine patients with muscle strains, contusions, ligament tears, and other injuries to the musculoskeletal system received 500 mg of bromelain three times daily, 30 minutes before meals.[31] Results from this study and others indicate that the use of bromelain led to a rapid improvement of pain at rest and during motion, swelling, inflammation, and tenderness on palpation.[63,64]

Seventy-seven otherwise healthy subjects completed a study investigating bromelain's effect on mild acute knee pain of less than 3 months' duration.[32] Two validated questionnaires (the Psychological Well-Being Index and the Western Ontario and McMaster Universities [WOMAC] Knee Health Index) were completed as a baseline and after 1 month of bromelain treatment (200 or 400 mg/day, allocated randomly to volunteers). In both bromelain groups, all WOMAC symptom scores decreased significantly compared with baseline. Reductions in the total symptom score were 41% for the low-dose group of bromelain recipients and 59% in the high-dose group. Additionally, those receiving 400 mg/day of bromelain experienced significantly greater improvement in total symptom score plus stiffness and physical function dimensions compared with those receiving low-dose (200 mg/day) bromelain. Both bromelain groups experienced a significant improvement in overall psychological well-being compared with baseline (researchers noted a dose-dependent relationship).

Researchers in a study on knee osteoarthritis found that 500 mg per day of bromelain (2000 GDU) taken for 4 weeks was as effective as diclofenac (a commonly prescribed NSAID) in reducing WOMAC scores.[4] The diclofenac arm of the study was terminated at week 4 because of ethical reasons relative to side effects, whereas the group receiving bromelain continued the study for 16 weeks. The researchers deemed bromelain treatment to be safe.

Surgery

Numerous studies indicate bromelain's effectiveness at reducing postsurgical inflammation.[28,30,93,94] A double-blind study of patients who underwent oral surgery found that bromelain was significantly superior to placebo in reducing swelling (3.8 days compared with 7 days for the placebo).[28] In addition, the duration of pain decreased to 5.1 days in the bromelain group compared with 8.1 days for the placebo group.[27,28] The researchers concluded that although postsurgical medication alone was effective, a regimen of presurgical and postsurgical bromelain was recommended to enhance the healing process.

Bromelain is also effective at relieving the pain and edema that typically follow wisdom tooth extraction.[65,94] Researchers in a study of 40 patients found that 70% experienced reduced swelling and pain after taking bromelain.[65] Researchers in another study of 46 patients found that bromelain was as effective as ketoprofen (an NSAID).[94]

Similar observations were made in studies of episiotomy cases. Several studies show that bromelain reduced pain and edema after episiotomy.[30,92,93] In addition, preoperative administration of bromelain improved the potential positive effects.[30,93]

Anti-inflammatory Action of Bromelain

Bromelain may function as an anti-inflammatory by (1) activating proteolytic activity at the inflammation site, (2) lysing fibrin at the site (fibrin is a protein required for blood clotting), (3) depleting kininogen (a kinin precursor; kinin causes blood vessels to dilate), and (4) inhibiting the biosynthesis of prostaglandins and the induction of prostaglandin E_1 accumulation (which, thereby, inhibits vasodilation).[33,48,81]

Bromelain's proteolytic activity demonstrates a delayed analgesic effect that involves the action of two mechanisms: (1) bromelain cleaves inflammatory mediators, including the kinins and prostaglandins, which directly stimulate the pain receptors; and (2) bromelain supports the breakdown of plasmin protein and immune complexes that stem from the tissues by cleaving them directly and by stimulating their phagocytosis. The reduction of edema that subsequently follows leads to a relief of pressure and, secondarily, a reduction in pain, thus inhibiting bradykinin formation (a chemical mediator of inflammation) at the inflammation site.

Of these processes, the primary pharmacological effects of bromelain are most likely plasmin activation and the decrease in kinin levels. As mentioned in the "Cancer" section, bromelain functions as an immunomodulator and effectively increases the levels of some cytokines while reducing the levels of others, thus leading to cytokine homeostasis and a reduction in inflammation.

Bromelain also exhibits anti-inflammatory activity when applied topically. Products are available containing bromelain (typically in combination with chondroitin and glucosamine) for the treatment of arthritis and joint/muscle pain. Other combinations containing bromelain are marketed for the relief of dry skin and eczema, back pain, and other inflammatory conditions.

Respiratory Tract Conditions

Respiratory tract conditions, including asthma, bronchitis, and sinusitis, are marked by increased mucus production, inflammation, and resulting pain. Bromelain has shown effectiveness against each of these symptoms.[42–44]

A double-blind study on patients with acute sinusitis found that 87% of those receiving bromelain experienced good to excellent results in symptom relief compared with 68% of the placebo group.[44] Most notable was the reduction of mucosal inflammation (83%–52%) and difficulty breathing (78%–68%) in the bromelain group compared with the placebo group. An epidemiological cohort study on children with acute sinusitis compared the symptom recovery time of those treated with bromelain, standard therapy (which included antibiotics, antihistamines, anti-inflammatories, and analgesics), or bromelain in combination with standard therapy.[45] The results of the study indicate that treatment with bromelain led to a statistically significant faster recovery time than with standard treatment or bromelain combined with standard treatment.

When given by intraperitoneal injection, bromelain has been found to exert anti-inflammatory effects in allergic airway disease in numerous studies involving murine models,[95–97] but it is also effective when given orally. Researchers hypothesize that bromelain may be beneficial in treating human asthma, as well.

In addition to its anti-inflammatory activity, bromelain may be effective in the treatment of respiratory tract conditions because of its mucolytic activity.[43] Bromelain appears to reduce sputum viscosity and possesses an antitussive effect in patients with chronic bronchitis. Spirometric examination of patients before and after bromelain treatment indicated increased FEV_1 (a measurement of the amount of air exhaled in a forced exhalation) and vital capacity, whereas residual volume was reduced. These favorable results were believed to be due to bromelain's ability to function as a mucolytic and decrease bronchial secretions, thereby reducing respiratory congestion.

Cosmetic and Dermatological Applications

Although bromelain supplements are effective against numerous conditions, bromelain's applications are not limited to its oral use. A number of topical bromelain preparations, including skin care products, dentifrices, and skin debridement formulations are available over the counter and also by prescription.

As a proteolytic enzyme, bromelain degrades proteins, including the dead cells in the stratum corneum—the outer layer of the epidermis. This ability is particularly beneficial in skin care products, including cosmetics, exfoliants, and shaving lotions.

Bromelain is also used in dentifrices for its tooth-whitening properties. Researchers in a study on bovine teeth found that treatment with a gel containing papain and bromelain effectively removed the stains that resulted after the teeth had soaked for 1 week in a coffee solution.[98] The researchers noted that unlike peroxide-based gels, which are commonly used in clinical dental bleaching products, the enzyme mixture does not produce reactive oxygen species (ROS), which can deleteriously affect the teeth and surrounding soft tissues.[99]

In another study, researchers subjected the enamel portions of human teeth to a mixture of human saliva, tea, instant coffee, areca nut, betel leaf, lime, tobacco leaf, smokeless tobacco, and chlorhexidine.[100] After 24 hours, the tooth portions were treated with either a control toothpaste (Colgate Regular) or with the test toothpaste (a commercial product containing papain, bromelain, miswak *[Salvadora persica]*, neem *[Azadirachta indica]*, and fluoride). Compared with the control product, the lightness value of the teeth treated with the bromelain-containing toothpaste had improved to a statistically significant level. According to the researchers, the enzymes' proteolytic activity removed the protein component of the plaque on the teeth to which the stains are known to bind.

Bromelain products are also effective in debriding dead and damaged tissue resulting from severe burns.[66–68] It is important to remove necrotic tissue because it can interfere with healing and potentially contaminate the wound. Debriding the damaged tissue exposes healthy tissue, promotes healing, and provides a clear foundation for skin grafting, when necessary.

One such enzyme formulation is a bromelain-based gel dressing. Several studies confirm its safety and efficacy in burn debridement.[66–68] Unlike surgery and chemical or biological methods, enzymatic debridement rapidly removes necrotic tissue without damaging underlying and adjacent healthy tissue.[66,68] Skin debridement with bromelain products is typically conducted in a burn center or other clinical setting.

A Brazilian study found that emulsion-based bromelain products remained more stable than gels and that heat and light had adverse effects on shelf life.[101]

DOSAGE

When used as a digestive aid, bromelain should be taken just before a meal (no more than 30 minutes before eating). When used systemically, bromelain should be taken between meals, on an empty stomach (about 1.5 hours before or after a meal). Because supplement potency varies by manufacturer, it is best to follow the recommendations on the label for dosage. Most bromelain currently marketed in the United States is between 600 and 2400 GDUs.

Bromelain is a hydrolytic enzyme, so supplements should be taken with plenty of water to place the enzyme into solution. As with all enzymes, bromelain's activity can be inhibited by cold or increased by heat; however, excessive heat will denature the enzyme, so avoid drinking hot liquids when taking bromelain supplements.

SIDE EFFECTS AND CONTRAINDICATIONS

High doses (nearly 2 g) of bromelain have been administered with no apparent side effects.[104] No lethal dose 50 (LD_{50}) exists up to 10 g/kg, so it is deemed to be virtually nontoxic.[103] Long-term use seems to be well tolerated. As with most therapeutic agents, allergic reactions may occur with prolonged occupational exposure or in sensitive individuals; however, no significant side effects have been noted.[103,106]

No teratogenic studies have been conducted in humans, so bromelain should not be used by pregnant women. In addition, studies have not been conducted on the safety of using bromelain in nursing mothers.

Although no anaphylactic reactions have been reported, bromelain can induce gastrointestinal allergic and immunoglobulin-E–mediated respiratory reactions, as well as cross-react with papain, rye flour, wheat flour, birch pollen, and grass pollen.[103,106] Although side effects are infrequently observed, bromelain sensitivity as manifested by skin rash or urticaria has occurred. Other possible but unconfirmed reactions include vomiting, nausea, metrorrhagia, menorrhagia, and diarrhea. Researchers hypothesize that bromelain's ability to inhibit platelet aggregation may increase the risk of bleeding[40]; however, there are no studies to support this assertion. Eckert et al. administered 3000 FIP units daily to patients with breast cancer, with no significant alterations in their prothrombin time or plasminogen.[80]

DRUG INTERACTIONS

There are few known interactions between bromelain and other medications. However, because bromelain is a potent inhibitor of platelet aggregation (both in vivo and in vitro),[5,12–18] caution should be exercised when used with anticoagulants (e.g., warfarin) as well as over-the-counter NSAIDs, including aspirin and ibuprofen. In addition, certain botanicals, including garlic, ginkgo biloba, and ginseng, may increase bleeding risk, so patients should be monitored when these are used in conjunction with bromelain.

Clinical studies show that bromelain can increase serum levels of several antibiotics (including tetracycline, amoxicillin, and penicillin) in body fluids and tissues.[54–56] This can be helpful when attempting to improve an antibiotic's efficacy; however, it is possible that there could be instances where elevated serum levels of antibiotics would be deleterious.

Any foods that contain protease inhibitors have the potential to decrease the proteolytic activity of bromelain. However, most of these foods (e.g., potatoes and soybeans) are rarely eaten raw (heat inactivates the protease inhibitors). In addition, many of the protease inhibitors found in these (and other) foods are actually trypsin inhibitors (bromelain contains no trypsin).

CONCLUSION

The future of oral enzyme therapy is already here; however, the rate at which this knowledge is used in restoring health to millions is deplorably slow. This concerns those scientists, doctors, biochemists, and pharmacists who recognize the potential of enzyme therapy. This chapter on bromelain and Chapter 100 on pancreatic enzymes have been written in the hope of hastening the day when enzyme therapy will be employed as a matter of course for every disorder in which it can be of assistance.

Bromelain has proven to be effective in treating the following diseases and conditions:

Angina[18,87]
Arthritis[4]
Athletic injuries[29]
Bronchitis[55]
Burn debridement[66–68]
Cancer[5–10,62]
Circulatory disorders[17,18,88]
Dermatological conditions[101]
Digestive disorders[51,52]
Dysmenorrhea[19,50,54]
Edema[30,65,92,93]
Infections[22–24,26]
Pancreatic insufficiency[51–53]
Sinusitis[44,45]
Staphylococcal infection[26,54,89]
Surgical trauma[28,30,92,93]
Thrombophlebitis[17,18]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rowan AD, Buttle DJ, Barrett AJ. The cysteine proteinases of the pineapple plant. *Biochem J*. 1990;266:869–875.
2. The biology of *Ananas comosus* var. *comosus* (pineapple). Australian Government Department of Health Office of the Gene Technology Regulator. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/pineapple-3/$FILE/biologypineapple08_2.pdf. Accessed May 29, 2010.
3. Ota S, Muta E, Katahira Y, et al. Reinvestigation of fractionation and some properties of the proteolytically active components of stem and fruit bromelains. *J Biochem*. 1985;98:219–228.
4. Kasemsuk T, Saengpetch N, Sibmooh N, et al. Improved WOMAC score following 16-week treatment with bromelain for knee osteoarthritis. *Clin Rheumatol*. 2016;35:2531–2540.
5. Goldstein N, Taussig SJ, Gallup JD, et al. Bromelain as a skin cancer preventive in hairless mice. *Hawaii Med J*. 1975;34:91–94.
6. Tysnes BB, Maurer HR, Porwol T, et al. Bromelain reversibly inhibits invasive properties of glioma cells. *Neoplasia*. 2001;3:469–479.
7. Amini A, Ehteda A, Masoumi S. Cytotoxic effects of bromelain in human gastrointestinal carcinoma cell lines (MKN45, KATO-III, HT29-5FIR, and HT29-5M21). *Onco Targets Ther*. 2013;6:403–409.
8. Amini A, Masoumi-Moghaddam S, Ehteda A, et al. Bromelain and N-acetylcysteine inhibit proliferation and survival of gastrointestinal cancer cells in vitro: significance of combination therapy. *J Exp Clin Cancer Res*. 2014;33.
9. Müller A, Barat S, Chen X, et al. Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines. *Int J Oncol*. 2016;48:2025–2034.
10. Chobotova K, Vernallis AB, Majid FA. Bromelain's activity and potential as an anti-cancer agent: current evidence and perspectives. *Cancer Lett*. 2010;290:148–156.
11. Taussig S, Goldstein N. Effect of bromelain on cancer. *Krebsgeschehen*. 1976;8:81–87.
12. Felton GE. Fibrinolytic and antithrombotic action of bromelain may eliminate thrombosis in heart patients. *Med Hypotheses*. 1980;6:1123–1133.
13. Gläser D, Hilberg T. The influence of bromelain on platelet count and platelet activity in vitro. *Platelets*. 2006;17:37–41.
14. Heinicke RM, van der Wal L, Yokoyama M. Effect of bromelain (Ananase) on human platelet aggregation. *Experentia*. 1972;28:844845.
15. Metzig C, Grabowska E, Eckert K, et al. Bromelain proteases reduce human platelet aggregation in vitro, adhesion to bovine endothelial cells and thrombus formation in rat vessels in vivo. *In Vivo*. 1999;13(1):7–12.
16. Seligman B. Bromelain: an anti-inflammatory agent. *Angiology*. 1962;13:508–510.
17. Seligman B. Oral bromelains as adjuncts in the treatment of acute thrombophlebitis. *Angiology*. 1969;20:22–26.
18. Taussig S, Nieper H. Bromelain: its use in prevention and treatment of cardiovascular disease, present status. *J Int Assoc Prev Med*. 1979;6:139–151.
19. Hunter RG, Henry GW, et al. The action of papain and bromelain on the uterus. *Am J Obstet Gynecol*. 1957;73:867–880.
20. Barth H, Guseo A, Klein R. In vitro study on the immunological effect of bromelain and trypsin on mononuclear cells from humans. *Eur J Med Res*. 2005;10:325–331.
21. Mynott TL, Ladhams A, et al. Bromelain, from pineapple stems, proteolytically blocks activation of extracellular regulated kinase-2 in T cells. *J Immunol*. 1999;163:2568–2575.
22. Mynott TL, Chandler DS, Luke RKJ. Efficacy of enteric-coated protease in preventing attachment of enterotoxigenic Escherichia coli and diarrheal disease in the RITARD model. *Infect Immun*. 1991;59:3708–3714.
23. Mynott TL, Guandalini S, Raimondi F, et al. Bromelain prevents secretion caused by *Vibrio cholerae* and *Escherichia coli* enterotoxins in rabbit ileum in vitro. *Gastroenterol*. 1997;113:175–184.
24. Chandler DS, Mynott TL. Bromelain protects piglets from diarrhoea caused by oral challenge with K88 positive enterotoxigenic *Escherichia coli*. *Gut*. 1998;43:196–202.
25. Mahajan S, Chandra V, Dave S, et al. Stem bromelain-induced macrophage apoptosis and activation curtail *Mycobacterium tuberculosis* persistence. *J Infect Dis*. 2012;206:366–376.
26. Maurer HR. Bromelain: biochemistry, pharmacology and medical use. *Cell Mol Life Sci*. 2001;58:1234–1245.
27. Tassman GC, Zafran JN, Zayon GM. Evaluation of a plant proteolytic enzyme for the control of inflammation and pain. *J Dent Med*. 1964;19:73–77.
28. Tassman GC, Zafran JN, Zayon GM. A double-blind crossover study of a plant proteolytic enzyme; in oral surgery. *J Dent Med*. 1965;20:51–54.
29. Blonstein JL. Control of swelling in boxing injuries. *Practitioner*. 1969:203–206.
30. Howat RC, Lewis GD. The effect of bromelain therapy on episiotomy wounds—a double blind controlled clinical trial. *J Obstet Gynaecol Br Commonw*. 1972;79:951–953.
31. Masson M. Bromelain in blunt injuries of the locomotor system. A study of observed applications in general practice. *Fortschr Med*. 1995;113:303–306. [German].
32. Walker AF, Bundy R, Hicks SM, et al. Bromelain reduces mild acute knee pain and improves well-being in a dose-dependent fashion in an open study of otherwise healthy adults. *Phytomedicine*. 2002;9:681–686.
33. Taussig SJ. The mechanism of the physiological action of bromelain. *Med Hypotheses*. 1980;6:99–104.
34. Fitzhugh DJ, Shan S, Dewhirst MW, et al. Bromelain treatment decreases neutrophil migration to sites of inflammation. *Clin Immunol*. 2008;128:66–74.
35. Hale LP, Haynes BF. Bromelain treatment of human T cells removes CD44, CD45RA, E2/MIC2, CD6, CD7, CD8, and Leu 8/LAM1 surface molecules and markedly enhances CD2-mediated T cell activation. *J Immunol*. 1992;149:3809–3816.
36. Hale LP Greer PK, Sempowski GD. Bromelain treatment alters leukocyte expression of cell surface molecules involved in cellular adhesion and activation. *Clin Immunol*. 2002;104:183–190.
37. Hale LP, Greer PK, Trinh CT, et al. Treatment with oral bromelain decreases colonic inflammation in the IL-10-deficient murine model of inflammatory bowel disease. *Clin Immunol*. 2005;116:135–142.
38. Hale LP, Greer PK, Trinh CT, et al. Proteinase activity and stability of natural bromelain preparations. *Int Immunopharmacol*. 2005;5(4):783–793.
39. Hale LP, Fitzhugh DJ, Staats HF. Oral immunogenicity of the plant proteinase bromelain. *Int Immunopharmacol*. 2006;6:2038–2046.
40. Secor ER, Carson WF, Singh A, et al. Oral bromelain attenuates inflammation in an ovalbumin-induced murine model of asthma. *Evid Based Complement Alternat Med*. 2008;5:61–69.
41. Rimoldi R, Ginesu F, Giura R. The use of bromelain in pneumological therapy. *Drugs Exp Clin Res*. 1978;4:55–66.
42. Ryan RE. A double-blind clinical evaluation of bromelains in the treatment of acute sinusitis. *Headache*. 1967;7:13–17.
43. Braun JM, Schneider B, Beuth HJ. Therapeutic use, efficiency and safety of the proteolytic pineapple enzyme Bromelain-POS in children with acute sinusitis in Germany. *In Vivo*. 2005;19:417–421.
44. Knill-Jones RP, Pearce H, Batten J, et al. Comparative trial of Nutrizym in chronic pancreatic insufficiency. *BMJ*. 1970;4:21–24.
45. Cichoke AJ. *Bromelain: The Active Enzyme That Helps Us Make the Most of What We Eat*. New York: Keats Publishing; 1998.
46. Ketnawa S, Chaiwut P, Rawdkuen S. Extraction of bromelain from pineapple peels. *Food Sci Technol Int*. 2011;17:395–402.
47. Pukrushpan T. *Role of Raw Material Conditions and Extraction in Bromelain Production*. Research report. Bangkok, Thailand. Kasetsart University, Faculty of Agro-Industry, Dept of Food Science and Technology; 1984.
48. Felton GE. Does kinin released by pineapple stem bromelain stimulate production of prostaglandin E1-like compounds? *Hawaii Med J*. 1977;36:39–47.
49. Taussig SJ, Batkin S. Bromelain, the enzyme complex of pineapple (Ananas comosus) and its clinical application. An update. *J Ethnopharmacol*. 1988;22:191–203.
50. Balakrishnan V, Hareendran A, Nair CS. Double-blind cross-over trial of an enzyme preparation in pancreatic steatorrhoea. *J Assoc Physicians India*. 1981;29:207–209.

51. Hale LP. Proteolytic activity and immunogenicity of oral bromelain within the gastrointestinal tract of mice. *Int Immunopharmacol*. 2004;4:255–264.
52. Agostinis C, Zorzet S, De Leo R, et al. The combination of N-acetyl cysteine, alpha-lipoic acid, and bromelain shows high anti-inflammatory properties in novel in vivo and in vitro models of endometriosis. *Mediators Inflam*. 2015. https://doi.org/10.1155/2015/918089.
53. Onken JE, Greer PK, Calingaert B, et al. Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies in vitro. *Clin Immunol*. 2008;126:345–352.
54. Neubauer RA. A plant protease for the potentiation of and possible replacement of antibiotics. *Exp Med Surg*. 1961;19:143–160.
55. Luerti M, Vignali M. Influence of bromelain on penetration of antibiotics in uterus, salpinx and ovary. *Drugs Exp Clin Res*. 1978;4:45–48.
56. Tinozzi S, Venegoni A. Effect of bromelain on serum and tissue levels of amoxicillin. *Drugs Exp Clin Res*. 1978;4:39–44.
57. Castell JV, Friedrich G, Kuhn CS, et al. Intestinal absorption of undegraded proteins in men: presence of bromelain in plasma after oral intake. *Am J Physiol*. 1997;273:G139–G146.
58. Castell JV. Intestinal absorption of undegraded bromelain in humans. In: Gardner MLG, Steffens KJ, eds. *Absorption of Orally Administered Enzymes*. Berlin: Springer Verlag; 1997:47–60.
59. Seifert J, Ganser R, Brendel W. Absorption of a proteolytic enzyme originating from plants out of the gastro-intestinal tract into blood and lymph of rats. *Z Gastroenterol*. 1979;17:1–8. [German].
60. Izaka KI, Yamada M, Kawano T, et al. Gastrointestinal absorption and antiinflammatory effect of bromelain. *Jpn J Pharmacol*. 1972;22:519–534.
61. Miller JM, Opher AW. The increased proteolytic activity of human blood serum after oral administration of bromelain. *Exp Med Surg*. 1964;22:277–280.
62. Pauzi AZM, Yeap SK, Abu N, et al. Combination of cisplatin and bromelain exerts synergistic cytotoxic effects against breast cancer cell line MDA-MB-231 in vitro. *Chin Med*. 2016;11:46. https://doi.org/10.1186/213020-016-0118-5.
63. Buford TW, Cooke MB, Redd LL, et al. Protease supplementation improves muscle function after eccentric exercise. *Med Sci Sports Exerc*. 2009;41:1908–1914.
64. Miller PC, Bailey SP, Barnes ME, et al. The effects of protease supplementation on skeletal muscle function and DOMS following downhill running. *J Sports Sci*. 2004;22:365–372.
65. Singh T, More V, Fatima U, et al. Effect of proteolytic enzyme bromelain on pain and swelling after removal of third molars. *J Int Soc Prev Community Dent*. 2016;6:S197–S204.
66. Rosenberg L, Shoham Y, Krieger Y, et al. Minimally invasive burn care: A review of seven clinical studies of rapid and selective debridement using a bromelain-based debriding enzyme (Nexobrid®). *Ann Burns Fire Disasters*. 2015;28:264–274.
67. Rosenberg L, Lapid O, Bogdanov-Berezovsky A, et al. Safety and efficacy of a proteolytic enzyme for enzymatic burn debridement: a preliminary report. *Burns*. 2004;30:843–850.
68. Singer AJ, McClain SA, Taira BR, et al. Rapid and selective enzymatic debridement of porcine comb burns with bromelain-derived Debrase; acute-phase preservation of noninjured tissue and zone of stasis. *J Burn Care Res*. 2010;31:304–309.
69. Kinsella K, Wan He. *An aging world: 2008*. Washington DC: US Census Bureau; 2009. International Population Reports, P95/09-1.
70. Batrinos ML. The aging of the endocrine hypothalamus and its dependent endocrine glands. *Hormones*. 2012;11:241–253.
71. Clegg A, Young J, Iliffe S, et al. Frailty in elderly people. *Lancet*. 2013;381. 752-652.
72. Rémond D, Shahar DR, Gille D, et al. Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget*. 2015;6:13858–13898.
73. Feldman M, Cryer B, McArthur KE, et al. Effects of aging and gastritis on gastric acid and pepsin secretin in humans: a prospective study. *Gastroenterology*. 1996;110:1043–1052.
74. Herzig KH, Purhonon AK, Räsänen, et al. Fecal pancreatic elastase-1 levels in older individuals without known gastrointestinal diseases or diabetes mellitus. *BMC Geriatr*. 2011;11. https://doi.org/10.1186/1471-2318-11-4.
75. Amini A, Masoumi-Moghaddam S, Ehteda A, et al. Potentiation of chemotherapeutics by bromelain and N-acetylcysteine: sequential and combination therapy of gastrointestinal cancer cells. *Am J Cancer Res*. 2016;6:350–369.
76. Beuth J, Van Leendert R, Schneider B, et al. Complementary medicine on side-effects of adjuvant hormone therapy in patients with breast cancer. *In vivo*. 2013;27:869–872.
77. Skin cancer statistics. Centers for Disease Control and Prevention. http://www.cdc.gov/cancer/skin/statistics/index.htm. Accessed September 18, 2017.
78. Renehan AG, Booth C, Potten CS. What is apoptosis, and why is it important? *BMJ*. 2001;322:1536–1538.
79. Báez R, Lopes MTP, Salas CE, et al. In vivo antitumoral activity of stem pineapple (Ananas comosus) bromelain. *Planta med*. 2007;73:1377–1383.
80. Eckert K, Grabowska E, Stange R, et al. Effects of oral bromelain administration on the impaired immunocytotoxicity of mononuclear cells from mammary tumor patients. *Oncol Rep*. 1999;6:1191–1199.
81. Lotz-Winter H. On the pharmacology of bromelain: an update with special regard to animal studies on dose-dependent effects. *Planta Med*. 1990;56:249–253.
82. Desser L, Holomanova D, Zavadova E, et al. Oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood. *Cancer Chemother Pharmacol*. 2001;47(suppl):S10–S15.
83. Zavadova E, Desser L, Mohr T. Stimulation of reactive oxygen reactive species production and cytotoxicity in human neutrophils in vitro and after oral administration of a polyenzyme preparation. *Cancer Biother*. 1995;10:147–152.
84. Fouz N, Amid A, Hashim YZH-Y. Cytokinetic study of MCF-7 cells treated with commercial and recombinant bromelain. *Asian Pac J Cancer Prev*. 2013;14:6709–6714.
85. Uhlenbruck G, Van Leendert R, Schneider B, et al. Reduced side-effects of adjuvant hormone therapy in breast cancer patients by complementary medicine. *In vivo*. 2010;24:799–802.
86. Schafer AI, Adelman B. Plasmin inhibition of platelet function and of arachidonic acid metabolism. *J Clin Invest*. 1985;75:456–461.
87. Nieper HA. Effect of bromelain on coronary heart disease and angina pectoris. *Acta Med Empirica*. 1978;5:274–278.
88. Juhasz B, Thirunavukkarasu M, Pant R, et al. Bromelain induces cardioprotection against ischemia-reperfusion injury through Akt/FOXO pathway in rat myocardium. *Am J Physiol Heart Circ Physiol*. 2008;294:H1365–H1370.
89. Watters CM, Burton T, Kirui DK, et al. Enzymatic degradation of in vitro *Staphylococcus aureus* biofilms supplemented with human plasma. *Infect Drug Resist*. 2016;9:71–78.
90. Russell DG, Cardona P-J, Kim M-J, et al. Foamy macrophages and the progression of the human TB granuloma. *Nat Immunol*. 2009;10: 943–948.
91. Praveen N, Rajesh a, Madan M, et al. Evaluation of antibacterial efficacy of pineapple extract (bromelain) on periodontal pathogens. *J Int Oral Health*. 2014;6:96–98.
92. Golezar S. Ananas comosus effect on perineal pain and wound healing after episiotomy: a randomized double-blind placebo-controlled clinical trial. *Iran Red Crescent Med J*. 2016;18:e21019.
93. Orsini RA. Bromelain. *Plast Reconstr Surg*. 2006;118:1640–1644.
94. Inchingolo F, Tatullo M, Marrelli M, et al. Clinical trial with bromelain in third molar exodontia. *Eur Rev Med Pharmacol Sci*. 2010;14:771–774.
95. Secor ER, Shah SJ, Guernsey LA. Bromelain limits airway inflammation in an ovalbumin-induced murine model of established asthma. *Altern Ther Health Med*. 2012;18:9–17.
96. Secor Jr ER, Carson WFIV, Cloutier MM, et al. Bromelain exerts anti-inflammatory effects in an ovalbumin-induced murine model of allergic airway disease. *Cell Immunol*. 2005;237:68–75.
97. Secor ER, Szczepanek SM, Castater CA, et al. Bromelain inhibits allergic sensitization and murine asthma via modulation of dendritic cells. *Evid Based Complement Alternat Med*. 2014. https://doi.org/10.1155/2013/702196.

98. Münchow EA, Hamann HJ, Carvajal MT, et al. Stain removal effect of novel papain- and bromelain-containing gels applied to enamel. *Clin Oral Invest*. 2016;20:2315–2320.
99. Sato C, Rodrigues FA, Garcia DM, et al. Tooth bleaching increases dentinal protease activity. *J Dent Res*. 2013;92:187–192.
100. Kalyana P, Shashidhar A, Meghashyam B, et al. Stain removal efficacy of a novel dentrifice containing papain and bromelain extracts—an in vitro study. *Int J Dent Hyg*. 2011;9:229–233.
101. Lourenço CB, Ataide JA, Cefali LC, et al. Evaluation of the enzymatic activity and stability of commercial bromelain incorporated in topical formulations. *Int J Cosmet Sci*. 2016;38:535–540.
102. Gutfreund AE, Taussig SJ, Morris AK. Effect of oral bromelain on blood pressure and heart rate of hypertensive patients. *Hawaii Med J*. 1978;37:143–146.
103. Kelly GS. Bromelain: a literature review and discussion of its therapeutic applications. *Altern Med Review*. 1996;1:243–257.
104. Baur X. Studies on the specificity of human IgE-antibodies to the plant proteases papain and bromelain. *Clin Allergy*. 1979;9:451–457.
105. Baur X, Fruhmann G. Allergic reactions, including asthma, to the pineapple protease bromelain following occupational exposure. *Clin Allergy*. 1979;9:443–450.
106. Rowe DJ, Baker AC. Perioperative risks and benefits of herbal supplements in aesthetic surgery. *Aesthetic Surg J*. 2009;29:150–157.
107. This chapter is dedicated to the memory of Dr. Max Wolf, the father of systemic enzyme therapy, as well as Dr. Karl Ransberger, who validated and marketed systemic enzyme therapy, making it a highly accepted worldwide treatment for a wide variety of systemic diseases and injuries.

FURTHER READING

For further information on enzymes and enzyme therapy, see the following:.

Cichoke AJ. *A New Look at Chronic Disorders and Systemic Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *A New Look at Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *Acute Trauma and Systemic Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *The Back Pain Bible*. Los Angeles: Keats; 1999.
Cichoke AJ. *Bromelain: The Active Enzyme That Helps Us Make the Most of What We Eat*. New Canaan, CT: Keats; 1998.
Cichoke AJ. *The Complete Book of Enzyme Therapy*. New York: Avery Publishing; 1999.
Cichoke AJ. *Enzymes: Nature's Energizers*. New Canaan, CT: Keats; 1997.
Cichoke AJ. *Enzymes: The Sparks of Life*. Canada: Alive Books; 2002.
Cichoke AJ. *Enzymes and Enzyme Therapy: How to Jump Start Your Way to Lifelong Good Health*. 2nd ed. New Canaan, CT: Keats; 2000.

60

Camellia sinensis (Green Tea)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Camellia sinensis (family: Theaceae)
Common name: green tea

GENERAL DESCRIPTION

Both green tea and black tea are derived from the same plant, *Camellia sinensis* (Fig. 60.1). The tea plant originated in China but is now grown and consumed worldwide. *C. sinensis,* an evergreen shrub or tree that can grow up to a height of 30 feet, is usually maintained at a height of 2 to 3 feet by regular pruning. The shrub is heavily branched, with young hairy leaves. The parts used are the leaf bud and the two adjacent young leaves together with the stem, broken between the second and third leaves (Fig. 60.2). Older leaves are considered of inferior quality.

Green tea is produced by lightly steaming the freshly cut leaf, whereas black tea is produced by allowing the leaves to oxidize. During oxidation, enzymes present in the tea convert many polyphenolic therapeutic substances to compounds with much less activity. With green tea, oxidation is not allowed to take place because the steaming process inactivates these enzymes. Green tea is high in polyphenols with potent antioxidant and anticancer properties. Oolong tea is a partially oxidized tea.

Of the nearly 2.5 million tons of dried tea produced each year, only 20% is green tea. India and Sri Lanka are the major producers of black tea. Green tea is produced and consumed primarily in China, Japan, and a few countries in North Africa and the Middle East (Table 60.1). Consumption is increasing in the United States.

CHEMICAL COMPOSITION

The chemical composition of green tea varies with climate, season, horticultural practices, and age of the leaf (i.e., position of the leaf on the harvested shoot). The major components of interest are the polyphenols.[1,2] The major polyphenols in green tea are the following flavonoids:

- Catechin
- Epicatechin
- Epicatechin gallate
- Epigallocatechin gallate (EGCG)
- Proanthocyanidins

EGCG is viewed as the most significant active component (Fig. 60.3). The leaf bud and the first leaves are richest in EGCG. The concentration of total polyphenols in dried green tea leaf is around 8% to 12%.

Other compounds of interest in dried green tea leaf include the following:

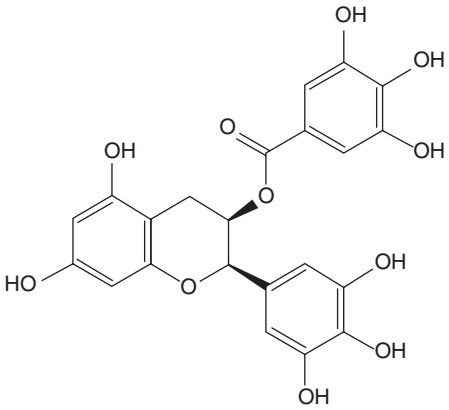
- Caffeine (3.5%)
- Theanine (one half of the total amino acid content, which is usually 4%)
- Lignin (6.5%)
- Organic acids (1.5%)
- Protein (15%)
- Chlorophyll (0.5%)

One cup of green tea contains about 300 to 400 mg of polyphenols and between 50 and 100 mg of caffeine. Commercial preparations that have been decaffeinated and have concentrated total polyphenols to between 60% and 80% are available.

PHARMACOLOGY

The primary pharmacological actions center around the antioxidant effects of the green tea polyphenols.[3] The degree of antioxidant protection produced by green tea is clearly dose dependent and requires a threshold dose. In a randomized crossover study, 15 healthy volunteers consumed 500 mL of green tea with different solid contents (1.4, 1.6, 1.8, and 2.0 g/L). Ingestion of the lowest dose produced no change in plasma antioxidant capacity. At the highest dose, the effects increased the plasma antioxidant capacity the greatest at 1 and 4 hours after ingestion.[4] This study is significant because it indicates that some in vivo studies that fail to demonstrate the increasing antioxidant activity of green tea may be due to insufficient dose.[5,6]

Fig. 60.1 Green tea sprig.



(–)-Epigallocatechin-3-gallate
(EGCG)

Fig. 60.2 Structure of epigallocatechin gallate (EGCG).

TABLE 60.1 World Tea Production by Type

Type	Dry Weight (×1000 tons)
Black	1940
Green	515
Oolong	60
Total	2515

In addition to exerting antioxidant activity on its own, green tea may increase the activity of antioxidant enzymes. In mice, oral feeding of a polyphenolic fraction isolated from green tea in drinking water for 30 days resulted in significantly increased activities of antioxidant and detoxifying enzymes (glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, and quinone reductase) in the small intestine, liver, lungs, and small bowel.[7]

Several in vitro and experimental models of cancer have shown that green tea polyphenols may offer significant protection.[8,9] Specifically, green tea polyphenols inhibit cancer via inhibition of metalloproteinases, various protein kinases, proteins that regulate DNA replication and transformation, tumor proteasomal activity, and the formation of cancer-causing compounds such as nitrosamines. Green tea polyphenols also upregulate or maintain intercellular–gap junction communication and increase apoptosis. Green tea acts as a carcinoma blocker by modulating the signal transduction pathways involved in cell proliferation, transformation, inflammation, and metastasis. The consumption of flavonoid-containing botanicals, like green tea, has demonstrated clear antioxidant benefit, and a diet high in polyphenols has demonstrated effectiveness at lowering 8-OHdG levels.[10]

L-Theanine

L-theanine (*N*-ethyl-L-glutamine) or theanine is a major amino acid uniquely found in green tea. In animal models, L-theanine increases brain serotonin, dopamine, and γ-aminobutyric acid (GABA) levels and has micromolar affinities for various brain receptor sites, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), kainate, and *N*-methyl-D-aspartic acid (NMDA). Theanine has been shown to exert neuroprotective effects in animal models, possibly through its antagonistic effects on group 1 metabotropic glutamate receptors. Behavioral studies in animals suggest improvement in learning and memory. Clinical studies demonstrated that L-theanine reduces stress, improves the quality of sleep, diminishes the symptoms of premenstrual syndrome, heightens mental acuity, and reduces the negative side effects of caffeine.[11,12] These clinical effects are directly related to L-theanine's ability to stimulate the production of α-brain waves (a state often achieved by meditation and characterized by relaxation with greater mental focus and mental alertness) as well as reduce β-waves (associated with nervousness, scattered thoughts, and hyperactivity).

L-theanine is a popular ingredient in functional foods and beverages as well as dietary supplements designed to produce mental and physical relaxation without inducing drowsiness. L-theanine is fast-acting. Generally, the effects are felt within the first 30 minutes and have been shown to last up to 8 to 12 hours. Based on the results of clinical studies, it has been established that L-theanine is effective in the range of 50 to 200 mg.

CLINICAL APPLICATION

The primary clinical applications for green tea are in the prevention of cancer and heart disease, as well as aiding weight loss. It may also offer benefit to bone health and may protect the skin from sun damage.

Cancer

Epidemiological studies demonstrate that green tea consumption might offer significant protection against many forms of cancer. However, epidemiological correlations regarding the relationship between tea consumption and human cancer are confounded by numerous variables. The forms of cancer that appear to be best prevented by green tea are those of the gastrointestinal tract, including cancers of the stomach, small intestine, pancreas, and colon. Green tea may also provide protection against lung and estrogen-related cancers, including most breast cancers, skin cancer, and prostate cancer.[13–18] It is generally held that a desirable green tea intake is 3 to 5 cups/day, providing a minimum of 250 mg/day catechins. However, based on clinical data, this dosage level may not be sufficient once neoplasia develops or in high-risk individuals.

Although a population-based study of women in Shanghai found that among nonsmokers, the consumption of green tea was associated with a clearly reduced risk of lung cancer, in a dose-dependent manner, 17 studies showed little benefit in antioxidant effects in smokers even at significant dosage levels (i.e., more than 3 grams green tea polyphenols per day).[19]

In preventing breast cancer, the main anticancer mechanism may be inhibition of the interaction of estrogen with its receptors in breast tissue. Polyphenol compounds in green tea extracts also block the interaction of tumor promoters, hormones, and growth factors with their receptors in breast tissue. Experimental and epidemiological studies demonstrated a protective effect against breast cancer,

and clinical results confirmed that green tea might be helpful for early-stage breast cancer. In an observational study, 1160 surgical cases of patients with invasive breast cancers were followed for 9 years. During a review of 5264 person-years follow-up, 133 subjects (12%) had a documented recurrence of breast cancer. A decreased hazard ratio for recurrence adjusted for stage was observed with the consumption of 3 or more cups a day of green tea. Particularly in Stage I, the hazard ratio was decreased quite significantly. A similar tendency was observed for Stage II subjects but was not present among more advanced stages.[20]

The coadministration of tamoxifen with green tea catechins may be an appealing strategy, especially in the case of estrogen receptor (ER)–negative breast cancer, where green tea catechins have been reported to reactivate ER. Indeed, it has been observed that the treatment of ER-alpha-negative breast cancer cells with green tea polyphenols led to the reactivation of ER-alpha expression.[21] Experimental trials suggest a synergistic interaction of green tea catechins with tamoxifen or raloxifene in the treatment of ER-positive and ER-negative breast cancer through ER-dependent and ER-independent mechanisms. No evidence of an interaction of green tea catechins with aromatase inhibitors or fulvestrant has been reported.[22]

Topical and oral treatments of green tea flavonoid components have also been explored to treat human papillomavirus (HPV)–related cervical cancer. A randomized, controlled clinical trial of 51 patients with cervical lesions, including chronic cervicitis, mild dysplasia, moderate dysplasia, and severe dysplasia, were divided into four treatment groups, whereas 39 untreated patients served as controls. Green tea components such as EGCG were shown to have antiproliferative properties through the induction of apoptosis and cell-cycle arrest in cervical cancer cell lines.[23]

A second trial used a polyphenol ointment made with EGCG applied locally to 27 patients twice a week for 12 weeks; 200 mg of the ointment or an EGCG capsule was taken orally every day for 8 to 12 weeks. Overall, a 69% response rate (35 of 51 patients) was noted for treatment with green tea extracts, with each treatment showing a similar response, compared with a 10% response rate (4 of 39 patients) in untreated controls. A response was considered positive when pretreatment and posttreatment biopsies and Pap smears detected an elimination of abnormal cells, and there was a decrease in or complete elimination of HPV or a decrease in the size of the cervical lesion. The authors of this study concluded that green tea extracts might be a potential therapy regimen for patients with HPV-infected cervical lesions.[24]

In vitro and in vivo studies showed that EGCG produces marked inhibition of both hormone-sensitive and hormone-insensitive prostate cancer cells. To demonstrate concentration within the prostate of EGCG after oral intake, men with clinically localized prostate cancer consumed six cups of green tea daily or water for 3 to 6 weeks before undergoing radical prostatectomy.[25] Using high-performance liquid chromatography, green tea polyphenols were not detected in prostate tissue or urine from men consuming water preoperatively. In contrast, both the methylated and nonmethylated forms of EGCG were detectable in prostate tissue and urine in men consuming green tea.

In an additional study of men undergoing radical prostatectomy for prostate cancer, subjects were given 1300 mg of green tea polyphenols daily until surgery. Serum was collected before initiation of the drug study and on the day of prostatectomy. Serum biomarkers, including hepatocyte growth factor, vascular endothelial growth factor, insulin-like growth factor (IGF), IGF binding protein-3, and prostate-specific antigen (PSA), were analyzed by enzyme-linked immunosorbent assay (ELISA). The results showed a significant reduction in serum levels of PSA, hepatocyte growth factor, and vascular endothelial growth factor in men with prostate cancer after brief treatment with green tea.[26] Even at this relatively high dosage of polyphenols, there was no elevation in liver enzymes.

A proof-of-principle clinical trial was designed to assess the safety and efficacy of green tea polyphenols for the chemoprevention of high-grade prostate intraepithelial neoplasia developing prostate cancer within 1 year after repeated biopsy.[27] Sixty volunteers with high-grade prostate intraepithelial neoplasia were given either a placebo or 600 mg of green tea polyphenol extract (GTPE) daily. After 1 year, only one tumor was diagnosed among the 30 GTPE-treated men (incidence, approximately 3%), whereas nine cancers were found among the 30 placebo-treated men (incidence, approximately 30%). Total PSAs did not change significantly between the two arms, but GTPE-treated men showed values constantly lower with respect to placebo-treated ones. The International Prostate Symptom Scores and quality-of-life scores of GTPE-treated men with coexistent benign prostate hyperplasia improved, reaching statistical significance in the case of International Prostate Symptom Scores. As a secondary observation, administration of GTPE also reduced lower urinary tract symptoms, suggesting that these compounds might also be of help for treating the symptoms of benign prostate hyperplasia. No significant side effects or adverse effects were documented.

Cancer Risk and Black Tea Consumption

In contrast to green tea, population studies analyzing black tea consumption are not as clear regarding cancer protection. Some early studies even indicated that black tea consumption might increase the risk for certain cancers (e.g., rectum, gallbladder, endometrium).[28,29]

For example, in one study, the relationship between black tea consumption and cancer risk was analyzed using data from an integrated series of case-control studies conducted in Northern Italy between 1983 and 1990.[28] The data set included 119 biopsy-confirmed cancers of the oral cavity and throat, 294 of the esophagus, 564 of the stomach, 673 of the colon, 406 of the rectum, 258 of the liver, 41 of the gallbladder, 303 of the pancreas, 149 of the larynx, 2860 of the breast, 567 of the endometrium, 742 of the ovary, 107 of the prostate, 365 of the bladder, 147 of the kidney, 120 of the thyroid, and a total of 6147 controls admitted to the hospital for acute noncancerous conditions. After allowance for age, sex, area of residence, education, smoking, and coffee consumption, results indicated an increased risk with tea consumption for cancers of the rectum, gallbladder, and endometrium. There was no association with cancers of the oral cavity, esophagus, stomach, bladder, kidney, prostate, or any other site considered.

In another study, data on black tea consumption from 1965 to 1968 were clinically examined in 7833 men of Japanese ancestry.[29] Since 1965, hundreds of newly diagnosed cancer cases have been identified: 152 colon, 151 lung, 149 prostate, 136 stomach, 76 rectum, 57 bladder, 30 pancreas, 25 liver, 12 kidney, and 163 at other (miscellaneous) sites. Compared with almost-never drinkers, men who habitually drank black tea more than once a day had a four-times-greater chance of developing rectal cancer.

Cardiovascular and Liver Disease

Epidemiological, clinical, and experimental studies have established a positive correlation between green tea consumption and cardiovascular health. Green tea polyphenols exert vascular protective effects through multiple mechanisms, including antioxidative, antihypertensive, anti-inflammatory, antiproliferative, antithrombogenic, and lipid-lowering effects.[30] The underlying mechanisms for these effects are listed in Table 60.2.

In a double-blind, placebo-controlled study, 111 healthy adult volunteers were given either a green tea preparation (GTP) containing 100 mg of L-theanine together with 200 mg of a decaffeinated catechin green tea extract twice a day or a placebo.[31] Before and after 3 weeks, blood

TABLE 60.2 Correlation Between Green Tea Consumption and Cardiovascular Health

Cardioprotective Effects	Mechanisms	Molecular targets
Antioxidant effect	Scavenge free radicals	↓ $O2^{\bullet-}$, $NO^{\bullet}$, 1O2, ONOO–
	Chelate metal ions	Chelate copper and iron
	↓ Redox active transcription factors	↓ NF-κB, AP-1
	↓ Prooxidant enzymes	↓ iNOS, XO
	Upregulate antioxidant enzymes	↑ Catalase, GPx, SOD
	Sparing of antioxidants	Sparing tocopherol
Improvement of lipid metabolism	↓ Cholesterol synthesis	↓ Squalene epoxidase
	↑ Cholesterol excretion	Bind to cholesterol
	↓ Cholesterol absorption	Bind to cholesterol
	↓ Triglycerides	↓ Phospholipase
	↓ Fatty acid synthesis	↓ Acetyl-CoA carboxylase
		↓ β-ketoacyl reductase of FAS
Improvement of endothelial function	↑ NO-dependent vasodilation	↑ eNOS
	↑ NO-independent vasodilation	↑ Prostacyclin
		↑ Cytosolic cAMP, cGMP
Anti-inflammatory effect	↓ Adhesion of leukocytes to ECs	↓ VCAM, MCP-1
	+ Apoptosis of monocytes	↑ Caspase 8 and 9
Antiproliferative effect	↓ VSMC growth	↓ PCNA
		↓ PDGF
	↓ VSMC migration	↓ MMP-2
Antiplatelet and antithrombotic effects	↓ Platelet aggregation	↓ Cytoplasmic Ca^{2+} release
		PAF
	↓ Thrombosis	↓ Thromboxane A2 synthase
		Scavenge free radicals

ApoA1, Apolipoprotein A1; *cAMP,* cyclic adenosine monophosphate; *cGMP,* cyclic guanosine monophosphate; *CoA,* coenzyme A; *EC,* endothelial cells; *eNOS,* endothelial nitric oxide synthase; *FAS,* fatty acid synthase; *GPx,* glutathione peroxidase; *iNOS,* inducible nitric oxide synthase; *MCP-1,* monocyte chemotactic protein-1; *MMP,* matrix metalloproteinase; *NF-κB,* nuclear factor-κB; *NO,* nitric oxide; *O2•–,* superoxide anion; *1O2,* singlet oxygen; *ONOO–,* peroxynitrite radical; *PAF,* platelet activating factor; *PCNA,* proliferating cell nuclear antigen; *PDGF,* platelet-derived growth factor; *SOD,* superoxide dismutase; *VCAM-1,* vascular cell adhesion molecule-1; *VSMC,* vascular smooth muscle cells; *XO,* xanthine oxidase.
Adapted from Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. *Curr Med Chem.* 2008;15:1840–1850.

pressure, serum lipids, serum amyloid-α (a marker of chronic inflammation), and serum malondialdehyde (a marker of oxidative stress) were measured. After 3 months, systolic blood pressure remained significantly lower. GTP lowered serum amyloid-α by 42% and lowered malondialdehyde by 11.9%. In men, there was a 9-mg/dL reduction in low-density lipoprotein cholesterol (LDL-c) and a 10-mg/dL reduction in total cholesterol (TC). In all subjects with a baseline LDL-c level greater than 99 mg/dL, there was a 9-mg/dL decrease of TC and LDL-c. A second double-blind, placebo-controlled trial enrolled 60 mildly hypercholesterolemic subjects (180–220 mg dL^{-1}) and divided them into three groups: catechin-enriched green tea (CEGT), catechin-enriched oolong tea (CEOT), or placebo. The subjects were instructed to drink 2 × 300 mL of CEGT (780.6 mg of catechin), CEOT (640.4 mg of catechin), or placebo beverage for 12 weeks. Drinking CEGT and CEOT significantly decreased subjects' body weight, fat, body mass index (BMI), lipid peroxidation, and lipid profile (TC, LDL-c, high-density lipoprotein cholesterol [HDL-c], and triglycerides [TG]). CEGT and CEOT also significantly improved ($p < 0.05$) the oxidative indices (TEAC and GSH) and antioxidant enzymes (SOD, CAT, GPx, and GR). Furthermore, ultrasound examination endorsed the hepatoprotective activity of CEGT and CEOT by reverting mild fatty liver to the normal hepatic condition because of antioxidant and hypolipidemic activities.[32]

Just as in the studies with cancer, cigarette smoking may nullify some of these protective effects against cardiovascular disease. A study from the Netherlands evaluating inflammatory markers, such as interleukin-6, interleukin-1β, tumor necrosis factor-α, C-reactive protein, and fibrinogen, found no benefit for 59 healthy smoking volunteers who drank tea with regard to inflammation, hemostasis, and the endothelial cardiovascular risk factors measured.[33] Given other studies of green tea that showed little protection for smokers and the strong body of literature regarding its efficacy in nonsmoking populations, it is possible that the level of oxidative damage sustained by regular smoking may be too great for tea's demonstrated antioxidant capacities. However, it may also be that the dosage of green tea polyphenols must be much higher in smokers. In one double-blind study, 30 healthy male smokers were divided into three groups and given green tea beverages containing 0 mg (control group), 80 mg (medium-dose group), or 580 mg (high-dose group) of green tea polyphenols daily for 2 weeks.[34] Endothelial-dependent and endothelial-independent vasodilatation were investigated by measuring the forearm blood flow (FBF) responses to acetylcholine and sodium nitroprusside using venous occlusion strain-gauge plethysmography. The FBF response to acetylcholine significantly increased at 2 hours and at 1 and 2 weeks after green tea polyphenol intake in the high-dose group, but no increase was observed in the other groups. FBF responses to sodium nitroprusside did not alter in any group at any time point. A significant increase in plasma nitric oxide and a decrease in asymmetrical dimethylarginine, malondialdehyde, and 4-hydroxynonenal, C-reactive protein, monocyte chemotactic protein-1, and soluble CD40 ligand levels were detected after long-term consumption of high-dose green tea polyphenol. This study indicates that higher dosages might be required to overcome the oxidative stress of cigarette smoke.

Weight Loss

The effects of green tea extracts on weight loss appear to require both the catechins and caffeine. Extracts containing both ingredients

decreased BMI (−0.55), body weight (−1.38 kg), and waist circumference (−1.93 cm) compared with caffeine alone. Studies that evaluated green tea catechins without concomitant caffeine administration did not show benefits on any of the assessed anthropometric end points. Green tea catechin and caffeine mixtures promote weight loss through thermogenesis and fat oxidation.[35,36]

Bone Health

Epidemiological evidence demonstrates an association between green tea consumption and the prevention of age-related bone loss in elderly individuals. There are five proposed mechanisms through which green tea protects bone health: (1) by mitigating bone loss through antioxidative stress action, (2) by mitigating bone loss through anti-inflammatory action, (3) by enhancing osteoblastogenesis, (4) by suppressing osteoclastogenesis, and (5) through its high content of fluoride and vitamin K_1.[37]

Neuroprotective Effects

EGCG has gained a lot of attention as a potential therapeutic agent for preventing neurodegenerative diseases, mostly attributed to the antioxidant, free-radical-scavenging, metal-chelating, antiapoptotic, and anti-inflammatory properties.[38] In support of this, a 13 year-long Finnish study with 30,000 adults aged 25 to 74 years reported a nearly 60% reduced risk of Parkinson's disease when 3 or more cups of tea were consumed per day.[39] In addition, a large-scale cohort 20-year follow-up analysis with approximately 50,000 men and 80,000 women showed that EGCG intake was associated with a 10% to 25% lower risk of Parkinson's, particularly in men.[40]

Animal research on EGCG suggests there may be potential in promoting healthy aging by improving the morphological and functional alterations that occur in a natural aging brain, suppressing cognitive dysfunction, increasing learning ability, and reducing oxidative damage in the brain.[41] Although EGCG shows promise as an iron-chelating, brain-permeable antioxidant agent, there is a need for examining this neuroprotective effect in depth through more human clinical trials.

PHOTOPROTECTIVE EFFECTS

Topical treatment or oral consumption of green tea polyphenols inhibits chemical carcinogen or ultraviolet (UV) radiation–induced skin tumorigenesis in different animal models. In one study, volunteers were treated with an extract of green tea or one of its constituents and subsequently exposed to graded doses of UV radiation 30 minutes later.[42] UV-treated skin was examined clinically for UV-induced erythema, looking histologically for the presence of sunburn cells or Langerhans cell distributions for UV-induced DNA damage. On histological examination, skin treated with green tea extracts had a reduced number of sunburn cells and protected epidermal Langerhans cells from UV damage. In a dose-dependent manner, EGCG and EGCG polyphenols proved to be the most efficient at inhibiting erythema. It was noted that the epigallocatechin and epicatechin fractions showed almost no effect. Green tea extracts also reduced the DNA damage that formed after UV radiation.

ORAL CAVITY DISEASES

Inflammatory gingival reactions, tooth decay, and oral cancer may also benefit from the use of green tea. By studying the amount of dental plaque present, as well as a bleeding index, green tea chews and rinses have been shown to positively affect the level of gum inflammation.[43] One trial evaluated volunteers who held 2 grams of brewed black tea or unbrewed green tea leaves in the mouth for 2 to 5 minutes. Results showed that this simple use of tea leaves, acted upon by salivary esterases, released high levels of catechins and theaflavin gallates into the oral cavity.[44] A controlled, clinical, double-blind, crossover study conducted to assess the efficacy of tablets containing green tea extract (equivalent of 1 mg polyphenols for three tablets) on oral volatile sulfur-containing compounds (VSCs) demonstrated statistically significant reductions in oral VSC levels immediately (55% reduction) and after 30 minutes (26% reduction).[45] Routine prophylaxis, as well as treatment of gum disease, may be well served by the addition of green tea support to standard brushing, flossing, and dental care regimens.

DOSAGE

The normal amount of green tea consumed by Japanese and other green tea–drinking cultures is about 3 cups daily or about 3 grams of soluble components, providing roughly 240 to 320 mg of polyphenols. For a green tea extract standardized for 80% total polyphenol and 55% EGCG content, this would mean a daily dose of 300 to 400 mg.

When selecting commercial green tea extracts, it is important to look for the level of EGCG as well as total polyphenol content.

TOXICITY

Green tea is not associated with any significant side effects or toxicity. As with any caffeine-containing beverage, overconsumption may produce a stimulant effect (e.g., nervousness, anxiety, insomnia, irritability). Consumption of green tea is not associated with liver damage in humans, and green tea infusion and GTE-based beverages are considered safe in the range of historical uses. Use of no-observed-adverse-effect-levels (NOAELs) from bolus administration to derive a tolerable upper intake level applying the margin-of-safety concept results in acceptable EGCG-doses lower than those from 1 cup of green tea. In clinical intervention studies, liver effects were not observed after intakes below 600 mg EGCG/person/day. Thus as a measure of safety, a tolerable upper intake level of 300 mg EGCG/person/day has been proposed for food supplements.[46]

DRUG INTERACTIONS

Populations with marginal or deficient iron status have been shown to have lower serum ferritin or hemoglobin levels, or both, presumably due to iron binding.[47] Theoretical concerns have been raised for interactions with several drug classes, including amphetamines and anticoagulants due to the caffeine content of green tea, but no interactions have been reported in humans.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Graham HN. Green tea composition, consumption, and polyphenol chemistry. *Prev Med*. 1992;21:334–350.
2. Min Z, Peigen X. Quantitative analysis of the active constituents in green tea. *Phytother Res*. 1991;5:239–240.
3. Ho CT, Chen Q, Shi H, et al. Antioxidative effect of polyphenol extract prepared from various chinese teas. *Prev Med*. 1992;21:520–525.
4. Pecorari M, Villaño D, Testa MF, et al. Biomarkers of antioxidant status following ingestion of green teas at different polyphenol concentrations and antioxidant capacity in human volunteers. *Mol Nutr Food Res*. 2010;54(suppl 2):S278–S283.
5. Hodgson JM, Croft KD, Mori TA, et al. Regular ingestion of tea does not inhibit in vivo lipid peroxidation in humans. *J Nutr*. 2002;132:55–58.
6. Rietveld A, Wiseman S. Antioxidant effects of tea: evidence from human clinical trials. *J Nutr*. 2003;133:3285S–3292S.
7. Khan SG, Katiyar SK, Agarwal R, et al. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res*. 1992;52:4050–4052.
8. Yang CS, Lambert JD, Hou Z, et al. Molecular targets for the cancer preventive activity of tea polyphenols. *Mol Carcinog*. 2006;45:431–435.
9. Dou QP. Molecular mechanisms of green tea polyphenols. *Nutr Cancer*. 2009;61:827–835.
10. Qian G, Xue K, Tang L, et al. Mitigation of oxidative damage by green tea polyphenols and tai chi exercise in postmenopausal women with osteopenia. *PLoS One*. 2012;7(10):e48090.PubMed PMID: 23118932 .
11. Nathan PJ, Lu K, Gray M, et al. The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent. *J Herb Pharmacother*. 2006;6:21–30.
12. Theanine—monograph. *Altern Med Rev*. 2005;10:136–138.
13. Boehm K, Borrelli F, Ernst E, et al. Green tea *(Camellia sinensis)* for the prevention of cancer. *Cochrane Database Syst Rev*. 2009;8:CD005004.
14. Khan N, Adhami VM, Mukhtar H. Review: green tea polyphenols in chemoprevention of prostate cancer: preclinical and clinical studies. *Nutr Cancer*. 2009;61:836–841.
15. Katiyar SK, Elmets CA. Green tea polyphenolic antioxidants and skin photoprotection (review). *Int J Oncol*. 2001;18:1307–1313.
16. Ji HT, Chow WH, Hsing A, et al. Green tea consumption and the risk of pancreatic and colorectal cancer. *Int J Cancer*. 1997;7:255–258.
17. Sun CL, Yuan JM, Lee MJ, et al. Urinary tea polyphenols in relation to gastric and esophageal cancers: a prospective study of men in Shanghai, China. *Carcinogenesis*. 2002;23:1497–1503.
18. Zhong L, Goldberg MS, Gao YT, et al. A population-based case-control study of lung cancer and green tea consumption among women living in Shanghai. *China Epidemiol*. 2001;12:695–700.
19. Ogunleye AA, Xue F, Michels KB. Green tea consumption and breast cancer risk or recurrence: a meta-analysis. *Breast Cancer Res Treat*. 2010;119:477–484.
20. Inoue M, Tajima K, Mizutani M, et al. Regular consumption of green tea and the risk of breast cancer recurrence: follow-up study from the hospital-based epidemiologic research program at aichi cancer center (HERPACC). *Japan Cancer Lett*. 2001;167:175–182.
21. Meeran SM, Patel SN, Li Y, Shukla S, Tollefsbol TO. Bioactive dietary supplements reactivate ER expression in ER-negative breast cancer cells by active chromatin modifications. *PLoS One*. 2012;7:e37748. PubMed PMID: 22662208.
22. Yiannakopoulou EC. Interaction of green tea catechins with breast cancer endocrine treatment: a systematic review. *Pharmacology*. 2014;94(5–6):245–248. PubMed PMID: 25471334.
23. Ahn WS, Huh SW, Bae SM, et al. A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, caski cells, through apoptosis, G(1) arrest, and regulation of gene expression. *DNA Cell Biol*. 2003;22:217–224.
24. Ahn WS, Yoo J, Huh SW, et al. Protective effects of green tea extracts (polyphenon E and EGCG) on human cervical lesions. *Eur J Cancer Prev*. 2003;12:383–390.
25. Wang P, Aronson WJ, Huang M, et al. Green tea polyphenols and metabolites in prostatectomy tissue: implications for cancer prevention. *Cancer Prev Res (Phila)*. 2010;3:985–993.
26. McLarty J, Bigelow RL, Smith M, et al. Tea polyphenols decrease serum levels of prostate-specific antigen, hepatocyte growth factor, and vascular endothelial growth factor in prostate cancer patients and inhibit production of hepatocyte growth factor and vascular endothelial growth factor in vitro. *Cancer Prev Res (Phila)*. 2009;2:673–682.
27. Bettuzzi S, Brausi M, Rizzi F, et al. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res*. 2006;66:1234–1240.
28. La Vecchia C, Negri E, Franceschi S, et al. Tea consumption and cancer risk. *Nutr Cancer*. 1992;17:27–31.
29. Heilbrun LK, Nomura A, Stemmermann GN. Black tea consumption and cancer risk: a prospective study. *Br J Cancer*. 1986;54:677–683.
30. Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. *Curr Med Chem*. 2008;15:1840–1850.
31. Nantz MP, Rowe CA, Bukowski JF, et al. Standardized capsule of *camellia sinensis* lowers cardiovascular risk factors in a randomized, double-blind, placebo-controlled study. *Nutrition*. 2009;25:147–154.
32. Venkatakrishnan K, Chiu HF, Cheng JC, et al. Comparative studies on the hypolipidemic, antioxidant and hepatoprotective activities of catechin-enriched green and oolong tea in a double-blind clinical trial. *Food Funct*. 2018;9(2):1205–1213. PubMed PMID: 29384173.
33. de Maat MP, Pijl H, Kluft C, et al. Consumption of black and green tea had no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. *Eur J Clin Nutr*. 2000;54:757–763.
34. Oyama J, Maeda T, Kouzuma K, et al. Green tea catechins improve human forearm endothelial dysfunction and have antiatherosclerotic effects in smokers. *Circ J*. 2010;74:578–588.
35. Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. *Int J Obes (Lond)*. 2009;33:956–961.
36. Phung OJ, Baker WL, Matthews LJ, et al. Effect of green tea catechins with or without caffeine on anthropometric measures: a systematic review and meta-analysis. *Am J Clin Nutr*. 2010;91:73–81.
37. Shen CL, Yeh JK, Cao JJ, et al. Green tea and bone metabolism. *Nutr Res*. 2009;29:437–456.
38. Weinreb O, Mandel S, Amit T, Youdim MB. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutri Biochem*. 2004;15(4):506–516. PubMed PMID: 15350981.
39. Hu G, Bidel S, Jousilahti P, Antikainen R, Tuomilehto J. Coffee and tea consumption and the risk of Parkinson's disease. *Mov Dis*. 2007;22(15):2242–2248. PubMed PMID: 17712848.
40. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. *Neurol*. 2012;78(15):1138–1145. PubMed PMID: 22491871.
41. Unno K, Takabayashi F, Yoshida H, et al. Daily consumption of green tea catechin delays memory regression in aged mice. *Biogerontol*. 2007;8(2):89–95. PubMed PMID: 16957869.
42. Elmets CA, Singh D, Tubesing K, et al. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J Am Acad Dermatol*. 2001;44:425–432.
43. Krahwinkel T, Willershausen B. The effect of sugar-free green tea chew candies on the degree of inflammation of the gingiva. *Eur J Med Res*. 2000;5:463–467.
44. Lee MJ, Lambert JD, Prabhu S, et al. Delivery of tea polyphenols to the oral cavity by green tea leaves and black tea extract. *Cancer Epidemiol Biomarkers Prev*. 2004;13:132–137.
45. Porciani PF, Grandini S. Effect of green tea-added tablets on volatile sulfur-containing compounds in the oral cavity. *J Clin Dent*. 2016;27(4):110–113. PubMed PMID: 28391664.
46. Dekant W, Fujii K, Shibata E, Morita O, Shimotoyodome A. Safety assessment of green tea-based beverages and dried green tea extracts as nutritional supplements. *Toxicol Lett*. 2017;277:104–108. PubMed PMID: 28655517.
47. Temme EH, Van Hoydonck PG. Tea consumption and iron status. *Eur J Clin Nutr*. 2002;56:379–386.

61

Cannabis (Marijuana) and Cannabinoids

Roy Upton, RH

OUTLINE

Cannabis spp. (family: Cannabaceae)
Common name: cannabis

GENERAL DESCRIPTION

Cannabis consists of the dried inflorescences and remains of subtending leaves of pistillate Cannabis plants (Fig. 61.1). It is an herbaceous annual growing from a taproot. Male and female flowers primarily occur on separate plants, but rarely male and female flowers occur on the same plant. There is considerable academic and practical debate regarding speciation of the genus, with some authorities recognizing a single highly varied species (*Cannabis sativa, C. sativa* var. *sativa; C. sativa* var. *indica;* etc.) and others recognizing separate species, specifically *C. sativa, C. indica,* and *C. ruderalis,* this latter species being a wild progenitor from central Asia. Species are often designated as narrow-leaf or broadleaf, botanically referring to *C. sativa* and *C. indica,* respectively, or fiber and drug types, respectively. Cannabis hemp types are generally nonintoxicating, yielding very low levels of the primary intoxicating cannabinoid tetrahydrocannabinol (THC) and, rather, yielding relatively high amounts of the nonintoxicating cannabidiol (CBD). (Technically, the forms in the raw, unprocessed plants are precursors THCA and DBDA.) Conversely, cannabis drug types yield much higher concentrations of THC. Many of the distinctions differentiating *C. sativa, C. indica,* narrow-leaf, broadleaf, drug type, and fiber type are no longer supported due to interbreeding, predominantly breeding for high-THC cultivars.

Based primarily on chemical analysis, three types of cannabis are generally recognized, irrespective of botanical origin or morphological characters, namely, drug type (THC precursor predominating), fiber types (CBD precursor predominating), and intermediate cultivars between the two. The manner in which the species is lumped or split has implications commercially and regulatorily, especially when making a distinction between intoxicating and fiber (hemp) types, a discussion that is beyond this review. For a detailed discussion of the taxonomic debate regarding the monotypic versus polytypic classification of cannabis, see Clarke and Merlin *Cannabis—Evolution and Ethnobotany* (2016).[1]

Confusing the popular nomenclature more is the manipulation of terpenes, which give cannabis its sensory characteristics of taste, feel, and aroma, giving rise to distinctive names such as "train wreck," "silver haze," "kush," "skunk," "lemon haze," and "lemon skunk," to name only a very few. Despite the many different descriptors appearing in cannabis trade, most of these can be categorized between types that yield one of several predominant terpene profiles (see "Constituents"). Although terpenes, and perhaps other compounds, can have a modulating effect on cannabinoids, the concentration and contributions of the activity of CBD or THC are generally far greater than the relatively small concentrations of individual terpenes. Still, most involved in the

Fig. 61.1 (A) Cannabis—whole plant. (B) *Cannabis sativa* habitat. (C) *C. sativa* inflorescence, female.

medicinal use of cannabis believe the effectiveness of the botanical is due to the entourage effects of all compounds present, including the numerous other cannabinoids beyond CBD and THC. For a detailed discussion of the concept of *entourage effects,* see Russo (2011).[2]

REGULATORY FRAMEWORK

Although recreational and medicinal use of cannabis is federally illegal, numerous states allow for its use. The federal scheduling of cannabis as a controlled substance is based on the legal grounds that the U.S. government considers cannabis (1) to have a high potential for abuse, (2) to have no accepted medical use, and (3) to be unsafe for use under medical supervision. None of these assertions has a scientific basis. The extensive historical medical use, modern clinical and pharmacological data supporting numerous uses of cannabis, its safety when used appropriately, and the recent approval by the U.S. Food and Drug Administration (FDA) of the cannabis drug Epidiolex (GW Pharmaceuticals, United Kingdom; 98% CBD) for the treatment of rare seizure disorders demonstrate that there is little evidence supporting such a prohibition—and likely never was. It is also clear that the recreational use of cannabis is far safer than that of other restricted, but allowed, substances such as cigarettes and alcohol, similarly suggesting such prohibitions based on safety are not scientifically based. Unfortunately, the U.S. prohibition against cannabis has influenced the manner in which the botanical is regulated in other countries and, more importantly, has limited meaningful research from taking place in North America. Fortunately, numerous states have minimally recognized the potential medical use of cannabis, and a few others have recognized the ability to use it safely recreationally. The laws governing the use of cannabis differ state by state and change frequently. Similarly, as evidenced by the FDA approval of Epidiolex, the regulatory framework of cannabis is evolving, making it necessary to refer to individual state laws and federal policies for current legal status.

HISTORICAL USE

Numerous medicinal preparations of cannabis have been used and prescribed since the earliest written records of medical history, including their formal recognition by medical authorities in official pharmacopeias, dispensatories, and *materia medicas*. Despite this, the plant has remained a source of controversy, possessing a reputation as both a pariah and medical panacea, depending on one's viewpoint. Fueling the former philosophy is the intoxicating nature of cannabis, which, historical and modern use demonstrates, has negative effects when

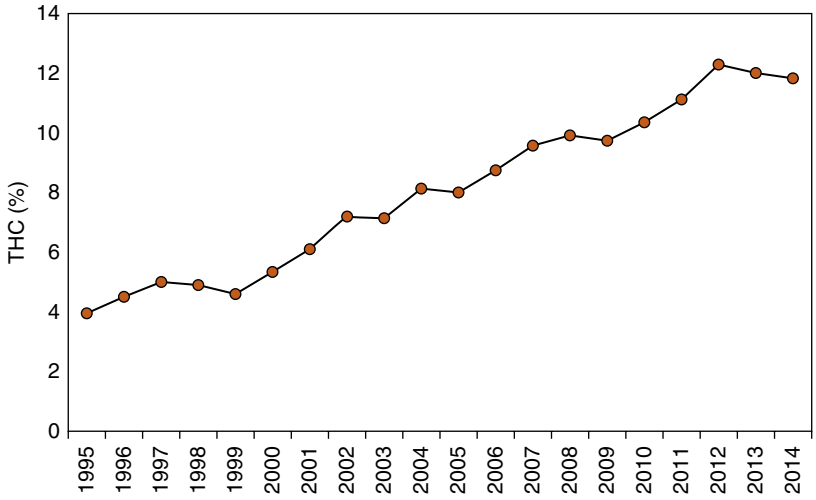


Fig. 61.2 Change in THC content over past two decades. (From ElSohly MA, Mehmedic Z, Foster S, et al. Changes in cannabis potency over the last 2 decades [1995–2014]: Analysis of current data in the United States. *Biol Psychiatry.* 2016 Apr 1;79[7]:613-61–9.)

used in excess and has been the subject of intense political and social demonization and religious prohibition. Representing the more positively biased side of the cannabis story is the discovery of the endocannabinoid system (ECS). The ECS is an integral part of human biology consisting of endogenous cannabinoids, most notably *anandamide,* named after the Sanskrit *ananda* for "bliss" or "joy" coupled with *amide,* and the widespread distribution of endocannabinoid receptors throughout the body (CB1 and CB2 receptors).

Endocannabinoid receptors occur in the central and peripheral nervous systems, including the brain, as well as the pancreas, uterus, bones, and liver.[3] Through a wide array of network signaling, cannabinoids have a modulating effect on many physiological systems that are both excitatory and inhibitory. There is a biphasic nature to the effects of cannabinoids on human physiology that is at least partially dependent on the receptor status of patients, that is often unpredictable until cannabis is administered. Anandamide is a neurotransmitter derived from arachidonic acid and is also synthesized in the uterus in pregnancy, eliciting a modulating effect at various stages of the developing embryo. The widespread distribution of cannabinoid receptors leads to the conclusion by some that anything that can modulate the ECS is beneficial for a myriad of diseases. Although this is mostly accurate, it leads lay advocates to believe that cannabis is a beneficial medicine for all people all the time in all diseases. In reality, beyond the general use of self-medicating with cannabis for the temporary relief of specific symptoms or long-term management of disease states (e.g., epilepsy), cannabinoid therapy should be tailored for individual needs, using preparations that favor specific cannabinoid profiles (i.e., CBD or THC prominence). Cannabis, as with other medications, should be integrated with lifestyle, behavioral, emotional, and/or other botanical or conventional medications that support cannabis therapy and normal physiology. Unfortunately, many resort to cannabis as a symptomatic Band-Aid to cope with mild to serious diseases, in the same manner as conventional drugs are used without addressing underlying or lifestyle factors that contribute to the disease state. That being said, aside from the ideal of using cannabis within the context of an integrative medical approach, it is a highly safe and effective medicine, with profound pharmacological activities that can be used to positively provide symptomatic relief and, in some cases, alter pathological states.

One important consideration regarding reference to the historical use of cannabis is the fact that the THC content of THC-prominent strains was historically much lower than plant material today. For example, in the 1960s and 1970s, the average percentage of THC in herbal cannabis was less than 1%, although anomalous samples reaching 9.5% were reported,[4] although this also has to be questioned based on analytical methodologies that were less well developed than they are today. In 1980 the average total THC concentration was approximately 1.5%,[5] which rose to approximately 3.3% in 1983 and 1984, fluctuated around 3% until 1992, and increased to 4.2% (average) in 1997. Since 1997, due to the increasing prevalence of cross-breeding for psychotropic effects, THC yields have steadily increased to today's average of approximately 13%.[6] In the same time period, other cannabinoid concentrations (e.g., CBD) remained relatively stable.[7,8] Another consistent theme reflected in the traditional herbal medical literature is that the efficacy of cannabis typically was evident below intoxicating doses, and much of the historical literature suggests that, if unwanted psychotropic effects occurred, too much was given (Fig. 61.2).

Another consideration in interpreting the therapeutic benefit of cannabis preparations is the differentiation between subjective and objective metrics of benefit and between short-term feelings of well-being that last only until the psychotropic effects of cannabis wear off (typically a few hours) and require repeated dosing versus long-term positive changes in physiology that last beyond the acute effects of cannabis. For example, the psychotropic effects of cannabis often contribute to a higher tolerance of or disassociation from subjective symptoms such as pain or depression but may not demonstrably alter pathology or lead to a worsening of symptoms. For integrated medical practitioners working with clients for whom cannabis may be used or indicated, these are important considerations. There are many potential benefits from the appropriate therapeutic use of cannabis and many more to be discovered. Following are the primary areas of application, but this discussion is by no means comprehensive.

Use in Traditional Healing Systems

Cannabis has been used throughout the world wherever consumers and medical practitioners had access to it. Its use dates to ancient times in Africa, China, Egypt, Greece, India, Rome, and Mesopotamia. The earliest writings of the medical use of cannabis occur on cuneiform bones. Some from approximately 800 BCE in Assyria record it as an ingredient in prescriptions. In Assyria, cannabis was named *gan-zi-gun-nu* ("the drug that takes away the mind") or *azallu* when used therapeutically.[9] During this time, cannabis was used to treat nocturnal epilepsy, a use relevant to clinical practice today.

Use in Traditional Chinese Medicine

The seed of *Cannabis* was discussed in China's earliest known text dedicated to medicinal therapies, the *Shennong Bencao Jing* (*Divine Farmer's Materia Medica*). In this *materia medica,* the psychotropic effects of cannabis were recognized in the statement that "excessive consumption causes one to see ghosts and walk frenetically." It was further recorded that consuming cannabis over a prolonged time "frees the spirit light and lightens the body."[10] In a 5th-century annotated edition of the *Shennong Bencao Jing,* the Taoist priest Tao Hongjing reported that *Cannabis* flower was "toxic," relieved pain due to "impediment," and was rarely used in formulas or by healthcare practitioners. Li Shizhen, regarded as the greatest compiler of Chinese *materia medica,* in his 16th-century *Bencao Gangmu,* recorded the use of cannabis for "hundred diseases of wind-withdrawal,"[11] which is a reference to mental disorders, a traditional category of mental illness characterized by either extreme withdrawal, mania, or both. The flowers, seeds, leaves, and stalk were later referenced in a number of other important historical Chinese texts on medicinals, but the vast majority of these and modern textbooks focus only on the use of the seeds.

In the modern era, the *Zhongyao Daci Dian* (Great Encyclopedia of Chinese Medicinals) is arguably the most complete single reference on all parts of the *Cannabis* plant. In this text, the following uses for the flowers were given: "pain wind," "withdrawal and mania," insomnia, and panting and cough. Although the seed is ubiquitous in modern Chinese *materia medica* and formula literature, relatively few texts include other parts of the *Cannabis* plant, and no other parts of the plant are commonly used today as part of traditional Chinese medicine (TCM) practice, either in the People's Republic of China or Taiwan. Some TCM specialists in North America are integrating the medical use of *Cannabis* into their practices based on the plant's modern use, however, not always according to TCM theory.

Use in Ayurveda

According to modern authorities,[12,13] cannabis is not featured in the foundational texts of Ayurveda, namely, *Caraka Samhita, Suśruta Samhita,* and *Vāgbhata's Aṣṭāngahrdayasamhitā.* The use of the leaves medicinally in oral preparations in India was definitively established around the 11th to 12th century CE in *Vangasena's Cikitsāsārasamgraha.* This text reports on the combination of opium and cannabis as a *vyavayin:* "a substance which pervades the whole body before being metabolized." The same text, naming the herb *indrāsana* ("food of Indra") and *tribhavanavijaya,* includes cannabis as an ingredient in two longevity formulas.

The pharmacodynamics of cannabis in Ayurveda *(dryavaguna)* is most consistently described in the historical literature. In the *Mahendrabhogika's Dhanvantarīyanighantu* of around the 11th century, cannabis is described as removing humoral phlegm, to be sharp, to aid digestion, and to be light. It is written that because cannabis is sharp and heating, it increases *pitta* (humorous bile) and provokes delusions, slows speech, and increases the digestive fire.[13] The *Rajanighantu* of the 13th century characterized cannabis properties and functions as acrid, astringent, heating, and pungent; as removing wind and phlegm, promoting speech, giving strength, and inspiring of mental power; and being an excellent excitant.[14] In Ayurveda, cannabis was used as a treatment for fevers to cool heated blood, soothe the overwakeful to sleep, give beauty, and secure length of days. It was also used to treat dysentery and sunstroke, clear phlegm, quicken digestion, sharpen the appetite, make the tongue of the lisper plain, freshen the intellect, and give alertness to the body and gaiety to the mind. In underscoring the need for appropriate doses, it was also stated that in moderation, cannabis is the best of gifts, but in excess, it causes abscess and even madness.[15]

The leaves of *Cannabis* are included in modern editions of the *Ayurvedic Pharmacopoeia of India* (2001)[16] under the name *vijayā,* where it is recorded for "sluggishness of the digestive fire" *(agnimāndya),* which often refers to dyspepsia and loss of appetite, acute diarrhea *(atisara),* digestive disturbances *(grahanīroga),* malabsorption, impotence *(klabiya),* and insomnia *(anidra).*

Use in Western Medical Traditions

Although cannabis is mentioned in the earliest writings of ancient Western medicine, detailed records of its clinical uses are lacking, focusing predominantly on its use for textiles, ropes, the seed as a cereal grain and providing fewer reports of its intoxicant and medical effects.

Irish physician William O'Shaughnessy, who learned of the herb's use in India, introduced cannabis into Western medical practice in the early 19th century. O'Shaughnessy provided detailed records and personal accounts of the effects and clinical use of cannabis that remain some of the most detailed guidance to date, which included the use of cannabis for relieving tetanus, rheumatic pain, cholera, and infantile convulsions. In this latter use, O'Shaughnessy's experience led him to believe that the anticonvulsant properties of cannabis had no equal and that it was the safest of the powerful narcotics of the time, for example, compared with opium.[17] The general effects of cannabis on humans as reported by O'Shaughnessy include alleviation of pain, antispasmodic, a remarkable increase in appetite, unequivocal aphrodisiac, great mental cheerfulness, and at the extreme continuum, delirium and a cataleptic state.

Cannabis came into use by the eclectic physicians of the United States around 1855 as practitioners learned of its use, partly from German physicians. The primary earliest actions were noted as anodyne, hypnotic, nervine, digestive, and antispasmodic, with indications including rheumatic pain accompanied by spasms and contraction that was only marginally responsive to other agents, as a narcotic to induce sleep, for menorrhagia, as a mind-altering drug, and even its use for "insanity" and reducing symptoms of withdrawal (delirium tremens), among other uses. As an anodyne, its action was considered to be less certain than opium but without the common side effects of opium or morphine,[18] a use of considerable importance today. This subsequently led to the inclusion of a comprehensive discussion of the herb's use in Felter and Lloyd's *King's American Dispensatory*,[19] in which both the inebriating and medicinal qualities of cannabis are noted. This foundational Eclectic work refers to cannabis as "one of the most important of our remedies" and additionally notes "but, like our best agents, it must not be used indiscriminately," providing sage advice for its medicinal use today. Cannabis was subsequently entered into the pharmacopeias of many European countries and the United States, where it was officially recognized as a medicinal agent, again attesting to the lack of scientific basis for today's current scheduling.

CHEMICAL COMPOSITION

There is a large number and variety of compounds in cannabis, most notably, cannabinoids and terpenes. There are more than 100 cannabinoids that potentially contribute to the activity of cannabis, of which several are prominent, the most well known being THC and CBD, accompanied by tetrahydrocannabivarin (THCV), cannabigerol (CBG), cannabichromene (CBC), and delta-8-tetrahydrocannabinol (delta-8-THC) and degradation compounds such as cannabinol (CBN). These compounds occur in the plant in carboxylic acid forms (e.g., as THC acid [THCA], CBD acid [CBDA], etc.) that are often considered to be relatively inactive but should be considered to possess a weaker or different activity than the decarboxylated forms of the parent compounds. Although possessing

some activity, conversion of the acids to their decarboxylated forms, usually by heating, is necessary for their full potential to be reached. For example, THCA lacks intoxicating effects and requires decarboxylation to the psychoactive THC. This conversion readily occurs in smoking or baking. Conversely, THCA possesses anti-inflammatory activity.[20]

Terpenes are volatile compounds that give cannabis its characteristic aroma, taste, and overall sensory characteristics. Although cannabis contains more than 200 terpenes and sesquiterpenes, like the cannabinoids, several predominate, most notably myrcene (reminiscent of cloves with hints of citrus), α- and β-pinene (pine aroma), and β-caryophyllene (reminiscent of pepper). Additional terpenoid chemovars include those containing limonene (citrus aroma), linalool (floral or sweet aroma), and humulene (reminiscent of hops), to name the primary types (for a more complete discussion, see Giese et al. [2015]).[21] Each terpene chemovar is generally believed to offer a unique suite of compounds beneficial for specific conditions and may modulate the effects of cannabinoids. To date, preference for one over another chemovar is highly subjective, based on patient or practitioner perspectives.

Whereas the cannabinoid profile and content in a particular chemovar are primarily genetically predetermined, terpenes are much more subject to variability through environmental and growing conditions, giving rise to the wide variety of chemovars, fanciful names, and sensory subtleties that exist in the market.

There is a host of additional compounds that occur in *Cannabis* that may contribute to the overall activity of the species. These include carbohydrates (monosaccharides, disaccharides, polysaccharides, sugar alcohols, cyclitols, and amino sugars), amino acids, amines (piperidine, hordenine, ammonia), noncannabinoid phenols (spiroindan-type, dihydrostilbene-type, cannabidihydrophenanthrene derivatives, simple phenols, simple phenolic glycosides, and phenol methyl esters), simple alcohols, aldehydes, ketones, acids, esters, lactones, steroids (phytosterols and brassinosteroids), vitamins, xanthones, coumarins, pigments, and alkaloids.[22]

Tetrahydrocannabinol

Heating THCA, the form found in the raw plant, results in decarboxylation to tetrahydrocannabinol (THC), which affects behavior by influencing both inhibitory GABAergic and excitatory glutamatergic terminals in the brain in a dose-dependent fashion. Gamma-aminobutyric acid (GABA) is a neurotransmitter involved in the communication between the brain and nervous system and is primarily responsible for promoting calmness by inhibiting neuronal activity. This has significant clinical implications for anxiety, depression, insomnia, various behavioral disorders, cognition, modulation of stress responses, and maintenance of muscle tone. GABA is also expressed in the immune system, where it has a modulating effect on immune responses and thus implications in autoimmune disease.[23,24]

In contrast to the inhibitory nature of the GABAergic system, the gluatmatergic system is excitatory, with glutamine being the primary excitatory neurotransmitter in the nervous system. Glutamate receptors are distributed throughout the spinal cord and brain in neurons and are linked to numerous other neurotransmitter pathways. There is also a close biological relationship between the GABAergic and glutamatergic systems in that GABA is synthesized by L-glutamic acid carboxylase, suggesting that, from an evolutionary perspective, the two systems work in tandem in modulating nervous system function between inhibitory and excitatory states.[25] There are various clinical implications of the effects of cannabinoids on the endocannabinoids system.

Cannabidiol

Like THC, CBD is not a naturally occurring cannabinoid in the raw *Cannabis* plant but rather has to be decarboxylated, usually by heat, from the acid form (CBDA) to CBD. Unlike THC, CBD does not possess the intoxicating activity of THC and is often referred to as lacking psychotropic activity. However, this compound has a marked effect on anxiety, so it can be considered to possess psychotropic effects but lacks the "high" that comes with THC consumption.

Like THC, CBD acts on both CB1 and CB2 receptors but to a much lesser degree than THC[26] and is often cited as potentially modulating the intoxicating effects of THC. Part of the activity of CBD is its ability to preserve the activity of the endogenous cannabinoid anandamide, either by preventing its degradation or increasing its uptake. Anandamide effects memory,[27] eating, sleep, and pain[28,29]; inhibits breast cancer cell proliferation[30]; and may be at least partially responsible for "runner's high."[30] Increasing the amount of anandamide in the brain has implications in the treatment of anxiety and depression.[31,32] CBD may also play a role in anxiety and depression through its effect as a serotonin (5HT1A) agonist, on pain through its interaction with TRP receptors, and in reducing inflammation through its ability to inhibit adenosine inactivation.[26,33–35] One primary area of investigation is the potential for CBD to positively affect numerous neurological and seizure disorders such as psychosis, multiple sclerosis, epilepsy, Dravet syndrome, Lennox-Gastaut syndrome, Huntington's disease, Tourette's, and numerous other neurological conditions. The use and often-remarkable effects of CBD for seizure disorders in children were popularized by the use of the CBD preparation Charlotte's Web (Stanley Brothers, Colorado) and the recently FDA-approved CBD preparation Epidiolex.

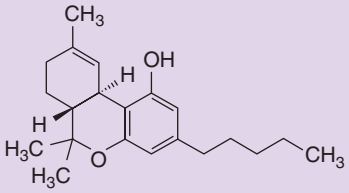
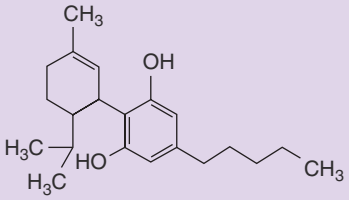
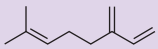
Other Nonintoxicating Cannabinoids

There is a host of other cannabinoids that, like CBD, lack the intoxicating effects associated with THC, including cannabigerol (CBG), cannabichromene (CBC), D9-tetrahydrocannabivarin (D9-THCV), and cannabidivarin (CBDV) and their acid forms D9-tetrahydrocannabinolic acid (D9-THCA) and cannabidiolic acid (CBDA).[36] These compounds occur in lower concentrations than THC and CBD and are often overshadowed by the more pronounced effects of THC and CBD. However, they are pharmacologically active compounds affecting both the endocannabinoid system, as well as systems outside of endocannabinoids, and they undoubtedly contribute to the entourage effect. CBN, for example, possesses approximately 10% of the activity of THC; D9-THCV is abundant in hashish from Pakistan and is reported to antagonize the effects of THC; and CBG effects a family of receptors (TRP) involved with sensations such as pain, hot, and cold but also exhibits antitumor activity and displays activity at very low doses. For a discussion of these minor compounds, see Izzo et al. (2009)[36] (Table 61.1).

PHARMACOLOGY

There are many indications for which cannabis is and can be used. Historically, the most common actions of cannabis were as an anesthetic, antidepressant, anodyne, calmative, appetite stimulant, and sedative. Based on these actions, cannabis was used for a wide array of conditions, including depression, pain management, spastic symptoms, acute mania, menorrhagia and dysmenorrhea, and wasting syndromes. Cannabis was also used topically for swelling, pain, bruises, burns, hemorrhoids, and fungal infections, applications not commonly employed today.

TABLE 61.1 Primary Compounds of Cannabis and Their Activity

 Delta-9-tetrahydrocannabinol (THC)	Primary psychotropic compound of cannabis Analgesic via CB1 and CB2 agonism (active at 20–40 nM)[191]; antiemetic[192,193]; anti-inflammatory, antioxidant[194]; antipruritic, cholestatic jaundice[195]; benefits duodenal ulcers[196]; bronchodilatory[197]; muscle relaxant[205]; reduces symptoms of Alzheimer's[199,200]
 Cannabidiol (CBD)	Anandamide (AEA) reuptake inhibitor,[29] analgesic,[201] anticonvulsant,[202] antidepressant in rodents,[203] antiemetic (5HT1A agonist; 5 mg/kg ip),[204] antifungal,[205] anti-inflammatory,[206] antagonizes effects of THC in humans,[207] antioxidant, anxiolytic via 5HT1A agonism,[34,208,209] decreases sebum/sebocyte proliferation,[210] effective against methicillin-resistant *Staphylococcus aureus* (MRSA),[211] increases adenosine A2A signaling,[35] proapoptotic against breast cancer cell lines,[212] treatment of addiction,[213] treatment of psychosis[214]
 Myrcene	Analgesic,[215,216] anti-inflammatory,[216] muscle relaxant,[217] sedative[218]

Most states that allow for the medical use of cannabis include cancer, HIV/AIDS, multiple sclerosis, glaucoma, and seizures/epilepsy as among the most commonly accepted of medical indications,[37,38] usually as adjuvants to other therapies and primarily for relieving symptoms associated with these conditions. Other surveys provide further evidence of additional uses of cannabis in states with medical marijuana laws and include pain, spasticity associated with multiple sclerosis, nausea, posttraumatic stress disorder, cachexia, glaucoma, and degenerative neurological conditions.[39,40] To date, the most comprehensive evidence-based reviews of cannabis use come from the National Academy of Science (NAS)[26] and the Canadian government (Health Canada).[41] The NAS review concluded there was conclusive evidence for an antiemetic effect in chemotherapy-induced nausea and vomiting; substantial evidence for moderate reduction of self-reported spasms; limited evidence for effect on clinician-measured spasms; limited evidence for reduction of depressive symptoms; moderate evidence for improvement of short-term sleep outcomes; and limited evidence for increasing appetite and decreasing weight loss in HIV. The NAS review failed to find supporting evidence for many of the other indications for which cannabis is popularly used.

The Health Canada review reports there are varying levels of evidence supporting the use of cannabis or cannabinoids in a myriad of conditions, including pain; movement disorders (epilepsy, multiple sclerosis, Parkinson's, Tourette's); adjunctive cancer and HIV care; mood, sleep, and psychiatric disorders; alcohol and opioid withdrawal; Alzheimer's; some gastrointestinal conditions; and potential anticancer activity.

Anecdotal reports of efficacy for use in conditions such as posttraumatic stress syndrome, cancer, and spastic conditions other than multiple sclerosis continue to drive cannabis use, and although lacking in strong formal evidence, these remain consistent uses for which the plant is employed.

The Endocannabinoid System

Cannabinoid receptors are often described as being separated between CB1 receptors in the central nervous system and CB2 receptors predominantly in the peripheral system. However, although both are preferentially concentrated in different tissues, both are broadly distributed throughout the body. CB1 receptors are highly concentrated in the brain and affect most areas and functions of the brain, affect neurotransmitters, and are highly expressed at GABAergic and glutamatergic neurons. CB1 is also highly expressed in nonneuronal tissues, including the gastrointestinal, reproductive, and cardiovascular systems, as well as in fat cells, the liver, the pancreas, and skeletal muscle (see Ligresti et al. [2016] and references therein).[42]

CB2 receptor expression and its functional physiological effects are less well understood than those for CB1 expression and activity. CB2 is highly expressed in various tissues of the immune system, including the spleen, intestines (gut lymphatic tissue), and lymph, and is also found in the brain and retina. One challenge in determining the specific tissue distribution and subsequent physiological activity of cannabinoid receptors is due to the fact that CB receptor expression can change with physiological stresses and disease states. For example, greater CB2 expression can occur in inflammatory conditions of the retina, more so than in healthy tissues.[42,43] Both receptor types have the ability to activate multiple signaling pathways that affect multiple systems and have the potential to positively influence numerous disease states. The endocannabinoids system has marked effects on neuronal activity, brain development in all stages of development, memory, emotions, appetite, and pain. Other cannabinoid receptors are also present (see Ligresti et al. [2016]),[42] and botanicals other than cannabis (e.g., echinacea) can influence cannabinoid receptors,[44] although both receptor types are highly activated specifically by the psychoactive THC and, to a lesser extent, the relatively minor cannabinoid cannabinol (CBN).

CLINICAL APPLICATIONS

Appetite Stimulation, Nausea, and Vomiting

Cannabis is well known for its ability to stimulate the appetite and is often used to sustain weight or promote weight gain in anorexia or weight loss associated with acquired immunodeficiency disorders (AIDS) and cancer. To date, no well-designed studies on the effects of cannabis on weight gain have been conducted. There is evidence for appetite stimulation, weight preservation, and weight gain from uncontrolled studies in patients with AIDS[44–47] and cancer,[48] and animal studies suggest that greatest efficacy is seen with relatively low doses[50] because the intoxication that accompanies high-dose cannabis use can impair the ability to initiate feeding. Some small investigational

studies suggest that smoking cannabis can increase caloric intake by 40%.[49,50] Other reports note that the increase in calories is mostly from snack foods, not increases in mealtime eating. This is predictable because CB1 receptors are highly expressed in sweet taste receptors.[51] Other investigations show an increase in caloric intake but no corresponding weight gain.[52] Numerous studies have shown efficacy for appetite stimulation and weight gain for synthetic THC (e.g., Jatoi et al. [2002],[53] Nelson et al. [1994],[54] Regelson et al. [1976],[48] Strasser et al. [2006],[55] Struwe et al. [1993], and Timpone et al. [1997][56] among others), but sometimes to a lesser degree than other therapies (e.g., megestrol acetate).[53,56] Most studies used orally consumed cannabis, whereas at least one study[47] reported comparable effects with smoked cannabis.

Cannabis is frequently used to allay nausea and vomiting, with THC generally considered as the primary active ingredient. The formal evidence regarding efficacy is reported as mixed and potentially positively biased, although cannabinoids are more effective than placebo and equally effective as conventional antiemetics.[26] Despite the potential for a positive bias in studies, the NAS review reported that there is conclusive or substantial evidence for the effectiveness of cannabis for allaying chemotherapy-induced nausea and vomiting. In one study, smoking was found to be more appropriate as a treatment option than pills, due to the inability to ingest oral forms when nauseous, but also due to higher blood levels of THC that are achieved with smoking.[57]

Synthetic cannabinoids (nabilone and dronabinol) were initially approved in 1985 for treating nausea and vomiting associated with cancer chemotherapy in patients who failed to respond adequately to conventional antiemetics. However, a number of studies and reviews since then report that THC or crude cannabis preparations are more effective than these approved THC analogs and are often equally effective as or more effective than other conventional antiemetic agents (for reviews, see NAS [2017],[26] Chang et al. [1979],[58] Lauritsen et al. [2016],[59] and Whiting et al. [2015][59]).

One important note regarding the use of cannabis as an appetite stimulant is that an increase in calories does not always result in an increase in weight gain because calories are often increased with simple sugars and snack foods, rather than increases at mealtime. Although weight gain has been reported in patients with HIV/AIDS using cannabis, cannabinoids are also reportedly less effective than progesterone megestrol acetate in improving appetite.[26]

The use of cannabis for allaying morning sickness has also been reported and is regarded by some as safe and others as contraindicated due to mixed evidence on the potential negative effects of cannabis on the developing fetus (see "Use in Pregnancy").

Neurological Disorders

Dravet and Lennox-Gastaut Syndromes

Cannabis, and in particular, CBD, has gained fame in recent years for its ability to dramatically decrease the severity of seizure disorders in children, conditions that in one third of cases are not effectively treated with conventional therapies. The use was stimulated by parental medication of their children with a CBD preparation (initially Charlotte's Web), in some cases with an almost complete control of seizures in some children with Dravet syndrome.

One group of investigators evaluated 214 patients with intractable Dravet and Lennox-Gastaut syndromes.[56] A 39% and 50% reduction in motor seizures was observed in subjects with Dravet and Lennox-Gastaut, respectively. There was a mean reduction in motor seizures of 30% over the 12-week period. The same research group[60] reported a 50% reduction in seizures in 43% of subjects with Dravet syndrome. At the end of the therapy, a small percentage (5%) was completely seizure-free. In subjects with Lennox-Gastaut syndrome, an almost 69% reduction in average monthly atonic seizures was observed, with 21% of subjects free of atonic seizures and 3% completely free of seizures during the last 4 weeks of therapy. The majority of caregivers (62%) reported improvement in the overall condition of the children given Epidiolex, compared with 34% of caregivers in the placebo group. There was no significant reduction in nonconvulsive seizures. Adverse effects were reported in 93% of treatment subjects, 89% of which were mild or moderate, and 75% of which were attributed to the medication. Some of the adverse effects were due to a potentiation of the effects of other medications taken. The most common adverse effects included sleepiness, vomiting, fatigue, elevated body temperature, decreased appetite, diarrhea, and elevated liver enzymes.[60]

In 2018 Epidiolex, a concentrated CBD oil (>98% CBD), was approved in the United States for the treatment of Dravet and Lennox-Gastaut syndromes that are resistant to standard treatments.

Epilepsy

Cannabis is widely employed in other types of seizure disorders, such as epilepsy, a long-standing historical indication[61] and an approved indication under the U.S. *compassionate drug use* program, in which select patients in the program are provided legally obtained cannabis as a seizure management tool. Numerous studies of a variety of designs demonstrate the efficacy of cannabis in reducing the incidence of epileptic seizures. In a case series of patients treated in U.S. epilepsy centers, a reduction in seizures of 36.5% was observed compared with baseline over a 12-week treatment period. In Israel, where the medical benefits of cannabis have been extensively studied for decades, 18% of children with epilepsy experienced a 75% to 100% reduction in seizure frequency, 34% experienced a 50% to 75% reduction, 12% reported a 25% to 50% reduction, and 26% reported a reduction of less than 25%. Aggravation of seizures was reported in 7% of subjects and led to a discontinuation of the treatment.[62] Tzadok et al.[63] reported on the use of cannabis in the treatment of seizure disorders represented by 272 cases in Washington state and California. Of these cases, cannabis was ineffective at reducing seizures in 14% (37) of subjects, 15% (29) experienced a 1% to 25% seizure reduction, 18% (60) a 26% to 50% reduction, 17% (45) a 51% to 75% reduction in seizures, 28% (75) a 76% to 99% reduction, and 10% (26) experienced a complete clinical response. Across patients studied in this review, 86% experienced some clinical benefit. Generally speaking, adverse effects were mild, and 4% of patients experienced an exacerbation of seizures. In these subjects, there was a wide range of effective cannabinoid doses, ranging from 0.05 to 9 mg/kg/day, and effective serum levels of CBD ranging from 1.8 to 80 ng/mL, showing a wide variation that makes it challenging to determine the most appropriate dose, which is highly individualized.[63] Some patients also included THC or THCA in their treatments. A similar reduction (84%) in seizures was reported in another survey of parents of children with seizure disorders who administered cannabinoid preparations to the children. Other beneficial effects included increased alertness, better mood, and improved sleep. Side effects included drowsiness and fatigue.[64] In a different report, seizures were exacerbated in 18% of subjects using a cannabis preparation[62] (see "Adverse Effects").

Although cannabis preparations are not effective for all subjects with seizure disorders, for those in which it is effective, the benefits can be remarkable. However, different subjects respond to different preparations and dosages. Although CBD has received a considerable amount of attention, some subjects require at least small amounts of THC. Others appear to respond to noncarboxylated preparations dominant in the nonpsychoactive THCA, whereas others may respond best to chemovars that favor one terpene over another (e.g., linalool).

Not treating patients who could benefit from cannabis as a novel therapy, treating with illicit or poorly characterized products in states where cannabis prohibition is still in place, and not having appropriate guidance in how to dose all carry a wide array of consequences in which the benefits must be weighed against the risk. One key consideration is the fact that cannabis has been widely effective in relatively large numbers of people, including children, with refractory seizure disorders. Their parents, often against great odds and with tremendous personal risk, are responsible for raising awareness about its potential benefit. For a more comprehensive review of the use of cannabis in seizure disorders and the pharmacological mechanisms involved, see Devinsky et al. (2016, 2017),[59,60] Russo (2017),[61] Stockings et al. (2018),[65] and Whalley (2014),[66] among others.

Multiple Sclerosis

Approximately 20% of patients with multiple sclerosis (MS) use cannabis for reducing MS symptoms,[67] and MS is one of the most commonly accepted diseases for the use of the plant in states where medical cannabis is approved. The NAS review concluded there was conclusive evidence for improving patient-reported symptoms associated with MS spasticity symptoms and limited clinical evidence for improvement of clinician-measured MS spasticity symptoms.[26] There is preclinical evidence of potential objective benefit based on anti-inflammatory properties associated with cannabis, with specific evidence suggesting a protective effect on neurons in MS models in mice.[68] Conversely, clinical evidence regarding the ability of cannabis to change disease progression is lacking.[69]

In formal clinical trials, the majority of evidence for efficacy is based on the investigation of the cannabis preparation Sativex, which is used as an oromucosal spray delivering 2.7:2.5 mg/100 μL THC:CBD, respectively. Most authoritative, evidence-based reviews report that information regarding the potential benefits of cannabis for MS is limited and that cannabis may be effective for relieving some symptoms (pain, spasticity, lack of bladder control, sleep) of MS.[69,198]

Other Movement Disorders

There are limited data on the use of cannabis in other movement disorders, including Huntington's disease,[70–72] Tourette's,[73] Parkinson's disease,[74–76] and amyotrophic lateral sclerosis.[77] Most evidence for these conditions comes from individual case reports, surveys, or pilot studies and is limited to a reduction of symptoms associated with these conditions, including anxiety, depression, obsessive-compulsive disorder, spasms, spastic rigidity, and mild psychosis. As a point of historical interest, a cannabis tincture (Squire's Extract) was used for the relief of symptoms in patients with Parkinson's disease beginning in the 1800s.

Pain

Cannabis has been used for the treatment of pain since as early as the 12th century. Formal reviews report there is conclusive or substantial evidence that cannabis or cannabinoids are effective for the treatment of chronic pain in adults. Pain is one of the most common conditions resulting in medical marijuana prescriptions, and an oromucosal spray (Sativex) of a whole *Cannabis* plant extract (THC:CBD, approximately 1:1) is approved for use in Europe, the United Kingdom, and Canada, specifically for the treatment of pain and spasticity associated with multiple sclerosis.[26]

Most studies, including a meta-analysis, investigating the use of cannabis in relieving pain, are in patients also suffering from other conditions, such as cancer, HIV/AIDS, or MS, and cannabis seems to be most effective for neuropathic pain.[78] Preclinical research on cannabis and pain has been carried out since 1899, at which time dogs exposed to cannabis inhalation failed to respond to pin-prick stimuli.[79] Relief of pain by cannabis appears to be due to multiple mechanisms that include direct analgesia, anti-inflammatory activity, and modulation of neurotransmitters and endogenous opioids.[80] Several studies, most with a placebo as a comparator and using different preparations, including smoking and extracts, reported significant reduction in neuropathic pain. Some subjects respond positively to low doses, whereas in others, high doses that are accompanied by intoxication were needed. Perhaps the most poignant observation suggesting the widespread efficacy of cannabis in pain management is the use of cannabis resulting in a 64% reduction in opioid use[81] and an almost 25% reduction in opioid fatalities in states with medical marijuana laws.[82]

Cancer

Cannabis is frequently used by cancer patients, both for allaying the symptoms associated with the disease, such as appetite loss, chemotherapy-induced nausea, depression, insomnia, and pain, as well as in an attempt to reduce tumor growth, based on a plethora of anecdotal reports and preclinical research suggesting direct anticancer activity (for reviews, see McAllister et al. [2015],[83] Patil et al. [2015],[84] Ramer and Hinz [2016],[85] Soliman et al. [2016],[86] Velasco et al. [2016][87]).

Cannabis is clearly beneficial for allaying symptoms associated with cancer and conventional cancer therapies.[88] Regarding pain control, the studies that have been conducted suggest that cannabis (smoked and vaporized) can alleviate chronic pain and can be effective when conventional therapies have failed. A single large-scale cohort study (the California Men's Health Study [CMHS]) reported an approximately 45% reduction in the incidence of bladder cancer in male subjects who reported cannabis use versus those who did not. The cohort included more than 82,000 subjects who were followed for 11 years. Conversely, an increase in bladder cancer incidence was reported in those who reported smoking tobacco only.[89] Although causality could not be ascertained, the results are suggestive of a potential protective effect of cannabis on the incidence of bladder cancer. Results of an open-label longitudinal study demonstrated that patients with chronic pain that was resistant to conventional therapies found relief with cannabis oil. As is typical of historical medical dosing, patients started at a low dose of one drop daily, increasing in increments of one drop per dose, three times daily, until satisfactory analgesia was achieved or until side effects appeared. In these oil preparations, the THC concentrations ranged from 11% to 19% and 0.5% to 5.5% CBD.[90] In some studies, plant extracts were reported to be preferred over synthetic cannabinoids.[88]

Numerous preclinical studies demonstrate that a number of cannabinoids (e.g., THC, CBD, CBG, CBC, CBDA) can inhibit cancer cell growth in vitro and also elicit a variety of antineoplastic effects in vivo. However, the in vivo doses used are generally magnitudes higher than is typically consumable to be clinically relevant.

A formal evidence-based review of the cannabis literature concluded there was substantial or conclusive evidence for the use of cannabinoids in chemotherapy-induced nausea.[26] There are mechanistic data suggesting that cannabis has direct anticancer activity, but there are no formal trials supporting a clinically relevant benefit. Conversely, there is widespread belief that cannabis holds great potential as an anticancer compound, leading to the popularization of high-dose THC protocols (e.g., Rick Simpson protocol) in an attempt to decrease tumor growth. No formal investigations of these protocols have been conducted.

Topical cannabis preparations are also popularly used for skin cancers but lack any formal evidence regarding efficacy.

Glaucoma

Glaucoma was one of the earliest conditions for which medical marijuana was approved in the United States in the compassionate drug

use program. There is an abundance of cannabinoid receptors in the tissues of the eyes. There is some evidence that THC reduces the intraocular pressure in the eye that accompanies glaucoma, but the effects seem to be transient (a few hours), and tolerance to the beneficial effects seems to develop over time.[91,92] Benefits were observed with an oral mucosal spray delivering 5 mg sublingual and up to 20 mg THC, and sublingual administration of 40 CBD caused a similarly transient increase in intraocular pressure.[93]

Addiction

Although cannabis has its own dependence-causing potential, both clinical and preclinical trials demonstrate it also has therapeutic application in the treatment of addictions to alcohol, cigarettes, cocaine, opioids, and stimulants, including the use of CBD (200–800 mg) for the treatment of cannabis dependence,[94] although the results of the available studies are mixed.

Anxiety, Depression, Posttraumatic Stress Disorder

There is a wide variety of evidence supporting the anxiolytic activity of cannabis. Although a small subset of the population experiences paranoia or agitation with cannabis use, and inexperienced users are more susceptible to agitation than experienced users, a large segment of the population experiences an anxiolytic effect. The benefit is especially evident for those suffering from other conditions, such as HIV/AIDS, cancer, and MS. Both THC-predominant chemovars and CBD are used. Some of the effects of THC are dose dependent, with low doses eliciting anxiolytic and mood-elevating effects and high doses and chronic use resulting in anxiety and an increased propensity for depression.

There are numerous anecdotal reports of the potential for both CBD and cannabis in general to relieve the symptoms of PTSD and, in some cases, to act as a reset that allows those with PTSD to reexperience joy and happiness, although again, results are mixed. Some investigations reveal that those with PTSD have relatively low concentrations of circulating endocannabinoids.[95] Much of the clinical work that is available was done with synthetic cannabinoids and shows benefit. A few studies investigated both THC and CBD.

In one study, patients with PTSD who received 2.5 to 10 mg THC mg experienced improvements in symptom severity, sleep quality, frequency of nightmares, and PTSD hyperarousal symptoms.[96] Targeting the endocannabinoid system has been described "as a possible ideal therapeutic target to treat both the emotional and cognitive dysfunctions characterizing PTSD."[97] The use of cannabis for PTSD is an approved indication in a number of states with medical marijuana laws, and there is generally a higher frequency of cannabis use in those suffering from PTSD, primarily for improving sleep.[98] Conversely, subjects with excessively high consumption of high-potency cannabis preparations, such as butane hash oil, report higher rates of anxiety and depression and are also prone to greater use of other illicit drugs and higher levels of physical dependence compared with those using crude high-potency herbal cannabis preparations.[99]

There is a biological relationship between cannabinoids and mental health. Anandamide levels in the cerebrospinal fluid and blood of patients are elevated in patients in early-stage psychosis, and there is an abundance of evidence of either a causal or associative relationship between excessive THC-predominant cannabis use and increased risk of psychosis and schizophrenia. There are numerous anecdotal reports of cannabis relieving the symptoms associated with schizophrenia, most notably for mood elevation and relief of anxiety and boredom.[100] However, despite subjective temporary relief of symptoms, ongoing cannabis use contributes to a worsening of schizophrenia, whereas cannabis cessation is associated with improved general health and cognition and a reduction in depression and psychotic symptoms.[101]

The negative effect of cannabis on psychosis and schizophrenia appears to be predominantly due to THC. Perhaps of greater relevance is that evidence suggests that substance abuse and symptoms associated with schizophrenia share similar neurological imbalances, making it difficult to determine whether the relationship is causal or associative.[102,103] Still, the available data suggest that cannabis use in those with schizophrenia or psychotic disorders results in a greater likelihood of relapse, longer hospital admissions, poorer adherence to treatment protocols, and greater need for psychiatric care, and the association is dose dependent, with strong strains producing the worst outcomes.[104] Conversely, CBD appears to lessen the negative effects of THC and even be of therapeutic benefit in relieving symptoms associated with psychosis and schizophrenia.[105–108]

In preclinical investigations, CBD at doses equivalent to 1.25 to 10 mg/kg in humans reduced the psychotic-producing effects of THC.[109,110] Similar effects were reported in human studies where increasing concentrations of CBD relative to THC (2:1 ratio of CBD:THC) reduced the intensity of the psychoactive effects of THC.[103] Another human study demonstrated that CBD blocked a number of negative effects associated with THC alone, including depersonalization, disconnected thoughts, paranoid ideas, and anxiety.

Alzheimer's Disease and Dementia

In recent years there have been reports of the potential use of cannabis for reducing symptoms associated with Alzheimer's and dementia, primarily based on preclinical research and case reports of symptomatic improvement. There is a relatively strong physiological basis for a potential benefit of cannabis in Alzheimer's because the brains of deceased patients with Alzheimer's contain lower levels of anandamide compared with matched control subjects.[111] A number of other physiological mechanisms associated with THC have been proposed as potential targeted therapies in Alzheimer's disease, including the ability of low-dose THC to stimulate mitochondrial activity.[112] In one animal study, CBD (2.5 and 10 mg/kg/day) inhibited inflammatory mediators in an experimental model of Alzheimer's-related neuroinflammation.[113] Conversely, a 2009 formal review of the use of cannabis for dementia found insufficient evidence of clinical efficacy,[114] whereas a more recent study of a medicated cannabis oil preparation reported a reduction of symptoms of delusions, agitation/aggression, irritability, apathy, sleep, and caregiver distress in patients with dementia.[115]

Sleep Disorders

Cannabis has been used to promote sleep for centuries. There is evidence that suggests the endocannabinoid system has a role in sleep. THC is associated with changes in slow-wave sleep, which is critical for learning and memory consolidation, both of which factor into normal sleep cycles. The effects of cannabis are dose dependent, decreasing time to sleep onset at low doses and increasing time to sleep onset at higher doses.[116] Most of the research has been conducted with synthetic cannabinoids. According to the NAS review (2017),[26] there is moderate evidence that cannabis or cannabinoids are effective for improving short-term sleep outcomes in individuals with sleep disturbance associated with obstructive sleep apnea syndrome, fibromyalgia, chronic pain, and multiple sclerosis, primarily synthetic THC.

DOSAGE

The dosing of cannabis preparations is highly variable.[117–119] Complicating the dosage determination is differentiating between the chemovar, terpene type and concentration, THC concentration, biphasic effects (low doses—1 mg THC or less—may stimulate, whereas larger doses can decrease activity, and vice versa), and the ratio of THC

TABLE 61.2 Typical Dosages of Cannabis and CBD Products[a,b]

Smoking	680 mg to 3 g dried herb
Vaporized	Dried herb: 1.5 g daily Concentrate: 5 puffs at 2 mg THC/CBD per puff
Tincture (1:5)	15–40 drops in single or divided doses[117–119] up to the equivalence of 1.5 g cannabis[119]
Fluid extract (1:1)	1 drop to 3–5 drops depending on extract
Tea	Equivalence of 1.5 g cannabis
Edible preparations	Equivalence of 1.5 g cannabis
Oil (1 g cannabis in 6.6 g oil)	13.9–16.5 g
Sativex	2.7 mg THC/2.5 mg CBD; titrate up as directed by a physician.
Epidiolex	Starting dose of 2.5 mg twice daily for 1 week; 5 mg twice daily week 2; titrate up as directed by a physician.

[a]Based on average medical use from a variety of demographics or as directed by physicians.
[b]Many of the effects of cannabis are biphasic and vary greatly in different individuals. Low doses are often stimulatory, whereas high or long-term use can decrease activity. It is best to start at the lowest possible dose and titrate up as tolerance and desired effect allow.
CBD, Cannabidiol; *THC,* tetrahydrocannabinol.

to CBD. Responses to cannabis are highly variable as well and very much dose dependent. When prescribing cannabis therapeutically, the dosage should start low (e.g., 1 mg THC) and move up as needed. Surveys of self-use suggest the average consumer taking cannabis for medicinal purposes uses from 1 to 3 g daily.[41] The U.S. national average for cannabis is 13% THC; this equates to a potential 130 mg to 390 mg of THC, although not 100% of the available THC is consumed. This is an extremely high dose compared with historical dosing regimens. Formal clinical trials of standardized preparations provide more exact doses for modern use.

The onset of effects differs between different preparations. The average time of onset for the various preparations is as follows:

- Smoking/vaporizing 6.5 to 7 minutes
- Tea 29 minutes
- Edibles and tinctures 46 minutes

However, response and detoxification rate vary widely. Another challenge is determining the dosage, which is greatly affected by form, concentration, and species. There is great variation in the literature between research studies and community use. In addition, the actual amount of THC and CBD in a product varies widely in the various types of extracts. Table 61.2 lists dosing regimens based on historical literature and surveys of typical use across studies internationally. These should be taken as relative guidelines. Individual patients and practitioners are recommended to start at the lowest dose and increase according to the desired effect and subject tolerability.

SAFETY

Adverse Effects

Historically, *Cannabis* was considered a safe herb to use for appropriate medical indications and with appropriate caution. Historical writers often reported that if symptoms of intoxication occurred, too much was given and the dose should be reduced. A similar observation has been made by experienced clinicians who often observe benefits at doses below intoxication thresholds (Sulak, personal communication, unreferenced). The safety profile of cannabis has changed over the decades as intensive interbreeding has increased THC levels from a historical concentration of 3% or less to a U.S. national average of 13%. Some preparations available today can be as high as 60% THC, which changes the landscape of cannabis safety with the side-effect potential first and foremost dose dependent and correlated with increasing THC concentrations. Despite increasing THC concentrations, when used with appropriate caution, cannabis is safe both when self-medicating and prescribed. The same adverse-effect profile is not evident with the use of nonintoxicating cultivars or preparations of cannabis, such as those based predominantly on hemp or CBD.

Details of the mechanisms associated with adverse effects are provided in the primary literature. Following is a basic overview of the primary safety considerations regarding cannabis. For more detailed and comprehensive information, refer to the primary literature.

The primary adverse effects that are correlated with THC intoxication include dizziness, sedation, euphoria, cognitive impairment, transient impairment of sensory and perceptual functions, clumsiness, dry mouth, hypotension, or increased heart rate. Most of these adverse effects are tolerable in healthy adults. The seriousness of most cannabis-induced adverse effects is similar to those experienced with other medications and is manageable with appropriate observation and care. More serious adverse effects associated with excessive THC exposure include panic attacks, severe anxiety, cannabis hyperemesis syndrome, psychosis, paranoia, hallucinations, and convulsions.[41]

Traditional Cautions

In the traditional Ayurvedic literature *(Ananadakanda),* nine stages of cannabis side effects were recognized: (1) red eyes and hot breath with dry tongue, lips, palate, and nose; (2) closed eyes and rigid body; (3) burning feeling in the feet, hands, eyes and a choking voice; (4) hunger and thirst, sleepy eyes, and rolling eyes; (5) a choking voice and forgetfulness of recent events; (6) complete amnesia; (7) weakness in upper limbs and body and prostration; (8) disorientation and weeping; and finally, (9) shouting, fainting, coma, eructation, groaning, rolling on the ground, difficulty speaking, incoherence, disclosure of secret feelings, misery, and collapse.[120] In traditional Chinese medicine, excessive consumption was said to cause one to see ghosts and walk frenetically.[10] In early American herbal traditions, the noted Eclectic physician John Uri Lloyd[117] described cannabis as "one of the most important of our remedies . . . but, like our best agents, it must not be used indiscriminately." Thus a number of the major herbal traditions recognized both the benefit and potential for adverse effects inherent in the use of cannabis. Acute symptoms subside with discontinued use. However, psychomotor impairment, specifically regarding attention and critical task performance, remain impaired despite three weeks of abstinence.[121]

Intoxication (the "High")—Typical Use

The most common effects that can be considered either desired or adverse, depending on the user and intent, are associated with intoxication when using THC-predominant strains. Short-term intoxication effects of recreational use include euphoria, relaxation, and loss of inhibition; time- and spatial distortion; and intensification of sensory experiences, such eating or listening to, music followed by a depressant period.[122,123] Cannabis use is associated with impaired function in a variety of cognitive and short-term memory tasks and can result in significant impairment of motor functions, such as slowed or distorted reaction times. Some experience dysphoria, agitation, anxiety, depression, and paranoia.[124] Increased appetite and rapid heart rate are relatively common physiological effects.[125–128]

Intoxication (the "High")—Excessive Chronic High-Dosage Use

There are significant adverse effects associated with high-dose chronic use of cannabis that persist beyond acute intoxication and have

anatomical, physiological, and psychological consequences. In addition to negative respiratory effects, excessive use of cannabis can result in structural changes in the white and gray matter of the brain,[129–131] increased risk of pancreatitis,[132] sperm abnormalities,[133] downregulation of CB1 receptors,[134] and dependence. Some of these changes reverse with discontinuation of cannabis use. However, the developing nervous system of young people is more susceptible to adverse effects. Psychologically, high-dose chronic cannabis use is associated with anxiety, depression, bipolar disorders, cognitive dysfunctions, and in adolescent users, increased risk of schizophrenia in subjects predisposed to schizophrenic disorders. Again, negative effects generally reverse quickly, although cognitive deficits can persist for up to 1 year.[135–139]

Respiratory Effects—Smoking

There are mixed data on the effects of cannabis smoking on the lungs. Some studies report that cannabis smoking has similar negative effects on the lungs as cigarette smoking, with the exception that there seems to be no definitive correlation between cannabis smoking and lung cancer. However, some studies suggest that smoking marijuana leaves is more carcinogenic than smoking tobacco leaves, and some studies report clear lung damage (e.g., Aldington [2007][140]). The comprehensive review of the available studies by Health Canada[41] reports there is no definitive risk, although the review provides a caution that smoking should generally be avoided. The most common respiratory symptoms self-reported for cannabis smoking by cannabis smokers include chronic bronchitis, cough, and phlegm.[141,142] Other respiratory symptoms that have been reported include shortness of breath and wheezing[143,144] and unusual conditions that include spontaneous pneumothorax,[145] bullous lung disease,[144] and barotrauma,[144,146] all of which are rare and for which causation is not definitive.[145] These unusual conditions are thought to result from the deep inhalation and holding of smoke in the lungs associated with cannabis smoking and are confounded by other factors, such as cigarette smoking. The holding of smoke in the lungs is linked to higher concentrations of tar, carbon monoxide, and THC in the lungs.[147] To date, no concerns have been reported for negative respiratory effects associated with nonsmoking exposure, and inhalation using a clean-technology vaporizer can minimize some of the risks of smoking.

Pediatric Intoxication (Intentional/Unintentional)

An unintended consequence with changing laws regarding cannabis has been an increased frequency in exposure to cannabis in children, or at least the reporting of such events. Perhaps the incidence of childhood exposure was the same before legalization, but the criminality of cannabis may have prevented parents from reporting accidental exposures. In children, the most common symptoms reported after acute ingestion include central nervous system (CNS) depression (e.g., lethargy, coma), confusion, agitation, and ataxia. Nausea and vomiting have also been reported, as well as drowsiness/lethargy, ataxia/dizziness, agitation, vomiting, tachycardia, dystonia/muscle rigidity, respiratory depression, bradycardia/hypotension, and seizures.[148] Exposures can be related to both medical use and, more commonly, unintentional consumption of edible preparations, which is of particular concern with children.[149] Unintentional exposures are primarily due to lack of supervision or poor product storage controls of parents. According to a review of poison control center data, the majority of exposures are provided palliative treatments, evaluated, and released without intensive medical care, although some are referred to intensive care for intubation. To date, there are no reports of unintentional intoxications causing permanent disability or death in children, and symptoms generally subside as the effects of cannabis wear off, usually within a few hours.

Effects on Driving

Cannabis significantly impairs motor functions, the impairment of which is proportional to the THC exposure and effects experienced, which are highly variable between consumers. There are mixed data regarding the incidence and fatalities associated with car accidents in states in the years following cannabis legalization. Some data suggest increases in accidents and fatalities, whereas others suggest decreases. In comparison, the risk of an accident is 5 to 30 times greater with impairment due to alcohol.[150]

The available data must also be interpreted cautiously. The incidence of cannabis-related accidents may be underestimated due to the time lapse between an accident, especially those that are fatal, and analysis of blood THC levels.[151] Conversely, the detection of small amounts of THC in the blood does not mean the driver was impaired or that cannabis was the cause of or contributed to an accident.

Results of laboratory experiments demonstrate that chronic use of cannabis negatively affects the long-term psychomotor and cognitive performance of drivers; the effects improve after 3 weeks of abstinence, but users do not recover completely.[152]

Cannabis Hyperemesis Syndrome

Chronic use of cannabis has been associated with severe cyclic vomiting in a small subset of cannabis users, leading to the development of a new clinical condition known as cannabinoid hyperemesis syndrome (CHS). Most subjects experiencing this are chronic heavy users smoking a few times weekly to several times daily. CHS is divided into three stages: (1) morning nausea, fear of vomiting, normal eating patterns, and abdominal discomfort that can last for years, as well as continued cannabis use, sometimes due to the belief that cannabis will reduce their nausea (preemetic or prodromal phase); (2) sudden onset of intense, profuse, persistent, and incapacitating nausea and vomiting up to five times per hour, with some patients reporting abdominal pain and weight loss (hyperemetic phase). When presenting to the emergency room, these subjects are generally dehydrated. (3) Treatment with antiemetics and fluids usually results in the cessation within 48 hours (recovery phase). Patients also often learn that frequent hot bathing provides temporary relief from the acute hyperemesis.[153] The mechanism associated with CHS is not definitively known but is related to a derangement of cannabinoid receptor signaling, and complete resolution of the condition with cannabis cessation has been reported.[154,155] Some patients experience relief by application of topical capsaicin (0.075% preparation) to the abdomen.[156]

Edibles—Special Caution

Oral consumption of cannabis in edible preparations requires particular caution due to a 35-minute to 2-hour latency between consumption and effects and effects that can last for up to 8 hours.[26] Unlike smoking, in which the effects occur within minutes and can be self-titrated, oral consumption can result in overconsumption of THC concentrations that are higher than expected or desired, resulting in negative adverse effects that can be severe, such as acute psychotic effects. Additionally, analytical methodologies for testing the THC concentration in edibles is not well established, and so the THC concentration of edible preparations may not be accurately disclosed, or the amount of THC delivered in edibles may have poor homogeneity control. Particular care must be taken when consuming edibles because fatalities with the consumption of edibles, predominantly due to psychotic episodes, have occurred.[156–158]

Treatment of Intoxication

In most cases, no treatment of cannabis intoxication is needed. Mild intoxication is primarily only troublesome for inexperienced users,

and the most marked effects wear off relatively quickly (typically 1.5 hours), requiring no more than reassurance and supportive therapy. More marked intoxication may benefit from pharmacological support with benzodiazepines. Severe intoxication, such as can occur with edibles, may require restraint in individuals experiencing acute psychotic episodes to prevent them causing harm to oneself or others.

Adverse Effects Based on Clinical Trials

The most detailed reports of adverse events associated with formal medical use can be discerned from controlled clinical trials conducted with standardized preparations and administered under medical supervision. In a review of cannabinoid studies over a period of 40 years, dizziness was reported as the primary adverse effect experienced by patients. In these studies, almost 97% of adverse effects experienced were considered nonserious.[162] Other common side effects experienced with cannabis use include lightheadedness, sleepiness, gastrointestinal upset, and dry mouth in short-term studies. In longer-term studies, many of these side effects subside, which is typical as patients become accustomed to (tolerant of) the effects of cannabis. A certain degree of tolerance for some of the symptoms associated with cannabis intoxication can occur in 4 to 10 days.[163] However, gastrointestinal (GI) upset and negative cognitive effects can persist, and subjects can experience more pain compared with those taking placebo.[64,159,160] Adverse events typically occur within 20 minutes of dosing and resolve completely within 3.5 hours.

According to one report, seizures were exacerbated in 18% of subjects using a cannabis preparation.[62] Inability to sleep, drowsiness, fatigue, loss of appetite, and GI upset were other common adverse effects, with an overall mean adverse-effects incidence of approximately 11% of subjects.[62] Assessing adverse events in children is particularly challenging, as is the use of illicit preparations in states where medical cannabis is not allowed.

Cannabis Dependence

There are a number of adverse effects that can occur with long-term excessive cannabis use. As with excessive use of other drugs, there is a potential for abuse, dependence, and withdrawal in some heavy users, which is most commonly designated as cannabis use disorder (CUD). Dependence has both physical and psychological aspects and is very much individualized between those with a propensity for dependence and those without such a propensity. There is evidence for both physical and psychological dependence to occur with excessive cannabis use, and dependence can be mild to severe. Mild withdrawal symptoms can include headache, lack of appetite, and restlessness; stronger withdrawal symptoms can include anxiety, insomnia, and depression, whereas more severe symptoms can include sweating, fever, chills, and hallucinations.[161] Mild symptoms usually subside in only a few days.[162]

Although there are no formally approved pharmaceutical therapies for the treatment of withdrawal, interestingly, there is evidence that CBD is potentially beneficial for cannabis dependence.[94]

Amotivational Syndrome

Long-term excessive cannabis use has been reported to contribute to amotivational syndrome, characterized by apathy and an inability to function effectively and, in some, long-term depression. Conversely, it can be debated that many excessive cannabis users are predisposed to exhibit amotivational tendencies rather than cannabis being causative, although abstinence results in a resolution of some symptoms.[163]

Toxicity of Cannabidiol

Most adverse effects associated with cannabis are both THC and dose dependent, with side effects worsening with excessive use of THC-rich preparations. In formal trials with the CBD preparation (Epidiolex), adverse events were reported in 75% to 93% of patients. The majority of these (89%) were reported as mild or moderate in severity. Adverse events reported with CBD (20 mg/kg body weight) in one study included diarrhea, vomiting, fatigue, increased body temperature, sleepiness, and abnormal results on liver-function tests, although some of these may have been associated with concomitant antiseizure medications.[60] A thorough review of CBD safety was published by Iffland and Grotenhermen (2017),[164] who report that CDB has a generally favorable side-effect profile and possesses less side effects than standard antiseizure medications and, because of this, helps in improving compliance with standard treatments.

Use in Pregnancy and Childhood Development

There is a long history of the use of cannabis in pregnancy, primarily for allaying morning sickness and facilitating childbirth by promoting labor, although in labor reportedly accompanied with much pain.[165] Cannabis is often used to help allay the nausea and vomiting due to morning sickness, with an estimate that 5% of pregnant women in the United Kingdom[166] and one in 20 pregnant women in the United States use cannabis.[167] In small doses for the acute relief of labor pain and nausea, cannabis use is generally believed to be safe for the mother. A formal investigation of the effects of low-dose cannabis in the neonate from conception to beyond delivery has not been undertaken.

Data suggest that pregnant women who use cannabis during pregnancy are more likely to be daily users and are more likely to meet the criteria for cannabis use disorders than nonpregnant women.[168,169] Those using cannabis during pregnancy also have numerous other behaviors that make the interpretation of effects difficult, such as other drug use or high-risk sociodemographic status. However, when such factors are taken into account, risks remain. Expectant mothers using cannabis are also reported to gain more weight than nonusers.[170]

THC can cross the placental barrier, raising the concern that excessive cannabis exposure can have a negative effect on the developing fetus.[171,172] In some studies, excessive cannabis exposure in pregnancy was associated with lower-than-average birth weight, height, and head circumference[170,173–175]; the differences were small but statistically significant.[176] Other studies confirmed the lower-than-average birth weight but failed to show changes in head circumference and height.[177] Other evidence suggests that using cannabis two or more times per week can increase the risk of premature birth.[174,178]

There are a number of studies establishing negative neonatal effects associated with excessive cannabis use, although the data are also mixed and of poor quality, making interpretation difficult. There is evidence to suggest that in utero cannabis exposure may negatively affect long-term growth and neurodevelopment, particularly in terms of cognition and behavior. Some of the primary adverse effects reported include lower memory scores, attention problems, hyperactivity and impulsivity in early childhood, problems with executive functioning later in life, and a greater likelihood of developing emotional and behavioral problems, such as depression and delinquent behavior.[168]

In the most comprehensive review to date, data on the developmental effects of cannabis from pregnancy ("first hit to the endocannabinoid system") to 22 years of age was summarized.[179] In one study, the gestational age of the neonate was decreased, whereas in another, cannabis exposure was associated with changes in birth length and weight in the first and third trimesters, respectively, but the findings between the studies were contradictory. In newborns, there was a decreased response to light and an increase in startle response and tremors, as well as a decrease in body length. The most effects were seen in the developing infant to 6 years old, with increases in motor skills, impulsivity, hyperactivity, nocturnal arousals, wake time after sleep onset,

delinquency, and aggression in girls and decreases in memory, verbal reasoning, mental development, short-term memory, sleep efficiency, concentration, IQ scores, and attention. The nature of these studies does not allow for definitive conclusions to be drawn. However, considering that the endocannabinoid system is integral to neurological development, it is not surprising that neurodevelopment changes can occur with exposure to cannabinoids.

Risk of Psychosis and Schizophrenia

In addition to the developmental effects reported previously, there appears to be a strong association between heavy adolescent cannabis use and an increased risk of schizophrenia. Some studies suggest there is a sixfold risk with heavy cannabis exposure, and there appears to be a greater risk for those genetically predisposed to schizophrenia.[180] Some consider cannabis an independent risk factor for psychosis as well, although there is evidence that also suggests that cannabis users who experience an initial psychotic episode have fewer neurological abnormalities than nonusers.[181] Animal data demonstrate that chronic exposure of cannabinoid agonists during the periadolescent period causes persistent behavioral alterations in adult animals. Although it is clear that cannabis can be used to provide symptomatic relief of symptoms associated with schizophrenia and psychosis, other researchers have continued to build a body of evidence suggesting caution in its use.[182]

One of the more interesting findings in the literature on cannabis and reproductive issues is an association with heavy prenatal, gestational, and postnatal cannabis use in males and an increased risk of sudden infant sudden syndrome (SIDS).[183] The reason for this has not been definitively ascertained. Cannabis use in pregnancy does not increase teratogenicity, stillbirths, or fetal distress, nor does it appear to elicit negative postnatal effects up to 1 year of age.[169,176,184,185]

One important principle in reviewing the childhood development data is to look at investigations at different stages of a child's life because certain cognitive deficits may not be evident, for example, until a child reaches school age. Over time, compensatory mechanisms can result in normalization after a cannabis-induced developmental deficit, followed by other developmental issues that become evident, such as an increased incidence of psychosis in susceptible populations, later in life. These cautions notwithstanding, the evidence for harm is more suggestive than definitive and must be interpreted carefully.

Because the endocannabinoid system plays a critical role in neurological development, there is concern that exposure to cannabinoids at critical times of gestational development can cause alterations that can have long-term negative consequences. This seems to be supported by animal data showing that exposure to low doses of THC during prenatal development can negatively affect cortical development in mice.[186]

Child Protection

There is a need to protect young children from accessing cannabis unintentionally. Reports of pediatric ingestion show that drowsiness, lethargy, "coma" that wears off as the effects of cannabis wear off, the need for respiratory intubation, agitation, an inability to walk, vomiting, respiratory depression, and aspiration pneumonia are among the most common adverse effects observed.[187] The dangers of accidental ingestion should also not be exaggerated because most children unintentionally exposed to cannabis recover from symptoms as the effects of cannabis wear off. Unlike opioids, which cause a depression in CNS function that can lead to respiratory failure, cannabis does not have the same effect. Although accidental ingestions do occur and reports to poison control centers increase in incidence in states in the first few years after states enact medical marijuana laws, the overwhelming majority of events resolve without the need for treatment, and relatively few serious events occur.[188–190] Even these reports of incidence must be considered carefully because increased reporting of unintentional ingestions does not necessarily mean there are more accidental ingestions in states where cannabis is legal but can also mean that there is a greater willingness to seek treatment, whereas before legalization, such reports carried a greater threat of childhood endangerment or neglect suspicions that may have caused parents to not seek medical care.

TOXICITY

Despite the widespread use of cannabis, including by youth, in the immunocompromised, and in pregnancy, there are no reports of death due to cannabis overdose.[41] This is predominantly due to the fact that, unlike opioids, cannabis use does not result in marked depression of respiratory and cardiovascular functions. When overdose does occur, subjects, even experienced users, can feel as if they have died or are at serious risk of dying, which can be very traumatic, and with edibles, this event can last for several hours, but full recovery can be expected. Conversely, there are individual reports of overdose resulting in psychotic behavior resulting in death. In at least two cases, edible preparations were implicated. In one, a young inexperienced user consumed a cannabis edible, did not feel as if any effect was occurring, due to the lag time between consumption and effects, and consumed many times the amount that he should have. This resulted in erratic behavior and his throwing himself over a balcony, falling to his death. His blood THC level was reported at 7.3 ng/mL, a little over the legal driving limit of 5 ng/mL. In another case, only a small amount of an edible preparation was eaten along with pain medications, causing a man to act erratically, asking his wife to get his gun and "kill him." While the wife called 911, the man got his gun and shot her. In the first case, causality appears clear. In the second case, a single case report such as this does not provide the entire picture of what could have contributed to this behavior. However, while on the call, the wife expressed that she had never seen her husband act in that manner and said he was acting more drunk than violent. There was a history of schizophrenia in the man's family, causing his defense attorneys to suggest he was particularly prone to cannabis-induced psychosis, a claim that was neither proven nor disproven.

There is a single case report of an 11-month-old who died of myocarditis subsequent to cannabis exposure, likely due to an edible preparation. Although causation cannot be assured, the physicians attempted to rule out other causes. Blood THC levels were 7.8 ng/mL,[220] which is very high for an 11-month-old, considering the 5-ng/mL limit established for adult intoxication. Cannabis also has marked cardiovascular-related effects, with tachycardia being one of the most physiological relevant effects of cannabis intoxication.[123] This still does not establish causality, but it is not unreasonable to consider cannabis as a contributing factor to such events.

As these reports suggest, the greatest risk of toxicity is with edible preparations because of the delay in onset of effects, and the risk is THC dependent. In contrast, the relatively immediate effects of respiratory administration allow consumers to self-regulate, and with the physical incapacitation that occurs in most users, it is very difficult to reach concentrations that would represent toxic overdose events.

Despite its relative safety, cannabis does result in reports to poison control centers and visits to emergency rooms, primarily due to dread of death, paranoia, anxiety, or convulsions. Although there are a number of pharmaceutical and mechanical interventions, the best policy is to provide support and reassurance to those suffering from excess cannabis use until the most acute effects wear off.

Based on rodent toxicology studies, the equivalent lethal dose of THC in humans has been extrapolated to be more than 15,000 mg or the equivalent of ingesting 7500 g of cannabis that contains 20% THC.[41]

DRUG INTERACTIONS

The most significant clinically relevant interactions can occur when cannabis is taken with CNS depressant drugs such as sedative-hypnotics or alcohol. THC is metabolized by a number of cytochrome enzymes (CYP2C9, 2C19, and 3A4) that are involved in the metabolization of the majority of conventional medications. Any substance that inhibits CYP enzymes can increase the bioavailability and action of THC and THC-related adverse effects. Concomitant use of cannabis with tricyclic antidepressants, amphetamines, and anticholinergic agents (e.g., antihistamines) has resulted in increased heart rate (tachycardia), high blood pressure, drowsiness, and cardiotoxicity. Substances that increase CYP activity can, conversely, potentially decrease the efficacy of cannabis. Of most clinical significance considering the recent approval of Epidiolex is the potential for interactions with antiseizure medications such as clobazam. CBD has been shown to inhibit the metabolization of this medication, resulting in a positive interaction associated with increases in plasma levels of clobazam metabolites and the ability to maintain seizure control with lower-than-normal clobazam doses. Conversely, CBD has been reported to decrease the anticonvulsive activity of other antiseizure medications.

Perhaps the most significant potential benefit of cannabis to date in relationship to other drug use is the reduction in opioid-related fatalities. In those states that enacted medical marijuana laws, a 24.8% reduction in opioid-related fatalities was observed.[82] Clinically, the use of cannabis resulted in a 64% reduction in opioid use.[81] In contrast, the intoxicating effects of cannabis can sometimes be enhanced with concomitant use of opioids.[41]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Clark R, Merlin M. *Cannabis Evolution and Ethnobotany*. Berkeley: University of California Press; 2016:456.
2. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br J Pharmacol*. 2011;163(7):1344–1364.
3. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev*. 2006;58(3):389–462.
4. Mikuriya TH, Aldrich MR. Cannabis Old drug, new dangers. The potency question. *J Psychoactive Drugs*. 1988;20(1):47–55.
5. Turner CE. Cannabis: the plant, its drugs, and their effects. *Aviat Space Environ Med*. 1983:363–368.
6. Brenneisen R. Chemistry and analysis of phytocannabinoids and other cannabis constituents. In: ElSohly M, ed. *(Marijuana and the Cannabinoids)*. Totowa, NJ: Human Press; 2007:322.
7. ElSohly MA, Rosa SA, Mehmedic Z, Arafat RYB, Banahan BF. Potency trends of delta9-THC and other cannabinoids in confiscated marijuana from 1980-1997. *J Foren Sci*. 2000;45:24–30.
8. Mehmedic Z, Chandra S, Slade D, et al. Potency trends of delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J Foren Sci*. 2010;55(5):1209–1217.
9. Mechoulam R, Ben-Shabat S. From gan-zi-gun-nu to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis. *Nature Prod Rep*. 1999;16:131–143.
10. Wilms S. *Shen Nong Bencao Jing*. Corbett, OR: Happy Goat Productions; 2016:515.
11. Li SZ. *Compendium of Materia Medica [in Chinese]Ben Cao Gang Mu. Book II.. Luo Xiwen, Trans*. Beijing: Foreign Language Pr; 1604.
12. Meulenbeld GJ. The search for clues to the chronology of Sanskrit medical texts as illustrated by the history of bhanga. *Studien zur Indologie und Iranistik*. 1989;15:59–70.
13. Wujastyk D. Cannabis in traditional Indian herbal medicine. In: Salema A, ed. Ayurveda at the crossroads of care and cure. *Proceedings of the Indo-European Seminar on Ayurveda; Ayurveda at the Crossroads of Care and Cure. Arrábida, Portugal, in November 2001*. Lisbon: Universidade Nova de Lisboa; 2002:45–73.
14. Grierson GA. The hemp plant in Sanskrit and Hindi literature. *Indian Antiquary*. 1894;23(10):260–262.
15. Campbell JM. Note on the religion of Hemp. In: *Report of the Indian Hemp Drugs Commission*. Bombay volumes: 1893–1894: 1–8.
16. Ayurvedic Pharmacopoeia India. *Ayurvedic Pharmacopoeia of India*. Vol. 1. New Delhi: Controller Pub Civil Lines; 2001.
17. O'Shaughnessy WB. On the preparations of the Indian hemp, or gunjah *(Cannabis indica)*. Their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *Prov Med J Retrosp Med Sci*. 1843;5(122):343–347.
18. Newton RS. *Eclectic Med J*. 1858;5(2):576.
19. Felter HW, Lloyd JU. 18th ed. *King's American Dispensatory*. Vols. 1–2. Portland: Eclectic Med Pub; 1898:2172. Reprinted:1983.
20. Nallathambi R, Mazuz M, Ion A, et al. Anti-inflammatory activity in colon models is derived from Δ9-tetrahydrocannabinolic acid that interacts with additional compounds in cannabis extracts. *Cannabis Cannabinoid Res*. 2017;2(1):167–182.
21. Giese MW, Lewis MA, Giese L, Smith KM. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in *Cannabis*. *J AOAC Int*. 2015;98(6):1503–1522.
22. Ross SA, ElSohly MA. Constituents of *Cannabis sativa* L. XXVIII. A review of the natural constituents: 1980–1994. *Zagazig J Pharmaceut Sci*. 1995;4(2):1–10.
23. Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL. Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *J Immunol*. 2004;173(8):5298–5304.
24. Tian J, Yong J, Dang H, Kaufman DL. Oral GABA treatment downregulates inflammatory responses in a mouse model of rheumatoid arthritis. *Autoimmunity*. 2011;44(6):465–470.
25. Institute of Medicine. *Glutamate-Related Biomarkers in Drug Development for Disorders of the Nervous System: A Workshop Summary*. Washington, DC: National Academics Press; 2011:74.
26. National Academies of Sciences, Engineering, and Medicine. *The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research*. Washington, DC: National Academies Press; 2017.
27. Mallet PE, Beninger RJ. The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. *Behav Pharmacol*. 1996;7:276–284.
28. Mahler SV, Smith KS, Berridge KC. Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacol*. 2007;32(11):2267–2278.
29. De Petrocellis L, Melck D, Palmisano A, et al. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proc Natl Acad Sci*. 1998;95:8375–8380.
30. Fuss J, Steinle J, Bindila L, et al. A runner's high depends on cannabinoid receptors in mice. *PNAS*. 2015;112(42):13105–13108.
31. Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. *Biol Psychiat*. 2011;70(5):479–486.
32. Evans RW, Ramadan NM. Are cannabis-based chemicals helpful in headache? *Headache*. 2004;44(7):726–727.
33. Bisogno T, Hanus L, De Petrocellis L, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol*. 2001;134(4):845–852.
34. Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res*. 2005;30(8):1037–1043.
35. Carrier EJ, Auchampach JA, Hillard CJ. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proceed Nat Acad Sci*. 2006;103(20):7895–7900.
36. Izzo AA, Piscitelli F, Capasso R, et al. Peripheral endocannabinoid dysregulation in obesity: relation to intestinal motility and energy processing induced by food deprivation and re-feeding. *Br J Pharmacol*. 2009;158:451–461.
37. Belendiuk KA, Baldini LL, Bonn-Miller MO. Narrative review of the safety and efficacy of marijuana for the treatment of commonly state-approved medical and psychiatric disorders. *Addict Sci Clin Pract*. 2015;10:10.
38. State medical marijuana laws. National Conference State Legislatures. http://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx. Accessed August 1, 2016.
39. Medical Marijuana Registry Statistics. Colorado Department of Public Health and Environment. https://www.colorado.gov/pacific/sites/default/files/CHED_MMR_Monthly_Report_Statistics_July_2016.pdf. Published July 31, 2016. Accessed October 12, 2016.
40. Oregon Medical Marijuana Program Statistical Snapshot July, 2016. Oregon Health Authority. https://www.oregon.gov/oha/PH/DISEASESCONDITIONS/CHRONICDISEASE/MEDICALMARIJUANAPROGRAM/Pages/index.aspx. Accessed October 12, 2016.
41. Health Canada. Cannabis (marihuana, marijuana). In: *Information for Health Care Professionals*. Ottawa: Government of Canada; 2018:259.
42. Ligresti A, De Petrocellis L, Di Marzo V. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiol Rev*. 2016;96:1593–1659.
43. Lu QJ, Straiker A, Lu QX, Maguire G. Expression of CB2 cannabinoid receptor mRNA in adult rat retina. *Vis Neuroscience*. 2000;17:91–95.
44. Abrams DI, Hilton JF, Leiser RJ, et al. Short-term effects of cannabinoids in patients with HIV-1 infection. *Ann Int Med*. 2003;139:258–266.
45. Beal JE, Olson R, Laubenstein L, et al. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *J Pain Sym Manag*. 1995;10:89–97.
46. Haney M, Rabkin J, Gunderson EW, Foltin RW. Dronabinol and marijuana in HIV+ marijuana smokers: acute effects on caloric intake and mood. *Psychopharmacol*. 2005;181:170–178.

47. Haney M, Gunderson EW, Rabkin J, et al. Dronabinol and marijuana in HIV-positive marijuana smokers. Caloric intake, mood and sleep. *JAIDS*. 2007;45:545–554.
48. Regelson W, Butler J, Schulz J, et al. Δ9-tetrahydrocannabinol as an effective antidepressant and appetite-stimulating agent in advanced cancer patients. In: Braude M, Szara S, eds. *The Pharmacology of Marijuana*. New York: Raven Press; 1976:865.
49. Abel EL. Cannabis: effects on hunger and thirst. *Behav Biol*. 1975;15: 255–281.
50. Foltin RW, Fischman MW, Byrne MF. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite*. 1988;11:1–14.
51. Jyotaki M, Shigemura N, Ninomiya Y. Modulation of sweet taste sensitivity by orexigenic and anorexigenic factors. *Endocrine J*. 2010;57: 467–475.
52. Struwe M, Kaempfer SH, Geiger CJ, et al. Effect of dronabinol on nutritional status in HIV infection. *Ann Pharmacother*. 1993;27:827–831.
53. Jatoi A, Windschitl HE, Loprinzi CL, et al. Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. *J Clin Oncol*. 2002;20:567–573.
54. Nelson K, Walsh D, Deeter P, Sheehan F. A phase II study of Δ9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *J Palliat Care*. 1994;10:14–48.
55. Strasser F, Luftner D, Possinger K, et al. Comparison of orally administered cannabis extract and Δ9-tetrahydrocannabinol in treating patients with cancer-related anorexia-cachexia syndrome: a multicenter, phase III, randomized, double-blind, placebo-controlled clinical trial from the Cannabis-In-Cachexia-Study-Group. *J Clin Oncol*. 2006;24: 3394–3400.
56. Timpone JG, Wright DJ, Li N, et al. The safety and pharmacokinetics of single-agent and combination therapy with megestrol acetate and dronabinol for the treatment of HIV wasting syndrome. *AIDS Res Hum Retroviruses*. 1997;13:305–315.
57. Bachhuber M, Saloner B, Cunningham CO, Barry CL. Medical cannabis laws and opioid analgesic overdose mortality in the United States, 1999-2010. *JAMA Intern Med*. 2014;174:1668–1673.
58. Chang AE, Shiling DJ, Stillman RC, et al. Δ9-tetrahydrocannabinol as an antiemetic in cancer patients receiving high-dose methotrexate. A prospective, randomized evaluation. *Ann Intern Med*. 1979;91:819–824.
59. Lauritsen KG, Rosenberg H. Comparison of outcome expectancies for synthetic cannabinoids and botanical marijuana. *Am J Drug Alcohol Abuse*. 2016;42(4):377–384.
60. Devinsky O, Marsh E, Friedman D, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol*. 2016;15:270–278.
61. Devinsky O, Cross H, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *NEJM*. 2017;376(21):2011–2020.
62. Russo EB. Cannabis and epilepsy: an ancient treatment returns to the fore. *Epilepsy Behavior*. 2017;70:292–297.
63. Tzadok M, Uliel-Siboni S, Linder I, et al. CBD-enriched medical cannabis for intractable pediatric epilepsy: the current Israeli experience. *Seizure*. 2016;35:41–44.
64. Zajicek J, Fox P, Sanders H, et al. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial. *Lancet*. 2003;362(9395):1517–1526.
65. Sulak D, Saneto R, Goldstein B. The current status of artisanal cannabis for the treatment of epilepsy in the United States. *Epilepsy Behav*. 2017;70(Pt B):328–333.
66. Stockings E, Zagic D, Campbell G, et al. Evidence for cannabis and cannabinoids for epilepsy: a systematic review of controlled and observational evidence. *J Neurol Neurosurg Psychiatry*. 2018;89(7):741–753.
67. Whalley BJ. *Cannabis in the Management and Treatment of Seizures and Epilepsy: A Scientific Review*. Scotts Valley, CA: American Herbal Pharmacopoeia; 2014:1–31.
68. Feinstein A, Banwell E, Pavisian B. What to make of cannabis and cognition in MS: in search of clarity amidst the haze. *Multiple Sclerosis J*. 2015;21(14):1755–1760.
69. Lyman WD, Sonett JR, Brosnan CF, Elkin R, Bornstein MB. Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 1989;(1):73–81.
70. Publication of interim decisions proposing to amend, or not amend, the current Poisons Standard, September 2018. Therapeutic Goods Administration. https://www.tga.gov.au/book-page/15-cannabidiol-and-tetrahydrocannabinols-thcs. Accessed October 10, 2019.
71. Curtis MA, Faulla RLM, Glass M. A novel population of progenitor cells expressing cannabinoid receptors in the subependymal layer of the adult normal and Huntington's disease human brain. *J Chem Neuroanatomy*. 2006;31:210–215.
72. Meisel K, Friedman JH. Medical marijuana in Huntington's disease: report of two cases. *Med Health*. 2012;95:178.
73. Sandyk R, Consroe P, Stern LZ, Snider SR. Effects of cannabidiol in Huntington's disease. *Neurology*. 1986;36:342.
74. Müller-Vahl KR. Treatment of Tourette syndrome with cannabinoids. *Behav Neurol*. 2013;27(2013):119–124.
75. Venderova K, Ruzicka E, Vorisek V, Visnovsky P. Survey on Cannabis use in Parkinson's disease: subjective improvement of motor symptoms. *Mov Disord*. 2004;19:1102–1106.
76. Zahodne LB, Fernandez HH. Pathophysiology and treatment of psychosis in Parkinson's disease: a review. *Drugs Aging*. 2008;25:665–682.
77. Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS. Cannabidiol, a *Cannabis sativa* constituent, as an antipsychotic drug. *Braz J Med Biol Res*. 2006;39:421–429.
78. Amtmann D, Weydt P, Johnson KL, Jensen MP, Carter GT. Survey of Cannabis use in patients with amyotrophic lateral sclerosis. *Am J Hosp Palliat Care*. 2004;21:95–104.
79. Iskedjian M, Bereza B, Gordon A, Piwko C, Einarson TR. Meta-analysis of Cannabis based treatments for neuropathic and multiple sclerosis-related pain. *Curr Med Res Opin*. 2007;23:17–24.
80. Hosking RD, Zajicek JP. Therapeutic potential of cannabis in pain medicine. *Br J Anesth*. 2008;101(1):59–68.
81. Russo EB, Hohmann AG. The role of cannabinoids in pain management. In: Deer T, Gordin V, eds. *Comprehensive Treatment of Chronic Pain by Medical, Interventional and Behavioral Approaches*. New York: Springer; 2013:181–197.
82. Boehnke KF, Litinas E, Clauw DJ. Medical cannabis associated with decreased opiate medication use in retrospective cross-sectional survey of chronic pain. *J Pain*. 2016;17:739–777.
83. Whiting PF, Wolff RF, Deshpande S, et al. Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA*. 2015;313(24):2456–2473.
84. McAllister SD, Soroceanu L, Desprez PY. The antitumor activity of plant-derived non-psychoactive cannabinoids. *J Neuroimmune Pharmacol*. 2015;10:255–267.
85. Patil KR, Goyal SN, Sharma C, Patil CR, Ojha S. Phytocannabinoids for cancer therapeutics: recent updates and future prospects. *Curr Med Chem*. 2015;22:3472–3501.
86. Ramer R, Hinz B. Antitumorigenic targets of cannabinoids—current status and implications. *Expert Opin Ther Targets*. 2016;20:1219–1235.
87. Soliman E, Ladin DA, Van Dross R. Cannabinoids as therapeutics for non-melanoma and melanoma skin cancer. *J Dermatol Clin Res*. 2016;4:1069–1076.
88. Velasco G, Sanchez C, Guzman M. Anticancer mechanisms of cannabinoids. *Curr Oncol*. 2016;23(suppl 2):S23–S32.
89. Bar-Sela G, Vorobeichik M, Drawsheh S, Omer A, Goldberg V, Muller E. The medical necessity for medicinal cannabis: prospective, observational study evaluating the treatment in cancer patients on supportive or palliative care. *Evid Based Complement Alternat Med*. 2013;2013:510392.
90. Thomas AA, Wallner LP, Quinn VP, et al. Association between cannabis use and the risk of bladder cancer: results from the California Men's Study. *Urology*. 2015;85:388–392.
91. Haroutounian S, Ratz Y, Ginosar Y, et al. The effect of medicinal cannabis on pain and quality of life outcomes in chronic pain: a prospective open-label study. *Clin J Pain*. 2016;32(12):1036–1043.

92. Chen J, Matias I, Dinh T, et al. Finding of endocannabinoids in human eye tissues: implications for glaucoma. *Biochem Biophys Res Comm*. 2005;330:1062–1067.
93. Tomida I, Pertwee RG, Azuara-Blanco A. Cannabinoids and glaucoma. *Br J Ophthalmol*. 2004;88:708–713.
94. Flach AJ. Δ9-tetrahydrocannabinol (THC) in the treatment of end stage open-angle glaucoma. *Trans Am Ophthalmol Soc*. 2002;100:215–224.
95. Prud'homme M, Cata R, Jutras-Aswad D. Cannabidiol as an intervention for addictive behaviors: a systematic review of the evidence. *Subst Abuse*. 2015;9:33–38.
96. Neumeister A, Seidel J, Ragen BJ, Pietrzak RH. Translational evidence for a role of endocannabinoids in the etiology and treatment of posttraumatic stress disorder. *Psychoneuroendocrinol*. 2015;51:577–584.
97. Roitman P, Mechoulam R, Cooper-Kazaz R, Shalev A. Preliminary, open-label, pilot study of add-on oral Delta9-tetrahydrocannabinol in chronic post-traumatic stress disorder. *Clin Drug Investig*. 2014;34(8):587–591.
98. Trezza V, Campolongo P. The endocannabinoid system as a possible target to treat both the cognitive and emotional features of post-traumatic stress disorder (PTSD). *Front Behav Neurosci*. 2013;7:100.
99. Bonn-Miller MO, Babson KA, Vandrey R. Using cannabis to help you sleep: Heightened frequency of medical cannabis use among those with PTSD. *Drug Alcohol Depend*. 2014;136:162–165.
100. Chan GCK, Hall W, Freeman TP, Ferris J, Kelly AB, Winstock A. User characteristics and effect profile of butane hash oil: an extremely high-potency cannabis concentrate. *Drug Alcohol Depend*. 2017;178:32–38.
101. Perez LG, Santacana AM, Baquero DB, Perez-Sola V. Reasons and subjective effects of cannabis use among people with psychotic disorders: a systematic review. *Actas Esp Psiquiatr*. 2014;42(2):83.
102. Rabin RA, Goodman MS, George TP, Barr MS. Neurobiology of comorbid substance use disorders in mental illness: a closer look at the underlying commonalities between cannabis and schizophrenia. *Curr Add Rep*. 2014;1:261.
103. van Winkel R, Kuepper R. Epidemiological, neurobiological, and genetic clues to the mechanisms linking cannabis use to risk for nonaffective psychosis. *Annu Rev Clin Psychol*. 2014;10:767–791.
104. Chambers RA, Krystal JH, Self DW. A neurobiological basis for substance abuse comorbidity in schizophrenia. *Biol Psychiatry*. 2001;50(2):71–83.
105. Schoeler T, Monk A, Sami MB, et al. Continued versus discontinued cannabis use in patients with psychosis: a systematic review and meta-analysis. *Lancet Psychiatry*. 2016;3(3):215–225.
106. Zuardi AW, Hallak JE, Dursun SM, et al. Cannabidiol monotherapy for treatment-resistant schizophrenia. *J Psychopharmacol*. 2006;20(5):683–686.
107. Karniol IG, Shirakawa I, Kasinski N, Pfeferman A, Carlini EA. Cannabidiol interferes with the effects of delta 9-tetrahydrocannabinol in man. *Eur J Pharmacol*. 1974;28(1):172–177.
108. Musty R. Cannabinoids and anxiety. In: Mechoulam R, ed. *Cannabinoids as Therapeutics*. Birkhau¨ser Basel; 2005:67–78.
109. Zuardi AW, Morais SL, Guimaraes FS, Mechoulam R. Antipsychotic effect of cannabidiol. *J Clin Psychiatry*. 1995;56(10):485–486.
110. Zuardi AW, Rodrigues JA, Cunha JM. Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology (Berl)*. 1991;104(2):260–264.
111. Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology (Berl)*. 1982;76(3):245–250.
112. Jung KM, Astarita G, Yasar S, et al. An amyloid β42-dependent deficit in anandamide mobilization is associated with cognitive dysfunction in Alzheimer's disease. *Neurobiol Aging*. 2012;33(8):1522–1532.
113. Cao C, Li Y, Liu H, et al. The potential therapeutic effects of THC on Alzheimer's disease. *J Alzheimer's Disord*. 2014;42:973–984.
114. Esposito G, Scuderi C, Savani C, et al. Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol*. 2007;151(8):1272–1279.
115. Krishnan S, Cairns R, Howard R. Cannabinoids for the treatment of dementia. *Cochrane Database Syst Rev*. 2009;(2):CD007204.
116. Shelef A, Barak Y, Berger U, et al. Safety and efficacy of medical cannabis oil for behavioral and psychological symptoms of dementia: an-open label, add-on, pilot study. *J Alzheimers Dis*. 2016;51(1):15–19. https://doi.org/10.3233/JAD-150915.
117. Garcia AN, Salloum IM. Polysomnographic sleep disturbances in nicotine, caffeine, alcohol, cocaine, opioid, and cannabis use: a focused review. *Am J Addict*. 2015;24(7):590–598.
118. Christison A. On the natural history, action, and uses of Indian hemp. *J Med Sci Edinburgh Scotland*. 1851;13:26–45. 117–121.
119. British Pharmaceutical Codex. Council of the Pharmaceutical Society of Great Britain. 1911.
120. Hazekamp A, Ware M, Muller-Vahl KR, Abrams D, Grotenhermen F. The medicinal use of cannabis and cannabinoids—an international cross-sectional survey on administration forms. *J Psychoactive Drugs*. 2013;45(3):199–210.
121. Bosker WM, Karschner EL, Lee D, et al. Psychomotor function in chronic daily cannabis smokers during sustained abstinence. *PLoS One*. 2013;8(1):e53127.
122. Dash VB. *Fundamentals of Ayurvedic Medicine*. Delhi: Sri Satguru Publications; 1999:346.
123. Ameri A. The effects of cannabinoids on the brain. *Prog Neurobiol*. 1999;58(4):315–348.
124. Hall W, Solowij N. Adverse effects of cannabis. *Lancet*. 1998;352(9140):1611–1616.
125. Institute of Medicine. First, do no harm: consequences of marijuana use and abuse. In: Joy JE, Watson SJ, Benson JA, eds. *Marijuana and Medicine: Assessing the Science Base*. Washington, DC: National Academy Press; 1999.
126. Aryana A, Williams MA. Marijuana as a trigger of cardiovascular events: Speculation or scientific certainty? *Int J Cardiol*. 2007;118(2):141–144.
127. Beaconsfield P, Ginsburg J, Rainsbury R. Marihuana smoking. Cardiovascular effects in man and possible mechanisms. *N Engl J Med*. 1972;287(5):209–212.
128. Perez-Reyes M. Marijuana smoking: factors that influence the bioavailability of tetrahydrocannabinol. *NIDA Res Monogr*. 1990;99:42–62.
129. Zuurman L, Ippel AE, Moin E, van Gerven JM. Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br J Clin Pharmacol*. 2009;67(1):5–21.
130. Batalla A, Bhattacharyya S, Yucel M, et al. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*. 2013;8(2):e55821.
131. Battistella G, Fornari E, Annoni JM, et al. Long-term effects of cannabis on brain structure. *Neuropsychopharmacology*. 2014;39(9):2041–2048. https://doi.org/10.1038/npp.2014.67.
132. Brumback T, Castro N, Jacobus J, Tapert S. Effects of marijuana use on brain structure and function: neuroimaging findings from a neurodevelopmental perspective. *Int Rev Neurobiol*. 2016;129:33–65.
133. Wargo KA, Geveden BN, McConnell VJ. Cannabinoid-induced pancreatitis: a case series. *JOP*. 2007;8(5):579–583.
134. Gundersen TD, Jørgensen N, Andersson AM, et al. 2015. Association between use of marijuana and Male reproductive Hormones and Semen quality: a study among 1,215 healthy young Men. *Am J Epidemiol*. 2015;182(6):473–481. https://doi.org/10.1093/aje/kwv135.
135. D'Souza DC, Cortes-Briones JA, Ranganathan M, et al. Rapid changes in CB1 receptor availability in cannabis dependent males after abstinence from cannabis. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2016;1(1):60–67.
136. Andreasson S, Allebeck P, Engstrom A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*. 1987;2(8574):1483–1486.
137. Crean RD, Crane NA, Mason BJ. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J Addict Med*. 2011;5(1):1–8.
138. McGrath J, Welham J, Scott J, et al. Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Arch Gen Psychiatry*. 2010;67:440–447.
139. Meier MH, Caspi A, Ambler A, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proc Natl Acad Sci U S A*. 2012;109(40):E2657–E2664.

140. Moore TH, Zammit S, Lingford-Hughes A, et al. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*. 2007;370(9584):319–328.
141. Aldington SM, Williams M, Nowitz M, et al. Effects of cannabis on pulmonary structure, function and symptoms. *Thorax*. 2007;62:1058–1063.
142. Bowles DW, O'Bryant CL, Camidge DR, Jimeno A. The intersection between Cannabis and cancer in the United States. *Crit Rev Oncol Hematol*. 2012;83:1–10.
143. Schrot RJ, Hubbard JR. Cannabinoids: medical implications. *Ann Med*. 2016;48(3):128–141.
144. Lee MH, Hancox RJ. Effects of smoking cannabis on lung function. *Expert Rev Respir Med*. 2011;5(4):537–546; quiz 547. https://doi.org/10.1586/ers.11.40.
145. Ribeiro LIG, Ind PW. Effect of cannabis smoking on lung function and respiratory symptoms: a structured literature review. *NPJ Prim Care Respir Med*. 2016;26:1–8.
146. Tashkin DP. Effects of marijuana smoking on the lung. *Ann Am Thorac Soc*. 2013;10(3):239–247.
147. Aujayeb A, Donald C, Doe S. Breath-holding in a marijuana smoker. *Respir Med Case Rep*. 2012;5:69–72.
148. Owen KP, Sutter ME, Albertson TE. Marijuana: respiratory tract effects. *Clin Rev Allerg Immunol*. 2014;46(1):65–81.
149. Wang GS, Le Lait MC, Deakyne SJ, Bronstein AC, Bajaj L, Roosevelt G. Unintentional pediatric exposures to marijuana in Colorado, 2009–2015. *JAMA Pediatr*. 2016;170(9):e160971. https://doi.org/10.1001/jamapediatrics.2016.0971.
150. Wang GS, Roosevelt G, Heard K. Pediatric marijuana exposures in a medical marijuana state. *JAMA Pediatr*. 2013;167(7):630–633.
151. Driving under the influence of drugs, alcohol and medicines in Europe: findings from the DRUID project. Thematic Papers, European Monitoring Centre for Drugs and Drug Addiction. http://www.emcdda.europa.eu/publications/thematic-papers/druid_en. Accessed October 10, 2019.
152. Gjerde H, Morland J. Risk for involvement in road traffic crash during acute cannabis intoxication. *Addiction*. 2016;111(8):1492–1495.
153. Allen JH, de Moore GM, Heddle R, et al. Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis use. *Gut*. 2004;53:1566–1570.
154. Galli JA, Sawaya RA, Friedenberg FK. Cannabinoid hyperemesis syndrome. *Curr Drug Abuse Rev*. 2011;4(4):241–249.
155. Soriano-Co M, Batke M, Cappell MS. The cannabis hyperemesis syndrome characterized by persistent nausea and vomiting, abdominal pain, and compulsive bathing associated with chronic marijuana use: a report of eight cases in the United States. *Dig Dis Sci*. 2010;55:3113–3119.
156. Moon AM, Buckley SA, Mark NM. Successful treatment of cannabinoid hyperemesis syndrome with Topical Capsaicin. *ACG Case Rep J*. 2018;5:e3. https://doi.org/10.14309/crj.2018.3.
157. Hancock-Allen JB, Barker L, VanDyke M, Holmes DB. Notes from the field: death following ingestion of an edible marijuana product—Colorado, March 2014. *MMWR Morb Mortal Wkly Rep*. 2015;64(28):771–772.
158. Wang T, Collet JP, Shapiro S, Ware MA. Adverse effects of medical cannabinoids: a systematic review. *CMAJ*. 2008;178(13):1669–1678.
159. Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following oral THC administration to humans. *Psychopharmacology (Berl)*. 1999;141(4):385–394.
160. Zajicek JP, Sanders HP, Wright DE, et al. Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatr*. 2005;76(12):1664–1669.
161. Porter BE, Jacobson C. Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy Behav*. 2013;29:574–577.
162. Budney AJ, Hughes JR, Moore BA, Vandrey R. Review of the validity and significance of cannabis withdrawal syndrome. *Am J Psychiatry*. 2004;161(11):1967–1977.
163. Lee D, Schroeder JR, Karschner EL, et al. Cannabis withdrawal in chronic, frequent cannabis smokers during sustained abstinence within a closed residential environment. *Am J Addict*. 2014;23(3):234–242.
164. Johns A. Psychiatric effects of cannabis. *Br J Psychiatry*. 2001;178:116–122.
165. Iffland K, Grotenhermen F. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. *Cannabis Cannabinoid Res*. 2017;21:139–154.
166. Christison A. *On the Natural History, Actions, and Uses of Indian Hemp*. London: John Churchill; 1851:26.
167. Substance Abuse and Mental Health Services Administration. *Key substance use and mental health indicators in the United States: Results from the 2016 National Survey on Drug Use and Health*. Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration, 2017. https://www.cdc.gov/marijuana/factsheets/pregnancy.htm#8. Accessed October 10, 2019.
168. Fergusson DM, Horwood LJ, Northstone K. Maternal use of cannabis and pregnancy outcome. *BJOG*. 2002;109(1):21–27.
169. El Marroun H, Brown QL, Lund IO, et al. An epidemiological, developmental and clinical overview of cannabis use during pregnancy. *Prev Med*. 2018;116:1–5.
170. Ko JY, Farr SL, Tong VT, Creanga AA, Callaghan WM. Prevalence and patterns of marijuana among pregnant and nonpregnant women of reproductive age. *Gynecol*. 2015;213(2):201.e1–201.e10.
171. Tennes K, Avitable N, Blackard C, et al. Marijuana: prenatal and postnatal exposure in the human. In: Pinkert TM, ed. *Consequences of Maternal Drug Abuse*. Rockville MD: National Institute on Drug Abuse; 1985. Research Monograph No. 59.
172. Blackard C, Tennes K. Human placental transfer of cannabinoids. *N Engl J Med*. 1984;311:797.
173. Bloch E. Effects of marihuana and cannabinoids on reproduction, endocrine function, development and chromosomes. In: Fehr KO, Kalant H, eds. *Cannabis and Health Hazards*. Toronto: Addiction Research Foundation; 1983.
174. Hatch EE, Bracken MB. Effect of marijuana use in pregnancy on fetal growth. *Am J Epidemiol*. 1986;124:986–993.
175. Sherwood RA, Keating J, Kavvadia V, Greenough A, Peters TJ. Substance misuse in early pregnancy and relationship to fetal outcome. *Eur J Pediatr*. 1999;158:488–492.
176. Zuckerman B, Frank DA, Hingson R, et al. Effects of maternal marijuana and cocaine use on fetal growth. *N Engl J Med*. 1989;320(0028–4793;12):762–768.
177. Fergusson DM, Horwood LJ, Swain-Campbell N. Cannabis use and psychosocial adjustment in adolescence and young adulthood. *Addiction*. 2002;97(9):1123–1135.
178. Gunn JK, Rosales CB, Center KE, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. *BMJ Open*. 2016;6(4):e009986.
179. Gibson GT, Baghurst PA, Colley DP. Maternal alcohol, tobacco and cannabis consumption and the outcome of pregnancy. *Aust NZ J Obstet Gynaecol*. 1983;23:15–19.
180. McLemore GL, Richardson KA. Data from three prospective longitudinal human cohorts of prenatal marijuana exposure and offspring outcomes from the fetal period through young adulthood. *Data Brief*. 2016;9:753–757.
181. Mueller-Vahl KR, Emrich HM. Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev Neurother*. 2008;8(7):1–12.
182. Cunha PJ, Rosa PG, Ayres Ade M, et al. Cannabis use, cognition and brain structure in first-episode psychosis. *Schizophr Res*. 2013;147(2–3):209.
183. Radhakrishnan R, Wilkinson ST, D'Souza DC. Gone to pot.-A review of the association between cannabis and psychosis. *Front Psychiat*. 2014;5(54):1–24.
184. Garry A, Rigourd V, Amirouche A, Fauroux V, Aubry S, Serreau R. Cannabis and breastfeeding. *J Toxicol*. 2009;2009:596149.
185. Varner MW, Silver RM, Rowland Hogue CJ, et al. Association between stillbirth and illicit drug use and smoking during pregnancy. *Obstet Gynecol*. 2014;123(1):113–125.
186. Warshak CR, Regan J, Moore B, Magner K, Kritzer S, Van Hook J. Association between marijuana use and adverse obstetrical and neonatal outcomes. *J Perinatol*. 2015;35(12):991–995.

187. de Salas-Quiroga A, Díaz-Alonso J, García-Rincón D, et al. Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB 1 receptors on developing cortical neurons. *Proc Nat Acad Sci U S A*. 2015;112(44):1–6. https://doi.org/10.1073/pnas.1514962112.
188. Lovecchio F, Heise CW. Accidental pediatric ingestions of medical marijuana: a 4-year poison center experience. *Am J Emerg Med*. 2015;33(6):844–845.
189. Onders B, Casavant MJ, Spiller HA, Chounthirath T, Smith GA. Marijuana exposure among children younger than six years in the United States. *Clin Pediatr*. 2016;55(5):428–436.
190. Wang M, Wang YH, Avula B, et al. Decarboxylation study of acidic cannabinoids: a novel approach using ultra-high-performance supercritical fluid Chromatography/Photodiode array-mass spectrometry. *Cannabis Cannabinoid Res*. 2016;1(1):262–271.
191. Rahn EJ, Hohmann AG. Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics*. 2009;6:713–737.
192. Hollister LE. Hunger and appetite after single doses of marihuana, alcohol, and dextroamphetamine. *Clin Pharmacol Ther*. 1971;12:44–49.
193. Machado Rocha FC, Stefano SC, De Cassia Haiek R, Rosa Oliveira LM, Da Silveira DX. Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *Eur J Can Care*. 2008;17:431–443.
194. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (−)Δ9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci*. 1998;95:8268–8273.
195. Neff GW, O'Brien CB, Reddy KR, et al. Preliminary observation with dronabinol in patients with intractable pruritus secondary to cholestatic liver disease. *Am J Gastroenterol*. 2002;97:2117–2119.
196. Douthwaite AH. Choice of drugs in the treatment of duodenal ulcers. *Br Med J*. 1947;2:43–47.
197. Williams SJ, Hartley JPR, Graham JDP. Bronchodilator effect of Δ1-tetrahydrocannabinol administered by aerosol to asthmatic patients. *Thorax*. 1976;31:720–723.
198. Kavia R, De Ridder D, Constantinescu CS, Stott CG, Fowler CJ. Randomised controlled trial of Sativex to treat detrusor overactivity in multiple sclerosis. *Mult Scler*. 2010;16:1349–1359.
199. Eubanks LM, Rogers CJ, Beuschner AE, Koob GF, Dickerson TJ, Janda KD. A molecular link between the active component of marijuana and Alzheimer's disease pathology. *Mol Pharmacol*. 2006;3:773–777.
200. Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *Int J Geriatr Psychiatry*. 1997;12(9):913–919.
201. Davis WM, Hatoum NS. Neurobehavioral actions of cannabichromene and interactions with delta 9-tetrahydrocannabinol. *Gen Pharmacol*. 1983;14:247–252.
202. Jones NA, Hill AJ, Smith I, et al. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *J Pharmacol Exp Ther*. 2010;332:569–577.
203. Deyo AR, Musty RE. *Cannabichromene (CBC) Extract Alters Behavioral Despair on the Mouse Tail Suspension Test of Depression*. Burlington (VT): International Cannabinoid Research Society; 2003:146.
204. Rock EM, Goodwin JM, Limebeer CL, et al. Interaction between non-psychotropic cannabinoids in marihuana: effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. *Psychopharmacology*. 2010;215:505–512.
205. Elsohly HN, Turner CE, Clark AM, Elsohly MA. Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. *J Pharm Sci*. 1982;71:319–323.
206. Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. *Free Rad Biol Med*. 2011;51:1054–1061.
207. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *Br J Pharmacol*. 2008;153:199–215.
208. Campos AC, Guimaraes FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacol*. 2008;199:223–230.
209. Resstel LB, Tavares RF, Lisboa SF, Joca SR, Correa FM, Guimaraes FS. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol*. 2009;156:181–188.
210. Biro T, Toth BI, Hasko G, Paus R, Pacher P. The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. *Trends Pharmacol Sci*. 2009;30:411–420.
211. Appendino G, Gibbons S, Giana A, et al. Antibacterial cannabinoids from Cannabis sativa: a structure-activity study. *J Nat Prod*. 2008;71:1427–1430.
212. Ligresti A, Moriello AS, Starowicz K, et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther*. 2006;318:1375–1387.
213. Xi ZX, Peng XQ, Li X, Zhang H, Li JG, Gardner EL. Brain cannabinoid CB2 receptors inhibit cocaine self-administration and cocaine-enhanced extracellular dopamine in mice. *Proceedings 20th Annual Symposium on the Cannabinoids*. Lund: International Cannabinoid Research Society; 2010: 32.
214. Russo EB. History of cannabis and its preparations in saga, science, and sobriquet. *Chem Biodivers*. 2007;4(8):1614–1648.
215. Mecha M, Torrao AS, Mestre L, Carrillo-Salinas FJ, Mechoulam R, Guaza C. Cannabidiol protects oligodendrocyte progenitor cells from inflammation-induced apoptosis by attenuating endoplasmic reticulum stress. *Cell Death Dis*. 2012;3:e331.
216. Lorenzetti B, Souza G, Sarti S, Filho D, Ferreira S. Myrcene mimics the peripheral analgesic activity of lemongrass tea. *J Ethnopharmacol*. 1991;34:43–48.
217. do Vale TG, Furtado EC, Santos Jr JG, Viana GS. Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from *Lippia alba* (Mill.) NE Brown. *Phytomedicine*. 2002;9:709–714.
218. Musty RE, Karniol IG, Shirikawa I, Takahashi RN, Knobel E. Interactions of Δ9-tetrahydrocannabinol and cannabinol in man. In: Braude MC, Szara S, eds. *The Pharmacology of Marihuana*. Vol. 2. New York: Raven Press; 1976:559–563.
219. Moreira FA, Guimaraes FS. Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *Eur J Pharmacol*. 2005;512(2–3):199–205.
220. Nappe TM, Hoyt CO. Pediatric death due to myocarditis after exposure to cannabis. *Clin Pract Cases Emerg Med*. 2017;1(3):166–170.

62

Capsicum frutescens (Cayenne Pepper)

Michael T. Murray, ND

OUTLINE

Capsicum frutescens (family: Solanaceae)
Common names: cayenne pepper, capsicum, chili pepper, red pepper, American pepper

GENERAL DESCRIPTION

Cayenne pepper (also known as chili or red hot pepper) is the fruit of *Capsicum annuum,* a shrubby, tropical plant that can grow up to 3 feet high. The fruit is technically a berry (Fig. 62.1). Paprika is a milder and sweeter-tasting fruit produced from a different variety of capsicum. Although cayenne pepper is native to tropical America, it is now cultivated in tropical locations throughout the world and has found its way into the cuisine of many parts of the world, particularly Southeast Asia, China, southern Italy, and Mexico.

CHEMICAL COMPOSITION

The most important constituents of cayenne pepper are the pungent compounds, with capsaicin being the most prominent (Fig. 62.2). Typically, cayenne pepper contains about 1.5% capsaicin and related principal components. Other active constituents present include carotenoids, vitamins A and C, and volatile oils.

HISTORY AND FOLK USE

The folk use of cayenne pepper is quite extensive. It has been used for the following:

- Asthma
- Fever
- Sore throats and other respiratory tract infections
- Digestive disturbances
- Poultices
- Cancer

It has also been used as a counterirritant in the topical treatment of arthritis and neuralgia.

PHARMACOLOGY

The pharmacology of cayenne pepper centers around its capsaicin content. Interestingly, capsaicin, although hot to the taste, has been shown to lower body temperature by stimulating the cooling center of the hypothalamus.[1] The ingestion of cayenne peppers by cultures native to the tropics may offer locals a way to deal with high temperatures.

When taken internally, cayenne pepper exerts several beneficial effects on the cardiovascular system. In addition to possessing antioxidant compounds, studies have shown that cayenne pepper reduces the likelihood of developing atherosclerosis by reducing blood cholesterol, triglyceride levels, and platelet aggregation, as well as increasing fibrinolytic activity.[2–4] (For the significance of these effects, see Chapter 149.) Cultures consuming large amounts of cayenne pepper have a much lower rate of cardiovascular disease.

When topically applied to the skin or mucous membranes, capsaicin is known to stimulate and then block small-diameter pain fibers by depleting them of the neurotransmitter substance P.[5] Substance P is thought to be the principal chemo-mediator of pain impulses from the periphery. In addition, substance P has been shown to activate inflammatory mediators into joint tissues in osteoarthritis and rheumatoid arthritis.[6]

CLINICAL APPLICATIONS FOR ORAL PREPARATIONS

Gastrointestinal Disorders

Cayenne pepper exerts several beneficial effects on gastrointestinal function, including acting as a digestant and carminative.[7] In addition, constituents of capsicum increase gastric emptying; stimulate gastromucosal defense and absorption; enhance permeability to micronutrients; and stimulate salivary, intestinal, hepatic, and pancreatic secretions.[8] Traditionally, *C. annuum* has been used against various gastrointestinal complaints, including dyspepsia, loss of appetite, gastrointestinal reflux disease (GERD), and gastric ulcer. Red pepper has been used therapeutically in atonic dyspepsia and flatulence because it increases the motility in the gastric antrum, duodenum, proximal jejunum, and colon.[9] Capsicum has been used as an antiseptic, counterirritant, appetite suppressor, antioxidant, and immunomodulator in the gastrointestinal system.

Double-blind studies show that red pepper consumption protects against aspirin-induced stomach damage and improves epigastric pain, fullness, and nausea scores in people with nonulcer dyspepsia.[10–12] In one study, the digestive-enhancing effects of capsicum were determined in 30 patients with functional dyspepsia and without GERD and irritable bowel syndrome (IBS). Patients randomly received 2.5 g/day of red pepper powder or placebo before meals for 5 weeks in a double-blind manner. Starting from the third week, overall symptom score and epigastric pain, fullness, and nausea scores were significantly lower than the placebo group. The decrease reached about 60% at the end of treatment in the red pepper group, whereas placebo scores decreased by less than 30%.

Capsicum does, however, lower the threshold for GERD, presumably by direct effects on sensory neurons, and may produce symptoms of GERD.[13,14] The therapeutic use of chili pepper and capsaicin may therefore act as a double-edged sword in many physiological circumstances, and further studies are warranted to determine the dose ceiling for their use as gastroprotective agents.

Fig. 62.1 *Capsicum annuum* fruit.

Thermogenic Aid

Capsicum ingestion may prove to be helpful in promoting weight loss in obese individuals. The antiobesity effects of capsicum occur via several mechanisms, including thermogenesis, satiety, fat oxidation, elevation of the basal metabolic rate, reduction of caloric intake, prevention of adipogenesis, restriction of the activity of lipoprotein lipase and pancreatic lipase, stimulation of lipolysis in adipose tissue, inhibition of the differentiation of adipocytes, and modulating adipokine release from adipose tissues.[15]

A study of 13 Japanese females showed that adding red pepper to high-fat meals increased diet-induced brown adipose tissue thermogenesis and lipid oxidation.[16] In a clinical trial, capsaicinoids from *Capsicum* were administered to subjects (age 42 years and body mass index [BMI] 30.4) for 12 weeks at a dose of 6 mg/day. At the end of the study, subjects in the treatment group had an average abdominal adiposity reduction of 21.1% compared with 20.2% in the placebo group,

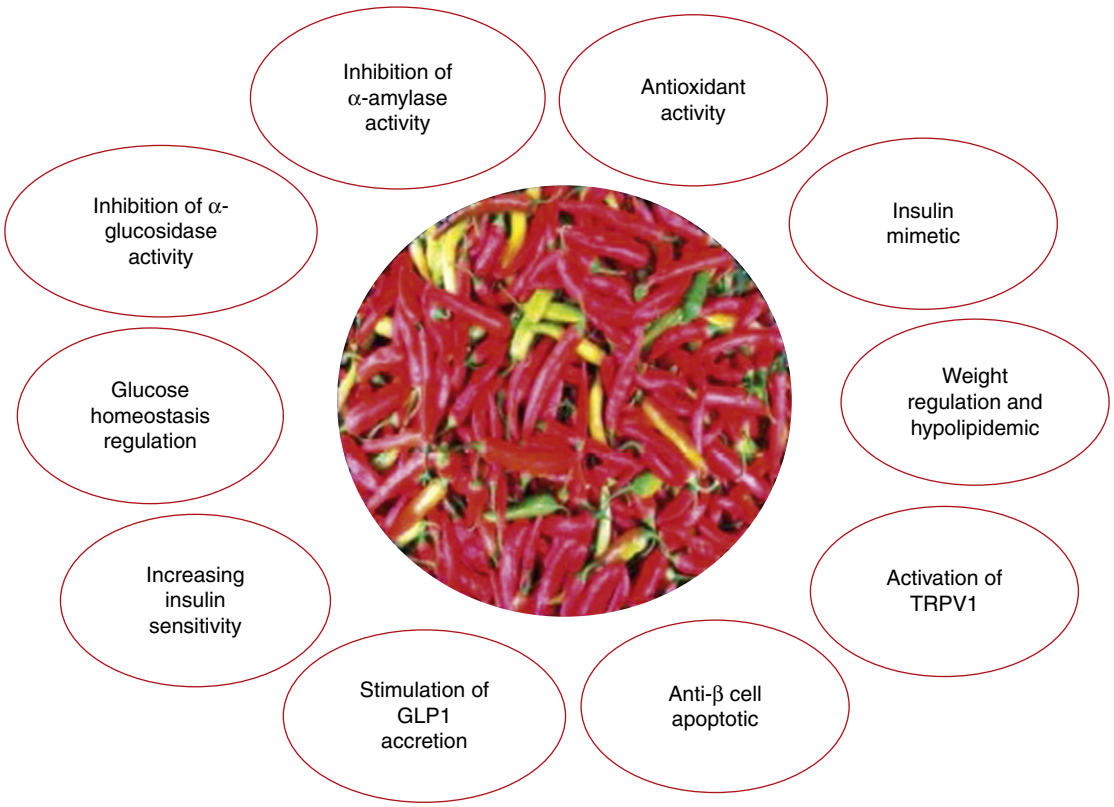


Fig. 62.2 Capsaicin.

and these effects were positively correlated with the change in body weight.[17] Researchers have also evaluated the effects of dietary red pepper added to high-fat and high-carbohydrate meals on subsequent feeding behavior and energy intake.[18] After ingesting a standardized dinner on the previous evening, the subjects ate one of the following for breakfast: a high-fat meal, a high-fat meal with red pepper (10 g), a high-carbohydrate meal, or a high-carbohydrate meal with red pepper (10 g). The addition of red pepper reduced appetite and hunger before lunch, and diet-induced thermogenesis was significantly enhanced by the addition of red pepper to either meal, but especially the high-fat meal. In another study, 40 women and 40 men (mean age of 42 years and BMI of 30.4) were randomly assigned to a capsinoid (6 mg/day) or placebo group.[19] Mean weight change was 0.9 and 0.5 kg in the capsinoid and placebo groups, respectively. There was no significant group difference in total change in adiposity, but abdominal adiposity decreased more in the capsinoid group (−1.11%) than in the placebo group (−0.18%), and this change correlated with the change in body weight. Changes in resting energy expenditure did not differ significantly between groups, but fat oxidation was higher at the end of the study in the capsinoid group.

Cardiovascular Disease and the Metabolic Syndrome

Capsicum exerts several effects beneficial in the prevention of cardiovascular disease, including inhibiting low-density lipoprotein cholesterol oxidation, acting as an antioxidant, inhibiting platelet aggregation, promoting fibrinolysis, and improving arterial function.[20] A randomized, double-blind, placebo-controlled clinical trial on hyperlipidemic subjects ingesting a traditional fermented red pepper paste (kochujang) for 12 weeks demonstrated significant reductions in total cholesterol (215.5 ± 16.1 mg/dL–194.5 ± 25.4 mg/dL) and LDL cholesterol (133.6 ± 14.8 mg/dL–113.5 ± 23.1 mg/dL) in the kochujang-supplemented group compared with placebo.[21] Capsaicin consumption 1 hour before low-intensity exercise is a valuable supplement for the treatment of individuals with hyperlipidemia and/or obesity because it improves lipolysis.[22] Besides being cardioprotective, the beneficial hypocholesterolemic influence of capsaicin extend to include the prevention of cholesterol gallstones and protection of the structural integrity of erythrocytes under conditions of hypercholesterolemia.[23]

It is known that insulin-like growth factor-1 (IGF-1) reduces arterial blood pressure. Because administration of capsaicin and isoflavone increases serum levels of IGF-1 by sensory neuron stimulation in subjects with alopecia, a study was conducted to evaluate the effects of capsaicin and isoflavone on blood pressure in patients with hypertension.[24] Systolic and diastolic blood pressure and serum levels of IGF-1 were measured before and at 1, 3, and 5 months after the administration of capsaicin and isoflavone. Although blood pressure was unaffected in normotensive subjects, systolic and diastolic blood pressure were significantly reduced in hypertensive volunteers after administration of capsaicin and isoflavone, and IGF-1 levels were significantly increased.

In healthy human subjects, a single meal with capsaicin caused an increase in postprandial GLP1 concentration while decreasing postprandial ghrelin concentration.[25] Chili consumption also lowers postprandial hyperinsulinemia.[26] In addition to the effects of capsaicin in type 2 diabetes, a randomized, double-blind, placebo-controlled trial on 44 pregnant women with gestational diabetes indicated that 5 mg/dL capsaicin for 4 weeks improved fasting lipid levels, improved postprandial hyperglycemia, improved hyperinsulinemia, and decreased the incidence of large-for-gestational-age newborns.[27] Fig. 62.3 describes several of the known mechanisms associated with the antidiabetic effects of *C. annuum*.

Transient receptor potential vanilloid subtype I (TRPV1) is the receptor for capsaicin and is widely expressed in the brain, sensory nerves, dorsal root ganglia, bladder, gut, and blood vessels. TRPV1

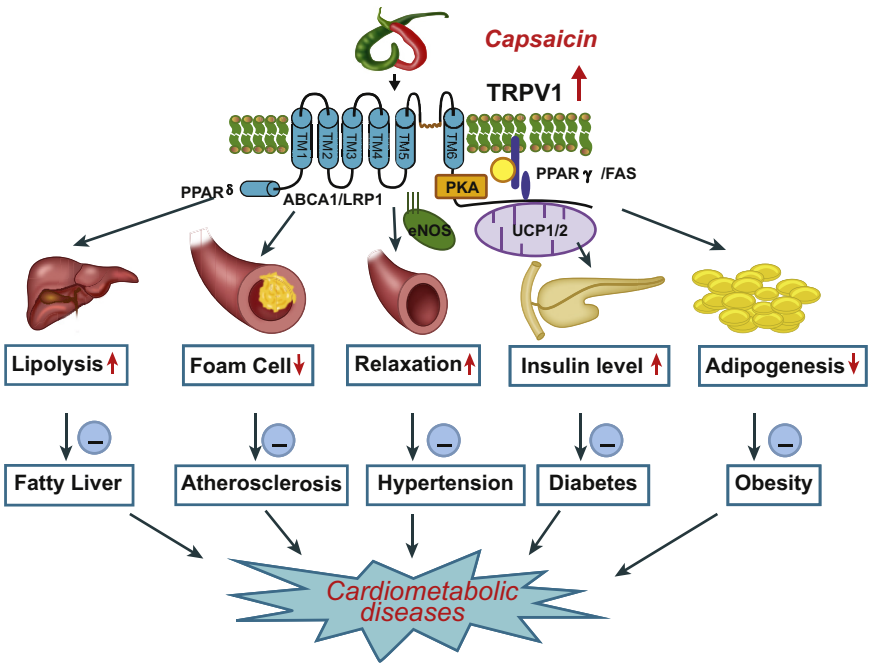


Fig. 62.3 Antidiabetic effects of *Capsicum annuum*. *GLP1,* glucagon-like peptide 1; *TRPV1*, transient receptor potential vanilloid subtype 1. (From Sanati S, Razavi BM, Hosseinzadeh H. A review of the effects of *Capsicum annuum L.* and its constituents, capsaicin, in metabolic syndrome. *Iran J Basic Med Sci.* 2018;21[5]:439–448.)

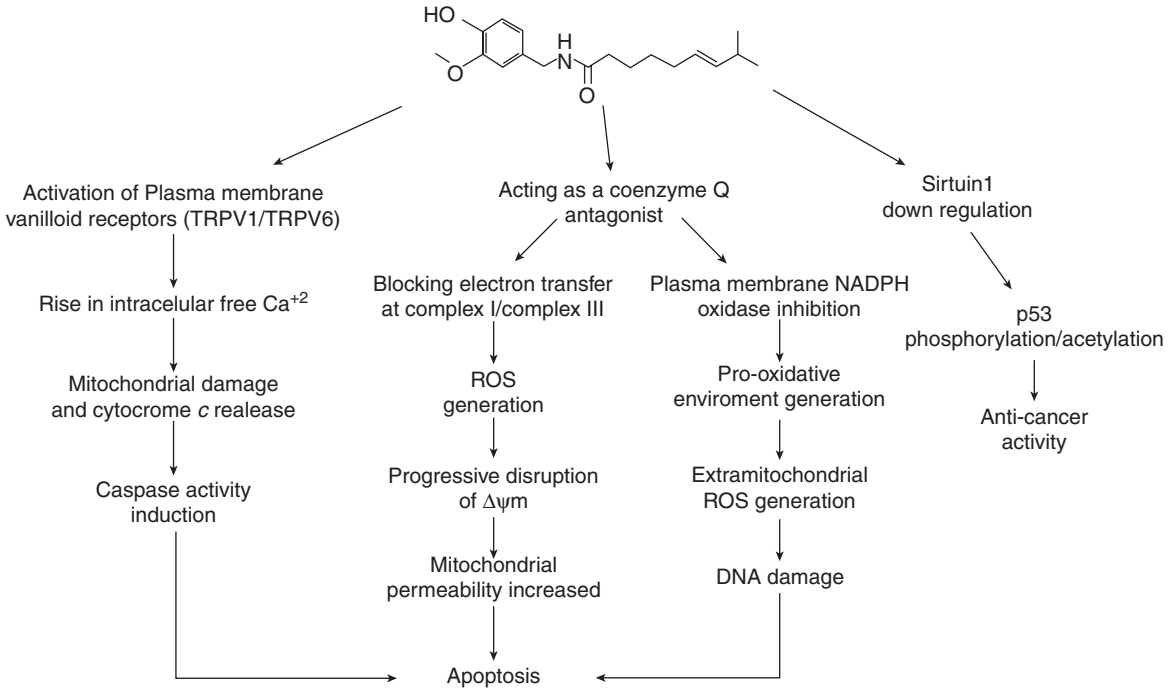


Fig. 62.4 Effects of capsaicin on cardiometabolic disease. (From Sun F, Xiong S, Zhu, Z. Dietary capsaicin protects cardiometabolic organs from dysfunction. *Nutrients*. 2016;8[5]:E174.)

is involved in inflammation, oxidative stress, and pain sensation and likely plays a critical role in the regulation of cardiovascular function and metabolic homeostasis. Activation of TRPV1 by capsaicin has been shown to prevent obesity, improve glucose homeostasis, alleviate hypertension, and antagonize dysfunction of cardiometabolic organs (Fig. 62.4).[28] This receptor provides a common link between the conditions associated with the metabolic syndrome and may be an optimal target for capsaicin in the treatment and management of cardiometabolic diseases.

Cancer

The preponderance of the data strongly indicates significant anticancer benefits of capsaicin. Capsaicin has been shown to alter the expression of several genes involved in cancer cell survival, growth arrest, angiogenesis, and metastasis. Capsaicin targets multiple signaling pathways, oncogenes, and tumor-suppressor genes in various types of cancer models. In addition, data suggest several benefits of combinational use of capsaicin with other dietary or chemotherapeutic compounds, leading to synergistic anticancer activity.

Capsicum has shown an antiproliferative effect on various human cancer cell lines. Capsaicin induced significant cytotoxicity with increases in oxidative stress, PARP cleavage, and apoptosis in gastric cancer cells (SNU-1).[29] Capsaicin-induced apoptosis in SNU-1 cells was associated with down-regulation of tumor-associated NADH oxidase (tNOX) mRNA and protein. This is significant because in cells in which tNOX was scarcely affected, capsaicin exhibited little apoptosis and low cytotoxicity. However, in tNOX-knockdown sensitized cells, capsaicin induced apoptosis and decreased growth, demonstrating that tNOX is essential for cancer cell growth. A similar mechanism was demonstrated in a study of human bladder cancer cells that indicated that capsaicin inhibits the growth of multiple bladder cancer cell phenotypes by inhibiting tNOX and SIRT1 (a deacetylase important in multiple cellular functions), thereby reducing proliferation, attenuating migration, and prolonging cell-cycle progression.[30]

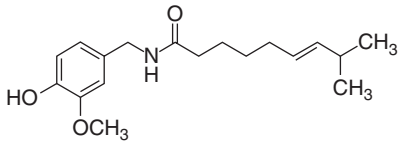


Fig. 62.5 Summary of apoptotic mechanisms and anticancer activity of capsaicin. (From Chapa-Oliver AM, Mejia-Teniente L. Capsaicin: from plants to a cancer-suppressing agent. *Molecules*. 2016;21[8]:E931.)

The various signaling pathways involved in capsaicin-mediated cellular responses include oxidative stress, which is known to trigger apoptosis. Capsaicin was shown to induce apoptosis in pancreatic cancer cells in association with a reduction in Complex I and Complex III activity, leading to reactive oxygen species (ROS) generation and mitochondrial damage.[31] The suppression of cyclooxygenase (COX), an ROS-generating enzyme, was shown to be involved in capsaicin-induced apoptosis of human neuroblastoma cells.[32] The plasma membrane–resident NADPH oxidase also responds to capsaicin, and the NADPH oxidase-mediated generation of ROS may be essentially involved in the mechanism of capsaicin-induced apoptosis in human hepatoblastoma cells.[33]

Other pathways are also relevant to capsaicin-mediated apoptosis. Caspase activation and nitric oxide (NO) elevation were found to be induced by capsaicin stimulating p53 and the apoptotic Bax expression through Mdm2 down-regulation, thus increasing mitochondrial-dependent apoptosis in colon carcinoma cells.[34] The transcription factor signal transducer and activator of transcription 3 (STAT3) has been closely linked with tumorigenesis. Capsaicin was found to inhibit the activation of STAT3 and alter protein expression of Bcl-2, Bcl-xL, and survivin in multiple myeloma cells, demonstrating a potential role in the prevention and treatment of multiple myeloma and other cancers.[35] Furthermore, in urothelial carcinoma cells, capsaicin exerts its apoptotic activity through overexpression of TRPV1.[36] Fig. 62.5 provides a summary of the apoptotic mechanisms and anticancer activity of capsaicin.

The development of resistance to anticancer drugs is a common clinical issue in the treatment of patients with cancer, and methods to address this complication are continuously sought. One solution is the coadministration of substances (chemosensitizers) that can reverse the resistance to anticancer drugs. Capsaicin not only exhibits apoptotic and preventive properties in cancer, but it has also shown significant potential as a chemosensitizing agent. Capsaicin significantly and synergistically increases the cytotoxicity of several chemotherapeutic drugs, including but not limited to doxorubicin,[37] sorafenib,[38] platinum drugs (e.g., cisplatin),[39] and vinblastine.[40]

CLINICAL APPLICATIONS FOR TOPICAL PREPARATIONS

Clinical use of cayenne pepper has focused on topical capsaicin-containing preparations. Commercial ointments containing 0.025% or 0.075% capsaicin are available over the counter. These preparations may offer significant benefits in many conditions, including pain disorders, diabetic neuropathy, cluster headache, osteoarthritis, and rheumatoid arthritis. In addition, topically applied capsaicin may be useful in psoriasis.

Postherpetic Neuralgia

The first studies and approved use for topically applied capsaicin were in relieving postherpetic neuralgia. Numerous studies now document this U.S. Food and Drug Administration (FDA)–approved application. For example, in one study, 39 patients with chronic postherpetic neuralgia (average duration 24 months) were treated with 0.025% capsaicin cream for 8 weeks.[41] During therapy, the patients rated their pain. Nineteen patients (48.7%) substantially improved after the 8-week trial; 5 (12.8%) discontinued therapy due to side effects, such as intolerable capsaicin-induced burning sensations (4) or mastitis (1); and 15 (38.5%) reported no benefit. The decrease in pain ratings was significant after 2 weeks of continuous application. Of the responders, 72.2% still reported improvement 10 to 12 months after the study, with most continuing to apply the cream regularly.

Higher concentration (0.075% vs. 0.025%) might produce better results (as high as 75% response).[42] Capsaicin responders were characterized by higher average daily pain, higher allodynia ratings, and relatively preserved sensory function at baseline compared with nonresponders.[43] In three of the "capsaicin responders," the area of allodynia expanded into previously nonallodynic and nonpainful skin that had normal sensory function and cutaneous innervation. A multicenter, randomized, double-blind, controlled study was conducted to confirm the efficacy, tolerability, and safety of NGX-4010, an 8% capsaicin dermal patch (capsaicin 640 μg/cm^2) in patients with postherpetic neuralgia.[44] In this study, 418 patients were randomized to receive a single 60-minute application of NGX-4010 or a 0.04% capsaicin control patch. NGX-4010 recipients had a significantly greater mean reduction from baseline in pain during weeks 2 to 8 compared with the control group (32.0% vs. 24.4%). Pain was significantly lower in the NGX-4010 group by week 2, and greater pain reduction was maintained throughout the remaining 12-week study period. In a 4-week, double-blind study, patients with postherpetic neuralgia were randomized to receive NGX-4010 or a control patch.[45] Efficacy was evaluated using a numerical pain rating scale (NPRS) scores. During days 8 to 28 after the double-blind treatment, NGX-4010 patients had a mean change in NPRS scores from baseline of –32.7% compared with –4.4% for control patients. Mean changes in NPRS scores from baseline during weeks 2 to 12 was –33.8% for NGX-4010 and +4.9% for control recipients. In both studies, transient increases in application site pain were adequately managed, and no increases in application reactions or adverse events were observed with repeated treatments. Capsaicin 8% patch is an effective, tolerable, and generally safe treatment in cases of postherpetic neuralgia.

Trigeminal Neuralgia

Topically applied capsaicin may be effective in reducing the pain of trigeminal neuralgia. In one study, 12 patients were followed up for 1 year after the topical application over the painful area of capsaicin 3 times a day for several days.[46] Six patients had complete and four patients had partial relief of pain; the remaining two patients had no relief of pain. Of the 10 patients who were responsive to therapy, 4 had relapses of pain within 95 to 149 days. No relapses followed the second therapy for the remainder of the year. These results are promising for a condition that usually does not respond to any therapy, short of surgery.

Postmastectomy Pain

Topically applied capsaicin may help in the relief of pain after breast reconstruction or mastectomy. In one double-blind study, 23 patients with postmastectomy pain syndrome applied either capsaicin (0.075%) or vehicle (placebo) only cream 4 times daily for 4 to 6 weeks.[47] There was a significant difference in jabbing pain, in category pain severity scales, and in overall pain relief scales in favor of capsaicin. Five of 13 patients on capsaicin were categorized as having good-to-excellent responses, with 8 patients (62%) having 50% or greater improvement. Only 1 of 10 cases had a good response to the vehicle, with 3 rated as 50% or better.

In another study, 14 patients with postmastectomy pain had significant pain relief after application of 0.025% capsaicin cream four times daily for 4 to 6 weeks.[48] Unpleasant or painful sensations to light touch or pressure in the painful area (hyperesthesia, allodynia) were also improved.

Mouth Pain Due to Chemotherapy or Radiation

In a study conducted at the Yale Pain Management Center, capsaicin was shown to dramatically reduce the pain from mouth sores resulting from chemotherapy or radiation treatment.[49] An interesting feature in this study was the vehicle used to deliver the capsaicin—taffy. The researchers chose taffy because it could be held in the mouth long enough to desensitize the neurons. The sugar decreased the initial burning sensation, and its soft edges would not aggravate sore mouths like a hard candy. All 11 patients in the Yale study had decreased pain, and in 2 cases the pain stopped entirely after eating the capsaicin-laced candy.

Diabetic Neuropathy

Topically applied capsaicin has been shown to be of considerable benefit in relieving the pain of diabetic neuropathy in numerous double-blind studies.[50–55] In one large double-blind, 8-week study, investigators at 12 sites enrolled 277 men and women with painful diabetic neuropathy of the hands and feet.[56] 69.5% of the group applying the capsaicin cream (0.075%) showed improvement compared with 53.4% in those applying only the vehicle cream.

In another study, 40 patients applied either 0.075% capsaicin cream or placebo to their affected extremities daily. After 4 weeks, 76% of treated patients had some pain relief compared with 50% of placebo patients. In addition, those responding to capsaicin had a 50% reduction in pain, whereas those in the placebo group averaged between 15% and 20% relief in pain symptoms.

A randomized, double-blind, placebo-controlled 12-week study evaluated the efficacy and safety of an 8% capsaicin patch versus placebo in patients with painful diabetic peripheral neuropathy.[57] In this

study, 369 patients were randomized to one 30-minute treatment with either capsaicin 8% patch or placebo to painful areas of the feet. The change in average daily pain score from baseline to between weeks 2 through 8 was statistically significant for the capsaicin patch versus placebo (–27.4% vs. –20.9%). In addition, patients treated with capsaicin 8% patch had a shorter median time to treatment response (19 vs. 72 days) and modest improvements in sleep interference scores compared with placebo. Results confirm the clinical utility of the capsaicin 8% patch in the diabetic population.

Nondiabetic Peripheral Neuropathy

Multiple studies have demonstrated the capsaicin 8% patch is an effective treatment option in patients with peripheral neuropathic pain (PNP) arising from different etiologies. The capsaicin 8% patch provides rapid and sustained pain reductions in patients with PNP and a significant reduction in prescribed concomitant neuropathic pain medications. In nondiabetic patients with PNP, an open-label, randomized, multicenter trial demonstrated the capsaicin 8% patch provided noninferior pain relief versus pregabalin, with a more rapid onset of pain relief and fewer systemic side effects.[58] A 12-week, noninterventional study of a single capsaicin 8% patch treatment demonstrated effectiveness for preexisting PNP and suggested that early initiation of topical treatment (within 6 months of diagnosis) may benefit patients to a greater extent than patients with a longer history of pain.[59] A prospective, open-label, observational safety study in patients with postherpetic neuralgia, posttraumatic or postsurgical nerve injury, HIV-associated distal sensory polyneuropathy, or other peripheral neuropathic pain concluded that capsaicin 8% patch repeat treatment over 52 weeks was well tolerated, with variable alteration in sensory function (newly emergent hyperesthesia or allodynia apparent in 1.1%–3.6% of cases) and minimal chance of complete sensory loss.[60]

ASCEND was an open-label, noninterventional study of patients with nondiabetes-related PNP who received capsaicin 8% patch treatment and were followed for ≤52 weeks.[61] Following the first treatment with the capsaicin 8% patch, there was an overall 26.6% reduction in mean NPRS "average pain" score from baseline to weeks 2 and 8. Overall, patients had a 24.5% reduction in their mean NPRS score from baseline to week 2 and a 37.0% reduction to week 52. A total of 44.4% and 26.2% of patients were classified as ≥30% and ≥50% responders, respectively, after the first treatment. Of responders at week 8, 86.9% retained responder status at week 12 after the first treatment. Patients had a median time from the first to second treatment of 191 days and a median time from the second to third treatment of 301 days.

Together, these studies confirm that the capsaicin 8% patch provides consistent pain relief in a broad range of PNP etiologies. A multicenter, randomized, semidouble-blind study indicated that 0.625% capsaicin patch may prove to be an effective and safe alternative with which to treat patients with PNP and could replace the high-concentration (8%) patch.[62] However, further studies are needed to definitively establish efficacy.

Cluster Headaches

Several studies found that intranasal application of capsaicin ointment by a physician might relieve cluster headaches. In one double-blind study, patients with acute cluster headaches were randomized to receive either capsaicin or placebo in the nostril for 7 days.[63] Patients recorded the severity of each headache for 15 days. Headaches on days 8 to 15 of the study were significantly less severe in the capsaicin group versus the placebo group. There was also a significant decrease in headache severity in the capsaicin group on days 8 to 15 compared with days 1 to 7. Episodic patients appeared to benefit more than chronic patients.

Arthritis

Topically applied capsaicin may be effective in relieving the pain of osteoarthritis and rheumatoid arthritis. One study showed it to be more effective in osteoarthritis, whereas another study showed more benefit for patients with rheumatoid arthritis.

In the double-blind study showing more effect in osteoarthritis, 7 patients with rheumatoid arthritis and 14 patients with osteoarthritis who had painful involvement of the hands applied either capsaicin 0.075% or vehicle-only cream to the hands four times daily. Capsaicin reduced tenderness and pain associated with osteoarthritis but not rheumatoid arthritis.[64]

In the study showing greater benefit for rheumatoid arthritis, 70 patients with osteoarthritis and 31 with rheumatoid arthritis received capsaicin or placebo for 4 weeks.[65] The patients were instructed to apply 0.025% capsaicin cream or its vehicle (placebo) to painful knees four times daily. Significantly more relief of pain was reported by the capsaicin-treated patients than the placebo patients throughout the study; after 4 weeks of capsaicin treatment, patients with rheumatoid and osteoarthritis demonstrated mean reductions in pain of 57% and 33%, respectively. These reductions in pain were statistically significant compared with those reported with placebo. According to overall evaluations, 80% of the capsaicin-treated patients experienced a reduction in pain after 2 weeks of treatment.

Psoriasis

Excessive substance P levels in the skin have been linked to psoriasis. This finding prompted researchers to study the effects of topically applied capsaicin. In one double-blind study, 44 patients with symmetrically distributed psoriasis lesions applied topical capsaicin to one side of their body and a placebo to the other side.[66] After 3 to 6 weeks, significantly greater reductions in scaling and redness were observed on the capsaicin side. Burning, stinging, itching, and skin redness were noted by nearly half of the patients initially, but these diminished or vanished upon continued application.

In a later study, 197 patients applied capsaicin 0.025% cream or placebo cream 4 times a day for 6 weeks.[67] Efficacy was based on a physician's evaluation and a combined psoriasis severity score, including scaling, thickness, erythema, and pruritus. Capsaicin-treated patients demonstrated significantly greater improvement at the physician's evaluation and in pruritus relief, as well as a significantly greater reduction in combined psoriasis severity.

Pruritus Ani

Pruritus ani is a common condition that can be difficult to treat. Results from a double-blind study indicated that capsaicin was a safe and highly effective treatment for severe, intractable idiopathic pruritus ani.[68] This study involved two 4-week treatment phases separated by a 1-week washout phase. Forty-four patients were randomized to receive locally either active capsaicin (0.006%) or placebo (menthol 1%) ointment over a 4-week period. After 4 weeks of treatment and a 1-week washout period, the placebo group began to receive capsaicin, whereas the treated group received placebo (menthol 1%), for another 4 weeks. At the end of the controlled study, responders from both groups continued with capsaicin treatment in an open-label manner. Results indicated that 31 of 44 patients experienced relief during capsaicin treatment periods and did not respond to menthol; all patients who did not respond to capsaicin also did not respond to menthol. In 13 patients, treatment with capsaicin was unsuccessful: 8 patients did not respond to capsaicin treatment, 1 responded equally to capsaicin and placebo, and 4 others dropped out because of side effects.

DOSAGE

Cayenne pepper can be used liberally in the diet. Creams containing 0.025% or 0.075% capsaicin or poultices can be applied to affected areas up to four times daily.

TOXICITY

Capsicum is generally recognized as safe by the U.S. Food and Drug Administration. Contraindications to oral ingestion include aggravation or appearance of GERD or other sensitivity. Studies showed that consumption of 3 g of red chilies per day during the postoperative period after hemorrhoidectomy or surgery for anal fissures increased the intensity of typical postoperative symptoms, stool frequency, and the consumption of analgesics.[69,70]

Topically applied capsaicin may produce a local burning sensation; however, this effect goes away with time and rarely is severe enough to warrant discontinuation of use of the cream.

DRUG INTERACTIONS

Although several theoretical interactions have been postulated, no clinical cases have been documented. The use of capsicum has been cautioned in patients on anticoagulants or antiplatelet therapy.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Dib B. Effects of intrathecal capsaicin on autonomic and behavioral heat loss responses in the rat. *Pharmacol Biochem Behavior*. 1987;28:65–70.
2. Kawada T, Hagihara K, Iwai K. Effects of capsaicin on lipid metabolism fed a high fat diet. *J Nutr*. 1986;116:1272–1278.
3. Wang JP, Hsu MF, Teng CM. Antiplatelet effect of capsaicin. *Thromb Res*. 1984;36:497–507.
4. Visudhiphan S, Poolsuppasit S, Piboonnukarintr, et al. The relationship between high fibrinolytic activity and daily capsicum ingestion in Thais. *Am J Clin Nutr*. 1982;35:1452–1458.
5. Szallasi A. Vanilloid (capsaicin) receptors in health and disease. *Am J Clin Pathol*. 2002;118:110–121.
6. Cordell GA, Araujo OE. Capsaicin: identification, nomenclature, and pharmacotherapy. *Ann Pharmacother*. 1993;27:330–336.
7. Horowitz M, Wishart J, Maddox A, et al. The effect of chili on gastrointestinal transit. *J Gastroenterol Hepatol*. 1992;7:52–56.
8. Maji AK, Banerji P. Phytochemistry and gastrointestinal benefits of the medicinal spice, Capsicum annuum L. (chilli): a review. *J Complement Integr Med*. 2016;13(2):97–122.
9. Pawar SS, Bharude NV, Sonone SS, Deshmukh RS, Raut AK, Umarker AR. Chiles as food, spice and medicine: a perspective. *Int J Pharma Bio Sci*. 2011;1:311–318.
10. Yeoh KG, Kang JY, Yap I, et al. Chili protects against aspirin-induced gastroduodenal mucosal injury in humans. *Dig Dis Sci*. 1995;40:580–583.
11. Bortolotti M, Coccia G, Grossi G, et al. The treatment of functional dyspepsia with red pepper. *Aliment Pharmacol Ther*. 2002;16:1075–1082.
12. Drewes AM, Schipper KP, Dimcevski G, et al. Gut pain and hyperalgesia induced by capsaicin: a human experimental model. *Pain*. 2003;104:333–341.
13. Rodriguez-Stanley S, Collings KL, Robinson M, et al. The effects of capsaicin on reflux, gastric emptying and dyspepsia. *Aliment Pharmacol Ther*. 2000;14:129–134.
14. Milke P, Diaz A, Valdovinos MA, et al. Gastroesophageal reflux in healthy subjects induced by two different species of chili *(Capsicum annum)*. *Dig Dis*. 2006;24:184–188.
15. Sanati S, Razavi BM, Hosseinzadeh H. A review of the effects of Capsicum annuum L. and its constituents, capsaicin, in metabolic syndrome. *Iranian J Basic Med Sci*. 2018;21(5):439–448. 29922422.
16. Yoshioka M, St-Pierre S, Suzuki M, et al. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *Br J Nutr*. 1998;80:503–510.
17. Gamboa-Gomez CI, Rocha-Guzman NE, Gallegos-Infante JA, Moreno-Jimenez MR, Vazquez-Cabral BD, Gonzalez-Laredo RF. Plants with potential use on obesity and its complications. *EXCLI J Experi Clin Sci*. 2015;14:809–831.
18. Yoshioka M, St-Pierre S, Drapeau V, et al. Effects of red pepper on appetite and energy intake. *Br J Nutr*. 1999;82:115–123.
19. Snitker S, Fujishima Y, Shen H, et al. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *Am J Clin Nutr*. 2009;89:45–50.
20. Ahuja KD, Ball MJ. Effects of daily ingestion of chili on serum lipoprotein oxidation in adult men and women. *Br J Nutr*. 2006;96:239–242.
21. Lim JH, Jung ES, Choi EK, et al. Supplementation with Aspergillus oryzae-fermented kochujang lowers serum cholesterol in subjects with hyperlipidemia. *Clin Nutr*. 2015;34(3):383–387.
22. Leung FW. Capsaicin as an anti-obesity drug. *Prog Drug Res*. 2014;68:171–179.
23. Srinivasan K. Biological activities of red pepper (*Capsicum annuum*) and its pungent principle capsaicin: a review. *Crit Rev Food Sci Nutr*. 2016;56(9):1488–1500.
24. Harada N, Okajima K. Effects of capsaicin and isoflavone on blood pressure and serum levels of insulin-like growth factor-1 in normotensive and hypertensive volunteers with alopecia. *Biosci Biotechnol Biochem*. 2009;73(6):1456–1459.
25. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy and substrate utilization, hormones, and satiety. *Eur J Nutr*. 2009;48(4):229–234.
26. Ahuja KD, Robertson IK, Geraghty DP, Ball MJ. Effects of chili consumption on postprandial glucose, insulin, and energy metabolism. *Am J Clin Nutr*. 2006;84(1):63–69.
27. Yuan LJ, Qin Y, Wang L, et al. Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia, and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns. *Clin Nutr*. 2016;35(2):388–393.
28. Sun F, Xiong S, Zhu Z. Dietary capsaicin protects cardiometabolic organs from dysfunction. *Nutrients*. 2016;8(5):E174.
29. Wang HM, Chuang SM, Su YC, Li YH, Chueh PJ. Down-regulation of tumor associated NADH oxidase, tNOX (ENOX2), enhances capsaicin-induced inhibition of gastric cancer cell growth. *Cell Biochem Biophys*. 2011;61(2):355–366.
30. Lin MH, Lee YH, Cheng HL, et al. Capsaicin inhibits multiple bladder cancer cell phenotypes by inhibiting tumor-associated NADH oxidase (tNOX) and Sirtuin1 (SIRT1). *Molecules*. 2016;21(7):E849.
31. Pramanik KC, Boreddy SR, Srivastava SK. Role of mitochondrial electron transport chain complexes in capsaicin mediated oxidative stress leading to apoptosis in pancreatic cancer cells. *PLoS One*. 2011;6(5):e20151.
32. Lee YS, Kwon EJ, Jin DQ, et al. Redox status-dependent regulation of cyclooxygenases mediates the capsaicin-induced apoptosis in human neuroblastoma cells. *J Environ Pathol Toxicol Oncol*. 2002;21(2):113–120.
33. Lee YS, Kang YS, Lee JS, Nicolova S, Kim JA. Involvement of NADPH oxidase-mediated generation of reactive oxygen species in the apoptotic cell death by capsaicin in HepG2 human hepatoma cells. *Free Radical Res*. 2004;38(4):405–412.
34. Kim MY, Trudel LJ, Wogan GN. Apoptosis induced by capsaicin and resveratrol in colon carcinoma cells requires nitric oxide production and caspase activation. *Anticancer Res*. 2009;29(10):3733–3740.
35. Bhutani M, Pathak AK, Nair AS, et al. Capsaicin is a novel blocker of constitutive and interleukin-6-inducible STAT3 activation. *Clin Cancer Res*. 2007;13(10):3024–3032.
36. Amantini C, Ballarini P, Caprodossi S, et al. Triggering of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner. *Carcinogenesis*. 2009;30(8):1320–1329.
37. Li H, Krstin S, Wang S, Wink M. Capsaicin and piperine can overcome multidrug resistance in cancer cells to doxorubicin. *Molecules*. 2018;23(3):E557.
38. Bort A, Spinola E, Rodriguez-Henche N, Diaz-Laviada I. Capsaicin exerts synergistic antitumor effect with soafenib in hepatocellular carcinoma cells through AMPK activation. *Oncotarget*. 2017;8(50):87684–87698.
39. Arzuman L, Beale P, Yu JQ, Huq F. Synthesis of tris(quinoline)monochloroplatinum(II) chloride and its activity alone and in combination with capsaicin and curcumin in human ovarian cancer cell lines. *Anticancer Res*. 2016;36(6):2809–2818.
40. Khan M, Maryam A, Mehmood T, Zhang Y, Ma T. Enhancing activity of anticancer drugs in multidrug resistant tumors by modulating P-glycoprotein through dietary nutraceuticals. *Asian Pac J Cancer Prev*. 2015;16(16):6831–6839.
41. Peikert A, Hentrich M, Ochs G. Topical 0.025% capsaicin in chronic post-herpetic neuralgia: efficacy, predictors of response and long-term course. *J Neurol*. 1991;238:452–456.
42. Bernstein JE, Bickers DR, Dahl MV, et al. Treatment of chronic postherpetic neuralgia with topical capsaicin. A preliminary study. *J Am Acad Dermatol*. 1987;17:93–96.
43. Petersen KL, Fields HL, Brennum J, et al. Capsaicin evoked pain and allodynia in post-herpetic neuralgia. *Pain*. 2000;88:125–133.
44. Irving GA, Backonja MM, Dunteman E, et al. A multicenter, randomized, double-blind, controlled study of NGX-4010, a high-concentration capsaicin patch, for the treatment of postherpetic neuralgia. *Pain Med*. 2011;12(1):99–109.
45. Backonja MM, Malan TP, Vanhove GF, Tobias JK. C102/106 study group. *Pain Med*. 2010;11(4):600–608.
46. Fusco BM, Alessandri M. Analgesic effect of capsaicin in idiopathic trigeminal neuralgia. *Anesth Analg*. 1992;74:375–377.
47. Watson CP, Evans RJ. The postmastectomy pain syndrome and topical capsaicin: a randomized trial. *Pain*. 1992;51:375–379.

48. Watson CP, Evans RJ, Watt VR. The post-mastectomy pain syndrome and the effect of topical capsaicin. *Pain*. 1989;38:177–186.
49. Nelson C. Heal the burn: pepper and lasers in cancer pain therapy. *J Natl Cancer Inst*. 1994;86:1381–1382.
50. The Capsaicin Study Group. Effect of treatment with capsaicin on daily activities of patients with painful diabetic neuropathy. *Diabetes Care*. 1992;15:159–165.
51. Tandan R, Lewis GA, Krusinski PB, et al. Topical capsaicin in painful diabetic neuropathy. Controlled study with long-term follow-up. *Diabetes Care*. 1992;15:8–14.
52. Tandan R, Lewis GA, Badger GB, et al. Topical capsaicin in painful diabetic neuropathy. Effect on sensory function. *Diabetes Care*. 1992;15:15–18.
53. Basha KM, Whitehouse FW. Capsaicin: a therapeutic option for painful diabetic neuropathy. *Henry Ford Hosp Med J*. 1991;39:138–140.
54. Pfeifer MA, Ross DR, Schrage JP, et al. A highly successful and novel model for treatment of chronic painful diabetic peripheral neuropathy. *Diabetes Care*. 1993;16:1103–1115.
55. Forst T, Pohlmann T, Kunt T, et al. The influence of local capsaicin treatment on small nerve fibre function and neurovascular control in symptomatic diabetic neuropathy. *Acta Diabetol*. 2002;39:1–6.
56. Biesbroeck R, Bril V, Hollander P, et al. A double-blind comparison of topical capsaicin and oral amitriptyline in painful diabetic neuropathy. *Adv Ther*. 1995;12:111–120.
57. Simpson DM, Robinson-Papp J, Van J, et al. Capsaicin 8% patch in painful diabetic peripheral neuropathy: a randomized, double-blind, placebo-controlled study. *J Pain*. 2017;18(1):42–53.
58. Haanpaa M, Cruccu G, Nurmikko TJ, et al. Capsaicin 8% patch versus oral pregabalin in patients with peripheral neuropathic pain. *Eur J Pain (London, England)*. 2016;20(2):316–328.
59. Malhofner CG, Heskamp ML. Treatment of peripheral neuropathic pain by topical capsaicin: impact of pre-existing pain in the QUEPP-study. *Eur J Pain (London, England)*. 2014;18(5):671–679.
60. Galvez R, Navez ML, Moyle G, et al. *Clin J Pain*. 2017;33(10):921–931.
61. Mankowski C, Poole CD, Ernault E, et al. Effectiveness of the capsaicin 8% patch in the management of peripheral neuropathic pain in European clinical practice: the ASCEND study. *BMC Neurol*. 2017;17(1):80.
62. Moon JY, Lee PB, Kim YC, Lee SC, Nahm FS, Choi E. Efficacy and safety of 0.625% and 1.25% capsaicin patch in peripheral neuropathic pain: multi-center, randomized, and semi-double blind controlled study. *Pain Physician*. 2017;20(2):27–35.
63. Marks DR, Rapoport A, Padla D, et al. A double-blind placebo-controlled trial of intranasal capsaicin for cluster headache. *Cephalalgia*. 1993;13:114–116.
64. McCarthy GM, McCarty DJ. Effect of topical capsaicin in the therapy of painful osteoarthritis of the hands. *J Rheumatol*. 1992;19:604–607.
65. Deal CL, Schnitzer TJ, Lipstein E, et al. Treatment of arthritis with topical capsaicin: a double-blind trial. *Clin Ther*. 1991;13:383–395.
66. Bernstein JE, Parish LC, Rapaport M, et al. Effects of topically applied capsaicin on moderate and severe psoriasis vulgaris. *J Am Acad Dermatol*. 1986;15:504–507.
67. Ellis CN, Berberian B, Sulica VI, et al. A double-blind evaluation of topical capsaicin in pruritic psoriasis. *J Am Acad Dermatol*. 1993;29:438–442.
68. Lysy J, Sistiery-Ittah M, Israelit Y, et al. Topical capsaicin—a novel and effective treatment for idiopathic intractable pruritus ani: a randomised, placebo controlled, crossover study. *Gut*. 2003;52:1323–1326.
69. Gupta PJ. Red hot chili consumption is harmful in patients operated for anal fissure—a randomized, double-blind, controlled study. *Dig Surg*. 2007;24:354–357.
70. Gupta PJ. Effect of red chili consumption on postoperative symptoms during the post-hemorrhoidectomy period: randomized, double-blind, controlled study. *World J Surg*. 2007;31:1822–1826.

63

Carnitine

Angela Sadlon, ND, and *Michael T. Murray, ND*

OUTLINE

INTRODUCTION

Carnitine is an essential nutrient for the transport of long-chain fatty acids into the mitochondrial matrix. Carnitine (β-hydroxy/γ-butyrobetaine) was originally isolated from meat extracts in 1905, and its exact chemical structure was determined in 1932 (Fig. 63.1). However, despite extensive physiological and pharmacological studies in the 1930s, no physiological role for carnitine was determined until 1955.[1–3]

The compound was virtually forgotten until Carter et al.[4] created new interest in carnitine in 1952 when they established it as a growth factor for the mealworm *Tenebrio molitor* (hence carnitine's other name, "vitamin BT"—*B* for the family of vitamins and *T* for *T. molitor*). When other species of organisms were also shown to be dependent on carnitine, researchers began to reexamine its role in humans.

Researchers soon found that carnitine was essential in the oxidation of lipids.[1–3] When the first carnitine-deficient human subjects were described in 1973, it stimulated greater investigation.[5]

BIOSYNTHESIS

Carnitine is synthesized in humans from lysine and methionine. In nonmammalians, carnitine synthesis begins with stepwise methylation of free lysine by S-adenosylmethionine to produce trimethyllysine. In mammals, however, protein-bound trimethyllysine, rather than free lysine, appears to be the major precursor for carnitine synthesis.[1–3]

Trimethyllysine is then converted through a series of enzymatic reactions (occurring in the liver, kidney, brain, heart, and skeletal muscle) to butyrobetaine. However, the conversion of butyrobetaine to carnitine can only occur in the liver, kidney, and brain because the enzyme required, butyrobetaine hydroxylase, is only present in these tissues.[1–3]

The synthesis of carnitine is largely controlled by the activity of butyrobetaine hydroxylase. This enzyme appears to be age dependent. In infancy, the activity of butyrobetaine hydroxylase has been shown to be only 12% of the normal adult mean. By 2.5 years, the activity is 30% of the adult mean, and by 15 years, the level is within a standard deviation of the adult mean.[1–3] These data would seem to indicate the importance of preformed carnitine in breast milk.

As apparent from Fig. 63.2, two essential amino acids (lysine and methionine), three vitamins (ascorbate, niacin, and vitamin B_6), and a metal ion (reduced iron) are required for the synthesis of carnitine. Obviously, a deficiency of any one of these nutrients would result in significantly impaired carnitine synthesis.[1–3] However, research using knockout mice unable to synthesize vitamin C endogenously showed normal carnitine values (comparable to their vitamin C–synthesizing counterparts), although they were vitamin C deficient. The researchers suggested that adequate levels of glutathione and glutathione peroxidase might compensate for or replace the deficient vitamin C in the carnitine biosynthesis pathway.[6]

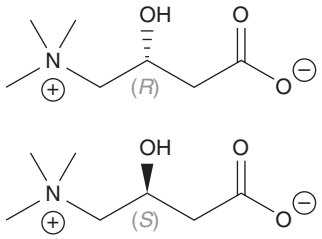


FIG. 63.1 Carnitine in both racemic forms.

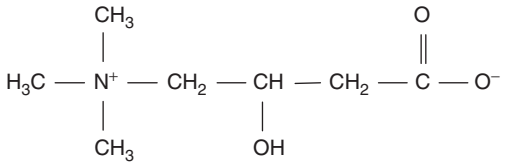


FIG. 63.2 Biosynthesis of carnitine.

METABOLISM

Pharmacokinetics

The heart and skeletal muscles, as well as many other tissues, depend primarily on fatty acid oxidation as a source of energy. Because they cannot synthesize carnitine, its transport into these tissues is of critical importance.

Specific carnitine-binding transport proteins were identified for several tissues (e.g., cardiac muscle, skeletal muscle, epididymis, liver, kidney) taking the serum carnitine into the cell.[1,3] Through this active transport mechanism, the tissues can concentrate carnitine up to 10 times greater than the concentration found in plasma.

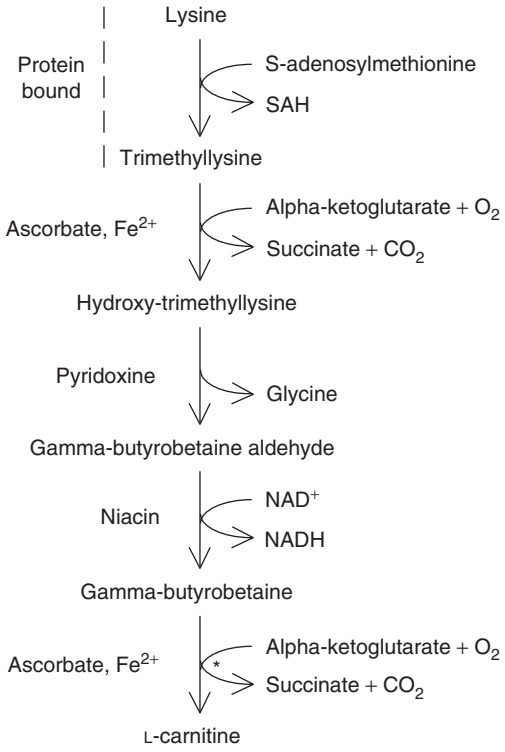
A small in vivo study measured the pharmacokinetics of carnitine in Chinese men and women participants. A 2000-mg single oral dose of L-carnitine (LC; Fig. 63.3) had a half-life of approximately 60.3 hours compared with L-acetylcarnitine (LAC) at 35.9 hours and L-propionylcarnitine (LPC) at 25.7 hours. The maximum plasma concentration of LC, LAC, and LPC occurred at 3.4, 2.4, and 3.8 hours after ingestion, respectively, and at quantities of 84.7, 12.9, and 5.1 µmol/L, respectively. The researchers found comparable levels of carnitine absorption and metabolism between men and women, suggesting that gender should not affect dosage.[7]

Urinary excretion of unchanged carnitine is the major route of elimination of carnitine. Because the tubular reabsorption of carnitine by the kidneys is extremely efficient, the daily turnover of carnitine is estimated to be only 4% to 6% of the total body pool of the healthy individual.[1–3,7] Factors that increase carnitine excretion and degradation are discussed later in the section on deficiency.

PHYSIOLOGICAL FUNCTIONS

Carnitine's basic function is in the transport of long-chain fatty acids into the mitochondrial matrix and the facilitation of β-oxidation.[1,3] Because acyl-coenzyme A formed in the endoplasmic reticulum or outer mitochondrial membrane cannot penetrate the inner mitochondrial membrane to the site of fatty acid β-oxidation, the acyl group must be transferred from coenzyme A to carnitine. The acyl-carnitine molecule then transports the fatty acid molecule to the mitochondrial surface of the inner mitochondrial membrane and releases the fatty acid into the matrix, where β-oxidation occurs. Fig. 63.4 summarizes this process.

Carnitine has several other physiological functions, including oxidation of the ketoacid analogs of the branched-chain amino acids valine, leucine, and isoleucine.[1–3] This function is extremely important during fasting, starvation, and exercise.



*In humans, the enzyme catalyzing this reaction occurs only in the liver, kidney, and brain

FIG. 63.3 L-carnitine.

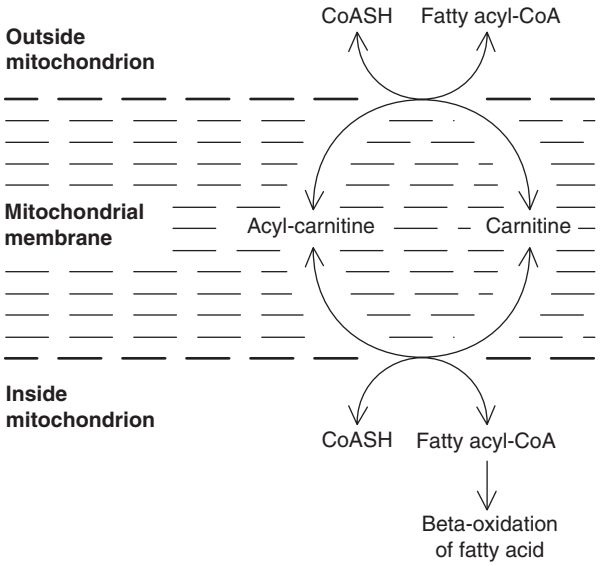


FIG. 63.4 Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane.

DEFICIENCY

Carnitine deficiency may arise from several causes, as listed in Box 63.1.

Carnitine deficiency states have been classified into two major groups:

- Systemic carnitine deficiency
- Myopathic deficiency

Diagnosis of systemic carnitine deficiency can be made using serum or 24-hour urine samples. Total, free, and esterified carnitine levels should be determined. In myopathic carnitine deficiency, diagnosis requires skeletal muscle biopsy.[8]

BOX 63.1 Causes of Carnitine Deficiency

- Dietary deficiency of the precursor amino acids lysine and methionine
- Deficiency of any cofactor (e.g., iron, ascorbic acid, pyridoxine, niacin) required by the enzymes of the lysine to carnitine pathway
- Genetic defect of carnitine biosynthesis
- Defective intestinal absorption of carnitine
- Liver or kidney dysfunction that impairs carnitine synthesis
- Increased metabolic losses of carnitine due to catabolism, impaired tubular resorption, or genetic defect
- Defective transport of carnitine from tissues of synthesis to tissues where it is maximally used
- Increased carnitine requirement due to a high-fat diet, drugs (e.g., valproic acid), metabolic stress, or disease

Until the late 1990s, there were no documented reports of patients with primary systemic carnitine deficiency. The systemic deficiency has always been secondary to some other factor rather than a defect in carnitine synthesis.[1–3,8,9] However, mutations in the organic cation/carnitine transporter gene *(OCTN2)* were identified in an infant. The infant presented with Reye-like symptoms including encephalopathy, hypoketotic hypoglycemia, elevated liver enzymes, and steatosis. Treatment with intravenous carnitine and then oral LC (200 mg/kg/day) produced a rapid recovery.[10] There are at least a half dozen more papers since then referencing the *OCTN2* gene mutation producing primary carnitine deficiency.

The consequences of systemic carnitine deficiency are impaired lipid metabolism and lipid accumulation in the skeletal muscles, myocardium, and liver. Progressive muscle weakness with lipid storage myopathy is found in all patients.[1–3,8] In adults, auxiliary nonmitochondrial oxidation mechanisms are apparently stimulated, resulting in some degree of adaptation. This adaptation occurs in starvation, diabetes, high-fat diets, and other causes of secondary carnitine deficiency. Systemic carnitine deficiencies usually respond dramatically to orally administered supplemental LC.[8,9]

Children are apparently unable to adapt to low carnitine levels as well as adults can.[8] Several cases of carnitine deficiency in children, presenting a clinical picture resembling Reye's syndrome (acute encephalopathy associated with altered liver function due to lipid accumulation), have been reported.[9,11,12]

The clinical presentation of secondary carnitine deficiency in children includes hypotonia, failure to thrive, recurrent infections, encephalopathy, nonketotic hypoglycemia, and cardiomyopathy.[8] Several fatal cases of systemic carnitine deficiency have been reported.[9,13]

In primary myopathic carnitine deficiency, there is an inborn error of carnitine metabolism that is limited to skeletal muscle.[8,9] The defect appears to be in the transport of carnitine into the skeletal muscle as serum carnitine; the carnitine levels in other tissues are normal. Severe lipid storage myopathy is the result. Supplemental carnitine is generally of no value in myopathic carnitine deficiency. Rather, improvements have been noted using diets high in medium-chain triglycerides and low in long-chain triglycerides.[8]

CARNITINE AS A NUTRIENT

Analysis of several hundred foods for carnitine content indicates that meat and dairy products are the major dietary sources of carnitine.[3] In general, the redder the meat, the higher the carnitine content. Cereals, fruits, and vegetables contain little or no carnitine. Preliminary studies indicate that the daily diet contains 5 to 100 mg of carnitine.[3] There is no evidence that a vegetarian or vegan diet promotes deficiency and would need supplementation. As long as the liver and kidney are able to synthesize carnitine from lysine and methionine, deficiency of carnitine typically does not occur.

Carnitine in the Infant Diet

Oxidation of long-chain fatty acids, which requires carnitine, is well known to be critical to the survival and normal development of the newborn.[3] Carnitine concentrations in fetal and umbilical cord blood are higher than in maternal blood, suggesting the placenta may actively transport carnitine to the fetus because carnitine synthesis is not fully developed.[3] The initial carnitine concentration in the newborn depends on maternal carnitine concentration.

Supplementation of carnitine during pregnancy may be necessary to ensure adequate tissue concentrations in the fetus, as well as the mother. Serum carnitine levels are typically lower in pregnant women than nonpregnant women, presumably due to increased excretion.[14,15]

The newborn infant is almost entirely dependent on external sources of carnitine.[3] Breastfed infants have the best chance of achieving optimal carnitine concentrations. The bioavailability of carnitine from breast milk is significantly greater than that in cow's milk–based formulas,[16] and soy-based infant formulas contain no detectable carnitine.[3] Formula feeding may necessitate supplemental carnitine to achieve normal carnitine concentrations in these infants.

Carnitine administration to preterm infants has potentiated weight gain and growth.[17] In preterm infants, serum values of carnitine decrease dramatically due to limited storage capacity coupled with a decreased ability to synthesize carnitine. Administration of LC to preterm infants is considered important.

CLINICAL APPLICATIONS

Many disease states, in addition to classic as well as secondary carnitine deficiency, may benefit from carnitine administration. Evidence supports the assertion that supplemental carnitine may benefit the conditions listed in Box 63.2 and discussed later.

Carnitine is available in several different forms. The form being used must be LC alone or bound to either acetic (LAC) or propionic acid (LPC). The D form of carnitine (discussed later in the section on "Toxicology") should never be used. The reason for using carnitine will dictate the form used. For Alzheimer's disease and brain and neurological effects, it appears that LAC may provide the greatest benefit. For angina, ulcerative colitis, and wound healing, LPC may be the best choice because the myocardium appears to prefer it to LAC, followed by LC.[18,19]

Cardiovascular Disease

Normal heart function is critically dependent on adequate concentrations of carnitine. A deficiency of carnitine in the heart would be similar to trying to run an automobile without a fuel pump. Despite plenty of fuel, there is no way to get it to the engine. Although the normal heart stores more carnitine than it needs, if the heart does not have a good supply of oxygen, carnitine levels quickly decrease. This lack of oxygen leads to decreased energy production in the heart and increased risk for angina and heart disease.

Oral LAC therapy (1 g twice daily) for 6 months showed a gradual but significant decrease in blood pressure in insulin-resistant participants who had higher risk factors for cardiovascular disease (hypertension, hypertriglyceridemia, obesity, and/or family history of diabetes type 2). In the patients with greater insulin resistance, the insulin sensitivity was significantly increased. The overall adiponectin values increased. These benefits were slowly reversed during an 8-week follow-up after LAC discontinuation. There were no changes to participants' diet or lifestyle during this study. LAC was well tolerated in all participants.[20] It appears that LAC can improve aspects that are characteristic of metabolic syndrome, but without lifestyle and dietary modifications, the patient may potentially need long-term supplementation with carnitine.

Carnitine exerts a beneficial effect on blood lipids by lowering triglycerides (TGs) and total cholesterol levels while raising high-density

BOX 63.2 Conditions That May Benefit From Carnitine Supplementation

- Acquired immunodeficiency syndrome
- Acute myocardial infarction
- Alcohol-induced fatty liver disease
- Alzheimer's disease, senile depression, and age-related memory defects
- Androgenetic alopecia
- Angina pectoris
- Arrhythmias and cardiotoxicity induced by drugs
- Beta thalassemia major
- Cardiac myopathy
- Cardiovascular diseases
- Celiac disease—fatigue
- Chronic obstructive pulmonary disease
- Congestive heart failure
- Depression
- Diabetes
- Down syndrome—visual memory and attention
- Elevated cholesterol levels
- Elevated triglyceride levels
- Enhancing physical performance
- Familial endocardial fibroelastosis
- Glutaric aciduria
- Hepatic cirrhosis—muscle cramps
- Hyperthyroidism
- Idiopathic mitral valve prolapse
- Inborn errors of amino acid metabolism
- Isovaleric acidemia
- Kidney disease and hemodialysis
- Liver cirrhosis
- Liver diseases
- Low sperm counts and decreased sperm motility
- Methylmalonic aciduria
- Muscular dystrophies
- Myocardial necrosis
- Neuropathy
- Organic aciduria
- Propionic acidemia
- Toxicity from various drugs
- Ulcerative colitis
- Wound healing

lipoprotein cholesterol (HDL-c). After 4 months of therapy with LC in patients with elevated blood lipids, typical changes observed were a 20% reduction for total cholesterol, a 28% decrease in TGs, and a 12% increase in HDL levels.[21,22] Due to the higher cost of carnitine compared with other natural agents (e.g., inositol hexaniacinate, garlic, berberine, and guggulipid), its use should be reserved for those cases unresponsive to these more cost-effective measures.

A study comparing LC (2 grams/day) with simvastatin (20mg/day) or simvastatin alone was conducted on patients with hyperlipidemia and elevated lipoprotein (a) (Lp[a]) levels. The results showed a significant reduction in Lp(a) with the combined therapy (LC + statin) versus simvastatin monotherapy. Both treatment groups lowered total cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B, and TGs significantly.[23]

Angina and Recovery From Myocardial Infarction

Carnitine is useful in angina due to its ability to improve oxygen usage and energy metabolism by the myocardium. As a result of improving fatty acid usage and energy production, carnitine also prevents the production of toxic fatty acid metabolites.[24] These compounds are extremely damaging because they disrupt cellular membranes. Changes in the properties of cell membranes throughout the heart are thought to contribute to impaired contraction of the heart muscle, increased susceptibility to irregular beats, and eventual death of heart tissue. Supplementing with carnitine increases heart carnitine levels and has been shown to prevent the production of toxic fatty acid metabolites, as well as boost antioxidant enzyme levels. In addition to angina, all of these effects make carnitine beneficial in recovery from a heart attack, cardiomyopathies, arrhythmias, and congestive heart failure.[25,26]

Numerous clinical trials demonstrated that carnitine improves angina and heart disease (note that all three commercial forms have been used).[25,27–34] Improvements were noted in exercise tolerance and heart function. The results indicated that carnitine is an effective alternative to drugs in cases of angina.

LPC may offer the greatest benefit in angina, as well as in other cardiovascular conditions. LPC is taken up by myocardial cells much more rapidly than other forms of carnitine.[18] In one study, LPC (15 mg/kg intravenously) significantly diminished myocardial ischemia as demonstrated by a significant 12% and 50% reduction in ST-segment depression and left ventricular end-diastolic pressure, respectively, during the atrial pacing test.[35] Left ventricular ejection fraction increased by 18%. Recovery of heart function after exercise occurred much quicker in the LPC group compared with the placebo group.

LC and LAC also showed good results. In one of the larger studies, 200 patients with exercise-induced stable angina received either standard therapy alone (e.g., nitroglycerin, calcium channel blockers, β-blockers, antihypertensives, diuretics, digitalis, antiarrhythmics, anticoagulants, hypolipidemics) or in combination with 2000 mg/day of LC over a 6-month period.[36] Compared with the control group, the patients on LC exhibited a significant reduction in premature ventricular contractions at rest, as well as an increased tolerance to exercise as demonstrated by an increased maximal cardiac frequency, increased maximal systolic blood pressure, cardiac output, and reduced ST-segment depression (70% reduction in the LC group versus no change in the control group). Reductions in LDL-c (8%) and TGs (12%) were also noted. These results are highly significant and provide a strong rationale for the inclusion of carnitine in patients using standard medical therapy.

In Italy, a large study involving 472 patients showed additional benefits.[37] The study was performed to evaluate the effects of LC administration on chronic left ventricular dilation in patients with acute anterior myocardial infarction. Placebo or LC was given at a dose of 9 g/day intravenously for the first 5 days and then 6 g/day orally for the next 12 months. Left ventricular volumes and ejection fraction were evaluated on admission, at discharge from hospital, and at 3, 6, and 12 months after acute myocardial infarction. A significant attenuation of left ventricular dilation in the first year after acute myocardial infarction was observed in patients treated with LC compared with those receiving placebo. The percent increase in both end-diastolic and end-systolic volumes from admission to 3-, 6-, and 12-month evaluation was significantly reduced in the LC group.

Congestive Heart Failure

Several double-blind clinical studies showed that carnitine (again, LPC appeared to be more effective than LC or LAC) improved cardiac function in patients with congestive heart failure.[25,38,39] In one double-blind study of LPC versus placebo in a group of 60 patients with mild to moderate (New York Heart Association [NYHA] classes II and III) congestive heart failure, LPC produced demonstrable benefit.[38] The group was made up of men and women between 48 and 73 years old

receiving long-term treatment with digitalis and diuretics for at least 3 months and who still displayed symptoms. Thirty of these patients were chosen randomly and for 180 days received 500 mg of LPC three times a day in addition to their usual treatment. At basal conditions and after 30, 90, and 180 days, the maximum exercise time was evaluated using an exercise tolerance test performed on an ergometer bicycle, and the left ventricular ejection fraction was tested by echocardiography. After 1 month of treatment, the patients treated with LPC, compared with the control group, showed significant increases in the values of both tests, increases that became even more evident after 90 and 180 days. At the stated times, the increases in the maximum exercise time were 16.4%, 22.9%, and 25.9%, respectively. The ventricular ejection fraction increased by 8.4%, 11.6%, and 13.6%, respectively.

Even more obvious benefits were seen in a 3-year study of 80 patients with moderate to severe heart failure (NYHA class III to IV) caused by dilated cardiomyopathy. After a period of stable cardiac function of up to 3 months, patients were randomly assigned to receive either carnitine (2 g/day orally) or placebo. After a mean of 33.7 months of follow-up (range 10–54 months), 70 patients remained in the study: 33 in the placebo group and 37 in the carnitine group. At the time of analysis, 63 patients were alive. Six deaths occurred in the placebo group, and one death in the carnitine group. Survival analysis showed that patients' survival was statistically significant in favor of the carnitine group.[40]

Peripheral Vascular Disease

All three forms of carnitine (2–4 g/day) were shown to improve the walking distance without pain in patients with intermittent claudication. Presumably this improvement was the result of improved energy metabolism within the muscle because carnitine was not shown to improve blood flow to the calf. LPC appeared to offer better effects than either LC or LAC.[41,42] However, in one double-blind study, LC at a dosage of 2 g twice daily demonstrated a 75% increase in walking distance after only 3 weeks of therapy.[43]

In the largest study with LPC, 485 patients with intermittent claudication were randomized to placebo or LPC (2 g/day) for 12 months. Maximal walking distance increased by 62% on LPC and by 46% on placebo in all patients. However, when only those patients with more severe disease status were analyzed, the maximal walking distance increased by 98% in the LPC group compared with only 54% in the placebo group.[44]

Enhancing Physical Performance and Relieving Fatigue

The ability to enhance exercise tolerance and physical performance with carnitine may not be limited to patients with cardiovascular disease because carnitine supplementation was also shown to be of benefit in healthy subjects and athletes. Efficient use of fatty acids by skeletal muscle, like the myocardium, also depends on an adequate supply of carnitine.

Carnitine supplementation (usually 2 g two to three times daily) resulted in significant improvements in cardiovascular function in response to exercise in several double-blind studies in both athletes and normal subjects.[45–47] Compared with control groups, the subjects on carnitine showed not only improvements in exercise intensity over time but also evidence of improved energy metabolism within the muscle (lowered blood lactic acid and free fatty acid levels). Obviously, the improved production of energy by the exercising muscle, as well as improved heart function, could be responsible for carnitine's ability to enhance physical performance.

Although at least three studies showed the benefits of carnitine on exercise performance to be of no more value than a placebo, carnitine supplementation should still be viewed as beneficial, especially in endurance-related events.[48–50] The reason behind this statement is the fact that studies demonstrated that carnitine improves energy-producing enzyme levels in long-distance runners.[51] These athletes received either a placebo or 2 g of LC twice daily for 4 weeks. Runners receiving LC showed a significant increase in enzymes involved in energy production (cytochrome C reductase and cytochrome oxidase). In contrast, there were no changes in the placebo group.

One study examined the influence of L-carnitine L-tartrate (LCLT) at a dosage of 2 g/day for 3 weeks on markers of purine catabolism, free radical formation, and muscle tissue disruption after squat exercise. Exercise-induced increases in plasma markers of purine catabolism (hypoxanthine, xanthine oxidase, and serum uric acid) and circulating cytosolic proteins (myoglobin, fatty acid-binding protein, and creatine kinase) were significantly reduced by LCLT supplementation. Exercise-induced increases in plasma malondialdehyde returned to resting values sooner during LCLT therapy compared with placebo. The amount of muscle disruption from magnetic resonance imaging scans during LCLT was 41% to 45% of the placebo area. These data indicated that LCLT supplementation was effective in assisting recovery from exercise.[52]

Interestingly, normal subjects taking carnitine have improved cardiovascular function and a more rapid return of heart rate to the resting rate after exercise.[53] The significance of these improvements is that carnitine apparently mimics the benefits in heart and vascular function produced by regular exercise training without working up a sweat.

Carnitine supplementation appeared to be beneficial in elderly subjects complaining of muscle fatigue.[54,55] In one double-blind study, 84 elderly subjects with onset of fatigue after even the slightest physical activity were randomized to receive carnitine 2 g twice daily or a placebo for 30 days. Fatigue scores decreased significantly by 40% (physical) and 45% (mental) in subjects taking carnitine, compared with 11% and 8%, respectively, in the placebo group. Other parameters improved significantly, including total fat mass, total muscle mass, total cholesterol, LDL cholesterol, HDL cholesterol, TGs, apolipoprotein-A1, and apolipoprotein-B.[54] In a similar study, researchers reported 2 g/day of LC given to centenarians had a comparable trend when looking at similar parameters.[55] The difference was 2 g twice daily showed greater improvement in most parameters measured.

Few clinical trials have been published regarding carnitine and fatigue relief in multiple sclerosis patients. Some studies reported significant benefit,[56,57] whereas another suggested better designed studies were needed to determine efficacy.[58] Because LC is well tolerated, it may be beneficial to try.

Cancer-Related Fatigue

A notable percentage of cancer patients are deficient in carnitine (serum total and free carnitine levels). Researchers have given a wide dosage range of LC and measured subjective results of fatigue with mixed results.[59–63]

An open-label, Phase I/II clinical trial of LC supplementation in carnitine deficient patients with advanced cancer was done. The results showed significant improvement in free carnitine levels. There were 27 patients and 7 treatment groups (250 mg/d, 750 mg/d, 1250 mg/d, 1750 mg/d, 2250 mg/d, 2750 mg/d, and 3000 mg/d) for 7 days. After the trial, all patients but two requested to continue treatment. The results showed "most of the patients who received LC experienced reduced fatigue and improved mood and sleep. The improvement in fatigue was dose dependent within the subgroup that received 7 days of supplementation and experienced a rise in carnitine-associated laboratory values."[60]

A small 2018 study used 500 mg three time daily for 8 weeks in cancer patients undergoing chemotherapy. There was improved

general fatigue in all cancer patients, and LC possibly improved the ability of cancer patient to cope with chemotherapy. Also, LC seemed to maintain the plasma levels and lymphocyte counts during chemotherapy, enabling patients to continue chemotherapy without dosage reduction.[63]

Another small study of LC supplementation in patients with advanced cancer and carnitine deficiency found no significant change in fatigue after 2 weeks. The patients were given a titrating dosage of LC (500 mg/day for 2 days, then 1 g/day for 2 days, then 2 g/day for 10 days). All carnitine deficiency normalized. The patients were then included in another 2-week open-label phase, and the patients who started the LC supplementation group reported significant improvement in fatigue, functional well-being, and performance status compared with the original placebo patients. Also, after the trial was over, 12 of 17 patients requested to stay on carnitine.[61]

Celiac Disease

Celiac disease can be considered a secondary cause of carnitine deficiency due to the characteristic malabsorption component.[64] In a small double-blind, placebo-controlled study ($n = 47$), tests were performed on participants with newly diagnosed celiac disease before and after 6 months of either a gluten-free diet (placebo) or a gluten-free diet plus LC (1 g twice daily; treatment group). Serum carnitine levels were significantly improved from baseline in both groups after implementing a gluten-free diet. In the treatment group, serum levels were comparable to the nonceliac control group. The LC group had significant improvement in fatigue compared with the placebo group as measured by the visual analog scale. The measurement of OCTN2 (cell membrane transport protein with strong specificity for carnitine) by intestinal biopsies increased by 83% in all celiac patients on a gluten-free diet; however, the amount was still significantly less than that in the nonceliac controls.[65]

Even with asymptomatic celiac disease, the individual breakdown of the serum carnitine esters may be significantly decreased, with LAC sometimes decreased by 50% to 80%.[66]

Ulcerative Colitis

Patients with ulcerative colitis (UC) showed similar levels of plasma LC compared with healthy controls; however, the levels of LPC were significantly lower.[66] Because short-chain fatty acids are the major fuel substrate for enterocytes and are theorized to be less available in UC, LPC has been studied as a potential treatment.[68–70]

One study of 10 males with mild UC used LPC enemas (6 g in 200 mL saline solution) twice daily for 14 days. Each enema took 120 minutes. Significant improvement was noted in all patients, both symptom-wise (abdominal pain, diarrhea, and/or mucus or blood in stool) and histologically (mucosal erosions, distortion of crypt architecture, epithelium inflammation, and interstitial inflammation). No adverse side effects were reported.[68]

In a multicenter, Phase II, double-blind, parallel-group trial of mild to moderate UC, patients using stable oral aminosalicylate or thiopurine therapy were also given oral LPC for 14 days. The group receiving 500 mg twice daily (1 hour before breakfast and dinner) produced the best effect. Seventy-five percent of patients saw improvement, and 55% had remission of disease, with the majority having mild UC.[69] A 2014 study used patients from the latter study to perform microscopical analysis of intestinal biopsies before PLC and after 4 weeks. The results showed a reduction of intestinal acute and chronic inflammation and amelioration of the damaged microvascular endothelium. The higher oxidative stress in UC was presumably from the intestinal microvascular endothelial dysfunction. The antioxidant effect of LPC mediates the reduction of inflammation.[70]

Thyroid Disease

L-carnitine's effect on thyroid hormone and its benefit in hyperthyroidism are reported in the literature as early as 1966 by Gilgore and De Felice.[71,72]

Since 1966, more researchers studied the benefit of LC on hyperthyroidism, regardless of the cause.[71–78] According to researchers, LC does not affect the thyroid gland itself; its action takes place in the peripheral cells. It inhibits thyroid hormone entry into the cell nucleus.[73,78] This may explain why patient symptoms (palpitations, tremors, nervousness, etc.) improve significantly, but the blood levels continue to be elevated. Dosages of oral LC ranged from 500 to 4000 mg daily.

In one study of 19 patients with subclinical hyperthyroidism (thyroid-stimulating hormone [TSH] = 0.1–0.4 mIU/L and positive antibodies), subjects took a combined daily oral dosage of 500 mg LC and 83 mcg selenium for 1 month. The results showed a drop in the score on a 9-item symptom scale from 25.61 ± 1.19 to 12.11 ± 1.15. The symptoms most improved included palpitations, tremor, and nervousness. However, after discontinuing the therapy, the symptom score increased to 23.33 ± 1.35 after 1 month. There were no significant changes in the TSH, free T3, or free T4 values throughout the study. The thyroid antibodies (TgAb and TPO Ab) had a drop of at least 100 points after 1 month of treatment and returned to pretreatment levels after discontinuing the treatment.[77]

L-carnitine prevented and reversed liver enzyme (alkaline phosphatase [ALP], aspartate transaminase [AST], alanine transaminase [ALT]) damage produced by the elevated thyroid hormone.[75]

L-carnitine was administered intravenously (75 mg/kg/d) to an 80-year-old man for severe hyperthyroidism with coma, induced by amiodarone. His Glasgow score was 8. The patient fully recovered 24 hours after starting intravenous (IV) LC administration. Even though he was asymptomatic, the thyroid profile was still classified as hyperthyroid. L-carnitine was administered for 2 months along with propylthiouracil and avlocardyl. The major improvement occurred when LC was administered.[76]

In another case report, a 24-year-old male was given 1 g of LC orally every 12 hours and low-dose methimazole (10–15 mg/d) for a thyroid storm. The LC reversed and prevented his hyperthyroid symptoms. While on the LC and methimazole, he had two more thyroid storms, which were preceded by triggering factors (influenza, emotional stress). Therefore it seems that LC does not prevent relapses of thyrotoxicosis. However, the second and third storms were clinically milder. The LC and methimazole were stopped 6 months after his third thyroid storm, and the patient was still euthyroid 10 months later.[74]

Alzheimer's Disease, Senile Depression, and Age-Related Memory Defect

Much research has been conducted over the past 20 years with LAC in the treatment of Alzheimer's disease, senile depression, and age-related memory defects. As described previously, LAC is a molecule composed of acetic acid and LC bound together. This reaction occurs naturally in the human brain; therefore it is not exactly known how much greater an effect is noted with LAC versus LC or LPC. However, LAC is thought to be substantially more active than these other forms of carnitine in conditions involving the brain.[79,80]

LAC is structurally related to acetylcholine, a major neurotransmitter responsible for memory and proper brain function. In Alzheimer's disease and, to a lesser extent, the normal aging human brain, there is a defect in the use of acetylcholine. The close structural similarity between LAC and acetylcholine led researchers to begin testing LAC in Alzheimer's disease. The results have been encouraging.

Researchers showed that LAC does mimic acetylcholine and is of benefit not only in patients with early-stage Alzheimer's disease but also in elderly patients who are depressed or who have impaired memory.[80] It has also been shown to act as a powerful antioxidant within the brain cell by stabilizing cell membranes, improving energy production within the brain cell, and enhancing or mimicking the function of acetylcholine.[81] In 2008, a study done on rats revealed a significant increase in the brain tissue's kinesin light-chain 1 gene expression with LAC administration. When the kinesin light-chain 1 gene was downregulated, amyloid deposition was accelerated.[82]

The results in delaying the progression of Alzheimer's disease have been promising. The studies have been well controlled and extremely thorough.[79,83–85]

Memory impairment need not be as severe as in Alzheimer's disease for LAC to demonstrate benefit.[86–88] In one double-blind study of 236 elderly subjects with mild mental deterioration, as evidenced by detailed clinical assessment, the group receiving 1500 mg of LAC daily demonstrated significant improvement in mental function, particularly in memory and constructional thinking.[88]

Many of the elderly have depression not only as a result of experiencing a great deal of loss in their lives but also because of the biochemical changes in the brain associated with aging. LAC was shown to improve depression in elderly subjects in double-blind studies using assessment scales standard to scientific research of antidepressant drugs (e.g., Hamilton Depression Scale, Clinical Global Impression, Sandoz Clinical Assessment). The usual dosage was 500 mg three times daily but has been researched as high as 3 g daily.[89–91] Elderly subjects with the highest depression scores were usually the ones who benefited the most from LAC.[89,91]

LAC also appears helpful in enhancing the effects of acetylcholinesterase inhibitors. In one open study of oral LAC (2 g/day for 3 months), 23 patients with mild Alzheimer's disease (unresponsive to donepezil or rivastigmine alone) showed an increased response rate with LAC treatment as an addition to the acetylcholinesterase inhibitor (38% vs. 50%, respectively). The response rate was a measurement of cognitive function, functional status, and behavioral symptoms.[92] This additive effect may be useful whenever a patient does not respond to either treatment alone.

Depression

Recently, animal studies examined the antidepressive activity of LAC.[93,94] LAC was shown to preserve brain plasticity in chronic restraint stress and to grow primary and secondary dendrites. Also, after LAC treatment, "dendrites of the medial amygdala stellate neurons were more complex and longer compared with the chronic restraint stress group that received just the vehicle." Behaviorally, LAC appears to enhance resilience to stress by reducing passive behavior and increasing the social interaction ratio in the social avoidance test. There was no change in behavior in nonstressed animals receiving LAC.[93]

A mouse model showed that LAC had an effect on the type 2 metabotropic glutamate receptors and improved depression symptoms within 3 days of daily dosing. No tolerance to dosage was seen by 21 days. The benefit from LAC lasted 2 weeks after discontinuation.[94]

There are many other theories as to the mechanism of action of LAC and depression. A thorough review can be found in Chiechio's review article.[95] In general, LAC seems to work as well as classic antidepressants, but in less time and without the adverse events. It was also reported that LAC is more effective in the older population compared with younger patients.[95]

Down Syndrome

Given that both Down syndrome and Alzheimer's disease are characterized by a deficit in cholinergic transmission, a study was conducted to assess the effect of a 90-day treatment with LAC in individuals with Down syndrome.[96] Treated patients with Down syndrome showed statistically significant improvements in visual memory and attention, both in absolute terms and in comparison with the other groups. No improvement was found in mentally deficient non–Down syndrome subjects, so the favorable effect of LAC appears to be specific for patients with Down syndrome. An effective dosage is 20 mg of LAC per 2 lb of body weight. The action of LAC in these pathologies may be related to its direct and indirect cholinomimetic effects.

Kidney Disease and Hemodialysis

Carnitine supplementation is much indicated in kidney diseases because the kidney is a major site of carnitine synthesis. Carnitine supplementation can be renoprotective. In one study, high amounts of dietary fructose had adverse effects (higher cholesterol and TGs, lower levels of antioxidants and hypertrophy) on rat kidneys, and concurrent carnitine supplementation (300 mg/kg per day intraperitoneal) was able to prevent the damage either partially or fully.[97]

Damage to the kidney or reduced kidney function has a profound effect on carnitine metabolism. It is well established that patients undergoing hemodialysis have carnitine deficiency due to the loss of considerable quantities of carnitine during dialysis, as well as decreased synthesis. Serum carnitine levels drop nearly 80% during hemodialysis.[1–3] Supplementation with LC (15 mg/kg of intravenous LC at the end of each hemodialysis) helps maintain carnitine status.[98]

Carnitine-treated dialyzed patients have also shown additional benefits, including the following[99–104]:

- Disappearance of angina pectoris and arrhythmias occurring during dialysis
- Reduction of muscle symptoms, including muscle cramps
- Increased muscle mass
- Significant improvement in chronic anemia, as demonstrated by increased hematocrit, hemoglobin, and red blood cell count
- Improved insulin sensitivity and reduced stress on pancreatic β cells
- Decreased serum C-reactive protein (29%) and serum interleukin-6 (61%) values

Since the early 1990s, a major advancement in the treatment of anemia associated with hemodialysis is recombinant human erythropoietin therapy. However, this therapy is expensive and has side effects. In one study, LC 1 g intravenously after every dialysis session for 6 months led to a significant reduction in dosage, as well as improvements in membrane fragility and endogenous erythropoietin secretion.[105] Given the high cost of erythropoietin, if doctors are unwilling to follow this procedure, insurance companies should get involved and force dialysis units to employ LC.

Diabetes

Patients with diabetes typically have deficient carnitine status. Studies revealed relationships between low carnitine status and increased plasma fatty acids, which negatively affect insulin action, so it has been recommended that free carnitine determinations should be made even if the patient has good metabolic control.[106] Given the risk of atherosclerotic cardiovascular disease and reduced kidney and liver function found in patients with diabetes, it may simply be more rational to supplement all patients with diabetes with LC.

Carnitine supplementation was shown to greatly improve peripheral vascular function, as well as nerve function, in patients with diabetes.[107–110] Carnitine supplementation might also affect blood lipids in patients with type 2 diabetes, particularly lipoprotein(a). In a study in type 2 diabetes, 2000 mg of carnitine daily lowered lipoprotein(a)

levels by roughly 20% after 6 months, whereas in another study of patients with markedly elevated lipoprotein(a) levels, even greater reductions were noted.[111,112]

Insulin sensitivity can be improved with LC supplementation, as shown in a 10-day investigation of newly diabetic men and women. The study consisted of two groups: the control group, which was given a hypocaloric diet to follow (55% carbohydrates, 25% lipids, 20% proteins); and the LC group, which consumed the same hypocaloric diet in addition to 2 g of oral LC twice daily. The LC group had no adverse reactions with 4 g/day for 10 days. Both groups had an improvement in fasting and postprandial serum glucose and decreased body weight. Insulin sensitivity improved significantly in the LC group (plasma insulin: 7.0–4.5 μU/mL; homeostasis model assessment of insulin resistance [HOMA-IR]: 1.9 to 1.1) compared with the control group, which had no change in plasma insulin or HOMA-IR levels.[113]

Neuropathy

Neuropathy can occur for many reasons and may be helped with different therapies that are specific to each individual. L-carnitine or LAC may be particularly beneficial if the patient is infected with HIV (associated with secondary deficiency of LC and also due to antiretroviral drugs), has used chemotherapy drugs (taxanes, platinum drugs and/or vinca alkaloids), or has neuropathy due to diabetes.[114]

In 21 HIV-positive patients with antiretroviral toxic neuropathy characterized as distal symmetrical polyneuropathy, 1500 mg of LAC twice daily was given for 33 months. Skin biopsies were done from the leg for drug-induced neuropathy. The nerve fibers were quantified and analyzed immunohistochemically. The small sensory fibers showed increased staining after 6 months, indicating nerve regrowth. Compared with controls, patients taking oral LAC for 6 months showed 92% epidural, 80% dermal, and 69% sweat gland innervation. The improvement continued at the 12-month biopsy.[115] Another similar study showed improvements in subjective measures such as pain, paresthesias, numbness, and vibration sensation on neurological examination.[116]

A multicenter, randomized, double-blind, placebo-controlled Phase II clinical trial was done on taxoid-induced peripheral neuropathy in 239 patients. The results showed LAC at 1000 mg thrice daily reduced the chemotherapy-induced neurotoxicity in 50.5% of the patients compared with 24.1% in the control group. The improvement was noticed by week 4 but became significant by week 8 with a decrease in grade 3+ neuropathy and an increase in grade 2. There was a significant improvement in the nerve conductive velocity testing with the LAC group versus control.[117]

A multicenter, randomized, double-blind, controlled trial of 204 patients with diabetic peripheral neuropathy compared oral LAC 500 mg or oral methylcobalamin 0.5 mg three times daily for 24 weeks after meals. The results showed that LAC is as effective as methylcobalamin using neuropathy symptom score and disability score. Both scores were reduced significantly at 12 and 24 weeks in both groups. Nerve conduction velocity and amplitude were improved in the LAC group at 24 weeks.[118] Another diabetic neuropathy study using oral 500 mg of LAC thrice daily showed a significant increase in fiber numbers and regenerating clusters. The group taking oral 1000 mg of LAC thrice daily noted improved vibratory parameters as well.[119] Intramuscular injections of 1000 mg of LAC daily for 10 days followed by oral LAC 2000 mg daily for 355 days was used for diabetic neuropathy and showed a mean pain score decrease of 39% at the end of the study, with 67% of those receiving LAC and 23% of placebo.[109]

Because animal studies exist showing benefit in compression neuropathy using LAC, a study of patients with carpal tunnel syndrome and LAC (3000 mg daily for 2 months after surgery) has been done, with results yet to come.[120]

Liver Disease

Carnitine plays an extremely important role in the usage and metabolization of fatty acids in the liver. Some evidence indicates that carnitine deficiency within the liver promotes fatty infiltration (also known as steatosis or liver congestion).[121]

Alcohol ingestion is a common cause of fatty infiltration of the liver. It has been suggested that chronic alcohol consumption results in a functional deficiency of carnitine. Carnitine was shown to significantly inhibit and reverse alcohol-induced fatty liver disease.[122]

Because carnitine normally assists fatty acid transport and oxidation in the mitochondria, a high liver carnitine level may be necessary to handle the increased fatty acid load produced by alcohol consumption or other liver injury.[123] Supplemental carnitine was shown to reduce levels of free fatty acids in patients with liver cirrhosis and to reduce serum TGs and liver enzyme levels while elevating HDL-c in alcohol-induced fatty liver disease.[121–123]

Carnitine has also proven helpful with hepatitis C in relieving the tremendous fatigue associated with interferon-α treatment. In one study, 50 patients with chronic hepatitis treated with interferon-α (3 million IU three times weekly) were given either carnitine (2 g/day) or placebo for 6 months. Patients treated with interferon plus carnitine showed a marked and significant reduction of both mental and physical fatigue levels.[124]

Muscular Dystrophies

Patients with various muscular dystrophies have reduced levels of carnitine in their skeletal muscles.[125–127] Although levels are not as low as those observed in patients with classic myopathic carnitine deficiency, the low carnitine levels are thought to contribute to the muscular weakness experienced by these patients. Unfortunately, it is undetermined whether supplemental carnitine would be of any value in patients with muscular dystrophy.

Low Sperm Counts and Decreased Sperm Motility

Carnitine concentrations are extremely high in the epididymis and spermatozoa, suggesting a role for carnitine in male reproductive function.[1–3] The epididymis derives the majority of its energy requirements from lipids, as do the spermatozoa during transport through the epididymis. After ejaculation, spermatocytes depend on glycolysis of glucose and fructose and on oxidation of lactate and pyruvate. Carnitine (in the form of acetylcarnitine, which is derived from pyruvate) serves as a readily available substrate. The motility of ejaculated sperm correlates positively with acetylcarnitine content.[1,3] In human sperm, high carnitine concentrations are critical to sperm energy metabolism. Several studies showed that the level of free carnitine in seminal fluid was strongly correlated with sperm count and motility.[128,129] The lower the carnitine content, the more likely it is that a man is infertile. A 2011 study measured seminal free LC and found in fertile men (447.6 mole/L), oligoastheno teratospermic men (157.6 mole/L), asthenospermic men (233.3 mole/L), and azoospermic men (46.5 mole/L).[130] Clearly, infertility is associated with decreased LC levels.

Given the known physiological role of carnitine in sperm function and its link to male infertility, a study was designed to assess the therapeutic effect of carnitine in men with low sperm counts and depressed sperm motility.[131] One hundred men selected from infertility clinics participated in the Italian Study Group on Carnitine and Male Infertility. Each subject was given 3000 mg of LC daily for 4 months.

The study results indicated that LC increased sperm counts and sperm motility, in both a qualitative and a quantitative manner:

- The number of ejaculated sperm increased from 142 to 163 billion.
- The percentage of motile sperm increased from 26.9% to 37.7%.

- The percentage of sperm with rapid linear progression increased from 10.8% to 18%.
- The mean sperm velocity increased from 28.4% to 32.5%.

The results were even more impressive when only patients with the poorest sperm motility were studied. This subgroup saw even more significant gains on all parameters. For example, the percentage of motile sperm increased from 19.3% to 40.9%, and the percentage of sperm with rapid linear progression increased from 3.1% to 20.3%.

Androgenetic Alopecia

There are promising in vitro studies on follicular keratinocytes and the effect of a combined natural product including saw palmetto or beta-sitosterol and stigmasterol, carnitine, and thioctic acid.[132–134] Carnitine was chosen for its anti-inflammatory activity and showed significant repression in LPS-activated inflammatory genes.[132] Another study suggested that "LC stimulates human scalp hair growth by upregulation of proliferation and down-regulation of apoptosis in follicular keratinocytes in vitro."[133] The goal of the researchers was to target both 5-alpha-reductase and inflammatory pathways as a new therapeutic approach to androgenetic alopecia.[132–134] One study demonstrated the natural product was more effective than finasteride at suppressing the mRNA expression of 5-alpha-reductase type 1, 2, and 3, DKK-1, FGF-1, 17beta-HSD-3, TGF-beta1, and beta2.[134]

A prospective, double-blind, randomized, placebo-controlled observational study was conducted on 51 healthy volunteers (19 males and 32 females) with clinically diagnosed mild to moderate androgenetic alopecia. The study used a 2% L-carnitine L-tartrate liposomal tonic applied locally twice daily for at least 5 hours each application for 6 months. The results showed a significant increase in the total number of terminal scalp hair shafts (170 hairs/cm^2 before therapy and 197 hairs/cm^2 posttherapy). The number or anagen hair follicles increased by 75%, and the number of telogen hair follicles was significantly downregulated (25%) in volunteers receiving topical 2% solution. In the placebo group, after 6 months, the telogen hair follicles were downregulated by 38%, and the anagen hair follicles increased by 62%.[135]

Chronic Obstructive Pulmonary Disease

Patients with chronic respiratory insufficiency are often severely affected by even the simplest physical activity. Treatment with LC (2 g three times daily) resulted in significant improvements in exercise capability.[136]

Acquired Immunodeficiency Syndrome

Several reports indicated that systemic carnitine deficiency might be a problem in patients with acquired immunodeficiency syndrome (AIDS). Reduced levels of serum carnitine are most often found in AIDS patients. However, more important is the carnitine depletion in peripheral blood mononuclear cells (PBMCs). Even AIDS patients with normal serum carnitine levels demonstrated low levels of carnitine in white blood cells.[137] Increasing the carnitine content of peripheral white blood cells strongly improved lymphocyte function, highlighting the importance of carnitine to immune function.

LC was shown to prevent the toxicity of the drug azidothymidine (AZT) on the mitochondria of the muscle cells.[138] AZT poisons the mitochondria of the muscle, leading to abnormal energy production within the muscle, which manifests clinically as muscle fatigue and pain. If LC can prevent this negative effect of AZT in human patients with AIDS, it would be a major improvement in the clinical management of AIDS.

Preliminary studies indicated that LC supplementation could improve immune function and reduce the level of human immunodeficiency virus-induced immune suppression. When AIDS patients treated with AZT were given 6 g/day of LC, it led to significantly increased PBMC proliferation and reduced blood levels of TGs and circulating tumor necrosis factor.[139] Given the suspected systemic carnitine deficiency, along with the tremendous safety of use, carnitine supplementation appears to be warranted in treating AIDS.

Inborn Errors of Amino Acid Metabolism

The use of carnitine to treat inborn errors of metabolism involving the urea acid cycle appears to be well justified. Preliminary studies showed impressive therapeutic response to LC supplementation in cases of glutaric aciduria, isovaleric acidemia, propionic acidemia, and methylmalonic aciduria.[140–143]

Protection Against Drug Toxicity

Carnitine was shown to protect against the damaging effects on the heart produced by the chemotherapy drug adriamycin.[144] Carnitine was also shown to improve the symptoms attributed to anticonvulsant medications, such as valproic acid (trade names: Depa, Depakene, Depakote, and Deproic) and carbamazepine (trade names: Epitol and Tegretol).[145,146] However, a subsequent study challenged the need to administer carnitine prophylactically because no significant differences were noted in well-being scores between the carnitine group and the placebo group.[147]

Attention Deficit Hyperactivity Disorder

Preliminary evidence indicated that carnitine might be beneficial in attention deficit hyperactivity disorder (ADHD). In one double-blind, placebo-controlled, double-crossover trial, 13 of 24 boys receiving carnitine demonstrated a significant positive response in school and home behavior.[148] However, researchers found no significant improvement in ADHD symptoms with LAC (500–1500 mg twice a day, weight dependent) in a U.S. multicenter, placebo-controlled trial of children 5 to 12 years old.[149]

In a double-blind, placebo-controlled trial on boys with fragile X syndrome ages 6 to 13 years with symptoms of ADHD, LAC (20–50 mg/kg per day) produced a reduction in hyperactivity and improved social behavior compared with placebo counterparts. These improvements were measured by Conners's Parent Global Index Scale and the Vineland Adaptive Behavior Scale.[150]

Miscellaneous Conditions

Beta-Thalassemia Major: A small study ($n = 30$) was done on children with beta-thalassemia major and short stature and the effect of supplemental LC (50 mg/kg/day divided into 3 doses) over 6 months. The growth hormone and IGF-1 values were significantly increased after LC therapy.[151]

Wound Healing: Wound healing improved significantly compared with placebo in a rat study with LPC at different dosages. The LPC-treated animals (100 mg/kg/day in drinking water) showed faster blood flow recovery of skin flap wound and improved viability. Also, the necrotic skin area was less than that of placebo-treated rats. Also, another part of the same study showed that the LPC group had dose-dependent (30, 60, 120 mg/kg/day) faster reepithelialization compared with the control group.[152]

Muscle Cramping: LC was studied in specific conditions to help lessen or resolve muscle cramping. In a small study of 42 patients with hepatic cirrhosis and painful muscle cramps, LC was given (300 mg three or four times daily) for 8 weeks. The muscle cramp frequency significantly decreased in all patients, with 29% having complete resolution. The visual analog scale score decreased in 27 patients (87%) and increased in 3 patients (10%) after treatment. The response was dose dependent, with better response in the 1200-mg

daily group.[153] In another study on patients with dialysis complications of muscle cramping and hypotension, LC (500 mg/day) was combined with vitamin E (200 IU/day) for 45 days and led to a significant improvement in both complications.[104]

Narcolepsy: A case report discussed the use of oral LC as a treatment for narcolepsy during pregnancy. A 32-year-old woman was titrated up to 500 mg twice daily. She had a progressive improvement in daytime somnolence and showed a regular night-time sleep schedule. She also reported a decrease in diurnal time for dozing off and the number of diurnal naps.[154]

DOSAGE

The daily dosage of LC in all of its forms has typically been between 1500 and 4000 mg in divided doses; however, a few studies have used up to 6000 mg daily. According to the Council for Responsible Nutrition, the "observed safety level" of LC for long-term supplementation is 2000 mg/day. However, it also stated that "much higher levels have been tested without adverse effects and may be safe."[155]

TOXICOLOGY

LC is extremely safe, with potential side effects being gastrointestinally related, such as nausea and diarrhea. Again, only LC should be used. The D form, the mirror image of the L form, has side effects, indicating that it interferes with the natural L form of carnitine. Patients undergoing hemodialysis given a mixture containing D,L-carnitine for 45 days experienced muscle pain and loss of muscle function, presumably due to lack of energy.[156] The symptoms disappeared on cessation of D,L-carnitine supplementation. Subsequent studies showed that D-carnitine produced an LC deficiency in cardiac and skeletal muscle.[157] Although LC resulted in significant improvement in exercise tolerance in patients with angina, D,L-carnitine actually dangerously reduced exercise tolerance in these patients.[158]

DRUG INTERACTIONS

There are two case reports of L-carnitine at 1000 mg/d potentiating the effect of the anticoagulant medication acenocoumarol.[159,160] Because acenocoumarol is similar to warfarin, it is advised to also avoid or use caution with carnitine and warfarin.

Carnitine and coenzyme Q_{10} appear to work synergistically when combined.[161] The same is true for pantethine.[162]

Perhaps the most important interaction is with choline. In young adult women, daily choline supplementation (20 mg/kg body weight) resulted in a 75% lower urinary carnitine excretion than in controls, without significantly altering plasma carnitine concentrations. Studies in guinea pigs demonstrated that choline supplementation resulted in a significantly lower urinary excretion and higher skeletal muscle carnitine concentrations. These studies indicate that choline supplementation results in a conservation of carnitine and might increase intracellular carnitine levels.[163]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bremer J. Carnitine—metabolism and functions. *Physiol Rev*. 1983;63(4):1420–1480.
2. Bamji MS. Nutritional and health implications of lysine carnitine relationship. *World Rev Nutr Diet*. 1984;44:185–211.
3. Borum PR. Carnitine. *Annu Rev Nutr*. 1983;3:233–259.
4. Carter HE, Bhattacharyya PK, Weidman KR, Fraenkel G. Chemical studies on vitamin BT isolation and characterization as carnitine. *Arch Biochem Biophys*. 1952;38:405–416.
5. Engel AG, Angelini C. Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: a new syndrome. *Science*. 1973;179(4076):899–902.
6. Furusawa H, Sato Y, Tanaka Y, et al. Vitamin C is not essential for carnitine biosynthesis in vivo: verification in vitamin C-depleted senescence marker protein-30/gluconolactonase knockout mice. *Biol Pharm Bull*. 2008;31(9):1673–1679.
7. Cao Y, Wang YX, Liu CJ, Wang LX, Han ZW, Wang CB. Comparison of pharmacokinetics of L-carnitine, acetyl- L-carnitine and propionyl-L-carnitine after single oral administration of L-carnitine in healthy volunteers. *Clin Invest Med*. 2009;32(1):E13–E19.
8. Gilbert EF. Carnitine deficiency. *Pathology*. 1985;17(2):161–171.
9. Winter SC, Szabo-Aczel S, Curry CJ, Hutchinson HT, Hogue R, Shug A. Plasma carnitine deficiency. Clinical observations in 51 pediatric patients. *Am J Dis Child*. 1987;141(6):660–665.
10. Mayatepek E, Nezu J, Tamai I, et al. Two novel missense mutations of the OCTN2 gene (W283R and V446F) in a patient with primary systemic carnitine deficiency. *Hum Mutat*. 2000;15(1):118.
11. Glasgow AM, Eng G, Engel AG. Systemic carnitine deficiency simulating recurrent Reye syndrome. *J Pediatr*. 1980;96(5):889–891.
12. Chapoy PR, Angelini C, Brown WJ, Stiff JE, Shug AL, Cederbaum SD. Systemic carnitine deficiency—a treatable inherited lipid-storage disease presenting as Reye's syndrome. *N Engl J Med*. 1980;303(24):1389–1394.
13. Rebouche CJ, Engel AG. Carnitine metabolism and deficiency syndromes. *Mayo Clin Proc*. 1983;58(8):533–540.
14. Scholte HR, Stinis JT, Jennekens FG. Low carnitine levels in serum of pregnant women. *N Engl J Med*. 1978;299(19):1079–1080.
15. Cederblad G, Fåhraeus L, Lindgren K. Plasma carnitine and renal-carnitine clearance during pregnancy. *Am J Clin Nutr*. 1986;44(3):379–383.
16. Warshaw JB, Curry E. Comparison of serum carnitine and ketone body concentrations in breast- and in formula-fed newborn infants. *J Pediatr*. 1980;97(1):122–125.
17. Ardissone P, Baccolla D, Berberis L, et al. The effects of treatment with L-carnitine of hypoglycemia in pre-term AGA infants. *Curr There Res*. 1985;38:256–264.
18. Siliprandi N, Di Lisa F, Pivetta A, Miotto G, Siliprandi D. Transport and function of L-carnitine and L-propionylcarnitine: relevance to some cardiomyopathies and cardiac ischemia. *Z Kardiol*. 1987;76(suppl 5):34–40.
19. Paulson DJ, Traxler J, Schmidt M, Noonan J, Shug AL. Protection of the ischaemic myocardium by L-propionylcarnitine: effects on the recovery of cardiac output after ischaemia and reperfusion, carnitine transport, and fatty acid oxidation. *Cardiovasc Res*. 1986;20(7):536–541.
20. Ruggenenti P, Cattaneo D, Loriga G, et al. Ameliorating hypertension and insulin resistance in subjects at increased cardiovascular risk: effects of acetyl- L-carnitine therapy. *Hypertension*. 2009;54(3):567–574.
21. Pola P, Savi M, Grilli R, et al. Carnitine in the therapy of dyslipidemic patients. *Curr There Res*. 1980;27:208–215.
22. Pola P, Tondi P, dal Lago A, et al. Statistical evaluation of long-term L-carnitine therapy in hyperlipoproteinemias. *Drugs Exp Clin Res*. 1983;9:925–934.
23. Florentin M, Elisaf MS, Rizos CV, et al. L-Carnitine/simvastatin reduces lipoprotein (a) levels compared with simvastatin monotherapy: a randomized double-blind placebo-controlled study. *Lipids*. 2017;52(1):1–9.
24. Opie LH. Role of carnitine in fatty acid metabolism of normal and ischemic myocardium. *Am Heart J*. 1979;97(3):375–388.
25. Goa KL, Brogden RN, L-Carnitine. A preliminary review of its pharmacokinetics, and its therapeutic use in ischaemic cardiac disease and primary and secondary carnitine deficiencies in relationship to its role in fatty acid metabolism. *Drugs*. 1987;34(1):1–24.
26. Gürlek A, Tutar E, Akçil E, et al. The effects of L-carnitine treatment on left ventricular function and erythrocyte superoxide dismutase activity in patients with ischemic cardiomyopathy. *Eur J Heart Fail*. 2000;2(2):189–193.
27. Silverman NA, Schmitt G, Vishwanath M, Feinberg H, Levitsky S. Effect of carnitine on myocardial function and metabolism following global ischemia. *Ann Thorac Surg*. 1985;40(1):20–24.
28. Cherchi A, Lai C, Angelino F, et al. Effects of L-carnitine on exercise tolerance in chronic stable angina: a multicenter, double-blind, randomized, placebo controlled crossover study. *Int J Clin Pharmacol Ther Toxicol*. 1985;23(10):569–572.
29. Orlando G, Rusconi C. Oral L-carnitine in the treatment of chronic cardiac ischemia in elderly patients. *Clin Trials J*. 1986;23:338–344.
30. Kamikawa T, Suzuki Y, Kobayashi A, et al. Effects of L-carnitine on exercise tolerance in patients with stable angina pectoris. *Jpn Heart J*. 1984;25(4):587–597.
31. Kosolcharoen P, Nappi J, Peruzzi P, et al. Improved exercise tolerance after administration of carnitine. *Curr Ther Res*. 1981;30:753–764.
32. Pola P, Savi L, Serricchio M, et al. Use of physiological substance, acetyl-carnitine, in the treatment of angiospastic syndromes. *Drugs Exp Clin Res*. 1984;10:213–217.
33. Lagioia R, Scrutinio D, Mangini SG, et al. Propionyl- L-carnitine: a new compound in the metabolic approach to the treatment of effort angina. *Int J Cardiol*. 1992;34(2):167–172.
34. Davini P, Bigalli A, Lamanna F, Boem A. Controlled study on L-carnitine therapeutic efficacy in post-infarction. *Drugs Exp Clin Res*. 1992;18(8):355–365.
35. Bartels GL, Remme WJ, Pillay M, Schönfeld DH, Kruijssen DA. Effects of L-propionylcarnitine on ischemia-induced myocardial dysfunction in men with angina pectoris. *Am J Cardiol*. 1994;74(2):125–130.
36. Cacciatore L, Cerio R, Ciarimboli M, et al. The therapeutic effect of L-carnitine in patients with exercise-induced stable angina: a controlled study. *Drugs Exp Clin Res*. 1991;17(4):225–235.
37. Iliceto S, Scrutinio D, Bruzzi P, et al. Effects of L-carnitine administration on left ventricular remodeling after acute anterior myocardial infarction: the L-Carnitine Ecocardiografia Digitalizzata Infarto Miocardico (CEDIM) Trial. *J Am Coll Cardiol*. 1995;26(2):380–387.
38. Mancini M, Rengo F, Lingetti M, Sorrentino GP, Nolfe G. Controlled study on the therapeutic efficacy of propionyl- L-carnitine in patients with congestive heart failure. *Arzneimittelforschung*. 1992;42(9):1101–1104.
39. Pucciarelli G, Mastursi M, Latte S, et al. The clinical and hemodynamic effects of propionyl- L-carnitine in the treatment of congestive heart failure. *Clin Ter*. 1992;141(11):379–384.
40. Rizos I. Three-year survival of patients with heart failure caused by dilated cardiomyopathy and L-carnitine administration. *Am Heart J*. 2000;139(2 Pt 3):S120–S123.
41. Brevetti G, Perna S, Sabbà C, et al. Superiority of L-propionylcarnitine vs L-carnitine in improving walking capacity in patients with peripheral vascular disease: an acute, intravenous, double-blind, cross-over study. *Eur Heart J*. 1992;13(2):251–255.
42. Sabbá C, Berardi E, Antonica G, et al. Comparison between the effect of L-propionylcarnitine, L-acetylcarnitine and nitroglycerin in chronic peripheral arterial disease: a haemodynamic double blind echo-Doppler study. *Eur Heart J*. 1994;15(10):1348–1352.
43. Brevetti G, Chiariello M, Ferulano G, et al. Increases in walking distance in patients with peripheral vascular disease treated with L-carnitine: a double-blind, cross-over study. *Circulation*. 1988;77(4):767–773.
44. Brevetti G, Diehm C, Lambert D. European multicenter study on propionyl- L-carnitine in intermittent claudication. *J Am Coll Cardiol*. 1999;34(5):1618–1624.
45. Drăgan AM, Vasiliu D, Eremia NM, Georgescu E. Studies concerning some acute biological changes after endovenous administration of 1 g L-carnitine, in elite athletes. *Physiologie*. 1987;24(4):231–234.
46. Drăgan GI, Vasiliu A, Georgescu E, Dumas I. Studies concerning chronic and acute effects of L-carnitine on some biological parameters in elite athletes. *Physiologie*. 1987;24(1):23–28.
47. Drăgan GI, Wagner W, Ploeşteanu E. Studies concerning the ergogenic value of protein supply and l-carnitine in elite junior cyclists. *Physiologie*. 1988;25(3):129–132.

48. Soop M, Björkman O, Cederblad G, Hagenfeldt L, Wahren J. Influence of carnitine supplementation on muscle substrate and carnitine metabolism during exercise. *J Appl Physiol (1985)*. 1988;64(6):2394–2399.
49. Greig C, Finch KM, Jones DA, Cooper M, Sargeant AJ, Forte CA. The effect of oral supplementation with L-carnitine on maximum and submaximum exercise capacity. *Eur J Appl Physiol Occup Physiol*. 1987;56(4):457–460.
50. Marconi C, Sassi G, Carpinelli A, Cerretelli P. Effects of L-carnitine loading on the aerobic and anaerobic performance of endurance athletes. *Eur J Appl Physiol Occup Physiol*. 1985;54(2):131–135.
51. Huertas R, Campos Y, Díaz E, et al. Respiratory chain enzymes in muscle of endurance athletes: effect of L-carnitine. *Biochem Biophys Res Commun*. 1992;188(1):102–107.
52. Volek JS, Kraemer WJ, Rubin MR, Gómez AL, Ratamess NA, Gaynor P. L-Carnitine L-tartrate supplementation favorably affects markers of recovery from exercise stress. *Am J Physiol Endocrinol Metab*. 2002;282(2):E474–E482.
53. Dal Negro R. Changes in physical performance of untrained volunteers: effects of L-carnitine. *Clin Trials J*. 1986;23:242–248.
54. Pistone G, Marino A, Leotta C, Dell'Arte S, Finocchiaro G, Malaguarnera M. Levocarnitine administration in elderly subjects with rapid muscle fatigue: effect on body composition, lipid profile and fatigue. *Drugs Aging*. 2003;20(10):761–767.
55. Malaguarnera M, Cammalleri L, Gargante MP, Vacante M, Colonna V, Motta M. L-Carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial. *Am J Clin Nutr*. 2007;86(6):1738–1744.
56. Tomassini V, Pozzilli C, Onesti E, et al. Comparison of the effects of acetyl L-carnitine and amantadine for the treatment of fatigue in multiple sclerosis: results of a pilot, randomised, double-blind, crossover trial. *J Neurol Sci*. 2004;218(1–2):103–108.
57. Lebrun C, Alchaar H, Candito M, Bourg V, Chatel M. Levocarnitine administration in multiple sclerosis patients with immunosuppressive therapy-induced fatigue. *Mult Scler*. 2006;12(3):321–324.
58. Tejani AM, Wasdell M, Spiwak R, Rowell G, Nathwani S. Carnitine for fatigue in multiple sclerosis. *Cochrane Database Syst Rev*. 2010;(2):CD007280.
59. Cruciani RA, Dvorkin E, Homel P, et al. L-carnitine supplementation for the treatment of fatigue and depressed mood in cancer patients with carnitine deficiency: a preliminary analysis. *Ann N Y Acad Sci*. 2004;1033:168–176.
60. Cruciani RA, Dvorkin E, Homel P, et al. Safety, tolerability and symptom outcomes associated with L-carnitine supplementation in patients with cancer, fatigue, and carnitine deficiency: a phase I/II study. *J Pain Symptom Manage*. 2006;32(6):551–559.
61. Cruciani RA, Dvorkin E, Homel P, et al. L-carnitine supplementation in patients with advanced cancer and carnitine deficiency: a double-blind, placebo-controlled study. *J Pain Symptom Manage*. 2009;37(4):622–631.
62. Cruciani RA, Zhang JJ, Manola J, Cella D, Ansari B, Fisch MJ. L-carnitine supplementation for the management of fatigue in patients with cancer: an eastern cooperative oncology group phase III, randomized, double-blind, placebo-controlled trial. *J Clin Oncol*. 2012;30(31):3864–3869.
63. Matsui H, Einama T, Shichi S, et al. L-Carnitine supplementation reduces the general fatigue of cancer patients during chemotherapy. *Mol Clin Oncol*. 2018;8(3):413–416.
64. Lerner A, Gruener N, Iancu TC. Serum carnitine concentrations in coeliac disease. *Gut*. 1993;34(7):933–935.
65. Ciacci C, Peluso G, Iannoni E, et al. L-Carnitine in the treatment of fatigue in adult celiac disease patients: a pilot study. *Dig Liver Dis*. 2007;39(10):922–928.
66. Bene J, Komlósi K, Gasztonyi B, Juhász M, Tulassay Z, Melegh B. Plasma carnitine ester profile in adult celiac disease patients maintained on long-term gluten free diet. *World J Gastroenterol*. 2005;11(42):6671–6675.
67. Bene J, Komlósi K, Havasi V, et al. Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. *World J Gastroenterol*. 2006;12(1):110–113.
68. Gasbarrini G, Mingrone G, Giancaterini A, et al. Effects of propionyl-L-carnitine topical irrigation in distal ulcerative colitis: a preliminary report. *Hepatogastroenterology*. 2003;50(53):1385–1389.
69. Mikhailova TL, Sishkova E, Poniewierka E, et al. Randomised clinical trial: the efficacy and safety of propionyl- L-carnitine therapy in patients with ulcerative colitis receiving stable oral treatment. *Aliment Pharmacol Ther*. 2011;34(9):1088–1097.
70. Scioli MG, Stasi MA, Passeri D, et al. Propionyl- L-carnitine is efficacious in ulcerative colitis through its action on the immune function and microvasculature. *Clin Transl Gastroenterol*. 2014;5:e55.
71. Gilgore SG, DeFelice SL. Evaluation of carnitine—an antagonist of thyroid hormone. Clinical pharmacology report. *J New Drugs*. 1966;6(6):349–350.
72. DeFelice SL, Gilgore SG. The antagonistic effect of carnitine in hyperthyroidism. Preliminary report. *J New Drugs*. 1966;6(6):351–353.
73. Benvenga S, Ruggeri RM, Russo A, Lapa D, Campenni A, Trimarchi F. Usefulness of L-carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab*. 2001;86(8):3579–3594.
74. Benvenga S, Lapa D, Cannavò S, Trimarchi F. Successive thyroid storms treated with L-carnitine and low doses of methimazole. *Am J Med*. 2003;115(5):417–418.
75. Chee R, Agah R, Vita R, Benvenga S. L-carnitine treatment in a seriously ill cancer patient with severe hyperthyroidism. *Hormones (Athens)*. 2014;13(3):407–412.
76. Kimmoun A, Munagamage G, Dessalles N, Gerard A, Feillet F, Levy B. Unexpected awakening from comatose thyroid storm after a single intravenous injection of L-carnitine. *Intensive Care Med*. 2011;37(10):1716–1717.
77. Nordio M. A novel treatment for subclinical hyperthyroidism: a pilot study on the beneficial effects of L-carnitine and selenium. *Eur Rev Med Pharmacol Sci*. 2017;21(9):2268–2273.
78. Benvenga S, Amato A, Calvani M, Trimarchi F. Effects of carnitine on thyroid hormone action. *Ann N Y Acad Sci*. 2004;1033:158–167.
79. Bowman BA. Acetyl-carnitine and Alzheimer's disease. *Nutr Rev*. 1992;50(5):142–144.
80. Carta A, Calvani M, Bravi D, Bhuachalla SN. Acetyl- L-carnitine and Alzheimer's disease: pharmacological considerations beyond the cholinergic sphere. *Ann N Y Acad Sci*. 1993;695:324–326.
81. Calvani M, Carta A, Caruso G, Benedetti N, Iannuccelli M. Action of acetyl- L-carnitine in neurodegeneration and Alzheimer's disease. *Ann N Y Acad Sci*. 1992;663:483–486.
82. Traina G, Federighi G, Brunelli M. Up-regulation of kinesin light-chain 1 gene expression by acetyl- L-carnitine: therapeutic possibility in Alzheimer's disease. *Neurochem Int*. 2008;53(6–8):244–247.
83. Pettegrew JW, Klunk WE, Panchalingam K, Kanfer JN, McClure RJ. Clinical and neurochemical effects of acetyl- L-carnitine in Alzheimer's disease. *Neurobiol Aging*. 1995;16(1):1–4.
84. Sano M, Bell K, Cote L, et al. Double-blind parallel design pilot study of acetyl levocarnitine in patients with Alzheimer's disease. *Arch Neurol*. 1992;49(11):1137–1141.
85. Spagnoli A, Lucca U, Menasce G, et al. Long-term acetyl- L-carnitine treatment in Alzheimer's disease. *Neurology*. 1991;41(11):1726–1732.
86. Passeri M, Cucinotta D, Bonati PA, Iannuccelli M, Parnetti L, Senin U. Acetyl- L-carnitine in the treatment of mildly demented elderly patients. *Int J Clin Pharmacol Res*. 1990;10(1–2):75–79.
87. Salvioli G, Neri M. L-acetylcarnitine treatment of mental decline in the elderly. *Drugs Exp Clin Res*. 1994;20(4):169–176.
88. Cipolli C, Chiari G. [Effects of L-acetylcarnitine on mental deterioration in the aged: initial results]. *Clin Ter*. 1990;132(suppl 6):479–510.
89. Garzya G, Corallo D, Fiore A, Lecciso G, Petrelli G, Zotti C. Evaluation of the effects of L-acetylcarnitine on senile patients suffering from depression. *Drugs Exp Clin Res*. 1990;16(2):101–106.
90. Bella R, Biondi R, Raffaele R, Pennisi G. Effect of acetyl- L-carnitine on geriatric patients suffering from dysthymic disorders. *Int J Clin Pharmacol Res*. 1990;10(6):355–360.

91. Tempesta E, Casella L, Pirrongelli C, Janiri L, Calvani M, Ancona L. L-acetylcarnitine in depressed elderly subjects. A cross-over study vs placebo. *Drugs Exp Clin Res*. 1987;13(7):417–423.
92. Bianchetti A, Rozzini R, Trabucchi M. Effects of acetyl- L-carnitine in Alzheimer's disease patients unresponsive to acetylcholinesterase inhibitors. *Curr Med Res Opin*. 2003;19(4):350–353.
93. Lau T, Bigio B, Zelli D, McEwen BS, Nasca C. Stress-induced structural plasticity of medial amygdala stellate neurons and rapid prevention by a candidate antidepressant. *Mol Psychiatry*. 2017;22(2):227–234.
94. Nasca C, Xenos D, Barone Y, et al. L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. *Proc Natl Acad Sci U S A*. 2013;110(12):4804–4809.
95. Chiechio S, Canonico PL, Grilli M, L-Acetylcarnitine. A mechanistically distinctive and potentially rapid-acting antidepressant drug. *Int J Mol Sci*. 2017;19(1).
96. De Falco FA, D'Angelo E, Grimaldi G, Scafuro F, Sachez F, Caruso G. Effect of the chronic treatment with L-acetylcarnitine in Down's syndrome. *Clin Ter*. 1994;144(2):123–127.
97. Rajasekar P, Viswanathan P, Anuradha CV. Renoprotective action of L-carnitine in fructose-induced metabolic syndrome. *Diabetes Obes Metab*. 2008;10(2):171–180.
98. Chazot C, Blanc C, Hurot JM, Charra B, Jean G, Laurent G. Nutritional effects of carnitine supplementation in hemodialysis patients. *Clin Nephrol*. 2003;59(1):24–30.
99. Bellinghieri G, Savica V, Mallamace A, et al. Correlation between increased serum and tissue L-carnitine levels and improved muscle symptoms in hemodialyzed patients. *Am J Clin Nutr*. 1983;38(4):523–531.
100. Donatelli M, Terrizzi C, Zummo G, al e. Effects of L-carnitine on chronic anemia and erythrocyte adenosine triphosphate concentration in hemodialyzed patients. *Curr Ther Res*. 1987;41:620–624.
101. Golper TA, Wolfson M, Ahmad S, et al. Multicenter trial of L-carnitine in maintenance hemodialysis patients. I. Carnitine concentrations and lipid effects. *Kidney Int*. 1990;38(5):904–911.
102. Vazelov E, Borissova AM, Kirilov G, Assenova B, Tchetirska M, Krivoshiev S. L-carnitine consecutively administered to patients on hemodialysis improves beta-cell response. *Int J Artif Organs*. 2003;26(4):304–307.
103. Shakeri A, Tabibi H, Hedayati M. Effects of L-carnitine supplement on serum inflammatory cytokines, C-reactive protein, lipoprotein (a), and oxidative stress in hemodialysis patients with Lp (a) hyperlipoproteinemia. *Hemodial Int*. 2010;14(4):498–504.
104. Tayebi Khosroshahi H, Habibi Asl B, Habibzadeh A, Chaichi P, Ghanbarpour A, Hossein Badie A. Comparison of vitamin e and L-carnitine, separately or in combination in patients with intradialytic complications. *Nephrourol Mon*. 2013;5(4):862–865.
105. Labonia WD. L-carnitine effects on anemia in hemodialyzed patients treated with erythropoietin. *Am J Kidney Dis*. 1995;26(5):757–764.
106. Coker M, Coker C, Darcan S, Can S, Orbak Z, Gökşen D. Carnitine metabolism in diabetes mellitus. *J Pediatr Endocrinol Metab*. 2002;15(6):841–849.
107. Greco AV, Mingrone G, Bianchi M, Ghirlanda G. Effect of propionyl-L-carnitine in the treatment of diabetic angiopathy: controlled double blind trial versus placebo. *Drugs Exp Clin Res*. 1992;18(2):69–80.
108. Morabito E, Serafini S, Corsico N, Martelli EA. Acetyl- L-carnitine effect on nerve conduction velocity in streptozotocin-diabetic rats. *Arzneimittelforschung*. 1993;43(3):343–346.
109. De Grandis D, Minardi C. Acetyl- L-carnitine (levacecarnine) in the treatment of diabetic neuropathy. A long-term, randomised, double-blind, placebo-controlled study. *Drugs R D*. 2002;3(4):223–231.
110. Evans JD, Jacobs TF, Evans EW. Role of acetyl- L-carnitine in the treatment of diabetic peripheral neuropathy. *Ann Pharmacother*. 2008;42(11):1686–1691.
111. Derosa G, Cicero AF, Gaddi A, Mugellini A, Ciccarelli L, Fogari R. The effect of L-carnitine on plasma lipoprotein(a) levels in hypercholesterolemic patients with type 2 diabetes mellitus. *Clin Ther*. 2003;25(5):1429–1439.
112. Sirtori CR, Calabresi L, Ferrara S, et al. L-carnitine reduces plasma lipoprotein(a) levels in patients with hyper Lp(a). *Nutr Metab Cardiovasc Dis*. 2000;10(5):247–251.
113. Molfino A, Cascino A, Conte C, Ramaccini C, Rossi Fanelli F, Laviano A. Caloric restriction and L-carnitine administration improves insulin sensitivity in patients with impaired glucose metabolism. *JPEN J Parenter Enteral Nutr*. 2010;34(3):295–299.
114. Head KA. Peripheral neuropathy: pathogenic mechanisms and alternative therapies. *Altern Med Rev*. 2006;11(4):294–329.
115. Hart AM, Wilson AD, Montovani C, et al. Acetyl- L-carnitine: a pathogenesis based treatment for HIV-associated antiretroviral toxic neuropathy. *AIDS*. 2004;18(11):1549–1560.
116. Valcour V, Yeh TM, Bartt R, et al. Acetyl- L-carnitine and nucleoside reverse transcriptase inhibitor-associated neuropathy in HIV infection. *HIV Med*. 2009;10(2):103–110.
117. Sun Y, Shu Y, Liu B, et al. A prospective study to evaluate the efficacy and safety of oral acetyl- L-carnitine for the treatment of chemotherapy-induced peripheral neuropathy. *Exp Ther Med*. 2016;12(6):4017–4024.
118. Li S, Chen X, Li Q, et al. Effects of acetyl- L-carnitine and methylcobalamin for diabetic peripheral neuropathy: a multicenter, randomized, double-blind, controlled trial. *J Diabetes Investig*. 2016;7(5):777–785.
119. Sima AA, Calvani M, Mehra M, Amato A, Group A-L-CS. Acetyl- L-carnitine improves pain, nerve regeneration, and vibratory perception in patients with chronic diabetic neuropathy: an analysis of two randomized placebo-controlled trials. *Diabetes Care*. 2005;28(1):89–94.
120. Curran MW, Olson J, Morhart M, Sample D, Chan KM. Acetyl-L-carnitine (ALCAR) to enhance nerve regeneration in carpal tunnel syndrome: study protocol for a randomized, placebo-controlled trial. *Trials*. 2016;17:200.
121. Sachan DS, Rhew TH, Ruark RA. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. *Am J Clin Nutr*. 1984;39(5):738–744.
122. Sachan DA, Rhew TH. Lipotropic effect of carnitine on alcohol-induced hepatic stenosis. *Nutr Rep Int*. 1983;27:1221–1226.
123. Noto R, Maugeri A, Grasso R, et al. Free fatty acids and carnitine in patients with liver disease. *Curr Ther Res*. 1986;40:35–39.
124. Neri S, Pistone G, Saraceno B, Pennisi G, Luca S, Malaguarnera M. L-carnitine decreases severity and type of fatigue induced by interferon-alpha in the treatment of patients with hepatitis C. *Neuropsychobiology*. 2003;47(2):94–97.
125. Borum PR, Broquist HP, Roelops RJ. Muscle carnitine levels in neuromuscular disease. *J Neurol Sci*. 1977;34(2):279–286.
126. Carrier HN, Berthillier G. Carnitine levels in normal children and adults and in patients with diseases muscle. *Muscle Nerve*. 1980;3(4):326–334.
127. Bresolin N, Freddo L, Tegazzin V, Bet L, Armani M, Angelini C. Carnitine and acyltransferase in experimental neurogenic atrophies: changes with treatment. *J Neurol*. 1984;231(4):170–175.
128. Bornman MS, du Toit D, Otto B, Müller II , Hurter P, du Plessis DJ. Seminal carnitine, epididymal function and spermatozoal motility. *S Afr Med J*. 1989;75(1):20–21.
129. Menchini-Fabris GF, Canale D, Izzo PL, Olivieri L, Bartelloni M. Free L-carnitine in human semen: its variability in different andrologic pathologies. *Fertil Steril*. 1984;42(2):263–267.
130. Ahmed SD, Karira KA, Jagdesh AS. Role of L-carnitine in male infertility. *J Pak Med Assoc*. 2011;61(8):732–736.
131. Costa M, Canale D, Filicori M, D'lddio S, Lenzi A. L-carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility. *Andrologia*. 1994;26(3):155–159.
132. Chittur S, Parr B, Marcovici G. Inhibition of inflammatory gene expression in keratinocytes using a composition containing carnitine, thioctic Acid and saw palmetto extract. *Evid Based Complement Alternat Med*. 2011;2011:985345.
133. Foitzik K, Hoting E, Förster T, Pertile P, Paus R. L-carnitine- L-tartrate promotes human hair growth in vitro. *Exp Dermatol*. 2007;16(11):936–945.
134. Chen L, Wang J, Mouser G, Li YC, Marcovici G. Blockade of androgen markers using a novel betasitosterol, thioctic acid and carnitine-containing compound in prostate and hair follicle cell-based assays. *Phytother Res*. 2016;30(6):1016–1020.
135. Foitzik K, Hoting E, Heinrich U, Tronnier H, Paus R. Indications that topical L-carnitine- L-tartrate promotes human hair growth in vivo. *J Dermatol Sci*. 2007;48(2):141–144.

136. Dal Negro R, Zoccatelli D, Pomari C, et al. L-carnitine and physiokinesiotherapy in chronic respiratory insufficiency. *Clin Trials J*. 1985;22:353–360.
137. De Simone C, Famularo G, Tzantzoglou S, Trinchieri V, Moretti S, Sorice F. Carnitine depletion in peripheral blood mononuclear cells from patients with AIDS: effect of oral L-carnitine. *AIDS*. 1994;8(5):655–660.
138. Semino-Mora MC, Leon-Monzon ME, Dalakas MC. Effect of L-carnitine on the zidovudine-induced destruction of human myotubes. Part I: L-carnitine prevents the myotoxicity of AZT in vitro. *Lab Invest*. 1994;71(1):102–112.
139. De Simone C, Tzantzoglou S, Famularo G, et al. High dose L-carnitine improves immunologic and metabolic parameters in AIDS patients. *Immunopharmacol Immunotoxicol*. 1993;15(1):1–12.
140. Seccombe DW, James L, Booth F. L-carnitine treatment in glutaric aciduria type I. *Neurology*. 1986;36(2):264–267.
141. de Sousa C, Chalmers RA, Stacey TE, Tracey BM, Weaver CM, Bradley D. The response to L-carnitine and glycine therapy in isovaleric acidaemia. *Eur J Pediatr*. 1986;144(5):451–456.
142. Roe CR, Bohan TP. L-carnitine therapy in propionicacidaemia. *Lancet*. 1982;1(8286):1411–1412.
143. Roe CR, Hoppel CL, Stacey TE, Chalmers RA, Tracey BM, Millington DS. Metabolic response to carnitine in methylmalonic aciduria. An effective strategy for elimination of propionyl groups. *Arch Dis Child*. 1983;58(11):916–920.
144. Furitano G, Paterna S, Perricone R, et al. Polygraphic evaluation of effects of carnitine in patients on adriamycin treatment. *Drugs Exp Clin Res*. 1984;10:107–111.
145. O'Connor JE, Costell M, Miguez MP, Grisolia S. Influence of the route of administration on the protective effect of L-carnitine on acute hyperammonemia. *Biochem Pharmacol*. 1986;35(18):3173–3176.
146. Matsuda I, Ohtani Y, Ninomiya N. Renal handling of carnitine in children with carnitine deficiency and hyperammonemia associated with valproate therapy. *J Pediatr*. 1986;109(1):131–134.
147. Freeman JM, Vining EP, Cost S, Singhi P. Does carnitine administration improve the symptoms attributed to anticonvulsant medications?: a double-blinded, crossover study. *Pediatrics*. 1994;93(6 Pt 1): 893–895.
148. Van Oudheusden LJ, Scholte HR. Efficacy of carnitine in the treatment of children with attention-deficit hyperactivity disorder. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67(1):33–38.
149. Arnold LE, Amato A, Bozzolo H, et al. Acetyl- L-carnitine (ALC) in attention-deficit/hyperactivity disorder: a multi-site, placebo-controlled pilot trial. *J Child Adolesc Psychopharmacol*. 2007;17(6):791–802.
150. Torrioli MG, Vernacotola S, Peruzzi L, et al. A double-blind, parallel, multicenter comparison of L-acetylcarnitine with placebo on the attention deficit hyperactivity disorder in fragile X syndrome boys. *Am J Med Genet A*. 2008;146A(7):803–812.
151. Beshlawy AE, Abd El Dayem SM, Mougy FE, Gafar EA, Samir H. Screening of growth hormone deficiency in short thalassaemic patients and effect of L-carnitine treatment. *Arch Med Sci*. 2010;6(1):90–95.
152. Scioli MG, Lo Giudice P, Bielli A, et al. Propionyl- L-carnitine enhances wound healing and counteracts microvascular endothelial cell dysfunction. *PLoS One*. 2015;10(10):e0140697.
153. Nakanishi H, Kurosaki M, Tsuchiya K, et al. L-Carnitine reduces muscle cramps in patients with cirrhosis. *Clin Gastroenterol Hepatol*. 2015;13(8):1540–1543.
154. Romigi A, Liguori C, Izzi F, et al. Oral L-carnitine as treatment for narcolepsy without cataplexy during pregnancy: a case report. *J Neurol Sci*. 2015;348(1–2):282–283.
155. Hathcock JN, Shao A. Risk assessment for carnitine. *Regul Toxicol Pharmacol*. 2006;46(1):23–28.
156. Bazzato G, Mezzina C, Ciman M, Guarnieri G. Myasthenia-like syndrome associated with carnitine in patients on long-term haemodialysis. *Lancet*. 1979;1(8124):1041–1042.
157. Paulson DJ, Shug AL. Tissue specific depletion of L-carnitine in rat heart and skeletal muscle by D-carnitine. *Life Sci*. 1981;28(26):2931–2938.
158. Watanabe S, Ajisaka R, Masuoka T, et al. Effects of L- and DL-carnitine on patients with impaired exercise tolerance. *Jpn Heart J*. 1995;36(3):319–331.
159. Martinez E, Domingo P, Roca-Cusachs A. Potentiation of acenocoumarol action by L-carnitine. *J Intern Med*. 1993;233(1):94.
160. Bachmann HU, Hoffmann A. Interaction of food supplement L-carnitine with oral anticoagulant acenocoumarol. *Swiss Med Wkly*. 2004;134(25–26):385.
161. Bertelli A, Ronca F, Ronca G, Palmieri L, Zucchi R. L-carnitine and coenzyme Q10 protective action against ischaemia and reperfusion of working rat heart. *Drugs Exp Clin Res*. 1992;18(10):431–436.
162. Gleeson JM, Wilson DE, Chan IF, et al. Effect of carnitine and pantetheine on the metabolic abnormalities of acquired total lipodystrophy. *Curr Ther Res*. 1987;41:83–88.
163. Daily JW, Sachan DS. Choline supplementation alters carnitine homeostasis in humans and Guinea pigs. *J Nutr*. 1995;125(7):1938–1944.

64

Centella asiatica (Gotu Kola)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Centella asiatica (family: *Umbelliferae* or *Apiaceae*)
Synonym: *Hydrocotyle asiatica* L.
Common names: gotu kola, Indian pennywort, South African pennywort, mandukaparni

GENERAL DESCRIPTION

Centella asiatica is an herbaceous perennial plant native to India, China, Indonesia, Australia, the South Pacific, Madagascar, and southern and middle Africa. This slender, creeping plant flourishes in and around water. Although it grows best in damp, swampy areas, centella is often observed growing along stone walls or other rocky, sunny areas at elevations of approximately 2000 feet in India and Sri Lanka.[1]

Depending on the environment, the form and shape of centella can change dramatically. In shallow water, centella forms floating leaves, whereas in dry locations, the leaves are small and thin, and numerous roots are formed.[1]

Typically, the constantly growing roots give rise to reddish stolons. The round-to-reniform, smooth-surfaced leaves, found on furrowed petioles, can reach a width of 1 inch and a length of 6 inches. The leaf margin may be smooth, crenate, or slightly lobed (Fig. 64.1). Usually three to six red flowers arise in a sessile manner or on short pedicels in axillary umbels at the end of 0.08- to 0.3-inch long peduncles. The fruit, formed throughout the growing season, is approximately 0.2 inches long, with seven to nine ribs and a curved, strongly thickened pericarp.[1]

Historically, the entire plant was used medicinally, with harvesting occurring any time during the year.[1]

CHEMICAL COMPOSITION

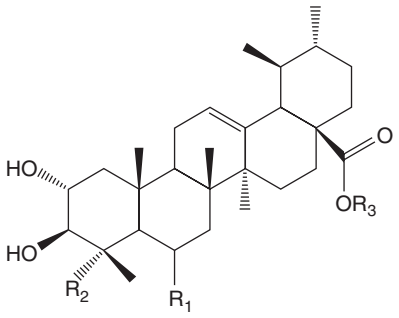
Although the primary pharmacologically active constituents of *C. asiatica* are known to be triterpenoid compounds,[2] its exact chemical profile is difficult to determine because of duplicate names and contradictory findings. In addition, centella samples from India, Sri Lanka, and Madagascar apparently do not contain the same constituents. In India, three (and possibly more) chemically different subspecies of *C. asiatica* have been found.

The concentration of triterpenes in centella can vary between 1.1% and 8%, with most samples yielding a concentration between 2.2% and 3.4%.[3] Fig. 64.2 illustrates the major triterpenoid components of *C. asiatica.*

The Madagascar variety is most commonly used to produce standardized extracts. It yields triterpene concentrations of asiatic acid (29%–30%), madecassic acid (29%–30%), asiaticoside (40%), and madecassoside (1%–2%).[2]

Centella also contains a green, volatile oil composed of an unidentified terpene acetate (which accounts for 36% of the total oil), camphor, cineole, and other essential oils. Centella oil also contains glycerides of

Fig. 64.1 Centella asiatica



	R_1	R_2	R_3
Asiatic acid	H	CH_2OH	H
Madecassic acid	OH	CH_2OH	H
Madasiatic acid	OH	CH_3	H
Asiaticoside	H	CH_2OH	Glucose-glucose-rhamnose
Madecassoside	OH	CH_2OH	Glucose-glucose-rhamnose

Fig. 64.2 The triterpene compounds of *Centella asiatica*.

fatty acids, various plant sterols (e.g., campesterol, stigmasterol, and sitosterol), and various polyacetylene compounds.[1,2]

Other notable compounds isolated from centella include the flavonoids kaempferol, quercetin, and their glycosides; myoinositol; sugars; a bitter substance (vellarin); amino acids; and resins.[1,2]

HISTORY AND FOLK USE

Centella has been used as a medicine in India since prehistoric times and is thought to be identical to the plant mandukaparni, listed in the *Susruta Samhita* (one of the valuable treatises in Ayurveda). Centella was also used extensively as a medicine, both internally and externally, by the people of Java and other islands of Indonesia. The medicinal use of centella in India and Indonesia centered around its ability to heal wounds and relieve leprosy, although it was also considered to be one of the "Rasayana" (rejuvenator) herbal medicines and was used to enhance memory and prolong life.[1,2]

In the 19th century, centella and its extracts were incorporated into the Indian pharmacopeia. In addition to being recommended for wound healing, it was recommended for the treatment of skin conditions such as leprosy, lupus, varicose ulcers, eczema, and psoriasis. It was also used to treat diarrhea, fever, amenorrhea, and diseases of the female genitourinary tract.[1]

In China, the leaves are prescribed for turbid leukorrhea and toxic fevers, whereas the shoots are used for boils and fevers. The plant is also used in the treatment of fractures, contusions, strains, and snakebites.[1] Centella was also used in China to delay senescence. One of the reported "miracle elixirs of life," centella's reputation as a promoter of longevity stems from the report of Chinese herbalist LiChing Yun, who reportedly lived 256 years. LiChing Yun's longevity was supposedly a result of his regular use of an herbal mixture chiefly composed of centella.[4]

C. asiatica was first accepted as a drug in France in the 1880s. Since then, centella extracts have been used to treat many of the previously listed conditions, along with those described in the section "Clinical Applications."

Centella, or gotu kola, has aroused much curiosity in American consumers. Many confuse gotu kola with kola nuts and assume gotu kola's rejuvenating activity is nothing more than the stimulant effect of caffeine. However, gotu kola is not related to the kola nut (*Cola nitida* or *Cola acuminata*), nor does it contain any caffeine.

BOX 64.1 Physiological Effects of *Centella asiatica* on Connective Tissue

- Stimulates hair and nail growth[1,4,6]
- Increases vascularization of connective tissue[1,4,6]
- Increases the formation of mucin and structural glycosaminoglycans, such as hyaluronic acid and chondroitin sulfate[1,4,6]
- Increases the tensile integrity of the dermis[1,4,6]
- Increases keratinization of epidermis through stimulation of the stratum germinativum[1,5,6]
- Possesses a eutrophic or balancing effect on connective tissue[1,6]

PHARMACOLOGY

The majority of pharmacological investigations on *C. asiatica* focused on the triterpene's wound-healing and venotonic activity.[1,2] Although the exact mechanism of action has not yet been fully determined, a number of interesting observations have been made:

- In one of the early pharmacological investigations of centella, Boiteau and Ratsimamanga[5] demonstrated that asiaticoside substantially hastened the healing of experimentally induced wounds. These authors concluded that asiaticoside worked selectively in stimulating the rapid and healthy activity of the reticuloendothelial system.
- Additional studies on the mechanisms of action of centella to enhance wound healing showed that asiaticoside, given orally, by intramuscular injection, or by implantation to rats, mice, guinea pigs, and rabbits, produced a wide range of effects (Box 64.1).
- The efficacy of centella in stimulating collagen synthesis was demonstrated in human tissue cultures.[3] Interestingly, this research demonstrated an additional benefit when vitamin C was added to the experimental cultures.
- The outcome of centella's complex actions is a balanced multiphasic effect on cells and tissues participating in the process of healing, particularly in connective tissues. Enhanced development of the normal connective tissue matrix is perhaps the prime therapeutic action of *C. asiatica.*
- Centella was shown to enhance the connective tissue structure of the perivascular sheath, reduce sclerosis, and improve blood flow through the affected veins.[1,6]

Research has also demonstrated positive effects of centella on the central nervous system, cognitive function, stress, and anxiety.[2] The major effects are an enhancement of cholinergic mechanisms and a significant antioxidant action via increasing glutathione levels.[7,8] Presumably, these mechanisms are responsible for the improvements in mental function noted in both animal and clinical trials. Excellent results in improving cognitive function and memory were seen in animal models of Alzheimer's disease.[9]

Ethanolic and methanolic extracts of *C. asiatica* have shown significant protection and lowered blood glucose to normal levels in glucose tolerance tests carried out in alloxan-induced diabetic rats.[10] Water extract has a chemopreventive effect on colon tumorigenesis,[11] and asiaticoside might be useful in cancer chemotherapy because it induces apoptosis and enhances the antitumor activity of vincristine in

TABLE 64.1 Clinical Applications of *Centella asiatica*

Conditions	References
Anal fissure	57
Bladder ulcer	58, 59
Burn	14, 15
Cellulite	16–20
Cirrhosis	21, 22
Dermatitis	18
Fibrocystic breast	60
Hemorrhoid	61
Keloid	23–26
Leprosy	29, 30
Lupus erythematosus	62
Peptic ulcer	63, 64
Perineal lesion	65
Periodontal disease	66
Retinal detachment	67
Scleroderma	41–44
Skin ulcer	68, 69
Surgical wound	45, 70
Tuberculosis	71
Venous disorder	45–51
Wound healing	53, 54, 70

cancer cells.[12] Animal studies also demonstrate cardioprotective activity against cardiomyopathy in rats, and results strongly suggest the cardioprotective activity of the plant in limiting ischemia-reperfusion-induced myocardial injury through preservation and restoration of the antioxidant defense system.[13] Centella was also shown to possess other diverse pharmacological effects in experimental models, including antiulcer, antimicrobial, immunomodulatory, and spasmolytic activity.[2]

CLINICAL APPLICATIONS

Centella is a valuable agent for the healing of wounds and the treatment of venous insufficiency and may also prove useful in improving mental function. Table 64.1 provides an abridged list of documented clinical applications of *C. asiatica*. Details on some of the more popular uses of centella are discussed in the following sections.

Burns

The standardized extract from *C. asiatica* has been used effectively in the treatment of patients with second- and third-degree burns caused by boiling water, electrical current, or gas explosion. Daily local application, intramuscular injections of the extract, or both, resulted in excellent results when treatment was started immediately after the accident.[14] The extract prevented or limited the shrinking and swelling of the skin caused by skin infection, and it inhibited scar formation, increased healing, and decreased fibrosis. A randomized controlled study compared the efficacy of silver sulfadiazine versus Centiderm (an ointment made from an ethanol extract of fresh *C. asiatica* leaves) in patients with partial-thickness burns from October 2014 to February 2015 in Iran.[15] Burn wounds were treated once daily, and patients followed up daily with a burn specialist until the wounds were completely healed, with subjective and objective variables recorded on days 3, 7, and 14. The use of Centiderm ointment not only improved the objective and subjective findings in less than 3 days but also improved the reepithelialization and complete healing times (about 7 days sooner compared with placebo).

Cellulite and Striae Gravidarum

Cellulite, known as liposclerosis, is a noninflammatory change within the subcutaneous adipose tissue caused by an increase in the volume of fat cells or by the increased division of connective tissue, which causes constriction of small blood vessels. Treatments for cellulite involve preparations that affect the adipose and connective tissue and improve microcirculation; they can be used topically, internally, and/or transdermally.

The effect of centella in the treatment of cellulite appears to be related to its ability to enhance connective tissue structure and reduce sclerosis by acting directly on fibroblasts. Madecassoside, an extract from *C. asiatica,* is known to induce collagen expression and/or modulate inflammatory mediators.[16] Triterpenes of *C. asiatica* increase the metabolism of lysine and proline, the amino acids that build collagen, and increase the synthesis of tropocollagen and mucopolysaccharide in the connective tissues, thus improving the nutrition of tissues and providing connective vascular stimulation.[17]

In several clinical studies, standardized extracts of *C. asiatica* demonstrated good results in the treatment of cellulite and the prevention of stretch marks during pregnancy. In the treatment of cellulite, Bourguignon observed the action of the extract on several types of cellulite in 65 patients who underwent other therapies without success.[18] Over a period of 3 months, good results were produced in 58% of the patients and satisfactory results in 20%. A study of 60 people with cellulite evaluated the influence of Madecassol® applied four times a day for 4 months.[19] Results clearly showed a beneficial effect of the gotu kola extract on inhibiting the progression of cellulite and a significant improvement in the skin condition in 85% of the experiment participants.

A randomized, double-blind, placebo-controlled trial of the cream Trofolastin®, containing an extract of *C. asiatica,* α-tocopherol, hydrolyzed collagen, and elastin, was carried out on 100 pregnant women.[20] The cohort was split evenly between the treatment group and the placebo group. After 30 months of treatment, striae occurred in 56% of the placebo group compared with 34% of the treated group; this difference was statistically significant. In women with a history of striae during puberty, the active cream induced a significant absolute prevention in 89% of the cases, whereas in the placebo group, all the women developed striae.

Cirrhosis of the Liver

Darnis et al. reported on the therapeutic use of an extract of *C. asiatica* in alcohol-induced cirrhosis (six patients), cirrhosis of unknown etiology (two patients), and chronic hepatitis.[21] In the cirrhosis patients, improvement in the histological findings and regression of inflammatory infiltration were observed. No effect was observed in the patients with chronic hepatitis. Other reports supported the use of centella in fibrotic conditions of the liver.[22]

Keloids

Keloids and hypertrophic scars are characterized by a prolonged inflammatory phase that may go on for months or even years without progressing to the maturation phase. The inflammatory phase is characterized histologically by large numbers of immature, swollen collagen bundles intermingled with inflammatory debris, whereas the maturation phase is characterized by mature fibrocytes, normal collagen fibers, and few inflammatory cell elements.

The standardized extract of *C. asiatica* demonstrated impressive clinical results in the treatment of keloids and hypertrophic scars.[23–25]

The mechanism of action appears to be multifaceted but is primarily the result of reducing the inflammatory phase of scar formation while simultaneously enhancing the maturation phase of scar formation. Asiaticoside has been shown to downregulate the expression of TGF-β1 mRNA and tissue inhibitors of metalloproteinases-1 and upregulate TGF-β3 mRNA expression, resulting in the ability to decompose the products of type I collagen and reduce hypertrophic scars.[26] It has been further proposed that asiaticoside interferes with scar formation by increasing the synthesis of acidic mucopolysaccharides and increasing the activity of myofibroblasts. In one study, 227 patients with keloids or hypertrophic scars were treated by oral administration with a standardized centella extract (effective dosage 60–90 mg). The centella extract was used alone in 139 patients (the curative group), and 88 used the extract along with surgical scar revision (preventive group).[24] In the curative group, 116 patients (82%) were found to have benefited from the extract after 2 to 18 months, either by relief of their symptoms or by the disappearance of the inflammatory phase. In a double-blind substudy of 46 of the 139 patients, 22 of 27 receiving the extract improved, whereas only 9 of 19 given a placebo improved. In the preventive group, the centella extract also demonstrated a significant positive effect. The therapeutic course in these patients began a few weeks before surgery. If a positive response was observed, the patient was brought to surgery and kept on the centella extract for 3 months. (This method of preselection allowed the researchers to offer other forms of therapy to unresponsive patients.) Clinical improvement was observed in 72 of the 88 patients (79%).

Kidney Disease

Traditional Chinese medicine has used gotu kola to treat kidney diseases for centuries. Its direct benefits for the kidneys have only been shown in animals but are encouraging. In rats, gotu kola showed a protective effect from Adriamycin-induced nephropathy, resulting in dramatically improved kidney function.[27] Another study combined gotu kola in conjunction with naringenin and showed decreased fibrosis formation in the kidneys.[28]

Leprosy

Several investigators reported impressive clinical results using *C. asiatica* and its extracts (oral, intramuscular, or topical) in the treatment of leprosy in both uncontrolled and controlled studies.[29,30] The therapeutic response was comparable to that of dapsone, the standard allopathic drug used in the treatment of leprosy.

In addition to its wound-healing activity, it appears that oxyasiaticoside, an oxidized form of asiaticoside, inhibited the growth of the tubercle bacillus in vitro and in vivo by dissolving the waxy coating of *Mycobacterium leprae*.[5]

Improving Mental Function and Quality of Life

The neuropharmacological properties of *C. asiatica* have been extensively studied. Mechanisms of action for its efficacy in memory disorders involve antioxidant, free radical scavenging, and cholinergic modulatory activities. Although long used to promote improved mental function, the first clinical investigation involving centella significantly increased the mental abilities of 30 developmentally disabled children.[31] After a 12-week period, the children were more attentive and better able to concentrate on assigned tasks.

Based on historical use and clinical studies, centella may also help improve mental function and quality of life in the elderly. Eighty elderly subjects participated in a double-blind study and were randomly assigned to receive placebo or standardized extract of *C. asiatica* at doses of 250, 500, and 750 mg once daily for 90 days.[32] The subjects were evaluated to establish baseline data of physical performance using the 30-second chair-stand test, hand-grip test, and 6-minute walk test. The health-related quality of life was assessed using a standard questionnaire. These assessments were repeated every month throughout the 3-month experimental period using the aforementioned parameters. Moreover, 1 month after the cessation of *C. asiatica* treatment, all subjects were also evaluated using these parameters again. The results showed that after 2 months of treatment, *C. asiatica* at doses of 500 and 750 mg/day increased lower extremity strength assessed via the 30-second chair-stand test. In addition, the higher doses of *C. asiatica* improved the life satisfaction subscale within the physical function subscale. Therefore the results from this study appear to support the traditional reputation of *C. asiatica* on strength improvement, especially in the lower extremities of the elderly. *C. asiatica* also possesses the potential to be a natural resource for increasing vigor and strength in healthy elderly persons.

Asiatic acid exerts a significant neuroprotective effect on cultured cortical cells by potentiation of the cellular oxidative defense mechanism, which may prove efficacious in protecting neurons from the oxidative damage caused by exposure to excessive glutamate.[33] The plant accelerates nerve regeneration and contains multiple active fractions that increase neurite elongation in vitro, suggesting that components in centella may be useful for accelerating repair of damaged neurons.[34]

Centella is also being investigated for its potential in Alzheimer's disease as an inhibitor of β-amyloid (Aβ) formation.[35,36] Water extracts of *C. asiatica* inhibit phospholipase A2 (PLA2) enzymes, particularly cPLA2 and sPLA2, which play key roles in Aβ-induced neurotoxicity.[37] Further studies on the water extract found that caffeoylquinic acids act as the active substances preventing Aβ-induced cell death by enhancing mitochondrial biogenesis in conjunction with activating antioxidant response genes and normalizing calcium homeostasis.[38]

Relieving Anxiety

Centella is viewed as an anxiolytic on the basis of in vitro models and emerging clinical evidence. An early study was conducted in humans to measure the effect of centella on reducing the acoustic startle response. After a single administration of centella, subjects experienced a significantly reduced acoustic startle response compared with the placebo—a clear indication of significant antianxiety action.[39] In an open clinical study in patients with generalized anxiety disorder, a 70% hydroethanolic extract of *C. asiatica* (500 mg/capsule, twice daily, after meal) was shown to significantly ($P < 0.01$) attenuate anxiety-related disorders and reduce the stress phenomenon and depression.[40]

Scleroderma

A standardized extract of *C. asiatica* was tested in several trials in the treatment of scleroderma (including systemic sclerosis).[41–44] In addition to decreasing skin induration, patients noticed a lessening of arthralgia and improved finger motility. Presumably, the positive therapeutic response was a result of centella's eutrophic effect on connective tissue, thereby preventing the excessive collagen synthesis observed in scleroderma.

Venous and Microcirculatory Disorders

The most well-documented clinical utility for a standardized extract of *C. asiatica* is in the treatment of chronic venous insufficiency, venous hypertension, and microcirculatory disorders.[45–51] The effect of *Centella* in venous insufficiency and varicose veins appears to be related to its ability to enhance connective tissue structure, reduce sclerosis, and improve blood flow through the affected limbs. Asiaticoside has been shown to improve microcirculation and reverse fibrosis in humans with varicose veins. 3, 5-Di-O-caffeoylquinic acid, a constituent of gotu kola, has been found to display an antithrombotic and

inhibitory effect on dynamic coagulation.[52] This is significant when one considers that fibrin clots play a pivotal role in cardiovascular conditions.

In the treatment of chronic venous insufficiency, significant improvement in symptomatology (e.g., feelings of heaviness in the lower legs, paresthesias, nocturnal cramps), physical findings (e.g., edema, telangiectasias, trophic ulcers, vein distensibility), and functional capacity (improved venous flow) was observed in approximately 80% of patients in the clinical trials.[1,45–51]

In studies of patients with venous hypertension (ambulatory venous pressure >42 mm Hg), detailed evaluations using laser Doppler flowmetry, transcutaneous oxygen, and carbon dioxide tension measurements and the determination of capillary filtration rate, ankle circumference, and ankle edema, along with detailed symptom evaluation, clearly demonstrated that centella extracts were clinically effective in dealing with this disorder.[45–47]

The standardized extract of *C. asiatica* also showed an ability to improve diabetic microangiopathy, it prevented the edema and microcirculation alterations seen during medium- to long-distance flights, and it normalized the echographic character of carotid arteries with significant plaque.[49–51]

Wound Healing

Standardized extracts of *C. asiatica* were shown in many clinical studies to greatly aid wound repair.[53,54] The types of wounds healed include the following:

- Surgical wounds, such as episiotomies and ear, nose, and throat surgeries
- Skin ulcers caused by arterial or venous insufficiency
- Traumatic injuries to the skin
- Gangrene
- Skin grafts
- Schistosomiasis lesions
- Perineal lesions produced during childbirth

DOSAGE

The majority of clinical studies on *C. asiatica* used proprietary formulas available in Europe (e.g., Madecassol, TECA, Centelase). These standardized extracts contain asiaticoside (40%), asiatic acid (29%–30%), madecassic acid (29%–30%), and madecassoside (1%–2%).

Because the concentration of triterpenes in centella varies between 1.1% and 8%, it is difficult to calculate an appropriate dosage when simply using the crude plant material. However, because most samples yield a concentration between 2.2% and 3.4%, approximately 2 to 4 g/day of crude plant material would contain an appropriate quantity of triterpenes, although it is unknown if this correlates with the clinical efficacy of the standardized extracts.

Daily dosages of the various forms of centella are as follows:

- Standardized extract (40% asiaticoside, 29%–30% asiatic acid, 29%–30% madecassic acid, and 1%–2% madecassoside): 60 to 180 mg/day
- Crude dried plant leaves: 2 to 4 g/day
- Tincture (1:5): 10 to 20 mL/day
- Fluid extract (1:1): 2 to 4 mL/day

TOXICOLOGY

C. asiatica and its extracts are well tolerated, especially orally.[1,2] There is one report of three case histories of women (61, 52, and 49 years old) who developed jaundice after taking *C. asiatica* for 30, 20, and 60 days, respectively.[55] However, there was little description or validation of the product in question. Nonetheless, the hepatic lesions were quite significant on biopsy (granulomatous hepatitis with marked necrosis and apoptosis, chronic hepatitis with cirrhotic transformation and intense necroinflammatory activity, and granulomatous hepatitis). All patients improved with *C. asiatica* discontinuation and ursodeoxycholic acid 10 mg/kg per day.

The topical application of a salve containing centella was reported to cause contact dermatitis, although quite infrequently.[1]

Although the oral administration of asiaticoside at a dose of 1 g/kg body weight was not proved toxic in toxicology studies, the toxic dose of asiaticoside by intramuscular application to mice and rabbits was reported as 40 to 50 mg/kg body weight.[1]

Asiaticoside was implicated as a possible skin carcinogen when repeated applications were used in an experimental animal model.[56] Teratologic studies using the extract in rabbits proved negative.[23]

DRUG INTERACTIONS

There are no known drug interactions at this time.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Kartnig T. Clinical applications of *Centella asiatica* (L) Urb. In: Craker LE, Simon JE, eds. *Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology*. Vol. 3. Phoenix: Oryx Press; 1989:145–173.
2. Brinkhaus B, Lindner M, Schuppan D, et al. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine*. 2000;7:427–448.
3. Bonte F, Dumas M, Chaudagne C, et al. Influence of asiatic acid, madecassic acid, and asiaticoside on human collagen I synthesis. *Planta Med*. 1994;60:133–135.
4. Duke JA. *Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1985.
5. Boiteau P, Ratsimamanga AR. Asiaticoside extracted from *Centella asiatica*, its therapeutic uses in the healing of experimental or refractory wounds, leprosy, skin tuberculosis, and lupus. *Therapie*. 1956;11:125–149.
6. Anonymous. Monograph. *Centella Asiatica*. Milan: Indena S.p.A; 1987.
7. Veerendra Kumar MH, Gupta YK. Effect of different extracts of *Centella asiatica* on cognition and markers of oxidative stress in rats. *J Ethnopharmacol*. 2002;79:253–260.
8. Lee MK, Kim SR, Sung SH, et al. Asiatic acid derivatives protect cultured cortical neurons from glutamate-induced excitotoxicity. *Res Commun Mol Pathol Pharmacol*. 2000;108:75–86.
9. Veerendra Kumar MH, Gupta YK. Effect of *Centella asiatica* on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats. *Clin Exp Pharmacol Physiol*. 2003;30:336–342.
10. Chauhan PK, Pandey IP, Dhatwalia VK. Evaluation of the antidiabetic effect of ethanolic and methanolic extracts of *Centella asiatica* leaves extract on alloxan induced diabetic rats. *Adv Biol Res*. 2010;4(1):27–30.
11. Bunpo P, Kataoka K, Arimochi H, et al. Inhibitory effects of *Centella asiatica* on azoxymethane-induced aberrant crypt focus formation and carcinogenesis in the intestines of F344 rats. *Food Chem Toxicol*. 2004;42(12):1987–1997.
12. Jamil SS, Nizami Q, Salam M. *Centella asiatica* (linn.) urban: a review. *Nat Prod Res*. 2007;6:158–170.
13. Gnanapragasam A, Ebenezar KK, Sathish V, Govindaraju P, Devaki T. Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. *Life Sci*. 2004;76:585–597.
14. Gravel JA. Oxygen dressings and asiaticoside in the treatment of burns. *Laval Med*. 1965;36:413–415. [French].
15. Saeidinia A, Keihanian F, Lashkari AP, et al. Partial-thickness burn wounds healing by topical treatment: a randomized controlled comparison between silver sulfadiazine and Centiderm. *Medicine (Baltimore)*. 2017;96(9):e6168. PubMed PMID:28248871.
16. Haftek M, Mac-Mary S, Le Bitoux MA, et al. Clinical, biometric and structural evaluation of the long-term effects of a topical treatment with ascorbic acid and madecassoside in photoaged human skin. *Exp Dermatol*. 2008;17(11):946–952. PubMed PMID:18503551.
17. Goldman MP, Bacci PA, Leibaschoff G. *Cellulite: Pathophysiology and Treatment*. New York, London: Taylor & Francis; 2006.
18. Bourguignon D. Study of the action of titrated extract of *Centella asiatica*. *Gaz Med Fr*. 1975;82:4579–4583.
19. Brinkhaus B, Lindner M, Schuppan D, Hahn EG. Chemical, pharmacological and clinical profile of the East Asian medical plant Centella asiatica. *Phytomedicine*. 2000;7(5):427–448. PubMed PMID:11081995.
20. Mallol J, Belda MA, Costa D, Noval A, Sola M. Prophylaxis of striae gravidarum with a topical formulation. A double blind trial. *Int J Cosmet Sci*. 1991;13(1):51–57. 19291041.
21. Darnis F, Orcel L, de Saint-Maur PP, et al. Use of a titrated extract of *Centella asiatica* in chronic hepatic disorders. *Sem Hop*. 1979;55:1749–1750. [French].
22. El Zawahry MD, Khalil AM, El Banna MH. Madecassol, a new therapy for hepatic fibrosis. *Bull Soc Int Chir (Belgium)*. 1975;34:573–577.
23. Bosse JP, Papillon J, Frenette G, et al. Clinical study of a new antikeloid agent. *Ann Plast Surg*. 1979;3:13–21.
24. Basset A, Ullmo A, Maleville J, et al. Treatment of keloids with Madecassol per os. *Bull Soc Fr Dermatol Syphiligr*. 1970;77:826–827. [French].
25. Ippolito F. Medical treatment of keloids. *G Ital Dermatol*. 1977;112:377–381.
26. Zhang T, Rong XZ, Yang RH, Li TZ, Xu YB. Effect of asiaticoside on the expression of transforming growth factor-beta mRNA and matrix metalloproteinases in hypertrophic scars. *Nan Fang Yi Ke Da Xue Bao*. 2006;26(1):67–70. PubMed PMID:16495179.
27. Wang Z, Liu J, Sun W. Effects of asiaticoside on levels of podocyte cytoskeletal proteins and renal slit diaphragm proteins in Adriamycin-induced rat nephropathy. *Life Sci*. 2013;93(8):352–358.
28. Meng XM, Zhang Y, Huang XR, et al. Treatment of renal fibrosis by rebalancing TGF-β/Smad signaling with the combination of asiatic acid and naringenin. *Oncotarget*. 2015. https://doi.org/10.18632/oncotarget.6100.
29. Chakrabarty T, Deshmukh S. Centella asiatica in the treatment of leprosy. *Sci Cult*. 1976;42:573.
30. Chaudhuri S, Ghosh S, Chakraborty T, et al. Use of a common Indian herb "Mandukaparni" in the treatment of leprosy. *J Indian Med Assoc*. 1978;70:177–180.
31. Appa Rao MVR, Srinivasan K, Koteswara RTL. The effect of *Centella asiatica* on the general mental ability of mentally retarded children. *Indian J Psychiatry*. 1977;19:54–59.
32. Mato L, Wattanathorn J, Muchimapura S, et al. *Centella asiatica* improves physical performance and health-related quality of life in healthy elderly volunteers. *Evid Based Complement Alternat Med*. 2011; Article ID 579467.
33. Park BC, Bosire ES, Lee YS, Kim JA. Asiatic acid induces apoptosis in SK-MEL-2 human melanoma cells. *Cancer Letters*. 2005;218(1):81–90.
34. Soumyanath A, Zhong YP, Gold SA, Yu X, Koop DR, Bourdette D, et al. *Centella asiatica* accelerates nerve regeneration upon oral administration and contains multiple active fractions increasing neurite elongation in vitro. *J Pharm Pharmacol*. 2005;57(9):1221–1229.
35. Dhanasekaran M, Holcomb LA, Hitt AR, et al. *Centella asiatica* extract selectively decreases amyloid beta levels in hippocampus of Alzheimer's disease animal model. *Phytother Res*. 2009;23:14–19.
36. Xu Y, Cao Z, Khan I, et al. Gotu kola *(Centella asiatica)* extract enhances phosphorylation of cyclic AMP response element binding protein in neuroblastoma cells expressing amyloid beta peptide. *J Alzheimers Dis*. 2008;13:341–349.
37. Defillipo PP, Raposo AH, Fedoce AG, et al. Inhibition of cPLA2 and sPLA2 activities in primary cultures of rat cortical neurons by *Centella asiatica* water extract. *Nat Prod Commun*. 2012;7(7):841–843. PubMed PMID:22908561.
38. Gray NE, Sampat H, Zweig JA, Quinn JF, Soumyanath A. *Centella asiatica* attenuates amyloid-β-induced oxidative stress and mitochondrial dysfunction. *J Alzheimers Dis*. 2015;45(3):933–946. PubMed PMID:25633675.
39. Bradwejn J, Zhou Y, Koszycki D, et al. A double-blind, placebo-controlled study on the effects of gotu kola *(Centella asiatica)* on acoustic startle response in healthy subjects. *J Clin Psychopharmacol*. 2000;20:680–684.
40. Jana U, Sur TK, Maity LN, Debnath PK, Bhattacharyya D. A clinical study on the management of generalized anxiety disorder with *Centella asiatica*. *Nepal Med Coll J*. 2010;12(1):8–11. PubMed PMID:20677602.
41. Bletry O. Comment on the treatment of scleroderma. *Gaz Med Fr*. 1980;87:1989–1990.
42. Fontan I, Rommel A, Geniaux M, et al. Localized scleroderma. *Concours Med*. 1987;109:498–504.
43. Sasaki S, Shinkai H, Akashi Y, et al. Experimental and clinical effects of asiaticoside (Madecassol) on fibroblasts, granulomas, and scleroderma. *Jap J Clin Dermatol*. 1971;25:585–593.
44. Sasaki S, Shinkai H, Akashi Y, et al. Studies on the mechanism of action of asiaticoside (Madecassol) on experimental granulation tissue and cultured fibroblasts and its clinical application in systemic scleroderma. *Acta Derm Venereol*. 1972;52:141–150.
45. Incandela L, Belcaro G, De Sanctis MT, et al. Total triterpenic fraction of *Centella asiatica* in the treatment of venous hypertension: a clinical, prospective, randomized trial using a combined microcirculatory model. *Angiology*. 2001;52(suppl 2):S61–S67.

46. De Sanctis MT, Belcaro G, Incandela L, et al. Treatment of edema and increased capillary filtration in venous hypertension with total triterpenic fraction of *Centella asiatica*: a clinical, prospective, placebo-controlled, randomized, dose-ranging trial. *Angiology*. 2001;52(suppl 2):S55–S59.
47. Cesarone MR, Belcaro G, Rulo A, et al. Microcirculatory effects of total triterpenic fraction of *Centella asiatica in* chronic venous hypertension: measurement by laser Doppler, TcPO2-CO_2, and leg volumetry. *Angiology*. 2001;52(suppl 2):S45–S48.
48. Cesarone MR, Belcaro G, De Sanctis MT, et al. Effects of the total triterpenic fraction of Centella asiatica in venous hypertensive microangiopathy: a prospective, placebo-controlled, randomized trial. *Angiology*. 2001;52(suppl 2):S15–S18.
49. Cesarone MR, Incandela L, De Sanctis MT, et al. Evaluation of treatment of diabetic microangiopathy with total triterpenic fraction of *Centella asiatica*: a clinical prospective randomized trial with a microcirculatory model. *Angiology*. 2001;52(suppl 2):S49–S54.
50. Cesarone MR, Incandela L, De Sanctis MT, et al. Flight microangiopathy in medium- to long-distance flights: prevention of edema and microcirculation alterations with total triterpenic fraction of *Centella asiatica*. *Angiology*. 2001;52(suppl 2):S33–S37.
51. Cesarone MR, Belcaro G, Nicolaides AN, et al. Increase in echogenicity of echolucent carotid plaques after treatment with total triterpenic fraction of *Centella asiatica*: a prospective, placebo-controlled, randomized trial. *Angiology*. 2001;52(suppl 2):S19–S25.
52. Satake T, Kamiya K, An Y, Oishi Nee Taka T, Yakamoto J, et al. The anti-thrombotic active constituents from *Centella asiatica*. *Biol Pharm Bull*. 2007;30(5):935–940.
53. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN. In vitro and in vivo wound healing activity of asiaticoside isolated from *Centella asiatica*. *J Ethnopharmacol*. 1999;65(1):1–11. 10350364.
54. Paocharoen V. The efficacy and side effects of oral *Centella asiatica* extract for wound healing promotion in diabetic wound patients. *J Med Assoc Thai*. 2010;93(suppl 7):S166–S170. PubMed PMID:21298840.
55. Jorge OA, Jorge AD. Hepatotoxicity associated with the ingestion of *Centella asiatica*. *Rev Esp Enferm Dig*. 2005;97:115–124.
56. Laerum OD, Iversen OH. Reticuloses and epidermal tumors in hairless mice after topical skin applications of cantharidin and asiaticoside. *Cancer Res*. 1972;32:1463–1469.
57. Bensaude A. The treatment of anal fissure. *Phleobologie*. 1980;33:683–688. [French].
58. Fam A. Use of titrated extract of *Centella asiatica* (TECA) in bilharzial bladder lesions. *Int Surg*. 1973;58:451–452.
59. Etrebi A, Ibrahim A, Zaki K. Treatment of bladder ulcer with asiaticoside. *J Egypt Med Assoc*. 1975;58:324–327.
60. Sterkers Desagnat M, Philbert M, Moreau L. Medical treatments for benign disease of the breast. *Therapeutique*. 1975;51:121–124.
61. Guarnerio F, Sansonetti G, Donzelli R, et al. Treatment of hemorrhoids with *Centella asiatica*. *G Ital Angiol*. 1986;6:46–52.
62. Gohil KJ, Patel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian J Pharma Sci*. 2010;72(5):546–556. PubMed PMID:21694984.
63. Kyoo WC. Medical treatment of peptic ulcer. *J Korean Med Assoc*. 1980;23:31–35.
64. Pergola F. Treatment of peptic ulcer with a titrated extract of *Centella asiatica*. *Med Chir Dig*. 1974;36:445–448.
65. Baudon-Glanddier B. Perineal lesions and asiaticoside. *Gaz Med Fr*. 1963;70:2463–2464.
66. Benedicenti A, Galli D, Merlini A. The clinical therapy of periodontal disease. The use of potassium hydroxide and the water-alcohol extract of *Centella asiatica* in combination with laser therapy in the treatment of severe periodontal disease. *Parodontol Stomatol*. 1985;24:11–26. [Italian].
67. Abou Shousha ES, Khalil HA. Effect of asiaticoside (Madecassol) on the healing process in cataract surgical wounds and retinal detachment operations (clinical and experimental study). *Bull Ophthalmol Soc Egypt*. 1967;60(64):451–470. PubMed PMID: 5602933.
68. Bazex J, Nogue J, Peyrot J. Periulcerous eczema type cutaneous reaction during and after ulcers of the leg. *Rev Med Toulouse*. 1982;18:171–174.
69. Dulauney MM. Postphlebitic leg ulcers and indications for therapy. *Bord Med*. 1979;12:1807–1810.
70. Sevin P. Some observations on the use of asiaticoside (Madecassol) in general surgery. *Progr Med (France)*. 1962;90:23–24.
71. King DS. Tuberculosis. *N Engl J Med*. 1950;243:530–536. 565–571.

65

Chinese Prepared Medicines

Mark Harrison Nolting, ND, EAMP

OUTLINE

INTRODUCTION

Chinese herbal medicine (CHM) is considered a key part of traditional Chinese medicine (TCM). TCM providers have spread across the world over the past 30 years in increasing numbers. As a result, those experienced in CHM have set up practices, become educators, established herb stores, and in some cases established growing operations and developed TCM trading companies.

This chapter focuses on one very popular and widely used segment of CHM: the Chinese prepared medicines (CPMs). These preparations were referred to as patent medicines for hundreds of years, then more properly as prepared medicines, and as the regulatory oversight of such products evolved (at least in the United States and Europe), as herbal supplements. The landscape of these "over-the-counter" (OTC) medicines (or supplements) is changing because of regulation, economics (large global profits), adulterants, endangered and extinct species, and basic supply and demand. Chinese "patent" medicines were exported from China to the tune of $160 million U.S. dollars in 2009, a growing number partly a result of the increased effort by the Chinese government to promote TCM globally.[1] The total value of exported traditional Chinese medicines in 2017 was $3.64 billion. Of that total, CPM exports had climbed to $250 million. That is just more than an 11% rise from 2016 and way up from the $160 million in 2009. The famous Tong Ren Tang TCM pharmacy company at the end of 2017 increased its international outlets to 140 in 27 different countries.[2] There was a sense in China (among some in the government) that the Chinese had lost ground over the years in the trading of herbal medicines and in the marketing of their traditional medicine. As the global herb market rapidly develops, China finds itself accounting for an increasing share of the market.[3]

East Asian medicine (EAM) is defined as a "medical system that arose in East Asia deriving from the traditional concepts of Qi and Yin-Yang Theory. EAM has developed various interventions such as acupuncture and moxibustion, massage and physical manipulation, diet therapy, herbal medicine, meditation and exercise."[4,5] Although acupuncture is the global face of Chinese or EAM, CHM is the therapeutic core. The "new face" of EAM is the hope, among Asian governments, "that high-volume screening and rigorous clinical trials will unlock the secrets of ancient herbal remedies and that the results will pass muster with Western scientists."[6]

The CHM (*Zhong-Yao* in Chinese pin-yin) pharmacy has evolved over many hundreds of years and is massive in scope and application, as the following "snapshot" of usage in China indicates:
Listed medicinal plants: 11,146
Animal drugs: 1581
Mineral drugs: 80
Processed remedies: 50
Clinically "proven" folk medicines: 5000
Traditional formulas: 84,464[7]

CHM is widely practiced across the world. In the United States, CHM is widely taught in most acupuncture school curriculums. However, for the most part, many students among the 62 (as of 2018) accredited schools in the country concentrate on acupuncture, with CHM as a secondary study,[8] but not all by any means. In California specifically, CHM is a major part of the study, licensure, and practice of acupuncturists.[8]

CPM is the OTC medicine of the traditional Chinese pharmacy, which includes thousands of various preparations of classic and conventional Chinese herbal formulas. Much of this medicine has been in constant use for hundreds, even thousands, of years, often maintaining original formulations.

One example is a frequently sold CPM called Jie Geng Wan (*Platycodon* root pills).[9] The source of this respiratory system medicine is the *Jing Kui Yao Lue Fang Lun* ("Synopsis of the Golden Chamber"), written by Zhang Zhong Jing in 219 A.D.[10] Zhu, writing in his highly useful and informative *Clinical Handbook of Chinese Prepared Medicines,* explained the following:

> *The overtaxed Sung-dynasty merchant, as well as the frazzled Ming mother, appreciated the simple convenience and economy of a well-prepared herbal pill, powder, or syrup as much as does his or her modern counterpart.*
>
> *Yan Bian Lian, the phrase commonly used when referring to these Zhong Cheng Yao ["Chinese Ready-to-Be-Taken Medicines"], may best express fundamental reason-for-being of ready-to-take medicaments in any tradition: "Effectiveness, Convenience, Economy."*[11]

Secret family (or company) recipes called patent medicines were first produced in the Song Dynasty (960–1234 AD) and were dispensed by government agencies, such as the Imperial Benevolent Prescriptions

of the Taiping Period.[12] Few true patent medicines exist anymore in the Chinese pharmacy. Many of these medicines have become part of the public domain, making it more appropriate to refer to them collectively as CPMs.

> *Keeping Chinese medicine formulae as trade secrets has led to some losses of knowledge and experience accumulated over generations. Drugs as well as dietary supplements are subject to regulations and disclosure requirements; it has become increasingly difficult for Chinese medicine (CM) practitioners to keep their prescriptions and treatment methods as trade secrets. In 1993 the Chinese government issued the Regulations on the Protection of Traditional Chinese Medicines which, to this date, provides administrative protection to certain prescriptions and manufacturing processes.*[13]

Unfortunately, there are too many dubious CPMs in the worldwide market that have been adulterated in various ways, some containing Western prescriptive medicines, toxic elements, and endangered species often hidden from the unsuspecting consumer.[14] In their quest for safe, natural, and effective medicine, consumers may find serious, unexpected medical consequences. This is an issue that deserves scrutiny by consumers, practitioners, and regulators alike. As with most natural products, CPM quality and purity are left to the discretion of the companies themselves.

Typical images of the U.S. "Wild West" include cowboys, American Indians, farms, and the frontier life. However, there was another, lesser-known presence in the West: the lives of Chinese immigrants. Many came to America seeking life on the "Gold Mountain," the view of America that had been created in China during the 1800s. According to Paul Buell, writing in his *Chinese Medicine on the Golden Mountain* book:

> *When in the midnineteenth century Chinese immigrants began to arrive in America, all kinds of traditional medical practitioners came with them; well-practiced Chinese medicine was often superior to contemporary western practice.*[15]

The immigrants, mostly men, left their families to mine for gold. Many ended up working as miners or other laborers. They brought with them their traditional form of Chinese medicine and their traditional herbal medicine, which included various forms of CPM. CHM is a highly evolved system of traditional medicine that draws from a *materia medica* composed of thousands of individual herbs (herbs in TCM include plant, mineral, and animal).[11,16] CPMs would generally be considered an adjunct to CHM in most practices because they are convenient and good alternatives to the strong, bitter-tasting decoctions often used in practice. Many practitioners, particularly in the West, prefer to use granulated Chinese herbs or powdered herbs.

Global Market

The market for CPMs is huge. More than 5000 "licensed patent medicines" are estimated to exist in China.[17] According to *China Today,* exports of CHMs worldwide reached $20 billion in 2002. About 5% of this amount was China's share, and prepared medicines comprised less than one-third of this. In China, the famous Tong Ren Tang factory, which exports the most CPMs in China, earned $14.5 million.[18] This amount continued to steadily rise into 2018.

Outside of the enormous "inside China market," the growing interest in and use of natural medicine worldwide are driving the consumption of prepared medicines rapidly upward. As mentioned previously, this growth has sparked abuse and problems with product labeling and safety that the entire profession is working to counteract.

Although Chinese medicine and pharmacy began to spread outside of China's borders nearly 2000 years ago, it is only in the past 30 years that interest has grown in North America. Much of this is a result of former President Richard Nixon's trip to the People's Republic of China in the early 1970s and subsequent reporters' visits and articles, especially those of James Reston of the *New York Times.* The growth of acupuncture and oriental medical practices, such as herbal medicine, in the United States has been steady since that time.

The traditional loose herb recipes used in CHM are cooked and made into medicinal soups, which are often bitter and difficult for the American palate. The Chinese solution to this dilemma was to increase the use of raw licorice in the formula and to offer sugar wafers to the patient to help offset the bitter taste, although most Chinese welcome a bitter taste as a normal part of Asian cuisine. The prepared medicines offer an alternative to traditional preparations. A number of companies, particularly in Taiwan and California, have begun to popularize the manufacture and sale of powder and tincture formulas in the single herb form to help "bridge" the traditional form and the prepared, more palatable, forms of medicine. This allows the practitioner to still use the traditional approach—that of mixing and adjusting the formulas tailored to the patient's individual diagnosis. Prepared formulas are fixed, of course, and do not allow any adjustments in the ingredients.

Before the mid-1970s, the U.S. Food and Drug Administration (FDA) restricted the import and sale of traditional medicines by ethnic groups. New legislation and court rulings since then have lifted these restrictions, which, coupled with the explosion of acupuncture on the American health scene, has opened the door to the spread of prepared medicines.[19]

Now, because of this rapid growth in the sale and use of prepared medicines, problems with adulterants and false labeling are increasing, especially in California, where a multiagency herbal medicine task force involving numerous California and federal government agencies was formed in response. The California Department of Health Services documents state that "most imported Asian patent medicines do not fully comply with California laws."[19] Although some of these violations are potentially dangerous and may involve toxic ingredients and the inclusion of endangered species, many involve labeling issues, which can often be sorted out by revising the labeling at the company of origin.

FORMULATIONS AND PREPARATIONS

CPMs are prepared in numerous different forms. As far back as 200 BC, Chinese pharmaceuticals had a surprising level of sophistication. Pills were bound together using a wide variety of animal and human substances.[20] Box 65.1 lists 15 of the most common dose forms of CPM. In China, prepared medicines are produced at factories located throughout the country. Reputation and awards are important to these mainland Chinese operations. Factories such as the Chongqing Tong Jun Ge Medicine Works in the Sichuan province, built in 1908, are proud of their products. This factory produces more than 200 medicines in 14 therapeutic categories.[20]

Roadside and rooftop billboards across China extol the benefits of "famous" CPMs and display the awards various formulas have received over the years. Longevity is an important virtue attributed to specific herbs and formulas in Asian medicine. Ginseng, in all its many species and preparations, is the most revered and used herb in China. The pursuit of longevity is central to its popularity, aside from the fact that it is a highly useful and effective herb. An interesting side note is that the American species of ginseng, *Panax quinquefolius,* is exported in great quantities to China, only to be repackaged and exported back to Western markets. American ginseng is considered a milder, more "supportive" ginseng in action, making it more suitable for the elderly and weak.

BOX 65.1 Common Dose Forms of Chinese Prepared Medicines

- Pills (honey, water, glue, extract)
- Paste
- Concentrated
- Powders
- Granules
- Pellet
- Tablets
- Syrup
- Mixtures
- Dripping pills
- Capsule
- Hard
- Soft extract
- Enteric
- Wine
- Tinctures
- Liquid extracts
- Plasters (adhesive)
- Ointments
- Medicinal distillates
- Tea
- Injections
- Suppositories
- Lozenges

Modified from the Pharmacopeia Commission of PRC. *Pharmacopeia of the People's Republic of China* (English edition), Vol. 1. Beijing: Chemical Industry Press; 1997:A3–A14; Chen JK, Chen TT. *Chinese Herbal Formulas and Applications. Pharmacological Effects & Clinical Research.* City of Industry, CA: Art of Medicine Press, Inc., 2009:16–18.

Ginsengs are part of the tonic herb family, a major classification that does not exist in the conventional Western pharmacy. The most famous factory for herbal medicine products in China is the Tong Ren Tang Pharmacy in Beijing. The Tong Ren Tang Pharmacy, first established in 1669, has been operated by the same family for more than 317 years. Prepared medicines produced in this factory have generally been held in the highest reputation in China, where the factory has supplied medicines to Chinese royalty over several centuries. The drug control acts implemented by the Chinese government in the 1970s and 1980s have helped further guarantee quality control of prepared medicines in China, and companies such as the Tong Ren Tang pride themselves on following these standards.[11] As noted earlier in this chapter, Tong Ren Tang has rapidly expanded its operations into many other countries. Indeed, the company is planning oversees factories and growing operations.

ADULTERANTS, ENDANGERED SPECIES, AND STANDARDS

The issue of adulterants and endangered species has increasingly threatened the reputation of CPM as safe and effective. The massive historical and growing contemporary usage of these products in Asia and the world often obscures these quality and ethical realities. The use of tiger bone, rhino horn, bear gallbladder, and seahorse are realities in a system of medicine that spans the centuries. Raising the consciousness of practitioners who grew up in that culture is the task of education to reshape that culture. Trafficking of illegal products is tied to big money, and separation between this practice and the legitimate market can be challenging. Western pharmaceuticals find their way into "herbal" products as "secret" ingredients that boost the effectiveness of the actions. Government control in the countries of origin is one thing, but controlling this globally, given widespread corruption and conflicting priorities, is a difficult task. The fundamental question remains: "Is the product purchased today in the Asian supermarket or local health food store free of adulterants and endangered species?"

Many prepared medicines made outside mainland China lack the same quality controls as the factories within China. Factories and sellers within China can be visited and found to have good-quality products determined to be safe and free of adulterants. In 1975 an herbal-based preparation called Toukuwan was manufactured in Hong Kong by the Nan-Lien Pharmaceutical Company and widely promoted in the United States as a treatment for rheumatism and arthritis. The FDA banned the importation of Toukuwan after it discovered four drugs, including valium, in the Chuifong preparation. Various prescription drugs have also been discovered in other prepared medicines. The *Journal of the American Medical Association* also reported four cases of agranulocytosis caused by prepared medicines in 1975, before the FDA ban. The Chuifong product has continued to appear under various names, such as "miracle herb," since 1975.[21,22] Caution must be used when purchasing and prescribing prepared herbal medicines, and we recommend using only well-known and reputable manufacturers.

If consumers are in doubt, we recommend using products produced at American, Canadian, or Australian companies, which typically are subject to far more quality control standards than those in Asia, and we suggest always consulting practitioners who research the origins of the various products they use and recommend.

In May 1994, Traffic USA and the World Wildlife Fund published "Prescription for Extinction: Endangered Species and Patented Oriental Medicines in Trade."[23] This report gave insight into the vast number of endangered species being used in natural medicine. The report indicated which products and traditional formulas contained endangered or threatened species, according to Appendices I, II, and III of the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora.[24]

Lu,[25] writing in the *China Daily,* described new European regulations in 2004 regarding the importation of CHMs into the European market. He stated the following:

> *The directive regulates that manufacturers who export herbal medicines to the EU market must get the union's GMP (Good Manufacturing Product) certificate, products quality must comply with EU pharmacopoeia, and importers should have import licenses.… But generally speaking, the directive, which for the first time grants TCM legal status as medicines, will benefit Chinese medicine makers to explore the European market in the future. Currently, most of the TCM products exported to the EU market are under the category of food, native produce, health products, or pharmaceutical ingredients, instead of medicine. Industry experts said that when one TCM product is registered as traditional herbal medicine in the EU, it indicates great market potentials. First, the market value of TCM will be greatly lifted compared to being sold as food or pharmaceutical ingredients. Second, the legal status as medicine would allow TCM to join EU countries' hospitalization insurance system. And EU's recognition of TCM will help reduce possible unfair treatment of Chinese medicines from other countries. Meanwhile, domestic makers should strengthen research and development, dismiss some ingredients which are unacceptable to western people, and develop products to cater for the huge European market.*

Hong Kong, long a stronghold of TCM despite a restrictive anti-TCM atmosphere under British rule, has upgraded its standards for Chinese herbs. The Chinese Medicine Council of Hong Kong was formed in 1999 to protect public health and consumers' rights and to ensure the professional standard of Chinese medicine practitioners and the trade of Chinese medicines. On the council's website, Shaw[17,26] discussed the trailblazing approach Australia took to create standards for the practice and sale of CHMs:

> *In that country [Australia], products were either "listed" or "registered." Listed products were of certified quality and were required to have supporting evidence of safety from the literature. No claims regarding efficacy could be made. Registered products equated to those with a license in the UK.*

Shaw also referred to the 1996 report "Towards a Safer Choice," which resulted from a review of the practice of TCM in Australia. The report concluded that regulation of CHMs and acupuncture practitioners was necessary and led to the Chinese Medicine Regulation Act of 1999, which set out statutory regulation for such practitioners in the state of Victoria. The intention was that regulation would eventually be brought in on a national scale. Shaw emphasized that this was being done for the benefit of the public, not practitioners.

Summing up, Shaw listed five elements for improving the safety of CHMs:

- Research into efficacy and safety
- Training of practitioners
- Quality herbs
- Adverse effect monitoring
- Appropriate regulation

Also on the council's website is a valuable list of safety concerns raised by Anderson[21] of the Hong Kong Medicines Control Agency, listed as follows:

- Lack of adequate authentication
- Intentional or accidental substitution
- Use of toxic substances and heavy metals (e.g., arsenic disulphide)
- Use of toxic animal materials (e.g., Bufo secretions)
- Use of synthetic drugs (e.g., corticosteroids, nonsteroidal anti-inflammatory drugs)
- Inadequate or inaccurate labeling

DISPENSARY GUIDE

CPM serves a number of different therapeutic purposes in and out of the practitioner's office. First and foremost, it is best prescribed within the principles of prescribing of traditional CHMs. For example, formulas for clearing heat or dispelling dampness require some understanding of the philosophy underlying these principles. If CHMs are just prescribed cookbook style, then individual patient reactions cannot be understood and tracked properly, and the full effect of the formulas will not be appreciated.

Secondly, CPM can augment and support traditional decoction-style prescribing. Thirdly, CPM is most valuable in acute prescribing and generally lends itself to OTC use. OTC use is best with input from trained Chinese medicine practitioners. In the case of Yin Chiao, described in the following, this widely used effective formula is most often prescribed at the onset of colds. Then there is the formula called Chuan Xin Lian (*Andrographis* anti-inflammatory tablets), which has tremendous effectiveness in inhibiting common bacterial infections, almost an "herbal antibiotic."[11] A final example of useful CPM in acute prescribing is the famous formula called Yunnan Baiyao. This is the most important CPM one should have at hand (according to general usage), at home and on the road. It is an emergency medicine stated as useful in all cases of injury. The signature of Yunnan Baiyao as traditionally dispensed involves little bottles of yellow powder with a tiny red "emergency" pill that is said to help revive critically injured individuals. Yang and Liang[27] described it as follows:

> *Yunnan Baiyao, literally meaning Yunnan White medicine, smells like a walk through an exotic forest. That's a clue to its composition: a dozen or so herbs from the southwestern flora-abundant province of Yunnan—but exactly which herbs remains a state secret. Successfully used for more than 80 years, Yunnan Baiyao, now available in several forms, is a veritable cure all and an essential, trusty family medicine in China.*

Because of the questionable standards of prepared medicines manufactured in Asia and generally outside China, it is suggested that practitioners scrutinize the labels of products from unknown sources or avoid them all together. With correct labeling, ingredients of prepared medicines should be obvious to anyone who can read Latin or Chinese herbal names.

In recent years, prepared medicine companies have become well established in the United States. Several high-quality manufacturers are now producing more suitable formulas. These tinctures, powders, and capsules are usually based on well-documented classic formulas.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. *Traditional Chinese Medicine*. People's Daily Online; 2010. http://english.people.com.cn. Accessed October 20, 2010.
2. *Medicine imports, exports rise as supply-side reform succeeds*. 2018-02-09 China Daily World. http://.ecns.cn/m/business/2018-02-09/detail-ifysszkh2355194.shtml. Wang Zihao. Accessed 08/19/2018.
3. *Huge challenges for China in overseas TCM market*. March 4, 2010. People's Daily Online. http://english.people.com.cn. Accessed 10/20/2010.
4. *East Asian Medicine Overview*. http://www.library.tufts.edu/hsl/subjectGuides/pain. Accessed 10/30/2011.
5. Kaptchuk TJ. East Asian medicine. *Ann Intern Med*. 2002;136:374–383.
6. Cooper EL. Complementary and alternative medicine, when rigorous, can be science. *Evid Based Complement Alternat Med*. 2004;1(1):261–264. www.ncbi.nlm.nih.gov/pmc/articles/PMC442104. Accessed January 11, 2010.
7. Pan SY, Chen SB, Dong HG, et al. New perspectives on Chinese herbal medicine (zhong-yao) research and development. *Evid Based Complement Alternat Med*. 2010;2011:403709.
8. Council of Colleges of Acupuncture and Oriental Medicine (CCAOM). http://ccaom.org. Accessed 11/10/2010.
9. *A Consumer's Guide to Acupuncture and Asian Medicine*. California Department of Consumer's Affairs. http://acupuncture.ca.gov. Accessed 11/01/2010.
10. The Pharmacopeia Commission of PRC. *Pharmacopeia of the People's Republic of China (English Ed)*. Vol. 1. Beijing: Chemical Industry Press; 1997.
11. Zhu CH. *Clinical Handbook of Chinese Prepared Medicines*. Brookline, MA: Paradigm Publications; 1986.
12. State Administration of Traditional Chinese Medicine. *Advanced Textbook on Traditional Chinese Medicine and Pharmacology*. Vol. 1. Beijing: New World Press; 2002.
13. Wang X, Chan AWK. Challenges and patenting strategies for Chinese herbal medicine. *Chin Med J*. 2010;16. http://cmjournal.org. Accessed January 11, 2010.
14. California Department of Health. *Asian Patent Medicines, Questions and Answers*. Sacramento, CA: California Department of Health Food & Drug Branch; 1991.
15. Buell P. *Chinese Medicine on the Golden Mountain: An Interpretive guide*. Seattle: Wing Luke Memorial Museum; 1984.
16. Barlow J, Richardson C. *China Doctor of John Day*. Portland, OR: Binford & Mort Publishing; 1979.
17. Traditional Chinese medicines. *The Chinese Market and International Opportunities*. London: Natural Medicine Marketing in Collaboration with Sino European Clinics Limited; 1996.
18. Li S. *Tong Ren Tang goes International: An Interview with Ding Yongling*, general manager of Beijing Tong Ren Tang International Co, Ltd and deputy general manager of Beijing Tong Ren Tang Group Co, Ltd. China #16 Today. www.chinatoday.com.cn. Accessed 8/27/2004.
19. State of California. Department of Health Services. *Important Information for the Sellers of Asian Patent Medicines*. State of California, Sacramento, CA: Department of Health Services; 1998.
20. Product catalog. *Chongqing Tong Jun Ge Medicine Works*. China: Chongqing, Sichuan
21. Rics CA, Sahud MA. Agranulocytosis caused by Chinese herbal medicines. *JAMA*. 1975;23:352–355.
22. McCaleb R, Blumenthal M. Black pearls lose luster. *HerbalGram*. 1990;22:4–5, 38–39.
23. Gaski AL, Johnson KA. *Prescription for extinction: Endangered Species and Patented Oriental Medicines in Trade* Washington, DC: Traffic USA; 1994.
24. C.I.T.E.S. Secretariat. *Convention Documents*. Geneva: Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)
25. Yu L. EU regulation affects TCM exports. *China Daily*. www.Chinadaily.com.cn. Accessed 8/7/2004.
26. Chinese Medicine Council of Hong Kong. *Regulation of Chinese medicine*. www.cmchk.org.hk. Accessed 8/12/2004.
27. Yang J, Liang S. A pharmacy in a tiny bottle. In: *Unbelievable Cures and Medicines from China*. Beijing: New World Press; 1997.

66

Cimicifuga racemosa (Black Cohosh)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Cimicifuga racemosa (family: *Ranunculaceae*)
Common names: black cohosh, macrotys, rattleweed, black snake root

GENERAL DESCRIPTION

Cimicifuga racemosa is a perennial herb native to North America that grows on hillsides and in woods at higher elevations from Maine and Ontario to Wisconsin, Georgia, and Missouri. The large, creeping rhizome produces stems up to 9 feet high. The ovate or oblong leaflets are 1 to 6 inches long and 4 inches wide, whereas the smaller leaflets are ternate, then pinnate, and sometimes even further divided. Small, white, fetid flowers grow in long racemes from May to August (Fig. 66.1). The rhizome is the portion of the plant used for medicinal purposes.

CHEMICAL COMPOSITION

C. racemosa contains at least three important natural product groups that contribute to its pharmacology: cycloartane triterpene glycosides, such as actein and 26-deoxyactein; phenylpropanoid esters; and phenolic compounds, such as caffeic acid derivatives, including ferulic acid and isoferulic acid (Figs. 66.2 and 66.3).[1–3] Early reports showed that it also contained the phytoestrogen flavonoid formononetin, but more recent analysis demonstrated that this compound was not contained in either the crude herb or standardized extracts. At this point, despite extensive chemical, biological, and clinical studies, neither the active constituents nor the exact mode of action of black cohosh have been determined.[4] Currently, the standardization of cimicifuga preparations is based on the content of triterpene glycosides, calculated as 26-deoxyactein. Other important triterpene glycosides include actein and cimicifugoside (aglycone cimegenol).

HISTORY AND FOLK USE

The generic name *cimicifuga* comes from the Latin *cimex,* a bug, and *fugo,* "to drive away," alluding to its use as a vermifuge. Native Americans used cimicifuga rhizomes for the relief of pain during menses and childbirth, as well as snakebite. The rhizome of *C. racemosa* was listed in the National Formulary from 1936 to 1950 and in the U.S. Pharmacopeia from 1820 to 1936. Eclectic physicians in the early part of the 20th century used cimicifuga in gynecological disorders, as well as rheumatoid and myalgic pain.

Although use in the United States declined dramatically from 1950 to 1995, cimicifuga preparations were used extensively in Europe during this same period, primarily as a natural alternative to hormone replacement therapy (HRT) during menopause. This popularity was based on substantial empiric and clinical evidence.

PHARMACOLOGY

The primary pharmacological effects of cimicifuga appear to revolve around its ability to affect endocrine regulatory mechanisms.[5–8] In the past, this activity was thought to be related to various phytoestrogenic components of cimicifuga, with formononetin perhaps being the most significant.[9] However, this line of reasoning has been questioned by the lack of formononetin in clinically studied cimicifuga extracts, as well as equivocal reports on phytoestrogen activity. The current thinking is that certain cimicifuga components exert selective estrogen receptor modulator (SERM) activity with no action in the uterus but that beneficial effects do occur in the hypothalamo-pituitary unit and in the bone.[5,10,11]

Cimicifuga's primary effect on endocrine regulatory mechanisms appears to be the result of complex synergistic actions of its key ingredients. Evidence suggests that these compounds act on both the hypothalamus and vasomotor centers to produce significant clinical benefits in menopause. For example, one study to determine the endocrinological actions of cimicifuga extract involved treating 110 women with either cimicifuga extract (supplying a total daily dose of 8 mg of 27-deoxyactein) or placebo.[8] After 2 months of treatment, luteinizing hormone (LH) decreased by 20% in the cimicifuga group compared with the placebo group. Unlike estrogens, cimicifuga does not affect the release of prolactin and follicle-stimulating hormone. Researchers then divided the extract into three distinct types of active compounds based on their ability to reduce LH secretion in ovariectomized rats and to compete in vitro with 17-β-estradiol for estrogen receptor binding sites:

- Constituents that did not bind to estrogen receptors but did suppress LH secretion
- Constituents able to bind to estrogen receptor sites and to inhibit LH secretion
- Compounds that did bind to estrogen receptors but that did not inhibit LH secretion

The authors concluded that "the LH suppressive effect of *C. racemosa* extracts observed in menopausal women and ovariectomized rats is caused by at least three different synergistically acting compounds."

One of cimicifuga's key pharmacological effects is inhibition of the secretion of LH by the pituitary gland. This effect is accomplished equally by components that do and do not bind to estrogen receptors. If cimicifuga was simply mimicking the effects of estrogen, it would certainly alter the secretion of other pituitary hormones just like estrogen, but it does not. It appears that enhancement of dopaminergic activity is the responsible mechanism for some of these central effects on menopausal symptoms.[5,12]

Cimicifuga extracts also appear to exert some beneficial effects in preventing bone loss. In an experiment carried out on ovariectomized rats, cimicifuga extract increased the expression of collagen I and osteocalcin. Similarly, other animal studies demonstrated that cimicifuga extract significantly reduced the urinary parameters of increased bone metabolism and bone loss. The effects in these animal models were similar to raloxifene, a well-confirmed SERM.

Importantly, although early studies reported a direct estrogenic effect with cimicifuga, the current perspective is that ethanol and isopropanol extracts of black cohosh do not contain formononetin or other estrogenic flavonoids and therefore do not bind to the estrogen receptor, upregulate estrogen-dependent genes, or stimulate the growth of estrogen-dependent tumors in in vitro and animal models.[5,13,14] Black cohosh (8% triterpene glycosides) was shown to induce a concentration-dependent decrease in both estrogen receptor alpha (ERα) and progesterone receptor (PR-A/B) protein levels, supporting the potential of black cohosh as a preventative measure against breast cancer initiation and progression.[15]

A systematic review conducted on black cohosh use in women with or at risk of breast cancer concluded that evidence does not support an association between black cohosh and increased risk of breast cancer.[16] Interestingly, of the studies reviewed, two studies reported significant reductions in the risk of primary breast cancer among postmenopausal women (adjusted odds ratio = 0.47, 95% confidence interval; 0.27–0.82), and risk of recurrence (adjusted hazard ratio = 0.75, 95% confidence interval; 0.63–0.89). Seventeen trials showed no significant effect on circulating hormone levels or proliferation in estrogen-responsive tissues.

Fig. 66.1 Black cohosh.

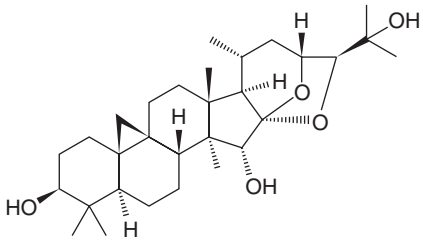


Fig. 66.2 Cimigenol.

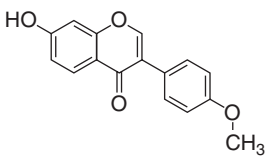


Fig. 66.3 Formononetin.

Stock photo

Stock photo

CLINICAL APPLICATIONS

C. racemosa extracts are by far the most widely used and thoroughly studied natural alternatives to HRT in menopause. Clinical studies showed that cimicifuga extracts relieved not only hot flashes but also depression and vaginal atrophy.[5,17–25]

Although evidence indicates that cimicifuga may provide benefit in other gynecological complaints, such as premenstrual syndrome, amenorrhea (both primary and secondary), dysmenorrhea, polymenorrhea, uterine fibroids, and fibrocystic breast disease, its primary clinical application is menopause.[24–27]

Menopause

In one of the first clinical studies, a large open study involving 131 doctors and 629 female patients, cimicifuga extract (two tablets twice a day, providing a daily dose of 4 mg 27-deoxyactein) produced clear improvement of menopausal symptoms in more than 80% of patients within 6 to 8 weeks.[20] As shown in Table 66.1, both physical and psychological symptoms improved. Most patients reported noticeable benefits within 4 weeks after the onset of cimicifuga therapy. After 6 to 8 weeks, complete resolution of symptoms was achieved in a large percentage of patients. Cimicifuga was well tolerated; there was no discontinuation of therapy, and only 7% of patients reported mild transitory stomach complaints.

In one of the first double-blind studies, 60 patients were given cimicifuga extract (two tablets twice a day providing a daily dose of 4 mg 27-deoxyactein), conjugated estrogens (0.625 mg/day), or diazepam (2 mg/day) for 12 weeks.[21] Results from standard indexes of menopausal symptoms indicated a clear advantage of cimicifuga extract over both drugs. Cimicifuga's effect on relieving depressive mood and anxiety associated with menopause was far superior to either diazepam or conjugated estrogens (Table 66.2).

TABLE 66.1 Cimicifuga in the Treatment of Menopause

Symptom	Percentage No Longer Present	Percentage Improved	Total Percentage Improved
Hot flashes	43.3	43.3	86.6
Profuse perspiration	49.9	38.6	88.5
Headache	45.7	36.2	81.9
Vertigo	51.6	35.2	86.8
Heart palpitation	54.6	35.2	90.4
Ringing in the ears	54.8	38.1	92.9
Nervousness/ irritability	42.4	43.2	85.6
Sleep disturbances	46.1	30.7	76.8
Depressive moods	46.0	36.5	82.5

TABLE 66.2 Effect on Kupperman Menopausal Index of Cimicifuga Compared With Conjugated Estrogens and Diazepam

Treatment Group	Beginning	At 12 Wk
Cimicifuga	35	14
Conjugated estrogens	35	16
Diazepam	35	20

One of the most used assessments in clinical studies in menopause is the Kupperman Menopausal Index. This quantitative assessment of menopausal symptoms is achieved by grading severity: severe = 3, moderate = 2, mild = 1, not present = 0. The symptoms assessed include the following:

- Hot flashes
- Depressive moods
- Profuse perspiration
- Feelings of vertigo
- Sleep disturbances
- Loss of concentration
- Headache
- Joint pain
- Nervousness/irritability
- Heart palpitation

The results of the Kupperman Menopausal Index from this trial clearly demonstrated cimicifuga extract's superiority over conjugated estrogens and diazepam, especially when safety and side effects were taken into consideration.

In another early double-blind study, 80 patients were given cimicifuga extract (two tablets twice a day, providing a daily dose of 4 mg 27-deoxyactein), conjugated estrogens (0.625 mg/day), or placebo for 12 weeks.[22] Cimicifuga produced better results in the Kupperman Menopausal Index, the Hamilton anxiety test, and the vaginal lining than estrogens or placebo. The number of hot flashes experienced each day dropped from an average of five to less than one in the cimicifuga group. In comparison, the estrogen group only dropped from 5 to 3.5. Even more impressive was the effect of cimicifuga on the vaginal lining. Although both conjugated estrogens and the placebo produced little effect, a dramatic increase in the number of superficial cells was noted in the cimicifuga group.

In a double-blind study of 110 women, cimicifuga extract (two tablets twice a day, providing a daily dose of 4 mg 27-deoxyactein) was shown to exert significant improvements in menopausal symptoms.[8] In addition to providing relief of hot flashes, cimicifuga once again demonstrated impressive results on the vaginal lining, as confirmed by vaginal smear.

In one study, 60 women less than 40 years old who had hysterectomies leaving at least one intact ovary received one of the following: cimicifuga extract (two tablets twice a day, providing a daily dose of 4 mg 27-deoxyactein), estriol (1 mg/day), conjugated estrogens (1.25 mg/day), or estrogen–progestin combination (Trisequens, one tablet a day).[23] Although the hormone therapies produced better results as determined by a modified Kupperman Menopausal Index, cimicifuga still displayed significant effects in relieving the symptoms of surgical menopause. These results indicated that cimicifuga could be a suitable alternative to estrogens in women having partial, and possibly even complete, hysterectomies.

In a double-blind study, cimicifuga extract was evaluated for its effect on menopausal symptoms, bone metabolism, and on the endometrium compared with those of conjugated estrogen and placebo.[24] The 62 postmenopausal women were treated with either black cohosh extract (40 mg/day), 0.6 mg conjugated estrogen, or matching placebo for 3 months. Results indicated that the black cohosh extract was equipotent to conjugated estrogen and superior to placebo in reducing menopausal complaints. Both black cohosh extract and conjugated estrogen produced beneficial effects on bone metabolism, but the black cohosh extract had no effect on endometrial thickness, which was significantly increased by conjugated estrogen. Vaginal superficial cells were increased with cimicifuga and conjugated estrogen treatment. These results seemed to confirm that cimicifuga extracts contain substances with SERM activity (i.e., with desired effects in the brain/hypothalamus, bone, and vagina, but without exerting uterotropic effects).

Women being treated with tamoxifen most often experience symptoms similar to menopause. To examine the effect of cimicifuga extract on hot flashes caused by tamoxifen adjuvant therapy in young premenopausal breast cancer survivors, 136 breast cancer survivors (ages 35–52 years) who had been treated with segmental or total mastectomy, radiation therapy, and adjuvant chemotherapy were randomly assigned to receive tamoxifen 20 mg/day orally or tamoxifen plus black cohosh extract (20 mg/day).[25] The duration of treatment was 5 years for tamoxifen, according to international standards for adjuvant therapies, and 12 months for the black cohosh extract. Follow-up included clinical assessment every 2 months; the primary goal was to record the number and intensity of hot flashes. Results indicated that almost half of the patients getting the black cohosh extract were free of hot flashes. Only 24.4% of patients taking black cohosh extract experienced severe hot flashes compared with 73.9% of the women taking tamoxifen alone. An isopropanolic extract of black cohosh was reviewed in a prospective observational study carried out in 50 patients with breast cancer with tamoxifen treatment. Patients were treated with one to four tablets (2.5 mg each) for 6 months and recorded their complaints before therapy and after 1, 3, and 6 months of therapy.[28] There was a statistically significant improvement in hot flashes, sweating, sleep problems, and anxiety, with 90% of participants reporting the tolerability of the black cohosh extract as good or very good.

A 2007 randomized, double-blind, controlled 3-month study was done in China, enrolling 244 menopausal women.[29] Women were assigned to either an isopropanolic extract of black cohosh containing 40 mg/day or 2.5 mg/day tibolone. There was a significant trend (57.08%) in the finding that women given Remifemin responded better than tibolone in terms of efficacy–risk balance, and black cohosh was clearly superior to tibolone if the safety profile for abnormal bleeding, endometrial thickening, breast pain, vaginal discharge, and edema was followed. Studies indicated that black cohosh might produce beneficial effects on bone metabolism by stimulating osteoblasts and may also have a weak effect on maturation of vaginal cells.[30,31]

Five studies showed no effect on menopausal symptoms with black cohosh use, including no effect on cognitive function.[32–36] Possible reasons included the use of an alcoholic extract versus an isopropanolic extract or the confounding health issues of the women studied.

Studies also evaluated the combination of black cohosh and St. John's wort. In one study, the mean Menopause Rating Scale score decreased 50% in the treatment group and 19.6% in the placebo group.[37] The Hamilton Depression Rating Scale score decreased 41.8% in the treatment group and 12.7% in the placebo group. In both measures, the results in the group using the St. John's wort and black cohosh were significantly superior to that of the placebo group.

Another black cohosh/St. John's wort trial was carried out in perimenopausal or postmenopausal Korean women.[38] Healthy perimenopausal women with typical climacteric symptoms and not on HRT for at least the previous 3 months were given a 264-mg tablet containing 0.364 mL of extract from black cohosh equivalent to 1 mg terpene glycosides and 84 mg of St. John's wort extract with 0.25 mg hypericin. Forty-two women in the treatment group and 35 women in the placebo group completed the study. Mean Kupperman index scores at 4 and 12 weeks were significantly lower in the treatment group ($P \leq 0.002$). At the end of the study, the average decrease in the Kupperman index score was 20 points in the treatment group and only 8.2 points in the placebo group ($P < 0.001$). Vaginal dryness and low libido were two symptoms that did not improve, but the average hot flash score was significantly lower in the black cohosh/St. John's wort group.

Despite some studies demonstrating no benefit of black cohosh, a meta-analysis of all nine placebo-controlled studies published until 2013 confirmed the reliable efficacy of *C. racemosa*–based medicinal products for menopausal symptoms.[39] The collective findings in black cohosh studies and long-term clinical anecdotal evidence on black cohosh indicate that it is most effective for menopause symptoms of day time or night time hot flashes, mood swings, sleep disorders, and body aches.

DOSAGE

The dosage of cimicifuga is based on its content of 27-deoxyactein, which serves as an important biochemical marker to indicate therapeutic effect. The dose of the cimicifuga extract used in the majority of clinical studies was 40 mg of black cohosh isopropanolic extract supplying 2 mg of 27-deoxyacteine twice daily. Approximate dosage recommendations using other (nonstandardized) forms of *C. racemosa* are as follows:

- Powdered rhizome: 1 to 2 g
- Tincture (1:5): 4 to 6 mL
- Fluid extract (1:1): 3 to 4 mL (1 tsp)
- Solid (dry powdered) extract (4:1): 250 to 500 mg

The German Commission E recommended that treatment with cimicifuga should be limited to 6 months (which is also the standard recommendation for HRT). However, this recommendation was made before detailed toxicology studies. Based on currently available data, cimicifuga is appropriate for long-term continued use.

TOXICOLOGY

The standardized extract of *C. racemosa* providing 1 mg of 27-deoxyactein in 40 mg of extract per tablet has been used in Germany since 1956 and has a remarkable safety record. A comprehensive review including preclinical and clinical research in estrogen-sensitive populations, including women at risk for breast cancer and breast cancer survivors, as well as human cell lines most relevant to breast cancer, confirmed this safety record. No serious side effects have ever been reported.[40] Cimicifuga offers a suitable natural alternative to HRT for menopause, especially when HRT is contraindicated (e.g., in women with a history of cancer, unexplained uterine bleeding, liver and gallbladder disease, pancreatitis, endometriosis, uterine fibroids, and fibrocystic breast disease).

Because cimicifuga extract shows some, albeit weak, estrogenic activity, researchers sought to determine Remifemin's effect on an established breast tumor cell line whose growth in vitro depends on the presence of estrogens. The results from these experiments showed no stimulatory effects, but rather inhibitory effects as well as an ability to promote apoptosis in these cells.[41,42] Furthermore, combining black cohosh with tamoxifen was shown to potentiate the inhibitory effects of tamoxifen.

In 2008 the Expert Committee of the U.S. Pharmacopeia's Council of Experts did an updated review of safety information for black cohosh.[43] Regulatory agencies in Australia, Canada, and Europe released statements and guidelines regarding the potential association between black cohosh products and hepatotoxicity. The committee analyzed human clinical case reports, adverse event reports, animal and toxicological data, historical uses, and regulatory status. Thirty reports on the use of black cohosh and possible liver damage were analyzed. All the reports of liver damage were considered to be possibly caused by black cohosh, and none were probable or certain. None of the animal toxicological or pharmacokinetic information revealed adverse information about black cohosh. The committee determined that the link between black cohosh and reports of liver damage was weak and "not of certain causality." They cited incomplete case information, unknown products, confounding variables (e.g., alcohol use

or concurrent medications), preexisting risk factors for liver damage, and no animal data or pharmacological data pointing to a mechanism of action that would cause a concern. Results of two meta-analyses of black cohosh confirmed these conclusions, finding no evidence of liver toxicity.[44,45] Despite these limitations, the increase in reports between 2002 and the present resulted in the committee's recommendation that black cohosh products should have a label with a cautionary statement, which is a change from the Expert Committee's previous decision.[43]

In 2012 black cohosh was nominated to the National Toxicology Program (NTP) for general testing by both the National Cancer Institute and National Institute of Environmental Health Sciences due to its widespread use and lack of human or animal studies in the published literature demonstrating its safety. In the study, female rodents (mice and rats) were given 0, 15 (rats only), 62.5 (mice only), 125, 250, 500, or 1000 mg/kg/day of black cohosh extract (BCE) by gavage for 90 days.[46] BCE induced dose-dependent hematological changes consistent with a nonregenerative macrocytic anemia and increased frequencies of peripheral micronucleated red blood cells indicative of chromosomal and hematological changes in both species. Both the chromosomal damage and hematological effects were more severe in mice than rats. Although this study provided the first comprehensive assessment of the subchronic toxicity of BCE in rodents, direct extrapolation of the results to humans is difficult because BCE preparations are variable natural mixtures of chemicals, with the potentially harmful compound(s) still unknown, and the dose used was significantly higher than a human subject would ingest. The lowest external dose that had an effect (62.5 mg/kg/day) was 125 times the recommended amount for daily human consumption (40 mg or ~0.5 mg/kg/day for a 70 kg human). Another research group conducted a study to investigate the potential of a functional cobalamin or folate deficiency as a possible explanation for the hematological changes observed in the NTP BCE subchronic toxicity study.[47] Female mice were exposed to 1000 mg/kg BCE for 92 days. The study confirmed the previous findings from the NTP study, in that exposure to BCE caused a decrease in the red blood cell count with an increase in MCV and MCH. In addition, although folate and cobalamin levels were unchanged, two biomarkers that are used for diagnosis or confirmation of folate and cobalamin deficiencies, homocysteine and MMA, were both elevated. The authors concluded that these findings suggested a BCE-induced functional deficiency of cobalamin, and possibly folate.

DRUG INTERACTIONS

As discussed previously, there is concern that black cohosh might harm the liver. Taking black cohosh along with medication that might also harm the liver requires proper monitoring.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Chen SN, Fabricant DS, Lu ZZ, et al. Cimiracemates A-D, phenylpropanoid esters from the rhizomes of Cimicifuga racemosa. *Phytochem*. 2002;61:409–413.
2. Li WK, Chen SN, Fabricant D, et al. High-performance liquid chromatographic analysis of black cohosh *(Cimicifuga racemosa)* constituents with in-line evaporative light scattering and photodiode array detection. *Anal Chim Acta*. 2002;471:61–75.
3. Shao Y, Harris A, Wang M, et al. Triterpene glycosides from *Cimicifuga racemosa*. *J Nat Prod*. 2000;63:905–910.
4. Kennelly EJ, Baggett S, Nuntanakorn P, et al. Analysis of thirteen populations of black cohosh for formononetin. *Phytomedicine*. 2002;9:461–467.
5. Borrelli F, Izzo AA, Ernst E. Pharmacological effects of *Cimicifuga racemosa*. *Life Sci*. 2003;73:1215–1229.
6. Jarry H, Harnischfeger G. Endocrine effects of constituents of *Cimicifuga racemosa*. 1. The effect on serum levels of pituitary hormones in ovariectomized rats. *Planta Med*. 1985;1:46–49.
7. Jarry H, Harnischfeger G, Duker E. The endocrine effects of constituents of Cimicifuga racemosa. 2. In vitro binding of constituents to estrogen receptors. *Planta Med*. 1985;4:316–319. [German].
8. Duker EM, Kopnski L, Jarry H, et al. Effects of extracts from *Cimicifuga racemosa* on gonadotropin release in menopausal women and ovariectomized rats. *Planta Med*. 1991;57:420–424.
9. Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol*. 1993;44:37–43.
10. Seidlova-Wuttke D, Hesse O, Jarry H, et al. Evidence for selective estrogen receptor modulator activity in a black cohosh *(Cimicifuga racemosa)* extract: comparison with estradiol-17beta. *Eur J Endocrinol*. 2003;149:351–362.
11. Nisslein T, Freudenstein J. Effects of an isopropanolic extract of Cimicifuga racemosa on urinary crosslinks and other parameters of bone quality in an ovariectomized rat model of osteoporosis. *J Bone Miner Metab*. 2003;21:370–376.
12. Jarry H, Metten M, Spengler B, et al. In vitro effects of the Cimicifuga racemosa extract BNO 1055. *Maturitas*. 2003;44:S31–S38.
13. Mahady GB. Is black cohosh estrogenic? *Nutr Rev*. 2003;61:183–186.
14. Zierau O, Bodinet C, Kolba S, et al. Antiestrogenic activities of Cimicifuga racemosa extracts. *J Steroid Biochem Mol Biol*. 2002;80:125–130.
15. Szmyd M, Lloyd V, Hallman K, et al. The effects of black cohosh on the regulation of estrogen receptor (ERα) and progesterone receptor (PR) in breast cancer cells. *Breast Cancer: Targets Ther*. 2018;10:1–11. PubMed PMID: 29403307.
16. Fritz H, Seely D, McGowan J, et al. Black cohosh and breast cancer: a systematic review. *Integr Cancer Ther*. 2014;13(1):12–29. PubMed PMID: 23439657.
17. Kligler B. Black cohosh. *Am Fam Physician*. 2003;68:114–116.
18. Borrelli F, Ernst E. *Cimicifuga racemosa:* a systematic review of its clinical efficacy. *Eur J Clin Pharmacol*. 2002;58:235–241.
19. McKenna DJ, Jones K, Humphrey S, et al. Black cohosh: efficacy, safety, and use in clinical and preclinical applications. *Altern Ther Health Med*. 2001;7:93–100.
20. Stolze H. An alternative to treat menopausal complaints. *Gynecology*. 1982;3:14–19.
21. Warnecke G. Influencing menopausal symptoms with a phytotherapeutic agent. *Med Welt*. 1985;36:871–874.
22. Stoll W. Phytopharmacon influences atrophic vaginal epithelium. Double-blind study—cimicifuga vs. estrogenic substances. *Therapeuticum*. 1987;1:23–31.
23. Lehmann-Willenbrock E, Riedel HH. Clinical and endocrinologic studies of the treatment of ovarian insufficiency manifestations following hysterectomy with intact adnexa. *Zentralbl Gynakol*. 1988;110:611–618. [German].
24. Wuttke W, Seidlova-Wuttke D, Gorkow C. The Cimicifuga preparation BNO 1055 vs. conjugated estrogens in a double-blind placebo-controlled study: effects on menopause symptoms and bone markers. *Maturitas*. 2003;44:S67–S77.
25. Hernandez Munoz G, Pluchino S. Cimicifuga racemosa for the treatment of hot flashes in women surviving breast cancer. *Maturitas*. 2003;44:S59–S65.
26. Bruker A. Essay on the phytotherapy of hormonal disorders in women. *Med Welt*. 1960;44:2331–2333.
27. Gorlich N. Treatment of ovarian disorders in general practice. *Arztl Prax*. 1962;14:1742–1743.
28. Rostock M, Fischer J, Mumm A, Stammwitz U, Saller R, Bartsch HH. Black cohosh *(Cimincifuga racemose)* in tamoxifen-treated breast cancer patients with climacteric complaints – a prospective observational study. *Gynecological Endocrinol*. 2011;27(10):844–848. PubMed PMID: 21231853.
29. Bai W, Henneicke-von Zepelin H, Wang S, et al. Efficacy and tolerability of a medicinal product containing an isopropanolic black cohosh extract in Chinese women with menopausal symptoms: randomized, double blind, parallel-controlled study versus tibolone. *Maturitas*. 2007;58:31–41.
30. Wuttke W, Gorkow C, Seidlova-Wuttke D. Effects of black cohosh on bone turnover, vaginal mucosa, and various blood parameters in postmenopausal women: a double-blind, placebo-controlled, and conjugated estrogens-controlled study. *Menopause*. 2006;13:185–196.
31. Spangler L, Newton K, Grothaus L, et al. The effects of black cohosh therapies on lipids, fibrinogen, glucose and insulin. *Maturitas*. 2007;57:195–204.
32. Pockaj B, Gallagher JG, Loprinzi CL, et al. Phase III double-blind, randomized, placebo-controlled crossover trial of black cohosh in the management of hot flashes: NCCTG Trial NO1CC1. *J Clin Oncol*. 2006;24:2836–2841.
33. Reed S, Newton K, LaCroix A, et al. Vaginal, endometrial, and reproductive hormone findings: randomized, placebo-controlled trial of black cohosh, multibotanical herbs, and dietary soy for vasomotor symptoms: the Herbal Alternatives for Menopause (HALT) Study. *Menopause*. 2008;15:51–58.
34. Van der Sluijs C, Bensoussan A, Change S, et al. A randomized placebo-controlled trial on the effectiveness of an herbal formula to alleviate menopausal vasomotor symptoms. *Menopause*. 2009;16:336–344.
35. Geller S, Shulman L, van Breemen R, et al. Safety and efficacy of black cohosh and red clover for the management of vasomotor symptoms: a randomized controlled trial. *Menopause*. 2009;16:1156–1166.
36. Maki P, Rubin L, Fornelli D, et al. Effects of botanicals and combined hormone therapy on cognition in postmenopausal women. *Menopause*. 2009;16:1167–1177.
37. Uebelhack R, B JU, Graubaum HJ, et al. Black cohosh and St. John's wort for climacteric complaints. *Obstet Gynecol*. 2006;107:247–255.
38. Chung D, Kim H, Park K, et al. Black cohosh and St. John's wort (GY-NO-Plus) for climacteric symptoms. *Yonsei Med J*. 2007;48:289–294.
39. Henneicke-von-Zepelin HH. (2017). 60 years of *Cimicifuga racemose* medicinal products: clinical research milestones, current study findings and current development. *Wiener Medizinische Wochenschrift*. 1946; 167(7–8):147–159. PubMed PMID: 28155126.
40. Dog TL, Powell KL, Weisman SM. Critical evaluation of the safety of *Cimicifuga racemosa* in menopause symptom relief. *Menopause*. 2003;10:299–313.
41. Hostanska K, Nisslein T, Freudenstein J, et al. *Cimicifuga racemosa* extract inhibits proliferation of estrogen receptor-positive and negative human breast carcinoma cell lines by induction of apoptosis. *Breast Cancer Res Treat*. 2004;84:151–160.
42. Nesselhut T, Borth S, Kuhn W. Influence of *Cimicifuga racemosa* extracts with estrogen-like activity on the in vitro proliferation of mammary carcinoma cells. *Arch Gynecol Obstet*. 1993;254:817–818.
43. Mahady G, Low Dog T, Barrett M, et al. United States Pharmacopeia review of the black cohosh case reports of hepatotoxicity. *Menopause*. 2008;15:628–638.
44. Naser B, Schnitker J, Minkin MJ, et al. Suspected black cohosh hepatotoxicity: no evidence by meta-analysis of randomized controlled clinical trials for isopropanolic black cohosh extract. *Menopause*. 2011;18(4):366–375. PubMed PMID: 21228727.
45. Teschke R, Schwarzenboeck A. Suspected hepatotoxicity by Cimicifuga racemose rhizome (black cohosh, root): critical analysis and structured causality assessment. *Phytomedicine*. 2009;16(1):72–84. PubMed PMID: 19010650.
46. Mercado-Feliciano M, Cora MC, Witt KL, et al. An ethanolic extract of black cohosh causes hematological changes but not estrogenic effects in female rodents. *Toxicol Appl Pharmacol*. 2012;263(2):138–147. PubMed PMID: 22687605.
47. Cora MC, Gwinn W, Wilson R, et al. A black cohosh extract causes hematologic and biochemical changes consistent with a functional cobalamin deficiency in female B6C3F1/N mice. *Toxicologic Pathol*. 2017;45(5):614–623. PubMed PMID: 28618975.

Citicoline (CDP-Choline)

Alexander G. Schauss, PhD

OUTLINE

INTRODUCTION

Citicoline (cytidine-5′-diphosphocholine; CDP-choline) is an endogenous mononucleotide, composed of ribose, cytosine, pyrophosphate, and choline, and an essential precursor for the synthesis of neuronal plasma membrane phospholipids, important as a rate-limiting step in phosphatidylcholine synthesis (Fig. 67.1). Citicoline can also be an exogenous source for the synthesis of acetylcholine, a key neurotransmitter, and is a member of the group of molecules that play important roles in cellular metabolism, such as nucleotides that form the basic structural units found in nucleic acids.[1]

Kennedy and colleagues first identified citicoline in 1955[2] and synthesized it in 1956. Since then it has been studied extensively in Europe, Japan, and the United States.

There are two forms of citicoline available as either a dietary supplement ingredient or as a pharmacotherapeutic: citicoline sodium and citicoline free-base. Whereas citicoline free-base has been available as a dietary supplement in the United States for three decades, citicoline sodium is primarily used in other countries as a drug or para-drug for the treatment of neurological disorders. Citicoline is also available for intravenous use and as a topical in solution, for use as eye drops.

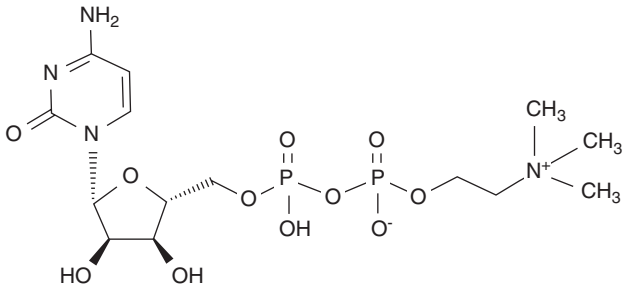


Fig. 67.1 Structure of citicoline.

Citicoline Versus Choline

Choline is a component of the diet and is produced in the brain, albeit in small amounts (Fig. 67.2). Because of its low endogenous production, it is considered an essential nutrient and classified with the B-vitamin complex. It plays several essential roles in human physiology, including enhancement of structural integrity and signaling for cell membranes; supporting acetylcholine synthesis; and the synthesis of betaine, a methyl donor.[3]

When taken orally, citicoline is hydrolyzed in the intestinal tract and in the circulation to form choline and cytidine, which is the nucleoside of cytosine. Citicoline provides the brain with a source of choline and cytidine, which are efficiently used in the Kennedy cycle to generate phospholipids. Although choline on its own is preferentially used for the synthesis of acetylcholine, cytidine is highly efficiently used in the brain for the synthesis of various nucleotides. Studies in neuronal cell lines showed that cytidine administration increased the incorporation of choline into membrane phosphatidylcholine.[4]

In terms of safety, choline is a substance with a low level of toxicological concern. Administering choline with cytidine, in the form of citicoline, lowers the toxicity index by twentyfold.[5] Furthermore, citicoline administration is significantly different from the administration of choline in cases of cerebral ischemia caused by stroke and other

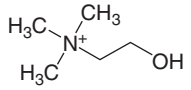


Fig. 67.2 Structure of choline.

conditions. Citicoline's therapeutic effects in such conditions stem from its ability to:

1. increase the synthesis of phosphatidylcholine, the primary component of neuronal membranes. Beyond phosphatidylcholine action in maintaining phospholipid metabolism, one of its mechanisms of action is to restore and preserve the structure/function integrity of neuronal membranes after an acute ischemic stroke or brain damage from traumatic head injuries;
2. enhance acetylcholine synthesis, thereby ameliorating the symptoms resulting from ischemic loss of cholinergic neurons;
3. promote the synthesis of several other membrane phospholipids, including phosphatidylethanolamine and phosphatidylserine, to contribute to the repair and regeneration of axons and synapses;
4. serve as agonists at nicotinic acetylcholine receptors, which play a critical role in maintaining normal cognition; and
5. prevent the accumulation of free fatty acids and generation of free radicals at the site of ischemia, thereby preventing the initiation of a proinflammatory cascade of events. However, as will be discussed in this chapter, citicoline has also been studied for its effects on eye health and visual function, substance abuse, infectious diseases, and metabolic diseases.

BIOAVAILABILITY/PHARMACOKINETICS

The pharmacokinetics of an oral dose of ^{14}C-labeled citicoline has been studied in humans. Administration of a single 300-mg dose to healthy adults was shown to have nearly complete absorption, with less than 1% of the labeled compound found in feces after a 5-day collection period. Absorption of citicoline gave rise to two chromatographic peaks in concentrations of radioactivity in plasma, the first at 1 hour and the second, larger peak at 24 hours after dosing. The main route of excretion was found to be via respiratory carbon dioxide, with significant excretion also occurring through urine. After 5 days, 16% of the administered dose was recovered, suggesting that the remainder was incorporated into tissues or was available for biosynthetic and biodegradative pathways.[6] Fig. 67.3 reports the metabolic pathways of citicoline.

A pharmacokinetic study in rats using ^{14}C-methyl-labeled citicoline confirmed almost complete absorption with oral administration, with calculated oral bioavailability being approximately 92% of that obtained from intravenous (IV) dosing. The absorption was categorized as slow and complete with sustained blood levels, the highest being at around 5.5 hours after administration. Radioactive labeling found citicoline and its metabolites widely distributed throughout tissues, including distribution of metabolites to the brain, confirming their ability to participate in the synthesis of phospholipids.[7]

A confirmatory study, again using radiolabeled citicoline in rats, found 62.8% of total radioactivity was distributed in brain tissue as phospholipids, including phosphatidylcholine and sphingomyelin. These results suggested that metabolites of orally administered citicoline were available in the brain for resynthesis of endogenous citicoline.[8] Although only a small percentage of the total citicoline dose crosses the blood–brain barrier as choline and cytidine, the utilization of these precursors in brain tissue for phospholipid biosynthesis is extremely efficient.[4]

A rapid liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method has been developed and validated for the pharmacokinetic investigation of choline, the active metabolite of citicoline in human plasma.[9] The method was effectively applied for evaluation in a study of the pharmacokinetic parameters and bioequivalence of citicoline administered to 12 healthy male volunteers.

MECHANISM OF ACTION

Much remains to be learned of citicoline's mechanisms of actions and biotransformation.[10] Much of the evidence shows that citicoline is sequentially hydrolyzed and dephosphorylated to uridine (in humans) and choline.

Citicoline has several important mechanisms of action, leading to a broad range of beneficial effects on neurological function. In cerebral ischemia, citicoline primarily acts by increasing the synthesis of phosphatidylcholine, the primary neuronal membrane phospholipid, and enhancing the production of acetylcholine. Oral citicoline administration increases plasma levels of choline and cytidine, which are building blocks used to restore neuronal membrane integrity.[5] These effects, in addition to apoptosis inhibition, neuroplasticity potentiation, acetylcholine (Ach) and phospholipid synthesis, contribute to our understanding of the mechanism of action that has demonstrated beneficial effects both in degenerative and vascular cognitive decline.

Interestingly, citicoline seems to have differential effects on phosphatidylcholine synthesis in younger versus older adults. Phosphatidylcholine is an essential compound for cell membrane integrity and repair. It is normally reduced in brain cell membranes as a result of aging. A study using protein magnetic resonance spectroscopy to measure brain concentrations of cytosolic choline-containing compounds before and after a single oral dose of citicoline found that the choline resonance in the brain of younger individuals increased,

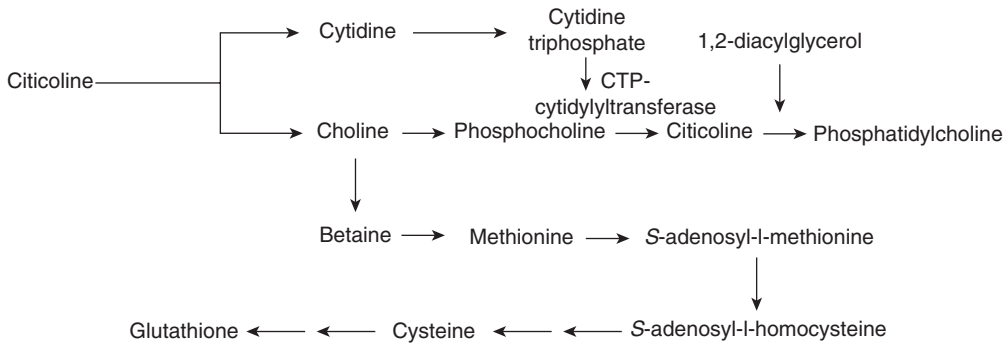


Fig. 67.3 Metabolic pathways of citicoline. (From ResearchGate. The role of citicoline in cognitive impairment: Pharmacological characteristics, possible advantages, and doubts for an old drug with new perspectives. <https://www.researchgate.net/Citicolines-metabolic-pathways-Abbreviation-CTP-cytidine-triphosphate_fig2_280776646>. Accessed August 22, 2018.)

whereas it decreased in older subjects. It was presumed that the cytidine component of citicoline enhanced the incorporation of brain choline into neural cell membrane phosphatidylcholine in older subjects, resulting in the decrease.[11]

The ability of citicoline to stimulate brain phospholipid synthesis in humans was further supported by studies showing that healthy subjects consuming 500 mg/day orally of citicoline for 6 weeks had increased levels of phosphodiesters in brain tissue, such as glycerophosphocholine and glycerophosphoethanolamine, as assessed by phosphorus magnetic resonance spectroscopy. These findings supported citicoline's ability to increase phosphatidylcholine synthesis.[12] Findings from a study of healthy middle-aged adults confirmed these results but suggested that the increase in phosphorus metabolites attributed to citicoline intake was regionally specific, with the frontal lobe being the preferred site of deposition, ultimately enhancing frontal lobe energetics and improving phospholipid membrane turnover. This area of the brain contributes to memory function by supporting vigilance, attention, and working memory capacity and by reducing mental fatigue. Because citicoline's effect was most prominent in this brain region, this is a likely explanation for its clinical benefit of improved cognitive function.[13]

Citicoline may further benefit patients experiencing ischemia by decreasing the accumulation of free fatty acids at the site of the lesion, which occurs as a result of neuronal cell damage and death. Soon after initiation of ischemia, there is a significant increase in proinflammatory arachidonic acid, glycerols, and free fatty acids, caused by the breakdown of neuronal membranes. Toxic metabolites, prostaglandins, thromboxanes, and free radicals, can accumulate, leading to further damage. Animal studies showed that intracerebral administration of citicoline before the induction of ischemia reduced the increase in free fatty acids, arachidonic acid, and other toxic metabolites, attenuating free radical damage and restoring membrane function.[5]

Some evidence points to the ability of citicoline to normalize neurotransmitter release patterns. In conditions of cerebral hypoxia, which exist in ischemia, norepinephrine release may decrease, whereas the release of dopamine may increase. In several animal models, citicoline was shown to inhibit the impairment of neurotransmitter release in hypoxic conditions. Furthermore, citicoline administration to rats kept in a chronic hypoxic state reduced behavioral deteriorations and increased survival time. Additional studies found that citicoline was able to increase the dilation of blood vessels in animals with cerebral microcirculation injury, significantly increasing cerebral blood flow.[4]

Citicoline shows neural restorative effects, presumably via action on the dopaminergic system of the central nervous system. Rats with substantia nigra lesions were shown to regenerate nerve cells after treatment with citicoline, indicating its protective effect in this region. Further studies found that citicoline administration to rats increased striatal dopamine synthesis. Several other investigations in animal models yielded evidence of citicoline's ability to enhance dopaminergic synthetic pathways.[4] This was a result of the activation of tyrosine hydroxylase and inhibition of dopamine reuptake, which is related to citicoline's activity on phospholipid synthetic pathways. Citicoline is also known to have effects on serotonin and norepinephrine.[14] Studies in rats showed that citicoline improved learning and memory capacity and enhanced motor performance and coordination in aged rats. These findings provided further evidence for citicoline's cholinergic activity.[15]

Studies have suggested that citicoline enhances the preservation of an inner mitochondrial membrane component known as cardiolipin, which is important for the preservation of mitochondrial function. Citicoline facilitates the preservation of sphingomyelin, which promotes signal transduction in nerve cells. Citicoline exhibits direct antioxidant effects; research has shown that it has an ability to stimulate glutathione synthesis and the activity of the enzyme glutathione reductase. Furthermore, citicoline attenuates lipid peroxidation. These downstream effects may be attributable to citicoline's larger function of attenuating the activation of phospholipase A_2, thus reducing inflammation in neural tissues and in general.[16] Citicoline was shown to have direct free radical suppressive effects, as seen in animal models of transient cerebral ischemia, in which citicoline had a suppressive effect on hydroxyl radical generation.[17]

Citicoline may significantly affect brain-remodeling activity. In an animal model, citicoline treatment significantly increased the length and branch points of dendrites, which led to increased efficiency of sensory information processing.[18] This mechanism of action could potentially account for a significant portion of citicoline's neurorestorative functions.

Diminished auditory sensory gating and associated cognitive deficits in schizophrenia have been studied relative to treatment with citicoline. In a randomized, placebo-controlled, double-blind design involving oral administration of 500 and 1000 mg of citicoline to 24 healthy volunteers assessed for auditory gating and for executive function, improvements were seen at the low dose.[19] The effectiveness of the lower dose of 500 mg was confirmed in a second study of schizophrenic patients using a randomized, placebo-controlled, double-blind design. In this study the orally administered dose was 500, 1000, and 2000 mg. As was found in the study of healthy subjects, the 500-mg dose was found to have a demonstrated treatment effect in increasing the suppression of P50 event-related potential (ERP) consistent with an α-7 nAChR mechanism.[20]

Choline is a selective α7-nAChR agonist. Twenty-four healthy male volunteers (mean age, 21.3 ± 0.99 years) were recruited to participate in a randomized, placebo-controlled, double-blind, crossover clinical trial to determine whether citicoline single dose administration of either 500 or 1000 mg would increase sensory gating and cognition. Citicoline exhibited changes in electroencephalographic (EEG) oscillations similar to those reported with nicotinic agonists and was observed to improve gating (1000 mg) and suppression of the S2 P50 response (500 and 1000 mg), with the effect being selective for individuals with low gating (suppression) levels.[21] These results have implications for cognitive treatments focused on specific functional roles such as coding of sensory information and cognitive disorders related to cortical and thalamic structural pathologies at sites with the highest α7-nAChR expression, seen in schizophrenia. The crossover trial also used EEG recordings to elucidate citicoline's mechanisms of action 4 hours after a single administration of either dose. Citicoline enhancement in the brain was found to facilitate multiple mechanisms, among which is its ability to facilitate nicotinic cholinergic activity (i.e., altered expression of the α-7 nicotinic acetylcholine receptors [nAChR]).[21]

To evaluate citicoline induction of an oscillatory response profile associated with nicotinic stimulation, a resting-state EEG is acquired to test the hypothesis. This kind of study was carried out in a pilot randomized, double-blind, placebo-controlled trial with 24 subjects, which showed that citicoline enhanced cognitive function.[19]

CLINICAL APPLICATION

Learning and Memory

Experiments in animals and humans provided evidence of citicoline's ability to promote important cognitive processes, including learning ability and memory functions. Clinical studies evaluating citicoline administration for cognitive enhancement have been conducted for several decades. A review of trials using citicoline as a treatment for senile alterations of memory in 1991 found significant benefits

in patients with cerebral insufficiency and chronic cerebrovascular disease.[22]

A randomized, double-blinded, placebo-controlled study was undertaken to assess the effects of citicoline supplementation on verbal memory function in 95 healthy subjects aged 50 to 85 years (47 women and 48 men) who were administered citicoline (500 mg orally twice daily) or placebo for 3 months. The study subjects were well educated (mean, 14.3 years of education). Baseline testing included a logical memory assessment test, which was used to classify those with relatively inefficient memories. At the end of the initial study, 32 individuals (16 from the citicoline group and 16 from the placebo group) from this pool were recruited to participate in a follow-up crossover study. The initial study found that citicoline improved delayed recall for only those with relatively inefficient memory at the beginning of the trial. In the follow-up crossover study, the dose of citicoline was increased to 2000 mg/day. In this subgroup, the higher dose of citicoline improved immediate and delayed logical memory.[23]

An open-label crossover trial consisting of 24 elderly individuals without dementia but with demonstrable memory impairment (assessed by comparison with 24 healthy young control subjects), showed that oral citicoline (500, 1000, or 300 mg/day combined with nimodipine [90 mg/day]) significantly improved memory performance compared with the no-treatment periods, as evidenced by reduced error scores on word recall tasks, immediate object recall, and delayed object recall.[24]

Attention

Attention and mental alertness play an important role in supporting cognitive function and performance. Attention is the cognitive process of selectively concentrating on discrete information. Paying attention is the first step in the learning process, especially the ability to focus attention at the right time on what is important and simultaneously ignoring irrelevant information occurring at the same time. Animal studies have shown that citicoline can enhance learning and memory.[25,26]

In a study using proton magnetic resonance spectroscopy to measure in vivo brain chemistry, investigators found that 3 hours after a single oral dose of citicoline was given to participants, plasma choline levels increased in younger and older adults. These results are particularly important to older adults because in this study, the investigators found that intracellular cytidine levels increased after the administration of citicoline, leading to increased incorporation of choline already present in the brain into membrane phosphatidylcholine.[11]

Citicoline may benefit healthy individuals by improving attentional ability. To test this hypothesis, a randomized, double-blind, placebo-controlled trial was carried out in 40- to 60-year old healthy females. Subjects were given either 250 or 500 mg/day for 28 days, or placebo. The groups receiving citicoline exhibited improved cognitive attentional performance by committing significantly fewer commission errors on the Conners' Continuous Performance Test II (CPT-II) compared with the placebo group.[27] A similar study was performed in healthy male individuals ranging from 13 to 18 years of age, in which either 250 or 500 mg/day of citicoline was administered, or placebo, for 28 days. Significant improvements in attentional task and motor function were measured.[28]

In a study by Bruce and colleagues, when citicoline was combined with caffeine to create a citicoline-caffeine based beverage, a generalized improvement in the ability to accommodate new and relevant information within working memory and sustained attention was seen, in addition to overall enhanced brain activitation.[29] The 8 ounces (227 g) of the beverage, containing 35 mg of caffeine and 250 mg of citicoline, or an identical placebo beverage without the caffeine-citicoline blend, was randomly given to 60 healthy adult participants (33 women and 27 men; $M = 24.2$) enrolled in a randomized, double-blind, placebo-controlled trial. Both measures of electrical brain activity using EEG 30 minutes after consuming the beverage and neuropsychological measures were used to measure electrical brain activity and neuropsychological performance changes in working memory, attention, behavioral inhibition, and executive functioning.

The observed increase in electrical brain activity seen in the Bruce study is in agreement with another study of a different beverage containing citicoline performed by the same lead author. In this study, 250 mg of citicoline was combined with lycopene, caffeine, and vitamin E.[30] Ten healthy adults (five women and five men; aged 25–32, $M = 28.1$) participated in a double-blind, placebo-controlled, crossover trial. Subjects undertook a noninvasive EEG that measured concomitant changes in ERPs in response to a range of cognitive tasks. The experimental activation tasks were designed to reflect the brain's core adaptive competencies and underlying neural networks. Thirty minutes after consuming 8 ounces of the beverage, EEG and ERP indices were significantly modified compared with the brains of the placebo group.

Alzheimer's Disease and Dementia

Citicoline supplementation has been well studied in Alzheimer's disease and vascular dementia. A study of 19 patients (mean age, 66.21 ± 1.48 years) given oral citicoline at a dosage of 1000 mg/day for 30 days found significant improvements in cognitive function in the subgroup of patients with early-onset Alzheimer's disease and a trend toward increased cognitive function in the overall group, as assessed by brain electrical mapping. Brain spectral data readings provided an indication that the brains of early-onset Alzheimer's patients showed greater damage than those of late-onset Alzheimer's patients, whereas both groups had the same degree of cognitive impairment. It was postulated that the therapeutic effects of citicoline might be mediated by an enhancement of cholinergic neural transmission, activation of repair mechanisms to rejuvenate neuronal membranes, a regulatory effect on parameters associated with blood flow and circulation, and the regulation of several immunological responses, which, if left unchecked, would lead to potential neuronal dysfunction and cell death.[31]

In further studies, oral administration of citicoline (1000 mg/day) to 20 patients (age range 57–78 years) with early- or late-onset Alzheimer's disease resulted in improvements in mental function, particularly in the early-onset group. This 1-month treatment with citicoline resulted in an increased blood-flow velocity from baseline measures (assessed by transcranial Doppler ultrasound) in the middle cerebral artery, which has been found to decrease with age, possibly resulting in neuropathologic changes. Citicoline's cholinergic effects and influence on cytokine production might also partially account for its benefits.[32]

Researchers investigated the regulatory effects of citicoline on blood histamine levels. Alterations in the histamine system are present in Alzheimer's disease, and high levels have been found in several central nervous system regions, cerebrospinal fluid, and serum. Histamine may also participate in the aging process, with histamine-related changes reported in several different tissues, including the central nervous system. In one study, 14 individuals with Alzheimer's disease (7 early onset, 7 late onset) were administered citicoline (1000 mg/day for 30 days). Blood histamine measurements were taken at baseline, at 2 hours after administration of the first dose, and after 30 days of treatment with citicoline. All participants experienced an acute reduction in blood histamine levels; after 30 days, early-onset Alzheimer's patients saw a decrease in blood levels of histamine of about 55% compared with baseline, whereas late-onset individuals saw a 45% decrease.

Early-onset patients clearly had higher baseline levels of histamine than late-onset patients. Reducing endogenous histamine excesses may support cognitive function because excessive histamine levels have been implicated in etiopathogenic events in Alzheimer's disease.[33]

The effects of citicoline administration (1000 mg/day orally for 3 months) were assessed in a trial in patients with senile dementia (Alzheimer's disease and multiinfarct dementia) to elucidate whether the nutrient was able to restore immune function and improve mental parameters. The study consisted of four groups: control subjects (n = 8), early-onset Alzheimer's subjects (n = 11), late-onset Alzheimer's subjects (n = 7), and multiinfarct dementia subjects (n = 10). After 3 months of treatment, citicoline supplementation improved mental performance in all groups (including controls), as assessed by several standard assessment tools (including the Mini-Mental State Examination and the Hamilton Rating Scale for Depression). Patients with early-onset Alzheimer's showed increased levels of interleukin-1β at baseline. Citicoline administration normalized these levels in the early-onset Alzheimer's group. The researchers concluded that citicoline showed benefit in senile dementia patients as a restorative and palliative treatment, improving vascular risk factors, stabilizing immune function, and improving mental performance.[34] Further studies corroborated these effects of citicoline.[35]

Citicoline was further studied in a double-blinded, placebo-controlled randomized trial in 30 patients with apolipoprotein-E (Apo-E) genotyped Alzheimer's disease. All 30 participants were categorized as having mild to moderate dementia. Citicoline (1000 mg/day orally) or placebo was administered daily for 12 weeks, and its efficacy was further evaluated on the basis of each of the individuals' Apo-E genotype. The development of certain symptoms of Alzheimer's disease is correlated with differing Apo-E genotypes. The results of the study showed that clinical interview-based impression of change scores worsened significantly in the placebo group, whereas a clear trend toward improvement in the citicoline group was observed. In those individuals bearing the ε4 allele of the Apo-E (Apo-E4), citicoline was found to induce significant improvements on the cognitive function subscale of the Alzheimer's Disease Assessment Scale. Furthermore, statistically significant improvements in Alzheimer's Disease Assessment Scale scores were found with citicoline administration in the subset of Apo-E4 patients with mild cognitive deterioration (as assessed by Geriatric Depression Scale scores of less than 5). An overall increase in cerebral blood flow velocity was also seen with citicoline compared with placebo, whereas beneficial changes were further noted in brain bioelectrical activity.[36] Recently two clinical studies were published on the effect of citicoline coadministered with cholinesterase inhibitors. In a retrospective, multicenter case-control study in Italy, involving 448 consecutive patients aged 65 years old or older affected with Alzheimer's disease, 197 patients were treated with an AChEI and 251 were treated with an AchEI + citicoline 1000 mg/day given orally. Patients treated with citicoline plus an AChEI showed a statistically significant increase in Mini-Mental State Examination (MMSE) and slowing disease progression.[37] The CITIRIVAD study was a retrospective case-control study on 174 consecutive outpatients aged 65 years or older affected with Alzheimer's disease (AD) or mixed dementia (MD). Of the 174 patients, 92 had been treated with rivastigmine + citicoline 1000 mg/day given orally; 82 patients had been treated with rivastigmine. Data show the effectiveness of combined administration versus the AchEI alone, mainly in slowing disease progression and consequently in disease management, both in AD and in MD.[38]

In a number of studies, coadministration of citicoline with prescription drugs has been proposed to lead to a greater increase in intrasynaptic levels of Ach than when either is given alone. Such a demonstration was reported in a retrospective multicenter case-control study of 448 patients aged 65 years of age or older with AD, of which 197 patients were treated with acetylcholinesterase inhibitors (AChEIs) and 251 were given AChEIs and 1000 mg/day of citicoline orally. Multiple functional cognitive assessments showed significant improvements of the combined administration after 3 and 9 months compared with AChEIs alone.[37] Similar results were reported in the CITIRIVAD study, a retrospective case-control study on 174 patients aged 65 years or older affected with AD or MD (mean age, 83.3 ± 4.5 yrs). In this study, 92 patients were treated with rivastigmine (an acetylcholinesterase inhibitor) and 1000 mg/day of citicoline, and 82 patients were treated with the ACHEIs alone. In both groups, a rivastigmine patch at the highest tolerated dosage had been used by the patients for at least 6 months. Multiple functional tests for cognition were administered to assess changes in both groups. The study found that the combination was well tolerated and that combined administration was effective in slowing disease progression and improving disease management.[38]

Parkinson's Disease

Citicoline may also benefit individuals with Parkinson's disease. Citicoline was administered by intramuscular (IM) injection to 20 patients (aged 52–76 years) at a dosage of 1000 mg/day for 15 days, followed by 500 mg/day for an additional 15-day period. (All of these patients received levodopa alone or in combination with other drugs before and during the trial.) The patients had improved scores on the Columbia Rating Scale (one of several validated and reliable rating tools for disability in Parkinson's[39]) by 7.3%, and also had measurable improvements in rigidity, time to walk 10 meters, time to turn over, and handwriting test scores. Results from a self-assessment revealed that symptomatology was improved in 15 of the 20 patients, including improvements in speech, gait, posture, tremor, agility, and slowness of movements. Five patients showed minor improvements in dyskinesia; however, this was otherwise unaffected by citicoline treatment.[40]

Additionally, citicoline has a levodopa-sparing effect and an ability to increase dopamine synthesis. In one trial, 85 Parkinson's disease patients were randomly assigned to two groups: patients received either their usual dose of levodopa (mean 381 mg/day) or half their usual dose (mean 196 mg/day). Both groups were simultaneously administered 1200 mg citicoline (400 mg orally three times daily); it was found that the group consuming half of their usual levodopa dose plus citicoline had significant improvements at week 6 (which was the end of the fourth week of citicoline administration) on the Webster Rating Scale, a measure of neurological and clinical symptoms. This trial indicated that citicoline has the ability to compensate for the reduction of levodopa dosage, potentially contributing to a reduction in side effects associated with long-term levodopa usage.[41]

A similar trial was performed in 30 individuals with Parkinson's disease in which the participants were treated with levodopa and concomitantly received 500 mg of citicoline by IM injection daily for 30 days. Significant improvements in neurological signs were noted (including moderate improvements in facial expression and digital skill and marked improvements in the ability to rise from a seated position, posture, and gait) in addition to improvement in certain electrophysiologic parameters. An increase in dyskinesia was noted as a side effect in this group. In the second phase of the trial, the levodopa dosage was reduced by one-third. This decrease restored the incidence of dyskinesia to pretreatment levels, whereas the therapeutic response remained stable.[42]

An additional double-blinded, placebo-controlled, crossover design trial, in which citicoline (500 mg/day IM) or placebo was administered to 30 Parkinson's disease patients already on levodopa and a dopa decarboxylase inhibitor found that citicoline induced improvements in bradykinesia. There was a 26.97% improvement from baseline on the

Webster Rating Scale. This improvement was highly statistically significant compared with baseline and compared with placebo. Significant improvements in rigidity were also noted with citicoline treatment, whereas no such improvement occurred with placebo.[43]

Stroke and Cerebral Ischemia

Stroke is one of the leading causes of physical disability and contributor to vascular cognitive impairment and vascular dementia that can significantly affect a patient's quality of life. Multiple trials have shown positive benefits after citicoline administration in patients who have suffered a stroke because of cerebrovascular ischemia.[44]

In one study, investigators conducted a multicenter, double-blinded, placebo-controlled trial to evaluate the efficacy of oral citicoline administration in patients who experienced an acute cerebral infarction. The study consisted of 272 patients with a confirmed diagnosis of cerebral infarction and a mild to moderately impaired level of consciousness. Patients were randomly assigned to receive either 1000 mg/day of citicoline administered intravenously or a placebo for 14 days. When these acute stroke patients were assessed at days 7 and 14, citicoline treatment resulted in significant improvements in the level of consciousness and neurological status.[45]

In a second multicenter, randomized trial conducted in the United States (the US Citicoline Stroke Treatment Study), positive results were reported for patients who experienced an acute stroke. Treatment was initiated within 24 hours of stroke onset with citicoline for 6 weeks, and patients were assigned to one of three dose groups that were administered either 500, 1000, or 2000 mg/day, then compared with a placebo group. The Barthel Index, an ordinal scale that assesses functional independence in stroke patients, was used as the primary outcome measure to assess functional improvement. In the 500-mg/day citicoline group, the odds ratio for improvement was 2.0, and in the 2000-mg/day group, the ratio was 2.1, signifying that individuals in these groups were twice as likely to achieve higher Barthel scores than those in the placebo group. Overall, the results showed that either 500 or 2000 mg/day of citicoline significantly improved functional recovery after 6 weeks of treatment compared with placebo by the 12th week of follow-up. Interestingly, the group taking 1000 mg/day of citicoline did not show a comparable benefit. This was puzzling in that the baseline characteristics of each group were essentially identical, except for weight, which was higher in this group than in the other treatment groups. The authors postulated this might have played a role in the outcome, suggesting that a high dose may be needed for patients with a body-mass index (BMI) greater than 30.[46]

In a third multicenter, randomized, placebo-controlled trial in acute stroke patients, patients were given citicoline for 6 weeks and assessed according to the National Institute of Health (NIH) Stroke Scale. No benefit was seen in terms of recovery, likely because of several significant confounding factors that affected the analysis, such as populating the placebo arm of the study with more patients with milder strokes.[47]

A larger Phase III, multicenter, randomized, placebo-controlled clinical trial was organized to assess the effectiveness of 2000 mg/day of citicoline taken for 6 weeks to determine whether this dosage would facilitate an accelerated rate of stroke recovery. This study featured recruitment from 118 stroke centers representing 899 patients randomized to receive either citicoline or placebo. Unlike earlier trials that assessed the efficacy of citicoline in moderate-stroke patients, this study recruited participants with NIH Stroke Scale scores of 8 or higher, characterized as having experienced a severe stroke. The major end point of this trial was a comparison of the proportion of individuals with an improvement of 7 or more points on the NIH Stroke Scale by week 12. Although no significant differences were noted between groups using the NIH Stroke Scale, a benefit was seen for citicoline on the Barthel Index at 6 weeks; a significantly higher proportion of those in the citicoline group returned to baseline function by then.[48]

An international, randomized, multicenter, placebo-controlled study, the International Citicoline Trial on Acute Stroke (ICTUS) trial, aimed to determine whether neurovascular protection and repair would improve the recovery of patients who experienced an acute ischemic stroke and received citicoline treatment. In this study, 2298 patients were randomly assigned to receive either citicoline or placebo within 24 hours after the onset of symptoms, after which they were given 1000 mg of citicoline every 12 hours intravenously in first 3 days after the stroke and orally thereafter for a total of 6 weeks. Of the patients, 1148 were assigned to the citicoline group versus 1150 placed in the placebo group. An analysis of the results found that global recovery was similar for both groups (odds ratio 1.03; 95% confidence interval [CI] 0.86–1.25; $p = 0.364$), and no significant differences were reported for adverse events.[49] A subsequent critique of the study pointed out that patients in the ICTUS trial were older by 4 years on average, had more severe strokes, and were more frequently treated with intravenous administration of tissue plasminogen activator to dissolve the clot to improve blood flow, along with heterogeneity among previous trials the ICTUS study compared itself to that could be a function of methodological and clinical differences and random error.[49] In an updated meta-analysis of the ICTUS trial, the authors showed an overall significant effect of citicoline (odds ratio 1.14; 95% CI 1.00–1.30) and a significant heterogeneity of effects ($p = 0.0029$) between previous studies and the ICTUS trial.[50]

Hazama and colleagues conducted a double-blinded, placebo-controlled trial in 1980 to assess the effect of citicoline administration as an adjunct to regular rehabilitation in post-stroke recovery from hemiplegia (paralysis of one side of the body).[51] In their study, citicoline was administered once daily for 8 weeks to patients continuing rehabilitation and assigned to one of three groups: a citicoline high-dose group (1000 mg/day IV), a citicoline low-dose group (250 mg/day IV), or placebo (isotonic saline). Upper and lower limb joint range of motion was assessed at intervals throughout the study, as were subjective symptoms, neurological signs, and mental symptoms. Significant improvements were noted in functional recovery in the upper limb in all groups, with dose-dependent improvements noted in both of the citicoline groups, which were significantly superior to placebo by week 8. No significant differences were noted between groups in the lower limb, although both citicoline groups showed a slightly higher rate of improvement compared with the placebo.[51]

Citicoline was evaluated in a randomized, placebo-controlled study consisting of 92 patients with chronic cerebrovascular conditions. Patients received 1000 mg/day of citicoline intramuscularly or a placebo for two treatment cycles of 4 weeks, each with a 1-week interval between cycles. The study outcome showed that citicoline significantly improved attention ability, as seen by the decreased number of wrong responses on the Toulouse-Piéron Test for nonverbal stimuli. Furthermore, a constant and progressive improvement was noted with citicoline treatment on memory tests and emotional and behavioral assessments.[52]

Citicoline treatment of patients with hemorrhagic, nontraumatic cerebral infarction was found to enhance the recovery of muscular strength associated with recovery. A double-blind, randomized trial conducted with 32 study subjects assigned to receive either 250 mg of citicoline IV twice daily or a placebo for 14 days showed that compared with baseline, muscular strength in the citicoline group increased significantly more so than in patients receiving placebo.[53]

It is noteworthy to mention the editorial that appeared in 2006 in the *Journal of Neurological Sciences* that declared citicoline to be the first

clinically effective neuroprotective agent in acute ischemic strokes.[54] The editorial recommended that citicoline be the agent of choice in trials of combination therapy for stroke with thrombolytic agents because of its safety profile and experimentally demonstrated efficacy in promoting recovery from ischemic conditions. This editorial followed by a year the review by the Cochrane Collaboration that analyzed the outcomes from clinical trials using citicoline in the treatment of cognitive and behavioral symptoms resulting from chronic cerebral conditions in the elderly, which concluded that citicoline showed benefits for improved memory function and behavior in elderly individuals with chronic cerebral disorders.[55]

To assess the value of citicoline administration over longer periods of time, two open-label, randomized, parallel studies were performed that compared citicoline with conventional treatments for the treatment of ischemic stroke patients was undertaken. In the first study, subjects were selected 6 weeks after suffering a stroke and recruited for an open-label, randomized, parallel study into one of two arms that either received 1000 mg/day of citicoline for 12 months or a control group that did not receive the supplement. All subjects underwent a series of neuropsychological tests and evaluations at months 1, 6 and 12. Of 347 subjects, 172 received citicoline, and 175 served as controls. Cognitive function improved at 6 and 12 months after the stroke in both groups. In comparison with controls, the patients receiving citicoline showed improvements in attention-executive functions ($p = 0.007$) and temporal orientation ($p = 0.045$) at the 12-month follow-up.[56] This study demonstrated that citicoline treatment for at least 12 months after an acute ischemic stroke is potentially effective and safe in preventing longer-term cognitive impairment in that citicoline treatment resulted in significant improvement in attention, executive function, and temporal orientation.

In the second open-label, randomized, parallel trial, patients were selected 6 weeks after they suffered a stroke and randomized into parallel arms. A series of neuropsychological examinations was performed at month 1, month 6, year 1, and year 2 after the stroke. Subjects in the study consisted of 163 patients who had suffered their first stroke. The mean age of subjects was 67.5 ± 10.5 years; 50.9% were females. After 2 years, long-term citicoline use was associated with a greater quality of life and significantly improved cognitive status compared with patients receiving conventional treatments.[57]

For patients with subjective memory complaints and neuroradiological evidence of vascular lesions, a 9-month study was performed in 349 subjects (79.9 ± 7.8 years) to assess the effectiveness and safety of 500 mg of citicoline given twice a day to 265 individuals with mild vascular cognitive impairment or a control group of 84 nonsupplemented patients (78.9 ± 7.01). Individuals recruited for the study with probable Alzheimer's disease were excluded. The study found that citicoline was effective and well tolerated.[58] Whereas the control group experienced a decline in scores over the 9-month period on the MMSE, used extensively to measure cognitive impairment, the citicoline group essentially remained unchanged over time, except for an insignificant increase of 0.5 points on the test.

In summary, the evidence in support of citicoline in the treatment of stroke continues unabated, as discussed by Martynov and Gusev in the *Journal of Experimental Pharmacology.*[59] In a review in the *Journal of Stroke and Cerebrovascular Diseases,* a similar conclusion stated, "Citicoline is the only drug that in a number of difference clinical stroke trials that continuously had some neuroprotective benefit," given its safety and beneficial effect in expediting the recovery of patients after experiencing an acute ischemic stroke.[60] Nevertheless, administration of tissue plasminogen activators (tPAs) remains the preferred method of treatment for patients experiencing an acute ischemic stroke, and conventional medicine remains sufficiently unimpressed with the experimental evidence of the benefits of citicoline to recommend its use concomitant with tPAs and other therapeutic agents. Unfortunately, this attitude comes from reliance on several cumulative meta-analyses that dilute the evidence for citicoline's benefits by mixing studies that are heterogeneous in dosage, administration, inclusion criteria, and outcome measures.[61,62] Nevertheless, the totality of evidence, when examining each study on citicoline's treatment effect in stroke and cerebral ischemic patients, supports the use of citicoline for its neuroregenerative potential benefit in preventing post-stroke cognitive impairment.

Traumatic Head Injuries

Traumatic head injuries are a major cause of long-term disability that may surpass many diseases as the major cause of death and neurobehavioral disability.

Research into the beneficial effects of citicoline for the treatment of traumatic head injuries and concussions has been ongoing for several years. Injuries to the head can result in decreased production of cell membrane phospholipids, resulting in an accumulation of intracellular water that leads to edema and possible deterioration of the hematoencephalic barrier. Citicoline can have therapeutic benefits in these conditions because it is a precursor for the synthesis of neuronal membrane phospholipids.

A single-blinded randomized study assessing the administration of 1000 mg of intravenous citicoline given every 6 hours for 2 days, followed by 1000 mg every 8 hours thereafter on the third and fourth days, in addition to conventional therapy, was undertaken in 216 patients with severe or moderate head injuries. The study found citicoline to be superior to conventional therapy alone for 3 months after injury as assessed by the Glasgow Outcome Scale, along with a trend toward shortening hospital stays for severe head injury patients while at the same time improving motor, cognitive, and mental symptoms.[63] The authors cited earlier research on citicoline in treating moderate to severe head injuries, which showed that citicoline increased the chances of recovery to a nondependent condition (including the ability to walk and perform activities of daily living) in improving quality of life. Citicoline also improved levels of consciousness assessed at 60 days after injury in traumatic coma patients while reducing the percentage of patients showing focal neurological signs at 60 and 90 days postinjury.[63]

In a study comparing 67 patients diagnosed with traumatic brain injury who received citicoline administration compared with 67 matched patients who did not, patients receiving citicoline experienced significantly reduced rates of intensive care unit mortality (5% vs. 24%, $p < 0.01$), in-hospital mortality (9% vs. 24%, $p = 0.035$), 6-month mortality (13% vs. 28%, $p = 0.031$), and observed versus expected mortality (0.42 vs. 0.84).[64]

Citicoline therapy may also help alleviate postconcussional symptoms, frequently associated with emotional disturbance of clinical proportions.[65] In one randomized trial with 14 young adults ($M = 25$ yr in citicoline group; $M = 20$ yr in placebo group) with mild to moderate head injuries, participants were administered 1 g of citicoline orally or a placebo. Assessments included tests of memory function, fluency, and attention. Although results at 1 month did not reach statistical significance, the group given citicoline trended toward higher improvement in several categories at follow-up, including improvements in recognition memory, and a decrease in the incidence of headaches, dizziness, and tinnitus.[66]

A review article examining trials of citicoline for the treatment of traumatic head injuries, including in children as young as age 5, espoused the benefits of citicoline therapy for improving neurological signs and symptoms, increasing the level of consciousness, enhancing

recuperation, and facilitating electroencephalographic improvements. The review highlighted several trials related to traumatic coma patients treated with citicoline, which led to better recovery of motor function and walking ability compared with placebo. The overall results of these trials indicate that citicoline treatment reduced the duration of coma and the incidence and severity of mental and motor deficits associated with traumatic head injuries. Citicoline was also shown to be safe and well tolerated in patient populations of several age ranges with various types of traumatic head injuries.[67]

To determine the possible benefit of citicoline in patients with severe traumatic brain injury, a double-blind, randomized clinical trial was conducted in 58 subjects with a diagnosis of diffuse axonal injury to the head that had a score of 8 or greater on the Glasgow Coma Scale (GCS). Although mean GSC levels improved in both groups, the difference bordered on insignificant ($p < 0.05$). Noteworthy was the increase in fetuin-A ($p = 0.012$), a negative-phase reactant, and matrix Gla-protein (MGP; $p = 0.046$), a calcification inhibitor, both of which are calcification modulators.[68] This outcome suggests that citicoline may have a protective effect against inflammation after vascular calcification secondary to traumatic brain injury by increasing serum levels of fetuin-A and MGP.

In the randomized, double-blind Citicoline Brain Injury Treatment Trial (CORBRIT), 1231 patients attending eight level I trauma centers were given either citicoline or a placebo to determine its neuroprotective properties to facilitate neurorepair after injury. Subjects were given either 2000 mg/day or placebo for 90 days. Functional and cognitive status were assessed, including the TBI-Clinical Trials Network Core Battery. Rates of improvement on the Glasgow Outcome Scale-Extended were similar for both groups, whereas other measures, such as the TBI Clinical Trials Network Core Battery (global odds ratio 0.98; 95% CI, 0.83–1.15) showed no difference between groups. The use of citicoline compared with placebo did not result in an improvement in functional or cognitive status.[69] Although the COBRIT study's outcome would not encourage the clinical use of citicoline in the treatment of patients with traumatic head injuries, it is important to keep in mind that there is insufficient evidence to support pharmacological treatments relying on drugs such as modafinil, atomoxetine, rivastigmine, and monoamine stabilizers; they have been found to be no more effective or better than placebo.[70] Hence, given the compelling evidence I support of citicoline's neuroprotective properties, consideration for its use is warranted for traumatic head injuries, especially when considering the evidence of its safety.

EYE HEALTH AND VISUAL FUNCTION

Several trials with citicoline showed beneficial effects on eye health, specifically in cases of amblyopia and glaucoma. Amblyopia, or lazy eye, is the leading cause of decreased visual acuity in children, resulting in poor depth perception. Glaucoma is a leading cause of blindness in U.S. adults and is a group of conditions resulting in damage to the optic nerve, usually as a result of elevated intraocular pressure.

Amblyopia

Amblyopia is a condition whose prevalence ranges between 1% to 5% of the population. The possibility that citicoline may be useful in the treatment of amblyopia is based on several studies that used citicoline in the treatment of this eye condition.[71]

In one study, 80 subjects were recruited to undergo amblyopia therapy with a Bangerter 0.8 filter or the filter with oral citicoline. Forty-eight of the subjects had exodeviation of the eyes, a type of strabismus in which the visual axes diverge, and 32 had esodeviation, characterized by a reduction in the vision of one or both eyes. Treatment with citicoline was administered daily, 5 days a week, for 12 months. After 1 year, those subjects who received citicoline along with the filter experienced a more rapid increase in visual acuity, along with reduced recovery time and improved visual acuity. This led the authors of the study to conclude that by combining the use of the filter with citicoline, both visual acuity and visual evoked potential latency improved, most likely because of enhanced transmission of the electric impulses from the retina to the visual cortex.[72]

An open trial and a pilot double-blinded follow-up study were conducted to assess the possible benefits of citicoline therapy in patients with amblyopia. The open trial consisted of 50 patients (mean age, 16.6 years) who were administered citicoline (1000 mg/day IM) for 15 days. The visual acuity of both eyes was tested 1 week after the initiation of treatment and continuing at weekly intervals for the first month, and then on a monthly basis for an additional 6 to 18 months. For the double-blinded portion of the study, 10 patients were divided into two groups: 1 group received citicoline (1000 mg/day IM), and the other group received placebo. Citicoline improved visual acuity in 92% of the patients in the open study. Improvements were noted in both the sound and amblyopic eyes and were highly statistically significant. In the double-blinded study, significant improvements were noted between groups, with the citicoline group showing enhanced visual acuity.[73]

Another trial of citicoline took place in 45 children with amblyopia aged 5 to 9 years old. Participants were divided into three treatment groups: group A received 500 mg of citicoline daily via IM injection for 10 days every 6 months; group B received the same dosage of citicoline as group A in combination with 1 hour of occlusion (of the sound eye) per day; and group C received daily occlusion therapy alone. Although visual acuity improved in all groups at the end of the treatment period, treatment with citicoline was found to enhance the effect of occlusion therapy. Visual acuity improved in 73% of participants in group A, 86.6% of group B, and 66.6% of group C.[74]

An open-label trial with oral citicoline (800–1200 mg/day of citicoline according to body weight for 30 days) plus partial occlusion therapy in 61 children (aged 5–10 years) found that citicoline contributed to stabilizing the gains obtained during the treatment period when assessed at the 60-day posttreatment follow-up visit. Those receiving occlusion therapy alone showed a decrease in visual acuity gains at the 60-day follow up, whereas those in the citicoline group maintained the gains achieved with occlusion therapy.[75]

A randomized controlled trial assessed the effectiveness of the addition of citicoline to patching in the treatment of amblyopia in the age group of 4 to 13 years. Patients received patching therapy until achieving a plateau. Thereafter, one group received citicoline plus patching while a second group continued on patching therapy. The primary outcome measurement was visual acuity on the logMAR chart, used to estimate visual acuity, after 12 months. The improvement in visual acuity with citicoline plus patching was significantly more than that with patching alone after 1 year of treatment.[76]

Citicoline likely influences improvements in visual acuity in amblyopic individuals by stimulating the availability of several neurotransmitters and neuromodulators. It also enhances the activity of endogenous dopamine while improving vascular aspects of neurological function.[77]

Glaucoma

Lulia and colleagues have reviewed the neuroprotective benefits of citicoline on glaucomatous disease and the retina,[78] and the review by Roberti and colleagues provides a critical summarization of the evidence for the use of citicoline in the treatment of glaucoma.[79] Included among the studies reviewed is a randomized, placebo-controlled,

clinical trial evaluating the effects of citicoline in 40 patients with open-angle glaucoma who received either daily IM injections containing 1000 mg of citicoline or placebo for 60 days. Citicoline significantly improved visual evoked potential (a measure of bioelectrical activity of the visual cortex in response to visual stimuli) and the pattern-electroretinographic (used to evaluate the functional integrity of the innermost retinal layers) parameters compared with placebo. Patients in the citicoline group were then divided into two age-matched groups after a 120-day washout period. In one of these groups, the washout period was extended for an additional 120 days, whereas the second group received a further 60-day treatment of citicoline. This second group showed further improvements in visual evoked potential and pattern-electroretinographic parameters, indicating citicoline's ability to enhance retinal function and visual cortical response in glaucoma patients.[80]

A double-blind RCT assessing the benefits of citicoline in glaucoma patients confirmed that administration of citicoline for 60 days (1000 mg/day IM) was superior to placebo and significantly improved retinal function and cortical bioelectrical responses. This particular trial included an analysis of 8 years of follow-up data.[81]

A review examining the potential mechanisms through which citicoline exerts its beneficial influence in glaucoma patients theorized that citicoline's ability to enhance the synthesis of phosphatidylcholine and other cell-membrane phospholipids could explain its observed improvements. Glaucoma is considered a neurodegenerative disease in which the pathology extends to retinal ganglion cells. The death of these cells is likely a result of apoptotic mechanisms. The enhancement of phosphatidylcholine synthesis as a result of citicoline intake countered the neuronal apoptotic mechanisms associated with glaucoma that conferred neuroprotection.[82]

The use of citicoline eye drops has also been investigated, starting with its encouraging effects on the vitreous and retina of animals,[83] after which human clinical trials did not take long to begin. In one trial that enrolled 34 patients, citicoline was found to delay the progression of glaucoma using a 1% and 2% administration of citicoline drops three times a day in the eye. In another trial, 53 patients were enrolled (47 completed the trial) with open-angle glaucoma (mean age 52.4 ± 4.72 years) and were treated with topical citicoline (3 drops/day) over a 2-month period and compared with 23 patients treated with beta-blocker monotherapy. Whereas the group treated with beta blockers showed no improvement, patients treated with citicoline drops experienced an improvement in both retinal bioelectrical response (increase of pattern electroretinogram) and bioelectrical activity of the visual cortex.[84] The conclusion of the investigators of both trials was that topical citicoline administered as drops in the eye had neuroprotective bioactivity.

That citicoline drops might significantly slow down the rate of progressive glaucoma was studied in 41 patients with a diagnosis of progressing glaucoma. The patients were given citicoline drops for 2 years, during which time visual field examinations were performed. After 2 years, the mean rate of progression was slowed and there was an increase in intraocular pressure, resulting in the investigators concluding that, "citicoline might significantly slow down glaucomatous rates of progression."[85] Frolov and colleagues administered the combined oral administration of 1000 or 500 mg/day of citicoline in patients with open-angle glaucoma with 10 days of intravenous citicoline. Following this treatment protocol, both dosage groups experienced improvements in visual function, perimetry, morphometric retinal tomography, and life quality. The authors believe the improvement seen in patients could be attributed to a decrease in apoptosis, supporting the evidence that citicoline is an ocular neuroprotective agent.[86]

Ischemic Optic Neuropathy

Nonarteritic ischemic neuropathy is an irreversible ischemic event associated with the intraocular optic nerve. The condition occurs acutely and painlessly, yet it induces a loss of visual acuity and visual field. In a pilot study designed to assess citicoline's effect on this condition, 26 patients with at least a 6-month history of nonarteritic ischemic optic neuropathy were divided into two groups: one received treatment consisting of oral citicoline (1600 mg/day) for 60 days, whereas the second group received no treatment. After the 60-day treatment cycle, there was a washout period of 120 days. After this, a second period of treatment with citicoline for 60 days was instituted in the original citicoline group. A third group of 14 age-matched healthy subjects provided control data. At the end of treatment, statistically significant improvements were noted in visual evoked potential, visual acuity, and pattern-electroretinographic parameters in the citicoline group compared with pretreatment values, whereas no such changes were observed in the untreated group of nonarteritic ischemic neuropathy subjects.[87]

Substance Abuse

Citicoline has been considered as an adjunct treatment for cocaine dependence by researchers in the field in recent years. The justification for its use stems from citicoline's ability to repair neuronal membranes, which are damaged by cocaine use, and its ability to increase central nervous system dopamine levels, attenuating cravings for cocaine and other abused substances.[88]

A small double-blinded, placebo-controlled trial in 14 subjects with a history of cocaine dependence found that oral citicoline (500 mg twice per day for 14 days) caused no adverse events and also attenuated some measures of cocaine craving and drug use.[89]

An additional experiment in eight healthy occasional cocaine users investigated the influence of citicoline pretreatment on cocaine-induced cardiovascular and behavioral effects and plasma levels of cocaine. The primary outcome measure was to determine the safety of coadministration of citicoline with cocaine. Because citicoline did not adversely affect cardiovascular end points associated with acute cocaine intake, the use of citicoline in this patient population was presumed safe. Although citicoline did not block the acute subjective effects of cocaine use, cocaine users experienced a higher incidence of major cerebrovascular events. Thus the authors speculated that citicoline could play a role in attenuating these undesirable consequences of cocaine use, although further studies are needed to investigate these potential benefits.[90] These studies have had mixed results to date. The possibility that citicoline could be used in the treatment of cocaine dependence was studied in 29 subjects in a randomized, double-blind, placebo-controlled trial. Cocaine-dependent outpatient participants were given either 500 mg twice daily of citicoline or placebo for 8 weeks. Citicoline had no effect on reducing cocaine craving or total use.[91] In contrast, a placebo-controlled study of 130 outpatients with bipolar 1 disorder (depressed or mixed mood state) and cocaine dependence found a significant reduction in cocaine dependence, which, however, diminished over time.[92] Also of interest was the study's completion rate, which favored the citicoline group (71% compared with 57% on placebo).

Methamphetamine dependence is a major public health problem. Because citicoline may decrease cocaine craving,[89,93] a preliminary study of citicoline treatment for methamphetamine dependence was conducted. Sixty participants with bipolar or unipolar depression and methamphetamine dependence participated in a randomized, double-blind, placebo-controlled trial. Subjects were given either 2000 mg/day of citicoline orally ($n = 28$) or a placebo ($n = 20$) for 12 weeks. Changes in mood state were assessed by several psychometric

instruments that found it had a measurable antidepressant effect.[94] However, despite a doubling of completion rates in the citicoline group, no differences were seen in drug use or other cognitive outcomes.

Individuals with bipolar disorder are at an increased risk for substance abuse, with cocaine use being particularly common in this condition. Both the disorder and cocaine use are associated with mood symptoms and cognitive deficits. Given these commonalities, a 12-week double-blinded randomized control trial was conducted to assess the effect of citicoline supplementation on 44 individuals with bipolar disorder and cocaine dependence. Citicoline resulted in an improvement in some aspects of declarative memory. However, no evidence for antidepressant properties was seen with the use of citicoline. There was also a significantly lower probability of a positive urine test for cocaine at the end of the study in the citicoline group.[95]

Although the legalization of medical marijuana is becoming widespread, along with the legalization of recreational marijuana in a number of states in the United States, there is concern that habitual use of marijuana can affect frontal/executive function in adolescents and young adults[96,97] and in long-term heavy users seeking treatment.[98] Because recreational marijuana use has been reported to be associated with a range of cognitive impairments and changes in brain structure and function, particularly after early-onset exposure (age 15 and earlier), a study was conducted to see if executive functioning, sustained attention, and impulse control would improve after 8 weeks of treatment with oral citicoline in a randomized, double-blind, placebo-controlled trial.[99] The study found that among 30 participants who received 2000 mg/day of citicoline or a placebo for 8 weeks, and who met eligibility criteria, of which 19 completed the study, those receiving citicoline demonstrated significantly lower levels of behavioral impulsivity, improved task accuracy on two measures of inhibitory function, and different patterns of brain activation patterns than those who received placebo. The later improvement in brain activation was based on a comparison of functional magnetic resonance imaging (fMRI) results of the anterior cingulate cortex, a region critical to inhibition processing. Other changes noted in the scans from baseline to 8 weeks of citicoline treatment included a shift from posterior/midcingulate to genual cingulate activation that corresponded with a reduction in total voxel cluster size. No activation in genual or near-genual regions was seen in the placebo group over the same time period.

Infectious Diseases

A recently published article highlighted the role citicoline might play as an adjunctive therapeutic agent for the treatment of disease arising from an infectious etiology. The pathology of infectious disease involves dysregulation of the host immune response. Although currently available treatments target the infectious agent, they do little to address the concurrent neurological abnormalities. These consequences, if unaddressed, lead to eventual mortality in a high percentage of cases. In the case of cerebral malaria, studies highlighted cytokine-induced endothelial inflammation and the compromised blood–brain barrier as major pathological factors leading to the associated neurological signs and symptoms. Ischemia is a large underlying mechanism for damage in sepsis and cerebral malaria. Because these underlying issues are similar to conditions such as stroke and myocardial infarction, it is likely that citicoline would benefit these patients as an adjunct treatment for sequelae of sepsis and cerebral malaria.[2]

Appetite Control and Satiety

Given citicoline's ability to enhance cognitive function, confer neuroprotection, and support neuroregenerative effects, and its action to support dopaminergic activity in the brain, researchers investigated the potential of citicoline administration for controlling appetite and promoting satiety. In this particular study, the effect of 2000 mg/day of oral citicoline given orally for 6 weeks was assessed by functional magnetic resonance imaging to elucidate cortico-limbic responses to images of various foods, along with subjective ratings of appetite and measures of weight. Sixteen healthy adults (age range of 40–57 years and a BMI range of 20.1–38.6 kg/m^2) were included in the study. Eight participants were assigned to the 500-mg/day citicoline group, and the other eight were assigned to 2000 mg/day. The results of this study indicated that self-assessment ratings for appetite declined significantly between visits in both groups, with the magnitude of decline in the high-dose group reaching statistical significance. No significant between-group differences were noted for the magnitude of weight change. The high-dose citicoline group also showed higher activation within the right lateral orbitofrontal cortex and left amygdala during visual perception of high-calorie foods than the low-dose group when assessed by fMRI. This might indicate that high-dose citicoline leads to appetite suppression and feelings of satiety by increasing the responsiveness of these regions to images of calorie-rich foods.[100]

Mental Health

Citicoline's influence on cognitive capacity and neurological health, including modulatory activity on neurotransmitter production and function, make it a logical choice to support mental health and cognitive functioning.

To evaluate the effect of citicoline administration in eight depressed patients, a randomized, double-blind, placebo-controlled trial was conducted to test its effectiveness as an adjuvant therapy in the treatment of major depression. In the study, patients were given 500 mg/day IM, as 300 mg at 8 AM and 200 mg at 5 PM, for 21 days or longer. On the day preceding the beginning of treatment, plasma growth hormone was measured, levels of which indicated reduced growth hormone secretion in these patients. Significant improvements were noted in seven of eight participants (treatment was discontinued in one participant because of a noted suicidal tendency) when assessed using the Hamilton Rating Scale for depression. Although the study was small, the results indicate the potential for significant benefit from citicoline therapy in depressed individuals.[101]

Another randomized, double-blind, placebo-controlled trial was assessed to determine whether 100 mg of citicoline taken twice a day for 6 weeks would alleviate depressive symptoms.[102] Fifty patients with major depressive disorder who were under treatment with citalopram were recruited for the study. Significantly greater improvement was observed in Hamilton Depression Rating Scale scores in the citicoline group compared with the placebo group over a 6-week period (p = 0.021). Citicoline was demonstrated to be an effective adjuvant to citalopram in the treatment of major depressive disorder.

In a preliminary study that examined the therapeutic effect of a combination of citicoline with galantamine for the treatment of schizophrenic patients, the results were encouraging. Evidence to date suggests that α7-nicotinic choline receptors have decreased functionality in schizophrenia. Because citicoline provides choline, a known α7-nicotinic choline-receptor agonist, and galantamine is a modulator of α7-nicotinic choline receptor function used to enhance the efficiency of choline binding to these receptors, it seemed reasonable to observe whether the combination would be of greater therapeutic value than either alone. To find out, a pilot study was conducted that included six schizophrenic patients given 2 g/day of citicoline in combination with 24 mg/day of galantamine daily in a 12-week open-label study. The combination proved to be well tolerated by all participants, with only minor transient side effects. No adverse cardiovascular events were reported. Five of the six participants had lower-than-baseline diastolic blood pressure readings at the end of the study, which was an unexpected benefit. Other improvements noted in five of six patients were

seen on the Clinical Global Impressions Inventory and the Positive and Negative Syndrome Scale. Total Positive and Negative Syndrome Scale scores and Clinical Global Impressions severity scale scores decreased over the study period, providing encouraging results suggesting that the combination therapy was potentially effective.[103]

Metabolic Diseases

Only recently has citicoline been the subject of investigations for its therapeutic potential in glucose management for patients with either type 1 or type 2 diabetes. Insulin-dependent diabetes mellitus is associated with a risk of potentially severe hypoglycemic episodes. The ability of citicoline to reduce neuronal death associated with such episodes has been demonstrated previously in animal studies.[104,105] An animal study showed that when citicoline is given by injection, it can significantly increase acetylcholine levels in the hippocampus after induction of severe hypoglycemia by insulin, suggesting that it could be used in the treatment of hypoglycemia-induced brain injuries.[106] In another animal study, the combination of S-Methylisothiourea (a selective iNOS inhibitor) and citicoline, alone or in combination, was investigated on neuropathic pain in the rat with type 2 diabetes mellitus because neuropathy is a common complication. Citicoline, and the combination, significantly attenuated the diabetic neuropathic pain associated with decreased nerve conduction velocity, mechanical and thermal hyperalgesia, and cold allodynia.[107]

TOXICOLOGY

Clinical investigations using citicoline revealed a favorable safety profile, with few reports of any major adverse events. The most common adverse reports were related to digestive disturbances. Citicoline has also been found to have a lack of significant adverse events in children, as evidenced by its use in clinical trials with pediatric subjects with amblyopia[74] and children with traumatic head injuries.[67]

Citicoline has undergone several toxicological evaluations in multiple animal species and has proven to have a high level of safety. Single-dose acute oral toxicity studies were performed in mice and rats, with a median lethal dose of 27.14 g/kg in mice and 18.5 g/kg in rats. Chronic oral toxicity tests in dogs (1.5 g/kg per day for 6 months) and subchronic intraperitoneal dosing studies in rats (1 g/kg per day for 12 weeks) showed no abnormal signs.[14]

An acute 14-day study and a 90-day subchronic toxicity evaluation of citicoline in rats revealed that the supplement was well tolerated. In the 14-day study, a single dose of 2000 mg/kg showed no abnormalities, and in the 90-day repeated oral dosing study, doses of 100, 350, and 1000 mg/kg per day resulted in no mortality in the animals. In male rats, slight increases in serum creatinine were noted in the two highest-dose groups, whereas in female rats, a dose-related increase in renal tubular mineralization was noted and attributed to an increase in phosphorus intake as a result of high citicoline consumption. Mineralization in female laboratory rats of all universally used strains is a common incidental finding as a result of a decreased calcium/phosphorus ratio in the diet. Because citicoline yields a significant amount of phosphorus—thus influencing the calcium/phosphorus ratio—this finding was not unexpected.[108]

A drug surveillance study was recently published examining the efficacy and safety of oral citicoline intake in acute ischemic stroke. The study of 4191 Korean patients confirmed a high level of safety for citicoline (500–4000 mg/day for 6 weeks or longer) with an incidence of 37 adverse events in 31 patients (only 0.73% of patients experienced adverse events). Adverse events in all but 1 of the 31 patients showed no relationship to citicoline administration. Thirty-two of the 37 (nearly 84%) patients reported events resolved over a mean of 9 days after onset. Furthermore, no dose-related effects of citicoline on the occurrence of adverse events were noted. The most frequent side effects included minor nervous system–related complaints ($n = 8$; numbness, headache, tingling sensations) followed by gastrointestinal symptoms ($n = 5$; abdominal discomfort, diarrhea).[109]

There remains limited information on the long-term use of citicoline in healthy individuals. What few transient side effects are reported in the literature have been minor and reportedly resolved over time. No reliable information to support the safety of citicoline supplementation in pregnant or breastfeeding women has been reported.

DOSAGE

Effective dosing of citicoline based on data from clinical trials ranges from 500 to 2000 mg/day. Based on evidence from trials in children as young as 5 years of age,[67,74] citicoline is safe for use in pediatric and adult populations. Oral doses of up to 1200 mg/day have been used in children.[75] Studies using oral, IM, and IV dosing of citicoline in children and adults, with minimal occurrence of adverse events, affirmed its high level of safety.

Because citicoline activates the biosynthesis of phospholipids in neuronal membranes and has neuroprotective effects during ischemia and hypoxia, the safety of oral administration of 500 mg of citicoline twice a day was of primary interest to investigators conducting the multicenter Italian studio di intervento nel decadimento vascolare lieve (IDEALE study) to assess the its effectiveness in 122 men and 143 women (mean age 79.9 ± 7.8 yrs) with mild vascular cognitive impairment. No adverse events were reported or recorded.[110]

DRUG INTERACTIONS

There are no reports of adverse drug interactions associated with the use of citicoline. Citicoline does have a levodopa-sparing effect of minimal clinical significance, given its potentiating benefits in reducing certain side effects associated with long-term levodopa or carbidopa use.[41–43]

CONCLUSIONS

Until recently, citicoline was only perceived to be a novel compound with a broad spectrum of benefits in conditions associated with neurological disorders and dysfunctions. This seemed reasonable in that citicoline acts at multiple levels to support and maintain neural health to support optimal cognitive function. It also promotes cholinergic and dopaminergic functions, supports phospholipid synthesis and its incorporation into cell membranes, and enhances antioxidant mechanisms in the body while suppressing the damaging effects of free radicals on neural tissue, facilitating anti-inflammatory activities, and facilitating the release of essential neurotransmitters. Given its widespread activity on neural tissue, citicoline should be considered a comprehensive therapeutic agent for supporting brain health.

Its broader clinical applications are growing beyond that of a neuroregenerative and neuroprotective agent. Citicoline treatment of conditions affecting the eye and visual functioning is an example of new therapeutic applications for this compound in ophthalmology. More research is needed on its potential to dampen the adverse effects of substances of abuse, such as cocaine and amphetamines. Recent interest in its attributes in treating metabolic diseases has just begun and awaits results. That citicoline is safe is supported by overwhelming evidence based on its use in dozens of controlled clinical trials, regardless of outcomes.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Secades JJ, Lorenzo JL. Citicoline: pharmacological and clinical review, 2006 update. *Methods Find Exp Clin Pharmacol*. 2006;28(suppl B):1–56.
2. Jambou R, El-Assaad F, Combes V, et al. Citicoline (CDP-choline): what role in the treatment of complications of infectious diseases. *Int J Biochem Cell Biol*. 2009;41:1467–1470.
3. Delage B. *Linus Pauling Institute at Oregon State University*. Choline. http://lpi.oregonstate.edu/mic/other-nutrients/choline. Accessed 3/6/2018.
4. Weiss GB. Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. *Life Sci*. 1995;56:637–660.
5. D'Orlando KJ, Sandage BW. Citicoline (CDP-choline): mechanisms of action and effects in ischemic brain injury. *Neurol Res*. 1995;17:281–284.
6. Dinsdale JR, Griffiths GK, Rowlands C, et al. Pharmacokinetics of 14C CDP-choline. *Arzneimittelforschung*. 1983;33:1066–1070.
7. Agut J, Font E, Sacristán A, Ortiz JA. Bioavailability of methyl-14C CDP-choline by oral route. *Arzneimittelforschung*. 1983;33:1045–1047.
8. Agut J, Font E, Sacristán A, Ortiz JA. Radioactivity incorporation into different cerebral phospholipids after oral administration of 14C methyl CDP-choline. *Arzneimittelforschung*. 1983;33:1048–1050.
9. Sakar AK, Ghosh D, Haldar D, et al. A rapid LC-ESI-MS/MS method for the quantitation of choline, an active metabolite of citicoline: application to in vivo pharmacokinetic and bioequivalence study in Indian healthy male volunteers. *J Pharm Biomed Anal*. 2012;71:144–147.
10. Grieb P. Neuroprotective properties of citicoline: facts, doubts, and unresolved issues. *CNS Drugs*. 2014;28(3):185–193.
11. Babb SM, Appelmans KE, Renshaw PF, et al. Differential effect of CDP-choline on brain cytosolic choline levels in younger and older subjects as measured by proton magnetic resonance spectroscopy. *Psychopharmacol (Berl)*. 1996;127:88–94.
12. Babb SM, Wald LL, Cohen BM, et al. Chronic citicoline increases phosphodiesters in the brains of healthy older subjects: an in vivo phosphorus magnetic resonance spectroscopy study. *Psychopharmacol (Berl)*. 2002;161:248–254.
13. Silveri MM, Dikan J, Ross AJ, et al. Citicoline enhances frontal lobe bioenergetics as measured by phosphorus magnetic resonance spectroscopy. *NMR Biomed*. 2008;21:1066–1075.
14. Secades JJ, Frontera G. CDP-choline: pharmacological and clinical review. *Methods Find Exp Clin Pharmacol*. 1995;17(suppl B):1–54.
15. Drago F, Mauceri F, Nardo L, et al. Effects of cytidine-diphosphocholine on acetylcholine-mediated behaviors in the rat. *Brain Res Bull*. 1993;31:485–489.
16. Adibhatla RM, Hatcher JF, Dempsey RJ. Citicoline: neuroprotective mechanisms in cerebral ischemia. *J Neurochem*. 2002;80:12–23.
17. Adibhatla RM, Hatcher JF. Citicoline decreases phospholipase A2 stimulation and hydroxyl radical generation in transient cerebral ischemia. *J Neurosci Res*. 2003;73:308–315.
18. Rema V, Bali KK, Ramachandra R, et al. Cytidine-5-diphosphocholine supplement in early life induces stable increase in dendritic complexity of neurons in the somatosensory cortex of adult rats. *Neuroscience*. 2008;155:556–564.
19. Knott V, Smith D, de la Salle, et al. CDP-choline: effects of the procholine supplement on sensory gating and executive function in healthy volunteers stratified for low, medium and high P50 suppression. *J Psychopharmacol*. 2014;28(12):1095–1108.
20. Aidelbaum R, Labelle A, Baddeley A, Knott V. Assessing the acute effects of CDP-choline on sensory gating in schizophrenia: a pilot study. *J Psychopharmacol*. 2018. https://doi.org/10.1177/0269881117746903. [Epub ahead of print].
21. Knott V, de la Salle S, Smith D, et al. Effects of acute CDP-choline treatment on resting state brain oscillations in healthy volunteers. *Neurosci Lett*. 2015;591:121–125.
22. de la Morena E. Efficacy of CDP-choline in the treatment of senile alterations in memory. *Ann N Y Acad Sci*. 1991;640:233–236.
23. Spiers PA, Myers D, Hochanadel GS, et al. Citicoline improves verbal memory in aging. *Arch Neurol*. 1996;53:441–448.
24. Alvarez XA, Laredo M, Corzo D, et al. Citicoline improves memory performance in elderly subjects. *Methods Find Exp Clin Pharmacol*. 1997;19:201–210.
25. Bruhwyler J, Liegeois JF, Geczy J. Facilitatory effects of chronically administrated citicoline on learning and memory processes in the dog. *Prog Neuro-psychopharmacol Biol Psychiatry*. 1998;22(1):115–128.
26. Zhao JJ, Liu Y, Chen XL, et al. Effect of citicoline on spatial learning and memory of rats after focal cerebral ischemia. *Nan Fang Yi Ke Da Xue Xue Bao*. 2006;26(2):174–176.
27. McGlade E, Locatelli A, Hardy J, et al. Improved attentional performance following citicoline administration in healthy adult women. *Food Nutr Sci*. 2012;3:769–773.
28. McGlade E, Agoston AM, DiMuzio J, et al. The effect of citicoline supplementation on motor speed and attention in adolescent males. *J Atten Disord*. 2015; pii: 1087054715593633. [Epub ahead of print].
29. Bruce SE, Werner KB, Preston BF, Baker LM. Improvements in concentration, working memory and sustained attention following consumption of a natural citicoline-caffeine beverage. *Int J Food Sci Nutr*. 2014;65(8):1003–1007.
30. Bruce SE. Improvements in quantitative EEG following consumption of a natural citicoline-enhanced beverage. *Int J Food Sci Nutr*. 2012;63(4):421–425.
31. Franco-Maside A, Caamaño J, Gómez MJ, Cacabelos R. Brain mapping activity and mental performance after chronic treatment with CDP-choline in Alzheimer's disease. *Methods Find Exp Clin Pharmacol*. 1994;16:597–607.
32. Caamaño J, Gómez MJ, Franco A, Cacabelos R. Effects of CDP-choline on cognition and cerebral hemodynamics in patients with Alzheimer's disease. *Methods Find Exp Clin Pharmacol*. 1994;16:211–218.
33. Fernández-Novoa L, Alvarez XA, Franco-Maside A, et al. CDP-choline-induced blood histamine changes in Alzheimer's disease. *Methods Find Exp Clin Pharmacol*. 1994;16:279–284.
34. Cacabelos R, Alvarez XA, Franco-Maside A, et al. Effect of CDP-choline on cognition and immune function in Alzheimer's disease and multi-infarct dementia. *Ann N Y Acad Sci*. 1993;695:321–323.
35. Cacabelos R, Caamaño J, Gómez MJ, et al. Therapeutic effects of CDP-choline in Alzheimer's disease. Cognition, brain mapping, cerebrovascular hemodynamics, and immune factors. *Ann N Y Acad Sci*. 1996;777:399–403.
36. Alvarez XA, Mouzo R, Pichel V, et al. Double-blind placebo-controlled study with citicoline in APOE genotyped Alzheimer's disease patients. Effects on cognitive performance, brain bioelectrical activity and cerebral perfusion. *Methods Find Exp Clin Pharmacol*. 1999;21:633–644.
37. Gareri P, Castagna A, Cotroneo AM, et al. The Citicholinage study: citicoline plus cholinesterase inhibitors in aged patients affected with Alzheimer's disease study. *J Alzheimers Dis*. 2017;56(2):557–565.
38. Castagna A, Cotroneo AM, Ruotolo G, Gareri P. The CITIRIVAD study: CITIcoline plus RIVAstigmine in elderly patients affected with dementia study. *Clin Drug Investig*. 2016;36(12):1059–1065.
39. Ramaker C, Marinus J, Stiggelbout AM, Van Hilten BJ. Systematic evaluation of rating scales for impairment and disability in Parkinson's disease. *Mov Disord*. 2002;17:867–876.
40. Martí Massó JF, Urtasun M. Citicoline in the treatment of Parkinson's disease. *Clin Ther*. 1991;13:239–242.
41. Eberhardt R, Birbamer G, Gerstenbrand F, et al. Citicoline in the treatment of Parkinson's disease. *Clin Ther*. 1990;12:489–495.
42. Cubells JM, Hernando C. Clinical trial on the use of cytidine diphosphate choline in Parkinson's disease. *Clin Ther*. 1988;10:664–671.
43. Agnoli A, Ruggieri S, Denaro A, Bruno G. New strategies in the management of Parkinson's disease: a biological approach using a phospholipid precursor (CDP-choline). *Neuropsychobiology*. 1982;8:289–296.
44. Davalos A, Castillo J, Alvarez-Sabin J, Secades JJ, Mercadal J, Lopez S, et al. Oral citicoline in acute ischemic stroke. An individual patient data pooling analysis of clinical trials. *Stroke*. 2002;33:2850–2857.
45. Tazaki Y, Sakai F, Otomo E, et al. Treatment of acute cerebral infarction with a choline precursor in a multicenter double-blind placebo-controlled study. *Stroke*. 1988;19:211–216.

46. Clark WM, Warach SJ, Pettigrew LC, et al. A randomized dose-response trial of citicoline in acute ischemic stroke patients. Citicoline Stroke Study Group. *Neurology*. 1997;49:671–678.
47. Clark WM, Williams BJ, Selzer KA, et al. A randomized efficacy trial of citicoline in patients with acute ischemic stroke. *Stroke*. 1999;30:2592–2597.
48. Clark WM, Wechsler LR, Sabounjian LA, Schwiderski UE. A phase III randomized efficacy trial of 2000 mg citicoline in acute ischemic stroke patients. *Neurology*. 2001;57:1595–1602.
49. Davalos A, Alvarez-Sabin J, Castillo J, Diez-Tejedor E, Ferro J, et al. Citicoline in the treatment of acute ischaemic stroke: an international, randomised, multicentre, placebo-controlled study (ICTUS trial). *Lancet*. 2012;380(9839):349–357.
50. Saver JL. Citicoline: update on a promising and widely available agent for neuroprotection and neurorepair. *Rev Neurol Dis*. 2008;5:167–177.
51. Hazama T, Hasegawa T, Ueda S, Sakuma A. Evaluation of the effect of CDP-choline on poststroke hemiplegia employing a double-blind controlled trial. Assessed by a new rating scale for recovery in hemiplegia. *Int J Neurosci*. 1980;11:211–225.
52. Piccoli F, Battistini N, Carbonin P, et al. CDP-choline in the treatment of chronic cerebrovasculopathies. *Arch Gerontol Geriatr*. 1994;18:161–168.
53. Iranmanesh F, Vakilian A. Efficiency of citicoline in increasing muscular strength of patients with nontraumatic cerebral hemorrhage: a double-blind randomized clinical trial. *J Stroke Cerebrovasc Dis*. 2008;17:153–155.
54. Overgaard K, Meden P. Citicoline—the first effective neuroprotectant to be combined with thrombolysis in acute ischemic stroke? *J Neurol Sci*. 2006;247:119–120.
55. Fioravanti M, Yanagi M. Cytidinediphosphocholine (CDP-choline) for cognitive and behavioural disturbances associated with chronic cerebral disorders in the elderly. *Cochrane Database Syst Rev*. 2005;(2): CD000269.
56. Alvarez-Sabin J, Ortega G, Jacas C, et al. Long-term treatment with citicoline may improve poststroke vascular cognitive impairment. *Cerebrovasc Dis*. 2013;35(2):136–154.
57. Alvarez-Sabin J, Santamarina E, Maisterra O, Jacas C, Molina C, Quintana M. Long-term treatment with citicoline prevents cognitive decline and predicts a better quality of life after a first ischemic stroke. *Int J Mol Sci*. 2016;17(3):390. https://doi.org/10.3390/ijms17030390.
58. Cotroneo AM, Castagna A, Putignano S, et al. Effectiveness and safety of citicoline in mild vascular cognitive impairment: the IDEALE study. *Clin Interven Aging*. 2013;8:131–137.
59. Martynov MY, Gusev EI. Current knowledge on the neuroprotective and neuroregenerative properties of citicoline in acute ischemic stroke. *J Exp Pharmacol*. 2015;7:17–28.
60. Overgaard K. The effects of citicoline on acute ischemic stroke: a review. *J Stroke Cerebrovas Dis*. 2014;23(7):1764–1769.
61. Fioravanti M, Yanagi M. Cytidinediphosphocholine (CDP-choline) for cognitive and behavioural disturbances associated with chronic cerebral disorders in the elderly. *Cochrane Database Syst Rev*. 2005;(2): CD000269.
62. Fioravanti M, Yanagi M, Cytidinediphosphocholine Secades JJ, et al. Citicoline for acute ischemic stroke: a systematic review and formal meta-analysis of randomized, double-blind, and placebo-controlled trials. *J Stroke Cerebrovasc Dis*. 2016;25(8):1984–1996.
63. Calatayud Maldonado V, Calatayud Pérez JB, Aso Escario J. Effects of CDP-choline on the recovery of patients with head injury. *J Neurol Sci*. 1991;103(suppl):S15–S18.
64. Trimmel H, Majdan A, Wodak A, Brazinova A. Citicoline in severe traumatic brain injury: indications for improved outcome. *Wiener Klinische Wochenschrift*. 2017;130(5):1–8.
65. Levin HS. Head trauma. *Curr Opin Neurol*. 1993;6(6):841–846.
66. Levin HS. Treatment of postconcussional symptoms with CDP-choline. *J Neurol Sci*. 1991;103(suppl):S39–S42.
67. Lozano R. CDP-choline in the treatment of cranio-encephalic traumata. *J Neurol Sci*. 1991;103(suppl):S43–S47.
68. Shokouhi G, Haghjoo AG, Sattarnezhad N, et al. Effects of citicoline on level of consciousness, serum level of fetuin-A and matrix Gla-protein (MGP) in trauma patients with diffuse axonal injury (DAI) and GSC≤8. *Ulus Travma Scil Cerrahi Derg*. 2014;20(6):410–416.
69. Zafonte RD, Bagiella E, Ansel BM, Novack TA, Friedewald WT, et al. Effect of citicoline on functional and cognitive status among patients with traumatic brain injury: citicoline brain injury treatment trial (CORBRIT). *JAMA*. 2012;308(19):1993–2000.
70. Dougall D, Poole N, Agrawal N. Pharmacotherapy for chronic cognitive impairment in traumatic brain injury. *Cochrane Database Syst Rev*. 2015. https://doi.org/10.1002/14651858.CD009221.pub2.
71. Sabetti L, Masedu F, Tresca C, Bianchi F, Valenti M. The use of choline in association with the Bangerter filters for the treatment of amblyopia. *Int J Ophthalmol*. 2017;10(11):1777–1778.
72. Mendonca RHFD, Ferreira EL. Visual evoked potentials (VEP) and visual acuity improvements after cytidine 5-diphosphocholine (CDP-choline) therapy in amblyopic patient. *Rev Bras Oftalmol*. 2012;71(5):328–330.
73. Campos EC, Schiavi C, Benedetti P, et al. Effect of citicoline on visual acuity in amblyopia: preliminary results. *Graefes Arch Clin Exp Ophthalmol*. 1995;233:307–312.
74. Campos EC, Bolzani R, Schiavi C, et al. Cytidin-5′-diphosphocholine enhances the effect of part-time occlusion in amblyopia. *Doc Ophthalmol*. 1997;93:247–263.
75. Fresina M, Dickmann A, Salerni A, et al. Effect of oral CDP-choline on visual function in young amblyopic patients. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:143–150.
76. Pawar PV, Mumbare SS, Patil MS, Ramakrishnan S. Effectiveness of the addition of citicoline in patching in the treatment of amblyopia around visual maturity: a randomized controlled trial. *Indian J Ophthalmol*. 2014;62(2):124–129.
77. Campos EC, Fresina M. Medical treatment of amblyopia: present state and perspectives. *Strabismus*. 2006;14:71–73.
78. Lulia C, Ruxandra T, Costin LB, Liliana-Mary V. Citicoline – a neuroprotector with proven effects on glaucomatous disease. *Rom J Ophthalmol*. 2017;61(3):152–158.
79. Roberti G, Tanga L, Michelessi M, et al. Cytidine 5′-diphosphocholine (citicoline) in glaucoma: rationale of its use, current evidence and future perspectives. *Int J Mol Sci*. 2015;16(12):28401–28417.
80. Parisi V, Manni G, Colacino G, Bucci MG. Cytidine-5′-diphosphocholine (citicoline) improves retinal and cortical responses in patients with glaucoma. *Ophthalmology*. 1999;106:1126–1134.
81. Parisi V. Electrophysiological assessment of glaucomatous visual dysfunction during treatment with cytidine-5′-diphosphocholine (citicoline): a study of 8 years of follow-up. *Doc Ophthalmol*. 2005;110:91–102.
82. Grieb P, Rejdak R. Pharmacodynamics of citicoline relevant to the treatment of glaucoma. *J Neurosci Res*. 2002;67:143–148.
83. Roberti G, Tanga L, Parisi V, Sampalmieri M, Centofanti M, Manni G. A preliminary study of the neuroprotective role of citicoline eye drops in glaucomatous optic neuropathy. *Indian J Ophthalmol*. 2014;62(5):549–553.
84. Parisi V, Centofanti M, Ziccardi L, et al. Treatment with citicoline eye drops enhances retinal function and neural conduction along the visual pathways in open angle glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 2015;253(8):1327–1340.
85. Ottobelli L, Manni GL, Centofanti M, Lester M, Allevena F, Rossetti L. Citicoline oral solution in glaucoma: is there a role in slowing disease progression? *Ophthalmologica*. 2013;229(4):219–226.
86. Frolov MA, Gonchar PA, Barashkov VI, et al. *Vestn Oftalmol*. 2011;127(5):18–21.
87. Parisi V, Coppola G, Ziccardi L, et al. Cytidine-5′-diphosphocholine (Citicoline): a pilot study in patients with non-arteritic ischaemic optic neuropathy. *Eur J Neurol*. 2008;15:465–474.
88. O'Leary G, Weiss RD. Pharmacotherapies for cocaine dependence. *Curr Psychiatry Rep*. 2000;2:508–513.
89. Renshaw PF, Daniels S, Lundahl LH, et al. Short-term treatment with citicoline (CDP-choline) attenuates some measures of craving in cocaine-dependent subjects: a preliminary report. *Psychopharmacology (Berl)*. 1999;142:132–138.
90. Lukas SE, Kouri EM, Rhee C, et al. Effects of short-term citicoline treatment on acute cocaine intoxication and cardiovascular effects. *Psychopharmacology (Berl)*. 2001;157:163–167.

91. Licata SC, Penetar DM, Ravichhandran C, et al. Effects of daily treatment with citicoline: a double-blind, placebo-controlled study in cocaine-dependent volunteers. *J Addict Med*. 2011;5(1):57–64.
92. Brown ES, Todd JP, Hu LT, et al. A randomized, double-blind, placebo-controlled trial of citicoline for cocaine dependence in bipolar 1 disorder. *Am J Psychiatry*. 2015;172(10):1014–1021.
93. Lukas SE, Kouri EM, Rhee C, et al. Effects of short-term citicoline treatment on acute cocaine intoxication and cardiovascular effects. *Psychopharmacology (Berl)*. 2001;157:163–167.
94. Brown ES, Gabrielson B. A randomized, double-blind, placebo-controlled trial of citicoline for bipolar and unipolar depression and methamphetamine dependence. *J Affect Disord*. 2012;143(1-3):257–260.
95. Brown ES, Gorman AR, Hynan LS. A randomized, placebo-controlled trial of citicoline add-on therapy in outpatients with bipolar disorder and cocaine dependence. *J Clin Psychopharmacol*. 2007;27:498–502.
96. Pope HG, Yurgelin-Todd D. The residual cognitive effects of heavy marijuana use in college students. *JAMA*. 1996;275:521–527.
97. Fontes MA, Bolla KI, Cunha PJ, Almeida PP, Jungerman F, et al. Cannabis use before age 15 and subsequent executive functioning. *Br J Psychiatry*. 2011;198:442–447.
98. Solowij N, Stephens RS, Roffman RA, Babor T, Kadden R, et al. Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA*. 2002;287:1123–1131.
99. Gruber SA, Sagar KA, Dahlgren MK, et al. Citicoline treatment improves measures of impulsivity and task performance in chronic marijuana smokers: a pilot BOLD fMR study. *J Neurol Neurother*. 2015;2(3):1–8.
100. Killgore WDS, Ross AJ, Kamiya T, et al. Citicoline affects appetite and cortico-limbic responses to images of high-calorie foods. *Int J Eat Disord*. 2010;43:6–13.
101. Salvadorini F, Galeone F, Nicotera M, et al. Clinical evaluation of CDP-choline (Nicholin): efficacy as antidepressant treatment. *Curr Ther Res Clin Exp*. 1975;18:513–520.
102. Salvadorini F, Galeone F, Nicotera M, et al. Clinical evaluation of CDP-choline (Nicholin): efficacy as antidepressant treatment. *Curr Ther Res Clin Exp*. 1975;18:513–520.
103. Deutsch SI, Schwartz BL, Schooler NR, et al. First administration of cytidine diphosphocholine and galantamine in schizophrenia: a sustained alpha7 nicotinic agonist strategy. *Clin Neuropharmacol*. 2008;31:34–39.
104. Adibhatla RM, Hatcher JF. Cytidine 5′- diphosphocholine (CDPcholine) in stroke and other CNS disorders. *Neurochem Res*. 2005;30:15–23.
105. Sahin S, Alkan T, Temel SG, Tureyen K, Tolunay S, Korfali E. Effects of citicoline used alone and in combination with mild hypothermia on apoptosis induced by focal cerebral ischemia in rats. *J Clin Neurosci*. 2010;17:227–231.
106. Kim JH, Choi BY, Kho AR, Lee SH, Jeong JH, et al. Acetylcholine precursor, citicoline (cytidine 5′-diphosphocholine), reduces hypoglycaemia-induced neuronal death in rats. *J Neuroendocrinol*. 2018;30(1). https://doi.org/10.1111/jne.12567.
107. Ahlawat A, Sharma S. A new promising simultaneous approach for attenuating type II diabetes mellitus induced neuropathic pain in rats: iNOS inhibition and neuroregeneration. *Eur J Pharmacol*. 2018;818:419–428.
108. Schauss AG, Somfai-Relle S, Financsek I, et al. Single- and repeated-dose oral toxicity studies of citicoline free-base (choline cytidine 5′-pyrophosphate) in Sprague-Dawley rats. *Int J Toxicol*. 2009;28:479–487.
109. Cho HJ, Kim YJ. Efficacy and safety of oral citicoline in acute ischemic stroke: drug surveillance study in 4,191 cases. *Methods Find Exp Clin Pharmacol*. 2009;31:171–176.
110. Cotroneo AM, Castagna A, Putignano S, et al. Effectiveness and safety of citicoline in mild vascular cognitive impairment: the IDEALE study. *Clin Interv Aging*. 2013;8:131–137.

68

Coenzyme Q_{10}

Joseph Katzinger, ND, Laurie Mischley, ND, PhD, MPH,* and *Jason Allen*, ND, MPH*

OUTLINE

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is an endogenous proenzyme synthesized naturally in the human body.[1] Because of its ubiquitous presence in nature and its quinone structure, CoQ_{10} is also known as ubiquinone (Fig. 68.1). The two major physiological actions CoQ_{10} has been recognized for are (1) as a cofactor in the production of adenosine triphosphate (ATP), and (2) as an antioxidant. In addition to these two well-known actions, recent evidence suggests it also has other physiological effects, including (3) an influence on the expression of genes involved in cellular signaling and metabolism, and (4) a modulation of the mechanical and permeability of lipid membranes (similar to cholesterol), both of which may explain some of its therapeutic effects. For example, in some human trials supplementation has been shown to downregulate expression of oxidized low-density lipoprotein receptor 1 (LDLR), several interleukin genes, and tumor necrosis factor alpha (TNF-α), while upregulating peroxisome proliferator-activated receptor gamma (PPAR-γ) expression.[2,3] This may help explain the anti-inflammatory effect of CoQ_{10}; for example, a 2017 meta-analysis of 17 randomized controlled trials found significant reductions in C-reactive protein, IL-6, and TNF-α with CoQ_{10} supplementation.[4]

Because most cellular functions depend on an adequate supply of ATP, CoQ_{10} is essential for the health of virtually all human tissues and organs. Cellularly, the highest concentration of CoQ_{10} is found in the inner mitochondrial membrane, where it facilitates energy production, but CoQ_{10} is found in the cell membranes of many organelles, where it plays a role in membrane stability.[5] CoQ_{10} is the only endogenously synthesized lipid soluble antioxidant.[6] In its role in electron transport, the CoQ_{10} molecule continuously goes through an oxidation-reduction cycle. As it accepts electrons, it becomes reduced to ubiquinol. As it gives up electrons, it becomes oxidized to ubiquinone. In contrast to other antioxidants, this compound inhibits both the initiation and the propagation of lipid and protein oxidation. In its reduced form, ubiquinol, the CoQ_{10} molecule holds electrons rather loosely, so the CoQ_{10} molecule will quite easily give up one or both electrons and thus act as an antioxidant. It is especially protective against the oxidation of bases of mitochondrial DNA. In addition, ubiquinol is responsible for regenerating vitamin E from the α-tocopheroxyl radical and, thereby, interfering with the propagation step of lipid peroxidation.

Biosynthesis of CoQ_{10} starts from acetyl coenzyme A (CoA) and flows through a multistep process of the mevalonate pathway to produce farnesyl-PP, the direct precursor for not only CoQ_{10} but also cholesterol, dolichol, and isoprenylated proteins (Fig. 68.2). The long isoprenoid side-chain of CoQ_{10} is synthesized by transprenyltransferase, which condenses farnesyl-PP with several molecules of isopentenyl-PP, all in the trans configuration. The next step involves condensation of this polyisoprenoid side-chain with 4-hydroxybenzoate, catalyzed by polyprenyl-4-hydroxybenzoate transferase. Hydroxybenzoate is synthesized from tyrosine or phenylalanine. In addition to their presence in mitochondria, these initial two reactions also occur in the endoplasmic reticulum and peroxisomes, indicating multiple sites of synthesis in human cells. Nonetheless, numerous conditions are now known to arise in which the body's synthetic capacity is insufficient to meet CoQ_{10} requirements. Susceptibility to CoQ_{10} deficiency appears to be greatest in cells that are the most metabolically active, such as the brain and heart. Tissue deficiencies or subnormal serum levels of CoQ_{10} have been reported to occur in a wide range of medical conditions and decline with advancing age.[6]

A need for supplemental CoQ_{10} could theoretically result from the following:

- Impaired CoQ_{10} synthesis as a result of nutritional deficiencies
- A genetic or acquired defect in CoQ_{10} biosynthesis or utilization
- Increased tissue needs resulting from a particular illness
- The requirement to prevent the side effects of medical intervention

*Previous edition contributor

Because oral administration of CoQ_{10} can increase tissue levels, it is possible to correct CoQ_{10} deficiency and its associated metabolic consequences by supplementation.[7]

Coenzyme Q_{10} Deficiency

Inherited CoQ_{10} deficiency has been associated with five major clinical phenotypes: (1) encephalomyopathy, (2) severe infantile multisystemic disease, (3) cerebellar ataxia, (4) isolated myopathy, and (5) nephrotic syndrome. In a few patients, pathogenic mutations have been identified in genes involved in the biosynthesis of CoQ_{10} (primary deficiencies) or in genes not directly related to CoQ_{10} biosynthesis (secondary deficiencies). Respiratory chain defects, reactive oxygen species production, and apoptosis contribute to the pathogenesis of primary CoQ_{10} deficiencies.[8,9] Considered quite rare, as of 2018, only 200 patients belonging to 130 families have been described in the medical literature.[10] However, an analysis published in 2017 using large-scale gene sequencing data estimates as many as ~124,000 individuals are likely to be affected worldwide (~1500 in the United States), with a minimum of 1665 if limited to known pathogenic variants (192 in the U.S.).[11]

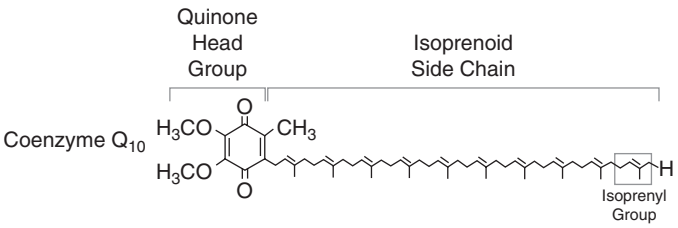


Fig. 68.1 Ubiquinone. (From Lee SQ, Tan TS, Kawamukai M, et al. Cellular factories for coenzyme Q_{10} production. Microb Cell Fact. 2017 Mar 2;16[1]:39. PMID: 28253886. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

Acquired CoQ_{10} deficiency is less well established. There are at least two major factors that lead to deficiency of CoQ_{10} in humans: reduced biosynthesis, and increased utilization or need by the body. The typical dietary intake of CoQ_{10} is 3 to 5 mg,[12] so dietary lack is probably not a significant contributor to CoQ_{10} deficiency. As mentioned previously, endogenous synthesis is a multistep process that can be affected by aging, disease status, and various medications. Some chronic disease conditions (cancer, heart disease, etc.) are associated not only with reduced biosynthesis but also increased demand for CoQ_{10}. Even a persistent food intolerance or allergy has been linked to an acquired CoQ_{10} deficiency, at least among children.[13] Additionally, a number of mitochondrial diseases may increase the degradation of CoQ_{10}, possibly by generating excessive reactive oxygen species as a result of a dysfunctional respiratory chain.[14]

Measurements of plasma CoQ_{10} levels have been used to detect deficiencies and are by far the most common clinical assessment of CoQ_{10} status.[15] Normal plasma levels are believed to range from 0.45 to 1.5 mcg/mL (or 0.46–1.78 μmol/L), with 93% to 100% being the reduced form, ubiquinol.[15,16] However, plasma measurement of CoQ_{10} has been criticized as having poor sensitivity (most patients with a primary CoQ_{10} deficiency still have normal plasma levels), and influenced by differences in plasma lipoprotein levels (which are CoQ_{10} carriers) and dietary intake.[17] Some studies use a functional assessment using an assay that measures the citric acid cycle (Krebs cycle) enzyme succinate

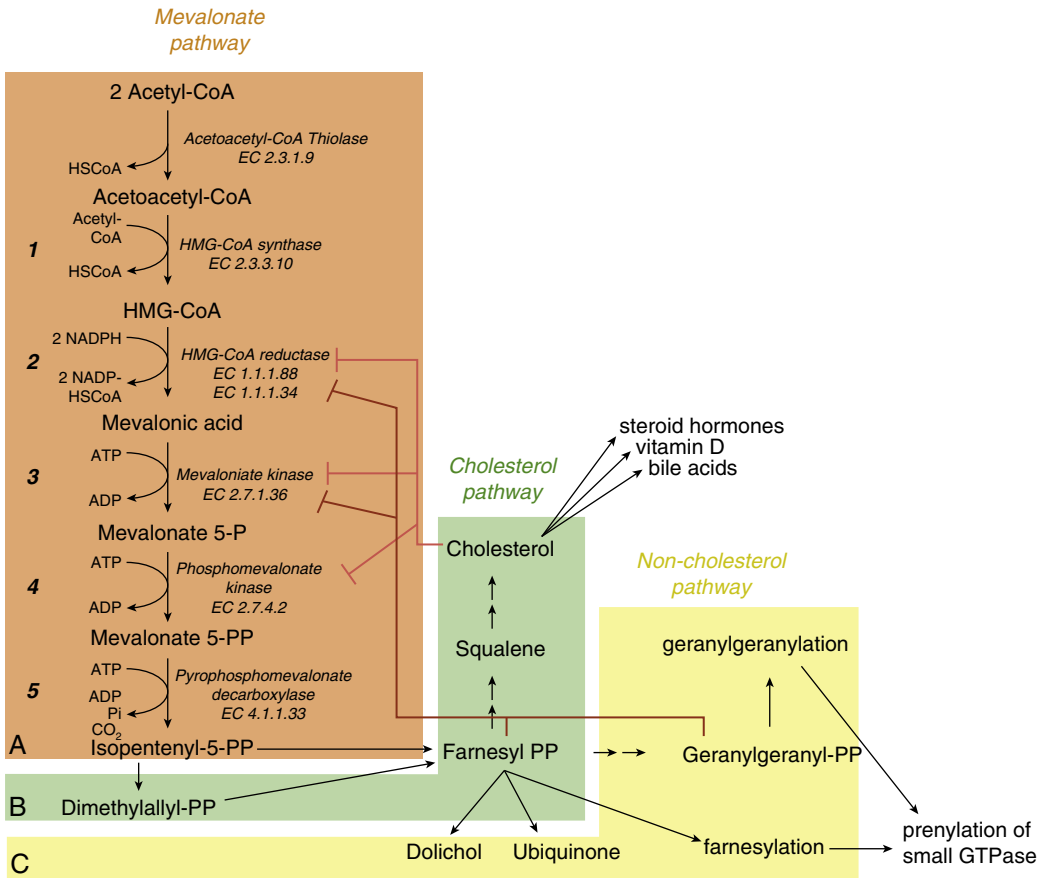


Fig. 68.2 Schematic representation of the mevalonate pathway. Divided into (a) The mevalonate pathway that produces mevalonate 5-PP and then isopentenyl 5-PP; (b) The cholesterol pathway that produces cholesterol, which in turn induces the formation of steroid hormones, vitamin D and bile acids; and (c) The non-cholesterol pathway important for the production of farnesyl-PP and geranylgeranyl-PP that induces respectively farnesylation and geranylgeranylation of small GTPase. (From Tricarico PM, Crovella S, Celsi F. Mevalonate pathway blockade, mitochondrial dysfunction and autophagy: a possible link. *Int J Mol Sci.* 2015;16(7):16067-84. PMID: 26184189. Reproduced from an open access article distributed under the terms and conditions of the Creative Commons Attribution license [http://creativecommons.org/licenses/by/4.0/]).

dehydrogenase-CoQ_{10} reductase.[18] CoQ_{10} acts as a critical coenzyme to this enzyme, so if the enzyme is fully saturated with CoQ_{10} in vivo, addition of exogenous CoQ_{10} does not increase enzyme activity. Nonetheless, muscle biopsy remains the best option for determining CoQ_{10} status in mitochondrial disorders, though additional methods for assessing CoQ_{10} status are also becoming available.[8,19,20] Leukocyte and skin fibroblasts, for example, appear to reasonably approximate muscle levels, though they are not widely available clinically, and urinary tract CoQ_{10} has emerged as a noninvasive technique for determining renal CoQ_{10} status.[21,22]

Coenzyme Q_{10} Absorption and Pharmacokinetics

After gastric emptying, CoQ_{10} is absorbed along with other lipids as chylomicron particles in the small intestine. The primary site of uptake of exogenous CoQ_{10} is the duodenum, followed by the colon, then the ileum, and then the jejunum. Absorption in the ileum and colon regions supports the possibility of an enterohepatic recirculation of CoQ_{10}. This possibility is further strengthened by the presence of a second peak in plasma levels about 24 hours after administration of either ubiquinone or ubiquinol. Absorption appears to be a complex process involving both active and passive mechanisms.

In the liver, CoQ_{10} is incorporated with lipoproteins and released into the blood. In plasma, CoQ_{10} is almost totally associated with lipoproteins, where it has greatest affinity for very low-density lipoprotein (VLDL) and LDL cholesterol. The CoQ_{10} is then distributed to peripheral tissues for uptake by cells and their mitochondria.

Commercial Forms and Dosage Considerations

Commercially CoQ_{10} is primarily produced via a yeast fermentation process using *Schizosaccharomyces pombe.* An alternate source of manufacturing is chemical synthesis using solanesol isolated from the tobacco plant.

A very important consideration in the clinical application of CoQ_{10} is its pharmacokinetics. CoQ_{10} as ubiquinone is a crystalline powder that is insoluble in water and has poor absorption characteristics as a result. Ubiquinol has greater solubility and has been promoted as having greater bioavailability that ubiquinone,[7,23] but there are limited data currently available and many questions remain to be answered, because ubiquinol is easily oxidized to ubiquinone and absorption studies to date have been suspect. Ubiquinone has an extensive history of use, particularly in oil-based soft-gelatin capsules. Several technologies are now used to enhance the bioavailability of ubiquinone, such as particle size reduction (nanonization) and solubility enhancement via use of emulsifying agents, carriers, and self-emulsifying systems.[24] For example, complexing ubiquinone to a soy peptide ($BioQ_{10}$ SA) has shown exceptional bioavailability because the soy peptide emulsifies the CoQ_{10} and helps usher it into the bloodstream.[25] Given the excellent absorption of this form of ubiquinone, the advantage of ubiquinol over regular ubiquinone appears to have more to do with its improved solubility than because it is in the ubiquinol form.

In light of the pharmacokinetic issues, proper clinical dosing is yet to be determined for CoQ_{10}. Therefore some have suggested that therapeutic targeting will likely be based on achieving specific plasma CoQ_{10} levels (e.g., more than 3.5 mg/mL) or tissue saturation.[15,26,27] Prescribing a set dosage, even a milligram per kilogram body weight dosage, at this time is a best guess scenario without confirmation by monitoring CoQ_{10} blood levels, given the differences noted in absorption rates among the different forms as well as the interindividual variability.

Numerous studies have now been conducted claiming enhanced absorption of one form or another.[7] However, there are shortcomings in most of these studies, and some of the studies appear to have been set up to show an advantage for commercial reasons. For example, ubiquinol is being promoted as the best absorbed form of CoQ_{10}, yet the published studies were set up in a curious fashion. In the one study examining ubiquinol absorption, the administration of the ubiquinol was always with a total of CoQ_{10} capsules that included the emulsification agents diglycerol monooleate, canola oil, soy lecithin, and beeswax, and although the study used a placebo, it did not compare ubiquinol with ubiquinone.[23] It is possible that ubiquinone might have fared just as well if emulsified as well as the ubiquinol in this study. Absorption studies showed that when CoQ_{10} was given with food, it was absorbed twice as fast and at least twofold greater than on an empty stomach.[28] It is believed that food-induced secretion of bile acids is responsible for the improved absorption.

What is known based on current absorption studies is that eventually a steady state is produced (usually after 3–4 weeks of constant dosing), and the absorption of CoQ_{10} may be limited in some individuals. Dosages that exceed an individual's absorptive capacity for CoQ_{10} may have minimal effect on efficacy and unnecessarily increase the cost of treatment. When single dosages of CoQ_{10} begin to exceed 300 mg, the percentage of CoQ_{10} absorbed declines. Plasma CoQ_{10} levels at a dosage of 900 mg/day (as ubiquinone in an oil suspension in a soft gel capsule) are not significantly greater than a 600 mg/day dosage. Divided dosages (e.g., two or three times a day) result in higher plasma levels compared with single dosages, especially at higher dosage levels.

Despite the challenges, based on existing data from published studies, an attempt can be made to calculate the approximate plasma levels of CoQ_{10} for different commercial forms at 100 and 300 mg doses for at least 30 days with several caveats. First, these are estimates only, based on a typical 75 kg body weight. Keep in mind that absorption of CoQ_{10} in any form is likely enhanced considerably if taken with a large meal that includes some fat. Lastly, considerable pharmacokinetic studies in humans have indicated significant interindividual variability in CoQ_{10} absorption, underscoring the need for monitoring CoQ_{10} plasma levels during clinical studies and perhaps clinical use of CoQ_{10} as well (Table 68.1).

TABLE 68.1 Coenzyme Q_{10} Plasma Levels

Dose and Form	Estimated Plasma Levels in Micrograms Per Milliliter
100 mg	
Ubiquinone powder in hard gelatin capsule	1.25
Ubiquinone suspended in oil in soft gelatin capsule with rice bran oil	1.8
Ubiquinone solubilized in soft gelatin capsule	2.25
Ubiquinone powder nanonized	2.25
Ubiquinone emulsified with soy peptide in soft or hard gelatin capsule	2.50
Ubiquinol in soft gel capsule	2.50
300 mg	
Ubiquinone powder in hard gelatin capsule	2.5
Ubiquinone suspended in oil in soft gelatin capsule with rice bran oil	3.5
Ubiquinone solubilized in soft gelatin capsule	5.0
Ubiquinone powder nanonized	5.0
Ubiquinone emulsified with soy peptide in soft or hard gelatin capsule	7.0
Ubiquinol in soft gel capsule	7.0

The optimal dose of CoQ_{10} for many clinical indications is not known, and plasma or target tissue levels of CoQ_{10} are likely the primary determinant of efficacy, rather than the dose.[26,29] Current opinion based on the scientific literature is that an acceptable therapeutic plasma target level of CoQ_{10} should be at least 2.5 mg/mL,[30] but levels higher than 3.5 mcg/mL may be necessary for optimum improvement in neurodegenerative diseases and myocardial function.[15,26,29] The usual starting dosage for CoQ_{10} is generally 100 to 200 mg/day. Where possible, the dosage of CoQ_{10} should be adjusted according to the response of the patient and, preferably, by monitoring plasma CoQ_{10} levels for 3 to 4 weeks of constant dosing, when steady-state plasma concentrations occur. Less is known about the ability of CoQ_{10} and the dose required to reach the central nervous system. Again, divided dosages result in higher plasma levels compared with single dosages (e.g., taking 100 mg twice a day produces higher plasma levels compared with taking 200 mg once a day).[31]

CLINICAL APPLICATIONS

Given the central role of CoQ_{10} in mitochondrial function and cellular antioxidant protection, its clinical applications are extensive. There are so many conditions where CoQ_{10} may offer benefit that there is no question that it should be considered a conditionally essential nutrient. The specific uses of CoQ_{10} are described in the following:

- General antioxidant
- Cardiovascular disease
- Cardiomyopathy
- Congestive heart failure
- Protection during cardiac surgery
- Hypertension
- Cancer
- Diabetes mellitus
- Male infertility
- Parkinson's disease
- Friedreich's ataxia
- Muscular dystrophy
- Immune function
- Acquired immunodeficiency syndrome (AIDS)
- Toxicant exposure
- Topical antiaging effects

General Antioxidant

Numerous studies have shown CoQ_{10} can reduce oxidative damage, DNA strand damage, LDL oxidation, and formation of lipid peroxides, thereby supporting its use as a general antioxidant.[5,32] In particular, CoQ_{10} is often used to counteract the reduced synthesis of CoQ_{10} associated with aging. After the age of 35 to 40 years, humans slowly begin to lose their ability to synthesize CoQ_{10}.[33] It has been proposed that the increase in age-associated diseases is due in part to decreased protection afforded by CoQ_{10} as both an antioxidant and a facilitator of energy production at a cellular level. Indeed, CoQ_{10} has been shown to activate the expression of the sirtuin and Pgc-1α genes, proposed as regulators of age and age-associated diseases, and linked to lower oxidative stress and upregulated glutathione production as well as mitochondrial biogenesis.[34]

Previous studies identified oxidative stress as a promoting factor for dry mouth (xerostomia) and the development of Sjögren's syndrome, a condition associated with significant dry mouth. Basically, oxidative damage leads to the inability of salivary cells to produce enough ATP to secrete sufficient amounts of water. CoQ_{10} exerts antioxidant effects, but its main action in relieving dry mouth may be by increasing energy (ATP) production, allowing the saliva-producing cells enough energy to secrete more saliva into the mouth. In one double-blind study, 66 patients, including 31 with dry mouth, were given either ubiquinol or ubiquinone orally at a dosage of 100 mg/day, or a placebo for 1 month.[35] Salivary secretion and salivary CoQ_{10} content were analyzed before and after treatment. Among the dry mouth patients treated with ubiquinone, salivary secretion increased significantly from 0.7 g/2 min before treatment to 1.2 g/2 min after 1 month of treatment. Among the patients treated with ubiquinol, salivary secretion also increased significantly from 0.8 g/2 min before treatment to 1.4 g/2 min after treatment. In normal subjects without dry mouth, salivary secretion increased with ubiquinone (from 4.9–5.7 g/2 min) at a statistically significant level, but did not differ significantly after treatment with ubiquinol (from 3.5–3.8 g/2 min). Either form of CoQ_{10} exhibited a marked increase in salivary CoQ_{10} concentration (ubiquinol more than ubiquionine in dry mouth, ubiquinone more than ubiquinol in normal subjects), suggesting that the observed increase in salivary secretion was attributable to the effect on salivary levels of CoQ_{10}.

Cardiovascular Disease—General Considerations

Enhancing myocardial function is an important, although frequently overlooked, component of the overall prevention and treatment of cardiovascular disease. CoQ_{10} plays a key role in energy production and is therefore essential for all energy-dependent processes, including heart muscle contraction. CoQ_{10} deficiency has been documented in patients with various types of cardiovascular disease. Whether a decline in CoQ_{10} levels is a cause or a consequence of heart disease is unclear. Cardiac cells are highly metabolically active, and thus have higher mitochondrial coenzyme requirements to maintain ATP production. In addition to its role in cellular energetics, exogenously administered CoQ_{10} acts as an antioxidant to inhibit LDL oxidation,[36] decreases proinflammatory cytokines interleukin-6 and tumor necrosis factor-α, and attenuates markers of oxidative and nitrative stress in a dose-dependent manner.[5]

CoQ_{10} deficiency is common in patients with various types of cardiovascular disease.[5,37] Approximately 75% of patients who had cardiac surgery were shown to be deficient in myocardial CoQ_{10}. Concentrations of CoQ_{10} declined progressively in both blood and myocardial tissue with increasing severity of heart disease.[38] Myocardial deficiencies of CoQ_{10} were also found in the majority of patients with aortic stenosis or insufficiency, mitral stenosis or insufficiency, diabetic cardiomyopathy, tetralogy of Fallot, atrial septal defects, and ventricular septal defects.[39] Deficiencies have now been documented in congestive heart failure, angina, coronary artery disease, cardiomyopathy, and hypertension.[40]

Cardiomyopathy

Cardiomyopathy can take several forms, but all are manifested by weakening of the heart muscle associated with inadequate heart pumping or other functional problems. The well-established role of CoQ_{10} as a facilitator of mitochondrial energy production lends strong rationale for a role of CoQ_{10} supplementation in all forms of cardiomyopathy.

A prospective, randomized, double-blind, placebo-controlled trial was conducted in 38 children with idiopathic dilated cardiomyopathy. After 6 months of supplementation, there was a statistically significant ($P = 0.011$) improvement in diastolic function, leading the authors to conclude that "administration of coenzyme Q_{10} is useful in ameliorating cardiac failure in patients with idiopathic dilated cardiomyopathy."[41]

In one study, 126 patients with dilated cardiomyopathy received 100 mg/day of CoQ_{10} for up to 66 months. After 6 months of treatment, the mean left ventricular ejection fraction (LVEF) increased from 41% to 59% ($P < 0.001$) and remained stable thereafter with

continued treatment. After 2 years, 84% of the patients were still alive, and at 5.5 years, 52% were alive.[42] These survival rates were considerably better than the published survival statistics of patients given conventional therapy (i.e., 2-year survival rate of 50% for symptomatic cardiomyopathy, and 1-year survival rate of 50% for decompensated cardiomyopathy).

In another study, 88 patients with cardiomyopathy received 100 mg/day of CoQ_{10} for 1 to 24 months. Significant improvements in at least two of three cardiac parameters (LVEF, cardiac output, and New York Heart Association [NYHA] class) were seen in 75% to 85% of the patients. Approximately 80% of the patients improved to a lower (i.e., more favorable) NYHA functional class.[30]

In a double-blind, crossover trial, 19 patients with cardiomyopathy received 100 mg/day of CoQ_{10} or a placebo, each for 12 weeks. Compared with placebo, CoQ_{10} treatment significantly increased cardiac stroke volume and LVEF. Eighteen patients reported improvement in activity while taking CoQ_{10}.[43] In 2014 the results of the Q-SYMBIO trial (Symptoms, Biomarker status [BNP], and Long-term Outcome) were published, a prospective, randomized, double-blind, placebo-controlled, multicenter trial of CoQ_{10} as an adjunctive treatment of chronic heart failure. With more than 400 participants enrolled, a 50% reduction in risk for a major adverse cardiovascular event within 2 years was found for those taking 100 mg CoQ_{10} three times per day versus placebo. Additionally, significant reductions in both cardiovascular mortality (9% vs. 16%) and all-cause mortality (10% vs. 18%) were seen with CoQ_{10} supplementation versus placebo, as were reductions in hospital stay and a significant improvement of NYHA class. Subgroup analysis found that those with dilated cardiomyopathy may have even greater benefits; when taking CoQ_{10} the hazard ratio for a major cardiovascular event for those without cardiomyopathy was 0.60 compared with placebo, but for those with dilated cardiomyopathy it was 0.28. Furthermore, the benefit attributed to CoQ_{10} was in addition to that associated with concurrent medications, including beta-blockers, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers.[44]

Congestive Heart Failure

The potential of CoQ_{10} as a treatment for congestive heart failure (CHF) was suggested as early as 1967 by Japanese researchers.[45] In 1976 these same investigators administered 30 mg/day of CoQ_{10} to 17 patients with CHF. All the patients improved, and 9 (53%) became asymptomatic after 4 weeks of treatment.[46] Numerous studies since have demonstrated that CoQ_{10} supplementation resulted in an improvement of stroke volume, LVEF, cardiac output, cardiac index, and end-diastolic volume index.[5,47,48]

In the largest multicenter trial completed to date, 2664 patients in NYHA classes II and III CHF received 50 to 150 mg/day of CoQ_{10} (78% of patients received 100 mg/day of CoQ_{10}). After 3 months of supplementation, the results showed a low incidence of side effects, and the following proportion of patients had improvements in clinical signs and symptoms[49]:

- Cyanosis, 71.8%
- Edema, 78.6%
- Pulmonary rales, 77.8%
- Enlargement of the liver area, 49.3%
- Jugular reflux, 71.81%
- Dyspnea, 54.7%
- Palpitations, 75.4%
- Sweating, 79.8%
- Subjective arrhythmia, 63.4%
- Insomnia, 60.8%
- Vertigo, 73.1%
- Nocturia, 53.6%

The results of these uncontrolled studies were confirmed in several double-blind trials. Some 641 patients with CHF (NYHA classes III or IV) were randomly assigned to receive placebo or CoQ_{10} (2 mg/kg per day) for 1 year while continuing conventional therapy. The number of patients requiring hospitalization during the study for worsening heart failure was 38% less in the CoQ_{10} group than in the placebo group ($P < 0.001$), and episodes of pulmonary edema were reduced by about 60% in the CoQ_{10} group compared with the placebo group ($P < 0.001$).[50]

These positive results with CoQ_{10}, however, have not been seen in all studies. In one double-blind study, 55 patients with CHF in NYHA classes III and IV, with ejection fractions less than 40%, and peak oxygen consumption less than 50% during standard therapy, were randomly assigned to receive CoQ_{10} (200 mg) or placebo. Analysis indicated that there were no changes in ejection fraction, peak oxygen consumption, and exercise duration in either group. Possible explanations for failure to achieve a therapeutic benefit in this study (and others) may be the result of CoQ_{10} not being strong enough to produce significant effects in more severe stages of CHF or the fact that blood levels of CoQ_{10} did not reach sufficient levels. Although the mean serum concentration of CoQ_{10} increased from 0.95 to 2.2 mcg/mL, in 19 of 22 patients on CoQ_{10}, blood levels were below the suggested threshold of 2.5 mcg/mL.[51]

A more recent study administered CoQ_{10} 100 mg three times a day for 4 weeks to 21 patients with CHF in a double-blind, placebo-controlled, crossover design. CoQ_{10} administration resulted in a threefold increase in plasma CoQ_{10} levels, improved left ventricular contractility, and subsequently enhanced functional capacity, without any side effects.[52]

The Q-SYMBIO trial, mentioned previously, was designed to address whether CoQ_{10} supplementation extends survival in patients with CHF.[53] As discussed previously, significant reductions in both cardiovascular and all-cause mortality were found, in addition to the benefit associated with conventional treatments. The Q-SYMBIO trial and other clinical trials were incorporated into a meta-analysis published in 2017. This analysis of 14 RCTs and more than 2000 patients found that the use of CoQ_{10} reduced mortality by 31% compared with placebo among those with heart failure (Fig. 68.3). Additionally, exercise capacity was increased with CoQ_{10} use, though no differences in the endpoints of left heart ejection fraction or NYHA classification were observed.[54]

An important consideration in patients with CHF in NYHA class IV is that they often fail to achieve adequate plasma CoQ_{10} levels (>2.5 mcg/mL) on supplemental ubiquinone at dosages up to 900 mg/day. In one study, 7 patients with subtherapeutic plasma CoQ_{10} levels (mean level, 1.6 mcg/mL, on an average dose of 450 mg of ubiquinone a day [150–600 mg/day]) were changed to an average of 580 mg/day of ubiquinol (450–900 mg/day) with follow-up plasma CoQ_{10} levels, clinical status, and ejection fraction measurements by echocardiography.[55] Mean plasma CoQ_{10} levels increased from 1.6 to 6.5 mcg/mL. Mean ejection fraction improved from 22% (10%–35%) to 39% (10%–60%) and NYHA class improved from a mean of class IV to a mean of class II (classes I to III). In this study, ubiquinol dramatically improved absorption in patients with severe heart failure, and the improvement in plasma CoQ_{10} levels was correlated with both clinical improvement and improvement in measurement of left ventricular function.

Protection During Cardiac Surgery

Cardiopulmonary bypass (CPB) is used in coronary artery bypass surgery, cardiac valve repair, and numerous other surgical procedures. CPB, although permitting life-saving surgical procedures, is known

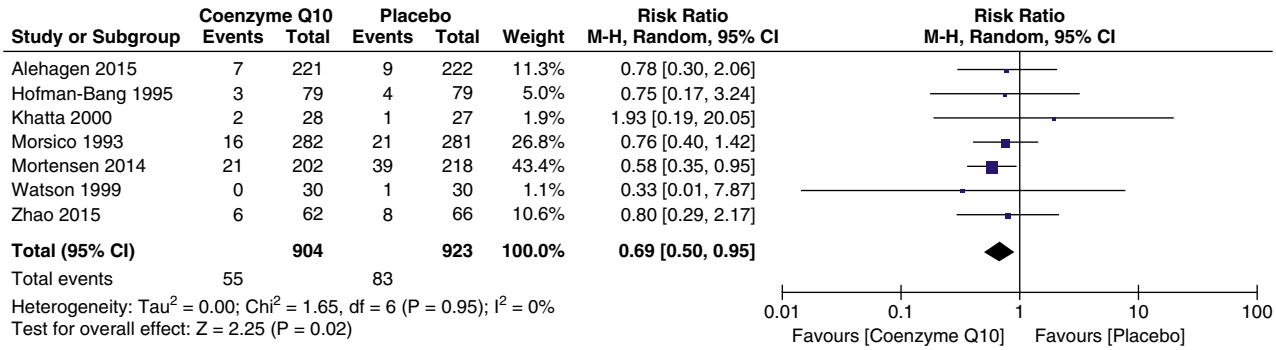


Fig. 68.3 Forest plot of mortality with CoQ_{10} supplementation for heart failure. (From Lei L, Liu Y. Efficacy of coenzyme Q_{10} in patients with cardiac failure: a meta-analysis of clinical trials. *BMC Cardiovasc Disord*. 2017 Jul 24;17[1]:196. PMID: 28738783. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

to induce oxidative stress. A prospective study of 30 individuals who underwent CPB randomized these patients to oral CoQ_{10} (150–180 mg/day) or placebo for 7 to 10 days preoperatively. The group that received CoQ_{10} had significantly fewer reperfusion arrythmias; lower requirements for inotropic agents, mediastinal drainage, and blood products; and shorter hospital stays compared with the control group.[56]

Postoperative low cardiac output is a major cause of early death after cardiac surgery. Fifty patients who underwent cardiac surgery for acquired valvular lesions were randomly assigned to receive 30 to 60 mg/day of CoQ_{10} for 6 days before surgery or to a control group that did not receive CoQ_{10}. Postoperatively, a state of severe low cardiac output developed in 48% of the patients in the control group, compared with only 12% of those in the CoQ_{10} group. These results suggested that preoperative administration of CoQ_{10} increased the tolerance of the heart to ischemia during aortic cross-clamping.[57]

A meta-analysis of eight clinical trials evaluating CoQ_{10} supplementation for patients undergoing cardiac surgery was published in 2015. CoQ_{10} supplementation was associated with a 53% reduced need for inotropic drugs (used to treat low cardiac output). Furthermore, supplementation was associated with a 95% reduction in the risk of developing a ventricular arrhythmia. No change in the cardiac index, length of hospital stay, or risk for atrial fibrillation was observed. Again, dosing may play an important role; in this meta-analysis, doses ranged from 30 mg to 600 mg per day, and in some cases CoQ_{10} was not given within 12 hours of surgery, whereas in others it was given orally for days or weeks before and after surgery, and via intravenous drip during surgery.[58]

Hypertension

The majority of studies exploring CoQ_{10} in the treatment of high blood pressure have been uncontrolled, or have used CoQ_{10} in combination with conventional antihypertensive medical treatments, making these studies difficult to interpret. In one study, 109 patients with essential hypertension received CoQ_{10} (average dose, 225 mg/day) in addition to their usual antihypertensive regimen. The dosage of CoQ_{10} was adjusted according to clinical response and blood CoQ_{10} levels (the aim was to attain blood levels >2 mg/mL). The need for antihypertensive medication declined gradually, and after a mean treatment period of 4.4 months, about half of the patients were able to discontinue between one and three drugs.[59] A review of studies on CoQ_{10} in the treatment of hypertension (12 clinical trials, 362 patients) concluded that in hypertensive patients, CoQ_{10} has the potential to lower systolic and diastolic blood pressure, without significant side effects. Among all included studies, decreases in systolic blood pressure ranged from 11 to 17 mm Hg and in diastolic blood pressure from 8 to 10 mm Hg.[60] In three of the 12 studies, CoQ_{10} was given in addition to existing antihypertensive medication, and in one of these, more than 50% of the patients were able to cease taking at least one antihypertensive medication during the trial. Large prospective and unbiased trials are needed, as concluded by a 2016 Cochrane review that found only two randomized trials sufficiently unbiased to be included in a meta-analysis and did not find a blood pressure–lowering effect of CoQ_{10}.[61] In a subsequent 2018 meta-analysis reviewing the effect of CoQ_{10} on blood pressure among participants with a metabolic disease (including diabetes, dyslipidemia, myocardial infarction, stroke, etc.), supplementation was associated with a statistically significant reduction in systolic blood pressure (standardized mean difference of −0.30; 95% CI −0.52, −0.08), on par with the effect of most antihypertensive medications.[62,63]

The mechanism for the antihypertensive action of CoQ_{10} is thought to be due to its counteracting vasoconstriction, resulting from either an impaired ability of the endothelium to induce nitric oxide–mediated relaxation of underlying smooth muscle, or via preservation of nitric oxide itself.[60]

Cancer

Because of its role in enhancing immune function, CoQ_{10} has been considered as a possible anticancer agent. In one study, 32 women with breast cancer, who were classified as "high risk" because of tumor spread to the axillary lymph nodes, received 90 mg/day of CoQ_{10}, along with vitamins C and E, β-carotene, and essential fatty acids. In six of these women, the tumor became smaller. During the 18-month treatment period, none of the patients died (the expected number of deaths was four), and none showed signs of further distant metastases. Six patients had an apparent partial remission. In addition, patients receiving CoQ_{10} required fewer painkillers.[64] In follow-up, a pilot study using this nutrient cocktail evaluated the survival of 40 patients with end-stage cancer over 9 years. Median survival of individuals receiving the CoQ_{10}-containing nutrient cocktail was 40% longer than median predicted survival using the calculated Kaplan-Meier curve.[65]

Anthracyclines are a class of drugs used in cancer chemotherapy for a wide range of tumors. The clinical value of these agents is limited by their cardiotoxicity, which is believed to be due to irreversible damage to heart cell mitochondria. These agents cause severe oxidative stress in cardiac inner mitochondrial membranes, resulting in damage to mitochondrial DNA and subsequent myocyte death.[66]

Research from the 1980s showed that cancer patients receiving doxorubicin (Adriamycin), a commonly used anthracycline, had lower myocardial levels of CoQ_{10} than did controls, and that the magnitude of CoQ_{10} depletion was directly related to the severity of cardiac

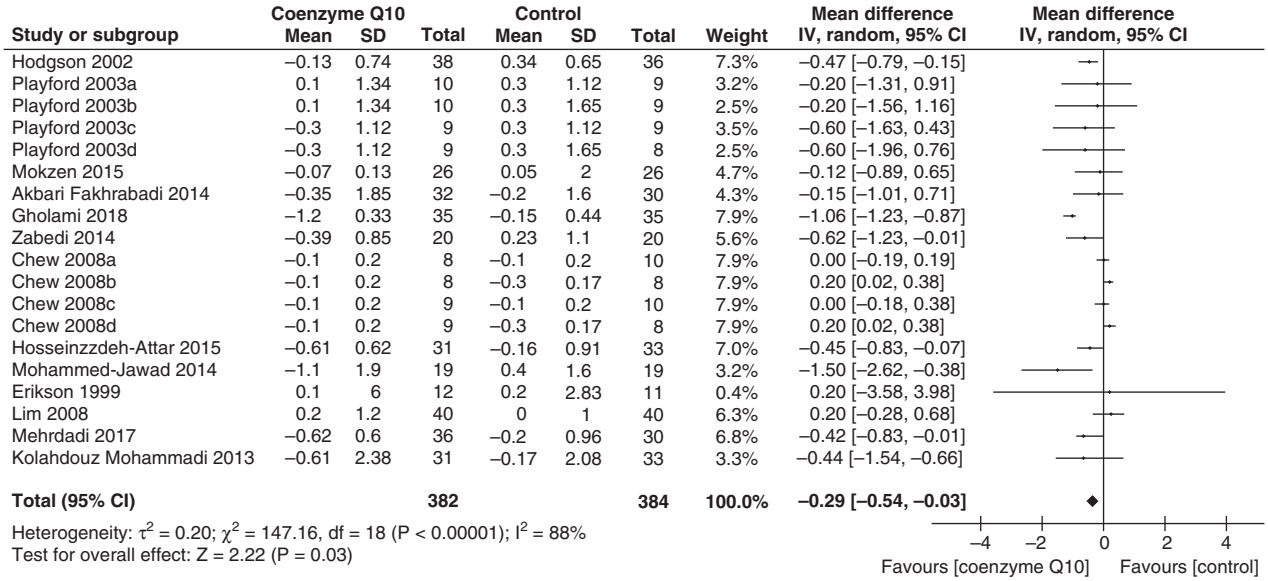


Fig. 68.4 Glycosylated hemoglobin with CoQ_{10} supplementation. (From Zhang SY, Yang KL, Zeng LT, et al. Effectiveness of coenzyme Q_{10} supplementation for type 2 diabetes mellitus: a systematic review and meta-analysis. *Int J Endocrinol*. 2018 Sep 16;2018:6484839. PMID: 30305810. Reproduced from an open access article distributed under the Creative Commons Attribution License.)

impairment.[67] To determine the effect of CoQ_{10} supplementation on doxorubicin cardiotoxicity, seven patients receiving doxorubicin were also given 100 mg/day of CoQ_{10}, beginning 3 to 5 days before doxorubicin was started. Another seven patients (control group) received doxorubicin without CoQ_{10}. Cardiac function deteriorated significantly in the control group, whereas patients given CoQ_{10} had little or no cardiotoxicity, although the cumulative dose of doxorubicin in the CoQ_{10} group was 50% greater than that in the control group.[68] These preliminary data, coupled with strong scientific rationale, suggest that CoQ_{10} supplementation should be considered for the prevention and treatment of anthracycline-induced cardiotoxicity.

Physical Performance

Because CoQ_{10} is involved in energy production and its concentration in muscle is correlated with performance, it is possible that supplementation may enhance aerobic capacity, muscle performance, and recovery.[69–71] In one study, six healthy sedentary men (mean age, 21.5 years) performed a bicycle ergometer test before and after taking CoQ_{10} (60 mg/day) for 4 to 8 weeks.[72] CoQ_{10} treatment improved certain performance parameters, including work capacity at submaximal heart rate, maximal workload, maximal oxygen consumption, and oxygen transport. These improvements ranged from 3% to 12% and were evident after about 4 weeks of supplementation. In a double-blind study in sedentary men, 100 mg/day of CoQ_{10} showed performance-enhancing effects during repeated bouts of supramaximal exercises.[73]

Although these studies suggest that administration of CoQ_{10} improves physical performance in sedentary individuals, other publications are conflicting. Larger studies are needed to confirm the performance-enhancing capacity of CoQ_{10}, and clarify any distinction between sedentary and athletic populations.[74] In a recent study that enrolled 21 male athletes, participants were given either placebo or 200 mg CoQ_{10} per day, for 30 days, before a single bout of intense exercise. Supplementation prevented the typical depletion of plasma CoQ_{10} levels, and reduced the cellular level of reactive oxygen species, though it did not alter any indexes of either physical performance or muscle damage.[75]

Diabetes Mellitus

Low tissue levels of CoQ_{10} have been consistently reported in diabetic patients, as well as a increased ratio of ubiquinone to ubiquinol.[76] There are several mechanisms by which CoQ_{10} may be of therapeutic value in diabetes.[77,78] Improved blood pressure and long-term glycemic control have been reported with CoQ_{10} supplementation (100 mg twice daily).[79] Enhancement of endothelial function may offer some protection against arteriopathies.[80] A study of 80 dyslipidemic type 2 diabetics found that CoQ_{10} (200 mg/day) in combination with fenofibrate (200 mg/day) significantly improved endothelial and nonendothelial forearm vasodilatory function.[81] This effect was not found with either therapy alone or in a placebo group. An accumulating body of research demonstrates a link between mitochondrial dysfunction and type 2 diabetes. The mitochondrial enhancement offered by supplemental CoQ_{10} appears to offer some therapeutic value against a defect in cellular energy production.[82,83]

In 2018 a systematic review and meta-analysis of CoQ_{10} use in diabetes was published, incorporating 13 randomized and controlled trials and 765 participants. CoQ_{10} was associated with a significant reduction in both hemoglobin A1c and fasting blood glucose levels (Fig. 68.4) but not with fasting insulin levels. It also was linked to a significant increase in HDL cholesterol and a reduction in triglyceride levels.[84]

Twenty-seven patients with ventricular premature beats (VPBs) and no evidence of organic heart disease received placebo for 3 to 4 weeks, followed by 60 mg/day of CoQ_{10} for 4 to 5 weeks. The reduction in VPBs was significantly greater after CoQ_{10} than after placebo. The beneficial effect of CoQ_{10} was seen primarily in diabetics, in whom the mean reduction in VPB frequency was 85.7%. A significant reduction in VPBs also occurred in 1 (11%) of 9 otherwise healthy patients and in 4 (36%) of 11 patients with hypertension.[85]

Male Infertility

Sperm dysfunction in infertile men has been associated with lipid peroxidation and decreased antioxidant defenses in spermatozoa.[86] A statistically significant correlation between CoQ_{10} levels and sperm count was found in 32 patients with a history of infertility.

Research suggested that sperm cells with low motility and abnormal morphology have low levels of CoQ_{10}.[87] Sperm cells with low CoQ_{10} concentrations might be less capable of quenching free radicals, and might also be compromised in their ability to produce ATP in these highly metabolically active cells. Some authors suggested using the reduced-to-oxidized CoQ_{10} ratio as a diagnostic test for asthenozoospermia.[88] In a 6-month, uncontrolled open trial, in which infertile men were supplemented orally with CoQ_{10}, sperm cells demonstrated a significant increase in motility, and both seminal plasma and sperm cell quantity of CoQ_{10} increased.[89] This was supported by other studies on both sperm motility and fertilization rates.[90,91] In 2013 a meta-analysis of 3 studies was published, which found that CoQ_{10} supplementation among men with infertility did not improve pregnancy rates (there were no data regarding live births), but it did improve both sperm motility and concentration.[92]

Parkinson's Disease

Excessive free radical production is largely responsible for dopaminergic cell death seen in Parkinson's disease (PD). Both as a cause and an effect of excessive oxidative stress, there is reduced activity of complex I of the mitochondrial electron transport chain. Given that CoQ_{10} is essential for the function of complex I and is also a powerful antioxidant, it holds therapeutic potential in the treatment of PD. Reduced levels of CoQ_{10} were demonstrated in the platelets of individuals with PD, and CoQ_{10} levels were strongly correlated with activity in complexes I and II/III. Clinically, it was demonstrated that oral CoQ_{10} increased complex I activity.[93]

A safety trial of CoQ_{10} supplementation in PD showed promising results.[29] All of the patients had the three primary features of PD—tremor, stiffness, and slowed movements—and were diagnosed with the disease within 5 years of the time they were enrolled. After initial screening and baseline blood tests, the patients were randomly divided into four groups. Three of the groups received CoQ_{10} at three different doses (300, 600, and 1200 mg/day), whereas a fourth group received a matching placebo for 16 months. The group that received the highest dose of CoQ_{10} (1200 mg/day) displayed a 44% reduction in PD progression compared with placebo. The groups that received 300 and 600 mg/day developed slightly less disability than the placebo group, but the effects were less than those in the group that received the highest dosage of CoQ_{10}. Average plasma levels were approximately 1.8, 2.1, and 4.5 mcg/mL, respectively, for the 300, 600, and 1200 mg dosages. These results indicated that the beneficial effects of CoQ_{10} in PD might require adequate plasma levels. It is important to point out in this study that CoQ_{10} was administered along with vitamin E at a dosage of 1200 international units (IU) per day. Because high dosages of vitamin E interfere with CoQ_{10} absorption,[12] it might have prevented higher levels of CoQ_{10} from being achieved. The researchers were aware of this issue but chose to include the vitamin E, given its apparent protective role against PD at the time (see Chapter 196 on Parkinson's Disease for more information).

Two recent studies cast doubt on the therapeutic efficacy of CoQ_{10} in PD. In a German study, a nanonized ubiquinone form of CoQ_{10} at a dosage of 100 mg three times daily or a matching placebo was given to 131 patients with PD for 3 months. Plasma levels of CoQ_{10} reached 4.6 mcg/mL in the treatment group, yet no effect on PD symptoms was shown. These results indicated that other factors might be responsible for determining the efficacy of CoQ_{10} in PD beyond achieving effective plasma levels. Two recent meta-analyses (published in 2016 and 2017) found that despite ample evidence of safety, there was no indication of a benefit from CoQ_{10} supplementation.[94,95]

On May 27, 2011 the National Institute of Neurological Diseases and Strokes (NINDS) stopped a Phase III study of CoQ_{10} for treatment of early-stage PD.[96] The study enrolled 600 patients with early PD at 67 sites throughout North America. Participants were randomized to receive one of the two dosing levels of active CoQ_{10} (1200 or 2400 mg/day) or matching placebo. All subjects also received vitamin E at a dosage of 1200 IU/day. Although CoQ_{10} was shown to be safe, results of an interim analysis showed that it was futile to complete the study because longer patient follow-up was not likely to demonstrate a statistically significant difference between active treatment and placebo. In 2014 the results of this Phase III trial were finally published, and no benefit was found.[97] Recently, a smaller study has suggested that when combined with creatine monohydrate, CoQ_{10} may slow the decline of cognitive function in Parkinson's patients.[98] Given the disappointing results from the Parkinson Study Group, it remains to be determined whether there is a role for CoQ_{10} in Parkinson's disease, either in combination with other therapies or for a subset of patients.

Friedreich's Ataxia

Friedreich's ataxia (FRDA) is a progressive neurological disease characterized by loss of myelin in the central nervous system. Evidence indicates that mitochondrial oxidative stress in FRDA may be responsible for the cardiomyocyte hypertrophy associated with this disease.[99] In a placebo-controlled trial, idebenone (a synthetic CoQ_{10} analog) significantly decreased heart hypertrophy (more than 20% decrease) in about 50% of study participants, with no adverse effects.[100,101]

A second study combined CoQ_{10} with vitamin E in an attempt to address two of the key features of FRDA—decreased mitochondrial respiratory chain function and increased oxidative stress. This therapy resulted in a rapid and sustained increase in the amount of energy generated by the diseased heart muscle, which returned to near-normal levels. The improvements in skeletal muscle energy generation paralleled those of the heart but were less substantial.[102]

A 2-year intervention study of CoQ_{10} with vitamin E in 50 FRDA patients demonstrated that low baseline levels of CoQ_{10} were the single best predictor of a positive clinical response.[103]

Muscular Dystrophy

The muscular dystrophies (MDs) are a group of hereditary diseases characterized by the progressive loss of muscle cells. In some types of MD, an impairment of mitochondrial function may contribute to the pathogenesis of the disease. Several studies demonstrated a deficiency of CoQ_{10} in muscle mitochondria of humans with MD.[38] In addition, serum CoQ_{10} levels were significantly ($P < 0.05$) inversely correlated with the degree of the genetic defect.[104] In addition to enhancing mitochondrial function, 90 days of CoQ_{10} supplementation resulted in markedly reduced inflammatory insult in animal models of MD.[105]

The first double-blind trial of CoQ_{10} in the treatment of individuals with MDs and neurogenic atrophies concluded, "Patients suffering from these muscle dystrophies and the like should be treated with vitamin CoQ_{10} indefinitely."[106] The same group conducted two studies on patients between 7 and 69 years of age who had MDs associated with cardiac diseases, including the Duchenne, Becker, and the limb-girdle dystrophies; myotonic dystrophy; Charcot-Marie-Tooth disease; and the Welander disease. Individuals were treated for 3 months with 100 mg/day CoQ_{10} or a matching placebo. In both trials, definite improvements in physical performance were recorded in the groups receiving CoQ_{10}. Cardiac function was monitored by technicians who were blinded to treatment group. In each case, they correctly identified the treatment group to which the patient had been assigned on the basis of improvement or lack of improvement in cardiac function.

In a smaller double-blind study, 100 mg of CoQ_{10} was given daily for 3 months to 12 patients with progressive MD. CoQ_{10} treatment resulted in significant improvements in cardiac output and stroke volume, as

well as increased physical well-being in 4 of 8 patients.[38] Subjective improvements included increased exercise tolerance, reduced leg pain, better control of leg function, and less fatigue. A small pilot trial of CoQ_{10} supplementation among participants with Duchenne muscular dystrophy receiving corticosteroid treatment also found benefit, with an 8.5% improvement in muscle strength among the CoQ_{10} group.[107]

Immune Function

A number of studies demonstrated immunomodulatory effects of CoQ_{10} or its analogs.[108–110] In a study of eight chronically ill patients, administration of 60 mg/day of CoQ_{10} was associated with significant increases in serum levels of immunoglobulin-G after 27 to 98 days of treatment.[111] In a cytokine analysis of white blood cells from 19 human volunteers, tumor necrosis factor-α levels were significantly decreased and interleukin-2 levels increased when incubated with CoQ_{10} in vitro.[112]

Acquired Immunodeficiency Syndrome

Because oxidative stress is believed to be involved in the pathogenesis of AIDS-related diseases, the antioxidant activity of CoQ_{10} may be of value for individuals with AIDS.[112,113]

Early studies suggested blood levels of CoQ_{10} were significantly lower in patients with AIDS and AIDS-related complex than in healthy controls, and that supplementation with CoQ_{10} improved ratios of lymphocytes.[114] Nucleoside reverse transcriptase inhibitor therapy, a mainstay of conventional human immunodeficiency virus management, has been associated with myopathy resulting from side effects on myocyte mitochondria. There is one report of CoQ_{10} alleviating this myopathy, thus permitting continuation of nucleoside reverse transcriptase inhibitor therapy.[115]

Toxicant Exposure

Many occupational and environmental toxicant exposures affect the mitochondrial respiratory chain.[116–120] Damage to the respiratory chain can decrease mitochondrial membrane potential, leading to upregulated apoptosis; CoQ_{10} demonstrated the ability to reduce this membrane-potential damage.[121,122] For example, chlorpyrifos, an organophosphorus pesticide associated with oxidative mitochondrial damage, may have some of its toxicity mitigated by CoQ_{10}.[123] It also appears that CoQ_{10} deficiency is associated with impaired detoxification via the sulfide oxidation pathway, as CoQ_{10} plays the role of an electron acceptor for the enzyme sulfide–quinone reductase (SQR).[124] This pathway is important for the detoxification of sulfide, which is endogenously produced via cysteine catabolism (Fig. 68.5). This pathway appears to be impaired among individuals with the nephrotic syndrome, for whom CoQ_{10} may be particularly important.[125] Although current diagnosis and treatment of environmentally related illnesses remain controversial, cytoprotection with CoQ_{10} may be an effective therapeutic method to reduce mitochondrial damage due to exposure to toxicants, both acute and chronic, and should be a focus of further research.[126]

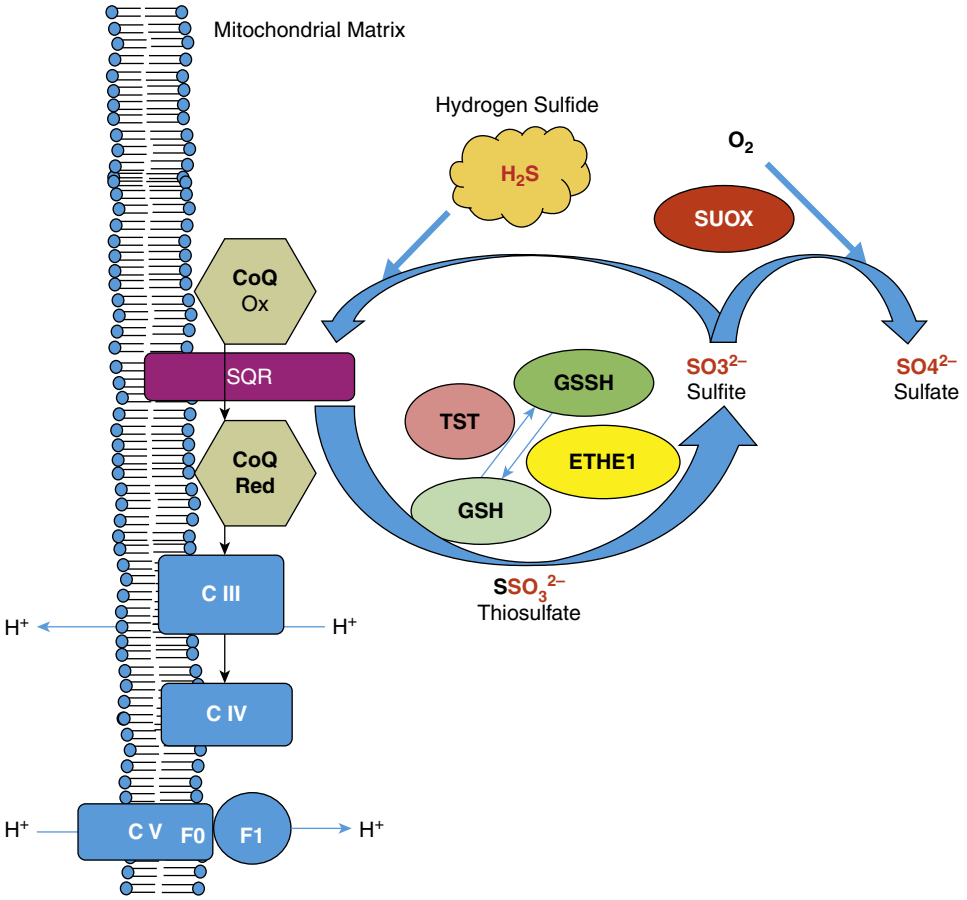


Fig. 68.5 Mitochondrial H2S oxidation pathway. SQR converts sulfide into thiosulfate by transferring two electrons from the H2S oxidation to ubiquinone (CoQ). Thiosulfate is then converted into sulfite by TST and ETHE1; this reaction requires a sulfur acceptor (glutathione, GSH). Excess of sulfite is converted into sulfate by SUOX. (From Ziosi M, Di Meo I, Kleiner G, et al. Coenzyme Q deficiency causes impairment of the sulfide oxidation pathway. *EMBO Mol Med.* 2017;9(1):96–111. PMID: 27856618. Reproduced from an open access article under the terms of the Creative Commons Attribution 4.0 License.)

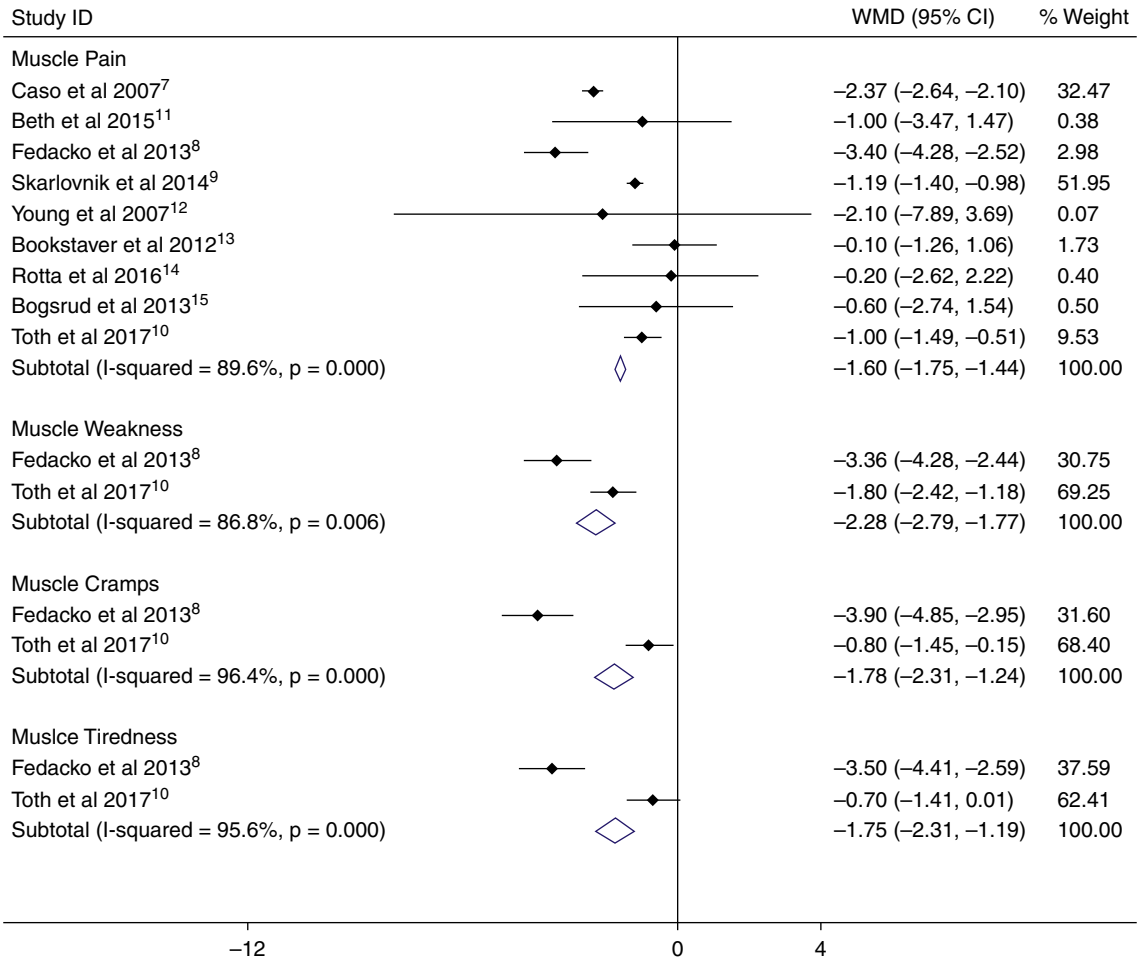


Fig. 68.6 Forest plot for statin-associated muscle symptoms: coenzyme Q_{10} versus placebo (fixed-effect model). CI indicates confidence interval; ID, identification; WMD, weighted mean difference. (From Qu H, Guo M, Chai H, et al. Effects of coenzyme Q10 on statin-induced myopathy: an updated meta-analysis of randomized controlled trials. *J Am Heart Assoc.* 2018;7(19):e009835. PMID: 30371340. Reproduced from an open access article under the terms of the Creative Commons Attribution–NonCommercial License.)

Topical Antiaging Effects

In recent years, makers of several over-the-counter skin creams have begun advertising the addition of CoQ_{10} as an ingredient capable of curbing the aging process. In a clinical trial, topical CoQ_{10} was able to protect against ultraviolet A–mediated oxidative stress, suppress the expression of collagenase, and cause a reduction in wrinkle depth.[33] Both ubiquinol and ubiquinone have been shown to increase in deeper epithelial layers (after topical application), and increase the skin's antioxidant capacity.[127]

Side Effects and Safety

CoQ_{10} appears to be safe and well tolerated in dosages up to 1200 mg/day in adults and up to 10 mg/kg body weight/day in children. Because safety during pregnancy and lactation has not been established, CoQ_{10} should not be used during these times unless the potential clinical benefit outweighs the risks. CoQ_{10} is contraindicated in cases of known hypersensitivity. In a series of 5143 patients treated with 30 mg/day of CoQ_{10}, the following incidence of side effects was reported[57]:

- Epigastric discomfort, 0.39%
- Loss of appetite, 0.23%
- Nausea, 0.16%
- Diarrhea, 0.12%

DRUG INTERACTIONS

Cholesterol-lowering statin drugs such as lovastatin, rosuvastatin, and pravastatin inhibit the enzyme 3-hydroxy-3-methylglutaryl CoA reductase, which is required for biosynthesis of both cholesterol and CoQ_{10}. Thus administration of these drugs might compromise CoQ_{10} status by decreasing its synthesis, and statin-associated myopathy has been hypothesized to be related to a depletion of CoQ_{10}. Although some trials demonstrated that statin therapy did reduce serum or muscle levels of CoQ_{10},[128,129] whether or not this CoQ_{10} depletion caused the myopathy remains controversial.[130] Given that statins are typically prescribed to lower cholesterol, with the intention being the prevention and treatment of cardiovascular disease, the concomitant use of CoQ_{10} seems well justified.

The question of whether or not CoQ_{10} therapy among statin users has additional benefit, however, may finally be answered after the results of two recent meta-analyses. In 2018 a meta-analysis of 12 randomized controlled trials of statin therapy found that statins were associated with decreased CoQ_{10} levels, regardless of the length of therapy, intensity, or type of statin.[131] That this decrease is consequential is well supported by a 2018 meta-analysis published in the *Journal of the American Heart Association;* this analysis of 12 randomized trials and 575 participants given CoQ_{10} or placebo (in addition to a statin) found that supplementation with CoQ_{10} significantly reduced muscle pain, muscle cramp, muscle tiredness, and muscle weakness, despite no reduction in plasma creatine kinase level (Fig. 68.6).[132]

The β-blockers propranolol and metoprolol were shown to inhibit CoQ_{10}-dependent enzymes.[133] The antihypertensive effect of these drugs might therefore be compromised in the long run by the development of CoQ_{10} deficiency. In one study, administration of 60 mg/day

of CoQ_{10} reduced the incidence of drug-induced malaise in patients receiving propranolol.[134]

A number of phenothiazines and tricyclic antidepressants were also shown to inhibit CoQ_{10}-dependent enzymes. It is therefore possible that CoQ_{10} deficiency might be a contributing factor to the cardiac side effects that are frequently seen with these drugs. In two clinical studies, supplementation with CoQ_{10} improved electrocardiographic changes in patients on psychotropic drugs.[135]

Several case reports describing potential interactions between CoQ_{10} and warfarin have been reported. CoQ_{10} is structurally related to menaquinone (vitamin K_2) and may have procoagulant effects.[136] In each of these patients, the international normalized ratio (INR), which had been stable and therapeutic, fell below the therapeutic range within 2 weeks of beginning CoQ_{10} supplementation (as low as 30 mg/day).[137] The INR returned to the therapeutic range after CoQ_{10} was discontinued. It is recommended that the INR be monitored closely if these agents are to be used concomitantly. However, in a double-blind trial, administration of 100 mg/day of CoQ_{10} for 4 weeks had no effect on the INR in 21 patients on long-term warfarin therapy.[138] Thus the sporadic case reports of an interaction between CoQ_{10} and warfarin might have been due to random fluctuations in INR values, rather than to CoQ_{10}.[15]

In a subchronic toxicity assessment, the no observed adverse effect level in rats was estimated to be 600 mg/kg per day for males and 200 mg/kg per day for females; in male and female dogs, the no observed adverse effect level was estimated to be more than 600 mg/kg per day.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bonakdar RA, Guarneri E. Coenzyme Q_{10}. *Am Fam Physician*. 2005;72:1065–1070.
2. Rahmani E1, Jamilian M2, Samimi M3, et al. The effects of coenzyme Q_{10} supplementation on gene expression related to insulin, lipid and inflammation in patients with polycystic ovary syndrome. *Gynecol Endocrinol*. 2018;34(3):217–222. PMID: 28949260.
3. Agmo V1 H, Eriksson EK1, Edwards K. Ubiquinone-10 alters mechanical properties and increases stability of phospholipid membranes. *Biochim Biophys Acta*. 2015;1848(10 Pt A):2233–2243. PMID: 25986530.
4. Fan L1, Feng Y2, Chen GC, et al. Effects of coenzyme Q_{10} supplementation on inflammatory markers: a systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res*. 2017;119:128–136. PMID: 28179205.
5. Kumar A, Kaur H, Devi P, et al. Role of coenzyme Q_{10} (CoQ_{10}) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacol Ther*. 2009;124:259–268.
6. Littarru GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol Biotechnol*. 2007;37:31–37.
7. Bhagavan HN, Chopra RK. Plasma coenzyme Q_{10} response to oral ingestion of coenzyme Q_{10} formulations. *Mitochondrion*. 2007;7(suppl):S78–S88.
8. Quinzii CM, DiMauro S, Hirano M. Human coenzyme Q_{10} deficiency. *Neurochem Res*. 2007;32:723–727.
9. Quinzii CM, Hirano M. Coenzyme Q and mitochondrial disease. *Dev Disabil Res Rev*. 2010;16:183–188.
10. Alcázar-Fabra M1, Trevisson E2, Brea-Calvo G3. Clinical syndromes associated with Coenzyme Q_{10} deficiency. *Essays Biochem*. 2018;62(3):377–398. PMID: 30030365.
11. Hughes BG1, Harrison PM1, Hekimi S2. Estimating the occurrence of primary ubiquinone deficiency by analysis of large-scale sequencing data. *Sci Rep*. 2017;7(1):17744. PMID: 29255295.
12. Pravst I, Zmitek K, Zmitek J. Coenzyme Q_{10} contents in foods and fortification strategies. *Crit Rev Food Sci Nutr*. 2010;50:269–280.
13. Miles MV, Putnam PE, Miles L, et al. Acquired coenzyme Q_{10} deficiency in children with recurrent food intolerance and allergies. *Mitochondrion*. 2011;11:127–135. PMID: 20817046.
14. Yubero D1, Montero R2, Martín MA, et al. Secondary coenzyme Q_{10} deficiencies in oxidative phosphorylation (OXPHOS) and non-OXPHOS disorders. *Mitochondrion*. 2016 Sep;30:51–58. PMID: 27374853.
15. Steele PE, Tang PH, DeGrauw AJ, et al. Clinical laboratory monitoring of coenzyme Q_{10} use in neurologic and muscular diseases. *Am J Clin Pathol*. 2004;121(suppl):S113–S120.
16. Miles MV, Horn PS, Tang PH, et al. Age-related changes in plasma coenzyme Q_{10} concentrations and redox state in apparently healthy children and adults. *Clin Chim Acta*. 2004;34:139–144.
17. Alcázar-Fabra M1, Trevisson E2, Brea-Calvo G3. Clinical syndromes associated with Coenzyme Q_{10} deficiency. *Essays Biochem*. 2018;62(3):377–398. PMID: 30030365.
18. Nakamura R, Littarru GP, Folkers K, et al. Study of CoQ_{10}-enzymes in gingiva from patients with periodontal disease and evidence for a deficiency of coenzyme Q_{10}. *Proc Natl Acad Sci U S A*. 1974;71:1456–1460.
19. Morre DJ, Morre DM. arNOX activity of saliva as a non-invasive measure of coenzyme Q_{10} response in human trials. *Biofactors*. 2008;32:231–235.
20. Abdul-Rasheed OF, Farid YY. Development of a new high performance liquid chromatography method for measurement of coenzyme Q_{10} in healthy blood plasma. *Saudi Med J*. 2009;30:1138–1143.
21. Yubero D1, Allen G2, Artuch R3, et al. The value of coenzyme Q_{10} Determination in mitochondrial patients. *J Clin Med*. 2017;6(4):pii: E37. PMID: 28338638.
22. Yubero D1, Montero R1, Ramos M, et al. Determination of urinary coenzyme Q_{10} by HPLC with electrochemical detection: reference values for a paediatric population. *Biofactors*. 2015;41(6):424–430. PMID: 26768296.
23. Hosoe K, Kitano M, Kishida H, et al. Study on safety and bioavailability of ubiquinol (Kaneka QH) after single and 4-week multiple oral administration to healthy volunteers. *Regul Toxicol Pharmacol*. 2007;47:19–28.
24. Beg S, Javed S, Kohli K. Bioavailability enhancement of coenzyme Q_{10}: an extensive review of patents. *Recent Pat Drug Deliv Formul*. 2010;4:245–255.
25. Takeda R, Sawabe A, Nakano R, et al. Effect of various food additives and soy constituents on high CoQ_{10} absorption. *Jpn J Med Pharma Sci*. 2011;64:614–620.
26. Sinatra ST. Coenzyme Q_{10} and congestive heart failure. *Ann Intern Med*. 2000;133:745–746.
27. Langsjoen PH, Langsjoen AM. Overview of the use of CoQ_{10} in cardiovascular disease. *Biofactors*. 1999;9:273–284.
28. Ochiai A, Itagaki S, Kurokawa T, et al. Improvement in intestinal coenzyme Q_{10} absorption by food intake. *Yakugaku Zasshi*. 2007;127:1251–1254.
29. Shults CW, Oakes D, Kieburtz K, et al. Effects of coenzyme Q_{10} in early Parkinson disease: evidence of slowing of the functional decline. *Arch Neurol*. 2002;59:1541–1550.
30. Langsjoen PH, Folkers K, Lyson K, et al. Effective and safe therapy with coenzyme Q_{10} for cardiomyopathy. *Klin Wochenschr*. 1988;66:583–590.
31. Singh RB, Niaz MA, Kumar A, et al. Effect on absorption and oxidative stress of different oral coenzyme Q_{10} dosages and intake strategy in healthy men. *Biofactors*. 2005;25:219–224.
32. Niklowitz P, Sonnenschein A, Janetzky B, et al. Enrichment of coenzyme Q_{10} in plasma and blood cells: defense against oxidative damage. *Int J Biol Sci*. 2007;3:257–262.
33. Hojerova J. Coenzyme Q_{10}—its importance, properties and use in nutrition and cosmetics. *Ceska Slov Farm*. 2000;49:119–123 [Slovak].
34. Tian G1, Sawashita J, Kubo H, et al. Ubiquinol-10 supplementation activates mitochondria functions to decelerate senescence in senescence-accelerated mice. *Antioxid Redox Signal*. 2014;20(16):2606–2620. PMID: 24124769.
35. Ryo K, Ito A, Takatori R, et al. Effects of coenzyme Q_{10} on salivary secretion. *Clin Biochem*. 2011;44(8–9):669–674.
36. Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. *Proc Natl Acad Sci U S A*. 1991;88:1646–1650.
37. Hanaki Y, Sugiyama S, Ozawa T, et al. Ratio of low-density lipoprotein cholesterol to ubiquinone as a coronary risk factor. *N Engl J Med*. 1991;325:814–815.
38. Folkers K, Wolaniuk J, Simonsen R, et al. Biochemical rationale and the cardiac response of patients with muscle disease to therapy with coenzyme Q_{10}. *Proc Natl Acad Sci U S A*. 1985;82:4513–4516.
39. Folkers K, Littarru GP, Ho L, et al. Evidence for a deficiency of coenzyme Q_{10} in human heart disease. *Int Z Vitaminforsch*. 1970;40:380–390.
40. Yang YK1, Wang LP1, Chen L, et al. Coenzyme Q_{10} treatment of cardiovascular disorders of ageing including heart failure, hypertension and endothelial dysfunction. *Clin Chim Acta*. 2015;450:83–89. PMID: 26254995.
41. Kocharian A, Shabanian R, Rafiei-Khorgami M, et al. Coenzyme Q_{10} improves diastolic function in children with idiopathic dilated cardiomyopathy. *Cardiol Young*. 2009;19:501–506.
42. Langsjoen PH, Folkers K. Long-term efficacy and safety of coenzyme Q_{10} therapy for idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1990;65:521–523.
43. Langsjoen PH, Vadhanavikit S, Folkers K. Effective treatment with coenzyme Q_{10} of patients with chronic myocardial disease. *Drugs Exp Clin Res*. 1985;11:577–579.
44. Mortensen SA1, Rosenfeldt F2, Kumar A, et al. The effect of coenzyme Q_{10} on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. *JACC Heart Fail*. 2014;2(6):641–649. PMID: 25282031.
45. Yamamura YIT, Yamagami T, Morita Y, et al. Clinical use of coenzyme-Q for treatment of cardiovascular disease. *Jpn Circ J*. 1967;31:168.
46. Ishiyama T, Morita Y, Toyama S, et al. A clinical study of the effect of coenzyme Q on congestive heart failure. *Jpn Heart J*. 1976;17:32–42.
47. Soja AM, Mortensen SA. Treatment of congestive heart failure with coenzyme Q_{10} illuminated by meta-analyses of clinical trials. *Mol Aspects Med*. 1997;18(suppl):S159–S168.

48. Sander S, Coleman CI, Patel AA, et al. The impact of coenzyme Q_{10} on systolic function in patients with chronic heart failure. *J Card Fail*. 2006;12:464–472.
49. Baggio E, Gandini R, Plancher AC, et al. Italian multicenter study on the safety and efficacy of coenzyme Q_{10} as adjunctive therapy in heart failure. CoQ_{10} Drug Surveillance Investigators. *Mol Aspects Med*. 1994;15(suppl):s287–s294.
50. Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q_{10} therapy in patients with congestive heart failure: a long-term multicenter randomized study. *Clin Investig*. 1993;71:S134–S136.
51. Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q_{10} in patients with congestive heart failure. *Ann Intern Med*. 2000;132:636–640.
52. Belardinelli R, Mucaj A, Lacalaprice F, et al. Coenzyme Q_{10} improves contractility of dysfunctional myocardium in chronic heart failure. *Biofactors*. 2005;25:137–145.
53. Molyneux SL, Florkowski CM, Richards AM, et al. Coenzyme Q_{10}; an adjunctive therapy for congestive heart failure? *N Z Med J*. 2009;122:74–79.
54. Lei L1, Liu Y2. Efficacy of coenzyme Q_{10} in patients with cardiac failure: a meta-analysis of clinical trials. *BMC Cardiovasc Disord*. 2017;17(1):196. PMID: 28738783.
55. Langsjoen PH, Langsjoen AM. Supplemental ubiquinol in patients with advanced congestive heart failure. *Biofactors*. 2008;32:119–128.
56. Makhija N, Sendasgupta C, Kiran U, et al. The role of oral coenzyme Q_{10} in patients undergoing coronary artery bypass graft surgery. *J Cardiothorac Vasc Anesth*. 2008;22:832–839.
57. Tanaka J, Tominaga R, Yoshitoshi M, et al. Coenzyme Q_{10}: the prophylactic effect on low cardiac output following cardiac valve replacement. *Ann Thorac Surg*. 1982;33:145–151.
58. de Frutos F1, Gea A2, Hernandez-Estefania R, et al. Prophylactic treatment with coenzyme Q_{10} in patients undergoing cardiac surgery: could an antioxidant reduce complications? A systematic review and meta-analysis. *Interact Cardiovasc Thorac Surg*. 2015;20(2):254–259. PMID: 25344142.
59. Langsjoen P, Willis R, Folkers K. Treatment of essential hypertension with coenzyme Q_{10}. *Mol Aspects Med*. 1994;15(suppl):S265–S272.
60. Ho MJ, Bellusci A, Wright JM. Blood pressure lowering efficacy of coenzyme Q_{10} for primary hypertension. *Cochrane Database Syst Rev*. 2009:CD007435.
61. Ho MJ1, Li EC, Wright JM. Blood pressure lowering efficacy of coenzyme Q_{10} for primary hypertension. *Cochrane Database Syst Rev*. 2016;3:CD007435. PMID: 26935713.
62. R1 T, Akbari M1, Sharifi N, et al. The effects of coenzyme Q_{10} supplementation on blood pressures among patients with metabolic diseases: a systematic review and meta-analysis of randomized controlled trials. *High Blood Press Cardiovasc Prev*. 2018;25(1):41–50. PMID: 29330704.
63. Conn VS1, Ruppar TM2, Chase JD3. Blood pressure outcomes of medication adherence interventions: systematic review and meta-analysis. *J Behav Med*. 2016;39(6):1065–1075. PMID: 26969094.
64. Lockwood K, Moesgaard S, Folkers K. Partial and complete regression of breast cancer in patients in relation to dosage of coenzyme Q_{10}. *Biochem Biophys Res Commun*. 1994;199:1504–1508.
65. Hertz N, Lister RE. Improved survival in patients with end-stage cancer treated with coenzyme Q(10) and other antioxidants: a pilot study. *J Int Med Res*. 2009;37:1961–1971.
66. Conklin KA. Coenzyme Q_{10} for prevention of anthracycline-induced cardiotoxicity. *Integr Cancer Ther*. 2005;4:110–130.
67. Karlsson JAH, Folkers K, Astrom H, et al. Effect of Adriamycin on heart and skeletal muscle coenzyme Q (CoQ_{10}) in man. In: Folkers K, ed. *Biomedical and clinical aspects of coenzyme Q.* Amsterdam: Elsevier Scientific.
68. Judy WVDW, Hall JH, Dugan W, et al. Coenzyme Q_{10} reduction of Adriamycin cardiotoxicity. In: Folkers K, ed. *Biomedical and clinical aspects of coenzyme Q.* Amsterdam: Elsevier Scientific.
69. Mizuno K, Tanaka M, Nozaki S, et al. Antifatigue effects of coenzyme Q_{10} during physical fatigue. *Nutrition*. 2008;24:293–299.
70. Jacobson BH, Smith DB, Warren AJ, et al. Assessment of the effectiveness of a sublingual, ergogenic spray on muscle strength and power. *J Strength Cond Res*. 2009;23:2326–2330.
71. Kon M, Kimura F, Akimoto T, et al. Effect of Coenzyme Q_{10} supplementation on exercise-induced muscular injury of rats. *Exerc Immunol Rev*. 2007;13:76–88.
72. Vanfraecchhem J. Coenzyme Q_{10} and physical performance. In: Folkers K, ed. *Biomedical and clinical aspects of coenzyme* Q_{10}. Amsterdam: Elsevier Scientific; 235–241.
73. Gökbel H, Gül I, Belviranl M, et al. The effects of coenzyme Q_{10} supplementation on performance during repeated bouts of supramaximal exercise in sedentary men. *J Strength Cond Res*. 2010;24:97–102.
74. Malm C, Svensson M, Ekblom B, et al. Effects of ubiquinone-10 supplementation and high intensity training on physical performance in humans. *Acta Physiol Scand*. 1997;161:379–384.
75. Orlando P1, Silvestri S1, Galeazzi R, et al. Effect of ubiquinol supplementation on biochemical and oxidative stress indexes after intense exercise in young athletes. *Redox Rep*. 2018;23(1):136–145. PMID: 29734881.
76. Hasegawa G1, Yamamoto Y, Zhi JG, et al. Daily profile of plasma %CoQ_{10} level, a biomarker of oxidative stress, in patients with diabetes manifesting postprandial hyperglycaemia. *Acta Diabetol*. 2005;42(4): 179–181. PMID: 16382305.
77. Kishi T, Kishi H, Watanabe T, et al. Bioenergetics in clinical medicine. XI. Studies on coenzyme Q and diabetes mellitus. *J Med*. 1976;7: 307–321.
78. Eriksson JG, Forsen TJ, Mortensen SA, et al. The effect of coenzyme Q_{10} administration on metabolic control in patients with type 2 diabetes mellitus. *Biofactors*. 1999;9:315–318.
79. Hodgson JM, Watts GF, Playford DA, et al. Coenzyme Q_{10} improves blood pressure and glycaemic control: a controlled trial in subjects with type 2 diabetes. *Eur J Clin Nutr*. 2002;56:1137–1142.
80. Watts GF, Playford DA, Croft KD, et al. Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in type II diabetes mellitus. *Diabetologia*. 2002;45:420–426.
81. Playford DA, Watts GF, Croft KD, et al. Combined effect of coenzyme Q_{10} and fenofibrate on forearm microcirculatory function in type 2 diabetes. *Atherosclerosis*. 2003;168:169–179.
82. Lamson DW, Plaza SM. Mitochondrial factors in the pathogenesis of diabetes: a hypothesis for treatment. *Altern Med Rev*. 2002;7:94–111.
83. Silvestre-Aillaud P, BenDahan D, Paquis-Fluckinger V, et al. Could coenzyme Q_{10} and L-carnitine be a treatment for diabetes secondary to 3243 mutation of mtDNA? *Diabetologia*. 1995;38:1485–1486.
84. Zhang 2 SY1, Yang KL1, Zeng LT, et al. Effectiveness of coenzyme Q_{10} supplementation for type 2 diabetes mellitus: a systematic review and meta-analysis. *Int J Endocrinol*. 2018;2018:6484839. PMID: 30305810.
85. Fujioka T, Sakamoto Y, Mimura G. Clinical study of cardiac arrhythmias using a 24-hour continuous electrocardiographic recorder (5th report)–antiarrhythmic action of coenzyme Q_{10} in diabetics. *Tohoku J Exp Med*. 1983;141(suppl):453–463.
86. Alleva R, Scararmucci A, Mantero F, et al. The protective role of ubiquinol-10 against formation of lipid hydroperoxides in human seminal fluid. *Mol Aspects Med*. 1997;18(suppl):S221–S228.
87. Mancini A, De Marinis L, Littarru GP, et al. An update of Coenzyme Q_{10} implications in male infertility: biochemical and therapeutic aspects. *Biofactors*. 2005;25:165–174.
88. Balercia G, Arnaldi G, Fazioli F, et al. Coenzyme Q_{10} levels in idiopathic and varicocele-associated asthenozoospermia. *Andrologia*. 2002;34:107–111.
89. Balercia G, Mosca F, Mantero F, et al. Coenzyme Q(10) supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study. *Fertil Steril*. 2004;81:93–98.
90. Lewin A, Lavon H. The effect of coenzyme Q_{10} on sperm motility and function. *Mol Aspects Med*. 1997;18(suppl):S213–S219.
91. Balercia G, Mancini A, Paggi F, et al. Coenzyme Q_{10} and male infertility. *J Endocrinol Invest*. 2009;32:626–632.
92. Lafuente R1, González-Comadrán M, Solà I, et al. Coenzyme Q_{10} and male infertility: a meta-analysis. *J Assist Reprod Genet*. 2013;30(9):1147–1156. PMID: 23912751.
93. Shults CW, Haas RH, Beal MF. A possible role of coenzyme Q_{10} in the etiology and treatment of Parkinson's disease. *Biofactors*. 1999;9:267–272.

94. Zhu ZG1, Sun MX2, Zhang WL, et al. The efficacy and safety of coenzyme Q_{10} in Parkinson's disease: a meta-analysis of randomized controlled trials. *Neurol Sci*. 2017;38(2):215–224. PMID: 27830343.
95. Negida A1, Menshawy A, El Ashal G, et al. Coenzyme Q_{10} for Patients with Parkinson's Disease: a systematic review and meta-analysis. *CNS Neurol Disord Drug Targets*. 2016;15(1):45–53. PMID: 26553164.
96. NINDS Website. Statement on the Termination of QE3 Study. http://www.ninds.nih.gov/disorders/clinical_trials/CoQ$_{10}$-Trial-Update.htm. Accessed June 15, 2011.
97. Parkinson Study Group QE3 Investigators, Beal MF, Oakes D, et al. A randomized clinical trial of high-dosage coenzyme Q_{10} in early Parkinson disease: no evidence of benefit. *JAMA Neurol*. 2014;71(5):543–552. PMID: 24664227.
98. Li Z1, Wang P, Yu Z, et al. The effect of creatine and coenzyme Q_{10} combination therapy on mild cognitive impairment in Parkinson's disease. *Eur Neurol*. 2015;73(3–4):205–211. PMID: 25792086.
99. Cooper JM, Schapira AH. Friedreich's ataxia: coenzyme Q_{10} and vitamin Etherapy. *Mitochondrion*. 2007;7(suppl):S127–S135.
100. Rustin P, Rotig A, Munnich A, et al. Heart hypertrophy and function are improved by idebenone in Friedreich's ataxia. *Free Radic Res*. 2002;36:467–469.
101. Hausse AO, Aggoun Y, Bonnet D, et al. Idebenone and reduced cardiac hypertrophy in Friedreich's ataxia. *Heart*. 2002;87:346–349.
102. Cooper JM, Schapira AH. Friedreich's ataxia: disease mechanisms, antioxidant and coenzyme Q_{10} therapy. *Biofactors*. 2003;18:163–171.
103. Cooper JM, Korlipara LV, Hart PE, et al. Coenzyme Q_{10} and vitamin E deficiency in Friedreich's ataxia: predictor of efficacy of vitamin E and coenzyme Q_{10} therapy. *Eur J Neurol*. 2008;15:1371–1379.
104. Siciliano G, Mancuso M, Tedeschi D, et al. Coenzyme Q_{10}, exercise lactate and CTG trinucleotide expansion in myotonic dystrophy. *Brain Res Bull*. 2001;56:405–410.
105. Potgieter M, Pretorius E, Van der Merwe CF, et al. Histological assessment of SJL/J mice treated with the antioxidants coenzyme Q_{10} and resveratrol. *Micron*. 2011;42(3):275–282.
106. Folkers K, Simonsen R. Two successful double-blind trials with coenzyme Q_{10} (vitamin Q_{10}) on muscular dystrophies and neurogenic atrophies. *Biochim Biophys Acta*. 1995;1271:281–286.
107. Spurney CF1, Rocha CT, Henricson E, et al. CINRG pilot trial of coenzyme Q_{10} in steroid-treated Duchenne muscular dystrophy. *Muscle Nerve*. 2011;44(2):174–178. PMID: 21698649.
108. Folkers K, Morita M, McRee Jr J. The activities of coenzyme Q_{10} and vitamin B6 for immune responses. *Biochem Biophys Res Commun*. 1993;193:88–92.
109. Bessler H, Bergman M, Blumberger N, et al. Coenzyme Q_{10} decreases TNF-alpha and IL-2 secretion by human peripheral blood mononuclear cells. *J Nutr Sci Vitaminol (Tokyo)*. 2010;56:77–81.
110. Gazdik F, Pijak MR, Borova A, et al. Biological properties of coenzyme Q_{10} and its effects on immunity. *Cas Lek Cesk*. 2003;142:390–393 [Czech].
111. Folkers K, Shizukuishi S, Takemura K, et al. Increase in levels of IgG in serum of patients treated with coenzyme Q_{10}. *Res Commun Chem Pathol Pharmacol*. 1982;38:335–338.
112. Sugiyama S, Kitazawa M, Ozawa T, et al. Anti-oxidative effect of coenzyme Q_{10}. *Experientia*. 1980;36:1002–1003.
113. Folkers K, Langsjoen P, Nara Y, et al. Biochemical deficiencies of coenzyme Q_{10} in HIV-infection and exploratory treatment. *Biochem Biophys Res Commun*. 1988;153:888–896.
114. Folkers K, Hanioka T, Xia LJ, et al. Coenzyme Q_{10} increases T4/T8 ratios of lymphocytes in ordinary subjects and relevance to patients having the AIDS-related complex. *Biochem Biophys Res Commun*. 1991;176:786–791.
115. Rosenfeldt FL, Mijch A, McCrystal G, et al. Skeletal myopathy associated with nucleoside reverse transcriptase inhibitor therapy: potential benefit of coenzyme Q_{10} therapy. *Int J STD AIDS*. 2005;16:827–829.
116. Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. *Parkinsonism Relat Disord*. 2003;9(suppl 2):S59–S64.
117. Dorta DJ, Leite S, DeMarco KC, et al. A proposed sequence of events for cadmium-induced mitochondrial impairment. *J Inorg Biochem*. 2003;97:251–257.
118. Peraza MA, Carter DE, Gandolfi AJ. Toxicity and metabolism of subcytotoxic inorganic arsenic in human renal proximal tubule epithelial cells (HK-2). *Cell Biol Toxicol*. 2003;19:253–264.
119. Robertson JD, Orrenius S. Molecular mechanisms of apoptosis induced by cytotoxic chemicals. *Crit Rev Toxicol*. 2000;30:609–627.
120. Robertson JD, Orrenius S. Role of mitochondria in toxic cell death. *Toxicology*. 2002;181–182:491–496.
121. Menke T, Gille G, Reber F, et al. Coenzyme Q_{10} reduces the toxicity of rotenone in neuronal cultures by preserving the mitochondrial membrane potential. *Biofactors*. 2003;18:65–72.
122. Kagan T, Davis C, Lin L, et al. Coenzyme Q_{10} can in some circumstances block apoptosis, and this effect is mediated through mitochondria. *Ann N Y Acad Sci*. 1999;887:31–47.
123. Ghayomi F1, Navaei-Nigjeh M2, M1 B, et al. A mechanistic approach for modulation of chlorpyrifos-induced toxicity in human lymphocytes by melatonin, coenzyme Q_{10}, and vinpocetine. *Hum Exp Toxicol*. 2016;35(8):839–850. PMID: 26519479.
124. Ziosi M, Di Meo I, Kleiner G, et al. Coenzyme Q deficiency causes impairment of the sulfide oxidation pathway. *EMBO Mol Med*. 2017;9(1):96–111. PMID: 27856618.
125. Kleiner G1, Barca E1, Ziosi M, et al. CoQ_{10} supplementation rescues nephrotic syndrome through normalization of H2S oxidation pathway. *Biochim Biophys Acta Mol Basis Dis*. 2018;1864(11):3708–3722. PMID: 30251690.
126. Merlo Pich M, Castagnoli A, Biondi A, et al. Ubiquinol and a coenzyme Q reducing system protect platelet mitochondrial function of transfusional buffy coats from oxidative stress. *Free Radic Res*. 2002;36:429–436.
127. Knott A1, Achterberg V1, Smuda C, et al. Topical treatment with coenzyme Q_{10}-containing formulas improves skin's Q_{10} level and provides antioxidative effects. *Biofactors*. 2015;41(6):383–390. PMID: 26648450.
128. Lamperti C, Naini AB, Lucchini V, et al. Muscle coenzyme Q_{10} level in statin-related myopathy. *Arch Neurol*. 2005;62:1709–1712.
129. Paiva H, Thelen KM, Van Coster R, et al. High-dose statins and skeletal muscle metabolism in humans: a randomized, controlled trial. *Clin Pharmacol Ther*. 2005;78:60–68.
130. Marcoff L, Thompson PD. The role of coenzyme Q_{10} in statin-associated myopathy: a systematic review. *J Am Coll Cardiol*. 2007;49:2231–2237.
131. Qu 2 H1, Meng YY3, Chai H, et al. The effect of statin treatment on circulating coenzyme Q_{10} concentrations: an updated meta-analysis of randomized controlled trials. *Eur J Med Res*. 2018;23(1):57. PMID: 30414615.
132. Qu 2 H1, Guo M3, Chai H, et al. Effects of coenzyme Q_{10} on statin-induced myopathy: an updated meta-analysis of randomized controlled trials. *J Am Heart Assoc*. 2018;7(19):e009835. PMID: 30371340.
133. Kishi T, Watanabe T, Folkers K. Bioenergetics in clinical medicine XV. Inhibition of coenzyme Q_{10}-enzymes by clinically used adrenergic blockers of beta-receptors. *Res Commun Chem Pathol Pharmacol*. 1977;17:157–164.
134. Kishi H, Kishi T, Folkers K. Bioenergetics in clinical medicine. III. Inhibition of coenzyme Q_{10}-enzymes by clinically used anti-hypertensive drugs. *Res Commun Chem Pathol Pharmacol*. 1975;12:533–540.
135. Kishi H. Inhibition of myocardial respiration by psychotherapeutic drugs and prevention by coenzyme Q. In: Folkers KYY, ed. *Biomedical and Clinical Aspects of Coenzyme Q*. Amsterdam: Elsevier Scientific; 139–154.
136. Morton RA. Ubiquinones, plastoquinones and vitamins K. *Biol Rev Camb Philos Soc*. 1971;46:47–96.
137. Spigset O. Reduced effect of warfarin caused by ubidecarenone. *Lancet*. 1994;344:1372–1373.
138. Engelsen J, Nielsen JD, Winther K. Effect of coenzyme Q_{10} and Ginkgo biloba on warfarin dosage in stable, long-term warfarin treated outpatients. A randomised, double blind, placebo-crossover trial. *Thromb Haemost*. 2002;87:1075–1076.

69

Coleus forskohlii

Michael T. Murray, ND

OUTLINE

Coleus forskohlii (family: *Labiatae*)
Synonyms: *Coleus barbatus, Plectranthus barbatus, Plectranthus forskohlii*
Common name: coleus

GENERAL DESCRIPTION

Coleus forskohlii, a perennial, is a small member of the mint *(Labiatae)* family. It grows on sun-exposed, dry hill slopes between an altitude of 1000 and 6000 feet in subtropical, temperate climactic zones. Thus it is found in India, Nepal, Sri Lanka, and Thailand. Its Latin name comes from the word *coleos,* which means "sheath," and refers to the fused filaments that form a sheath around the stylus of the flower. The epithet *forskohlii* commemorates the Finnish botanist Forskal, who traveled extensively in Egypt and Arabia in the 18th century.

The radially spread rootstock is the portion of the plant that is used for medicinal purposes. The rootstock is also the source of a compound of unique biological importance, forskolin. No other species of *Coleus* contains forskolin.

CHEMICAL COMPOSITION

The primary chemical of clinical interest contained in *C. forskohlii* is the diterpene forskolin (Fig. 69.1). In 1974 forskolin was discovered during a large-scale screening of medicinal plants by the Indian Central Drug Research Institute. The screening revealed the presence of a hypotensive and spasmolytic component, which was initially named coleanol.[1] Additional investigation determined the exact chemical structure, and the name was changed to forskolin. From 1981 to 2010, forskolin was used in more than 15,000 in vitro and in vivo experimental studies designed to better understand the cellular processes governed by cyclic adenosine monophosphate (cAMP). Although most of these studies used this isolated constituent, there is evidence that other components within the plant extract may have biological activity, as well as enhance the absorption and action of forskolin.

HISTORY AND FOLK USE

C. forskohlii has a long history of use in Ayurvedic, Siddha, and Unani systems of medicine. Studies of the pharmacological activity of forskolin substantiate the traditional uses of *C. forskohlii* in such conditions as the following[1]:

- Cardiovascular disease
- Eczema
- Abdominal colic
- Respiratory disorders
- Painful urination
- Insomnia
- Convulsions

PHARMACOLOGY

As noted in a landmark experiment in 1981, the basic mechanism of action of forskolin is the activation of adenylate cyclase, which increases cAMP in cells.[2] cAMP is perhaps the most important cell-regulating compound. Once formed, it activates many other enzymes involved in diverse cellular functions.[3]

Under normal situations, cAMP is formed when an activating hormone (e.g., epinephrine) binds to a receptor site on the cell membrane and stimulates the activation of adenylate cyclase. This enzyme is found in all cellular membranes, and only the specificity of the receptor site determines which hormone will activate it in a particular cell. In contrast, forskolin appears to directly activate adenylate cyclase, bypassing hormonal transmembrane activation of adenylate cyclase.

The physiological and biochemical effects of a raised intracellular cAMP level include the following:

- Inhibition of platelet activation and degranulation
- Inhibition of mast cell degranulation and histamine release
- Increased force of contraction of heart muscle
- Relaxation of the arteries and other smooth muscles
- Increased insulin secretion
- Increased thyroid function

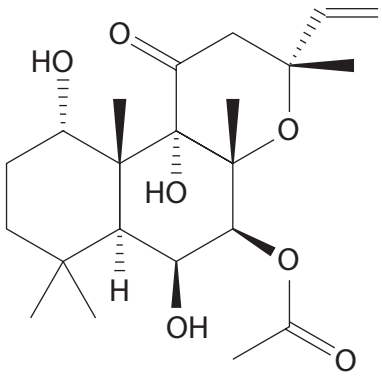


Fig. 69.1 Forskolin.

- Lipolysis

Forskolin possesses additional mechanisms of action independent of its ability to directly stimulate adenylate cyclase and cAMP-dependent physiological responses.[4] Specifically, forskolin was shown to inhibit a number of membrane transport proteins and channel proteins through a mechanism that did not involve the production of cAMP. The result was transmembrane signaling that resulted in activation of other cellular enzymes. Research is under way to determine the exact receptors to which forskolin binds.

Another action of forskolin is the inhibition of platelet-activating factor (PAF) by interfering with PAF binding to receptor sites.[5] PAF plays a central role in many inflammatory and allergic processes, including neutrophil activation, increased vascular permeability, smooth muscle contraction (e.g., bronchoconstriction), and reduction in coronary blood flow. Treatment of platelets with forskolin before PAF exposure results in a 30% to 40% decrease in PAF binding. This decrease in PAF binding caused by forskolin was concomitant with a decrease in the physiological responses of platelets induced by PAF. However, this forskolin-induced decrease in PAF binding was not a consequence of cAMP formation because the addition of a cAMP antagonist did not inhibit the action of forskolin. In addition, the inactive analog of forskolin, dideoxyforskolin, which does not activate adenyl cyclase, also reduced PAF binding to its receptor. Researchers speculated that the action of forskolin on PAF binding was due to a direct effect of this molecule and its analog on the PAF receptor itself or to components of the postreceptor signaling for PAF.

CLINICAL APPLICATIONS

The therapeutic ramifications of *C. forskohlii* based on the pharmacology of forskolin are immense. In many conditions, a decreased intracellular cAMP level is thought to be a major factor in the development of the disease process. *C. forskohlii* appears to be especially well indicated for the following:

- Atopic dermatitis
- Asthma
- Psoriasis
- Angina
- Hypertension
- Weight loss
- Glaucoma

Although *C. forskohlii* can be used alone, it may prove to be most useful when combined with other botanicals or measures, or both.

Inflammatory Conditions

Allergic conditions such as asthma and eczema are characterized by a relative decrease in cAMP in both the bronchial smooth muscle and the skin. As a result, mast cells degranulate and smooth muscle cells contract more readily. In addition, these allergic conditions are also characterized by excessive levels of PAF.

Asthma and Eczema

Current drug therapy for allergic conditions like asthma and eczema is largely designed to increase cAMP levels by using substances that either bind to receptors to stimulate adenylate cyclase (e.g., corticosteroids) or inhibit the enzyme phosphodiesterase, which breaks down cAMP once it is formed (e.g., methylxanthines). These actions are different than forskolin's ability to increase the production of cAMP via transmembrane activation of adenylate cyclase. The cAMP-elevating action of forskolin supports the use of *C. forskohlii* extracts alone or in combination with standard drug therapy in the treatment of virtually all allergic conditions.

C. forskohlii extracts may be particularly useful in asthma because increasing intracellular levels of cAMP results in relaxation of bronchial muscles and relief of respiratory symptoms. Forskolin was shown to have remarkable effects in relaxing constricted bronchial muscles in patients with asthma.[6–8] This type of smooth muscle is also found in the gastrointestinal tract, uterus, bladder, and arteries. Forskolin was shown to have tremendous antispasmodic action on these various smooth muscles. This antispasmodic action of forskolin supports the folk medicine use of *C. forskohlii* in the treatment of not only asthma but also intestinal colic, uterine cramps (menstrual cramps), painful urination, angina, and hypertension. In addition to forskolin's ability to relax smooth muscle, its other antiallergic activities, such as inhibiting the release of histamine and the synthesis of allergic compounds, are also beneficial in treating asthma.[9]

One double-blind clinical study sought to compare the antiasthmatic effects of forskolin with the drug fenoterol. Sixteen patients with asthma were studied using three different preparations:

- Single inhalation dose of fenoterol (as a dry-powder capsule [0.4 mg])
- Metered doses of fenoterol (0.4 mg)
- Forskolin dry-powder capsules (10 mg)

All three preparations led to a significant improvement in respiratory function and bronchodilation. However, although the fenoterol preparations caused tremors and decreased blood potassium levels, no such negative effects were seen with forskolin.

In another study, the bronchodilating effect (after 5 minutes) of forskolin was as good as that produced by fenoterol in 12 healthy volunteers (nonsmokers), as determined by whole-body plethysmography.[10] Both substances were administered by metered-dose inhalers. At the beginning (after 3 and 5 minutes) of the study, the protective effect of forskolin against inhaled acetylcholine was as good as that produced by fenoterol, whereas later on (after 15 and 30 minutes), fenoterol provided stronger protection.

Whether orally administered forskolin in the form of *C. forskohlii* extract would produce similar bronchodilatory effects is yet to be determined. However, based on the plant's historical use and additional mechanisms of action, it appears likely.

Psoriasis

Psoriasis is a common skin disorder that seems to be caused by a relative decrease in cAMP compared with cyclic guanosine monophosphate (cGMP). The result is a tremendous increase in cell division. Cells divide in psoriasis at a rate 1000 times greater than normal. Preliminary studies indicated that forskolin might be of great benefit in individuals with psoriasis via its ability to reestablish the normal balance between cAMP and cGMP.[1]

Cardiovascular Effects

The most useful clinical applications of *C. forskohlii* extracts that will emerge in the future will likely be for cardiovascular diseases such as hypertension, congestive heart failure, and angina. The cardiovascular effects of *C. forskohlii* and its components have been studied in great detail.[1,11,12] Its basic cardiovascular actions involve the lowering of blood pressure, along with improving the contractility of the heart. Again, this is related to increasing cAMP levels throughout the cardiovascular system, which results in relaxation of the arteries and increased force of contraction. The net effect is a significant improvement of cardiovascular function.

Hypertension and Cardiac Failure

Several clinical and animal studies supported the use of forskolin in hypertension and cardiac failure.[11–15] In one human study involving seven patients with dilated cardiomyopathy, forskolin was shown to improve left ventricular function primarily via reduction of preload and without raising metabolic costs.[13] This study confirmed earlier animal studies showing that forskolin increased the contractile force of heart muscle.[12]

In another human study, the hemodynamic effects of intravenous (3 mcg/kg per minute) forskolin given to patients with dilated cardiomyopathy were evaluated.[14] Although systemic vascular resistance and diastolic pressure decreased, forskolin had no effect on the cardiac index, ejection fraction, or myocardial oxygen consumption at this low dosage. However, when a small dosage of dobutamine was given along with the forskolin, an increase in all four parameters was observed. At a higher dosage (4 mcg/kg per minute), forskolin increased heart function by 19% and produced a 16% rise in heart rate. However, these changes were associated with symptomatic flush syndromes. These results indicated that forskolin might best be used in congestive heart failure in combination with other botanicals, such as *Crataegus* (see Chapter 71).

Forskolin was also shown to be a direct cerebral vasodilator, indicating that it might prove to be useful in cerebral vascular insufficiency and poststroke recovery.[15]

An additional mechanism of action particularly beneficial in a wide range of cardiovascular conditions is inhibition of platelet aggregation. In this area, the evidence indicated that the standardized *C. forskohlii* extract was superior to pure forskolin.[16] In an animal model evaluating in vivo inhibition of platelet aggregation, rats were divided into four groups: group 1 received *C. forskohlii* extract (480 mg/kg supplying 20 mg/kg of forskolin), group 2 received forskolin (20 mg/kg), group 3 received dipyridamole, and group 4 served as the controls. All treatments were given orally once daily. Adenosine diphosphate–induced platelet aggregation was measured on odd days 1 through 15. All three treatments produced significant inhibition of platelet aggregation. On day 15, the inhibitions were approximately 42% for group 1, 37% for group 2, and 52% for group 3. Hence, the extract of *C. forskohlii* produced greater inhibition than the pure forskolin.

Weight Loss Programs

Forskolin was shown to stimulate lipolysis, as well as inhibit the synthesis of fat in adipocytes.[17–20] Forskolin was also shown to counteract the age-related decreased response of fat cells to lipolytic hormones like epinephrine.[21] In one double-blind clinical investigation, oral ingestion of forskolin (250 mg of 10% forskolin extract twice a day) for a 12-week period was shown to favorably alter body composition while concurrently increasing bone mass and serum free testosterone levels in overweight and obese men.[22] However, a similar study in slightly overweight females showed little benefit over a placebo.[23] In the most recent double-blind study, there were no significant advantages with a *C. forskohlii* extract over the placebo in terms of weight loss or waist-to-hip ratio in the 12-week study, but results showed that supplementation with the extract in conjunction with a hypocaloric diet significantly improved insulin and insulin resistance and thus may produce greater effects over a longer period of time and be useful in the management of metabolic risk factors. Significant increases in high-density lipoprotein cholesterol (HDL-c) were observed in both groups after the 12-week intervention.[24]

Glaucoma

In clinical studies, forskolin was shown to greatly reduce intraocular pressure (IOP) when it was applied directly to the eyes.[24–27] This effect indicated that topical forskolin preparations might be of benefit in the treatment of glaucoma. Unlike current drug therapy, forskolin actually increased intraocular blood flow, had no side effects, and did not induce miosis. Oral administration might prove effective as well. In an open-label pilot study, 16 patients with glaucoma and stable IOP underwent treatment with different topical drugs and were given a supplement containing 15 mg of forskolin along with rutin (200 mg), thiamin (0.7 mg), and riboflavin (0.8 mg) for 40 days, which produced a further decrease in IOP by roughly 20% of the initial value.[28]

OTHER CLINICAL APPLICATIONS

C. forskohlii extracts concentrated and standardized for forskolin content may prove to be useful in a number of other clinical applications, including the following:

- Hypothyroidism
- Malabsorption and digestive disorders
- Depression
- Prevention of cancer metastases
- Immune system enhancement

Hypothyroidism

Forskolin was shown to increase thyroid hormone production, as well as to stimulate thyroid hormone release.[29]

Malabsorption and Digestive Disorders

Forskolin stimulated digestive secretions, including the release of hydrochloric acid, pepsin, amylase, and pancreatic enzymes.[30,31] Forskolin was shown to promote nutrient absorption in the small intestine.[32] *C. forskohlii* extracts might prove to be quite useful in treating dry mouth because forskolin was found to increase salivation.[33]

Depression

Forskolin was shown to exert antidepressant activity in animal studies.[34]

Cancer

The ability to increase cAMP is very relevant to cancer. Forskolin has shown a number of antitumor effects, including the induction of mesenchymal-to-epithelial transition, inhibition of cell growth and migration, and enhancement of sensitivity to conventional antitumor drugs in cancer cells. Forskolin was also shown to be a potent inhibitor of cancer metastasis in mice injected with malignant cells. As little as 82 mcg administered to mice inhibited metastasis by over 70%.[35]

Immune System Enhancement

Forskolin exhibited potent immune system enhancement (primarily through activation of macrophages and lymphocytes) in several models.[36–38]

DOSAGE

The recommended dosage should be based on the level of forskolin. Because the forskolin content of coleus root is typically only 0.2% to 0.3%, crude coleus products may not be sufficient to produce a pharmacological effect. The safety of the whole root at high dosages is not as well studied. It is best to use standardized extracts that have known forskolin content.

Daily dosages:

- Forskolin: 5 to 10 mg two to three times a day
- Standardized extract (10% forskolin): 250 mg one to two times a day
- Dried root: 2 to 5 g two to three times a day

TOXICOLOGY

The animal studies on forskolin indicated low toxicity. Inclusion of *C. forskohlii* extract in the diet at a 1% level is, however, associated with the inducement of fatty liver, presumably due to nonforskolin components stimulating the synthesis and accumulation of triglycerides within the liver.[39] Although this level of intake is unlikely in humans, it does indicate that screening for liver function is indicated with any long-term use.

The pharmacology of forskolin suggests it would be wise to restrict the use of *C. forskohlii* preparations in patients with low blood pressure and peptic ulcers.

DRUG INTERACTIONS

C. forskohlii preparations should be used cautiously in patients on prescription medications, especially antiasthmatics and antihypertensives, due to its ability to potentiate these and other drugs' effects.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ammon HPT, Muller AB. Forskolin: from Ayurvedic remedy to a modern agent. *Planta Med*. 1985;51:473–477.
2. Seamon KB, Daly JW. Forskolin: a unique diterpene activator of cyclic AMP-generating systems. *J Cyclic Nucleotide Res*. 1981:7201–7224.
3. Insel PA, Ostrom RS. Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. *Cell Mol Neurobiol*. 2003;23:305–314.
4. Laurenza A, Sutkowski EM, Seamon KB, et al. A specific stimulator of adenyl cyclase or a diterpene with multiple sites of action. *Trends Pharmacol Sci*. 1989;10:442–447.
5. Wong S, Mok W, Phaneuf S, et al. Forskolin inhibits platelet-activating factor binding to platelet receptors independently of adenylyl cyclase activation. *Eur J Pharmacol*. 1993;245:55–61.
6. Wong S, Mok W, Phaneuf S. Forskolin inhibits platelet-activating factor binding to platelet receptors independently of adenylyl cyclase activation. *Eur J Pharmacol*. 1993;245:55–61.
7. Lichey J, Friedrich T, Priesnitz M, et al. Effect of forskolin on methacholine-induced bronchoconstriction in extrinsic asthmatics. *Lancet*. 1984;2:167.
8. Bauer K, Dietersdorfer F, Sertl K, et al. Pharmacodynamic effects of inhaled dry powder formulations of fenoterol and colforsin in asthma. *Clin Pharmacol Ther*. 1993;53:76–83.
9. Marone G, Columbo M, Triggiani M, et al. Forskolin inhibits the release of histamine from human basophils and mast cells. *Agents Actions*. 1986;18:96–99.
10. Kaik G, Witte PU. Protective effect of forskolin in acetylcholine provocation in healthy probands. Comparison of 2 doses with fenoterol and placebo. *Wien Med Wochenschr*. 1986;136:637–641.
11. Dubey MP, Srimal RC, Nityand S, et al. Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*. *J Ethnopharmacology*. 1981;3:1–13.
12. Lindner E, Dohadwalla AN, Bhattacharya BK. Positive inotropic and blood pressure lowering activity of a diterpene derivative isolated from *Coleus forskohlii*. *Forskolin. Arzneimittelforschung*. 1978;28:284–289.
13. Kramer W, Thormann J, Kindler M, et al. Effects of forskolin on left ventricular function in dilated cardiomyopathy. *Arzneimittelforschung*. 1987;37:364–367.
14. Schlepper M, Thormann J, Mitrovic V. Cardiovascular effects of forskolin and phosphodiesterase-III inhibitors. *Basic Res Cardiol*. 1989;84:197–212.
15. Wysham DG, Brotherton AF, Heistad DD. Effects of forskolin on cerebral blood flow. Implications for a role of adenylate cyclase. *Stroke*. 1986;17:1299–1303.
16. Wysham DG, Brotherton AF, Heistad DD. Effects of forskolin on cerebral blood flow. Implications for a role of adenylate cyclase. *Stroke*. 1986;17:1299–1303.
17. Allen DO, Quesenberry JT. Quantitative differences in the cyclic AMP-lipolysis relationships for isoproterenol and forskolin. *J Pharmacol Exp Ther*. 1988;244:852–858.
18. Allen DO, Ahmed B, Naseer K. Relationships between cyclic AMP levels and lipolysis in fat cells after isoproterenol and forskolin stimulation. *J Pharmacol Exp Ther*. 1986;238:659–664.
19. Okuda H, Morimoto C, Tsujita T. Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells. *J Lipid Res*. 1992;33:225–231.
20. Bianco AC, Kieffer JD, Silva JE. Adenosine 3',5'-monophosphate and thyroid hormone control of uncoupling protein messenger ribonucleic acid in freshly dispersed brown adipocytes. *Endocrinology*. 1992;130:2625–2633.
21. Hoffman BB, Chang H, Reaven GM. Stimulation and inhibition of lipolysis in isolated rat adipocytes. Evidence for age-related changes in responses to forskolin and PGE1. *Horm Metab Res*. 1987;19:358–360.
22. Godard MP, Johnson BA, Richmond SR. Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. *Obes Res*. 2005;13:1335–1343.
23. Henderson S, Magu B, Rasmussen C. Effects of Coleus forskohlii supplementation on body composition and hematological profiles in mildly overweight women. *J Int Soc Sports Nutr*. 2005;2:54–62.
24. Loftus HL, Astell KJ, Mathai ML, Su XQ. Coleus forskohlii extract supplementation in conjunction with a hypocaloric diet reduces the risk factors of metabolic syndrome in overweight and obese subjects: a randomized controlled trial. *Nutrients*. 2015;7(11):9508–9522.
25. Potter DE, Burke JA, Temple JR. Forskolin suppresses sympathetic neuron function and causes ocular hypotension. *Curr Eye Res*. 1985;4:87–96.
26. Meyer BH, Stulting AA, Muller FO. The effects of forskolin eye drops on intra-ocular pressure. *S Afr Med J*. 1987;71:570–571.
27. Seto C, Eguchi S, Araie M, et al. Acute effects of topical forskolin on aqueous humor dynamics in man. *Jpn J Ophthalmol*. 1986;30:238–244.
28. Pescosolido N, Librando A. Oral administration of an association of forskolin, rutin and vitamins B1 and B2 potentiates the hypotonising effects of pharmacological treatments in POAG patients. *Clin Ter*. 2010;161:e81–e85.
29. Saunier B, Dib K, Delemer B, et al. Cyclic AMP regulation of Gs protein. Thyrotropin and forskolin increase the quantity of stimulatory guanine nucleotide-binding proteins in cultured thyroid follicles. *J Biol Chem*. 1990;265:19942–19946.
30. Roger PP, Servais P, Dumont JE. Regulation of dog thyroid epithelial cell cycle by forskolin, an adenylate cyclase activator. *Exp Cell Res*. 1987;172:282–292.
31. Haye B, Aublin JL, Champion S, et al. Chronic and acute effects of forskolin on isolated thyroid cell metabolism. *Mol Cell Endocrinol*. 1990;43: 41–50.
32. Reymann A, Braun W, Woermann C. Proabsorptive properties of forskolin. Disposition of glycine, leucine and lysine in rat jejunum. *Naunyn Schmiedebergs Arch Pharmacol*. 1986;334:110–115.
33. Larsson O, Detsch T, Fredholm BB. VIP and forskolin enhance carbachol-induced K+ efflux from rat salivary gland fragments by a Ca2(+)-sensitive mechanism. *Am J Physiol*. 1990;259:C904–C910.
34. Wachtel H, Loschmann PA. Effects of forskolin and cyclic nucleotides in animal models predictive of antidepressant activity: interactions with rolipram. *Psychopharmacol (Berl)*. 1986;90:430–435.
35. Sapio L, Gallo M, Illiano M. The Natural cAMP elevating compound forskolin in cancer therapy: is it time? *J Cell Physiol*. 2017;232(5):922–927.
36. Schorlemmer HU. Forskolin for immune stimulation. *Chem Abstr*. 1985;102:1009.
37. Krall JF, Fernandey EI, Connolly-Filtingoff M. Human aging: effect on the activation of lymphocyte cyclic AMP-dependent protein kinase by forskolin. *Proc Soc Exp Biol Med*. 1987;184:396–402.
38. Chang J, Cherney ML, Moyer JA. Effect of forskolin on prostaglandin synthesis by mouse resident peritoneal macrophages. *Eur J Pharmacol*. 1984;103:303–312.
39. Umegaki K, Yamazaki Y, Yokotani K, et al. Induction of fatty liver by Coleus forskohlii extract through enhancement of de novo triglyceride synthesis in mice. *Toxicol Rep*. 2014;1:787–794.

Commiphora mukul (Mukul Myrrh Tree)

Michael T. Murray, ND

OUTLINE

Commiphora mukul (family: Burseraceae)
Common name: mukul myrrh tree

GENERAL DESCRIPTION

Commiphora mukul, a small, thorny tree that grows 4 to 6 feet tall, is native to Saudi Arabia and India. In its natural setting, the tree remains essentially free of foliage for most of the year. Its bark is ash colored and comes off in rough flakes, exposing the underbark, which also peels off. When injured, the tree exudes a yellowish gum resin that has a balsamic odor. This oleoresin is referred to as "gum guggul" or "guggulu." This resin is used for medicinal purposes. When tapped during the winter, the average tree yields 1.5 to 2 lb of resin.[1]

CHEMICAL COMPOSITION

Guggulu contains a mixture of diverse chemical constituents that can be separated into several fractions.[1] The first step in the fractionation process involves mixing guggulu with ethyl acetate to yield soluble and insoluble fractions. The insoluble fraction, containing the carbohydrate constituents, is regarded as toxic and is the major reason why extracts of the soluble portion are preferred to crude gum guggul for medical use. The insoluble portion has no demonstrable pharmacological activity other than toxicity.[1]

In contrast, the soluble portion possesses significant cholesterol-lowering and anti-inflammatory activity. The soluble portion can be further separated into base, acid, and neutral fractions. The neutral portion possesses almost all of the cholesterol-lowering activity, whereas the acid portion possesses the anti-inflammatory components.[1]

On further purification of the neutral portion, it was determined that the ketone fraction contains the most potent cholesterol-lowering components. The ketone fraction is composed of C_{21} or C_{27} steroids, with the major components being Z- and E-guggulsterone (Fig. 70.1). These compounds are considered the major active components of gum guggul and its extracts.[1]

For medicinal purposes, a standardized extract known as guggulipid, which is standardized to contain a minimum of 50 mg of guggulsterones per gram, is regarded as the most beneficial in terms of safety and effectiveness.[1,2] In addition to guggulsterones, guggulipid contains various diterpenes, sterols, esters, and fatty alcohols. These accessory components appear to exert a synergistic effect.[1,2]

HISTORY AND FOLK USE

Guggulu is a highly valued botanical medicine in the Indian system of medicine, Ayurveda. It is included in formulas for various health conditions, including rheumatoid arthritis and lipid disorders. The classic Ayurvedic medical text, the *Sushruta Samhita,* describes in detail the usefulness of guggul in the treatment of obesity and other disorders of fat metabolism, including "coating and obstruction of channels."[1,2]

Inspired by this description, researchers began examining, in well-designed scientific studies, the clinical effectiveness of gum guggul and its extracts in disorders of lipid metabolism—specifically, its ability to lower cholesterol and triglyceride levels and promote weight loss. This research resulted in the development of a natural cholesterol-lowering substance that is safer and more effective than many cholesterol-lowering drugs, including niacin. Guggulipid was granted approval in India for marketing as a lipid-lowering drug in 1986.[1,2]

PHARMACOLOGY

Lipid Disorders

Numerous studies in humans and animals showed that gum guggul (both crude and purified alcohol extract),[3–7] its petroleum ether extract (referred to as fraction A),[8–11] and guggulipid (standardized ethyl acetate extract)[12,13] all exert effective lipid-lowering activity. All lower both elevated cholesterol and triglyceride levels. The effect on cholesterol is particularly beneficial, as guggul lowers very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol, simultaneously elevating high-density lipoprotein (HDL) cholesterol, thus offering protection against heart disease as a result of atherosclerosis.

Guggul preparations appear most indicated in type IIb (increased LDL, VLDL, and triglycerides) and type IV (increased VLDL and triglycerides) hyperlipidemias. Preliminary human clinical trials using guggulipid found that cholesterol levels showed typical reductions of 14% to 27% in total cholesterol levels in a 4- to 12-week period, whereas triglyceride levels dropped from 22% to 30%.[12–14] However, a larger

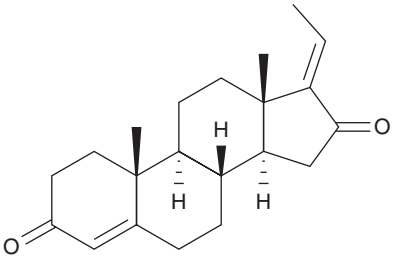


Fig. 70.1 Guggulsterone.

double-blind study did not show that either of two doses of a standardized guggul extract (guggulipid, containing 2.5% guggulsterones) lowered LDL cholesterol levels in healthy adults with hyperlipidemia.[15] Subjects received either standard-dose guggulipid (1000 mg), high-dose guggulipid (2000 mg), or matching placebo three times daily for 8 weeks. LDL cholesterol levels decreased by 5% in the placebo group. Both standard-dose guggulipid and high-dose guggulipid raised levels of LDL cholesterol by 4%, respectively. There were no significant changes in levels of total cholesterol, HDL cholesterol, triglycerides, or VLDL cholesterol in response to treatment with guggulipid in the intention-to-treat analysis. Although guggulipid was generally well tolerated, 6 participants treated with guggulipid developed a hypersensitivity rash compared with none in the placebo group. These results call into question the clinical effectiveness of guggulipid despite plausible mechanisms of action.

In vitro studies showed that the primary mechanism of action involves guggulsterones acting as an antagonist ligand for the farnesoid X receptor (FXR) and upregulating the bile salt export pump. As a result, guggulsterones promote the conversion of cholesterol to bile acids and increase the uptake of LDL cholesterol from the blood by the liver.[14,16–19] Another possible action of guggulsterones in affecting lipid levels is their ability to stimulate thyroid function.[20,21]

Despite these noted in vitro effects, more research is needed to clarify the clinical usefulness of guggul as a lipid-lowering agent. However, guggul may have other benefits against atherosclerosis beyond lowering lipid levels. Gum guggul and its extracts, including guggulipid, have been shown to prevent the formation of atherosclerosis and aid in the regression of preexisting atherosclerotic plaques in animals. This implies that they may have similar effects in humans.

Gum guggul and guggulipid were also shown to prevent heart damage by free radicals and to improve heart metabolism.[9,14] Gum guggul and its extracts have a mild effect in inhibiting platelet aggregation and promoting fibrinolysis, implying that it may also prevent the development of a stroke or embolism.[2,14]

Stock photo

Anti-Inflammatory Effects

The guggulsterone fraction of gum guggul was shown to exhibit significant anti-inflammatory action in experimental models of inflammation (e.g., raw paw edema and adjuvant arthritis method).[22–24] Its activity in models of acute inflammation is comparable to approximately one fifth that of hydrocortisone and equal to phenylbutazone and ibuprofen.[22] In models of chronic inflammation, it was shown to be more effective than hydrocortisone, phenylbutazone, and ibuprofen in reducing the severity of secondary lesions. The anti-inflammatory action is thought to be caused by inhibition of delayed hypersensitivity reactions.[23,24] Guggulsterones exert a number of anti-inflammatory effects, including inhibition of nuclear factor-κβ.[25]

The clinical application of these effects may include osteoarthritis. In an open trial of 30 patients with osteoarthritis of the knee, 500 mg of the concentrated extract three times daily produced significant improvements on the Western Ontario and McMaster University Osteoarthritis Index Total Score and subscales, visual analog scale, and 6-minute walk test. Western Ontario and McMaster University Osteoarthritis Index subscales were used as outcome measures.[26]

Anticancer Effects

Guggulsterone was shown to exert a wide range of anticancer effects, including an ability to modulate expression of proteins with antiapoptotic, cell survival, cell, angiogenic, and metastatic activities in tumor cells. As a result, evidence suggested that guggulsterone could suppress tumor initiation, promotion, and metastasis.[27] The clinical usefulness of these effects has not yet been determined.

DOSAGE

Although the crude oleoresin (gum guggul), alcohol extract, and petroleum ether extract all exert lipid-lowering and anti-inflammatory action, they are associated with side effects (e.g., skin rashes, diarrhea) at the doses required to produce a clinical effect. Interestingly, in classic Ayurvedic texts, the purification of crude guggul in Triphala kashaya is recommended to eliminate these side effects.[2]

Guggulipid, the standardized ethyl acetate extract of the gum guggul, demonstrated not only greater clinical efficacy but also much greater patient tolerance than crude or purified gum guggul. The dosage of guggulipid is based on its guggulsterone content. The typical dosage, 25 mg of guggulsterone three times per day, is an effective treatment for elevated cholesterol levels, elevated triglyceride levels, or both. For a 2.5% guggulsterone content extract, this translates to an effective dose of 1000 mg three times/day.

For comparison, the daily dosages of the other forms follow:

- Crude gum guggul—10 g
- Alcoholic extract—4.5 g
- Petroleum ether extract—1.5 g

TOXICOLOGY

The side effects of crude gum guggul and alcoholic and petroleum ether extracts were discussed previously. In clinical studies, guggulipid did not display any untoward side effects, nor did it adversely affect liver function, blood sugar control, kidney function, or hematologic parameters.[11–13]

Safety studies in rats, rabbits, and monkeys demonstrated guggulipid to be nontoxic.[14] It does not possess any embryotoxic or fetotoxic effects and is therefore considered safe to use in pregnancy. In mice, the oral and intraperitoneal mean lethal dose values were 1600 mg/kg.[1]

DRUG INTERACTIONS

Although there are no reported guggulipid–drug interactions, one in vitro study with hepatocytes showed that guggulipid could induce the expression of CYP3A enzyme activity, suggesting that guggulipid therapy should be used cautiously in patients taking prescription medications that are metabolized by CYP3A family members, including such drugs as statins, ketoconazole (Nizoral), itraconazole (Sporanox), fexofenadine (Allegra), triazolam (Halcion), and many others.[28] In addition, guggulsterones demonstrated agonist activity against the estrogen receptor α isoform and the progesterone receptor; therefore it should be used with caution with estrogen and/or progesterone preparations.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Satyavati GV. Gugulipid. a promising hypolipidaemic agent from gum guggul (Commiphora wightii). *Econ Med Plant Res*. 1991;5:47–82.
2. Satyavati GV. Gum guggul (Commiphora mukul)—the success story of an ancient insight leading to a modern discovery. *Indian J Med Res*. 1988;87:327–335.
3. Satyavati GV, Dwarakanath C, Tripathi SN. Experimental studies of the hypocholesterolemic effect of Commiphora mukul. *Indian J Med Res*. 1969;57:1950–1962.
4. Khana DS, Agarwal OP, Gupta SK, et al. A biochemical approach to anti-atherosclerotic action of Commiphora-mukul. An Indian indigenous drug in Indian domestic pigs. *Indian J Med Res*. 1969;57:900–906.
5. Nityand S, Kapoor NK. Hypocholesterolemic effect of Commiphora mukul resin (guggal). *Indian J Exp Biol*. 1971;9:376–377.
6. Kuppurajan K, Rajagopalan SS, Koteswara RT, et al. Effect of guggul (Commiphora mukul-Engl.) on serum lipids in obese, hypercholesterolemic and hyperlipidemic cases. *J Assoc Physicians India*. 1978;26:367–371.
7. Baldwa VS, Bhasin V, Ranka PC, et al. Effects of Commiphora mukul (Guggul) in experimentally induced hyperlipidemia and atherosclerosis. *J Assoc Physicians India*. 1981;29:13–17.
8. Malhotra SC, Ahuja MMS. Comparative hypolipidaemic effectiveness of gum guggulu (Commiphora mukul) fraction "A," ethyl-p-chlorophenoxy-isobutyrate and Ciba-13437-Su. *Indian J Med Res*. 1971;10:1621–1632.
9. Arora RB, Das D, Kapoor SC, et al. Effect of some fractions of Commiphora mukul on various serum lipid levels in hypercholesterolemic chicks and their effectiveness in myocardial infarction in rats. *Indian J Exp Biol*. 1973;11:166–168.
10. Malhotra SC, Ahuja MMS, Sundaram KR. Long term clinical studies on the hypolipidaemic effect of Commiphora mukul (guggulu) and clofibrate. *Indian J Med Res*. 1977;65:390–395.
11. Verna SK, Bordia A. Effect of Commiphora mukul (gum guggulu) in patients of hyperlipidemia with special reference to HDL-cholesterol. *Indian J Med Res*. 1988;87:356–360.
12. Agarwal RC, Singh SP, Saran RK, et al. Clinical trial of gugulipid—a new hypolipidemic agent of plant origin in primary hyperlipidemia. *Indian J Med Res*. 1986;84:626–634.
13. Nityanand S, Srivastava JS, Asthana OP. Clinical trials with gugulipid. A new hypolipidaemic agent. *J Assoc Physicians India*. 1989;37:323–328.
14. Anonymous. Gugulipid. *Drugs Future*. 1988;13:618–619.
15. Szapary PO, Wolfe ML, Bloedon LT, et al. Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial. *JAMA*. 2003;290: 765–772.
16. Singh V, Kaul S, Chander R, et al. Stimulation of low density lipoprotein receptor activity in liver membrane of guggulsterone treated rats. *Pharmacol Res*. 1990;22:37–44.
17. Cui J, Huang L, Zhao A, et al. Guggulsterone is a farnesoid X receptor antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump. *J Biol Chem*. 2003;278:10214–10220.
18. Urizar NL, Liverman AB, Dodds DT, et al. A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science*. 2002;296:1703–1706.
19. Deng R. Therapeutic effects of guggul and its constituent guggulsterone: cardiovascular benefits. *Cardiovasc Drug Rev*. 2007;25:375–390.
20. Tripathi YB, Tripathi P, Malhorta OP, et al. Thyroid stimulatory action of (Z)-guggulsterone: mechanism of action. *Planta Med*. 1988;54:271–277.
21. Panda S, Kar A. Guggulu (Commiphora mukul) potentially ameliorates hypothyroidism in female mice. *Phytother Res*. 2005;19:78–80.
22. Arora RB, Kapoor V, Gupta SK, et al. Isolation of a crystalline steroidal compound from Commiphora mukul and its anti-inflammatory activity. *Indian J Exp Biol*. 1971;9:403–404.
23. Arora RB, Taneja V, Sharma RC, et al. Anti-inflammatory studies on a crystalline steroid isolated from Commiphora mukul. *Indian J Med Res*. 1972;60:929–931.
24. Sharma JN, Sharma JN. Comparison of the anti-inflammatory activity of Commiphora mukul (an indigenous drug) with those of phenylbutazone and ibuprofen in experimental arthritis induced by mycobacterial adjuvant. *Arzneimittelforschung*. 1977;27:1455–1457.
25. Lv N, Song MY, Kim EK, et al. Guggulsterone, a plant sterol, inhibits NF-kappaB activation and protects pancreatic beta cells from cytokine toxicity. *Mol Cell Endocrinol*. 2008;289:49–59.
26. Singh BB, Mishra LC, Vinjamury SP, et al. The effectiveness of Commiphora mukul for osteoarthritis of the knee: an outcomes study. *Altern Ther Health Med*. 2003;9:74–79.
27. Shishodia S, Harikumar KB, Dass S, et al. The guggul for chronic diseases: ancient medicine, modern targets. *Anticancer Res*. 2008;28:3647–3664.
28. Brobst DE, Ding X, Creech KL, et al. Guggulsterone activates multiple nuclear receptors and induces CYP3A gene expression through the pregnane X receptor. *J Pharmacol Exp Ther*. 2004;310:528–535.

71

Crataegus oxyacantha (Hawthorn)

Michael T. Murray, ND

OUTLINE

Crataegus oxyacantha (family: *Rosacea*)
Synonym: *Crataegus laevigata*
Common names: hawthorn, may bush, whitethorn, haw

GENERAL DESCRIPTION

Crataegus oxyacantha is a spiny tree or shrub that is native to Europe. It may reach a height of 30 ft but is often grown as a hedge plant. Its common name, hawthorn, is a corruption of "hedgethorn" because it was used in Germany to divide plots of land. Its botanical name, *C. oxyacantha,* is from the Greek *kratos,* meaning "hardness (of the wood)"; *oxus,* meaning "sharp"; and *akantha*, meaning "a thorn." The fruit and blossoms are used medicinally.[1]

Other species of crataegus (e.g., *C. monogyna, C. pentagyna*) contain similar constituents and likely have identical pharmacological actions to *C. oxyacantha,* so they are suitable alternatives.[2,3]

CHEMICAL COMPOSITION

Hawthorn leaves, berries, and blossoms contain many biologically active flavonoid compounds, particularly anthocyanidins and proanthocyanidins (polymers of anthocyanidins, also known as biflavans or procyanidins; Fig. 71.1).[2–5] These flavonoids are responsible for the red to blue colors not only of hawthorn berries but also of blackberries, cherries, blueberries, grapes, and many flowers as well. These compounds are highly concentrated in hawthorn berry and flower extracts.

High-performance liquid chromatography and thin-layer chromatography (of crataegus extracts) demonstrated that extracts of the flowers are particularly rich in flavonoids (e.g., quercetin, quercetin-3-galactoside, vitexin, vitexin-4′-rhamnoside), procyanidins (Fig. 71.2), and oligomers of procyanidins (Fig. 71.3).[5,6]

In addition to flavonoids, crataegus extracts also contain the following[7]:

- Cardiotonic amines (e.g., phenylethylamine, o-methoxyphenylethylamine, tyramine, isobutylamine)
- Choline and acetylcholine
- Purine derivatives (e.g., adenosine, adenine, guanine, caffeic acid)
- Amygdalin
- Pectins
- Triterpene acids (ursolic, oleanolic, and crategolic acids)

HISTORY AND FOLK USE

Crataegus flowers and berries have been used primarily as cardiac tonics and mild diuretics in organic and functional heart disorders. They are also used for their astringent qualities to relieve sore throat pain.[1]

PHARMACOLOGY

The pharmacology of crataegus centers on its flavonoid components.[2–4] The proanthocyanidins in crataegus are largely responsible for its cardiovascular activities.

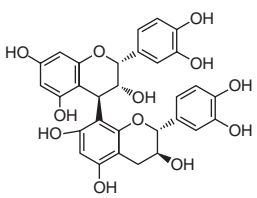


Fig. 71.1 Proanthocyanidin B2.

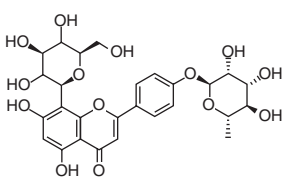


Fig. 71.2 Vitexin-4′-rhamnoside.

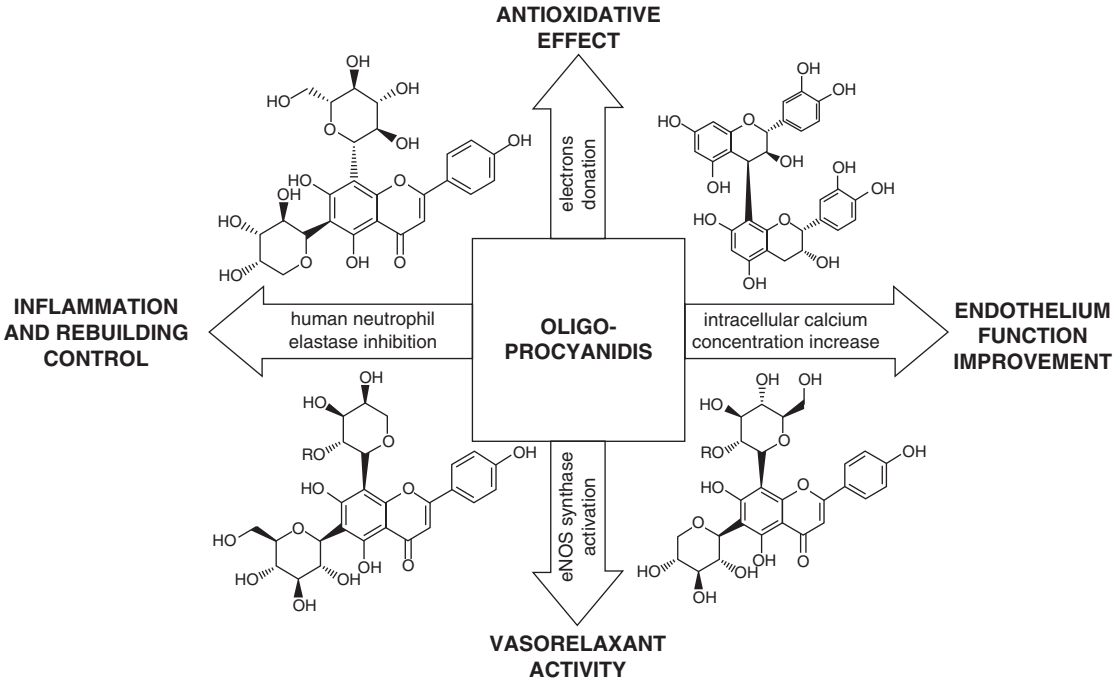


Fig. 71.3 Main mechanisms of action of oligopolycyanidins in *Crataegus*. (From Zorniak M, Szydlo B, Krzeminski TF. (2017). *Crataegus* special extract WS 1442: up-to-date review of experimental and clinical experiences. *J Physiol Pharmacol*. 2017;68[4]:521–526.)

Synergism with Vitamin C

As stated earlier, crataegus is particularly rich in anthocyanidins and proanthocyanidins. These flavonoids have strong "vitamin P" activity. Included in their effects are the abilities to increase intracellular vitamin C levels, stabilize vitamin C (by protecting it from oxidation), and decrease capillary permeability and fragility.[8,9]

Collagen-Stabilizing Action

Similar to other proanthocyanidin-rich extracts, crataegus possesses significant collagen-stabilizing action, including the following:

- Reinforcement of the natural cross-linking of collagen that forms the collagen matrix of connective tissue (e.g., ground substance, cartilage, tendon)
- Prevention of free radical damage because of potent antioxidant and free radical–scavenging action
- Inhibition of enzymatic cleavage by enzymes secreted by white blood cells during inflammation
- Prevention of the release and synthesis of compounds that promote inflammation, such as histamine, serine proteases, prostaglandins, and leukotrienes

Cardiovascular Effects

The beneficial effects of crataegus in the treatment of cardiovascular conditions appear to be a result of the following pharmacological actions:

- Improvement of the blood supply to the heart by dilating the coronary vessels[2,3,10–15]
- Improvement of the metabolic processes in the heart, which results in an increase in the force of contraction of the heart muscle and elimination of some types of rhythm disturbances[2,3,11,16–18]
- Inhibition of angiotensin-converting enzyme (ACE)[19]
- Decreased expression of microRNA-155, resulting in an increase in endothelial nitric oxide synthase (eNOS) expression and improved endothelial function.[42]
- Donation of electrons producing antioxidative free radical–scavenging effects
- Inhibition of human neutrophil elastase
- Increases intracellular calcium concentrations, protecting endothelial permeability dysfunction

The ability of crataegus to dilate coronary blood vessels has been repeatedly demonstrated in experimental studies. This effect appears to be a result of relaxation of vascular smooth muscle by a combination of direct effects on both the vascular endothelium and smooth muscle. In addition, various flavonoid components in crataegus have been shown to inhibit vasoconstriction by various substances, including hypophysin, histamine, and acetylcholine. In addition, procyanidins have been shown to inhibit ACE.[2,3,20]

Improvement in cardiac metabolism has been demonstrated in humans and animals that have received crataegus extracts.[2,3] The improvement is not only a result of increased blood and oxygen supply to the myocardium but also a result of flavonoid–enzyme interactions. Crataegus extracts and various flavonoid components have been shown to inhibit cyclic adenosine monophosphate phosphodiesterase (cAMP-PDE).[21] This results in increased levels of cAMP within the myocardium, leading to a positive inotropic effect (i.e., an increase in the force of contraction). This is particularly beneficial in cases of congestive heart failure (CHF; discussed in the section "Congestive Heart Failure").

The procyanidins of crataegus demonstrated a specific inhibition of ACE similar to the synthetic ACE inhibitors widely used in the treatment of hypertension.[20] The proanthocyanidins that appear to have the highest activity are proanthocyanidins B-5 3,3′-di-O-gallate and C-1 3,3′,3″-tri-O-gallate. It is not surprising that these proanthocyanidins are found in relatively high concentrations in hawthorn berries, flowers, and their extracts.[5,6]

Mitochondrial Effects

Many cardiovascular diseases (e.g., arrhythmias, heart failure, myocardial weakness) are associated with structural and functional

disturbances of the mitochondria. Because mitochondria produce 95% of the energy necessary for heart function, therapeutic agents that could influence mitochondrial dysfunction are of special importance. Considering the cardioprotective and cardiotonic effects of hawthorn, studies have been done to investigate the effect of crataegus fruit extracts (CE) on mitochondrial function. One animal study performed an oxygraphic investigation of the effect of CE (in concentration range from 70 ng/mL to 13.9 μg/mL of crataegus phenolic compounds [PC]) on isolated rat heart mitochondria.[22] At concentrations from 278 ng/mL to 13.9 μg/mL of PC, CE stimulated stage 2 respiration by 11% to 34% and decreased mitochondrial membrane potential by 1.2 to 4.4 mV, as well as H_2O_2 production. The highest CE concentration also slightly reduced the maximal adenosine diphosphate (ADP)-stimulated and uncoupled respiration, which may be because of inhibition of the mitochondrial respiratory chain between flavoprotein and cytochrome c. An additional animal study investigated the effect of CE on mitochondrial function during experimentally induced myocardial infarction in rats.[23] For 30 days, CE was administered orally at a dosage of 0.5 mL/100 g body weight. At the end of the 30 days, the animals were administered isoproterenol for 2 days at an interval of 24 hours. Pretreatment with CE maintained mitochondrial antioxidant status and prevented mitochondrial lipid peroxidative damage and a decrease in Krebs's cycle enzymes induced by isoproterenol. Although further studies are necessary, the promotion of mitochondrial function may be a significant mechanism of efficacy.

CLINICAL APPLICATIONS

The clinical use of crataegus revolves around its cardiovascular effects. Its use in atherosclerosis, hypertension, and CHF is discussed in the following subsections.

Atherosclerosis

Crataegus preparations, although in a supplement form, should be thought of as a necessary food in the prevention and treatment of atherosclerosis. Increasing the intake of flavonoid compounds by taking crataegus extracts has numerous health-promoting effects, including reducing cholesterol levels and decreasing the size of existing atherosclerotic plaques.[24] This effect is probably a result of collagen stabilization.

A decrease in the integrity of the collagen matrix of the artery results in cholesterol deposition. Many researchers believe that if the collagen matrix of the artery remains strong, atherosclerotic plaque will never develop. Crataegus flavonoids, by increasing the integrity of collagen structures, may offer significant protection against atherosclerosis. In addition, feeding proanthocyanidin extracts to animals resulted in the reversal of atherosclerotic lesions and decreased serum cholesterol levels.

Flavonoids contained in hawthorn extracts appear to offer significant prevention, and potential reversing effects, in the treatment of atherosclerotic processes, which are still the major causes of death in the United States.

Hypertension

Crataegus exerts a mild antihypertensive effect, which has been demonstrated in both experimental and clinical studies.[2] Its action in lowering blood pressure is unique in that it produces a number of diverse pharmacological effects. Specifically, it dilates the coronary vessels, inhibits ACE, acts as an inotropic agent, and possesses mild diuretic activity. Its clinical effects in lowering blood pressure, however, are generally mild.[19]

In one double-blind study evaluating crataegus hypotensive effects, 79 patients with type 2 diabetes were randomized to either crataegus extract (1200 mg/day) or placebo for 16 weeks. Hypotensive drugs were used by 71% of the study population, with a mean intake of 4.4 hypoglycemic and/or hypotensive drugs. Although there was no effect on systolic blood pressure, the diastolic blood pressure in the crataegus group dropped from a baseline of 85.6 to 83.0 mm Hg compared with the placebo group (baseline: 84.5 mm Hg; outcome: 85.0 mm Hg). No herb–drug interaction was found.[25]

The effects of crataegus generally require prolonged administration, and in many instances, it may take up to 2 weeks before adequate tissue concentrations are achieved. It should be kept in mind that because beta blockers lower blood pressure by reducing cardiac output, crataegus administration to patients on these drugs may produce a mild hypertensive response.

Congestive Heart Failure

Crataegus has a long history of use in the treatment of CHF, particularly in combination with digitalis or other herbs containing cardiac glycosides such as *Cereus grandiflorus,* also known as *Cactus grandiflorus,* and *Convallaria majalis.* It potentiates the action of the cardiac glycosides, presumably via its ability to inhibit cAMP-PDE and to interact with calcium channels.

Because of this enhancing effect, lower doses of cardiac glycosides can be used. In addition, magnesium has also been shown to augment digitalis action. For mild to moderate cases of CHF, crataegus extract used alone may be sufficient, but for moderate to severe CHF, it should be used in combination with other cardiac glycosides.

The effectiveness of crataegus in early or mild stages of CHF was repeatedly demonstrated in double-blind studies.[2,3,26–33] In a meta-analysis of 10 trials including 855 patients with chronic heart failure (New York Heart Association [NYHA] classes I to III), it was concluded that hawthorn extract provided significant benefit in symptom control and physiological outcomes as an adjunctive treatment for chronic heart failure. Exercise tolerance, the pressure–heart rate product, and symptoms (e.g., shortness of breath and fatigue) all improved significantly with hawthorn treatment compared with placebo.[34]

In one double-blind study, 30 patients with CHF (NYHA stage II) were assessed in a randomized, double-blind study.[29] Treatment consisted of a crataegus extract standardized to contain 15 mg procyanidin oligomers per 80-mg capsule. Treatment duration was 8 weeks, and the substance was administered at a dose of one capsule taken twice a day. The group receiving the crataegus extract showed a statistically significant advantage over placebo in terms of changes in heart function as determined by standard testing procedures. Systolic and diastolic blood pressures were also mildly reduced. Like all other studies with crataegus extracts, no adverse reactions occurred.

In another study, 78 patients with CHF (NYHA stage II) were given either 600 mg of extract standardized to contain 18.8% procyanidolic oligomers or placebo daily.[30] The parameter used to measure effectiveness was the patient's working capacity (W) on a bicycle ergometer. After 56 days of treatment, the crataegus group had a mean increase of 25 W compared with the placebo group's increase of only 5 W. In addition, the crataegus group also experienced a mild, but significant, reduction in systolic blood pressure (from 171–164 mm Hg) and heart rate (from 115–110 beats/min). There was no change in blood pressure or heart rate in the placebo group.

Crataegus provides a definite dose response. Individuals with more severe CHF require higher dosages to experience the most benefit. This comment is based on an important finding in a double-blind

study of 209 patients with NYHA class III CHF. Although those receiving 900 mg of a crataegus extract standardized to contain 18.8% procyanidolic oligomers demonstrated improvement in all parameters tested, those receiving 1800 mg showed even better results.[32] The failure of some studies to demonstrate a therapeutic benefit with crataegus extracts may reflect inadequate dosages being administered.[35–37]

DOSAGE

Clearly, the dosage depends on the type of preparation and source material. The amount of crataegus extract used in various clinical studies on CHF was equivalent to 30 to 169 mg of epicatechin or 3.5 to 19.8 mg of flavonoids, usually administered in two or three doses. Standardized extracts are preferred because the flavonoid content in crude preparations can vary tremendously. The dosages listed for the various crataegus formulas are for use three times a day:

- Berries or flowers (dried): 3 to 5 g or as infusion
- Tincture (1:5): 4 to 5 mL (alcohol may elicit pressor response in some individuals)
- Fluid extract (1:1): 1 to 2 mL
- Freeze-dried berries: 1 to 1.5 g
- Flower extract (standardized to contain 1.8% vitexin-4′-rhamnoside or 18% procyanidolic oligomers): 200 to 600 mg

TOXICOLOGY

Crataegus was shown to have low toxicity, with a mean lethal dose of 25 mg/kg.[38] In a human study in patients with CHF, dosages at levels 100 times the typical dosage did not produce any evidence of toxicity. Although some studies showed that proanthocyanidins might be carcinogenic, more careful evaluation indicated that the carcinogenicity was probably a result of nitrosamines found in the extracts used.[39] Purified proanthocyanidins were found to be nonmutagenic, according to the *Salmonella* mutagenicity assay system.

In total, 166 adverse events were reported in a review of 24 clinical studies and data from 5577 patients who received the daily dose (range, 160–1800 mg) and had 3 to 24 weeks of treatment with hawthorn monopreparations. Most of these adverse events were, in general, mild to moderate, with the most frequent adverse events being dizziness/vertigo, gastrointestinal complaints, headache, and heart palpitation.[40] Postmarketing surveillance studies reported only mild and infrequent side effects with crataegus extract. In a study of 1011 patients, 14 adverse events (1.4%) occurred after the administration of 900 mg of hawthorn extract for 24 weeks. In two patients, a causal relation with crataegus was suspected, but it was regarded by the treating doctors as unlikely. In another postmarketing surveillance study of 3664 patients who were treated with 900 mg of crataegus extract for 8 weeks, 48 patients (1.3%) reported adverse events, including hot flushes, stomach complaints, palpitations, dizziness, dyspnea, headache, and epistaxis. In 19 patients, this resulted in discontinuation of the treatment.[26]

DRUG INTERACTIONS

Hawthorn may potentiate or inhibit the actions of anticoagulants, antihypertensives, and cardiac glycosides. This concern is based primarily on theoretical grounds rather than clinical reports. Importantly, in a randomized crossover trial with eight healthy volunteers, hawthorn did not significantly alter the pharmacokinetic parameters for digoxin. This suggests that the combination of hawthorn and digoxin may be administered safely.[41]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Grieve M. *A Modern Herbal*. Vol. 1. New York: Dover Publications; 1971.
2. Chang Q, Zuo Z, Harrison F, et al. Hawthorn. *J Clin Pharmacol*. 2002;42:605–612.
3. Rigelsky JM, Sweet BV. Hawthorn: pharmacology and therapeutic uses. *Am J Health Syst Pharm*. 2002;59:417–422.
4. Zorniak M, Szydlo B, Krzeminski TF. Crataegus special extract WS 1442: up-to-date review of experimental and clinical experiences. *J Physiol Pharmacol*. 2017;68:521–526.
5. Svedstrom U, Vuorela H, Kostiainen R, et al. Isolation and identification of oligomeric procyanidins from *Crataegus* leaves and flowers. *Phytochemistry*. 2002;60:821–825.
6. Ficarra P, Ficarra R, Tommasini A, et al. High-performance liquid chromatography of flavonoids in *Crataegus oxyacantha* L. I. Reversed-phase high-pressure liquid chromatography. *Farmaco [Prat]*. 1983;39:148–157.
7. Wagner H, Grevel J. Cardiotonic drugs IV. Cardiotonic amines from *Crataegus oxyacantha*. *Planta Medica*. 1982;45:98–101. [German].
8. Quettier-Deleu C, Voiselle G, Fruchart JC, et al. Hawthorn extracts inhibit LDL oxidation. *Pharmazie*. 2003;58:577–581.
9. Bahorun T, Aumjaud E, Ramphul H, et al. Phenolic constituents and antioxidant capacities of *Crataegus monogyna* (Hawthorn) callus extracts. *Nahrung*. 2003;47:191–198.
10. Nijveldt RJ, van Nood E, van Hoorn DE, et al. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr*. 2001;74:418–425.
11. Schussler M, Holzl J, Fricke U. Myocardial effects of flavonoids from *Crataegus* species. *Arzneimittelforschung*. 1995;45:842–845.
12. Kim SH, Kang KW, Kim KW, et al. Procyanidins in *Crataegus* extract evoke endothelium-dependent vasorelaxation in rat aorta. *Life Sci*. 2000;67:121–131.
13. Mavers VWH, Hensel H. Changes in local myocardial blood flow following oral administration of a *Crataegus* extract in non-narcotized dogs. *Arzniemittelforschung*. 1974;24:783–785. [German].
14. Roddewig C, Hensel H. Reaction of local myocardial blood flow in non-anesthetized dogs and anesthetized cats to oral and parenteral application of a *Crataegus* fraction (oligomere procyanidins). *Arzneimittelforschung*. 1977;27:1407–1410.
15. Vierling W, Brand N, Gaedcke F, et al. Investigation of the pharmaceutical and pharmacological equivalence of different Hawthorn extracts. *Phytomedicine*. 2003;10:8–16.
16. Schwinger RH, Pietsch M, Frank K, et al. *Crataegus* special extract WS 1442 increases force of contraction in human myocardium cAMP-independently. *J Cardiovasc Pharmacol*. 2000;35:700–707.
17. Muller A, Linke W, Klaus W. *Crataegus* extract blocks potassium currents in guinea pig ventricular cardiac myocytes. *Planta Med*. 1999;65:335–339.
18. Schussler M, Holzl J, Fricke U. Myocardial effects of flavonoids from *Crataegus* species. *Arzneimittelforschung*. 1995;45:842–845.
19. Walker AF, Marakis G, Morris AP, et al. Promising hypotensive effect of hawthorn extract: a randomized double-blind pilot study of mild, essential hypertension. *Phytother Res*. 2002;16:48–54.
20. Lacaille-Dubois, Franck U, Wagner H. Search for potential angiotensin converting enzyme (ACE)-inhibitors from plants. *Phytomedicine*. 2001;8:47–52.
21. Petkov E, Nikolov N, Uzunov P. Inhibitory effect of some flavonoids and flavonoid mixtures on cyclic AMP phosphodiesterase activity of rat heart. *Planta Medica*. 1981;43:183–186.
22. Bernatoniene J, Trumbeckaite S, Majiene D, et al. The effect of crataegus fruit extract and some of its flavonoids on mitochondrial oxidative phosphorylation in the heart. *Phytotherapy Res*. 2009;23(12):1701–1707. PubMed PMID: 19441016.
23. Jayalakshmi R, Thirupurasundari CJ, Devaraj SN. Pretreatment with alcoholic extract of *Crataegus oxycantha* (AEC) activates mitochondrial protection during isoproterenol-induced myocardial infarction in rats. *Mol Cell Biochem*. 2006;292(1–2):59–67. PubMed PMID: 16733799.
24. Wegrowski J, Robert AM, Moczar M. The effect of procyanidolic oligomers on the composition of normal and hypercholesterolemic rabbit aortas. *Biochem Pharm*. 1984;33:3491–3497.
25. Walker AF, Marakis G, Simpson E, et al. Hypotensive effects of hawthorn for patients with diabetes taking prescription drugs: a randomised controlled trial. *Br J Gen Pract*. 2006;56:437–443.
26. Pittler MH, Schmidt K, Ernst E. Hawthorn extract for treating chronic heart failure: meta-analysis of randomized trials. *Am J Med*. 2003;114:665–674.
27. O'Conolly VM, Jansen W, Bernhoft G, et al. Treatment of decreasing cardiac performance. Therapy using standardized crataegus extract in advanced age. *Fortschr Med*. 1986;104:805–808. [German].
28. O'Conolly VM, Jansen W, Bernhoft G, et al. Treatment of decreasing cardiac performance. Therapy using standardized crataegus extract in advanced age. *Fortschr Med*. 1986;104:805–808. [German].
29. Leuchtgens H. *Crataegus* Special Extract WS 1442 in NYHA II heart failure. A placebo controlled randomized double-blind study. *Fortschr Med*. 1993;111:352–354. [German].
30. Schmidt U, Kuhn U, Ploch M, et al. Efficacy of the hawthorn *(Crataegus)* preparation LI 132 in 78 patients with chronic congestive heart failure defined as NYHA functional class II. *Phytomedicine*. 1994;1:17–24.
31. Zapfe jun G. Clinical efficacy of crataegus extract WS 1442 in congestive heart failure NYHA class II. *Phytomedicine*. 2001;8:262–266.
32. Tauchert M. Efficacy and safety of crataegus extract WS 1442 in comparison with placebo in patients with chronic stable New York Heart Association class-III heart failure. *Am Heart J*. 2002;143:910–915.
33. Degenring FH, Suter A, Weber M, et al. A randomised double blind placebo controlled clinical trial of a standardised extract of fresh *Crataegus* berries (Crataegisan) in the treatment of patients with congestive heart failure NYHA II. *Phytomedicine*. 2003;10:363–369.
34. Pittler MH, Guo R, Ernst E. Hawthorn extract for treating chronic heart failure. *Cochrane Database Syst Rev*. 2008;1:CD005312.
35. Zick SM, Vautaw BM, Gillespie B, et al. Hawthorn extract randomized blinded chronic heart failure (HERB CHF) trial. *Eur J Heart Fail*. 2009;11:990–999.
36. Holubarsch CJ, Colucci WS, Meinertz T, et al. The efficacy and safety of Crataegus extract WS 1442 in patients with heart failure: the SPICE trial. *Eur J Heart Fail*. 2008;10:1255–1263.
37. Zick SM, Gillespie B, Aaronson KD. The effect of *Crataegus oxycantha* Special Extract WS 1442 on clinical progression in patients with mild to moderate symptoms of heart failure. *Eur J Heart Fail*. 2008;10:587–593.
38. Schlegelmilch R, Heywood R. Toxicity of *Crataegus* (hawthorn) extract (WS 1442). *J Am Coll Toxicol*. 1994;13:103–111.
39. Yu CI, Swaminathan B. Mutagenicity of proanthocyanidins. *Food Chem Toxicol*. 1987;25:135–139.
40. Daniele C, Mazzanti G, Pittler MH, et al. Adverse-event profile of *Crataegus* spp.: a systematic review. *Drug Saf*. 2006;29:523–535.
41. Tankanow R, Tamer HR, Streetman DS, et al. Interaction study between digoxin and a preparation of hawthorn *(Crataegus oxyacantha)*. *J Clin Pharmacol*. 2003;43:637–642.
42. Wang X, Liang Y, Shi J, Zhu HJ, Bleske BE. *Crataegus* special extract WS 1442 effects on eNOS and microRNA 155. *Planta Med*. 2018. https://doi.org/10.1055/a-0601-7083.

72

Croton lechleri (Dragon's Blood)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Croton spp. (family: Euphorbiaceae)

Synonym: Many Croton species are identified as dragon's blood, including Croton lechleri, C. sordidus, C. urucurana, C. draco (C. draconoides, C. palanostigma), C. xalapensis, and C. erythrochilus.[1-4]

Common names: dragon's blood, sangre de drago, sangre de grado, Lora sangre (sp.), sangre de perro (sp.)

GENERAL DESCRIPTION

Dragon's blood comes from the species of Euphorbiaceae trees native to Bolivia, Brazil, Colombia, Ecuador, and Peru that produce a latex ranging in color from faint to deep red, and occasionally a slight orange.[1]

CHEMICAL COMPOSITION

The latex is used medicinally.[5] It consists primarily of proanthocyanidins (90%), including crofelemer, but also contains an important alkaloid, taspine, simple phenols, diterpenes, and phytosterols (Fig. 72.1 and Fig. 72.2).[5-8]

HISTORY AND FOLK USE

Dragon's blood is used widely by the indigenous peoples of the Amazon basin, and the Spaniards reported that it was used widely in the Amazon region in the 1600s.[5,7] The latex is applied topically to abrasions, cuts, scratches, blisters, bites, and stings. It is taken internally in dilute form for gastrointestinal distress (e.g., gastritis, gastric ulcer, intestinal infections and inflammation, diarrhea). It is used as a gargle for sore throat; a vaginal antiseptic; a hemostatic after childbirth; a topical hemostatic for severe cuts and lacerations; and an analgesic applied directly to molar toothaches.[2] It is also taken internally for wound healing. The latex is used for various respiratory infections (tonsillitis, pharyngitis, lung infections/pneumonia, influenza, and colds).

PHARMACOLOGY

Analgesic Effect

Dragon's blood balm (1% concentration)[9] had a significant analgesic effect compared with a placebo balm in a series of animal experiments.[3] Dragon's blood completely prevented hyperalgesia induced by a protease and blocked hyperalgesia induced by prostaglandin. Dragon's blood was analgesic even if the hyperalgesic stimuli were applied intradermally, suggesting that its active components can penetrate the skin. Dragon's blood inhibited the responses of mesenteric arteries to calcitonin generated peptide (a primary neurotransmitter of sensory afferent nerves) and attenuated the epithelial secretory response to capsaicin in guinea pigs, but not that of neurokinin-1 antagonist,

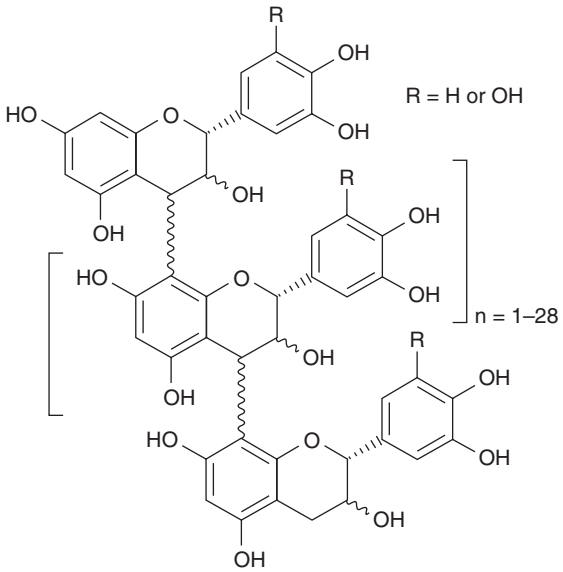


Fig. 72.1 Crofelemer.

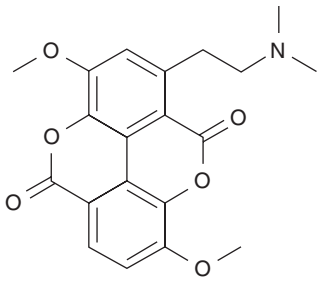


Fig. 72.2 Taspine.

suggesting a unique prejunctional and postjunctional effect on sensory afferent nerves. These pharmacological results tend to confirm the traditional uses of dragon's blood to relieve itching, pain, edema, redness, and discomfort.

Antidiarrheal Effect

Crofelemer is an extract of the proanthocyanidins and tannins from dragon's blood latex approved as a drug by the Food and Drug Administration (FDA) in 2012 (tradename Mytesi previously Fulyzac, originally studied as an investigational drug Provir or Virend or SP-303 hereinafter referred to as crofelemer). It is a first-in-class agent useful for different types of noninfectious secretory diarrhea as it prevents chloride and fluid secretion into the bowel by directly inhibiting two distinct chloride channels.

Crofelemer restored intestinal fluid accumulation to near-normal levels in mice administered cholera toxin.[10]

Antimicrobial Effect

Several phenolic compounds and diterpenes isolated from dragon's blood demonstrated potent activity against *Bacillus subtilis* and *Escherichia coli* in vitro.[11] Dragon's blood latex significantly inhibited growth of gram-positive and gram-negative organisms in vitro, including *Proteus vulgaris, E. coli,* and *Staphylococcus aureus.*[12]

Antiproliferative or Cytotoxic Effect, or Both

Dragon's blood latex and taspine were as effective as paclitaxel and vinblastine on human melanoma cells in vitro.[13] Taspine was cytotoxic against cervical cancer and hamster lung cells in vitro.[14] Dragon's blood induced apoptosis in human gastrointestinal cells in a dose-dependent manner, and cell proliferation decreased in all cells exposed to dragon's blood for 48 hours.[15] Overall, the effects were deemed similar to those observed with paclitaxel. Dragon's blood significantly reduced cancer cell adhesion, with more than 85% of cells remaining in suspension.

Taspine is also a topoisomerase inhibitor, but unlike other inhibitors such as doxorubicin and etoposide, taspine was cytotoxic to cells overexpressing drug efflux transporters and induced widespread apoptosis in spheroids in vitro and, to some extent, in vivo.[16] However, Dragon's blood showed no cytotoxicity and actually increased survival in 89% of acute leukemia cells.[17]

Ulcer Healing Effect

Dragon's blood administered at dilutions of 1:1000 and 1:10,000 in drinking water assisted the healing of ulcers in rats with acetic acid–induced gastric ulcers.[7] These doses were equivalent to doses used in traditional medicine for the treatment of gastric disorders. It healed ulcers comparably to a combination of penicillin and streptomycin and resulted in ulcers that were smaller, less inflamed, and with lower bacterial content. Whole dragon's blood latex was effective at lower doses than was crofelemer. The latex contains trace amounts of compounds that are 30 times more potent than penicillin.

Antiviral Effect

Various compounds in dragon's blood showed antiviral activity against influenza, parainfluenza, herpes simplex viruses (HSV) types I and II, and hepatitis A and B in vitro.[18] Crofelemer showed antiviral activity against respiratory syncytial virus (RSV) and inhibited viral penetration of the host cell.[19] Crofelemer also showed antiviral activity against two strains of HSV type 1, as well as a strain of HSV type 2 at a dose comparable to that of acyclovir.[20] The compound significantly reduced HSV lesion formation in the mouse vaginal model (applied topically in a 5%–10% preparation).

At aerosol doses of 0.5 to 9.4 mg/kg per day, crofelemer increased the survival of mice lethally infected with RSV.[21] At aerosol doses of 1.3 to 9.8 mg/kg, it reduced lung titers of RSV in infected rats. Crofelemer administered intraperitoneally also significantly reduced pulmonary RSV titers in infected rats with a minimum efficacious dose of 3 mg/kg.[22]

Crofelemer administered once daily for 8 days beginning before or after influenza A infection in mice reduced lung consolidation, but was lethally toxic at the dose of 30 mg/kg per day. The drug administered as an aerosol (2.5, 5, and 10 mg/mL) for 1 hour twice daily also reduced lung consolidation in animals. However, when administered before virus exposure, crofelemer treatment lengthened mean day to death, but did not inhibit lung virus titer.[23]

Wound-Healing Effect

In rats, crude dragon's blood rapidly stimulated wound contraction, formation of both a crust and new collagen along with quick and complete regeneration of the epithelial layer. The isolated polyphenol fraction stimulated contraction of the wound and formation of the crust but delayed wound repair and had no influence on the growth of new blood vessels.[24]

Dragon's blood precipitates proteins and other matrix elements, fostering accelerated wound healing with reduced pain, inflammation, and scarring.[3] Taspine showed a dose-related cicatrizant effect when administered in vivo to mice.[25]

CLINICAL APPLICATIONS

Analgesic

Ten pest control workers exposed to frequent insect bites applied either dragon's blood balm or placebo balm to bites and recorded the results over a 3-month period.[3] When workers had multiple simultaneous bites, they used both balms on different bites and compared the results. Dragon's blood latex offered rapid symptomatic relief in all cases and all applications. It was deemed far more effective than placebo by participants.

Diarrhea

Crofelemer is approved by the FDA for symptomatic relief of noninfectious diarrhea in adult patients with HIV/AIDS or antiretroviral therapy (ART). Approval was based on a multicenter trial that randomized 376 patients with HIV/AIDS on ART to take either oral crofelemer 125 mg twice daily or placebo.[26] Crofelemer significantly reduced secretory diarrhea compared with placebo.

No significant benefit in stool consistency was seen in a double-blind, randomized, placebo-controlled clinical trial in patients with diarrhea predominant irritable bowel syndrome (IBS; n = 242). The lack of benefit seen may possibly be a result of the diarrhea in IBS not being caused by a derangement of chloride secretion.[27]

Crofelemer 125 and 250 mg doses four times a day was effective in improving symptoms of acute-onset traveler's diarrhea in a multicenter, double-blind, randomized, placebo-controlled study of 184 adults. However, a 500-mg dose was no more effective than placebo, suggesting additional studies are needed.[28]

Clinical research on the benefit of crofelemer specifically in infectious diarrhea are largely lacking. A review of crofelemer in secretory diarrhea noted two foreign studies currently only available in abstract form. In one, a single 125-mg dose of crofelemer administered after rehydration and oral azithromycin significantly reduced watery stool output in the first 24 hours in patients with cholera compared with placebo in a preliminary trial of 100 adults.[29] In a second, 98 patients with acute diarrhea caused by various pathogens were treated with crofelemer (250 mg four times daily) or placebo for 2 days. Those taking crofelemer had better overall clinical success resolving watery stools in 48 hours but statistical details were lacking. (FN: *Ibid.*) Further clinical studies on the benefit of crofelemer in infectious diarrhea are definitely needed.

Herpes Simplex Viral Lesions

Virend (a topical formulation of crofelemer) showed a trend toward decreased lesion pain in a double-blind, placebo-controlled study of 45 HIV-positive patients with active-phase, culture-positive genital or perineal HSV lesions, or both. Virend was more active in patients with smaller lesion areas. Two patients experienced pain and burning after applying Virend, and one withdrew from the study for this reason. In another open-label study, 9 AIDS patients with HSV unresponsive to acyclovir were treated with crofelemer.[30] The drug showed a transient positive effect but failed to completely heal or stop virus shedding. Several patients complained that crofelemer caused pain or burning on application.

Stretch Marks

In an open trial of 10 women with stretch marks at hip level and 10 without, a cream combining *Punica granatum* (pomegranate) seed oil and dragon's blood latex extract was applied twice daily for 6 weeks.[31] The cream improved skin thickness, hydration, and elasticity, suggesting it might be helpful in preventing or improving stretch marks.

Wound Healing

Dragon's blood powder in a cream base applied twice daily significantly improved wound healing duration from the third day in a double-blind, placebo-controlled, randomized clinical trial of 60 patients undergoing skin tag repair (a total of 100 wounds).[32] The polyphenolic compounds create a protective layer on the wound surface, preventing microbial contamination and appear to shorten the inflammation process in wound healing.

DOSAGE

In traditional medicine, the internal dose is 3 to 20 drops mixed in a beverage, one to three times daily for up to 3 weeks.[2,7] The pure latex is applied topically as needed.

Although the usual study dose of crofelemer for diarrhea is 125 to 250 mg four times daily, the manufacturer Napa Pharmaceuticals recommends a 125-mg delayed release tablet twice daily (https://www.mytesi.com).

Doses of 125 and 250 mg of crofelemer, administered every 6 hours, were more effective at reducing the duration of traveler's diarrhea than the 500 mg dose.[33]

TOXICOLOGY

Dragon's blood, used topically or at low internal doses, is considered nontoxic.[34] Neither dragon's blood nor taspine showed carcinogenic or tumor-promoting effects in a 17-month long mouse study.[35] Taspine (<150 ng) was nontoxic to human foreskin fibroblasts.[21] It had no cell proliferative, carcinogenic, or tumor-promoting effects. 3′,4-0-Dimethycedrusin, isolated from dragon's blood, was nonproliferative and instead inhibited thymidine incorporation while protecting cells against degradation in a starvation medium.[15] In vitro, dragon's blood had a negligible effect on proliferation of endothelial cells and was not cytotoxic.[36] Crofelemer at doses of 16 to 18.7 mg/kg per day caused weight loss and decreased survival time in mice and rats.[21] Crofelemer injected intraperitoneally caused significant weight loss and death in rats at doses of 30 mg/kg per day. However, no toxicity was observed after oral administration of up to 270 mg/day of crofelemer.[22]

DRUG INTERACTIONS

Very little information is available about drug interactions with dragon's blood, but none has been reported in the existing clinical studies. However, crofelemer significantly worsened dacomitinib-induced diarrhea without altering blood levels of dacomitinib in rats.[37] Whether this would be a problem in humans should be investigated in a clinical trial.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Desmarchelier C, Schaus FW. *Sixty Medicinal Plants From the Peruvian Amazon, Ecology, Ethnomedicine and Bioactivity*. Lima, Peru: Grafica Bellido.
2. Borges JR, King SR, Hughes K, et al. *Conservation of Biocultural Diversity and Benefit Sharing Mechanisms in the Amazon: Croton Lechleri, a Traditional Indigenous Resource*. Brazil: Manaus: Presented at the INPI/EC meeting "Role of intellectual property protection in the field of biodiversity and traditional knowledge; 2001:9–11.
3. Miller MJ, Vergnolle N, McKnight W, et al. Inhibition of neurogenic inflammation by the Amazonian herbal medicine sangre de grado. *J Invest Dermatol*. 2001;117:725–730.
4. Pieters L, de Bruyne T, Claeys M, et al. Isolation of a dihydrobenzofuran lignan from South American dragon's blood (Croton spp.) as an inhibitor of cell proliferation. *J Nat Prod*. 1993;56:899–906.
5. Milanowski DJ, Winter RE, Elvin-Lewis MP, et al. Geographic distribution of three alkaloid chemotypes of Croton lechleri. *J Nat Prod*. 2002;65:814–819.
6. Cai Y, Evans FJ, Roberts MF, et al. Polyphenolic compounds from Croton lechleri. *Phytochem*. 1991;30:2033–2040.
7. Miller MJ, MacNaughton WK, Zhang XJ, et al. Treatment of gastric ulcers and diarrhea with the Amazonian herbal medicine sangre de grado. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G192–G200.
8. Lopes Pereira Peres MT, Delle Monache F, Pizzolatti MG, et al. Analgesic compounds of Croton urucurana Baillon. Pharmaco-chemical criteria used in their isolation. *Phytother Res*. 1998;12:209–211.
9. One percent Zangrado Bute Bite Balm, Rainforest Phytoceuticals, LLC, Delmar, NY.
10. Orozco-Topete R, Sierra-Madero J, et al. Safety and efficacy of Virend for topical treatment of genital and anal herpes simplex lesions in patients with AIDS. *Antiviral Res*. 1997;35:91–103.
11. Chen ZP, Cai Y, Phillipson JD. Studies on the anti-tumour, anti-bacterial, and wound-healing properties of dragon's blood. *Planta Med*. 1994;60:541–545.
12. Carpenter MR, Goodman-Snitkoff GW. Antibacterial effects of sangre de grado on gram-positive and gram-negative bacteria. *Abstr Gen Meet Am Soc Microbiol*. 2001;101:38.
13. Montopoli M, Bertin R, Chen Z, et al. Croton lechleri sap and isolated alkaloid taspine exhibit inhibition against human melanoma SK23 and colon cancer HT29 cell lines. *J Ethnopharmacol*. 2012;144:747–753.
14. Itokawa H, Ichihara Y, Mochizuki M, et al. A cytotoxic substance from sangre de grado. *Chem Pharm Bull (Tokyo)*. 1991;39:1041–1042.
15. Sandoval M, Okuhama NN, Clark M, et al. Sangre de grado Croton palanostigma induces apoptosis in human gastrointestinal cancer cells. *J Ethnopharmacol*. 2002;80:121–129.
16. Fayad W, Fryknas M, Brnjic S, et al. Identification of a novel topoisomerase inhibitor effective in cells overexpressing drug efflux transporters. *PLoS One*. 2009;4:e7238.
17. Stlkyczynski J, Wysocki M. Alternative medicine remedies might stimulate viability of leukemic cells. *Pediatr Blood Cancer*. 2006;46:94–98.
18. Williams JE. Review of antiviral and immunomodulating properties of plants of the Peruvian rainforest with a particular emphasis on una de gato and sangre de grado. *Alt Med Rev*. 2001;6:567–579.
19. Barnard DL, Huffman JH, Meyerson LR, et al. Mode of inhibition of respiratory syncytial virus by a plant flavonoid, SP-303. *Chemotherapy*. 1993;33:212–217.
20. Barnard DL, Smee D, Huffman JH, et al. Antiherpesvirus activity and mode of action of SP-303, a novel plant flavonoid. *Chemotherapy*. 1993;39:203–211.
21. Gilbert BE, Wyde PR, Wilson SZ, et al. SP-303 small-particle aerosol treatment of influenza A virus infection in mice and respiratory syncytial virus in cotton rats. *Antiviral Res*. 1993;21:37–45.
22. Wyde PR, Ambrose MW, Meyerson LR, et al. The antiviral activity of SP-303, a natural polyphenolic polymer, against respiratory syncytial and parainfluenza type 3 viruses in cotton rats. *Antiviral Res*. 1993;20:145–154.
23. Sidwell RW, Huffman JH, Moscon BJ, et al. Influenza virus-inhibitory effects of intraperitoneally and aerosol-administered SP-303, a plant flavonoid. *Chemotherapy*. 1994;40:42–50.
24. Pieters L, De Bruyne T, Van Poel B, et al. In vivo healing activity of dragon's blood (Croton spp.), a traditional South American drug and its constituents. *Phytomedicine*. 1995;2:17–22.
25. Vaisberg AJ, Milla M, Planas MC, et al. Taspine is the cicatrizant principle in sangre de grado extracted from Croton lechleri. *Planta Med*. 1989;55:140–143.
26. Frampton JE, Crofelemer. A review of its use in the management of non-infectious diarrhea in adult patients with HIV/AIDS on antiretroviral therapy. *Drugs*. 2013;73:1121–1129.
27. Mangel AW, Chaturvedi P. Evaluation of crofelemer in the treatment of diarrhea-predominant irritable bowel syndrome patients. *Digestion*. 2008;78:180–186.
28. DiCesare D, DuPont HL, Mathewson JJ, et al. A double blind, randomized, placebo-controlled study of crofelemer in the symptomatic treatment of acute diarrhea among travelers to Jamaica and Mexico. *Am J Gastroenterol*. 2002;97:2585–2588.
29. Crutchley RD, Miller J, Garey KW. Crofelemer, a novel agent for treatment of secretory diarrhea. *Annals Pharmacol*. 2010;44:878–884.
30. Safrin S, McKinley G, McKeough M, et al. Treatment of acyclovir-unresponsive cutaneous herpes simplex virus infection with topically applied SP-303. *Antiviral Res*. 1994;25:185–192.
31. Bogdan C, Iurian S, Tomuta I, Moldovan M. Improvement of skin condition in striae distensae: development, characterization and clinical efficacy of a cosmetic product containing Punica granatum seed oil and Croton lechleri resin extract. *Drug Des Dev Ther*. 2017;11:521–531.
32. Namjoyan F, Kiashi F, Moosavi ZB, et al. Efficacy of dragon's blood cream on wound healing: a randomized, double-blind, placebo-controlled clinical trial. *J Trad Comp Med*. 2016;6:37–40.
33. DiCesare D, DuPont HL, Mathewson JJ, et al. A double blind, randomized, placebo-controlled study of SP-303 (Provir) in the symptomatic treatment of acute diarrhea among travelers to Jamaica and Mexico. *Am J Gastroenterol*. 2002;97:2585–2588.
34. Duke JA. *Handbook of Medicinal Herbs*. 2nd ed. Boca Raton, FL: CRC Press.
35. Villegas LF, Frenandez ID, Maldonado H, et al. Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. *J Ethnopharmacol*. 1997;55:193–200.
36. Chen ZP, Cai Y, Phillipson JD. Studies on the anti-tumour, anti-bacterial, and wound-healing properties of dragon's blood. *Planta Med*. 1994;60:541–545.
37. Van Sebille YZA, Gibson RJ, Wardill HR, et al. Dacomitinib-induced diarrhea: Targeting chloride secretion with crofelemer. *Int J Cancer*. 2018;142(2):369–380. https://doi.org/10.1002/ijc.31048.

Curcuma longa (Turmeric)

Michael T. Murray, ND

OUTLINE

Curcuma longa (family: *Zingiberaceae*)
Common names: turmeric, curcuma, Indian saffron

GENERAL DESCRIPTION

Curcuma longa, a perennial herb of the ginger family, is cultivated extensively in India, China, Indonesia, and other tropical countries. It has a thick rhizome from which arise large, oblong, and long-petioled leaves. The rhizome is the part used; it is usually cured (boiled, cleaned, and sun dried) and polished (Fig. 73.1).

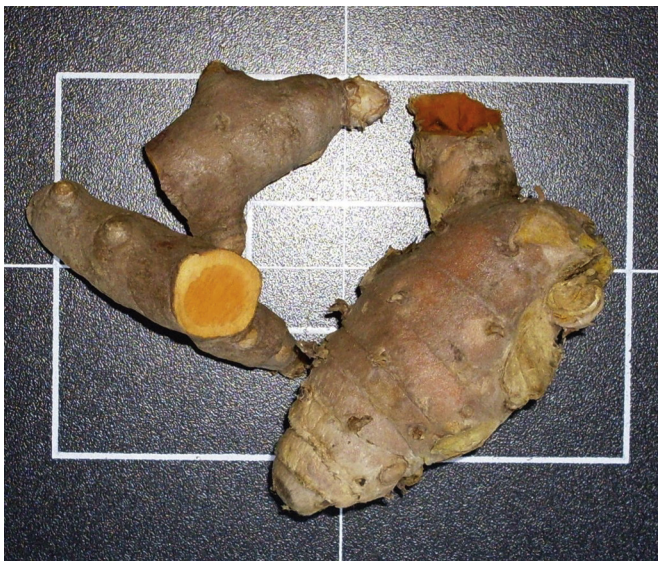


Fig. 73.1 *Curcuma longa* rhizome.

CHEMICAL COMPOSITION

Turmeric, like many spices, is full of many active phytochemicals. The key components of turmeric include the following[1]:

- Curcuminoids: 0.3% to 5.4%
- Volatile oils (e.g., turmerone, atlantone, zingiberene, etc.): 3% to 7%
- Resins (including terpenoids, triterpenoids, phenylpropenes, etc.): trace
- Alkaloids: trace
- Carbohydrates: 60% to 70%
- Fat: 5% to 10%
- Protein: 6% to 8%
- Fiber: 2% to 7%
- Vitamins: trace
- Minerals: trace
- Water: 6% to 13%

Fig. 73.2 shows the chemical structure of turmeric.

The reported consumption of turmeric in Indian and Asian cultures is in the range of 200 to 1000 mg/day, with consumption in urban areas being closer to the lower level (i.e., 200 mg/day), whereas in rural areas, the consumption is in the range of 600 to 1000 per day. Based on an average curcumin level of about 3%, even at 1000 mg of turmeric a day, the curcumin intake would be only 30 mg daily.

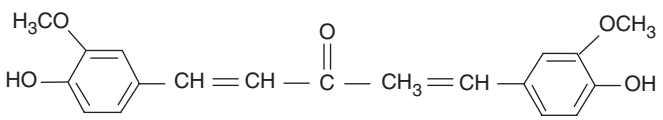


Fig. 73.2 Curcumin.

HISTORY AND FOLK USE

Turmeric is the major ingredient of curry powder and is also used in prepared mustard. It is extensively used in foods for both its color and flavor. In addition, turmeric is used in both the Chinese and Indian (Ayurvedic) systems of medicine as an anti-inflammatory agent and for the treatment of numerous conditions, including flatulence, jaundice, menstrual difficulties, bloody urine, hemorrhage, toothache, bruises, chest pain, and colic. Turmeric poultices are often applied locally to relieve inflammation and pain.

PHARMACOLOGY

Turmeric and its derivatives have undergone a great deal of pharmacological investigation.[1,2] Although a number of components demonstrate activity, curcumin and the volatile oil components are believed to be the most active components. Since 1990, more than 9000 scientific investigations on turmeric and curcumin have been published. Turmeric and curcumin-rich extracts have been shown to possess the following effects and many more:

- Antioxidant
- Anticarcinogenic
- Anti-inflammatory
- Antimicrobial
- Cardiovascular protective
- Hepatoprotective
- Gastrointestinal carminative and protectant
- Neuroprotective

These beneficial effects and actions, however, are largely based on in vitro or experimental models. There are significant pharmacokinetic issues with curcumin because it is poorly absorbed and rapidly metabolized and eliminated from the body. Pharmacokinetic studies in animals showed that 40% to 85% of an oral dose of curcumin passes through the gastrointestinal tract unchanged.[3,4] Several studies have looked at relatively high dosages of curcumin powder in humans. In one study, 24 healthy volunteers were administered escalating doses from 500 to 12,000 mg.[5] No curcumin was detected in the serum of participants administered 500, 1000, 2000, 4000, 6000, or 8000 mg. Table 73.1 shows the concentrations of curcumin in two subjects (one taking 10,000 mg and one taking 12,000 mg). No plasma concentrations of curcumin were detected in the remaining subjects at the 10,000- or 12,000-mg dose levels. Similar results were seen in other studies looking at dosages ranging from 3.6 to 12 g.

Another challenge with curcumin and other polyphenols is that they circulate bound to glucuronic acid. The theory is that when cells or mitochondria experience cellular stress or damage, they release glucuronidase. This enzyme subsequently liberates the curcumin, flavonoid, or other polyphenol to exert its effect locally or on a cellular basis.

Antioxidant Effects

Turmeric and curcumin exert significant antioxidant activity. Because of its bright yellow color and antioxidant properties, turmeric is often used to protect against lipid peroxidation in butter, margarine, cheese, and other food products. Although turmeric powder as well as both water- and fat-soluble extracts have been shown to be effective antioxidants in various in vitro and in vivo models, curcumin is the most potent component.[2] For active oxygen species, curcumin is slightly weaker than vitamin C but stronger than vitamin E and superoxide dismutase. Against hydroxyl radicals, curcumin offers greater effectiveness than these vitamins.[6] Not all of the antioxidant properties of turmeric are due to curcumin alone, because the aqueous extract of turmeric is more effective against superoxide than curcumin and is much stronger in inhibiting oxidative damage to DNA.[7,8] The antioxidant activities of curcumin may in part explain the anti-inflammatory, anticarcinogenic, and cardioprotective capacity of this spice. In vitro and in vivo studies have also shown this antioxidant action to be neuroprotective as well.[9]

TABLE 73.1 Serum Curcumin Levels for Two Subjects (in ng/mL)

Dose	Baseline	1 Hour	2 Hours	4 Hours
10 g	Approx. 6.0	30.4	39.5	50.5
12 g	Trace	29.7	57.6	51.2

Anticarcinogenic Effects

The antineoplastic effects of turmeric and curcumin have been demonstrated at all steps of carcinogenesis: initiation, promotion, and progression. In addition to inhibiting the development of cancer, several studies suggest that curcumin can also promote cancer regression. The protective effects of turmeric and its derivatives are only partially explained by its direct antioxidant and free radical–scavenging effects. It also inhibits nitrosamine formation, enhances the body's natural antioxidant system, increases the levels of glutathione and other nonprotein sulfhydryls, and acts directly on several enzymes and gene loci.

Curcumin's ability to protect against damage to DNA was demonstrated in a study in a community with a high content of groundwater arsenic.[10] Arsenic is extremely carcinogenic because it causes severe oxidative damage to DNA. Blood samples before curcumin supplementation showed severe DNA damage, with increased levels of free radicals and lipid peroxidation. Three months of curcumin intervention reduced the DNA damage, retarded free-radical formation and lipid peroxidation, and raised the level of antioxidant activity. In another study, cigarette smokers receiving turmeric demonstrated a significant reduction in the level of urinary-excreted mutagens—an indication of the ability of the body to rid itself of cancer-causing compounds via detoxification mechanisms (Fig. 73.3). For many reasons, curcumin is emerging as a very important agent in the battle against cancer.[11]

Data also suggest that curcumin causes cancer to regress. Some of curcumin's benefits come from its antioxidant activity, but it also:

- Inhibits the formation of cancer-causing nitrosamines
- Enhances the body's production of cancer-fighting compounds, such as glutathione
- Promotes the liver's proper detoxification of cancer-causing compounds
- Prevents overproduction of cyclooxygenase-2, an enzyme that may contribute to the development of tumors

In addition to these preventative actions, curcumin has also been shown to inhibit tumor growth in several ways:

- Inhibiting epidermal growth factor (EGF) receptor sites: EGF stimulates cells to proliferate by connecting to a receptor on the cell surface. About two thirds of all cancers produce an abundance of these receptors, which makes them highly sensitive to EGF. By reducing the number of EGF receptors, curcumin decreases the cell's tendency to proliferate.
- Inhibiting angiogenesis: Fibroblast growth factor is a protein that promotes the formation of new blood vessels to feed the growing tumor. Curcumin inhibits the production of this growth factor.
- Inhibiting nuclear factor-κβ (NF-κβ): This is a protein that many cancer cells produce to block the signals commanding them to stop proliferating.

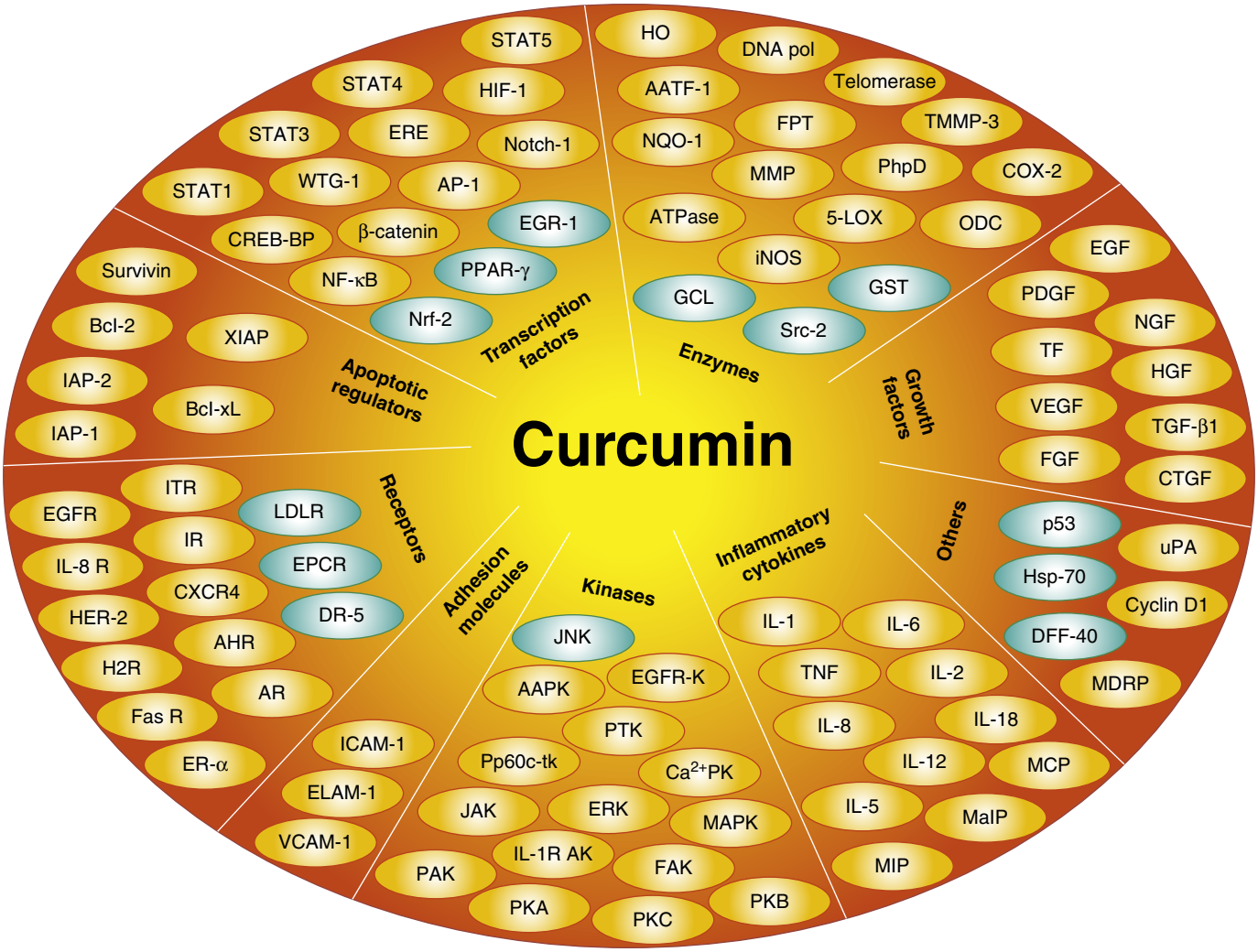


Fig. 73.3 Molecular targets of curcumin. 5–LOX, 5–lipoxygenase; AAPK, autophosphorylation–activated protein kinase; AATF–1, arylamine *N*–acetyltransferases–1; AHR, aryl hydrocarbon receptor; AP–1, activating protein–1; AR, androgen receptor; Bcl–2, beta–cell lymphoma protein 2; Bcl–xL, beta–cell lymphoma extra large; Ca2+PK, Ca2+–dependent protein kinase; CXCR4, chemokine (C–X–C motif) receptor 4; CREB–BP, CREB–binding protein; CTGF, connective tissue growth factor; DFF–40, DNA fragmentation factor 40–kd subunit; DR5, death receptor–5; ELAM–1, endothelial leukocyte adhesion molecule–1; EPCR, endothelial protein C–receptor; ERE, electrophile response element; ER–α, estrogen receptor–alpha; FAK, focal adhesion kinase; FPT, farnesyl protein transferase; FR, Fas receptor; GCL, glutamyl cysteine ligase; GST, gluthathione–S–transferase; H2R, histamine (2)–receptor; HER–2, human epidermal growth factor receptor–2; HGF, hepatocyte growth factor; HIF–1, hypoxia inducible factor–1; HO, haem oxygenase 1; HSP–70, heat–shock protein 70; IAP–1, inhibitory apoptosis protein–1; ICAM–1, intracellular adhesion molecule–1; iNOS, inducible NOS; IR, integrin receptor; MaIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; MDRP, multi–drug resistance protein; MIP, migration inhibition protein; NGF, nerve growth factor; NQO–1, NAD(P)H:quinoneoxidoreductase–1; Nrf, nuclear factor 2–related factor; ODC, ornithine decarboxylase; PAK, protamine kinase; PhpD, phospholipase D; Pp60c–tk, pp60c–src tyrosine kinase; PTK, protein tyrosine kinase; Src–2, Src homology 2 domain–containing tyrosine phosphatase 2; STAT, signal transducer and activator of transcription; TF, tissue factor; TMMP–3, tissue inhibitor of metalloproteinase–3; uPA, urokinase–type plasminogen activator; VCAM–1, vascular cell adhesion molecule–1; WTG–1, Wilms' tumor gene 1.

- Increasing the expression of the nuclear p53 protein: This protein is essential for apoptosis, the normal process of cell "suicide."
- Inhibiting enzymes that promote cancer cell growth.

Anti-inflammatory Effects

The volatile-oil fraction of *C. longa* has been demonstrated to possess anti-inflammatory activity in various experimental models (e.g., Freund's adjuvant-induced arthritis, formaldehyde- and carrageenan-induced paw edema, and cotton pellet and granuloma pouch tests).[12,13] Its effects in these studies were comparable to cortisone and phenylbutazone. Even more potent in acute inflammation is curcumin. In animal models of acute inflammation, curcumin is as effective as cortisone or phenylbutazone, but it is only half as effective in chronic models.[14,15] However, although phenylbutazone and cortisone are associated with significant toxicity, curcumin displays virtually no toxicity (see later discussion under "Toxicology").

The rank in order of potency of curcumin analogs, cortisone, and phenylbutazone in carrageenan-induced paw edema is as follows: sodium curcuminate > tetrahydrocurcumin > curcumin > cortisone > phenylbutazone > triethylcurcumin.[15,16]

Based on in vitro studies, curcumin exhibits many direct anti-inflammatory effects, including the following[17]:

- Inhibition of leukotriene formation
- Inhibition of platelet aggregation
- Promotion of fibrinolysis
- Inhibition of neutrophil response to various stimuli involved in the inflammatory process
- Stabilization of lysosomal membranes
- Inhibition of NF-κB
- Inhibition of tumor necrosis factor

The blocking the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) by curcumin is especially important.[18] Curcumin also inhibits tumor necrosis factor α (TNF-α), a major mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, NF-κβ.[19]

Curcumin's counterirritant effect may also be a major factor in its topical anti-inflammatory action. Sodium curcuminate can be produced by mixing turmeric with slaked lime. This mixture, applied as a poultice, is an ancient household remedy for sprains, muscular pain, and inflamed joints. Capsaicin, a similar pungent principal from *Capsicum frutescens* (cayenne pepper), has been shown to be quite effective as a topical pain reliever in cases of postherpetic neuralgia and arthritis. Both capsaicin and curcumin deplete nerve endings of the neurotransmitter of pain, substance P.[20]

In addition to its direct anti-inflammatory effects, curcumin also appears to exert some indirect effects. In models of chronic inflammation, curcumin was much less active in adrenalectomized animals. Possible mechanisms of action include the following:

- Stimulating the release of adrenal corticosteroids
- "Sensitizing" or priming cortisol receptor sites, thereby potentiating cortisol action
- Increasing the half-life of endogenous cortisol through alteration of hepatic degradation

Cardiovascular Effects

The effects of turmeric and curcumin on the cardiovascular system include the prevention of oxidative damage to low-density lipoprotein cholesterol, inhibiting platelet aggregation, and reducing fibrinogen levels.[21–24] These effects are of great significance in preventing atherosclerosis and its complications.

In animal studies, adding as little as 0.1% curcumin to a high-cholesterol rat diet decreased cholesterol levels to one half of those found in rats fed cholesterol but no curcumin. Curcumin's cholesterol-lowering actions include interfering with intestinal cholesterol uptake; increasing the conversion of cholesterol into bile acids by increasing the activity of hepatic cholesterol-7-α-hydroxylase, the rate-limiting enzyme of bile acid synthesis; and increasing the excretion of bile acids via its choleretic effects.[24]

Effects on blood lipids from human studies are equivocal. In one small study, 10 healthy volunteers received 500 mg/day of curcumin for 7 days.[21] A significant decrease in the level of serum lipid peroxides of 33%, an increase in high-density lipoprotein cholesterol of 29%, and a decrease in total serum cholesterol of 11.63% were observed. However, two other studies failed to show any effect on blood lipids. One study used escalating doses (15, 30, and 60 mg three times daily),[25] and another used dosages of up to 4 g/day for 6 months, but these studies only raised the plasma curcumin concentration to 490 nmol/L.[26]

TABLE 73.2 Lowering Effects of the Treatment With a Curcuma Extract (Turmeric) on Abnormally High Plasma Fibrinogen Levels (Values in Milligrams per Deciliter Before and After a 15-Day Treatment)

Subject Number	Sex	Age (Years)	Before	After
1	M	68	809	241
2	M	31	476	272
3	M	64	584	290
4	F	54	351	282
5	F	59	480	268
6	F	62	690	240
7	F	69	490	272
8	F	66	402	251
Means ± SD			535.6 ± 151.4[a]	271.1 ± 31

[a]As shown by the analysis of variance test, the decrease had a statistical significance of α = 0.05.

Modified from Ramirez Bosca A, Soler A, Carrion-Gutierrez MA, et al. An hydroalcoholic extract of Curcuma longa lowers the abnormally high values of human-plasma fibrinogen. *Mech Ageing Dev.* 2000;114:207–210.

Curcumin's effect on fibrinogen and platelet aggregation, which are known predictors of future coronary heart disease, may be more significant than its effects on cholesterol. In a preliminary study of 30 apparently healthy volunteers, baseline levels of fibrinogen were recorded, and 8 subjects were found to have high plasma fibrinogen (above 350 ng/mL).[27] These subjects were given a hydroalcoholic extract of *C. longa* containing 20 mg of curcumin for 15 days. Impressively, curcumin administration decreased the fibrinogen levels to the range of 240 to 290 mg/dL (Table 73.2).

Turmeric's and curcumin's action on inhibiting platelet aggregation appears to be mediated by inhibiting the formation of thromboxanes (a promoter of aggregation) while simultaneously increasing prostacyclin (an inhibitor of aggregation).

Hepatic Effects

Curcumin exhibits hepatoprotection similar to that of glycyrrhizin and silymarin (see Chapters 96 and 123, respectively, for further discussion) against carbon tetrachloride and galactosamine-induced liver injury.[28] This protection is largely a result of its potent antioxidant activity. Similar results were seen with Javanese turmeric *(Curcuma xanthorrhiza)*. Mice given intraperitoneal injections of the hepatoxic drugs carbon tetrachloride (32 mg/kg) and acetaminophen (600 mg/kg) experienced significantly decreased liver damage, as measured by serum glutamate oxaloacetate and serum glutamate pyruvate transaminase, when treated with 100 mg/kg of turmeric.[29]

The antioxidant and hepatoprotective effects alone support turmeric's historical use in liver disorders; however, turmeric and curcumin also exert anti-inflammatory and choleretic effects. The increases of serum glutamate oxaloacetate and serum glutamate pyruvate transaminase commonly seen in experimental models of inflammation were prevented by curcumin. Curcumin is an active choleretic, increasing bile acid output by more than 100%. In addition to increasing biliary excretion of bile salts, cholesterol, and bilirubin, curcumin also increases the solubility of bile.[28] This suggests a benefit in the prevention and treatment of cholelithiasis.

Gastrointestinal Effects

Turmeric and its components exert a number of beneficial effects on the gastrointestinal system. Turmeric's long use as a carminative is supported in preclinical studies showing it to inhibit gas formation by *Clostridium perfringens* and in rats given diets rich in flatulence-producing foods. In addition, sodium curcuminate was shown to inhibit intestinal spasm, and another compound from turmeric, *p*-tolymethylcarbinol, was shown to increase the secretion of secretin, gastrin, bicarbonate, and pancreatic enzymes.[2]

As a component of curries and spicy foods, there is some concern that turmeric may be irritating to the stomach. However, several studies showed turmeric to be beneficial to gastric integrity. Turmeric and curcumin were shown to increase the mucin content of the stomach and exert gastroprotective effects against ulcer formation induced by stress, alcohol, indomethacin, pyloric ligation, and reserpine.[30] However, at high doses, curcumin or turmeric may be ulcerogenic (see later discussion on "Toxicology").

Antiaging and Neuroprotective Effects

As far as slowing down the aging process, in addition to the effects discussed previously, there is considerable evidence that curcumin protects against age-related brain damage, in particular, Alzheimer disease. Researchers began exploring this effect after noting that elderly (aged 70–79) residents of rural India, who eat large amounts of turmeric, were shown to have the lowest incidence of Alzheimer disease in the world, 4.4 times lower than that of Americans. In addition, researchers also demonstrated that curcumin was able to prevent the development of amyloid plaque and the neurofibrillary tangles that are the hallmark features of the brain lesions in mice specifically bred to develop the disease.[31,32]

Antimicrobial Effects

Alcohol extracts and the essential oil of *C. longa* were shown in one study to inhibit the growth of most organisms occurring in cholecystitis (i.e., *Sarcina, Gaffkya, Corynebacterium,* and *Clostridium*).[2] Other microorganisms that were inhibited include *Staphylococcus, Streptococcus, Bacillus, Entamoeba histolytica,* and several pathogenic fungi.[33] The concentrations used in these studies were relatively high: 0.5 to 5 mg/mL of the alcohol extract and essential oil and 5 to 100 mg/mL of curcumin.

CLINICAL APPLICATIONS

Turmeric and curcumin preparation have extensive potential clinical applications. The most popular uses of curcumin are for three primary purposes: (1) as a general antioxidant to prevent heart disease and slow down the aging process; (2) as a chemoprotector against cancer and as a treatment adjunct; and (3) to reduce inflammation, particularly in osteoarthritis and inflammatory bowel disease.

General Antioxidant and Prevention of Atherosclerosis

The benefits of curcumin as a general antioxidant and in the prevention of atherosclerosis and slowing down the aging process have been previously discussed. Curcumin's antioxidant activity may be particularly helpful in preventing exercise-induced oxidative damage as well as protecting against low-density lipoprotein cholesterol from becoming oxidized and damaging arteries. In addition, curcumin exerts other beneficial effects in preventing atherosclerosis, including possibly lowering cholesterol levels, preventing plaque formation, inhibiting platelet aggregation, and reducing fibrinogen levels (see previous discussion "Cardiovascular Effects").[24]

In regard to its effects as a general antioxidant, several clinical studies with forms of curcumin with enhanced absorption provide clinical support. For example, in one double-blind study, 40 patients with mild-to-moderate primary knee osteoarthritis were given Curcumin C3 Complex + Bioperine capsules (1500 mg of curcuminoids/day in 3 divided doses) or matched placebo capsules for a period of 6 weeks. Curcumin supplementation resulted in a significant elevation in serum SOD activities (mean change: 2.94 vs. –0.38; $p < 0.001$) and a significant reduction in MDA concentrations (mean change: –5.26 vs. –2.49; $p = 0.044$) in the curcuminoids compared with the placebo group. These changes in the serum activities of SOD and concentrations of GSH and MDA during the course of the trial were significantly correlated. Short-term supplementation with curcuminoids attenuates systemic oxidative stress in patients with osteoarthritis. These antioxidant effects may account for the reported therapeutic effects of curcuminoids in relieving osteoarthritis symptoms.[34]

Theracurmin was also shown to reduce oxidative stress in a double-blind clinical trial. It also increased the antioxidant capacity in response to acute endurance exercise. In the study, 10 male participants, ages 26.8 ± 2.0 years, completed three trials in a random order: (1) placebo (control), (2) single (only before exercise), and (3) double (before and immediately after exercise) curcumin supplementation trials. Each participant received oral administration of 90 mg of curcumin (from Theracurmin) or the placebo 2 hours before exercise and immediately after exercise. Each participant walked or ran at 65% of VO_2 max on a treadmill for 60 min. Blood samples were collected preexercise, immediately after exercise, and 2 hours after exercise. The concentrations of serum derivatives of reactive oxygen metabolites measured immediately after exercise were significantly higher than preexercise values in the placebo trial (308.8), but not in the single (259.9) or double (273.6) curcumin supplementation trials. Serum biological antioxidant potential concentrations measured immediately after exercise were significantly elevated in the single and double curcumin supplementation trials compared with preexercise values ($p < 0.05$). These findings indicate that curcumin supplementation can reduce exercise-induced oxidative stress by increasing blood antioxidant capacity. [35]

In another double-blind study, Theracurmin (150 mg curcumin) was shown to dramatically enhance the benefits of exercise on improving the health of the aorta and the entire arterial system. In the study, 45 postmenopausal women were randomly assigned to four interventions: placebo ingestion ($n = 11$), curcumin ingestion ($n = 11$), exercise training with placebo ingestion ($n = 11$), or exercise training with curcumin ingestion ($n = 12$). Very detailed assessments were used to evaluate cardiovascular function in all subjects. The aim of the study was to test the hypothesis that the regular endurance exercise combined with daily curcumin ingestion lowers the age-related increase in left ventricular (LV) afterload to a greater extent than either intervention alone in postmenopausal women. LV afterload is the tension against which the heart must contract to eject blood. Arterial stiffness, especially in the aorta, is the major determinant of LV afterload. The stiffer the artery due to atherosclerosis (hardening of the artery), the greater the LV afterload. Not surprisingly, an increase in LV is an independent risk for heart failure and death due to heart disease mortality. LV afterload tends to increase with advancing age. Regular physical exercise has been found to decrease the LV afterload to an extent, but in this study, the effect of exercise plus Theracurmin (equivalent to 5 capsules per day) reduced LV afterload, but neither therapy on its own was as effective. These results indicate that some of the cardiovascular benefits associated with exercise are greatly enhanced when exercise is combined with Theracurmin, and vice versa.[36]

In another evaluation in postmenopausal women, the effect of Theracurmin (150 mg curcumin) on vascular endothelial function was determined by using flow-mediated dilation as an indicator. In total,

32 postmenopausal women were assigned to three groups: control, exercise, and curcumin groups. The curcumin group ingested curcumin orally for 8 weeks. The exercise group underwent moderate aerobic exercise training for 8 weeks. Before and after each intervention, flow-mediated dilation was measured. Results indicated that curcumin ingestion was equal to aerobic exercise training in increasing flow-mediated dilation in postmenopausal women.[37]

The effects of curcumin (1.5 g per day) on risk factors for atherosclerosis were investigated in a 6-month randomized, double-blinded and placebo-controlled clinical trial that included subjects diagnosed with type 2 diabetes. The primary parameter assessed to measure atherosclerotic processes was pulse wave velocity (PWV), a well-accepted surrogate marker used for assessing atherosclerosis status. Results showed that curcumin intervention significantly reduced PWV, increased the level of serum adiponectin, and decreased the level of leptin. These results are associated with reduced levels of homeostasis-model-assessed insulin resistance, triglycerides, uric acid, visceral fat, and total body fat. In summary, a 6-month curcumin intervention in the type 2 diabetic population lowered the atherogenic risks. In addition, the extract helped improve relevant metabolic profiles in this high-risk population.[38]

Cancer Prevention and Treatment Adjunct

The anticancer effects of curcumin have been demonstrated in preclinical studies at all steps of cancer formation: initiation, promotion, and progression. Even dietary consumption of turmeric has been shown to exert some protective effects against cancer. In one human study, 16 long-term smokers were given 1.5 g of turmeric daily, whereas a group of 6 nonsmokers served as a control group.[39] At the end of the 30-day trial, the smokers receiving the turmeric demonstrated a significant reduction in the level of mutagens excreted in the urine. These results are quite significant because the level of urinary mutagens is thought to correlate with the systemic load of carcinogens and the efficacy of detoxification mechanisms. Due to widespread exposure to smoke, aromatic hydrocarbons, and other environmental carcinogens, the frequent use of turmeric as a spice appears warranted.

In one study, 62 patients with either ulcerating oral or cutaneous squamous-cell carcinomas who failed to respond to the standard treatments of surgery, radiation, and chemotherapy were given either an ethanol extract of turmeric (for oral cancers) or an ointment containing 0.5% curcumin in Vaseline.[40] The ointment or extract was applied topically three times daily. At the end of the 18-month study, the treatment was found to be effective in reducing the smell of the lesion (90%), itching and exudate (70%), pain (50%), and the size of the lesion (10%). Although these were not spectacular results, it must be pointed out that this patient population failed to respond to standard medical treatment.

Two studies have been conducted in patients with advanced pancreatic cancer given 8000 mg of regular curcumin daily. These studies showed some positive results.[41,42] For example, in one study of 26 patients with advanced pancreatic cancer, 8000 mg of curcumin produced clinically relevant biological activity in two patients. The failure to affect more patients in these studies is thought to be the result of poor absorption of curcumin. Formulations with much greater bioavailable curcumin may offer better results. For example, in one study, Theracurmin was given to 16 patients with advanced pancreatic cancer who were unresponsive to conventional chemotherapy.[43] This Phase 1 study sought to determine safety and dosage parameters. The patients produced no significant adverse effects even at relatively high dosage levels for Theracurmin (200 mg/day and 400 mg/day). The results showed that Theracurmin produced significant increases in the blood concentrations of curcumin in a dose-dependent fashion—the higher the dosage, the greater the increase in curcumin in the blood. Median plasma curcumin levels 2 hours after Theracurmin administration (representing peak levels) were as follows:

- 324 ng/mL at Level 1 (Theracurmin containing 200 mg of curcumin)
- 440 ng/mL at Level 2 (Theracurmin containing 400 mg of curcumin)

These values were significantly higher than the median values (85 ng/ mL) achieved in the authors' previous study using 8 g of conventional curcumin.

The big finding from the study was that Theracurmin produced a significant improvement in key quality-of-life (QOL) scores, such as the following:

- Fatigue
- Functional improvement (emotional, role, cognitive, physical, and social functions)
- Diarrhea
- Appetite loss

In addition, the median survival time (MST) was 132 days, and three patients (21%) survived more than 12 months. As a reminder, these patients were unresponsive to conventional cancer treatment, and with such advanced cancer, they were generally regarded as terminal with an average survival time of less than 2 months, so these results are quite promising.

General Inflammation

C. longa has been used in Ayurvedic medicine as a general anti-inflammatory. This use seems to be substantiated not only by the experimental studies described previously but also by clinical investigations in a variety of inflammatory conditions. In an early study, the postoperative inflammation model for evaluating nonsteroidal anti-inflammatory drugs (NSAIDs) was used, and it showed curcumin to exert comparable anti-inflammatory action to phenylbutazone.[44] However, although curcumin has an anti-inflammatory effect similar to phenylbutazone and various NSAIDs, it does not possess direct analgesic action. It is also important to point out that phenylbutazone and NSAIDs are associated with significant adverse effects, whereas curcumin is not.

As far as validation of systemic effects, there is a study that involved a rather interesting condition, chronic inflammation of the uvea—the front portion of the eye including the iris.[45] The study group consisted of 106 patients divided into three main groups of different uveitis origin: group 1 (autoimmune uveitis), group 2 (uveitis due to herpes), and group 3 (different causes of uveitis). The patients were given 1200 mg of Meriva for 1 year. In the previous year, there were a total of 275 relapses. In the 1-year treatment group with Meriva, there were only 36 relapses at the end of the 12-month follow-up, an 88% improvement. This study provides considerable evidence that curcumin can affect inflammation in a chronic inflammatory condition.

Again, the theory is that because curcumin circulates bound to glucuronic acid, when cells or mitochondria experience cellular stress or damage, they release glucuronidase. This enzyme subsequently liberates the curcumin to exert its effect locally or on a cellular basis.

Osteoarthritis

Curcumin exerts a number of mechanisms that address much of the underlying pathophysiology of osteoarthritis (OA). In addition to its antioxidant effects, in vitro studies demonstrated that curcumin exerts a number of beneficial effects through its ability to block the activation of the NF-κB system, such as suppressing the release of proteoglycans and metal metalloproteases and the expression of cyclooxygenase, prostaglandin E-2, and inflammatory cytokines in chondrocytes.[46]

Clinical studies also show some benefits in osteoarthritis. In one double-blind study, 40 patients with mild to moderate primary knee OA were given Curcumin C3 Complex + Bioperine capsules (1500 mg of curcuminoids/day in 3 divided doses) or matched placebo capsules for a period of 6 weeks. Treatment with curcuminoids was associated with significantly greater reductions in WOMAC ($p=0.001$), visual analog scale (VAS; $p<0.001$), and LPFI ($p=0.013$) scores compared with placebo. With respect to WOMAC subscales, there were significant improvements in the pain and physical function scores but not in the stiffness score.[47]

In a second analysis of this clinical trial, serum levels of interleukins 4 (IL-4) and 6 (IL-6), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and high-sensitivity C-reactive protein (hs-CRP) and erythrocyte sedimentation rate (ESR) were determined at baseline as well as at the end of the trial. The results did not indicate any significant difference between the study groups, and the authors concluded: "Significant improvement in clinical symptoms of OA in curcuminoid-treated subjects cannot be attributed to the systemic anti-inflammatory effects of these phytochemicals."[48] Effects may be more localized at sites of inflammation, as described previously, although this preparation and more bioavailable forms of curcumin have shown systemic anti-inflammatory effects.

In the first double-blind study with curcumin in OA, 50 patients over 40 years old with knee osteoarthritis confirmed by x-ray took either 180 mg/day of curcumin (as Theracurmin) or a placebo daily for 8 weeks. Blood biochemistry analyses were performed before and after 8 weeks of each intervention to evaluate safety. The patients' knee symptoms were evaluated at 0, 2, 4, 6, and 8 weeks by the knee-scoring system of the Japanese Orthopedic Association and also the Japanese Knee Osteoarthritis Measure, the knee pain VAS, and the need for NSAIDs. The results showed that knee pain scores were significantly lower in the Theracurmin group than in the placebo group in those patients with moderate to severe symptoms. Theracurmin also lowered the use of celecoxib (Celebrex) much more significantly than placebo. Although 60% of the placebo group still relied on Celebrex for adequate pain relief at the 8-week mark, only 32% of the Theracurmin group still needed the NSAID, and there was a definite strong trend for eventual discontinuation. No major side effects were observed in the patients taking Theracurmin. This study is significant because it showed such a significant advantage over a placebo in a short-term study. Generally, in OA, this requires a much larger study group and much longer periods of time. Therefore, for Theracurmin to show such clear benefit in this relatively small, short-term study bodes well for people with OA gaining immediate and noticeable benefits with curcumin.[49]

Meriva has also been used with success in several studies.[50] In the first study, 50 patients were given 1000 mg Meriva (providing 200 mg of curcumin) for 3 months, after which symptom scores decreased by 58%, walking distance on the treadmill test was prolonged from 76 to 332 m, and the level of an inflammatory marker (C-reactive protein) in the blood decreased from 168 to 11.3 mg/L in the subpopulation with high C-reactive protein (CRP).[51] In another study, 100 patients with osteoarthritis were given the same dosage of Meriva for 8 months. Just as in the previous study, symptom scores, walking distance, and blood measurements of inflammation were all significantly improved.[52]

Rheumatoid Arthritis

Curcumin has been studied in rheumatoid arthritis (RA), with positive outcomes. For example, in one double-blind crossover clinical trial in patients with RA, curcumin (1200 mg/day) was compared with phenylbutazone (300 mg/day) (Fig. 73.4). The improvements in the

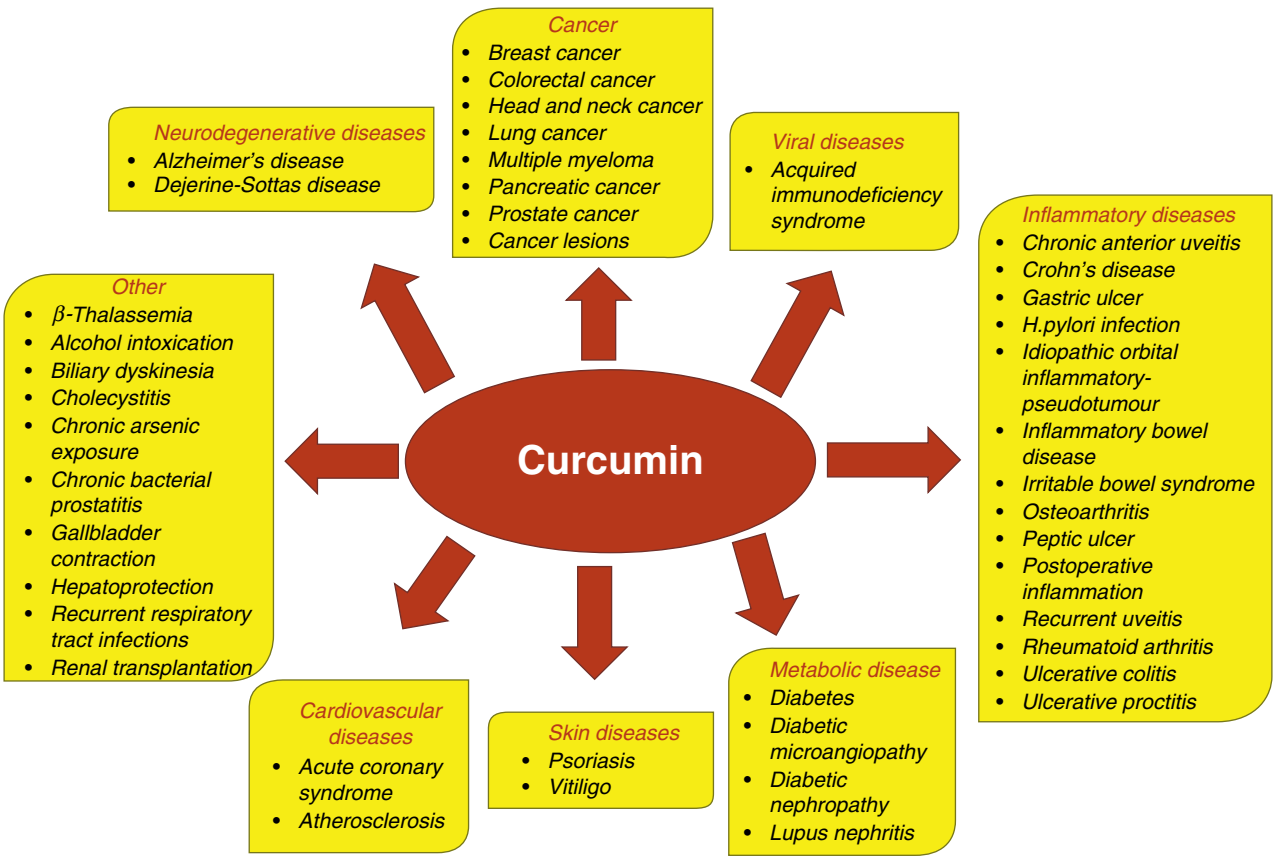


Fig. 73.4 Activity of curcumin against different human diseases based on clinical findings.

duration of morning stiffness, walking time, and joint swelling were comparable in both groups.[53]

In a more recent study in patients with active RA, 45 patients diagnosed with RA were randomized into three groups, with patients receiving regular, nonenhanced curcumin (500 mg) and diclofenac sodium (50 mg) alone or their combination. The primary end points were a reduction in Disease Activity Score (DAS) 28. The secondary end points included American College of Rheumatology (ACR) criteria for reduction in tenderness and swelling of joint scores. Patients in all three treatment groups showed statistically significant changes in their DAS scores. Interestingly, the curcumin group showed the highest percentage of improvement in overall DAS and ACR scores (ACR 20, 50, and 70), and these scores were significantly better than those of the patients in the diclofenac sodium group.[45]

Another small clinical trial used a more bioavailable form (Cureit). The 36 patients were divided into three groups, 250 mg curcumin, 500 mg curcumin, or a placebo twice daily. Significant changes in ESR, CPR, and rheumatoid factor (RF) values in patients receiving the study product compared with baseline and placebo. The results indicate that this novel curcumin in a turmeric matrix acts as an analgesic and anti-inflammatory agent for the management of RA at a dose as low as 250 mg twice daily, as evidenced by significant improvement in the ESR, CRP, VAS, RF, DAS, and ACR responses compared with placebo.[54]

Gastrointestinal Inflammation

Absorption of curcumin may not be as significant of a factor when dealing with gastrointestinal inflammation. There have been three randomized controlled trials in the treatment of ulcerative colitis that included a total of 142 patients. The use of curcumin along with mesalamine was associated with increased odds of clinical remission. Clinical improvement, endoscopic remission, and improvement rate also trended higher in the curcumin group compared with placebo.[55]

In one of the double-blind studies with ulcerative colitis, 43 patients received regular curcumin, 1 g after breakfast and 1 g after the evening meal, plus sulfasalazine or mesalamine, and 39 patients received placebo plus sulfasalazine or mesalamine for 6 months. Of the patients who received curcumin, 2 relapsed during 6 months of therapy (4.65%), whereas 8 of 39 patients (20.51%) in the placebo group relapsed. Furthermore, curcumin improved both the clinical signs and symptoms with ulcerative colitis via standard activity indexes.[56] In another study, 50 people with mild to moderate ulcerative colitis not responsive to mesalamine were given 1.5 g of curcumin twice a day (3 g per day of curcumin) or placebo for 1 month with continued mesalamine treatment. At week 4, 53.8% of those receiving curcumin went into remission, whereas none of the patients who received placebo achieved remission.[57] In another study, 1000 mg of curcumin taken twice daily in addition to mesalamine or sulfasalazine for 6 months helped maintain remission from ulcerative colitis compared with standard medications plus placebo.[58] But a lower dose (450 mg of curcumin daily) did not improve remission rates or improve mucosal healing compared with placebo in men and women with active ulcerative colitis taking mesalamine in another study.[59] This difference in response may be related to dosage.

In a study using an enhanced form of curcumin similar to Theracurmin, 56 men and women with mild to moderate ulcerative colitis found that 80 mg curcumin taken three times daily (240 mg per day) in addition to mesalamine for 1 month modestly decreased urgency of bowel movements and improved self-reported well-being, but did not decrease blood in the stool or colitis-related skin ulcers, compared with mesalamine alone.[60]

DOSAGE

On the basis of the evidence presented previously, it is clear that turmeric can be consumed liberally in the diet for its health-promoting effects. When specific medicinal effects are desired, due to the current understanding of curcumin pharmacokinetics, extracts of *C. longa* or curcumin are more feasible in achieving sufficient absorption.

Turmeric extracts, and more specifically curcumin preparations, are now major sellers in North America, with sales well above $300 million in 2018. The total global market for curcumin is growing as well and is expected to reach $1.3 billion by 2025. When natural products become commercially popular, it is not uncommon for synthetic versions to be introduced. That appears to have begun in early 2015. Plant-derived products can be distinguished from synthetic products by their content of an exceedingly small but accurately determinable amount of radioactivity. Plants assimilate carbon dioxide (CO_2) from the atmosphere, which contains a small quantity of radioactive CO_2. When made synthetically, the material has zero radioactivity. Any adulteration of an herbal extract, even partially, with synthetic equivalents can be detected accurately as to the relative ratios of each of the constituents.

The commercial landscape with curcumin has focused on improving bioavailability. Historically, turmeric and curcumin have been consumed in a lipid base, such as lecithin, fish oils, or essential fatty acids (with meals) or formulated in conjunction with Piperine or bromelain (on an empty stomach). A number of new methods now exist to enhance the absorption of curcumin. Table 73.3 provides several formulas reported to have enhanced bioavailability. These preparations deserve special mention because of their clinical investigations, most notably Meriva and Theracurmin. Meriva is produced by complexing curcuminoids with phosphatidylcholine to Phytosome.[49] Theracurmin is a surface-controlled particle dispersion within gum ghatti as the carrier.[61] It has an average particle size of curcumin of 0.19 μm compared with an average particle size of 22.75 μm in curcumin powder. This represents a reduction of over 100 times.

TABLE 73.3 Selected Formulations Reported to Improve Curcumin Bioavailability With Some Clinical Validation

Formulation	Manufacturer	Formulation Description
Biocurcumax (BCM-95)	Arjuna Natural Extracts Ltd. India (Dolcas Biotech)	Curcuminoid, essential oil of turmeric (45% ar-turmerone), and curcuminoids
Curcumin C3 Complex + Bioperine	Sabinsa, USA	Bioperine, and curcuminoids
Meriva	Indena SpA, Italy	Phytosome technology (curcumin, soy lecithin, microcrystalline cellulose, and 18%–20% curcuminoids)
Theracurmin	Theravalues Corp., Japan	Colloidal suspension of micronized curcumin (36% curcuminoids, 14.6% gum ghatti, 48.7% maltodextrin, 0.7% citrate)

Many brands/formulations will offer comparisons to curcumin. However, this practice is irrelevant given the poor oral absorption of curcumin. If the absorption of curcumin nears zero, how is any multiple of that number relevant clinically? Based on existing data from clinical studies, what is more important than comparing it to curcumin is the ability of a preparation to produce clinical results based on achieving targeted blood values. Here are the recommended targets:

- For general anti-inflammatory support, the therapeutic target is 50 to 100 ng/mL
- For more severe inflammation and in Alzheimer disease, the therapeutic target is 150 to 200 ng/mL
- For cancer and serious inflammatory conditions, the therapeutic target maybe 300 ng/mL or more

When evaluating absorption data from published human trials or the marketing claims from a manufacturer, the question comes down to what dosage of the preparation is required to produce the targeted blood levels listed here.

TOXICOLOGY

Toxicity has not been reported at standard dosage levels. The oral mean lethal dose levels for turmeric, its alcohol extracts, and curcumin have not been determined because 2.5 g/kg fed to mice, rats, guinea pigs, and monkeys and 3 g/kg sodium curcuminate fed to rats resulted in neither mortality nor chromosomal aberrations in teratology tests. At high doses, curcumin or turmeric may damage the gastrointestinal system because curcumin, with doses of 100 mg/kg body weight, was ulcerogenic in rats. Some studies found a sensitivity of mice to turmeric that resulted in hepatotoxicity. Curcumin toxicity has not been found in rats or other mammals, even at very high doses (5%–10% by weight of diet). Human studies suggest that curcumin is nontoxic to humans up to 8000 mg/day when taken by mouth for 3 months. Even the more bioavailable formulations of curcumin have been shown to be safe for humans.[62]

DRUG INTERACTIONS

Curcumin has several possible drug interactions, only a few of which have been confirmed. In one human study, 300 mg of curcumin reduced the absorption of the β-blocker talinolol by roughly 35%.[63]

In rabbits, pretreatment with curcumin resulted in increased plasma elimination half-life, thereby reducing the dosage of norfloxacin.[64]

In an experimental model, curcumin was shown to inhibit apoptosis produced by a number of chemotherapy agents, including camptothecin, mechlorethamine, and doxorubicin.[62]

Based upon in vitro studies, curcumin may inhibit the following drug-metabolizing enzymes: CYP3A4, CYP1A2, and CYP2A6.[65]

Curcumin was also shown to downregulate intestinal P-glycoprotein levels, thereby increasing the concentrations of Celiprolol and midazolam. This action may have significance for other drugs influenced by intestinal P-glycoprotein downregulation, including colchicine; various chemotherapy agents, including etoposide, doxorubicin, and vinblastine; digoxin; and immunosuppressive agents.[66]

Some theoretic concern has been raised that curcumin might increase the risk of bleeding of anticoagulant drugs due to its ability to decrease platelet aggregation.[67]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Sun W, Wang S, Zhao W, et al. Chemical constituents and biological research on plants in the genus Curcuma. *Crit Rev Food Sci Nutr*. 2017;57(7):1451–1523.
2. Kunnumakkara AB, Bordoloi D, Padmavathi G, et al. Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *Br J Pharmacol*. 2017;174(11):1325–1348.
3. Shankar TN, Shantha NV, Ramesh HP, et al. Toxicity studies on turmeric *(Curcuma longa)*: acute toxicity studies in rats, guinea pigs & monkeys. *Indian J Exp Biol*. 1980;18:73–75.
4. Wahlstrom B, Blennow G. A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol (Copenh)*. 1978;43:86–92.
5. Vareed SK, Kakarala M, Ruffin MT, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev*. 2008;17(6):1411–1417.
6. Sharma OP. Antioxidant properties of curcumin and related compounds. *Biochem Pharmacol*. 1976;25:1811–1825.
7. Shalini VK, Srinivas L. Lipid peroxide induced DNA damage: protection by turmeric *(Curcuma longa)*. *Mol Cell Biochem*. 1987;77:3–10.
8. Srinivas L, Shalini VK. DNA damage by smoke. Protection by turmeric and other inhibitors of ROS. *Free Radical Biol Med*. 1991;11:277–283.
9. Pluta R, Bogucka-Kocka A, Ułamek-Kozioł M, et al. Neurogenesis and neuroprotection in postischemic brain neurodegeneration with Alzheimer phenotype: is there a role for curcumin? *Folia Neuropathol*. 2015;53(2):89–99.
10. Biswas J, Sinha D, Mukherjee S, et al. Curcumin protects DNA damage in a chronically arsenic-exposed population of West Bengal. *Hum Exp Toxicol*. 2010;29:513–524.
11. Shehzad A, Wahid F, Lee YS. Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. *Arch Pharm (Weinheim)*. 2010;343:489–499.
12. Chandra D, Gupta SS. Anti-inflammatory and anti-arthritic activity of volatile oil of *curcuma longa* (Haldi). *Indian J Med Res*. 1972;60:138–142.
13. Arora RB, Kapoor V, Basu N, et al. Anti-inflammatory studies on *Curcuma longa* (turmeric). *Indian J Med Res*. 1971;59:1289–1295.
14. Srimal R, Dhawan B. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J Pharm Pharmacol*. 1973;25:447–452.
15. Mukhopadhyay A, Basu N, Ghatak N, et al. Anti-inflammatory and irritant activities of curcumin analogues in rats. *Agents Actions*. 1982;12:508–515.
16. Ghatak N, Basu N. Sodium curcuminate as an effective anti-inflammatory agent. *Indian J Exp Biol*. 1972;10:235–236.
17. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. *Altern Med Rev*. 2009;14:141–153.
18. Buhrmann C, Mobasheri A, Busch F, et al. Curcumin modulates nuclear factor kappaB (NF-kappaB)-mediated inflammation in human tenocytes in vitro: role of the phosphatidylinositol 3-kinase/Akt pathway. *J Biol Chem*. 2011;286(32):28556–28566.
19. Sahebkar A, Cicero AFG, Simental-Mendía LE, Aggarwal BB, Gupta SC. Curcumin downregulates human tumor necrosis factor-á levels: a systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res*. 2016;107:234–242.
20. Patacchini R, Maggi CA, Meli A. Capsaicin-like activity of some natural pungent substances on peripheral ending of visceral primary afferents. *Arch Pharmacol*. 1990;342:72–77.
21. Soni KB, Kuttan R. Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. *Indian J Physiol Pharmacol*. 1992;36:273–275.
22. Srivastava R, Dikshit M, Srimal RC, et al. Anti-thrombotic effect of curcumin. *Thromb Res*. 1985;40:413–417.
23. Srivastava R, Puri V, Srimal RC, et al. Effect of curcumin on platelet aggregation and vascular prostacyclin synthesis. *Arzneimittelforschung*. 1986;36:715–717.
24. Wongcharoen W, Phrommintikul A. The protective role of curcumin in cardiovascular diseases. *Int J Cardiol*. 2009;133:145–151.
25. Alwi I, Santoso T, Suyono S, et al. The effect of curcumin on lipid level in patients with acute coronary syndrome. *Acta Med Indones*. 2008;40:201–210.
26. Baum L, Cheung SK, Mok VC, et al. Curcumin effects on blood lipid profile in a 6-month human study. *Pharmacol Res*. 2007;56:509–514.
27. Ramirez Bosca A, Soler A, Carrion-Gutierrez MA, et al. An hydroalcoholic extract of *Curcuma longa* lowers the abnormally high values of human-plasma fibrinogen. *Mech Ageing Dev*. 2000;114:207–210.
28. Kiso Y, Suzuki Y, Watanabe N, et al. Antihepatotoxic principles of *Curcuma longa* rhizomes. *Planta Med*. 1983;49:185–187.
29. Lin SC, Lin CC, Lin YH, et al. Protective and therapeutic effects of *Curcuma* xanthorrhiza on hepatotoxin-induced liver damage. *Am J Chi Med*. 1995;23:243–254.
30. Rafatullah S, Tariq M, Al-yahya MA, et al. Evaluation of turmeric *(Curcuma longa)* for gastric and duodenal antiulcer activity in rats. *J Ethnopharmacol*. 1990;29:25–34.
31. Lim GP, Chu T, Yang F, et al. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci*. 2001;21:8370–8377.
32. Sikora E, Bielak-Zmijewska A, Mosieniak G, et al. The promise of slow down ageing may come from curcumin. *Curr Pharm Des*. 2010;16:884–892.
33. Lutomski J, Kedzia B, Debska W. Effect of an alcohol extract and active ingredients from *Curcuma longa* on bacteria and fungi. *Planta Med*. 1974;26:9–19. [German].
34. Panahi Y, Alishiri GH, Parvin S, Sahebkar A. Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: results of a randomized controlled trial. *J Diet Suppl*. 2016;13(2):209–220.
35. Takahashi M, Suzuki K, Kim HK, et al. Effects of curcumin supplementation on exercise-induced oxidative stress in humans. *Int J Sports Med*. 2013;34:1–7.
36. Sugawara J, Akazawa N, Miyaki A, et al. Effect of endurance exercise training and curcumin intake on central arterial hemodynamics in postmenopausal women: pilot study. *Am J Hypertens*. 2012;25(6):651–656. https://doi.org/10.1038/ajh.2012.24. Epub 2012 Mar 15.
37. Akazawa N, Choi Y, Miyaki A, et al. Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutr Res*. 2012;32(10):795–799.
38. Chuengsamarn S, Rattanamongkolgul S, Phonrat B, et al. Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: a randomized controlled trial. *J Nutr Biochem*. 2014;25(2):144–150.
39. Polasa K, Raghuram TC, Krishna TP, et al. Effect of turmeric on urinary mutagens in smokers. *Mutagenesis*. 1992;7:107–109.
40. Kuttan R, Sudheeran PC, Josph CD. Turmeric and curcumin as topical agents in cancer therapy. *Tumori*. 1987;73:29–31.
41. Dhillon N, Aggarwal BB, Newman RA, et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res*. 2008;14(14):4491–4499.
42. Kanai M, Yoshimura K, Asada M, et al. A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer. *Cancer Chemother Pharmacol*. 2011;68(1):157–164.
43. Kanai M, Otsuka Y, Otsuka K, et al. A phase I study investigating the safety and pharmacokinetics of highly bioavailable curcumin (Theracurmin) in cancer patients. *Cancer Chemother Pharmacol*. 2013;71(6):1521–1530.
44. Satoskar RR, Shah SJ, Shenoy SG. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int J Clin Pharmacol Ther Toxicol*. 1986;24:651–654.
45. Chandran B, Goel A. A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis. *Phytother Res*. 2012;26(11):1719–1725.
46. Chin KY. The spice for joint inflammation: anti-inflammatory role of curcumin in treating osteoarthritis. *Drug Des Devel Ther*. 2016;10:3029–3042.
47. Panahi Y, Rahimnia AR, Sharafi M, et al. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. *Phytother Res*. 2014;28(11):1625–1631.
48. Rahimnia AR, Panahi Y, Alishiri G, Sharafi M, Sahebkar A. Impact of supplementation with curcuminoids on systemic inflammation in patients with knee osteoarthritis: findings from a randomized double-blind placebo-controlled trial. *Drug Res (Stuttg)*. 2015;65(10):521–525.
49. Nakagawa Y, Mukai S, Yamada S, et al. Short-term effects of highly-bioavailable curcumin for treating knee osteoarthritis: a random-

ized, double-blind, placebo-controlled prospective study. *J Orthop Sci*. 2014;19(6):933–939.
50. Mirzaei H, Shakeri A, Rashidi B, Jalili A, Banikazemi Z, Sahebkar A. Phytosomal curcumin: a review of pharmacokinetic, experimental and clinical studies. *Biomed Pharmacother*. 2017;85:102–112.
51. Belcaro G, Cesarone MR, Dugall M, et al. Product-evaluation registry of Meriva®, a curcumin-phosphatidylcholine complex, for the complementary management of osteoarthritis. *Panminerva Med*. 2010;52(2 suppl 1):55–62.
52. Appendino G, Belcaro G, Cesarone MR, et al. Efficacy and safety of Meriva, a curcumin-phosphatidylcholine complex, during extended administration in osteoarthritis patients. *Altern Med Rev*. 2010;15:337–344.
53. Deodhar SD, Sethi R, Srimal RC. Preliminary studies on anti-rheumatic activity of curcumin (diferuloyl methane). *Indian J Med Res*. 1980;71:632–634.
54. Amalraj A, Varma K, Jacob J, et al. A novel highly bioavailable curcumin formulation improves symptoms and diagnostic indicators in rheumatoid arthritis patients: a randomized, double-blind, placebo-controlled, two-dose, three-arm, and parallel-group study. *J Med Food*. 2017;20(10):1022–1030.
55. Iqbal U, Anwar H, Quadri AA. Use of curcumin in achieving clinical and endoscopic remission in ulcerative colitis: a systematic review and meta-analysis. *Am J Med Sci*. 2018;356(4):350–356.
56. Hanai H, Iida T, Takeuchi K, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol*. 2006;4:1502–1506.
57. Lang A, Salomon N, Wu JC, et al. Curcumin in combination with mesalamine induces remission in patients with mild-to-moderate ulcerative colitis in a randomized controlled trial. *Clin Gastroenterol Hepatol*. 2015;13(8):1444–1449.
58. Hanai H, Iida T, Takeuchi K, Watanabe F, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol*. 2006;4(12):1502–1506.
59. Kedia S, Bhatia V, Thareja S, et al. Low dose oral curcumin is not effective in induction of remission in mild to moderate ulcerative colitis: results from a randomized double blind placebo controlled trial. *World J Gastrointest Pharmacol Ther*. 2017;8(2):147–154.
60. Masoodi M, Mahdiabadi MA, Mokhtare M, et al. The efficacy of curcuminoids in improvement of ulcerative colitis symptoms and patients' self-reported well-being: a randomized double-blind controlled trial. *J Cell Biochem*. 2018;119(11):9552–9559.
61. Imaizumi A. Highly bioavailable curcumin (Theracurmin): its development and clinical application. *Pharma Nutrition*. 2015;3:123–130.
62. Soleimani V, Sahebkar A, Hosseinzadeh H. Turmeric *(Curcuma longa)* and its major constituent (curcumin) as nontoxic and safe substances: review. *Phytother Res*. 2018;32(6):985–995.
63. Juan H, Terhaag B, Cong Z, et al. Unexpected effect of concomitantly administered curcumin on the pharmacokinetics of talinolol in healthy Chinese volunteers. *Eur J Clin Pharmacol*. 2007;63:663–668.
64. Pavithra BH, Prakash N, Jayakumar K. Modification of pharmacokinetics of norfloxacin following oral administration of curcumin in rabbits. *J Vet Sci*. 2009;10:293–297.
65. Zhang W, Tan TM, Lim LY. Impact of curcumin-induced changes in P-glycoprotein and CYP3A expression on the pharmacokinetics of peroral celiprolol and midazolam in rats. *Drug Metab Dispos*. 2007;35:110–115.
66. Zhang W, Lim LY. Effects of spice constituents on P-glycoprotein-mediated transport and CYP3A4-mediated metabolism in vitro. *Drug Metab Dispos*. 2008;36:1283–1290.
67. Shah BH, Nawaz Z, Pertani SA. Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca2+ signaling. *Biochem Pharmacol*. 1999;58:1167–1172.

74

Dehydroepiandrosterone (DHEA)

Cory Szybala, ND, Mollie Parker Szybala, ND, MPH, Laurie Mischley, ND, PhD, MPH, Paul Nicolai*, ND,* and *Alan R. Gaby*, MD*

OUTLINE

INTRODUCTION

Dehydroepiandrosterone (DHEA) is a steroid hormone secreted primarily by the adrenal glands and, to a lesser extent, by the testes and ovaries, brain, and gastrointestinal tract (Fig. 74.1).[1,2] Circulating levels of DHEA and its ester, DHEA sulfate (DHEAS), are 20 times higher than those of any other adrenal steroid. In the past, no DHEA-specific receptors have been found. Thus this hormone has long been considered to only function as a reservoir from which the body could draw as a precursor for the formation of other hormones. Elucidating a physiological role for DHEA and possible clinical applications for DHEA are an ongoing topic of exploration in human medical research. This has paid off with new research showing direct neurotransmitter regulation, specifically the neurosteroids associated with neuronal excitability.[3]

Although DHEA was first identified in 1934, its clinical use did not achieve prominence until 1993 when French scientists claimed it was a "cure" for aging[4] based on the observation that human DHEA levels peak in the late 20s and decline steadily thereafter. Epidemiological evidence suggests that higher DHEA levels are associated with increased life expectancy[5] and enhanced well-being.[6–8] Persons with a steeper decline and greater variability in DHEAS levels over time have been shown to have a higher death rate, regardless of baseline DHEAS levels.[9] It has therefore been postulated that some of the manifestations of aging may be caused by a decline in DHEA production. DHEA has often been and continues to be used as part of an "antiaging" treatment program.

DHEA production varies profoundly throughout life. Levels begin to increase in children at 8 to 10 years of age (adrenarche) and peak in the second decade of life. Beginning in the early 30s, levels then progressively decline with age, approaching a plateau at approximately 65 to 70 years, until only 20% of peak levels remain.[6,7,10] This decline in DHEA production, often referred to as adrenopause, ultimately results in an 80% loss of total DHEA production compared with that of a young adult. Interestingly, this age-related decline does not occur with other adrenal hormones such as glucocorticoids or mineralocorticoids.[11] In addition to the normal decline associated with aging, other possible causes for a decline in DHEA production include inflammation, many illnesses, and both chronic and subchronic stress.[12]

DHEA BIOCHEMISTRY

DHEA and its sulfate, DHEAS, are the most abundant steroid hormones in the human body and can be found in blood, saliva, urine, and cerebrospinal fluid (CSF).[12] Most DHEAS synthesis occurs in the adrenals via the action of hydroxysteroid sulfotransferase, otherwise known as DHEA sulfotransferase. It is this sulfated form of DHEA that has the longest half-life and is found in the bloodstream in the highest amount, thus leading to the commonly accepted theory that DHEAS serves as a precursor or "buffer" reservoir of DHEA to be used for the intracellular synthesis of various estrogens and androgens throughout the human body. DHEA and DHEAS undergo continuous interconversion, via the sulfation and desulfation process, but because DHEAS represents approximately 80% of the total DHEA pool, has a longer half-life, and lacks the circadian fluctuation seen with DHEA, it is more reliably used in clinical laboratory assessment.[13,14]

Despite DHEA being the major secretory product of the human adrenals, an understanding of DHEA's many physiological roles remains elusive. That is until new research revealed the modulating effects of DHEA and DHEAS on the excitatory neurotransmitter system, as neurosteroids, by modulating the NMDA glutamate receptor, dopamine and serotonin signaling, and inhibition of GABA A receptor activity.[3] Still, assessing whether the many effects of DHEA are a direct result of the hormone, its metabolites, or a combination of the two has been difficult.[15] Although often chemically classified as a weak

*Previous edition contributor

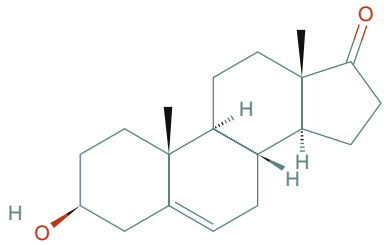


Fig. 74.1 DHEA. (From https://pubchem.ncbi.nlm.nih.gov/compound/Dehydroepiandrosterone.)

androgen, little evidence of direct androgenic activity exists, so any androgenic activity is most likely mediated through its conversion to other more potent androgens. As a person ages and both ovarian and testicular production of hormones declines, this peripheral conversion of DHEA/DHEAS becomes increasingly more important as a source of these androgens.[12] In women, about two thirds of circulating testosterone is produced from peripheral conversion of ovarian and/or adrenal precursors. This percentage is higher in women who have had bilateral oophorectomy.[16]

The biosynthesis of all sex steroids in humans proceeds through DHEA.[17] As a steroid precursor, DHEA is converted to both estrogens and androgens, depending on the requirements of the target tissue. The majority of DHEA conversion to metabolites occurs within peripheral cells via the enzymes aromatase and steroid sulfatase (STS), both of which are present in the majority of human cells (Fig. 74.2).

The effect of endogenous DHEA in humans varies significantly depending on the individual's age, gender, menopausal status, and DHEA levels. On a cellular level, both the target tissue and intracellular enzyme availability shape the fate of the molecule.[18] This process, often referred to as intracrinology, allows for local action of the various hormonal metabolites of DHEA on target tissues such as the brain, bones, breasts, and ovaries without either a specific DHEA receptor or a significant increase in the circulating levels of these metabolites. A specific high-affinity DHEA receptor has not yet been discovered, and there is little evidence that DHEA is able to exert any physiological action without either modulating another receptor type or being converted into an active metabolite or another hormone, with the exception of its recently discovered direct effects on the neurotransmitter system.[3,19,20] In the brain, for example, endogenous DHEA's effects appear to be mediated via a combination of sex steroids, metabolites such as 7 α-hydroxy-DHEA, γ-aminobutyric acid, *N*-methyl-D-asparate, or other receptors.[12] In vitro studies demonstrated that DHEA has the ability to activate estrogen receptors in the prostate at reasonable physiological concentrations, as well as demonstrating an inhibitory effect of DHEA on androgen receptors at much higher concentrations.[19]

DHEA IMMUNOLOGY

Research has shown a significant immune modulation effect, including both immune stimulation and antiglucocorticoid effects.[21] Most of this information must be approached with caution in terms of a clinical perspective, however, due primarily to the fact that a preponderance of the data are from either in vitro studies or in vivo murine studies.

DHEA appears to have a significant regulatory effect on cytokine production in the form of increasing interleukin-2 (IL-2) secretion from TH-1 cells and decreasing production of IL-6 and IL-10 from TH-2 cells.[21] It has also been shown to significantly increase serum insulin-like growth factor-1 (IGF-1) and natural killer (NK) cell numbers in healthy age-advanced men with low DHEAS levels.[22] IL-6 stimulates hepatocytes to produce acute-phase reactants and B-lymphocytes to produce immunoglobulin. In rheumatoid arthritis, levels of IL-6 correlated strongly with both erythrocyte sedimentation rate and rheumatoid factor titers.[23] Both IL-6 and IL-10 were shown to be mediators of inflammatory- and age-related diseases, such as polymyalgia rheumatica, rheumatoid arthritis, inflammatory bowel disease, osteoporosis, and atherosclerosis. These same mediators are implicated as possible contributors to the pathophysiology of Alzheimer's disease, Parkinson's disease, and β-cell malignancies.[24]

It has been theorized that the increase in IL-6 production during the process of aging might be due in part to diminished DHEA secretion.[24] This theory is based on research demonstrating that serum DHEA levels correlated negatively with serum IL-6. Exogenously administered DHEA inhibits IL-6 secretion in a U-shaped fashion, with serum concentrations equaling that of healthy controls exerting the greatest effect.[24]

In one double-blind study, administration of 50 mg/day of DHEA to postmenopausal women produced a twofold increase in NK cell activity and a 6% decrease in the proportion of T-helper cells.[25] The significance of these changes is not entirely clear. Although the increase in NK cell activity might be expected to enhance immune surveillance against cancer and viral infections, the decline in T-helper cells could have adverse consequences. However, because DHEA is known to mediate T-cell responses,[26] the decline in T-helper cells could merely be a reflection of enhanced T-cell function.

DHEA was also shown to have a measurable positive effect in terms of mitigating the immune-suppressive effects of both endogenously produced and exogenously administered glucocorticoids.[21]

CLINICAL APPLICATIONS

Early studies hinted at a wide variety of beneficial effects in conditions such as primary and secondary adrenal insufficiency, mood and self-esteem, diabetes, obesity, decreased immune function, atherosclerosis, and many of the disorders typically associated with normal aging.[27] Although promising, the clinical utility of many DHEA studies may be limited in that they have been overly reliant on animal models, use inconsistent dosing protocols (in some cases doses unachievable in humans), and do not consider the rapid metabolism of DHEA, test subject comorbidities, or organ-specific differences.[28] Rodents have little endogenous DHEA, and their DHEA levels do not decrease with age. DHEA in rodent studies is often administered at supraphysiological doses, which may also make it difficult to determine the clinical relevance of using physiological doses of DHEA in humans. It is the intention of this chapter to base recommendations for the clinical use of DHEA on human studies to the greatest extent possible.

Adrenal Hypofunction/Addison's Disease

Despite the well-documented decline in DHEA levels seen in true adrenal hypofunction, adrenal hormone replacement protocols do not routinely recommend the administration of DHEA.[29–31] Even in the context of adequate glucocorticoid replacement, individuals with reduced adrenal function commonly report symptoms of depression and dysthymia, as well as a general reduction in overall quality of life. Several studies in patients with adrenal insufficiency demonstrated that DHEA replacement might result in an improvement in mood and fatigue, an increase in sexual satisfaction, and a better overall sense of well-being.[32–36]

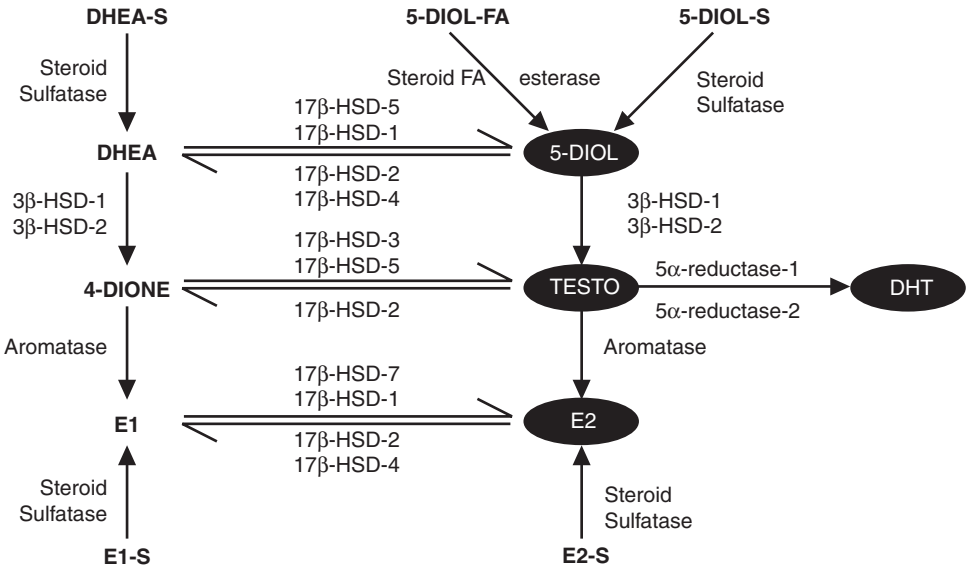


Fig. 74.2 Dehydroepiandrosterone metabolism to other steroid molecules in peripheral tissues. (From Labrie F, Luu-The V, Labrie C, Simard J. DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. *Frontiers Neuroendocrinology* 2001;22:185-212.)

Aging

Preliminary data suggest that DHEA might retard the aging process. For example, in a study of 75 healthy subjects, aged 90 to 106 years, men with the highest DHEAS levels had the highest level of functioning, as assessed by the Katz's scale of Activities of Daily Living (ADL).[37] Several other studies in elderly humans supported an inverse correlation between DHEA levels and the degree of dependence in activities of daily living and mobility. In one study, plasma DHEA levels were low in 80% of male nursing home residents who required total care, compared with 40% in other nursing home residents and 6% in independently living men of comparable age.[7,38]

In men older than 90 years of age, higher DHEAS levels were associated with a higher body mass index and waist-to-hip ratio, taken as indices of the body's energy (fat) reserves. In women older than 90 years of age, DHEAS levels were positively correlated with serum-free triiodothyronine and inversely with triglycerides.[37]

In an attempt to determine whether DHEA supplementation would counteract defects associated with aging, 280 healthy individuals between the ages of 60 and 79 years old were given 50 mg/day of DHEA or placebo for a year. In women older than 70 years of age, there was a reduction in the amount of bone loss and an increase in most libido parameters. Women also showed improvements of skin status, including greater hydration, epidermal thickness, sebum production, and pigmentation.[39]

Although DHEA levels do not appear to correlate significantly with cognitive function,[6,7,40] low serum DHEAS was associated with decreased psychological well-being and symptoms of depression.[7]

Depression

Elevated cortisol levels are well documented in depression, occurring in approximately 50% of cases.[41] Hypercortisolemia has also been shown to impair learning and memory in humans, and prolonged exposure to excess glucocorticoids leads to neuronal atrophy.[42,43] Therefore the neurocognitive deficits observed in depressive disorders may be partially attributable to glucocorticoid hypersecretion. DHEA possesses significant antiglucocorticoid activity,[44] and it may be via this mechanism that administration of DHEA reduces neurocognitive deficits in major depression.[45,46]

An extensive study of depression in adolescents showed approximately 30% had abnormally low DHEA levels,[47] and mean levels of DHEA in the saliva of depressed adults were lower than those in normal control subjects.[41] Improvements in cognition were observed after oral administration of DHEA to middle-aged and elderly depressed patients,[48] and the antidepressant actions of DHEA were demonstrated in several randomized controlled trials.[49,50–53] The ability of DHEA to affect depressive symptomatology seems to be most substantial where DHEA levels are low or in those with an elevated cortisol-to-DHEA ratio.

CSF DHEAS levels are highly correlated with serum levels,[7] and DHEA easily crosses the blood–brain barrier. Evidence also suggests the brain synthesizes DHEA from cholesterol.[54] The ability of DHEA to enhance mood may be related to its role in the central nervous system as a neurosteroid. DHEA supplementation was shown to increase levels of β-endorphins, bind *N*-methyl-D-asparate receptors, act as an antagonist of γ-aminobutyric acid receptors, regulate the excitatory neurotransmitter system, and protect against the deleterious effects of glucocorticoids.[3,49,50,55] In rats, DHEA was shown to increase hypothalamic serotonin levels.[56] These pharmacological properties might help explain the efficacy of DHEA in mood disorders.

New research looking at the effects of DHEA on mood disorders, particularly minor and major depression, has revealed a significant improvement of symptoms in just 3 weeks when subjects were supplementing with 90 mg/day of DHEA. In each study, few adverse events were reported, suggesting this readily available intervention might have clinical utility in some populations.[50–52,57]

Alzheimer's Disease

Unlike peripheral DHEA, which is predominantly converted to estrogens and testosterone, DHEA metabolism in the central nervous system appears to be somewhat unique.[58] A decreased concentration of plasma DHEAS in patients with Alzheimer's disease has been reported,[59–61] and low plasma DHEAS levels appears to be a risk factor for the development of Alzheimer's disease.[59] However, elevated brain tissue and CSF levels of this steroid also appeared to be a risk factor

for the development of Alzheimer's disease.[62] Reduced plasma DHEAS levels did not appear to be associated with the degree of cognitive decline or risk of mortality.[63]

Several small studies using DHEA in the treatment of Alzheimer's disease produced unimpressive results,[64–67] and it remains unclear as to whether different study designs might produce positive results. For instance, one proposed mechanism was that DHEA's antiglucocorticoid effects might offset hippocampal damage associated with elevated basal cortisol levels, and yet another looked at the inhibition of monoamine oxidase (MAO) and NMDA-induced nitric oxide (NO) production in the brain, suggesting an antioxidant effect.[64,65] Despite its unlikely role in cognitive improvement, there continues to be a substantial amount of enthusiasm for the potential role of DHEA as a neuroprotective agent.

Menopause

Practitioners of complementary and alternative medicine (CAM) often recommend including DHEA as part of a postmenopausal hormone therapy regimen to promote well-being, mental acuity, and enhance sexual function. A closer look at the current medical literature revealed that low sexual function in women was associated with low DHEAS more than any other androgen[68] and suggested several possible uses for DHEA in this population.

That said, any discussion pertaining to the use of DHEA in menopausal patients is likely handicapped by the fact that there is often great disparity between the way CAM practitioners prescribe DHEA and the way it is used in studies. CAM practitioners typically recommend smaller daily doses of 5 to 25 mg rather than the 50 mg/day (or more) that is often used in clinical research. Most clinical research also uses DHEA as the sole intervention, something most CAM practitioners would be unlikely to do with actual patients, as they would often employ some combination of estrogens, progesterone, DHEA, and testosterone, while attempting to help a patient improve their diet, exercise patterns, and other aspects of their lifestyle. One study that attempted to explore the use of DHEA in this type of combined manner demonstrated that oral dosing of DHEA at 10 mg/day for 12 months, either alone or in combination with estradiol and micronized progesterone, produced a significant rise in both androgens and β-endorphins, as well as a decrease in cortisol levels. Although intellectually promising and noteworthy, the researchers' decision to measure only biochemical outcomes instead of the physiological outcomes that are useful in clinical practice leaves much still to be determined.[11]

Topical application of 1% DHEA for 4 months resulted in an improvement in several parameters of skin health compared with topical application of the same formulation without the DHEA.[69]

Although evidence regarding the oral administration of DHEA does not tend to demonstrate consistent efficacy in terms of improving libido in postmenopausal women,[70,71] there is promising evidence regarding the use of vaginal DHEA for this purpose. Clinical trial data on DHEA vaginal suppositories demonstrated significant positive effects on libido, arousal, pleasure, and orgasm, as well as improvement in measures of vaginal atrophy, parabasal and superficial cells, pain at sexual activity (dyspareunia), and a lowering of vaginal pH. Also of potential clinical significance is the fact that 3 months of intravaginal DHEA administration did not affect endometrial histology and did not increase serum levels of estradiol, testosterone, or DHEA over those found in normal postmenopausal women.[72–76]

Osteoporosis

A possible relationship between DHEA deficiency and osteoporosis was suggested by a study of women with Addison's disease in whom the onset of menopause was followed by an unusually rapid rate of bone loss. This accelerated bone loss was associated with marked reductions in plasma concentrations of both DHEA and testosterone.[77] A study examining women aged 45 to 69 years determined that 55 of 105 women had low bone mineral density (BMD), and that the average serum DHEAS level was 60% lower in women with low BMD compared with those with normal bones. Also of note is the fact that, although there was no relationship between estrogen levels and BMD in this study, women with low DHEAS values were 40 times more likely to have osteoporosis than were women with normal DHEAS levels.[78] Another study of 29 postmenopausal women demonstrated significant positive correlation between bone mineral content in the distal radius and ulna and age-adjusted serum DHEA levels.[79] More recent studies continue to expand upon these findings by documenting the positive correlation between androgen levels and BMD in postmenopausal women who do not have Addison's disease, thus suggesting an essential role for DHEA, testosterone, or both, in the maintenance of bone mass in postmenopausal women. It appears that free testosterone levels are more significantly correlated with increased bone mass in the lumbar spine, whereas DHEAS levels tend to correlate with increased bone mass in the femoral neck, both notable areas of concern in terms of the prevention and treatment of osteoporosis.[75]

Although testosterone was shown to have receptor sites on osteoblasts, the lack of an analogous receptor for DHEA leaves the mechanism by which DHEA contributes to bone production and maintenance as a matter of some speculation. Possible theories include functioning as a precursor molecule for the formation of estradiol and various androgens in systemic circulation, a direct promotion of bone formation by increasing levels of IGF-1 while inhibiting skeletal catabolic IL-6,[80,81] or possibly an intracrine mechanism involving an interaction with vitamin D3 to enhance the conversion of DHEA to estrone in osteoblasts.[82]

Recommendations pertaining to the clinical use of DHEA for the prevention and treatment of osteoporosis are less clear due to mixed results in controlled trials and a lack of both long-term studies and good data in terms of fracture risk reduction. One double-blind, placebo-controlled study of 50 mg/day DHEA supplementation demonstrated improved bone turnover and decreased osteoclastic activity in women older than 70 years.[39] In another, treatment using transdermal DHEA cream for 1 year in postmenopausal women demonstrated significantly increased serum osteocalcin levels and BMD of the femur, while simultaneously demonstrating a decrease in bone alkaline phosphatase levels.[83] A recent randomized, placebo-controlled trial in 225 healthy adults age 55 to 85 years showed that oral dosing of 50 mg/day DHEA for a year increased lumbar spine BMD in women only. There was no effect on femoral neck or total body BMD in women and no significant changes in BMD were noted at any site in the men studied.[84] A study that selected exclusively men and women with lower levels of DHEA demonstrated a notably different result using the same 50 mg/day dose, in that BMD in the lumbar spine was improved in both men and women compared with placebo, as was hip BMD in women.[85] Unfortunately, neither of these studies looked at or directly mentioned fractures or fracture risk in their subjects, although there was one indirect mention of DHEA supplementation leading to increased serum IGF-1 levels and the positive correlation between lower IGF-1 levels and risk of hip fracture in postmenopausal women.[86]

Erectile Dysfunction

The Massachusetts Male Aging Study, conducted from 1987 to 1989, was the first to describe an inverse correlation between DHEAS levels and the incidence of erectile dysfunction. More than half of the men in this study had minimal, moderate, or complete impotence,[87] and the degree of impotence was directly correlated with lower serum DHEAS

levels. Follow-up research suggested that this was especially true for men younger than 60 years.[88] More recently, it has also been shown that men with higher levels of testosterone and DHEA and decreased levels of interleukin-6 (IL-6) reportedly perceived better general health, emotional support, intimacy, satisfaction with life, resilience, and a better relationship quality, all of which should be considered clinically in any CAM practice.[89]

One double-blind, placebo-controlled study demonstrated that oral DHEA supplementation at 50 mg/day for 6 months resulted in improvement in all five domains of the International Index of Erectile Function, which quantitatively describes an individual's ability to achieve or maintain an erection sufficient for satisfactory sexual performance.[90,91] Response was most substantial in individuals with hypertension or lack of an organic etiology for their condition.[90] There was no effect of DHEA treatment on serum prostate specific antigen, prolactin, testosterone, mean prostate volume, or mean postvoid residual urine volume.[91] A more recent placebo-controlled randomized trial of 50 mg DHEA twice a day demonstrated no benefit compared with either testosterone or placebo.[92]

Cardiovascular Disease

Administration of DHEA reduced the severity of atherosclerosis in cholesterol-fed rabbits.[60] In another rabbit model, low-dose DHEA mitigated the damaging biochemical and structural effects of a high-fat diet.[93] DHEAS was also shown to have digitalis-like activity, accounting for 62% to 100% of the total plasma digitalis-like factors in 11 healthy adults.[64] A study reviewing serum DHEA and DHEA supplementation found that DHEA has antiremodeling and vasorelaxant qualities.[94]

Mean plasma DHEAS levels are significantly lower in men with a history of heart disease than in men without such a history. One study found that in men with no history of heart disease at baseline, a low plasma DHEAS level (<140 mcg/dL) was associated with a more than threefold increase in the age-adjusted risk of death from cardiovascular disease.[95] Similar findings were reported by others,[96–100] although some investigators described only a modest protective effect of DHEA.[101,102]

The association between DHEAS and cardiovascular disease in women is less clear.[103] Cardiovascular death rates might actually be greatest in women with both low and high DHEAS compared with those with intermediate levels.[104,105] Although studies exploring the relationship between endogenous DHEA levels and cardiovascular risk factors have been encouraging in terms of demonstrating decreased carotid intima-media thickness[106] and less atherogenic lipid profiles,[107] recent randomized double-blind trials in hypoadrenal women did not demonstrate this benefit with the administration of oral DHEA. Several studies actually showed a tendency toward more atherogenic lipid profiles in terms of statistically significant decreases in the participant's high-density lipoprotein levels,[102,108] whereas another randomized, double-blind, placebo-controlled study of longer duration found no significant changes in the lipid values of postmenopausal women who took 50 mg/day of oral DHEA for an entire year.[109]

There appear to be numerous reasons to continue the study of DHEA administration for the prevention and treatment of cardiovascular disease, but there are currently few clinical data that support the routine use of DHEA, particularly when given the myriad of both pharmaceutical and complementary and alternative options that are available for this purpose.

Cancer

DHEA supplementation for cancer, although mostly positive, does have a few concerns, particularly in regards to breast cancer and potentially other hormone-related cancers.[110–113] There are premenopausal women with breast cancer that had significantly lower plasma levels of DHEA than age-matched controls without breast cancer, whereas postmenopausal women had significantly higher DHEA levels than age-matched controls.[114] In another study, women with DHEA levels in the highest tertile were 60% less likely to develop breast cancer than were women in the lowest tertile.[115] In a prospective case–control study, serum DHEA and DHEAS levels were significantly lower in individuals who subsequently developed bladder cancer than in those who did not.[116]

These findings suggest that DHEA has anticancer activity and that low DHEA levels may be a risk factor for cancer, except in postmenopausal women. However, it is obvious that further research is required before guidelines can be developed regarding DHEA therapy and cancer. The observation that some postmenopausal women with breast cancer have elevated DHEA levels, as well as the fact that DHEA is converted in part to estrogen and testosterone, should be cause for concern. It is unknown whether the anticancer effects of DHEA are stronger than the potential prostate cancer–promoting effects of additional testosterone or the breast cancer–promoting effects of additional estrogen. This is a complicated issue, because the metabolism of DHEA is usually "cross-gender" (i.e., women get an increase in testosterone whereas estrogen levels often do not change, and vice versa for men). Until these questions can be answered, DHEA therapy should be approached with caution in patients who have either been diagnosed with or who are at risk for developing hormone-dependent cancers.

Rheumatoid Arthritis

Although a 2009 case–control study demonstrated no significant correlation between serum DHEAS levels and the future likelihood of developing rheumatoid arthritis (RA), decreased serum DHEAS concentrations were demonstrated in patients with RA,[117–119] possibly due to increased aromatase activity in the synovial tissue and the subsequent conversion of DHEA into estrogens, which may in turn stimulate the inflammatory process in patients with RA.[120–122] From a clinical perspective, the real issue at hand continues to be whether these low DHEA levels are a consequence of RA rather than a predisposing factor to the disease and whether or not DHEA administration is beneficial in patients with RA.[118]

It is worth noting that several studies on RA demonstrated protective or even therapeutic effects of DHEA in rodents with experimentally induced arthritis.[123,124] One study noted a clear benefit from the administration of about 10 mg/day, an amount roughly equivalent to 2500 mg/day for a 75-kg human being.[124] Despite these potentially encouraging results and the fact that some practitioners have observed patients with RA who exhibited clinical improvement after treatment with DHEA, there are no controlled trials in humans demonstrating either a lack of efficacy or the same positive effects on disease activity that have been shown in murine models of RA.[125]

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic inflammatory connective tissue disorder that occurs predominantly in women. A positive effect of DHEA administration in autoimmune conditions such as SLE is likely due to a decrease in the production of proinflammatory cytokines, such as IL-6, IL-10, and cytokine-mediated antibody, thus DHEA has the potential for a positive effect on the clinical manifestations of antibody-mediated diseases.[24,126,127]

Several studies demonstrated efficacy in patients with SLE when treated with 50 to 200 mg/day DHEA.[127–133] A double-blind, placebo-controlled trial of women with SLE demonstrated that the group receiving DHEA had a significant reduction in the number of disease

flares and serious lupus-related adverse events, as well as improvement in the person's global assessment.[132] However, a recent review of randomized controlled trials comparing DHEA with placebo in persons with SLE demonstrated little to no effect on measures of disease activity, but did demonstrate an improvement in quality-of-life measures.[134] It was also noted that DHEA-treated subjects reported twice the rate of androgenic side effects compared with those given placebo.[135]

Inflammatory Bowel Disease

DHEAS concentrations are decreased in patients with both Crohn's disease and ulcerative colitis (UC).[136] In one study, 200 mg/day DHEA was given orally for 56 days to 7 patients with active Crohn's disease and 13 patients with active UC, all of whom were refractory to conventional drugs. The treatment response rate was 85.7% (6 of 7) for Crohn's disease and 61.5% (8 of 13) for UC. No patient withdrew from the study because of side effects.[137]

The mechanism of action may result from the ability of DHEA to decrease mediators of inflammation. It was shown that DHEA inhibits the activation of nuclear factor-κβ and the secretion of IL-6 and IL-12.[24]

DOSAGE

Although DHEA appears to be safe, the long-term effects of moderate to high dosages are unknown. In the elderly, 50 mg/day will increase DHEA levels into the reference range of young adults.[6] Although some practitioners routinely prescribe 50 mg/day for healthy women and 100 mg/day for healthy men, such doses may be supraphysiological. Dosages of 5 to 25 mg/day for women and 10 to 50 mg/day for men appear to be safe.[35] Topical DHEA can be safely applied to the skin or inserted vaginally, at a dosage of 0.25% to 1%, for up to 3 to 4 months.[76,138] Patients with SLE or other autoimmune diseases sometimes need as much as 200 mg/day or more to obtain benefit. Large doses should be split into twice-daily dosing, usually morning and evening.

Studies show that orally administered DHEA is well absorbed,[139] and that once in circulation, DHEA readily penetrates the blood–brain barrier.[140,141]

TOXICITY

With some exceptions, both oral and topical administration of DHEA appears to be relatively safe for clinical use.[35] In one study, administration of 1600 mg/day for 28 days to healthy volunteers resulted in some degree of insulin resistance but no other significant side effects. A 2009 study giving postmenopausal women 50 mg/day of oral DHEA for a year demonstrated no significant effects on either insulin, glucose, or lipid values.[109] The same study also documented no significant difference in endometrial proliferation compared with placebo. In several SLE studies, 200 mg/day given for a number of months was generally well tolerated, with the exception of mild to moderate acne and occasional mild hirsutism. Another study reported intermittent nausea, perioral dermatitis, subjective feelings of aggressiveness, and intermittent hoarseness.[137] According to several reports, there is a possibility that cardiac arrhythmias and anxiety may be exacerbated by DHEA supplementation.[142,143] A positive association was reported between adrenal androgen levels and breast cancer risk in premenopausal women, particularly those over age 45.[144] Because DHEA is a known precursor to more potent sex hormones, its use should probably be avoided in individuals with hormone-sensitive cancers.

DRUG INTERACTIONS

Estrogen (oral contraceptive pills, hormone replacement therapy), free testosterone, and DHEA levels were reduced in women taking estrogen.[145]

All medications used to treat hormone-sensitive cancers including estrogen receptor downregulators (Fulvestrant) and aromatase inhibitors. These drugs are used to interrupt the growth of hormone-dependent cancers. Aromatase is the enzyme responsible for many of the conversions in the steroid pathway. When taken with DHEA, aromatase inhibitors may increase testosterone levels and reduce estrogen levels. More well-designed studies are needed to confirm this theory.[146]

DHEA may affect how insulin works in the body, and vice versa. Supplementation should be used with caution for those with conditions related to blood sugar dysregulation.[147]

Steroid sulfatase (STS) inhibitors are currently being developed for use in breast cancer therapy. STS controls the hydrolysis of DHEAS.[148]

DHEA may potentiate the action of thyroid hormones.[149]

Antidepressant medications, specifically those that increase serotonin levels. DHEA modulates the release of serotonin in hippocampal neurons and it is hypothesized that an increase in serotonin from supplementation may lead to serious side effects.[150]

SUMMARY

In conclusion, DHEA appears to have potential as a therapy for numerous conditions. However, much remains to be clarified in terms of human applications, so it would seem appropriate to treat this powerful hormone with caution and respect to maximize its benefits and minimize its risks.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Zwaine IH, Yen SS. Dehydroepiandrosterone: biosynthesis and metabolism in the brain. *Endocrinology*. 1999;140(2):880–887.
2. Huang K, Wu LD, Bao JP. Dehydroepiandrosterone and experimental osteoarthritis. *Vitam Horm*. 2018;108:367–384.
3. Perez-Neri I, Montes S, Ojeda-Lopez C, Ramirez-Bermudez J, Rios C. Modulation of neurotransmitter systems by dehydroepiandrosterone and dehydroepiandrosterone sulfate: mechanism of action and relevance to psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32:1118–1130.
4. Holden C. Interest grows in anti-aging drug. *Science*. 1995;269:33.
5. Lasley BL, Santoro N, Randolf JF, et al. The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. *J Clin Endocrinol Metab*. 2002;87:3760–3767.
6. Dhatariya KK, Nair KS. Dehydroepiandrosterone: is there a role for replacement? *Mayo Clin Proc*. 2003;78:1257–1273.
7. Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Res Brain Res Rev*. 1999;30:264–288.
8. Zorumski CF, Paul SM, Izumi Y, Covey DF, Mennerick S. Neurosteroids, stress and depression: potential therapeutic opportunities. *Neurosci Biobehav Rev*. 2013;37:109–122.
9. Cappola AR, O'Meara ES, Guo W, et al. Trajectories of dehydroepiandrosterone sulfate predict mortality in older adults: the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2009;64:1268–1274.
10. Williams PA. *Basic Geriatric Nursing*. 6th ed. Elselvier; 2016:66–68.
11. Pluchino N, Ninni F, Stomati M, et al. One-year therapy with 10 mg/day DHEA alone or in combination with HRT in postmenopausal women: effects on hormonal milieu. *Maturitas*. 2008;59:293–303.
12. Maninger N, Wolkowitz OM, Reus VI, et al. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol*. 2009;30:65–91.
13. Baulieu EE, Robel P. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc Natl Acad Sci U S A*. 1998;95:4089–4091.
14. Liu CH, Laughlin GA, Fischer UG, et al. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for a reduced 17,20-desmolase enzymatic activity. *J Clin Endocrinol Metab*. 1990;71:900–906.
15. Regelson W, Kalimi M. Dehydroepiandrosterone (DHEA)—the multifunctional steroid. II. Effects on the CNS, cell proliferation, metabolic and vascular, clinical and other effects. Mechanism of action? *Ann N Y Acad Sci*. 1994;719:564–575.
16. Shifren JL. The role of androgens in female sexual dysfunction. *Mayo Clin Proc*. 2004;79(suppl 4):S19–S24.
17. Allolio B, Arlt W. DHEA treatment: myth or reality? *Trends Endocrinol Metab*. 2002;13:288–294.
18. Corrigan B. DHEA and sport. *Clin J Sport Med*. 2002;12:236–241.
19. Arnold JT, Blackman MR. Does DHEA exert direct effects on androgen and estrogen receptors, and does it promote or prevent prostate cancer? *Endocrinology*. 2005;146:4565–4567.
20. Webb SJ, Geoghegan TE, Prough RA, et al. The biological actions of dehydroepiandrosterone involves multiple receptors. *Drug Metab Rev*. 2006;38:89–116.
21. Hazeldine J, Arlt W, Lord JM. Dehydroepiandrosterone as a regulator of immune cell function. *J Steroid Biochem Mol Biol*. 2010;120:127–136.
22. Khorram O, Vu L, Yen SS. Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. *J Gerontol A Biol Sci Med Sci*. 1997;52(1):M1–M7.
23. Dasgupta B, Corkill M, Karkham B, et al. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. *J Rheumatol*. 1992;19:22–25.
24. Straub RH, Konecna L, Hrach S, et al. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence. *J Clin Endocrinol Metab*. 1998;83:2012–2017.
25. Casson PR, Andersen RN, Herrod HG, et al. Oral dehydroepiandrosterone in physiologic doses modulates immune function in postmenopausal women. *Am J Obstet Gynecol*. 1993;169:1536–1539.
26. Regelson W, Loria R, Kalimi M. Dehydroepiandrosterone (DHEA)—the mother steroid. I. Immunologic action. *Ann N Y Acad Sci*. 1994;719:553–563.
27. Cameron DR, Braunstein GD. The use of dehydroepiandrosterone therapy in clinical practice. *Treat Endocrinol*. 2005;4(2):95–114.
28. Rutkowski K, et al. Dehydroepiandrosterone (DHEA): hypes and hopes. *Drugs*. 2014;74(11):1195–1207.
29. Chakera AJ, Vaidya B. Addison disease in adults: diagnosis and management. *Am J Med*. 2010;123:409–413.
30. Neary N, Nieman L. Adrenal insufficiency: etiology, diagnosis and treatment. *Curr Opin Endocrinol Diabetes Obes*. 2010;17:217–223.
31. Arlt W. The approach to the adult with newly diagnosed adrenal insufficiency. *J Clin Endocrinol Metab*. 2009;94:1059–1067.
32. Hunt PJ, Gurnell EM, Curran SE, et al. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. *J Clin Endocrinol Metab*. 2000;85:4650–4656.
33. Allolio B, Arlt W, Hahner S. DHEA: why, when, and how much—DHEA replacement in adrenal insufficiency. *Ann Endocrinol (Paris)*. 2007;68(4):268–273.
34. Johannsson G, Burman P, Wirén L, et al. Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. *J Clin Endocrinol Metab*. 2002;87:2046–2052.
35. Gurnell EM, Hunt PJ, Curran SE, et al. Long-term DHEA replacement in primary adrenal insufficiency: a randomized, controlled trial. *J Clin Endocrinol Metab*. 2008;93:400–409.
36. Alkatib AA, Cosma M, Elamin MB, et al. A systematic review and meta-analysis of randomized placebo-controlled trials of DHEA treatment effects on quality of life in women with adrenal insufficiency. *J Clin Endocrinol Metab*. 2009;94:3676–3681.
37. Ravaglia G, Forti P, Maioli F, et al. The relationship of dehydroepiandrosterone sulfate (DHEAS) to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds. *J Clin Endocrinol Metab*. 1996;81:1173–1178.
38. Rudman D, Shetty KR, Mattson DE. Plasma dehydroepiandrosterone sulfate in nursing home men. *J Am Geriatr Soc*. 1990;38:421–427.
39. Baulieu EE, Thomas G, Legrain S, et al. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci U S A*. 2000;97:4279–4284.
40. Barrett-Connor E, Edelstein SL. A prospective study of dehydroepiandrosterone sulfate and cognitive function in an older population: the rancho bernardo study. *J Am Geriatr Soc*. 1994;42:420–423.
41. Michael A, Jenaway A, Paykel ES, et al. Altered salivary dehydroepiandrosterone levels in major depression in adults. *Biol Psychiatry*. 2000;48:989–995.
42. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry*. 2000;57:925–935.
43. Young AH, Sahakian BJ, Robbins TW, et al. The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. *Psychopharmacology (Berl)*. 1999;145:260–266.
44. Kaminska M, Harris J, Gijsbers K, et al. Dehydro-epiandrosterone sulfate (DHEAS) counteracts decremental effects of corticosterone on dentate gyrus LTP. Implications for depression. *Brain Res Bull*. 2000;52:229–234.
45. Young AH, Gallagher P, Porter RJ. Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patients. *Am J Psychiatry*. 2002;159:1237–1239.
46. Gallagher P, Young A. Cortisol/DHEA ratios in depression. *Neuropsychopharmacology*. 2002;26:410.
47. Goodyer IM, Herbert J, Altham PM. Adrenal steroid secretion and major depression in 8- to 16-year-olds, III. Influence of cortisol/ DHEA ratio at presentation on subsequent rates of disappointing life events and persistent major depression. *Psychol Med*. 1998;28:265–273.

48. Wolkowitz OM, Reus VI, Roberts E, et al. Dehydroepiandrosterone (DHEA) treatment of depression. *Biol Psychiatry*. 1997;41:311–318.
49. Wolkowitz OM, Reus VI, Keebler A, et al. Double-blind treatment of major depression with dehydroepiandrosterone. *Am J Psychiatry*. 1999;156:646–649.
50. Demirgoren S, Majewska MD, Spivak CE, et al. Receptor binding and electrophysiological effects of dehydroepiandrosterone sulfate, an antagonist of the GABAA receptor. *Neuroscience*. 1991;45:127–135.
51. Peixoto C, Devicari Cheda JN, Nardi AE, et al. The effects of dehydroepiandrosterone (DHEA) in the treatment of depression and depressive symptoms in other psychiatric and medical illnesses: a systematic review. *Curr Drug Targets*. 2014;15(9):901–914.
52. Schmidt PJ, Daly RC, Bloch M, et al. Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. *Arch Gen Psychiatry*. 2005;62(2):154–162.
53. Bloch M, Schmidt PJ, Danaceau MA, et al. Dehydroepiandrosterone treatment of midlife dysthymia. *Biol Psychiatry*. 1999;45(12):1533–1541.
54. Khorram O. DHEA: a hormone with multiple effects. *Curr Opin Obstet Gynecol*. 1996;8:351–354.
55. Genazzani AR, Stomati M, Bernardi F, et al. Dehydroepiandrosterone as neurosteroid: neuroendocrine effects in post-menopausal women. *J Endocrinol Invest*. 1999;22(suppl 10):19–23.
56. Abadie JM, Wright B, Correa G, et al. Effect of dehydroepiandrosterone on neurotransmitter levels and appetite regulation of the obese Zucker rat. The obesity research program. *Diabetes*. 1993;42:662–669.
57. Rabkin JG, McElhiney MC, Rabkin R, et al. Placebo-controlled trial of dehydroepiandrosterone (DHEA) for treatment of nonmajor depression in patients with HIV/AIDS. *Am J Psychiatry*. 2006;163:59–66.
58. Weill-Engerer S, David JP, Sazdovitch V, et al. In vitro metabolism of dehydroepiandrosterone (DHEA) to 7alpha-hydroxy-DHEA and Delta5-androstene-3beta, 17beta-diol in specific regions of the aging brain from Alzheimer's and non-demented patients. *Brain Res*. 2003;969:117–125.
59. Hillen T, Lun A, Reischies FM, et al. DHEAS plasma levels and incidence of Alzheimer's disease. *Biol Psychiatry*. 2000;47:161–163.
60. Yanase T, Fukahori M, Taniguchi S, et al. Serum dehydroepiandrosterone (DHEA) and DHEASulfate (DHEAS) in Alzheimer's disease and in cerebrovascular dementia. *Endocr J*. 1996;43:119–123.
61. Aldred S, Mecocci P. Decreased dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) concentrations in plasma of Alzheimer's disease (AD) patients. *Arch Gerontol Geriatr*. 2010;51:e16–e18.
62. Brown RC, Han Z, Cascio C, et al. Oxidative stress-mediated DHEA formation in Alzheimer's disease pathology. *Neurobiol Aging*. 2003;24:57–65.
63. Bo M, Massaia M, Zannella P, et al. Dehydroepiandrosterone sulfate (DHEAS) and Alzheimer's dementia in older subjects. *Int J Geriatr Psychiatry*. 2006;21:1065–1070.
64. Leblhuber F, Haller H, Steiner K, et al. DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled trial. *Neurology*. 2004;62:1030.
65. Aly HF, Metwally FM, Ahmed HH. Neuroprotective effects of dehydroepiandrosterone (DHEA) model of Alzheimer's disease. *ACTA Biochimica Polonica*. 2011;58(4):513–520.
66. Wolkowitz OM, Kramer JH, Reus VI, et al. DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. *Neurology*. 2003;60:1071–1076.
67. Knopman D, Henderson VW. DHEA for Alzheimer's disease: a modest showing by a superhormone. *Neurology*. 2003;60:1060–1061.
68. Davis SR, Davison SL, Donath S, et al. Circulating androgen levels and self-reported sexual function in women. *JAMA*. 2005;294:91–96.
69. Nouveau S, Bastien P, Baldo F, et al. Effects of topical DHEA on aging skin: a pilot study. *Maturitas*. 2008;59:174–181.
70. Mendivil Dacal JM, Borges VM. Dehydroepiandrosterone (DHEA), review of its efficiency in the managing of the libido decrease and other symptoms of aging. *Actas Urol Esp*. 2009;33:390–401. [Spanish].
71. Panjari M, Bell RJ, Jane F, et al. The safety of 52 weeks of oral DHEA therapy for postmenopausal women. *Maturitas*. 2009;63:240–245.
72. Labrie F, Archer D, Bouchard C, et al. Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women. *Menopause*. 2009;16:923–931.
73. Labrie F, Archer D, Bouchard C, et al. Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy. *Menopause*. 2009;16:907–922.
74. Labrie F, Archer D, Bouchard C, et al. Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration. *Menopause*. 2009;16:897–906.
75. Labrie F, Cusan L, Bouchard C, et al. Effect of intravaginal DHEA on serum DHEA and eleven of its metabolites in postmenopausal women. *J Steroid Biochem Mol Biol*. 2008;111:178–194.
76. Labrie F, Archer DF, Koltun W, et al. Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause*. 2016;23(3):243–256.
77. Devogelaer JP, Crabbe J, Nagant de Deuxchaisnes C. Bone mineral density in Addison's disease: evidence for an effect of adrenal androgens on bone mass. *BMJ (Clin Res Ed)*. 1987;294:798–800.
78. Szathmari M, Szucs J, Feher T, et al. Dehydroepiandrosterone sulphate and bone mineral density. *Osteoporos Int*. 1994;4:84–88.
79. Brody S, Carlstrom K, Lagrelius A, et al. Adrenal steroids in post-menopausal women: relation to obesity and to bone mineral content. *Maturitas*. 1987;9:25–32.
80. Tok EC, Ertunc D, Oz U, et al. The effect of circulating androgens on bone mineral density in postmenopausal women. *Maturitas*. 2004;48:235–242.
81. Zhou S, Glowacki J. Dehydroepiandrosterone and bone. *Vitam Horm*. 2018;108:251–271.
82. Takayanagi R, Goto K, Suzuki S, et al. Dehydroepiandrosterone (DHEA) as a possible source for estrogen formation in bone cells: correlation between bone mineral density and serum DHEASulfate concentration in postmenopausal women, and the presence of aromatase to be enhanced by 1,25-dihydroxyvitamin D3 in human osteoblasts. *Mech Ageing Dev*. 2002;123:1107–1114.
83. Labrie F, Diamond P, Cusan L, et al. Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women. *J Clin Endocrinol Metab*. 1997;82:3498–3505.
84. von Muhlen D, Laughlin GA, Kritz-Silverstein D, et al. Effect of dehydroepiandrosterone supplementation on bone mineral density, bone markers, and body composition in older adults: the DAWN trial. *Osteoporos Int*. 2008;19:699–707.
85. Jankowski CM, Gozansky WS, Van Pelt RE, et al. Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial. *J Clin Endocrinol Metab*. 2006;91:2986–2993.
86. Garnero P, Sornay-Rendu E, Claustrat B, et al. Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women. *Lancet*. 2000;355:898–899.
87. Feldman HA, Goldstein I, Hatzichristou DG, et al. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol*. 1994;151:54–61.
88. Reiter WJ, Pycha A, Schatzl G, et al. Serum dehydroepiandrosterone sulfate concentrations in men with erectile dysfunction. *Urology*. 2000;55:755–758.
89. Walther A, Mahler F, Debelak R, Ehlert U. Psychobiological protective factors modifying the association between age and sexual health in men: findings from the Men's health 40+ study. *Am J Mens Health*. 2017;11(3):737–747.
90. Reiter WJ, Schatzl G, Mark I, et al. Dehydroepiandrosterone in the treatment of erectile dysfunction in patients with different organic etiologies. *Urol Res*. 2001;29:278–281.
91. Reiter WJ, Pycha A, Schatzl G, et al. Dehydroepiandrosterone in the treatment of erectile dysfunction: a prospective, double-blind, randomized, placebo-controlled study. *Urology*. 1999;53:590–595.

92. Morales A, Black A, Emerson L, et al. Androgens and sexual function: a placebo-controlled, randomized, double-blind study of testosterone vs. dehydroepiandrosterone in men with sexual dysfunction and androgen deficiency. *Aging Male*. 2009;12:104–112.
93. Aragno M, Meineri G, Vercellinatto I, et al. Cardiac impairment in rabbits fed a high-fat diet is counteracted by dehydroepiandrosterone supplementation. *Life Sci*. 2009;85:77–84.
94. Savineau JP, Marthan R, Dumas de la Roque E. Role of DHEA in cardiovascular diseases. *Biochem Pharmacol*. 2013;85(6):718–726.
95. Barrett-Connor E, Khaw KT, Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med*. 1986;315:1519–1524.
96. Mitchell LE, Sprecher DL, Borecki IB, et al. Evidence for an association between dehydroepiandrosterone sulfate and nonfatal, premature myocardial infarction in males. *Circulation*. 1994;89:89–93.
97. Ohlsson C, Labrie F, Barrett-Connor E, et al. Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. *J Clin Endocrinol Metab*. 2010;95:4406–4414.
98. Ponholzer A, Madersbacher S, Rauchenwald M, et al. Vascular risk factors and their association to serum androgen levels in a population-based cohort of 75-year-old men over 5 years: results of the VITA study. *World J Urol*. 2010;28:209–214.
99. Tivesten A, Vandenput L, Carlzon D, et al. Dehydroepiandrosteron and its sulfate predict the 5-year risk of coronary heart disease events in elderly men. *J Am College Cardiol*. 2014;64(17):1801–1810.
100. Wu TT, Chen Y, Zhou Y, et al. Prognostic value of dehydroepiandrosterone sulfate for patients with cardiovascular disease: a systematic review and meta-analysis. *J Am Heart Assoc*. 2017;6(5).
101. Newcomer LM, Manson JE, Barbieri RL, et al. Dehydroepiandrosterone sulfate and the risk of myocardial infarction in US male physicians: a prospective study. *Am J Epidemiol*. 1994;140:870–875.
102. Srinivasan M, Irving BA, Frye RL, et al. Effects on lipoprotein particles of long-term dehydroepiandrosterone in elderly men and women and testosterone in elderly men. *J Clin Endocrinol Metab*. 2010;95:1617–1625.
103. Barrett-Connor E, Goodman-Gruen D. Dehydroepiandrosterone sulfate does not predict cardiovascular death in postmenopausal women. The rancho bernardo study. *Circulation*. 1995;91:1757–1760.
104. Shufelt C, Bretsky P, Almeida CM, et al. DHEAS levels and cardiovascular disease mortality in postmenopausal women: results from the National institutes of health–National heart, lung, and blood institute (NHLBI)-sponsored women's ischemia syndrome evaluation (WISE). *J Clin Endocrinol Metab*. 2010;95:4985–4992.
105. Cappola AR, Xue QL, Walston JD, et al. DHEAS levels and mortality in disabled older women: the Women's Health and Aging Study I. *J Gerontol A Biol Sci Med Sci*. 2006;61:957–962.
106. Jian ZW, Wu SZ, Ruan YJ. Relationship between dehydroepiandrosterone and arteriosclerosis in premenopausal and postmenopausal women. *Nan Fang Yi Ke Da Xue Xue Bao*. 2008;28:942–943. [Chinese].
107. Bednarek-Tupikowska G, Tworowska-Bardzinska U, Tupikowski K, et al. The correlations between endogenous dehydroepiandrosterone sulfate and some atherosclerosis risk factors in premenopausal women. *Med Sci Monit*. 2008;14:CR37–CR41.
108. Srinivasan M, Irving BA, Dhatariya K, et al. Effect of dehydroepiandrosterone replacement on lipoprotein profile in hypoadrenal women. *J Clin Endocrinol Metab*. 2009;94:761–764.
109. Panjari M, Bell RJ, Jane F, et al. A randomized trial of oral DHEA treatment for sexual function, well-being, and menopausal symptoms in postmenopausal women with low libido. *J Sex Med*. 2009;6:2579–2590.
110. Key T, Appleby P, Barnes I, et al. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst*. 2002;94(8):606–616.
111. Morris KT, Toth-Fejel S, Schmidt J, et al. High dehydroepiandrosterone-sulfate predicts breast cancer progression during new aromatase inhibitor therapy and stimulates breast cancer cell growth in tissue culture: a renewed role for adrenalectomy. *Surgery*. 2001;130(6):947–953.
112. Calhoun K, Pommier R, Cheek J, et al. The effect of high dehydroepiandrosterone sulfate levels on tamoxifen blockade and breast cancer progression. *Am J Surg*. 2003;185(5):411–415.
113. Kensler KH, Eliassen AH, Rosner BA, et al. Pre-diagnostic sex hormone levels and survival among breast cancer patients. *Breast Cancer Res Treat*. 2019.
114. Zumoff B, Levin J, Rosenfeld RS, et al. Abnormal 24-hr mean plasma concentrations of dehydroisoandrosterone and dehydroisoandrosterone sulfate in women with primary operable breast cancer. *Cancer Res*. 1981;41:3360–3363.
115. Helzlsouer KJ, Gordon GB, Alberg AJ, et al. Relationship of prediagnostic serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate to the risk of developing premenopausal breast cancer. *Cancer Res*. 1992;52:1–4.
116. Gordon GB, Helzlsouer KJ, Comstock GW. Serum levels of dehydro-epiandrosterone and its sulfate and the risk of developing bladder cancer. *Cancer Res*. 1991;51:1366–1369.
117. Sambrook PN, Eisman JA, Champion GD, et al. Sex hormone status and osteoporosis in postmenopausal women with rheumatoid arthritis. *Arthritis Rheum*. 1988;31:973–978.
118. Deighton CM, Watson MJ, Walker DJ. Sex hormones in postmenopausal HLA-identical rheumatoid arthritis discordant sibling pairs. *J Rheumatol*. 1992;19:1663–1667.
119. Imrich R, Rovensky J, Malis F, et al. Low levels of dehydroepiandrosterone sulphate in plasma, and reduced sympathoadrenal response to hypoglycaemia in premenopausal women with rheumatoid arthritis. *Ann Rheum Dis*. 2005;64:202–206.
120. Castagnetta LA, Carruba G, Granata OM, et al. Increased estrogen formation and estrogen to androgen ratio in the synovial fluid of patients with rheumatoid arthritis. *J Rheumatol*. 2003;30:2597–2605.
121. Rovensky J, Kvetnansky R, Imrich R, et al. Hormone concentrations in synovial fluid of patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 2005;23:292–296.
122. Macho L, Rovensky J, Imrich R, et al. Levels of hormones in plasma and in synovial fluid of knee joint of patients with rheumatoid arthritis. *Cas Lek Cesk*. 2007;146:292–296. [Slovak].
123. Williams PJ, Jones RH, Rademacher TW. Reduction in the incidence and severity of collagen-induced arthritis in DBA/1 mice, using exogenous dehydroepiandrosterone. *Arthritis Rheum*. 1997;40:907–911.
124. Rontzsch A, Thoss K, Petrow PK, et al. Amelioration of murine antigen-induced arthritis by dehydroepiandrosterone (DHEA). *Inflamm Res*. 2004;53:189–198.
125. Offner H, Firestein GS, Boyle DL, et al. An orally bioavailable synthetic analog of an active dehydroepiandrosterone metabolite reduces established disease in rodent models of rheumatoid arthritis. *J Pharmacol Exp Ther*. 2009;329:1100–1109.
126. Linker-Israeli M, Deans JR, Wallace DJ, et al. Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. *J Immunol*. 1991;147:117–123.
127. Chang DM, Chu SJ, Chen HC, et al. Dehydroepiandrosterone suppresses interleukin 10 synthesis in women with systemic lupus erythematosus. *Ann Rheum Dis*. 2004;63:1623–1626.
128. van Vollenhoven RF. Dehydroepiandrosterone for the treatment of systemic lupus erythematosus. *Expert Opinion on Pharmacotherapy*. 2002;3(1):23–31.
129. van Vollenhoven RF, Engleman EG, McGuire JL. An open study of dehydroepiandrosterone in systemic lupus erythematosus. *Arthritis Rheum*. 1994;37:1305–1310.
130. van Vollenhoven RF, Engleman EG, McGuire JL. Dehydro-epiandrosterone in systemic lupus erythematosus. Results of a double-blind, placebo-controlled, randomized clinical trial. *Arthritis Rheum*. 1995;38:1826–1831.
131. van Vollenhoven RF, Park JL, Genovese MC, et al. A double-blind, placebo-controlled, clinical trial of dehydroepiandrosterone in severe systemic lupus erythematosus. *Lupus*. 1999;8:181–187.
132. Chang DM, Lan JL, Lin HY, et al. Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 2002;46:2924–2927.

133. Zhang L, Li B, Ma W, et al. Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEAS) regulate apoptosis during neurogenesis by triggering the Akt signaling pathway in opposing ways. *Brain Res Mol Brain Res*. 2002;98:58–66.
134. Sawalha AH, Kovats S. Dehydroepiandrosterone in systemic lupus erythematosus. *Curr Rheumatol Rep*. 2008;10(4):286–291.
135. Crosbie D, Black C, McIntyre L, et al. Dehydroepiandrosterone for systemic lupus erythematosus. *Cochrane Database Syst Rev*. 2007;4:CD005114.
136. Straub RH, Vogl D, Gross V, et al. Association of humoral markers of inflammation and dehydroepiandrosterone sulfate or cortisol serum levels in patients with chronic inflammatory bowel disease. *Am J Gastroenterol*. 1998;93:2197–2202.
137. Andus T, Klebl F, Rogler G, et al. Patients with refractory Crohn's disease or ulcerative colitis respond to dehydroepiandrosterone: a pilot study. *Aliment Pharmacol Ther*. 2003;17:409–414.
138. Nouveau S, Bastien P, Baldo F, de Lacharriere O. Effects of topical DHEA on aging skin: a pilot study. *Maturitas*. 2008;59(2):174–181.
139. Drucker WD, Blumberg JM, Gandy HM, et al. Biologic activity of dehydroepiandrosterone sulfate in man. *J Clin Endocrinol Metab*. 1972;35:48–54.
140. Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res*. 1997;52:1–32.
141. Schwarz S, Pohl P. Steroid hormones and steroid hormone binding globulins in cerebrospinal fluid studied in individuals with intact and with disturbed blood-cerebrospinal fluid barrier. *Neuroendocrinology*. 1992;55:174–182.
142. Nicolai P. *Personal Communication*; 2004.
143. Sahelian R, Borken S. Dehydroepiandrosterone and cardiac arrhythmia. *Ann Intern Med*. 1998;129:588.
144. Tworoger SS, Missmer SA, Eliassen AH, et al. The association of plasma DHEA and DHEA sulfate with breast cancer risk in predominantly premenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2006;15:967–971.
145. Tazuke S, Khaw KT, Barrett-Connor E. Exogenous estrogen and endogenous sex hormones. *Medicine (Baltimore)*. 1992;71:44–51.
146. Baulieu EE, Thomas G, Legrain S, et al. Dehydroepiandrosterone DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *PNAS*. 2000;97(8):4279–4284.
147. Weiss EP, Villareal DT, Fontana L. Dehydroepiandrosterone (DHEA) replacement decreases insulin resistance and lowers inflammatory cytokines in aging humans. *Aging (Albany NY)*. 2011;3(5):533–542.
148. Purohit A, Woo LW, Chander SK, et al. Steroid sulphatase inhibitors for breast cancer therapy. *J Steroid Biochem Mol Biol*. 2003;86:423–432.
149. McIntosh MK, Berdanier CD. Influence of dehydroepiandrosterone (DHEA) on the thyroid hormone status of BHE/cdb rats. *J Nutr Biochem*. 1992;3:194–199.
150. Sripada RK, Marx CE, King AP, et al. DHEA enhances emotion regulation neurocircuits and modulates memory for emotional stimuli. *Neuropsychopharmacology*. 2013;38(9):1798–1807.

75

Echinacea Species (Narrow-Leafed Purple Coneflower)

Michael T. Murray, ND

OUTLINE

Echinacea spp. (family: *Asteraceae*)
Echinacea angustifolia
Common names: narrow-leafed purple coneflower, black sampson, snakeroot
Echinacea purpurea
Common name: purple coneflower
Echinacea pallida
Common name: pale purple coneflower

GENERAL DESCRIPTION

Echinacea spp. are perennial herbs native to midwestern North America, from Saskatchewan to Texas. The genus derives its name from the Greek *echinos,* meaning "sea urchin." This refers to the prickly scales of the dried seed head portion of the flower. Nine species of echinacea have been taxonomically classified by McGregor on the basis of comparative anatomy and morphology (Table 75.1).[1]

Of the nine species, *Echinacea angustifolia, E. purpurea,* and *E. pallida* are the most commonly used clinically. *E. angustifolia,* with a typical height of up to 2 ft, is shorter than *E. purpurea* (1.5–5 ft) and *E. pallida* (1–3 ft). Another key to species identification is that *E. angustifolia* and *E. purpurea* have yellow pollen, whereas *E. pallida* is noticeably paler and has white pollen. The portions of the plant used for medicinal purposes include the aerial portion, the whole plant including the root, and the root itself. The taproot of *E. angustifolia* can reach a length of 3 to 4 ft.

E. angustifolia has thick, hairy, 1- to 3-inch-long leaves found at the base of a purple seed head shaped like a cone. The only exception to the family of "purple" coneflower is *Echinacea paradoxa,* which has a yellow flower.

CHEMICAL COMPOSITION

Analysis of *Echinacea* spp. has yielded a wide assortment of chemical constituents with pharmacological activities.[2,3] From a pharmacologic perspective, the important constituents of *Echinacea* spp. can be divided into seven categories:

- Polysaccharides
- Alkylamides
- Caffeic acid derivatives
- Flavonoids
- Essential oils
- Polyacetylenes
- Miscellaneous chemicals

In the case of echinacea, it appears that although individual immune-enhancing compounds produce significant effects when they are combined in meaningful amounts, there is an additive effect. The immune-enhancing components of echinacea work together in

TABLE 75.1 Taxonomic Formation of the Genus *Echinacea*

Species	Synonyms
Echinacea angustifolia	*Brauneria angustifolia*
E. atrorubens	*Rudbeckia atrorubens*
E. laevigata	*B. laevigata*
E. pallida	*R. pallida, B. pallida*
E. paradoxa	*B. paradoxa*
E. purpurea	*R. purpurea*
R. hispida	
R. serotina	
E. speciosa	*E. simulata*
E. intermedia	
E. sanguinea	
E. tennesseensis	*B. tennesseensis*

Modified from McGregor RL. The taxonomy of the genus *Echinacea (Compositae). Univ Kansas Sci Bull.* 1968;48:113–142.

a harmonious fashion to produce the phenomena of synergy. A key manner in which echinacea affects immune function is by enhancing the ability of macrophages to engulf and destroy particulate matter. The specific components of echinacea that possess this action are the polysaccharides, alkylamides, and cichoric acid. Although each component is effective alone, the greatest degree of enhancement was noted when the three active components were used in a specific ratio (0.25, 2.5, and 25 mg/mL).[4] This phenomenon of synergy was noted with a clear dose-dependent effect. In other words, the effects with the three actives were greater than any individual active and the higher the dosage, the greater the effect on enhancing macrophage function. A similar effect was noted in the ability of macrophages to detect the presence of foreign matter in the blood and signal the other components of the immune system to mount an attack via interleukin-1 (IL-1) and granulocyte colony-stimulating factor.

Because echinacea contains a wide assortment of chemical constituents with confirmed immune-enhancing effects, this is important for manufacturers to recognize to ensure sufficient levels of all these active compounds. Unfortunately, most echinacea products on the market do not specify the levels of active compounds because they have not been analyzed for them. In addition, when manufacturers do state the level of a particular marker compound, most consumers fail to realize that concentrating only for one particular active compound of echinacea results in the loss of other constituents and, as a result, all of the synergistic effects. For example, some manufacturers standardize for "total phenolic content" or the compound echinacoside.[5] Although these types of echinacea extracts were found to have some antioxidant properties, recent studies found them to have no effect on enhancing immune function in experimental animal studies.[6,7]

The growing understanding of the chemical composition of echinacea requires manufacturers to perform quality control tests not only on the finished product but also on the plant to ensure that it is being grown properly and harvested at the exact time for maximal levels of all active compounds. It is imperative that echinacea be treated properly after harvesting. In addition, studies indicate that a significant amount of the active ingredients are destroyed in the drying process. If the fresh plant material is not processed immediately, the content of several key components—especially cichoric acid and alkylamides—will be low (as much as 80% will be lost).[8–10] Chemical analysis of commercial echinacea preparations demonstrated tremendous variation in the levels of key compounds. For example, one analysis of various commercial echinacea products found that there was not only tremendous variation in the level of cichoric acid, with most products containing either none or little, but even within the same product, there was tremendous interbatch variation.[11]

Polysaccharides

A number of immunostimulatory and mild anti-inflammatory polysaccharides have been isolated from *Echinacea* spp.[2,3,12–15] Most notable are inulin, which is found in a high concentration (5.9%) in *E. angustifolia* root, and the high-molecular-weight (25,000–50,000) polysaccharides found in the aerial part of *E. purpurea.* These components possess significant immune-enhancing properties. Typically, the most potent immune-enhancing polysaccharides are the water-soluble, acidic, branched-chain heteroglycans composed of many types of sugars rather than the polyfructose content of inulin.

Alkylamides

Alkylamides typically exert a tingling sensation on the tongue, which is representative of their mild anesthetic effect. These compounds are found in highest concentrations in the roots. The roots of *E. angustifolia* contain higher concentrations (0.004%–0.039%) than *E. purpurea* (0.009%–0.151%) and *E. pallida* (0.001%).[16,17] Alkylamides are among the most active constituents of echinacea on macrophage function.[18] In a human pharmacokinetic study, alkylamides were detected in plasma 20 minutes after ingestion of an echinacea preparation and indicated that dosing at least three times a day was required to maintain plasma concentrations.[19]

Caffeic Acid Derivatives

Caffeic acid serves as the backbone for a number of important medicinal plant compounds in other plants and *Echinacea* spp. (Fig. 75.1). The first compound believed to be unique to *Echinacea* is echinacoside, a compound eventually shown to be composed of caffeic acid, a caffeic acid derivative (similar to catechol), glucose, and rhamnose, all attached to a central glucose molecule.[20] Echinacoside accumulates in the roots but is also found in smaller concentrations in the flowers. The roots *of E. angustifolia* contain 0.3% to 1.3%, whereas the roots of *E. pallida* contain a similar concentration of 0.4% to 1.7%.[12] It is assumed that *E. purpurea* has similar echinacoside levels as well.

Other caffeic acid derivatives important in the pharmacology of *Echinacea* include cichoric acid, chlorogenic acid, and cynarin.3 Cichoric acid was originally isolated from *E. purpurea* and is found in much higher concentrations in this species compared with *E. angustifolia* and *E. pallida.*[3,21] However, *E. angustifolia* and *E. pallida* have higher amounts of other types of caffeic acid derivatives. These differences are not thought to have much clinical significance; rather, they may prove to be valuable in quick chemical differentiation of species.

Flavonoids

The leaves and stems of *E. angustifolia* and *E. purpurea* have been shown to contain numerous flavonoids, with rutoside being the most abundant.[3] The total flavonoid content (calculated as quercetin) for *E. angustifolia* and *E. purpurea* was 0.48% and 0.38%, respectively.[3]

Essential Oils

The essential oil content varies among the three common species[22]:

- *E. angustifolia* root and leaves contain less than 0.1%.
- *E. purpurea* root contains 0.2%, and flowers and leaves contain 0.6%.
- *E. pallida* root contains up to 2%, and the leaves contain less than 1%.

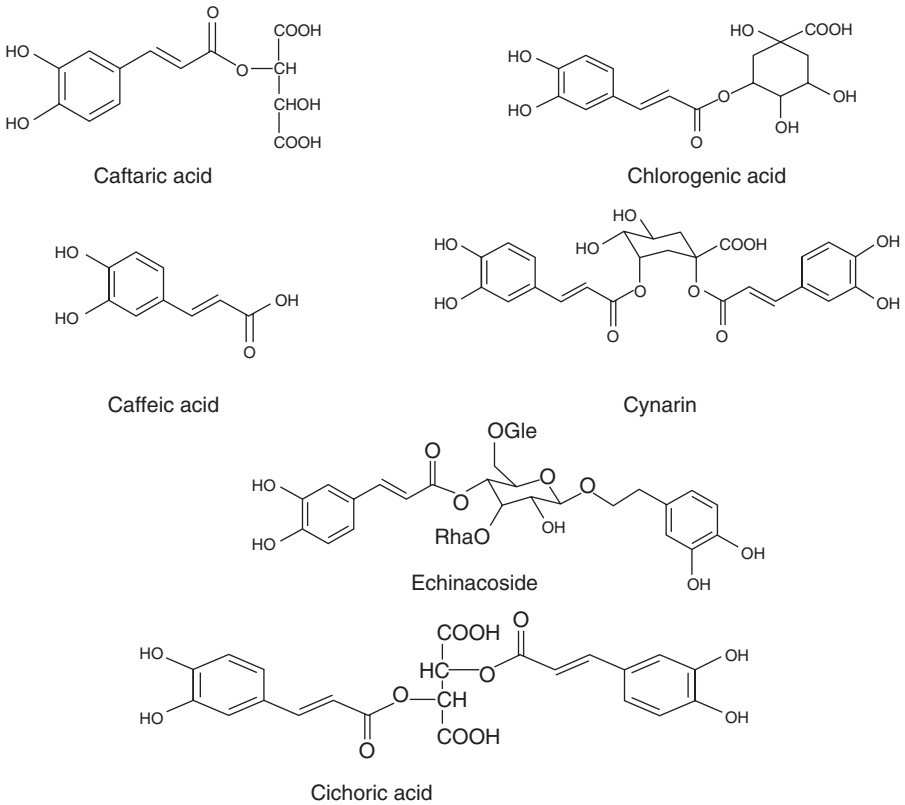


Fig. 75.1 Caffeic acid, Echinacoside, and other caffeic acid derivatives.

Interestingly, in one study, the essential oil content of *E. pallida* root was found to rise to 3.5% to 4% in April and May but fall to 1% to 1.5% for the rest of the year.[23] The major essential oil components are sesquiterpene derivatives, borneol, α-pinine, and related aromatic compounds.[3]

Polyacetylenes

A number of polyacetylenes have been identified from the roots of all three commercial species.[24] The difference in the type of polyacetylene and susceptibility to breakdown may help differentiate which species is best for commercial use. Because the polyacetylenes of *E. pallida* are quite susceptible to autoxidation, *E. angustifolia* may be better for commercial products.[25] Research showed that long-term storage greatly decreases the content of polyacetylenes to only trace levels at best. However, the polyacetylene derivatives of autoxidation of *E. pallida* are quite characteristic and useful in differentiating *E. pallida* from *E. angustifolia*.

MISCELLANEOUS

Undoubtedly, other constituents contribute to the pharmacology of echinacea. The occurrence of a "colorless alkaloid" was first reported by the great John Uri Lloyd in 1897 and substantiated recently by the isolation of the alkaloids tussilagine and isotussilagine.[26] Other compounds isolated from *Echinacea* spp. include the following[3]:

- Resins
- Glycoproteins
- Sterols
- Minerals
- Fatty acids

HISTORY AND FOLK USE

Echinacea was used extensively by the Native Americans living in areas where it grew. American Indians used echinacea against more illnesses than any other plant. The root was used externally for the healing of wounds, burns, abscesses, and insect bites; internally for infections, toothache, and joint pains; and as an antidote for rattlesnake bites.[27]

H.C.F. Meyer, a German lay healer, introduced a commercial product containing echinacea to Americans around 1870. He recommended "Meyer's blood purifier"[3] as a wonder cure for almost every conceivable malady, and there were numerous case reports of successful treatments for snakebites, typhus, diphtheria, and other infections.

E. angustifolia became a favorite with eclectic physicians because it was thought to be greater in activity than other species. Eclectics used it externally as a local antiseptic, stimulant, deodorant, and anesthetic and internally for "bad blood" (i.e., to correct "fluid deprivation with tendency to sepsis and malignancy").[28,29]

Although many physicians began to investigate and use echinacea as a serious medicine, in 1909 the Council on Pharmacy and Chemistry of the American Medical Association refused to recognize echinacea as an active drug, stating: "In view of the lack of any scientific scrutiny of the claims made for it, *Echinacea* is deemed unworthy of further consideration until more reliable evidence is presented in its favor." Despite this opposition, echinacea was included in the National Formulary of the United States and remained there until 1950.[3]

With the demise of the eclectic movement, the popularity of echinacea in the United States waned except among naturopathic physicians until around 1980, when it was rediscovered because of increased consumer interest in immune system disorders such as candidiasis, chronic fatigue syndrome, acquired immunodeficiency syndrome (AIDS), and cancer. Although interest in echinacea decreased in America between the 1930s and 1980s, European physicians continued research. Much

of this research was initiated by a 1932 study by Gerhard Madaus, who demonstrated immune-enhancing effects of a preparation from the fresh juice of the aerial portion of *E. purpurea.* This was followed by the development of a commercial product (Echinacin) and a great deal of scientific study. Thus *E. purpurea* began to be as respected as *E. angustifolia* among herbal practitioners in Europe.[3]

PHARMACOLOGY

The chemistry, pharmacology, and clinical applications of echinacea have been the subject of more than 300 scientific studies.[2,3,30] This section summarizes some of the pharmacologic information on *Echinacea,* with attention to the species used, part of the plant used, solvent used for extraction, and other relevant features. When no species delineation is made, the activity described is similar in all species.

Tissue Regeneration and Anti-inflammatory Properties

Echinacin (a commercial product consisting of the freshly pressed juice of *E. purpurea* stabilized in ethanol) and polysaccharide components of echinacea have been shown to promote tissue regeneration and reduce inflammation in experimental studies.[31] This effect is apparently largely because of inhibition of the enzyme hyaluronidase via formation of a polysaccharide complex with hyaluronic acid, thereby maintaining the structure and integrity of the collagen matrix in connective tissue and ground substance. In addition to increased hyaluronic acid stabilization, echinacea also stimulates fibroblast growth and the manufacture of glycosaminoglycans, a critical goal in wound healing. Echinacea exerts a mild, direct cortisone-like effect and enhances the secretion of adrenal cortex hormones.[3] The polysaccharide portion appears to be responsible for the direct anti-inflammatory effects, although the alkylamide fraction has also demonstrated some activity.[32]

Immunostimulatory Properties

Echinacea possesses a broad spectrum of effects on the immune system as a result of its content of a diverse range of active components affecting different aspects of immune function.[2,3] For example, inulin, the major component in the root of *E. angustifolia,* activates the alternative complement pathway and thus promotes chemotaxis of neutrophils, monocytes, and eosinophils; solubilization of immune complexes; neutralization of viruses; and bacteriolysis. Echinacea also increases the levels of properdin, the normal serum globulin that stimulates the alternative complement pathway.[2,3,33] Another nonspecific immune enhancement is echinacea's enhancement of serum leukocyte and granulocyte counts.[2,3,30–34]

The high-molecular-weight heteroglycan polysaccharide components of echinacea have profound immunostimulatory effects. The majority of these effects appear to be mediated by the binding of active echinacea polysaccharides to carbohydrate receptors on the cell surface of macrophages and T lymphocytes. However, some of the T-cell activation found in early studies is now thought to be a result of a contaminant protein. Later studies using a purer polysaccharide fraction did not show significant results.[3]

Echinacea promotes nonspecific T-cell activation (i.e., transformation, production of interferon, and secretion of lymphokines). The resultant effect is enhanced T-cell mitogenesis, macrophage phagocytosis, antibody binding, natural killer cell activity, and increased numbers of circulating neutrophils.[3,30]

Echinacea polysaccharides were also shown to enhance macrophage phagocytosis and to stimulate macrophages to produce increased amounts of tumor necrosis factor (TNF), interferon, and IL-1; destroy tumor cells in tissue culture; and inhibit *Candida albicans* infection in rats infected intravenously with a lethal dose (3×10^5 cells) of *C. albicans.*[3,34] The interactions with macrophages were most likely responsible for much of the immune system enhancement of echinacea polysaccharides.

In addition to the polysaccharides, lipophilic alkylamides and caffeic acid derivatives like cichoric acid are thought to contribute to the immunostimulatory aspects of echinacea, especially alcoholic extracts.[2,3,30] Although most research has been devoted to the water-soluble components such as polysaccharides, the lipophilic fraction yields the most potent enhancement of macrophage phagocytosis.[3,4]

The carbon clearance test is often used to measure systemic macrophage activation. The method involves measuring the rate of disappearance of carbon granules from the blood at varying intervals after administration of the test substance. Root extracts of echinacea administered orally tend to yield greater effects on phagocytic activity than the aerial portion, with *E. purpurea* greater than *E. angustifolia,* which is greater than *E. pallida.*[3]

Many studies used injectable preparations, but oral preparations are generally thought to yield similar or even better results, although direct comparisons are apparently not available. For example, intramuscular Echinacin administered to healthy males on 4 successive days was shown to increase granulocytic phagocytosis by nearly 50%, whereas the oral administration of an *E. purpurea* root extract at a dose of 30 drops three times a day to healthy males for 5 consecutive days resulted in an increase of 120%.[3] However, this difference might be because of the differing constituents of the forms used. The expressed juice of the aerial portion *E. purpurea,* as found in Echinacin, has lower concentrations of several of the phagocytosis-stimulating compounds characteristic to echinacea, including polysaccharides, alkylamides, and caffeic acid derivatives like cichoric acid, compared with the alcoholic extract.[3]

In general, echinacea appears to offer benefits for all infectious conditions. An exception to this statement may be AIDS. It is unclear at this time if echinacea should be recommended for AIDS. Although this condition is associated with widespread depression of the immune system, presumably because of the human immunodeficiency virus (HIC), stimulation of T-cell replication may also stimulate replication of the virus as well. In addition, echinacea was shown to lower T-helper cells and decrease the ratios of T-helper cells to suppressor cells.[2,3] Although there are anecdotal reports of echinacea's efficacy in HIV-infected individuals, more research is necessary.

Antiviral Properties

The juice of the aerial portion of *E. purpurea,* along with alcoholic and aqueous extracts of the roots, were shown to possess antiviral activity. Some of the viruses inhibited in cell cultures include influenza, herpes, and vesicular stomatitis viruses.[3,35] Although certain echinacea components (e.g., echinacoside, other caffeic acid derivatives, polysaccharides) might block virus receptors on the cell surface, the antiviral effects might also be a result of inhibition of hyaluronidases. The viral-inhibiting action of echinacea was significantly diminished when hyaluronidase was added to the cell cultures.[3] Many organisms secrete hyaluronidase, which increases connective tissue permeability and allows the organism to become more invasive.[36]

Clinically, the inhibition of hyaluronidase coupled with general immunostimulation of echinacea is probably more important than direct antiviral activity. The nonspecific antiviral action of echinacea enhances cytotoxic killing of virus-infected cells and the release of interferon. Interferons bind to cell surfaces, where they stimulate the synthesis of intracellular proteins that block the transcription of viral RNA.

Antibacterial Properties

The direct antibacterial activity of echinacea is quite mild. This is somewhat surprising because echinacea has a long history of effective use in both internal and external bacterial infections. Possibly, it possesses antiinfective properties that prevent bacterial adherence, although this has yet to be determined. Clearly, its clinical efficacy is because of its strong immune-potentiating actions.

Echinacea possesses mild antibacterial action due largely to echinacoside, the complex caffeic acid derivative, found in highest concentrations in the root of *E. angustifolia.* Echinacoside and caffeic acid were shown to have antibacterial action against *Staphylococcus aureus, Corynebacterium diphtheriae,* and *Proteus vulgaris.* Approximately 6.3 mg of echinacoside is equivalent to 10 Oxford units of penicillin.[3,20]

Antineoplastic Activity

Obviously, echinacea possesses indirect, antineoplastic activity via its general immuno-enhancing effects. Specifically important is its stimulation of macrophages to greater cytotoxic activity against tumor cells. (Z)-1,8-pentadecadiene, a lipid-soluble component found in the roots of *E. angustifolia* and *E. pallida,* was shown in vivo to possess significant direct antineoplastic activity.[37]

Endocannabinoid System

The alkamides of echinacea have been reported to have cannabinomimetic properties on both cannabinoid CB1 and CB2 receptors.[2] This effect is related to the structural similarity to the endogenous cannabinoid receptor ligand anandamide. In addition, these alkamides also inhibit the breakdown of anandamide. The clinical application of these effects on the ECS is in the treatment of anxiety and atopic dermatitis (both discussed later in the chapter).

CLINICAL APPLICATIONS

At present, although the results from clinical research are mixed, given the long history, safety, and confirmed immune-enhancing effects in experimental models, and the numerous clinical studies that showed positive results, the clinical applications of echinacea include the following:

- Treatment of the common cold and other viral respiratory tract infections
- Possible prevention of the common cold and viral respiratory tract infections
- Treatment of temporary immune deficiency and increased susceptibility to infections
  - Children attending daycare
  - Adults experiencing undue stress
  - Sport-induced immunodeficiency
- Supportive therapy to enhance the effectiveness of antibiotics in bacterial infections
- Chemotherapy and radiation-induced immune suppression
- Herpes simplex infections
- Anxiety
- Atopic dermatitis

Upper Respiratory Tract Infections

Mixed results from clinical studies with echinacea were most likely because of lack of or insufficient quantity of active compounds. The axiom for the effectiveness of any herbal product is its ability to deliver an effective dose of active compounds. If the product, by chance, had sufficient levels of active compounds, it would be effective. If not, it would likely be no more effective than a placebo. For example, in one double-blind study, 160 subjects were given either echinacea or placebo and then exposed to a common cold virus. Infection occurred in 44 (57%) and illness occurred in 36 (43%) of the echinacea- and placebo-treated subjects, respectively. However, the preparation contained no echinacosides or alkamides and contained only 0.16% cichoric acid.[38] In contrast, in a clinical trial using a well-defined echinacea extract containing alkamides, cichoric acid, and polysaccharides at concentrations of 0.25, 2.5, and 25 mg/mL, respectively, prepared from freshly harvested *E. purpurea* plants (commercially available as Echinilin or Echinamide), showed excellent results.[39] In this randomized, double-blind, placebo-controlled trial, 282 subjects, 18 to 65 years old, with a history of two or more colds in the previous year but otherwise in good health, were randomized to receive either echinacea extract or placebo. They were instructed to start the echinacea or placebo at the onset of the first symptom related to a cold, consuming 10 doses the first day and 4 doses per day on subsequent days for 7 days. The severity of symptoms (10-point scale: 0, minimum; 9, maximum) and dosing were recorded daily. A nurse examined the subjects on the mornings of days 3 and 8 of their cold. A total of 128 subjects contracted a common cold (59 echinacea, 69 placebo).

The total daily symptom scores were found to be 23.1% lower in the echinacea group than in the placebo group. Throughout the treatment, the response rate to treatments was greater in the echinacea group. This study indicated that early intervention with a standardized formulation of echinacea resulted in reduced symptom severity in subjects with naturally acquired upper respiratory tract infection.

Again, to highlight the issue of quality control and source of preparation, several studies with less well-defined echinacea products showed little benefit, especially in experimentally induced rhinovirus infections. In another double-blind study, 302 volunteers from four military institutions and one industrial plant in Germany were given either a placebo or alcohol-based tinctures from either *E. purpurea* or *E. angustifolia* dried root for 12 weeks.[40] The main outcome measure was time until the first upper respiratory tract infection. The secondary outcome measures were the number of participants with at least one infection, global assessment, and adverse effects. The time until occurrence of the first upper respiratory tract infection was 66 days in the *E. angustifolia* group, 69 days in the *E. purpurea* group, and 65 days in the placebo group. In the placebo group, 36.7% had an infection, whereas in the *E. angustifolia* group, it was 32%, and in the *E. purpurea* group, it was 29.3%. These results indicated that there was no significant benefit with either form of echinacea, although there was an approximately 20% reduced risk of infection in the echinacea groups. In addition, 70% of the *E. purpurea* and 78% of the *E. angustifolia* group felt they had benefited from treatment compared with 56% in the placebo group. The *E. angustifolia* group had a slightly higher percentage of subjects experiencing side effects (18%) compared with the *E. purpurea* (10%) and placebo (11%) groups.

In one of the most detailed clinical trials, 719 patients were assigned to 1 of 4 parallel groups: no pills, placebo pills (blinded), echinacea pills (blinded), or echinacea pills (unblinded, open-label). Echinacea groups received 8 tablets on the first day and 4 tablets per day on the subsequent 4 days, each containing the equivalent of 675 mg of alcoholic extract of *E. purpurea* root (of dried roots) and 600 mg of alcoholic extract *E. angustifolia* root; the placebo group received the same number of tablets. The primary end point was defined as the "area under the curve" for global severity with duration and severity. Severity was assessed by the Wisconsin Upper Respiratory Symptom Survey-21 questionnaire and the duration by the participants' impression of having a cold. The results showed only a statistically insignificant trend in reduction of the cold duration by half a day and a reduction of severity of approximately 10%.[41]

The result from this trial indicated that a major issue with some of the echinacea research might be the echinacea preparations used (i.e., weak ethanol-based tinctures derived from dried root). Clinical studies in upper respiratory tract infections done on extracts of fresh *E. purpurea* whole plant or aerial plant, especially in liquid form, are consistently positive compared with those using dried echinacea species extracts (or powdered herbs), especially in solid forms (tablets or capsules). It is possible that echinacea may exert direct local effects and that its contact with the oropharyngeal lymphatic tissue is extremely important in acute upper respiratory tract infection conditions. Reasonably large and well-designed, double-blind, placebo-controlled studies found that preparations of echinacea from the aerial portion of the plant produced modest effects in aborting, and reducing, the symptoms and duration of colds.[30,40,42]

In addition to the previously described study with a well-defined echinacea preparation from the aerial portion of *E. purpurea,* another study showed good results when 108 patients with the initial symptoms suggesting a cold received either EchinaGuard—an extract of the fresh-pressed juice of *E. purpurea*—at a dosage of 4 mL twice daily or placebo for 8 weeks.[43] The percentages of patients who remained healthy were 35.2% of the echinacea group and 25.9% of the placebo group. The length of time between infections was 40 days for the echinacea group and 25 days for the placebo group. When infections did occur in patients receiving echinacea, they were less severe and resolved more quickly. Patients showing evidence of a weakened immune system (CD4/CD8 ratio <1.5) benefited the most from echinacea.

The results from another trial were especially encouraging because it also suggested that echinacea could not only make colds shorter and less severe, but it could also sometimes stop a cold that was just starting.[44] In this study, 120 people were given a preparation from the fresh-pressed juice of *E. purpurea* or a placebo as soon as they started showing signs of getting a cold. Participants took either echinacea or placebo at a dosage of 20 drops every 2 hours for 1 day, then 20 drops 3 times a day for 9 more days. Fewer people in the echinacea group felt that their initial symptoms actually developed into "real" colds (40% of those taking echinacea vs. 60% taking the placebo actually became ill). Also, among those who did come down with "real" colds, improvement in the symptoms started sooner in the echinacea group (4 days instead of 8 days). Both results were statistically significant. However, echinacea's ability to shorten the duration of colds was more dramatic.

Not all studies of the fresh-pressed juice of *E. purpurea* or EchinaGuard showed positive effects in reducing the duration or severity, or both, of upper respiratory infections. For example, a study in children 2 to 11 years old was particularly disappointing because the results indicated that it was not only ineffective, but its use was also associated with an increased risk of rash.[45]

In another double-blind trial, 128 patients received 100 mg of the freeze-dried pressed juice from the aerial portion of *E. purpurea* or a placebo three times daily until cold symptoms were relieved or until the end of 14 days, whichever came first.[46] Symptoms (sneezing, nasal discharge, nasal congestion, headache, sore or scratchy throat, hoarseness, muscle aches, and cough) were scored subjectively by the patient and recorded daily in a diary. No statistically significant difference was observed between treatment groups for either total symptom scores or mean individual symptom scores. The time to resolution of symptoms was also not statistically different. The failure in this trial might have been a result of the previously mentioned lack of direct contact with the oropharyngeal lymphatic system.

Clearly, more research using well-characterized echinacea preparations at appropriate dosages is necessary in well-designed trials. Currently, the "gold standard" for evaluating cold remedies involves inoculating healthy individuals with rhinovirus. Although the concentration of viral assault is much greater than what one might encounter in the real world, any substance showing efficacy in this model is regarded as being highly efficacious. In one study, 48 healthy adults received the fresh-pressed juice of *E. purpurea* (EchinaGuard) or placebo, 2.5 mL 3 times per day, for 7 days before and 7 days after intranasal inoculation with rhinovirus (RV-39).[47] A total of 92% of echinacea recipients and 95% of placebo recipients were infected. However, colds developed in 58% of echinacea recipients compared with 82% of placebo recipients. Although administration of echinacea before and after exposure to rhinovirus did not decrease the rate of infection, it did appear to reduce the clinical development of a cold. However, because of the small sample size, statistical hypothesis testing had relatively poor power to detect statistically significant differences in the frequency and severity of illness. As mentioned previously, the only other experimental rhinovirus infection and echinacea study was seriously marred because the preparation used contained no active components.[38]

Candidiasis

Echinacea's effect against *C. albicans* noted in animal studies was confirmed in several human clinical studies.[3] A study featured in Table 75.2 demonstrated that Echinacin greatly accentuated the efficacy of a topical antimycotic agent (econazole nitrate), decreasing reoccurrence from 60.5% to 5% to 16.7%. The researchers used standardized skin tests to show that this enhancement was a result of echinacea's boosting of cell-mediated immunity.[48] Also of interest was the similarity in the efficacies of both the oral and injectable forms.

Wound Healing

Several uncontrolled clinical studies substantiated echinacea's wound-healing activities.[3,37,49,50] The largest (4598 patients) demonstrated that a salve of the juice of the aerial portion of *E. purpurea* had an 85% overall success rate in the treatment of inflammatory skin conditions, such as abscesses, folliculitis, wounds of all kinds, eczema, burns, herpes, and varicose ulcers of the leg.[3]

Arthritis

Echinacea's anti-inflammatory activity was shown in uncontrolled studies to be useful in rheumatoid arthritis. In one study, 15 drops of Echinacin three times daily resulted in a 21.8% decrease in inflammation. Although this improvement was less than cortisone (42%) and prednisone (49.2%), no side effects were associated with Echinacin, whereas both of these drugs have well-known side effects.[51]

TABLE 75.2 Treatment of Recurrent Candidiasis with Echinacin—Rate of Reoccurrence at 6 Months

Therapeutic Scheme	No. Of Patients	Recurrence Rate (%)
Topical antimycotic alone	43	60.5
Topical antimycotic + subcutaneous Echinacin	20	15
Topical antimycotic + intramuscular Echinacin	60	5
Topical antimycotic intravenous Echinacin	20	15
Topical antimycotic oral Echinacin	60	16.7

Modified from Coeugniet EG, Kuhnast R. Recurrent candidiasis: adjuvant immunotherapy with different formulations of echinacin. *Therapiewoche*. 1986;36:3352–3358.

Cancer

Several studies noted a stimulatory effect of echinacea on leukocyte counts in patients receiving radiation for cancer therapy.[49,52] A study using the commercial preparation Esberitox demonstrated that 85% of 55 patients showed a stabilization of leukocyte counts compared with the control group, which showed a steady decline in levels (starting at 6000 and decreasing to 2500 after 45 days).[49] This strongly supports the recommendation of echinacea to patients undergoing orthodox cancer treatments. In an open prospective study with matched historical controls, an injection of polysaccharide fraction isolated from the herb *E. purpurea* was shown to counteract the undesired effects of chemotherapy.[53] Fifteen patients with advanced gastric cancer, who underwent palliative chemotherapy with etoposide, leucovorin, and 5-fluorouracil, received daily intravenous injections of 2 mg of a polysaccharide fraction isolated from *E. purpurea* herb cell cultures for 10 days (beginning 3 days before chemotherapy). The median number of leukocytes 14 to 16 days after chemotherapy was 3630/mL in the patients who received echinacea polysaccharide compared with 2370/mL (870–3950) in the patients of the historical control group. These results suggest that echinacea might be effective in reducing chemotherapy-induced leukopenia.

Anxiety

The anxiolytic effects of echinacea alkamides were demonstrated in animal studies. In rats, an alkamide-rich preparation of *E. angustifolia* root decreased anxiety in the elevated plus-maze and ameliorated contextual conditioned fear models with no toxicity or adverse effects on behavior noted even when rats treated at dosages of 1000 and 3000 mg/kg. The extract was without effect in tests of locomotion (open-field), memory (object recognition), and rewarding potential (conditioned place preference) within a wide dose range. In human studies, 20 or 40 mg of the same *E. angustifolia* extract were administered for 1 week to volunteers scoring high on the State-Trait Anxiety Inventory (STAI). The dosage of 40 mg per day decreased STAI scores within 3 days in human subjects, an effect that remained stable for the duration of the treatment (7 days) and for the 2 weeks that followed treatment. The lower dose, 20 mg per day, did not affect anxiety significantly.[54]

Atopic Dermatitis

In the skin, the main physiological function of the ECS is to control the proliferation, differentiation, survival, and immune competence and/or tolerance of skin cells. Loss of ECS exacerbates allergic skin reactions, whereas enhanced ECS function leads to alleviation of these same symptoms. To prove the benefit of a topically applied alkamide-rich *E. purpurea* extract, an in vitro study was conducted on keratinocytes followed by an assessment of cutaneous tolerability, clinical efficacy, and the effects on stratum corneum structure and lipid in subjects suffering from atopic dermatitis.[55]

The study demonstrated a significant anti-inflammatory activity of the alkamide-rich extract, a low allergenic potential in the study of volunteers similar to placebo skin creams, no phototoxicity, and an ability to reduce the main dermatological symptoms associated with atopic dermatitis (erythema, edema/papules, weeping/crusts, excoriation, lichenification, dryness, pruritus) in a 3-month double-blind study involving 60 subjects with subacute or chronic atopic dermatitis. The improvements were sustained for up to 85 days.

An explanation of the long-standing improvements was offered in the clinical assessment of skin barrier function, levels of overall lipids, ceramide, and cholesterol. These were all significantly increased from baseline at day 15 following a twice-daily application of the alkamide-rich cream.

Snakebites

Echinacea has quite a reputation among naturopathic physicians and Native American healers for the treatment of snakebites. No controlled clinical studies of this use have been reported, but echinacea's inhibition of hyaluronidase might account for much of its reputed efficacy because most snake venoms permeate the system as a result of hyaluronidase in the venom breaking down the connective tissue of the ground substance.

COMMERCIAL PREPARATIONS

As evident from the previous discussion, determining which echinacea preparation is best is difficult. It is not only difficult to determine which species is most effective, but also the portion of the plant used and how it is prepared are serious issues. Dosage recommendations for all currently available forms follow, along with a few observations regarding the "preparations controversy."

Another problem that needs to be addressed is quality control. Since as early as 1904, many commercial sources of echinacea have contained adulterants and no echinacea. For example, it was estimated that because of supplier errors in collection, more than 50% (and possibly as high as 90% at times) of the echinacea sold in the United States from 1908 to 1991 was actually *Parthenium integrifolium,* or Missouri snakeroot.[54] Some suggested that this adulteration was because of confusion of the common names. Others pointed out that although the *P. integrifolium* plant looks quite different, "once the root is cut and sifted it has an uncanny resemblance to *E. angustifolia* or *E. pallida* roots, though it possesses its own characteristic flavor and fragrance."[56] From practical and clinical viewpoints, physicians should require adequate documentation from suppliers that they are, in fact, supplying echinacea and its species.

Species

Although studies have shown various echinacea species, or components found in higher concentrations in one species, to be more effective than others, each commercial species has its advantages and disadvantages. No "best" species can be recommended at this time because differing experimental models have yielded inconsistent results. Rather, the clinician must recognize the unique value of each species. Although *E. angustifolia* has long been considered the best species and to possess the greatest activity, some studies dispute this. For example, several studies showed *E. purpurea* to demonstrate greater enhancement of phagocytosis. In one study, an aqueous extract of *E. angustifolia* did not demonstrate any effect on phagocytic function in rats, whether it was administered orally, intraperitoneally, or intravenously.[57] Because *E. purpurea* is the easiest to grow commercially, it may become the most used in the United States, as it is in Europe.

Part of the Plant to Use

The portion of the plant that possesses the greatest immune-enhancing properties depends on the experimental model. The key points, as described earlier, are that various echinacea components exert immune-enhancing effects, and there is clearly a synergistic effect among these constituents.

Preparations

Echinacea products are available in many different forms:

- Crude plant in either ground or powdered form
- Freeze-dried
- Alcohol-based tinctures and liquid extracts
- Aqueous tinctures and liquid extracts
- Dry, powdered alcoholic or aqueous

It is popular to standardize hydro-/alcoholic extracts for echinacoside (for *E. angustifolia*) or cichoric acid (for *E. purpurea*). Although these extracts are thought by their proponents to be the most potent, it must be noted that other components have shown greater effects in some experimental models, and even 4 to 10× homeopathic preparations have been shown to produce activity.[32]

Solvents

Which solvent is best to use? Again, this question is extremely difficult to answer because both hydrophilic and lipophilic components have been shown to possess immune-enhancing activities. Even a small amount of ethanol results in the precipitation or breakdown of the immuno-active polysaccharides, suggesting that aqueous extracts may be best. However, an aqueous extract would leave behind valuable lipophilic immune-enhancing alkylamides and caffeic acid derivatives. To optimize an extract's immune-enhancing effects, many manufacturers use low-ethanol (10%–20%) hydro-/alcoholic mixtures or combine a low-ethanol extract with a high-ethanol extract. Both products typically contain both polar and lipophilic compounds.

Dosage

Box 75.1 lists dosages of echinacea as a general immune stimulant during infection. The question of whether echinacea should be used on a long-term or continual basis really depends on the need. In a healthy individual with no apparent depression of the immune system, continual administration is certainly not indicated. However, it appears that individuals with impaired immune function do benefit from long-term use. The usual recommendation with long-term use is 8 weeks on, followed by 1 week off, but there is no real basis for the week off.

Toxicology

When used at the recommended doses, there is no danger of toxicity because no studies have reported acute or chronic toxicity reactions caused by echinacea extracts. Echinacin, given intravenously, resulted in fever (0.5°C–1°C elevation in body temperature) on occasion. This was presumably a result of secretion of interferon-α and IL-1 by activated macrophages.[3]

The median lethal dose (LD_{50}) of intravenous Echinacin was determined to be 50 mL/kg body weight in mice and rats. The polysaccharides in *E. purpurea* (aerial portion) were shown to have an LD_{50} of 1000 to 2500 mg/kg when given peritoneally to mice. Long-term administration of Echinacin to rats at doses many times the human therapeutic doses gave no evidence of any toxic effects. Mutagenic tests have demonstrated no mutagenic activity.[2,3]

BOX 75.1 Dosages (Three Times/Day) as a General Immune Stimulant During Infection

Dried root (or as tea): 0.5 to 1 g
Freeze-dried plant: 325 to 650 mg
Juice of aerial portion of *Echinacea purpurea* stabilized in 22% ethanol: 2 to 3 mL (0.5–0.75 tsp)
Tincture (1:5): 2 to 4 mL (1–2 tsp)
Fluid extract (1:1): 1 to 2 mL (½-1 tsp)
Solid (dry-powdered) extract (6.5:1 or 3.5% echinacoside): 150 to 300 mg

Modern reviews of the safety and efficacy of *Echinacea* spp. found no toxicity in both adults and children in cases of both acute and long-term administration.[2,30,58–60] Given the number of echinacea doses consumed yearly (>10 million) and the number of adverse events (<100), echinacea appears extremely safe. Reported side effects are also uncommon and usually limited to minor gastrointestinal symptoms, increased urination, and mild allergic reactions. However, severe allergic reactions have occurred occasionally, some of them life threatening. Allergic reactions were often reported in people who were also allergic to other members of plants in the daisy *(Compositae)* family (e.g., daisy, ragweed, marigolds).

The German Commission E warns against using echinacea in cases of autoimmune disorders, such as multiple sclerosis, lupus, and rheumatoid arthritis. These warnings are theoretical because there is no evidence that echinacea use has actually harmed anyone with these diseases.

Echinacea appears to be safe even for pregnant or lactating women based on both animal studies and evaluation studies in women using echinacea during pregnancy showing no harmful effects.[61]

Drug Interactions

Echinacea preparations appear to have a low potential for drug interactions.[62] Currently, there are no verifiable reports of drug–herb interactions with any echinacea product. Theoretically, echinacea may interfere with drugs that are purposely used to suppress the immune system. These include cyclosporin, which is used in patients who have had organ transplants to prevent the immune system from rejecting the transplanted organ.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. McGregor RL. The taxonomy of the genus *Echinacea (Compositae)*. *Univ Kansas Sci Bull*. 1968;48:113–142.
2. Manayi A, Vazirian M, Saeidnia S. *Echinacea purpurea*: pharmacology, phytochemistry and analysis methods. *Pharmacogn Rev*. 2015;9(17):63–72.
3. Bauer R, Wagner H. Echinacea species as potential immunostimulatory drugs. In: Wagner H, Farnsworth NR, eds. *Economic and Medicinal Plant Research*. Vol. 5. New York: Academic Press; 1986:253–321.
4. Goel V, Chang C, Slama J, et al. Echinacea stimulates macrophage function in the lung and spleen of normal rats. *J Nutr Biochem*. 2002;13:487.
5. Perry NB, Burgess EJ, Glennie VL. Echinacea standardization: analytical methods for phenolic compounds and typical levels in medicinal species. *J Agric Food Chem*. 2001;49:1702–1706.
6. Pellati F, Benvenuti S, Magro L, et al. Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. *J Pharm Biomed Anal*. 2004;35:289–301.
7. Rininger JA, Kickner S, Chigurupati P, et al. Immunopharmacological activity of echinacea preparations following simulated digestion on murine macrophages and human peripheral blood mononuclear cells. *J Leukoc Biol*. 2000;68:503–510.
8. Perry NB, van Klink JW, Burgess EJ, et al. Alkamide levels in *Echinacea purpurea*: effects of processing, drying and storage. *Planta Med*. 2000;66:54–56.
9. Kim HO, Durance TD, Scaman CH, et al. Retention of caffeic acid derivatives in dried *Echinacea purpurea*. *J Agric Food Chem*. 2000;48:4182–4186.
10. Stuart DL, Wills RB. Effect of drying temperature on alkylamide and cichoric acid concentrations of *Echinacea purpurea*. *J Agric Food Chem*. 2003;51:1608–1610.
11. Bauer R. Standardization of *Echinacea purpurea* expressed juice with reference to cichoric acid and alkamides. *J Herbs Spices Medicinal Plants*. 1999;6:51–61.
12. Wagner H, Proksch A, Riess-Maurer I, et al. Immunostimulating action of polysaccharides (heteroglycans) from higher plants. *Arzneimittelforschung*. 1985;35:1069–1075. [German].
13. Stimpel M, Proksch A, Wagner H, et al. Macrophage activation and induction of macrophage cytotoxicity by purified polysaccharide fractions from the plant *Echinacea purpurea*. *Infect Immun*. 1984;46:845–849.
14. Luettig B, Steinmuller C, Gifford GE, et al. Macrophage activation by the polysaccharide arabinogalactan isolated from plant cell cultures of *Echinacea purpurea*. *J Natl Cancer Inst*. 1989;81:669–675.
15. Tubaro A, Tragni E, Del Negro P, et al. Anti-inflammatory activity of a polysaccharide fraction of *Echinacea angustifolia*. *J Pharm Pharmacol*. 1987;39:567–569.
16. Clifford LJ, Nair MG, Rana J, et al. Bioactivity of alkamides isolated from *Echinacea purpurea* (L.) Moench. *Phytomedicine*. 2002;9:249–253.
17. Bauer R, Remiger P. TLC and HPLC analysis of alkylamides in echinacea drugs. *Planta Med*. 1989;55:367–371.
18. Goel V, Chang C, Slama JV, et al. Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. *Int Immunopharmacol*. 2002;2:381–387.
19. Matthias A, Penman KG, Matovic NJ, et al. Bioavailability of echinacea constituents: Caco-2 monolayers and pharmacokinetics of the alkylamides and caffeic acid conjugates. *Molecules*. 2005;10:1242–1251.
20. Stoll A, Renz J, Brack A. Antibacterial substances II. Isolation and constitution of echinacoside, a glycoside from the roots of *Echinacea angustifolia*. *Helv Chim Acta*. 1950;33:1877–1893.
21. Bauer R, Reminger P, Alstat E. Alkamides and caffeic acid derivatives from the roots of *Echinacea tennesseensis*. *Planta Med*. 1990;56:533–534.
22. Neugebauer H. The constituents of echinacea. *Pharmazie*. 1949;4:137–140.
23. Heinzer F, Meusy JP, Chavanne M. *Echinacea pallida*, *Echinacea purpurea*. Follow-up of weight development and chemical composition for the first two culture years. *Proceedings of the 36th Annual Congress of the Society of Medicinal Plant Research*. Freiburg, Germany; 1988.
24. Schulte KE, Rucker G, Perlick J. The presence of polyacetylene compounds in *Echinacea purpura* Mnch and *Echinacea angustifolia* DC. *Arzneimittelforschung*. 1967;17:825–829. [German].
25. Bauer R, Khan IA, Wagner H. TLC and HPLC analysis of *Echinacea pallida* and *E. angustifolia* roots. *Planta Med*. 1988;54:426–430.
26. Roder E, Wiedenfeld H, Hille T, et al. Pyrrolizidine in *Echinacea angustifolia* DC, und *Echinacea purpurea* MOENCH-Isolierung und Analytik. *Dtsch Apoth Ztg*. 1984;124:2316–2318.
27. Vogel VJ. *American Indian Medicine*. Norman, OK: University of Oklahoma Press.
28. Felter H. *The Eclectic Materia Medica, Pharmacology and Therapeutics*. Portland, OR: Eclectic Medical Publications; 1970.
29. Kuts-Cheraux AW. *Naturae Medicina and Naturopathic Dispensatory*. Yellow Springs, OH: Antioch Press; 1953.
30. Percival SS. Use of echinacea in medicine. *Biochem Pharmacol*. 2000;60:155–158.
31. Tragni E, Tubaro A, Melis S, et al. Evidence from two classic irritation tests for an anti-inflammatory action of a natural extract, Echinacina B. *Food Chem Toxicol*. 1985;23:317–319.
32. Wagner H, Breau W, Willer F, et al. In vitro inhibition of arachidonate metabolism by some alkamides and phenylated phenols. *Planta Med*. 1989;55:566–567.
33. Mose JR. Effect of Echinacin on phagocytosis and natural killer cells. *Med Welt*. 1983;34:1463–1467. [German].
34. Roesler J, Steinmuller C, Kiderlen A, et al. Application of purified polysaccharides from cell cultures of the plant *Echinacea purpurea* to mice mediates protection against systemic infections with *Listeria monocytogenes* and *Candida albicans*. *Int J Immunopharmacol*. 1991;13:27–37.
35. Wacker A, Hilbig W. Virus-inhibition by *Echinacea purpurea*. *Planta Med*. 1978;33:89–102. [German].
36. Hopp ES, Burn HF. Ground substance in the nose in health and infection. *Ann Otol Rhinol Laryngol*. 1958;67:480–490.
37. Voaden D, Jacobson M. Tumor inhibitors. 3. Identification and synthesis of an oncolytic hydrocarbon from American coneflower roots. *J Med Chem*. 1972;15:619–623.
38. Turner RB, Riker DK, Gangemi JD. Ineffectiveness of echinacea for prevention of experimental rhinovirus colds. *Antimicrob Agents Chemother*. 2000;44:1708–1709.
39. Goel V, Lovlin R, Barton R, et al. Efficacy of a standardized echinacea preparation (Echinilin) for the treatment of the common cold: a randomized, double-blind, placebo-controlled trial. *J Clin Pharm Ther*. 2004;29:75–83.
40. Melchart D, Linde K, Worku F, et al. Immunomodulation with echinacea—a systematic review of controlled clinical trials. *Phytomedicine*. 1994;1:245–254.
41. Barrett B, Brown R, Rakel D, et al. Echinacea for treating the common cold: a randomized trial. *Ann Intern Med*. 2010;153:769–777.
42. M1 Karsch-Völk, Barrett B, Kiefer D, et al. Echinacea for preventing and treating the common cold. *Cochrane Database Syst Rev*. 2014;(2):CD000530.
43. Schoneberger D. The influence of immune-stimulating effects of pressed juice from *Echinacea purpurea* on the course and severity of colds. Results of a double-blind study. *Forum Immunol*. 1992;8:2–12.
44. Hoheisel O, Sandberg M, Bertram S, et al. Echinagard treatment shortens the course of the common cold: a double-blind, placebo-controlled clinical trial. *Eur J Clin Res*. 1997;9:261–268.
45. Taylor JA, Weber W, Standish L, et al. Efficacy and safety of echinacea in treating upper respiratory tract infections in children: a randomized controlled trial. *JAMA*. 2003;290:2824–2830.
46. Yale SH, Liu K. *Echinacea purpurea* therapy for the treatment of the common cold: a randomized, double-blind, placebo-controlled clinical trial. *Arch Intern Med*. 2004;164:1237–1241.
47. Sperber SJ, Shah LP, Gilbert RD, et al. *Echinacea purpurea* for prevention of experimental rhinovirus colds. *Clin Infect Dis*. 2004;38:1367–1371.
48. Coeugniet EG, Kuhnast R. Recurrent candidiasis: adjuvant immunotherapy with different formulations of Echinacin. *Therapiewoche*. 1986;36:3352–3358.
49. Pohl P. On the therapy of irradiation-induced leukopenia with Esberitox. *Med Klin*. 1969;64:1546–1547. [German].
50. Kinkel HJ, Plate M, Tullner HU. Effect of Echinacin ointment in healing of skin lesions. *Med Klin*. 1984;79:580–583. [German].

51. Seidel K, Knobloch H. Determination & comparison of the antiphlogistic effects of antirheumatic drugs. *Z Rheumaforsch*. 1957;16:231–238. [German].
52. Chone B, Manidakis G. Echinacin test for the provocation of leukocytes in effective radiotherapy. *Dtsch Med Wochenschr*. 1969;94:1406–1410. [German].
53. Melchart D, Clemm C, Weber B, et al. Polysaccharides isolated from *Echinacea purpurea* herbal cell cultures to counteract undesired effects of chemotherapy—a pilot study. *Phytother Res*. 2002;16:138–142.
54. Haller J, Freund TF, Pelczer KG, et al. The anxiolytic potential and psychotropic side effects of an echinacea preparation in laboratory animals and healthy volunteers. *Phytother Res*. 2013;27(1):54–61.
55. Knie U, Dähnhardt-Pfeiffer S, Abels C, Bíró T. *Echinacea purpurea*-derived alkylamides exhibit potent anti-inflammatory effects and alleviate clinical symptoms of atopic eczema. *J Dermatol Sci*. 2017;88(1):67–77.
56. Foster S. *Echinacea: Nature's Immune Enhancer*. Rochester, VT: Healing Arts Press; 1991.
57. Schumacher A, Friedberg KD. The effect of *Echinacea angustifolia* on non-specific cellular immunity in the mouse. *Arzneimittelforschung*. 1991;41:141–147. [German].
58. Mengs U, Clare CB, Poiley JA. Toxicity of *Echinacea purpurea.* Acute, subacute and genotoxicity studies. *Arzneimittelforschung*. 1991;41:1076–1081.
59. Parnham MJ. Benefit-risk assessment of squeezed sap of the purple coneflower *(Echinaea purpurea)* for long-term oral immunostimulation. *Phytomedicine*. 1996;3:95–102.
60. Mullins RJ, Heddle R. Adverse reactions associated with echinacea: the Australian experience. *Ann Allergy Asthma Immunol*. 2002;88:42–51.
61. Gallo M, Koren G. Can herbal products be used safely during pregnancy? Focus on echinacea. *Can Fam Physician*. 2001;47:1727–1728.
62. Freeman C, Spelman K. A critical evaluation of drug interactions with *Echinacea* spp. *Mol Nutr Food Res*. 2008;52:789–798.

76

Eleutherococcus senticosus (Siberian Ginseng)

Michael T. Murray, ND

OUTLINE

Eleutherococcus or *Acanthopanax senticosus* (family: *Araliaceae*)
Common names: Siberian ginseng, touch-me-not, devil's shrub, eleuthero ginseng

GENERAL DESCRIPTION

Eleutherococcus senticosus (Siberian ginseng) is a shrub that grows 5 to 8.5 ft high. Its erect, spiny shoots, 1.5 to 2.5 inches in diameter, are covered with a light gray or brownish bark. The leaves are long petioled in a compound, palmate configuration. The five leaflets are elliptic and finely serrated at the margins on both sides, with scattered, minute spinules along the veins.[1]

Eleuthero grows abundantly in parts of Russia, the Far East, Korea, China, and Japan, north of latitude 38. Its distribution is much greater than that of *Panax ginseng* (see Chapter 110).[1]

The root is the most widely used component, with the highest concentration of biologically active substances occurring in the fall, just before defoliation. The leaves are also used medicinally, with their highest concentration of biologically active substances occurring in July, just before flowering.

CHEMICAL COMPOSITION

The initial phytochemical report on eleuthero was published in 1965 by members of the Institute of Biologically Active Substances in Vladivostok, Russia.[1] Seven compounds, termed eleutherosides A through G, were isolated from a physiologically active fraction of the methanol extract of eleuthero. The total eleutheroside content of the root ranges from 0.6% to 0.9%, and the content of the stems ranges from 0.6% to 1.5%. The ratio of the eleutherosides A through G obtained is approximately 8:30:10:12:4:2:1, respectively. Fig. 76.1 illustrates the structure of a key type of constituent. Table 76.1, modified from reviews of eleuthero, summarizes what is currently known about the components of *E. senticosus*.[1,2]

It is important to recognize that the ginsenosides characteristic of *Panax* sp. (American, Chinese, Korean, Japanese ginsengs) are not present in the roots of *E. senticosus*.

HISTORY AND FOLK USE

Ginseng plants, members of the family *Araliaceae*, including *E. senticosus*, are among the most ancient and esteemed of all medicinal herbs. Their use in Chinese herbal medicine dates back more than 4000 years.[1] References in ancient documents to members of the *Araliaceae* family are imprecise, giving rise to some confusion in modern interpretation. Nonetheless, in China, it has been long believed that the regular use of eleuthero increases longevity, improves general health and appetite, and restores memory.

The Russians have a separate history of eleuthero beginning in 1855 when a pair of Russian scientists, C. I. Maximovich and L. I. Shrenk, traveled from St. Petersburg to the Ussuri region of Russia on the Amur River. It was in this area that Maximovich observed a vast thicket of unusual plants, with leaves resembling horse chestnut and young shoots resembling ginseng. Unable to identify the plant, the two scientists brought back samples to St. Petersburg for classification. The plant was given the genus name of *Eleuthero,* or "free-berried shrub," and the species name of *senticosus,* which means "thorny" in Latin.

Not until the middle of the 20th century was eleuthero again "discovered," when Russian scientists began investigating substances that produce a "state of nonspecific resistance" in the body. Substances with this effect were termed *adaptogens.* In 1958 Brekhman described an adaptogen as follows[1,3]:

- It must be innocuous and cause minimal disorders in the physiological functions of an organism.
- It must have a nonspecific action (i.e., it should increase the resistance to adverse influences by a wide range of physical, chemical, and biochemical factors).
- It usually has a normalizing action regardless of the direction of the pathological state (alterative action).

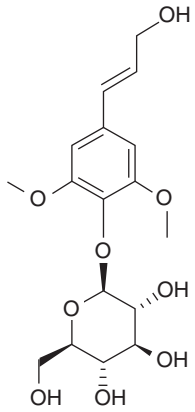


Fig. 76.1 Eleutheroside B.

TABLE 76.1 Compounds Found in *Eleutherococcus senticosus*

Compound	Type	Location
Eleutheroside A (daucosterol)	Sterol	Roots, stems
Eleutheroside B (syringin)	Phenylpropanoid	Roots, stems
Eleutheroside B_1 (isofraxidin-7–0-α-l-glucoside; also known as β-calycanthoside)	Coumarin	Roots
Eleutheroside B_2	Unknown	Roots
Eleutheroside B_3	Unknown	Roots
Eleutheroside B_4 [(–)-sesamin]	Lignan	Roots
Eleutheroside C (methyl-α-d-galactoside)	Sugar	Roots, stems
Eleutheroside D [(–)-Syringaresinol di-0-β-d-glucoside]	Lignan	Roots, stems
Eleutheroside E (different crystalline form of D; also known as acanthoside D)	Lignan	Roots, stems
Eleutheroside F	Unknown	Roots
Eleutheroside G	Unknown	Roots
Eleutheroside I (= mussenin B)	Triterpene	Leaves
Eleutheroside K	Triterpene	Leaves
Eleutheroside L	Triterpene	Leaves
Eleutheroside M (= hedera saponin B)	Triterpene	Leaves
Senticosides A–D (may be identical to eleutherosides I, K, L, and M)	Triterpene	Leaves
Vitamin E	Benzofuran	Roots
Beta-carotene	Carotenoid	Roots
Isofraxidin	Coumarin	Roots
Coumarin X	Coumarin	Roots
Complex mixture	Essential oil	Roots
Copper	Mineral	Roots
(–)-Syringaresinol	Lignan	Roots
Caffeic acid	Phenylpropanoid	Roots
Caffeic acid ethyl ester	Phenylpropanoid	Roots
Coniferyl aldehyde	Phenylpropanoid	Roots
Sinapyl alcohol	Phenylpropanoid	Roots
Beta-sitosterol	Sterol	Roots
Polysaccharides	Sugar	Roots, fruit
Galactose	Sugar	Roots
Glucose (α and β)	Sugar	Roots
Maltose (α and β)	Sugar	Roots
Sucrose	Sugar	Roots
Oleanolic acid	Triterpene	Roots

Modified from Davydov M, Krikorian AD. *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. *(Araliaceae)* as an adaptogen: a closer look. *J Ethnopharmacol.* 2000;72:345–393.

Brekhman's research with adaptogens began with *P. ginseng* because this was the best-known natural adaptogen. After confirming the adaptogenic action of panax in human studies, Brekhman began searching for an alternative to this plant because of the difficulty and expense in obtaining panax. Initially, all six species of *Araliaceae* native to Russia were investigated, and eleuthero was found to be the most promising. Numerous studies (in vivo, in vitro, and human studies) have been conducted since the late 1950s, nearly all in the Soviet Union. They are not referenced here because they are not translated and not widely available. Instead, review articles and original articles in English are cited.[1–7]

PHARMACOLOGY

As mentioned earlier, several experimental and clinical studies demonstrated that eleuthero possesses adaptogenic properties (i.e., the ability to increase nonspecific body resistance to stress, fatigue, and disease). Additional experimental and clinical research supports additional therapeutic applications of *E. senticosus.*

Adaptogenic Activity

In experimental models, *E. senticosus* was found to do the following:

- Impede adrenal hypertrophy induced by adrenocorticotropic hormone and adrenal atrophy induced by cortisone
- Impede thyroid hypertrophy induced by thyroidin and thyroid gland atrophy induced by 6-methylthiouracil
- Reduce blood glucose levels in alimentary and adrenal hyperglycemia and increase glucose levels in insulin-induced hypoglycemia
- Reduce leukocytosis induced by the parenteral administration of milk and leukopenia induced by endotoxins
- Reduce erythrocytosis induced by cobaltous nitrate and erythropenia induced by phenylhydrazine

Adaptogenic activities are summarized in Table 76.2. Similar results were obtained with *P. ginseng* (see Chapter 110). In addition to adaptogenic activities, eleuthero, like panax, has been shown to do the following[1]:

- Increase resistance to infection in animals
- Reduce hepatic cholesterol biosynthesis
- Increase reproductive capacity and sperm counts in bulls
- Possess significant antioxidant activity
- Stimulate DNA synthesis and cellular repair enzymes

Stress Control

Another important action of adaptogens is the inhibition of the alarm phase of the stress reaction. Eleuthero was shown to increase the swimming time of rats, reduce activation of the adrenal cortex in response to stress (alarm-phase reaction), and prevent stress-induced thymic and lymphatic involution.[1–6]

Radiation Protection

Eleuthero has demonstrated both protective and therapeutic actions in animals exposed to both single and prolonged radiation. In one study, both *E. senticosus* and *P. ginseng* were found to double the life span of rats exposed to prolonged radiation (total doses of 1620–7000 rads).[7] When eleuthero was combined with antibiotics, the lifetime of irradiated rats (total dose of 3000 rads over 60 days) increased threefold. Eleuthero may therefore be of benefit in protecting against harmful radiation and as an adjunctive aid in radiation therapy in oncology.

Antiedema Effects

Proper lymphatic function is necessary for the transport of fluids, macromolecules, antigens, and immune cells out of the interstitium. Alterations in lymphatic function have long been associated

TABLE 76.2 Abnormalities Normalized by *Eleutherococcus senticosus*

Function	Normalization Action of *Eleutherococcus Senticosus*
Adrenal	Impedes hypertrophy induced by ACTH
	Impedes atrophy induced by cortisone
Thyroid	Impedes hypertrophy induced by thyroidin
	Impedes atrophy induced by 6-methylthiouracil
Kidney	Increases renal capacity in pyelonephritis
Blood pressure	Decreases high blood pressure through pressure improvement of atherosclerosis
	Increases pressure in hypotension
Blood glucose	Reduces alimentary and adrenal hyperglycemia glucose
	Increases blood sugar in insulin-induced hypoglycemia
Leukocyte	Reduces leukocytosis induced by the administration of milk
	Reduces leukopenia induced by endotoxins
Erythrocyte	Reduces erythrocytosis induced by cobaltous nitrate
	Reduces erythropenia induced by phenylhydrazine
Stress	Reduces activation of the adrenal cortex in response to stress
	Prevents stress-induced thymic and lymphatic involution
Radiation	Protects against radiation exposure
Cancer	Inhibits carcinogenesis from urethane, 6-methylthiouracil indole
Cholesterol	Reduces hepatic biosynthesis
DNA	Stimulates synthesis

ACTH, Adrenocorticotropic hormone.

with edema and inflammation, and certain inflammatory signals induce large increases in permeability by disrupting the integrity of the lymphatic endothelial barrier. Tie2 is an endothelial-specific receptor that, when activated, stabilizes lymphatic vessels. In vitro, E. *senticosus* and its component eleutheroside E induce phosphorylation of Tie2.[8] In addition, Siberian ginseng was found to stabilize lymphatic endothelial cells (LECs) by promoting the intercellular localization of vascular endothelial cadherin (an endothelial-specific cell–cell adhesion molecule) and induce the phosphorylation of endothelial nitric oxide synthase by LECs, effects mediated by the activation of Tie2. Researchers in this same study also investigated whether eleuthero powder improves edema in a two-way, randomized, crossover study in 50 healthy female volunteers. Compared with the control group, edema of the lower limbs was significantly attenuated at 2 and 4 hours after ingestion of Siberian ginseng powder.

Carcinogenesis Inhibition

Eleuthero preparations were shown to inhibit several cancer types in experimental studies in animals.[1,3] Some of the anticancer effects of eleuthero may be because of its immune-stimulating effects. In vitro, Siberian ginseng increased phagocytosis of *Candida albicans* by granulocytes and monocytes from healthy donors by 30% to 45%.[9,10] In vitro studies showed that whole ethanolic fluid extract of *E. senticosus* could induce and enhance the production of interleukin-1 and interleukin-6 but not interleukin-2.[11] A methanolic extract of Siberian ginseng conferred growth inhibitory effects against a number of lung and colon cancer cell lines in vitro, with increasing concentration displaying a nonlinear inhibition in cancer cell differentiation from 12.5 to 200 μg/mL.[12]

CLINICAL APPLICATIONS

Adaptogenic Activity in Healthy Individuals

A fluid extract (33% ethanol) of *E. senticosus* root was administered to more than 2200 human subjects in clinical trials conducted in Russia designed to evaluate the adaptogenic effects of eleuthero. The male and female subjects ranged in age from 19 to 72 years. Dosages of the fluid extract (33% ethanol) ranged from 2 to 16 mL, one to three times a day, for periods of up to 60 consecutive days. The data indicated that eleuthero had the following effects:

- Increased the ability of humans to withstand many adverse physical conditions (e.g., heat, noise, motion, workload increase, exercise, decompression)
- Increased mental alertness and work output
- Improved the quality of work under stressful conditions and improved athletic performance

A double-blind, randomized, placebo-controlled, crossover study was designed to examine the effects of *E. senticosus* supplementation (800 mg/d) on the endurance capacity, cardiovascular functions, and metabolism of recreationally trained males for 8 weeks.[13] Although the cohort was small (9 subjects), there was a significant improvement in VO_2 peak (12% elevation) and endurance time (23% improvement) and a 4% increase in the highest heart rate. It was concluded that 8 weeks of eleuthero supplementation enhances endurance capacity, elevates cardiovascular functions, and alters the metabolism for sparing glycogen. Although several studies reported no effect of *E. senticosus* supplementation on endurance performance, it seems that the ergogenic effects are dependent on the duration of supplementation and not just the dose.

Adaptogenic Activity in Disease States

A fluid extract (33% ethanol) of *E. senticosus* root was administered to more than 2200 human subjects with various illnesses, including angina, hypertension, hypotension, acute pyelonephritis, various types of neuroses, acute craniocerebral trauma, rheumatic heart disease, chronic bronchitis, and cancer.[1] Eleuthero appeared to be effective in atherosclerotic conditions, as evidenced by its ability to lower elevated serum cholesterol and prothrombin levels, reduce blood pressure, and eliminate anginal symptoms in human subjects. Its action on blood pressure was found to be truly adaptogenic because eleuthero was also shown to increase blood pressure in subjects with hypotension.

Its effect in regulating blood pressure might be indicative of improved renal function. Patients with acute pyelonephritis given eleuthero extract had increased renal capacity, as measured by an increased secretion of phenol red.[1]

Eleuthero appears to have some psychotropic action because it was proven effective in the treatment of various psychological disturbances. Eleuthero consistently demonstrated an ability to increase one's sense of well-being, regardless of the psychological complaint (e.g., insomnia, hypochondriasis, various neuroses). This may be the result of improved balance of the biogenic amines, including serotonin, dopamine, norepinephrine, and epinephrine, because eleuthero extract administered to rats was shown to increase biogenic amine content in the brain, adrenals, and urine.[1]

Extracts of *E. senticosus* have been reported to reduce urinary excretion of calcium and hydroxyproline in glucocorticoid-induced osteoporotic rats.[14] An additional study investigated the prevention of bone loss by a standardized extract of dried *E. senticosus* stem bark (DES) in an ovariectomized (OVX) rat model of osteoporosis.[15] Eight weeks of treatment with DES significantly decreased the bone mineral density loss in the femur and inhibited bone markers such as serum alpha-fetoprotein (ALP), osteocalcin, and telopeptides of collagen type I levels

compared with the OVX control group without the influence of hormones such as estrogen. Oral administration of DES did not affect body weight gain, uterotrophic activity, or serum estradiol concentrations.

Data are insufficient to fully evaluate eleuthero's action in other disease states. However, it must be kept in mind that an adaptogen is nonspecific in its action and possesses a normalizing action regardless of the direction of the changes from physiological norms.

DOSAGE

The standard dosage of the fluid extract (33% ethanol) of *E. senticosus* roots used in most studies ranged from 2 to 4 mL (up to 16 mL), one to three times a day, for periods of up to 60 consecutive days. In multiple-dosing regimens, there is usually a 2- to 3-week interval between courses.[1,4]

Dosages include the following:

- Dried root: 2 to 4 g
- Tincture (1:5): 10 to 20 mL
- Fluid extract (1:1): 2 to 4 mL
- Solid (dry-powdered) extract (20:1): 100 to 200 mg

TOXICOLOGY

Toxicity studies in animals demonstrated that eleuthero extracts are virtually nontoxic. The median lethal dose of the 33% ethanol extract of eleuthero is 14.5 mL/kg in mice and greater than 20 mL/kg in rats. No long-term toxicity was observed when a daily dose of 5 mL/kg of the fluid extract was administered to rats. Teratogenicity was studied in three species (rats, rabbits, and minks), with no adverse effects observed.[1]

In human clinical studies, it was demonstrated that eleuthero extracts (33% ethanol) in the recommended dosage range were well tolerated, and side effects were infrequent. A few studies found mild side effects at higher dosages (4.5–6 mL three times daily) when used for long periods of time (≥60 days). Symptoms included insomnia, irritability, melancholy, and anxiety. Pericardial pain, headaches, palpitations, and elevations in blood pressure were reported in individuals with rheumatic heart disease.[1] These symptoms were likely a result of the mild stimulating effects of eleuthero and, although not serious, indicated the need to decrease the dosage, allow a washout period, or both.

DRUG INTERACTIONS

A clinical study in healthy humans indicated that extracts of eleuthero at generally recommended doses are unlikely to alter the levels of drugs primarily dependent on the CYP2D6 or CYP3A4 pathways for elimination.[16] However, in a case report, although a clear relationship could not be established, one person taking eleuthero with digoxin (Lanoxin) developed dangerously high serum digoxin levels.[17] Therefore simultaneous use with digoxin requires close medical supervision and regular monitoring of blood digoxin levels. In addition, eleutheroside B and eleutheroside E may inhibit the metabolism of drugs metabolized via CYP2C9 and CYP2E1 and have the potential to increase the toxicity of the drugs.[18]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Davydov M, Krikorian AD. *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. *(Araliaceae)* as an adaptogen: a closer look. *J Ethnopharmacol*. 2000;72:345–393.
2. Huang L, Zhao H, Huang B, et al. *Acanthopanax senticosus*: review of botany, chemistry and pharmacology. *Pharmazie*. 2011;66(2):83–97.
3. Baranov AI. Medicinal uses of ginseng and related plants in the Soviet Union. Recent trends in the Soviet literature. *J Ethnopharmacol*. 1982;6:339–353.
4. Brekhman II, Dardymov IV. New substances of plant origin which increase nonspecific resistance. *Ann Rev Pharmacol*. 1969;9:419–430.
5. Brekhman II, Dardymov IV. Pharmacological investigation of glycosides from ginseng and *Eleutherococcus*. *Lloydia*. 1969;32:46–51.
6. Brekhman II, Kirillov OI. Effect of *Eleutherococcus* on alarm-phase of stress. *Life Sci*. 1969;8:113–121.
7. Ben-Hur E, Fulder S. Effect of *Panax ginseng saponins* and *Eleutherococcus senticosus* on survival of cultured mammalian cells after ionizing radiation. *Am J Chin Med*. 1981;9:48–56.
8. Fukada K, Kajiya-Sawane M, Matsumoto Y, Hasegawa T, Fukaya Y, Kajiya K. Antiedema effects of Siberian ginseng in humans and its molecular mechanism of lymphatic vascular function in vitro. *Nutrition Research*. 2016;36(7):689–695. PubMed PMID: 27333960.
9. Windfeuer A, Mayerhofer D. The effects of plant preparations on cellular functions in body defense. *Arzneimittelforschung*. 1994;44:361–366. [German].
10. Wagner H, Proksch A, Riess-Maurer I, et al. Immunostimulating action of polysaccharides (heteroglycans) from higher plants. *Arzneimittelforschung*. 1985;35:1069–1075. [German].
11. Steinmann GG, Esperester A, Joller P. Immunopharmacological in vitro effects of *Eleutherococcus senticosus* extracts. *Arzneimittelforschung*. 2001;51:76–83.
12. Cichello SA, Yao Q, Dowell A, Leury B, He XQ. Proliferative and inhibitory activity of Siberian ginseng *(Eleutherococcus senticosus)* extract on cancer cell lines: A-549, XWLC-05, HCT-116, CNE and Beas-2b. *Asian Pac J Cancer Prev*. 2015;16(11):4781–4786. PubMed PMID: 26107240.
13. Kuo J, Chen KW, Cheng IS, Tsai PH, Lu YJ, Lee NY. The effect of eight weeks of supplementation with *Eleutherococcus senticosus* on endurance capacity and metabolism in human. *Chin J Physiol*. 2010;53(2):105–111. PubMed PMID: 21793317.
14. Kropotov AV, Kolodnyak OL, Koldaev VM. Effects of Siberian ginseng extract and IpriFlavone on the development of glucocorticoid-induced osteoporosis. *Bull Exp Biol Med*. 2002;133(3):252–254.
15. Lim DW, Kim JG, Lee Y, Cha SH, Kim YT. Preventive effects of *Eleutherococcus senticosus* bark extract in OVX-induced osteoporosis in rats. *Molecules*. 2013;18(7):7998–8008. PubMed PMID: 23884131.
16. Donovan JL, DeVane CL, Chavin KD, et al. Siberian ginseng *(Eleutherococcus senticosus)* effects on CYP2D6 and CYP3A4 activity in normal volunteers. *Drug Metab Dispos*. 2003;31:519–522.
17. McRae S. Elevated serum digoxin levels in a patient taking digoxin and Siberian ginseng. *CMAJ*. 1996;155:293–295.
18. Guo S, Liu Y, Lin Z, Tai S, Yin S, Liu G. Effects of eleutheroside B and eleutheroside E on activity of cytochrome P450 in rat liver microsomes. *BMC Complement Altern Med*. 2014;14:1. PubMed PMID: 24383621.

77

Ephedra Species[1]

Joseph E. Pizzorno, ND, and *Michael T. Murray, ND*

OUTLINE

Ephedra sinica (family: Ephedraceae)
Common names: Chinese ephedra, Ma Huang, Chinese joint fir
Related species:
Ephedra distachya (European ephedra)
Ephedra trifurca or *Ephedra viridis* (desert tea)
Ephedra nevadensis (Mormon tea, cay note, canutillo, whorehouse tea, tapopote, teamster's tea)
Ephedra americana (American ephedra)
Ephedra gerardiana (Pakistani ephedra)

GENERAL DESCRIPTION

Ephedra spp. are erect, branching shrubs found in desert or arid regions throughout the world. The 1.5- to 4-ft shrubs typically grow on dry, rocky, or sandy slopes. The many slender, yellow-green branches of ephedra have two small leaf scales at each node. The mature, double-seeded cones are visible in the fall.

CHEMICAL COMPOSITION

The chemical analysis of the stems and branches of *Ephedra* spp. are focused on their alkaloid content. In *Ephedrea sinica,* the total alkaloid content can be up to 3.3%, with 40% to 90% of this being ephedrine (Fig. 77.1). The remaining alkaloids are primarily pseudoephedrine and norpseudoephedrine.[2,3] In *E. gerardiana,* the alkaloid content usually varies from 0.8% to 1.4%, with about 50% ephedrine and 50% other alkaloids (e.g., pseudoephedrine, *N*-methylephedrine, norephedrine).[3,4] *E. nevadensis* contains little or no ephedrine.

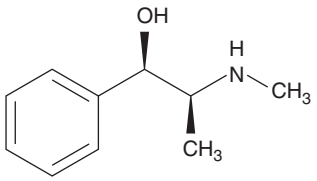


Fig. 77.1 Ephedrine.

Depending on species, parts used (aerial, stem, leaf, or a combination of stem and leaf), harvesting, and extraction techniques, the alkaloid content of commercially available ephedra products varies considerably. In a series of studies, the alkaloid content in ephedra-based products varied by as much as fivefold, with different brands exhibiting lot-to-lot differences ranging from 44% to 260%. Although some products listed the ephedrine content on the label, many did not. In addition to the variations in alkaloid content, commercial ephedra products usually contained other highly active botanicals, including kola nut, guarana, and St. John's wort, as well as many contaminants (e.g., heavy metals).[4,5]

HISTORY AND FOLK USE

E. sinica has been used for medicinal purposes in China since approximately 2800 BC. Ma Huang (the stem and branch) was used primarily in the treatment of the common cold, asthma, hay fever, bronchitis, edema, arthritis, fever, hypotension, and urticaria.[2]

The Chinese believed the effect of the root and rhizome (Ma Huanggen) to be the opposite of the stem and branches and limited its use to the treatment of profuse night sweating.[2] Two hypotensive principles (ephedradine A and B) have since been isolated from ephedra root, along with a hypertensive compound (1-tyrosine betaine or maokine).[2]

Western medicine's interest in ephedra began in 1923 with the demonstration that the isolated alkaloid ephedrine possessed several pharmacological effects. Ephedrine was synthesized in 1927 and has since been used extensively for clinical conditions in which sympathomimetic effects are desired.[4]

After ephedra-containing dietary supplements were linked to a high rate of serious side effects and numerous deaths, the US Food and Drug Administration (FDA) banned the sale of ephedra-containing supplements on April 12, 2004. However, this ban is not without controversy (see the "Toxicology" section).

PHARMACOLOGY

The pharmacology of ephedra centers around its ephedrine content. Ephedrine and pseudoephedrine have been extensively investigated

and are widely used in both prescription and over-the-counter (OTC) medications for asthma, hay fever, and rhinitis. In 1973 more than 20 million prescriptions contained one of these alkaloids. In 2000 ephedra was found in at least 200 OTC products, and it was estimated that as many as two to three billion doses were consumed that year in the United States.

Ephedrine

Ephedrine's basic pharmacological action is that of a sympathomimetic. Ephedrine stimulates both α- and β-adrenergic receptors, as well as the release of norepinephrine. Ephedrine shares many pharmacological actions with epinephrine, although ephedrine is much less active. Ephedrine also differs from epinephrine in its ability to be absorbed orally, its longer duration of action, and its more pronounced effect upon the central nervous system (CNS). The CNS effects of ephedrine are similar to those of amphetamines but, again, much less potent.[2–5] Fig. 77.1 illustrates its chemical structure.

The cardiovascular effects of ephedrine are also similar to those of epinephrine. It increases both diastolic and systolic blood pressure, cardiac output, and heart rate, but for a longer (about 10 times) period of time. Like epinephrine, ephedrine increases coronary, cerebral, and muscle blood flow at the expense of renal and splanchnic blood flow.[2–6]

The bronchial muscle relaxation induced by ephedrine is much less than epinephrine, but, again, the duration of action is much longer. Other smooth muscles, except for the human uterus, are generally affected by ephedrine in the same manner (mild relaxation) as epinephrine. Ephedrine relaxes the human uterus, whereas the effects of epinephrine are more complex.[2–5]

Extracts of ephedra were shown to inhibit complement in vitro, and a Chinese herbal medicinal prescription, *Makyo-kanseki-to*, was found to inhibit cyclic adenosine monophosphate activity.[7,8]

The principle adverse effects of ephedrine are CNS stimulation, nausea, tremors, tachycardia, and urinary retention.[2,3]

The major route of elimination of ephedrine is in the urine as the unchanged drug. The average half-life is 6 hours, although acidifying the urine decreases the half-life considerably. Alkalinization increases the half-life.[9,10]

Pseudoephedrine

Pseudoephedrine exhibits bronchodilating activity similar to ephedrine but has weaker pressor, cardiac, and CNS effects. Pseudoephedrine is often recommended over ephedrine in the treatment of chronic asthma because it has fewer side effects.

Pseudoephedrine demonstrated significant anti-inflammatory effects in various experimental models.[9–11] Other ephedra alkaloids, including ephedrine, also exhibited anti-inflammatory activity, although at much lower potency. Because the anti-inflammatory effect of pseudoephedrine is essentially identical in normal and adrenalectomized mice, the anti-inflammatory activity is not exerted via the adrenal glands. Instead, it appears that the anti-inflammatory activity of pseudoephedrine and other ephedra alkaloids is caused by inhibition of prostaglandin E_2 synthesis.

CLINICAL APPLICATIONS

Asthma and Hay Fever

Ephedra and its alkaloids have proven effective as bronchodilators for the treatment of mild to moderate asthma and hay fever. The peak bronchodilation effect occurs in 1 hour and lasts about 5 hours after administration.

The therapeutic effect of ephedra diminishes if used over a long period of time because of the weakening of the adrenal gland caused by ephedrine. Therefore it is often necessary to use ephedra in combination with herbs such as *Glycyrrhiza glabra* and *Panax ginseng* and nutrients such as vitamin C, magnesium, zinc, vitamin B_6, and pantothenic acid, which all support the adrenal glands.

The folklore herbal treatment of asthma involved the use of ephedra in combination with herbal expectorants. Expectorants are herbs that modify the quality and quantity of secretions of the respiratory tract, resulting in the expulsion of the secretions and an improvement in respiratory tract function. Commonly used expectorants include:

- *Glycyrrhiza glabra* (licorice)
- *Grindelia camporum* (grindelia)
- *Euphorbia hirta* (euphorbia)
- *Drosera rotundifolia* (sundew)
- *Polygala senega* (senega)

Common Cold

Ephedrine and pseudoephedrine are components of many OTC products for the self-treatment of the common cold. In China, ephedra, in combination with various other herbs, was found to be clinically effective in the treatment of cold symptoms, as well as those of influenza, pneumonia, whooping cough, and bronchitis.[2] Polysaccharide from *Ephedra sinica* lowers the recruitment of inflammatory cells, decreases the production of TNF-α, IL-6, IL-8, and MMP-9, and significantly reduces the expression of TGF-β1, P-Smad2, and P-Smad3, thus reducing airway and pulmonary inflammation.[12]

Weight-Loss Aid

In both human and animal studies, ephedrine was shown to promote weight loss.[3,13–21] Although ephedrine demonstrated an anorectic effect,[13] its main mechanism for promoting weight loss appeared to be increasing the metabolic rate of adipose tissue.[13–18] Therefore the weight-reducing effects of ephedra are most significant in those individuals with a low basal metabolic rate.[15] These effects can be greatly enhanced when used in combination with methylxanthines, caffeine, theophylline, and aspirin,[15–20,22] which potentiate the action of ephedrine and other ephedra compounds.

In one animal study, when ephedrine was used alone, it resulted in a 14% decrease in body weight and a 42% decrease in body fat. However, when used in combination with caffeine or theophylline, the decreases were 25% and 75%, respectively.[16] When either caffeine or theophylline was used alone, however, there was no significant loss in body weight. The greater decrease in body weight is likely a result of the increased metabolic rate and fat cell breakdown promoted by ephedrine and enhanced by caffeine and theophylline.

It is recommended that, for methylxanthines, *Camellia sinensis* (green tea) or extracts of *Cola* sp. be used rather than coffee or black tea.

One of the better designed studies on the use of an ephedrine/caffeine combination determined the safety and efficacy of an herbal supplement containing Ma Huang (90 mg/day ephedrine) and kola nut (192 mg/day caffeine) given to subjects for 6 months for weight loss.[21] A total of 167 subjects were randomized to receive either a placebo or ephedrine/caffeine combination. The subjects were monitored primarily for changes in blood pressure, heart function, and body weight. In addition to these parameters, body composition and metabolic changes were studied. Results demonstrated significant beneficial effects on body weight, body fat, and blood lipids of the herbal supplement in overweight men and women who were otherwise healthy. Herbal versus placebo treatment decreased body weight (−5.3 vs −2.6 kg), body fat (−4.3 vs −2.7 kg), and low-density lipoprotein cholesterol (−8 vs 0 mg/dL), and increased high-density lipoprotein cholesterol (+2.7 vs −0.3 mg/dL; $P = 0.004$). The ephedrine/caffeine mixture

produced small changes in blood pressure variables (+3 to −5 mm Hg) and increased heart rate (4 ± 9 beats/min), but cardiac arrhythmias were not increased. Compared with placebo treatment, the herbal supplement produced no adverse events and only minimal side effects consistent with the known stimulant action of ephedrine and caffeine (e.g., dry mouth, insomnia).

A 12-week, multicenter, double-blind, placebo-controlled, randomized study conducted at three clinical sites in New York state evaluated the efficacy and side effects of an herbal formulation containing ephedrine, caffeine, and other ingredients to promote weight loss.[23] One hundred and two overweight/obese volunteers between the ages of 18 and 65 years were randomized to receive either the active product or a placebo supplement. Subjects receiving the active treatment experienced, on average, 1.5 kg of weight loss and greater reductions in BMI and waist circumference compared with subjects receiving the placebo. The benefits were achieved in the absence of any lifestyle, dietary, or exercise change. In addition, lower doses of ephedrine alkaloids and caffeine were used, and subjects experienced results without an increase in blood pressure, pulse, or the rate of adverse events.

Obesity related human gut microbiota have been studied for identification of correlated bacteria and their mechanisms. It has been hypothesized that ephedra can have an influence on gut microbiota, and that influence may be associated with weight loss or metabolic markers or body composition indices. The antiobesity effects of ephedra via gut microbiota modulation was evaluated in a study of females between the ages of 40 and 65 who were weight-stable but obese.[24] The subjects were asked to take 2 g of ephedra extract two times per day for 8 weeks. After ephedra intake, mean value of body weight was decreased by 1.86 kg, mean BMI was decreased by 0.79 kg/m^2, and body fat was reduced by 1.17%. In correlation analysis, *Subdoligranulum*, *Oscillibacter*, and *Akkermansia* showed an association with changes of body weight and BMI. However, the influences of gut microbiota are unique according to indigenous microbiota and differences in individual sensitivity to ephedra.

DOSAGE

The optimum dosage of ephedra depends on the alkaloid content in the form used. The average total alkaloid content of *E. sinica* is 1% to 3%. When used in the treatment of asthma or as a weight-loss aid, the ephedra dose should have an ephedrine content of 12.5 to 25 mg and be taken two to three times daily. For the crude herb, an equal dose would be approximately 500 to 1000 mg three times a day. Standardized preparations are often preferred because they have more dependable therapeutic activity.

TOXICOLOGY

Ephedra can produce the same side effects as ephedrine (e.g., increased blood pressure and heart rate, insomnia, anxiety).

The FDA advisory review panel on nonprescription drugs recommended that ephedrine not be taken by patients with heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland. Nor should ephedrine be used in patients on antihypertensive or antidepressant drugs.

Pregnant women should also avoid the use of ephedra and ephedrine. Ephedrine administered to chick embryos resulted in cardiovascular teratogenicity and embryotoxicity at doses as low as 1 μmol/egg.[25] The teratogenic effect of ephedrine is potentiated by caffeine.[26] Presumably, this activity of ephedrine is the result of the production of nitrosamines.[27] Simultaneous vitamin C administration might reduce the formation of nitrosamines from ephedra alkaloids.[28,29]

A key reason the FDA issued a ban on ephedra products was the tremendous amount (more than 2000) of adverse event reports (AERs) filed with the FDA related to the use of supplements containing ephedra alkaloids.[30,31] However, when the Council of Responsible Nutrition studied the AERs filed with the FDA, it found that 98% of the AERs did not contain complete information, and in 81% of the reports, information on total daily intake of ephedra was not provided.[32] Of the 1173 reports, 121 were selected for further evaluation. Of the 121 selected cases, 47 were considered to contain serious adverse events, including 15 cases of stroke and stroke-like symptoms, 13 cases of seizures, 15 cases of cardiac arrest, and 2 cases of individuals who collapsed.

From the selected 121 cases, there were 8 reports of death: 6 were cardiovascular, 1 occurred in an automobile accident, and 1 was a spontaneous abortion. Among the 6 cardiovascular-related deaths, 5 were males and 1 was female. All 5 males who died from cardiovascular problems were using dietary supplements containing ephedra as an athletic performance enhancer. Additionally, 4 of the 5 were simultaneously using other performance-enhancing products such as caffeine. Therefore their deaths cannot be directly attributed to the use of ephedra. After extensive examination of these AERs, the report could not conclusively determine whether there were any unexpected toxicological effects due to the ephedra contained in the dietary supplements based solely on the information presented in the AERs.

Although there have been no large controlled studies on the possible association between prescribed ephedrine/caffeine and cardiovascular events in general, one study linked data from four different sources within Statistics Denmark on 257,364 users of prescribed ephedrine/caffeine for the period 1995 to 2002. The researchers analyzed the data using a case-crossover technique with a composite endpoint: death outside of a hospital, myocardial infarction, or stroke. To account for effects of chronic exposure and effects in naïve users, the authors performed a secondary case-control study nested within the cohort of ephedrine/caffeine ever users. Among 2316 case subjects, 282 (12.2%) were current users of ephedrine/caffeine. The case-crossover analysis yielded an odds ratio (OR) of 0.84. After adjustment for trends in ephedrine/caffeine use, the OR was 0.95. Subgroup analyses revealed no strata with significantly elevated risk. In the case-control substudy, there was no increased risk among naïve users or users with large cumulative doses. The analysis concluded that prescribed ephedrine/caffeine was not associated with a substantially increased risk of adverse cardiovascular outcomes.[33]

DRUG INTERACTIONS

As mentioned earlier, ephedrine should not be taken by patients with heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland. Nor should ephedrine be used in patients on antihypertensive or antidepressant drugs. Ephedrine will potentiate other stimulant/sympathomimetic drugs and will likely counteract antihypertensives.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ephedra was once an extremely popular herbal product. That popularity ended on February 6, 2004, when the U.S. Food and Drug Administration (FDA) issued a ban on the sale of dietary supplements containing ephedra. This ban was finalized on April 6, 2004. The FDA made this decision based on increased reports of adverse events. Dietary supplements containing ephedrine alkaloids, like other sympathomimetics, raise blood pressure and increase heart rate. The FDA believed ephedrine-based products exposed users to several risks, including the consequences of a sustained increase in blood pressure (e.g., serious illnesses or injuries including stroke and heart attack that can result in death). There was also a risk of increased morbidity and mortality from worsened heart failure and proarrhythmic effects in susceptible individuals.
2. Chang HM, But PP. *Pharmacology and Applications of Chinese Materia Medica*. Philadelphia: World Scientific.
3. Abourashed EA, El-Alfy AT, Khan IA, et al. Ephedra in perspective—a current review. *Phytother Res*. 2003;17:703–712.
4. Gurley BJ, Gardner SF, Hubbard MA. Content versus label claims in ephedra-containing dietary supplements. *Am J Health Syst Pharm*. 2000;57:963–969.
5. Haller CA, Duan M, Benowitz NL, et al. Concentrations of ephedra alkaloids and caffeine in commercial dietary supplements. *J Anal Toxicol*. 2004;28:145–151.
6. White LM, Gardner SF, Gurley BJ, et al. Pharmacokinetics and cardiovascular effects of ma-huang (Ephedra sinica) in normotensive adults. *J Clin Pharmacol*. 1997;37:116–122.
7. Ling M, Piddlesden SJ, Morgan BP. A component of the medicinal herb ephedra blocks activation in the classical and alternative pathways of complement. *Clin Exp Immunol*. 1995;102:582–588.
8. Nikaido T, Iizuka S, Okada N, et al. The study of Chinese herbal medicinal prescription with enzyme inhibitory activity. VI. The study of makyo-kanseki-to with adenosine 3',5'-cyclic monophosphate phosphodiesterase. *Yakugaka Zasshe*. 1992;112:124–128. [Japanese].
9. Wilkinson GR, Beckett AH. Absorption metabolism and excretion of the ephedrines in man. I. The influence of urinary pH and urine volume output. *J Pharmacol Exp Ther*. 1968;162:139–147.
10. Pickup ME, May CS, Sendagire R, et al. The pharmacokinetics of ephedrine after oral dosage in asthmatics receiving acute and chronic treatment. *Br J Clin Pharmacol*. 1976;3:123–134.
11. Hikino H, Konno C, Takata H, et al. Anti-inflammatory principle of ephedra herbs. *Chem Pharm Bull*. 1980;28:2900–2904.
12. Liang S, Meng X, Wang Z, Liu J, Kuang H, Wang Q. Polysaccharide from Ephedra sinica Stapf inhibits inflammation expression by regulating Factor-β1/Smad2 signaling. *Int J Biol Macromol*. 2018;106:947–954. PubMed PMID:28830773.
13. Kasahara Y, Hikino H, Tsuru S, et al. Anti-inflammatory actions of ephedrines in acute inflammations. *Planta Med*. 1985;54:325–331.
14. Zarrindast MR, Hosseini-Nia, Farnoodi F. Anorectic effect of ephedrine. *Gen Pharmacol*. 1987;18:559–561.
15. Astrup A, Madsen J, Holst JJ, et al. The effect of chronic ephedrine treatment on substrate utilization, the sympathoadrenal activity, and expenditure during glucose-induced thermogenesis in man. *Metabolism*. 1986;35:260–265.
16. Bailey CJ, Thornburn CC, Flatt PR. Effects of ephedrine and atenol on the development of obesity and diabetes in ob/ob mice. *Gen Pharmacol*. 1986;17:243–246.
17. Dulloo AG, Miller DS. The thermogenic properties of ephedrine/methylxanthine mixtures: animal studies. *Am J Clin Nutr*. 1986;43:388–394.
18. Pasquali R, Cesari MP, Melchionda N, et al. Does ephedrine promote weight loss in low-energy-adapted obese women? *Int J Obesity*. 1987;11:163–168.
19. Miller DS. A controlled trial using ephedrine in the treatment of obesity. *Int J Obesity*. 1986;10:159–160.
20. Dulloo AG, Miller DS. Aspirin as a promoter of ephedrine-induced thermogenesis: potential use in the treatment of obesity. *Am J Clin Nutr*. 1987;45:564–569.
21. Boozer CN, Daly PA, Homel P, et al. Herbal ephedra/caffeine for weight loss: a 6-month randomized safety and efficacy trial. *Int J Obes Relat Metab Disord*. 2002;26:593–604.
22. Daly PA, Krieger DR, Dulloo AG, et al. Ephedrine, caffeine and aspirin. Safety and efficacy for the treatment of human obesity. *Int J Obes Relat Metab Disord*. 1993;17(suppl 1):S73–S78.
23. Coffey CS, Steiner D, Baker BA, Allison DB. A randomized double-blind placebo-controlled clinical trial of a product containing ephedrine, caffeine, and other ingredients from herbal sources for treatment of overweight and obesity in the absence of lifestyle treatment. *Int J Obesity Rel Metabol Disorders*. 2004;28(11):1411–1419. PubMed PMID:15356670.
24. Kim BS, Song MY, Kim H. The anti-obesity effect of Ephedra sinica through modulation of gut microbiota in obese Korean women. *J Ethnopharmacol*. 2014;152(3):532–539. PubMed PMID:24556223.
25. Kalix P. The pharmacology of psychoactive alkaloids from ephedra and catha. *J Ethnopharmacol*. 1991;32:201–208.
26. Nishikawa T, Bruyere Jr HJ, Takagi Y, et al. Cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol Lett*. 1985;29:59–63.
27. Nishikawa T, Bruyere Jr HJ, Gilbert EF, et al. Potentiating the effects of caffeine on the cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol Lett*. 1985;29:65–68.
28. Alwan SM, Al-Hindawi MK, Abdul-Rahman SK, et al. Production of nitrosamines from ephedrine, pseudoephedrine and extracts of ephedra foliata under physiological conditions. *Cancer Lett*. 1986;31:221–226.
29. Sever PS, Dring LG, Williams RT. The metabolism of (-)-ephedrine in man. *Eur J Clin Pharmacol*. 1975;9:193–198.
30. Soni MG, Carabin IG, Griffiths JC, et al. Safety of ephedra: lessons learned. *Toxicol Lett*. 2004;150:97–110.
31. Schulman S. Addressing the potential risks associated with ephedra use: a review of recent efforts. *Public Health Rep*. 2003;118:487–492.
32. Council for Responsible Nutrition. Safety assessment and determination of a tolerable upper limit for ephedra. *Cantox Health Sci Int*. 2000:1–169.
33. Hallas J, Bjerrum L, Støvring H, Andersen M. Use of a prescribed ephedrine/caffeine combination and the risk of serious cardiovascular events: a registry-based case-crossover study. *Am J Epidemiol*. 2008;168(8):966–973.

Epilobium Species (Fireweed)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Epilobium spp. (family: Onagraceae)
Synonym: *Chamaenerion* spp.
Common names: fireweed, willow herb, great willow herb *(Epilobium angustifolium),* rose-bay *(Epilobium angustifolium),* wickup *(Epilobium angustifolium);* small-flowered willow herb *(Epilobium parviflorum);* marsh epilobium *(Epilobium palustre),* wickop *(Epilobium palustre),* swamp willow herb *(Epilobium palustre)*

GENERAL DESCRIPTION

Epilobium is a genus of some 200 species that typically grow at relatively high latitudes or high altitudes.[1] This perennial is found in all parts of the world. It has tall, erect, and somewhat woody stems, from 2 to 7 feet tall, crowded with long, narrow, alternate leaves that are from 2 to 6 in. long. Its flowers range from lavender to pink to carmine-purple, and its pods are long, narrow, and filled with feathery seeds.[2] Most species have small flowers (e.g., *Epilobium parviflorum,* known as small-flowered willow herb). A few have large flowers (e.g., *E. angustifolium,* known as great willow herb). Despite the use of the term "willow," there is no relationship between *Epilobium* spp. and *Salix* spp. (willow) (Fig. 78.1).

CHEMICAL COMPOSITION

The aerial parts are rich in flavonoids[3] and flavonol glycosides based on kaempferol, quercetin, and myricetin skeletons.[4] The plants contain complex tannins such as ellagitannins (oenothein A and B) and gallotannins[5]; β-sitosterol; triterpenes[6]; and gallic, chlorogenic, and ellagic acids.[7] In the small-flowered species, myricetin is the predominant flavonoid. The large-flowered species have a completely different flavonoid pattern, with isoquercitrin being predominant (Fig. 78.2). The plant contains the highest content of the flavonoid myricetin 3-0-β-D-glucuronide shortly after flowering. All fireweed species contain oenothein B, which plays an important role in the plant's efficacy in vitro and in vivo.[8,9] Studies indicate that oenothein B is metabolized to urolithin by gut microbes. Urolithins are bioavailable and have demonstrated antioxidant, antiestrogenic, estrogenic, antimalarial, anti-inflammatory, and anticancer activities in a variety of cell models.[10]

HISTORY AND FOLK USE

Fireweed has been widely used as a medicine and as a food in many parts of the world. Native Americans used it for burning urination, male urination problems, coughs and sore throats, stomachaches and intestinal discomfort, bowel hemorrhages, gastritis, tuberculosis, and as a panacea for pain.[11] It was used as a poultice for boils; abscesses; carbuncles; bruises; infected sores, cuts, and wounds; and other skin ailments. Various Eskimo and Siberian tribes also used the plant to treat sores.[12] Young shoots were widely consumed as food and fodder, as were the roots and leaves. The seed fluff was used for weaving cloth, making thread, and starting fires. Fireweed is named *mjoelke* in Scandinavia, a derivation of the word *milk*, because of centuries of observation that cows grazing on the plant produce more milk.

Eclectic physicians considered fireweed unequaled as a treatment for summer bowel troubles and also used it in other types of diarrhea, including cholera infantum and typhoid dysentery.[13] The Eclectics often administered fireweed as an infusion, using frequent small doses (up to every 10 minutes). *E. hirsutum* was used in Egyptian and European folk medicine to treat inflammation, adenoma, and prostate tumors.[14] Europeans also used the plant to treat eczema, seborrhea, other skin conditions, and menstrual disorders.[15] The plant was also used for the treatment of benign prostatic hyperplasia.[16]

PHARMACOLOGY

Analysis of studies on fireweed is complex, given the subtle differences among species and differences in the actions of aqueous and alcoholic extracts. Overall, most fireweed species appear to have analgesic, antidiarrheal, anti-inflammatory, antimicrobial, antineoplastic, and prostate-related activities.

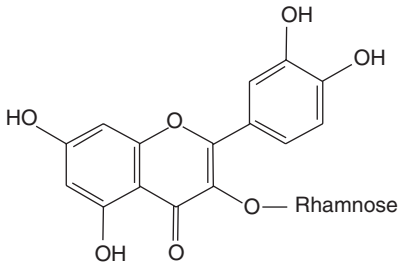


Fig. 78.1 *Chamerion angustifolium* infloresence.

Fig. 78.2 Quercitrin.

Analgesic Effect

Ethanolic extracts of *E. angustifolium* injected subcutaneously had a weak analgesic effect on mice in the hot plate test, whereas its analgesic effect was greater than that of acetylsalicylate in the acetic acid test.[17] In mice, an extract of *E. hirsutum* had an analgesic effect comparable to morphine and diclofenac.[18]

Antidiarrheal Effect

Ethanolic extracts of fireweed showed significant antidiarrheal and antimotility effects in a variety of animal models.[19]

Anti-Inflammatory Effect

Aqueous extracts of *E. angustifolium* (40 mg/kg orally) strongly reduced the release of prostaglandins and depressed edema in vivo.[20] Its effect on edema was equivalent to indomethacin (2 mg/kg). *E. parviflorum* did not suppress edema and had about 20% of the effect on prostaglandin release. Isolated myricetin-3-0-β-D-glucuronide was 10 times more effective than indomethacin at inhibiting edema in rats, was equally effective at inhibiting prostaglandin biosynthesis, and had a dose-related antiarthritic effect.[21,22] It inhibited both cyclooxygenase-1 and -2 nearly equivalently and inhibited 5-lipooxygenase comparably to nordihydroguaiaretic acid. It dose-dependently inhibited platelet aggregation equivalently to indomethacin, but in contrast to indomethacin, it induced no gastric ulcers. The compound may be safer than nonsteroidal anti-inflammatory drugs, because it prevents the arachidonic shunting that results in the formation of leukotrienes.

Antimicrobial Effect

Dilute extracts of fireweed are antimicrobial in vitro.[23] *E. angustifolium* and *E. rosmarinifolium* have the broadest spectrum of action, inhibiting bacteria, yeast, and fungi. *E. angustifolium* and *E. hirsutum* inhibited *Microsporum canis* at concentrations as low as 10 mcg/mL. In another study, *E. angustifolium* failed to inhibit *Aspergillus niger* but somewhat inhibited *Candida albicans* while strongly inhibiting *Staphylococcus aureus* and *Escherichia coli.*[24]

Antitumor Effect

Oenothein B, isolated from fireweed, showed some antitumor activity against sarcoma 180 in mice and some selectivity against lung cancer cells and two colon cancer lines, but did not have a noticeable effect on the prostate cancer lines tested.[25] In other studies, *E. angustifolium* had a specific and significant antiproliferative effect on human prostate epithelial cells.[26] In addition, several of its flavonoids were potent growth inhibitors when tested on various hormone-sensitive and hormone-nonsensitive cell lines.

Effect on Prostate and Prostatic Enzymes

Extracts of various fireweed species inhibited aromatase in vitro.[27] This action was primarily attributed to oenothein A and B, but other fireweed flavonoids also inhibited aromatase. Both oenothein A and B had a considerably greater inhibitory action on 5-α-reductase in vitro than the reference drug finasteride. The aqueous extract and ultrafiltrate of *E. angustifolium* had an antiandrogenic effect in intact rats, and the aqueous extract had a proandrogenic effect in castrated rats.[28] In another study, alcoholic extracts of fireweed failed to inhibit 5-α-reductase, whereas the aqueous extract showed significant inhibition.[29] This action was attributed to the compound oenothein B. Although data on the bioavailability of oenothein B are lacking, it appears that aqueous extracts of *E. hirsutum* are metabolized by gut microbes to dibenzopyran-6-one or urolithins.[30] Urolithins are bioavailable and have been shown to accumulate in mice prostate glands and inhibit cell proliferation[31]

CLINICAL APPLICATIONS

One randomized, double-blind, placebo-controlled trial of 57 healthy males diagnosed with benign prostatic hyperplasia (BPH) found that a combination product, ProstateEZE Max, significantly reduced daytime and nighttime urinary frequency and reduced the international prostate specific score (36% reduction vs. 8% in placebo group). ProstateEZE Max combines *Curcurbita pepo* (pumpkin) seed, *E. parviflorum,* lycopene, *Prunus africanum* (pygeum) bark, and *Serenoa repens* (saw palmetto) fruit.[32] However, no clinical studies were found specifically looking at any fireweed species by themselves. This is unfortunate, given preliminary studies indicated that fireweed might be more effective than drugs such as finasteride and indomethacin, as discussed earlier. The existing pharmacological research supports fireweed's well-established use in traditional medicine as a treatment for prostate disorders and as a topical medicine in various skin conditions.[33]

DOSAGE

Dosages for fireweed are not well established. Fireweed is traditionally administered as a standard infusion using 2 to 3 teaspoons of herb to 1 cup of boiling water, taken as needed.[34] The suggested dose for an aqueous/alcoholic tincture is 2 to 6 mL three times a day.

Aqueous/alcoholic tinctures appear to be more effective than tinctures with a high alcohol concentration.[35,36] No widely accepted standardization exists to ensure quality fireweed extracts, although flavonoid profiles can be used to distinguish the various species.[37,38]

Species grown in Africa appear to have a significantly higher content of oenothein B than the same species grown in Europe.[39]

TOXICOLOGY

Fireweed has no known toxic effects, and its lack of toxicity is underscored by a worldwide use as both food and fodder. The median lethal dose for injected ethanol extract in mice is 1.4 g/kg.[40]

DRUG INTERACTIONS

There are no documented drug interactions with fireweed. However, oral doses of *E. angustifolium* slightly increased the expression of CYP2D2 in rats and significantly decreased expression of CYP3A1 in one study; another found intraperitoneal delivery of an extract of *E. hirsutum* decreased CYP2E1 and CYP1A1, indicating a potential risk of interactions with drugs metabolized by these enzymes.[41,42]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Averett JE, Kerr BJ, Raven PH. The flavonoids of onagraceae, tribe epilobieae: epilobium sect. *Epilobium Am J Bot*. 1978;65:567–570.
2. Moore M. *Medicinal plants of the Pacific West*. Santa Fe, NM: Red Crane Books.
3. Ducrey B, Marston A, Gohring S, et al. Inhibition of 5 alpha-reductase and aromatase by the ellagitannins oenothein a and oenothein b from epilobium species. *Planta Med*. 1997;63:111–114.
4. Averett JE, Kerr BJ, Raven PH. The flavonoids of onagraceae, tribe epilobieae: epilobium sect. *Epilobium Am J Bot*. 1978;65:567–570.
5. Ducrey B, Marston A, Gohring S, et al. Inhibition of 5 alpha-reductase and aromatase by the ellagitannins oenothein A and oenothein B from epilobium species. *Planta Med*. 1997;63:111–114.
6. Glowniak K, Zareba S, Kozyra M, et al. P23 sterols and triterpenoids from epilobium sp. and some other medicinal plants (poster). *Eur J Pharm Sci*. 1994;2:124.
7. Lesuisse D, Berjonneau J, Ciot C, et al. Determination of oenothein b as the active 5-alpha-reductase-inhibiting principle of the folk medicine epilobium parviflorum. *J Nat Prod*. 1996;59:490–492.
8. Granica S, Bazylko A, Kiss AK. Determination of macrocyclic ellagitannin oenothein b in plant materials by HPLC-DAD-MS: method development and validation. *Phytochem Anal*. 2012;23:582–587.
9. Granica S, Piwowarski JP, Czerwinska ME, Kiss AK. Phytochemistry, pharmacology and traditional uses of different epilobium species onagraceae:a review. *J Ethnopharmacol*. 2014;156:316–346.
10. Larrosa M, Gonzalez-Sarrias A, Garcia-Conesa ME, et al. Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and anti-estrogenic activities. *J Agri Food Chem*. 2006;54:1611–1620.
11. Moerman DE. *Native American Ethnobotany*. Portland, OR: Timber Press.
12. Kallman S. *Wild Plants as Food & Medicine*. Vasteras, Sweden: ICA Bokforlag.
13. Felter HW, Lloyd JU. *King's American Dispensatory*. Sandy, OR: Eclectic Medical Publications; 1.
14. Barakat HH, Hussein SAM, Marzouk MS, et al. Polyphenolic metabolites of epilobium hirsutum. *Phytochem*. 1997;46:935–941.
15. Battinelli L, Tita B, Evandri MG, et al. Antimicrobial activity of epilobium spp. extracts. *Farmaco*. 2001;56:345–348.
16. Hevesi BT, Houghton PJ, Habtemariam S, et al. Antioxidant and anti-inflammatory effect of epilobium parviflorum schreb. *Phytother Res*. 2009;23:719–724.
17. Tita B, Abdel-Haq H, Vitalone A, et al. Analgesic properties of epilobium angustifolium, evaluated by the hot plate test and the writhing test. *Farmaco*. 2001;56:341–343.
18. Pourmorad F, Ebrahimzadeh MA, Mahmoudi M, et al. Antinociceptive effect of methanolic extract of epilobium hirsutum. *Pakistan J Biol Sci*. 2007;10:2764–2767.
19. Vitali F, Fonte G, Saija A, et al. Inhibition of intestinl motility and secretion by extracts of epilobium spp. in mice. *J Ethnopharmacol*. 2006;107:342–348.
20. Hiermann A, Juan H, Sametz W. Influence of epilobium extracts on prostaglandin biosynthesis and carrageenin induced oedema of the rat paw. *J Ethnopharmacol*. 1986;17:161–169.
21. Hiermann A, Schramm HW, Laufer S. Anti-inflammatory activity of myricetin-3-0-beta-d-glucuronide and related compounds. *Inflamm Res*. 1998;47:421–427.
22. Hiermann A, Reidlinger M, Juan H, et al. [Isolation of the antiphlogistic principle from epilobium angustifolium.]. *Planta Med*. 1991;57:357–360. [German].
23. Battinelli L, Tita B, Evandri MG, et al. Antimicrobial activity of epilobium spp. extracts. *Farmaco*. 2001;56:345–348.
24. Rauha JP, Remes S, Heinonen M, et al. Antimicrobial effects of finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol*. 2000;56:3–12.
25. Ducrey B, Marston A, Gohring S, et al. Inhibition of 5 alpha-reductase and aromatase by the ellagitannins oenothein a and oenothein b from epilobium species. *Planta Med*. 1997;63:111–114.
26. Vitalone A, Bordi F, Baldazzi C, et al. Anti-proliferative effect on a prostatic epithelial cell line (PZ-HPV-7) by epilobium angustifolium L. *Farmaco*. 2001;56:483–489.
27. Ducrey B, Marston A, Gohring S, et al. Inhibition of 5 alpha-reductase and aromatase by the ellagitannins oenothein a and oenothein b from epilobium species. *Planta Med*. 1997;63:111–114.
28. Hiermann A, Bucar F. Studies of epilobium angustifolium extracts on the growth of accessory sexual organs in rats. *J Ethnopharmacol*. 1997;55:179–183.
29. Lesuisse D, Berjonneau J, Ciot C, et al. Determination of oenothein b as the active 5-alpha-reductase inhibiting principle of the folk medicine Epilobium parviflorum. *J Nat Prod*. 1996;59:490–492.
30. Stoarczyk M, Piwowarski JP, Granica S, et al. Extracts from epilobium sp. herbs, their components and gut microbiota metabolites of epilobium ellagitannins, urolithins, inhibit hormone-dependent prostate cancer cells—(LNCaP) proliferation and PSA secretion. *Phtyother Res*. 2013;27:1842–1848.
31. Seeram NP, Henning SM, Zhang VJ, et al. Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine up to 48 hours. *J Nutr*. 2008;136:2481–2485.
32. Coulson S, Rao A, Beck SL, et al. A phase II randomized double-blind placebo-controlled clinical trial investigating the efficacy and safety of prostateeze max: a herbal medicine preparation for the management of symptoms of benign prostatic hypertrophy. *Complement Ther Med*. 2013;21:172–179.
33. Granica S, Piwowarski JP, Czerwinska ME, Kiss AK. Phytochemistry, pharmacology and traditional uses of different epilobium species (Onagraceae): a review. *J Ethnopharmacol*. 2014;156:316–346.
34. Moore M. *Medicinal plants of the Pacific West*. Santa Fe, NM: Red Crane Books.
35. Hiermann A, Bucar F. Studies of epilobium angustifolium extracts on the growth of accessory sexual organs in rats. *J Ethnopharmacol*. 1997;55:179–183.
36. Lesuisse D, Berjonneau J, Ciot C, et al. Determination of oenothein b as the active 5-alpha-reductase inhibiting principle of the folk medicine epilobium parviflorum. *J Nat Prod*. 1996;59:490–492.
37. Slacanin I, Marston A, Hostettmann K, et al. Isolation and determination of flavonol glycosides from epilobium species. *J Chromatol*. 1991;557:391–398.
38. Averett JE, Kerr BJ, Raven PH. The flavonoids of onagraceae, tribe epilobieae: epilobium sect. *Epilobium*. *Am J Bot*. 1978;65:567–570.
39. Ducrey B, Marston A, Gohring S, et al. Inhibition of 5 alpha-reductase and aromatase by the ellagitannins oenothein A and oenothein B from epilobium species. *Planta Med*. 1997;63:111–114.
40. Tita B, Abdel-Haq H, Vitalone A, et al. Analgesic properties of epilobium angustifolium, evaluated by the hot plate test and the writhing test. *Farmaco*. 2001;56:341–343.
41. Kujawski R, Ozarowski M, Derebecka-Holysz N, et al. Effect of willow herb (Epilobium angustifolium L.) extract on gene expression of selected p450 cytochromes in rat iver—preliminary study. *Herba Polonica*. 2009;55:52–64.
42. Karakut S, Semiz A, Celik G, et al. Epilobium hirsutum alters xenobiotic metabolizing CYP1A1, CYP2E1, NQO1 And GPx activities, mRNA and protein levels in rats. *Pharmaceut Biol*. 2013;51:650–658.

79

Fatty Acid Metabolism

Lahnor Powell, ND, MPH, and *Edward C. Wallace, ND, DC*

OUTLINE

FATTY ACID STRUCTURE AND METABOLISM

Fatty acids derived from digestion and small intestine absorption or endogenous production are either converted into energy, stored as triglycerides, incorporated into cellular membranes, or they give rise to longer fatty acids.[1] The effect of fatty acids on health depends on their structure and quantity, plus the balance in relationship to other fatty acids. Fatty acids affect membrane permeability, the production of inflammatory molecules (eicosanoids), and free-radical generation, which are underlying processes thought to play a role in the pathophysiology of mood and cognition, cardiovascular disease, inflammatory conditions, obesity, and cancer.[2–12] This chapter focuses on fatty acid nomenclature, metabolism, and a review of their importance in health and disease.

NOMENCLATURE

The basic structure of a fatty acid consists of a hydrogen-carbon (hydrocarbon) open-chain molecule with a terminal carboxylic group (–COOH; Fig. 79.1).[11] Each carbon atom forms four bonds, either to another carbon or to a hydrogen atom. Nomenclature systems describe the number of carbon atoms and indicates the type and location of the bonds.[11,13] There are several nomenclature systems. In general, a numerical prefix describes the length of the hydrocarbon chain, and the suffix indicates the type and location of the bonds.[13] To describe the relative location of the hydrogen atoms for fatty acids containing double bonds, a cis- or trans- notation is used. When the hydrogen atoms and double bond are present on opposite sides, the trans- notation is used.[11] If the hydrogen atoms and double bond are present on the same side, the cis- notation is used.[11] The structural variances of fatty acids result in important differences in the form, function, and energetics of the molecule.

Saturated Fatty Acids

A saturated fatty acid carbon chain contains the maximum number of hydrogen bonds with a terminal carboxylic group (–COOH).[11] A familiar nomenclature system, from the International Union of Pure and Applied Chemists (IUPAC), uses a Latin numerical system to describe the number of carbon atoms.[13] A 16-carbon saturated fatty acid is termed *hexadecanoic acid,* with *hexa-* as the prefix for the number 16.[13] Lipid numbers nomenclature is an abbreviated system listing the number of carbon atoms. The lipid numbers nomenclature for hexadecanoic acid is 16:0.[11] The common nomenclature system, frequently used in the literature, aims to simplify the naming system. For example, hexadecanoic acid's common name is palmitic acid.[11]

Saturated fatty acids can range from 2 to 30 carbons (Fig. 79.2). The most common and important fatty acids are 12 to 22 carbons in length: stearic acid (octadecanoic acid C18:0), palmitic acid (hexadecanoic acid C16:0), myristic acid (tetradecanoic acid C14:0), and lauric acid (dodecanoic acid C12:0).[11,14–16] They are commonly found in plant and animal fats such as coconut, nutmeg, palm kernel oil, fish oil, and certain animal products such as dairy.[11,15] Because of their structure, saturated fatty acids are solid at room temperature and have better oil stability.[11] Oil stability describes the oil's heat index, or smoke temperature, and resistance to oxidation.[17] When an oil reaches its smoke point or has a low resistance to oxidation, it degrades or generates free radicals.[17] Accumulation of free radicals, also termed oxidative stress, can lead to changes in cell membranes and other structures, such as DNA, proteins, carbohydrates, and lipids.[18,19] If free radicals are not regulated by antioxidants, they can induce a variety of chronic and degenerative illnesses.[18,19] Compared with corresponding unsaturated fatty acids, saturated fats have a higher heat index and better oxidative stability, making them an ideal oil for cooking at higher temperatures.[17]

The saturated fatty acid structure, with its molecules packed tightly together, makes it challenging for the body to convert into energy. Fatty acids have been associated with many chronic conditions, including

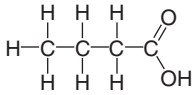
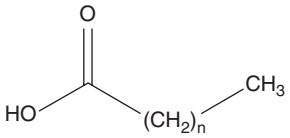


Fig. 79.1 Fatty acid structure.



Caprylic acid	n = 6
Capric acid	n = 8
Lauric acid	n = 10
Myristic acid	n = 12
Palmitic acid	n = 14
Stearic acid	n = 16

Fig. 79.2 Structure of saturated fatty acids.

cardiovascular disease, cancers, and systemic inflammation.[12] This led influential health and nutrition agencies, including the World Health Organization and the American Heart Association, to recommend that saturated fatty acid intake be limited to 7% to 10% of daily calorie energy consumption or 24 grams.[15] Recent advances in nutrition research focused on individual saturated fatty acid structure, metabolism, and function. The research highlighted the importance of certain saturated fatty acids in gene expression, through the alteration of messenger RNA (mRNA; conveys genetic information from DNA), plus lipid metabolism by altering the production, secretion, and clearance of cholesterol.[12] Evidence of the effects of saturated fatty acids on cardiovascular health, insulin resistance, and stroke is still mixed.[14,16,20] Therefore careful incorporation of these specific fatty acids in the diet may be potentially beneficial for health.[11,14-16]

Unsaturated Fatty Acids

Unsaturated fatty acid carbon chains contain one or more double bonds with a terminal carboxylic group (–COOH), unlike saturated fatty acids, which contain no double bonds.[11] These fatty acids are subdivided into two groups depending on the number of double bonds. A single double bond is termed *monounsaturated,* and those with more than one double bond are termed polyunsaturated. The IUPAC name for an 18-carbon fatty acid with a double bond at position 9 is called octadec-9-enoic acid.[11,13] In addition to nomenclature systems describing the number of carbon atoms and location of the double bond(s), a "cis-" or "trans-" notation describes the configuration. A cis- notation means hydrogen atoms are on the same side as the carbon double bond, whereas a trans- notation means hydrogen atoms are on the opposite side of the carbon double bonds (Fig. 79.3).[11,21] The shape of the trans fatty acid creates a kink in the molecule that determines its fluidity state at room temperature and its molecular stability.[11] Lipid number nomenclature for the 18-carbon fatty acid with a single double bond at position 9 and hydrogen atoms on the same side of the double bond is 18:1cis-9.[11] The common name is oleic acid. The common name for an 18-carbon polyunsaturated fatty acid with double bonds at positions 9, 12, and 15 is alpha-linolenic acid (ALA).[11] The IUPAC name is 9, 12, 15-octadecatrienoic acid, and the lipid number is 18:3cis-9,12,15.[13]

Monounsaturated Fatty Acids

Monounsaturated fatty acids tend to be solid when chilled and are liquid at warmer temperatures. Compared with corresponding saturated fatty acids, monounsaturated fats have a lower heat index and

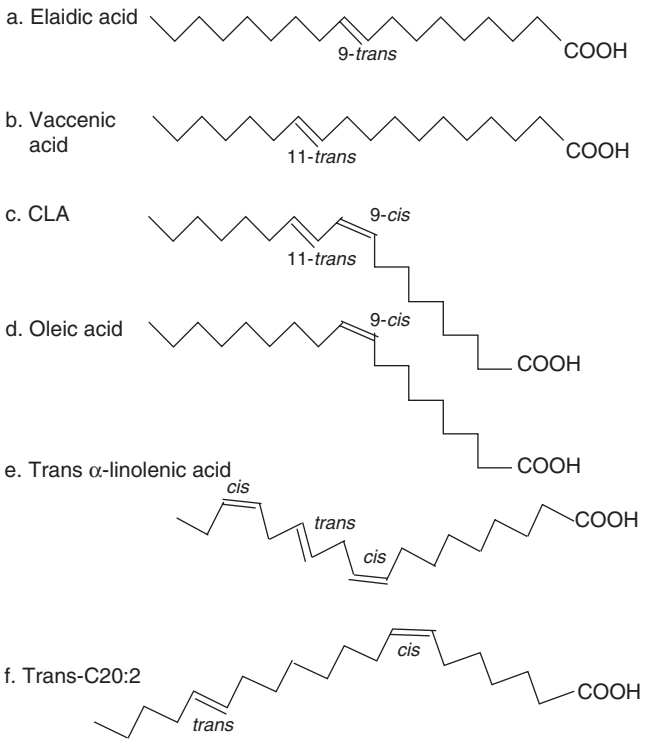


Fig. 79.3 Structure and examples of unsaturated fatty acids.

oxidative stability, making them less ideal for cooking at high temperatures.[17] In descending order, the most common monounsaturated fatty acids in the diet are oleic acid (18:1 cis-9/omega-9), palmitoleic acid (16:1 cis-7/omega-7), and vaccenic acid (18:1 cis-7/omega-7).[21] These fatty acids are found in olive oil, sesame, oil, canola oil, avocados, and many nuts and seeds.[11,21] The main sources of monounsaturated fatty acids have the cis-configuration. The most common trans-configuration monounsaturated fatty acid is elaidic acid (18:1 trans-9), found in hydrogenated vegetable oils and naturally in some meats in small amounts.[21]

The Mediterranean diet, which is high in monounsaturated fatty acids, continues to receive attention because of its association with lower cardiovascular disease risk, improvement in insulin sensitivity, decreased risk for certain cancers, and positive effects on cognition.[21–23] Two specific monounsaturated fatty acids consumed in great quantities in the Mediterranean diet are omega-7 and omega-9 fatty acids. Omega-7 fatty acids are found in fish (salmon and anchovy), olive oil, and macadamia oil, and omega-9 fatty acids are found in olive oil, canola oil, and sunflower oil. Because these fatty acids can assist with the regulation of blood sugar and fat metabolism or breakdown, they are receiving positive attention for the management of metabolic disorders (diabetes, cardiovascular disease, and obesity). [24–26]

Health and nutrition organizations recommend that 20% to 25% of total caloric energy be derived from monounsaturated fatty acids, although debate on dietary amounts continues.[12,21] National nutritional guidelines emphasize balance—by increasing monounsaturated fatty acid intake at the expense of saturated fatty acids—to gain the greatest health benefits.[21,23]

Polyunsaturated Fatty Acids

Because of the structure of polyunsaturated fatty acids, they tend to be liquids. Compared with monounsaturated fatty acids, polyunsaturated fats have a lower heat index and oxidative stability, making them less ideal for cooking at high temperatures.[17] The biological function of a

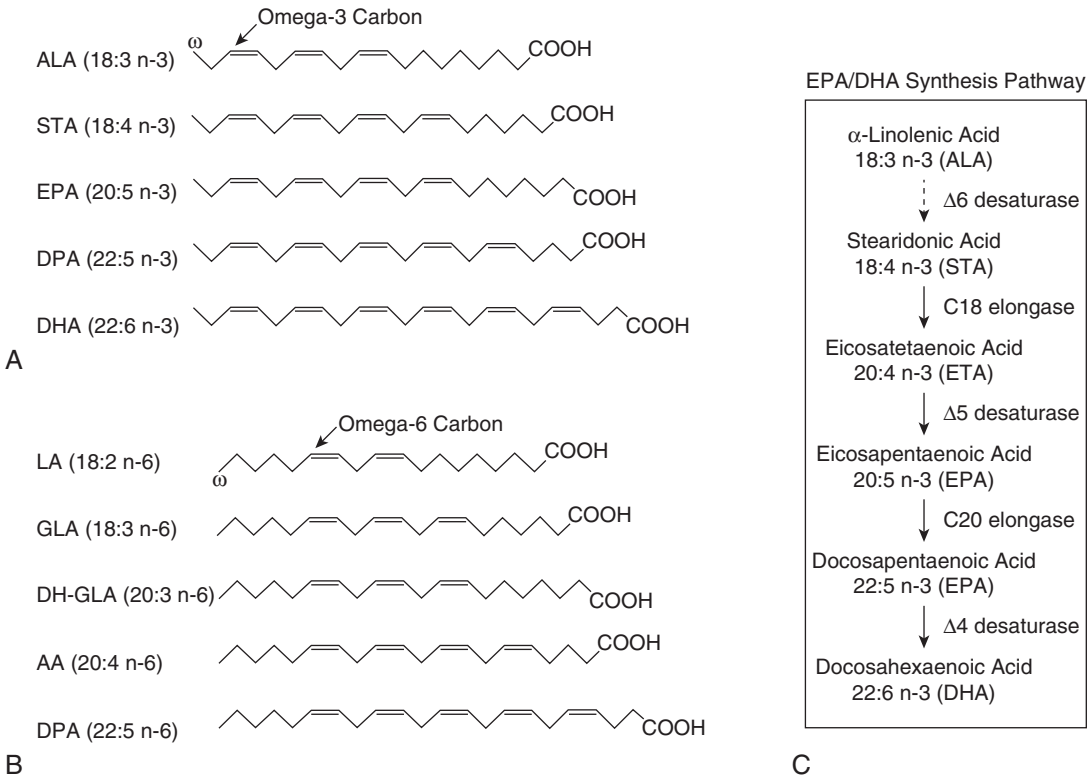


Fig. 79.4 Omega-3 and omega-6 fatty acid structures and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) synthesis pathway. (A) Omega-3 fatty acids include alpha-linolenic acid (ALA), stearidonic acid (STA), EPA, docosapentaenoic acid n-3 (DPA n-3), and DHA. (B) Omega-6 fatty acids include linoleic acid (LA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DH-GLA), arachidonic acid (AA), and docosapentaenoic acid n-6 (DPA n-6). Additionally, the arrows in A and B point to the omega-3 and omega-6 carbon, respectively. (C) The EPA/DHA synthesis pathway is given with fatty acids and respective enzymes for each step. The omega-6 synthesis of LA (18:2 n-6) to DPA n-6 (22:5 n-6) uses the same enzymes. (From ResearchGate. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. <https://www.researchgate.net/Fig-1-Omega-3-and-Omega-6-Fatty-Acid-Structures-and-EPA-DHA-Synthesis-Pathway-A_fig1_5630480>. Accessed August 22, 2018.)

polyunsaturated fatty acid depends on the location of the last double bond respective to total carbon atoms.[7] Alpha-linolenic acid (ALA) lipid number nomenclature 18:3cis-9,12,15 describes an 18-carbon cis-polyunsaturated fat with double bonds at positions 9, 12, and 15.[11] The location of the last double bond is carbon 15, which is three carbons away from the tail of the 18-carbon fatty acid. Therefore this is considered an omega-3 fatty acid. If the last double bond is six carbons from the tail of the fatty acid, it is classified as an omega-6 fatty acid.[11] Polyunsaturated fatty acids are unique because the human body cannot synthesize precursors to the omega-3 and omega-6 series of fatty acids (Fig. 79.4).[7,11] These precursors are termed essential fatty acids. Therefore they must be consumed from food sources such as nuts and seeds and plant-based oils.[7,12,27]

Essential Fatty Acids

The essential precursor to the omega-3 series of fatty acids is 18:3cis-9,12,15 or ALA, found in green leafy vegetables and various nuts and seeds (walnuts, flaxseed, chai seeds).[7,12,27,28] The majority of ALA is used to generate energy. Only a small portion of ALA is converted to eicosapentaenoic acid (20:5cis-5,8,11,14,17 or EPA) and docosahexaenoic acid (22:6cis-4,7,10,13,16,19 or DHA); therefore dietary intake is important.[8,27,29] Direct sources of EPA and DHA are marine fatty fish (salmon, mackerel, and sardines), enriched foods (milk, yogurt, and margarines), and supplements.[27]

The essential precursor to the omega-6 series of fatty acids is 18:2cis-9,12 or linoleic acid (LA), found in plant oils (sunflower, safflower, grape seed, sesame, and corn oils), cereals, eggs, some grains, and poultry.[7,12,30] Polyunsaturated fatty acids are precursors to eicosanoids, or signaling molecules. Those of the omega-6 series are generally considered proinflammatory. However, certain omega-6 fatty acids—specifically dihomo-gamma-linolenic acid (C20:3cis-8,11,14 or DGLA)-derived metabolites—play an essential role in cognition, the reduction of inflammation, the promotion of cardiovascular health, and healthy growth and development.[7,8,12,30] Conversely, proinflammatory arachidonic acid (C20:4cis5,8,11,14 or AA) metabolites are associated with chronic inflammation, cardiovascular disease, and cancer.[7,30]

Polyunsaturated fatty acids can be metabolized into eicosanoids via cyclooxygenase enzymes (COX) and lipoxygenase (LOX) enzymes.[7] Eicosanoids are inflammatory mediators and include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), and hydroxyeicosatetraenoic acids (HETEs).[6–8] AA (an omega-6 fatty acid) converts to 2-series prostaglandins (PGI2, PGE2, PGF2, and PGD2) and thromboxanes (TXA2), plus 4-series leukotrienes (LTA4, LTB4, LTC4, LTD4, and LTE4), contributing to inflammatory conditions like arthritis, obesity, cardiovascular disease, cancer, and inflammatory bowel disease (IBD).[7,12,30] EPA and DHA (omega-3 fatty acids), plus DGLA (omega-6), convert to the anti-inflammatory eicosanoids

3-series prostaglandins (PGI3, PGE3, PGF3, and PGD3) and thromboxanes (TXA3), plus 5-series leukotrienes (LTA5, LTB5, LTC5, and LTD5).[7,30] Because of the varied actions of polyunsaturated fatty acids, dietary intake should be based on balance. The current consumption of omega-6 to omega-3 is a ratio of 20:1, whereas our Paleolithic ancestors consumed equal amounts (1:1).[31] This dietary imbalance of omega-6 to omega-3 increases proinflammatory eicosanoids and their associated health risks.[7,12] Dietary recommendations suggest a decrease in omega-6 consumption or a balance of 2:1 to 5:1 (omega-6:omega-3) to manage and prevent chronic disease.[27,31,32]

Trans Fatty Acids

Trans fatty acids contain hydrogen atoms on the opposite side of the carbon double bond(s).[11] This slight change in configuration causes the fatty acid to be solid at room temperature, with a longer shelf-life than other unsaturated fatty acids.[11,15] A naturally occurring 18-carbon trans fatty acid with a single double bond at position 11 is called octadec-11-enoic acid (IUPAC), C18:1trans11 (lipid number), or vaccenic acid (common name).[33] Naturally occurring trans fatty acids are found in predominantly non–grass-fed meat and dairy products.[33,34] The industrial partial hydrogenation of liquid plant oils is a common source of synthetic trans fatty acids.[15] This process involves the addition of a hydrogen atom to a fatty acid, converting the double bond to a single bond.[33] Industrially manufactured, or synthetic, trans fatty acids are found in fried foods, potato chips, baked deserts, margarine, and shortening.[33,34] Although both trans fatty acids have a similar structure, industrially produced trans fatty acids have a greater negative effect on health than naturally occurring sources.[35] Synthetic trans fatty acids contribute to cardiovascular disease, stroke, and diabetes through the inhibition of anti-inflammatory polyunsaturated fatty acids.[35,36] Because of the health effect, current recommendations for the consumption of trans fatty acids are less than 1% of total caloric energy intake.[15,33,34]

METABOLISM AND BIOCHEMISTRY

Fatty acids derived from digestion and small intestine absorption or endogenous production are either converted into energy, stored as triglycerides, incorporated into cellular membranes, or they give rise to longer fatty acids.[1] In contrast to other macronutrients (carbohydrates and proteins), fatty acids yield the greatest energy per gram: 9 kcal/gram.[1] To generate energy, fatty acids enter the mitochondria through a series of transport proteins, enzymatic reactions, and free diffusion.

The first step combines the fatty acid with a coenzyme A molecule to generate acyl-CoA (fatty acid–CoA).[37] This process occurs within the cytosol of a cell. Specific transmembrane proteins are used to transport fatty acids into the cytosol, notably fatty acid transport proteins (FATPs) and fatty acid translocase (FAT).[37–39] FATPs are a family of six proteins (FATP1–FATP6), which are expressed differently in various tissues (i.e., adipose, skeletal muscle, brain, liver, kidney, lung, enterocyte, and brain).[38,40] FAT is expressed in macrophages, fat, muscle, intestine, and liver cells.[39,41] In addition to lipid metabolism, FAT is involved in angiogenesis, atherosclerosis, and inflammation.[41] Coenzyme A derived from pyruvate, a metabolite of glucose metabolism, enters the cytosol through translocation.[1] Once the fatty acid and coenzyme A are in the cytosol of the cell, fatty acyl-CoA synthase (FACS) generates an acyl-CoA molecule[37]:

$$\text{Fatty Acid (cytosol)} + \text{Coenzyme A (cytosol)} + \text{ATP} \xrightarrow{\text{FACS}} C_n - \text{Acyl - CoA} + \text{AMP}$$

Next, the acyl-CoA molecule moves from the cytosol into the mitochondria, either by diffusion or through transport by carnitine mitochondria transport proteins.[37] The length of the fatty acid chain determines how it enters the mitochondria. Short- and medium-chain fatty acids—shorter than 12 carbons—diffuse through the mitochondria membranes. Longer-chained fatty acids require the assistance of carnitine mitochondria transport proteins.[37]

Once the acyl-CoA molecule is within the mitochondrial matrix, it is shortened through a sequence of enzymatic reactions termed beta-oxidation.[37,42] This is a multistep catabolic process. The end products of beta-oxidation are an acetyl group, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide F(ADH_2)[37,42]. All of these molecules enter the citric acid cycle (also known as the tricarboxylic acid cycle or Krebs cycle) or the electron transport chain to generate energy in the form of adenosine triphosphate (ATP)[37,42]:

$$C_n\text{ - Acyl CoA} + \text{FAD} + \text{NAD}^+ + H_2O + \text{CoA} \longrightarrow C_{n-2}\text{ Acyl CoA} + FADH_2 + \text{NADH} + \text{acetyl CoA} + H^+$$

Carnitine Mitochondria Transport Proteins and Deficiencies

Movement of the acyl-CoA molecule from the cytosol through the outer mitochondrial membrane is catalyzed by carnitine palmitoyltransferase I (CPT1). There are three isoforms of CPT1—CPT1a (liver), CPT1b (muscles), and CPT1c (neurons).[43] Initially, the enzyme splits the acyl-CoA into two molecules, acyl and CoA. The acyl group is joined to a carnitine molecule forming acyl-carnitine or palmitoyl-carnitine. This molecule is then transferred across the inner mitochondrial membrane, or the mitochondria matrix, by carnitine-acylcarnitine translocase (CACT).[43] Once the acyl-carnitine or palmitoyl-carnitine molecule is within the matrix, it is converted back into acyl-CoA by carnitine palmitoyltransferase II (CPT2).[43] This process releases a carnitine molecule, which is moved back into the cytosol of the cell, using CACT and diffusion, where it can then assist another acyl molecule to enter the mitochondria.[43] Once the acyl-CoA molecule is within the mitochondria, it can undergo beta-oxidation to generate ATP:

$$\text{Cytosol: } C_n\text{–Acyl} + \text{CoA} + \text{carnitine} \xrightarrow{\text{CPT1}} C_n\text{–Acyl-carnitine or palmitoyl-carnitie}$$

$$\text{Mitochondria: } C_n\text{–Acyl-carnitine or palmitoyl-carnitine} \xrightarrow{\text{CPT2}} C_n\text{–Acyl-CoA} + \text{carnitine}$$

Carnitine palmitoyltransferase I (CPT1) is considered the rate-limiting, or slowest, step needed to move acyl-CoA into the mitochondria for beta-oxidation. There is a rare genetic polymorphism for CPT1 that makes it difficult to use longer-chained fatty acids for energy production. It is generally diagnosed in childhood, and the primary symptom is hypoketotic hypoglycemia (low ketone and blood sugar levels).[44] Affected individuals are at risk of hepatomegaly, liver dysfunction, and/or elevated serum carnitine, potentially causing nervous system damage, liver failure, seizures, coma, and sudden death.[44]

Some individuals have a genetic polymorphism for CPT2, or the enzyme that assists with the conversion of acyl-carnitine or palmitoyl-carnitine into acyl-CoA energy production. There are several mutations of the *CPT2* gene: the lethal neonatal form is associated with cardiac damage, liver failure, seizures, and death shortly after birth; the severe infantile form is associated with increased risk of liver failure, nervous system damage, coma, and sudden death; and a benign adult form is characterized by rhabdomyolysis.[44]

Management for CPT1 and CPT2 genetic polymorphisms include frequent feedings or avoiding fasting, avoiding sustained exercise, stress management, maintaining optimal immune health, and a low-fat diet.[44]

Another genetic polymorphism that affects fatty acid and carnitine movement through the inner mitochondrial membrane is carnitine-acylcarnitine translocase (CACT) deficiency. Metabolic consequences include hypokinetic hypoglycemia (low ketone and blood sugar levels), elevated serum ammonia, elevated creatinine kinase and transaminases, dicarboxylic aciduria, and an abnormal acylcarnitine profile (low free carnitine and acylcarnitine species).[44] Symptomatic neonates present with a rapidly progressive form of CACT deficiency that is often fatal, whereas presentation later in life is generally milder. Dysfunction or damage can occur to the brain, heart, skeletal muscles, and liver, leading to progressive neurological, cardiac, and liver deterioration; coma; and sudden death.[44] The therapeutic approach is to avoid periods of fasting and hypoglycemia, restrict long-chain fatty foods, and implement carnitine supplementation as needed.

The transport of longer-chained fatty acids across the mitochondria membranes for subsequent beta-oxidation is dependent on carnitine. Carnitine can be synthesized by the body, obtained from the consumption of animal products, or acquired through exogenous supplementation. Endogenous carnitine synthesis occurs in the liver and kidney and, to a lesser extent, the brain. The essential amino acids methionine and lysine are joined to form carnitine with the assistance of several nutrient cofactors: pyridoxal phosphate (B_6), niacin (B_3), vitamin C, and iron.[43]

People on strict nonanimal product diets maintain carnitine levels, in part, because of efficient renal tubular resorption. Organic cation/carnitine transporter 2 (OCTN2) assists with reabsorption and the accumulation of carnitine in the liver, kidney, and muscles.[43] A genetic polymorphism of OCTN2 leads to urinary carnitine wasting and decreased accumulation within cells.[44] Unlike other carnitine genetic defects, OCTN2 affects plasma carnitine levels.[44] This autosomal-recessive disorder is aggravated by periods of fasting and low dietary carnitine intake.[44] Symptoms range from hypoglycemia and hepatic encephalopathy, experienced in early life, to skeletal or cardiac myopathy and cardiac-related sudden death.[43,44] Clinical management consists of carnitine monitoring and supplementation, monitoring cardiac and liver function, and avoiding hypoglycemic episodes (i.e., fasting).[43,44]

Beta-Oxidation

Georg Franz Knoop is credited with discovering fatty acid beta-oxidation in 1904.[37,42] His landmark experiment involved feeding specific fatty acid compounds to dogs and analyzing their urine. He noticed that the initial compound fed to the dog was shortened by two carbons when analyzed in urine. He concluded that fatty acid metabolism via beta-oxidation is the successive removal of two carbon fragments.[37,42] Other influential scientists confirmed and further developed Knoop's beta-oxidation theory. They identified genetic polymorphism enzyme deficiencies, clarified the role of carnitine in fatty acid metabolism, purified various beta-oxidation enzymes, and cloned genes responsible for disease-causing mutations.[37,42] Beta-oxidation is an important metabolic pathway for liver, heart, and skeletal muscle energy production.[42] During fasting, most tissues, excluding the brain, rely on beta-oxidation to generate energy.[42] The brain uses ketones—organic compounds created by the liver from fatty acids—as its energy source.[42]

As Georg Knoop discovered, beta-oxidation is a cyclic process, cleaving two carbon atoms from the acyl-CoA chain. Beta-oxidation is a series of four reactions, including oxidation, hydration, and cleavage of the acyl-CoA molecule.[37,42] Each cycle produces acetyl-CoA, nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide ($FADH_2$), and water.[37] The total energy produced from a fatty acid depends on the carbon length. Each cycle of beta-oxidation has the potential to yield 17 ATP molecules using the products of beta-oxidation in the electron transport chain and the citric acid cycle.[1]

Oxidation

The initial step of the beta-oxidation cycle introduces a double bond between the second and third carbon (in relation to the terminal carboxylic group) of the acyl-CoA molecule. This process is catalyzed by acyl-CoA dehydrogenase (ACD), generating 2-trans-enoyl-CoA.[37,45,46] Flavin adenine dinucleotide (FAD) is a cofactor for this reaction. In humans, FAD is made from dietary riboflavin or vitamin B_2.[47] This molecule accepts the two hydrogen molecules released from the acyl-CoA molecule—$FADH_2$—which can be used to generate ATP:

$$C_n - \text{Acyl-CoA} + \text{FAD} \xrightarrow{\text{ACD}} \text{2-trans-enoyl-CoA} + FADH_2$$

There are several variations of ACD, based on the length of the acyl-CoA molecule: short-chain ACD (SACD), medium-chain ACD (MACD), long-chain ACD (LACD), and very long-chain ACD (VLACD).[48] Although the enzyme's active site varies in amino acid sequencing, the mechanism of action is similar. Unfortunately, ACD genetic polymorphisms can result in ACD enzyme deficiencies.[49] This autosomal-recessive inborn error of mitochondrial fatty acid oxidation is commonly diagnosed through newborn screening.[50–53] ACD deficiency prevents the body from converting fats into energy through beta-oxidation, which is essential, especially during periods of restricted caloric intake. Mortality and morbidity are highest in undiagnosed individuals.[49] Each ACD deficiency manifests slightly differently (see following discussion); however, the management principles are the same: frequent feeding or avoidance of fasting, consumption of a low-fat diet, and improving immune health to minimize infections.[49–53]

Medium-chain acyl-CoA dehydrogenase deficiency (MACDD) is the most common fatty acid oxidation disorder.[49,51] If not identified with newborn screening, symptoms of hypoketotic hypoglycemia (low ketone and blood sugar levels) typically manifest by age 3 or later in adulthood.[52] Although variable, individuals can experience episodes of vomiting and lethargy, mainly triggered by fasting or illness.[51,52]

Short-chain acyl-CoA dehydrogenase deficiency (SCADD), if not fatal, typically presents in early childhood by age 5.[50,54] Symptoms include developmental delay, seizures, behavioral disorders, and hypoglycemia.[50,54,55] People with SCADD can remain asymptomatic or experience muscle-related weakness and wasting in adulthood.

The last two ACD genetic polymorphisms, long-chain and very-long-chain dehydrogenase deficiencies, are rare. As early as days or weeks after birth, infants are lethargic and hypoglycemic, have hypertrophic cardiomyopathy, irregular heart rhythms, and respiratory failure, potentially leading to death.[53] With late-onset presentation, symptoms include lethargy, hypoglycemia, an enlarged liver, metabolic acidosis, coma, and respiratory or cardiac arrest.[53]

Hydration

The second reaction of the beta-oxidation cycle hydrates 2-trans-enoyl-CoA into 3-hydroxy-acyl-CoA. A water molecule adds a hydroxyl group (–OH) to the double bond at C2.[37] This process is catalyzed by the enzyme enoyl-CoA hydratase[37,56]:

$$\text{2-trans-enoyl-CoA} + H_2O \xrightarrow{\text{Enoyl-CoA hydratase}} \text{3-hydroxy-acyl-CoA}$$

Dehydrogenase

The hydroxyl group (–OH) from 3-hydroxy-acyl-CoA is dehydrogenated by NAD^+, which removes a hydride ion (H^+) from the hydroxyl group on the third carbon.[37] In humans, NAD is made from the essential amino acid tryptophan or salvaged from niacin or vitamin B_3.[57]

The enzyme that catalyzes the third step of beta-oxidation is 3-hydroxylacyl-CoA-dehydrogenase, resulting in NADH, H^+ molecules, and 3-ketoacyl-CoA[37]:

$$\text{3-hydroxy-acyl-CoA} \xrightarrow{\text{3-hydroxylacyl-CoA-dehydrogenase}} \text{3-keto-acyl-CoA} + \text{NADH} + \text{H}^+$$

Thiolase

In the final step of beta-oxidation, ketoacyl-CoA thiolase attaches a CoA group to 3-ketoacyl-CoA, yielding two molecules: an acetyl-CoA and a 2-carbon-shorter acyl-CoA.[37]

If the initial acyl-CoA molecule is an even-number carbon chain, it reenters the beta-oxidation cycle until two carbon acetyl-CoA molecules are formed as the end product.[37,56] The acetyl-CoA molecules are further used to generate energy from the citric acid cycle. Odd-number carbon chain acyl-CoA molecules result in the formation of a three-carbon propionyl-CoA and a two-carbon acetyl-CoA.[56] The propionyl-CoA is converted into succinyl-CoA through the action of propionyl-CoA carboxylase and methylmalonyl-CoA mutase.[56] Both the acetyl-CoA and succinyl-CoA molecules enter into the citric acid cycle to generate ATP:

$$\text{3-keto-acyl-CoA} + \text{CoA} \xrightarrow{\text{ketoacyl-CoA thiolase}} \text{C}_{n-2}\text{Acyl-CoA} + \text{Acetyl-CoA}$$

Peroxisome Beta-Oxidation

If a fatty acid or derivative is unable to be oxidized by mitochondrial enzymes, beta-oxidation can occur in peroxisomes.[58] Peroxisomes, a type of organelle, are located within the cytoplasm of cells.[59] They contain a variety of enzymes involved in various metabolic reactions, including energy production from certain fatty acids.[59] Peroxisome beta-oxidation is reserved for very long-chain fatty acids and long-chain fatty acids.[43,58] A CoA molecule is removed from the longer-chained fatty acyl-CoA through ATP-binding cassette (ABC) transporters, allowing entry into the peroxisomal membrane.[43,60] Similar to mitochondrial beta-oxidation, peroxisome beta-oxidation consists of oxidation, hydration, dehydrogenase, and thiolase enzymes.[58,60] However, as the fatty acid chain shortens, it can be exported to the mitochondria for the completion of beta-oxidation.[43,60] The primary function of peroxisome beta-oxidation is chain-shortening, whereas mitochondria beta-oxidation is focused on the generation of energy.[37,43]

MODIFICATION AND SYNTHESIS OF FATTY ACIDS

Fatty acids are predominately derived from the diet; however, the body can synthesize fatty acids from acetyl-CoA or change the structure and function of a fatty acid, by either introducing a double bond (desaturation) or adding carbon atoms (elongation).[61,62] Modification or endogenous production of fatty acids leads to functionally diverse fatty acids for energy production, incorporation into cellular membranes, or conversion into eicosanoid signaling molecules.[1]

De Novo Lipogenesis

De novo lipogenesis—or endogenous fatty acid synthesis—gives rise to fatty acids within the cytoplasm of cells.[62] The precursor, acetyl-CoA, is derived from pyruvate (a carbohydrate metabolite), fatty acid beta-oxidation, citrate from the citric acid cycle, and the breakdown of branched-chain amino acids (isoleucine, leucine, and valine).[1,62] Acetyl-CoA carboxylase, a biotin-dependent enzyme, catalyzes the first step, generating malonyl-CoA from acetyl-CoA, and requires carbon dioxide and ATP.[1,5] There are two isoforms of acetyl-CoA carboxylase (ACC)—ACC1 and ACC2—with expressions in different tissues (adipose, skeletal muscles, and heart); however, both are found in the liver.[5] Malonyl-CoA from ACC1 adds 2 carbon atoms to fatty acids, whereas malonyl-CoA from ACC2 is an inhibitor of carnitine palmitoyltransferase I (CPTI)[5,63]:

$$\text{Acetyl-CoA} + \text{CO}_2 + \text{ATP} \xrightarrow{\text{ACC}} \text{Malonyl-CoA}$$

ACC is regulated by several molecules. Insulin activates carboxylase, whereas epinephrine, an end product of de novo lipogenesis (acyl-CoA), and glucagon inactivate carboxylase.[1,5,37] Diet can also regulate ACC synthesis. A prolonged high-carbohydrate diet leads to an increase in ACC synthesis, and a prolonged low-calorie diet decreases ACC synthesis.[1,5]

Malonyl-CoA, generated from acetyl-CoA, extends the length of fatty acids through the addition of two carbons. This process is catalyzed by fatty acid synthase (FASN), a multienzyme protein, generating a saturated acyl chain with additional two carbon groups.[5,62,64] The primary end product of de novo lipogenesis is palmitate (C16:0 or hexadecanoic acid), a 16-carbon saturated fatty acid and stearic acid (C18:0 or octadecanoic acid), in smaller quantities.[64,65] The endogenous fatty acids are stored as triglycerides, incorporated into cell membranes as phospholipids, converted to ATP, or converted to longer fatty acids.[5]

Desaturation

Desaturation is the introduction of a double bond into a fatty acid chain. This process is catalyzed by endoplasmic reticulum membrane-bound enzymes: NADH-cytochrome b5 reductase, cytochrome b5, and a terminal desaturase.[2] Through a complex process of transferring electrons and reduced iron to interact with oxygen, a double bond is formed, releasing two molecules of water.

The location of the double bond depends on the type of enzyme. In humans, the name of the enzyme is delta (Δ) desaturase, which introduces a double bond, counted from the carboxyl end of the fatty acid.[2,8] Three Δ desaturases are found in mammals: Δ-9 desaturase, Δ-6 desaturase, and Δ-5 desaturase.[8] Delta-9 desaturase introduces a double bond between carbons 9 and 10, mainly generating monounsaturated fatty acids.[2,3,8] The primary product of this pathway is oleic acid (18:1 cis-9), which is incorporated into triglycerides and used for phospholipid synthesis.[2] Delta-5 and Δ-6 desaturase enzymes introduce a double bond between carbons 5 and 6 or carbons 6 and 7. These enzymes are responsible for the synthesis of highly unsaturated fatty acids (HUFAs).[2]

HUFAs function to maintain membrane fluidity, triglyceride synthesis, eicosanoid signaling (modulating inflammation and protecting the digestive tract epithelium), and gene expression regulation.[2,3] Unlike plants and lower eukaryotes, mammals lack the ability to add double bonds beyond carbon number 9, making dietary consumption of certain fatty acids metabolically essential.[8] Alpha-linolenic acid (ALA) is an 18-carbon fatty acid containing three double bonds at positions 9, 12, and 15.[11] The location of the double bonds makes this omega-3 fatty acid essential, meaning it must be supplied through dietary intake. ALA gives rise to other omega-3 series fatty acids: eicosapentaenoic acid (20:5cis-5,8,11,14,17 or EPA) and docosahexaenoic acid (22:6cis-4,7,10,13,16,19 or DHA) using desaturase enzymes.[8,11] Linoleic acid (LA) is also an 18-carbon essential fatty acid containing two double bonds at location 9 and 12.[11] LA gives rise to other omega-6 series fatty acids: dihomo-gamma-linolenic acid (C20:3cis-8,11,14 or DGLA) and arachidonic acid (C20:4cis5,8,11,14 or AA) using desaturase enzymes.[8,11]

There are several conditions that influence delta-desaturase activity: genetic polymorphisms, vitamin and mineral deficiencies, and insulin resistance. Delta-9 desaturase is encoded by stearoyl-CoA desaturase (SCD), whereas Δ-6 and Δ-5 desaturase are

encoded by fatty acid desaturase 1 (FADS1) and fatty acid desaturase 2 (FADS2).[2,8,30] These enzyme deficiencies lead to a reduction in beneficial fatty acids (i.e., oleic acid and EPA) while perpetuating chronic diseases (inflammation and cardiovascular disease) associated with the presence or deficiency of nonbeneficial fatty acids.[8] These enzymes are also influenced by the deficiency in cofactors: magnesium, vitamin C, vitamin B_2, vitamin B_6, and vitamin B_9. Therefore appropriate cofactors will improve the availability of various omega-3 and omega-6 series fatty acids.[66] Lastly, insulin enhances Δ-6 and Δ-5 desaturase, increasing eicosanoid production, which, in turn, enhances insulin's action.[2,66] Insulin resistance, therefore, affects these enzymes, leading to a decrease in eicosanoid production and inefficient insulin activity.[2,66]

Chain Elongation

Fatty acid elongation occurs within different compartments of cells, mainly the cytosol, mitochondria, and endoplasmic reticulum or microsomes.[4] In the cytosol, the addition of two carbons occurs as a part of de novo lipogenesis, producing palmitate (C16:0 or hexadecanoic acid) as the primary end product.[4] Within the mitochondria, acetyl-CoA and fatty acyl-CoA increase the carbon length of fatty acids catalyzed by enoyl-CoA reductase.[4] The microsomal fatty acid pathway, the principal pathway for elongation, uses products of de novo lipogenesis and dietary fatty acids to generate longer-chain fatty acids, catalyzed by elongase enzymes (Elovl 1–7).[4,30,67]

In combination with desaturation, elongation enzymes can generate longer-chained fatty acids from the metabolically essential omega-6 (DGLA and AA) and omega-3 fatty acids (EPA and DHA).[30,67] AA converts to 2-series prostaglandins (PGI2, PGE2, PGF2, and PGD2) and thromboxanes (TXA2), plus 4-series leukotrienes (LTA4, LTB4, LTC4, LTD4, and LTE4), contributing to inflammatory conditions.[7,12,30] EPA, DHA, and DGLA convert to anti-inflammatory eicosanoids: 3-series prostaglandins (PGI3, PGE3, PGF3, and PGD3) and thromboxanes (TXA3)s, plus 5-series leukotrienes (LTA5, LTB5, LTC5, and LTD5).[7,12,30]

HEALTH AND DISEASE

Saturated Fatty Acids in Health and Disease

Saturated fatty acids are associated with many chronic conditions, including cardiovascular disease, cancers, and systemic inflammation.[12] However, certain saturated fatty acids, ranging from 12 to 22 carbons, may play an important role in hormone production, cardiovascular health, gene transcription, lipogenesis, apoptosis, cellular membrane structure, and protein signaling.[12,14,16,20]

Dementia and Cognitive Decline

The saturated fatty acid link to dementia, elevated serum low-density lipoproteins (LDLs), and other chronic conditions, was based on an association with elevated serum cholesterol and elevated SFA intake.[68] However, a recent scientific trial failed to identify an association between SFA consumption and an increase in LDL cholesterol, visceral fat, or the incidence of metabolic syndrome, all of which are postulated to contribute to cognitive decline and dementia.[21] This trial evaluated the effects of specific SFAs on lipid metabolism and noticed an alteration in the secretion and clearance of cholesterol.[12] In descending order, the following SFAs raised total cholesterol and LDL cholesterol: myristic acid (tetradecanoic acid C14:0), lauric acid (dodecanoic acid C12:0), and palmitic acid (hexadecanoic acid C16:0), but not stearic acid (octadecanoic acid C18:0) or medium-chain saturated fatty acids. [11,14–16]

Cardiovascular Disease

Because of the link between saturated fatty acids and elevated serum low-density lipoproteins, it was postulated that the dietary intake of saturated fatty acids contributes to cardiovascular disease.[69] However, a comparison of two populations with similar intakes of saturated fatty acids and cholesterol—France and Finland—revealed different cardiovascular mortality rates.[70] This study highlights that certain saturated fatty acids (stearic acid and medium-chained saturated fatty acids less than 12 carbons), and balanced consumption with monounsaturated fatty acids, play an important role in cardiovascular health.[70,71]

Literature focusing on the quality of saturated fatty acids links the intake of stearic acid and medium-chained fatty acids (less than 12 carbons) to increases in larger, more buoyant LDL particles, which present a lower risk for cardiovascular disease compared with smaller, dense LDL particles, despite cholesterol content.[72–74] Therefore common cardiovascular risk factors, such as dyslipidemia, obesity, and metabolic syndrome, are not perpetuated by certain SFAs. Specifically, stearic acid (octadecanoic acid C18:0) and medium-chain saturated fatty acids decrease intestinal cholesterol absorption.[11,14–16,75]

Monounsaturated Fats in Health and Disease

The Mediterranean diet is often considered to be a healthy approach because of the dietary focus on monounsaturated fatty acids (MUFAs) and their association with lower cardiovascular disease risk, improvement in insulin sensitivity, a decreased risk for certain cancers, and positive effects on cognition.[21–23] Increasing MUFA intake and decreasing saturated fatty acids is recommended to achieve the greatest health benefits, specifically oleic acid (18:1 cis-9/omega-9), palmitoleic acid (16:1 cis-7/omega-7), and vaccenic acid (18:1 cis-7/omega-7).[21]

Cardiovascular Health and Metabolic Syndrome

Monounsaturated fatty acids demonstrate cardio-protective effects by promoting a healthy lipid profile, mediating blood pressure, and improving insulin sensitivity and glucose levels.[21,23,76] When MUFAs replace dietary saturated fatty acid intake, improvements in cardiovascular lipid profiles are noted, namely, lowering the ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol.[21,23] In addition, when MUFAs replace dietary carbohydrates and specific saturated fatty acids (myristic acid, lauric acid, and palmitic acid), hypotensive effects, a reduction in heart rate, and improvement in insulin and glycemic control are noted.[21,23] Additionally, dietary MUFAs lead to a decrease in central adiposity, a reduction in visceral fat accumulation, and enhanced beta-cell function.[22,23,77]

Polyunsaturated Fats in Health and Disease

Alpha-linolenic acid (ALA) and linoleic acid (LA) are metabolically essential polyunsaturated fatty acids derived from dietary consumption or supplementation. ALA gives rise to EPA and DHA, whereas LA gives rise to DGLA and AA, after the combined efforts of desaturation and elongation enzymes.[8,11] EPA, DHA, and DGLA convert to anti-inflammatory eicosanoids—3-series prostaglandins (PGI3, PGE3, PGF3, and PGD3) and thromboxanes (TXA3), plus 5-series leukotrienes (LTA5, LTB5, LTC5, and LTD5)—which have been shown to decrease signaling proteins involved in inflammation, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6).[7,12,30] Arachidonic acid contributes to inflammatory conditions through conversion to 2-series prostaglandins (PGI2, PGE2, PGF2, and PGD2) and thromboxanes (TXA2), plus 4-series leukotrienes (LTA4, LTB4, LTC4, LTD4, and LTE4).[7,12,30] Because of the varied actions of polyunsaturated fatty acids, dietary intake guidelines focus on balance: favoring anti-inflammatory PUFAs (EPA, DHA, and DGLA) compared with the inflammatory PUFA (AA) because inflammation is considered a contributing factor, or a mediator, for many chronic conditions.[7,12,31]

Mood and Cognition

Polyunsaturated fatty acids (PUFAs) are present in all cellular membranes. Arachidonic acid and DHA are the PUFAs found in high concentrations within the brain.[78–80] Altered PUFA concentrations in the brain, resulting from dietary deficits in omega-3 and an abundance of omega-6 fatty acids, are thought to play a role in the pathophysiology of mood and cognition. Within brain membranes, PUFAs regulate neurotransmission, inflammation and oxidation, and neuroplasticity.[81] The unsaturated nature of omega-3 fatty acids encourages an increase in membrane fluidity that is necessary for proper functioning of cell membrane proteins, such as neurotransmitter receptors. A change in the omega-3 concentration in the brain has been shown to lead to an increase in serotonin receptors (5-HT_2) and a decrease in dopamine receptors (D_2).[81,82] This leads to altered neurotransmitter metabolism, release, and uptake.[82] Furthermore, proinflammatory cytokines (TNF-alpha and interleukin-6) alter serotonin metabolism and contribute to oxidative damage.[82] Anti-inflammatory omega-3 fatty acids counteract these cytokines, whereas AA contributes to an increase in the production of reactive oxygen species, inducing oxidative damage.[81–84] An increase in oxidative stress also leads to neuronal loss, cognitive impairment, and neurodegenerative diseases.[9] Lastly, omega-3 fatty acids have been shown to improve neuroplasticity through their incorporation into membranes and the generation of neurons.[80,81] Therefore alterations in the balance of AA and omega-3 contribute to mood and cognition. In fact, decreasing the ratio of omega-6 to omega-3 has been shown to reduce symptoms of anxiety and depression in mood disorders.[80–84]

Cardiovascular

Atherosclerosis is characterized by inflammation-derived eicosanoid production from arachidonic acid (AA). Thromboxane-A2 and leukotrienes (LTC4, LTD4, and LTE4) induce vasoconstriction, whereas thromboxane-A2 and leukotriene-B4 activate platelet aggregation and induce free radicals.[7,85,86] Furthermore, these eicosanoids stimulate proinflammatory cytokines (IL-1, IL-2, IL-6, and TNF-α), which contribute to obesity development and poor insulin sensitivity, which are associated with several comorbidities, including cardiovascular disease (coronary artery disease), high blood pressure, peripheral artery disease, and stroke.[31,87,88] The American Heart Association recommends that adults maintain a low ratio of AA to omega-3 fatty acids by consuming fatty fish a minimum of twice per week or 1 gram of EPA and DHA daily.[89]

Cancer

Three-series PG (PGI3, PGE3, PGF3, and PGD3), derived from EPA, DHA, and DGLA, can inhibit the growth of cancer cells through the induction of apoptosis; this is beneficial in proliferative diseases, like cancer.[90] Cancer cells evade apoptosis through several proposed mechanisms: modulation of eicosanoids, influencing gene expression, generation of intracellular oxidative stress, and affecting cellular signaling.[10] A balance of inflammatory-promoting fatty acids, AA, and anti-inflammatory omega-3 plus DGLA may affect cancer-cell–specific apoptosis and enhance conventional medical therapies.[10,91]

Inflammatory Conditions

Omega-3 fatty acids and DGLA give rise to anti-inflammatory eicosanoids (3-series prostaglandins (PGI3, PGE3, PGF3, and PGD3) and thromboxanes (TXA3), plus 5-series leukotrienes (LTA5, LTB5, LTC5, and LTD5).[17,22] Omega-6 AA contributes to inflammation through conversion to 2-series prostaglandins (PGI2, PGE2, PGF2, and PGD2) and thromboxanes (TXA2), plus 4-series leukotrienes (LTA4, LTB4, LTC4, LTD4, and LTE4).[7,12,15,30] Production of proinflammatory eicosanoids (AA) leads to an increase in inflammatory cytokine (IL-6, IL-2, IL-1, and TNF-alpha) production.[7] This contributes to inflammation, which is considered a contributing factor, or mediator, for several chronic conditions, including inflammatory bowel disease (IBD), asthma, osteoporosis/osteopenia, Alzheimer's disease, autoimmune conditions, and psoriasis.[7,27,92–97] Therefore anti-inflammatory fatty acid support can assist in the reduction of disease severity by decreasing proinflammatory cytokine production.

Trans Fatty Acids in Health and Disease

Increased intake of trans fatty acids, specifically those that are synthetic or industrially produced (in foods that contain partially hydrogenated oil, such as packaged snacks, fried foods, and margarine), coincides with adverse cardiovascular lipid profiles, increased inflammation, and endothelial dysfunction.[35] Trans fatty acids inhibit the Δ-6-desaturase enzyme needed to convert metabolically essential fatty acids into longer-chained anti-inflammatory fatty acids (EPA, DHA, and DGLA).[7] Because these anti-inflammatory fatty acids are also incorporated into phospholipids, trans fatty acid consumption negatively affects cell-membrane composition.

Cardiovascular Disease

Trans fatty acid intake corresponds to adverse cardiovascular lipid profile markers, including an elevation in the low-density lipoprotein (LDL) to high-density lipoprotein (HDL)–cholesterol ratio, an increase in triglycerides, an increase in lipoprotein (a), a reduction in LDL particle size, and an increase in LDL particle number.[35,36,98] Trans fatty acids are associated with the proinflammatory markers interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), and C-reactive protein (CRP).[35,36,99] These inflammatory substances potentiate atherosclerotic changes, and negatively affect measures of vascular endothelial function.[35] Their contribution to an increase in adipose tissue promotes further inflammatory changes and increased cardiovascular risk.[35]

Liver Dysfunction

An increase in lipid accumulation in hepatocytes, along with oxidative damage, can contribute to the pathophysiology of liver dysfunction.[100] EPA and DHA give rise to anti-inflammatory eicosanoids (3-series prostaglandins [PGI3, PGE3, PGF3 and PGD3] and thromboxanes [TXA3], plus 5-series leukotrienes [LTA5, LTB5, LTC5, and LTD5]), reducing lipid accumulation (fat deposits) and attenuating oxidative damage through up regulation of nuclear factor-like 2 (Nrf2), an anti-oxidant regulating protein protective against oxidative damage.[7,30,101] Additional evidence suggests that functional liver changes (increasing cholesterol accumulation, cholesterol secretion, and lipoproteins), systemic inflammation, and visceral fat accumulation can also contribute to insulin resistance.[102,103]

Mood and Cognition

Increased trans fatty acids, and the resulting decrease in omega-3 fatty acids, can lead to an increase in aggression, irritability, and poor word-recall performance.[104,105] The proposed mechanism of action is the inhibition of Δ-6 desaturase.[105] Because omega-3 fatty acids are incorporated into brain cellular membranes, decreased production can negatively affect neurotransmission, inflammation and oxidation, neuroplasticity, and, thereby, mood and cognition.[81,82,106] These same mechanisms are postulated to contribute to cognitive disorders, including dementia.[107]

TABLE 79.1 Fatty Acid Therapeutic Considerations in Disease

Condition	Therapeutic Considerations
Asthma	Decrease omega-6 and increase omega-3,[108–111] increase GLA,[112,113] and avoid trans fats.[35,36,114]
Cancer	Decrease omega-6 and increase omega-3.[115–119] Some evidence supports a protective effect with increased MUFAs[120,121]; otherwise, opinion is mixed.[122] Decrease saturated FAs[123,124] (opinion remains mixed and association unclear); avoid trans fats.[121,123,125]
Cardiovascular disease	Decrease omega-6 and increase omega-3,[108,126,127] decrease saturated FAs,[128,129] and substitute with MUFAs.[21,130,131]
Cognitive impairment	Decrease omega-6[132] and increase omega-3,[9,132] decrease saturated FAs,[107,132,133] substitute or increase MCTs,[134] increase MUFAs,[133] and avoid trans fats.[105,107]
Depression	Decrease omega-6 and increase omega-3[78,80,84,135]; avoid trans fats.[106]
Diabetes	Omega-6 may benefit,[136] avoid trans fats,[35] omega-3 may benefit (n-3 FAs have been found to positively affect concomitants of diabetes but not the condition directly), decrease saturated FAs,[137,138] and substitute or increase MUFAs.[130,139]
Eczema	Increase n-6 GLAs.[112,113] Some evidence exists for benefits from n-3 fish oils, but the studies are small and mixed. Consider zinc deficiency affecting the conversion of LA into GLA.[140,141]
Irritable bowel disease	Decrease omega-6[142]; increase omega-3.[142–144]
Obesity	Decrease omega-6,[88,145] increase omega-3,[87] increase MUFAs,[146] increase MCFAs.[147–149] SFA has mixed studies. Some show SFA increases adiposity; some show SFA decreases adiposity.
Osteoporosis	Decrease omega-6 and increase omega-3[27,93,150,151]; decrease saturated FAs.[152]
Psoriasis	Decrease omega-6 and increase omega 3.[96,108,126]

FA, fatty acid; *GLA,* gamma-linolenic acid; *MCFA,* medium-chain fatty acid; *MCT,* medium-chain triglyceride; *MUFA,* monounsaturated fatty acid; *SFA,* saturated fatty acid.

CONCLUSION

The basic structure of a fatty acid is a hydrogen-carbon chain with a terminal carboxylic group. Fatty acids are grouped into two main categories, saturated fatty acids and unsaturated fatty acids, based on the presence or absence of double bonds.[11] The number of carbon atoms plus the location of a double bond is the basis for the nomenclature system of fatty acids. A common system is the International Union of Pure and Applied Chemists (IUPAC) system, which uses a Latin numerical system to describe the number of carbon atoms plus the location of double bonds.[13] Although very descriptive, this system can be cumbersome, leading many to use simplified common names.[11]

Fatty acids derived from digestion and small intestine absorption or endogenous production are either converted into energy, stored as triglycerides, incorporated into cellular membranes, or they give rise to longer fatty acids.[1] They yield the greatest energy per gram, compared with other macronutrients, through mitochondria or peroxisome beta-oxidation.[1,37,42] In addition, the body is able to generate fatty acids using acetyl-CoA (de novo lipogenesis) and modify existing fatty acids by adding a double bond (desaturase) or increasing the carbon-chain length (elongation).[2,4,5] The process of elongation and desaturation gives rise to important omega-3 fatty acids—EPA and DHA—from essential alpha-linolenic acid. Omega-6 fatty acids—DGLA and AA—are formed from linoleic acid.[30,67]

The effect of fatty acids on health depends on their structure and quantity, plus the balance in relationship to other fatty acids. The structure, quantity, and balance of fatty acids help ensure proper fluidity and membrane permeability of cells, balance of eicosanoid production, and free-radical generation.[2–10] An impermeable and stiff cellular membrane, inflammation, and oxidative damage are underlying processes that are thought to play a role in the pathophysiology of mood and cognition, cardiovascular disease, inflammatory conditions, obesity, and cancer.[11,12] Therefore the strategic incorporation of fatty acids, through dietary intake or supplementation, can positively influence health. Examples of conditions that may be helped by a proper balance of fatty acids, along with recommendations for achieving a better balance, can be found in Table 79.1.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Champe PC, Harvey RA, Ferrier DR. *Biochemistry*. 3rd ed. Philadelphia: Lippincott/Williams & Wilkins; 2005.
2. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu Rev Nutr*. 2004;24:345–376.
3. Chirala SS, Wakil SJ. Structure and function of animal fatty acid synthase. *Lipids*. 2004;39(11):1045–1053.
4. Jump DB. Mammalian fatty acid elongases. *Methods Mol Biol*. 2009;579:375–389.
5. Tong L. Acetyl-coenzyme A carboxylase: crucial metabolic enzyme and attractive target for drug discovery. *Cell Mol Life Sci*. 2005;62(16):1784–1803.
6. Levant B. N-3 (omega-3) polyunsaturated Fatty acids in the pathophysiology and treatment of depression: pre-clinical evidence. *CNS Neurol Disord Drug Targets*. 2013;12(4):450–459.
7. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab*. 2012;2012:539426.
8. Lee JM, Lee H, Kang S, Park WJ. Fatty acid desaturases, polyunsaturated fatty acid regulation, and biotechnological advances. *Nutrients*. 2016;8(1).
9. Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the atherosclerosis risk in communities study. *Am J Clin Nutr*. 2007;85(4):1103–1111.
10. D'Eliseo D, Velotti F. Omega-3 fatty acids and cancer cell cytotoxicity: implications for multi-targeted cancer therapy. *J Clin Med*. 2016;5(2).
11. Chow CK. *Fatty Acids in Foods and Their Health Implications*. 3rd ed. Boca Raton: CRC Press; 2008.
12. Calder PC. functional roles of fatty acids and their effects on human health. *JPEN J Parenter Enteral Nutr*. 2015;39(suppl 1):18S–32S.
13. The nomenclature of lipids. recommendations (1976) IUPAC-IUB commission on biochemical nomenclature. *Lipids*. 1977;12(6):455–468.
14. Legrand P, Rioux V. The complex and important cellular and metabolic functions of saturated fatty acids. *Lipids*. 2010;45(10):941–946.
15. de Souza RJ, Mente A, Maroleanu A, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ*. 2015;351:h3978.
16. Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids*. 2010;45(10):893–905.
17. Perez-Herrera A, Rangel-Zuniga OA, Delgado-Lista J, et al. The antioxidants in oils heated at frying temperature, whether natural or added, could protect against postprandial oxidative stress in obese people. *Food Chem*. 2013;138(4):2250–2259.
18. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev*. 2010;4(8):118–126.
19. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci*. 2008;4(2):89–96.
20. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am J Clin Nutr*. 2010;91(3):535–546.
21. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. *Nutrients*. 2012;4(12):1989–2007.
22. van Dijk SJ, Feskens EJ, Bos MB, et al. Consumption of a high monounsaturated fat diet reduces oxidative phosphorylation gene expression in peripheral blood mononuclear cells of abdominally overweight men and women. *J Nutr*. 2012;142(7):1219–1225.
23. Gillingham LG, Harris-Janz S, Jones PJ. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids*. 2011;46(3):209–228.
24. Sales-Campos H, Souza PR, Peghini BC, da Silva JS, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. *Mini Rev Med Chem*. 2013;13(2):201–210.
25. Bernstein AM, Roizen MF, Martinez L. Purified palmitoleic acid for the reduction of high-sensitivity C-reactive protein and serum lipids: a double-blinded, randomized, placebo controlled study. *J Clin Lipidol*. 2014;8(6):612–617.
26. Bolsoni-Lopes A, Festuccia WT, Farias TS, et al. Palmitoleic acid (n-7) increases white adipocyte lipolysis and lipase content in a PPARalpha-dependent manner. *Am J Physiol Endocrinol Metab*. 2013;305(9):E1093–E1102.
27. Lavado-Garcia J, Roncero-Martin R, Moran JM, et al. Long-chain omega-3 polyunsaturated fatty acid dietary intake is positively associated with bone mineral density in normal and osteopenic Spanish women. *PLoS One*. 2018;13(1):e0190539.
28. Harris W. Omega-6 and omega-3 fatty acids: partners in prevention. *Curr Opin Clin Nutr Metab Care*. 2010;13(2):125–129.
29. Huang YS, Pereira SL, Leonard AE. Enzymes for transgenic biosynthesis of long-chain polyunsaturated fatty acids. *Biochimie*. 2004;86(11):793–798.
30. Tosi F, Sartori F, Guarini P, Olivieri O, Martinelli N. Delta-5 and delta-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease. *Adv Exp Med Biol*. 2014;824:61–81.
31. Simopoulos AP. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*. 2016;8(3):128.
32. Sheppard KW, Cheatham CL. Executive functions and the omega-6-to-omega-3 fatty acid ratio: a cross-sectional study. *Am J Clin Nutr*. 2017;105(1):32–41.
33. Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2012;29(6):861–874.
34. Kris-Etherton PM, Lefevre M, Mensink RP, Petersen B, Fleming J, Flickinger BD. Trans fatty acid intakes and food sources in the U.S. population: NHANES 1999-2002. *Lipids*. 2012;47(10):931–940.
35. Mozaffarian D, Aro A, Willett WC. Health effects of trans-fatty acids: experimental and observational evidence. *Eur J Clin Nutr*. 2009;63(suppl 2):S5–S21.
36. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med*. 2006;354(15):1601–1613.
37. Kunau WH, Dommes V, Schulz H. beta-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. *Prog Lipid Res*. 1995;34(4):267–342.
38. Stahl A. A current review of fatty acid transport proteins (SLC27). *Pflugers Arch*. 2004;447(5):722–727.
39. Bonen A, Campbell SE, Benton CR, et al. Regulation of fatty acid transport by fatty acid translocase/CD36. *Proc Nutr Soc*. 2004;63(2):245–249.
40. Anderson CM, Stahl A. SLC27 fatty acid transport proteins. *Mol Aspects Med*. 2013;34(2-3):516–528.
41. Bonen A, Chabowski A, Luiken JJ, Glatz JF. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. *Physiology (Bethesda)*. 2007;22:15–29.
42. Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid beta-oxidation. *J Inherit Metab Dis*. 2010;33(5):469–477.
43. Longo N, Frigeni M, Pasquali M. Carnitine transport and fatty acid oxidation. *Biochim Biophys Acta*. 2016;1863(10):2422–2435.
44. Longo N, Amat di San Filippo C, Pasquali M. Disorders of carnitine transport and the carnitine cycle. *Am J Med Genet C Semin Med Genet*. 2006;142C(2):77–85.
45. Thorpe C, Kim JJ. Structure and mechanism of action of the acyl-CoA dehydrogenases. *FASEB J*. 1995;9(9):718–725.
46. Ghisla S, Thorpe C. Acyl-CoA dehydrogenases. a mechanistic overview. *Eur J Biochem*. 2004;271(3):494–508.
47. Devlin TM. *Textbook of Biochemistry: with Clinical Correlations*. 7th ed. Hoboken, NJ: John Wiley & Sons; 2011.
48. Lea W, Abbas AS, Sprecher H, Vockley J, Schulz H. Long-chain acyl-CoA dehydrogenase is a key enzyme in the mitochondrial beta-oxidation of unsaturated fatty acids. *Biochim Biophys Acta*. 2000;1485(2-3):121–128.

49. Gregersen N, Bross P, Andresen BS. Genetic defects in fatty acid beta-oxidation and acyl-CoA dehydrogenases. Molecular pathogenesis and genotype-phenotype relationships. *Eur J Biochem*. 2004;271(3):470–482.
50. Jethva R, Bennett MJ, Vockley J. Short-chain acyl-coenzyme A dehydrogenase deficiency. *Mol Genet Metab*. 2008;95(4):195–200.
51. Grosse SD, Khoury MJ, Greene CL, Crider KS, Pollitt RJ. The epidemiology of medium chain acyl-CoA dehydrogenase deficiency: an update. *Genet Med*. 2006;8(4):205–212.
52. Matern D, Rinaldo P. Medium-chain acyl-coenzyme a dehydrogenase deficiency. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews(R)*. Seattle (WA); 1993.
53. Schiff M, Mohsen AW, Karunanidhi A, McCracken E, Yeasted R, Vockley J. Molecular and cellular pathology of very-long-chain acyl-CoA dehydrogenase deficiency. *Mol Genet Metab*. 2013;109(1):21–27.
54. Bhala A, Willi SM, Rinaldo P, Bennett MJ, Schmidt-Sommerfeld E, Hale DE. Clinical and biochemical characterization of short-chain acyl-coenzyme a dehydrogenase deficiency. *J Pediatr*. 1995;126(6):910–915.
55. van Maldegem BT, Wanders RJ, Wijburg FA. Clinical aspects of short-chain acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis*. 2010;33(5):507–511.
56. Agnihotri G, Liu HW. Enoyl-CoA hydratase. reaction, mechanism, and inhibition. *Bioorg Med Chem*. 2003;11(1):9–20.
57. Belenky P, Bogan KL, Brenner C. NAD+ metabolism in health and disease. *Trends Biochem Sci*. 2007;32(1):12–19.
58. Schrader M, Costello J, Godinho LF, Islinger M. Peroxisome-mitochondria interplay and disease. *J Inherit Metab Dis*. 2015;38(4):681–702.
59. Cooper GM. *The cell: a Molecular Approach*. 2nd ed. Washington, D.C: Sunderland, Mass: ASM Press; Sinauer Associates; 2000.
60. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr*. 2001;21:193–230.
61. Hellerstein MK. De novo lipogenesis in humans: metabolic and regulatory aspects. *Eur J Clin Nutr*. 1999;53(suppl 1):S53–S65.
62. Mashima T, Seimiya H, Tsuruo T. De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy. *Br J Cancer*. 2009;100(9):1369–1372.
63. Foster DW. Malonyl-CoA: the regulator of fatty acid synthesis and oxidation. *J Clin Invest*. 2012;122(6):1958–1959.
64. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. *Biol Rev Camb Philos Soc*. 2016;91(2):452–468.
65. Ameer F, Scandiuzzi L, Hasnain S, Kalbacher H, Zaidi N. De novo lipogenesis in health and disease. *Metabolism*. 2014;63(7):895–902.
66. Das UN. A defect in Delta6 and Delta5 desaturases may be a factor in the initiation and progression of insulin resistance, the metabolic syndrome and ischemic heart disease in South Asians. *Lipids Health Dis*. 2010;9:130.
67. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res*. 2006;45(3):237–249.
68. Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. *Dement Geriatr Cogn Disord*. 2009;28(1):75–80.
69. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet (London, England)*. 2016;388(10053):1659–1724.
70. Artaud-Wild SM, Connor SL, Sexton G, Connor WE. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in france and finland. a paradox. *Circulation*. 1993;88(6):2771–2779.
71. Sacks FM, Lichtenstein AH, Wu JHY, et al. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. *Circulation*. 2017;136(3):e1–e23.
72. Hoogeveen RC, Gaubatz JW, Sun W, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the atherosclerosis risk in communities (ARIC) study. *Arterioscler Thromb Vasc Biol*. 2014;34(5):1069–1077.
73. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res*. 2002;43(9):1363–1379.
74. Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. *Am J Clin Nutr*. 2006;83(5):1025–1031;quiz 1205.
75. Wong ND. Epidemiological studies of CHD and the evolution of preventive cardiology. *Nat Rev Cardiol*. 2014;11(5):276–289.
76. Negele L, Schneider B, Ristl R, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. *Eur J Clin Nutr*. 2015;69(3):337–343.
77. Lopez S, Bermudez B, Pacheco YM, Villar J, Abia R, Muriana FJ. Distinctive postprandial modulation of beta cell function and insulin sensitivity by dietary fats: monounsaturated compared with saturated fatty acids. *Am J Clin Nutr*. 2008;88(3):638–644.
78. Levant B. N-3 (Omega-3) Polyunsaturated fatty acids in the pathophysiology and treatment of depression: pre-clinical evidence. *CNS Neurol Disord Drug Targets*. 2013;12(4):450–459.
79. Liperoti R, Landi F, Fusco O, Bernabei R, Onder G. Omega-3 polyunsaturated fatty acids and depression: a review of the evidence. *Curr Pharm Des*. 2009;15(36):4165–4172.
80. Stahl LA, Begg DP, Weisinger RS, Sinclair AJ. The role of omega-3 fatty acids in mood disorders. *Curr Opin Investig Drugs*. 2008;9(1):57–64.
81. Su KP, Matsuoka Y, Pae CU. Omega-3 Polyunsaturated fatty acids in prevention of mood and anxiety disorders. *Clin Psychopharmacol Neurosci*. 2015;13(2):129–137.
82. Grosso G, Galvano F, Marventano S, et al. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxid Med Cell Longev*. 2014;2014:313570.
83. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. *Brain Behav Immun*. 2011;25(8):1725–1734.
84. Colin A, Reggers J, Castronovo V, Ansseau M. Lipids, depression and suicide. *Encephale*. 2003;29(1):49–58.
85. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol*. 2011;58(20):2047–2067.
86. Bowen KJ, Harris WS, Kris-Etherton PM. Omega-3 fatty acids and cardiovascular disease: are there benefits? *Curr Treat Options Cardiovasc Med*. 2016;18(11):69.
87. Liu HQ, Qiu Y, Mu Y, et al. A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. *Nutr Res (New York, NY)*. 2013;33(10):849–858.
88. Naughton SS, Mathai ML, Hryciw DH, McAinch AJ. Linoleic acid and the pathogenesis of obesity. *Prostaglandins Other Lipid Mediat*. 2016;125:90–99.
89. Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Stroke*. 2000;31(11):2751–2766.
90. Wang X, Lin H, Gu Y. Multiple roles of dihomo-gamma-linolenic acid against proliferation diseases. *Lipids Health Dis*. 2012;11:25.
91. Murphy RA, Mourtzakis M, Chu QS, Baracos VE, Reiman T, Mazurak VC. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. *Cancer*. 2011;117(16):3774–3780.
92. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J*. 2007;6:2.
93. Hogstrom M, Nordstrom P, Nordstrom A. n-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO2 Study. *Am J Clin Nutr*. 2007;85(3):803–807.
94. Li J, Xun P, Zamora D, et al. Intakes of long-chain omega-3 (n-3) PUFAs and fish in relation to incidence of asthma among American young adults: the CARDIA study. *Am J Clin Nutr*. 2013;97(1):173–178.

95. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res*. 2008;52(8):885–897.
96. Rahman M, Beg S, Ahmad MZ, et al. Omega-3 fatty acids as pharmacotherapeutics in psoriasis: current status and scope of nanomedicine in its effective delivery. *Curr Drug Targets*. 2013;14(6):708–722.
97. Dobryniewski J, Szajda SD, Waszkiewicz N, Zwierz K. The gamma-linolenic acid (GLA)—the therapeutic value. *Przegl Lek*. 2007;64(2):100–102.
98. Makarewicz-Wujec M, Dworakowska A, Kozlowska-Wojciechowska M. Replacement of saturated and trans-fatty acids in the diet v. CVD risk in the light of the most recent studies. *Public Health Nutr*. 2018:1–10.
99. Shen J, Ordovas JM. Impact of genetic and environmental factors on hsCRP concentrations and response to therapeutic agents. *Clin Chem*. 2009;55(2):256–264.
100. Sullivan S. Implications of diet on nonalcoholic fatty liver disease. *Curr Opin Gastroenterol*. 2010;26(2):160–164.
101. Sakai C, Ishida M, Ohba H, et al. Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. *PLoS One*. 2017;12(11):e0187934.
102. Wang Q, Imamura F, Ma W, et al. Circulating and dietary trans fatty acids and incident type 2 diabetes in older adults: the Cardiovascular Health Study. *Diabetes Care*. 2015;38(6):1099–1107.
103. Micha R, Mozaffarian D. Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. *Nat Rev Endocrinol*. 2009;5(6):335–344.
104. Golomb BA, Evans MA, White HL, Dimsdale JE. Trans fat consumption and aggression. *PLoS One*. 2012;7(3):e32175.
105. Golomb BA, Bui AK. A Fat to forget: trans fat consumption and memory. *PLoS One*. 2015;10(6):e0128129.
106. Sanchez-Villegas A, Verberne L, De Irala J, et al. Dietary fat intake and the risk of depression: the SUN Project. *PLoS One*. 2011;6(1):e16268.
107. Barnard ND, Bunner AE, Agarwal U. Saturated and trans fats and dementia: a systematic review. *Neurobiol Aging*. 2014;35(suppl 2):S65–S73.
108. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharm*. 2013;75(3):645–662.
109. Miyata J, Arita M. Role of omega-3 fatty acids and their metabolites in asthma and allergic diseases. *Allergol Int Off J Japan Soc Allergol*. 2015;64(1):27–34.
110. Black PN, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J*. 1997;10(1):6–12.
111. Kremmyda LS, Vlachava M, Noakes PS, Diaper ND, Miles EA, Calder PC. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. *Clin Rev Allerg Immunol*. 2011;41(1):36–66.
112. Horrobin DF. Essential fatty acid metabolism and its modification in atopic eczema. *Am J Clin Nutr*. 2000;71(suppl 1):367s–372s.
113. Simon D, Eng PA, Borelli S, et al. Gamma-linolenic acid levels correlate with clinical efficacy of evening primrose oil in patients with atopic dermatitis. *Adv Ther*. 2014;31(2):180–188.
114. Iqbal MP. Trans fatty acids – A risk factor for cardiovascular disease. *Pakistan J Med Sci*. 2014;30(1):194–197.
115. Maillard V, Bougnoux P, Ferrari P, et al. N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. *Int J Cancer*. 2002;98(1):78–83.
116. de Lorgeril M, Salen P. Helping women to good health: breast cancer, omega-3/omega-6 lipids, and related lifestyle factors. *BMC Med*. 2014;12:54.
117. Hidaka BH, Li S, Harvey KE, et al. Omega-3 and omega-6 Fatty acids in blood and breast tissue of high-risk women and association with atypical cytomorphology. *Cancer Prev Res (Phila)*. 2015;8(5):359–364.
118. Bagga D, Anders KH, Wang HJ, Glaspy JA. Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer. *Nutr Cancer*. 2002;42(2):180–185.
119. Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Assessment of risk associated with specific fatty acids and colorectal cancer among French-Canadians in Montreal: a case-control study. *Int J Epidemiol*. 2003;32(2):200–209.
120. Kohlmeier L. Biomarkers of fatty acid exposure and breast cancer risk. *Am J Clin Nutr*. 1997;66(suppl 6):1548S–1556S.
121. Voorrips LE, Brants HA, Kardinaal AF, Hiddink GJ, van den Brandt PA, Goldbohm RA. Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the netherlands cohort study on diet and cancer. *Am J Clin Nutr*. 2002;76(4):873–882.
122. Khodarahmi M, Azadbakht L. The association between different kinds of fat intake and breast cancer risk in women. *Int J Prevent Med*. 2014;5(1):6–15.
123. Patel VH. Nutrition and prostate cancer: an overview. *Exp Rev Anticancer Ther*. 2014;14(11):1295–1304.
124. Shapira N. The potential contribution of dietary factors to breast cancer prevention. *Eur J Cancer Prevent*. 2017;26(5):385–395.
125. Laake I, Carlsen MH, Pedersen JI, et al. Intake of trans fatty acids from partially hydrogenated vegetable and fish oils and ruminant fat in relation to cancer risk. *Int J Cancer*. 2013;132(6):1389–1403.
126. Mori TA, Beilin LJ. Omega-3 fatty acids and inflammation. *Curr Atherosclerosis Rep*. 2004;6(6):461–467.
127. Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA, Lands WE. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. *Am J Clin Nutr*. 2006;83(suppl 6):1483s–1493s.
128. Li Y, Hruby A, Bernstein AM, et al. Saturated fat as compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. *J Am Coll Cardiol*. 2015;66(14):1538–1548.
129. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Med*. 2010;7(3):e1000252.
130. Schwingshackl L, Strasser B, Hoffmann G. Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. *Annals Nutr Metabol*. 2011;59(2-4):176–186.
131. Kris-Etherton P, Eckel RH, Howard BV, Jeor S St, Bazzarre TL. Lyon diet heart study. *Circulation*. 2001;103(13):1823.
132. Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes—The EVA Study. *Am J Clin Nutr*. 2003;77(4):803–808.
133. Solfrizzi V, Capurso C, D'Introno A, et al. Dietary fatty acids, age-related cognitive decline, and mild cognitive impairment. *J Nutr Health Aging*. 2008;12(6):382–386.
134. Page KA, Williamson A, Yu N, et al. Medium-chain fatty acids improve cognitive function in intensively treated type 1 diabetic patients and support in vitro synaptic transmission during acute hypoglycemia. *Diabetes*. 2009;58(5):1237–1244.
135. Grosso G, Galvano F, Marventano S, et al. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxidat Med Cellular Longevity*. 2014;2014:313570.
136. Yary T, Voutilainen S, Tuomainen TP, Ruusunen A, Nurmi T, Virtanen JK. Serum n-6 polyunsaturated fatty acids, Delta5- and Delta6-desaturase activities, and risk of incident type 2 diabetes in men: the kuopio ischaemic heart disease risk factor study. *Am J Clin Nutr*. 2016;103(5):1337–1343.
137. Nicholas DA, Zhang K, Hung C, et al. Palmitic acid is a toll-like receptor 4 ligand that induces human dendritic cell secretion of IL-1beta. *PloS one*. 2017;12(5):e0176793.
138. Nicholas DA, Salto LM, Boston AM, et al. Identification of anti-long chain saturated fatty acid IgG antibodies in serum of patients with Type 2 diabetes. *Mediators Inflamm*. 2015;2015:196297.
139. Schwingshackl L, Strasser B. High-MUFA diets reduce fasting glucose in patients with type 2 diabetes. *Annals Nutr Metabol*. 2012;60(1):33–34.
140. Knez M, Stangoulis JCR, Glibetic M, Tako E. The linoleic acid: dihomo-gamma-linolenic acid ratio (LA:DGLA)-an emerging biomarker of Zn Status. *Nutrients*. 2017;9(8).
141. Knez M, Stangoulis JCR, Zec M, et al. An initial evaluation of newly proposed biomarker of zinc status in humans - linoleic acid: dihomo-gamma-linolenic acid (LA:DGLA) ratio. *Clin Nutr ESPEN*. 2016;15:85–92.
142. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary Omega-6 polyunsaturated fatty acids. *J Nutr Metabol*. 2012;2012:539426.
143. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids*. 2001;36(9):1007–1024.

144. Lorenz R, Weber PC, Szimnau P, Heldwein W, Strasser T, Loeschke K. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease—a randomized, placebo-controlled, double-blind cross-over trial. *J Int Med Suppl*. 1989;731:225–232.
145. Kim DH, Puri N, Sodhi K, et al. Cyclooxygenase-2 dependent metabolism of 20-HETE increases adiposity and adipocyte enlargement in mesenchymal stem cell-derived adipocytes. *J Lipid Res*. 2013;54(3):786–793.
146. Kaippert VC, dos Santos Lopes MCO, de Carvalho PD, Rosado EL. Effects of unsaturated fatty acids on weight loss, body composition and obesity related biomarkers. *Diabetol Metabol Syndrome*. 2015;7(suppl 1): A139.
147. Turner N, Hariharan K, TidAng J, et al. Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent: potent tissue-specific effects of medium-chain fatty acids. *Diabetes*. 2009;58(11):2547–2554.
148. St-Onge MP, Mayrsohn B, O'Keeffe M, Kissileff HR, Choudhury AR, Laferrere B. Impact of medium and long chain triglycerides consumption on appetite and food intake in overweight men. *Eur J Clin Nutr*. 2014;68(10):1134–1140.
149. St-Onge MP, Bosarge A. Weight-loss diet that includes consumption of medium-chain triacylglycerol oil leads to a greater rate of weight and fat mass loss than does olive oil. *Am J Clin Nutr*. 2008;87(3):621–626.
150. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J*. 2007;6(1):2.
151. Weiss LA, Barrett-Connor E, von Mühlen D. Ratio of n–6 to n–3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study1–3. *Am J Clin Nutr*. 2005;81(4):934–938.
152. Corwin RL, Hartman TJ, Maczuga SA, Graubard BI. Dietary saturated fat intake is inversely associated with bone density in humans: analysis of NHANES III. *J Nutr*. 2006;136(1):159–165.

Fish Oils and Omega-3 Fatty Acids

John Nowicki, ND, Cristiana I. Paul, MS Nutrition, Alexander G. Schauss*, PhD,* and *Joseph E. Pizzorno*, ND*

OUTLINE

INTRODUCTION

Thousands of epidemiological, observational, experimental, and randomized controlled studies have been published on the effects of the omega-3 (ω-3) fatty acids in fish and fish oil on human health. The benefit of these fatty acids extends to an extraordinary number of chronic diseases and to many physiological systems and health outcomes, likely because of the cellular and physiological mechanisms of action. Fish oil is well established as an anti-inflammatory, perhaps one of the most critical, because it supplies the building blocks for the natural resolution phase of inflammation, it favorably affects the genetic expression of nuclear receptors and nuclear transcription factors related to inflammation, and it shifts the production of prostaglandin and leukotrienes to less inflammatory mediators. Fish oil also modulates cellular membrane structures, including their fluidity, which affects protein function, ion permeability, the activity of membrane-associated proteins, and signaling pathways with other cells and intracellular organelles. Rather than treating one specific target, fish oil modulates a variety of cellular pathways to improve function and reduce inflammation, giving it broad-based multisystem benefit. Research has helped clarify which individuals are more likely to benefit from fish oil, as well as more optimal dosing.

Initial scientific evidence of fish oil's benefits first appeared in the late 1970s, when it was claimed that fish oil consumption among Greenland Eskimos and other fish-eating populations might convey a lifetime protective effect against coronary heart disease (CHD).[1–3] A large study in the United States, the Diet and Reinfarction Trial (DART),[4,5] and two studies in Europe, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione Trial and the Lyon Diet Heart Study,[6–8] supported the observational evidence from these indigenous populations and demonstrated that by increasing dietary ω-3 fatty acid intake, a significant decrease in ischemic heart disease occurred. In addition, the Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study (JELIS) trial randomized nearly 20,000 individuals to either a statin or a statin plus EPA and found a 19% reduction in major coronary events in patients using supplemental EPA, as well as a significant reduction in stroke.[9,10] In 2010 the Heart and Soul study documented a significantly lower risk of overall mortality in individuals with higher blood levels of EPA and docosahexaenoic acid (DHA).[11] This latter study is particularly important because it suggested that we have a biomarker for risk assessment, one that may avoid some of the biases (e.g., food frequency questionnaires, variability in fish oil products, etc.).

These studies and many others have had a profound effect on public health policymakers' perceptions of the importance of fish and fish oil to human health. For example, on February 8, 2002, the U.S. Food and Drug Administration (FDA) announced that it would permit the claim "consumption of omega-3 fatty acids may reduce the risk of CHD" to appear on labels of ω-3 fatty acid supplements containing a dose level of

* Previous edition contributor

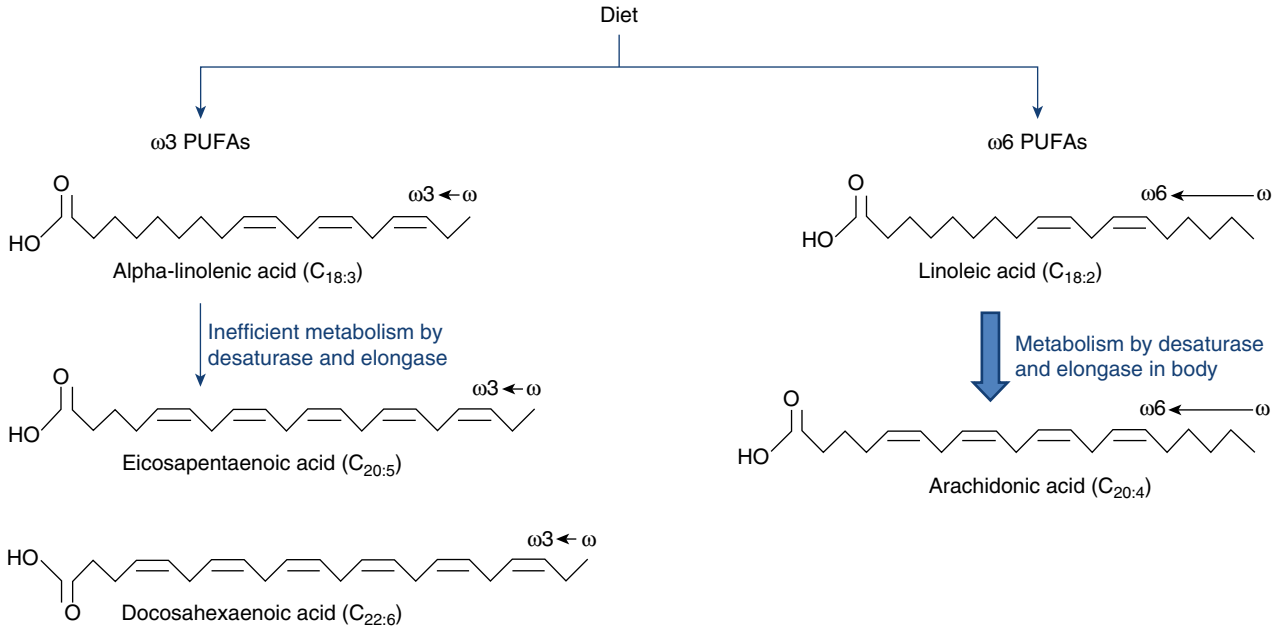


Fig. 80.1 Structures of omega-3 and omega-6 fatty acids. (From Goel A, Pothineni NV, Singhal M, Paydak H, Saldeen T, Mehta JL. Fish, fish oils and cardioprotection: promise of fish tale? *Int J Molecular Sci.* 2018;19[12]:ii: E3003.)

DHA and EPA of up to 2000 mg/day. The FDA's decision to permit such a claim is significant and timely, in that by 2000, 1 million Americans were reported to have heart attacks annually. Based on these and several other studies, it is now estimated that this one change in diet could save at least 150,000 people annually from fatal heart attacks.[12–17] Of course, increased daily intake of 5 to 7 servings of fruits, vegetables, and nuts rich in antioxidants will also reduce the incidence of heart attacks.

In 2002 the American Heart Association (AHA) released a position paper advocating the consumption of oily fish twice per week among those without heart disease and at least 1 g/day of EPA/DHA among those with established coronary artery disease (CAD).[18] This marks the first time the AHA recommended a nutritional supplement for CAD prevention.

Although most clearly established for the prevention and treatment of cardiovascular disease, the benefit of dietary fish oil and fish oil supplements extends much more broadly to other conditions, including autoimmune, inflammatory, neurological, gastrointestinal, and respiratory conditions. For example, the EPA and DHA found in fish oil were shown in various studies to reduce the pain of rheumatoid arthritis (RA), menstrual cramps by decreasing inflammation, the risk of premature delivery in pregnancy, and low birth weights. Beneficial effects were also reported in the treatment or management of bipolar (manic-depressive) disorder, mild to moderate depression, Raynaud disease, systemic lupus erythematosus (SLE), immunoglobulin-A (IgA) nephropathy, chronic fatigue syndrome, irritable bowel disease, kidney stones, cystic fibrosis, psoriasis, and cancer. Additionally, DHA was shown to be important for neurogenesis of neuronal synapses and healthy eye development of fetuses and infants. Perhaps most importantly, fish oil has the potential to improve overall physiological function and well-being, not because it targets one specific abnormality but because it reduces systemic inflammation and supports optimal cellular function.

DESCRIPTION

Marine life is generally rich in two ω-3 fatty acids (also referred to as n-3 fatty acids), EPA and DHA, which enter the food chain through phytoplankton. These long-chained and highly polyunsaturated fatty acids (PUFAs) contain, respectively, 20 and 22 carbons and 5 and 6 double bonds, also referred to as 20:5n-3 PUFA and 22:6n-3 PUFA (Fig. 80.1). The 20:5n-3 PUFAs and 22:6n-3 PUFAs are abundant in shellfish, sea mammals, and fish, hence the reason Greenland Eskimos' diet is rich in EPA and DHA. By comparison, EPA and DHA levels are low or absent in domesticated land animals, in part because mammals lack the enzymes needed to insert a double bond in the n-6 or n-3 position. Thus, linoleic acid and α-linolenic acid (ALA), rich in several plant oils, are essential fatty acids and, slowly and with significant genetic variation, are enzymatically converted to EPA and DHA. Fish oil from herring, cod liver, wild salmon, mackerel, and sardines is particularly rich in ω-3 fatty acids containing EPA and DHA. Table 80.1[19] lists the relative concentration of fatty acids in various common fish species.

Wild-Caught Versus Farmed Fish

The habitat in which fish grow has a major effect on their fatty acid composition.[20] In the wild, fish consume food sources that contain high levels of ALA (18:3n-3). Fish raised in "fish farms" deserve mention. It has been reported that salmon raised in fish farms receive a steady diet of synthetic pigment to give them a rich pink hue; otherwise, they would be unappetizing given their rather morbid, pale-gray appearance. In the natural ocean environment, phytoplankton, which are rich in EPA and DHA, form the basis of the food chain for salmon and contribute to its pink color. Commercial fish foods contain less DHA and EPA and therefore lower concentrations of ω-3 fatty acids. Studies showed that wild fish have higher concentrations of ω-3 fatty acids than pond-reared and/or cultured fish grown in fish farms and fed commercial feedstuffs that are devoid of EPA and DHA.[21] Additionally, although wild fish are also increasingly contaminated, farmed fish often contain higher levels of toxins, such as organochlorines, brominated flame retardants, and methylmercury, particularly when they are also given fish meal as a food source.[22,23] Of particular concern, farmed fish typically have substantial contamination with polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs). Research has shown that this contamination is from their feed, as well demonstrated in Fig. 80.2.

TABLE 80.1 Relative Concentration of Fatty Acids in Fish Oils and Mercury (Hg) in Fish

OIL	CHOLESTEROL (mg/100 g)	UNSATURATED			N-3 PUFAS		Hg (mcg/g)
		Mono- (%)	Poly- (%)	18:3 (%)	20:5 (%)	22:6 (%)	
Cod liver	570	18	51	0.7	9	9.5	0.111
Herring	760	19	60	0.6	7.1	4.3	0.04
Menhaden	600	34	32	1	12.7	8	—
Salmon	485	24	40	1	8	11	0.015
Pilchard	—	25	29	Trace	17	9	—
Mackerel, King	—	21	43	Trace	11	11	0.73
Anchovy	—	28	29	Trace	17	9	0.017
Sardine	—	24	34	Trace	15	10	0.013

PUFAs, Polyunsaturated fatty acids; —, not measured.
Data from Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr.* 1990 Jul;52(1):1–28; Food Standards Agency. Oily fish advice: your questions answered. http://.www.food.gov.uk/news/newsarchive/2004/jun/oilyfishfaq. Accessed July 7, 2005;
U.S. Food and Drug Administration. Methyl mercury. http://www.fda.gov/food/foodsafety/product-specificinformation/seafood/foodbornepathogenscontaminants/methylmercury/ucm115644.htm. Accessed August 30, 2011.

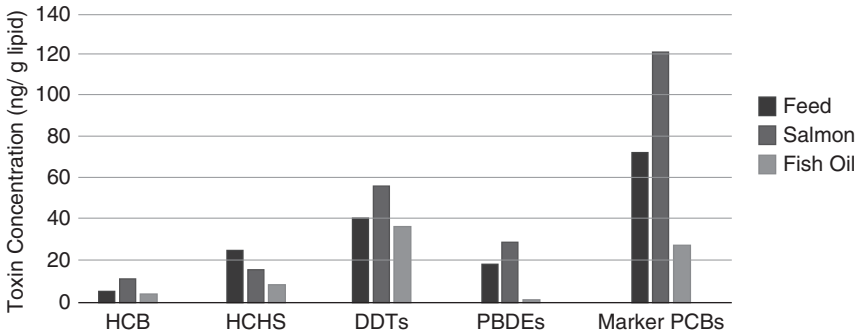


Fig. 80.2 The persistent organic pollutants in farmed fish are from their feed. (From Jacobs MN, Covaci A, Schepens P. Investigation of selected persistent organic pollutants in farmed Atlantic salmon *(Salmo salar),* salmon aquaculture feed, and fish oil components of the feed. *Environ Sci Technol.* 2002 Jul 1;36[13]:2797–2805.)

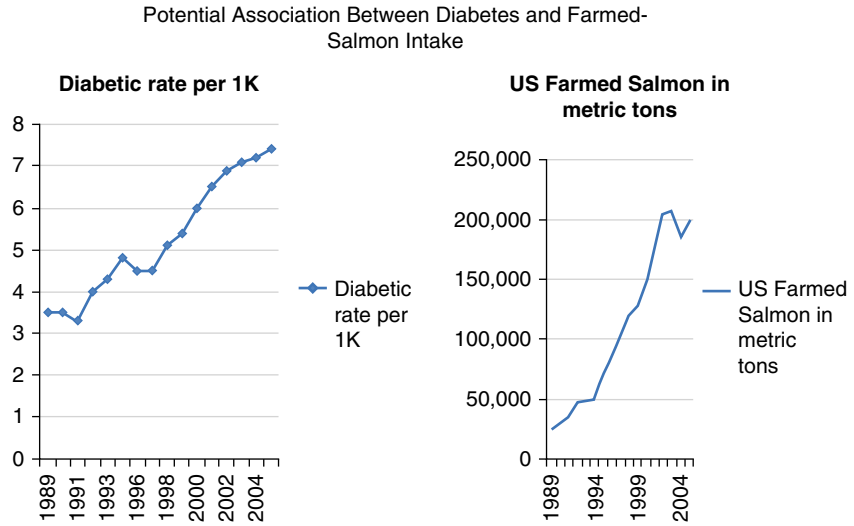


Fig. 80.3 Association between polychlorinated biphenyl (PCB) exposure from farmed-salmon and diabetes. (From Ibrahim MM, Fjaere E, Lock EJ, Naville D, Amlund H, et al. Chronic consumption of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. *PLoS One.* 2011;6[9]:e25170.)

Although fish oil is associated with a reduction in insulin resistance, fish intake is now associated with an increased risk of diabetes.[24] Mice fed either a very high-fat diet or a Western diet, both of which included farmed salmon fillets, developed insulin resistance and obesity, and these negative effects were shown to be, in part, mediated by the presence of POPs in fatty fish.[25] Fig. 80.3 shows a compelling association between PCB exposure and diabetes incidence.

Other Sources of Omega-3 Oils

For individuals who do not or cannot consume seafood, foods such as tofu, canola oil, black currant oil, flaxseed oil (the best nonfish source), nuts, and soybeans are important sources of ALA (18:3n-3). However, soy-derived oils and foods and most nuts contain large amounts of ω-6 fatty acids that can reduce some of the therapeutic benefits of the ω-3 fatty acids, so finding a prudent balance between ω-3 and ω-6 fatty acids is suggested. Additionally, one analysis indicated that a dramatic 1000-fold increase in the consumption of soybean oil over the past century increased ALA intake, but it was offset by the increase in linoleic acid and therefore likely to depress tissue levels of EPA and DHA.[26] A review found that the fatty acids found in fish oil, particularly DHA, are substantially lower in tissues of vegetarians and vegans, including plasma levels, breast milk, and blood cells.[27] Fortunately, organically derived supplemental DHA and EPA from algae is now available for those wishing to avoid fish consumption.[28]

Dietary Requirement

The first suspected case of ω-3 fatty acid deficiency in humans was not reported until 1988, and the optimal intake of ω-3 fatty acids, both plant and marine based, is still not well established.[29] No Adequate Intakes (AIs) or Dietary Reference Intakes (DRIs) have been set so far by the Institute of Medicine for EPA and DHA or the long-chain ω-6 arachidonic acid (AA), although it has established AIs for the short-chain ω-3 (ALA of 1.1 g for women and 1.6 g for men). There are several factors that complicate the establishment of clear dietary requirements, and several lines of evidence offer some guidance. Evolutionary intakes of ω-3 fatty acids ALA and EPA+DHA estimated by Kuipers et al.[30] suggest a probable value of 9 g/day (range 8.7–9.3g) of ALA and 3.9 g/day (range 0.8–9.7g) of EPA+DHA per 2000 Kcal/day.

One of the factors involved is the proportional intake of ω-6 fatty acids to ω-3 fatty acids. It appears that the ratio of these two types of fatty acids, as well as their total intake, is important in determining health outcomes, and a change in both variables has occurred in Western diets over the past 150 years. Currently, the ratio of n-6:n-3 fatty acids in a typical Western diet is 15 to 20:1, whereas in wild animals, as well as humans throughout most of evolutionary history, it has been approximately 1:1.[31] This dramatically shifted ratio has skewed the metabolic products (prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins) derived from AA (vs. n-3 acids) to favor a more inflammatory, prothrombotic, proaggregatory, provasoconstrictive, and proproliferative physiological state. The n-6:n-3 ratio was found to be important not only to the pathogenesis of cardiovascular diseases but also in cancer, in inflammatory and autoimmune diseases, and in brain function.[32]

One example that highlights the importance of the ratio between oils was demonstrated in a study of more than 400 healthy men and 450 healthy women. Researchers found that only at high n-6 intakes were DHA and EPA intakes inversely associated with some markers of inflammation, and a combination of both was associated with the lowest inflammation.[33] Similarly, the intake of n-3 fatty acids in women with a high intake of n-6 fatty acids has an inverse relationship with the risk of breast cancer.[34] This was confirmed in a very large prospective cohort study of Chinese women (Shanghai Women's Health Study), in which the relative intake was more important to the risk of breast cancer than total intake.[35] In other words, the consequence of low n-3 intake is particularly important given a background of high n-6 intake.

Another contributing factor may be variations in the activity of enzymes involved in eicosanoid synthesis. For example, there is some conversion from ALA to EPA and DHA, but this conversion is limited and has significant interindividual variation.[36] This conversion from ALA is estimated to be approximately 5% for EPA and less than 0.5% for DHA, and the desaturase enzymes involved are influenced by smoking, gender, age, trans fat intake, and n-6 fatty acid intake. One study found that despite large differences in the intake of n-3 fatty acids between fish eaters and nonfish eaters, the plasma levels were much closer than expected, likely due to increased conversion.[37] Also of importance is the individual variability of the activity of enzymes involved in mediating the physiological effects of fish oil. For example, variants in the 5-lipoxygenase *(ALOX5)* gene appear to modify the effects of fish oil on cardiovascular risk, as do polymorphisms in peroxisome proliferator–activated receptor-α (PPAR-α).[38–40]

Despite these limitations, most recommendations suggest a desired DHA and EPA total intake of approximately 0.5 to 1 g/day as preventative therapy, with higher doses indicated for treating specific conditions. A ratio of 2:1 to 1:2 EPA/DHA is often advocated, depending on therapeutic goals. For example, the Technical Committee on Dietary Lipids of the International Life Sciences Institute (North America) suggests an intake between 250 and 500 mg DHA+EPA per day for CAD prevention. Currently, even a 500-mg target would require at least a tripling of the current intake in most Western countries.[41]

Because the concentration of EPA and DHA in fish can vary depending on the growth environment, fish oil supplements can offer a viable and reliable source of ω-3 fatty acids standardized around the content of EPA and DHA (see Table 80.3). Most fish oil capsules contain between 300 and 500 mg EPA+DHA fatty acids per gram, whereas some are concentrated to contain as much as 700 mg EPA+DHA per gram. Patients using fish oil at the therapeutic levels discussed herein may require between 15 and 30 capsules daily to derive the described benefits, depending on their DHA+EPA content. At 9 calories/g of oil, this can represent a significant increase in caloric intake and may necessitate an increase in energy expenditure to avoid weight gain. We recommend using fish oil supplements that are highly concentrated to decrease unnecessary calorie intake.

EPA- and DHA-rich fish oil should be kept in capsules (e.g., soft gelatin with minimal plasticizer content[42]; see later discussion under "Oxidation") capable of providing an oxygen barrier. Otherwise, toxic lipid peroxides (e.g., malondialdehyde) and anisidine isomers may form. Encapsulated liquid sources generally do not have this protection unless they contain antioxidants. Encapsulated products typically contain fish oil, not fish liver oil. This distinction is important because fish liver oil contains the fat-soluble vitamins that, if taken in excessive amounts, can cause vitamin A toxicity, although some brands are reducing the vitamin A content during processing.

Explaining Inconsistent Results of Fish Oil Supplementation Trials

Several articles in the fish oil literature have reported equivocal or contradictory results. Many of these differences in outcomes can be explained by the use of olive oil as a placebo or the lack of control for saturated or ω-6 fatty acid intake, as well as an insufficient dose of n-3 fatty acids. For example, in the Alpha Omega trial, nearly 5000 patients with a previous myocardial infarction were given only 400 mg DHA/EPA, a dose less than one half that used in previous trials.[43] Not surprisingly, this low dose did not improve the rate of major cardiovascular events. It also raised plasma levels to a much lower degree than in previous trials that used a higher dosage.

Olive oil cannot be considered an inert placebo in trials that investigate effects on platelet function or CHD risk. Several studies showed that olive oil supplementation has similar inhibitory effects on various aspects of platelet function, including decreased platelet aggregation and thromboxane A_2 (TXA_2) release, increased platelet membrane oleic acid content, and decreased platelet membrane AA content.[44,45]

Further, an excess of oleic acid impairs incorporation of AA into platelet phospholipids.

An additional consideration that is often ignored is the background intake of the enrolled study population. For example, an increase in n-3 intake in Western populations showed a reduction in the risk of sudden death, a benefit not consistently shown in Japanese populations, where the background intake is much higher.[46]

The lack of a relevant biomarker, such as the ω-3 index (discussed in the following section), and the lack of consideration of an individual's genetic response are also variables that certainly contribute to the appearance of ineffectiveness. For example, studies that use food frequency questionnaires often do not accurately gauge n-3 intake. Additionally, they do not consider differences in fatty acid metabolism that are vital to determining more functional measures. An analysis of more than 700 individuals found that supplements and the intake of EPA-/DHA-rich food explained less than one half of the variability in red blood cell content, which may be due to the individual intake of linoleic and/or arachidonic acid because they all compete for incorporation in the cell membranes.[47]

Another possible complicating factor is the squalene found in olive oil and some deep-water fishes but not in other vegetable oils.[48] Other problems include the differing ratios of fatty acids, the position of the fatty acids on the glycerol backbone, and the susceptibility to peroxidation of the various fish oil preparations.[49]

Also, overprocessing of fish oil supplements can result in a loss of the fish oil's active constituents and, hence, an attendant decrease in its efficacy. This was demonstrated in at least two studies that looked at such variables as how processing affects levels of triglycerides, cholesterol, lipoprotein(a), atherogenic index, and fibrinogen after fish oil supplementation.[50,51]

PHARMACOLOGY

The effects of supplemental and dietary unsaturated fatty acids appear to be mediated through changes in serum lipids, altered ratios of prostaglandins, decreased platelet aggregation, and modification of cell membrane activity.[49] Omega-3 polyunsaturated fatty acids cause translocation and activation of endothelial nitric oxide synthase (eNOS) into the cytosol, resulting in vasodilation and improved endothelial function.[52] Additionally, n-3 fatty acids have been shown to modify gene expression and to directly reduce inflammation by binding to n-3–specific receptors. Omega-3 fatty acids (EPA, DHA, and docosapentaenoic acid [DPA]) are also the metabolic precursors to the resolvins, lipoxins, and protectins now shown to be essential to the resolution of inflammation.

In 2010 researchers published a breakthrough study in the journal *Cell,* which for the first time identified a new G-protein–coupled receptor, GPR120, that "functions as an n-3 FA receptor/sensor in proinflammatory macrophages and mature adipocytes."[53] Through this receptor, DHA and EPA mediated robust anti-inflammatory signaling, inhibiting both the toll-like receptor and tumor necrosis factor-α (TNF-α) pathways. This finding was quite important, in part because insulin resistance is modified by these pathways, and because these same researchers were able to document a direct insulin-sensitizing effect by EPA and DHA mediated via this receptor. This provides a direct and specific mechanism for improving insulin sensitivity.

Fish oils have also been shown to be the precursors for the modulators of the resolution phase of inflammation.[54] Resolvins, derived from EPA, and protectins, derived from DHA, have been shown to be crucial to both the resolution of inflammation and the initiation of tissue repair in multiple body systems. This modulation of inflammation by fish oil has also dramatically changed the way inflammation is viewed. It is now understood to be a carefully orchestrated active process, dependent on adequate intake of eicosanoid precursors, rather than a passive process.

Fish oils also appear to reduce triglycerides and decrease the production of the prothrombotic substance TXA_2 by occupying TXA_2 receptors and enhancing the production of the platelet antiaggregatory substance prostacyclin. These actions play an important role in inflammation, atherogenesis, thrombosis, and CHD. Fish meals that provide an average of 3.6 g/day of ω-3 fatty acids reduce platelet aggregation and platelet TXB_2 responses.[55] Significant decreases in platelet sensitivity to collagen, serum TXB_2 levels, and urinary TXB_2 metabolites were observed after ω-3 fatty acid treatment.[56] EPA supplementation was shown to decrease synthesis of the ω-6 platelet agonist TXA_2 coincident with formation of the inactive ω-3 form, TXA_3.[57,58] A longitudinal study showed a pronounced action of dietary ω-3 fatty acids to diminish platelet formation and endothelial deposition, which could lead to thrombosis.[59]

Through the vasodilatory effects of prostaglandin I3 (PGI_3), fish oils may improve peripheral circulation, thereby assisting very low-density lipoprotein (VLDL) cholesterol removal. This may be accomplished by altering membrane fluidity in a specific manner, thus affecting the activity of membrane-bound enzymes and resulting in changes in receptor activity, specificity, and signal transduction. The incorporation of fish oil into membranes was shown to have direct clinical consequences. For example, in a case-control study of more than 300 patients, a higher ω-3 fatty acid content in red blood cell membranes was associated with a 70% risk reduction in cardiac arrest.[60] Evidence also suggests that fish oils decrease hepatic synthesis of fatty acids and triglycerides and reduce secretion of VLDL cholesterol while displacing AA from tissue phospholipids. This results in ω-3 fatty acid levels that inhibit thromboxane synthesis.

Researchers noted that fish oil effects are selective. EPA and DHA not only displace AA from phospholipid pools and inhibit cyclooxygenase, but EPA also becomes a preferred substrate for cyclooxygenase when peroxide tone is high. This results in decreased production of the vasoactive and aggregatory prostacyclin (PGI_2) and increased production of PGI_3, which has more potent antiaggregatory effects. According to some researchers, increased bleeding time is due to either less TXA_2 or higher prostacyclin I_3 levels,[57] although others contend that EPA conversion to PGI_3 is the primary cause.[61] Many researchers believe this change is one of the primary factors that decrease the risk of atherosclerosis and thrombosis.[62–68] These findings may explain some of the epidemiological evidence of decreased CAD and prolonged bleeding time seen in some Eskimos and in those Japanese who eat a diet rich in fish.[69] Analyses also indicated that increases in bleeding time might have been exaggerated, with data pointing to minimal, if any, effect.

Fish oils rich in EPA and DHA were also found to suppress the production of inflammatory mediators found in patients with RA and psoriasis. The anti-inflammatory effect of the ω-3 fatty acids is probably due to reduced production of leukotrienes, interleukin-2 (IL-2), and TNF-α, all principal mediators of inflammation. One study found that the ability of fish oil to decrease TNF-α production was influenced by an inherent TNF-α production and by polymorphisms in the TNF-α and lymphotoxin α genes (also known as TNF-β).[70]

In relation to CAD, ingestion of fish oil and its effect on platelets, erythrocytes, neutrophils, monocytes, and liver cells are important in explaining its benefits. The increased concentrations of EPA and DHA from the ingestion of fish oil were shown to do the following:

- Decrease production of prostaglandin E2 (PGE2) metabolites
- Decrease production of TXA_2 (an active vasoconstrictor and platelet aggregator)

- Increase prostacyclin PGI3 (an active vasodilator and inhibitor of platelet aggregation)
- Increase production of leukotriene B5 (a weak inducer of inflammation; weak chemotactic agent)
- Decrease production of leukotriene B4 (a weak inducer of inflammation; inducer of leukocyte adherence and chemotaxis)

Fish oil, particularly DHA, was shown to modify mitochondrial membrane composition, improving mitochondrial function. DHA reduced the vulnerability to the opening of the mitochondrial permeability transition pore, a pathological feature of heart failure.[71,72]

CLINICAL APPLICATIONS

Cardiovascular Disease

No other clinical application for fish oil has been as well studied as cardiovascular disease, including hyperlipidemia, atherosclerosis, stroke, myocardial infarction, and atrial fibrillation. Perhaps because n-3 fatty acids inhibit the development and progression of atherosclerosis and reduce the systemic inflammation that underlies so much of cardiovascular pathology, fish oil has demonstrated benefit for a wide range of applications.[73] What has emerged as a potential biomarker for heart disease is the use of the ω-3 index, the sum of EPA and DHA expressed as a percentage of the total fatty acid content of red blood cell membranes. It was shown to be inversely associated with the risk for both fatal and nonfatal cardiac events, allowing clinicians to assess individual risk for their patients, and might serve as a tool for monitoring therapeutic efficacy.[74,75]

In a study of nearly 800 patients, the ω-3 index was associated with a 69% risk reduction in those with the highest compared with lowest levels for acute coronary syndrome, with an inverse linear relationship between risk and DHA/EPA levels. This amounts to a threefold greater risk in those with a low ω-3 index than those with a high index. In this study, "low" levels were defined as less than 4%, whereas "high" levels were more than 8%, suggesting a possible therapeutic target.[76] In the Heart and Soul study mentioned previously, those with an ω-3 index above the median had a 27% decreased risk of death compared with those with a baseline ω-3 index below the median, which was unaffected by adjustment for both traditional cardiovascular risk factors and inflammatory markers.[11] This was a prospective cohort study of nearly 1000 patients with stable CHD. Interestingly, in a study published in *Journal of the American Medical Association* in 2010, a subset of patients recruited from the Heart and Soul study was found to have an inverse relationship between the ω-3 index and the rate of telomere shortening over a 5-year period.[77]

Certainly, one population that warrants increased intake of DHA and EPA is those with an uncommon (6% of the population) genetic variant that predisposes them to rapid atherosclerotic progression. Individuals with two variants of the ALOX5 promoter have a greater risk for atherosclerosis, marked by an increase in carotid intima–media thickness. Increased consumption of n-6 fatty acids exacerbated this condition, whereas a higher intake of n-3 fatty acids blunted this atherogenic effect.[39]

Mechanisms of Cardiovascular Benefit

The EPA and DHA in fish oil inhibit the development of atherosclerosis, which can reduce the risk of multiple permutations of cardiovascular disease.[60] As discussed in the "Pharmacology" section, fish oil acts through diverse physiological mechanisms, related to resolution and inhibition of inflammatory pathways, as well as modulation of cellular membrane fluidity (Fig. 80.4). For example, EPA and DHA were shown to increase cell membrane fluidity, affecting intra- and intercellular signaling and ultimately improving cell function.[78,79] EPA and DHA were also shown to stimulate endothelial production of nitric acid, decrease production of inflammatory cytokines and IL-1, and inhibit monocyte migration into atherosclerotic plaques. EPA and DHA also affect gene expression, at least partly via activation of PPARs. In a randomized and double-blinded trial that enrolled more than 100 participants, supplementation with 1800 mg/day of EPA/DHA was shown to change the expression of more than 1040 genes in peripheral blood mononuclear cells, including genes involved in atherogenesis and inflammation, such as nuclear factor-κβ target genes, proinflammatory cytokines, nitric oxide synthase 3, and genes involved in eicosanoid synthesis.[80]

Several biologically active compounds derived from EPA/DHA have been elucidated through newly discovered pathways. These are potent mediators of cellular function, with anti-inflammatory actions and actions that promote the resolution of inflammation, and are thus known as "resolution-phase interaction products" or resolvins, as well as maresins.[81,82] The anti-inflammatory component of fish oil may at least partly explain its ability to slow the progression of atherosclerosis, as well as to stabilize atherosclerotic plaques against rupture.[83,84] Atherosclerotic plaques have been shown to incorporate EPA, and a higher EPA content was associated with greater plaque stability and a reduced number of foam and T cells.[85]

Fish oil also demonstrated lipid-lowering effects, likely mediated partly through the inhibition of VLDL and triglyceride synthesis without reducing the production of high-density lipoprotein (HDL) cholesterol.[86,87] The triglyceride-lowering effect appears to be dose dependent, with a greater effect on those with highly elevated triglycerides. This benefit is not seen with plant-source oils containing land-based α-linolenic fatty acid–rich polyunsaturated fat sources (e.g., flaxseed oil) or via reduction of intake of animal-source saturated fatty acids.[68] In a review of more than 40 studies that examined the use of fish oils for either primary or secondary prevention, fish oils were found to reduce the rates of all-cause mortality, cardiac and sudden death, and perhaps stroke.[88]

As mentioned previously, the AHA for the first time endorsed a nutritional supplement for cardiovascular disease prevention in 2002, advocating consumption of oily fish twice per week among those without heart disease, at least 1 g/day of EPA/DHA among those with established CAD, and 2 to 4 g EPA/DHA for individuals with hypertriglyceridemia.[18] However, there appears to be substantial variability between dietary intake and the ω-3 index (ω-3 blood levels), the latter of which was inversely associated with total mortality among individuals with CAD.[11] Optimal intake might be more appropriately determined using the ω-3 index as a benchmark, with evidence suggesting a target of 8% or higher.[89]

Elevated Serum Lipids

The most consistent effect of fish oils on serum lipids is a reduction in total triglycerides and VLDL cholesterol, especially in patients with severe hypertriglyceridemia. In general, dietary fish oils appear to both reduce undesirable serum-circulating fats and decrease the production of the prothrombotic thromboxanes. In most studies, fish oil supplementation causes a significant reduction in VLDL cholesterol, plasma triglycerides, plasma cholesterol, and LDL cholesterol. In hypertriglyceridemic patients, fish oil supplementation typically results in a significant decline in VLDL cholesterol levels because large decreases in triglyceride levels lead to a decrease in VLDLs.[67,90–93]

Studies also showed that fish oil induced favorable changes in LDL size. It is well established that plasma levels of small, dense LDL cholesterol are associated with plasma triglyceride concentrations, are more atherogenic, and are markers for cardiovascular disease risk.[94,95] Data from the Quantification of the Optimal n-6/n-3 ratio in the UK Diet

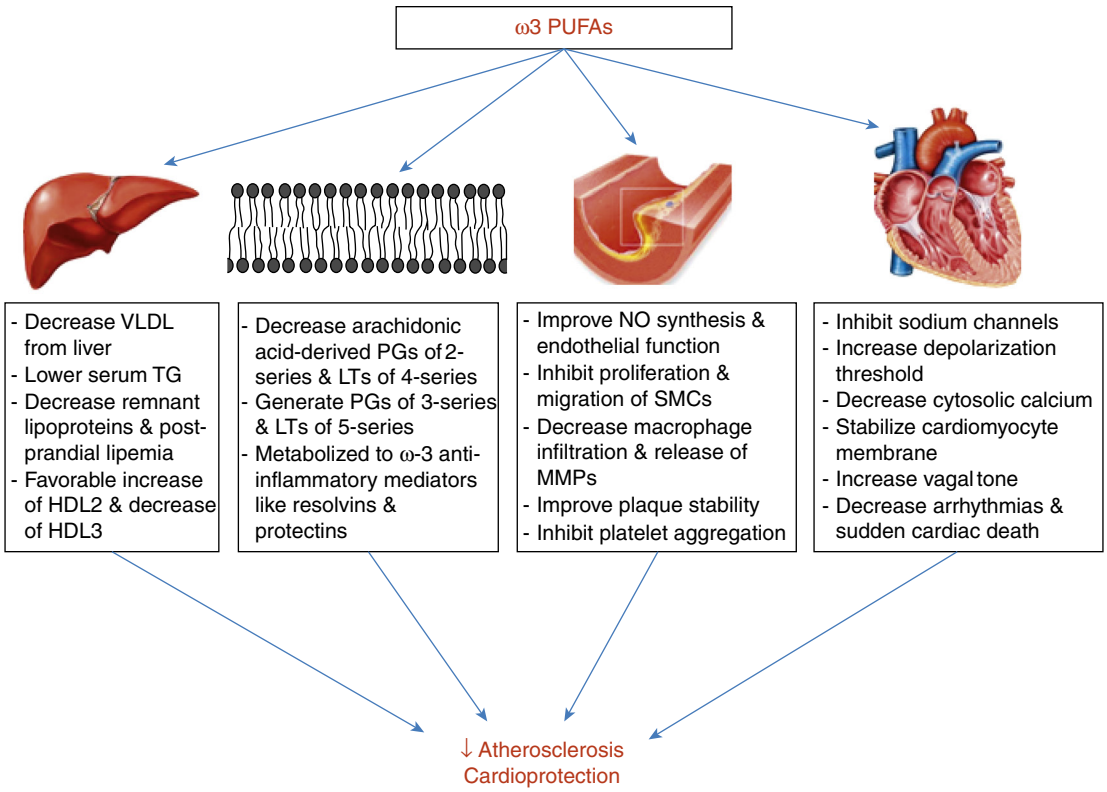


Fig. 80.4 Mechanisms of omega-3 fatty acids in cardioprotection. (From Goel A, Pothineni NV, Singhal M, Paydak H, Saldeen T, Mehta JL. Fish, fish oils and cardioprotection: promise of fish tale? *Int J Molecular Sci.* 2018;19[12]:ii: E3003.)

(OPTILIP) study indicated that DHA and EPA reduced the proportion of the small, dense LDL cholesterol, even if no changes were seen in total LDL cholesterol levels.[96] Data from JELIS, a large-scale intervention trial, found that EPA (1800 mg/day) was particularly effective in patients with the potent combination of risk factors of low HDL cholesterol and elevated triglycerides, reducing the risk of CAD in this group by 53%.[97]

Some studies did not find decreases of total cholesterol or LDL cholesterol after fish oil supplementation.[98–101] However, these equivocal studies used olive oil as a placebo, which, as discussed earlier, is a confounder. Additionally, beneficial changes to subfractions and particle size with DHA supplementation (3 g/day) were cited in one randomized trial, despite no significant effect on total LDL cholesterol levels.[102] Furthermore, an inverse relationship was found between serum EPA levels and oxidized LDL. Oxidized LDL is emerging as an important biomarker of cardiovascular risk and is a very early and critical step in the development of atherosclerosis.[103,104]

For patients with elevated serum cholesterol (>7.75 mmol/L = 300 mg/dL) or triglycerides (>5.64 mmol/L = 500 mg/dL), there is sufficient evidence to consider fish oil supplementation of 5 to 10 g/day if lower dosages (2–4 g/day) are not effective.[105] In one study of 365 patients, supplementation with 10 mL/day (9.2 g) of MaxEPA resulted in significant reductions in triglyceride levels, which were maintained over 4 years.[106] Continuing reductions were observed in persons remaining in the study for more than 4 years. The authors concluded that for triglycerides to remain depressed, it seems necessary to maintain a daily intake of 9.2 g MaxEPA (1 g MaxEPA contains 180 mg EPA + 180 mg DHA).

Fish oil (4 g/day) was also shown to improve the non-HDL cholesterol–lowering effect when combined with statin medications. Increases in HDL cholesterol, as well as decreases in total cholesterol and triglyceride levels, were seen when combined with atorvastatin versus atorvastatin alone.[107] Similar results were also documented when used with simvastatin.[105]

Hypertension

In one double-blind placebo-controlled randomized clinical trial, healthy men and women consumed a control oil or fish oil providing 0.7 g or 1.8 g EPA + DHA per day (intakes achievable through diet), in random order, each for 8 weeks to examine the effect of fish oil on systolic and diastolic blood pressure (BP).[108] Findings indicated that in adults with isolated systolic hypertension, daily doses of EPA + DHA as low as 0.7 g showed clinically meaningful BP reductions.

A meta-analysis of more than 30 trials (22 double blinded) investigating the effect of fish oils in reducing blood pressure found that higher doses (median dose 3.7 g/day) were associated with a reduction in systolic blood pressure of 2.1 mm Hg and diastolic blood pressure of 1.6 mm Hg, although lower doses (<0.5 g/day) might not have an effect. The BP-lowering effect of fish oil was found to be greater among those aged 45+, as well as among those with hypertension versus normotension.[109] Another review cited a number of mechanisms for a hypotensive effect of fish oil, including changes in eicosanoids, blood viscosity, hormonal cellular response, and renin secretion, as well as a decreased response to vasopressors,[110] although they also concluded the effect was likely to be small at a dose of 2 to 4 g fish oil per day.

Epidemiological evidence indicates a hypotensive effect of dietary fish consumption. The largest study is probably the International Study of Macro- and Micronutrients and Blood Pressure (INTERMAP) trial, an international cross-sectional epidemiological study of nearly 5000 men and women living in China, Japan, United Kingdom, and the United States. Researchers found an inverse relationship between total dietary n-3 fatty acids and systolic and diastolic BP, in both those with

hypertension and those with normotension, although the effect was considered small.[111]

Fish oil seems to have hypotensive effects, ranging from limited (dosages of 5 g/day or less) to substantially larger dosages,[55,68,112–119] although there is some conflicting evidence.[105] It has been proposed that fish oil depresses vascular response to the hormones involved in hypertension.[105] Another suggestion is that fish oil acts by increasing vasodilatory prostaglandins PGI_1 and PGI_3 and that this increase accounts for observed reductions in blood pressure.[105]

To test this hypothesis, one study examined the effect of fish oil on BP in men with mild essential hypertension.[120] One group received 10 mL of fish oil (3 g of ω-3 fatty acids), a second group received 50 mL (9 g of EPA and 6 g of DHA), a third group received 50 mL of safflower oil (39 g of ω-6 fatty acids), and a fourth group received 50 mL of a mixture of coconut, olive, and safflower oils. The latter group represented the approximate amount and ratio of fatty acids consumed in the average American diet (39% saturated fat, 46% monounsaturated fat, and 15% polyunsaturated fat). The group receiving the highest dose of fish oil (50 mL) had an average reduction of 6.5 mm Hg in systolic pressure and 4.4 mm Hg in diastolic pressure. None of the other groups, including the low fish oil–supplemented group, demonstrated BP reductions in the aggregate. The study did not find the expected association between increased production of PGI_1 and PGI_3 and sustained reduction in BP. This suggests that vasodilatory prostaglandins are probably not the primary mediators of BP reduction by fish oil consumption, although they may play a role.

Another proposed mechanism is that fish oils may facilitate excretion of sodium and fluid by the kidneys. This was supported by an unpublished double-blind, crossover study in which researchers placed healthy, nonhypertensive men and women on a diet supplemented with approximately 1 g/day of PUFAs for 28 days.[121] Each participant received capsules with either fish oil or safflower oil. The fish oil dose was similar to the amount consumed in a single daily serving of tuna, lake trout, or salmon. After 2 weeks, the subjects crossed over to the other supplement. The study found an average drop of 2 to 3 mm Hg in both diastolic and systolic BP in those who received fish oil supplements. The researchers also found that fish oil increased urine output by approximately 10%, performing much like a low-sodium diet. This resulted in a reduction in fluid volume. Unlike diuretics, the fish oil supplementation did not increase potassium excretion.

Stroke

In the Health Professional Follow-up Study, a prospective cohort with more than 12 years of follow-up, a significantly lower multivariate relative risk (RR = 0.57) of ischemic stroke was documented for men who ate fish one to three times a month compared with men who consumed fish less than once a month. Interestingly, higher levels of consumption did not further reduce the risk, and no effect was seen on hemorrhagic stroke.[122] A reduction in the risk of ischemic stroke by 30% was also shown in the Cardiovascular Health Study. This trial had nearly 5000 adults aged 65+ years and found a 30% lower risk of ischemic stroke with a fish intake of five or more times a week and 27% lower risk with a fish intake of one to four times a week, both compared with an intake of less than once a month. In this study, the benefit was limited to tuna or other broiled/baked fish. Consumption of fried fish or fish sandwiches was associated with an increased risk for total and ischemic stroke.[123]

A study with more than 30,000 participants in the United States examined the risk of stroke as it relates to fish consumption. This study examined possible explanations for the increased stroke risk in the "Stroke Belt and Buckle" in the southeastern United States, a region known for having at least a 50% higher incidence of stroke. The authors of this study concluded that regional and racial differences in fish consumption might help explain this anomaly; that is, those living in the Stroke Belt are less likely to have two or more servings of fish per week.[124]

An earlier study published in 1995 found that men who ate fish five or more times a week had a 40% lower risk of experiencing a stroke than men who ate fish less than once a week. A similar study in women by researchers at Harvard Medical School found even more impressive results for women.[125] In this study, 79,839 female nurses between the ages of 34 and 59 years were followed for 14 years, by which time 574 had experienced a stroke; 303 of the strokes were caused by blood clots, whereas another 181 were caused by a ruptured artery, and the remaining 90 were of undetermined origin. It was determined that women who ate fish once a week lowered their risk of a stroke of any kind by 22%. However, women who consumed fish five or more times per week reduced their risk by 52%. The investigators concluded that women whose intake of fish oils was 0.5 g/day or more had a 30% lower risk of a stroke than women whose intake was less than 0.1 g/day. Of interest was the finding that in those women who had a high fish or fish oil consumption, there was no evidence of an increased risk of hemorrhagic stroke. The researchers concluded that the protective effect of fish oils was due to their ability to inhibit platelet aggregation, lower blood viscosity, suppress formation of leukotrienes, reduce fibrinogen levels, reduce blood pressure, and reduce insulin resistance. Curiously, the beneficial effects were substantially more likely in women who did not take aspirin on a regular basis, although subsequent studies have not shown a relationship with aspirin intake.

In a subanalysis of the JELIS trial, a 20% reduction in stroke risk was shown for individuals taking EPA and a statin compared with those only taking a statin, although these results were limited to participants who had already experienced a stroke (i.e., secondary vs. primary prevention).[10] Currently, more direct intervention trials using fish oil for stroke reduction as the primary outcome are lacking.

Bypass Patients

Some studies demonstrated that fish oil supplementation might help prevent reclosing (restenosis) of the arteries after angioplasty. In one such study, bypass patients were supplemented with 4 g/day of fish oil.[126] One year later, those patients who consumed the fish oil supplements had an occlusion rate of 27%, whereas control patients had a 33% rate of occlusion, a relative improvement of 23%. Based on a postoperative evaluation 3 weeks after patients had cardiac transplantation, it was found that patients who consumed fish oil supplements had normal endothelium-dependent coronary vasodilation, which remained abnormal in patients who did not consume fish oil.

A much larger and well-conducted study published in the *Annals of Thoracic Surgery* documented a robust protective effect of fish oil after coronary artery bypass grafting (CABG). Of 2100 participants, 44% were given 850 to 882 mg of EPA and DHA as ethyl esters, in the average ratio of EPA/DHA of 1:2. In addition to a lower risk for late mortality (hazard ratio = 0.51–0.55), those consuming fish oil had roughly one half the need for repeat revascularization. Furthermore, they had lower adjusted risk for the composite of death, Q-wave myocardial infarction, or cerebrovascular events. Those with poor left ventricular function had a quite large 64% reduction in mortality when taking fish oil.[127] The authors of this study supported the use of fish oil supplementation for all patients as part of standard medical therapy after CABG.

Atrial Fibrillation

A link has long been suspected between fish oil consumption and atrial fibrillation based on epidemiological and experimental evidence,

although the benefit is not clearly established. A prospective cohort study (described previously in the "Stroke" section) found a reduction in the risk of stroke with broiled or baked fish, as well as a roughly 30% lower risk of atrial fibrillation for those consuming fish regularly, although not fried fish.[128] The association between fish oil intake and reduced fatal CHD and sudden cardiac death also lends strong support for its use in preventing ventricular arrhythmias.[129]

Additionally, fish oil targets several of the underlying abnormalities found in atrial fibrillation, including:

1. modulation of electrophysiologic and metabolic heterogeneities secondary to atherosclerotic disease;
2. modulation of cardiac myocyte metabolic activity and cardiovascular oxidant stress;
3. direct modulation of ion channel and transporter activity;
4. indirect modulation of ion channel and transporter activity, via modulation of autonomic nervous system activity; and
5. modulation of inflammatory pathways that promote ectopic electric activity and abnormal conduction.[130]

Also, atrial fibrillation is known to occur more frequently in damaged hearts, which may contribute to mitochondrial pathology and insufficient adenosine triphosphate production, an effect possibly modulated by fish oil.[131]

However, not all epidemiological studies found a protective effect, including the Rotterdam study and the Danish Diet, Cancer, and Health Study, two very large prospective cohort studies.[132,133] The Women's Health Initiative, a large cohort of healthy women, also reported no apparent benefit of increased fish consumption.[134]

A meta-analysis of randomized clinical trials published in the journal *Heart* also did not find benefit for the prevention of atrial fibrillation. However, this study had important limitations, including significant heterogeneity and methodological flaws in the included trials, and limited statistical power to detect a benefit. Its authors also acknowledged that most of the studies used a dose that might have been too low (3–4 g/day fish oil) to have a clinical benefit.[135] A randomized and placebo-controlled study published in *Journal of the American Medical Association* also found no benefit when giving 8 g of prescription ω-3 fatty acids (1 g prescription ω-3 fatty acids contains 375 mg of DHA and 465 mg of EPA) during the first 7 days and 4 g/day for 24 weeks.[136]

The optimal dosage and composition of fish oil for the prevention of atrial fibrillation is not well established. One prospective study of more than 2000 men found that blood levels of n-3 fatty acids appeared to be inversely associated with the risk of atrial fibrillation. However, they found that this protective effect was limited to DHA.

It is thus difficult to determine the role fish oil has in the incidence and the recurrence of atrial fibrillation, given the conflicting evidence published so far. It may be that optimal administration, dosage, and related factors play a role, but given the complexity underlying atrial fibrillation, it may also depend on the specific etiology and the timing. For example, some data suggest that fish oil is more likely to be of benefit for ventricular arrhythmias if CAD is also present, whereas those without heart disease were unlikely to benefit.[137]

Not all atrial fibrillations have the same pathology, with different pathophysiologic mechanisms characterizing distinct clinical presentations. Some authors suggested that n-3 fatty acids might be more likely to be of benefit for preventing the structural remodeling that leads to resistant and/or permanent atrial fibrillation, which is more likely with underlying heart conditions or after myocardial infarction.[138]

In summary, doses used clinically so far may not be sufficient for benefit, and not all presentations of atrial fibrillation may respond to treatment. Earlier treatment with fish oil is more likely to have an effect, before significant structural remodeling has taken place. Furthermore, given the mechanisms involved with fish oil, a longer time until benefit is seen might be expected, an effect not considered in many trials. For example, in the randomized trial cited previously by Kowey et al.,[136] almost half of recurrences occurred within the first 2 weeks of follow-up (of 24 total), suggesting insufficient time for the buildup of tissue levels.

Myocardial Infarction and Acute Coronary Syndrome

One of the largest trials to document a protective benefit for individuals after a myocardial infarction was the GISSI study.[139] In this large secondary-prevention trial, 1 g of n-3 fatty acids (containing 850–882 mg EPA/DHA ethyl esters) greatly reduced sudden cardiac death within 4 months of starting therapy and resulted in a reduction in all-cause and cardiovascular-related mortality. The reduction in the incidence of sudden cardiac death accounted for about 57% of the total improvement in mortality rates. At the end of the study, 2.7% of the placebo group participants died from sudden cardiac death compared with 2% in the fish oil group. Overall, cardiovascular death (including stroke) at the end of the study was 6.5% in the placebo group versus 5.5% in the fish oil group. There was no statistically significant difference in the incidence of nonfatal heart attacks between the fish oil and placebo groups. The researchers concluded that fish oils exerted their protective effect by preventing fatal ventricular arrhythmias rather than through an improvement in cholesterol profile. They did note a small drop in triglyceride levels of 4.6% in the fish oil group but found no significant differences in LDL and HDL cholesterol between the two groups. They also pointed out that the number of lives per 1000 patients that could be saved every year by giving heart attack survivors fish oil exceeded the number of lives per 1000 patients estimated to be saved by treating heart disease patients with high cholesterol levels with pravastatin. This puts fish oil supplements squarely in the category of a highly effective unpatentable heart drug, although Lovaza, a prescription-only form of fish oil, is now available.

Furthermore, a second trial with more than 9000 participants from the GISSI study found that treatment significantly reduced sudden death, with greater benefit in those with left ventricular systolic dysfunction (fourfold greater risk reduction), which increases the risk for mortality.[140]

The JELIS trial, a prospective trial that combined 1800 mg EPA with a statin for hypercholesterolemic patients, found a 51% reduction in risk for myocardial infarction or cardiac death over 5 years. A reduced risk for myocardial infarction was found for those with and without a previous myocardial infarction, although the effect was greater in those with the former. This study was particularly important because they were able to examine adherence to therapy and found a greater effect in those with complete adherence.[141,142]

These studies indicate that fish oil reduces the risk for myocardial infarction and cardiac death and that some populations receive quite significant benefit.

However, some studies did not find benefit for survivors of myocardial infarction in terms of reducing the rate of major cardiovascular effects. For example, a double-blinded, randomized trial with nearly 5000 participants reported no benefit with 400 mg/day of EPA+DHA. Participants in this study were also treated aggressively with other antihypertensive, antithrombotic, and lipid-modifying therapies, which greatly reduced their risk for subsequent cardiovascular events, which might have reduced any potential benefit of fish oil therapy. Additionally, this study was criticized for using too low a dose of fish oil for a clinically meaningful effect.[43] Similarly, the German OMEGA trial (a randomized, placebo-controlled trial to test the effect of highly purified ω-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction), which also used aggressive therapies and

a fairly low dose of fish oil (460 mg EPA, 380 mg DHA), did not report a therapeutic effect.[143] The authors of this study reported limitations, including its low power to detect benefit, given the risk reduction associated with other therapies. Also, they reported that both groups (those receiving fish oil and those not) significantly increased their dietary fish consumption during the study period. Given the low dose used, it was not clear that those receiving supplements had a significantly higher intake of EPA/DHA than those who did not.

Considerable animal-based and experimental evidence shows that dietary fats modulate the electrical stability of the myocardium and that the appropriate fatty acids can reduce the vulnerability to arrhythmia during myocardial ischemia.[144–146] Raising the ω-3 fatty acids to higher levels in the myocardium may also prevent postinfarction ventricular fibrillation.[147–149] Data in humans with acute myocardial infarction found that 465 mg/day EPA and 375 mg/day DHA improved ultrasound indexes of endothelial function without affecting serum asymmetrical dimethylarginine levels, suggesting preclinical benefit.[150]

One early study (DART) evaluated the effect of dietary intervention in 2033 men recovering from myocardial infarction.[4] Patients were randomly allocated to receive one of four types of dietary advice:

1. Lowered intake of dietary fat
2. Consumption of at least two portions of fatty fish (200–400 g) a day
3. Supplementation with three capsules (1.5 g) of MaxEPA (1 g Max EPA contains 180 mg EPA + 180 mg DHA) a day
4. Increased dietary fiber intake

At the end of 2 years, those in the group consuming fish were found to have increased their EPA intake to four times that of other subjects. This group also experienced significantly lower mortality. The fat and fiber advice groups showed no differences in mortality. Although the rate of recurrence of heart attack was similar in all groups, the fish and fish oil group had a 29% reduction in risk of death compared with the other groups. These findings are in direct conflict with an earlier study showing no such benefits.[151] However, it may be significant that the earlier study was conducted over a short period of 6 weeks.

Even in patients with no apparent clinical evidence of arterial disease, fish consumption was shown to improve arterial wall function. One study found that in both healthy patients and those with non–insulin-dependent diabetes mellitus, those who ate fish showed significantly better compliance of their left subclavian artery and femoral arteries.[152]

Fish and fish oil intake were also associated with the risk of angina and acute coronary syndrome. In one case-control study, individuals with acute coronary syndrome were found to have a 20% lower EPA/DHA content in their red blood cell membranes than controls. Those with the highest levels had a nearly 70% lower risk for acute coronary syndrome than those with the lowest levels.[76] Another epidemiological study suggested that the benefit of dietary fish consumption might be limited to fatty fish (i.e., not lean fish), with a more apparent benefit in men.[153] Long-term fish consumption might be particularly protective, providing another rationale for fish oil use in those with increased cardiovascular risk.[154]

Interestingly, one study of men with acute coronary syndrome found that symptoms of depression increased as blood levels of EPA/DHA decreased, supporting a possible link between depression and cardiovascular outcomes.[155] Given the underlying pathophysiology, this seems quite plausible from a systems biology perspective.

A meta-analysis published in the *Annals of Medicine* in 2009 found an overall 29% and 23% reduction in risk for cardiac death and all-cause mortality, respectively, when using fish or fish oil. In patients with a previous myocardial infarction, the reduction in risk was even greater (57%). Surprisingly, they found an increased risk for sudden cardiac death in patients with angina with higher fish/fish oil intake.[156] This increased risk for participants with angina was unexpected because previous studies showed that fish oil supplementation reduced the number of angina attacks and reduced mortality in men recovering from myocardial infarction.[4,157] Significant rheologic improvements in patients with stable angina pectoris might occur after daily fish oil supplementation with 2.8 g of EPA and 1.8 g of DHA.[158] In a double-blind, placebo-controlled study, fish oil supplementation resulted in increased red blood cell deformability, reduced whole blood viscosity, and prolonged bleeding time compared with olive oil supplementation. The frequency of angina attacks decreased in both groups. However, neither type of oil affected exercise capacity or hemodynamic response to exercise.

Finally, as mentioned previously, a comprehensive review of the use of fish oils for either primary or secondary prevention concluded that they reduced the rates of all-cause mortality, cardiac and sudden death, and perhaps stroke.[88] Additionally, an analysis of more than 15,000 patients from the JELIS trial published in 2011 in the *Journal of Atherosclerosis and Thrombosis* found a significant reduction in major coronary events, defined as sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris, and angioplasty/stenting or coronary artery bypass grafting. Those with higher plasma levels of EPA (participants were supplemented with EPA only, not DHA) had the greatest reduction in risk.[159]

Immune Function

Several of the mechanisms by which fish oil modulates immune function have now been clarified, although much remains to be determined, including optimal dosing and the proportions of DHA/EPA necessary for a clinical effect. Additionally, how in vitro or ex vivo changes translate into clinical significance has not been well established. In healthy subjects, the effect of EPA and DHA on ex vivo lymphocyte proliferation was reported in at least 14 articles, at 27 different dose levels, ranging from 0.2 to 7 g EPA+DHA per day, whereas the influence on cytokine production by monocytes was evaluated in 24 studies in 46 treatment cohorts.[160] It was shown that increasing the EPA/DHA content of immune cells affected a number of immune functions and that DHA and EPA had varying effects. For example, in one double-blinded study of healthy volunteers, a marker of T-lymphocyte activation was inhibited by approximately 5 g/day of DHA, whereas EPA and olive oil had no effect.[161] Most studies, however, did not show any effect on lymphocyte activation, although differences were cited between different age groups and genders. Similarly, although some reports suggested an inhibitory effect on natural killer cell function and/or modulation of cytokine production by lymphocytes, there was considerable inconsistency among the studies, and no clear relationship exists.[160]

Per an excellent review published in 2007, the greatest modulation of immune function by fish oil was mediated via alterations in inflammatory cytokine production by monocytes, including IL-1β, TNF-α, and IL-6.[160] This ties in with an increased awareness of the links between inflammatory and immune function. Even here, however, there are inconsistencies. Not all studies documented an inhibitory effect (although none showed an increase in inflammatory cytokine production), and the expected dose-dependent relationship was not always observed. One very plausible hypothesis for these inconsistencies is that highly relevant genetic polymorphisms are often not considered, such as those that affect TNF-α production.[162]

Decreased production of the proinflammatory mediator PGE_2, as well as the incorporation of EPA and DHA into human inflammatory cells, does appear to follow a dose-dependent relationship, along with an inverse relationship between EPA and TNF-α and IL-1β in most studies. One study suggested that at supplementation levels of 1.65 g EPA+DHA per day, no effect was seen, and a threshold existed between 1.65 and 3.3 g EPA+DHA per day.[163] Data also suggest that

older individuals, as well as those with specific inflammatory conditions, might experience a greater therapeutic effect of fish oil than young, healthy individuals.

Some research also suggests that a higher n-6:n-3 ratio has an inhibitory effect on phagocytic function, suggesting a higher intake of n-3 fatty acids might improve this activity.[164] This was confirmed in one trial of healthy volunteers given 3 g/day fish oil (26% EPA and 54% DHA), which found an increase in phagocytic activity of 62% and 145% in neutrophils and monocytes, respectively.[165] Data has described resolvin D1 (derived from DHA) recognition sites on phagocytes (G-protein–coupled receptors), which mediate its actions, leading to enhanced phagocyte and clearance functions, as well as the resolution of acute inflammation.[166] Research published in 2009 in *Nature* also pointed to critical immune roles for other resolvins related to microbial sepsis and immune vigilance.[167]

Autoimmune and Inflammatory Diseases

Fish oils may play a role in the treatment of autoimmune disease (e.g., SLE, dermatomyositis, autoimmune nephritis, multiple sclerosis, celiac disease)[168–171] and inflammatory disorders (e.g., RA,[172] psoriasis,[173] atopic dermatitis[1]). This may be in part due to the chronic inflammation that often plays a role in the development and perpetuation of these conditions. For example, DHA may directly inhibit the release of AA in intestinal epithelial cells when stimulated by gliadin.[174] Furthermore, fish oils may help improve the comorbidities that often accompany autoimmune disease, such as the premature incidence and acceleration of atherosclerosis associated with SLE. Clinical trials in humans demonstrated a benefit not only for disease activity but also for improving markers of cardiovascular function. In one trial, patients with SLE taking 3 g MaxEPA (1g Max EPA contains 180 mg EPA + 180 mg DHA) had a significant reduction in disease activity (measured by Systemic Lupus Activity Measure [SLAM-R][175]). In a second randomized and double-blinded, placebo-controlled trial, participants given Omacor (1.8 g EPA and 1.2 g DHA per day) showed significant improvement in two markers of disease activity (British Isles Lupus Assessment Group and SLAM-R). Additionally, markers of endothelial function and oxidative stress improved after therapy.[176] Importantly, this study used olive oil as a placebo, which also showed benefit for several cardiovascular risk factors, yet fish oil provided a statistically significant benefit in comparison.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is in many ways a representative chronic inflammatory autoimmune disorder and may serve as a model for other conditions. The enzyme COX-2 is overexpressed in the synovium of patients, as are products of the enzyme 5-LOX. Interestingly, a study from Spain found decreased levels of ω-3 fatty acids in the blood and synovial fluids of male and female RA patients compared with healthy controls.[177] Epidemiological evidence suggested that RA might be linked to n-3 fatty acid intake. For example, it is rare among Eskimos, yet by comparison, 2% of the world's population is affected. Studies of the Japanese population confirmed an inverse relationship between high dietary fish consumption and a low incidence of RA.[178] Further support comes from a population-based, case-controlled study in women that found a decreased risk of RA in those who consumed the most fish.[172]

A number of reviews concluded that fish oil supplementation was associated with a number of benefits; it reduced pain, the number of tender joints, the duration of morning stiffness, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with RA and was associated with improved physical performance.[179] A meta-analysis of 17 randomized controlled trials published in 2007 in the journal *Pain* supported these conclusions, although the authors concluded that it might take several months of therapy for an effect.[180] This slow building effect might also explain the results of an earlier study that found that the anti-inflammatory effect of the fish oils continued for up to 4 weeks after cessation of supplementation.

The reduction in the use of NSAIDs is no small benefit. Growing concern about the adverse effects and safety of long-term NSAID use makes any therapy that reduces their necessity quite attractive. For example, in an extremely large meta-analysis published in 2011, every single NSAID evaluated, including ibuprofen and celecoxib, was associated with an increased risk for cardiovascular death.[181] In one small but well-controlled study, cod liver oil was able to reduce NSAID use more than 30% in 39% of patients versus only 10% of controls.[182] Fish oil was also shown to enhance the COX-inhibiting effect of other medications, specifically of paracetamol, offering additive clinical benefit.[183] Similar benefit for reducing disease activity was also cited for indomethacin.[184]

Interestingly, in a review of the efficacy of fish oil for RA, researchers looked at the combination of benefits, including a reduction in symptoms, decreased use of medications, and improvement in comorbidities, and concluded that an argument for the use of fish oil in RA is strong.[185] However, they also concluded that:

> *It is probable that the main barrier to clinician acceptance is the promotion of pharmaceutical use as the dominant treatment modality by the pharmaceutical industry sales force that attends to the "detailing" of doctors. In the absence of an equivalent marketing effort for fish oil, rheumatologists are not inclined to consider, or even be aware of fish oil as a potential component of routine therapy for RA patients, despite the efficacy for symptom relief, the NSAID sparing and the benefits for cardiovascular health, which is compromised in RA patients due to their disease.*

Sixty patients with active RA were involved in a prospective, randomized trial of a 12-week supplementation with fish oil (group I), fish oil with primrose evening oil (group II), or with no supplementation (group III).[186] The Disease Activity Score 28 (DAS 28 score), number of tender joints, and visual analog scale (VAS) score decreased notably after supplementation in groups I and II ($p < 0.001$). In plasma phospholipids, the n-6/n-3 fatty acid ratio declined from 15.47 ± 5.51 to 10.62 ± 5.07 ($p = 0.005$) and from 18.15 ± 5.04 to 13.50 ± 4.81 ($p = 0.005$) in groups I and II, respectively. The authors concluded that daily supplementation with n-3 fatty acids alone or in combination with GLA exerted significant clinical benefits and certain changes in disease activity in patients with RA.

As discussed earlier, the intake of n-3 fatty acids should probably not be considered in isolation because the ratio to n-6 fatty acid intake is quite significant. This is particularly true for RA. A low-AA diet, often vegan and gluten-free, has been used with considerable success for individuals with RA, possibly by supplying reduced amounts of inflammatory precursors. The beneficial effect of fish oil appears to be amplified by a low-AA diet.[187] The authors also cited a time-dependent effect (2 months before improvement) and the EPA/AA ratio as being decisive for clinical effectiveness.

Psoriasis

Despite its underlying inflammatory process, very little recent research has been conducted for the use of fish oil to benefit individuals with psoriasis. Psoriasis is accompanied by high concentrations of AA in the plaques and profound changes in the metabolism of eicosanoids, leading to an increase in proinflammatory agents. Fish oils presumably improve the condition by decreasing the levels of inflammatory leukotriene compounds, especially leukotriene B_4, a lipoxygenation

product of AA. The EPA in fish oil replaces the AA in phospholipids, leading to the formation of leukotriene B_5 rather than B_4, resulting in a much weaker inflammatory response.[188] This effect was demonstrated in neutrophils isolated from the peripheral blood of patients given fish oil to treat their psoriasis.[189] Patients who showed evidence of clinical response to fish oil therapy for their psoriasis were shown to have higher levels of leukotriene B_5 than those failing to improve.

In a controlled observational study, participants were given 2 capsules of Oravex per day (280 mg of EPA, 40 mg of DHA, 50 mg of thyme extract, 50 mg of olive leaf extract, 20 mg of green tea extract, 7.5 mg of zinc, 27.5 mcg of selenium per capsule), for a total of 560 mg EPA and 80 mg DHA. Compared with those treated topically with tacalcitol, those given Oravex and tacalcitol had a significantly greater improvement in several markers of disease severity and quality of life.[190]

Patients with psoriasis (vulgaris) were successfully treated with a low-fat diet supplemented with fish oil.[189] An impressive 77% (23% were nonresponders) of the patients reported either excellent, moderate, or mild improvement. It was interesting to note that several patients did not show improvement until at least 4 months after initiation of supplementation. Again, this may indicate the importance of allowing adequate time for clinical improvement after initiating fish oil therapy.

Etretinate is a powerful drug used to treat skin disorders such as psoriasis. It can cause serious adverse effects, however, when used in the regularly prescribed dose of about 1 mg/kg per day. One study showed that a combination of EPA and etretinate at a lower dose (0.3–0.5 mg/kg per day) worked as well as the pure, high dose and had significantly fewer side effects.[191] Forty-five percent of the patients in the combination group showed excellent improvement (more than 75%) compared with 15% in the pure etretinate group. The time to achieve a 50% improvement in symptoms was also considerably shorter in the combination group (5.1 weeks) than in the monotherapy group (7.6 weeks). Adverse reactions such as inflammation of the lips, dry mouth and eyes, and scaling were observed in both groups but were mild and tolerable. The researchers concluded that the combination regimen was effective in the treatment of psoriasis without marked adverse reactions.

Another study investigated whether topical application of fish oil to skin areas affected by psoriasis would alleviate symptoms. The clinical trial involved 25 patients with psoriasis who were randomly assigned to apply either fish oil or liquid paraffin to their psoriatic plaques and leave them covered for 6 hours overnight under an occlusive dressing.[192] The treatment was repeated daily for a 4-week period. Fish oil proved highly effective in reducing scaling (severity of scaling went from an average rating of 2.91 to 0.32 on a scale from 0–4), plaque thickness (from a rating of 2.21 to 0.52), and erythema (from a rating of 2.71 to 0.90). Itching was not relieved by the fish oil treatment. The 4-week liquid paraffin treatment was also effective in reducing erythema but was significantly inferior to the fish oil treatment in reducing scaling and had no significant effect on itching or plaque thickness. Both treatments were well accepted by the patients, and the researchers concluded that they were both clinically effective, with the fish oil treatment being superior to the paraffin treatment.

Asthma

Elevated intakes of n-6 fatty acids combined with low intakes of n-3 fatty acids may play a causative role in increasing asthma incidence. A recent review cited five epidemiological studies that all found a protective effect of maternal n-3 fatty acid consumption for asthma and atopic diseases. Intake during infancy and childhood was not as consistently shown to be beneficial, but the majority found a protective benefit.[193] The authors suggested that early life programming might help regulate immune system function. For example, children of mothers receiving fish oil during pregnancy had a nearly 40% lower risk of asthma in the 16 years after their birth compared with mothers receiving olive oil.[194] However, a long-term study found that dietary fatty acid modification, as implemented in a study from birth to age 5 years, did not reduce the prevalence of asthma, atopy, or other atopic disorders at age 8 years. However, the dose used in this study contained less than 200 mg n-3 fatty acids, along with a nearly identical amount of saturated fatty acids, per capsule. Compliance was also poorly monitored.[195]

The effectiveness of fish oil for treating established asthma is controversial. In one study, a small number of participants with exercise-induced asthma were given 3.2 g EPA and 2.0 g DHA per day and shown to have equal benefit when given the 5-LOX inhibitor montelukast.[196] However, not all trials documented benefit, although the parameters of some studies seem designed to better evaluate drug-type interventions than nutritional ones. For example, a controlled trial comparing placebo with n-3 supplementation reported no benefit on lung function or asthma control.[197] This study was only a 2-week study, and considering that the delay before benefit in other inflammatory conditions, such as RA, may be 2 to 3 months, it is worth bearing in mind the slower onset of action expected of fish oil therapy. Many of the clinical trials that did not find benefit were less than 2 months' duration.[198]

Lastly, a study documented increased asthma severity with a polymorphism of the ALOX5 promoter. Given that individuals with genetic variants in this promoter have been found to have an increase in cardiovascular disease risk that is mitigated by a diet high in n-3, future studies seem warranted to assess the potential benefit in asthmatic patients with or without this genetic predisposition.[199]

A note of caution: aspirin-intolerant asthmatics may have a worsening of symptoms, associated with the inhibition of COX by EPA.

Cancer

Fish and fish oil demonstrated multiple beneficial roles related to cancer, specifically both its prevention and as an adjunct to cancer treatment. They were associated with a reduced risk of several cancers, and both might improve the efficacy of other interventions, as well as reduce adverse effects associated with cancer therapy. In some studies, fish oil was also shown to increase survival times. Although specific mechanisms vary from various cancer sites, the ratio of n-6 to n-3 fatty acids might also be an important feature for cancer development. For example, AA was shown to be elevated in a variety of cancer cell types, whereas DHA and EPA were both depressed.[200–202] The role of resolvins and other eicosanoid mediators might include regulating inflammatory precursors to some cancers, such as colorectal cancer.[203]

Cancer Prevention

Most epidemiological studies showed some benefit of n-3 consumption on either cancer incidence or cancer-related mortality. A meta-analysis of fish consumption and prostate cancer risk found that although there was no association between the risk of developing prostate cancer and fish consumption, there was a 63% reduction in prostate cancer–specific mortality.[204,205] A very important case-control study of 466 men found that not only did those with the highest n-3 consumption have a 63% lower risk for aggressive prostate cancer compared with those with the lowest n-3 consumption, but a genetic polymorphism in the COX gene (SNP rs4648310) strongly modified this risk. Those with the polymorphism and a low n-3 intake had 5.5-fold increased risk for aggressive prostate cancer, a risk that was reversed with a high n-3 intake.[206]

A large epidemiological study of 23 dietary factors in countries with high and low risks of cancer found a strong association between the percentage of calories from fat and the risk of breast cancer.[207] Fish consumption was found to have the next most significant association, a negative correlation. As mentioned previously, for breast cancer, the ratio of n-3:n-6 fatty acids might be particularly important, as demonstrated by two large trials. One trial, which enrolled more than 70,000 women, found that those with high intake of n-6 combined with a low intake of n-3 fatty acid had more than two times the risk of developing breast cancer.[34,35]

A previous study of 4052 postmenopausal women found that not only was the type of dietary fat associated with breast cancer but also that this risk might be modified by the activity of the enzyme δ-9-desaturase. These women were followed for an average of 5.5 years, and those with DHA concentrations in the highest tertile had less than half the risk of breast cancer than did women in the lowest tertile. PUFAs overall were also protective, with ω-3 acids being somewhat more protective than ω-6 acids. Saturated fatty acid concentrations were not significantly related to breast cancer risk. A higher concentration of monounsaturated fats, especially oleic acid, was associated with a significantly increased risk. The researchers pointed out that most oleic acid in mammalian tissue is derived from saturated stearic acid through a process involving the enzyme δ-9-desaturase. Saturated fatty acids, cholesterol, carbohydrates, insulin, testosterone, and estrogen all activate this enzyme, whereas dietary PUFAs and fasting deactivate it. The researchers concluded that the δ-9-desaturase enzyme might be an important link between breast cancer risk and dietary fat consumption.[208]

A meta-analysis that examined 19 prospective cohort studies found a 12% decrease in the relative risk of colorectal cancer comparing high fish consumption with low fish consumption.[209] The largest study (nearly 500,000 men and women) contributing to this meta-analysis was the European Prospective Investigation into Cancer (EPIC) study, which found a 31% lower risk of colorectal cancer comparing highest with lowest fish intake.[210] An intervention trial using 2 g/day of EPA compared with placebo was shown to reduce the number and size of polyps in familial adenomatous polyposis, further strengthening the evidence for its effectiveness.[211]

This builds on a previous study in which 34 men and 26 women who had just undergone surgery to remove benign polyps from their colon were followed.[212] Patients were divided into four groups. Group 1 was supplemented with 1.4 g/day of EPA and 1.1 g/day of DHA, group 2 with 2.7 g of EPA and 2.4 g of DHA, and group 3 with 4.1 g of EPA and 3.6 g of DHA. Group 4 received placebo capsules containing olive oil. Biopsy samples from the lower part of the colon and blood samples were taken and analyzed at the start of the trial and at 30 days. Overall, patients in the fish oil groups experienced a significant decline in the number of abnormal cells in their colon lining compared with the placebo group. Further analysis showed that the reduction in the number of abnormal cells was limited to patients who had a large number of abnormal cells at the beginning of the trial. The researchers also noted a significant increase in EPA and DHA levels and a significant drop in AA levels in the biopsy samples from the fish oil–supplemented patients. A separate 6-month trial involving 15 patients taking 1.4 g/day of EPA and 1.1 g/day of DHA also showed a significant drop in the number of abnormal colon-lining cells. The researchers concluded that low-dose supplementation with fish oils inhibited the proliferation of those abnormal cells associated as precursors to polyps in patients at risk for colon cancer and that this effect could be maintained with long-term treatment. Similar to several other studies concerned with oxidation of fish oil in vivo, the authors cautioned that it might be advisable to increase vitamin E intake during fish oil administration.

A British investigation of the association between high dietary fat intake and the risk of developing breast and colon cancer revealed interesting findings concerning the incidence of cancer. The study compared cancer mortality rates in 24 European countries, Canada, and the United States with fish consumption and the intake of animal fats.[213] In countries where the animal fat intake was high, the researchers found a clear inverse correlation between the ratio of fish fat to animal fat and the risk of developing breast cancer in women and colon cancer in both men and women. A similar correlation was found between cancer risk and the ratio of fish fat to total fat intake. This led the investigators to conclude that fish and fish oils not only protect against colon cancer in men but also against colon and breast cancer in women. This protective effect, however, is only apparent in countries where the intake of animal fats is high. In other words, a high intake of fish or fish oils counteracts the detrimental effects of high animal fat consumption. The researchers further concluded that a 15% decrease in animal fat intake combined with a threefold increase in fish oil intake could possibly reduce male colon cancer risk by as much as 30% in countries with a high animal fat intake. A threefold increase in fish oil intake could be achieved by eating fish three times a week or by taking two standard fish oil daily supplements containing EPA and DHA.

A prospective cohort study published in the *Journal of the American Medical Association* in 2006 also found a protective effect of fish consumption on renal cancer. For women consistently reporting high consumption of fatty fish (once per week or more), a risk reduction of as high as 74% was observed compared with women reporting no fish consumption. Lean fish consumption did not reduce the risk of more than 60,000 women in this 15-year study.[214]

Cachexia and Treatment Efficacy

Some studies showed that fish oil supplementation, especially at higher doses, might help with weight stabilization in participants with cancer. For example, one early study found that supplementation with approximately 2.2 g of EPA and 1.4 g of DHA assisted stabilization of weight in patients with inoperable pancreatic cancer. Researchers not only documented weight gain and improved appetite, but these patients also survived an average of 8 months, compared with the expected survival time of 4.1 months typically reported for patients given chemotherapy.[215] In another study, 18 patients with inoperable pancreatic cancer, including 9 patients with Stage 4 tumors, were started on a dose of 2 g of fish oils (containing 360 mg/day of EPA and 240 mg/day of DHA).[216] The dose was subsequently increased by 2 g/day every week until the patients' body tolerance was reached, with an average final intake of 12 g/day. Before entering the trial, the mean weight loss among the patients was 2.9 kilos per month. After 3 months of fish oil supplementation, an average weight gain of 0.3 kilos a month was observed. Overall, 11 patients (61%) gained weight, 3 became weight stable, and 4 continued to lose weight, but at a significantly reduced rate. The concentration of EPA in plasma phospholipids increased from 0% to 5.3% of total fatty acids after 1 month of supplementation, whereas the concentration of DHA increased to 6.6% from a base level of 3.5%. The researchers concluded that fish oil supplementation arrested weight loss in cancer patients with cachexia.

A study published in the journal *Cancer* found a very significant benefit when supplementing patients with non–small-cell lung cancer (NSCLC) at a dose of 2.2 g/day of EPA. These patients also received chemotherapy and were compared with a group receiving standard of care only (i.e., chemotherapy without fish oil). Despite having a mean weight loss of 6.3% over the previous 6 months before chemotherapy, patients receiving EPA maintained weight, muscle mass, and adipose tissue throughout approximately 10 weeks of chemotherapy. Those who received standard of care only lost on average 2.3 kg over the same

period. This study also might have been the first to monitor body composition directly using computed tomographic imaging. They found that gains were made in muscle and adipose tissue, an important distinction from the accelerated weight gains sometimes observed in lean tissues (liver and spleen) in terminal stages. Additionally, those with the greatest increases in plasma levels of EPA had the greatest gains in muscle mass, suggesting again that a biomarker may be worth incorporating into fish oil therapy. Although this was a very small study, these gains were potentially quite significant, especially given the low dose of fish oil used.[217]

These same researchers also published a trial that enrolled patients with advanced NSCLC, and although the study was also small, it suggested a significant benefit of fish oil therapy. Patients received 2.2 g/day of EPA and 240 to 500 mg/day of DHA, along with standard-of-care chemotherapy, and were compared with those receiving chemotherapy alone. In this trial, fish oil supplementation appeared to increase the efficacy of chemotherapy, without increasing its toxicity. The authors reported an approximately twofold increase in response rates and clinical benefit compared with standard of care alone. Additionally, a greater portion of patients who received fish oil were still alive at the time of reporting than the control group, suggesting a likely increase in survival.[218] Other studies also reported benefits for treating patients with NSCLC with fish oil.[219]

Similar improvements in survival time were also seen in earlier studies of patients with other end-stage cancers with generalized malignancies. In one study, 60 patients with generalized solid tumors were divided into two groups: one group received 18 g of fish oil containing 170 mg of EPA and 115 mg of DHA per capsule, whereas the other received a placebo.[220] The fish oil group also received 200 mg of vitamin E daily to reduce oxidation in vivo from the effect of so much fish oil. Each group included 15 well-nourished and 15 malnourished patients. None of the well-nourished patients had cancer cachexia (abnormally low weight and general weakness). The researchers measured the levels of T cells, natural killer cells, and the synthesis of IL-1, IL-6, and TNF before the start of the supplementation and on day 40 of the trial. The study followed all patients until they died. Malnourished patients were found to have a considerably impaired immune function and a decreased production of TNF; both parameters were restored through fish oil supplementation. Malnourished patients overall had a much shorter survival time than well-nourished patients (mean of 213 vs. 481 days). Both malnourished and well-nourished patients who received fish oil and vitamin E survived significantly longer than did patients on placebo. The researchers speculated that fish oils exert their beneficial effect by decreasing the body's production of PGE_2, which is believed to play an important role in the initiation and progression of cancer. They concluded that supplementation with dietary ω-3 polyunsaturated fatty acids, specifically fish oils with an antioxidant such as vitamin E, might offer significant palliative support to cancer patients with end-stage metastatic disease.

Potential mechanisms for benefit were outlined in several reviews, including one published in the journal *Nutrition and Cancer* that called for major clinical trials to be conducted using fish oil as adjunctive therapy. The authors reviewed previous research and concluded that long-chain PUFAs not only had cytotoxic anticancer effects by themselves but also modulated the effectiveness of particular chemotherapeutic agents.[221] For example, DHA was shown in vitro to have synergistic toxicity with taxanes toward cancer cells and downregulated Her-2/neu oncogene expression, an effect confirmed in an animal model of breast cancer.[222,223] Other mechanisms included modulation of eicosanoid production, modulation of gene expression and of transcription factor activity (e.g., nuclear factor-κB), and alteration of membrane-associated signal transduction, to name just a few.[224] Interestingly, one study even suggested that fish oil might offer benefit for both breast cancer as well as cardiac arrhythmias through a similar mechanism, the regulation of a sodium channel, NaV1.5, although this has not yet been proven.[225] Lastly, one review cited both a cytotoxic effect of cancer cells and a protective effect toward healthy cells by the downstream products of DHA (protectins), allowing them to work "as both a sword and a shield."[226]

Despite considerable evidence for a beneficial effect of fish oil in the prevention and treatment of various cancers, not all research showed a positive effect. Although some studies might have been too short-term or used too low of a dose for a clinical effect,[227] others found benefit for only a subset of patients, even at higher doses.[228] Many older studies used fish oil with a very low concentration of DHA/EPA, requiring an intolerable number of pills, a limitation recent advances in available products might remedy. Also, the use of biomarkers, such as the ω-3 index, and genetic polymorphisms might help identify those most likely to benefit, as well as those who need higher dose supplementation. For example, a Phase II trial of DHA (1.8 g/day) given to patients with metastatic breast cancer receiving chemotherapy found that those who incorporated it the most (into plasma and/or red blood cells) delayed time to tumor progression and had longer overall survival compared with those who incorporated it the least.[229]

Migraine Headache

A role for inflammation in migraine was established for at least some migraineurs, yet surprisingly few studies have been conducted with fish oil for its prevention (benefit for an acute migraine seems unlikely). Two placebo-controlled studies were performed, both finding no benefit compared with placebo, yet methodological flaws are found in both. In both studies, olive oil was used as a placebo, and a high placebo effect was observed.[230,231] Well-designed clinical trials are early in the research process.

A systematic review and meta-analysis of randomized controlled trials showed that ω-3 intake had no effect on the frequency and severity of migraine but had a reduction effect of approximately 3.4 hours on the duration of migraine attacks.[232] Anecdotal reports indicate that patients with migraine headaches who were given 1 g/day of MaxEPA (1 g MaxEPA contains 180 mg EPA + 180 mg DHA), particularly males, had significantly less frequent episodes, less intense episodes, or both. These results might be due to changes in prostaglandin synthesis or reduction, or both, in platelet serotonin release, with a resultant reduction in cerebral vasospasm.

Diabetes

The relationship between fish consumption and diabetes incidence and treatment is not as simple as previously believed. As mentioned previously, a G-protein–coupled receptor was identified that modulates many of the physiological effects of DHA/EPA, including an increase in insulin sensitivity, and most epidemiological evidence suggests either no effect or a reduced incidence of diabetes among populations with higher fish intake.[233] For example, the incidence of diabetes is low in Greenland Eskimos.[234]

However, recent studies suggest that greater intake of fish might pose a risk for diabetes. One of the largest was a prospective study of more than 35,000 women (Women's Health Study), which found a linear increase in the risk for type 2 diabetes (T2D) with fish intake and particularly high risk for those consuming the highest portions (two or more servings of fish per day). This increase in risk with increasing n-3 fatty acid intake from fish was not observed with n-3 fatty acids from plant sources, which provides ALA.[235] This increase in risk with fish intake was also observed in three other prospective cohorts of both men and women in the United States (152,700 women and 42,504 men), with a 22% increase in risk in those consuming fish more than five times per week compared with those consuming it less than

once per month.[236] In a large prospective trial in China (the Singapore Chinese Health Study), no association between n-3 fatty acid intake from fish was seen, although ALA from plant sources reduced the risk of diabetes.[237] As discussed previously, contamination of the fish and fish oils with diabetes-inducing persistent and nonpersistent organic pollutants likely accounts for this discrepancy, especially in highly polluted China.

In one large prospective study of older adults, researchers measured the plasma phospholipid levels of EPA/DHA as well as ALA and examined the risk for diabetes. They speculated that many of the previous trials that found an increased risk for diabetes were prone to error, such as the error associated with food frequency questionnaires. Using a more objective biomarker, they found that phospholipid levels of EPA+DHA along with ALA were not associated with an increased risk of diabetes, and instead, those individuals with the highest concentrations of EPA+DHA or ALA had a lower risk of diabetes.[238] Earlier studies that used biomarkers in younger populations also found no increase in risk.[239,240]

Although further studies may help clarify the relationship, it is worth noting two additional points. One, as noted earlier, is that fish are becoming contaminated with compounds such as POPs, which have been associated with a surprisingly large increase in the risk for diabetes. Some studies showed fish consumption to be an important predictor for POPs serum levels.[241–243]

The second consideration is the favorable effect of fish oil on cardiovascular parameters, an important cause of morbidity and mortality among diabetics. In individuals with T2D, fish oil ingestion was demonstrated to favorably alter arterial wall compliance without adversely affecting cholesterol levels, blood pressure, or fasting blood sugar levels, thereby contributing to a reduced risk of the vascular complications seen in patients with insulin-dependent diabetes mellitus and non–insulin-dependent diabetes mellitus.[244,245] One study even showed a desirable significant decrease in VLDL triglyceride and cholesterol concentrations in T2D patients consuming three fish oil capsules a day containing 320 mg EPA and 530 mg DHA.[246] In another study, 2 g of fish oil per day given to the offspring of individuals with diabetes for 12 weeks was associated with reduced inflammation and markers of endothelial function. Thus, in those at an increased risk for diabetes, fish oil might have an important cardiovascular and metabolic benefit.[247] Interestingly, increased fish oil consumption might also reduce the risk of type 1 diabetes in those with increased susceptibility.[248]

A randomized double-blinded, placebo-controlled trial was conducted to determine the effect of fish oil administration on gene expression related to insulin action, blood lipids, and inflammation in women with gestational diabetes mellitus (GDM).[249] Participants with GDM (n = 40), aged 18 to 40 years, were randomized to take either 1000 mg fish oil capsules, containing 180 mg eicosapentaenoic acid and 120 mg docosahexaenoic acid (n = 20), or placebo (n = 20) twice a day for 6 weeks. Overall, compared with placebo, fish oil supplementation for 6 weeks in women with GDM significantly improved gene expression of peroxisome proliferator-activated receptor gamma (PPAR-γ), IL-1, and TNF-α but not gene expression of IL-8. Interestingly, these benefits were observed with relatively low doses of n-3 fatty acids.

Although fish oil has not always been shown to improve markers of diabetes and glucose control among those with T2D, its cardiovascular benefit warrants consideration for use in all patients with diabetes.[88,250]

Neurodegenerative Disease

Several lines of evidence point to a protective role for EPA and DHA for neurodegenerative diseases. DHA specifically is the predominant n-3 fatty acid in the brain and appears to play a key role in structures involved in the formation of new memories. Additionally, DHA is the precursor for neuroprotectin 1, an important neuronal modulator of function, with anti-inflammatory as well as antiapoptic functions. N-3 fatty acids, particularly DHA and its downstream metabolites, have been shown to act via several pathways associated with neurological function, including the following[251]:

- Facilitating the interaction of α-secretase with amyloid precursor protein to produce nontoxic fragments and prevent the formation of Aβ
- Shielding the essential recognition sequence and intramembrane cleavage site for γ-secretase
- Serving as a local sink for free radicals that reduce the enzymatic augmentation of γ-secretase activity, which can be induced by free radical damage to the protein complex, which is important for the regulation of normal γ-secretase function
- Directly inhibit fibrillation and formation of toxic oligomeric species of Aβ

Observational data also implied an important protective role of n-3 fatty acids. For example, participants in the Rancho Bernardo study had plasma levels of DHA, as well as dietary fish and dietary DHA levels, evaluated. Each of these variables was associated with the subsequent risk of developing either dementia or Alzheimer's disease. Having the highest levels of plasma DHA reduced the odds of all-cause dementia by 65% and Alzheimer's disease by 60%. Dietary DHA had similar benefits, and although fish consumption followed this trend, it was not a statistically significant association.[252] A study published in 2007 that enrolled more than 8000 participants found a quite important association. Overall, fruits and vegetables, as well as weekly consumption of fish, was associated with a reduced risk for dementia. However, although the protective effects of fruit and vegetable intake applied to all participants, fish consumption was only associated with those who did not carry the apolipoprotein-E ε-4 allele (*APOE*-ε4 allele). Similarly, excess n-6 consumption increased the risk of dementia more than twofold, but it was modified by two factors. It increased risk only if not compensated by high n-3 intake, and only among *APOE*-ε4 noncarriers. Thus the relationship between n-3 consumption was not only modified by other dietary factors, such as n-6 consumption, but also by genetic variables.[253] A similar association was found in the Cardiovascular Health Cognition Study, which found that fatty fish, but not lean fish, was associated with reduced risk for dementia, but again, only in noncarriers of the *APOE*-ε4 allele.[254]

A double-blind, randomized controlled trial has demonstrated that n-3 PUFA supplementation (480 mg DHA and 720 mg EPA per day) is associated with improved cognitive function compared with control group with placebo (olive oil) in patients with mild cognitive impairment.[255] In addition, several studies have reported that the effect of n-PUFAs in patients with Alzheimer's disease may be through an increase in the clearance of amyloid-β peptide, neurotrophic and neuroprotective factors, and anti-inflammatory effects.[256]

Regarding the treatment of neurodegenerative disease, efficacy also appears to be modulated by genetic predisposition. In an analysis of n-3 fatty acid erythrocyte membrane content and cognitive variation in a birth cohort study of cognitively normal volunteers, cognitive benefits were associated with higher erythrocyte n-3 content, but again, this was only significant in *ApoE*-ε4 noncarriers.[257]

Not all studies have shown benefit, perhaps because the optimal dose and composition have not yet been clearly established. For example, as evidenced in trials for major depression, although DHA is the predominant n-3 fatty acid in the brain, EPA appears to have a greater clinical effect. This may be why a controlled trial published in the *Journal of the American Medical Association* in 2010 that supplemented participants with 2 g/day of DHA (no EPA) did not find any evidence of benefit. This study also did not report *APOE* status.[258]

The first large-scale, well-designed trial was published in 2006 and used 1.7 g DHA and 0.6 g EPA per day or placebo among participants with mild to moderate Alzheimer's disease, all of whom were also taking a cholinesterase inhibitor. Although no difference in primary outcome was observed, a subgroup with mild disease demonstrated a significant reduction in decline compared with placebo. Although *APOE* status was reported, the treatment effects per *APOE* status were not. Importantly, only 31% of those in the treatment group were non–*APOE*-ε4 carriers. This study suggests those most likely to benefit are at earlier stages of disease.[259]

Overall, the bulk of the data suggest that those with earlier disease are more likely to benefit, but this benefit is mediated by *APOE* status (and perhaps other genetic polymorphisms) and perhaps by n-6 fatty acid intake. The optimal dosage and DHA/EPA composition are not known, but DHA by itself may not be effective. N-3 fatty acids from fish also appear to have a protective effect on other neurodegenerative diseases, including Parkinson's disease, likely through similar mechanisms.[260,261]

Depression

Several epidemiological studies showed a reduced risk for major depression, perinatal depression, and bipolar disorder with diets high in n-3 fatty acids.[262,263] Also, a meta-analysis of 14 trials found that lower levels of EPA, DHA, and total n-3 fatty acids levels in plasma and erythrocytes (data were combined) were associated with depression. When these studies restricted their analysis to studies that used *Diagnostic and Statistical Manual of Mental Disorders* criteria for major depressive disorder diagnosis, the magnitude of differences in EPA/DHA and total n-3 became larger.[264]

Regarding trials for the treatment of depression, results have been quite varied, often due to differences in methodology and the type and dose of fish oil used. However, most have shown a beneficial effect. A review of published randomized controlled trials indicated that studies that found positive effects generally included a high ratio of EPA to DHA, and 1 to 2 g/day might be necessary for effect, but higher levels were not associated with greater benefit.[265] Also, the earlier the supplementation of DHA/EPA is started during pregnancy, the greater the chance for benefit.[266] Despite DHA being a much more prevalent structural component of phospholipids in neuronal cell membranes compared with EPA, it is EPA that has more apparent effectiveness in clinical trials.[267] A second meta-analysis of randomized trials over a 40-year period also found a positive effect of n-3 fatty acids on both major depression and bipolar disorder.[268]

A small but important study also compared a fairly low dose of EPA (1 g/day) with fluoxetine for major depression and found that both were equally effective. Furthermore, when used together, they were more effective than either agent used alone. Given the likely differences in mechanisms of action, this is not surprising, and combination therapy should be considered in those not responding to monotherapy.[269]

A case-control study published in 2010 examined some of the links between cardiovascular disease and depression, which are known to have a high comorbidity rate. Among individuals with major depressive disorder, several cardiovascular risk factors indicated heightened risk, including a low ω-3 index.[270] This biomarker might provide a useful tool for determining the efficacy of n-3 fatty acid therapy for depression as well as cardiovascular disease.

Lastly, little attempt has been made to examine the role of genetic polymorphisms regarding the interaction between n-3 fatty acids and depression. This may be a promising area of investigation. A published study found that patients with chronic hepatitis C were more likely to experience depression when given interferon-α if they had polymorphisms in the *COX-2* gene or the phospholipase-A2 gene. Both of these genes are involved in eicosanoid metabolism and may modulate the influence of n-3 fatty acid therapy.[271]

Raynaud Disease

Preliminary evidence indicated that symptoms of Raynaud disease improved in primary but not secondary Raynaud phenomenon, based on a double-blind, placebo-controlled clinical trial involving 32 patients.[272] Patients were given either 12 1-g fish oil capsules daily for 12 weeks containing 4 g of EPA and 2.6 g of DHA, or a placebo. The group receiving the fish oil supplements reported significant alleviation of symptoms associated with Raynaud disease at the end of the study, including 5 patients who developed symptoms before the experiment began but could not induce symptoms at all after either 6 or 12 weeks of supplementation.

Malaria

Malaria afflicts more than 500 million people worldwide, with about 5% of victims dying each year. Unpublished studies at the U.S. Department of Agricultural Research Service Center in Beltsville, Maryland, found evidence that the ω-3 fatty acids in fish oil may be beneficial in the treatment of malaria.[273] In their research, mice were fed dietary fish oils for 4 weeks and then inoculated with parasites, either *Plasmodium yoelii* or *Plasmodium berghei.* As the mice continued to eat the diet high in fish oil, the parasites multiplied as usual. However, after 3 to 4 weeks, the mice were free of the parasites. The researchers suspected that the cause of the parasites' death was rupture of the parasites' cell membranes or red blood cell hosts. The researchers theorized that infected cells were more susceptible to rupture because the parasites fostered several destructive oxidative reactions to which a diet high in fish oils makes them more vulnerable. Subsequent studies are lacking, although one animal-based study found that fish oil without vitamin E may improve survival and reduce reinfection.[274] Human trials are lacking.

Renal Disease/Immunoglobulin-A Nephropathy

Fish oil supplementation was shown to be effective for several renal diseases, including IgA nephropathy, the nephrotic syndrome, and a deficit of n-3 fatty acids, which was demonstrated in several studies for those with end-stage renal disease. In a small study of continuous ambulatory peritoneal dialysis patients, a depletion of reduced n-3 fatty acids and a high ratio of n-6:n-3 fatty acids were observed.[275] Lower EPA and DHA in the plasma/serum phospholipids of those with chronic kidney disease was also documented, and intravenous n-3 therapy was used to restore normal levels.[276,277]

Both hypertriglyceridemia and hypercholesterolemia are common in patients with nephrotic syndrome. This association results in an increased risk of cardiovascular disease. Fish oils were found to lower serum triglycerides in patients with nephrotic syndrome and might therefore be of clinical benefit.[278] Unfortunately, no recent trials have examined this benefit.

IgA nephropathy is a kidney disease that can follow a viral infection of the gastrointestinal or upper respiratory tract. About 20% to 40% of all IgA nephropathy patients develop renal failure 5 to 25 years after diagnosis. One study at the Mayo Clinic found that fish oil supplementation could be effective in slowing down the progression of the disease.[272] The progression of the disease was judged by regularly measuring the level of creatinine in blood serum during the 2 years of the trial. A clear difference was observed. The patients in the fish oil group who received 12 fish oil capsules a day, containing 1.9 g of EPA and 1.4 g of DHA, had an average median increase in serum creatinine of only 0.03 mg/dL, whereas the patients in the placebo group experienced an increase of 0.14 mg/dL annually, indicating that their disease

was progressing significantly faster. After 4 years, 40% of the patients in the placebo group died or developed end-stage renal disease compared with only 10% in the fish oil group. No adverse effects of fish oil supplementation were observed. A recent reanalysis of this data did not find that body size was helpful in predicting optimal dosage.[279]

An additional study using 1.8 g/day of EPA for 1 year found small but significant benefits, including an improvement in the estimated creatinine clearance.[280]

Muscle and Bone

N-3 fatty acids have emerged as being of potential clinical importance for the health of both muscle and bone tissue. In a study of more than 1500 men and women from the Rancho Bernardo Study, a cohort of community-dwelling individuals, a higher n-6:n-3 fatty acid ratio (based on food frequency questionnaires) was associated with a lower bone mineral density of the hip in both men and women.[281] A second smaller study published in 2007 found that among young men (women were not studied) serum phospholipid levels of n-3 fatty acids, particularly DHA, were associated with peak bone mineral density in the total body and spine, and with bone accrual in the spine.[282]

Intervention data are still lacking, however. In one small trial that enrolled adults with depression, no change in C-terminal cross-linking telopeptide of type 1 collagen, a bone resorption marker, was seen after 1.45 g EPA/DHA for 12 weeks. However, this study could be criticized for using a low dose over a short period of time and evaluating only 1 marker of bone resorption, and the patient population did not necessarily start with increased bone resorption. This was a sample of patients of a wide range of ages with depression, not osteoporosis or other risk factors for bone loss. The authors' conclusion that n-3 supplementation might not be useful for preventing bone loss may only be appropriate to the population they studied, not more generally, and perhaps not using different doses or markers of bone health.[283] A study published in 2007 found just the opposite. Although researchers used plant-based n-3 fatty acids, which might not be equivalent to DHA/EPA, they did find that supplementation with ALA did have a protective effect and inhibited bone resorption. This indicates that in principle, n-3 fatty acids play an important role.[284] The effect of fish oil supplementation as an intervention to prevent and/or treat osteoporosis awaits well-designed clinical trials.

In a study published in 2008, a retrospective analysis was done of a cohort of nearly 3000 men and women aged 59 to 73 years. When examining multiple dietary influences on grip strength, a potential marker for muscle strength and function, fatty fish consumption emerged as having the greatest effect.[285] Although very small, a second interventional study compared the effects of either corn oil or n-3 fatty acid supplementation on muscle synthesis in older adults; 1.86 g EPA and 1.50 g DHA were shown to increase muscle protein synthesis. Particularly, supplementation augmented the hyperaminoacidemia–hyperinsulinemia induced synthesis of muscle, suggesting it may be beneficial for the treatment and/or prevention of sarcopenia.[286]

Pregnancy and Lactation

Many studies have been published on the effects of DHA and/or fish oil during pregnancy and lactation, with several indicating that maternal intake of n-3 fatty acids reduces the chance of premature birth, improves children's neurological development, and also reduces the risk of maternal depression.[262,287,288] No significant adverse effects were cited with intakes of up to 1 g/day DHA or 2.7 g/day long-chain n-3 fatty acids in randomized trials. As mentioned earlier, the sooner supplementation begins during (or before) pregnancy, the greater the chance for benefit, at least regarding maternal depression.[266]

A study published in *Journal of the American Medical Association,* however, did not find a reduction in maternal depression or improved cognition and/or language in the children of mothers given 800 mg DHA and 100 mg EPA during pregnancy and challenged existing thought regarding supplementation.[289] One possible limitation of this study was the failure to assess the n-3 intake of participants in the study, aside from the provided supplement, although cord blood determinations suggested a low intake in the control group. The median gestational age was 19 weeks in this study. Supplementation earlier or before conception may have had a greater effect. In contrast, a review of interventional and epidemiological studies supported the use of DHA and EPA supplementation for childhood cognition and development, as well as preventing childhood psychiatric disorders.[290]

Mercury toxicity is also of concern and may accompany increased fish consumption. A study published in 2008 found that although maternal fish consumption was associated with a benefit on children's cognition at age 3, mercury levels were associated with poorer test scores. This certainly suggests that obtaining fish oil from purified supplements or low-mercury fish is preferable and may provide greater benefit than is often cited due to fish consumption (which may be contaminated with mercury).[291] Recommendations to avoid fish (perhaps due to mercury) may be associated with worse outcomes, as suggested by an observational study published in the *Lancet* in 2007.[292]

Finally, an article published in 2011 suggested that genetic variation might significantly modulate n-3 fatty acid levels in pregnancy, children, and breast milk composition.[293] Here, too, the use of a biomarker such as the ω-3 index might be of clinical value.

TOXICITY AND TOXIN CONTAMINATION

Despite widespread use, toxicity with fish oil is quite rare, with the most frequent side effects being mild dyspepsia, nausea, and belching ("fish burps"). Of growing concern, however, is the increasing contamination by environmental toxins such as mercury and POPs (e.g., PCBs and organochlorine pesticides). An excellent risk and/or benefit analysis of fish consumption was published that offers a species-specific analysis of ω-3 content as well as mercury contamination of each species.[294] The authors of this study suggest that in theory, fish oil supplementation from a high-quality source avoids the contamination of consuming fish. Previous studies found the commercial fish oil to be free of mercury, PCBs, and organochlorines, but quality control is essential.[295] Additionally, supplements that use fish oil derived from small, cold-water fatty fish are much less likely to be contaminated.[296]

Care must also be taken to use fish oil, not fish liver oil, because the latter can be excessively high in vitamins A and D and possibly result in toxicity.

Finally, large dosages of fish oil supplements may result in a significant increase in total caloric intake, which can be ameliorated by an increase in energy expenditure.

Bleeding Time

Concerns have been expressed about prolonged bleeding time in populations having a relatively high intake of fish. Several studies showed that this effect appears to be dose dependent, although collagen-induced platelet aggregation was not shown to be inhibited.[297] Many studies showed that fish oil supplements prolong bleeding time, inhibit platelet aggregation, and decrease TXA_2 production.[62,112,298–308]

However, many of these studies are several decades old. In an editorial published in March 2007 in the *American Journal of Cardiology,* the author concluded that current research was nearly unanimous: "omega-3 fatty acid supplements do not increase the risk for clinically significant bleeding, even in patients also being treated with

antiplatelet or antithrombotic medications. Anecdotal reports of an increased bruising tendency have not been tested in a controlled setting." He also considered this to be at the "A" level of evidence (i.e., based on well-designed, randomized controlled clinical trials). One caution was that interactions with newer antiplatelet drugs such as clopidogrel have not been evaluated.[309] A second review in the same issue also concluded that clinical trials showed high-dose fish oil consumption to be safe, even when concurrently administered with other agents that may increase bleeding, including aspirin and warfarin. This review also suggested that the reported antiatherothrombotic effects of fish oils might outweigh the unproven bleeding risks, particularly for those patients at high risk for thrombosis.[310]

Some early research suggested that the effect of fish oil supplementation on bleeding time was largely determined by the dosage, duration, and composition of the supplement, and the typical treatment regimens are safe. In one double-blind, placebo-controlled trial, daily supplementation with 30 mL of a Scandinavian fish oil formulation (ESKIMO-3: 35% n-3 fatty acids, 18% EPA, and 12% DHA) given for both short (4 weeks) and long (6 months) durations resulted in no changes in bleeding time.[65] Another controlled study used 1.5, 3, or 6 g of fish oils (SuperEPA) as a supplement for 3 months and found no effect on bleeding time in 45 healthy male volunteers with normal triglycerides.[311] In the large Enoxaparin MaxEPA Prevention of Angioplasty Restenosis (EMPAR) trial (of more than 800 patients receiving fish oil and low-molecular-weight heparin), a decrease in bleeding frequency was seen in the fish oil group compared with placebo.[312]

Evidence as to the degree to which fish oil may affect fibrinolysis is conflicting. Plasma plasminogen activator inhibitor (PAI-1) is an inhibitor of fibrinolysis. Increased PAI-1 activity was linked to the development of myocardial infarction and thrombosis by several investigators. In at least one double-blind, randomized study conducted on an untreated essential hypertensive population, a modest increase in fibrinogen levels was observed after fish oil and corn oil intake by 4 g of ω-3 PUFAs.[313] However, no change in PAI-1 activity or tissue plasminogen activator activity was found. A crossover study found fish oil to have the same effect on PAI-1 activity as olive oil.[314]

No major bleeding events had been reported in trials as of 2018.

Oxidation

It was reported in an animal-based study that a diet rich in fish oil taken for many months can induce a deficiency of vitamin E, leading to cardiac necrosis.[315,316] For this reason, periodic vitamin E (mixed tocopherols or γ-tocopherol) supplementation may be warranted. However, there is no evidence that the incidence of cardiac necrosis is higher in Eskimos.

The ω-3 fatty acids found in fish oil are susceptible to oxidative breakdown. For this reason, they must be protected from oxidation by proper extraction and storage, encapsulation, or stabilization with an antioxidant such as vitamin E. Inappropriately stored fish oils (i.e., exposure to oxygen) may result in the formation of toxic lipid peroxides over time.

Measurement of Lipid Oxidation Products Is Essential in Fatty Acid Supplements

The consumption of essential fatty acid supplements and vegetable oils is increasing, with fish oil now one of the most frequently consumed nutritional supplements. However, the high concentration of unsaturated fatty acids in these products makes them more susceptible to spoilage and oxidative damage. Several factors accelerate spoilage of these oils, including the presence of reactive metals, high water content, or high temperatures during processing and storage.

Consumers perceive oxidative damage as rancidity or off-flavors and off-odors. The health implications of this damage are important. Oxidative damage diminishes the nutritional value of these oils by destroying vitamins and other nutrients. Oxidative products may also interact with protein and carbohydrates. In addition, the by-products of lipid oxidation may pose a health risk to consumers.

Some edible oils are more resistant to oxidative damage. For example, vegetable oils may naturally contain antioxidants such as tocopherols or vitamin E. Antioxidants prevent the oxidative reactives that can lead to rancidity. Many pathways of oxidation of lipids are not completely understood. Two pathways that have received attention are oxidative and hydrolytic rancidity.

Oxidative reactions tend to be more complex, and their by-products are difficult to measure. Initially, oxygen combines with free fatty acids to form hydroperoxides and free radicals. These reactions are influenced by many factors: the percentage of unsaturated fatty acids present in the oil; processing and storage temperatures; oxygen concentration; light exposure; presence of antioxidants in the oil; moisture content of the oil; and the presence of reactive transition metals, such as iron, nickel, or copper.

The primary oxidative products are unstable. Hydroperoxides are rapidly degraded to aldehydes, ketones, alcohols, and hydrocarbons. These secondary products are relatively stable and are responsible for the characteristic flavor and odor associated with rancid oils.

Historically, manufacturers of edible oils relied on two analytic tests to determine the level of oxidation in oil: the free fatty acid percentage and peroxide value. Two additional measures, the anisidine value and the total oxidation value, have become the focus of attention.

The percentage of free fatty acids in oil is an indication of freshness. As oil ages, triglycerides are cleaved to glycerol and free fatty acids through hydrolysis. Free fatty acids are more prone to oxidation than triglycerides, so their presence in oils increases the possibility of rancidity. A fresh, carefully processed oil has a free fatty acid percentage of less than 0.05%.

The peroxide value measures the level of hydroperoxides in oil. Hydroperoxides are the primary by-product of oxidation. For example, 4-hydroxy-hexenal is a by-product of DHA peroxidation known to be elevated in disease states.[317] Peroxide value alone is not indicative of the actual oxidative state of the oil because hydroperoxides decompose rapidly to secondary by-products such as aldehydes. Therefore an oil might also have a low peroxide value as a result of extensive oxidation rather than due to low levels of oxidation. In theory, fresh oil would have a peroxide value of zero. In practice, most vegetable oils have peroxide values ranging from 0.1 to 1. Besides the peroxide value, the anisidine value must also be calculated.

The anisidine value is a measure of the amount of α- and β-unsaturated aldehydes. Anisidine is an aromatic amine. Synonyms for anisidine include aminoanisole, methoxyaniline, methoxyphenylamine, and methoxybenzenamine. Anisidine has two isomeric forms. Both were reported to enhance mutagenicity in bacterial strains.[318] The ortho-anisidine isomer was associated with bladder tumors. Data on carcinogenicity are almost exclusively on the ortho-isomer, not the para-isomer, which most companies focus on when they measure anisidine values in fish oils.

The total oxidative value used to express the extent of oxidation of an oil is the sum of the peroxide value and the anisidine value. Tests for peroxide value should comply with the *American Oil Chemists, Society* (AOCS) *Official Method* Cd 8-53 and not exceed a maximum of 5 mEq/kg, whereas the para-anisidine value should comply with *AOCS Official Method* 18-90 and not exceed a maximum of 20.

Many commercially available oils contain high levels of hydroperoxides and secondary by-products of oxidation. In the 1990s, Shukla

TABLE 80.2 Analytic Constants of Various Encapsulated Essential Fatty Acid Oils (1985–1988)

Product	Peroxide Value (Px)	Anisidine Value (Av)	Total Oxidation (2 Px + Av)	Iodine Value	Percentage of Free Fatty Acids
Vegetable oils					
A	5.4	6	16.8	151.5	1.02
B	1.5	4.4	7.4	146.2	4.1
C	6.9	4.8	18.6	178.6	0.14
D	3.4	0	6.8	152	0.80
Fish oils					
E	3.7	30.2	37.6	189	0.10
F	2.7	14.3	19.7	196.5	0.09
G	20.8	17.9	59.5	241.7	0.25
H	2.2	29.2	33.6	199.4	0.31

Data from Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr.* 1990 Jul;52(1):1–28.

TABLE 80.3 Analytic Constants of the Same Brand of Fish Oil Capsules Bought in Four Different Countries

Country	Peroxide Value (Px)	Anisidine Value (Av)	Total Oxidation Value (2 Px + Av)
England	3.8	16.6	24.2
The Netherlands	1.8	34	37.6
Denmark	3.7	30.2	37.6
United States	2.2	20.6	25

Data from Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr.* 1990 Jul;52(1):1–28.

and Perkins[319] analyzed various oils for oxidative stability. Their results showed that fish oils were more prone to oxidation than vegetable oils. The total oxidation values of fish oils ranged from 20 to 60 compared with 5 to 20 for vegetable oils (Tables 80.2 and 80.3).[19]

Shukla and Perkins made the following recommendations to prevent oxidation during the manufacturing process[42]:

- Always use top-quality, freshly produced raw materials stored in temperature- and humidity-controlled facilities.
- Prevent exposure to oxygen by using closed, impermeable containers.
- Avoid exposure of raw materials to sunlight.
- Use stringent quality control.
- Use stainless-steel equipment.
- Minimize heating of oil during processing.
- Use antioxidants to prolong shelf life.
- When using soft gelatin capsules, choose a shell with the lowest concentration of plasticizer possible.

By the early 21st century, many manufacturers of fatty acid supplements and vegetable oils took the lead to protect consumers by producing oils with the lowest levels of oxidative by-products possible. Consumer education and accurate measurement of peroxide and anisidine values are vital to this effort.

DOSAGE

A daily dose of 1000 mg EPA+DHA may be sufficient as a general health adjunct. However, for more therapeutic indications, the dosage range based on most clinical studies is between 3 and 4 g/day of EPA+DHA. Research also suggests that biomarker assessment may be of value for determining the optimal dosage for a variety of conditions, especially protection against cardiovascular disease. In a small study, supplementation with 1296 mg EPA + 864 mg DHA per day increased the erythrocyte ω-3 index, a potential biomarker for at least cardiovascular disease risk, to near 8%.[320] Interindividual differences in eicosanoid metabolism as well as digestive and/or absorptive function, along with genetic polymorphisms, may call for more individualized dosing. As clearly evidenced by clinical trials such as those on RA, a benefit may not be seen for 2 to 3 months.

The optimal ratio of EPA/DHA is not well established and may vary for individuals as well as conditions. Research in depression suggests that although DHA is the more prevalent fatty acid in brain tissue, EPA may be more clinically effective. This should be kept in mind when considering other conditions, such as fish oil supplementation for neurodegenerative disease.

Also, the form of fish and fish oil supplements is an important consideration. In one small trial comparing cod liver oil supplementation with actual fish consumption, cooked salmon increased serum levels to a greater extent than cod liver oil, despite providing less total DHA/EPA.[321] This may be because in fish, the fatty acids are triacylglycerols and phospholipids, whereas in almost all refined fish oils used in studies, EPA and DHA were in the ethyl ester form. Currently, EPA/DHA supplements are widely available in the triglyceride, phospholipid, and ethyl ester forms. Although several studies have shown similar absorption for ethyl esters, phospholipids, or triglycerides, other studies have shown that EPA/DHA consumed as triacylglycerides increased the ω-3 index more rapidly and to a greater degree than the ethyl esters of DHA/EPA.[322–324] However, the clinical significance of this advantage has not yet been demonstrated, especially with chronic administration.

DRUG INTERACTIONS

Individuals known or suspected to have a bleeding disorder, as well as those prescribed therapeutic levels of aspirin or warfarin, should have their bleeding time parameters measured, although as previously described (see "Bleeding Time"), this risk is not likely to exist. Patients may benefit from fish oil when taking cyclosporine.[325] Fish oil may also have synergistic or additive effects with the cholesterol- and triglyceride-lowering drugs pravastatin and simvastatin and have broader therapeutic value.[326] Fish oil is likely to modulate the cytotoxicity of many chemotherapeutic agents, as previously described, to increase therapeutic efficacy without increasing toxicity. It may also reduce the adverse

effects of many chemotherapeutic regimens, including the unintentional loss of weight. It may increase the efficacy of some antidepressants without an increase in toxicity.[269] A thorough review by Stargrove et al.[327] found nearly every drug interaction to be a beneficial one.

CONCLUSION

A substantial body of evidence continues to grow, documenting the safety and efficacy of *uncontaminated* n-3 fatty acids from fish and fish oil for a broad range of diseases, as well as for improving optimal cellular and physiological function. The mechanisms by which EPA and DHA from fish oil modulate cellular function and membrane structure continue to be elucidated in greater detail, and the potential of biomarkers, such as the ω-3 index, and genetic variants related to fish oil metabolism highlight the need for individualized and targeted fish oil supplementation.

The list of conditions for which fish oil is of benefit extends well beyond this chapter because its use has been researched for most medical conditions. Many of the common and most well-established uses for fish oil have been highlighted, such as for cardiovascular disease and modulation of inflammatory activity. The growing contamination of fish and the potential decline in worldwide fish stocks are of considerable concern, especially given the broad-ranging benefit of this underconsumed nutrient. Also, a growing awareness of the importance of not just n-3 intake but also of the ratio of n-6 fatty acids may help increase the efficacy of its nutritional modulation. One article cited that despite a 0.005% change in human genes over the past 10,000 years, an extraordinarily significant shift in the ratio of n-6:n-3 has occurred, which might help explain so many of today's modern illnesses.[31]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Kromann N, Green A. Epidemiological studies in the Upernavik district. Greenland. *Acta Med Scand*. 1980;208:401–406.
2. Yamori Y, Nara Y, Iritani N, et al. Comparison of serum phospholipid fatty acids among fishing and farming Japanese populations and American inlanders. *J Nutr Sci Vitaminol (Tokyo)*. 1985;31:417–422.
3. Bulliyya G, Reddy KK, Reddy GP, et al. Lipid profiles among fish-consuming coastal and non-fish-consuming inland populations. *Eur J Clin Nutr*. 1990;44:481–485.
4. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial. *Lancet*. 1989;2:757–761.
5. Burr ML, Fehily AM, Rogers S, et al. Diet and reinfarction trial (DART): design, recruitment, and compliance. *Eur Heart J*. 1989;10:558–567.
6. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet*. 1999;354:447–455.
7. Brown M. Do vitamin E and fish oil protect against ischaemic heart disease? *Lancet*. 1999;354:441–442.
8. de Lorgeril M, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon diet heart study. *Circulation*. 1999;99:779–785.
9. Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet*. 2007;369:1090–1098.
10. Tanaka K, Ishikawa Y, Yokoyama M, et al. Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial. *Stroke*. 2008;39:2052–2058.
11. Pottala JV, Garg S, Cohen BE, et al. Blood eicosapentaenoic and docosahexaenoic acids predict all-cause mortality in patients with stable coronary heart disease: the Heart and Soul study. *Circ Cardiovasc Qual Outcomes*. 2010;3:406–412.
12. Hock CE, Holahan MA, Reibel DK. Effect of dietary fish oil on myocardial phospholipids and myocardial ischemic damage. *Am J Physiol*. 1987;252:H554–H560.
13. Reibel DK, Holahan MA, Hock CE. Effects of dietary fish oil on cardiac responsiveness to adrenoceptor stimulation. *Am J Physiol*. 1988;254:H494–H499.
14. Lands WE, Libelt B, Morris A, et al. Maintenance of lower proportions of n-6 eicosanoid precursors in phospholipids of human plasma in response to added dietary n-3 fatty acids. *Biochim Biophys Acta*. 1992;1180:147–162.
15. Lands WE. Long-term fat intake and biomarkers. *Am J Clin Nutr*. 1995;61:721S–772S.
16. Nair SS, Leitch JW, Falconer J, et al. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. *J Nutr*. 1997;127:383–393.
17. Nestel P, Shige H, Pomeroy S, et al. The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *Am J Clin Nutr*. 2002;76:326–330.
18. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002;106:2747–2757.
19. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr*. 1990;52:1–28.
20. Erasmus U. *Fats and Oils*. Vancouver, BC: Alive; 1986.
21. van Vliet T, Katan MB. Lower ratio of n-3 to n-6 fatty acids in cultured than in wild fish. *Am J Clin Nutr*. 1990;51:1–2.
22. Bustnes JO. Salmon farms as a source of organohalogenated contaminants in wild fish. *Environ Sci Technol*. 2010;44:8736–8743.
23. Dórea JG. Studies of fish consumption as source of methylmercury should consider fish-meal-fed farmed fish and other animal foods. *Environ Res*. 2009;109:131–132.
24. Van Woudenbergh GJ, van Ballegooijen AJ, Kuijsten A, Sijbrands EJ, et al. Eating fish and risk of type 2 diabetes: a population-based, prospective follow-up study. *Diabetes Care*. 2009 Nov;32(11):2021–2026. PubMed PMID: 19675200.
25. Ibrahim MM, Fjaere E, Lock EJ, Naville D, Amlund H, et al. Chronic consumption of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. *PLoS One*. 2011;6(9):e25170. PubMed PMID: 21966444.
26. Blasbalg TL, Hibbeln JR, Ramsden CE, et al. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am J Clin Nutr*. 2011;93:950–962.
27. Sanders TA. DHA status of vegetarians. *Prostaglandins Leukot Essent Fatty Acids*. 2009;81:137–141.
28. Doughman SD, Krupanidhi S, Sanjeevi CB. Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. *Curr Diabetes Rev*. 2007;3:198–203.
29. Bjerve KS, Thoresen L, Borsting S. Linseed and cod liver oil induce rapid growth in a 7-year old girl with omega-3 fatty acid deficiency. *J Parenter Enteral Nutr*. 1988;12:521–525.
30. Kuipers RS, Luxwolda MF, Dijck-Brouwer DA, et al. Estimated macronutrient and fatty acid intakes from an East African Paleolithic diet. *Br J Nutr*. 2010;104(11):1666–1687.
31. Simopoulos. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pac J Clin Nutr*. 2008;17(S1):131–134.
32. Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem Pharmacol*. 2009;77:937–946.
33. Pischon T, Hankinson SE, Hotamisligil GS, et al. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation*. 2003;108:155–160.
34. Thiébaut AC, Chajès V, Gerber M, et al. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. *Int J Cancer*. 2009;124:924–931.
35. Murff HJ, Shu XO, Li H, et al. Dietary polyunsaturated fatty acids and breast cancer risk in Chinese women: a prospective cohort study. *Int J Cancer*. 2011;128:1434–1441.
36. Burdge GC, Calder PC, et al. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev*. 2005;45:581–597.
37. Welch AA. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the product-precursor ratio [corrected] of α-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. *Am J Clin Nutr*. 2010;92:1040–1051.
38. Stephensen CB, Armstrong P, Newman JW, et al. ALOX5 gene variants affect eicosanoid production and response to fish oil supplementation. *J Lipid Res*. 2011;52:991–1003.
39. Dwyer JH, Allayee H, Dwyer KM, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med*. 2004;350:29–37.
40. Rudkowska I, Caron-Dorval D, Verreault M, et al. PPARalpha L162V polymorphism alters the potential of n-3 fatty acids to increase lipoprotein lipase activity. *Mol Nutr Food Res*. 2010;54:543–550.
41. Harris WS, Mozaffarian D, Lefevre M, et al. Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. *J Nutr*. 2009;139:804S–819S.
42. Shukla VK, Perkins EG. The presence of oxidative polymeric materials in encapsulated fish oils. *Lipids*. 1991;26:23–26.
43. Kromhout D, Giltay EJ, Geleijnse JM, et al. N-3 fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med*. 2010;363:2015–2026.
44. Barradas MA, Christofides JA, Jeremy JY, et al. The effect of olive oil supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations. *Nutr Res*. 1990;10:403–411.
45. Tichelaar HY. Eicosapentaenoic acid composition of different fish oil concentrates. *Lancet*. 1990;336:1450.

46. De Caterina R. N-3 fatty acids in cardiovascular disease. *N Engl J Med*. 2011;364:2439–2450.
47. Block RC, Harris WS, Pottala JV, et al. Determinants of blood cell omega-3 fatty acid content. *Open Biomarkers J*. 2008;1:1–6.
48. Budiarso IT. Fish oil versus olive oil. *Lancet*. 1990;336:1313–1314.
49. Allard JP, Kurian R, Aghdassi E, et al. Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids*. 1997;32:535–541.
50. Haglund O, Luostarinen R, Wallin R, et al. Effects of fish oil on triglycerides, cholesterol, lipoprotein(a), atherogenic index and fibrinogen. Influence of degree of purification of the oil. *Nutr Res*. 1992;12:445–468.
51. Saldeen AS, Engstrom K, Ronneberg R, et al. Stable fish oil has a better effect on cholesterol and joint stiffness than ordinary fish oil. *Hygie*. 1996;105:373. [Swedish].
52. Yagi S, Fukuda D, Aihara KI, Shimabukuro M, Sata M. N-3 polyunsaturated fatty acids: promising nutrients for preventing cardiovascular disease. *J Atherosclerosis Thrombosis*. 2017;24(10):999–1010. PubMed PMID: 28835582.
53. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*. 2010;142:687–698.
54. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol*. 2007;25:101–137.
55. Mori TA, Beilin LJ, Burke V, et al. Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 1997;17:279–286.
56. Prisco D, Filippini M, Francalanci I, et al. Effect of n-3 fatty acid ethyl ester supplementation on fatty acid composition of the single platelet phospholipids and on platelet functions. *Metabolism*. 1995;44:562–569.
57. Needleman P, Raz A, Minkes MS, et al. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci USA*. 1979;76:944–948.
58. Knapp HR, Reilly IA, Alessandrini P, et al. In vivo indexes of platelet and vascular function during fish-oil administration in patients with atherosclerosis. *N Engl J Med*. 1986;314:937–942.
59. Lands WE, Culp BR, Hirai A, et al. Relationship of thromboxane generation to the aggregation of platelets from humans: effects of eicosapentaenoic acid. *Prostaglandins*. 1985;30:819–825.
60. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA*. 1995;274:1363–1367.
61. Dyerberg J. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr Rev*. 1986;44:125–134.
62. Thorngren M, Gustafson A. Effects of 11-week increases in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. *Lancet*. 1981;2:1190–1193.
63. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in northwestern Greenland. *Am J Clin Nutr*. 1980;33:2657–2661.
64. Sinclair HM. Advantages and disadvantages of an Eskimo diet. In: Fumagalli R, Kritchevsky D, Paoletti R, eds. *Drugs Affecting Lipid Metabolism*. Amsterdam: Elsevier; 1980:363–370.
65. Haglund O, Wallin R, Luostarinen R, et al. Effects of a new fluid fish oil concentrate, ESKIMO-3, on triglycerides, cholesterol, fibrinogen and blood pressure. *J Intern Med*. 1990;227:347–353.
66. Conner WE. The impact of dietary omega-3 fatty acids on the synthesis and clearance of apo B lipoproteins and chylomicrons. In: *Proceedings of Scientific Conference on Omega-3 Fatty Acids in Nutrition. Vascular Biology, and Medicine*. Houston, TX: American Heart Association; 1995.
67. Hwang DH, Chanmugam PS, Ryan DH, et al. Does vegetable oil attenuate the beneficial effects of fish oil in reducing risk factors for cardiovascular disease? *Am J Clin Nutr*. 1997;66:89–96.
68. Kestin M, Clifton P, Belling GB, et al. N-3 fatty acids of marine origin lower systolic blood pressure and triglycerides but raise LDL cholesterol compared with n-3 and n-6 fatty acids from plants. *Am J Clin Nutr*. 1990;51:1028–1034.
69. Weisburger JH. Dietary fat and risk of chronic disease: mechanistic insights from experimental studies. *J Am Diet Assoc*. 1997;97:S16–S23.
70. Grimble RF, Howell WM, O'Reilly G, et al. The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. *Am J Clin Nutr*. 2002;76:454–459.
71. Khairallah RJ, Sparagna GC, Khanna N, et al. Dietary supplementation with docosahexaenoic acid, but not eicosapentaenoic acid, dramatically alters cardiac mitochondrial phospholipid fatty acid composition and prevents permeability transition. *Biochim Biophys Acta*. 2010;1797:1555–1562.
72. Khairallah RJ, O'Shea KM, Brown BH, et al. Treatment with docosahexaenoic acid, but not eicosapentaenoic acid, delays Ca2+-induced mitochondria permeability transition in normal and hypertrophied myocardium. *J Pharmacol Exp Ther*. 2010;335:155–162.
73. Wilson PW. Evidence of systemic inflammation and estimation of coronary artery disease risk: a population perspective. *Am J Med*. 2008;121(10 suppl 1):S15–S20.
74. von Schacky C. The Omega-3 Index as a risk factor for cardiovascular diseases. *Prostaglandins Other Lipid Mediat*. 2011;96(1-4):94–98.
75. Harris WS. The omega-3 index as a risk factor for coronary heart disease. *Am J Clin Nutr*. 2008;87:1997S–2002S.
76. Block RC, Harris WS, Reid KJ, et al. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. *Atherosclerosis*. 2008;197:821–828.
77. Farzaneh-Far R, Lin J, Epel ES, et al. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA*. 2010;303:250–257.
78. Horrocks LA, Farooqui AA. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:361–372.
79. Yang X, Sheng W, Sun GY, et al. Effects of fatty acid unsaturation numbers on membrane fluidity and α-secretase-dependent amyloid precursor protein processing. *Neurochem Int*. 2011;58:321–329.
80. Bouwens M, van de Rest O, Dellschaft N, et al. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. *Am J Clin Nutr*. 2009;90:415–424.
81. Serhan CN, Chiang N, Van Dyke TE, et al. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol*. 2008;8:349–361.
82. Norling LV, Serhan CN, et al. Profiling in resolving inflammatory exudates identifies novel anti-inflammatory and pro-resolving mediators and signals for termination. *J Intern Med*. 2010;268:15–24.
83. Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. *Eur J Pharmacol*. 2011 Sep;668(suppl 1):S50–S58. Epub 2011 Jul 28.
84. Calder PC, Yaqoob P. Omega-3 (n-3) fatty acids, cardiovascular disease and stability of atherosclerotic plaques. *Cell Mol Biol (Noisy-le-grand)*. 2010;56:28–37.
85. Cawood AL, Ding R, Napper FL, et al. Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. *Atherosclerosis*. 2010;212:252–259.
86. Balk EM, Lichtenstein AH, Chung M, et al. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis*. 2006;189:19–30.
87. Fetterman Jr JW, Zdanowicz MM, et al. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. *Am J Health Syst Pharm*. 2009;66:1169–1179.
88. Wang C, Harris WS, Chung M, et al. N-3 fatty acids from fish or fish oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr*. 2006;84:5–17.
89. Harris WS. The omega-3 index: clinical utility for therapeutic intervention. *Curr Cardiol Rep*. 2010;12:503–508.
90. Connor WE. Do the n-3 fatty acids from fish prevent deaths from cardiovascular disease? *Am J Clin Nutr*. 1997;66:188–189.

91. Phillipson BE, Rothrock DW, Conner WE, et al. Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oil in patients with hypertriglyceridemia. *N Engl J Med*. 1985;312:1210–1216.
92. Nestel PJ, Connor WE, Reardon MF, et al. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J Clin Invest*. 1984;74:82–89.
93. Simons LA, Hickie JB, Balasubramaniam S. On the effects of dietary n-3 fatty acids (MaxEPA) on plasma lipids and lipoproteins in patients with hyperlipidemia. *Atherosclerosis*. 1985;54:75–88.
94. Davidson MH. Apolipoprotein measurements: is more widespread use clinically indicated? *Clin Cardiol*. 2009;32:482–486.
95. Shin MJ, Krauss RM, et al. Apolipoprotein CIII bound to apoB-containing lipoproteins is associated with small, dense LDL independent of plasma triglyceride levels in healthy men. *Atherosclerosis*. 2010;211(1): 337–341.
96. Griffin MD, Sanders TA, Davies IG, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. *Am J Clin Nutr*. 2006;84:1290–1298.
97. Saito Y, Yokoyama M, Origasa H, et al. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis*. 2008;200:135–140.
98. Dart AM, Riemersma RA, Oliver MF. Effects of MaxEPA on serum lipids in hypercholesterolemic subjects. *Atherosclerosis*. 1989;80:119–124.
99. Riemersma RA, Sargent CA, Abraham RA, et al. Fish and the heart. *Lancet*. 1989;2:1450–1452.
100. Demke DM, Peters GR, Linet OI, et al. Effects of a fish oil concentrate in patients with hypercholesterolemia. *Atherosclerosis*. 1988;70:73–80.
101. Bilo HJ, Gans RO, Donker AJ. Fish oil for preventing coronary restenosis. *Lancet*. 1989;2:693–694.
102. Kelley DS, Siegel D, Vemuri M, et al. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr*. 2007;86:324–333.
103. Garrido-Sánchez L, García-Fuentes E, Rojo-Martínez G, et al. Inverse relation between levels of anti-oxidized-LDL antibodies and eicosapentanoic acid (EPA). *Br J Nutr*. 2008;100:585–589.
104. Matsuura E, Hughes GR, Khamashta MA, et al. Oxidation of LDL and its clinical implication. *Autoimmun Rev*. 2008;7:558–566.
105. Yetiv JZ. Clinical applications of fish oils. *JAMA*. 1988;260:665–670.
106. Saynor R, Gillott T. Fish oil revisited. *Lancet*. 1989;2:810–812.
107. Bays HE, McKenney J, Maki KC, et al. Effects of prescription omega-3-acid ethyl esters on non-high-density lipoprotein cholesterol when co-administered with escalating doses of atorvastatin. *Mayo Clin Proc*. 2010;85:122–128.
108. Minihane AM, Armah CK, Miles EA, et al. Consumption of fish oil providing amounts of eicosapentanoic acid and docosahexanoic acid that can be obtained from the diet reduces blood pressure in adults with systolic hypertension: a retrospective analysis. *J Nutri*. 2016;146(3):516–523. PubMed PMID: 26817716.
109. Geleijnse JM, Giltay EJ, Grobbee DE, et al. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *J Hypertens*. 2002;20:1493–1499.
110. Cicero AF, Ertek S, Borghi C, et al. Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. *Curr Vasc Pharmacol*. 2009;7:330–337.
111. Ueshima H, Stamler J, Elliott P, et al. Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension*. 2007;50:313–319.
112. Lorenz R, Spengler U, Fisher S, et al. Platelet function, thromboxane formation and blood pressure control during supplementation of the Western diet with cod liver oil. *Circulation*. 1983;67:504–511.
113. Singer P, Wirth M, Voigt S, et al. Clinical studies on lipid and blood pressure lowering effect of eicosapentaenoic acid-rich diet. *Biomed Biochim Acta*. 1984;43:S421–S425.
114. Rylance PB, Gordge MP, Saynor R, et al. Fish oil modifies lipids and reduces platelet aggregability in haemodialysis patients. *Nephron*. 1986;43:196–202.
115. Lorenz R, Spengler U, Seiss W, et al. Membrane fatty acids, platelet aggregation and thromboxane formation on dietary cod liver oil supplementation. In: *Prostaglandins, Fifth International Conference*. Florence, Italy: Fondazione Giovanni Lorenzini; 1982:695–699.
116. Singer P, Jaeger W, Wirth M, et al. Lipid and blood pressure lowering effect of mackerel diet on man. *Atherosclerosis*. 1983;49:99–108.
117. van Houwelingen AC, Hornstra G. Effect of a moderate fish intake on blood pressure, platelet function and safety aspects. *Agents Actions*. 1987;22:371–372.
118. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis Rheum*. 1990;33:810–820.
119. Margolin G, Huster G, Glueck CJ, et al. Blood pressure lowering in elderly subjects: a double-blind crossover study of omega-3 and omega-6 fatty acids. *Am J Clin Nutr*. 1991;53:562–572.
120. Knapp HR, Fitzgerald GA. The antihypertensive effects of fish oils. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. *N Engl J Med*. 1989;320:1037–1043.
121. Raloff J. Fish oil lowers even normal blood pressure. *Sci News*. 1989;136:181.
122. He K. Fish consumption and risk of stroke in men. *JAMA*. 2002;288:3130–3136.
123. Mozaffarian D, Longstreth Jr WT, Lemaitre RN, et al. Fish consumption and stroke risk in elderly individuals. *Arch Intern Med*. 2005;165: 200–206.
124. Nahab F, Le A, Judd S, et al. Racial and geographic differences in fish consumption: the REGARDS study. *Neurology*. 2011;76: 154–158.
125. Iso H, Rexrode KM, Stampfer MJ, et al. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA*. 2001;285:304–312.
126. Von Schacky C. N-3 fatty acids and the prevention of coronary atherosclerosis. *Am J Clin Nutr*. 2000;71:S224–S227.
127. Benedetto U, Melina G, di Bartolomeo R, et al. N-3 polyunsaturated fatty acids after coronary artery bypass grafting. *Ann Thorac Surg*. 2011;91:1169–1175.
128. Mozaffarian D, Psaty BM, Rimm EB, et al. Fish intake and risk of incident atrial fibrillation. *Circulation*. 2004;110:368–373.
129. Mozaffarian D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. *Am J Clin Nutr*. 2008;87:1991S–1996S.
130. Rennison JH, Van Wagoner DR. Impact of dietary fatty acids on cardiac arrhythmogenesis. *Circ Arrhythm Electrophysiol*. 2009;2:460–469.
131. Flachs P, Horakova O, Brauner P, et al. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia*. 2005;48:2365–2375.
132. Brouwer IA, Heeringa J, Geleijnse JM, et al. Intake of very long-chain n-3 fatty acids from fish and incidence of atrial fibrillation. The Rotterdam Study. *Am Heart J*. 2006;151:857–862.
133. Frost L, Vestergaard P, et al. N-3 fatty acids consumed from fish and risk of atrial fibrillation or flutter: the danish diet, cancer, and health study. *Am J Clin Nutr*. 2005;81:50–54.
134. Berry JD. Dietary fish intake and incident atrial fibrillation (from the Women's Health Initiative). *Am J Cardiol*. 2010;105:844–848.
135. Liu T, Korantzopoulos P, Shehata M, et al. Prevention of atrial fibrillation with omega-3 fatty acids: a meta-analysis of randomised clinical trials. *Heart*. 2011;97:1034–1040.
136. Kowey PR, Reiffel JA, Ellenbogen KA, et al. Efficacy and safety of prescription omega-3 fatty acids for the prevention of recurrent symptomatic atrial fibrillation: a randomized controlled trial. *JAMA*. 2010;304:2363–2372.
137. Brouwer IA, Raitt MH, Dullemeijer C, et al. Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators. *Eur Heart J*. 2009;30:820–826.

138. Nattel S, Van Wagoner DR, et al. Atrial fibrillation: therapy with omega-3 fatty acids-is the case closed? *Nat Rev Cardiol*. 2011;8: 126–128.
139. Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the gruppo italiano per lo studio della sopravvivenza nell'infarto miocardico (GISSI)-prevenzione. *Circulation*. 2002;105:1897–1903.
140. Macchia A. Left ventricular systolic dysfunction, total mortality, and sudden death in patients with myocardial infarction treated with n-3 polyunsaturated fatty acids. *Eur J Heart Fail*. 2005;7: 904–909.
141. Matsuzaki M, Yokoyama M, Saito Y, et al. Incremental effects of eicosapentaenoic acid on cardiovascular events in statin-treated patients with coronary artery disease. *Circ J*. 2009;73:1283–1290.
142. Origasa H, Yokoyama M, Matsuzaki M, et al. Clinical importance of adherence to treatment with eicosapentaenoic acid by patients with hypercholesterolemia. *Circ J*. 2010;74:510–517.
143. Rauch B, Schiele R, Schneider S, et al. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation*. 2010;122:2152–2159.
144. McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. *Am Heart J*. 1988;116:709–717.
145. Riemersma RA, Sargent CA. Dietary fish oil and ischaemic arrhythmias. *J Intern Med*. 1989;225:111–116.
146. Lepran I, Nemecz G, Koltai M, et al. Effect of a linoleic acid-rich diet on the acute phase of coronary artery occlusion in conscious rats: influence of indomethacin and aspirin. *J Cardiovasc Pharmacol*. 1981;3:847–853.
147. De Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343:1454–1459.
148. Myerburg RJ. Epidemiology of ventricular tachycardia/ventricular fibrillation and sudden cardiac death. *Pacing Clin Electrophysiol*. 1986;9: 1334–1338.
149. Buxton AE. Sudden cardiac death. *Ann Intern Med*. 1986;104:716–718.
150. Haberka M, Mizia-Stec K, Mizia M, et al. N-3 polyunsaturated fatty acids early supplementation improves ultrasound indices of endothelial function, but not through NO inhibitors in patients with acute myocardial infarction: N-3 PUFA supplementation in acute myocardial infarction. *Clin Nutr*. 2011;30:79–85.
151. Hardarson T, Kristinsson A, Skuladottir G, et al. Cod liver oil does not reduce ventricular extrasystoles after myocardial infarction. *J Intern Med*. 1989;226:33–37.
152. Wahlqvist ML, Lo CS, Myers KA. Fish intake and arterial wall characteristics in healthy people and diabetic patients. *Lancet*. 1989;2:944–946.
153. Bjerregaard LJ, Joensen AM, Dethlefsen C, et al. Fish intake and acute coronary syndrome. *Eur Heart J*. 2010;31:29–34.
154. Pounis GD, Panagiotakos DB, Chrysohoou C, et al. Long-term fish consumption is associated with lower risk of 30-day cardiovascular disease events in survivors from an acute coronary syndrome. *Int J Cardiol*. 2009;136:344–346.
155. Amin AA, Menon RA, Reid KJ, et al. Acute coronary syndrome patients with depression have low blood cell membrane omega-3 fatty acid levels. *Psychosom Med*. 2008;70:856–862.
156. Zhao YT, Chen Q, Sun YX, et al. Prevention of sudden cardiac death with omega-3 fatty acids in patients with coronary heart disease: a meta-analysis of randomized controlled trials. *Ann Med (Helsinki)*. 2009;41:301.
157. Aucamp AK, Schoeman HS, Coetzee JH. Pilot trial to determine the efficacy of a low dose of fish oil in the treatment of angina pectoris in the geriatric patient. *Prostaglandins Leukot Essent Fatty Acids*. 1993;49:687–689.
158. Solomon SA, Cartwright I, Pockley G, et al. A placebo-controlled, double-blind study of eicosapentaenoic acid-rich fish oil in patients with stable angina pectoris. *Curr Med Res Opin*. 1990;12:1–11.
159. Itakura H, Yokoyama M, Matsuzaki M, et al. Relationships between plasma fatty acid composition and coronary artery disease. *J Atheroscler Thromb*. 2011;18:99–107.
160. Sijben JW. Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. *Proc Nutr Soc*. 2007;66:237–259.
161. Kew S, Mesa MD, Tricon S, et al. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *Am J Clin Nutr*. 2004;79:674–681.
162. Grimble RF, Howell WM, O'Reilly G, et al. The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. *Am J Clin Nutr*. 2002;76:454–459.
163. Rees D, Miles EA, Banerjee T, et al. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr*. 2006;83:331–342.
164. Kew S, Banerjee T, Minihane AM, et al. Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. *Am J Clin Nutr*. 2003;77:1278–1286.
165. Gorjão R, Verlengia R, Lima TM, et al. Effect of docosahexaenoic acid-rich fish oil supplementation on human leukocyte function. *Clin Nutr*. 2006;25:923–938.
166. Krishnamoorthy S, Recchiuti A, Chiang N, et al. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. *Proc Natl Acad Sci USA*. 2010;107:1660–1665.
167. Spite M, Norling LV, Summers L, et al. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature*. 2009;461:1287–1291.
168. Rudin DO. The dominant disease of modernized societies as omega-3 essential fatty acid deficiency syndrome: substrate beriberi. *Med Hypotheses*. 1982;8:17–47.
169. Voorhees JJ. Leukotrienes and other lipoxygenase products in the pathogenesis and therapy of psoriasis and other dermatoses. *Arch Dermatol*. 1983;119:541–547.
170. Prickett JD, Robinson DR, Steinberg AD. Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB X NZW/F1 mice. *Arthritis Rheum*. 1983:26133–26139.
171. Shinto L, Marracci G, Baldauf-Wagner S, et al. Omega-3 fatty acid supplementation decreases matrix metalloproteinase-9 production in relapsing-remitting multiple sclerosis. *Prostaglandins Leukot Essent Fatty Acids*. 2009;80:131–136.
172. Shapiro JA, Koepsel TD, Voigt LF, et al. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. *Epidemiology*. 1996;7:256–263.
173. Isseroff RR. Fish again for dinner! The role of fish and other dietary oils in the therapy of skin disease. *J Am Acad Dermatol*. 1988;19:1073–1080.
174. Vincentini O, Quaranta MG, Viora M, et al. Docosahexaenoic acid modulates in vitro the inflammation of celiac disease in intestinal epithelial cells via the inhibition of cPLA(2). *Clin Nutr*. 2011;30:541–546.
175. Duffy EM, Meenagh GK, McMillan SA, et al. The clinical effect of dietary supplementation with omega-3 fish oils and/or copper in systemic lupus erythematosus. *J Rheumatol*. 2004;31:1551–1556.
176. Wright SA, O'Prey FM, McHenry MT, et al. A randomised interventional trial of omega-3-polyunsaturated fatty acids on endothelial function and disease activity in systemic lupus erythematosus. *Ann Rheum Dis*. 2008;67:841–848.
177. Navarro E, Esteve M, Olive A, et al. Abnormal fatty acid pattern in rheumatoid arthritis. A rationale for treatment with marine and botanical lipids. *J Rheumatol*. 2000;27:298–303.
178. Cleland LG, James MJ, et al. Rheumatoid arthritis and the balance of dietary N-6 and N-3 essential fatty acids. *Br J Rheumatol*. 1997;36:513–514.
179. Ruggiero C, Lattanzio F, Lauretani F, et al. Omega-3 polyunsaturated fatty acids and immune-mediated diseases: inflammatory bowel disease and rheumatoid arthritis. *Curr Pharm Des*. 2009;15:4135–4148.
180. Goldberg RJ, Katz J, et al. A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain*. 2007;129:210–223.

181. Trelle S, Reichenbach S, Wandel S, et al. Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. *BMJ*. 2011;342:c7086.
182. Galarraga B, Ho M, Youssef HM, et al. Cod liver oil (n-3 fatty acids) as an non-steroidal anti-inflammatory drug sparing agent in rheumatoid arthritis. *Rheumatology (Oxford)*. 2008;47:665–669.
183. Caughey GE, James MJ, Proudman SM, et al. Fish oil supplementation increases the cyclooxygenase inhibitory activity of paracetamol in rheumatoid arthritis patients. *Complement Ther Med*. 2010;18:171–174.
184. Das Gupta AB, Hossain AK, Islam MH, et al. Role of omega-3 fatty acid supplementation with indomethacin in suppression of disease activity in rheumatoid arthritis. *Bangladesh Med Res Counc Bull*. 2009;35:63–68.
185. James M, Proudman S, Cleland L, et al. Fish oil and rheumatoid arthritis: past, present and future. *Proc Nutr Soc*. 2010;69:316–323.
186. Veselinovic M, Vasiljevic D, Vucic V, et al. Clinical benefits of n-3 PUFA and γ-linolenic acid in patients with rheumatoid arthritis. *Nutrients*. 2017;9(4):E325. PubMed PMID: 28346333.
187. Adam O. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatol Int*. 2003;23:27–36.
188. Shils ME, Young VR. In: *Modern Nutrition in Health and Disease.* Lea & Febiger: Philadelphia; 198: 1473–1474.
189. Kragballe K, Fogh K. A low-fat diet supplemented with dietary fish oil (Max-EPA) results in improvement of psoriasis and in formation of leukotriene B5. *Acta Derm Venereol*. 1989;69:23–28.
190. Balbás GM, Regaña MS, Millet P, et al. Study on the use of omega-3 fatty acids as a therapeutic supplement in treatment of psoriasis. *Clin Cosmet Investig Dermatol*. 2011;4:73–77.
191. Danno K, Sugie N. Combination therapy with low-dose etretinate and eicosapentaenoic acid for psoriasis vulgaris. *J Dermatol*. 1998;25:703–705.
192. Escobar SO, Achenbach R, Iannantuono R, et al. Topical fish oil in psoriasis: a controlled and blind study. *Clin Exp Dermatol*. 1992;17:159–162.
193. Calder PC, Kremmyda LS, Vlachava M, et al. Is there a role for fatty acids in early life programming of the immune system? *Proc Nutr Soc*. 2010;69:373–380.
194. Olsen SF, Østerdal ML, Salvig JD, et al. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am J Clin Nutr*. 2008;88:167–175.
195. Toelle BG. Eight-year outcomes of the childhood asthma prevention study. *J Allergy Clin Immunol*. 2010;126:388–389.
196. Tecklenburg-Lund S, Mickleborough TD, Turner LA, et al. Randomized controlled trial of fish oil and montelukast and their combination on airway inflammation and hyperpnea-induced bronchoconstriction. *PLoS One*. 2010;5:e13487.
197. Moreira A, Moreira P, Delgado L, et al. Pilot study of the effects of n-3 polyunsaturated fatty acids on exhaled nitric oxide in patients with stable asthma. *J Investig Allergol Clin Immunol*. 2007;17:309–313.
198. Mickleborough TD, Rundell KW, et al. Dietary polyunsaturated fatty acids in asthma- and exercise-induced bronchoconstriction. *Eur J Clin Nutr*. 2005;59:1335–1346.
199. Kalayci O, Birben E, Sackesen C, et al. ALOX5 promoter genotype, asthma severity and LTC production by eosinophils. *Allergy*. 2006;61:97–103.
200. Koki AT, Masferrer JL. Celecoxib: a specific COX-2 inhibitor with anticancer properties. *Cancer Control*. 2002;9:28–35.
201. Hammamieh R, Chakraborty N, Miller S, et al. Differential effects of omega-3 and omega-6 fatty acids on gene expression in breast cancer cells. *Breast Cancer Res Treat*. 2007;101:7–16.
202. Berquin IM, Edwards IJ, Chen YQ, et al. Multi-targeted therapy of cancer by omega-3 fatty acids. *Cancer Lett*. 2008;269:363–377.
203. Janakiram NB, Rao CV, et al. Role of lipoxins and resolvins as anti-inflammatory and proresolving mediators in colon cancer. *Curr Mol Med*. 2009;9:565–579.
204. Szymanski KM, Wheeler DC, Mucci LA, et al. Fish consumption and prostate cancer risk: a review and meta-analysis. *Am J Clin Nutr*. 2010;92:1223–1233.
205. Thiebaut AC, Chajès V, Gerber M, et al. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. *Int J Cancer*. 2009;124:924–931.
206. Fradet V, Cheng I, Casey G, et al. Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. *Clin Cancer Res*. 2009;15:2559–2566.
207. Kaizer L, Boyd NF, Kriukov V, et al. Fish consumption and breast cancer risk: an ecological study. *Nutr Cancer*. 1989;12:61–68.
208. Pala V, Krogh V, Muti P, et al. Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. *J Natl Cancer Inst*. 2001;93:1088–1095.
209. Geelen A, Schouten JM, Kamphuis C, et al. Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. *Am J Epidemiol*. 2007;166:1116–1125.
210. Norat T, Bingham S, Ferrari, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *J Natl Cancer Inst*. 2005;97:906–916.
211. West NJ, Clark SK, Phillips RK, et al. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut*. 2010;59:918–925.
212. Anti M, Armelao F, Marra G, et al. Effects of different doses of fish oil on rectal cell proliferation in patients with sporadic colonic adenomas. *Gastroenterology*. 1994;107:1709–1718.
213. Caygill CP, Charlett A, Hill MJ. Fat, fish, fish oil and cancer. *Br J Cancer*. 1996;74:159–164.
214. Wolk SC, Larsson JE, Johansson JE, et al. Long-term fatty fish consumption and renal cell carcinoma incidence in women. *JAMA*. 2006;296:1371–1376.
215. Barber MD, Ross JA, Voss AC, et al. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br J Cancer*. 1999;81:80–86.
216. Wigmore SJ, Ross JA, Falconer JS, et al. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition*. 1996;12:S27–S30.
217. Murphy RA, Mourtzakis M, Chu QS, et al. Nutritional intervention with fish oil provides a benefit over standard of care for weight and skeletal muscle mass in patients with nonsmall cell lung cancer receiving chemotherapy. *Cancer*. 2011;117:1775–1782.
218. Murphy RA, Mourtzakis M, Chu QS, et al. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. *Cancer*. 2011;117:3774–3780.
219. van der Meij BS, Langius JA, Smit EF, et al. Oral nutritional supplements containing (n-3) polyunsaturated fatty acids affect the nutritional status of patients with stage III non-small cell lung cancer during multimodality treatment. *J Nutr*. 2010;140:1774–1780.
220. Gogos CA, Ginopoulos P, Salsa B, et al. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy. *Cancer*. 1998;82:395–402.
221. Shaikh IA, Brown I, Wahle KW, et al. Enhancing cytotoxic therapies for breast and prostate cancers with polyunsaturated fatty acids. *Nutr Cancer*. 2010;62:284–296.
222. Menendez JA, Lupu R, Colomer R, et al. Exogenous supplementation with omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA; 22:6n-3) synergistically enhances taxane cytotoxicity and downregulates Her-2/neu (c-erbB-2) oncogene expression in human breast cancer cells. *Eur J Cancer Prev*. 2005;14:263–270.
223. Manna S. Fish oil regulates cell proliferation, protect DNA damages and decrease HER-2/neu and c-Myc protein expression in rat mammary carcinogenesis. *Clin Nutr*. 2010;29:531–537.
224. Biondo PD, Brindley DN, Sawyer MB, et al. The potential for treatment with dietary long-chain polyunsaturated n-3 fatty acids during chemotherapy. *J Nutr Biochem*. 2008;19:787–796.
225. Gillet L, Gillet L, Roger S, et al. Beneficial effects of omega-3 long-chain fatty acids in breast cancer and cardiovascular diseases: voltage-gated sodium channels as a common feature? *Biochimie*. 2011;93:4–6.
226. Gleissman H, Johnsen JI, Kogner P, et al. Omega-3 fatty acids in cancer, the protectors of good and the killers of evil? *Exp Cell Res*. 2010;316:1365–1373.
227. Bruera E, Strasser F, Palmer JL, et al. Effect of fish oil on appetite and other symptoms in patients with advanced cancer and anorexia/cachexia: a double-blind, placebo-controlled study. *J Clin Oncol*. 2003;21:129–134.

228. Burns CP, Halabi S, Clamon G, et al. Phase II study of high-dose fish oil capsules for patients with cancer-related cachexia. *Cancer*. 2004;101:370–378.
229. Bougnoux P, Hajjaji N, Ferrasson MN, et al. Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: a phase II trial. *Br J Cancer*. 2009;101:1978–1985.
230. Harel Z, Gascon G, Riggs S. Supplementation with omega-3 polyunsaturated fatty acids in the management of recurrent migraines in adolescents. *J Adolesc Health*. 2002;31:154–161.
231. Pradalier A, Bakouche P, Baudesson G, et al. Failure of omega-3 polyunsaturated fatty acids in prevention of migraine: a double-blind study versus placebo. *Cephalalgia*. 2001;21:818–822.
232. Maghsoumi-Norouzabad L, Mansoori A, Abed R, Shishehbor F. Effects of omega-3 fatty acids on the frequency, severity, and duration of migraine attacks: a systemic review and meta-analysis of randomized controlled trials. *Nutri Neurosci*. 2018;21(9):614–623. PubMed PMID: 28665211.
233. Feskens EJ, Virtanen SM, Räsänen L, et al. Dietary factors determining diabetes and impaired glucose tolerance: a 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care*. 1995;18:1104–1112.
234. Scott EM, Griffith IV, et al. Diabetes mellitus in Eskimos. *Metabolism*. 1957;6:320–325.
235. Djoussé L, Gaziano JM, Buring JE, et al. Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes. *Am J Clin Nutr*. 2011;93:143–450.
236. Kaushik M, Mozaffarian D, Spiegelman D, et al. Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. *Am J Clin Nutr*. 2009;90:613–620.
237. Brostow DP, Odegaard AO, Koh WP, et al. Omega-3 fatty acids and incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Clin Nutr*. 2011;94:520–526.
238. Djoussé L, Biggs ML, Lemaitre RN. Plasma omega-3 fatty acids and incident diabetes in older adults. *Am J Clin Nutr*. 2011;94:527–533.
239. Wang L, Folsom AR, Zheng ZJ, et al. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr*. 2003;78:91–98.
240. Laaksonen DE, Lakka TA, Lakka HM, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med*. 2002;19:456–464.
241. Lee DH. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. *Diabetes Care*. 2006;29(7):1638–1644.
242. Gallo MV, Schell LM, DeCaprio AP, et al. Levels of persistent organic pollutant and their predictors among young adults. *Chemosphere*. 2011;83:1374–1382.
243. Schecter A, Colacino J, Haffner D, et al. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. *Environ Health Perspect*. 2010;118:796–802.
244. McVeigh GE, Brennan GM, Cohn JN, et al. Fish oil improves arterial compliance in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb*. 1994;14:1425–1429.
245. Luo J, Rizkalla SW, Vidal H, et al. Moderate intake of n-3 fatty acids for 2 months has no detrimental effect on glucose metabolism and could ameliorate the lipid profile in type 2 diabetic men. *Diabetes Care*. 1998;21:717–724.
246. Rivellese AA, Maffettone A, Iovine C, et al. Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. *Diabetes Care*. 1996;19:1207–1213.
247. Rizza S, Tesauro M, Cardillo C, et al. Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. *Atherosclerosis*. 2009;206:569–574.
248. Norris JM, Yin X, Lamb MM, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. *JAMA*. 2007;298:1420–1428.
249. Jamilian M, Samimim M, Mirhosseini N, et al. A randomized double-blinded, placebo-controlled trial investigating the effect of fish oil supplementation on gene expression related to insulin action, blood lipids, and inflammation in gestational diabetes mellitus-fish oil supplementation and gestational diabetes. *Nutrients*. 2018;10(2):E163. PubMed PMID: 29385062.
250. Kabir M, Skurnik G, Naour N, et al. Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. *Am J Clin Nutr*. 2007;86:1670–1679.
251. Jicha GA, Markesbery WR, et al. Omega-3 fatty acids: potential role in the management of early Alzheimer's disease. *Clin Interv Aging*. 2010;5:45–61.
252. Lopez LB, Kritz-Silverstein D, Barrett Connor E, et al. High dietary and plasma levels of the omega-3 fatty acid docosahexaenoic acid are associated with decreased dementia risk: the Rancho Bernardo study. *J Nutr Health Aging*. 2011;15:25–31.
253. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. *Neurology*. 2007;69:1921–1930.
254. Huang TL, Zandi PP, Tucker KL, et al. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology*. 2005;65:1409–1414.
255. Bo Y, Zhang X, Wang Y, et al. The n-3 polyunsaturated fatty acids supplementation improved the cognitive function in the Chinese elderly with mild cognitive impairment: a double-blind randomized controlled trial. *Nutrients*. 2017;9(1):E54. PubMed PMID: 28075381.
256. Yanai H. Effects of n-3 polyunsaturated fatty acids on dementia. *Journal of Clinical Medicine Research*. 2017;9(1):1–9. PubMed PMID: 27924168.
257. Whalley LJ, Deary IJ, Starr JM, et al. N-3 fatty acid erythrocyte membrane content, APOE varepsilon4, and cognitive variation: an observational follow-up study in late adulthood. *Am J Clin Nutr*. 2008;87:449–454.
258. Quinn JF, Raman R, Thomas RG, et al. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *JAMA*. 2010;304:1903–1911.
259. Freund-Levi Y, Eriksdotter-Jönhagen M, Cederholm T, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol*. 2006;63:1402–1408.
260. de Lau LM, Bornebroek M, Witteman JC, et al. Dietary fatty acids and the risk of Parkinson disease: the Rotterdam study. *Neurology*. 2005;64:2040–2045.
261. Ozsoy O, et al. The influence and the mechanism of docosahexaenoic acid on a mouse model of Parkinson's disease. *Neurochem Int*. 2011 Oct;59(5):664–670.
262. Golding J, Steer C, Emmett P, et al. High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish. *Epidemiology*. 2009;20:598–603.
263. Tanskanen JR, Hibbeln JR, Tuomilehto J, et al. Fish consumption and depressive symptoms in the general population in Finland. *Psychiatr Serv*. 2001;52:529–531.
264. Lin PY, Huang SY, Su KP, et al. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biol Psychiatry*. 2010;68:140–147.
265. Freeman MP, Rapaport MH, et al. Omega-3 fatty acids and depression: from cellular mechanisms to clinical care. *J Clin Psychiatry*. 2011;72:258–259.
266. Wojcicki JM, Heyman MB. Maternal omega-3 fatty acid supplementation and risk for perinatal maternal depression. *J Matern Fetal Neonatal Med*. 2011;24:680–686.
267. Martins JG. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. *J Am Coll Nutr*. 2009;28:525–542.
268. Lin PY, Su KP, et al. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *J Clin Psychiatry*. 2007;68:1056–1061.
269. Jazayeri M, Tehrani-Doost M, Keshavarz SA, et al. Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine,

separately and in combination, in major depressive disorder. *Aust NZ J Psychiatry*. 2008;42:192–198.
270. Baghai TC, Varallo-Bedarida G, Born C, et al. Major depressive disorder is associated with cardiovascular risk factors and low Omega-3 index. *J Clin Psychiatry*. 2011;72(9):1242–1247.
271. Su KP, Huang SY, Peng CY, et al. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-alpha-induced depression by regulating polyunsaturated fatty acids levels. *Biol Psychiatry*. 2010;67: 550–557.
272. DiGiacomo RA, Kremer JM, Shah DM. Fish-oil dietary supplementation in patients with Raynaud's phenomenon: a double-blind, controlled, prospective study. *Am J Med*. 1989;86:158–164.
273. Anonymous. Fish oil: new hope in fighting malaria. *Sci News*. 1989;135:237.
274. Fevang P, Sääv H, Høstmark AT, et al. Dietary fish oils and long-term malaria protection in mice. *Lipids*. 1995;30:437–441.
275. Yerlikaya FH. Plasma fatty acid composition in continuous ambulatory peritoneal dialysis patients: an increased omega-6/omega-3 ratio and deficiency of essential fatty acids. *Ren Fail*. 2011;33(8):819–823.
276. Madsen T, Christensen JH, Svensson M, et al. Marine n-3 polyunsaturated fatty acids in patients with end-stage renal failure and in subjects without kidney disease: a comparative study. *J Ren Nutr*. 2011;21: 169–175.
277. Madsen T, Christensen JH, Toft E, et al. Effect of intravenous omega-3 fatty acid infusion and hemodialysis on fatty acid composition of free fatty acids and phospholipids in patients with end-stage renal disease. *J Parenter Enteral Nutr*. 2011;35:97–106.
278. Bakker D, Haberstroh B, Philbrick D, et al. Triglyceride lowering in nephrotic syndrome patients consuming a fish oil concentrate. *Nutr Res*. 1989;9:27–34.
279. Donadio JV, Bergstralh EJ, Bibus DM, et al. Is body size a biomarker for optimizing dosing of omega-3 polyunsaturated fatty acids in the treatment of patients with IgA nephropathy? *Clin J Am Soc Nephrol*. 2006;1:933–939.
280. Uchiyama-Tanaka Y, Mori Y. Effects of eicosapentaenoic acid supplementation on immunoglobulin A nephropathy. *Ther Apher Dial*. 2010;14:303–307.
281. Weiss LA, Barrett-Connor E, von Mühlen D, et al. Ratio of n-6 to n-3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study. *Am J Clin Nutr*. 2005;81:934–938.
282. Högström M, Barrett-Connor E, von Mühlen D, et al. N-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO2 Study. *Am J Clin Nutr*. 2007;85:803–807.
283. Appleton KM, Fraser WD, Rogers P, et al. Supplementation with a low-moderate dose of n-3 long-chain PUFA has no short-term effect on bone resorption in human adults. *Br J Nutr*. 2011;105:1145–1149.
284. Griel AE, Kris-Etherton PM, Hilpert KF, et al. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. *Nutr J*. 2007; 6–2. https://doi.org/10.1186/1475-2891-6-2.
285. Robinson SM, Jameson KA, Batelaan SF, et al. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. *J Am Geriatr Soc*. 2008;56:84–90.
286. Smith GI, Atherton P, Reeds DN, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clin Nutr*. 2011;93:402–412.
287. Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*. 2003;111:e39–e44.
288. Koletzko B, Cetin I, Brenna JT, et al. Dietary fat intakes for pregnant and lactating women. *Br J Nutr*. 2007;98:873–877.
289. Makrides M, Gibson RA, McPhee AJ, et al. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. *JAMA*. 2010;304: 1675–1683.
290. Ryan AS, Astwood JD, Gautier S, et al. Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: a review of human studies. *Prostaglandins Leukot Essent Fatty Acids*. 2010;82:305–314.
291. Oken E. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am J Epidemiol*. 2008;167:1171–1181.
292. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet*. 2007;369:578–585.
293. Glaser C, Lattka E, Rzehak P, et al. Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. *Matern Child Nutr*. 2011;7(suppl 2):27–40.
294. Ginsberg Toal BF, et al. Quantitative approach for incorporating methylmercury risks and omega-3 fatty acid benefits in developing species-specific fish consumption advice. *Environ Health Perspect*. 2009;117: 267–275.
295. Melanson SF, Lewandrowski EL, Flood JG, et al. Measurement of organochlorines in commercial over-the-counter fish oil preparations: implications for dietary and therapeutic recommendations for omega-3 fatty acids and a review of the literature. *Arch Pathol Lab Med*. 2005;129:74–77.
296. Bourdon JA, Bazinet TM, Arnason TT, et al. Polychlorinated biphenyls (PCBs) contamination and aryl hydrocarbon receptor (AhR) agonist activity of omega-3 polyunsaturated fatty acid supplements: implications for daily intake of dioxins and PCBs. *Food Chem Toxicol*. 2010;48: 3093–3097.
297. Ahmed AA, Holub BJ. Alteration and recovery of bleeding times, platelet aggregation and fatty acid composition of individual phospholipids in platelets of human subjects receiving a supplement of cod-liver oil. *Lipids*. 1984;19:617–624.
298. Goodnight Jr SH, Harris WS, Connor WE. The effects of dietary omega 3 fatty acids on platelet composition and function in man: a prospective, controlled study. *Blood*. 1981;58:880–885.
299. Sanders TA, Roshanai F. The influence of different types of omega-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clin Sci (Lond)*. 1983;64:91–99.
300. Siess W, Roth P, Scherer B, et al. Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet*. 1980;1:441–444.
301. Brox JH, Killie JE, Gunnes S, et al. The effect of cod-liver oil and corn oil on platelets and vessel wall in man. *Thromb Haemost*. 1981;46:604–611.
302. Hirai A, Terano T, Hamazaki T, et al. The effects of the oral administration of fish oil concentrate on the release and the metabolism of 14C-arachidonic acid and 14C-eicosapentaenoic acid by human platelet. *Thromb Res*. 1982;28: 285–298.
303. Bradlow BA, Chetty N, van der Westhuyzen J, et al. The effects of a mixed fish diet on platelet function, fatty acids and serum lipids. *Thromb Res*. 1983;29:561–568.
304. Schimke E, Hildebrandt R, Beitz J, et al. Influence of a cod liver oil diet in diabetics type I on fatty acid patterns and platelet aggregation. *Biomed Biochim Acta*. 1984;43:S351–S353.
305. Von Schacky C, Fischer S, Weber PC. Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest*. 1985;76: 1626–1631.
306. Srivastava KC. Docosahexaenoic acid (C22: 6 omega 3) and linoleic acid are anti-aggregatory and alter arachidonic acid metabolism in human platelets. *Prostaglandins Leukot Med*. 1985;17:319–327.
307. Sanders TA, Hochland MC. A comparison of the influence on plasma lipids and platelet function of supplements of omega 3 and omega 6 polyunsaturated fatty acids. *Br J Nutr*. 1983;50:521–529.
308. Croft KD, Beilin LJ, Vandongen R. The effect of dietary fish oil on platelet metabolism of 14C arachidonic acid. *Thromb Res*. 1986;42:99–104.
309. Harris WS. Expert opinion: omega-3 fatty acids and bleeding-cause for concern? *Am J Cardiol*. 2007;99(suppl 6A):44C–46C.
310. Bays HE. Safety considerations with omega-3 fatty acid therapy. *Am J Cardiol*. 2007;99(suppl 6A):35C–43C.
311. Blonk MC, Bilo HJ, Nauta JJ. Dose-response effects of fish-oil supplementation in healthy volunteers. *Am J Clin Nutr*. 1990;52:120–127.
312. Cairns JA, Gill J, Morton B, et al. Fish oils and low-molecular-weight heparin for the reduction of restenosis after percutaneous transluminal

coronary angioplasty. The EMPAR Study. *Circulation*. 1996;94:1553–1560.
313. Toft I, Bonaa KH, Ingebretsen OC, et al. Fibrinolytic function after dietary supplementation with omega-3 polyunsaturated fatty acids. *Arterioscler Thromb Vasc Biol*. 1997;17:814–819.
314. Jones PJ, Demonty I, Chan YM, et al. Fish-oil esters of plant sterols differ from vegetable-oil sterol esters in triglycerides lowering, carotenoid bioavailability and impact on plasminogen activator inhibitor-1 (PAI-1) concentrations in hypercholesterolemic subjects. *Lipids Health Dis*. 2007;6:28. https://doi.org/10.1186/1476-511X-6-28.
315. Ruiter A, Jongbloed AW, van Gent CM, et al. The influence of dietary mackerel oil on the condition of organs and on the blood lipid composition in the young growing pig. *Am J Clin Nutr*. 1978;31:2159–2166.
316. Gudbjarnason S, Hallgrimsson J. The role of myocardial membrane lipids in the development of cardiac necrosis. *Acta Med Scand Suppl*. 1976;587:17–27.
317. Long EK, Picklo Sr MJ, et al. Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: make some room HNE. *Free Radic Biol Med*. 2010;49:1–8.
318. Thompson DC, Josephy PD, Chu JW, et al. Enhanced mutagenicity of anisidine isomers in bacterial strains containing elevated N-acetyltransferase activity. *Mutat Res*. 1992;279:83–89.
319. Shukla VK, Perkins EG. Rancidity in encapsulated health food oils. *Inform*. 1998;9:955–961.
320. Cao J, Schwichtenberg KA, Hanson NQ, et al. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem*. 2006;52:2265–2272.
321. Elvevoll EO, Barstad H, Breimo ES, et al. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. *Lipids*. 2006;41:1109–1114.
322. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. *Biochem Biophys Res Commun*. 1988;156:960–963.
323. Neubronner J, Schuchardt JP, Kressel G, et al. Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur J Clin Nutr*. 2011;65:247–254.
324. Dyerberg J, Madsen P, Møller JM, et al. Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids*. 2010;83:137–141.
325. Andreassen AK, Hartmann A, Offstad J, et al. Hypertension prophylaxis with omega-3 fatty acids in heart transplant recipients. *J Am Coll Cardiol*. 1997;29:1324–1331.
326. Nakamura N, Hamazaki T, Ohta M, et al. Joint effects of HMG-CoA reductase inhibitors and eicosapentaenoic acids on serum lipid profile and plasma fatty acid concentrations in patients with hyperlipidemia. *Int J Clin Lab Res*. 1999;29:22–25.
327. Stargrove MB, Treasure J, McKee DL. *Herb, Nutrient, and Drug Interactions*. Mosby Elsevier: St Louis, MO.

81

Flavonoids—Quercetin, Citrus Flavonoids, and Hydroxyethylrutosides

Michael T. Murray, ND

OUTLINE

INTRODUCTION

Flavonoids, a group of polyphenolic plant pigments, are largely responsible for the colors of many fruits and flowers. Considerable research suggests that flavonoids may be useful in the treatment and prevention of many health conditions. Many of the medicinal actions of foods, juices, herbs, and bee pollen are now known to be directly related to their flavonoid content. More than 8000 flavonoid compounds have been characterized and classified according to their chemical structure. Flavonoids are a bit of an enigma to scientists because they are quite reactive compounds. They can enter into almost any type of reaction known to organic chemistry, such as oxidation-reduction reactions, carbonyl reaction, acid–base reactions, free-radical reactions, hydrophobic interactions, tautomery, and isomerizations.[1] As such, characterization of the many diverse physiological properties of flavonoids is a considerable challenge to biochemists and researchers alike. This chapter discusses a few representatives of this class of useful clinical agents (quercetin, citrus bioflavonoids, and hydroxyethylrutosides).

Another beneficial group of plant flavonoids is the proanthocyanidins (also referred to as procyanidins). Collectively, mixtures of proanthocyanidin dimers, trimers, tetramers, and larger molecules are referred to as procyanidolic oligomers (PCOs). Chapter 117 discusses PCOs.

HISTORICAL PERSPECTIVE

Flavonoids, and vitamin C, were discovered by Albert Szent-Györgyi (1893–1986), one of the most respected and honored biochemists of the 20th century. Szent-Györgyi received the Nobel Prize in 1937 for his discovery of some of the properties of these molecules.

Szent-Györgyi discovered the flavonoids while isolating vitamin C. A friend with bleeding gums had stopped the bleeding by taking a crude vitamin C preparation isolated from lemon. When the problem reappeared, Szent-Györgyi gave his friend a purer form of vitamin C. He expected to observe an even more impressive result, but the purer form of vitamin C did not work. Szent-Györgyi then isolated the flavonoid fraction from the original crude vitamin C preparation, gave it to his friend, and observed complete healing.

Szent-Györgyi termed his discovery "vitamin P" because of its ability to reduce vascular permeability, one of the hallmark features of scurvy. He went on to show that the clinical symptoms of scurvy are the result of a combined deficiency of vitamin C and flavonoids. However, because flavonoids could not fulfill all the requirements of a vitamin, the designation as vitamin P was abandoned. Although flavonoids are often referred to as "semiessential" nutrients, their importance in human nutrition appears to be as important to good health as the essential vitamins and minerals.

Good dietary sources of flavonoids include citrus fruits, berries, onions, parsley, legumes, green tea, and red wine. The average daily intake in the United States for flavonoids is estimated to be between 150 and 200 mg.

CHEMICAL DESCRIPTIONS

Quercetin

Quercetin (Fig. 81.1) is a flavonoid that serves as the aglycone for many other flavonoids, including the citrus flavonoids rutin, quercitrin, and hesperidin. These derivatives differ from quercetin in that they have sugar molecules attached to the quercetin backbone. Quercetin is

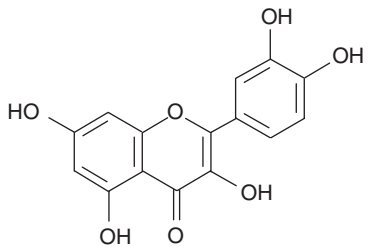


Fig. 81.1 Quercetin.

consistently one of the most active of the flavonoids in experimental studies, and many medicinal plants owe much of their activity to their high quercetin content.

Citrus Bioflavonoids

Citrus bioflavonoid preparations can include rutin, hesperidin, quercitrin, and naringin. Most of the clinical research on rutin and crude bioflavonoid complexes occurred before 1970. Since then, most of the clinical research has used a standardized mixture of rutinosides known as hydroxyethylrutosides (HERs). Impressive clinical results have been obtained in the treatment of capillary permeability, excessive bruisability, hemorrhoids, and varicose veins with HERs (discussed in the section "Collagen Matrix Support"). Citrus bioflavonoids can be viewed as providing similar effects to, but probably not as potent as, HERs or quercetin.

PHARMACOLOGY

As a class of compounds, flavonoids have been referred to as "nature's biological response modifiers" because of their ability to modify the body's reaction to other compounds, such as allergens, viruses, and carcinogens, as evidenced by their anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties.[2] In addition, flavonoids act as powerful antioxidants, providing remarkable protection against oxidative and free-radical damage[3]; they also have the ability to traverse the blood–brain barrier, thus exerting a neuroprotective effect.[4] Flavonoids are known to form strong ligand complexes with heavy-metal ions and may prove to be a good agent for heavy-metal detoxification.[1]

The practical aspect of this antioxidant activity was highlighted by the results of a study in 805 men designed to determine the effect of dietary flavonoids on protecting against heart disease. The study demonstrated an inverse correlation between flavonoid intake and death from a heart attack.[5] This effect was probably a result of the potent antioxidant effects of the flavonoids preventing, similar to vitamins C and E, the formation of oxidized cholesterol. However, the antioxidant activity of flavonoids is generally more potent and effective against a broader range of oxidants than the traditional antioxidant nutrients like vitamins C and E, selenium, and zinc.[6,7]

Because different flavonoids tend to provide different benefits, additional and more specific beneficial effects are discussed under the key selected flavonoid categories. There is significant overlap among these flavonoids, however.

Quercetin

Quercetin consistently demonstrates the greatest activity among the flavonoids studied in experimental models, particularly in vitro studies. The primary actions that are briefly reviewed here are its anti-inflammatory effects, inhibition of aldose reductase, antiviral activity, and anticancer properties.

Anti-inflammatory Effects

Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several of the initial processes of inflammation via interaction with calcium channels or calmodulin (the intracellular calcium-binding protein), or both. It works through other mechanisms as well, such as inhibiting mast cell and basophil degranulation, neutrophil and monocyte lysosomal secretion, prostaglandin (most notably, leukotriene) formation, lipid peroxidation, and the resultant cascade of effects that is often a result of these processes. For example, it inhibits both the manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent antioxidant activity and vitamin C–sparing action.[6–9]

Effect on Histamine Release

The release of histamine and other inflammatory mediators from mast cells and basophils is involved in the pathogenesis of acute allergic and inflammatory responses. Mast cells are widely distributed throughout the human body but are found in higher concentrations in the blood vessels of the subepithelial connective tissue of the respiratory tract, conjunctiva, gastrointestinal tract, and skin. Mast cell and basophil degranulation is an active process that requires calcium influx. Quercetin and many other flavonoids have been shown to be potent inhibitors of mast cell, neutrophil, and basophil degranulation. A generally accepted hypothesis for this action is that quercetin inhibits receptor-mediated calcium influx, thereby inhibiting the primary signal for degranulation. However, quercetin is also active under conditions in which the calcium channel mechanism is not operative, indicating that other mechanisms are responsible as well.[6–9]

Membrane Stabilization, Antioxidant Activity, and Hyaluronidase Inhibition

Quercetin inhibits many of the inflammatory processes attributed to activated neutrophils. This effect is probably a result of its membrane-stabilizing action, potent antioxidant effect (which prevents the production of free radicals and inflammatory leukotrienes), inhibition of the enzyme hyaluronidase (thus preventing the breakdown of the collagen matrix of connective tissue and ground substance), and finally inhibition of the proinflammatory cytokines. Quercetin's membrane-stabilizing effect could also account for its action in preventing mast cell and basophil degranulation. This effect also inhibits inflammation by decreasing neutrophil lysosomal enzyme secretion.[10] Neutrophils and monocytes contain lysosomes that, on secretion of their contents, contribute greatly to the inflammatory process.

Effects on Eicosanoid Metabolism

Excessive leukotriene formation has been linked to asthma, psoriasis, atopic dermatitis, gout, ulcerative colitis, and possibly cancer. Quercetin has been shown to inhibit many steps in eicosanoid metabolism. Its inhibition of phospholipase A2 and lipoxygenase enzymes is probably its most significant action (see Chapter 149 for diagram). The net result is a significant reduction in the formation of leukotrienes. The leukotrienes C4, D4, and E4 (composing the slow-reacting substances of anaphylaxis) are derived from arachidonic acid and are 1000 times as potent as histamine in promoting inflammation. Leukotrienes promote inflammation by causing vasoconstriction (thereby increasing vascular permeability) and bronchoconstriction (thus inducing asthma) and by promoting white blood cell chemotaxis and aggregation. The reduction of leukotriene formation by quercetin has significant anti-inflammatory effects.

Inhibition of Aldose Reductase

Quercetin is a strong inhibitor of aldose reductase, the enzyme responsible for the conversion of blood glucose to sorbitol. This compound is strongly implicated in the development of diabetic complications such

as diabetic cataracts, neuropathy, and retinopathy.[11] The mechanism by which sorbitol is involved in the development of diabetic complications is best understood by considering its involvement in cataract formation. Although the lens does not have any blood vessels, it is an actively metabolizing tissue that continuously grows throughout life. Elevated blood sugar levels result in shunting of glucose to the sorbitol pathway.

Because the lens membranes are virtually impermeable to sorbitol and lack the enzyme required to break down sorbitol (polyol dehydrogenase), sorbitol accumulates to high concentrations. These high concentrations persist even if glucose levels return to normal. This accumulation creates an osmotic gradient that results in water being drawn into the cells to maintain osmotic balance. As the water is pulled in, the cell must release small molecules like amino acids, inositol, glutathione, niacin, vitamin C, magnesium, and potassium to maintain osmotic balance. Because these latter compounds function to protect the lens from damage, their loss results in an increased susceptibility to damage. As a result, the delicate protein fibers within the lens become opaque, and a cataract forms.

Quercitrin, which is hydrolyzed by gut bacteria to yield quercetin and a sugar moiety, was shown to significantly decrease the accumulation of sorbitol in the lens of diabetic animals, effectively delaying the onset of cataracts.[12] In addition to its effect on aldose reductase, quercetin is also of value in diabetes for its ability to enhance insulin secretion and protect the pancreatic β cells from the damaging effects of free radicals, and for its inhibition of platelet aggregation.[6,7]

Antiviral Activity

Flavonoids as a group possess significant antiviral activity, with quercetin having the greatest antiviral activity against herpes virus type I, parainfluenzae 3, poliovirus type I, and respiratory syncytial virus.[13] Quercetin was shown, in vitro, to inhibit both viral replication and infectivity. In vivo studies in animals also showed quercetin to inhibit viral infection.[14] This would suggest that quercetin might be of some benefit in viral infections, including the common cold.

Anticancer Properties

Many flavonoids have also been shown to inhibit tumor formation, but again quercetin has consistently been the most effective. In experimental models, quercetin demonstrated significant antitumor activity against a wide range of cancers, including squamous-cell carcinoma; leukemia; and cancers of the breast, ovaries, colon, rectum, and brain. The cancer-preventive effects of quercetin have been attributed to various mechanisms, including antioxidative activity, inhibition of enzymes that activate carcinogens, modification of signal transduction pathways, and interactions with receptors and other proteins, such as the androgen receptor involved in the development and progression of prostate cancer.[15–17]

Clinically, quercetin holds great promise in reversing multidrug resistance (MDR) in cancer cells. Transporter-mediated active efflux of cytotoxic agents is one of the best-characterized mechanisms by which cancer cells develop MDR. Quercetin demonstrated multitargeted effects in reversing MDR.[17]

Citrus Flavonoids

Collagen Matrix Support

In addition to possessing antioxidant activity and an ability to increase intracellular levels of vitamin C, rutin, hesperidin, and HER exert many beneficial effects on capillary permeability and blood flow, primarily via strengthening endothelial cells and supporting collagen structures. Collagen, the most abundant protein of the body, is responsible for maintaining the integrity of "ground substance" and the integrity of tendons, ligaments, and cartilage. Collagen is also the support structure of the skin and blood vessels. Citrus flavonoids affect collagen metabolism in several ways.

They reinforce the natural cross-linking of collagen that forms the so-called collagen matrix of connective tissue and protect against free-radical damage with their potent antioxidant and free-radical–scavenging action. They also inhibit enzymatic cleavage of collagen by enzymes secreted by leukocytes during inflammation and microbes during infection. Like quercetin, citrus flavonoids also prevent the release and synthesis of compounds that promote inflammation and allergies, such as histamine, serine proteases, prostaglandins, and leukotrienes.[8,9] It is believed that the citrus bioflavonoid hesperidin possesses antihistaminic activity through its metabolite heparitin, a weak inhibitor of cyclooxygenase-2 enzymes. This metabolite is produced as a product of intestinal bacteria, which underscores the need for a balanced intestinal flora to gain the antihistamine benefits of citrus bioflavonoids.[18]

Bone Metabolism Effects

Histomorphometric research on ovariectomized mice showed that marked decreases in trabecular bone volume and trabecular thickness of the femoral distal metaphysis were significantly prevented by hesperidin. Additionally, calcium, phosphorus, and zinc concentrations in the femur were significantly higher in the hesperidin-fed group, whereas serum and hepatic lipids were lower in mice that consumed hesperidin-containing diets.[19]

PHARMACOKINETICS

Before discussing the clinical applications of quercetin, it is important to address absorption and metabolism. Previous pharmacokinetic studies in animals and humans indicated that little quercetin is absorbed intact, with the majority of the oral dose (53%) being excreted in the feces.[20,21] One of the main problems in studying the absorption of quercetin and other flavonoids is their degradation by microorganisms in the colon. To sidestep this issue, one study examined the absorption of quercetin in healthy ileostomy patients with complete small intestines.[22] The study examined the absorption of quercetin from fried onions (a rich source of quercetin glycosides), rutin, or 100 mg of pure quercetin. Absorption was defined as oral intake minus ileostomy excretion and corrected for degradation within the ileostomy bag. Absorption results were as follows: 52% from onions, 17% from quercetin rutinoside, and 24% for pure quercetin. These results indicate that humans do absorb appreciable amounts of quercetin and that absorption (but not necessarily pharmacological activity) may be enhanced when quercetin is bound to glucose. In other words, citrus bioflavonoid preparations or HERs, or both, may prove to be more effective clinically.

Subsequent studies shed additional light on the absorption and metabolism of quercetin. In a study of 35 healthy volunteers, the volunteers were randomly assigned to take 50, 100, or 150 mg/day (groups Q50, Q100, and Q150, respectively) of quercetin for 2 weeks. Fasting blood samples were collected at the beginning and end of the supplementation period. Compared with baseline, quercetin supplementation significantly increased plasma concentrations of quercetin by 178% (Q50), 359% (Q100), and 570% (Q150). The pharmacokinetics of quercetin were investigated in a subgroup of 15 volunteers. The areas under the plasma concentration–time curves ranged from 76.1 to 305.8 μmol/min/L(−1) (50- and 150-mg doses, respectively). Median maximum plasma concentrations of quercetin (431 nmol/L) were observed 360 minutes after intake of 150 mg of quercetin.[23]

In a much larger study, 1002 subjects were randomized to one of three groups: Q-500 (500 mg/day), Q-1000 (1000 mg/day), or placebo.

Quercetin supplementation over 12 weeks caused a significant increase in overnight-fasted plasma quercetin, with a net increase of 332 and 516 mcg/L for Q-500 and Q-1000 compared with 53.6 mcg/L for placebo. However, the increase in plasma quercetin was highly variable within each quercetin supplementation group.[24]

Quercetin is significantly metabolized into conjugates and smaller molecules. When healthy adults were given 1000 mg of quercetin daily for 3 months, it was extensively converted to isorhamnetin-3-glucuronide, which had the highest concentration at 3 months, followed by quercetin-3-glucuronide, quercetin-3-sulfate, and quercetin diglucuronide.[25]

Quercetin should be administered with dietary fat because it leads to improved absorption through intestinal micellization.[26]

Given the relatively poor bioavailability and high variability of absorption among subjects, improved clinical results may be achieved with newer forms of quercetin, such as enzymatically modified isoquercitrin (EMIQ) or phosphatidylcholine-bound quercetin to overcome these shortcomings (discussed in the section "Commercial Forms").

CLINICAL APPLICATIONS

Allergic and Inflammatory Conditions

Largely on the basis of in vitro studies, quercetin appears to be indicated in virtually all inflammatory and allergic conditions, including asthma, hay fever, rheumatoid arthritis, and lupus, and in diabetes and cancer. Clinical documentation in these areas, however, is lacking. In a study using a highly bioavailable EMIQ, very good results were achieved. In a parallel-group, double-blind, placebo-controlled study design, 20 subjects with hay fever caused by Japanese cedar pollinosis took two capsules daily of 100 mg EMIQ or a placebo for 8 weeks during the pollen season. During the entire study, the total ocular score and the ocular itching score for the EMIQ group were significantly lower than those of the placebo group. When limited to the individual periods, the total symptom score for the EMIQ group was significantly lower than that for the placebo group. The levels of serum cytokines, such as interleukin (IL)-4, IL-5, IL-12, IL-13, interferon-γ, and eotaxin and immunoglobulin-E, were not significantly downregulated by the intake of EMIQ, but the serum concentrations of oxidized low-density lipoprotein and thymus and activation-regulated chemokines were reduced.[27]

In another study of EMIQ in subjects with hay fever caused by Japanese cedar pollinosis, 24 subjects took 100 mg EMIQ or a placebo for 8 weeks, starting 4 weeks before the onset of pollen release. During the entire study period, the ocular symptom plus medication score for the EMIQ group was significantly lower than that of the placebo group. When limited to the pollen release period, ocular symptom scores and ocular congestion scores for the EMIQ group were significantly lower than that for the placebo group, whereas other scores for the EMIQ group, such as ocular itching scores, lacrimation scores, and ocular congestion scores, all tended to be lower. However, no significant differences were found in nasal symptoms between the two groups. These results indicate that EMIQ is useful in reducing the ocular symptoms of hay fever, especially ocular congestion.[28]

Topical application of quercetin showed anti-inflammatory effects. In a study of 40 patients with aphthous stomatitis randomly divided into two groups (group 1 patients used a benzydamine hydrochloride mouthwash three times daily; group 2 patients placed two to three dabs of quercetin three times daily directly on their ulcers), the topical application of quercetin cream to minor mouth ulcers relieved pain and produced complete healing in seven of the group 2 patients (35%) in 2 to 4 days, in 18 patients (90%) in 4 to 7 days, and in 20 patients (100%) in 7 to 10 days. Although aphthous ulcers typically resolve on their own in 1 to 2 weeks, the daily topical application of quercetin might be useful in accelerating the healing process of minor aphthous ulcers.[29]

Performance, Ergogenic, and Mitochondrial Effects

By far the most popular application, quercetin is in beverages designed for athletic performance. The commercial success of this application led to significant scientific investigation of quercetin's ability to improve physical performance. In a number of clinical trials, quercetin was shown to improve mental/physical performance and reduce infection risk after intense exercise.[30] In one small trial, 12 volunteers were randomly assigned to one of two treatments: (1) 500 mg of quercetin twice daily dissolved in vitamin-enriched Tang or (2) a nondistinguishable placebo (Tang). Baseline maximal oxygen consumption (VO_2max) and bike-ride times to fatigue were established. Treatments were administered for 7 days using a randomized, double-blind, placebo-controlled, crossover study design. After treatment, both VO_2max and ride time to fatigue were determined. Seven days of quercetin feedings were associated with a modest increase in VO_2max (3.9% vs. placebo) along with a substantial (13.2%) increase in ride time to fatigue. These data suggest that as little as 7 days of quercetin supplementation can increase endurance without exercise training in untrained participants. However, a similar study using treadmill testing failed to show the same sort of effect.[31]

In a more detailed study, 26 young adult males were given quercetin (1 g/day) or placebo for 2 weeks and then crossed over.[32] Subjects provided blood and muscle biopsy samples before and after supplementation periods and underwent 12-minute time trials on 15% graded treadmills after 60 minutes of moderate exercise preloads at 60% VO_2max. Plasma quercetin levels rose significantly during the 2-week quercetin supplementation period. Quercetin produced a 2.9% increased net distance in the 12-minute trial; skeletal muscle messenger RNA expression tended to increase (range, 16%–25%) during quercetin use for sirtuin 1, peroxisome proliferator-activated receptor γ coactivator-1α, cytochrome-c oxidase, and citrate synthase. Muscle mitochondrial DNA (relative copy number per diploid nuclear genome) increased by 4.1% with quercetin compared with a 6.0% decrease with the placebo. This result seems to indicate that quercetin does provide some benefit in improving physical performance in untrained volunteers.

In regard to moderately trained men and women, the data are not clear on indicators of performance, but quercetin showed other benefits in this group. In one study, 58 subjects were randomly assigned to quercetin (1 g/day) or placebo groups. Six weeks of dietary quercetin supplementation did not improve VO_2max or other indicators of performance.[33] In double-blind studies, however, quercetin (1 g/day) was shown to reduce upper respiratory infections in moderately trained individuals.[34,35] Of 20 trained male cyclists who received quercetin or placebo before, during, and for 2 weeks after a 3-day period in which subjects cycled for 3 hours/day at approximately 57% maximal workload, only 1 of 20 developed upper respiratory infection symptoms in the quercetin group compared with 9 of 20 in the placebo group.[34] In another study, a reduction in upper respiratory tract infection total sick days and severity was noted in middle-aged and older subjects ingesting 1000 mg/day quercetin for 12 weeks who rated themselves as physically fit.[35]

Although one small study of 16 male soldiers supplemented with 500 mg of quercetin twice daily showed no benefit in improving aerobic athletic performance (i.e., 75 minutes of loaded treadmill marching and a subsequent cycling time trial to complete 200 kJ of work,[36] a study with phosphatidylcholine-bound quercetin (Quercetin Phytosome), a more bioavailable form of quercetin, has been shown to be of particular benefit to athletes. For example, in a study in triathletes, Quercetin

Phytosome was shown to improve performance time and reduce postexercise muscular pain, cramps, and recovery time.[37] These benefits were attributed to its ability to reduce oxidative stress. Quercetin also exerts significant support for the immune system and antiallergy effects. This product is best for allergies, food sensitivities, and athletes.

Cardiovascular Effects

Various studies indicated quercetin and citrus bioflavonoids beneficially affected cardiovascular risk factors. In animal studies, increases in high-density lipoprotein and decreases in low-density lipoprotein cholesterol, total lipid, and triglyceride plasma levels in normolipidemic rats and in rats with diet and induced hyperlipidemia were observed.[38] Animal studies also showed antihypertensive effects in spontaneously hypertensive rats and normotensive rats.[39] EMIQ was even more effective based on animal studies that evaluated the antihypertensive effects and antiatherogenic effects in spontaneously hypertensive rats.[40,41]

A variety of human studies substantiated some of these effects and many more. Hesperidin and quercetin both improved endothelial function.[42,43] In particular, quercetin at a dosage of 200 mg/day augmented nitric oxide status and reduced endothelin-1 concentrations to improve endothelial function. In a pilot study with quercetin-4′-O-β-D-glucoside, which, like EMIQ, is a prodrug for quercetin, subjects given either 50 or 300 mg had significant inhibition of platelet aggregation at 30 and 120 minutes after ingestion.[44]

In one study, the effect of quercetin supplementation on blood pressure, lipid metabolism, markers of oxidative stress, inflammation, and body composition was studied in an at-risk population of 93 overweight-obese volunteers (aged 25–65 years of age) with metabolic syndrome traits in relation to apolipoprotein-E (Apo-E) genotype.[45,46] Participants were randomized to receive 150 mg/day quercetin in a double-blinded, placebo-controlled, crossover trial with 6-week treatment periods separated by a 5-week washout period. Participants were classified into the following three Apo-E phenotypes: Apo-E2 ($n = 3$), Apo-E3 ($n = 60$), and Apo-E4 ($n = 26$). Data were analyzed for the Apo-E3 and Apo-E4 subgroups. Quercetin decreased systolic blood pressure by 3.4 mm Hg in the Apo-E3 group, whereas no significant effect was observed in the Apo-E4 group. Quercetin decreased serum high-density lipoprotein cholesterol (i.e., from 52–47 mg/dL), resulting in a slightly increased ratio of low-density lipoprotein to high-density lipoprotein cholesterol in the Apo-E4 subgroup, whereas the Apo-E3 subgroup had no significant changes in these variables. Quercetin significantly decreased plasma oxidized low-density lipoprotein and tumor necrosis factor-α in the Apo-E3 and Apo-E4 groups, whereas no significant intergroup differences were found. Serum C-reactive protein and nutritional status (body weight, waist circumference, fat mass, fat-free mass) were unaffected compared with placebo. In conclusion, quercetin exhibited blood pressure–lowering effects in overweight-obese carriers of the Apo ε3/ε3 genotype but not in carriers of the ε4 allele. Although the degree of blood pressure reduction was not clinically meaningful in this study, in another randomized, double-blind, placebo-controlled, crossover study, 730 mg/day of quercetin for 28 days was shown to lead to meaningful reductions in systolic (−7 mm Hg), diastolic (−5 mm Hg), and mean arterial pressures (−5 mm Hg) in stage 1 hypertensive patients.[47]

The exact mechanism for quercetin is not known, although one study showed that it is not related to inhibiting the activity of angiotensin-converting enzyme or endothelin-1 nitric oxide production.[48] Quercetin is definitely cardioprotective; at a dosage of 120 mg per day, it was shown to reduce the total time and number of episodes of ST-segment depression and the number of premature ventricular contractions (PVCs) as demonstrated with 24 hour Holter electrocardiogram (ECG) monitoring.[49]

Gout

Quercetin may be helpful in gout through inhibition of xanthine oxidoreductase, the final step in intracellular uric acid production. In a double-blind, placebo-controlled, crossover trial, 22 healthy males (19–60 years) with baseline plasma uric acid concentration in the higher, but still considered healthy, range (339 [SD 51] μmol/l) were given either a tablet containing 500 mg of quercetin or a placebo daily for 4 weeks, with a 4-week washout period between crossover treatment. After quercetin treatment, plasma uric acid concentrations were significantly lowered by –26·5 μmol/l. This amount of 500 mg of quercetin contains the same bioavailable amount of quercetin as present in approximately 100 g of red onions.[50]

Chronic Venous Insufficiency, Capillary Fragility, Excessive Bruisability, and Hemorrhoids

A deficiency of hesperidin in the diet was linked with abnormal capillary leakiness, and pain in the extremities, causing aches, weakness, and night leg cramps.[51] Early studies demonstrated rutin to be effective in reducing capillary fragility, easy bruising, swelling and bruising after sports injuries, and nosebleeds.[52–54] More recent and much more extensive studies were performed with HERs. Positive double-blind clinical studies exist in the treatment of venous insufficiency, including varicose veins, hemorrhoids, diabetic vascular disease, and diabetic retinopathy.[55–67]

In double-blind studies of patients with chronic venous insufficiency, HER improved microvascular blood flow and clinical symptoms (pain, tired legs, night cramps, and restless legs) in 73% to 100% of patients.[55–66] Several studies were conducted on pregnant women, in whom HER was shown to be of great benefit in improving venous function and in helping relieve hemorrhoidal signs and symptoms. In one study, 90% of the women given HER (1000 mg/day for 4 weeks) had improved symptoms compared with only 12% in the placebo group.[66] Similar results in hemorrhoids not associated with pregnancy were also reported.[65]

In a very practical study, the effects of HER at a dose of 1 g/day on the prevention and control of flight microangiopathy and edema were studied in subjects with varicose veins and moderate chronic venous insufficiency who flew for more than 11 hours.[64] Measurements of skin laser Doppler resting flux venoarteriolar response, ankle swelling, and edema were made within 12 hours before and within 3 hours after the flights. The resulting edema after the flights was evaluated with a composite edema score (analog scale line). A group of 20 subjects was treated with HER (1 g/day, starting 2 days before the flight and 1 g for every 12 hours on day of travel). Another group of 18 subjects formed the control group. The length of the flights was between 11 and 13 hours; all seats were in coach class. Fifty patients were enrolled, and 38 patients were evaluable at the end of the trial. The results showed clearly that HER was useful for reducing the level of microangiopathy and the increased capillary filtration and in controlling edema in patients with venous disease during long flights. The higher level of flux and venoarteriolar response and the reduction in edema indicated a positive effect of HER on microcirculation.

There are a number of natural therapeutic agents to choose from in chronic venous insufficiency. In a double-blind study, the effects of HER were compared with micronized diosmin plus hesperidin (D+H). A first group of 90 patients with severe venous hypertension was randomized to treatment with HER or D+H. The HER group received oral HER (2 g/day); the D+H group received three 500-mg tablets daily every 8 hours for 8 weeks. A second group of 122 comparable patients was included in a registry following the same study format. The results show that HER was more effective in relieving signs and symptoms of chronic venous insufficiency and improving the quality-of-life scale in this study.[63]

Diabetes

Flavonoids appear to be important in the long-term care of diabetes for many reasons. One of the hallmark features of diabetes is a significant disturbance in blood flow through the small blood vessels. HERs appear to improve blood flow in diabetics significantly and can be useful in the treatment of diabetic microvascular disease and retinopathy. Flavonoids can also stimulate an otherwise weak insulin effect in several ways. For example, they can influence the protein phosphokinases, which modulate second-messenger pathways known to upregulate the gene encoding the glucose transporters.[1] PCOs or bilberry extracts may be better than quercetin, citrus flavonoids, and HER in diabetics.

Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) is associated with low levels of the adipose-derived hormone adiponectin, even in the absence of adiposity. Because quercetin may reduce serum glucose, insulin, triglycerides, and cholesterol levels and increases the expression and secretion of adiponectin, researchers conducted a double-blind study in 84 women with PCOS. The treatment group received 1 g of quercetin (two 500-mg capsules) daily for 12 weeks, and the control group received a placebo. Quercetin slightly increased the level of total adiponectin by 5.56% and high-molecular-weight adiponectin by 3.9% compared with placebo. It also reduced the level of testosterone (0.71 ng/dL in quercetin vs. 0.77 ng/dL in placebo) and luteinizing hormone (LH; 8.42 IU/l in quercetin vs. 8.68 IU/l in placebo). HOMA-IR levels were also significantly lower in the quercetin (1.84) group compared with the placebo group (2.21). These results indicate that quercetin may have some benefit in the treatment of PCOS.[68]

Thalassemias

Although it may be difficult to treat the long-term outcomes of thalassemia conditions, it is possible that quercetin may serve to protect abnormally sensitive lymphocytes from common dietary substances. An in vitro study evaluated the effect of food mutagens on the lymphocytes from three different types of thalassemia patients: β-thalassemia major, β-thalassemia/Hb E, and a β-thalassemia trait with a 3.7-kb deletion. When the mutagen exposure was combined with quercetin, reduced sensitivities among the various thalassemic genotypes were observed in a dose-dependent manner.[69] Although human studies are necessary to confirm this beneficial effect, quercetin might be worth a try to attenuate the toxic mutagenic effects commonly found in these susceptible patients.

COMMERCIALLY AVAILABLE FORMS

Quercetin

Quercetin is available alone in powder and capsule form. However, if quercetin is being used for its anti-inflammatory properties, products that combine it with the pineapple enzyme bromelain may provide additional benefit. Bromelain (see Chapter 71) exerts antiallergy and anti-inflammatory activity on its own and may also enhance the absorption of quercetin. Combination preparations of protein-digesting enzymes, like bromelain, and flavonoids have been shown to potentiate each other's anti-inflammatory activity.[70]

EMIQ is a new form of quercetin that showed greater absorption in both human and animal studies. It is prepared by enzymatic deglycosylation and the subsequent α-oligoglucosylation of quercetin-3-O-rutinoside (rutin). The plasma level of quercetin conjugates is instantly increased by oral intake of EMIQ.[71,72] In a rat study, the absorption of EMIQ was compared with quercetin, quercetin-3-O-rutinoside (rutin), quercetin-3-O-glucoside (isoquercitrin [IQC]), quercetin-3-O-maltoside (Q3M), quercetin-3-O-gentiobioside (Q3G), α-monoglucosyl rutin (α-MR), and α-oligoglucosyl rutin (α-OR). Bioavailability (F value) was calculated from the concentrations of total quercetin in plasma from 0 to 12 hours after administration. The F value of quercetin was 2.0%, and those of IQC, Q3M, and EMIQ were 12%, 30%, and 35%, respectively. Quercetin Phytosome® (phosphatidylcholine-bound quercetin) has shown even greater bioavailability.[73] On a weight-equivalence basis, 500 mg of Quercetin Phytosome®, containing 200 mg of quercetin, versus 500 mg of quercetin produced blood levels 20 times greater than the quercetin.

Citrus Bioflavonoids

Mixed preparations of citrus bioflavonoids are the most widely used and least expensive flavonoid sources. However, mixed citrus flavonoids are the least active and generally the least quantified source of flavonoids because most commercially available sources of mixed citrus flavonoids only contain 50% flavonoids. Preparations containing pure rutin and hesperidin or those that clearly state the levels of rutin and hesperidin are superior to products that do not quantify the amount of the individual flavonoid components. HERs are probably the better choice when opting for the benefits in this class of flavonoids.

Dosages

The usual recommended dosage for quercetin is 500 mg two times daily. If quercetin is being used for its anti-inflammatory properties and bromelain supplementation is also indicated, administration with bromelain may enhance absorption. Combination preparations of proteolytic enzymes and flavonoids have been shown to have significant anti-inflammatory activity in experimental studies.[70] If used with bromelain, the amount of bromelain (1800 milk clotting units [mcu]) should be equal to the amount of quercetin.

Dosages used for HERs in double-blind clinical studies for the treatment of venous insufficiency and hemorrhoids ranged from 1000 to 3000 mg/day. This translates to a dosage of citrus bioflavonoids, rutin, and hesperidin of 3000 to 6000 mg/day.

Toxicity

Quercetin appears to be well tolerated in humans. A Phase I dose-escalation study was performed to evaluate the safety of quercetin in 30 untreated patients with chronic hepatitis C virus infection. Quercetin exhibited safety (up to 5 g daily), and there was a potential for antiviral activity in some hepatitis C patients. Quercetin also produced a "clinically meaningful" 0.41-log viral load decrease in eight of the subjects.[74]

Carcinogenic and teratogenic studies in rats and rabbits showed that quercetin does not have apparent side effects, even when consumed in large quantities (2000 mg/kg body weight and 5%–10% of total diet) for long periods of time (up to 2 years).[75–81] In addition, quercetin administration (up to 2000 mg/kg body weight) to pregnant rats had no teratogenic effects.[80] The weight of the available evidence supports the safety of quercetin.[82] As is true of any other compound, allergic reactions may occur. Although uncommon, if they occur, discontinue use. EMIQ has the status of generally recognized as safe in the United States.

Citrus bioflavonoids, rutin, hesperidin, and HER appear to be extremely safe and without side effects even during pregnancy.

Drug Interactions

Rutin, hesperidin, and HER do not appear to interact with any drug. Quercetin was shown to elevate the plasma concentrations of fexofenadine in healthy subjects, probably via the phosphoglycoprotein (Pgp)-mediated efflux in humans.[82] The Pgp enhances the uptake from the intestine of many drugs, including vinblastine, cyclosporine, digoxin, fexofenadine, and losartan, and can also increase the

oral bioavailability of drugs like nifedipine, felodipine, verapamil, and terfenadine, and inhibit the breakdown of various drugs, particularly caffeine, coumarin, and estrogens. Quercetin significantly induced the activity of Pgp, and this induced effect was more obvious in MDR1 3435 TT individuals.[83]

Unlike naringin from grapefruit, quercetin does not appear to affect CYP2C8 activity and has little possibility of interacting with drugs that are metabolized by this enzyme.[84]

Citrus bioflavonoid preparations generally are not an issue. However, if derived from grapefruit, they may contain naringin and interact with many drugs. Naringin activates Pgp and suppresses the expression of the cytochrome *P450 3A4* gene that can enhance oxidative decomposition of some drugs, including drugs used frequently in the therapy of cancer, human immunodeficiency virus, immune disorders, hypertension, and other serious conditions.[85] Avoidance of grapefruit juice and flavonoid preparations containing naringin is recommended where drug interactions are likely.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Havsteen BH. The biochemistry and medical significance of the flavonoids. *Pharmacol Ther*. 2002;96:67–202.
2. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev*. 2000;52:673–751.
3. Rice-Evans C. Flavonoid antioxidants. *Curr Med Chem*. 2001;8:797–807.
4. Youdim KA, Dobbie MS, Kuhnle G, et al. Interaction between flavonoids and the blood-brain barrier: in vitro studies. *J Neurochem*. 2003;85:180–192.
5. Hertog MG, Feskens EJ, Hollman PC, et al. Dietary antioxidant flavonoids and risk of coronary heart disease: the zutphen elderly study. *Lancet*. 1993;342:1007–1011.
6. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. *Curr Opin Clin Nutr Metab Care*. 2008;11:733–740.
7. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol*. 2008;585:325–337.
8. Middleton Jr E, Drzewieki G. Naturally occurring flavonoids and human basophil histamine release. *Int Arch Allergy Appl Immunol*. 1985;77:155–157.
9. Amella M, Bronner C, Briancon F, et al. Inhibition of mast cell histamine release by flavonoids and bioflavonoids. *Planta Med*. 1985;51:16–20.
10. Busse WW, Kopp DE, Middleton E. Flavonoid modulation of human neutrophil function. *J Allergy Clin Immunol*. 1984;73:801–809.
11. Chaudhry PS, Cabrera J, Juliani HR, et al. Inhibition of human lens aldose reductase by flavonoids, sulindac and indomethacin. *Biochem Pharmacol*. 1983;32:1995–1998.
12. Varma SD, Mizuno A, Kinoshita JH. Diabetic cataracts and flavonoids. *Science*. 1977;195:205–206.
13. Kaul TN, Middleton E, Ogra PL. Antiviral effects of flavonoids on human viruses. *J Med Virol*. 1985;15:71–79.
14. Guttner J, Veckenstedt A, Heinecke H, et al. Effect of quercetin on the course of mengo virus infection in immunodeficient and normal mice. A histologic study. *Acta Virol*. 1982;26:148–155.
15. Hirpara KV, Aggarwal P, Mukherjee AJ, et al. Quercetin and its derivatives: synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. *Anticancer Agents Med Chem*. 2009;9:138–161.
16. Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. *Cancer Lett*. 2008;269:315–325.
17. Chen C, Zhou J, Ji C. Quercetin: a potential drug to reverse multidrug resistance. *Life Sci*. 2010;87:333–338.
18. Lee NK, Choi SH, Park SH, et al. Antiallergic activity of hesperidin is activated by intestinal microflora. *Pharmacology*. 2004;71:174–180.
19. Chiba H, Uehara M, Wu J, et al. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. *J Nutr*. 2003;133:1892–1897.
20. Petrakis PL, Kallianos AG, Wender SH, et al. Metabolic studies of quercetin labeled with C14. *Arch Biochem Biophys*. 1959;85:264–271.
21. Gugler R, Leshik M, Dengler HJ. Disposition of quercetin in man after single oral and intravenous doses. *Eur J Clin Pharmacol*. 1975;9:229–234.
22. Hollman PC, de Vries JH, van Leeuwen SD, et al. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am J Clin Nutr*. 1995;62:1276–1282.
23. Egert S, Wolffram S, Bosy-Westphal A, et al. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *J Nutr*. 2008;138:1615–1621.
24. Jin F, Nieman DC, Shanely RA, et al. The variable plasma quercetin response to 12-week quercetin supplementation in humans. *Eur J Clin Nutr*. 2010;64:692–697.
25. Cialdella-Kam L, Nieman DC, Sha W, et al. Dose-response to 3 months of quercetin-containing supplements on metabolite and quercetin conjugate profile in adults. *Br J Nutr*. 2013;109(11):1923–1933.
26. Guo Y, Mah E, Davis CG, et al. Dietary fat increases quercetin bioavailability in overweight adults. *Mol Nutr Food Res*. 2013;57(5):896–905.
27. Kawai M, Hirano T, Arimitsu J, et al. Effect of enzymatically modified isoquercitrin, a flavonoid, on symptoms of Japanese cedar pollinosis: a randomized double-blind placebo-controlled trial. *Int Arch Allergy Immunol*. 2009;149:359–368.
28. Hirano T, Kawai M, Arimitsu J, et al. Preventative effect of a flavonoid, enzymatically modified isoquercitrin on ocular symptoms of Japanese cedar pollinosis. *Allergol Int*. 2009;58:373–382.
29. Hamdy AA, Ibrahem MA. Management of aphthous ulceration with topical quercetin: a randomized clinical trial. *J Contemp Dent Pract*. 2010;11:E009–E016.
30. Davis JM, Carlstedt CJ, Chen S, et al. The dietary flavonoid quercetin increases VO(2max) and endurance capacity. *Int J Sport Nutr Exerc Metab*. 2010;20:56–62.
31. Ganio MS, Armstrong LE, Johnson EC, et al. Effect of quercetin supplementation on maximal oxygen uptake in men and women. *J Sports Sci*. 2010;28:201–208.
32. Nieman DC, Williams AS, Shanely RA, et al. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. *Med Sci Sports Exerc*. 2010;42:338–345.
33. Bigelman KA, Fan EH, Chapman DP, et al. Effects of six weeks of quercetin supplementation on physical performance in ROTC cadets. *Mil Med*. 2010;175:791–798.
34. Nieman DC, Henson DA, Gross SJ, et al. Quercetin reduces illness but not immune perturbations after intensive exercise. *Med Sci Sports Exerc*. 2007;39:1561–1569.
35. Heinz SA, Henson DA, Austin MD, et al. Quercetin supplementation and upper respiratory tract infection: a randomized community clinical trial. *Pharmacol Res*. 2010;62:237–242.
36. Sharp MA, Hendrickson NR, Staab JS, et al. Effects of short-term quercetin supplementation on soldier performance. *J Strength Cond Res*. 2012;26(suppl 2):S53–S60.
37. Riva A, Vitale JA, Belcaro G, et al. Quercetin Phytosome® in triathlon athletes: a pilot registry study. *Minerva Med*. 2018;109(4):285–289.
38. Monforte MT, Trovato A, Kirjavainen S, et al. Biological effects of hesperidin, a citrus flavonoid. (Note II): hypolipidemic activity on experimental hypercholesterolemia in rat. *Farmaco*. 1995;50:595–599.
39. Ohtsuki K, Abe A, Mitsuzuwi H, et al. Effects of long-term administration of hesperidin and glucosyl hesperidin to spontaneously hypertensive rats. *J Nutr Sci Vitaminol (Tokyo)*. 2002;48:420–422.
40. Emura K, Yokomizo A, Toyoshi T, et al. Effect of enzymatically modified isoquercitrin in spontaneously hypertensive rats. *J Nutr Sci Vitaminol (Tokyo)*. 2007;53:68–74.
41. Motoyama K, Koyama H, Moriwaki M, et al. Atheroprotective and plaque-stabilizing effects of enzymatically modified isoquercitrin in atherogenic apoE-deficient mice. *Nutrition*. 2009;25:421–427.
42. Morand C, Dubray C, Milenkovic D, et al. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. *Am J Clin Nutr*. 2011;93:73–80.
43. Loke WM, Hodgson JM, Proudfoot JM, et al. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr*. 2008;88:1018–1025.
44. Hubbard GP, Wolffram S, Lovegrove JA, et al. Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J Thromb Haemost*. 2004;2:2138–2145.
45. Egert S, Boesch-Saadatmandi C, Wolffram S, et al. Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. *J Nutr*. 2010;140:278–284.
46. Egert S, Bosy-Westphal A, Seiberl J, et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. *Br J Nutr*. 2009;102:1065–1074.
47. Edwards RL, Lyon T, Litwin SE. Quercetin reduces blood pressure in hypertensive subjects. *J Nutr*. 2007;137:2405–2411.
48. Larson A1, Witman MA, Guo Y, et al. Acute, quercetin-induced reductions in blood pressure in hypertensive individuals are not secondary to lower

plasma angiotensin-converting enzyme activity or endothelin-1: nitric oxide. *Nutr Res*. 2012;32(8):557–564.
49. Chekalina NI, Shut SV, Trybrat TA, et al. Effect of quercetin on parameters of central hemodynamics and myocardial ischemia in patients with stable coronary heart disease. *Wiad Lek*. 2017;70(4):707–711.
50. Shi Y, Williamson G. Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomised, double-blinded, placebo-controlled, cross-over trial. *Br J Nutr*. 2016;115(5):800–806.
51. Garg A, Garg S, Zaneveld LJ, et al. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother Res*. 2001;15:655–669.
52. Lagrue G, Behar A, Maurel A. Edematous syndromes caused by capillary hyperpermeability. *Diffuse angioedema. J Mal Vasc*. 1989;14:231–235. [French].
53. Horoschak A. Nocturnal leg cramps, easy bruisability and epistaxis in menopausal patients: treated with hesperidin and ascorbic acid. *Del Med J*. 1959;31:19–22.
54. Cragin RB. The use of bioflavonoids in the prevention and treatment of athletic injuries. *Med Times*. 1962;90:529–532.
55. Wadworth AN, Faulds D. Hydroxyethylrutosides. A review of its pharmacology, and therapeutic efficacy in venous insufficiency and related disorders. *Drugs*. 1992;44:1013–1032.
56. Poynard T, Valterio C. Meta-analysis of hydroxyethylrutosides in the treatment of chronic venous insufficiency. *Vasa*. 1994;23:244–250.
57. Boisseau MR, Taccoen A, Garreau C, et al. Fibrinolysis and hemorheology in chronic venous insufficiency: a double blind study of troxerutin efficiency. *J Cardiovasc Surg (Torino)*. 1995;36:369–374.
58. Neumann HA, van den Broek MJ. A comparative clinical trial of graduated compression stockings and O-(beta-hydroxyethyl)-rutosides (HR) in the treatment of patients with chronic venous insufficiency. *Z Lymphol*. 1995;19:8–11.
59. Renton S, Leon M, Belcaro G, et al. The effect of hydroxyethylrutosides on capillary filtration in moderate venous hypertension: a double blind study. *Int Angiol*. 1994;13:259–262.
60. MacLennan WJ, Wilson J, Rattenhuber V, et al. Hydroxyethylrutosides in elderly patients with chronic venous insufficiency: its efficacy and tolerability. *Gerontology*. 1994;40:45–52.
61. Cesarone MR, Belcaro G, Pellegrini L, et al. HR, 0-(beta-hydroxyethyl)-rutosides; (Venoruton): rapid relief of signs/symptoms in chronic venous insufficiency and microangiopathy: a prospective, controlled study. *Angiology*. 2005;56:165–172.
62. Cesarone MR, Belcaro G, Ricci A, et al. Prevention of edema and flight microangiopathy with Venoruton (HR), (0-[beta-hydroxyethyl]-rutosides) in patients with varicose veins. *Angiology*. 2005;56:289–293.
63. Cesarone MR, Belcaro G, Pellegrini L, et al. Venoruton vs Daflon: evaluation of effects on quality of life in chronic venous insufficiency. *Angiology*. 2006;57:131–138.
64. Bergstein NA. Clinical study on the efficacy of O-(beta-hydroxyethyl)rutoside (HR) in varicosis of pregnancy. *J Int Med Res*. 1975;3:189–193.
65. Annoni F, Boccasanta P, Chiurazzi D, et al. Treatment of acute symptoms of hemorrhoid disease with high dose oral O-(beta-hydroxyethyl)-rutosides. *Minerva Med*. 1986;77:1663–1668. [Italian].
66. Wijayanegara H, Mose JC, Achmad L, et al. A clinical trial of hydroxyethylrutosides in the treatment of haemorrhoids of pregnancy. *J Int Med Res*. 1992;20:54–60.
67. Yildiz CE, Conkbayir C, Huseynov E, et al. The efficiency of O-(beta-hydroxyethyl)-rutosides in reducing the incidence of superficial venous insufficiency in patients with calf muscle pump dysfunction. *Phlebology*. 2017;32(3):179–184.
68. Rezvan N, Moini A, Janani L, et al. Effects of quercetin on adiponectin-mediated insulin sensitivity in polycystic ovary syndrome: a randomized placebo-controlled double-blind clinical trial. *Horm Metab Res*. 2017;49(2):115–121.
69. Ruf AA, Webb J, Anderson D. Modulation by flavonoids of the effects of a food mutagen in different thalassaemia genotypes in the Comet assay. *Teratog Carcinog Mutagen*. 2003;23(suppl 2):93–102.
70. Tarayre JP, Lauressergues H. Advantages of a combination of proteolytic enzymes, flavonoids and ascorbic acid in comparison with non-steroid anti-inflammatory agents. *Arzneimittelforschung*. 1977;27:1144–1149.
71. Murota K, Matsuda N, Kashino Y, et al. Alpha-oligoglucosylation of a sugar moiety enhances the bioavailability of quercetin glucosides in humans. *Arch Biochem Biophys*. 2010;501:91–97.
72. Makino T, Shimizu R, Kanemaru M, et al. Enzymatically modified isoquercitrin, alpha-oligoglucosyl quercetin 3-O-glucoside, is absorbed more easily than other quercetin glycosides or aglycone after oral administration in rats. *Biol Pharm Bull*. 2009;32:2034–2040.
73. Quercetin Phytosome Absorption Study to come.
74. Lu NT, Crespi CM, Liu NM, et al. A phase I dose escalation study demonstrates quercetin safety and explores potential for bioflavonoid antivirals in patients with chronic hepatitis C. *Phytother Res*. 2016;30(1):160–168.
75. Stoewsand GS, Anderson JL, Boyd JN, et al. Quercetin: a mutagen, not a carcinogen, in Fischer rats. *J Toxicol Environ Health*. 1984;14:105–114.
76. Hirono I, Ueno H, Hosaka S, et al. Carcinogenicity examination of quercetin and rutin in ACI rats. *Cancer Lett*. 1981;13:15–21.
77. Kato K, Mori H, Fujii M, et al. Lack of promotive effect of quercetin on methylazoxymethanol acetate carcinogenesis in rats. *J Toxicol Sci*. 1984;9:319–325.
78. Kato K, Mori H, Tanaka T, et al. Absence of initiating activity by quercetin in the rat liver. *Ecotoxicol Environ Saf*. 1985;10:63–69.
79. Hosaka S, Hirono I. Carcinogenicity test of quercetin by pulmonary-adenoma bioassay in strain A mice. *Gann*. 1981;72:327–328.
80. Hirose M, Fukushima S, Sakata T, et al. Effect of quercetin on two-stage carcinogenesis of the rat urinary bladder. *Cancer Lett*. 1983;21:23–27.
81. Willhite CC. Teratogenic potential of quercetin in the rat. *Food Chem Toxicol*. 1982;20:75–79.
82. Kim KA, Park PW, Park JY. Short-term effect of quercetin on the pharmacokinetics of fexofenadine, a substrate of P-glycoprotein, in healthy volunteers. *Eur J Clin Pharmacol*. 2009;65:609–614.
83. Wang SY, Duan KM, Li Y. Effect of quercetin on P-glycoprotein transport ability in Chinese healthy subjects. *Eur J Clin Nutr*. 2013;67(4):390–394.
84. Kim KA, Park PW, Kim HK, et al. Effect of quercetin on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in healthy subjects. *J Clin Pharmacol*. 2005;45:941–946.
85. Fuhr U, Kummert AL. The fate of naringin in humans: a key to grapefruit juice-drug interactions? *Clin Pharmacol Ther*. 1995;58:365–373.

82

Ginkgo biloba (Ginkgo Tree)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Ginkgo biloba (family: *Ginkgoaceae*)
Common names: ginkgo tree, maidenhair tree

GENERAL DESCRIPTION

Ginkgo biloba is a deciduous tree that lives up to 1000 years old and grows to a height of 100 to 122 ft and a diameter of 3 to 4 ft (Fig. 82.1). Ginkgo has short horizontal branches with short shoots bearing fan-shaped leaves that measure 2 to 4 inches across. Because the leaf resembles a maidenhair fern, the ginkgo tree has been called "maidenhair tree." Ginkgo bears an inedible foul-smelling fruit and an edible ivory-colored inner seed that is sold in marketplaces in the Orient. Extracts from the leaves of the ginkgo tree are used medicinally.

CHEMICAL COMPOSITION

The active components of ginkgo leaves are the ginkgo-flavone glycosides or ginkgo heterosides (flavonoid molecules with sugars attached, which are unique to the ginkgo), several terpene molecules unique to ginkgo (ginkgolides and bilobalide), and organic acids.

Ginkgo biloba extract (GBE) is often standardized to contain 24% flavone glycosides because these molecules represent a convenient analytic reference group. Although they play a major role in the pharmacological activity of GBE, other components are also important. The three major backbone flavonoids of the *G. biloba* flavonols are quercetin, kaempferol, and isorhamnetin. The glucoside components are glucose and rhamnose, which are present as single sugars or disaccharides.

Other significant flavonoid components of the extract include proanthocyanidins, largely composed of dimers and oligomers of delphinoidine and cyanidine. The major terpene molecules of GBE, which account for 6% of the extract, are the ginkgolides and bilobalide

Fig. 82.1 *Ginkgo biloba.*

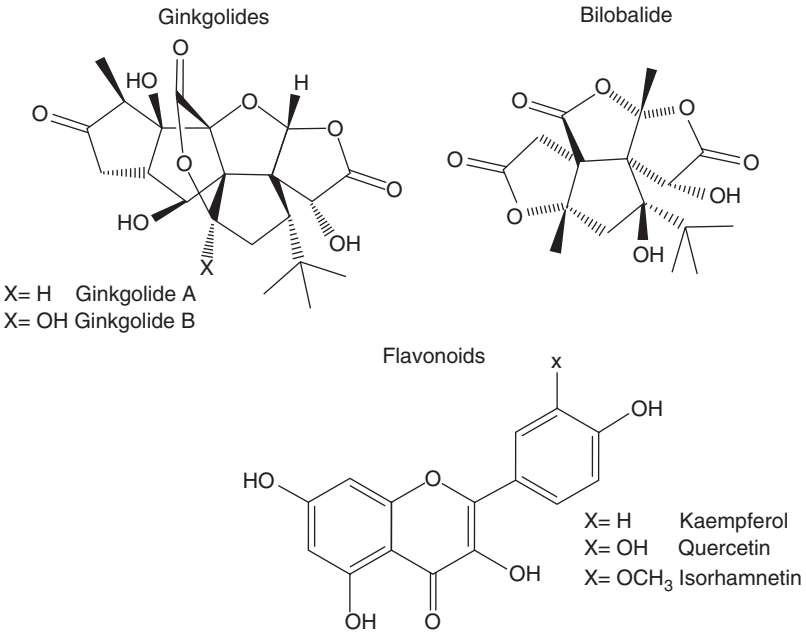


Fig. 82.2 Flavonoids and terpenes of *Ginkgo biloba*.

(Fig. 82.2). These substances are unique to ginkgo and are not found in any other plants. Other constituents of GBE include several organic acids. These compounds contribute valuable properties to the extract by making the usually water-insoluble flavonoid and terpene molecules of ginkgo water soluble.

HISTORY AND FOLK USE

G. biloba is the world's oldest living tree species. The sole surviving species of the family *Ginkgoaceae,* the ginkgo tree can be traced back more than 200 million years to the fossils of the Permian period, and for this reason, it is often referred to as the "living fossil."

Once common in North America and Europe, the ginkgo was almost destroyed during the Ice Age in all regions of the world except China, where it has long been cultivated as a sacred tree.

In the late 17th century, Engelbert Kaempfer, a German physician and botanist, became the first European to discover and catalog the ginkgo tree. The flavonoid kaempferol is named after Kaempfer. In 1771 Linnaeus named the tree *G. biloba.*

The ginkgo tree was brought to America in 1784 to the garden of William Hamilton near Philadelphia. The ginkgo tree is now planted throughout much of the United States as an ornamental tree. The ginkgo tree, which is the tree most resistant to insects, disease, and pollution, is frequently planted along streets in cities. The first green growth to reappear at the center of Hiroshima in 1946 was the sprout of a ginkgo tree that grew to be a normal, full-size tree.

G. biloba's medicinal use can be traced back to the oldest Chinese materia medica (2800 BC). Ginkgo leaves have been used in traditional Chinese medicine for their ability to "benefit the brain," relieve the symptoms of asthma and coughs, and help the body eliminate filaria.

In the late 1990s, ginkgo leaf extracts were among the leading prescription medicines in both Germany and France, where they accounted for 1% and 1.5%, respectively, of total prescription sales. In America, it was the top-selling botanical medicine in 1999, with $148 million in sales.[1] Ginkgo's popularity has waned a bit in the 21st century, but it remains among the top-selling natural products in Europe and North America.

PHARMACOLOGY

The GBE extract standardized to contain 24% ginkgo flavone glycosides has demonstrated remarkable diverse pharmacological effects. Interestingly, the total extract is more active than single isolated components. This suggests synergism between the various components of GBE, an explanation that is well supported in more than 2000 clinical and experimental studies using the extract.

General Effects

GBE exerts profound, widespread tissue effects, including membrane-stabilizing, antioxidant, and free-radical–scavenging effects. GBE also enhances the use of oxygen and glucose.[2,3,4]

Red blood cells provide excellent models for evaluating the effects of substances on membrane functions. Red blood cell studies using GBE demonstrated that in addition to directly stabilizing membrane structures and scavenging free radicals, GBE also enhanced membrane transport of potassium into the cell and sodium out of the cell by activating the sodium pump. In essence, GBE leads to better membrane polarization. This effect is particularly important in excitable tissues such as nerve cells.[2,3] GBE prevents metabolic and neuronal disturbances in experimental models of cerebral ischemia.[2,3,5–8] It accomplishes this by enhancing oxygen use and increasing cellular uptake of glucose, thus restoring energy production.

GBE also helps reestablish effective tissue perfusion during hypoxia. Particularly interesting is GBE's ability to normalize the circulation in areas most affected by microembolization, namely, the hippocampus and striatum. GBE promotes an increased nerve transmission rate, improves synthesis and turnover of brain neurotransmitters, normalizes acetylcholine receptors in the hippocampus (the area of the brain most affected by Alzheimer's disease), and inhibits β-amyloid deposition.[9]

Although the sample size was small, a study was conducted to evaluate the effects of a 6-week supplementation with 160 mg/day of a standardized extract of *G. biloba* or a matching placebo on aerobic performance, blood antioxidant capacity, and the level of brain-derived neurotrophic factor (BDNF) in healthy, physically active young men,

TABLE 82.1 Cellular and Membrane Mechanisms of the Vasoregulatory Effects of *Ginkgo biloba* Extract

Site of action	Upstream flow (arteriole)			Exchange area (capillary)			Downstream flow (venous)	
Tissue pathological condition	Arterial spasm	Arterial thrombosis	Vascular atony	Hypoxic vasoparalysis	Capillary hyperpermeability and plasma	Debilitated capillary walls	Venous relaxation and vasoparalysis extravasation	Venular spasms
Effects found with *Ginkgo biloba* extract	Vasorelaxation	Diminished platelet hyperaggregability	Restitution of arterial tone	Diminished accumulation of toxic wastes: K^+, CO_2, and lactate	Amelioration of capillary hyperpermeability	Increased capillary resistance	Venous tonic effect	Antagonism of experimental venous spasms
Cellular and membrane mechanisms explaining *G. biloba*'s effects	Release of EDRF from endothelium	Release of PGI_2	Direct effect	Consequence of venous tonic effect	Direct membrane effect	Direct membrane effect	Direct effect	Inhibition of PDE (?)
	Inhibition of PDE	Direct membrane effect	Potentiation of α-adrenergic effects[a]	Mitochondrial metabolic restart			Indirect effect via α-adrenergic effects	Release of dilating prostaglandins
	Potentiation of α-adrenergic effects[a]							

CO_2, Carbon dioxide; *EDRF,* endothelium-derived relaxing factor; *K,* potassium; *PDE,* phosphodiesterase; *PGI_2,* prostaglandin I_2.
[a]Partly through catechol-o-methyl transferase inhibition.

randomly allocated to two groups (n = 9 each). At baseline, and on the day after the treatment, the participants performed an incremental cycling test for the assessment of maximal oxygen uptake. Results showed that six weeks' supplementation with *G. biloba* extract in physically active young men may provide some marginal improvements in their endurance performance, expressed as VO_2max and blood antioxidant capacity, and elicit somewhat better neuroprotection through increased exercise-induced production of BDNF.[10]

Vascular Effects

The mechanisms of GBE's vascular effects were investigated using a number of in vivo and in vitro techniques (Table 82.1). Isolated vessel techniques allowed for the separation of GBE's effects on different parts of the vascular system (e.g., arterial, arteriolar, microcirculatory, venular, and venous components), whereas in vivo studies provided information on the total circulatory phenomena (i.e., GBE's ability to increase the perfusion rate to various regions).

In general, GBE exerts its vascular effects primarily through its effects on the lining of the blood vessels (vascular endothelium) and the system that regulates blood vessel tone. Its vasodilating action is explained by direct stimulation of the release of endothelium-derived relaxing factor and prostacyclin (a beneficial prostaglandin). In addition, GBE inhibits 3′,5′-cyclic guanosine monophosphate phosphodiesterase, an enzyme that results in relaxation of blood vessels.[2,11]

On the venous system, GBE stimulates greater tone, thus aiding the dynamic clearing of toxic metabolites that accumulate during ischemia (Fig. 82.3).[2,11]

GBE normalizes circulation by producing tonic effects. These effects are much more apparent in an ischemic vascular area than in a normally perfused area. However, despite intense investigation, many of GBE's tonic effects on vascular components are still largely unexplained. GBE can simultaneously combat the phenomena resulting from vascular spasm and, with the same efficiency, restore circulation to areas subject to vasomotor paralysis.

The importance of this dual action is becoming more apparent in cerebral insufficiency because single-direction drugs (i.e., vasodilators) can often aggravate the condition by preferentially dilating the healthy areas, thereby deflecting blood and oxygen away from the ischemic area.

Another prime clinical application of GBE's effects on vascular endothelium is early-stage diabetic nephropathy. In one 8-week clinical trial, in the GBE group, the brachial arterial endothelium-dependent dilation increased from 4.91% before treatment to 6.78% after treatment, whereas the level of von Willebrand factor decreased from 182% to 128.5%, and nitrous oxide increased from 50.16 to 70.65 μmol/L.[12]

GBE also protects against endothelial damage caused by oxidized low-density lipoprotein cholesterol, thereby preserving endothelial adhesive properties and disruption of ionic homeostasis.[13]

Platelet Effects

GBE and isolated ginkgolides have profound effects on platelet function, including inhibition of platelet aggregation, adhesion, and degranulation.[4] These effects appear to be a result of direct membrane and antioxidant effects, increased synthesis of prostacyclin, and antagonism of platelet-activating factor (PAF).

GBE and the ginkgolides were shown to be potent inhibitors of PAF.[4,14–16] PAF is a potent stimulator of platelet degranulation and is involved in many inflammatory and allergic processes, including neutrophil activation, increased vascular permeability, smooth muscle contraction (e.g., bronchoconstriction), and reduction in coronary blood flow. GBE and ginkgolides compete with PAF for binding sites and inhibit the various events induced by PAF.[2,3,17,18] These actions may be responsible for many of GBE's clinical effects. Interestingly,

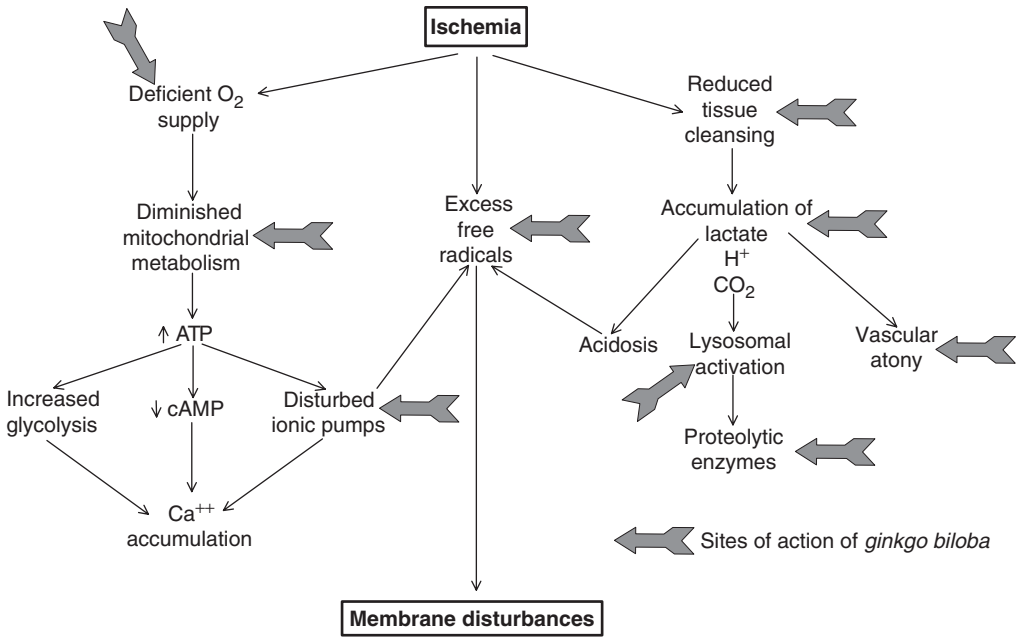


Fig. 82.3 The effect of *Ginkgo biloba* extract on ischemia.

despite these effects on PAF, GBE exerts no inhibition of platelet aggregation in humans.

ABSORPTION AND DISTRIBUTION OF *GINKGO BILOBA* EXTRACT

The pharmacokinetics (absorption, distribution, and elimination) of GBE were studied in rats using radiolabeled extracts.[2,3,4] After oral administration, at least 60% of the radiolabeled extract was absorbed. Because blood levels peaked after 1.5 hours, upper gastrointestinal tract absorption was suspected.

The flavonoids appear to have an affinity for organs rich in connective tissues, such as the aorta, eyes, skin, and lungs. Levels of radioactivity in these tissues are two to three times higher than those in blood and decrease little over time. Retained specific activity in the heart is twice that found in the skeletal muscles. Of the glands, the adrenals retain the greatest level of radioactivity.

At 72 hours, the hippocampus and striated bodies show radioactivity five times greater than that of the blood. This deposition pattern, which parallels the circulation improvement observed after ischemia caused by blood clotting in rats, is alleviated by GBE extracts. Other areas of the brain, such as the cerebral cortex, brainstem, and cerebellum, do not show such high levels of radioactivity.

Complexing GBE with phospholipids showed that the bioavailability of quercetin, kaempferol, and isorhamnetin was increased significantly, indicating these preparations might offer the best clinical response.[19] For central nervous system effect, complexing GBE with phosphatidylserine may prove superior to phosphatidylcholine (discussed in the section "Cerebral Vascular Insufficiency and Impaired Mental Performance").[20]

CLINICAL APPLICATIONS

GBE's primary clinical application has been in the treatment of vascular insufficiency. In more than 50 double-blind clinical trials, both patients with chronic cerebral arterial insufficiency and those with peripheral arterial insufficiency responded favorably to GBE. As described earlier, GBE exerts an extraordinary array of pharmacological activities that imply a broad spectrum of possible clinical applications, an implication borne out by the new applications of GBE, which are constantly being discovered.

Cerebral Vascular Insufficiency and Impaired Mental Performance

Cerebral vascular insufficiency is an extremely common condition in the elderly of developed countries because of the high prevalence of atherosclerosis. In well-designed studies, GBE displayed a statistically significant regression of the major symptoms of cerebral vascular insufficiency and impaired mental performance, including the following:

- Short-term memory loss
- Vertigo
- Headache
- Ringing in the ears
- Lack of vigilance
- Depression

Regression of these symptoms by GBE suggests that vascular insufficiency may be the major causative factor accounting for these so-called age-related cerebral disorders versus a true degenerative process.

In a comprehensive review, the quality of research on more than 40 clinical studies with GBE in the treatment of cerebral insufficiency was analyzed. The results indicated that GBE was effective in reducing all symptoms of cerebral insufficiency, including impaired mental function (senility). The quality of research was comparable to Hydergine (dihydroergotamine), a drug approved by the Food and Drug Administration (FDA) for use in the treatment of cerebral vascular insufficiency and Alzheimer's disease. Eight studies stood out as being extremely well designed and are summarized in Table 82.2.[14–17,21–23] It appears that by increasing cerebral blood flow, and therefore oxygen and glucose use, GBE offers relief from these effects of aging and may offer significant protection against their development.

G. biloba's antiaggregatory effect on platelets offers additional protection against a stroke. This was supported in a clinical study of post-stroke patients, which demonstrated that GBE improved blood flow and blood viscosity.[24] In another study using dynamic susceptibility

TABLE 82.2 Studies Demonstrating the Significant Regression of the Major Symptoms of Cerebral Vascular Insufficiency Through the Use of *Ginkgo biloba* Extract

Principal author	Year	Diagnosis	No. of patients	Age (yr)	Duration (wks)	Dosage (mg)	Type	Compared with:	Efficacy
Agnoli	1984	CCI	30	60	4	120	DB, CS	Placebo ($n = 30$)	76%
Arrigo	1984	CCI	80	40–80	7	120	DB, CS	Placebo ($n = 40$)	65%
Augustin	1976	Misc	99	77	24	120	DB	Placebo ($n = 90$)	44%
Bono	1975	CCI	14	65	5	120	DB	Placebo ($n = 14$)	65%
		CCI	40	67	5	120	Open	ED ($n = 19$)	90%
Boudouresques	1975	CCI, CVA	47	35–80	3	120	Open		80%
Choussat	1977	CCI	48	69–95	8	360	Open		60%
Dieli	1981	CCI	20	62	5	160	DB	Placebo ($n = 20$)	80%
Eckmann	1982	CVA	25	60	4	120	DB	Placebo ($n = 20$)	92%
Gessner	1983	Senescence	19	57–88	12	120	DB	Placebo ($n = 19$) Nicergoline ($n = 19$)	69%
Hofferberth	1989	COS	36	53–69	8	120	DB	Placebo ($n = 18$)	83%
Israel	1977	D	48	72	8	240	Open		NS
Leroy	1978	CVA	27	78	8	120	Open	Raubasine + ED ($n = 24$)	74%
Moreau	1975	CCI	30	84	12	120	DB	Placebo ($n = 30$) ED ($n = 30$)	79%
Pidoux	1983	CCI	12	87	12	160	DB	Placebo	85%
Safi	1977	CCI	20	47–86	2	35 (IV)	Open		76%
Tea	1979	CVA	19	67	8	160	Open		
Terasse	1976	Misc	20	59–84	1–3	17.5 (IV)	Open		55%
Vorberg	1985	CCI	112	55–94	52	120	Open, M		68%
Wackenheim	1977	CCI	50	50	27	160	Open		76%
		CCT	50	50	27	160	Open		72%

CCI, Chronic cerebral insufficiency; *CCT,* cerebral circulation time; *COS,* cerebral organic syndrome; *CS,* crossover; *CVA,* cerebral vascular accident; *D,* dementia; *DB,* double-blind; *ED,* ergot derivatives; *M,* multicenter; *NS,* not specified.

contrast-enhanced magnetic resonance imaging, elderly human subjects taking a modest dosage of 60 mg of GBE for 4 weeks experienced a significant increase of global cerebral blood flow, 15% in white matter and 13% in gray matter.[25]

In addition to improving blood supply to the brain, experimental and clinical studies showed that GBE increased the rate at which information was transmitted at the nerve cell level.[26,27] One study investigated the effects of acute doses of standardized GBE on memory and psychomotor performance in 31 healthy volunteers who were 30 to 59 years old.[28] It was a randomized, double-blind, placebo-controlled study with a five-way crossover design. Ginkgo improved working memory in all volunteers, especially those who were 50 to 59 years old. Fifty patients aged 50 to 85 years with mild cognitive impairment (MCI) and associated dual-task-related gait impairment participated in a randomized, double-blind, placebo-controlled, exploratory Phase IV drug trial of a *G. biloba* extract (Symfona forte 120 mg).[29] Subjects were divided into two groups. Patients allocated to the intervention group (IG) received standardized GBE LI 1370 (Symfona forte 120 mg; 25% standardized flavone glycoside, 6% terpene lactone content) twice daily for 6 months, whereas patients in the control group (CG) received identically appearing placebo capsules. After 6 months, dual-task-related cadence increased in the IG compared with the CG. This suggests that in patients with MCI, 120 mg of GBE twice daily for at least 6 months may improve dual-task-related gait performance. In the open-label phase, the IG was able to maintain most effects on the spatiotemporal gait parameters, indicating that long-term GBE intake over 1 year may be required to elicit more pronounced effects.[30]

GBE's memory-enhancing effects are not limited to the elderly. In another double-blind study, the reaction time in healthy young women performing a memory test was improved significantly after administration of GBE.[31] GBE complexed with phosphatidylserine might show even better results in these sorts of applications. In one study, 28 healthy young participants received 120 mg GBE, 120 mg GBE complexed with phosphatidylserine (Virtiva), 120 mg GBE complexed with phosphatidylcholine, and a matching placebo, on separate days 7 days apart.[19] Cognitive performance was assessed using the Cognitive Drug Research computerized test battery and Serial Subtraction tasks immediately before dosing and at 1, 2.5, 4, and 6 hours thereafter. In keeping with previous research utilizing the same methodology, 120 mg of GBE was not associated with markedly improved performance on the primary outcomes. However, administration of Virtiva resulted both in improved secondary memory performance and significantly increased speed in memory-task performance across all postdose testing sessions. Enhancement after GBE complexed with phosphatidylcholine was restricted to a modest improvement in secondary-memory performance, which was restricted to one postdose time point.

Cognitive Dysfunction

The prefrontal cortex (PFC) plays a central role in cognitive control functions, and dopamine in the PFC modulates cognitive control, thereby influencing attention, impulse inhibition, prospective memory, and cognitive flexibility. Because reduced prefrontal dopamine has been associated with impaired cognitive control,[32] interventions that improve prefrontal dopaminergic functions are of interest. A randomized, placebo-controlled, double-blind, pilot trial in 61 elderly volunteers with subjective memory impairment was conducted to evaluate the effects of GBE on cognitive functions related to prefrontal dopamine.[33] Indications for improved cognitive flexibility without

TABLE 82.3 Effect of *Ginkgo biloba* Extract on Cardiovascular Performance Measures

	TIME OF MEASUREMENT	
Parameter	**Week 0**	**Week 104**
Pain-free walking distance, m	62.9	172.4
Total walking distance, m	113.8	384
Rest flow, mL/100 mL/min	1.6	2.7
Peak flow, mL/100 mL/min	3.7	6.9
Doppler measurement after strain, mm Hg	46.5	72.6

changes in brain activation suggested increased processing efficiency with GBE, and together with a trend for improved response inhibition, results were compatible with mild enhancement of prefrontal dopamine. Ginkgo's putative neuroprotective and cognitive-enhancing properties have provided support for its use in treating neurological, psychiatric, functional, and physiological symptoms, including problems with memory, information processing, attention and concentration, psychomotor function, mood, fatigue, and activities of daily living. Clinical studies have shown that the cognitive domains yielding the largest proportion of significant effects of GBE versus placebo are in the following areas[33–38]:

- Fluid intelligence
- Selective attention
- Short-term and long-term verbal and visual memory
- Executive functions (e.g., planning, working memory, flexibility, and processing speed)

Alzheimer's Disease

GBE has been extensively investigated in cases of dementia, including Alzheimer's disease (AD). In addition to GBE's ability to increase functional brain capacity via the mechanisms described earlier, it was shown to normalize the acetylcholine receptor in the hippocampus of aged animals, increase cholinergic transmission, inhibit β-amyloid deposition,[9] and address many of the other major elements of AD.[2,3,39] Although preliminary studies in established AD patients were quite promising, at this time it appears that at best GBE only helps reverse or merely delay mental deterioration in the early stages of AD. However, even this application is fraught with conflicting results in double-blind studies. That is, in several studies, no benefit over placebo was observed in halting cognitive decline.[40] In more advanced cases of AD, GBE does not offer any significant advantage over placebo.

The benefits of GBE in early-stage AD were quite evident in several double-blind studies and a meta-analysis of studies of more than 6 months' duration.[41] In one study, 216 patients with AD or multiinfarct dementia were given either 240 mg/day of GBE or placebo for 24 weeks.[42] Improvements were noted in several clinical parameters, including the Clinical Global Impressions scale (Table 82.3). Similar results were seen in another double-blind study, in which the 240-mg dose was administered once daily.[42]

One study that deserves special mention is the first U.S. clinical study on GBE published in the *Journal of the American Medical Association*.[43] The study was conducted at six research centers. Harvard Medical School and the New York Institute for Medical Research approved the design of the study, in which 202 patients with AD were given either a modest dose of GBE (120 mg/day) or a placebo for 1 year. GBE not only stabilized AD but also led to significant improvements in mental function in 64% of the patients. There were no side effects with GBE.

The results from these positive studies indicate that GBE in early-stage AD or other causes of dementia may lead to some mild cognitive improvement and help enable patients maintain a normal life and avoid being institutionalized.

It is important to point out that direct comparison studies on ginkgo versus standard pharmaceutical regimens indicated similar efficacy in AD. A comparative analysis of studies of at least 6 months in duration demonstrated that GBE and second-generation cholinesterase inhibitors (e.g., tacrine, donepezil, rivastigmine, metrifonate) were equally effective in treating mild to moderate AD.[44] In a meta-analysis of 50 articles that examined the effect of ginkgo on objective measures of cognitive function in patients with AD, using standardized measures of cognition including the Alzheimer's Disease Assessment Scale–cognitive subscale, it was concluded that GBE produced comparable benefits to standard cholinesterase inhibitors.[45]

In addition to possibly being beneficial in early-stage AD, if the mental deficit is a result of vascular insufficiency or depression and not AD, GBE is usually effective in reversing the deficit.[2,3,4,46] Importantly, GBE should be taken consistently for at least 12 weeks to determine its effectiveness. Although some people with AD report benefits within a 2- to 3-week period, most will need to take GBE for a longer period of time.

Tinnitus

Permanent, severe tinnitus is an extremely difficult condition to treat. Previous studies showed contradictory results of GBE in the treatment of tinnitus. For example, in Meyer's study,[47] GBE improved the condition in all patients regardless of prognostic factor. However, in Coles's study,[48] 21 patients with tinnitus took GBE for 12 weeks: 11 reported no change, 4 reported slight to very slight improvement, and 5 reported that their tinnitus was worse. A possible explanation for these different results is that in Meyer's study, the patients had recent-onset tinnitus, whereas in Coles's study, 18 patients had tinnitus for at least 3 years.

A 1994 study of GBE in tinnitus used a two-part design: the first part was an open part, without a placebo control, and the second part was a double-blind, placebo-controlled, crossover study.[49] The 80 patients in the open study were referred to the Department of Audiology, Sahlgren's Hospital, Goteborg, Sweden, because of permanent severe tinnitus. Twenty patients reporting a positive effect with GBE (14.6 mg twice daily) after 2 weeks were recruited for the double-blind study. Patients were given either GBE or placebo for 2 weeks and then crossed over into the other group. The evaluation indicated that six patients preferred GBE, seven preferred placebo, and seven had no preference.

On the surface, this study seemingly indicated that GBE was ineffective for permanent, severe tinnitus. However, the study was designed for the GBE to fail. First, the dosage used (14.6 mg twice daily or 29.2 mg/day) was far less than the standard dosage of 40 mg three times daily (or roughly four times the daily dosage used in the study). Secondly, in studies on patients with cerebral vascular insufficiency, it is well established that GBE often takes at least 2 weeks before benefits become apparent. The longer that GBE is used, the more obvious the benefit. In a condition like permanent, severe tinnitus, 2 to 4 weeks is simply not enough time. However, given the small, insufficient dosage, it probably would not have mattered if the subjects had been studied for a longer period of time.

A study of 1121 healthy people between 18 and 70 years old with tinnitus that was comparatively stable showed no benefit over placebo when given 50 mg of standardized ginkgo three times a day.[50] Although these data were discouraging, it should be noted that outcome measures were obtained through questionnaires and phoned-in patient reports, without objective audiometric metering.

A review study of randomized, placebo-controlled clinical trials of GBE was conducted to determine the effectiveness of standardized extract (EGb 761) in the treatment of tinnitus.[51] The authors concluded there was evidence of efficacy for the standardized extract in the treatment of tinnitus from three trials in patients in whom tinnitus was the primary complaint, and supportive evidence exists from five trials in patients with age-associated cognitive impairment or dementia in whom tinnitus was present as a concomitant symptom. In all identified and retrieved studies using the standardized GBE (EGb 761®), this specific preparation was found to be superior to placebo in the treatment of tinnitus. The degree to which GBE is of benefit in permanent, severe tinnitus remains to be determined, but given GBE's excellent safety profile, it is certainly worth a try.

Cochlear Deafness/Ototoxicity

Ischemia is usually the underlying factor in acute cochlear deafness. GBE improved recovery in cases of acute cochlear deafness caused by unknown factors or caused by sound trauma or pressure (barotrauma).[52] GBE was also shown to be a protective agent against ototoxicity from treatment with the chemotherapeutic agent cisplatin, without decreasing antineoplastic efficacy.[53]

Senile Macular Degeneration and Diabetic Retinopathy

GBE appears to effectively address the multifactorial pathophysiology of senile macular degeneration, the most common cause of blindness in adults. In double-blind studies, GBE demonstrated a statistically significant improvement in long-distance visual acuity in both macular degeneration and diabetic retinopathy.[54,55] GBE has also demonstrated impressive protective effects against free-radical damage to the retina in experimental studies. Furthermore, GBE was shown to prevent diabetic retinopathy in rats, suggesting it might have a protective effect in humans as well.[2]

Peripheral Arterial Insufficiency

The primary lesion of peripheral arterial disease is the same cholesterol-containing plaque that is responsible for other conditions associated with atherosclerosis (e.g., coronary artery disease, cerebral vascular insufficiency).

The arterial obstruction or narrowing causes a reduction in blood flow during exercise or at rest. Clinical symptoms are caused by the consequent ischemia. The most common symptom of peripheral arterial disease is pain during exertion as a result of intermittent claudication. The pain usually occurs in the calf and is described as cramping, tightness, or severe fatigue. The pain is usually bilateral. The cause of the pain is not only reduced oxygen delivery but also an increase in the production of toxic metabolites and cellular free radicals. These free radicals accumulate and react with the lipid constituents of the cell membrane.

Pain at rest indicates a serious reduction in resting blood flow. It is obviously a sign of severe disease. The pain may be localized to one or more toes, or it may have a stocking-type distribution. The character of the pain is usually described as burning or gnawing and is generally worse at night. Cyanosis or pallor of the extremity is usually apparent. In moderate to severe narrowing of the artery, there are trophic changes, including a dry, scaly, and shiny epidermis. Hair may disappear, and the toenails may become brittle, ridged, and deformed.

In nine double-blind, randomized clinical trials of GBE versus placebo in two matched groups of patients with peripheral arterial insufficiency of the leg, GBE was shown to be quite active and superior to placebo (eight studies) and equal to pentoxifylline (one study).[2,3,4,55–60] Not only did measurements of pain-free walking distance (75%–110%) and maximum walking distance (52.6%–119%) dramatically increase, but plethysmographic and Doppler ultrasound measurements also demonstrated increased blood flow through the affected limb. Blood lactate levels also dropped.

In studies following strict methodology and with a sufficient number of patients for reliable evaluation, the demonstration that GBE improves limb blood flow, and improves walking tolerance, indicates that GBE is far superior to pentoxifylline, cilostazol, and standard medical therapy in peripheral arterial insufficiency. This includes other peripheral vascular disorders such as diabetic peripheral vascular disease, Raynaud's disease, acrocyanosis, and postphlebitis syndrome. In the treatment of intermittent claudication, the effects are somewhat modest, especially compared with exercise programs.[61,62]

The longer GBE is used, the greater the benefit. Table 82.3 summarizes a 2-year trial of GBE (160 mg/day) in the treatment of peripheral arterial disease (Fontaine stage IIb). Pain-free walking distance increased by more than 270%.[2]

The usual daily dosage of GBE is 120 mg (40 mg three times a day); however, some of the studies employed a dosage of 160 mg/day, including the study summarized in Table 82.3. Given the excellent safety profile of GBE, dosages similar to those used in cerebral vascular insufficiency (e.g., 240 mg/day) may prove to be more effective.

Sexual Dysfunction

Most cases of impotence (erectile dysfunction) are caused by impaired blood flow to erectile tissue. Evidence indicates that GBE might be extremely beneficial in the treatment of erectile dysfunction caused by lack of blood flow. Sixty patients with proven erectile dysfunction who had not reacted to papaverine injections up to 50 mg were treated with 60 mg/day GBE for 12 to 18 months.[63] The penile blood flow was reevaluated by duplex sonography every 4 weeks. The first signs of improved blood supply were seen after 6 to 8 weeks. After 6 months' therapy, 50% of the patients regained potency, and in 20%, a new trial of papaverine injection was then successful; 25% of the patients showed an improved blood flow, but papaverine was still not successful. The remaining 5% were unchanged. The improvement of the arterial inflow to erectile tissue is assumed to be because of the known effect of GBE on enhancing blood flow through both arteries and veins without any change in systemic blood pressure. Ginkgo's effects are more apparent with long-term therapy, and better results may have been obtained with a 120-mg/day dose to take full advantage of its effect on improving blood flow.

Antidepressant-induced sexual dysfunction is a prevalent side effect of most conventional antidepressant drugs. A study of 33 women and 30 men on selective serotonin-reuptake inhibitors (SSRIs) showed an overall 84% effectiveness rate at curbing symptoms, with GBE exhibiting a positive effect on all four phases of the sexual response cycle: desire, excitement (erection and lubrication), orgasm, and resolution (afterglow).[64] This research group also demonstrated the efficacy of ginkgo for these side effects after other management trials of cyproheptadine, yohimbine, amantadine, and buspirone hydrochloride failed.

In a study in women with sexual arousal disorder, a single dose of 300 mg GBE had a small but significant facilitatory effect on physiological, but not subjective, sexual arousal compared with placebo in 99 women with sexual dysfunction.[65] Long-term GBE administration at this dosage did not significantly enhance arousal responses beyond placebo. The authors concluded that (1) neither short- nor long-term administration of GBE alone substantially affected sexual function in women, (2) a substantial placebo effect on sexual function existed in women with sexual concerns, and (3) teaching women to focus on genital sensations during sex enhanced certain aspects of women's sexual functioning.

Premenstrual Syndrome and Idiopathic Cyclic Edema

Premenstrual syndrome (PMS) is often characterized by fluid retention, vascular congestion, increased capillary permeability, and breast tenderness. A double-blind, placebo-controlled study sought to determine the effectiveness of GBE in treating these symptoms.[66] The population studied was a group of 165 women between the ages of 18 and 45 years who had congestive symptoms for at least three cycles. The patients were then assigned to receive either GBE (80 mg twice daily) or placebo from day 16 of the cycle to day 5 of the next. On the basis of extensive symptom evaluation by patients and physicians, it was concluded that GBE was effective against the congestive symptoms of PMS, particularly breast pain or tenderness. Patients taking GBE also noted improvements in neuropsychologic assessments. These results indicate that GBE might hold some promise in the treatment of PMS.

Antidepressant Effects

The ability of GBE to improve general mood in patients with cerebral vascular insufficiency in double-blind studies led researchers to begin investigating GBE's antidepressive effects. In a double-blind study, 40 elderly patients (ranging from 51–78 years old) with depression who had not benefited fully from standard antidepressant drugs were given either 80 mg of GBE three times daily or a placebo.[67] By the end of the eighth week, the total score of the Hamilton Rating Scale for Depression in the GBE group dropped from 14 to 4.5. In comparison, the placebo group dropped from 14 to only 13. This study indicated two things:

1. GBE can be used with standard antidepressants.
2. GBE enhanced the effectiveness of standard antidepressants, particularly in patients older than 50 years of age.

Importantly, the dosage used in the study (80 mg three times daily) was higher than the standard dosage of 40 mg three times daily.

See the previous section "Sexual Dysfunction" regarding ginkgo's ability to decrease unwanted side effects of standard antidepressant medications.

Allergies/Asthma

Mixtures of ginkgolides, and GBE standardized to contain 24% ginkgoflavonglycosides, showed clinical effects in allergic conditions because of their inhibition of PAF, a key chemical mediator in asthma, inflammation, and allergies.[16,17]

In one double-blind placebo study, the ability of a mixture of ginkgolides to block the effects of PAF when PAF was injected into the skin was investigated.[16] Normally, when PAF is injected, it causes an immediate formation of a hive (classic wheal and flare reaction). However, if the ginkgolide mixture (120 mg) was given before PAF injection, it effectively counteracted the wheal and flare reaction. Specifically, the ginkgolide reduced the flare (reddened) area by a mean of 62.4% and the wheal (hive) volume by a mean of 60%.

A study from the Qingdao Hospital of Integrated Traditional and Western Medicine in Shandong, China, assessed the efficacy of an oral ginkgo liquor to alleviate airway hyperreactivity. It was found that the treatment significantly reduced airway inflammation and improved the pulmonary function and clinical symptoms of asthmatic patients.[68]

Mixtures of ginkgolides, and purified ginkgolides, are under investigation in several European countries. The hope is that they will be proven clinically effective in eczema, allergies, and many other conditions in which PAF plays a central role.[16,17]

Raynaud's Disease

Most common in young women (60%–90% of reported cases), Raynaud's disease is a condition of vasospastic response that causes areas of the body, including the fingers, toes, and tips of the nose and ears, to feel numb and cool in response to cold temperatures or stress. During a Raynaud attack, these blood vessels that supply the skin narrow, limiting blood circulation to affected areas. Ginkgo's vasodilating activity is most likely responsible for its effectiveness in treating this condition.

Given the side-effect profile of pharmaceutical treatments for Raynaud's disease, a double-blinded, placebo-controlled study evaluated the use of GBE to treat this difficult condition. A 10-week trial revealed a 56% reduction in the number of weekly attacks, whereas placebo reduced the number by 27%.[69] The World Health Organization recommends the use of ginkgo in Raynaud's disease, acrocyanosis, and postphlebitic syndrome.[70]

High-Altitude Sickness

In a study involving a Himalayan expedition of moderate altitude with gradual exposure, 44 volunteers were divided into a placebo group and a group that received 80 mg of ginkgo twice a day.[71] No subject in the ginkgo group developed acute mountain sickness, unlike 40.9% of subjects in the placebo group. Vasomotor disorders of the extremities were also prevented in the ginkgo group.

Future Applications of *Ginkgo biloba* Extract

There are many preliminary studies indicating possible clinical applications for GBE. For example, several studies have also shown the benefits of GBE on kidney function. One animal study showed improved kidney blood flow and function in hypertensive rats.[72] Another study found that ginkgo protects the kidneys from glyphosate.[73] Other animal studies have shown protection of the kidneys from mercury, uranium, naphthalene, and many other toxins.[74–76] Ginkgo also protects the kidneys from gut-derived endotoxins.[77] Ginkgo also has the ability to protect against numerous mitochondrial toxins, which strengthens the case for its use in kidney disease because mitochondrial damage in the kidneys mediates much of the nephrotoxicity.[78]

Other potential applications of GBE include angina, congestive heart failure, and acute respiratory distress syndrome. Its action on PAF may also make it useful for a great number of other applications in addition to allergies, including various types of shock, thrombosis, graft protection during organ transplantation, multiple sclerosis, and burns.

DOSAGE

Most of the clinical research on *G. biloba* used a standardized extract, containing 24% ginkgo flavone glycosides, has utilized dosages of 120 to 240 mg per day, usually in divided doses.

Devising a dosage schedule using other forms of ginkgo is difficult because of extreme variation in the content of active compounds in dried leaf and crude extracts. Whatever form of ginkgo is used, it appears to be essential that it is standardized for content and activity. For example, a standard 1:5 tincture obtained from crude ginkgo leaf with the highest possible flavonoid content would require 1 oz/day to provide the equivalent dosage level of the standardized extract.

Clinical research clearly shows that GBE should be taken consistently for at least 12 weeks to determine effectiveness. Although most people report benefits within 2 to 3 weeks, some may take longer to respond.

TOXICITY

GBE is extremely safe, and side effects are uncommon. In 44 double-blind studies involving 9772 patients taking GBE, the number of side effects reported was extremely small. The most common side

effect, gastrointestinal discomfort, occurred in only 21 cases, followed by headache (7 cases) and dizziness (6 cases).[4,46] Because of its inhibition of PAF, many practitioners are concerned that ginkgo may increase bleeding risk when used concurrently with warfarin (Coumadin), aspirin, and other antiplatelet therapies. However, a study evaluating the use of ginkgo and warfarin concurrently revealed no change in the international normalized ratio (INR), which is the blood test chosen to monitor the patient's possible bleeding risk in response to anticoagulation therapy.[79] Results from controlled studies consistently indicated that ginkgo did not significantly affect bleeding time or platelet aggregation, and it did not adversely affect the safety of coadministered aspirin or warfarin.[80]

In contrast to the tolerance of the leaf extract, contact with or ingestion of the fruit pulp produced severe allergic reactions.[81,82] Contact with the ginkgolic acids in the fruit pulp causes erythema and edema, with the rapid formation of vesicles accompanied by severe itching. This is similar to an allergic reaction to the poison ivy, oak, and sumac group, suggesting cross-reactivity between *G. biloba* fruit and this family. Ingestion of as little as two pieces of fruit pulp was reported to cause severe gastrointestinal irritation from the mouth to the anus. Because the ginkgo leaf does have a low level of irritative ginkgolic acids, animal studies were conducted to examine the possibility of adverse reactions from using ginkgo leaf extract. It was concluded that extracts of *G. biloba* taken orally were considered safe.[81]

DRUG INTERACTIONS

Ginkgo has been shown to have antagonistic effects on anesthetics, analgesics, anticoagulants, and antiplatelet agents because of ginkgo's inhibition of platelet aggregation.[82] As a result of these concerns, ginkgo should be used cautiously in patients taking these medications.

Because of its inhibition of PAF, many practitioners are concerned that ginkgo may increase bleeding risk when used concurrently with warfarin (Coumadin), aspirin, and other antiplatelet therapies. However, a study evaluating the use of ginkgo and warfarin concurrently revealed no change in the international normalized ratio (INR), which is the blood test chosen to monitor the patient's possible bleeding risk in response to anticoagulation therapy.[83] Results from controlled studies consistently indicated that ginkgo did not significantly affect bleeding time or platelet aggregation, and it did not adversely affect the safety of coadministered aspirin or warfarin.[84] In addition, a double-blind, placebo-controlled, randomized, two-way crossover trial found that *G. biloba* extract had no significant pharmacodynamic effects on warfarin and had no effects on prothrombin time and activated partial thromboplastin time.[85] Prudent caution suggests that patients taking warfarin should have their INRs closely monitored or refrain from ginkgo use.

Human clinical trials have demonstrated no clinically significant effects on CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.[86,87]

Ginkgo should be used with caution, or avoided, concomitantly with antiretrovirals because it may decrease the levels of selected antiviral therapies.[88,89]

Regarding the pharmacokinetic herb–drug interactions, the intake of standardized *G. biloba* extract together with synthetic drugs appears to be safe if daily doses up to 240 mg are consumed.[90]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Blumenthal M. Herb sales down 3% in mass market retail stores—sales in natural food stores still growing, but at lower rate. *Herbal Gram*. 2000;49:68.
2. DeFeudis FV, ed. *Ginkgo biloba extract (EGb 761). Pharmacological activities and clinical applications*. Paris: Elsevier; 2000.
3. Zuo W, Yan F, Zhang B, Li J, Mei D. Advances in the studies of *Ginkgo biloba* leaves extract on aging-related diseases. *Aging Dis*. 2017;8(6):812–826.
4. Kleijnen J, Knipschild P. *Ginkgo biloba*. *Lancet*. 1992;340:1136–1139.
5. Schaffler VK, Reeh PW. Double-blind study of the hypoxia protective effect of a standardized Ginkgo biloba preparation after repeated administration in healthy subjects. *Arzneimittelforschung*. 1985;35:1283–1286. [German].
6. Nash KM, Shah ZA. Current perspectives on the beneficial role of Ginkgo biloba in neurological and cerebrovascular disorders. *Integr Med Insights*. 2015;10:1–9.
7. Le Poncin Lafitte M, Rapin J, Rapin JR. Effect of *Ginkgo biloba* on changes induced by quantitative cerebral microembolization in rats. *Arch Int Pharmacodyn Ther*. 1980;243:236–244.
8. Karcher L, Zagerman P, Krieglstein J. Effect of an extract of *Ginkgo biloba* on rat brain energy metabolism in hypoxia. *Naunyn Schmiedebergs Arch Pharmacol*. 1984;327:31–35.
9. Watanabe CM, Wolffram S, Ader P, et al. The in vivo neuromodulatory effects of the herbal medicine *Ginkgo biloba*. *Proc Natl Acad Sci U S A*. 2001;98:6577–6580.
10. Sadowska-Krepa E, Klapcinska B, Pokora I, Domaszewski P, Kempa K, Podgorski T. Effects of six-week *Ginkgo biloba* supplementation on aerobic performance, blood pro/antioxidant balance, and serum brain-derived neurotrophic factor in physically active men. *Nutrients*. 2017;9(8). pii: E803. PubMed PMID:28933745.
11. Tian J, Liu Y, Chen K. *Ginkgo biloba* extract in vascular protection: molecular mechanisms and clinical applications. *Curr Vasc Pharmacol*. 2017;15(6):532–548.
12. Li XS, Zheng WY, Lou SX, et al. Effect of ginkgo leaf extract on vascular endothelial function in patients with early stage diabetic nephropathy. *Chin J Integr Med*. 2009;15:26–29.
13. Pierre SV, Lesnik P, Moreau M, et al. The standardized Ginkgo biloba extract Egb-761 protects vascular endothelium exposed to oxidized low density lipoproteins. *Cell Mol Biol (Noisy-le-grand)*. 2008;54(suppl): OL1032–OL1042.
14. Bruchert E, Heinrich SE, Ruf-Kohler P, et al. 1370 bei alteren patienten mit himleistungsschwache. Multizentrische doppelblindstudie des fachverbandes Deutscher Allegemeinaezte. *Munch Med Wochenschr*. 1991;133:S9–S14.
15. Meyer B. Multicenter randomized double-blind drug vs. placebo study of the treatment of tinnitus with *Ginkgo biloba* extract. *Presse Med*. 1986;15:1562–1564. [French].
16. Taillandier J, Ammar A, Rabourdin JP, et al. Treatment of cerebral aging disorders with Ginkgo biloba extract. A longitudinal multicenter double-blind drug vs. placebo study. *Presse Med*. 1986;15:1583–1587. [French].
17. Koltai M, Hosford D, Guinot P, et al. Platelet activating factor (PAF). A review of its effects, antagonists and possible future clinical implications (Part I). *Drugs*. 1991;42:9–29.
18. Koltai M, Hosford D, Guinot P, et al. PAF. A review of its effects, antagonists and possible future clinical implications (Part II). *Drugs*. 1991;42:174–204.
19. Chen ZP, Sun J, Chen HX, et al. Comparative pharmacokinetics and bioavailability studies of quercetin, kaempferol and isorhamnetin after oral administration of *Ginkgo biloba* extracts, *Ginkgo biloba* extract phospholipid complexes and *Ginkgo biloba* extract solid dispersions in rats. *Fitoterapia*. 2010;81:1045–1052.
20. Kennedy DO, Haskell CF, Mauri PL, et al. Acute cognitive effects of standardised *Ginkgo biloba* extract complexed with phosphatidylserine. *Hum Psychopharmacol*. 2007;22:199–210.
21. Haguenauer JP, Cantenot F, Koskas H, et al. Treatment of equilibrium disorders with *Ginkgo biloba* extract. A multicenter double-blind drug vs. placebo study. *Press Med*. 1986;15:1569–1572.
22. Vorberg G, Schmidt U, Schenk N. Wirksamkeit eines neuen Ginkgo biloba extraktes bei 100 patienten mit zerebraler insuffizienz. *Herg Gefasse*. 1989;9:936–941. [German].
23. Eckmann F. Cerebral insufficiency-treatment with *Ginkgo-biloba* extract. Time of onset of effect in a double-blind study with 60 inpatients. *Fortschr Med*. 1990;108:557–560.
24. Wesnes K, Simmons D, Rook M, et al. A double-blind placebo-controlled trial of Tanakan in the treatment of idiopathic cognitive impairment in the elderly. *Hum Psychopharmacol*. 1987;2:159–171.
25. Anadere I, Chmiel H, Witte S. Hemorrheological findings in patients with completed stroke and the influence of *Ginkgo biloba* extract. *Clin Hemorheol*. 1985;5:411–420.
26. Mashayekh A, Pham DL, Yousem DM, et al. Effects of Ginkgo biloba on cerebral blood flow assessed by quantitative MR perfusion imaging: a pilot study. *Neuroradiology*. 2011;53(3):185–191.
27. Gessner B, Voelp A, Klasser M. Study of the long-term action of a Ginkgo biloba extract on vigilance and mental performance as determined by means of quantitative pharmaco-EEG and psychometric measurements. *Arzneimittelforschung*. 1985;35:1459–1465.
28. Hofferberth B. Effect of *Ginkgo biloba* extract on neurophysiological and psychometric measurement in patients with organic brain syndrome. A double-blind study against placebo. *Arzneimittelforschung*. 1989;39:918–922.
29. Rigney U, Kimber S, Hindmarch I. The effects of acute doses of standardized Ginkgo biloba extract on memory and psychomotor performance in volunteers. *Phytother Res*. 1999;13:408–415.
30. Gschwind YJ, Bridenbaugh SA, Reinhard S, Granacher U, Monsch AU, Kressig RW. *Ginkgo biloba* special extract LI 1370 improves dual-task walking in patients with MCI: a randomized, double-blind, placebo-controlled exploratory study. *Aging Clin Exp Res*. 2017;29(4):609–619. PubMed PMID: 28181206.
31. Vellas B, Coley N, Ousset PJ, et al. GuidAge Study Group. Long-term use of standardized *Ginkgo biloba* extract for the prevention of Alzheimer's disease (GuidAge): a randomized placebo-controlled trial. *Lancet Neurol*. 2012;11(10):851–859. PubMed PMID:22959217.
32. Subhan Z, Hindmarch I. The psychopharmacological effects of Ginkgo biloba extract in normal healthy volunteers. *Int J Clin Pharmacol Res*. 1984;4:89–93.
33. Floresco SB. Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Front Neurosci*. 2013;7:62. PubMed PMID:23626521.
34. Beck SM, Ruge H, Schindler C. Effects of *Ginkgo biloba* extract EGb 761 on cognitive control functions, mental activity of the prefrontal cortex and stress reactivity in elderly adults with subjective memory impairment – a randomized double-blind placebo-controlled trial. *Hum Psychopharmacol*. 2016;31(3):227–242. PubMed PMID:27147264.
35. Moeller CK, Kurt S, Scheich H, Schulze H. Improvement of auditory discrimination learning by *Ginkgo biloba* extract EGb 761. *Neurosci Lett*. 2009;463(3):219–222. PubMed PMID:19660527.
36. Rojas P, Ruiz-Sanchez E, Rojas C, Ogren SO. *Ginkgo biloba* extract (EGb 761) modulates the expression of dopamine-related genes in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice. *Neuroscience*. 2012;223:246–257. PubMed PMID:22885234.
37. Klement S, Bock N, Rothenberger A. *Ginkgo biloba* extract EGb 761 is effective and safe in the treatment of ADHD in children. *World Ferderation on ADHD, 3rd International Congress on ADHD – From Childhood to Adult Disease. 26-May 29, 2011*. Berlin, Germany; 2011: P-35-06.
38. Kaschel R. *Ginkgo biloba*: specificity of neuropsychological improvement? A selective review in search of differential effects. *Hum Psychopharmacol*. 2009;24:345–370.
39. Tan MS, Yu JT, Tan CC, Wang HF, et al. Efficacy and adverse effects of *Ginkgo biloba* for cognitive impairment and dementia: a systematic review and meta-analysis. *J Alzheimers Dis*. 2015;43(2):589–603.
40. Shi C, Liu J, Wu F, et al. *Ginkgo biloba* extract in Alzheimer's disease: from action mechanisms to medical practice. *Int J Mol Sci*. 2010;11:107–123.
41. Weinmann S, Roll S, Schwarzbach C, et al. Effects of *Ginkgo biloba* in dementia: systematic review and meta-analysis. *BMC Geriatr*. 2010;10:14–26.

42. Wang BS, Wang H, Song YY, et al. Effectiveness of standardized Ginkgo biloba extract on cognitive symptoms of dementia with a six-month treatment: a bivariate random effect meta-analysis. *Pharmacopsychiatry*. 2010;43:86–91.
43. Kanowski S, Hermann WM, Stephan K, et al. Proof of efficacy of the Ginkgo biloba special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. *Phytomedicine*. 1997;4:3–13.
44. Le Bars PL, Katz MM, Berman N, et al. A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. North American EGb Study Group. *JAMA*. 1997;278:1327–1332.
45. Wettstein A. Cholinesterase inhibitors and ginkgo extracts—are they comparable in the treatment of dementia? Comparison of published placebo-controlled efficacy studies of at least six months' duration. *Phytomedicine*. 2000;6:393–401.
46. Kleijnen J, Knipschild P. *Ginkgo biloba* for cerebral insufficiency. *Br J Clin Pharmacol*. 1992;34:352–358.
47. Oken BS, Storzbach DM, Kaye JA. The efficacy of *Ginkgo biloba* on cognitive function in Alzheimer disease. *Arch Neurol*. 1998;55:1409–1415.
48. Meyer B. A multicenter randomized double-blind study of *Ginkgo biloba* extract versus placebo in the treatment of tinnitus. In: Funfgeld EW, ed. *Rokan (Ginkgo Biloba)—Recent Results in Pharmacology and Clinic*. New York: Springer-Verlag; 1988:245–250.
49. Coles RRA. Trial of an extract of *Ginkgo biloba* (EGB) for tinnitus and hearing loss. *Clin Otolaryngol*. 1988;13:501–504.
50. Holgers KM, Axelsson A, Pringle I. *Ginkgo biloba* extract for the treatment of tinnitus. *Audiology*. 1994;33:85–92.
51. Drew S, Davies E. Effectiveness of *Ginkgo biloba* in treating tinnitus: double blind, placebo controlled trial. *BMJ*. 2001;332:73–75.
52. von Boetticher A. *Ginkgo biloba* extract in the treatment of tinnitus: a systematic review. *Neuropsychiatr Dis Treat*. 2011;7:441–447. PubMed PMID:21857784.
53. Bascher V, Steinert W. Differential diagnosis of sudden deafness and therapy with high dose infusions of Ginkgo biloba extract. In: Clausen CF, Kirtane MV, Schlitter K, eds. *Vertigo, Nausea, Tinnitus, and Hypoacusia in Metabolic Disorders*. Amsterdam: Elsevier Science; 1988:575–582.
54. Fukaya H, Kanno H. Experimental studies of the protective effect of Ginkgo biloba extract (GBE) on cisplatin-induced toxicity in rats. *Nippon Jibiinkoka Gakkai Kaiho*. 1999;102:907–917. [Japanese].
55. Lanthony P, Cosson JP. The course of color vision in early diabetic retinopathy treated with Ginkgo biloba extract. A preliminary double-blind versus placebo study. *J Fr Ophtalmol*. 1988;11:671–674. [French].
56. Schneider B. *Ginkgo biloba* extract in peripheral arterial diseases. Meta-analysis of controlled clinical studies. *Arzneimittelforschung*. 1992;42:428–436. [German].
57. Thomson GJ, Vohra RK, Carr MH, et al. A clinical trial of Ginkgo biloba extract in patients with intermittent claudication. *Int Angiol*. 1990;9:75–78.
58. Saudreau F, Serise JM, Pillet J, et al. Efficacy of Ginkgo biloba extract in the treatment of chronic obliterating arteriopathies of the lower limbs in stage III of Fontaine's classification. *J Mal Vasc*. 1989;14:177–182.
59. Rudofsky VG. Effect of *Ginkgo biloba* extract in arterial occlusive disease. Randomized placebo controlled crossover study. *Fortschr Med*. 1987;105:397–400. [German].
60. Bauer U. Ginkgo biloba extract in the treatment of arteriopathy of the lower limbs. A 65-week trial. *Presse Med*. 1986;15:1546–1549.
61. Bauer U. 6-month double-blind randomized clinical trial of Ginkgo biloba extract versus placebo in two parallel groups in patients suffering from peripheral arterial insufficiency. *Arzneimittelforschung*. 1984;34:716–721.
62. Pittler MH, Ernst E. Ginkgo biloba extract for the treatment of intermittent claudication: a meta-analysis of randomized trials. *Am J Med*. 2000;108:276–281.
63. Nicolaï SP, Gerardu VC, Kruidenier LM, et al. From the Cochrane Library: Ginkgo biloba for intermittent claudication. *Vasa*. 2010;39:153–158.
64. Sikora R, Sohn M, Deutz FJ, et al. *Ginkgo biloba* extract in the therapy of erectile dysfunction. *J Urol*. 1989;141:188A.
65. Cohen AJ, Bartlik B. *Ginkgo biloba* for antidepressant-induced sexual dysfunction. *J Sex Marital Ther*. 1998;24:139–143.
66. Meston CM, Rellini AH, Telch MJ. Short- and long-term effects of *Ginkgo biloba* extract on sexual dysfunction in women. *Arch Sex Behav*. 2008;37:530–547.
67. Tamborini A, Taurelle R. Value of standardized *Ginkgo biloba* extract (Egb 761) in the management of congestive symptoms of premenstrual syndrome. *Rev Fr Gynecol Obstet*. 1993;88:447–457. [French].
68. Schubert H, Halama P. Depressive episode primarily unresponsive to therapy in elderly patients. Efficacy of *Ginkgo biloba* (Egb 761) in combination with antidepressants. *Geriatr Forsch*. 1993;3:45–53.
69. Li M, Yang B, Yu H, et al. Clinical observation of the therapeutic effect of ginkgo leaf concentrated oral liquor on bronchial asthma. *Chinese J Integr Med*. 1997;3:264–267.
70. Muir AH, Robb R, McLaren M, et al. The use of *Ginkgo biloba* in Raynaud's disease: a double-blind placebo-controlled trial. *Vasc Med*. 2002;7:265–267.
71. World Health Organization. *Who Monographs on Selected Medicinal Plants*. vol. 1. Geneva: World Health Organization; 1999.
72. Roncin JP, Schwartz F, D'Arbigny P. EGb 761 in control of acute mountain sickness and vascular reactivity to cold exposure. *Aviat Space Environ Med*. 1996;67:445–452.
73. Mansour SM, Bahgat AK, El-Khatib AS, Khayyal MT. *Ginkgo biloba* extract (EGb 761) normalizes hypertension in 2K, 1C hypertensive rats: role of antioxidant mechanisms, ACE inhibiting activity and improvement of endothelial dysfunction. *Phytomedicine*. 2011;18(8-9):641–647.
74. Cavuşoğlu K, Yapar K, Oruç E, Yalçın E. Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*. 2011;14(10):1263–1272.
75. Sener G, Sehirli O, Tozan A, et al. Ginkgo biloba extract protects against mercury(II)-induced oxidative tissue damage in rats. *Food Chem Toxicol*. 2007;45(4):543–550.
76. Yapar K, Cavuşoğlu K, Oruç E, Yalçin E. Protective role of *Ginkgo biloba* against hepatotoxicity and nephrotoxicity in uranium-treated mice. *J Med Food*. 2010;13(1):179–188.
77. Tozan A1, Sehirli O, Omurtag GZ, et al. *Ginkgo biloba* extract reduces naphthalene-induced oxidative damage in mice. *Phytother Res*. 2007;21(1):72–77.
78. Coskun O, Armutcu F, Kanter M, Kuzey GM. Protection of endotoxin-induced oxidative renal tissue damage of rats by vitamin E or/and EGb 761 treatment. *J Appl Toxicol*. 2005;25(1):8–12.
79. Eckert A. Mitochondrial effects of Ginkgo biloba extract. *Int Psychogeriatr*. 2012;24(suppl 1):S18–S20.
80. Becker LE, Skipworth GB. Ginkgo-tree dermatitis, stomatitis, and proctitis. *JAMA*. 1975;231:1162–1163.
81. Hausen BM. The sensitizing capacity of ginkgolic acids in guinea pigs. *Am J Contact Dermat*. 1998;9:146–148.
83. Engelsen J, Nielsen JD, Winther K. Effect of coenzyme Q10 and Ginkgo biloba on warfarin dosage in stable, long-term warfarin treated outpatients. A randomised, double blind, placebo-crossover trial. *Thromb Haemost*. 2002;87:1075–1076.
84. Bone KM. Potential interaction of Ginkgo biloba leaf with antiplatelet or anticoagulant drugs: what is the evidence? *Mol Nutr Food Res*. 2008;52:764–771.
85. Zhou Y, Zeng R. Effects of Ginkgo biloba extract on anticoagulation and blood drug level of warfarin in healthy volunteers. *Zhongguo Zhong Yao Za Zhi*. 2011;36(16):2290–2293.
86. Gurley BJ, Gardner SF, Hubbard MA, et al. Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly: St John's wort, garlic oil, Panax ginseng and Ginkgo biloba. *Drugs Aging*. 2005;22(6):525–539.
87. Zadoyan G, Rokitta D, Klement S, et al. Effect of Ginkgo biloba special extract EGb 761 on human cytochrome P450 activity: a cocktail interaction study in healthy volunteers. *Eur J Clin Pharmacol*. 2012;68(5):553–560.
88. Fasinu PS, Gurley BJ, Walker LA. Clinically relevant pharmacokinetic herb-drug interactions in antiretroviral therapy. *Current Drug Metabolism*. 2015;17(1):52–64.
89. Jalloh MA, Gregory PJ, Hein D, et al. Dietary supplement interactions with antiretrovirals: a systematic review. *International Journal of STD & AIDS*. 2017;28(1):4–15.
90. Unger M. Pharmacokinetic drug interactions involving Ginkgo biloba. *Drug Metabolism Reviews*. 2013;45(3):353–385.

Glucosamine

Michael T. Murray, ND

OUTLINE

GENERAL DESCRIPTION

Glucosamine is a simple molecule, manufactured in the body from glucose and an amine. One of the primary physiological roles of glucosamine is in the joints, where it stimulates the manufacture of glycosaminoglycans (GAGs), key structural components of cartilage. Glucosamine also promotes the incorporation of sulfur into cartilage. Because of this effect, glucosamine sulfate (GS) is thought to be the best source of glucosamine (Fig. 83.1).

As some people age, they apparently lose the ability to manufacture adequate levels of glucosamine. The result is that the synthesis of GAGs does not keep up with degradation. The inability to manufacture glucosamine at an adequate rate has been suggested to be the major factor leading to osteoarthritis (OA).

There are no food sources of glucosamine. Commercially available sources of glucosamine are derived from chitin—the exoskeleton of shrimp, lobsters, and crabs.

AVAILABLE FORMS

Glucosamine is commercially available as GS, glucosamine hydrochloride, and *N*-acetylglucosamine (NAG). GS, the only form of glucosamine that has been the subject of more than 300 scientific investigations and more than 20 double-blind studies, is the preferred form. GS has also been used by millions of people worldwide and is registered as an aid in OA in 70 countries. In fact, there may even be differences between the patented crystalline glucosamine sulfate preparation (Rottapharm/Meda) and other glucosamine formulations. Glucosamine products may vary substantially in molecular form, pharmaceutical formulation, and dose regimens.[1]

The patented crystalline glucosamine sulfate preparation is given as a highly bioavailable once-daily dose (1500 mg). Single high-dosage levels may prove more effective to allow glucosamine to reach the tissue levels required to affect mechanisms involved in the pathophysiology or healing of OA. Studies conducted with the crystalline form of GS are generally quite positive, whereas noncrystalline GS preparations and glucosamine hydrochloride (GH) have not shown the same degree of benefit, and in most studies, no benefit was noted. The European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) recommends the patented crystalline GS formulation over other glucosamine preparations because of a much stronger evidence base.[1,2]

Furthermore, when authors or manufacturers discuss GH or NAG, they often cite references of clinical studies that used GS or the combination of glucosamine hydrogen chloride (HCl) and chondroitin sulfate. On the basis of currently available clinical evidence, it appears that only GS provides proven clinical effectiveness when administered as an isolated agent (numerous studies showed the combination of glucosamine HCl and chondroitin sulfate to be effective). It does not appear to matter, however, if the GS is in true crystalline form whether it is stabilized with either sodium or potassium.[3] It also appears that in terms of symptom improvement, no further benefit is offered to GS if the formulation also includes chondroitin sulfate.[4] However, the combination of GS with methylsufonylmethane (MSM) may offer some additive effect to GS alone, and it is definitely useful to include MSM in combinations of glucosamine HCl plus chondroitin sulfate because of the sulfur.[5]

Glucosamine Sulfate Versus *N*-Acetylglucosamine

NAG differs from GS in that instead of a sulfur molecule, NAG has a portion of an acetic acid molecule attached to it (Fig. 83.2). GS and NAG are different molecules and appear to be handled by the body differently. Companies marketing NAG claim that this form is better absorbed, more stable, and better used than GS. These contentions are without support in the scientific literature. Detailed human studies on the absorption, distribution, and elimination of orally administered GS show an absorption rate as high as 98% and that, once absorbed, it is then distributed primarily to joint tissues, where it is incorporated into the connective tissue matrix of cartilage, ligaments, and tendons.[6] In addition, there are impressive clinical studies on thousands of patients. In contrast, the only clinical study with NAG used was as a polymer that is not yet available commercially.[7] Polymerization is thought to be necessary because of the poor oral availability of unbound NAG.

Further evidence of the superiority of GS to NAG is offered by studies on laboratory animals. Several studies demonstrated that glucosamine absorption and utilization are at least twice that of NAG.[8] It is well established that glucosamine is a more efficient precursor

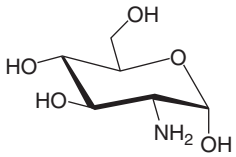


Fig. 83.1 Glucosamine.

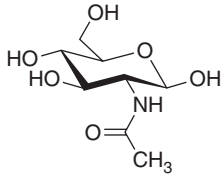


Fig. 83.2 *N*-acetylglucosamine.

of macromolecular hexosamine (GAG) than NAG. It is possible that NAG does not penetrate the cell membranes and, as a result, is unavailable for incorporation into glycoproteins and mucopolysaccharides.[7] The body preferentially uses GS rather than NAG. This preference appears largely because of the active processes that enhance absorption of GS in the intestines.[9]

The absorption of NAG by humans is poor for several reasons:

- NAG is quickly digested by intestinal bacteria.
- NAG binds with dietary lectins in the gut, resulting in a lectin–NAG complex, which is excreted in the feces.
- A large percentage of NAG is metabolized by intestinal cells.

In addition to the question of absorption, articular tissue cannot use NAG as well as it does glucosamine. These absorption and utilization problems suggest NAG is highly unlikely to possess the same kind of antiarthritic and anti-inflammatory properties that GS has been shown to possess.

Glucosamine Sulfate Versus Glucosamine Hydrogen Chloride

Sulfur is an extremely important component in the therapeutic effect of GS, and its substitution is likely to decrease the efficacy of supplemental glucosamine. Sulfur is an essential nutrient for joint tissue, where it functions in the stabilization of the connective tissue matrix of cartilage, tendons, and ligaments. Even healthy humans have low serum sulfate (0.3–0.4 mM) and synovial sulfur levels, but in OA, these concentrations are even lower. As far back as the 1930s, researchers demonstrated that individuals with arthritis were commonly deficient in this essential nutrient.[10,11] Restoring sulfur levels brought about significant benefit to these patients. In addition to sulfur playing a critical role in the manufacture of GAGs like chondroitin sulfate and keratan sulfate, sulfur was shown to inhibit the various enzymes that lead to cartilage destruction in OA (e.g., collagenase, elastases, and hyaluronidase).[12]

Because one of the primary effects of GS is to promote the manufacture of GAGs, a lack of the sulfur moiety may mean less GAG synthesis when glucosamine HCl is used. Therefore it is unlikely that glucosamine HCl will show the same excellent clinical results achieved with GS because it lacks this critical element.

Results from double-blind studies indicated that glucosamine HCl might be no more effective than a placebo in relieving OA. One double-blind, placebo-controlled, 10-week study examined the effects of glucosamine HCl in patients with OA of the knee.[13] Patients received either 500 mg of glucosamine HCl or a placebo three times daily. Forty-five patients received glucosamine HCl, whereas 53 received the placebo. The results indicated that 49% of patients who received glucosamine HCl felt they had improved compared with 45% of the placebo group. However, the difference between the two groups was not statistically significant. What these results call into question is the viability of glucosamine HCl as an effective form of glucosamine. Unfortunately, several large, well-publicized studies utilized this form. For example, the Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT) study involved 1583 patients with symptomatic knee OA.[14] Patients were randomized to receive 1500 mg of glucosamine daily, 1200 mg of chondroitin sulfate daily, both glucosamine and chondroitin sulfate (same dosages as the single arms), 200 mg of celecoxib daily, or placebo for 24 weeks. The mean age of the patients was 59 years, and 64% were women. Overall, glucosamine and chondroitin sulfate were not significantly better than placebo in reducing knee pain by 20%. Compared with the rate of response to placebo (60.1%), the rate of response to glucosamine was 3.9% higher, the rate of response to chondroitin sulfate was 5.3% higher, and the rate of response to combined treatment was 6.5% higher. The rate of response in the celecoxib control group was 10.0% higher than that in the placebo control group. For patients with moderate to severe pain at baseline, the rate of response was significantly higher with combined therapy than with placebo (79.2% vs. 54.3%).

In an analysis of GAIT subjects with a radiographic confirmation of OA, the odds of achieving a 20% reduction in Western Ontario and McMaster Osteoarthritis Index (WOMAC) pain score compared with placebo were as follows: celecoxib, 1.21; glucosamine, 1.16; combination glucosamine/chondroitin sulfate, 0.83; and chondroitin sulfate alone, 0.69. These rates were not statistically significant.[15,16]

CLINICAL APPLICATIONS

Osteoarthritis

The primary use for GS is in the treatment of OA. This application has significant support in the medical literature because numerous double-blind studies have shown GS to produce much better results compared with nonsteroidal anti-inflammatory drugs (NSAIDs), placebo, or acetaminophen in relieving the pain and inflammation of OA.[17–21] Although some of the studies comparing GS with NSAIDs or acetaminophen showed similar reductions in pain and symptom scores, only GS improved indexes of joint function or markers showing improvement of cartilage structure. Typically, the advantages of GS over these other treatments were seen after 2 to 4 weeks of use, but there is some evidence that the longer GS is used, the greater the therapeutic benefit.

Not all studies showed clear positive results; a few exist that showed no greater benefit for GS over placebo in improving symptom scores.[22–26] However, it must be kept in mind that the placebo response in OA is quite high and may confound the true benefit of GS and other approaches to OA. Fortunately, there were several studies that showed objective improvements (discussed in the immediately following paragraphs).

The two longest placebo-controlled trials were 3 years in duration. The results from these studies showed, quite convincingly, that GS slowed down the progression of OA; produced regression of the disease in many cases, as noted by radiological improvements; and significantly reduced the incidence of total joint replacement even after as much as 5 years after GS discontinuation.[27–30]

In the first long-term study, 212 patients with knee OA were randomly assigned 1500 mg oral GS or placebo once daily for 3 years. Weight-bearing anteroposterior radiographs of each knee in full extension were taken at enrollment and after 1 and 3 years. The mean joint-space width of the medial compartment of the tibiofemoral joint was assessed by digital image analysis, whereas the minimum joint-space width (i.e., at the narrowest point) was measured by visual inspection with a magnifying lens. Symptoms were scored by the WOMAC OA index. The 106 patients on placebo had a progressive joint-space

narrowing, with a mean joint-space loss after 3 years of −0.31 mm. No significant joint-space loss occurred in the 106 patients on GS (−0.06 mm). Similar results were reported with minimum joint-space narrowing. As assessed by WOMAC scores, symptoms worsened slightly in patients on placebo compared with the clinical improvement observed in the group that received GS.[27]

To investigate the relationship between baseline radiographic severity of knee OA and the importance of long-term joint-space narrowing in more detail, a subanalysis of data from the 3-year trial was performed. Measurements of mean joint-space width, assessed by a computer-assisted method, were performed at baseline and after 3 years on weight-bearing anteroposterior knee radiographs. Those who received GS demonstrated a trend for significant reduction in joint-space narrowing in patients in the highest quartile of baseline mean joint-space width (>6.2 mm). In these patients, a joint-space narrowing of 14.9% occurred in the placebo group after 3 years, whereas patients from the GS group experienced a narrowing of only 6%.[31]

Of 414 participants randomized in the two long-term studies, 319 were postmenopausal women. In a subset analysis, after 3 years, postmenopausal participants in the GS group showed no joint-space narrowing, whereas participants in the placebo group experienced a narrowing of −0.33 mm. Percent changes after 3 years in the WOMAC index showed an improvement in the GS group (−14.1%) and a trend for worsening in the placebo group (5.4%). These results indicated that postmenopausal women might be especially responsive to GS.[27]

Additional insight into who might best respond to GS was provided in another analysis from this 3-year study that examined the ability of GS to improve a biochemical marker of collagen type II degradation (CTX-II).[32] At baseline, the 212 patients had an average concentration of urinary CTX-II of 222.4 ng/mmol creatinine. This was significantly above the CTX-II levels measured in urine samples from 415 healthy controls (169.1 ng/mmol). Although there was no significant difference in the CTX-II response in the placebo group and the glucosamine-treated group, those with high cartilage turnover presented a significant decrease in CTX-II after 12-month glucosamine treatment.

The group with CTX-II concentrations above normal average plus 1 standard deviation decreased 15.5% after 12-month therapy. The change in CTX-II in these patients correlated with the change in average joint-space width observed after 36 months. Increased baseline levels of CTX-II in the placebo group were associated with a worsening of the WOMAC index. These data indicate that measurement of urinary collagen type II C-telopeptide fragments enabled the identification of patients with high cartilage turnover, who appeared to be most responsive to GS therapy.

In the second study, 202 patients with knee OA were randomized to receive oral GS (1500 mg once a day) or placebo. Changes in computed tomographic radiographic minimum joint-space width were measured in the medial compartment of the tibiofemoral joint, and symptoms were assessed using the Lequesne (an index of severity of osteoarthritis) and WOMAC. Although symptoms improved more significantly in the GS group, the most telling result was the fact that progressive joint-space narrowing with placebo use was −0.19 mm after 3 years, whereas there was no average change with GS use (an increase of 0.04 mm was the average).[29]

Although a 2-year double-blind study failed to show any difference with either GS (1500 mg) or chondroitin sulfate (CS 800 mg) either alone or in combination versus the placebo group in terms of pain reduction, the combination of both GS and CS produced a statistically significant reduction of joint-space narrowing compared with placebo, with a mean difference of 0.10 mm. These results indicate that even if symptomatic improvement is not observed, GS may be exerting positive effects on joint structure.[33]

Another study sought to determine changes in levels of serum cartilage oligomeric matrix protein (COMP) and urine c-telopeptide of CTX-II as markers for cartilage turnover in patients with OA of the knee, in response to muscle strength training in combination with treatment with glucosamine, ibuprofen, or placebo. All three groups increased their muscle strength after 12 weeks of strength training. Glucosamine's reduction of serum COMP was statistically significant compared with both placebo and ibuprofen; the mean reduction with glucosamine was 13% versus placebo and 17% versus ibuprofen. This suggested an effect by glucosamine on the response of the OA cartilage to a period of joint loading in humans with knee OA. No effect was noted on CTX-II.[34]

Several head-to-head, double-blind studies also showed that GS produced much better results compared with NSAIDs and analgesics in relieving the pain and inflammation of OA, despite the fact that GS exhibited little direct anti-inflammatory effect and no direct analgesic or pain-relieving effects.[35–39] Although NSAIDs and analgesics, like acetaminophen, offer purely symptomatic relief, and NSAIDs may actually promote the disease process, GS appears to address the cause of OA. By promoting cartilage synthesis, thus treating the root of the problem, GS not only relieves the symptoms but also helps the body repair damaged joints. The clinical effect is impressive, especially when glucosamine's safety and lack of side effects are considered.

In one of the earlier comparative studies in which GS (1500 mg/day) was compared with ibuprofen (1200 mg/day), pain scores decreased faster in the first 2 weeks in the ibuprofen group. However, by week 4, the group that received GS experienced a significantly better improvement than the ibuprofen group.[35] Physicians rated the overall response as good in 44% of the GS-treated patients compared with only 15% of the ibuprofen group.

Additional studies designed to further evaluate the comparative effectiveness of GS versus NSAIDs provided even better evidence.[36–38,40] One study consisted of 200 subjects with OA of the knee given either GS (500 mg three times daily) or ibuprofen (400 mg three times daily) for 4 weeks.[36] Consistent with previous studies, the ibuprofen group experienced quicker pain relief. However, by the end of the second week, the group taking GS experienced results as good as those in the ibuprofen group, with one major exception: although the side effects with glucosamine were mild and affected only 6% of the group, ibuprofen produced more significant side effects much more frequently, with 35% of the group experiencing them.

A total of 319 patients with symptomatic OA of the knee received GS (1500 mg/day), piroxicam (20 mg/day), both drugs, or a placebo for 12 weeks followed by 8 weeks without treatment.[37] The main efficacy variable was represented by the Lequesne index, a standard method of assessing disease activity. In the GS group, the Lequesne index decreased by 4.8 points during treatment, for a decrease of 2.9 and 0.7 points, in the piroxicam and placebo groups, respectively ($p < 0.001$). The association did not differ from GS alone. GS did not differ in safety (14.8% incidence of adverse events during treatment) from placebo (23.7%) but was significantly better tolerated than piroxicam (40.9%) or the association (35%). The improvement in GS-treated patients persisted during the 8-week follow-up period, whereas the improvement with piroxicam did not. These impressive results with GS were achieved without side effects. Patients on GS had fewer side effects than the placebo and no dropouts. Table 83.1 provides the side effect and dropout values among the four groups.

In another study on OA of the knee (Table 83.2), GS showed comparable symptomatic relief but was better tolerated and produced residual benefit after discontinuation.[38]

TABLE 83.1 Side Effects and Dropouts From Glucosamine Compared With Piroxicam and Placebo

	Placebo	GS	Piroxicam	GS + Piroxicam
Incidence of side effects	24.4%	14.8%	40.9%	5.9%
Dropouts	3	0	20	3

TABLE 83.2 Results From a Double-Blind Study of Glucosamine Sulfate Versus Ibuprofen

Time	Glucosamine Sulfate	Ibuprofen
Knee Pain (Average Score)		
Before treatment	8.42	8.46
Wk 2	5.54	5.63
Wk 4	3.60	4.18
2 wk after treatment	3.26	3.84
Knee Swelling (Average Score)		
Before treatment	1.43	1.48
Wk 2	0.77	0.89
Wk 4	0.47	0.48
2 wk after treatment	0.36	0.54

	GLUCOSAMINE SULFATE		IBUPROFEN	
Clinical Improvement (Effectiveness)	After 4 wk	After 6 wk	After 4 wk	After 6 wk
Symptom-free	45%	55%	32%	36%
Improved	39%	32%	45%	41%
Unchanged	11%	7%	15%	14%
Worsened	5%	6%	8%	9%

Side Effects	Glucosamine Sulfate	Ibuprofen
Side effects	6%	16%
Dropouts	0%	10%

Data from Qiu GX, Gao SN, Giacovelli G, et al. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittelforschung.* 1998;48:469–474.

Another study showed an obvious advantage of GS over ibuprofen in patients diagnosed with temporomandibular joint (TMJ) OA. In the double-blind study, 40 women and 5 men received either GS (500 mg) or ibuprofen (400 mg) three times daily for 90 days. Fifteen patients taking GS (71%) and 11 taking ibuprofen (61%) improved, with positive clinical response taken as a 20% decrease in TMJ pain and function. Between-group comparison revealed that patients taking GS had a significantly greater decrease in TMJ pain with function, effect of pain, and acetaminophen used between days 90 and 120 compared with patients taking ibuprofen.[40]

In addition to showing benefit in double-blind studies, oral GS was shown to offer significant benefit in an open trial involving 252 doctors and 1506 patients in Portugal.[41] This large study provided valuable clinical information on the appropriate use of GS. The patients received 500 mg of GS three times a day over a mean period of 50 days. Symptoms of pain at rest, on standing, on exercise, and limited active and passive movements, all improved steadily throughout the treatment period. Objective therapeutic efficacy was rated by doctors as "good" in 59% of the patients and "sufficient" in a further 36%. Although this was not a controlled study, a 95% response rate is impressive. The results with GS were rated by both doctors and patients as being significantly better than those obtained with previous treatment, including NSAIDs, vitamin therapy, and cartilage extracts. GS produced good benefit in a significant portion of patients who did not respond to any other medical treatment.

In the study, obesity was associated with a significant shift from good to fair. This finding may indicate that higher dosages may be required for obese individuals or that oral glucosamine is not enough to counteract the added stress of obesity on the joints. Patients with peptic ulcers and individuals taking diuretics were also associated with a shift from good to sufficient in terms of efficacy and tolerance. Individuals with peptic ulcers should take GS with foods. Individuals taking diuretics may need to increase the dosage to compensate for reduced effectiveness. The improvement with glucosamine lasted for 6 to 12 weeks after the end of treatment.

Use in Athletes: Prevention, Acute Injury, and Surgical Recovery

GS may have a role as a preventive measure against the development of OA, especially in athletes subjected to joint strain, and an aid to recover from minor acute joint injuries (i.e., sprains and strains). One study investigated the chondroprotective action of GS on articular cartilage in athletes, the levels of biomarkers for CTX-II, and type II collagen synthesis (CPII) compared between soccer players and non-athlete controls before and after taking GS or placebo. CTX-II and CPII levels were substantially elevated in soccer players compared with those in controls, indicating that cartilage metabolism (CTX-II and CPII) was increased in soccer players. GS administration (1.5 and 3 g/day for 3 months) significantly decreased the CTX-II level; however, the effect disappeared after withdrawal of administration. In contrast, GS administration did not essentially affect the increased level of CPII. Furthermore, cartilage damage was evaluated using the ratio of CTX-II/CPII. The ratio in soccer players was significantly higher than that in controls, suggesting that CTX-II was relatively more enhanced compared with CPII in soccer players than in control students. Of tremendous importance, the ratio was reduced by glucosamine administration but returned to the preadministration level after GS supplementation ended. Together, these observations suggest that glucosamine is expected to exert a chondroprotective action in athletes (soccer players) by preventing CTX-II but maintaining CPII, although the effect is transient and disappears after stopping use.[42]

In a study designed to examine the effects of 4 weeks of glucosamine administration on functional ability and the degree of pain intensity in competitive male athletes after acute knee injury, 106 patients with an acute knee injury were randomized to either take GS (1500 mg/day) or a placebo for 28 days.[43] No significant difference was found between the glucosamine and placebo groups in mean pain intensity scores for resting and walking and the degree of knee swelling at the 7-, 14-, 21-, and 28-day assessments. There was no significant difference between passive knee flexibility at the 7-, 14-, and 21-day assessments, but after 28 days of treatment, the patients from the glucosamine group demonstrated significant improvement in knee flexion and extension compared with the placebo group. These results showed modest benefits and suggest use, but additional measures certainly should be used to speed recovery.

GS was also assessed for its ability to aid recovery in athletes undergoing anterior cruciate ligament (ACL) reconstruction. Thirty patients took either GS (1000 mg) or a placebo daily in addition to engaging in identical physical therapy. Results revealed significant improvements in both groups after 8 weeks, but no significant difference was detected between groups in any of the parameters. Although GS supplementation did not improve the rehabilitation outcomes of athletes after ACL reconstruction, the study may not have used a sufficient dosage to observe an effect (see "Dosage" section).[44]

DOSAGE

The standard dosage for GS is 1500 mg/day. It appears that administration as a single dosage may produce better results. Obese individuals may need higher dosages based on body weight (e.g., 20 mg/kg body weight daily). Individuals taking diuretics may also need to take higher dosages. Athletes or individuals who are subjecting their joints to greater wear and tear may need to increase the dosage to 3000 mg to maintain positive cartilage synthesis.

SIDE EFFECTS AND TOXICITY

GS has an excellent safety record in animal and human studies. Because of these studies, many experts have recommended that GS "be considered as a drug of choice for prolonged oral treatment of rheumatic disorders." Side effects, when they do appear, are generally limited to light to moderate gastrointestinal symptoms, including stomach upset, heartburn, diarrhea, nausea, and indigestion. If these symptoms occur, GS should be taken with meals.

Regarding people who are "sulfur sensitive," an important distinction must be made. When patients report they are allergic to sulfur, what they usually mean is that they are allergic to the so-called sulfa drugs or sulfite-containing food additives. It is impossible to be allergic to sulfur because sulfur is an essential mineral. The sulfate form of sulfur is present in human blood. In short, GS is extremely well tolerated, and no allergic reactions have been reported.

Concern has been expressed that glucosamine may influence insulin secretion or action, or both, based primarily on in vitro studies with concentrations of glucosamine not possible to achieve with oral supplementation or at recommended dosages. Detailed human studies showed GS had no effect on insulin secretion or action in either healthy subjects, those with type 2 diabetes, or those with insulin resistance.[45–48] Long-term studies with GS actually produced a nonsignificant lowering of fasting blood glucose concentrations in all groups of subjects.[48]

One side effect that has come up in elderly subjects is that GS may increase intraocular pressure (IOP).[49] In a double-blind study,[44] patients with osteoarthritis were randomized into either GS (750 mg three times daily) or a placebo group. A comprehensive ophthalmological examination including IOP was performed at baseline, month 1, and month 3. At 3 months, about 34% in the treatment group and 12.5% in the placebo group had clinically significant (defined as ≥2 mm Hg) rise in IOP. These results need to be confirmed, but physicians should monitor IOP in glaucoma patients taking GS.

DRUG INTERACTIONS

GS may potentiate the effect of warfarin (Coumadin). The World Health Organization's adverse drug reactions database documents 21 spontaneous reports of increased international normalized ratio associated with glucosamine use, 17 of which resolved when glucosamine was stopped. Two additional case reports also exist. Given the widespread use of GS, it does not appear to be a significant concern. Nonetheless, the use of warfarin and glucosamine may lead to an increased international normalized ratio, and physicians should monitor accordingly.[50]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bruyere O, Cooper C, Pelletier JP, et al. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: a report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin Arthritis Rheum*. 2014;44:253–263.
2. Kucharz EJ, Kovalenko V, Szántó S, et al. A review of glucosamine for knee osteoarthritis: why patented crystalline glucosamine sulfate should be differentiated from other glucosamines to maximize clinical outcomes. *Curr Med Res Opin*. 2016;32(6):997–1004.
3. Wangroongsub Y, Tanavalee A, Wilairatana V, et al. Comparable clinical outcomes between glucosamine sulfate-potassium chloride and glucosamine sulfate sodium chloride in patients with mild and moderate knee osteoarthritis: a randomized, double-blind study. *J Med Assoc Thai*. 2010;93:805–811.
4. Simental-Mendía M, Sánchez-García A, Vilchez-Cavazos F, et al. Effect of glucosamine and chondroitin sulfate in symptomatic knee osteoarthritis: a systematic review and meta-analysis of randomized placebo-controlled trials. *Rheumatol Int*. 2018. https://doi.org/10.1007/s00296-018-4077-2.
5. Lubis AMT, Siagian C, Wonggokusuma E, Marsetyo AF, Setyohadi B. Comparison of glucosamine-chondroitin sulfate with and without methylsulfonylmethane in grade i-ii knee osteoarthritis: a double blind randomized controlled trial. *Acta Med Indones*. 2017;49(2):105–111.
6. Setnikar I, Palumbo R, Canali S, et al. Pharmacokinetics of glucosamine in man. *Arzneimittelforschung*. 1993;43:1109–1113.
7. Rubin BR, Talent JM, Kongtawelert P, et al. Oral polymeric N-acetyl-D-glucosamine and osteoarthritis. *J Am Osteopath Assoc*. 2001;101:339–344.
8. Capps JC, Shetlar MR, Bradford RH. Hexosamine metabolism. I. The absorption and metabolism, in vivo, of orally administered D-glucosamine and N-acetyl-D-glucosamine in the rat. *Biochim Biophys Acta*. 1966;127:194–204.
9. Tesoriere G, Dones F, Magistro, et al. Intestinal absorption of glucosamine and N-acetylglucosamine. *Experientia*. 1972;28:770–771.
10. Sullivan MX, Hess WC. Cystine content of fingernails in arthritis. *J Bone Joint Surg Am*. 1935;16:185–188.
11. Senturia BD. Results of treatment of chronic arthritis and rheumatoid conditions with colloidal sulphur. *J Bone Joint Surg Am*. 1934;16:119–125.
12. Vignon E, Mazières B, Richard M, et al. In vitro evaluation of a chondro-protector. *Rev Rhum Mal Osteoartic*. 1990;57(9 Pt 2):S15–S18. [French].
13. Houpt JB, McMillan R, Wein C, et al. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. *J Rheumatol*. 1999;26:2423–2430.
14. Clegg DO, Reda DJ, Harris CL, et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. *N Engl J Med*. 2006;354:795–808.
15. Sawitzke AD, Shi H, Finco MF, et al. The effect of glucosamine and/or chondroitin sulfate on the progression of knee osteoarthritis: a report from the glucosamine/chondroitin arthritis intervention trial. *Arthritis Rheum*. 2008;58:3183–3191.
16. Sawitzke AD, Shi H, Finco MF, et al. Clinical efficacy and safety of glucosamine, chondroitin sulphate, their combination, celecoxib or placebo taken to treat osteoarthritis of the knee: 2-year results from GAIT. *Ann Rheum Dis*. 2010;69:1459–1464.
17. Crolle G, D'Este E. Glucosamine sulfate for the management of arthrosis: a controlled clinical investigation. *Curr Med Res Opin*. 1980;7:104–109.
18. Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthrosis. *Curr Med Res Opin*. 1980;7:110–114.
19. Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity of oral glucosamine sulfate in osteoarthrosis: a placebo-controlled double-blind investigation. *Clin Ther*. 1980;3:260–272.
20. D'Ambrosia E, Casa B, Bompani R, et al. Glucosamine sulphate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica*. 1982;2:504–508.
21. Braham R, Dawson B, Goodman C. The effect of glucosamine supplementation on people experiencing regular knee pain. *Br J Sports Med*. 2003;37:45–49.
22. Hughes R, Carr A. A randomized, double-blind, placebo-controlled trial of glucosamine sulphate as an analgesic in osteoarthritis of the knee. *Rheumatology*. 2002;41:279–284.
23. Rindone JP, Hiller D, Collacott E, et al. Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. *West J Med*. 2000;172:91–94.
24. Rozendaal RM, Koes BW, van Osch GJ, et al. Effect of glucosamine sulfate on hip osteoarthritis: a randomized trial. *Ann Intern Med*. 2008;148:268–277.
25. Cibere J, Thorne A, Kopec JA, et al. Glucosamine sulfate and cartilage type II collagen degradation in patients with knee osteoarthritis: randomized discontinuation trial results employing biomarkers. *J Rheumatol*. 2005;32:896–902.
26. Wilkens P, Scheel IB, Grundnes O, et al. Effect of glucosamine on pain-related disability in patients with chronic low back pain and degenerative lumbar osteoarthritis: a randomized controlled trial. *JAMA*. 2010;304:45–52.
27. Bruyere O, Pavelka K, Rovati LC, et al. Glucosamine sulfate reduces osteoarthritis progression in postmenopausal women with knee osteoarthritis: evidence from two 3-year studies. *Menopause*. 2004;11:138–143.
28. Reginster JY, Deroisy R, Rovati LC, et al. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet*. 2001;357:251–256.
29. Pavelka K, Gatterova J, Olejarova M, et al. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med*. 2002;162:2113–2123.
30. Bruyere O, Pavelka K, Rovati LC, et al. Total joint replacement after glucosamine sulphate treatment in knee osteoarthritis: results of a mean 8-year observation of patients from two previous 3-year, randomised, placebo-controlled trials. *Osteoarthritis Cartilage*. 2008;16:254–260.
31. Bruyere O, Honore A, Ethgen O, et al. Correlation between radiographic severity of knee osteoarthritis and future disease progression. Results from a 3-year prospective, placebo-controlled study evaluating the effect of glucosamine sulfate. *Osteoarthritis Cartilage*. 2003;1:1–5.
32. Christgau S, Henrotin Y, Tanko LB, et al. Osteoarthritic patients with high cartilage turnover show increased responsiveness to the cartilage protecting effects of glucosamine sulphate. *Clin Exp Rheumatol*. 2004;22:36–42.
33. Fransen M, Agaliotis M, Nairn L, et al. Glucosamine and chondroitin for knee osteoarthritis: a double-blind randomised placebo-controlled clinical trial evaluating single and combination regimens. *Ann Rheum Dis*. 2015;74(5):851–858.
34. Petersen SG, Saxne T, Heinegard D, et al. Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training. *Osteoarthritis Cartilage*. 2010;18:34–40.
35. Lopes Vaz A. Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulfate in the management of osteoarthrosis of the knee in out-patients. *Curr Med Res Opin*. 1982;8:145–149.
36. Muller-Fassbender H, Bach GL, Haase W, et al. Glucosamine sulfate compared to ibuprofen in osteoarthritis of the knee. *Osteoarthritis Cartilage*. 1994;2:61–69.
37. Rovati L. The clinical profile of glucosamine sulfate as a selective symptom modifying drug in osteoarthritis: current data and perspective. *Osteoarthr Cartil*. 1997;5(SA):72.
38. Qiu GX, Gao SN, Giacovelli G, et al. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittelforschung*. 1998;48:469–474.
39. Herrero-Beaumont G, Ivorra JA, Del Carmen Trabado M, et al. Glucosamine sulfate in the treatment of knee osteoarthritis symptoms: a randomized, double-blind, placebo-controlled study using acetaminophen as a side comparator. *Arthritis Rheum*. 2007;56:555–567.
40. Thie NM, Prasad NG, Major PW. Evaluation of glucosamine sulfate compared to ibuprofen for the treatment of temporomandibular joint osteoarthritis: a randomized double blind controlled 3 month clinical trial. *J Rheumatol*. 2001;28:1347–1355.

41. Tapadinhas MJ, Rivera IC, Bignamini AA. Oral glucosamine sulfate in the management of arthrosis: report on a multi-centre open investigation in Portugal. *Pharmatherapeutica*. 1982;3:157–168.
42. Yoshimura M, Sakamoto K, Tsuruta A, et al. Evaluation of the effect of glucosamine administration on biomarkers for cartilage and bone metabolism in soccer players. *Int J Mol Med*. 2009;24:487–494.
43. Ostojic SM, Arsic M, Prodanovic S, et al. Glucosamine administration in athletes: effects on recovery of acute knee injury. *Res Sports Med*. 2007;15:113–124.
44. Eraslan A, Ulkar B. Glucosamine supplementation after anterior cruciate ligament reconstruction in athletes: a randomized placebo-controlled trial. *Res Sports Med*. 2015;23(1):14–26.
45. Salazar J, Bello L, Chávez M, et al. Glucosamine for osteoarthritis: biological effects, clinical efficacy, and safety on glucose metabolism. *Arthritis*. 2014;2014:432463. https://doi.org/10.1155/2014/432463.
46. Scroggie DA, Albright A, Harris MD. The effect of glucosamine-chondroitin supplementation on glycosylated hemoglobin levels in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Arch Intern Med*. 2003;163:1587–1590.
47. Tannis AJ, Barban J, Conquer JA. Effect of glucosamine supplementation on fasting and non-fasting plasma glucose and serum insulin concentrations in healthy individuals. *Osteoarthritis Cartilage*. 2004;12:506–511.
48. Simon RR, Marks V, Leeds AR, et al. A comprehensive review of oral glucosamine use and effects on glucose metabolism in normal and diabetic individuals. *Diabetes Metab Res Rev*. 2011;27:14–22.
49. Esfandiari H, Pakravan M, Zakeri Z, et al. Effect of glucosamine on intraocular pressure: a randomized clinical trial. *Eye (Lond)*. 2017;31(3):389–394.
50. Knudsen JF, Sokol GH. Potential glucosamine-warfarin interaction resulting in increased international normalized ratio: case report and review of the literature and MedWatch database. *Pharmacotherapy*. 2008;28:540–548.

84

Glutamine

Kristaps Paddock, ND, and *Peter B. Bongiorno*, ND, MSAc, LAc*

OUTLINE

INTRODUCTION

Glutamine (Fig. 84.1) is the most abundant amino acid in blood and muscle tissue. It comprises approximately 6% of mixed whole body protein and is unique among amino acids in that it is a preferred fuel of rapidly dividing cells, such as intestinal and immune cells, and is important in maintaining pancreatic function.[1-3] Glutamine is involved in the transport of circulating amino nitrogen and is an important intermediary that allows for accelerated gluconeogenesis from amino acids that are released by the skeletal muscle during stress states.[4] In addition, glutamine is used as a precursor for DNA and glutathione synthesis.[5] As one of the principal fuels used by the cells of the intestinal lining, it accounts for 35% of enterocyte energy production.

Although readily available in the diet and synthesized in the body from glutamate and ammonia, supplementation is known to enhance the energy metabolism of the gastrointestinal mucosa, thus stimulating regeneration.[6] Although glutamine is not considered essential in healthy people, there is evidence that the increased need for glutamine in stressed states such as burns, septicemia, endotoxemia, intestinal failure, and critical illness may result in it being "conditionally essential."[3,7,8]

Food Sources

Typical food sources of glutamine include animal and plant proteins. Typical foods are cabbage, beef, chicken, fish, legumes, miso (a salty, fermented bean product), and dairy products.

Forms

The nomenclature of L-glutamine and glutamine are used interchangeably. D-glutamine is the stereoisomer of L-glutamine and does not have any known biological activity. L-glutamine is not soluble in water, and aqueous solutions are unstable at temperatures of 22°C to 24°C. As a result, the more soluble and more stable dipeptides such as alanyl-glutamine are used as delivery forms of L-glutamine in total parenteral nutrition solutions.[9,10]

Physiological Effects

Intestinal Repair and Protection

Animal and human studies suggest that glutamine stimulates intestinal mucosal growth[11] and protects from mucosal atrophy. Glutamine prevents intestinal mucosal damage and was shown to decrease bacterial leakage across the intestines after they are damaged, presumably by stimulating repair.[12] Glutamine is thought to accomplish this by strengthening epithelial tight junctions and also by preventing paracellular permeabilities through an epidermal growth factor receptor–dependent mechanism (Fig. 84.2).

In one tissue culture experiment, intestinal epithelium cells were treated with acetaldehyde to compromise barrier function. These cells were treated with L-glutamine, D-glutamine, L-asparagine, L-arginine, L-lysine, or L-alanine. Only the L-glutamine demonstrated a benefit by decreasing aldehyde effects on transepithelial resistance. Furthermore, L-glutamine–treated cells decreased permeability that was dose dependent. L-glutamine reduced the acetaldehyde-induced disturbance of transmembrane structures, such as occludin, zonula occludens-1, E-cadherin, and β-catenin from the intercellular junctions. Lastly, L-glutamine induced a rapid increase in the tyrosine phosphorylation of the epidermal growth factor receptor. No other amino acids demonstrated this effect.[13]

Acid Base Balance

Glutamine plays an important role in acid–base homeostasis.[14] Glutamine is synthesized from glutamate and the toxic alkaline waste product ammonia by the enzyme glutamine synthetase, which requires magnesium and adenosine triphosphate. When ammonia levels are elevated, the body effectively removes ammonia from the blood by synthesizing glutamine. Conversely, if the blood is too acidic, the glutamine

*Previous edition contributor

can be broken down into glutamate and ammonia, which increases blood pH. Ammonia can bind hydrogen ions to produce ammonium cations, which are excreted in the urine along with chloride anions. Bicarbonate ions are simultaneously released into the bloodstream. Clinical studies showed that relatively small oral doses of glutamine can elevate plasma bicarbonate concentrations in healthy adults.

In one study, 2 g of glutamine was dissolved in a cola drink and ingested over a 20-minute period 45 minutes after a light breakfast. Control subjects were given soda only. Blood samples were taken 1 week before, at baseline, and subsequently at three separate 30-minute intervals after ingestion of the glutamine drink or placebo. Eight of 9 subjects responded to the oral glutamine load with a significant increase in plasma glutamine at 30 and 60 minutes before returning to the baseline value at 90 minutes. Ninety minutes after the glutamine was administered, plasma bicarbonate concentration was found to be increased. Circulating plasma growth hormone concentration was elevated as well. Concomitant with enhanced renal acid secretion, glutamine ingestion also caused an increase in the glomerular filtration rate.[15]

The authors of this study explained that their results showed that it was unlikely L-glutamine was a direct precursor of bicarbonate. Instead, L-glutamine appeared to play an indirect role in accelerating acid secretion through mechanistic changes in the kidneys. Human studies showed that urinary ammonium excretion is altered by changes in glutamine intake.[16]

Chronic metabolic acidosis is a common clinical problem encountered in catabolic states such as sepsis, shock, and diabetes, and is a major factor in many biological derangements.[17] Because glutamine becomes an essential amino acid in catabolic states when the increased demand exceeds the body's capability to synthesize it,[18] glutamine supplementation may be quite useful to maintain pH homeostasis in patients with acidotic conditions.

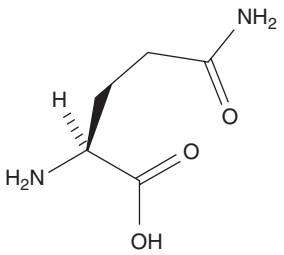


Fig. 84.1 Glutamine.

Glutathione Repletion

Glutathione (GSH) is a tripeptide consisting of glutamate, cysteine, and glycine (Fig. 84.3). As a reservoir source for glutamate in the body, the availability of glutamine appears crucial for the regeneration of glutathione stores in the liver during hepatic injury; in skeletal muscle after major trauma, sepsis, or surgery; and in chemotherapy-injured heart muscle.[19-21] Glutamine can enhance intracellular repletion of glutathione, an important scavenger of reactive oxygen species.[22] Rat studies demonstrated that during 5-fluorouracil–induced free radical–mediated hepatic injury, glutamine increased glutathione biosynthesis and preserved the glutathione stores in hepatic tissue.[19] A more recent study found that glutamine protected porcine enterocytes from cell death from oxidative damage by regulating glutathione synthesis.[23]

The promise of these in vitro and animal studies has also been verified by human studies. Seventeen patients who underwent a standardized surgical procedure were prospectively given 0.56 g/kg body weight/day of glutamine or a placebo. Using percutaneous muscle biopsies and blood samples, there were no significant decreases in total or reduced glutathione in the glutamine-supplemented group 24 and 72 hours after the operation. In contrast, the placebo group experienced total muscle glutathione losses of 47 ± 8% and 37 ± 11%, as well as reduced glutathione decreases of 53 ± 10% and 45 ± 16% at 24 and 72 hours, respectively. An in vivo study of 12 HIV+ patients found that daily supplementation of high-dose glutamine (20 g/day) increased plasma measurements of GSH; this same study also found that *N*-acetylcysteine was effective at increasing GSH levels.[24]

Protein Sparing

Glutamine is a regulator of muscle proteolysis,[25] and supplementation can attenuate loss of protein in the muscle. Experiments using animal cancer models demonstrated decreased protein loss and simultaneous protection of immune and gut-barrier function during radiation therapy in patients with advanced cancer.[5] In children with severe muscle

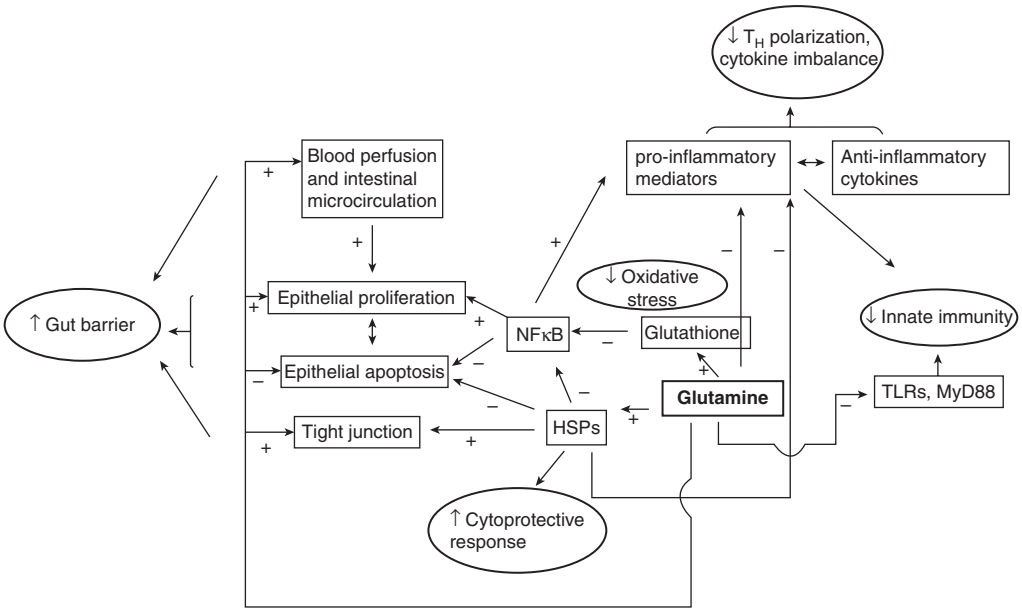


Fig. 84.2 The role of glutamine in intestinal repair and protection. (From Xue H. Glutamine therapy in colitis models. In: Rajendram R, Preedy V, Patel V, eds. *Glutamine in Clinical Nutrition. Nutrition and Health*. New York, NY: Humana Press; 2015.)

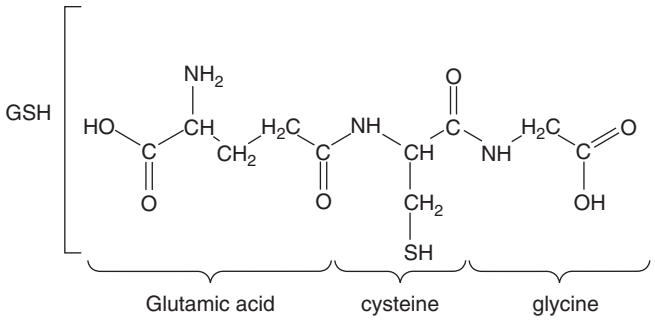


Fig. 84.3 Glutathione.

wasting, 5 hours of oral glutamine was shown to have protein-sparing effect (see later discussion on "Cachexia").[26]

Immune Support

Although poorly understood, it appears that glutamine has an immune-modulating effect by enhancing interleukin (IL)-6 levels[27] and lymphocyte function.[28] IL-6 plays an essential role in the final differentiation of β-cells into immunoglobulin-secreting cells, nerve cell differentiation, and acute phase reactants in hepatocytes. Exercise by itself is known to induce an elevenfold increase in plasma IL-6. Glutamine supplementation further enhances IL-6 levels.[27] The ability of lymphocytes to proliferate and generate lymphokine-activated killer cell activity in vitro was found to be glutamine dependent.[29] Additionally, glutamine-enriched parenteral nutrition demonstrated enhanced lymphocyte activity in patients who received high doses of chemotherapy after stem cell transplantation for hematologous malignancy.

CLINICAL APPLICATIONS

Intestinal Permeability–Related Conditions

A number of conditions are linked to intestinal permeabilities, including chronic urticaria,[30] inflammatory bowel disease (Crohn's disease),[31-33] celiac disease,[34] liver and biliary cirrhosis and cases of portal hypertension,[35,36] systemic sclerosis,[37] diabetes,[38] rheumatologic disorders,[39,40] cystic fibrosis,[41] alcohol overuse,[42] adult and child asthma,[43] HIV/AIDS,[44] nonsteroidal anti-inflammatory drug–treated arthritis patients,[45] moderate to major burn injuries,[46] corticosteroid use,[47] cardiopulmonary bypass patients,[48,49] and acute metal toxicities.[50] To evaluate these permeabilities, sucrose serves as a marker for gastroduodenal permeability and the urinary lactulose/mannitol ratio for intestinal permeability, after administration of these sugars.[30] From a naturopathic perspective, the underlying cause of many of these conditions may stem from food allergies that contribute first to chronic inflammation in the intestinal tract[30] and then to systemic endotoxemia. Certain conditions such as cardiopulmonary bypass can cause intestinal ischemia,[51] which is then the primary insult that causes permeabilities in these patients. The use of glutamine can help heal these permeabilities, thus removing a mode of pathogenesis in these variegated conditions.

Infectious Diarrhea

Animal models showed the usefulness of glutamine in diarrhea to augment sodium and water absorption and to enhance blood glucose and body weight.[52] A rat model of cholera toxin–induced diarrhea showed that glutamine was able to improve water and electrolyte intestinal absorption even better than traditional glucose solutions.[10] One placebo-controlled, double-blind, randomized trial human study evaluated glutamine to treat acute diarrhea in 128 otherwise healthy children. Of these 6- to 24-month-olds, 63 received 0.3 g/kg per day of glutamine and 65 controls received a placebo for 7 days. The average duration of diarrhea in the glutamine-treated group was significantly shorter than that of the placebo group (3.40 ± 1.96 vs 4.57 ± 2.48 days, respectively). However, no differences in serum IL-8 and secretory immunoglobulin-A were found between groups at the beginning of treatment or 1 week later.[53]

Clearly, glutamine holds promise for enhancing repair of mucosal injury caused by a wide range of infections or toxic agents and thus has great potential as a nutritional therapeutic for patients with enteric infection.[54]

Postsurgical Complications of the Gastrointestinal Tract

Patients undergoing abdominal surgeries such as gastrectomies, sigmoidectomies, cholecystectomies, colectomies, and rectal resections are at risk for the development of intestinal failure or short bowel syndrome (SBS). In SBS, a serious malabsorption of fluid, electrolytes, and other nutrients can occur, placing the patient at higher morbidity and mortality risk.[55] Trauma from abdominal surgery may also compromise the intestinal mucosa to the point where bacteria and endotoxins can easily transfer through the intestinal wall and invade tissue and blood in an event called bacterial translocation. Through inflammatory mechanisms, bacteria, and endotoxic septic conditions, the intestinal mucosal barrier can be adversely affected and cause further damage, thus forming a vicious circle. Severe cases result in systemic inflammatory response syndrome and multiple organ dysfunction syndrome.[56]

In a regimen that includes growth hormone and diet changes, glutamine can help difficult cases to enhance bowel adaptation. In one study of 10 patients with SBS who previously failed to adapt to enteral nutrients, 8 subjects received exogenous growth hormone, supplemental glutamine, and a modified high-carbohydrate, high-fiber diet. Two patients were treated with the modified diet alone. Three weeks of treatment with growth hormone, glutamine, and a modified diet significantly increased total caloric absorption from approximately 60% to 75%, protein absorption from 49% to 63%, and carbohydrate absorption from 60% to 82%. Water absorption increased from 46% to 65%, and sodium from 49% to 69%. Fat absorption did not change. Diet alone did not influence nutrient absorption or stool output. After 28 days of therapy, the patients were discharged and instructed to continue the diet and glutamine treatment.[57] It is unknown whether glutamine and diet changes alone, without concomitant growth hormone administration, would have the same positive effect.

In a second study, 20 patients who underwent abdominal surgery were randomized into two groups receiving oral administration of 30 g of glutamine or a placebo in divided doses for 7 days. Serum glutamine concentration was significantly decreased in the placebo group and increased in the glutamine group after 7 days. Markers of intestinal permeability were significantly increased in the placebo group and decreased in the glutamine group. Additionally, the serum markers of endotoxin, diamine oxidase, and malondialdehyde concentrations were significantly decreased in the glutamine group compared with those in the placebo group. Temperatures, heart rates, and white blood cell counts were also significantly lower in the glutamine group.[56]

Ischemia reperfusion of the gut is also a common event in various clinical conditions, such as trauma, burn, septic shock, cardiac or aortic surgery, and liver or small bowel transplantation, and is associated with a high death rate. Intestinal ischemia reperfusion can cause edema and disruption of the structural integrity and function of the intestinal mucosa and associated vascular tissue. It may set the stage for endotoxemic translocation of a number of bacteria, including *Escherichia coli, Enterococcus, Pseudomonas, Proteus,* and *Staphylococcus.* Studies of animal models demonstrated that glutamine, when supplemented as total parenteral nutrition, protected the intestines from morphological

and functional mucosal injury after intestinal ischemia reperfusion. Furthermore, intestinal permeabilities and the incidence of bacterial translocation in intestinal ischemia reperfusion animals were also prevented in a dose-dependent manner by glutamine supplementation.[58,59]

The gastrointestinal tract is susceptible to SBS, severe intestinal permeabilities, ischemia perfusion damage, systemic inflammatory response during trauma, various medical conditions, and abdominal postoperative periods. Glutamine can decrease intestinal permeability, maintain an intestinal barrier, and attenuate systemic inflammatory response in early postoperative patients.

Chemotherapy and Radiation Side Effects

Standard cancer therapies often include the use of chemotherapy and radiation, which can injure rapidly dividing intestinal cells. It was shown that during chemotherapeutic and radiotherapy insult, glutamine reduced degeneration of intestinal mucosa in rats, prevented intestinal mucosal injury,[58] protected liver function through enhanced glutathione biosynthesis and storage in hepatic tissue, increased immune function, and reduced permeability of the gut.[19,28]

In one investigation, 70 patients with colorectal cancer were randomly assigned to oral glutamine at 18 g/day or placebo before the first regimen of 5-fluorouracil and folinic acid administered intravenously for 5 days. Glutamine was given 5 days before, during, and after chemotherapy. Using D-xylose urinary excretion and cellobiose/mannitol evaluation, damage to the intestines was assessed at baseline, and 4 and 5 days after the end of the first cycle of chemotherapy. After one cycle of chemotherapy, the reduction in D-xylose absorption and reduction of mannitol was significantly greater in the placebo group (7.1% vs. 3.8% and 9.2% vs. 4.5%, respectively). Urinary recovery of cellobiose was not different between the study arms. Accordingly, the cellobiose/mannitol ratio increased more in the placebo treatment group. Furthermore, diarrhea parameters, and the average number of antidiarrheal opiate loperamide tablets needed, were reduced in the glutamine arm, thus supporting the positive clinical effect of this low-cost supplement.[11]

Oropharyngeal mucositis, or mouth sores, and accompanying swallowing difficulty are other untoward results of radiotherapy and can be a major source of suffering in patients with head and neck cancer. Glutamine during and after chemotherapy appears to be an excellent way to safely decrease the incidence of mouth sores. One investigation of 17 patients with head and neck cancer who received primary or adjuvant mouth irradiation for 5 days a week were randomized to either adjunctive glutamine suspension of 16 g in 240 mL normal saline or a saline placebo. Patients were instructed to swish the test solutions (30 mL) four times daily. The duration of objective oral mucositis was significantly shorter in the glutamine arm.[60] A second randomized, double-blind crossover trial observed 24 patients who were given a glutamine or placebo suspension to swish and swallow on days of chemotherapy administration and for at least 14 days after therapy. Significant improvement was observed in the glutamine group. Additionally, the duration of mouth pain was 4.5 days less in chemotherapy courses with concomitant glutamine supplementation. The severity of oral pain was reduced so significantly when glutamine was used that a patient could venture past soft foods 4 days sooner compared with placebo.[61]

Glutamine studies validating its use are also beginning to emerge in other areas of oncology. In a study of esophageal cancer patients, 13 patients were randomized into two groups, controls and a group that received oral glutamine supplemented at a dosage of 30 g/day for 4 weeks. It was observed that supplementation of glutamine enhanced lymphocyte mitogenic function and reduced permeability of the gut during radiochemotherapy.[5] Patients who underwent bone marrow transplant and myelosuppressive chemotherapy for acute myeloid leukemia also found that parenteral glutamine therapy could improve neutrophil recovery, although no change in neutropenic fever was shown.[62] Given that glutamine improves the structure and function of the gut, it is understandable that multiple parameters and markers of healthy physiological function will improve with its use.

It should be noted that glutamine's efficacy may depend on a number of other factors, including the specific chemotherapeutic prescribed and dosage. A study of 65 patients with advanced breast cancer receiving doxifluridine were prescribed 30 g/day of glutamine in three divided doses of 10 g each or a placebo for 8 consecutive days during each interval between chemotherapy, which was administered from days 1 to 4. In this case there was no statistical difference with regard to diarrhea morbidity, nor did glutamine affect the severity and duration of tumor growth.[63] Interestingly, a study of bone marrow transplantation patients found that allogeneic transplantation patients (those receiving bone marrow from another individual) did not have the same beneficial mouth pain reduction that autologous transplantation patients (those who donated their own marrow) experienced when receiving glutamine support. However, in the work mentioned previously, the amounts of glutamine were less than those used in other studies.

It was also theorized that methotrexate use in the allogeneic patients might have been responsible for the decreased protection. Nevertheless, in the allogeneic patients, the 28-day survival was still increased.[64] A third multicenter study of 129 patients found no protection against diarrhea when used adjunctively with pelvic radiation therapy. These patients received 4 g of glutamine or a placebo by mouth, which was also a significantly lower dose than the more successful studies employed.[65]

Although intestinal function is greatly compromised with chemotherapy and radiation treatment, cardiac function is commonly affected as well. The use of doxorubicin therapy for breast cancer is often limited by cardiomyopathic heart changes that often result in congestive heart failure. One rat study simulated doxorubicin treatment with and without glutamine support and found that oxidative damage to the heart was diminished in the glutamine-treated group, probably as a result of glutamine's ability to maintain cardiac tissue glutathione levels (see later discussion on "Cardiac Disease").[21]

Cachexia

Cancer-related cachexia is caused by a diverse combination of accelerated protein breakdown and slowed protein synthesis.[66] There has been considerable interest in giving supplemental glutamine to cancer patients because glutamine is taken up by the growing tumor, and any subsequent deficiency of glutamine in the host may cause cancer cachexia.[5]

One animal study found that glutamine levels in plasma and skeletal muscle were decreased in tumor-bearing rats, whereas glutamine production and the conversion of arginine to glutamine were increased. In rats supplemented with glutamine, total parenteral nutrition demonstrated a reduced whole body protein breakdown rate during chemotherapy.[5] A clinical study of patients with stage IV solid malignancies who had weight loss of at least 5% randomly assigned the participants in a double-blind fashion to either a control mixture of nonessential amino acids or treatment of 14 g/day of glutamine, along with the leucine metabolite β-hydroxy-β-methylbutyrate (3 g/day) and L-arginine (14 g/day). Within 4 weeks, the patients supplemented with the glutamine mixture gained 0.95 ± 0.66 kg of body mass, whereas control subjects lost 0.26 ± 0.78 kg during the same period. This effect continued over the 24 weeks, with no negative effect of treatment on the incidence of adverse effects or quality of life measures.[66]

Because the glutamine derived from skeletal muscle is trapped by the tumor, there is a theoretical concern that glutamine supplementation in cancer patients could potentially encourage tumor growth. One research group, however, showed that glutamine supplementation does not appear to enhance DNA content in tumor cells.[5,67] Additionally, some tissue culture studies provided evidence that glutamine might even inhibit cancer promotion.[68] Although research is necessary to clarify this point, given the immediate risk of mortality due to protein loss in cancer patients, it still would seem prudent to administer glutamine to those patients at greater immediate risk of cachexia-related death.

Human Immunodeficiency Virus

Loss of body cell mass and drug-associated gastrointestinal problems often occur in patients with HIV infection. In these cases, the patient's ability to survive can be affected in the long term. Given the role glutamine plays in cachexic body mass loss (see previous section on "Cachexia"), reversal of malabsorption,[69] and protection of the small intestine,[70] glutamine deficiency is a probable causal factor in HIV-associated wasting.[71]

Preliminary clinical studies suggested improvements in HIV-positive patients dosed at 8 g/day with regard to intestinal permeability and intestinal absorption. The authors of this study correctly suggested that at least 20 g/day might be necessary for more significant improvements.[72] A double-blind, placebo-controlled trial of 26 patients with greater than 5% weight loss since their disease onset used a glutamine and antioxidant regimen, including 40 g/day of glutamine in divided doses or 40 g of a glycine placebo for 12 weeks. Over 3 months, the glutamine/antioxidant group gained 2.2 kg in body weight (3.2%), whereas the control group gained only 0.3 kg (0.4%).

The glutamine-antioxidant group gained 1.8 kg in body cell mass, whereas the control group gained 0.4 kg. Of note, the intracellular water increased in the glutamine-antioxidant group but not in the control group.[73] Glutamine can help HIV patients decrease the severity of iatrogenic diarrhea. Twenty-five patients suffering for more than a month from nelfinavir-associated diarrhea were randomized in a double-blind, placebo-controlled, crossover trial to receive L-glutamine at 30 g/day or a placebo for 10 days. In this study, the L-glutamine significantly reduced the severity of nelfinavir-associated diarrhea and produced improved quality of life compared with placebo.[74]

Peptic Ulcers

Cabbage juice, a key source of glutamine, has been well documented as having remarkable success in treating peptic ulcers. One liter per day of the fresh juice, taken in divided doses, resulted in total ulcer healing in an average of only 10 days. Further research showed that the high glutamine content of the juice is probably responsible for the efficacy of cabbage in treating these ulcers. In a double-blind clinical study of 57 patients, 24 using 1.6 g/day of glutamine and the rest using conventional therapy (antacids, antispasmodics, milk, and bland diet), glutamine proved to be the more effective treatment. Half of the glutamine patients showed complete healing (according to radiographic analysis) within 2 weeks, and 22 of the 24 showed complete relief and healing within 4 weeks.[101] Although the mechanism for these results is unknown, it was postulated by the authors to be due to the role of glutamine in the biosynthesis of the hexosamine moiety in certain mucoproteins. These moieties may stimulate mucin synthesis, which would benefit peptic ulcer patients.

Severe Burns

Plasma glutamine levels were demonstrated to be profoundly decreased after severe burns in adults. This may at least partially explain the impaired cellular immunity that is seen in burn patients. Burn victims given glutamine showed better intestinal repair, a higher quality of wound healing, and reduced hospitalization. In one study, 48 severe burn patients were randomly divided into two groups: a control group that took a placebo, and a glutamine-treated group that received 0.5 g of glutamine/kg body weight/day, both for 14 days. After taking glutamine for 14 days, plasma glutamine concentration was significantly increased in the glutamine group compared with the control group. In addition, a greater quality of wound healing as well as shorter hospital stays were experienced in the glutamine-treated burn patients.[75]

In another study, dosage of L-glutamine at 0.6 g/kg per day did not result in an immediate whole body protein gain (an important factor in a burn patient's convalescence) and resulted in an insignificant increase in plasma glutamine.[4] However, this study measured only the first 48 hours, which might not have been enough time to show a long-term benefit.

Another study of 45 severely burned adults found that those randomized to receive enteral glutamine experienced a reduction in blood infection by a factor of three, and mortality risk was lowered.[76] Another investigation of burned patients whose total body surface burns ranged from 50% to 80% and third-degree burns ranged from 20% to 40%, but who did not have respiratory injuries, found improved gut permeability, initially decreased plasma endotoxin levels, and reduced hospitalization.[77]

Low-Birth-Weight Infants

Infants with a birth weight of less than 1000 to 1500 g may be especially susceptible to glutamine depletion, as nutritional supply of glutamine is limited in the first weeks after birth. This may increase morbidity by contributing to problems with gut integrity, as well as immune suppression.[3] One study of 35 ill preterm neonates of less than 1000 g were randomized to receive either glutamine-supplemented parenteral nutrition or standard parenteral nutrition. Although there were no significant differences between the groups in white cell count, differential white cell count, blood urea nitrogen, plasma ammonia, lactate, pyruvate, plasma glutamine, or glutamate, the median time to achieving full enteral nutrition was shorter in the glutamine group (13 vs. 21 days). Parenteral glutamine was well tolerated and considered safe for these preterm neonates.[78]

However, other studies found that formula supplemented with glutamine in growing preterm infants was entirely metabolized in the gut and did not have a discernable effect on whole-body protein and nitrogen kinetics.[79] A large multicenter, double-blind, randomized trial of infants with a birth weight of 401 to 1000 g were given either a control or 20% isonitrogenous solution by parenteral nutrition for up to either 120 days of age, death, or discharge from the hospital. Of the 721 infants who were assigned to glutamine supplementation, 370 (51%) died or developed late-onset sepsis, compared with 343 of the 712 infants (48%) assigned to control. Although no adverse effects were noted as a result of being given glutamine, this study demonstrated that glutamine did not decrease mortality. This study and others also found no reduction in the incidence of sepsis in these young patients.[80,81]

Exercise and Weight Lifting

Glutamine is considered to have an anabolic effect on skeletal muscle. Given the benefits on glutathione reserves, protein catabolism, and intestinal integrity, some glutamine enthusiasts believe that glutamine supplementation may be useful for exercise and strength training as well (Fig. 84.4). One small study suggested that oral glutamine increases growth hormone release.[15] Even so, clinical trials studying glutamine as an exercise performance enhancer are not encouraging.

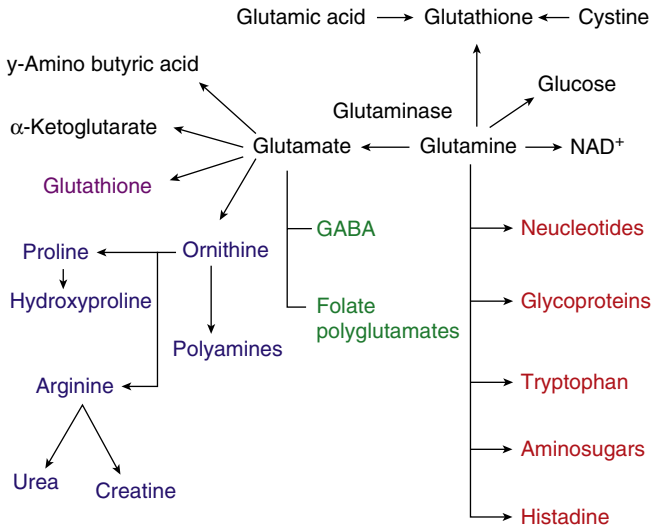


Fig. 84.4 The potential roles of glutamine in exercise.

A double-blind, placebo-controlled, crossover study had 6 resistance-trained men lift weights after the ingestion of glutamine or glycine at 0.3 g/kg body weight or a placebo. One hour after ingestion, subjects performed four total sets of exercise to momentary muscular failure, including two sets of leg presses at 200% of body weight and two sets of bench presses at 100% of body weight. Despite glutamine's possible role in exercise, there were no actual differences in the average number of maximal repetitions performed in the leg press or bench press exercises among these three groups.[82] Other studies using 0.3 to 0.9 g/kg of body weight also demonstrated no changes in exercise performance, body composition, or muscle protein degradation in young healthy adults.[83,84] It is possible that beneficial effects of glutamine are best detected only in patients with chronic illness and those with compromised physiology instead of normal, healthy individuals.

Cardiac Disease

Cardiac disease is a recognized stress on the physiology of the gastrointestinal and immune system. Animal studies supported the notion that glutamine can help recovery from cardiac ischemia and help the heart recover after reperfusion injury.[85,86] As noted earlier, glutamine can protect the heart from damage due to chemotherapy regimens by its glutathione-replenishing effect (see earlier discussion on "Chemotherapy and Radiation Side Effects").[19] The benefits to the gastrointestinal system in those heart patients experiencing ischemic gut episodes after cardiac bypass were also previously noted.[48,49] Animal research showed that greater levels of plasma glutamine also prevented decreases in the ratio between adenosine triphosphate to adenosine diphosphate in myocardial tissue.[86]

Unfortunately, little research has been done to evaluate the clinical use of glutamine in various cardiac situations. One investigation of patients with chronic stable angina received a single 80 mg/kg oral dose of glutamine or a placebo in a double-blind, random fashion 40 minutes before a standard exercise test. This single dose of glutamine significantly increased plasma glutamine concentration from 419 to 649 μmol/L. Moreover, the glutamine appeared to encourage positive changes in ST depression on the echocardiogram.[86] Clearly, more research is required, but given that heart disease is the leading reason for mortality, natural and safe treatments like glutamine should be further explored.

Crohn's Disease

The effect of glutamine on Crohn's disease might be expected to be positive, given its role as a nutrient source for enterocytes. However, studies have been mixed. One study failed to show an effect from a 4-week course of a glutamine-enriched diet in the treatment of active Crohn's disease.[87] Another failed to show an effect on small intestine permeability after long-term supplementation of 21 g/day of glutamine; patients were screened for plasma glutamine, plasma glutamate, plasma ammonium, Crohn's disease activity index, C-reactive protein, and nutritional status.[88] However, a more recent study found that glutamine did improve intestinal permeability and morphology in patients with Crohn's disease. Patients were administered 0.5 g/kg ideal body weight/day for 2 months and had significant improvements in permeability, as measured by lactulose mannitol excretion, and in morphology.[89] This study also included an active control group who were administered whey protein, and also experienced improvements in permeability and morphology. A review of the available literature available until November 2015 determined that there was insufficient evidence to draw conclusions on the efficacy or safety of glutamine in Crohn's disease.[90] The role of glutamine in Crohn's disease thus remains unresolved.

Sickle Cell Disease

Sickle cell disease is a complex condition in which increased oxidative stress causes deformation of the red blood cell protein hemoglobin, which then causes deformation of red blood cells themselves. These deformed cells are then prone to hemolysis by the spleen, causing the well-known anemia, as well as obstruction of capillaries, causing pain, necrosis, and organ damage. Starting in the 1990s, it had been observed that glutamine improved NAD redox potential and NADH levels in sickled RBCs, and that these changes might decrease oxidative susceptibility of red blood cells in the disease.[91] Later research also found that oral administration of 30 g of glutamine per day for at least 4 weeks decreased adhesion of sickle RBCs to endothelial cells.[92] More recently, a drug consisting only of l-glutamine has been approved to treat sickle cell anemia.

Other Conditions

A number of other medical conditions, such as alcoholism, pancreatitis, and brain injury, will further adversely affect the health of a patient who already has protein and energy needs and is more susceptible to infection. Although more studies are necessary, the following includes a few interesting conditions not mentioned earlier.

In alcoholics, glutamine supplementation (1 g/day) was shown to reduce voluntary alcohol consumption in uncontrolled human studies and experimental animal studies.[93-96] Despite the fact that this research is about 50 years old, there has never been any follow-up to these preliminary studies. This is unfortunate, because the results were quite promising, finding glutamine to be safe and relatively inexpensive. Related to alcoholism in many patients, individuals with acute pancreatitis also benefited from parenteral glutamine treatment with improvement in immune function, decreased systemic inflammation, and a trend toward shorter hospital stays.[1]

In an interesting study of 20 brain injury patients, 10 subjects were randomized to receive either an early enteral diet or the same formula with glutamine and probiotic added for a range of 5 to 14 days. The infection rate was found to be 100% in the control group, but only 50% in the glutamine group. The median number of infections per patient was significantly greater in the control group compared with the study group. Critical care stay and ventilation requirements were more than halved in the treatment group (10 vs. 22 days, and 14 vs. 7

days, respectively). Interestingly, probiotics were also used in the treatment group. This synergistic enhancement in gut flora may be useful to augment the already known benefits of glutamine.[97]

DOSAGE

The typical minimum oral dosage of glutamine is 100 mg three times a day, although the delivery method and actual effective dosage should be condition specific, meaning dosages are often much higher. The following points serve as guidelines:

- Severe burns: enteral dosage for individuals with severe burns was approximately 0.5 g/kg per day in most studies.
- Preventing and treating the side effects of chemotherapy involves the following:
- Patients treated with 5-fluorouracil received up to 18 g/day 5 days before, then during, and 5 days after treatment.[11]
- High-dose chemotherapy after stem cell transplantation: total parenteral nutrition enriched with glutamine 20 g.[28]
- Oral mucositis: 16 g mixed with 240 mL normal saline, and 30 mL is swished four times a day.[60]
- Esophageal cancer radiotherapy was given with a successful adjunctive oral glutamine supplement of 30 g/day for 4 weeks.[5]
- Cachexia patients were tested with dosages of 14 g/day combined with other amino acids.[66]
- Pediatric oncology patients: 0.65 g/kg was found to be a safe dose of glutamine to use in a clinical study in pediatric oncology patients.[98]
- Children with acute diarrhea: 0.3 g/kg per day was used successfully.[53]
- Peptic ulcers: drinking 1 L a day in divided doses was sufficient or 1.6 g/day for 1 month.[101]
- HIV patients: 30 to 40 g/day of glutamine to prevent medication-associated diarrhea and to improve intestinal permeability[73,74]; coadministration of antioxidants might also be helpful.
- Postabdominal surgeries: glutamine can be dissolved in warm water and taken orally or by gastric tube after the operation at 30 g/day for 7 days.[56]

TOXICITY

Glutamine, even at high doses, is without apparent side effects and is well tolerated.[4,54] Glutamine is synthesized from glutamate and the toxic alkaline waste product ammonia. If the blood is too acidic, the glutamine can be broken down into glutamate and ammonia, which will increase blood pH. Glutamate levels in the blood can increase slightly with high doses of supplemental glutamine administration (around 15 g in a single dose), but not with moderate doses (of about 5 g in a single dose). The higher doses may contribute to glutamate levels, and should be used with caution in patients with neurodegenerative diseases such as amyotrophic lateral sclerosis and multiple sclerosis.[99] In one pediatric oncology patient, a single dose of 0.75/kg was found to raise the blood ammonia level to an unacceptably high limit. Related to this, it was difficult to disperse the glutamine adequately at this dose, resulting in the suspension being found unpalatable.

DRUG INTERACTIONS

In cancer treatment, glutamine does not appear to change the efficacy of cancer drugs, rate of relapse, or progression of malignancy.[64] Some animal studies suggested that glutamine supplementation might even preferentially increase tumor retention of methotrexate, thus increasing the therapeutic window of this drug.[100] Many antiseizure medications, including phenobarbital, phenytoin, carbamazepine, primidone, and valproic acid, work to block glutamate activity in the brain. Because glutamine can convert to glutamate, clinicians should be cautious when using glutamine in patients using these medications.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ockenga J, Borchert K, Rifai K, et al. Effect of glutamine-enriched total parenteral nutrition in patients with acute pancreatitis. *Clin Nutr*. 2002;21:409–416.
2. Souba WW. Cytokine control of nutrition and metabolism in critical illness. *Curr Probl Surg*. 1994;31:577–643.
3. van den Berg A, van Elburg RM, Twisk JW, et al. Glutamine-enriched enteral nutrition in very low birth weight infants. Design of a double-blind randomised controlled trial. *BMC Pediatr*. 2004;4:17.
4. Sheridan RL, Prelack K, Yu YM, et al. Short-term enteral glutamine does not enhance protein accretion in burned children: a stable isotope study. *Surgery*. 2004;135:671–678.
5. Yoshida S, Kaibara A, Ishibashi N, et al. Glutamine supplementation in cancer patients. *Nutrition*. 2001;17:766–768.
6. Souba WW. Glutamine: a key substrate for the splanchnic bed. *Annu Rev Nutr*. 1991;11:285–308.
7. Souba WW, Klimberg VS, Plumley DA, et al. The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *J Surg Res*. 1990;48:383–391.
8. Qin HL, Cui HG, Zhang CH, et al. Effects of glutamine on structure and function of gut in endotoxemic rats. *China Natl J New Gastroenterol*. 1996;2:69–72.
9. PDR health. *L-Glutamine*. Available online at http://www.pdrhealth.com/drug_info/nmdrugprofiles/nutsupdrugs/lgl_0125.shtml. Accessed 9/23/2004.
10. Carneiro-Filho BA, Bushen OY, Brito GA, et al. Glutamine analogues as adjunctive therapy for infectious diarrhea. *Curr Infect Dis Rep*. 2003;5:114–119.
11. Daniele B, Perrone F, Gallo C, et al. Oral glutamine in the prevention of fluorouracil induced intestinal toxicity: a double blind, placebo controlled, randomised trial. *Gut*. 2001;48:28–33.
12. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. *Arch Surg*. 1990;125:1040–1045.
13. Seth A, Basuroy S, Sheth P, et al. L-Glutamine ameliorates acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayer. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G510–G517.
14. Welbourne T, Claville W, Langford M. An oral glutamine load enhances renal acid secretion and function. *Am J Clin Nutr*. 1998;67:660–663.
15. Welbourne TC. Increased plasma bicarbonate and growth hormone after an oral glutamine load. *Am J Clin Nutr*. 1995;61:1058–1061.
16. Welbourne T, Weber M, Bank N. The effect of glutamine administration on urinary ammonium excretion in normal subjects and patients with renal disease. *J Clin Invest*. 1972;51:1852–1860.
17. Pan M, Meng Q, Choudry HA, et al. Stimulation of intestinal glutamine absorption in chronic metabolic acidosis. *Surgery*. 2004;136:127–134.
18. Souba WW. *Glutamine: Physiology, Biochemistry, and Nutrition in Critical Illness.* Austin, TX: RG Landes Publishing
19. Yu JC, Jiang ZM, Li DM. Glutamine: a precursor of glutathione and its effect on liver. *World J Gastroenterol*. 1999;5:143–146.
20. Flaring UB, Rooyackers OE, Wernerman J, et al. Glutamine attenuates post-traumatic glutathione depletion in human muscle. *Clin Sci (Lond)*. 2003;104:275–282.
21. Cao Y, Kennedy R, Klimberg VS. Glutamine protects against doxorubicin-induced cardiotoxicity. *J Surg Res*. 1999;85:178–182.
22. Harward TR, Coe D, Souba WW, et al. Glutamine preserves gut glutathione levels during intestinal ischemia/reperfusion. *J Surg Res*. 1994;56:351–355.
23. Liu N, Ma X, Luo X, et al. L-glutamine attenuates apoptosis in porcine enterocytes by regulating glutathione-related redox homeostasis. *J Nutr*. 2018;148(4):526–534.
24. Borges-Santos MD, Moreto F, Pereira PC, Ming-Yu Y, Burini RC. Plasma glutathione of HIV+ patients responded positively and differently to dietary supplementation with cysteine or glutamine. *Nutrition*. 2012;28(7–8):753–756.f.
25. May PE, Barber A, D'Olimpio JT, et al. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. *Am J Surg*. 2002;183:471–479.
26. Jensen GL, Miller RH, Talabiska DG, et al. A double-blind, prospective, randomized study of glutamine-enriched compared with standard peptide-based feeding in critically ill patients. *Am J Clin Nutr*. 1996;64:615–621.
27. Hiscock N, Petersen EW, Krzywkowski K, et al. Glutamine supplementation further enhances exercise-induced plasma IL-6. *J Appl Physiol*. 2003;95:145–148.
28. Piccirillo N, De Matteis S, Laurenti L, et al. Glutamine-enriched parenteral nutrition after autologous peripheral blood stem cell transplantation: effects on immune reconstitution and mucositis. *Haematologica*. 2003;88:192–200.
29. Noyer CM, Simon D, Borczuk A. A double-blind placebo-controlled pilot study of glutamine therapy for abnormal intestinal permeability in patients with AIDS. *Am J Gastroenterol*. 1998;93:972–975.
30. Buhner S, Reese I, Kuehl F, et al. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. *Allergy*. 2004;59:1118–1123.
31. Danese S, Sans M, Fiocchi C. Inflammatory bowel disease: the role of environmental factors. *Autoimmun Rev*. 2004;3:394–400.
32. Peeters M, Geypens B, Claus D, et al. Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterol*. 1997;113:802–807.
33. Puspok A, Oberhuber G, Wyatt J, et al. Gastroduodenal permeability in Crohn's disease. *Eur J Clin Invest*. 1998;28:67–71.
34. Smecuol E, Bai JC, Vazquez H, et al. Gastrointestinal permeability in celiac disease. *Gastroenterol*. 1997;112:1129–1136.
35. Di Leo V, Venturi C, Baragiotta A, et al. Gastroduodenal and intestinal permeability in primary biliary cirrhosis. *Eur J Gastroenterol Hepatol*. 2003;15:967–973.
36. Campillo B, Pernet P, Bories PN, et al. Intestinal permeability in liver cirrhosis: relationship with severe septic complications. *Eur J Gastroenterol Hepatol*. 1999;11:755–759.
37. Catanoso M, Lo Gullo R, Giofre MR, et al. Gastro-intestinal permeability is increased in patients with limited systemic sclerosis. *Scand J Rheumatol*. 2001;30:77–81.
38. Vaarala O. The gut immune system and type 1 diabetes. *Ann N Y Acad Sci*. 2002;958:39–46.
39. Katz JP, Lichtenstein GR. Rheumatologic manifestations of gastrointestinal diseases. *Gastroenterol Clin North Am*. 1998;27:533–562.
40. Parke AL. Gastrointestinal disorders and rheumatic diseases. *Curr Opin Rheumatol*. 1991;3:160–165.
41. Van Elburg RM, Uil JJ, van Aalderen WM, et al. Intestinal permeability in exocrine pancreatic insufficiency due to cystic fibrosis or chronic pancreatitis. *Pediatr Res*. 1996;39:985–991.
42. Keshavarzian A, Holmes EW, Patel M, et al. Leaky gut in alcoholic cirrhosis: a possible mechanism for alcohol-induced liver damage. *Am J Gastroenterol*. 1999;94:200–207.
43. Hijazi Z, Molla AM, Al-Habashi H, et al. Intestinal permeability is increased in bronchial asthma. *Arch Dis Child*. 2004;89:227–229.
44. Uil JJ, van Elburg RM, van Overbeek FM, et al. Clinical implications of the sugar absorption test: intestinal permeability test to assess mucosal barrier function. *Scand J Gastroenterol Suppl*. 1997;223:70–78.
45. Weber P, Brune T, Ganser G, et al. Gastrointestinal symptoms and permeability in patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2003;21:657–662.
46. Deitch EA. Intestinal permeability is increased in burn patients shortly after injury. *Surgery*. 1990;107:411–416.
47. Kiziltas S, Imeryuz N, Gurcan T, et al. Corticosteroid therapy augments gastroduodenal permeability to sucrose. *Am J Gastroenterol*. 1998;93:2420–2425.
48. Riddington DW, Venkatesh B, Boivin CM, et al. Intestinal permeability, gastric intramucosal pH, and systemic endotoxemia in patients undergoing cardiopulmonary bypass. *JAMA*. 1996;275:1007–1012.

49. Sinclair DG, Haslam PL, Quinlan GJ, et al. The effect of cardiopulmonary bypass on intestinal and pulmonary endothelial permeability. *Chest*. 1995;108:718–724.
50. Gotteland M, Araya M, Pizarro F, et al. Effect of acute copper exposure on gastrointestinal permeability in healthy volunteers. *Dig Dis Sci*. 2001;46:1909–1914.
51. Videm V, Svennevig JL, Fosse E, et al. Plasma endotoxin concentrations during cardiac surgery may be related to atherosclerosis. *Perfusion*. 2000;15:421–426.
52. Brooks HW, White DG, Wagstaff AJ, et al. Evaluation of a glutamine-containing oral rehydration solution for the treatment of calf diarrhea using an Escherichia coli model. *Vet J*. 1997;153:163–169.
53. Yalcin SS, Yurdakok K, Tezcan I, et al. Effect of glutamine supplementation on diarrhea, interleukin-8 and secretory immunoglobulin A in children with acute diarrhea. *J Pediatr Gastroenterol Nutr*. 2004;38:494–501.
54. Majamaa H, Isolauri E, Saxelin M, et al. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr*. 1995;20:333–338.
55. Matarese LE, Seidner DL, Steiger E. Growth hormone, glutamine, and modified diet for intestinal adaptation. *J Am Diet Assoc*. 2004;104:1265–1272.
56. Quan ZF, Yang C, Li N, et al. Effect of glutamine on change in early postoperative intestinal permeability and its relation to systemic inflammatory response. *World J Gastroenterol*. 2004;10:1992–1994.
57. Byrne TA, Morrissey TB, Nattakom TV, et al. Growth hormone, glutamine, and a modified diet enhance nutrient absorption in patients with severe short bowel syndrome. *J Parenter Enteral Nutr*. 1995;19:296–302.
58. Wu GH, Wang H, Zhang YW, et al. Glutamine supplemented parenteral nutrition prevents intestinal ischemia-reperfusion injury in rats. *World J Gastroenterol*. 2004;10:2592–2594.
59. Zhang WX, Zhou LF, Zhang L, et al. Protective effects of glutamine preconditioning on ischemia-reperfusion injury in rats. *Hepatobiliary Pancreat Dis Int*. 2011;10:78–82.
60. Huang EY, Leung SW, Wang CJ, et al. Oral glutamine to alleviate radiation-induced oral mucositis: a pilot randomized trial. *Int J Radiat Oncol Biol Phys*. 2000;46:535–539.
61. Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. *Cancer*. 1998;83:1433–1439.
62. Scheid C, Hermann K, Kremer G, et al. Randomized, double-blind, controlled study of glycyl-glutamine-dipeptide in the parenteral nutrition of patients with acute leukemia undergoing intensive chemotherapy. *Nutrition*. 2004;20:249–254.
63. Bozzetti F, Biganzoli L, Gavazzi C, et al. Glutamine supplementation in cancer patients receiving chemotherapy: a double-blind randomized study. *Nutrition*. 1997;13:748–751.
64. Anderson PM, Ramsay NK, Shu XO, et al. Effect of low-dose oral glutamine on painful stomatitis during bone marrow transplantation. *Bone Marrow Transplant*. 1998;22:339–344.
65. Kozelsky TF, Meyers GE, Sloan JA. North Central Cancer Treatment Group. Phase III double-blind study of glutamine versus placebo for the prevention of acute diarrhea in patients receiving pelvic radiation therapy. *J Clin Oncol*. 2003;21:1669–1674.
66. May PE, Barber A, D'Olimpio JT, et al. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. *Am J Surg*. 2002;183:471–479.
67. Klimberg VS, Souba WW, Salloum RM, et al. Glutamine-enriched diets support muscle glutamine metabolism without stimulating tumor growth. *J Surg Res*. 1990;48:319–323.
68. Kaufmann Y, Kornbluth J, Feng Z, et al. Effect of glutamine on the initiation and promotion phases of DMBA-induced mammary tumor development. *J Parenter Enteral Nutr*. 2003;27:411–418.
69. Dwyer JT. Nutrition support of HIV+ patients. *Henry Ford Hosp Med J*. 1991;39:60–65.
70. Klimberg VS, Souba WW, Dolson DJ, et al. Prophylactic glutamine protects the intestinal mucosa from radiation injury. *Cancer*. 1990;66:62–68.
71. Shabert JK, Wilmore DW. Glutamine deficiency as a cause of human immunodeficiency virus wasting. *Med Hypotheses*. 1996;46:252–256.
72. Noyer CM, Simon D, Borczuk A, et al. A double-blind placebo-controlled pilot study of glutamine therapy for abnormal intestinal permeability in patients with AIDS. *Am J Gastroenterol*. 1998;93:972–975.
73. Shabert JK, Winslow C, Lacey JM, et al. Glutamine-antioxidant supplementation increases body cell mass in AIDS patients with weight loss: a randomized, double-blind controlled trial. *Nutrition*. 1999;15:860–864.
74. Huffman FG, Walgren ME. L-glutamine supplementation improves nelfinavir-associated diarrhea in HIV-infected individuals. *HIV Clin Trials*. 2003;4:324–329.
75. Peng X, Yan H, You Z, et al. Effects of enteral supplementation with glutamine granules on intestinal mucosal barrier function in severe burned patients. *Burns*. 2004;30:135–139.
76. Garrel D, Patenaude J, Nedelec B, et al. Decreased mortality and infectious morbidity in adult burn patients given enteral glutamine supplements: a prospective, controlled, randomized clinical trial. *Crit Care Med*. 2003;31:2444–2449.
77. Zhu M, Tang D, Zhao X, et al. Impact of glutamine of gut permeability and clinical prognosis on the aging patients undergoing gastric-intestinal operation. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*. 2000;22:425–427 ([Chinese]).
78. Thompson SW, McClure BG, Tubman TR. A randomized, controlled trial of parenteral glutamine in ill, very low birth-weight neonates. *J Pediatr Gastroenterol Nutr*. 2003;37:550–553.
79. Parimi PS, Devapatla S, Gruca LL, et al. Effect of enteral glutamine or glycine on whole-body nitrogen kinetics in very-low-birth-weight infants. *Am J Clin Nutr*. 2004;79:402–409.
80. Poindexter BB, Ehrenkranz RA, Stoll BJ, et al. National Institute of Child Health and Human Development Neonatal Research Network. Parenteral glutamine supplementation does not reduce the risk of mortality or late-onset sepsis in extremely low birth weight infants. *Pediatrics*. 2004;113:1209–1215.
81. Vaughn P, Thomas P, Clark R, et al. Enteral glutamine supplementation and morbidity in low birth weight infants. *J Pediatr*. 2003;142:662–668.
82. Antonio J, Sanders MS, Kalman D, et al. The effects of high-dose glutamine ingestion on weightlifting performance. *J Strength Cond Res*. 2002;16:157–160.
83. Candow DG, Chilibeck PD, Burke DG, et al. Effect of glutamine supplementation combined with resistance training in young adults. *Eur J Appl Physiol*. 2001;86:142–149.
84. Haub MD, Potteiger JA, Nau KL, et al. Acute L-glutamine ingestion does not improve maximal effort exercise. *J Sports Med Phys Fitness*. 1998;38:240–244.
85. Khogali SE, Harper AA, Lyall JA, et al. Effects of L-glutamine on post-ischaemic cardiac function: protection and rescue. *J Mol Cell Cardiol*. 1998;30:819–827.
86. Khogali SE, Pringle SD, Weryk BV, et al. Is glutamine beneficial in ischemic heart disease? *Nutrition*. 2002;18:123–126.
87. Akobeng AK, Miller V, Stanton J, Elbadri AM, Thomas AG. Double-blind randomized controlled trial of glutamine-enriched polymeric diet in the treatment of active Crohn's disease. *J Pediatr Gastroenterol Nutr*. 2000;30(1):78–84.
88. Den Hond E, Hiele M, Peeters M, Ghoos Y, Rutgeerts P. Effect of long-term oral glutamine supplements on small intestinal permeability in patients with Crohn's disease. *J Parenter Enteral Nutr*. 1999;23(1):7–11.
89. Benjamin J, Makharia G, Ahuja V, et al. Glutamine and whey protein improve intestinal permeability and morphology in patients with Crohn's disease: a randomized controlled trial. *Dig Dis Sci*. 2012;57(4):1000–1012.
90. Akobeng AK, Elawad M, Gordon M. Glutamine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev*. 2016;2:CD007348.
91. Oral l-glutamine therapy for sickle cell anemia: subjective clinical improvement and favorable change in red cell nad redox potential. *Am J Hematol*. 1998;58(2):117–121.
92. Niihara Y, Matsui NM, Shen YM, et al. L-glutamine therapy reduces endothelial adhesion of sickle red blood cells to human umbilical vein endothelial cells. *BMC Blood Disord*. 2005;5:4.
93. Rogers LL, Pelton RB, Williams RJ. Voluntary alcohol consumption by rats following administration of glutamine. *J Biol Chem*. 1955;214:503–506.

94. Rogers LL, Pelton RB. Glutamine in the treatment of alcoholism; a preliminary report. *Q J Stud Alcohol*. 1957;18:581–587.
95. Ravel JM, Felsing B, Lansford EM, et al. Reversal of alcohol toxicity by glutamine. *J Biol Chem*. 1955;214:497–501.
96. Rogers LL, Pelton RB, Williams RJ. Voluntary alcohol consumption by rats following administration of glutamine. *J Biol Chem*. 1955;214:503–506.
97. Falcao de Arruda IS, de Aguilar-Nascimento JE. Benefits of early enteral nutrition with glutamine and probiotics in brain injury patients. *Clin Sci (Lond)*. 2004;106:287–292.
98. Ward E, Picton S, Reid U, et al. Oral glutamine in pediatric oncology patients: a dose finding study. *Eur J Clin Nutr*. 2003;57:31–36.
99. Dharmananda S. Amino acid supplements I: glutamine with Reference to the Related Compound Glutamate. *Director, Institute for Traditional Medicine, Portland, Oregon*. http://www.itmonline.org/arts/glutamine.htm. Accessed 4/7/2009.
100. Rubio IT, Cao Y, Hutchins LF, et al. Effect of glutamine on methotrexate efficacy and toxicity. *Ann Surg*. 1998;227:772–778.
101. Shive W, Snider RN, Dubilier, et al. Glutamine in treatment of peptic ulcer; preliminary report. *Tex State J Med*. 1957;53(11):840–842.

Glycyrrhiza glabra (Licorice)

Michael T. Murray, ND

OUTLINE

Glycyrrhiza glabra (family: Leguminosae)
Common names: licorice, glycyrrhiza

GENERAL DESCRIPTION

Glycyrrhiza glabra is a perennial, temperate-zone herb or subshrub, 3 to 7 feet high, with a long, cylindrical, branched, flexible, and burrowing rootstock with runners (Fig. 85.1). The parts used are the dried runners and roots, which are collected in the fall.

CHEMICAL COMPOSITION

The major active component of licorice root is the triterpenoid saponin glycyrrhizin (also known as glycyrrhizic acid or glycyrrhizinic acid), which is usually found in concentrations ranging from 6% to 10% (Fig. 85.2). The intestinal flora is believed to hydrolyze glycyrrhizin, yielding the aglycone molecule (glycyrrhetinic acid) and a sugar moiety, resulting in absorption of both.[1]

A processed licorice extract, deglycyrrhizinated licorice (DGL), which is used in the treatment of peptic and aphthous ulcers, is made by removing the glycyrrhizin molecule. The active components of DGL are flavonoids. These compounds demonstrated impressive protection against chemically induced ulcer formation in animal studies.[2]

Other active constituents of licorice include isoflavonoids (e.g., isoflavonol, kumatakenin, licoricone, glabrol); chalcones; coumarins (e.g., umbelliferone, herniarin); triterpenoids; and sterols, lignins, amino acids, amines, gums, and volatile oils.[3]

HISTORY AND FOLK USE

The medicinal use of licorice in both Western and Eastern cultures dates back several thousand years. It was used primarily as a demulcent, expectorant, antitussive, and mild laxative. Licorice is one of the most popular components of Chinese medicines. Its traditional uses include treating peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, insomnia, and infections.[3]

PHARMACOLOGY

Licorice is known to exhibit many pharmacological actions, including the following[3]:

- Estrogenic
- Aldosterone-like action
- Anti-inflammatory (cortisol-like action)
- Antiallergic
- Antibacterial, antiviral, and antitrichomonas
- Antihepatotoxic
- Anticonvulsive
- Choleretic
- Anticancer

- Expectorant
- Antitussive activities

Although much of the pharmacology focuses on glycyrrhizin and glycyrrhetinic acid, it is worth remembering that licorice has many other components, such as flavonoids, which may have significant pharmacological effects.

Estrogenic Activity

Most herbalists generally believe that glycyrrhiza exhibits alterative action on estrogen metabolism (i.e., when estrogen levels are too high, it inhibits estrogen action, and when estrogens are too low, it potentiates estrogen action when used in greater amounts).[4] Glycyrrhetinic acid has been shown to antagonize many of the effects of estrogens, particularly exogenous estrogens.[5] The estrogenic action of glycyrrhiza is a result of its isoflavone content, as many isoflavone structures (e.g., daidzein and genistein from soy) are known to possess estrogenic effect. The estrogenic activity of the isoflavones appears to be more significant than the estrogen antagonism of glycyrrhetinic acid.[6] Interestingly, these same components inhibit breast cancer cell growth.[7]

Fig. 85.1 *Glycyrrhiza glabra*. (From Grigorii_Pisotckii/iStock.com.)

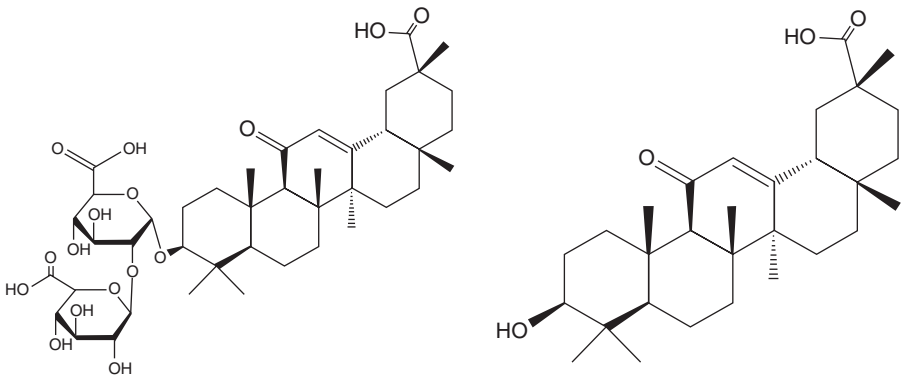
Pseudoaldosterone Activity

Long-term ingestion of glycyrrhiza in large doses leads to a well-documented pseudoaldosteronism syndrome (i.e., hypertension, hypokalemia, sodium and water retention, low plasma renin activity, and suppressed urine and serum aldosterone levels).[8-13] In normal subjects, the amount of glycyrrhizin needed to produce these side effects is between 0.7 and 1.4 g, which corresponds to approximately 10 to 14 g of the crude herb.[9] Although glycyrrhiza possesses mineralocorticoid activity (about four orders of magnitude lower than aldosterone) and binds to aldosterone receptors, it is largely without effect in adrenalectomized animals or in patients with severe adrenocorticoid insufficiency. Therefore it can be concluded that its primary effects are largely a result of glycyrrhetinic acid inhibiting the breakdown of aldosterone in the liver.[14] Glycyrrhizin and glycyrrhetinic acid were shown to suppress 5-β-reductase, the main enzyme in humans responsible for inactivating cortisol, aldosterone, and progesterone. These effects can be put to good use in the treatment of Addison's disease, a severe disease of adrenal insufficiency.[13]

Anti-Inflammatory and Antiallergic Activity

Glycyrrhiza has significant anti-inflammatory and antiallergic activity.[15,16] Although both glycyrrhizin and glycyrrhetinic acid bind to glucocorticoid receptors, and much of glycyrrhiza's anti-inflammatory activity has been explained by its "cortisol-like effects," many of the effects of glycyrrhiza actually antagonize or counteract cortisol.[17] Antagonism to such actions of cortisol includes activation of tryptophan oxygenase, accumulation of hepatic glycogen, stimulation of hepatic cholesterol synthesis, inhibition of thymus atrophy, and inhibition of adrenocorticotropic hormone synthesis and secretion. Glycyrrhizin does, however, reinforce cortisol's inhibition of antibody formation, stress reaction, and inflammation. Like its mineralocorticoid effect, glycyrrhiza's major influence on glucocorticoid metabolism is probably related to its suppression of 5-β-reductase activity, thus increasing the half-life of cortisol. Glycyrrhetinic acid can also increase the conversion of cortisol to the more powerful cortisone.[18]

Glycyrrhiza's major cortisol-like effect relates to its ability to inhibit phospholipase A_2.[19] This enzyme is responsible for cleaving lipids from biomembranes for eicosanoid metabolism. In addition to this effect, glycyrrhizin was also shown to inhibit cyclic adenosine monophosphate phosphodiesterase, thereby raising cyclic adenosine monophosphate levels and prostaglandin formation by activated peritoneal macrophages from rats.[20,21] Glycyrrhizin was shown to inhibit experimentally induced allergenic reactions, such as the Arthus phenomenon, the Shwartzman phenomenon, and Forssman anaphylaxis, and



Structure of Glycyrrhizin **Structure of Glycyrrhetinic acid**

Fig. 85.2 Chemical structures of glycyrrhizin and glycyrrhetinic acid. (Retrieved from https://www.sciencedirect.com/science/article/pii/S001429991730345X [accessed October 22, 2018]).

to be an antidote against many toxins, including diphtheria, tetanus, and tetrodotoxin.[21,22]

Glycyrrhizin exerts antithrombotic effects but does not potentiate the inhibitory activity of antithrombin III or heparin cofactor II toward thrombin.[23]

Immunostimulatory and Antiviral Effects

Glycyrrhizin and glycyrrhetinic acid were shown to induce interferon.[24] The induction of interferon leads to significant antiviral activity, because interferons bind to cell surfaces, where they stimulate synthesis of intracellular proteins that block the transcription of viral DNA. The induction of interferon is also followed by activation of macrophages and augmentation of natural killer cell activity.

Glycyrrhizin was shown to directly inhibit the growth of several DNA and RNA viruses in cell cultures (vaccinia, Epstein-Barr, herpes simplex, Newcastle disease, vesicular stomatitis viruses, severe acute respiratory syndrome [SARS]-associated coronavirus, and HIV) and to inactivate herpes simplex virus 1 (HSV-1) irreversibly.[25-28] Administration of glycyrrhizin to mice with herpetic encephalitis increased their survival rate on average about 2.5 times, whereas it reduced HSV-1 replication in the brain to 45.6% of the controls.[29] Glycyrrhizin, as stated earlier, also inhibited the thymolytic and immunosuppressive action of cortisone. Other licorice components exerted immunomodulatory effects as well.[30]

Anticancer Effects

Licorice components exert a wide range of anticancer effects.[31] The most active appear to be the flavonoids and coumarins. For example, isoliquiritigenin was shown to suppress colon cancer in mice via markedly decreasing both prostaglandin E_2 and nitric oxide production in mouse macrophage cells.[32] Isoliquiritigenin was also shown to significantly inhibit the proliferation of prostate and breast cancer cell lines in dose- and time-dependent manners.[7,33] Isoliquiritigenin also significantly reduced pulmonary metastasis in mouse renal cell carcinoma and prevented the leukocytopenia caused by administration of 5-fluorouracil.[34] A coumarin compound, identified as licocoumarone, was shown to be the factor in licorice that induces apoptosis.[35]

Antibacterial Activity

Alcohol extracts of glycyrrhiza displayed antimicrobial activity in vitro against *Helicobacter pylori, Staphylococcus aureus* (including antibiotic resistant strains), *Streptococcus mutans, Mycobacterium smegmatis, Bacillus subtilis, S. pyogenes, Haemophilus influenzae, Moraxella catarrhalis,* and *Candida albicans.*[36-39] The majority of the antimicrobial effects are due to isoflavonoid components, with the saponins having a lesser antibacterial effect.

Antihepatotoxic Activity

Glycyrrhetinic acid inhibits carbon tetrachloride and galactosamine-induced liver damage. The mechanism of action is prevention of nonenzymatic lipid peroxidation and inhibition of the production of free radicals by the enzymatic action of nicotine adenine disphosphonucleotide, reduced–cytochrome P450 reductase on CCl4.[40]

Memory-Enhancing Effect

Licorice may exert some memory-enhancing effects. In a study in mice, licorice was shown to enhance learning and memory in mice as determined by the elevated plus-maze and passive avoidance paradigm. Furthermore, licorice significantly reversed the amnesia induced by diazepam and scopolamine. Although anti-inflammatory and antioxidant properties may contribute favorably to the memory-enhancing effect, because scopolamine-induced amnesia was reversed as well, it is possible that the beneficial effect on learning and memory was a result of facilitation of cholinergic transmission.[41]

Antinephritic Activity

Glabridin, an isoflavan isolated from *G. glabra,* improved urinary protein excretion, total cholesterol, serum creatinine, and blood urea nitrogen levels after its oral administration to mice with glomerular disease.[42]

CLINICAL APPLICATIONS

Licorice is a component of more traditional Chinese and Japanese herbal formulas than any other herb and has been commonly used in Western natural medicine and herbalism for centuries. Although extremely pharmacologically diverse, the current clinical applications of licorice can be divided into four main categories:

- Use of DGL
- Use of oral licorice preparations containing glycyrrhizin
- Use of licorice flavonoid oil (LFO)
- Use of topical preparations containing glycyrrhetinic acid

The key use of DGL is in ulcerative conditions of the gastrointestinal tract (e.g., peptic ulcers, canker sores, inflammatory bowel disease), whereas the key uses of oral licorice containing glycyrrhizin include viral infections (e.g., the common cold, HIV and AIDS, viral hepatitis); premenstrual syndrome (PMS) and menopause; acute intermittent porphyria; Addison's disease; inflammation; syndrome X; and as a sweetening agent. Topical preparations containing glycyrrhetinic acid can be used in eczema, psoriasis, herpes, and melasma.

DEGLYCYRRHIZINATED LICORICE

Although glycyrrhetinic acid was the first drug proven to promote healing of gastric and duodenal ulcers,[43] most physicians using licorice in the treatment of peptic ulcers now use DGL. DGL was actually shown to be more effective than glycyrrhetinic acid, without side effects.[44]

DGL's mode of action is different than that of current drugs, such as antacids and H_2-receptor antagonists, which focus on reducing gastric acidity. Although effective, these treatments can be expensive, carry some risk of toxicity, disrupt normal digestive processes, and alter the structure and function of the cells that line the digestive tract. The latter factor is just one of the reasons why peptic ulcers develop again if antacids, cimetidine, ranitidine, and similar drugs are used.

Rather than inhibit the release of acid, DGL stimulates the normal defense mechanisms that prevent ulcer formation and stimulate healing of the damaged mucous membranes. Specifically, DGL increases the following[45,46]:

- The blood supply to the damaged mucosa
- The number of cells producing the mucus that protects the mucous membranes
- The amount of mucus the cells produce
- The life span of the intestinal cell

In addition, several flavonoid components of *G. glabra* have shown significant activity against *H. pylori,* including antibiotic-resistant strains.[38] To evaluate the effect of licorice in *H. pylori* eradication in 120 patients suffering from dyspepsia either with peptic ulcer disease (PUD) or nonulcer dyspepsia (NUD), licorice (380 mg twice daily) was given in addition to clarithromycin-based standard triple regimen for 2 weeks.[47] *H. pylori* eradication was assessed 6 weeks after therapy. Response to treatment was 83.3% in the licorice group and 62.5% in the control group.

Gastric Ulcers

Numerous clinical studies over the years found DGL to be an effective antiulcer compound. DGL was shown to be extremely effective in the

treatment of gastric ulcers.[48-52] In one study, 33 gastric ulcer patients were treated with either DGL (760 mg, three times a day) or a placebo for 1 month.[50] There was a significantly greater reduction in ulcer size in the DGL group (78%) than in the placebo group (34%). Complete healing occurred in 44% of those receiving DGL but only in 6% of the placebo group.

In several head-to-head comparison studies, DGL was shown to be more effective than cimetidine (Tagamet), ranitidine (Zantac), or antacids in both short-term treatment and maintenance therapy of peptic ulcers.[48,49,52] For example, in a head-to-head comparison with Tagamet, 100 patients received either DGL (760 mg, three times a day between meals) or Tagamet (200 mg, three times a day, and 400 mg at bedtime).[49] The percentage of ulcers healed after 6 and 12 weeks were similar in both groups. Although Tagamet is associated with some significant side effects, DGL is extremely safe to use.

Gastric ulcers are often a result of using alcohol, aspirin, or other nonsteroidal anti-inflammatory drugs, caffeine, and other factors that decrease the integrity of the gastric lining. Because DGL was shown in human studies to reduce the gastric bleeding caused by aspirin, DGL is strongly indicated for the prevention of gastric ulcers in patients requiring long-term treatment with ulcerogenic drugs such as aspirin, nonsteroidal anti-inflammatory agents, and corticosteroids.[51]

Duodenal Ulcers

DGL is also effective in duodenal ulcers. This is perhaps best illustrated by one study in patients with severe duodenal ulcers: 40 patients with chronic duodenal ulcers of 4 to 12 years' duration and more than six relapses during the previous year were treated with DGL.[53] All of the patients had been referred for surgery because of relentless pain, sometimes with frequent vomiting, despite treatment with bed rest, antacids, and anticholinergic drugs. Half of the patients received 3 g/day of DGL for 8 weeks; the other half received 4.5 g/day for 16 weeks. All 40 patients showed substantial improvement, usually within 5 to 7 days, and none required surgery during the 1-year follow-up. Although both dosages were effective, the higher dosage was significantly more effective than the lower dosage.

In another more recent study, the therapeutic effect of DGL was compared with that of antacids or cimetidine in 874 patients with confirmed chronic duodenal ulcers.[52] Ninety-one percent of all ulcers healed within 12 weeks; there was no significant difference in healing rate in the groups. However, there were fewer relapses in the DGL group (8.2%) than in those receiving cimetidine (12.9%) or antacids (16.4%). These results, coupled with DGL's protective effects and very low toxicity, suggest that DGL is a superior treatment of duodenal ulcers.

Aphthous Ulcers

Recurrent aphthous stomatitis (canker sores) is a common problem. DGL may be effective in promoting healing. In one study, 20 patients were instructed to use a solution of DGL as a mouthwash (200 mg powdered DGL dissolved in 200 mL warm water) four times daily.[54] Fifteen of the 20 (75%) patients experienced 50% to 75% improvement within 1 day, followed by complete healing of the ulcers by the third day. DGL in tablet form may produce even better results.

ORAL LICORICE PREPARATIONS CONTAINING GLYCYRRHIZIN

The most popular use of oral licorice preparations containing glycyrrhizin is in the treatment of viral illnesses, particularly the common cold. Licorice has long been used in this application. This historical use is justified by its immune-enhancing and antiviral effects. In addition, licorice components were shown to exert antibacterial action against the common pathogens *S. pyogenes, Haemophilus influenzae,* and *Moraxella catarrhalis.*[55]

Another popular use of licorice is in the treatment of gynecological issues, primarily PMS and menopause. Regarding PMS, because glycyrrhizin and glycyrrhetinic acid possess antiestrogenic effects and suppress the breakdown of progesterone, administration of licorice 2 weeks before the onset of menstruation (the midluteal phase) may help reduce PMS symptomatology. Clinical trials showed that taking licorice containing herbal combinations was useful in dysmenorrhea.[56] Isoflavones from glycyrrhiza showed an ability to inhibit serotonin reuptake, and therefore might also exhibit some antidepressant effects in PMS.[57]

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Glycyrrhizin-containing preparations are showing promise in the treatment of HIV-related diseases, including AIDS. Although much of the research featured intravenous administration, this route of administration might not be necessary, as glycyrrhizin and glycyrrhetinic acid are easily absorbed orally and are well tolerated. This was most evident in a recent double-blind study on the clinical effectiveness of glycyrrhizin by long-term oral administration to 16 hemophiliac patients with evidence of HIV infection.[58] Patients received daily doses of 150 to 225 mg of glycyrrhizin for 3 to 7 years.

Helper and total T-lymphocyte numbers, other immune system parameters, and glycyrrhizin and glycyrrhetinic acid levels in the blood were monitored. The results indicated that orally administered glycyrrhizin was converted into glycyrrhetinic acid, which was detected in sera, without manifesting any side effects. None of the patients given the glycyrrhizin had progression of immunological abnormalities or development to AIDS. In contrast, the group not receiving glycyrrhetinic acid showed decreases in helper and total T-cell counts and antibody levels. Two of the 16 patients in the control group developed AIDS.

In another study, 10 HIV positive patients without AIDS took 150 to 225 mg/day of glycyrrhizin.[59] After 1 to 2 years, none developed symptoms associated with AIDS or AIDS-related complex, whereas 1 of 10 patients of a matched control group developed AIDS-related complex, and 2 progressed to AIDS and subsequently died.

The result of glycyrrhizin in HIV-positive and AIDS patients is almost immediate improvement in immune function. In one study, 9 symptom-free HIV-positive patients received 200 to 800 mg/day of glycyrrhizin intravenously. After 8 weeks, the groups had increased T-helper cells, improved helper/suppressor ratios, and improved liver function.[60]

In another study, 6 AIDS patients received 400 to 1600 mg/day of glycyrrhizin intravenously.[61] After 30 days, 5 of the 6 showed a reduction or disappearance of the P24 antigen, which indicates active disease. The results of these studies and others in HIV-positive and AIDS patients are encouraging.

Hepatitis

Some studies of HIV patients used an intravenous glycyrrhizin-containing product, Stronger Neo-Minophagen C (SNMC), consisting of 0.2% glycyrrhizin, 0.1% cysteine, and 2.0% glycine in physiological saline solution. This product is used in Japan primarily for the treatment of hepatitis. The other components, glycine and cysteine, appear to modulate glycyrrhizin's actions. Glycine was shown to prevent the aldosterone effects of glycyrrhizin, whereas cysteine aids the liver in detoxification reactions.

In addition to AIDS, SNMC demonstrated beneficial results in treating chronic hepatitis B and C, often difficult infections for the body to clear.[22,62-64] Specifically, SNMC was shown to improve liver function and lower levels of liver enzymes. Glycyrrhizin therapy appears particularly helpful in patients with chronic hepatitis C who fail to respond to interferon and in those who cannot be treated with it for various reasons.

Acute Intermittent Porphyria

This disorder of heme biosynthesis is characterized by recurrent attacks of neurological and psychiatric dysfunction. The symptoms include the following:

- Abdominal complaints of nausea, vomiting, and colicky pain, occasionally severe enough to present as an acute abdomen without fever or leukocytosis
- Variable neurological signs and symptoms (e.g., paresthesia, hypesthesia, neuritic pain, wrist or foot drop, loss of deep tendon reflexes)
- Variable mental and emotional disturbances, typically restlessness, disorientation, and visual hallucinations (seen in one third of patients)

Because estrogens are known to exacerbate or induce acute intermittent porphyria (AIP), it is quite possible that some of the so-called PMS symptoms are exacerbations of AIP caused by the midcycle estrogen surge.

A partial (50%) deficiency of uroporphyrinogen I synthase results in increased inducibility of aminolevulinic acid synthase by drugs and foreign chemicals and by 5-β-reductase steroid metabolites (potent inducers of aminolevulinic acid synthase). AIP is also associated with a marked deficiency in the activity of 5-α-reductase, resulting in increased 5-β-reductase activity.[65] Glycyrrhetinic acid and glycyrrhizin were shown to significantly reduce 5-β-reductase while increasing 5-α-reductase.[66] (Lead also increases 5-β-reductase activity, resulting in a presenting picture similar to AIP.[66] Chronic or acute lead toxicity must be ruled out in these patients.)

Obesity and Metabolic Syndrome

Preparations containing glycyrrhetinic acid may be effective in reducing various issues related to syndrome X or metabolic syndrome. For example, in a preliminary study, 15 normal-weight subjects (7 males, 22–26 years old, and 8 females, 21–26 years old), who consumed 3.5 g/day of a commercial preparation of licorice containing glycyrrhetinic acid for 2 months, had reduced body fat mass of 1.2% in men and 2.8% in women.[67] This weight loss might have been mediated not only via suppressing renin activity and aldosterone levels via inhibition of 11-β-hydroxysteroid dehydrogenase, but also via improving blood glucose control—a key goal in syndrome X.[68-70]

In another study, supplementation of a licorice root extract to moderately hypercholesterolemic patients for 1 month reduced plasma susceptibility to oxidation (by 19%). It also increased resistance of plasma low-density lipoprotein against three major atherogenic modifications: oxidation (by 55%), aggregation (by 28%), and retention (by 25%). It reduced plasma cholesterol levels (by 5%), which were caused by a 9% reduction in plasma low-density lipoprotein cholesterol levels, and reduced (by 14%) plasma triglyceride levels. Licorice extract supplementation also reduced systolic blood pressure by 10%.[71]

Addison's Disease

As described later in "Pseudoaldosterone Activity," licorice exerts an aldosterone-like effect that is useful in treating Addison's disease.

Inflammation

Virtually any inflammatory or allergic condition may be reduced by licorice by the mechanisms discussed earlier in the section on "Pharmacology." Historically, licorice was successfully used for treating asthma and other atopic conditions.[3,15]

Licorice was shown to enhance the action of corticosteroids like prednisone and prednisolone, as well as the levels of the body's own corticosteroids.[72,73] In one study, six subjects received an intravenous dose of prednisolone with or without 200 mg glycyrrhizin. Glycyrrhizin was found to significantly increase the concentration of total and free prednisolone by inhibiting its breakdown. Furthermore, the effects of prednisolone appeared to be potentiated by glycyrrhizin.[73]

One interesting application shown with positive clinical results in a double-blind study was reduction of postoperative sore throat.[74] Forty adults who underwent elective lumbar laminectomy were randomized into two groups of 20 patients each. One group received water (Group C); the other received 0.5 g licorice in water (Group L). Both groups gargled 5 minutes before anesthesia. Postoperative sore throat incidence and severity as well as postextubation cough were reduced for all time points in the licorice group compared with the water group at rest and on swallowing. Postextubation cough was reduced in Group L compared with Group C ($P < 0.05$). There was no difference in side effects between groups ($P > 0.05$).

A review study evaluated the possible application of the active components of licorice, glycyrrhizin (GL) and glycyrrhetinic acid (GA), in rheumatoid arthritis (RA) treatment based on the cyclooxygenase (COX)-2/thromboxane A2 (TxA2) pathway (Fig. 85.3).[75] The COX-2/TxA2 pathway, an auto-regulatory feedback loop, has been found to be a crucial mechanism underlying the pathogenesis of RA. Both nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs) are typically prescribed medications for treatments of patients with RA. TxA2 is believed to be the nontarget of NSAIDs and DMARDs, and the limitations and adverse effects of those drugs may be, at least in part, caused by lack of the effects on the COX-2/TxA2 pathway. The active components of licorice, GL and GA, could not only potentiate the therapeutic effects but also decrease the adverse effects of NSAIDs or DMARDs through suppressing the COX-2/TxA2 pathway and hold the potential as a novel add-on therapy in the treatment of RA.

Sweetening Agent

Because glycyrrhizin is 50 to 100 times sweeter than sucrose, licorice can be used as a sweetening or flavoring agent to mask the bitter taste of other medications.[3]

LICORICE FLAVONOID OIL

LFO shows promise as an antiobesity and weight loss agent. It is standardized to contain 30% polyphenols with glabridin standardized at 3%. The flavonoids are extracted with ethanol and then solubilized in medium chain triglycerides oil (hence 70% of LFO is medium chain triglycerides). The extracted licorice flavonoids are hydrophobic compounds and virtually free of the hydrophilic compounds glycyrrhizin and glycyrrhizinic acid (there is less than 0.005% glycyrrhizic acid in LFO).

LFO decreases the activity of acetyl coenzyme A carboxylase and fatty acid synthase, the rate-limiting enzymes in the fatty acid synthetic pathway, while increasing the enzymatic activity of acyl coenzyme A dehydrogenase, the rate-limiting enzyme in the fatty acid oxidative pathway. These effects are thought to be responsible for the reduction in abdominal fat in animal and human studies.[76–79]

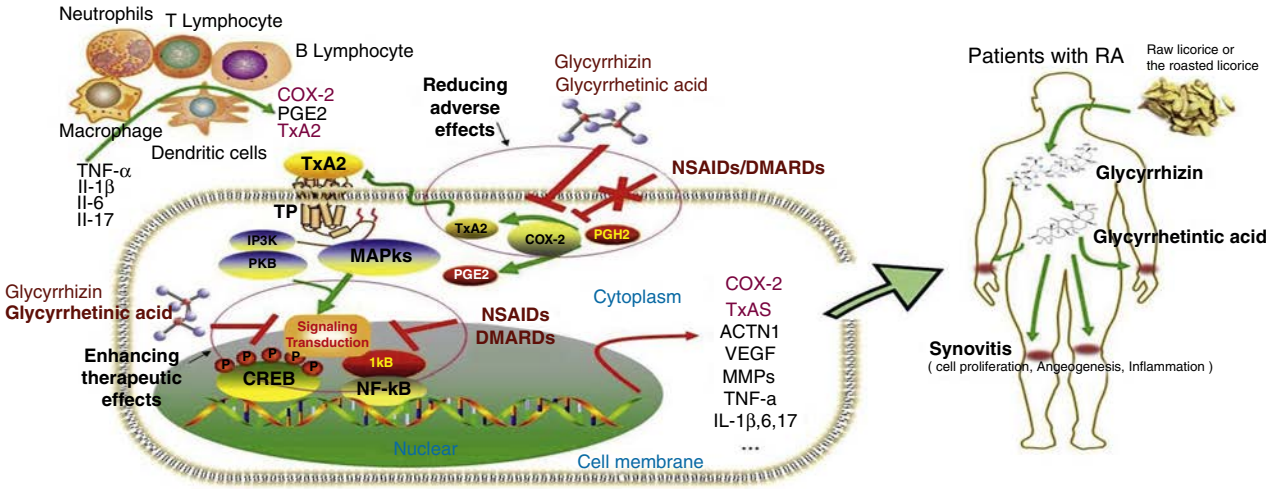


Fig. 85.3 The COX-2/TxA2 pathway is a crucial mechanism underlying the toxicity reducing and efficacy enhancing effects of glycyrrhizin (GL) and glycyrrhetinic acid (GA) to NSAIDs/DMARDs. *TNF*, Tumor necrosis factor; *IL*, interleukin; *RA FLS*, rheumatoid arthritis fibroblast-like synoviocytes; *COX*, cyclooxygenase; *TxA2*, thromboxane A2; *TP*, thromboxane A2 receptor; *PGH2*, prostaglandin H2; *PGE2*, prostaglandin E2; *NSAIDs*, nonsteroidal anti-inflammatory drugs; *DMARDs*, disease modifying antirheumatic drugs; *NF-κB*, nuclear factor κB; *CREB*, cAMP response element-binding protein; *MAPKs*, mitogen activated protein kinases; *PI3K*, phosphoinositide-3-kinase; *VEGF*, vascular endothelial growth factor; *ACTN1*, α-actinin-1; *MMPs*, matrix metalloproteinase. (From Huang QC, Wang MJ, Chen XM, et al. Can active components of licorice, glycyrrhizin and glycyrrhetinic acid, lick rheumatoid arthritis? *Oncotarget*. 2016;7[2]:1193-1202. PubMed PMID: 26498361.)

TABLE 85.1 Body Weight and Mass Compared With Baseline After Licorice Flavonoid Oil Treatment

Group	Baseline	4 Weeks	8 Weeks
Body Weight (kg)			
Placebo	73.13	73.07	73.39
900 mg	72.79	72.20	72.05
Body Mass Index (kg/m^2)			
Placebo	26.51	26.49	26.59
900 mg	26.22	26.01	25.97

In a double-blind study, 56 men and 28 postmenopausal women, aged 40 to 60 years with body mass indexes of 24 to 30 kg/m^2, were randomized into four groups: the placebo group took three placebo capsules per day, the low-dose group took one LFO capsule and two placebos per day (300 mg/day LFO), the middle-dose group took two LFO capsules and one placebo per day (600 mg/day LFO), and the high-dose group took three LFO capsules per day (900 mg/day LFO). The subjects in the 900 mg LFO group experienced significant decreases from baseline in body weight and body mass index compared with the placebo group (Table 85.1).[79]

The subjects in all three LFO groups, but not the placebo group, had significantly lower body fat masses compared with baseline after 8 weeks of treatment. Computed tomographic scans showed that visceral fat mass decreased significantly compared with baseline in the 900 mg LFO group (122.37–113.02 cm^2).

Another study with LFO was conducted to determine its ability to increase the muscle mass of elderly patients. Fifty subjects aged 54 to 90 years (7 men, 43 women), who underwent rehabilitation treatment for osteoarthritis of the knee, were assigned to either the LFO (300 mg per day) or placebo group. In the LFO group, muscle mass in the body trunk increased significantly after 16 weeks of LFO intake (+0.38 kg). In addition, the body fat percentage and body trunk fat percentage of the LFO group were also reduced.[80]

A safety study demonstrated that LFO is safe when administered once daily up to 1200 mg/day.[81] There were no clinically noteworthy changes in hematologic or related biochemical parameters.

TOPICAL APPLICATIONS

Eczema and Psoriasis

Glycyrrhetinic acid exerts an effect similar to that of topical hydrocortisone in the treatment of eczema, contact and allergic dermatitis, and psoriasis.[82–85] In several studies, glycyrrhetinic acid was shown to be superior to topical cortisone, especially in chronic cases. For example, in one study of patients with eczema, 93% of the patients applying glycyrrhetinic acid demonstrated improvement compared with 83% using cortisone.[86] In another study, a topical gel containing 2% glycyrrhetinic acid was shown to be effective for treatment of atopic dermatitis and was more effective than preparations containing 1% glycyrrhetinic acid in reducing the scores for erythema, edema, and itching over 2 weeks.[82]

Glycyrrhetinic acid can also be used to potentiate the effects of topically applied hydrocortisone by inhibiting 11-β-hydroxysteroid dehydrogenase, which catalyzes the conversion of hydrocortisone to an inactive form.[83] It also increases the permeation of topically applied steroids. In one study, glycyrrhetinic acid in a concentration of 0.1% in gel increased diclofenac sodium flux value tenfold compared with a control gel.[84]

Herpes Simplex

Clinical studies showed topical glycyrrhetinic acid and derivatives to be quite helpful in reducing the healing time and pain associated with cold sores and genital herpes.[87,88] As mentioned previously, glycyrrhizin inactivates HSV-1 irreversibly and stimulates the synthesis and release of interferon.[25]

Melasma

Two components, glabrene and isoliquiritigenin, can inhibit tyrosinase—a key enzyme in melanin biosynthesis.[89] Dermatological disorders such as melasma, age spots, and sites of actinic damage arise from the accumulation of melasma. Glabrene and isoliquiritigenin may serve as candidates for skin-lightening agents.

DOSAGE

The dosage of licorice for most clinical applications is based on the content of glycyrrhetinic acid. The exception is in the treatment of peptic ulcer. In this application, DGL is preferred, as it produces equally effective results compared with glycyrrhetinic acid but is free from any side effects.

For most purposes, the goal is to achieve a high level of glycyrrhetinic acid in the blood without producing side effects (discussed later in "Toxicology"). In general, the following doses three times a day are safe and effective in raising glycyrrhetinic acid levels:

- Powdered root: 1 to 2 g
- Fluid extract (1:1): 2 to 4 mL
- Solid (dry powdered) extract (4:1): 250 to 500 mg

In the treatment of AIDS, pure glycyrrhetinic acid products or extracts standardized for glycyrrhetinic acid are recommended. Toxicity can become a problem for patients taking licorice for any period longer than 1 month (see "Toxicology" and "Drug Interactions").

Dosage Instructions for Deglycyrrhizinated Licorice

To be effective in healing peptic ulcers, it appears that DGL must mix with saliva. DGL may promote the release of salivary compounds, which stimulate the growth and regeneration of stomach and intestinal cells. DGL in capsule form has not been shown to be effective.[90,91]

The standard dosage for DGL is two to four 380-mg chewable tablets between or 20 minutes before meals. Taking DGL after meals is associated with poor results.[92] DGL should be continued for 8 to 16 weeks, depending on the response.

Dosage Instructions for Licorice Flavonoid Oil

The standard dosage for LFO is 900 mg/day usually administered as 300 mg three times a day.

TOXICOLOGY

The main hazards of licorice administration are due to the aldosterone-like effects of glycyrrhetinic acid. If ingested regularly, licorice root (>3 g/day for more than 6 weeks) or glycyrrhizin (>100 mg/day) may cause sodium and water retention, hypertension, and hypokalemia.[8,9,93-95] Individuals with existing hypertension may be more predisposed to this effect via increased sensitivity to the inhibition of 11-β-hydroxysteroid-dehydrogenase by glycyrrhetinic acid.[18,82,96] Monitoring of blood pressure and electrolytes and increasing dietary potassium intake is suggested, as the pseudoaldosterone effects can be quite significant. The maximal effect on blood pressure with long-term ingestion is observed after 2 weeks of use.[97]

There is great individual variation in the susceptibility to the symptom-producing effects of glycyrrhizin, primarily due to differences in pharmacokinetics and conversion to the more potent glycyrrhetinic acid (100–200 times more active in suppressing 11-β-hydroxysteroid-dehydrogenase).[98] Adverse effects are rarely observed at levels below 100 mg/day, whereas they are quite common at levels above 400 mg/day.[9] However, some persons may be susceptible to long-term dosages at even lower levels, especially if the more potent glycyrrhetinic acid is available in free form. One study determined a no-effect level of glycyrrhetinic acid at 2 mg/kg, from which an acceptable daily intake of 0.2 mg/kg body weight can be extrapolated with a safety factor of 10. This translates to a consumption of 12 mg/day of glycyrrhetinic acid for a person with a body weight of 60 kg.[99]

Prevention of the side effects of glycyrrhizin may be possible by following a high-potassium, low-sodium diet. Although no formal trial has been performed, patients who normally consume high-potassium foods and restrict sodium intake, even those with high blood pressure and angina, have been reported to be free from the aldosterone-like side effects of glycyrrhizin.[100]

Licorice should probably not be used in patients with a history of hypertension or renal failure or in those who currently use digitalis preparations.

Licorice preparations containing glycyrrhizin may reduce serum and salivary testosterone levels in men. In one study, men consuming the equivalent of 500 mg of glycyrrhizin experienced a drop of 26% in serum testosterone levels.[101] However, in another study, no significant effect was noted.[102]

Licorice intake during pregnancy is generally regarded as safe, unless hypertension becomes an issue. There was one detailed study on maternal consumption of glycyrrhizin and how it affected birth weight.[103] Glycyrrhizin intake was calculated from detailed questionnaires on licorice consumption. Glycyrrhizin exposure was grouped into three levels: low (<250 mg/week), moderate (250–499 mg/week), and heavy (≥500 mg/week). Birth weight and gestational age (from ultrasound measurements) were obtained from hospital records. Babies with heavy exposure to glycyrrhizin were not significantly lighter at birth, but they were significantly more likely to be born earlier—2.52 days earlier. No other associations could be made.

DRUG INTERACTIONS

No significant reports of drug interactions have appeared, although on theoretical grounds, licorice components have shown considerable interactions with various enzyme systems. Licorice root extract and purified glabridin were shown to inhibit P450 3A4, a major human drug metabolizing P450 enzyme, in time- and concentration-dependent manners, thereby potentiating the action of many drugs.[104] Glycyrrhizin intake may be problematic for people on digitalis, diuretics, or antihypertensive medications. Also, individuals using oral hypoglycemic drugs or insulin need to monitor blood sugar levels closely when using glycyrrhiza.

Glycyrrhetinic acid can reduce the prevalence of side effects related to the diuretic activity of spironolactone. In a study of 32 women with polycystic ovarian syndrome, women who received 3.5 g of licorice a day with spironolactone eliminated symptoms related to volume depletion, and the activation of the renin-aldosterone system was significantly lower during spironolactone plus licorice than with spironolactone alone. The prevalence of metrorrhagia was also lower with the combined therapy.[105]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Hattori M, Sakamoto T, Kobashi K, et al. Metabolism of glycyrrhizin by human intestinal flora. *Planta Med*. 1983;48:38–42.
2. Yamamoto K, Kakegawa H, Ueda H, et al. Gastric cytoprotective anti-ulcerogenic actions of hydroxychalcones in rats. *Planta Med*. 1992;58:389–393.
3. Chandler RF. Licorice, more than just a flavour. *Can Pharm J*. 1985;118:421–424.
4. Kumagai A, Nishino K, Shimomura A, et al. Effect of glycyrrhizin on estrogen action. *Endocrinol Jpn*. 1967;14:34–38.
5. Kraus S, Kaminskis A. The anti-estrogenic action of beta-glycyrrhetinic acid. *Exp Med Surg*. 1969;27:411–420.
6. Tamir S, Eizenberg M, Somjen D, et al. Estrogen-like activity of glabrene and other constituents isolated from licorice root. *J Steroid Biochem Mol Biol*. 2001;78:291–298.
7. Maggiolini M, Statti G, Vivacqua A, et al. Estrogenic and antiproliferative activities of isoliquiritigenin in MCF7 breast cancer cells. *J Steroid Biochem Mol Biol*. 2002;82:315–322.
8. Farese Jr RV, Biglieri EG, Shackleton CH, et al. Licorice-induced hypermineralocorticoidism. *N Engl J Med*. 1991;325:1223–1227.
9. Stormer FC, Reistad R, Alexander J. Glycyrrhizic acid in liqourice—evaluation of health hazard. *Food Chem Toxicol*. 1993;31:303–312.
10. Takeda R, Morimoto S, Uchida K, et al. Prolonged pseudoaldosteronism induced by glycyrrhizin. *Endocrinol Jpn*. 1979;26:541–547.
11. Baron J. Side-effects of carbenoxolone. *Acta Gastroenterol Belg*. 1983;46:469–484.
12. Epstein MT, Espiner EA, Donald RA, et al. Effect of eating liquorice on the renin-angiotensin aldosterone axis in normal subjects. *BMJ*. 1977;1:488–490.
13. Armanini D, Karbowiak I, Funder JW. Affinity of liquorice derivatives for mineralocorticoid and glucocorticoid receptors. *Clin Endocrinol (Oxf)*. 1983;19:609–612.
14. Tamura Y, Nishikawa T, Yamada K. Effects of glycyrrhetinic acid and its derivatives on delta 4-5 alpha- and 5 beta-reductase in rat liver. *Arzneimittelforschung*. 1979;29:647–649.
15. Kuroyanagi T, Saito M. Effect of prednisolone and glycyrrhizin on passive transfer of experimental allergic encephalomyelitis. *Arerugi*. 1966;15:67–74. ([Japanese]).
16. Cyong J, Otsuka Y. A pharmacological study of the anti-inflammatory activity of Chinese herbs. A review. *Acupunct Electrother Res*. 1982;7:173–202.
17. Kumagai A, Nanaboshi M, Asanuma Y, et al. Effects of glycyrrhizin on thymolytic and immunosuppressive action of cortisone. *Endocrinol Jpn*. 1967;14:39–42.
18. Van Uum SH, Walker BR, Hermus AR, et al. Effect of glycyrrhetinic acid on 11 beta-hydroxysteroid dehydrogenase activity in normotensive and hypertensive subjects. *Clin Sci (Colch)*. 2002;102:203–211.
19. Okimasa E, Moromizato Y, Watanabe S, et al. Inhibition of phospholipase A2 by glycyrrhizin, an anti-inflammatory drug. *Acta Med Okayama*. 1983;37:385–391.
20. Amer MS, Mckinney GR, Akcasu A. Effect of glycyrrhetinic acid on the cyclic nucleotide system of the rat stomach. *Biochem Pharmacol*. 1974;23:3085–3092.
21. Ohuchi K, Kamada Y, Levine L, et al. Glycyrrhizin inhibits prostaglandin E2 production by activated peritoneal macrophages from rats. *Prostaglandins Med*. 1981;7:457–463.
22. Suzuki H, Ohta Y, Takino T, et al. Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis—double blind trial. *Asian Med J*. 1984;26:423–438.
23. Mendes-Silva W, Assafim M, Ruta B, et al. Antithrombotic effect of Glycyrrhizin, a plant-derived thrombin inhibitor. *Thromb Res*. 2003;112:93–98.
24. Abe N, Ebina T, Ishida N. Interferon induction by glycyrrhizin and glycyrrhetinic acid in mice. *Microbiol Immunol*. 1982;26:535–539.
25. Pompei R, Pani A, Flore O, et al. Antiviral activity of glycyrrhizic acid. *Experientia*. 1980;36:304.
26. Lin JC. Mechanism of action of glycyrrhizic acid in inhibition of Epstein-Barr virus replication in vitro. *Antiviral Res*. 2003;59:41–47.
27. Cinatl J, Morgenstern B, Bauer G, et al. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet*. 2003;361:2045–2046.
28. Sasaki H, Takei M, Kobayashi M, et al. Effect of glycyrrhizin, an active component of licorice roots, on HIV replication in cultures of peripheral blood mononuclear cells from HIV-seropositive patients. *Pathobiology*. 2003;70:229–236.
29. Sekizawa T, Yanagi K, Itoyama Y. Glycyrrhizin increases survival of mice with herpes simplex encephalitis. *Acta Virol*. 2001;45:51–54.
30. Barfod L, Kemp K, Hansen M, et al. Chalcones from Chinese liquorice inhibit proliferation of T cells and production of cytokines. *Int Immunopharmacol*. 2002;2:545–555.
31. Wang ZY, Nixon DW. Licorice and cancer. *Nutr Cancer*. 2001;39:1–11.
32. Takahashi T, Takasuka N, Iigo M, et al. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci*. 2004;95:448–453.
33. Kanazawa M, Satomi Y, Mizutani Y, et al. Isoliquiritigenin inhibits the growth of prostate cancer. *Eur Urol*. 2003;43:580–586.
34. Yamazaki S, Morita T, Endo H, et al. Isoliquiritigenin suppresses pulmonary metastasis of mouse renal cell carcinoma. *Cancer Lett*. 2002;183:23–30.
35. Watanabe M, Hayakawa S, Isemura M, et al. Identification of licocoumarone as an apoptosis-inducing component in licorice. *Biol Pharm Bull*. 2002;25:1388–1390.
36. Mitscher L, Park Y, Clark D. Antimicrobial agents from higher plants. Antimicrobial isoflavonoids and related substances from Glycyrrhiza glabra L. var. typica. *J Nat Products*. 1980;43:259–269.
37. Fukai T, Marumo A, Kaitou K, et al. Antimicrobial activity of licorice flavonoids against methicillin-resistant Staphylococcus aureus. *Fitoterapia*. 2002;73:536–539.
38. Fukai T, Marumo A, Kaitou K, et al. Anti-Helicobacter pylori flavonoids from licorice extract. *Life Sci*. 2002;71:1449–1463.
39. Tsukiyama R, Katsura H, Tokuriki N, et al. Antibacterial activity of licochalcone A against spore-forming bacteria. *Antimicrob Agents Chemother*. 2002;46:1226–1230.
40. Kiso Y, Tohkin M, Hikino H, et al. Mechanism of antihepatotoxic activity of glycyrrhizin, I: effect on free radical generation and lipid peroxidation. *Planta Medica*. 1984;50:298–302.
41. Dhingra D, Parle M, Kulkarni SK. Memory enhancing activity of Glycyrrhiza glabra in mice. *J Ethnopharmacol*. 2004;91:361–365.
42. Fukai T, Satoh K, Nomura T, et al. Preliminary evaluation of antinephritis and radical scavenging activities of glabridin from Glycyrrhiza glabra. *Fitoterapia*. 2003;74:624–629.
43. Doll R, Hill I, Hutton C, et al. Clinical trial of a triterpenoid liquorice compound in gastric and duodenal ulcer. *Lancet*. 1962;2:793–796.
44. Wilson JA. A comparison of carbenoxolone sodium and deglycyrrhizinated liquorice in the treatment of gastric ulcer in the ambulant patient. *Br J Clin Pract*. 1972;26:563–566.
45. Van Marle J, Aarsen PN, Lind A, et al. Deglycyrrhizinised liquorice (DGL) and the renewal of rat stomach epithelium. *Eur J Pharmacol*. 1981;72:219–225.
46. Goso Y, Ogata Y, Ishihara K, et al. Effects of traditional herbal medicine on gastric mucin against ethanol-induced gastric injury in rats. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*. 1996;113:17–21.
47. Hajiaghamohammadi AA, Zargar A, Oveisi S, Samimi R, Reisian S. To evaluate of the effect of adding licorice to the standard treatment regimen of Helicobacter pylori. *Braz J Infect Dis*. 2016;20(6):534–538. https://doi.org/10.1016/j.bjid.2016.07.015. Epub 2016 Sep 8.
48. Morgan AG, Pacsoo C, McAdam WA. Maintenance therapy. A two year comparison between Caved-S and cimetidine treatment in the prevention of symptomatic gastric ulcer. *Gut*. 1985;26:599–602.
49. Morgan AG, McAdam WA, Pacsoo C, et al. Comparison between cimetidine and Caved-S in the treatment of gastric ulceration, and subsequent maintenance therapy. *Gut*. 1982;23:545–551.

50. Turpie AG, Runcie J, Thomson TJ. Clinical trial of deglycyrrhizinised liquorice in gastric ulcer. *Gut*. 1969;10:299–303.
51. Rees WD, Rhodes J, Wright JE, et al. Effect of deglycyrrhizinated liquorice on gastric mucosal damage by aspirin. *Scand J Gastroent*. 1979;14:605–607.
52. Kassir ZA. Endoscopic controlled trial of four drug regimens in the treatment of chronic duodenal ulceration. *Ir Med J*. 1985;78:153–156.
53. Tewari SN, Wilson AK. Deglycyrrhizinated liquorice in duodenal ulcer. *Practitioner*. 1973;210:820–823.
54. Das SK, Das V, Gulati AK, et al. Deglycyrrhizinated liquorice in aphthous ulcers. *J Assoc Physicians India*. 1989;37:647.
55. Tanaka Y, Kikuzaki H, Fukuda S, et al. Antibacterial compounds of licorice against upper airway respiratory tract pathogens. *J Nutr Sci Vitaminol (Tokyo)*. 2001;47:270–273.
56. Tanaka TA. Novel anti-dysmenorrhea therapy with cyclic administration of two Japanese herbal medicines. *Clin Exp Obstet Gynecol*. 2003;30:95–98.
57. Ofir R, Tamir S, Khatib S, et al. Inhibition of serotonin re-uptake by licorice constituents. *J Mol Neurosci*. 2003;20:135–140.
58. Ikegami N, Akatani K, Yamazaki S, et al. Prophylactic effect of long-term oral administration of glycyrrhizin on AIDS development of asymptomatic patients. *Int Conf AIDS*. 1993;9:234. (abstract).
59. Ikegami N, Akatani K, Yamazaki S, et al. *Clinical Evaluation of Glycyrrhizin on HIV-Infected Asymptomatic Hemophiliac Patients in Japan. Fifth International Conference on AIDS*. Abstract W.B.P; 1989:298.
60. Mori K, Sakai H, Suzuki S, et al. The present status in prophylaxis and treatment of HIV infected patients with hemophilia in Japan. *Rinsho Byhori*. 1989;37:1200–1208. ([Japanese]).
61. Hattori T, Ikematsu S, Koito A, et al. Preliminary evidence for inhibitory effect of glycyrrhizin on HIV replication in patients with AIDS. *Antiviral Res*. 1989;11:255–261.
62. Eisenburg J. Treatment of chronic hepatitis B. Part 2. Effect of glycyrrhizinic acid on the course of illness. *Fortschr Med*. 1992;110:395–398 ([German]).
63. Acharya SK, Dasarathy S, Tandon A, et al. A preliminary open trial on interferon stimulator (SNMC) derived from Glycyrrhiza glabra in the treatment of subacute hepatic failure. *Indian J Med Res*. 1993;98:69–74.
64. Kumada H. Long-term treatment of chronic hepatitis C with glycyrrhizin [stronger neo-minophagen C (SNMC)] for preventing liver cirrhosis and hepatocellular carcinoma. *Oncology*. 2002;62:94–100.
65. Anderson K, Bradlow H, Sassa S, et al. Studies in porphyria VII. Relationship of the 5 alpha-reductive metabolism of steroid hormones to clinical expression of the genetic defect in acute intermittent porphyria. *Am J Med*. 1979;66:644–650.
66. Tomita T, Sato T, Saito K, et al. Effects of lead and arsenic on the formation of 5 beta-H steroids. *Toxicol Lett*. 1979;3:291–297.
67. Armanini D, De Palo CB, Mattarello MJ, et al. Effect of licorice on the reduction of body fat mass in healthy subjects. *J Endocrinol Invest*. 2003;26:646–650.
68. Kuroda M, Mimaki Y, Sashida Y, et al. Phenolics with PPAR-gamma ligand-binding activity obtained from licorice (Glycyrrhiza uralensis roots) and ameliorative effects of glycyrin on genetically diabetic KK-A(y) mice. *Bioorg Med Chem Lett*. 2003;13:4267–4272.
69. Mae T, Kishida H, Nishiyama T, et al. A licorice ethanolic extract with peroxisome proliferator-activated receptor-gamma ligand-binding activity affects diabetes in KK-Ay mice, abdominal obesity in diet-induced obese C57BL mice and hypertension in spontaneously hypertensive rats. *J Nutr*. 2003;133:3369–3377.
70. Armanini D, Fiore C, Mattarello MJ, et al. History of the endocrine effects of licorice. *Exp Clin Endocrinol Diabetes*. 2002;110:257–261.
71. Fuhrman B, Volkova N, Kaplan M, et al. Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. *Nutrition*. 2002;18:268–273.
72. MacKenzie MA, Hoefnagels WH, Jansen RW, et al. The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. *J Clin Endocrinol Metab*. 1990;70:1637–1643.
73. Chen MF, Shimada F, Kato H, et al. Effect of glycyrrhizin on the pharmacokinetics of prednisolone following low dosage of prednisolone hemisuccinate. *Endocrinol Jpn*. 1990;37:331–341.
74. Agarwal A, Gupta D, Yadav G, et al. An evaluation of the efficacy of licorice gargle for attenuating postoperative sore throat: a prospective, randomized, single-blind study. *Anesth Analg*. 2009;109:77–81.
75. Huang QC, Wang MJ, Chen XM, et al. Can active components of licorice, glycyrrhizin and glycyrrhetinic acid, lick rheumatoid arthritis? *Oncotarget*. 2016;7(2):1193–1202. PubMed PMID: 26498361.
76. Honda K, Kamisoyama H, Tominaga Y, et al. The molecular mechanism underlying the reduction in abdominal fat accumulation by licorice flavonoid oil in high fat diet-induced obese rats. *Anim Sci J*. 2009;80:562–569.
77. Kamisoyama H, Honda K, Tominaga Y, et al. Investigation of the anti-obesity action of licorice flavonoid oil in diet-induced obese rats. *Biosci Biotechnol Biochem*. 2008;72:3225–3231.
78. Aoki F, Honda S, Kishida H, et al. Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high-fat diet-induced obese C57BL/6J mice. *Biosci Biotechnol Biochem*. 2007;71:206–214.
79. Tominaga Y, Nakagawa K, Mae T, et al. Licorice flavonoid oil reduces total body fat and visceral fat in overweight subjects: a randomized, double-blind, placebo-controlled study. *Obes Res Clin Prac*. 2009;3:159–178.
80. Kinoshita T, Matsumoto A, Yoshino K, Furukawa S. The effects of licorice flavonoid oil with respect to increasing muscle mass: a randomized, double-blind, placebo-controlled trial. *J Sci Food Agric*. 2017;97(8):2339–2345. https://doi.org/10.1002/jsfa.8044. Epub 2016 Nov 11.
81. Aoki F, Nakagawa K, Kitano M, et al. Clinical safety of licorice flavonoid oil (LFO) and pharmacokinetics of glabridin in healthy humans. *J Am Coll Nutr*. 2007;26:209–218.
82. Saeedi M, Morteza-Semnani K, Ghoreishi MR. The treatment of atopic dermatitis with licorice gel. *J Dermatolog Treat*. 2003;14:153–157.
83. Teelucksingh S, Mackie AD, Burt D, et al. Potentiation of hydrocortisone activity in skin by glycyrrhetinic acid. *Lancet*. 1990;335:1060–1063.
84. Nokhodchi A, Nazemiyeh H, Ghafourian T, et al. The effect of glycyrrhizin on the release rate and skin penetration of diclofenac sodium from topical formulations. *Farmaco*. 2002;57:883–888.
85. Saeedi M, Morteza-Semnani K, Ghoreishi MR. The treatment of atopic dermatitis with licorice gel. *J Dermatolog Treat*. 2003;14:153–157.
86. Evans FQ. The rational use of glycyrrhetinic acid in dermatology. *Br J Clin Pract*. 1958;12:269–274.
87. Partridge M, Poswillo D. Topical carbenoxolone sodium in the management of herpes simplex infection. *Br J Oral Maxillofac Surg*. 1984;22:138–145.
88. Csonka GW, Tyrrell DA. Treatment of herpes genitalis with carbenoxolone and cicloxolone creams. A double blind placebo controlled trial. *Br J Vener Dis*. 1984;60:178–181.
89. Nerya O, Vaya J, Musa R, et al. Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. *J Agric Food Chem*. 2003;51:1201–1207.
90. Bardhan KD, Cumberland DC, Dixon RA, et al. Clinical trial of deglycyrrhizinised liquorice in gastric ulcer. *Gut*. 1978;19:779–782.
91. Multicentre Trial. Treatment of duodenal ulcers with glycyrrhinizin acid-reduced liquorice. *BMJ*. 1973;3:501–503.
92. Feldman H, Gilat T. A trial of deglycyrrhizinated liquorice in the treatment of duodenal ulcer. *Gut*. 1971;12:499–555.
93. Nazari S, Rameshrad M, Hosseinzadeh H. Toxicological effects of Glycyrrhiza glabra (licorice): a review. *Phytother Res*. 2017;31(11):1635–1650. https://doi.org/10.1002/ptr.5893. Epub 2017 Aug 18.
94. Belhadj-Tahar H, Nassar B, Coulais Y, et al. Acute pseudo-aldosteronism syndrome induced by liquorice. *Therapie*. 2003;58:375–378.
95. Al-Qarawi AA, Abdel-Rahman HA, Ali BH, et al. Liquorice (Glycyrrhiza glabra) and the adrenal-kidney-pituitary axis in rats. *Food Chem Toxicol*. 2002;40:1525–1527.
96. Tanahashi T, Mune T, Morita H, et al. Glycyrrhizic acid suppresses type 2 11 beta-hydroxysteroid dehydrogenase expression in vivo. *J Steroid Biochem Mol Biol*. 2002;80:441–447.

97. Sigurjonsdottir HA, Franzson L, Manhem K, et al. Liquorice-induced rise in blood pressure: a linear dose-response relationship. *J Hum Hypertens*. 2001;15:549–552.
98. Ploeger B, Mensinga T, Sips A, et al. The pharmacokinetics of glycyrrhizic acid evaluated by physiologically based pharmacokinetic modeling. *Drug Metab Rev*. 2001;33:125–147.
99. Van Gelderen CE, Bijlsma JA, van Dokkum W, et al. Glycyrrhizic acid: the assessment of a no effect level. *Hum Exp Toxicol*. 2000;19:434–439.
100. Baron J, Nabarro J, Slater J, et al. Metabolic studies, aldosterone secretion rate, and plasma renin after carbenoxolone sodium as biogastrone. *BMJ*. 1969;2:793–795.
101. Armanini D, Bonanni G, Mattarello MJ, et al. Licorice consumption and serum testosterone in healthy man. *Exp Clin Endocrinol Diabetes*. 2003;111:341–343.
102. Josephs RA, Guinn JS, Harper ML, et al. Liquorice consumption and salivary testosterone concentrations. *Lancet*. 2001;358:1613–1614.
103. Strandberg TE, Jarvenpaa AL, Vanhanen H, et al. Birth outcome in relation to licorice consumption during pregnancy. *Am J Epidemiol*. 2001;153:1085–1088.
104. Kent UM, Aviram M, Rosenblat M, et al. The licorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450S 3A4, 2B6, and 2C9. *Drug Metab Dispos*. 2002;30:709–715.
105. Armanini D, Castello R, Scaroni C, et al. Treatment of polycystic ovary syndrome with spironolactone plus licorice. *Eur J Obstet Gynecol Reprod Biol*. 2007;131:61–67.

86

Hydrastis canadensis (Goldenseal) and Other Berberine-Containing Botanicals

Roy Upton, RH

OUTLINE

Hydrastis canadensis (family: *Ranunculaceae*)
Common names: goldenseal, yellow root, Indian turmeric, eye root, jaundice root
Berberis vulgaris (family: *Berberidaceae*)
Common name: barberry
Berberis aquifolium, Berberis nervosa (family: *Berberidaceae*)
Common names: Oregon grape, trailing mahonia
Coptis chinensis (family: *Ranunculaceae*)
Common name: coptis

INTRODUCTION

Berberine-containing plants have played a major role in the world's herbal traditions. The primary berberine-containing botanicals used in North America include goldenseal *(Hydrastis canadensis),* barberry *(Berberis vulgaris),* Oregon grape *(Berberis aquifolium),* and coptis *(Coptis chinensis).* The roots of these plants share similar indications and effects due to their high content of isoquinoline alkaloids, primarily berberine, which is common to these species. Additionally, goldenseal contains hydrastine, canadine, and tetrahydropalmatine. In addition to berberine, coptis contains coptisine, jatrorrhizine, and palmatine.[1,2] Barberry and Oregon grape, which botanically are alternately assigned to either the genus *Berberis* or *Mahonia,* in addition to berberine, contain jatrorrhizine, magnoflorine, and palmatine.[3] Other closely related berberine-containing species of North American and Eastern herbal traditions feature prominently as herbal medicines and include yellow root *(Xanthorrhiza simplissima)* and other species of *Berberis* or *Mahonia* (e.g., *Berberis nervosa* and *B. repens* from North America, *B. teeta* from India, and *Mahonia bealei* from China, among others). Most of these latter species of *Berberis* and *Mahonia* should be considered interchangeable.

The chief berberis alkaloid, berberine, has been studied extensively in both experimental and clinical settings. The general description, history and folk use, chemical composition, and specific clinical indications for the primary berberine-containing plants used in North America and pharmacology of berberine are presented here. Although the pharmacology of these plants is primarily discussed in terms of the activity of berberine, it is valuable to recognize that constituents other than alkaloids contribute to the efficacy of medicinal plants. In these particular cases, polysaccharides (in coptis and Oregon grape root)[4,5] and phenolic compounds (in Oregon grape root) are also present and are pharmacologically relevant (see Table 86.1).[6]

TABLE 86.1 Primary Alkaloids of Berberine-Containing Plants

Hydrastis canadensis[7,8]	*Berberis aquifolium*[1,2]	*Berberis vulgaris*[7,8]	*Coptis chinensis*[1]
Berberine (0.5%–6%)	Berberine	Berberine	Berberine (3.6%–7%)
Hydrastine (1.5%–4%)	Berbamine	Jatrorrhizine	Jatrorrhizine (0.1%–0.4%)
Berberastine (2%–3%)	Canadine	Berberubine	Palmatine (0.7%–1.5%)
Canadine (0.5%–1%)	Corypalmine	Berbamine	
Candaline	Mahonine	Bervulcine	
Corypalmine	Oxyacanthine	Palmatine	
Hydrastinine (degradation compound of hydrastine)	Isocorydine	Columbamine	

Fig. 86.1 *Hydrastis canadensis*. (From https://www.istockphoto.com/photo/goldenseal-leaves-and-flowers-gm475712354-65496923. Accessed November 24, 2018.)

GENERAL DESCRIPTION

Hydrastis canadensis

Goldenseal is native to eastern North America and is cultivated in Oregon and Washington. It is a perennial herb with a knotty yellow rhizome from which arises a single leaf and an erect hairy stem. In early spring, it bears two five- to nine-lobed rounded leaves near the top, which are terminated by a single greenish-white flower (Fig. 86.1). The primary parts used are the dried rhizome and roots, although the leaf is used in the southeastern parts of the United States.[7,8]

Berberis vulgaris

The common barberry is a deciduous spiny shrub that may reach 16 ft in height (Fig. 86.2). Native to Europe, it has been naturalized in eastern North America. The parts used are the whole rhizomes and roots and, less frequently, the bark of the stem and root.[7,8]

Berberis aquifolium

The Oregon grape is an evergreen, spineless, ornamental shrub that is 3 to 7 ft in height (Fig. 86.3). It is native to the Rocky Mountains from British Columbia to California. The parts used are whole rhizomes and roots and, less frequently, the bark of the stem and root.[8]

Fig. 86.2 *Berberis vulgaris*. (From https://www.istockphoto.com/photo/barberry-gm488402720-74107579. Accessed November 24, 2018.)

Fig. 86.3 *Berberis aquifolium*. (From https://www.istockphoto.com/photo/oregon-grape-gm485322828-71877603. Accessed November 24, 2018.)

Coptis chinensis

Coptis, also known as goldthread, is a perennial herb native to China. The parts used are the rhizomes and root.[9]

CHEMICAL COMPOSITION

The chemical structure of berberine, jatrorrhizine, and palmatine are shown in Fig. 86.4.

Hydrastis canadensis

Alkaloids isolated from *Hydrastis* include the following:

- Hydrastine (1.5%–4%)
- Berberine (0.5%–6%)
- Berberastine (2%–3%)
- Canadine
- Candaline
- Hydrastinine
- Other related alkaloids

Other constituents include meconin, chlorogenic acid, phytosterins, and resins.[1,2]

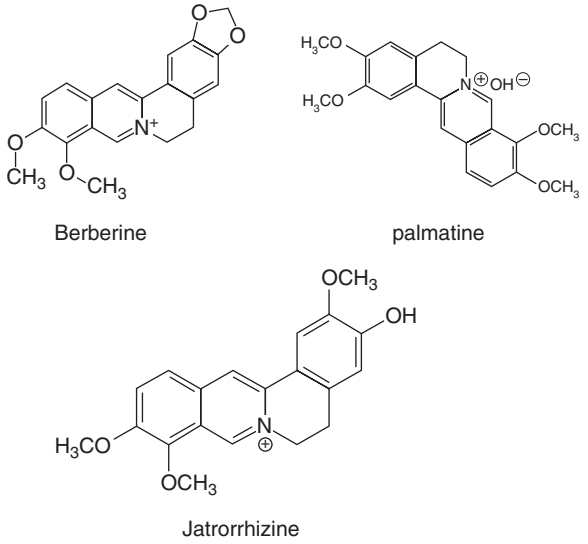


Fig. 86.4 Primary berberis alkaloids. (From https://en.wikipedia.org/wiki/Jatrorrhizine https://en.wikipedia.org/wiki/Berberine https://en.wikipedia.org/wiki/Palmatine. Accessed November 24, 2018.)

Berberis vulgaris

Barberry contains several alkaloids in its rhizomes and roots.

- Jatrorrhizine
- Berberine
- Berberrubine
- Berbamine
- Bervulcine
- Palmatine
- Columbamine
- Oxyacanthine

Barberry also contains chelidonic, citric, malic, and tartaric acids.[7,8]

Berberis aquifolium

Oregon grape contains the alkaloids berbamine, berberine, canadine, corypalmine, hydrastine, isocorydine, mahonine, and oxyacanthine. Resins and tannins have also been reported.[1,2]

Coptis chinensis

Goldthread root contains berberine (5%–8%) and other alkaloids similar to those found in goldenseal.[3]

HISTORY AND FOLK USE

Hydrastis canadensis

Native to North America, goldenseal root was used extensively by American Indians as an herbal medication and clothing dye. Its medicinal use centered around its ability to soothe the mucous membranes of the respiratory, digestive, and genitourinary tracts in inflammatory conditions induced by allergy or infection. The Cherokee and other tribes used goldenseal in disorders of the eye and skin.[7,8] Berberine was isolated from goldenseal root by early American naturalist Constantine Rafinesque in 1828. Goldenseal root featured prominently in the Eclectic medical practice of the mid-1800s, where it was recorded as among "their best articles"[10] and was used for gastrointestinal complaints, jaundice, infections, as a bitter tonic to regulate bile production, and as a uterine tonic. For many of these purposes, goldenseal was considered to have no equal.[11]

Berberis vulgaris

Barberry is native to most of Europe. Closely related species are found in North Africa and Asia. Barberry's historical use is as an antidiarrheal agent, bitter tonic, antipyretic, and antihemorrhagic.[7,8]

Berberis aquifolium

Oregon grape's historical and folk use is like that of goldenseal. In addition, Oregon grape was used in the treatment of chronic skin conditions such as acne, psoriasis, and eczema.[7,8]

Coptis chinensis

In China, coptis was used in the traditional medicine system to "drain fire," which refers to the treatment of conditions associated with infections and toxemia. It was used primarily in infectious conditions, similar to the historical use of goldenseal. Specific uses included fever, dysentery, gastrointestinal infection, furuncles, boils, and eye infections.[9]

PHARMACOLOGY

The primary medicinal value of goldenseal, barberry, Oregon grape root, and coptis is thought to be due to their high content of isoquinoline alkaloids, of which berberine has been the most widely studied. Berberine demonstrates antibiotic, anticonvulsant, antidiarrheal, anti-inflammatory, choleretic, carminative, hepatoprotectant, hypolipidemic, hypotensive, immunostimulatory, sedative, and uterotonic activity. Berberine's pharmacological activities strongly support the historical use of berberine-containing herbs.

Antimicrobial, Anti-infective Activity, and Gut Health

The most broadly celebrated of berberine's effects has been its antimicrobial activity. Although not as potent as many prescription antibiotics, berberine exhibits a broad spectrum of antimicrobial activity against a wide range of human pathogens that include bacteria, protozoa, and fungi, including the specific strains listed in Table 86.2.[7–9,12–21]

Most of these microbes have a significant effect on human health, with many affecting the gastrointestinal system. *E. histolytica* alone is responsible for approximately 55,000 deaths annually. Berberine exhibits broad-spectrum antibacterial activity against numerous strains of *Staphylococcus,* and additionally it enhances the efficacy of conventional antibiotic agents such as linezolid, cefoxitin, and erythromycin.[21]

Perhaps berberine's greatest potential contribution to human health is that pathogens generally fail to develop resistance to its antimicrobial effects.[22] As a result of the excessive use of antibiotics for decades, both used clinically for the treatment of infections and through exposure through the food chain from commercially raised foods, numerous bacteria have become resistant to standard treatment with antibiotics. Of these, methicillin-resistant *Staphylococcus aureus* (MRSA) is most well known and can cause serious skin infections and pneumonia that can result in death or cause other systemic infections that are resistant to treatment. MRSA most commonly occurs in hospital settings, as a result of the prevalence of patients with infectious disease, but can also arise from contact with generally healthy people.

Experiments were conducted to determine whether a variety of bacteria, including *E. coli, S. aureus, Bacillus subtilis, Proteus vulgaris, Salmonella typhimurium,* and *Pseudomonas aeruginosa*, could develop resistance to berberine. No resistance was observed after 200 generations of exposure of these bacteria to berberine.[23] Prevention of resistance was also demonstrated for berberine analogs against *Acinetobacter baumannii,* a bacterial pathogen that can cause serious infections of the blood, lungs, and brain.[24]

TABLE 86.2 In Vitro Sensitivity of Microorganisms to Berberine Sulfate

Organism	Inhibitory Concentration (mg/mL) (unless otherwise specified[a])
Bacteria	
Acinetobacter baumannii	2 nmol/mL
Bacillus cereus	25
Bacillus subtilis	25
Corynebacterium diphtheriae	6.2
Enterobacter aerogenes	2500.05
Escherichia coli	600.05
Klebsiella spp.	>100
Klebsiella pneumoniae	25
Proteus spp.	>100
Pseudomonas mangiferae	>100
Pseudomonas pyocyanea	>100
Salmonella paratyphi	>100
Salmonella typhimurium	>100
Shigella boydii	12.5
Staphylococcus aureus	6.2–50
Streptococcus pyogenes	12.5
Vibrio cholera	25
Fungi	
Candida utilis	12.5
Candida albicans	12.5
Candida glabrata	16 5 g/mL
Candida guilliermondii	16 µg/mL
Candida krusei	16 µg/mL
Candida tropicalis	31.3 µg/mL
Cryptococcus neoformans	150[b]
Microsporum gypseum	50[b]
Saccharomyces cerevisiae	100[b]
Sporothrix schenckii	6.2
Trichophyton mentagrophytes	100[b]
Other	
Entamoeba histolytica	200
Erwinia carotovora	100
Leishmania donovani	5.08
Mycobacterium tuberculosis	200[b]
Xanthomonas citri	3.1

[a]Minimum concentration that totally inhibits growth in a liquid medium at pH 8. Maximum concentration tested was 100 mg/mL, unless otherwise noted.

[b]Tested in a solid medium, which typically requires a concentration that is 4 to 10 times greater for the same level of inhibition.

Data from Kost'alova D, Kardosova A, Hajnicka V. Effect of *Mahonia aquifolium* stem bark crude extract and one of its polysaccharide components on production of IL-8. *Fitoterapia*. 2001;72(7):802–806. Wirth C, Wagner H. Pharmacologically active phenolic compounds from the bark of *Mahonia aquifolium*. *Phytomedicine*. 1997;4(4):357–358. Chang HM, But PPH. *Pharmacology and Applications of Chinese Materia Medica*. Vol. 2. Teaneck, NJ: World Scientific. He JM, Mu Q. The medicinal uses of the genus *Mahonia* in traditional Chinese medicine: an ethnopharmacological, phytochemical and pharmacological review. *J Ethnopharmacol*. 2013;175(2015):668–683.

Part of the ability of berberine to avoid developing bacterial resistance against it is that it works through multiple mechanisms of action. When an anti-infective agent, or any therapeutic agent, has a single mode of action, such as direct bactericidal activity, it is relatively easy for a pathogen, whose nature it is to survive and multiply, to quickly become resistant to the killing effects of the antibiotic. When an antimicrobial works through multiple actions, it is much more difficult for the pathogen to become resistant because it must find multiple ways to overcome the myriad of disparate killing effects of the agent.

In one study, researchers demonstrated berberine's ability to inhibit the adherence of group A *Streptococci* to host cells on the basis that the therapeutic effect of berberine appeared to be greater than its direct antibiotic effects.[25] Other studies showed that certain antimicrobial agents can block the adherence of microorganisms to host cells at doses much lower than those needed to kill cells or to inhibit cell growth. Berberine's ability to inhibit the adhesion of *Streptococci* to host cells has several modes of action. First, berberine causes *Streptococci* to lose lipoteichoic acid. Lipoteichoic acid is the major substance responsible for adhesion of bacteria to host tissues. If the bacteria cannot adhere, it cannot become infectious. Berberine also exhibited direct bactericidal activity against *Streptococci agalactiae,* a human pathogen that can cause meningitis, septicemia, and pneumonia. Another important action of berberine is preventing the adhesion of fibronectin to the *Streptococci,* as well as eluting already-bound fibronectin. The binding of fibronectin to *Streptococci* increases the virulence of the bacteria. In contrast, inhibition of fibronectin binding has clinical relevance in both treating and preventing select infectious diseases.[26,27]

A unique mechanism associated with berberine's antimicrobial activity is its ability to damage the DNA of bacteria.[21] Numerous other studies elucidate other mechanisms of action that contribute to berberine's antimicrobial activity, which include the inhibition of bacterial DNA duplication, bacterial RNA transcription, and bacterial protein synthesis; the inhibition of enzymes needed for bacterial proliferation; and the destruction of the bacterial biofilm and cell surface that make bacteria more susceptible to destruction by immune cells.[23] This multitude of actions makes berberine the ideal antibiotic for modern use, especially in MRSA. Moreover, berberine has been shown to restore the efficacy of previously resistant antibiotics, such as penicillin, ampicillin, and oxacillin.[28] The antimicrobial activity of berberine was also found to increase with pH in a number of organisms studied.[13] At a pH of 8, the antimicrobial activity of berberine in vitro is typically two to four times greater than it is at pH 7, which in turn is one to four times greater than at pH 6. This suggests that alkalinization will improve the clinical efficacy of berberine, particularly in the treatment of urinary tract infections.

Berberine also exhibits significant antifungal activity against *Candida albicans,* the overgrowth of which can result in both systemic and vaginal yeast infections. Excessive use of conventional antibiotics is a very common contributing factor to yeast overgrowth. The fact that berberine both acts as an antibiotic and kills *Candida,* again, makes it an ideal antibiotic of choice. Other fungi against which berberine exhibits antimicrobial activity include *Aspergillus, Cryptococcus,* and *Penicillium.*[21]

In addition to the direct actions of berberine, other studies show that other compounds in berberine-containing plants also contribute to overall antimicrobial activity because crude extracts often display greater antimicrobial activity than a comparable amount of isolated berberine.[29,30] In contrast to the previous report of berberine being resistant to bacterial resistance, one study showed *E. coli* to become resistant to pure berberine; however, no resistance was developed for the methanolic extract, demonstrating the synergism that exists in whole-plant extracts.[31] This underscores the strongly held belief of

medical herbalists that the activity of a botanical cannot be defined by a single compound.

A unique contribution of berberine to human health is its ability to promote healthy intestinal microflora. Most anti-infectious agents, such as conventional antibiotics, have a negative effect on gut flora and therefore digestion, assimilation, and gut-mediated immunity. Berberine promotes the growth of the *Akkermansia muciniphila, Bacteroides,* and *Ruminococcus* bacterial species normally found in healthy intestinal flora. Together, these mechanisms help reduce intestinal permeability,[32–34] which can reduce exposure to endotoxins that can trigger inflammatory responses, damage the liver and kidneys,[35] and contribute to the development of atherosclerosis.[36] In part, this broad spectrum of positive effects on gut health may be the primary underlying reason berberine-containing plants have been so highly regarded as traditional medicines for so long.

In addition to the effects already mentioned, berberine may have a positive effect on reducing the severity or incidence of colitis via an anti-inflammatory activity through modulation of interferon, interleukins, and prostaglandins in the gut and systemically. This was demonstrated in colitis induced in an animal model in which berberine improved body weight, reduced colon shortening, prevented colon damage, and reduced histological markers of inflammation.[37]

Immunostimulatory Activity and Anticancer Effects

There is an abundance of preclinical evidence for the effects of berberine in modulating immune responses and eliciting anticancer activity that suppresses the growth of a wide spectrum of tumors, including leukemia, melanoma, hepatoma, epidermoid carcinoma, glioblastoma, and oral carcinoma, as well as breast, lung, gastric, prostate, and cervical cancer. Berberine was also found to suppress chemical-induced carcinogenesis, tumor promotion, and tumor invasion in some animal models.[38]

In one study, berberine was shown to increase the blood supply to the spleen.[39] This improved blood supply may promote the optimal activity of the spleen by increasing the release of compounds, such as tuftsin, that potentiate immune function. Research shows that berberine influences splenic antibody response.[40] Berberine was also shown to activate macrophages, via both enhanced priming and triggering.[41] Other research demonstrated that total extract of barberry and its alkaloid fractions (berberine and oxyacanthine) elicits anti-inflammatory activity in both acute and chronic animal models of inflammation (Fig. 86.5). As in other studies reported, the total extract elicited a stronger anti-inflammatory response than the isolated fractions.[42] One study suggested the anti-inflammatory activity of berberine to be associated with a modulation, not an inhibition, of cyclooxygenase (COX) protein.[43]

Berberine exhibits potent anticancer activity both directly by killing tumor cells and indirectly by stimulating white blood cells.[39,41,44,45] Whereas most other actions attributed to berberine-containing plants are well established in the historical literature, anticancer effects seem to be a relatively modern discovery. Most anticancer data for berberine comes from in vitro and animal studies. One of the most significant effects was reported in a study demonstrating antitumor activity against human and rat malignant brain tumors.[44] Several experimental approaches were used in the study. In vitro studies were performed on a series of six human malignant brain tumor cell lines and rat 9L brain tumor cells. Berberine used alone at a dose of 150 mg/mL showed an average cancer cell kill of 91%. This kill rate was more than twice that of 1,3-*bis*(2-chloro-ethyl)-1-nitrosourea (BCNU), the standard chemotherapeutic agent for brain tumors, which had a cell kill rate of 43%. In vitro results do not always match the clinically relevant effects in vivo, so such reports have to be interpreted carefully. However, in this case, follow-up studies in rats with solid 9L brain tumors also demonstrated that berberine had antitumor effects. Rats treated with berberine (10 mg/kg) had an 81% cell kill. However, the combination treatment of berberine and BCNU exhibited additive effects on killing cancer cells. These results indicate that berberine may prove to be more effective than BCNU or, at the least, a valuable therapeutic addition in the treatment of difficult brain cancers.

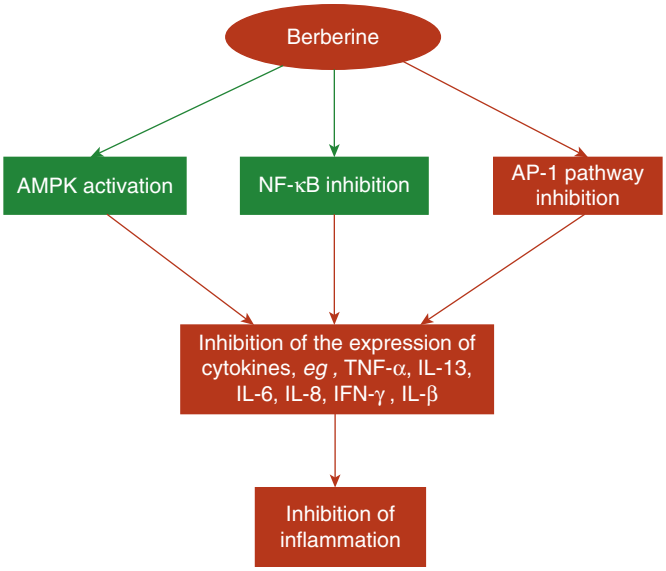


Fig. 86.5 Summary of the anti-inflammatory effect of berberine. (From Zou K, Li Z, Zhang Y, Zhang HY, Li B, Zhu WL, Shi JY, Jia Q, Li YM. Advances in the study of berberine and its derivatives: a focus on anti-inflammatory and anti-tumor effects in the digestive system. *Acta Pharmacologica Sinica.* 2017;38(2):157–167. PubMed PMID: 27917872.)

Another study demonstrated that berberine exhibited cytotoxic activity against nasopharyngeal carcinoma cells and inhibited the repair of cancer cell DNA that subsequently resulted in the death of cancer cells.[46] Berberine has also been shown to be cytotoxic against prostate cancer cells and to inhibit their proliferation in vitro, mediated primarily through the G1 phase-cycle arrest and caspase-dependent pathway.[47] Berberine has been shown to possess cytotoxic activity against numerous other cancer cell lines in vitro.[48] Whether this has any clinical relevance requires further in vivo research. In one such study, berberine combined with radiation demonstrated a synergistic anticancer effect in a lung cancer animal model.[49] The ability for berberine to increase the anticancer activity of numerous chemotherapeutic drugs has similarly been determined in animal models. Of particular interest is that berberine not only enhanced the anticancer effects of numerous chemotherapeutic agents but also decreased cardiac, renal, and hepatic toxicity associated with the chemotherapy.[38] Berberine exhibits antioxidant and antimutagenic effects that may have relevance in carcinogenesis.[50,51] If the in vitro and preclinical in vivo effects of berberine enhancing the efficacy of conventional chemotherapeutic drugs is realized in human trials, berberine will represent a novel adjunct to standard cancer therapy.

Antipyretic Activity

Historically, berberine-containing plants were used as febrifuges. Berberine produces an antipyretic effect three times as potent as aspirin in a pyretic model in rats.[52] However, although aspirin suppresses fever through its action on prostaglandins, berberine appears to lower

fever by increasing the immune system's handling of fever-producing compounds from microorganisms.

Type 2 Diabetes and Metabolic Syndrome

In another novel use, berberine possesses a wide range of antidiabetic effects, including an ability to increase insulin-receptor expression and improve glucose utility both in in vitro and in animal models, reduce insulin resistance through protein kinase C–dependent upregulation of insulin receptor expression, stimulate insulin secretion, block glucose absorption via inhibition of α-glucosidase and epithelial glucose transport, and reduce serum lipid levels in animal models of impaired glucose tolerance.[53–55] One insulin-independent hypoglycemic activity is associated with the stimulation of glycolysis and activation of AMP-activated protein kinase, representing a relatively novel herbal approach to the management of diabetes.[56]

In addition to the myriad of hypoglycemic activity elicited by berberine, the formation of advanced glycosylation end products and oxidative stress that leads to diabetic nephropathy is suppressed by the administration of berberine.[57]

Cardiovascular Effects

In more recent years there has been active research investigating the cardiac effects associated with berberine, most notably derived from *Coptis chinensis.* In animal models of cardiac hypertrophy and heart failure, berberine decreased plasma noradrenaline and adrenaline in the heart, increasing cardiac contractility and decreasing diastolic blood pressure and vascular resistance.[58]

CLINICAL APPLICATIONS

The broad antimicrobial effects of berberine, combined with the anti-infective and immune-stimulating actions reported previously, support the historical use of berberine-containing plants in infections of the mucous membranes as well as the gastrointestinal system. Although the primary focus of this activity is typically correlated with antimicrobial effects, berberine also has a protective effect on the epithelial cells of the gastrointestinal tissues, supporting one of most unique aspects of berberine-containing plants, decreasing intestinal permeability, which has significantly positive and broad-reaching effects on improving human health.[59,60] Lastly, clinical research on the use of berberine in the treatment of type 2 diabetes, dyslipidemia, and metabolic syndrome is extremely promising.[61]

Infectious Diarrhea and Bowel Health

Berberine showed significant success in the treatment of acute diarrhea in several clinical studies. It was found to be effective against diarrheas caused by *E. coli* (traveler's diarrhea), *Shigella dysenteriae* (shigellosis), *Salmonella paratyphi, Salmonella typhimurium* (food poisoning, infectious diarrhea), *Klebsiella species, G. lamblia* (giardiasis), and *V. cholerae* (cholera).[62–71]At least one clinical study demonstrated that patients with irritable bowel syndrome (IBS) taking berberine had better symptom control compared with placebo.[59]

Similar positive findings of the effects of berberine on supporting healthy gastrointestinal functioning are reported in preclinical research. Studies in hamsters and rats showed that berberine had significant activity against *Entamoeba histolytica,* the causative organism of amebiasis.[15,16] In another study of animals with hemorrhagic diarrhea, berberine successfully inhibited multidrug-resistant enterovirulent *E. coli.*[72]

There is good evidence that berberine is effective in treating the majority of common gastrointestinal infections, as evidenced by the widespread antimicrobial activity against major gastrointestinal pathogens such as *H. pylori, Salmonella typhi, G. lamblia, Entamoeba histolytica,* and *C. albicans.* In many cases, clinical studies demonstrate that berberine produces comparable effects to standard antibiotics. In several studies, the efficacy of berberine was found to be greater than that of conventional therapies.[64,67,68] For example, in a study of 65 children younger than 5 years of age with acute diarrhea caused by *E. coli, Shigella, Salmonella, Klebsiella,* or *Fecalis aerogenes,* those given berberine tannate (25 mg/6 hours) responded better than those treated with standard antibiotic therapy.[67]

In another study, 40 children ages 1 to 10 years and infected with the parasite *Giardia* received either berberine (5 mg/kg body weight each day), the drug metronidazole (10 mg/kg body weight each day), or a placebo of vitamin B syrup in three divided doses.[68] After 6 days, 48% of patients treated with berberine were symptom-free, and on stool analysis, 68% were *Giardia*-free. In the metronidazole (Flagyl) group, 33% of patients were without symptoms, and on stool analysis, all were *Giardia*-free. In comparison, 15% of patients on placebo were asymptomatic, and on stool analysis, 25% were *Giardia*-free. These results indicate that berberine was actually more effective than metronidazole in relieving symptoms at half the dose but less effective than the drug in clearing the organism from the intestines.

Finally, in a study of 200 adult patients with acute diarrhea, the subjects were given standard antibiotic treatment with or without berberine hydrochloride (150 mg/day). The patients receiving berberine recovered quicker.[64] An additional 30 cases of acute diarrhea were treated with berberine alone. Berberine arrested diarrhea in all of these cases, with no mortality or toxicity.

Despite these results, because of the serious consequences of an ineffectively treated infectious diarrhea due to highly pathogenic organisms, it is best to consider using berberine or berberine-containing plants as an adjunct to standard antibiotic therapy.

Much of berberine's effectiveness is undoubtedly due to a combination of its direct antimicrobial activity, inhibition of microbial attachment to mucous membranes, and blocking of the action of toxins produced by several pathogenic bacteria.[72–74] The toxin-blocking effect is most evident in diarrheas caused by enterotoxins (e.g., *V. cholerae, E. coli*), cholera, and traveler's diarrhea.[69–73]

Although cholera is a serious disorder that needs standard antibiotic therapy, traveler's diarrhea is usually self-limiting. Good results with berberine in the treatment of traveler's diarrhea have been obtained. In one study, patients with traveler's diarrhea randomly received 400 mg of berberine sulfate in a single dose or served as controls.[72] In treated patients, the mean stool volumes were significantly less than those of controls during three consecutive 8-hour periods after treatment. At 24 hours after treatment, diarrhea ceased in significantly more treated patients compared with controls (42% vs. 20%).

For people planning to travel to an underdeveloped country or an area of poor water quality or sanitation, the prophylactic use of berberine-containing herbs 1 week before, during, and 1 week after visiting may be useful.

Trachoma

Water extracts of berberine-containing plants have been employed to treat various eye complaints, including infectious processes, by cultures throughout the world. In older studies, berberine demonstrated a remarkable effect in the treatment of trachoma in patients with active lesions. Patients were treated with either 0.2% aqueous berberine chloride ophthalmic solution (two drops per eye, three times daily for 8 weeks), 20% sulfacetamide ophthalmic solution, or both. Although the 20% sulfacetamide solution relieved clinical symptoms completely, it did not eradicate the pathogen, *Chlamydia trachomatis.* By the end of treatment in the berberine group, patients were symptom-free and

tested negative for *C. trachomatis.* In 5 of 17 patients in the sulfacetamide treatment group, symptoms recurred 4 to 6 months after treatment. None of the berberine-treated trachoma patients suffered from recurrence up to 1 year after treatment. The combination of berberine and sulfacetamide ophthalmic solutions provided only slightly better improvement than did berberine alone; this group was bacteria-free after treatment, with no recurrent infections.[75,76] Trachoma is a major cause of blindness and impaired vision in underdeveloped countries, affecting approximately 500 million people worldwide, and results in blindness in 2 million of those affected. The drug sulfacetamide is currently the most widely used antitrachoma drug. In the aforementioned study, although sulfacetamide showed the best improvement (decrease in conjunctival discharge, edema, and papillary reactions), the conjunctival scrapings of all patients receiving sulfacetamide remained positive for *C. trachomatis,* showing the conventional treatment was primarily symptomatic. These patients had a high rate of recurrence of symptoms. In contrast, patients treated with the berberine solution showed mild ocular symptoms, which disappeared more gradually, but their conjunctival scrapings were always negative for *C. trachomatis.* These patients did not have relapse even 1 year after treatment, which suggests that berberine is curative for trachoma.[75,76]

Berberine's efficacy is believed to be due to stimulation of some host defense mechanism, rather than only a direct cytotoxic action on the organism. Because the berberine concentration used in these studies was 100 times less than the concentration of sulfacetamide, and berberine is much cheaper, it may be more cost-effective than other treatments for trachoma. This suggests that berberine (0.2% solution) is an appropriate therapy for many types of conjunctivitis.

Berberine in Liver Health

Cholecystitis and Cirrhosis of the Liver

Several clinical studies demonstrate that berberine stimulates the secretion of bile (choleretic effect) and bilirubin.[77–79] This is significant because bile production and secretion are the primary physiological mechanisms by which chemical waste products, hormones, and fat and cholesterol are removed from the body. Without this detoxification mechanism, precancerous hormones recirculate, and fats as cholesterol saturate the cardiovascular system. Bitter substances, and specifically berberine-containing plants, are among the oldest traditional herbal detoxification therapies used worldwide. There are also many health benefits of bilirubin, including antioxidant activity.[80]

In one clinical study of 225 patients, oral berberine at doses of 5 to 20 mg three times a day before meals for 24 to 48 hours resolved symptoms of chronic cholecystitis (gallbladder inflammation), decreased the bilirubin level, and increased the bile volume of the gallbladder.[77–79]

Berberine was shown to have a beneficial effect on liver cirrhosis. Cirrhosis results in an elevation of the amino acid tyramine, a sign of liver damage. Berberine both reduced tyramine in cirrhosis and prevented the elevation of serum tyramine after oral tyrosine load by inhibiting the enzyme tyrosine decarboxylase found in bacteria in the large intestine.[78] Excessive tyramine levels are also believed to be responsible for some of the cardiovascular and neurological complications of liver disease, such as hepatic encephalopathy. The accumulation of tyramine and its derivatives may cause lowering of peripheral resistance, with resultant high cardiac output, reduction in renal function, and cerebral dysfunction. This tyramine-lowering effect of berberine may have significance in other conditions as well.

Liver-Protective Effects of Berberine

Berberine has marked protective effects against chemical-induced liver damage, most notably against acetaminophen (Tylenol)-induced hepatotoxicity, whose excessive use remains one of the primary causes of liver failure worldwide. In the United States, acetaminophen is responsible for more than 50% of drug-induced liver injury (DILI) and more than 20% of liver transplants and has been for almost 40 years.[81] Berberine significantly ameliorates acetaminophen-induced elevations in liver enzymes, another sign of liver damage, and increases concentrations of endogenous antioxidants (e.g., glutathione), which protect against liver damage.[82] In one study, a high dose (100 mg/kg per day for 10 days) was protective against liver damage caused by methotrexate, a drug used in serious forms of psoriasis and rheumatoid arthritis.[83]

Cancer

Berberine and its derivatives have high activity against cancer and inflammation (Fig. 86.6). Berberine and another alkaloid found in berberine-containing plants, berbamine, were shown to exert beneficial effects as adjuncts in cancer therapy. Berbamine has been used in China since 1972 in the treatment of depressed white blood cell (WBC) counts resulting from chemotherapy and radiation. In one study, 405 patients with WBC counts less than 4000 were given 150 mg/day of berbamine (50 mg orally three times daily) for 1 to 4 weeks. Berbamine was viewed as "significantly effective" if WBC count increased to greater than 4000 after 1 week or increased to greater than 1000 after 2 weeks; "effective" if WBC count increased to greater than 4000 after 2 weeks or increased greater than 1000 after 4 weeks; and "ineffective" if there was no change in WBC count after 4 weeks of treatment. The overall results for the 405 patients were as follows:

- Significantly effective in 163 cases (40.2%)
- Effective in 125 cases (38.8%)
- Ineffective in 117 cases (29%)

The total effective rate was 71%. However, the WBC count before therapy was related to overall effectiveness. The effective rate was only 54.8% in 31 cases in which the WBC count was less than 1000 and 82.7% in cases in which the WBC count was between 3100 and 3800.[84]

Heart Disease

Several human investigations provide evidence for the benefit of berberine in heart disease. In one double-blind study, 156 patients with chronic congestive heart failure (CHF) and more than 90 ventricular premature complexes (VPCs) or nonsustained ventricular tachycardia, or both, on 24-hour Holter monitoring were randomly divided into two groups. All patients were given conventional therapy for CHF, consisting of angiotensin-converting enzyme inhibitors, digoxin, diuretics, and nitrates, along with 1.2 to 2 g of berberine per day or a placebo. Symptoms, a 6-minute walk test, left ventricular ejection fraction (LVEF), frequency and complexity of VPCs, and quality of life were assessed after 8 weeks of treatment and during a mean 24-month follow-up. The addition of berberine resulted in a significantly greater increase in LVEF and exercise capacity, improvement of the dyspnea-fatigue index, and a decrease of frequency and complexity of VPCs compared with the control group. There was also a significant decrease in mortality in the berberine-treated patients during long-term follow-up (7 patients receiving treatment died vs. 13 on placebo). There were no apparent side effects in the berberine group. Researchers concluded that berberine improved quality of life and decreased VPCs and mortality in patients with CHF.[85]

Similar findings were reported in another group of patients with refractory CHF in older studies. In this investigation, intravenous administration of berberine (0.2 mg per minute for 30 minutes) significantly decreased systemic and pulmonary vascular resistance, decreased blood pressure, and increased the cardiac and stroke index, both measures of increased cardiac competency and overall cardiac performance.[86]

A number of studies conducted between 2004 and 2008 establish beneficial antilipidemic effects associated with 500 mg berberine one

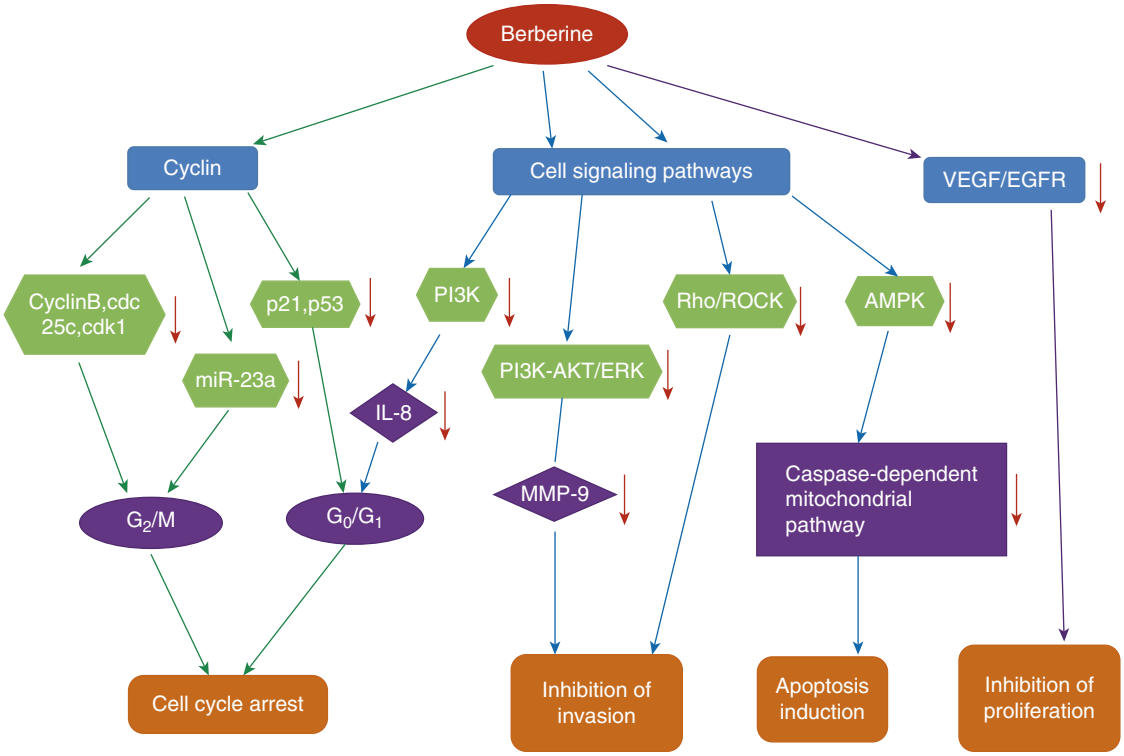


Fig. 86.6 Summary of the antitumor effect of berberine. "→" stands for inhibition or down-regulation. (From Zou K, Li Z, Zhang Y, Zhang HY, Li B, Zhu WL, Shi JY, Jia Q, Li YM. Advances in the study of berberine and its derivatives: a focus on anti-inflammatory and anti-tumor effects in the digestive system. *Acta Pharmacologica Sinica*. 2017;38(2):157–167. PubMed PMID: 27917872.)

to three times daily in patients with mild to moderate hyperlipidemia, including high cholesterol levels resistant to treatment with statin drugs. Most studies were 4 to 24 weeks in duration and resulted in reductions in low-density lipoprotein-cholesterol (LDL-C) levels of 20% to 32%, with positive results experienced within 4 weeks and greater results occurring with the longest duration of treatment.[87]

Type 2 Diabetes and Metabolic Syndrome

Human clinical studies validate the antidiabetic effects noted in in vitro and in vivo models showing that berberine (500 mg three times a day) significantly lowered fasting blood glucose, hemoglobin A_{1C} (HbA_{1c}), triglycerides, and insulin levels in patients with type 2 diabetes mellitus.[88–90] The efficacy of berberine in lowering the fasting blood glucose and HbA_{1c} was similar to that of metformin and rosiglitazone. For example, in one study, berberine reduced HbA_{1c} from 9.5% to 7.5%, fasting blood glucose from 10.6 to 6.95 mmol/L, postprandial blood glucose from 19.8 to 11.1 ± 0.9 mmol/L, and plasma triglycerides from 1.13 to 0.89 mmol/L.[91] In another study, berberine at a dosage of 1.0 g/day for 3 months decreased fasting blood glucose from 7.0 to 5.6 mmol/L, HbA_{1c} from 7.5% to 6.6%, triglycerides from 2.51 to 1.61 mg/dL, total cholesterol from 5.31 to 4.35 mg/dL, and LDL-C from 3.23 to 2.55 mg/dL.[90]

A meta-analysis of 27 human clinical trials of the effects of berberine on a variety of metrics in patients with diabetes similarly supported the potential benefits of berberine in diabetes. Berberine lowered fasting blood sugar levels, postprandial plasma glucose, glycosylated hemoglobin, hyperlipidemia, blood pressure, and total high-density lipoprotein cholesterol (HDL-C) and triglycerides and increased LDL-C. Berberine had a greater effect in lowering both fasting and postprandial blood sugar levels and glycosylated hemoglobin than lifestyle modification or placebo. In this study, berberine was equally as effective as oral hypoglycemic agents. No serious side effects were reported in these studies.[92] As for the treatment of hyperlipidemia, berberine with lifestyle intervention was better than lifestyle intervention alone, and berberine with oral lipid-lowering drugs was better than lipid-lowering drugs alone, in reducing the level of total cholesterol (TC) and LDL-C and raising the level of HDL-C. In the comparative study between berberine and/or lipid-lowering drugs, there was no statistical significance in reducing the level of TC and LDL-C, but berberine showed better effect in lowering the level of triglycerides and raising the level of HDL-C. In the treatment of hypertension, berberine with lifestyle intervention tended to lower the level of blood pressure more than the lifestyle intervention or placebo alone.[92]

Consistent with the in vitro and in vivo models, berberine-treated patients experienced a statistically significant increase in the percentages of peripheral blood lymphocytes that express insulin receptor expression.[88] In patients with chronic viral hepatitis (B and C), liver function was improved greatly through the reduction of liver enzymes. Additional mechanisms of value to patients with type 2 diabetes include the ability to inhibit aldose reductase, lower blood lipids levels (including the ability to reduce free fatty acid levels), and protect and improve endothelial function.[93] In a lipid-lowering trial in 32 hypercholesterolemic patients for 3 months, berberine (500 mg three times daily) reduced serum cholesterol by 29%, triglycerides by 35%, and LDL-C by 25%.[91] Berberine lowers LDL-C by upregulating hepatic LDL-C receptor expression through a mechanism of mRNA stabilization.[94] In recent years, considerable investigation of the potential use of berberine in treating metabolic syndrome has been conducted. Numerous studies demonstrate improvement in metabolic syndrome in vivo. In one study of humans with newly diagnosed type 2 diabetes, 0.5 g of berberine was given three times daily at the beginning of each meal. A reduction in fasting and postprandial blood

glucose was observed. Other beneficial effects, such as a reduction in plasma triglycerides, TC, and LDL-C, were also reported.[56] In at least two other clinical studies, similar reductions in triglycerides (35% and 22%), serum cholesterol (29% and 16%), and LDL-C (25% and 20%) were observed.[91,95] A meta-analysis of clinical trials in patients with dyslipidemia, including three trials of patients with type 2 diabetes, demonstrated that berberine significantly reduced TC, LDL-C, and triglyceride levels and significantly increased HDL-C.[96] Another systematic review of subjects with dyslipidemia, including patients with diabetes, similarly reported improvements in a number of metrics. Specifically, in patients with diabetes, significant reductions in TC, LDL-C, and triglycerides were observed with 500 mg berberine twice daily for 3 months.[97]

Bioavailability

Modern researchers tend to lack confidence in the ability of berberine to elicit any therapeutic effect because of early pharmacokinetic evidence that suggested it is poorly absorbed. For example, in an early study, it was found that only 0.043% of a 100-mg oral dose of berberine was recoverable from the urine of normal volunteers after 24 hours.[98] This is partly due to poor permeation of berberine through healthy intestinal tissues. Interestingly, intestinal permeability increases when tissues are inflamed, allowing for greater berberine absorption across diseased tissues. More recent data demonstrate that berberine and its four primary metabolites—berberrubine, thalifendine, demethyleneberberine, and jatrorrhizine—are detected in the liver 30 minutes after oral administration of berberine, and in bile they are detected 1 hour after oral administration.[99] Additionally, the absorption of berberine when consumed in berberine-containing plants or as whole-plant decoctions is 39% to 43% greater than when isolated berberine is consumed,[100] and it also increases with continued use.[101]

Although detailed pharmacokinetic data are lacking, berberine-containing plants have been used for many millennia for many of the indications for which berberine has been shown to be effective. The available clinical and preclinical evidence provides a strong basis for efficacy. Also, these plants have predominantly been used for a variety of antimicrobial activities that, in many cases, do not require systemic absorption for efficacy, instead eliciting an effect in tissues with which berberine comes in contact, such as in the gastrointestinal system.

Dosage

Because no detailed clinical studies have differentiated which berberine-containing herb to use for specific conditions, the following is offered as a guideline based on experimental studies and historical use. In general, the plants can be viewed as interchangeable.

H. canadensis

- Infective, congestive, and inflammatory states of the mucous membranes
- Digestive disorders
- Gastritis
- Peptic ulcers
- Colitis
- Anorexia
- Painful menstruation

Berberis vulgaris

- The treatment of gallbladder disease, including gallstones, and as a less expensive form of berberine in the treatment of the conditions listed earlier for goldenseal.

Berberis aquifolium

- Best used in the treatment of chronic skin diseases and in the conditions listed earlier for goldenseal.

Coptis chinensis

Can be used in the treatment of infective, congestive, and inflammatory states of the mucous membranes, fever, and infectious disorders of the skin

Dosages three times a day are as follows:

- Dried root or infusion (tea): 2 to 4 g
- Tincture (1:5): 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content): 250 to 500 mg
- Berberine sulfate: see dosage levels used in clinical studies referenced previously (e.g., in type 2 diabetes: 500 mg two to three times a day).

Quality Considerations

The quality of berberine-containing plants, most notably goldenseal, can vary widely. For example, goldenseal is very costly, which leads to widespread adulteration of the botanical in the herbal products industry. Although such adulterations are against the law, they nevertheless occur as extract manufacturers add lower-cost berberine-containing plants to increase the profitability of diluted goldenseal. Generally speaking, the dosage of these plants should be minimally based on berberine content, which is correlated with the intense bitterness of the plants. Because there is a wide range of quality in goldenseal preparations, standardized extracts are generally recommended. In one study of 20 products purchased at local pharmacies or health food stores, 17 were labeled as containing goldenseal root, and three contained goldenseal herb as the sole active herbal ingredient.[102] The berberine concentration varied from 0.82% to 5.86%, whereas the hydrastine concentration ranged from 0% to 2.93%. Only 10 of 17 products met the proposed U.S. Pharmacopeia standards for the hydrastine and berberine content of goldenseal root. Five products contained little or no hydrastine, unusual berberine-to-hydrastine ratios, and additional peaks not observed with other products. Luckily, the majority of adulterating species do not present a health hazard and have similar activity, but conversely, they may not yield optimal amounts of berberine.

ADVERSE EFFECTS AND TOXICITY

Berberine and berberine-containing plants are generally nontoxic at the recommended dosages. In the aforementioned meta-analysis of 27 human clinical trials of the effects of berberine in patients with diabetes, no noticeable adverse effects were reported.[92] However, berberine-containing plants are not recommended for use during pregnancy, and higher dosages may interfere with B vitamin metabolism. The oral median lethal dose in rats for berberine is greater than 1000 mg/kg body weight, indicating that the toxicity is extremely low.[103]

DRUG INTERACTIONS

Both positive and negative interactions between berberine and conventional medications can occur. Berberine may interfere with the absorption of tetracycline and related antibiotics. In one double-blind study, giving subjects 100 mg of berberine at the same time as 500 mg of tetracycline four times daily led to a reduction in the efficacy of tetracycline in subjects

with cholera.[68] However, another double-blind study showed no such interaction.[69] Nonetheless, simultaneous use of berberine and antibiotics should only be done with great care to ensure efficacy and safety.

Berberine produces significant inhibition of CYP3A enzymes in humans.[94,104] Because these enzymes metabolize most drugs, berberine may decrease the clearance of many medications, thereby increasing the amount that remains in the bloodstream and altering efficacy and safety. CYP3A is involved in the metabolism of approximately half the drugs that are used today, including acetaminophen, codeine, cyclosporine, diazepam, and erythromycin. Sometimes this inhibitory effect of berberine on CYP3A may be useful for improving a therapeutic benefit. For example, the combined use of cyclosporine with berberine markedly increased the blood concentration of cyclosporine in heart transplantation recipients, reduced the dosage of cyclosporine required, and showed no obvious adverse reaction.[105] There are a number of other potential positive herb–drug interactions associated with berberine. Berberine increases the effectiveness of statin drugs to lower cholesterol; in one study, the effect of a simvastatin and berberine combination was 30% greater than simvastatin alone.[106]

Berberine-containing plants may enhance the effects of oral hypoglycemic drugs through its multitude of antidiabetic effects. Patients on oral hypoglycemic drugs should take extra care in monitoring blood glucose levels if taking berberine-containing plants. Adjustment of the dose of conventional hypoglycemic agents may be required to maintain healthy blood sugar levels.

As noted, positive interactions between a number of chemotherapeutic drugs are recorded in the literature. Specifically, berberine decreased the toxicity of 5-fluorouracil, arsenic trioxide, nitrogen mustard, camptothecin, cetuximab, erlotinib, lapatinib, gefitinib, cisplatin, doxorubicin, paclitaxel, and tamoxifen and additionally increased the efficacy of radiation therapy.[38] Although not established in human clinical trials, the potential for such a positive interaction in the absence of demonstrable toxicity warrants clinical investigation.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Chen JH, Wang FW, Liu J. Analysis of alkaloids in *coptis chinensis* Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Analytica Chimica Acta*. 2008;613(2):184–195.
2. Wu D, Wen W, Qi CL, et al. *Phytomedicine*. 2012;19(8-9):712–718. https://doi.org/10.1016/j.phymed.2012.03.003. Epub 2012 Apr 4.
3. Galle K, Muller-Jakic B, Proebstle A, Jurcic K, Bladt S, Wagner H. Analytical and pharmacological studies on *mahonia aquifolium*. *Phytomed Int J Phytother Phytopharm*. 1994;1:59–62.
4. Cui L, Liu M, Chang X, Sun K. The inhibiting effect of the *coptis chinensis* polysaccharide on the type II diabetic mice. *Biomed Pharmacother*. 2016;81:111–119.
5. Kost'alova D, Kardosova A, Hajnicka V. Effect of *mahonia aquifolium* stem bark crude extract and one of its polysaccharide components on production of IL-8. *Fitoterapia*. 2001;72(7):802–806.
6. Wirth C, Wagner H. Pharmacologically active phenolic compounds from the bark of *mahonia aquifolium*. *Phytomedicine*. 1997;4(4):357–358.
7. Duke JA. *Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1985.
8. Leung AY. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. New York: John Wiley; 1996.
9. Chang HM, But PPH. *Pharmacology and Applications of Chinese Materia Medica*. Vol. 2. Teaneck, NJ: World Scientific; 1987.
10. King J, Newton RS. *The Eclectic Dispensatory of the United States*. Cincinnati, OH: HW Derby; 1852:708.
11. Jones LE, Scudder JM. *The American Eclectic Materia Medica and Therapeutics*. Vol. 1–2. Cincinnati, OH: Moore, Wilstach, Keys; 1858:1010.
12. Hahn FE, Ciak J. *Antibiorirb (Berlin)*. 1967;3:577–580.
13. Amin AH, Subbaiah TV, Abbasi KM. Berberine sulfate. Antimicrobial activity, bioassay, and mode of action. *Can J Microbiol*. 1969;15: 1067–1076.
14. Johnson CC, Johnson G, Poe CF. Toxicity of alkaloids to certain bacteria. II. Berberine, physostigmine, and sanguinarine. *Acta Pharmacol Toxicol*. 1952;8:71–78.
15. Kaneda Y, Torii M, Tanaka T. In vitro effects of berberine sulfate on the growth of *entamoeba histolytica, giardia lamblia* and *trichomonas vaginalis*. *Ann Trop Med Parasitol*. 1991;85:417–425.
16. Subbaiah TV, Amin AH. Effect of berberine sulfate on *entamoeba histolytica*. *Nature*. 1967;215:527–528.
17. Ghosh AK, Bhattacharyya FK, Ghosh DK. *Leishmania donovani:* amastigote inhibition and mode of action of berberine. *Exper Parasitol*. 1985;60:404–413.
18. Mahajan VM, Sharma A, Rattan A. Antimycotic activity of berberine sulphate: an alkaloid from an Indian medicinal herb. *Sabouraudia*. 1982;20(3):79–81.
19. Hwang BY, Roberts SK, Chadwick LR, et al. Antimicrobial constituents from goldenseal (the rhizomes of *Hydrastis canadensis*) against selected oral pathogens. *Planta Med*. 2003;69:623–627.
20. Kumar A, Ekavali Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: an update. *Eur J Pharmacol*. 2015;761:288–297.
21. Tillhon M, Guaman Ortiz LM, Lombard P, Ivana A. Berberine: new perspectives for old remedies. *Biochem Pharmacol*. 2012;84:126–1267.
22. Wojtyczka RD, Dziedzic A, Kępa M, et al. Berberine enhances the antibacterial activity of selected antibiotics against coagulase-negative *Staphylococcus* strains in vitro. *Molecules*. 2014;19(5):6583–6596. https://doi.org/10.3390/molecules19056583.
23. Jin JL, Hua GQ, Meng Z, Gao PJ. Antibacterial mechanisms of berberine and reasons for little resistance of bacteria. *Chinese Herbal Medicine*. 2010;3(1):27–35.
24. Gao WW, Gopala L, Bheemanaboina RRY, Zhang GB, Li S, Zhou CH. Discovery of 2-aminothiazolyl berberine derivatives as effectively antibacterial agents toward clinically drug-resistant gram-negative *acinetobacter baumanii*. *Eur J Med Chem*. 2018;146:15–37.
25. Sun D, Courtney HS, Beachey EH. Berberine sulfate blocks adherence of *Streptococcus pyogenes* to epithelial cells, fibronectin, and hexadecane. *Antimicrob Agents Chemother*. 1988;32(9):1370–1374.
26. Peng L, Kang S, Yin ZQ, et al. Antibacterial activity and mechanism of berberine against *Streptococcus agalactiae*. *Int J Clin Exp Pathol*. 2015;8(5):5217–5223.
27. Xie Q, Johnson BR, Wenckus CS, Fayad MI, Wu CD. Efficacy of berberine, an antimicrobial plant alkaloid, as an endodontic irrigant against a mixed-culture biofilm in an in vitro tooth model. *J Endod*. 2012;38(8):1114–1117.
28. Yu HH, Kim KJ, Cha JD, et al. Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *staphylococcus aureus*. *J Med Food*. 2005;8(4):454–461.
29. Gentry EJ, Jampani HB, Gentry EJ, Jampani HB, Keshavarz-Shokri A, et al. Antitubercular natural products: berberine from the roots of commercial *Hydrastis canadensis* powder. *J Nat Prod*. 1998;61:1187–1193.
30. Scazzocchio F, Cometa MF, Palmery M. Antimicrobial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. *Fitoterapia*. 1998;69(suppl 5):58–59.
31. Malik TA, Kamili AN, Chishti MZ, et al. Breaking the resistance of *Escherichia coli*: antimicrobial activity of *berberis lycium* royle. *Microb Pathog*. 2017;102:12–20.
32. Gu L, Li N, Gong J, Li Q, Zhu W, Li J. Berberine ameliorates intestinal epithelial tight-junction damage and down-regulates myosin light chain kinase pathways in a mouse model of endotoxinemia. *J Infect Dis*. 2011;203:1602–1612.
33. Han J, Lin H, Huang W. Modulating gut microbiota as an anti-diabetic mechanism of berberine. *Med Sci Monit*. 2011;17(7):Ra164–167.
34. Tan S, Yu W, Lin Z, et al. Berberine ameliorates intestinal mucosal barrier damage induced by peritoneal air exposure. *Biol Pharm Bull*. 2015;38(1):122–126.
35. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. *J Immunol*. 2002;168(11):5817–5823.
36. Konev IuV, et al. Endotoxin (LPS) in the pathogenesis of atherosclerosis. *Eksp Klin Gastroenterol*. 2011;11:15–26.
37. Li YH, Zhang M, Xiao HT, et al. Addition of berberine to 5-aminosalicylic acid for treatment of dextran sulfate sodium-induced chronic colitis in C57BL/6 mice. 2015. https://doi.org/10.1371/journal.pone.0144101.
38. Bharti AC, Rajan P, Pande D, Singh T, Bhat A. Berberine as an adjuvant and sensitizer to current chemotherapy. Role of nutraceuticals in cancer chemosensitization. In: *Cancer Sensitizing Agents for Chemotherapy*. Vol. 2. Cambridge, MA: Academic Press; 2018:221–240.
39. Sabir M, Akhter MH, Bhide NK. Antagonism of cholera toxin by berberine in the gastrointestinal tract of adult rats. *Indian J Med Res*. 1977;65(3):305–313.
40. Ivanovska N, Philipov S. Study on the anti-inflammatory action of *Berberis vulgaris* root extract, alkaloid fractions and pure alkaloids. *Int J Immunopharmacol*. 1996;18(10):553–561.
41. Kumazawa Y, Itagaki A, Fukomoto M, Fujisawa H, Nishimura C, Nomoto K. Activation of peritoneal macrophages by berberine-type alkaloids in terms of induction of cytostatic activity. *Int J Immunopharm*. 1984;6:587–592.
42. Singh A, Duggal S, Kaur N, Singh J. Berberine: alkaloid with wide spectrum of pharmacological activities. *J Nat Prod*. 2010;3:64–75.
43. Kuo CL, Chi CW, Liu TY. The anti-inflammatory potential of berberine in vitro and in vivo. *Cancer Lett*. 2004;203(2):127–137.
44. Zhang RX, Dougherty DV, Rosenblum ML. Laboratory studies of berberine used alone and in combination with 1, 3-bis(2- chloroethyl)-1-nitrosourea to treat malignant brain tumors. *Chin Med J*. 1990;103(8):658–665.
45. Cordero CP, Gomez-Gonzalez S, Leon-Acosta CJ, et al. Cytotoxic activity of five compounds isolated from colombian plants. *Fitoterapia*. 2004;75:225–227.
46. Szeto S, Yow CMN, Fung KW. Characterization of berberine on human cancer cells in culture. *Turk J Med Sci*. 2002;32:363–368.
47. Mantena SK, Sharma SD, Katiyar SK. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. *Mol Cancer Ther*. 2006;5(2):296–308.

48. Sun Y, Xun K, Wang Y, Chen X. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. *Anticancer Drugs*. 2009;20(9):757–769.
49. Peng PL, Kuo WH, Tseng HC, Chou FP. Synergistic tumor-killing effect of radiation and berberine combined treatment in lung cancer: the contribution of autophagic cell death. *Int J Radiat Oncol Biol Phys*. 2008;70(2):529–542.
50. Cernakova M, Kost'alova D, Kettmann V, et al. Potential antimutagenic activity of berberine, a constituent of *Mahonia aquifolium*. *BMC Complement Altern Med*. 2002;19:2–6.
51. Yokozawa T, Ishida A, Kashiwada Y, et al. *Coptidis rhizoma:* protective effects against peroxynitrite-induced oxidative damage and elucidation of its active components. *J Pharm Pharmacol*. 2004;56:547–556.
52. Nishino H, Kitagawa K, Fujiki H, Iwashima A. Berberine sulfate inhibits tumor-promoting activity of teleocidin in two-stage carcinogenesis on mouse skin. *Oncology*. 1986;43(2):131–134.
53. Leng SH, Lu FE, Xu LJ. Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. *Acta Pharmacol Sin*. 2004;25:496–502.
54. Pan GY, Huang ZJ, Wang GJ, et al. The antihyperglycemic activity of berberine arises from a decrease of glucose absorption. *Planta Med*. 2003;69:632–636.
55. Kong WJ, Zhang H, Song DQ, et al. Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. *Metabolism*. 2009;58:109–119.
56. Yin J, Ye JP, Jia WP. Effects and mechanisms of berberine in diabetes treatment. *Acta Pharmaceutica Sinica B*. 2012;2(4):327–334.
57. Wu D, Wen W, Qi CL, Zhao RX, Lü JH, Zhong CY, Chen YY. *Phytomedicine*. 2012;19(8-9):712–718. https://doi.org/10.1016/j.phymed.2012.03.003. Epub 2012 Apr 4.
58. Xia LM, Luo MH. Study progress of berberine for treating cardiovascular disease. *Chronic Dis Transl Med*. 2015;1(4):231–235.
59. Habtemariam S. Berberine and inflammatory bowel disease: a concise review. *Pharmacol Res*. 2016;113(A):592–599.
60. Zhang X, Zhao Y, Zhang M, et al. Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats. *PLoS One*. 2012;7(8):e42529.
61. Gu Y, Zhang Y, Shi X, et al. Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. *Talanta*. 2010;81:766–772.
62. Gupte S. Use of berberine in the treatment of giardiasis. *Am J Dis Child*. 1975;129:866.
63. Bhakat MP, Nandi N, Pal HK, et al. Therapeutic trial of berberine sulphate in non-specific gastroenteritis. *Indian Med J*. 1974;68:19–23.
64. Kamat SA. Clinical trial with berberine hydrochloride for the control of diarrhoea in acute gastroenteritis. *J Assoc Physicians India*. 1967;15:525–529.
65. Desai AB, Shah KM, Shah DM. Berberine in the treatment of diarrhoea. *Ind Pediatr*. 1971;8:462–465.
66. Sharma R, Joshi CK, Goyal RK. Berberine tannate in acute diarrhoea. *Ind Pediatr*. 1970;7:496–501.
67. Sack RB, Froehlich JL. Berberine inhibits intestinal secretory response of *Vibrio cholerae* toxins and *Escherichia coli* enterotoxins. *Infect Immun*. 1982;35:471–475.
68. Choudry VP, Sabir M, Bhide VN. Berberine in giardiasis. *Indian Pediatr*. 1972;9:143–146.
69. Khin-Maung-U, Myo-Khin, Nyunt-Nyunt-Wai, et al. Clinical trial of berberine in acute watery diarrhoea. *BMJ*. 1985;291:1601–1605.
70. Rabbani GH, Butler T, Knight J, et al. Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic *Escherichia coli* and *Vibrio cholerae*. *J Infect Dis*. 1987;155:979–984.
71. Chu M, Ding R, Chu ZY, et al. Role of berberine in anti-bacterial as a high-affinity LPS antagonist binding to TLR4/MD-2 receptor. *BMC Complement Altern Med*. 2014;14:89.
72. Bandyopadhyay S, Patra PH, Mahanti A, Mondal DK, Dandapat P, et al. Potential antibacterial activity of berberine against multi drug resistant enterovirulent *Escherichia coli* isolated from yaks (*Poephagus grunniens*) with haemorrhagic diarrhea. *Asian Pac J Trop Med*. 2013;6(4):315–319.
73. Akhter MH, Sabir M, Bhide NK. Possible mechanism of antidiarrhoeal effect of berberine. *Indian J Med Res*. 1979;70:233–241.
74. Tai YH, Feser JF, Marnane WG, Desjeux JF. Antisecretory effects of berberine in rat ileum. *Am J Physiol*. 1981;241:G253–G258.
75. Babbar OP, Chhatwal VK, Ray IB, et al. Effect of berberine chloride eye drops on clinically positive trachoma patients. *Indian J Med Res*. 1982;76:83–88.
76. Mohan M, Pant CR, Angra SK, et al. Berberine in trachoma. *Indian J Ophthalmol*. 1982;30:69–75.
77. Preininger V. The pharmacology and toxicology of the papaveraceae alkaloids. *Alkaloids*. 1975;15:207–251.
78. Chan MY. The effect of berberine on bilirubin excretion in the rat. *Comp Med East West*. 1977;5:161–168.
79. Watanabe A, Obata T, Nagashima H. Berberine therapy of hypertyraminemia in patients with liver cirrhosis. *Acta Med Okayama*. 1982;36:277–281.
80. Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci U S A*. 2009;106(13):5171–5176.
81. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-induced hepatotoxicity: a comprehensive update. *J Clin Transl Hepatol*. 2016;4(2):131–142.
82. Zhao Z, Weib QY, Huab WW, Liu YX, Liu X, Zhua YB. Hepatoprotective effects of berberine on acetaminophen-induced hepatotoxicity in mice. *Biomed Pharmacother*. 2018;103:1319–1326.
83. Mehrzadi S, Fatemi F, Esmaeilizadeh M, Ghaznavi H, Kalantarf H, Goudarzif M. Hepatoprotective effect of berberine against methotrexate induced liver toxicity in rats. *Biomed Pharmacother*. 2018;97:233–239.
84. Liu CX, Xiao PG, Liu GS. Studies on plant resources, pharmacology and clinical treatment with berbamine. *Phytother Res*. 1991;5:228–230.
85. Zeng XH, Zeng XJ, Li YY. Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol*. 2003;92:173–176.
86. Marin-Neto JA, Maciel BC, Secches AL, Gallo Jr L. Cardiovascular effects of berberine in patients with severe congestive heart failure. *Clin Cardiol*. 1988;11(4):23–60.
87. Cicero A, Etrek S. Metabolic and cardiovascular effects of berberine: from preclinical evidences to clinical trial results. *Clini Lipidol*. 2009;4(5):553–563. https://doi.org/10.2217/clp.09.41.
88. Zhang H, Wei J, Xue R. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. *Metabolism*. 2010;59:285–292.
89. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism*. 2008;57:712–717.
90. Zhang Y, Li X, Zou D, et al. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. *J Clin Endocrinol Metab*. 2008;93:2559–2565.
91. Kong W, Wei J, Abidi P, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med*. 2004;10:1344–1351.
92. Lan J, Zhao Y, Dong F, et al. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. *J Ethnopharmacol*. 2015;161:69–81.
93. Wang JM, Yang Z, Xu MG, et al. Berberine-induced decline in circulating CD31+/CD42- microparticles is associated with improvement of endothelial function in humans. *Eur J Pharmacol*. 2009;614:77–83.
94. Gurley BJ, Swain A, Hubbard MA, et al. Supplementation with goldenseal (*Hydrastis canadensis*), but not kava kava (*Piper methysticum*), inhibits human CYP3A activity in vivo. *Clin Pharmacol Ther*. 2008;83:61–69.
95. Cicero AF, Rovati LC, Setnikar I. Eulipidemic effects of berberine administered alone or in combination with other natural cholesterol-lowering agents. A single-blind clinical investigation. *Arzneimittel-Forschung*. 2007;57:26–30.

96. Ju JQ, Li JG, Lin Q, Hao X. Efficacy and safety of berberine for dyslipidaemias: a systematic review and meta-analysis of randomized clinical trials. *Phytomedicine*. 2018;50(15):25–34.
97. Koppen, et al. Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: a systematic review. *Evid Based Complement Alternat Med*. 2017;22(4):956–968.
98. Miyazaki H, Shirai E, Ishiashi M, Nizima K. Quantitative analysis of berberine in urine samples by chemical ionization mass fragmentography. *J Chromatogr*. 1978;152(1):79–86.
99. Zuo F, Nakamura N, Akao T, Hattori M. Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats determined by liquid chromatography/ion trap mass spectrometry. *Drug Metab Dispos*. 2006;34(12):2064–2072.
100. Chen G, Lu F, Xu LJ, et al. The anti-diabetic effects and pharmacokinetic profiles of berberine in mice treated with Jiao-Tai-Wan and its compatibility. *Phytomedicine*. 2003;20(20):780–786.
101. Spinozzi S, Colliva C, Camborata C, et al. Berberine and its metabolites: relationship between physicochemical properties and plasma levels after administration to human subjects. *J Natural Products*. 2014;77(4):766–772.
102. Edwards DJ, Draper EJ. Variations in alkaloid content of herbal products containing goldenseal. *J Am Pharm Assoc (Wash)*. 2003;43:419–423.
103. Kowalewski Z, Mrizikiewicz A, Bobkiewicz T, et al. Toxicity of berberine sulfate. *Acta Pol Pharm*. 1975;32:113–120.
104. Xin HW, Wu XC, Li Q, et al. The effects of berberine on the pharmacokinetics of cyclosporin A in healthy volunteers. *Methods Find Exp Clin Pharmacol*. 2006;28:25–29.
105. Zhong Xi Zhongguo. Effect of berberine hydrochloride on blood concentration of cyclosporine A in cardiac transplanted recipients. *Yi Jie He Za Zhi*. 2008;28:702–724.
106. Kong WJ, Wei J, Zuo ZY. Combination of simvastatin with berberine improves the lipid-lowering efficacy. *Metabolism*. 2008;57:1029–1037.
107. He JM, Mu Q. The medicinal uses of the genus *Mahonia* in traditional Chinese medicine: an ethnopharmacological, phytochemical and pharmacological review. *J Ethnopharmacol*. 2013;175(2015):668–683.
108. Freile ML, Giannini F, Pucci G, et al. Antimicrobial activity of aqueous extracts and of berberine isolated from *Berberis heterophylla*. *Fitoterapia*. 2003;74:602–705.
109. Kim SH, Shin DS, Oh MN, et al. Inhibition of the bacterial surface protein anchoring transpeptidase sortase by isoquinoline alkaloids. *Biosci Biotechnol Biochem*. 2004;68:421–424.
110. Swabb EA, Tai YH, Jordan L. Reversal of cholera toxin-induced secretion in rat ileum by luminal berberine. *Am J Physiol*. 1981;241:G248–G252.

87

5-Hydroxytryptophan

Michael T. Murray, ND

OUTLINE

INTRODUCTION

5-Hydroxytryptophan (5-HTP) is the intermediate between tryptophan and serotonin (Fig. 87.1).[1] Although the use of 5-HTP may be relatively new to most clinicians, it has been available through pharmacies for several years and has been intensely researched for the past three decades. It has been used clinically since the 1970s.

TRYPTOPHAN AND 5-HYDROXYTRYPTOPHAN METABOLISM

Once tryptophan is absorbed from the intestines, it is carried by the blood to the liver, along with other amino acids consumed during the meal. Ingested tryptophan can pass into the general circulation, metabolize into blood proteins, or convert to kynurenine (which then goes on to form nicotinic acid, picolinate, and other important metabolites) in the liver (Fig. 87.2). After conversion to kynurenine, it cannot be converted to serotonin. The same is likely true if the tryptophan is incorporated into blood proteins. Unchanged tryptophan can be converted to 5-HTP and then to serotonin. However, if this conversion occurs outside the brain, brain chemistry will not be influenced. Even under the best-case scenario, only 3% of a dosage of L-tryptophan in supplemental or dietary form is likely to be converted to serotonin in the brain.[2]

The manufacture of serotonin from tryptophan within the brain is highly dependent on the level of tryptophan or 5-HTP that crosses the blood–brain barrier. Although 5-HTP easily crosses the blood–brain barrier, the delivery of tryptophan into the brain depends on several factors. The first factor of importance is the level of free tryptophan in the blood. In a number of situations, the liver's conversion of tryptophan to kynurenine occurs at an elevated rate (i.e., stress, elevated cortisol levels, low B-vitamin status, and high doses of L-tryptophan [greater than 2000 mg]). These situations lead to increased activity of tryptophan oxidase and kynurenine formamidase, which convert tryptophan to kynurenine. Elevated levels of kynurenine block the entry of tryptophan into the brain and lower brain serotonin levels. Increasing tryptophan intake makes matters worse when tryptophan oxidase activity is increased.

Unlike 5-HTP, which easily enters the brain, the transport of tryptophan across the blood–brain barrier involves the binding of tryptophan to a transport molecule. Because tryptophan shares this transport vehicle with several other amino acids, when the ratio of tryptophan to these other amino acids is low, little tryptophan is transported into the brain. The protein in almost all foods contains relatively small amounts of tryptophan and larger proportions of other amino acids. This generally leads to low serotonin levels with a high-protein meal. The opposite occurs with a high-carbohydrate meal.

PHARMACOLOGY

Several pharmacokinetic studies showed that about 70% of a dose of 5-HTP taken orally is delivered to the bloodstream.[3,4] The remaining 30% is metabolized by intestinal cells.

Ample evidence from these pharmacokinetic studies, and clinical studies, indicates that once absorbed, 5-HTP is delivered to the brain, resulting in increased formation of not only serotonin but also other brain chemicals (e.g., the monoamines melatonin, endorphins, dopamine, and norepinephrine; Fig. 87.3). By raising brain serotonin levels, and through other effects, 5-HTP showed positive effects in the various conditions associated with low serotonin levels.

Besides raising serotonin and melatonin levels, 5-HTP was shown to raise β-endorphin levels. Many pain-relieving and mood-elevating

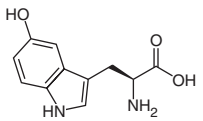


Fig. 87.1 Structure of 5-hydroxytryptophan.

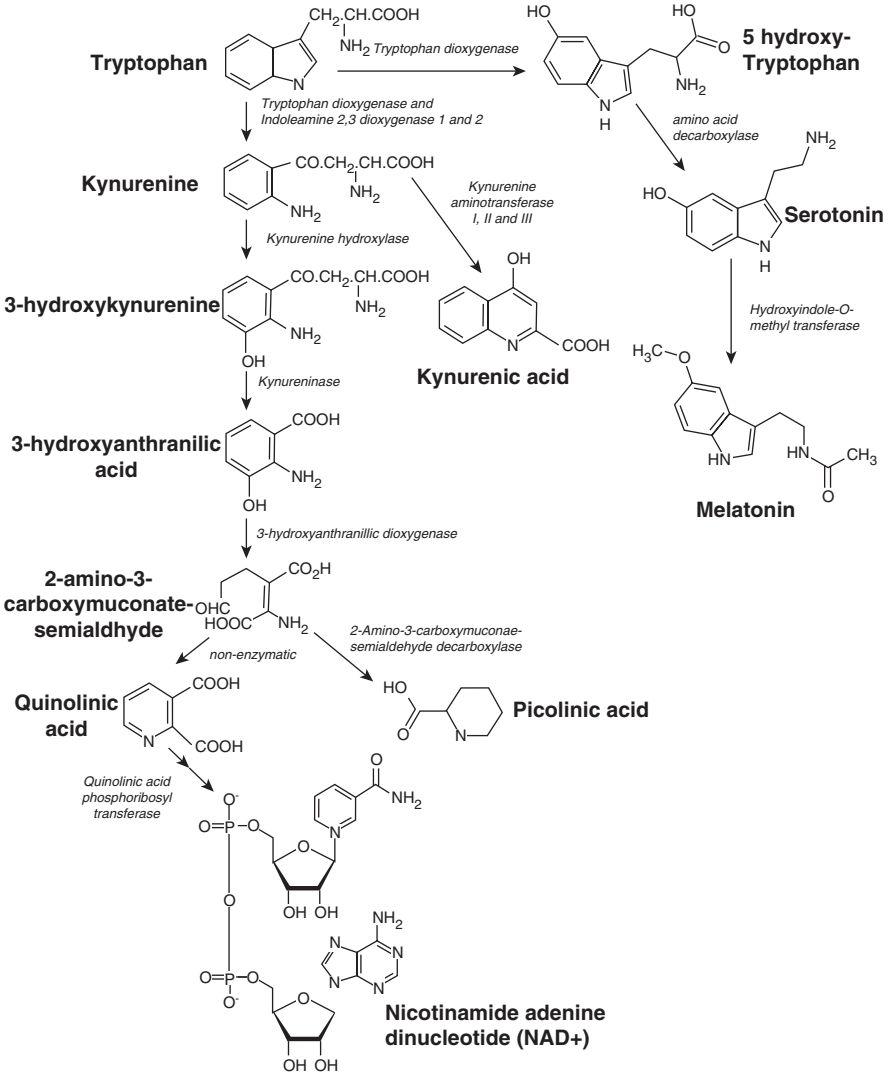


Fig. 87.2 Tryptophan metabolism pathways.

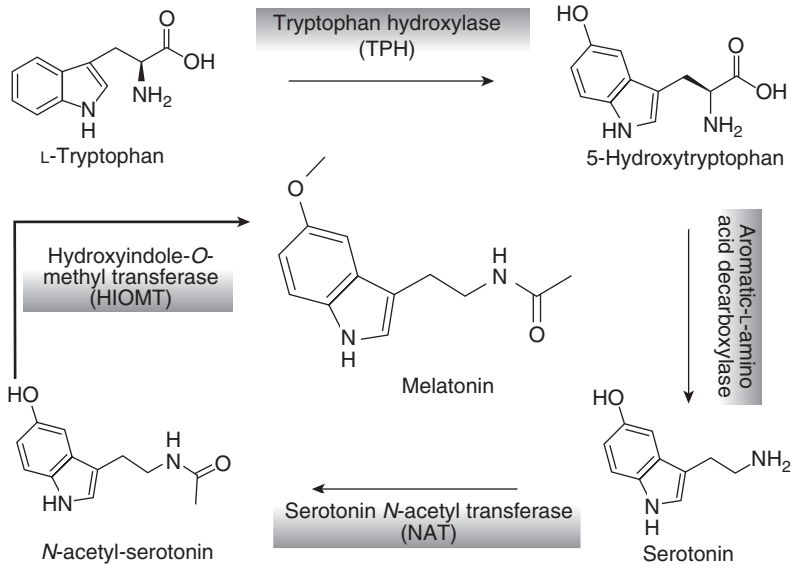


Fig. 87.3 Conversion of 5-hydroxytryptophan to serotonin and melatonin.

benefits of 5-HTP might be related more to its ability to enhance endorphin levels than to its ability to increase serotonin levels. This endorphin-increasing action is useful for both migraine and tension headaches, fibromyalgia, and other painful situations. In addition, raising endorphin levels produces significant effects on mood and behavior.

By raising serotonin, melatonin, and β-endorphin levels, 5-HTP has a significant effect on helping regulate and improve brain chemistry. 5-HTP was also shown to raise the levels of other important neurotransmitters, such as dopamine and norepinephrine.[5] The ability of 5-HTP to increase both serotonin (and other indolamines) and catecholamines is quite significant and unique to 5-HTP. It is an effect that 5-HTP does not share with L-tryptophan. The effective treatment of depression requires more than simply raising serotonin levels; catecholamine levels must also be increased; 5-HTP provides the brain with both sets of tools.

5-Hydroxytryptophan Versus l-Tryptophan

Nutrition-oriented physicians have long used precursor therapy for affecting brain chemistry. Unfortunately, L-tryptophan produced inconsistent results. These results were likely a result of its varying elevation of brain serotonin levels.

In a head-to-head comparison study of 5-HTP and L-tryptophan in the treatment of depression, 5-HTP proved superior.[6] The proposed reason is the fact that 5-HTP easily crosses the blood–brain barrier and is not affected by competing amino acids. 5-HTP affects brain chemistry in a broader and more positive fashion. L-Tryptophan is often effective in cases of low serotonin, especially insomnia, but 5-HTP is more broadly effective.

There are many advantages of 5-HTP over L-tryptophan. Chief among them is that 5-HTP easily crosses the blood–brain barrier and is one step further on the path to serotonin synthesis. The conversion of tryptophan to 5-HTP by tryptophan hydroxylase is the most important step in the manufacture of serotonin. This enzyme is inhibited by several factors, including the following:

- Stress
- Vitamin B_6 insufficiency
- Low magnesium levels
- Insensitivity to insulin
- Various hormones
- Genetic factors

In addition, as noted earlier, these same factors and others are known to increase the activity of tryptophan oxygenase, increasing the conversion of L-tryptophan to kynurenine.

Perhaps the biggest advantage of 5-HTP over L-tryptophan is that it is safer.[7] Although L-tryptophan is safe if properly prepared and free of the contaminants linked to eosinophilia myalgia syndrome (EMS), 5-HTP is inherently safer. The reasons are that taking L-tryptophan to produce positive effects in the treatment of depression, insomnia, and other low-serotonin conditions requires a relatively high dose (e.g., a minimum of 2000 mg in insomnia and 6000 mg in depression). At high doses such as these, L-tryptophan is potentially problematic because more L-tryptophan will be shunted toward the kynurenine pathway, and L-tryptophan promotes oxidative damage. Excessive levels of dietary tryptophan or high doses of L-tryptophan result in tryptophan actually acting as a free radical.[8] In contrast, 5-HTP is an antioxidant.[9] This antioxidant difference is a result of the additional molecule of oxygen and hydrogen in 5-HTP. This simple change in molecular structure allows the phenolic ring structure to effectively accept or quench the unpaired electron of a free radical.

BOX 87.1 Conditions Associated With Low Serotonin Levels

- Depression
- Anxiety
- Obsessive-compulsive disorder
- Obesity
- Carbohydrate craving
- Bulimia
- Insomnia
- Narcolepsy
- Sleep apnea
- Migraine headaches
- Tension headaches
- Chronic daily headaches
- Premenstrual syndrome
- Fibromyalgia
- Epilepsy
- Myoclonus
- Chronic pain disorders

CLINICAL APPLICATIONS

A massive amount of evidence suggests that low serotonin levels are a common consequence of modern living. The lifestyle and dietary practices of many people living in this stress-filled era result in lowered levels of serotonin within the brain. As a result, many people are overweight, crave sugar and other carbohydrates, experience bouts of depression, get frequent headaches, and have vague muscle aches and pains. All of these maladies are correctable by raising brain serotonin levels. The primary therapeutic applications for 5-HTP are low-serotonin states, as listed in Box 87.1.

Depression

Some of the first clinical studies on 5-HTP for the treatment of depression began in the early 1970s in Japan. The first study involved 107 patients with either unipolar depression or manic bipolar depression.[10] These patients received 5-HTP at dosages ranging from 50 to 300 mg/day. The researchers observed a quick response (within 2 weeks) in more than half of the patients. Seventy-four of the patients either experienced complete relief or significantly improved, and none experienced significant side effects. These promising results were repeated in several other Japanese studies. An interesting aspect in two of these studies was the fact that 5-HTP was shown to be effective in some patients (50% in one study, 35% in another) who had not responded positively to any other antidepressant agent.[11,12]

The most detailed of the Japanese studies was conducted in 1978.[13] The study enrolled 59 patients with depression (30 males and 29 females). The groups were mixed, in that both unipolar and bipolar depressions were included, along with a number of other subcategories of depression. The severity of the depression in most cases was moderate to severe. Patients received 5-HTP in dosages of 50 or 100 mg three times a day for at least 3 weeks.

The antidepressant activity and clinical effectiveness of 5-HTP were determined using a rating scale developed by the Clinical Psychopharmacology Research Group in Japan. The improvements among the various patients are detailed in Table 87.1. These results indicated that 5-HTP was helpful in 14 of 17 patients with unipolar depression and 12 of 21 patients with bipolar depression. The degree of improvement in most cases ranged from excellent to very good.

TABLE 87.1 Improvement in Various Subtypes of Depression

Subtype	IMPROVEMENT[a] 1	2	3	4	5, 6, 7	8	1 + 2 + 3/total[b]
First-episode depression	1	1	0	1	0	0	2/3
Unipolar depression	3	8	3	1	2	0	14/17
Bipolar depression	6	4	2	3	3	3	12/21
Mixed depression	0	1	0	1	0	0	1/2
Presenile or senile depression	3	2	0	3	1	0	5/9
Neurotic depression	0	1	2	1	0	0	3/4
Reactive depression	0	1	1	0	0	0	2/2
Schizophrenic depression	0	1	0	0	0	0	1/1
Total	13	19	8	10	6	3	40/59
% of total	22	32.2	13.6	16.9	10.2	5.1	67.8

[a]Improvement: 1, marked improvement; 2, moderately improved; 3, slightly improved; 4, unchanged; 5, 6, 7, felt worse; 8, dropped out.
[b]The number of subjects who improved (improvement scores 1, 2, or 3) compared with the total number of subjects in that subtype.

TABLE 87.2 Day When Improvements Were First Noticed

Improvement Group	DAY 1	2	3	4–7	8–14	15
Marked	1	3	2	5	2	0
Moderate	0	1	13	1	4	0
Slight	0	2	2	2	1	1
Total	1	6	17	8	7	1

The results achieved in this open study were quite good, given how rapidly they were achieved. Thirty-two of the 40 patients who responded to 5-HTP did so within the first 2 weeks of therapy. Typically, in most studies with antidepressant drugs, the benefits are not apparent until after 2 weeks to 1 month of use. For this reason, the length of study when assessing antidepressant drugs should be at least 6 weeks because it may take that long to significantly affect brain chemistry in a positive manner. In contrast, many of the studies with 5-HTP were shorter than 6 weeks because statistically significant results were achieved so soon (Table 87.2). However, the longer 5-HTP is used, the better the results. Some people may need to be on 5-HTP for at least 2 months before they experience benefits.

The only major side effect noted in this study was mild nausea. The occurrence of nausea caused by 5-HTP is actually less frequent than that experienced with other antidepressant drugs (roughly 10% of subjects taking 5-HTP at a daily dose of more than 300 mg experience nausea compared with about 23% taking Prozac) and about the same as that which occurs with a placebo. In double-blind studies, about 10% or so of people taking the placebo typically complain of nausea. Nonetheless, mild nausea may be a natural consequence of elevated serotonin levels with 5-HTP. About 30% of the 5-HTP taken orally is converted to serotonin in the intestinal tract. This can lead to a mild case of nausea. Fortunately, this effect wears off after a few weeks of use.

A 5-HTP dosage of 150 to 300 mg/day is sufficient in most cases. For example, in one study, it was shown that 13 of 18 subjects with depression given 5-HTP at a level of 150 or 300 mg/day experienced good to excellent results.[14] This percentage of responders is quite good, but if the level of serotonin in the blood is viewed as a rough indicator of brain serotonin levels, some interesting conclusions can be made (Table 87.3). In some cases, a higher dosage may be necessary.

TABLE 87.3 Level of Serotonin in Blood (nanograms per milliliter): Controls, Responders, and Nonresponders

	Before	After 1 Wk (150 OR 300 mg/day 5-HTP)
Normal subjects	150	
Responders	78	148
Nonresponders	56	77

5-HTP, 5-Hydroxytryptophan.

TABLE 87.4 Hamilton Depression Rating Scale Scores From a Comparative Study of 5-Hydroxytryptophan (5-HTP), L-Tryptophan, and Placebo

	5-HTP	L-tryptophan	Placebo
Beginning of the study	26	25	23
End of the study (30 days)	9	15	19

The measurements in Table 87.3 suggest that serotonin levels in depressed individuals are considerably lower than those found in normal subjects and that individuals who respond to 5-HTP show a rise in serotonin to levels consistent with normal subjects. The level of serotonin in those who do not respond to 5-HTP remains quite low. These results imply that nonresponders may require higher doses to raise serotonin levels or that additional support may be necessary. When prescribing higher doses, it is important that the 5-HTP be taken in divided doses not only to reduce the problem with nausea but also because the rate of brain-cell uptake of 5-HTP is limited.

The first studies of 5-HTP were open trials.[15] The antidepressive effects of 5-HTP were also compared with L-tryptophan in the early 1970s.[6] In one study, 45 subjects with depression were given L-tryptophan (5 g/day), 5-HTP (200 mg/day), or a placebo. The patients were matched in clinical features (e.g., age, sex) and severity of depression. The main outcome measure was a rating scale called the Hamilton Depression Rating Scale (HAM-D), the most widely used assessment tool in clinical research on depression.

The HAM-D score is determined by having the test subject complete a series of questions in which he or she rates the severity of symptoms on a numerical basis, as follows:

- 0—not present
- 1—present but mild
- 2—moderate
- 3—severe
- 4—very severe

Symptoms assessed by the HAM-D include depression, feelings of guilt, insomnia, gastrointestinal symptoms and other bodily symptoms of depression (e.g., headaches, muscle aches, heart palpitations), and anxiety. The HAM-D is popular in research because it provides a good assessment of the overall symptoms of depression. Table 87.4 shows the results of the study.

A review of head-to-head comparison studies showed that 5-HTP, at a dosage of 200 mg/day, produced therapeutic success on par with

TABLE 87.5 Change in Hamilton Depression Rating Scale Scores

	5-HTP + MAO	MAO + PLACEBO
Initial measurement	28.67	26.33
After 8 days	16.67	19.23
After 15 days	11.77	6.03

5-HTP, 5-Hydroxytryptophan; *MAO,* monoamine oxidase.
Modified from Alino JJ, Gutierrez JL, Iglesias ML. 5-Hydroxytryptophan (5-HTP) and a MAOI (nialamide) in the treatment of depressions. A double-blind controlled study. *Int Pharmacopsychiatry*. 1976;11:8–15.

tricyclic antidepressant drugs.[16] Research also showed that combining 5-HTP with clomipramine and other types of antidepressant drugs produced better results than any of the compounds given alone.[17–22] For example, in one study, 5-HTP combined with a monoamine oxidase inhibitor demonstrated significant advantages compared with the monoamine oxidase inhibitor alone (Table 87.5).[21]

This line of research suggests that 5-HTP might also be used in conjunction with St. John's wort extract and *Ginkgo biloba* extract, two herbal medicines with proven antidepressant activity.

Because 5-HTP was expensive in 1972, researchers developed a test to determine who was most likely to respond to it so that it would not be wasted on people who were unlikely to respond.[15,23] The test involved the patients first having a spinal tap to measure the level of 5-hydroxyindoleacetic acid (5-HIAA, the breakdown product of serotonin) in the cerebrospinal fluid (CSF). The drug probenecid, which prevents the transport of 5-HIAA from the CSF to the bloodstream, was administered for the next 3 days. As a result of this blocking action, the amount of serotonin produced over a 4-day period could be calculated by a repeat spinal tap on day 4. Because the 5-HIAA could not leave the CSF, it accumulated and provided a measure of serotonin manufacture.

The researchers discovered that the average level of 5-HIAA after 3 days of probenecid was significantly lower in depressed individuals than in controls matched for age, sex, and weight. This low level of serotonin reflected a decreased rate of manufacture within the brain. 5-HTP was most effective in patients with a low 5-HIAA response to 3 days of probenecid.[23–25] In other words, 5-HTP is most effective as an antidepressant when the amount of serotonin manufactured in the brain is reduced.

As stated earlier, 5-HTP often produces good results in patients who are unresponsive to antidepressant drugs. One of the more impressive studies involved 99 patients described as having "therapy-resistant" depression.[26] These patients did not respond to any previous therapy, including all available antidepressant drugs and electroconvulsive therapy. These therapy-resistant patients received 5-HTP at dosages averaging 200 mg/day but ranging from 50 to 600 mg/day. Complete recovery was seen in 43 of the 99 patients, and significant improvement was noted in 8 more. Such a significant improvement in patients with long-standing, unresponsive depression is quite impressive, prompting the author of another study to state the following[27]:

> *L-5-HTP merits a place in the front ranks of antidepressants instead of being used as a last resort. I have never in 20 years used an agent that (1) was effective so quickly; (2) restored patients so completely to the persons they had been and their partners had known; and (3) was so entirely without side effects.*

In an open-label study, 15 women with major depressive disorder who had failed selective serotonin-reuptake inhibitor (SSRI) or serotonin–norepinephrine-reuptake inhibitor (SNRI) monotherapy were treated with 5 g of creatine monohydrate daily and 100 mg of 5-HTP twice daily for 8 weeks. Mean HAM-D scores declined from 18.9 at pretreatment visits to 7.5 at the end of 8 weeks.[28]

TABLE 87.6 5-Hydroxytryptophan (5-HTP) Versus Fluvoxamine in Percentage Changes in the Hamilton Depression Rating Scale (HAM-D) Score

	Decrease in HAM-D	5-HTP (*n* = 34)	Fluvoxamine (*n* = 29)
After 2 wk	Mean decrease (%)	23	18.9
	<35% decrease	20	19
	35%–50% decrease	10	8
	50%–75% decrease	4	2
After 4 wk	Mean decrease (%)	46.2	46.1
	<35% decrease	2	8
	35%–50% decrease	7	3
	50%–75% decrease	12	13
	>75% decrease	3	5
After 6 wk	Mean decrease (%)	60.7	56.1
	<35% decrease	4	5
	35%–50% decrease	8	3
	50%–75% decrease	12	8
	>75% decrease	10	13

A 1987 review article on 5-HTP in depression highlighted the need for well-designed, double-blind, head-to-head studies of 5-HTP versus standard antidepressant drugs.[29] Although 5-HTP was viewed as an antidepressant agent with few side effects, the authors of this review felt that the big question to answer was how 5-HTP compared with the new breed of antidepressant drugs, the SSRIs like Prozac, Paxil, and Zoloft. In 1991 a double-blind study comparing 5-HTP with an SSRI was conducted in Switzerland.[30] In the study, 5-HTP was compared with the SSRI fluvoxamine (Luvox). Fluvoxamine is used primarily in the United States as a treatment for obsessive-compulsive disorder, an anxiety disorder characterized by obsessions and compulsions affecting an estimated 5 million Americans. Fluvoxamine exerts antidepressant activity comparable to (if not better than) other SSRIs like Prozac, Zoloft, and Paxil.

In the study, subjects received either 5-HTP (100 mg) or fluvoxamine (50 mg) three times a day for 6 weeks. The assessment methods used to judge effectiveness included the HAM-D, Self-Assessment Depression Scale (SADS), and physician's assessment (Clinical Global Impression). As indicated in Table 87.6, the percentage decrease in depression was slightly better in the 5-HTP group (60.7% vs. 56.1%). 5-HTP was quicker acting than the fluvoxamine, and a higher percentage of patients responded to 5-HTP than to fluvoxamine.

The advantages of 5-HTP over fluvoxamine are evident when looking at the subcategories of the HAM-D: depressed mood, anxiety, physical symptoms, and insomnia. For depressed mood, 5-HTP produced a 65.7% reduction in severity compared with 61.8% for fluvoxamine; for anxiety, 5-HTP produced a 58.2% reduction in severity compared with 48.3% for fluvoxamine; for physical symptoms, 5-HTP produced a 47.6% decrease in severity compared with 37.8% for fluvoxamine; and for insomnia, 5-HTP produced a 61.7% decrease in severity compared with a 55.9% decrease for fluvoxamine. However, perhaps more important than simply relieving insomnia was 5-HTP's ability to improve the quality of sleep. In contrast, antidepressant drugs greatly disrupt sleep processes. On the SADS, 5-HTP produced a 53.3% drop in SADS values compared

TABLE 87.7 5-Hydroxytryptophan (5-HTP) Versus Antidepressant Drugs: Comparison of Side Effects

	PERCENTAGE OF PATIENTS EXPERIENCING SIDE EFFECTS		
Side Effects	**5-HTP**	**Tricyclics**	**SSRIs**
Nausea	9	15	23
Headache	5	16	20
Nervousness	2.5	11	16
Insomnia	2.5	7	17
Anxiety	2.5	9	14
Drowsiness	7	23	11
Diarrhea	2.5	4	12
Tremor	0	18	11
Dry mouth	7	64.5	12
Sweating	2.5	15	9
Dizziness	5	25.5	7
Constipation	5	25	5.5
Vision changes	0	14.5	4

SSRI, Selective serotonin-reuptake inhibitor.

with a drop of 47.6% for the fluvoxamine group. A drop greater than 50% is an excellent result. A 50% drop is the best SSRIs generally produce.

In another head-to-head comparison study, this time with fluoxetine, 5-HTP also showed q good antidepressant effect.[31] The study consisted of 60 patients suffering from their first depressive episode who were randomly divided to receive 5-HTP (group A) or fluoxetine (group B) for a period of 8 weeks. 5-HTP was given at 150 mg in three divided dosages during the first 2 weeks, and then the dose was doubled (300 mg) after the second week. The dosages were increased to 400 mg in three divided dosages after the fourth week. Thereafter, the same dosages were continued. All patients in group B were given fluoxetine 20-mg capsules along with two placebo dosages during the first 2 weeks and then increased to 30 mg along with placebo dosages after the second week. All patients were administered the HAM-D to assess the severity of depression at baseline, 2 weeks, 4 weeks, and 8 weeks. A final evaluation of both efficacy and tolerance was conducted using the Clinical Global Impression (CGI) scale at the end of the study.

Results showed that both treatment groups experienced a significant and nearly equal reduction in HAM-D scores beginning at week 2 and continuing through week 8. Twenty-two patients (73.33%) in the 5-HTP group and 24 patients (80%) in the fluoxetine group showed a positive response at the end of the study. 5-HTP was very well tolerated, and it was concluded that 150 mg represents a minimal effective dosage in depression for 5-HTP.

The existing data indicate that 5-HTP is equal to or better than standard antidepressant drugs, and the side effects are much less severe (Table 87.7). In the study comparing 5-HTP with fluvoxamine, this is how the physicians described the differences among the two groups: Whereas the two treatment groups did not differ significantly in the number of patients sustaining adverse events, the interaction between the degree of severity and the type of medication was highly significant: fluvoxamine predominantly produced moderate to severe side effects; oxitriptan (5-HTP) produced primarily mild forms of adverse effects.

Fourteen (38.9%) of the patients receiving 5-HTP reported side effects compared with 18 patients (54.5%) in the fluvoxamine group. The most common side effects with 5-HTP were nausea, heartburn, and gastrointestinal problems (flatulence, feelings of fullness, and rumbling sensations). These side effects were rated as being very mild to mild. In contrast, most of the side effects experienced in the fluvoxamine group were of moderate to severe intensity. The only subject to drop out of the 5-HTP group did so after 35 days (5 weeks), whereas four subjects in the fluvoxamine group dropped out after only 2 weeks. On the basis of studies on weight loss, the longer that 5-HTP is used (e.g., after 4–6 weeks of use), the less the problem with mild nausea.

5-HTP has been shown to have "equipotency" with SSRIs and tricyclic antidepressants in terms of effectiveness, but it offers several advantages in that it is better tolerated and associated with fewer and much milder side effects. In addition, many people prefer to use a natural substance like 5-HTP over synthetic drugs.

l-Tyrosine: an Adjunct to 5-Hydroxytryptophan

In the early 1970s, researchers discovered that in about 20% of patients who responded well to 5-HTP, the results tended to decrease after 1 month of treatment. The antidepressant effects of 5-HTP in these subjects began to wear off gradually after the first month even though the level of 5-HTP in the blood, and presumably the level of serotonin in the brain, remained at the same level as when they were experiencing benefits.[5]

The researchers discovered that although serotonin levels appeared to stay at the same levels after 1 month of treatment, the levels of the other important monoamine neurotransmitters, dopamine and norepinephrine, declined. As discussed earlier, when depressed patients are treated with 5-HTP, they experience a rise not only in serotonin but also in catecholamines like dopamine and norepinephrine. In about 20% of subjects, the catecholamine-enhancing effects of 5-HTP tended to wear off. Providing these patients with L-tyrosine, the amino acid precursor to the catecholamines, helped reestablish the efficacy of 5-HTP.[5] The dosage was 200 mg/day for 5-HTP and 100 mg/kg body weight for L-tyrosine. This dosage for L-tyrosine is quite high and requires substantial clinical supervision.

Anxiety and Panic Disorder

5-HTP showed an ability to relieve anxiety in studies in depressed individuals. Lowering the availability of serotonin to the brain by tryptophan depletion increased the vulnerability of patients with panic disorder to an experimental carbon dioxide panic challenge, and increasing the availability of serotonin inhibited the response to such a challenge. When 5-HTP or placebo was given to 24 patients with panic disorder and 24 healthy volunteers before a panic challenge, 5-HTP significantly reduced the reaction to the panic challenge in patients with panic disorder regarding subjective anxiety, panic symptom score, and number of panic attacks, as opposed to placebo.[32] These results indicate that 5-HTP might be helpful in relieving panic attacks.

Weight Loss

A considerable body of scientific evidence documents the major role serotonin in the brain plays in influencing eating behavior. A key finding is that when animals and humans are fed tryptophan-free diets, appetite is significantly increased, resulting in binge eating—carbohydrates would be preferable, but animals binge on whatever is available.[2,3] A diet low in tryptophan leads to low brain serotonin levels; as a result, the brain senses it is starving and stimulates the appetite control centers in a powerful way. This stimulation results in a preference for carbohydrates. When animals or humans are fed a carbohydrate meal, more tryptophan is delivered to the brain, resulting in more serotonin being manufactured. This scenario led to the idea that low serotonin levels lead to "carbohydrate craving" and play a major role in the development of obesity and bulimia.

Cravings for carbohydrates can be mild or quite severe. They may range in severity from the desire to nibble one piece of bread or cookie

TABLE 87.8 Effect of 5-Hydroxytryptophan (5-HTP) on Food Intake

	Food Intake (calories/day)	Protein Intake (g/day)	Carbohydrate Intake (g/day)
Pretreatment	2903	101	274
Placebo	2327	85	223
5-HTP	1819	79	176

to uncontrollable binging. At the upper end of the spectrum of carbohydrate addiction is bulimia, a potentially serious eating disorder characterized by binge eating and purging of the food through forced vomiting or the use of laxatives. The serotonin theory of bulimia is that low serotonin levels trigger binge eating, which leads to a rush of serotonin being produced and released in the brain.[33,34] This increased serotonin effect produces a brief reduction in feelings of stress and tension. This serotonin "fix" is short-lived and followed by feelings of guilt and low self-esteem. The current medical treatment for bulimia is the use of drugs that enhance the effects of serotonin. Although there are no reports in the medical literature of 5-HTP being used in the treatment of bulimia, given its effects on serotonin levels, it merits consideration.

5-HTP may help prevent the decline in serotonin levels associated with reduced calorie intake. Concentrations of tryptophan in the bloodstream and subsequent brain serotonin levels plummet with dieting.[35] In response to severe drops in serotonin levels, the brain puts out a strong message to eat. This situation sets up the scenario to explain why most diets do not work.

As far back as 1975, researchers demonstrated that giving 5-HTP to rats who were genetically bred to overeat and be obese resulted in a significant reduction in food intake.[36] It turns out that these rats bred to be fat had decreased activity of the enzyme that converts tryptophan to 5-HTP and subsequently to serotonin.

There is circumstantial evidence that many humans are genetically predisposed to obesity. This predisposition may involve the same mechanism as rats genetically predisposed to obesity. By providing preformed 5-HTP, this genetic defect is bypassed, and more serotonin is manufactured.

The early animal studies with 5-HTP as a weight-loss aid were followed by a series of three human clinical studies. The first study involved 19 significantly overweight female subjects with a body mass index (BMI) ranging from 30 to 40 kg/m^2.[37] Analysis of the pretreatment dietary intake concluded that these women tended to overeat carbohydrates. Food intake and eating behavior were assessed using a 3-day diet diary at the beginning and end of the two treatment periods. All food was carefully weighed before meals and weighed again if there were any leftovers. Participants also filled out a self-evaluation of appetite and satiety twice a week, and mood was evaluated using standard psychological tests.

The daily dosage of 5-HTP used in the study was 8 mg/kg body weight. Patients were given either 5-HTP or placebo 20 minutes before meals for 5 weeks, and after a 1-week interval, they were switched to receive the other treatment. No dietary restrictions were prescribed because the researchers wanted to answer the question, "Does 5-HTP reduce appetite and promote weight loss without any conscious effort?" To make sure that the women actually took the 5-HTP, researchers measured the level of the serotonin breakdown product, 5-HIAA, in the urine. Table 87.8 lists the results of the study.

These results with 5-HTP were achieved without the women making any conscious effort to reduce food consumption. The average amount of weight loss during the 5-week period of 5-HTP supplementation

TABLE 87.9 Effect of 5-Hydroxytryptophan (5-HTP) on Weight Loss

	Placebo	5-HTP
Weight (lb)		
Baseline	207.68	229.46
After 6 wk	206.58	225.94
After 12 wk	205.4	219.12
Total weight loss (lb)		
After 6 wk	1.1	3.52
After 12 wk	2.28	10.34

TABLE 87.10 Effect of 5-Hydroxytryptophan (5-HTP) on Appetite and Satiety

	5-HTP		PLACEBO	
	Wk 1–6	Wk 7–12	Wk 1–6	Wk 7–12
Taste alteration	2/7	1/7	0/7	0/7
Smell alteration	2/7	1/7	0/7	0/7
Meat aversion	3/7	1/7	0/7	0/7
Early satiety	7/7	6/7	2/7	2/7
Nausea	5/7	0/7	1/7	2/7

was a little more than 3 lb, compared with less than 1 lb of total weight loss during the placebo period.

Interestingly, evaluation of the various self-tests indicated that appetite or degree of initial hunger did not differ between the two groups. What differed was satiety. In other words, the 5-HTP did not reduce appetite before a meal, but after consuming an adequate amount of food, the satiety centers in the brain were stimulated, and the women did not feel hungry. As a result, their caloric intake was dramatically reduced.

The level of 5-HIAA, the breakdown product of serotonin, in the group receiving the 5-HTP increased by more than fiftyfold over the control group. This increase provided two things: (1) it assured researchers that subjects actually took the 5-HTP, and (2) it clearly indicated that 5-HTP increased serotonin manufacture.

The next study sought to determine whether 5-HTP helped overweight individuals adhere to dietary recommendations.[38] Fourteen overweight female subjects with a BMI between 30 and 40 kg/m^2 were enrolled in the double-blind study.[10] Again, analysis of the pretreatment dietary intake concluded that these women tended to overeat. The women were randomly assigned to receive either 5-HTP (300 mg) or placebo 30 minutes before meals. The 12-week study was divided into two 6-week periods. For the first 6 weeks, there were no dietary recommendations, and for the second 6 weeks, the women were placed on a 1200-calorie diet.

The women were seen every 2 weeks to evaluate body weight, diet diaries, self-evaluations of appetite, and satiety. The women were also asked if they experienced the presence of meat aversion; taste or smell alterations; early satiety; and nausea or vomiting, or both. To verify patient compliance, urinary measurement of 5-HIAA was again determined. As shown in Table 87.9, the women taking the placebo lost 2.28 lb, whereas the women taking the 5-HTP lost 10.34 lb.

Like the previous study, 5-HTP appeared to promote weight loss by promoting satiety. Although some women reported some aversion to meat or altered taste and smell, each woman (100%) reported early satiety (Table 87.10). Most of the women receiving 5-HTP also

experienced mild nausea during the first 6 weeks of the trial, but during the last 6 weeks, none complained of nausea. The fact that weight loss was accelerated during the second 6-week period makes it highly unlikely that 5-HTP promoted weight loss as a result of producing nausea.

One study with 5-HTP enrolled overweight women with a BMI ranging between 30 and 40 kg/m^2 and an overactive appetite.[39] The 28 subjects of the study were given either 5-HTP (300 mg three times daily before meals) or a placebo. For the first 6 weeks, there were no dietary restrictions, and for the second 6 weeks, the women were placed on a diet of 1200 calories/day. Carbohydrates contributed 53% of the calories, fats comprised 29%, and proteins provided 18%. No carbohydrate-rich foods were permitted between meals. Subjects were examined every 2 weeks to evaluate food intake and body weight. Routine blood measurements were also performed at the beginning, at 6 weeks, and at the end of the study. To verify patient compliance, urinary measurement of 5-HIAA was determined.

The results from this study were even more impressive than the previous studies, for several reasons. The group receiving the 5-HTP lost an average of 4.39 lb after the first 6 weeks and an average of 11.63 lb after 12 weeks. In comparison, the placebo group lost an average of only 0.62 lb after the first 6 weeks and 1.87 lb after 12 weeks. The lack of weight loss during the second 6-week period in the placebo group obviously reflected the fact that the women had difficulty adhering to the diet.

Early satiety was reported by 100% of the subjects during the first 6-week period. During the second 6-week period, even with severe caloric restriction, 90% of the women taking 5-HTP reported early satiety. Once again, many of the women receiving the 5-HTP reported mild nausea during the first 6 weeks of therapy. However, the symptom was never severe enough for any of the women to drop out of the study. No other side effects were reported.

On the basis of the urinary measurements of 5-HIAA, the women took their 300 mg of 5-HTP with meals and, as a result, achieved weight loss. The amount of weight loss was amplified by a better capability to adhere to a 1200-calorie diet. The structure of the dietary changes reflected primarily a reduction in pasta, bread, and other carbohydrate-rich foods (the study was conducted in Rome).

In the latest study, 25 overweight outpatients with non–insulin-dependent diabetes were enrolled in a double-blind, placebo-controlled study and randomized to receive either 5-HTP (750 mg/day) or a placebo for 2 consecutive weeks, during which no dietary restrictions were prescribed.[40] Results again indicated that patients receiving 5-HTP significantly decreased their daily energy intake by reducing carbohydrate and fat intake and reduced their body weight.

Insomnia

Several clinical studies showed 5-HTP produced good results in promoting and maintaining sleep in normal subjects and in those experiencing insomnia.[39–46] One of the key benefits with 5-HTP in the treatment of insomnia is its ability to increase sleep quality. This effect is evident by its ability to increase rapid eye movement (REM) sleep (typically by about 25%) while simultaneously increasing deep sleep stages 3 and 4 without increasing total sleep time.[39,42] The sleep stages that are reduced by 5-HTP to compensate for the increases are non-REM stages 1 and 2, the least important stages of sleep. In one of the studies, the subjects receiving 200 mg of 5-HTP increased their amount of REM sleep by 15.5 minutes during the 5-night study.[39] Subjects taking 600 mg of 5-HTP increased REM sleep time by an average of 20 minutes for the 5-night study. These results indicated that 5-HTP increased the amount of dream time by about 3 to 4 minutes a night.

Although there was a clear dose-related effect, the lower dosage is sufficient in most cases. In addition, taking too much 5-HTP may increase REM sleep to an abnormal level, leading to an increased risk for nightmares. That said, 5-HTP might prove effective in sleep terrors in children. To test this hypothesis, an open trial in a group of children with sleep terrors was compared with a group of children with the same disorder but without 5-HTP treatment.[47] 5-HTP was administered (2 mg/kg per day) at bedtime to 31 randomly selected patients for 20 consecutive days. After 1 month of treatment, 29 of 31 (93.5%) patients showed a positive response. In the comparison group without drug therapy, after 1 month, the episodes disappeared in only 4 children (28.6%), whereas 10 children (71.4%) showed persistence of episodes with the same frequency as before. After 6 months, 26 of 31 (83.9%) children treated with 5-HTP were sleep terror–free, whereas in five children (16.1%), sleep terror episodes persisted. Of the children in the comparison group, 10 (71.4%) continued to show sleep terrors at 6-month follow-up. These results represent preliminary evidence that treatment with 5-HTP is able to modulate the arousal level in children and induce a long-term improvement in sleep terrors.

Migraine and Tension Headaches

The relationship between serotonin and headaches is fully described in Chapter 198. Because patients with migraines have low levels of serotonin in their tissues, some researchers refer to migraine headaches as a "low-serotonin syndrome."[46] Although the primary benefit of 5-HTP in the prevention of both migraine and tension headaches is related to its ability to normalize underlying imbalances in the serotonin system, it also influences the endorphin system in a positive way.

Several clinical studies on the use of 5-HTP for headaches, both vascular and nonvascular, showed excellent results. In particular, the use of 5-HTP in the prevention of migraine headaches offers considerable advantages over drug therapy. Although a number of drugs were shown to be useful in the prevention of migraine headaches, all of them carry significant side effects.

The problem with drug therapy in the prevention of migraine headaches is perhaps best exemplified by one of the most commonly used drugs, methysergide (Sansert). Methysergide therapy for the prevention of migraine attacks is effective in about 60% to 80% of cases. However, this effectiveness is not without a high price because side effects are quite common and can be severe. Retroperitoneal fibrosis, pleuropulmonary fibrosis, and fibrotic thickening of cardiac valves may occur in patients receiving long-term methysergide maleate therapy. Therefore this preparation must be reserved for prophylaxis in patients whose vascular headaches are frequent or severe and uncontrollable and for those who are under close medical supervision.

Several studies compared 5-HTP with methysergide in the prevention of migraine headaches. In one of the largest double-blind studies, 124 patients received either 5-HTP (600 mg/day) or methysergide (3 mg/day) in identical pills for 6 months.[48] Treatment was determined successful if there was a reduction of greater than 50% in the frequency of attacks or in the number of severe attacks. Although 75% of the patients (30 of the remaining 40 patients) taking methysergide demonstrated significant improvement compared with 71% of the patients (32 of the 45 patients) taking 5-HTP, this difference was not viewed as statistically significant (Table 87.11). The advantage of 5-HTP over methysergide was demonstrated when researchers looked at side effects. Side effects were more frequent in the group receiving methysergide than in the 5-HTP group. Five patients in the methysergide group withdrew during the trial because of side effects.

Two other studies comparing 5-HTP with drugs used in the prevention of migraine headaches (pizotifen and propranolol) demonstrated that 5-HTP compared quite favorably in terms of effectiveness.[49,50]

TABLE 87.11 5-Hydroxytryptophan (5-HTP) Versus Methysergide: Clinical Effects of Treatment in 124 Patients

	Methysergide	5-HTP
No attacks (100% reduction)	35%	25%
Improvement (>50% reduction)	40%	46%
No improvement	12.5%	29%
Withdrawal because of side effects	12.5%	0%

Although these drugs have significant side effects, 5-HTP is extremely well tolerated even at dosages as high as 600 mg/day. One of the other key differences noted in these studies between 5-HTP and the other drugs was 5-HTP's ability to improve mood and relieve feelings of depression.

In a double-blind study of 5-HTP in chronic tension headache, 78 patients with chronic tension-type headaches were treated with 5-HTP (300 mg/day) or a placebo for 8 weeks.[48] In comparison with the group treated with a placebo, there were no statistically significant changes in the number of days with headache or in headache intensity in the group treated with 5-HTP; however, there was a significant decrease in the consumption of analgesics.[51] During the 2 weeks after treatment, there was also a significant decrease in the number of days with headache. Subjective opinion during this latter period was also favorable to 5-HTP.

Juvenile Headache

One of the best uses of 5-HTP is in chronic headaches in children. These headaches are a big problem because of the tremendous risk for side effects of the current drugs used to treat, and prevent, them. Several studies of 5-HTP in the treatment of chronic headaches in children and adolescents showed excellent results.[52–54] Given the risks of current drugs used in chronic childhood headaches, a trial of 5-HTP for 2 months certainly seems reasonable. If the headaches are also accompanied by sleep disorders, 5-HTP appears to be especially well suited.

In one study, 48 elementary and junior high students with recurrent headaches (at least one headache every 2 weeks) and sleep disorders, including difficulty in getting to sleep, frequent awakenings, sleepwalking, nightmares, and bedwetting, were divided into two groups.[52] Group A was given 5-HTP for 2 months, followed by a placebo for 2 months, whereas group B received just the opposite. It was necessary to divide group A into a nine-patient subgroup, group C. These nine patients did so well on the 5-HTP that they did not want to switch over to the other medication, although they had no idea whether they were, in fact, taking 5-HTP or were on a placebo. The dosage of 5-HTP was based on the child's weight (4.5 mg/kg per day).

The headache index was reduced by about 70% when the kids were taking 5-HTP compared with an 11.5% drop when they were taking the placebo. In the nine patients in group C, there was an 81.8% decrease in the headache index after the second month. Interestingly, these same patients only exhibited an 18.2% reduction after the first month. These results indicate that an evaluation of the benefits of 5-HTP in the treatment of headaches requires at least a 2-month trial. The failure to show benefit with 5-HTP in some studies in headaches might be because of the fact that they lasted less than 2 months. The 25 patients experienced a modest reduction in frequent awakenings, nightmares, sleepwalking, and talking while asleep and no change in difficulty falling asleep or in bedwetting.

TABLE 87.12 Serotonin and β-Endorphin Levels in Juvenile Patients with Headaches Before and After Administration of 5-Hydroxytryptophan (5-HTP)

	Serotonin (serum, mg/L)	Beta-Endorphin (plasma, pmol/L)	Beta-Endorphin (white blood cells, pmol/106 GB/L)
Migraine (13 subjects)			
Before	104.6	16.2	110.5
After	115.7	19.4	120.3
Tension-type (7 subjects)			
Before	90.7	14.5	142.3
After	97.2	17.6	152.4
Total (20 subjects)			
Before	100.5	15.7	129.3
After	108.3	18.4	140.4
Controls (17 subjects)			
	96	21.3	359.3

Overall, this study demonstrated very good effects in these children. Perhaps the most impressive aspect to consider, however, was the fact that these benefits were achieved without side effects. Not a single child reported a side effect while taking 5-HTP. Interestingly, for some reason, children rarely experience even mild nausea from 5-HTP.

The possible benefits of using 5-HTP in children with recurrent headaches or sleep disorders, or both, is far-reaching. Evaluation of the 48 children in the trial demonstrated inadequate school progress compared with their classmates. The children were shown to be of normal intellectual capacity but demonstrated inattentiveness similar to that observed in depression. Many of the children might have had depression. The unwillingness of the nine subjects in group C to switch to the other unknown medication (which was the placebo) is a strong indicator that these children and their parents noted some rather dramatic improvements beyond simply a reduction in headaches or improved sleep.

The manner in which 5-HTP may be of benefit in migraine headaches may not simply be the overcoming of some defect in serotonin synthesis. As noted previously, part of the clinical benefit of 5-HTP may be via an ability to increase the levels of β-endorphin. A decrease in β-endorphin levels in patients with migraine headaches, and tension headaches, was demonstrated by several investigators.[55,56]

A clinical trial measured the effects of 5-HTP on serotonin and β-endorphin levels in 20 juvenile patients with migraine or tension-type headaches.[57] Patients were monitored and evaluated for the frequency and intensity of headache attacks for 3 months before 5-HTP treatment and during 3 months of therapy. The researchers reported a statistically significant reduction in the headache score with 5-HTP treatment in both migraine and tension-type patients. These improvements were likely a result of increased β-endorphin levels, as noted in Table 87.12. However, the level of β-endorphins achieved with 5-HTP, especially as measured in the white blood cell, was still far less than that observed in control patients without recurrent headaches. These results imply that longer periods of 5-HTP supplementation may be required before there is a normalization of β-endorphin levels in children prone to headaches.

TABLE 87.13 Patients' and Physicians' Opinions on the Effectiveness of 5-Hydroxytryptophan (5-HTP) Versus Placebo in Fibromyalgia

Response	5-HTP	Placebo
Good	11	1
Fair	8	5
Poor	4	8
None	0	9

These results may indicate that 5-HTP alone is not able to raise β-endorphin levels sufficiently to reduce or eliminate headaches, suggesting that other therapies designed to increase β-endorphin levels should be used along with 5-HTP. Examples of other therapies that have been shown to raise β-endorphin levels are exercise, acupuncture, and biofeedback.

Fibromyalgia

The history of the development of 5-HTP as an effective treatment for fibromyalgia began with studies on the drug fenclonine.[58] This drug blocks the enzyme that inhibits the conversion of tryptophan to 5-HTP and, as a result, blocks serotonin production. During the late 1960s and early 1970s, it was thought that increased serotonin formation might promote migraine headaches (the opposite of what was later proved, i.e., increasing serotonin levels reduced the occurrence of migraine headaches). The researchers discovered that providing headache patients with fenclonine resulted in severe muscle pain. This effect was the exact opposite of what was expected, but it led to some important advances in the understanding of fibromyalgia—a way to induce its severe symptoms of (and symptoms nearly identical to) eosinophilia myalgia syndrome (EMS), the condition caused by contaminated L-tryptophan. The researchers also discovered that migraine patients had a greater reaction to the drug than nonheadache subjects. In most normal subjects, fenclonine produced no fibromyalgia. These occurrences highlighted just how sensitive migraine patients are to low serotonin levels.

Migraine headaches and fibromyalgia share a common feature: both are low-serotonin syndromes.[59] After more than 25 years of research, one of the lead researchers stated: "In our experience, as well as in that of other pain specialists, 5-HTP can largely improve the painful picture of primary fibromyalgia."[60]

A double-blind study in 50 patients with fibromyalgia found that 100 mg of 5-HTP three times per day significantly improved their symptoms.[61] As shown in Table 87.13, 5-HTP was rated substantially better than placebo by subjects and evaluating physicians. Improvements were noted in all symptom categories: number of painful areas, morning stiffness, sleep patterns, anxiety, and fatigue. In another study, 100 mg of 5-HTP taken three times a day demonstrated maximum results by day 30 of the 90-day trial.[62]

One of the primary benefits with 5-HTP in fibromyalgia may be its ability to improve sleep quality. A key finding in patients with fibromyalgia is a reduced REM sleep and an increase in non-REM sleep.[63] In addition, the deeper levels (stages III and IV) are not achieved for long enough periods. As a result, people with fibromyalgia wake up feeling fatigued and in pain. The severity of the pain of fibromyalgia correlates with the rating of sleep quality. For example, a study of 50 women with fibromyalgia syndrome recorded their sleep quality, pain intensity, and attention to pain for 30 days, using palm-top computers programmed as electronic interviewers.[64] They described their previous night's sleep quality within 30 minutes of awakening each day and rated their present pain at randomly selected times in the morning, afternoon, and evening. The researchers found that a night of poor sleep was followed by a significantly more painful day, and a more painful day was followed by a night of even poorer sleep. 5-HTP might help break the cycle by addressing the low serotonin level and by promoting restful sleep.

TABLE 87.14 Effect of 5-Hydroxytryptophan (5-HTP) on Depression in Parkinson's Disease

Patient No.	l-Dopa (mg/day)	Carbidopa (mg/day)	5-HTP (mg/day)	HAM-D	
				Before	After
1	1000	175	125	22	11
2	300	75	75	14	3
3	400	150	100	21	13
4	1000	100	100	12	6
5	500	125	500	18	22
6	1125	112.5	300	18	7
7	500	50	100	17	13

HAM-D, Hamilton Depression Rating Scale.

Parkinson's Disease

The use of 5-HTP in Parkinson's disease provides some benefit but only if used in combination with the drug Sinemet (the combination of L-dopa with the decarboxylase inhibitor carbidopa). Although brain levels of serotonin are decreased in Parkinson's disease, the reduction in dopamine receptors is more severe. Increasing serotonin levels with 5-HTP in patients not taking Sinemet is associated with worsening of symptoms, especially rigidity.[65]

One of the key benefits of taking 5-HTP in Parkinson's disease is that it can help counteract the negative effects that the L-dopa in the Sinemet has on sleep and mood.[66–68] In addition, 5-HTP was also shown to improve the physical symptoms of Parkinson's disease.

About 9 of 10 people with Parkinson's disease have depression. The degree of depression in Parkinson's disease is a reflection of the individual's serotonin levels. The lower the level of serotonin, the more severe is the individual's depression. One study examined the effect of 5-HTP in seven patients with Parkinson's disease, all of whom were on Sinemet.[65] The initial dosage of 5-HTP was 75 mg, which was increased by 25 mg every 3 days until the patients reported relief of their depression, or up to a maximum of 500 mg/day for 4 months. The impressive results obtained in these patients are shown in Table 87.14.

Six of seven patients responded to 5-HTP. It should be noted that the doses of 5-HTP in five of the six patients who responded ranged from only 75 to 125 mg. The only patient who did not respond took 500 mg of 5-HTP.

Seizure Disorders

Most of the recent research on 5-HTP focused on its use in the treatment of several seizure disorders.[69–74] 5-HTP showed good results in most (but not all) studies in patients with diseases characterized by myoclonus, with the exception of epilepsy, which is not helped by 5-HTP.

The best response to 5-HTP for myoclonus occurs in people who have intention myoclonus, which is most often produced as a result of ischemic damage to the brain. Intention myoclonus can be a problem after a stroke or heart attack, overdosage of a drug such as heroin, a severe asthma attack, or an adverse reaction to anesthesia or another chemical. Improvements with 5-HTP were demonstrated in patients with intention myoclonus. 5-HTP also

produced good results in patients with progressive myoclonus epilepsy, essential myoclonus, palatal myoclonus, and Friedreich's ataxia.

In a 1983 article, one researcher of 5-HTP stated: "Some helpless bedridden patients dramatically improved to the extent that they could walk again and resume independent living."[74] Because of the phenomenal results, 5-HTP was the first compound to be evaluated as an orphan drug by the Pharmaceutical Manufacturing Associations Commission on Drugs for Rare Diseases.

Irritable Bowel Syndrome and the "Leaky Gut"

Irritable bowel syndrome (IBS) is often accompanied by impaired intestinal barrier function and "leaky gut" syndrome. Decreased serotonergic function appears to play a part in the intestinal barrier dysfunction. In an interesting double-blind study, 15 patients with IBS and 15 healthy volunteers ingested either 100 mg 5-HTP or a placebo.[75] Oral 5-HTP administration significantly increased mucosal levels of 5-HIAA in both healthy controls and patients with IBS, with the latter group showing a significantly larger increase. Urinary lactulose/L-rhamnose ratios were significantly lower after administration of 5-HTP in healthy controls and were accompanied by redistribution of zonula occludens-1, pointing to reinforcement of the barrier. In IBS, expression of the tight-junction proteins was significantly lower compared with healthy controls, and 5-HTP resulted in a further decrease in occludin expression. These results indicate that in healthy controls, a reinforcement of the intestinal barrier was seen with 5-HTP intake, whereas such reaction was absent in patients with IBS. This could indicate the presence of a serotonin-mediated mechanism aimed to reinforce the function of the intestinal barrier, which seems to be dysfunctional in patients with IBS.

DOSAGE

Enteric-coated preparations are required to avoid nausea. The dosage should be started at 50 mg three times daily. If the response is inadequate after 2 weeks, increase the dosage to 100 mg three times daily. This recommendation will greatly reduce the mild symptoms of nausea often experienced during the first few weeks of 5-HTP therapy. Because 5-HTP does not rely on the same transport vehicle as L-tryptophan, it can also be taken with food. Because the half-life of 5-HTP is relatively short (3–4 hours, with peak serum levels achieved at 1–2 hours), more frequent dosing may be more effective than single higher doses.

For insomnia, the general recommendation is 100 to 300 mg, 30 to 45 minutes before retiring. Start with the lower dose for at least 3 days before increasing the dose.

TOXICOLOGY

The major concern with 5-HTP is a possible link to L-tryptophan and EMS. However, an important distinction must be made in the manufacturing process. Although L-tryptophan is produced via bacterial fermentation and filtration, 5-HTP is commercially available through an extraction process from the seed of *Griffonia simplicifolia,* an African plant. 5-HTP extracted from this natural source avoids the contamination problem associated with past manufacturing of L-tryptophan.

Detailed analyses of all evidence by the Centers for Disease Control and Prevention and other experts led to the conclusion that the cause of the EMS epidemic could be traced to a single Japanese manufacturer, Showa Denko.[76,77] Of the six Japanese companies that supplied L-tryptophan to the United States, Showa Denko was the largest (50%–60% of all the L-tryptophan). The L-tryptophan was used not only as a nutritional supplement but also in infant formulas and nutrient mixtures for intravenous feeding.

A single case report linked 5-HTP to a condition like EMS in 1980.[78] However, this case involved the use of high dosages of 5-HTP (1400 mg) over a 20-month period. Further examination of the patient indicated a defect in tryptophan metabolism that resulted in elevations in kynurenine. Such defects in tryptophan metabolism are common in patients with scleroderma, which shares many common features with EMS. It appeared that either the 5-HTP might have contained a contaminant to which this man was sensitive or that taking such high doses of 5-HTP over a prolonged period of time aggravated his abnormal handling of L-tryptophan.

There was also a report of a 28-year-old woman, her husband, and her two sons, 33 and 13 months old, who developed an EMS-like illness in response to contaminated 5-HTP.[76] The young boys inherited the inability to convert tryptophan to 5-HTP. As a result, they required daily administration of 5-HTP (5–7 mg/kg). Both boys received the 5-HTP almost from birth.

The mother was not taking 5-HTP, but she prepared it for the young boys by opening the capsules, mixing the powder in juice or water, and giving it to them orally with a syringe. She never took the 5-HTP; she only touched it with her hands as she emptied the capsules.

When the second boy was about 9 months old, the mother began experiencing symptoms of EMS. After consulting a physician in July 1991, it was noted that her eosinophil count was well over 30%. She continued to worsen and was hospitalized in August 1991 with a tentative diagnosis of EMS. At this time, she was referred to the National Institutes of Health for further evaluation.

Because of the possible link between the mother's symptoms and the 5-HTP, the boys and the father were also evaluated. The older boy had an eosinophil count of 9% (normal is 1%–4%), and the younger boy had a count of 6%. The father had no abnormalities.

The 5-HTP that the boys used was analyzed by high-pressure liquid chromatography and found to contain an impurity not found in the 5-HTP that the National Institutes of Health used in its studies for ataxia and myoclonus. Switching the boys to the contaminant-free 5-HTP brought about a normalization of the eosinophil counts. The mother's case was interesting because she was the most severely affected, and skin exposure was the only contact with the contaminated 5-HTP.

In 1998, researchers at the Mayo Clinic identified trace levels of a compound termed "peak X" as the key contaminant using a sensitive laboratory technique.[79] As a result, manufacturers of 5-HTP now screen for the presence of this compound to ensure that all of the 5-HTP on the marketplace is free from peak X as outlined in current Food and Drug Administration methodology. A minor chromatographic peak (peak X) reported in some 5-HTP samples lacks credibility because of chromatographic artifacts and infinitesimal concentrations and has raised undue speculations concerning its chemistry and toxicity. It might also be a moot point because extensive analyses of several sources of 5-HTP showed no toxic contaminants similar to those associated with L-tryptophan, nor the presence of any other significant impurities.[80]

There have been no reports of a single person developing EMS from 5-HTP despite its popularity over the past 20 years. Nonetheless, to be on the safe side, some experts recommend that long-term continual use of 5-HTP be monitored by regular (every 6 months) eosinophil determination.

Evidence that uncontaminated 5-HTP does not cause EMS is also provided by researchers who have been using 5-HTP for more than 25 years. They state: "EMS has never appeared in the patients of ours who received only uncontaminated L-tryptophan or 5-hydroxytryptophan

(5-HTP)."[81] Furthermore, researchers at the National Institutes of Health studying the effects of uncontaminated 5-HTP on various metabolic conditions have not observed a single case of EMS, nor has a case of elevated eosinophils been attributed to 5-HTP in these studies.[82] In short, there has never been a report of uncontaminated 5-HTP causing EMS.

Although there has never been a single person developing EMS from 5-HTP products proven to be free from the contaminants, long-term continual use of 5-HTP should be monitored by regular (every 6 months) eosinophil determination. For any person with scleroderma resulting from the problem with tryptophan metabolism noted in these patients, an eosinophil determination after the first month of 5-HTP use is indicated, especially if dosages are greater than 500 mg/day. In addition is the following advice:

- Do not use 5-HTP during pregnancy or lactation.
- Do not use 5-HTP in Parkinson's disease unless the patient is on Sinemet.
- Do not use 5-HTP in patients with scleroderma.

DRUG INTERACTIONS

Because 5-HTP is the direct precursor to serotonin, it should not be used by individuals taking antidepressant drugs without close medical supervision. Although 5-HTP has been used safely in combination with prescription antidepressant drugs in clinical studies, taking this combination could result in too much serotonin in the body. The result is a condition known as the "serotonin syndrome," characterized by confusion, fever, shivering, sweating, diarrhea, and muscle spasms.

5-HTP may antagonize the effects of drugs used in migraine headaches like methysergide and cyproheptadine, which block serotonin effects.

The drug carbidopa greatly enhances the half-life and produces an apparent clearance at least 14 times smaller, and an area under the curve that is 15.4 times greater, compared with 5-HTP 100 mg given without carbidopa.[83] By combining 5-HTP with carbidopa, increased bioavailability for brain penetration and decreased peripheral side effects would be expected because of reduced peripheral decarboxylation of 5-HTP to serotonin. However, in a double-blind study in healthy male volunteers, nausea and vomiting occurred dose-dependently as the most frequent side effects, resulting in dose-related dropout of 6.6% at 100 mg and 45.5% at 300 mg 5-HTP.[80]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Turner EH, Loftis JM, Blackwell AD. Serotonin a la carte: supplementation with the serotonin precursor 5-hydroxytryptophan. *Pharmacol Ther*. 2006;109:325–338.
2. Filippini GA, Costa CVL, Bertazzo A, eds. *Recent Advances in Tryptophan Research, Tryptophan and Serotonin Pathways*. New York, NY: Plenum; 1996.
3. Magnussen IE, Nielsen-Kudsk FL. Bioavailability and related pharmacokinetics in man of orally administered L-5-hydroxytryptophan in steady state. *Acta Pharmacol Toxicol (Copenh)*. 1980;46:257–262.
4. Magnussen I, Jensen TS, Rand JH, et al. Plasma accumulation of metabolism of orally administered single dose L-5-hydroxytryptophan in man. *Acta Pharmacol Toxicol*. 1981;49:184–189.
5. Van Praag HM, Lemus C. Monoamine precursors in the treatment of psychiatric disorders. In: Wurtman RJ, Wurtman JJ, eds. *Nutrition and the Brain*. Vol. 7. New York: Raven Press; 1977:89–139.
6. Van Praag HM. Studies on the mechanism of action of serotonin precursors in depression. *Psychopharmacol Bull*. 1984;20:599–602.
7. Das YT, Bagchi M, Bagchi D, et al. Safety of 5-hydroxy-L-tryptophan. *Toxicol Lett*. 2004;150:111–122.
8. Aviram M, Cogan U, Mokady S. Excessive dietary tryptophan enhances plasma lipid peroxidation in rats. *Atherosclerosis*. 1991;88:29–43.
9. Simic MG, al-Sheikhly M, Jovanovic SV. Inhibition of free radical processes by antioxidants—tryptophan and 5-hydroxytryptophan. *Bibl Nutr Dieta*. 1989;43:288–289.
10. Sano I. L-5-hydroxytryptophan-(L-5-HTP) therapy. *Folia Psychiatr Neurol Japan*. 1972;26:7–17. [Japanese].
11. Takahashi S, Kondo H, Kato N. Effect of L-5-hydroxytryptophan on brain monoamine metabolism and evaluation of its clinical effect in depressed patients. *J Psychiatr Res*. 1975;12:177–187.
12. Fujiwara J, Otsuki S. Subtype of affective psychosis classified by response on amine precursors and monoamine metabolism. *J Oral Pathol*. 1973;2:93–100.
13. Nakajima T, Kudo Y, Kaneko Z. Clinical evaluation of 5-hydroxy-L-tryptophan as an antidepressant drug. *Folia Psychiatr Neurol Jpn*. 1978;32:223–230.
14. Kaneko M, Kumashiro H, Takahashi Y, et al. L-5-HTP treatment and serum 5-HTP level after L-5-HTP loading on depressed patients. *Neuropsychobiology*. 1979;5:232–240.
15. van Praag HM, Korf J, Dols LC, et al. A pilot study of the predictive value of the probenecid test in application of 5-hydroxytryptophan as antidepressant. *Psychopharmacologia*. 1972;25:14–21.
16. van Praag HM. Management of depression with serotonin precursors. *Biol Psychiatry*. 1981;16:291–310.
17. Robie TR, Flora A. Anti-depressant chemotherapy, 1965. Rapid response to serotonin precursor potentiated by Ritalin. *Psychosomatics*. 1965;6:351–354.
18. Nardini M, De Stefano R, Iannuccelli M, et al. Treatment of depression with L-5-hydroxytryptophan combined with chlorimipramine, a double-blind study. *Int J Clin Pharmacol Res*. 1983;3:239–250.
19. Rousseau JJ. Effects of a levo-5-hydroxytryptophan-dihydroergocristine combination on depression and neuropsychic performance. A double-blind placebo-controlled clinical trial in elderly patients. *Clin Ther*. 1987;9:267–272.
20. Mendlewicz J, Youdim MB. Antidepressant potentiation of 5-hydroxytryptophan by L-deprenil in affective illness. *J Affect Disord*. 1980;2:137–146.
21. Alino JJ, Gutierrez JL, Iglesias ML. 5-Hydroxytryptophan (5-HTP) and a MAOI (nialamide) in the treatment of depressions. A double-blind controlled study. *Int Pharmacopsychiatry*. 1976;11:8–15.
22. Angst J, Woggon B, Schoepf J. The treatment of depression with L-5-hydroxytryptophan versus imipramine. Results of two open and one double-blind study. *Arch Psychiatr Nervenkr*. 1977;224:175–186.
23. Van Praag HM, Korf J. 5-Hydroxytryptophan as an antidepressant. The predictive value of the probenecid test. *J Nerv Ment Dis*. 1974;158:331–337.
24. Van Praag HM, Korf J. Serotonin metabolism in depression: clinical application of the probenecid test. *Int Pharmacopsychiatry*. 1974;9:35–51.
25. Van Praag HM. Central monoamine metabolism in depressions. I. Serotonin and related compounds. *Compr Psychiatry*. 1980;21:30–43.
26. Van Hiele JJ. L-5-hydroxytryptophan in depression. The first substitution therapy in psychiatry? *Neuropsychobiology*. 1980;6:230–240.
27. Kielholz P. Treatment for therapy-resistant depression. *Psychopathology*. 1986;19:194–200.
28. Kious BM, Sabic H, Sung YH, Kondo DG, Renshaw P. An open-label pilot study of combined augmentation with creatine monohydrate and 5-hydroxytryptophan for selective serotonin reuptake inhibitor- or serotonin-norepinephrine reuptake inhibitor-resistant depression in adult women. *J Clin Psychopharmacol*. 2017;37(5):578–583.
29. Byerley WF, Judd LL, Reimherr FW, et al. 5-Hydroxytryptophan. A review of its antidepressant efficacy and adverse effects. *J Clin Psychopharmacol*. 1987;7:127–137.
30. Poldinger W, Calanchini B, Schwarz W. A functional-dimensional approach to depression: serotonin deficiency as a target syndrome in a comparison of 5-hydroxytryptophan and fluvoxamine. *Psychopathology*. 1991;24:53–81.
31. Jangid P, Malik P, Singh P, Sharma M, Gulia AK. Comparative study of efficacy of l-5-hydroxytryptophan and fluoxetine in patients presenting with first depressive episode. *Asian J Psychiatr*. 2013;6(1):29–34.
32. Schruers K, van Diest R, Overbeek T, et al. Acute L-5-hydroxytryptophan administration inhibits carbon dioxide-induced panic in panic disorder patients. *Psychiatry Res*. 2002;113:237–243.
33. Weltzin TE, Fernstrom MH, Kaye WH. Serotonin and bulimia nervosa. *Nutr Rev*. 1994;52:399–408.
34. Weltzin TE, Fernstrom MH, Fernstrom JD, et al. Acute tryptophan depletion and increased food intake and irritability in bulimia nervosa. *Am J Psychiatry*. 1995;152:1668–1671.
35. Goodwin GM, Cowen PJ, Fairburn CG, et al. Plasma concentrations of tryptophan and dieting. *BMJ*. 1990;300:1499–1500.
36. Blundel JE, Leshem MB. The effect of 5-HTP on food intake and on the anorexic action of amphetamine and fenfluramine. *J Pharm Pharmacol*. 1975;27:31–37.
37. Ceci F, Cangiano C, Cairella M, et al. The effects of oral 5-hydroxytryptophan administration on feeding behavior in obese adult female subjects. *J Neural Transm*. 1989;76:109–117.
38. Cangiano C, Ceci F, Cairella M, et al. Effects of 5-hydroxytryptophan on eating behavior and adherence to dietary prescriptions in obese adult subjects. *Adv Exp Med Biol*. 1991;294:591–593.
39. Cangiano C, Ceci F, Cascino A, et al. Eating behavior and adherence to dietary prescriptions in obese adult subjects treated with 5-hydroxytryptophan. *Am J Clin Nutr*. 1992;56:863–867.
40. Cangiano C, Laviano A, Del Ben M, et al. Effects of oral 5-hydroxy-tryptophan on energy intake and macronutrient selection in non-insulin dependent diabetic patients. *Int J Obes Relat Metab Disord*. 1998;22:648–654.
41. Guilleminault C, Cathala HP, Castaigne P. Effects of 5-HTP on sleep of a patient with brain stem lesion. *Electroencephalog Clin Neurophysiol*. 1973;34:177–184.
42. Wyatt RJ, Zarcone J, Engelman K. Effects of 5-hydroxytryptophan on the sleep of normal human subjects. *Electroencephalogr Clin Neurophysiol*. 1971;30:505–509.
43. Autret A, Minz M, Bussel B, et al. Human sleep and 5-HTP. Effects of repeated high doses and of association with benserazide. *Electroencephalogr Clin Neurophysiol*. 1976;41:408–413.
44. Zarcone Jr VP, Hoddes E. Effects of 5-hydroxytryptophan on fragmentation of REM sleep in alcoholics. *Am J Psychiatry*. 1975;132:74–76.
45. Soulairac A, Lambinet H. Effect of 5-hydroxytryptophan, a serotonin precursor, on sleep disorders. *Ann Med Psychol (Paris)*. 1977;1:792–798. [French].
46. Sicuteri F. Hypothesis: migraine, a central biochemical dysnociception. *Headache*. 1976;16:145–149.
47. Bruni O, Ferri R, Miano S, et al. L-5-Hydroxytryptophan treatment of sleep terrors in children. *Eur J Pediatr*. 2004;163:402–407.
48. Titus F, Davalos A, Alom J, et al. 5-Hydroxytryptophan versus methysergide in the prophylaxis of migraine. Randomized clinical trial. *Eur Neurol*. 1986;25:327–329.

49. Bono G, Criscuoli M, Martignoni E, et al. Serotonin precursors in migraine prophylaxis. *Adv Neurol*. 1982;33:357–363.
50. Maissen CP, Ludin HP. Comparison of the effect of 5-hydroxytryptophan and propranolol in the interval treatment of migraine. *Schweiz Med Wochenschr*. 1991;121:1585–1590. [German].
51. Ribeiro CA. L-5-Hydroxytryptophan in the prophylaxis of chronic tension-type headache: a double-blind, randomized, placebo-controlled study. For the Portuguese Head Society. *Headache*. 2000;40:451–456.
52. De Giorgis G, Miletto R, Iannuccelli M, et al. Headache in association with sleep disorders in children. A psychodiagnostic evaluation and controlled clinical study—L-5-HTP versus placebo. *Drugs Exp Clin Res*. 1987;13:425–433.
53. Santucci M, Cortelli P, Rossi PG, et al. L-5-hydroxytryptophan versus placebo in childhood migraine prophylaxis: a double-blind crossover study. *Cephalalgia*. 1986;6:155–157.
54. Longo G, Rudoi I, Iannuccelli M. Treatment of essential headache in developmental age with L-5-HTP (cross over double-blind study versus placebo). *Pediatr Med Chir*. 1984;6:241–245. [Italian].
55. Fettes, Gawel M, Kuzniak S. Endorphin levels in headache syndromes. *Headache*. 1985;25:37–39.
56. Leone M, Sacerdote P, D'Amico, et al. Beta-endorphin levels are reduced in peripheral mononuclear cells of cluster headache patients. *Cephalalgia*. 1993;13:413–416.
57. Battistella PA, Bordin A, Cernetti R, et al. Beta-endorphin in plasma and monocytes in juvenile headache. *Headache*. 1996;36:91–94.
58. Sicuteri F. The ingestion of serotonin precursors (L-5-hydroxytryptophan and L-tryptophan) improves migraine. *Headache*. 1973;13:19–22.
59. Nicolodi M, Sicuteri F. Fibromyalgia and migraine, two faces of the same mechanism. Serotonin as the common clue for pathogenesis and therapy. *Adv Exp Med Biol*. 1996;398:373–379.
60. Nicolodi M, Sicuteri F. Eosinophilia myalgia syndrome. The role of contaminants, the role of serotonergic metabolism set up. *Adv Exp Med Biol*. 1996;398:351–357.
61. Caruso I, Sarzi Puttini P, Cazzola M, et al. Double-blind study of 5-hydroxytryptophan versus placebo in the treatment of primary fibromyalgia syndrome. *J Int Med Res*. 1990;18:201–209.
62. Puttini PS, Caruso I. Primary fibromyalgia syndrome and 5-hydroxy-L-tryptophan: 90-day open study. *J Int Med Res*. 1992;20:182–189.
63. White KP, Harth M. An analytical review of 24 controlled clinical trials for fibromyalgia syndrome (FMS). *Pain*. 1996;64:211–219.
64. Affleck G, Urrows S, Tennen H, et al. Sequential daily relations of sleep, pain intensity, and attention to pain among women with fibromyalgia. *Pain*. 1996;68:363–368.
65. Chase TN, Ng LK, Watanabe AM. Parkinson's disease. Modification by 5-hydroxytryptophan. *Neurology*. 1972;22:479–484.
66. Mayeux R, Stern Y, Sano M, et al. The relationship of serotonin to depression in Parkinson's disease. *Mov Disord*. 1988;3:237–244.
67. Bastard J, Truelle JL, Emile J. Effectiveness of 5 hydroxytryptophan in Parkinson's disease. *Nouv Presse Med*. 1976;5:1836–1837. [French].
68. Sano I, Taniguchi K. L-5-hydroxytryptophan (L-5-HTP) therapy of Parkinson's disease. *Munch Med Wochenschr*. 1972;114:1717–1719. [German].
69. Pranzatelli MR, Tate E, Galvan I, et al. A controlled trial of 5-hydroxy-L-tryptophan for ataxia in progressive myoclonus epilepsy. *Clin Neurol Neurosurg*. 1996;98:161–164.
70. Trouillas P, Serratrice G, Laplane D, et al. Levorotatory form of 5-hydroxytryptophan in Friedreich's ataxia. Results of a double-blind drug-placebo cooperative study. *Arch Neurol*. 1995;52:456–460.
71. Wessel K, Hermsdorfer J, Deger K, et al. Double-blind crossover study with levorotatory form of hydroxytryptophan in patients with degenerative cerebellar diseases. *Arch Neurol*. 1995;52:451–455.
72. Pranzatelli MR, Tate E, Huang Y, et al. Neuropharmacology of progressive myoclonus epilepsy. Response to 5-hydroxy-L-tryptophan. *Epilepsia*. 1995;36:783–791.
73. Trouillas P, Brudon F, Adeleine P. Improvement of cerebellar ataxia with levorotatory form of 5-hydroxytryptophan. *Arch Neurol*. 1988;45:1217–1222.
74. Van Woert MH. Myoclonus and L-5-hydroxytryptophan (L-5-HTP). *Prog Clin Biol Res*. 1983;127:43–52.
75. Keszthelyi D, Troost FJ, Jonkers DM, et al. Serotonergic reinforcement of intestinal barrier function is impaired in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2014 August;40(4):392–402.
76. Kilbourne EM. Eosinophilia-myalgia syndrome: coming to grips with a new illness. *Epidemiologic Rev*. 1992;14:16–36.
77. Kilbourne EM, Philen RM, Kamb ML. Tryptophan produced by Showa Denko and epidemic eosinophilia-myalgia syndrome. *J Rheumatol Suppl*. 1996;46:81–88.
78. Sternberg EM, Van Woert MH, Young SN, et al. Development of a scleroderma-like illness during therapy with L-5-hydroxytryptophan and carbidopa. *N Engl J Med*. 1980;303:782–787.
79. Klarskov K, Johnson KL, Benson LM, et al. Eosinophilia-myalgia syndrome case-associated contaminants in commercially available 5-hydroxytryptophan. *Adv Exp Med Biol*. 1999;467:461–468.
80. Smarius LJ, Jacobs GE, Hoeberechts-Lefrandt DH, et al. Pharmacology of rising oral doses of 5-hydroxytryptophan with carbidopa. *J Psychopharmacol*. 2008;22:426–433.
81. Nicolodi M, Sicuteri F. Eosinophilia myalgia syndrome. The role of contaminants, the role of serotonergic metabolism set up. *Adv Exp Med Biol*. 1996;398:351–357.
82. Michelson D, Page SW, Casey R, et al. An eosinophilia-myalgia syndrome related disorder associated with exposure to L-5-hydroxytryptophan. *J Rheumatol*. 1994;21:2261–2265.
83. Gijsman HJ, van Gerven JM, de Kam ML, et al. Placebo-controlled comparison of three dose-regimens of 5-hydroxytryptophan challenge test in healthy volunteers. *J Clin Psychopharmacol*. 2002;22:183–189.

88

Hypericum perforatum (St. John's Wort)

Peter B. Bongiorno, ND, MSAc, LAc

OUTLINE

Hypericum perforatum (family: Hypericaceae)
Common names: St. John's wort, Klamath weed, hypericum

GENERAL DESCRIPTION

Likely the most well-scientifically studied botanical of all time, St. John's wort *(Hypericum perforatum)* is a shrubby perennial plant with numerous bright yellow flowers (Fig. 88.1). It is commonly found in dry, gravelly soils, fields, and sunny places. St. John's wort is native to many parts of the world, including Europe, Asia, and the United States. It grows especially well in northern California and southern Oregon.[1]

The plant is glabrous throughout, green or sometimes glaucescent; the stems are erect, branched at the top, and 30 to 100 cm long. The leaves are (1) oval or elliptic; (2) oblong-ovate or rather narrow, oblong-linear, subobtuse, and flat; or (3) more or less revolute—marginated with numerous pellucid and a few black granular dots. The yellow flowers are numerous, forming a broad, paniculate, almost corymbose inflorescence, 7 to 11 cm long and 5 to 11 cm broad. The lanceolate bracts are 0.5 cm long and acute. The calyx is deeply parted, 5 mm long, and about two to three times shorter than the corolla. The sepals are lanceolate or narrow lanceolate 4 to 5 mm long, 1 mm broad, as long as the ovary, acute or acuminate, sparingly furnished with black oval dots, with a smooth or sparsely toothed margin. The petals are oblong to oblong-elliptic, 1.2 to 1.5 cm long and 0.5 to 0.6 cm broad, with or without numerous black granular dots and lines on the margin in the upper part, whereas the surface is full of yellow glandular dots, thin lines, and stripes. The three-bundled stamens are numerous; the ovary is ovoid, 3 to 5 mm long. The seed is 1 mm long, cylindric, brown, and minutely pitted longitudinally.[2]

The whole plant is used medicinally. Harvesting time is generally July through August. The plant must be dried immediately to prevent degradation of active principles.[3]

CHEMICAL COMPOSITION

Hypericum contains the acylphoroglucinol hyperforin, the naphthodianthones (hypericin and pseudohypericin, isophypericin, and protohypericin), flavonoids (such as hyperocide, quercitrin, and isoquecitrin), and numerous other compounds.[4] The most studied compounds are hypericin (Fig. 88.2), pseudohypericin, and hyperforin. These compounds are typically found in low concentrations, ranging from 0.0095% to 0.466% in the leaves and as much as 0.24% in the flowers.

Given its pleiotropic actions, researchers have been interested in the other chemical constituents (especially the various flavonoids and xanthones). The interest in these other components stems largely from pharmacological studies with commercially available extracts demonstrating effects and benefits beyond hypericin, pseudohypericin, and hyperforin. Studies looking at multifractionated extracts with higher levels of botanical components show better clinical outcomes.[5] For example, amentoflavone was shown to bind to benzodiazepine receptors and to act as a modulator of γ-aminobutyric acid, which may be a major anxiolytic mechanism.[2] The active components include[1]:

- Flavonoids (flowers 16%, leaves 12%, and whole herb 9%)
- Xanthones
- Phenolic carboxylic acids (caffeic, chlorogenic, ferulic, and gentisic acids)
- Essential oils (whole herb content 0.13%)
- Carotenoids
- Alkanes
- Phloroglucinol derivatives
- Phytosterols
- Medium-chain fatty acid alcohols

Fig. 88.1 *Hypericum perforatum.* (From iStock.com/alexmak72427.)

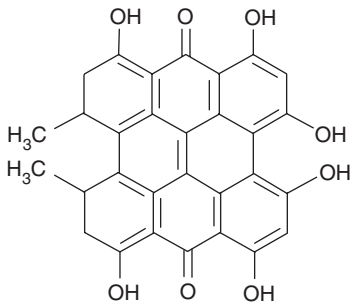


Fig. 88.2 Hypericin.

HISTORY AND FOLK USE

St. John's wort has a long history of folk use. Dioscorides, the foremost physician of ancient Greece, as well as Pliny and Hippocrates, used St. John's wort in the treatment of many illnesses. Its Latin name, *H. perforatum,* is derived from Greek and means "over an apparition," a reference to the belief that the herb was so obnoxious to evil spirits that a whiff of it would cause them to depart.

The naming of St. John's wort has its origins in folk traditions. One claims that the red spots, symbolic of the blood of St. John, appeared on the leaves of the plant on the anniversary of the saint's beheading. Another comes from a common medieval belief that if one slept with a piece of the plant under a pillow on St. John's Eve, "the Saint would appear in a dream, give his blessing, and prevent one from dying during the following year."

Many people from the time of the ancient Greeks through the Middle Ages believed St. John's wort to have magical powers. Recent research on St. John's wort appears to offer some explanation of this "magical" power. On the basis of a long history of use as a mood-elevating substance and preliminary in vitro experiments and clinical studies, in 1984, the German Commission E permitted the medicinal use of St. John's wort for depression, anxiety, or nervous excitement.

The Commission E evaluates efficacy of herbal medicines on the basis of a doctrine of reasonable certainty, in contrast to the U.S. Food and Drug Administration's doctrine of absolute proof. Herbal products can be marketed in Germany with drug claims if they are proven to be safe and effective. Whether the herbal product is available by prescription or over the counter is based on its application and safety of use. Herbal products sold in German pharmacies are reimbursed by insurance if they are prescribed by a physician.

Because the German system allowed companies to market their products according to the guidelines of the Commission E, many companies achieved commercial success allowing them to fund the necessary research to gain greater acceptance within mainstream conventional medicine. St. John's wort extract in the treatment of depression is a perfect case in point to illustrate how the Commission E monographs fueled the science of botanical medicine. For example, it was originally thought that hypericin acted as an inhibitor of the enzyme monoamine oxidase (MAO), thereby resulting in the increase of central nervous system (CNS) monoamines such as serotonin and dopamine. However, newer research indicates that St. John's wort possesses no in vivo inhibition of MAO. Apparently, the antidepressant activities are related more to modulating the relationship between the immune system and mood, as well as by inhibiting serotonin reuptake. In addition, it appears that although hypericin is an important marker that correlates with clinical efficacy, other compounds such as flavonoids are thought to play a major role in the pharmacology of St. John's wort.

More than 27 double-blind randomized trials, involving more than 3800 patients with mild to moderately severe depression, showed that St. John's wort extracts standardized for hypericin had excellent results in the treatment in mild to moderate depression, with far fewer side effects than standard antidepressant medications.[6] For patients with preexisting conductive heart dysfunction or elderly patients, high-dose hypericum extract is safer with regard to cardiac function than tricyclic antidepressants.[7]

PHARMACOLOGY

St. John's wort extracts (primarily of the flowering tops) have shown a wide variety of effects in experimental and clinical studies. Some of the activities demonstrated include the following[1]:

- Antidepressant effects
- Antiviral effects
- Antibiotic effects
- Increased healing of wounds and burns

Antidepressant Activity

As a testament to the wise complexity of the natural world, hypericum has a number of pleiotropic effects that are probably, in a synergistic fashion, responsible for its antidepressant activity. These include, but are not limited to, MAO A and B inhibition, modulation of IL-6 activity, serotonin reuptake inhibition, sigma 1-receptor activation, gamma amino butyric acid (GABA) inhibition, and hypothalamic gene transcription.

MAO Inhibition

Among the different biological hypotheses for depression, the biogenic amine hypothesis is the most widely accepted. This hypothesis suggests that depression is the result of a deficiency in function of the biogenic amines (e.g., serotonin, catecholamines, dopamine). These neurotransmitters are stored in granules within neurons. After stimulation of the neurons, these neurotransmitters are released into the synaptic cleft via exocytosis. After binding to postsynaptic receptors, the neurotransmitters are either taken up again and restored in the vesicles or they are catabolized by the enzymes MAO or catechol-O-methyltransferase

(COMT). Most antidepressant drugs increase the availability of these amines, particularly serotonin, by either inhibiting the reuptake or blocking MAO.

As stated earlier, initial studies indicated that St. John's wort extract's antidepressant action was based on the ability of crude hypericin preparations to inhibit both types A and B MAO.[8,9] As a result of this inhibition, there is an increase in the level of neurotransmitters within the brain that maintain normal mood and emotional stability, including serotonin, catecholamines, and dopamine. These preliminary results identified hypericin as the supposed active constituent. However, later chemical analysis of these crude hypericin preparations identified the active content as being as much as 20% of St. John's wort's other constituents, with flavonoids being the most important.[1] In other words, it is unknown to what extent hypericin or the flavonoids individually really contribute to any MAO inhibition.

A study was conducted to better understand the influence of hypericin, hypericum total extract, and hypericum fractions on the activity of MAO.[10] An inhibition of MAO was shown using the following concentrations:

- Hypericin to 10^{-3} mol/L
- Hypericum total extract to 10^{-4} mol/L
- One extract fraction up to 10^{-5} mol/L

The key result from this study, as well as in another in vitro/ex vivo study, was the demonstration that the concentrations of inhibition shown, particularly with regard to the inhibition of MAO activity, were likely insufficient to explain the clinically proven antidepressive effect of St. John's wort extract.[10,11] Therefore additional mechanisms are likely responsible for these clinical benefits. The same group also looked at effect on COMT and found no inhibition either.[10]

Modulation of IL-6 Activity

The modulating effect of St. John's wort extract on IL-6 is the most interesting, as it proposes a mechanism by which St. John's wort interacts with the link between the immune system and mood. The cytokine IL-6 is heavily involved in the communication between cells within and outside the immune system. With regard to the nervous system, IL-6 is known to modulate hypothalamic-pituitary-end organ axes, especially the hypothalamic-pituitary-adrenal axis. The hypothesis is that an elevation in IL-6 results in activation of the hypothalamic-pituitary-adrenal axis, leading to elevations in corticotropin-releasing hormone and other adrenal regulatory hormones—hallmark features in depression. St. John's wort extract showed an ability to reduce IL-6 levels; therefore this action may explain the clinical effectiveness of St. John's wort extract.[12]

Animal studies comparing the effects of hypericum in mice who produce normal IL-6 levels versus IL-6 knockout mice (those with no IL-6 expression) found higher levels of serotonin produced in the former group.[13] An in vitro study looked at blood in five healthy volunteers and four depressive patients. The release of IL-6, IL-1β, and tumor necrosis factor-α was measured quantitatively after an incubation time of 24 hours on microtiter plates. A massive suppression of IL-6 release was found with samples exposed to phytohemagglutinin-stimulated St. John's wort extract.[12] If these effects can be duplicated in vivo, it would provide a mechanism by which St. John's wort extract modulates release of corticotropin-releasing hormone and, subsequently, mood.

Serotonin Reuptake Inhibition

St. John's wort extract was also shown to inhibit the reuptake of serotonin in a similar fashion to drugs like fluoxetine (Prozac), paroxetine (Paxil), and sertraline (Zoloft). The study that demonstrated a 50% serotonin reuptake inhibition used the 0.3% hypericin content standardized extract at a concentration of 6.2 mg/mL and did not attempt to identify the active inhibitors. The authors of the study concluded that "the antidepressant activity of hypericum extract is due to inhibition of serotonin uptake by postsynaptic receptors." However, results were conflicting with regard to the effect of acute doses of St. John's wort extracts on the serotonergic system in rodents.[14] A marked increase of both serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) was reported in rat brain cortex. Other researchers reported high 5-HIAA and serotonin levels in the mouse hypothalamus and hippocampus, but not in the cerebral cortex, where only 5-HIAA increased. However, other research found no significant changes in either serotonin or 5-HIAA after a schedule of treatment during the forced swimming test. One study of the rat locus coeruleus showed that systemic hyperforin induced a lasting and marked (100%–200%) increase in the extracellular concentrations of serotonin, but not 5-HIAA.[15]

Sigma 1-Receptor Activation and GABA Inhibition

There is growing research that St. John's wort may affect function via Sigma$_1$ σ receptors and GABA receptors. σ receptors were shown to play an important role in antidepressive effects because selective σ_1-receptor agonists, as well as typical antidepressants, reduced the immobility time in the forced swimming and tail suspension tests.[16] Research demonstrated that the pretreatment of rats with St. John's wort extracts or hyperforin trimethoxybenzoate could block other ligand binding to σ-receptors. The antidepressant-like activity of hyperforin trimethoxybenzoate was completely antagonized by pretreating rats with BD 1047, a selective σ_1 antagonist. These results, together with the previous observation that agonists at σ_1 receptors are active in antidepressant models in rats, suggest that St. John's wort's antidepressant effects might be mediated in part by an indirect action on σ-receptors.[14] Looking at lung vasculature in cats, St. John's wort showed a GABA receptor vasodepressant effect.[17] Other in vitro studies have also shown a GABA receptor inhibition by hypericum. This finding may indicate that GABA inhibition may also be partially responsible for its antidepressant action.

Hypothalamic Gene Transcription

A fascinating study evaluated the effect of both St. John's wort and antidepressant imipramine on gene transcription in the rat hypothalamus. The depressed patient's hypothalamus is known to manifest changes associated with food intake, decreased libido, and abnormal circadian rhythms. This research found a significant correlation of six genes directly modulated by both these test compounds. The probability of this occurring by chance was 1.14×10^{-23}. The functions of these specific genes include protein synthesis and degradation, cellular scaffolding and intracellular transport, mitochondrial and glycolytic energy metabolism, and cellular signaling through modulation of calcium-binding proteins. Although the implication of this research is not totally clear, the authors postulated that these modulated gene functions might be related to immune and inflammatory downregulation with neuronal protection from cellular injury.[18]

Amentoflavone, one of the many flavone contents in St. John's wort, was shown to cross the blood–brain barrier by passive diffusion.[19] This might account for the ability of this botanical to travel into the brain and affect CNS gene transcription. Nevertheless, one point regarding the probable synergistic effects of the St. John's wort constituents should be underscored: given the multitude of active components in this botanical medicine, it is likely that synergistic sites of action outside the CNS, in accordance with CNS effects, account for the complete and effective global actions of St. John's wort.

Extracts of St. John's wort were tested in various animal models designed to study its antidepressant effects. In these studies, St. John's wort extract was found to enhance the exploratory activity of mice in a foreign environment, extend the narcotic sleeping time in a dose-dependent fashion, antagonize the effects of reserpine, and decrease aggressive behavior in socially isolated male mice.[20] These activities are consistent with the expected effects of antidepressant compounds and appear to be the result of increased monoamine activity.

Antiviral Activity

In vitro studies showed that hypericin and pseudohypericin exhibit strong antiviral activity against herpes simplex virus I and II, as well as influenza types A and B and vesicular stomatitis virus.[21] These compounds also demonstrated remarkable antiviral activity against Epstein-Barr virus.[22]

Researchers from New York University Medical Center and the Weizmann Institute of Science in Israel generated a tremendous amount of excitement when they demonstrated the antiretroviral activity of hypericin and pseudohypericin.[23] This preliminary study examined the effect of these compounds on two animal retroviruses, Friend leukemia virus and radiation leukemia virus, both in vitro and in vivo (in mice). The researchers found the effective dose of hypericin in mice to be 1.5 to 2 mg/mL confirming the potency of a relatively small dose of this herbal extract.

Two possible mechanisms were described to explain the antiviral activity of both hypericin and pseudohypericin.[24] First was inhibition of assembly or processing of intact virions from infected cells—released virions contain no detectable activity of reverse transcriptase. Second, these compounds also directly inactivate mature and properly assembled retroviruses.

The antiviral activity of hypericin against HIV appears to require interaction with light to activate the hypericin.[25,26] Another requirement is sufficient concentrations, as entry of hypericin into infected cells depends on the concentration of hypericin in the blood. At sufficient concentrations, hypericin incubated with HIV-infected whole blood decreases culturable HIV, indicating significant antiviral activity.[27]

Antibacterial Activity

St. John's wort extracts have broad-spectrum antimicrobial activity against both gram-negative and gram-positive bacteria. The organisms studied included *Staphylococcus aureus, Streptococcus mutans, Proteus vulgaris, Escherichia coli,* and *Pseudomonas aeruginosa.*[28]

Pharmacokinetic Studies

Pharmacokinetic studies indicate that hyperforin, as well as hypericin and pseudohypericin, have adequate absorption to produce serum levels that correspond to the levels showing benefit in in vitro studies.[29] However, hyperforin is the only ingredient of *H. perforatum* that could be determined in the brains of rodents after oral administration of alcoholic extracts. This result and others seem to indicate that it may be the chief constituent responsible for the antidepressant effects of *H. perforatum.*

CLINICAL APPLICATIONS

As the only truly recognized herbal treatment for the condition, St. John's wort is most recognized for the treatment of depression. It can also be of benefit in the treatment of chronic viral infections, restless legs, and topically for various skin and pain conditions.

Depression

Extracts of St. John's wort standardized for hypericin content (most studies used the 0.3% hypericin content extract) have significant support in the treatment of mild to moderate depression. The official German Commission E monograph for St. John's wort lists psychovegetative disturbances, depressive states, fear, and nervous disturbances as clinical indications for St. John's wort.[30]

St. John's wort extract improved many psychological symptoms, including the following:

- Depression
- Anxiety
- Apathy
- Sleep disturbances
- Insomnia
- Anorexia
- Feelings of worthlessness

Even more impressive is that St. John's wort extract was able to achieve these benefits without producing significant side effects.

The history of meta-analyzing hypericum studies has been full of tumult and contradiction. One meta-analysis in 1996 of 23 randomized trials included 1757 outpatients with mainly mild or moderately severe depressive symptoms. It showed that hypericum extracts were significantly superior to placebo and as effective as standard antidepressants. This analysis revealed 0.8% dropouts for side effects with hypericum compared with 3.0% for standard antidepressant drugs. In this research, side effects occurred in 19.8% of patients on hypericum and 52.8% of patients on standard antidepressants.[31]

However, there were two well-publicized clinical studies claiming that hypericum was ineffective in treating depression.[32] One 8-week trial employed suboptimal doses of hypericum, using 900 mg/day for patients with severe depression. If there was no response, doses were increased to only 1200 mg/day. In a previous severe depression study, patients improved significantly on hypericum, compared with placebo and the antidepressant drug imipramine, on a dose of 1800 mg/day.[33,34] Given that the trial was funded by a company that manufactures antidepressant medications, and in light of a strong history of positive hypericum trials, it is doubtful that this accurately reflects the clinical ability of such a well-studied botanical.

A second 8-week study from 2002 made a similar suboptimal dosing error, and subsequently deemed hypericum to be ineffective. A valuable note in this study was that the comparison drug, sertraline (with a much stronger side effect profile than hypericum), was not any more effective than hypericum or the placebo.[35] The study generated considerable media attention as showing SJW is ineffective—ignoring or underplaying the other finding that the standard antidepressant was also ineffective.

A third meta-analysis in 2008 included only trials from 1995 to 2006 that were randomized and double-blinded, involving patients with major depression. Extracts of hypericum were compared with placebo or standard antidepressants, including fluoxetine, sertraline, imipramine, citalopram, paroxetine, and amitriptyline. In all, 29 studies involving 5489 patients were analyzed. Hypericum extracts tested in the trials were superior to placebo and equally effective as standard antidepressants. Patients given hypericum extracts dropped out of trials less frequently because of lower adverse effects than those given standard antidepressants. It was concluded that the results "imply that an attempt of treating mild to moderate major depression with one of the hypericum preparations positively tested in clinical trials is clearly justified," but added that evidence was "still insufficient to draw conclusions about the efficacy of hypericum for treating severe major depression." The authors cited that the mild physical side effects associated with hypericum might be enhancing the placebo effect. A second concern was that hypericum was popular in Germany, so the more robust effects in the studies from German-speaking countries might be attributable to an "allegiance" effect. A third concern was that, although positive, the larger-scale trials used in the analysis

produced overall smaller effects.[36] Another meta-analysis published in 2016 was consistent with past reviews. This review of randomized controlled trials examined 35 studies, evaluating a total of 6993 patients looking at hypericum extracts including 0.3% hypericin and 1% to 4% hyperforin. The herb exhibited over 50% greater response compared with placebo, with an effect at least as good as antidepressants and with significantly fewer adverse events.[37]

Hypericum has been compared head-to-head with leading antidepressant medications multiple times.[38,39] In an example randomized, controlled, double-blind trial, 70 patients with mild to moderate depression received one tablet of either hypericum extract or fluoxetine twice a day for 6 weeks. As evaluated by the 17-item Hamilton Rating Scale for Depression (HAMD), the von Zerssen depression scale (DS), and patients' response, there were significant decreases (P <0.001) in symptoms in the St. John's wort group (50%) and in the fluoxetine group (58%) on their HAMD scores. The hypericum extract achieved 83% of the efficacy of fluoxetine on the HAMD and 78% on the DS. Assessments by physicians and patients indicated considerable improvement with no between-treatment differences.[38] The authors concluded that the hypericum tested in this study was therapeutically equivalent to fluoxetine and that it was a reasonable alternative to synthetic antidepressants.

Another recent meta-analysis of St. John's wort reviewed 27 clinical trials with a total of 3808 patients comparing the use of St. John's wort and selective serotonin reuptake inhibitor (SSRI) medications. Study durations varied from a 4- to 12-week period, and revealed St. John's wort had comparable efficacy. Even more, there was also a significantly lower discontinuation and dropout rate compared with the drugs. Safety compared with SSRIs showed significantly lower discontinuation of the herb.[40]

Hypericum extract was similarly tested and proven at least as effective as sertraline in the treatment of mild to moderate depression in a small group of outpatients.[39] Efficacy and tolerability of hypericum extract was also compared with imipramine and was found equivalent to the drug in treating mild to moderate depression. In addition, as expected, patients tolerated the hypericum better.[41]

Although preliminary, St. John's wort may be a safe recommendation during pregnancy and postpartum depression (see "Pregnancy" and "Nursing" for more information). St. John's wort has also been studied in children. Three open-label trials in depressed children found benefit with minimal side effects. The first study of 101 children, less than 12 years of age, were administered 300 to 1800 mg/day at a standardized dose of 900 mcg hypericin.[42] A second, 8-week open-label trial in 33 youths from 6 to 16 years of age used a dose of 150 to 900 mg/day, and enjoyed an 80% response rate.[43] A third trial of 26 adolescents given 300 mg three times a day had an 82% response rate in the 11 patients who completed the study. However, in this study, 15 patients withdrew either because of noncompliance or worsening of symptomology.[44,45]

Seasonal Affective Disorder

Seasonal affective disorder (SAD) represents a subgroup of major depression with a regular occurrence of symptoms in autumn/winter and full remission in spring/summer (see Chapter 142 Affective Disorders for more discussion). Light therapy has become the standard treatment for this type of depression. Apart from this, pharmacotherapy with antidepressants also seems to provide an improvement of SAD symptoms.[46] SAD patients with major depression were randomized in a 4-week treatment study with 900 mg/day of St. John's wort extract (0.3% hypericin content) combined with either bright (3000 lux; n = 10) or dim light (less than 300 lux therapy). The significant reduction in the Hamilton Depression Scale in both groups (72% and 60%, respectively) indicated that St. John's wort extract might benefit patients with SAD as a sole therapeutic agent, as well as in combination with light therapy.

Insomnia

St. John's wort was shown to improve sleep quality and well-being in healthy elderly subjects.[47] With antidepressant drugs, particularly tricyclic antidepressants and MAO inhibitors, rapid eye movement sleep is reduced. St. John's wort did not interfere with rapid eye movement sleep like other antidepressants and was shown to increase the intensity of deep sleep during the total sleeping period as demonstrated by brainwave studies. Although St. John's wort improved sleep quality, it did not act as a sedative (i.e., it did not reduce sleep onset), nor did it change total sleep duration.

Mental Function

One of the most interesting comparative studies was a double-blind study in which St. John's wort extract (0.3% hypericin content) was compared with maprotiline in 24 healthy volunteers by measuring resting brainwave (electroencephalography) tracings and mental activity (visual and acoustic evoked potentials).[48] Interpretation of the differences in reactions indicated that, unlike maprotiline, which interferes with mental function, St. John's wort actually improved memory and other mental activities. Long-term administration of St. John's wort in rats improved learning and spatial memory with significant changes in the content of monoamines in several brain regions.[49]

Acquired Immunodeficiency Syndrome and Other Viral Infections

St. John's wort may be of value in the treatment of AIDS. In response to in vitro and animal studies, many AIDS patients began self-administering St. John's wort where patients reported feeling better with a more positive outlook, more energy, and less fatigue.[50,51] Hypericum seems to suppresses HIV-1 expression and inhibits its replication.[52] A St. John's wort protein called P27SJ has also been shown to minimize HIV-1 induced neurotoxicity in the brain by inhibiting viral replication and calming inflammatory response.[53] Small studies looking at AZT treatment with St. John's showed modest T-cell count increases of 13% from baseline. Another open pilot study of 18 HIV patients showed stable or even increasing counts of absolute T-helper cells over the 40 months, with lowered opportunistic infections[54] (for further information about HIV/AIDS, see Chapter 173).[55,56] St. John's wort may have negative reaction with protease inhibitor drugs and simultaneous use with HIV medications should be avoided.

Research suggests that St. John's wort may be a useful adjunctive treatment for herpes simplex, mononucleosis, and influenza, although further human studies are necessary to establish the optimal dosage of the standardized extract. Combined with its antidepressant activity, St. John's wort also appears to be a promising treatment for chronic fatigue syndrome.

Restless Legs Syndrome

Restless legs syndrome (RLS), also known as Willis-Ekbom disease, was treated with a low dose (300 mg qd) of St. John's wort extract in 21 patients with a concentrated extract for 3 months. Seventeen of these patients found significant relief. Researchers suggested it decreased thyroid levels by increasing thyroid hormone degradation through the CYP3A4 enzyme isoform and, perhaps, by increasing stimulates the expression of P-glycoprotein.[57]

Topical Application

St. John's wort has a historical use as an aid in wound healing and dermatological support. Research demonstrated antibacterial and wound healing activity. Although research is relatively scant,

hyperforin encourages growth and differentiation of keratinocytes, while hypericin acts as a photosensitizer that can be employed for selective treatment of nonmelanoma skin cancer.[58] St. John's wort preparations have also been used in burns, as a sunscreen, and in the treatment of muscular pain.[3] Oil-based preparations are preferred for topical applications.

DOSAGE

The dosages of St. John's wort preparations are based on their hypericin content. The overwhelming majority of the studies in depression used St. John's wort extract standardized to contain 0.3% hypericin and 3% to 5% hyperforin. This extract is produced via an extraction with 80% methanol (which is subsequently removed). Although hypericin and hyperforin are key components, this extract is composed of a wide range of compounds constituting the remaining 95% of the extract. Manufacturers of these standardized extracts employ high-performance liquid chromatography techniques to identify not only the hypericin, pseudohypericin, and hyperforin, but also related compounds, flavonoid components, xanthones, cinnamic acid, and several other key components. The point is that although the dosage is based on hypericin levels, assuring appropriate levels of these other constituents is also vitally important.

Dosing of three times a day is recommended because of its short half-life. To achieve the benefits noted in the clinical trials, it is difficult to recommend any other forms beyond standardized extracts. Nonetheless, here are dosage recommendations for various forms of St. John's wort:

- Dried flowers: 2 to 4 g three times a day
- Tincture (1:5): 3 to 6 mL three times a day
- Fluid extract (1:1): 1 to 2 mL three times a day
- Standardized solid (dry-powdered) extract (0.3% hypericin and 3%–5% hyperforin): 900 to 1800 mg a day

SAFETY/TOXICITY

Although the conventional medical community focuses on concerns for herbal and natural remedies in terms of safety, a balanced look at botanicals suggests both benefits and risk. Looking at St. John's wort, there seems to be an exemplary beneficial profile and definite concerns in terms of photosensitivity and drug interactions. There are also some beneficial drug interactions to consider. The literature on pregnancy and breast feeding suggests possible safety, but there is not enough literature to be clear.

The side effect profile of hypericum extract is minor, and is safer compared with the well-known side effects of antidepressant medications.[59] Also notable, there seems to be no memory impairment, an advantage for senior patients over conventional drugs.[60] St. John's wort also seems to be less toxic on the wallet: an Australian group studied the economic effects of St. John's wort versus antidepressants, and found the herbal remedy to have reduced cost profile compared with generic antidepressants with advantages as a result of its low incidence of adverse effects.[61]

Serotonin Syndrome Concern

A main concern in the literature is regarding excessive levels of serotonin called "serotonin syndrome" where symptoms can include mental status changes (e.g., agitation, hallucinations, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, and hyperthermia), neuromuscular aberrations (e.g., hyperreflexia and incoordination), and/or gastrointestinal tract symptoms (e.g., nausea, vomiting, and diarrhea). Severe cases can resemble neuroleptic malignant syndrome, which includes hyperthermia, muscle rigidity, autonomic instability with possible rapid fluctuation of vital signs, and mental status changes. An example of this was reported in a study of four elderly patients who ostensibly developed serotonin syndrome as a result of an interaction between tramadol and mirtazapine.[62] Although polypharmacy of antidepressants caused this syndrome, no reports of natural substances causing this syndrome have been reported to date. Because its action on serotonin is minor at best, it is unlikely that hypericum would cause this syndrome. Nevertheless, monitoring is prudent when working with hypericum, and serotonin precursors tryptophan or 5-hydroxytryptophan along with SSRIs. There are case reports linking St. John's wort to mania, suggesting it may not be a good choice for bipolar disorder.[63]

Photosensitivity

Although hypericum was demonstrated to induce photosensitivity in some patients (less than 1% of people taking 900 mg once a day or more), this is not likely with standard dosages. Cases of photosensitivity occurred mainly in HIV patients using higher than normal quantities for an antiviral effect.[56] Because of the photosensitivity concern, avoidance of strong sunlight and other sources of ultraviolet light when using St. John's wort is often recommended to individuals, especially those with fair skin. However, although this recommendation may be appropriate, it must be pointed out that the therapeutic dosage of 2.7 mg/day of hypericin is about 30 to 50 times below the level found to produce phototoxicity.[64]

Pregnancy

Despite the ample research on St. John's wort, relatively little research has focused on safety in pregnancy and breast feeding. Animal pregnancy toxicology studies showed no change in maternal weight gain or gestational length[65] and no change in offspring anomaly rates.[66,67] These negative animal studies can be reassuring because they suggest a lack of structural or functional deficits attributable to hypericum exposure during gestation.[68] However, we must always keep in mind that rodents and humans have varying detoxification abilities.

Human studies are few. In 1998 there were reports of two women who took hypericum during their pregnancies to avoid using conventional synthetic medications. Both cases seemed to reveal no concerns. One of these cases was of a 38-year-old woman who started hypericum at 24 weeks' gestation. The pregnancy was unremarkable, with the exception of late onset of thrombocytopenia (which the author did not attribute to hypericum). The offspring was born healthy, had a normal birth weight, normal APGAR scores, and physical examination and laboratory results were normal. Infant behavioral assessment at 4 and 23 days was normal.[69]

One 2006 review by naturopathic physicians looked at the evidence on the use, safety, and pharmacology of hypericum. This review searched seven databases, including the Cochrane bases, MedLine, Natural Database, and Natural Standard. Some unpublished research and bibliographies were also included. Data were compiled according to grade of evidence. The researchers found varying levels of scientific evidence. They concluded that there was in vitro evidence from animal studies that hypericum during pregnancy does not affect cognitive development or cause long-term behavioral defects but found some evidence of lower offspring birth weight. It is important to remember that rodents have very different detoxification abilities, and extrapolation to humans may not be prudent. This review also pointed to weak scientific evidence that hypericum induced CYP450 enzymes, which may lower serum medication levels below therapeutic range, which would be of concern when administering medications during pregnancy. The authors concluded that caution is warranted with the

use of hypericum during pregnancy until further high-quality human research is conducted to determine its safety.[70]

The first study of hypericum looking to determine whether exposure could be associated with major malformations prospectively followed 54 subjects using hypericum and compared them with a matched group of 108 pregnant women taking other pharmacological therapy for depression, as well as a third group of healthy women, who were not exposed to any known teratogens. The study showed that malformation rates were similar across the three groups, with 5%, 4%, and 0% in hypericum, pharmaceutical subjects, and healthy groups, respectively. This was not different than the 3% to 5% risk expected in the general population. Live birth and prematurity rates were also not different among the three groups. The authors suggested this first human study on pregnancy the did provide some evidence of fetal safety.[71] More studies are very welcome.

Nursing

Albeit in its own infancy, studies using hypericum during breast feeding are starting to support its use and safety for depressed mood in mothers while breast feeding. Animal studies using doses up to 25 times the equivalent recommended human dose throughout lactation did not show any neurobehavioral or developmental effects in offspring.[65] One study evaluating the safety of St. John's wort while nursing studied 33 breast-feeding women over a 2-year period. Compared with 101 disease-matched and 33 age-matched controls, there were no statistically significant differences found in maternal or infant demographics or maternal adverse events. No significant difference was observed in the frequency of maternal report of decreased milk production among the groups, nor was a difference found in infant weight over the first year of life.[72] A review of multiple databases also suggested that hypericum consumption during lactation did not affect maternal milk production or infant weight, but could cause colic, drowsiness, or lethargy. The authors of this review concluded that hypericum use during lactation appeared to be of minimal risk but could cause some mild side effects.[70] St. John's wort may be a choice if other natural recommendations are not sufficient for the lactating female, although more data would be welcome to confirm safety.

DRUG INTERACTIONS

Historically, those taking St. John's wort have been advised to avoid foods and medications that are known to interact with MAO-inhibiting drugs (such as tyramine-containing foods like cheeses, beer, wine, pickled herring, and yeast). In addition, drugs such as L-dopa and SSRIs were categorically recommended to avoid. However, given recent research demonstrating the lack of in vivo MAO inhibition, this recommendation is no longer justified.

Strong evidence warrants precaution when combining St. John's wort with several pharmaceutic therapies. St. John's wort was found to decrease the plasma concentrations of alprazolam, amitriptyline, digoxin, cyclosporine, indinavir, irinotecan, methadone, nevirapine, simvastatin, tacrolimus, theophylline, warfarin, phenprocoumon, and oral contraceptives. In the case of cyclosporine, the interaction resulted in some transplantation graft rejections. Hypericum was also shown to decrease the bioavailability of the R and S forms of verapamil.[73,74]

When combined with serotonin reuptake inhibitors, antidepressants (e.g., sertraline, paroxetine, nefazodone), or buspirone, hypericum can theoretically contribute to "serotonergic syndrome," a syndrome caused by excess serotonin levels. This syndrome has been iatrogenically induced by psychotropic and nonpsychotropic pharmaceutic coadministration and may include symptoms such as agitation, confusion, severe shivering, diaphoresis, myoclonus, hyperreflexia, mydriasis, tachycardia, and fever.[75]

Women on oral contraception should be informed regarding the possible decrease in birth control efficacy with hypericum. St. John's wort causes an induction of ethinyl estradiol and norethindrone metabolism consistent with increased CYP3A activity and can contribute to intracyclic bleeding.[76] One study evaluated the use of St. John's wort and oral contraception. St. John's wort appeared to contribute to breakthrough bleeding in 7 of 12 women (vs. 2 of 12 women in the oral contraception alone group). When taking oral contraceptive pills with St. John's wort, women may experience breakthrough bleeding and should consider adding a barrier method of contraception.[77]

Although there is great concern for negative effects on drug metabolism, hypericum is now being studied for its positive effects on medication outcomes as well. One study using the platelet inhibitor clopidogrel found that 2 of 10 patients using clopidogrel were poor responders but use of 300 mg hypericum for 2 weeks in these patients resulted in an increase in platelet inhibition of 20%. A previous study using 300 mg three times daily demonstrated an increase of 36%. Furthermore, no negative changes were seen in patients on statin medications.[78] Hypericum may be a good choice in poor responders, or to help lower the dose necessary in normal responders experiencing side effects. A small open trial of 20 patients looked at concomitant use of metformin alone (1 gram) on glucose tolerance and insulin action compared with using with the herb. There was no apparent effect on the steady-state pharmacokinetics of metformin, save for a minor renal clearance reduction of the medication.[79]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Barnes J, Anderson LA, Phillipson JD. St John's wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol*. 2001;53:583–600.
2. Hanrahan JR, Chebib M, Davucheron NL, et al. Semisynthetic preparation of amentoflavone: a negative modulator at GABA(A) receptors. *Bioorg Med Chem Lett*. 2003;13:2281–2284.
3. Hobbs C. St John's wort. *Hypericum perforatum L. HerbalGram.* 1989;18/19:24–33.
4. Oliveira AI, Pinho C, Sarmento B, Dias AC. Neuroprotective activity of Hypericum perforatum and its major components. *Front Plant Sci*. 2016;7:1004. https://doi.org/10.3389/fpls.2016.01004.
5. Di Pierro F, Risso P, Settembre R. Role in depression of a multi-fractionated versus a conventional Hypericum perforatum extract. *Panminerva Med*. 2018;60(4):156–160. https://doi.org/10.23736/S0031-0808.18.03518-8. Epub 2018 Jul 10.
6. Ng Q, Venkatanarayanan N, Ho C. Clinical use of Hypericum perforatum (St John's wort) in depression: a meta-analysis. *J Affect Disord*. 2017;210:211-221. https://doi.org/10.1016/j.jad.2016.12.
7. Czekalla J, Gastpar M, Hubner WD, et al. The effect of hypericum extract on cardiac conduction as seen in the electrocardiogram compared to that of imipramine. *Pharmacopsychiatry*. 1997;30(suppl 2):86–88.
8. Suzuki O, Katsumata Y, Oya M, et al. Inhibition of monoamine oxidase by hypericin. *Planta Medica*. 1984;50:272–274.
9. Holzl J, Demisch L, Gollnik B. Investigations about antidepressive and mood changing effects of Hypericum perforatum. *Planta Med*. 1989;55:643.
10. Thiede HM, Walper A. Inhibition of MAO and COMT by hypericum extracts and hypericin. *J Geriatr Psychiatry Neurol*. 1994;7(suppl 1): S54–S56.
11. Bladt S, Wagner H. Inhibition of MAO by fractions and constituents of hypericum extract. *J Geriatr Psychiatry Neurol*. 1994;7:S57–S59.
12. Thiele B, Brink I, Ploch M. Modulation of cytokine expression by hypericum extract. *J Geriatr Psychiatry Neurol*. 1994;7:S60–S62.
13. Calapai G, Crupi A, Firenzuoli F, et al. Interleukin-6 involvement in antidepressant action of Hypericum perforatum. *Pharmacopsychiatry*. 2001;34(suppl 1):S8–S10.
14. Mennini T, Gobbi M. The antidepressant mechanism of Hypericum perforatum. *Life Sci*. 2004;75:1021–1027.
15. Kaehler ST, Sinner C, Chatterjee SS, et al. Hyperforin enhances the extracellular concentrations of catecholamines, serotonin and glutamate in the rat locus coeruleus. *Neurosci Lett*. 1999;262:199–202.
16. Noda Y, Kamei H, Nabeshima T. Sigma-receptor ligands and anti-stress actions. *Nippon Yakurigaku Zasshi*. 1999;114:43–49. [Japanese].
17. Hoover JM1, Kaye AD, Ibrahim IN, Fields AM, Richards TA. Analysis of responses to St. John's Wort in the feline pulmonary vascular bed. *J Herb Pharmacother*. 2004;4(3):47–62.
18. Wong ML, O'Kirwan F, Hannestad JP, et al. St John's wort and imipramine-induced gene expression profiles identify cellular functions relevant to antidepressant action and novel pharmacogenetic candidates for the phenotype of antidepressant treatment response. *Mol Psychiatry*. 2004;9:237–251.
19. Gutmann H, Bruggisser R, Schaffner W, et al. Transport of amentoflavone across the blood-brain barrier in vitro. *Planta Med*. 2002;68:804–807.
20. Okpanyi SN, Weischer ML. Animal experiments on the psychotropic action of a Hypericum extract. *Arzneim Forsch*. 1987;37:10–13.
21. Muldner H, Zoller M. Antidepressive effect of a Hypericum extract standardized to an active hypericine complex. Biochemical and clinical studies. *Arzneimittelforschung*. 1984;34:918–920.
22. Lavie D. Antiviral Pharmaceutical Compositions Containing Hypericin or Pseudohypericin. European Patent Application, No. 87111467.4, filed 8/8/87, *European Patent Office.* Publ No. 0 256 A2. 175–177. 1987.
23. Someya H. Effect of a constituent of Hypericum erectum on infection and multiplication of Epstein-Barr virus. *J Tokyo Med Coll*. 1985;43:815–826.
24. Meruelo D, Lavie G, Lavie D. Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses. Aromatic polycyclic diones hypericin and pseudohypericin. *Proc Natl Acad Sci U S A*. 1988;85:5230–5234.
25. Lavie G, Valentin F, Levin B, et al. Studies of the mechanism of action of the antiretroviral agents hypericin and pseudohypericin. *Proc Natl Acad Sci U S A*. 1989;86:5963–5967.
26. Degar S, Prince AM, Pascual D, et al. Inactivation of the human immunodeficiency virus by hypericin. Evidence for photochemical alterations of p24 and a block in uncoating. *AIDS Res Human Retroviruses*. 1992;8:1929–1936.
27. Valentine FT, Lavie G, Levin B, et al. Synthetic hypericin enters blood lymphocytes and monocytes in vitro and decreases culturable HIV in blood obtained from infected individuals. *Int Conf AIDS*. 1991;7:97.
28. Barbagallo C, Chisari G. Antimicrobial activity of three Hypericum species. *Fitoterapia*. 1987;58:175–177.
29. Wurglics M, Schubert-Zsilavecz M. Hypericum perforatum: a 'modern' herbal antidepressant: pharmacokinetics of active ingredients. *Clin Pharmacokinet*. 2006;45(5):449–468.
30. *The Complete German Commission E Monographs Therapeutic Guide to Herbal Medicines.* M. Blumenthal, W.R. Busse, A. Goldberg, J. Gruenwald, T. Hall, C.W. Riggins, R.S. Rister (eds.) S. Klein and R.S. Rister (trans.). 1998. Austin: American Botanical Council; Boston: Integrative Medicine Communications.
31. Linde K, Ramirez G, Mulrow CD, et al. St John's wort for depression—an overview and meta-analysis of randomised clinical trials. *BMJ*. 1996;313:253–258.
32. Shelton RC, Keller MB, Gelenberg A, et al. Effectiveness of St John's wort in major depression: a randomized controlled trial. *JAMA*. 2001;285:1978–1986.
33. Vorbach EU, Arnoldt KH, Hubner WD. Efficacy and tolerability of St. John's wort extract LI 160 versus imipramine in patients with severe depressive episodes according to ICD-10. *Pharmacopsychiatry*. 1997;30:S81–S85.
34. Miller AL. Vitamin C causes cancer! St. John's wort useless for depression! *Altern Med Rev*. 2001;6:353–354.
35. Hypericum Depression Trial Study Group. Effect of Hypericum perforatum (St John's wort) in major depressive disorder: a randomized controlled trial. *JAMA*. 2002;287:1807–1814.
36. Linde K, Berner MM, Kriston L. St John's wort for major depression. *Cochrane Database Syst Rev*. 2008;(4):448.
37. Apaydin EA, Maher AR, Shanman R, et al. A systematic review of St. John's wort for major depressive disorder. *Syst Rev*. 2016;5(1):148. https://doi.org/10.1186/s13643-016-0325-2.
38. Behnke K, Jensen GS, Graubaum HJ, et al. Hypericum perforatum versus fluoxetine in the treatment of mild to moderate depression. *Adv Ther*. 2002;19:43–52.
39. Brenner R, Azbel V, Madhusoodanan S, et al. Comparison of an extract of hypericum (LI 160) and sertraline in the treatment of depression: a double-blind, randomized pilot study. *Clin Ther*. 2000;22:411–419.
40. Ng QX1, Venkatanarayanan N2, Ho CY3. Clinical use of Hypericum perforatum (St John's wort) in depression: a meta-analysis. *J Affect Disord*. 2017;210:211–221. https://doi.org/10.1016/j.jad.2016.12.048. Epub 2017 Jan 3.
41. Woelk H. Comparison of St John's wort and imipramine for treating depression: randomised controlled trial. *BMJ*. 2000;321:536–539.
42. Hubner WD, Kirste T. Experience with St John's Wort (Hypericum perforatum) in children under 12 years with symptoms of depression and psychovegetative disturbances. *Phytother Res*. 2001;15:367–370.
43. Findling RL, McNamara NK, O'Riordan MA, et al. An open-label pilot study of St John's wort in juvenile depression. *J Am Acad Child Adolesc Psychiatry*. 2003;42:908–914.
44. Simeon J, Nixon MK, Milin R, et al. Open-label pilot study of St. John's wort in adolescent depression. *J Child Adolesc Psychopharmacol*. 2005;15:293–301.
45. Potter M, Moses A, Wozniak J. Alternative treatments in pediatric bipolar disorder child and adolescent. *Psychiatric Clin North Am*. 2009;18:483–514.
46. Martinez B, Kasper S, Ruhrmann S, et al. Hypericum in the treatment of seasonal affective disorders. *J Geriatr Psychiatry Neurol*. 1994;7(suppl 1):S29–S33.
47. Schulz H, Jobert M. Effects of hypericum extract on the sleep EEG in older volunteers. *J Geriatr Psychiatry Neurol*. 1994;7(suppl 1):S39–S43.
48. Johnson D, Ksciuk H, Woelk H, et al. Effects of hypericum extract LI 160 compared with maprotiline on resting EEG and evoked potentials in 24 volunteers. *J Geriatr Psychiatry Neurol*. 1994;7(suppl 1):S44–S46.

49. Widy-Tyszkiewicz E, Piechal A, Joniec I, et al. Long term administration of Hypericum perforatum improves spatial learning and memory in the water maze. *Biol Pharm Bull*. 2002;25:1289–1294.
50. James JS. Report. Hypericin/St. John's wort: experience so far. *AIDS Treat News*. 1989:74.
51. James JS. Report. Hypericin/St. John's wort: experience so far. *AIDS Treat News*. 1989:91.
52. Darbinian-Sarkissian N1, Darbinyan A, Otte J, et al. p27(SJ), a novel protein in St John's Wort, that suppresses expression of HIV-1 genome. *Gene Ther*. 2006;13(4):288–295.
53. Schulz V. Safety of St. John's Wort extract compared to synthetic antidepressants. *Phytomedicine*. 2006;13:199–204.
54. Steinbeck-Klose A, Wernet P. Successful long term treatment over 40 months of HIV-patients with intravenous hypericin. *Int Conf AIDS*. 1993;9:470.
55. Furner V, Bek M, Gold J. A phase I/II unblinded dose ranging study of hypericin in HIV-positive subjects. *Int Conf AIDS*. 1991;7:199.
56. Gulick R, Lui H, Anderson R, et al. Human hypericism: a photosensitivity reaction to hypericin (St John's wort). *Int Conf AIDS*. 1992;8:B90.
57. Pereira JC, Pradella-Hallinan M, Alves RC. Saint John's wort, an herbal inducer of the cytochrome P4503A4 isoform, may alleviate symptoms of Willis-Ekbom's disease. *Clinics (Sao Paulo)*. 2013;68(4):469–474.
58. Wölfle U1, Seelinger G2, Schempp CM1. Topical application of St. John's wort (Hypericum perforatum). *Planta Med*. 2014;80(2–3):109–120.
59. Cui YH, Zheng Y. A meta-analysis on the efficacy and safety of St John's wort extract in depression therapy in comparison with selective serotonin reuptake inhibitors in adults. *Neuropsychiatr Dis Treat*. 2016;12:1715–1723. https://doi.org/10.2147/NDT.S106752.
60. Wang YS, Li YL, Yan GQ, et al. Clinical study of neurostan in treatment of encephalosis secondary depression. *Chin J Pract Nerv Dis*. 2009;21:84–85.
61. Solomon D, Adams J, Graves N. Economic evaluation of St. John's wort (Hypericum perforatum) for the treatment of mild to moderate depression. *J Affect Disord*. 2013;148(2–3):228–234.
62. Gnanadesigan N, Espinoza RT, Smith R, et al. Interaction of serotonergic antidepressants and opiod analgesics: is serotonin syndrome going undetected? *J Am Med Dir Assoc*. 2005;6:265–269.
63. Y1 G, Scholte WF, Assies J, Becker HE. Mania during the use of a combination preparation with St. John's wort (Hypericum perforatum). *Ned Tijdschr Geneeskd*. 2001;145(40):1943–1945.
64. Siegers CP, Biel S, Wilhelm KP. Zur frage der phototoxizitat von hypericum. *Nervenheilkunde*. 1993;12:320–322.
65. Cada AM, Hansen DK, LaBorde JB, et al. Minimal effects from developmental exposure to St. John's wort (Hypericum perforatum) in Sprague–Dawley rats. *Nutr Neurosci*. 2001;4:135–141.
66. Rayburn WF, Gonzalez CL, Christensen HD, et al. Impact of hypericum (St. John's-wort) given prenatally on cognition of mice offspring. *Neurotoxicol Teratol*. 2001;23:629–637.
67. Rayburn WF, Christensen HD, Gonzalez CL. Effect of antenatal exposure to Saint John's wort (Hypericum) on neurobehavior of developing mice. *Am J Obstet Gynecol*. 2000;183:1225–1231.
68. Rayburn WF, Gonzalez CL, Christensen HD, et al. Effect of prenatally administered hypericum (St John's wort) on growth and physical maturation of mouse offspring. *Am J Obstet Gynecol*. 2001;184:191–195.
69. Grush LR, Nierenberg A, Keefe K, et al. St John's wort during pregnancy. *JAMA*. 1998;280:1566.
70. Dugoua JJ, Mills E, Perri D, et al. Safety and efficacy of St. John's wort (hypericum) during pregnancy and lactation. *Can J Clin Pharmacol*. 2006;13:e268–e276.
71. Moretti ME, Maxson A, Hanna F, et al. Evaluating the safety of St. John's wort in human pregnancy. *Reprod Toxicol*. 2009;28:96–99.
72. Lee A, Minhas R, Matsuda N, et al. The safety of St. John's wort (Hypericum perforatum) during breastfeeding. *J Clin Psychiatry*. 2003;64:966–968.
73. Tannergren C, Engman H, Knutson L, et al. St John's wort decreases the bioavailability of R- and S-verapamil through induction of the first-pass metabolism. *Clin Pharmacol Ther*. 2004;75:298–309.
74. Izzo AA. Drug interactions with St. John's wort (Hypericum perforatum): a review of the clinical evidence. *Int J Clin Pharmacol Ther*. 2004;42:139–148.
75. Houlihan DJ. Serotonin syndrome resulting from coadministration of tramadol, venlafaxine, and mirtazapine. *Ann Pharmacother*. 2004;38:411–413.
76. Pfrunder A, Schiesser M, Gerber S, et al. Interaction of St John's wort with low-dose oral contraceptive therapy: a randomized controlled trial. *Br J Clin Pharmacol*. 2003;56:683–690.
77. Hall SD, Wang Z, Huang SM, et al. The interaction between St John's wort and an oral contraceptive. *Clin Pharmacol Ther*. 2003;74:525–535.
78. Lau WC, Gurbel PA. *Annual Scientific Session of the American College of Cardiology*. Elsevier global medical news; 2010.
79. Stage TB, Pedersen RS, Damkier P, et al. Intake of St John's wort improves the glucose tolerance in healthy subjects who ingest metformin compared with metformin alone. *Br J Clin Pharmacol*. 2015;79(2):298–306.

Lobelia inflata (Indian Tobacco)

Michael T. Murray, ND

OUTLINE

Lobelia inflata (family: Campanulaceae)
Common names: Indian tobacco, pukeweed, asthma weed, gagroot, vomitwort

GENERAL DESCRIPTION

Lobelia is an indigenous North American annual or biennial plant with an erect, angular, hairy stem that contains a milky sap and grows from 6 inches to 3 feet in height. Numerous small, two-lipped blue flowers grow in spike-like racemes from July to November.

CHEMICAL COMPOSITION

Lobelia contains about 0.48% pyridine (piperidine) alkaloids composed mainly of lobeline (Fig. 89.1), with lesser amounts of lobelanine, lobelanidine, and other alkaloids.[1] Other constituents include resin, gum, lipids, and chelidonic acid.[2]

HISTORY AND FOLK USE

Lobelia was named after Matthias de Lobel (1570–1616), a famous French botanist and physician to the court of King James I. After chewing the plant, early settlers noted that the taste was similar to tobacco and produced effects like that of nicotine, the principal alkaloid in domestic tobacco, *Nicotiana tobaccum*. Native Americans also smoked the dried leaves to obtain the central nervous system effects of the alkaloids in the plant. Thus lobelia was commonly referred to as "Indian tobacco."

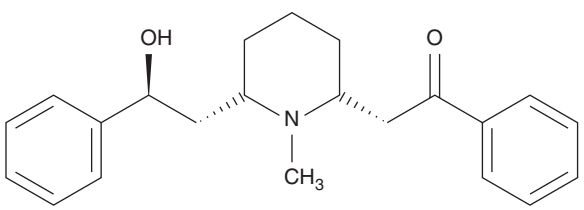


Fig. 89.1 Lobeline.

Lobelia became one of the most commonly used herbs by Thomsonians, who used it as an emetic, diaphoretic, expectorant, sedative, antispasmodic, and antiasthmatic. It has been used for the following conditions:

- Asthma
- Whooping cough
- Bruises
- Sprains
- Ringworm
- Insect bites
- Poison ivy symptoms

Thomson stated: "There is no vegetable which the earth produces more harmless in its effect on the human system, and none more powerful in removing disease and promoting health than lobelia."[3]

PHARMACOLOGY

Lobeline possesses many of the same pharmacological actions as nicotine, only less potent. However, its complex actions cannot be explained simply as being a weaker version of nicotine. Detailed pharmacological investigation comparing the effects of lobeline with nicotine indicate that lobeline possesses novel effects on nicotinic receptors.[4,5]

Respiratory Effects

One of the earliest known uses of lobeline was as a safe, short-acting respiratory stimulant. This respiratory stimulation was shown to be caused by direct stimulation of the carotid body chemoreceptors. The powerful respiratory stimulant action of lobeline has been used for stimulation of respiration in fever cases, cases of paralysis of respiratory centers caused by narcotic poisoning, alcohol, soporifics, morphine, narcosis, or spinal anesthesia. Lobeline has also been used to treat accident victims who have been buried, nearly drowned, hit by lightning or electrically shocked, and poisoned by asphyxiating gases.

Gastrointestinal Effects

The emetic action of lobeline is mediated by its stimulation of the emetic chemoreceptor trigger zone in the area postrema of the medulla oblongata (outside the blood–brain barrier) and activation of the vagal

and spinal afferent nerves that form the sensory input of the reflex pathways involved in vomiting.[6]

Central Nervous System Effects

One of the more promising lines of research with lobeline is that it appears to disrupt the fundamental mechanisms of dopamine storage and release. This action may help in antagonizing the neurochemical and behavioral effects of the psychostimulants amphetamine and methamphetamine. Lobeline was found to inhibit the amphetamine-induced release of dopamine in vitro and amphetamine-induced hyperactivity, drug discrimination, and self-administration in animal experiments.[7,8] Because lobeline is not addictive itself, it may reduce the abuse liability of these psychostimulants.

CLINICAL APPLICATIONS

Lobelia is primarily used as an expectorant in such conditions as pneumonia, asthma, and bronchitis. It appears that some of its actions may be mediated by the adrenal cortex.[9] Experimentally induced lung edema in rats was responsive to lobeline in many models that were unresponsive to any other medication. Furthermore, although lobeline causes bronchoconstriction in dogs and rats, in guinea pigs and (presumably) humans, the opposite—bronchodilation—occurs.[10]

Although effective when used alone in the treatment of asthma, it has traditionally been used in combination with other botanical agents, including *Capsicum frutescens* and *Symphlocarpus factida.*

Lobelia has also been used as an aid in stopping smoking with equivocal results from clinical trials.[11]

DOSAGE

- Dried herb: 0.2 to 0.6 g three times a day
- Tincture: 15 to 30 drops three times a day
- Fluid extract: 8 to 10 drops three times a day

TOXICOLOGY

Although lobelia has a reputation for being toxic, a thorough review of the medical literature was unable to find any well-documented case of serious problems or death caused by lobelia.[12] The main reason may be that lobelia causes nausea and vomiting when the amount used is too high, thereby avoiding absorption of toxic amounts. In general, a dosage of more than 1 mL of tincture one time is enough to produce significant nausea and possibly vomiting. Lobelia should not be used for more than 1 month consecutively and should be avoided during pregnancy and breastfeeding.

Like nicotine poisoning, toxic symptoms of lobelia include the following:

- Nausea
- Salivation
- Diarrhea
- Disturbed hearing and vision
- Mental confusion
- Marked weakness

Faintness and prostration ensue; blood pressure falls; the pulse becomes weak, rapid, and irregular; breathing is difficult; and collapse occurs, followed by convulsions. Death may conceivably result from respiratory failure. The antidote in acute poisoning is 2 mg of atropine, which is given subcutaneously.

DRUG INTERACTIONS

There are no known drug interactions with lobelia.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Karawya MS, Abdel-Wahab SM, Zaki AY. Colorimetric method for the estimation of alkaloids in lobelia and its pharmaceutical preparations. *J Assoc Off Anal Chem*. 1971;54:1423–1425.
2. Leung A. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. New York: Wiley.
3. Christopher J. *School of Natural Healing*. Provo, UT: BiWorld Publishers.
4. Damaj MI, Patrick GS, Creasy KR, et al. Pharmacology of lobeline, a nicotinic receptor ligand. *J Pharmacol Exp Ther*. 1997;282:410–419.
5. Miller DK, Harrod SB, Green TA, et al. Lobeline attenuates locomotor stimulation induced by repeated nicotine administration in rats. *Pharmacol Biochem Behav*. 2003;74:279–286.
6. Laffan RJ, Borison HL. Emetic action of nicotine and lobeline. *J Pharmacol Exp Ther*. 1957;121:468–476.
7. Dwoskin LP, Crooks PA. A novel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. *Biochem Pharmacol*. 2002;63:89–98.
8. Harrod SB, Dwoskin LP, Crooks PA, et al. Lobeline attenuates d-methamphetamine self-administration in rats. *J Pharmacol Exp Ther*. 2001;298:172–179.
9. Halmagyi DF, Kovacs A, Neumann P. Adrenocortical pathway of lobeline protection in some forms of experimental lung edema of the rat. *Dis Chest*. 1958;33:285–296.
10. Cambar PJ, Shore SR, Aviado DM. Bronchopulmonary and gastrointestinal effects of lobeline. *Arch Int Pharmacodyn Ther*. 1969;177:1–27.
11. Stead LF, Hughes JR. Lobeline for smoking cessation. *Cochrane Database Syst Rev*. 2000;2:CD000124.
12. Bergner P. Is lobelia toxic? *Med Herbal*. 1998;10(1):15–32.

90

Medicinal Mushrooms

Anna Sitkoff, BS, ND

OUTLINE

INTRODUCTION

Mushrooms have a millennia-long history of consumption as food and medicine all over the world with their use documented in ancient writings and even Asztec pictographs. Scientific Western herbal materia medicas such as King's *American Dispensatory*, first published in 1854, and *Kuts-Cheraux's Naturae Medicina and Naturopathic Dispensatory*, published in 1953, have long provided clinical guidance for several mushrooms, few of which are mentioned here. Chinese medicine has explored their use far more extensively, which has led recently to substantial laboratory and clinical research. The astute reader will notice that the majority of the over 200 references in this chapter have been published in the past 10 years.

Medicinal mushrooms are one of the fastest growing categories of supplement sales and their market is projected to hit $50 billion in the coming years.[1] In vitro and in vivo research on medicinal fungi has revealed many promising ways to integrate this medicine into our current health care. The physiological activities are vast and target many of the physiological dysfunctions that are the root cause of disease: protecting mitochondria, decreasing inflammation, and reducing oxidative stress in the human system.

GENERAL DESCRIPTION

The fungi kingdom has been both feared and revered by mankind. Fungi come in many forms, from mold to jelly, giant underground mycelial mats to claws and phalluses. "Mushroom" is a commonly used name to describe macrofungi with a unique fruiting body, and they are categorized based on the morphology of the spores they produce, with a majority being basidiomycetes and some ascomycetes. Intriguingly, humans are more related to fungi than to florae, respiration and digestion being two fundamental and fascinating similarities that we have with them.

There are 14,000 to 22,000 known species of mushrooms, with an estimated 140,000 species worldwide, and about 7000 species that have known benefits for humans.[2] In relation to the number of known species, the number of investigated species is small. Medicinal mushrooms as a whole have immune-centric qualities, making them a valuable medicinal resource for common debilitating ailments such as cancer and autoimmunity.

CHEMICAL COMPOSITION

Mushrooms are all built from the same general chemical symphony. Among the most researched constituents are polysaccharides, triterpenes, phenolic compounds, ergosterol, ergothioneine, fatty acids, proteins, and trace elements. Each of these components plays a role in the human system to facilitate healing and general wellness. See Fig. 90.1 for a description of each class of chemical and its medicinal mechanism.

Polysaccharides

Biologic Response Modifiers

The fungal cell wall is composed of many layers—mannoproteins, β-(1,6)-glucan and β-(1,3)-glucan (Fig. 90.1), and chitin. Humans do not

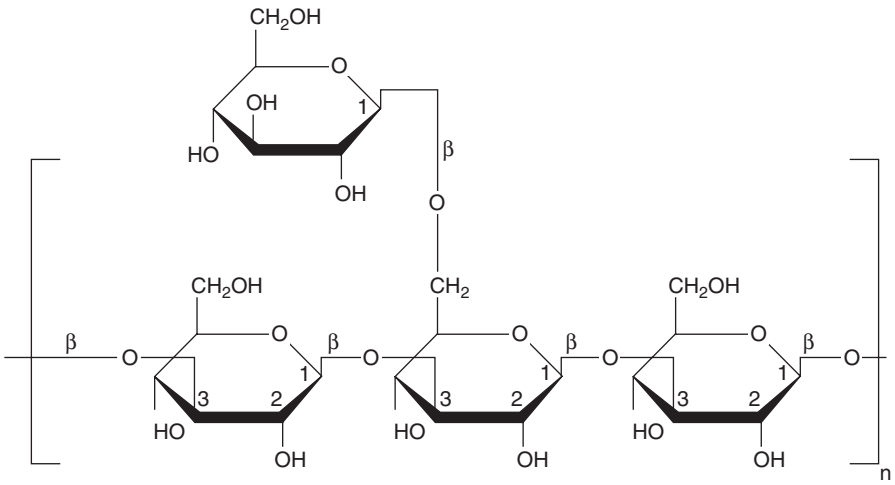


Fig. 90.1 Pleuran, a polysaccharide isolated from *Pleurotus ostreatus* (Oyster mushroom).

make chitinase and cannot use chitin for anything other than nondigestible fiber. Chitin can be broken down by different processing techniques, including heat. Once this is performed, the mannan and beta-glucan layers can be used as medicine. From an immunological perspective, these layers are considered pathogen associated molecular patterns (PAMPs) and bind to pathogen recognition receptors (PRRs) on cells in the innate immune system—macrophages, granulocytes, dendritic cells, and natural killer cells. The majority of binding occurs in the gut associated lymphoid tissue (GALT), where Peyer's patches contain cells of the innate immune system. Specific receptors that bind these β-glucans include dectin-1 and TLR-2.[3] Binding has a hormetic effect, creating a stress response in the immune system, stimulating the release of cytokines with anti-inflammatory, proinflammatory, antiviral, and anticancer activity. These cytokines stimulate an increase in natural killer cells and cytotoxic T cells—overall, generating a stress signal leading to a more primed immune system.

Blood Sugar Regulation

Polysaccharides inhibit the enzyme alpha-glucosidase, which is known to participate in the rise in blood sugar after a meal. Additionally, polysaccharides have also been shown to upregulate GLUT 4, an insulin-response glucose transporter, while downregulating NF-κB, a nuclear transcription factor that regulates inflammation.[4-6]

Antioxidant

Polysaccharides increase the activity of hepatic oxidative enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, effectively increasing levels of glutathione and decreasing malondialdehyde levels (a marker of lipid peroxidation). Generally, these compounds support the innate antioxidant systems in relieving oxidative stress and free radical damage.[7]

Prebiotic

The oligosaccharides and polysaccharides contained in mushrooms are consumed by *Bifidobacterium* and *Lactobacillus*, two important bacteria in a healthy microbiome.[4,8,9]

Triterpenoids

As the mushroom matures from primordia to fruiting body, the enzyme CYP450 stimulates synthesis of triterpenes (Fig. 90.2); therefore, there are more of these compounds found in the fruiting bodies of mushrooms. The triterpenes are commonly lanostane triterpenes and may be referred to as "lanostanoids." Triterpenes are major compounds of interest in cancer research because they have been shown to be directly cytotoxic to many

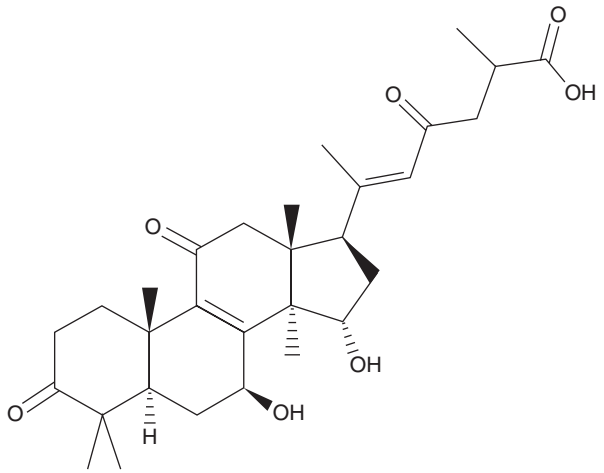


Fig. 90.2 Ganoderic acid, a triterpene isolated from *Ganoderma* spp. (Reishi).

cancer cell lines.[10-14] They also have specific antiviral activity through inhibition of the enzyme, neuraminidase.[15-17] These compounds are also beneficial in the treatment of hypersensitivity reactions, including allergy, asthma, dermatitis, and rhinitis by inhibiting histamine release from mast cells.[10]

Phenolic Compounds

Phenolic compounds are the building blocks for pigments in mushrooms, and once absorbed in the small intestine, they act as antioxidant, anti-inflammatory, and antiviral agents. The most well researched phenolic compounds among mushrooms are hispolon (Fig. 90.3) and phelligridin, both found in *Phellinus* spp. and *Inonotus obliquus*.[18-20]

Ergosterol

Ergosterol (Fig. 90.4) is as ubiquitous in mushrooms as cholesterol is in humans. It is formed by an almost identical metabolic process—the mevalonate pathway. When mushrooms are exposed to ultraviolet light, ergosterol is converted to ergocalciferol, or vitamin D_2. Vitamin D_2 is then converted to calcidiol in the liver and eventually calcitriol, active vitamin D_3, in the kidneys. There is clinical evidence to show that ergocalciferol-D2, although not as bioavailable as calcitriol-D3, could significantly improve vitamin D deficiency in human patients.[21] However, as many of the metabolic activities of vitamin D_2 are different from the vitamin D_3 produced in the skin of mammals, they should not be used interchangeably.

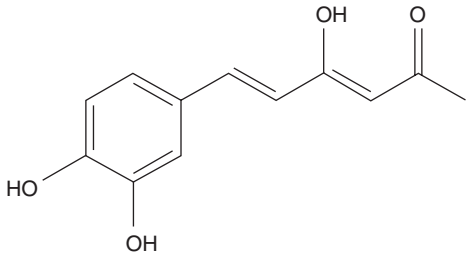


Fig. 90.3 Hispolon, a phenolic compound isolated from *Phellinus* spp. (Fire Sponge) and *Inonotus obliquus* (Chaga).

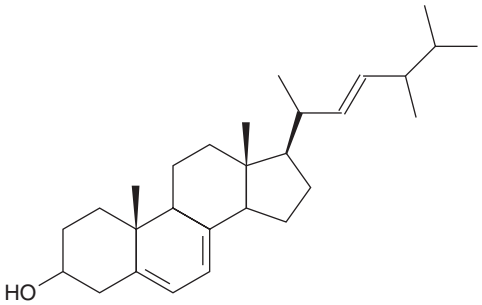


Fig. 90.4 Ergosterol.

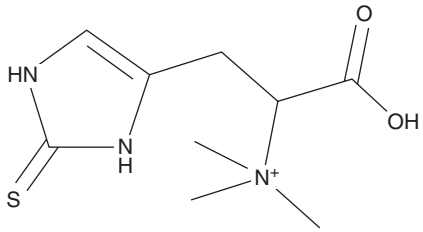


Fig. 90.5 Ergothioneine.

Ergothioneine

Ergothioneine (ERG; Fig. 90.5) is an amino acid derivative, a crystalline betaine, derived from histidine. The ERG transporter, OCTN1 (gene: SLC22A4)[22] is found on erythrocytes, fetal liver and bone marrow, ileum of the small intestine, trachea, kidney, cerebellum, lung, monocytes, seminal vesicles, and the lens and cornea of the eye.[23] Interestingly, OCTN1 is concentrated in the mitochondria, suggesting a role in protecting mitochondrial components from DNA damage.[23,24] When cells are depleted of OCTN1, they are more susceptible to oxidative stress, leading to mitochondrial damage, protein oxidation, and lipid peroxidation.

After ingestion, whether as an isolated molecule or in a whole mushroom, ERG is absorbed into the bloodstream, where it is highly bioavailable, and retained for up to a month. In a human trial observing ERG bioavailability, subjects consumed either 8 g or 16 g of mushrooms, and increases in red blood cell (RBC) ERG were observed. After 1 and 4 hours of consumption, the 16 g mushroom dose increased RBC ERG concentration compared with the control, and after 2 hours, RBC ERG concentration was significantly higher than the control.[25]

ERG chelates divalent metal cations: copper, mercury, zinc, cadmium, cobalt, iron, and nickel.[26-28] Cation binding may help prevent their participation in the generation of reactive oxygen species; for example, ERG has been found to protect DNA and protein against copper induced oxidative damage through formation of a redox-inactive EGT-Cu complex.

ERG enhances glutathione activity in rat liver cytosol in a dose dependent manner, leading researchers to postulate that declining ERG may play a role in age-related decline of glutathione and glutathione peroxidase. Furthermore, ERG levels are lower in the elderly with early stages of dementia and in Parkinson's disease patients relative to age-matched healthy controls.[29]

Of all the mushrooms analyzed, ERG was highest in the fruiting body of *Pleurotus ostreatus* (Oyster Mushroom), containing 26.4 mg per 85 g mushroom.[30]

Fig. 90.6 *Ganoderma lucidum.*

Fig. 90.7 *Ganoderma oregonense.*

I. MUSHROOMS WITH HUMAN CLINICAL STUDIES

Reishi (*Ganoderma lucidum, G. applanatum, G. tsugae,* Lingzhi, 灵芝, 영지)

History and Folk Use

Reishi has been used in Chinese medicine for thousands of years and is considered one of the most important herbs; the name, Linghzi, translates to "herb of spiritual potency." It is said that the medicine of Lingzhi nourishes the heart, and in Chinese medicine the heart is the home of the "Shen," or "spirit." Aqueous extracts of the fruiting body were traditionally used to support the Shen by quieting the mind and relieving fatigue (Fig. 90.6 and Fig. 90.7).

Pharmacology

Antigout. *Ganoderma* spp. decrease uric acid accumulation through multiple mechanisms. In vitro, 2, 5 Dihydroxacetophenone, a bioactive compound from *G. applanatum,* and lanostanoids from *G. tsugae* bind to and decrease xanthine oxidase activity.[31,32] Significantly, xanthine oxidase is the terminal enzyme in the formation of uric acid. In an animal model, ethanol and water extracts of *G. applanatum* were administered to hyperuricemic mice, and the extracts lowered serum uric acid levels and increased both mRNA and protein levels of organic anion transporter (OAT1), which induced uric acid excretion. Additionally, renal uric acid reabsorption was blocked through inhibition of glucose transporter 9 (GLUT 9) and uric acid transporter 1(URAT1).[33]

Antiandrogenic. In vitro research and animal studies suggest that Reishi exhibits antiandrogenic effects via inhibition of the enzyme 5-α-reductase, which converts testosterone to dihydrotestosterone, an androgen hormone that is correlated with benign prostatic hyperplasia in older men. Fruiting body preparations of the mushroom significantly inhibited the growth of the ventral prostate in rats, and decreased levels of prostate specific antigen.[34-36] The ethanol extract of *G. lucidum* fruiting body containing ganoderol B and ganoderic acid DM was found to bind androgen receptors, inhibiting androgen induced lymph node carcinoma of the prostate as well as inhibiting 5-alpha reductase, respectively.[36]

Antiatherogenic. Lipid peroxidation of low-density lipoproteins (LDL) particles in the macrovasculature is a major contributor to the pathophysiology of atherosclerosis. Animal studies have demonstrated the antiatherogenic effects of *G. lucidum* polysaccharides, revealing that after administration, treated rats had decreased levels of triglycerides and LDL-c, lower liver weight, and increased high-density lipoprotein (HDL)-c, serum superoxide dismutase, and glutathione peroxidase compared with the control.[37] Other studies have explored beneficial cardiovascular effects via the quenching of free radicals that are responsible for lipid peroxidation; it is postulated that the antioxidant triterpenoids may be responsible for this effect.[38]

Blood sugar regulation. Ganoderic acids isolated from Reishi inhibit aldose reductase and alpha-glucosidase, two essential enzymes in the treatment of hyperglycemia.[39] *G. lucidum* also contains a protein, LZ-8, that demonstrates immunomodulatory and antitype 1 diabetes activity via decreasing lymphocyte infiltration and increasing antibody detection of insulin in pancreatic β-cells. Some studies have found that this immunomodulatory action is a result of LZ-8 stimulating FOXP3 regulatory T cells,[40] indicating a possible benefit for other autoimmune disease.

Antineoplastic. *G. applanatum* triterpenoids and polyketids have anticancer effects in vitro and in vivo in animal models. These triterpenoid compounds are anti-inflammatory, antioxidant, and have overall cytotoxic effects via upregulation of caspase-3 and increased P53 tumor suppressor gene expression.[41] In vitro, triterpenoids extracted from *G. lucidum* ganoderic acids have a number of anticancer actions that have been observed in cervical, breast, liver, lymphoma, melanoma, leukemia, lung, and colon cancers, including cancer cell-specific cytotoxicity, inhibition of cell proliferation, induction of apoptosis via caspase-3, suppression of cell adhesion, inhibition of angiogenesis, and inhibition of migration and invasion.[42,43]

Antiviral. *Ganoderma* triterpenoids also pose antiviral effects in vitro to H1N1 and H5N1 influenza viruses via inhibition of neuraminidase enzymes.[44] In addition to the antiviral terpenes, water soluble compounds exhibit antiviral activity; specifically, the hot water extract of *G. lucidum* significantly inhibits plaque formation of HSV-2.[45]

Clinical Applications

Antiandrogenic. In a randomized, double-blind, placebo-controlled and dose ranging study, *G. lucidum* was administered at a dose of 6 mg per day to men with lower urinary tract symptoms caused by prostate hyperplasia. Extracts of *G. lucidum* at this dose were found to be safe and effective in relieving urinary symptoms in men with symptoms of bladder outlet obstruction.[46]

Antineoplastic. *Ganoderma* polysaccharide, "Ganopoly," positively modulates the immune function of advanced stage cancer patients with lung, breast, colon, liver, or prostate cancer. Patients were treated with 1800 mg of 'Ganopoly' three times a day orally before meals for 12 weeks (5.4 g of isolated Ganopoly is equivalent to 30 g of fruiting body per day), resulting in an increase in interleukin 2 (IL-2), IL-6, and interferon γ (IFN-γ), while significantly decreasing IL-1 and tumor necrosis factor α (TNF-α). Additionally, there was a significant increase in mean natural killer (NK) cell activity compared with baseline.[47] Taken together, these findings suggest an immunomodulatory effect that could be of great benefit as an adjunct therapy in cancer treatment.

Antiviral. A human study found the combination of *G. lucidum* and *T. versicolor* at 400 mg/day for 2 months cleared oral human papilloma virus in 87.8% of cases compared with the control, which only cleared 5% of cases.[48]

Chronic fatigue syndrome. Female volunteers with chronic fatigue syndrome receiving 2 g of *G. lucidum* extract daily had a significant benefit in quality-of-life parameters compared with the control group. Average serum cortisol level, measured 12 weeks after the first dose, was increased up to 1.5 times from baseline measurement, leading the authors to conclude that women with chronic fatigue syndrome who generally have low cortisol levels can benefit from Reishi intake, and may also see a rise in serum cortisol.[49]

Dosage

Condition	Dose of Whole Mushroom	Timing
Maintenance[50]	1 g	daily
Skin allergies[50]	1.5 g	3x/day
Insomnia[50]	1.5 g	3x/day
Cholesterol lowering[50]	2 g	3x/day
Chemotherapy adjunct[50]	3 g	3x/day
Immune potentiating[50]	2 g	3x/day
Chronic fatigue[49]	2 g	daily
Immune support in advanced stage cancer patients[47]	30 g 1.8 g polysaccharide extract	daily 3x/day

Cordyceps (*C. sinensis, C. militaris,* Caterpillar fungus, dong chong xia cao, 冬虫夏草)

History and Folk Use

Cordyceps is an extraordinary mushroom and is considered an endoparasite—the mycelium takes over the life of insects, zombifies them, and grows an erect fruiting body, usually through the caput of an arthropod. According to ancient descriptions, *C. sinensis* possesses important pharmacological activities for protecting lung and kidney function and nourishes essential and vital energy. The Nepalese people observed livestock consuming Cordyceps and saw an increase in the animal's sexual vitality and general stamina. The people then began to consume the mushrooms as the other animals did and found that it improved their own vitality as well— increasing stamina, endurance, and treating impotence.

Pharmacology

Renal protective. *C. sinensis* significantly improves tubulointerstitial renal fibrosis, in vitro,[51] and polysaccharide extracts relieve the formation of reactive oxygen species and reduce kidney damage through inhibition of tumor growth factor β (TGF-β).[52]

Endurance. Though used widely by athletes, few ergogenic mechanisms have been demonstrated through in vivo animal models. *Cordyceps* spp. have been shown to stimulate the enzyme lactic acid dehydrogenase (LDH), accelerating the removal of lactic acid and increasing available adenosine triphosphate for anaerobic exercise.[53] *Cordyceps* spp. also contain the nucleoside "adenosine,"[54] which in exercise relaxes vascular smooth muscle, contributing to the local vasodilation that accompanies muscle contraction. Studies have found that during exercise-induced, higher-frequency contractions, adenosine contributes around 14% to 29% of the necessary vasodilation.[55]

Respiratory protection. *C. sinensis* may be beneficial for decreasing smoking-related cellular damage in lung tissue. In vitro research shows that Cordyceps decreases cigarette smoke extract (CSE)-induced cellular senescence. Activation of the oxidative, inflammatory, and oncogene signaling pathway iROS/PI3K/AKT/mTOR is enhanced by CSE treatment, and decreases when *C. sinensis* is administered.[56] Cordycepin, a derivative of the nucleoside adenosine, significantly reduces airway inflammatory cell infiltration via inhibition of ERK/NF-κB signaling pathway, which suppresses inflammatory mediators.[57-61] Cordyceps inhibits airway remodeling of asthma by downregulating TGF-β and suppressing p38MAPK signaling pathways, which indicates possible use as a treatment for allergic asthma.[62]

Antineoplastic. Cordycepin has been widely recognized for its therapeutic potential against many types of cancers through numerous mechanisms, inducing apoptosis, cell cycle arrest, and causing DNA damage in cancer cells, thereby killing cancer cells and controlling growth. Cordycepin also induces autophagy, inhibits tumor metastasis, and modulates the immune system. Although there have been many successful cases of cordycepin in anticancer research in vitro and in animal models, clinical trials have yet to be reported.[63] Cordycepin also reduces migration of human glioblastoma cells in vitro and decreases brain tumor size in mice, and actively downregulates the expression of integrin β1, FAK, and p-FAK, subsequently reducing cancer cell migration.[64]

Erectile dysfunction. *Cordyceps* has a history of use as an aphrodisiac, specifically aiding sexual endurance and erectile dysfunction. Studies indicate this mechanism is via a protein with vasorelaxant effects linked to the production of endothelial nitric oxide synthase (eNOS). These are preliminary results and the authors stress that further work on the mechanism of action is necessary.[65]

Clinical Applications

Exercise recovery. A double-blind placebo-controlled trial assessed the oxidative stress biomarkers in athletes supplementing with Cordyceps and Reishi mushrooms. The researchers found that after 3 months of supplementation with 1335 mg Cordyceps extract and 1170 mg Reishi extract per day, the athletes had significantly more free radical scavenging activity after a race than the placebo group.[54] These results could be an indication that these mushrooms are also beneficial for recovery after endurance exercise.

Chronic kidney disease. In a review of 22 studies that involved 1746 participants with chronic kidney disease (CKD) not receiving dialysis, Cordyceps preparations were found to significantly decrease serum creatinine, an important marker of CKD severity. Cordyceps preparation, as an adjunctive therapy to conventional medicine, can decrease serum creatinine, increase creatinine clearance, reduce proteinuria, and alleviate CKD-associated complications, such as increased hemoglobin and serum albumin.[66]

Another systematic review compared 3 to 12 g daily doses of Cordyceps-based immunosuppressant therapy with azathioprine-based immunosuppressant therapy. Both immunosuppressant therapies included cyclosporine A and prednisolone. Patients who took the Cordyceps-based immunosuppressant therapy had significantly lower blood urea nitrogen and serum creatinine, decreased serum uric acid, total cholesterol, alanine transaminase and aspartate transaminase liver enzymes, and increased HDL-cholesterol. Urinary leukocytes and erythrocytes were notably lower in the Cordyceps group along with lower infection rates. Based on these studies, Cordyceps is a safe adjunctive treatment that could be beneficial for patients with CKD.[67]

Immune-stimulating. A placebo-controlled trial observed the immune-stimulating effects of *C. militaris* fruiting body extract in healthy adult males: 1.5 g of mushroom was administered daily, and blood was drawn after 2 weeks and after 4 weeks. After 4 weeks, there was a significant increase in NK cells, IFN-γ, and IL-2 compared with the control group.[68]

Dosage

Condition	Dose	Timing
Chinese tonic dose	2–9 g	daily
Respiratory viral infection[69]	6–9 g	daily
Kidney support[67]	4 g	daily
Enhance cell-mediated immunity[68]	1.5 g	daily

Turkey Tail (*Trametes versicolor, Coriolus versicolor,* Kawaratake, Yun Zhi, 云芝)

History and Folk Use

In traditional Chinese medicine, Turkey Tail is called *Cloud Fungus.* It is said to replenish essence and qi, and regulate immune function. Traditionally, it was used to invigorate the spleen and eliminate dampness, arrest cough, and help with breathing difficulty (Fig. 90.8).

Fig. 90.8 *Trametes versicolor.*

Pharmacology

The majority of research that has been done on Turkey Tail has been focused on polysaccharide krestin (PSK) and polysaccharide peptide (PSP).

Gut microbiome. Turkey Tail has demonstrated prebiotic-like activity in vitro and in vivo. In vitro research has shown that the fermentation of PSP extract by gut bacteria increases the beneficial bacteria *Bifidobacterium* and *Lactobacillus* spp. while reducing less desirable bacteria, *Clostridium difficile, Staphylococcus* spp. and *Enterococcus* spp. This study also saw an increased level of lactate and beneficial short-chain fatty acids.[70]

Antineoplastic. PSK extract modulates the immune response through stimulation of both extracellular PRR (TLR2) and intracellular pathogen sensors (NLRP3 inflammasome).[71] There have been many peer-reviewed publications on Turkey Tail in cancer treatment, including 37 in vitro articles, 55 animal studies, 43 published human clinical studies, and 11 review articles in gastrointestinal, breast, and lung cancer. In the past 2 years, five more PSK trials in colorectal cancer have been published. *Trametes* water extract is made up of 62% polysaccharide and 38% PSK. After ingestion, PSK is highly bioavailable and can be found in the bone marrow, salivary gland, brain, liver, spleen, and pancreas in mice and rabbits within 24 hours. Once absorbed, PSK induces cytokine modulation, increasing TNF-α, IFN-γ, IL-2, and IL-8.[72]

Clinical Applications

Microbiome. In a preclinical trial with 21 women, PSP gel was administered intravaginally. There was improved epithelialization of the cervical mucosa and the concentration of *Lactobacillus* increased 54.5% while the pH decreased from 4.2 to 4.09.[9]

In a randomized clinical trial, 1200 mg of PSP was administered three times a day on an empty stomach alongside the antibiotic amoxicillin. Although amoxicillin alone led to an increase in *Escherichia* and *Shigella,* the PSP group had distinctive positive changes in the human microbiome, consistent with its activity as a prebiotic.[73]

Antineoplastic. PSK improves immune function, reduced tumor-associated symptoms, and extended survival time in lung cancer patients. In six randomized controlled trials, 3 g a day of PSK or PSP alongside standard chemotherapy showed benefit for immune function, performance status and body weight, and overall survival. PSK increases immune surveillance and offsets chemotherapy-induced bone marrow toxicity, and reduces depression of immune cells and immune cell activity during chemotherapy. PSK also increases NK cells and phagocytic activity, reduces TGF-β, and increases the antitumor response of peripheral blood mononuclear cells.[74]

In a randomized, double blind, placebo-controlled trial exploring palliative treatment for hepatocellular carcinoma, Turkey Tail was given as a standard, continuous daily dose of 2.4 g. These patients reported better physical, emotional, cognitive, and social functions compared with the placebo during treatment. They experienced higher cognitive function, less pain and increased appetite, and had reduced IL-17 and monocyte chemoattractant protein-1.[75]

In a Phase I clinical trial of *T. versicolor* in women with breast cancer, researchers found that up to 9 g per day of a Turkey Tail preparation is safe and tolerable and the women taking 6 g a day had a significant trend upward in NK cell counts.[76]

Dosage

Condition	Dose	Timing
Microbiome support[73]	1200 mg	3x/day
Cancer therapy adjunct[74-76]	3–9 g	daily

Chaga (*Inonotus obliquus*, Kabanoanatake)

History and Folk Use

Chaga sclerotium has been used as medicine in Eastern Europe at least since the 12th century. Historical accounts suggest that a Russian duke, Vladimis Monomach, cured himself of lip cancer using Chaga. The Khanty people, an ethnic group from Siberia, used this mushroom as medicine for digestive ailments and to prevent heart and liver disease. Chaga sclerotium grows out from the core of old birch trees, and consequently has medicinal compounds, betulin and betulinic acid, derived from these trees.

Pharmacology

The triterpenoid compounds extracted from Chaga inhibit proliferation of cancer cells, induce cell cycle arrest at various cell cycle checkpoints, enhance apoptosis, and enhance regulation of signal transduction pathways.[77] The triterpene inotodiol, specifically, has been shown to have an antihistamine effect via the inhibition of mast cell degranulation in the small intestine when administered to mice with food allergies.[78]

Antioxidant. The polysaccharides extracted from Chaga have antioxidant effects through their superoxide scavenging activity[79,80] The antioxidant mechanism may be as result of the supply of hydrogen by the polysaccharides, which combines with radicals and forms a stable radical to terminate the damaging radical chain reaction.

Anti-Inflammatory. Aqueous extract containing polysaccharides, proteins, and phenolic compounds was administered to mice with intestinal inflammation. The extract suppressed edema, mucosal damage, and inhibited nMRNA expression of proinflammatory cytokine TNF-α, and there was an overall inhibition of inflammatory transcription factor NF-κB.[81]

Hypoglycemic. Polysaccharide-rich aqueous extract exhibits hypoglycemic activities in vitro and in vivo. The animals in this study showed significant alpha-glucosidase activity inhibition, slowing the release of glucose in the intestine, and reducing postprandial hyperglycemia.[82]

Clinical Applications

Psoriasis and GI inflammation. A group of 50 patients suffering from psoriasis were treated with Chaga extract paste. Forty-three patients started the treatment with Chaga during the acute stage of psoriasis, and 7 started during the steady-state. Chaga extract was heated and one tablespoon of the extract was diluted in a glass of boiled water at room temperature. Chaga extract was administered three times a day 20 to 30 minutes before meals. Psoriatic rashes were significantly improved after 3 months of regular intake, and extensive psoriasis was completely cured in 16 patients. Overall, Chaga was found to be an especially useful treatment for patients with psoriasis co-occurring with chronic inflammatory diseases of the gastrointestinal tract and liver.[83,84]

Dosage

Condition	Dose	Timing
Traditional general dose[83]	3 g	daily
Psoriasis[84]	1 tbs extract	3x/day

Maitake (*Grifola frondosa*, Hen of the Woods)

History and Folk Use

Traditionally, Maitake has been used in China to improve the spleen, appease stomach ailments, treat hemorrhoids, and calm the mind and nerves. Also a delicious culinary mushroom, it is commonly used as

food. Some medicinal mushrooms are much too woody to eat (Reishi, Turkey Tail, and Chaga), but Maitake is easy to cook and tastes rather good. As a general principle: mushrooms should be eaten if palatable.

Pharmacology

β-D-glucans from Maitake have been named Maitake D fraction, or MD fraction, and have been the focus of many Maitake studies. Maitake also contains a significant amount of ergothioneine, and so possesses the beneficial antioxidant and neuroprotective qualities of ergothioneine described previously.

Immune modulation. In an animal study, Maitake extract decreased TNF-α and enhanced interferon activity in rats with invasive bladder cancer. MD fraction relieves colon inflammation via suppression of both TNF-α and signaling through NF-κB in vitro,[85] and enhances both innate and adaptive immunity, indicating use for defense against foreign pathogens and protection for general, healthy immune function.[86]

Antineoplastic. MD fraction has direct antiproliferative and cytotoxic effects in human cancer cells, including prostate, bladder, liver, brain, blood and breast. The mechanisms include a decrease in cell viability, increase in cell adhesion, and a reduction in the migration and invasion of human lung cancer cells. (Rossi) Fruiting body extract, which contains polysaccharides and phenolic compounds, displays an antiangiogenic effect in rats via inhibition of vaso-endothelial growth factor (VEGF) and extracellular signal–related kinase (ERK) through suppression of reactive oxygen species (ROS).[87] These in vitro and in vivo findings are meaningful when it comes to angiogenesis-associated diseases including retinopathies, benign and malignant angiogenic tumors, and progression of malignant tumors.

Clinical Applications

Neoplasia. Maitake polysaccharide extract was administered to postmenopausal women who had a history of breast cancer. At a dose of 5 mg/kg per day, increases in both suppressive and stimulatory cytokine blood markers were observed, including IL-10, IL-2, TNF-α, and IFN-γ,[88] which suggests an immunomodulatory action. Because this was a polysaccharide fraction and Maitake contains about 45% polysaccharides, if taking whole mushroom rather than polysaccharide extract, the dose should be doubled. In another human trial of patients with stage II to IV cancer, either cancer regression or significant symptom improvement was observed in 58% of liver cancer patients, 68.8% of breast cancer patients, and 62.5% of lung cancer patients.[88]

Polycystic ovarian syndrome. Insulin resistance is a prominent feature of polycystic ovarian syndrome (PCOS), and insulin sensitizing drugs are used to induce ovulation, typically clomiphene citrate (CC). In a clinical trial with 72 patients with PCOS, 26 women took Maitake extract, 31 women took clomiphene citrate, and 18 women received combined therapy. The ovulation rates were 76.9% for the Maitake patients and 93.5% for the CC patients, while combination therapy increased the ovulation rate to 87%. These results indicate the possibility of using Maitake as a monotherapy to induce ovulation in PCOS patients as well as a combination therapy with clomiphene citrate.[89]

Dosage

Condition	Dose	Timing
Chemotherapy adjunct[88]	5–7 mg/kg polysaccharide extract, 10 mg/kg whole mushroom	daily
Insulin regulation/ PCOS[89]	~1 g	3x/day

Fig. 90.9 *Pleurotus.*

Pleurotus ostreatus (Hiratake, píng gū, 平菇)

History and Folk Use

Oyster mushrooms have no boundaries—they grow everywhere, on most anything, and are used as food throughout the world. According to some resources, they were first cultivated in Germany during World War I as an important food source. Oyster mushrooms are an especially fascinating mushroom because they are considered carnivorous—they are one of the few mushrooms known to consume critters, specifically nematodes, as a nitrogen source (Fig. 90.9).

Pharmacology

Oyster mushroom contains the most ergothioneine of any known mushroom, and so the antioxidant and neuroprotective actions of ergothioneine are a major component of the medicinal benefit. Other unique compounds found in Oyster mushroom include chrysin, lovastatin, and GABA. The β-D-glucan on which the majority of the Oyster mushroom cancer research is focused is called "pleuran."

Respiratory support. Active treatment in children for recurrent respiratory tract infections with pleuran resulted in a significant reduction of peripheral blood eosinophils and stabilized levels of total IgE in serum. A potential mechanism proposed in the study was that allergic children have respiratory infections more often and through stabilization of IgE, these may be prevented.[90]

Antiatherogenic. There are many factors that aggregate and result in atherosclerosis. These include and are not limited to hypertension, hyperglycemia, hypercholesterolemia, and lipid peroxidation. Oyster mushroom research has demonstrated protection against all of those factors. In an animal study, rats were administered chrysin-rich *Pleurotus* extract. After administration there was a significant decrease in mean blood serum levels of glucose, lipid profile parameters, and hepatic marker enzymes and a simultaneous increase in enzymatic and nonenzymatic antioxidant parameters.[91]

P. ostreatus is the richest known source of ergothioneine, containing 118.91 mg/kg. As mentioned previously, ergothioneine protects against DNA damage and lipid oxidation. For hypertension, Oyster mushroom water extract inhibits angiotensin-converting enzyme (ACE)—a common mechanism in hypertensive medication.

Oyster mushroom also contains lovastatin, a naturally occurring statin compound that reduces LDL cholesterol through inhibition of HMG-CoA reductase.[92]

Clinical Applications

Atopic dermatitis. In a split-body study of 80 patients, topical *P. ostreatus*-based β-glucan cream application resulted in improvement

of both subjective and objective symptoms of atopic dermatitis. The patients applied the cream on one segment of the body with atopic dermatitis and no treatment on another atopic dermatitis segment. On the application site there was a significant decrease in the number of days and severity of atopic dermatitis.[93]

Respiratory disease. Pleuran extract from Oyster mushrooms has clinical evidence for application with various respiratory diseases. In a human study, chronic obstructive pulmonary disease (COPD) patients treated with 100 mg pleuran, 60 mg vitamin C, and 5 mg zinc had significantly lower incidence and shorter duration of exacerbations compared with the control (60 mg vitamin C and 5 mg zinc).[94]

In another double-blinded, placebo-controlled, randomized multicentric study, 175 children were treated with either pleuran or placebo over a 12-month period. Children treated with pleuran experienced a significant reduction in the frequency of recurrent respiratory tract infections.[90,95] These findings agreed with a Spanish study investigating 166 children aged 1 to 10 years old who were also treated with pleuran for recurrent respiratory infection.[96]

Advantageous respiratory effects of pleuran were also observed in adult athletes. A study included 50 athletes treated with pleuran over a 3-month period of time and found a significant reduction in the frequency of upper respiratory tract infections compared with athletes treated with placebo. Blood samples of the athletes showed significantly higher levels of circulating NK cells in the pleuran group compared with the placebo group.[97]

Antihyperlipidemic. Twenty subjects were randomized to take either one portion of soup containing 30 g dried oyster mushrooms or a tomato soup (placebo) daily for 21 days. Standardized blood concentrations of lipid parameters and oxidized LDLs were measured at baseline and after 21 days. Treatment with Oyster mushroom soup decreased both triacylglycerol and oxidized LDL levels significantly, and showed a significant tendency toward lowering total cholesterol values.[98]

Dosage

Condition	Dose	Timing
Atherosclerosis[98]	30 g dried mushroom	daily
Respiratory support[95,96]	10 mg/kg pleuran (Oyster mushrooms contain ~40% polysaccharide, so whole mushroom dose would be about 25 mg/kg)	daily

Lion's Mane (*Hericium erinaceus*, Yamabushitake, Hedgehog Mushroom, 山伏茸, 猴头菇)

History and Folk Use

Although Lion's Mane is now widely known for its nootropic effects, it was traditionally used in Chinese medicine to reduce gastrointestinal heat. This toothed mushroom can be cooked as food or taken as an extract. Historically, its major use was to lull gastrointestinal inflammation and cool gastrointestinal ulcers (Fig. 90.10).

Pharmacology

The most studied constituents in Lion's Mane are erinacines and hericenones, found in the mycelium and fruiting body, respectively. Erinacines are cyathin diterpenoids and have thus far only been found in mycelium extracts of Lion's Mane. Erinacine A and S have been shown to increase nerve growth factor mRNA expression whereas erinacine E is a kappa opioid receptor agonist, prompting further investigation of potential benefit for opioid addiction.[99] Hericenones are meroterpenoids that have anti-inflammatory effects through the inhibition of the inflammatory transcription factor, NF-κB. Hericerins are aromatic, anti-inflammatory compounds that inhibit COX-2, and reduce iNOS, PGE2, TNF-α, IL-6, and IL-1.[100,101]

Fig. 90.10 *Hericium erinaceus.*

Antifatigue. Animal studies demonstrated significant antifatigue activity through an increase in tissue glycogen content and a decrease in both blood lactic acid and serum urea nitrogen.[102]

Nervous system. There is speculation about the use of Lion's Mane for diabetic peripheral neuropathy, and although animal trials are promising, there is not a known treatment dose for humans. In an animal model, polysaccharides extracted from fresh fruiting bodies of Lion's Mane were administered to rats with peroneal nerve injury, and the rat's ability to respond to heat stimulus and general recovery was significantly improved compared with the control.[103,104] This aqueous extract contains neuroactive compounds that induce nerve growth factor synthase, which is important for promoting the growth and differentiation of neurons.

Another animal model demonstrated that aqueous extract of Lion's Mane fruiting body administered to rats with alloxan-induced diabetic neuropathic pain reversed diabetes-induced thermal hyperalgesia and mechanical allodynia. These diabetic rats also showed significant decrease in serum and urine glucose.[105]

Aqueous extracts also have been shown to ameliorate various Alzheimer's disease-related pathologies. Mycelial extracts containing erinacine A and S have been shown to inhibit plaque growth, diminish activation of glial cells, and promote hippocampal neurogenesis.[106]

An animal study found that Lion's Mane extract had anxiolytic effects with a chronic high-dose administration at 60 mg/kg. After 4 weeks at this dose, there was significantly less depressive behavior in the mice compared with the control group, and an increase in the number of proliferating cells in the subgranular zone of the dentate gyrus, thereby enhancing hippocampal neurogenesis. This proliferation is associated with an increase in nerve growth factor production stimulated by the mushroom extract.[107]

Gastrointestinal effects. In an animal study, mice with alcohol-induced gastric ulcers were administered a fruiting body polysaccharide extract of *H. erinaceus*. There was a significant improvement in treated mice compared with the control—fewer ulcerations, less inflammatory cytokines, and improved overall regulation of gastric secretions—suggesting use as an adjunct therapy for gastric ulcers.[108]

Clinical Applications

Depression and anxiety. The combined anti-inflammatory and nerve growth factor-enhancing actions have proven to reduce depression and anxiety, specifically in menopausal females. In one randomized, double-blind, placebo-controlled clinical trial, women

Fig. 90.11 *Lentinus edodes.*

were given four cookies with .5 g of powdered fruiting body or placebo for 4 weeks. The women who consumed the Lion's Mane cookies experienced significantly less anxiety and depression compared with the placebo.[109]

Gastrointestinal inflammation. Traditionally, Lion's Mane has been used to lull gastrointestinal inflammation and preliminary clinical research suggests that taking *H. erinaceus* before meals daily for 3 months improves symptoms of GI inflammation. In this study, patients who presented with chronic atrophic gastritis were given either Lion's Mane extract or placebo. The Lion's Mane group had significant improvement in upper abdominal pain, dysplasia, and an overall reduction in inflammatory infiltration.[110]

Dosage

Condition	Dose	Timing
Cognitive impairment[111]	1 g	3x/day
Depression and anxiety[109]	2 g	daily

Shiitake (*Lentinula edodes*, 椎茸, 香菇, 표고)

History and Folk Use

The legend goes that 5000 years ago a deity, Shennong, bestowed the world with Shiitake as well as all other medicinal mushrooms. Logs would be inoculated with Shiitake spores during a child's birth, and the child and the mushrooms would mature together until adulthood. At adulthood, the child would be lucky enough to inherit the fortune of the shiitake flush (Fig. 90.11).

Pharmacology

The majority of research regarding Shitake has explored a specific β-D-glucan, lentinan. Lentinan, like other β-D-glucans, has immune-centric qualities including antineoplastic and anti-inflammatory activity.

Antineoplastic. Lentinan inhibits both tumor angiogenesis and the growth of lung and colon cancers by increasing IFN-γ production.[112] Lentinan exerts antitumor effects in colon cancer cells both in vitro and in vivo. Cancer cell apoptosis is induced through two major pathways: the intrinsic pathway via overproduction of reactive oxygen species, and activation of caspase-9 and the extrinsic pathway through the increase of TNF-α, NF-κB inhibition, and activation of caspase-8 and 3.[113] Other studies have concluded that lentinan also inhibits tumor cell proliferation through targeting p53 tumor suppressor gene, increasing tumor cell apoptosis through caspase-3-dependent signaling pathways, and inhibiting angiogenesis through VEGF depression.[114]

Anti-Inflammatory. NF-κB is an important regulatory element for IL-8 expression in human epithelial cells; in an animal study, lentinan suppressed IL-8 gene expression, which indicates lentinan could be beneficial for intestinal inflammatory diseases, like irritable bowel disease (IBD), in humans.[115]

Oral antimicrobial. In vitro studies have explored the effect of Shiitake extracts containing adenine and oxalic acid on common bacteria implicated in caries and gingivitis. Adenine showed 50% biofilm inhibition with *Streptococcus mutans,* and 20% biofilm disaggregation, whereas oxalic acid showed biofilm inhibition of 80% for *Actinomyces naeslundii.*[116,117] The Shiitake mushroom aqueous extract (1 mg/mL) demonstrated antimicrobial activity against 84.6% of 39 microorganisms tested.[118] Sesquiterpenes, steroids, anthraquinone, benzoic acid derivatives and quinolones in shiitake extract all inhibit growth of *S. mutans.* Oil extract is the most efficacious in biofilm inhibition and disruption, potentially due to the carvicrol content in the oil extract, which significantly inhibited and disrupted *P. gingivalis.*[119]

Clinical Applications

Antineoplastic. Patients with gastric cancer were administered either chemotherapy alone or chemo-immunotherapy with lentinan. There was a significantly longer median overall survival time in the lentinan group and the researchers concluded that lentinan should be widely accepted for use as adjunctive chemo-immunotherapy treatment for advanced gastric cancer. The lentinan treated group was administered 2 mg/body (JP: This unclear dosage is what was reported in the study) weight via IV for 30 minutes every 2 to 3 weeks.[120] Lentinan provides synergistic actions with a molecular targeting agent and cytotoxic drugs through the modulation of antibody-dependent cellular toxicity and programmed cell death, which may support the idea that the chemo-immunotherapy prolongs the survival of metastatic gastric cancer patients, compared with chemotherapy alone.[121]

A case of recurrent ovarian cancer was successfully treated with immunotherapy alongside 2 mg IV lentinan administered every 2 weeks. In this case study, lentinan changed the Th1/Th2 balance such that the Th1 level exceeded Th2. The authors proposed that lentinan worked on cervical lymph node metastatic cancer by evading the immune surveillance system.[122]

Other studies exploring the use of lentinan as an adjunct to chemotherapy found less adverse side effects from the chemotherapy, improved IFN-γ production, and increased NK cell count.[123]

Dosage

Few clinical trials have been done with humans consuming whole mushroom or whole mushroom extract, and most research on Shiitake focuses on lentinan and cancer. This research is hopeful, yet it leaves the dosage for whole Shiitake mushroom unknown. Based on research with other mushrooms, dosage is most likely to be at least 3 g/day for immune-centric effects.

Agaricus blazei (Kawarihiratake, Himematsutake, 姬松茸)

History and Folk Use

Agaricus blazei is a mushroom originally native to a small village Piedade, in the highland areas in the province of Sao Paulo, Brazil.

Although originally from Brazil, this mushroom is now used commonly throughout Asia as food and medicine and is traditionally believed to fight physical and emotional stress.

Pharmacology

Antineoplastic. Antineoplastic activity was observed in three separate mouse studies, showing an increase in CD69 and CD49 T-cells in mouse colon cancer and an increase in CD3, CD19 and NK cell activity in mouse leukemia.[125,126,127] In vivo animal models and in vitro research have elucidated many antineoplastic mechanisms with an array of various malignancies; the most notable in vivo mechanisms were a decrease in metastases of both ovarian and lung cancers.[127]

In vitro human hepatocarcinoma and osteosarcoma cells treated with *A. blazei* polysaccharide extract had an increase in percent of apoptosis, and a decrease in cell growth.[128,129]

Antimicrobial. Hot water extract shows antiquorum sensing activity against *Pseudomonas aeruginosa,* an important antibacterial mechanism that should be further explored.[130]

Antioxidant. Phenolic compounds from *A. blazei* showed higher antioxidant activity compared with Shiitake.[131]

Clinical Applications

Cancer. *A. Blazei* mushroom extract was administered to gynecological cancer patients undergoing chemotherapy. Blood was drawn a few weeks after administration, and NK cell count was significantly higher in the mushroom extract group compared with the placebo group, and there was a significant reduction in chemotherapy-associated side effects.[132]

Diabetes. Either *Agaricus blazei* fruiting body extract (AbM) or placebo (cellulose) was administered in a 5-month trial with 536 registered patients with diabetes who had been taking gliclazide and metformin for more than 6 months. Subjects took either 500 mg of AbM extract or cellulose three times a day for 12 weeks; the capsule was taken with glicliazide 30 minutes before eating and metformin was taken 30 minutes after eating. The AbM group had improved insulin resistance, possibly due to an increase in adiponectin concentration after the 12 week treatment.[133]

Dosage

Condition	Dose	Timing
Diabetes adjunct[133]	500 mg	3x/day

Enokitake (*Flammulina velutipes*, えのき茸, 팽이버섯)

History and Folk Use

Enokitake has been consumed as food for millennia throughout Asia. Not surprisingly, an epidemiological survey of cancer deaths found that Enokitake mushroom farmers (who were consuming a lot of enokitake mushrooms) had lower rates of cancer deaths than controls who were not involved in farming.

Pharmacology

Similar to other medicinal fungi, Enokitake has an affinity for the immune system and contributes to cytotoxicity in cancer cells. The isolated constituent proflamin prolongs the survival of cancer-stricken mice by 85%.[134]

Microbiome modulation. An intriguing in vivo animal study found that Enokitake polysaccharide extract improves scopolamine-induced learning and memory impairment in mice by modulating gut microbiota composition. Through modulating the gut microbiome to a more favorable composition, there was a reduction in neuroinflammation and, therefore, an antagonizing effect of the scopolamine-induced memory deficit.[135]

Antimicrobial. In vitro research has demonstrated antioxidant and antimicrobial activity against *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtillus.*[136]

Anti-Inflammatory. A recent review article explains that the immunomodulatory protein, FIP-fve, elicits its immunomodulatory effects by shifting toward a Th1 immune response. FVP-fve also successfully suppresses airway inflammation and hyper-responsiveness via inhibition of inflammatory cell infiltration and Th2 cytokines. This protein also inhibits host dust mite-induced asthma inflammation in mice.[137]

The water-soluble polysaccharide FVP-2 enhanced growth of primary hepatocytes from mice in vitro via inhibition of intracellular alanine aminotransferase (ALT) and portrayed anti-inflammatory effects via inhibition of iNOS, TNF-α, and COX-2 in vitro.[137]

Fig. 90.12 *Fomitopsis pinicola.*

Dosage

There are not enough clinical studies to propose specific dosage.

II. MUSHROOMS WITH LABORATORY AND ANIMAL RESEARCH BUT INSUFFICIENT HUMAN CLINICAL TRIALS

The following mushrooms have encouraging in vitro research and in vivo animal studies. Similar to the previously mentioned mushrooms, they have antineoplastic, anti-inflammatory, immune-modulating, and antioxidant qualities. These mushrooms have a long history of use, but a lack of human clinical trials.

Red Belted Polypore (*Fomitopsis pinicola*)

History and Folk Use

F. pinicola is one of the most commonly found polypore mushrooms throughout the world. Traditionally, it has been used for treatment of headache, nausea, liver problems, as a styptic, and as an anti-inflammatory agent. Interestingly, during the 1800s, eclectic physicians soaked the polypore in whiskey as a remedy for malaria-like symptoms (Fig. 90.12).

Fig. 90.13 *Phellinus igniarius.*

Pharmacology

Antineoplastic. In vitro, *F. pinicola* extract proves to employ many antineoplastic actions inhibiting cell migration, inducing ROS-dependent apoptosis, and causing p53 mediated G1 phase arrest in human colorectal cancer cells. The researchers postulate that ergosterol may play a role in these processes.[138]

Antioxidant. In vitro, both extracellular and intracellular polysaccharides consisting of mannose, rhamnose, xylose, and galactose portray strong free radical scavenging abilities, reducing UV- and H_2O_2-induced oxidative damage.[139] Another study investigating the antioxidant properties of *F. pinicola* uncovered that the antioxidant effects were mostly likely caused by the phenolic compounds contained in the mushroom extract.[140]

Anti-Inflammatory. Ethanol extract of Red Belted Polypore fresh fruiting bodies contains a number of lanostane triterpenes,[141] fomitopinic acids, and lanostane triterpene glycosides, fomitosides. In vitro, this extract selectively inhibits COX-2.[142]

Fire Sponge (*Phellinus linteus, Phellinus igniarius,* Mesima, Willow bracket)

History and Folk Use

The Yup'ik of Western Alaska called the fungus *arak,* and the mixture of tobacco and the ash *iqmik*—"thing to put in the mouth." It has been reported that 52% of First Nations people used this fungus. The mushroom was traditionally burnt to an ash and smoked with tobacco[143] (Fig. 90.13).

Pharmacology

Phellinus spp. contain two noteworthy phenolic compounds—phelligridin and hispolon. Phelligridins are antiviral,[144] whereas hispolon has analgesic, anti-inflammatory, and antineoplastic qualities.[19,145]

Antineoplastic. In vitro, hispolon has significant antitumor activity.[146,147] In one study exploring antineoplastic mechanisms of hispolon on human lung cancer cells, the proposed mechanism of action was an induction of G0/G1 cell cycle arrest.[146]

Ethanol extracts inhibit the proliferation of human hepatocarcinoma cell lines as well as rat heart vascular endothelial cells. When the extract was given in combination with chemotherapy, there was a synergistic inhibition of the proliferation of hepatocarcinoma.[148] This study and others suggest hepatoprotective qualities for the ethanol extract.[146]

Antiviral. The water extract is effective against influenza virus A and B, including H1N1, H2N3, and Avian flu. The extract interferes with events in the virus replication cycle, including viral attachment to the target cell.[149] Fruiting body extracts inhibit neuraminidase from H3N2, H1N1, and H5N1 influenza viruses.[144]

Immune modulation. The biologically active compounds that modulate the immune system have been found to have therapeutic value for slowing multiple sclerosis progression in mice.[150] After 3 weeks of being injected with the extract every other day, demyelination and immune cell infiltrations in the spinal cord were examined and there was a significant decrease in the daily incidence rate and clinical score of autoimmune encephalomyelitis.

Witch's Butter (*Tremella fuciformis, Tremella aurantia, Tremella mesenterica,* 雪耳)

History and Folk Use

In Chinese medicine this slimy mushroom is used for cooling, moistening, and nourishing the lungs, stomach, and kidneys. It has been used in body care products throughout Asia and is said to provide proper moisture to the skin. The traditional use for skin elasticity is to brew fruiting bodies as a thick gelatinous tea and apply to the skin with a cotton pad, rinsing after 30 minutes.

Pharmacology

Pharmacologically active polysaccharides make up the bulk of the fruit body at 60% to 90%, whereas with other medicinal mushrooms, polysaccharides make up a much smaller part of the biomass (closer to 10%–40%). These polysaccharides, like all fungal polysaccharides, demonstrate antioxidant and immune-modulating activities.[151]

Antidiabetic. Intraperitoneal and oral administration with a water-soluble polysaccharide from *T. fuciformis* and *T. aurantia* had significant hypoglycemic activity in normal mice and had significant antihyperglycemic activity in mice with streptozotocin-induced and genetic diabetes. These antidiabetic effects result from the increase in insulin secretion and the acceleration in glucose metabolism in the liver.[151]

Animal models administered with *T. mesenterica* had significantly decreased 2-hour postprandial serum concentrations of insulin without altering blood glucose concentrations. These results suggest that *T. mesenterica* has the ability to increase the insulin sensitivity, instead of increasing the insulin secretion, in normal rats.[153]

Clinical Applications

Cognitive impairment. Seventy-five subjects were randomly allocated to receive supplementation with *T. fuciformis;* this randomized clinical trial demonstrated that oral administration of *T. fuciformis* improved subjective memory complaints and cognitive performance in individuals with subjective cognitive impairment. Treatment was given in low doses of 600 mg per day or high doses of 1200 mg per day and there was significant improvement with short-term memory and executive performance in the high-dose treatment group. *T. fuciformis* supplementation was also associated with increases in gray matter volumes of several brain regions.[154]

Dosage

Condition	Dose	Timing
Subjective cognitive impairment[154]	1200 mg	daily

Fig. 90.14 *Psilocybe cyanescens.*

Psilocybe spp.

Psilocybe mushrooms have been used as ceremonial entheogens by cultures all over the world. In recent years, there has been a significant number of in vivo human trials exploring the benefits of psilocybin, an alkaloid derived from this mushroom. Psilocybin is being investigated for more complex psycho-emotional disorders such as addiction,[155-158] depression,[159] and end-of-life cancer-related psychological distress[160,161] (Fig. 90.14).

TOXICOLOGY

Many of these medicinal mushrooms are consumed as food in various parts of the world and are considered safe even when consumed in large quantities on a regular basis. One should be aware, however, that because some of these mushrooms can chelate heavy metals from their substrate, growing conditions must always be considered. Medicinal mushrooms must be obtained from dependable, certified organic sources.

Although mild side effects were reported in the literature, as with most natural health products, a practitioner will rarely run into these problems.

There were two reported cases of hepatic toxicity linked to the long-term use of Reishi.[162,163] There was also a case of chronic diarrhea in a 49-year-old man with non-Hodgkin lymphoma after prolonged consumption of Reishi extract.[164]

Prolonged consumption of Shiitake was linked to a few cases of dermatitis and photosensitivity.[165,166] Eosinophilia and gastrointestinal upsets were also observed with the use of Shiitake.[167]

DRUG INTERACTIONS

Drug interactions with medicinal mushrooms are more theoretical than clinically proven. The most common category of these drug interactions is one of "theoretical accumulation." Many of these mushrooms work on blood sugar, blood pressure, and blood lipid issues—the type of concerns for which patients are often already prescribed pharmaceuticals. Frequently, after consuming mushroom preparations together with the drugs for a month or so, the patient will require less or no pharmaceuticals for these conditions moving forward. These effects become less about drug–mushroom interactions and more about the mushroom preparation exerting its effects. Using mushroom preparation in diabetic or hypertensive patients requires close blood sugar monitoring during the first few months of treatment as the pharmaceutical dosage may require adjustment. The mushroom that has been highlighted the most in this area is Reishi because of its large range of therapeutic uses and the large number of people consuming it. Although anticoagulant, antiplatelet, immunosuppressant, and cytochrome p450 interaction concerns can be found in the literature, it is difficult to find research on actual cases of these problems. Again, concerns seem to lie more in the realms of theory and caution than actual cases.

SUMMARY

We have discussed a wide variety of potential medicinal applications for a wide variety of mushrooms, and many have extensive in vitro and in vivo research to support their efficacy. There is, however, a great need for more human clinical trials, though the trials that have been completed show positive therapeutic outcomes with few, if any, harmful side effects. There is wide use of mushroom polysaccharides as adjunctives to cancer therapies throughout Asia, though these strategies are underused in the United States. Recent interest in medicinal mushrooms has improved availability for lay people, but there continues to be little clinical use. As a consequence, there is still much to learn about specific dosage for many conditions, and further research is warranted.

A NOTE ABOUT PURCHASING MUSHROOM SUPPLEMENTS

When purchasing medicinal mushroom products, it is important to understand the extraction methods used in production. Mushrooms must be heated to properly extract the water-soluble polysaccharides, and cold ethanol extracts are not an appropriate delivery method for these compounds. When purchasing mushroom products as a liquid, the extract should have gone through a dual extraction process—with hot water and ethanol. When purchasing capsules, there should be powdered extract within the capsules, and if the label simply reads "mushroom powder," it is a good indication that the contents will be unprocessed mushroom powders that are generally indigestible. If a product is mycelium-based, the dose will most likely need to be higher because it will be diluted by the substrate the mycelium was growing on.

REFERENCES

See www.expertconsult.com for a complete list of references.

SUMMARY OF RESEARCH (TABLES 90.1 AND 90.2)

TABLE 90.1 Human Trials

Condition	Mushrooms	Dose Used in Trial
Bladder outlet obstruction	Reishi[46]	6 mg extract/day
Cancer treatment adjunct	Reishi[50]	1800 mg extract/day
	Turkey Tail[74-76]	3–9 g extract/day
	Maitake[86]	5–7 mg extract/kg/day
	Shiitake[121-123]	2 mg lentinan injections
	Agaricus[132]	
Oral HPV	Reishi and Turkey Tail[48]	400 mg/day
Chronic fatigue syndrome	Reishi[49]	2 g extract/day
Endurance support	Reishi and Cordyceps[54]	1170 mg/day (taken together)
		1335 mg/day
Chronic kidney disease	Cordyceps[66,67]	3–12 g/day
Microbiome dysregulation	Turkey Tail[73]	1200 mg PSP 3x/day
Psoriasis with GI inflammation	Chaga[84]	1 Tbs extract 3x/day
PCOS	Maitake[89]	1 g 3x/day
COPD	Oyster[94]	100 mg pleuran/day
Respiratory tract infections	Oyster[96,97]	10 mg pleuran/kg/day
Hyperlipidemia	Oyster[98]	30 g whole mushroom daily
Cognitive impairment	Lion's mane[111]	1 g 3x/day
	Tremella[154]	1200 mg/day
Depression and anxiety	Lion's Mane[109]	2 g daily
Diabetes	Agaricus[133]	500 mg 3x/day

TABLE 90.2 Proposed Actions From In Vitro Research and In Vivo Animal Studies

Action	Mushrooms	Microbes/Cancer Type	Compounds
Antioxidant	*Agaricus blazei*[169]		Ascorbic acid, carotenoids, ergothioneine, phenolic compounds, superoxide dismutase, tocopherols[168]
	Chaga[170-172]		
	Enokitake[173]		
	Maitake[174]		
	Reishi[175,176]		
	Shiitake[177,178]		
	Oyster[91]		
Antimicrobial	*Ganoderma spp.*[179-181]	*Staphylococcus aureus*	Sesquiterpene hydroquinones
	Shiitake[182,183,116,118,119]	*Streptococcus mutans*	
	Enokitake[136]	*Actinomyces naeslundii*	
	Agaricus blazei[130]	*Streptococcus mutans*	
		Porphyromonas gingivalis	
		Staphylococcus aureus	
		Escherichia coli	
		Bacillus subtilus	
		Pseudomonas aeruginosa	
Antiviral	Reishi[184]	HIV-1, H1N1, H5N1, HSV-2	Triterpenes
	Chaga[185]	Influenza A and B, HIV	PSK
	Shiitake[186-188]	HIV	Phelligridins
	Maitake[189]	HIV	
	Turkey Tail[190,191]	HIV	
	Phellinus spp.[144,149]	H1N1, H2N3, Avian flu, H3N2, H5N1	
Antitumor	Shiitake[112-114]	Lung, colon	Lentinan
	Cordyceps[63,64]	Glioblastoma	Cordycepin
	Red Belted Polypore[13,138]	Colorectal	Triterpenes
	Phellinus spp.[146,147]	Lung, liver	Hispolon
	Ganoderma spp.[41-43]	Cervical, breast, liver, lymphoma, leukemia, lung, colon	Triterpenes
	Turkey Tail[71,72]	Colorectal, breast	PSK
	Agaricus blazei[124-129]	Colon, leukemia, ovarian, lung, hepatocarcinoma, osteocarcinoma	Polysaccharide
	Maitake[85,87]	Prostate, bladder, liver, brain, blood, breast	MD fraction

Continued

TABLE 90.2 Proposed Actions From In Vitro Research and In Vivo Animal Studies—cont'd

Action	Mushrooms	Microbes/Cancer Type	Compounds
Antiallergic	*Ganoderma* spp.[39,192,193]		Triterpenes
Antiatherogenic	*Ganoderma* spp.[194,195]		Triterpenes
	Agaricus blazei[196,197]		β-glucans
	Shiitake[198]		Eritadine
	Oyster[91,199]		Chrysin, Lovastatin
Anti-inflammatory	Reishi[41]		Terpenes
	Chaga[81]		Phenolics, β-glucans
	Lion's Mane[100,101]		Hericenones, Hericerins
	Shiitake[115]		Lentinan
	Enokitake[137]		FVP-fve, FVP-2
	Red Belted Polypore[140]		Fomitopinic acid, Fomitosides
	Phellinus spp.[19,145]		Hispolon
	Maitake[174]		Ergosterol
Hypoglycemic	Maitake[200-202]		β-glucans
	Reishi[203-205]		Ganoderan A and B
	Turkey Tail[206]		PSP
	Chaga[207]		β-glucans
	Agaricus blazei[133,197,208]		β-glucans
	Cordyceps[209-211]		β-glucans
	Tremella spp.[152,153]		
Hepato-protective	*Ganoderma* spp.[211-214]		Triterpenes
Nootropic	Lion's Mane[103-105]		Erinacines, polysaccharides

REFERENCES

1. *Mushroom Market Analysis, Market Size, Application Analysis, Regional Outlook, Competitive Strategies, and Forecasts*—2015 to 2022. (n.d.). Retrieved from: https://www.grandviewresearch.com/industry-analysis/mushroom-market. Accessed 9 Sept 2019.
2. Hawksworth DL. Mushrooms: the extent of the unexplored potential. *Int J Med Mushrooms*. 2001;3:333–337.
3. Baert K, Sonck E, Goddeeris BM, Devriendt B, Cox E. Cell type-specific differences in β glucan recognition and signalling in porcine innate immune cells. *Dev Comp Immunol*. 2015;48(1):192–203. https://doi.org/10.1016/j.dci.2014.10.005.
4. Friedman M. Mushroom polysaccharides. *Chemistry*. 2016. https://doi.org/10.3390/foods5040080.
5. Vitak T, Yurkiv B, Wasser S, Nevo E, Sybirna N. Effect of medicinal mushrooms on blood cells under conditions of diabetes mellitus. *World J Diabetes*. 8(5):187–201. https://doi.org/10.4239/wjd.v8.i5.187.
6. Chen Y, Liu Y, Rahman M, Yan X, Yang C. Structural characterization and antidiabetic potential of a novel heteropolysaccharide from grifola frondosa via IRS1 / PI3K-JNK signaling pathways. *Carbohydr Polym*. 2018;198(15):452–461. https://doi.org/10.1016/j.carbpol.2018.06.077.
7. Wang H, Liu YM, Qi ZM, et al. An overview on natural polysaccharides with antioxidant properties. *An Overview on Natural Polysaccharides with Antioxidant Properties*. 2015. https://doi.org/10.2174/0929867311320230006.
8. Yu Z, Liu B, Mukherjee P, Newburg DS. Trametes versicolor extract modifies human fecal microbiota composition. *In Vitro*. 2013:107–112. https://doi.org/10.1007/s11130-013-0342-4.
9. Palacios S, Losa F, Dexeus D, Cortés J. Beneficial effects of a *Coriolus versicolor*-based vaginal gel on cervical epithelization, vaginal microbiota and vaginal health: a pilot study in asymptomatic women. *BMC Womens Health*. 2017:4–9. https://doi.org/10.1186/s12905-017-0374-2.
10. Ríos JL. Effects of triterpenes on the immune system. *J Ethnopharmacol*. 2010;128(1):1–14. https://doi.org/10.1016/j.jep.2009.12.045.
11. Grienke U, Kaserer T, Pfluger F, et al. Accessing biological actions of ganoderma secondary metabolites by in silico profiling. *Phytochemistry*. 2015;114:114–124. https://doi.org/10.1016/j.phytochem.2014.10.010.
12. Bhattarai G, Lee Y-H, Lee N-H, et al. Fomitoside-K from fomitopsis nigra induces apoptosis of human oral squamous cell carcinomas (YD-10B) via mitochondrial signaling pathway. *Biol Pharm Bull*. 2012;35(10):1711–1719. https://doi.org/10.1248/bpb.12-00297.
13. Ren G, Liu XY, Zhu HK, Yang SZ, Fu CX. Evaluation of cytotoxic activities of some medicinal polypore fungi from China. *Fitoterapia*. 2006;77(5):408–410. https://doi.org/10.1016/j.fitote.2006.05.004.
14. Jin X, Ruiz Beguerie J, Sze DM-Y, Chan GCF. Ganoderma lucidum (Reishi mushroom) for cancer treatment. *Cochrane Database Syst Rev*. 2016;4:CD007731. https://doi.org/10.1002/14651858.CD007731.pub3.
15. Zhu Q, Bang TH, Ohnuki K, Sawai T, Sawai K, Shimizu K. Inhibition of neuraminidase by ganoderma triterpenoids and implications for neuraminidase inhibitor design. *Sci Rep*. 2015;5:13194. https://doi.org/10.1038/srep13194.
16. Teplyakova TV, Psurtseva NV, Kosogova TA, Mazurkova NA, Khanin VA, Vlasenko VA. Antiviral activity of polyporoid mushrooms (higher basidiomycetes) from altai mountains (Russia). *Int J Med Mushrooms*. 2012;14(1):37–45. https://doi.org/10.1615/IntJMedMushr.v14.i1.40.
17. Gao L, Sun Y, Si J, et al. Cryptoporus volvatus extract inhibits influenza virus replication in vitro and in vivo. *PLoS One*. 2014;9(12). https://doi.org/10.1371/journal.pone.0113604.
18. Lee I, Kim Y, Jang Y, Jung J, Yun B. New antioxidant polyphenols from the medicinal mushroom Inonotus obliquus. *Bioorg Med Chem Lett*. 17:6678–6681. https://doi.org/10.1016/j.bmcl.2007.10.072.
19. Chang H, Sheu M, Yang C, et al. Analgesic effects and the mechanisms of anti-inflammation of hispolon in mice. *Evid Based Complement Alternat Med*. 2011;2011:478246. https://doi.org/10.1093/ecam/nep027.
20. Hong D, Park MINJU, Jang EUNH, Jung BOM, Kim NAMJ, Kim JHO. Hispolon as an inhibitor of TGF-β-induced epithelial-mesenchymal transition in human epithelial cancer cells by co-regulation of TGF-β-snail/twist axis. *Oncol Lett*. 2017:4866–4872. https://doi.org/10.3892/ol.2017.6789.
21. Bashardoust B, Zakaeri A, Fouladi N, Izadi Z, Hosseini F. Treatment effect of ergocalciferol on bone metabolism indexes and parathyroid hormone in hemodialysis patients. *Iran J Kidney Dis*. 2018;12(6):359–363.
22. Gründemann D. The ergothioneine transporter controls and indicates ergothioneine activity—a review. *Prev Med (Baltim)*. 2012. https://doi.org/10.1016/j.ypmed.2011.12.001.
23. Halliwell B, Cheah IK, Drum CL. Biochemical and biophysical research communications ergothioneine, an adaptive antioxidant for the protection of injured tissues? A hypothesis. *Biochem Biophys Res Commun*. 2016;470(2):245–250. https://doi.org/10.1016/j.bbrc.2015.12.124.
24. Paul BD, Snyder SH. The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death Differ*. 2010. https://doi.org/10.1038/cdd.2009.163.
25. Weigand-heller AJ, Kris-etherton PM, Beelman RB. The bioavailability of ergothioneine from mushrooms (Agaricus bisporus) and the acute effects on antioxidant capacity and biomarkers of inflammation. *Prev Med (Baltim)*. 2018;54(2012):S75–S78. https://doi.org/10.1016/j.ypmed.2011.12.028.
26. Cheah IK, Tang RMY, Yew TSZ. Administration of pure ergothioneine to healthy human subjects: uptake, metabolism, and effects on biomarkers of oxidative damage and inflammation. *Antioxid Redox Signal*. 26(5):193–207. https://doi.org/10.1089/ars.2016.6778.
27. Song TY, Chen CL, Liao JW, Ou HC, Tsai MS. Ergothioneine protects against neuronal injury induced by cisplatin both in vitro and in vivo. *Food Chem Toxicol*. 2010. https://doi.org/10.1016/j.fct.2010.09.030.
28. Kerley RN, McCarthy C, Kell DB, Kenny LC. The potential therapeutic effects of ergothioneine in pre-eclampsia. *Free Radic Biol Med*. 2018. https://doi.org/10.1016/j.freeradbiomed.2017.12.030.
29. Cheah IK, Halliwell B. Ergothioneine; antioxidant potential, physiological function and role in disease. *Biochim Biophys Acta-Mol Basis Dis*. 2012. https://doi.org/10.1016/j.bbadis.2011.09.017.
30. Dubost NJ, Ou B, Beelman RB. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chem*. 2007. https://doi.org/10.1016/j.foodchem.2007.01.030.
31. Liang D, Yong T, Chen S, et al. Hypouricemic effect of 2 , 5-dihydroxyacetophenone, a computational screened bioactive compound from ganoderma applanatum, on hyperuricemic Mice. *Int J Mol Sci*. 2018. https://doi.org/10.3390/ijms19051394.
32. Lin K, Chen Y, Yang S, Wei B, Hung C, Lin C. Fitoterapia xanthine oxidase inhibitory lanostanoids from ganoderma tsugae. *Fitoterapia*. 2013;89:231–238. https://doi.org/10.1016/j.fitote.2013.06.006.
33. Yong T, Chen S, Xie Y, et al. Hypouricemic effects of ganoderma applanatum in hyperuricemia mice through OAT1 and GLUT9. *Front Pharmacol*. 2018;8:1–11. https://doi.org/10.3389/fphar.2017.00996.
34. Nahata A, Dixit VK. Ganoderma lucidum is an inhibitor of testosterone-induced prostatic hyperplasia in rats. *Andrologia*. 2012;44(suppl 1):160–174. PubMed PMID: 21651601. https://doi.org/10.1111/j.1439-0272.2010.1155.x. Epub 2011 Jun 8.
35. Shrivastava A, Gupta VB. Various treatment options for benign prostatic hyperplasia: a current update. *J Midlife Health*. 2012;3(1):10–19. PubMed PMID: 22923974; PubMed Central PMCID: PMC3425142. https://doi.org/10.4103/0976-7800.98811.
36. Liu J, Shimizu K, Konishi F. The anti-androgen effect of ganoderol B isolated from the fruiting body of Ganoderma lucidum. *Bioorg Med Chem*. 2007;15(14):4966–4972. https://doi.org/10.1016/j.bmc.2007.04.036.
37. Wu S. International journal of Biological macromolecules hypolipidaemic and anti-lipidperoxidant activities of ganoderma lucidum polysaccharide. *Int J Biol Macromol*. 2018;118:2001–2005. https://doi.org/10.1016/j.ijbiomac.2018.07.082.
38. Hsu P, Lin Y, Ni H, Mo F. Ganoderma triterpenoids exert antiatherogenic effects in mice by alleviating disturbed flow-induced oxidative stress and Inflammation. *Oxid Med Cell Longev*. 2018; 2018:1–11. https://doi.org/10.1155/2018/3491703.
39. Chen ML, Hsieh CC, Chiang BL, Lin BF. Triterpenoids and polysaccharide fractions of ganoderma tsugae exert different effects on antiallergic activities. *Evidence-based Complement Altern Med*. 2015;2015. https://doi.org/10.1155/2015/754836.

40. Ma H, Hsieh J, Chen S. Phytochemistry anti-diabetic effects of ganoderma lucidum. *Phytochemistry*. 2015;114:109–113. https://doi.org/10.1016/j.phytochem.2015.02.017.
41. Elkhateeb WA, Zaghlol GM, El-Garawani IM, Ahmed EF, Rateb ME, Abdel Moneim AE. Ganoderma applanatum secondary metabolites induced apoptosis through different pathways: in vivo and in vitro anticancer studies. *Biomed Pharmacother*. 2018;101:264–277. https://doi.org/10.1016/j.biopha.2018.02.058.
42. Cheng S, Sliva D. Ganoderma lucidum for cancer treatment: we are close but still not there. *Integr Cancer Ther*. 2015;14(3):249–257. https://doi.org/10.1177/1534735414568721.
43. Nguyen VT, The N, Dao T, et al. Cytotoxic and anti-angiogenic effects of lanostane triterpenoids from Ganoderma lucidum. *Phytochem Lett*. 2015;12:69–74. https://doi.org/10.1016/j.phytol.2015.02.012.
44. Zhu Q, Bang TH, Ohnuki K, Sawai T, Sawai K, Shimizu K. Inhibition of neuraminidase by ganoderma triterpenoids and implications for neuraminidase inhibitor design. *Sci Rep*. 2015;5:13194. https://doi.org/10.1038/srep13194.
45. Eo SK, Kim YS, Lee CK, Han SS. Antiviral activities of various water and methanol soluble substances isolated from ganoderma lucidum. *J Ethnopharmacol*. 1999;68(1–3):129–136. https://doi.org/10.1016/S0378-8741(99)00067-7.
46. Noguchi M, Kakuma T, Tomiyasu K, Kurita Y, Kukihara H, Konishi F. Effect of an extract of ganoderma lucidum in men with lower urinary tract symptoms: a double-blind, placebo-controlled randomized and dose-ranging study. *Asian J Androl*. 2008;10(4):651–658. https://doi.org/10.1111/j.1745-7262.2008.00336.x.
47. Gao Y, Zhou S, Jiang W, Huang M. Effects of ganopoly (A ganoderma lucidum polysaccharide extract) on the immune functions in advanced-stage cancer patients. *Immunol Invest*. 2003;32(3):201–215. https://doi.org/10.1081/IMM-120022979.
48. Donatini B. Control of oral human papillomavirus (HPV) by medicinal mushrooms, trametes versicolor and ganoderma lucidum: a preliminary clinical trial. *Int J Med Mushrooms*. 2014;16(5):497–498. https://doi.org/10.1615/IntJMedMushrooms.v16.i5.80.
49. Soksawatmakhin S, Boonyahotra W. Preliminary study of the applications of *Ganoderma lucidum* in chronic fatigue syndrome. *Journal of Asian Association of Schools of Pharmacy*. 2013:262–268.
50. Soo TS. Effective dosage of the extract of ganoderma lucidum in the treatment of various ailments. *Mushroom Biol Mushroom Prod*. 1996:177–185.
51. Du F, Li S, Wang T, et al. Cordyceps sinensis attenuates renal fibrosis and suppresses BAG3 induction in obstructed rat kidney. *Am J Transl Res*. 2015;7(5):932–940.
52. Wang Y, Yin H, Lv X, Wang Y, Gao H, Wang M. Fitoterapia protection of chronic renal failure by a polysaccharide from cordyceps sinensis. *Fitoterapia*. 2010;81(5):397–402. https://doi.org/10.1016/j.fitote.2009.11.008.
53. Song J, Wang Y, Teng M, et al. Studies on the antifatigue activities of cordyceps militaris fruit body extract in mouse model. *Evid Based Complement Alternat Med*. 2015;2015. https://doi.org/10.1155/2015/174616.
54. Rossi P, Buonocore D, Altobelli E, et al. Improving training condition assessment in endurance cyclists: effects of ganoderma lucidum and ophiocordyceps sinensis dietary supplementation. *Evid Based Complement Alternat Med*. 2014;2014. https://doi.org/10.1155/2014/979613.
55. Ballard HJ. Invited Review ATP and adenosine in the regulation of skeletal muscle blood flow during exercise. *Sheng Li Xue Bao*. 2014;66(1):67–78. https://doi.org/10.13294/j.aps.2014.0009.
56. Liu, Ailling Wu J. The inhibitory mechanism of Cordyceps sinensis on cigarette smoke extract-induced senescence in human bronchial epithelial cells. *Int J COPD*. 2016;11:1721–1731.
57. Chiou Y, Lin C. The Extract of cordyceps sinensis Inhibited airway inflammation by blocking NF-κ B activity. *Inflammation*. 2012;35(3). https://doi.org/10.1007/s10753-011-9402-9.
58. Hsu C, Sun H, Sheu J, et al. Effects of the immunomodulatory agent cordyceps militaris on airway inflammation in a mouse asthma model. *Pediatr Neonatol*. 2008;49(5):171–178. https://doi.org/10.1016/S1875-9572(09)60004-8.
59. Singh M, Tulsawani R, Koganti P, Chauhan A, Manickam M, Misra K. Cordyceps sinensis increases hypoxia tolerance by inducing heme oxygenase-1 and metallothionein via Nrf2 activation in human lung epithelial cells. *Biomed Res Int*. 2013;2013:1–13. https://doi.org/10.1155/2013/569206.
60. Yang X, Li Y, He Y, et al. International immunopharmacology cordycepin alleviates airway hyperreactivity in a murine model of asthma by attenuating the inflammatory process. *Int Immunopharmacol*. 2015;26(2):401–408. https://doi.org/10.1016/j.intimp.2015.04.017.
61. Lei J, Wei Y, Song P, Li Y, Zhang T, Feng Q. Cordycepin inhibits LPS-induced acute lung injury by inhibiting in fl ammation and oxidative stress. *Eur J Pharmacol*. 2018;818:110–114. https://doi.org/10.1016/j.ejphar.2017.10.029.
62. Fei X, Zhang X, Zhang G, Bao W, Zhang Y, Zhang M. Sciencedirect cordycepin inhibits airway remodeling in a rat model of chronic asthma. *Biomed Pharmacother*. 2017;88:335–341. https://doi.org/10.1016/j.biopha.2017.01.025.
63. Khan MA, Tania M. Cordycepin in anticancer research: molecular mechanism of therapeutic effects. *Curr Med Chem*. 2018. PubMed PMID: 30277143. https://doi.org/10.2174/0929867325666181001105749. [Epub ahead of print].
64. Hueng DY, Hsieh CH, Cheng YC, Tsai WC, Chen Y. Cordycepin inhibits migration of human glioblastoma cells by affecting lysosomal degradation and protein phosphatase activation. *J Nutr Biochem*. 2017;41:109–116. PubMed PMID: 28068557. https://doi.org/10.1016/j.jnutbio.2016.12.008. Epub 2016 Dec 29.
65. Drewes SE, George J, Khan F. Recent findings on natural products with erectile-dysfunction activity. *Phytochemistry*. 2003;62:1019–1025.
66. Zhang Y, Du Y, Yu H, Zhou Y, Ge F. Protective effects of ophiocordyceps lanpingensis on glycerol-induced acute renal failure in mice. *J Immunol Res*. 2017;2017:2012585. PubMed PMID: 29159186; PubMed Central PMCID: PMC5660786. https://doi.org/10.1155/2017/2012585. Epub 2017 Oct 12.
67. Ong BY, Aziz Z. Efficacy of Cordyceps sinensis as an adjunctive treatment in kidney transplant patients: a systematic-review and meta-analysis. *Complement Ther Med*. 2017;30:84–92. https://doi.org/10.1016/j.ctim.2016.12.007.
68. Kang HJ, Baik HW, Kim SJ, et al. Cordyceps militaris enhances cell-mediated immunity in healthy Korean men. *J Med Food*. 2015;18(10):1164–1172. https://doi.org/10.1089/jmf.2014.3350.
69. Buhner SH. *Herbal Antivirals*. North Adams, MA: Storey Publishing; 2013.
70. Yu Z, Liu B, Mukherjee P, Newburg DS. Trametes versicolor extract modifies human fecal microbiota composition in vitro. *Plant Foods Hum Nutr*. 2013:107–112. https://doi.org/10.1007/s11130-013-0342-4.
71. Yang Y, Inatsuka C, Gad E, et al. Protein-bound polysaccharide-K induces IL-1 b via TLR2 and NLRP3 inflammasome activation. *Innate Immun*. 2014. https://doi.org/10.1177/1753425913513814.
72. Standish LJ, Wenner CA, Sweet ES, et al. Trametes versicolor mushroom immune therapy in breast cancer. *J Soc Integr Oncol*. 2008;6(3):122–128. https://doi.org/10.2310/7200.2008.0016.
73. Pallav K, Dowd SE, Villafuerte J, et al. Effects of polysaccharopeptide from Trametes versicolor and amoxicillin on the gut microbiome of healthy volunteers: a randomized clinical trial. *Gut Microbes*. 2014;5(4):458–467. PubMed PMID: 25006989. https://doi.org/10.4161/gmic.29558. Epub 2014 Jul 9.
74. Fritz H, Kennedy DA, Ishii M, et al. Extracts for lung cancer: a systematic review. *Integr Cancer Ther*. 2015. https://doi.org/10.1177/1534735415572883.
75. Chay WY, Tham CK, Toh HC, et al. Coriolus versicolor (Yunzhi) use as therapy in advanced hepatocellular carcinoma patients with poor liver. *J Altern Complement Med*. 2017;23(8):648–652. https://doi.org/10.1089/acm.2016.0136.
76. Torkelson CJ, Sweet E, Martzen MR, et al. Phase 1 clinical trial of Trametes versicolor in women with breast cancer. *Int Sch Res Netw*. 2012:2012. https://doi.org/10.5402/2012/251632.
77. Zhao F, Xia G, Chen L, Zhao J, Xie Z. Chemical constituents from Inonotus obliquus and their antitumor activities. *J Nat Med*. 2016:721–730. https://doi.org/10.1007/s11418-016-1002-4.

78. Minh T, Nguyet N, Lomunova M, Vinh B, Sun J, Kyu S. International Immunopharmacology the mast cell stabilizing activity of chaga mushroom critical for its therapeutic effect on food allergy is derived from inotodiol. *Int Immunopharmacol*. 2018;54:286–295. https://doi.org/10.1016/j.intimp.2017.11.025.
79. Huang S, Ding S, Fan L. International journal of biological macromolecules antioxidant activities of five polysaccharides from inonotus obliquus. *Int J Biol Macromol*. 2012;50(5):1183–1187. https://doi.org/10.1016/j.ijbiomac.2012.03.019.
80. Mu H, Zhang A, Zhang W, Cui G, Wang S. Antioxidative properties of Crude polysaccharides from inonotus obliquus. *Int J Mol Sci*. 2012:9194–9206. https://doi.org/10.3390/ijms13079194.
81. Kumar S, Kang J, Kim D, Hyun S, Kyung M. Orally administered aqueous extract of Inonotus obliquus ameliorates acute inflammation in dextran sulfate sodium (DSS)-induced colitis in mice. *J Ethnopharmacol*. 2012;143(2):524–532. https://doi.org/10.1016/j.jep.2012.07.008.
82. Liu P, Xue J, Tong S, Dong W, Wu P. Structure Characterization and Hypoglycaemic activities of two polysaccharides from inonotus obliquus. *Molecules*. 2018. https://doi.org/10.3390/molecules23081948.
83. Shikov AN, Pozharitskaya ON, Makarov VG, Wagner H, Verpoorte R, Heinrich M. Medicinal plants of the russian pharmacopoeia; their history and applications. *J Ethnopharmacol*. 2014;154(3):481–536. https://doi.org/10.1016/j.jep.2014.04.007.
84. Dosychev EA, Bystrova VN. [Treatment of psoriasis using "chaga" fungus preparations]. *Vestn Dermatol Venerol*. 1973;47(5):79–83. Russian. PubMed PMID: 4755970.
85. Rossi P, Difrancia R, Quagliariello V, Savino E, Randazzo CL, Berretta M. B-glucans from *Grifola frondosa* and *Ganoderma lucidum* in breast cancer: an example of complementary and integrative medicine. *Oncotarget*. 2018;9(37):24837–24856.
86. Kodama N, Murata Y, Nanba H. Administration of a polysaccharide from *Grifola frondosa* stimulates immune function of normal mice. *J Med Food*. 2004;7(2):141–145.
87. Lee J, Park BC, Ko YJ, et al. Grifola frondosa (maitake mushroom) water extract inhibits vascular endothelial growth factor-induced angiogenesis through inhibition of reactive oxygen species Phosphorylation, extracellular signal-regulated kinase. *J Med Food*. 2008;11(4):643–651. https://doi.org/10.1089/jmf.2007.0629.
88. Deng G, Lin H, Seidman A, et al. A phase I/II trial of a polysaccharide extract from Grifola frondosa (Maitake mushroom) in breast cancer patients: immunological effects. *J Cancer Res Clin Oncol*. 2009;135:1215. https://doi.org/10.1007/s00432-009-0562-z.
89. Chen J-T, Tominaga K, Sato Y, et al. Maitake mushroom (grifola frondosa) extract induces ovulation in patients with polycystic ovary Syndrome: a possible monotherapy and a combination therapy. *J Altern Complement Med*. 2010;16(12):1295–1299. https://doi.org/10.1089/acm.2009.0696.
90. Jesenak M, Hrubisko M, Majtan J, Rennerova Z, Banovcin P. Anti-allergic effect of pleuran (b-glucan from pleurotus ostreatus) in children with recurrent respiratory tract infections. *Phytother Res*. 2014;474:471–474.
91. Anandhi R, Annadurai T, Anitha TS, et al. Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, Pleurotus ostreatus, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. *J Physiol Biochem*. 2013;69(2):313–323. PubMed PMID: 23104078.https://doi.org/10.1007/s13105-012-0215-6. Epub 2012 Oct 27.
92. Hamdi M, Abidin Z, Abdullah N, et al. Therapeutic properties of pleurotus species (oyster mushrooms) for atherosclerosis: a review Therapeutic properties of Pleurotus species (oyster mushrooms). *Int J Food Prop*. 2017;20(6):1251–1261. https://doi.org/10.1080/10942912.2016.1210162.
93. Jesenak M, Urbancek S, Majtan J, Banovcin P, Hercogova J. β-Glucan-based cream (containing pleuran isolated from pleurotus ostreatus) in supportive treatment of mild-to-moderate atopic dermatitis. *J Dermatolog Treat*. 6634(4):351–354. https://doi.org/10.3109/09546634.2015.1117565.
94. Minov J, Bislimovska-karadzhinska J, Petrova T, Vasilevska K, Stoleski S. Effects of pleuran (B–glucan from *Pleurotus ostreatus*) supplementation on incidence and duration of COPD exacerbations. *Open Access Maced J Med Sci*. 5(7):893–898.
95. Jesenak M, Urbanclkova I, Banovcin P. Respiratory tract infections and the role of biologically active polysaccharides in their. *Nutrients*. 2017:1–12. https://doi.org/10.3390/nu9070779.
96. Pico Sirvent L, Sapena Grau J, Morera Ingles M, Rivero Urgell M. Effect of supplementation with β–glucan from Pleurotus ostreatus in children with recurrent respiratory infections. *Ann Nurr Metab*. 2013;63(1):1378.
97. Bergendiova K, Tibenska E. *Pleuran (*β-glucan from pleurotus ostreatus) Supplementation, Cellular Immune Response and Respiratory Tract Infections in Athletes *Eur J Appl Physiol*. 2011:2033–2040. https://doi.org/10.1007/s00421-011-1837-z.
98. Schneider I, Kressel G, Meyer A, et al. Lipid lowering effects of oyster mushroom (pleurotus ostreatus) in humans. *J Funct Foods*. 2011;3(1):17–24. https://doi.Org/10.1016/J.Jff.2010.11.004.
99. Saito T, Aoki F, Hirai H, et al. Erinacine E as a kappa opioid receptor agonist and its new analogs from a basidiomycete, hericium ramosum. *J Antibiot*. 1998;51:983–990.
100. Friedman M. Chemistry, Nutrition, and health-promoting properties of hericium erinaceus (Lion's Mane) mushroom fruiting bodies and mycelia and their bioactive compounds. *J Agric Food Chem*. 2015. https://doi.org/10.1021/acs.jafc.5b02914.
101. Ma B-J, Shen J-W, Yu H, Ruan Y, Wu T-T, Zhao X. Hericenones and erinacines.pdf. *Mycology*. 2010;1(2):92–98.
102. Liu J, Du C, Wang Y, Yu Z. Anti-fatigue activities of polysaccharides extracted from Hericium erinaceus. *Exp Ther Med*. 2015;9(2):483–487. https://doi.org/10.3892/etm.2014.2139.
103. Wong KH, Naidu M, David RP, Bakar R, Sabaratnam V. Neuroregenerative potential of lion's mane mushroom, hericium erinaceus (Bull. Fr.) Pers. (higher Basidiomycetes), in the treatment of peripheral nerve injury (review). *Int J Med Mushrooms*. 2012;14(5):427–446. Review PubMed PMID: 23510212.
104. Wong K, Kanagasabapathy G, Bakar R, Phan C, Sabaratnam V. Restoration of sensory dysfunction following peripheral nerve injury by the polysaccharide from culinary and medicinal mushroom, *Hericium erinaceus* (Bull.: Fr.) Pers. through Its Neuroregenerative Action. *Food Sci. Technol (Campinas)* 2015;35(4):712–721.
105. Yi Z, Shao-long Y, Ai-hong W, et al. Protective effect of ethanol extracts of *Hericium erinaceus* on alloxan-induced diabetic neuropathic pain in rats. *Evid Based Complement Alternat Med*. 2015;2015.
106. Tzeng TT, Chen CC, Chen CC, et al. The cyanthin diterpenoid and sesterterpene constituents of hericium erinaceus mycelium ameliorate alzheimer's disease-related pathologies in APP/PS1 transgenic mice. *Int J Mol Sci*. 2018;19(2):E598. pii: E598. PubMed PMID: 29463001; PubMed Central PMCID: PMC5855820. https://doi.org/10.3390/ijms19020598.
107. Ryu S, Kim HG, Kim JY, Kim SY, Cho K. Hericium erinaceus extract reduces anxiety and depressive behaviors by promoting hippocampal neurogenesis in adult mouse brain. *J Med Food*. 2018;21(2):174–180. https://doi.org/10.1089/jmf.2017.4006.
108. Wang X, Yin J, Zhao M, Liu S, Nie S, Xie M. Gastroprotective activity of polysaccharide from hericium erinaceus against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer , and its antioxidant activities. *Carbohydr Polym*. 2018;186:100–109. https://doi.org/10.1016/j.carbpol.2018.01.004.
109. Nagano M, Shimizu K, Kondo R, et al. Reduction of depression and anxiety by 4 weeks Hericium erinaceus intake. *Biomed Res*. 2010;31(4):231–237. PubMed PMID: 20834180.
110. Xu CP, Liu WW, Liu FX, et al. A double-blind study of effectiveness of hericium erinaceus pers therapy on chronic atrophic gastritis. A preliminary report. *Chin Med J (Engl)*. 1985;98(6):455–456. PubMed PMID: 3932005.
111. Mori K, Inatomi S, Ouchi K, Azumi Y, Tuchida T. Improving effects of the mushroom yamabushitake (Hericium erinaceus) on mild cognitive impairment: a double-blind placebo-controlled clinical trial. *Phytother Res*. 2009;23(3):367–372. PubMed PMID: 18844328. https://doi.org/10.1002/ptr.2634.
112. Deng S, Zhang G, Kuai J, et al. Lentinan inhibits tumor angiogenesis via interferon γ and in a T cell independent manner. *J Exp Clin Cancer Res*. 2018;1–12.
113. Wang J, Li W, Huang X, Liu Y, Li Q, Zheng Z. A polysaccharide from Lentinus edodes inhibits human colon cancer cell proliferation and suppresses tumor growth in athymic nude mice. *Oncotarget*. 2017;8(1):610–623.

114. Xu H, Zou S, Xu X, Zhang L. Anti-tumor effect of β-glucan from Lentinus edodes and the underlying mechanism. *Nat Publ Gr*. 2016;(2):1–13. https://doi.org/10.1038/srep28802.
115. Nishitani Y, Zhang L, Yoshida M, Azuma T, Kanazawa K. Intestinal anti-inflammatory activity of lentinan: influence on IL-8 and TNFR1 expression in intestinal epithelial cells. 2013;8(4):e62441. https://doi.org/10.1371/journal.pone.0062441.
116. Papetti A, Signoretto C, Spratt DA, et al. Components in lentinus edodes mushroom with anti-biofilm activity directed against bacteria involved in caries and gingivitis. *Food Funct*. 2018;9(6):3489–3499. PubMed PMID: 29882939. https://doi.org/10.1039/c7fo01727h.
117. Gazzani G. Components in Lentinus edodes mushroom with anti-biofilm activity directed against bacteria involved in caries and gingivitis. *Food Funct*. 2018;9(6):3489–3499. PubMed PMID: 29882939. https://doi.org/10.1039/c7fo01727h.
118. Hearst R, Nelson D, Mccollum G, et al. Complementary therapies in clinical practice An examination of antibacterial and antifungal properties of constituents of Shiitake (Lentinula edodes) and oyster (Pleurotus ostreatus) mushrooms. *Complement Ther Clin Pract*. 2019;15(1):5–7. https://doi.org/10.1016/j.ctcp.2008.10.002.
119. Avinash J, Vinay S, Jha K, Das D, Goutham BS, Kumar G. *The Unexplored Anticaries Potential of Shiitake Mushroom*; 2016. https://doi.org/10.4103/0973-7847.194039.
120. Ina Kfuruta, Ryuichi kataoka T. Lentinan prolonged survival in patients with gastric cancer receiving S-1-based chemotherapy. *World J Clin Oncol*. 2011;2(10):339–343. https://doi.org/10.5306/wjco.v2.i10.339.
121. Ina K, Furuta R, Kataoka T, Kayukawa S, Ina H, Yoneda M. *Medical Sciences Chemo-Immunotherapy Using Lentinan for the Treatment of Gastric Cancer with Liver Metastases*; 2016. https://doi.org/10.3390/medsci4020008.
122. Fujimoto K, Tomonaga M, Goto S. A case of recurrent ovarian cancer successfully treated with adoptive immunotherapy and lentinan. *Anticancer Res*. 2006;4018:4015–4018.
123. Okuno K, Uno K. Efficacy of orally administered lentinula edodes mycelia extract for advanced gastrointestinal cancer patients undergoing cancer chemotherapy: a pilot study. *Asian Pac J Cancer Prev*. 2011;12:1671–1674.
124. Takimoto H, Wakita D, Kawaguchi K, Kumazawa Y. Potentiation of cytotoxic activity in naïve and tumor-bearing mice by oral administration of hot-water extracts from Agaricus brazei fruiting bodies. *Biol Pharm Bull*. 2004;27(3):404–406.
125. Ishii PL, Prado CK, Mauro Mde O, et al. Evaluation of Agaricus blazei in vivo for antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities. *Regul Toxicol Pharmacol*. 2011;59(3):412–422. PubMed PMID: 21295629. https://doi.org/10.1016/j.yrtph.2011.01.004. Epub 2011 Feb 3.
126. Lin JG, Fan MJ, Tang NY, et al. An extract of agaricus blazei murill administered orally promotes immune responses in murine leukemia BALB/c mice in vivo. *Integr Cancer Ther*. 2012;11(1):29–36. PubMed PMID: 22637937.0221101 https://doi.org/10.1177/1534735411400314.
127. Hetland G, Johnson E, Lyberg T, et al. Effects of the medicinal mushroom agaricus blazei murill on immunity, infection and cancer. *Scand J Immunol*. 2008;68:363–370.
128. Lee JS, Hong EK. Agaricus blazei murill enhances doxorubicin-induced apoptosis in human hepatocellular carcinoma cells by NFκB-mediated increase of intracellular doxorubicin accumulation. *Int J Oncol*. 2011;38(2):401–408. PubMed PMID: 21132265. https://doi.org/10.3892/ijo.2010.852. Epub 2010 Dec 3.
129. Wu B, Cui J, Zhang C, Li Z. A polysaccharide from agaricus blazei inhibits proliferation and promotes apoptosis of osteosarcoma cells. *Int J Biol Macromol*. 2012;50(4):1116–1120. PubMed PMID: 22390851. https://doi.org/10.1016/j.ijbiomac.2012.02.023. Epub 2012 Feb 27.
130. Soković M, Ćirić A, Glamočlija J, Nikolić M, van Griensven LJ. Agaricus blazei hot water extract shows anti quorum sensing activity in the nosocomial human pathogen Pseudomonas aeruginosa. *Molecules*. 2014;19(4):4189–4199. PubMed PMID: 24705563; PubMed Central PMCID: PMC6271851. https://doi.org/10.3390/molecules19044189.
131. Carneiro AAJ, Ferreira ICFR, Barros L, Gomes E, Santos-buelga C. Chemical composition and antioxidant activity of dried powder formulations of Agaricus blazei and Lentinus edodes. *Food Chem*. 2013;138(4):2168–2173. https://doi.org/10.1016/j.foodchem.2012.12.036.
132. Ahn WS, Kim DJ, Chae GT, et al. Natural killer cell activity and quality of life were improved by consumption of a mushroom extract, agaricus blazei murill kyowa, in gynecological cancer patients undergoing chemotherapy. *Int J Gynecol Cancer*. 2004;14(4):589–594. PubMed PMID: 15304151.
133. Hsu CH, Liao YL, Lin SC, Hwang KC, Chou P. The mushroom agaricus blazei murill in combination with metformin and gliclazide improves insulin resistance in type 2 diabetes: a randomized, double-blinded, and placebo-controlled clinical trial. *J Altern Complement Med*. 2007;13(1):97–102. PubMed PMID: 17309383.
134. Ikekawa T, Maruyama H, Miyano T, et al. Proflamin, a new antitumor agent: preparation, physicochemical properties and antitumor activity. *Jpn J Cancer Res*. 1985;76(2):142–148. PubMed PMID: 3920103.
135. Su A, Yang W, Zhao L, et al. Flammulina velutipes polysaccharides improve scopolamine-induced learning and memory impairment in mice by modulating gut microbiota composition. *Food Funct*. 2018;9(3):1424–1432. https://doi.org/10.1039/c7fo01991b.
136. Dong YR, Cheng SJ, Qi GH, Yang ZP, Yin SY, Chen GT. Antimicrobial and antioxidant activities of flammulina velutipes polysaccharides and polysaccharide-iron(III) complex [corrected]. *Carbohydr Polym*. 2017Vol. 161:26–32. https://doi.org/10.1016/j.carbpol.2016.12.069. Epub 2016 Dec 29. Erratum in: *Carbohydr Polym*. 2017;165:470. PubMed PMID: 28189237.
137. Tang C, Hoo PC, Tan LT, Pusparajah P, Chan K. Golden needle mushroom: a culinary medicine with evidenced-based biological activities and health promoting properties. *Front Pharmacol*. 2016;7:474. https://doi.org/10.3389/fphar.2016.00474.
138. Wang Y, Cheng X, Wang P, et al. Investigating migration inhibition and apoptotic effects of Fomitopsis pinicola chloroform extract on human colorectal cancer SW-480 cells. *PLoS One*. 2014;9(7):1–13. https://doi.org/10.1371/journal.pone.0101303.
139. Hao L, Sheng Z, Lu J, Tao R, Jia S. Characterization and antioxidant activities of extracellular and intracellular polysaccharides from Fomitopsis pinicola. *Carbohydr Polym*. 2016;141:54–59. PubMed PMID: 26876995. https://doi.org/10.1016/j.carbpol.2015.11.048. Epub 2015 Nov 19.
140. Reis FS, Pereira E, Barros L, Sousa MJ, Martins A, Ferreira ICFR. Biomolecule profiles in inedible wild mushrooms with antioxidant value. *Molecules*. 2011:4328–4338. https://doi.org/10.3390/molecules16064328.
141. Petrova A, Popov S, Gjosheva M, Bankova V. A new triterpenic alcohol from fomitopsis pinicola. *Nat. Prod. Res.* 2007;21:401–405.
142. Yoshikawa K, Inoue M, Matsumoto Y, et al. Lanostane triterpenoids and triterpene glycosides from the fruit body of Fomitopsis pinicola and their inhibitory activity against COX-1 and COX-2. *J Nat Prod*. 2005:69–73.
143. Blanchette RA. *Fungus Ashes and Tobacco: The Use of Phellinus Igniarius by the Indigenous People of North America*. St. Paul: Department of Plant Pathology, University of Minnesota; 2001;15(1).
144. Kim J, Kim D, Hwang BS, et al. Mycobiology neuraminidase inhibitors from the fruiting body of *Phellinus igniarius. Mycobiology*. 2016:117–120.
145. Hsieh M, Chien S, Chou Y, Chen C, Chen J, Chen M. Phytomedicine hispolon from phellinus linteus possesses mediate caspases activation and induces human nasopharyngeal carcinomas cells apoptosis. *Eur J Integr Med*. 2014;21(12):1746–1752. https://doi.org/10.1016/j.phymed.2014.07.013.
146. Wu, X. Lin S, Zhu C, et al. Studies on constituents of cultures of fungus Phellinus igniarius. Zhongguo Zhong Yao Za Zhi. 2011;37(7):874–880.
147. Hsiao PC, Hsieh YH, Chow JM, et al. Hispolon induces apoptosis through JNK1/2-mediated activation of a caspase-8, -9, and -3-dependent pathway in acute myeloid leukemia (AML) cells and inhibits AML xenograft tumor growth in vivo. *J Agric Food Chem*. 2013;61(42):10063–10073. PubMed PMID: 24093560. https://doi.org/10.1021/jf402956m. Epub 2013 Oct 15.
148. Song T, Lin H, Yang N, Hu M. Antiproliferative and antimetastatic effects of the ethanolic extract of phellinus igniarius (Linnearus: Fries). *Quelet*. 2008;115:50–56. https://doi.org/10.1016/j.jep.2007.09.001.

149. Lee S, Kim JI, Heo J, et al. The anti-influenza virus effect of phellinus igniarius extract. *J Microbiol (Seoul, Korea)*. 2013;51(5):676-681.
150. Li L, Wu G, Choir BY, et al. A Mushroom extract piwep from phellinus igniarius ameliorates experimental autoimmune encephalomyelitis by inhibiting immune cell infiltration in the spinal cord. *BioMed Res Int*. 2014;(2014):1–11.
151. Jeong, S.-C., S. Rao Koyyalamudi, J. M. Hughes, et al. Antioxidant and immunomodulating activities of exo-and endopolysaccharide fractions from submerged mycelia cultures of culinary-medicinal mushrooms. *Int J Med Mush*. 2013;15(3):251-266.
152. Kiho T, Kochi M, Usui S, Hirano K, Aizawa K, Inakuma T. Antidiabetic effect of an acidic polysaccharide (TAP) from Tremella aurantia and its degradation product (TAP-H). *Biol Pharm Bull*. 2001;24(12):1400–1403. PubMed PMID: 11767110.
153. Lo H-C, Hsu T-H, C-H, Lin F-Y, Wasser Sp. The fruiting bodies, submerged culture biomass, and acidic polysaccharide glucuronoxylomannan of yellow brain mushroom tremella mesenterica modulate the immunity of peripheral blood leukocytes and splenocytes in rats with impaired glucose tolerance. *J Trad Complement Med*. 2014;0.0.
154. Ban S, Lee SL, Jeong HS, Park S. Efficacy and safety of Tremella fuciformis i n individuals with subjective cognitive impairment: a randomized controlled trial. *J Med Food*. 2018;21(4):400–407. https://doi.org/10.1089/jmf.2017.4063.
155. Johnson MW, Griffiths RR. Potential therapeutic effects of psilocybin. *Neurotherapeutics*. 2017;14(3):734–740. https://doi.org/10.1007/s13311-017-0542-y. ReviewPubMed PMID: 28585222; PubMed Central PMCID: PMC5509636.
156. Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PC, Strassman RJ. Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *J Psychopharmacol*. 2015;29(3):289–299. PubMed PMID: 25586396. https://doi.org/10.1177/0269881114565144. Epub 2015 Jan 13.
157. Bogenschutz MP. It's time to take psilocybin seriously as a possible treatment for substance use disorders. *Am J Drug Alcohol Abuse*. 2017 Jan;43(1):4–6. https://doi.org/10.1080/00952990.2016.1200060. Epub 2016 Aug 24.
158. Bogenschutz MP, Podrebarac SK, Duane JH, et al. Clinical interpretations of patient experience in a trial of psilocybin-assisted psychotherapy for alcohol use disorder. *Front Pharmacol*. 2018;9:100. PubMed PMID: 29515439; PubMed Central PMCID: PMC5826237. https://doi.org/10.3389/fphar.2018.00100. eCollection 2018.
159. Carhart-Harris RL, Bolstridge M, Day CMJ, et al. Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology (Berl)*. 2018;235(2):399–408. PubMed PMID: 29119217; PubMed Central PMCID: PMC5813086. https://doi.org/10.1007/s00213-017-4771-x. Epub 2017 Nov 8.
160. Ross S, Bossis A, Guss J, et al. Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *J Psychopharmacol*. 2016;30(12):1165–1180. PubMed PMID: 27909164; PubMed Central PMCID: PMC5367551.
161. Griffiths R, Johnson M, Carducci M, et al. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: a randomized double-blind trial. *J Psychopharmacol*. 2016;30(12):1181–1197. https://doi.org/10.1177/0269881116675513.
162. Yuen MF, Ip P, Ng WK, et al. Hepatotoxicity due to a formulation of ganoderma lucidum (lingzhi). *J Hepatol*. 2004;41:686–687.
163. Wanmuang H, Leopairut J, Kositchaiwat C, et al. Fatal fulminant hepatitis associated with ganoderma lucidum (Lingzhi) mushroom powder. *J Med Assoc Thai*. 2007;90:179–181.
164. Wanachiwanawin D, Piankijagum A, Chaiprasert A, et al. Ganoderma lucidum: a cause of pseudoparasitosis. *Southeast Asian J Trop Med Public Health*. 2006;37:1099–1102.
165. Hanada K, Hashimoto I. Flagellate mushroom (Shiitake) dermatitis and photosensitivity. *Dermatology*. 1998;197:255–257.
166. Garg S, Cockayne SE. Shiitake dermatitis diagnosed after 16 years! *Arch Dermatol*. 2008;144:1241–1242.
167. Levy AM, Kita H, Phillips SF, et al. Eosinophilia and gastrointestinal symptoms after ingestion of shiitake mushrooms. *J Allergy Clin Immunol*. 1998;101:613–620.
168. Ferreira I, Barros L, Abreu RM. Antioxidants in wild mushrooms. *Curr Med Chem*. 2009;16:1543–1560.
169. Ker YB, Chen KC, Chyau CC. Antioxidant capability of polysaccharides fractionated from submerge-cultured agaricus blazei mycelia. *J Agricult Food Chem*. 2005;53:7052–7058.
170. Cui Y, Kim DS, Park KC. Antioxidant effect of Inonotus obliquus. *J Ethnopharmacol*. 2005;96:79–85.
171. Park YK, Lee HB, Jeon EJ, et al. Chaga mushroom extract inhibits oxidative DNA damage in human lymphocytes as assessed by comet assay. *BioFactors*. 2004;21:109–112.
172. Babitskaia VG, Shcherba VV, Ikonnikova NV. Melanin complex of the fungus Inonotus obliquus. *Prikladnaia Biokhimiia I Mikrobiologiia*. 2000;36:439–444.
173. Bao HN, Ushio H, Ohshima T. Antioxidative activity and antidiscoloration efficacy of ergothioneine in mushroom (Flammulina velutipes) extract added to beef and fish meats. *J Agricult Food Chem*. 2008;56:10032–10040.
174. Zhang Y, Mills GL, Nair MG. Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of the edible mushroom Grifola frondosa. *J Agricult Food Chem*. 2002;50:7581–7585.
175. Kim MY, Seguin P, Ahn JK. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from korea. *J Agricult Food Chem*. 2008;56:7265–7270.
176. Wu Y, Wang D. A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from Ganoderma lucidum fruiting bodies. *J Proteome Res*. 2009;8:436–442.
177. Ye SF, Wakame K, Ichimura K, et al. Amelioration by active hexose correlated compound of endocrine disturbances induced by oxidative stress in the rat. *Endocr Regulat*. 2004;38:7–13.
178. Ye SF, Ichimura K, Wakame K, et al. Suppressive effects of active hexose correlated compound on the increased activity of hepatic and renal ornithine decarboxylase induced by oxidative stress. *Life Sci*. 2003;74:593–602.
179. Lindequist U, Truscher E, Narbe G. Neue wirkstoffe aus basidiomyceten. *Z. Phytother*. 1990;11:139–149.
180. Mothana RAA, Jansen R, Julich WD, et al. Ganomycin A and B new antimicrobial farnesyl hydroquinones from basidiomycete ganoderma pfeifferi. *J Nat Prod*. 2000;63:416–418.
181. Lindequist U, Niedermeyer THJ, Jülich W-D. The pharmacological potential of mushrooms. *Evid Based Complement Alternat Med*. 2005;2:285–289.
182. Bender S, Dumitrache CN, Backhaus J, et al. A case for caution in assessing the antibiotic activity of extracts of culinary-medicinal Shiitake mushroom [lentinus edodes (Berk.) Singer] (Agaricomycetidae). *Int J Med Mushrooms*. 2003;5:31–35.
183. Badalyan SM. Antiprotozoal activity and mitogenic effect of mycelium of culinary-medicinal shiitake mushroom Lentinus edodes (Berk.) Singer (Agaricomycetidae). *Int J Med Mushrooms*. 2004;6:131–138.
184. Mothana RAA, Awadh NAA, Jansen R, et al. Antiviral lanostanoid triterpenes from the fungus Ganoderma pfeifferi BRES. *Fitoterapia*. 2003;74:177–180.
185. Ichimura T, Watanabe O, Maruyama S. Inhibition of HIV-1 protease by water-soluble lignin-like substance from an edible mushroom, Fuscoporia obliqua. *Biosci Biotechnol Biochem*. 1998;62:575–577.
186. Tochikura TS, Nakashima H, Ohashi Y, et al. Inhibition (in vitro) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of lentinus edodes mycelia. *Med Microbiol Immunol*. 1988;177:235–244.
187. Suzuki H, Okubo A, Yamazaki S, et al. Inhibition of the infectivity and cytopathic effect of human immunodeficiency virus by water-soluble lignin in an extract of the culture medium of lentinus edodes mycelia (LEM). *Biochem Biophys Res Commun*. 1989;160:367–373.
188. Yoshida O, Nakashima H, Yoshida T, et al. Sulfation of the immunomodulating polysaccharide lentinan: a novel strategy for antivirals to human immunodeficiency virus (HIV). *Biochem Pharmacol*. 1988;37:2887–2891.
189. Nanba H, Kodama N, Schar D, et al. Effects of maitake (Grifola frondosa) glucan in HIV-infected patients. *Mycoscience*. 2000;41:293–295.
190. Tochikura TS, Nakashima H, Hirose K, et al. A biological response modifier, PSK, inhibits human immunodeficiency virus infection in vitro. *Biochem Biophys Res Commun*. 1987;148:726–733.
191. Collins RA, Ng TB. Polysaccharopeptide from Coriolus versicolor has potential for use against human immunodeficiency virus type 1 infection. *Life Sci*. 1997;60:PL383–PL387.

192. Tasaka K, Mio M, Izushi K, et al. Anti-allergic constituents in the culture medium of Ganoderma lucidum. (II). The inhibitory effect of cyclooctasulfur on histamine release. *Agents Actions*. 1988;23:157–160.
193. Kohda H, Tokumoto W, Sakamoto K, et al. The biologically-active constituents of Ganoderma lucidum (Fr.) Karst.Histamine release-inhibitory triterpenes. *Chem Pharm Bull*. 1985;33:1367–1373.
194. Komoda Y, Shimizu M, Sonoda Y, et al. Ganoderic acid and its derivatives as cholesterol synthesis inhibitors. *Chem Pharma Bull*. 1989;37:531–533.
195. Hajjaj H, Macé C, Roberts M, et al. Effect of 26-oxygenosterols from Ganoderma lucidum and their activity as cholesterol synthesis inhibitors. *Appl Environ Microbiol*. 2005;71:3653–3658.
196. Liu Y, Fukuwatari Y, Okumura K, et al. Immunomodulating activity of agaricus brasiliensis KA21 in mice and in human volunteers. *Evid Based Complement Alternat Med*. 2008;5:205–219.
197. Kim YW, Kim KH, Choi HJ, et al. Anti-diabetic activity of beta-glucans and their enzymatically hydrolyzed oligosaccharides from Agaricus blazei. *Biotechnol Lett*. 2005;27:483–487.
198. Enman J, Rova U, Berglund KA. Quantification of the bioactive compound eritadenine in selected strains of shiitake mushroom (Lentinus edodes). *J Agricult Food Chem*. 2007;55:1177–1180.
199. Gunde-Cimerman N, Cimerman A. Pleurotus fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase-lovastatin. *Exp Mycol*. 1995;19:1–6.
200. Konno S, Aynehchi S, Dolin DJ, et al. Anticancer and hypoglycemic effects of polysaccharides in edible and medicinal maitake mushroom Grifola frondosa (Dicks.:Fr.) S.F.Gray. *Int J Med Mushrooms*. 2002;4:185–195.
201. Konno S, Tortorelis DG, Fullerton SA, et al. A possible hypoglycaemic effect of maitake mushroom on Type 2 diabetic patients. *Diabetic Med*. 2001;18:1010.
202. Manohar V, Talpur NA, Echard BW, et al. Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. *Diabetes Obes Metab*. 2002;4:43–48.
203. Hikino H, Konno C, Mirin Y, et al. Isolation and hypoglycaemic activities of ganoderans A and B, glucans of Ganoderma lucidum fruit bodies. *Planta Med*. 1985;51:339–340.
204. Zhang HN, Lin ZB. Hypoglycemic effect of ganoderma lucidum polysaccharides. *Acta Pharmacol Sinica*. 2004;25:191–195.
205. Ang BK, Jung YS, Song CH. Hypoglycemic effects of ganoderma applanatum and collybia confluens exo-polymers in streptozotocin-induced diabetic rats. *Phytother Res*. 2007;21:1066–1069.
206. Ikuzawa M, Oguchi Y, Matsunaga K, et al. Pharmaceutical preparation containing a glycoprotein. *German Patent DE*. 1985;3:429–551.
207. Mizuno T. Antitumor and hypoglycemic activities of polysaccharides from the sclerotia and mycelia of Inonotus obliquus. *Int J Med Mushrooms*. 1999;1:301–316.
208. Fortes RC, Novaes MR, Recôva VL, et al. Immunological, hematological, and glycemia effects of dietary supplementation with agaricus sylvaticus on patients' colorectal cancer. *Exp Biol Med*. 2009;234:53–62.
209. Kiho T, Hui J, Yamane A, et al. Polysaccharides in fungi. XXXII. hypoglycemic activity and chemical properties of a polysaccharide from the cultural mycelium of cordyceps sinensis. *Biol Pharma Bull*. 1993;16:1291–1293.
210. Zhao CS, Yin WT, Wang JY. CordyMax Cs-4 improves glucose metabolism and increases insulin sensitivity in normal rats. *J Alter Complement Med*. 2002;8:309–314.
211. Li SP, Zhang GH, Zeng Q. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured cordyceps mycelia. *Phytomedicine*. 2006;13:428–433.
212. Hirotani M, Ino C, Furuya T, et al. Ganoderic acids T, S and R, new triterpenoids from the cultured mycelia of ganoderma lucidum. *Chem Pharm Bull*. 1986;34:2282–2285.
213. Chen RY, Yu DQ. Studies on the triterpenoid constituents of the spores from ganoderma lucidum karst. *J Chin Pharm Sci*. 1993;2:91–96.
214. Wang MY, Liu Q, Che QM, et al. Effects of total triterpenoids extract from Ganoderma lucidum (Curt.:Fr.) P.Karst. (Reishi Mushroom) on experimental liver injury models induced by carbon tetrachloride or D-galactosamine in mice. *Int J Med Mushrooms*. 2002;4:337–342.

Melaleuca alternifolia (Tea Tree)

Michael T. Murray, ND

OUTLINE

Melaleuca alternifolia (family: *Myrtaceae*)
Common name: tea tree

GENERAL DESCRIPTION

Tea tree *(Melaleuca alternifolia)* is a small tree native to only one area of the world: the northeast coastal region of New South Wales, Australia. The leaves, the portion of the plant that is used medicinally, are the source of a valuable therapeutic oil. Although there are more than 50 members of the *Melaleuca* family, the oil from the leaf of *M. alternifolia* has received the most research attention.

CHEMICAL COMPOSITION

Tea tree leaves contain about 1.8% of oil obtained via steam distillation.[1] This oil contains more than 48 compounds but is chiefly composed of the following[2]:

- 1-terpinen-4-ol
- 1,8-cineol
- Gamma-terpinene (see Fig. 91.1)
- *p*-cymene
- Other terpenes

The Australian standard (AS 2782–1985) "Oil of Melaleuca (Terpinen-4-ol Type)" sets a minimum content of terpinen-4-ol at 30% and a maximum 1,8-cineol content of 15%.[1]

HISTORY AND FOLK USE

The medicinal properties of crushed tea tree leaves were known to the Bundjalung Aborigines of northern New South Wales, Australia. The waters of a lagoon where tea tree leaves had fallen and decayed for hundreds of years were viewed as having tremendous healing properties.[1]

The popular name of tea tree was first reported in 1777, in Captain Cook's account of his second voyage, entitled *A Voyage to the South Pole.* The leaves of *M. alternifolia* were also used by the early settlers of Australia to make tea—hence, the further use of the popular name "tea tree."[1]

The first report of tea tree's medicinal use appeared in the *Medical Journal of Australia* in 1930.[3] A surgeon in Sydney reported impressive results using a solution of tea tree oil to clean surgical wounds. According to this report[3]:

> *The results obtained in a variety of conditions when it [tea tree oil] was first tried were most encouraging, a striking feature being that it dissolved pus and left the surface of infected wounds clean so that its germicidal action became more effective without any apparent damage to the tissues. This was something new, as most efficient germicides destroy tissue as well as bacteria.*

During World War II, tea tree oil was issued to soldiers to use as a disinfectant. The Australian Army went so far as to commandeer supplies of the oil and exempt leaf cutters from national service to maintain production. The production of tea tree oil during World War II was regarded as an "essential" industry.[1]

After World War II, the tea tree oil industry stagnated for more than 30 years. There were a number of reasons for this, including the general trend away from natural medicines and toward synthetic medical drugs. However, during the late 1970s and early 1980s, the Australian tea tree oil industry was reborn as successful plantations growing *M. alternifolia* were established.[1]

Tea tree oil has been used in the treatment of the following conditions[1]:

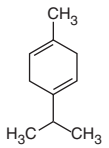


Fig. 91.1 Gamma-terpinene.

- Acne
- Aphthous stomatitis
- Boils
- Burns
- Carbuncles
- Corns
- Gingivitis
- Herpes
- Impetigo
- Infections of the nail bed
- Insect bites
- Lice
- Mouth ulcers
- Pharyngitis
- Psoriasis
- Ringworm
- Root canal treatment
- Sinus infections
- Skin and vaginal infections
- Thrush
- Tinea pedis (athlete's foot)
- Tonsillitis

A variety of tea tree oil–based cosmetic products exist in the marketplace, including toothpastes, shampoos and conditioners, creams, hand and body lotions, soaps, gels, liniments, and nail polish removers.

PHARMACOLOGY

Tea tree oil possesses significant antiseptic properties and is regarded by many as the ideal skin disinfectant. This claim is supported by its efficacy against a wide range of organisms, its good penetration, and its lack of skin irritation.[1] The therapeutic uses of tea tree oil are based largely on its antiseptic and antifungal properties. Bacteria and fungal organisms inhibited by tea tree oil are listed in Tables 100.1 and 100.2.[1,4–6]

CLINICAL APPLICATIONS

The historical uses of tea tree oil demonstrate its wide range of applications as an antiseptic. Three of the more popular and documented uses are in the treatment of skin infections, vaginal infections, and common foot complaints.

Skin Infections

Tea tree oil is useful in a broad range of dermal infections, not only because of its broad-spectrum antiseptic properties but also because of its capacity to mix with sebaceous secretions and penetrate the epidermis.

A clinical trial in patients with furuncles demonstrated that tea tree oil encouraged more rapid healing without scarring than was seen in matched control subjects.[7] Presumably, the positive clinical effects were a result of the oil's germicidal activity against *Staphylococcus aureus.* Tea tree oil efficiently kills *S. aureus* in the stationary growth phase and within biofilms, making it a promising tool for *S. aureus* eradication.[8] Furthermore, other studies have shown that tea tree oil is antimicrobial against antibiotic-resistant strains of *S. aureus.*[9,10] In the clinical trial, the method of application consisted of cleaning the site followed by painting the surface of the furuncle freely with tea tree oil two or three times a day.

For most skin infections, the most effective treatment appears to be direct application of full-strength, undiluted oil at the site of infection. If irritation occurs, diluted preparations may be tried.

TABLE 91.1 Bacteria Inhibited by *Melaleuca alternifolia* Oil

Bacterial Species	% (vol/vol)	
	MIC	MBC
Acinetobacter baumannii	1	1
Actinomyces viscosus	0.6	>0.6
Actinomyces spp.	1	1
Bacillus cereus	0.3	
Bacteroides spp.	0.06–0.5	0.06–0.12
Corynebacterium spp.	0.2–2	2
Enterococcus faecalis	0.5–>8	>8
E. faecium (vancomycin resistant)	0.5–1	0.5–1
Escherichia coli	0.08–2	0.25–4
Fusobacterium nucleatum	0.6–>0.6	0.25
Klebsiella pneumoniae	0.25–0.3	0.25
Lactobacillus spp.	1–2	2
Micrococcus luteus	0.06–0.5	0.25–6
Peptostreptococcus anaerobius	0.2–0.25	0.03–>0.6
Porphyromonas endodontalis	0.025–0.1	0.025–0.1
P. gingivalis	0.11–0.25	0.13–>0.6
Prevotella spp.	0.03–0.25	0.03
Prevotella intermedia	0.003–0.1	0.003–0.1
Propionibacterium acnes	0.05–0.63	0.5
Proteus vulgaris	0.08–2	4
Pseudomonas aeruginosa	1–8	2–>8
Staphylococcus aureus	0.5–1.25	1–2
S. aureus (methicillin resistant)	0.04–0.35	0.5
S. epidermidis	0.45–1.25	4
S. hominis	0.5	4
Streptococcus pyogenes	0.12–2	0.25–4
Veillonella spp.	0.016–1	0.03–1

MBC, Minimal bactericidal concentration; *MIC,* minimum inhibitory concentration.

Data from Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev.* 2006;19:50–62.

Lice Infestations

Pediculosis capitis (head lice infestation) treatment typically involves the application of topical insecticides such as a pyrethrin or permethrin. An in vitro study showed that tea tree oil exerted the greatest effect of essential oils tested against head lice.[11] A clinical study compared the efficacy and safety of three topical pediculicides. A pediculicide containing tea tree and lavender oil was compared with a product containing pyrethrins and piperonyl butoxide; the percentage of subjects who were louse-free 1 day after the last treatment with the tea tree oil and lavender product was 97.6% (41 of 42 patients) compared with 25% (10 of 40 patients) of those using the product containing pyrethrins and piperonyl butoxide.[12] These clinical results, along with in vitro studies, indicate that products containing tea tree oil are effective alternatives to pyrethrin-based products. However, it should also be mentioned that in an in vitro study looking at essential oils from tea tree, wild bergamot, clove, lavender, and Yunnan verbena, by far the best mite destruction was produced by clove oil, diluted either in coconut oil or sunflower oil.[13] Clove oil produced greater than 90% mortality within 2 hours in lice submitted to a 30-minute contact. The high content of eugenol was thought to be the main factor.

TABLE 91. 2 Fungi Inhibited by *Melaleuca alternifolia* Oil

Fungal Species	% (vol/vol)	
	MIC	MFC
Alternaria spp.	0.016–0.12	0.06–2
Aspergillus flavus	0.31–0.7	2–4
A. fumigatus	0.06–>2	1–2
A. niger	0.016–0.4	2–8
Blastoschizomyces capitatus	0.25	
Candida albicans	0.06–8	0.12–1
C. glabrata	0.03–8	0.12–0.5
C. parapsilosis	0.03–0.5	0.12–0.5
C. tropicalis	0.12–2	0.25–0.5
Cladosporium spp.	0.008–0.12	0.12–4
Cryptococcus neoformans	0.015–0.06	
Epidermophyton flocossum	0.008–0.7	0.12–0.25
Fusarium spp.	0.008–0.25	0.25–2
Malassezia furfur	0.03–0.12	0.5–1.0
M. sympodialis	0.016–0.12	0.06–0.12
Microsporum canis	0.03–0.5	0.25–0.5
M. gypseum	0.016–0.25	0.25–0.5
Penicillium spp.	0.03–0.06	0.5–2
Rhodotorula rubra	0.06	0.5
Saccharomyces cerevisiae	0.25	0.5
Trichophyton mentagrophytes	0.11–0.44	0.25–0.5
T. rubrum	0.03–0.6	0.25–1
T. tonsurans	0.004–0.016	0.12–0.5
Trichosporon spp.	0.12–0.22	0.12

MBC, Minimal bactericidal concentration; *MIC,* minimum inhibitory concentration.

Data from Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006;19:50–62.

Oral Infections

Tea tree oil was shown to exert significant activity against oral pathogens, both bacterial and fungal, and was suggested to be a suitable alternative to chlorhexidine.[14] From a clinical perspective, these effects may best be used in the treatment of oral candidiasis (thrush). In one open study, 27 patients with acquired immunodeficiency syndrome (AIDS) and oral candidiasis that was clinically refractory to fluconazole were randomly assigned to receive either an alcohol-based or an alcohol-free melaleuca oral solution four times daily for 2 to 4 weeks. Overall, 60% of patients demonstrated a clinical response to the melaleuca oral solution (seven cases were cured, and eight cases were clinically improved) at 4-week evaluation.[15]

Tea tree oil appears to be quite useful in oral candidiasis in cancer patients. Often these yeasts are resistant to azole antifungal drugs. In one study, 301 yeasts isolated from the mouths of 199 patients with advanced cancer were shown to be susceptible to tea tree oil, including 41 yeasts that were known to be resistant to both fluconazole and itraconazole.[16]

A sublethal concentration of tea tree oil or terpinen-4-ol was shown to dramatically increase the activity of fluconazole against 32 clinical strains of fluconazole-resistant *Candida albicans.* The 24-hour exposure of fluconazole-resistant *C. albicans* strains to fluconazole with tea tree oil resulted in 62.5% of isolates being classified as susceptible, 25.0% as intermediately susceptible, and only 12.5% as resistant. Even better results were seen with terpinen-4-ol. These results demonstrate that combining tea tree oil with fluconazole may be the best approach in treating difficult yeast infections.[17]

Dandruff

There is evidence that dandruff appears to be related to the yeast *Pityrosporum ovale.* Because tea tree oil has antifungal activity against *P. ovale,* a 4-week study was designed to investigate the efficacy and tolerability of 5% tea tree oil shampoo versus a placebo in 126 male and female patients, aged 14 years and older, with moderate dandruff. The 5% tea tree oil shampoo group showed a 41% improvement in the quadrant-area-severity score, compared with an 11% improvement in the placebo group. Statistically significant improvements were also observed in the score for total area of involvement, the total severity score, and the itchiness and greasiness components of the patients' self-assessments. The scaliness component of patient self-assessment improved but was not statistically significant. There were no adverse effects.[18]

Acne

Topical application of tea tree oil is a suitable alternative to benzoyl peroxide preparations.[19,20] In one study, 124 patients with mild to moderate acne randomly received either a 5% gel of tea tree oil or 5% benzoyl peroxide lotion to be applied topically every day. After 3 months, both treatments produced a significant improvement in the mean number of both noninflamed and inflamed lesions, although with noninflamed lesions, benzoyl peroxide was found to be more effective. An important finding was that there were fewer reports of side effects (dryness, pruritus, stinging, burning, and skin redness) with tea tree oil (44% vs. 79%).[20]

Demodicosis

Demodex folliculorum and *Demodex brevis* are skin mites primarily found in the face, near the nose, the eyelashes, and eyebrows, but also occur elsewhere on the body. *D. folliculorum* is found in hair follicles; *D. brevis* in sebaceous glands. When these mite populations dramatically increase, it results in a condition known as demodicosis. There is also a link between *Demodex* infection with acne vulgaris and rosacea. Topical application of tea tree oil has been shown to be effective in improving demodex-related diseases.[21] In particular, tea tree oil at 50% concentration is effective in reducing *Demodex* mite counts and the surface inflammation associated with blepharitis, conjunctivitis, and keratitis. However, tea tree oil can also cause ocular irritation, so it has been suggested that because terpinen-4-ol is the most potent ingredient, using terpinen-4-ol alone would not only enhance its potency in killing *Demodex* mites but also reduce the adverse effects noted with the other ingredients in tea tree.[22]

Common Foot Problems

Tea tree oil, in emollient form (8% tea tree oil) or in solution (40%), can be massaged into the feet daily for the treatment of tinea pedis, foot irritation, and bromhidrosis (severely foul-smelling feet).

One researcher concluded, after 6 years of using different concentrations and preparations, that tea tree oil eradicated or improved the symptoms of tinea pedis when used daily by patients at home.[4] He also reported that even undiluted forms had little effect on onychomycosis (discussed further in "Fungal Nail Infection"). Diluted tea tree oil in solution was found to reduce foot irritation and promote wound healing with surgical incision in cases of corns, calluses, bunions, and hammer toes and was extremely effective in diminishing bromhidrosis.

In tinea pedis, one double-blind study found that 10% tea tree oil cream compared quite favorably with the antifungal tolnaftate in relieving symptoms but was less effective in eliminating the fungi from cultures.[5] Specifically, both the tea tree group (24 of 37 patients) and the tolnaftate group (19 of 33 patients) showed significant improvement in the four clinical parameters of scaling, inflammation, itching, and burning, but only 30% of the subjects who applied tea tree oil cream tested culture negative, compared with 85% in the tolnaftate group.

In another double-blind trial, 158 patients with tinea pedis were randomly assigned to receive either placebo or a 25% or 50% tea tree oil solution.[23] Patients applied the solution twice daily to affected areas for 4 weeks and were assessed after 2 and 4 weeks of treatment. A marked clinical response was seen in 68% of the 50% tea tree oil group and in 72% of the 25% tea tree oil group, compared with 39% in the placebo group. Mycologic cure was assessed by culture of skin scrapings taken at baseline and after 4 weeks of treatment. The mycologic cure rate was 64% in the 50% tea tree oil group compared with 31% in the placebo group. Four (3.8%) patients applying tea tree oil experienced moderate to severe dermatitis that improved quickly upon cessation of the study medication.

Fungal Nail Infection

Fungal nail infections (onychomycosis) are the most common cause of nail disease, affecting approximately 2% to 13% of the population. Standard medical treatments include débridement, topical antifungals, and systemic antifungals. All current therapies have high recurrence rates. Oral therapy has the added disadvantages of high cost and potentially serious adverse effects.

One study compared the efficacy and tolerability of the topical application of 1% clotrimazole (CL) solution with that of 100% tea tree oil for the treatment of toenail onychomycosis.[24] The 117 patients received twice daily applications of either 1% CL solution or 100% tea tree oil for 6 months. Débridement and clinical assessment were performed at 0, 1, 3, and 6 months. Culture specimens were obtained at 0 and 6 months. Each patient's subjective assessment was also obtained 3 months after the conclusion of therapy. After 6 months of therapy, the two treatment groups were comparable on the basis of culture cure (CL = 11%, tea tree = 18%) and clinical assessment documenting partial or full resolution (CL = 61%, tea tree = 60%). Three months later, about half of each group reported continued improvement or resolution (CL = 55%, tea tree = 56%). These results indicate that topical therapy with tea tree oil, in conjunction with débridement, provides excellent improvement in nail appearance and symptomatology.

Vaginal Infections

Tea tree oil demonstrates germicidal activity against a number of common vaginal pathogens and opportunistic organisms, including *Trichomonas vaginalis* and *Candida albicans.*[25]

A 40% solution of tea tree oil emulsified with isopropyl alcohol and water was found to be highly effective for the treatment of cervicitis, chronic endocervicitis, trichomonal vaginitis, and vaginal candidiasis.[25] Weekly in-office treatment (usually four to six were necessary) involved thorough washing of the perineum, labia, and vagina with a suitable scrub (the commercial product pHisoHex was used in the study). After drying, the affected areas were washed with a 1% tea tree oil solution. This was followed by insertion of a tampon (three 4 × 4–inch sponges) saturated with the 40% tea tree oil solution. Patients were instructed to remove the tampon after 24 hours.

For infectious processes—trichomonas and candidiasis—daily vaginal douches containing 1 quart of water with a 0.4% concentration of the oil were prescribed. No irritation, burning, or other side effects were reported or observed with either the office applications or the douches.

DOSAGE

A number of commercial products contain tea tree oil. Tea tree oil can be used as a topical antiseptic for reducing microbial counts in wounds, surgical incisions, and skin and vaginal infections. In addition, tea tree leaves can be used to make teas that may be of benefit in cases of sore throat, tonsillitis, sinus infections, and colitis.

TOXICITY

Tea tree oil appears to be very safe for use as a topical antiseptic.[26] However, it may cause allergic contact dermatitis in some individuals. During a 3-year period, seven patients were seen in an outpatient dermatology clinic in Kona, Hawaii, for contact dermatitis because of the use of commercially available product containing 100% tea tree oil.[27] The patients were treating preexisting skin conditions, which included foot fungus, dog scratches, insect bites, and rashes. All patients presented with an eczematous dermatitis. Patch tests indicated that all patients were sensitive to a 1% solution of tea tree oil. Individuals should apply the oil to a small area of skin before using tea tree oil for the first time so as to avoid contact dermatitis over a larger area.

The oral ingestion of tea tree oil cannot be recommended because it could lead to a toxic reaction. However, folk use suggests that oral ingestion of tea from the leaves is reasonably safe.

DRUG INTERACTIONS

Tea tree oil may produce a synergistic effect with other topical antimicrobials. In an in vitro study, tea tree oil and tobramycin in combination produced significantly lower minimum inhibitory concentrations against *Escherichia coli* and *Staphylococcus aureus.*[28] A similar synergism was demonstrated between tea tree oil and the antifungal compound amphotericin in inhibiting *Candida* species.[29]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006;19:50–62.
2. Swords G, Hunter GLK. Composition of Australian tea tree oil *(Melaleuca alternifolia)*. *J Agric Food Chem*. 1978;26:734–737.
3. Humphery EM. A new Australian germicide. *Med J Australia*. 1930;1:417–418.
4. Walker M. Clinical investigation of Australian *Melaleuca alternifolia* oil for a variety of common foot problems. *Current Podiatry*. 1972;18:30–35.
5. Tong MM, Altman PM, Barnetson RS. Tea tree oil in the treatment of tinea pedis. *Australas J Dermatol*. 1992;33:145–149.
6. Carson CF, Riley TV. The antimicrobial activity of tea tree oil. *Med J Aust*. 1994;160:236.
7. Feinblatt HM. Cajeput-type oil for the treatment of furunculosis. *J Nat Med Assoc*. 1960;52:32–34.
8. Kwieciński J, Eick S, Wójcik K. Effects of tea tree *(Melaleuca alternifolia)* oil on *Staphylococcus aureus* in biofilms and stationary growth phase. *Int J Antimicrob Agents*. 2009;33:343–347.
9. Falci SP, Teixeira MA, Chagas PF, et al. Antimicrobial activity of *Melaleuca* sp. oil against clinical isolates of antibiotics resistant *Staphylococcus aureus*. *Acta Cir Bras*. 2015;30(6):401–406.
10. Falci SP, Teixeira MA, Chagas PF, et al. Antimicrobial activity of *Melaleuca* sp. oil against clinical isolates of antibiotics resistant *Staphylococcus aureus*. *Acta Cir Bras*. 2015;30(7):491–496. https://doi.org/10.1590/S0102-865020150070000007.
11. Williamson EM, Priestley CM, Burgess IF. An investigation and comparison of the bioactivity of selected essential oils on human lice and house dust mites. *Fitoterapia*. 2007;78:521–525.
12. Barker SC, Altman PM. A randomised, assessor blind, parallel group comparative efficacy trial of three products for the treatment of head lice in children–melaleuca oil and lavender oil, pyrethrins and piperonyl butoxide, and a "suffocation" product. *BMC Dermatol*. 2010;10:6.
13. Candy K, Nicolas P, Andriantsoanirina V, Izri A, Durand R. In vitro efficacy of five essential oils against *Pediculus humanus capitis*. *Parasitol Res*. 2018;117(2):603–609.
14. Groppo FC, Ramacciato JC, Simoes RP, et al. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. *Int Dent J*. 2002;52:433–437.
15. Vazquez JA, Zawawi AA. Efficacy of alcohol-based and alcohol-free melaleuca oral solution for the treatment of fluconazole-refractory oropharyngeal candidiasis in patients with AIDS. *HIV Clin Trials*. 2002;3:379–385.
16. Bagg J, Jackson MS, Petrina Sweeney M, et al. Susceptibility to *Melaleuca alternifolia* (tea tree) oil of yeasts isolated from the mouths of patients with advanced cancer. *Oral Oncol*. 2006;42:487–492.
17. Mertas A, Garbusińska A, Szliszka E, et al. The influence of tea tree oil *(Melaleuca alternifolia)* on fluconazole activity against fluconazole-resistant *Candida albicans* strains. *Biomed Res Int*. 2015;2015:590470. https://doi.org/10.1155/2015/590470. Epub 2015 Feb 4.
18. Satchell AC, Saurajen A, Bell C, et al. Treatment of dandruff with 5% tea tree oil shampoo. *J Am Acad Dermatol*. 2002;47:852–855.
19. Hammer KA. Treatment of acne with tea tree oil (melaleuca) products: a review of efficacy, tolerability and potential modes of action. *Int J Antimicrob Agents*. 2015;45(2):106–110.
20. Bassett IB, Pannowitz DL, Barnetson RS. A comparative study of tea-tree oil versus benzoylperoxide in the treatment of acne. *Med J Aust*. 1990;153:455–458.
21. Lam NSK, Long XX, Griffin RC, Chen MK, Doery JC. Can the tea tree oil (Australian native plant: *Melaleuca alternifolia Cheel*) be an alternative treatment for human demodicosis on skin? *Parasitology*. 2018:1–11.
22. Tighe S, Gao YY, Tseng SC. Terpinen-4-ol is the most active ingredient of tea tree oil to kill *Demodex* mites. *Transl Vis Sci Technol*. 2013;2(7):2.
23. Satchell AC, Saurajen A, Bell C, et al. Treatment of interdigital tinea pedis with 25% and 50% tea tree oil solution: a randomized, placebo-controlled, blinded study. *Australas J Dermatol*. 2002;43:175–178.
24. Buck DS, Nidorf DM, Addino JG. Comparison of two topical preparations for the treatment of onychomycosis. *Melaleuca alternifolia* (tea tree) oil and clotrimazole. *J Fam Pract*. 1994;38:601–605.
25. Pena EF. *Melaleuca alternifolia* oil: its use for trichomonal vaginitis and other vaginal infections. *Obstet Gynecol*. 1962;19:793–795.
26. Hammer KA, Carson CF, Riley TV, et al. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food Chem Toxicol*. 2006;44:616–625.
27. Knight TE, Hausen BM. Melaleuca oil (tea tree oil) dermatitis. *J Am Acad Dermatol*. 1994;30:423–427.
28. D'Arrigo M, Ginestra G, Mandalari G, et al. Synergism and postantibiotic effect of tobramycin and *Melaleuca alternifolia* (tea tree) oil against *Staphylococcus aureus* and *Escherichia coli*. *Phytomedicine*. 2010;17:317–322.
29. Rosato A, Vitali C, Gallo D, et al. The inhibition of *Candida* species by selected essential oils and their synergism with amphotericin B. *Phytomedicine*. 2008;15:635–638.

92

Melatonin

Ron Reichert, BA, ND

OUTLINE

INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone synthesized in reply to neuronal signals arriving from the suprachiasmatic nucleus (SCN) of the anterior hypothalamus.[1,2] Its action in promoting sleep and the associated sleep/wake cycle is primarily the result of two G protein–coupled receptors within the SCN called MT1 and MT2.[3] Both receptors modulate neuronal activity within the SCN and influence circadian rhythm activation.[4] Biochemically, melatonin is synthesized as follows: l-tryptophan to 5-hydroxytryptophan to serotonin. The biogenic amine serotonin is then converted to *N*-acetylserotonin and ultimately into melatonin with the rate-limiting steps for melatonin production catalyzed by two enzymes, arylalkylamine *N*-acetyl transferase and hydroxyindole-*O*-methyltransferase (Fig. 92.1).[5] Melatonin is secreted by the pineal gland in response to darkness. This latter action has been elegantly described as the "opening of the sleep gate."[6]

PHARMACOKINETICS

Peak plasma concentrations of endogenous melatonin in adults reach a high of 60 to 70 pg/mL and typically occur between 2:00 and 4:00 AM.[5] Supplementary exogenous melatonin, according to the result of one human study, has a 15% absolute bioavailability at the 2- and 4-mg oral doses.[7] In another study using 250 mcg of daytime melatonin in healthy volunteers, it was noted that melatonin had a rapid absorption, with a mean time to reach maximal concentration (T_{max}) of 23 minutes. The mean terminal half-lives of melatonin were 36 ± 6 minutes and 45 ± 14 minutes, in men and women, respectively.[8] Sustained-release melatonin (2 mg), in contrast, has a T_{max} of 3 hours, which is delayed in the presence of food (T_{max} 0.75 hours). Terminal half-life is 3.5 to 4 hours.[9]

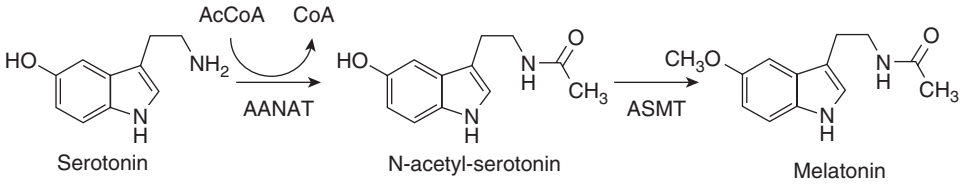


Fig. 92.1 Synthesis of melatonin.

CLINICAL APPLICATIONS

Jet Lag

Melatonin for the treatment of jet lag has been extensively studied in controlled studies and proven to be remarkably successful in reducing jet lag symptoms.[10] In one early double-blind, placebo-controlled study, 20 healthy volunteers (8 women, 12 men; 28–68 years) engaging in long-haul air flights across five time zones were invited to participate to explore the effects of melatonin on their jet lag symptoms. Subjects were randomly assigned to 5 mg of melatonin or placebo for 3 days before their outward flight (London to New Zealand) and during the flight between 10:00 AM and 12:00 PM local time, as well as for 3 days after their arrival between 10:00 PM and 12:00 AM . According to the results of visual analog scale (VAS) testing, those using the melatonin had less overall jet lag symptoms at day 10 (mean score 2.15) versus placebo (3.40; $P < 0.01$). Moreover, the melatonin group experienced a better adaptation pattern in terms of sleep pattern, daytime tiredness, and normal energy levels than the placebo group ($P < 0.05$).[11] Although this latter study suggested an optimal time pattern for melatonin use in an eastbound flight, another author noted that bedtime may not be an ideal time to employ melatonin on a westbound flight that crosses less than six to eight time zones. He suggested employing a lower dose of melatonin (0.5 mg) on the westbound flight later in the evening as "melatonin has the least phase shifting effects when it overlaps with endogenous secretion."[12]

Acute Sleep

It is estimated that 50 to 70 million American adults have difficulty sleeping.[13] This issue of poor sleep quality can have profound consequences for the individual and society at large, including an increased risk of accidents and mood and behavioral changes, as well as a reduction in work productivity.[14] One of the numerous options available to help improve overall sleep is melatonin, both in healthy adults as an acute remedy for insomnia and in the elderly dealing with issues of chronic insomnia. The acute use of melatonin in healthy adults without insomnia at doses of 0.3 to 1 mg at 8:00 or 9:00 PM significantly reduces sleep-onset latency and latency to stage 2 sleep compared with the placebo (P <0.001). Moreover, melatonin does not produce any "sleep hangover" symptoms (according to the Profile of Mood States and Four Choice Reaction Time Test) commonly associated with benzodiazepine-type drug use.[15]

Insomnia in the Elderly

Most elderly individuals report sleeping an average of 7 hours a night. Although the total amount of sleep time does not change as we age, alterations in sleep architecture are common.[16] This includes a decrease in both deep sleep (stages 3 and 4 slow wave sleep) and rapid eye movement (REM) sleep, as well as an increase in stage 1 (light) sleep.[17] Older individuals also have a reduction in endogenous melatonin production (likely due to a deterioration in the neuronal functioning of the SCN), which in turn disrupts the normal wake/sleep cycle.[18]

In a controlled clinical trial, 30 men and women over 50 years of age with both chronic insomnia and normal sleep patterns were randomized to receive capsules with either 0.1, 0.3, or 3 mg of melatonin or placebo 30 minutes before bedtime for 7 days. Treatments were separated by a 1-week washout period. Using polysomnography, the researchers concluded that although melatonin did not improve sleep efficiency in normal subjects, those with chronic insomnia had significant improvements in sleep efficiency at all three melatonin doses used, with the 0.3 mg dose triggering the strongest effect ($P <$ 0.0001). The physiological dose acted primarily in the middle portion of the night and raised plasma melatonin levels to normal. The authors noted that 3 mg of melatonin significantly raised plasma levels throughout a portion of the day and triggered reductions in core body temperature after ingestion of the hormone.[6]

Prolonged Release Melatonin in the Elderly

After a 2-week single blind run-in period, 354 elderly men and women (55–80 years of age) with primary insomnia were randomized to receive 2 mg of a prolonged release (PR) melatonin or placebo 2 hours before bedtime, for 3 weeks. Subjects enrolled in the study were asked to complete a sleep diary measuring quality of day and night, plus a battery of sleep questionnaires including the Pittsburgh Sleep Quality Index (PSQI), Leeds Sleep Evaluation Questionnaire (LSEQ), as well as quality-of-life (World Health Organization-5 [WHO-5] Well Being Index) and clinician Clinical Global Impression (CGI) scores. At the conclusion of the trial, those who took the melatonin had improved (26%) quality of sleep and morning alertness as recorded by the LSEQ in contrast to placebo (15%) ($P = 0.014$). Sleep latency, as noted by the PSQI results, improved in subjects employing the PR melatonin by 24.3 minutes compared with 12.9 minutes for the placebo group ($P = 0.028$). Quality of life as recorded by the WHO-5 Index was also improved in the active group ($P = 0.034$).[19] A similar study supported the latter conclusion with a positive effect found on the restorative value of sleep in elderly patients using 2 mg of PR melatonin. Moreover, melatonin users did not experience any rebound insomnia or withdrawal symptoms at the conclusion of the controlled study. The authors noted that plasma cortisol increased in middle-aged adults, and this phenomenon might impair sleep. Administration of PR melatonin in older adults with primary insomnia may delay the production of nighttime cortisol, with subsequent improvements in both sleep quality and morning alertness.[20]

Magnesium, Melatonin, and Zinc on Primary Insomnia and the Elderly

Magnesium, along with zinc, is crucial for the endogenous synthesis of melatonin according to the results of one study. Elderly men and women (n = 43; average age 78.3 years) living in a long-term care facility were invited to participate in a double-blind, placebo-controlled study of 60 days' duration on melatonin, zinc, and magnesium on primary insomnia. Each volunteer was randomized to receive a 100 g pear pulp food supplement with 5 mg melatonin, 225 mg of magnesium, and 11.25 mg of zinc, or placebo 1 hour before bedtime for 2 months. At the conclusion of the trial, those employing the active supplement experienced an improvement in their overall PSQI scores compared with placebo ($P < 0.001$). Moreover, the LSEQ results confirmed that

those employing the food mixture not only had better quality of sleep but had greater ease getting to sleep ($P < 0.001$). Quality of life was measured by the Medical Outcomes Study Short-Form 36 scale, which noted that the active group had better physical and mental functioning after 8 weeks of treatment ($P = 0.006$). Adverse effects were minimal, with only two participants in the treatment group complaining of headache, whereas one volunteer in the placebo group complained of epigastric pain.[21]

Insomnia in Perimenopausal Women

In a series of case reports, 11 perimenopausal women (45–52 years) with insomnia were treated with 7.5 to 15 mg of mirtazapine for 2 to 4 weeks. Two milligrams of melatonin were then added to the treatment regimen, along with a concomitant reduction and discontinuation of mirtazapine for 30 to 90 days. Global PSQI scores decreased from 17.45 at baseline to 8.55 at the second visit (mirtazapine and PR melatonin) to 6 by the third visit (PR melatonin) ($P < 0.01$). These latter changes were paralleled by a similar drop in PSQI sleep latency scores, with 52.73, 21.36, and 18.64 minutes at baseline, visit 2, and visit 3, respectively ($P < 0.01$). Volunteers also noted that their quality of life as measured by the WHO-5 score improved significantly by 89% from baseline ($P < 0.01$).[22]

Chronic Sleep Onset Insomnia in Children

Pediatric insomnia is estimated to affect 1% to 6% of children, but can be significantly elevated to 50% to 75% if there are other associated psychiatric or neurodevelopmental issues such as attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (ASDs), and epilepsy.[23]

A group of children (49 boys, 13 girls; aged 6–12 years) with chronic sleep-onset insomnia of more than 12 months' duration were randomized to receive 5 mg melatonin or placebo at 7 pm for 30 days. Using several questionnaires measuring overall health status (RAND General Health Rating Index and Functional Status II), children who used the melatonin had better outcomes in terms of eating, sleeping, response to attention, fatigue, illness, and overall health than those who received the placebo ($P < 0.05$). In addition, melatonin users had an advancement in their sleep onset by 57 minutes ($P = 0.003$), a reduction in sleep latency by 17 minutes ($P = 0.048$), and a decrease in their dim light melatonin onset (DLMO) by 82 minutes ($P < 0.001$).[24]

Insomnia, Melatonin, and Chronic Obstructive Pulmonary Disorder

Approximately 40% of patients with chronic obstructive pulmonary disorder (COPD) complain of sleep disorders.[25] Treatment of sleep disorders in this population group may help improve their overall quality of life as noted in one study employing melatonin.[26] Thirty-nine patients (64–67 years of age on average) with stage II to IV COPD were randomized to receive 3 mg of melatonin or placebo 1 hour before bedtime for 21 days. In the 25 patients who completed the study, the overall PSQI score was significantly improved in those employing the melatonin ($P = 0.012$). In addition, components of the PSQI including an improvement in sleep latency ($P = 0.008$) and an increase in sleep duration ($P = 0.046$) were noted in the active group. There was no difference between active and placebo groups in the Epworth Sleepiness Scale (ESS), lung function parameters (forced expiratory volume in 1 second [FEV1], forced vital capacity [FVC], and FEV1/FVC ratio), or functional exercise capacity as measured by the 6-minute walking distance test. Four of the 25 cases reported adverse events including mild headache and dryness of mouth in the melatonin group and tongue swelling, mouth dryness, and loss of appetite in the placebo group.[27] A subsequent controlled study in patients with COPD has shown that a similar dose of melatonin decreases 8-isoprostane from a baseline measure of 20.41 ± 2.92 pg/mL to 12.70 ± 2.18 pg/mL ($P = 0.04$) by month 3. Moreover, dyspnea was improved significantly by melatonin treatment ($P = 0.01$) without changes in lung function or exercise capacity. A significant increase in interleukin-8 (IL-8) was seen in the placebo group only ($P = 0.03$).[28]

Tetraplegia, Sleep Disruption, and Melatonin

Individuals with spinal cord injury, especially tetraplegia, have higher rates of sleep disturbances and subsequently poorer quality of life.[29] Eight individuals with chronic complete tetraplegia (average age 49.5 years) were randomized to receive 3 mg of melatonin capsules or placebo 2 hours before bedtime for 3 weeks. In those taking the melatonin, the proportion of light sleep (stages 1 and 2 sleep) significantly increased after melatonin use ($P < 0.05$). In addition, quality of life as measured by Assessment of Quality of Life Questionnaire (AQoL) subscale 'psychological wellbeing' was significantly improved after melatonin use compared with placebo ($P < 0.01$). Similarly, values on the Basic Nordic Sleepiness Questionnaire (BNSQ) item number 12 (i.e., how many hours of sleep per night the participant was experiencing), were significantly elevated after melatonin use in contrast to placebo ($P < 0.05$).[30]

Melatonin, Beta-Blockers, and Insomnia

In the U.S. an estimated 22 million people use beta-blockers on a regular basis. As this class of medications suppresses nighttime melatonin secretion, it could explain the insomnia linked to this drug.[31] Sixteen patients (age 45–64 years) using beta-blockers (i.e., atenolol or metoprolol) were randomized to receive 2.5 mg melatonin or placebo every night for 3 weeks. In comparison with placebo, 3 weeks of melatonin supplementation significantly increased total sleep time (+36 min; $P = 0.046$), sleep efficiency (+7.6%; $P = 0.046$), and reduced sleep onset latency to Stage 2 (-14 min; $P = 0.001$) as recorded by polysomnography. In addition, and in comparison to placebo, melatonin also significantly increased stage 2 sleep time (+41 min; $P = 0.037$) but did not alter the duration of other sleep stages.[31]

Fibromyalgia

Fibromyalgia (FM) is a challenging condition with characteristic symptoms that include widespread and variable chronic pain, as well as fatigue, stiffness, cognitive disturbances, depression, and insomnia.[32] Although alterations in the secretion of melatonin have been observed in FM patients, whether or not this latter phenomenon contributes to the pathophysiology of this disease remains controversial.[33] Several studies suggested, however, that melatonin is of value in treating this chronic musculoskeletal condition.

Twenty-one female patients (average age 51 years) who met the American College of Rheumatology (ACR) criteria for primary FM for at least 6 months before their participation in an uncontrolled pilot study were invited to help evaluate the effects of a 3 mg capsule of melatonin 30 minutes before bedtime for 1 month. Of the 21 patients who completed the study, 19 were found to have a significant reduction from baseline in musculoskeletal tender point count (−28.6%), severity of pain (−33.4%), and sleep disturbances (−67.2%) as measured by VAS ($P < 0.05$). Although fatigue, depression, and anxiety did not show statistical improvement on VAS, the overall physician and patient VAS global treatment assessment scores of FM disease status (with 10 representing the worst score) improved from 7.6 to 5.7 and 7 to 4.7, respectively. Four patients experienced mild and transient side effects during the trial, including tremor, heartburn, anxiety, and somnolence.[34]

In another randomized, double-blind placebo-controlled trial, 101 patients (6 men, 95 women; 18–65 years) with ACR confirmed primary FM were randomized to one of four treatment groups: (A) 20 mg/day fluoxetine and placebo; (B) 5 mg/day melatonin and placebo; (C) 20 mg/day fluoxetine with 3 mg/day melatonin; and (D) 20 mg/day fluoxetine and 5 mg/day melatonin for 2 months. Using the Fibromyalgia Impact Questionnaire (FIQ) to evaluate the clinical outcomes, the researchers determined that compared with baseline, both fluoxetine and melatonin as single therapies significantly reduced within-group symptom scores by 21.5% and 18.9%, respectively ($P < 0.05$). However, those volunteers combining fluoxetine and melatonin at both 3 and 5 mg doses experienced nearly comparable (28.8% vs. 28.9%) and highly significant reductions in their FIQ scores ($P < 0.001$). Although all treatments reduced FIQ pain scores, it was interesting to note the reduction in fatigue scores in groups B, C, and D by 23.7%, 20.3%, and 34.7%, respectively, compared with only 9.9% in the fluoxetine-only treatment group. Fluoxetine as a single agent did not alter the rest/sleep score compared with the significant improvements seen in groups B, C, and D.[35]

Chronic Fatigue Syndrome

Much like FM, chronic fatigue syndrome (CFS) is associated with a number of symptoms, including intense and disabling fatigue that does not improve with rest.[36] One of the factors contributing to fatigue and sleep disturbances in CFS is disruption in the hypothalamic-pituitary-adrenal axis.[37] However, under research scrutiny, both adults and adolescents with CFS have increased levels of melatonin, suggesting the hormone has no role to play in this disorder.[37] Research on DLMO concluded that a certain subset of CFS patients may respond to the therapeutic use of melatonin. DLMO is a naturally occurring event that may account for delayed sleep phase syndrome in 10% of chronic insomniacs.[38]

Twenty-nine patients (24 women, 5 men; average age 33.2 years) with CFS were asked to take 5 mg of melatonin at bedtime for 3 months. Using the checklist individual strength (CIS) questionnaire to measure several variables, the authors determined that the total score ($P = 0.006$), as well as the subscores of fatigue ($P = 0.017$), concentration ($P = 0.031$), motivation ($P = 0.010$), and activity ($P = 0.008$), improved significantly after 90 days of melatonin use. Moreover, those patients with late DLMO (>21.30 hours) overall CIS and its subscores were better than in those CFS patients with early DLMO.[39]

Children With Epilepsy

Seizure-free epileptic children (n = 31; 3–12 years of age) with a history of either partial or generalized seizures and using 10 mg/kg per day of sodium valproate participated in a double-blind, placebo-controlled study of 8 weeks' duration. The children were randomized to receive either melatonin tablets (6 mg for those less than 9 years of age and less than 30 kg body weight or 9 mg for those more than 9 years of age and more than 30 kg body weight, to be taken in the evening) or comparable placebo 1 hour before bedtime. The primary end point of the study was evaluated using the parental Sleep Behavior Questionnaire. By the conclusion of the controlled trial, those children using the additional melatonin had a significantly greater average percentage reduction in their total sleep scores (24.4%) compared with placebo (14.4%) ($P < 0.05$). Moreover, the median reduction in the parasomnia scores significantly favored those who took melatonin (60%) relative to the placebo (36.4%) ($P < 0.05$). No adverse effects were noted during the trial.[40] In another placebo-controlled trial, 11 children aged 6 to 11 years with epilepsy were randomized to receive 9 mg of sustained release melatonin or placebo 30 minutes before bedtime for 1 month. At the conclusion of the trial, melatonin decreased mean sleep latency by 11 minutes ($P = 0.02$) and mean wake after sleep onset (WASO) by 22 minutes ($P = 0.04$) in contrast to placebo. Sleep diaries revealed that children who employed melatonin slept 11 minutes more ($P = 0.1$) and woke up 18 minutes later ($P = 0.048$) in contrast to those employing placebo. Furthermore, and compared with placebo, the use of melatonin increased stage 3 by 5.5% ($P = 0.04$) and REM latency by 58 minutes ($P = 0.04$) and decreased REM % by 5.8% ($P = 0.01$). Melatonin did not alter seizure frequency. Four children reported adverse events while taking melatonin compared with two children on placebo.[41]

Autism/Fragile X Syndrome

In a recent review and meta-analysis, the authors noted that children with ASDs not only have a higher incidence of sleep-associated problems, but that melatonin use led to significant improvements in sleep duration and sleep-onset latency compared with placebo ($P < 0.01$). This poor sleep pattern in turn can lead to a worsening of overall autistic behavior symptoms.[23]

In one double-blind, placebo-controlled, crossover trial, 18 subjects (16 boys, 2 girls; mean age 6 years) with ASD or fragile X syndrome, or both, and insomnia were randomized to receive 3 mg melatonin or placebo 30 minutes before bedtime for 14 days. After 2 weeks, subjects were crossed over, such that the active group received placebo and vice versa. Children were evaluated using actigraphy and parentally recorded sleep diaries. After 4 weeks and in 12 of 18 children who had complete data sets, the authors noted that those using the melatonin had an improvement in the total night's average sleep duration (+21 minutes longer than placebo; $P = 0.02$), a reduction in sleep latency time (28 minutes vs placebo; $P = 0.0001$), and a 42-minute earlier sleep-onset time in contrast to the placebo group ($P = 0.02$).[42]

A controlled clinical trial supports the use of melatonin in children with ASD and neurogenetic disorders (NGD). In this study, 125 children and adolescents (2–17.5 years of age; 92 males, 33 female consisting of 96.8% ASD, 3.2% Smith-Magenis syndrome) whose sleep did not improve while employing behavioral interventions were randomized to receive prolonged-release melatonin (2 mg escalated to 5 mg) or placebo for 13 weeks. At the conclusion of the trial, participants who employed sustained-release melatonin slept on average 57.5 minutes longer at night compared with 9.14 minutes with placebo ($P = 0.034$). Moreover, sleep latency (SL) decreased on average by 39.6 minutes on average in the active group and 12.5 minutes with placebo ($P = 0.011$).[43]

Children With Insomnia and Attention Deficit Hyperactivity Disorder

Twenty-seven children (6–14 years of age) diagnosed with ADHD and insomnia and employing stimulant medications (i.e., methylphenidate, dextroamphetamine), participated in a controlled trial. Those subjects who did not respond to a 10-day sleep hygiene regimen were randomized to receive 5 mg of melatonin or placebo 20 minutes before bedtime for 1 month. Compared with the placebo, children who took melatonin had a significant reduction in sleep-onset latency as measured by somnolog (46.4 minutes vs. 62.1 minutes placebo) and actigraphy ($P < 0.01$) at the conclusion of the trial. Adverse events were considered mild by the investigators. Melatonin treatment and the subsequent improvement in sleep did not, however, improve ADHD symptoms as noted by Conner's Attention Deficit Scale, Parent version scores.[44]

One hundred and five medication-free children (aged 6–12 years) with ADHD were randomized to receive 3 mg of melatonin or 6 mg of melatonin if body weight was less than 30 kg or greater than 30 kg, respectively, or placebo at 7:00 PM for 30 days. Those subjects who used

melatonin had advancements in sleep onset as measured by actigraphy (26.9 ± 47.8 minutes), compared with a delay (10.5 ± 37.4 minutes) in the placebo group ($P < 0.0001$). Additionally, there was a significant increase in total sleep time (19.8 ± 61.9 minutes) versus a reduction (13.6 ± 50.6) seen in the placebo group ($P = 0.01$). Children treated with the active compound showed an advance in DLMO of 44.4 ± 67.9 minutes compared with a delay in children who received the placebo ($P < 0.001$). No changes were noted in behavior and cognition. Adverse events did not differ between active groups and placebo.[45] A long-term 3.7-year follow-up using the same group of children (93% response rate) verified that melatonin use was not associated with any serious side effects but also did not provide a permanent cure for insomnia in ADHD children.[46]

Children With Migraine/Tension-Type Headaches

Tension headaches and migraines are two of the more likely reasons that will cause children to complain of pain. Annual prevalence for migraine and tension-type headaches can vary with between a 3% to 11% and a 10% to 24% occurrence, respectively.[47]

In an uncontrolled pilot study, 22 children (10 boys and 12 girls; age range 6–16 years) with various types of long-standing (average 40.05 ± 30.2 months) headache-type discomfort (13 recurrent migraines/no aura; 8 chronic tension-type headaches; 1 migraine with aura) participated in a 12-week study on the effects of oral melatonin. After a 1-month washout period in which all preventive therapy was discontinued, the children were asked to take 3 mg of melatonin at bedtime for 90 days. At the conclusion of the study and according to the results of a structured daily headache diary, the frequency (i.e., the number of attacks per month) and the duration of attacks (hours) decreased from 12.3 to 5.7 and 13.5 to 9.7, respectively ($P < 0.001$). Pragmatically, and as the authors noted, 14 of the 21 subjects (one child stopped the melatonin due to excessive somnolence) had a greater than 50% reduction in headache attacks. Notably, four of the children had complete resolution of their headaches.[48]

Functional Dyspepsia

Postprandial fullness and early satiation, along with epigastric pain and burning without evidence of upper gastrointestinal structural pathology on endoscopy, are the hallmark of Rome III diagnostic criteria for functional dyspepsia.[49] One group of researchers suggested that melatonin use stimulates enterocytes to produce bicarbonate, which in turn may help neutralize gastric hydrogen chloride and, therefore, reduce symptoms as evidenced by the following study.[50] Sixty patients (18 men, 42 women; ages 19–39 years) with a 3- to 12-year history of nonulcer dyspeptic symptoms (i.e., chronic or recurrent epigastric pain) were randomized to receive 5 mg of melatonin or placebo in the evening for 3 months. After 12 weeks of treatment and using a 10-point scale (0 = no symptoms; 10 = maximum symptoms) for therapeutic evaluation, 17 of 30 (56.6%) patients using the melatonin had their condition resolve completely, such that they did not require further treatment, whereas an additional 9 of 30 (30%) patients experienced a partial improvement in symptoms (i.e., reduction in nighttime gastric pain) ($P < 0.01$). Although 4 patients in the active treatment group noted no change in symptoms, a larger percentage of subjects (93.3%) using placebo noted no change in their condition. The authors noted that those who had a previous infection with *Helicobacter pylori* were less likely to respond to melatonin treatment.[50]

Irritable Bowel Syndrome

It is estimated that 7% to 15% of the U.S. population has irritable bowel syndrome (IBS), a painful abdominal condition that is primarily associated with diarrhea and/or constipation, along with a number of other symptoms, including gas and bloating.[51] Recent research showed that the excretion of the urinary metabolite of melatonin (6-sulphatoxymelatonin) was significantly lower in subjects with IBS than controls ($P = 0.0004$), suggesting that melatonin is involved in the pathogenesis of IBS by acting on the enterochromaffin cells in the gastrointestinal tract.[52]

In a double-blind, placebo-controlled study, 18 patients (12 men, 6 women; ages 18–65 years) with IBS were randomized to receive 3 mg of melatonin at bedtime or a matching placebo for 2 months. By the conclusion of the trial, those subjects taking melatonin had a 45% improvement in their overall IBS symptom scores (i.e., pain severity and frequency, bloating) compared with 16.7% in those taking placebo ($P < 0.05$). Moreover, the extracolonic symptom scores, including headache, backache, body aches, thigh pain, and lethargy, were significantly lower in the melatonin group compared with pretreatment values ($P < 0.001$) and the placebo group ($P < 0.05$). Overall, those subjects taking the melatonin experienced an improved quality of life score (43.63%) in contrast to those taking placebo (14.64%) ($P < 0.05$). Three subjects experienced side effects, including decreased libido and drowsiness.[53]

In another controlled study, 80 postmenopausal women (mean age 55.3 ± 6.4 years) with constipation-predominant IBS (IBS-C) (n = 40) and diarrhea-predominant IBS (IBS-D) (n = 40) received 3 mg of melatonin in the morning and 5 mg of melatonin in the evening or a placebo for 6 months. Clinical outcomes were evaluated using a 10-point VAS (mild [1–4 points], moderate [5–7 points], and severe [8–10 points]) along with patient observations recording the changes in visceral pain, abdominal bloating, constipation and/or diarrhea along with a urinary marker (urinary 6-sulfatoxymelatonin [6-HMS]) of melatonin metabolism.

After 6 months of treatment, the researchers determined that in those with IBS-C, there was a negative correlation ($r = -0.714$) between symptom scores and the excretion of 6-HMS. In contrast, in those patients with IBS-D, this correlation was positive ($r = 0.409$). As such, only the IBS-C group employing the melatonin had statistically significant reductions in IBS symptoms at both the 4- ($P < 0.05$) and 6-month ($P < 0.01$) mark. In the IBS-C group, melatonin decreased the intensity of visceral pain and abdominal bloating in 70% of patients ($P < 0.01$) and constipation in 50% of patients ($P < 0.05$).[54]

Gastroesophageal Reflux Disease

In 2004 it was estimated that 20% of the American population has gastroesophageal reflux disease (GERD), which is responsible for 64.6 million prescriptions annually.[55] According to several studies, melatonin is of therapeutic value with or without omeprazole.

In this study, 36 individuals with GERD were selected and equally placed into four groups: control; 3 mg melatonin at bedtime; 20 mg of omeprazole twice daily; and 3 mg of melatonin and 20 mg of omeprazole twice daily for 2 months. After 8 weeks, those using the melatonin alone had a significant reduction in GERD symptoms, increase in lower esophageal sphincter (LES) pressure (better LES tone), increase in serum gastrin, a reduction in gastric basal acid output, and an increase in serum melatonin levels ($P < 0.05$). In contrast, although omeprazole was also effective in reducing GERD symptoms ($P < 0.05$), it did not alter LES tone in a significant fashion. The combination of melatonin and omeprazole had similar results in the single melatonin group, but had a better reduction in basal acid output and an increase in serum gastrin ($P < 0.05$).[56]

Individuals (141 men, 210 women; mean age 44 years) with at least one episode of moderate to severe heartburn within the previous week before the onset of treatment within a clinical trial were randomized to receive 6 mg melatonin plus vitamin B_6 (25 mg), vitamin B_{12} (50 mcg),

folic acid (10 mg), tryptophan (100 mg), methionine (100 mg), and betaine (100 mg) or one capsule (20 mg) omeprazole per day in the evening for 40 days. After 40 days, individuals using the natural product combination had 100% reduction in symptoms as measured by a five-point severity scale, compared with the reduction in symptoms seen (65.7%) in the omeprazole group ($P < 0.05$). As an observation, those 34.3% of the subjects who experienced a persistence of their GERD symptoms with the use of omeprazole had complete resolution of their symptoms after 40 days with the natural combination of vitamins, melatonin, and amino acids.[57]

The authors suggested that the efficacy for their combination medication was a result of several factors. Melatonin may block the production of nitric oxide synthase, which may help lower the degree of LES relaxation. Melatonin also stimulates the production of bicarbonate, which protects against the action of gastric acid production. Moreover, the combination of vitamin B_6, tryptophan, and vitamin B_{12} may decrease acute gastrointestinal pain as a result of serotonin inhibiting the nociceptive system. Methionine and betaine may induce S-adenosyl methionine production, which has both anti-inflammatory and analgesic properties.[57,58]

Cancer

According to the results of a systemic review, melatonin decreased the risk of death from solid tumors at the 1-year mark.[59] This was supported by several independently published articles, primarily by one of the premier researchers on melatonin use and cancer, Professor Paolo Lissoni.

Nonsmall Cell Metastatic Lung Cancer

One hundred patients (59 men, 41 women; average age 39–81 years) with metastatic nonsmall cell lung cancer were randomized to receive chemotherapy (cisplatin and etoposide) every 21 days for four cycles or chemotherapy plus 20 mg of melatonin in the evening. Patients in the melatonin group continued to use melatonin after chemotherapy cessation. Reviewing the clinical response after 60 to 72 months of follow-up, the authors determined that those using the melatonin and chemotherapy combination had a significantly better complete and partial response (35%; 17 of 49 patients) to treatment versus the chemotherapy group (18%; 9 of 51 patients) ($P < 0.05$). Moreover, a progressive disease state was seen in 20 of 51 patients in the chemotherapy group in contrast to 6 of 49 patients in the chemotherapy/melatonin group ($P < 0.01$). Side effects of chemotherapy, including neurotoxicity, thrombocytopenia, weight loss of more than 10%, and asthenia, were notably reduced in the melatonin group. Overall 5-year survival rate was significantly longer in those concomitantly treated with melatonin ($P < 0.001$). Three of the 49 patients were still alive 5 years from the date of therapy onset in the combination group, compared with no patients in the chemotherapy alone section.[60]

Metastatic Solid Tumors: Gastric, Colorectal, and Nonsmall Cell Lung Cancer

Three hundred and seventy patients with metastatic nonsmall cell lung cancer, colorectal cancer, or gastric cancer were randomized and treated with the appropriate chemotherapy alone or chemotherapy plus melatonin 20 mg/day in the evening. After 24 months, the overall response rate (complete and partial remission) was higher in the melatonin and chemotherapy group (68 of 167 patients; 36%) versus chemotherapy alone (37 of 183 patients; 20%) ($P < 0.001$). In addition, the 2-year survival rate in those using melatonin adjunctively was better (47 of 187 patients; 25%) versus those employing chemotherapy as a sole treatment (24 of 183 patients; 13%) ($P < 0.05$).[61]

Metastatic Breast Cancer

Melatonin may enhance the effects of the drug tamoxifen according to the results of one preliminary study. Fourteen women (age 42–80 years) with metastatic breast cancer whose breast cancer did not respond to tamoxifen therapy (n = 3) or who had progressed after their disorder had stabilized (n = 11) on tamoxifen participated in this Phase II trial. After a 1-month washout of tamoxifen, patients were given melatonin 20 mg in the evening, 1 week before starting tamoxifen and 20 mg midday for at least 12 months. Although no complete response to the combination therapy was noted in any of the patients, 28.5% (4 of 14 patients) did achieve a partial response. Moreover, 10 of 14 patients were still alive after 1 year. Serum levels of insulin-like growth factor (IGF-1) were reduced in response to the treatment at both 1 ($P < 0.05$) and 3 months ($P < 0.01$). Prolactin levels were also reduced from 25 ± 3 ng/ml^{-1} to 13 ± 2 ng/ml^{-1} after 12 weeks ($P < 0.05$). No toxicity was noted.[62]

Metastatic Prostate Cancer

Fourteen men (mean age of 71.5 years) with metastatic prostate cancer (tumor grades G2 [n = 9] and G3 [n = 5]) whose prostate-specific antigen (PSA) scores had worsened using a combination of triptorelin (3.75 mg intramuscular every 28 days) and flutamide (750 mg/day by mouth for 1 month only) participated in this open clinical trial. After a 30-day washout period in which no triptorelin was given, patients were asked to begin melatonin 20 mg at night 7 days before starting their monthly injections of luteinizing hormone-releasing hormone (LHRH) analog drug. After 90 days, 8 of 14 patients had a greater than 50% reduction in their PSA scores compared with pretreatment values. In addition, average PSA serum concentrations were reduced from a pretreatment value of 421 ± 118 ng/mL to 108 ng/mL ($P < 0.05$) after 3 months of melatonin and triptorelin. IGF-1 and prolactin serum values were significantly reduced in patients using the combination of the two drugs ($P < 0.05$).[63]

Taxane-Related Neuropathy

Although the two types of taxanes (paclitaxel and docetaxel) are widely used in oncology, they have several side effects, including neuropathy.[64] A recent study suggested that melatonin might help with this issue. In this open-label Phase II pilot study, 22 women with breast cancer (33–68 years of age) who underwent chemotherapy with taxanes were instructed to take 21 mg of melatonin at bedtime for up to 6 months. Using the National Cancer Institute-Cancer Treatment Center's scale for neuropathy, the authors noted that, "in our trial, patients receiving melatonin during taxane chemotherapy had a reduced incidence of all grade neuropathy (46%) compared with historical controls (around 60%)." Fifty-five percent of the patients (n = 11) reported no neuropathy (grade zero). Adverse events included nausea, vomiting, and fatigue. None of the patients reported any incidence of daytime sedation.[65]

Cachexia

Progressive weight loss, anorexia, and reduction in the body's cell mass are hallmarks of cancer-related cachexia.[66] The cytokine tumor necrosis factor-alpha (TNF-α) plays a role in cancer cachexia according to the conclusions of one trial. Patients (56 men, 30 women; 39–76 years) with untreatable solid tumors (i.e., breast, colorectal, gastric, nonsmall cell lung cancer, etc.) and metastasis to various sites were stratified according to tumor type and randomized to either standard care (nonsteroidal anti-inflammatory drugs and opioids for pain) or standard care plus melatonin (20 mg in the evening) for at least 3 months. The authors noted that at the end of 16 weeks, weight loss of

greater than 10% occurred in 32% of the patients treated with conventional care versus only 4% of the patients treated with melatonin and conventional care ($P < 0.01$). Concomitantly with the lower reduction in weight loss, serum values of TNF-α were also significantly lower in patients using the standard care and melatonin compared with the standard care group alone ($P < 0.01$).[67]

Melatonin and Solid Tumor Cancer Therapy: Meta-Analysis

Melatonin has shown to be an effective adjunctive therapy in cancer patients with solid tumors using 20 mg per day. In a meta-analysis, melatonin significantly improved complete and partial remissions (16.5 vs. 32.6%; $P < 0.00001$) as well as the 1-year survival rate (28.4 vs. 52.2%; $P = 0.001$). Melatonin use was also associated with a reduction in radio-and chemotherapy-related side effects including thrombocytopenia (19.7 vs. 2.2%; $P < 0.00001$), neurotoxicity (15.2 vs. 2.5%; $P < 0.0001$), and fatigue (49.1 vs. 17.2%; $P < 0.00001$). No severe adverse events were noted in the randomized control trials.[68]

Breast Cancer and Insomnia

Sleep disruptions in those with cancer are a significant clinical issue and require intervention.[69] In a controlled trial, 48 patients (aged 30–75 years) scheduled for a lumpectomy or mastectomy for breast cancer were randomized to receive 6 mg of melatonin or placebo 1 hour before bedtime 3 days preoperatively until 2 weeks postoperatively.[70] At the conclusion of the trial and according to actigraphy, the administration of oral melatonin significantly improved sleep efficiency ($P = 0.007$) and reduced wake after sleep onset ($P = 0.01$) during the entire 14 day postoperative period only.[70] No changes were noted with VAS and Karolinska Sleepiness Scale outcomes along with other actigraphy variables.[70] A second controlled study found that 95 postmenopausal breast cancer survivors who completed active cancer therapy had improved their sleep quality with 3 mg of melatonin or placebo at bedtime over a 4-month time period. Compared with placebo, those who were given melatonin had significantly greater improvements in subjective sleep quality as measured by the PSQI score (i.e., −0.1 in the placebo group compared with −1.9 in the melatonin group; $P < 0.001$).[71]

Topical Melatonin and Breast Irradiation Dermatitis

Forty-seven patients median age 54 to 55 years of age with stage 0-II breast cancer treated with radiation therapy were randomized to receive a melatonin-containing emulsion or placebo cream and apply the cream twice a day over the treated breast (not less than 2 hours before radiation treatment) for the 5 weeks during the radiotherapy and 2 weeks thereafter. At the conclusion of the study (2 weeks postradiation), those taking melatonin had favorable results: 59% (13 patients) had no toxicity (grade 0) and 41% (9 patients) had grade 1/2 dermatitis. This is in contrast to the placebo group, which had 11% with grade 0 (2 patients) and 90% with grade 1/2 (17 patients) dermatitis ($P = 0.03$). Four women treated with melatonin developed a localized and regional cutaneous allergic reaction presenting as maculopapular rash that resolved with treatment.[72]

Fertility

It is estimated that about 6.1 million women in the U.S. between the ages of 15 to 44 years have difficulty with either becoming pregnant or staying pregnant. Because the success rate with in vitro fertilizations (IVFs) and subsequent pregnancies can vary,[73] researchers have considered other options, including melatonin. One hundred fifteen women (24–45 years) who had not become pregnant using IVF were divided into two groups and given 3 mg of melatonin at 10:00 PM from the fifth day of the previous menstrual cycle until the day of oocyte retrieval. Those using the melatonin in conjunction with the second IVF had a higher fertilization rate (50.0 ± 38.0%; $P < 0.01$) compared with the first IVF trial (20.2 ± 19.0%), with a higher number of pregnancies (19.6%; 11 of 56 patients) being reported. In contrast, the nonmelatonin users had no improvement in their fertilization rates (20.9 ± 16.5% first IVF vs. 22.8 ± 19.0% second IVF), resulting in a lower number of successful pregnancies (10.2%; 6 of 59 patients) during the second IVF-ET trial. The difference between the two groups' pregnancy rates was not statistically significant. In an additional analysis of 54 infertile women, it was determined by the authors that 3 mg of melatonin at 10:00 PM or 200 mg of vitamin E (given from the fifth day of the previous menstrual cycle until oocyte retrieval) three times a day significantly decreased the intrafollicular oxidative stress by decreasing the 8-OHdG concentrations compared with the first IVF cycle ($P < 0.05$).[74]

Forty-six infertile women (38–42 years of age) who had not conceived previously using IVF were recruited to receive 2 g of myoinositol plus 200 mg of folic acid in the morning and the latter combination in the evening plus 3 mg of melatonin daily for 90 days. After 3 months of using the treatment, a second cycle of IVF was instituted, resulting in a significant improvement in the number of embryos (0.48 ± 0.51 vs. 0.80 ± 0.69; $P = 0.01$) and the quality of the embryos transferred (0.13 ± 0.48 vs. 0.35 ± 0.48; $P = 0.01$). The implantation rate during the second IVF cycle was 22.8% compared with 0% in the initial IVF cycle, resulting in 13 pregnancies. Four of the 13 pregnancies were not successful and spontaneously aborted.[75]

Depression

Although 6 mg of slow release melatonin for 4 weeks did not show significant improvement in major depressive disorder symptoms, some individuals may benefit from melatonin use, such as those with depression and delayed sleep phase syndrome and women during perimenopause or menopause.[76]

Depression and Delayed Sleep Phase Syndrome

In a double-blind, placebo-controlled crossover trial, 13 men (age 35.6 ± 14 years) and 7 women (aged 30.8 ± 12.4 years) with established delayed sleep phase syndrome were divided into groups: those with (group I; n = 8; 3 men and 5 women) or without depression (group II; n = 12; 4 women and 8 men) based on a clinical assessment and the results of the Center for Epidemiological Studies Depression Scale (CES-D) and the Hamilton Depression Rating Scale (HDRS). Patients were randomly assigned to either 5 mg of melatonin or placebo and asked to consume the medication between 7:00 PM and 9:00 PM for 4 weeks followed by a 1-week washout before treatment crossover for another 4 weeks. Those in group I had a significant reduction in their HDRS and CES-D scores in comparison to placebo ($P < 0.05$). Moreover, according to polysomnographic results, sleep-onset latency was reduced in both groups I and II compared with both placebo and baseline measures ($P = 0.03$).[77]

Depression in Menopause/Perimenopause

Women with either perimenopause (n = 36), premenopause (n = 25), and postmenopause (n = 18), ranging in age from 42 to 62 years, were divided into two age groups (42–49 years and 50–62 years) and given either 3 mg of synthetic melatonin or placebo at bedtime between 10:00 PM and 11:00 PM for 6 months. Subjects were evaluated for salivary melatonin and serum levels of several hormones, including thyroid-stimulating hormone (TSH), T3, T4, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estrone, estradiol, and progesterone at time 0, 3, and 6 months. After 6 months of treatment, melatonin use significantly increased T3 and T4 ($P < 0.0001$) compared with time 0. The placebo group also had increases in T3 after 3

months ($P < 0.05$) but not at 6 months. TSH was not affected by either treatment group. Melatonin use also reduced plasma LH levels in the younger group of women (28.6%) but not in the older group ($P < 0.05$). FSH levels were also significantly reduced, especially in women with low basal melatonin levels ($P < 0.05$). In contrast to placebo, women who took melatonin had an improvement in their mood state, with only 6.7% of the active group reporting morning depression compared with 21% of the placebo users ($P < 0.05$).[78]

Alzheimer's Disease

Alzheimer's dementia (AD) is a growing concern both in the U.S. and throughout the global community. It is estimated that there were 24.3 million people worldwide with dementia in 2001, and that this figure would rise to 81.1 million by the year 2040.[79] Similarly in America, AD is expected to increase to an estimated 13 million by the year 2050.[80] In addition to a deterioration in memory and day-to-day functioning, sundowning syndrome or nocturnal delirium are a common behavior, found in 2.4% to 25%, respectively, of those diagnosed with AD.[81] Sundowning can occur typically between 3:00 PM and 7:10 PM and is associated with a heightened degree of agitation, irritation, and confusion.[82] Because this problem can cause great distress for both caregivers and health care professionals, several researchers noted that sundowning is a form of circadian rhythm disturbance, and that the associated delirium would respond well to melatonin.[83]

Sundowning Phenomenon in Alzheimer's Disease

In a retrospective study, 14 patients (8 women, 6 men; average age 72 years) diagnosed with AD received 9 mg melatonin daily at bedtime for 22 to 35 months. The researchers concluded that although melatonin use significantly improved their sleep quality ($P = 0.001$), none of the other neuropsychiatric test parameters (i.e., Functional Assessment Staging [FAST] or Alzheimer's Disease Assessment Scale [ADAS]) showed any difference between initial and final assessment. However, the condition of sundowning was no longer clinically detectable in 12 of 14 patients. Moreover, the melatonin users did not exhibit the typical cognitive and behavioral deterioration normally seen in these patients over a 1- to 3-year evolution of dementia. No side effects were noted.[84]

In a preliminary pilot study, 11 nursing home participants (8 women, 3 men; 79–82 years) with dementia received one 3 mg tablet of melatonin in the evening for 3 weeks. Using the Nurses Ratings of Sleep Patterns Questionnaire as an assessment tool, sleepiness during the daytime hours (how long and how often the residents napped) was significantly reduced ($P = 0.0245$ and $P = 0.0105$), but no changes were noted in the evening or nighttime hours. Frequency of agitation, as recorded by the Cohen-Mansfield Agitation Inventory, was significantly reduced in five subjects ($P = 0.05$), especially during the evening hours when sundowning is most prevalent.[85]

Alzheimer's Dementia

In this randomized controlled trial, 20 patients with confirmed AD (3 men, 17 women; 67–90 years of age) were randomized to receive either 3 mg of melatonin or placebo at 8:30 PM daily for 1 month. In those using the melatonin, actigraphic data demonstrated that the melatonin users had an increase in the amount of mean evening sleep time (33.2 ± 37.6%) compared with a reduction in placebo users (0.2 ± 13.7%) ($P = 0.017$). Similarly, there was a reduction in the nighttime activity counts in the active group (−44.9 ± 21.9%) compared with the increase (29.8 ± 77%) seen with placebo use ($P = 0.014$). There was no change in daytime sleep or activity between the two groups. Those using the melatonin also had a significantly improved ADAS cognition and noncognition scores (−4.3 ± 3.6 and −4.1 ± 2.2) versus placebo (−0.3 ± 3.7 and −0.8 ± 1.0), respectively ($P = 0.017$ and $P = 0.002$).[86]

In another controlled study, 80 patients (50.7% women; 49.3% men; average age 75.3 years) diagnosed with mild to moderate Alzheimer's dementia with or without insomnia and receiving standard therapy (i.e., acetylcholinesterase inhibitors) were treated for 2 weeks with placebo and then randomized to receive 2 mg of prolonged release melatonin or placebo nightly for 24 weeks, followed by 2 weeks placebo. Overall and at the conclusion of the trial (24 weeks), those patients treated with prolonged release melatonin had significantly better cognitive performance than placebo, as measured by the Instrumental Activities of Daily Living (IADL) ($P = 0.004$) and Mini Mental State Examination (MMSE) ($P = 0.044$). Sleep efficiency (as measured by the PSQI subscale 4) was also improved with the use of the prolonged release melatonin ($P = 0.017$). Mean AD Assessment Scale-Cognition (ADAS-Cog) scores did not differ between the groups. On further analysis of the comorbid insomnia (PSQI ≥ 6) subgroup, the use of prolonged-release melatonin resulted in significantly different outcomes versus the placebo in mean IADL ($P = 0.032$), MMSE score (+1.5 vs. −3 points) ($P = 0.0177$), and sleep efficiency ($P = 0.04$). Moreover, the reduction in median ADAS-Cog values in the active group (−3.5) were significantly better than placebo (+3 points) ($P = 0.045$). Adverse events that were noted during were not considered to be related to the use of prolonged release melatonin.[87]

Headache

Migraine

Individuals with chronic migraines have decreased levels of urinary 6-sulphatoxymelatonin levels, supporting melatonin's role in the pathophysiology of migraine. In an open study, 34 patients (29 men, 5 women) with episodic migraines were given 3 mg of melatonin 30 minutes before bedtime for 3 months. Compared with baseline and after 12 weeks of therapy, melatonin use decreased headache frequency, intensity, and duration ($P < 0.001$). Complete resolution of the migraine was seen in 25% of the patients. The use of medication (i.e., analgesics and triptan) for the treatment of migraine was also reduced ($P < 0.001$).[88] These promising initial results were confirmed in a placebo-controlled trial comparing melatonin with amitriptyline for migraine prevention. One hundred and seventy-eight men (n = 83) and women (n = 95) (average age 36.6–37.2 years) with two to eight migraine attacks per month were randomized to one of three groups for 12 weeks: placebo, melatonin 3 mg, or amitriptyline 25 mg taken at bedtime. After 3 months of treatment and compared with placebo, both melatonin and amitriptyline significantly reduced the number of days with migraine headaches by 1.6 and 1.1 days, respectively ($P < 0.05$). The mean attack duration was also reduced by 4.8 hours in the melatonin group and by 4.4 hours in the amitriptyline group in contrast to placebo (−2.5 hours) ($P < 0.05$). Moreover, the number of analgesic medications and headache intensity were reduced similarly in both active groups. However, the proportion of responders (defined as patients with a higher than 50% improvement in headache frequency along with the number of migraine-associated headache days) was superior in the melatonin group (54.4%) versus amitriptyline (39.1%) ($P < 0.05$) and placebo (20.4%) ($P < 0.01$). During the course of the trial, there were no serious adverse events. Daytime sleepiness, dry mouth, gastralgia, weight gain, and constipation were the most common side effects reported by the participants.[89]

Cluster Headache

Twenty cluster headache (2 chronic; 18 episodic) patients (15 men, 5 women; 30–40 years) who met the International Headache Society (IHS) criteria were invited to participate in this trial. After a 1-week washout period in which prophylactic treatment was stopped, patients were randomized to receive 10 mg of melatonin in the evening or

placebo for 14 days. Those employing the melatonin had a significant reduction in daily attacks, from 3.31 to 1.51, by the end of week 2 ($P < 0.03$). There was also an observed decrease in daily analgesic use by the melatonin group, but this was not statistically significant ($P < 0.06$). Additionally, and in contrast to placebo, headache frequency was reduced in both the first ($P < 0.03$) and second weeks of melatonin treatment ($P = 0.01$). However, the 2 chronic cluster headache patients did not respond to melatonin use. The authors speculated that melatonin could act by modulating 5HT2 receptors, inhibit the synthesis of prostaglandin E_2, or increase the activation threshold level of aminobutyric acid pain pathways.[90]

Primary Headache (Tension and Migraine)

In this open clinical trial, 49 patients (11 men, 38 women; average age range 38.65–45.25 years) (12 with chronic tension-type headache and 37 with migraine) were given 4 mg of prolonged release melatonin, 30 minutes before bedtime for 6 months. At the termination of the study, melatonin had a favorable effect on attack frequency, reducing the number of migraine and tension headache episodes from baseline 4.72 ± 0.73 and 15.91 ± 6.88 ($P = 0.033$) to 2.18 ± 0.84 and 5.33 ± 2.57 ($P < 0.001$), respectively. The Headache Impact Test score (HIT) measuring quality of life was significantly reduced in both tension type ($P = 0.002$) and migraine headaches ($P < 0.001$).[91]

Tinnitus

A perception of a ringing or roaring or a humming sound within the ears without any acoustic stimulation is known as tinnitus.[92] It was speculated by researchers that melatonin could assist in the reduction of tinnitus symptoms by reducing labyrinth pressure.[93] Based on case reports in which melatonin use symptomatically improved tinnitus patients, one group of medical investigators embarked on a double-blind, placebo-controlled, crossover study in 30 patients (15 men, 8 women; average age 64.4 years) with this condition. Patients were randomized to either 3 mg of melatonin 1 to 2 hours before bedtime or placebo for 30 days. After a 7-day washout, patients were crossed over for another 30 days. Of the 23 patients who completed the study, there was no statistical difference between active and placebo groups in terms of Tinnitus Handicap Inventory (THI) scores. However, those melatonin users (n = 9) with an initial pretreatment THI score of 47.6 and who reported an overall improvement in their symptoms had significantly higher than the average pretreatment scores (25.1) compared with melatonin users (n = 14) who did not respond to therapy ($P = 0.02$). This suggests that patients with higher initial THI scores are more likely to respond to melatonin use. In addition, 7 of 15 patients who reported difficulty in falling asleep secondary to tinnitus symptoms were able to fall asleep more readily, in contrast to only 3 of 15 patients in the placebo group ($P = 0.04$). Bad dreams were noted as a side effect in 17% of both the active and placebo users.[94]

A controlled trial employing 102 subjects with tinnitus determined that only the combination of 3 mg melatonin in the evening with a sulodexide (250 mg twice daily for 40 days; then 250 mg/day for another 40 days) significantly improved their symptoms as measured by THI scores and acufenometry evaluation compared with melatonin alone.[95]

Melatonin's effectiveness is confirmed in adults with chronic tinnitus according to the results of a prospective randomized, double-blind, crossover clinical trial. In this study participants (n = 61) were randomized to 3 mg oral melatonin or placebo on a nightly basis for 1 month followed by a 30-day washout period and then each group was crossed over into the opposite arm. At the conclusion of the trial, in those taking melatonin, there was a significantly greater reduction in both audiometric tinnitus matching (TM) and Self Rated Tinnitus (SRT) scores ($P < 0.05$) from baseline in contrast to the placebo group. The authors note that, "melatonin is most effective in men, those without a history of depression, those who have not undergone prior tinnitus treatments, those with more severe and bilateral tinnitus, and those with a history of noise exposure."[96]

Melatonin, Zinc, and Type 2 Diabetes: Lipid and Glycemic Control

Type 2 diabetes is a global epidemic and the number of those afflicted with this disorder has quadrupled over the past 3 decades.[97] Although there are many therapeutic options, melatonin and zinc may be of value in stabilizing blood glucose and reducing cardiovascular risk in this population group. In one randomized controlled trial, 46 patients (21 men, 25 women aged 40–64 years) with type 2 diabetes and poor glycemic control using metformin were selected and allocated into three groups: group A (n = 15) dietary changes and 10 mg of metformin per day and placebo; group B (n = 18) dietary changes and 10 mg of melatonin plus 50 mg of zinc acetate per day plus 10 mg of melatonin per day; or group C (n = 13) dietary changes along with 10 mg of melatonin plus 50 mg of zinc acetate per day for 3 months. Seventeen healthy subjects (9 males and 8 females) in the same age range as that of patients served as controls.

At the conclusion of the trial, group A patients had a significant decrease in fasting plasma glucose levels (9%) after 90 days ($P < 0.01$), whereas hemoglobin A1C (HbA_{1c}) had a significant reduction ($P < 0.01$) after 30 days of treatment (14%) but did not change at 90 days in contrast to baseline. Group B had a significant decrease ($P < 0.01$) in fasting plasma glucose (25%) and HbA_{1C} (17%) levels after both 30 days of treatment. In this latter group, glycated hemoglobin levels showed a further 26% reduction after 3 months. Compared with baseline, Group C treatment resulted in a 23% decrease in fasting plasma glucose levels after 90 days ($P < 0.01$) along with a 15% and 29% reduction ($P < 0.01$) in HbA_{1C} levels after 30 and 90 days, respectively. C peptide levels did not change in any of the three groups ($P > 0.05$). Comparative analysis determined that the adjunct use of melatonin and zinc with metformin (group B) produces the highest reduction in fasting plasma glucose levels compared with the other tested groups ($P < 0.05$).[98]

Using the same study design noted previously, the combination of zinc and melatonin with or without metformin resulted in improvements in lipid levels. Overall and after 90 days, both group B and group C had significant reductions ($P < 0.01$) in total cholesterol levels (14% and 11%, respectively), compared with group A, which had elevations in total cholesterol (13%). Compared with baseline, group A recorded a 7% increase in triglycerides whereas groups B (−18%) and C (−21%) had a reduction in triglycerides after 3 months of treatment ($P < 0.05$). In addition, groups B (+47%) and C (+32%) had a significant increase in high-density lipoprotein cholesterol levels compared with baseline ($P < 0.01$). Although low-density lipoprotein (LDL) levels did not change in group A, both groups B and C recorded a decrease of 22 and 26%, respectively, in contrast to baseline ($P < 0.01$). Treatment with melatonin and zinc plus metformin (group B) or melatonin and zinc (group C) resulted in significant reduction in microalbuminuria (MAU) (−37 and −36%, respectively), compared with baseline ($P < 0.01$).[99]

Metabolic Syndrome

In a clinical study, 33 patients (12 men, 18 women) with metabolic syndrome who had not responded to 3 months of lifestyle changes were given 5 mg of melatonin 2 hours before bedtime for 2 months. After 1 month of treatment and compared with baseline, those with metabolic syndrome had a significant reduction in body mass index (BMI) (kg/m^2)

($P < 0.05$), systolic blood pressure (mm Hg) ($P < 0.01$), diastolic blood pressure ($P < 0.05$), thiobarbituric acid reactive substances (um/gHb) ($P < 0.05$), and an increase in fibrinogen levels ($P < 0.05$). At the 60-day mark, there was no change in BMI or fibrinogen levels, but the results showed a decrease in LDL-C ($P < 0.05$) along with further reductions in systolic ($P < 0.001$) and diastolic ($P < 0.01$) blood pressures. There was also a reduction in thiobarbituric acid reactive substances ($P < 0.01$) and an increase in catalase (U/g Hb) ($P < 0.01$).[100]

Atopic Dermatitis (AD) in Children

In this controlled clinical trial, 48 subjects (median age, 7 years; age range, 1.8–17.1 years) with atopic dermatitis and sleep disturbances were randomized to receive 3 mg of melatonin or placebo at bedtime for 4 weeks. Melatonin treatment not only resulted in a decrease of the SCORing Atopic Dermatitis (SCORAD) index by 9.1 compared with placebo ($P < 0.001$), but also a reduction of the sleep-onset latency by 21.4 minutes ($P = 0.02$). No side effects were noted from the use of melatonin.[101]

Cataract Surgery

Forty patients (21 men 19 women aged 68.5–72.8 years) scheduled for cataract surgery were randomized to receive 10 mg of oral melatonin or placebo 90 minutes before surgery. Compared with the placebo group, those employing the melatonin had significant reductions in anxiety after premedication ($P = 0.04$) and intraoperatively ($P = 0.005$). Pain scores were significantly lower in the melatonin group than in the control group at the end of surgery (6 minutes; $P < 0.002$) and in the recovery room (7 minutes; $P < 0.032$). The authors note that there was a lower need for fentanyl during surgery as 15 patients in the control group and only 7 patients in the melatonin group required additional fentanyl boluses ($P = 0.007$). In addition, intraocular pressure decreased in the melatonin group and this reduction was maintained until the end of the surgical procedure ($P < 0.001$). Adverse events were minimal, with 1 patient in the melatonin group complaining of dizziness, and another patient in the control group had nausea.[102]

BMI, Melatonin, Fluoxetine, and Postmenopausal Women

Sixty-four women, aged 54 to 65 years with a BMI approximately 30 (kg/height in m^2), were randomly allocated into two groups: Group I (n = 30) was administered fluoxetine (1 × 20 mg in the morning) and placebo (in the evening) for 6 months. Group II (n = 34), in contrast, was given fluoxetine (1 × 20 mg in the morning) and melatonin (5 mg in the evening) for 6 months. Although both groups were effective in improving anxiety and decreasing depression scores from baseline, there were no significant differences between groups. However, in group II taking the fluoxetine combined with melatonin, the improvement of the sleep quality index was faster and resulted in highly statistically significant reductions from 15.8 ± 2.4 points to 9.4 ± 2.0 points ($P < 0.01$) after 12 weeks and 7.7 ± 1.5 points ($P < 0.001$) after 15 weeks. In group I, the sleep quality index in group decreased from 14.9 ± 2.5 points to 12.9 ± 2.6 points after 12 weeks ($P < 0.05$) and to 10.9 ± 1.9 points after 24 weeks ($P < 0.01$). In group II, the BMI decreased non-significantly from 30.9 ± 3.1 to 29.6 ± 2.8 ($P > 0.05$) after 12 weeks decreased significantly to 26.3 ± 3.2 ($P < 0.05$) after 24 weeks. By comparison, in group I, BMI decreased slightly from 30.1 ± 3.5 to 28.5 ± 2.9 ($P > 0.05$), but after 24 weeks, the BMI returned to the baseline value of 29.5 ± 3.1 ($P > 0.05$).[103]

Nicotine Withdrawal

Despite the fact that smoking is strongly associated with increased cardiovascular disease risk, many individuals continue to smoke.[104] One of the factors contributing to engaging in smoking cessation is the symptoms associated with acute nicotine withdrawal. In one study, 12 subjects (19–53 years) who had smoked on an average for 3 to 27 years were given 0.3 mg of melatonin or placebo at 11:30 PM on the second night of the experiment, 3.5 hours after nicotine withdrawal. Those using the melatonin had significant reductions in VAS self-rating scores for anxiety ($P < 0.01$), irritability ($P = 0.001$), anger ($P < 0.05$), and craving for cigarettes ($P < 0.005$), compared with placebo.[105]

Vascular Protection in Smokers

In this controlled study, 63 volunteers (46 men, 17 women; average age 31.7–34.6 years) were split into two groups (smokers and nonsmokers) and then randomly divided into four groups: (a) nonsmoker with oral placebo (n = 15); (b) nonsmoker with oral melatonin (n = 16); (c) smoker with oral placebo (n = 17); and (d) smoker with oral melatonin (n = 15). Each group was given 3 mg/day of melatonin or placebo 1 hour before bedtime for 2 weeks. Compared with placebo group, melatonin not only lowered the concentration of fibrinogen ($P = 0.04$) and free fatty acids ($P = 0.04$) but also decreased the expression of the markers for vascular injury including intercellular adhesion molecule-1 ($P = 0.004$), vascular cell adhesion molecule-1 ($P = 0.001$), and endothelin-1 ($P < 0.0001$) in smokers.[106]

Nonalcoholic Steatohepatitis

In this preliminary study, 42 patients with histologically confirmed nonalcoholic steatohepatitis were asked to consume 2 x 5 mg of melatonin (n = 30) or placebo (n = 12) daily for 12 weeks. All patients were enrolled in a lifestyle program consisting of walking for 30 to 60 min/day along with dietary interventions.

In those taking the melatonin, alanine aminotransferase (IU/L) levels decreased significantly from 118.0 (79.2–158.5) at baseline to 66.0 (55.0–79.5) at week 12 ($P < 0.001$). In contrast, alanine aminotransferase (IU/L) levels in the placebo group also decreased significantly from 120.0 (81.3–135.0) at baseline to 83.5 (70.7–100.0) at week 12 ($P < 0.05$). Aspartate aminotransferase levels (IU/L) decreased significantly in only the melatonin group from 76.5 (64.2–114.2) at baseline to 47.0 (38.2–58.7) at week 12 ($P < 0.001$). In addition, and unlike placebo, the melatonin group had significant reductions in gamma-glutamyl transferase (IU/L) from 113 (75.8–210.8) at baseline to 60 (47.3–85.3) by week 12 ($P < 0.001$).[107]

Ulcerative Colitis

Sixty patients (38 women and 22 men, aged 26–49 years) with left-sided ulcerative colitis who were in clinical remission for the past 12 months were divided into two equal groups of 30 patients. Patients were given the drug mesalazine in daily doses 2 x 1.0 g and melatonin 5 mg daily at bedtime (group I) or placebo (group II) for 1 year. At the end of the study, using the Mayo Clinic Disease Activity Index (MCDAI) score, group I increased from 1.50 ± 0.51 at baseline to 2.75 ± 1.86 points ($P < 0.01$) compared with placebo, which had significantly higher MCDAI (1.61 ± 0.68 points to 5.10 ± 2.22 points) scores ($P < 0.05$). Similarly, C-reactive protein (CRP) levels in the melatonin group remained within the normal range (from 3.49 ± 1.40–4.1 ± 72.10 mg/dL) in contrast to placebo, which increased from 3.85 ± 1.29 to 13.13 ± 6.08 mg/dL ($P < 0.01$). Additionally, in the placebo group, there was a significant reduction in serum hemoglobin from 12.05 ± 0.69 to 10.93 ± 0.81 g/dL. In comparison, the melatonin group experienced a slight decrease in serum hemoglobin levels from 12.29 ± 0.87 to 11.76 ± 1.09 g/dL ($P < 0.01$). Although there was a strong placebo effect, melatonin may have value in maintaining remission in ulcerative colitis.[108]

DOSAGE

Melatonin has been used at a variety of different dosages as described in the Clinical Applications section. For its most popular use to enhance

sleep quality the typical dosage is 3 mg 30 to 45 minutes before bedtime. Dosage ranges for the clinical applications are given in Table 92.1. In general, use the same dosage level used in the positive clinical studies for the specific application.

Side Effects and Contraindications

Common side effects of high dosage melatonin use include headaches, dizziness, nausea, and drowsiness.[109] One author notes that, "despite the well-recognized role of melatonin in the regulation of reproduction in photoperiodic species, it seems unlikely that chronic ingestion of moderate melatonin doses will have a profound impact on reproductive function in humans."[110] However, without further clinical evidence of safety, melatonin should not be employed in pregnancy and lactation. One study in women noted that high-dose melatonin (300 mg/day for 4 months) could significantly reduce mean LH levels in contrast to nonmedicated controls ($P < 0.001$) (see Table 92.1).[111]

DRUG INTERACTIONS

Although melatonin overall has an excellent safety record, clinicians should be cognizant of several drug interactions, especially zolpidem and melatonin.

Zolpidem

In a controlled study, 16 healthy subjects (12 men, 4 women; average age 59.4 years) were randomized to receive placebo, 2 mg prolonged release melatonin, 10 mg zolpidem, or both active medications for 2 days in the evenings (8:00 PM) with a 2- to 10-day washout period between treatments. Psychomotor functions (i.e., reaction time, vigilance), memory, dexterity, sedation, and simulated car driving were evaluated at 1 to 4 hours, the next morning after drug administration. The results demonstrated that compared with placebo, PR melatonin on its own did not impair any of the variables tested. However, the use of zolpidem and zolpidem with PR melatonin significantly impaired memory and psychomotor performance 1 to 4 hours after administration. In a simulated driving task, the number of collisions significantly increased at the 1- to 4-hour mark by 1.4 and 2.8 in the zolpidem ($P < 0.05$) and zolpidem-PR melatonin ($P < 0.001$) groups compared with placebo, respectively. No changes were noted by the investigators the next morning, except that the decline in memory recall was still evident with zolpidem.[112]

Fluvoxamine

The bioavailability of melatonin is increased by the coadministration of the selective serotonin reuptake inhibitor fluvoxamine. When 50 mg of fluvoxamine was used in concert with 5 mg of melatonin, on average, there was a seventeenfold increase ($P < 0.05$) in the area under concentration-time curve (AUC) and a twelvefold higher ($P < 0.01$) serum peak concentration (C_{max}) of melatonin. The terminal elimination half-life was not significantly affected.[113]

Fluoxetine

Melatonin was employed in concert with fluoxetine to reduce the incidence of dyskinesia. In a case report, one 23-year-old male with obsessive-compulsive disorder and major depression developed shoulder and neck dyskinesia after taking 60 mg fluoxetine for 4 months. Because the patient and the doctors did not want to stop the medication, which had proven clinically helpful, they added 6 mg of melatonin twice a day. Within 2 weeks the dyskinesia resolved. Secondary to experiencing daytime drowsiness, the patient discontinued the melatonin with a reappearance and worsening of the dyskinesia. Resumption of the melatonin 6 mg at nighttime again resolved the problem within 2 weeks of onset. The authors noted that the antioxidant effects of melatonin might help scavenge the toxic free radicals that may trigger dyskinesia.[114]

Caffeine

Caffeine intake can affect the bioavailability of melatonin, which is likely caused by the inhibition of CYP1A2 activity. In 12 healthy volunteers, when 600 mg of caffeine was coadministered with 6 mg of melatonin, the C_{max} and AUC of melatonin were increased on average by 142% ($P = 0.001$) and 120% ($P < 0.001$), respectively. The inhibitory effect of caffeine was more pronounced in nonsmokers and in individuals with the *1F/*1F genotype.[115]

CYP1A

As melatonin is metabolized by CYP1A in vitro, there are a number of potential drug interactions that could occur, including coadministration of acetaminophen, omeprazole, theophylline, warfarin, and estradiol. Caution should be exercised by clinicians when administering melatonin and prescription medications metabolized by CYP1A.[116]

Progesterone

In women, 300 mg melatonin and 75 mg of melatonin combined with 0.3 mg of the synthetic progestin norethisterone caused a significant decrease in LH, estradiol, and progesterone levels compared with a nonmedicated control group in the first and fourth months of the investigation ($P < 0.05$). This strongly suggests that melatonin and progesterone have an additive effect and should not be used by women taking progesterone.[117]

REFERENCES

See www.expertconsult.com for a complete list of references.

TABLE 92.1 Dose Ranges

Condition	Dose
Alzheimer's dementia (+ sundowning symptoms)	3–9 mg in the evening 2 mg of prolonged release melatonin
Acute insomnia	0.3–1 mg at bedtime
Atopic dermatitis (children)	3 mg of melatonin at bedtime
Beta-blockers and insomnia	2.5 mg at night
Cancer (solid tumors: breast, lung, prostate)	20 mg in the evening
Breast irradiation dermatitis and topical melatonin	Cream applied twice daily not less than 2 hours before radiation treatment
Cachexia	20 mg in the evening

Continued

TABLE 92.1 **Dose Ranges—cont'd**

Condition	Dose
Taxane-related neuropathy	21 mg at bedtime
Breast cancer and insomnia	3–6 mg at bedtime
Cataract surgery	10 mg of melatonin 90 minutes before surgery
CFS and insomnia	5 mg at bedtime
Chronic insomnia	0.3–3 mg 30 minutes before bedtime
Children and insomnia	5 mg at 7 PM
Depression	
Delayed sleep phase syndrome	5 mg at 7–9 PM
Perimenopausal/menopausal	3 mg at 10–11 PM
COPD and insomnia	3 mg 1 hour before bedtime
Epilepsy and insomnia	6 mg for those less than 9 years of age and less than 30 kg in the evening; 9 mg for those greater than 9 years of age and greater than 30 kg
ASD and insomnia	2–5 mg before bedtime
ADHD and insomnia	3 mg for those less than 30 kg and 6 mg for those greater than 30 kg at 7 PM
Fibromyalgia	3 mg 30 minutes before bedtime
Functional dyspepsia	5 mg in the evening
GERD	3–6 mg in the evening
Headache	
Children: migraine prevention	3 mg at bedtime
Adults: migraine	3 mg at bedtime
Adults: cluster headache	10 mg at bedtime
Adults: tension headache/migraine	4 mg prolonged release melatonin 30 minutes before bedtime
IBS	3 mg in the evening
IBS-C	3 mg in the morning and 5 mg in the evening
Infertility in women with IVF	3 mg in the evening
Jet lag	
Eastbound	5 mg, 3 days before and 3 days after, in the evening local time
Westbound	0.5 mg in the evening
Metabolic syndrome	5 mg 2 hours before bedtime
NASH	5 mg twice a day
Nicotine withdrawal symptoms	0.3 mg in the evening
Tinnitus	3 mg in the evening
Type 2 diabetes (glycemic control and cholesterol)	10 mg of melatonin and 50 mg of zinc acetate
Tetraplegia and insomnia	3 mg 2 hours before bedtime
Ulcerative colitis (maintain remission)	5 mg daily at bedtime
Vascular protection (in smokers)	3 mg 1 hour before bedtime

ADHD, Attention deficit hyperactivity disorder; *ASD*, autism spectrum disorder; *CFS*, chronic fatigue syndrome; *COPD*, chronic obstructive pulmonary disease; *GERD*, gastroesophageal reflux disease; *IBS*, irritable bowel syndrome; *IVF*, in vitro fertilization; *NASH*, nonalcoholic steatohepatitis.

REFERENCES

1. Rios ERV, Venancio ET, Rocha NFM, et al. Melatonin: pharmacological aspects and clinical trends. *Int J Neurosci*. 2010;120:583–590.
2. Srinivasan V, Trahkt SR, Spence DW, et al. Melatonin and melatonergic drugs on sleep: possible mechanisms of action. *Int J Neurosci*. 2009;119:821–846.
3. Dubocovich ML, Markowska M. Functional MT1 and MT2 receptors in mammals. *Endocrine*. 2005;27:101–110.
4. Dubocovich ML. Melatonin receptors: role on sleep and circadian rhythm regulation. *Sleep Med*. 2007;8(suppl 3):34–42.
5. Doyen C, Mighiu D, Kaye K, et al. Melatonin in children with autistic spectrum disorders: recent and practical data. *Eur Child Adolesc Psychiatry*. 2011;20:231–239.
6. Zhdanova IV, Wurtman RJ, Regan MM, et al. Melatonin treatment for age-related insomnia. *J Clin Endocrinol Metab*. 2001;86:4727–4730.
7. DeMuro RL, Nafziger AN, Blask DE, et al. The absolute bioavailability of oral melatonin. *J Clin Pharmacol*. 2000;40:781–784.
8. Fourtillan JB, Brisson AM, Gobin P, et al. Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. *Biopharm Drug Dispos*. 2000;21:15–22.
9. European Medicines Agency, "*Circadin,* ." http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000695/human_med_000701.jsp&murl=menus/medicines/medicines.jsp. Accessed 8/29/2011.
10. Herxheimer A, Petrie KJ. Melatonin for the prevention and treatment of jet lag. *Cochrane Database Sys Rev*. 2002;2:CD001520.
11. Petrie K, Conaglen JV, Thompson L, et al. Effect of melatonin on jet lag after long haul flights. *BMJ*. 1989;298:705–707.
12. lag SRLJ. *N Engl J Med*. 2010;362:440–447.
13. Insufficient sleep is a public health epidemic. http://www.cdc.gov/Features/dsSleep. Accessed February 16, 2011.
14. Pandi-Perumal SR, Verster JC, Kayumov L, et al. Sleep disorders, sleepiness and traffic safety: a public health menace. *Braz J Med Biol Res*. 2006;39:863–871.
15. Zhdanova IV, Wurtman RJ, Lynch HJ, et al. Sleep-inducing effects of low doses of melatonin ingested in the evening. *Clin Pharmacol Therap*. 1995;57:552–558.
16. Neikrug AB, Ancoli-Israel S. Sleep disorders in the older adult-a mini-review. *Gerontology*. 2010;56:181–189.
17. Fetveit A. Late-life insomnia: a review. *Geriatr Gerontol Int*. 2009;9:220–234.
18. Pandi-Perumal SR, Zisapel N, Srinivasan V, et al. Melatonin and sleep in aging population. *Exp Gerontol*. 2005;40:911–925.
19. Wade A, Ford I, Crawford G, et al. Efficacy of prolonged released melatonin in insomnia patients aged 55-80 years: quality of sleep and next day alertness outcomes. *Curr Med Res Opinion*. 2007;23:2597–2605.
20. Lemoine P, Nir T, Laudon M, et al. Prolonged-release melatonin improves sleep quality and morning alertness in insomnia patients aged 55 years and older and has no withdrawal effects. *J Sleep Res*. 2007;16:372–380.
21. Rondanelli M, Opizzi A, Montefarrario F, et al. The effect of melatonin, magnesium, and zinc on primary insomnia in long term care facility residents in Italy: a double-blind, placebo-controlled clinical trial. *J Am Geriatr Soc*. 2011;59:82–90.
22. Dolev Z. Case series of perimenopausal women with insomnia treated with mirtazapine followed by prolonged release melatonin add-on monotherapy. *Arch Womens Ment Health*. 2011;14:269–273.
23. Rossingol DA, Frye RE. Melatonin in autistic spectrum disorders: a systematic review and meta-analysis. *Dev Med Child Neurol*. 2011;53:782–792.
24. Smits MG, Van Stel HF, van der Heijden K, et al. Melatonin improves health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled trial. *J Am Acad Child Adolesc Psychiatry*. 2003;42(11):1286–1293.
25. McNicholas WT, Verbraecken J, Marin JM. Sleep disorders in COPD: the forgotten dimension. *Eur Respir Rev*. 2013;22:365–375.
26. Tsai SC. Chronic obstructive pulmonary disease and sleep related disorders. *Curr Opin Pulm Med*. 2017;23:124–128.
27. Nunes DM, Mota RM, Machado MO, Pereira ED, Bruin VM, Bruin PF. Effect of melatonin administration on subjective sleep quality in chronic obstructive pulmonary disease. *Braz J Med Biol Res*. 2008;41:926–931.
28. de Matos Cavalcante AG, de Bruin PF, de Bruin VM, et al. Melatonin reduces lung oxidative stress in patients with chronic obstructive pulmonary disease: a randomized, double-blind, placebo-controlled study. *J Pineal Res*. 2012;53:238–244.
29. Spong J, Graco M, Brown DJ, Schembri R, Berlowitz DJ. Subjective sleep disturbances and quality of life in chronic tetraplegia. *Spinal Cord*. 2015;53:636–640.
30. Spong J, Kennedy GA, Tseng J, Brown DJ, Armstrong S, Berlowitz DJ. Sleep disruption in tetraplegia: a randomised, double-blind, placebo-controlled crossover trial of 3 mg melatonin. *Spinal Cord*. 2014;52:629–634.
31. Scheer FAJL, Morris CJ, Garcia JI, et al. Repeated melatonin supplementation improves sleep in hypertensive patients treated with beta-blockers: a randomized controlled trial. *SLEEP*. 2012;35:1395–1402.
32. McCarberg BH. Clinical overview of fibromyalgia. *Am J Therap*. 2011.
33. Wilhelmsen M, Amirian I, Reiter RJ, et al. Analgesic effects of melatonin: a review of current evidence from experimental and clinical studies. *J Pineal Res*. 2011;51(3):270–277.
34. Citera G, Arias MA, Maldonado-Cocco JA, et al. The effect of melatonin in patients with fibromyalgia: a pilot study. *Clin Rheumatol*. 2000;19:9–13.
35. Hussain SAR, Al-Khalifa II , Jasim NA, et al. Adjuvant use of melatonin for the treatment of fibromyalgia. *J Pineal Res*. 2011;50:267–271.
36. Avellaneda FA, Pérez Martín A, Izquierdo MM, et al. Chronic fatigue syndrome: aetiology, diagnosis and treatment. *BMC Psychiatry*. 2009;9(suppl 1):S1.
37. Cleare AJ. The neuroendocrinology of chronic fatigue syndrome. *Endocr Rev*. 2003;24:236–252.
38. Rahman SA, Kayumov L, Tchmoutina EA, et al. Clinical efficacy of dim light melatonin onset testing in diagnosing delayed sleep phase syndrome. *Sleep Med*. 2009;10:549–555.
39. Van Heukelom RO, Prins JB, Smits MG, et al. Influence of melatonin on fatigue severity in patients with chronic fatigue syndrome and late melatonin secretion. *Eur J Neurol*. 2006;13:55–60.
40. Gupta M, Aneja S, Kohli K. Add-on melatonin improves sleep behaviour in children with epilepsy: randomized, double-blind, placebo-controlled trial. *J Child Neurol*. 2005;20:112–115.
41. Jain SV, Horn PS, Simakajornboon N, et al. Melatonin improves sleep in children with epilepsy: a randomized, double-blind, crossover study. *Sleep Med*. 2015;16:637–644.
42. Wirojanan J, Jacquement S, Diaz R, et al. The efficacy of melatonin for sleep problems in children with autism, fragile X syndrome, or autism and fragile X syndrome. *J Clin Sleep Med*. 2009;5:145–150.
43. Gringras P, Nir T, Breddy J, Frydman-Marom A, Findling RL. Efficacy and safety of pediatric prolonged-release melatonin for insomnia in children with autism spectrum disorder. *Am Acad Child Adolesc Psychiatry*. 2017;56:948–957.
44. Weiss MD, Wasdell MB, Bomben MM, et al. Sleep hygiene and melatonin treatment for children and adolescents with ADHD and initial insomnia. *J Am Acad Child Adolesc Psychiatry*. 2006;45:512–519.
45. Van der Heijden KB, Smits MG, Van Someren EJW, et al. Effect of melatonin on sleep, behavior, and cognition in ADHD and chronic sleep-onset insomnia. *J Am Acad Child Adolesc Psychiatry*. 2007;46:233–241.
46. Hoebert M, Van der Heijden KB, Van Geijiswijk KB, et al. Long term follow-up of melatonin treatment of children with ADHD and chronic sleep onset insomnia. *J Pineal Res*. 2009;47:1–7.
47. Kernick D, Reinhold D, Campbell JL. Impact of headache on young people in a school population. *Br J Gen Pract*. 2009;59:678–681.
48. Milano S, Parisi P, Pellicia A, et al. Melatonin to prevent migraine or tension-type headache in children. *Neurol Sci*. 2008;29:285–287.
49. Loyd RA, McClellan DA. Update on the evaluation and management of functional dyspepsia. *Am Fam Physician*. 2011;83:547–552.

50. Klupinska G, Poplawski T, Drzewoski J, et al. Therapeutic effect of melatonin in patients with functional dyspepsia. *J Clin Gastroenterol*. 2007;41:270–274.
51. Khan S, Chang L. Diagnosis and management of IBS. *Nature Rev Gastroenterol Hepatol*. 2010;7:565–581.
52. Radwan P, Skrzydlo-Radomanska B, Radwan-Kwiatek K, et al. Is melatonin involved in the irritable bowel syndrome? *J Physiol Pharmacol*. 2009;60(suppl 3):67–70.
53. Saha L, Malhotra S, Rana S, et al. A preliminary study of melatonin in irritable bowel syndrome. *J Clin Gastroenterol*. 2007;41:29–32.
54. Chojnacki C, Walecka-Kapica E, Lokieć K, et al. Influence of melatonin on symptoms of irritable bowel syndrome in postmenopausal women. *Endokrynol Pol*. 2013;64:114–120.
55. Digestive diseases Statistics for the United States. http://digestive.niddk.nih.gov/statistics/statistics.htm. Accessed February 22, 2012.
56. Kandil TS, Mousa AA, El-Gendy AA, et al. The potential therapeutic effect of melatonin in gastro-esophageal reflux disease. *BMC Gastroenterol*. 2010;10:7–16.
57. De Souza Pereira R. Regression of gastroesophageal reflux disease symptoms using dietary supplementation with melatonin, vitamins and amino acids: comparison with omeprazole. *J Pineal Res*. 2006;41:195–200.
58. De Oliveira Torres JDF, de Souza Pereira R. Which is the best choice for gastroesophageal disorders: melatonin or proton pump inhibitors? *World J Gastrointest Pharmacol Ther*. 2010;1:102–106.
59. Mills E, Wu P, Seely D, et al. Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. *J Pineal Res*. 2005;39:360–366.
60. Lissoni P, Chilelli M, Villa S, et al. Five years in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. *J Pineal Res*. 2003;35:12–15.
61. Lissoni P. Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. *Path Biologie*. 2007;55:201–204.
62. Lissoni P, Barni S, Meregalli S, et al. Modulation of cancer endocrine therapy by melatonin: a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone. *Br J Cancer*. 1995;71:854–856.
63. Lissoni P, Cazzaniga M, Tancini G, et al. Reversal of clinical resistance to LHRH analogue in metastatic prostate cancer by the pineal hormone melatonin: efficacy of LHRH analogue plus melatonin in patients progressing on LHRH analogue alone. *Eur Urol*. 1997;31:178–181.
64. Hagiwara H, Sunada Y. Mechanism of taxane neurotoxicity. *Breast Cancer*. 2004;11:82–85.
65. Nahleh Z, Pruemer J, Lafollette J, et al. Melatonin, a promising role in taxane-related neuropathy. *Clin Med Insights Oncol*. 2010;4:35–41.
66. Kern KA, Norton JA. Cancer cachexia. *J Parenter Enteral Nutr*. 1988;12:286–298.
67. Lissoni P, Paolorossi F, Tancrini G, et al. Is there a role for melatonin in the treatment of neoplastic cachexia. *Eur J Cancer*. 1996;32A:1340–1343.
68. Wang YM, Jin BZ, Ai F, et al. The efficacy and safety of melatonin in concurrent chemotherapy or radiotherapy for solid tumors: a meta-analysis of randomized controlled trials. *Cancer Chemother Pharmacol*. 2012;69:1213–1220.
69. Langford DJ, Lee K, Miaskowski C. Sleep disturbance interventions in oncology patients and family caregivers: a comprehensive review and meta-analysis. *Sleep Med Rev*. 2012;16:397–414.
70. Madsen MT, Hansen MV, Andersen LT, et al. Effect of melatonin on sleep in the perioperative period after breast cancer surgery: a randomized, double-blind, placebo-controlled trial. *J Clin Sleep Med*. 2016;12:225–233.
71. Chen WY, Giobbie-Hurder A, Gantman K, et al. A randomized, placebo-controlled trial of melatonin on breast cancer survivors: impact on sleep, mood, and hot flashes. *Breast Cancer Res Treat*. 2014;145:381–388.
72. Ben-David MA, Elkayam R, Gelernter I, Pfeffer RM. Melatonin for prevention of breast radiation dermatitis: a phase II, prospective, double-blind randomized trial. *Isr Med Assoc J*. 2016;18:188–192.
73. Infertility Fact Sheet. http://www.womenshealth.gov/faq/infertility.cfm. Accessed 2/22/2012.
74. Tamura H, Takasaki A, Miwa I, et al. Oxidative stress impairs oocyte quality and melatonin protects from free radical damage and improves fertilization rate. *J Pineal Res*. 2008;44:280–287.
75. Unfer V, Raffone E, Rizzo P, et al. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. *Gynecol Endocrin*. 2011;27:857–861.
76. Serfaty MA, Osborne D, Buszewicz MJ, et al. A randomized double-blind placebo-controlled trial of treatment as usual plus exogenous slow-release melatonin (6 mg) or placebo for sleep disturbance and depressed mood. *Int Clin Psychopharmacol*. 2010;25:132–142.
77. Rahman SA, Kayumov L, Shapiro CM. Antidepressant action of melatonin in the treatment of delayed sleep phase syndrome. *Sleep Med*. 2010;11:131–136.
78. Bellipanni G, Bianchi P, Pierpaoli W, et al. Effects of melatonin in perimenopausal and menopausal women: a randomized and placebo controlled study. *Exp Gerontol*. 2001;36:297–310.
79. Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. *Disease-a-Month*. 2010;56:484–546.
80. Ballard C, Gauthier S, Corbett A, et al. Alzheimer's disease. *Lancet*. 2011;19(377):1019–1031.
81. Sharer J. Tackling sundowning in patient with Alzheimer's disease. *Med-Surg Nursing*. 2008;17:27–29.
82. Burney-Puckett M. Sundown syndrome: etiology and management. *J Psychosoc Nurs Ment Health Serv*. 1996;34:40–43.
83. De Jonghe A, Korevaar JC, van Munster BC, et al. Effectiveness of melatonin treatment on circadian rhythm disturbances in dementia. Are there implications for delirium? A systematic review. *Int J Geriatr Psychiatry*. 2010;25:1201–1208.
84. Brusco LI, Marquez M, Cardinali DP. Melatonin treatment stabilizes chronobiologic and cognitive symptoms in Alzheimer's disease. *Neuroendocrinol Lett*. 2000;21:39–42.
85. Cohen-Mansfield J, Garfinkel D, Lipson S. Melatonin for treatment of sundowning in elderly persons with dementia—a preliminary study. *Arch Gerontol Geriatr*. 2000;31:65–76.
86. Asayama K, Yamadera H, Ito T, et al. Double-blind study of melatonin effects on sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. *J Nippon Med Sch*. 2003;70:334–341.
87. Wade AG, Farmer M, Harari G, et al. Zisapel Add-on prolonged-release melatonin for cognitive function and sleep in mild to moderate Alzheimer's disease: a 6-month, randomized, placebo-controlled, multicenter trial. *Clin Interv Aging*. 2014;9:947–961.
88. Peres MFP, Zukerman E, da Cunha Tanuri F, et al. Melatonin, 3 mg, is effective for migraine prevention. *Neurology*. 2004;63:757–758.
89. Gonçalves AL, Martini Ferreira A, Ribeiro RT, Zukerman E, Cipolla-Neto J, Peres MF. Randomised clinical trial comparing melatonin 3 mg, amitriptyline 25 mg and placebo for migraine prevention. *J Neurol Neurosurg Psychiatry*. 2016;87:1127–1132.
90. Leone M, D'Amico D, Moschiano F, et al. Melatonin versus placebo in the prophylaxis of cluster headache: a double-blind study with parallel groups. *Cephalalgia*. 1996;16:494–496.
91. Bougea A, Spantideas N, Lyras V, Avramidis T, Thomaidis T. Melatonin 4 mg as prophylactic therapy for primary headaches: a pilot study. *Funct Neurol*. 2016;31:33–37.
92. Megwalu UC, Finnell JE, Piccirillo JF. The effects of melatonin on tinnitus and sleep. *Otolaryngol Head Neck Surg*. 2006;134:210–213.
93. Rudin DO. Glaucoma, auditory glaucoma, articular glaucoma, and the third eye. *Med Hypotheses*. 1980;6:427–430.
94. Rosenberg SI, Silverstein H, Rowan PT, et al. Effect of melatonin on tinnitus. *Laryngoscope*. 1998;108:305–310.
95. Neri G, DeStefano A, Baffa C, et al. Treatment of central and sensorineural tinnitis with orally administered melatonin and sulodexide: personal experience from a randomized controlled study. *Acta Otorhinolaryngol Ital*. 2009;29:86–91.

96. Hurtuk A, Dome C, Holloman CH, et al. Melatonin: can it stop the ringing? *Ann Otol Rhinol Laryngol*. 2011;120:433–440.
97. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2017. https://doi.org/10.1038/nrendo.2017.151.
98. Hussain SA, Khadim HM, Khalaf BH, Ismail SH, Hussein KI, Sahib AS. Effects of melatonin and zinc on glycemic control in type 2 diabetic patients poorly controlled with metformin. *Saudi Med J*. 2006;27:1483–1488.
99. Kadhim HM, Ismail SH, Hussein KI, et al. Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. *Pineal Res*. 2006;41:189–193.
100. Koziróg M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J. Broncel Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. *J Pineal Res*. 2011;50:261–266.
101. Chang YS, Lin MH, Lee JH, et al. Melatonin supplementation for children with atopic dermatitis and sleep disturbance: a randomized clinical trial. *JAMA Pediatr*. 2016;170:35–42.
102. Ismail SA, Mowafi HA. Melatonin provides anxiolysis, enhances analgesia, decreases intraocular pressure, and promotes better operating conditions during cataract surgery under topical anesthesia. *Anesth Analg*. 2009;108:1146–1151.
103. Chojnacki C, Walecka-Kapica E, Klupinska G, Pawlowicz M, Blonska A, Chojnacki J. Effects of fluoxetine and melatonin on mood, sleep quality and body mass index in postmenopausal women. *J Physiol Pharmacol*. 2015;66:665–671.
104. Lakier JB. Smoking and cardiovascular disease. *Am J Med*. 1992;3(suppl 1):S8–S12.
105. Zhdanova IV, Piotrovskaya VR. Melatonin treatment attenuates symptoms of acute nicotine withdrawal in humans. *Pharmacol Biochem Behav*. 2000;67:131–135.
106. Wang Z, Ni L, Wang J, et al. The protective effect of melatonin on smoke-induced vascular injury in rats and humans: a randomized controlled trial. *J Pineal Res*. 2016;60:217–227.
107. Gonciarz M, Gonciarz Z, Bielanski W, et al. The pilot study of 3-month course of melatonin treatment of patients with nonalcoholic steatohepatitis: effect on plasma levels of liver enzymes, lipids and melatonin. *J Physiol Pharmacol*. 2010;61:705–710.
108. Chojnacki C, Wisniewska-Jarosinska M, Walecka-Kapica E, Klupinska G, Jaworek J, Chojnacki J. Evaluation of melatonin effectiveness in the adjuvant treatment of ulcerative colitis. *J Physiol Pharmacol*. 2011;62:327–334.
109. Buscemi N, Vandermeer B, Hooton N, et al. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. *BMJ*. 2006;18:385–393.
110. Weaver DR. Reproductive safety of melatonin: a "wonder drug" to wonder about. *J Biol Rhythms*. 1997;12:682–689.
111. Voordouw BC, Euser R, Verdonk RE, et al. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J Clin Endocrinol Metab*. 1992;74:108–117.
112. Otmani S, Demazieres AS, Tanner C, et al. Effects of a prolonged release melatonin, zolpidem, and their combination on psychomotor functions, memory recall, and driving skills in healthy middle aged and elderly volunteers. *Human Psychopharmacol Clin Exp*. 2008;23:693–705.
113. Härtter S, Grözinger M, Weigmann H, et al. Increased bioavailability of oral melatonin after fluvoxamine coadministration. *Clin Pharmacol Ther*. 2000;67:1–6.
114. Savita GB, Andrade C. Melatonin treatment of shoulder-and-neck dyskinesia possibly related to fluoxetine treatment. *World J Biol Psych*. 2009;10:1047–1048.
115. Härtter S, Nordmark A, Rose DM, et al. Effects of caffeine intake on the pharmacokinetics of melatonin, a probe drug for CYP1A2 activity. *Br J Clin Pharmacol*. 2003;56:679–682.
116. Yeleswaram K, Vachharajani N, Santone K. Involvement of cytochrome P-450 isozymes in melatonin metabolism ad clinical implications. *J Pineal Res*. 1999;26:190–191.
117. Voordouw BC, Euser R, Verdonk RE, et al. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J Clin Endocrinol Metab*. 1992;74:108–117.

Melissa officinalis (Lemon Balm)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Melissa officinalis (family: *Lamiaceae*)
Common names: lemon balm, balm, melissa, balm mint

GENERAL DESCRIPTION

Melissa officinalis, a mint, has square stems and opposite leaves (Fig. 93.1). The leaves are used medicinally and exude a lemony odor when brushed or crushed. Lemon balm is native to southern Eurasia. The word *balm* is derived from the term "balsam," referring to the delightful odor of the plant. The genus name *Melissa* is derived from a Greek word for bees, which are strongly attracted to this plant.

CHEMICAL COMPOSITION

Lemon balm leaves contain volatile oil, giving the plant its scent of lemon. Two major classes of terpenoids are found in this oil—hydrocarbon terpenes, particularly citrals a and b (Fig. 93.2), and sesquiterpenes, particularly β-caryophyllene.[1] Lemon balm is also a rich source of flavonoid glycosides.[2,3] More than 3% of the dry weight of lemon balm is composed of rosmarinic acid, a phenylpropanoid derivative (Fig. 93.3).[4]

HISTORY AND FOLK USE

Lemon balm has been used for millennia in Europe, primarily for dyspepsia and nervousness.[5] The ancient Greeks also used it for wound healing and insect bites.[6] The 17th-century herbalist Nicholas Culpeper lauded the benefits of lemon balm in enhancing digestion, relieving melancholy, and "opening the brain."[7]

The Basque people used lemon balm as a digestive for nervous stomachs and as a sedative for insomnia, hysteria, depression, and for heart palpitations caused by nerves. They also used it for neuralgias and to regulate menses and combined it with cloves as a topical treatment for toothaches.[8]

The eclectic physicians in North America used lemon balm as a moderate stimulant, diaphoretic, and antispasmodic.[9] The standard infusion was often combined with lemon juice to treat fevers and painful menstruation. The Cherokee used the plant similarly, and the Costanoan tribe used the decoction for infants' colic and stomachaches.[10]

PHARMACOLOGY

The pharmacodynamics of lemon balm are complex because of the large number of active and secondary constituents present in the plant. The volatile oil fraction appears to have antimicrobial, antiviral, and antiparasitic activity and contributes to the anxiolytic and relaxing effects of lemon balm. The flavonoids and rosmarinic acid are antioxidant, anxiolytic, sedative, and antiviral, and they inhibit both

Fig. 93.1 *Melissa officinalis.*

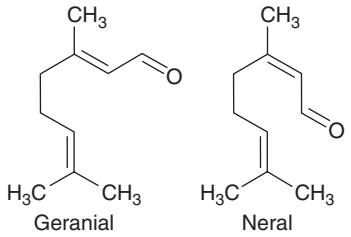


Fig. 93.2 Citral a (geranial) and citral b (neral).

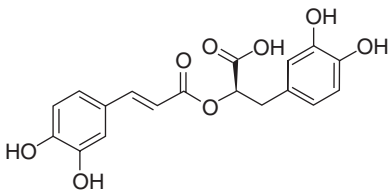


Fig. 93.3 Rosmarinic acid.

complement and protein synthesis. Various extracts of these constituents show similar properties and antithyroid effects.

Sedative Effects

Animal studies confirmed that lemon balm has a calming effect. For instance, a tincture of lemon balm was sedative, analgesic, and hypnotic in mice and potentiated phenobarbital.[11] In vitro studies on human neurons with ethanolic extracts of lemon balm showed that it has cholinergic activity.[12]

Antiviral Effects

Lemon balm consistently showed antiviral and antiretroviral effects in vitro, particularly against herpes simplex virus but also against HIV-1.[13–16] The volatile oil of lemon balm was active against *Trypanosoma brucei* but had minimal activity against *Leishmania major.*[17]

Thyroid Effects

Lemon balm has diverse effects on the thyroid gland and thyroid hormones. It inhibits rat iodothyronine deiodinase and prevents bovine thyroid-stimulating hormone (TSH) from stimulating human thyroid membranes in vitro.[18,19] The latter effect was a result of adduct formation between polyphenols and TSH, thereby preventing TSH receptor binding. Adducts also formed with immunoglobulin-G antibodies formed in patients with Graves' disease, which mimicked TSH, blocking their binding to the TSH receptor in vitro.[20]

Other Effects

Preliminary investigations suggest a wealth of other miscellaneous effects: rosmarinic acid inhibited C3 and C5 convertases, suggesting an anti-inflammatory effect.[21,22] Lemon balm flavonoids show potent antiperoxidative activity.[23] Lemon balm was antiulcerogenic in rats in one trial, both alone and in combination with various other herbs.[24]

CLINICAL APPLICATIONS

Herpes Simplex Virus

Two clinical trials investigated the effect of lemon balm on herpes labialis lesions. In one double-blind trial, 116 patients with an acute outbreak of herpes were randomly assigned to receive treatment with a cream containing a 70:1 extract of lemon balm or placebo.[25] Redness was reduced more in the lemon balm group, but other symptoms were not significantly different from the placebo group. No major adverse effects occurred in either group.

In a second double-blind trial, 66 patients were randomly assigned to apply either a lemon balm cream or placebo.[26] Average daily symptomatic relief was significantly better in those given lemon balm. Other measures of efficacy were not significantly different between the groups, although there was a consistent trend in favor of lemon balm.

Anxiety, Agitation, Insomnia, Memory

Lemon balm has been studied in people with mild anxiety and as a relaxant in healthy people. In a double-blind study, 102 individuals with mild anxiety were randomly assigned to receive either a lemon balm combination product or placebo for 8 weeks.[27] Those taking the lemon balm product had significantly less overall anxiety, nervousness, excitability, and emotional lability than subjects taking the placebo. In a study of 24 healthy individuals, a mixture of lemon balm and *Valeriana officinalis* (valerian) had a dose-dependent anxiolytic effect.[28]

Another randomized, double-blind crossover trial administered varying doses of lemon balm extract to 20 healthy individuals.[29] At lower doses, lemon balm had a calming effect, and at moderate doses, it increased alertness, an effect lost at the highest dose tested. In vitro investigations suggested that these results might have been mediated by the cholinergic effects of lemon balm constituents.

A combination product of valerian, passion flower, lemon balm, and butterbur extracts (Ze 185) was administered to 72 healthy men in a randomized, placebo-controlled, double-blind study with three parallel groups (Ze 185, placebo, or no treatment) for 4 days. The active treatment did not affect salivary cortisol levels but significantly attenuated the subjective anxiety response to a stress test.[30]

A randomized, placebo-controlled clinical trial (n = 100 female teenagers) found that lemon balm capsules (600 mg/2 capsules/day for 3 months) improved the psychosomatic symptoms score, anxiety, depression, sleep, and "social function disorder" significantly compared with a placebo.[31]

In a double-blind study of 72 patients with clinically severe agitation and severe dementia, lemon balm lotion applied topically twice daily significantly reduced agitation compared with a sunflower oil placebo.[32] Lemon balm oil, placebo, and donepezil were not found to have a significant sedative effect in a rigorous multicenter study of Alzheimer's patients with severe agitation (n = 81).[33]

A randomized, double-blind, placebo-controlled 4-month trial of children diagnosed with attention deficit hyperactivity disorder (ADHD) found a highly significant improvement in all four dimensions of the Test of Variables of Attention (TOVA) in those getting an herbal formula containing lemon balm. However, the exact formula was not provided, and it appears that other herbs (e.g., *Paeonia lactiflora, Withania somnifera, Centella asiatica, Bacopa monnieri,* and *Spirulina platensis*) that could plausibly improve ADHD were present in greater amounts in the formula.[34]

Young children (>12 y.o., n = 914) diagnosed with pathological restlessness and/or nervous dyssomnia participated in an open, multicenter study of a mixture of 160 mg valerian and 80 mg lemon balm per tablet. For 4 to 5 weeks, each child took between 1 and 4 tablets per day, and the core symptoms of restlessness and dyssomnia improved significantly. There were no adverse events related to the test medicine.[35]

A study of 24 children compared a lemon balm tincture with a placebo over a month's time and found it did not reduce muscle activity in children with bruxism.[36]

In a double-blind, randomized, placebo-controlled study of healthy adults older than 40 years (n = 44), 5 mL of a tincture of salvia *(Salvia officinalis),* rosemary *(Rosmarinus officinalis),* and lemon balm did

not differ from placebo in either immediate or delayed word recall, although a somewhat younger subgroup showed some improvements in verbal episodic memory.[37]

A double-blind trial in 98 individuals with mild insomnia compared a combination of valerian root and lemon balm with placebo for 1 month.[38] The extract was significantly more effective than placebo in improving subjectively rated sleep quality, although recordings of sleep quality on a visual analog scale did not show any differences between the two groups. A similar combination product had a positive action in a study of 918 children with restlessness and dyssomnia.[39] Lemon balm was approved by the German Commission E for the treatment of nervous sleeping disorders.[40]

One hundred women (50–60 years old) with a score of ≥5 on the Pittsburgh Sleep Quality Index were randomized to take either 2 capsules daily containing 160 mg of valerian and 80 mg of lemon balm or starch in a triple-blinded approach. One month postintervention, a statistically significant improvement in sleep was noted.[41]

Premenstrual Syndrome

A double-blind, randomized, placebo-controlled trial of high school girls ($n = 100$) found that 1200 mg of lemon balm "essence" daily from day 1 to the last day of their menstrual cycle for three cycles significantly reduced premenstrual syndrome (PMS) symptoms.[42]

Dyspepsia and Irritable Bowel Syndrome

Lemon balm leaf was approved by the German Commission E for the treatment of dyspepsia and possibly irritable bowel syndrome (IBS).[40] No clinical trials documenting the efficacy of lemon balm alone for digestive disorders could be found. All trials included lemon balm only as a component of herbal combination products. A systematic review of the formula Iberogast I (Iberogast, in addition to lemon balm, contains *Iberis amara* [bitter candytuft], *Matricaria recutita* [chamomile], *Mentha x piperita* [peppermint], *Carum carvi* [caraway], *Glycyrrhiza glabra* [licorice], *Angelica archangelica* [garden angelica], *Chelidonium majus* [greater celandine], and *Silybum marianum* [milk thistle]) found the formula efficacious in dyspepsia.[43] Another preliminary study of 32 patients with IBS found that a mixture of lemon balm, *Mentha spicata* (spearmint), and *Coriandrum sativum* (coriander) reduced abdominal pain and bloating.[44] In infants, a mixture of lemon balm, *Matricaria recutita* (chamomile), and *Foeniculum vulgare* (fennel) reduced colic symptoms.[45] Although these trials did not address the efficacy of lemon balm in isolation, they do support the historical use of lemon balm in patients with dyspepsia or IBS.

Infantile Colic

In a multicenter randomized comparative study, 176 infants with colic were given 1 mL of a mixture of lemon balm, chamomile (*Matricaria chamomilla* L.), and *Lactobacillus acidophilus,*[46] 10^8 units of *L. reuteri* DSM 17938 in 5 mL oil, or 60 mg of simethicone daily for 28 days. The herbal mixture and the probiotic treatment were equally effective and superior to simethicone in reducing the mean daily crying time.[47]

Hyperlipidemia

In a parallel, randomized, double-blind, placebo-controlled trial, 58 patients with hyperlipidemia (total cholesterol [TC] = 200–260 mg/mL, low-density lipoprotein [LDL] = 100–160 mg/dL) took 1000 mg of lemon balm leaf or starch placebo 3×/day for 2 months. LDL decreased significantly, as did aspartate aminotransferases. No changes were seen in other tested serum parameters.[48]

Arterial Stiffness

In an open-label, parallel-group, comparative trial, 28 healthy individuals drank 200 mL/day of lemon balm or barley tea for 6 weeks. The improved brachial ankle pulse wave velocity in the active group suggests that lemon balm tea may reduce arterial stiffness, especially in those with significant stiffness. Lemon balm tea also suppressed the reduction of skin elasticity in female test subjects and generally appeared to improve glycation-associated tissue damage.[49]

Cardiac Palpitations

Individuals ($n = 55$) with benign palpitations received 500 mg of freeze-dried lemon balm twice daily for 14 days in this double-blind, randomized, placebo-controlled clinical trial. Lemon balm significantly reduced the frequency and self-reported intensity of episodes of palpitations compared with placebo.[50]

Radiation Protection

In a single open study, workers in a medical radiology department used lemon balm tea (1.5 g in 100 mL water twice per day) for 30 days.[51] Plasma catalase, superoxide dismutase, and glutathione levels rose, whereas DNA damage, myeloperoxidase, and lipid peroxidation product levels all fell significantly compared with baseline. Controlled trials are warranted in people exposed to chronic low-dose ionizing radiation.

DOSAGE

Lemon balm is traditionally employed as a tea or tincture. The dried leaf should still smell strongly of lemon and should be dark green; if not, it is of inferior quality. For a tea, 2 to 3 teaspoons of herb are steeped in 1 cup hot water for 10 to 15 minutes; three cups of this are drunk daily.[51] The dose of a tincture, made preferably from fresh material and as concentrated as possible (1:2–1:3 weight/volume ratio), is taken 2 to 6 mL three times daily.[52]

There is no widely accepted standardization to ensure quality lemon balm extracts, although a minimum 0.05% volatile oil content is recommended by the German Commission E.40 Pure volatile oil of lemon balm may also be used at a dose of 1 to 3 drops taken internally three times daily in adults, or by topical application to the skin in a cream, lotion, or fixed oil base. Because of the expense of extracting the oil from this plant, however, there are many adulterated products on the market that contain lemon scent, lemongrass, or other lemon-scented herbs and not lemon balm.

TOXICOLOGY

In a pharmacokinetic study, lemon balm containing 500 mg rosmarinic acid caused no adverse effects and did not affect liver, kidney, or blood cell function parameters.[53] An ethanolic lemon balm extract was antigenotoxic in mice, whereas the aqueous extract was not. Neither extract was genotoxic.[54] However, in an Ames assay, a water extract of lemon balm was found to have a significant genotoxic and mutagenic effect, whereas the methanol and chloroform extracts lacked mutagenicity.[55]

Despite the theoretical concern that it might suppress thyroid function in euthyroid or hypothyroid patients, there are no published reports of this occurrence. Controlled trials in humans established that lemon balm, combined with extracts of *V. officinalis* (valerian) root, does not interfere with the ability to drive or operate heavy machinery.[56] In one case study, a patient arrived at the emergency room (ER) with restlessness, tremor, distractibility, and sweating after discontinuing lemon balm, raising the possibility that lemon balm may present a risk of dependency and can lead to withdrawal symptoms.[57] In contrast, a review found that lemon balm dose-dependently reduced opioid withdrawal syndrome, with no mention of the herb itself causing issues.[58]

DRUG INTERACTIONS

There are no documented drug interactions for lemon balm. It does not interact with alcohol.[55] Given its effects on thyroid function in vitro, there is a theoretical concern that the herb may have additive or synergistic effects with thyrosuppressive drugs and may interact in unpredictable ways with thyroid hormone replacement agents. Lemon balm should be combined with these classes of drugs only under careful supervision by an experienced health-care professional.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Mrlianova M, Tekel'ova D, Felklova M, et al. The influence of the harvest cut height on the quality of the herbal drugs *Melissae folium* and *Melissae herba*. *Planta Med*. 2002;68:178–180.
2. Heitz A, Carnat A, Fraisse D, et al. Luteolin 3′-glucuronide, the major flavonoid from *Melissa officinalis* subsp. *officinalis. Fitoterapia*. 2000;71:201–202.
3. Patora J, Klimek B. Flavonoids from lemon balm *(Melissa officinalis L, Lamiaceae)*. *Acta Pol Pharm*. 2002;59:139–143.
4. Lamaison JL, Petitjean-Freytet C, Carnat A. Rosmarinic acid, total hydroxycinnamic derivatives and antioxidant activity of *Apiaceae. Boraginaceae* and *Lamiaceae* medicinals. *Ann Pharm Fr*. 1990;48:103–108. [French].
5. Weiss RF. *Herbal Medicine*. Gothenburg, Sweden: Ab Arcanum and Beaconsfield.
6. Grieve MA. *A Modern Herbal*. New York: Dover Publications; 1996.
7. Culpeper N. *Culpeper's Complete Herbal*. Hertfordshire, UK: Wordsworth Reference; 1995.
8. Molina GV. *Medicinal Plants of the Basque Country*. San Sebastian, Spain: Editorial Txertoa; 1999.
9. Felter HW, Lloyd JU. *King's American Dispensatory*. Vol. 2. Sandy, OR: Eclectic Medical Publications; 1983.
10. Moerman DE. *Native American Ethnobotany*. Portland, OR: Timber Press; 1998.
11. Soulimani R, Fleurentin J, Mortier F, et al. Neurotropic action of the hydroalcoholic extract of *Melissa officinalis* in the mouse. *Planta Med*. 1991;57:105–109.
12. Wake G, Court J, Pickering A, et al. CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. *J Ethnopharmacol*. 2000;69:105–114.
13. Kucera LS, Cohen RA, Herrmann Jr EC. Antiviral activities of extracts of the lemon balm plant. *Ann N Y Acad Sci*. 1965;130:474–482.
14. Dimitrova Z, Dimov B, Manolova N, et al. Antiherpes effect of *Melissa officinalis* L. extracts. *Acta Microbiol Bulg*. 1993;29:65–72.
15. Yamasaki K, Nakano M, Kawahata T, et al. Anti-HIV-1 activity of herbs in *Labiatae*. *Biol Pharm Bull*. 1998;21:829–833.
16. Nolkemper S, Reichling J, Stintzing FC, et al. Antiviral effect of aqueous extracts from species of the Lamiaceae family against herpes simplex virus type 1 and type 2 in vitro. *Planta Med*. 2006;72:1378–1382.
17. Mikus J, Harkenthal M, Steverding D, et al. In vitro effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med*. 2000;66:366–368.
18. Auf'mkolk M, Kohrle J, Gumbinger H, et al. Antihormonal effects of plant extracts: iodothyronine deiodinase of rat liver is inhibited by extracts and secondary metabolites of plants. *Horm Metab Res*. 1984;16:188–192.
19. Auf'mkolk M, Ingbar JC, Amir SM, et al. Inhibition by certain plant extracts of the binding and adenylate cyclase stimulatory effect of bovine thyrotropin in human thyroid membranes. *Endocrinology*. 1984;115:527–534.
20. Auf'mkolk M, Ingbar JC, Kubota K, et al. Extracts and auto-oxidized constituents of certain plants inhibit the receptor-binding and the biological activity of Graves' immunoglobulins. *Endocrinology*. 1985;116:1687–1693.
21. Englberger W, Hadding U, Etschenberg E, et al. Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory activity. *Int J Immunopharmacol*. 1988;10:729–737.
22. Peake PW, Pussell BA, Martyn P, et al. The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *Int J Immunopharmacol*. 1991;13:853–857.
23. Hohmann J, Zupko I, Redei D, et al. Protective effects of the aerial parts of *Salvia officinalis, Melissa officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. *Planta Med*. 1999;65:576–578.
24. Khayyal MT, el-Ghazaly MA, Kenawy SA, et al. Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. *Arzneimittelforschung*. 2001;51:545–553.
25. Wölbling RH, Leonhardt K. Local therapy of herpes simplex with dried extract of *Melissa officinalis*. *Phytomedicine*. 1994;1:25–31.
26. Koytchev R, Alken RG, Dundarov S. Balm mint extract (Lo-701) for topical treatment of recurring herpes labialis. *Phytomedicine*. 1999;6:225–230.
27. Büchner KH, Hellings H, Huber M, et al. Double blind study as evidence of the therapeutic effect of Melissengeist on psycho-vegetative syndromes. *Med Klin*. 1974;69:1032–1036. [German].
28. Kennedy DO, Little W, Haskell CF, et al. Anxiolytic effects of a combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. *Phytother Res*. 2006;13:383–387.
29. Kennedy DO, Scholey AB, Tildesley NT, et al. Modulation of mood and cognitive performance following acute administration of *Melissa officinalis* (lemon balm). *Pharmacol Biochem Behav*. 2002;72:953–964.
30. Meier S, Haschke M, Zahner C, et al. Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men – an explorative randomized placebo-controlled double-blind study. *Phytomedicine*. 2018;39:85–92.
31. Heydari N, Dehghani M, Emamghoreishi M, Zkbarzadeh M. Effect of *Melissa officinalis* capsule on the mental health of female adolescents with premenstrual syndrome: a clinical trial study. *Int J Adolesc Med Health*. 2018. Epub ahead of print. https://doi.org/10.1515/ijamh-2017-0015.
32. Ballard CG, O'Brien JT, Reichelt K, et al. Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of a double-blind, placebo-controlled trial with *Melissa*. *J Clin Psychiatry*. 2002;63:553–558.
33. Burns A, Perry E, Holmes C, et al. A double-blind placebo-controlled randomized trial of *Melissa officinalis* oil and donepezil for the treatment of agitation in Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2011;31:158–164.
34. Katz M, Adar Levine A, Kol-Degani H, Kav-Venaki L. A compound herbal preparation (CHP) in the treatment of children with ADHD: a randomized controlled trial. *J Atten Disorders*. 2010;14:281–291.
35. Muller SF, Klement S. A combination of valerian and lemon balm is effective in the treatment of restlessness and dyssomnia in children. *Phytomedicine*. 2006;13:383–387.
36. Bortoletto CC, Cordeiro da Silva F, Salgueiro Mda C, et al. Evaluation of electromyographic signals in children with bruxism before and after therapy with *Melissa officinalis* L—a randomized controlled clinical trial. *J Phys Ther Sci*. 2016;28:738–742.
37. Perry NSL, Menzies R, Hodgson F, et al. A randomized double-blind placebo-controlled pilot trial of a combined extract of sage, rosemary and lemon balm, traditional herbal medicines, on the enhancement of memory in normal health subjects, including influence of age. *Phytomedicine*. 2018;39:42–48.
38. Cerny A, Schmid K. Tolerability and efficacy of valerian/lemon balm in healthy volunteers (a double-blind, placebo-controlled, multicentre study). *Fitoterapia*. 1999;70:221–228.
39. Muller SF, Klement S. A combination of valerian and lemon balm is effective in the treatment of restlessness and dyssomnia in children. *Phytomed*. 2006;13:383–387.
40. In: Blumenthal M, Busse WR, Goldberg A, et al., eds. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*. Austin, TX: American Botanical Council:160–161.
41. Taavoni S, Nazem ekbatani N, Haghani H. Valerian/lemon balm use for sleep disorders during menopause. *Comp Ther Clin Prac*. 2013: 193–196.
42. Akbarzadeh M, Dehghani M, Moshfeghy Z, et al. Effect of *Melissa officinalis* capsule on the intensity of premenstrual syndrome symptoms in high school girl students. *Nurs Midwifery Stud*. 2015;4:e27001.
43. Melzer J, Iten F, Reichling J, et al. Iberogast-results of a systematic review concerning functional dyspepsia. *J Herbal Pharmacol*. 2004;4:51–59.
44. Vejdani R, Shalmani HRM, Mir-Fattahi M, et al. The efficacy of an herbal medicine, Carmint, on the relief of abdominal pain and bloating in patients with irritable bowel syndrome: a pilot study. *Dig Dis Sci*. 2006;51:1501–1508.
45. Savino F, Cresi F, Castagno E, et al. A randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita, Foeniculum vulgare* and *Melissa officinalis* (ColiMil) in the treatment of breastfed colicky infants. *Phytother Res*. 2005;19:335–340.
46. A commercial product where 2 ml contains 130 mg lemon balm, 18 mg chamomile, and 2 × 109 tyndalized (dead) L. acidophilus..

47. Martinelli M, Ummarino D, Giugliano FP, et al. Efficacy of a standardized extract of *Matricariae chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) in infantile colic: an open randomized trial. *Neurogastroenterol Motil*. 2017 Dec;29(12):E13145.
48. Jandaghi P, Noroozi M, Ardalani H, Alipour M. Lemon balm: a promising herbal therapy for patients with borderline hyperlipidemia – a randomized double-blind placebo-controlled clinical trial. *Complement Ther Med*. 2016;26:136–140.
49. Yui S, Fujiwara S, Harada K, et al. Beneficial effects of lemon balm leaf extract on in vitro glycation of proteins, arterial stiffness, and skin elasticity in healthy adults. *J Nutr Sci Vitaminol (Tokyo)*. 2017;63:59–68.
50. Alijaniha F, Naseri M, Afsharypuor S, et al. Heart palpitation relief with *Melissa officinalis* leaf extract: double blind, randomized, placebo controlled trial of efficacy and safety. *J Ethnopharmacol*. 2015;164:378–384.
51. Zeraatpishe A, Oryan S, Bagheri MH, et al. Effects of *Melissa officinalis* L. on oxidative status and DNA damage in subjects exposed to long-term low-dose ionizing radiation. *Toxicol Ind Health*. 2011;27(3):205–212.
52. Hoffmann D. *The New Holistic Herbal*. New York: Barnes & Noble Books; 1990.
53. Noguchi-Shinohara M, Ono K, Hamaguchi T, et al. Pharmacokinetics, safety and tolerability of *Melissa officinalis* extract which contained rosmarinic acid in healthy individuals: a randomized controlled trial. *PLoS One*. 2015;10:e0126422.
54. Cssettari de Carvalho N, Correa-Angeloni MJF, Dimer Leffa D, et al. Evaluation of the genotoxic and antigenotoxic potential of *Melissa officinalis* in mice. *Genet Mol Biol*. 2011;34:290–297.
55. Abudayyak M, Ozdemir Nath E, Ozhan G. Toxic potentials of ten herbs commonly used for aphrodisiac effect in turkey. *Turk J Med Sci*. 2015;45:496–506.
56. Albrecht M, Berger W, Laux P, et al. Psychopharmaceuticals and safety in traffic. *Z Allegmeinmed*. 1995;71:1215–1221. [German].
57. Demici K, Akgonul M, Demirdas A, Akpinar A. Does *Melissa officinalis* cause withdrawal or dependence? *Med Arch*. 2015;69:60–61.
58. Ebrahimie M, Bahmani M, Shirzad H, et al. A review study on the effect of Iranian herbal medicines on opioid withdrawal syndrome. *J Evid Based Complementary Altern Med*. 2015;20:302–309.

Mentha piperita (Peppermint)

Michael T. Murray, ND

OUTLINE

Mentha piperita (family: *Labitae*)
Common name: peppermint

GENERAL DESCRIPTION

Peppermint is a natural hybrid of garden spearmint *(Mentha spicata)* and water mint *(Mentha aquatica)*. First described in England in 1696, peppermint now grows all over the world. The two most popular varieties are white peppermint (*Mentha piperita* var. *officinalis*) and black peppermint (*M. piperita* var. *vulgaris*). Both are typical members of the mint family, that is, herbs with square stems, horizontal rhizomes, and lanceolate leaves with a serrated edge. Black peppermint has deep red stems with purplish-tinged dark green leaves, whereas white peppermint has green stems with lighter green leaves. Both varieties produce purple flowers during the summer months. For medicinal effects, the aerial portion of the plant is the most widely used.

CHEMICAL COMPOSITION

The major medicinal component of peppermint is its volatile oil, which can be found in concentrations of up to 1.5% in the herb but is usually present in the range of 0.3% to 0.4%. The principal components of the oil are menthol (28%–29%; Fig. 94.1), menthone (20%–31%), and menthyl acetate (3%–10%), although gas chromatographic analysis of peppermint oil typically shows more than 40 different peaks. Most of the volatile oil components are terpenoids.[1]

The composition of menthol and other volatile oil components is sensitive to climate and latitude and also to the maturity of the plant. Pharmaceutical-grade peppermint oil is produced by distilling the fresh aerial parts of the plant harvested at the very beginning of the flowering cycle. The oil is standardized to contain no less than 44% free menthol and a minimum of 5% esters calculated as menthyl acetate. The ketone component (calculated as menthone) usually ranges from 15% to 30%, with the remainder of the oil being composed of various terpenoids.[1] Menthol is also synthesized by hydrogenation of thymol.

Other components of peppermint herb that may contribute to its medicinal effects are polymerized polyphenols (19% of dry weight), flavonoids (12%), tocopherols, carotenes, betaine, and choline.[2]

HISTORY AND FOLK USE

Although peppermint was not officially recognized until the 17th century, mints have been used for their medicinal effects for thousands of years. Records from the ancient Egyptian, Greek, and Roman eras show that other members of the mint family, particularly spearmint *(M. spicata)*, were used.[1]

The most popular uses of peppermint for medicinal purposes were in the treatment of indigestion and intestinal colic, colds, fever, and headache.

PHARMACOLOGY

The pharmacology of peppermint is attributed almost entirely to its menthol components. The major categories of actions for peppermint and menthol are as follows:

- Carminative
- Antispasmodic
- Choleretic
- External analgesic
- Nasal decongestant

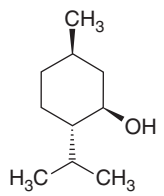


Fig. 94.1 Menthol.

Carminative Effects

Carminatives promote the elimination of intestinal gas. Peppermint and peppermint oil are well-accepted carminatives. Although the exact mechanism of action has not been determined, one proposed mechanism is relaxation of the esophageal sphincter, which leads to the release of gas pressure in the stomach.[3]

Antispasmodic Effects

The mechanism behind peppermint oil's antispasmodic effects was determined. Researchers concluded that peppermint oil inhibits contractions of isolated smooth muscles by blocking the influx of calcium into the muscle cells.[4,5] Researchers hypothesized that the clinical effectiveness of peppermint oil in the treatment of irritable bowel syndrome (IBS) is a result of inhibition of the hypercontractility of intestinal smooth muscle, thereby returning the muscle to its proper tone.

Choleretic Effects

Choleretics stimulate the flow of bile. Menthol and related terpenes were shown to exert a choleretic effect and to improve the solubility of the bile.[6–10]

External Analgesic Effects

The external analgesic and counterirritant effects of menthol are well accepted. When applied to the skin, peppermint oil or menthol stimulates the nerves that perceive cold and simultaneously depresses those for pain. The initial cooling effect is followed by a period of warmth.

CLINICAL APPLICATIONS

Peppermint oil is the most extensively used of all the volatile oils. Pharmaceutic preparations often use peppermint oil or menthol for its therapeutic and flavoring properties. For example, it is used extensively in antacid products and irritant laxatives for both its flavor and its therapeutic effects. The same is true for its inclusion in mouthwash preparations and after-dinner mints.

The pharmacological effects of peppermint and peppermint oil are useful in a number of clinical situations. The most notable are as follows:

- IBS
- Intestinal colic
- Gallstones
- Musculoskeletal pain
- The common cold

Irritable Bowel Syndrome

Enteric-coated peppermint oil (ECPO) was shown to be quite helpful in improving gastrointestinal (GI) function in individuals with IBS, a common functional disorder of the large intestine characterized by some combination of (1) abdominal pain; (2) altered bowel function, constipation, or diarrhea; (3) hypersecretion of colonic mucus; (4) dyspeptic symptoms (flatulence, nausea, anorexia); and (5) varying degrees of anxiety or depression.

Several double-blind studies showed ECPO to be effective in relieving all symptoms of IBS in approximately 70% to 85% of cases within 2 to 4 weeks.[11–18] In one trial, 42 children between 8 and 10 years old with IBS were given ECPO or a placebo for 2 weeks. The dosage was one capsule three times daily for children weighing 30 to 45 kg and two capsules three times daily for children weighing more than 45 kg. After 2 weeks, 76% of the ECPO group reported significant improvements, compared with only 19% in the placebo group.[15]

In one double-blind study, 57 patients with IBS, with normal lactose and lactulose breath tests and negative antibody screening for celiac disease, were treated with peppermint oil (two enteric-coated capsules twice per day or placebo) for 4 weeks in a double-blind study. Results showed that 75% of the patients in the ECPO group showed a more than 50% reduction of basal total IBS symptoms score compared with 38% in the placebo group.[16]

In an 8-week randomized double-blind, placebo-controlled study on 90 outpatients with IBS, the number of subjects free from abdominal pain or discomfort changed from 0 at week 0 to 14 at week 8 in the ECPO group and from 0 to 6 in controls. The severity of abdominal pain was also reduced significantly in the ECPO group compared with controls.[17]

One of the central findings in IBS is a hypercontractility (excessive contraction) of intestinal smooth muscle. Peppermint oil, especially when combined with caraway oil, inhibits the hypercontractility of intestinal smooth muscle, making it useful in IBS and in esophageal spasm and intestinal colic.[19] It may also affect gallbladder and small intestine function. A pharmacodynamic study of 90 mg peppermint oil and 50 mg caraway oil demonstrated that complete inhibition of gallbladder emptying was caused by both oils, and the transit time in the small intestine and the orocecal transit time were significantly prolonged by peppermint oil.[20]

Although effective on its own, ECPO is best used within a comprehensive treatment protocol for IBS (see Chapter 183).

Nonulcer Dyspepsia

In addition to its effects in IBS, ECPO has benefits in nonulcer dyspepsia (NUD), gastroesophageal reflux disorder (GERD), and intestinal overgrowth of *Candida albicans* (a common yeast implicated in many cases of IBS) and *Helicobacter pylori.*

NUD and GERD are basically catch-all terms that reflect a kind of wastebasket diagnosis that doctors use when they cannot find any real reason for a patient's upper gastrointestinal (GI) dysfunction, just as IBS is used as a wastebasket diagnosis for lower GI dysfunction. Symptoms of NUD and GERD include heartburn, difficulty swallowing, feelings of pressure or heaviness after eating, sensations of bloating after eating, stomach or abdominal pains and cramps, and the symptoms of IBS. About 3 of every 10 patients with NUD and GERD also meet the criteria for IBS.

Several of the clinical studies in patients with IBS featured the combination of peppermint oil and caraway oil in an enteric-coated capsule (ECPCO). The results of these trials indicated that this combination produced better results than peppermint oil alone for symptoms of IBS. Later studies also indicated that the combination of peppermint and caraway oils was quite helpful in improving NUD.[20–22] In one double-blind study, 120 patients with NUD were given either the ECPCO combination or cisapride (Propulsid) for 4 weeks.[21] The mean reduction of pain score was comparable in the two groups (4.62 for ECPCO; 4.6 for cisapride). Other symptoms of NUD also improved in a similar fashion. Positive results were also found in *H. pylori*–positive individuals.

Although the ECPCO combination is extremely safe at recommended levels, cisapride caused fatal heart rhythm problems. According to the U.S. Food and Drug Administration, at least 111 people died as a result of cisapride use, and nearly 400 experienced heart abnormalities. Cisapride (Propulsid) was subsequently taken off the market.

Cholelithiasis

Several studies showed that a formula containing menthol and related terpenes (menthone, pinene, borneol, cineol, and camphene) demonstrated efficacy in dissolving gallstones.[6–10] This approach to gallstone removal offers an effective alternative to surgery and was shown to be

safe even when the formula was consumed for prolonged periods (up to 4 years). Terpenes, like menthol, help dissolve gallstones by reducing bile cholesterol levels while increasing bile acid and lecithin levels in the gallbladder. Because menthol was the major component of this formula, peppermint oil, especially if enteric-coated, may offer similar benefits.

External Analgesic

Menthol and related substances can be used as counterirritants in the treatment of arthritis, fibromyositis, tendonitis, and other inflammatory conditions involving the musculoskeletal system.

The Common Cold

Menthol and peppermint oils are often employed in the treatment of the common cold as components of topical nasal decongestants, cough and throat lozenges, ointments, salves, and inhalants. Whether the use of these products is of benefit has not been proven in clinical studies. However, their popularity appears to represent their ability to help make breathing easier during the common cold. The best method for using menthol or peppermint oil is to apply commercial preparations to the upper chest during periods of rest so that the vapors can be inhaled continuously.

Peppermint tea may also be of benefit during the common cold. Peppermint and other members of the mint family demonstrated significant antiviral activity in addition to a mild diaphoretic effect.[23] The most active antiviral components, the polyphenols, are concentrated in the tea.[2] Peppermint oil also showed antiviral activity against Newcastle disease, herpes simplex, and vaccinia.

Headache

In a double-blind, placebo-controlled, randomized crossover trial, topical application of peppermint oil to the forehead and temples resulted in a statistically significant decrease in muscle tension as reported subjectively and measured objectively by electromyographic activity of the temporal muscle. In addition, the oil application decreased sensitivity to pain.[24]

Endoscopy and Colonoscopy

A 20-mL oral solution containing 0.8% L-menthol suppresses peristalsis in patients undergoing endscopy.[25] Taking ECPO capsules before colonoscopy has also shown benefits. In a double-blind study, 65 adult patients undergoing colonoscopy were randomized to receive either ECPO or a placebo 4 hours before the procedure.[26] Premedication with ECPO was beneficial in terms of the time required for cecal intubation and total procedure time, reducing colonic spasm, increasing endoscopist satisfaction, and decreasing pain in patients during colonoscopy.

DOSAGE

Peppermint is most widely used as a tea (infusion), on its own or in combination with other botanicals. The infusion is usually prepared with 1 to 2 teaspoons of the dried leaves per 8 oz of water.

The dosage of peppermint oil administered in an enteric-coated capsule for the treatment of the IBS is one to two capsules (0.2 mL per capsule) three times daily between meals. This dosage is also appropriate for the treatment of gallstones.

The dosage of menthol as an external analgesic is 1.26% to 16% applied to the affected area no more than three or four times daily.

TOXICOLOGY

Peppermint herb is generally regarded as safe when used as a tea; however, hypersensitivity reactions have been reported. Adverse reactions to ECPO capsules are rare but include hypersensitivity reactions (rash), heartburn, bradycardia, and muscle tremor.

When applied topically, peppermint oil or menthol can induce contact dermatitis and hypersensitivity reactions. The likelihood of the development of such a reaction is enhanced when heating pads are used in conjunction with topically applied preparations containing menthol.[27]

In rats, the lethal dose of menthol for 50% of subjects is 3280 mg/kg. The fatal oral dose in humans is calculated at 1 g/kg. In one study, repeated high-dose feeding of rats for 28 days with peppermint oil produced signs of dose-related brain lesions, but the dosage (40 mg/kg) given to the rats far exceeded that used in humans.[1,28,29]

DRUG INTERACTIONS

Peppermint oil may enhance the oral bioavailability of certain drugs. Most notably, in a study in rats, peppermint oil (100 mg/kg) tripled the mean cyclosporine maximum concentration and increased the area under the concentration-versus-time curve.[30] This dosage does not reflect the dosage used in clinical studies; however, physicians should be aware that peppermint oil may raise serum levels of certain drugs when the agents are coadministered.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Briggs C. Peppermint: medicinal herb and flavoring agent. *Can Pharm J*. 1993;126:89–92.
2. Duband F, Carnat AP, Carnat A, et al. Aromatic and polyphenolic composition of infused peppermint, *Mentha x piperita* L. *Ann Pharm Fr*. 1992;50:146–155. [French].
3. Giachetti D, Taddei E, Taddei I. Pharmacological activity of essential oils on Oddi's sphincter. *Planta Med*. 1988;54:389–392.
4. Hills JM, Aaronson PI. The mechanism of action of peppermint oil in gastrointestinal smooth muscle: an analysis using patch clamp electrophysiology and isolated tissue pharmacology in rabbit and guinea pig. *Gastroenterology*. 1991;101:55–65.
5. Hawthorne M, Ferrante J, Luchowski E, et al. The actions of peppermint oil and menthol on calcium channel dependent processes in intestinal, neuronal and cardiac preparations. *Aliment Pharmacol Ther*. 1988;2:101–118.
6. Hordinsky BZ. Terpenes in the treatment of gallstones. *Minn Med*. 1971;54:649–652.
7. Bell GD, Doran J. Gall stone dissolution in man using an essential oil preparation. *BMJ*. 1979;1:24.
8. Doran J, Keighley RB, Bell GD. Rowachol—a possible treatment for cholesterol gallstones. *Gut*. 1979;20:312–317.
9. Ellis WR, Bell GD. Treatment of biliary duct stones with a terpene preparation. *BMJ*. 1981;282:611.
10. Somerville KW, Ellis WR, Whitten BH, et al. Stones in the common bile duct: experience with medical dissolution therapy. *Postgrad Med J*. 1985;61:313–316.
11. Mascher H, Kikuta C, Schiel H. Pharmacokinetics of menthol and carvone after administration of an enteric coated formulation containing peppermint oil and caraway oil. *Arzneimittelforschung*. 2001;51:465–469.
12. Somerville K, Richmond C, Bell G. Delayed release peppermint oil capsules (Colpermin) for the spastic colon syndrome: a pharmacokinetic study. *Br J Clin Pharmacol*. 1984;18:638–640.
13. Rees W, Evans B, Rhodes J. Treating irritable bowel syndrome with peppermint oil. *BMJ*. 1979;2:835–836.
14. Lech Y, Olesen KM, Hey H, et al. Treatment of irritable bowel syndrome with peppermint oil: a double-blind study with a placebo. *Ugeskr Laeger*. 1988;150:2388–2389. [Danish].
15. Kline RM, Kline JJ, Di Palma J, et al. Enteric-coated, pH-dependent peppermint oil capsules for the treatment of irritable bowel syndrome in children. *J Pediatr*. 2001;138:125–128.
16. Cappello G, Spezzaferro M, Grossi L, et al. Peppermint oil (Mintoil) in the treatment of irritable bowel syndrome: a prospective double blind placebo-controlled randomized trial. *Dig Liver Dis*. 2007;39:530–536.
17. Merat S, Khalili S, Mostajabi P, et al. The effect of enteric-coated, delayed-release peppermint oil on irritable bowel syndrome. *Dig Dis Sci*. 2010;55:1385–1390.
18. Cash BD, Epstein MS, Shah SM. A novel delivery system of peppermint oil is an effective therapy for irritable bowel syndrome symptoms. *Dig Dis Sci*. 2016;61(2):560–571.
19. Goerg KJ, Spilker T. Effect of peppermint oil and caraway oil on gastrointestinal motility in healthy volunteers: a pharmacodynamic study using simultaneous determination of gastric and gall-bladder emptying and orocaecal transit time. *Aliment Pharmacol Ther*. 2003;17:445–451.
20. May B, Kuntz HD, Kieser M, et al. Efficacy of a fixed peppermint oil/caraway oil combination in non-ulcer dyspepsia. *Arzneimittelforschung*. 1996;46:1149–1153.
21. May B, Kohler S, Schneider B. Efficacy and tolerability of a fixed combination of peppermint oil and caraway oil in patients suffering from functional dyspepsia. *Aliment Pharmacol Ther*. 2000;14:1671–1677.
22. Rich G, Shah A, Koloski N, et al. A randomized placebo-controlled trial on the effects of Menthacarin, a proprietary peppermint- and caraway-oil-preparation, on symptoms and quality of life in patients with functional dyspepsia. *Neurogastroenterol Motil*. 2017;29(11).
23. Herrmann EC, Kucera L. Antiviral substances in plants of the mint family (Labiatae). 3. peppermint *(Mentha piperita)* and other mint plants. *Proc Soc Exp Biol Med*. 1967;124:874–878.
24. Gobel H, Schmidt G, Dworshak M, et al. Essential plant oils and headache mechanisms. *Phytomedicine*. 1995;2:93–102.
25. Hiki N, Kaminishi M, Yasuda K, et al. Multicenter phase II randomized study evaluating dose-response of antiperistaltic effect of L-menthol sprayed onto the gastric mucosa for upper gastrointestinal endoscopy. *Dig Endosc*. 2012;24(2):79–86.
26. Shavakhi 1, Ardestani SK, Taki M, Goli M, Keshteli AH. Premedication with peppermint oil capsules in colonoscopy: a double blind placebo-controlled randomized trial study. *Acta Gastroenterol Belg*. 2012;75(3):349–353.
27. Heng MC. Local necrosis and interstitial nephritis due to topical methyl salicylate and menthol. *Cutis*. 1987;39:442–444.
28. Olsen P, Thorup I. Neurotoxicity in rats dosed with peppermint oil and pulegone. *Arch Toxicol*. 1984;(suppl 7):408–409.
29. Thorup I, Wurtzen G, Carstensen J, et al. Short term toxicity study in rats dosed with peppermint oil. *Toxicol Lett*. 1983;19:211–215.
30. Wacher VJ, Wong S, Wong HT. Peppermint oil enhances cyclosporine oral bioavailability in rats: comparison with d-alpha-tocopheryl poly(ethylene glycol 1000) succinate (TPGS) and ketoconazole. *J Pharm Sci*. 2002;91:77–90.

95

Microbial Enzyme Therapy

Corey Resnick, ND

OUTLINE

INTRODUCTION

Enzymes derived from fungal species such as *Aspergillus oryzae, A. niger,* and *Rhizopus spp*. have been shown to be safe and effective in the treatment of a variety of clinical disorders. In some cases, fungal enzymes may be more effective than animal-derived enzymes or other available therapies. Some fungal enzyme preparations are particularly well suited for clinical application because of their inherent ability to resist degradation by stomach acid and their ability to hydrolyze physiologically or pathologically important substrates over a wide pH range.

Clinically, fungal enzyme preparations are most often administered orally at mealtime to aid digestion by hydrolyzing dietary substrates such as fats, carbohydrates, and proteins. They have also been used intravenously for the treatment of vascular obstruction, thrombotic disorders, and ischemic disease. Controlled studies in humans and animals have demonstrated efficacy of various fungal enzyme preparations administered orally or parenterally in a wide range of conditions, including the following:

- Maldigestion and malabsorption
- Pancreatic insufficiency
- Functional gastrointestinal disorders
- Steatorrhea
- Gluten-related disorders
- Lactose intolerance
- Oligosaccharide-induced gastrointestinal symptoms
- Arterial obstruction
- Ischemic disease
- Thrombotic disease

This chapter reviews evidence of efficacy for fungal enzyme preparations in a number of the previously mentioned conditions. It also presents indirect evidence that some portion of orally administered fungal enzymes may be absorbed intact in an enzymatically active form. To the extent that such absorption does occur, fungal enzyme supplements administered orally between meals may be of possible benefit as an adjunct in the treatment of systemic conditions, such as the following:

- Inflammatory conditions
- Food allergies

DIGESTIVE ENZYME DEFICIENCIES

Adequate digestion is a prerequisite for normal gastrointestinal function and overall health. Deficiencies in digestive enzymes and imbalances in gastrointestinal pH can lead to impaired digestion, which can cause or aggravate a number of conditions, including the following[1–6]:

- Pancreatic insufficiency
- Maldigestion and malabsorption
- Intestinal permeability defects
- Food intolerance
- Gastrointestinal discomfort
- Food allergy
- Autoimmune conditions
- Bacterial overgrowth

Oral administration of supplemental digestive enzymes can help treat enzyme deficiencies and improve conditions that are related to impaired digestion such as these.

ACID-STABLE FUNGAL ENZYMES

Oral supplementation with acid-stable fungal enzymes, also known as plant enzymes or microbial enzymes, has been shown to be safe and effective in the treatment of a number of disorders in humans and animals. In individuals with gastrointestinal pH imbalances, these enzymes may offer advantages over conventional pancreatin and enteric-coated pancreatic enzyme preparations.[2,7–10]

Pancreatic enzymes have optimal activity in neutral to alkaline conditions around pH 7 and above. They are unstable in acidic conditions. As a result, exposure to gastric acid can destroy up to 90% of orally administered pancreatic lipase and 65% of pancreatic trypsin

according to some studies.[10] Enteric-coated preparations are designed to protect pancreatic enzymes from gastric acidity; however, these do not work reliably in all cases.[2,10,11] This is because most patients with pancreatic insufficiency are not able to concentrate bicarbonate sufficiently to alkalinize the upper small intestine. As a result, enteric-coated tablets or capsules often fail to dissolve and deliver their enzymes in the duodenum or jejunum as intended. The resulting jejunal hyperacidity can also inhibit pancreatic enzyme activity even if enteric coatings do dissolve as intended.[2,7,10,12] Gastric hyperacidity can also lead to impaired fat digestion by causing acidic jejunal pH.[12] Although H_2 receptor antagonists, such as cimetidine, are often used along with pancreatin to lessen intragastric inactivation, this approach is unsuccessful in improving lipid digestion in many patients and carries the possible risk of adverse side effects.[13]

Studies show that certain acid-stable fungal enzyme preparations are naturally stable and enzymatically active in both acid and alkaline pH conditions. For example, lipase preparations derived from both *A. oryzae* and *R. arrhizus* were shown to be active and stable in the broad range of pH 2 to pH 10.[7,8] As a result, such preparations do not require enteric coatings or coadministration of pH-altering drugs. They are effective even in patients with gastrointestinal pH imbalances, such as pancreatic insufficiency, jejunal hyperacidity, and gastric hyperacidity, which can limit the effectiveness of pancreatic enzymes.

As a result of their broad pH range of activity, acid-stable fungal enzymes begin digesting food in the acid environment of the stomach and continue working in the alkaline pH of the small intestine.[2,10,14] As discussed in this chapter, the following fungal enzymes have been shown to aid digestion in humans when administered orally at the time of food consumption:

- Lipase
- Protease
- Amylase
- Prolyl endopeptidase
- Dipeptidyl peptidase IV
- Lactase
- Alpha-galactosidase
- Phytase
- Cellulase

CLINICAL APPLICATIONS

Pancreatic Insufficiency and Other Digestive Disorders

Studies have compared the efficacy of pancreatic enzymes and acid-stable fungal lipase in the treatment of exocrine pancreatic insufficiency in humans and animals. Some studies showed that acid-stable fungal lipase is effective at a substantially lower dose of enzyme activity than that required for pancreatin to reduce pathologically elevated fecal fat levels, stool weight, and diarrhea.[7,8]

A controlled, crossover-design clinical trial in 17 patients with severe pancreatic insufficiency compared the effects of an acid-stable fungal enzyme preparation with conventional pancreatic enzymes and enteric-coated pancreatin. One group of nine patients with pancreatoduodenectomy and bowel resection (Whipple procedure) was found to have pancreatic enzyme levels less than 10% of normal on stimulated secretion before the trial. A second group of eight nonsurgical patients had stimulated pancreatic enzyme levels less than 29% of normal. Both groups were placed on a diet containing 100 g/day of fat. Stools were collected for 72 hours before treatment beginning 5 days after discontinuing all medications and supplemental enzymes. Thereafter all patients were placed on consecutive 2-week periods of treatment, beginning with enteric-coated pancreatin (100,000 International Pharmaceutical Federation [FIP] lipase units [LU]), followed by conventional pancreatin (360,000 FIP LU), and finally, acid-stable fungal lipase (75,000 FIP LU). The fungal enzyme preparation and both of the pancreatic enzyme preparations also contained protease and amylase activity. Stools were collected for the last 72 hours of each treatment period and analyzed for fecal fat content and stool weight. All three of the enzyme treatment protocols led to a significant reduction in fecal fat excretion in both groups ($P < 0.05$), and all patients became virtually symptom-free with regard to diarrhea and abdominal discomfort. Of note, the enteric-coated pancreatin dosage was one-third higher and the conventional pancreatin dosage was nearly five times higher than that required for fungal lipase to produce comparable clinical improvement.[8]

A similar randomized, placebo-control, crossover-design study in dogs compared the effectiveness of 4000 LU of acid-stable fungal lipase derived from *A. oryzae* to 60,000 LU of lipase from pancreatin in the treatment of surgically induced pancreatic insufficiency and steatorrhea. Dogs in the placebo group had significant weight loss as a result of malabsorption and pathologically elevated fecal fat content and stool weight. Compared with placebo, dogs in both enzyme treatment groups experienced significant reductions in fecal fat and stool weight, with no significant weight loss. In this study, the enzyme dose of pancreatin was 15 times higher than that required for the acid-stable fungal lipase to produce comparable clinical results.[7]

One controlled clinical trial showed that an acid-stable fungal lipase derived from *Rhizopus arrhizus* was effective at reducing steatorrhea by 56.5% and stool weight by 45.2% compared with placebo in seven patients with pancreatic insufficiency. The study also demonstrated that the fungal lipase retained greater than 80% of its enzyme activity under conditions of simulated gastric acidity at pH 3 for 1 hour.[15]

Another study in 100 outpatients evaluated the efficacy of a fungal enzyme preparation containing lipase derived from *R. arrhizus* and protease and amylase from *A. oryzae* in a range of gastrointestinal complaints, including epigastric pain and pressure, flatulence, heartburn, belching, and nausea. Investigators reported that 96% of patients experienced some relief of symptoms and rated treatment outcomes as good to very good in 65% of patients.[16]

Some clinical trials reported on the efficacy of enzyme preparations containing protease, amylase, cellulase, and hemicellulase derived from *A. oryzae* in combination with pancreatic enzymes in the treatment of a range of digestive disorders.[17,18] One double-blind, placebo-controlled, crossover design study in a group of 31 outpatients (ages 17–75 years) and 23 geriatric hospital inpatients (ages 70–90 years) found that this enzyme combination was significantly effective in the global improvement of diverse gastrointestinal symptoms ($P < 0.05$) that included pain, nausea, heartburn, bloating, flatulence, constipation, and diarrhea.[17]

Gluten-Related Disorders

Adverse reactions to gluten in the diet can lead to a broad range of gastrointestinal and systemic conditions caused by several different pathophysiological mechanisms that have been referred to collectively as "gluten-related disorders."[19–21] Among the most widely recognized, celiac disease is caused by an autoimmune reaction to dietary gluten. It affects about 1% of the Western population.[22] Other less well-known forms of gluten-induced autoimmune disease include dermatitis herpetiformis and gluten ataxia. Nonautoimmune allergic reactions to gluten can also occur as in the case of wheat allergy.[19,20] Other forms of gluten intolerance include nonceliac gluten sensitivity, which can produce a variety of gastroenterological[21] and neurological symptoms.[19,23,24] The actual prevalence of all forms of gluten-related disorders combined is not known with certainty. However, data from one

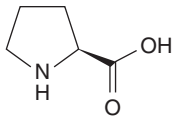


Fig. 95.1 Proline.

review study indicate that adverse reactions to gluten in the diet may affect about 10% of the population.[19]

Gluten is a type of protein found in wheat, barley, and rye. It consists of two main fractions: gliadins and glutenins. Adverse reactions to gluten appear primarily as a result of unusually high amounts of the amino acid proline that it contains, comprising as much as 20% of the molecule.[25] The proline-rich peptide bonds in gluten make it resistant to digestion by proteolytic enzymes in the gastrointestinal tract (Fig. 95.1). As a result, the intestinal mucosa is exposed to undigested gluten-derived polypeptides. A number of these peptides have been shown to cause pathological reactions in sensitive individuals.[22,25,26]

The most effective way to manage gluten-related disorders is for affected individuals to maintain a strict gluten-free diet. However, that can often be hard to accomplish. In addition to the difficulty of compliance and potential social challenges of avoiding foods known to contain gluten, exposure to hidden sources of gluten in the diet is a common problem. One study estimated that unsuspected sources of gluten contained in many prepared foods such as sauces, salad dressings, flavorings, thickeners, etc., may result in daily exposure to 10 to 1000 mg of hidden gluten—even among individuals trying to maintain a gluten-free diet.[27] This has led to a search for therapeutic interventions to ease the burdens of a gluten-free diet and the consequences of accidental exposure to hidden gluten. Beginning as early as the 1950s, studies have examined the efficacy of various proteolytic enzymes derived from fungal and bacterial sources capable of hydrolyzing and thereby degrading the gluten molecule with the goal of reducing its toxicity to sensitive individuals. Some of these have shown great promise in mitigating the challenges of gluten-related disorders by reducing the risk of exposure to hidden gluten. This section reviews evidence of efficacy for some of these enzymes, alone and in combination.

Prolyl Endopeptidase

Celiac disease is an autoimmune condition triggered by reactions that occur in the small intestine to immunogenic gluten-derived peptide fragments, or epitopes, in genetically susceptible individuals. Signs and symptoms can be severe and include abdominal pain and distension, chronic diarrhea, malabsorption, weight loss, anemia, fatigue, and increased risk of osteoporosis and intestinal cancers.[22] Some of these immunogenic peptides are described by the number of amino acids in the peptide chain followed by the suffix "-mer." For example, a 33-mer from α-gliadin and a 26-mer from γ-gliadin are known to trigger proinflammatory T-cell reactions leading to mucosal damage. Antibodies to immunogenic gluten-derived peptides that are produced by activated T-cells and B-cells cross-react with host tissues leading to inflammation, mucosal atrophy, loss of digestive function, nutrient malabsorption, and other GI and systemic disorders.[22,28]

Recent studies have shown that a specific type of protease derived from *Aspergillus niger* is capable of hydrolyzing proline bonds within these immune-reactive gluten epitopes. This enzyme is known as *Aspergillus niger*-derived prolyl endopeptidase or prolyl endoprotease (AN-PEP). It is a proline-specific endopeptidase that primarily cleaves the hard-to-digest proline peptide bonds in the interior portions of gluten peptides.

A 2006 study measured the effectiveness of AN-PEP at detoxifying immunogenic gluten epitopes in vitro. After intact gluten and gluten predigested with pepsin and trypsin were treated with AN-PEP they no longer triggered reactions in gluten-sensitive T-cells. Destruction of all 14 of the tested immune-reactive gluten peptides was confirmed by mass spectrometry, enzyme-linked immunosorbent assay, high-performance liquid chromatography, and Western blot testing. AN-PEP was found to be active and stable from pH 2 to 8 with optimal activity at pH 4 to 5.[29]

An in vitro study in 2008 measured the effectiveness of AN-PEP at breaking down immunogenic gluten peptides from a slice of bread and, in a second experiment, from a gluten-containing "fast food" test meal. Samples of digested food were taken from the stomach and intestinal compartments of a simulated gastrointestinal model (TIM system). These were analyzed for the presence of immunogenic peptides from gliadins and glutenins using T-cell proliferation assays, monoclonal antibody-based assays, and Western blot testing. AN-PEP degraded almost all the immune-active gluten peptides in the stomach before they could reach the duodenum.[30]

A randomized, double-blind, placebo-controlled, crossover study in 2015 measured the ability of AN-PEP to digest gluten in the stomach of 12 healthy volunteers. Each subject was given a liquid low- or high-calorie meal containing 4 g of gluten with either AN-PEP or placebo administered to the stomach via catheter. Gastric and duodenal samples were aspirated over a period of 4 hours and analyzed for content of an immunodominant α-gliadin epitope using enzyme-linked immunosorbent assay and Western blot analysis. Compared with placebo, AN-PEP significantly lowered α-gliadin concentrations in the stomach and duodenum ($P < 0.001$) and lowered α-gliadin in the duodenum from low ($P < 0.001$) and high ($P = 0.013$) calorie meals. Almost all the gluten was degraded in the stomach within a 1-hour period from both low and high calorie meals. A dosage of 1,600,000 Protease Picomole International (PPI) units of AN-PEP significantly enhanced digestion 4 g of dietary gluten in the stomach before entering the duodenum in this study.[21]

Dipeptidyl Peptidase IV

Unlike proteases from the stomach and pancreas that are unable to hydrolyze proline-peptide bonds, the brush border membrane of the intestinal mucosa produces a protease, dipeptidyl peptidase IV (DPP-IV), which can degrade some of the proline-rich immunogenic peptides in gluten.[28,31] Clinical studies have shown that DPP-IV levels are substantially depressed in celiac patients. Other brush border digestive enzymes are also depressed in celiac patients during periods of active disease (e.g., lactase, sucrase, maltase). However, other brush border enzymes return to normal levels in celiac patients who are in remission and DPP-IV remains consistently depressed in these patients.[28,32]

In contrast to AN-PEP, which cleaves proline-peptide bonds mainly in the interior of gluten-derived peptides, DPP-IV is a proline-specific exopeptidase capable of cleaving proline-containing dipeptides at the N-terminal end of gluten peptides.[28] In vitro studies have shown that porcine-derived DPP-IV is able to break down immunodominant epitopes from gliadin.[28,32] DPP-IV derived from *Aspergillus oryzae* has also been shown to enhance the breakdown of immunogenic gluten epitopes in vitro.[27]

Some research has suggested that gluten may also cause neurological symptoms in susceptible individuals. A 2006 literature review found several studies showing a drastic or complete remission of schizophrenic symptoms after withdrawal of gluten from the diet in a subset of patients.[33] In addition to larger gluten-derived immunogenic peptides such as 33-mer and 26-mer that can cause autoimmune reactions found in celiac disease, other smaller gluten-derived peptides may cause neurological symptoms not mediated by immune

mechanisms. A 1992 study isolated four different peptides from wheat gluten with chain lengths of 4 to 5 amino acids that were highly specific for delta opioid receptors found in the brain. These were termed gluten exorphins, or glumorphins. One of these peptide sequences, known as glumorphin A5, was found at 15 locations in wheat glutenin.[23]

It has been hypothesized that opioid peptides derived from gluten and casein may also play a role in autism spectrum disorders (ASD), although this has not been conclusively proven. Children with autism have significantly higher serum levels of IgG antibody to gliadin compared with healthy controls, particularly in those with gastrointestinal symptoms.[34] A two-stage, randomized, controlled study of children with ASD on a gluten-free and casein-free diet reported significant group improvements in core autistic and related behaviors after 8 and 12 months on the diet.[35] A study in 2012 found that glumorphin A5 was present in the urine of children with autism but not in normal control subjects.[24] Glumorphin A5 contains an N-terminal proline dipeptide like other gluten derived peptides that can be degraded by DPP-IV; however, this has not been confirmed in controlled clinical studies.

Efficacy of Enzyme Combinations

In addition to the individual activities of AN-PEP and DPP-IV, there is evidence that some combinations of proline-specific fungal peptidases are more effective at detoxifying dietary gluten than single enzymes alone. As an exopeptidase, DPP-IV has been shown to hydrolyze proline dipeptide bonds at the N-terminal ends of immunogenic gluten epitopes. As an endopeptidase, AN-PEP has primarily been shown to hydrolyze proline-peptide bonds in the interior of gluten peptides. AN-PEP has also been shown to have exopeptidase activity capable of hydrolyzing some proline-peptide bonds at the ends of these peptides; however, it does not cleave all such bonds.[29]

One study showed that a proline-specific bacterial oligopeptidase used in combination with AN-PEP was able to hydrolyze the N-terminal proline-dipeptide bonds that were resistant to digestion by AN-PEP alone.[29] Another study showed that the combination of DPP-IV derived from *Aspergillus oryzae* with an *Aspergillus niger*-derived protease was more effective than either enzyme alone in detoxifying gluten. The exopeptidase activity of DPP-IV accelerated the clearance of short peptides, thereby facilitating access of longer gluten fragments to the active site of the other enzyme. The combination of enzymes resulted in significantly enhanced degradation of immunogenic gluten epitopes.[27] These in vitro studies indicate that oral supplementation with a combination of proline-specific fungal peptidases may provide synergistic benefits by hydrolyzing different portions of the molecule, resulting in more complete and efficient gluten degradation. Future studies may help determine which combinations of such enzymes may offer greater efficacy for detoxifying dietary gluten in sensitive individuals.

Lactose Intolerance

Deficient secretion of intestinal lactase (β-galactosidase) can produce signs and symptoms of dietary lactose intolerance, including abdominal pain, diarrhea, bloating, flatulence, and an increase in breath hydrogen excretion. Studies indicate that lactase deficiency occurs in more than half of the adult human population.[36] Some degree of lactose maldigestion is also a common problem in children, occurring in 76% of apparently healthy children in one study and 56% in another controlled trial.[37] Maldigestion of lactose can result from genetic nonpersistence of intestinal lactase some time after weaning as well as from acquired lactase deficiencies. Deficient lactase production may or may not produce clinical symptoms of lactose intolerance.[3] A study of 232 children with intestinal biopsies found that lactase activity decreased significantly with age and correlated with degree of intestinal injury. Other studies also showed that intestinal secretion of lactase, sucrase, and maltase were decreased in conditions with intestinal mucosal injury and morphological changes, including those seen in celiac disease and chronic diarrhea.[38–41]

A number of controlled human studies showed that fungal lactase administered orally at the time of milk consumption or added to milk at mealtime was each effective at preventing or treating signs and symptoms of intolerance in lactose-intolerant individuals.[3,37,42] One double-blind, placebo-controlled study showed that oral supplementation with fungal lactase taken at the time of lactose consumption was significantly effective at reducing breath hydrogen excretion and treating clinical symptoms in 18 children with lactose intolerance (ages 8–14 years). Test subjects were given tablets containing β-galactosidase derived from *A. oryzae* coingested with a lactose solution (3000 Food Chemicals Codex [FCC] lactase units [ALU]/5 g lactose) after a minimum fast of 8 hours. Breath hydrogen was measured every 30 minutes and clinical symptoms were monitored for the 120-minute test period. Lactase treatment successfully lowered breath hydrogen to below lactose malabsorption threshold levels in 89% of patients ($P < 0.001$). In the placebo group, 89% experienced abdominal pain, 83% bloating, 61% diarrhea, and 44% experienced flatulence. In the lactase group, only 6% experienced abdominal pain, 6% had diarrhea, and none of the test subjects experienced bloating or flatulence.[3]

Oligosaccharide Intolerance

Oligosaccharides contained in legumes and other vegetables can produce symptoms such as flatulence, bloating, abdominal pain, and diarrhea in many individuals. These carbohydrates are resistant to digestion by gastrointestinal enzymes and are therefore available for uptake and fermentation by colonic microflora resulting in the production of gases such as hydrogen. Studies have shown that oral administration of alpha-galactosidase enzyme derived from *Aspergillus niger* is safe and effective at breaking down these dietary oligosaccharides and preventing or reducing flatulence and related abdominal symptoms in sensitive individuals.

A double-blind crossover trial compared the effects of alpha-galactosidase and placebo administered with a test meal of vegetarian chili in 19 subjects. They were asked to keep a careful record of gastrointestinal symptoms for 6 hours after the meal, including the passing of intestinal gas. The number of flatulence events was significantly reduced during the follow-up period in those taking alpha-galactosidase versus placebo ($P = 0.016$).[43]

A double-blind, placebo-controlled study in 2007 measured the effect of alpha-galactosidase on intestinal gas production and symptoms of abdominal pain, discomfort, flatulence, and diarrhea in 8 healthy volunteers. Doses of 300 GalU or 1200 GalU of alpha-galactosidase or placebo were administered with a test meal containing 420 g of cooked beans and breath hydrogen and gastrointestinal symptoms were monitored for 8 hours. Compared with placebo, both doses of alpha-galactosidase decreased breath hydrogen excretion and significantly reduced total symptom scores.[44]

IMPROVING NUTRIENT BIOAVAILABILITY

Dietary supplementation with fungal enzymes has been shown to improve nutrient bioavailability in humans and animals. The effect of supplementation with lipase, amylase, protease, cellulase, and lactase from *Aspergillus niger* in combination with bromelain was studied in 16 clinically stable nursing-home patients receiving enteral nutrition by intubation before and after three consecutive 15-day feeding

periods. Supplementation with the enzyme mixture improved nutritional status as measured by significantly improved blood protein levels compared with the nonsupplemented diet ($P = 0.02$). Albumin concentration and lymphocyte count also tended to improve with enzyme administration.[45]

Bioavailability of minerals can be considerably reduced by dietary phytate. Humans and monogastric animals produce little or no endogenous phytase in the gastrointestinal tract.[46] A controlled clinical trial in humans showed that dietary supplementation with an acid-stable fungal phytase from *A. niger* increased absorption of iron from the diet. The study also showed that the fungal phytase was stable and active across a broad range from pH 1.0 to 7.5, and that it initiated digestion of dietary phytate beginning in the stomach.[14] Animal studies showed that supplementation with fungal phytase helped improve zinc, calcium, and phosphorus bioavailability, and increased bone strength in a dose-dependent manner.[47,48]

Studies showed that supplementation with fungal cellulase helped increase nutritional value of dietary grains in animals. Cellulase significantly improved digestibility of dietary cell wall components and increased solubility of calcium, phosphorus, iron, zinc, and copper associated with cell walls.[49] Studies using multienzyme combinations, including fungal amylase, protease, invertase, phytase, and cellulase, showed increased digestibility of nutrients and improved growth in pigs and chickens.[50,51]

VASCULAR DISEASE

A number of placebo-controlled clinical studies confirmed the effectiveness of a proteolytic fungal enzyme in the treatment of chronic arterial obstruction in humans.[52–55] A single-blind, placebo-controlled, crossover study evaluated the effectiveness of protease from *A. oryzae* versus placebo in 18 patients (ages 63–75 years) with stable intermittent claudication.[52] Patients were divided into an experimental group and a placebo group of nine subjects each, and all patients were assessed before testing. Translumbar aortogram, Doppler ultrasound, and peripheral systolic blood pressure were used to assess the patency of eight different arterial segments in each patient (a total of 72 arterial segments in each group). Anticoagulant therapy with warfarin was introduced after assessment and continued throughout an observation period of 3 months and a subsequent trial period of 2 weeks. Patients were reassessed after the 3-month observation period. Thereafter a series of six intravenous infusions of fungal protease or normal saline placebo was given to experimental and control groups, respectively, over the 2-week trial period. Assessment of peripheral circulation was repeated at the end of the trial period. No other form of therapy was given, except warfarin for anticoagulation. Before treatment, patients in the experimental group were found to have 27 obstructed segments (completely obstructed), 34 stenosed segments (partially obstructed), and only 11 patent arterial segments. Patients in the placebo group had 25 obstructed, 26 stenosed, and 21 patent arterial segments before treatment.

At the end of the 3-month observation period, no changes in Doppler ultrasound findings or peripheral blood pressure were found in either group. At the end of the 2-week trial period, three of the stenosed arterial segments in the placebo group had progressed to complete obstruction, and none of the obstructed segments had become patent (i.e., 4% worse). In the protease group, 16 of the 17 arterial segments that were completely obstructed became patent and one improved to stenosed status during the 2-week treatment period. Overall, treatment with the fungal protease infusion improved patency of 33 of 72 arterial segments, a 46% improvement ($P < 0.001$).

Further analysis of results from this study suggested that an increase in the number and/or dosage of protease infusions might have resulted in even greater improvement in obstructed and stenosed arterial segments. Other studies showed intravenous administration of fungal protease to be effective in the treatment of arterial obstruction in patients with more advanced conditions, such as gangrene and other severe ischemic disease.[53,55]

PHARMACOLOGY OF FUNGAL PROTEASES

Fungal proteases have been used for many years in food production and medical applications. The most common fungal proteases used in medical and dietary supplement applications are derived from *Aspergillus oryzae* and *A. niger*. *A. oryzae*–derived protease preparations are used orally for the prevention and treatment of digestive disorders and are treated as drugs for this purpose in some countries.[17,18,56] *A. oryzae* produces three main proteases that are naturally acid-resistant and that exhibit aggregate proteolytic enzyme activity over a broad range from pH 3 to 9.[56,57] One of these three proteases, sometimes referred to in the literature as *A. oryzae* protease I, was used for treatment of vascular disease in the previously cited clinical trials.[52,53] Studies showed that the thrombolytic effects it exhibited were caused by its fibrinolytic activity.[58,59] It also has hydrolytic enzyme activity against fibrinogen. It does not affect the plasminogen–plasmin system nor does it interfere with normal blood clotting in therapeutic doses.[60]

A. oryzae protease I has a molecular weight of about 35 kDa.[59] It is carried in the blood bound to protease inhibitor proteins α_2-macroglobulin and α_1-antitrypsin.[52,59] These are the same plasma proteins that act as carriers for trypsin, chymotrypsin, and bromelain.[61–63] These protease inhibitors help maintain a protective balance by modulating proteolytic activity in the blood while also maintaining the proteases they carry in an enzymatically active form.

As mentioned previously, acid-stable fungal protease has been used and studied clinically to aid digestion of dietary protein in the gastrointestinal tract on oral administration at mealtime.[17,18,56] Anecdotal reports suggested that some clinicians also administer fungal protease supplements orally between meals as an adjunct in the nutritional treatment of inflammatory conditions and food allergies. A possible mechanism of action for these applications is supported by indirect evidence that some portion of orally administered fungal enzymes is probably absorbed intact and retains enzymatic activity in the blood.

Absorption of Orally Administered Enzymes

Contrary to long-held theories that the healthy intestinal mucosa is an essentially impermeable barrier to intact proteins, including enzymes, there is compelling evidence that such macromolecules can and do pass intact from the human gut into the bloodstream under both normal and pathological conditions.[1,64–67]

Numerous whole proteins, including animal and plant-derived enzymes, were shown in human and animal studies to be absorbed intact into the bloodstream after oral administration. Several human studies showed that exogenous pancreatic trypsin (molecular weight approximately 23 kDa) and chymotrypsin (25 kDa) were absorbed intact in an enzymatically active form after oral administration.[68–70] Studies showed that an enteropancreatic circulation normally exists, in which pancreatic enzymes are absorbed from the gut intact and while enzymatically active, travel in the circulation, are reabsorbed by the pancreas, and resecreted back into the gut mixed with newly synthesized enzymes.[71,72]

Other examples of enzymes and other proteins that are absorbed intact on enteral administration include amylase (54 kDa), elastase, chymotrypsinogen, human albumin and lactalbumin, bovine

albumin, ovalbumin, lactoglobulin, and other large molecules, such as ferritin (500 kDa) and botulism toxin (1000 kDa).[64–67,70,73–75] Studies also demonstrated the intact absorption of orally administered plant enzymes, such as bromelain (24 kDa), papain (39 kDa), and peroxidase from horseradish (40 kDa).[61,70,76]

Some studies examined the quantitative significance of the absorption of orally administered enzymes. A randomized, double-blind, placebo-controlled trial measured the absorption of orally administered bromelain in 19 healthy men (ages 18–45 years). Enteric-coated bromelain was given to the test group in six daily doses, averaging 660 mg each over a 2-day period. Bromelain was found to be absorbed intact in an enzymatically active form and in time-dependent concentrations in the blood of all subjects in the test group. Individuals in the placebo group had no detectable bromelain levels. Total bromelain measured in the blood of test subjects over a 4-day monitoring period was equivalent to approximately 0.7% of the administered oral dose.[61]

Studies in animals looked at the percentage of total administered doses of pancreatic enzymes and papain that were absorbed intact after enteral administration. One study in rats found that approximately 27% of the total administered dose of trypsin, 14% of chymotrypsin, 8% of amylase, and 6% of the administered dose of papain were absorbed into the bloodstream as intact macromolecules in an enzymatically active form after enteral administration.[70]

Based on indirect evidence in humans and animals such as this, it appears likely that some portion of *A. oryzae*–derived protease and other fungal enzymes is absorbed intact after oral administration between meals. To the extent that such absorption does occur, orally administered fungal proteases may exhibit therapeutic properties in the systemic circulation and tissues. These properties include fibrinolytic activity that may be of potential benefit in the treatment of inflammatory conditions in various tissues throughout the body. They also include the ability to hydrolyze dietary proteins and polypeptides that may be of potential benefit in the treatment of food allergies.

FOOD ALLERGIES

Under healthy conditions, adequate protein digestion, normal intestinal permeability, and healthy immune function are important physiological mechanisms that act together to limit the amount of undegraded dietary proteins and polypeptides that is absorbed into the general circulation. Under pathological conditions, these food-derived macromolecules can act as antigens and contribute to food allergies.[1,67,77] Fungal protease administered orally at mealtime helps hydrolyze dietary protein in the gut[17,18,56] and may therefore help reduce the supply of foreign macromolecules available to leak into the bloodstream. In addition, it is possible that fungal protease administered orally between meals may be partially absorbed in an enzymatically active form and help to some extent "digest" dietary protein-derived antigens in the blood. This could potentially reduce the quantity of circulating antigens and be of benefit in the treatment of food allergies or perhaps even food-linked autoimmune disorders. Further research is needed to evaluate these proposed mechanisms of action and confirm whether fungal enzyme supplementation may be a useful adjunct in the treatment of food allergies.

SUMMARY

Research has shown that certain enzymes derived from fungal species such as *A. oryzae, A. niger,* and *Rhizopus spp*. are safe and effective at increasing digestion and treating certain digestive disorders when administered orally at mealtime. These enzymes are inherently stable on exposure to stomach acid and exhibit digestive enzyme activity on a wide variety of dietary substrates over a broad pH range. Because of these properties, fungal enzymes may offer clinical advantages over pancreatic enzymes and pH-altering drugs in some conditions such as pancreatic insufficiency.

Indirect evidence suggests that fungal enzymes may be partially absorbed in an intact and enzymatically active form when administered orally between meals. To the extent that such absorption does occur, it is possible that orally administered *A. oryzae* protease may have potential benefit as an anti-inflammatory agent as a result of its known fibrinolytic activity; however, any efficacy in this application remains unproven. Further research is also needed to confirm a possible benefit for the use of fungal enzymes in the treatment of food allergies.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Husby S, Jensenius JC, Svehag SE. Passage of undegraded dietary antigen into the blood of healthy adults: further characterization of the kinetics of uptake and the size distribution of the antigen. *Scand J Immunol*. 1986;24:447–455.
2. Roberts IM. Enzyme therapy for malabsorption in exocrine pancreatic insufficiency. *Pancreas*. 1989;4:496–503.
3. Medow MS, Thek KD, Newman LJ, et al. Beta-galactosidase tablets in the treatment of lactose intolerance in pediatrics. *Am J Dis Child*. 1990;144:1261–1264.
4. Hvatum M, Kanerud L, Hallgren R, et al. The gut-joint axis: cross reactive food antibodies in rheumatoid arthritis. *Gut*. 2006;55:1240–1247.
5. Mack DR, Flick JA, Durie PR, et al. Correlation of intestinal lactulose permeability with exocrine pancreatic dysfunction. *J Pediatr*. 1992;120:696–701.
6. Mancilla A, Madrid S, Hurtado H, et al. Small intestine bacterial overgrowth in patients with chronic pancreatitis. *Rev Med Chil*. 2008;136:976–980. [Spanish].
7. Griffin SM, Alderson D, Farndon JR. Acid resistant lipase as replacement therapy in chronic pancreatic exocrine insufficiency: a study in dogs. *Gut*. 1989;30:1012–1015.
8. Schneider MU, Knoll-Ruzicka ML, Domschke S, et al. Pancreatic enzyme replacement therapy: comparative effects of conventional and enteric-coated microspheric pancreatin and acid-stable fungal enzyme preparations on steatorrhea in chronic pancreatitis. *Hepatogastroenterol*. 1985;32:97–102.
9. Nakamura T, Takeuchi T, Tando Y. Pancreatic dysfunction and treatment options. *Pancreas*. 1998;16:329–336.
10. Lebenthal E, Rolston DD, Holsclaw DS. Enzyme therapy for pancreatic insufficiency: present status and future needs. *Pancreas*. 1994;9:1–12.
11. Zentler-Munro PL, Fitzpatrick WJ, Batten JC, et al. Effect of intrajejunal acidity and aqueous bile acid and lipid concentrations in pancreatic steatorrhea due to cystic fibrosis. *Gut*. 1984;25:500–507.
12. Go VL, Poley JR, Hofmann AF, et al. Disturbances in fat digestion induced by acidic jejunal pH due to gastric hypersecretion in man. *Gastroenterol*. 1970;58:638–646.
13. Staub JL, Sarles H, Soule JC, et al. No effects of cimetidine on the therapeutic response to oral enzymes in severe pancreatic insufficiency. *N Engl J Med*. 1981;304:1364–1365.
14. Sandberg AS, Hulthen LR, Turk M. Dietary Aspergillus Niger phytase increases iron absorption in humans. *J Nutr*. 1996;126:476–480.
15. Pointner H, Flegel U. Treatment of exocrine pancreatic insufficiency with fungal lipase. *Arzneimittelforschung*. 1975;25:1833–1835. [German].
16. Zorn J. Experiences with substitution therapy using a new pancreatic enzyme of plant origin. *Fortschr Med*. 1978;96:1941–1943. [German].
17. Karani S, Kataria MS, Barber AE. A double-blind clinical trial with a digestive enzyme product. *Br J Clin Pract*. 1971;25:375–377.
18. Kataria MS, Bhaskarrao D. A clinical double-blind trial with a broad spectrum digestive enzyme product (Combizym) in geriatric practice. *Br J Clin Pract*. 1969;23:15–17.
19. Catassi C, Bai JC, Bonaz B, et al. Non-celiac gluten sensitivity: the new Frontier of gluten related disorders. *Nutrients*. 2013;5(10):3839–3853.
20. Sapone A, Bai JC, Ciacci C, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med*. 2012;10:13.
21. Salden BN, Monserrat V, Troost FJ, et al. Randomised clinical study: Aspergillus Niger-derived enzyme digests gluten in the stomach of healthy volunteers. *Aliment Pharmacol Ther*. 2015;42:273–285.
22. Hausch F, Shan L, Santiago NA, et al. Intestinal digestive resistance of immunodominant gliadin peptides. *Am J Physiol Gastrointest Liver Physiol*. 2002;283(4):G996–G1003.
23. Fukudome S, Yoshikawa M. Opioid peptides derived from wheat gluten: their isolation and characterization. *FEBS Lett*. 1992;296(1):107–111.
24. Reichelt KL, Tveiten D, Knivsberg A-M, Brønstad G. Peptides' role in autism with emphasis on exorphins. *Microb Ecol Health Dis*. 2012;23.
25. Piper JL, Gray GM, Khosla C. Effect of prolyl endopeptidase on digestive-resistant gliadin peptides in vivo. *J Pharmacol Exp Ther*. 2004;311(1):213–219.
26. Shan L, Ø M, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. 2002;297(5590):2275–2279.
27. Ehren J, Morón B, Martin E, et al. A food-grade enzyme preparation with modest gluten detoxification properties. *PLoS One*. 2009;4(7):e6313.
28. Koch S, Anthonsen D, Skovbjerg H, Sjöström H. On the role of dipeptidyl peptidase IV in the digestion of an immunodominant epitope in celiac disease. *Adv Exp Med Biol*. 2003;524:181–187.
29. Stepniak D, Spaenij-Dekking L, Mitea C, et al. Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. *Am J Physiol Gastrointest Liver Physiol*. 2006;291(4):G621–G629.
30. Mitea C, Havenaar R, Drijfhout JW, et al. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut*. 2008;57(1):25–32.
31. Detel D, Persić M, Varljen J. Serum and intestinal dipeptidyl peptidase IV (DPP IV/CD26) activity in children with celiac disease. *J Pediatr Gastroenterol Nutr*. 2007;45(1):65–70.
32. Sjöström H, Norén O, Krasilnikoff PA, Gudmand-Høyer E. Intestinal peptidases and sucrase in coeliac disease. *Clin Chim Acta*. 1981;109(1):53–58.
33. Kalaydjian AE, Eaton W, Cascella N, Fasano A. The gluten connection: the association between schizophrenia and celiac disease. *Acta Psychiatr Scand*. 2006;113(2):82–90.
34. Lau NM, Green PH, Taylor AK, et al. Markers of celiac disease and gluten sensitivity in children with autism. *PLoS One*. 2013;8:e66155.
35. Whiteley P, Haracopos D, Knivsberg AM, et al. The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. *Nutr Neurosci*. 2010;13:87–100.
36. Jarvela I, Torniainen S, Kolho KL. Molecular genetics of human lactase deficiencies. *Ann Med*. 2009;41:568–575.
37. Barillas C, Solomons NW. Effective reduction of lactose maldigestion in preschool children by direct addition of beta-galactosidases to milk at mealtime. *Pediatrics*. 1987;79:766–772.
38. O'Grady JG, Stevens FM, Keane R. Intestinal lactase, sucrase, and alkaline phosphatase in 373 patients with celiac disease. *J Clin Pathol*. 1984;37:298–301.
39. Simadibrata M, Wanders RJ, Jan G, et al. Examination of small bowel enzymes in chronic diarrhea. *J Gastroenterol Hepatol*. 2003;18:53–56.
40. Langman JM, Rowland R. Activity of duodenal disaccharidases in relation to normal and abnormal mucosal morphology. *J Clin Pathol*. 1990;43:537–540.
41. Gupta SK, Chong SK, Fitzgerald JF. Dissacharidase activities in children: normal values and comparison based on symptoms and histologic changes. *J Pediatr Gastroenterol Nutr*. 1999;28:246–251.
42. Corazza GR, Benati G, Sorge M, et al. Beta-galactosidase from Aspergillus Niger in adult lactose malabsorption: a double-blind crossover study. *Aliment Pharmacol Ther*. 1992;6:61–66.
43. Ganiats TG, Norcross WA, Halverson AL, et al. Does Beano prevent gas? A double-blind crossover study of oral alpha-galactosidase to treat dietary oligosaccharide intolerance. *J Fam Pract*. 1994;39:441–445.
44. Di Stefano M, Miceli E, Gotti S, et al. The effect of oral alpha-galactosidase on intestinal gas production and gas-related symptoms. *Dig Dis Sci*. 2007;52:78–83.
45. Glade MJ, Kendra D, Kaminski MV. Improvement in protein utilization in nursing-home patients on tube feeding supplemented with an enzyme product derived from Aspergillus Niger and bromelain. *Nutrition*. 2001;17:348–350.
46. Pallauf J, Rimbach G. Nutritional significance of phytic acid and phytase. *Arch Tierernahr*. 1997;50:301–319.
47. Lei X, Ku PK, Miller ER, et al. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. *J Nutr*. 1993;123:1117–1123.
48. Venum TL, Ellersieck MR. Effect of low doses of Aspergillus Niger phytase on growth performance, bone strength, and nutrient absorption and excretion by growing and finishing swine fed corn-soybean meal diets deficient in available phosphorus and calcium. *J Anim Sci*. 2008;86:858–870.
49. Friesen OD, Guenter W, Rotter BA, et al. The effects of enzyme supplementation on the nutritive value of rye grain (Secale cereale) for the young broiler chick. *Poult Sci*. 1991;70:2501–2508.

50. Omogbenigun FO, Nyachoti CM, Slominski BA. Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. *J Anim Sci*. 2004;82:1053–1061.
51. Cowieson AJ, Acamovic T, Bedford MR. Supplementation of diets containing pea meal with exogenous enzymes: effects on weight gain, feed conversion, nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks. *Br Poult Sci*. 2003;44:427–437.
52. Fitzgerald DE, Frisch EP, Milliken JC. Relief of chronic arterial obstruction using intravenous brinase: a control study. *Scand J Thorac Cardiovasc Surg*. 1979;13:327–332.
53. Verhaeghe R, Verstraete M, Schetz J, et al. Clinical trial of brinase and anticoagulants as a method of treatment for advanced limb ischemia. *Eur J Clin Pharmacol*. 1979;16:165–170.
54. Frisch EP, Fitzgerald DE. Relief of chronic peripheral artery obstruction by intravenous brinase. *J Ir Med Assoc*. 1973;66:313–318.
55. Lund F, Ekestrom S, Frisch EP, et al. Thrombolytic treatment with i.v. brinase of advanced arterial obliterative disease of the limbs. *Angiology*. 1975;26:534–556.
56. Haussner K, Hilgendorf P, Hofbauer C, et al. New international F.I.P method for the determination of the activity of Aspergillus oryzae proteases. *Pharmazie*. 1996;51:946–950.
57. Bergkvist R. The proteolytic enzymes of Aspergillus oryzae: II. Properties of the proteolytic enzymes. *Acta Chem Scand*. 1963;17:1541–1551.
58. Kiessling H, Svensson R. Influence of an enzyme from Aspergillus oryzae, protease I, on some components of the fibrinolytic system. *Acta Chem Scand*. 1970;24:569–579.
59. Larsson LJ, Frisch EP, Torneke K, et al. Properties of the complex between alpha-2-macroglobulin and brinase, a proteinase from Aspergillus oryzae with thrombolytic effect. *Thromb Res*. 1988;49:55–68.
60. Vanhove P, Donati MB, Claeys H, et al. Action of brinase on human fibrinogen and plasminogen. *Thromb Haemost*. 1979;42:571–581.
61. Castell JV. Intestinal absorption of undegraded bromelain in humans. In: (eds. Gardner MLG, Steffens K-J.) *Absorption of Orally Administered Enzymes*. Berlin: Springer-Verlag; 46–60.
62. Cohen AB. The interaction of alpha-1-antitrypsin with chymotrypsin, trypsin and elastase. *Biochem Biophys Acta*. 1975;391:193–200.
63. Protsenko AV. Antiproteinase activity of alpha 2-macroglobulin. *Ukr Biokhim Zh*. 1984;56:546–549. [Russian].
64. Gardner ML. Gastrointestinal absorption of intact proteins. *Ann Rev Nutr*. 1988;8:329–350.
65. Gardner ML. Intestinal assimilation of intact peptides and proteins from the diet—a neglected field? *Biol Rev Camb Philos Soc*. 1984;59:289–331.
66. Warshaw AL, Walker WA, Isselbacher KJ. Protein uptake by the intestine: evidence for absorption of intact macromolecules. *Gastroenterol*. 1974;66:987–992.
67. Husby S, Foged N, Host A, et al. Passage of dietary antigens into the blood of children with coeliac disease: quantification and size distribution of absorbed antigens. *Gut*. 1987;28:1062–1072.
68. Ambrus JL, Lassman HB, DeMarchi JJ. Absorption of exogenous and endogenous proteolytic enzymes. *Clin Pharmacol Ther*. 1967;8:362–368.
69. Kabacoff BL, Wohlman A, Umhey M, et al. Absorption of chymotrypsin from the intestinal tract. *Nature*. 1963;199:815.
70. Seifert J, Siebrecht D, Lange J-P, et al. The quantitative absorption of orally administered proteins and histological evidence of enzymes in the wound. In: (eds. Gardner MLG, Steffens K-J.) *Absorption of Orally Administered Enzymes*. Berlin:Springer-Verlag; 29–38.
71. Leibow C, Rothman SS. Enteropancreatic circulation of digestive enzymes. *Science*. 1975;189:472–474.
72. Rothman S, Liebow C, Isenman L. Conservation of digestive enzymes. *Physiol Rev*. 2002;82:1–8.
73. Jakobsson I, Lindberg T, Lothe L, et al. Human alpha-lactalbumin as a marker of macromolecular absorption. *Gut*. 1986;27:1029–1034.
74. Andre C, Lambert R, Bazin H, et al. Interference of oral immunization with the intestinal absorption of heterologous albumin. *Eur J Immunol*. 1974;4:701–704.
75. Dannaeus A, Inganas M, Johansson SG, et al. Intestinal uptake of ovalbumin in malabsorption and food allergy in relation to serum IgG antibody and orally administered sodium cromoglycate. *Clin Allergy*. 1979;9:263–270.
76. McLean E, Ash R. The time-course of appearance and net accumulation of horseradish peroxidase (HRP) presented orally to juvenile carp, cyprinus carpio. *Comp Biochem Physiol A Comp Physiol*. 1986;84:687–690.
77. Hemmings WA. The absorption of large breakdown products of dietary proteins into the body tissues including the brain. In: (eds. Hemmings G, Hemmings WA.) *The Biological Basis of Schizophrenia*. Lancaster PA: MTP Press; 239–257.

96

Natural Medicines Quality Control

Joseph E. Pizzorno, ND

OUTLINE

BACKGROUND

Dietary supplements (DSs) are obviously beneficial to promote health, restore balance, and treat disease. People take them for long periods of time, often for years and years. A sensible strategy for limiting contaminants in DSs, making sure that they are authentic and the potency they claim to be, is to require suppliers to prove that they routinely do thorough testing to verify and assure those quality parameters. DS prescribers and consumers need to ask for legitimate proof of quality from each manufacturer.

Before launching into the details and challenges of product quality control, the author and editors want to acknowledge and commend those nutritional supplement manufacturing companies that perform most, or all, of the processes necessary for quality verification. Unfortunately, too many in the industry—retail, mail order, professional, and online—fall short in providing legitimately verified quality DSs.

Abbreviations and Definitions

COA: Certificate of analysis.

DS: Dietary supplement(s).

DS CGMPs: Dietary Supplement Current Good Manufacturing Practices. The "current" is included because they are subject to updating and changes, and DS manufacturers are responsible for staying current with the updates in the regulations.

DS manufacturer: A company that obtains raw materials from domestic or foreign suppliers and uses those materials to manufacture, label, and sell capsules, tablets, powders, liquids, etc.

EMA: Economically motivated adulteration.

FDA: Food and Drug Administration.

FP: Finished product.

HPLC: High-performance liquid chromatography. An instrument used to analyze and check for the potency level(s) of botanical and nonbotanical raw materials and FPs.

HPTLC: High-performance thin layer chromatography. An instrument used to analyze and check for the correct genus and species (identity) of botanical raw materials.

OOS: Out-of-specification (studies).

RM: Raw material, also known as a component or DS ingredient used to make the final DS.

Scientifically valid method: This is from the preamble section of the FDA CGMP final rule for Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements: "Consistent with the view that we expressed in the 2003 CGMP Proposal, we believe a scientifically valid method is one that is accurate, precise, and specific for its intended purpose. In other words, a scientifically valid test is one that consistently does what it is intended to do."

SOPs: Standard operating procedures.

Supplier: A domestic or foreign company that sells DS raw materials, as a distributor, broker, agent, or it may be the original direct manufacturer.

Verify: To prove the truth of, as by evidence; confirm; substantiate; to ascertain the truth or correctness of, as by examination; to act as ultimate proof or evidence of; serve to confirm.

STATE OF THE INDUSTRY

There are approximately 1500 DS manufacturing companies in the United States. In addition, there are a number of marketing companies that have their DSs made by a private label contract manufacturer and sell their brand nationally or worldwide. Many of these manufacturing

companies historically have performed little or no testing on their raw materials (RM) or their finished DSs. They accept a certificate of analysis (COA) from their supplier. The COA is just a piece of paper that says the material is what it is supposed to be. When a DS manufacturer blindly accepts a COA as the "sole" proof of authenticity, potency, and purity, they relinquish their verification role. Why do they do this? Because it is quick, easy, cheap, and saves money, hassle, delay, and personnel. In short, they take the easy way out. This practice is likely to continue until practitioners and consumers knowledgeably demand quality or the Food and Drug Administration (FDA) clamps down and puts noncompliant manufacturers out of business or makes them comply under the threat of constant scrutiny, steep fines, or shutting down their business. The chain of events needed to produce high-quality DSs is too long and complicated to "trust" it will happen with consistent integrity without comprehensive testing of raw materials and finished products (FPs).

Pharmaceutical drugs are all too frequently sold as DSs. Unscrupulous DS manufacturers or marketing companies will sell products for weight loss, erectile dysfunction, fatigue, athletic performance, etc. These products are advertised as DSs, but the active ingredient is a pharmaceutical drug. In addition, drug and disease claims are often used in the marketing of DSs. These two practices give the DS industry a bad name, tarnishing the legitimate manufacturers and leading the FDA to mistrust companies that produce DSs.

THE FOOD AND DRUG ADMINISTRATION

The FDA has had regulatory oversight of the DS industry for 15 years or longer. In 2007 they put into place Current Good Manufacturing Practices (CGMPs) regulations. The regulations are basically rules to follow when manufacturing DSs, much like a strict recipe that has to be followed to get the intended outcome. They are "the law," and every manufacturer has to follow them. For about a decade the FDA has been conducting inspections of DS companies to see if they are complying with regulations. Word on the street is that many manufacturers are not complying. Maybe they will at some point in the future, but how will you know if a particular company is, or is not, complying with DS CGMPs?

Points regarding the FDA:

- The FDA's mission is to promote and protect the public health by helping safe and effective products reach the market in a timely way, and monitoring products for continued safety after they are in use.
- The FDA is probably underfunded and overburdened. At present, they probably do not have the capacity to strictly oversee the DS industry and enforce wide compliance in a short period of time.
- The DS CGMP regulations were written loosely so as to be flexible. This is a serious problem because it allows each manufacturer to apply "their own" interpretation of what it will take to comply with the regulations, possibly resulting in poor-quality DSs in the marketplace.
- Because the regulations were written to be flexible, the FDA gives little to no guidance on the detailed specifics of "Expected Best Practices" to DS manufacturers. The lack of FDA specific guidance parameters keeps everybody guessing as to whether or not the way "they" interpret the regulations will be acceptable to the FDA.
- The FDA will strictly enforce the DS CGMPs. As they do, it is likely to bring significant positive change to the DS industry that will ultimately benefit the consumer.

If a DS manufacturer says that they are in compliance with FDA CGMPs, it does not mean they have done enough to adequately test and verify their raw materials and FPs and to ensure that the final product is authentic, potent, and has maximum freedom from contamination. In addition, it is important to know that the FDA has mandated by law that if a product has an expiration date or best used by date on the label, the DS manufacturer has to have data to support the expiration date time period.

What data do DS manufacturers have to show? The FDA is silent on this point. The FDA CGMPs do not give any guidance to the industry as to what is acceptable to meet this requirement. This is a perfect example of the FDA providing no guidance on an important part of the DS CGMPs and leaving it to the DS manufacturer to guess and interpret what may be adequate.

Basics of Food and Drug Administration Current Good Manufacturing Practices

Companies that supply DS ingredients (domestic or foreign) to DS manufacturers do not have to follow the FDA DS CGMPs. According to the FDA, this means that the responsibility for the quality of raw material ingredients falls on the FP DS manufacturer and not on the supplier of the materials. This is unfortunate and a major deficiency in the DS CGMPs. Why? Because companies that supply ingredients to DS manufacturers can produce and sell poor-quality or tainted ingredients and are not held accountable. If the supplier of the ingredients and the manufacturer that uses the ingredients had to follow the same regulations, there would be a synergy to bring quality ingredients to the marketplace.

The following information comes from the FDA's website (www.fda.gov), and it gives enough detail to see what DS manufacturers must do to comply with these legally required regulations. The FDA DS CGMPs are not voluntary but must be followed because they are the law. All DS manufacturers are required to follow these rules as of June 2010.

CGMP Final Rule

- The current good manufacturing practices (CGMPs) final rule will require that proper controls are in place for dietary supplements so that they are processed in a consistent manner, and meet quality standards.
- The CGMPs apply to all domestic and foreign companies that manufacture, package, label, or hold dietary supplements, including those involved with the activities of testing, quality control, packaging and labeling, and distributing them in the United States.
- The rule establishes CGMPs for industry-wide use that are necessary to require that dietary supplements are manufactured consistently as to identity, purity, strength, and composition.
- The requirements include provisions related to:
  - The design and construction of physical plants that facilitate maintenance
  - Cleaning
  - Proper manufacturing operations
  - Quality control procedures
  - Testing final product or incoming and in-process materials
  - Handling consumer complaints, and
  - Maintaining records

Consumer Benefits

- Consumers should have access to dietary supplements that meet quality standards and that are free from contamination and are accurately labeled.
- The rule will give consumers greater confidence that the dietary supplement they use has been manufactured to ensure its identity, purity, strength, and composition.

- The rule addresses the quality of manufacturing processes for dietary supplements and the accurate listing of supplement ingredients. It does not limit consumers' access to dietary supplements, or address the safety of their ingredients, or their effects on health when proper manufacturing techniques are used.

Manufacturers

- Under the Dietary Supplement Health and Education Act (DSHEA), manufacturers have an essential responsibility to substantiate the safety of their products and for determining that any representations or claims made about their products are substantiated by adequate evidence to show that they are not false or misleading.
- The CGMPs will help ensure manufacturers produce unadulterated and properly labeled dietary supplements.
- Under the CGMP rule, manufacturers are required to:
  - Employ qualified employees and supervisors.
  - Design and construct their physical plant in a manner to protect dietary ingredients and dietary supplements from becoming adulterated during manufacturing, packaging, labeling, and holding.
  - Use equipment and utensils that are of appropriate design, construction, and workmanship for the intended use.
  - Establish and use master manufacturing and batch production records.
  - Establish procedures for quality control operations.
  - Hold and distribute dietary supplements and materials used to manufacture dietary supplements under appropriate conditions of temperature, humidity, light, and sanitation so that the quality of the dietary supplement is not affected.
  - Keep a written record of each product complaint related to CGMPs.
  - Retain records for 1 year past the shelf life date, if shelf life dating is used, or 2 years beyond the date of distribution of the last batch of dietary supplements associated with those records.
- Examples of product quality problems that the rule will help prevent are:
  - Dietary supplements that contain ingredients in amounts that are greater than those listed on the label
  - Dietary supplements that contain ingredients in amounts that are less than those listed on the label
  - Wrong ingredient
  - Other contaminant (e.g., bacteria, pesticide, glass, lead)
  - Foreign material in a dietary supplement container
  - Improper packaging
  - Mislabeling

The regulations mandate that each DS manufacturer set, document, and meet quality specifications for raw material ingredients and FPs. Each manufacturer gets to set its own specifications based on what it feels and/or thinks is important and complies with the regulation to ensure the four buzz words: identity, purity, strength, and composition. Herein lies a major problem with the CGMPs. The FDA gives little if any guidance on setting specifications.

"Purity" is one of the buzz words; one aspect of purity is contamination. Part 111.70 of the regulation states, "You must establish limits on those types of contamination that may adulterate or may lead to adulteration of the finished batch of the dietary supplement to ensure the quality of the dietary supplement." No guidance is given as to the types of contamination to consider. In CGMP seminars conducted around the country in the past few years, the FDA mentioned some examples of contamination and ultimately stated that the DS manufacturer should know globally what contaminants to consider.

Here is an example to illustrate:

- DS manufacturer "A" sets a specification for testing for a wide panel of chemical solvent residues in each batch of received RMs, examines those results for acceptable limits, and approves or rejects materials based on those limits.
- DS manufacturer "B" sets a specification for testing for chemical solvent residue only if they are listed on the suppliers' COA, examines those results for acceptable limits, and approves or rejects materials based on those limits.
- DS manufacturer "C" sets a specification that does not include chemical solvent residue testing.

Each DS manufacturer set a specification per the CGMPs, but each was different. Are they all acceptable to the FDA? We do not know. Which manufacturer's products would you prefer to prescribe?

THE IMPORTANCE OF QUALITY CONTROL

The first reason quality control is very important is clinical effectiveness. In other words, will it have the intended physiological effect on the body? Great quality does not always ensure significant clinical response, but most would agree that a low potency product or one that was not even what it claimed to be is not likely to help patients.

The second reason quality control is very important is that some sort of contamination may be present in the product. Long-term use of contaminated DSs may possibly cause harm. Depending on whether it is botanical or nonbotanical, the contaminants present could be lead, mercury, cadmium, arsenic, an array of chemical solvents, aflatoxins (liver toxins from mold growth), herbicides and pesticides, harmful bacteria, and industrial chemicals.

Unfortunately, with RMs coming from all over the world, contamination is a risk that is unavoidable.

Food and Drug Administration Definition of Quality

The FDA DS CGMP regulation has specific definitions for quality and quality control.

- Quality means that the dietary supplement consistently meets the established specifications for identity, purity, strength, composition, and limits on contaminants, and has been manufactured, packaged, labeled, and held under conditions to prevent adulteration under section 402(a)(1), (a)(2), (a)(3), and (a)(4) of the act.
- Quality control means a planned and systematic operation or procedure for ensuring the quality of a dietary supplement.

The only test specifically mandated by the FDA that must be performed is identity testing. The need for further testing is open to interpretation by the DS manufacturer.

A More Specific and Robust Definition of Quality

A DS is a quality product or not based on three parameters.

Is It Real?

In other words, is the RM authentic (i.e., has it been properly identified via a test method that is widely accepted as scientifically valid)? If no testing was performed to verify or identify the RM, and it was used in an FP anyway, its inclusion in the product not only could have resulted in decreased response; it may have actively caused harm.

Identifying and authenticating botanical products can be challenging especially if they are powdered extracts versus plant parts or powdered plant parts that have not been extracted. Plant parts (leaves, roots, stems) when whole or in pieces can be identified more easily by

gross and microscopic examinations to ensure compliance with published criteria that are specific to the plant. Most powdered botanical extracts need to undergo some sort of chemical or technical testing to verify correct genus and species.

Vitamins, amino acids, and many other nonbotanical materials are somewhat easier to chemically identify than botanicals, and botanicals are much more prone to adulteration.

Mineral forms can also present a serious challenge to truly authenticate. For example, magnesium glycinate is thought to have some absorption advantage over magnesium oxide and other magnesium forms. Magnesium glycinate is supposed to be magnesium chemically bonded in a particular way to glycine. This is a true chelate (i.e., one molecule). Some mineral RM suppliers skip the bonding part either in part or completely and just mix magnesium carbonate or magnesium oxide with glycine and call it magnesium glycinate. In essence, it is a dry blend of two compounds with only partial or no bonding.

Is It the Potency It Claims to Be?

In most cases (there are some exceptions for skip lot testing if an RM supplier is properly qualified; see "Supplier Certification and Skip Lot Testing") testing every lot of material received for potency helps ensure a consistently potent FP. If most lots are solely identified as authentic and not tested for potency, this does not document the potency of the FP.

Much of the potency issue has to do with label claims as well. Some examples:

Scenario 1: If a licorice extract product has a label stating licorice extract 12% 200 mg (i.e., the licorice extract contains 12% glycyrrhizin), one would have to verify the potency (by testing) of the RM going into the FP and would also have to verify by testing that there was 12% glycyrrhizin in the FP.

If a label has a potency claim, the DS manufacturer has to prove that the claim was met and the FP contained 100% of the labeled potency claim. Always meeting 100% of a potency claim is stipulated by current DS labeling law.

Scenario 2: Another option could be a licorice extract 200 mg with no glycyrrhizin claim. The label just says licorice extract 200 mg. The amount of glycyrrhizin does not have to be verified at the RM or the FP stage. The only thing that has to be verified is that 200 mg is in each capsule, and that it is really licorice. In this scenario, the potency of the product has not been verified. It could be high, low, or really low.

Scenario 3: The same label of licorice extract 200 mg product as in Scenario 2. The label just says licorice extract 200 mg. The amount of glycyrrhizin does not have to be verified as RM or at FP stage, but the conscientious manufacturer tested anyway to ensure the potency of the material at the RM stage (i.e., how much glycyrrhizin is in the RM). To stay consistent, let us say the potency was 12% glycyrrhizin, because this is the level known to be clinically effective. No need to test at the FP stage because no label claim for a specific potency is made. The only need is to verify that 200 mg is in each capsule. In the end, the potency was verified by the RM testing.

The difference between Scenarios 2 and 3 is the DS manufacturer in case 3 had full knowledge and verified the potency of the licorice extract RM, whereas in Scenario 2, the DS manufacturer may not have had a clue as to how potent the FP licorice extract is (high or low) because no testing was performed on the RM or FP.

Clearly, no matter what the obligation is regarding potency testing per label claim, it is always best to know the strength of a botanical going into an FP. This can only be accomplished through potency testing the RM batch. A point of clarification must be made. Many botanical RM products do not have marker compounds to test, or there is no scientifically valid method that exists to perform the potency testing. In this case no potency/strength testing is the only option.

Ideally, one should ask and know if a DS manufacturer is routinely conducting potency/strength testing on RMs and/or FPs.

Does the Product Contain Any Contaminants?

Another way of saying this is: does it have maximum freedom from contamination? Usually, contaminants can only be found through testing the RM. It is generally not possible to detect low level contamination by relying on physical inspection and no testing. A physical inspection may find plastic, metal, glass, bug parts, and other filth.

As noted previously, the FDA CGMP regulations leave it up to the DS manufacturer to determine whether contaminant testing will be done and, if so, to set the acceptable specification(s) for such testing. Some DS manufacturers perform comprehensive contaminant testing, some perform partial testing, and some perform none. As a prescriber of DSs, which contaminant testing profile makes you feel most comfortable and safe?

CONTAMINANT TESTING

Nonbotanical Raw Materials

Heavy Metal(s) Where Indicated

An example of heavy metal contamination is lead content in calcium and magnesium or products made from the shells of shellfish.

Solvent Residue

Many vitamins, minerals, amino acids, enzymes, and glandular products are manufactured using one or more chemicals or chemical solvents. Currently, the natural products industry has no limits on chemical or solvent residue allowed in natural products. One could use and comply with the allowable chemical and solvent limits for the pharmaceutical industry. Testing a panel of multiple solvents is recommended when trying to find residual solvent levels in botanicals or nonbotanicals. Why a panel? Most DS suppliers do not give adequate, accurate, or often any information regarding residual solvent levels on the COA for an RM under consideration for purchase. Often DS manufacturers in the industry will do no testing for any solvents if there are no solvents listed on the COA. This is a mistake, because when a wide net is cast looking for solvent residue, one or more solvents are frequently found.

Bacterial, Yeast, Mold

All batches of RMs and FPs should be tested for these microbiological parameters.

Miscellaneous Items to Consider Where Appropriate

For example, test for melamine levels in protein powders or foods.

Botanicals

Heavy Metal(s)

Every batch of RM should be tested for lead, mercury, cadmium, and arsenic.

Solvent Residue

A panel of multiple solvents is recommended when trying to find residual solvent levels in botanicals as previously explained.

Bacterial, Yeast, Mold

All batches of RMs and FPs should be tested for these microbiological parameters. Some forms of botanical materials are more prone to having higher levels of these items compared with nonbotanical materials.

Herbicide, Pesticide, and Fungicide Residue

Ideally, every batch of botanical product purchased should be tested for residual amounts of a variety of herbicides, pesticides, and fungicides. Certified organic botanical products can be exempted. There are a number of testing profiles to choose from: FDA, United States Pharmacopoeia (USP), European Pharmacopeia, or the California Department of Food and Agriculture. Testing broadly for organochlorines, organophosphates, organonitrogens, and carbamates is ideal and covers a wide spectrum. Every batch should be tested because plant medicines come from all over the world, and various countries have strict or lax rules on what is allowed or not allowed to be applied on crops. Despite rules and regulations in some third-world countries, there could be a lack of enforcement, which creates a big hole for skirting the rules and even more of a reason to test comprehensively every batch received. For example, testing of a sample of kava extract from Indonesia could reveal residues of dichlorodiphenyltrichloroethane (DDT), which is banned in the United States and has been for years. The FDA has a zero-tolerance limit for DDT and its metabolites.

Aflatoxin Residue

Aflatoxins are toxic metabolites produced by certain fungi that are in or on foods and plant materials. Four different aflatoxins, B1, B2, G1, and G2, have been identified, with B1 being the most toxic, carcinogenic, and prevalent. Aflatoxins are probably the best known and most intensively researched fungal toxins in the world. Aflatoxins have been associated with various diseases, such as aflatoxicosis in livestock, domestic animals, and humans throughout the world. Aflatoxins have received greater attention than any other fungal toxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans. Many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on food and feed for animals. The FDA has set a limit of less than 20 ppb for total aflatoxins in food and feed. The European Union has a much lower overall tolerance (10 ppb), and is more specific for limits on individual aflatoxins, especially B1 at 2 ppb or 5 ppb depending on the food.

Fish Oil and Vegetable Oil Products

Heavy Metal(s)

Every batch of RM should be tested for lead, mercury, cadmium, and arsenic.

Bacterial, Yeast, Mold

All batches of RM and FP should be tested for these microbiological parameters.

Dioxins, Dioxin-Like Compounds

Testing every batch received is ideal because levels of these chemicals are present from batch to batch. The same can be said for polychlorinated biphenyls (PCBs), as explained in the following. Fish oil processing can remove many of these residues if the crude material is cleaned. Some portion of the crude fish oil sold worldwide and used to make capsules or liquid fish oil is not cleaned and may contain higher levels of these chemicals.

Dioxin is a generic term used to describe a family of 210 compounds. The most dangerous of this family are 17 members of this group characterized by the presence of chlorine atoms in the 2, 3, 7, and 8 positions. Each of those 2, 3, 7, 8-substituted congeners has been assigned a Toxic Equivalent Factor, which is used for the computation of the Toxic Equivalency Quotient. This scale is used in risk assessment studies to calculate the probability of causing cancer and other life-threatening diseases in humans. The European Union has set a fish oil for human consumption tolerance limit for dioxins.

Polychlorinated Biphenyls

PCBs are a mixture of individual chemicals that are no longer produced in the United States, but are still found in the environment. Health effects that are associated with exposure to PCBs include acne-like skin conditions in adults and neurobehavioral and immunological changes in children. PCBs are known to cause cancer in animals. The Department of Health and Human Services concluded that PCBs may reasonably be anticipated to be carcinogens. The Environmental Protection Agency and the International Agency for Research on Cancer determined that PCBs are probably carcinogenic to humans.

Rancidity Testing

Oil products ideally should be tested for primary oxidation byproducts (peroxide levels) and secondary oxidation byproducts (anisidine levels). A high level of any of either is indicative of rancidity of long-chain fatty acids in the oil product. It is critical to test for both because peroxides are transitory and can drop to a low level as they transform into secondary oxidation byproducts (anisidines). Once an oil has started down the path of rancidity, it usually proceeds to higher levels of rancidity.

FINISHED PRODUCT EXPIRATION DATE AND STABILITY TESTING

Many DS companies have no data to support their expiration date or "best if used by" claims. The following information is taken from the preamble portion of the FDA DS GMP regulations:

> *"Because the final rule does not require that you establish an expiration date, we (the FDA) decline to offer guidance on the type of data that are acceptable to support an expiration date, other than to repeat that any expiration date that you place on a product label (including a "best if used by" date) should be supported by data."*

Because the FDA gives no guidance on the matter, what should be asked of a DS manufacturer? Strength/potency testing is probably most important because the potency of the product over time might be critical to the product producing intended results.

Some questions to ask are:

1. Are you conducting any stability testing on your FPs?
2. If so, what testing? Strength/potency? Bacteria, yeast, mold testing?
3. If no testing is taking place, are you performing physical parameter inspections (appearance; weight; integrity of the capsule, tablet, or powder, etc.)?
4. If no testing is taking place, how do you verify the strength/potency of the product over the shelf life expiration dating period? What data do you have to support the expiration date period and stability of your product(s)?

ASSESSING QUALITY VIA COMPREHENSIVE TESTING

Start with the concept that "you only find what you look for." This can and should be applied to DS RM ingredients. When a DS manufacturer buys an RM (domestic or foreign), generally they cannot just tell that the material is of good quality. The measure of quality of a DS FP is a function of the quality of the ingredients that went into it. If a DS manufacturer looks at the quality of an ingredient in a cursory manner and only performs the

most minimal of tests, some essential quality parameters may be missed, and the ingredient goes unverified overall. If comprehensive testing is performed for an array of quality parameters, the ingredient may be deemed verified as a quality ingredient if the test results are acceptable.

The definition of verification is repeated to make a point about the value of assessing quality via comprehensive testing. To verify means:

- To prove the truth of, as by evidence; confirm; substantiate
- To ascertain the truth or correctness of, as by examination
- To act as ultimate proof or evidence of; serve to confirm.

Verification of quality via testing provides evidence, proof, substantiation, and confirmation of quality parameters. How else can it be achieved? Testing is king when it comes to confirming and assessing the quality parameters of DS ingredients and FPs.

If a DS manufacturer does extensive and comprehensive testing on all RM ingredients that go into the FP, the overall quality of the FP is mostly assured. To fully ensure quality in this scenario, the only items left to test on the FP would be strength/potency (if potency claims are made) and microbiological profile to make sure it did not become contaminated with bacteria, yeast, or mold as it went through manufacturing. Ideally, the FP would also be run through a metal detector to rule out the possible introduction of metal pieces from the manufacturing process. Finally, if any of the active constituents could be damaged by the manufacturing process, such as a heat labile substance, its potency should be checked before quality control release.

SUPPLIER CERTIFICATION AND SKIP LOT TESTING

The FDA DS CGMPs allow for certifying a DS supplier. Once a DS supplier is certified, the DS manufacturer can perform reduced testing of RM batches (i.e., skip certain testing parameters because they have done enough due diligence to "trust" the DS supplier's quality).

The FDA CGMP regulation in Subpart E, §111.75 states:

A firm may rely upon a certificate of analysis (COA) from its supplier of a component (in other words, not test), provided that certain criteria are met, which include the following:

- The firm first qualifies the supplier by establishing the reliability of the supplier's COA through confirmation of the results of the supplier's tests or examinations
- Maintains documentation of how it qualified the supplier
- Periodically reconfirms the supplier's COA
- Quality control personnel review and approve the documentation setting forth the basis for qualification (and requalification) of any supplier
- Some questions remain regarding these guidelines. The FDA gives no acceptable criteria on establishing reliability of the supplier—does this need to happen once, twice, six times? How was this confirmed? How often is the COA from the supplier reconfirmed? Every year, 2, 3, or 5 years?

Officials from the FDA have said the following at numerous trade shows and conferences. Unfortunately, this provides no guidance for DS manufacturers. "We anticipate that Industry will be creative in qualifying the global supply chain." In addition, the FDA has told the DS industry that qualifying a supplier must include an onsite comprehensive audit of the supplier's facility (wherever it may be) and manufacturing and quality assurance processes for the ingredients in question.

LABORATORY TESTING AND LABORATORY QUALITY

DSs are tested by contract laboratories to ensure identity (assure authenticity without adulteration), purity (assess contamination levels), and strength (verify the claimed potency). Laboratories use "methods" to test DSs. Ideally, a validated method would be used for the specific item in the specific mix of ingredients. However, few laboratories have adequately validated methods to cover all situations.

The most pressing issue regarding laboratory quality assurance is, by far, the lack of available validated methods. Method validation shows that a method is suitable and fit for its purpose—demonstrating accuracy and reproducibility. It is easy to define and hard to implement because many different, time-consuming experiments need to be performed to accomplish it. Method validation is extremely important in the DS industry, especially because the FDA requires manufacturers to support their ingredient label claims.

Validating a method proves beyond a reasonable doubt that the results are accurate and reproducible. Method validation is absolutely necessary to ensure the highest degree of reliability. In addition, method validation is needed to settle variant results from laboratory to laboratory. If the laboratory has a variety of quality systems in place, then this may not be completely necessary within a single laboratory.

The quality systems of any laboratory are paramount to achieving the goal of consistently producing accurate, precise, and reproducible results. However, there is no systematic way to know that laboratories are producing accurate results. Many of them do not have the necessary systems or methods in place to ensure quality results. Some laboratories start out using a validated method, but later "modify" the method to suit their purposes. By doing this, they invalidate the validation because the method has been changed. This "method rigging" may introduce inaccuracies and unreliability in the results.

Quality systems every contract laboratory should employ include:

- Use of appropriate reference standards
- Repeatability studies
- Ensuring system suitability requirements are performed, such as:
  a. Determining whether the instrument is calibrated properly and operating correctly
  b. Following written standard operating procedures (SOPs)
  c. Performing out-of-specification (OOS) studies. An OOS study is triggered when a laboratory gets a test value that is either substantially lower or higher than the anticipated value. The OOS study verifies that the laboratory had everything correct (by rechecking all necessary parameters) when the analysis was run. Then the laboratory repeats the analysis to see if the same value is obtained or not
  d. Regularly performing recovery studies using pure compound for recovery to test extraction performance and equipment. A recovery study is when the analyst prepares a sample (extraction) with a known concentration of substance and runs it in the machine. The recovery of the result should be the concentration expected. If not, something is wrong with the equipment or sample preparation (extraction)
  e. Ensuring the method used is based on good, valid science, and is applicable for the DS being tested
  f. Allowing independent audits of procedures, practices, and data

If an officially approved validated method is not available, a scientifically valid analytic method is one that is based on scientific data or results published in, for example, scientific journals, references, textbooks, or proprietary research. These methods can yield good results if they are not changed to any significant degree and they are appropriate for the analysis. The author had a schisandra extract (*Schisandra chinensis* or *S. sphenanthe*) tested at one laboratory that yielded a 10% schisandrins result and at another laboratory that yielded a less than

2% result. The first laboratory was using a method that was designed to test material in blood or serum and not a botanical extract. Clearly, it was not a "good science" method because it was not appropriate for the intended purpose.

Validated methods are the gold standard for obtaining accurate laboratory results. Unfortunately, official (meaning recognized by the government) or unofficial validated methods do not exist for many dietary supplements. The bottom line: only trust the laboratories that follow and adhere to the quality systems outlined previously and strive to use scientifically valid methods when validated methods are not available.

ECONOMICALLY MOTIVATED ADULTERATION

Economically motivated adulteration (EMA) is the fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production (i.e., for economic gain). EMA includes dilution of products with increased quantities of an already present substance (e.g., increasing inactive ingredients of a drug with a resulting reduction in strength of the FP, or watering down of juice) to the extent that such dilution poses a known or possible health risk to consumers, as well as the addition or substitution of substances to mask dilution.

Examples of Products That Are Prone to Economically Motivated Adulteration

- Ginkgo biloba extract: The most common method of adulterating ginkgo is to add an inexpensive flavonoid source that artificially inflates the total flavonoid level in the extract to meet the required 24%. Rutin, a flavonoid source extracted from buckwheat with a low cost, has been the most common additive used for this purpose. *Sophora japonica* (Japanese Pagodatree) is also used to artificially elevate the flavonoid level of ginkgo extract.
- Panax and American Ginseng extract: Ginseng root is widely considered the source of the herb's therapeutic activity; however, large quantities of the leaf can be added to surreptitiously and artificially increase levels of some ginsenosides.
- Goldenseal root extract: Goldenseal root naturally contains a compound called berberine at a concentration of approximately 4% to 8%. Goldenseal root extract can be spiked with added pure berberine or substituted with other berberine containing plants.
- *Coptis chinensis* extract: This can be spiked with berberine or substituted with other berberine containing plants.
- Bilberry extract 25% anthocyanosides: The anthocyanoside content can be manipulated by blending in cheaper berries, such as black currant and elderberries; reports showed even amaranth dye has been used to spike bilberry in an attempt to fool the testing and pass as a quality product.[1]
- Saw palmetto extract: This extract may be spiked with exhausted plant effluent (powder left over after the plant is extracted) or adulterated with palm oil. Addition of palm oil allows for the fatty acid content to meet the claim, but it throws off the natural balance of fatty acids in the extract, which can be detected with proper testing. In addition, unripe berries can be used versus the traditional use of ripe fruit.
- Grape seed extract: Addition of cheap flavonoids can allow a poor-quality product to pass as high-quality product.
- Black cohosh extract: Other cohosh species are used rather than *Cimicifuga racemosa*. The substituted species are not therapeutically active or have a different therapeutic activity.
- Pomegranate extract: Ellagic acid can be added to the pomegranate extract to fraudulently meet the guaranteed amount in the final extract without spending the money on expensive pomegranate dry material.
- Fish oil: This can be adulterated with vegetable oils. Proper analytic testing of the fatty acid profile and examination of fat ratios can usually detect if a fish oil product has had vegetable oil added.
- Protein powders: Addition of a plastic-like agent called melamine can artificially raise the apparent protein level. A test is available to detect the adulteration of protein powders with melamine. Melamine can cause serious health problems, including death.

RESIDUAL CHEMICAL SOLVENTS IN DIETARY SUPPLEMENTS

It is important to note that solvent residue testing is not mandated as part of the FDA guidelines for CGMPs in regard to DSs. It is up to the DS manufacturers themselves to determine whether solvent residue testing is needed.

This is a big hole in the FDA CGMPs because many RM components used to manufacturer DSs have some level of chemical solvent residue. The question is how much chemical residue is present, and is the level acceptable, safe, and will not cause harm over a long period of ingestion? Hence unacceptable residual solvent levels will be an ongoing quality challenge for many DS manufacturers and thus an ongoing quality concern.

A Solvent Primer

The issue of solvents is complex and involved. Official US policy began in 1997 when a joint initiative involving both regulators and research-based industry members from numerous countries published the *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Impurities: Guideline for Residual Solvents.* This was then adopted by the FDA. Based on these guidelines, the USP revised Chapter 467, "Residual Solvents," in 2007. This then became official policy on July 1, 2008. Basically, there are four classes of solvents. The following is a simple primer based on the more current USP revisions.[2]

Note that these guidelines are for pharmaceutical products. There are no guidelines for DSs. So, in the absence of DS residual solvent guidelines, it seems obvious to use the existing standard for pharmaceuticals rather than do nothing and completely ignore the issue.

Class I Solvents

These solvents should not be employed in the manufacture of drug substances, excipients, and drug products (and hence natural medicines, either) because of their unacceptable toxicity or their deleterious environmental effects. However, to produce a drug product (or natural medicine), if their use is unavoidable, then their levels should be restricted, as shown in Table 96.1.

Class II Solvents

These solvents should be limited in use because of their inherent toxicity. There are 27 in this category, with acceptable limits from 50 to 4840 ppm. Some examples are chloroform, cyclohexane, hexane, methanol, and toluene.

Class III Solvents

These solvents are regarded as less toxic and of lower risk to human health. There are also 27 in this category, all with limits of 5000 ppm. Some examples are acetone, dimethyl sulfoxide, ethanol, ethyl acetate, and methyl acetate.

TABLE 96.1 Class I Solvents (Solvents That Should Be Avoided)

Solvent	Acceptable Limit (ppm)	Concern
1,1,1 Trichloroethane	1500	Environmental hazard
1,1 Dichloroethene	8	Toxic
1,2 Dichloroethane	5	Toxic
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard

Class IV Solvents

These solvents may be of interest, but no toxicity data were found.

THE BUYING DECISION

Most consumers and practitioners do little or nothing to assess and verify product quality before they choose a supplier or make a buying decision. All too often our buying decisions are based on what other people, clinicians, educators, books, magazines, and DS company marketing materials have told us. We may like an ad, the packaging, the salesperson, believe the company's quality story, be influenced by their marketing claims, or recognize that the company has good clinical education that ties their products to the education piece. In essence, usually everything but evidence of quality verification drives our decision. It is critical to verify quality claims.

To verify is to prove the truth of something by presentation of evidence. People do not usually ask for that evidence to verify that a manufacturer is providing verified quality products. Why not? Probably because we are too busy, we assume companies are providing the quality we seek, we are not educated enough to know how to separate the marketing hype from the real evidence of quality verification, and lastly the average person may be too intimidated to call the company and ask very pointed quality questions.

This is a huge missing piece. Health care professionals would be wise to demand to see the evidence that supports the marketing claims of quality products. If we choose not to, we must realize that we could be prescribing subpotent, inauthentic, and/or contaminated products.

The author (and editors) has seen far too many examples of subpotency, lack of authenticity, contamination, and a general lack of quality assurance all across the supply chain that feeds the natural products industry. If we think that all the companies we purchase from have done all their quality assurance homework and have applied it, we need to think again. We do not know because most companies do not divulge what they do or, more importantly, what they do not do regarding the full story of the quality assurance they apply to their products.

The bottom line take home message is this: step out of your comfort zone and call companies you intend to purchase from and do your best to determine their quality practices by asking very pointed questions and requesting some proof to support their answers. You can read and become familiar with the questionnaire provided in this chapter to give you some idea of what to ask for. Asking for some level of accountability from the company you plan to buy from is better than blindly assuming they are performing adequate quality assurance to protect you and your patients.

Verifying the quality of a brand has to be done by asking the company if they have in place and consistently follow a comprehensive testing protocol to test RMs and FPs at release and conduct FP stability testing. You can use the questionnaire provided here to give you information and guidance on what questions to ask and the necessary information to gather.

DIETARY SUPPLEMENT PRICING AND QUALITY

A specific brand of DS may carry a high price. A high or low priced DS does not necessarily correlate with product quality. Two thoughts to consider regarding price and quality: First, a low or very low priced DS product is not likely to have been put through comprehensive quality testing. The cost of consistently performing comprehensive quality testing on DS products is very steep, and product pricing needs to reflect that financial investment. Second, full adherence with DS FDA CGMPs is very, very costly, as it requires financial investments in testing, personnel education and training, facility system changes, and experienced quality managers to run the quality department and comply with developing and maintaining the volumes of documentation dictated by FDA CGMPs. The days of cheap DSs are probably coming to an end.

CURRENT GOOD MANUFACTURING PRACTICE CERTIFIED PROGRAMS

In the United States and abroad, there are government and nongovernment agencies that conduct audits of DS manufacturers to assess their CGMP compliance with the standard set by the auditing agency. If the DS manufacturer successfully passes one or more necessary audits, it gets a "CGMP Compliant" or "CGMP Certified" certificate from the auditing agency. DS manufacturers use these certifications to trumpet their quality. The comprehensive nature of the audit process and criteria applied to receive a pass varies from auditing body to auditing body. All certification programs contain some value, but some may have serious limitations and may not tell clinicians all they need to know to trust a DS manufacturer and their products. Be aware that certification does not guarantee quality, and also that lack of certification does not mean lack of quality.

There are items to consider regarding these independent CGMP certifying bodies. First, the FDA CGMPs trump all of these auditing bodies because compliance with the FDA legally required regulations are currently most important. I have already described some of the "confusing and not spelled out" FDA CGMP interpretation and compliance issues. Until those issues are defined and specified, any outside auditing agency is making their best guess and applying their interpretation of what will pass with the FDA. Second, the most important quality practice to verify and understand with any DS manufacturer is how comprehensively they conduct testing to verify identity, strength/potency, purity (contamination), and shelf life dating. None of these CGMP Certified seals will give you that information. You must attempt to get that information directly from the DS company.

Quality Questionnaire, a Tool to Help Verify Quality Claims and Testing Practices

The road to ensuring high quality is successfully traveled when clinicians ask for, obtain, and evaluate DS manufacturer supplied information on CGMP compliance and valid evidence (test results) of a product's identity (authenticity), purity (maximum freedom from contamination), strength/potency, and shelf-life strength.

A questionnaire entitled, "The Manufacturer Quality Assurance Self-Audit Form," is available for use.[3] It is reprinted in the Appendix. The form is intended to give clinicians a basis upon which to question or request documentation from manufacturers and/or suppliers about

their quality control/quality assurance practices and testing programs. There is also an attending compliance document that can be used to evaluate some of the responses obtained from DS manufacturers.

> *"Not having the information you need when you need it leaves you wanting. Not knowing where to look for that information leaves you powerless. In a society where information is king, none of us can afford that."*
> ***Lois Horowitz***

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Penman KG, Halstead CW, Matthias A, et al. Bilberry adulteration using the food dye amaranth. *J Agric Food Chem*. 2006;54:7378–7382.
2. US Pharmacopeia. <467>Residual solvents. Second Supplement, USP–NF. http://www.usp.org/pdf/EN/USPNF/generalChapter4672ndSupp.pdf. Accessed April 6, 2012.
3. The IMCJ Supplement Quality Audit Form. Integrative Medicine: A Clinician's Journal. http://www.imjournal.com/resources/web_pdfs/0609_auditform.pdf. Accessed April 6, 2012.

97

Naturally Occurring Antioxidants

Robert A. Ronzio, PhD

OUTLINE

A growing body of research implicates excessive oxidative damage in multiple disease processes as well as aging. This chapter first examines free radicals and reactive oxygen species, in oxidative stress as participants in cell signaling, then considers the role of antioxidants in redox regulation in human health.

FREE RADICALS AND NONRADICAL OXIDANTS

Free Radicals

These molecules possess an unpaired electron; therefore they participate in single-electron oxidations. In the body, the hydroxyl radical (•OH) is one of the most reactive free radicals, precluding any useful physiological role. Less reactive radicals, nitric oxide (NO•) and superoxide (O_2•−), are able to participate in autocrine and paracrine functions. In isolated systems, a chain initiation event generates free radicals, such as alkyl (RO•) and peroxyl (ROO•) radicals. Radical propagation steps repeated many times can lead to chain reactions. However, it is unlikely that free radical chain reactions are propagated in vivo, due to abundant molecular targets, radical scavenging enzymes, and low-molecular-weight antioxidants.

In addition to free radicals, the body generates an array of nonradical oxidizing agents that participate in electron-pair oxidations. This group includes hydrogen peroxide (H_2O_2), lipid peroxides (ROOH), hypochlorite, peroxynitrite, quinones, and disulfides. Nonradical oxidants vary in their reactivity, with hypochlorite and peroxynitrite representing potent oxidizers and H_2O_2 a much less reactive, diffusible signaling molecule.

Reactive Oxygen Species

Reactive oxygen species (ROS) represent more or less reactive oxidizing agents, whether or not they are free radicals. They include superoxide and H_2O_2. H_2O_2 is not very reactive; however, it spontaneously forms hydroxyl radicals in the presence of iron and copper ions. Superoxide is not highly reactive, either, and superoxide concentrations in the cytoplasm and mitochondria are usually held in check by superoxide dismutases (SODs), which yield H_2O_2. Nonspecific usage of the term *ROS* is not useful because "ROS" cannot be induced, measured, or inhibited. As oxidizing agents, individual rate constants vary from 2×10^{-2} to 2×10^{9}.

Normal metabolism generates ROS according to several mechanisms and initiates a variety of toxic reactions, including lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3 phosphate dehydrogenase, inhibition of membrane sodium/potassium ATP-ase activity, and inactivation of membrane sodium channels, which all play a role in the pathophysiology of inflammation (Fig. 97.1).

Oxidases

Nicotine adenine disphosphonucleotide (NADPH) oxidase is the only enzyme that exclusively produces superoxide; therefore it is carefully regulated. Peroxisomes oxidize fatty acids while producing ROS. Xanthine oxidase oxidizes purines from DNA, RNA, and adenosine triphosphate (ATP). Microsomal mixed-function oxidases (cytochrome P450) generate ROS in the detoxification of metabolites and xenobiotics. Drugs that cause peroxisome proliferation, such as clofibrate, can stimulate the production of H_2O_2 by this mechanism.

Cyclooxygenase and Lipoxygenase

Inflammation activates the arachidonic acid cascade, which converts arachidonate to eicosanoids that mediate inflammation and activate NADPH oxidase to increase the production of ROS.[1]

Redox Cycling

Several xenobiotics, such as paraquat and alloxan, catalyze the formation of superoxide through cyclic reactions, promoting auto-oxidation.

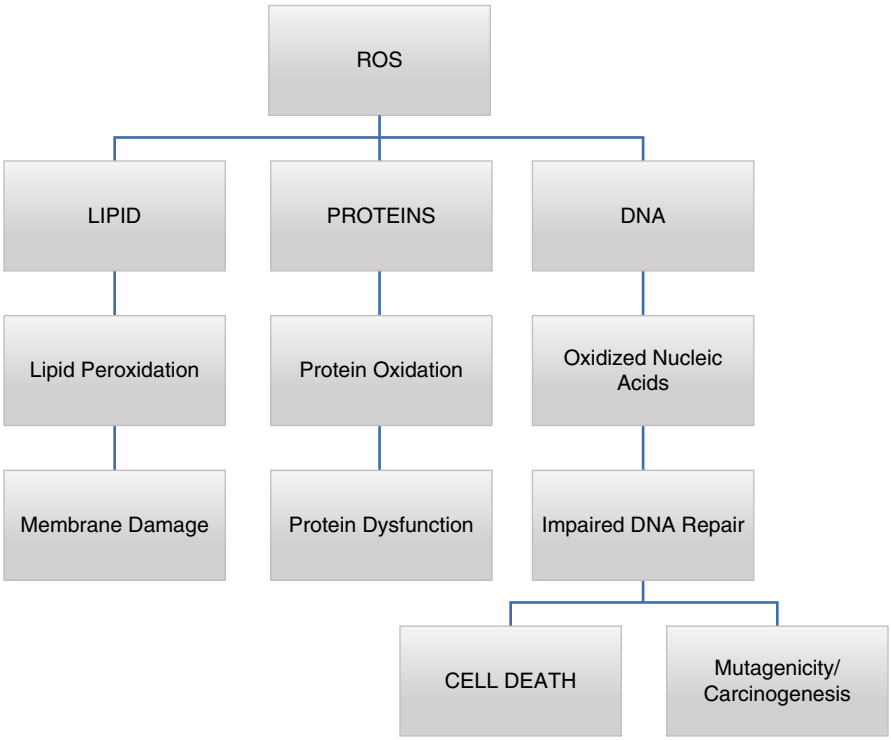


Fig. 97.1 Potential effects of reactive oxygen species.

Reactive Oxygen Species Generated Directly from Oxygen

In the presence of reduced iron or copper ions, oxygen can produce H_2O_2 and hydroxyl radicals. Oxygen can also react spontaneously with heme proteins such as myoglobin, hemoglobin, and cytochrome c to generate superoxide. Excessive iron and iron overload may cause hydroxyl radical production. Consequently, the release of iron from storage sites during inflammation and injury may promote the spontaneous production of free radicals.[2]

Oxidative Stress

The term *oxidative stress* refers to a shift in the ratio of reducing agents to oxidants and its consequences in the body. In this sense, oxidative stress occurs when oxidants outweigh the various antioxidant systems as a result of excessive ROS production and/or limited antioxidant defenses. An expanded definition of oxidative stress now includes dysfunctional redox signaling and imbalanced regulatory mechanisms, which emphasizes the role in signal transduction. During oxidative stress, endogenous defenses may be consumed, or they may not be replenished by recycling. Decreased dietary intake of antioxidant nutrients or malabsorption syndromes can reduce antioxidant defenses and create chronic imbalances, setting the stage for disease.

OXIDATIVE STRESS AND TOXICANT EXPOSURE

Toxic Metals

Arsenic, cadmium, mercury, and lead have all been shown to cause increased DNA oxidation, measurable with 8-OHdG. Iron, copper, chromium, vanadium, and cobalt undergo redox-cycling reactions producing toxicity, whereas mercury, cadmium, and nickel deplete glutathione and bond to sulfhydryl groups of proteins.[3] Lead not only generates ROS but also causes a reduction in the activity of ROS-quenching enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, resulting in diminished antioxidant defense. At the cellular level, cadmium provokes the generation of ROS, which indirectly modulates gene expression and signal transduction and reduces the activities of proteins involved in antioxidant defenses.[4] The carcinogenic effect of arsenic may be related to the activation of redox-sensitive transcription factors involving NF-kβ and AP-1.[5]

Persistent Organic Pollutants

Exposure to organophosphate pesticides will lead to DNA oxidative damage, resulting in an increase of 8-OHdG. Malathion, a commonly used organophosphate pesticide, generates free radicals that induce oxidative stress in human erythrocytes through the inhibition of catalase, glutathione peroxidase, and superoxide dismutase.[6] Oxidative stress from malathion exposure may also be a result of damage to mitochondrial complexes by inhibiting the activity of Complex IV.[7] A positive correlation has been found between farmers exposed to pesticides and oxidative stress biomarkers.[8]

Air Pollution

Diesel exhaust particles, probably the most toxic component of urban outdoor air pollutants, are a mixture of carbon particles, organic chemicals, heavy metals, and free radicals, causing DNA oxidative damage that is measurable with elevated 8-OHdG levels. Persons exposed to traffic in their work (e.g., traffic officers) have higher levels of 8-OHdG, indicative of increased oxidative damage.[9]

Urban air pollution has long been positively associated with respiratory and cardiac problems and increased mortality rates. Particulate matter (PM) is one of the major components of urban air pollution and has been linked to increased levels of 8-OHdG. PM causes significant oxidative damage in the tissues and organs to which it is distributed and has been associated with increased mortality, primarily from cardiovascular,[10] respiratory,[11] and neoplastic diseases.[12] A study in Taiwan demonstrated that exposure to indoor air pollutants caused an increase in oxidative damage, aggravating sick building syndrome–related symptoms.[13] Peruvian women exposed to wood smoke from indoor cooking fires also had higher urinary 8-OHdG levels.[14]

Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to induce ROS in cardiac and cardiovascular-related cells, which may explain the increased risk of heart attack and stroke associated with NSAID use. Rofecoxib, a cyclooxygenase-2 (COX-2) inhibitor, was removed from the market because its extended use was found to increase the risk of atherothrombotic events. It is now known that the cardiotoxicity of rofecoxib was not related to COX-2 inhibition but rather to the oxidative modification of low-density lipoproteins and cellular membrane lipids, which contributed to plaque instability and thrombus formation.[15]

Radiation

Ionizing radiation can cause DNA damage, gene mutation, apoptosis, and cancer. The toxic effects of radiation result from rapid generation of ROS through radiolysis of water molecules as well as secondary reactions leading to increased levels of ROS, which can diffuse within the cell and delay the toxic effects.

Tobacco

Chronic tobacco smoke inhalation induces an intracellular oxidative environment characterized by decreased concentrations of circulating antioxidants, increased oxidation of glutathione, and increased levels of DNA damage. Tobacco smoke has long been linked with elevated levels of 8-OHdG in smokers and in those exposed to secondhand smoke. One study of smokers showed 50% higher 8-OHdG levels compared with nonsmokers,[16] with cessation of smoking resulting in a 21% drop in 8-OHdG levels after 4 weeks.[17]

OXIDATIVE STRESS, AGING, AND CHRONIC DISEASE

Aging refers to the time-dependent alteration in function in the form of cellular and systemic functional decline, with increased morbidity and mortality. The increased prevalence of chronic degenerative diseases can be viewed in terms of irreversible cellular deficits.

Free Radical Theory of Aging

According to the free radical theory of aging, free radicals from normal metabolism or external sources gradually overcome antioxidant mechanisms, leading to cumulative oxidative damage to essential cellular elements: proteins, DNA, and lipids.[18]

More than 100 conditions provide circumstantial evidence linking oxidative damage to disease processes, although these correlations do not distinguish cause or consequence. This list includes several forms of cancer,[19,20] atherosclerosis,[21,22] hypertension, diabetes mellitus,[23] cataracts,[24] inflammation and autoimmune disease,[25,26] lung disease,[27] neurological disorders including Alzheimer's disease and Parkinson's disease,[28,29] hepatitis,[30] obesity,[31] and fibromyalgia and chronic fatigue syndrome,[32] in addition to cell death[33] and attributes of aging.[34,35] Psychological stress and responses to social stressors can affect antioxidant enzymes and oxidative stress, and vice versa.[36]

Oxidative stress is linked to aging and senescence in two lines of research. One is based on increased levels of oxidative products in senescent cells and organs. As an example, carbonylated proteins were markedly elevated in the last third of life in human lens, brain, and skin fibroblasts and rat liver.[37] The second, stress-induced premature senescence, demonstrates that a chronic stress response, induced in cells using subtoxic levels of H_2O_2 or other oxidants, can lead to a senescence phenotype (gene overexpression).

The consequences of oxidative stress are often subtle: Increased membrane fluidity and damage to membrane receptor proteins may alter cellular regulatory mechanisms such as signal transduction, inactivation of proteins required for ATP production, or calcium homeostasis. The more or less continuous production of ROS by activated phagocytes during chronic, possibly low-level inflammation may eventually deplete antioxidant defenses, allowing ROS to attack cells, with ensuing tissue injury.

Recognition of mitochondrial dysfunction led to an update of free radical theory.[38] An estimated 3% of oxygen molecules passing through mitochondria are converted to superoxide due to leakage of the electron transport chain.[39] Thus mitochondria can be considered a prominent source of ROS. Mitochondria also produce H_2O_2 and lipid peroxides, which can damage highly susceptible mitochondrial DNA and alter mitochondrial membrane permeability to release apoptogenic substances such as cytochrome c. H_2O_2 can act as a powerful inducer of a senescent phenotype across multiple cell types.[40] Elevated ROS produced by impaired mitochondria has been suggested as a primary cause of aging.[41]

Hydroxyl Radicals, Nitric Oxide, and Peroxynitrite

Hydroxyl radicals are extremely powerful oxidants, and they probably diffuse only several angstroms before attacking cell constituents and forming characteristic decomposition products of lipids, proteins, and DNA.[42] Superoxide rapidly forms hydroxyl radicals in the presence of transition metal ions.

Reactive Nitrogen Species: Linkage to Superoxide

Reactive nitrogen species (RNS) include peroxynitrite and its reaction products, such as NO_2. High, sustained levels of NO and superoxide, precursors of peroxynitrite ($ONOO^-$), are associated with tissue toxicity, cancer, and inflammatory conditions, such as arthritis, juvenile diabetes, and ulcerative colitis.[43] Under inflammatory conditions, simultaneous production of superoxide and NO can increase 1000-fold, and production of peroxynitrite can increase by a million-fold.[44] Associated oxidative stress can result from increased production of superoxide, which reacts spontaneously with NO to produce ($ONOO^-$). Excessive superoxide can come from upregulated xanthine oxidase or cytochrome P450. ROS uncouple nitric oxide synthase (NOS) to produce superoxide rather than NO. Thus exposure of human endothelial cells to lysophosphatidylcholine leads to downregulation of endothelial NOS and SOD and thus a superoxide overload.[45,46] As a highly reactive peroxide, $ONOO^-$ yields secondary free radicals as nitrogen dioxide. A preponderance of NO and superoxide toxicity is due to $ONOO^-$, triggering nitration reaction and cell death.[47]

In nonalcoholic fatty liver disease, an isoform of NADPH oxidase, NOX1, is up-regulated. Mice deficient in NOX1 had increased protein nitrotyrosine adducts in hepatic sinusoids, as did wild-type mice fed a high-fat and high-cholesterol diet. Up-regulation of NOX1 in sinusoidal endothelial cells may be responsible for peroxynitrite-mediated cell injury.[48]

Reactive Oxygen Species: Broad-Spectrum Antibiotics

Infection, toxic exposure, ischemia, and trauma activate phagocytic cells—macrophages, monocytes, neutrophils, and eosinophils—to create ROS.[49] The binding of immune complexes, bacterial endotoxins, or other inflammatory agents to cell-surface receptors triggers a respiratory burst, a localized production of ROS able to oxidize viruses and bacteria. Excessive superoxide from NADPH oxidase undergoes dismutation to H_2O_2 via SOD. Myeloperoxidase in lysosomes then converts H_2O_2 and the chloride ion to hypochlorite, which spontaneously produces highly reactive chloramines from amines.

Oxidative Stress: Redox Regulation of Cell-Signaling Cascades

Physiological levels of ROS influence cell regulatory processes as diverse as apoptosis, cell growth, and chemotaxis.[50,51] Many signal cascades are sensitive to redox balance and can be modulated by ROS and antioxidants. Essential to these pathways are multiple protein kinases, whose phosphorylation products are often other kinases or kinase inhibitors. Related protein phosphatases reverse those effects. Rather than isolated enzyme systems, cross talk is achieved via ROS and RNX signaling to control responses, such as inflammation.

Transcription Factors

Nuclear factor-κB (NF-κB) and activating protein-1 (AP-1) help regulate inflammation and the response to oxidative stress. These factors bind to antioxidant response elements (AREs) in promoter regions to induce transcription of proinflammatory molecules. Transcription factors can boost defenses against ROS, depending on which signaling cascade is activated, to increase transcription of glutathione-S transferase (GSH S-transferase), metallothionein-1, and manganese-dependent SOD.

Nuclear Factor-κB

The NF-κB pathway represents a premier proinflammatory signaling pathway by proinflammatory cytokines as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). Proinflammatory cytokines and lipopolysaccharide (LPS) promote NF-κB activation. NF-κB upregulation leads to the production of cytokines, chemokines, and adhesion molecules. As such, it may contribute to chronic oxidative stress and diseases, such as rheumatoid arthritis, hypertension, and even overweight and obesity.[52] With age, NF-κB expression in the body increases, a link to chronic systemic inflammation.

Nuclear Factor Erythroid-2 Related Factor-2

Nuclear factor erythroid-2 related factor-2 (Nrf2) acts as a master redox switch in cellular defense against oxidative stress. It induces antioxidative enzymes, Phase II detoxifying enzymes, and stress-responsive proteins, as well as NADPH:quinone reductase, glutamate cysteine ligase, GSH S-transferase, GSH peroxidase (GPx), and thioredoxin. Nrf2 also controls genes involved in metabolism, such as NADPH generation and oxidation of fatty acids, while suppressing those involved in gluconeogenesis and lipogenesis.[53] Release from its inactive cytoplasmic complex is redox-dependent.[54]

Activating Protein-1

The transcription factor activating protein-1 (AP-1) controls additional processes affecting cell proliferation and apoptosis suppression, including COX-2 expression. As an example of AP-1 activation, epidermal growth factor and platelet-derived growth factor bind to their receptors, activating phosphatidylinositol kinase, followed by Rac (guanosine triphosphatase [GTPase]) activation, in turn stimulating NADPH oxidase to produce superoxide.[55]

Protein Kinases and Protein Phosphatases

The kinase family includes the protein kinase C (PKC) group, which includes serine/threonine kinases that are dependent on calcium and

phospholipids, the mitogen-activated protein kinase (MAPK) family (extracellular signal-regulated kinases, Jun N-terminal kinase [JNK], and p38 kinases), and tyrosine protein kinases. Related phosphatases reverse their actions. Members of these broad enzyme families participate in posttranscriptional control due to their redox-sensitive cysteinyl domains.[56] As cell-signaling proteins, PKCs are especially sensitive to redox stress and to ROS as a second messenger during the cell proliferation and differentiation essential for cardiovascular health.[57] As an example, vasocontraction occurs when angiotensin II binds to its receptor and activates PKC, in turn activating NADPH oxidase to produce superoxide, which destroys NO. As another example, H_2O_2 from SOD can oxidize catalytic cysteinyl moieties of tyrosine phosphatases, thereby preventing inactivation of tyrosine kinases, including Src, to stimulate AP-1.

Proinflammatory Cytokines

Transcription factors can be activated by inflammatory cytokines, such as TNF-α. AP-1 activation (with NF-κB inhibition) blocks the production of proinflammatory cytokines, including interleukin (IL)-1β, IL-2, IL-4, IL-6, and TNF-α.

Proinflammatory Eicosanoids

The arachidonate cascade features proinflammatory hydroperoxides and endoperoxide eicosanoids: prostaglandins such as PGG_2, PGH_2, and PGE_2 (via COX); leukotrienes; and hydroxyeicosatetraenoic acid (via lipoxygenase). COX-2 can be induced by superoxide, H_2O_2, and inflammatory cytokines such as IL-1 and TNF-α. Stimulated lipoxygenase can trigger ROS production through sequential activation of GTPase (Rac), PKC, and phosphorylation of the NOx subunit of NADPH oxidase, leading to activation of plasma membrane NADPH oxidase and ultimately to superoxide synthesis.[58]

Calcium Homeostasis

Transcription factors can also be regulated through calcium-signaling mechanisms, and H_2O_2 can stimulate calcium release from mitochondria. Thus H_2O_2 increases L-type calcium channels and calcium influx by vascular smooth muscle cells linked to hypertension.[59]

Apoptosis and Cell-Cycle Regulation

Programmed cell death is regulated by complex pathways involving the transcription factors described previously; therefore, it, too, relies on the cellular redox balance. Oxidative stress triggers apoptosis in several model systems, and apoptosis may be regulated by antioxidants.[60,61] Conditions ranging from diabetes mellitus and heart failure to HIV infection may entail altered apoptosis.[62]

Reactive Oxygen Species: Xenobiotic, Phytochemical, and Carcinogen Metabolism

ROS modulate components of the cytochrome P450 system and can activate potential carcinogens. In contrast, Phase II detoxification enzymes (sulfotransferases, quinone reductase, GSH S-transferase, uridine diphosphate glucuronyl transferase) can block carcinogenic effects and modulate detoxification.[63]

Reactive Oxygen Species: Secondary Messengers in Signaling Cascades

Nitric Oxide

Physiological NO levels regulate multiple processes; thus NO produced by the endothelial isoform of NOS (eNOS) regulates vasodilation. In response to proinflammatory cytokines, large amounts of NO are produced during inflammation by phagocytic cells via the inducible isoform (iNOS).[64] NO participates in protein S-nitrosylation. NO reacts with cysteinyl residues to form S-nitrosothiols. As an example, increased NO production yields reversible cysteinyl S-nitrosylation at the active site of protein tyrosine phosphatases.[65] High levels of NO induce apoptosis by stimulating the release of cytochrome c from mitochondria. This activates the AKDK1, cyclic-dependent kinase-1 (CDK1), and JNK pathways, ultimately leading to active caspases that trigger cell death.[66]

Hydrogen Peroxide

The primary source of H_2O_2 is superoxide, constitutively produced by mitochondria and NADPH oxidases. To control H_2O_2, catalase and peroxidases specifically degrade H_2O_2, whereas SOD limits superoxide to curtail H_2O_2 accumulation. A high steady-state level of H_2O_2, associated with chronic inflammation, increases the production of NF-κB and AP-1 via posttranscriptional modification. H_2O_2 oxidizes cysteinyl moieties in redox-switching proteins; this process activates tyrosine kinases and inhibits related phosphatases,[67] leading to nuclear factor translocation to promote neutrophil binding with increased expression of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). ROS, like H_2O_2, can oxidize GSH peroxidase, peroxiredoxins (Prx), and inactive transcription factors directly by oxidizing cysteinyl moieties, leading to the formation of protein disulfides or sulfenic acid derivatives.[68] Chronic vascular oxidative stress may reflect nonspecific host responses with induction of the NF-κB pathway and repression of the Nrf2 pathway.[69]

LABORATORY ASSESSMENT OF OXIDATIVE STRESS

Current methods measure a small fraction of potential oxidation products, given the wide range of ROS and possible cell targets. Measurement of oxidized macromolecules due to ROS provides three classes of biomarkers: protein oxidation products, lipid oxidation products, and nucleic acid oxidation products. These include products of protein degradation as protein carbonyls[70] and nitrosylated adducts; urinary and/or plasma products of lipid peroxidation, such as F_2 isoprostanes[70] and aldehydes, such as malondialdehyde from lipid fragmentation; as well as products of purine oxidation, such as 8-oxo-2-deoxyguanosine.[34,71]

Antibody-based methods have been used to identify and quantify oxidative posttranslational modified proteins. The most common include Western blotting, immune staining, and immunocytochemistry, although accuracy relies on the specificity of antibodies used.[72] As an example, systemic nitro-oxidative stress has been studied in perinatal asphyxia and neonatal hypoglycemia.[73,74] Polyunsaturated fatty acids are susceptible to free radical attack, yielding hydroperoxides in situ, which are released from membranes by phospholipases. Other lipid oxidation products include ketones such as 4-hydroxynonenal. The accuracy of analysis of unmodified or derivatized lipids by chromatography/mass spectroscopy far exceeds that of the commonly used thiobarbituric acid (TBAR) assays.

The guanine moiety of DNA is particularly susceptible to oxidation, yielding a premutagenic lesion involved in GC:TA transversion mutations.[75] Inflammation and oxidative stress are related mechanistically, and certain markers of systemic inflammation offer a secondary window to examine oxidative stress. C-reactive protein can serve as a marker for systemic inflammation, as well as liver dysfunction,[76] and levels can be reduced by antioxidant supplementation.[77]

Gamma-glutamyltransferase (γ-glutamyltranspeptidase [GTT]) is linked to the redox state and to the GSH/oxidized GSH (GSSH) balance; therefore it promotes GSH production. GTT may be useful in

assessing oxidative stress associated with type 2 diabetes, metabolic syndrome,[78] and Alzheimer's disease,[79] in addition to coronary heart disease and stroke.[80]

ANTIOXIDANTS

Generally, antioxidants inhibit the oxidation of molecules targeted by radicals and ROS.[81] To prevent oxidative stress, organisms possess an elegant antioxidative defense network. Broadly, antioxidants can be classified as endogenous or exogenous. Endogenous antioxidants include proteins as blood constituents, metal ion–binding proteins and enzymes, as well as low-molecular-weight molecules produced by metabolism. Exogenous antioxidants can be considered dietary constituents in animal products, fruits, vegetables, and other plant-based foods. Endogenous and exogenous antioxidant systems cooperate and rely on each other for optimal effectiveness.

There is an apparent "pecking order" among antioxidants; some are more readily oxidized than others and will be consumed rapidly unless replenished or recycled.[82] Certain antioxidants are preventive inhibitors that block the initiation of free radical attack. Preventive inhibitors include defensive enzymes such as catalase, SOD, and peroxidases (GPx), as well as low-molecular-weight compounds, including reduced GSH. Beta-carotene, chelating agents such as organic acids, and plant polyphenols represent preventive antioxidants when they quench singlet oxygen or sequester metal ion catalysts. Antioxidants can function as chain breakers, which convert free radicals to stable products and thus block free radical chain reactions. Vitamin E and ascorbic acid are chain-breaking antioxidants.

Laboratory Assessment of Antioxidant Activity

Successful preventive measures for ROS-related disease rely on the early assessment of the presence of oxidative stress and its molecular sequelae. As a starting point, accurate measurement of the total antioxidant capacity may assist in evaluating both physiological and nutritional factors involved.[83] The ferric ion reducing ability (FRAP) assay has been used to correlate serum total antioxidant capacity (TAC) with metabolic risk factors. Ingestion of foods with high TAC values was found to reduce markers of systemic inflammation and liver dysfunction.[76,84] Other methods rely on peroxyl radical scavenging (ORAC), total reactive antioxidant potential (TRAP), total oxidant scavenging capacity (TOSC), hydroxyl radical scavenging (deoxyribose assay), and organic radical scavenging (DPPH). Although international standards are unavailable, these protocols continue to be used, for example, to study the effects of omega-3 fatty acids and vitamin D supplementation on pregnancy outcomes in patients with gestational diabetes.[85] As a further example, the ORAC is often used to compare the antioxidant activity of foods and complex mixtures. This assay measures the decay in fluorescence (possibly from peroxyl radicals), and results are expressed as "trolox equivalents."[86]

ORAC values for foods were published by the U.S. Food and Drug Administration (FDA).[87] However, the USDA removed the ORAC database from its website after concluding that values of antioxidant capacity are not relevant to physiological effects of putative bioactive compounds. The TAC content (FRAC) of more than 3000 foods and supplements has also appeared.[88] It can be misleading to market the health benefits of foods based on measures of total antioxidant content. Yet some evidence suggests the utility of ORAC values to assess dietary and oxidant intake and guide choices of foods and natural products, with the goal of reducing the risk of cancer, cardiovascular disease, and metabolic syndrome.[89,90] Cellular antioxidant activity (CAA), as measured by the ability to quench peroxyl radical-induced fluorescence of a fluorescein probe in cultured hepatoma cells, was developed to account for update and metabolism of antioxidants.[91] It is worth noting that CAA values of flavonoids do not correlate with ORAC values.

Studies of ROS and radical quenching by antioxidants frequently employ single time points (end-point assays). A more reliable approach evaluates the IC_{50}, the concentration of antioxidant yielding 50% inhibition of a given oxidant or radical. It also is important to compare antioxidant activities in several assay systems. Studies showed that procyanidolic oligomers (PCOs) found in numerous foods and used in supplements (e.g., pine bark and grape seed extract) effectively quench diphenyl picrylhydrazyl radicals.[93] Galloyl catechins were shown to be more effective than simple catechins in the inhibition of NADPH-dependent lipid peroxidation of rat liver microsomes.[92]

Endogenous Antioxidants: Enzyme Systems

Superoxide Dismutase: Antioxidant Role for Manganese, Copper, and Zinc

SODs efficiently convert superoxide to H_2O_2. The mitochondrial isoform (SOD2) requires manganese, whereas the cytoplasmic (SOD1) and extracellular (SOD3) enzymes require both copper and zinc. SOD2 is induced during acute inflammation,[94] whereas SOD3 in vessel walls plays a major role in regulating vascular ROS.[95] Knockout mice lacking SOD2 die several days after birth due to massive oxidative damage. Mice lacking SOD1 develop ROS-related pathologies with reduced life span. Several lines of evidence suggest an involvement of SOD in neurological abnormalities. Mutations in the SOD1 gene account for 20% of patients with a familial dominant form of amyotrophic lateral sclerosis, although the mechanism is unknown.[96] Overexpression of SOD2 in a mouse model of Alzheimer's disease was shown to reduce brain superoxide and prevent memory losses.[97]

SOD may be effective in treating experimental ulcerative colitis.[98] High-risk premature infants treated with prophylactic recombinant human SOD1 had reduced early pulmonary injury at 1 year.[99] SOD supplementation may also improve stress and fatigue in healthy adults.[100] Intravenous administration of derivatized SOD can raise SOD activity in adults. [101] Micronutrient supplementation can increase endogenous SOD among patients with type 2 diabetes administered a combination of vitamins E and C.[102] In a rabbit model, copper supplementation inhibited the progression of atherosclerosis via increased SOD expression and potentiating NO-mediated pathways.[103] Indeed, serum SOD is linked to vascular function in hypersensitive and diabetic patients.[104]

Catalase

The iron-dependent enzyme catalase converts H_2O_2 to diatomic oxygen extremely efficiently. It occurs widely in cells and is a component of peroxisomes. Animal studies with catalase, usually in conjunction with SOD, suggest protection against ischemic injury to the lung,[105] intestinal ROS damage,[106] and radiation.[107] Examination of human atherosclerotic coronary arteries revealed that vascular antioxidant enzymes, including catalase, were selectively elevated in smooth muscle cells and macrophages in atherosclerotic lesions.[108] In contrast, knockout mice lacking catalase were phenotypically normal, possibly due to effective scavenging by peroxidases.[109] In the cardiac proteome of wild-type and transgenic mice, overexpression of catalase was linked to decreased cysteinyl oxidation among 82 proteins, including mitochondrial and contractile proteins, implicated in pathways of cardiac disease.[110]

The Peroxidase Family

Glutathione Peroxidase (GPx): Antioxidant Role for Selenium. GPxs reduce H_2O_2 as well as lipid peroxides with GSH as the reducing agent. Unlike catalase and SOD, GPxs require selenocysteine. GPxs exist as

several isoforms. GPx-1 is a cytoplasmic isoform that prefers H_2O_2 as a substrate. GPx-4 has a high affinity for lipid hydroperoxides, reducing membrane lipid peroxides to nontoxic fatty acid alcohols.[111]

GPx-1 expression is regulated in part by the availability of selenium and by selenocysteine during protein synthesis. By limiting H_2O_2 accumulation, GPx-1 can modulate mitochondrial function and growth-factor–mediated regulation. GPx has been implicated in age-related diseases, including cancer and cardiovascular disease. Antioxidant enzymes, including extracellular GSH peroxidase, are induced by the oxidative stress associated with lung diseases.[112] GPx-1 can block oxidative stress induced by cigarette smoke. Among patients with chronic obstructive pulmonary disease, exposure to cigarette smoke provoked protein unfolding in lung epithelial cells, which was reversed in vitro by GPx-1.[113] Mice lacking GPx-1 have a normal life span; however, they develop early cataracts. In contrast, GPx-4 knockout mice do not survive early embryonic development.[114]

Peroxiredoxins (Prx). This group of six thiol peroxidases reduces H_2O_2, lipid peroxides, and peroxynitrite via an active-site cysteinyl, –SH. The PrxII isoform is one of the most abundant proteins in erythrocytes. Prxs control intracellular levels of H_2O_2 to regulate cell signaling in various cell types and have been implicated in carcinogenesis, tumor metastasis, and drug resistance. Mice lacking PrxI or PrxII develop severe hemolytic anemia and are prone to cancer due to elevated H_2O_2. Prx1 knockout mice exhibit atherosclerotic lesions due to endothelial cell dysfunction.[115] NO may protect macrophages against oxidative and nitrosative stress by inducing Prx.[116] PrxII can express dual roles in cancer progression. Decreased expression is correlated with enhanced proliferation and metastasis of melanoma cells. In contrast, PrxII has been shown to be overexpressed in prostate, cervical, and esophageal cancer. PrxII also plays a role in colorectal cancer, apparently by helping maintain colorectal cancer stem cell–like behavior.[117]

Storage and Transport Proteins

Ferritin, Transferrin, and Ceruloplasmin

Free iron and copper ions catalyze the conversion of H_2O_2 to hydroxyl radicals; therefore proteins that bind these ions help protect tissues against ROS. Transferrin (which has a high affinity for iron) and ceruloplasmin (which binds copper) can be considered part of the antioxidant defenses.[118] Iron stored in ferritin does not participate in the generation of free radicals. Under normal circumstances, little unbound iron is present in cells. However, with chronic inflammation, unbound iron may be released from ferritin, posing a potential hazard. Iron storage disease is linked to oxidative damage. Studies of H_2O_2-resistant cell lines suggest that adaptation to chronic oxidative stress includes increased cellular antioxidant defenses, including elevated levels of mitochondrial ferritin, GSH, and GPx.[119]

Metallothionein

Metallothionein, a small, cysteine-rich protein, binds many metals. It exists as isoforms, induced by cytokines, toxic metals, oxidative stress, inflammation, axonal development, and apoptosis. Mitochondrial-specific ROS generators can increase the production of metallothionein in the liver by 3.7- to 11.8-fold in mice, far more than SOD or GSH peroxidase.[120] Similarly, acute ethanol-induced hepatotoxicity and associated oxidative stress were curtailed in mice that had been genetically manipulated to overexpress metallothionein, compared with wild-type mice.[121] The isoform MT-II is mainly expressed in neurons and is induced by oxidative stress and metals. It has been implicated in neuroprotection and after brain injury. Using an in vitro model of stroke as well as animals with cerebral artery occlusion, exogenous application of MT-II was shown to protect against ischemic injury.[122]

Selenoprotein P

Nearly 60% of plasma selenium is represented by selenoprotein P, which transports selenium to tissues. In vitro, selenoprotein P protects against peroxynitrite-induced damage and reduces phospholipid hydroperoxides.[123]

Redox Regulation: The Glutathione System

Glutathione (GSH)

GSH is a sulfhydryl-reducing agent containing cysteine that occurs in millimolar concentrations in most cells, where it acts as a detoxifying agent, assists amino acid transport, and quenches free radicals, in addition to regulating the internal redox environment of cells. Together with ascorbate, GSH participates in the regeneration of vitamin E, which emphasizes the intracellular cooperation of antioxidants. Reduced GSH reacts directly with singlet oxygen, hydroxyl radicals, and superoxide radicals to form oxidized glutathione (GSSG).

GSH Reductase

GSH reductase reduces GSSG with NADPH, linking thiol regulation to robust glucose metabolism. GSH reductase helps maintain a high GSH/GSSG ratio. Oxidative stress reduces this ratio, activates transcription factors, and increases production of IL-1 and TNF.[124] NADPH and GSH are cofactors for other reductases that help regenerate tocopherol and ascorbate, demonstrating the principle that overall metabolic balance is a prerequisite for adequate antioxidant defense.

GSH Transferase

GSH S-transferase adds GSH to endogenous and exogenous electrophilic compounds and is considered a component of Phase II detoxification enzymes. Modified products are cleared from the cell with multidrug-resistance–associated proteins. This transferase also functions in the synthesis of prostaglandin E2 and leukotriene C4 and D4. Like GSH peroxidases and Prxs, GSH S-transferase requires an ample supply of reduced GSH for optimal activity.

GSH and Neuroprotection

With aging, there is a general decline in GSH levels, and low GSH levels are associated with a variety of chronic conditions, such as diabetes, age-related macular degeneration, gastrointestinal disorders, and neurodegenerative diseases.[125]

In the brain, GSH serves to detoxify xenobiotics and has an important role in antioxidant defense and the regulation of intracellular redox status. This extends its reach to gene expression and cell differentiation in the brain. Dysregulation of glutathione homeostasis has been implicated in neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's disease, as well as amyotrophic lateral sclerosis.[126] In a transgenic mouse model of Alzheimer's disease, the formation of mixed disulfide protein on brain and blood preceded amyloid plaque appearance.[127] In a neuronal-like cell line, exposure to oxidized low-density lipoprotein cholesterol (LDL-C) revealed increased cytotoxicity, lowered the GSH/GSS6 ratio, and increased β-amyloid production.[128]

Redox Regulation: The Thioredoxin System

The thioredoxin system contains two components: thioredoxin (Trx) and Trx reductase. Trx is a small sulfhydryl protein that acts as a reducing agent for Prxs and ribonucleotide reductase. Trx also promotes the activation of several transcription factors.[129] Trx reductase is a selenocysteine-containing enzyme that specifically catalyzes the reduction of Trx. Together, Trx and Trx reductase function as a widespread redox-regulating mechanism with multiple roles in intracellular signaling and resistance to oxidative stress. Thus thioredoxin activates apoptosis signal-regulating kinase-1. It reduces redox factor-1, which

then reduces oxidized (inactive) transcription factors such as NF-κB and Nref-2.[130] Apurinic/apyrimidinic endonuclease 1 (APE-1), a dual-functional DNA repair enzyme that can also reduce transcription factors (such as AP-1 and p53), is regenerated by thioredoxin, linking DNA repair to the cellular redox balance.[131]

Antioxidant Enzymes: Transactivation of Antioxidant Response Elements

Nuclear transcription factors such as Nfr2 can be regarded as a cell-sensing system for oxidative and electrophilic stress, which is responsive within 15 minutes after exposure to a stressor.[132] Transactivation of AREs is regulated by the redox balance, in which nuclear factor Nrf2 migrates to the nucleus in response to ROS, where it activates genes responsible for a battery of antioxidant enzymes, including Prx-6, GPx, GSH transferase, and Trx, as well as Phase II detoxifying enzymes.[133] The Nrf2–ARE pathway represents a master antioxidant mechanism especially relevant to diabetic dysfunction.[134]

Antioxidant enzyme levels tend to decrease with age-related conditions.[135,136] Several antioxidant enzymes are often induced during the oxidative stress associated with age-related diseases. As an example, in human atherosclerotic coronary arteries, catalase, GSH peroxidase, and SOD were upregulated in atherosclerotic lesions, possibly as the result of oxidative stress.[108] Upregulation of Trx1, Trx2, and TrxR2 can occur in aging skeletal muscle, although their role in redox-regulated adaptions to failed muscle functioning remains unclear.[137]

Micronutrients as Antioxidants

Vitamin C

Ascorbic acid can react with a wide range of ROS, including superoxide, singlet oxygen, hypochlorite, and sulfur radicals.[138] Ascorbic acid is an efficient chain-breaking antioxidant in human plasma,[139] where it protects lipids and membranes by scavenging peroxyl and hydroxyl radicals.[140] Vitamin C can reduce heavy-metal toxicity.[141] In animal models, high levels of ascorbate compensated for low GSH production, and vice versa. A combination of ascorbate with vitamin E is possibly more effective than ascorbate alone for older adults.[142] Ascorbic acid functions with GSH and lipoic acid to regenerate α-tocopherol. To recycle ascorbate, dehydroascorbate is reduced back to ascorbate via GSH and NADPH.

The recommended dietary allowance (90 mg for men >19 years old; 75 mg for women >19 years old) is probably an underestimate. Healthy young women may require at least 400 mg/day of ascorbic acid, according to tissue-saturation studies.[143]

Cardiovascular Disease. Observational studies suggest that ascorbic acid has, at best, modest effects on the risk of coronary heart disease. Although a meta-analysis found no benefit with vitamin C supplementation on survival and cardiovascular disease (CVD) risk,[144] others reported benefits with high supplemental vitamin C.[145] The Nurses' Health Study suggested that vitamin C intake of more than 359 mg/day (diet plus supplements) reduced the risk of CVD.[146] For diabetic postmenopausal women, high vitamin C supplementation correlated with an increased risk of CVD mortality.[147] The Physicians' II study found that vitamin C supplementation at 500 mg/day was not cardioprotective for middle-aged men.[148] Another randomized controlled study (the MRC/BHF Heart Protection Study) did not find that vitamin C supplementation reduced the risk of CVD.[149]

Hypertension. In young healthy women, a higher level of plasma vitamin C was associated with decreased blood pressure.[150] A meta-analysis of 29 short-term trials indicated that a median supplementation dose of 500 mg/day significantly reduced blood pressure in healthy and hypertensive adults.[151]

Cancer. High ascorbic acid intake has been linked to a reduced risk of cancers of the oral cavity and esophagus,[152] ovaries,[153] stomach,[154] and colon. However, a pooled analysis of eight prospective studies found that vitamin C intake was unrelated to lung cancer.[155] Treatment of male physicians aged equal to or more than 50 years with 500 mg ascorbic acid daily for 8 years found no reduced risk of prostate cancer or total cancer.[156] Several studies suggested that extracellular ascorbate at millimolar concentrations (achieved via intravenous or intraperitoneal administration) can help increase survival rates.[157] A plausible mechanism involves ascorbate reduction of transition metals (cupric to cuprous, ferric to ferrous), which react with oxygen, yielding superoxide and H_2O_2, to which tumor cells are susceptible.[158]

Neurodegeneration. The brain possesses one of the highest ascorbic acid concentrations in the body. The Rotterdam Study suggested that a high intake of ascorbic acid and vitamin E was associated with a reduced risk of Alzheimer's disease, especially among cigarette smokers.[159] Although the ascorbate levels in the plasma or cerebrospinal fluid (CSF) of 32 patients with Alzheimer's disease did not correlate with cognitive decline over 1 year, the ratio of CSF ascorbate/plasma ascorbate increased, which was possibly related to an impaired ability of the brain to concentrate neuroprotective nutrients.[160] Supplementation of ascorbic acid was associated with a lower incidence of severe cognitive impairment among elderly volunteers.[161] In an animal model, intraperitoneal injection of ascorbic acid prevented disruption of the blood–brain barrier, resulting in sustained somatosensory function.[162]

Cataract. Although some investigations reported the risk of cataracts and retinal damage with lowered vitamin C status, a prospective study employing 500 mg of vitamin C, 400 IU of vitamin E, and 15 mg of β-carotene found no effect on the development or progression of cataracts.[163]

Diabetes. A 12-year prospective study of nondiabetic participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk Study noted that those with the highest 20% of plasma vitamin C had a 62% lower risk of developing type 2 diabetes compared with those in the lowest 20%.[157] Another cross-sectional study noted an inverse relationship between the levels of ascorbic acid and hemoglobin A1c.[164]

Vitamin C: A Cell Response Modifier? Generally, vitamin C appears to function primarily as an antioxidant in modulating the redox balance in a variety of situations, such as attenuating cytochrome P450 detoxification or plasma C-reactive protein levels. Vitamin C was able to increase the efficiency of generating mouse and human pluripotent stem cells from somatic cells, in part by attenuating cell senescence.[165] In other research, the expression of five functional protein groups related to signaling, apoptosis, and activated transcription, among others, was detected in vitamin C–treated T cells.[166] As an additional factor relevant to neurodegenerative disorders, ascorbic acid can switch central nervous system (CNS) metabolism from glucose consumption over to lactate oxidation to maintain synaptic functioning.[167]

Vitamin E (Tocopherols)

Vitamin E refers to four tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) that possess an unsaturated isoprenoid side chain. Vitamin E represents a primary chain-breaking antioxidant of lipids, lipoproteins, and membranes, where it acts as a peroxyl radical scavenger, creating a tocopheryl radical. This radical will decompose unless converted back to tocopherol by ascorbic acid, GSH, and coenzyme Q_{10} (CoQ_{10}), illustrating that antioxidant defenses complement one another.[139,168] Vitamin E can exacerbate hypertension in susceptible people. High levels may antagonize other fat-soluble vitamins, thus decreasing bone mineralization. This vitamin may be contraindicated in patients receiving anticoagulants or in those with a vitamin K deficiency.

Vitamin E supplements often incorporate synthetic α-tocopherol, a mixture of D and L isomers, but only the D form (RRR-α-tocopherol) is active in the body. Esterified α-tocopherol is considerably more stable than unesterified tocopherol, and supplements often use esters of vitamin E, which are readily hydrolyzed and absorbed. As with other fat-soluble vitamins, supplementation is prudent in patients with malabsorption syndromes.

Immunity. α-Tocopherol may increase the T-cell–mediated response, which is impaired with aging. Supplementing healthy elderly people (consuming an otherwise typical diet) with 60 to 800 mg vitamin E improved several aspects of cell-mediated immunity within 6 to 12 months.[169] Among older adults (mean age 70), supplementation with 200 mg/day of synthetic vitamin E for 3 months increased neutrophil chemotaxis and proliferation, natural killer cell activity, and IL-2 production.[170] An RCT of nursing home residents found that supplementation with 200 IU synthetic vitamin/day for 1 year reduced the risk of upper respiratory tract infections.[171] In other research, ingestion of 268 mg/day of natural vitamin E for 8 months significantly reduced serum immunoglobulin-E, with apparent normalization of most lesions in patients with atopic dermatitis.[172]

Cardiovascular Disease. Hypothetically, the oxidation of LDL-C and other lipoproteins can initiate atherosclerosis. Increasing dietary vitamin E intake to more than 20 times the usual intake in a Western-type diet was suggested to reduce the risk of heart disease and its manifestations, such as myocardial infarction, among low-risk populations.[173] However, randomized, placebo-controlled clinical trials with α-tocopherol (200–800 IU/day) in Western populations have been mainly negative.[174]

Only 5 of 12 clinical studies on vitamin antioxidants and CVD reported reduced cardiovascular death and nonfatal myocardial infarction. These include the Cambridge Heart and Antioxidant Study (CHAOS), the Women's Health Study (reduced risk of sudden death from CVD), and the Secondary Prevention With Antioxidants of CVD in End-Stage Renal Disease (SPACE) study of hemodialysis patients who were at high risk of oxidative stress. Inconsistent results with vitamin E may reflect compounding variables, including the heterogeneity of risk factors for coronary heart disease; treatment for the late course of disease versus treatment for early-onset oxidative stress[175]; and genotypic differences among subgroups, for example, the haptoglobin 2-2 genotype in patients with type 2 diabetes.[176] Potentially harmful effects of supplemental vitamin E were reported in three of these (increased risks of stroke and heart failure). As is typical of reductionistic approaches to medicine, α-tocopherol is only one of eight tocopherols found in nature, with others, such as gamma and delta tocopherols, having many important health benefits beyond simply antioxidant activity. This is discussed in more detail later in the chapter.

Neurodegenerative Disease. A high intake of vitamin E and vitamin C was found to be associated with a reduced risk of Alzheimer's disease according to the Rotterdam study.[159] A meta-analysis concluded that circulating vitamin E, C, and A were significantly reduced in patients with Alzheimer's disease versus healthy individuals.[177] In a controlled trial of patients with moderately severe Alzheimer's disease, α-tocopherol administered at a daily level of 2000 IU delayed the onset of severe dementia or death in comparison with controls.[178] However, a controlled clinical study of patients with mild cognitive impairment found that treatment with 2000 IU vitamin E failed to slow mental decline.[179] A multicenter RCT found that supplementation with 2000 IU vitamin E daily for 2 years delayed the decline in daily functioning, yet it failed to alter cognition according to standardized tests.[180] Little evidence suggests that long-term supplementation with vitamin E alone improves cognition among healthy adults.

More generally, it is unrealistic to expect that dosing with one or two antioxidants can substitute for multiple synergistic factors that support interlocking regulatory pathways in reducing risks of chronic degenerative disease.

Cancer. Most clinical studies have not demonstrated the benefits of vitamin E supplementation in reducing the risk of most cancers. Effects of antioxidant supplementation often depend on the subject's nutritional status. Clinical studies with vitamin E illustrate this principle. The Shanghai Breast Cancer Study observed a 20% reduction in breast cancer risk with vitamin E supplementation among women with low dietary intake.[181] The Linxian General Population Nutrition Intervention Trial, which included adults deficient in several micronutrients, examined the effects of 50 mcg/day of selenium, 30 mg/day of vitamin E, and 15 mg/day of β-carotene. Supplementation led to decreased all-cause mortality, including overall cancer risk.[182] In contrast, U.S. studies of vitamin E on cancer rates reported negative results for breast cancer,[183] endometrial cancer,[184] lung cancer (pooled analysis of eight studies),[185] and cancer incidence and cancer mortality.[186]

Although earlier studies suggested an inverse association between vitamin E intake and the risk of prostate cancer, the Physician's Health Study II of men aged 50 years or older found no reduction in the risk of prostate cancer and other site-specific cancers or the risk of total cancer after synthetic vitamin E supplementation (400 IU daily for 8 years).[187] Supplementation of men (ages >55 years) with vitamin E 400 IU per day for up to 12 years increased the risk of prostate cancer by 17%. Men who took vitamin E together with selenomethionine (200 mcg per day) had no higher risk than those taking a placebo.[188] Unknown compounding variables in such studies include the uncertain status of vitamin D, ω-6 fatty acids, and γ-tocopherol, and male hormones in addition to possible polymorphisms for the α-tocopherol–associated protein.[189]

Diabetes. Whether α-tocopherol can benefit diabetes has not been established through large, well-controlled clinical studies. A meta-analysis of 14 small RCTs of patients with type 2 diabetes found that vitamin E supplementation of 200 to 1000 IU/day for 6 to 27 weeks had no significant effects on glycemic control (assessed by fasting glucose and insulin levels and by hemoglobin A1c).[190]

Gamma-Tocopherol. Although the typical U.S. diet supplies twice as much γ-tocopherol as the α form or tocotrienols, α-tocopherol predominates in the body, due to selection by liver tocopherol-transfer protein, which preferentially incorporates α-tocopherol into lipoproteins. Gamma-tocopherol is also rapidly degraded. It selectively blocks reactive nitrogen species and complements antioxidant actions of α-tocopherol.[191] Gamma-tocopherol can protect pancreatic β-cells against NO inhibition in vitro.[192] Mice supplementation with a mixture of α- and γ-tocopherol (not α-tocopherol alone) reduced age-related transcriptional changes in the brain.[193] Clinical findings using γ-tocopherol are limited. Patients with metabolic syndrome treated with γ-tocopherol 800 mg and α-tocopherol 800 mg/day for 6 weeks had significantly decreased markers of inflammation and oxidative stress (high-sensitivity C-reactive protein and nitrotyrosine) compared with placebo.[194] The EPIC Study found no correlation between plasma levels of either α- or γ-tocopherol and the risk of prostate cancer.[195] Cigarette smokers supplemented with γ-tocopherol experienced short-term benefits on vascular endothelial activity during smoking cessation.[196]

Tocotrienols. Tissue distributions of tocotrienols and tocopherols differ, which is perhaps indicative of selective mechanisms in various tissues.[197] Tocotrienols can induce apoptosis in prostate cancer and breast cancer cells.[198] Tocotrienols can suppress inflammatory markers, such as IL-6, TNF-α, and NO, by stimulated macrophages.[199] Clinical studies are in their infancy. Hypercholesterolemic subjects treated with mixtures of tocotrienols daily for 28 days did not have

improved serum lipid or glucose concentrations or lipid peroxidation (urinary 8-iso-prostaglandin F2a).[200] In contrast, treatment of healthy adults with tocotrienols 160 mg/day for 6 months according to a randomized, double-blind protocol resulted in reduced DNA damage.[201]

Vitamin E: A Cellular Response Modifier? Several studies suggest that vitamin E administration can increase the expression of genes related to Th1/Th2 ratios in older mice.[202] Alpha-tocopherol can block upregulation of NF-κB, PKC, and p38 MAPK pathways to prevent inflammatory cytokine production in stimulated human monocytes and in human dendritic cells.[203] The variation in cytokine responses to vitamin E in elderly populations seems to reflect polymorphisms in cytokine genes.[204] Physiologically relevant concentrations of α-tocopherol were found to interfere with the cytotoxic and cytostatic actions of several protein kinase inhibitors, whereas other antioxidants did not affect the action of these inhibitors on cell-cycle arrest or cell death.[205] Nonetheless, such observations reflect the altered redox state of cells and tissues. For example, vitamin E may protect membrane domains and polyunsaturated fatty acid pathways from ROS as its underlying mechanism of action, rather than its involvement in specific signaling actions.[206]

Carotenoids

Carotenoids, plant pigments, are conveniently divided into carotenes and xanthophylls (oxygenated carotenes). The best-known carotenoid, β-carotene, represents only 25% to 33% of plasma carotenoids. In general, carotenoids are versatile antioxidants. Beta-carotene is especially effective at low oxygen tension, as found in tissues.[207] Increased carotenoid levels have been associated with decreased LDL-C oxidation.[208] They can quench ROS produced during inflammation and modulate levels of proinflammatory prostaglandins and leukotrienes.[209,210]

Lung Cancer. Although retrospective studies suggested an inverse correlation, analysis of pooled results from six prospective cohort studies found no relationship between dietary β-carotene and the reduction of lung cancer risk.[211] A systematic review of prospective studies concluded that dietary intake of total carotenoids did not significantly reduce the risk of lung cancer.[212] Supplementation has been studied in large RCTs. The two studies (the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial [ATBC] and the Beta-Carotene and Retinol Efficacy Trial [CARET]) reported an increased risk of lung cancer in high-risk groups supplemented with 30 mg β-carotene/day.[213] In contrast, the Physicians' Health Study (only 11% smokers) found no effect with β-carotene supplements at a level of 50 mg every other day.[214]

Prostate Cancer. A meta-analysis of 15 case-control studies showed an inverse relationship between blood levels of lycopene and the risk of advanced-stage cancer. There was no risk association in nonaggressive disease.[215] Dietary lycopene intake was not related to prostate cancer risk in a large prospective study.[195] The question remains whether consumption of lycopene-rich foods can lower the risk of prostatic cancer.

Breast Cancer. An inverse relationship between the consumption of carotenoid-rich fruits and vegetables and the risk of breast cancer was found to be strongest among premenopausal women.[216] For women who were smokers and did not use supplements, dietary α-carotene and β-carotene intake was inversely associated with the risk of breast cancer.[217] Pooled analysis of 14 case-control studies reported a reduction in breast cancer risk with increased blood levels of total carotenoids, α-carotene, and lutein.[218] In contrast, the Women's Health Study found no link between lycopene intake and a reduced risk of breast cancer.

Cardiovascular Disease. Although several prospective studies found that higher intake of carotenoid-rich foods correlated with reduced risk of CVD, others did not.[145] Four RCTs found no effect of oral β-carotene, ranging from 20 to 50 mg/day, in preventing CVD.[214] Thus, although diets rich in β-carotene are often linked to reduced risk of cardiovascular disease, the benefits may derive from other constituents in fruits and vegetables.

Adult Macular Degeneration. The xanthophylls lutein and zeaxanthin are the only carotenoids in the retina and macula, where they filter blue light. Cross-sectional and retrospective case-control studies suggest that increased consumption of carotenoids, especially lutein and zeaxanthin, is linked with a lower risk of advanced age-related macular degeneration (AMD).[219] Yet another study of more than 4000 subjects found no evidence that β-carotene supplementation prevented or delayed AMD with intermediate risk of adult macular degeneration or with advanced AMD in one eye when supplemented with lutein (10 mg/day) and zeaxanthin (2 mg/day) in combination with vitamin C, vitamin E, β-carotene, and zinc according to the Eye Disease Study (AREDS-2) protocol.[220] Supplementation did not slow the progression to advanced AMD. Research extending this study to various combinations of carotenoids suggests that lutein and zeaxanthin can reduce disease progression compared with β-carotene alone.

Carotenoids: Cell Response Modifiers? The interpretation of the effects of β-carotene and other provitamin A carotenoids is complicated by their potential formation of retinoids, which are well-known regulatory species. Although they are effective antioxidants, non–provitamin A carotenoids (lycopene, lutein, canthaxanthin, and astaxanthin) do not present this complication. Much of lycopene's activity relates to its antioxidant actions; however, its metabolites could provide regulatory effects.[221] The nonprovitamin A carotenoids may affect cancer cells through increased apoptosis, decreased cell-cycle progression, or decreased production of cytokines. Alternatively, lycopene's inhibition of cancer cell proliferation may involve increased gap-junction–mediated intercellular communication.[222]

Coenzyme Q_{10} (Ubiquinone)

Ubiquinones contain side chains with isoprene units, and the predominant form in humans is ubiquinone 10 (CoQ_{10}). It functions as an essential electron carrier in mitochondrial ATP production. As the only lipophilic antioxidant synthesized in the body, CoQ_{10} also stabilizes membranes, and it helps recycle α-tocopherol.[223] Ubiquinol, the reduced form of CoQ_{10}, protects LDL-C against lipid peroxidation.[224] CoQ_{10} synthesis requires vitamins B_2, B_6, B_{12}, and folate, and synthesis may be suboptimal in people with a low intake of these micronutrients.

Immunity. CoQ_{10} may enhance the immune system. Supplementation of healthy volunteers with CoQ_{10} was found to increase white blood cell levels and decrease lymphocyte DNA damage (3 mg/kg daily for 12 weeks).[225]

Cardiovascular Disease. In an RCT of patients at risk for CVD, 6-month treatment with a combination of vitamins C and E, CoQ_{10}, and selenium increased arterial elasticity, lowered glycosylated hemoglobin, and increased high-density lipoprotein cholesterol (HDL-C) compared with controls.[226] CoQ_{10} has been used with conventional therapy in the treatment of congestive heart failure. Studies employing doses ranging from 100 to 200 mg/day for up to 3 months reported minor improvements.[227] Drugs such as β-blockers and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors lower serum levels of CoQ_{10}.[228] However, the necessity of CoQ_{10} supplementation in these situations remains uncertain. A meta-analysis of 14 RCTs of various CoQ_{10} protocols suggests an improvement in exercise capacity, with a significant difference in left heart ejection as the end point.[229] Conversely, an RCT of 420 patients with cardiac failure receiving standard therapy and 300 mg of CoQ_{10} daily for 2 years found that CoQ_{10} significantly reduced major adverse cardiovascular events.[230]

Thiols in Redox Balance

Alpha-Lipoic Acid

As a coenzyme of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, α-lipoic acid (ALA) is required in the mitochondrial catabolism of carbohydrates and fatty acids. ALA also scavenges hydroxyl radicals, peroxynitrite, superoxide, and peroxyl radicals, and it can regenerate endogenous antioxidants, including ascorbic acid and GSH.

Diabetes. A meta-analysis of four RCTs with intravenous ALA of 600 mg/day indicated a significant improvement in diabetic neuropathy.[231] Whether oral ALA supplementation offers benefits is less clear. The highest tissue level of lipoic acid likely attainable from oral doses is less than 10% of other intracellular antioxidants such as GSH.[232] The effect of lipoic acid supplementation on long-term glycemic control remains uncertain. For example, a controlled study of 72 patients with type 2 diabetes reported that oral supplementation with 600 mg ALA daily for 4 weeks improved insulin sensitivity by 25%.[233]

Aging and Neurological Disease. Animal studies suggest that high-dose ALA and/or acetyl-L-carnitine can improve brain function,[234] reduce brain lipid peroxidation, and increase brain antioxidant enzyme activities in the brains of aged rats,[235] especially in the hippocampus.[236] Aged mice supplemented orally with ALA had increased glucose tolerance, energy expenditure, and skeletal muscle mitochondria biogenesis, but they also had increased loss of lean body mass.[237] It is not clear that these results can be extrapolated clinically. Patients with varying stages of Alzheimer's disease administered 600 mg/day of ALA for 48 months showed slowed disease progression in patients with mild, not moderate, dementia.[238]

Cardiovascular Disease. ALA supplementation was used in mouse models of ischemia-reperfusion and atherosclerosis, where it slowed the formation of atherosclerotic lesions.[239] In a rat model of diabetes, intraperitoneal ALA reduced cardiac apoptosis and caspase expression and enhanced MnSOD activity and GSH levels.[240] Feeding high-dose ALA reduced myocardial oxidant production to levels observed in healthy young rats.[241] Limited research has focused on the question of oral administration of ALA to improve vascular function in diabetes. As an example, supplementation of patients with diabetic neuropathy with 300 mg ALA/day for 4 weeks improved flow-mediated vasodilation.[242]

Obesity. Preliminary data for metabolic syndrome are suggestive. A study of 1127 of obese and preobese subjects supplemented for 4 months with ALA 800 mg/day reported a significant reduction in weight, blood pressure, body mass index, and abdominal circumference.[243]

Alpha-Lipoic Acid: A Cell Response Modifier? ALA can induce several Phase II antioxidant and thiol-protective enzymes, including enzymes needed for GSH synthesis. Old rats treated with ALA intraperitoneally were found to have increased γ-glutamylcysteine ligase (GGL) activity, reflecting induced Nrf2 binding to ARE and increased transcriptional levels of GGL subunits, thus reversing the age-related losses.[244] ALA activated protein kinase B (Akt), increasing the survival of cultured neuronal cells exposed to oxidative stress.[245]

N-ACETYLCYSTEINE AND GLUTATHIONE

As a supplement, *N*-acetylcysteine is an effective precursor for GSH. GSH synthesis is regulated in part by cysteine availability. It is deacetylated in the gut to free cysteine, and oral supplementation can efficiently raise intracellular GSH[246] in model systems and in healthy individuals.[247] *N*-acetylcysteine may play a supportive role in improving GSH status and maintaining cell redox balance, countering ROS, and attenuating drug toxicity as a conjugating agent in drug metabolism.[248] Orally administered GSH may increase intestinal GSH levels via a GSH transport system.[249]

Oxidative Stress. Supplementation of healthy, trained men with *N*-acetylcysteine (1800 mg/day for 3 days before exhaustive resistance exercise) significantly increased plasma thiols and TAC and reduced lipid peroxidation and protein oxidation.[250] An RCT of hypertensive patients with type 2 diabetes reported that 6-month treatment with *N*-acetylcysteine and arginine significantly reduced systolic blood pressure and lowered levels of oxidized LDL-C, C-reactive protein, VCAM-1, and protein oxidation (plasma nitrotyrosine), suggesting improved endothelial function, along with reduced oxidative stress.[251]

To examine the role of glutathione and Nrf2-dependent detoxification in age-related sensitivity to redox cycling, hepatocytes from young and old mice were studied. Exposure to xenobiotic insult led to a marked decline in GPx4 and GSH and a concomitant increase in lipid peroxidation. These differences were accentuated in hepatocytes from old mice. Pretreatment with *N*-acetylcysteine reduced cell death by more than twofold. These results suggest that *N*-acetylcysteine might help maintain glutathione levels and attenuate age-related decline in Nrf2–GSH dependent detoxification.[252]

MINERALS AND REDOX BALANCE

Magnesium

As a cofactor for nearly all ATP-requiring reactions, magnesium is integral to intermediary metabolism. Low magnesium status is linked to inflammation and activation of the systemic stress response. Subclinical magnesium deficiency is common in the United States, and magnesium deficiency is correlated with chronic diseases associated with aging.[253]

In rodents, low magnesium status caused the release of substance P, leading to increased circulating inflammatory cells, cytokines, and ROS, with depletion of antioxidants associated with cardiomyopathy.[254] Furthermore, magnesium deficiency promoted senescence in primary human fibroblasts.[255] Experimental magnesium deficiency created systemic inflammation with leukocyte and macrophage activation, inflammatory cytokine release, the formation of acute-phase proteins, and oxidative stress. A study of young male athletes and sedentary controls suggested that supplementation with 500 mg magnesium daily for 28 days reduced DNA oxidation in peripheral blood lymphocytes.[256] Possible mechanisms include opening calcium channels and activation of NF-κB.[257] High fructose intake, for example, from excessive consumption of high-fructose corn syrup, coupled with low magnesium status could set the stage for oxidative stress, insulin resistance, and metabolic syndrome in susceptible individuals.[258] A meta-analysis concluded that dietary magnesium intake is inversely related to the prevalence of metabolic syndrome.[259]

ZINC

Beyond its role in SOD, zinc is required by many transcription factors, including tumor-suppression protein p53. "Zinc fingers"—domains that bind multiple zinc atoms—stabilize these proteins. In addition to stabilizing membranes, zinc is involved in apoptosis, hormone release, and nerve transmission.[260] Given the high prevalence of zinc deficiency, subclinical zinc deficiency manifests as inflammation and oxidative stress, and it often accompanies aging.[261] That zinc deficiency diminishes immune function, especially Th1 activity, is well established. Zinc is essential for IL-2–mediated T-cell activation. The mechanism seems to rely on the activation of NF-κB signaling, resulting in increased expression of cytokines as TNF-α, IL-1B, IL-2, and IL-6.[262] The ability of zinc supplementation to influence cytokine production is consistently reported. However, attenuation or potentiation seems to be dose dependent. Thus daily supplementation with 45 mg or more

zinc may decrease in vivo–generated proinflammatory cytokine production in stimulated mononuclear cells.[263] Reduced cytokine production and T-cell activation was reported for elderly men and women supplemented with less than 20 mg zinc/day.[264]

METABOLITES AS SECONDARY ANTIOXIDANTS

L-Carnitine

L-Carnitine is synthesized mainly by the liver and kidney, then transported to tissues using fatty acids and carbohydrate as fuel. Fatty acid transport into mitochondria relies on a carnitine shuttle for β-oxidation. A decline in carnitine correlates with the age-related decline in mitochondrial energy production and increased ROS.[265] After demonstrations that supplementation with acetyl-L-carnitine reversed age-related decline in tissue carnitine levels in rats, later experiments showed that animals supplemented with high doses of ALA and acetyl-L-carnitine for 1 month exhibited improved substrate binding of brain carnitine acetyltransferase,[234,266] improved memory,[267] and decreased DNA damage.[268] Whether such improvements with high doses of acetyl-L-carnitine translate into long-term benefits in preventing age-related declines in memory and energy for patients remains to be investigated. Supplementation with 1000 mg/day for 12 weeks in patients with coronary artery disease increased HDL-C and Apo-A1 levels.[269] In a related study, this protocol led to reduced lipid peroxidation and increased levels of antioxidant enzymes (CAT, SOD, and GPx), suggesting a potential increase in antioxidant defenses with *N*-acetyl-carnitine supplements.[270]

URIC ACID (URATE)

Uric acid is a waste product of purine metabolism that occurs in high levels in plasma. Urate is a broad-spectrum antioxidant capable of scavenging free radicals, and it can chelate transition metals.[271] Uric acid is responsible for 21% to 34% of the total plasma antioxidant activity, in which it appears to protect α-tocopherol from peroxyl radicals.[272] Also, assays of TAC indicated that 49% of the TAC of human plasma is due to uric acid.[273] Although a ubiquitous antioxidant, elevated urate is not necessarily beneficial.

MELATONIN: A HORMONE WITH ANTIOXIDANT POTENTIAL

In addition to helping set the circadian rhythm and sleep patterns, melatonin acts as an antioxidant in vitro and in vivo.[274] In senescence-accelerated mice, long-term administration of physiological levels of melatonin corrected hepatic mitochondrial dysfunction, suggesting that melatonin may reduce the oxidative damage associated with aging.[275] Melatonin can act by inhibiting the expression of eNOS in laboratory animals.[276] It can also upregulate SOD and catalase in the brain and liver of rats exposed to carcinogens.[277] In an experimental model of diabetes, melatonin was shown to reduce lipid peroxidation and upregulate GSH reductase, GPx, and γ-glutamylcysteine synthetase.[278] As applied to oxidative stress in obesity, melatonin may play a role in benefiting calorie-restricted diets. In a small study, patients were supplemented with 10 mg melatonin daily or placebo for 30 days. After melatonin supplementation, levels of adiponectin and GPx increased, whereas lipid peroxidation was decreased. Furthermore, melatonin facilitated weight loss.[279] In keeping with its putative role as an anti-inflammatory agent, melatonin has a protective effect in the activation of the NLRP3 inflammasome, which responds to environmental agents, including pathogens, as well as to endogenous danger signals such as mitochondria-derived ROS.[280]

NONNUTRITIVE ANTIOXIDANTS

Polyphenols

Phytochemical antioxidants include polyphenols, which are composed of phenolic units. They are classified according to the number of ring structures as flavonoids, stilbenes, lignans, and phenolic acids. The persistent interest in polyphenols is due to their probable contribution to the health benefits of fruit- and vegetable-rich diets.

FLAVONOIDS

Flavonoids (bioflavonoids) represent one of the largest classes of polyphenols, and more than 5000 different flavonoids have been identified. They fall into 12 major subclasses, differing according to their antioxidant properties, physiological activities, and bioavailability.[281]

Dietary Patterns. For typical Western diets, the estimated daily consumption of total flavonoids is in the range of 100 to 200 mg,[282] although intake reflects cultural and even regional preferences. As examples, simple phenolic acids (caffeic acid, ferulic acid, gallic acid, and coumaric acids) accounted for 75% of total phenolic intake among Finnish adults, whereas flavonoids accounted for 24% of the total.[283] In an adult Spanish population, the mean intake of total flavonoids was shown to be 313 mg/day, with proanthocyanidins accounting for 60% of the total.[284]

Flavonoid Bioavailability/Biotransformation. The uptake of individual flavonoids including polymers such as proanthocyanidins is generally quite limited. Most flavonoids have very low water solubility. Phosphatidylcholine complexes of flavonoids may improve uptake.[285] Absorbed aglycone flavonoids are often conjugated (sulfates, glucuronides) or methylated by intestinal and liver systems, then excreted. Such flavonoid derivatives may have different biological effects than parent compounds. Additionally, colonic bacteria can degrade polyphenols by ring session, and absorbed monophenols often act differently than parent compounds. Enterohepatic and enteric recycling of flavonoid metabolites is another complication.[286] Analysis of the plasma and urine of healthy volunteers 2 hours after ingesting 200 mg of quercetin or green tea flavonoids failed to detect increases in any of 71 aromatic metabolites observable at 5 hours after ingestion, suggesting that flavonoid metabolites may not be responsible for rapid responses to ingested flavonoid-rich foods or beverages.[287]

Direct Antioxidant Activity. In vitro, various flavonoids scavenge a variety of radicals[288,289] and block LDL-C oxidation.[290] As chelators, flavonoids can inhibit spontaneous generation of hydroxyl radicals[291] catalyzed by copper and iron ions. Although data are conflicting, it appears that flavonoids and their derivatives probably make small contributions as direct antioxidants in vivo.[292] The observed increased antioxidant capacity in plasma after ingestion of fruits, fruit juice, and flavonoid-rich foods may be due to increased production of the antioxidant uric acid rather than due to flavonoids.[293,294] Peak plasma concentrations of flavonoids and their metabolites after acute ingestion are often less than 1 μmol/L, several orders of magnitude lower than plasma or intracellular concentrations of ascorbic acid, uric acid, and GSH.[295]

Effects on Cell-Signaling Pathways. Results of cell-culture studies and animal testing indicate that antiinflammation, antidiabetic, neuroprotective, and anticancer activities depend on modification of cell-signaling pathways. As examples, potential targets include the protein kinase/protein phosphatase families that ultimately regulate transcription factors. Additionally, flavonoids can alter growth-factor signaling by preventing receptor binding.

Flavonoids in Cancer Prevention. The different flavonoid subclasses provide multiple compounds with varying biological

activities. Therefore it is difficult to generalize the activities of specific flavonoids. Evidence for a relationship between flavonoids and cancer incidence is conflicting. Among healthy postmenopausal women, there was an inverse association between the incidence of lung cancer and the intake of flavanones and proanthocyanidins for current or past smokers—those with the highest intake of quercetin had a lower incidence of lung cancer—and in men, those with the highest intake of myricetin had a reduced risk of prostate cancer.[296] In contrast, among elderly Dutchmen, dietary catechins mainly from tea were not associated with a reduced risk of lung cancer or epithelial cancers.[297] In earlier studies, flavonoid consumption was not associated with reduced cancer risk in this population,[298] although it was inversely related to mortality due to coronary heart disease (CHD).[299]

Flavonoids can reduce inflammation by blocking the expression of inflammatory mediators. Screening flavonoid, flavonoid metabolites, and combinations of flavonoids plus metabolites according to their ability to inhibit induction of TNF-α by isolated human monocytes revealed that combinations of flavonoid metabolites can be more effective than individual flavonoids or individual metabolites used singly.[300] Using a coculture system of intestinal epithelial and endothelial cells, researchers demonstrated that cyanidin-o-glucoside, one of the most common flavonoids, inhibited inflammation signaling, including selective inhibition of the NF-κB pathway.[301]

Flavonoids in Cardiovascular Protection. Variable results have been obtained from studies of dietary flavonoids and antioxidants in medicinal plants and the risk of chronic disease, such as cardiovascular disease.[302] Whether increased consumption of flavonoid-rich foods can reduce the risk of CVD is not clear. For example, a study of postmenopausal American women found significant reductions in the risk of CVD and all-cause mortality with increased flavonoid intake.[303] Results of the Cancer Prevention Study II Nutrition Cohort suggested an inverse relationship between total dietary flavonoid intake and the risk of stroke and total CVD, especially for men.[304] In contrast, a study of U.S. women reported no significant relationship between consumption of flavanols and flavones and CHD.[305] There was no strong relationship between flavonoid intake and total CHD among American male health professionals.[306] Caution is needed in interpreting these prospective studies due to omissions, such as the failure to (1) include all flavonoid subclasses, (2) calculate flavonoid intake based on more complete databases for flavonoid content of foods, and (3) make adjustments for the overall quality of diets. Other dietary factors besides flavonoids (vitamins, fiber, other phytochemicals) contribute to potential bias.

Specific Antioxidant Mechanisms for Polyphenols in Vivo? Interpretation of polyphenolic "antioxidant effects" is complicated by several factors. Most data regarding polyphenol activities come from cultured cells and tissues and often test concentrations of individual flavonoids that far exceed those found in vivo. Flavonoids such as quercetin and green tea catechins rapidly produce H_2O_2 as an artifact under typical cell-culture conditions, thereby complicating interpretation. The identity of the polyphenolic species affecting cells and tissues is uncertain due to their biotransformation. Localized concentrations of active species in vivo are also uncertain.

Depending on the experimental design, polyphenols can inhibit COX, lipooxygenase, telomerase, xanthine oxidase, cytochrome P450, and protein glycation, as well as modulate drug transport systems and platelet function. Flavonoids may influence multiple signaling pathways, making it difficult to assign specific actions. As an example, red wine polyphenols administered to rats for 16 weeks inhibited chemically induced colon cancer.[307] In the process, responses to oxidative stress were downregulated. Microarray analysis of cellular changes after polyphenol treatment for 14 days revealed the following frequencies of downregulated genes: receptors and signal transduction, 22%; transport and binding proteins, 14%; inflammatory and immune response, 11%; metabolic enzymes, 4%; gene expression/control, 4%; xenobiotic metabolism, 3%; and cell-cycle regulation, 3%. In the final analysis, the observed protective effects of flavonoid-rich foods in humans may be independent of flavonoids.

Gastrointestinal Tract as Primary Target for Polyphenols? The gastrointestinal (GI) tract is continuously exposed to ROS and RNS from multiple sources. The gut epithelium may not adapt to long-term oxidative stress, and because of low initial levels of defensive enzymes, it may be susceptible to oxidative damage with even moderate inflammation.[308] After ingestion of flavonoid-rich foods, phenolic compounds are present in the stomach and intestinal lumen at much higher concentrations than those found in plasma.[309] Of particular interest are inflammatory bowel disease, gastric ulcers, and liver disease.[310,311] ROS are present at high levels in the colons of patients with ulcerative colitis.[312] Green tea polyphenols can reduce inflammation in models of inflammatory bowel disease.[313]

Polyphenolic by-products of intestinal metabolism, such as hydroxybenzoic acid and protocatechuic acid, exhibit important properties. As an example, they can induce apoptosis in gastric cancer cells.[314] Bioactive metabolites from dietary polyphenols can affect both the commensal gut microbiome and the host immune system. Animal studies implicate direct interaction with the gut epithelium as a prerequisite. In a rodent model of chemically induced colitis, oral administration of apple polyphenols downregulated intracellular signal cascades in the gut epithelium and infiltrated neutrophils.[315] This effect was not observed with polyphenols during peritoneal administration.

FLAVONOIDS FROM FOOD SOURCES

Soy

Studies with vascular cells suggest that soy isoflavones can increase eNOS activity[316] while activating the redox-regulated Nfr2–Keap1 signal pathway to increase the transcription of Phase II enzymes and antioxidant enzymes.[317]

Cardiovascular Disease. Some epidemiological studies correlated soy, especially soy protein, with a reduced risk of CHD.[318] Several RCTs reported cardiovascular benefits with improved systemic arterial function after a meal enriched with soy isoflavones.[319] In an RCT, supplementation of postmenopausal women with genistein for up to 36 months decreased homocysteine and fasting glucose.[320] However, the results were inconsistent.

Cancer. A meta-analysis of soy isoflavone consumption and risk of breast cancer observed a protective effect only among studies of Asian populations.[321] This analysis failed to find a dose–response relationship between total isoflavone consumption and breast cancer incidence. Interventional studies using high doses of soy isoflavones have found altered breast fluid, a predictor of breast cancer risk.[322]

Soy isoflavones can suppress tumors in animal models. Genistein can induce apoptosis via downregulation of Bcl-2 and Bcl-XL, anti-apoptotic proteins in human hepatoma cell lines, as well as in breast, prostate, pancreatic, and non–small-cell lung cancer cells.[323] Genistein also inhibits activation of the NF-κB and Akt signaling pathways that normally balance cell survival and apoptosis.[324] A meta-analysis suggested a significant association between the consumption of soy-based foods and a reduction in the risk of prostate cancer.[325] Nonetheless, epidemiological studies on the relationship between a reduced risk of breast cancer and soy intake have yet to provide definitive evidence for taking soy supplements.

BERRIES

Berries, currants, grapes, and certain tropical fruits and vegetables contain high levels of polyphenols, including flavanols, flavan-3-ols, tannins, and hydroxycinnamates and anthocyanins. Cyanidin-3-glucoside is the most prevalent anthocyanidin. Cell-culture studies, animal models, and limited clinical trials suggest anthocyanins may help prevent age-related deterioration and disease, including CVD and neurological disease.[326]

Cardiovascular Disease. Small-scale RCTs suggest that short-term consumption of blueberries, strawberries, cranberries, and other berries can improve several cardiovascular parameters, such as blood pressure, endothelial function, and serum lipids. A review of clinical trials using grape juice found an inverse relationship between dose and flow-mediated dilation.[327] In another study, healthy volunteers (50–70 years) consumed a mixed-berry beverage (blueberries, black-currant, elderberry, lingonberries, strawberries, and tomatoes [total polyphenols = 295 mg]) or control (polyphenol-free) beverage daily for 5 weeks. The berry intervention significantly reduced LDL-C and blood glucose levels compared with controls. Test subjects with the berry beverage also performed better on a test of working memory.[328]

Wine contains abundant polyphenols, and drinking red wine can increase the antioxidant capacity of serum.[329,330] A meta-analysis of 133 RCTs studying the effects of flavonoids and flavonoid-rich foods suggests that red wine consumption did not improve flow-mediated dilation, alter blood pressure, or change LDL-C levels.[318] Results for grape juice consumption have also been mixed. Overweight volunteers who consumed 480 mL/day of grape juice for 12 weeks did not have improved plasma antioxidant capacity or lipid profiles.[331]

Cancer. Black raspberries inhibited induced esophageal carcinogenesis in a rodent model, apparently by suppressing oxidative stress via NF-κB/MAPK signaling.[332] Analysis of the Nurses' Health Study and the Health Professionals Follow-Up Study found no association between the risk of colorectal cancer and consumption of flavonoid classes in tea, blueberries, and oranges.[333] Another meta-analysis found no significant association between high flavonoid consumption and the risk of lung cancer.[334]

Diabetes. Several intervention studies suggest beneficial effects of berries and fruits for patients with diabetes as well as for at-risk individuals. As an example, an 8-week RCT found that treating healthy obese or overweight volunteers with 2 g grape polyphenols daily for 8 weeks prevented a fructose-induced increase in oxidative stress and insulin resistance.[335]

Patients with diabetes supplemented with cherry extracts providing 600 mg anthocyanins/day for 6 weeks experienced reduced levels of HbA1c and triglycerides.[336] Compared with placebo, ingestion of 320 mg anthocyanins daily for 24 weeks improved lipid profiles, decreased markers of oxidative stress, and improved TAC while reducing insulin resistance in patients with type 2 diabetes.[337]

Neurodegeneration. Regular consumption of berries may help preserve cognitive health during aging. Analysis of the Nurses' Health Study concluded that a high intake of blueberries and strawberries could delay cognitive aging by approximately 2.5 years.[338] Furthermore, increased consumption of total flavonoids as well as anthocyanidins correlated with reduced rates of cognitive decline.

Flavonoids may promote neurogenesis and synaptic growth for memory-related regions such as the hippocampus, via increased production of neurotrophins.[339] As antioxidants, they may provide protection for inflammatory mediators due to activated microglia or hypertrophic astrocytes. Additionally, flavonoids can increase NO production to augment cerebral blood flow.

Blueberries have been implicated in the preservation of cognition and brain health. Anthocyanins or their metabolites seem to cross the blood–brain barrier. Supplementation of rodents with blueberry preparations reduced NF-κB (indicator of oxidative stress), augmented the cyclic adenosine monophosphate (AMP) response element-binding protein (central to neuronal growth and maintenance), and increased the production of brain-derived nerve factor, all of which correlated with enhanced memory.[340] An RCT indicated that blueberry supplementation can activate blood oxygen-dependent signaling in specific brain regions in older adults with mild cognitive impairment.[341] Clinical trials with berries and anthocyanins are limited, and carefully designed studies of larger groups are needed.

COCOA

Cocoa is a source of flavan-3-ols, especially (-)–epicatechin and procyanidins. Their relationship to oxidative stress and TNF-α–mediated signaling have been studied.

Cardiovascular Disease. Cocoa consumption is linked to a reduced incidence of CVD in the elderly population, and it is associated with increased peripheral blood flow.[342] Overweight adults who ingested cocoa beverages experienced significantly improved flow-mediated dilation in a randomized trial.[343] Furthermore, administration of 40 g/day cocoa powder for 4 weeks significantly reduced ICAM-1 and P-selectin in high-risk patients with CVD.[344] An RCT of healthy male adults observed short-term benefits of cocoa-flavored consumption, as assessed by increased HDL-C, lower LDL-C, and reduced blood pressure.[345] A meta-analysis of 18 RCTs found that consumption of cocoa beverages and chocolate bars for up to 18 months significantly increased flow-mediated dilation.[346]

In terms of mechanisms, (-)–epicatechin elevates NO in endothelial cells via inhibitors of NADPH-oxidase. Among human cohorts, cocoa and cocoa flavonols reduced markers of inflammation, including decreased IL-1B, VCAM-1, IL-10, and ICAM, in hypertensive adults.[347]

Neuroprotection. Cocoa flavonoids have been associated with cognitive improvements in healthy elderly adults. The Cocoa, Cognition and Aging Study examined this question in detail. Older adults consuming an intermediate dosage of cocoa flavanols (520 mg/day) or a high dosage (993 mg/day) for 8 weeks showed improved cognitive function.[348] Nonetheless, negative results on cognition from cocoa supplementation have also been reported. Possible mechanisms include improved glucose metabolism, insulin sensitivity, improvements in endothelial function, and improved cerebral blood flow.[349]

Diabetes. Results of epidemiological studies of dietary intake of flavanols from various sources have been inconsistent. Even so, a meta-analysis of RCTs concluded that moderate consumption of chocolate (up to 6 servings/week) is associated with reduced risk of CVD, stroke, and type 2 diabetes.[350] As an example of an intervention trial, daily consumption of 100 g of dark chocolate bars (147 mg catechins/day) improved insulin sensitivity and reduced blood pressure in glucose-intolerant hypertensive patients.[351] Cell-culture studies to define the antidiabetic mechanisms of flavonoids can improve redox states and increase GPx and Gr via Nfr2 signaling, yielding increased reductions in GSH.

TEA

The health benefits of tea are likely related to polyphenolic content. The total flavonoid content in green tea and black tea is 138 mg and 118 mg per 100 mL, respectively. Flavanol-3-ols (catechins) are a major class of

tea flavonoids, represented by epigallocatechin, epigallocatechin-3 gallate (EGCG), epicatechin-3 gallate (ECG), and (-)–epicatechin (EC). Tea flavonoids comprise 30% to 40% by weight in brewed tea extract. Increased consumption of flavon-3-ols in U.S. adults correlated with reduced proinflammatory cytokines and oxidative stress, although tea consumption per se did not correlate with a composite inflammation score.[352] Theaflavins are additional derivatives found in black tea. They are formed from green tea catechins by oxidation and fermentation. These condensation products are poorly absorbed; consequently, systemic effects probably reflect the actions of microbial metabolites.

Cancer. Tea flavonoids may block signal transduction pathways and induce apoptosis in various tumor cells, including skin, prostate, colon, breast, and lung. EGCG blocked the growth of human cervical cancer cells via cell-cycle arrest and induced apoptosis.[517] It did so by inhibiting tyrosine kinases, thus blocking MAPK signaling pathways and NF-κB and AP-1.[518] Green tea catechins can limit DNA oxidation, lipid peroxidation, and free radical production in smokers.[519]

Analysis of nearly 200 epidemiological studies examined associations between green tea consumption and risks of cancer at different sites and found that the majority of reports did not show a preventive effect. Inhibition with green tea consumption was more frequent among Asian populations, according to case-control studies.[353,354] However, tea consumption may help specific subgroups, such as lowering the risk of breast cancer only among women with low catechol-*O*-methyl transferase.[355]

Intervention studies are a more effective way to establish the protective effects of tea on cancer risk. In a double-blind study, patients at risk for prostate cancer treated with green tea catechins 600 mg/day for 1 year had a cancer rate of 3% compared with a 30% rate for the placebo group.[356] A prospective study of patients after resection for colon cancer or polypectomy employed a daily dose of EGCG 20 mg and apigenin 20 mg for up to 4 years. The recurrence rate of neoplasia was 7% for treated patients versus 47% for untreated patients.[357]

Cardiovascular disease. In apolipoprotein-E gene knockout mice, an animal model of atherosclerosis, theaflavin with quercetin reduced the formation of atherosclerotic lesions and vascular superoxide and ILB-4 while increasing vascular endothelial NOS activity, NO production, and vasodilation.[358] A meta-analysis of seven cohort and case-control studies reported an 11% decrease in the risk of myocardial infarction with daily consumption of three cups of tea.[359] A later meta-analysis of nine observational studies suggested that consumption of the equivalent of three cups of green or black tea daily was associated with a significantly reduced risk of ischemic stroke.[360]

Diabetes. A meta-analysis of RCTs regarding the possible health effects of green tea paints a conflicting picture. Analysis of seven trials with patients with diabetes and prediabetes found no effect of green tea or green tea extracts on fasting blood glucose and insulin levels or on glycemic control (as glycated hemoglobin, HbA1c, or insulin sensitivity).[361] Another meta-analysis found that green tea extract given to individuals with prediabetes, individuals with diabetes, or overweight individuals for 4 to 6 weeks improved fasting glucose and HbA1c levels, especially with high doses of catechins.[362]

BOTANICAL EXTRACTS AND ISOLATED FLAVONOIDS

Pine Bark Extract

Pine bark extract (PBE) represents a complex mixture of polyphenols enriched in procyanidolic oligomers (PCOs), containing two to seven monomers of catechin and epicatechin. PCOs are effective antioxidants in vitro and can protect and/or regenerate α-tocopherol and vitamin C. PBE can upregulate antioxidative enzymes in cultured cells[363] and also protect rodents against hepatotoxicity and oxidative stress.[364]

Inflammation. PBE can inhibit the production of ROS by macrophages blocking the expression of proinflammatory cytokines, such as IL-1β.[365] Plasma from subjects treated with PBE can block NF-κB activation and the production of matrix metalloproteinases in human monocytes.[366] Rodents fed diets supplemented with PBE had increased plasma antioxidant capacity and reduced lipid peroxidation (thiobarbituric acid reactive substances).[367] Plasma taken from healthy subjects 30 minutes after ingestion of a single dose of 300 mg of PBE had inhibited COX-1 and COX-2 in human monocytes.[368] These observations suggest that PCOs may be useful in reducing inflammation; standardized PBE has been used to treat capillary dysfunction in patients with diabetes, venous abnormalities,[369] and knee osteoarthritis.[370] As an example, the San Valentino Osteoarthritis Study found that a standardized PBE (Pycnogenol) reduced pain and stiffness and improved the flexibility of osteoarthritic joints.[371]

GRAPE SEED EXTRACT

Wine flavonoids including PCOs are effective antioxidants in vitro. They can reduce lipid peroxides and decrease the susceptibility of isolated LDL-C to oxidation. Grape seed extract (GSE) has been employed as a source of monomeric and oligomeric favon-3-ols.

Oxidative Stress. GSE treatment led to a significant postprandial reduction in plasma lipid hydroperoxides and increased antioxidant capacity in healthy volunteers.[372] Male smokers administered GSE had significantly decreased platelet reactivity compared with control subjects given a placebo.[373] Healthy volunteers consuming 430 mg GSE showed a 33% improvement in redox states 24 hours after ingestion.[374]

Diabetes. Several studies suggest that GSE may benefit patients with diabetes. Patients with type 2 diabetes received GSE 600 mg/day according to a controlled double-blind protocol. After 4 weeks, significant decreases were observed for plasma fructosamine and high-sensitivity C-reactive protein, and significant increases were seen for whole-blood GSH compared with placebo.[375] A meta-analysis of 16 RCTs concluded that GSE is associated with beneficial effects on blood pressure, especially in obese subjects and patients with metabolic disorders.[376]

Neurological Disease. Animal experiments indicate that GSE can limit lipid peroxidation in the brain.[377] GSE polyphenols have been shown to inhibit protofibril formation and protofiber oligomerization in a mouse model of Alzheimer's disease.[378]

Chemoprevention. GSE can inhibit the proliferation of a variety of cancer cell lines involving regulatory targets, such as MAPKs, NF-κB, and phosphoinositide 3-kinases (PI 3-kinases), and inhibit angiogenesis.[379] Furthermore, pretreatment with GSE protected rodents against experimentally induced hepatotoxicity, pulmonary toxicity, and doxorubicin-induced cardiotoxicity by suppressing oxidative stress.[377,380]

GINKGO BILOBA

A variety of evidence suggests the benefits of *Ginkgo biloba* extract for vascular insufficiency, cerebral insufficiency, and improved cognition. Most studies utilized a standardized extract, EGb 761.

Oxidative Stress. In part, the beneficial effects appear to be related to antioxidant and anti-inflammatory actions, and it is likely that active constituents, ginkgolides, and related flavonoids reduce oxidative stress.[381,382] Cell-culture studies suggest that EGb 761 can raise GSH levels and induce γ-glutamylcysteinyl synthetase, the rate-limiting enzyme in GSH synthesis.[383] Damage to human endothelial cells

exposed to oxidized LDL-C could be prevented by EGb 761.[384] Rats treated with EGb 761 had increased catalase and SOD activity and reduced lipid peroxidation in the hippocampus, striatum, and substantia nigra compared with controls.[385]

Aging and Dementia. Mice supplemented with EGb 761 also had less ROS-induced apoptosis in lymphocytes than untreated mice; this result was greater in old mice than in young animals.[386] In clinical studies, patients with type 2 diabetes treated with EGb 761 for 3 months were found to have reduced markers of lipid peroxidation in platelets, possibly via inhibition of the COX-1–mediated cascade.[387] Large randomized trials (the GEM Study and the Guid Age Study) found no benefits of EGb 761 supplementation for age-related cognitive decline.[388,389] A recent RCT of patients with vascular cognitive impairment found that EGb 761 treatment for 6 months slowed cognitive deterioration in only one of four neuropsychological tests.[390]

SILYMARIN

Silymarin, a flavonoid concentrate from milk thistle, and silibinin, a principal constituent of silymarin, can scavenge ROS and decrease inflammation.

Hepatoprotection. Silymarin reduced oxidative stress and prevented liver necrosis in mice exposed to toxic agents.[391] Silymarin blocked the production of TNF-α, interferon-γ, and IL-2 by stimulated peripheral blood mononuclear cells and T cells isolated from hepatitis C virus–infected and noninfected subjects.[295] Phase I to II trials of silymarin treatment have not demonstrated a consistent reduction in liver function tests and viral load or improved liver histology in patients with hepatitis C,[392] a conclusion supported by a systematic review and meta-analysis.[393] Another meta-analysis of silymarin in the treatment of nonalcoholic fatty liver disease found a significant reduction in only a single marker of liver status (aspartate transaminase and alanine transaminase).[394]

Diabetes. An open trial with patients with cirrhotic diabetes treated with 600 mg/day silymarin for 12 months reported a significant reduction in glucosuria, glycosylated hemoglobin, and malondialdehyde levels compared with controls.[395] Similar positive results were found in an RCT of treatment of patients with type 2 diabetes with 600 mg/day silymarin for 4 months.[396] A meta-analysis of 5 RCTs found that silymarin administration can lead to a significant reduction in fasting blood glucose levels and HbA1c levels but not in lipid profiles.[397]

Cancer. Androgen-responsive prostate cancer cells can be inhibited by silymarin. Using a transgenic mouse prostate cancer model, a dietary phosphatidylcholine–silibinin complex inhibited tumor growth and metastasis by reducing the expression of metalloproteinases and vascular endothelial growth factor and its receptor.[398]

QUERCETIN

Quercetin, a well-studied flavonol, acts as a strong antioxidant in vitro and possesses anti-inflammatory properties in cultured cells.

Inflammation. Experiments with guinea pig epidermal cells suggest that flavonoids, such as quercetin, can inhibit COX-2 and 5-lipoxygenase pathways to curtail production of inflammatory eicosanoids.[399] Flavonoids may reduce the expression of adhesion molecules needed to recruit white blood cells to arterial walls, a property unrelated to their antioxidant potential. Quercetin and kaempferol inhibited adhesion molecule expression induced by TNF-α in human aortic endothelial cells.[400] Preexposure of hepatocytes to flavonoids blocked this inhibitory effect, suggesting that liver biotransformation reduces the ability of flavonoids to influence endothelial cells. Quercetin supplementation of healthy volunteers with quercetin (500 or 1000 mg) together with vitamin C (125 or 250 mg) daily over 12 weeks significantly increased plasma quercetin without changing plasma oxidant capacity (FRAP and ORAC).[401]

Exercise-Induced Oxidative Stress. Stress induced by strenuous exercise increased plasma cortisone and lipid peroxides and decreased SOD, GPx, and brain catalase in rats. Treatment with quercetin significantly increased these activities while lowering plasma peroxides.[402] In contrast, long-term quercetin supplementation (1000 mg/day) in athletes did not protect against exercise-induced oxidative stress, although supplementation led to a modest improvement in maximal oxygen uptake in untrained individuals.[369,403]

Cardiovascular Disease. The Zutphen study reported an inverse relationship between the incidence of stroke and CAD and the consumption of flavonoids, particularly quercetin.[299] Short-term consumption of quercetin may improve CVD risk factors, including endothelial function, in healthy volunteers and patients with CAD.

In an RCT with a crossover design, healthy men who ingested 200 mg of quercetin experienced reduced levels of plasma endothelin-1 and increased levels of plasma nitrite and S-nitrosothiols, biomarkers of NO production.[404] Hypertensive patients supplemented with 730 mg quercetin/day had significantly reduced systolic blood pressure.[405]

Chemoprevention. Quercetin can induce apoptosis and stimulate the production of antioxidant enzymes, such as NADPH, and quinone oxidoreductase activity in human breast cancer cells.[401,406] In a study of induced lung carcinogenesis in mice, quercetin pretreatment effectively prevented losses of SOD, catalase, GPx, GSH S-transferase, and GSH reductase while limiting tumor formation.[407] Quercetin can increase the life span of tumor-bearing mice by up to fivefold through the activation of mitochondrial apoptosis.[408] A case-control study found an inverse relationship between the intake of quercetin-rich foods and lung cancer risk, especially for heavy smokers. This effect corresponded to up-regulation of GSH transferase isozymes and downregulation of cytochrome P450 isoforms.[409]

RESVERATROL

Grapes, blueberries, and peanuts contain resveratrol and methylated resveratrol, members of the stilbene family of polyphenols. Resveratrol can act as an antioxidant to scavenge free radicals and other antioxidants in vitro. It can also protect human erythrocytes against H_2O_2-induced lipid peroxidation.[410] Stilbenes are rapidly cleared, and ingestion of acute doses of resveratrol by healthy subjects achieves only nanomolar plasma concentrations, levels several orders of magnitude less than those used for in vitro or animal studies.

Cardiovascular Protection. Exposure to resveratrol correlated with decreased myocardial damage associated with ischemia-reperfusion and inhibition of LDL-C oxidation.[411] Possible mechanisms relating to anti-inflammatory effects include inhibition of COX and lipoxygenase and blocking the action of NK-κB and AP-1 in cultured cells.[412] A proteomic and immunoblot study revealed differential up-regulation of proteins related to energy metabolism in diabetic rat hearts compared with normal hearts.[413] Resveratrol may also promote vasodilation. Resveratrol was found to activate human platelet eNOS, increase NO production, and reduce NOx activity, thus lowering superoxide production.[414] It also increases NO production in vascular endothelial cells.[415]

Supplementation with a resveratrol-rich grape preparation was examined in an RCT involving patients at high risk for CVD. Consumption of 8 mg resveratrol/day for 6 months followed by 16 mg/day for another 6 months reduced levels of C-reactive protein (CRP), TNF-α, and

plasminogen activator 1, together with decreased oxidized lipoprotein levels.[416] Although preliminary human studies suggest that resveratrol may benefit cardiovascular health, it is doubtful that consuming up to 2 glasses of red wine daily can provide enough resveratrol to achieve this effect.

Diabetes. A study using experimentally induced diabetes in rats found that resveratrol reduced glucose levels compared with untreated animals. Cardioprotection of diabetic rats involved induction of eNOS, vascular endothelial growth factor, and heme oxygenase.[417] In humans, resveratrol has been linked to improved glucose and lipid metabolism. As an example, patients with type 2 diabetes were treated with 1000 mg of resveratrol daily for 45 days. Comparison with baseline and placebo measures in patients with type 2 diabetes revealed improved HbA1c levels and improved insulin sensitivity. HDL-C levels increased, and LDL-C levels decreased.[418]

Chemoprevention. In various cancer cell lines, resveratrol exposure can stimulate apoptosis and inhibit cell proliferation.[419] A study of rats fed resveratrol demonstrated that high-dose resveratrol suppressed a transgenic rat model of spontaneous prostate cancer.[420] Yet several animal studies have reported a lack of effect of oral treatment with resveratrol on lung and colon cancer.

Aging and Neuro-Protection. Resveratrol can increase longevity in a variety of organisms, including mice.[421] It may modulate transcription of the sirtuin gene family implicated in senescence, endothelial function, and angiogenesis. Sirt1, a deacetylase that silences transcription, can be induced by resveratrol in vitro. It is worth noting that low doses of resveratrol can improve cell survival by up-regulating anti-apoptotic and redox-regulating proteins, whereas high doses induce cell death by downregulating redox proteins and inducing production of apoptotic proteins.[422] Although increased cerebral blood flow in healthy young adults given an oral dose of resveratrol (250 mg) has been reported in several investigations, reproducible benefits on cognition in humans remain to be established.[423] Questions of bioavailability, long-term safety, effective dosage, and optimal duration of treatment have not yet been defined.[424] It is worth pointing out that the administration of a resveratrol preparation (SRT501) led to serious side effects, including kidney failure. Safe ways of administering high doses of resveratrol would seem imperative before forming conclusions about clinical benefits.[425]

CURCUMIN

Curcuminoids are bright yellow pigments found in turmeric and represent a family of lipophilic diketones. Curcumin, the principal curcuminoid, is poorly absorbed. Serum concentrations in the nanomolar to micromolar range can occur with a single oral dose of up to 8 g.[426] Curcumin possesses pleiotropic activities due to its ability to modify diverse signaling molecules, including proinflammatory cytokines, apoptotic proteins, NF-κB, cyclooxygenases, C-reactive protein, adhesion molecules, and more.

Inflammation. Curcumin can scavenge ROS and RNS in vitro. In cultured colonic cells, curcumin inhibited iNOS; blocked lipoxygenase, COX-2, and phospholipase A; and reduced the production of proinflammatory prostaglandins, thromboxanes, and leukotrienes by inhibiting NF-κB activation.[427] Consumption of 200 mg/day of curcuminoids decreased serum lipid peroxide levels and LDL-C lipid peroxidation in healthy subjects.[428]

Oral administration of emulsified curcumin to arthritic rats attenuated inflammation, implicating the gut–brain axis. Curcumin apparently corrected an imbalance between sympathetic and parasympathetic activity, increased vagus nerve excitability, and enhanced gut–brain acetylcholine synthesis.[429]

Clinical studies of curcumin on inflammatory conditions are preliminary. For example, an RCT of patients experiencing remission of ulcerative colitis examined high-dose curcumin supplementation in addition to standard treatment. After 6 months, 2 of 43 patients relapsed compared with 8 of 39 patients in a placebo group.[430]

Cancer. Curcumin can induce apoptosis and inhibit angiogenesis and the production of matrix metalloproteinases in several cancer cell lines. Oral curcumin administration inhibited a mouse model of familial adenomatous polyposis,[431] and it inhibited the growth of human tumors in xenotransplant animal models.

In clinical trials, curcumin has exhibited activity against multiple types of cancer. These studies demonstrated that curcumin is well tolerated at doses up to 8 g/day. As examples, treatment of patients with colorectal cancer with curcumin at a dose of 1.08 g/day for 30 days improved weight gain while reducing serum TNF-α and inducing p53 expression.[432] When patients with lung cancer who were smokers were treated with 1.5 g/day for 30 days, they had lowered urinary excretion of mutagens.[433] A Phase I trial of patients with advanced breast cancer treated with curcumin 6 g/day according to a rotation schedule demonstrated that combination with docetaxel was well tolerated.[434]

Neurodegeneration. In mouse models, curcumin can reduce Alzheimer's disease pathology. In vitro studies have shown that curcumin can block amyloid beta (Aβ) aggregation. Curcumin can cause major structural changes in amyloid aggregates and potentially reduce Aβ toxicity.[435] A mouse model of Alzheimer's disease treated with curcumin for 7 days reduced existing plaques and structural irregularities in dystrophic dendrites.[436] Of four clinical trials of curcumin supplementation for Alzheimer's disease, the two that have been reported found no significant differences in cognitive performance between treated and placebo groups.[437] Thus RCTs of patients with Alzheimer's disease that incorporated doses of curcumin ranging from 1 to 4 g per day for up to 6 months found no differences between curcumin and placebo.[438,439] Several studies of curcumin supplementation in Alzheimer's disease are ongoing or results have yet to be published.

THERAPEUTIC USE OF ANTIOXIDANTS

Immunodeficiency and Oxidative Stress

Adequate levels of proinflammatory cytokines help balance anti-inflammatory Th2-mediated responses; consequently, restoring balance is a logical therapeutic goal.[440,441] Supplementation with antioxidants, including vitamins C and E and carotenoids, along with selenium, copper, and zinc, may enhance aspects of innate and humoral immunity when used alone or in combination. These nutrients may improve lymphocyte proliferation, delayed hypersensitivity responses, and immune cell functions such as Th1 cytokine-mediated responses. However, an RCT of allergic individuals found no change in immune responses, serum antioxidant capacity, or markers of oxidative stress after 4-week supplementation with vitamins C and E, β-carotene, selenium, and zinc at levels several-fold higher than the recommended dietary allowances.[442]

A growing literature suggests that HIV infection is associated with oxidative stress. Sero-positive HIV patients were shown to have significantly reduced plasma TAC compared with healthy controls, a finding that correlated with significantly elevated lipid peroxidation and reduced levels of vitamins C and E, SOD, and oxidative stress, which may relate to HIV dementia.[443,444] A cross-sectional study demonstrated increased plasma lipid peroxide levels along with depleted GSH levels in HIV-infected individuals compared with healthy controls.[445] Low levels of reduced GSH could activate transcription factor NF-κB to increase transcription of the HIV genome.[446]

It is logical to propose that antioxidant treatment could benefit HIV-positive individuals, and polyphenols and other antioxidants can inhibit HIV infectivity of cells in vitro.[447] However, the results of clinical studies are mixed. An RCT found that oral supplementation with 1200 mg/day ALA for 10 weeks provided no benefit in treating HIV-related cognitive impairment.[448] A review of six RCTs concluded that β-carotene/vitamin A did not change overall mortality, morbidity, or CD4 and CD8 counts, whereas four RCTs of other micronutrients did find improved overall mortality.[449] Benefits of micronutrient supplementation in improving mortality among HIV-positive patients appears to involve correcting nutrient deficiencies.[450] As an added consideration, high levels of vitamin C or vitamin E can potentially interfere with indinavir therapy.[451]

EXERCISE AND OXIDATIVE STRESS

The health benefits of regular physical activity are well established. Physical inactivity is regarded as one of the most common risk factors for CVD. However, the exact mechanisms remain unclear. Some data suggest that long-term exercise increases antioxidant defenses as an adaptation. Clearly, effects vary depending on the type, intensity, frequency, and duration of exercise regimens, as well as individual fitness and medical history. Strenuous physical exercise increases ROS production and muscle fiber damage.

Regular physical activity promotes up-regulation of antioxidant defenses. Anti-inflammatory actions of exercise focus on skeletal muscle, with increased mitochondria biogenesis, increased IL-6 and IL-10, and decreased TNF-α; on adipose tissue, with decreased visceral fat, IL-6, and TNF-α and increased adiponectin; and on vasculature, with decreased NF-κB and VCAM-1.[452] The interplay of exercise-induced ROS and muscle redox-sensitive protein signaling seems to be linked to glucose homeostasis via stress-activated protein kinase and mitogen-activated protein kinase pathways.[453] Although vitamin C, vitamin E, ALA, L-carnitine, and flavonoids can attenuate certain signs and symptoms due to resistance training, they do not eliminate tissue injury.

The field is confusing because of differing markers of antioxidant status and differing training levels. Furthermore, many studies use blood levels of antioxidants and ROS-induced by-products as end points to assess antioxidant status after exercise, a practice that contributes to variable test results. It is questionable whether these end points represent tissue-level changes.

The benefits of micronutrient supplementation are most often seen in untrained subjects, rather than in athletes, especially when there are nutrient deficiencies. For example, supplementing with antioxidant vitamins such as E, C, and CoQ_{10} may decrease exercise-induced oxidative damage in untrained individuals, especially in older people.[454]

In resistance-trained men, supplementation with 1000 mg/day of vitamin C and 378 mg/day of mixed tocopherols or placebo for 14 days did not change markers of muscle injury or oxidative stress (blood protein carbonyls and peroxides).[455] Nonetheless, optimal doses of putative beneficial antioxidants and pretreatment intervals are unknown.[456] Currently, there does not appear to be enough clinical evidence for recommending antioxidant supplements for well-trained and well-nourished athletes.[457,458] Antioxidant supplementation may actually be counterproductive. As an example, an RCT of kayakers given standard antioxidants (vitamin C 400 mg, α-tocopherol 272 mg, β-carotene 30 mg, lutein 2 mg, selenium 400 mcg, zinc 30 mg, magnesium 600 mg) daily for 4 weeks found that supplementation failed to lower lipid peroxidation induced by strenuous exercise, and it reduced muscle recovery times.[459]

Do Antioxidants Explain the Health Benefits of Plant-Based Foods?

Consumption of plant-based foods rich in antioxidants was reported to decrease oxidative damage in humans by some authors,[309] although others have found little effect.[460] Analysis of data from the EPIC study found that eating five portions of fruits and vegetables daily lowered the overall risk of cancer by 9%, far less than expected.[461] Nonetheless, previous studies concluded that five servings could reduce the risk of stroke; of cancers of the oral cavity, bowel, and lung; and obesity.

Do antioxidants account for such benefits? Results of assays of antioxidant activity and assessments of oxidative stress reduction are suggestive, but as yet, there is no firm demonstration that in vitro measurements translate into specific health benefits. The FDA and the European Food Safety Authority concluded that claims cannot be made linking foods and food constituents to the protection of the body, cells, and molecules, such as DNA, proteins, and lipids, from oxidative damage.[462]

Because short-term interventions with vitamins C or E, β-carotene, or flavonoids have not demonstrated consistent health benefits, the data suggest that other plant food factors may account for such protection.[463] As an example, flavonoids seem to account for only a small percentage of the oxidant capacity of apple extracts.[294]

Antioxidant Supplementation for Disease Prevention?

A primary goal of supplementation is to correct any imbalances in an individual's nutritional status. Overall, studies suggest that the recommended dietary allowances for antioxidants are inadequate for optimal immune function for certain groups. A second goal of antioxidant supplementation is to support the body's defense systems and increase resilience. Antioxidant supplementation should be balanced against the oxidant burden.

The prevalence of subclinical deficiencies of nutrients with antioxidant roles may be quite high—magnesium (56%), zinc (12%), vitamin E (93%), and vitamin C (31%) for general populations, as judged by intakes less than an estimated average requirement.[34] More than 90% of Americans do not consume the dietary reference intake (DRI) for vitamin E of 15 mg, which is judged as adequate to prevent myopathy and neuropathy.[464] Success with antioxidant supplementation is most notable when there are deficiencies, overt or not. According to a metabolic "triage" hypothesis, low micronutrient intake can increase the risk of chronic degenerative diseases without overt deficiencies because the body's homeostatic mechanisms direct essential nutrients to support the most critical functions, leaving other functions with suboptimal levels.[34]

Multiple, complementary antioxidants are likely to be far more effective than large amounts of a single antioxidant because antioxidants often work synergistically.[465] Animal studies suggest that the consumption of a greater diversity of antioxidants is more effective than the use of single antioxidants. Daily supplementation with vitamin C (500 mg), vitamin E (400 IU), β-carotene (15 mg), zinc (80 mg), and copper (2 mg) significantly slowed the progression of advanced AMD, although this regimen did not affect the risk of cataracts or loss of visual acuity.[466] In other research, the combination of CoQ_{10}, vitamin E, and ALA was shown to act synergistically in protecting LDL-C against lipid peroxidation in vitro.[467]

Table 97.1 provides a summary of the safe upper limits of antioxidant nutrients as well as recommended allowances for adults (ages >50 years). Notably, the levels of vitamins and minerals sometimes prescribed in protocols may be considerably higher than these recommendations.

Increase Antioxidant Intake Without Supplements?

In general, the consumption of a broad spectrum of antioxidants in amounts geared to meet an individual's oxidant burden and nutritional

TABLE 97.1 Micronutrient Antioxidants for Older Adults (>50 years): Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Limits (TUILs)

Micronutrient	RDA Men	RDA Women	TUIL[a]
Vitamin A	900 μg/day	700 μg/day	3000 μg/day[b]
Vitamin C	90 mg/day	75 mg/day	200 0mg/day[b] (none established)[c]
Vitamin E	15 mg/day	15 mg/day	1000 mg/day[b] from supplements OR fortified foods (300 mg/day[c])
Beta-carotene	None established	None established	None established
Copper	900 μg/day	900 μg/day	10 μg/day (5 mg/day[c])
Magnesium	420 mg/day (adequate intake)	320 mg/day (adequate intake)	350 mg/day from supplements OR fortified foods[b]
Manganese	2.3 mg/day	1.8 mg/day	11 mg/day[b]
Selenium	55 μg/day	55 μg/day	400 μg[b]

[a]Tolerable upper intake levels represent intake from foods and supplements unless noted otherwise.
[b]U.S. Food and Nutrition Board, Institute of Medicine, *Tolerable Upper Intake Levels*.
[c]European Food Safety Authority. *Tolerable Upper Intake Levels for Vitamins and Minerals*.

status may minimize the effects of genetic predisposition that compromise defenses against degenerative disease, promote optimal health, and slow the aging process. A safe and effective way to increase the intake of a broad spectrum of defined and undefined antioxidants relies on increased consumption of a variety of antioxidant-rich foods, especially colored fruits, vegetables, and legumes and beverages such as green tea. The utility of this strategy in designing clinical trials is illustrated by the MIND diet, based on the Mediterranean and DASH diets, which emphasizes 10 brain-healthy food groups, including green leafy vegetables, berries, nuts, and wine. This hybrid diet was shown to slow cognitive decline related to aging and to reduce the incidence of Alzheimer's disease.[468] Target dietary reference intakes for phytochemicals may eventually reflect composites beyond the currently recommended five daily servings of fruits and vegetables.[469]

LIMITS OF ANTIOXIDANT SUPPLEMENTATION

The frequent negative or conflicting results from clinical trials of antioxidant supplements may reflect wide variations in the nutritional status and heterogeneity of antioxidant intake in study populations, as well as biochemical individuality (genetic polymorphisms) and medical history, especially for an aging population for whom multiple pharmaceuticals are prescribed.

No single supplement, nutrient, or food can maintain the body's antioxidants and prevent disease, nor can single ingredients or foods cure a disease state. There are simply too many oxidants to be neutralized and too many layers of antioxidant defenses to be sustained, and the range of reactivity of water- and lipid-soluble ROS is far too great. Nor can antioxidant supplements substitute for a prudent diet and supportive lifestyle changes, including cessation of smoking, regular exercise, and effective stress management.

A personalized approach to nutritional interventions generally, and to antioxidant usage specifically, must consider individual variability. Yet major challenges remain: For most antioxidants, used singly or in combinations, the dosage, duration, efficacy, safety, and levels needed to sustain optimal health remain unknown. This is especially true for polyphenols.[470] In addition, a therapeutic window for beneficial effects is often inferred rather than clearly demonstrated.

PROOXIDANT EFFECTS

A further caveat is that antioxidant supplementation can be counterproductive. As discussed earlier, physiological levels of ROS are essential in maintaining homeostasis for multiple systems. Thus superoxide and NO play essential roles in maintaining the body's defenses and homeostatic mechanisms. Yet imbalanced antioxidants can create "antioxidative stress," in which antioxidants, especially exogenous species, can overwhelm the body's essential free radicals and ROS. Prooxidant effects of antioxidants depend on several factors, including their concentration and redox potential, the presence of other antioxidants or transition metals, and the activities of endogenous antioxidants.[471]

As examples of potential benefits versus harmful effects, acute supplementation with vitamins C, E, and α-lipid acid negated the reduction of blood pressure and the improvement in flow-mediated vasodilation due to exercise in elderly hypertensive men.[472] Transient oxidative stress, induced by short-term physical activity, can ameliorate insulin resistance. However, pretreatment with α-tocopherol 400 IU/day and vitamin C 1000 mg/day for 4 weeks prevented exercise-induced insulin sensitivity and blocked induction of endogenous antioxidant enzymes in healthy volunteers.[473] Quercetin and myricetin efficiently inhibited Trx reductase to induce apoptosis and cell-cycle arrest in tumor cell lines by direct binding and by semiquinone radical oxidation.[474] The Trx system broadly affects redox balance in normal cells and tissues, and its inhibition may be detrimental to health. Finally, moderate concentrations of ROS mediate defenses against microorganisms, as well as damaged or cancerous cells. Excessive antioxidant supplements could interfere with these essential processes.

VITAMIN E AND MORTALITY RISK?

A meta-analysis of 68 randomized trials found that supplementation up to 800 IU/day did not change the risk of all-cause mortality.[144] A more recent metaregression analysis of 29 clinical trials representing a total of 138,462 participants and vitamin E dosages ranging from 16.5 to 5000 IU/day found no causal relationship between high-dose vitamin E and mortality.[475] Indiscriminant use of high doses of vitamin E is not recommended.[188,476] Vitamin E can interfere with vitamin K–dependent clotting factors, and high doses increase the risk of bleeding in at-risk individuals.

Beta Carotene and Lung Cancer and Coronary Heart Disease?

As a further consideration, β-carotene may act as a prooxidant for those at risk for oxidative stress (smoking, alcohol consumption); β-carotene may also raise the risk of lung cancer in high-risk

populations unless protected by antioxidants like vitamin E.[477] When β-carotene is supplemented during oxidative stress, genotoxic cleavage products are produced, and these may account for the carcinogenic effects observed in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) and Beta-Carotene and Retinol Efficacy Trial (CARET).[478] High levels of β-carotene may be contraindicated in smokers with myocardial infarction. In one study, the rate of fatal CHD rose in patients receiving 20 mg/day β-carotene (with or without α-tocopherol).[479] Carotenoids generally lose their effectiveness at high concentrations. This feature may account for the possible benefits of consumption of carotenoid-rich foods, although therapeutic doses of isolated carotenoids may be deleterious.[480]

Polyphenols and Mutagenicity?

The potential prooxidant actions of polyphenolic radicals are well documented.[481] Flavonoids are metabolized and detoxified by liver enzymes. Pharmacological doses of flavonoids can induce Phase I detoxification enzymes, increasing their ability to modify antioxidant metabolites for excretion. The trade-off lies in the possibly greater sensitivity to mutagens and carcinogens when toxins are transformed into potential mutagens.

Vitamin C, Toxicity, and Hypertension?

In vitro studies suggest that ascorbic acid can decompose lipid peroxides to genotoxins.[482] In vivo, these effects would likely require high ascorbate concentrations coupled with the depletion of tocopherol.[483] Vitamins C and E exhibit prooxidant activity in vitro under certain conditions. When exposed to catalytic levels of iron or copper ions, ascorbate promotes the formation of H_2O_2 and hydroxyl radicals.[484,485] Excessive dietary iron or iron accumulation could promote ascorbate-induced oxidation, and ascorbate could act as an oxidant in the presence of iron or copper released during inflammation or injury. In an RCT, hypertensive patients were treated with 500 mg ascorbic acid and/or 1000 mg grape seed polyphenols daily for 6 weeks. Although ascorbic acid alone reduced elevated systolic pressure, the combination significantly increased both systolic and diastolic blood pressure.[486]

LIMITATIONS OF ANTIOXIDANTS AS ADJUVANT CANCER THERAPY

Although antioxidant intake can influence the effectiveness of anticancer therapy and related adverse effects, results depend on the type of cancer, the mechanism of action of the prescribed drug, and the type and dosage of antioxidants.[487]

A systematic review found that 24 of 33 RCTs reported decreased toxicity of chemotherapy with concomitant use of antioxidants, including vitamin E and vitamin C, with one study of vitamin A reporting increased toxicity.[488] A study of pediatric patients with acute lymphoblastoma being treated with chemotherapy and concurrent supplementation with 400 IU vitamin E and 600 mg *N*-acetylcysteine daily for 28 days found that children taking the supplements experienced less liver toxicity, fewer blood abnormalities, and fewer blood transfusions.[489] The use of α-tocopherol succinate to inhibit tumor growth in vivo remains unproven. Because this derivative possesses no antioxidant activity, presumably its inhibition of cancer cell proliferation and induction of apoptosis in tumor cells relies on other mechanisms.

POTENTIAL NUTRIENT–DRUG INTERACTIONS AND PHYTOCHEMICAL–DRUG INTERACTIONS

The popularity of antioxidant supplementation comes with additional concerns regarding interactions with over-the-counter and prescription medications. The literature in these areas is steadily growing, and several useful compendia are available.[490–493] In addition, the editors of the *Pharmacist's Letter* publish the Natural Medicines Comprehensive Database, which is updated frequently.

Botanical supplements can promote hepatoxicity via inhibition of cytochrome P450 induction of their expression. As examples, green tea extract and isolated catechins can inhibit multiple cytochrome P450 isozymes in human liver, as well as in animal models. In addition, turmeric can inhibit drug metabolism in human liver microsomes, although human subject data are lacking.[494]

RESEARCH FRONTIERS

Redox Balance and the Gut Microbiome

Close relationships among commensals, pathogens, and the intestinal epithelium implicate ROS. As examples, *Lactobacilli* can activate NOX1 to enhance intestinal stem-cell proliferation. Disruption of the epithelial barrier can stimulate H_2O_2 release from the mucosal lining to alter the microbial redox balance and reduce virulence. H_2O_2 can directly kill pathogens, and it plays a role in intercellular microbial communication.[495]

REDOX BALANCE AND GASO-TRANSMITTER SYSTEMS

The ROS and RNS help regulate hydrogen sulfate (H_2S) and carbon monoxide, as well as NO. In particular, H_2S most likely acts as a signaling molecule to increase antioxidant defenses.[496] Lower levels of H_2S tend to be anti-inflammatory and neuroprotective. Hypothetically, H_2S regulates antioxidant genes while reducing NOX expression directly via thiol modifications, creating further linkage among H_2S, GSH, cysteine metabolism, and NO.[497]

REDOX BALANCE AND AGING

As described earlier, oxidative stress is a well-established factor in age-associated diseases. Clearly, protein dysfunction occurs during aging, and proteasome-mediated degradation of oxidized species is essential for homeostasis.[498] An increase in markers of systemic oxidative stress, such as protein carbonylation and the production of proinflammatory cytokines and lipid peroxides in older individuals, is matched by reduced expression of genes governing antioxidant responses.[499]

Any mechanisms must account for the linkage among calorie restriction, adipocyte SIRT1 activation, altered insulin sensitivity, and dysfunction of glucose regulation. Of the many proposals to account for the action of diet restriction on longevity, several relate to mitochondrial oxidative stress.[500] Cardiovascular risk factors (e.g., diabetes, hypertension, hypercholesterolemia, smoking) induce eNOS uncoupling and ROS/RNS production; eNOS glutathionylation is a possible mechanism. Strong evidence indicates that increased expression and/or activation of the NOX family, especially NOX4, plays a role in age-related disease.[501] There is interest in the role of ROS in a "geriatric syndrome," characterized by reduced functional reserve and increased vulnerability to oxidative stress.[502]

REDOX BALANCE AND MITOCHONDRIAL FUNCTION

Although global cellular oxidative stress would be expected to depend on ROS production versus ROS elimination (antioxidant defenses), ROS in mitochondria are much more dependent on highly localized ROS products than on antioxidants. Accordingly, ROS may not be viewed as by-products of the mitochondrial respiratory chain. Instead, mitochondrial ROS seem to be carefully regulated at more or

less constant levels while endogenous antioxidants and DNA repair enzymes are temporarily induced as needed.[500]

FUTURE DIRECTIONS: CONCEPTS AND STRATEGIES

Nutrigenomics

Redox homeostasis and antioxidant status depend on how the individual genetic makeup affects dietary responses and how gene–diet interactions promote disease, especially ways that food compounds affect gene expression regulating central pathways. The large-scale analysis of gene expression, individual proteins, and lipid markers, as well as the integration of metabolic pathways, can utilize functional analysis software. This approach demonstrated that a diet enriched in putative antioxidants can modulate low-grade oxidative stress and inflammation in overweight men.[503] The potential of herbs and botanicals in the prevention and treatment of conditions involving free radical pathology further enriches the future of antioxidant research.[504]

Nutrigenomics includes an examination of epigenetic processes, such as the role of microRNAs in chronic disease.[505] MicroRNAs typically regulate gene silencing, and they can be controlled by isolated nutrients and bioactive molecules, further indicating the importance of diet as related to inflammation, oxidative stress, and antioxidant defenses.

Polyphenols, particularly flavonoids, possess epigenome-regulating actions involving DNA methylation, histone acetylation, and deacetylation, as shown by studies of cultured cell lines. Quercetin, curcumin, EGCG, and berberine can function as sirtuin activators (histone acetyltransferase) while inhibiting histone deacetylase.[506] Genistein can act as an anticancer flavonoid in various cancer cell types via epigenetic control. Curcumin's pleiotropic effects in reducing liver damage in experimental cirrhosis include up-regulation of SIRT3, AMPK, and MnSOD.[507] Whether epigenetic regulation is caused by prooxidant effects or by the antioxidant nature of these flavonoids has not been established.

NUTRITIONAL COGNITIVE NEUROSCIENCE

The emerging discipline of nutritional cognitive neuroscience incorporates nutrient biomarker patterns and indices of brain health based on high-resolution magnetic resonance imaging (MRI) together with nutritional epidemiology.[508] The latter employs principal component analysis to discover nutrient biomarker patterns. With this method, a pattern of antioxidants, vitamins C and E, the B complex vitamins, and vitamin D has been associated with increased cognitive function.[509] High-throughput analytical chemistry permits examination of up to thousands of metabolites of the metabolome.[510] This approach has identified possible biomarkers for individual foods and dietary patterns, such as raspberries, citrus fruits, and overall fruit and vegetable intake.[511] Neuroimaging has been used to examine the role of diet and nutrients in structural brain changes during aging. Through this method, preservation of brain volume during aging has been associated with α-tocopherol, tocotrienol status, and vitamin C status, as well as reliance on a Mediterranean-style diet.[512]

HORMESIS

The concept of hormesis has been recommended to understand how substances such as endogenous and exogenous antioxidants promote ROS production, especially in mitochondria. In this context, these agents may act as mild prooxidants at low concentrations (picomolar/submicromolar), as antioxidants at micromolar ranges, or as toxic prooxidants at high dosages (micromolar to millimolar). Theoretically, closely regulated constitutive ROS production could create a dynamic oscillating system, in which mitochondria and calcium flux play central regulatory roles.[513]

ROS INTERACTOME

As a conceptual approach to integrating the complexity of redox balance in metabolic control, a "reactive species interactome" has been proposed.[514] Accordingly, a multilevel redox regulatory system allows the rapid adaptation to stressors to enhance resilience. System-level analysis may help sort out the ROS precursors, reaction products, and antioxidant signatures. Currently, there are no standards for nutrigenetic research outcomes in guiding the development of dietary recommendations. Criteria for evaluating the validity of this research and developing dietary guidelines have been proposed.[515]

CONCLUSION

The broad outlines for antioxidant defenses against oxidative stress are established. However, our understanding of the inner workings of redox regulation remains incomplete, and details of the body's response to oxidative stress remain poorly understood. Hundreds of redox switches have been uncovered using large-scale proteomic analysis.[516] It is not clear how ROS are compartmentalized within a given cell type; indeed, monitoring ROS in vivo remains challenging. The translation of experiential findings into a therapeutic setting requires knowledge of how the same molecules mediate both pathological events and normal physiological responses. Threshold levels transforming "good" ROS into "bad" ones are likely to be different in healthy and diseased cells. These considerations apply to the development of personalized therapeutic protocols, advanced diagnostic procedures, and precise monitoring.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Cho KJ, Seo JM, Kim JH. Bioactive lipoxygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. *Mol Cells*. 2011;32:1–5.
2. Li L, Frei B. Prolonged exposure to LPS increases iron, heme, and p22phox levels and NADPH oxidase activity in human aortic endothelial cells: inhibition by desferrioxamine. *Arterioscler Thromb Vasc Biol*. 2009;29:732–738.
3. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. *Curr Med Chem*. 2005;12(10):1161–1208. PubMed PMID: 15892631.
4. Bertin Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie*. 2006;88(11):1549–1559. PubMed PMID: 15892631.
5. Yang C, Frenkel K. Arsenic-mediated cellular signal transduction, transcription factor activation, and aberrant gene expression: implications in carcinogenesis. *J Environ Pathol Toxicol Oncol*. 2002;21(4):331–342. PubMed PMID: 12510962.
6. Durak D, Uzun FG, et al. Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. *Environ Toxicol*. 2009;24(3):235–242. PubMed PMID: 18655177.
7. Delgado EH, Streck EL, et al. Mitochondrial respiratory dysfunction and oxidative stress after chronic malathion exposure. *Neurochem Res*. 2006;31(8):1021–1025. PubMed PMID: 16865556.
8. Lee KM, Park SY, et al. Pesticide metabolite and oxidative stress in male farmers exposed to pesticide. *Ann Occup Environ Med*. 2017;29:5. PubMed PMID: 28265414.
9. Prasad SB, Vidyullatha P, Vani GT, et al. Association of gene polymorphism in detoxification enzymes and urinary 8-OHdG levels in traffic policemen exposed to vehicular exhaust. *Inhal Toxicol*. 2013;25(1):1–8. PubMed PMID: 23293967.
10. Ito K, Mathes R, Ross Z, Nadas A, Thurston G, Matte T. Fine particulate matter constituents associated with cardiovascular hospitalizations and mortality in New York City. *Environ Health Perspect*. 2011;119:467–473. PubMed PMID: 21463978.
11. Oh SM, Kim HR, Park YJ, Lee SY, Chung KH. Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells). *Mutat Res*. 2011;723(2):142–151. PubMed PMID: 21524716.
12. Katanoda K, Sobue T, Satoh H, et al. An association between long-term exposure to ambient air pollution and mortality from lung cancer and respiratory diseases in Japan. *J Epidemiol*. 2011;21(2):132–143. PubMed PMID: 21325732.
13. Lu CY, Ma YC, Lin JM, Li CY, Lin RS, Sung FC. Oxidative stress associated with indoor air pollution and sick building syndrome-related symptoms among office workers in Taiwan. *Inhal Toxicol*. 2007;19(1):57–65. PubMed PMID: 17127643.
14. Commodore AA, Zhang JJ, Chang Y, et al. Concentrations of urinary 8-hydroxy-2'-deoxyguanosine and 8-isoprostane in women exposed to woodsmoke in a cookstove intervention study in San Marcos, Peru. *Environ Int*. 2013;60:112–122. PubMed PMID: 24041735.
15. Mason RP, Walter MF, McNulty HP, et al. Rofecoxib increases susceptibility of human LDL and membrane lipids to oxidative damage: a mechanism of cardiotoxicity. *J Cardiovasc Pharmacol*. 2006;47(suppl 1):S7–S14. PubMed PMID: 16785833.
16. Loft S, Vistisen K, Ewertz, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*. 1992;13:2241–2247. PubMed PMID: 1473230.
17. Prieme H, Loft S, Klarlund M, Gronbaek K, Tonnesen P, Poulsen HE. Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7,8-dihyro-2'-deoxyguanosine excretion. *Carcinogenesis*. 1998;19:347–351. PubMed PMID: 9498287.
18. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1957;2:298–300.
19. Kovacic P, Jacintho JD. Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. *Curr Med Chem*. 2001;8:773–796.
20. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2002;82:47–95.
21. Ross JS, Stagliano NE, Donovan MJ, et al. Atherosclerosis and cancer: common molecular pathways of disease development and progression. *Ann N Y Acad Sci*. 2001;947:271–292.
22. Iuliano L. The oxidant stress hypothesis of atherogenesis. *Lipids*. 2001;36:S41–S44.
23. Wei W, Liu Q, Tan Y, et al. Oxidative stress, diabetes and diabetic complications. *Hemoglobin*. 2009;33:370–377.
24. Ottonello S, Foroni C, Carta A, et al. Oxidative stress and age-related cataract. *Ophthalmologica*. 2000;214:78–85.
25. D'Odorico A, Bortolan S, Cardin R, et al. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in inflammatory bowel disease. *Scand J Gastroenterol*. 2001;36:1289–1294.
26. Tak PP, Zvaifler NJ, Green DR, et al. Rheumatoid arthritis and p53: how oxidative stress might alter the course of inflammatory diseases. *Immunol Today*. 2000;21:78–82.
27. Rahman I. Oxidative stress, transcription factors and chromatin remodeling in lung inflammation. *Biochem Pharmacol*. 2002;64:935–942.
28. Esposito E, Rotilio D, Di Matteo V, et al. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiol Aging*. 2002;23:719–735.
29. Moosmann B, Behl C. Antioxidants as treatment for neurodegenerative disorders. *Expert Opin Investig Drugs*. 2002;11:1407–1435.
30. Jain SK, Pemberton PW, Smith A, et al. Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. *J Hepatol*. 2002;36:805–811.
31. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidative stress in humans. *Int J Obes (Lond)*. 2006;30:400–418.
32. Maes M, Twisk FN. Why myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) may kill you: disorders in the inflammatory and oxidative and nitrosative stress (IO&NS) pathways may explain cardiovascular disorder in ME/CFS. *Neuro Endocrinol Lett*. 2009;30:677–693.
33. Curtin JF, Donovan M, Cotter TG. Regulation and measurement of oxidative stress in apoptosis. *J Immunol Methods*. 2002;265:49–72.
34. Ames BN. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci USA*. 2006;103:17589–17594.
35. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med*. 2002;227:671–682.
36. Goncalves L, Dafre AL, Carobrez SG, et al. A temporal analysis of the relationships between social stress, humoral immune response and glutathione-related antioxidant defenses. *Behav Brain Res*. 2008;192:226–231.
37. Stadtman ER, Levine RL. Protein oxidation. *Ann NY Acad Sci*. 2000;899:191–208.
38. Miquel J. An update on the oxygen stress-mitochondrial mutation theory of aging: genetic and evolutionary implications. *Exp Gerontol*. 1998;33:113–126.
39. Lanza IR, Nair KS. Mitochondrial function as a determinant of life span. *Pflugers Arch*. 2010;459:277–289.
40. Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. *Int J Biochem Cell Biol*. 2005;37:961–976.
41. Barja G. The mitochondrial free radical theory of aging. *Prog Mol Biol Transl Sci*. 2014;127:1–27.
42. Davies MJ. Protein oxidation and peroxidation. *Biochem J*. 2016;473:805–825.
43. Keshavarzian A, Sedghi S, Kanotsky J, et al. Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe. *Gastroenterol*. 1992;103:177–185.
44. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007;87:315–424.
45. Droge W. Free radicals in the physiological control of cell function. *Phys Rev*. 2002;82:47–95.
46. Choi S, Park S, Laing GH, et al. Superoxide generated by lysophosphatidylcholine induces endothelial nitric oxide synthase downregulation in human endothelial cells. *Cell Physiol Biochem*. 2010;25:233–240.
47. Radi R. Peroxynitrite, a stealthy biological oxidant. *J Biol Chem*. 2013;288:26464–26472.

48. Matsumoto M, Zhang J, Zhang X, et al. The NOX1 isoform of NADPH oxidase is involved in dysfunction of liver sinusoids in nonalcoholic fatty liver disease. *Free Radic Biol Med*. 2017;115:412–420.
49. Roos D. The involvement of oxygen radicals in microbicidal mechanisms of leukocytes and macrophages. *Klin Wochenschr*. 1991;69:975–980.
50. Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med*. 1997;22:269–285.
51. Lam GY, Huang J, Brumell JH. The many roles of NOX2 NADPH oxidase-derived ROS in immunity. *Semin Immunopathol*. 2010;32: 415–430.
52. Pierce GL, Lesniewski LA, Lawson BR, et al. Nuclear factor kappaB activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation*. 2009;119. 1294–1292.
53. Tebay LE, Robertson H, Durant ST, et al. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic Biol Med*. 2015;88:108–146.
54. Suhr YJ, Kundu JK, Na HK. Nfr2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemoprotective phytochemicals. *Planta Med*. 2008;74:1526–1539.
55. Fruefhauf JP, Meyskens Jr FL. Reactive oxygen species: a breath of life or death? *Clin Cancer Res*. 2007;13:789–794.
56. Giorgi C, Agnoletto C, Baldini C, et al. Redox control of protein kinase C: cell and disease specific aspects. *Antioxid Redox Signal*. 2010;13:1051–1058.
57. Consentino-Gomes D, Rocco-Machado N, Meyer-Fernandes JR. Cell signaling through protein kinase C oxidation and activation. *Int J Mol Sci*. 2012;13:10697–10721.
58. Edderkoui M, Hong P, Vaquero EC, et al. Extracellular matrix stimulates reactive oxygen specific production and increases pancreatic cell survival through 5-lipoxygenase and NADPH oxidase. *Am J Physiol Gastrointest Liver Physiol*. 2005;289:G1137–G1147.
59. Tabet F, Savoia C, Schiffrin EL, et al. Differential calcium regulation by hydrogen peroxide and superoxide in vascular smooth muscle cells from spontaneously hypertensive rats. *J Cardiovasc Pharmacol*. 2004;44: 200–208.
60. Vissers MC, Lee WG, Hampton MB. Regulation of apoptosis by vitamin C. Specific protection of the apoptotic machinery against exposure to chlorinated oxidants. *J Biol Chem*. 2001;276:46835–46840.
61. Roberts RA, Soames AR, James NH, et al. Dosing-induced stress causes hepatocyte apoptosis in rats primed by the rodent nongenotoxic hepatocarcinogen cyproterone acetate. *Toxicol Appl Pharmacol*. 1995;135:192–199.
62. Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end stage heart failure. *N Engl J Med*. 1996;335:1182–1189.
63. Moon YJ, Wang X, Morris ME. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicol In Vitro*. 2006;20:187–210.
64. Pfeilschifter J, Eberhardt W, Huwiler A. Nitric oxide and mechanisms of redox signaling. *J Am Soc Nephrol*. 2003;14:S237–S240.
65. Barrett DM, Black SM, Todor H, et al. Inhibition of protein-tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation. *J Biol Chem*. 2005;280:14453–14461.
66. Snyder CM, Shroff EH, Jing L, et al. Nitric oxide induces cell death by regulating anti-apoptotic BCL-2 family members. *PloS ONE*. 2009;4:e7059.
67. Cho SH, Lee CH, Ahn Y, et al. Redox regulation of PTEN and protein tyrosine phosphatases in H2O2 mediated cell signaling. *FEBS Lett*. 2004;560:7–13.
68. Forman HJ, Maiorino M, Ursini F. Signaling functions of reactive oxygen species. *Biochemistry*. 2010;49:835–842.
69. Gajos-Draus A, Duda M, Beresewicz A. Cardiac and renal upregulation of Nox2 and NF-κB and repression of Nox4 and Nrf2 in season- and diabetes-mediated models of vascular oxidative stress in guinea-pig and rat. *Physiol Rep*. 2017;5:e13474. PMCID: PMC5661235.
70. Chevion M, Berenshtein E, Stadtman ER. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic Res*. 2000;33:S99–S108.
71. Roberts LJ. 2nd, Morrow JD. Products of isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell Mol Life Sci*. 2002;59:808–820.
72. Houee-Levin C, Bobrowski K, Horakova L, et al. Exploring oxidative modifications of tyrosine: an update on mechanisms of formation, advances in analysis and biological consequences. *Free Radic Res*. 2015;49:347–373.
73. Wayenberg JL, Ransy V, Vermeylen E, et al. Nitrated plasma albumin as a marker of nitrative stress and neonatal encephalopathy in perinatal asphyxia. *Free Radic Biol*. 2011;100:387–397.
74. Wayenberg JL, Cavedon C, Ghaddhab C, et al. Early transient hypoglycemia is associated with increased albumin nitration in the preterm infant. *Neonatology*. 2011;100:387–397.
75. Kasai H, Crain PF, Kuchino Y, et al. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis*. 1986;7:1849–1851.
76. Valtuena S, Pellegrini N, Franzini L, et al. Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. *Am J Clin Nutr*. 2008;87:1290–1297.
77. Block G, Jensen C, Dietrich M, et al. Plasma C-reactive protein concentrations in active and passive smokers: influence of antioxidant supplementation. *J Am Coll Nutr*. 2004;23:141–147.
78. Forlani G, Di Bonito P, Mannucci E, et al. Prevalence of elevated liver enzymes in type 2 diabetes mellitus and its association with the metabolic syndrome. *J Endocrinol Invest*. 2008;31:146–152.
79. Yavus BB, Yavuz B, Halil M, et al. Serum elevated gamma-glutamyltransferase levels may be a marker for oxidative stress in Alzheimer's disease. *Int Psychogeriatr*. 2008;20:815–823.
80. Fraser A, Harris R, Sattar N, et al. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol content: analysis of the British Women's Heart and Health Study and meta-analysis. *Arterioscler Thromb Vasc Biol*. 2007:2729–2735.
81. Smith CV. Free radical mechanisms in tissue injury. In: Moslen MY, Smith CV, eds. *Free Radical Mechanisms of Tissue Injury*. Boca Raton: CRC Press; 1992:2–22.
82. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys*. 1993;300:535–543.
83. Prasetyo EN, Otto K, Nyanhongo GS, et al. Developing SyrinOX total antioxidant capacity assay for measuring antioxidants in humans. *Int J Exp Pathol*. 2013;94:25–33.
84. Kwak HK, Yoon S. Relation of serum total antioxidant status with metabolic risk factors in Korean adults. *Nutr Res Pract*. 2007;1:335–340.
85. Razavi M, Jamilian M, Samimi M, et al. The effects of vitamin D and omega-3 fatty acids co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in patients with gestational diabetes. *Nutr Metab (Lond)*. 2017;14:80.
86. Garrett AR, Murray BK, Robinson RA, et al. Measuring antioxidant capacity using the ORAC and TOSC assays. *Methods Mol Biol*. 2010;594:251–262.
87. Nutrient Data Laboratory, Agricultural Research Service. *USDA Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods*. USDA: Washington, DC.
88. Carlsen MH, Halvorsen BL, Holte K, et al. The total antioxidant content of more than 3100 foods, herbs, beverages, spices, and herbs and supplements used worldwide. *Nutr J*. 2010;9:3. PMCID: PMC2841576.
89. Prior R. Antioxidant food databases? valuable or not? brunswick laboratories. http://www.brunswicklabs.com/Portals/153979/docs/A_Response_to_the_USDA_ORAC_Statement.pdf. Accessed December 1, 2012.
90. Gifkins D, Olson SH, Demissie K, et al. Total and individual antioxidant intake and endometrial cancer risk: results from a population-based case-control study in New Jersey. *Cancer Causes Control*. 2012;239:887–895.
91. Wofe KL, Liu RH. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *J Agric Food Chem*. 2007;55:8896–8907.

92. Yang B, Kotani A, Arai K, et al. Relationship of electrochemical oxidation of catechins on their antioxidant activity in microsomal lipid peroxidation. *Chem Pharm Bull*. 2001;49:747–751.
93. Sandoval M, Ronzio RA, Muanza DN, et al. Peroxynitrite-induced apoptosis in epithelial (T84) and macrophage (RAW 264.7) cell lines: effect of legume-derived polyphenols (phytolens). *Nitric Oxide*. 1997;1:476–483.
94. Visner GA, Dougall WC, Wilson JM, et al. Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor. *J Biol Chem*. 1990;265:2856–2864.
95. Fukai T, Folz RJ, Landmesser U, et al. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res*. 2002;55:239–249.
96. Deng HX, Hentati A, Tainer JA, et al. Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. *Science*. 1993;261:1047–1051.
97. Massaad CA, Washington TM, Paulter RG, et al. Overexpression of SOD2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA*. 2009;106:13576–13581.
98. Segui J, Gironella M, Sans M, et al. Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine. *Leukoc Biol*. 2004;76:537–544.
99. Davis JM, Parad RB, Michele T, et al. Pulmonary outcome at 1 year corrected age in premature infants treated at birth with recombinant human CuZn superoxide dismutase. *Pediatrics*. 2003;111:469–476.
100. Carillon J, Notin C, Schmitt K, et al. Dietary supplementation with a superoxide dismutase-melon concentrate reduces stress, physical and mental fatigue in healthy people: a randomized, double-blind, placebo-controlled trial. *Nutrients*. 2014;6:2348–2359.
101. Broeyer FJF, van Aken BE, Suzuki J, et al. The pharmacokinetics and effects of a long-acting preparation of superoxide dismutase (PC-SOD) in man. *Br J Clin Pharmacol*. 2008;65:22–29.
102. Rafighi Z, Shiva A, Arab S, et al. Association of dietary vitamin c and e intake and antioxidant enzymes in type 2 diabetes mellitus patients. *Glob J Health Sci*. 2013;5:183–187.
103. Lamb DJ, Tickner ML, Hourani SMO, et al. Dietary copper supplements modulate aortic superoxide dismutase, nitric oxide and atherosclerosis. *Int J Exp Pathol*. 2005;86:247–255.
104. Gomez-Marcos MA, Blazquez-Medela AM, Gamella-Pozuelo L, et al. Serum superoxide dismutase is associated with vascular structure and function in hypertensive and diabetic patients. *Oxid Med Call Longev*. 2016:9124676. PMCID: PMC4655282.
105. Muzykantov VR. Delivery of antioxidant enzyme proteins to the lung. *Antioxid Redox Signal*. 2001;3:39–62.
106. Sanders LM, Henderson CE, Hong MY, et al. Pro-oxidant environment of the colon compared to the small intestine may contribute to greater cancer susceptibility. *Cancer Lett*. 2004;208:155–161.
107. Jones JB, Cramer HM, Inch WR, et al. Radioprotective effect of free radical scavenging enzymes. *J Otolaryngol*. 1990;19:299–306.
108. Kobayashi S, Inoue N, Azumi H, et al. Expressional changes of the vascular antioxidant system in atherosclerotic coronary arteries. *J Atheroscler Thromb*. 2002;9:184–190.
109. Ho YS, Xiong Y, Ma W, et al. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *J Biol Chem*. 2004;279:32804–32812.
110. Yao C, Behring JB, Shao D, et al. Overexpression of catalase diminishes oxidative cysteine modifications of cardiac proteins. *PLoS One*. 2015;10:e0144025.
111. Lu J, Holmgren A. *Selenoproteins. J Biol Chem*. 2009;284:723–727.
112. Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. *Am J Physiol Lung Cell Mol Physiol*. 2002;283:L246–L255.
113. Geraghty P, Baumin N, Salathe MA, et al. Glutathione peroxidase-1 suppresses the unfolded protein response upon cigarette smoke exposure. *Mediators Inflamm*. 2016;9461289. PMCID: PMC5187475.
114. Muller FL, Lustgarten MS, Jang Y, et al. Trends in oxidative aging theories. *Free Radic Biol Med*. 2007;43:477–503.
115. Kisucka J, Chauhan AK, Patten IS, et al. Peroxiredoxin-1 prevents excessive activation and early atherosclerosis. *Circ Res*. 2008;103:598–605.
116. Diet A, Abbas K, Bouton C, et al. Regulation of peroxiredoxins by nitric oxide in immunostimulated macrophages. *J Biol Chem*. 2007;282:36199–36205.
117. Lv Zhenbing, Wei J, You W, et al. Disruption of the c-Myc/miR-200b-3p/PRDX2 regulatory loop enhances tumor metastasis and chemotherapeutic resistance in colorectal cancer. *J Transl Med*. 2017;15:257.
118. Krsek-Staples JA, Webster RO. Ceruloplasmin inhibits carbonyl formation in endogenous cell proteins. *Free Radic Biol Med*. 1993;14:115–125.
119. Al-Qenaei A, Yiakouvaki A, Reelfs O, et al. Role of intracellular labile iron, ferritin, and antioxidant defence in resistance of chronically adapted Jurkat T cells to hydrogen peroxide. *Free Radic Biol Med*. 2014;68:87–100.
120. Kondoh M, Inoue Y, Atagi S, et al. Specific induction of metallothionein synthesis by mitochondrial oxidative stress. *Life Sci*. 2001;69:2137–2146.
121. Zhou Z, Sun X, Kang YJ. Metallothionein protection against alcoholic liver injury through inhibition of oxidative stress. *Exp Biol Med*. 2002;227:214–222.
122. Eidizadeh A, Khajehalichalehshtari M, Freyer D, Trendelenburg G. Assessment of the therapeutic potential of metallothionein-II application in focal cerebral ischemia in vitro and in vivo. *PLoS One*. 2015;10:e0144035. PMCID: PMC4682799.
123. Moschos MP, Selenoprotein P. *Cell Mol Life Sci*. 2000;57:1836–1845.
124. Peristeris P, Clark BD, Gatti S, et al. N-acetylcysteine and glutathione as inhibitors of tumor necrosis factor production. *Cell Immunol*. 1992;140:390–399.
125. Ballatori N, Krance SM, Notenboom S, et al. Glutathione dysregulation and the etiology and progression of human diseases. *Biol Chem*. 2009;390:191–214.
126. Johnson WM, Wilson-Delfosse AL, Mieyal JJ. Dysregulation of glutathione homeostasis in neurodegenerative diseases. *Nutrients*. 2012;4:1399–1440.
127. Zhang C, Rodriguez C, Spaulding J, et al. Age-dependent and tissue-related glutathione redox status in a mouse model of Alzheimer's Disease. *J Alzheimers Dis*. 2012;28:655–666.
128. Dias IH, Mistry J, Fell S, et al. Oxidized LDL lipids increase β-amyloid production by SH-SY5Y cells through glutathione depletion and lipid raft formation. *Free Radic Biol Med*. 2014;75:48–59.
129. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med*. 2001;31:1287–1312.
130. Hanschmann EM, Godoy JR, Berndt C, et al. Thioredoxins, glutaredoxins, and peroxiredoxins—molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxid Redox Signal*. 2013;19:1539–1605.
131. Luo M, He H, Kelley MR, et al. Redox regulation of DNA repair: implications for human health and cancer therapeutic development. *Antioxid Redox Signal*. 2010;12:1247–1269.
132. Kaspar JW, Niture SK, Jaiswal AK. Nrf2: Inrf2(keap1) signaling in oxidative stress. *Free Radic Biol Med*. 2009;47:1304–1309.
133. Zhang H, Davies KJ, Forman HJ. Oxidative stress response and Nrf2 signaling in aging. *Free Radic Biol Med*. 2015;88:314–336.
134. David JA, Rifkin WJ, Rabbani PS, Ceradini DJ. The Nrf2/Keap1/ARE Pathway and oxidative stress as a therapeutic target in type II diabetes mellitus. *J Diabetes Res*. 2017:4826724. PMCID: PMC5585663.
135. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med*. 2002;227:671–682.
136. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. *J Neuropathol Exp Neurol*. 2010;69:155–167.
137. Dimauro I, Pearson T, Caporossi D, et al. In vitro susceptibility of thioredoxins and glutathione to redox modification and aging-related changes in skeletal muscle. *Free Radic Biol Med*. 2012;53:2017–2027.
138. Krinsky NI. Mechanism of action of biological antioxidants. *Proc Soc Exp Biol Med*. 1992;200:248–254.
139. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci U S A*. 1989;86:6377–6381.
140. Chakraborty S, Nandi A, Mukhopadhyay M, et al. Ascorbate protects guinea pig tissues against lipid peroxidation. *Free Radic Biol Med*. 1994;16:417–426.

141. Calabrese EJ, Stoddard A, Leonard DA, et al. The effect of vitamin C supplementation on blood and hair levels of cadmium, lead, and mercury. *Ann N Y Acad Sci*. 1987;498:347–353.
142. Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the established populations for epidemiologic studies of the elderly. *Am J Clin Nutr*. 1996;64:190–196.
143. Levine M, Wang Y, Padayatty SJ, et al. A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci U S A*. 2001;98:9842–9846.
144. Bjelakovic G, Nikolova D, Gluud LL, et al. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systemic review and meta-analysis. *JAMA*. 2007;297:842–857.
145. Knekt P, Ritz J, Pereiara MA, et al. Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. *Am J Clin Nutr*. 2004;80:1508–1520.
146. Osganian SK, Stampfer MJ, Rimm E, et al. Vitamin C and risk of coronary heart disease in women. *J Am Coll Cardiol*. 2003;42:246–252.
147. Lee DH, Folsom AR, Harnack L, et al. Does supplemental vitamin C increase cardiovascular disease risk in women with diabetes? *Am J Clin Nutr*. 2004;80:1194–1200.
148. Sesso HD, Buring JE, Christen WG, et al. Vitamins E and C in the prevention of cardiovascular disease and cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA*. 2008;300:2123–2133.
149. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomized placebo-controlled trial. *Lancet*. 2002;360:23–33.
150. Block G, Jensen CD, Norkus EP, et al. Vitamin C in plasma is inversely related to blood pressure and change in blood pressure during the previous year in young black and white women. *Nutr J*. 2008;7:35. PMCID: PMC2621233.
151. Juraschek SP, Guallar E, Appel LJ, et al. Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2012;95:1079–1088.
152. Negri E, Franceschi S, Bosetti C, et al. Selected micronutrients and oral and pharyngeal cancer. *Int J Cancer*. 2000;86:122–127.
153. Fleischauer AT, Olson SH, Mignone L, et al. Dietary antioxidants, supplements, and risk of epithelial ovarian cancer. *Nutr Cancer*. 2002;40:92–98.
154. You WC, Zhang L, Gail MH, et al. Gastric dysplasia and gastric cancer: Helicobacter pylori, serum vitamin C, and other risk factors. *J Natl Cancer Inst*. 2000;92:1607–1612.
155. Cho E, Hunter DJ, Spiegelman D, et al. Intakes of vitamin A, C and E and folate and multivitamins and lung cancer: a pooled analysis of 8 prospective studies. *Int J Cancer*. 2006;118:970–978.
156. Gaziaono JM, Glynn RJ, Christen WG, et al. Vitamins E and C in the prevention of prostate and total cancer in men: The Physicians' Health Study II randomized controlled trial. *JAMA*. 2009;301:52–62.
157. Harding AH, Wareham NJ, Bingham SA, et al. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new onset type 2 diabetes mellitus: European prospective investigation of cancer—Norfolk Prospective Study. *Arch Intern Med*. 2008;168:1493–1499.
158. Chen Q, Espey MG, Sun AY, et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. *Proc Natl Acad Sci USA*. 2008;105:11105–11109.
159. Engelhart MJ, Geerlings MI, Ruitenberg A, et al. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*. 2002;287:3223–3229.
160. Bowman GL, Dodge H, Frei B, et al. Ascorbic acid and rates of cognitive decline in Alzheimer's disease. *J Alzheimers Dis*. 2009;16:93–98.
161. Paleologos M, Cumming RG, Lazarus R, et al. Cohort study of vitamin C intake and cognitive impairment. *Am J Epidemiol*. 1998;148:45–50.
162. Lin JL, Huang YH, Shen YC, et al. Ascorbic acid prevents blood-brain barrier disruption and sensory deficit caused by sustained compression of primary somatosensory cortex. *J Cerb Blood Flow Metab*. 2010;30:1121–1136.
163. Age-Related Eye Disease Study Research Group. AREDS report no. 9. A randomized, placebo-controlled trial of high dose supplementation with vitamins C, E and beta carotene for age-related cataract and vision loss. *Arch Ophthalmol*. 2001;119:1439–1452.
164. Kositsawat J, Freeman VL. Vitamin C and A1c relationship in the National Health and Nutrition Examination Survey (NHANES) 2003-2006. *J Am Coll Nutr*. 2011;30:477–483.
165. Estaban MA, Wang I, Qin B, et al. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. *Cell Stem Cell*. 2010;6:71–79.
166. Grant MM, Mistry N, Lunec J, et al. Dose-dependent modulation of the T cell proteome by ascorbic acid. *Br J Nutr*. 2007;97:19–26.
167. Covarrubias-Pinto A, Acuna AI, Beltran FA, et al. Old things new view: ascorbic acid protects the brain in neurodegenerative disorders. *Int J Mol Sci*. 2015;16:28194–28217.
168. Chan AC. Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol*. 1993;71:725–731.
169. Meydani SN, Meydani M, Blumberg JB, et al. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. *JAMA*. 1997;277:1380–1386.
170. De la Fuente M, Hernanz A, Guayerbas N, et al. Vitamin E ingestion improves several immune functions in elderly men and women. *Free Radic Res*. 2008;42:272–280.
171. Meydani SN, Leka LS, Fine BC, et al. Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA*. 2004;292:828–836.
172. Tsoureli-Nikita E, Hercogova J, Lotti T, et al. Evaluation of dietary intake of vitamin E in the treatment of atopic dermatitis: a study of the clinical course and evaluation of the immunoglobulin E serum levels. *Int J Dermatol*. 2002;41:146–150.
173. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med*. 1993;328:1444–1449.
174. Sesso HD, Buring JE, Christen WG, et al. Vitamins E and C in the prevention of cardiovascular disease and cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA*. 2008;300:2123–2133.
175. Frei B. Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. *J Nutr*. 2004;134:S3196–S3198.
176. Milman U, Blum S, Shapira C, et al. Vitamin E supplementation reduces cardiovascular events in a sub group of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin G2-2 genotype. *Arterioscler Thromb Vasc Biol*. 2008;28:341–347.
177. Lopes da Silva S, Vellas B, Elemans S, et al. Plasma nutrient status of patients with Alzheimer's disease: Systematic review and meta-analysis. *Alzheimers Dement*. 2014;10:485–502.
178. Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *N Engl J Med*. 1997;336:1216–1222.
179. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med*. 2005;352:2379–2388.
180. Dysken MW, Sano M, Asthana S, et al. Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial. *JAMA*. 2014;311:33–44.
181. Dorjgochoo T, Shrubsole MJ, Suh XO, et al. Vitamin supplement use and risk of breast cancer: the shanghai breast cancer study. *Breast Cancer Res Treat*. 2008;111:269–278.
182. Qiao Y-L, Dawsey SM, Kamangar F, et al. Total and cancer mortality after supplementation with vitamins and minerals: follow-up of the Linxian General Population Intervention Trial. *J Natl Cancer Inst*. 2009;101:507–518.
183. Hunter DJ, Manson JE, Colditz GA, et al. A prospective study of the intake of vitamins C, E and A and the risk of breast cancer. *N Engl J Med*. 1993;329:234–240.
184. Cui X, Rosner B, Wilett WC, et al. Antioxidant intake and risk of endometrial cancer: results from the Nurses' Health Study. *Int J Cancer*. 2011;128:1169–1178.
185. Slatore CG, Littman AJ, Au DH, et al. Long term use of supplemental multivitamins vitamin C, vitamin E, and folate does not reduce the risk of lung cancer. *Am J Respir Crit Care Med*. 2008;177:524–530.

186. Lin J, Cook NR, Albert C, et al. Vitamins C and E and beta carotene supplementation and cancer risk: a randomized, controlled trial. *J Natl Cancer Inst*. 2009;101:14–23.
187. Wang L, Sesso HD, Glynn RJ, et al. Vitamin E and C supplementation and risk of cancer in men: posttrial follow-up in the Physicians' Health Study II randomized trial. *Am J Clin Nutr*. 2014;100:915–923.
188. Klein EA, Thompson IM, Tangen Jr IM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 2011;306:1549–1556.
189. Major JM, Yu K, Weinstein SJ, et al. Genetic variants reflecting higher vitamin e status in men are associated with reduced risk of prostate cancer. *J Nutr*. 2014;144:729–733.
190. Xu R, Zhang S, Tao A, Zhang M. Influence of vitamin E supplementation on glycaemic control: a meta-analysis of randomized controlled trials. *PLoS One*. 2014;9:e95008.
191. Christen S, Woodall AA, Shigenaga MK, et al. Gamma tocopherol traps mutagenic electrophiles such as Nox and complements alpha tocopherol. Physiological implications. *Proc Natl Acad Sci U S A*. 1997;94:3217–3222.
192. Sjoholm A, Berggren PO, Coney RV. Gamma tocopherol partially protects insulin-secreting cell against functional inhibition by nitric oxide. *Biochem Biophy Res Commun*. 2000;277:334–340.
193. Park SK, Page GP, Kim K, et al. Differential effect of alpha- and gamma-tocopherol supplementation in age-related transcriptional alterations in heart and brain of B6/C3H mice. *J Nutr*. 2008;138:1010–1018.
194. Devaraj S, Leonard S, Traber MG, et al. Gamma-tocopherol supplementation alone or in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radica Biol Med*. 2008;44:1203–1208.
195. Key TJ, Appleby PN, Allen NE, et al. Plasma carotenoids, retinol, and tocopherols and the risk of prostate cancer in the European Prospective Investigation into Cancer and Nutrition Study. *Am J Clin Nutr*. 2007;86:672–681.
196. Mah E, Pei R, Guo Y, et al. γ-tocopherol-rich supplementation additively improves endothelial function during smoking cessation. *Free Radic Biol Med*. 2013;65:1291–1299.
197. Podda M, Weber C, Traber MG, et al. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols and ubiquinones. *J Lipid Res*. 1996;37:893–901.
198. Bachawal SV, Wali VB, Sylvester PW. Enhanced antiproliferative and apoptotic response to combined treatment of gamma-tocotrienol with erlotinib or gefitinib in mammary tumor cells. *BMC Cancer*. 2010;10:84–97.
199. Yam ML, Abdul Haifid SR, Cheng HM, et al. Tocotrienols suppress proinflammatory markers and cyclooxygenase-2 expression in RAW264.7 macrophages. *Lipids*. 2009;44:787–797.
200. Mustad VA, Smith CA, Ruey PP, et al. Supplementation with 3 compositionally different tocotrienol supplements does not improve cardiovascular disease risk factors in men and women with hypercholesterolemia. *Am J Clin Nutr*. 2002;76:1237–1243.
201. Chin SF, Hamid NA, Latiff AA, et al. Reduction of DNA damage in older healthy adults by Tri E Tocotrienol supplementation. *Nutrition*. 2008;24:1–10.
202. Han SN, Adolfsson O, Lee CK, et al. Age- and vitamin E-induced changes in gene expression profiles of T cells. *J Immunol*. 2006;177:6052–6061.
203. Tan BH, Sagoo P, Chan C, et al. Inhibition of NF-kB and oxidative pathways in human dendritic cells by antioxidant vitamins generates regulatory T cells. *J Immunol*. 2005;174:7633–7644.
204. Belisle SE, Leka LS, Delgado-Lista J, et al. Polymorphisms at cytokine genes may determine the effect of vitamin E on cytokine production in the elderly. *J Nutr*. 2009;139:1855–1860.
205. Pedeboscq S, Rey C, Petit M, et al. Non-antioxidant properties of α-tocopherol reduce the anticancer activity of several protein kinase inhibitors in vitro. *PLoS One*. 2012;7:e36811.
206. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med*. 2007;43:4–15.
207. Vile GF, Winterbourn CC. Inhibition of adriamycin-promoted microsomal lipid peroxidation by beta-carotene, alpha-tocopherol and retinal at high and low oxygen partial pressures. *FEBS Lett*. 1988;238:353–356.
208. Jialal I, Norkus EP, Cristol L, et al. Beta carotene inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta*. 1991;1086:134–138.
209. Rock CL. Carotenoids: biology and treatment. *Pharmacol Ther*. 1997;75:185–197.
210. Bendich A. Carotenoids and the immune response. *J Nutr*. 1989;119:112–115.
211. Mannisto S, Smith-Warner SA, Spiegelman D, et al. Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. *Cancer Epidemiol Biomarkers Prev*. 2004;13:40–48.
212. Gallichio L, Boyd K, Matanoski G, et al. Carotenoids and the risk of developing lung cancer: a systematic review. *Am J Clin Nutr*. 2008;88:372–383.
213. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst*. 1996;88:1550–1559.
214. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with β-carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med*. 1996;334:1145–1149.
215. Key TJ, Appleby PN, Travis RC, et al. Carotenoids, retinol, tocopherols, and prostate cancer risk: pooled analysis of 15 studies. *Am J Clin Nutr*. 2015;102:1142–1157.
216. Zhang S, Hunter DJ, Forman MR, et al. Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. *J Natl Cancer Inst*. 1999;91:547–556.
217. Larsson SC, Bergkvist L, Wolk A. Dietary carotenoids and risk of hormone-receptor defined breast cancer in a prospective cohort of Swedish women. *Eur J Cancer*. 2010;46:1079–1085.
218. Aune D, Chan DS, Vieira AR, et al. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systemic review and meta-analysis of prospective studies. *Am J Clin Nutr*. 2012;96:356–373.
219. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C and E, and advanced age-related macular degeneration: eye disease case-control study group. *JAMA*. 1994;272:1413–1420.
220. Age-Related Eye Disease Study 2 Research Group, Chew EY, Clemons TE, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA Ophthalmol*. 2014;132:142–149.
221. Erdman Jr JW, Ford NA, Lindshield BL. Are the health attributes of lycopene related to its antioxidant function? *Arch Biochem Biophysics*. 2009;483:229–235.
222. Chalab N, Delort L, Satih S, et al. Immunohistochemical expression of RARalpha, RARbeta and CX43 in breast tumor cell lines after treatment with lycopene and correlation with RT-QPCR. *J Histochem Cytochem*. 2007;55:877–883.
223. Maguire JJ, Kagan V, Ackrell BA, et al. Succinate-ubiquinone reductase linked recycling of alpha-tocopherol in reconstituted systems and mitochondria: requirement for reduced ubiquinone. *Arch Biochem Biophys*. 1992;292:47–53.
224. Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoproteins more efficiently against lipid peroxidation than does alpha tocopherol. *Proc Natl Acad Sci USA*. 1991;88:1646–1650.
225. Niklowitz P, Sonnenshein A, Janetzky B, et al. Enrichment of coenzyme Q10 in plasma and blood cells. Defense against oxidative damage. *Int J Biol Sci*. 2007;3:257–262.
226. Shargorodsky M, Debbi O, Matas Z, et al. Effect of long term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q10 and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risk factors. *Nutr Metab*. 2010;7:55. PMCID: PMC2911454.
227. Molyneux SL, Florkowski CM, Richards AM, et al. Coenzyme Q10: an adjunctive therapy for congestive heart failure? *NZ Med J*. 2009;122:74–79.
228. De Pinieux G, Chariot P, Ammi-Said M, et al. Lipid-lowering drugs and mitochondrial function: effects of HMG-CoA reductase inhibitors on

serum ubiquinone and blood lactate/pyruvate ratio. *Br J Clin Pharmacol*. 1996;42:333–337.
229. Lei L, Liu Y. Efficacy of coenzyme Q10 in patients with cardiac failure: a meta-analysis of clinical trials. *BMC Cardiovasc Disord*. 2017;17:196.
230. Mortensen SA, Rosenfeldt F, Kumar A, et al. The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. *JACC Heart Fail*. 2014;2:641–649.
231. Ziegler D, Nowak H, Kempler P, et al. Treatment of symptomatic diabetic neuropathy with the antioxidant alpha lipoic acid: a meta-analysis. *Diabetic Med*. 2004;21:114–121.
232. Smith AR, Shenvi SV, Widlansky M, et al. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem*. 2004;11:1135–1146.
233. Jacob S, Rett K, Henriksen EJ, Haring HU. Thioctic acid—effects on insulin sensitivity and glucose-metabolism. *Biofactors*. 1999;10:169–174.
234. Liu J, Killilea DW, Ames BN. Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate binding affinity and activity in brain by feeding old rats acetyl-L-carnitine and/or R-alpha-lipoic acid. *Proc Natl Acad Sci USA*. 2002;99:1876–1881.
235. Arivazhagan P, Shila S, Kumaran S, et al. Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and antioxidant enzymes in various brain regions of aged rats. *Exp Gerontol*. 2002;37:803–811.
236. Aliev G, Liu J, Shenk JC, et al. Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats. *J Cell Mol Med*. 2009;13:320–333.
237. Wang Y, Li X, Guo Y, et al. Alpha lipoic acid increases energy expenditure by enhancing adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor gamma coactivator-1-alpha signaling in the skeletal muscle of aged rats. *Metabolism*. 2010;59:967–976.
238. Maczurek A, Hager K, Kenlies M, et al. Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease. *Adv Drug Deliv Rev*. 2008;60:1463–1470.
239. Zhang WJ, Bird KE, McMillen TS, et al. Dietary lipoic acid supplementation inhibits atherosclerotic lesion development in apolipoprotein E deficient and apolipoprotein E/low density lipoprotein receptor deficient mice. *Circulation*. 2008;117:421–428.
240. Li C-J, Zhang QM, Zhang J, et al. Attenuation of myocardial apoptosis by alpha lipoic acid through suppression of oxidative stress to reduce diabetic cardiomyopathy. *Chinese Med J*. 2009;122:2580–2586.
241. Suh JH, Shigeno ET, Morrow JD, et al. Oxidative stress in the aging rat heart is reversed by dietary supplementation with (R)-(alpha)-lipoic acid. *FASEB J*. 2001;15:700–706.
242. Ziegler D. Thioctic acid for patients with symptomatic diabetic polyneuropathy: a critical review. *Treat Endocrinol*. 2004;3:173–189.
243. Carbonelli MG, Di Renzo L, Bigioni M, et al. Alpha lipoic acid supplementation: a tool for obesity therapy? *Curr Pharm Des*. 2010:16840–16846.
244. Suh JH, Shenvi SV, Dixon BM, et al. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible by lipoic acid. *Proc Natl Acad Sci USA*. 2004;101:3381–3386.
245. Zhang L, Xing GQ, Barker JL, et al. Alpha lipoic acid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Akt signaling pathway. *Neurosci Lett*. 2001;312:125–128.
246. Yim CY, Hibbs Jr JB, McGregor JR, et al. Use of N-acetyl cysteine to increase intracellular glutathione during induction of antitumor responses by IL-2. *J Immunol*. 1994;152:5796–5805.
247. Roes EM, Raijmakers MT, Peters WH, et al. Effects of oral N-acetylcysteine on plasma homocysteine and whole blood glutathione levels in healthy, non-pregnant women. *Clin Chem Lab Med*. 2002;40:496–498.
248. Wu G, Fang YZ, Yang S, et al. Glutathione metabolism and its implications for health. *J Nutr*. 2004;134:489–492.
249. Iantomasi T, Favilli F, Marraccini P, et al. Glutathione transport system in human small intestine epithelial cells. *Biochim Biophys Acta*. 1997;1330:274–283.
250. Zembron-Lacny A, Ostapiuk J, Szyszka K. Effects of sulfur-containing compounds on plasma redox status in muscle damaging exercise. *Chin J Physiol*. 2009;52:289–294.
251. Martina V, Masha A, Gigliardi VR, et al. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. *Diabetes Care*. 2008;31:940–943.
252. Thomas NO, Shay KP, Kelley AR, et al. Glutathione maintenance mitigates age-related susceptibility to redox cycling agents. *Redox Biol*. 2016;10:45–52.
253. Ames BN. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci USA*. 2006;103:17589–17594.
254. Tejero-Taldo MI, Kramer JH, Mak I, et al. The nerve-heart connection in the pro-oxidant response to Mg-deficiency. *Heart Fail Rev*. 2006;11:35–44.
255. Killilea DW, Ames BN. Magnesium deficiency accelerates cellular senescence in culture human fibroblasts. *Proc Natl Acad Sci USA*. 2008;105:5768–5773.
256. Petrovic J, Stanic D, Dmitrasinovic G, et al. Magnesium supplementation diminishes peripheral blood lymphocyte DNA oxidative damage in athlete and sedentary young man. *Oxid Med Cell Longev*. 2016;2019643. PMCID: PMC4799816.
257. Mazur A, Maier JA, Rock E, et al. Magnesium and the inflammatory response: potential physiopathological implications. *Arch Biochem Biophys*. 2007;458:48–56.
258. Rayssiguier Y, Gueux E, Nowacki W, et al. High fructose consumption combined with low dietary magnesium intake may increase the incidence of the metabolic syndrome by inducing inflammation. *Magnes Res*. 2006;19:237–243.
259. Dibaba DT, Xun P, Fly AD, et al. Dietary magnesium intake and risk of metabolic syndrome: a meta-analysis. *Diabet Med*. 2014;31:1301–1309.
260. Truong-Tan AQ, Ho LH, Chai F, et al. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. *J Nutr*. 2000;130(suppl 5):S1459–S1466.
261. Prasad AS. Zinc: mechanisms of host defense. *J Nutr*. 2007;137:1345–1349.
262. Shen H, Oesterling E, Stromberg A, et al. Zinc deficiency induces vascular pro-inflammatory parameters associated with NF-kappaB and PAR signaling. *J Am Coll Nutr*. 2008;27:577–587.
263. Bao B, Prasad AS, Beck FWJ, et al. Zinc supplementation decreases oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients. *Transl Res*. 2008;152:67–80.
264. Kahmann L, Uciechowski P, Warmuth S, et al. Zinc supplementation in the elderly reduces spontaneous inflammatory cytokine release and restores T cell functions. *Rejuvenation Res*. 2008;11:227–237.
265. Ames BN, Liu J. Delaying the mitochondrial decay of aging with acetylcarnitine. *Ann NY Acad Sci*. 2004;1033:108–116.
266. Savitha S, Sivarajan K, Haripriya D, et al. Efficacy of levo-carnitine and alpha lipoic acid in ameliorating the decline in mitochondrial enzymes during aging. *Clin Nutr*. 2005;24:794–800.
267. Liu J, Head E, Gharib AM, et al. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or alpha-lipoic acid. *Proc Natl Acad Sci USA*. 2002;99:2356–2361.
268. Savitha S, Panneerselvan C. Mitigation of age-dependent oxidative damage to DNA in rat heart by carnitine and lipoic acid. *Mech Ageing Dev*. 2007;128:206–212.
269. Lee BJ, Lin JS, Lin YC, Lin PT. Effects of L-carnitine supplementation on lipid profiles in patients with coronary artery disease. *Lipids in Health and Disease*. 2016;15:107.
270. Lee BJ, Lin JS, Lin YC, Lin PT. Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial. *Nutri J*. 2014;13:79.
271. Ames BN, Cathcart R, Schwiers E, et al. Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA*. 1981;78:6858–6862.

272. Wayner DD, Burton GW, Ingold KU, et al. Quantitative measurement of the total peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. *FEBS Lett*. 1985;187:33–37.
273. Kampa M, Nistikaki A, Tsaousis V, et al. A new automated method for the determination of the total antioxidant capacity (TAC) of human plasma, based on the crocin bleaching assay. *BMC Clin Pathol*. 2002;2:3. PMCID: PMC128814.
274. Stetinova V, Smetanova L, Grossmann V, et al. In vitro and in vivo assessment of the antioxidant activity of melatonin and related indole derivatives. *Gen Physiol Biophys*. 2002;21:153–162.
275. Okatani Y, Wakatsuki A, Reiter RJ, et al. Hepatic mitochondrial dysfunction in senescence-accelerated mice: correction by long-term, orally administered physiological levels of melatonin. *J Pineal Res*. 2002;33:127–133.
276. Aydogan S, Yerer MB, Goktas A. Melatonin and nitric oxide. *J Endocrinol Invest*. 2006;29:281–287.
277. Murawska-Cialowicz E, Jethon Z, Magdalen J, et al. Effects of melatonin on lipid peroxidation and antioxidative enzyme activities in the liver, kidneys and brain of rats administered with benzo(a)pyrene. *Exp Toxicol Pathol*. 2009;63:97–103.
278. Winiarska K, Fraczyk T, Malinska D, et al. Melatonin attenuates diabetes-induced oxidative stress in rabbits. *J Pineal Res*. 2006;40:168–176.
279. Szewczyk-Golec K, Rajewski P, Gackowski M, et al. Melatonin supplementation lowers oxidative stress and regulates adipokines in obese patients on a calorie-restricted diet. *Oxidative Medicine and Cellular Longevity*. 2017. https://doi.org/10.1155/2017/8494107.
280. Favero G, Franceschetti L, Bonomini F, et al. Melatonin as an anti-inflammatory agent modulating inflammasome activation. *Int J Endocrinol*. 2017. https://doi.org/10.1155/2017/1835195.
281. Tapiero H, Tew KD, Ba GN, et al. Polyphenols: do they play a role in the prevention of human pathologies? *Biomed Pharmacother*. 2002;56:200–207.
282. Manach C, Williamson G, Morand C, et al. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(suppl):S230–S242.
283. Ovaskainen ML, Torronen R, Koponen JM, et al. Dietary intake and major food sources of polyphenols in Finnish adults. *J Nutr*. 2008;138:562–566.
284. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, et al. Estimation of dietary source of flavonoid intake in Spanish adult population (EPIC-Spain). *J Am Diet Assoc*. 2010;110:390–398.
285. Kidd PM. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea and grape seed extracts. *Alt Med Rev*. 2009;14:226–246.
286. Liu Z, Hu M. Natural polyphenol disposition via coupled metabolic pathways. *Expert Opin Drug Metab Toxicol*. 2007;3:389–406.
287. Loke WM, Jenner AM, Proudfoot JM, et al. A metabolic profiling approach to identify biomarkers of flavonoid intake in humans. *J Nutr*. 2009;139:2309–2314.
288. Rice-Evans CA, Miller NJ, Pagangu G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Bio Med*. 1996;20:933–956.
289. Roy MK, Coide M, Rao TP, et al. ORAC & DPPH assay comparison to assess antioxidant capacity of tea infusions: relationship between total polyphenol and individual catechin content. *Int J Food Sci Nutr*. 2010;61:109–124.
290. De Whalley CV, Rankin SM, Hoult JR, et al. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem Pharmacol*. 1990;39:1743–1750.
291. Afanas'ev IB, Dorozhko AI, Brodskii AV, et al. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol*. 1989;38:1763–1769.
292. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence or epiphenomenon? *Free Radic Biol Med*. 2006;41:1727–1746.
293. Lotito SB, Frei B. Relevance of apple polyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. *Free Radic Biol Med*. 2004;36:201–211.
294. Lotito SB, Frei B. The increase in plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. *Free Radic Biol Med*. 2004;37:251–258.
295. Rothwell JA, Urpi-Sarda M, Boto-Ordonez M, et al. Systematic analysis of the polyphenol metabolome using the Phenol-Explorer database. *Mol Nutr Food Res*. 2016;60:203–211.
296. Cutler GJ, Nettleton JA, Ross JA, et al. Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. *Int J Cancer*. 2008;123:664–671.
297. Arts IC, Hollman PC, Bueno De Mesquita HB, et al. Dietary catechins and epithelial cancer incidence: the Zutphen elderly study. *Int J Cancer*. 2001;92:298–302.
298. Hertog MG, Feskens EJ, Hollman PC, et al. Dietary flavonoids and cancer risk in the Zutphen elderly study. *Nutr Cancer*. 1994;22:175–184.
299. Hertog MG, Feskens EJ, Hollman PC, et al. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet*. 1993;342:1007–1011.
300. di Gesso JL, Kerr JS, Zhang Q, et al. Flavonoid metabolites reduce tumor necrosis factor-α secretion to a greater extent than their precursor compounds in human THP-1 monocytes. *Mol Nutr Food Res*. 2015;59:1143–1154.
301. Ferrari D, Cimino F, Fratantonio D, et al. Cyanidin-3-O-glucoside modulates the in vitro inflammatory crosstalk between intestinal epithelial and endothelial cells. *Mediators Inflamm*. 2017. https://doi.org/10.1155/2017/3454023. PMCID: PMC5360945.
302. Hassan W, Noreen H, Rehman S, et al. Oxidative stress and antioxidant potential of one hundred medicinal plants. *Curr Top Med Chem*. 2017;17:1336–1370.
303. Mink PJ, Scrafford CG, Barraj LM, et al. Flavonoid intake and the risk of cardiovascular disease in postmenopausal women. *Am J Clin Nutr*. 2007;85:895–909.
304. McCullough ML, Peterson JJ, Patel R, et al. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *AM J Clin Nutr*. 2012;95:454–464.
305. Lin J, Rexrode KM, Hu F, et al. Dietary intakes of flavanols and flavones and coronary heart disease in US women. *Am J Epidemiol*. 2007;165:1305–1313.
306. Rimm EB, Katan MB, Ascherio A, et al. Relation between intake of flavonoids and risk of coronary heart disease in male health professionals. *Ann Intern Med*. 1996;125:384–389.
307. Dolara P, Luceri C, De Filippo C, et al. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutat Res*. 2005;591:237–246.
308. Grisham MB, MacDermott RP, Deitch EA. Oxidant defense mechanisms in the human colon. *Inflammation*. 1990;14:669–680.
309. Halliwell B, Rafter J, Jenner A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *Am J Clin Nutr*. 2005;81(suppl):S268–S276.
310. Dryden GW, Song M, McClain C. Polyphenols and gastrointestinal disease. *Curr Opin Gastroenterol*. 2006;22:165–170.
311. Romier B, Schneider YJ, Larondelle Y, et al. Dietary polyphenols can modulate the intestinal inflammatory response. *Nutr Rev*. 2009;67:363–378.
312. Keshavarzian A, Sedghi S, Kanotsky J, et al. Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe. *Gastroenterol*. 1992;103:177–185.
313. Mota KS, Dias ME, Luiz-Ferreira A, et al. Flavonoids with gastroprotective activity. *Molecules*. 2009;14:979–1012.
314. Hoensch HP, Krich W. Potential role of flavonoids in the prevention of intestinal neoplasia: a review of their mode of action and their primary clinical perspectives. *Int J Gastroenterol Cancer*. 2005;35:187–195.
315. Skyberg JA, Robison A, Golden S, et al. Apple polyphenols require T cells to ameliorate dextran sulfate sodium-induced colitis and dampen proinflammatory cytokine expression. *J Leukoc Biol*. 2011;90:1043–1054.
316. Joy S, Siow RC, Rowlands DJ, et al. The isoflavone equol mediates rapid vascular relaxation: Ca-independent activation of endothelial nitric oxide synthase involving ERK1/2 and Akt phosphorylation in human endothelial cells. *J Biol Chem*. 2006;281:27335–27345.

317. Mann GE, Bonacasa B, Ishi TY, et al. Targeting the redox sensitive Nrf2-Keap1 pathway in cardiovascular disease: protection afforded by dietary isoflavones. *Curr Opin Pharmacol*. 2009;9:139–145.
318. Hooper L, Kroon PA, Rimm EB, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2008;88:38–50.
319. Hall WL, Formanuik NL, Harnparich DM, et al. A meal enriched with soy isoflavones increases nitric oxide mediated vasodilation in healthy postmenopausal women. *J Nutr*. 2008;138:1288–1292.
320. Marini H, Bitto A, Altavilla D, et al. Efficacy of genistein aglycone on some cardiovascular risk factors and homocysteine levels: a follow-up study. *Nutr Metab Cardiovasc Dis*. 2010;20:332–340.
321. Dong JY, Qin LQ. Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Res Treat*. 2011;125:315–323.
322. Willett W. Lessons from dietary studies in Adventists and questions for the future. *Am J Clin Nutr*. 2003;78:539S–543S.
323. Su SJ, Chow NH, Kung ML, et al. Effects of soy isoflavones on apoptosis induction and G2-M arrest in human hepatoma cells involvement of capsase-3 activation, Bcl-2 and Bcl-XL downregulation and Cdc2 activity. *Nutr Cancer*. 2003;45:113–123.
324. Sarkar FH, Li Y. Soy isoflavones and cancer prevention. *Cancer Invest*. 2003;21:744–757.
325. Yan L, Spitznagel EL. Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am J Clin Nutr*. 2009;89:1156–1163.
326. Figueira I, Menezes R, Macedo D, et al. Polyphenols beyond barriers: a glimpse into the brain. *Curr Neuropharmacol*. 2017;15:562–594.
327. Blumberg JG, Vita JA, Chen CY. Concord grape juice polyphenols and cardiovascular risk factors: dose-response relationships. *Nutrients*. 2015;7:10032–10052.
328. Nilsson A, Salo I, Plaza M, Bjorck I. Effects of a mixed berry beverage on cognitive functions and cardiometabolic risk markers; A randomized cross-over study in healthy older adults. *PLoS*. 2017;12:e0188173. PMCID: PMC5687726.
329. Whitehead TP, Robinson D, Allaway S, et al. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin Chem*. 1995;41:32–35.
330. Simonetti P, Gardana C, Pietta P. Plasma levels of caffeic acid and antioxidant status after red wine intake. *J Agric Food Chem*. 2001;49:5964–5968.
331. Hollis JH, Houchins JA, Blumberg JB, et al. Effects of Concord grape juice on appetite, diet, body weight, lipid profile and antioxidant status in adults. *J Am Coll Nutr*. 2009;289:574–582.
332. Shi N, Chen F, Zhang X, et al. Suppression of oxidative stress and NFκB/MAPK signaling by lyophilized black raspberries for esophageal cancer prevention in rats. *Nutrients*. 2017;9:413.
333. Nimptsch K, Zhang X, Cassidy, et al. Habitual intake of flavonoid subclasses and risk of colorectal cancer in 2 large prospective cohorts. *Am J Clin Nutr*. 2016;103:184–191.
334. Woo HD, Kim J. Dietary flavonoid intake and smoking-related cancer risk: a meta-analysis. *PLoS One*. 2013;8:e75604.
335. Hokayem M, Blond E, Vidal H, et al. Grape polyphenols prevent fructose-induced oxidative stress and insulin resistance in first-degree relatives of type 2 diabetic patients. *Diabetes Care*. 2013;36:1454–1461.
336. Soltani R, Gorji A, Asgary S, et al. Evaluation of the effects of *Cornus mas* L. fruit extract on glycemic control and insulin level in type 2 diabetic adult patients: a randomized double-blind placebo-controlled clinical trial. *Evid Based Complement Alternat Med*. 2015:740954.
337. Zhang LD, Liu Y, Sun R, et al. Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. *J Nutr*. 2015;145:742–748.
338. Devore EE, Kang JH, Breteler MM, Grodstein F. Dietary intake of berries and flavonoids in relation to cognitive decline. *Ann Neurol*. 2012;72:135–143.
339. Moosavi F, Hosseini R, Saso L, Firuzi O. Modulation of neurotrophic signaling pathways by polyphenols. *Drug Des Devel Ther*. 2016;10:23–42.
340. Pribis P, Shukitt-Hale B. Cognition: the new frontier for nuts and berries. *Am J Clin Nutr*. 2014;100(suppl 1):347S–352S.
341. Boespglug EL, Eliassen JC, Dudley JA, et al. Enhanced neural activation with blueberry supplementation in mild cognitive impairment. *Nutr Neurosci*. 2017. https://doi.org/10:1080/1028415X.2017.1287833.
342. West SG, McIntyre MD, Piotrowski MJ, et al. Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults. *Br J Nutr*. 2014;111:653–661.
343. Njike VY, Faridi Z, Shuval K, et al. Effect of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults. *Int J Cardiol*. 2011;149:83–88.
344. Monagas M, Kahan N, Andres-Lacueve C, et al. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients of cardiovascular disease. *Am J Clin Nutr*. 2009;90:1144–1150.
345. Sansone R, Rodriguez-Mateos A, Heuel J, et al. Cocoa flavanol intake improves endothelial function and Framingham Risk Score in healthy men and women: a randomized, controlled, double-masked trial: the Flaviola Health Study. *Br J Nutr*. 2015;114:1246–1255.
346. Hooper L, Kay C, Abdelhamid A, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *Am J Clin Nutr*. 2012;95:740–751.
347. Dower JI, Geleijnse JM, Gijsbers L, et al. Supplementation of the pure flavonoids epicatechin and quercetin affects some biomarkers of endothelial dysfunction and inflammation in (pre) hypertensive adults: a randomized double-blind, placebo-controlled, crossover trial. *J Nutr*. 2015;145:1459–1463.
348. Mastroiacovo D, Kwik-Uribe C, Grassi D, et al. Cocoa flavanol consumption improves cognition function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study—a randomized controlled trial. *Am J Clin Nutr*. 2015;10:538–548.
349. Sorond FA, Lipsitz LA, Hollenberg NK, et al. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatr Dis Treat*. 2008;4:433–440.
350. Lin X, Zhang I, Li A, et al. Cocoa flavanol intake and biomarkers for cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *J Nutr*. 2016;146:2325–2333.
351. Grassi D, Desideri G, Necozione S, et al. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr*. 2008;138:1671–1676.
352. Cassidy A, Rogers G, Peterson JJ, et al. Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults. *Am J Clin Nutr*. 2015;102:172–181.
353. Ju J, Lu G, Lambert JD, et al. Inhibition of carcinogenesis by tea constituents. *Semin Cancer Biol*. 2007;17:395–402.
354. Yang CS, Wang X, Lu G, et al. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat Rev Cancer*. 2009;9:429–439.
355. Wu AH, Tseng CC, Van Den Berg D, et al. Tea intake, COMT genotype, and breast cancer in Asian-American women. *Cancer Res*. 2003;63:7526–7529.
356. Bettuzzi S, Brausi M, Rizzi F, et al. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high grade prostate interepithelial neoplasia: a preliminary report from a one year proof of principle study. *Cancer Res*. 2006;66:1234–1240.
357. Hoensch H, Groh B, Edler L, Kirch W. Prospective cohort comparison of flavonoid treatment in patients with resected colorectal cancer to prevent recurrence. *World J Gastroenterol*. 2008;14:2187–2193.
358. Loke WM, Proudfoot JM, Hodgson JM, et al. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler Thromb Vasc Biol*. 2010;30:749–757.
359. Peters U, Poole C, Arab L. Does tea affect cardiovascular disease? A meta-analysis. *Am J Epidemiol*. 2001;154:495–503.
360. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. *Stroke*. 2009;40:1786–1792.
361. Wang X, Tian J, Jiang J, et al. Effects of green tea or green tea extract on insulin sensitivity and glycaemic control in populations at risk of type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *J Hum Nutr Diet*. 2014;27:501–512.

362. Liu K, Zhou R, Wang B, et al. Effect of green tea on glucose control and insulin sensitivity: a meta-analysis of 17 randomized controlled trials. *Am J Clin Nutr*. 2013;98:340–348.
363. Rohdewald P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse pharmacology. *Int J Clin Pharmacol Ther*. 2002;40:158–168.
364. Ko JW, Lee IC, Park SH, et al. Protective effects of pine bark extract against cisplatin-induced hepatotoxicity and oxidative stress in rats. *Lab Anim Res*. 2014;30:174–180.
365. Cho KJ, Yun CH, Packer L, et al. Inhibition mechanisms of bioflavonoids extracted from the bark of Pinus maritima on the expression of proinflammatory cytokines. *Ann N Y Acad Sci*. 2001;928:141–156.
366. Grimm T, Chovanova Z, Muchova J, et al. Inhibition of NF-kappaB and MM-9 secretion by plasma of human volunteers after ingestion of maritime pine bark extract (Pycnogenol). *J Inflamm*. 2006;3:1. PMCID: PMC1413525.
367. Busserolles J, Geux E, Balasinska B, et al. In vivo antioxidant capacity of procyanidin-rich extracts from grape seed and pine (Pinus maritima) bark in rats. *Int J Vitamin Nutr Res*. 2006;76:22–27.
368. Schafer A, Chovanova Z, Muchova J, et al. Inhibition of COX-1 and COX-2 activity by plasma of human volunteers after ingestion of French maritime pine bark extract (Pycnogenol). *Biomed Pharmacother*. 2006;60:5–9.
369. McAnulty SR, McAnulty LS, Nieman DC, et al. Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. *Appl Physiol Nutr Metab*. 2008;33:254–262.
370. Cisar P, Jany R, Waczulikova I, et al. Effect of pine bark extract (Pycnogenol) on symptoms of knee osteoarthritis. *Phytother Res*. 2008;22:1087–1092.
371. Belcaro G, Cesarone MR, Errichi S, et al. Treatment of osteoarthritis with Pycnogenol®. The SVOS (San Valentino Osteo-Arthrosis Study). Evaluation of signs, symptoms, physical performance and vascular aspects. *Phytother Res*. 2007;22:518–523.
372. Natella F, Belelli F, Gentili V, et al. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. *J Agric Food Chem*. 2002;50:7720–7725.
373. Polagruto JA, Gross HB, Kamangar F, et al. Platelet reactivity in male smokers following acute consumption of flavonol-rich grapeseed extract. *J Med Food*. 2007;10:725–730.
374. Grases F, Prieto RM, Fernandez-Cabot RA, et al. Effect of consuming a grape seed supplement with abundant phenolic compounds on the oxidative status of healthy human volunteers. *Nutr J*. 2015;14:94.
375. Kar P, Laight D, Rooprai HK, et al. Effects of grape seed extract in type 2 diabetic subjects at high cardiac risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. *Diabet Med*. 2009;26:526–531.
376. Zhang H, Liu S, Li L, et al. The impact of grape seed extract treatment on blood pressure changes. *Med*. 2016;95:e4247. PMCID: PMC5370781.
377. Bagchi D, Bagchi M, Stohs SJ, et al. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicol*. 2000;148:187–197.
378. Ono K, Condron MM, Ho L, et al. Effects of grape seed-derived polyphenols on amyloid beta-protein self-assembly and cytotoxicity. *J Biol Chem*. 2008;283:32176–32187.
379. Nandakumar V, Singh T, Katiyar SK. Multi-targeted prevention and therapy by proanthocyanidins. *Cancer Lett*. 2008;269:378–387.
380. Li W, Zu B, Xu J, et al. Procyanidins produce significant attenuation of doxorubicine-induced cardiotoxicity via suppression of oxidative stress. *Basic Clin Pharmacol Toxicol*. 2009;104:192–197.
381. Rong Y, Geng Z, Lau BH. Ginkgo biloba attenuates oxidative stress in macrophages and endothelial cells. *Free Radic Biol Med*. 1996;20:121–127.
382. Otamiri T, Tagesson C. *Ginkgo biloba* extract prevents mucosal damage associated with small intestinal ischemia. *Scand J Gastroenterol*. 1989;24:666–670.
383. Rimbach G, Gohil K, Matsugo S, et al. Induction of glutathione synthesis in human keratinocytes by Ginkgo biloba extract (EGb 761). *Biofactors*. 2001;15:39–52.
384. Pierre SV, Lesnik P, Bonello L, et al. The standardized Ginkgo biloba extract Egb-761 protects vascular endothelium exposed to oxidized low density lipoproteins. *Cell Mol Biol*. 2008;54(suppl):1032–1042.
385. Bridi R, Crossetti FP, Steffen VM, et al. The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. *Phytother Res*. 2001;15:449–451.
386. Schindowski K, Leutner S, Kressmann S, et al. Age-related increase of oxidative stress-induced apoptosis in mice prevention by Ginkgo biloba extract (EGb 761). *J Neural Transm*. 2001;108:969–978.
387. Kudolo GR, Delaney D, Blodgett J. Short-term ingestion of Ginkgo biloba extract (EGb 761) reduces malondialdehyde levels in washed platelets of type 2 diabetic patients. *Diabetes Res Clin Pract*. 2005;68:29–38.
388. Snitz BE, O'Meara ES, Carson MC, et al. Ginkgo Evaluation of Memory (GEM) Study Investigators. Gingko biloba for preventing cognitive decline in older adults: a randomized trial. *JAMA*. 2009;302:2663–2670.
389. Schneider LS. Ginkgo and AD: key negatives and lessons from Guid-Age. *Lancet Neurol*. 2012;11:836–837.
390. Demarin V, Basic Kes V, Trkanjec Z, et al. Efficacy and safety of Ginkgo biloba standardized extract in the treatment of vascular cognitive impairment: a randomized, double-blind, placebo-controlled clinical trial. *Neuropsychiatr Dis Treat*. 2017;13:483–490.
391. Patel J, Joseph C, Corcoran GB, et al. Silymarin modulates doxorubicine-induced oxidative stress, Bcl-xL and p35 expression while preventing apoptotic and necrotic cell death in the liver. *Toxicol Appl Pharmacol*. 2010;245:143–152.
392. Hawke RL, Schrieber SJ, Soule TA, et al. Silymarin ascending multiple oral dosing phase I studies in noncirrhotic patients with chronic hepatitis C. *J Clin Pharmcol*. 2010;50:434–449.
393. De Avelar CR, Pereira EM, de Farias Costa PR, et al. Effect of silymarin on biochemical indicators in patients with liver disease: systemic review with meta-analysis. *World J Gastroenterol*. 2017;23:5004–5017.
394. Zhong S, Fan Y, Yan Q, et al. The therapeutic effect of silymarin in the treatment of nonalcoholic fatty disease. *Med*. 2017;96:e9061. PMCID: PMC5728929.
395. Velussi M, Cerrigoi AM, De Monte A, et al. Long term (12 months) treatment with an antioxidant drug, silymarin, is effective on hyperinsulinemia, exogenous insulin need and malondialdehyde levels in cirrhotic diabetic patients. *J Hepatol*. 1997;26:871–879.
396. Huseini HF, Larijani B, Heshmat R, et al. The efficacy of Silybum marianum (L) Gaertn. (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled clinical trial. *Phytother Res*. 2006;20:1036–1039.
397. Voroneanu L, Nistor I, Dumea R, et al. Silymarin in type 2 diabetes mellitus: a systemic review and meta-analysis of randomized controlled trials. *J Diabetes Res*. 2016;5147468. PMCID: PMC4908257.
398. Singh RP, Raina K, Sharma G, et al. Silibinin inhibits established prostate tumor growth, progression, invasion, and metastasis and suppresses tumor angiogenesis and epithelial-mesenchymal transition in transgenic adenocarcinoma of the mouse prostate model. *Clin Cancer Res*. 2008;14:7773–7780.
399. Kim HP, Mani I, Iversen L, et al. Effects of naturally-occurring flavonoids and bioflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea pigs. *Prostaglandins Leukot Essent Fatty Acids*. 1998;58:17–24.
400. Lotito SB, Frei B. Dietary flavonoids attenuate tumor necrosis factor-alpha induced adhesion molecule expression in human aortic endothelial cells. *J Biol Chem*. 2006;281:37102–37110.
401. Shanely RA, Knab AM, Nieman DC, et al. Quercetin supplementation does not alter antioxidant status in humans. *Free Radica Res*. 2010;44:224–231.
402. Haleagrahara N, Radhakrishnan A, Lee N, et al. Flavonoid quercetin protects against swimming stress-induced changes in oxidative biomarkers in the hypothalamus of rats. *Eur J Pharmacol*. 2009;621:46–52.
403. Davis JM, Carlstedt CJ, Chen S, et al. The dietary flavonoid quercetin increases VO(2max) and endurance capacity. *Int J Sport Nutr Exerc Metab*. 2010;20:56–62.
404. Loke WM, Hodgson JM, Proudfoot JM, et al. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr*. 2008;88:1018–1025.
405. Edwards RL, Lyon T, Litwin SE, et al. Quercetin reduces blood pressure in hypertensive subjects. *J Nutr*. 2007;137:2405–2411.

406. Valerio Jr LG, Kepa JK, Pickwell GV, et al. Induction of human NADPH quinone oxidoreductase (NQO1) gene expression by the flavonol quercetin. *Toxicol Lett*. 2001;119:49–57.
407. Kamaraj S, Vinodhkumar R, Anandakumar R, et al. The effects of quercetin on antioxidant status and tumor markers in lung and serum of mice treated with benzo(a)pyrene. *Biol Pharmac Bull*. 2007;30:2268–2273.
408. Srivastava S, Somasagara RR, Hegde M, et al. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. *Sci Rep*. 2016;6:24049. PMCID: PMC4828642.
409. Lam TK, Rotunno M, Lubin JH, et al. Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue and lung cancer risk. *Carcinogenesis*. 2010;31:634–642.
410. Mijkstacka R, Rimando AM, Ignatowicz E. Antioxidant effect of trans resveratrol, pterostilbene, quercetin and their combinations in human erythrocytes in vitro. *Plant Foods Hum Nutr*. 2010;65:57–63.
411. Wu JM, Wang ZR, Hsieh TC, et al. Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine. *Int J Mol Med*. 2001;8:3–17.
412. Das S, Das DK. Anti-inflammatory responses of resveratrol. *Inflamm Allergy Drug Targets*. 2007;6:168–173.
413. Dekkers DHW, Bezstarosti K, Gurusamy N, et al. Identification by a differential proteomic approach of the induced stress and redox proteins by resveratrol in the normal and diabetic rat heart. *J Cell Mol Med*. 2008;12:1677–1689.
414. Gresele P, Pignatelli P, Guglielmini G, et al. Resveratrol at concentrations attainable with moderate wine consumption stimulates human platelet nitric oxide production. *J Nutr*. 2008;138:1602–1608.
415. Takahashi S, Nakashima Y. Repeated and long-term treatment with physiological concentrations of resveratrol promotes NO production in vascular endothelial cells. *Br J Nutr*. 2012;107:774–780.
416. Tome-Carneiro J, Gonzalvez M, Larrosa M, et al. One-year consumption of a grape nutraceutical containing resveratrol improves the inflammatory and fibrinolytic status of patients in primary prevention of cardiovascular disease. *Am J Cardiol*. 2012;110:356–363.
417. Thirunavukkarasu M, Penumathsa SV, Koneru S, et al. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of nitric oxide, thioredoxin and heme oxygenase. *Free Radic Biol Med*. 2007;43:720–729.
418. Movahed A, Nabipour I, Lieben LX, et al. Antihyperglycemic effects of short term resveratrol supplementation in type 2 diabetic patients. *Evid Based Complement Alternat Med*. 2013:851267.
419. Udenigwe CC, Ramprasath VR, Aluko RE, et al. Potential of resveratrol in anticancer and anti-inflammatory therapy. *Nutr Rev*. 2008;66:445–454.
420. Harper CE, Cook LM, Patel BB, et al. Genistein and resveratrol, alone and in combination, suppress cancer in SV-40 tag rats. *Prostate*. 2009;69:1668–1682.
421. Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high calorie diet. *Nature*. 2006;444:337–342.
422. Dudley J, Das S, Mukherjee S, et al. Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium, depending on dose. *J Nutr Biochem*. 2009;20:443–452.
423. Evans HM, Howe PRC, Wong RHX. Effects of resveratrol on cognitive performance, mood and cerebrovascular function in post-menopausal women; A 14-week randomized placebo-controlled intervention trial. *Nutrients*. 2017;9:27.
424. Cottart CH, Nivet-Antoine V, Laguillier-Morizot C, et al. Resveratrol bioavailability and toxicity in humans. *Mol Nutr Food Res*. 2010;54:7–16.
425. Popat R, Plesner T, Davies F, et al. A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. *Br J Haematol*. 2013;160:714–717.
426. Cheng AL, Hsu Ch, Lin JK, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high risk or premalignant lesions. *Anticancer Res*. 2001;21:2895–2900.
427. Plummer SM, Holloway KA, Manson MM, et al. Inhibition of cyclooxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappa activation via the NIK/IKK signaling complex. *Oncogene*. 1999;18:6013–6020.
428. Miquel J, Bernd A, Sempere JM, et al. The curcuma antioxidants: pharmacological effects and prospects for future chemical use. A review. *Arch Gerontol Geriatr*. 2002;34:37–46.
429. Dou Y, Luo J, Wu X, et al. Curcumin attenuates collagen-induced inflammatory response through the "gut-brain axis. *J Neuroinflammation*. 2018;15:6. PMCID: PMC5756354.
430. Hanai H, Iida T, Takeuchi K, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind placebo controlled trial. *Clin Gastroenterol Hepatol*. 2006;4:1502–1506.
431. Perkins K, Vershoyle RD, Hill K, et al. Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+mouse, a model of familial adenomatous polyposis. *Cancer Epidemiol Biomarkers Prev*. 2002;11:535–540.
432. He ZY, Shi CB, Wen H, et al. Upregulation of p53 expression in patients with colorectal cancer by administration of curcumin. *Cancer Investig*. 2011;29:208–213.
433. Polasa K, Raghuram TC, Krishna TP, et al. Effect of turmeric on urinary mutagens in smokers. *Mutagenesis*. 1992;7:107–109.
434. Bayet-Robert M, Kwiatkowski F, Leheurteur M, et al. Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer. *Cancer Biol Ther*. 2010;9:8–14.
435. Mithu VS, Sarkar B, Bhowmik D, et al. Curcumin alters the salt bridge-containing turn region in amyloid β (1-42) aggregates. *J Biol Chem*. 2014;289:11122–11131.
436. Garcia-Alloza M, Borrelli LA, Rozkalne A, et al. Curcumin labels amyloid pathology in vivo, disrupts existing plaques and partially restores distorted neurite in an Alzheimer mouse model. *J Neurochem*. 2007;102:1095–1104.
437. Hamaguchi T, Ono K, Yamada M. Curcumin and Alzheimer's disease. *CNS Neurosci Ther*. 2010;16:285–297.
438. Baum L, Lam WK, Cheung SK, et al. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. *J Clin Psychopharmacol*. 2008;28:110–113.
439. Ringman JM, Frautschy A, Teng E, et al. Oral curcumin for Alzheimer's disease: tolerability and efficacy in a 24-week randomized, double-blind, placebo-controlled study. *Alzheimers Res Ther*. 2012;4:43.
440. Webb AL, Villamor E. Update: effects of antioxidant and non-antioxidant supplementation on immune function. *Nutr Rev*. 2007;65:181–217.
441. Wintergerst ES, Maggini S, Horrig DH. Contribution of selected vitamins and trace minerals to immune function. *Ann Nutr Metab*. 2007;51:301–323.
442. Dunstan JA, Breckler L, Hale J, et al. Supplementation with vitamins C, E, beta-carotene and selenium has no effect on anti-oxidant status and immune responses in allergic individuals. A randomized controlled trial. *Clin Exp Allergy*. 2007;37:180–187.
443. Suresh DR, Annam V, Pratibha K, et al. Total antioxidant capacity—a novel early biochemical marker of oxidative stress in HIV infected individuals. *J Biomed Sci*. 2009;16:61. PMCID: PMC2714592.
444. Steiner J, Haugey N, Li W, et al. Oxidative stress and therapeutic approaches in HIV dementia. *Antiox Redox Signal*. 2006;8:2089–2100.
445. Wanchu A, Rana SV, Pallikkuth S, et al. Short communication: oxidative stress in HIV-infected individuals: a cross-sectional study. *AIDS Res Hum Retroviruses*. 2009;25:1307–1311.
446. Greenspan HC. The role of reactive oxygen species, antioxidants and phytopharmaceuticals in human immunodeficiency virus activity. *Med Hypotheses*. 1993;40:85–92.
447. Nance CL, Siwak EB, Shearer WT. Preclinical development of the green tea catechin epigallocatechin gallate as an HIV-1 therapy. *J Allergy Clin Immunol*. 2009;123:459–465.
448. Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders. A randomized, double-blind, placebo-controlled trial of deprenyl and thioctic acid in human immunodeficiency virus-associated cognitive impairment. *Neurology*. 1998;50:645–651.

449. Irlam JH, Visser ME, Rollins N, et al. Micronutrient supplementation in children and adults with HIV infection. *Cochrane Database Syst Rev*. 2005;19:CD003650.
450. Austin J, Singhal N, Voight R, et al. A community randomized controlled clinical trial of mixed carotenoids and micronutrient supplementation of patients with acquired immunodeficiency syndrome. *Eur J Clin Nutr*. 2006;60:1266–1276.
451. Slain D, Amsden JR, Khakoo RA, et al. Effect of high dose vitamin C on the steady state pharmacokinetics of the protease inhibitor indinavir in healthy volunteers. *Pharmacotherapy*. 2005;25:165–170.
452. Gondim OS, de Camargo VT, Gutierrez FA, et al. Benefits of regular exercise on inflammatory and cardiovascular risk markers in normal weight, overweight and obese adults. *PLoS One*. 2015;10:e0140596. PMID 26474157.
453. Parker L, Shaw CS, Stepto NK, Levinger I. Exercise and glycemic control: focus on redox homeostasis and redox-sensitive protein signaling. *Front Endocrinol*. 2017. https://doi.org/10-3389/fendo.2017.00087.
454. Goldfarb AH. Antioxidants: role of supplementation to prevent exercise induced oxidative stress. *Med Sci Sports Exerc*. 1993;25:232–236.
455. Bloomer RJ, Falvo MJ, Schilling BK, et al. Prior exercise and antioxidant supplementation: effect on oxidative stress and muscle injury. *J Int Soc Sports Nutr*. 2007;4:9. PMCID: PMC2131751.
456. Bloomer RJ. The role of nutritional supplements in the prevention of resistance-induced skeletal muscle injury. *Sports Med*. 2007;37:519–532.
457. Williams SL, Strobel NA, Lexis LA, et al. Antioxidant requirements of endurance athletes: implications for health. *Nutr Rev*. 2006;64:93–108.
458. Margaritis I, Rousseau AS. Does physical exercise modify antioxidant requirements? *Nutr Res Rev*. 2008;21:2–12.
459. Teixeria VH, Valente HF, Casal SI, et al. Antioxidants do not prevent post-exercise peroxidation and may delay muscle recovery. *Med Sci Sports Exerc*. 2009;49:1752–1760.
460. McAnulty SR, McAnulty LS, Morrow JD, et al. Effect of daily fruit ingestion on angiotensin converting enzyme activity, blood pressure, and oxidative stress in chronic smokers. *Free Radic Res*. 2005;39:1241–1248.
461. Boffetta P, Couto E, Wichmann J, et al. Fruit and vegetable intake and overall cancer risk in the european prospective investigation into cancer and nutrition (EPIC). *J Natl Cancer Inst*. 2010;102:529–537.
462. European Food Safety Authority Scientific Opinion. EFSA panel on dietetic products, nutrition and allergies (NDA). *EFSA*. 2011;9:2474–2486.
463. Halliwell B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Radic Biol Med*. 2002;32:968–974.
464. Maras JE, Bermudez OI, Qiano N. Intake of alpha-tocopherol is limited among US adults. *J Am Diet Assoc*. 2004;104:567–575.
465. May JM, Qu ZC, Whitesell RR, et al. Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. *Free Radic Biol Med*. 1996;20:543–551.
466. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled clinical trial of high-dose supplementation with vitamins C and E, beta carotene and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119:1417–1436.
467. Schneider D, Elstner EF. Coenzyme Q10, vitamin E, and dihydrothioctic acid cooperatively prevent diene conjugation in isolated low density lipoprotein. *Antioxid Redox Signal*. 2000;2:327–333.
468. Morris MC, Tangney CC, Wang Y, et al. MIND diet slows cognitive decline with aging. *Alzehimers Dement*. 2015;11:1015–1022.
469. Williamson G, Holst B. Dietary reference intake (DRI) value for dietary polyphenols: are we heading in the right direction? *Br J Nutr*. 2008;99(suppl 3):S55–S58.
470. Canada AT, Watkins WD, Nguyen TD. The toxicity of flavonoids to guinea pig enterocytes. *Toxicol Appl Pharmacol*. 1989;99:357–361.
471. Villanueva C, Cross RD. Antioxidant-induced stress. *Int J Mol Sci*. 2012;13:2091–2109.
472. Wray DW, Uberoi A, Lawrenson L, et al. Oral antioxidants and cardiovascular health in exercise trained and untrained elderly: a radically different outcome. *Clin Sci (Lond.)*. 2009;116:433–441.
473. Ristow M, Zarse K, Oberback A, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci USA*. 2009;106:8665–8670.
474. Lu J, Papp LV, Fang J, et al. Inhibition of mammalian thioredoxin reductase by some flavonoids: implication for myricetin and quercetin. *Cancer Res*. 2006;66:4410–4418.
475. Gerss J, Kopcke W. The questionable association of vitamin E supplementation and mortality—inconsistent results from different meta-analytic approaches. *Cell Mol Biol*. 2009;55(suppl O):L1111–L1120.
476. Dolan Y, Lichtenberg D, Pincuk I. No evidence supports vitamin E indiscriminate supplementation. *Biofactors*. 2009;35:469–473.
477. Paiva SA, Russell RM. Beta carotene and other carotenoids as antioxidants. *J Am Coll Nutr*. 1999;18:426–433.
478. Alija AJ, Bresgen N, Sommerburg O, et al. Cyto- and genotoxic potential of beta carotene and cleavage products under oxidative stress. *Biofactors*. 2005;24:159–163.
479. Rimm EB, Stampfer MJ, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease. *N Engl J Med*. 1993;328:1450–1456.
480. Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys*. 2001;385:20–27.
481. Galati G, Sabzevari O, Wilson JX, et al. Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. *Toxicology*. 2002;177:91–104.
482. Lee SH, Oe T, Blair IA. Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science*. 2001;292:2083–2086.
483. Childs A, Jacobs C, Kaminski T, et al. Supplementation with vitamin C and N-acetylcysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. *Free Radic Biol Med*. 2001;31:745–753.
484. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA*. 1993;90:7915–7922.
485. Chen LH. Interaction of vitamin E and ascorbic acid. *In Vivo*. 1989;3:199–209.
486. Ward NC, Hodgson JM, Croft KD, et al. The combination of vitamin C and grape seed polyphenols increases blood pressure: a randomized, double blind, placebo-controlled trial. *J Hypertens*. 2005;23:427–434.
487. Mut-Salud N, Alvarez PJ, Garrido JM, et al. Antioxidant intake and antitumor therapy: Toward nutritional recommendations for optimal results. *Oxid Med Cell Longev*. 2016. https://doi.org/10.1155/2016/6719534.
488. Block KI, Koch AC, Mead MN, et al. Impact of antioxidant supplementation on chemotherapeutic toxicity: a systematic review of evidence from randomized controlled trials. *Int J Cancer*. 2008;123:1227–1239.
489. Al-Tonbary Y, Al-Haggar M, El-Ashry R, et al. Vitamin E and N-acetylcysteine as antioxidant adjuvant therapy, in children with acute lymphoblastic leukemia. *Adv Hematol*. 2009;689639. PMCID: PMC2778172.
490. Cassileth BR, Yeung KS, Gubili J, Herb-Drug Interactions in Oncology. *Sloan Kettering Cancer Center, Integrative Medicine Service*. 2nd ed. China: People's Medical Publishing House; 2010.
491. Harkness R. *Mosby's Handbook of Drug-Herb and Drug-Supplement Interactions*. St. Louis: Mosby; 2002.
492. Stargrove MB, Treasure J, McKee DL. *Herb, Nutrient, and Drug Interactions: Clinical Implications and Therapeutic Strategies*. St. Louis: Mosby; 2007.
493. *PDR for Herbal Medicines. Physicians' Desk Reference*. 3rd ed. Montvale, NJ: Thompson PDR; 1998.
494. Brewer CT, Chen T. Hepatotoxicity of herbal supplements mediated by modulation of cytochrome P450. *Int J Mol Sci*. 2017;18:2353.
495. Ito A, Sato Y, Kudo S, et al. The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychotropic food-borne pathogens. *Curr Microbiol*. 2003;47:231–236.
496. Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol Rev*. 2012;92:1386–1392.
497. Mistry RK, Brewer AC. Redox-dependent regulation of sulfur metabolism in biomolecules: implications for cardiovascular health. *Antioxid Redox Signal*. 2017. https://doi.org/10.1089/ars.2017.7224.
498. Chondrogianni N, Stratford FL, Trougakos LP, et al. Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. *J Biol Chem*. 2003;278:28026–28037.

499. Ingles M, Gambini J, Carnicero JA, et al. Oxidative stress is related to frailty, not to age or sex, in a geriatric population: lipid and protein oxidation as biomarkers of frailty. *J Am Geriatr Soc*. 2014;62:1324–1328.
500. Barja G. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. *Antioxid Redox Signal*. 2013. https://doi.org/10.1089/ars.2012.5148.
501. Ago T, Matsushima S, Kuroda J, et al. The NADPH oxidase Nox4 and aging in the heart. *Aging*. 2010;2:1012–1016.
502. Liu CK, Lyass A, Larson MG, et al. Biomarkers of oxidative stress are associated with frailty: the Framingham offspring study. *Age*. 2016;38:1.
503. Bakker GC, van Erk MJ, Pellis L, et al. An anti-inflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *Am J Clin Nutr*. 2010;91:1044–1059.
504. Hasani-Ranibar S, Larijani B, Abdollahi M. 2009. A systematic review of the potential herbal sources of future drugs effective in oxidant-induced diseases. *Inflamm Allergy Drug Targets*. 2009;8:2–10.
505. Quintanilha BJ, Reis BZ, Graziela B, et al. Nutrimiromics: role of microRNAs and nutrition in modulating inflammation and chronic diseases. *Nutrients*. 2017;9:1168.
506. Trevino-Saldana N, Garcia-Rivas G. Regulation of sirtuin-mediated protein deacetylation by cardioprotective phytochemicals. *Oxid Med Cell Longev*. 2017. https://doi.org/10.1155/2017/1750306.
507. Chenari S, Safari F, Moradi A. Curcumin enhances liver SIRT3 expression in the rat model of cirrhosis. *Iran J Basic Med Sci*. 2017;20:1306–1311.
508. Zamroziewicz M, Barbey AK. Nutritional cognitive neuroscience: innovations for healthy brain aging. *Front Neurosci*. 2016. https://doi.org/10.3389/fnins.2016.00240.
509. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. *Neurology*. 2012;78:241–249.
510. Scalbert A, Brennan L, Manach C, et al. The food metabolome: a window over dietary exposure. *Am J Clin Nutr*. 2014;99:1286–1308.
511. Lloyd AJ, Fave F, Beckmann M, et al. Use of mass spectrometry fingerprinting to identify urinary metabolites after consumption of specific foods. *Am J Clin Nutr*. 2011b;94:981–991.
512. Mangialasche F, Westman E, Kivipelto M, et al. Classification and prediction of clinical diagnosis of Alzheimer's disease based on MRI and plasma measures of alpha-/gamma-tocotrienols and gamma-tocopherol. *J Intern Med*. 2013;273:602–621.
513. Chirumbolo S, Bjorklund G. Perm Hypothesis: the fundamental machinery able to elucidate the role of xenobiotics and hormesis in cell survival and homeostasis. *Int J Mol Sci*. 2017;18:165.
514. Cortese-Krott MM, Koning A, Gunter GC, et al. The reactive species interactome: Evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine. *Antioxidants & Redox Signaling*. 2017. https://doi.org/10.1089/ars.2017.7083.
515. Grimaldi KA, van Ommen B, Ordovas JM, et al. Proposed guidelines to evaluate scientific validity and evidence for genotype-based dietary advice. *Genes & Nutrition*. 2017. https://doi.org/10.1186/s12263-017-0584-0.
516. Go YM, Jones DP. The redox proteome. *J Biol Chem*. 2013:26512–26520.
517. Qiao Y, Cao J, Xie L, et al. Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells. *Arch Pharm Res*. 2009;32:1309–1315.
518. Shimizu M, Shirakami Y, Moriwaki H. Targeting receptor tyrosine kinases for chemoprevention by green tea catechin, EGCG. *Int J Mol Sci*. 2008;9:1034–1049.
519. Klaunig JE, Xu Y, Han C, et al. The effect of tea consumption on oxidative stress in smokers and nonsmokers. *Proc Soc Exp Biol Med*. 1999;220:249–254.
520. Chevion M, Berenshtein E, Stadtman ER. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic Res*. 2000;33:S99–S108.

98

Opuntia Species (Prickly Pear)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Opuntia spp. (family: *Cactaceae*)
Common names: prickly pear, nopal

GENERAL DESCRIPTION

There are about 260 species of *Opuntia*.[1] These cacti are shrubs, and their flat, pear-shaped pads are stems or branches, not leaves, as many suppose. Some prickly pear varieties form thickets many feet tall and many feet across. Prickly pear's flowers range from yellow to orange and red to rose (Fig. 98.1). Their fruits come in shades of yellow through red to purple. Most species have glochids, sometimes referred to as tiny spines, although they are actually leaf hairs with tiny barbs.[2] The stems, fruits, and flowers are all used as medicines.

CHEMICAL COMPOSITION

Comparisons of the constituents of the many prickly pear species are sparse, and there is no indication that any particular species is a better medicinal plant. The pad is high in mucilage, consisting of carbohydrate-containing polymers.[3] The pads also contain a mixture of saturated and unsaturated long-chain aliphatic esters; daidzein; genistein; β-sitosterol; and vanillic, ferulic, myristic, palmitic, and stearic acids.[3–6] Prickly pear fruits are high in carbohydrates and contain the pigments indicaxanthin and betanin.[7] The flowers contain flavonoids such as quercetin and isorhamnetin-3 glucosides in varying amounts.[8]

HISTORY AND FOLK USE

Prickly pear has a long history of medicinal use. The Aztecs used prickly pear root with *Geranium* spp. (cranesbill) as a febrifuge, to alleviate hernias, and to soothe irritated livers.[9] The fruit and seeds were used to prevent diarrhea. Indigenous peoples along the Yaqui River used the fluid from roasted prickly pear pads to relieve pain.[10] In New Mexico, prickly pear pads were used as poultices for painful, inflamed skin conditions; for swollen glands in the neck; and for congested, purplish breasts in lactating women. The pads were also used as emollients for tumors, warts, and calluses. The Tarahumara used the pads for the pain of bites and burns, and the Paipai used them to heal festering wounds. The Yaqui and the Hispanics in New Mexico soak diced pads in water and drink the liquid for thirst and diabetes; they also use the roasted pad to treat diabetic infections. Prickly pear was brought from the Americas to Africa and Europe, and its medicinal uses followed.[11]

Today, the Moors apply the heated pads to swellings on the body, the South African Bantu use prickly pear for a variety of tumors, and Zimbabweans use prickly pear internally as a treatment for pain in gouty arthritis.[12,13] In the Canary Islands, prickly pear fruits are used topically for a variety of inflamed wounds and internally for gastrointestinal and bronchial problems. In the Mediterranean region, prickly pear pads are used to treat gastric ulcers, and in Sicilian folk medicine, a flower infusion is used for its diuretic and relaxant action on the kidneys.[14]

PHARMACOLOGY

Analgesic Effect

Prickly pear fruits and pads were found to have significant analgesic effects in mice.[15] Prickly pear fruit injected intraperitoneally (400 mg/kg) had an analgesic effect comparable to that of aspirin (70 mg/kg) in vivo.

Anti-inflammatory Effect

Prickly pear significantly reduced edema in rat paws and showed an anti-inflammatory effect in a mouse model of chronic inflammation.[16] The active constituent was identified as β-sitosterol. Another study showed that prickly pear *(O. dillenii)* fruit extract injected intraperitoneally had anti-inflammatory action.[17] Pretreatment with prickly pear

Fig. 98.1 *Opuntia* spp. (From RinoCdZ/iStock.com. Accessed November 23, 2018.)

significantly reduced edema dose-dependently, and its maximal effect was similar to that of indomethacin.

Antiviral Effect

After reports that a woman stopped frequent recurrent outbreaks of herpes genitalis by taking 2 g prickly pear a day, studies investigated the plant's antiviral effects.[18] In hamster kidney cells, prickly pear specifically reduced herpes simplex virus type 2 (HSV-2) replication at 3.5 mg/mL and completely inhibited replication at 15 mg/mL. Prickly pear also inhibited other herpes viruses (pseudorabies virus, bovine mammillitis virus, equine herpesvirus type 1, as well as the more cell-associated human herpes virus, cytomegalovirus, and varicella-zoster virus).

Prickly pear inhibited replication by HSV-2 in infected human cervix tissue and inhibited RNA virus replication.[18] Testing included a laboratory strain of influenza A virus, an isolate of the respiratory syncytial virus, and strains of encephalomyocarditis (EMC) virus and HIV-1. The only virus that prickly pear failed to inhibit was the picornavirus EMC. The researchers found prickly pear to be a promising antiviral agent because it combines a breadth of in vitro reactivity with a high index of clinical safety. However, prickly pear pads combined with fresh *Capsicum frutescens* (cayenne) fruit and fresh *Citrus limon* (lemon) did not protect chickens from Newcastle virus.[19]

CICATRIZANT EFFECT

When administered by mouth, dried prickly pear pad *(O. ficus indica)* significantly inhibited hydrochloride-induced, aspirin-induced, and ethanol-induced gastric lesions in rats.[20,21] Prickly pear did not affect gastric juice secretion, acid output, or stomach pH in the rats. Extracts of *O. ficus indica* showed significant wound-healing activity in rats.[22]

Diuretic and Other Renal Effects

Fruit and flower infusions of *O. ficus indica* significantly increased diuresis in rats, and the fruit infusion had an antiuric effect.[14] Prickly pear pads (20 mg/100 g body weight daily for 5 weeks) significantly decreased uric acid levels and increased water intake while only slightly raising urine output in rats.[23] Although the rise in urine flow was slight, the investigators suggested that it could account for the greater excretion of uric acid. *O. megacantha* pads (20 mg/kg daily for 5 weeks) significantly increased urinary sodium output in diabetic rats and normal rats compared with controls.[24] Prickly pear did not alter plasma aldosterone concentrations. In earlier studies, prickly pear extract administered orally (20 mg/100 g body weight) increased creatinine clearance in diabetic rats and raised plasma creatinine and urea levels in all rats.[23,25]

Hypoglycemic Effect

Animal studies showed prickly pear pad had a hypoglycemic effect in streptozotocin-induced diabetes in rats and rabbits, although one study showed no effect if the rabbits' pancreatic β-cells were completely obliterated.[25–28] Prickly pear appears to improve glucose resistance/sensitivity and glucose uptake through a mechanism that involves AMPK/p38 MAPK signaling pathway and GLUT4 translocation from intracellular storage vesicles to the plasma membrane in muscle cells (Fig. 98.2). In one study, prickly pear's hypoglycemic effect was comparable to that of tolbutamide.[26] Prickly pear and insulin equivalently reduced, but did not normalize, glucose and insulin levels in diabetic rats. Prickly pear combined with insulin had a synergistic effect. Diabetic rats given the combination rapidly achieved normal glucose levels, and at 7 weeks, they became hypoglycemic. At that point, prickly pear alone was sufficient to maintain normal glucose levels.[27]

Hypocholesterolemic Effects

Prickly pear pectin was found to have a hypocholesterolemic effect in guinea pigs.[29,30] Prickly pear consistently reduced blood cholesterol levels, reduced low-density lipoprotein (LDL) cholesterol, and increased LDL density.

CLINICAL APPLICATIONS

Alcohol Intoxication

Prickly pear extract administered 5 hours before alcohol consumption reduced symptoms of a hangover. It appeared to act by inhibiting the production of inflammatory mediators.[31] However, a subsequent systematic review found no compelling evidence that prickly pear reduced hangovers.[32]

Diabetes (Adult-Onset Non–Insulin-Dependent)

There are a number of clinical trials on the effect of prickly pear pad in non–insulin-dependent diabetes mellitus (NIDDM).[33–39] A preliminary meta-analysis of the clinical trials on prickly pear's benefit

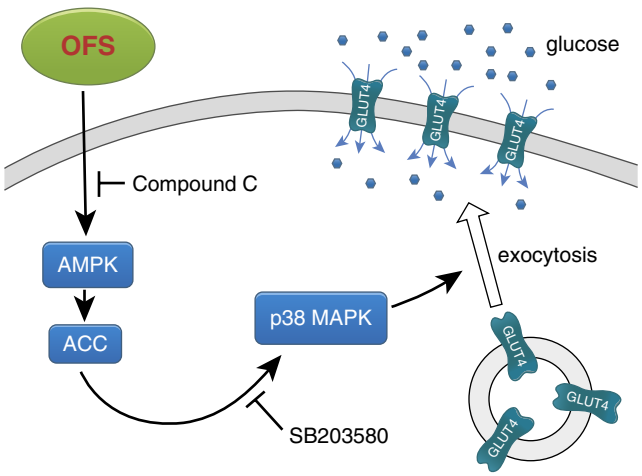


Fig. 98.2 Schematic summary of the possible molecular mechanism by which prickly pear regulates glucose uptake in muscle cell. (From Leem KH, Kim MG, Hahm YT, Kim HK. Hypoglycemic effect of *Opuntia ficus-indica* var. *saboten* is due to enhanced peripheral glucose uptake through activation of AMPK/p38 MAPK pathway. *Nutrients*. 2016;8[12], pii: E800. PubMed PMID: 27941667.)

in NIDDM concluded that the intervention data showed it to lower blood glucose levels by 10 to 30 mg/dL and supported claims that prickly pear had a true metabolic effect in diabetes.[40] Although the control data were insufficient for a full meta-analysis, a statistically significant reduction of blood glucose and, when tracked, in insulin levels was seen consistently when broiled prickly pear was administered. The magnitude of the hypoglycemic effect varied from mild to moderate depending on the dose. For example, a 300-g dose of broiled pad acutely lowered blood glucose levels by 30 mg/dL in eight subjects, whereas 500 g lowered levels by an average of 45 mg/dL in the same subjects. In another open study of 14 subjects with type 2 diabetes, 300 g of steamed pad as part of a high-carbohydrate (HC) or high-soy-protein (HS) breakfast had antihyperglycemic and antihyperinsulinemic effects in both. Prickly pear prevented postprandial blood glucose peaks in the HS breakfast.[41]

Most studies used broiled pads. One that used homogenized prickly pear pad preparation did not find a hypoglycemic effect.[39] However, when the pad was ground in a regular blender rather than ultrahomogenized, it had an effect equivalent to that of broiled pads.[33] Capsules of dried prickly pear did poorly in a small, crossover, single-blind study, in which a single dose of 30 capsules had no hypoglycemic effect.[42] Only a mild effect was seen in volunteers taking 30 capsules per day for 10 days—an impractical dose for most people. In a randomized, placebo-controlled, double-blind, parallel-arm study of 67 individuals with metabolic syndrome, a mixture of dehydrated prickly pear, chia seeds, oats, soy protein, Splenda, and flavoring did not affect blood glucose or insulin levels, although it did reduce the glycemic index after a glucose tolerance test and reduced serum triglycerides.[43]

In contrast, prickly pear has been shown to affect glucose levels mildly or not at all in normal individuals.[34,36,44] In two studies, prickly pear pad did not reduce glucose or insulin levels in healthy volunteers. In a third study, nondiabetic individuals who ate broiled prickly pear pads before meals for 10 days showed a mild but significant reduction in fasting glucose levels, but prickly pear pad had a significant hypoglycemic effect in volunteers in an induced hyperglycemic state.[42–46] In a fourth small study, a proprietary extract of prickly pear pad and fruit skin had an acute blood-glucose–lowering effect.[47]

Hyperlipidemia

Prickly pear pads lowered cholesterol and triglyceride levels and improved cholesterol ratios in a small, non–placebo-controlled trial.[36] Eight healthy patients, 14 obese patients, and 7 patients with NIDDM ate 100 g of broiled prickly pear pads before meals three times a day for 10 days. Each group experienced a significant reduction in total cholesterol levels; triglyceride levels also diminished significantly in obese and diabetic patients (23%–27%). An 8-week pilot study found that prickly pear pulp normalized blood fats as well as other indicators of metabolic syndrome in 24 hypercholesterolemic men.[48] In another 6-week randomized, placebo-controlled, double-blind study of 68 women with metabolic syndrome, an extract of dehydrated prickly pear (NeOpuntia, 4.8 g/day) was reported to significantly increase high-density-lipoprotein (HDL) cholesterol levels.[49] Based on this study, an application was made to the European Food Safety Authority (EFSA) for permission to make a cardiovascular disease reduction claim for NeOpuntia. The application was denied because EFSA found that the evidence failed to demonstrate that NeOpuntia had a significant effect on blood parameters.[50] A meta-analysis found that although prickly pear may have beneficial effects on total cholesterol, the effect sizes are small. It also found that studies are warranted on prickly pear's ability to lower both systolic and diastolic blood pressure.[51]

In 10 patients with familial hypercholesterolemia, prickly pear upregulated LDL binding.[52]

Osteopenia

A 2-year-long, blinded, randomized, quasi-experimental study (n = 181) compared women with normocalciuria taking 2.5 grams/day of prickly pear, women with hypercalciuria also taking 2.5 grams/day, women with hypercalciuria taking 15 grams/day, and untreated women with normal bone density. The high dose of dehydrated, mature prickly pear improved bone density (total hip and lumbar spine regions) and normalized calciuria levels in premenopausal women with low bone density and hypercalciuria.[53]

Prediabetes

In a double-blind, placebo-controlled study, 29 obese prediabetic individuals were given either 400 mg/day of a proprietary product, OpunDia consisting of 75% prickly pear pads and 25% fruit skins, or a placebo for 16 weeks. OpunDia was no more effective than placebo in lowering blood glucose concentrations.[54]

Prostatic Hyperplasia

Dried prickly pear flowers improved the subjective symptomology of prostatic hyperplasia in a preliminary open study.[55] Eighty-eight patients at two centers took two 250-mg capsules of flower extract three times daily for 2 to 6 months. Many patients reported decreases in urgency, emergency urination, and the sensation of fullness in the bladder, but not all patients experienced relief.

Weight Loss

In a small, open-label study, prickly pear pads significantly reduced the body weight of obese and diabetic subjects and showed a trend toward weight loss in normal subjects.[36] Fourteen obese patients, 7 patients with NIDDM, and 8 healthy patients ate 100 g of broiled prickly pear pad before meals three times a day for 10 days. Each group showed a mean reduction in weight of 1.5 kg while maintaining their usual diets. In another double-blind, placebo-controlled, multicenter study, two 500-mg capsules of IQP G-002AS (a prickly pear fiber derivative with added soluble fiber from *Acacia* species) three times daily triggered statistically significant weight loss in the active group (n = 123). Some 75% of those on the active treatment lost at least 3% of their body weight compared with just 28% of the placebo group, a statistically significant difference.[56] Nonetheless, a subsequent meta-analysis did not find that prickly pear supplementation had a statistically significant effect on body weight, although significant reductions in body mass index, percentage body fat, systolic and diastolic blood pressure, and cholesterol levels were observed.[57]

DOSAGE

Studies of the use of this plant have used a variety of forms of prickly pear pad. Most have used broiled or grilled pads, whereas some studies have used juiced slurries. These studies used 100 to 500 g (3–17 oz) of pads three times a day, taken before meals. There is little information on dried preparations, but as mentioned previously, such preparations did not achieve results in some small trials.

TOXICOLOGY

Prickly pear showed a high degree of safety in studies of mice, horses, and humans.[28] It has a long history of use as a food, a factor that also indicates a high level of safety. Most adverse events involving the plant are the result of its glochids (tiny, nearly invisible spines), which can

become embedded in the skin.[58] Sabra dermatitis is an occupational disease among prickly pear fruit handlers, in which the glochids cause a widespread skin eruption resembling scabies, which in chronic cases involves granuloma formation. However, in two animal studies, prickly pear significantly raised blood urea and creatinine levels in rats and increased urinary creatinine clearance rates in diabetic rats only.[23,24] The researchers expressed concern that prickly pear perhaps caused an early stage of kidney dysfunction in rats. However, the same researchers did not report similar results in a subsequent study, and no other studies have reported such effects.[25]

DRUG INTERACTIONS

No human data on drug interactions are available for prickly pear. All herbs rich in complex carbohydrates may affect transit time and thus may alter the absorption of practically any medication.

Many of the diabetes trials of this plant included patients who were taking oral hypoglycemic agents (tolbutamide, glyburide, chlorpropamide), and no adverse interactions were reported.[34–36,38,59] Prickly pear and insulin had a synergistic effect in rats, first normalizing blood sugar levels and then inducing hypoglycemia.[27] The effect of combining prickly pear and insulin in human subjects has not been studied.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Thornber JJ, Bonker F. *The Fantastic Clan: The Cactus Family*. New York: Macmillan; 1932.
2. Moore M. *Medicinal Plants of the Desert and Canyon West*. Santa Fe: Museum of New Mexico Press; 1989.
3. Smestad PB, Steinar LP. Water-soluble polysaccharides of opuntia ficus-indica cv "Burbank's spineless". *Phytochemistry*. 1979;18:569–571.
4. Munoz EC, Millan AL, Braverman V, et al. Isoflavones in Mexican foods and soy products. *FASEB J*. 2001;15:A286.
5. Abramovitch RA, Coutts RT, Knaus EE. Identification of constituents of *Opuntia fragilis*. *Planta Med*. 1968;16:147–157.
6. McGarvie D, Parolis H. The mucilage of *Opuntia aurantiaca*. *Carbohydrate Res*. 1981;94:67–71.
7. Fernandez-Lopez JA, Almela L. Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. *J Chromatog A*. 2001;913:415–420.
8. Clark DW, Parfitt BD. Flower flavonoids of *Opuntia* series *Opuntiae*. *Phytochemistry*. 1980;19:1856–1857.
9. Dunmire WW, Tierney GD. *Wild Plants of the Pueblo Province*. Santa Fe: Museum of New Mexico Press; 1995.
10. Curtin LSM, Moore M, eds. *Healing Herbs of the Upper Rio Grande: Traditional Medicine of the Southwest*. Santa Fe: Western Edge Press; 1997:116–120.
11. Kay MA. *Healing With Plants in the American and Mexican West*. Tucson: University of Arizona Press; 1996.
12. Dimmitt MA. Genus *Opuntia* (incl. *Cylindropuntia, Grusonia, and Corynopuntia)*. In: Phillips SJ, Comus PW, eds. *A Natural History of the Sonoran Desert*. Tucson: Arizona-Sonoran Desert Museum Press; 2000:209–218.
13. Hartwell JL. *Plants Used Against Cancer*. Lawrence, MA: Quarterman Publications; 1982.
14. Galati EM, Tripodo MM, Trovato A, et al. Biological effect of *Opuntia ficus indica* (L.) Mill. *(Cactaceae)* waste matter. Note 1: diuretic activity. *J Ethnopharmacol*. 2002;79:17–21.
15. Choi J, Chung KL, Young CL, et al. Screening on biological activities of the extracts from fruit and stem of prickly pear (*Opuntia ficus indica* var. *saboten*). *J Pharmacogn*. 2001;32:330–337. [Korean].
16. Park EH, Kahng JH, Lee SH, et al. An anti-inflammatory principle from cactus. *Fitoterapia*. 2001;72:288–290.
17. Loro JF, del Rio I, Perez-Santana L. Preliminary studies of analgesic and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. *J Ethnopharmacol*. 1999;67:213–218.
18. Ahmad A, Davies J, Randall S, et al. Antiviral properties of extract of *Opuntia streptacantha*. *Antiviral Res*. 1996;30:75–80.
19. Mtambo MM, Mushi EJ, Kinabo LD, et al. Evaluation of the efficacy of the crude extracts of *Capsicum frutescens, Citrus limon* and *Opuntia vulgaris* against Newcastle disease in domestic fowl in Tanzania. *J Ethnopharmacol*. 1999;68:55–61.
20. Lee EB, Hyun JE, Li da W, et al. Effects of *Opuntia ficus-indica* var. *Saboten* on gastric damage in rats. *Arch Pharmacal Res*. 2002;25:67–70.
21. Galati EM, Monforte MT, Tripodo MM, et al. Antiulcer activity of *Opuntia ficus indica* (L.) Mill. (*Cactaceae)*: Ultrastructural study. *J Ethnopharmacol*. 2001;76:1–9.
22. Park EH, Chun MJ. Wound healing activity of *Opuntia ficus indica*. *Fitoterapia*. 2001;72:165–167.
23. Bwititi P, Zamurawo M, Mabhachi G, et al. Toxic and hypericaemic effects of *Opuntia megacantha* extract in rats. *Phytother Res*. 1997;11:389–391.
24. Bwititi PT, Machakaire T, Nhachi CB, et al. Effects of *Opuntia megacantha* leaves extract on renal electrolyte and fluid handling in streptozotocin (STZ)-diabetic rats. *Renal Fail*. 2001;23:149–158.
25. Bwititi P, Musabayane CT, Nhachi CF. Effects of *Opuntia megacantha* on blood glucose and kidney function in streptozotocin diabetic rats. *J Ethnopharmacol*. 2000;69:247–252.
26. Trejo-Gonzalez A, Gabriel-Ortiz G, Puebla-Perez AM, et al. A purified extract from prickly pear *(Opuntia fuliginosa)* controls experimentally induced diabetes in rats. *J Ethnopharmacol*. 1996;55:27–33.
27. Ibanez-Camacho R, Roman-Ramos R. Hypoglycemic effect of *Opuntia* cactus. *Arch Invest Med (Mex)*. 1979;10:223–230.
28. Ibanez-Camacho R, Meckes-Lozoya M, Mellado-Campos V. The hypoglycemic effect of Opuntia streptacantha studied in different animal experimental models. *J Ethnopharmacol*. 1983;7:175–181.
29. Fernandez ML, Trejo A, McNamara DJ. Pectin isolated from prickly pear (*Opuntia* sp.) modifies low density lipoprotein metabolism in cholesterol-fed guinea pigs. *J Nutr*. 1990;120:1283–1290.
30. Fernandez ML, Lin EC, Trejo A, et al. Prickly pear (*Opuntia* sp.) pectin reverses low density lipoprotein receptor suppression induced by a hypercholesterolemic diet in guinea pigs. *J Nutr*. 1992;122:2330–2340.
31. Weise J, McPherson S, Odden MC, et al. Effect of *Opuntia ficus* on symptoms of the alcohol hangover. *Arch Intern Med*. 2004;164:1334–1340.
32. Pittler MH, Verser JC, Ernst E. Interventions for preventing or treating alcohol hangover: systematic review of randomized controlled trials. *BMJ*. 2005;331:1515–1518.
33. Frati AC, Jimenez E, Ariza CR. Hypoglycemic effect of *Opuntia ficus-indica* in non insulin-dependent diabetes mellitus patients. *Phytotherapy Res*. 1990;4:195–197.
34. Frati AC, Gordillo BE, Altamirano P, et al. Acute hypoglycemic effect of *Opuntia streptacantha lemaire* in NIDDM. *Diabetes Care*. 1990;13:455–456.
35. Frati-Munari AC, Gordillo BE, Altamirano P, et al. Hypoglycemic effect of *Opuntia streptacantha lemaire* in NIDDM. *Diabetes Care*. 1988;11:63–66.
36. Frati-Munari AC, Fernandez-Harp JA, De La Riva H, et al. Effects of nopal (*Opuntia* sp.) on serum lipids, glycemia and body weight. *Arch Invest Med (Mex)*. 1983;14:117–125. [Spanish].
37. Frati-Munari AC, Del Valle-Martinez LM, Ariza-Andraca CR, et al. Hypoglycemic effect of different doses of nopal *(Opuntia streptacantha lemaire)* in patients with type II diabetes. *Arch Invest Med (Mex)*. 1989;20:197–201. [Spanish].
38. Frati-Munari AC, Gil UR, Ariza-Andraca CR, et al. Duration of hypoglycemic action of *Opuntia streptacantha Lem*. *Arch Invest Med (Mex)*. 1989;20:297–300.
39. Frati-Munari AC, Altamirano-Bustamante E, Rodriguez-Barcenas N, et al. Hypoglycemic action of *Opuntia streptacantha Lemaire*: study using raw extracts. *Arch Invest Med (Mex)*. 1989;20:321–325. [Spanish].
40. Aguilar C, Ramirez C, Castadeda-Andrade I, et al. *Opuntia* (prickly pear cactus) and metabolic control among patients with diabetes mellitus. *Annu Meet Int Soc Technol Assess Health Care*. 1996;12:14.
41. Lopez-Romero P, Pichardo-Ontiveros E, Avila-Nava A, et al. The effect of nopal (*Opuntia ficus indica*) on postprandial blood glucose, incretins, and antioxidant activity in Mexican patients with type 2 diabetes after consumption of two different composition breakfasts. *J Acad Nutr Diet*. 2014;114:1811–1818.
42. Frati-Murnari AC, Vera-Lastra O, Ariza-Andraca CR. Evaluation of nopal capsules in diabetes mellitus. *Gac Med Mex*. 1992;128:431–436. [Spanish].
43. Guevara-Cruz M, Tovar AR, Aguilar-Salinas CA, et al. A dietary pattern including nopal, chia seed, soy protein and oat reduces serum triglycerides and glucose intolerance in patients with metabolic syndrome. *J Nutr*. 2012;142:64–69.
44. Frati-Munari AC, Yever-Garces A, Islas-Andrade S, et al. Studies on the mechanism of 'hypoglycemic' effect of nopal (*Opuntia* sp.). *Arch Invest Med (Mex)*. 1987;18:7–12.
45. Frati-Munari AC, Quiroz-Lazaro JL, Altamirano-Bustamante P, et al. The effect of various doses of nopal *(Opuntia streptacantha Lemaire)* on the glucose tolerance test in healthy individuals. *Arch Invest Med (Mex)*. 1988;19:143–148.
46. Frati-Munari AC, Licona-Quesada R, Araiza-Andraca C, et al. Activity of *Opuntia streptacantha* in healthy individuals with induced hyperglycemia. *Arch Invest Med (Mex)*. 1990;21:99–102. [Spanish].
47. Godard MP, Ewing BA, Ziegler A, et al. Acute blood glucose lowering effects and long-term safety of OpunDia supplementation in pre-diabetic males and females. *J Ethnopharmacol*. 2010;130:631–634.
48. Wolfram RM, Kritz H, Efthimiou Y, et al. Effect of prickly pear *(Opuntia robusta)* on glucose- and lipid-metabolism in non-diabetics with hyperlipidemia—a pilot study. *Weiner Klinische Wochenschrift*. 2002;114:19–20.
49. Linares E, Thimonier C, Degre M. The effect of NeOpuntia on blood lipid parameters—risk factors for the metabolic syndrome (syndrome X). *Advances Ther*. 2007;24:1115–1125.

50. Scientific opinion of the panel of dietetic products, nutrition and allergies on a request from BIO SERAE on NeOpuntia and improvement of blood lipid parameters associated with cardiovascular risks, especially HDL-cholesterol. *EFSA J*. 2008;788:1–10.
51. Onakpoya IJ, O'Sullivan Heneghan CJ. The effect of cactus pear *(Opuntia ficus-indica)* on body weight and cardiovascular risk factors: a systemic review and meta-analysis of randomized clinical trials. *Nutrition*. 2015;31:640–646.
52. Palumbo B, Efthimiou Y, Stamatopoulos J, et al. Prickly pear induces upregulation of liver LDL binding in familial heterozygous hypercholesterolemia. *Nuclear Med Rev*. 2003;6:35–39.
53. Aguilera-Barreiro MA, Rivera-Marquez JA, Trujillo-Arriaga HM, et al. Intake of dehydrated nopal (*Opuntia ficus indica*) improves bone mineral density and calciuria in adult Mexican women. *Food Nutr Res*. 2013;57:19106. https://doi.org/10.3402/fnr.v57i0.
54. Godard MP, Ewing BA, Pischel I, et al. Acute blood glucose lowering effects and long-term safety of OpunDia™ supplementation in pre-diabetic males and females. *J Ethnopharmacol*. 2010;130:631–634.
55. Palevitch D, Earon G, Levin I. Treatment of benign prostatic hypertrophy with *Opuntia ficus-indica* (L.) *Miller*. *J Herbs Spices Med Plants*. 1993;2:45–49.
56. Grube B, Chong PW, Lau KZ, orzechowski HD. A natural fiber complex reduces body weight in the overweight and obese: a double-blind, randomized, placebo-controlled study. *Obesity*. 2013;21:58–64.
57. Onakpoya IJ, O'Sullivan Heneghan CJ. The effect of cactus pear *(Opuntia ficus-indica)* on body weight and cardiovascular risk factors: a systemic review and meta-analysis of randomized clinical trials. *Nutrition*. 2015;31:640–646.
58. Whiting DA, Bristow JH. Dermatitis and keratoconjunctivitis caused by a prickly pear *(Opuntia microdasys)*. *S Afr Med J*. 1975;49:1445–1448.
59. Meckes-Lozyoa M, Roman-Ramos R. *Opuntia streptacantha*: a coadjutor in the treatment of diabetes mellitus. *Am J Chin Med*. 1986;14:116–118.

99

Panax ginseng (Korean Ginseng)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Panax ginseng C.A. Meyer (family: Araliaceae)
Synonym: *Panax schinseng Nees*
Common names: Korean ginseng, Chinese ginseng, Asiatic ginseng, Oriental ginseng

GENERAL DESCRIPTION

Korean or Chinese ginseng is a small perennial plant that originally grew wild in the damp woodlands of northern China, Manchuria, and Korea. Wild ginseng is now extremely rare. However, ginseng is a widely cultivated plant, especially in Korea, but also in Russia, China, and Japan. In addition to *Panax ginseng* C.A. Meyer, four other, closely related species are often used:

- *Panax quinquefolius* (American ginseng)
- *Panax japonicus* C.A. Meyer (Japanese ginseng)
- *Panax pseudoginseng* (Himalayan ginseng)
- *Panax trifolium*
- *P. ginseng* C.A. Meyer is the most widely used and most extensively studied species.[1,2]

Its pharmacology is the major focus of this chapter.

Fully mature, Korean ginseng is an herbaceous plant with a taproot, five-lobed palmate leaves, and greenish-white flowers in an umbel (Fig. 99.1). In its first year, ginseng bears only a single leaf with three leaflets. In the second year, it bears a single leaf with five leaflets, and in its third year, it bears two leaves with five leaflets. It usually starts flowering in its fourth year, while bearing three leaves. The roots of the cultivated plant are 3 to 4 mm in diameter and 10 cm long, and the roots of wild plants may attain 10 cm in diameter and a length of 50 to 60 cm.

Ginseng is often processed in two forms, white and red. White ginseng is the dried root whose peripheral skin is frequently peeled off. Red ginseng is the steamed root, which has a caramel-like color (Fig. 99.2).[2]

There are many types and grades of ginseng and ginseng extracts, which vary according to the source, age, and parts of the root used, where it was grown, the time of harvesting, and the methods of preparation.[1-3] Old, wild, well-formed roots are the most valued, whereas rootlets of cultivated plants are considered the lowest grade. High-quality preparations are usually in the form of extracts of the main root of plants between 4 and 6 years old that have been standardized for ginsenoside content (see later) and ratio to ensure optimum pharmacological effect.

CHEMICAL COMPOSITION

Ginseng contains at least 30 different triterpenoid saponins, collectively known as ginsenosides, which are believed to be the most important active constituents. The usual concentration of ginsenosides is between 2% and 3%. The ginsenosides have been designated R_0, R_{b1}, R_{b2}, R_{b3}, R_c, R_d, R_e, R_f, 20-gluco-R_f, R_{g1}, and R_{g2}. The ginsenosides originate from the following three fundamental aglycones:

- Oleanolic acid (ginsenoside R_0)
- 20-S-protopanaxadiol (ginsenosides R_{b1} to R_d)
- 20-S-protopanaxatriol (ginsenosides R_e to R_{g2})

As can be seen from Fig. 99.3, the ginsenosides differ primarily in their sugar groups.

Ginsenosides R_{b1}, R_{b2}, R_c, R_e, and R_{g1} are present in significant concentrations in Korean ginseng. In contrast, American ginseng (*P. quinquefolius*) contains primarily ginsenosides R_{b1} and R_e and does not contain ginsenosides R_f, R_{b2}, or, in some instances, R_{g1}.[4] These features allow for easy detection of species with high-pressure liquid chromatography.

Other components of ginseng are[1,2]:

- Panacene, a volatile oil
- Free and glucoside-bound sterols (e.g., β-sitosterol and its β-glucoside)

Fig. 99.1 *Panax ginseng.*

Fig. 99.2 Ginseng root preparations.

- Polyacetylene derivatives β-elemene and panaxynol
- 8% to 32% starch
- Low-molecular-weight polysaccharides
- Pectin
- Vitamins (e.g., thiamine, riboflavin, B_{12}, nicotinic acid, pantothenic acid, biotin)
- 0.1% to 0.2% choline
- Minerals (including germanium)[5]
- Simple sugars (glucose, fructose, sucrose, maltose, trisaccharides, etc.)
- Unique proteins (e.g., panaxagin, a protein that possesses antifungal, antiviral, translation-inhibiting, and ribonuclease activities)[5]
- Various flavonoids

Although it was reported that ginseng contains large amounts of germanium (i.e., 300 ppm), a follow-up study using highly sensitive (detection limit of 1 ppb), flameless atomic absorption spectrometry combined with solvent extraction demonstrated that the highest concentration of germanium measured in samples of ginseng purchased in the Osaka market was only 6 ppb.[6] More research is needed to accurately determine the germanium content of botanical medicines, because the reported concentrations vary widely. Such low levels suggest that a connection between the pharmacology of ginseng and its germanium content is unlikely.

HISTORY AND FOLK USE

Perhaps the most famous medicinal plant of China, ginseng has been generally used alone or in combination with other herbs to restore the "Yang" quality. It has also been used as a tonic for its revitalizing properties, especially after a long illness. Conditions for which ginseng is used in folk medicine are shown in Box 99.1. It has been used as an alterative, anodyne, aperitif, aphrodisiac, cardiotonic, carminative, emetic, estrogenic, expectorant, gonadotrophic, nervine, sedative, sialogogue, stimulant, stomachic, and tranquilizer.[1,2] As can be seen, ginseng reflects a broad range of nutritional and medicinal properties.

PHARMACOLOGY AND CLINICAL INDICATIONS

Since the 1950s, significant research has been conducted worldwide to determine whether the therapeutic properties attributed to ginseng are legend or fact. Unfortunately, inconsistent outcomes resulting from different procedures in the preparation of extracts, use of nonofficial parts of the plant, use of adulterants, and lack of quality control in the ginseng used, have made the determination of ginseng's true properties difficult. Nonetheless, research does exist to indicate that ginseng possesses pharmacological activity, especially when high-quality extracts, standardized for active constituents, are used (Fig. 99.4).

Over the years, ginseng has been reported to have numerous pharmacological effects in humans and laboratory animals, including[1,2]:

- General stimulatory effects during stress
- Decrease in sensitivity to stress
- Increase in mental and physical capacity for work
- Improved endocrine system function
- Amelioration of radiation sickness, experimental neurosis, and cancer
- Enhanced protein synthesis and cell reproduction
- Improved glucose control in diabetes
- Modulation of various immune system parameters
- Lowering of serum cholesterol
- Protection of the liver from hepatotoxins

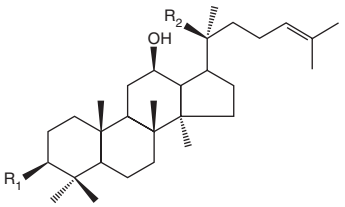
Some of these actions are discussed in greater detail in the following sections.

Adaptogenic and Antistress Activity

Ginseng was originally investigated for its adaptogenic qualities. An adaptogen was defined in 1957 by the Russian pharmacologist I. I. Brekhman[2] as a substance with the following properties:

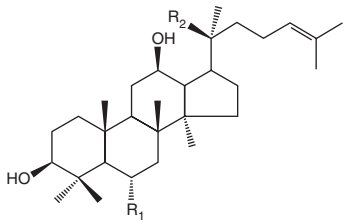
- It must be innocuous and cause minimal disorders in the physiological functions of an organism.
- It must have a nonspecific action (i.e., it should increase resistance to adverse influences through a wide range of physical, chemical, and biochemical factors).
- It usually has a normalizing action irrespective of the direction of the pathological state.

According to tradition and scientific evidence, ginseng possesses this kind of equilibrating, tonic, antistress action. Thus the term *adaptogen* is quite appropriate to describe its effects.[2]



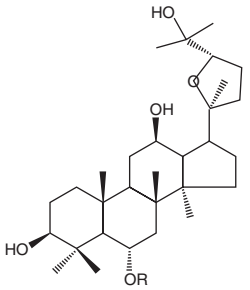
Ginsenoside PPD type

PPD type	R_1	R_2
Ginsenoside Ra_1	glc-glc-O-	xyl-ara(P)-glc-O-
Ginsenoside Rb_1	glc-glc-O-	glc-glc-O-
Ginsenoside Rc	glc-glc-O-	ara(f)-glc-O-
Ginsenoside Rd	glc-glc-O-	glc-O-
(20S)-ginsenoside Rg3	glc-glc-O-	OH
Ginsenoside Rh_2	glc-O-	OH
Ginsenoside Rs_1	ac-glc-glc-O-	ara(P)-glc-O-
Compound K	OH	glc-O-



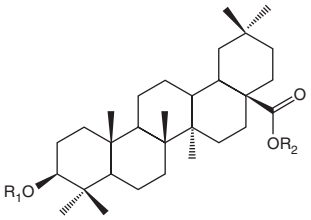
Ginsenoside PPT type

PPT type	R_1	R_2
Ginsenoside Re	rha-glc-O-	glc-O-
Ginsenoside Rf	glc-glc-O-	OH
Ginsenoside Rg_1	glc-O-	glc-O-
(20S)-ginsenoside Rg_2	rha-glc-O-	OH
Ginsenoside Rh_1	glc-O-	OH
20-glucoginsenoside Rf	glc-glc-O-	glc-O-
Notoginsenoside R_1	xyl-glc-O-	glc-O-
Notoginsenoside R_2	xyl-glc-O-	OH



Ginsenoside ocotillol type

Ocotillol type	R
Majonoside R_2	xyl-glc-



Ginsenoside oleanolic acid type

Oleanic type	R_1	R_2
Ginsenoside Ro	glcA-glc-	glc-
Chikusetsusaponin IVa	glcA-	glc-
Chikusetsusaponin IV	ara(f)-glcA-	glc-

Fig. 99.3 Ginsenosides. (From Zhang L, Virgous C, Si H. Ginseng and obesity: observations and understanding in cultured cells, animals, and humans. *J Nutr Biochem.* 2017;44:1-10.)

From a clinical perspective, ginseng can be used as a general tonic, especially in debilitated and feeble individuals. This use is consistent with its historic application. It can also be used as an antistress aid because it has been shown to enhance the ability to cope with various stressors, both physical and mental.

Ginseng delays the alarm phase response in Selye's classic model of stress. Animal studies found that adrenal cholesterol levels were many times higher in animals given ginseng than in their matched controls, indicating greater tolerance of stress and delayed alarm phase response.[7-9]

Italian researchers studied the effect of a standardized ginseng extract, the ginsenoside composition of which was accurately determined, on the adrenal functions of rats exposed to cold.[8] The ginseng extract significantly counteracted body temperature decline without affecting blood glucose or cortisone levels. In a group of adrenalectomized rats, the ginseng extract had no significant effects. Administration of hydrocortisone to the adrenalectomized rats did, however, cause body temperature to be maintained when the rats were exposed to cold.

Histological findings in this study were as follows:

- Evidence of hyperfunctioning in the supraoptic and paraventricular nuclei of the hypothalamus in rats fed the ginseng extract
- Remarkable increase in corticotropic basophilic cells (adrenocorticotropic hormone [ACTH] producing) in the pars distalis of the pituitary gland
- Hyperplasia of the adrenal zona fasciculata, indicating that hyperfunctioning of the adrenal was promoted by the administration of the ginseng extract.

Other researchers demonstrated that ginseng saponins significantly increased plasma ACTH and corticosteroids (in a parallel kinetic pattern).[10,11] Because this effect could be blocked by dexamethasone (which acts on the hypothalamus and pituitary to prevent ACTH release), it was concluded that ginsenosides act predominantly on the hypothalamus or pituitary to promote secretion of ACTH. This conclusion was confirmed further by indirect studies. ACTH first stimulates an increase in cyclic adenosine monophosphate (cAMP) in the adrenal and then promotes corticosteroid synthesis. Ginseng administration was shown to increase adrenal cAMP in normal rats but not in hypophysectomized rats.

These investigations indicate that ginseng's antistress action is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, as follows:

- The antistress action of ginseng is greatly reduced by adrenalectomy.
- Ginseng continues to exert its antistress action after hypophysectomy only if ACTH is administered.

BOX 99.1 Conditions for Which Ginseng is Used in Folk Medicine

- Amnesia
- Anemia
- Anorexia
- Asthma
- Atherosclerosis
- Boils
- Bruises
- Cachexia
- Cancer
- Convulsions
- Cough
- Debility
- Diabetes
- Diuretic
- Divination
- Dysentery
- Dysmenorrhea
- Dyspepsia
- Enterorrhagia
- Epilepsy
- Epistaxis
- Fatigue
- Fear
- Fever
- Forgetfulness
- Gastritis
- Hangover
- Headache
- Heart
- Hemoptysis
- Hemorrhage
- Hyperglycemia
- Hypertension
- Hypotension
- Impotence
- Insomnia
- Intestinal complaints
- Longevity promotion
- Malaria
- Menorrhagia
- Nausea
- Neurasthenia
- Palpitations
- Polyuria
- Pregnancy
- Puerperium
- Rectocele
- Rheumatism
- Rhinitis
- Shortness of breath
- Sores
- Spermatorrhea
- Splenitis
- Swelling
- Vertigo

- Histological and chemical evidence demonstrate a strong link between ginseng and the HPA axis.
- Dexamethasone blocks the effects of ginseng.

ACTH and corticosteroids have been shown to bind directly to brain tissue to increase mental activities during stress. Therefore the release of ACTH and associated pituitary substances (e.g., β-lipoprotein, endorphins, enkephalins) coupled with their end organ effects are likely responsible for many of the antifatigue and antistress actions of ginseng. From a clinical perspective, it is apparent that ginseng has a balancing effect or alterative action on the HPA axis through adjustment of metabolic and functional systems governing hormonal control of homeostasis. Ginseng assists the body's response to the challenge of stress and, therefore, is indicated when there is a disruption in the HPA axis. A double-blind, placebo-controlled, counterbalanced within-group human study investigated the effect on constitutive and stress-induced effects of a Korean ginseng supplement on HPA and antioxidant activity.[12] Ten women and nine men completed three 14-day treatment cycles with different doses (high: 960 mg; low: 160 mg; placebo: 0 mg) separated by a 1-week washout period. Ginseng supplementation produced stress-inducible dose-dependent reductions in circulating cortisol and increased enzymatic and nonspecific antioxidant activity. Twenty-four hours after intense exercise, a high-dose bioactive ginsenoside metabolite (GINST15) significantly reduced muscle damage and HPA responses to physical stress.

Ginseng may be effective in restoration of normal adrenal function and prevention of adrenal atrophy associated with corticosteroid administration. In rats, ginseng was found to inhibit cortisone-induced adrenal and thymic atrophy.[13]

Antifatigue (Mental and Physical) Activity

Some of the first studies of ginseng's adaptogenic activities were performed during the late 1950s and early 1960s by Brekhman and Dardymov[14,15] in the Soviet Union and by Petkov[16-18] in Bulgaria.

In one of Brekhman's experiments, Soviet soldiers given an extract of ginseng ran faster in a 3-km race than those given a placebo. In another, radio operators tested after administration of ginseng extract transmitted text significantly faster and with fewer mistakes than those given placebo. These and similar results reported by European researchers, who demonstrated improvement in human

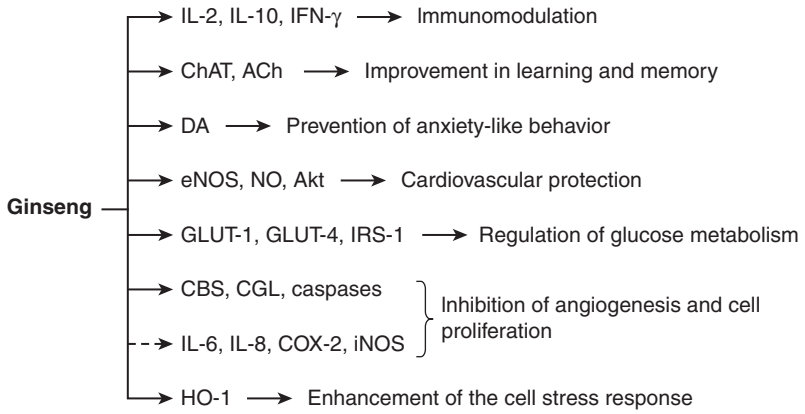


Fig. 99.4 Some of the intracellular targets involved in the pharmacological effects of ginseng. Straight arrow, increase/stimulation; dashed arrow, decrease/inhibition. *ACh*, Acetylcholine; *CBS*, cystathionine-β-synthase; *CGL*, cystathionine-γ-lyase; *ChAT*, choline acetyl transferase; *COX-2*, cyclooxygenase-2; *DA*, dopamine; *eNOS*, endothelial nitric oxidesynthase; *GLUT*, glucose transporter; *HO-1*, heme oxygenase-1; *IFN*, interferon; *IL*, interleukin; *iNOS*, inducible nitric oxide synthase; *IRS-1*, insulin receptor substrate-1. (From Mancuso C. Santangelo R. Panax ginseng and Panax quinquefolius: from pharmacology to toxicology. *Food Chem Toxicol.* 2017;107[Pt A]:362-372.)

physical and mental performance after administration of ginseng extracts, prompted researchers to confirm the results in experimental models using mice.[2,14,15,19]

In perhaps the best known of these experiments, mice were subjected to swimming in cold water or running up an apparently endless rope to determine whether ginseng could lengthen the time to exhaustion. A dose-dependent increase in time to exhaustion was noted in mice receiving ginseng, indicating that ginseng possessed significant antifatigue activity.[2,15,20-22] In one study, the time to exhaustion was lengthened by up to 183% in mice given ginseng 30 minutes before exercising, compared with controls.[15]

Experimental animal studies indicated that much of the antifatigue action of ginseng was due to the stimulant effect of ginseng on the central nervous system (CNS). Ginseng has been shown to improve locomotor activity,[23] modify electroencephalographic (EEG) tracings,[17] improve metabolic activity in the CNS,[24] and affect the HPA axis (discussed later), all of which could be responsible for ginseng's antifatigue activity in mental and physical performance. The CNS activity of ginseng is essentially different from that of typical stimulants. Although stimulants are active under most situations, ginseng reveals its stimulatory action only with the challenge of stress.[24]

On the physical level, stress coupled with ginseng ingestion induced alterations in energy metabolism during prolonged exercise. Exercise physiologists established that during prolonged exercise, the development of fatigue is closely related to the depletion of glycogen stores and the buildup of lactic acid, both in skeletal muscle and in the liver. If an adequate supply of oxygen is available to the working muscle, nonesterified fatty acids are the preferential energy substrate, thus sparing use of muscle glycogen, blood glucose, and, consequently, liver glycogen. The greater the ability to conserve body carbohydrate stores through mobilization and oxidizing of fatty acids, the greater the amount of time to exhaustion. Ginseng enhances fatty acid oxidation during prolonged exercise, thereby sparing muscle glycogen stores.[9]

Mental and physical antifatigue activity effects have been demonstrated in animal studies and double-blind clinical trials in humans. In one double-blind clinical study, nurses who switched from day to night duty rated themselves for competence, mood, and general well-being, and were evaluated with an objective test of psychophysical performance, blood cell counts, and blood chemistry analysis. The group administered ginseng demonstrated higher scores in competence, mood parameters, and objective psychophysical performance than those who received a placebo.[25] The antifatigue effects of enzyme-modified ginseng extract (EMGE) were investigated in healthy adults in a randomized, double-blind, placebo-controlled trial. Fifty-two healthy subjects were randomly allocated into one of two groups: EMGE (2000 mg/day) or placebo. EMGE or placebo were administered to each group for 4 weeks.[26] Fatigue scores using the Visual Analog Fatigue Scale (VAFS) and Revised Piper Fatigue Scale (RPFS) were considered as the primary outcome measure. Life-quality scores were investigated using the Short-Form Health Survey (SF-36). A repeated-measures analysis of variance showed that there was a significant difference in the VAFS scores between the treatment and placebo groups after 4 weeks, with the treatment group's score decreasing more than that of the placebo group. Although there was no difference in the RPFS and SF-36 scores between the two groups, EMGE treatment for 4 weeks decreased fatigue severity in a healthy population.

From a clinical standpoint, cancer patients who underwent chemotherapy, patients with chronic obstructive pulmonary disease (COPD), and athletes were all shown to benefit from ginseng use. In one double-blind study, 92 adults with COPD were randomly assigned to receive either ginseng or placebo.[27] Pulmonary function tests, maximum voluntary ventilation, maximum inspiratory pressure, and maximal oxygen consumption were studied before treatment and every 2 weeks throughout the 3-month study. In the ginseng group, but not in the control group, all parameters were significantly higher than baseline and higher than the placebo group. Maximum increases, in comparison with baseline, were forced vital capacity (32.5%), forced expiratory volume$_{1.0}$ (27.0%), maximum voluntary ventilation (40.4%), maximum inspiratory pressure (47.0%), and maximal oxygen consumption (37.5%). No side effects were observed.

Cancer-related fatigue (CRF) is the most common and severe symptom in patients with cancer. In a large double-blind study of 290 cancer patients, subjects were randomized to receive American ginseng in doses of 750, 1000, or 2000 mg/day or placebo given in twice daily dosing over 8 weeks. Over twice as many patients on ginseng perceived a benefit and were satisfied with treatment compared with those on placebo.[28] In another double-blind trial, 53 cancer patients were randomly assigned to receive ginseng (3000 mg/day) or placebo for 12 weeks. Quality of life was assessed using the World Health Organization Quality of Life Assessment-BREF (WHOQOL-BREF) and the General Health Questionnaire-12.[29] After 12 weeks of therapy, the "psychological domain" score of the WHOQOL-BREF was significantly improved in patients randomized to ginseng, compared with those randomized to placebo. There was a tendency for ginseng to improve the "physical health" and "environment" domain scores of the WHOQOL-BREF, compared with placebo. The General Health Questionnaire-12 total score was significantly improved in patients treated with ginseng than in those with placebo.

In a prospective, open-label study, 30 patients with CRF (≥4/10) received high-dose *Panax ginseng* (PG) at 800 mg orally daily for 29 days.[30] Scores on the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) scale, Edmonton Symptom Assessment System (ESAS), and Hospital Anxiety and Depression Scale (HADS) were assessed at baseline, day 15, and day 29. Global Symptom Evaluation (GSE) was assessed at day 29. Of the 24 evaluable patients, 21 (87%) had an improved (by ≥3 points) FACIT-F score by day 15. The mean ESAS score (standard deviation) for well-being improved from 4.67 (2.04) to 3.50 (2.34) ($P = 0.01374$), and mean score for appetite improved from 4.29 (2.79) to 2.96 (2.46) ($P = 0.0097$). GSE score of PG for fatigue was ≥3 in 15/24 patients (63%) with median improvement of 5. Thus PG is safe and improves CRF fatigue as well as overall quality of life, appetite, and sleep at night.

Cognitive Performance

Standardized extracts of *P. ginseng* alone or in combination with *Ginkgo biloba* (a formula called Gincosan) were shown to improve cognitive performance in animal and clinical studies.[31-41] In university students in Italy (a double-blind, crossover design), ginseng extract alone was compared with placebo in various tests of psychomotor performance.[33]A favorable effect of ginseng relative to baseline performance was observed in attention (cancellation test), mental arithmetic, logical deduction, integrated sensorimotor function (choice reaction time), and auditory reaction time. However, statistically significant superiority of the ginseng group over the placebo group was noted only for mental arithmetic. It was interesting to note that during the trial, the students taking ginseng reported a greater sensation of well-being. Later studies in college-aged students showed similar results with a dose dependence response.[34-36]

The benefits of ginseng on cognitive performance, mood, and quality of life assessment are considerably greater in middle-aged adults than in younger adults. A double-blind, placebo-controlled, balanced, crossover study examined the effect of a ginsenoside-rich extract of American ginseng (*Panax quinquefolius* L.) on working memory and

mood in healthy middle-aged individuals.[37] Fifty-two healthy volunteers (40–60 years old, mean age 51.63) received 200 mg of *P. quinquefolius* or a matching placebo. The Cognitive Drug Research battery and the Computerised Mental Performance Assessment System were used to evaluate cognitive performance at baseline then 1, 3, and 6 hours after treatment. Compared with placebo, *P. quinquefolius* improved cognitive performance on 'Working Memory' factor at 3 hours. Similar effects were observed in one of the two tasks making up this factor: spatial working memory.

Unlike studies in older subjects, no positive effect on mood or quality of life has yet been demonstrated with the administration of ginseng to healthy young adults. In several studies of the administration of ginseng alone or in combination with *G. biloba* extract, significant improvements in tests of cognition and memory were noted, in addition to social functioning and mental health.[38-40] One study demonstrated attenuation of ginseng's effects after 4 weeks of use, indicating that cotherapy with *G. biloba* may be more efficacious or that cycling of the ginseng dosage may be necessary to produce a prolonged effect.[40]

G. biloba and *P. ginseng* have been shown to modulate aspects of cognitive performance, including effects on EEG recordings. One double-blind, placebo-controlled, balanced, crossover experiment of 15 healthy volunteers assessed the effects of single doses of *G. biloba* extract (360 mg), a proprietary *P. ginseng* extract (200 mg G115), and an identical placebo on auditory-evoked potentials, contingent negative variation, and resting power within the δ, θ, α, and β wavebands.[41] The results showed that ginseng use led to a significant shortening of the latency of the P300 component of the evoked potential. Both ginseng and ginkgo also led to significant reductions in frontal "eyes closed" θ and β activity, with additional reduction for ginseng in the α waveband. These findings were the first to demonstrate that *P. ginseng* can directly modulate EEG activity, and that these effects are more pronounced than those that follow ingestion of *G. biloba.* Additional studies with G115 also showed an ability to enhance cognitive function in normal subjects.[42-44]

Results from an open-label study suggest ginseng may improve cognitive performance in Alzheimer's disease (AD).[45] In the study, consecutive AD patients were randomly assigned to the ginseng (n = 58) or the control group (n = 39). The ginseng group was treated with *P. ginseng* powder (4.5 g/day) for 12 weeks. Cognitive performances were monitored using the Mini-Mental State Examination (MMSE) and Alzheimer's disease assessment scale (ADAS) during 12 weeks of the ginseng treatment and for 12 weeks after discontinuing ginseng. MMSE and ADAS scales showed no baseline difference between the groups. After ginseng treatment, the cognitive subscale of ADAS and the MMSE scores began to show improvements and continued up to 12 weeks. After discontinuing ginseng, the improved ADAS and MMSE scores declined to the levels of the control group. These results suggest that *P. ginseng* is clinically effective in the cognitive performance of AD patients.

Ergogenic Aid

The benefits of ginseng on athletic performance are not clear, and results at this time do not support claims that ginseng improves athletic performance. However, other benefits have been noted, such as an ability to protect against muscle injury and inflammation in athletes, reduced exercise-induced oxidative stress, reduced postexercise plasma creatine kinase levels, as well as improved psychomotor performance at rest during a graded exercise test.[46-49]

Diabetes

Ginseng, alone or in combination with other botanicals, has a long folk use in the treatment of diabetes. Hypoglycemic activity was confirmed in both experimental and clinical trials. In one double-blind study in type 2 diabetes, ginseng therapy reduced fasting blood glucose levels, elevated mood, improved psychophysical performance, and lowered body weight and glycosylated hemoglobin.[50] The chief constituents responsible for this effect are[51-55]:

- Five glycans (polysaccharides designated panaxans A to E)
- Adenosine
- A carboxylic acid
- A peptide
- A fraction designated DPG-3-2

The ratio of ginsenosides appears to be an important factor. In one human study, the ratio of protopanaxadiol/protopanaxatriol was the sole predictor of whether a ginseng preparation would exert hypoglycemic effects.[56] It may be important to use crude, standardized extracts having a higher protopanaxadiol/protopanaxatriol ratio, such as those from American ginseng extract, to produce the desired benefit.

Powdered American ginseng root *(P. quinquefolius)* at a dosage of about 3 g before each meal was shown to significantly reduce postprandial blood sugar in patients with type 2 diabetes. It was determined that American ginseng stimulated pancreatic β-cells, with a subsequent increase in insulin secretion. American ginseng also has significant antioxidant properties, improves cognitive function, and possesses nerve protection and regeneration properties. This could prove to be valuable in diabetes considering peripheral and autonomic nerve damage often occurs.[57-59]

It is interesting to note that ginseng raises serum cortisol levels in nondiabetic individuals but reduces serum cortisol levels in patients with diabetes.[60] Because cortisol antagonizes insulin, this is presumably a beneficial effect. This again demonstrates ginseng's nonspecific balancing effect, which is baffling to researchers accustomed to investigating compounds with consistent pharmacological effects.

In a double-blind, randomized, crossover design study of 19 patients with well-controlled type 2 diabetes, each participant received the selected ginseng preparation (rootlets) or placebo at the selected dose (2 g/meal = 6 g/day) and mode of administration (preprandial oral agent [−40 minutes]) for 12 weeks as an adjunct to their usual antidiabetic therapy (diet and/or medications).[61] Although there was no change in glycosylated hemoglobin, ginseng treatment decreased 75 g oral glucose tolerance test plasma glucose (OGTT-PG) indexes by 8% and fasting plasma insulin (PI) and 75 g OGTT-PG indexes by 33%, and increased the fasting insulin sensitivity index and 75 g OGTT-insulin sensitivity index by 33%, compared with placebo.

In another double-blind study, improvements were seen with American ginseng supplementation on fasting blood sugar levels and insulin resistance in type 2 diabetic patients.[62] Proposed mechanisms included effects on insulin release from pancreatic β-cells, insulin-stimulated glucose disposal, and increasing insulin sensitivity related to the peroxisome proliferator-activated receptor.

A systematic review and meta-analysis of randomized clinical trials comparing ginseng supplementation versus control in patients with T2DM or impaired glucose tolerance found no significant difference in Hb A_{1c} levels between the ginseng supplementation and the control groups (pooled standardized difference in means = −0.148, 95% CI: −0.637 to 0.228, P = 0.355).[63] Ginseng supplementation improved fasting glucose, postprandial insulin, and HOMA-IR levels, though no difference in postprandial glucose or fasting insulin was observed among the groups. Similarly, triglycerides, total cholesterol, and low-density lipoprotein levels showed significant difference between the treatment groups, whereas no difference in high-density lipoprotein was seen. In addition, ginseng-related therapy was ineffective in decreasing the fasting glucose levels in patients treated with oral hypoglycemic agents or insulin. The authors concluded that these results

establish the benefit of ginseng supplementation in improving glucose control and insulin sensitivity in patients with type 2 diabetes mellitus or impaired glucose intolerance.

Ginseng appears useful as an adjunctive therapy in the treatment of diabetes, both for its antihyperglycemic effect and for its ability to decrease the atherogenic index. Although the root has received the most attention, animal studies indicate that extracts from the ginseng berry may be more beneficial as a result of a higher concentration of ginsenoside R_e.[60,62,64]

Reproductive Effects

Although ginseng is claimed to be a "sexual rejuvenator" and aphrodisiac, human studies supporting these effects are limited. However, in animal studies, ginseng was shown to[65,66]:

- Promote the growth of the testes and increase spermatogenesis in rabbits
- Accelerate the growth of the ovary and enhance ovulation in frogs
- Stimulate egg-laying in hens
- Facilitate lordotic response in female rats
- Increase gonadal weight in both male and female rats
- Raise testicular nucleic acid content in rats
- Increase sexual activity and mating behavior in male rats

Ginseng was also shown to increase testosterone levels while reducing prostate weight in animals.[67] This suggests that ginseng should have favorable effects in the treatment of benign prostatic hyperplasia. However, no clinical trials have been reported.

Ginsenosides were shown to bind to human myometrial receptor proteins and exert estrogen-like action on the vaginal epithelium significantly enough to prevent the atrophic vaginal changes associated with postmenopause and other menopausal symptoms.[68] Korean red ginseng (KRG) is known to have beneficial effects on women's health as a result of its estrogen-like function. Bisphenol A (BPA) is a significant endocrine disrupting chemical having estrogen-like effects. In a single-blind randomized clinical trial, the efficacy and safety outcomes of KRG against BPA were evaluated, focusing on female quality of life (QOL).[69] Young women (N = 22) consumed 2.7 g of KRG or placebo per day for 2 weeks and completed questionnaires regarding gynecological complaints at four time spots. Urinary levels of total BPA and malondialdehyde (MDA), an oxidative stress biomarker, were also analyzed. KRG consumption decreased urinary BPA and MDA levels and alleviated "menstrual irregularity," "menstrual pain," and "constipation."

Based on historical use and experimental evidence, other clinical indications involving the reproductive system include decreased sperm counts, testicular atrophy or hypofunction, organic causes of male infertility, ovarian atrophy or hypofunction, amenorrhea, and organic causes of female infertility. It should be noted that several reports of mastalgia were reported in women taking ginseng.[70,71]

Many clinical studies involving reproductive effects focused on ginseng's effect on erectile dysfunction (ED). Ginsenosides can facilitate penile erection by directly inducing the vasodilatation and relaxation of penile corpus cavernosum mediated by the release and/or modification of the release of nitric oxide from endothelial cells and perivascular nerves.[72-74]

In one double-blind, crossover study, 45 patients with clinically diagnosed ED showed significantly higher mean International Index of Erectile Function (IIEF) scores with Korean red ginseng than those who received placebo.[74] In response to the global efficacy question, 60% of the patients answered that Korean red ginseng improved erection.

In a double-blind, placebo-controlled trial, a tissue-cultured mountain ginseng extract (TMGE) or placebo was given to 86 male patients with ED.[75] Over the course of 8 weeks, one group took 1000 mg of TMGE twice a day, and the other group took 1000 mg of placebo twice a day. The effects of the TMGE and the placebo were analyzed using the IIEF questionnaire. All 86 patients completed 8 weeks of treatment. The scores on the five domains of the IIEF after medication were significantly higher than the baseline scores in the group treated with TMGE, whereas no significant improvement was observed in the placebo group. Erectile function and overall satisfaction scores after medication were significantly higher in the TMGE group compared with the placebo group.

In another double-blind study, 60 patients presenting with mild or mild to moderate ED were given either *P. ginseng* at a dosage of 1000 mg three times daily or a placebo.[76] The IIEF score after treatment was significantly higher in the ginseng group compared with before the treatment. In contrast, there was no difference before and after the treatment in the placebo group.

Menopause

Ginseng is a popular treatment for menopausal symptoms. However, clinical research is unconvincing. A double-blind study of 384 menopausal women was performed to compare the effects of a standardized ginseng extract with those of a placebo on quality of life, menopausal symptoms, and physiological markers of menopause (follicle-stimulating hormone and estradiol levels, endometrial thickness, maturity index, and vaginal pH).[77] Although the ginseng extract showed no statistically significant effects on the physiological parameters, including vasomotor symptoms (hot flashes), it was associated with statistically significant improvements in mood and well-being. A systematic review of double-blind, randomized, placebo-controlled trials provided positive evidence of ginseng for sexual function and KRG for sexual arousal and total hot flashes score in menopausal women, but the results of KRG or ginseng failed to show specific effects on hot flash frequency, hormones, biomarkers, or endometrial thickness.[78]

Cell-Proliferating, Antioxidant, and Antiaging Effects

Ginseng has a dual effect on cell growth. It stimulates cell division in an adequate nutritional environment but acts cytostatically under adverse conditions. Furthermore, ginseng yielded impressive results in lengthening the life span of cells in culture.[79]

Enhancement of cellular proliferation and function has been shown in a variety of cell types (e.g., epithelial, hepatic, lymphocyte, fibroblast, thymic), especially nerve cells, which may be a result of potentiation of nerve growth factor by ginsenosides.[3,80-82] Clinically, these results indicate a potential use of ginseng in healing damage to virtually all tissue types, particularly the brain.

In animal studies, ginseng was shown to reduce oxidative stress, scavenge free radicals directly, protect endothelial cells from damage, and increase cellular antioxidant enzymes like superoxide dismutase.[83-88]

Immunomodulating Effects

Ginseng enhances the following immune functions[89-96]:

- Antibody plaque-forming cell response
- Circulatory antibody titer against sheep erythrocytes
- Cell-mediated immunity
- Natural killer (NK) cell activity
- The production of interferon
- Lymphocyte mitogenesis
- Reticuloendothelial system proliferative and phagocytic functions

In addition to these immunostimulatory aspects, ginseng components were shown to exert antiallergy and anti-inflammatory effects in experimental models.[97,98] Ginsenosides regulate inflammatory responses primarily through the inhibition of the NF-κβ

signaling pathways. In lipopolysaccharide (LPS)-stimulated macrophages and microglial cells, ginsenosides suppress the production of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, and interleukin-6, as well as inflammatory enzymes such as inducible nitric oxide synthase and cyclooxygenase-2.[99] Furthermore, the biological activities of ginsenoside metabolites have been shown to be effective in ameliorating inflammation associated with several conditions including edema, colitis, atopic dermatitis, and asthma.

The long-term ingestion of ginseng by individuals with mild immunodeficiency may reduce the risk of viral infection. This is consistent with the historic use of ginseng by debilitated individuals. Clinical evidence is also supportive. Extracts of *P. ginseng* were found to stimulate NK function in normal individuals and patients with either chronic fatigue syndrome or AIDS.[100] Ginseng was shown to prevent respiratory viral infections in a nursing home environment (89% relative risk reduction) and potentiate influenza vaccinations.[101,102]

Large doses of ginseng may be contraindicated in acute infections considering its in vitro inhibition of lymphocyte transformation (similar to cortisone) at high (more than 1 mg/mL) but not low concentrations.[103,104] In vitro, ginseng at 1.6 mcg/mL was shown to inhibit phytohemagglutinin-induced transformation of peripheral blood lymphocytes to a greater degree than cortisone at 500 mcg/mL.[103] The greatest level of inhibition was observed when ginseng was used in combination with cortisone. These results demonstrate that ginseng at high doses may be effective against T-cell–mediated inflammatory diseases without producing glucocorticoid-like side effects. It also suggests that a lower dose of cortisone could be used if ginseng were given simultaneously.

When recommending ginseng, the clinician must remember: (1) ginseng's in vitro effect on lymphocyte proliferation is biphasic (i.e., it exerts a strong inhibition at high concentrations and a moderate stimulation at low concentrations); and (2) although ginseng has demonstrated significant inhibition of lymphocyte proliferation in vitro, observed effects in vivo show enhancement of lymphocyte proliferation.[91] These effects may be related to a dose-dependent ginseng-induced elevation of interferon, which inhibits lymphocyte proliferation.

Anticancer Properties

In vivo and in vitro studies have demonstrated the anticancer effect of ginseng in various types of cancer, including breast, lung, liver, colon, and skin cancer. Ginseng increases the mitochondrial accumulation of apoptosis protein and downregulates the expression of antiapoptotic protein, thus reducing the proliferation of cancerous cells and stopping the angiogenesis process (Fig. 99.5).[105] It also aids in the reduction of alopecia, fatigue, and nausea, improves cognitive function, enhances psychomotor performance, and prevents normal cell damage when used with chemotherapeutic drugs. Long-term oral administration of ginseng to newborn mice was shown to reduce the incidence and inhibit the proliferation of tumors induced by various chemical carcinogens, including 7,12-dimethylbenz[*a*]anthracene, urethane, and aflatoxin B_1. In two large population studies, the risk for development of cancer was significantly lower among people who consumed ginseng on a regular basis. Ginseng extract and powder were shown to be more effective than fresh sliced ginseng, ginseng juice, or ginseng tea in reducing cancer risk. A statistically, highly significant dose–response relationship between ginseng intake and cancer risk was observed. These results support the preventive and anticancer effects of ginseng demonstrated in animal and in in vitro studies.[106-108]

The anticancer effects of ginseng can be summarized as[108-112]:

- Ginseng promotes apoptosis.
- The effect is observed only in slow-growing tumors, such as Ehrlich and sarcoma 180 ascites tumor.
- The effect is not observed in rapidly growing tumors, such as l1210, p388, and Walker carcinoma.

In addition to a possible role in cancer prevention, some researchers are investigating *P. ginseng* extracts as a possible adjunct in cancer treatment.[113] Exerting some direct and indirect anticancer actions, ginseng may also offer protection against chemotherapy-induced damage to normal cells. In one animal study, *P. ginseng* extract was shown to protect against cisplatin nephrotoxicity.[114] A multicenter, large-sample, randomized, double-blind trial enrolled 414 patients with advanced nonsmall cell lung cancer to examine the efficacy of the combination of chemotherapy and

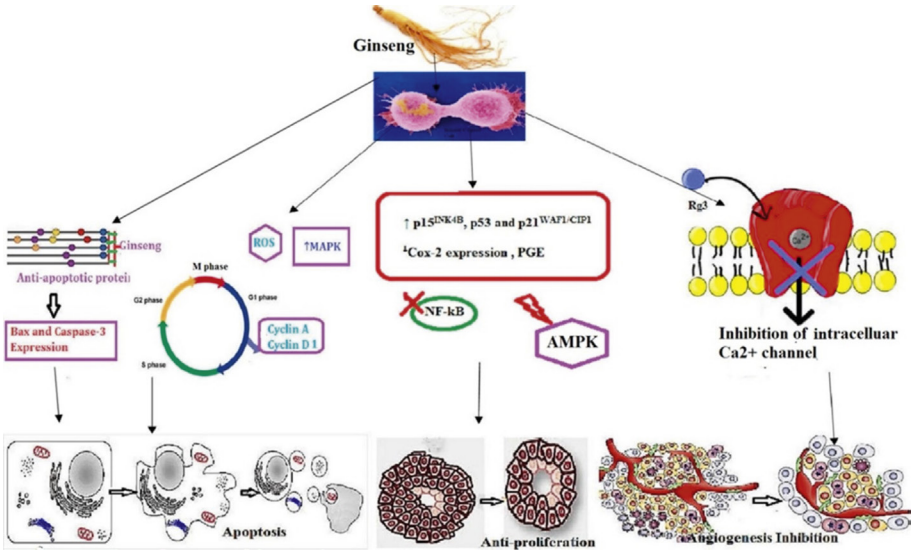


Fig. 99.5 The mechanisms of ginseng in cancer. (From Majeed F, Malik FZ, Ahmed Z, Afreen A, Afzal MN, Khalid N. Ginseng phytochemicals as therapeutics in oncology: recent perspectives. *Biomed Pharmacother.* 2018;100:52-63.)

Ginseng Rg3.[115] One hundred and ninety-nine patients were in the experimental group and treated with the standard first-line chemotherapy combined with Ginseng Rg3, and the 215 patients in the control group were treated with the same chemotherapy combined with placebo. The median overall survival was 12.03 months in the experimental group, which was significantly better than that in the control group (8.46 months). In addition, the combined therapy improved patients' symptoms and reduced chemotherapy-induced myelosuppression.

A randomized, double-blind, placebo-controlled study evaluated the effect of red ginseng on toxicity, health-related quality of life (HRQL), and survival after adjuvant chemotherapy in patients with epithelial ovarian cancer (EOC).[116] A total of 30 patients with EOC were randomly assigned to placebo (n = 15) and red ginseng groups (n = 15). All patients took placebo or red ginseng (3000 mg/day) for 3 months. Changes of genotoxicity, HRQL, and survival were compared between the two groups. In terms of HRQL, red ginseng was associated with improved emotional functioning and decreased symptoms of fatigue, nausea and vomiting, and dyspnea, reduced anxiety and interference affecting life, and improved daytime somnolence. However, there was no effect of red ginseng on prognosis of EOC. There were no differences in adverse events between placebo and red ginseng groups.

Cardiovascular Effects

In vitro and in vivo results show that ginseng has beneficial effects on cardiac and vascular diseases via several mechanisms including antioxidation, control of vasomotor function, modulation of ion channels and signal transduction, improvement of lipid profiles, adjustment of blood pressure, improvement in cardiac function and blood circulation, stimulation of nitric oxide generation, enhancement of vasomotor tone, and reduction in platelet aggregation (Fig. 99.6).[117]

From a clinical perspective, ginseng may offer some protection against atherosclerotic disease, further supporting its use as a general tonic. Its effect on improving arterial endothelial function is especially novel,[118] and may possess a blood pressure–regulating effect via improvement in the endothelial dysfunction underlying many cases of hypertension.[119] A systematic review that included nine randomized, double-blind, placebo-controlled clinical trials of ginseng provides positive evidence for the efficacy of KRG on reducing blood pressure in patients with prehypertension and hypertension in acute and long term.[120]

Ginseng administered to human subjects with hyperlipidemia was shown to reduce total serum cholesterol, triglycerides, and nonesterified fatty acid levels while raising serum high-density lipoprotein cholesterol levels. Platelet adhesiveness was also decreased.[121] These results in humans confirmed earlier studies on rats fed high-cholesterol diets.[122,123] The mechanism of action

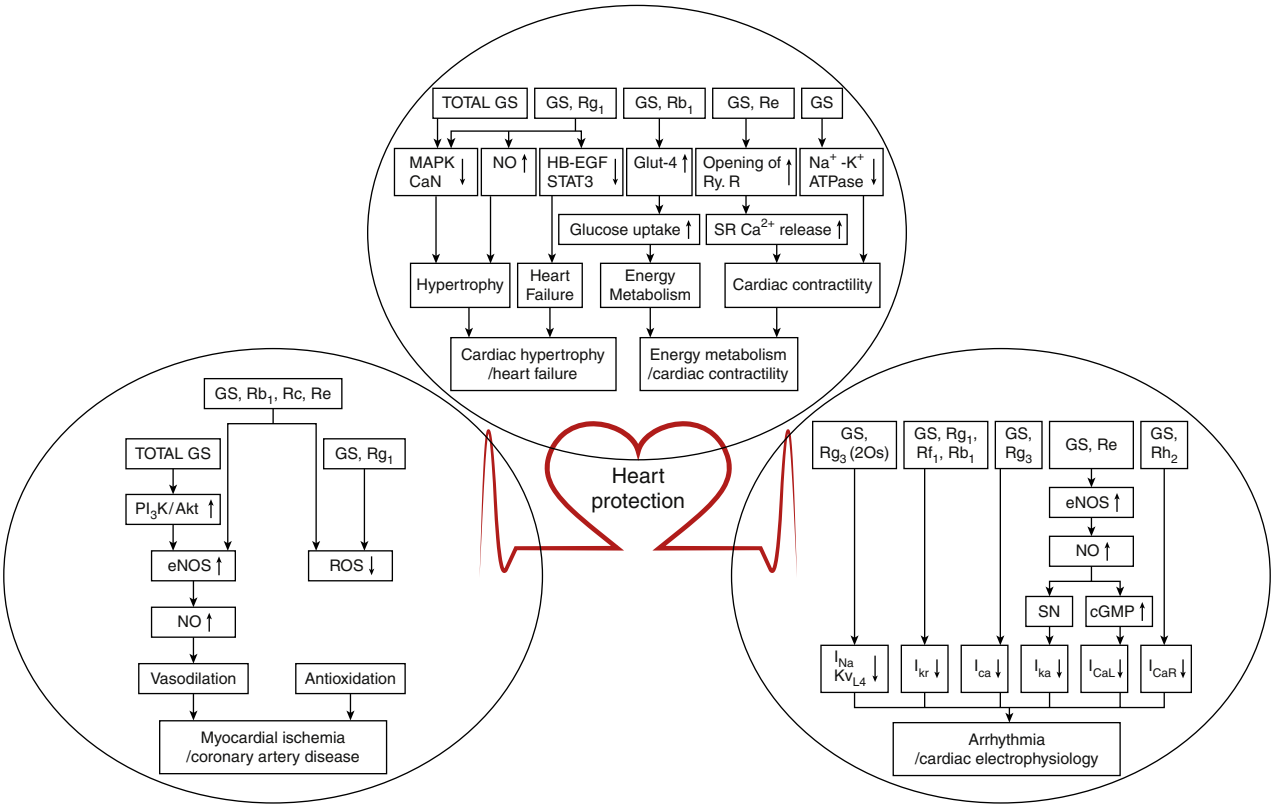


Fig. 99.6 Cardiovascular protection by ginseng and ginsenosides. *eNOS*, Endothelial nitric oxide synthase; *GLUT-4*, glucose transporter-4; *GS*, ginsenoside; *HB-EGF*, heparin-binding EGF-like growth factor; *ICa,L*, L-type calcium channel current; *ICa,R*, R-type calcium channel current; *IKr*, rapidly activating component of delayed rectifier K+ current; *IKs*, slowly activating component of delayed rectifier K+ current; *MAPK*, mitogen-activated protein kinase; *PI3K/Akt*, phosphoinositide 3-kinase/protein kinase B; *ROS*, reactive oxygen species; *RyRs*, ryanodine receptors; *SN*, S-nitrosylation of channel protein; *SR*, sarcoplasmic reticulum; *STAT3*, signal transducer and activator of transcription. (From Kim JH. Pharmacological and medical applications of panax ginseng and ginsenosides: a review for use in cardiovascular diseases. *J Ginseng Res.* 2018;42[3]:264-269.)

appears to be accelerated degradation, conversion, and excretion of cholesterol and triglycerides despite increased lipogenesis and cholesterogenesis. Ginseng was also shown to be effective in inhibiting platelet aggregation and the conversion of fibrinogen to fibrin.[124]

Hepatic Effects

Adaptogenic substances must affect the liver because of this organ's central role in metabolic and detoxification reactions. Ginseng affects the liver in several ways. Several mechanisms of attenuating the damage to hepatocytes have been suggested by experimental and clinical studies of ginseng, including inhibition of cytotoxicity, inhibition of oxidative damage, and anti-inflammatory effects by reducing proinflammatory cytokines.[125] These complicated mechanisms can simultaneously affect the hepatoprotection. Perhaps most important is its ability to produce a marked hyperplasia of the Kupffer cells of the liver and of the folliculi in the spleen and lymph nodes. The hyperplastic folliculi show an increase in the number and volume of light centers, thus demonstrating morphological evidence of increased host defense capacity against a wide variety of external assaults. Because these cells are responsible for filtering out much of the toxins and debris from the portal circulation, increasing their number and activity could have profound effects.

Ginseng was shown to reverse diet-induced fatty liver in animals and to possess significant antihepatotoxic action.[126] In a clinical study that enrolled 26 Egyptian patients with hepatocellular carcinoma (HCC), the therapeutic effect of Korean red ginseng (KRG) was evaluated.[127] Liver function was assessed at 6 weeks and 11 weeks after oral KRG administration (600 mg/day). A significant decrease in the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were found in patients who received KRG, but not in the control group. In addition, oral administration of KRG resulted in elevated serum albumin levels after 6 weeks of therapy. The same study also revealed KRG administration was associated with a decrease of alpha fetoprotein levels and a decrease in viral loads in patients with hepatitis C compared with control hepatitis C patients. A prospective, randomized clinical trial conducted to evaluate the antifatigue and anti-inflammatory effects of KRG in patients with nonalcoholic fatty liver disease found a significant decrease in the serum levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, and proinflammatory cytokines in the KRG group compared with placebo.[128] The clinical indications of these hepatic actions of ginseng are quite broad and support its general tonic/adaptogen properties.

Ginseng was also shown to increase nuclear RNA biosynthesis, indicating greater protein synthesis.[3,115] Ginseng increased nuclear RNA synthesis ribosomal and messenger RNA, the amount of rough endoplasmic reticulum, and the activity of RNA polymerase.[3,129-131] These results indicate that ginseng activates virtually every step in protein biosynthesis. Because protein synthesis is often reduced in the elderly, the significance of the effects on enhancement of hepatic protein synthesis could be extremely high. However, results have yet to be confirmed by clinical studies.

Radiation-Protecting Effects

Ginseng was shown to offer some protection against harmful radiation, both in vivo and in vitro, and to hasten recovery from radiation sickness.[132,133] In the presence of ever-increasing environmental radiation contamination, ginseng may be an appropriate prophylactic against radiation exposure.

Antidepressant and Anxiolytic Effects

As a traditional Chinese medicine, *Panax notoginseng* has a long history of clinical value producing effects such as neuronal protection and inhibition of neuronal apoptosis. Research indicates that ginseng may also have antiinsomnia and antidepression effects, alleviate anxiety, and decrease neural network excitation. The main chemical components involved are *Panax notoginseng* saponins (PNS). A thorough review study showed PNS and its components may exert these effects by promoting the release of neurotransmitters (serotonin, dopamine, norepinephrine), modulation of gamma-amino butyric acid neurotransmission, increasing brain-derived neurotrophic factor by regulating multiple upstream neural signal transduction pathways in the CNS, and modulation of the HPA axis.[134] Moreover, PNS exerted antidepressant-like effects and neuronal protection by regulating the release of interleukin-1β, interleukin-6, tumor necrosis factor-α, and anti-inflammatory cytokines interleukin-4 and interleukin-10, leading to the preservation of brain nerves.

In an 8-week prospective study, 35 female outpatients aged 18 to 65 years (45.1 ± 9.5) who were remitted from major depression with residual symptoms were given KRG at doses of 3 g/day to determine the effectiveness and tolerability of KRG as an adjuvant treatment.[135] The Depression Residual Symptom Scale (DRSS) and Montgomery-Åsberg Depression Rating Scale (MADRS) were administrated to evaluate depressive symptoms. The general severity of symptoms was assessed by a clinician using the Clinical Global Impressions Scale for Severity (CGI-S), and the Depression and Somatic Symptom Scale (DSSS) was also used to evaluate somatic symptoms in the subjects. KRG treated individuals reported significant decrease in depressive symptoms on the DRSS, and MADRS decreased significantly over the 8-week period. The scores on the CGI-S, an objective measurement of symptoms, showed significant improvement in the severity of illness. Somatic symptoms on the DSSS also attenuated significantly during the study period. These results suggest that KRG is efficacious as an adjuvant treatment for patients experiencing residual symptoms of major depression.

Antiobesity

In cultured adipocytes, animals, and humans, ginseng and its ginsenosides may prevent/reduce obesity through several effects and mechanisms. Ginseng and ginsenosides may affect appetite, food digestion and absorption, gut microbiota, inhibit fat tissue formation (adipogenesis and angiogenensis), promote fat oxidation, improve leptin resistance, and promote energy expenditure (Fig. 99.7).[136] The adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a key sensor of cellular energy. Once activated, it switches on catabolic pathways generating adenosine triphosphate (ATP) while switching off biosynthetic pathways consuming ATP. Inhibition of peroxisome proliferator-activated receptor-gamma/CCAAT/enhancer-binding protein-alpha (PPAR-γ/C/EBP-α) by ginseng contributes to its antiadipogenic effect, and through the activation of AMPK, metabolism is switched from anabolism to catabolism. Furthermore, peroxisome proliferator-activated receptor-alpha (PPAR-α) is activated downstream by AMPK and stimulates lipid oxidation and energy expenditure.

The antiobesity effect of ginseng may come from both American ginseng and Asian ginseng, but most studies using whole extract/juice in cultured cells, animals, and humans are from Asian ginseng. To investigate the effect of ginseng on gut microbiota, 10 obese middle-aged Korean women took *Panax ginseng* extracts (4 g/tablet, 2 tablets/day) for 8 weeks and assessment of body composition parameters, metabolic biomarkers, and gut microbiota composition was performed at baseline and at 8 weeks.[137] Significant changes were observed in body weight and body mass index; however, slight changes were observed in gut microbiota. The participants were then divided into two groups, the effective and the ineffective

weight loss groups, depending on weight loss effect, to determine whether the antiobesity effect was influenced by the composition of gut microbiota. The composition of gut microbiota was compared between the two groups. Before ginseng intake, significant differences of gut microbiota were observed between both at phyla and genera levels. Results of this study indicate that ginseng exerted a weight loss effect and slight effects on gut microbiota in all participants. In addition, its antiobesity effects differed depending on the composition of gut microbiota before ginseng intake. However, in another double-blind, placebo-controlled, randomized study, KRG powder (6 g/person per day, 12 weeks) did not change the body weight, percent body fat, and insulin sensitivity of overweight and obese adults who did not have diabetes or hypertension.[138]

Interestingly, ginsenosides have a very low bioavailability after oral intake, and only the deglycosylated forms of ginsenosides can be absorbed into the circulatory system. The transformation of ginsenosides is largely dependent on intestinal bacteria, which release various glycosidases to hydrolyze the sugar moieties of ginsenosides. Intestinal microflora varies among individuals, and approximately 20% of people cannot partially or fully transform ginsenosides.[139] This may explain the differing results attained in human-based research. More clinical trials are required to confirm the antiobesity effect of ginseng and individual ginsenosides.

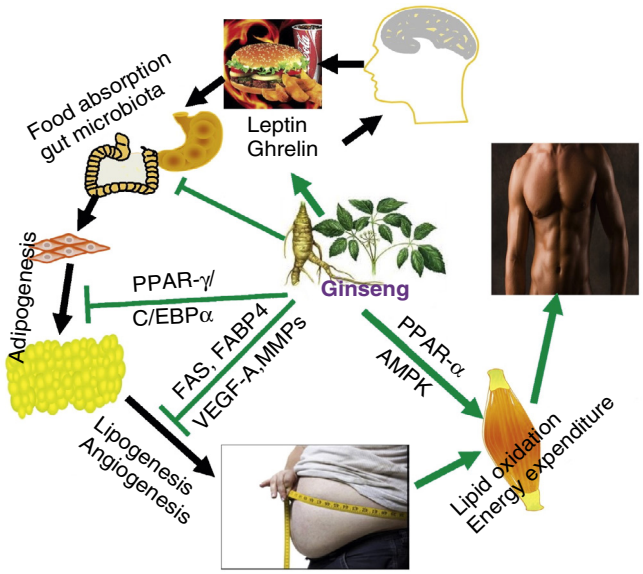


Fig. 99.7 Hypothetical physiological, cellular, and molecular mechanisms of the antiobesity effect of ginseng and its ginsenosides. (From Zhang L, Virgous C, Si H. Ginseng and obesity: observations and understanding in cultured cells, animals, and humans. *J Nutr Biochem.* 2017;44:1-10.)

Central Nervous System Effects

Animal models and cell cultures have demonstrated ginseng and its constituents produce beneficial effects on the CNS, including neuroprotection, improved cognition, and memory performance enhancement (Fig. 99.8). In models of neurodegenerative disorders, ginseng and ginsenosides have shown several benefits including[140]:

- Decrease Aβ production and aggregation, increase Aβ clearance, decrease of tau hyperphosphorylation, and improve cholinergic function in Alzheimer's disease
- Protection against neurotoxic damage and inhibition of α-synuclein aggregation in Parkinson's disease
- Protection against neurotoxic damage and inhibition of Ca^{2+} signaling in Huntington disease

TOXICOLOGY

The problem of quality control makes toxicology difficult to address. This is exemplified by a 1979 article in the *Journal of the American Medical Association* titled "Ginseng Abuse Syndrome."[141] In this

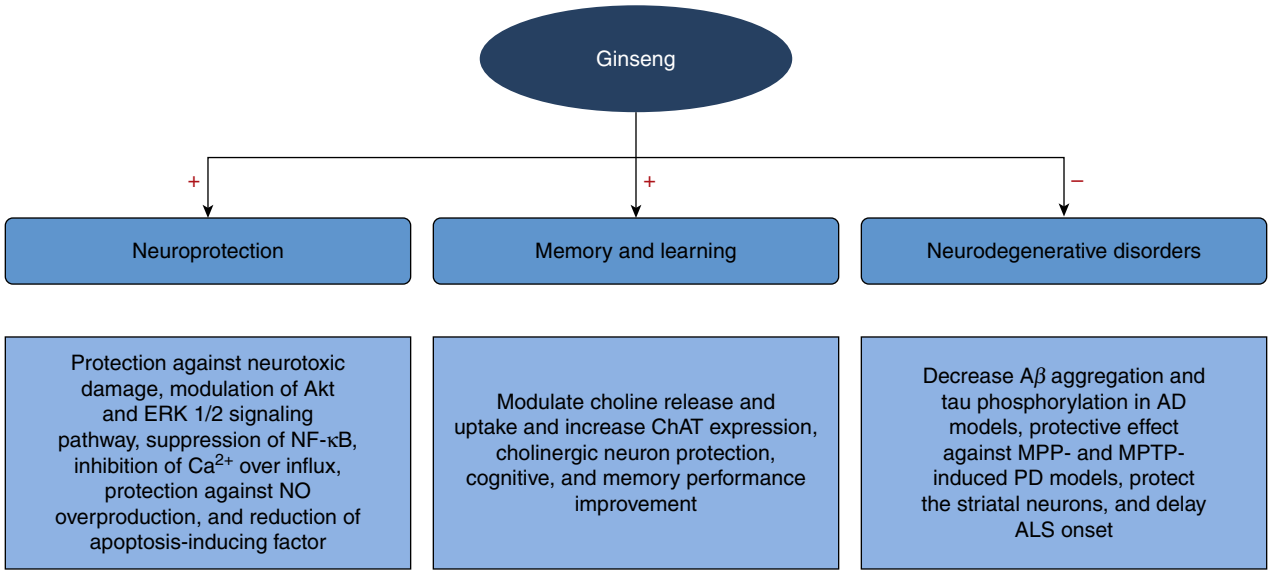


Fig. 99.8 Multiple therapeutic targets of ginseng and its metabolites in the central nervous system. *ERK 1/2*, Extracellular-signal-regulated kinases 1 and 2; *NF-κB*, nuclear factor-kappa B; *NO*, nitric oxide; *ChAT*, choline acetyltransferase; *Aβ*, β-amyloid; *AD*, Alzheimer's disease; *MPTP*, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; *MPP*, 1-methyl-4-phenylpyridium; *PD*, Parkinson's disease; *ALS*, amyotrophic lateral sclerosis. (From Rokot NT, Kairupan TS, Cheng KC, et al. A role of ginseng and its constituents in the treatment of central nervous system disorders. *Evid Based Complement Alternat Med.* 2016;2614742. PubMed PMID: 27630732.)

article, several side effects of commercial preparations of ginseng were reported, including:

- Hypertension
- Euphoria
- Nervousness
- Insomnia
- Skin eruptions
- Morning diarrhea

Given the extreme variation in quality of ginseng in the American marketplace and the use of both nonofficial parts of the plant and adulterants, it is not surprising that side effects were noted. None of the commercial preparations used in the trial were subjected to controlled analysis. Furthermore, the species of ginseng used included *P. ginseng, Panax quinquefolius, Eleutherococcus senticosus,* and *Rumex hymenosepalus* in a variety of different forms—roots, capsules, tablets, teas, extracts, cigarettes, chewing gum, and candies. It is virtually impossible to derive any firm conclusions from the data presented in the *JAMA* article. An important caveat is that these ginseng abuse syndrome effects are neither uniformly negative nor uniformly predictable. Nevertheless, long-term ingestion of large amounts of ginseng should be avoided, as even a panacea can cause problems if abused.

Studies performed on standardized extracts of ginseng demonstrated the absence of side effects and mutagenic or teratogenic effects.[142-144] These studies differed markedly from the trial reported in *JAMA,* in that high-quality extracts were used. An extensive review of the safety of *P. ginseng* concluded, "Data from clinical trials suggest that the incidence of adverse events with ginseng monopreparations is similar to that with placebo."[141] In addition, a systematic review conducted to assess the safety of ginseng in randomized controlled clinical trials concluded that *Panax ginseng* showed a very safe profile with no statistical significance between ginseng and placebo groups on the frequency or symptoms of adverse events, and no serious or severe adverse events reported.[145]

DOSAGE

The dosage of ginseng is inversely proportional to the ginsenoside content; that is, if an extract or ginseng preparation contains high concentrations of ginsenosides (and presumably other active components), a lower dose suffices. The standard dose for ginseng ranges between 4.5 to 6 g/day.

Currently, there is almost a total lack of quality control in ginseng products marketed in the United States. Independent research and published studies clearly documented a tremendous variation in the ginsenoside content of commercial preparations. Many products on the market contain only trace amounts of ginsenosides, and some formulations contain no ginseng at all. This situation has led to several problems, ranging from toxicity reactions to lack of medicinal effect. The widespread disregard for quality control in the health food industry has done much to tarnish the reputation of ginseng as well as other important botanicals.

We recommend the use of standardized ginseng preparations to ensure sufficient ginsenoside content, consistent therapeutic results, and reduced risk of toxicity. Products should be standardized in their ginsenoside content. The typical dose (taken one to three times daily) for general tonic effects should contain a saponin content of at least 5 mg of ginsenosides with a R_{b1}/R_{g1} ratio of 2:1. For example, for a high-quality ginseng root powder containing 5% ginsenosides, the dose would be 100 mg.[144]

Because each individual's response to ginseng is unique, the patient should be monitored for signs of possible ginseng toxicity. It is best to begin at a lower dose and increase it gradually. The Russian approach for long-term administration is to use ginseng cyclically for a period of 15 to 20 days followed by a 2-week interval without any ginseng.

DRUG INTERACTIONS

Taking ginseng preparations may increase the effectiveness of insulin and drugs that lower blood sugar levels such as glyburide (Diabeta, Micronase). It is important to discuss proper monitoring of blood sugar levels with patients who have diabetes before prescribing ginseng. *P. ginseng* may potentiate the monoamine oxidase inhibitor phenelzine (Nardil) to produce manic-like symptoms.[144]

Ginseng may reduce the effectiveness of Coumadin (warfarin). In one double-blind study designed to evaluate the interactions between American ginseng and Coumadin (warfarin), 20 healthy volunteers received warfarin for 3 days during weeks 1 and 4.[146] Beginning in week 2, patients were assigned to receive either American ginseng or placebo. The peak international normalized ratio statistically significantly decreased after 2 weeks of ginseng administration compared with placebo, with the difference between ginseng and placebo being zero. The international normalized ratio area under the curve, peak plasma warfarin level, and warfarin area under the curve were also statistically significantly reduced in the ginseng group compared with the placebo group. These results indicate a potential for ginseng reducing the effectiveness of warfarin. However, in a study in stroke patients, no interaction was noted.[147] Korean ginseng and American ginseng have different ginsenoside profiles, and the heterogeneous composition of compounds among the types of ginseng might have led to different results. Until this interaction is further clarified, ginseng should only be used with careful monitoring, if at all, in patients on Coumadin.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Leung AY, Foster S. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics*. New York: John Wiley.
2. Shibata S, Tanaka O, Shoji J, et al. In: Wagner H, Hikino H, Farnsworth NR. *Economic and Medicinal Plant Research*. Vol. 1. London: Academic Press: 217–284.
3. Dong TT, Cui XM, Song ZH, et al. Chemical assessment of roots of Panax notoginseng in China: regional and seasonal variations in its active constituents. *J Agric Food Chem*. 2003;51:4617–4623.
4. Assinewe VA, Baum BR, Gagnon D, et al. Phytochemistry of wild populations of Panax quinquefolius L. (North American ginseng). *J Agric Food Chem*. 2003;51:4549–4553.
5. Ng TB, Wang H. Panaxagin, a new protein from Chinese ginseng, possesses anti-fungal, anti-viral, translation-inhibiting and ribonuclease activities. *Life Sci*. 2001;68:739–749.
6. Mino Y, Oto N, Sakao S, et al. Determination of germanium in medicinal plants by atomic absorption spectrometry with electrothermal atomization. *Chem Pharm Bull (Tokyo)*. 1980;28:2687–2691.
7. Avakian Jr EV, Evonuk E. Effect of Panax ginseng extract on tissue glycogen and adrenal cholesterol depletion during prolonged exercise. *Planta Med*. 1979;36:43–48.
8. Bombardelli E, Cristoni A, Lietti A. The effect of acute and chronic (Panax) ginseng saponins treatment on adrenal function; biochemical and pharmacological. In: *Proceedings of the 3rd International Ginseng Symposium*. Seoul: Korean Ginseng Research Institute.
9. Fulder SJ. Ginseng and the hypothalamic-pituitary control of stress. *Am J Chin Med*. 1981;9:112–118.
10. Hiai S, Yokoyama H, Oura H. Features of ginseng saponin-induced corticosterone secretion. *Endocrinol Jpn*. 1979;26:737–740.
11. Hiai S, Yokoyama H, Oura H, et al. Evaluation of corticosterone secretion-inducing activities of ginsenosides and their prosapogenins and sapogenins. *Chem Pharm Bull (Tokyo)*. 1983;31:168–174.
12. Flanagan SD, DuPont WH, Caldwell LK, et al. The effects of a Korean ginseng, GINST15, on hypo-pituitary-adrenal and oxidative activity induced by intense work stress. *J Med Food*. 2018;21(1):104–112. PubMed PMID: 28981384.
13. Tanizawa H, Numano H, Odani T, et al. Study of the saponin of Panax ginseng C.A. Meyer. I. Inhibitory effect on adrenal atrophy, thymus atrophy and the decrease of serum K+ concentration induced by cortisone acetate in unilateral adrenalectomized rats. *Yakugaku Zasshi*. 1981;101:169–173. ([Japanese]).
14. Brekhman II, Dardymov IV. New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol*. 1969;9:419–430.
15. Brekhman II, Dardymov IV. Pharmacological investigation of glycosides from ginseng and Eleutherococcus. *Lloydia*. 1969;32:46–51.
16. Petkov W. Pharmacology of the drug *Panax ginseng*. *Arzneimittelforschung*. 1959;9:305–311. ([German]).
17. Petkov W. On the mechanism of action of Panax ginseng C.A. Mey: on the problem of a pharmacology of reactivity.2. *Arzneimittelforschung*. 1961;11:288–295. 418-422: [German].
18. Petkov V. Effect of ginseng on the brain biogenic monoamines and 3'-5'-AMP system. Experiments in rats. *Arzneimittelforschung*. 1978;28:388–393. ([German]).
19. Park HS, Cho JH, Kim KW, et al. Effects of Panax *ginseng* on obesity in animal models: a systematic review and meta-analysis. *Evid Based Complement Alternat Med*. 2018;2018:2719794.
20. Saito H, Yoshida Y, Takagi K. Effect of Panax ginseng root on exhaustive exercise in mice. *Jpn J Pharmacol*. 1974;24:119–127.
21. Kaku T, Miyata T, Uruno T, et al. Chemico-pharmacological studies on saponins of Panax ginseng C.A. Meyer. II. Pharmacological part. *Arzneimittelforschung*. 1975;25:539–547.
22. Sterner W, Kirchdorfer AM. Comparative work load tests on mice with standardized ginseng extract and a ginseng containing pharmaceutical preparation. *Z Gerontol*. 1970;3:307–312.
23. Cicero AF, Bandieri E, Arletti R. Orally administered Panax notoginseng influence on rat spontaneous behaviour. *J Ethnopharmacol*. 2000;73:387–391.
24. Samira MM, Attia MA, Allam M, et al. Effect of the standardized ginseng extract G115 on the metabolism and electrical activity of the rabbit's brain. *J Int Med Res*. 1985;13:342–348.
25. Hallstrom C, Fulder S, Carruthers M. Effect of ginseng on the performance of nurses on night duty. *Comp Med East West*. 1982;6:277–282.
26. Lee N, Lee SH, Yoo HR, Yoo HS. Anti-fatigue effects of enzyme-modified ginseng extract: a randomized, double-blind, placebo-controlled trial. *J Alternat Complement Med*. 2016;22(11):859–864. PubMed PMID: 27754709.
27. Gross D, Shenkman Z, Bleiberg B, et al. Ginseng improves pulmonary functions and exercise capacity in patients with COPD. *Monaldi Arch Chest Dis*. 2002;57:242–246.
28. Barton DL, Soori GS, Bauer BA, et al. Pilot study of Panax quinquefolius (American ginseng) to improve cancer-related fatigue: a randomized, double-blind, dose-finding evaluation: NCCTG trial N03CA. *Support Care Cancer*. 2010;18:179–187.
29. Kim JH, Park CY, Lee SJ. Effects of sun ginseng on subjective quality of life in cancer patients: a double-blind, placebo-controlled pilot trial. *J Clin Pharm Ther*. 2006;31:331–334.
30. Yennurajalingam S, Reddy A, Tannir NM, et al. High-dose Asian ginseng (Panax ginseng) for cancer-related fatigue: a preliminary report. *Integrative Cancer Ther*. 2015;14(5):419–427. PubMed PMID: 25873296.
31. Petkov VD, Belcheva S, Petkov VV. Behavioral effects of ginkgo biloba L., panax ginseng C.A. Mey. And *Gincosan*. *Am J Chin Med*. 2003;31:841–855.
32. Petkov VD, Kehayov R, Belcheva S, et al. Memory effects of standardized extracts of Panax ginseng (G115), Ginkgo biloba (GK 501) and their combination Gincosan (PHL-00701). *Planta Med*. 1993;59:106–114.
33. D'Angelo L, Grimaldi R, Caravaggi M, et al. A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. *J Ethnopharmacol*. 1986;16:15–22.
34. Scholey AB, Kennedy DO. Acute, dose-dependent cognitive effects of Ginkgo biloba, Panax ginseng and their combination in healthy young volunteers: differential interactions with cognitive demand. *Hum Psychopharmacol*. 2002;17:35–44.
35. Kennedy DO, Scholeya AB, Wesnes KA. Dose dependent changes in cognitive performance and mood following acute administration of ginseng to healthy young volunteers. *Nutr Neurosci*. 2001;4:295–310.
36. Cardinal BJ, Engels HJ. Ginseng does not enhance psychological well-being in healthy, young adults: results of a double-blind, placebo-controlled, randomized clinical trial. *J Am Diet Assoc*. 2001;101:655–660.
37. Ossoukhova A, Owen L, Savage K, et al. Improved working memory performance following administration of a single dose of American ginseng (Panax quinquefoliuys L.) to healthy middle-age adults. *Hum Psychopharmacol*. 2015;30(2):108–122. PubMed PMID: 25778987.
38. Coleman CI, Hebert JH, Reddy P. The effects of Panax ginseng on quality of life. *J Clin Pharm Ther*. 2003;28:5–15.
39. Ellis JM, Reddy P. Effects of Panax ginseng on quality of life. *Ann Pharmacother*. 2002;36:375–379.
40. Wesnes KA, Ward T, McGinty A, et al. The memory enhancing effects of a Ginkgo biloba/Panax ginseng combination in healthy middle-aged volunteers. *Psychopharmacol (Berl)*. 2000;152:353–361.
41. Kennedy DO, Scholey AB. Ginseng: potential for the enhancement of cognitive performance and mood. *Pharmacol Biochem Behav*. 2003;75:687–700.
42. Reay JL, Scholey AB, Kennedy DO. Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. *Hum Psychopharmacol*. 2010;25:462–471.
43. Reay JL, Kennedy DO, Scholey AB. Effects of Panax ginseng, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks. *J Psychopharmacol*. 2006;20:771–781.
44. Reay JL, Kennedy DO, Scholey AB. Single doses of Panax ginseng (G115) reduce blood glucose levels and improve cognitive performance during sustained mental activity. *J Psychopharmacol*. 2005;19:357–365.
45. Lee ST, Chu K, Sim JY, et al. Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2008;22:222–226.
46. Cabral de Oliveira AC, Perez AC, Merino G, et al. Protective effects of Panax ginseng on muscle injury and inflammation after eccentric exercise. *Comp Biochem Physiol C Toxicol Pharmacol*. 2001;130:369–377.

47. Ziemba AW, Chmura J, Kaciuba-Uscilko H, et al. Ginseng treatment improves psychomotor performance at rest and during graded exercise in young athletes. *Int J Sport Nutr*. 1999;9:371–377.
48. Kim SH, Park KS, Chang MJ, et al. Effects of Panax ginseng extract on exercise-induced oxidative stress. *J Sports Med Phys Fitness*. 2005;45:178–182.
49. Hsu CC, Ho MC, Lin LC, et al. American ginseng supplementation attenuates creatine kinase level induced by submaximal exercise in human beings. *World J Gastroenterol*. 2005;11:5327–5331.
50. Sotaniemi EA, Haapakoski E, Rautio A. Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care*. 1995;18:1373–1375.
51. Ng TB, Yeung HW. Hypoglycemic constituents of *Panax ginseng*. *Gen Pharmacol*. 1985;16:549–552.
52. Waki I, Kyo H, Yasuda M, et al. Effects of a hypoglycemic component of ginseng radix on insulin biosynthesis in normal and diabetic animals. *J Pharmacobiodyn*. 1982;5:547–554.
53. Konno C, Sugiyama K, Kano M, et al. Isolation and hypoglycaemic activity of panaxans A, B, C, D and E, glycans of Panax ginseng roots. *Planta Med*. 1984;50:434–436.
54. Kimura M, Waki I, Tanaka O, et al. Pharmacological sequential trials for the fractionation of components with hypoglycemic activity in alloxan diabetic mice from ginseng radix. *J Pharmacobiodyn*. 1981;4:402–409.
55. Kimura M, Waki I, Chujo T, et al. Effects of hypoglycemic components in ginseng radix on blood insulin level in alloxan diabetic mice and on insulin release from perfused rat pancreas. *J Pharmacobiodyn*. 1981;4:410–417.
56. Sievenpiper JL, Arnason JT, Leiter LA, et al. Decreasing, null and increasing effects of eight popular types of ginseng on acute postprandial glycemic indices in healthy humans: the role of ginsenosides. *J Am Coll Nutr*. 2004;23:248–258.
57. Vuksan V, Sievenpiper J, Koo VY, et al. American ginseng (Panax quinquefolius L) reduces postprandial glycemia in nondiabetic subjects with type 2 diabetes mellitus. *Arch Intern Med*. 2000;160:1009–1013.
58. Vuksan V, Stavro MP, Sievenpiper JL, et al. Similar postprandial glycemic reductions with escalation of dose and administration time of American ginseng in type 2 diabetes. *Diabetes Care*. 2000;23:1221–1226.
59. Franz MJ, Bantle JP, Beebe CA, et al. Nutrition principles and recommendations in diabetes. *Diabetes Care*. 2004;27(suppl 1):S36–S46.
60. Dey L, Xie JT, Wang A, et al. Anti-hyperglycemic effects of ginseng: comparison between root and berry. *Phytomedicine*. 2003;10:600–605.
61. Vuksan V, Sung MK, Sievenpiper JL, et al. Korean red ginseng (Panax ginseng) improves glucose and insulin regulation in well-controlled, type 2 diabetes: results of a randomized, double-blind, placebo-controlled study of efficacy and safety. *Nutr Metab Cardiovasc Dis*. 2008;18:46–56.
62. Ma SW, Benzie IF, Chu TT, et al. Effect of Panax ginseng supplementation on biomarkers of glucose tolerance, antioxidant status and oxidative stress in type 2 diabetic subjects: results of a placebo-controlled human intervention trial. *Diabetes Obes Metab*. 2008;10:1125–1127.
63. gui QF, Xu ZR, Xu KY, Yang YM. The efficacy of ginseng-related therapies in type 2 diabetes mellitus: an updated systematic review and meta-analysis. *Med (Baltimore)*. 2016;95(6):e2584. PubMed PMID: 26871778.
64. Attele AS, Zhou YP, Xie JT, et al. Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component. *Diabetes*. 2002;51:1851–1858.
65. Nocerino E, Amato M, Izzo AA. The aphrodisiac and adaptogenic properties of ginseng. *Fitoterapia*. 2000;71(suppl 1):S1–S5.
66. Kim C, Choi H, Kim CC, et al. Influence of ginseng on mating behavior of male rats. *Am J Chin Med*. 1976;4:163–168.
67. Fahim MS, Fahim Z, Harman JM, et al. Effect of Panax ginseng on testosterone level and prostate in male rats. *Arch Androl*. 1982;8:261–263.
68. Punnonen R, Lukola A. Oestrogen-like effect of ginseng. *BMJ*. 1980;281:1110.
69. Yang M, Lee HS, Hwang MW, Jin M. Effects of Korean red ginseng (Panax ginseng Meyer) on bisphenol A exposure and gynecologic complaints: single blind, randomized clinical trial of efficacy and safety. *BMC Complement Alternat Med*. 2014;14:265. PubMed PMID: 25063041.
70. Yonezawa M. Restoration of radiation injury by intraperitoneal injection of ginseng extract in mice. *J Radiat Res (Tokyo)*. 1976;17:111–113.
71. Palmer BV, Montgomery AC, Monteiro JC. Gin Seng and mastalgia. *BMJ*. 1978;1:1284.
72. Murphy LL, Lee TJ. Ginseng, sex behavior, and nitric oxide. *Ann N Y Acad Sci*. 2002;962:372–377.
73. Price A, Gazewood J. Korean red ginseng effective for treatment of erectile dysfunction. *J Fam Pract*. 2003;52:20–21.
74. Hong B, Ji YH, Hong JH, et al. A double-blind crossover study evaluating the efficacy of Korean red ginseng in patients with erectile dysfunction: a preliminary report. *J Urol*. 2002;168:2070–2073.
75. Kim TH, Jeon SH, Hahn EJ, et al. Effects of tissue-cultured mountain ginseng (Panax ginseng CA Meyer) extract on male patients with erectile dysfunction. *Asian J Androl*. 2009;11:356–361.
76. De Andrade E, de Mesquita AA, Claro Jde A, et al. Study of the efficacy of Korean Red Ginseng in the treatment of erectile dysfunction. *Asian J Androl*. 2007;9:241–244.
77. Wiklund IK, Mattsson LA, Lindgren R, et al. Effects of a standardized ginseng extract on quality of life and physiological parameters in symptomatic postmenopausal women: a double-blind, placebo-controlled trial. Swedish Alternative Medicine Group. *Int J Clin Pharmacol Res*. 1999;19:89–99.
78. Lee HW, Choi J, Kil KJ, Lee MS. Ginseng for managing menopausal women's health: a systematic review of double-blind, randomized, placebo-controlled trials. *Med (Baltimore)*. 2016;95(38):e4914. PubMed PMID: 27661038.
79. Fulder SJ. The growth of cultured human fibroblasts treated with hydrocortisone and extracts of the medicinal plant *Panax ginseng*. *Exp Gerontol*. 1977;12:125–131.
80. Yamamoto M, Masaka M, Yamada K, et al. Stimulatory effect of ginsenosides on DNA, protein and lipid synthesis in bone marrow and participation of cyclic nucleotides. *Arzneimittelforschung*. 1978;28:2238–2241.
81. Radad K, Gille G, Moldzio R, et al. Ginsenosides Rb1 and Rg1 effects on survival and neurite growth of MPP+-affected mesencephalic dopaminergic cells. *J Neural Transm*. 2004;111:37–45.
82. Mizumaki Y, Kurimoto M, Hirashima Y, et al. Lipophilic fraction of Panax ginseng induces neuronal differentiation of PC12 cells and promotes neuronal survival of rat cortical neurons by protein kinase C dependent manner. *Brain Res*. 2002;950:254–260.
83. Fu Y, Ji LL. Chronic ginseng consumption attenuates age-associated oxidative stress in rats. *J Nutr*. 2003;133:3603–3609.
84. Liu ZQ, Luo XY, Sun YX, et al. Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? *Biochim Biophys Acta*. 2002;1572:58–66.
85. Siddique MS, Eddeb F, Mantle D, et al. Extracts of Ginkgo biloba and Panax ginseng protect brain proteins from free radical induced oxidative damage in vitro. *Acta Neurochir Suppl*. 2000;76:87–90.
86. Kim YK, Guo Q, Packer L. Free radical scavenging activity of red ginseng aqueous extracts. *Toxicology*. 2002;172:149–156.
87. Kwan CY, Kwan TK. Effects of Panax notoginseng saponins on vascular endothelial cells in vitro. *Acta Pharmacol Sin*. 2000;21:1101–1105.
88. Kim SH, Park KS. Effects of Panax ginseng extract on lipid metabolism in humans. *Pharmacol Res*. 2003;48:511–513.
89. Jie YH, Cammisuli S, Baggiolini M. Immunomodulatory effects of Panax ginseng C.A. Meyer in the mouse. *Agents Actions*. 1984;15:386–391.
90. Gupta S, Agarwal SS, Epstein LB, et al. Panax. A new mitogen and interferon producer. *Clin Res*. 1980;28:504A.
91. Singh VK, Agarwal SS, Gupta BM. Immunomodulatory activity of Panax ginseng extract. *Planta Med*. 1984;50:462–465.
92. Shin JY, Song JY, Yun YS, et al. Immunostimulating effects of acidic polysaccharides extract of Panax ginseng on macrophage function. *Immunopharmacol Immunotoxicol*. 2002;24:469–482.
93. Assinewe VA, Amason JT, Aubry A, et al. Extractable polysaccharides of Panax quinquefolius L. (North American ginseng) root stimulate TNFalpha production by alveolar macrophages. *Phytomedicine*. 2002;9:398–404.
94. Lim DS, Bae KG, Jung IS, et al. Anti-septicaemic effect of polysaccharide from Panax ginseng by macrophage activation. *J Infect*. 2002;45:32–38.

95. Cho JY, Kim AR, Yoo ES, et al. Ginsenosides from Panax ginseng differentially regulate lymphocyte proliferation. *Planta Med*. 2002;68:497–500.
96. Lim TS, Na K, Choi EM, et al. Immunomodulating activities of polysaccharides isolated from Panax ginseng. *J Med Food*. 2004;7:1–6.
97. Park EK, Choo MK, Han MJ, et al. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. *Int Arch Allergy Immunol*. 2004;133:113–120.
98. Choo MK, Park EK, Han MJ, et al. Antiallergic activity of ginseng and its ginsenosides. *Planta Med*. 2003;69:518–522.
99. Kim JH, Yi YS, Kim MY, Cho JY. Role of ginsenosides, the main active components of panax ginseng, in inflammatory responses and diseases. *J Ginseng Res*. 2017;41(4):435–443. PubMed PMID: 29021688.
100. See DM, Broumand N, Sahl L, et al. In vitro effects of Echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. *Immunopharmacol*. 1997;35:229–235.
101. McElhaney JE, Gravenstein S, Cole SK, et al. A placebo-controlled trial of a proprietary extract of North American ginseng (CVT-E002) to prevent acute respiratory illness in institutionalized older adults. *J Am Geriatr Soc*. 2004;52:13–19.
102. Scaglione F, Cattaneo G, Alessandria M, et al. Efficacy and safety of the standardised ginseng extract G115 for potentiating vaccination against the influenza syndrome and protection against the common cold. *Drugs Exp Clin Res*. 1996;22:65–72.
103. Chong SK, Brown HA, Rimmer E, et al. In vitro effect of Panax ginseng on phytohaemagglutinin-induced lymphocyte transformation. *Int Arch Allergy Appl Immunol*. 1984;73:216–220.
104. Yeung HW, Cheung K, Leung KN. Immunopharmacology of Chinese medicine. 1. Ginseng induced immunosuppression in virus-infected mice. *Am J Chin Med*. 1982;10:44–54.
105. Majeed F, Malik FZ, Ahmed Z, Afreen A, Afzal MN, Khalid N. Ginseng phytochemicals as therapeutics in oncology: recent perspectives. *Biomed Pharmacother*. 2018;100:52–63. PubMed PMID: 29421582.
106. Yun TK, Choi SY. Non-organ specific cancer prevention of ginseng: a prospective study in Korea. *Int J Epidemiol*. 1998;27:359–364.
107. Yun TK. Panax ginseng—a non-organ-specific cancer preventive? *Lancet Oncol*. 2001;2:49–55.
108. Shin HR, Kim JY, Yun TK, et al. The cancer-preventive potential of Panax ginseng: a review of human and experimental evidence. *Cancer Causes Control*. 2000;11:565–576.
109. Kim HS, Lee EH, Ko SR, et al. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. *Arch Pharm Res*. 2004;27:429–435.
110. Liu WK, Xu SX, Che CT. Anti-proliferative effect of ginseng saponins on human prostate cancer cell line. *Life Sci*. 2000;67:1297–1306.
111. Yun TK, Yun YS, Han IW. Anticarcinogenic effect of long-term oral administration of red ginseng on newborn mice exposed to various chemical carcinogens. *Cancer Detect Prev*. 1983;6:515–525.
112. Lee KD, Huemer RP. Antitumoral activity of Panax ginseng extracts. *Jpn J Pharmacol*. 1971;21:299–302.
113. Chang YS, Seo EK, Gyllenhaal C, et al. Panax ginseng: a role in cancer therapy? *Integr Cancer Ther*. 2003;2:13–33.
114. Liu SJ, Zhou SW. Panax notoginseng saponins attenuated cisplatin-induced nephrotoxicity. *Acta Pharmacol Sin*. 2000;21:257–260.
115. Zhang Y, Wang XQ, Liu H, Liu J, Hou W, Lin HS. A multicenter, large-sample, randomized clinical trial on improving the median survival time of advanced non-small cell lung cancer by combination of Ginseng Rg3 and chemotherapy. *Zhonghua Zhong Liu Za Zhi*. 2018;40(4):295–299. PubMed PMID: 29730918.
116. Kim HS, Kim MK, Lee M, Kwon BS, Suh DH, Song YS. Effect of red ginseng on genotoxicity and health-related quality of life after adjuvant chemotherapy in patients with epithelial ovarian cancer: a randomized, double-blind, placebo-controlled trial. *Nutrients*. 2017;9(7):E772. PubMed PMID: 28753932.
117. Kim JH. Pharmacological and medical applications of panax ginseng and ginsenosides: a review for use in cardiovascular diseases. *J Ginseng Res*. 2018;42(3):264–269. PubMed PMID: 29983607.
118. Jovanovski E, Jenkins A, Dias AG, et al. Effects of Korean red ginseng (Panax ginseng C.A. Mayer) and its isolated ginsenosides and polysaccharides on arterial stiffness in healthy individuals. *Am J Hypertens*. 2010;23:469–472.
119. Sung J, Han KH, Zo JH, et al. Effects of red ginseng upon vascular endothelial function in patients with essential hypertension. *Am J Chin Med*. 2000;28:205–216.
120. Lee HW, Lim HJ, Jun JH, Choi J, Lee MS. Ginseng for treating hypertension: a systematic review and meta-analysis of double blind, randomized, placebo-controlled trials. *Curr Vascular Pharmacol*. 2017;15(6):549–556. PubMed PMID: 28707603.
121. Yamamoto M, Uemura T, Nakama S, et al. Serum HDL-cholesterol-increasing and fatty liver-improving actions of Panax ginseng in high cholesterol diet-fed rats with clinical effect on hyperlipidemia in man. *Am J Chin Med*. 1983;11:96–101.
122. Yamamoto M, Kumagai A, Yamamura Y. Plasma lipid-lowering actions of ginseng saponins and mechanisms of the action. *Am J Chin Med*. 1983;11:84–87.
123. Joo CN. The preventative effect of Korean (P. ginseng) saponins on aortic atheroma formation in prolonged cholesterol-fed rabbits. In: *Proceedings of the 3rd International Ginseng Symposium*. Seoul: Korean Ginseng Research Institute.
124. Matsuda H, Namba K, Fukuda S, et al. Pharmacological study on Panax ginseng C.A. Meyer. III. Effects of red ginseng on experimental disseminated intravascular coagulation. (2). Effects of ginsenosides on blood coagulative and fibrinolytic systems. *Chem Pharm Bull (Toyko)*. 1986;34:1153–1157.
125. Park TY, Hong M, Sung H, Kim S, Suk KT. Effect of Korean red ginseng in chronic liver disease. *J Ginseng Res*. 2017;41(4):450–455. PubMed PMID: 29021690.
126. Hikino H, Kiso Y, Kinouchi J, et al. Antihepatotoxic actions of ginsenosides from Panax ginseng roots. *Planta Med*. 1985;1:62–64.
127. Abdel-Wahhab MA, Gamil K, El-Kady AA, El-Nekeety AA, Naguib KM. Therapeutic effects of Korean red ginseng extract in Egyption patients with chronic liver diseases. *J Ginseng Res*. 2011;35:69–79.
128. Hong M, Lee YH, Kim S, et al. Anti-inflammatory and antifatigue effect of Korean red ginseng in patients with nonalcoholic fatty liver disease. *J Ginseng Res*. 2016;40(3):203–210. PubMed PMID: 27616896.
129. Oura H, Hiai S, Seno H. Synthesis and characterization of nuclear RNA induced by radix ginseng extract in rat liver. *Chem Pharm Bull (Tokyo)*. 1971;19:1598–1605.
130. Oura H, Hiai S, Nabetani S, et al. Effect on ginseng extract on endoplasmic reticulum and ribosome. *Planta Med*. 1975;28:76–88.
131. Oura H, Nakashima S, Tsukada K, et al. Effect of radix ginseng extract on serum protein synthesis. *Chem Pharm Bull (Tokyo)*. 1972;20:980–986.
132. Kim TH, Lee YS, Cho CK, et al. Protective effect of ginseng on radiation-induced DNA double strand breaks and repair in murine lymphocytes. *Cancer Biother Radiopharm*. 1996;11:267–272.
133. Ben-Hur E, Fulder S. Effect of Panax ginseng saponins and Eleutherococcus senticosus on survival of cultured mammalian cells after ionizing radiation. *Am J Chin Med*. 1981;9:48–56.
134. Xie W, Meng X, Zhai Y, et al. Panax notoginseng saponins: a review of its mechanisms of antidepressant or anxiolytic effects and network analysis on phytochemistry and pharmacology. *Molecules*. 2018;23(4):E940. PubMed PMID: 29673237.
135. Jeong HG, Ko YH, Oh SY, Han C, Kim T, Joe SH. Effect of Korean red ginseng as an adjuvant treatment for women with residual symptoms of major depression. *Asia-Pacific Psychiatry*. 2015;7(3):330–336. PubMed PMID: 25504813.
136. Zhang L, Virgous C, Si H. Ginseng and obesity: observations and understanding in cultured cells, animals, and humans. *J Nutr Biochem*. 2017;44:1–10. PubMed PMID: 27930947.
137. Song MY, Kim BS, Kim H. Influence of Panax ginseng on obesity and gut microbiota in obese middle-aged Korean women. *J Ginseng Res*. 2014;38:106–115.
138. Cho YH, Ahn SC, Lee SY, et al. Effect of Korean red ginseng on insulin sensitivity in non-diabetic healthy overweight and obese adults. *Asia Pacific J Clin Nutri*. 2013;22(3):365–371.
139. Li Z, Ji GE. Ginseng and obesity. *J Ginseng Res*. 2018;42(1):1–8. PubMed PMID: 29348715.

140. Rokot NT, Kairupan TS, Cheng KC, et al. A role of ginseng and its constituents in the treatment of central nervous system disorders. *Evidence-Based Complement Alternat Med*. 2016;2016:2614742. PubMed PMID: 27630732.
141. Siegel RK. Ginseng abuse syndrome: problems with the panacea. *JAMA*. 1979;241:1614–1615.
142. Hess Jr FG, Parent RA, Stevens KR, et al. Effects of subchronic feeding of ginseng extract G115 in beagle dogs. *Food Chem Toxicol*. 1983;21:95–97.
143. Hess Jr FG, Parent RA, Cox GE, et al. Reproduction study in rats of ginseng extract G115. *Food Chem Toxicol*. 1982;20:189–192.
144. Coon JT, Ernst E. Panax ginseng: a systematic review of adverse effects and drug interactions. *Drug Safety*. 2002;25:323–344.
145. Kim YS, Woo JY, Han CK, Chang IM. Safety analysis of Panax ginseng in randomized clinical trials: a systematic review. *Med (Basel)*. 2015;2(2):106–126. PubMed PMID: 28930204.
146. Yuan CS, Wei G, Dey L, et al. Brief communication: American ginseng reduces warfarin's effect in healthy patients: a randomized, controlled trial. *Ann Intern Med*. 2004;141:23–27.
147. Lee SH, Ahn YM, Ahn SY, et al. Interaction between warfarin and Panax ginseng in ischemic stroke patients. *J Altern Complement Med*. 2008;14:715–721.

100

Pancreatic Enzymes[213]

Anthony J. Cichoke Jr., BS, BS, MA, MA, PhD, DC, DACBN

OUTLINE

INTRODUCTION

Pancreatic enzymes produced by the body are well known for the integral role they play in the digestion of the foods we eat. Pancreatic juice contains numerous enzymes, including amylase, lipase, cholesterol esterase, and phospholipase, and the proenzymes trypsinogen, chymotrypsinogen, and procarboxypolypeptidase, which are converted in the small intestine to their active forms trypsin, chymotrypsin, and carboxypeptidase, respectively.[1]

Foods in the human diet are composed primarily of macronutrients, that is, protein, carbohydrates, and fats. Protein leaves the stomach predominantly in the form of proteoses, peptones, and large polypeptides.[1] Upon reaching the small intestine, these are further digested by the proteolytic enzymes trypsin, chymotrypsin, and carboxypolypeptidase. Protein digestion primarily occurs in the duodenum and jejunum. Carbohydrates are digested by α-amylase in the pancreatic juice, which breaks starches (converting them into maltose and other small glucose polymers), whereas pancreatic lipase, cholesterol esterase, and phospholipase digest fats.[1]

Pancreatic enzyme supplements (which include chymotrypsin, trypsin, amylase, lipase, pancreatin, and combinations of one or more) not only aid digestion but also assist in a surprising variety of bodily functions including detoxification, immunity, aging, blood fluidity, and tissue repair. Unfortunately, an inadequate production of, or an excessive requirement for, pancreatic enzymes can occur for a variety of reasons including genetics, illness (including cystic fibrosis, chronic pancreatitis, and pancreatic cancer), surgery (such as after pancreatectomy or gastrointestinal bypass surgery), injury, exercise, aging, and toxins (both endogenous and exogenous). A deficiency of pancreatic enzymes, for whatever reason, may be the cause of numerous illnesses and degenerative conditions. When a deficiency occurs, enzymes from an external source may be necessary.

History

Pancreatic enzymes have a long history of clinical use. In the early 20th century, John Beard, a Scotch embryologist, successfully treated cancer using a pancreatic extract, which he described in his book *The Enzyme Treatment of Cancer and Its Scientific Basis.* Beard injected pancreatic juice (freshly extracted from young animals) into cancer patients and, when possible, directly into their tumors. He found that the pancreatic juice could inhibit the growth of cancer cells.

In 1934, Dr. Ernst Freund, a Viennese physician, studied the blood of people who were free from cancer and discovered a substance that had the ability to dissolve cancer cells. Patients with cancer did not have this material, which Freund called "normal substance." In the early 1930s, Professor Doctor Max Wolf worked with Freund in Vienna and successfully identified "normal substance" as an enzyme that decomposes fatty materials and proteins. For his work in the field of enzymes Wolf is generally considered the father of modern enzyme therapy. The work of Freund and John Beard sparked Wolf's interest in the possibilities of treating malignant diseases with enzymes. He subsequently founded the Biological Institute of New York City and, after studying various enzymes and enzyme combinations, developed what he regarded as an optimal preparation for the treatment of various acute and chronic conditions. His preparation was a combination of a

fractionated hydrolysate of beef pancreas, calf thymus, *Pisum sativum* (common pea), *Lens esculenta* (edible lentil), mannitol, and *Carica papaya* (papaya, a source of the enzyme papain).

In the 1960s, Irving Innerfield conducted landmark research in the area of pancreatic enzymes, primarily relating to the clinical use of trypsin, chymotrypsin, and pancreatin as well as streptokinase (a microbial proteolytic enzyme). Professor Heinrich Wrba, who for many years was head of the Austrian Cancer Research Institute at the University of Vienna, believed that enzyme therapy should be considered a highly effective causal anticancer compound. Dr. Wrba's interest in cancer was piqued when he lost a daughter to leukemia. He devoted his life to educating oncologists in Germany and around the world about enzyme therapy.

It was the late Karl Ransberger, however, who continued and refined Wolf's research, bringing it to doctors, hospitals, and patients throughout the world. Ransberger encouraged and funded research projects in numerous hospitals and universities in Europe, the Americas, and elsewhere. His research, and that of others, validated enzyme therapy's effectiveness in treating numerous conditions, including arthritis,[2,3,4] cancer,[5,6] multiple sclerosis,[5–9] cardiovascular disease,[6,10,11] and human immunodeficiency virus (HIV).[12–14]

PANCREATIC ENZYME SUPPLEMENTS

The pancreatic enzyme supplements commonly used in health care are chymotrypsin, trypsin, pancrelipase, and pancreatin. Chymotrypsin and trypsin are proteolytic enzymes that break proteins into peptides. Chymotrypsin liberates the amino acids L-tyrosine, L-tryptophan, and L-phenylalanine and other molecules, including several synthetic esters and amides.[15] Trypsin hydrolyzes primarily lysyl and arginyl residues. Pancreatin contains amylase (which breaks down starch), lipase (which breaks down fats), and protease (which breaks down proteins). Pancrelipase is similar to pancreatin, but with a higher concentration of lipase.

These enzymes are primarily obtained from hog or ox pancreas, but some (such as lipase) can also be obtained from microbial sources (e.g., *Aspergillus niger* and *Aspergillus oryzae*). Nevertheless, only enzymes isolated from animal pancreatic glands can be considered pancreatic enzymes.

According to the *U.S. Pharmacopeia* (USP), chymotrypsin and trypsin are routinely crystallized from ox pancreas gland extract, and pancreatin from both hog and ox sources, whereas pancrelipase is derived from hog pancreas. [16]Porcine pancreas is especially rich in amylase and lipase and is similar to the human pancreas.[15] Bovine pancreas contains considerable amounts of proteolytic enzymes but substantially lower amounts of lipase and amylase.[15] Germany, Japan, England, India, and other countries use their own pharmacopeia, and foreign companies may use other sources to formulate their enzyme products.

Enzyme concentration and activity levels can vary depending on the age, sex, and species of pork or ox used to produce the supplement. For example, sow glands (from pork) are high in lipase, whereas butcher hogs (young male hogs, up to 90 kg in weight and 6 months of age) are high in protease. Beef cows and bulls have different enzyme levels from those in steers or heifers. Beef, although it provides all three basic enzyme types, does not exhibit the activity levels of pork (which has an activity level one-third to one-half higher). Furthermore, the physiology of hogs is more similar to that of humans than to that of any other animal.

Enzymes extracted from animal sources are sensitive to environmental changes, so manufacturers take particular care during extraction to control pH (usually with buffers), temperature (using precooled solutions and apparatus), substrate, and proteolysis (controlled through the use of inhibitors) to render a product that is enzymatically active.[17]

Enzyme Standardization

In 2010, the U.S. Food and Drug Administration (FDA) began requiring manufacturers of pancreatic enzyme products to test them in clinical trials if those products were prescribed to treat individuals with pancreatic diseases. Until that time, there were frequent inconsistencies in enzyme formulation. For example, a study of six different enzyme preparations found that, in some cases, the actual lipase activity in a product was more than twice that listed on the label.[18] Another study on nine pancreatic enzyme products found that, although within USP limits, the percentage of lipase activity after dissolution was not always equal.[19] These inconsistencies in content and activity level could unexpectedly alter patient enzyme requirements.

The FDA's ruling ensures that pancreatic enzymes are standardized with a consistent activity level. Pancreatic enzymes marketed as dietary supplements, however, do not require testing or approval by the FDA. The requirements regarding activity level apply only to those pancreatic enzyme products prescribed for exocrine pancreatic insufficiency due to cystic fibrosis (CF), chronic pancreatitis, and other conditions.

ABSORPTION OF PROTEINS

In the past, it was believed that the intestinal epithelial mucosa was impermeable to large protein molecules.[20] However, research over the past several decades has shown that the intestinal epithelium can be crossed by macromolecules, including intact proteins such as proteolytic enzymes.[21]

These macromolecules normally penetrate the mucosal surface via the transcellular route because, in healthy mucosa, the tight junctions (zonula occludens) between the enterocytes prohibit paracellular passage.[22] Binding to the luminal membrane of the enterocyte is followed by phagocytosis.[23] Some of the vacuole membrane vesicles formed fuse with lysosomes, and within the resulting phagolysome the peptides and proteins may be hydrolyzed by lysosomal enzymes.[24] Other macromolecules avoid intracellular digestion and are passed from the enterocytes through the basolateral membrane into the interstitial space.[25] In the interstitial space, the macromolecules become available to macrophages and lymphoid cells.[26] Those molecules not taken up by macrophages or lymphatic cells eventually pass from the interstitial space into the blood or lymph.[27]

The exact level of the intestinal absorption of intact molecules or large breakdown products of dietary proteins is not yet totally clear and can vary by individual.[28] Although it is generally assumed that, apart from a very small proportion, all protein is hydrolyzed into amino acids or low-molecular-weight peptides before absorption by the mucosa, some research supports the hypothesis that a considerable proportion of dietary protein is taken up in the form of macromolecules and is only then hydrolyzed intercellularly in the peripheral tissue into amino acids (a process called *distributed digestion*).[29]

Understanding the Absorption of Enzymes

Understanding the intestinal transport of macromolecules is especially important for understanding the functions and absorption of enzymes, specifically. Hydrolases, such as trypsin or elastase, can be transported functionally intact into the bloodstream from the lumen of the gut. These circulating proteinases are bound to antiproteinases, such as $alpha_2$ macroglobulin or $alpha_1$ antiproteinase,[30] and can be resorbed

from the main bloodstream by pancreatic cells (enteropancreatic circulation as an enzyme conservation process).[31] Thus the intestinal absorption of intact enzymes appears to be important for the balance between hydrolases and antiproteinases in the intracellular space[32] and is an important factor for the establishment and maintenance of the internal stability in the body.

Although there are a number of absorption mechanisms, the primary mechanism for the enteral absorption of enzymes and other macromolecules is pinocytotic transfer by the M-cells of the small intestinal epithelium.[33] The enzymes connect to a receptor in the intestinal wall mucosa and are then absorbed into the wall by pinocytosis, guided through the intestinal cells in vesicles, and finally released into the blood by exocytosis.[33]

To clarify the rate of absorption, Steffen et al.[34] investigated the absorption of an enzyme mixture "A" (EMA), which contained 100 mg of pancreatin, 60 mg of papain, 10 mg of lipase, 10 mg of amylase, 24 mg of trypsin, 1 mg of chymotrypsin, 45 mg of bromelain, and 50 mg of the bioflavonoid rutin, in rabbits. Using electrophoresis, these researchers found that entire enzyme molecules were absorbed. Although enzyme particles were also present, the ratio to the entire amount administered was not measured. EMA was found in both the lungs and the liver after 1 to 2 hours. After 1 to 4 hours, approximately twice as much EMA was found in the liver as in the lungs. The absorption maximum in all animals occurred approximately 1 hour after administration. Within 6 hours, the absorption rate of orally ingested EMA was about 20%.[35] After 24 hours, EMA was no longer found in either the lungs or the liver.

FACTORS AFFECTING ENZYME ACTIVITY

Numerous factors, including pH, temperature, substrate (and substrate concentration), cofactors, metal ions, inhibitors, and coating, can affect the activity of supplemental enzymes.

Optimal pH Range

Each enzyme has an optimal pH range, depending on such variables as the temperature and substrate concentration at which the enzymatic catalytic reaction occurs most rapidly. Chymotrypsin has an optimum pH of 8.0, has reversible denaturation at a pH below 3.0, and becomes inactive at a pH above 9.0.[15] Trypsin has an optimum pH between 7.0 and 9.0, is stable at a pH of 3.0 (and at low temperature), and is irreversibly denatured at a pH of 9.0 or higher.[15] The pH of the normal human stomach is 1.5 to 3.0,[36] low enough to denature or inactivate some or all of a pancreatic enzyme supplement if it is not enterically coated or otherwise treated to protect it from a low-pH environment.

The Effects of Temperature

In general, an increase of 18°F (10°C) in the enzymatic environment approximately doubles the rate of the chemical reaction.[37] However, because enzymes are proteins, excessively high temperatures can denature them, thus destroying their activity. The optimum temperature for an enzyme is the temperature at which the catalyzed enzymatic reaction progresses most rapidly without damage to the enzyme. This temperature can vary by enzyme. This is a good rationale for avoiding hot beverages when taking enzyme supplements.

The enzymes in the human body develop high levels of activity at about body temperature, increasing to maximum at about the temperature of a severe fever, that is, 104°F (40°C).[37]

Substrate Concentration

The rate of any reaction is accelerated by raising the substrate concentration until the enzyme is saturated by substrate. At this level, the rate of reaction becomes independent of substrate concentration and is no longer accelerated by the addition of more substrate. This is why it is particularly important to ingest sufficient quantities of supplemental enzymes (which vary according to the condition being treated).

Cofactors

Although all enzymes consist of protein, some are complex proteins; that is, they have a protein component and a *cofactor*. If the cofactor is removed, the protein (no longer active enzymatically) is called the apoenzyme. A cofactor might be a metal (e.g., iron, magnesium, copper, or zinc), a prosthetic group (a moderately sized organic molecule), or a coenzyme (small organic compound). Prosthetic groups and metals can aid in the catalytic function of the enzyme, whereas coenzymes take part in the enzymatic reaction. Many vitamins, trace elements, and minerals essential to human bodily function are part of enzymatic cofactors. Thus the physician must ensure that his or her patients take multivitamins and multiminerals to "feed" their enzymes.

Coenzymes are essential for the activity of many enzymes and serve as a type of substrate in certain reactions. In these reactions, the coenzyme is converted to a form no longer active in catalyzing the reaction.

Metal Ions

Specific metal ions are required for the activity of many enzymes. Some metal ions increase enzyme activity, and others decrease or inhibit it. Calcium, cobalt, copper, iron, magnesium, manganese, molybdenum, potassium, and zinc are the most common enzyme activators in humans. Certain heavy-metal ions inhibit enzyme reactions; they are barium, lead, and mercury, and they combine with the sulfhydryl reactive group (−SH) that is part of the active site of many enzymes.

Inhibitors

Ions, atoms, or molecules that terminate or retard enzyme activity are called inhibitors. They are classified as either noncompetitive or competitive. A noncompetitive inhibitor combines with the enzyme at a location other than the active site. The noncompetitive inhibitor retards the conversion of the substrate by the enzyme, although it does not affect the bonding of the substrate of the enzyme. An inhibitor is classified as competitive if it combines with the active site of the enzyme, preventing the substrate from having access to the active site.

Supplement Coating

The pH of the stomach's hydrochloric acid secretions is about 0.8.[1] This low pH inhibits bacterial growth and activates certain enzymes. This acidic nature, however, can destroy pH-sensitive supplemental enzymes. For this reason, many enzyme products are enterically coated. This coating allows the product to reach the small intestine before disintegrating. Other products are encapsulated in "microspheres," delaying disintegration. For example, pancreatic protease encapsulated with a mixture of cellulose acetate phthalate and maize starch can remain stable in simulated gastric conditions (pH of 3.97) for at least 3 hours.[38] This would theoretically provide enough time for the capsule to pass through each part of the gastrointestinal tract. The capsule then disintegrates rapidly under pH 6.82 and temperature of 39.5°C (as would occur in the small intestine).[38]

Nanotechnology is opening a new field for the delivery of enzymes and other small proteins. Nanotechnology is the study of matter as small as 1 billionth of a meter. According to the National Nanotechnology Initiative, nanoparticles are being used in timed-release drug delivery.[39] Enzymes can be attached to nanoparticles and actually maneuvered to destroy diseased cells. Research on nondegradable nanocapsules showed that proteins can be efficiently transported to individual cells, surviving different pH levels.[40] So, it is no wonder that enzymes (which are proteins) can also be attached to nanoparticles and used to treat disease at the cellular level.

MEASURING ENZYME ACTIVITY

When considering enzymes and enzyme applications, the physician must understand the variables affecting their performance. The selection of an enzyme for therapeutic purposes requires more than knowing whether a given product contains amylase, protease, lipase, or other enzymes. The activity levels of the enzymes are critical.

As mentioned previously, the manufacturers of pancreatic enzymes prescribed to treat specific conditions must clearly disclose content and enzyme activity levels. Unfortunately, the same is not true of enzyme products sold as dietary supplements, whose labels may not indicate enzyme activity levels. In addition, even when the activity is stated, the consumer has no way of knowing which enzyme assay the manufacturer used unless the label also indicates that the product conforms to the guidelines of the USP. This is particularly confusing because activity levels are greatly affected by the conditions under which the assay was performed (including temperature, pH, and substrate).

Adding to the confusion, enzyme manufacturers use diverse assay methodologies, making a direct comparison of competing products difficult, if not impossible. Using a single assay system (such as detailed in the USP) is necessary to directly compare competitive products. Several standardized assay systems are available for enzyme suppliers and are found in the USP (for a definitive assay), the *NFIA Laboratory Methods Compendium,* and the *Food Chemical Codex.*

Incomplete labeling and the inconsistent use of standardized assay methodologies make evaluating competitive products extremely challenging. Price could be the first indication of inequities in assay procedures. For example, if company A is selling a product at 1000 U/g for $30 a bottle, and company B is selling a product at 5000 U/g for $10 a bottle, the units are most likely not the same.

For clinical reliability, one must use only appropriately labeled products or obtain the assay procedures from each of the manufacturers. If possible, competitor products should be compared by means of assays performed in an independent laboratory.

CLINICAL APPLICATIONS

Historically, enzyme therapy has been used in a wide variety of applications, ranging from oral supplementation to treat pancreatic insufficiency to the centuries-old external application of enzymes to treat leg ulcers, topical wounds, wrinkles, blemishes, episiotomies, scars, and so on. Usually administered in capsules or tablets, enzymes are also available as lozenges (dissolved in the mouth) or in powder form. Topical enzyme ointment is currently used to debride necrotic tissue and other wound debris. Enzymes can also be administered by injection (normally in a hospital setting because of the risk of anaphylactic reaction) or rectally, by retention implant.

Enzymes can be used individually but are typically more efficacious when used in combination with other enzymes. An enzyme combination has numerous therapeutic advantages over a preparation with only one or two components. Combining enzymes from different sources—animal, plant, and fungi—results in a wider range of optimal pH, synergism of the combined enzymes, greater absorption, higher level of efficacy, and broader range of application. For example, one German product contains an enzyme extract consisting of proteinases, triacylglycerol lipase, and alpha-glycosidase (amylase); minor amounts of elastase, nuclease, and carboxypeptidase; and calcium ions to boost activity.

Dr. Peter Streichhan, a well-known enzyme researcher, stated that certain enzymatic mixtures have a broader range of action than pancreatin, bromelain, or any other standardized monohydrolytic preparation—this is because certain enzyme mixtures characteristically possess differences in optimal pH and also differences in reactive properties of the proteolytic, lipolytic, and/or amylolytic-acting hydrolases.[41]

BOX 100.1 Clinical Applications of Chymotrypsin

- Debridement, treatment of abscesses and ulcerations, liquefaction of mucous secretions[95]
- Ophthalmic cataract surgeries and therapy of eyeball hematomas and ophthalmorrhagias[95,96]
- Before and after tooth extractions, as well as in operative dentistry[97,98]
- After episiotomy procedures[99]
- As an anthelmintic against enterozoic worms[100]
- Early recognition of tumor cells[101]
- Histologic gastroenterology diagnostics[102]
- Inflammatory conditions (local and systemic) to promote the dispersion of blood extravasates and effusions from fractures[96,103–110]
- Surgical trauma[99,108]
- Sporting injuries[103–117]
- Accidental soft tissue trauma[103–117,110]
- Intervertebral disc lesions[111]
- Uveitis vitreous hemorrhage, diabetic retinopathy, and asthmatic symptoms[112]

BOX 100.2 Clinical Applications of Trypsin

- Debridement of necrotizing wounds, ulcerations, abscesses, empyemas, hematomas, fistulas, and decubitus[113-116]
- To accelerate healing in injuries, inflammations, phlogistic edemas, and traumatic changes[103–110]
- As an auxiliary agent in meningitis therapy[117]
- As an ointment or dressing (wet or dry)[15]
- As a liquid or an aerosol to liquefy sputum in bronchial disorders and in the preparation of sputum for cytological examination[15]
- As an anti-inflammatory agent; oily suspensions are injected intramuscularly[15]
- As an aid in the treatment of intraocular hemorrhage, thrombophlebitis, intestinal obstruction (due to cirrhosis or carcinoma)[118]

BOX 100.3 Clinical Applications of Amylase

- Needs calcium ions for enzymatic activity
- Acts on starch, glycogen, and related poly- and oligosaccharides[119]
- In combination with other enzymes, as a digestant[95,120,121]
- As an anti-inflammatory[120]
- Treatment of deficiencies of exocrine pancreas, amylaceous dyspepsia, and cystic fibrosis[15]

It should be remembered that at the beginning of therapy, an individual's symptoms may occasionally become more severe. This is a sign that a therapeutic reaction is occurring and should be evaluated positively. The medication does not need to be discontinued, although a temporary reduction in dose might be advisable.

Clinical uses of individual enzymes can be found in Boxes 100.1 through 100.5, whereas clinical uses of combinations can be found in Box 100.6. For more information on how enzymes can treat more than 150 conditions, please see the author's book, *The Complete Book of Enzyme Therapy.*

Most conditions treated by enzymes can be assigned to one or more of the following categories:

- Digestive conditions
- Inflammatory conditions
- Cancer

BOX 100.4 Clinical Applications of Lipase

- In pancreatin-containing remedies to increase pancreatic/lipolytic activities (replacement therapy)[122–125]
- When given with pancreatin (in combined preparations), reduces fat level in stools[126–128]
- Synergistically intensifies the activity of lipoprotein lipase in the blood[129] and migration of agranulocytes[130]
- As a digestive aid[128]

BOX 100.5 Clinical Applications of Pancreatin

- In pancreatic insufficiency, inadequate secretion of exocrine pancreas, disturbed digestion, and after gastrectomy[66,123,131-142]
- In chronic pancreatitis[143] and after surgery for chronic pancreatitis[144]
- After pancreatectomy[15,143]
- In ductal obstruction from neoplasm (e.g., of the pancreas or common bile duct)[143]
- To treat severe cases of steatorrhea (as found in cystic fibrosis)[145–150]

BOX 100.6 Clinical Applications of Enzyme Combinations

- Soft tissue injuries[73,151]
- Sprained ankle[73,152,153]
- Tendonitis[47]
- Reabsorption of hematomas[151,154]
- Sports medicine[50,155–162]
- Meniscectomy (pre- and postoperative therapy)[163,164]
- Traumatology[165]
- Fractures[48,49]
- Pancreatitis[166]
- Surgery[151,167]
- Lower extremity bypass surgery[168]
- Operative dentistry[169]
- Proctology[170]
- Sinusitis[2,171–173]
- Acute and chronic bronchitis[6,70,174]
- Cystitis and lower urinary tract infections[2,175,176]
- Prostatitis[2,176,177]
- Pelvic inflammatory disease[178,179]
- Postthrombotic syndrome[3,180–183]
- Pathological venous processes[180,184–188]
- Occlusive arterial disease[189]
- Lymphedema[4,63,190–194]
- Soft tissue rheumatism (nonarticular rheumatoid syndrome)[71,72]
- Rheumatoid arthritis (chronic polyarthritis)[2–4,52,69,70,74–76,195–206]
- Ankylosing spondylitis (Bekhterev's disease)[73–76]
- Degenerative rheumatic joint disease[2,158]
- Monoarticular, activated osteoarthrosis[158,174,207]
- Multiple sclerosis[5–9,79,208,209]
- HIV infections[12–14,210,211]
- Sepsis[83]
- Fibrocystic breast disease[193,212]
- Ulcerative colitis and Crohn's disease[2,6,9,70]
- Autoimmune or immune disorders
- Cardiovascular disorders
- Viral and bacterial disorders
- Other conditions

Digestive Disorders

Supplementing the diet with pancreatic enzymes can improve food digestion and therefore nutrient assimilation. This is especially true if the body's own pancreatic enzyme production and/or supply is deficient, as can occur with age or because of an underlying health condition, such as cystic fibrosis (CF), chronic pancreatitis, pancreatic insufficiency, steatorrhea, biliary tract disease, or celiac disease. Pancreatic enzymes have a long history of use in treating those conditions, as well as indigestion, heartburn, gas, diarrhea, constipation—the list seems endless.

Patients with CF are routinely prescribed pancreatin or pancrelipase capsules containing enterically coated beads. A hereditary disease, CF involves a disorder of the exocrine glands and causes the production of thick mucus, which leads to obstructions in various glands and ducts in the body. When the mucus affects the lungs, it leads to severe coughing and repeated lung infections, including bronchitis and pneumonia. When the mucus obstructs the pancreatic duct, it prevents the enzyme-rich pancreatic juice from reaching the intestine, negatively affecting food digestion and nutrient absorption. This can result in malnutrition, weight loss, steatorrhea (fatty stools), or intestinal obstruction. Poor digestion affects approximately 90% of all CF patients.[42]

Although critical for proper digestion in those with CF, enzyme supplementation can also improve digestion and nutrient absorption in those with chronic pancreatitis, a condition marked by weight loss, steatorrhea, and abdominal pain and swelling. By improving nutrient absorption, enzymes can improve serum albumin levels (low levels reflect protein-calorie malnutrition),[43] improve steatorrhea,[44] and, thereby, increase weight gain[43] in these subjects.

Even healthy individuals can benefit from taking enzymes because enzyme supplementation can improve the digestion of carbohydrates, proteins, and fats. For example, a study on healthy individuals found that ingesting an acid-resistant lipase supplement immediately after eating a high-fat meal (comprising 55% fat) reduced the feeling of stomach fullness that typically follows such a meal.[45]

Inflammatory Diseases and Conditions

The inflammatory process is involved in numerous diseases and conditions, from sports injuries to arthritis to sinusitis to fibrositis. Whenever an injury or infection (regardless of the cause) occurs, a number of repair mechanisms are activated, resulting in microthrombi and fibrin surrounding the traumatized region. The fibrin web cuts off normal circulation, resulting in stasis and inhibiting the repair process (nutrients cannot get into the damaged area, and waste products cannot be removed). This, in turn, leads to increased pressure in the capillaries, causing fluid to be forced out into the surrounding tissue, resulting in swelling and pain. Supplemental enzymes, after absorption, circulate through the bloodstream to the affected area and have been shown to reduce several of the proinflammatory cytokines while increasing anti-inflammatory cytokines.[46] The enzymes attack the microclots and fibrin formation, effectively lysing the fibrin, breaking open the clogged vessels, and reestablishing circulation. The resulting restoration of normal blood flow leads to a faster resolution of postinflammatory pain and edema. Equally important, the essential physiological inflammatory repair process is not blocked or diminished (as with anti-inflammatory agents), but rather is accelerated and reinforced.

In a Canadian study, postal workers with rotator cuff tendonitis were treated with either naturopathic care (NC), which included

dietary counseling, acupuncture, and an enzyme supplement (containing bromelain, trypsin, and rutin), or with physical exercise (PE), which included exercise and placebo.[47] Those participants on the NC therapy saw a decrease in their shoulder pain and disability of over 54% (as measured by Shoulder Pain and Disability Index) compared with 18% in the PE group. The NC group also had significant improvement in range of motion compared with the PE group.[47]

Fractures often require surgery to repair the broken bone. Czech researchers followed the progress of 60 patients after surgery for repair of long-bone fractures. Thirty patients received an enzyme mixture (containing trypsin, bromelain, and rutin), whereas another 30 patients were treated by standard antiedema drugs. Both groups received the same pain medicine. The group that received the enzyme mixture had a significantly faster reduction in posttraumatic and postoperative swelling. In addition, those taking enzymes consumed significantly fewer painkillers.[48]

A similar study in Russia using an enzyme mixture on children with fractured long bones found that swelling and pain subsided twice as fast in the enzyme group as in the group receiving traditional therapy.[49]

Intense exercise can lead to soft tissue injury, inflammation, and muscle soreness. Researchers found that supplementing with protease mixtures could help ease muscle soreness and assist muscle healing after exercise.[50] A similar study[51] conducted a randomized, placebo-controlled clinical trial to determine the effect of systemic enzyme therapy on exercise-induced inflammation.[51] Compared with placebo, the enzyme therapy was superior in reducing biomarkers of inflammation in all study subjects.

Even inflammatory diseases, such as arthritis[52] and herpes zoster,[53] were shown to respond to the systemic application of enzymes. A double-blind, randomized study compared an oral enzyme mixture (containing trypsin, bromelain, and the bioflavonoid, rutin) with diclofenac (a nonsteroidal anti-inflammatory drug frequently prescribed for osteoarthritis and rheumatoid arthritis).[54] Ninety patients received either the enzyme mixture or the diclofenac for a 6-week period. Results indicated that the enzyme mixture was just as effective as diclofenac.

A similar study on 150 patients with moderate to severe knee osteoarthritis compared the effect of an enzyme combination (containing trypsin, bromelain, and rutin) with that of diclofenac.[55] Those patients taking the enzyme combination experienced a significant improvement in joint pain, function, and joint flexibility. According to the researchers, the enzyme treatment was as effective as diclofenac with fewer potential side effects.

Individual enzymes and enzyme combinations (particularly those including trypsin, chymotrypsin, pancreatin, amylase, lipase, papain, and bromelain, with rutin) are effective in treating inflammation because they help limit the injury, aid its rectification, and promote new, healthy tissue formation. Enzymes effectively accelerate the inflammatory process (a necessary component of wound healing). This acceleration means, on the one hand, that the work of damage control, damage repair, and new tissue construction is carried out more actively, and thus completed more swiftly. On the other hand, it means that there can be a temporary increase in the visual and sensory effects produced by the inflammation (i.e., more redness, swelling, heat, and pain).

Despite the supportive research, the administration of enzymes in traumatology and injuries is not widely used in the United States, which is in contrast to Europe, where all types of injuries—sprains, strains, hematomas, dislocations, and even postoperative conditions—are effectively treated with enzymes. Although the inflammatory process is involved in viral and bacterial infections, those disorders are covered in the "Viral and Bacterial Disorders" section within this chapter.

Cancer

Just as Beard researched the effects of enzymes on cancer, so have modern researchers. Dr. William Kelley devised an anticancer program that employed detoxification, pancreatic enzymes (and other nutritional supplements), improved enzyme-rich nutrition, and coffee enemas.[56] He initiated his treatment program in response to his own bout with pancreatic cancer. Building on Kelley's research, Dr. Nicholas Gonzales treated various types of cancer with diet, nutritional supplements, and detoxification methods. Many physicians around the world use systemic enzyme therapy to treat cancer; however, it remains a controversial therapy. Widely accepted, however, is the practice of using enzyme therapy to treat the side effects of cancer and cancer treatment.

Standard cancer therapy can often result in serious side effects. For instance, radiation therapy of the head and neck is often accompanied by acute side effects, including difficulty swallowing, mucous membrane inflammation, and skin redness or soreness. Oral enzyme preparations seem to protect against these acute side effects, reducing their severity and duration.[57]

A study on patients with laryngeal cancer receiving radiation treatment found that those patients receiving an enzyme mixture (containing trypsin, bromelain, and rutin) had no cases of radiomucositis of the second degree and were able to continue on the radiation therapy.[58] Researchers also noted that those on enzyme therapy had a mean weight loss of only 5% compared with 10% in the control group.

In another study, researchers tested an enzyme combination in patients who underwent radiation therapy for cervical cancer.[59] Radiation therapy can cause numerous side effects, including bladder irritation, loose bowels, nausea, vomiting, and vaginal dryness. Results indicated that patients who received the enzyme mixture (which contained trypsin, chymotrypsin, and papain) had a significant reduction in vaginal mucosa reactions, genitourinary symptoms, and subcutaneous changes.[59]

Researchers in another study on patients with lung cancer who underwent chemotherapy found that an enzyme mixture containing papain, trypsin, chymotrypsin, and thymic peptides reduced the incidence of leukopenia, nausea, vomiting, sensory neuropathy, and treatment-related depression.[60]

Breast Cancer

It is estimated that one of every eight women will be diagnosed with breast cancer sometime during her life.[61] Unfortunately, standard treatment (which can include radiation, chemotherapy, and surgery) can cause serious side effects, including gastrointestinal symptoms, headache, pain, skin disorders, and infections. Researchers in Germany evaluated whether treatment with oral enzymes could reduce some of the typical side effects of breast cancer therapy.[62] They found that 74% of the enzyme group experienced a clear reduction in chemotherapy and radiotherapy side effects and that oral enzyme therapy could improve disease signs and symptoms, reduce side effects, and improve quality of life.[62]

Lymphedema

Treatment for breast cancer can sometimes lead to lymphedema (swelling caused by a buildup of lymph). This typically occurs after a lymph node is removed or after radiation treatment and can develop slowly. Symptoms include swelling in the arm, hand, shoulder, chest, or breast and may be accompanied by changes in skin texture, aching, and restricted mobility.

Typical medical therapy for lymphedema includes massage, exercising, special bandaging, manual lymphatic drainage, and the use of diuretic therapy. One clinical trial compared therapy with enzymes

(the product contained pancreatin, papain, bromelain, trypsin, chymotrypsin, and rutosid) with a diuretic.[63] After 7 weeks, significantly more of the patients receiving enzymes were pain-free compared with the patients receiving diuretics.

Pancreatic Cancer

It is estimated that pancreatic cancer will kill more than 43,000 in the United States this year alone.[64] In the past, survival rates for this disease were extremely low because treatment options were limited, and diagnosis usually did not occur until the disease was quite advanced. However, treatment improvements and earlier diagnosis have dramatically improved survival rates.[65] The standard medical treatment for pancreatic cancer involves surgery, radiation therapy, chemotherapy, and immunotherapy (also called biological therapy).

Pancreatic cancer can block the pancreatic duct and prevent enzymes from reaching the small intestine, resulting in malnutrition and weight loss. It is estimated that 80% to 90% of all patients with pancreatic cancer have malabsorption and weight loss.[66] Supplementing with pancreatic enzymes is an important supportive step in treating pancreatic cancer because it can improve digestion and therefore nutrient absorption.[67,44,68]

Autoimmune and Immune Complex–Mediated Diseases

In autoimmune disease, tissue-bound immune complexes activate the complement system. Activation of the enzyme cascade results in an intense protein-destroying inflammatory response, leading to significant local tissue destruction. For instance, when immune complexes collect in the kidneys, complement activation causes inflammation, resulting in glomerulonephritis.

Research showed that some enzymes can inhibit immune complex–mediated diseases, such as glomerulonephritis and rheumatoid arthritis,[2,3,4,52,69] by interrupting the complement cascade. Other disorders with similar mechanisms also respond to supplemental enzymes. Some are conditions such as Crohn's disease,[2,6,9,70] chronic rheumatism,[71,72] and ankylosing spondylitis,[73–76] which do not respond well to conventional medical treatments.

Multiple Sclerosis

According to the Multiple Sclerosis Foundation, there may be as many as 1 million Americans living with multiple sclerosis (MS).[77] Unfortunately, the government does not track MS cases, so the frequency can only be estimated. Although its cause is unknown, it is generally believed to be an autoimmune disease. In MS, antibodies attack the myelin sheath that protects and covers nerve fibers. This leads to inflammation and myelin sheath damage, which in turn interferes with nerve communications.

MS patients typically have higher concentrations of circulating immune complexes (CICs) than healthy individuals. Research showed that enzyme therapy can help reduce those CICs and thereby reduce inflammation.[78]

A Ukrainian study on an enzyme combination containing trypsin, bromelain, and rutin found that patients with MS taking the combination for 1 to 3 years had fewer and less severe complications and longer remissions and also experienced a slowing of their disease progression.[79]

Cardiovascular Disorders

Cardiovascular disorders include diseases of the heart and blood vessels (including strokes). In the United States, heart disease and stroke kill nearly three-quarters of a million people every year and account for nearly one third of all deaths.[80]

In normal circulation, there is a constant dynamic balance between blood clotting and fibrinolysis.[81] If fibrinolysis is impaired, clot formation is abnormal. If fibrinolysis increases, a tendency toward excessive bleeding results. Therefore maintenance of proper equilibrium is extremely important for the circulatory system.

One study examined the effects of various enzyme combinations on fibrinolysis and fibrin formation.[6] The researchers induced fibrin deposition with calcium ions or staphylocoagulase in the plasma of centrifuged, acellular citrated blood from humans or rabbits and treated the clot with various concentrations of enzyme suspensions. They found that the higher the enzyme content of the solution, the more rapidly clots were degraded. Inflammation is considered to play a central role in all atherosclerotic processes. Therefore the use of enzymes is indicated because of their anti-inflammatory effect.

A German study measured the effects of a proteolytic enzyme combination (containing trypsin, bromelain, and rutin) on blood fluidity.[10] Researchers found that even a minimum enzyme concentration resulted in a significant reduction in plasma viscosity and erythrocyte aggregation.

Systemic enzyme therapy appears to be particularly effective in the treatment of phlebitis and thrombophlebitis,[11] as well as chronic venous insufficiency (also called postphlebitic syndrome), which can occur because of phlebitis. In one study, treatment with systemic enzyme therapy led to a decrease in several symptoms, including pain, edema, and trophic ulcers.[82]

Viral and Bacterial Disorders

Enzymes have been used to treat many viral and bacterial conditions, including abscesses, acne, adenoiditis, adnexitis, bladder infection, boils, conjunctivitis, diarrhea, ear infections, empyemas, epididymitis, gingivitis, kidney disorders, laryngitis, pneumonia, rheumatic fever, sinusitis, staphylococcal infection, tonsillitis, and sepsis.

Sepsis is a particularly dangerous systemic response to infection. Researchers in India found that proteolytic enzyme therapy improved recovery time in children with sepsis.[83] Thirty young boys (ages 1 month to 12 years) with sepsis were given proteolytic enzymes. Another group of 30 boys received placebo tablets. Both groups received supportive treatment and the appropriate antibiotics. Fever subsided faster in the enzyme group (3 days) than in the placebo group (4 days), and those in the enzyme group were able to begin oral feeding in 4 days as opposed to 5 days in the placebo group. In addition, hemodynamic support was only needed for 2 days in the enzyme group (as opposed to 3 days in the placebo group), and the modified Glasgow Coma Scale normalized for enzyme participants in 3 days (as opposed to 5.5 days in the placebo group).[83]

As more and more bacteria become resistant to antibiotics, researchers and physicians are investigating supportive treatments, including systemic enzyme therapy. As mentioned previously, enzymes not only reduce inflammation, improve circulation, and stimulate and balance immune system function, but they are also able to enhance the ability of antibiotics when their use is required.[84,83] Enzymes fortify the action of antibiotics, leading to complete healing and preventing the progression of an acute infection into its chronic form. A good example is urogenital chlamydiosis, which is a sexually transmitted disease and a major cause of infertility. According to the Centers for Disease Control and Prevention, there were more than 1 million cases reported in the United States in 2008. By 2016 that number had increased to 1½ million cases.[85] Standard treatment is with antibiotics, including azithromycin or doxycycline. Although antibiotic treatment appears to be highly efficacious, in some individuals the infection becomes persistent and chronic, leading to long-term disorders in females, including pelvic inflammatory disease, ectopic pregnancy, and sterility.[86] A Russian study of 227 patients with urogenital chlamydiosis found

that treatment with antibiotics led to complete recovery in only 61.4% of patients, whereas enzyme therapy in conjunction with antibiotics resulted in over 90% treatment success.[84]

In addition to its use in treating bacterial infections, enzyme therapy can also aid in the treatment of viruses, including HIV, chancre sores, chickenpox, colds and coughs, hepatitis, herpes simplex, herpes zoster, influenza, measles, pneumonia (viral), and warts. The human body defends against these, and other, viruses with the help of macrophages or natural killer (NK) cells. Oral enzyme combinations (e.g., papain, trypsin, and chymotrypsin) synergistically increase the antiviral effects of tumor necrosis factor (TNF), macrophages, and NK cells and the breakdown of CICs.

DOSAGE

Regardless of the condition being treated, a wide range in daily dosage has historically been used for the following reasons:

- There are individual differences in patient health.
- The level of effective absorption in tablet strength can vary.
- There is a wide variety of influencing ranges in pH.
- Enzyme activity levels vary between products.

The dosage of any pancreatic enzyme will depend on the condition being treated, the patient's diet and digestive requirements, and the composition and activity level of the supplement. Dosage can also vary because of the supplement's susceptibility to inactivation in the stomach and duodenum. For best results, follow the label for dosage recommendations.

TOXICOLOGY

In general, the side effects of orally administered enzymes are few, short term, and due primarily to higher dosages or hypersensitivity reactions. According to Wolf and Ransberger, high dosages (70 tablets of 17.5 g each) of a proteolytic enzyme mixture have been given without long-term side effects.[87] Studies and literature searches commissioned by regulatory authorities such as the FDA apparently confirmed that enzyme preparations obtained from suitable sources (e.g., nontoxic, nonpathogenic sources) are safe to consume.[88]

The potential side effects of pancreatic enzyme therapy include diarrhea; intestinal gas; abdominal pain or cramping; and greasy, pale, or pungent stools. Reducing the dose can usually reduce or eliminate these side effects. Hyperuricosuria (excess uric acid in the urine) and hyperuricemia (excess uric acid in the blood) have been associated with extremely high doses. Sneezing, lacrimation, rash, and other hypersensitivity reactions have been reported in sensitive individuals.

Pancreatin preparations held in the mouth or chewed before swallowing can result in stomatitis, or ulcerations and irritation of the mucosa, particularly in infants, in whom pancreatin may also cause perianal soreness.[89] However, some of the capsules containing microencapsulated enzyme beads can be opened and dispensed by the spoonful to infants or mixed into foods such as apple sauce or gelatin.

Prolonged skin contact with proteolytic enzymes (i.e., in ointment form) may cause skin irritation, whereas enzyme enemas can sometimes cause itching or burning in the rectum or anus. Care should be exercised when using powdered enzymes or enzyme capsules to avoid inhaling dust particles, which could irritate the mucous membranes of the throat, nose, and eyes.[88] In addition, inhalation may trigger an asthma attack.[90]

It is not known whether pancreatin or pancrelipase given to pregnant women can harm the fetus because animal reproductive studies have not been conducted. Therefore pancreatin should not be used during pregnancy.[89] Also, it is not known if pancreatin or pancrelipase is distributed into a mother's milk. Therefore caution should be exercised with the use of this substance in nursing women.[89]

Enzymes should not be used by those with hemophilia or those on blood thinners (unless under a physician's care), nor immediately before or after surgical procedures with an increased risk of bleeding. Enzymes should also be avoided by those with pulmonary emphysema or thrombocytopenia. Pancrelipase (and any other enzyme derived from pork sources) is contraindicated in those who are hypersensitive or allergic to pork products.

There is some indication that patients with CF who are undergoing long-term high-dose pancreatic enzyme therapy may demonstrate colonic wall thickening, a condition called fibrosing colonopathy (FCP). It was once believed that the copolymer coating on the tablets might be the culprit,[91] and there have been instances of FCP in CF patients not taking any type of enzyme preparation.[92] However, it is generally believed that FCP occurs because of prolonged treatment with very high doses of pancreatic enzymes.[93] Many of the individuals who were first diagnosed with FCP were taking tens of thousands of units in excess of label recommendations.[94] To reduce the risk of FCP, it is extremely important that patients closely follow their physicians' recommendations regarding dosage.

A note about supplement safety: During the past few decades, there has been increased concern regarding mad cow disease (bovine spongiform encephalopathy [BSE]). Called Creutzfeldt–Jakob disease (CJD) in humans, it can be caused by eating beef that is infected with BSE, but it can also be transmitted through corneal transplant tissue, from hormone extracts (e.g., human growth hormone), and possibly from ingesting contaminated glandular products. Unfortunately, it may take as long as 20 years after infection for the characteristic symptoms of CJD to manifest. Since 1995, the FDA has required that dietary supplement manufacturers ensure that any "high-risk" animal products used in their supplements do not originate in BSE countries, in countries with inadequate surveillance, or in countries with requirements less strict than those that would be acceptable for importation into the United States. The list is constantly updated; for the most current list of BSE countries, refer to the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service website (https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/import-live-animals/ct_bovine_spongiform_encephalopathy). Contact the supplement manufacturer for concerns about pancreatic supplement safety or to see what measures they employ to ensure BSE-free products.

DRUG INTERACTIONS

In addition to lipases and amylases, pancreatic enzymes contain proteases, so they are subject to some of the same drug interaction warnings as is bromelain (see Chapter 59 on bromelain). As with bromelain, caution should be exercised when pancreatic enzymes are used with anticoagulants (blood thinners), as well as over-the-counter nonsteroidal anti-inflammatory drugs, including aspirin and ibuprofen. In addition, certain botanicals, including garlic, gingko biloba, and ginseng, may increase bleeding risk and so should be monitored when used in conjunction with pancreatic enzymes.

Certain diabetes drugs, such as acarbose (brand names Glyset and Precose), function as alpha-glucosidase inhibitors, lowering blood sugar levels by inhibiting the digestion of dietary carbohydrates. This, in turn, limits the rise of blood sugar levels. However, the amylase in pancreatic enzymes has the potential to reduce the effectiveness of alpha-glucosidase inhibitors because amylase improves carbohydrate

digestion. Therefore pancreatic enzymes should not be taken concomitantly with acarbose and other alpha-glucosidase inhibitors.

Similarly, drugs that function as lipase inhibitors (e.g., Orlistat) should be avoided while taking pancreatic enzymes. Orlistat is available in prescription (as Xenical) and nonprescription (as Alli) forms. It functions by inhibiting lipase in both the lumen of the stomach and the small intestine. Although there are no known studies on Orlistat given concomitantly with pancreatic enzymes, it would seem counterproductive to take a drug designed to inhibit lipase activity while also taking a drug containing lipase. In addition, because Orlistat inhibits the activity of lipase, it may interfere with the action of pancreatic enzymes.

Any foods that contain enzyme inhibitors have the potential to decrease the activity of pancreatic enzymes. Heat inactivates enzyme inhibitors (such as those found in potatoes, legumes, and soybeans, which are rarely eaten raw).

CONCLUSION

As we can see, the use of pancreatic enzymes has been found to be extremely effective in treating a wide variety of conditions, from digestive diseases to inflammation, cancer, autoimmune diseases, viral and bacterial conditions, and more. It is my firm belief that future research will not only continue to validate but will also reveal new and exciting applications for systemic enzyme therapy.

This chapter is dedicated to the memory of Dr. Max Wolf, the father of systemic enzyme therapy, as well as Dr. Karl Ransberger, who validated and marketed systemic enzyme therapy, making it a highly accepted worldwide treatment for a wide variety of systemic diseases and injuries.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Guyton GC, Hall JE, eds. *Textbook of Medical Physiology*. 11th ed. Philadelphia: WB Saunders; 2006.
2. Riede NU, Schaefer HE, Wehner H. *Allgemeine und Spezielle Pathologie*. Stuttgart: Thieme Verlag; 1989.
3. Klein K. Proteolytisches enzympraparat erfolgreich. *Therapiewoche Osterreich*. 1989;39:448.
4. Streichhan P, Inderst R. Konventionelle und enzymtherapeutische massnahmen bei der behandlung brustkrebsbedingter armlymphodeme. *Der Prakt Arzt*. 1991;13:37–38.
5. Ransberger K, van Schaik W. Enzymtherapie bei multipler sklerose. *Der Kassenarzt*. 1986;41:42–45.
6. Inderst R. Enzymtherapie—grundlagen und anwendungsmoglichkeiten. *Natur-und Ganzheitsmedizin*. 1991;3.
7. Tilwe GH, Beria S, Tutrakhia NH, et al. Efficacy and tolerability of oral enzyme therapy as compared to diclofenac in active osteoarthritis of knee joint: an open randomized controlled clinical trial. *J Assoc Physicians I*. 2001;49:617–621.
8. Masuhr KF. *Neurologie*. Stuttgart: Hippokrates; 1989.
9. Neuhofer Ch, van Schaik W, Stauder G, et al. *Pathogenetic Immune Complexes in MS: Their Elimination by Hydrolytic Enzymes. A Therapeutic Approach. International Multiple Sclerosis Conference*. Bologna: Monduzzi Editore SPA; 1988.
10. Saradeth T, Quittan M, Ghanem AH, et al. Improvement in blood fluidity characteristics via Phlogenzym—an in vitro pilot study. *Perfusion*. 1995;8:196–198.
11. Koshkin VM, Kirienko AI, Leontjev SG, et al. Systemic enzyme therapy of lower limb postphlebitis syndrome. *Angiol Vasc Surg*. 2000;6:61–64.
12. Ransberger K. *Naturheilkundliche therapie von AIDS mit enzympraparaten. Forum des Prakt und Allgemeinarztes Heft 4.27*. Jahrgang; 1988.
13. Diringer H. *Unkonventionelle viruserkrankungen*. *Bundesgesundhbl.* 1990;33:187–194.
14. Inderst R, Ransberger K, Brand G. Fortschritte in der therapie der erworbenen immunschwache durch naturheilkundliche methoden. *Naturheilpraxis*. 1998;41:1050.
15. Ruyssen R, Lauwers A. eds. Pharmaceutical enzymes: properties and assay methods. *Gent:Story-Scientia*. 1978:34–35.
16. *US Pharmacopeia XXII*. Rockville, MD: United States Pharmacopeial Convention; 1990.
17. Eisenthal R, Danson MJ. *Enzyme Assays: A Practical Approach*. Oxford: IRL Press; 1992.
18. O'Hare MM, McMaster C, Dodge JA. Stated versus actual lipase activity in pancreatic enzyme supplements; implications for clinical use. *J Pediatr Gastr Nutr*. 1995;21:59–63.
19. Case CL, Henniges F, Barkin JS. Enzyme content and acid stability of enteric-coated pancreatic enzyme products in vitro. *Pancreas*. 2005;30:180–183.
20. Gardner ML. Intestinal assimilation of intact peptides and proteins from the diet—a neglected field? *Biol Rev Camb Philos*. 1984;59:289–331.
21. Brambell FWR. *The Transmission of Passive Immunity from Mother to Young*. Vol. 18. Amsterdam: North-Holland Publishing; 1970.
22. Bjarnason I, Peters TJ. Helping the mucosa make sense of macromolecules. *Gut*. 1987;28:1057–1061.
23. Adibi SA, Mercer DW. Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. *J Clin Invest*. 1973;52:1586–1594.
24. Georgopoulou U, Dabrowski MF, Vernier JM. Absorption of intact proteins by the intestinal epithelium of trout, Salmo gairdneri. *Cell Tissue Res*. 1988;251:145–152.
25. Borgstrom B, Dahlqvist A, Lundh G, et al. Studies of intestinal digestion and absorption in humans. *J Clin Invest*. 1957;36:1521–1536.
26. Seifert J, Sellschopp CH, Sass W. Werden Makromolekule resorbiert und welchen Einfluss hat dabei die Immunitatslage? *Z Hautkrankheiten*. 1987;62:55–59.
27. Chung YC, Kim YS, Shadchehr A, et al. Protein digestion and absorption in human small intestine. *Gastroenterol*. 1979;76:1415–1421.
28. Hemmings WA, Williams EW. Transport of large breakdown products of dietary protein through the gut wall. *Gut*. 1978;19:715–723.
29. Hemmings WA. Distributed digestion. *Med Hypotheses*. 1980;6:1209–1213.
30. LeFevre ME, Joel DD. Intestinal absorption of particulate matter. *Life Sci*. 1977;21:1403–1408.
31. Lake-Bakaar G, Smith-Laing G, Summerfield JA. Origin of circulating serum immunoreactive trypsin in man. *Dig Dis Sci*. 1982;27:143–148.
32. Seifert J, Ganser R, Brendel W. Die resorption eines proteolytischen enzyms pflanzlichen ursprungs aus dem magen-darm-trakt in das blut und die lymphe von erwachsenen ratten. *Z Gastroenterol*. 1979;17:1–8.
33. Gebert G. *Physiologie*. Stuttgart: Schattauer; 1987.
34. Steffen C, Menzel J, Smolen H. Untersuchungen uber intestinale resorption mit 3H-markiertem enzymgemisch (Wobenzym). *Acta Med Aust*. 1979;6:13–18.
35. Streichhan P. Resorption enteraler makromolekule. *Verdauungs-krankheiten*. 1989;7:28–38.
36. Burtis G, Davis J, Martin S. *Applied Nutrition and Diet Therapy*. Philadelphia: WB Saunders; 1988.
37. Hasselberger FX. *Uses of Enzymes and Immobilized Enzymes*. Chicago: Nelson-Hall; 1978.
38. Lin CW, Lee TG. A note on the micro-encapsulation of pancreatic protease for protection against gastric digestion. *Anim Prod*. 1993;56:413–417.
39. Small wonders, endless frontiers: A review of the National Nanotechnology Initiative. National Academy of Science. 2002;36. http://www.nap.edu/openbook.php?record_id=10395&page=36. Accessed November 1, 2011.
40. Yan M, Du J, Gu Z, et al. A novel intracellular protein delivery platform based on single-protein nanocapsules. *Nat Nanotechnol*. 2010;5:48–53.
41. Wobenzym Streichan P. An orally administered combination preparation consisting of hydrolytic enzymes and rutin acting in circulating body fluids. *Inventory Text Part A, Preclinical Results*. Geretsried: Mucos Pharma GmbH & Co.; 1993.
42. Munck A, Duhamel JF, Lamireau T, et al. Pancreatic enzyme replacement therapy for young cystic fibrosis patients. *J Cyst Fibros*. 2009;8:14–18.
43. Trolli PA, Conwell DL, Zuccaro Jr G. Pancreatic enzyme therapy and nutritional status of outpatients with chronic pancreatitis. *Gastroenterol Nurs*. 2001;24:84–87.
44. Safdi M, Bekal PK, Martin S, et al. The effects of oral pancreatic enzymes (Creon 10 capsule) on steatorrhea: a multicenter, placebo-controlled, parallel group trial in subjects with chronic pancreatitis. *Pancreas*. 2006;33:156–162.
45. Levine ME, Koch SY, Koch KL. Lipase supplementation before a high-fat meal reduces perceptions of fullness in healthy subjects. *Gut Liver*. 2015;9:464–469.
46. Minaev SV, Nemilova TK, Knorring GI. Polyenzymatic therapy in prevention of adhesive processes in the abdominal cavity in children. *Vestn Khir ImGrekov*. 2006;165:49–54. [Russian].
47. Szczurko O, Cooley K, Mills EJ, et al. Naturopathic treatment of rotator cuff tendonitis among Canadian postal workers: a randomized controlled trial. *Arthritis Rheum*. 2009;61:1037–1045.
48. Kamenícek V, Holán P, Franěk P. Systemic enzyme therapy in the treatment and prevention of post-traumatic and postoperative swelling. *Acta Chir Orthop*. 2001;68:45–49. [Czech].
49. Isaeva AV, Minaev SV, Sternin IUI, et al. Modern approach to the rehabilitation of children with fractured long tubular bones. *Vopr Kurortol Fizioter Lech Fiz Kult*. 2009;3:29–31. [Russian].
50. Miller PC, Bailey SP, Barnes ME, et al. The effects of protease supplementation on skeletal muscle function and DOMS following downhill running. *J Sports Sci*. 2004;22:365–372.
51. Marzin T, Lorkowski G, Reule C. Effects of a systemic enzyme therapy in healthy active adults after exhaustive eccentric exercise: a randomized, two-stage, double-blinded, placebo-controlled trial. *BMJ Open Sport Exerc Med*. 2017. https://doi.org/10.1136/bmjsem-2016-000191.
52. Miehlke K. Enzymtherapie bei rheumatoider arthritis. *Natur-und Ganzheits-medizin*. 1988:108–111.

53. Kleine MW. Therapie des herpes zoster mit proteolytischen. *enzymen. Therapiewoche.* 1987;37:1108–1112.
54. Klein G, Kullich W, Schnitker J, et al. Efficacy and tolerance of an oral enzyme combination in painful osteoarthritis of the hip. A double-blind, randomized study comparing oral enzymes with non-steroidal anti-inflammatory drugs. *Clin Exp Rheumatol.* 2006;24:25–30.
55. Bolten WW, Glade MJ, Raum S, et al. The safety and efficacy of an enzyme combination in managing knee osteoarthritis pain in adults: a randomized, double-blind, placebo-controlled trial. *Arthritis.* 2015. https://doi.org/10.1155/2015/251521.
56. Kelley WD. *One Answer to Cancer*. Ontario, OR: The Kelley Foundation; 1974.
57. Gujral MS, Patnaik PM, Kaul R, et al. Efficacy of hydrolytic enzymes in preventing radiation therapy-induced side effects in patients with head and neck cancers. *Cancer Chemoth Pharm*. 2001;47(suppl):S23–S28.
58. Pluzhnikov MS, Ryabova MA, Karpiscenko SA. The effect of systemic enzyme therapy (Phlogenzym) in the treatment of radiomucositis in patients with laryngeal cancer. *Folia Otorhinolaryngologiae et Pathologiae Respiratoriae.* 1999;5:73–75.
59. Dale PS, Tamhankar CP, George D, et al. Co-medication with hydrolytic enzymes in radiation therapy of uterine cervix: evidence of the reduction of acute side effects. *Cancer Chemoth Pharm*. 2001;4(suppl). S29–S34.
60. Bahl A, Chander S, Julka PK, et al. Micronuclei evaluation of reduction in neoadjuvant chemotherapy related acute toxicity in locally advanced lung cancer: an Indian experience. *J Assoc Physician I.* 2006;54:191–195.
61. American Cancer Society. How common is breast cancer? 2017. https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html. Accessed December 6, 2017.
62. Beuth J, Ost B, Pakdaman A, et al. Impact of complementary oral enzyme application on the postoperative treatment results of breast cancer patients-results of an epidemiological multicentre retrolective cohort study. *Cancer Chemoth Pharm*. 2001;47(suppl):S45–S54.
63. Korpan MI, Fialka V. Wobenzyme and diuretic therapy in lymphedema after breast operation. *Wien Med Wochenschr*. 1996;146:67–72. [German].
64. American Cancer Society. *Estimates*. 2017. https://cancerstatisticscenter.cancer.org/?&_ga=2.198327919.1711288936.1512591049-1982717732.1505488095#!/. Accessed December 6, 2017.
65. American Cancer Society. *Cancer Facts & Figures 2009*. Atlanta: American Cancer Society; 2009.
66. Ihse I, Arnesjö B, Kugelberg C, et al. Intestinal activities of trypsin, lipase, and phospholipase after a test meal. An evaluation of 474 examinations. *Scand J Gastroentero*. 1977;12:663–668.
67. DiMagno EP, Malagelada JR, Go VLW, et al. Fate of orally ingested enzymes in pancreatic insufficiency; comparison of two dosage schedules. *N Engl J Med*. 1977;296:1318–1322.
68. Bruno MJ, Haverkort EB, Tijssen GP, et al. Placebo controlled trial of enteric coated pancreatin microsphere treatment in patients with unresectable cancer of the pancreatic head region. *Gut*. 1998;42:92–96.
69. Inderst R. *Enzymtherapie. Erfahrungsheilkunde.* 1989;38:305.
70. Zech R, Domagk G. *Enzyme—Biochemie, Pathobiochemie, Klinik, Therapie*. Weinheim: VCH Verlagsgesellschaft mbH; 1986.
71. Uffelmann K, Vogler W, Fruth C. Der eisatz hydrolytischer enzyme beim extraartikularen rheumatismus. *Allgemeinmedizin*. 1990;19:151–153.
72. Vogler W. Der stellenwert der enzymtherapie bei entzundlich-rheumatischen erkrankungen. Systemische Enzymtherapie, Medizinische Woche, Baden-Baden, 1990. *Natur-und Ganzheitsmedizin*. 1991;2:23.
73. Baumüller M. Der einsatz von hydrolytischen enzymen bei steumpfen weichteilverletzungen und sprunggelenksdistorsionen. *Allgemeinmedizin*. 1990;19:178–182.
74. Reinbold H. Die biologische alternative in der therapie entzundlicher rheumatischer erkrankungen. *Z Allgemeinmed.* 1981;34.
75. Reinbold H. Die therapie des morbus bechterew mit enzymen. *Erfahrungsheilkunde*. 1980;10.
76. Goebel KM. *Enzymtherapie Bei Spondylitis Ankylosans*. Vienna: Lecture given at 17th Systemische Enzymtherapie Symposium; 1991.
77. Multiple Sclerosis Foundation. Common Questions; 2016. https://msfocus.org/Get-Educated/Common-Questions.aspx. Accessed December 29, 2017.
78. Neuhofer CH. Autoimmunerkrankungen: Multiple sklerose, amyotrophe lateralsklerose, colitis ulcerosa. *Erfahrungsheilkunde*. 1988;38:451–454.
79. Mialovyts'ka OA. Effect of phlogenzym in long-term treatment of patients with multiple sclerosis. *Lik Sprava*. 2003;(3–4):109–113. [Ukrainian].
80. Murphy SL, Xu J, Kochanek KD, et al. Deaths: Final data for 2015. *National Vital Statistics Reports*. 2017;66.
81. Haid-Fischer F, Haid H. *Venenerkrankungen*. Stuttgart: Thieme; 1985.
82. Kopadze TSh, Natsvlishvili GA, Tvaladze MG, et al. Application of wobenzym and phlogenzym to the angiology and vascular surgery. *Georg Med News*. 2001;2:27–29.
83. Shahid SK, Turakhia NJ, Kunda M, et al. Efficacy and safety of phlogenzym—a protease formulation, in sepsis in children. *J Assoc Physicians I*. 2002;50:527–531.
84. Sukhikh GT, Loginova NS, Faizullin LZ, et al. The use of Wobenzym to facilitate interferon synthesis in the treatment of chronic urogenital chlamydiosis. *Int J Immunotherapy*. 1997:131–133.
85. Department of Health and Human Services, Centers for Disease Control and Prevention. *Chlamydia—CDC Fact Sheet*. https://www.cdc.gov/std/stats16/chlamydia.htm. Accessed December 29, 2017.
86. Askienazy-Elbhar M, Henry-Suchet J. Persistent "silent" Chlamydia trachomatis female genital tract infections. *Infect Dis Obst Gyn*. 1999;7: 31–34.
87. Wolf M, Ransberger K. *Enzyme Therapy*. Los Angeles: Regent House; 1972.
88. Sears A, Walsh G. *Biotechnology in the Feed Industry: Proceedings of Alltech's Ninth Annual Symposium*. Nicholasville, KY: Alltech Technical Publ; 1993.
89. McEvoy GK, ed. *AHFS '94 Drug Information, American Hospital Formulary Service*. Bethesda, MD: American Society of Hospital Pharmacists; 1994.
90. Park HS, Kim HY, Suh YJ, et al. Alpha amylase is a major allergenic component in occupational asthma patients caused by porcine pancreatic extract. *J Asthma*. 2002;39:511–516.
91. Ramsden WH, Moya EF, Littlewood JM. Colonic wall thickness, pancreatic enzyme dose and type of preparation in cystic fibrosis. *Arch Dis Child*. 1998;79:339–343.
92. Serban DE, Florescu P, Miu N. Fibrosing colonopathy revealing cystic fibrosis in a neonate before any pancreatic enzyme supplementation. *J Pediatr Gastr Nutr*. 2002;35:356–359.
93. FitzSimmons SC, Burkhart GA, Borowitz D. High-dose pancreatic-enzyme supplements and fibrosing colonopathy in children with cystic fibrosis. *N Engl J Med*. 1997;336:1283–1289.
94. Dodge JA. Fibrosing colonopathy: recent advances. *J Roy Soc Med Suppl.* 1996;89(suppl 27):19–23.
95. Reynolds JEP, ed. *Martindale—the Extra Pharmacopoeia*. London: Pharmaceutical Press; 1996.
96. Konotey-Ahulu FID. Enzyme treatment of vitreous haemorrhage. *Lancet*. 1972;2:714–715.
97. Sowray JH. An assessment of the value of lyophilised chymotrypsin in the reduction of post-operative swelling following the removal of impacted wisdom teeth. *Brit Dent J*. 1961;110:30–133.
98. Wrba F, Messer E. Enzyme treatment of traumatic swelling in oral and maxillofacial surgery. *Clin Med*. 1967;74:29–31.
99. Bumgardner HD, Zatuchni GI. Prevention of episiotomy pain with oral chymotrypsin. *Am J Obstet Gynecol.* 1965;92:514–517.
100. Fiel RA. Tratamiento experimental de la trichuriasis masiva infantil con quimotripsina. *Trop Dis Bull Bd*. 1968;65:917.
101. Takahashi M, Hashimoto K, Osada H. Parenteral administration of chymotrypsin for the early detection of cancer cells in sputum. *Acta Cytol Bd*. 1967;11:61–63.
102. Brandborg LL, Tankersley CB, Uyeda F. "Low" versus "high" concentration chymotrypsin in gastric exfoliative cytology. *Gastroenterol.* 1969;57:500–505.

103. Soule SD, Wasserman HC, Burstein R. Oral proteolytic enzyme therapy (Chymoral) in episiotomy patients. *Am J Obstet Gynecol*. 1966;95:820–833.
104. Boyne PS, Medhurst H. Oral anti-inflammatory enzyme therapy in injuries in professional footballers. *Practitioner*. 1967;198:543–546.
105. Blonstein JL. Oral enzyme tablets in the treatment of boxing injuries. *Practitioner*. 1967;198:547–548.
106. Buck JE, Phillips N. Trial of Chymoral in professional footballers. *Brit J Clin Pract*. 1970;24(9):375–377.
107. Rathgeber WF. The use of proteolytic enzymes (Chymoral) in sporting injuries. *S Afr Med J*. 1971;45:181–183.
108. De N'Yeurt A. The use of Chymoral in vasectomy. *J R Coll Gen Pract*. 1972;22:633–637.
109. Winsor T. Inhibition of the response to thermal injury by oral proteolytic enzymes. *J Clin Pharmacol N*. 1972;12:325–330.
110. Rathgeber WF. The use of proteolytic enzymes in tenosynovitis. *Clin Med*. 1973;80:39–41.
111. Gibson T, Dilke TF, Grahame R. Chymoral in the treatment of lumbar disc prolapse. *Rheumatol Rehabil*. 1975;14:186–190.
112. *Transcript from the American Academy Ophthalmology and Otolaryngology*. Chicago; 1959.
113. Wrba H. *Trypsin und chymotrypsin—klinisches sachverstan-digengutachten zur systemischen wirkung. Inst f angewante u exptl Onkologie d Univ Wien*. Working Paper. 1988.
114. Stille G, Tuluwett K. Pharmakologisch-toxikologisches sach-verstandigengutachten zut trypsin/chymotrypsin. *Working Paper*. 1988.
115. Gordon B. The use of topical proteolytic enzymes in the treatment of post-thrombotic leg ulcers. *Brit J Clin Pract*. 1975;29:143–146.
116. Sather MR, Weber Jr CE, George J. Pressure sores and the spinal cord injury patient. *Drug Intel Clin Phar*. 1977;11:154–169.
117. Marquez OHD, Segur FG. The intrathecal use of proteolytic enzymes in tuberculous meningoencephalitis. Preliminary Communication. *Abstr Wld Med*. 1968;42:800–801.
118. Lichtman AL. Traumatic injury in athletes. *Rec Med*. 1957;170:322–325.
119. Dixon M, Webb E. *Enzymes*. New York: Academic Press; 1979:28–29.
120. Windholz M, ed. *The Merck Index—An Encyclopedia of Chemicals, Drugs, and Biologicals*. Rahway, NJ: Merck; 1983.
121. Auterhoff H, Knage J. *Lehrouch der pharmazueutischen chemie*. Stuttgart: Verlagsgesellschaft Wissenschafel; 1983.
122. Graham DY. Enzyme replacement therapy of exocrine pancreatic insufficiency in man. *N Engl J Med*. 1977;296:1314–1317.
123. Meyer JH. The ins and outs of oral pancreatic enzymes. *N Engl J Med*. 1977;296:1347–1348.
124. Yeh TL, Rubin ML. Potency of pancreatic enzyme preparations. *N Engl J Med*. 1977;297:615–616.
125. Kirshen R. Letter to the editor. *N Engl J Med*. 1977;297:616.
126. Mackie RD, Levine AS, Levitt MD. Malabsorption of starch in pancreatic insufficiency. *Gastroenterol*. 1981;80:1220.
127. Lankisch PG, Creutzfeldt W. Therapy of exocrine and endocrine pancreatic insufficiency. *Clin Gastroenterol*. 1984;13:985–999.
128. Schneider MU, Knoll-Ruzicka ML, Domschke S, et al. Pancreatic enzyme replacement therapy. Comparative effects of conventional and enteric-coated microspheric pancreatin and acid-stable fungal enzyme preparations on steatorrhoea in chronic pancreatitis. *Hepato-gastroenterol*. 1985;32:97–102.
129. Hall DA, Zajac AR, Cox R, et al. The effect of enzyme therapy on plasma lipid levels in the elderly. *Artherosclerosis*. 1982;43:209–215.
130. Tylewska S, Tyski S, Hrynie-Wicz W. Effect of Staphylococcus aureus lipase on granulocyte chemotaxis. *Med Dosw Mikrobiol*. 1983;35:171–174. [Polish].
131. LeBauer E, Smith K, Greenberger NJ. Pancreatic influence and vitamin B_{12} malabsorption. *Arch Intern Med*. 1968;122:423–425.
132. Kataria MS, Bhaskarrao D. A clinical double-blind trial with a broad spectrum digestive enzyme product (Combinzym) in geriatric practice. *Brit J Clin Pract*. 1969;23:15–17.
133. Karani S, Kataria MS, Barber AE. A double-blind clinical trial with a digestive enzyme product. *Brit J Pract*. 1971;25:75–377.
134. Knill-Jones RP, Pearce H, Batten H, et al. Comparative trial of Nutrizym in chronic pancreatic insufficiency. *BMJ*. 1970;4:21–24.
135. DiMagno EP, Go VLW, Summerskill WHJ. Relations between pancreatic enzyme outputs and malabsorption in severe pancreatic insufficiency. *N Engl J Med*. 1973;288:813–815.
136. Saunders JHB, Wormsley KG. Progress report. Pancreatic extracts in the treatment of pancreatic exocrine insufficiency. *Gut*. 1975;16:157–162.
137. Bank S, Marks IN, Barbezat GO. Treatment of acute and chronic pancreatitis. *Drugs*. 1977;13:373–381.
138. Saunders JHB, Drummond S, Wormsley KG. Inhibition of gastric secretion in treatment of pancreatic insufficiency. *BMJ*. 1977;1:418–419.
139. Anonymous Pancreatic extracts. *Lancet*. 1977;2:73–74.
140. Regan PT, Malagelada JR, DiMagno EP, et al. Comparative effects of antacids, cimetidine and enteric coating on the therapeutic response to oral enzymes in severe pancreatic insufficiency. *N Engl J Med*. 1977;297:854–858.
141. Regan PT, Malagelada JR, Dimagno EP, et al. Rationale for the use of cimetidine in pancreatic insufficiency. *Mayo Clin Proc*. 1978;53:79–88.
142. Austad WJ. Pancreatitis; the use of pancreatic supplements. *Drugs*. 1979;17:480–487.
143. *Physicians' Desk Reference*. Vol. 47. Medical Economics; 1993.
144. Van Hoozen CM, Peeke PG, Taubeneck M, et al. Efficacy of enzyme supplementation after surgery for chronic pancreatitis. *Pancreas*. 1997;14:174–180.
145. Anonymous Pancreatic extracts. *BMJ*. 1970;2:161–163.
146. Anderson CM. Pancreatic enzyme replacement in the treatment of cystic fibrosis. *Prescribers J*. 1972;12:45–49.
147. Roy CC, Weber AM, Morin CL, et al. Abnormal biliary lipid composition in cystic fibrosis. *N Engl J Med*. 1977;197:1301–1305.
148. Smalley AC, Brown GA, Parkes ME, et al. Reduction of bile acid loss in cystic fibrosis by dietary means. *Arch Dis Child*. 1978;53:477–482.
149. Goodschild MC, Sagaro E, Brown GA, et al. Comparative trial of Pancrex V. forte and Nutrizym in treatment of malabsorption in cystic fibrosis. *BMJ*. 1974;3:712–714.
150. Nakamura T, Takebe K, Kuoh K, et al. Effects of pancreatic digestive enzymes, sodium bicarbonate, and a proton pump inhibitor on steatorrhoea caused by pancreatic disease. *J Int Med Res*. 1995;23:37–47.
151. Kleine M-W, Pabst H. Die wirkung einer oralen enzymtherapie auf experimentell erzeugte hamatoma. *Forum des Prakt und Allgemeinarztes*. 1988;27:42–48.
152. Baumüller M. Enzyme zur wiederherstellung nach sprunggelenkdistorsionen. *Z Allg Med*. 1992;68:61–65.
153. Baumüller M. Therapy of ankle joint distortions with hydrolytic enzymes—Results from a double blind clinical trial. In: Hermans GPH, Mosterd WL, eds. *Sports, Medicine and Health*. Amsterdam: Excerpta Medica; 1990:1137.
154. Cichoke AJ, Marty L. The use of proteolytic enzymes with soft tissue athletic injuries. *Am Chiro*. 1981:32–33.
155. Helpap B. *Leitfaden der Allgemeinen Entzundungslehre*. Berlin: Springer; 1987.
156. Kleine M-W. Systemische enzymtherapie in der sportmedizin. *Deut Z Sportmed*. 1990;41:126–134.
157. Müller-Hepburn W. Anwendung von enzymen in der sportmedizin. *Forum d Prakt Arztes*. 1970;18:7–10.
158. Niethard FU, Pfeil J. *Orthopadie*. Stuttgart: Hippokrates Verlag; 1989.
159. Hiss WF. Enzyme in der sport- und unfallmedizin. *Z Naturheilmethoden: Contincationars*. Müller-Hepburn; 1979:12.
160. Doenicke A, Hoernecke R. Wirksame behandlung von traumen mit schwellung und/oder hamatom im eishockeysport durch enzymtherapie. *Deut Z Sportmed*. 1993;5:214–219.
161. Kleine M-W. Introduction to systemic enzyme therapy and results of experimental trials. In: Hermans GPH, Mostered WL, eds. *Sports, Medicine and Health*. Amsterdam: Excerpta Medica; 1990:1131.
162. Worschhauser S. Konservative Therapie der Sportverletzungen. Enzympraparate fur Therapie und Prophylaxe [Conservative therapy for sports injuries. Enzyme preparations for therapy and prophylaxis]. *Allgemein Medizin*. 1990;19:173–177.
163. Rahn H-D, Kilic M. Die wirksamkeit hydrolytischer enzyme in der traumatologie. Ergebnise nach 2 prospektiven randomisierten doppelblindstudien. *Allgemeinarzt*. 1990;19:183–187.

164. Rahn H-D. *Enzyme verkurzen rekonvaleszenz. Lecture given at 13th Systemische Enzymtherapie Symposium*. Lindau; 1990.
165. Carillo AR. Klinische untersuchung eines enzymatischen entzundungshemmers in der unfallchirurgie. *Arztl Praxis*. 1972;24:2307–2308.
166. Chappa-Alvarez R. *Pankreatitisbehandlung mit Wobenzym*. Working paper. 1992.
167. Guggenbichler JP. Einfluss hydrolytischer enzyme auf thrombusbildung und thrombolyse. *Med Welt*. 1988;39:277–280.
168. Rahn H-D. *Wobenzymnach Gefassbypassoperationen am bein*. Lecture given at 17th Systemische Enzymtherapie Symposium, Vienna, 1991.
169. Vinzenz K. Ödembehandlung bei zahnchirurgischen eingriffen mit hydrolytischen enzymen. *Die Quintessenz*. 1991;42(7):1053–1064.
170. Werk W. Ein Polyenzympraparat zur Beschleunigung der Narbenbildung. *Proktologie*. 1979;3:28–29.
171. Ryan RE. A double-blind clinical evaluation of bromelain in the treatment of acute sinusitis. *Headache*. 1967;7:13–17.
172. Zollner N, ed. *Innere Medizin*. Heidelberg: Springer; 1991.
173. Wohlrab R. Enzymkombinationspraparat zur therapie der sinusitis acuta. *Der Allgemeinarzt*. 1993;15:104–114.
174. Grimminger A. Enzymtherapie bei thoraxerkrankungen. *Erfahrungsheilkunde*. 1971;1:18.
175. Barsom S, Sasse-Rollenhagen K, Bettermann A. Zur behandlung von zystitden und zystopyelitiden mit hydrolytischen enzymen. *Acta Medica Empirica*. 1983;32:25–129.
176. Barsom S, Sasse-Rollenhagen K, Bettermann A. Erfolgreiche prostatitisbehandlung mit hundrolytischen enzymen. *Erfarungsheilkunde*. 1982;31:2.
177. Rugendorff EW, Burghele A, Schneider H-J. Behandlung der chronischen abakteriellen prostatitis mit hydrolytischen enzymen. *Der Kassenarzt*. 1986;14:43–51.
178. Dittmar F-W, Weissenbacher ER. Therapie der adnexitis—unterstutzung der antibiotischen basisbehandlung durch hydrolytische enzyme. *Int J Exper Clin Chemother*. 1992;5:73–82.
179. Dittmar F-W. Enzymtherapie in der gynakologie. *Allgemeinmedizin*. 1990;19:158–159.
180. Rahn H-D. *Wirksamkeit von enzymen bei gefasserkrankungen*. Lecture given at 2nd Systemische Enzymtherapie Symposium, Germany: Dusseldorf; 1987.
181. Ernst E, Matrei A. Orale therapie mit proteolytischen enzymen modifiziert die blutrheologie. *Klin Wsch*. 1987;65:994.
182. Mörl H. Behandlung des postthrombotischen syndroms mit einem enzymgemisch. *Therapiewoche*. 1986;36:2443.
183. Mörl H. *Therapie und prophylze des postthrombotischen syndroms mit Wobenzym*. Lecture given at 17th Systemische Enzymtherapie Symposium, Vienna, 1991.
184. Valls-Serra J. Proteolytische enzyme in der behandlung von thrombophlebitis. *Med Clin*. 1967.
185. Mahr H. Zur enzymtherapie entzundlicher venenerkrankungen der tiefen beinvenenthrombose und des postthrombotischen syndromes. *Erfahrungsheilkunde*. 1983;117.
186. Maehder K. Enzymtherapie venoser gefasserkrankungen. *Die Arztpraxis*. 1972;2.
187. Kluken N. Venose krankheiten in klinik und praxis, systemische enzymtherapie, medizinische woche Baden-Baden, 1990. *Natur-und Ganzheitsmedizin*. 1991;2:8–13.
188. Vogler W. *Enzymtherapie. Hessen: Hausarzt.* 1989;4:116–119.
189. Rokitansky O. *Ozontherapie und enzyme bei der chronisch-arteriellen verschlusskrankeit. Systemische Enzymtherapie am 31.10.90. Medizinische Woche, Baden-Baden, 1990. Natur-und Ganzheitsmedizin*. 1991;(suppl 1):14–16.
190. Keim H, Al-Yousef S, Wachter K. Methode zur linderung der lymphstauung am arm nach behandlung des mammakarzinoms. *Rontgenberichte*. 1972;1:45–41.
191. Scheef W, Pischnamazadeh M. Proteolytische enzyme als einfache und sichere methode zur verhutung des lymphodems nach ablatio mammae. *Med Welt*. 1984;35:1032–1033.
192. Ransberger K, Stauder G, Streichhan P. *Wissenschaftliche monographie zur praklinik Wobenzym N*, Mulsal N, Phlogenzym. *Forum Medizin*. 1991.
193. König W. Erfahrungen der Robert-Janker-Klinik, Bonn, mit systemischer enzymtherapie und emulgierten vitaminen. *Acta Medica Empirica*. 1988;37:11–17.
194. König W. Proteolytische enzyme verhindern lymphodem. In: Medizinische Enzym-Forschungesgesellschaft e.V., ed. Systemische Enzymtherapie, Symposium Munich, Medizin Aktuell (Enzyme Series): Informed International Congress Report 53; 1986.
195. Hörger I, Moro V, van Schaik W. Zirkulierende Immunkomplexe bei polyarthritis-patienten. *Natur-und Ganzheitsmedizin.* 1988;1:117–122.
196. Steffen C, Smolen J, Miehlke K, et al. Enzymtherapie im vergleich mit immunkiomplexbestimmungen bei chonischler polyarthritis. *Z Rheumatol*. 1985;44:51–56.
197. Ekerot L, Ohlsson K, Necking L. Elimination of protease-inhibitor complexes from the arthritic joint. *Int J Tissue React*. 1985:391–395.
198. Ballachi G. Hydrolytische enzyme bei aktivierten polyarthrosen. *Rheuma*. 1988;8.
199. Hörger I. Enzymtherapie bei einem rheumakollektiv. *Therapiewoche*. 1983;33:3948–3957.
200. Panijel M. Entzundlich-rheumatische erkrankungen. *Z Allgemeinmedizin*. 1985;61:1305–1307.
201. Steffen C, Zeitlhofer J, Menzel J, et al. Die antigen-induzierte experimentelle arthritis als prufverfahren fur entzundungshemmung durch oral appliqierte substanzen. *Z Rheumatol*. 1979;38:264–278.
202. Miehlke K. Enzymtherapie bei chronischer polyarthritis. 46. Der Kassenarzt; 1989.
203. Miehlke K. *Rheumabahandlung Mit Enzymen Ist Mehr Als Nur Antiphlogistische Therapie*. Munich: Lecture given at 17th Systemische Enzymtherapie Symposium; 1991.
204. Klein G, Schwann H, Kullich W. Enzymtherapie bei chronischer polyarthritis. *Natur-und Ganzheitsmedizin.* 1988;1:112–116.
205. Klein G, Pollmann G, Kullich W. Klinische Erfahrungen mit der Enzymtherapie bei Patienten mit Chronischer Polyarthritis im Vergleich Zur Oralen Goldtherapie [Clinical experience with enzyme therapy in patients with rheumatoid arthritis in comparison with oral gold]. *Allgemein Medizin*. 1990;19:144–147.
206. Klein K. *Behandlung der rheumatoiden arthritis mit Wobenzym im vergleich zur basistherapie mit gold*. Lecture given at 17th Systemische Enzymtherapie u Vienna, 1991.
207. Singer F. *Aktivierte arthrosen knorpelschonend behandeln*. Lecture given at 10th Systemische Enzymtherapie Symposium, Frankfurt, 1990.
208. Neuhofer CH. Enzymtherapie bei multipler sklerose. *Hufeland J*. 1986:47–50.
209. Neuhofer CH. *Systemische enzymtherapie bei encephalomyelitis disseminata*. Der Prakt Arzt. 1991;702.
210. Jäger H, Popescu M, Samtleben W, et al. Hydrolytic enzymes as biological response modifiers (BRM) in HIV-infection. In: *San Marino Conferences—Highlights in Medical Virology, Immunology and Oncology*. Vol. 1. Oxford: Pergamon Press; 1988.
211. Jäger H. Hydrolytische enzyme in der therapie der HIV-erkrankung. *Z Allgemeinmedizin*. 1990;19:160–164.
212. Scheef W. Gutartige veranderungen der weiblichen brust. *Therapiewoche*. 1985;35:5909–5912.
213. The author wishes to express his deep appreciation in writing these two enzyme chapters for the cooperation of the following researchers from the United States, Germany, the Czech Republic, and other Eastern European countries: Dr. Barry Ritz, Dr. Winfried Miller, Dr. Claudia Loyall, Dr. Zinovij Masinovsky, Dr. Michaela Lysikova, Dr. Joseph Pizzorno, Dr. Michael Murray, and Ms. Karen Hood..

FURTHER READING

Cichoke AJ. *A New Look at Chronic Disorders and Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *A New Look at Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *Acute Trauma and Systemic Enzyme Therapy*. Portland, OR: Seven C's Publishing; 1993.
Cichoke AJ. *Bromelain*. New Canaan, CT: Keats; 1998.
Cichoke AJ. *Enzymes and Enzyme Therapy: How to Jump Start Your Way to Lifelong Good Health*. New Canaan, CT: Keats; 2000.
Cichoke AJ. *The Complete Book of Enzyme Therapy*. New York: Avery Publishing; 1999.

Phage Therapy: Bacteriophages as Natural, Self-Limiting Antibiotics

Elizabeth Kutter, BS, PhD, Naomi Hoyle, MD, William Eisner, BSc, Sarah Kuhl, MD, PhD, Zemphira Alavidze, PhD, and *Bob G. Blasdel, PhD*

OUTLINE

INTRODUCTION

Phage therapy involves the use of bacteriophages—viruses that only attack bacteria and are very host specific—to kill pathogenic microorganisms. The art was first developed at the Pasteur Institute in Paris in 1917, practiced to some extent in France until the 1980s, and is still fairly extensively used and further developed in such places as Russia and the Republic of Georgia.[1] After the advent of chemical antibiotics in the 1940s, it was largely forgotten in the Western world. Today, however, the increased prevalence of bacteria that are resistant to most or all available antibiotics is precipitating a major health crisis, as is passionately stressed by the World Health Organization and by governments, scientists, and health practitioners worldwide.[2] In particular, extensive research work has been published by the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland's center of phage therapy, complementing the ongoing routine use of phage in the Republic of Georgia.[3] Numerous patients from around the world have been treated in recent years in Phage International's Phage Therapy Center and in the Eliava Phage Therapy Center in Tbilisi, Georgia, long the epicenter of therapeutic phage production and application, as further discussed later in the following section.

Today, the one major western European center carrying out phage therapy is at the LabMCT, which is part of the burn wound center of the Queen Astrid Military Hospital in Brussels, Belgium. The LabMCT provides phage active ingredients to pharmacists who produce preparations for patients in Belgium under the magistral framework (Pirnay et al., 2018).[4] This framework, roughly analogous to the compounding framework in the United States or the Specials framework in the UK, allows the Belgian phage ecosystem to fill prescriptions with phage preparations that are tailor-made to each patient. In Belgium, as at the Hirszfeld Institute, phage cocktails are prepared from phages that have been determined to be active against a patient's clinical isolate through the use of a companion diagnostic method.

In the United States, the Food and Drug Administration (FDA) now also quite routinely approves compassionate use of phage therapy in individual very serious cases where there are no other options. One such case, involving a near-fatal *Acinetobacter* infection of a UC San Diego faculty member and a massive worldwide effort that found the phages that rescued him after 4 months in a coma, is described in some detail later in this chapter. The spectacular success there led the UC San Diego clinic to apply phage therapy in half a dozen cases where organ transplants were being blocked by intransigent infections. The UC San Diego has just made a million-dollar investment in developing a phage therapy center and relevant sources of potential therapeutic phages are springing up.

This chapter was written to put phage therapy into historical and ecological perspective for health practitioners and to explore very interesting early research in France, the United States, and Eastern Europe, as well as growing recent studies worldwide, that provide the

foundation for growing interest today in its possibilities. The hope is that this modality will soon be more readily available for external applications, while physicians and researchers occasionally take advantage of "Compassionate Use" guidelines and deal with the challenges of getting funding for full-scale clinical trials of more invasive approaches. Here, we explore available data that strongly support expedited evaluation of phage therapy to help address the serious menace of antibiotic resistant bacteria.[6-18]

Extensively documented results of early French and Eastern European therapeutic phage applications are very encouraging, but most involved individualized applications to infections recalcitrant to all other available treatments, not double-blind clinical trials. There have been encouraging developments. In 2006 the US FDA and the European Medicines Agency (EMA) both approved phage preparations targeting *Listeria monocytogenes* on ready-to-eat foods. Intralytix (Baltimore, Maryland) in the United States now markets similar products targeting *E. coli* O157, other pathogenic *E. coli*, *Salmonella* (PLSV-1) and *Clostridium perfringens* (INT-401) in poultry, or a wide range of pathogenic *Salmonella* serotypes on food: Typhimurium, Enteritidis, Heidelberg, Newport, Hadar, Kentucky, Thompson, Georgia, Agona, Grampian, Senftenberg, Alachua, Infantis, Reading, and Schwarzengrund. SalmoFresh is specifically designed for treating foods that are at high risk for *Salmonella* contamination. Red meat and poultry can be treated before grinding for significant reductions in *Salmonella* contamination. SalmoFresh received Generally Recognized As Safe (GRAS) recognition from the FDA in 2013 (GRAS Notice No. GRN 000435), for direct applications onto poultry, fish and shellfish, and fresh and processed fruits and vegetables.

Designing and successfully carrying out successful double-blind trials has been very challenging; safety has been established, but difficulties involving design have made proof of efficacy unavailable. For example, the Nestlé corporation carried out an extensive project in Dhaka, Bangladesh, to study the safety and efficacy of phage therapy in treating infant diarrhea believed to have been caused by *E. coli*.[19] Either a novel cocktail of T4-like phages used in earlier safety trials, a commercially available Russian anti-*E. coli* phage cocktail (Microgen), or a placebo were added in blinded fashion to standard oral rehydration solution. The phages being used for the main experimental arm of the trial had been isolated from the stools of pediatric diarrhea patients in Bangladesh, with the broad-spectrum ones applicable for phage therapy all turning out to be members of the highly studied T4 family of phages, described in the following section.[20] They also reported the details of their very extensive genomic, mouse, and human safety studies of representative phages from their set.[21] The trial very effectively established safety. However, for several technical reasons that are now much clearer, despite the enormous expense, it was unable to establish efficacy. Apparently, the true culprits were, at least partly, a pair of previously unknown streptococci. Also, as our laboratory established at about that time, T4-infected stationary-phase *E. coli* goes into a sort of hibernation-mode state, killing the bacteria but not completing the cycle, and releasing a large burst of phages until glucose and amino acids are added, as much as 48 hours later (Bryan et al., 2016).[5]

PROPERTIES OF BACTERIOPHAGES

Bacteriophages, like other viral particles, are like spaceships that can transfer their genomes into susceptible cells where they can reproduce. In the case of bacteriophages, the targets are bacterial cells, specific to varying degrees for each phage; they cannot infect the cells of eukaryotic organisms. Each virion consists of DNA or RNA, containing the information that determines the properties of the virus, which is encased in a protein coat (Fig. 101.1). Unlike eukaryotic viruses, most

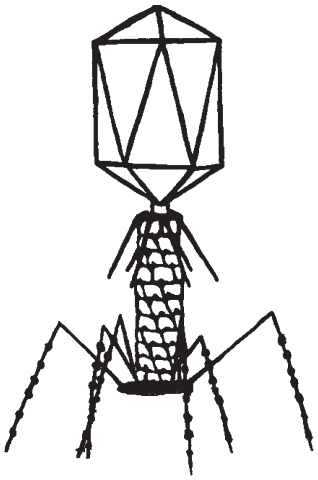


Fig. 101.1 Phage diagram (bacteriophage T4).

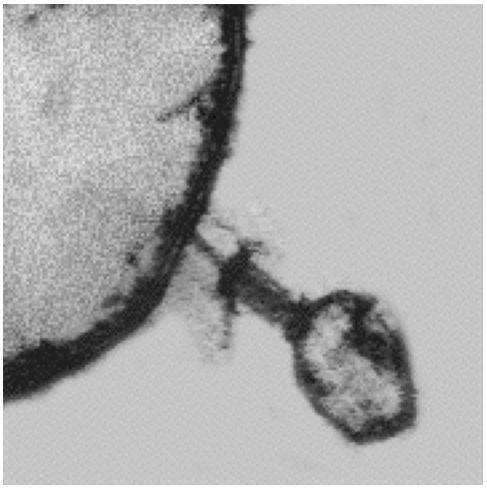


Fig. 101.2 Electron micrograph of phage infecting a bacterium.

DNA phages have tails, the tips of which can bind to specific molecules on the surfaces of their target bacteria (Fig. 101.2). Many have 4 to 6 tail fibers that initiate the bacterial recognition process and help the virion position itself in a manner facilitating the injection of its DNA into the cell. Once in the cell, the DNA is replicated, creating numerous copies of the entire virion. At human body temperature, many phages produce more than 100 virions per cell in about half an hour while for phages targeting oceanic bacteria, for example, that process may take 24 hours.

Each strain of bacteria has characteristic protein, carbohydrate, and lipopolysaccharide molecules present in very large quantities on its surface. These molecules are involved in forming pores, conferring motility, and binding of the bacteria to particular surfaces. Each such molecule can act as a receptor for particular phages. Development of resistance to a particular phage generally reflects mutational alteration or loss of its specific receptor; this loss frequently has negative effects on the bacterium, often making such mutants less virulent, and does not confer protection against the many other phages that use different receptors. Each kind of bacterium has its own phages, which can generally be isolated wherever that bacterium grows: from sewage, feces, soil, and even ocean depths or hot springs. However, finding phages suitable for therapeutic applications is much easier for some species than for others; this relates significantly to the ability to grow the host bacterium in the laboratory. The process of isolation is straightforward for

phages targeting many of the best-studied pathogens. An environmental sample is combined with an appropriate nutrient-fortified solution and one or more kinds of targeted bacteria, incubated overnight, and lysed with chloroform. A series of tenfold dilutions is made and 10 μL of each dilution is spotted on a nutrient-agar plate prepared with a single strain of target bacteria. The next day, a dense bacterial lawn is seen, hopefully with cleared spots at each dilution, until small, clear individual plaques are seen. Each plaque contains over a million phages, all of them progeny of a single initial phage. An individual plaque is plucked from that furthest dilution and put through a redilution to a concentration giving about 10 to 30 plaques. Finally, one plaque is picked and grown up in an appropriate host to give a stock of a phage whose host range, purity, and other properties can then be studied.

One major source of confusion in the early phage work was that each laboratory isolated its own new phages, often infecting different bacteria, so there was little continuity or basis for comparison. Key technical developments in the 1930s to 1940s that helped clarify the general nature and properties of bacteriophages were (1) the concentration and purification of a few large phages using the newly available high-speed centrifugation and the demonstration that they contained roughly equal amounts of DNA and protein; and (2) visualization of phages by the newly developed electron microscopes (EM).[22,23] Phages specific for virtually every well-studied bacterial species have now been isolated and studied in substantial detail in laboratories all over the world. Many are becoming well classified.

The early EM work helped resolve the major disputed question as to whether the lytic principle termed "bacteriophage" simply reflected an inherent property of the specific bacteria, as strongly proposed by eminent immunologist Jules Bordet, 1919 winner of a Nobel Prize, or required regular reinfection by an external agent, as indicated by d'Hérelle's research (see "Historical Context of Phage Therapy"). During the 1930s and 1940s, it gradually became clear that in some senses both were true—that there were two quite fundamentally different groups of bacteriophages. Obligatorily lytic phages always have to infect from outside, reprogram the host cell, and release a burst of phage through breaking open or lysing the cell after a relatively short fixed interval. Temperate phages, in contrast, have another option; they can actually integrate their DNA into the host DNA, much as HIV can integrate the DNA copy of its RNA. For several reasons, such temperate phages are not appropriate for therapeutic applications. They generally make the host resistant to related phages, blocking treatment efficacy, and may carry genes that actually increase the pathogenicity of the host; very specific prophages are a key factor in such diseases as cholera and diphtheria.

Using the EM, each phage family was found to have its own specific shape and size, from "lunar lander"–style complexity with a contractile tail and long tail fibers attached to a baseplate, to globular heads with long or short tails, to the small filamentous phages that looked much like bacterial pili (Fig. 101.3). With recent advances in DNA sequencing techniques, our understanding of various phages is exploding in powerful and important ways; the genomes of several hundred phages, infecting a variety of organisms, have now been sequenced, revealing a remarkable variety of types and properties that can be very important when considering potential therapeutic applications. In general appearance, 95% of the studied phages belong to one of the three-tailed morphotypes: the short-tailed podoviridae, the siphoviridae with long, often flexible tails, and the myoviridae, with tails composed of an inner tube and an outer contractile sheath attached to a complex baseplate. There are both obligatorily lytic and temperate phage families with each of these three general morphotypes, so other kinds of data are also needed to determine whether a newly isolated phage is temperate or lytic.

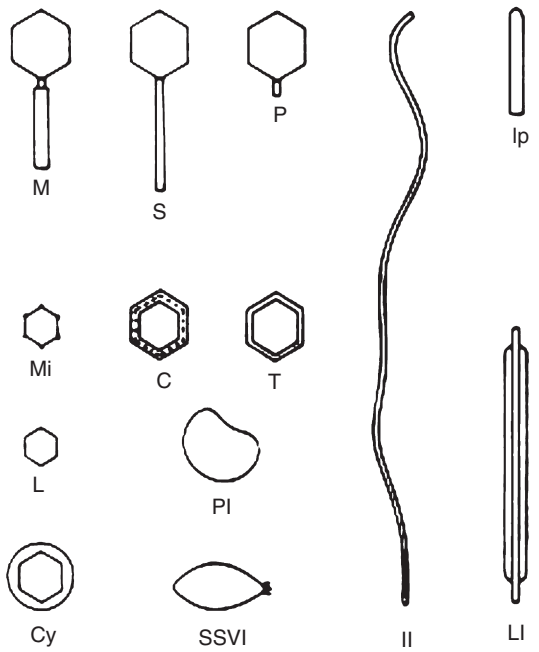


Fig. 101.3 Various phages.

Obligatorily Lytic Phages and the Development of Molecular Biology

A much better understanding of the interactions between the lytic life cycle of phages and bacteria began with one-step growth curve experiments. These demonstrated an eclipse period, during which the DNA began replicating and there were no free phages in the cell; a latent period of accumulation of intracellular phages; and a precisely timed lysis process that released the phage to go in search of new hosts. This phage infection cycle is illustrated in Fig. 101.4 for coliphage T4, which does a particularly effective job of shutting off all host functions and whose family is very widespread in nature and in therapeutic phage preparations for enteric bacteria.

In 1943 an event occurred that had a major effect on the orientation of phage research in the United States and much of western Europe, strongly shifting the emphasis from practical applications to basic science. Physicist-turned–phage biologist Max Delbruck met with Alfred Hershey and Salvador Luria to form a "Phage Group" and establish a long-lasting annual phage course and meeting at Cold Spring Harbor, Long Island. A major element of the success of phages as model systems for working out fundamental biological principles at the molecular level was that Delbruck persuaded most US phage biologists to focus on one bacterial host (*E. coli* B) and seven of its highly lytic phages, arbitrarily named types T1 through T7. Fortuitously, T2, T4, and T6 are quite similar to one another, defining a family of myoviridae now called the T-even phages, which were key in demonstrating that DNA is the genetic material, that viruses can encode enzymes, that gene expression is mediated through special "messenger RNA," that the genetic code is triplet in nature, and other fundamental concepts. The negative side of this focus on a few phages growing on one host under rich laboratory conditions was that there was very little study or awareness of the ranges, roles, and properties of bacteriophages in the natural environment or of potential applications. On the positive side, most of the strongly lytic phages selected for therapeutic applications targeting enteric phages have turned out to belong to one or another of these three very well-studied families of phages (as confirmed by sequencing data as well as morphological details), which is very useful as we work to ensure their safety and to understand the physiological properties involved.

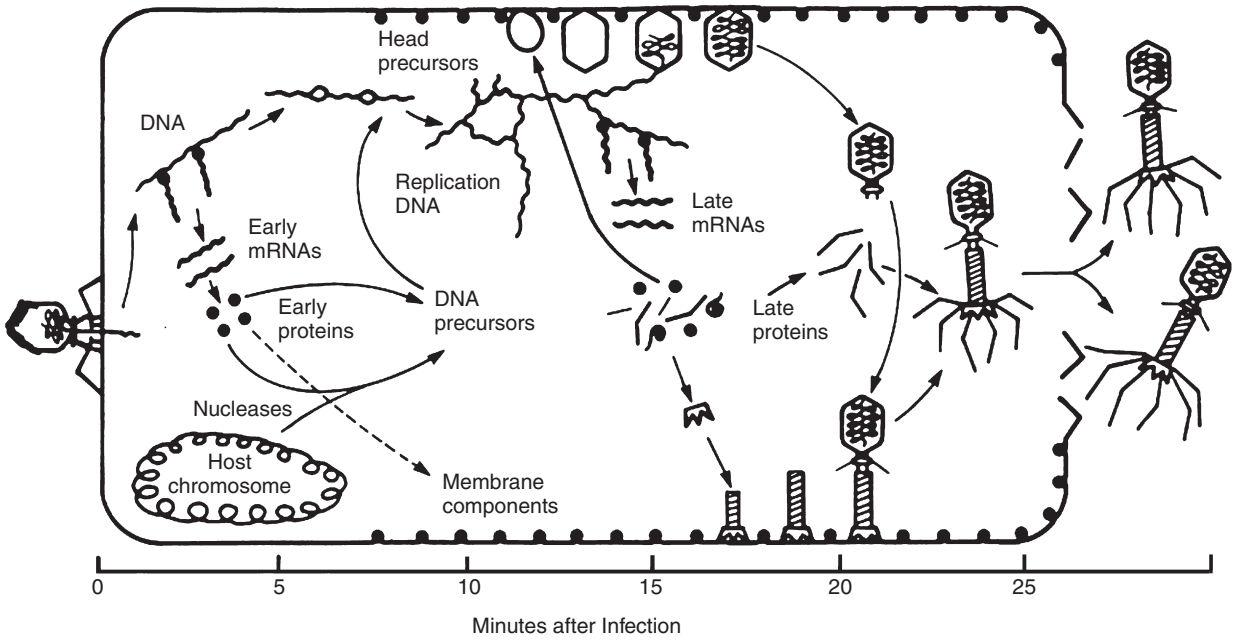


Fig. 101.4 Bacteriophage intracellular growth cycle. Noteworthy features: both nucleolytic action on host chromosome and new phage enzymes furnish DNA precursors; replicating DNA is much longer than virion DNA; a number of phage-coded proteins become associated with the host membrane to alter host functions and to facilitate phage assembly and maturation.

The temperate phages generally encode repressors to turn off most of their own genes when they are in the temperate prophage state, integrases to let them insert themselves into the DNA of their hosts, and excision enzymes to cut their DNA back out to enter a lytic cycle. Obligately lytic phages generally have much more extensive mechanisms for shutting off the host, often involving nucleases and transcription factors and a variety of small host-lethal "monkey-wrench" proteins made under the control of strong promoters very early in infection to redirect cellular metabolism; T4, for example, encodes at least 50 such proteins (Miller et al., 2003).[105]

HISTORICAL CONTEXT OF PHAGE THERAPY

Edward Twort and Félix d'Hérelle independently reported the isolation of filterable entities capable of lysing bacterial cultures. When diluted sufficiently, they made small round cleared areas d'Herelle called "plaques" on plates of bacterial cultures, implying that discrete particles were involved. It was d'Hérelle, a Canadian working at the Pasteur Institute in Paris, who gave the name *bacteriophage* to these entities, which he discovered in the stools of soldiers recovering from dysentery—using the suffix *phage* "not in its strict sense of *to eat*, but in that of *developing at the expense of*."[24] He soon also isolated phages targeting avian typhosis and tested their application during an outbreak in French chickens, showing that three-quarters of the untreated chickens died, whereas all of the phage-treated infected chickens survived. He then accepted an urgent request to treat several children battling dysentery at the Paris Hôpital des Enfantes Malades; the day before treating the first child, he and several assistants swallowed far more phages than they would be administering, thus carrying out the first known Phase I clinical trial. The treatment of the children was fully successful, but d'Hérelle turned to intense study of the nature of bacterial infection by phages before publishing the results or carrying out further therapeutic applications. In a 300-page 1922 book, *The Bacteriophage: Its Role in Immunity*,[24] d'Hérelle wrote the original, classic descriptions of bacteriophage formation of "plaques" (clear round holes in lawns of susceptible bacteria), isolation of phages from various sources, the lysis process, host specificity of adsorption and multiplication, the dependence of phage production on the precise state of the host, and the factors controlling stability of the free phage. He quickly became fascinated with the apparent role of phages in the natural control of microbial infections and discussed the frequent specificities of the phages isolated from recuperating patients for the disease organisms infecting them, as well as the rather rapid variations over time of their phage populations. Throughout his life, he worked to develop the therapeutic potential of properly selected phages against the most devastating health problems of the day, traveling to many parts of the world, then teaching at Yale from 1928 to 1933 while establishing his own Laboratoire du Bactériophage in Paris, where they produced the first commercial phage cocktails such as Bacté-Intesti-Phage and Bacté-Pyo-Phage, which form the basis for today's complex cocktails in Eastern Europe.

Unfortunately, most of the English-language literature reviews of phage therapy ignored the successful application of phage therapy in France using these phage preparations, which were very carefully produced and tested by d'Hérelle's laboratory or by the Bacteriophage Service of the Lyon and Paris branches of the Institut Pasteur. Effectively complementing other antimicrobials, some of this French use continued until the early 1990s, when the AIDS scare led the French Parliament to ban the inclusion of any sort of viruses in any kind of medications.

Key to d'Hérelle's many successes was that he focused intensely on first understanding phage biology and on applying that knowledge in his production and application of phages, including careful ongoing quality control and close work with physicians. The depth of his insights became more accessible via publication of a translation of this book's appendix.[24]

Through much of the early developmental time, he worked closely with George Eliava, director of the Georgian Institute of Microbiology. Over the years, the two developed plans for an Institute of Bacteriophage Research in Tbilisi as a world center of phage therapy.

In 1926 the current large campus on the river Mtkvari was allotted for the project, and d'Hérelle sent supplies, equipment, and library materials. In 1934 to 1935 he visited Tbilisi for 6 months, set up his laboratory, and wrote a book, *The Bacteriophage and the Phenomenon of Recovery*.[25] He intended to move to Georgia, but Eliava was arrested in 1937 as a "people's enemy" by Beria, and was executed, sharing the tragic fate of many Georgian and Russian progressive intellectuals of the time, and d'Hérelle, disillusioned, never returned to Georgia. However, their Bacteriophage Institute survived under the leadership of a group of Institute women well trained by Eliava and d'Hérelle. The Institute was transferred to the All-Union Ministry of Health in 1951, and hundreds of thousands of pathogenic bacterial samples were sent to the Institute from throughout the Soviet Union to isolate more effective phage strains and better characterize their usefulness. A new five-story building on the Eliava grounds provided facilities for making up to 2 tons of phage products twice a week, with 80% of the preparations going to the Soviet military. Products included tablets against dysentery, a serious problem among soldiers, pyophage cocktails targeting wound and other purulent infections, and Intestiphage, still used today for a wide range of enteric pathogens. Research laboratories continually isolated new phages, upgraded therapeutic cocktails, and collaborated closely with physicians to test the phage preparations and ways of administering them. In 1988 an official Scientific Industrial Union "Bacteriophage" was formed, centered in Tbilisi with branches in Ufa, Perm, and Gorki (now called Nizhny Novgorod). After the 1991 breakup of the Soviet Union, the three Russian sites were pulled into the developing pharmaceutical giant, Microgen (eng.microgen.ru), which produces an even wider range of phage preparations.

What is now the Eliava Institute lost most of its markets and funding and struggled for survival as the factory portions were privatized. Lead research scientists switched to preparing their two most important phage cocktails for Georgian use in 30-L batches in their own laboratories, packaged them by hand in 5-mL ampules, and sold them in the Eliava diagnostic clinic, where laboratory leaders continued to do the actual patient testing and diagnosis, supporting the laboratories while continuing to acquire new pathogenic strains for research and production purposes. By 1996 help became available from the International Science and Technology Centers and the NATO Civilian Research and Development Fund, the PhageBiotics Foundation, and other sources, and the institute is again thriving at its original site.

Initial Western Explorations

Phage therapy was explored extensively in Europe and the United States in the 1920s through 1940s, with early successes being reported for a variety of diseases, including dysentery, typhoid and paratyphoid fevers, staphylococcal, pyogenic and urinary tract infections, and cholera.[8] Phages have been given orally, through colon infusion, as injections and aerosols, or infused directly into lesions. The early strong interest in phage therapy is reflected in some 800 papers published on the topic between 1917 and 1956. The reported results were quite variable. Unfortunately, many of the physicians, entrepreneurs, and pharmaceutical firms who initially became very excited by the potential clinical implications, especially in the preantibiotic era, jumped into application efforts with very little understanding of phages, microbiology in general, or the basic scientific process. Thus many of the early studies were anecdotal and/or poorly controlled; many of the failures were predictable; and some of the reported successes did not make sense in light of current knowledge. Too often, uncharacterized phages at unknown concentrations were given to patients without previous specific bacteriologic diagnosis, and there is no mention of follow-up, controls, or placebos. Much of the understanding being gained by d'Hérelle was ignored in this early work, and inappropriate methods of preparation, "preservatives," and storage procedures were often used. On one occasion, d'Hérelle reported testing 20 preparations from various companies and finding that not one of them contained active phages! In general, little of the essential quality control was carried out except in a few research centers, mainly in France, the Soviet Union, and the United States.

Specific Problems of Early Phage Therapy Work

Some still believe (erroneously) that phage therapy was proven not to work during that period; however, it simply was never adequately researched for a variety of reasons, and the work that was done well is not widely enough known. It is thus important to carefully consider the reasons for the early problems and for the questioning of efficacy:

- Total lack of understanding of the heterogeneity and ecology of the phages and the bacteria involved
- Lack of availability or reliability of bacterial laboratories for carefully identifying the pathogens involved (important considering the relative specificity of phage therapy)
- Use of too few of the needed different phages in infections that involved mixtures of different bacterial species and strains
- Failure to select obligatorily lytic phages against the target bacteria before using them in patients
- Emergence of phage-resistant bacterial strains, through selection of resistant mutants, or through lysogenization (in cases where temperate phages were used, as discussed in "Properties of Bacteriophages")
- Failure to appropriately characterize or titer phage preparations, many of which, even from major companies, were shown to be totally inactive[26,27]
- Failure to neutralize gastric pH before oral phage administration
- Inactivation of phages by both specific and nonspecific factors in bodily fluids

All of these factors need to be taken into consideration as we now work to formally document phage efficacy and integrate phages into medical practice worldwide.

PHAGE INTERACTIONS IN THE BODY

A number of early experiments involving phage injection into animals led to widespread belief that phage therapy could not succeed because the phages were too rapidly cleared by the immune system. Early experiments in rabbits, rats, and mice indeed showed rapid disappearance of phage from the blood and organs but long-term survival in the spleen.[28,29] However, these experiments were done in the absence of host bacteria in which the phage could multiply and were often carried out by the unnatural mode of intravenous injection, exposing the phage almost immediately to the reticuloendothelial system. Many later studies made it clear that phages are seen in the mammalian circulatory system for prolonged periods when they are entering it from some sort of reservoir in other tissues and the host is dealing with infection by a bacterium in which they can replicate—precisely the sort of situation usually seen in phage therapy as currently practiced.

This pattern is demonstrated particularly well in research published in 1943 by noted Harvard bacteriologist René Dubos[30] (Fig. 101.5). Dubos et al.[30] injected mice *intracerebrally* with a dose of a smooth *Shigella dysenteriae* strain that was sufficient to kill more than 95% of the mice in 2 to 5 days and then treated them with *intraperitoneal* injection of a mixture of phages that had been isolated from New York City sewage, grown in the same bacteria, and purified only by sterile filtration. With no treatment, or when treated with filtrates of bacterial cultures or with *heat-killed* phage, only three of 84 mice (3.6%) survived; in contrast, 46 of 64 (72%) of the mice given 10^7 to 10^9 of

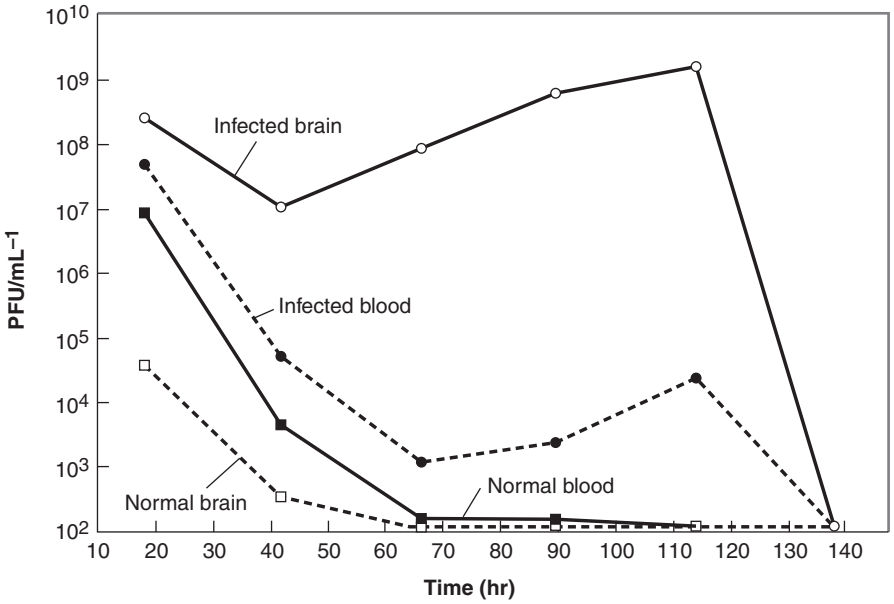


Fig. 101.5 This figure, based on the data in the 1943 mouse studies of Dubos et al.,[30] provides significant insight into why phage therapy works well even in treating infections that antibiotics cannot treat. When the mice were injected intraperitoneally with 10^9 phage, they quickly appeared in the bloodstream and some even crossed the blood–brain barrier, but they were rapidly cleared. However, when the mice were also injected intracerebrally with *Shigella dysenteriae*, the host for these phages, 46 of 64 of the mice survived (compared with 3 of 84 in the absence of appropriate viable phage), and the brain level of phage climbed to over 10^9/g. Once the bacteria cleared, the level of phage dropped below detection limits.

the appropriate phages survived. Pharmacokinetic studies showed that when phages were given to *uninfected* mice, they appeared in the bloodstream almost immediately, but the levels started to drop within hours and very few were seen in the brain, as shown in Fig. 101.5. However, in the *infected* animals, brain levels of the viable phage appropriate to those bacteria soon far exceeded blood levels; around 10^7 to 10^9 phages/g were often seen between 8 and 114 hours after administration, with the level dropping between 75 and 138 hours after phage addition. After the first 18 hours, blood levels were far lower than brain levels, but phages were still present in blood at 10^4 to 10^5 phages/mL in those animals in which the brain levels were still high.

Equally well-controlled experiments performed by Henry Morton and Enrique Perez-Otero from 1943 to 1945 at the University of Pennsylvania supported those of Dubos et al.[30] and further showed the lack of any protection when phages with inappropriate host specificities were used. These results clearly established that (1) the phages themselves were responsible, not something in the lysate that just stimulated normal immune mechanisms; (2) phages could rapidly find and multiply in foci of infection anywhere in the body, including crossing the blood–brain barrier; and (3) phages could be maintained in the circulation as long as there was a privileged reservoir of infection where phages were continually being produced. A final review authorized by the Council on Pharmacy and Chemistry discussed the major advantages of phages, such as their ability to replicate into problem areas and treat localized infections that are relatively inaccessible via the circulatory system, and the fact that their high specificity greatly aided in reducing later resistance problems.[31] The review also emphasized that most of the earlier phage research had been so poorly conceived and/or carried out that it offered no proof either for or against the promise of phages as antibiotics; therefore the negative conclusions of the earlier American Medical Association reviews were neither unexpected nor very relevant to the potential for eventual success.

The context of these studies sheds enlightening insights into the historical course of phage therapy in the United States.[32] In 1942 both *The Lancet* and the *British Medical Journal* published editorials about the apparently successful use of anti-dysentery phages by the Soviet military in the Middle and Far East. By November 1942 the US National Research Council Committee on Medical Research began supporting research possibilities offered by anti-dysenteric phages for dealing with this perpetual scourge of armies (including the previous studies) in top US bacteriology laboratories new to phage work, initially requiring them to keep the results secret. This promising work ended in 1944, when the end of World War II made penicillin generally available. The military secrecy, the end of the war emergency funding, the rapid rise in antibiotic availability and their broad-spectra "wonder-drug" status, and Max Delbruck's success in persuading the phage community to strongly shift its focus to basic molecular research involving a few model systems all contributed to the fact that there was little US follow-up to these very interesting and successful results. Although the results were published in major journals, few people even knew about them, or about any of the highly successful ongoing human applications, until they were recently rediscovered in medical journals by Thomas Häusler during an intensive search at major libraries around the world, such as those at the National Institutes of Health and the Pasteur Institute.[6]

Penicillin works only against gram-positive bacteria, so it cannot treat typhoid fever, and early phage efforts against typhoid had very mixed success. It was then found that the major pathogenic strains of *Salmonella typhi* all carried one particular antigen, named Vi (for "virulence"). In 1936 a pair of Canadians identified a number of phages specific against the Vi antigen; these are still used as "typing phages" in rapidly identifying and following outbreaks. In the late 1930s and early 1940s, physicians at the Los Angeles County Hospital used phage treatments to help deal with repeated serious outbreaks of typhoid that were killing one in five of those afflicted.[33] Walter Ward tested the

Vi-specific phages against mouse typhus and found that the death rate fell to 6% versus 93% in controls.[33] When these phages were then used to treat patients with typhoid, only three of 56 treated patients died compared with the 20% mortality for the other treatments available at the time.[34] Most impressively, this study reported that the patients who received phage therapy rapidly changed from being largely comatose to full of vigor, with renewed appetite, in 24 to 48 hours. In 1948 and 1949 near Montreal, Desranleau treated nearly 100 patients with dysentery by giving them a cocktail of six different Vi-specific phages, and the death rate dropped from 20% to 2%.[35] By then, however, chloramphenicol had been shown to work well against typhoid, and it was much easier for pharmaceutical companies to deal with, so that seems to have been the end of phage clinical trials in the western hemisphere.

The high specificity of phages still plays a strong role in the phage typing sets used for detecting and following problem strains of such bacteria as *Shigella, Salmonella,* and *E. coli,* but phage therapy itself is only beginning to stage a comeback.

CLINICAL APPLICATION

The growing understanding of phage biology had the potential to facilitate more rational thinking about the therapeutic process and the selection of therapeutic phages. However, there was generally little interaction between those who were so effectively using phages as tools to understand molecular biology and those working on phage ecology and therapeutic applications. Many in the latter group were spurred on by a concern about the rising incidence of nosocomial infections and of bacterial resistance against most or all known antibiotics, as well as by the fact that phages are far more effective than antibiotics in areas of the body where circulation is poor and does not disrupt normal flora, such as in treating foot infections in diabetics. This strong sense of the potential importance of phages was particularly seen in Poland, France, Switzerland, and the former Soviet Union, where use of therapeutic phages never died out and where there has been ongoing research and clinical experience, if not the double-blind experiments that are now the gold standard. In France, Dr. Jean-François Vieu led the therapeutic phage efforts until 1997. He worked in the Service des Entérobactéries of the Pasteur Institute in Paris and, for example, prepared *Pseudomonas* phages on a case-by-case basis for patients. His experience there is discussed in two articles.[36,37] In Vevey, Switzerland, the small pharmaceutical firm Saphal made "Coliphagine," "Intestiphagine," "Pyophagine," and "Staphagine" in drinkable and injectable forms, salves, and sprays into the 1960s.[32] The owner, Harrmann Glauser, had been encouraged and trained by d'Hérelle's old colleague Paul Hauduroy, who had become a professor of microbiology at the University of Lausanne during World War II. These phage preparations were officially approved and were paid for by insurance.

Phage therapy was used extensively in many parts of eastern Europe as a regular part of clinical practice, and companies in Russia now make phages for this purpose. However, most of the research and much of the phage preparation came under the direction of key centers in Tbilisi, Georgia, and Wroclaw, Poland. In both cases, the close interactions between research scientists and physicians played an important role in the high degree of success obtained, just as was the case for d'Hérelle's early work.

Clinical Research at the Institute of Immunology and Experimental Medicine, Polish Academy of Sciences

The most detailed publications documenting phage therapy are from the Polish group originally led by Director Stefan Slopek at the Wroclaw Institute of Immunology and Experimental Medicine. Slopek's initial series of papers described work completed in 1981 to 1986 with 550 patients in 10 regional Polish medical centers, under the careful supervision of the physicians there and with access to new phages from the Institute as needed.[38-40] The patients ranged in age from 1 week to 86 years, and venues included the Wroclaw Medical Academy Institute of Surgery's Cardiosurgery Clinic, Children's Surgery Clinic and Orthopedic Clinic, the Institute of Internal Diseases Nephrology Clinic, and the Clinic of Pulmonary Diseases. In 518 cases, phage use followed unsuccessful treatment with all then-available methods, including antibiotics. The major categories of infections included long-persisting suppurative fistulas and abscesses, septicemia, respiratory tract suppurative infections, pneumonia, and purulent peritonitis. In a final summary article, the results were carefully analyzed with regard to such factors as nature and severity of the infection and mono infection versus infection with multiple bacteria.[40] Rates of success under these ideal conditions ranged from 75% to 100% (92% overall), as measured by marked improvement in relevant physical condition, wound healing, and disappearance of titratable bacteria; 84% of subjects demonstrated full elimination of the suppurative process and healing of local wounds. Infants and children did particularly well. Not surprisingly, the poorest results occurred in elderly patients and those in the final stages of extended serious illnesses.

In the first study alone, 259 of their phages were tested (116 targeting *Staphylococcus*, 42 for *Klebsiella*, 11 for *Proteus*, 39 for *Escherichia*, 30 for *Shigella*, 20 for *Pseudomonas*, and one for *Salmonella*); 40% were selected to be used for therapy. All treatments were conducted in research mode, with the phage prepared at the Institute and tested for sterility. Treatment generally involved 10 mL of sterile phage lysate given orally half an hour before each meal, with gastric juices neutralized by ingesting (basic) Vichy water, baking soda, or gelatin. Phage-soaked compresses were generally applied three times a day where dictated by localized infection. Treatment ran for 1.5 to 14 weeks (mean, 5.3 weeks). For intestinal problems, short treatment usually sufficed, whereas months-long use was necessary for such problems as pneumonia with pleural fistula and pyogenic arthritis. Bacterial levels and phage sensitivity were continually monitored, and the phage(s) were changed if the bacteria lost their sensitivity. Therapy was generally continued for 2 weeks beyond the last positive test result for the bacteria. Few side effects were observed; those that were seen seemed to be directly associated with the therapeutic process.[38]

Various methods of administration were successfully used, including oral, aerosols, and infusion, either rectally or into surgical wounds. Intravenous administration was not recommended for fear of possible toxic shock from bacterial debris in the lysates.[38] However, it was clear that the phages readily entered the body from the digestive tract or wounds and multiplied internally wherever appropriate bacteria were present, as measured by their presence in blood and urine and by therapeutic effects.[41] This interesting and rather unexpected finding has been replicated in many studies and systems.[42-45] The final evaluating therapist filled out a special inquiry form that was also sent to the Polish Academy of Science research team. The Computer Center at Wroclaw Technical University carried out extensive analyses of the data, using the categories established in the World Health Organization's (1977) *International Classification of Diseases*. They also looked at the effects of age, severity of initial condition, type(s) of bacteria involved, length of treatment, and other concomitant treatments. After Slopek's retirement, his prime assistant Dr. Beatta Weber-Dabrowska carried on with his phage therapy work with patients in regional hospitals, publishing a summary in English of the results for the next 1600 patients.[46] In 1998 immunologist A. Górski took over as Institute director and revived a strong focus on phage work, with special emphasis on the immunological consequences of phage treatment. These researchers have been

working with the basic phage group of Dr. Malgorzata Lobocka in Warsaw to sequence and further characterize their key phages since I introduced them to each other during my first visit to the Institute in 2001.

Clinical Research at the Bacteriophage Institute, Tbilisi

Particularly extensive efforts for phage therapy were carried out over many decades by scientists at the Bacteriophage Institute in Tbilisi, Georgia, working closely with local physicians. Phage therapy is an accepted component of the general standard of care in Georgia, and was used extensively in pediatric, burn, and surgical hospital settings. Phage preparation was carried out on an enormous scale before the breakup of the Soviet Union, employing 700 people in the factory and several hundred more in the research arm of the Institute, often making 2 tons of phage products weekly to ship throughout the former Soviet Union. They were available both over the counter and through physicians; 80% went to the military for wound and burn infections and, largely as tablets, for preventing debilitating gastrointestinal epidemics. In hospitals, they were used to treat both primary and nosocomial infections, alone or in conjunction with other antimicrobials. The International Science and Technology Centers program, set up jointly by the United States, Europe, and Japan to give constructive opportunities to scientists formerly working with Soviet military projects, became one of the strongest supporters of basic and applied research in this area in Tbilisi.

From the Bacteriophage Institute's inception, the industrial part was run on a self-supporting basis, whereas its scientific branch was government supported. The Institute carried out the extensive studies needed for approval by the Ministry of Health in Moscow of each new strain, therapeutic cocktail, and means of delivery. This careful study of the host range, lytic spectrum, cross-resistance, and other fundamental properties of the phages being used was a major factor in the reported successes of their phage therapy work, as were their methods for selecting highly virulent phages from among the many available against any given host. Where necessary, new cocktails were prepared with broader host ranges. The depth and extent of the work involved are impressive. For example, in 1983 to 1985, the Institute's Laboratory of Morphology and Biology of Bacteriophages carried out studies of growth, biochemical features, and phage sensitivity on 2038 strains of *Staphylococcus,* 1128 of *Streptococcus,* 328 of *Proteus,* 373 of *P. aeruginosa,* and 622 of *Clostridium* received from clinics and hospitals in towns across the former Soviet Union. New broader acting phage strains were isolated and were included in a reformulation of their extensively used Pyophage preparation. A good deal of work went into developing the documentation for Ministry of Health approval of specialized new delivery systems, such as a spray for use in respiratory tract infections, in treating the incision area before surgery, and in sanitation of hospital problem areas. An enteric-coated pill was also developed, using phage strains that could survive the drying process, and accounted for the bulk of the shipments to other parts of the former Soviet Union. Much work focused on combating nosocomial infections, in which multidrug-resistant organisms have become a particularly lethal problem.

Staphylococcus aureus Infections, Whether or Not Methicillin-Resistant

Methicillin-resistant *S. aureus* (MRSA) is a particular concern given its reduced susceptibility to antibiotic treatment, wide prevalence in hospital-acquired infections and in the community, and potentially lethal and otherwise serious consequences. These pathogens are targeted by the anti-*S. aureus* activity of phage preparations such as Pyophage (which include potent anti-*Staphylococcus* phages of the broad-spectrum Sb1-staph phage K family). Here as elsewhere, there is no cross-resistance between phages and antibiotics. Furthermore, very little development of resistance to this family of phages has been observed, presumably implying that the still unidentified primary receptor is a molecule of significant importance to the bacteria. Thus as far as phages are concerned, MRSA is simply another strain of *Staphylococcus aureus.* Treatment of MRSA using phages can be accomplished by local application for local infections or, if necessary, with substantially more caution, more systemic dosing such as intraperitoneally for systemic infections.[47] The use of phage treatment for local infections, including particularly those resulting from *S. aureus*, has the distinction of being one of only two phage therapy strategies that were deemed to be convincingly efficacious by the Eaton and Bayne-Jones report in 1934,[48-50] and an otherwise phage therapy skeptical publication.[51]

The first human phage therapy publication reported on treatment of *S. aureus* skin infections.[10,52,53] The first publication on successful phage treatment in the United States in about 40 years also involved phage targeting *S. aureus*, in this case in diabetic toe ulcers.[54] The Eliava commercial staph phage Sb1 was used to treat a series of 11 patients where antibiotics had failed and whose only other option was amputation, and all of them healed in a matter of weeks; details for six of the cases are reported here, and further information on both this work and diabetic foot phage work in Novosibirsk is provided in Fish et al.[55] and Morozova et al.[56] A follow-up 2 years later for one of Fish's patients with osteoporosis showed that there was also significant bone regeneration.[54] In France, the Pherecydes corporation now has a clinical trial underway, approval for which by their board was based on this successful work, they have told us[57] (Fig. 101.6).

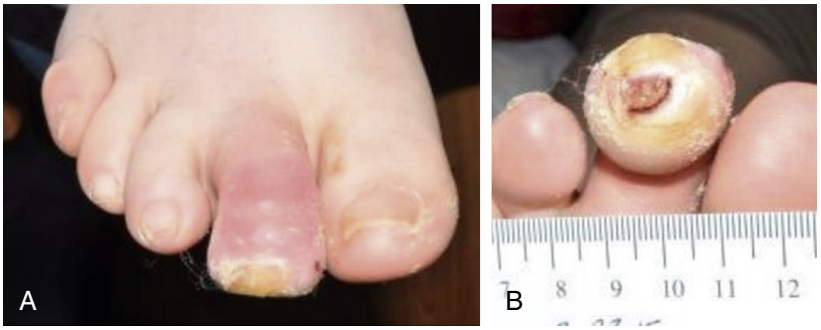


Fig. 101.6 Diabetic toe ulcer healed with phage. Images of the patient's ulcer on presentation. (A) Top view of foot. (B) View of ulcer at the distal tip. (From https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6316425/figure/antibiotics-07-00087-f001.)

Leszczynski et al.[58] described the use of oral phage therapy for targeting MRSA in a nurse who was a carrier. This individual had MRSA colonized in her GI tract and also had a urinary tract infection. The result was complete elimination of culturable MRSA. The same group argued that MRSA treatment using phages could be economically preferable to MRSA treatment using antibiotics.[59] Jikia et al.[60] described phage treatment of MRSA infecting radiation burns. Slopek et al.[40] reported 92.4% positive cases for phage treatment of 550 single and mixed-etiology infections involving *S. aureus*. Slopek et al.[39] specifically used phage treatment for suppurative staphylococcal infections, with a reported 93% "effective" rate "based on case history and data contained in a special questionnaire," while also using this treatment of various kinds of *Staphylococcus* infections in children (95.5% positive results for the 90 children treated). Working in New York, MacNeal and Frisbee[47] described their relatively successful treatment of staphylococcal bacteremia in 100 patients. MacNeal et al. subsequently reported very positive results in the cumulative treatment of 500 patients with staphylococcal bacteremia, using cocktails of phages that were lytic in vitro.[61]

Urogenital Tract Infections

Phages have been applied to treat various infections of the urogenital systems either systemically, via direct injection into the bladder, or via topical application. Phage treatment of urogenital tract infections could potentially be complimented by current naturopathic protocols involving alkalinization of the urine with citrates and minerals. Eaton and Bayne-Jones[48-50] in their otherwise-critical 1934 report were convinced of the efficacious use of phage therapy against cystitis. Letwiewicz et al.[62] described phage application rectally to target *Enterococcus faecalis* infection of the prostate, with substantial success in eliminating the target bacteria. In this case, the phages were presumed to be taken up through the rectal wall. Letarov et al.[63] noted that rectal phage suppositories are available on the Russian market. Slopek et al.[40] reported 92.9% positive results for phage treatment of 42 "diseases of the genitourinary tract." Chanishvili and Sharp's[64] book has chapters on "Phage Therapy in Urology" and "Phage Therapy in Gynecology." They summarize the results there as follows: "In cases of acute cystitis a therapeutic effect was observed within 4 to 5 hours of the first administration and resulted in relief of pain, a decrease in the frequency of urination and a normalization of the composition of the urine. Full recovery was achieved within 1 to 3 days in all 13 cases (100%), but treatment of chronic forms of cystitis was less successful, with only a moderate improvement observed."[64] Supported by a 3-year grant from the International Science and Technology Centers, the Eliava Institute developed a new phage cocktail specifically targeted against a large pool of bacteria from prostatitis and urinary tract infections. At the 2009 Evergreen International Phage Biology Meeting, Alavidze reported on its production and on very successful preliminary trials involving more than 100 patients. A collaborative Western-style double-blind, placebo-controlled trial involving addition of this phage to the standard treatment at one of the major urology hospitals in Tbilisi is currently underway, supported by Swiss funding of a collaborative research project.[65]

Gastrointestinal Infections

From the beginning, acute diarrheal infections were a major and very successful part of phage therapy practice, and in both Russia and the Republic of Georgia. However, most GI problems, such as irritable bowel syndrome, diverticulitis, and Crohn's disease, typically involve factors other than bacteria, and may include long-term chronic infections and immune system challenges. Although there is no body of older literature to build on from work in Tbilisi or Poland on the latter application, this would appear to be an area well worth exploration. Intestinal phage communities change in patients with inflammatory bowel disease (IBD) and other gut-related diseases.[66] There are some reports of disease specific alterations that could potentially contribute to high trend inflammation.[67] Other reports indicate specific targeting of pathogenic bacteria in IBD patients may be beneficial. The use of phage as treatment for IBD is an area that requires further study. Some research indicates the potential for targeting invasive *E. coli* strains implicated in Crohn's disease,[68] while some authors have observed an abnormal enteric virome in patients with IBD.[69] As we begin to better understand the depth of the human microbiome through metagenomic analysis, we also recognize the importance of the virome, and its role in maintaining normal function.[70] Extensive clinical experience using phage cocktails such as Intestiphage to treat functional gastrointestinal disorders has shown a positive effect in a large portion of patients being treated in the Republic of Georgia (unpublished data), with the idea of targeting the overgrowth of pathobionts that produce by-products and that are a potential trigger for visceral pain.[71] As work towards making fecal transplant a standard of care, the idea that phage preparations may contain those essential elements for restoration of normal function is an important one to consider.[72] A recent American controlled clinical study used phage as a prebiotic with the intent to modify gut microbiota. Its general conclusions were that phage was both safe and tolerable for consumption.[73] Much additional highly relevant gastrointestinal-related work has just been published.[74-78]

Respiratory Tract Infections

Respiratory infections can be differentiated into numerous types; however, phage therapy is limited in efficacy to those with a bacterial etiology. Weber-Dabrowska et al.[79] reported successful treatment of pneumonia in 6 cancer patients. Similarly, Slopek et al.[80] reported 86.7% positive results for phage treatment in 180 "diseases of the respiratory system" (see also Slopek et al.[38]). The first case studies of phages used to treat people for chronic lung infections of *S. aureus* and *P. aeruginosa* in Tbilisi used Pyophage and their fully sequenced *S. aureus* phage, phage Sb-1, which is now sold commercially, delivered by standard cystic fibrosis (CF) nebulizers.[81,82] The latter study included a detailed description of the successful treatment of *P. aeruginosa* infection in the lungs of a 7-year-old patient (using Pyophage) along with treatment of an *S. aureus* coinfection in the same patient using phage Sb-1. Success in treating infections in animal models of CF associated infection was also reported by Debarbieux et al.[83] and Carmody et al.,[84] in addition to the exploration of using nebulization as a phage delivery strategy. Work with CF patients has continued at the Eliava Phage Therapy Center, with more than 20 CF patients being treated over a 5-year period. Clinical improvement and a reduction in hospitalization has been seen consistently in most patients.[85] Although complete eradication of pathogens from the CF lung is not expected as a result of the well-established and complex microbiota of the CF lung, they have observed several cases of eradication of particularly problematic bacterial infections such as the *Achromobacter* infection in a 17-year-old girl using a specially prepared phage preparation. In addition to general clinical improvement, lung function also significantly improved. Treatment of these infections via nebulizer is generally tolerable, although some patients do observe increased sputum production and cough or dyspnea, and all patients should be closely monitored during treatment.

Phage treatments of lung infections can also be used effectively from systemic circulation, in at least some circumstances, as animal models have also shown.[84]

Ear Infections

Chronic otitis externa, known less formally as swimmer's ear, is often caused by a *P. aeruginosa* ear infection that resists antibiotic treatment. Otitis media is often caused by *Streptococcus pneumoniae* and is the leading cause of physician visits from children. The British company Biocontrol (now part of AmpliPhi Biosciences) developed anti-*P. aeruginosa* phages targeting otitis externa, after publishing similar studies on dogs.[86,87] In 2009 they published the results of their double-blinded Phase 1/2a (safety and small-number efficacy) trial in human patients with this condition.[88] Increases in phage numbers in situ, microbiological improvements (reductions in bacterial presence), and reduction in disease symptoms in the phage-treated cohort, but not the phage-negative controls, were observed. No side effects were seen. Complete bacterial eradication was not observed, but the extent of success was particularly notable considering that only a single phage dose was administered. Weber-Dabrowska et al.[79] also reported phage therapy success in treated purulent otitis media, and Slopek et al.[80] reported 93.8% positive results for phage treatment of 16 cases of conjunctivitis, blepharoconjunctivitis, and otitis media.

TOXICOLOGY

From a clinical standpoint, all indications are that phages are very safe. This feature is not surprising, given that humans are exposed to a very wide variety of phages from birth. Bergh et al.[89] reported that nonpolluted water contains about 10^8 phages/mL. Phages are normally found in the GI tract, skin, urine, and mouth, where they are harbored in saliva and dental plaque.[90-92] They have also been shown to be unintentional contaminants of sera and therefore of commercially available vaccines,[93-96] which were given dispensation to be sold despite this discovery, because of the general consensus that phages are safe for humans.

Extensive preclinical animal testing was required for approving new phage formulations in the former Soviet Union, but only a few of these studies were published. For example, Bogovazova et al.[97,98] evaluated the safety and efficacy of *Klebsiella* phages produced by the Russian company Immunopreparat. Pharmacokinetic and toxicologic studies using intramuscular, intraperitoneal, or intravenous administration of phages were carried out in mice and guinea pigs. The researchers found no signs of acute toxicity or histologic changes, even using a dose 3500-fold higher than the projected human dose. They then evaluated the safety and efficacy of the phages in treating 109 patients. The phage preparation was reported to be nontoxic to humans and to be effective in treating *Klebsiella* infections, as manifested by marked clinical improvements and bacterial clearance in the phage-treated patients.

Occasional mild side effects such as liver pain and fever reported in the early days of Western phage therapy may have been caused least in part by bacterial byproducts in preparations used intravenously.[99-101] Concerned about this possibility, the Polish phage therapy group does not administer their phages intravenously. The same is true for almost all of the therapeutic work carried out in Tbilisi and may help explain the apparent lack of any significant problems in both places; their many years of experience, careful attention to detail, and supportive infrastructure are presumably also important factors. Because phages readily enter the bloodstream after infusion in or near wounds and other sites of localized infection and travel to sites of infection throughout the body,[102] there seems to be no reason for undergoing the extra risks of intravenous administration in most cases.

Drug Interactions

No negative effects on the efficacy or safety of other drugs have been reported anywhere as a result of phage administration. No systematic studies have been carried out in this regard, but phages are so specific in their actions that it is hard to determine where such interactions might occur. In contrast, at least some antibiotics can interfere with phage treatment of localized infections by killing off the most accessible of the bacteria in which the phages could multiply as they work their way deeper into the site of infection; this would be a particular problem in cases in which the phages still attach and infect but cannot complete their replication cycle. Georgian physicians generally believe that antibiotics should never be used topically for wounds and deep-seated infections, because the decrease in antibiotic concentration below the surface provides a strong selection for antibiotic resistance; this problem does not occur with phages.

DOSING STRATEGY

Phage cocktails can be designed in two distinctly different ways. The major style currently used in Georgia, Russia, and Poland, termed "active treatment," uses a low concentration of phages and relies on in situ phage replication to achieve a therapeutically relevant concentration at all sites of infection by that pathogen encountered by progeny phage in the body. Titers of the many individual phages in each commercial cocktail are typically about 10^5 to 10^6 PFU/mL and in general 5 to 10 mL is used per dose. This approach is preferred when antibiotics fail because of poor circulation or surgical inaccessibility. "Passive treatment" ignores the self-replicating nature of phages when the infected area is directly accessible and enough phage particles are applied to treat the infection in a single dose.[103]

CONCLUSION

Phages have many potential advantages:

- They are self-replicating but also self-limiting, because they multiply only in the presence of susceptible bacteria.
- They can be targeted much more specifically than most antibiotics to the problem bacteria, causing far less of the bacterial imbalance or "dysbiosis" that are major problems with antibiotics, which often lead to serious secondary infections involving relatively resistant bacteria that can increase hospitalization time, expense, and mortality. Particular resultant problems involve *Pseudomonads*, which are especially difficult to treat, and *Clostridium difficile*, the cause of serious diarrhea and membranous colitis[104]; though no therapeutic phage cocktails directly targeting the anaerobe *C. difficile* are currently available, Intestiphage can help, presumably through rebalancing of the gastrointestinal flora.
- Phages can often be targeted to receptors on the bacterial surface that are involved in pathogenesis, so any resistant mutants are less problematic.
- No serious side effects have been reported for phage therapy.
- Phage therapy is particularly useful for people with allergies to antibiotics.
- Appropriately selected phages can easily be used prophylactically to help prevent bacterial disease at times of exposure or to sanitize hospitals and help protect against hospital-acquired (nosocomial) infections.
- At least for external applications, useful phages can potentially be prepared for administration fairly inexpensively and locally, facilitating their applications to underserved populations.
- Phages can be used either independently or in conjunction with other antimicrobials to help reduce the development of bacterial resistance.

The time has come to look more carefully at the potential of phage therapy for future practice, both by strongly supporting new research

and by scrutinizing the research already available, such as the very interesting human anti-typhoid phage research carried out in the United States in the 1940s,[34] as well as the extensive earlier work in France, the United States, Georgia, Poland, and Russia.[8,9]

With the enormous possibilities and decreasing costs of genomic analysis, it is now possible to sequence the phages included in cocktails to ascertain more about the phage families involved and be sure that phages from temperate families are excluded, which is generally necessary or at least highly desirable because they may carry or acquire genes related to pathogenicity or toxin production. Sequencing is now standard procedure for therapeutic phages being developed in the West. Such modern techniques are also beginning to be applied to some of the Georgian phage preparations with help from grants from the International Science and Technology Centers and Civilian Research and Development Foundation programs, both of which were set up to support civilian applications of science formerly funded by the Soviet military, and we are also doing some cocktail sequencing and detailed analysis here at Evergreen. This is an important step in considering the importation of such phages for use in the Western world.

The attitude toward phage therapies and its potential are at last beginning to change in both the United States and Europe, with more and more people now becoming aware of its existence and potential applications. The 2016 case discussed in the introduction in which a UC San Diego faculty member was cured with US Navy phage of a life-threatening *Acinetobacter* infection obtained while visiting Egypt has been key in creating far more US attention to the efficacy of phage as a medicine. It has had wide publicity, and the many articles, mass-media releases and recent book about the experience have led to an explosion of interest in phage therapy and in expediting its compassionate use.

That successful treatment was made possible by the ability of the practitioners to send patient samples to several groups working with *Acinetobacter* and their ability to very rapidly identify and obtain multiple phages against the very unusual infective strain. Due to the recent strong interest in *Acinetobacter* as a result of its frequent appearance in wounds from the conflicts in Iraq and the ways the Navy and others were isolating and handling relevant phages, it was possible to very quickly test them, identify several effective phages, grow them up and purify them sufficiently with the help of phage labs in Texas and San Diego, and use them sequentially to eradicate this infection. Building libraries with several iterations of phages targeting particularly threatening pathogens, as some have begun to do across the world, is facilitating a very rapid potential response and collaborative preparation of cocktails for treatment of such cases.

The professor and his wife have developed a new phage therapy center at UC San Diego, taking advantage of their experience and the subsequent treatment there of six candidates for organ transplants, which were being seriously delayed by infectious complications. This is the first actual phage therapy center in the Americas, and is already collaborating with Ampliphi and the new Adaptive Phage Therapeutics company, set up in D.C. to work with the Navy and others to very rapidly identify and prepare appropriate phages for emergency compassionate use cases.

In the U.S. any newly developed medicine attempting to make its way to market must go through the FDA, which is now accepting the concept and potential importance of phage therapy and working to adapt to its very unique aspects. The three phases of FDA clinical trials are designed to prove safety, efficacy, and marketability in respect to other approved treatments. To date, there have been just three completed Phase II clinical phage trials. The first took place in 2009 in England and dealt with very long-term chronic *Pseudomonas* osteitis, including a total of 12 patients; it was deemed successful in demonstrating safety and efficacy of the phage preparation used. Nestlé funded a 2016 trial of phage for acute infant diarrhea patients, developed over 15 years of selecting and intensively studying phages targeting infant diarrheal *E. coli* strains from the central hospital in Bangladesh and testing them in adults and older children in Switzerland and Bangladesh, as well as in the laboratory. The French firm Pherecydes led the Phagoburn trial for topical treatment of severe *Pseudomonas* infections in burn victims at 14 hospitals in France, Belgium, and Switzerland. Both of the latter trials confirmed safety but were deemed unsuccessful in proving efficacy, largely due to various design complications that became apparent.

Although it seems premature to broadly introduce injectable phage preparations in the West without further extensive research, their successful use in the extreme San Diego cases has helped stimulate FDA approval for very serious cases. More broadly, the carefully implemented use of phage in external applications and for a variety of agricultural purposes should help reduce the emergence of antibiotic-resistant strains and deal with problems with which we have serious difficulty handling today. Compassionate use of appropriate phages seems now warranted and increasingly widely accepted in cases in which bacteria resistant to all available antibiotics are causing life-threatening illness. Phages are especially useful in dealing with recalcitrant nosocomial infections, in which large numbers of particularly vulnerable people are being exposed to the same strains of bacteria in a closed hospital setting. In these cases especially, the environment as well as the patients can be effectively treated with phage preparations.

Acknowledgments

Special thanks to Drs. Guram Gvasalia, Ramaz Katsarava, Mzia Kutateladze, Rezo Adamia, Teona Danelia, and their colleagues in Tbilisi, and to Beata Weber-Dabrowski and Andre Gorski in Wroclaw for their hospitality, hard work on phage therapy, and efforts through the years to help us understand the extensive therapeutic work carried out there. We express our thanks to the many phage biologists, companies and health care personnel around the world who are now working hard to bring phage therapy back into the Western world. Special thanks also to the Phagebiotics Research Foundation and to The Evergreen State College for their long-time support of phage therapy research (since 1997) and of our bacteriophage research here at The Evergreen State College since 1972, respectively.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. *Bacteriophage*. 2011;1(2):66–85.
2. World Health Day- 7 April 2011, Bacterial resistance: no action today, no cure tomorrow. www.who.int/world-health-day/2011/en. Accessed 9/19/2011.
3. Kutter E, Borysowski J, Miedzybrodzki R, et al. Clinical phage therapy. In: Borysowski J, Miedzybrodzki R, Gorski A, Phage, eds. *Therapy: Current Research and Applications*. Caister Academic Press; 2014:257–288.
4. Pirnay JP, Verbeken G, Ceyssens PJ, Huys I, De Vos D, Ameloot C, Fauconnier A. The magistral phage. Viruses. 2018;10(2):64.
5. Bryan D, El-Shibiny A, Hobbs Z, Porter J, Kutter EM. Bacteriophage T4 infection of stationary phase E. coli: life after log from a phage perspective. *Front Microbiol*. 2016;7:1391.
6. Häusler T. *Viruses vs. Superbugs: A Solution to the Antibiotic Crisis*. Macmillan: New York.
7. Kutter E. Bacteriophage therapy: past and present. In: Schaechter M. ed. *Encyclopedia of Microbiology*. Elsevier: New York; 258–266.
8. Abedon S, Kuhl S, Blasdel B, et al. Phage treatment of human infections. *Bacteriophage*. 2011;1:66–85.
9. Kutter E, De Vos D, Gvasalia G, et al. Phage therapy in clinical practice: treatment of human infections. *Curr Pharm Biotechnol*. 2010;11:69–86.
10. Sulakvelidze A, Kutter E. Bacteriophage therapy in humans. In: (editors: Kutter E, Sulakvelidze A.) *Bacteriophages: Biology and Application*. Boca Raton, FL: CRC Press; 381–436.
11. Brüssow H. Phage therapy: the Western perspective. In: Mc Grath S, van Sinderen D. ed. *Bacteriophage: Genetics and Microbiology*. Norfolk, UK: Caister Academic Press; 159–192.
12. Górski A, Borysowski J, Miedzybrodzki R, et al. Bacteriophages in medicine. In: McGrath S, van Sinderen D. eds. *Bacteriophage: Genetics and Microbiology*. Norfolk, UK: Caister Academic Press; 125–158.
13. Harper DR, Kutter E. Bacteriophage: therapeutic use. In: *Encyclopedia of Life Sciences*. Hoboken, NJ: John Wiley & Sons, Ltd.
14. Górski A, Miedzybrodzki R, Borysowski J, et al. Bacteriophage therapy for the treatment of infections. *Curr Opin Investig Drugs*. 2009;10:766–774.
15. Alisky J, Iczkonski K, Rapoport A, et al. Bacteriophage shows promise as antimicrobial agents. *J Infection*. 1998;36:5–3613.
16. Sulakvelidze A, Alavidze A, Morris J. Bacteriophage therapy. *Antimicrob Agents Chemother*. 2001;45:649–659.
17. Summers WC. *Felix d'Herelle and the Origins of Molecular Biology*. New Haven, CT: Yale University Press.
18. Kutter E, Sulakvelidze A. *Bacteriophages: Biology and Applications*. Boca Raton, FL: CRC Press.
19. Antibacterial treatment against diarrhea in oral rehydration solution. http://www.clinicaltrials.gov/ct2/show/NCT00937274. Accessed April 3, 2012.
20. Chibani-Chennoufi S, Sidoti J, Bruttin A, et al. Isolation of T4-like bacteriophages from the stool of pediatric diarrhea patients in Bangladesh. *J Bacteriol*. 2004;186:8287–8294.
21. Bruttin A, Brüssow H. Human volunteers receiving Escherichia coli phage T4 orally: a safety test of phage therapy. *Antimicrob Agents Chemother*. 2005;49:2874–2878.
22. Ruska H. Die sichtbarmachung der bakteriophagen lyse im ubermikroskop. *Naturwissenschaften*. 1940;28:45.
23. Pfankuch E, Kausche G, Isolierung U. Uebermikroskopische abbildung eines bakteriophagen. *Naturwissenschaften*. 1940;28:46.
24. D'Hérelle F. *The Bacteriophage: Its Role in Immunity*. Smith GH (trans). Williams & Wilkins: Baltimore.
25. Eliava G. *Bakteriofagi Fenomenyz Dorovieniya*. Tbilisi, Georgia: Tbilis National University Publications .
26. Summers WC. Bacteriophage therapy. *Ann Rev Microbiol*. 2001;55:437–451.
27. Straub ME, Appelbaum M. Studies on commercial bacteriophage products. *JAMA*. 1933;100:110–113.
28. Appelmans R. Le bactériophage dans l'organisme. *C R Seances Soc Biol Fil*. 1921;85:722–724.
29. Evans A.C. Inactivation of antistreptococcus bacteriophage by animal fluids. *Public Health Reports*. 1933;48:411–446. Cited in Merril C, S Adhya S. Phage therapy. In: (editor: Calendar R.) *The Bacteriophages*. Oxford University Press: Oxford, pp. 725–741.
30. Dubos RJ, Straus JH, Pierce C. The multiplication of bacteriophage in vivo and its protective effects against an experimental infection with Ishigella dysenteriae. *J Exp Med*. 1943;78:161–168.
31. Morton HE, Engely FB. Dysentery bacteriophage: review of the literature on its prophylactic and therapeutic uses in man and in experimental infections in animals. *JAMA*. 1945;17:584–891.
32. Häusler T. Gesund durch Viren. München. Piper: Germany.
33. Ward WE. Protective action of VI bacteriophage in Eberthella typhi infections in mice. *J Infect Dis*. 1943;72:172–176.
34. Knouf EG, Ward WE, Reichle PA, et al. Treatment of typhoid fever with type specific bacteriophage. *JAMA*. 1946;132:134–138.
35. Desranleau JM. Progress in the treatment of typhoid fever with Vi bacteriophages. *Can J Public Health*. 1949;40(11):473–478.
36. Vieu JF. Les bactériphages. In: Fabre J ed. *Traité de thérapeutique, Sérums et vaccins*. Paris: Flammarion; 337–430.
37. Vieu JF, Guillermet F, Minck R, et al. Données actuelles sur les applications thérapeutiques des bactériophages. *Bull Acad Natl Med*. 1979;163:61–66.
38. Slopek S, Durlakova I, Weber-Dabrowska B, et al. Results of bacteriophage treatment of suppurative bacterial infections. I. General evaluation of the results. *Arch Immunol Ther Ezp*. 1983;31:267–291.
39. Slopek S, Kucharewica-Krukowska A, Weber-Dabrowska B, et al. Results of bacteriophage treatment of suppurative bacterial infections. VI. Analysis of treatment of suppurative staphylococcal infections. *Arch Immunol Ther Exp*. 1985;33:261–273.
40. Slopek S, Weber-Dabrowska B, Dabrowski M, et al. Results of bacteriophage treatment of suppurative bacterial infections in the years 1981-1986. *Arch Immunol Ther Exp*. 1987;35:569–583.
41. Weber-Dabrowska B, Dabrowski M, Slopek S. Studies on bacteriophage penetration in patients subjected to phage therapy. *Rch Immunol Ther Exp (Warsz)*. 1987;35:363–368.
42. Smith HW, Huggins RB. Successful treatment of experimental E. coli infections in mice using phage: its general superiority over antibiotics. *J Gen Microbiol*. 1982;128:307–318.
43. Smith HW, Huggins RB. Effectiveness of phages in treating experimental E. coli diarrhoea in calves, piglets and lambs. *J Gen Microbiol*. 1983;129:2659–2675.
44. Smith HW, Huggins RB. The control of experimental E. coli diarrhea in calves by means of bacteriophage. *J Gen Microbiol*. 1987;133:1111–1126.
45. Smith HW, Huggins RB, Shaw KM. Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *J Gen Microbiol*. 1987;133:1127–1135.
46. Weber-Dabrowska B, Mulczyk M, Gorski A. Bacteriophage therapy of bacterial infections: an update of our institute's experience. *Arch Immunol Ther Exp*. 2000;48:547–551.
47. MacNeal WJ, Frisbee FC. One hundred patients with Staphylococcus septicemia receiving bacteriophage service. *Am J Med Sci*. 1936;191:179–195.
48. Eaton MD, Bayne-Jones S. Bacteriophage therapy: review of the principles and results of the use of bacteriophage in the treatment of infections (I). *JAMA*. 1934;103:1769–1776.
49. Eaton MD, Bayne-Jones S. Bacteriophage therapy: review of the principles and results of the use of bacteriophage in the treatment of infections (II). *JAMA*. 1934;103:1847–1853.
50. Eaton MD, Bayne-Jones S. Bacteriophage therapy: review of the principles and results of the use of bacteriophage in the treatment of infections (III). *JAMA*. 1934;103:1934–1939.
51. MacNeal WJ, Frisbee FC, McRae MA. Staphylococcemia 1931–1940. Five hundred patients. *Am J Clin Pathol*. 1942;12:281–294.

52. Kutter E. Phage therapy: bacteriophages as naturally occurring antimicrobials. In: (editors: Goldman E, Green L.H.) *Practical Handbook of Microbiology*. Boca Raton, FL: CRC Press: 713–730.
53. Bruynoghe R, Maisin J. Essais de thérapeutique au moyen du bactériophage du Staphylocoque. *Compt Rend Soc Biol*. 1921;85:1120–1121.
54. Fish R, Kutter E, Bryan D, Wheat G, Kuhl S. Resolving digital staphylococcal osteomyelitis using bacteriophage-A case report. *Antibiotics (Basel, Switzerland)*. 2018;7(4):87. https://doi.org/10.3390/antibiotics7040087.
55. Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Bacteriophage treatment of intransigent diabetic toe ulcers: a case series. *J. Wound Care*. 2016;25:S27–S33. https://doi.org/10.12968/jowc.2016.25.7.S2.
56. Morozova VV, Kozlova YN, Ganichev DA, Tikunova NV. Bacteriophage Treatment of Infected Diabetic Foot Ulcers. In: Azeredo J, Sillankorva S. eds. *Bacteriophage Therapy*. Methods in Molecular Biology. Vol. 1693. New York, NY: Humana Press; 2018
57. Jault P, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis*. 2019;19(1):35–45.
58. Leszczynski P, Weber-Dabrowska B, Kohutnicka M, et al. Successful eradication of methicillin-resistant Staphylococcus aureus (MRSA) intestinal carrier status in a healthcare worker - a case report. *Folia Microbiol*. 2008;51:236–238.
59. Miedzybrodzki R, Fortuna W, Weber-Dabrowska B, et al. Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. *Postepy Higieny I Medycyny Doswiadczalnej*. 2007;61:461–465.
60. Jikia D, Chkhaidze N, Imedashvili E, et al. The use of a novel biodegradable preparation capable of the sustained release of bacteriophages and ciprofloxacin, in the complex treatment of multidrug-resistant Staphylococcus aureus-infected local radiation injuries caused by exposure to Sr90. *Clin Exp Dermatol*. 2005;30:23–26.
61. MacNeal WJ, et al. Conjoined action of penicillin and bacteriophages. *J Lab Clin Med*. 1946;31(9):974–981.
62. Letkiewicz S, Miedzybrodzki R, Fortuna W, et al. Eradication of Enterococcus faecalis by phage therapy in chronic bacterial prostatitis—case report. *Folia Microbiol (Praha)*. 2009;54:457–461.
63. Letarov AV, Golomidova AK, Tarasyan KK. Ecological basis of rational phage therapy. *Acta Naturae*. 2010;2:60–71.
64. Chanishvili N. Phage therapy in urology. In: Chanishvili N, Sharp R. ed. *A Literature Review of the Practical Application of Bacteriophage Research*. Tbilisi: Georgia: Eliava Institute; 59–60.
65. Ujmajuridze A, Chanishvili N, Goderdzishvili M, et al. Adapted bacteriophages for treating urinary tract infections. 2018;9(1832). https://doi.org/10.3389/fmicb.2018.01832.
66. De Sordi L, Lourenco M, Debarbieux L. The battle within: interactions of bacteriophages and bacteria in the gastrointestinal tract. *Cell Host & Microbe*. 2019;25(2):210–218.
67. Gogokhia L, Buhrke K, Bell R, Casjens SR, Longman RS, Round JL. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. *Cell Host & Microbe*. 2019;25(2). 285-29.
68. Galtier M, De Sordi L, Sivignon A, et al. Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease. 2017. Advance publication online. https://doi:10.1093/ecco-jcc/jjw224.
69. Norman JM, Handley SA, Baldridge MT, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell*. 2015;160(3):447–460.
70. Rascovan N, Duraisamy R, Desnues C. *Metagenomics and the Human Virome in Asymptomatic Individuals*. 2016;70(1):125–141. https://doi.org/10.1146/annurev-micro-102215-095431.
71. Ghoshal UC, Shukla R, Ghoshal U, Gwee KA, Ng SC, Quigley EM. The gut microbiota and irritable bowel syndrome: friend or foe? *Int J Inflam*. 2012:151085.
72. Distrutti E, Monaldi L, Ricci P, Fiorucci S. Gut microbiota role in irritable bowel syndrome: new therapeutic strategies. *World J Gastroenterol*. 2016;22(7):2219–2241.
73. Gindin M, Febvre HP, Rao S, Wallace TC, Weir TL. Bacteriophage for gastrointestinal health (PHAGE) study: evaluating the safety and tolerability of supplemental bacteriophage consumption. *J Am Colleg Nutri*. 2019;38(1):68–75. https://doi.org/10.1080/07315724.2018.1483783.
74. Moossavi S, et al. Composition and variation of the human milk microbiota are influenced by maternal and early-life factors. *Cell Host & Microbe*. 2019;25(2). 324–335.e324.
75. Moreno-Gallego JL, et al. Virome diversity correlates with intestinal microbiome diversity in adult monozygotic twins. *Cell Host & Microbe*. 2019;25(2):261–272.e265.
76. Bollyky PL, Secor PR. The innate sense of bacteriophages. *Cell Host & Microbe*. 2019;25(2):177–179.
77. Caballero-Flores G, et al. Maternal Immunization confers protection to the offspring against an attaching and effacing pathogen through delivery of IgG in breast milk. *Cell Host & Microbe*. 2019;25(2):313–323.e314.
78. Elhenawy W, et al. Host-specific adaptive diversification of Crohn's disease-associated adherent-invasive Escherichia coli. *Cell Host & Microbe*. 2019;25(2):301–312.e305.
79. Weber-Dabrowska B, Mulczyk M, Górski A. Bacteriophage therapy for infections in cancer patients. *Clin Appl Immunol Rev*. 2001;1:131–134.
80. Slopek S, Weber-Dabrowska B, Dabrowski M, et al. Results of bacteriophage treatment of suppurative bacterial infections in the years 1981–1986. *Arch Immunol Ther Exp (Warsz)*. 1987;35:569–583.
81. Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends Biochem*. 2010;28:591–595.
82. Kvachadze L, Balarjishvili N, Meskhi T, et al. Evaluation of lytic activity of staphylococcal bacteriophage Sb-1 against freshly isolated clinical pathogens. *Microb Biotechnol*. 2011;4:1–8.
83. Debarbieux L, Leduc D, Maura D, et al. Bacteriophages can treat and prevent Pseudomonas aeruginosa lung infections. *J Infect Dis*. 2010;201:1096–1104.
84. Carmody LA, Gill JJ, Summer EJ, et al. Efficacy of bacteriophage therapy in a model of Burkholderia cenocepacia pulmonary infection. *J Infect Dis*. 2010;201:264–271.
85. Hoyle N, Zhvaniya P, Balarjishvili N, et al. Phage therapy against Achromobacter xylosoxidans lung infection in a patient with cystic fibrosis: a case report. *Research in Microbiology*. 2018;169(9):540–542. https://doi.org/10.1016/j.resmic.2018.05.001.
86. Marza JAS, Soothill JS, Boydell P, et al. Multiplication of therapeutically administered bacteriophages in Pseudomonas aeruginosa infected patients. *Burns*. 2006;32:644–646.
87. Hawkins C, Harper D, Burch D, et al. Topical treatment of Pseudomonas aeruginosa otitis of dogs with a bacteriophage mixture: a before/after clinical trial. *Vet Microbiol*. 2010;146:309–313.
88. Wright A, Hawkins CH, Anggård EE, et al. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clin Otolaryngol*. 2009;34:349–357.
89. Bergh O, Borsheim KY, Bratbak G, et al. High abundance of viruses found in aquatic environments. *Nature*. 1989;340:467–468.
90. Caldwell JA. Bacteriologic and bacteriophagic study of infected urines. *J Infect Dis*. 1928;43:353–362.
91. Yeung MK, Kozelsky CS. Transfection of Actinomyces spp. by genomic DNA of bacteriophages from human dental plaque. *Plasmid*. 1997;35:141–153.
92. Bachrach G, Leizerovici-Zigmond M, Zlotkin A, et al. Bacteriophage isolation from human saliva. *Lett Appl Microbiol*. 2003;36:50–53.
93. Merril CR, Friedman TB, Attallah AF, et al. Isolation of bacteriophages from commercial sera. *Vitro*. 1972;8:91–93.
94. Geier MR, Attallah AF, Merril CR. Characterization of escherichia coli bacterial viruses in commercial sera. *Vitro*. 1975;11:55–58.
95. Milch H, Fornosi F. Bacteriophage contamination in live poliovirus vaccine. *J Biol Stand*. 1975;3:2307–2310.
96. Moody EE, Trousdale MD, Jorgensen JH, et al. Bacteriophages and endotoxin in licensed live-virus vaccines. *J Infect Dis*. 1975;131:588–591.
97. Bogovazova GG, Voroshilova NN, Bondarenko VM. The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental

Klebsiella infection. *Zh Mikrobiol Epidemiol Immunobiol*. 1991;4:5–8. [Russian].
98. Bogovazova GG, Voroshilova NN, Bondarenko VM, et al. Immunobiological properties and therapeutic effectiveness of preparations from Klebsiella bacteriophages. *Zh Mikrobiol Epidemiol Immunobiol*. 1992;3:30–33. [Russian].
99. Larkum NW. Bacteriophage as a substitute for typhoid vaccine. *J Bacteriol*. 1929;17:42.
100. Larkum NW. Bacteriophage from public health standpoint. *Am J Pub Health*. 1929;19:31–36.
101. King WE, Boyd DA, Conlin JH. The cause of local reactions following the administration of Staphylococcus bacteriophage. *Am J Clin Pathol*. 1934;4:336–345.
102. Górski A, Ważna E, Weber-Dąbrowska B, et al. Bacteriophage translocation. *FEMS Immunol Med Microbiol*. 2006;46:313–319.
103. Abedon ST, Thomas-Abedon C. Phage therapy pharmacology. *Curr Pharm Biotechnol*. 2010;11:28–47.
104. Fekety R. Antibiotic-associated diarrhea and colitis. *Cur Opin Infect Dis*. 1995;8:391–397.
105. Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Rüger W. Bacteriophage T4 genome. *Microbiol Mol Biol Rev*. 2003;67(1):86–156.

Phosphatidylserine (PS)

Parris M. Kidd, BSc, PhD

OUTLINE

INTRODUCTION

Phosphatidylserine (PS) is a phospholipid nutrient that occurs naturally in all cells; it is an orthomolecule ("orthodox to the body") according to Linus Pauling's definition.[1] In humans, PS is most highly concentrated in the brain, and as a dietary supplement its clinical benefits are most apparent for memory, attention, and other higher brain-related functions, such as mood and coping with stress.[2-6]

PS is fundamentally important for all known life forms. PS is an essential building block for cell membranes, which occur in all known cells and are the sites of most of the cell's functional activity.[7] Cell membranes are thin, continuous structures composed mainly of a double molecular layer of phospholipids (the "lipid bilayer"), into which are inserted diverse arrays of enzymes and other catalytic proteins. The presence of PS makes essential contributions to the physical and chemical *milieu* required for the optimal functioning of numerous membrane proteins.[2,7]

The PS molecule has a characteristic layout (Fig. 102.1)—featuring a head piece, a middle piece, and two tail pieces ("tails").[2,7] The head piece contains serine attached to a phosphoryl chemical group, has a net negative charge, and is positioned on the membrane's inner (cytoplasmic) surface.[7] The middle piece is a three-carbon glyceryl sequence. The two tail pieces are constructed from fatty acids, extend deep into the membrane, and help create a water-free, oily, semifluid membrane interior that is crucial for membrane protein catalytic activity.[2]

Biochemically, PS is not a single molecule but a family of molecules.[2,8] The head and middle pieces are standard, but each of the two tail pieces can be constructed from many different fatty acids. Thus there are as many PS molecules as there are fatty acid permutations. Further, all cells carry enzymes ("acylases") that can detach fatty acid tails and substitute them with others, thereby modifying the membrane's fluidity or other biochemical characteristics.[8] In the brain's highly active gray matter, the PS molecules carry more of the highly fluidizing ω-3 fatty acids, consistent with high membrane metabolic activity. In the less active white matter, by contrast, the PS molecules carry less ω-3 and more saturated and monounsaturated (ω-9) fatty acid tails, which are less fluidizing and support lower membrane metabolic activity.[8]

PHYSIOLOGICAL ROLES

The physiological activity of PS seemingly is based exclusively in cell membranes. In the membrane systems of nerve cells, for example, PS enables energy generation, electrical conduction activity, and neurotransmission across synaptic junctions.[2,7–11] PS is also intrinsic to the blood coagulation cascade, again as a constituent of cell membranes, and is central to physiological disposal of dying and dead cells.[2,7] At the cell membrane level, activities specifically linked to PS include[8,9,12–14]:

- Membrane/cell stabilization: PS binds with structural proteins to stabilize the cell's outer membrane and overall cell shape.[9]
- Energetics: Mitochondrial production of adenosine triphosphate (ATP) requires the membrane phospholipid phosphatidylethanolamine (PE), which local enzymes derive almost exclusively from membrane PS.[8]
- Ion pumping: The sodium, potassium ATPase enzyme, which moves sodium in and potassium out of our cells, uses some 25% of all the body's energy. It requires PS for its activity.[7]
- Signal transduction: The conversion of external signals to internal cell transformations, which often requires G-protein coupled receptors (GPCR) and protein kinase C enzymes (PKC), protein families both likely dependent on PS for optimal activity.[10]
- Electrical signal transmission: The fusion of nerve cells' synaptic vesicles with the outer cell membrane to release neurotransmitters requires the presence of PS.[11]
- The "eat me" message: In all tissues, as cells lose function and become moribund, PS becomes relocated to the outer half of their outer membrane. This serves to "tag" such cells for disposal by circulating immune cells. Immune phagocytes have PS receptor proteins that interpret the PS "tag" as an "eat me" message.[12]

PHARMACOLOGY

The pharmacology of PS is consonant with its diverse actions in cell membranes.[2,10,11] Through its presence in synaptic membranes, PS fundamentally enables the brain's diverse neurotransmitter systems.[10] PS facilitates stress management by enhancing hypothalamic-pituitary-adrenal axis (HPAA) function.[11,15]

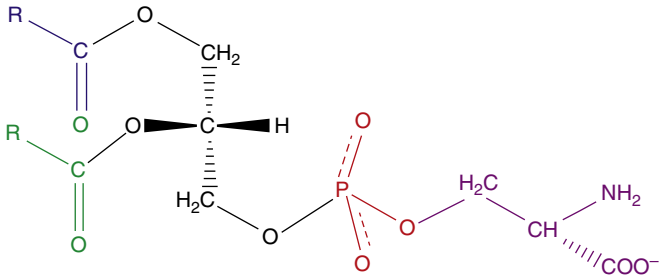


Fig. 102.1 Phosphatidylserine.

Other animal findings indicate PS has an overall trophic (restorative) effect in the brain. Feeding PS to aging rats significantly slowed the usual age-related decline of forebrain cholinergic synapses[16] and of nerve cell dendritic densities in the hippocampus.[17,18] Also in aging rats, PS given by mouth significantly slowed the age-associated decline of receptors for nerve growth factor, a protein factor that stimulates brain circuit maintenance and renewal.[18]

Consistent with its importance for mitochondrial function, PS has enhanced human brain energetics. A positron emission tomography (PET) imaging study of dementia patients found that orally administered PS improved energy production and utilization by an average 14% across the brain.[19]

CLINICAL APPLICATIONS

Memory Decline

The primary clinical application of PS is to improve memory and other cognitive functioning in the middle-aged and elderly. In multiple randomized, double-blind, placebo-controlled trials, PS as a dietary supplement consistently improved memory, learning, word recall, and other cognitive functions.[2–5,20–28] In one such trial conducted with healthy subjects at an early measurable stage of cognitive decline (age-associated memory impairment), PS partially reversed the decline in the subgroup that was worst afflicted at the outset. The researchers estimated PS may have reversed approximately 12 years of decline and stated, "the magnitude of effect may be considered significant by many patients and clinicians."[3]

For patients with full-blown dementia, PS has shown mild benefits for memory and concentration, along with improvement in sociability and sometimes also in activities of daily living.[2,4,5,20,21,23] In the largest controlled dementia trial, a total of 425 elderly patients (aged 65–93 years) received either PS (300 mg/day) or a placebo for 6 months.[5] The PS group showed improvements in verbal learning and memory as well as in withdrawal/apathy that were highly statistically significant (P <0.01) compared with the placebo group. These patients were maintained on a variety of drugs, and no clinically meaningful adverse interactions with PS were observed.

In May 2003 PS acquired landmark regulatory status as a brain nutrient. The U.S. Food and Drug Administration granted two "qualified health claims" that PS may: (1) reduce the risk of dementia in the elderly; and (2) reduce the risk of cognitive dysfunction in the elderly.[29]

There have been indications PS also can improve mood and anxiety, but the trials conducted to date have been very small and poorly designed.[30–32] Larger and better designed clinical trials are needed to clarify the mood benefits of PS.

Stress

There is better evidence that PS can improve coping with stress, even in young, healthy men. British male undergraduate students were randomized to receive PS (300 mg/day) or a placebo for 30 days, then subjected to a challenging timed mental arithmetic test.[33] The PS group reported feeling less stressed after the test, compared with the placebo group. On visual analog mood scales, the PS group also surpassed placebo for feeling clear-headed, composed, and confident. Among subgroups with higher neuroticism scores, the PS subgroup reported significantly better mood after taking the test and were less stressed.

Regarding stress imposed by physical challenge, a low dose of PS (200 mg/day for 42 days) was found to significantly improve golfers' driving accuracy compared with placebo.[34] High daily doses of PS (750–800 mg/day) may also improve physical performance in cycling, running, and weight training.[35] Physical challenge can raise blood cortisol, and in two small trials with healthy men subjected to cycling stress, PS may have blunted this cortisol response.[6,36] High doses were needed: 600 mg/day[6] or 800 mg/day[36] for 10 days. However, both these trials had a crossover design that may not be optimal for PS. Because PS is a cell membrane nutrient, it may require a longer "washout" time to depart the membranes than the 10 or 14 days these trials allowed. Further, well-designed trials are needed to refine the utility of PS for countering mental or physical stress.

Other clinical trials suggest PS may work better against stress when combined with phosphatidic acid (PA), a phospholipid known to have cell membrane and cell-to-cell messenger functions in humans. In a randomized, double-blind trial, three groups of healthy men received two different doses of PS+PA (called PAS, in a ratio of 1:1 PS:PA by weight), or a placebo, for 42 days.[37] Then they were subjected to acute stress from the Trier Social Stress Test: make an unrehearsed job application speech THEN perform a complex mental arithmetic task. This increased their salivary and serum cortisol and adrenocorticotropic hormone (ACTH) levels. The high PAS dose (400 mg PS + 400 mg PA) maintained their ACTH level (but not cortisol) significantly lower, versus either the low PAS (200 mg PS + 200 mg PA) or the placebo. However, a subgroup with higher stress response to the Trier Test did also display both cortisol and ACTH blunting on the high PAS dose.

Evolution of PS Sourcing

PS as a dietary ingredient has evolved. PS used in early clinical trials came from cow brain as bovine cortex PS (BC-PS), produced by a laborious and costly process. By the 1990s the advent of prion-related "mad cow disease" made this source no longer guaranteed safe. Soy became the new source. Skeptics suggested this plant-source PS would not work because its tail fatty acid profiles differed from BC-PS. BC-PS had a small content of omega-3 docosahexaenoic acid (DHA) in its tails (well below 10%), whereas soy has none.[38] However, soy PS preparations have since produced clinical benefits in at least 16 double-blind trials.[38] Sunflower PS was also developed to avoid the perceived allergenicity of soy PS.

Advances with enzyme technology have resulted in commercial PS preparations with omega-3 DHA and eicosapentaenoic acid (EPA) contained in the molecules' tail pieces. In a 2008 double-blind trial, a group of children with attention deficit hyperactivity disorder (ADHD) showed improved visual sustained attention after 3 months on a "PL-Omega3" that provided 300 mg PS and 168 mg other phospholipids that altogether carried 156 mg EPA and 95 mg DHA.[39] This group's improvement surpassed the other groups that received fish oil (triglyceride) EPA+DHA or placebo. Subsequently a larger ADHD trial was conducted with a "PS-Omega3" that provided 300 mg PS carrying 80 mg EPA and 40 mg DHA per day for 15 weeks. Then all the children were continued on half that dose for another 15 weeks.[40] Restlessness and impulsivity improved significantly more in the PS-Omega3 group, as did a measure termed "Parent Impact-emotional." During the extension phase, those children switched from placebo to PS improved significantly on oppositional and hyperactivity scores.

Another omega-3, PS-DHA, was tried on nondemented elderly subjects with memory complaints.[41] After 15 weeks, this preparation (300 mg PS, carrying about 59 mg DHA and about 20 mg EPA per day) had improved verbal immediate recall superior to a placebo. The trial was then extended for another 15 weeks,[42] during which all the subjects received one third of the previous PS-DHA dose (100 mg PS, about 20 mg DHA, about 6 mg EPA) each day. Those subjects who were continuers on the PS-DHA maintained their improvement, whereas those who switched to PS-DHA from placebo attained significant improvement in attention and memory.

These technologically advanced omega-3 PS preparations all proved safe and well tolerated.[39–42] But their positive trial outcomes are tempered by findings that soy PS seemingly can perform just as well without omega-3 fatty acids attached. Thus in a double-blind trial with elderly subjects, soy PS at either 100 or 300 mg/day taken for 6 months significantly improved delayed word recall compared with placebo.[43] Also, in a double-blind trial with ADHD children, soy PS at 200 mg/day for 2 months displayed significant improvements in their overall ADHD symptoms, attention, and auditory memory, versus a placebo group.[44] The researchers commented that the children who received soy PS were noticeably better behaved at school and more tidy at home. Animal research had previously indicated that the head piece of the PS molecule is the key to its cell biology and clinical benefits.[2]

DOSAGE

The standard dosage recommendation for PS is 100 mg taken with meals one to three times daily. For optimal memory results, daily doses of 300 mg are more predictably beneficial.

TOXICOLOGY AND DRUG INTERACTIONS

After more than 3 decades' use as a dietary supplement, PS has shown no evidence of toxic effects or adverse drug interactions at dosages up to 800 mg/day.[2,5,21,39–44] The superb benefit-risk profile of this cell membrane nutrient makes it a premier option for anyone desiring to rescue, upgrade, or optimize their memory and overall brain wellness.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Pauling L. Orthomolecular psychiatry. Varying the concentrations of substances normally present in the human body may control mental disease. *Science*. 1968;160:265–271.
2. Kidd PM. Phosphatidylserine, membrane nutrient for memory. A clinical and mechanistic assessment. *Alternative Med Rev*. 1996;1:70–84.
3. Crook TH, Tinklenberg J, Yesavage J, et al. Effects of phosphatidylserine in age-associated memory impairment. *Neurology*. 1991;41:644–649.
4. Crook T, Petrie W, Wells C. Effects of phosphatidylserine in Alzheimer's disease. *Psychopharmacol Bull*. 1992;28:61–66.
5. Cenacchi T, Bertoldin T, Farina C, et al. Cognitive decline in the elderly: a double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. *Aging (Milano)*. 1993;5:123–133.
6. Starks MA, Starks SL, Kingsley M, et al. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. *J Intl Soc Sports Nutr*. 2008;5:11–17.
7. Alberts B, Johnson A, Lewis J, et al. *Molecular Biology of the Cell*. 6th ed. New York: Garland Science; 2015.
8. Vance JE. Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids. *J Lipid Res*. 2008;49:1377–1387.
9. Manno S, Takakuwa Y, Mohandas N. Identification of a functional role for lipid asymmetry in biological membranes. *Proc Natl Acad Sci U S A*. 2002;99:1943–1948.
10. Salem N, Niebylski CD. The nervous system has an absolute molecular species requirement for proper function. *Mol Membr Biol*. 1995;12: 131–134.
11. Samson JC. The biological basis of phosphatidylserine pharmacology. *Clin Trials J*. 1987;24:1–8.
12. Jastrzebska B, Goc A, Golczak M, Palczewski K. Phospholipids are needed for proper formation, stability and function of the photoactivated rhodopsin-transducin complex. *Biochemistry*. 2009;48:5159–5170.
13. Lai Y, Shin YK. The importance of an asymmetric distribution of acidic lipids for synaptotagmin 1 function as a Ca2+ sensor. *Biochem J*. 2012;443:223–229.
14. Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harb Perspect Biol*. 2013;5(19):a008748.
15. Masturzo P, Murialdo G, de Palma D, et al. TSH circadian secretions in aged men and effect of phosphatidylserine treatment. *Chronobiologia*. 1990;17:267–274.
16. Nunzi MG, Milan F, Guidolin D, et al. Therapeutic properties of phosphatidylserine in the aging brain. In: (eds. Hanin I, Pepeu G.)*Phospholipids: biochemical, pharmaceutical, and analytical considerations*. New York: Plenum Press.
17. Nunzi MG, Milan F, Guidolin D. Dendritic spine loss in hippocampus of aged rats. Effect of brain phosphatidylserine administration. *Neurobiol Aging*. 1987;8:501–510.
18. Nunzi MG, Guidolin D, Petrelli L, et al. Behavioral and morpho-functional correlates of brain aging: a preclinical study with phosphatidylserine. *Adv Exp Med Biol*. 1992;318:393–398.
19. Klinkhammer P, Szelies B, Heiss WD. Effect of phosphatidylserine on cerebral glucose metabolism in Alzheimer's disease. *Dementia*. 1990;1:197–201.
20. Delwaide PJ, Gyselynck-Mambourg AM, Hurlet A. Double-blind randomized controlled study of phosphatidylserine in demented patients. *Acta Neurol Scand*. 1986;73:136–140.
21. Amaducci L. Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. *Psychopharmacol Bull*. 1988;24:130–134.
22. Fuenfgeld EW. Double-blind study with phosphatidylserine (PS) in parkinsonian patients with senile dementia of Alzheimer's type (SDAT). *Prog Clin Biol Res*. 1989;317:1235–1246.
23. Hershkowitz M, Fisher M, Bobrov D, et al. Long-term treatment of dementia Alzheimer type with phosphatidylserine: effect on cognitive functioning and performance in daily life. In:(eds. Bazan NG, Horrocks LA, Toffano G.) *Phospholipids in the Nervous System: Biochemical and Molecular Pathology*. Padova: Liviana Press; 279–288.
24. Engel RR, Satzger W, Gunther W, et al. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. *Eur Neuropsychopharmacol*. 1992;2:149–155.
25. Palmieri G, Palmieri R, Inzoli MR, et al. Double-blind controlled trial of phosphatidylserine in patients with senile mental deterioration. *Clin Trials J*. 1987;24:73–83.
26. Nerozzi D, Aceti F, Melia E, et al. Phosphatidylserine and memory disorders in the aged. *Clin Ter*. 1987;120:399–404.
27. Ransmayr G, Plörer S, Gerstenbrand F. Double-blind placebo-controlled trial of phosphatidylserine in elderly patients with arteriosclerotic encephalopathy. *Clin Trials J*. 1987;24:62–72.
28. Villardita C, Grioli S, Salmeri G, et al. Multicentre clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. *Clin Trials J*. 1987;24:84–93.
29. U.S. Food and Drug Administration/Center Food Safety & Applied Nutrition. *Phosphatidylserine and Cognitive Dysfunction and Dementia (Qualified Health Claim: Final Decision Letter)*; 2003.
30. Maggioni M, Picotti GB, Bondiolotti GP, et al. Effects of phosphatidylserine therapy in geriatric patients with depressive disorders. *Acta Psychiatr Scand*. 1990;81:265–270.
31. Brambilla F, Maggioni M, Panerai AE, et al. Beta-endorphin concentration in peripheral blood mononuclear cells of elderly depressed patients-effects of phosphatidylserine therapy. *Neuropsychobiol*. 1996;34:18–21.
32. Gindin J, Novikov M, Kedar D, et al. The effect of plant phosphatidylserine on age-associated memory impairment and mood in the functioning elderly. *Geriatric Institute for Education and Research and Department of Geriatrics, Rehovot, Israel.* Internal Report of Lipogen Ltd: Kaplan Hospital.
33. Benton D, Donohue RT, Sillance B. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. *Nutr Neurosci*. 2001;4:169–178.
34. Jaeger R, Purpura M, Geiss K-R, et al. The effect of phosphatidylserine on golf performance. *J Intl Soc Sports Nutr*. 2007;4:23–28.
35. Jaeger R, Purpura M, Kingsley M. Phospholipids and sports performance. *J Intl Soc Sports Nutr*. 2007;4:5–13.
36. Monteleone P, Maj M, Beinat L, et al. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. *Eur J Clin Pharmacol*. 1992;41:385–388.
37. Hellhammer J, Vogt D, Franz N, et al. A soy-based phosphatidylserine/phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamic-pituitary-adrenal axis in chronically stressed male subjects. *Lipids Hlth Dis*. 2014;13:121 (11 pages).
38. Kidd PM. *PS Double-Blind Clinical Trials Compilation*; 2019. Available upon request to drpkidd@gmail.com.
39. Vaisman N, Kaysar N, Zaruk-Adasha Y, et al. Correlation between changes in blood fatty acid composition and visual sustained attention performance in children with inattention: effect of dietary n-3 fatty acids containing phospholipids. *Am J Clin Nutr*. 2008;87:1170–1180.
40. Manor I, Magen A, Keidar D, et al. The effect of phosphatidylserine containing Omega3 fatty-acids on attention-deficit hyperactivity disorder symptoms in children: a double-blind placebo-controlled trial, followed by an open-label extension. *Eur Psychiatr*. 2012;27:335–342.
41. Vakhapova V, Cohen T, Richter Y, et al. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: a double-blind placebo-controlled trial. *Dement Geriatr Cogn Disord*. 2010;29:467–474.
42. Vakhapova V, Cohen T, Richter Y, et al. Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: results from an open-label extension study. *Dement Geriatr Cogn Disord*. 2014;38:39–45.
43. Kato-Katoaka A, Sakao M, Ebina R, et al. Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *J Clin Biochem Nutr*. 2010;47:246–255.
44. Hirayama S, Terasawa K, Rabeler R, et al. The effect of phosphatidylserine administration on memory and symptoms of attention-deficit hyperactivity disorder: a randomised, double-blind, placebo-controlled clinical trial. *J Hum Nutr Diet*. 2014;27(suppl 2):284–291.

Piper methysticum (Kava)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Piper methysticum (family: Piperaceae)
Common name: kava

GENERAL DESCRIPTION

Kava is a hardy, slow-growing perennial that generally resembles other members of the pepper family. It is an attractive shrub and can attain heights of more than 9 ft. The plant does not have many leaves, but those it does have are thin, single, heart-shaped, alternate, petiolate, 4 to 10 in. long, and sometimes wider than they are long. Although *Piper methysticum* does flower, it is incapable of self-reproduction; its propagation is vegetative and now caused solely by human effort.[1,2]

For medicinal purposes, it is the rootstock that is used. The rootstock is knotty, thick, and sometimes tuberous with holes or cracks created by partial destruction of the parenchyma. In other words, the rootstock is often somewhat pithy (Fig. 103.1). From the main rootstock, there are extensions of lateral roots up to 9 ft long.[1,2]

CHEMICAL COMPOSITION

Analysis of the composition of the dried kava rootstock indicates that it contains approximately 43% starch, 12% water, 3.2% simple sugars, 3.6% proteins, 3.2% minerals (primarily potassium), and 15% kavalactones (Table 103.1).[1,2]

Based on detailed analysis of the active ingredients of kava, a laborious process over the past 120 years, many experts now believe that the pharmacological activities of kava are caused mostly, if not entirely, by the presence of compounds known as kavalactones (also referred to as kava α-pyrones). Although the kavalactones are the primary active components, other components appear to contribute to the sedative and anxiolytic activities of kava, as one study found the sedative activity of a crude preparation to be more effective than the isolated kavalactones (Fig. 103.2).[3] The kavalactone content of the root can vary between 3% and 20%.

HISTORY AND FOLK USE

Oceania, the Pacific island communities of Micronesia, Melanesia, and Polynesia, is one of the few geographic areas of the world that did not have alcoholic beverages before European contact in the 18th century. However, these islanders did possess a "magical" drink used in ceremonies and celebrations because of its calming effect and ability to promote sociability. The drink, kava, is still used today in this region of the world, where the people are often referred to as the happiest and friendliest in the world.

The origins of kava use are not known, as it predates written history in Oceania.[1,2] It was first described for the Western world by Captain James Cook in the account of his voyage to the South Seas in 1768.

Many myths and legends surround the early use of kava. The plant itself probably originated in the New Guinea/Indonesia area and was spread along with other plants from island to island by early Polynesian explorers in canoes. Each culture has its own story on the origins of kava. For example, in Samoa, a story is told about the origins of kava and sugar cane. The story involves a Samoan girl who went to Fiji, where she married a great chief. After some time, she returned to Samoa, but before doing so, she noticed two plants growing on a hill. She saw a rat chewing on one of the plants and noticed that the rat seemed to go to sleep. She concluded that the plant was a comforting food. She decided she would take this plant, sugar cane, back to Samoa, but then she noticed that the rat awoke and began to chew the root of another plant, kava. The animal that had been weak and shy became bold, strong, and more energetic. She decided that she would take both plants back with her to plant in Samoa. The plants grew very well in Samoa, and soon a chief from a neighboring island exchanged two laying hens for roots of the two plants. Hence, the Samoans take credit for the spread of both sugar cane and kava.

In Tonga, a legend is told about a great chief named Loau who lived on the island of Euaiki. He went to visit his servant Feva'anga, who wanted to give a feast in honor of the chief, but it was a time of

Fig. 103.1 Dried root of *Piper methysticum*. (From HeikeRau/iStock.com.)

TABLE 103.1 Kavalactones

Compound	R	R′	R″	R‴	C5–6	C7–8
Kavain					—	==
7,8-Dihydrokavain					—	—
5,6-Dihydrokavain					—	==
Yangonin	OCH_3				==	==
5,6,7,8-Tetrahydroyangonin	OCH_3				—	—
Methysticin	O-CH_2-O				—	==
Dihydromethysticin	O-CH_2-O				—	==
5,6-Dihydromethysticin	O-CH_2-O				==	==
5,6-Dihydroyangonin	OCH_3				—	==
7,8-Dihydroyangonin	OCH_3				—	==
10-Methoxy-yangonin	OCH_3		OCH_3		==	==
11-Methoxy-yangonin	OCH_3	OCH_3			==	==
11-Hydroxy-yangonin	OCH_3	HO			==	==
Hydroxykavain				OH	—	==
11-Methoxy-12-hydroxy dehydrokavain	OH	OCH_3			==	==

Single dashes represent a single bond, and double dashes represent a double bond.

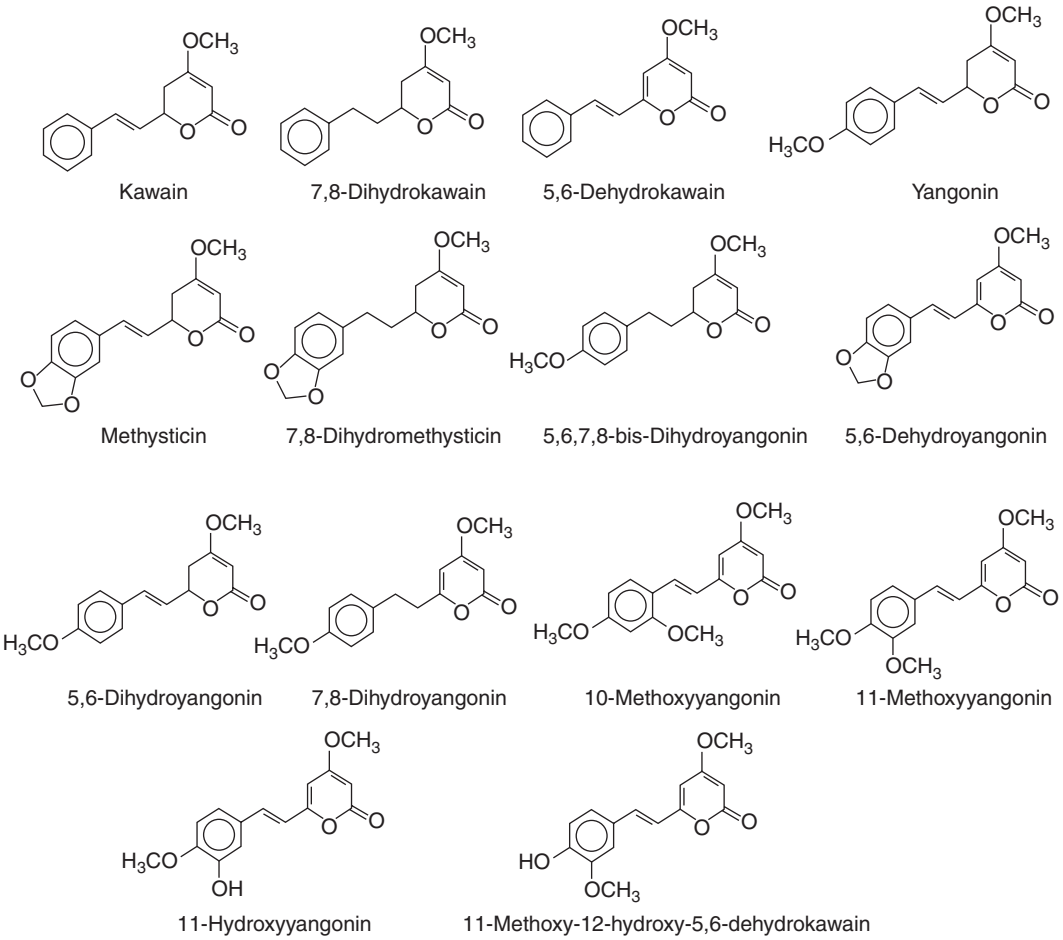


Fig. 103.2 Kavalactones.

great famine. In desperation, he and his wife killed and cooked their only daughter to be served to the chief. However, Loau recognized the human flesh in the food when it was served and would not eat it. He instructed Feva'anga to plant the food in the ground and to bring him the plant that would spring forth. On receiving the mature plant, Loau instructed that a drink be prepared from it and consumed with due ceremony.

The Kava Ceremony

Regardless of exactly how kava originated, it has been used in ceremonies by the Oceanic people for thousands of years. There are three basic kava ceremonies: the full ceremonial enacted on every formal occasion; the one performed at the meeting of village elders, chiefs, and nobles and for visiting chiefs and dignitaries; and the less formal kava circle common on social occasions.[1,2]

The first step of any kava ceremony was the preparation of the beverage. The following description of the classic process was written in 1777 by Georg Forster, a young naturalist on James Cook's second Pacific voyage:

> *[Kava] is made in the most disgustful manner that can be imagined, from the juice contained in the roots of a species of pepper-tree. This root is cut small, and the pieces chewed by several people, who spit the macerated mass into a bowl, where some water (milk) of coconuts is poured upon it. They then strain it through a quantity of fibres of coconuts, squeezing the chips, till all their juices mix with the coconut-milk; and the whole liquor is decanted into another bowl. They swallow this nauseous stuff as fast as possible; and some old topers value themselves on being able to empty a great number of bowls.*

As this traditional method of preparation became frowned upon or made illegal by colonial governments and missionaries, more sanitary methods of preparation, involving grinding or grating, took its place in many parts of Oceania.

The full kava ceremony, reserved for very highly honored guests, involves leading all the guests to a platform. The ceremony begins with the arrival of a group of young men dressed in ceremonial attire and carrying a bowl of the kava drink and necessary utensils. The bowl is placed between the kava preparers and the visitors. The kava is poured in a cup by a specially selected individual who then turns and faces the visitor and delivers the beverage to the chief guest. The guest is instructed to hold the cup with both hands and drink from it. If the whole cup is drained without stopping, everyone says "a maca" (pronounced "a matha," meaning "it is empty") and claps three times with cupped hands. The cupbearer then returns to the kava bowl and proceeds to serve the person next in rank or importance.

Important people who visit Fiji and other islands of Oceania still participate in the kava ceremonies. For example, during a 1992 presidential campaign visit to Hawaii, Hillary Clinton participated in a kava ceremony conducted by the Samoan community on O'ahu.

The Effects of Drinking Kava

Kava drinkers relate a pleasant sense of tranquility and sociability upon consumption. Subjective reports given by scientists who have sampled kava themselves are relatively abundant. One of the first scientific studies of kava was performed by the noted pharmacologist Louis Lewin in 1886. A later description written in 1927 is as follows[1]:

> *When the mixture is not too strong, the subject attains a state of happy unconcern, well-being and contentment, free of physical or psychological excitement. At the beginning, conversation comes in a gentle, easy flow and hearing and sight are honed, becoming able to perceive subtle shades of sound and vision. Kava soothes temperaments. The drinker never becomes angry, unpleasant, quarrelsome or noisy, as happens with alcohol. Both natives and whites consider kava as a means of easing moral discomfort. The drinker remains master of his consciousness and his reason. When consumption is excessive, however, the limbs become tired, the muscles seem no longer to respond to the orders and control of the mind, walking becomes slow and unsteady and the drinker looks partially inebriated. He feels the need to lie down. He is overcome by somnolence and finally drifts off to sleep.*

A later description was provided by researcher R.J. Gregory, who wrote the following from his own experience[1]:

> *Kava seizes one's mind. This is not a literal seizure, but something does change in the processes by which information enters, is retrieved, or leads to actions as a result. Thinking is certainly affected by the kava experience, but not in the same ways as are found from caffeine, nicotine, alcohol, or marijuana. I would personally characterize the changes I experienced as going from lineal processing of information to a greater sense of "being" and contentment with being. Memory seemed to be enhanced, whereas restriction of data inputs was strongly desired, especially with regard to disturbances of light, movements, noise and so on. Peace and quiet were very important to maintain the inner sense of serenity. My senses seemed to be unusually sharpened, so that even whispers seemed to be loud while loud noises were extremely unpleasant.*

Drinking about half a coconut shell (100–150 mL) of certain varieties of kava is enough to put most people into a deep, dreamless sleep within 30 minutes. Unlike alcohol and other sedatives, kava does not produce a morning hangover. The kava drinker awakens having fully recovered normal physical and mental capacities.

PHARMACOLOGY

Many of the first comprehensive studies on the activities of kavalactones were conducted by a team of scientists from the Freiburg University Institute of Pharmacology in Germany, led by Hans J. Meyer, during the 1950s and 1960s.[3] This research determined that kavalactones exhibit sedative, analgesic, anticonvulsant, and muscle-relaxing effects in laboratory animals. These studies seemed to confirm earlier empirical and subjective observations. Later studies used better-defined kava extracts.

Isolated Kavalactones Compared With Crude Extracts

Some evidence suggests that the whole complex of kavalactones and other compounds naturally found in kava produce greater pharmacological activity. In addition, studies showed that kavalactones are more rapidly absorbed when given orally as an extract of the root rather than as the isolated kavalactones. The bioavailability of lactones, as measured by peak plasma concentrations, is three to five times higher from the extract than when given as isolated substances.[3] Further evidence that kava root extracts are superior to isolated kavalactones was offered by an animal study demonstrating that although isolated kavalactones were well absorbed by the brain, crude kava preparations produced brain concentrations of lactones 2 to 20 times higher.[4] This evidence suggests that crude extracts standardized for kavalactone content may offer the greatest therapeutic benefit.

Several clinical trials featured a kava extract standardized to contain 70% kavalactones. However, this high percentage of kavalactones might be sacrificing some of the other constituents that may contribute

to the pharmacology of kava. More important than the actual percentage of kavalactones is the total dosage of the kavalactones and the assurance that the full range of kavalactones and other important constituents are present.

Sedative Effects

Numerous studies confirmed and/or elaborated on the sedative effects of kava. Most notable were studies that demonstrating that kavalactones exert many of their effects through nontraditional mechanisms. For example, most sedative drugs, including the benzodiazepines (e.g., diazepam, triazolam, clorazepate dipotassium), work by binding to specific receptors (benzodiazepine or γ-aminobutyric acid [GABA] receptors) in the brain, leading to the neurochemical changes (potentiation of GABA effects) that promote sedation. Studies in animals showed that the kavalactones do not bind to benzodiazepine or GABA receptors.[5] Instead, the kavalactones are thought to somehow modify receptor domains rather than interact specifically with receptor-binding sites. In addition, other studies indicated that the kavalactones appear to act primarily on the limbic system, the ancient part of the brain that affects all other brain activities and is the principal seat of the emotions.[6] It is thought that kava may also promote sleep by altering the way in which the limbic system modulates emotional processes. It appears that many of the laboratory models of identifying how a substance works to promote a calming effect are simply not sophisticated enough to evaluate the physiological effects of kava.

Analgesic Effects

In another example of the unusual pharmacological qualities of kava, a study designed to evaluate its pain-relieving effects could not demonstrate any binding to opiate receptors.[7] The significance of this finding was that the study used experimental models in which nonopiate analgesics like aspirin and other nonsteroidal anti-inflammatory drugs were ineffective. In addition, it was determined that the sedative or muscle-relaxing effects were not responsible for the pain-relieving effects. These findings indicate that kava reduces pain in a manner unlike morphine, aspirin, or any other pain reliever.

Anxiolytic Effects

An interesting difference of kava from other anxiolytics is that kava does not lose effectiveness with time. Kavalactones, even when administered in large doses, demonstrated no loss of effectiveness in animal studies.[8] This is another example of the unusual qualities of kava. Despite the long history of kava consumption and the wealth of clinical evidence in favor of the efficacy of kavalactones in treating anxiety, there is a severe gap in our understanding of the molecular target(s) and the mechanism(s) of action of these psychoactive compounds.

Anti-Ischemia Effects

Another important pharmacological activity of kava is its ability to protect against brain damage resulting from ischemia.[9] This effect was demonstrated in two animal models of focal cerebral ischemia. The effectiveness of the kavalactones was a result of their ability to limit the infarct area and to provide a mild anticonvulsant effect. Kava extract may prove useful in the recovery from a stroke.

Anti-Inflammatory Effects

In animal studies, the compounds extracted from *Piper methysticum* show promise in the prevention and treatment of inflammatory conditions. Kava-241, an optimized kavain derived compound, significantly reduced epithelial downgrowth and alveolar bone loss in a model of periodontitis.[10] Kava-241 treatment also significantly decreased tumor necrosis factor-α in serum from mice, emphasizing the novel properties of Kava-241 in the management of tumor necrosis factor-α–related diseases such as infective rheumatoid arthritis.[11]

CLINICAL APPLICATIONS

The primary clinical application of kava is in the treatment of anxiety. This application is well supported by clinical research.[12] However, the medicinal use of kava is shrouded in uncertainty as a result of reports of hepatoxicity (discussed in the "Toxicology" section).

Anxiety

Early clinical trials used D,L-kavain, a purified kavalactone, at a dosage of 400 mg/day. For example, in one double-blind, placebo-controlled study of 84 patients with anxiety symptoms, kavain was shown to improve vigilance, memory, and reaction time.[13] In another double-blind study, kavain was compared with oxazepam (a drug similar to diazepam [Valium]) in 38 patients.[14] Both substances caused progressive improvements in two different anxiety scores (Anxiety Status Inventory and the Self-rating Anxiety Scale) over a 4-week period. However, whereas oxazepam and similar drugs are addictive and cause side effects, kavain appeared to be free of these complications.

In one of the early studies with kava extracts, a 70% kavalactone extract was shown to exhibit significant therapeutic benefit in patients with anxiety.[15] The study was double-blind, and 29 patients were assigned to receive 100 mg of the kava extract three times a day whereas 29 other patients received a placebo. Therapeutic effectiveness was assessed using several standard psychological assessments, including the Hamilton Anxiety Scale (HAMA). The results of this 4-week study indicated that individuals taking the kava extract had a statistically significant reduction in symptoms of anxiety, including feelings of nervousness, and somatic complaints, such as heart palpitations, chest pains, headache, dizziness, and feelings of gastric irritation. No side effects were reported with the kava extract.

Studies also compared the effects of a kava extract standardized to contain 30% kavalactones with buspirone and opipramol. In the double-blind study, 129 patients with generalized anxiety disorder (GAD) were given either 400 mg kava (30% kavalactones), 10 mg buspirone, or 100 mg opipramol daily for 8 weeks.[16] Detailed analysis showed that no significant differences were observed regarding all efficacy and safety measures. About 75% of patients were classified as responders (50% reduction of the HAMA score) in each treatment group, and about 60% achieved full remission.

Kava was also shown to be particularly effective in relieving anxiety in perimenopausal and postmenopausal women.[17–19] In one double-blind study, two groups of 20 women with menopause-related symptoms were treated for 8 weeks with the 70% kavalactone extract (100 mg three times daily) or placebo.[20] The measured variable was once again the HAMA. The group receiving the kava extract demonstrated significant improvement at the end of the very first week of treatment. Scores continued to improve over the course of the 8-week study. In addition to symptoms of stress and anxiety, a number of other symptoms also improved. Most notably there was an overall improvement in subjective well-being, mood, and general symptoms of menopause, including hot flashes. As with previous studies, no side effects were noted.

Additional studies showed that unlike benzodiazepines, alcohol, and other drugs, kava extract was not associated with depressed mental function or impairment in driving or the operation of heavy equipment.[21,22] In one of these studies, 12 healthy volunteers were tested

in a double-blind crossover manner to assess the effects of oxazepam (placebo on days 1–3, 15 mg on the day before testing, 75 mg on the morning of the experiment), the extract of kava standardized at 70% kavalactones (200 mg three times daily for 5 days), and a placebo on behavior and event-related potentials (ERP) in electroencephalographic readings on a recognition memory task. The subjects' task was to identify within a list of visually presented words those that were shown for the first time and those that were being repeated. Consistent with other benzodiazepines, oxazepam inhibited the recognition of both new and old words as noted by ERP. In contrast, kava allowed a slightly greater recognition rate and a larger ERP difference between old and new words. The results of this study once again demonstrated the unusual effects of kava. In this case it improved anxiety, but unlike standard anxiolytics, kava actually improved mental function and, at the recommended levels, did not promote sedation. Another study indicated that kava helped improve reflex vagal control of heart rate in GAD patients.[23]

In 2009 the first documented human clinical trial assessing the anxiolytic and antidepressant efficacy of an aqueous extract of kava was published.[24] The World Health Organization suggested the investigation of aqueous kava extracts as a way to reduce the risk of hepatotoxicity with kava preparations. The Kava Anxiety Depression Spectrum Study was a 3-week, placebo-controlled, double-blind crossover trial that recruited 60 adult participants with 1 month or more of elevated generalized anxiety. The kava preparation produced significant anxiolytic and antidepressant activity and raised no hepatoxicity or safety concerns at the dose and duration studied. Specifically, kava reduced participants' HAMA scores in the first controlled phase by −9.9 versus −0.8 for placebo and in the second controlled phase by −10.3 versus +3.3. Pooled analyses also revealed highly significant relative reductions in anxiety and depression scale scores. In a subsequent 6-week, parallel, double-blind, randomized, controlled trial involving 75 participants with GAD (58 randomized to 120 mg daily kavalactones titrated to 240 mg for nonresponse), results revealed a significant reduction in HAMA scores in favor of kava over placebo.[25] For participants with moderate-to-severe level of anxiety, the treatment effect was more pronounced. As part of this trial, five GABA transporter polymorphisms were examined as potential pharmacogenetic markers of kava response. Within the kava group, GABA transporter polymorphisms rs2601126 and rs2697153 were associated with significant reductions in HAMA scores, suggesting specific GABA transporter polymorphisms may potentially modify anxiolytic response to kava.

DOSAGE

In clinical studies using pure kavalactones or kava extracts standardized for kavalactones, the dosage was based on the level of kavalactones. Because the kavalactone content of the root varies between 3% and 20%, preparations standardized for kavalactone content are preferred to crude preparations. A standard bowl of traditionally prepared kava drink contains approximately 250 mg of kavalactones, and in Oceania, several bowls may be consumed at one sitting. Dosages vary according to the desired therapeutic effect.

- Anxiolytic dosage: 45 to 80 mg of kavalactones three times a day
- Sedative dosage: 180 to 250 mg of kavalactones 1 hour before retiring

SIDE EFFECTS AND TOXICOLOGY

In November 2001 German health authorities announced that 24 cases of liver disease (including hepatitis, liver failure, and cirrhosis) associated with the use of kava had been reported. Of the affected persons, one died and three required a liver transplant. As a result, in December 2001, the US Food and Drug Administration began advising consumers of the potential risk of severe liver injury associated with the use of kava-containing dietary supplements. Kava was subsequently withdrawn from the market in the European Union, United Kingdom, and Canada. In 2007 Germany reevaluated the data, and the ban imposed on kava was overturned in 2014 after the decisions of two administrative German courts.[26] In the initial report, the true nature of kava-induced liver damage was clouded by the fact that in 18 of the cases, conventional prescription or over-the-counter pharmaceutical drugs with known or potential liver toxicity were also being used. Proponents of kava quickly argued that it was entirely possible that the use of kava by these individuals was a coincidence rather than the cause of the liver problems, resulting from excessive dosage or some other factor. As of 2007, of the approximately 100 cases of hepatotoxicity that were reported worldwide, causality deemed to be "probable" occurred in only 14 cases.[27] Two drug-monitoring studies, including a total of 7078 patients taking 120 to 150 mg kava extract per day, did not find a single case of kava-induced hepatotoxicity. A thorough review concluded that kava-induced hepatotoxicity would be extremely rare, highlighting the fact that even if all the cases of reported toxicity were causally linked to kava intake, as drug-induced hepatotoxicity frequency ranges from 1 to 10 per 100,000 exposed individuals, kava would be under 1 per 100,000.[28]

The existing data are complex, but it appears that the major factor in any kava-induced hepatotoxicity was the use of aerial parts, such as stems and leaves as well as stem peelings.[29] Once the demand for kava skyrocketed, there was not enough kava root to fill the demand. Suppliers then knowingly or unknowingly purchased the leaves and peelings of kava. The only parts of the kava plant that were traditionally used throughout its 3000-year history were the roots, never the peelings or the leaves. According to a World Health Organization report, German pharmaceutical industries preferred to buy kava stem peelings to extract kavalactones to make kava drugs; kava stem peelings were sold at almost one tenth of the price of kava roots.[30] In addition, dosage might also have been a factor in some of the cases of hepatotoxicity. A survey of 400 German medical practices showed that 78% of the kava prescriptions that were written before 2001 significantly exceeded the recommended intake.[31] Nonetheless, there were reports of hepatitis in patients using kava at dosages equal to or only slightly higher than recommended levels, indicating other factors beyond dosage.[32] Flavokawain B, a chalcone from kava root, was identified as a potent hepatocellular toxin.[33]

Measures suggested to address the hepatotoxicity issue include: (1) use of a noble kava cultivar such as Borogu, which is at least 5 years old at time of harvest; (2) use of peeled and dried rhizomes and roots; (3) dosage recommendation of less than or equal to 250 mg kavalactones per day (for medicinal use); and (4) a Pan Pacific quality control system enforced by strict policing.[29] Another important step may be determination of flavokawain B. It should be mentioned that although it has been suggested that traditional aqueous extracts should be used over ethanolic or acetonic extracts, the toxicity is linked to the kava plant itself, with a possibly low quality of the used kava cultivar or kava plant part rather than the method of extraction or solvent.[34]

High daily dosages of kava consumed over a prolonged period (a few months to a year) are associated with a number of side effects beyond liver damage, including the development of "kava dermopathy," a condition of the skin characterized by a peculiar generalized scaly eruption known as kani.[35] The skin becomes dry and covered with scales, especially the palms of the hands, soles of

the feet, forearms, the back, and shins. It was thought at one time that kava dermopathy might be caused by interference with niacin. However, in a double-blind, placebo-controlled study, niacinamide (100 mg/day) demonstrated no therapeutic effect.[36] It appears that the only effective treatment for kava dermopathy is reduction or cessation of kava consumption. No cases of kava dermopathy have been reported in persons taking standardized kava extracts at recommended levels.

Other reported adverse effects of extremely high doses of kava (e.g., more than 310 g/week) for prolonged periods are[37]:

- Biochemical abnormalities (low levels of serum albumin, protein, urea, and bilirubin)
- Elevated liver enzymes
- Presence of blood in the urine
- Increased red blood cell volume
- Decreased platelet and lymphocyte counts
- Shortness of breath

At this time, kava is not recommended for use by anyone who has any liver problems or who is a regular consumer of alcohol. Use of kava for more than 4 weeks requires close monitoring of liver enzymes (once every 4–6 weeks). Patients should be instructed to discontinue use of kava if symptoms of jaundice (e.g., dark urine, yellowing of the eyes) occur. Nonspecific symptoms of liver disease include nausea, vomiting, light-colored stools, unusual tiredness, weakness, stomach or abdominal pain, and loss of appetite.

Kava is not recommended for use by pregnant or breastfeeding women.

DRUG INTERACTIONS

Kava may potentiate the effects of benzodiazepines, barbiturates, and prescription sedatives. Kava also inhibits a number of the cytochrome P450 enzymes that play a role in the breakdown of many medications.[38] Therefore it has the potential to interact with a wide range of medications. There is evidence that kava interferes with dopamine or other drugs used in the treatment of Parkinson's disease; therefore until this issue is resolved, kava extract should not be used by patients with Parkinson's disease.[35]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lebot V, Merlin M, Lindstrom L, Kava: *The Pacific Drug*. New Haven, CT: Yale University Press.
2. Singh Y. Kava: an overview. *J Ethnopharmacol*. 1992;37:13–45.
3. Meyer HJ. Pharmacology of kava. In: (eds: Efron D. Holmstedt B. Kline NS.) *Ethnopharmacologic Search for Psychoactive Drugs*. New York: Raven Press; 133–140.
4. Keledjian J, Duffield PH, Jamieson DD. Uptake into mouse brain of four compounds present in the psychoactive beverage kava. *J Pharm Sci*. 1988;77:1003–1006.
5. Davies LP, Drew CA, Duffield P, et al. Kava pyrones and resin: studies on GABAA, GABAB and benzodiazepine binding sites in rodent brain. *Pharmacol Toxicol*. 1992;71:120–126.
6. Holm E, Staedt U, Heep J, et al. The action profile of d,l-kavain. Cerebral sites and sleep-wakefulness-rhythm in animals. *Arzneimittelforschung*. 1991;41:673–683. [German].
7. Jamieson DD, Duffield PH. The antinociceptive action of kava components in mice. *Clin Exp Pharmacol Physiol*. 1990;17:495–507.
8. Duffield PH, Jamieson D. Development of tolerance to kava in mice. *Clin Exp Pharmacol Physiol*. 1991;18:571–578.
9. Backhauss, Krieglstein J. Extract of kava (Piper methysticum) and its methysticum constituents protect brain tissue against ischemic damage in rodents. *Eur J Pharmacol*. 1992;215:265–269.
10. Alshammari A, Patel J, Al-Hashemi J, et al. Kava-241 reduced periodontal destruction in a collagen antibody primed Prophyromonas gingivalis model of periodontitis. *J Clin Periodontol*. 2017;44(11):1123–1132. PubMed PMID: 28746780.
11. Huck O, You J, Han X, Cai B, Panek J, Amar S. Reduction of articular and systemic inflammation by Kava-241 in Porphyromonas gingivalis-induced arthritis murine model. *Infection Immunity*. 2018. pii: IAI.00356-18 PubMed PMID: 29914930.
12. Pittler MH, Ernst E. Efficacy of kava extract for treating anxiety: systematic review and meta-analysis. *J Clin Psychopharmacol*. 2000;20:84–89.
13. Scholing WE, Clausen HD. On the effect of d,l-kavain: experience with neuronika (author's transl). *Med Klin*. 1977;72:1301–1306. [German].
14. Lindenberg D, Pitule-Schodel H d. l-kavain in comparison with oxazepam in anxiety disorders. A double-blind study of clinical effectiveness. *Forschr Med*. 1990;108:49–50. 53-54. [German].
15. Kinzler E, Kromer J, Lehmann E. Clinical efficacy of a kava extract in patients with anxiety syndrome. Double-blind placebo controlled study over 4 weeks. *Arzneimittelforschung*. 1991;41:584–588. [German].
16. Boerner RJ, Sommer H, Berger W, et al. Kava-Kava extract LI 150 is as effective as opipramol and buspirone in generalised anxiety disorder—an 8-week randomized, double-blind multi-centre clinical trial in 129 out-patients. *Phytomedicine*. 2003;10(suppl 4):38–49.
17. Cagnacci A, Arangino S, Renzi A, et al. Kava-Kava administration reduces anxiety in perimenopausal women. *Maturitas*. 2003:44 103–109.
18. De Leo V, La Marca A, Morgante G, et al. Evaluation of combining kava extract with hormone replacement therapy in the treatment of postmenopausal anxiety. *Maturitas*. 2001;39:185–188.
19. De Leo V, La Marca A, Lanzetta D, et al. Assessment of the association of Kava-Kava extract and hormone replacement therapy in the treatment of postmenopause anxiety. *Minerva Ginecol*. 2000;52:263–267. [Italian].
20. Warnecke G. Psychosomatic dysfunctions in the female climactericzClinical effectiveness and tolerance of kava extract WS 1490. *Fortschr Med*. 1991;109:119–122. [German].
21. Herberg KW. Effect of kava-special extract WS 1490 combined with ethyl alcohol on safety-relevant performance parameters. *Blutalkohol*. 1993;30:96–105. [German].
22. Munte TF, Heinze HJ, Matzke M, et al. Effects of oxazepam and an extract of kava roots (Piper methysticum) on event-related potentials in a word recognition task. *Neuropyschobiol*. 1993;27:46–53.
23. Watkins LL, Connor KM, Davidson JR. Effect of kava extract on vagal cardiac control in generalized anxiety disorder: preliminary findings. *J Psychopharmacol*. 2001;15:283–286.
24. Sarris J, Kavanagh DJ, Byrne G, et al. The Kava Anxiety Depression Spectrum Study (KADSS): a randomized, placebo-controlled crossover trial using an aqueous extract of Piper methysticum. *Psychopharmacol (Berl)*. 2009;205:399–407.
25. Sarris J, Stough C, Bousman CA, et al. Kava in the treatment of generalized anxiety disorder: a double-blind, randomized, placebo-controlled study. *J Clin Psychopharmacol*. 2013;33(5):643–648. PubMed PMID: 23635869.
26. Kuchta K, Schmidt M, Nahrstedt A. German kave ban lifted by court: the alleged hepatotoxicity of kave (Piper methysticum) as a case of ill-defined herbal drug identity, lacking quality control, and misguided regulatory politics. *Planta Medica*. 2015;81(18):1647–1653. PubMed PMID: 26695707.
27. Ernst E. A re-evaluation of kava (Piper methysticum). *Br J Clin Pharmacol*. 2007;64:415–417.
28. Pantano F, Tittarelli R, Mannocchi G, et al. Hepatotoxicity induced by "the 3Ks": kava, kratom and khat. *x*. 2016;17(4):580. PubMed PMID: 27092496.
29. Teschke R, Sarris J, Lebot V. Kava hepatotoxicity solution: a six-point plan for new kava standardization. *Phytomedicine*. 2011;18(2–3):96–103.
30. WHO (World Health Organization). *Assessments of the Risk of Hepatotoxicity with Kava Products*. Geneva, Switzerland: WHO Document Production Services.
31. Schroder-Bernhardi D, Dietlein G. Compliance with prescription recommendations by physicians in practices. *Int J Clin Pharmacol Ther*. 2001;39:477–479.
32. Escher M, Desmeules J, Giostra E, et al. Hepatitis associated with kava, a herbal remedy for anxiety. *BMJ*. 2001;322:139.
33. Zhou P, Gross S, Liu JH, et al. Flavokawain B, the hepatotoxic constituent from kava root, induces GSH-sensitive oxidative stress through modulation of IKK/NF-kappaB and MAPK signaling pathways. *FASEB J*. 2010;24:4722–4732.
34. Teschke R, Genthner A, Wolff A. Kava hepatotoxicity: comparison of aqueous, ethanolic, acetonic kava extracts and kava-herbs mixtures. *J Ethnopharmacol*. 2009;123:378–384.
35. Schelosky L, Raffauf C, Jendroska K, et al. Kava and dopamine antagonism (lett). *J Neurol Neurosurg Psychiatry*. 1995;58:639–640.
36. Norton SA, Ruze P, dermopathy K. *J Am Acad Dermatol*. 1994;31:89–97.
37. Ruze P. Kava-induced dermopathy. A niacin deficiency. *Lancet*. 1990;335:1442–1445.
38. Mathews JM, Etheridge AS, Black SR. Inhibition of human cytochrome P450 activities by kava extract and kavalactones. *Drug Metab Dispos*. 2002;30:1153–1157.

104

Prebiotics, Synbiotics, and Colonic Foods

Jason A. Hawrelak, ND, BNat(Hons), PhD

OUTLINE

INTRODUCTION

Two of the main tools commonly used to beneficially modify the gastrointestinal tract (GIT) microbiota are probiotics and prebiotics. The focus of this chapter is to review the beneficial actions and safety profile of the latter. A *prebiotic* is defined as "*a substrate that is selectively utilized by host microorganisms conferring a health benefit.*"[113] Prebiotics are typically prescribed to modify the GIT microbiota; however, they are also now being used to modify the vaginal ecosystem and, in the future, will undoubtedly be used to modify the microbiota of other body sites, such as the skin, nose, and oral cavity.

Gastrointestinal prebiotics must meet several criteria:

1. Neither be digested nor absorbed in the stomach or small intestine[1]
2. Act as a selective food source for one or a limited number of potentially beneficial native bacterial species in the intestines[1]
3. Change the microbiota ecosystem toward a healthier composition[1]
4. Induce luminal or systemic changes that improve the health of the host[2]

Most emphasis at this stage has been on finding and studying food sources that are used by lactic acid-producing bacteria. This is a result of the health-promoting properties of these organisms.[1] The best-known lactic acid-producing bacteria belong to the genera *Lactobacillus* and *Bifidobacterium.* However, more recently, there has also been an expansion of focus onto substrates that enhance the growth of other beneficial bacterial groups, such as *Faecalibacterium prausnitzii, Akkermansia muciniphila,* and butyrate-producing species, such as *Anaerostipes* spp., *Eubacterium* spp., and *Roseburia* spp.[113]

Not all food ingredients that make it to the colon undigested will be prebiotics. Only compounds that are selectively consumed by species generally considered to be beneficial members of the microbiota, while being unused by potentially pathogenic ones, can truly be termed prebiotics. When prebiotics reach the colon (or distal small bowel), they are preferentially used by those organisms that have the capacity to hydrolyze their bonds. The metabolism of the prebiotic results in the increased growth and activity of the beneficial organism(s), often at the expense of other components of the microbiota.[3] Fig. 104.1 illustrates the prebiotic concept using two types of FOS as examples. Food ingredients that make it to the colon undigested but lack the selectivity of fermentation are best termed "colonic foods" and are discussed in more depth at the end of this chapter.

There are numerous compounds with the potential to be termed gastrointestinal prebiotics (Fig. 104.2). Table 104.1 lists these compounds and the type of microorganisms whose growth they promote. However, the best-researched prebiotics are fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose.

FRUCTOOLIGOSACCHARIDES

Description

FOS are linear or branched chains of fructose and glucose molecules.[8] The number of fructose units contained in the compound determines the name of the FOS. Oligofructose is generally composed of between 2 and 7 units, whereas inulin is composed of up to 60.[9] FOS are found in varying percentages in foods and have been discovered in more than 36,000 plant species, where they function primarily as storage carbohydrates.[10] FOS are found in many common vegetables, including asparagus, onion, leek, garlic, artichoke, Jerusalem artichoke, and chicory

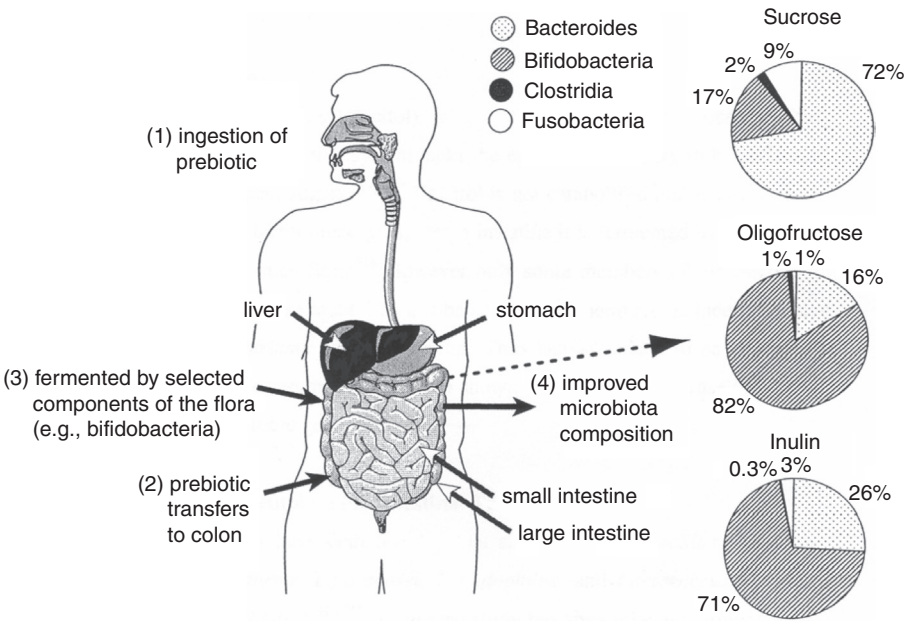


Fig. 104.1 The prebiotic concept: the effects of sucrose (placebo), oligofructose, and inulin on the intestinal ecosystem. Pie diagrams illustrate how the microbiota can develop during the feeding of sucrose and the prebiotics, oligofructose and inulin. (From Gibson GR. Dietary modulation of the human gut microflora using prebiotics. *Br J Nutr.* 1998;80[suppl 2]:S209-S212, used with permission.)

root. However, it is from the chicory root (*Cichorium intybus*) that most of the commercially produced inulin and oligofructose is manufactured. Short-chain fructans, such as oligofructose, are produced from inulin through a process of partial enzymatic hydrolysis.[8]

Commercial Forms

There are two ways in which FOS can be consumed, in supplements and in foods.

Fructooligosaccharide Supplements

The most commonly employed method to purify and concentrate FOS for supplement use is via a hot water extraction of fresh chicory roots. This process results in inulin (a large-chain FOS) as the end-product. Some manufacturers use enzymatic hydrolysis to produce oligofructose (a medium-chain FOS) from inulin,[9] although other manufacturers synthesize FOS from sucrose using the fungal enzyme fructosyltransferase (from *Aspergillus niger*). This latter process involves chemical synthesis of a new compound (called Neosugar or Actilight, a short-chain FOS) from two other natural compounds (fructose and glucose). The finished compound is similar to naturally obtained FOS, only smaller in size.[8]

FOS are resistant to digestion in the upper GIT because of the β-configuration of the bonds between the fructose units. Human digestive enzymes are specific in requiring α-linkages; thus, FOS are classified as nondigestible oligosaccharides.[8]

Food Sources of Fructooligosaccharides

As previously mentioned, FOS are common food ingredients. Individuals consuming the standard Western diet consume an average of 5.1 g/day of FOS.[11] However, this can easily be increased if foods rich in FOS are consumed daily. Foods containing FOS are outlined in Table 104.2.

There is no advantage to the consumption of FOS in supplements compared with FOS-rich foods. If the amount of FOS consumed in a food is similar to that used in clinical studies, it will promote similar effects, as all FOS consumed reach the colon intact, whether ingested in whole foods or in supplements.

Clinical Applications

The health benefits claimed for FOS mainly stem from their ability to increase numbers of beneficial organisms in the colon, to reduce numbers of potentially pathogenic microorganisms, and to stimulate short-chain fatty acid (SCFA) production. These health benefits include:

- Enhanced resistance to enteric pathogens due to colonization resistance provided by the increased growth of lactic acid bacteria[12]
- Increased resistance to infections due to the nonspecific stimulation of the immune system[12]
- Modification of carcinogen metabolism[12]
- Enhanced absorption of minerals[12]
- Improved serum lipid parameters[12]
- Improved satiety[13]
- Improved metabolic health[114]
- Treatment of atopic eczema and prevention of atopy development[14]
- Increased intestinal mucin production and trophic effects on the colonic epithelium, secondary to the increased production of SCFAs[15]

This review focuses primarily on the ability of FOS to improve the microbiota composition, enhance the immune response, improve mineral absorption, treat and prevent atopic diseases, improve satiety, and improve metabolic health.

Microbiota Composition

A number of trials have now verified the capacity of FOS to beneficially modify the microbiota-increasing levels of beneficial bacterial species while simultaneously decreasing levels of pathobionts. Table 104.3 contains an overview of some of these trials showing the effects observed at different dosage points.

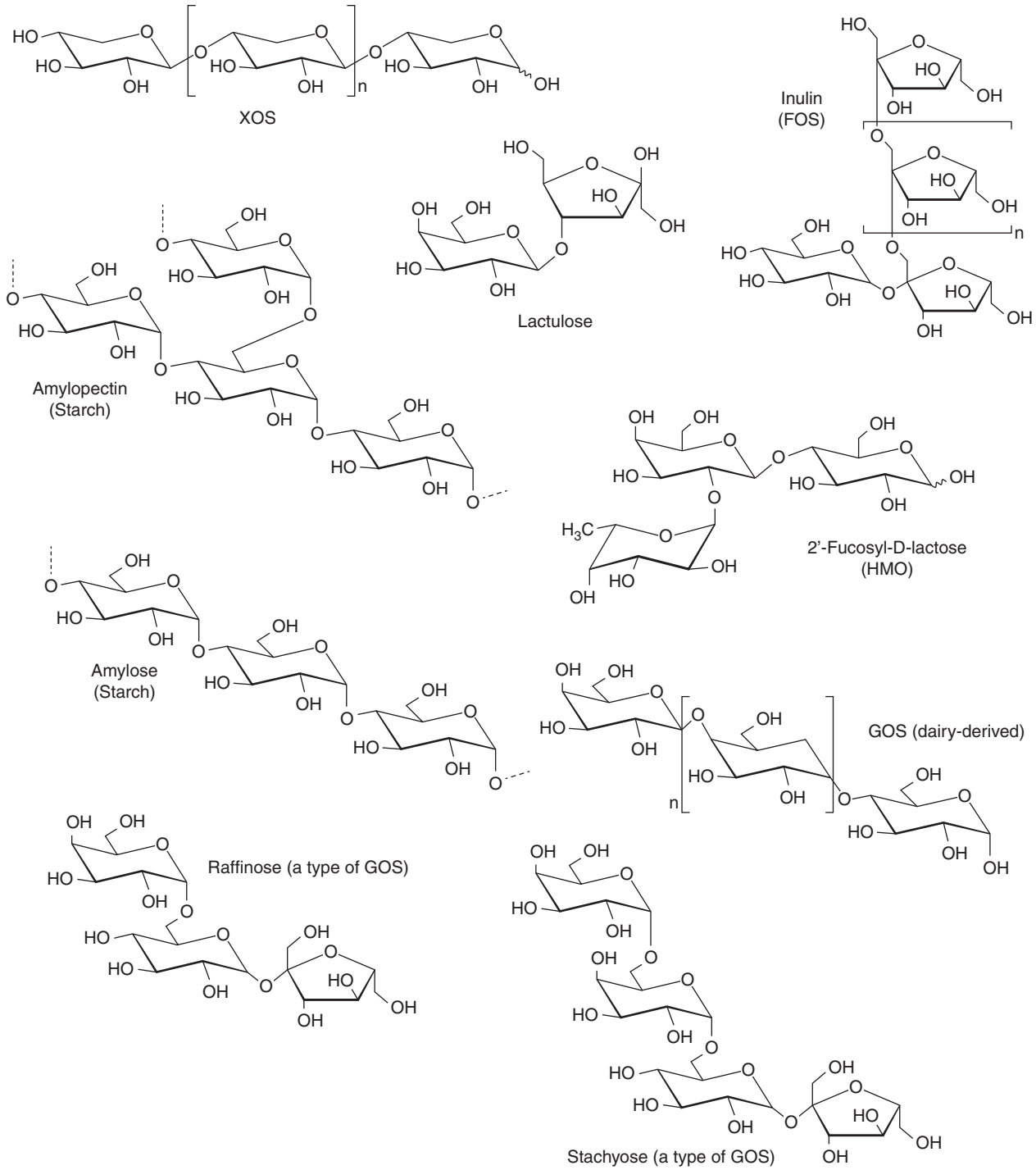


Fig. 104.2 Chemical structures of some of the main prebiotics and resistant starch. (From Healthy effects of prebiotics and their metabolites against intestinal diseases and colorectal cancer - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/Chemical-structures-of-the-different-prebiotics-families-FOS-fructo-oligosaccharides_fig1_292963145 [accessed Aug 27, 2018].)

In an attempt to determine the optimal dose of FOS, in terms of maximizing bifidobacteria numbers and minimizing side effects, Bouhnik et al.[16] designed a trial that used five different dosage levels. The dosages used were 20, 10, 5, and 2.5 g/day of oligofructose and 0 g/day as the placebo. The trial lasted 7 days. The data indicated that bifidobacteria counts did not change in subjects who received 0 or 2.5 g/day of oligofructose, but that concentrations in those subjects who ingested 5, 10, and 20 g/day were significantly greater ($P < 0.05$) at day 8 than at baseline. A significant correlation between the ingested dose of oligofructose and fecal bifidobacterial counts was observed at day 8 ($P < 0.01$). In terms of side effects, all groups, including the placebo group, experienced mild abdominal symptoms, such as bloating, excess flatus, borborygmi, or mild abdominal pain. In general, the higher the dose of oligofructose, the more side effects experienced.

TABLE 104.1 Potential Prebiotics and Their Target Organisms

Prebiotic Compound	Food Source	Targeted Microorganisms
Acacia gum	Gum Arabic (*Acacia senegal*)	*Bifidobacterium* spp.
Fructooligosaccharides	See Table 104.2	*Bifidobacterium* spp.
Galactooligosaccharides	See Table 104.4	*Bifidobacterium* spp.
Glucomannan	Konjac root (*Amorphophallus konjac*)	*Lactobacillus* spp.
Human milk Oligosaccharides	Human milk	*Bifidobacterium* spp.
Lactitol	None known	*Lactobacillus* spp.; *Bifidobacterium* spp.
Lactulose	UHT milk	*Lactobacillus* spp.; *Bifidobacterium* spp.
Larch arabinogalactans	Western larch (*Larix occidentalis*)	*Lactobacillus* spp.
Partially hydrolyzed guar gum	Guar bean (*Cyamopsis tetragonoloba*)	*Bifidobacterium* spp.; Butyrate-producing species
Polydextrose	None known	*Lactobacillus* spp.; *Bifidobacterium* spp.
Xylooligosaccharides	Oats	*Bifidobacterium* spp.

UHT, Ultrahigh-temperature. Potential prebiotic substances and the organisms whose growth they promote.[162-167,5-8]

TABLE 104.2 Fructooligosaccharide-Containing Foods

Foodstuff	Form	Average percentage of FOS Fresh Weight	Typical Serving Size	Approximate Dose of FOS per Serving
Asparagus	raw	2.5%	½ bunch (~250 g)	6.3 g
	boiled	1.7%	½ bunch (~250 g)	4.3 g
Banana	raw	0.5%	Medium sized banana	0.6 g
Barley	cooked kernels	0.15%	1 cup	0.2 g
Burdock (gobo)	root (raw)	14.5%	1 cup chopped	18.0 g
Chicory	roasted (as 'coffee')	41.7%	1 teaspoon powder	1.3 g
Dandelion	leaves (raw)	13.5%	½ cup chopped	2.0 g
	root (raw)	7.9%	1 cup chopped	8.0 g
Garlic	raw	12.9%	1 medium-sized clove	0.4 g
Globe artichoke	boiled	4.4%	1 medium-sized bud (assuming 45% edible portion)	3.0 g
Jerusalem artichoke	tubers (raw)	18.0%	Medium tuber	9.0 g
Leek	raw	3.0%	1 cup chopped	2.7 g
Onion	raw	4.3%	½ cup chopped	3.5 g
	cooked	3.1%	½ medium-sized onion	3.0 g
Rye	flour (raw)	0.7%	1 cup	0.9 g
Salsify	root (raw)	4.2%	1 cup chopped	4.3 g
Wheat	flour (baked)	2.4%	1 cup	2.9 g
Yacon	root (raw)	11.0%	1 cup chopped	13.2 g

The richest food sources of FOS.[151,152]

Bouhnik et al.[16] concluded that 10 g/day was well tolerated and that this dose was probably the optimal dose of oligofructose because it led to a significant increase in colonic bifidobacteria with minimal side effects in most participants.

Immune Response

Prebiotics, like FOS, have long been suggested to have immune enhancing effects.[18,19] It is, however, only recently that human clinical trials with hard outcomes have been completed. One of the first of these trials investigated the effects of a prebiotic mixture (containing a combination of FOS and GOS) in protecting against infections during the first 6 months of life in formula-fed infants. In this randomized, double-blind, placebo-controlled trial, infants allocated to the prebiotic group experienced significantly fewer episodes of all types of infections combined ($P = 0.01$) compared with those in the placebo group. There was also a trend for fewer episodes of upper respiratory tract infection ($P = 0.07$) and fewer infections requiring antibiotic treatment ($P = 0.10$) in the prebiotic group. Additionally, the cumulative incidence of recurring infection and recurring respiratory tract infection was 3.9% and 2.9% in the prebiotic group versus 13.5% and 9.6% in the placebo group, respectively ($P < 0.05$).[20]

This same infant cohort was followed up over the next 1.5 years. Those infants that received the prebiotic-enhanced formula for the first 6 months of life experienced significantly fewer episodes of

TABLE 104.3 The Effects of Various Doses of FOS on the Microbiota

Dose of FOS	Effects of FOS on the GIT Microbiota
4 g/day	Increase in bifidobacteria; decrease in β-glucuronidase activity[153]
8 g/day	Increase in bifidobacteria; decrease in enterobacteria populations[154]
10 g/day	Increase in bifidobacteria and *Faecalibacterium prausnitzii* populations[155]
12 g/day	Increase in bifidobacteria and the butyrate-producing species *Anaerostipes;* decrease in *Bilophila* abundance (an H_2S producer)
15 g/day	Increase in bifidobacteria; decrease in fecal bacteroides, clostridia, and fusobacteria concentrations[156]
15.4 g/day	Increase in bifidobacteria; decrease in fecal concentrations of Bacteroides/Prevotella and clostridia[157]
40 g/day	Increase in bifidobacteria; decrease in fecal concentrations of enterococci and enterobacteria[158]

physician-diagnosed respiratory tract infections ($P < 0.01$), fever episodes ($P < 0.00001$), and fewer antibiotic prescriptions ($P < 0.05$) compared with those in the placebo group.[21]

In a randomized, placebo-controlled, open-label study, Bruzzese et al.[22] also investigated the efficacy of a prebiotic-enhanced infant formula (a combination of GOS and FOS) on infection incidence in infants. The prebiotic-enhanced formula or a standard infant formula was consumed over the initial 12 months of life. During this period, the incidence of gastroenteritis was 59% lower in the prebiotic group ($P = 0.015$). Additionally, the number of children with recurrent upper respiratory tract infections tended to be lower in the prebiotic group (28% vs. 45%; $P = 0.06$), and the number of children prescribed multiple antibiotic courses per year was also lower (40% vs. 66%; $P = 0.004$).[22]

In a randomized, double-blind, placebo-controlled trial looking at older children (aged 7–19 months) attending daycare centers, short-term administration (21 days) of FOS was found to significantly reduce the number of infectious diseases requiring antibiotic treatment ($P < 0.001$), episodes of diarrhea and vomiting ($P < 0.001$), and episodes of fever ($P < 0.05$) compared with controls.[23]

In another randomized, controlled trial investigating the effects of FOS in toddlers (4–24 month olds) attending daycare, FOS administration was found to significantly reduce antibiotic use (32% reduction; $P = 0.001$) and daycare absenteeism (61% decrease; $P = 0.025$) compared with those in the control group. There was also a 34% reduction in episodes of fever in combination with any cold symptoms ($P = 0.001$) and a 61% decrease in episodes of fever in association with diarrhea ($P < 0.05$) in the FOS-supplemented group.[24]

Mineral Absorption

Studies using animal models have demonstrated that microbiota degradation of FOS significantly increases calcium and magnesium absorption.[25-27] Human studies have also shown that FOS consumption improves calcium absorption.[28-30] The proposed mechanism by which mineral absorption is enhanced by FOS is via the action of the protonated SCFA. Protonated SCFAs are absorbed across the apical membrane of colonocytes by direct diffusion. Due to the low pK_a values of the SCFA in relation to the intracellular pH, the SCFA dissociates once it enters the cell. This results in the release of a hydrogen ion. The hydrogen ion is subsequently secreted from the cell in return for a cation, which may be a magnesium or calcium ion. The hydrogen ion is then available to protonate another SCFA and enable it to diffuse into the cell.[28] However, other mechanisms may also be involved, such as the decrease in colonic pH, which results in increased solubility of calcium, increased colonic venous blood flow, enlarged colonic villi, and enhanced expression of calbindin-D9k (the active calcium transport route).[31] These effects are more pronounced in the colon, some of which are calcium-specific, explaining why calcium absorption is increased, whereas there is very little effect on the absorption of other minerals (e.g., iron and zinc).[32]

Bioavailability of Phytoestrogens

An interesting animal (rat) study recently found that concurrent consumption of FOS and the soy isoflavones genistein and daidzein significantly improved the bioavailability of these compounds. The relative absorption of genistein was approximately 20% higher in FOS-fed rats than in controls. In addition, the presence of both phytoestrogens in serum was maintained longer in FOS-fed rats than in controls, suggesting that FOS enhanced colonic absorption of these compounds.[33]

This result may be especially relevant to women after antibiotic therapy, when the metabolism and subsequent absorption of phytoestrogens appears to be impaired.[34] Bifidobacteria were demonstrated to possess β-glucosidase activity, and FOS administration resulted in enhanced β-glucosidase activity in animal models[35] and improved phytoestrogen bioavailability.[36] Therefore FOS consumption not only aids in the reestablishment of a healthy gut microbiota, but its consumption also increases colonic β-glucosidase activity, resulting in enhanced deglycosylation and, thus, increased colonic concentrations of the medicinally active aglycones.

Atopic Eczema and Prevention of Atopy Development

A number of studies found an aberrant composition of the GIT microflora in infants who later developed food allergies and atopic eczema.[37-39] More specifically, the development of atopic eczema was correlated with increased colonic concentrations of *Bacteroides* spp., *Clostridia* spp., and *Escherichia coli,* with a decreased concentration of bifidobacteria. This change in microbial composition was theorized to deprive the developing immune system from counterregulatory signals against T-helper 2 (Th2) mediated responses, and therefore promote Th2-type immunity.[39] Infantile atopic eczema is characterized by a Th2-dominated immune response, as well as excessive intestinal inflammation and aberrant macromolecular absorption across the intestinal mucosa.[40-42] These latter characteristics may also be caused by the dysbiotic condition. Bacteroides, clostridia, and *E. coli* all have the potential to trigger inflammatory responses in the gut and can release toxins that can impair intestinal permeability, leading to increased exposure to potential antigens.[39] Supplementation with FOS was demonstrated to decrease colonic concentrations of both bacteroides and clostridia, as well as promoting a bifidobacteria-dominated colonic flora.[17] FOS use may thus bring the aberrant intestinal flora back into balance and improve gut barrier function. The promotion of an intestinal flora dominated by gram-positive bacteria may also promote a shift toward Th1 immunity via enhanced production of interleukin-12 and interferon-γ.[43]

No trials appear to have assessed FOS in isolation in the treatment or prevention of atopic eczema. One trial was conducted, however, which assessed the efficacy of a FOS and GOS combination in the prevention of atopic dermatitis in infants.

In a randomized, double-blind, placebo-controlled trial, Moro et al.[44] evaluated the effects of a prebiotic-enhanced infant formula

on the incidence of atopic dermatitis during the first 6 months of life in formula-fed infants at high risk of atopy development. Atopic dermatitis developed in 23% of infants in the control group compared with 10% in the prebiotic group ($P = 0.014$) over the 6-month intervention period.[44] Subjects in the prebiotic group were also found to have significantly reduced plasma levels of total immunoglobulin (IgE) ($P = 0.007$), IgG2 ($P = 0.029$), and IgG3 ($P = 0.0343$), as well as cow's milk protein-specific IgG1 ($P = 0.015$), suggesting that FOS and GOS supplementation induced an antiallergic antibody profile.[45]

These same infants were followed up over the next 18 months. Those infants that received the prebiotic-enhanced formula for the first 6 months of life were at reduced risk of developing atopic disease over the follow-up period. Infants in the control group experienced a significantly higher rate of atopic disease, such as atopic dermatitis (27.9% vs. 13.6%), recurrent wheezing (20.6% vs. 7.6%), and allergic urticaria (10.3% vs. 1.5%), compared with infants in the prebiotic group (all $P < 0.05$).[21]

The capacity of prebiotics (as a class of agents) to prevent allergic diseases was assessed in a recent systematic review and meta-analysis. Prebiotic supplementation to formula-fed infants significantly reduced the risk of developing eczema (RR = 0.68, 95% CI: 0.40–1.15), wheezing/asthma (RR = 0.37; 95% CI: 0.17–0.80), and food allergy (RR = 0.28, 95% CI: 0.08–1.00). Nineteen of the 22 studies included in the review evaluated the effects of a FOS-GOS combination (like that previously mentioned) typically added to formula.[115]

Satiety

In the wake of some interesting preliminary animal research,[46] a single-blind, crossover, placebo-controlled trial was performed to assess the effects of FOS on satiety and energy intake in humans. Subjects ingested either 8 g of FOS twice daily (with breakfast and dinner) or a placebo. FOS supplementation was found to significantly increase satiety at breakfast and dinner (both $P = 0.04$), but not lunch. At dinner, FOS supplementation was also found to reduce hunger ($P = 0.04$) and prospective food consumption ($P = 0.05$). Energy intake at breakfast ($P = 0.01$) and lunch ($P = 0.03$) were also found to be significantly reduced after FOS supplementation, resulting in a 5% decrease in total energy intake per day.[13]

A randomized, double-blind, placebo-controlled trial investigated the effects of FOS supplementation in overweight and obese subjects. Over a 12-week period, subjects consumed either a placebo or 21 g/day of FOS. Subjects in the FOS group experienced a mean reduction of 1.03 kg body weight compared with a 0.45 kg increase in weight in the control group ($P = 0.01$). FOS consumption was also associated with a lower area under the curve for ghrelin ($P = 0.004$) and a higher area under the curve for peptide YY ($P = 0.03$), suggesting an upregulation of satiety hormone secretion. These changes coincided with a reduction in self-reported caloric intake ($P \leq 0.05$). Serum glucose concentrations and insulin levels also significantly improved in the FOS group compared with baseline measures (both $P \leq 0.05$).[47]

Metabolic Health and Type 2 Diabetes

Inulin and Jerusalem artichokes have a long history of use in the treatment of diabetes, with the first recommendations of their use in the medical literature dating back to 1925.[116] More recently, there have been a number of animal and human trials that have substantiated this use.

A systematic review and meta-analysis of animal models that evaluated the effect of FOS supplementation on glucose homeostasis found the consumption of FOS to consistently decrease fasting blood glycemia levels, irrespective of the animal's metabolic status (e.g., in healthy, obese, or diabetic animals) and diet (low-fat or high-fat). A global reduction of 18% in fasting glycemia was observed in the FOS-treated animals compared with controls. There was a clear dose-dependent effect. FOS supplementation also increased fasting and postprandial glucagon-like peptide 1 concentrations, as well as peptide YY concentrations.[117]

A number of recent randomized controlled trials have shown FOS supplementation to aid in the management of type 2 diabetes. In one of the first of these trials, 10 g daily FOS supplementation over a period of 2 months to women with type 2 diabetes resulted in significant decreases in fasting plasma glucose levels of 8.5%, glycosylated hemoglobin levels of 10.4%, and malondialdehyde levels of 37.2% (all $P < 0.05$). Additionally, there were significant increases in total antioxidant capacity (18.8%) and superoxide dismutase activity (4.4%) in the FOS group compared with controls (both $P < 0.05$). However, changes in fasting insulin, homeostasis model assessment of insulin resistance, and catalase activity were not significant compared with the placebo group.[118]

In a follow-up study, the same dose of FOS given over 8 weeks to women with type 2 diabetes resulted in significant decreases in fasting blood sugar (8.5%), hemoglobin A1c (10.4%), fasting insulin (34.3%), homeostasis model assessment of insulin resistance (39.5%), high-sensitivity C-reactive protein (35.6%), tumor necrosis factor (TNF)-α (23.1%), and plasma lipopolysaccharide (27.9%) compared with the placebo group (all $P < 0.05$). The authors concluded that FOS supplementation improved blood sugar regulation, modulated inflammation, and decreased metabolic endotoxemia in diabetic women.[119]

In another similarly designed trial, 10 g FOS/day given to overweight women with type 2 diabetes for 8 weeks resulted in significant decreases in levels of fasting plasma glucose of 9.5%, glycosylated hemoglobin of 8.4%, interleukin-6 of 8.2%, TNF-α of 19.8%, and plasma lipopolysaccharide of 22.0% compared with placebo controls (all $P < 0.05$). Decreases in levels of interferon-γ and high-sensitivity C-reactive protein and increases in the level of interleukin-10 were not significant in the FOS group compared with controls.[114]

A further study in a similar population of overweight diabetic patients evaluating the anti-inflammatory effect of FOS found 10 g/day over 45 days to significantly decrease TNF-α mRNA expression, in addition to decreasing high-sensitivity CRP levels and diastolic blood pressure (all $P < 0.05$ vs. placebo). Gastrointestinal populations of the beneficial microbe *Akkermansia muciniphila* significantly increased with FOS supplementation.[120]

Dosage

Studies showed a bifidogenic effect in dosages of 4 to 40 g/day of FOS. The optimum dosage in adults, in terms of side effect profile and increases in bifidobacteria, is considered to be 10 g/day.[16] However, it may be a good idea to start off with a lower dose (e.g., 3 g/day) and slowly increase it to reduce chances of adverse GIT reactions. Dosages of less than 3 g/day in adults are unlikely to cause significant alterations in the GIT microecology. Studies in infants and toddlers generally administered between 1 and 3 g/day of FOS.

Toxicity

FOSs are components of many common foods. There are no genotoxic, carcinogenic, mutagenic, teratogenic, or toxicological effects associated with the ingestion of any FOS.[12,48] Oligofructose and inulin are officially recognized as natural food ingredients in most European countries and have a self-affirmed "Generally Regarded as Safe" status in the United States.[8]

Recently a published case study described an instance of anaphylaxis attributed to inulin found in vegetables and processed foods. This

TABLE 104.4 Galactooligosaccharide-Containing Foods

Foodstuff	Form	Average Percentage of GOS Fresh Weight	Typical Serving Size	Dose of GOS per Serving
Beetroot	tuber (raw)	0.14%	1 cup grated	0.20 g
Borlotti beans	canned	1.00%	½ cup	0.91 g
Broccoli	raw	0.13%	½ cup chopped	0.05 g
Chick peas	boiled	1.25%	½ cup	1.08 g
Fennel	Bulb (raw)	0.10%	1 cup sliced	0.09 g
Haricot beans	boiled	1.09%	½ cup	1.09 g
Lentils - green	boiled	0.46%	½ cup	0.43 g
Lentils - red	boiled	0.46%	½ cup	0.43 g
Lima beans	boiled	1.34%	½ cup	1.22 g
LSA mix	raw and ground	0.58%	1 tablespoon	0.07 g
Oats	dry	0.34%	½ cup	0.12 g
Onion	raw	0.19%	½ medium onion	0.11 g
Radicchio	raw	0.11%	1 cup chopped	0.06 g
Red kidney beans	boiled	1.44%	½ cup	1.33 g
Rye bread (sourdough)	sourdough	0.33%	2 slices	0.17 g
Soy beans	boiled	0.79%	½ cup	0.67 g
Split peas	boiled	1.88%	½ cup	1.70 g
Wheat (wholemeal)	bread	0.5%	2 slices	0.24 g

The richest food sources of GOS.[159,160]

was later confirmed with skin-prick testing and blinded food-provocation testing.[49] This allergy does appear to be extremely rare, however, considering the widespread consumption of FOS-containing foods.

The only side effects noted with administration are mild digestive symptoms, such as flatulence, borborygmi, abdominal bloating, and abdominal discomfort. However, these effects are dose-dependent and occur less regularly in smaller doses. Over time, these symptoms will diminish as the intestinal flora adjusts to the greater amount of substrate available.[50] However, some individuals may continue to experience mild abdominal bloating and discomfort even with continued use.

There have been some concerns in the literature regarding the ability of *Klebsiella pneumoniae* to use FOS as a growth substrate.[51,52] FOS was shown to stimulate the growth of *K. pneumoniae* in Petri dishes. However, this occurred only when *Klebsiella* was grown in isolation, with no other competing organisms present. In mixed culture experiments, where *Klebsiella* was grown in the presence of many other human GIT microorganisms, this did not occur.[9] In these situations, which more closely resemble the environment of the human GIT (where more than 500 species of bacteria compete for available growth substrates),[53] FOS did not stimulate the growth of *Klebsiella*.[8] In addition, no human or animal experiment has ever reported an increase in the *Klebsiella* concentration in the GIT after FOS consumption.

Given the efficacy of a diet low in oligosaccharides (e.g., the low fermentable oligosaccharide, disaccharide, monosaccharide and polyol [FODMAP] diet) in the management of irritable bowel syndrome (IBS),[121] it would be prudent to be cautious with FOS supplementation in patients with IBS. Not all patients react negatively to FOS supplementation, however.[122] Additionally, some research has found FOS supplementation in IBS patients to decrease rectal hypersensitivity and reduce anxiety levels; thus providing benefit to these patients.[123] Therefore in IBS patients where FOS supplementation is warranted, it may be a case of starting low and slowly increasing the dose over a few weeks to achieve the therapeutic dose.

GALACTOOLIGOSACCHARIDES

Description

Galactooligosaccharides (GOS) are chains of galactose molecules, typically containing between three and eight units, with a glucose molecule at the reducing terminus. Galactose-containing oligosaccharides are found in all mammalian milks.[54] Commercially, they are produced synthetically from lactose, typically using the bacterially derived enzyme β-galactosidase.[55] Like all prebiotics, GOS are neither broken down nor absorbed in the upper GIT.

Commercial Forms

GOS are available in two main forms: syrup and powder. As galactose-containing oligosaccharides are abundant in human breastmilk, GOS have recently been added to some artificial infant formulas in efforts to more closely approximate breastmilk.[56,57] Foods naturally rich in GOS are outlined in Table 104.4.

Clinical Applications

GOSs have a number of clinical applications, including:

- Promotion of bifidobacterial growth
- Treatment of constipation
- Prevention of atopic disease
- Treatment of irritable bowel syndrome
- Increased resistance to infections
- Prevention of gastrointestinal infections
- Improved absorption of calcium
- Promoting skin health
- Modulating mood and the stress response

Bifidobacterial Growth

A number of studies have now shown the ability of GOS to beneficially modify the gastrointestinal microbiota—both increasing levels of beneficial bacterial species while simultaneously decreasing levels of

TABLE 104.5 The Effects of Various Doses of GOS on the Microbiota

Dose of GOS	Effects of GOS on the GIT Microbiota
2.5 g/day	Increase in bifidobacteria ($P < 0.05$); decreased fecal concentrations of protein putrefactive byproducts (indoles and isovaleric acid)[161]
2.6 g/day	Fecal bifidobacteria counts increased significantly, as did fecal levels of *Lactobacillus-Enterococcus* spp. (both $P < 0.001$ compared with placebo); concentrations of *Bacteroides* spp., the *Clostridium histolyticum* group, *E. coli,* and *Desulfovibrio* spp. decreased compared with placebo treatment (all $P < 0.001$)[162]
5 g/day	Increase in bifidobacteria and *Faecalibacterium prausnitzii* (both $P < 0.05$); decrease in *Bacteroides* spp. ($P < 0.01$)[163]
10 g/day	Increase in bifidobacteria ($P = 0.007$)[129]

pathobionts. Table 104.5 contains an overview of some of these trials showing the effects observed at different dosage points.

A randomized, double-blind, placebo-controlled trial attempted to determine the optimal dose of GOS by investigating the effects of four different doses: 2.5, 5, 7.5, and 10 g/day. After 7 days of consumption of 10 g/day, fecal bifidobacteria levels increased 0.4 log units ($P = 0.007$ compared with placebo). In the dose–response phase of the trial, there were no significant differences found between the different GOS doses in their capacity to increase bifidobacteria numbers (i.e., there was a lack of dose response).[58]

Treatment of Constipation

A number of studies were conducted to evaluate the effects of GOS administration on bowel function. Consuming 9 g/day of GOS was found to increase defecation frequency (from 5.9–7.1 movements per week) in elderly subjects over a 2-week treatment period. There was also a trend for easier defecation ($P = 0.07$) during the GOS phase of the trial.[59]

Randomized controlled trials evaluated the laxative effects of GOS in healthy adult subjects with a tendency to constipation. At a daily dose of 5 and 10 g, there were significant improvements in defecation frequency, from 0.92 to 1.07 movements daily ($P < 0.05$) and from 0.85 to 0.97 times per day ($P < 0.05$), respectively.[60]

Atopic Disease

No trials appear to have assessed GOS in isolation for the treatment or prevention of atopic eczema. One trial was conducted, however, assessing the efficacy of a GOS and FOS combination in the prevention of atopic dermatitis in infants. This trial is discussed in the FOS section under "Treatment of Atopic Eczema and Prevention of Atopy Development."

Irritable Bowel Syndrome

In a randomized, single-blind, placebo-controlled, crossover trial, supplementation of 3.5 g/day of GOS to subjects with IBS was found to significantly improve stool consistency, flatulence scores, bloating scores, and overall IBS symptom scores compared with placebo (all $P < 0.05$) over a 4-week treatment period. Supplementation of 7 g/day resulted in significant improvements in overall IBS symptom scores and a reduction in anxiety levels compared with placebo (both $P < 0.05$). However, there was also a significant increase in bloating scores relative to baseline when subjects were taking the higher dosage ($P < 0.05$).[61]

Immune Function

No human studies appear to have assessed GOS on their own for the improvement of immune function. A number of trials were conducted assessing the efficacy of FOS and GOS combinations, however. These trials are discussed in the FOS section.

Gastrointestinal Infections

Animal and in vitro models suggested that GOS supplementation might have a protective effect against a range of gastrointestinal pathogens. GOS was demonstrated to reduce adherence of pathogenic strains of *E. coli* to gastrointestinal cells,[62] inhibit binding of *Vibrio cholerae* toxin to its receptor in the human GIT,[63] and reduce colonization and pathology associated with *Salmonella typhimurium* infection in a murine model.[64]

These preliminary results inspired a recent randomized, double-blind, placebo-controlled trial evaluating the potential of GOS administration to prevent travelers' diarrhea. Subjects ingested 2.6 g GOS once daily, starting 7 days before reaching their holiday destination and continued taking it daily throughout their holiday. Compared with the placebo group, subjects in the GOS group experienced a 40% decrease in the incidence ($P < 0.05$) and a 48% reduction in duration of travelers' diarrhea ($P < 0.05$). Additionally, they had less abdominal pain ($P < 0.05$) and experienced improved quality of life ($P < 0.05$).[65]

Calcium Absorption

Research found supplemental GOS significantly increased calcium absorption in a randomized, double-blind, crossover trial in postmenopausal women. Administration of 20 g/day was found to increase true calcium absorption from 16% during placebo administration to 24% during GOS treatment ($P = 0.04$). This increase in absorption was not accompanied by increases in urinary calcium excretion.[66]

Skin Health

In a randomized, double-blind, placebo-controlled trial, investigators examined the effects of GOS ingestion (2 g/day) on the condition of the skin. After 12 weeks of supplementation, the increase in corneometer values from baseline to week 12 was significantly greater in the GOS group than in the placebo group (6.9 vs. 2.9 arbitrary units, $P < 0.05$). A corneometer uses the high dielectric constant of water to analyze the water-related changes in the electrical capacitance of the skin. An increase in corneometer values is interpreted as improved skin hydration. The transepidermal water loss in the GOS group was also significantly reduced after 12 weeks of GOS treatment (20.1 g/h/m^2 at baseline vs. 17.5 g/h/m^2 at week 12; $P < 0.05$). Additionally, GOS treatment significantly reduced total wrinkle area and the percentage of wrinkle area compared with baseline ($P < 0.05$), whereas both these values increased slightly in the placebo group.[124]

A further randomized, double-blind, placebo-controlled trial evaluated the effects of a prebiotic combination (GOS + lactulose; 4.5 g/day total) on skin health. At the end of the 8-week trial, subjects in the prebiotic group showed a reduction in mean wrinkle length and depth as measured via a quantitative skin evaluation, whereas the placebo group showed slight increases in these parameters ($P < 0.001$). The wrinkle severity rating scale significantly improved in the prebiotic group, versus a worsening in the placebo group ($P < 0.001$). The global esthetic improvement scale was also significantly higher for the prebiotic group ($P < 0.001$). The mechanisms by which orally ingested prebiotics improve skin condition and hydration are not currently known.[125]

Mood and Stress Response

As detailed in the previously mentioned IBS GOS trial, the 7 g/day dose was found to significantly reduce anxiety scores in IBS patients.[126] This

finding has stimulated other researchers to examine the potential of prebiotics, and GOS treatment in particular, to modify mood.

Using a mouse model of chronic stress, investigators found GOS or a GOS-FOS combination to attenuate acute stress-induced corticosterone release and reduce the number of stress-induced bowel movements. The combination also significantly increased hippocampal brain-derived neurotrophic factor gene expression, GABA receptor gene expression, and serotonin levels in the prefrontal cortex. Additionally, the GOS-FOS combination reduced stress-induced depression-like and anxiety-like behavior in the mice.[127]

In a double-blind, placebo-controlled trial, healthy volunteers were randomized to receive either FOS (5.5 g/day), GOS (5.5 g/day), or placebo for 3 weeks. At the end of the 3 weeks, salivary morning cortisol was significantly lower only in the GOS group ($P < 0.05$). This group also experienced increased processing of positive versus negative attentional vigilance. The results suggest the capacity of GOS to beneficially modify neuroendocrine stress responses in humans.[128]

Dosage

A bifidogenic effect was observed in doses of 2.5 to 15 g/day. With no apparent dose response, there appears to be little benefit in dosing higher than the minimum required dose (i.e., 2.5 g/day) for this purpose. For the treatment of IBS, 3.5 g/day seems to be the optimal dose. For anxiety management and to modify the stress response, 5.5 to 7.0 g/day appears efficacious, whereas for the prevention of travelers' diarrhea 2.6 g/day has been shown to be effective. To enhance calcium absorption, higher doses may be required (e.g., 20 g/day).

The Golden Rule of prebiotic dosing is to start low and slowly increase the dose over a period of weeks to achieve the therapeutic or optimal dose. Most individuals tolerate a starting dose of 2.5 g/day with few issues—only minor digestive discomfort (if any). However, if a patient is very prone to bloating and distension, one can start at one-quarter to one-half this dose. It is worth noting, however, that GOS supplements have been found to decrease bloating, distension, and abdominal pain scores in patients with irritable bowel syndrome,[126] and some research has found GOS to actually decrease intestinal gas (hydrogen) production.[129]

Toxicity

Galactose-containing oligosaccharides are found in all mammalian milks. Animal research has consistently demonstrated a lack of genotoxic, mutagenic, or toxicologic effects with GOS ingestion.[67] The nontoxic dose level of GOS was found to be over 2000 mg/kg body weight per day in murine models.[68] Therapeutic use of GOS is considered very safe and GOS themselves are components of many commonly consumed foods. Clinical trials have generally found GOS to be very well tolerated (in infants, adolescents, adults, the elderly, and during pregnancy),[126,130-133] with GIT side effects (e.g., bloating) only noted with higher doses in most research.[126] Supplemental GOS is generally very well tolerated, even in individuals who react to other prebiotics (e.g., FOS and lactulose) and even in those patients who react to food sources of GOS (i.e., legumes).

LACTULOSE

Introduction

Lactulose is a semisynthetic disaccharide composed of the monosaccharides fructose and galactose. It was first synthesized from lactose in 1929 by Montgomery and Hudson.[69] The human digestive system lacks the ability to break down lactulose into its component hexose and pentose moieties. Hence, lactulose is neither catabolized nor absorbed in the small intestine.

Once lactulose reaches the large intestine, it is fermented by the normal intestinal microflora. Fermentation is the process through which the normal intestinal microflora catabolizes carbohydrates to obtain energy for growth and maintenance of cellular functions. The end products of lactulose fermentation include SCFA, lactic acid, hydrogen, and carbon dioxide.[70] In vitro experiments demonstrated that overall SCFA production is increased two- to threefold by the addition of lactulose, whereas acetate synthesis is increased four- to sixfold.[71]

Within the large intestine, these SCFAs are avidly absorbed by colonocytes and used as a source of energy, either locally or systemically. The production of SCFA also results in the acidification of the colonic contents.[70] Bown et al.[72] demonstrated that a daily dose of 30 to 40 g of lactulose decreased the pH of the proximal colon from 6.0 to 4.85.

Commercial Forms

Lactulose is available in two forms: syrup and crystalline powder. Lactulose syrup generally contains between 5% and 67% lactulose (in addition to some lactose and galactose), whereas the crystalline powder is composed of 99% lactulose. The only food source of lactulose thus far identified is ultraheat treated milk; however, lactulose is present only in insignificant amounts.[4]

Clinical Applications

Lactulose has a wide variety of clinical applications, including:

- Promotion of lactobacilli and bifidobacteria growth
- Decreasing growth of intestinal pathogens
- Constipation
- Prevention of colon cancer
- Liver disease
- Endotoxemia
- Prevention of urinary tract infections (UTIs)
- Enhancing calcium and magnesium absorption

Lactobacilli and Bifidobacterial Growth

In one of the original studies using lactulose to change the GI microbiota, MacGillivray et al.[73] assessed the effects of lactulose feeding on infants. All infants were formula-fed and under 4 months old. After a short feeding period of 2 days, the lactulose-containing formula feed produced a preponderance of *Lactobacillus bifidus* (now known as *Bifidobacterium bifidum*) in the stools of 88% of infants. In 66% of the infants, *B. bifidum* dominated the microbiota in concentrations of greater than 90%. None of these infants had a bifidobacteria-dominated flora at baseline.[73]

Terada et al.[74] reported that 3 g/day of lactulose taken over a 2-week period altered intestinal microbiota and fecal bacterial metabolism in adults. The study was conducted in eight healthy volunteers. During the intake of lactulose, the number of bifidobacteria increased significantly ($P < 0.001$), as did the lactobacilli population ($P < 0.05$), whereas the number of Bacteroidaceae and clostridia decreased ($P < 0.05$) compared with baseline. Bifidobacteria became the numerically dominant microorganism in the fecal flora after 14-day administration. The fecal metabolites skatole, indole, and phenols were also significantly decreased with lactulose intake ($P < 0.05$). Fecal β-glucuronidase, azoreductase, and nitroreductase activities also decreased significantly ($P < 0.05$) after 14 days of treatment. These latter results indicated decreased metabolism of putrefactive (protein fermenting) organisms of the microbiota. The mean fecal pH also decreased from 7.0 to 6.4.[74]

Ballongue et al.[75] conducted a double-blind, randomized, placebo-controlled study, assessing the effects of a daily dose of 20 g of lactulose (10 g bid) on the gastrointestinal microbiota. After a 4-week treatment period, populations of *Bacteroides, Clostridium,*

and coliforms decreased by 4.1, 2.3, and 1.8 log units, respectively. *Bifidobacterium* and *Lactobacillus* populations increased by 3.0 and 1.9 log units (both $P < 0.01$ compared with placebo), respectively. The fecal pH also decreased from a baseline of 6.9 to 5.8 by the end of the treatment period ($P < 0.01$). Acetic acid production was increased by 33% and lactic acid production by 30% ($P < 0.01$) via lactulose administration. Gastrointestinal production of putrefactive by-products, cresol, indole, phenol, and skatol were all significantly reduced by lactulose administration (all $P < 0.05$), as were the production of the bacterial enzymes, azoreductase, 7α-dehydroxylase, β-glucuronidase, nitroreductase, and urease (all $P < 0.05$).[75]

Inhibition of Potentially Pathogenic Microorganisms

Research suggests that lactulose not only enhances the growth of beneficial members of the GIT microbiota, but also inhibits the growth of potential intestinal pathogens. Studies demonstrated that lactulose consumption can decrease colonic concentrations of clostridia, *Bacteroides* spp., and coliforms,[75] as well as eradicating carrier states of *Salmonella* spp.[76] Some preliminary evidence also suggests a possible role for lactulose therapy in the treatment of small intestinal bacterial overgrowth.[77]

The ability of lactulose to increase numbers of lactobacilli and bifidobacteria and to decrease numbers of gram-negative bacteria is believed to be a result of two mechanisms. The first is lactulose's ability to act directly as a food source for lactobacilli and bifidobacteria organisms, while being only weakly metabolized by gram-negative organisms.[78] The second mechanism is the change in colonic pH caused by the end products of lactulose metabolism—lactic acid and SCFAs. The coupled effect of production of SCFAs and pH decrease has been shown to inhibit the growth of gram-negative bacteria. The decreased pH resulting from lactulose administration was shown to increase the total concentration of the undissociated forms of SCFA.[75] These lipophilic acids can penetrate the microbial cell membrane, and at the higher intracellular pH, dissociate to produce hydrogen ions. The hydrogen ions interfere with essential metabolic functions, such as substrate translocation and oxidative phosphorylation, and hence inhibit the growth of gram-negative microbes.[79]

In vitro experiments also demonstrated the ability of lactulose to inhibit the growth of *Candida albicans.* A continuous flow culture was used as a model of the human GIT ecosystem. Within 24 hours of the input of 0.25% lactulose, *C. albicans* numbers were reduced by 97%. The reduction in *Candida* numbers was believed to be caused by the increase in the growth of beneficial lactic acid bacteria and the overall decrease in pH.[80]

Constipation

Lactulose is used in conventional medicine mainly for its laxative effects. It works primarily as an osmotic laxative, but the increased production of SCFAs may also play a role.[70,81] Ingestion of lactulose was found to cause statistically significant increases in the frequency, weight, volume, and water content of stools and to produce stools of softer consistency compared with both baseline and placebo.[82]

Colon Cancer Prevention

Lactulose may protect against the development of colon cancer via a number of different mechanisms. Firstly, lactulose administration was found to decrease the production of secondary bile acids in the intestinal tract (secondary bile acids have been postulated to be promoters of colonic carcinogenesis). This effect was thought to be mediated by the reduction in luminal pH caused by lactulose fermentation.[83]

Secondly, lactulose also has the ability to decrease the metabolism of PPMs. This action appears to be directly related to lactulose's ability to decrease the colonic pH. The low pH suppresses overall metabolism of these organisms, which can be indirectly measured by the decrease in fecal enzymatic activity by these microorganisms. In a human trial, Ballongue et al.[75] demonstrated a statistically significant decrease in the specific activity of a number of bacterial enzymes that are believed to be involved in the genesis of colon cancer. Azoreductase activity was lowered by 45%, 7-α-dehydroxylase by 40%, β-glucuronidase by 38%, nitroreductase by 36%, and urease by 27% (all $P < 0.01$). Facultative anaerobes like coliforms and anaerobes like *Clostridium* and *Bacteroides* normally produce β-glucuronidase, 7-α-dehydroxylase, and nitroreductase. The decreased activity of these enzymes correlated with the decreased numbers of bacteroides, clostridia, and coliforms found in this clinical trial after administration of lactulose.[75]

Thirdly, lactulose appears to inhibit the formation of ammonia within the intestinal tract.[84] Ammonia was shown to alter the morphology and intermediary metabolism of intestinal cells, as well as increasing DNA synthesis in, and reducing the life span of mucosal cells.[85] It is also considered to be more toxic to healthy mucosal cells than transformed cells and, thus, it may potentially select for neoplastic growth.[86] Thus any inhibition of ammonia production should be of benefit in the prevention of colon tumorigenesis.

Additionally, a randomized clinical trial conducted by Roncucci et al.[87] demonstrated the ability of lactulose to prevent the growth of resected colorectal polyps. Lactulose administration (20 g/day) reduced the recurrence rates of colonic polyps by 66% ($P < 0.02$) compared with controls.[87]

Hepatic Encephalopathy

Hepatic encephalopathy is often found as a complication of liver cirrhosis. It is considered the totality of nervous system manifestations of liver failure. Symptoms include tremors, mental confusion, memory loss, and personality changes. The exact cause of hepatic encephalopathy is not known. However, it is commonly believed that an accumulation of neurotoxins (particularly ammonia) in the cerebral circulation is the main causative factor.[88] A number of studies have found lactulose to be effective in the management of this condition[89-91] and in its prevention.[92]

Endotoxemia

Endotoxins (also known as lipopolysaccharides) are constituents of the outer membranes of gram-negative bacteria. Gram-negative bacteria continuously shed components of their outer cell membranes, particularly as they die. Gram-negative bacteria can comprise a significant portion of the intestinal microbiota (e.g., members of the Bacteroidetes and Proteobacteria phyla can make up more than 50% of the microbiota in some individuals)[134]; thus, this membrane shedding results in significant quantities of endotoxins being released into the intestinal lumen. In healthy individuals, with good intestinal integrity, luminal endotoxin causes minimal systemic effects. However, in individuals with a compromised intestinal barrier or suboptimal liver function, this intestinal endotoxin can have pathological consequences.[94]

In fact, a number of diseases are now being linked to excessive absorption of GI-derived endotoxins, which has a negative effect on the systemic inflammatory milieu, including: alcoholic liver disease,[135] Alzheimer's disease,[136] atherosclerosis,[137] congestive cardiac failure,[138] chronic fatigue syndrome,[139] depression,[140] metabolic syndrome,[141] nonalcoholic fatty liver disease,[142] obesity,[143] and type 2 diabetes.[144] Hence, there is a great need for agents capable of both decreasing endotoxin load in the lumen, as well as having other antiendotoxin effects.

Research by Liehr et al.[95] has demonstrated that lactulose possesses antiendotoxin activity. When rats were fed lactulose over 4 or 8 days before intravenous administration of galactosamine, the liver damage

that normally develops was prevented. Because galactosamine-induced liver necrosis and inflammation is mediated by systemic endotoxemia of intestinal origin, the prevention of liver inflammation and necrosis after lactulose consumption suggests an antiendotoxin effect. This effect was most likely mediated by an alteration of the intestinal microecology, which indirectly diminished the intestinal pool of endotoxin. However, the authors also suggested a direct antiendotoxin effect, as intravenous administration of lactulose also prevented galactosamine-induced hepatitis.[95]

This work was followed by a prospective, controlled trial assessing the use of lactulose in endotoxemia secondary to obstructive jaundice. Oral lactulose (30 mL/6 h) was given to 12 patients for 3 days before surgery, whereas another 12 patients served as controls. Lactulose administration significantly reduced the incidence of endotoxemia in the portal blood during the operation and in the systemic blood postoperatively (both $P < 0.05$). Interestingly, lactulose appeared to both decrease the luminal pool of endotoxin and directly prevent endotoxin absorption.[96]

Urinary Tract Infections

Two human studies demonstrated the efficacy of lactulose therapy in the prevention of UTIs. McCutcheon and Fulton[97] conducted a retrospective study using 45 elderly, long-term hospital patients as subjects. The study found that daily lactulose therapy for 6 months (30 mL lactulose syrup) resulted in a significant reduction in UTIs compared with controls ($P < 0.025$). Sixteen of the 17 lactulose-treated patients (94%) remained infection free over the 6 months compared with 16 of the 28 control patients (57%) ($P < 0.005$). In addition, there was a significant reduction in the number of antibiotic prescriptions ($P < 0.05$) and in the number of patients who received antibiotics ($P < 0.005$) in the lactulose group.[97]

In the second study, Mack et al. enrolled 75 elderly, hospitalized patients in a randomized, placebo-controlled trial. Thirty-eight patients received the placebo and 58 received lactulose therapy. Twelve percent of the lactulose group developed UTIs during follow-up compared with 32% in the control group ($P < 0.01$). Thus lactulose consumption demonstrated significant protection against the development of UTIs.[98]

The intestinal microbiota appears to act as a reservoir for urinary tract pathogens. Microorganisms such as *E. coli, Enterococcus faecalis, E. faecium,* and *K. pneumoniae* are all frequent urinary tract pathogens, and these organisms are also commonly found in the GIT. These organisms can escape the confines of the large bowel, and in susceptible individuals colonize the vagina, periurethral area, and distal urethra. From these areas they can ascend into the bladder. Thus interventions that can inhibit the growth of these organisms in the GIT should result in decreased numbers of these organisms colonizing the genitourinary tract.[93] This is likely to be the mechanism by which lactulose therapy prevents UTIs. Ingestion of lactulose acidifies the large bowel and significantly increases the production of SCFAs.[72,75] At a low pH, these SCFAs (particularly butyrate) inhibit the growth and metabolism of enterococci and *E. coli,* substantially reducing their overall populations in the large bowel,[93] and thus decreasing the intestinal reservoir of urinary tract pathogens.

Calcium and Magnesium Absorption

In a randomized, controlled trial, low-dose lactulose consumption (2 g/day) was found to significantly improve magnesium absorption, and high dose (4 g/day) administration significantly improved both calcium and magnesium absorption in adult males (all $P < 0.01$ compared with placebo). There appeared to be a clear dose-dependent effect, with the higher lactulose dose being more effective than the lower for both magnesium and calcium.[99]

Dosage

Doses as low as 3 g/day (equivalent to about 5 mL of the syrup) have been shown to cause beneficial alterations in the intestinal microbiota.[74] However, higher doses (e.g., 10 g twice a day) appeared to produce more substantial positive changes.[75] As with FOS and GOS, therapy should commence with a lower dose and slowly increase over time. For the treatment of constipation, the recommended dose is from 15 to 40 g/day.[82,100] Higher dosages are usually used (~35 g/day) for the management of liver disease.[101]

Toxicity

Therapeutic use of lactulose is considered to be extremely safe, with adverse reactions generally being mild and few. Side effects consist of vomiting, nausea, diarrhea, and abdominal cramping.[102] However, these reactions tend to occur only in single doses of more than 60 g. This appears to be the maximum load of lactulose that the intestinal flora is able to metabolize to SCFAs at one time. When the bacterial fermentation capacity is exceeded, osmotic diarrhea, abdominal cramping, and nausea may result. These symptoms are most likely caused by the interference with net fluid absorption in the colon as a result of the osmotic effect of malabsorbed and intact sugars.[70] Nonetheless, flatulence, abdominal bloating, and discomfort are common symptoms when lactulose therapy is commenced. Gastrointestinal symptoms, however, diminish as the colonic microbiota adapt to the greater amount of fermentable substrate.[103]

SYNBIOTICS

Synbiotics are products that contain both probiotic and prebiotic agents.[104] The combination is supposed to enhance the survival of the probiotic bacteria through the upper GIT, improve implantation of the probiotic in the colon, and have a stimulating effect on the growth and/or activities of both the exogenously provided probiotic strain(s) and the endogenous inhabitants of the bowel.[105] Over the past 10 years, research in this area has been steadily growing.

When considering the therapeutic potential of a product claimed to be a synbiotic, a number of factors should be assessed:

1. Does the product use well-characterized and researched probiotic strains? (See Chapter 105 on Probiotics.)
2. Does the "prebiotic" substance meet the requirements to be truly considered a prebiotic?
3. Has the "prebiotic" been demonstrated to enhance the growth of the exact probiotic strain(s) contained in the product?
4. Are both agents included in therapeutic doses?

Ideally, a synbiotic product should meet all four of these criteria. Unfortunately, many currently on the market do not.

Strain-Specific Synbiotic Combinations

Individual probiotic strains (see Chapter 105 on Probiotics) have variable capacities to use different prebiotic substrates. For example, *Bifidobacterium lactis* Bb12 can use GOS, lactulose, and FOS, but is unable to use lactitol.[106] *Lactobacillus rhamnosus* GG is unable to use lactulose, lactitol, or FOS.[107,108] Both *L. acidophilus* NCFM and *L. acidophilus* DDS-1 can use FOS as a growth substrate.[108] Sadly, there is currently a paucity of research in this area to guide decision-making.

COLONIC FOODS

Introduction

Colonic foods are defined as "foods entering the colon and serving as substrates for the endogenous colonic bacteria, thus indirectly providing the host with energy, metabolic substrates and essential micronutrients."[2] By definition, colonic foods escape digestion and absorption in

TABLE 104.6 Other Foods and Food Constituents That Have Been Shown to Cause Beneficial Shifts in the GIT Microbiota

Food (dose)	Effects on the GIT Microbiota
Almonds (roasted with skins; 56 g/day = ~1/3 cup)	Increased fecal concentrations of bifidobacteria and lactobacilli; decreased fecal β-glucuronidase, nitroreductase and azoreductase activities[164]
Apples (2 medium apples/day)	Increased fecal concentrations of bifidobacteria; trend for increased concentrations of lactobacilli; decrease in fecal clostridia; decreased fecal sulfide concentrations[165]
Black currants (4 capsules/day black currant powdered extract)	Increased fecal concentrations of bifidobacteria and lactobacilli; decreased counts of *Bacteroides* spp., and *Clostridium* spp.; decrease in fecal pH; decreased fecal β-glucuronidase activity; increased β-glucosidase activity[166]
Blueberries (wild) (25 g of powdered wild blueberries in water/day)	Increased fecal concentrations of bifidobacteria[167]
Brown rice (~550 g/day = 4 cups cooked)	Increased numbers of bifidobacteria; decreased counts of *Bacteroides* spp., *Collinsella aerofaciens, Escherichia coli,* and *Clostridium* spp.[168]
Cocoa powder (~14 g dark cocoa powder/day)	Increased fecal concentrations of bifidobacteria and lactobacilli; decrease in fecal clostridia[169]
Green tea (300 mg catechins/day; equivalent to ~5–6 cups/day)	Increased fecal concentrations of bifidobacteria and lactobacilli; decreased concentrations of bacteroides, clostridia, and enterobacteria; decrease in fecal pH; decrease in fecal concentrations of potentially toxic protein putrefactive byproducts ammonia, sulfide, skatol, indole, and cresol; increased production of short-chain fatty acids (SCFAs)[170]
Resistant starch (30g/day; from potato tubers; 70% type 2 resistant starch)	Increased fecal concentrations of bifidobacteria; decrease in fecal Proteobacteria populations[171]

the upper GIT to reach the colon intact. Here members of the colonic microbiota ferment them to produce SCFAs, hydrogen, methane, and carbon dioxide. Through the production of SCFAs, the ingestion of colonic foods plays a pivotal role in the health of the host.[81]

Colonic foods lack the specificity of fermentation that prebiotics possess. Thus their ingestion promotes the growth and/or metabolic activity of a number of different bacterial species within the large bowel, including species that are considered potentially harmful. Colonic foods include plant cell wall polysaccharides (e.g., cellulose), hemicelluloses, pectins, and many gums.[2]

Commonly used fibers, such as slippery elm, pectin, psyllium husks, and guar gum should all be considered colonic foods, not prebiotics, because they are used by a number of different bacterial species in the bowel. For example, guar gum is metabolized by *Bacteroides* spp. and *Ruminococcus* spp.[109]; pectin is fermented by *Bacteroides* spp., *Bifidobacterium* spp., *Eubacterium* spp., and *Clostridium* spp.[110]; and psyllium husks are fermented primarily by *Bacteroides* spp.[109]

Some foods, however, have demonstrated the ability to selectively promote the growth of beneficial species of bacteria. Foods and food constituents that have shown this selective fermentation capacity are listed in Table 104.6.

Drug Interactions

FOSs and GOSs do not appear to interact with any pharmaceuticals. When used in prebiotic dosages, lactulose is also unlikely to elicit any drug interactions. When administered in larger doses for use as a laxative over prolonged periods of time, however, there is a theoretical risk of increased electrolyte loss, which could affect electrolyte balance. These electrolyte alterations could potentiate the risk of more severe electrolyte imbalances, such as hypokalemia, which has been associated with the use of some medications (e.g., corticosteroids, diuretics, and drugs that prolong the QT interval). However, lactulose is less likely to elicit electrolyte disturbances with long-term use than other laxatives because of the capacity of the colonic microflora to adapt to lactulose ingestion, with long-term ingestion resulting in a reduction in lactulose-induced gastrointestinal symptoms and diarrhea.[103,111]

When used in laxative dosages, lactulose was found to significantly increase the relative risk of over anticoagulation in patients taking coumarin anticoagulants, presumably via decreased intestinal absorption.[112]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr*. 1999;69(suppl):S1052–S1057.
2. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*. 1995;125:1401–1412.
3. Goldin BR, Gorbach SL. Prebiotics: a review of their effects on the intestinal flora and health benefits. *Gastroenterol Int*. 1998;11(suppl 1):1–3.
4. Hudson MJ, Marsh PD. Carbohydrate metabolism in the colon. In: Gibson GR, Macfarlane GT, eds. *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology.* Boca Raton: CRC Press:61–73.
5. Teramoto F, Rokutan K, Kawakami Y. Effect of 4-B-D-galactosylsucrose (lactosucrose) on fecal microflora in patients with chronic inflammatory bowel disease. *J Gastroenterol*. 1996;31:33–39.
6. Teuri U, Korpela R. Galacto-oligosaccharides relieve constipation in elderly people. *Ann Nutr Metabol*. 1998;42:319–327.
7. Van Loo J, Cummings J, Delzenne N, et al. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). *Br J Nutr*. 1998;81:121–132.
8. Roberfroid MB, Delzenne N. Dietary fructans. *Ann Rev Nutr*. 1998;18:117–143.
9. Roberfroid MB, Van Loo J, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. *J Nutr*. 1998;128:11–19.
10. Niness KR. Inulin and oligofructose: what are they? *J Nutr*. 1999;129:S1402–S1406.
11. Moshfegh AJ, Friday JE, Goldman JP, et al. Presence of inulin and oligofructose in the diets of Americans. *J Nutr*. 1999;129:S1407–S1411.
12. Macfarlane GT, Cummings JH. Probiotics and prebiotics: can regulating the activities of the intestinal bacteria benefit health. *BMJ*. 1999;318: 999–1003.
13. Cani PD, Joly E, Horsmans Y, et al. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr*. 2006;60:567–572.
14. Laiho K, Ouwehand A, Salminen S, et al. Inventing probiotic functional foods for patients with allergic disease. *Ann Allergy Asthma Immunol*. 2002;89(suppl):75–82.
15. Latella G. Effects of SCFA on human colonocytes. *Gastroenterol Int*. 1998;11(suppl 1):76–79.
16. Bouhnik Y, Vahedi K, Achour L, et al. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *J Nutr*. 1999;129:113–116.
17. Gibson GR, Beatty ER, Wang X, et al. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*. 1995;108:975–982.
18. Watz B, Girrbach S, Roller M. Inulin, oligofructose and immunomodulation. *Br J Nutr*. 2005;93:S49–S55.
19. Bornet FR, Brouns F. Immune-stimulating and gut health-promoting properties of short-chain fructo-oligosaccharides. *Nutr Rev*. 2002;60:326–334.
20. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J Nutr*. 2007;137:2420–2424.
21. Arslanoglu S, Moro GE, Schmitt J, et al. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J Nutr*. 2010;138:1091–1095.
22. Bruzzese E, Volpicelli M, Squeglia V, et al. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. *Clin Nutr*. 2009;28:156–161.
23. Waligora-Dupriet AJ, Campeotto F, Nicolis J, et al. Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. *Int J Food Microbiol*. 2007;113:108–113.
24. Saavedra JM, Tschernia A. Human studies with probiotics and prebiotics: clinical implications. *Br J Nutr*. 2002;87(suppl 2):S241–S246.
25. Ohta A, Ohtsuki M, Baba S, et al. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. *J Nutr*. 1995;125:2417–2424.
26. Younes H, Coudray C, Bellanger J, et al. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *Br J Nutr*. 2001;86:479–485.
27. Takahara S, Morohashi T, Sano T, et al. Fructooligosaccharide consumption enhances femoral bone volume and mineral concentrations in rats. *J Nutr*. 2000;130:1792–1795.
28. Trinidad P, Wolever TM, Thompson LU. Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans. *Am J Clin Nutr*. 1996;63:574–578.
29. van den Heuvel E, Muys T, van Dokkum W, et al. Oligofructose stimulates calcium absorption in adolescents. *Am J Clin Nutr*. 1999;69:544–548.
30. Griffin IJ, Davila PM, Abrams SA. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. *Br J Nutr*. 2002;87(suppl 2):S187–S191.
31. Scholz-Ahrens KE, Schaafsma G, van den Heuvel E, et al. Effects of prebiotics on mineral metabolism. *Am J Clin Nutr*. 2001;73(suppl):S459–S464.
32. Coudray C, Bellanger J, Castiglia-Delavaud C, et al. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur J Clin Nutr*. 1997;51:375–380.
33. Uehara M, Ohta A, Sakai K, et al. Dietary fructooligosaccharides modify intestinal bioavailability of a single dose of genistein and daidzein and affect their urinary excretion and kinetics in blood of rats. *J Nutr*. 2001;131:787–795.
34. Kilkkinen A, Pietinen P, Klaukka T, et al. Use of oral antimicrobials decreases serum enterolactone concentration. *Am J Epidemiol*. 2002;155:472–477.
35. Rowland IR, Rumney CJ, Coutts JT, et al. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis*. 1998;19:281–285.
36. Ohta A, Uehara M, Sakai K, et al. A combination of dietary fructooligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. *J Nutr*. 2002;132:2048–2054.
37. Bjorksten B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol*. 2001;108:516–520.
38. Kalliomaki M, Kirjavainen PV, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol*. 2001;107:129–134.
39. Kirjavainen PV, Arvola T, Salminen S, et al. Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? *Gut*. 2002;51:51–55.
40. Eigenmann PA, Calza AM. Diagnosis of IgE-mediated food allergy among Swiss children with atopic dermatitis. *Pediatr Allergy Immunol*. 2000;11:95–100.
41. Majamaa H, Laine S, Miettinen A. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. *Clin Exp Allergy*. 1999;29: 1502–1506.
42. Van Der Velden VH, Laan MP, Baert MR, et al. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-g, IL-4 and IL-10. *Clin Exp Allergy*. 2001;31:997–1006.
43. Kirjavainen PV, Apostolou E, Arvola T, et al. Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease. *FEMS Immunol Med Microbiol*. 2001;32:1–7.
44. Moro G, Arslanoglu S, Stahl B, et al. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Childhood*. 2006;91:814–819.
45. Nauta AJ, Arslanoglu S, Boehm G, et al. A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induced an anti-allergic Ig profile in infants at risk for allergy. *Proc Nutr Soc*. 2008;67:E82.
46. Cani PD, Neyrinck AM, Maton N, et al. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1. *Obes Res*. 2005;13:1000–1007.

47. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr*. 2009;89:1751–1759.
48. Roberfroid MB. Chicory fructooligosaccharides and the gastrointestinal tract. *Nutr*. 2000;16:677–679.
49. Gay-Crosier F, Schreiber G, Hauser C. Anaphylaxis from inulin in vegetables and processed food (lett). *N Engl J Med*. 2000;342:1372.
50. Buddington RK, Williams CH, Chen S, et al. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *Am J Clin Nutr*. 1996;63:709–716.
51. Trenev N. *Probiotics: Nature's Internal Healers.* New York: Avery Publishing.
52. Miller AL. The pathogenesis, clinical implications, and treatment of intestinal hyperpermeability. *Altern Med Rev*. 1997;2:330–345.
53. Moore WEC, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol*. 1974;27:961–979.
54. Stahl B, Zens Y, Boehm G. Prebiotic with sPecial emphasis on fructo-, galacto-, galacturono-, and xylooligosaccharides. In: Kamerling P, ed. *Comprehensive Glycoscience.* Oxford: Elsevier: Oxford:725–742.
55. Gosling A, Stevens GW, Barber AR, et al. Recent advances refining galactooligosaccharide production from lactose. *Food Chem*. 2010;121:307–318.
56. Boehm G, Moro G. Structural and functional aspects of prebiotics used in infant nutrition. *J Nutr*. 2008;138:S1818–S1828.
57. Macfarlane GT, Steed H, Macfarlane S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J Appl Microbiol*. 2008;104:305–344.
58. Bouhnik Y, Raskine L, Simoneau G, et al. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr*. 2004;80:1658–1664.
59. Teuri U, Korpela R. Galacto-oligosaccharides relieve constipation in elderly people. *Ann Nutr Metab*. 1998;42:319–327.
60. Niittyen L, Kajander K, Korpela R. Galacto-oligosaccharides and bowel function. *Scand J Food Nutr*. 2007;51:62–66.
61. Silk DB, Davis A, Vulevic J, et al. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Therap*. 2009;29:508–518.
62. Shoaf K, Mulvey GL, Armstrong GD, et al. Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infect Immun*. 2006;74:6920–6928.
63. Sinclair HR, de Slegte J, Gibson GR, et al. Galactooligosaccharides (GOS) inhibit *Vibrio cholerae* toxin binding to its GM1 receptor. *J Agric Food Chem*. 2009;57:3113–3119.
64. Searle LE, Best A, Nunez A, et al. A mixture containing galactooligosaccharide, produced by the enzymic activity of *Bifidobacterium bifidum,* reduces *Salmonella enterica* serovar *Typhimurium* infection in mice. *J Med Microbiol*. 2009;58:37–48.
65. Drakoularakou A, Tzortzis G, Rastall RA, et al. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. *Eur J Clin Nutr*. 2009;64:146–152.
66. van den Heuvel E, Schoterman MHC, Mujis T. Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women. *J Nutr*. 2000;130:2938–2942.
67. Kobayashi M, Yasutake N, Uchida K, et al. Safety of a novel galacto-oligosaccharide: genotoxicity and repeated oral dose studies. *Human Exp Toxicol*. 2009;28:619–630.
68. Toshihide K, Kazumi U, Kimiyuki K, et al. Ninety days repeated oral dose toxicity study of GOS in rats. *Annual Rep Yakult Ins Microbiol Res*. 2004;23:25–42.
69. Montgomery E, Hudson CS. Transformation of lactose to a new disaccharide, lactoketose. *Science*. 1929;69:556–567.
70. Clausen MR, Mortensen PB. Lactulose, disaccharides and colonic flora. *Drugs*. 1997;53:930–942.
71. Mortensen PB, Rasmussen HS, Holtug K. Lactulose detoxifies *in vitro* short-chain fatty acid production in colonic contents induced by blood: implications for hepatic coma. *Gastroenterology*. 1988;94:750–754.
72. Bown RL, Gibson JA, Sladen GE, et al. Effects of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. *Gut*. 1974;15:999–1004.
73. MacGillivray PC, Finlay HVL, Binns TB. Use of lactulose to create a preponderance of lactobacilli in the intestine of bottle-fed infants. *Scot Med J*. 1959;4:182–189.
74. Terada A, Hara H, Kataoka M, et al. Effect of lactulose on the composition and metabolic activity of the human faecal flora. *Microb Ecol Health Dis*. 1992;5:43–50.
75. Ballongue J, Schumann C, Quignon P. Effects of lactulose and lactitol on colonic microflora and enzymatic activity. *Scand J Gatroenterol*. 1997;32(suppl 222):41–44.
76. Knothe H, Knapp G, Meyer M, et al. Therapy of salmonella enteritis with special reference to lactulose (author's translation). *Infection*. 1980;8(suppl 3):294–298. [German].
77. Kurtovic J, Segal I, Riordan SM. Culture-proven small intestinal bacterial overgrowth as a cause of irritable bowel syndrome: response to lactulose but not broadspectrum antibiotics. *J Gastroenterol*. 2005;40:767–770.
78. Salminen S, Salminen E. Lactulose, lactic acid bacteria, intestinal microecology and mucosal protection. *Scand J Gastroenterol*. 1997;32(suppl 222):45–48.
79. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of lactic acid bacteria. *Crit Rev Food Sci Nutr*. 1999;38:13–126.
80. Bernhardt H, Knoke M. Mycological aspects of gastrointestinal microflora. *Scand J Gastroenterol*. 1997;32(suppl 222):102–107.
81. Topping DL. Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific J Clin Nutr*. 1996;5(suppl):15–19.
82. Bass P, Dennis S. The laxative effects of lactulose in normal and constipated subjects. *J Clin Gastroenterol*. 1981;3(suppl 1):23–28.
83. Nagengast FM, Hectors MPC, Buys WAM, et al. Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups. *Eur J Clin Invest*. 1988;18:56–61.
84. Bird SP, Hewitt D, Ratcliffe B, et al. Effects of lactulose and lactitol on protein digestion and metabolism in conventional and germ free animal models: relevance of the results to their use in the treatment of portosystemic encephalopathy. *Gut*. 1990;31:1403–1406.
85. Macfarlane GT, Gibson GR. Metabolic activities of the normal colonic flora. In: Gibson GR, Macfarlane GT, eds. *Human Health: The Contribution of Microorganisms.* London: Springer-Verlag:17–53.
86. Macfarlane S, Macfarlane GT. Proteolysis aNd amino acid fermentation. In: Gibson GR, Macfarlane GT, eds. *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology.* Boca Raton: CRC Press:75–100.
87. Roncucci L, Di Donato P, Carati L, et al. Antioxidant vitamins or lactulose for the prevention of the recurrence of colorectal polyps. *Dis Colon Rectum*. 1993;67:227–234.
88. In: Porth C, ed. *PathophysIology: Concepts of Altered Health States.* Philadelphia: Lippincott.
89. Horsmans Y, Solbreux PM, Daenens C, et al. Lactulose improves psychometric testing in cirrhotic patients with subclinical encephalopathy. *Aliment Pharmacol Therap*. 1997;11:165–170.
90. Watanabe A, Sakai T, Sato S, et al. Clinical efficacy of lactulose in cirrhotic patients with and without subclinical hepatic encephalopathy. *Hepatology*. 1997;26:1410–1414.
91. Dhiman RK, Sawhney MS, Chawla YK, et al. Efficacy of lactulose in cirrhotic patients with subclinical hepatic encephalopathy. *Dig Dis Sci*. 2000;45:1549–1552.
92. Sharma BC, Sharma P, Agrawal A, et al. Secondary prophylaxis of hepatic encephalopathy: an open-label randomized controlled trial of lactulose versus placebo. *Gastroenterol*. 2009;137:885–891.
93. Tannock GW. The bowel microflora: an important source of urinary tract pathogens. *World J Urol*. 1999;17:339–344.
94. Houdijk APJ, Meijer C, Cuesta MA, et al. Perioperative anti-endotoxin strategies. *Scand J Gastroenterol*. 1997;32(suppl 222):93–97.
95. Liehr H, Englisch G, Rasenack U. Lactulose - a drug with antiendotoxin effect. *Hepato-Gastroenterology*. 1980;27:356–360.
96. Pain JA, Bailey ME. Experimental and clinical study of lactulose in obstructive jaundice. *Br J Surg*. 1986;73:775–778.

97. McCutcheon J, Fulton JD. Lowered prevalence of infection with lactulose therapy in patients in long-term hospital care. *J Hosp Infect*. 1989;13:81–86.
98. Mack DJ, Erwin L, Fulton JD. Chronic constipation in elderly patients. *BMJ*. 1993;307(6916):1425–1426.
99. Seki N, Hamano H, Iiyama Y, et al. Effect of lactulose on calcium and magnesium absorption: a study using stable isotopes in adult men. *J Nutr Sci Vitaminol*. 2007;53:5–12.
100. Sanders JF. Lactulose syrup assessed in a double-blind study of elderly constipated patients. *J Am Geriatr Soc*. 1978;26:236–239.
101. Riggio O, Varriale M, Testore GP, et al. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. *J Clin Gastroenterol*. 1990;12:433–436.
102. Liao W, Cui XS, Jin XY, et al. Lactulose- a potential drug for the treatment of inflammatory bowel disease. *Med Hypotheses*. 1994;43:234–238.
103. Flourie B, Briet F, Florent C, et al. Can diarrhea induced by lactulose be reduced by prolonged ingestion of lactulose? *Am J Clin Nutr*. 1993;58:369–375.
104. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. *Am J Clin Nutr*. 2001;73:361–364.
105. Casiraghi MC, Canzi E, Zanchi R, et al. Effects of a synbiotic milk product on human intestinal ecosystem. *J Appl Microbiol*. 2007;103:499–506.
106. Vernazza CL, Gibson GR, Rastall RA. Carbohydrate preference, acid tolerance and bile tolerance in five strains of *Bifidobacterium*. *J Appl Microbiol*. 2006;100:846–853.
107. Kontula P, Suihko ML, Von Wright A, et al. The effect of lactose derivatives on intestinal lactic acid bacteria. *J Dairy Sci*. 1999;82:249–256.
108. Kaplan H, Hutkins RW. Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Appl Exp Microbiol*. 2000;66:2682–2684.
109. Salyers AA, Leedle JAZ. CarbohydrAte metabolism in the human colon. In: Hentges DJ, ed. *Human Intestinal Microflora in Health and Disease.* New York: Academic Press:129–146.
110. Bernalier A, Dore J, Durand M. BiochemistRy of Fermentation. In: Gibson GR, Roberfroid M, eds. *Colonic Microbiota, Nutrition and Health.* Dordoecht: Kluwer Academic Publishers:37–54.
111. Szilagyi A, Rivard J, Shrier I. Diminished efficacy of colonic adaptation to lactulose occurs in patients with inflammatory bowel disease. *Dig Dis Sc*. 2002;47:2811–2822.
112. Visser LE, Penning-van Beest FJA, Wilson JHP, et al. Overanticoagulation associated with combined use of lactulose and acenocoumarol or phenprocoumon. *Br J Clin Pharmacol*. 2004;57:522–524.
113. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14(8):491–502.
114. Dehghan P, Pourghassem Gargari B, Asghari Jafar-abadi M. Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized controlled clinical trial. *Nutrition*. 2014;30(4):418–423.
115. Cuello-Garcia C, Fiocchi A, Pawankar R, et al. Prebiotics for the prevention of allergies: a systematic review and meta-analysis of randomized controlled trials. *Clin Exp Allerg*. 2017;47(11):1468–1477.
116. Root HF, Baker ML. Inulin and artichokes in the treatment of diabetes. *Archiv Int Med*. 1925;36(1):126–145.
117. Le Bourgot C, Apper E, Blat S, Respondek F. Fructo-oligosaccharides and glucose homeostasis: a systematic review and meta-analysis in animal models. *Nutri Metabol*. 2018;15(1):9.
118. Pourghassem Gargari B, Dehghan P, Aliasgharzadeh A, Asghari Jafar-abadi M. Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes. *Diabetes Metab J*. 2013;37(2):140–148.
119. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutri*. 2014;65(1):117–123.
120. Roshanravan N, Mahdavi R, Alizadeh E, et al. The effects of sodium butyrate and inulin supplementation on angiotensin signaling pathway via promotion of Akkermansia muciniphila abundance in type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *J Cardiovasc Thorac Res*. 2017;9(4):183–190.
121. Varju P, Farkas N, Hegyi P, et al. Low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet improves symptoms in adults suffering from irritable bowel syndrome (IBS) compared to standard IBS diet: a meta-analysis of clinical studies. *PLoS One*. 2017;12(8):e0182942.
122. Major G, Pritchard S, Murray K, et al. Colon hypersensitivity to distension, rather than excessive gas production, produces Carbohydrate-related symptoms in individuals with irritable bowel syndrome. *Gastroenterology*. 152(1):124–133.e122.
123. Azpiroz F, Dubray C, Bernalier-Donadille A, et al. Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. *Neurogastroenterol Motil*. 2017;29(2):e12911-n/a.
124. Hong YH, Chang UJ, Kim YS, Jung EY, Suh HJ. Dietary galacto-oligosaccharides improve skin health: a randomized double blind clinical trial. *Asia Pac J Clin Nutr*. 2017;26(4):613–618.
125. Jung EY, Kwon JI, Hong YH, Suh HJ. Evaluation of anti-wrinkle effects of DuOligo, composed of lactulose and galactooligosaccharides. *Prev Nutr Food Sci*. 2017;22(4):381–384.
126. Silk DB, Davis A, Vulevic J, et al. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2009;29:508–518.
127. Burokas A, Arboleya S, Moloney RD, et al. Targeting the microbiota-gut-brain Axis: prebiotics have Anxiolytic and Antidepressant-like effects and Reverse the impact of chronic stress in mice. *Biol Psychiatry*. 2017.
128. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berl)*. 2015;232(10):1793–1801.
129. Bouhnik Y, Flourié B, D'Agay-Abensour L, et al. Administration of Trans-galacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutri*. 1997;127(3):444–448.
130. Whisner CM, Martin BR, Schoterman MH, et al. Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. *Br J Nutr*. 2013;110(7):1292–1303.
131. Fanaro S, Marten B, Bagna R, et al. Galacto-oligosaccharides are bifidogenic and safe at weaning: a double-blind randomized multicenter study. *J Pediatr Gastroenterol Nutr*. 2009;48(1):82–88.
132. Shadid R, Haarman M, Knol J, et al. Effects of galactooligosaccharide and long-chain fructooligosaccharide supplementation during pregnancy on maternal and neonatal microbiota and immunity—a randomized, double-blind, placebo-controlled study. *Am J Clin Nutr*. 2007;86(5):1426–1437.
133. Teuri U, Korpela R. Galacto-oligosaccharides relieve constipation in elderly people. *Annals Nutri Metabol*. 1998;42:319–327.
134. Guarner F. The gut microbiome: what do we know? *Clin Liver Dis*. 2015;5(4):86–90.
135. Malaguarnera G, Giordano M, Nunnari G, Bertino G, Malaguarnera M. Gut microbiota in alcoholic liver disease: pathogenetic role and therapeutic perspectives. *World J Gastroenterol*. 2014;20(44):16639–16648.
136. Lukiw WJ. Bacteroides fragilis lipopolysaccharide and inflammatory signaling in Alzheimer's disease. *Front Microbiol*. 2016;7:1544.
137. Wang J, Si Y, Wu C, et al. Lipopolysaccharide promotes lipid accumulation in human adventitial fibroblasts via TLR4-NF-κB pathway. *Lipids Health Dis*. 2012;11(1):139.
138. Charalambous BM, Stephens RC, Feavers IM, Montgomery HE. Role of bacterial endotoxin in chronic heart failure: the gut of the matter. *Shock*. 2007;28(1):15–23.
139. Giloteaux L, Goodrich JK, Walters WA, Levine SM, Ley RE, Hanson MR. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome*. 2016;4(1):30.
140. Grigoleit JS, Kullmann JS, Wolf OT, et al. Dose-dependent effects of endotoxin on neurobehavioral functions in humans. *PLoS One*. 2011;6(12):e28330.

141. Jialal I, Rajamani U. Endotoxemia of metabolic syndrome: a pivotal mediator of meta-inflammation. *Metabol Syndrome Relat Disord*. 2014;12(9):454–456.
142. Alisi A, Ceccarelli S, Panera N, Nobili V. Causative role of gut microbiota in non-alcoholic fatty liver disease pathogenesis. *Front Cellul Infect Microbiol*. 2012;2:132.
143. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia Initiates obesity and insulin resistance. *Diabetes*. 2007;56(7):1761–1772.
144. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes Care*. 2011;34(2):392–397.
145. Robinson RR, Feirtag J, Slavin JL. Effects of dietary arabinogalactan on gastrointestinal and blood parameters in healthy human subjects. *J Am Coll Nutri*. 2001;20(4):279–285.
146. Chen HL, Cheng HC, Wu WT, Liu YJ, Liu SY. Supplementation of konjac glucomannan into a low-fiber Chinese diet promoted bowel movement and improved colonic ecology in constipated adults: a placebo-controlled, diet-controlled trial. *J Am Coll Nutr*. 2008;27(1):102–108.
147. Asakuma S, Hatakeyama E, Urashima T, et al. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. *J Biol Chem*. 2011;286(40):34583–34592.
148. Kaneko T, Kohmoto T, Fukui F, et al. Acute and chronic toxicity and Mutagenicity studies on Isomaltooligosaccharides, and the effects on Peripheral blood Lymphocytes and intestinal microflora. *Food Hyg Safety Sci (Shokuhin Eiseigaku Zasshi)*. 1990;31(5):394–403_391.
149. Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. Acacia gum is a bifidogenic dietary Fibre with high digestive tolerance in healthy humans. *Microb Ecol Health Dis*. 2003;15(1):43–50.
150. Ohashi Y, Sumitani K, Tokunaga M, Ishihara N, Okubo T, Fujisawa T. Consumption of partially hydrolysed guar gum stimulates Bifidobacteria and butyrate-producing bacteria in the human large intestine. *Benef Microbes*. 2015;6(4):451–455.
151. Moshfegh AJ, Friday JE, Goldman JP, et al. Presence of inulin and oligofructose in the diets of Americans. *J Nutri*. 1999;129:1407S–1411S.
152. Van Loo J, Coussement P, De Leenheer L, et al. On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Crit Rev Food Sci Nutri*. 1995;35:525–552.
153. Buddington RK, Williams CH, Chen S, et al. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *Am J Clin Nutri*. 1996;63:709–716.
154. Mitsuoka T, Hidaka H, Eida T. Effect of fructo-oligosaccharides on intestinal microflora. *Die Nahrung*. 1987;31:5–6.
155. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr*. 2009;101(4):541–550.
156. Gibson GR, Beatty ER, Wang X, et al. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*. 1995;108:975–982.
157. Kleessen B, Schwarz S, Boehm A, et al. Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. *Br J Nutr*. 2007;98(3):540–549.
158. Kleesen B, Sykura B, Zunft HJ, et al. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *Am J Clin Nutri*. 1997;65:1397–1402.
159. Muir JG, Rose R, Rosella O, et al. Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *J Agricul Food Chem*. 2009;57(2):554–565.
160. Biesiekierski JR, Rosella O, Rose R, et al. Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. *J Hum Nutri Dietet Offl J Br Dietet Ass*. 2011;24(2):154–176.
161. Ito M, Deguchi Y, Matsumoto K, et al. Influence of galactooligosaccharides on the human fecal microflora. *J Nutr Sci Vitaminol*. 1993;39:635–640.
162. Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson GR. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *Am J Clin Nutr*. 2008;88(5):1438–1446.
163. Davis LMG, Martinez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One*. 2011;6(9):e25200.
164. Liu Z, Lin X, Huang G, Zhang W, Rao P, Ni L. Prebiotic effects of almonds and almond skins on intestinal microbiota in healthy adult humans. *Anaerobe*. 2014;26:1–6.
165. Shinohara K, Ohashi Y, Kawasumi K, Terada A, Fujisawa T. Effect of apple intake on fecal microbiota and metabolites in humans. *Anaerobe*. 2010;16(5):510–515.
166. Molan AL, Liu Z, Plimmer G. Evaluation of the effect of Blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother Res*. 2014;28(3):416–422.
167. Vendrame S, Guglielmetti S, Riso P, Arioli S, Klimis-Zacas D, Porrini M. Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. *J Agric Food Chem*. 2011;59(24):12815–12820.
168. Benno Y, Endo K, Miyoshi H, Okuda T, Koishi H, Mitsuoka T. Effect of rice fiber on human fecal microflora. *Microbiol Immunol*. 1989;33(5):435–440.
169. Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Uribe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *Am J Clin Nutr*. 2011;93(1):62–72.
170. Goto K, Kanaya S, Nishikawa T, et al. The influence of tea catechins on fecal flora of elderly residents in long-term care facilities. *Ann Long-Term Care*. 1998;6:43–48.
171. Alfa MJ, Strang D, Tappia PS, et al. A randomized trial to determine the impact of a digestion resistant starch composition on the gut microbiome in older and mid-age adults. *Clin Nutri*. 2017;37(3):797–807.

Probiotics

Jason A. Hawrelak, ND, BNat(Hons), PhD

OUTLINE

INTRODUCTION

The term *probiotic* (from the Greek *for life*) has had a number of different meanings over recent history. First coined by Lilley and Stillwell, they used the term to describe substances secreted by one microorganism that stimulated the growth of another.[1] In 1974 this definition was modified by Parker to mean "…*organisms and substances which contribute to intestinal microbial balance,*" which more closely approximates the contemporary use of the term.[2] The current definition comes from the International Scientific Association for Probiotics and Prebiotics.[3] They define probiotics as "*live microorganisms that, when administered in adequate amounts, confer a health benefit to the host.*"

The current definition of probiotics includes preparations that contain viable, microbial agents that have been demonstrated to improve health. Typically, these products will contain freeze-dried (lyophilized) or live bacteria or yeasts, most commonly from the genera *Lactobacillus* and *Bifidobacterium.*[4]

The original definitions of probiotics were inclusive of traditional fermented foods such as yogurt (nonmedicinal varieties), sauerkraut, and kefir, but the most recent interpretation of the definition has, somewhat controversially, excluded these traditional ferments. These are now considered food sources of "*live and active cultures*" but not probiotics.[3] The reasoning behind this decision is that these foods may contain a diverse community of microbes that are not well defined in terms of strain composition or stability, both of which can also differ from batch to batch. Additionally, the strains contained in these foods may also lack specific therapeutic qualities (e.g., they may not confer any health benefit on the host, beyond the enhanced nutritional profile of the fermented food). It is for these reasons that traditional fermented foods (wild ferments) cannot be relied upon for specific therapeutic effects in the same way that probiotic preparations containing well-defined strains, with well-characterized clinical effects, in precise doses can.

Currently, there is an explosion of interest in the fields of fermented foods and probiotics. This interest has been stimulated by the ongoing expansion of research in these areas. This chapter focuses on the health benefits and therapeutic uses of probiotics.

DESCRIPTION

At the turn of the century, Metchnikoff[5] asserted that yogurt was the elixir of life. He theorized that putrefactive bacteria in the large intestine produce toxins that cause disease and shorten life. He believed that eating yogurt would cause lactobacilli to become dominant in the colon and displace the putrefactive bacteria. For years, these claims

of healthful effects from fermented foods were considered unscientific folklore. However, a substantial and growing body of scientific evidence has now demonstrated that lactobacilli, bifidobacteria, and, more broadly, fermented foods, can play a significant role in promoting human health and treating disease.

The bulk of probiotic research to date has focused on strains from two genera—*Lactobacillus* and *Bifidobacterium*. The genus *Lactobacillus* is characterized by considerable heterogeneity. They are gram-positive, nonsporing, and rod-shaped bacteria that produce lactic acid as the major end product of carbohydrate fermentation. Lactobacilli appear to be fairly unique, in that they have been isolated from a number of diverse environments, such as fermented vegetables and dairy foods, as well as the human gastrointestinal tract (GIT) and vagina.[6]

In contrast, bifidobacteria are not found in natural fermentative processes but are native to the GIT.[7] Bifidobacteria are also gram-positive, nonsporing bacteria; however, they are Y-shaped instead of rod-shaped, and their major fermentative end product is acetic acid.[8]

Research is currently underway on the isolation and commercialization of next-generation probiotics, using novel strains isolated from important gastrointestinal species, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*.[6,9] Other research has evaluated microbiologically complex "probiotic" products derived from human fecal specimens.[6,7] A number of so-called "soil-based probiotics" are now also commercially available and have a growing research base,[8] so expect the marketplace to expand dramatically in the variety of probiotic products over the coming decade.

PROPOSED MECHANISMS OF ACTION

The exact mechanisms by which probiotics accomplish their myriad beneficial actions have become clearer over the past two decades and we now know of several mechanisms that explain many of their favorable effects.

Immune Modulation

A growing body of evidence indicates that some probiotic strains are capable of modulating the immune system at both the systemic and the mucosal levels, affecting many cell types (e.g., dendritic cells, epithelial cells, natural killer cells, etc.). This immune response may take the form of increased secretion of immunoglobulin A via interaction with mesenteric lymph nodes,[10,11] enhanced phagocytic activity of macrophages, or elevated numbers of natural killer cells.[12] Recent research has demonstrated that dendritic cells in the lamina propria can extend their appendices between epithelial cells, and, via toll-like receptors on their surface, sample probiotic-bacterial molecular patterns. This interaction leads to the maturation of the dendritic cells and to the release of cytokines, which orchestrate the conversion of naive T-helper cells (Th0) into a mature, balanced response of T-helper cells (Th1, Th2, and Th3/Tr1).[11]

Anti-Inflammatory Activity

Probiotic strains have been shown to have anti-inflammatory effects via a number of different mechanisms. Some can secrete metabolites with anti-inflammatory properties (e.g., antitumor necrosis factor [TNF]-α effects),[13] some can interact with toll-like receptors,[14] others down-regulate the transcription of a number of genes encoding proinflammatory effectors,[15] and others upregulate production of anti-inflammatory cytokines.[16]

GIT Transit Time Modification

Some probiotic strains can modify GIT transit. Two strains of bifidobacteria have been found to significantly speed colonic transit time,[17,18] and a strain of propionibacteria has been shown to slow descending colon transit.[19] The mechanisms by which probiotics alter GIT transit time have not yet been fully elucidated; however, it is postulated that a bacterial metabolite may affect sigmoid tone and alter colonic motility.[20]

Induction of Oral Tolerance

The GIT microbiota has been shown to play a crucial role in generating an adequate population of Th2 cells that are capable of oral tolerance induction.[21] In the presence of intestinal dysbiosis, some probiotic strains can also help induce oral tolerance and help protect against the development of food allergies.[22] Other strains have been shown to induce oral tolerance even in the presence of anaphylactic reactions.[23]

Decrease Visceral Hypersensitivity

Animal research has suggested that some probiotic strains are capable of decreasing visceral hypersensitivity, believed to be one of the main contributing factors to irritable bowel syndrome (IBS) and other functional gastrointestinal disorders.[24-26] The exact mechanisms are unknown, but another lactobacilli strain has been shown to decrease visceral hypersensitivity by inducing cannabinoid and opioid receptor expression in the colonic epithelium.[27]

Competition for GI Adhesion Sites

Many pathogenic organisms must associate with the GIT epithelium to colonize effectively. However, some strains of bifidobacteria and lactobacilli can adhere to the epithelium and act as "colonization barriers" by preventing pathogens from adhering to the mucosa.[28] This effect has been demonstrated with *Lactobacillus rhamnosus* GG and *L. plantarum* 299v. Both of these organisms have shown the ability to inhibit attachment of *Escherichia coli* to human colon cells.[29]

Antagonism Against Potentially Pathogenic Microorganisms (PPMs) and Viruses

One of the mechanisms by which probiotics exert their beneficial effects is via inducing changes to the GIT microbiota, specifically by inhibiting the growth of potentially pathogenic organisms. Some probiotic strains are capable of producing inhibitory substances such as bacteriocins, lactic acid, and oxygen metabolites, such as hydrogen peroxide.[30,31] A number of probiotic strains have demonstrated in vitro inhibitory activity against a range of potentially pathogenic microorganisms, such as *Helicobacter pylori, Clostridium difficile, E. coli, Candida albicans, Salmonella enteric, Shigella sonnei,* and *Vibrio cholerae*.[32-35] Some probiotic strains (*L. rhamnosus* GG and *Bifidobacterium lactis* Bb12) can also bind to viruses, such as rotaviruses, helping prevent mucosa-associated viral infections.[36] Several probiotic strains have also demonstrated the ability to bind or remove toxins, such as aflatoxins and cyanotoxins,[36] as well as inhibit the effects of bacterial toxins, such as *C. difficile* toxins A and B.[37] Specific strains have also been found to reduce the expression of virulence factors via inhibition of pathogen gene encoding.[38]

Selective GI Antimicrobial Activity

Ingestion of selected probiotic strains has been found to significantly increase GI populations of beneficial bacteria (i.e., lactobacilli or bifidobacteria) while simultaneously decreasing populations of less health-promoting genera.[39-41]

Production of Beneficial Compounds

The production of short-chain fatty acids (SCFAs) as metabolic byproducts is a characteristic that almost all probiotic strains share. These SCFAs help create a healthier colonic milieu by decreasing the

luminal pH, and some (e.g., butyrate) are used as energy sources by colonocytes.[42,43] Other probiotic strains can help restore normal small intestinal architecture and upregulate intestinal brush border enzyme expression via the luminal release of polyamines.[44-46] These strains will have clinical utility in situations of small intestinal damage and decreased brush border enzyme activity, such as celiac disease, Crohn's disease, or after small intestinal infections.

Strengthen the Intestinal Barrier

A number of probiotic strains have been found to increase mucin production in the gut via increases in mucin gene expression, which provides a protective coating between the lumen and intestinal epithelial cells.[47,48] Probiotics are also capable of directly strengthening the intestinal barrier. A strain of *L. plantarum* (WCSF1) has been found to decrease paracellular intestinal permeability by increasing the relocation of occludin and zonulin into the tight junction between duodenal epithelial cells.[49] Other strains appear to enhance barrier function via the preservation of enterocyte cytoskeleton architecture and enhancement of tight junctional protein structures.[43] Such strains should prove useful in the treatment and prevention of intestinal permeability.

PROBIOTIC CHARACTERISTICS

Probiotic organisms require certain characteristics to enable them to exert maximum therapeutic effects. These qualities are summarized in Table 105.1.

Of these characteristics, there are some that are considered almost a prerequisite for a probiotic strain to have therapeutic effects. These are: (1) gastric acid and bile salt stability; (2) an ability to adhere to the intestinal mucosa; and (3) an ability to temporarily colonize the intestinal tract.[34] Unfortunately, many commercially available probiotic supplements and yogurts contain strains that do not exhibit these vital characteristics. If a probiotic strain does not exhibit these characteristics, then it will have only a limited capacity to interact with host cells.

Probiotics in Use

There are many different microorganisms currently used as probiotics. Table 105.2 lists commonly used probiotic species.

To better understand how bacteria are named and classified, the following discussion may be helpful. Genus is the first name of a bacterium (e.g., *Lactobacillus*). It is somewhat general and refers to a grouping of organisms based on similarity of qualities, such as physical characteristics, metabolic needs, and metabolic end products. Species is a bacterium's second name (e.g., *acidophilus*). It is a much more narrow classification based on shared common characteristics that distinguish them from other species. Strain is an even more specific classification that divides members of the same species into subgroups based on several properties that these bacteria have in common that are distinct from other members of the species (e.g., strain LA5).[37]

Issues in Probiotic Nomenclature

There are some changes in nomenclature, some more recent and some fairly antiquated, that should be noted to make better sense of probiotic literature.

- The species *Lactobacillus bulgaricus* is now referred to as *Lactobacillus delbrueckii* ssp. *bulgaricus*.[38]

TABLE 105.1 The Desirable Characteristics of Effective Probiotic Strains

Characteristics	Functional Benefit
Human origin	Human origin should translate to ability to survive conditions in the human GIT, as well as the possibility of species-specific health effects
Gastric acid and bile salt stability	Survival through stomach and small intestine
Adherence to intestinal mucosa	Believed to be essential for immune cell modulation and competitive inhibition of pathogens
Temporary colonization of intestinal tract	Multiplication in the intestines suggests that daily ingestion may not be needed; immune cell modulation
Safety in food and documented clinical safety	Adverse effects absent or minimal; accurate identification (genus, species, strain)
Production of antimicrobial compounds	Normalization of the GIT ecosystem; suppressed growth of pathogens
Antagonism against pathogenic organisms	Prevention of adhesion and toxin production by pathogens
Clinically documented and validated health effects	Clinicians can be confident of therapeutic effects; dose–response data for minimum effective dosage in different formulations is known

GIT, Gastrointestinal tract.
(Modified from Mattila-Sandholm T, Salminen S. Up-to-date on probiotics in Europe. *Gastroenterol Int.* 1998;11[suppl 1]:8-16.)[33]

TABLE 105.2 Common Probiotic Microorganisms

Lactobacillus spp.	*Bifidobacterium* spp.	*Bacillus* spp.	*Streptococcus* spp.	*Enterococcus* spp.	*Saccharomyces* spp.
acidophilus	*adolescentis*	*clausii*	*salivarius*	*faecium*	*cerevisiae*
brevis	*animalis*	*coagulans*	*thermophilus*		
casei	*bifidum*	*subtilis*			
crispatus	*breve*				
delbrueckii	*infantis*				
fermentum	*lactis*				
gasseri	*longum*				
johnsonii	*thermophilum*				
lactis					
paracasei					
plantarum					
reuteri					
rhamnosus					

The most common species currently used as probiotics.[33,35,36,153-156]

- *Lactobacillus bifidus* (also known as "bifidus") was renamed *Bifidobacterium bifidum* more than 30 years ago, yet the improper nomenclature is still sometimes used.[38]
- Many strains of bacteria that were once classified as *Lactobacillus casei* have been reclassified as strains of *Lactobacillus rhamnosus* (e.g., *L. rhamnosus* GG) or *Lactobacillus paracasei.*[50]
- Strains of *Lactobacillus sporogenes* have been renamed *Bacillus coagulans* (they are not true lactobacilli because they form spores).[40]
- Bacterial strains that were once classified as *Lactobacillus acidophilus* (often referred to as "acidophilus") have now been divided into six species: *L. acidophilus*, *L. gasseri*, *L. amylovorus*, *L. gallinarum*, *L. johnsonii*, and *L. crispatus.*[39]
- Strains of *Saccharomyces boulardii* are now definitively regarded as a distinct group within the species *Saccharomyces cerevisiae* should be referred to as *Saccharomyces cerevisiae* var. *boulardii.*[41,42] The improper nomenclature of *S. boulardii* is still widely used.

Importance of Strain

Within each species of bacteria there is a multitude of strains. Some probiotic strains are resilient and strong, with a demonstrated capacity to survive passage through the upper GIT and have therapeutic actions, whereas others cannot even survive transit through the stomach.[51] It is vital to note that just because one strain of bacteria in a given species has a proven action or characteristic, it does not mean that another strain will, too, even if they are closely related.

Strains of bacteria within the same species can have significantly different actions, properties, and characteristics, as these are all essentially strain-specific qualities.[52,53] For example, *L. plantarum* strain 299v has been shown to effectively reduce IBS symptoms,[54] whereas administration of *L. plantarum* strain MF1298 actually worsens IBS symptoms.[55] In another study, two strains of *L. rhamnosus* were used in a trial assessing their efficacy in the treatment of viral gastroenteritis. One strain was *L. rhamnosus* strain GG (LGG); the other was a strain found in a supplemental product (Lactophilus). LGG accelerated recovery from diarrhea, whereas the closely related strain did not.[44] Thus to achieve the desired therapeutic result, it is imperative to prescribe the precise probiotic strains that have demonstrated therapeutic and clinical efficacy in the condition in question.

Additionally, strains that work in one condition will not necessarily be effective in other conditions. For example, the multistrain preparation commonly referred to as Lab4 (containing *L. acidophilus* strains CUL21 and CUL60, *B. bifidum* CUL20, and *B. lactis* CUL34) appears effective in the management of IBS[56] but is ineffective in the prevention of antibiotic-associated side effects.[57] As another example, *L. rhamnosus* GG appears to be effective in the prevention of antibiotic-associated side effects[58] but not of any demonstrable benefit in urinary tract infections.[59]

Unfortunately, this strain specificity is not well known, leading to inaccurate extrapolations from the literature. For example, some supplement manufacturers will quote a study that used *L. rhamnosus* strain GG and then say that their probiotic supplement containing a strain of *L. acidophilus* or another strain of *L. rhamnosus* will do the same. This is quite incorrect. Unless proven, one cannot assume that a given strain of *L. acidophilus*, *B. bifidum*, or any other species of lactic acid bacteria will survive transit through the upper GIT, let alone colonize the intestines or have specific therapeutic actions. They might, but, unless proven, it is impossible to know.

Thus clinicians are urged to use well-researched probiotic strains whenever possible. By choosing well-researched strains, one can be assured of getting probiotics that have documented gastric acid and bile tolerance, can adhere to the intestinal mucosa, and can temporarily colonize the intestinal tract, as well as having proven therapeutic actions. This latter point is particularly important in improving the probability of achieving good clinical outcomes.

TABLE 105.3 The Pros and Cons of Different Probiotic Delivery Systems

Delivery System	Pros	Cons
Fermented dairy	Affordability and easy availability Ease of incorporation into daily patterns Additional nutritional benefits Enhanced bacterial survival through upper GIT (100 times less bacteria can be given per dose)[66] Effective in the upper GIT	Contains dairy proteins and lactose Taste can be issue Not suitable when traveling Not suitable for vegans
Capsules	Ease of administration Contain no binders	Not therapeutic in upper GIT (unless opened or chewed) May contain allergenic excipients Higher cost
Tablets	Ease of administration Effective in the upper GIT	May contain allergenic or otherwise problematic binders and excipients (e.g., gluten) Higher cost Chewable tablets may contain potentially problematic sweeteners
Oils	Ease of administration Effective in the upper GIT Dosages can be easily adjusted Can be incorporated into foods or drinks Contain no binders	
Powders	Effective in the upper GIT Dosages can be easily adjusted Can be incorporated into foods or drinks Contain no binders	

GIT, Gastrointestinal tract.

COMMERCIAL FORMS

Probiotic organisms can be incorporated into supplements (powders, capsules, tablets, oils, wafers) and foods (milk drinks, medicinal yogurts, fruit juices, confectionery bars, ice cream). All of these mediums essentially work as carriers for the probiotic organisms. Common probiotic delivery systems are compared in Table 105.3. Regardless of the form in which the microorganisms are consumed, for clinical efficacy, products containing probiotic organisms must provide live organisms in sufficient numbers to exert therapeutic effects.

The quality of probiotic preparations depends on two main factors: (1) the characteristics of the strains contained in the supplement; and (2) adequate viability, so that sufficient numbers of bacteria are viable at the point of consumption. Bacterial strains used in probiotic preparations should ideally demonstrate all the characteristics outlined in Table 105.1 and have evidence of efficacy in treating the clinical condition in question (see "Clinical Applications" section later).

Viability at consumption depends on a number of factors, such as proper manufacturing and the "hardiness" of the strain and packaging and storing the product in the right amount of moisture and at the correct temperature. Some strains of lactobacilli and bifidobacteria do not respond well to freeze-drying (lyophilization), spray drying, or conventional frozen storage, and excessive temperature during packaging or storage can dramatically reduce viability. Typically, unless the product has been shown to be stable, refrigeration is necessary during storage and ideally during transport. Some products may not have to be refrigerated until after the bottle has been opened, however, and others not at all.

Some manufacturers use enteric coatings on their tablets and capsules to improve survival through the acidic medium of the stomach. Research suggests that this practice does enhance survival through the upper GIT,[60] although enteric coatings are not necessary if the strain has demonstrated satisfactory tolerance to gastric acid.

Although there are a number of companies providing high-quality probiotic products, it can be difficult to sort through all of the manufacturers' claims of superiority. Additionally, market-basket surveys found that some supplements contain potentially pathogenic contaminants,[61] whereas others fail to contain the species and quantity of bacteria listed on the label.[62]

Clearly, the clinician needs documentation of strain designation, strain characteristics, product viability, microbiological content, and strain-specific research before prescribing to his or her patients.

CLINICAL APPLICATIONS

The clinical applications of probiotics have grown myriad in the past decade—from solely gastrointestinal conditions to a wide range of diseases and conditions affecting other body systems. Although they can still be used to promote optimal digestive health, they are more often being used to treat specific disease conditions.

Antibiotic Use

Antibiotic use frequently results in significant perturbations in the GIT microbiota and GI adverse events, such as diarrhea.[63] It was once believed that taking probiotics concurrently with antibiotics would be a waste of time and money, as the antibiotic would likely destroy all the administered probiotic bacteria. However, research conducted over the past 20 years clearly shows that concurrent administration of specific probiotic strains alongside antibiotics significantly decreases the incidence of antibiotic-related side effects. A number of meta-analyses have now shown conclusively that probiotics are an effective class of tools to decrease antibiotic-associated side effects.[64-67] Table 105.4 highlights the research examining the effect of specific probiotic strains and strain combinations on antibiotic-related GI adverse events, primarily antibiotic-associated diarrhea (AAD). Not all probiotic preparations have been found to be effective, however. For example, the multistrain preparation commonly referred to as Lab4 (containing *L. acidophilus* strains CUL21 and CUL60, *B. bifidum* CUL20, and *B. lactis* CUL34) has been found to be ineffective in the prevention of antibiotic-associated side effects.[57]

Abdominal Pain (Functional)

A meta-analysis of trials evaluating *L. rhamnosus* GG (LGG) in the treatment of functional abdominal pain in children found LGG effective. LGG supplementation was associated with a significantly higher rate of treatment responders (defined as no pain or a decrease in pain intensity) in the overall population of children with abdominal pain-related functional gastrointestinal disorders (three trials; $n = 290$; relative risk [RR] 1.31, 95% CI 1.08–1.59) and in the IBS subgroup (three randomized controlled trials [RCTs], $n = 167$; RR 1.70, 95% CI 1.27–2.27, NNT 4, 95% CI 3–8). There was also a significant decrease in the perception of pain intensity in children with functional abdominal pain.[62]

Other probiotic strains, such as *B. infantis* 35624 and the combination of *B. lactis* Bi-07 and *L. acidophilus* NCFM, have been found to be ineffective in reducing functional abdominal pain, although the latter combination did improve abdominal bloating scores.[68,69]

Atopic Eczema

The intestinal microbiota plays a major protective role against the development of allergy through its ability to reduce antigen transport through the intestinal mucosa and induce oral tolerance.[70] Consequently, probiotics have been theorized to have a protective role in the prevention and/or management of atopic dermatitis (AD) and eczema.[71] A number of clinical trials have investigated probiotic therapy to prevent atopic eczema development, and some have evaluated the efficacy of probiotic therapy in the treatment of atopic eczema.

Prevention of Atopic Eczema

The initial ground-breaking research detailing the efficacy of probiotics in the prevention of atopic eczema used the probiotic strain *L. rhamnosus* GG (LGG).[72] This initial positive result led to a number of follow-up trials—some using the same strain and others evaluating other probiotic strains. A 2015 meta-analysis found that probiotics, as a class of agents, are effective in reducing the risk of atopic eczema. Meta-analysis found a reduced risk of eczema when used by women during the last trimester of pregnancy (RR = 0.71; 95% CI 0.60–0.84), when probiotics were used by breastfeeding mothers (RR = 0.57; 95% CI 0.47–0.69), or when given directly to infants (RR = 0.80; 95% CI 0.68–0.94).[73] The conclusions of this review have been criticized, however, because the authors combined the data from a number of different probiotic strains into a single analysis—a process that does not provide meaningful guidance to consumers or practitioners about which probiotic strain should be used in this clinical scenario. In fact, a meta-analysis by strain on the only probiotic preparation with more than one RCT for this application (LGG) found a nonsignificant decreased relative risk of eczema at 12 to 24 months (RR = 0.68; 95% CI 0.42–1.10).[74]

A number of other probiotic strains have been evaluated for their effectiveness in the prevention of atopic eczema with differing results. In a randomized, double-blind, placebo-controlled trial, Taylor et al. administered *L. acidophilus* strain LAVRI-A1 (3×10^9 CFU/day) to infants at high risk of allergic disease for the first 6 months of life. At both 6 and 12 months, the incidence of atopic dermatitis was similar in both the probiotic and placebo groups. However, the proportion of children with a positive skin prick test and atopic eczema was significantly higher in the probiotic group ($P = 0.045$) at 12 months, as was the rate of allergen sensitization ($P = 0.030$). Hence, this strain was not only ineffective in decreasing the incidence of atopic dermatitis development, it appears to have actually increased allergen sensitization, despite promising preliminary in vitro results.[75]

Abrahamsson et al. conducted a double-blind, randomized, placebo-controlled trial investigating the role of *L. reuteri* strain BioGaia in the prevention of atopic dermatitis development ($n = 232$). The mothers received *L reuteri* BioGaia (1×10^8 CFU) daily from gestational week 36 until delivery. Their babies then continued with the same product from birth until 12 months of age. The incidence of eczema was found to be similar in both groups at 2 years of age. Infants in the probiotic group did, however, have less immunoglobulin E-associated eczema (8 vs. 20%; $P = 0.02$) and in infants with allergic mothers, less skin-prick test reactivity (14 vs. 31%; $P = 0.02$).[76]

Wickens et al. performed a randomized, double-blind, placebo-controlled trial to assess the effect of two different probiotic strains

TABLE 105.4 Effect of Probiotics on Antibiotic-Related GI Adverse Events

Trial Methodology	Strain(s) Used	Results
Meta-analysis 5 studies *n* = 445 subjects of all ages	*Lactobacillus rhamnosus* GG	Pooled data from randomized, controlled trials found this strain reduced the risk of AAD in children from 23%–9.6% (RR = 0.48; 95% CI 0.26–0.89) and in a subset of adult patients receiving antibiotics as part of *Helicobacter pylori* eradication therapy (RR = 0.26; 95% CI 0.11–0.59).[67]
Meta-analysis 5 studies *n* = 1076 subjects of all ages	*Saccharomyces cerevisiae* var. *boulardii* Biocodex	Supplementation reduced the risk of AAD from 17.2% in controls to 6.7% (RR = 0.43; 95% CI 0.23–0.78).[160]
R, DB, PC *n* = 162 adult subjects receiving antibiotic treatment for *H. pylori* infection	*L. acidophilus* strains CUL-60 and CUL-21, *Bifidobacterium lactis* CUL-34, and *B. bifidum* CUL-20 (Lab4)	Coadministration of the probiotic combination with antibiotics prevented the increase in fecal *Candida albicans* populations immediately after antibiotic therapy ($P = 0.049$).[161]
R, DB, PC *n* = 239	*L. plantarum* 299v	Thirty-one percent reduced risk of developing loose or watery stools (95% CI 0.52–0.92; $P = 0.012$); 49% reduced risk of experiencing nausea (95% CI 0.30–0.85; $P = 0.0097$).[162]
R, DB, PC *n* = 40 pediatric subjects	*L. reuteri* DSM 17938	Reduced incidence of a number of GI symptoms relative to placebo in triple therapy-treated children—epigastric pain (15% vs. 45%; $P < 0.04$), abdominal distension (0% vs. 25%; $P < 0.04$), disorders of defecation (15% vs. 45%; $P < 0.04$), and halitosis (5% vs. 35%; $P < 0.04$).[163]
R, DB, PC *n* = 87	*L. rhamnosus* GG, *L. acidophilus* LA5, and *B. lactis* Bb12	Seventy-nine percent reduced risk of developing AAD ($P = 0.035$).[164]
R, DB, PC *n* = 72 pediatric subjects	*L. rhamnosus* GG, *L. acidophilus* LA5, and *B. lactis* Bb12	Concurrent ingestion of these three strains in a yogurt base resulted in a reduced risk of AAD in children. Severe diarrhea occurred in 17% of controls versus 0% of the probiotic group ($P = 0.025$). Minor diarrhea occurred in 58% of controls compared with 3% of the probiotic-treated children ($P < 0.001$).[138]
R, DB, PC *n* = 135 hospital inpatients	*L. casei* DN-114 001	Patients treated with *L. casei* DN-114 001 had a decreased risk of developing AAD compared with placebo (12% vs. 34%; $P = 0.007$). There was zero incidence of *Clostridium difficile*-associated diarrhea in the probiotic group versus 17% in placebo group ($P = 0.001$).[60]
R, DB, PC *n* = 437	*L. acidophilus* CL 1285 and *L. casei* LBC80R	Patients receiving antibiotic therapy had reduced incidence ($P = 0.037$) and duration ($P = 0.045$) of AAD compared with placebo-controls.[61]

AAD, Antibiotic-associated diarrhea; *DB,* double-blind; *GI,* gastrointestinal; *PC,* placebo-controlled; *R,* randomized.

on the development of atopic eczema. Pregnant women with an atopic family history were randomized to take *L. rhamnosus* HN001 or *B. lactis* strain HN019 or placebo daily from 35 weeks' gestation until 6 months if breastfeeding, and their infants were randomized to receive the same treatment from birth to 2 years ($n = 474$). Infants in the *L. rhamnosus* HN001 group had a 49% reduced risk of eczema development at 2 years of age (95% CI 0.30–0.85; $P = 0.01$). On the other hand, there was no reduction in eczema risk in infants receiving *B. lactis* strain HN019.[77]

In another randomized, placebo-controlled, double-blind trial, the efficacy of *L. paracasei* F-19 was evaluated. In this trial, the probiotic was administered to the infant ($n = 179$) directly at the time of weaning (mixed into food). Infants consumed the probiotic agent (1×10^8 CFU/day) from 4 to 13 months of age. Probiotic supplementation resulted in a significantly reduced cumulative incidence of eczema at 13 months of age (11 vs. 22%; $P < 0.05$) and an improved Th1/Th2 ratio.[78]

In a further double-blind trial, women ($n = 415$) were randomized to receive either placebo or a probiotic milk (containing *L. rhamnosus* GG, *L. acidophilus* La5, and *B. lactis* Bb12) from 36 weeks' gestation to 3 months postnatally during breastfeeding. At 2 years old, the odds ratio (OR) for the cumulative incidence of atopic eczema was 0.51 in the probiotic group compared with the placebo (95% CI 0.30–0.87; $P = 0.013$). There were no significant effects on rates of asthma or atopic sensitization.[79]

Treatment of Atopic Eczema

To determine whether probiotics, as a class of agents, are efficacious in treating atopic eczema (AE), Kim et al. performed a meta-analysis of randomized, controlled trials. Twenty-five eligible studies were located, including 1599 subjects. Significant differences in eczema severity (SCORAD values) favoring probiotics over placebo were observed for the total population (mean −4.51; 95% CI −6.78 to −2.24), in children aged 1 to 18 years old (mean −5.74; 95% CI −7.27 to −4.20), and in adults (mean −8.26; 95% CI −13.28 to −3.25). However, the effectiveness of probiotics as a whole in infants (< 1 year old) was not demonstrated.[80] Individual probiotic preparations that have been evaluated in the treatment of atopic eczema are highlighted in the following section.

A randomized, double-blind study of 56 young children (aged 6–18 months) with moderate or severe atopic AE found that treatment with *L. fermentum* VRI-003 PCC (1×10^9 CFU; twice daily) produced a significant reduction in the SCORAD index. At week 16, 92% of children receiving the probiotic had a SCORAD index that was significantly better than baseline compared with 63% in the placebo group ($P = 0.01$).[81]

A randomized, controlled trial conducted by Sistek et al. found that a combination of two probiotic strains (*L. rhamnosus* HN001 and *B. lactis* HN019) given to children with established AE effectively reduced the SCORAD index among the food-sensitized children ($P = 0.047$) but not in the group as a whole. Children in this study received 2×10^{10} CFU/day of the probiotic combination or placebo.[82]

Gastrointestinal Infections

Probiotics have a role in the management of various gastrointestinal infections, such as bacterial and viral-induced diarrhea, *H. pylori,* travelers' diarrhea prevention, and recurrent *C. difficile*-associated disease.

Diarrhea

Various probiotic strains have been subjected to clinical trials to evaluate their efficacy in diarrhea of different origins.

Viral Gastroenteritis—Prevention

In a randomized, double-blind, placebo-controlled trial, infants admitted to a chronic medical hospital (average duration of stay 80 days) ingested formula that was supplemented with either placebo or a combination probiotic. The probiotic contained *B. lactis* Bb12 (1.9×10^8 CFU/g powdered formula) and the TH-4 strain of *Streptococcus thermophilus* (0.14×10^8 CFU/g powdered formula). Probiotic supplementation was found to significantly reduce diarrhea incidence (7 vs. 31%; $P = 0.035$) and rotavirus shedding (10 vs. 39%; $P = 0.025$).[83]

Another randomized, controlled trial found the administration of *L. rhamnosus* GG to hospitalized children to reduce the risk of rotavirus gastroenteritis by 87% ($P = 0.02$).[84]

Viral Gastroenteritis—Treatment

A meta-analysis of eight RCTs ($n = 988$) by Szajewska et al. found that supplementation of *L. rhamnosus* GG significantly reduced duration of rotavirus diarrhea by 2.1 days in children ($P = 0.006$), and the risk of diarrhea lasting > 7 days was reduced by 75% ($P = 0.01$). The authors did caution, however, that the heterogeneity and methodological considerations of the studies limited the strength of the conclusions.[85]

To determine the dose-dependent effect of *L. rhamnosus* Lcr35 (Lcr35) on fecal rotavirus shedding, Fang et al. conducted an open-label randomized study. Twenty-three children with rotavirus gastroenteritis were treated for 3 days with either a placebo, a low dose of Lcr35 (2×10^8 CFU/day), or a high dose (6×10^8 CFU/day). Only the high-dose group experienced a significant reduction of rotavirus levels in stool samples (an 86% decrease after 3 days).[86]

A randomized, placebo-controlled trial comparing the efficacy of two different doses of *L. reuteri* DSM 17938 (1×10^{10} CFU/day or 1×10^7 CFU/day) with placebo in children with rotavirus-associated diarrhea ($n = 66$) found that probiotic treatment reduced the duration of viral gastroenteritis-induced diarrhea from 2.5 days in the placebo group to 1.9 days in the low-dose group and 1.5 days in high dose group ($P = 0.01$). By the second day of treatment, watery diarrhea persisted in 80% of the placebo, 70% of the low-dose group, and 48% of the large-dose group ($P = 0.04$, large dosage vs. placebo).[87]

Another double-blind, randomized, placebo-controlled study evaluated the efficacy of the multistrain probiotic preparation (VSL#3) in the treatment of rotavirus diarrhea in children. Two hundred and thirty children were enrolled in the trial. By day 2, a lower mean stool frequency and improved stool consistency was noted in the VSL#3 group (both $P \leq 0.05$). On day 4 of treatment, 89% of VSL#3-treated children were recovered versus 40% of controls ($P < 0.001$).[88]

Travelers' Diarrhea

Travelers' diarrhea (TD) is the most common health problem in those visiting developing countries, affecting between 20% and 50% of tourists. Although it is usually short-lived and self-limiting, travelers' diarrhea represents a considerable socioeconomic burden for both the traveler and the host country. The most common enteropathogen is *E. coli,* but a number of other microorganisms are also implicated.[89] Recent research has highlighted the causative role of TD in the development of postinfectious irritable bowel syndrome.[90] Thus agents capable of preventing the development of TD are much needed.

Clinical trials with probiotics have thus far produced mixed results. In an attempt to clarify the role of probiotics in the prevention of TD, McFarland conducted a systematic review and meta-analysis on the area. Combining the data from the 12 included RCTs indicated that probiotics (as a class of agents) appear to significantly prevent the development of TD, with a pooled relative risk of 0.85 (95% CI 0.79–0.91; $P < 0.001$). Although the data for probiotics as a whole were positive, there were a number of preparations found ineffective—*L. fermentum* VRI-003,[91] Lactinex (unspecified strains of *L. acidophilus* and *L. helveticus* in combination),[92] and an unspecified strain of *L. acidophilus* (Antibiophilus-Kapseln).[93] Only strains with proven efficacy in TD should be used clinically (see later).

In a randomized, double-blind, placebo-controlled trial by Black et al., the combination of *L. acidophilus* La5 and *B. lactis* Bb12 was evaluated for the prevention of TD. Ninety-five Danish travelers touring Egypt took part in the trial and consumed either placebo or a probiotic preparation containing 1.8×10^{10} CFU/day of a combination of *L. acidophilus* La5, *B. lactis* Bb12, *L. delbrueckii* ssp. *bulgaricus,* and *S. thermophilus* starting 2 days before travel. The first two strains constituted 90% of the mixture. The number of tourists developing diarrhea was significantly reduced in the probiotic group—from 71% in controls to 43% ($P = 0.019$). This equated to a protection rate of 39.4%.[94]

In another randomized, placebo-controlled trial investigating the efficacy of LGG (2×10^9 CFU/day) in the prophylaxis of TD, 245 subjects traveling from Finland to developing nations were enrolled. The risk of TD development for subjects taking LGG was reduced by 47% compared with the placebo-treated controls ($P = 0.05$).[95]

Using a randomized, placebo-controlled, double-blind trial design, Kollaritsch et al. investigated the efficacy of two different doses of *S. cerevisiae* var. *boulardii* strain Biocodex (250 mg/day and 500 mg/day) in the prevention of TD. Four hundred and six Austrian subjects traveling to tropical locales were enrolled. The rate of TD in the placebo group was 42.6%, versus 33.6% in the low-dose group and 31.8% in the high-dose group. This equates to a 21% reduction in incidence in the 250 mg/day group ($P < 0.007$) and 25% reduction with 500 mg/day ($P < 0.002$) compared with placebo.[93]

In another placebo-controlled double-blind study, two doses (250 and 1000 mg) of *S. cerevisiae* var. *boulardii* strain Biocodex (ScB) were administered prophylactically to 3000 Austrian travelers. A significant reduction in the incidence of diarrhea was observed, with success depending directly on the rigorous use of the preparation. A tendency was noted for ScB to have a regional effect, which was particularly marked in North Africa and in Turkey. The effect was also dose-dependent, with participants taking the higher dose of probiotics experiencing the lowest incidence of travelers' diarrhea (29%); little difference was observed between low-dose supplementation (34%) and placebo (39%). The treatment was well tolerated.[96]

Clostridium-difficile-Associated Diarrhea (CDAD)

Clostridium difficile is a common cause of diarrhea associated with treatment with antimicrobial and/or antibiotic medication and can potentially progress to colitis, pseudomembranous colitis, toxic megacolon, and death. Despite antimicrobial therapy, recurrence is common, and increasingly, probiotic supplementation has been investigated as a potential treatment for CDAD.

A meta-analysis by Johnston et al. assessed 20 RCTs ($n = 3818$ patients) that used probiotic preparations in the management of CDAD. Pooling the data on all the probiotic preparations found a 66% reduced incidence of CDAD (RR = 0.34; 95% CI 0.24–0.49) in patients taking probiotics concurrently with antibiotics. However, not all probiotic preparations were found to be effective.

Probiotic preparations that have demonstrated efficacy in randomized, controlled trials for the prevention or treatment of CDAD include *S. cerevisiae* var. *boulardii* Biocodex,[98] *L. plantarum* 299v,[99] *L. casei* DN-114 001,[100] and the combination of *L. acidophilus* CL 1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2.[101]

Vancomycin-Resistant Enterococci

Over the past 15 to 20 years, there has been a rapid increase in the prevalence of vancomycin-resistant enterococci (VRE). This has been associated with the widespread use of broad-spectrum antibiotics. VRE can be involved in the pathogenesis of persistent, nosocomial infections that often have poor outcomes. Additionally, they have the capacity to transfer their antibiotic resistance genes to other organisms. Nonantibiotic control measures of VRE are thus much needed.

This randomized, double-blind, placebo-controlled trial was performed to assess the efficacy of a medicinal yogurt containing *L. rhamnosus* GG (LGG), *L. acidophilus* La5, and *B. lactis* Bb12 in the eradication of VRE. Twenty-seven VRE-positive hospital inpatients were randomly allocated to consume either a yogurt containing LGG (100 g daily for 4 weeks) or an equivalent amount of pasteurized yogurt. All subjects who received the probiotic yogurt were cleared of VRE compared with only 8% of controls ($P < 0.001$).[102] On the other hand, two trials investigating the capacity of LGG on its own to decrease VRE colonization found this strain in isolation unable to clear or reduce VRE colonization.[103,104]

Chemotherapy-Induced Diarrhea

In an open-label trial, subjects ($n = 150$) undergoing 5-fluorouracil-based chemotherapy for colorectal cancer were randomly assigned to receive supplementation with *L. rhamnosus* GG ($1–2 \times 10^{10}$ CFU/day). *L. rhamnosus* GG supplementation was associated with a 41% reduced frequency of severe diarrhea ($P = 0.027$), an 83% reduction in abdominal discomfort scores ($P = 0.025$), and 55% decreased frequency in bowel toxicity-induced dose reductions ($P = 0.0008$) compared with untreated controls.[105]

AIDS-Related Diarrhea

In a small double-blind, placebo-controlled study, 24 women with AIDS/HIV, aged 18 to 44 years, who were not being treated with antiretrovirals and had moderate diarrhea, were given either a yogurt supplemented with the probiotic strains *L. rhamnosus* GR-1 and *L. reuteri* RC-14 or an unsupplemented yogurt for 15 days. Women receiving the supplemented probiotic–yogurt experienced less diarrhea, flatulence, and nausea than those receiving the control yogurt. All probiotic-treated subjects were free of diarrhea after 15 days' treatment versus 9% of controls. Additionally, there was an increase in CD4 counts observed in probiotic-treated subjects compared with a decrease in controls ($P < 0.02$).[106]

In an open-label trial, 17 HIV-positive patients with chronic diarrhea were given *S. cerevisiae* var. *boulardii* Biocodex strain (3 g/day) for 15 days. The mean number of stools per day decreased from 9.0 on enrollment to 2.1 on day 15. Patients gained a mean of 3.6 kg during the trial.[107]

Constipation

Constipation affects a significant proportion of the population. Probiotics have long been touted as useful for the treatment of constipation, and recent meta-analyses of randomized controlled trials have demonstrated efficacy for some probiotic strains. In a systematic review of randomized controlled trials (14 trials, including 1182 subjects), combining the data from all of the different probiotic strains found a significant reduction in whole-gut transit time (12.4 h reduction; 95% CI: −22.3 to −2.5 h) and increased stool frequency by an extra 1.3 bowel movements/week (95% CI: 0.7–1.9 bowel movements/week). Stool consistency was also significantly improved.[108]

Strains that have shown consistent efficacy in trials to date include *B. lactis* DN-173 010,[109] *E. coli* Nissle 1917,[110] *B. lactis* Bb12,[111] *L. reuteri* DSM 17938,[112] and the combination of *B. breve* PXN 25, *B. longum* PXN 30, *L. acidophilus* PXN 35, *L. casei* PXN 37, *L. rhamnosus* PXN 54, and *S. thermophilus* PXN 66.[113]

Another systematic review evaluating the capacity of probiotics to specifically speed intestinal transit time found that probiotics, as a whole, could significantly reduce intestinal transit time ($P < 0.0001$). Large effect sizes were seen in particular with two probiotic strains—*B. lactis* HN019 and *B. lactis* DN-173 010.[114]

Immune Enhancement

The potential role of probiotics in the enhancement of immune function has been under investigation over the past few decades. The process started with in vitro and animal research, followed by studies evaluating the effects of probiotic administration on immune cell function in human subjects. More recently, well-conducted, human trials with hard outcomes have finally been published. In a recent Cochrane review, Hao et al. assessed the data from randomized controlled trials (12 trials; $n = 3720$) that had investigated the role of probiotics for the prevention and treatment of acute upper respiratory tract infections (URTIs). Probiotics were found to be better than placebo in reducing the number of participants experiencing episodes of acute URTIs ([OR] of at least one URTI = 0.53; 95% CI 0.37 to 0.76; $P < 0.001$) and in reducing the mean duration of an acute URTI episode (mean difference [MD] = −1.89 days; 95% CI −2.03 to −1.75 days; $P < 0.001$). Antibiotic prescription rates for acute URTIs was also significantly reduced (OR = 0.65; 95% CI 0.45–0.94), as was the incidence of cold-related school absence (OR = 0.10; 95% CI 0.02–0.47).[115]

Data suggest, however, that some, but not all, probiotic preparations will demonstrate the capacity to enhance immune function and decrease risk and duration of respiratory tract infections. A number of probiotic strains that have demonstrated the capacity to reduce the incidence of infections and/or shorten their duration are detailed in Table 105.5.

Infantile Colic

Infantile colic is defined as excessive crying or fussing in infants without apparent cause. Probiotics have been suggested as potential strategies to treat this common clinical scenario. In a systematic review and meta-analysis, Bird et al. evaluated the effect of probiotics on the symptoms of infantile colic. Five trials ($n = 317$) were included in this systematic review. Only two probiotic strains were evaluated—*L. reuteri* DSM 17938 and its nearly identical parent strain *L. reuteri* ATCC 55730. Analysis of response rates showed that infants receiving the *L. reuteri* strains had a 2.3-fold greater chance of having a 50% or greater decrease in crying/fussing time compared with controls ($P = 0.01$).[116]

Irritable Bowel Syndrome

Although the etiology of IBS is still debated, there is growing evidence that there is a persistent, mild inflammatory state with changes in mucosal function or structure and an associated imbalance in the gastrointestinal microbiota.[117] Dysbiosis has long been theorized to play a role in the pathophysiology of IBS, and the first trial conducted investigating the efficacy of probiotics in IBS was published in 1955.[118] Many trials have been conducted since this time, as have a number of systematic reviews.

Didari et al. performed a systematic review and meta-analysis of randomized controlled trials that investigated the efficacy of probiotics in the treatment of IBS. Twenty-four RCTs were included in the review, but only 15 of these ($n = 1793$) had data that could be combined into a meta-analysis. Subjects taking probiotics were nearly twice as likely to respond with a reduction in abdominal pain as those taking placebo (RR = 1.96; 95% CI 1.14–3.36; $P = 0.001$) and more than twice as likely to have an improvement in their global IBS symptom score (RR = 2.43; 95% CI 1.13–5.21; $P = 0.02$). They were also twice as likely to report adequate relief of their IBS symptoms (RR = 2.14; 95% CI 1.08–4.26; $P = 0.03$).[119]

Once again, not all probiotic strains have been shown to be effective, with studies using *L. rhamnosus* GG (in isolation),[120] *L. salivarius* UCC4331,[121] and *L. acidophilus* NCFM[122] failing to demonstrate

TABLE 105.5 Probiotics in the Prevention of Infections

Trial Methodology	Strain(s) Used	Results
R, DB, PC *n* = 201; infants 4–10 months old	*Bifidobacterium lactis* Bb12	Over a 3-month period, there was a reduction in fever episodes by 34% ($P < 0.001$), diarrhea episodes by 58% ($P < 0.001$), and duration of diarrhea episodes by 37% ($P < 0.001$).[166]
R, DB, PC *n* = 326; children 3–5 years of age	*Lactobacillus acidophilus* NCFM	Over a 6-month period, fever incidence was reduced by 53% ($P = 0.0085$) and coughing incidence by 41% ($P = 0.027$); use of antibiotics reduced by 68% ($P = 0.0002$); 32% reduction in days absent from childcare ($P = 0.002$).[167]
R, DB, PC *n* = 326; children 3–5 years of age	*L. acidophilus* NCFM and *B. lactis* Bi07	Over a 6-month period, fever incidence was reduced by 73% ($P = 0.0009$), coughing incidence by 62% ($P = 0.005$), and rhinorrhea incidence by 59% ($P = 0.03$); use of antibiotics was reduced by 84% ($P < 0.0001$); 28% reduction in days absent from childcare ($P < 0.001$).[167]
R, DB, PC *n* = 215 infants 6–12 months old	*L. fermentum* CECT5716	Over a 6-month period, there was a 46% reduced incidence of gastrointestinal infections ($P = 0.032$), a 26% reduction in respiratory tract infections ($P = 0.022$), and a 30% reduction in the total number of infections ($P = 0.003$); the incidence of recurrent respiratory tract infections was reduced by 72%.[168]
R, DB, PC, CO *n* = 20; elite, male distance runners	*L. fermentum* VRI-003	Over a 4-month winter period, distance runners taking the probiotic reported less than half the number of days of respiratory symptoms (30 vs. 72; $P = 0.00006$) compared with placebo; illness severity was also decreased ($P = 0.06$).[169]
R, DB, PC *n* = 201; infants 4–10 months old	*L. reuteri* DSM 17938	Over a 3-month period, fever episodes were reduced by 73% ($P < 0.001$), diarrhea episodes by 94% ($P < 0.001$), duration of diarrhea by 75% ($P < 0.001$), and childcare absences by 67% ($P = 0.015$) in infants.[166]
R, DB, PC *n* = 262; healthy adults	*L. reuteri* DSM 17938	Over the 80-day trial period, there was a 58% reduction in number of subjects reporting sick days in the DSM 17938 group compared with placebo ($P < 0.01$); among shift workers, 33% of those in the placebo group reported sick over the study period vs. none in the DSM 17938 group ($P < 0.005$).[170]
R, DB, PC *n* = 571; children aged 1–6 years	*L. rhamnosus* GG	Over the 7-month winter period, there were 16% fewer days absent from daycare in children in the LGG group ($P = 0.03$) and a 19% reduction in antibiotic use for respiratory tract infections ($P = 0.03$).[171]
R, DB, PC *n* = 281 day-care-aged children	*L. rhamnosus* GG	Over the 3-month intervention period, there was a 34% reduced risk of upper respiratory tract infections in toddlers in the LGG group, a 43% reduced risk of respiratory tract infections lasting longer than 3 days, and significantly fewer number of days with respiratory tract symptoms (all $P < 0.001$). There was a trend for reduced number of days with gastrointestinal symptoms ($P = 0.06$).[172]
R, DB, PC *n* = 81 infants < 2 months old	*L. rhamnosus* GG and *B. lactis* Bb12	Over the 10-month period, there was a 56% reduced risk of otitis media in infants ($P = 0.014$), a 48% reduced risk of antibiotic prescription ($P = 0.015$), and a 49% reduced risk of recurrent respiratory tract infection ($P = 0.022$).[173]

CO, Crossover; *DB,* double-blind; *PC,* placebo-controlled; *R,* randomized.

positive results. Probiotic preparations that have shown efficacy in the treatment of IBS are detailed in Table 105.6.

Inflammatory Bowel Disease

Probiotics are also being used as adjunctive therapy for the two main inflammatory bowel diseases—Crohn's disease (CD) and ulcerative colitis (UC). Overall, current research indicates a limited role for probiotics in CD, whereas the results for UC are more promising.

Crohn's Disease

Derwa et al. in their systematic review and meta-analysis of RCTs found no benefit of probiotics (as a whole) in inducing remission of active CD, in preventing relapse of quiescent CD, or in preventing relapse of CD after surgically induced remission.[123]

However, there is some preliminary supportive evidence for one probiotic strain. In an open-label study, Guslandi et al. compared *S. cerevisiae* var. *boulardii* Biocodex plus mesalazine versus mesalazine alone and found that adjunctive probiotic treatment resulted in fewer relapses (6% vs. 38%; $P = 0.04$).

Given this promising preliminary research on *S. cerevisiae* var. *boulardii* Biocodex (ScB) in CD, the strain was evaluated using a more rigorous randomized, double-blind, placebo-controlled trial design. One hundred and sixty-five subjects with CD that was currently in remission after treatment with steroids or salicylates were randomly allocated to receive either placebo or probiotic (1 g/day) for 12 months. Over the 12 months, the relapse rates were 47.5% in the probiotic group and 53.2% in the placebo group (a nonsignificant difference). The median time to relapse did not differ significantly between patients given probiotics (40.7 weeks) versus placebo (39.0 weeks). There were also no significant differences between groups in mean Crohn's disease activity index scores or erythrocyte sedimentation rates or in median levels of C-reactive protein. A post-hoc analysis did reveal, however, that nonsmokers given ScB were less likely to experience a relapse of CD than nonsmokers given placebo (OR 0.22, 95% CI 0.07–0.70; $P = 0.01$).[124] The latter result requires confirmation in future research to ensure it was not a random finding, but in the meantime, a trial of this probiotic strain would be warranted in nonsmoking CD patients.

Ulcerative Colitis

A recent meta-analysis of randomized, controlled trials assessed the effect of probiotics on remission induction and maintenance in UC. Thirteen RCTs were included in the analysis. Seven reports evaluated remission rates ($n = 399$). When combined (8 studies; $n = 709$), the adjunct use of probiotics alongside standard care did not significantly alter remission rates. There was, however, marked heterogeneity in the results. Compared with the placebo group, the use of probiotics was found to significantly reduce the UC recurrence rate (recurrence rate: 0.69, 95% CI 0.47–1.01;

TABLE 105.6 Probiotics in the Treatment of Irritable Bowel Syndrome

Trial Methodology	Strain(s) Used	Results
R, DB, PC *n* = 34 female subjects with C-IBS	*Bifidobacterium lactis* DN-173 010	After a 4-week treatment period, there was a significant reduction in abdominal distension ($P = 0.02$), an acceleration of gut transit time ($P = 0.049$), and a reduction in overall IBS symptom severity ($P = 0.032$). There were also reductions in abdominal pain/discomfort ($P = 0.044$), bloating ($P = 0.059$), and flatulence scores ($P = 0.092$).[174]
R, DB, PC *n* = 362 female subjects with IBS	*B. infantis* 35624	After a 4-week treatment phase, there were significant reductions in abdominal pain/discomfort ($P = 0.023$), bloating/distension ($P = 0.046$), feeling of incomplete evacuation ($P < 0.04$), sense of straining at stool ($P < 0.02$), passage of gas ($P < 0.04$), and composite IBS symptom scores ($P = 0.013$) compared with controls. There was also a significant improvement in bowel habit satisfaction ($P < 0.02$).[147]
R, DB, PC *n* = 52 subjects with IBS	*Lactobacillus acidophilus* strains CUL-60 and CUL-21, *B. lactis* CUL-34, and *B. bifidum* CUL-20 (Lab4)	After an 8-week treatment period, there were significant reductions in composite IBS symptom scores ($P = 0.0217$) and improvements in quality-of-life scores ($P = 0.0068$) compared with controls.[175]
R, DB, PC *n* = 214 subjects with IBS	*L. plantarum* 299V	After a 4-week treatment phase, the frequency and severity of abdominal pain, bloating, and feeling of incomplete evacuation scores were significantly reduced in the 299V group compared with controls (all $P < 0.05$). Stool frequency was significantly reduced in the 299V group ($P < 0.05$), and 78% of subjects in the 299V group scored the symptomatic effect as excellent or good versus 8% of controls ($P < 0.01$).[54]
R, DB, PC *n* = 48 subjects with IBS and bloating	VSL#3	After either a 4- or 8-week treatment phase, there was a significant reduction in flatulence scores ($P = 0.011$). There were no significant changes in other IBS symptoms or bowel function, although colonic transit time was retarded in the VSL#3 group.[176]

C-IBS, Constipation-predominant irritable bowl syndrome; *CO,* crossover; *DB,* double-blind; *IBS,* irritable bowel syndrome; *PC,* placebo-controlled; *R,* randomized.

$P = 0.05$). Heterogeneity was again observed. The results suggest that concurrent us of probiotics provides little additional benefit in inducing remission in a UC flare but that probiotic auxiliary therapy is much better than nonprobiotics therapy for maintenance of remission.[125] The significant heterogeneity observed in this study, which should have precluded the conduction of a meta-analysis, was most likely caused by the all-too-common error of combining research conducted on different probiotic strains, as each strain must be viewed as a separate therapeutic agent. Probiotic strains and specific preparations that have shown efficacy in the management of UC are detailed in Tables 105.7 and 105.8.

Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease comprises a spectrum of diseases ranging from simple steatosis to nonalcoholic steatohepatitis, fibrosis, and cirrhosis. Probiotics have been proposed as a treatment option because of their modulating effect on the gut flora that could influence the gut-liver axis, and there are now considerable data from animal models in support of this idea and some promising human data.

In a randomized, double-blind, placebo-controlled trial, supplementation of obese children ($n = 20$; mean age 10.7 years) with persisting hypertransaminasemia and ultrasonographic changes suggestive of NAFLD, with *L. rhamnosus* GG (1.2×10^{10} CFU/day) for 8 weeks resulted in a decrease in alanine aminotransferase concentration ($P = 0.03$) and in antipeptidoglycan-polysaccharide antibody levels ($P = 0.03$). Alanine aminotransferase levels normalized in 80% of LGG-treated subjects.[126]

Colon Cancer

In vitro and animal data have supported the potential use of probiotics, as a general class of agents, to prevent colorectal cancer—mainly via their antimicrobial effects against carcinogen-producing microorganisms, direct antimutagenic properties, and via alteration of tumor differentiation processes.[127]

One strain with supportive human data is *L. casei* Shirota. A randomized, controlled trial evaluated the efficacy of this probiotic strain in the prevention of colorectal cancer in patients ($n = 398$) who had had at least two colorectal tumors removed previously. Daily consumption of the probiotic over a 4-year period resulted in a significant reduction in the occurrence of colorectal tumors with moderate or severe atypia compared with the dietary instruction–only group ($P < 0.05$).[128]

Mastitis

Mastitis is an inflammatory condition of the breast that may, or may not, be associated with infection. It is, however, a common reason for premature cessation of breastfeeding.[129] Recent research has found some probiotic strains to be of benefit in the treatment of mastitis and the prophylaxis of recurrent mastitis.

In a randomized, double-blind, placebo-controlled trial, 352 women with lactational mastitis were allocated into one of three groups—antibiotics, *L. fermentum* CECT5716, or *L. salivarius* CECT (both at 1.0×10^9 CFU/day). After 3 weeks' treatment, both probiotic strains were found to be superior to antibiotics in decreasing levels of pathogenic bacteria in breast milk, increasing lactobacilli counts in breast milk, and decreasing breast pain scores (all $P < 0.001$). They also significantly reduced the rate of recurrence compared with antibiotics—10.5% in the *L. fermentum* CECT5716 group and 7.1% in the *L. salivarius* CECT5713 group versus 30.7% in the antibiotic group (both $P < 0.001$).[130]

In another randomized, placebo-controlled trial, women ($n = 20$) with antibiotic-resistant mastitis were allocated to receive either placebo or a probiotic preparation (*L. salivarius* CECT5713 and *L. gasseri* CECT5714; 2.0×10^{10} CFU/day) for 14 days. All mastitis signs were eliminated by day 14 in the probiotic group, whereas mastitis persisted in all women in the control group. There was also an ~100-fold decrease in milk staphylococcal counts in the probiotic group.[131]

Postpartum Obesity

Another novel area of research is using probiotics to prevent postpartum obesity. Ilmonen et al. investigated the use of a probiotic combination product (containing *L. rhamnosus* GG and *B. lactis* Bb12) in the prophylaxis of

TABLE 105.7 Probiotics in the Treatment of Ulcerative Colitis (UC)—Induction of Remission

Trial Methodology	Strain(s) Used	Results
R, DB, C *n* = 116 patients with active UC	*Escherichia coli* Nissle 1917	Over a 12-month treatment period, *E. coli* Nissle 1917 supplementation in conjunction with standard IBD therapy (corticosteroids) was equivalent to mesalazine in terms of remission rate ($P = 0.05$) and time to remission ($P = 0.009$). The duration of remission was also equivalent ($P = 0.017$).[177]
R, DB, PC *n* = 90 patients with moderately active distal UC	*Escherichia coli* Nissle 1917	Intrarectal administration (via enema) of three different doses of *E. coli* Nissle 1917 (40, 20, or 10 mL) daily for 2 weeks did not significantly affect rates of remission in ITT analysis. There was a large number of protocol violations, however, and a per protocol analysis demonstrated a significant dose-dependent effect ($P = 0.0446$). Remission rates in the 40 mL group were 53%, 44% in the 20 mL group, and 27% in the 10 mL group compared with 18% in the placebo group.[178]
R, DB, PC *n* = 147 subjects with mild-to-moderate active UC	VSL#3	At 6 weeks, 33% of VSL#3-treated subjects achieved a > 50% in the UC disease activity index versus 10% of controls ($P = 0.001$). At week 12, 43% of subjects in the VSL#3 group were in remission versus 16% of controls ($P < 0.001$).[179]
R, DB, PC *n* = 29 children with active UC	VSL#3	In conjunction with standard UC therapy (steroids and mesalamine), remission was achieved in 93% of VSL#3-treated children versus 36% of controls ($P < 0.001$). Twenty-one percent of VSL#3 treated subjects relapsed within 1 year versus 73% of controls ($P = 0.014$).[180]

DB, Double-blind; *IBD,* irritable bowel disease; *ITT,* intention to treat; *PC,* placebo-controlled; *R,* randomized.

TABLE 105.8 Probiotics in the Treatment of Ulcerative Colitis (UC)—Maintenance of Remission

Trial Methodology	Strain(s) Used	Results
R, DB, C *n* = 327 UC patients in remission	*Escherichia coli* Nissle 1917	Over the 12-month trial period, *E. coli* Nissle 1917 treatment was found to be equally effective as mesalazine in preventing relapse (36.4% relapse vs. 33.9%; $P = 0.003$).[181]
R, DB, C *n* = 120 UC patients in remission	*E. coli* Nissle 1917	Over a 12-week period, relapse rates were 11.3% under mesalazine and 16.0% under *E. coli* Nissle 1917 (not significantly different). The mean relapse-free time was 103 ± 4 days for mesalazine and 106 ± 5 days for *E. coli* Nissle 1917 (not significantly different).[182]
OL *n* = 34 UC patients in remission aged 11–18 years	*E. coli* Nissle 1917	Over the 12-month treatment period, the relapse rate was 25% in probiotic-treated subjects versus 30% in the mesalazine group.[183]
R, OL *n* = 187 UC patients in remission	*Lactobacillus rhamnosus* GG	Subjects were randomized to receive either *L. rhamnosus* GG (LGG) alone or in combination with mesalazine, or mesalazine alone. Over the 12-month trial period, there was no difference in relapse rates between the three groups. LGG supplementation was as equally effective as mesalazine in maintaining clinical remission but significantly more effective than mesalazine in prolonging the relapse-free time ($P < 0.05$).[184]
OL *n* = 20 UC patients in remission	VSL#3	After 12 months, 15 of the 20 participants remained in remission, which compares favorably with rates of remission observed during long-term mesalazine therapy.[185]

C, Controlled; *DB,* double-blind; *OL,* open-label; *PC,* placebo-controlled; *R,* randomized.

postpartum obesity. In the first trimester of pregnancy, 256 women were randomly assigned to receive no dietary counseling or nutritional counseling (low-fat and high-fiber diet) plus probiotics (LGG and Bb12; 2.0×10^{10} CFU/day) or counseling plus placebo. Interventions lasted until the end of exclusive breastfeeding for up to 6 months. At 6 months' postpartum, the risk of central adiposity (defined as waist circumference 80 cm or more) was lowered in women in the diet/probiotics group compared with the control/placebo group (OR 0.30, 95% CI 0.11–0.85; $P = 0.023$ adjusted for baseline body mass index), whereas the diet/placebo group did not differ from the controls (OR 1.00, 95% CI 0.38–2.68; $P = 0.994$). The number needed to treat with diet/probiotics to prevent one woman from developing a waist circumference of 80 cm or more was four.[132] At 12 months postpartum, central obesity occurred in 25% of diet/probiotic subjects versus 43% in the diet/placebo group. The proportion of body fat was also 3.5% lower in the diet/probiotic group ($P = 0.018$).[133]

Urogenital Infections

Probiotics are widely used in the treatment and prevention of urogenital infections. They can be administered both orally and locally, with multiple trials supporting their use. There are additional criteria needed for probiotic strains to be efficacious in urogenital applications. Strains must demonstrate the capacity to adhere to uroepithelial and vaginal cells, temporarily colonize the vagina, inhibit urogenital pathogen growth and/or attachment, and ideally should produce hydrogen peroxide and lactic acid. Strains that will be administered orally must also have sufficient tolerance to gastric acid and bile salts and the verified capacity to colonize the vagina after oral intake.[134] Probiotic strains that do not exhibit these characteristics are unlikely to be therapeutic for urogenital applications.

The mechanisms by which some *Lactobacillus* strains reduce bacterial vaginosis, vaginal candidiasis, and UTIs appear to involve a combination of antiadhesion factors, byproducts such as hydrogen peroxide, bacteriocins, and lactic acid (both D- and L-forms), and destruction of urogenital pathogen biofilms, as well as immune modulation or modification of vaginal epithelial cell cytokine production.[30,135-137]

Bacterial Vaginosis

Bacterial vaginosis (BV) is the most prevalent vaginal infection worldwide and is characterized by an altered vaginal ecosystem (i.e.,

TABLE 105.9 Probiotics in the Treatment of Bacterial Vaginosis (BV)

Trial Methodology	Strain(s) Used	Results
R, DB, PC *n* = 64 women	*Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14	After a single dose of tinidazole and 4 weeks of oral probiotic treatment, subjects in the probiotic group had a higher rate of BV cure (88% vs. 50%; *P* = 0.001) and vaginal flora normalized in 75% of probiotic-treated subjects versus 34% of controls (*P* = 0.011).[186]
R, DB, PC *n* = 40 women	*L. rhamnosus* GR-1 and *L. reuteri* RC-14	Intravaginal application of probiotics nightly for 5 days resulted in a 90% cure rate by day 30; follow-up at day 6, 15, and 30 showed cure of BV in significantly more probiotic-treated subjects (16, 17, and 18/20, respectively) compared with metronidazole gel treatment (9, 9, and 11/20, respectively; *P* = 0.016 at day 6, *P* = 0.002 at day 15, and *P* = 0.056 at day 30).[187]
R, DB, PC *n* = 106 women	*L. rhamnosus* GR-1 and *L. reuteri* RC-14	Women receiving an antibiotic prescription for BV (metronidazole 500 mg twice daily for 7 days) who were given concurrent probiotics (*L. rhamnosus* GR-1 and *L. reuteri* RC-14) for 30 days had an eradication rate of 88% versus 40% in the antibiotic/placebo group (*P* < 0.001).[188]
R, DB, PC *n* = 80 women	*Bifidobacterium breve* PXN 25, *B. longum* PXN 30, *L. acidophilus* PXN 35, *L. casei* PXN 37, *L.* delbrueckii ssp. *bulgaricus* PXN 39, *L. rhamnosus* PXN 54, and *Streptococcus thermophilus* PXN 66	In women receiving oral metronidazole, oral supplementation with this probiotic combination resulted in an 87.5% treatment success rate compared with 67.5% treatment success in placebo group (*P* = 0.032).[189]

CO, Crossover; *DB,* double-blind; *OL,* open-label; *PC,* placebo-controlled; *R,* randomized.

dysbiosis)—specifically a depletion of the indigenous lactobacilli population. Administration of exogenous lactobacilli strains has thus been an area of research interest.[138]

A Cochrane systematic review conducted in 2009 included four RCTs that examined probiotic agents in the treatment of BV. Analysis suggested that probiotics were effective in enhancing microbiological cure rates with the oral metronidazole/probiotic (*L. rhamnosus* GR-1 and *L. reuteri* RC-14) regimen (OR 0.09, 95% CI 0.03–0.26) and the probiotic (unspecified strain of *L. acidophilus*)/estriol preparation (OR 0.02, 95% CI 0.00–0.47) showing effectiveness. The authors cautioned, however, that larger, well-designed trials were needed before any firm conclusions could be made.[139] Research on specific strains that have demonstrated efficacy are highlighted later in Table 105.9.

Urinary Tract Infections

Grin et al. performed this systematic review and meta-analysis to review the data on the potential effectiveness of lactobacilli in the prevention of recurrent urinary tract infections in women. Five randomized, controlled trials were included in the analysis. Combining the data from all the trials (294 patients) found no preventative effect of lactobacilli supplementation (RR = 0.85, 95% CI 0.58–1.25; *P* = 0.41). However, a sensitivity analysis was performed, excluding studies using ineffective strains and studies testing for safety only. Data from 127 patients in two trials were included. A statistically significant decrease in recurrent UTI was found in patients given lactobacilli, denoted by the pooled risk ratio of 0.51 (95% CI 0.26–0.99; *P* = 0.05).[140]

A significant reduction in UTI recurrence rate was reported in a randomized double-blind study involving 55 premenopausal women. The study investigated the effectiveness of treatment for 1 year with a weekly suppository containing either a combination of *L. rhamnosus* GR-1 and *L. fermentum* B-54 or a *Lactobacillus* growth factor (skim milk). Treatment resulted in the UTI rate decreasing by 73% and 79%, respectively, with no adverse effects reported.[141]

A recent, randomized, double-blind, noninferiority trial compared the efficacy of 12 months of prophylaxis with antibiotics (trimethoprim-sulfamethoxazole; 480 mg/day) or oral probiotics (2×10^9 CFU/day of a combination of *L. rhamnosus* GR-1 and *L. reuteri* RC-14) in the prevention of UTIs in postmenopausal women with recurrent cystitis (*n* = 252). The number of symptomatic UTIs over the 12-month treatment period was 2.9 in the antibiotic group and 3.3 in the lactobacilli group—a between-treatment difference of 0.4 episodes (95% CI −0.4–1.5) or 13.8%. In the year preceding the trial, the subjects experienced a mean of 6.9 UTIs. The percentage of patients with at least one UTI at 12 months was 69.3% in the trimethoprim-sulfamethoxazole group and 79.1% in the lactobacilli group. The median times to first recurrence were 6 and 3 months, respectively. After 12 months of trimethoprim-sulfamethoxazole prophylaxis, all urinary *E. coli* isolates of asymptomatic women were resistant to trimethoprim-sulfamethoxazole and trimethoprim. Probiotic use, however, was not associated with increased antibiotic resistance. The authors stated that this lactobacilli combination may be an acceptable alternative for the prevention of UTIs, especially in women who dislike taking antibiotics.[142] Although probiotic therapy appeared slightly less effective than antibiotic prophylaxis in postmenopausal women with recurrent UTIs, their use does not damage the GIT microbiota or create antibiotic resistant organisms.[143]

Vaginal Candidiasis

In theory, supplying specific lactobacilli strains to the vagina in women with vaginal candidiasis (VC) or at risk of VC should help manage or prevent the infection. Lactobacilli can impair the growth of *C. albicans* via the production of lactic acid, which creates a low-pH environment resulting in suppressed fungal growth. Cocultures of some lactobacilli strains with *C. albicans* has revealed inhibitory effects. Transcriptome analyses showed increased expression of stress-related genes and lower expression of genes involved in antifungal resistance.[136] Research to date, however, has produced mixed results.

Some probiotic preparations have been shown to be clearly ineffective in the treatment of VC. For example, a combination of *L. rhamnosus* HN001 and *B. longum* Bl-05 and a propriety preparation, Femilac (unspecified strains of *L. rhamnosus, L. delbrueckii* ssp *bulgaricus, L. acidophilus*, and *S. thermophilus*).[144] Other preparations have shown some degree of efficacy, however. Those preparations that have shown efficacy in the treatment of VC are detailed in Table 105.10, although it should be noted that most of these trials were small in size.

TABLE 105.10 Probiotics in the Treatment of Vulvovaginal Candidiasis (VC)

Trial Methodology	Strain(s) Used	Results
R, DB, PC *n* = 27 women with a history of recurrent VC	*Lactobacillus acidophilus* NAS as a vaginal suppository in isolation or with *L acidophilus* NAS strain; *Bifidobacterium bifidum* Malyoth strain, and *L. bulgaricus* LB-51 strain taken orally	In women who used the *L. acidophilus* NAS vaginal suppository, the incidence of recurrent VC was significantly reduced compared with controls ($P = 0.005$). Subjects who took both the suppository and oral probiotic supplement also had a significant reduction in the number of infections compared with controls ($P = 0.011$). There was, however, no extra benefit seen from taking the oral probiotic preparation.[189]
R, DB, PC *n* = 55 women with VC	*L. rhamnosus* GR-1 and *L. reuteri* RC-14	After a single dose of fluconazole and 4 weeks of oral treatment with either probiotic or placebo, subjects in the probiotic group had reduced *Candida* colonization (10% vs. 39%; $P = 0.014$) and decreased vaginal discharge (10% vs. 35%; $P = 0.03$).[190]
R, PC, CO *n* = 33 women with a history of recurrent VC	*L. acidophilus* LA5	The mean number of infections per 6 months decreased while in the probiotic phase compared with the control phase of the trial (0.38 vs. 2.54; $P = 0.001$). The incidence of *Candida* colonization also decreased during probiotic treatment (0.84 vs. 3.23 per 6 months; $P = 0.001$).[191]
OL *n* = 28 women with a history of recurrent VC	*L. rhamnosus* GG	Twice-daily intravaginal application of LGG resulted in decreased symptoms of VC, as well as less erythema and discharge; 4/5 women who were positive for *Candida* at baseline had negative cultures at the end of 7 days.[178]

CO, Crossover; *DB*, double-blind; *OL*, open-label; *PC*, placebo-controlled; *R*, randomized.

Toxin Reduction

Some *Lactobacillus* strains are able to break down aldehydes in the body through enhancement of the enzyme aldehyde dehydrogenase.[145] In addition, some probiotic strains can bind to aflatoxin B_1 in the intestine, preventing the toxin from causing damage to the liver and other organs.[146]

DOSAGE

The dosage of probiotic foods and supplements is based solely upon the number of live organisms present in the product. Successful results have been attained in clinical trials using between 10^7 and 10^{11} CFU per day.[147-149] Interestingly, it appears that 100 times fewer viable bacteria need to be given in a dairy medium than in a freeze-dried supplement to achieve similar numbers of live bacteria in the lower bowel.[63] Dairy appears to work as an ideal transport medium for the bacteria, enhancing their survival through the upper GIT.[50]

Supplements

Supplements are best consumed with meals to take advantage of the increased alkalinity of the gastric environment (which equates to greater bacterial survival).[150] A dosage of 10^8 bacteria per sitting is often mentioned in the probiotic literature as the minimum quantity of bacteria needed to produce therapeutic effects.[50,150] Additionally, there have been a handful of studies that demonstrated therapeutic effects using 10^7 to 10^8 viable bacteria per dose.[147,148,151] However, most of the successful probiotic research used greater than or equal to 10^9 CFU per dose.

The minimum concentration of probiotic bacteria needed to achieve therapeutic effects appears to be strain dependent, in that for some strains (e.g., *L. reuteri* DSM 17938) 10^7 bacteria is a sufficient quantity to produce beneficial effects,[148] whereas for other strains, 10^9 viable bacteria is needed (e.g., *L. rhamnosus* GG).[63] This situation, unfortunately, makes it hard to give firm dosage recommendations, as the minimum effective dosage appears to differ by strain. Thus it is best practice to ensure that supplements contain bacteria in concentrations greater than or equal to 10^9 CFU per dose, unless research has demonstrated that the specific strain contained in the supplement is effective in smaller amounts.

Therefore the dosage of viable bacteria given in supplemental forms should generally be 10^9 to 10^{11} bacteria per dose. If a formulation contains multiple strains, each strain must be present in amounts greater than or equal to 10^9, because dosages of less than 10^9 CFU may not produce therapeutic effects.

Studies evaluating a potential dose-response have to date been extremely limited. Results do suggest, however, that at least for the areas of acute viral diarrhea and antibiotic-associated diarrhea, there is a probable dose-dependent effect—with doses of $> 10^{10}$ CFU/day generally more effective. Perhaps not surprisingly, fecal concentrations also correlated well with oral dosing regimens for most probiotic strains, with higher oral dosing equating to higher fecal concentrations.[152]

Dosage in Dairy Mediums

The minimum dosage of viable bacteria needed in a dairy medium is 10^8 CFU of each strain per dose. Therapeutic yogurts contain greater than or equal to 10^6 viable bacteria per milliliter, thus a 100 g serving (approximately 1/2 cup) will provide sufficient probiotic bacteria for therapeutic effects.[150] Unfortunately, many so-called "acidophilus" and/or "bifidus" yogurts do not contain this minimum level.[153] Only yogurt brands that are guaranteed to contain this level of viable bacteria or those that have done so in market-basket surveys should be used. A serving of yogurt containing less than 10^8 viable bacteria is unlikely to have any medicinal effects beyond its inherent nutritional content.

A 100 g serving of yogurt contains only 3.1 to 3.5 g of lactose,[154] which is well below the threshold level in individuals with lactose intolerance. Hence, lactose-intolerant individuals should be able to consume the minimum amount of yogurt without ill effects.[155]

TOXICITY

Lactobacilli have been consumed in large numbers throughout recorded history. The fermentation of foodstuffs is one of the oldest known uses of biotechnology, and even today, fermented foods and beverages constitute 20% to 40% of the human food supply worldwide. Thus lactobacilli have a long history of safe ingestion.[5]

In a recent review done to assess the safety of probiotics, Hempel et al. evaluated the adverse event profiles of 622 human trials that used probiotics from the genera *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus* and *Bacillus.* Combining the data from those studies that reported adverse events, no increased risk of adverse events was observed in those subjects receiving probiotics

versus those taking a placebo (RR = 1.00; 95% CI 0.93–1.07). Nor was there an increase in gastrointestinal side effects, infections, or serious adverse events in probiotic-consuming subjects (RR = 1.06; 95% CI 0.97–1.16).[149]

Another systematic review looking specifically at probiotic safety in children evaluated the data from 74 clinical trials. Studies included healthy, immune-compromised, and obese subjects, as well as subjects with intestinal disorders, infections, and inflammatory disorders. Probiotics were found to be well-tolerated, with adverse events occurring more frequently in the placebo arm compared with those taking probiotics. The authors caution, however, that there was inadequate reporting and classification of adverse events in many of the trials.[150]

Serious adverse events (such as bacteremia and fungemia) occur very rarely with probiotic use. Most cases have been associated with an immune-compromised state, impaired intestinal barrier function (such as in multiorgan failure), and the use of a central venous catheter. As these conditions are often present in critically ill patients, probiotic treatment is contraindicated in this population.[151]

Despite a substantial increased use of probiotic supplements worldwide, epidemiological evidence suggests there has been no corresponding increase in cases of bacteremia or fungemia as a consequence—further supporting their excellent safety profile.[156]

DRUG INTERACTIONS

Lactobacilli and bifidobacteria are negatively affected by alcohol and antibiotics.[157,158] Although there is no evidence that the organism interferes with the activity of most antibiotics, the metabolism of sulfasalazine, chloramphenicol palmitate, and phthalylsulfathiazole may be affected by some strains of *L. acidophilus.*[159]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lilley DM, Stillwell RH. Probiotics: growth promoting factors produced by microorganisms. *Science*. 1965;147:747–748.
2. Parker RB. Probiotics, the other half of the antibiotic story. *Animal Nut Hlth*. 1974;29:4–8.
3. Hill C, Guarner F, Reid G, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506–514.
4. Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr*. 1999;69(suppl):1052S–1057S.
5. Cani PD, Van Hul M. Novel opportunities for next-generation probiotics targeting metabolic syndrome. *Curr Opin Biotechnol*. 2015;32:21–27.
6. Petrof EO, Gloor GB, Vanner SJ, et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. *Microbiome*. 2013;1(1):3.
7. Khanna S, Pardi DS, Kelly CR, et al. A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent clostridium difficile infection. *J Infect Dis*. 2016;214(2):173–181.
8. McFarlin B, Hennin A, Bowman E, al E. Oral spore-based probiotic supplementation was associated with reduced incidence of post-prandial dietary endotoxin, triglycerides, and disease risk biomarkers. *World J Gastrointest Pathophysiol*. 2017;8(3):117–126.
9. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat Microbiol*. 2017;2:17057.
10. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschliman JM. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immunol Med Microbiol*. 1994;10:55–64.
11. Walker WA. Mechanisms of action of probiotics. *Clin Infect Dis*. 2008;46(suppl 2):S87–S91.
12. Schiffrin EJ, Brassart D, Servin AL, et al. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. *Am J Clin Nutr*. 1997;66:515S–520S.
13. Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut*. 2004;53(6):821–828.
14. Rachmilewitz D, Katakura K, Karmeli F, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology*. 2004;126(2):520–528.
15. Tien MT, Girardin SE, Regnault B, et al. Anti-Inflammatory effect of lactobacillus casei on Shigella-Infected human intestinal epithelial cells. *J Immunol*. 2006;176(2):1228–1237.
16. Imaoka A, Shima T, Kato K, et al. Anti-inflammatory activity of probiotic Bifidobacterium: enhancement of IL-10 production in peripheral blood mononuclear cells from ulcerative colitis patients and inhibition of IL-8 secretion in HT-29 cells. *World J Gastroenterol*. 2008;14(16):2511–2516.
17. Waller PA, Gopal PK, Leyer GJ, et al. Dose-response effect of Bifidobacterium lactis HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. *Scand J Gastroenterol*. 2011;46(9):1057–1064.
18. Meance CC, Turchet P, Raimondi A, Lucas C, Antoine J-M. Séverine: a fermented milk with a bifidobacterium probiotic strain DN-173 010 shortened oro-fecal gut transit time in elderly. *Microb Ecol Health Dis*. 2001;13(4):217–222.
19. Bougle D, Roland N, Lebeurrier F, Arhan P. Effect of propionibacteria supplementation on fecal bifidobacteria and segmental colonic transit time in healthy human subjects. *Scand J Gastroenterol*. 1999;34(2):144–148.
20. Marteau P, Cuillerier E, Meance S, et al. Bifidobacterium animalis strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther*. 2002;16(3):587–593.
21. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol*. 1997;159(4):1739–1745.
22. Prioult G, Fliss I, Pecquet S. Effect of probiotic bacteria on induction and maintenance of oral tolerance to β-Lactoglobulin in gnotobiotic mice. *Clin Diagn Lab Immunol*. 2003;10(5):787–792.
23. Hsiao KC, Ponsonby AL, Axelrad C, Pitkin S, Tang MLK. Long-term clinical and immunological effects of probiotic and peanut oral immunotherapy after treatment cessation: 4-year follow-up of a randomised, double-blind, placebo-controlled trial. *Lancet Child Adolesc Health*. 2017;1(2):97–105.
24. McKernan DP, Fitzgerald P, Dinan TG, Cryan JF. The probiotic Bifidobacterium infantis 35624 displays visceral antinociceptive effects in the rat. *Neurogastroenterol Motil*. 2010;22(9):1029–1035. e1268.
25. Johnson AC, Greenwood-Van Meerveld B, McRorie J. Effects of Bifidobacterium infantis 35624 on post-inflammatory visceral hypersensitivity in the rat. *Dig Dis Sci*. 2011;56(11):3179–3186.
26. Ait-Belgnaoui A, Han W, Lamine F, et al. Lactobacillus farciminis treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. *Gut*. 2006;55(8):1090–1094.
27. Rousseaux C, Thuru X, Gelot A, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med*. 2007;13(1):35–37.
28. Fuller R, Gibson GR. Modification of the intestinal microflora using probiotics and prebiotics. *Scand J Gastroentero*. 1997;32(suppl 222):28–31.
29. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. *Am J Physiol*. 1999;276(4):G941–G950.
30. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic Lactobacillus species in vaginal health. *Res Microbiol*. 2017;168(9):782–792.
31. Mogha KV, Prajapati JB. Selection of promising strain of Lactobacillus for treating vaginal infections. *Rev Med Microbiol*. 2017;28(2):49–55.
32. Hasslof P, Hedberg M, Twetman S, Stecksen-Blicks C. Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli—an in vitro study. *BMC Oral Health*. 2010;10:18.
33. Naaber P, Smidt I, Štšepetova J, Brilene T, Annuk H, Mikelsaar M. Inhibition of Clostridium difficile strains by intestinal Lactobacillus species. *J Med Microbiol*. 2004;53(6):551–554.
34. Sgouras D, Maragkoudakis P, Petraki K, et al. Vitro and in vivo inhibition of Helicobacter pylori by lactobacillus casei strain Shirota. *Appl Envl Microbiol*. 2004;70(1):518–526.
35. Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human-derived probiotic Lactobacillus reuteri demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. *Anaerobe*. 2008;14(3):166–171.
36. Salminen S, Nybom S, Meriluoto J, Collado MC, Vesterlund S, El-Nezami H. Interaction of probiotics and pathogens—benefits to human health? *Curr Opin Biotechnol*. 2010;21(2):157–167.
37. Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. *Infect Immun*. 1999;67(1):302–307.
38. Corr SC, Hill C, Gahan CG. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. *Adv Food Nutr Res*. 2009;56:1–15.
39. Benno Y, He F, Hosoda M, et al. Effects of Lactobacillus GG yoghurt on human intestinal microecology in Japanese subjects. *Nutr Today*. 1996;31:9S–12S.
40. Mohan R, Koebnick C, Schildt J, et al. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *J Clin Microbiol*. 2006;44:4025–4031.
41. Ahmed M, Prasad J, Gill H, et al. Impact of consumption of different levels of Bifidobacterium lactis HN019 on the intestinal microflora of elderly human subjects. *J Nutr Health Aging*. 2007;11:26–31.

42. D'Argenio G, Mazzacca G. Short-chain fatty acid in the human colon. In: Zappia V, Ragione F, Barbarisi A, Russo G, eds. *Advances in Nutrition and Cancer 2*. Vol. 472. Iacovo R: Springer US; 1999:149–158.
43. Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanisms of action of probiotics: recent advances. *Inflamm Bowel Dis*. 2009;15(2):300–310.
44. Buts JP, Bernasconi P, Van Craynest MP, Maldague P, De Meyer R. Response of human and rat small intestinal mucosa to oral administration of Saccharomyces boulardii. *Pediatr Res*. 1986;20(2):192–196.
45. Buts JP, De Keyser N, De Raedemaeker L. Saccharomyces boulardii enhances rat intestinal enzyme expression by endoluminal release of polyamines. *Pediatr Res*. 1994;36(4):522–527.
46. Guillot CC, Bacallao EG, Santa Cruz Dominguez M, Garcia MF, Gutierrez PM. Effects of *Saccharomyces boulardii* in children with chronic diarrhea, especially cases due to giardiasis. *Rev Mex de Puercultura y Pediatr*. 1995;2(12):1–5.
47. Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. *Gut*. 2003;52(6):827–833.
48. Caballero-Franco C, Keller K, De Simone C, Chadee K. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. 2007;292(1):G315–G322.
49. Ahrne S, Hagslatt ML. Effect of lactobacilli on paracellular permeability in the gut. *Nutrients*. 2011;3(1):104–117.
50. Bottari B, Felis GE, Salvetti E, et al. Effective identification of Lactobacillus casei group species: genome-based selection of the gene mutL as the target of a novel multiplex PCR assay. *Microbiology*. 2017;163(7):950–960.
51. Bao Y, Zhang Y, Zhang Y, et al. Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. *Food Control*. 2010;21(5):695–701.
52. Guarner F, Khan AG, Garisch J, et al. *Probiotics and prebiotics*. World Gastroenterol Organisation Global Guideline; 2011.
53. Marteau P. Evidence of probiotic strain specificity makes extrapolation of results impossible from a strain to another, even from the same species. *Annals Gastroenterol Hepatol*. 2011;2(1):34–36.
54. Ducrotte P, Sawant P, Jayanthi V. Clinical trial: lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. *World J Gastroenterol*. 2012;18(30):4012–4018.
55. Ligaarden SC, Axelsson L, Naterstad K, Lydersen S, Farup PG. A candidate probiotic with unfavourable effects in subjects with irritable bowel syndrome: a randomised controlled trial. *BMC Gastroenterol*. 2010;10:16.
56. Williams EA, Stimpson J, Wang D, et al. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Aliment Pharmacol Ther*. 2009;29(1):97–103.
57. Allen SJ, Jordan S, Storey M, et al. Probiotics in the prevention of eczema: a randomised controlled trial. *Archiv Dis Childhood*. 2014.
58. Arvola T, Laiho K, Torkkeli S, et al. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics*. 1999;104(5):1–4.
59. Kontiokari T, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. *Br Med J*. 2001;322:1571.
60. Hickson M, D'Souza AL, Muthu N, et al. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ*. 2007;335(7610):80.
61. Sampalis J, Psaradellis E, Rampakakis E. Efficacy of BIO K+ CL1285 in the reduction of antibiotic-associated diarrhea—a placebo controlled double-blind randomized, multi-center study. *Arch Med Sci*. 2010;6(1):56–64.
62. Horvath A, Dziechciarz P, Szajewska H. Meta-analysis: lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. *Aliment Pharmacol Ther*. 2011;33(12):1302–1310.
63. Rafii F, Sutherland JB, Cerniglia CE. Effects of treatment with antimicrobial agents on the human colonic microflora. *Ther Clin Risk Manag*. 2008;4(6):1343–1358.
64. Hempel S, Newberry SJ, Maher AR, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA*. 2012;307(18):1959–1969.
65. Blaabjerg S, Artzi D, Aabenhus R. Probiotics for the prevention of antibiotic-associated diarrhea in outpatients—a systematic review and meta-analysis. *Antibiotics*. 2017;6(4):21.
66. Cai J, Zhao C, Du Y, Zhang Y, Zhao M, Zhao Q. Comparative efficacy and tolerability of probiotics for antibiotic-associated diarrhea: systematic review with network meta-analysis. *United European Gastroenterology Journal*. 2018;6(2):169–180.
67. Szajewska H, Kołodziej M. Systematic review with meta–analysis: lactobacillus rhamnosus GG in the prevention of antibiotic–associated diarrhoea in children and adults. *Aliment Pharmacol Ther*. 2015;42(10):1149–1157.
68. Ringel-Kulka T, McRorie J, Ringel Y. Multi-center, double-blind, randomized, placebo-controlled, parallel-group study to evaluate the benefit of the probiotic bifidobacterium infantis 35624 in non-patients with symptoms of abdominal discomfort and bloating. *Am J Gastroenterol*. 2017;112(1):145–151.
69. Ringel-Kulka T, Palsson OS, Maier D, et al. Probiotic bacteria Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol*. 2011;45(6):518–525.
70. Tanaka K, Ishikawa H. Role of intestinal bacterial flora in oral tolerance induction. *Histol Histopathol*. 2004;19(3):907–914.
71. Drago L, Toscano M. Probiotics: immunomodulatory properties in allergy and eczema. In: Watson R, Preedy V, ed. *Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion*. Elsevier; 2015:831–837.
72. Kalliomaki M, Salminen S, Arvilommi H, et al. Probiotics in primary prevention of atopic disease: a randomised, placebo-controlled trial. *Lancet*. 2001;357:1076–1079.
73. Cuello-Garcia CA, Brożek JL, Fiocchi A, et al. Probiotics for the prevention of allergy: a systematic review and meta-analysis of randomized controlled trials. *J Allerg Clin Immunol*. 136(4):952–961.
74. Szajewska H, Shamir R, Turck D, van Goudoever JB, Mihatsch WA, Fewtrell M. Recommendations on probiotics in allergy prevention should not be based on pooling data from different strains. *J Allerg Clin Immunol*. 136(5):1422.
75. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allerg Clin Immunol*. 2007;119(1):184–191.
76. Abrahamsson TR, Jakobsson T, Bottcher MF, et al. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2007;119:1174–1180.
77. Wickens K, Black PN, Stanley TV, et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2008;122:788–794.
78. West CE, Hammarstrom ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol*. 2009;20:430–437.
79. Dotterud CK, Storro O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. *Br J Dermatol*. 2010;163(3):616–623.
80. Kim SO, Ah YM, Yu YM, Choi KH, Shin WG, Lee JY. Effects of probiotics for the treatment of atopic dermatitis: a meta-analysis of randomized controlled trials. *Ann Allergy Asthma Immunol*. 2014;113(2):217–226.
81. Weston S, Halbert A, Richmond P, et al. Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child*. 2005;90:892–897.
82. Sistek D, Kelly R, Wickens K, Stanley T, Fitzharris P, Crane J. Is the effect of probiotics on atopic dermatitis confined to food sensitized children? *Clin Exp Allerg*. 2006;36(5):629–633.
83. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet*. 1994;344:1046–1049.

84. Szajewska H, Kotowska M, Mrukowicz JZ, et al. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. *J Pediatr*. 2001;138:361–365.
85. Szajewska H, Skorka, Rszczynski M, et al. Meta-analysis: lactobacillus GG for treating acute diarrhoea in children. *Aliment Pharmacol Ther*. 2007;25:871–881.
86. Fang SB, Lee HC, Hu JJ, Hou SY, Liu HL, Fang HW. Dose-dependent effect of Lactobacillus rhamnosus on quantitative reduction of faecal rotavirus shedding in children. *J Trop Pediatr*. 2009;55(5):297–301.
87. Shornikova AV, Casas IA, Mykkanen N, Salo E, Vesikari T. Bacteriotherapy with Lactobacillus reuteri in rotavirus gastroenteritis. *Pediatr Infect Dis*. 1997;16:1103–1107.
88. Dubey AP, Rajeshwari K, Chakravarty A, et al. Use of VSL#3 in the treatment of rotavirus diarrhea in children. *J Clin Gastroenterol*. 2008;42:S126–S129.
89. Ardley C, Wright S. Travellers' diarrhoea. *Medicine*. 2010;38(1):26–29.
90. Stermer E, Lubezky A, Potasman I, Paster E, Lavy A. Is traveler's diarrhea a significant risk factor for the development of irritable bowel syndrome? A prospective study. *Clin Infect Dis*. 2006;43(7):898–901.
91. Katelaris PH, Salam I. Lactobacilli to prevent traveler's diarrhea. *New England J Med*. 1995;333(20):1360–1361.
92. de dios Pozo-Olano J, Warram Jr JH, Gomez RG, Cavazos MG. Effect of a lactobacilli preparation on traveler's diarrhea. A randomized, double blind clinical trial. *Gastroenterology*. 1978;74(5 Pt 1):829–830.
93. Kollaritsch H, Kremsner P, Wiedermann G, et al. Prevention of traveller's diarrhea: comparison of different nonantibiotic preparations. *Travel Med Int*. 1989:9–17.
94. Black F, Anderson P, Orskov J, et al. Prophylactic efficacy of Lactobacilli on travellers' diarrhea. *Travel Med*. 1989;7:333–335.
95. Hilton E, Kolakowski P, Singer C, et al. Efficacy of Lactobacillus GG as a diarrheal preventive in travelers. *J Travel Med*. 1997;4:41–43.
96. Kollaritsch H, Holst H, Grobara P, Wiedermann G. Prevention of traveler's diarrhea with Saccharomyces boulardii. Results of a placebo controlled double-blind study. *Fortschr Med*. 1993;111(9):152–156.
97. McFarland LV, Surawicz CM, Greenberg RN, et al. A randomized placebo-controlled trial of saccharomyces boulardii in combination with standard antibiotics for clostridium difficile disease. *JAMA: J Am Med Ass*. 1994;271(24):1913–1918.
98. Surawicz CM, McFarland LV, Greenberg RN. The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. *Clin Infect Dis*. 2000;31(4):1012–1017.
99. Klarin B, Wullt M, Palmquist I, et al. Lactobacillus plantarum 299v reduces colonisation of Clostridium difficile in critically ill patients treated with antibiotics. *Acta Anaesthesiol Scand*. 2008;52:1096–1102.
100. Hickson M, D'Souza AL, Muthu N, et al. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ*. 2007;335(7610):80.
101. Johnson S, Maziade PJ, McFarland LV, et al. Is primary prevention of Clostridium difficile infection possible with specific probiotics? *Int J Infect Dis*. 2012;16(11):e786–e792.
102. Manley KJ, Fraenkel MB, Mayall BC, et al. Probiotic treatment of vancomycin-resistant enterococci: a randomised controlled trial. *MJA*. 2007;186:454–457.
103. Doron S, Hibberd PL, Goldin B, Thorpe C, McDermott L, Snydman DR. Effect of lactobacillus rhamnosus GG administration on vancomycin-resistant Enterococcus colonization in adults with comorbidities. *Antimicrob Agents Chemother*. 2015;59(8):4593–4599.
104. Kwon JH, Bommarito KM, Reske KA, et al. Randomized controlled trial to determine the impact of probiotic administration on colonization with multidrug-resistant organisms in critically ill patients. *Infect Control Hosp Epidemiol*. 2015;36(12):1451–1454.
105. Osterlund P, Ruotsalainen T, Korpela R, et al. Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. *Br J Cancer*. 2007;97:1028–1034.
106. Anukam KC, Osazuwa EO, Osadolor HB, et al. Yogurt containing probiotic Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 helps resolve moderate diarrhea and increases CD4 count in HAV-AIDS patients. *J Clin Gastroenterol*. 2008;42:239–243.
107. McFarland LV, Bernasconi P. Saccharomyces boulardii. A review of an innovative biotherapeutic agent. *Microb Ecol Health Dis*. 1993;6(4):157–171.
108. Dimidi E, Christodoulides S, Fragkos KC, Scott SM, Whelan K. The effect of probiotics on functional constipation in adults: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2014;100(4):1075–1084.
109. Yang YX, He M, Hu G, et al. Effect of a fermented milk containing Bifidobacterium lactis DN-173010 on Chinese constipated women. *World J Gastroenterol*. 2008;14(40):6237–6243.
110. Mollenbrink M, Bruckschen E. Treatment of chronic constipation with physiologic Escherichia coli bacteria. Results of a clinical study of the effectiveness and tolerance of microbiological therapy with the E. coli Nissle 1917 strain (Mutaflor). *Med Klin (Munich)*. 1994;89(11):587–593.
111. Eskesen D, Jespersen L, Michelsen B, Whorwell PJ, Muller-Lissner S, Morberg CM. Effect of the probiotic strain Bifidobacterium animalis subsp. lactis, BB-12(R), on defecation frequency in healthy subjects with low defecation frequency and abdominal discomfort: a randomised, double-blind, placebo-controlled, parallel-group trial. *Br J Nutr*. 2015;114(10):1638–1646.
112. Ojetti V, Petruzziello C, Migneco A, Gnarra M, Gasbarrini A, Franceschi F. Effect of Lactobacillus reuteri (DSM 17938) on methane production in patients affected by functional constipation: a retrospective study. *Eur Rev Med Pharmacol Sci*. 2017;21(7):1702–1708.
113. Fateh R, Iravani S, Frootan M, Rasouli MR, Saadat S. Synbiotic preparation in men suffering from functional constipation: a randomised controlled trial. *Swiss Med Wkly*. 2011;141:w13239.
114. Miller LE, Ouwehand AC. Probiotic supplementation decreases intestinal transit time: meta-analysis of randomized controlled trials. *World J Gastroenterol*. 2013;19(29):4718–4725.
115. Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev*. 2015;(2):CD006895.
116. Bird AS, Gregory PJ, Jalloh MA, Cochrane ZR, Hein DJ. Probiotics for the treatment of infantile colic: a systematic review. *J Pharm Pract*. 2017;30(3):366–374.
117. Mayer EA, Labus JS, Tillisch K, Cole SW, Baldi P. Towards a systems view of IBS. *Nat Rev Gastroenterol Amp Hepatol*. 2015;12:592.
118. Rafsky HA, Rafsky JC. Clinical and bacteriological studies of a new Lactobacillus acidophilus concentrate in functional gastrointestinal disturbances. *Am J Gastroenterol*. 1955;24:87–92.
119. Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: updated systematic review with meta-analysis. *World J Gastroenterol*. 2015;21(10):3072–3084.
120. O'Sullivan MA, O'Morain CA. Bacterial supplementation in the irritable bowel syndrome: a randomised double-blind placebo-controlled crossover study. *Digestiv Liver Dis*. 2000;32:294–301.
121. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and Bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterol*. 2005;128:541–551.
122. Newcomer AD, Park HSP, O'Brien PC, McGill DB. Response of patients with irritable bowel syndrome and lactase deficiency using unfermented acidophilus milk. *Am J Clin Nutr*. 1983;38(2):257–263.
123. Derwa Y, Gracie DJ, Hamlin PJ, Ford AC. Systematic review with meta−analysis: the efficacy of probiotics in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2017;46(4):389–400.
124. Bourreille A, Cadiot G, Le Dreau G, et al. Saccharomyces boulardii does not prevent relapse of Crohn's disease. *Clin Gastroenterol Hepatol*. 2013;(0).
125. Sang LX, Chang B, Zhang WL, Wu XM, Li XH, Jiang M. Remission induction and maintenance effect of probiotics on ulcerative colitis: a meta-analysis. *World J Gastroenterol*. 2010;16(15):1908–1915.
126. Vajro P, Mandato C, Licenziati MR, et al. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. *J Pediatr Gastroenterol Nutr*. 2011;52(6):740–743.
127. Liong MT. Roles of probiotics and prebiotics in colon cancer prevention: postulated mechanisms and in-vivo evidence. *Int J Mol Sci*. 2008;9(5):854–863.

128. Ishikawa H, Akedo I, Otani T, et al. Randomized trial of dietary fiber and Lactobacillus casei administration for prevention of colorectal tumors. *Int J Cancer*. 2005;116(5):762–767.
129. Academy of Breastfeeding Medicine. ABM clinical protocol #4: mastitis. Revision. *Breastfeed Med*. 2008;3(3):177–180.
130. Arroyo R, Martin V, Maldonado A, Jimenez E, Fernandez L, Rodriguez JM. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacilli isolated from breast milk. *Clin Infect Dis*. 2010;50(12):1551–1558.
131. Jimenez E, Fernandez L, Maldonado A, et al. Oral administration of Lactobacillus strains isolated from breast milk as an alternative for the treatment of infectious mastitis during lactation. *Appl Environ Microbiol*. 2008;74:4650–4655.
132. Ilmonen J, Isolauri E, Poussa T, Laitinen K. Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: a randomized placebo-controlled trial. *Clin Nutr*. 2011;30(2):156–164.
133. Laitinen K, Ilmonen J, Isolauri E. Dietary counselling and probiotic intervention initiated in early pregnancy modifies maternal adiposity over 12 months postpartum. *Obesity Facts*. 2009;2(suppl 2):4.
134. Reid G, Bruce AW. Selection of Lactobacillus strains for urogenital applications. *J Infect Dis*. 2001;183(suppl 1):S77S80.
135. McMillan A, Dell M, Zellar MP, et al. Disruption of urogenital biofilms by lactobacilli. *Colloids Surfaces B: Biointerfaces*. 2011;86(1):58–64.
136. Kohler GA, Assefa S, Reid G. Probiotic interference of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the opportunistic fungal pathogen Candida albicans. *Infect Dis Obstet Gynecol*. 2012;2012:636474.
137. Wagner RD, Johnson SJ. Probiotic lactobacillus and estrogen effects on vaginal epithelial gene expression responses to Candida albicans. *J Biomed Sci*. 2012;19:58.
138. Martinez RC, Franceschini SA, Patta MC, et al. Improved cure of bacterial vaginosis with single dose of tinidazole (2 g), Lactobacillus rhamnosus GR-1, and Lactobacillus reuteri RC-14: a randomized, double-blind, placebo-controlled trial. *Can J Microbiol*. 2009;55:133–138.
139. Senok AC, Verstraelen H, Temmerman M, Botta GA. Probiotics for the treatment of bacterial vaginosis. *Cochrane Database Syst Rev*. 2009;4:CD006289.
140. Grin PM, Kowalewska PM, Alhazzan W, Fox-Robichaud AE. Lactobacillus for preventing recurrent urinary tract infections in women: meta-analysis. *Can J Urol*. 2013;20(1):6607–6614.
141. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. *FEMS Immunol Med Microbiol*. 2001;30:49–52.
142. Beerepoot MA, ter Riet G, Nys S, et al. Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women. *Arch Intern Med*. 2012;172(9):704–712.
143. Trautner BW, Gupta K. The advantages of second best: comment on "Lactobacilli vs antibiotics to prevent urinary tract infections." *Arch Intern Med*. 2012;172(9):712–714.
144. Pirotta M, Gunn J, Chondros P, et al. Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. *BMJ*. 2004;329(7465):548.
145. Nosova T, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M. Acetaldehyde production and metabolism by human indigenous and probiotic Lactobacillus and Bifidobacterium strains. *Alcohol Alcohol*. 2000;35(6):561–568.
146. Gratz S, Mykkänen H, El-Nezami H. Aflatoxin B1 binding by a mixture of Lactobacillus and Propionibacterium: in vitro versus ex vivo. *J Food Prot*. 2005;68(11):2470–2474.
147. Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy—a randomised, double-blind, placebo-controlled study. *Br J Nutr*. 2009;101:1722–1726.
148. Hilton E, Rindos P, Isenberg HD. Lactobacillus GG vaginal suppositories and vaginitis. *J Clin Microbiol*. 1995;33:1433.
149. Hempel S, Newberry S, Ruelaz A, et al. Safety of probiotics used to reduce risk and prevent or treat disease. *Evidence Rep Technol Assess*. 2011;200:1–645.
150. Mvd N, Brummer RJ, Guarner F, Morelli L, Cabana M, Claassen E. Safety of probiotics and synbiotics in children under 18 years of age. *Benef Microb*. 2015;6(5):615–630.
151. Sanders ME, Akkermans LM, Haller D, et al. Safety assessment of probiotics for human use. *Gut Microbes*. 2010;1(3):164–185.
152. Patton TJ, Guandalini S. Are probiotic effects dose-related? In: Guarino A, Quigley EM, eds. *Probiotic Bacteria and Their Effect on Human Health and Well-Being*. Vol. 107. Walker WA: Karger; 2013:151–160.
153. Patras KA, Wescombe PA, Rösler B, Hale JD, Tagg JR, Doran KS. Streptococcus salivarius K12 Limits group B Streptococcus vaginal colonization. *Infect Immun*. 2015;83(9):3438–3444.
154. Hemmerling A, Harrison W, Schroeder A, et al. Phase 1 Dose-ranging safety trial of lactobacillus crispatus CTV-05 for the prevention of bacterial vaginosis. *Sexually Transmitt Dis*. 2009;36(9):564–569.
155. Hatanaka M, Yamamoto K, Suzuki N, et al. Effect of Bacillus subtilis C-3102 on loose stools in healthy volunteers. *Benef Microb*. 0(0):1–10.
156. Urdaci MC, Bressollier P, Pinchuk I. Bacillus clausii probiotic strains: antimicrobial and immunomodulatory activities. *J Clin Gastroenterol*. 2004;38(suppl 6):S86–S90.
157. Daikos GK, Kontomichalou P, Bilalis D, Pimenidou L. Intestinal flora ecology after oral use of antibiotics: terramycin, chloramphenicol, ampicillin, neomycin, paromomycin, aminodidin. *Chemotherapy*. 1968;13:146–160.
158. Finegold SM, Mathisen GE, George WL. Changes in human intestinal flora related to the administration of antimicrobial agents. In: Hentges DJ, ed. *Human Intestinal Microflora in Health and Disease*. London: Academic Press; 1983: 355–448.
159. Pradhan A, Majumdar MK. Metabolism of some drugs by intestinal lactobacilli and their toxicological considerations. *Acta Pharmacol Toxicol (Copenh)*. 1986;58:11–15.
160. Szajewska H, Mrukowicz J. Meta-analysis: non-pathogenic yeast Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther*. 2005;22(5):365–372.
161. Plummer SF, Garaiova I, Sarvotham T, et al. Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. *Int J Antimicrob Agents*. 2005;26(1):69–74.
162. Lonnermark E, Friman V, Lappas G, et al. Intake of Lactobacillus plantarum reduces certain gastrointestinal symptoms during treatment with antibiotics. *J Clin Gastroenterol*. 2010;44:106–112.
163. Lionetti E, Miniello VL, Castellaneta SP, et al. Lactobacillus reuteri therapy to reduce side-effects during anti- Helicobacter pylori treatment in children: a randomized placebo controlled trial. *Aliment Pharmacol Ther*. 2006;24:1461–1468.
164. Wenus C, Goll R, Loken EB, et al. Prevention of antibiotic-associated diarrhoea by a fermented probiotic milk drink. *Eur J Clin Nutr*. 2008;62:299–301.
165. Fox MJ, Ahuja KDK, Robertson IK, Ball MJ, Eri RD. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. *BMJ Open*. 2015;5(1).
166. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics*. 2005;115:5–9.
167. Leyer GJ, Li S, Mubasher ME, et al. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics*. 2009;124:e172–e179.
168. Maldonado J, Canabate F, Sempere L, et al. Human milk probiotic Lactobacillus fermentum CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. *J Pediatr Gastroenterol Nutr*. 2012;54(1):55–61.
169. Cox AJ, Pyne DB, Saunders PU, et al. Oral administration of the probiotic Lactobacillus fermentum VRI-003 and mucosal immunity in endurance athletes. *Br J Sports Med*. 2008. https://doi.org/10.1136/bjsm.2007.044628.
170. Tubelius P, Stan V, Zachrisson A. Increasing work-place healthiness with the probiotic Lactobacillus reuteri: a randomised, double-blind placebo-controlled study. *Environ Health Global Access Sci Source*. 2005;4. https://doi.org/10.1186/1476-1069X-1184-1125.

171. Hatakka K, Savilahti E, Ponka A, et al. Effect of long-term consumption of probiotic milk on infections in children attending day care centres: double-blind, randomised trial. *Br Med J*. 2001;322:1327.
172. Hojsak I, Snovak N, Abdovic S, et al. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. *Clin Nutr*. 2010;29:312–316.
173. Agrawal A, Houghton LA, Morris J, et al. Clinical trial: the effects of a fermented milk product containing Bifidobacterium lactis DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. *Aliment Pharmacol Ther*. 2009;29(1):104–114.
174. Whorwell PJ, Altringer L, Morel J, et al. Efficacy of an encapsulated probiotic bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol*. 2006;101(7):1581–1590.
175. Williams EA, Stimpson J, Wang D, et al. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Aliment Pharmacol Ther*. 2008;29:97–103.
176. Kim HJ, Vazquez Roque MI, Camilleri M, et al. A randomized controlled trial of a probiotic combination VSL#3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motility*. 2005;17:1–10.
177. Rembacken BJ, Hawkey PM, Chalmers DM, et al. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomized trial. *Lancet*. 1999;354:635–639.
178. Matthes H, Krummenerl T, Giensch M, Wolff C, Schulze J. Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered Escherichia coli Nissle 1917 (EcN). *BMC Complement Altern Med*. 2010;10:13.
179. Sood A, Midha V, Makharia GD, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol*. 2009;7:1202–1209.
180. Miele E, Pascarella F, Giannetti E, et al. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol*. 2009;104:437–443.
181. Kruis W, Fric P, Pokrotnieks J, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut*. 2004;53:1617–1623.
182. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther*. 1997;11(5):853–858.
183. Henker J, Muller S, Laass MW, et al. Probiotic Escherichia coli Nissle 1917 (EcN) for successful remission maintenance of ulcerative colitis in children and adolescents: an open-label pilot study. *Z Gastroenterol*. 2008;46:874–875.
184. Zocco MA, Zileri Dal Verme L, Cremonini F, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther*. 2006;23:1567–1574.
185. Venturi A, Gionchetti P, Rizzello F, et al. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther*. 1999;13:1103–1108.
186. Anukam KC, Osazuwa EO, Osemene GI, et al. Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microb Infect*. 2006;8:2772–2776.
187. Anukam K, Osazuwa E, Ahonkhai I, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. *Microb Infect*. 2006;8(6):1450–1454.
188. Tafazzoli-Harandi H, Akbari SA, Afrakhteh H, al e. Comparison of metronidazole versus a combination of metronidazole plus probiotics in the treatment of bacterial vaginosis. *J Womens Health Issues*. 2014;3(3):2.
189. Metts J, Famula T, Trenev N, Clemens R. Lactobacillus acidophilus, strain NAS (H2O2 Positive), in reduction of recurrent candidal vulvovaginitis. *J Appl Res*. 2003;3(4):340–348.
190. Martinez RC, Franceschini SA, Patta MC, et al. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14. *Lett Appl Microbiol*. 2009;48:269–274.
191. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing Lactobacillus acidophilus as prophylaxis for candidal vaginitis. *Annals Int Med*. 1992;116(5):353–357.

Procyanidolic Oligomers

Michael T. Murray, ND

OUTLINE

GENERAL DESCRIPTION

The proanthocyanidins (also referred to as "procyanidins") are one of the most beneficial groups of plant flavonoids. The most active proanthocyanidins are those bound to other proanthocyanidins. Collectively, mixtures of proanthocyanidin dimers, trimers, tetramers, and larger molecules are referred to as procyanidolic oligomers (PCOs) or oligomeric proanthocyanidins (OPCs).[1,2] Although PCOs exist in many plants as well as red wine, commercially available sources of PCOs include extracts from grape seed skin *(Vitex vinifera)* and the bark of the maritime (Landes) pine.[1,2] This chapter reviews the benefits of PCOs from grape seeds and pine bark interchangeably because of their similar chemical composition and pharmacological profiles.

CHEMICAL COMPOSITION

Grape seed and pine bark PCO extracts are well defined chemically. Grape seed extracts are available that contain 92% to 95% PCOs, whereas the PCO content of pine bark extracts varies from 80% to 85% (Fig. 106.1).

HISTORY AND FOLK USE

In 1534, French explorer Jacques Cartier led an expedition up the Saint Lawrence River in what would become North America. Trapped by ice, Cartier and his crew were forced to survive on a ration of salted meat and biscuits. Cartier's crew began to exhibit signs and symptoms of scurvy, the cause of which was unknown at that time. Fortunately for Cartier and the surviving members of his crew, they met a Native American who advised them to make a tea from the bark and needles of pine trees. As a result, Cartier and his men survived.

More than 400 years later, Professor Jacques Masquelier of the University of Bordeaux, France, read the book Cartier wrote detailing his expedition. Intrigued by Cartier's story, Masquelier and others concluded that pine bark must contain some vitamin C as well as bioflavonoids, which can exert vitamin C–like effects.

Masquelier termed the active components of the pine bark *pycnogenols.*[1,3] This term was used to describe an entire complex of proanthocyanidin complexes found in a variety of plants, including pine bark, grape seeds, lemon tree bark, peanuts, cranberries, and citrus peels. The term *pycnogenol* has been replaced in the scientific community by the terms *proanthocyanidins, OPC,* and *PCO.* In the United States, Pycnogenol is a registered trademark of Horphag Ltd of Guernsey, United Kingdom, and refers to the PCO extracted from the bark of the French maritime pine.

Masquelier patented the method of extracting PCOs from pine bark in France in 1951 and from grape seeds in 1970. The two PCO sources have volleyed back and forth in popularity, based largely on which one is receiving research focus and marketing support.

PHARMACOLOGY

Extracts of PCOs demonstrate a wide range of activity, as listed in Box 106.1. Pharmacokinetic studies showed that proanthocyanidins and other breakdown products of PCOs from either grape seed extract or maritime pine are detectable in the serum after oral intake.[4,5] Pharmacokinetic studies in humans and animals given C^{14}-labeled PCOs also showed efficient oral absorption.[6,7]

Antioxidant and Free-Radical-Scavenging Activity

Perhaps the most celebrated effects of PCOs in the United States are their potent antioxidant and free-radical-scavenging activities. The

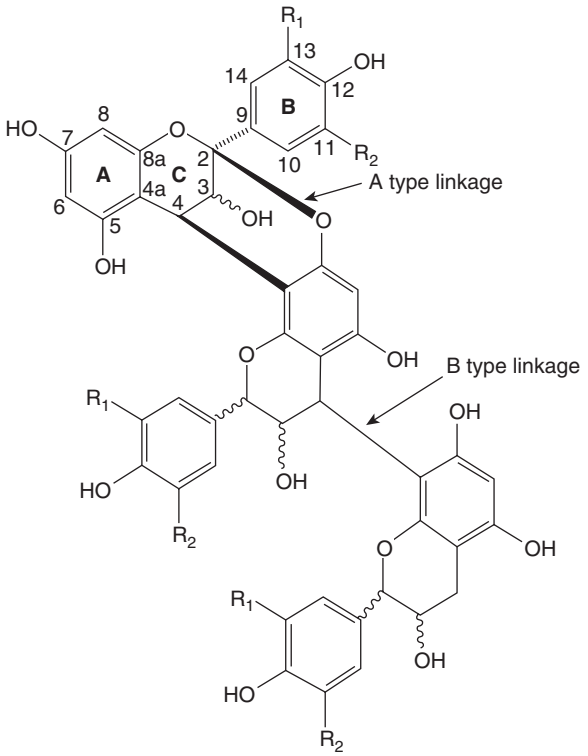


Fig. 106.1 General structure of proanthocyanidins. (R1, R2 = H, propelargonidin; R1 = OH, R2 = H, procyanidin; R1, R2 = OH, prodelphinidin). (From Ly Q, Luo F, Zhao X, Liu Y, Hu G, Sun C, Li X, Chen K. Identification of proanthocyanidins from litchi *[Litchi chinensis Sonn.]* pulp by LC-ESI-Q-TOF-MS and their antioxidant activity. *PLoS One.* 2015;10[3]:e0120480. PubMed PMID: 25793378.)

BOX 106.1 Basic Pharmacological Activity of Proanthocyanidins

- Increase intracellular vitamin C levels
- Decrease capillary permeability and fragility
- Scavenge oxidants and free radicals
- Inhibit destruction of collagen
- Reduce inflammation

Data from Rohdewald P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int J Clin Pharmacol Ther.* 2002;40:158–168; and Schwitters B, Masquelier J. *OPC in practice: biflavanols and their application.* Rome: Alfa Omega; 1993.

antioxidant and free-radical-scavenging effects of PCOs were discovered by Masquelier in 1986.[1]

One study evaluated the free-radical-scavenging activity of PCOs and determined their inhibitory effects on xanthine oxidase (a primary generator of oxygen-derived free radicals) and the lysosomal enzyme system (which governs the release of enzymes that can damage the connective tissue framework acting as a protective sheath around capillary walls).[8] This research, summarized in Box 106.2, provides a detailed explanation of the vascular protective action of PCOs and a strong rationale for their use in vascular disease.

In experimental models, the antioxidant activity of PCOs is much greater (approximately 50 times) than that of vitamin C and vitamin E. PCOs reduce lipid peroxidation[9] and oxidation of low-density lipoprotein (LDL),[10] increase basal levels of α-tocopherol in endothelial cells,

BOX 106.2 Antioxidant and Free-Radical-Scavenging Activities of Procyanidolic Oligomers

- Trap hydroxyl free radicals
- Trap lipid peroxides and free radicals
- Markedly delay the onset of lipid peroxidation
- Chelate free iron molecules, thereby preventing iron-induced lipid peroxidation
- Inhibit production of free radicals by noncompetitively inhibiting xanthine oxidase
- Inhibit the damaging effects of the enzymes (e.g., hyaluronidase, elastase, collagenase) that can degrade connective tissue structures

protect endothelium from oxidative stress induced by reactive nitrogen species,[11,12] and protect endothelial cells from activated macrophage-induced glutathione depletion.[13] From a cellular perspective, one of the most advantageous features of PCOs' free-radical-scavenging activity is that, because of their chemical structure, they are incorporated into cell membranes. This physical characteristic, along with their ability to protect against both water- and fat-soluble free radicals, provides significant cellular protection against damage by free radicals.[8]

Ant-Iinflammatory Activity

PCOs affect the activity or expression of many inflammatory mediators. In particular, PCOs suppress the nuclear factor (NF)-κB pathway and activate the Nrf2 pathway. NF-κB is a transcription factor activated by cell stress and in turn regulates the expression of many genes involved in the inflammatory response, such as immune receptors (interferon-gamma [IFN-γ] receptor, major histocompatibility complex [MHC], interleukin [IL]-2 receptor), cytokines (tumor necrosis factor-alpha [TNF-α], IL-6, IL-1), adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1]), prooxidant enzymes (cyclooxygenase-2 [COX-2], inducible nitric oxide synthase [iNOS]), and chemokines (monocyte chemoattractant protein [MCP]-1, MIP-1α, IL-8). PCOs reduce oxidative stress and inflammation by suppressing NF-κB and the mRNA expression of proinflammatory cytokines like TNF-α and IL-1β as well as the inflammatory prostaglandin products of COX-2. All of these effects support an anti-inflammatory and cancer preventive action for PCOs. Further supporting this action is that PCOs also activate Nrf2, a crucial transcriptional regulator needed to manufacture cytoprotective compounds in response to oxidative stress and inflammation.

In vitro and in vivo studies also show that PCOs significantly directly inhibit COX-1 and COX-2 enzymes as well as the formation of proinflammatory cytokines, IL-1β, and TNF-β, as well as histamine release from mast cells.[14–17] When 10 volunteers received a single dose of 300 mg Pycnogenol, within 30 minutes, serum samples showed statistically significant inhibition of both COX-1 and COX-2 ($P < 0.002$). Blood samples before and after 5 days of administration of 200 mg Pycnogenol to five healthy humans showed similar inhibition.

Anticancer Activity

PCOs also prevent oxidative-induced DNA damage and promote DNA repair through direct antioxidant protection of DNA and prevention of the formation of highly mutagenic cyclobutane pyrimidine dimers (CPDs), enhancing the functions of DNA and CPD repair enzymes, promoting nucleotide excision repair mechanisms, and inhibiting DNA hypomethylation.[18] PCOs exert a number of anticancer effects beyond their general antioxidant actions. PCOs were shown to selectively induce apoptosis in human breast cancer cells, inhibit cell

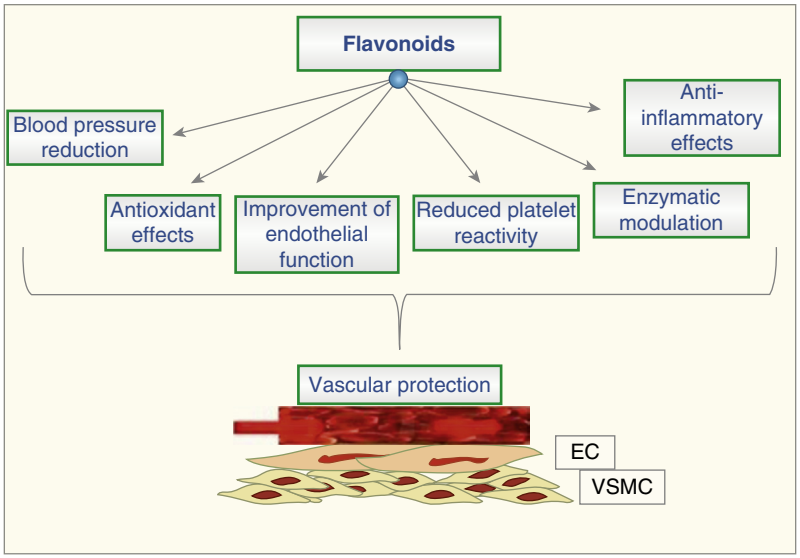


Fig. 106.2 Potential effects of flavonoids on cardiovascular protection. (From Grassi D, Desideri G, Ferri C. Flavonoids: antioxidants against atherosclerosis. *Nutrients.* 2010;2[8]:889–902. PubMed PMID: 22254061.)

proliferation and induce differentiation and apoptosis in leukemia cells, and inhibit nitrosamine activation in lung microsomes.[19–22]

Cardiovascular Protection

In addition to protecting against oxidative damage to low-density lipoprotein (LDL) and endothelial cells, PCOs increase erythrocyte membrane fluidity, reduce platelet aggregation, enhance endothelial nitric oxide synthase (eNOS) activity to increase nitric oxide levels, and inhibit angiotensin-I–converting enzyme (ACE) (Fig. 106.2).[23–26] PCO extracts were shown to lower blood cholesterol levels and to shrink the size of cholesterol deposits in arteries in animal and human studies.[1,27,28]

PCOs also significantly lower C-reactive protein (CRP), a major risk factor for cardiovascular disease. In a double-blind study, 29 subjects in the Pycnogenol group and 26 patients in the placebo group had CRP levels higher than 3 mg/L at baseline.[16] After 3-month treatment with Pycnogenol, at a dose of 100 mg/day, CRP significantly decreased plasma free radicals to 70.1% of baseline values. Specifically, plasma CRP levels decreased from 3.9 mg/L at baseline to 1.1 mg/L in the Pycnogenol group, whereas the control group had initial values of 3.9 mg/L that decreased to 3.6 mg/L. Fibrinogen levels were found to be lowered to 62.8% of initial values in the Pycnogenol group.

Protection of Collagen and Cartilage

Collagen, the most abundant protein of the body, is responsible for maintaining the integrity of ground substance as well as the integrity of tendons, ligaments, and cartilage. Collagen is also the support structure of the dermis and blood vessels. PCOs are remarkable in their effects of supporting collagen structures and preventing collagen destruction. They affect collagen metabolism in several ways. They have the unique ability to cross-link collagen fibers, resulting in reinforcement of the natural cross-linking of collagen that forms the so-called collagen matrix of connective tissue.[29,30] They also protect against free radical damage with their potent antioxidant and free-radical-scavenging action, and they inhibit enzymatic cleavage of collagen by enzymes secreted by leukocytes during inflammation and microbes during infection.[8,31] PCOs also prevent the release and synthesis of compounds that promote inflammation and allergies, such as histamine, serine proteases, prostaglandins, and leukotrienes.[1,2,32,33]

PCOs also exert significant chondroprotective effects. In one controlled trial, 33 patients with severe osteoarthritis (OA) scheduled for a knee arthroplasty either received 100 mg of Pycnogenol twice daily or no treatment (control group) 3 weeks before surgery. Results demonstrated that the intake of Pycnogenol downregulated the gene expression of various cartilage degradation markers in the patients' chondrocytes; the decreases of MMP3, MMP13, and the proinflammatory cytokine IL-1β were statistically significant.[34]

CLINICAL APPLICATIONS

The primary clinical applications of PCOs are in the treatment of the following conditions:

- Antioxidant therapy
- Asthma
- Atherosclerosis, hypertension, metabolic syndrome, and type 2 diabetes
- Attention deficit disorder and poor cognition
- Male infertility and erectile function
- Osteoarthritis
- Periodontal disease
- Venous insufficiency and capillary fragility
- Visual function, retinopathy, and macular degeneration

Antioxidant Therapy

PCOs exert broad-spectrum antioxidant activity and are clinically useful in many health conditions because of this action alone. In healthy human subjects, supplementation with PCOs for 6 weeks was shown to considerably improve the plasma oxygen radical absorbance capacity score (ORAC).[28] In one double-blind study, the effect of a standardized formulation of a PCO bound to phosphatidylcholine (Leucoselect Phytosome) was determined on the susceptibility of LDL to oxidation in a group of heavy smokers.[35] Among oxidative indexes, the concentration of thiobarbituric acid reactive substances was significantly reduced in subjects taking the PCO extract, and the lag phase was prolonged compared with the values in subjects taking a placebo, as well as with baseline values.

In another double-blind study, a group of 155 smokers was supplemented with 50 mg Pycnogenol or a placebo for 8 weeks followed by

2 weeks' cessation. Results showed that even at this dosage, Pycnogenol was able to significantly lower reactive oxygen species.[36]

Athletes can also gain benefits from PCOs. One study was designed to evaluate the effects of Pycnogenol on improving physical fitness in normal individuals and athletes training for a triathlon. The study was divided into two parts. In part 1 (Pycnogenol 100 mg/day), the Army Physical Fitness Test (APFT) was used to assess improvement in physical fitness during an 8-week preparation and training program. In part 2 (Pycnogenol 150 mg/day), the study evaluated the effects of Pycnogenol supplementation in athletes in training for a triathlon.

In part 1, results showed that there was a significant improvement in both males and females in the 2-mile running time, sit-ups, and push-ups within both groups, but the group using Pycnogenol performed statistically better than controls. In part 2, the swimming, biking, and running scores in both groups improved with training. However, the Pycnogenol group had more benefits in comparison with controls. The total triathlon time was 89 minutes and 44 seconds in Pycnogenol subjects versus 96 minutes and 5 seconds in controls. Controls improved their performance time by 4.6 minutes on average in comparison with an improvement of 10.8 minutes in Pycnogenol subjects. A significant decrease in cramps and running and postrunning pain was seen in the Pycnogenol group; there were no significant differences in controls. In Pycnogenol subjects, there was a lower increase in oxidative stress and a faster recovery.[37]

The performance benefits are not limited to the body; cognitive function can also improve with PCO supplementation. In a double-blind, placebo-controlled study in 101 elderly participants (60–85 years), consuming a daily dose of 150 mg of Pycnogenol for 3 months produced significant improvements in several memory-based cognitive assessments and lipid peroxidation products (F2-isoprostanes) relative to the control group.[38]

Asthma

PCOs appear to have an affinity for the lungs, indicating a possible benefit in lung disorders, including asthma. In a double-blind study involving 60 subjects (aged 6–18 years old) with mild to moderate asthma, patients were given either Pycnogenol (1 mg/lb body weight per day) or placebo for 3 months. Subjects were instructed to record their peak expiratory flow with an Assess Peak Flow Meter each evening. Compared with subjects taking a placebo, the group that took Pycnogenol had significantly more improvement in pulmonary function and asthma symptoms. Those in the Pycnogenol group were able to reduce or discontinue their use of rescue inhalers more often than the placebo group. There was also a significant reduction of urinary leukotrienes in the Pycnogenol group. The results of this study demonstrate the efficacy of Pycnogenol as an adjunct in the management of mild to moderate childhood asthma. Given the safety of PCOs, higher dosages (e.g., 2–3 mg/lb body weight) may produce better results than the lower dosage used in the clinical trial.[39]

Atherosclerosis, Hypertension, Metabolic Syndrome, and Type 2 Diabetes

PCO extracts, although in a supplement form, should be regarded as a necessary food in the prevention and treatment of atherosclerosis, metabolic syndrome, and type 2 diabetes. PCOs are similar to red wine components that are protective against heart disease, but they are more concentrated.[40] The active components in red wine are thought to be proanthocyanidins. Flavonoid consumption, in general, has an inverse correlation with death due to heart attack.[41] Both Pycnogenol and grape seed extract showed considerable ability to reduce cardiovascular risk factors, especially in patients with type 2 diabetes.[42–44] However, even healthy subjects can take advantage of the beneficial effects of PCOs against cardiovascular risk factors. In one study, when 25 healthy subjects received Pycnogenol (150 mg/day) for 6 weeks, a significant increase in ORAC in plasma was observed throughout the supplementation period (returning to baseline after a 4-week washout period).[28] Pycnogenol also significantly reduced LDL cholesterol levels and increased high-density lipoprotein (HDL) cholesterol levels in the plasma of two thirds of the subjects. Although the LDL changes reversed during washout, the HDL increase did not. Like other flavonoid sources, PCO extracts appear to be very helpful in improving endothelial function. Again, this effect may be particularly important in patients with hypertension, metabolic syndrome, and diabetes. In one double-blind study in 58 patients with hypertension who received supplementation with 100 mg PCO over 12 weeks, endothelin-1 concentrations were significantly lower, and concentrations of 6-keto prostaglandin F_{1a} in plasma were significantly higher, in the PCO group than in the placebo group. Additionally, the PCOs helped reduce the effective dose of the calcium antagonist nifedipine in a statistically significant manner.[41] In another double-blind study, PCOs from grape seed extract were shown to produce a mild hypotensive effect (−11 mm Hg for both systolic and diastolic readings) in patients with metabolic syndrome at dosages of 150 and 300 mg/day.[45]

The benefits of PCOs in hypertension appear to be most significant in pre- to moderate hypertension. In one study, 36 subjects with prehypertension were randomized to ingest a juice containing 0 mg (placebo) or 150 mg PCOs twice daily for 6 weeks preceded by a 2-week period where both groups took the placebo beverage and followed by 4-week no-beverage follow-up. Results showed that subjects taking the GSE for 6 weeks significantly reduced both their systolic (–5.6%) and diastolic blood pressure (BP) (–4.7%). However, subjects with higher initial BP experienced greater BP reduction, nearly double the effect size. The majority of subjects were able to achieve normal BP measurements with PCO supplementation. In contrast, no effect was noted in the placebo group. BP returned to baseline levels after the 4-week discontinuation period of the PCO beverage, indicating that the results are only apparent while the PCOs are being consumed.[46]

PCOs may prove to be vitally important in preserving renal function in hypertensive patients. One double-blind study evaluated the effects of Pycnogenol as an adjunct to ACE inhibitor ramipril treatment of hypertensive patients presenting with early signs of renal failure. One group of 26 patients was medicated with 10 mg/day ramipril only; a second group of 29 patients took Pycnogenol (150 mg/day) in addition to the ACE inhibitor over 6 months. At the end of the trial, lower systolic and diastolic BPs were found in both groups, with a significant further reduction of diastolic pressure in the group given Pycnogenol in addition to ramipril. The study also showed that Pycnogenol was effective in improving BP in patients with metabolic syndrome. The study found that taking Pycnogenol as an adjunct to ramipril significantly further lowered systolic and diastolic BPs compared with the group taking ramipril alone. Although average blood pressure in the ramipril group was lowered to a borderline high of 128.2/90.2 mm Hg, the values in the group taking Pycnogenol with ramipril reached essentially normal values (122.2/85.3 mm Hg) after 6 months of treatment. Kidney function improved in both groups, as judged by a significant reduction of protein detected in collected urine. With ramipril alone, urinary protein decreased by 22%, and with the addition of Pycnogenol, it decreased by 52.7%. Further, the group taking Pycnogenol had a lower fasting blood glucose level, which was reduced from a high average value of 135.6 mg/dL at baseline to an essentially healthy reference value of 102.3 mg/dL after 6 months of treatment. Pycnogenol also led to a remarkable improvement of blood-flow velocity in the kidney arteries. Blood-flow velocity in the kidneys significantly increased with ramipril from 17.2 to 23.8 cm/s for systolic

and 4.2 to 2.0 cm/s for diastolic; the addition of Pycnogenol was more effective, improving blood flow from 18.2 to 27.2 cm/s for systolic and 4.1 to 9.8 cm/s for diastolic.[47]

Other studies showed that PCOs might lower fasting glucose levels. In a study of patients with type 2 diabetes, patients received, in succession, 50, 100, 200, and 300 mg Pycnogenol in intervals of 3 weeks.[48] Every 3 weeks, fasting and postprandial glucose, endothelin-1, glycosylated hemoglobin (HbA_{1c}), and insulin were analyzed. Fasting blood glucose was lowered dose-dependently until a dose of 200 mg Pycnogenol was administered. Increasing the dose from 200 to 300 mg did not further decrease blood glucose. Compared with baseline, 100 to 300 mg of Pycnogenol lowered fasting glucose significantly from 8.64 to 7.54 mmol/L (155–135 mg/dL). The maximum decrease of postprandial glucose was observed with 200 mg of Pycnogenol (0.07 ± 2.69 mmol/dL [180 mg/dL]). HbA_{1c} levels decreased continuously from 8.02% to 7.37%. Endothelin-1 decreased significantly after 100 to 300 mg Pycnogenol, from 104 to 91 pg/mL. Stimulation of insulin secretion was excluded as a cause for lower glucose levels because insulin levels were not affected.

These effects were confirmed in a double-blind, placebo-controlled study with 77 patients with type 2 diabetes. After 8 weeks of treatment, the median drop in fasting blood glucose levels in the Pycnogenol group was 1.96 mmol/L (35.28 mg/dL).[49]

PCOs are particularly useful in addressing the microvascular pathology of diabetes. In one study in patients with severe diabetic microangiopathy, 30 patients received oral Pycnogenol (50 mg capsules, three times daily, for a total of 150 mg/day for 4 weeks), whereas 30 comparable patients were observed as controls.[50] After 4 weeks, microcirculatory and clinical evaluations showed a progressive decrease in skin flux at rest in the foot (indicating an improvement in the level of microangiopathy), a significant decrease in capillary filtration, and a significant improvement in the venoarteriolar response in all treated subjects. There were no visible effects in controls, except a slight reduction in skin flux at rest in the foot.

PCOs are also very much indicated in the treatment of diabetic retinopathy. Early intervention in diabetic retinopathy led to significant improvements in microcirculation, retinal edema, and visual acuity.[51]

Menopause and Postmenopause

PCOs appear clinically indicated in women during and beyond menopause because they have been shown to exert benefit in reducing menopausal symptoms and offering significant cardiovascular benefits during and after menopause. Pycnogenol, 100 mg/day, was used as a supplement for 8 weeks by a group of 35 women. A comparable group of 35 women with identical cardiovascular risk factors was included as the control group.

Almost all menopausal symptoms, scored by the Menopausal Symptoms Questionnaire 34, improved significantly in the Pycnogenol group. Supplementation with Pycnogenol also decreased the slightly elevated cholesterol and triglycerides after 8 weeks ($P < 0.05$). Also, the fasting glucose levels were normalized. The borderline increased BP was reduced to normal values at 8 weeks ($P < 0.05$). Plasma free radicals dropped significantly by 22%. Homocysteine and CRP levels decreased sharply by 43% and 60%, respectively. No significant changes in these risk factors were noted in the control group.[52]

In a double-blind, placebo-controlled study, 170 perimenopausal women were enrolled and treated with 30 mg Pycnogenol or placebo twice daily over a period of 3 months. Menopausal symptoms were evaluated by the Women's Health Questionnaire (WHQ) and by the Kupperman index, accompanied by an investigation of sex hormones and routine blood chemistry. A significant placebo effect was apparent in this study, suggesting an improvement of a majority of the WHQ categories. Compared with baseline, Pycnogenol significantly ($P < 0.05$) improved all symptoms with the exception of formication sensation and abnormal perceptions. Pycnogenol was found to be especially effective in improving vasomotor and insomnia/sleep problem symptoms, which were significantly better after 4 and 12 weeks than with placebo. The total Kupperman's index for perimenopausal symptom severity score decreased significantly by 56% compared with placebo (–39%) after 12 weeks of treatment ($P < 0.05$). The symptom score was also significantly better after only 4 weeks of treatment with Pycnogenol compared with placebo.[53]

In another double-blind study, 96 menopausal women aged 40 to 60 years were randomized to receive grape seed extract tablets containing either low-dose (100 mg/d) or high-dose (200 mg/d) PCOs, or placebo, for 8 weeks. The following significant changes were observed during the course of the study: (1) physical symptom score, hot flash score, and (2) Athens Insomnia Scale score decreased in the high-dose group after 8 weeks of treatment; (3) Hospital Anxiety and Depression Scale Anxiety score and (4) systolic and diastolic BP decreased in the low-dose and high-dose groups after 4 weeks; and lastly, (5) muscle mass increased in the low-dose and high-dose groups after 8 weeks of treatment.[54]

Attention Deficit Disorder

Increased oxidative damage is believed to be a central factor in attention deficit hyperactivity disorder (ADHD), indicating a possible application for PCO therapy. To date, four studies have been conducted in this patient population. In one study, children with ADHD supplementation with PCO (1 mg/kg body weight per day) had an improved ratio of reduced glutathione to oxidized glutathione (an indication of improved antioxidant status).[55] A second study confirmed this effect and also showed a reduced urinary catecholamine concentration, leading to less hyperactivity.[56] Another study showed that PCOs reduced the level of oxidized purines represented by 8-oxo-7,8-dihydroguanine in the urine and also increased total antioxidant status.[57] Finally, 61 children with ADHD supplemented with 1 mg/kg per day Pycnogenol or placebo over 4 weeks showed that 1 month of Pycnogenol administration caused a significant reduction in hyperactivity, improved attention and visual-motoric coordination, and improved the concentration of children with ADHD.[58] No positive effects were found in the placebo group. Standard questionnaires, the Child Attention Problems teacher rating scale, Conner's Teacher Rating Scale, the Conner's Parent Rating Scale, and a modified Wechsler Intelligence Scale for children were used as assessments. These results point to an option for the use of Pycnogenol as a nutritional adjunct in children with ADHD.

Mild Cognitive Impairment

Various flavonoids and polyphenols have been shown to exert beneficial effects in improving cognitive function. There is some evidence that PCOs also exert these benefits, especially in subjects with oxidative stress. In a study with 44 subjects with oxidative stress, the use of Pycnogenol at a dosage of 100 mg/day was associated with a decline in the Informant Questionnaire on Cognitive Decline in the Elderly (IQ Code), along with improvements in daily tasks, cognitive function, and oxidative stress.[59]

Male Infertility and Erectile Function

Pycnogenol alone[60] and in combination with L-arginine[61] was shown to be helpful in improving sperm quality and function (see Chapter 180, Male Infertility, for more information). Benefits have also been noted in erectile dysfunction.[62]

Osteoarthritis

Two double-blind studies have been conducted assessing the effect of PCOs in OA. In the first study, Pycnogenol (100 mg) or placebo was given daily for 3 months to 156 patients with osteoarthritis.[63] The global Western Ontario and McMasters University questionnaire for OA score decreased by 56% in the treatment group versus 9.6% in the placebo group. Walking distance on the treadmill test was prolonged from 68 m at the start of the study to 198 m in the Pycnogenol group compared with 65 to 88 m in the placebo group. The use of drugs decreased by 58% in the Pycnogenol group versus 1% in the placebo group. Foot edema decreased in 79% of Pycnogenol-treated patients versus 1% in controls. Similar results were seen in the second study when Pycnogenol was given at a dosage of 150 mg/day for 3 months.[64]

A pharmacokinetic study showed that procyanidins distribute into the synovial fluid of patients with OA, which supports rationalizing the results of clinical efficacy studies. Thirty-three patients with severe OA scheduled for a knee arthroplasty were randomized to receive either 200 mg per day Pycnogenol or no treatment over 3 weeks before surgery. Catechin and taxifolin primarily resided within the blood cells, whereas the microbial catechin metabolite δ-(3,4-dihydroxy-phenyl)-γ-valerolactone, ferulic, and caffeic acid were mainly present in synovial fluid samples. Taxifolin was detected in serum and synovial fluid exclusively in the Pycnogenol group.[65]

Periodontal Disease

Given the importance of supporting periodontal collagen structures in periodontal disease, PCOs are an important clinical consideration (see Chapter 199 on periodontal disease for more information).

Venous Insufficiency and Capillary Fragility

PCOs are very much indicated for the treatment of varicose veins, venous insufficiency, and capillary fragility because of their ability to:

- Reduce microvascular edema
- Increase the integrity of the venous wall
- Inhibit the breakdown of the compounds composing the ground substance
- Improve the muscular tone of the vein

Numerous double-blind studies with Pycnogenol validated the effectiveness of PCOs in chronic venous insufficiency, including studies showing an ability to decrease venous ulcer size,[66–68] reduce the edema and thrombosis associated with airline travel or prolonged sitting,[69–71] reduce nighttime claudication, and reduce other signs and symptoms of chronic venous insufficiency and microangiopathy.[72–74]

Visual Function, Retinopathy, and Macular Degeneration

Increased intake of PCO is likely to benefit almost everyone. This suggestion is perhaps best illustrated by research evaluating the effects of grape seed PCO extract on visual function in healthy subjects.[75,76] In one of the studies, 100 normal volunteers with no retinal disorder received either 200 mg/day of PCOs or placebo for 5 or 6 weeks. The group receiving PCOs demonstrated significant improvement in visual performance in dark and after-glare tests compared with the placebo group. PCO extracts were also shown to be of benefit in treating retinopathies, especially diabetic retinopathy. In one study, 40 patients with diabetes, atherosclerosis, and other vascular diseases involving the retina were randomly treated with placebo or PCO extract (50 mg three times daily for 2 months). The results demonstrated a beneficial effect of PCOs on the progression of retinopathy. In patients without any treatment (placebo group), the retinopathy progressively worsened during the trial, and the visual acuity significantly decreased. In contrast, the PCO-treated patients showed no deterioration of retinal function, and a significant recovery of visual acuity was also obtained. Fluorangiography showed an improvement of retinal vascularization and reduced endothelial permeability and leakage in the PCO group but not in the placebo-treated patients. Ophthalmoscopy and electroretinography also confirmed the beneficial effects of PCOs. In addition to the PCOs exerting free-radical-scavenging, anti-inflammatory, and capillary protective activities, it was suggested that PCOs might bind to the blood vessel wall proteins and mucopolysaccharides and produce a capillary "sealing" effect, leading to reductions in capillary permeability and edema formation.[77]

Psoriasis

Pycnogenol significantly improved the painful and visible symptoms of psoriasis, including redness, flaking, and the thickness of the total surface area of the affected skin patches.[78] Seventy-three patients (age range 30–45) with moderate/severe plaque psoriasis were included in a 12-week study. Patients either received "standard drug management" alone or with Pycnogenol at a dosage of 150 mg/day (50 mg three times daily). Over 12 weeks, the addition of Pycnogenol was shown to:

- Significantly decrease the area of skin affected by psoriasis in all body regions (Pycnogenol 20%; standard management [SM] 8%)
- Significantly reduce redness (Pycnogenol 44%; SM 28%), skin hardening (Pycnogenol 45%; SM 21%), and flaking (Pycnogenol 45%; SM 16%) of body area affected by psoriasis
- Significantly increase the content of water and oil/lipids in all areas of the skin
- Significantly reduce the need for standard management drugs
- Significantly reduce oxidative stress

In addition to the physical results just listed, supplementation with Pycnogenol was shown to significantly decrease the cost of treatment (decreased 36.4% on average in comparison with SM) and reduce the number of side effects reported for other treatments.

DOSAGE

As antioxidant support, a daily dose of 50 to 100 mg of either the grape seed or pine bark extract is suitable. When PCO extracts are being used for therapeutic purposes, the daily dosage should be increased to 150 to 300 mg/day.

TOXICITY

PCO extracts are without known side effects. In a clinical study with PCOs from grape seed extract, 29 subjects daily received oral dosages of 1000, 1500, or 2500 mg daily for 4 weeks. At this level, PCOs were found to be generally safe and well tolerated in humans. Research with a larger number of subjects is required to confirm these findings.[79]

DRUG INTERACTIONS

There are no documented drug interactions for the procyanidins.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rohdewald P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int J Clin Pharmacol Ther*. 2002;40:158–168.
2. Schwitters B, Masquelier J. *OPC in Practice: Biflavanols and Their Application*. Rome: Alfa Omega; 1993.
3. Masquelier J. [Procyanidolic oligomers.]. *J Parfums Cosmetiques Aromes*. 1990;95:89–97. [French].
4. Grimm T, Skrabala R, Chovanova Z, et al. Single and multiple dose pharmacokinetics of maritime pine bark extract (pycnogenol) after oral administration to healthy volunteers. *BMC Clin Pharmacol*. 2006;6:4. https://doi.org/10.1186/1472-6904-6-4.
5. Sano A, Yamakoshi J, Tokutake S, et al. Procyanidin B1 is detected in human serum after intake of proanthocyanidin-rich grape seed extract. *Biosci Biotechnol Biochem*. 2003;67:1140–1143.
6. Benakis A, Schopfer C, Ritschard J. Disposition of 14C-labelled ENDOTELON in humans. *Eur J Drug Metab Pharmacokinet*. 2008;33:37–43.
7. Tsang C, Auger C, Mullen W, et al. The absorption, metabolism and excretion of flavan-3-ols and procyanidins following the ingestion of a grape seed extract by rats. *Br J Nutr*. 2005;94:170–181.
8. Maffei Facino R, Carini M, Aldini G, et al. Free radicals scavenging action and anti-enzyme activities of procyanidines from *Vitis vinifera*. A mechanism for their capillary protective action. *Arzneimitteforschung*. 1994;44:592–601.
9. Virgili F, Kim D, Packer L. Procyanidins extracted from pine bark protect alpha-tocopherol in ECV 304 endothelial cells challenged by activated RAW 264.7 macrophages: role of nitric oxide and peroxynitrite. *FEBS Lett*. 1998;431:315–318.
10. Virgili F, Kobuchi H, Packer L. Procyanidins extracted from *Pinus maritima* (Pycnogenol): scavengers of free radical species and modulators of nitrogen monoxide metabolism in activated murine RAW 264.7 macrophages. *Free Radic Biol Med*. 1998;24:1120–1129.
11. Rimbach G, Virgili F, Park YC, et al. Effect of procyanidins from *Pinus maritima* on glutathione levels in endothelial cells challenged by 3-morpholinosydnonimine or activated macrophages. *Redox Rep*. 1999;4:171–177.
12. Devaraj S, Vega-Lopez S, Kaul N, et al. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids*. 2002;37:931–934.
13. Dvorakova M, Sivonova M, Trebaticka J, et al. The effect of polyphenolic extract from pine bark, Pycnogenol on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD). *Redox Rep*. 2006;11:163–172.
14. Schäfer A, Chovanová Z, Muchová J, et al. Inhibition of COX-1 and COX-2 activity by plasma of human volunteers after ingestion of French maritime pine bark extract (Pycnogenol). *Biomed Pharmacother*. 2006;60:5–9.
15. Blazso G, Gabor M, Rohdewald P. Antiinflammatory activities of procyanidin-containing extracts from Pinus pinaster Ait. after oral and cutaneous application. *Pharmazie*. 1997;52:380–382.
16. Belcaro G, Cesarone MR, Errichi S, et al. Variations in C-reactive protein, plasma free radicals and fibrinogen values in patients with osteoarthritis treated with Pycnogenol. *Redox Rep*. 2008;13:271–276.
17. Sharma SC, Sharma S, Gulati OP. Pycnogenol inhibits the release of histamine from mast cells. *Phytother Res*. 2003;17:66–69.
18. Yang L, Xian D, Xiong X. Proanthocyanidins against oxidative stress: from molecular mechanisms to clinical applications. *Biomed Res Int*. 2018;2018:8584136. https://doi.org/10.1155/2018/8584136.
19. Huynh HT, Teel RW. Selective induction of apoptosis in human mammary cancer cells (MCF-7) by pycnogenol. *Anticancer Res*. 2000;20:2417–2420.
20. Huang WW, Yang JS, Lin CF, et al. Pycnogenol induces differentiation and apoptosis in human promyeloid leukemia HL-60 cells. *Leuk Res*. 2005;29:685–692.
21. Huynh HT, Teel RW. Effects of pycnogenol on the microsomal metabolism of the tobacco-specific nitrosamine NNK as a function of age. *Cancer Lett*. 1998;132:135–139.
22. Huynh HT, Teel RW. Effects of intragastrically administered Pycnogenol on NNK metabolism in F344 rats. *Anticancer Res*. 1999;19(3A):2095–2099.
23. Chang WC, Hsu FL. Inhibition of platelet aggregation and arachidonate metabolism in platelets by procyanidins. *Prostagland Leukot Essent Fatty Acids*. 1989;38:181–188.
24. Meunier MT, Villie F, Jonadet M. Inhibition of angiotensin I converting enzyme by flavanolic compounds: in vitro and in vivo studies. *Planta Med*. 1987;54:12–15.
25. Sivonova M, Waczulikova I, Kilanczyk E, et al. The effect of Pycnogenol on the erythrocyte membrane fluidity. *Gen Physiol Biophys*. 2004;23:39–51.
26. Fitzpatrick DF, Bing B, Rohdewald P. Endothelium-dependent vascular effects of Pycnogenol. *J Cardiovasc Pharmacol*. 1998;32:509–515.
27. Wegrowski J, Robert AM, Moczar M. The effect of procyanidolic oligomers on the composition of normal and hypercholesterolemic rabbit aortas. *Biochem Pharmacol*. 1984;33:3491–3497.
28. Devaraj S, Vega-Lopez S, Kaul N, et al. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids*. 2002;37:931–934.
29. Masquelier J, Dumont MC, Dumas J. Stabilization of collagen by procyanidolic oligomers. *Acta Therap*. 1981;7:101–105. [French].
30. Tixier JM, Godeau G, Robert AM. Evidence by in vivo and in vitro studies that binding of pycnogenols to elastin affects its rate of degradation by elastases. *Biochem Pharmacol*. 1984;33:3933–3939.
31. Meunier MT, Duroux E, Bastide P. Free-radical scavenger activity of procyanidolic oligomers and anthocyanosides with respect to superoxide anion and lipid peroxidation. *Plant Med Phytother*. 1989;4:267–274.
32. Sharma SC, Sharma S, Gulati OP. Pycnogenol inhibits the release of histamine from mast cells. *Phytother Res*. 2003;17:66–69.
33. Cho KJ, Yun CH, Packer L, et al. Inhibition mechanisms of bioflavonoids extracted from the bark of *Pinus maritima* on the expression of proinflammatory cytokines. *Ann N Y Acad Sci*. 2001;928:141–156.
34. Jessberger S, Högger P, Genest F, Salter D, Seefried L. Cellular pharmacodynamic effects of Pycnogenol® in patients with severe osteoarthritis: a randomized controlled pilot study. *BMC Complement Altern Med*. 2017;17(1):537. https://doi.org/10.1186/s12906-017-2044-1.
35. Vigna GB, Costantini F, Aldini G, et al. Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers. *Metabolism*. 2003;52:1250–1257.
36. Belcaro G, Hu S, Cesarone MR, Dugall M. A controlled study shows daily intake of 50 mg of french pine bark extract (Pycnogenol®) lowers plasma reactive oxygen metabolites in healthy smokers. *Minerva Med*. 2013;104(4):439–446.
37. Vinciguerra G, Belcaro G, Bonanni E, et al. Evaluation of the effects of supplementation with Pycnogenol® on fitness in normal subjects with the Army Physical Fitness Test and in performances of athletes in the 100-minute triathlon. *J Sports Med Phys Fitness*. 2013;53(6):644–654.
38. Ryan J, Croft K, Mori T, et al. An examination of the effects of the antioxidant Pycnogenol on cognitive performance, serum lipid profile, endocrinological and oxidative stress biomarkers in an elderly population. *J Psychopharmacol*. 2008;22:553–562.
39. Lau BH, Riesen SK, Truong KP, et al. Pycnogenol as an adjunct in the management of childhood asthma. *J Asthma*. 2004;41:825–832.
40. Frankel EN, Kanner J, German JB, et al. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*. 1993;341:454–457.
41. Hertog MG, Feskens EJ, Hollman PC, et al. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet*. 1993;342:1007–1011.
42. Zibadi S, Rohdewald PJ, Park D, et al. Reduction of cardiovascular risk factors in subjects with type 2 diabetes by Pycnogenol supplementation. *Nutr Res*. 2008;28:315–320.
43. Kar P, Laight D, Rooprai HK, et al. Effects of grape seed extract in type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. *Diabet Med*. 2009;26:526–531.
44. Belcaro G, Cesarone MR, Ricci A, et al. Control of edema in hypertensive subjects treated with calcium antagonist (nifedipine) or angiotensin-converting enzyme inhibitors with Pycnogenol. *Clin Appl Thromb Hemost*. 2006;12:440–444.

45. Sivaprakasapillai B, Edirisinghe I, Randolph J, et al. Effect of grape seed extract on blood pressure in subjects with the metabolic syndrome. *Metabolism*. 2009;58:1743–1746.
46. Park E, Edirisinghe I, Choy YY, Waterhouse A, Burton-Freeman B. Effects of grape seed extract beverage on blood pressure and metabolic indices in individuals with pre-hypertension: a randomised, double-blinded, two-arm, parallel, placebo-controlled trial. *Br J Nutr*. 2015;16:1–13.
47. Cesarone MR, Belcaro G, Stuard S, et al. Kidney flow and function in hypertension: protective effects of pycnogenol in hypertensive participants—a controlled study. *J Cardiovasc Pharmacol Ther*. 2010;15(1):41–46.
48. Liu X, Zhou HJ, Rohdewald P. French maritime pine bark extract Pycnogenol dose-dependently lowers glucose in type 2 diabetic patients. *Diabetes Care*. 2004;27:839.
49. Cesarone MR, Belcaro G, Rohdewald P, et al. Improvement of diabetic microangiopathy with pycnogenol: a prospective, controlled study. *Angiology*. 2006;57:431–436.
50. Liu X, Wei J, Tan F, et al. Antidiabetic effect of Pycnogenol French maritime pine bark extract in patients with diabetes type II. *Life Sci*. 2004;75:2505–2513.
51. Steigerwalt R, Belcaro G, Cesarone MR, et al. Pycnogenol improves microcirculation, retinal edema, and visual acuity in early diabetic retinopathy. *J Ocul Pharmacol Ther*. 2009;25:537–540.
52. Luzzi R, Belcaro G, Hosoi M, et al. Normalization of cardiovascular risk factors in peri-menopausal women with Pycnogenol®. *Minerva Ginecol*. 2017;69(1):29–34.
53. Kohama T, Negami M. Effect of low-dose French maritime pine bark extract on climacteric syndrome in 170 perimenopausal women: a randomized, double-blind, placebo-controlled trial. *J Reprod Med*. 2013;58(1–2):39–46.
54. Terauchi M, Horiguchi N, Kajiyama A, et al. Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: a randomized, double-blind, placebo-controlled pilot study. *Menopause*. 2014;21(9):990–996.
55. Dvoráková M, Sivonová M, Trebatická J, et al. The effect of polyphenolic extract from pine bark, Pycnogenol on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD). *Redox Rep*. 2006;11:163–172.
56. Dvoráková M, Jezová D, Blazícek P, et al. Urinary catecholamines in children with attention deficit hyperactivity disorder (ADHD): modulation by a polyphenolic extract from pine bark (pycnogenol). *Nutr Neurosci*. 2007;10:151–157.
57. Chovanová Z, Muchová J, Sivonová M, et al. Effect of polyphenolic extract, Pycnogenol, on the level of 8-oxoguanine in children suffering from attention deficit/hyperactivity disorder. *Free Radic Res*. 2006;40:1003–1010.
58. Trebatická J, Kopasová S, Hradecná Z, et al. Treatment of ADHD with French maritime pine bark extract. *Pycnogenol. Eur Child Adolesc Psychiatry*. 2006;15:329–335.
59. Belcaro G, Dugall M, Ippolito E, Hu S, Saggino A, Feragalli B. The COFU3 Study. Improvement in cognitive function, attention, mental performance with Pycnogenol® in healthy subjects (55-70) with high oxidative stress. *J Neurosurg Sci*. 2015;59(4):437–446.
60. Roseff SJ. Improvement in sperm quality and function with French maritime pine tree bark extract. *J Reprod Med*. 2002;47:821–824.
61. Stanislavov R, Nikolova V, Rohdewald P. Improvement of seminal parameters with Prelox: a randomized, double-blind, placebo-controlled, cross-over trial. *Phytother Res*. 2009;23:297–302.
62. Stanislavov R, Rohdewald P. Improvement of erectile function by a combination of French maritime pine bark and roburins with aminoacids. *Minerva Urol Nefrol*. 2015;67(1):27–32.
63. Belcaro G, Cesarone MR, Errichi S, et al. Treatment of osteoarthritis with Pycnogenol. The SVOS (San Valentino Osteo-arthrosis Study). Evaluation of signs, symptoms, physical performance and vascular aspects. *Phytother Res*. 2008;22(4):518–523.
64. Cisár P, Jány R, Waczulíková I, et al. Effect of pine bark extract (Pycnogenol) on symptoms of knee osteoarthritis. *Phytother Res*. 2008;22(8):1087–1092. https://doi.org/10.1002/ptr.2461.
65. Mülek M, Seefried L, Genest F, Högger P. Distribution of constituents and metabolites of maritime pine bark extract (Pycnogenol®) into serum, blood cells, and synovial fluid of patients with severe osteoarthritis: a randomized controlled trial. *Nutrients*. 2017;58;9(5):E443. https://doi.org/10.3390/nu9050443.
66. Belcaro G, Cesarone MR, Errichi BM, et al. Venous ulcers: microcirculatory improvement and faster healing with local use of Pycnogenol. *Angiology*. 2005;56:699–705.
67. Cesarone MR, Belcaro G, Rohdewald P, et al. Comparison of Pycnogenol and Daflon in treating chronic venous insufficiency: a prospective, controlled study. *Clin Appl Thromb Hemost*. 2006;12(2):205–212.
68. Toledo RR, Santos MERC, Schnaider TB. Effect of pycnogenol on the healing of venous ulcers. *Ann Vasc Surg*. 2017;38:212–219.
69. Belcaro G, Cesarone MR, Rohdewald P, et al. Prevention of venous thrombosis and thrombophlebitis in long-haul flights with pycnogenol. *Clin Appl Thromb Hemost*. 2004;10:373–377.
70. Cesarone MR, Belcaro G, Nicolaides AN, et al. Prevention of venous thrombosis in long-haul flights with Flite Tabs: the LONFLIT-FLITE randomized, controlled trial. *Angiology*. 2003;54:531–539.
71. Sano A, Tokutake S, Seo A. Proanthocyanidin-rich grape seed extract reduces leg swelling in healthy women during prolonged sitting. *J Sci Food Agric*. 2013;93(3):457–462.
72. Cesarone MR, Belcaro G, Rohdewald P, et al. Improvement of signs and symptoms of chronic venous insufficiency and microangiopathy with Pycnogenol: a prospective, controlled study. *Phytomedicine*. 2010;17:835–839.
73. Cesarone MR, Belcaro G, Rohdewald P, et al. Rapid relief of signs/symptoms in chronic venous microangiopathy with pycnogenol: a prospective, controlled study. *Angiology*. 2006;57:569–576. *Erratum Angiology* 2008;59:385.
74. Nuzum DS, Gebru TT, Kouzi SA. Pycnogenol for chronic venous insufficiency. *Am J Health Syst Pharm*. 2011;68(17):1589–1590, 1599–1601.
75. Corbe C, Boissin JP, Siou A. Light vision and chorioretinal circulation: study of the effect of procyanidolic oligomers Endotelon. *J Fr Ophtalmol*. 1988;11:453–460. [French].
76. Boissin JP, Corbe C, Siou A. Chorioretinal circulation and dazzling: use of procyanidol oligomers. *Bull Soc Ophtalmol Fr*. 1988;88:173–174, 177–179.
77. Spadea L, Balestrazzi E. Treatment of vascular retinopathies with Pycnogenol. *Phytother Res*. 2001;15:219–223.
78. Belcaro G, Luzzi R, Hu S, et al. Improvement in signs and symptoms in psoriasis patients with Pycnogenol® supplementation. *Panminerva Med*. 2014;56(1):41–48.
79. Sano A. Safety assessment of 4-week oral intake of proanthocyanidin-rich grape seed extract in healthy subjects. *Food Chem Toxicol*. 2017;108(Pt B):519–523.

Pygeum africanum (Bitter Almond)

Michael T. Murray, ND

OUTLINE

Pygeum africanum (family: *Rosaceae*)
Synonym: *Prunus africanum*
Common names: bitter almond, red stinkwood

GENERAL DESCRIPTION

Pygeum africanum is an evergreen tree native to Africa that can grow to a height of 120 to 150 ft. It has pendulous branches with thick, oblong, leather-like, matte leaves and creamy white flowers. The fruit (drupe) resembles a cherry when ripe. The dark brown to gray bark of the trunk is the part used for medicinal purposes.

CHEMICAL COMPOSITION

The major active components of the bark are as follows:

- Lipid-soluble pentacyclic triterpenes
- Sterolic triterpenes
- Fatty acids
- Esters of ferulic acid (Fig. 107.1)

The pentacyclic triterpenic components are ursolic acid (Fig. 107.2), oleanolic acid, crataegolic acid, and their derivatives. The sterolic fraction is composed mainly of β-sitosterol and β-sitosterone (Fig. 107.3). The fatty acids range from C12 to C24, and the important ferulic acid esters are those bound to *n*-tetracosanol and *n*-docosanol.[1–4]

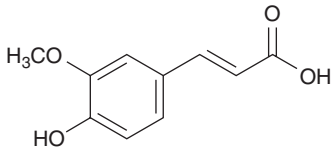


Fig. 107.1 Ferulic acid.

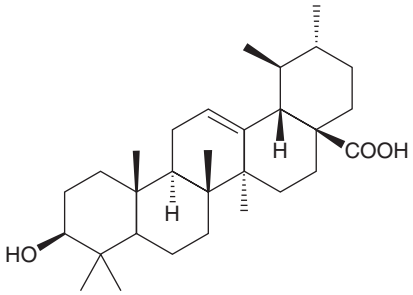


Fig. 107.2 Ursolic acid.

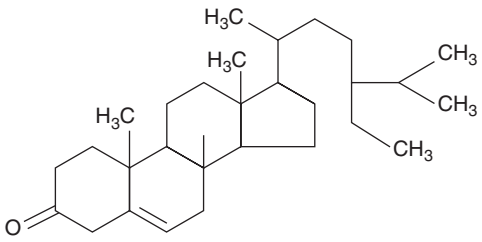


Fig. 107.3 Beta-sitosterone.

HISTORY AND FOLK USE

The powdered bark of *P. africanum* was used by the natives of tropical Africa as a treatment for urinary disorders. It was often given with palm oil or milk. Since about 1970, the bark has been entirely wild-collected, with the major exporters being Cameroon, Madagascar, Equatorial Guinea, and Kenya. Two companies, Groupe Fournier of France and Indena of Italy, produce 86% of the world's bark extract, both for their own products and for the free market. Worldwide exports of dried bark in 2000 were estimated at 1350 to 1525 metric tons per year, down from its peak of 3225 tons in 1997. Bark extracts (6370–7225 kg/year) are worth an estimated $4.36 million per year. However, wild collection of the bark is no longer sustainable. Since 1995, *P. africanum* has been

declared an endangered species. Alternatives to wild collection to meet future market demand include conservation practices, enrichment plantings, and small- and large-scale production. Currently, the species is at the beginning of a transition from an exclusively wild-collected species to that of a cultivated medicinal tree.[5]

PHARMACOLOGY

Pharmacological screening of various extracts prepared with solvents of differing degrees of polarity indicated that the highest activity was found in lipophilic extracts.[1] This finding is interesting in light of pygeum's historical administration in lipophilic media (palm oil or milk). Virtually all of the pharmacological research featured a pygeum extract standardized to contain 14% triterpenes, including β-sitosterol and 0.5% *n*-docosanol. This extract was extensively studied in both experimental animal studies and clinical trials with humans.

The primary target organ for pygeum's effects in men is the prostate. The three major active components of pygeum appear to exert different, yet complementary, effects in benign prostatic hyperplasia (BPH). In addition, pygeum was shown to enhance the secretions of the prostate and bulbourethral glands, in terms of both quantity and quality.

Ferulic Acid Esters

The esters of ferulic acid act primarily on the endocrine system. Studies in animals showed docosanol reduced levels of luteinizing hormone and testosterone while raising adrenal steroid secretion of both adrenal androgens and corticosteroids.[6,7] Docosanol also significantly lowers serum prolactin levels. This reduction of prolactin is quite significant because prolactin increases both the uptake of testosterone and the synthesis of dihydrotestosterone within the prostate. The accumulation of testosterone within the prostate and its subsequent conversion to the more potent dihydrotestosterone is thought to be the major contributing factor to the hyperplasia of the prostatic cells observed in BPH.[8] Although traces of docosanol are present in pygeum, the esterification with ferulic acid results in greater bioavailability and activity.[2,4,9]

Ferulic acid esters and the sterol fraction of pygeum exert cholesterol-lowering action systemically as well as within the prostate.[9] Breakdown products of cholesterol were shown to accumulate in prostate tissue affected with either BPH or cancer.[8] These metabolites of cholesterol initiate degeneration of prostatic cells, which can promote prostatic enlargement. Drugs that lower cholesterol levels were shown to have a favorable influence on BPH, preventing the accumulation of cholesterol in prostatic cells and limiting subsequent formation of damaging cholesterol metabolites. The lowering of intraprostatic cholesterol content is an important aspect of the pharmacology of pygeum.

The sterolic fraction is also endowed with competitive action against testosterone accumulation within the prostate. In addition, the sterols of pygeum were also shown to reduce inflammation by preventing the intraprostatic formation of inflammatory prostaglandins.[9,10]

Other Components

Other components of pygeum are also important. For example, the pentacyclic triterpenes exhibit anti-inflammatory effects within the prostatic epithelium and may be responsible for stimulation of the secretory cells of the prostate, seminal vesicles, and bulbourethral glands.[9–11] Finally, the fatty acid components are similar to those of *Serenoa repens* (see Chapter 122) and may exert similar effects and also improve the oral bioavailability of other components of the lipophilic extract.

CLINICAL APPLICATIONS

Prostate Disorders

The pharmacological actions of the standardized pygeum extract support its use in prostate disorders, BPH in particular. Adding further support are the results of numerous clinical trials in more than 600 patients.[12–34] These studies consistently demonstrated that pygeum effectively reduces the symptoms and clinical signs of BPH, especially in early cases. However, it must be pointed out that improvement is largely symptomatic because the reductions in the size of the prostate and in residual urine content of the bladder are modest. The results of the clinical trials on pygeum are given in Table 107.1. A discussion of some of the most important aspects of these studies follows.

One of the major findings in evaluating the effectiveness of pygeum in BPH was the high rate of response to placebo. This was well demonstrated in one of the larger double-blind studies.[30] As in other double-blind studies, pygeum extract was shown to be statistically superior to placebo in reducing the major symptoms of BPH (nocturnal frequency, difficulty in starting micturition, and incomplete emptying of the bladder). However, there was a high percentage of responses to the placebo (Table 107.2). It seems that simply taking a capsule provides relief to many sufferers.

Another study highlighted the importance of double-blind studies that featured both objective and subjective findings. In the study, both patients and physicians rated the placebo and pygeum extract to be effective in improving subjective symptoms, which were daytime frequency, nocturia, weak stream, after-dribbling, hesitation, and interruption of flow.[29] However, urodynamic variables (flow, frequency, and histogram) clearly demonstrated the superiority of pygeum over placebo.

One of the shortcomings of some of the clinical research on pygeum in BPH was the lack, in many of the studies, of objective measures, such as urine flow rate (milliliters per second), residual urine content, and prostate size. Studies that used objective measurements showed some good results. For example, in one open trial, 30 patients with BPH who were given 100 mg/day of the pygeum extract for 75 days demonstrated significant improvements in objective parameters: maximum urine flow rate increased from 5.43 to 8.20 mL/s, and the residual urine volume dropped from 76 to 33 mL.[23]

Pygeum may emerge as a significant protector against prostate cancer (PCa) because pygeum extract and its components (e.g., atraric acid and *N*-butylbenzene-sulfonamide) inhibit the transactivation mediated by the ligand-activated human androgen receptor (AR). Inhibition of human AR by androgen ablation therapy and synthetic antiandrogens is the primary goal in the treatment of PCa. Both in vitro and in vivo studies showed pygeum components inhibited the nuclear transport of AR and endogenous prostate-specific antigen expression and efficiently repressed the growth of both the androgen-dependent and some types of androgen-independent prostate cancer cells.[35–38] Currently used synthetic AR antagonists bind to the AR and induce a conformational change that leads to the dissociation of key proteins in the AR, but the antagonist-bound AR is still translocated to the nucleus, where it recruits corepressors to block its transcriptional activity. Pygeum components act a little differently, in that they selectively bind to the AR and inhibit the translocation to the nucleus, and in consequence, it functions as an androgen-activated transcription factor. This difference in mechanism may overcome a major shortcoming with antihormone therapy in PCa. Typically, antihormone treatment is effective only for a limited period of about 16 to 24 months, after which PCa becomes androgen independent. Different mechanisms seem to be involved in this process, but AR mutations during

TABLE 107.1 Results of the Most Significant Open and Double-Blind Studies of the Past 20 Years on Outpatients Using *Pygeum africanum* for 1 to 3 Months

Study	Dosage (mg/day)	No. of Days	No. of Patients in Study	PATIENTS SHOWING REDUCTION (%)				
				Dysuria	Nocturia	Frequency	Residual Urine	Prostate Volume
Open Trials								
Guillemin[12]	100	30	25	80	80	80	80	NC
Lange and Muret[13]	100	30	25	72	NC	72	NC	NC
Wemeau et al[14]	100	45	27	60	NC	71	NC	NC
Viollet[15]	75	60	20	64	NC	64	NC	NC
Lhez and Leguevague[16]	75	90	52	69	NC	NC	NC	NC
Thomas and Rouffilange[17]	75	50	33	60	57	57	NC	—
Huet[18]	50	30	55	85	85	85	NC	20
Rometti[19]	100	50	25	72	72	72	NC	25
Gallizia and Gallizia[20]	100	60	19	90	85	70	20	NC
Durval[21]	100	90	23	72	72	72	72	NC
Pansadoro and Benincasa[22]	75	90	35	94	94	94	94	—
Double-Blinded Trials								
Maver[24]	100	60	60	77	70	57	23	—
Bongi[25]	75	60	50	88	88	88	88	88
Doremieux et al.[26]	100	60	77	85	NC	NC	NC	NC
Del Valio[27]	100	60	30	—	—	48	—	—
Colpi and Farina[28]	150	45	47	—	70	—	76	NC
Donkervoort et al.[29]	150	90	20	80	80	80	NC	NC
Dufour et al.[30]	100	45	120	—	78	45	65	NC
Legramandi et al.[31]	100	45	104	89	89	89	NC	NC
Ranno et al.[32]	200	60	39	75	75	75	NC	NC
Frasseto et al.[33]	200	60	20	—	—	—	—	—
Bassi et al.[34]	200	60	40	70	70	70	70	70

—, Not measured; *NC,* no change.

TABLE 107.2 Patients Showing Response to Placebo and Pygeum

Symptom	Placebo Group	Pygeum Group
Nocturia	26/52 (50%)	44/56 (78%)
Daytime frequency	16/50 (33%)	27/54 (50%)
Incomplete voiding	14/40 (35%)	21/32 (66%)
Dribbling	15/34 (44%)	13/33 (39%)
Urine flow rate	11/43 (26%)	21/38 (55%)

Data from references 12–22 and 24–34.

PCa progression render therapeutically used antiandrogens into AR agonists. In consequence, those therapeutics then become useless, and even counterproductive, because they can promote PCa progression.

One of the most interesting studies with pygeum was an investigation on its effect on prostate cell growth in vitro using human serum collected before and after pygeum extract intake at a dosage of at 50 mg twice per day for 5 days. The in vitro analysis consisted of primary and organotypic cultures of human prostatic stromal myofibroblast cell line WPMY and prostatic epithelial cell line PNT2 along with fresh benign prostatic tissue. Results showed that the serum of a treated man induced decreases in the proliferation of primary cells, organotypic cells and WPMY cells but not PNT2 cells. Researchers also analyzed the effect of treated serum on the gene expression profile of WPMY cells. The transcriptome analysis revealed an upregulation of genes involved in multiple tumor-suppression pathways and a downregulation of genes involved in inflammation and oxidative-stress pathways. Hence, the oral intake of pygeum extract resulted in serum levels of active substances that were sufficient to inhibit the proliferation of cultured prostatic cells. This inhibition was associated with changes in the transcriptome.[39]

Male Infertility and Impotence

Pygeum may be effective in improving fertility when diminished prostatic secretion plays a significant role in the problem. Pygeum was shown to increase prostatic secretions and improve the composition of seminal fluid.[40–42] Specifically, pygeum administration to men with decreased prostatic secretion led to higher levels of total seminal fluid plus increases in alkaline phosphatase and protein levels. Pygeum appears to be most effective in cases in which the level of alkaline phosphatase activity is reduced (i.e., less than 400 IU/cm^3) and in which there is no evidence of inflammation or infection (i.e., absence of white blood cells and immunoglobulin-A [IgA]). The lack of IgA in the semen is a good predictor of clinical success. In one study, the patients with no IgA in the semen demonstrated an alkaline phosphatase increase from 265 to 485 IU/cm^3[39] In contrast, subjects whose semen contained IgA showed only a modest increase, from 213 to 281 IU/cm^3.

Pygeum extract also showed an ability to improve the capacity to achieve an erection in patients with BPH or prostatitis, as determined by nocturnal penile tumescence in a double-blind clinical trial.[43] BPH and prostatitis are often associated with erectile dysfunction and other sexual disturbances. Presumably by improving the underlying condition, pygeum can improve sexual function.

Pygeum Versus Serenoa

The standardized liposterolic extract of *Serenoa repens* is another popular botanical treatment for BPH (see Chapter 122). In a double-blind study that compared pygeum extract with extract of serenoa, the serenoa extract produced a greater reduction of symptoms and was better tolerated.[44] In addition, the improvement of objective parameters, especially urine flow rate and residual urine content, was better in the clinical studies of serenoa. However, there may be circumstances in which pygeum is more effective than serenoa. For example, serenoa has not been shown to have the effects on prostate secretion that pygeum has, and the anticancer effects of pygeum may be more significant. Although the two extracts have somewhat overlapping mechanisms of actions, they can be used in combination.

DOSAGE

The dosage of the lipophilic extract of *P. africanum* standardized to contain 14% triterpenes, including β-sitosterol and 0.5% *n*-docosanol, is 100 to 200 mg/day. Typically, the dosage has been divided for twice-a-day administration; however, results were similar in a double-blind study with 100 mg given once a day and 50 mg twice a day, indicating that taking pygeum once a day is equivalent to using divided doses.[45] The crude herb is not used.

TOXICOLOGY

Tests for acute and long-term toxicity in the rat and mouse showed that the standardized extract of *P. africanum* bark is nontoxic. Raising the dosage from 1 to 6 g/kg in the mouse and from 1 to 8 g/kg in the rat caused no deaths within 48 hours. In long-term toxicity studies, giving the animals from 60 to 600 mg/kg for 11 months did not produce any negative effects.

Pygeum extract also demonstrated no significant toxicity in human clinical trials. The most common side effect is gastrointestinal irritation, resulting in symptoms ranging from nausea to severe stomach pains; however, the presence of these side effects rarely leads to discontinuation of therapy.

DRUG INTERACTIONS

There are no documented drug interactions for *P. africanum.*

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Longo R, Tira S. Steroidal and other components of *Pygeum africanum* bark. *Farmaco*. 1983;38:288–292.
2. Martinelli EM, Seraglia R, Pifferi G. Characterization of *Pygeum africanum* bark extracts by HRGC with computer assistance. *J High Resolut Chrom*. 1986;9:106–110.
3. Pierini N, Citti F, Di Marzio S, et al. Identification and determination of n-docosanol in the bark extract of *Pygeum africanum* and in patent medicines containing it. *Boll Chim Farm*. 1982;121:27–34.
4. Uberti E, Martinelli EM, Pifferi G, et al. HPLC analysis of n-docosyl ferulate in *Pygeum africanum* extracts and pharmaceutical formulations. *Fitoterapia*. 1990;61:342–347.
5. Stewart KM. The African cherry *(Prunus africana)*: can lessons be learned from an over-exploited medicinal tree? *J Ethnopharmacol*. 2003;89:3–13.
6. Muntzing J, Eneroth P, Gustafsson JA, et al. Direct and indirect effects of docosanol (IK.2), the active principle in Tadenan, on the rat prostate. *Invest Urol*. 1979;17:176–180.
7. Thieblot L. Preventive and curative action of a bark extract of an African plant, *Pygeum africanum*: experimental prostatic adenoma in the rat. *Therapie*. 1971;26:575–580.
8. Hinman F. *Benign Prostatic Hyperplasia*. New York: Springer-Verlag; 1983.
9. Bombardelli E. Methods, composition and compounds for the treatment of prostatic adenoma. *EP Appl 8330491*. 1985;10:3.
10. Marcoli M. Anti-inflammatory and antiedemigenic activity of extract of *Pygeum africanum* in the rat. *New Trends Androl Sci*. 1985;1:89–93.
11. Latalski M. The ultrastructure of the epithelium of bulbourethral glands after administration of the Tadenan preparation. *Folia Morphol*. 1979;1:193–201.
12. Guillemin P. Clinical trials of V1326, or Tadenan, in prostatic adenoma. *Med Prat*. 1970;386:75–76.
13. Lange J, Muret P. Clinical trial of V1326 in prostatic disease. *Med*. 1970;11:2807–2811.
14. Wemeau L, Delmay J, Blankaert J. Tadenan in prostatic adenoma. *Vie Medicale*. 1970:585–588.
15. Viollet G. Clinical experimentation of a new drug from prostatic adenoma. *Vie Medicale*. 1970:3457–3458.
16. Lhez A, Leguevague G. Clinical trials of a new lipid-sterolic complex of vegetal origin in the treatment of prostatic adenoma. *Vie Medicale*. 1970:5399–5404.
17. Thomas JP, Rouffilange F. The action of Tadenan in prostatic adenoma. *Rev Int Serv*. 1970;43:43–45. [French].
18. Huet JA. Prostatic disease in old age. *Med Intern*. 1970;5:405–408.
19. Rometti A. Medical treatment of prostatic adenoma. *Prov Med*. 1970;38:49–51.
20. Gallizia F, Gallizia G. Medical treatment of benign prostatic hypertrophy with a new phytotherapeutic principle. *Recent Med*. 1972;9:461–468.
21. Durval A. On the use of a new drug in the therapy of prostatic adenoma: Tadenan. Clinical considerations on 23 cases. *Minerva Urol*. 1970;22:106–111. [Italian].
22. Pansadoro V, Benincasa A. Prostatic hypertrophy. Results obtained with *Pygeum africanum* extract. *Minerva Med*. 1972;11:119–144.
23. Zurita IE, Pecorini M, Cuzzoni G. Treatment of prostatic hypertrophy with *Pygeum africanum* extract. *Rev Bras Med*. 1984;41:364–366.
24. Maver A. Medical treatment of fibroadenomatous hypertrophy of the prostate with a new plant substance. *Minerva Med*. 1972;63:2126–2136.
25. Bongi G. Tadenan in the treatment of prostatic adenoma: anatomo-clinical study. *Minerva Urol*. 1972;24:129–139.
26. Doremieux J, Masson JC, Bollack C. Prostatic hypertrophy, clinical effects and histological changes produced by a lipid complex extracted from *Pygeum africanum*. *J Med Strasbourg*. 1973;4:253–257. [French].
27. Del Valio B. Use of a new drug in the treatment of chronic prostatitis. *Minerva Urol*. 1974;26:81–94.
28. Colpi G, Farina U. Study of the activity of chloroformic extract of *Pygeum africanum* bark in the treatment of urethral obstructive syndrome caused by non-cancerous prostapathy. *Urologia*. 1976;43:441–448.
29. Donkervoort T, Sterling J, van Ness J, et al. A clinical and urodynamic study of Tadenan in the treatment of benign prostatic hypertrophy. *Eur Urol*. 1977;8:218–225.
30. Dufour B, Choquenet C, Revol M, et al. Controlled study of the effects of *Pygeum africanum* extract on the functional symptoms of prostatic adenoma. *Ann Urol*. 1984;18:193–195.
31. Legramandi C, Ricci-Barbini V, Fonte A. The importance of *Pygeum africanum* in the treatment of chronic prostatitis void of bacteria. *Gazz Med Ital*. 1984;143:73–76. [Italian].
32. Ranno S, Minaldi G, Viscusi G, et al. Efficacy and tolerability in the treatment of prostatic adenoma with Tadenan 50. *Progr Med*. 1986;42:165–169.
33. Frasseto G, Bertoglio S, Mancuso S, et al. Study of the efficacy and tolerability of Tadenan 50 in patients with prostatic hypertrophy. *Progr Med*. 1986;42:49–52.
34. Bassi P, Artibani W, De Luca V, et al. Standardized extract of *Pygeum africanum* in the treatment of benign prostatic hypertrophy: controlled clinical study vs. placebo. *Minerva Urol*. 1987;39:45–50.
35. Shenouda NS, Sakla MS, Newton LG, et al. Phytosterol *Pygeum africanum* regulates prostate cancer in vitro and in vivo. *Endocrine*. 2007;31:72–81.
36. Papaioannou M, Schleich S, Prade I, et al. The natural compound atraric acid is an antagonist of the human androgen receptor inhibiting cellular invasiveness and prostate cancer cell growth. *J Cell Mol Med*. 2009;13:2210–2223.
37. Papaioannou M, Schleich S, Roell D, et al. NBBS isolated from *Pygeum africanum* bark exhibits androgen antagonistic activity, inhibits AR nuclear translocation and prostate cancer cell growth. *Invest New Drugs*. 2010;28:729–743.
38. Quiles MT, Arbós MA, Fraga A, et al. Antiproliferative and apoptotic effects of the herbal agent *Pygeum africanum* on cultured prostate stromal cells from patients with benign prostatic hyperplasia (BPH). *Prostate*. 2010;70:1044–1053.
39. Larré S, Camparo P, Comperat E, et al. Biological effect of human serum collected before and after oral intake of *Pygeum africanum* on various benign prostate cell cultures. *Asian J Androl*. 2012;14(3):499–504.
40. Lucchetta G, Weill A, Becker N, et al. Reactivation of the secretion from the prostatic gland in cases of reduced fertility: biological study of seminal fluid modifications. *Urol Int*. 1984;39:222–224.
41. Menchini-Fabris GF, Giorgi P, Andreini F, et al. New perspectives on the use of *Pygeum africanum* in prostato-bladder pathology. *Arch Int Urol Nefrol Androl*. 1988;60:313–322.
42. Clavert A, Cranz C, Riffaud JP, et al. Effects of an extract of the bark of *Pygeum africanum* (V.1326) on prostatic secretions in the rat and man. *Ann Urol*. 1986;20:341–343.
43. Carani C, Salvioli C, Scuteri A, et al. Urological and sexual evaluation of treatment of benign prostatic disease using *Pygeum africanum* at high doses. *Arch Ital Urol Nefrol Androl*. 1991;63:341–345.
44. Duvia R, Radice GP, Galdini R. Advances in the phytotherapy of prostatic hypertrophy. *Med Praxis*. 1983;4:143–148.
45. Chatelain C, Autet W, Brackman F. Comparison of once and twice daily dosage forms of *Pygeum africanum* extract in patients with benign prostatic hyperplasia: a randomized, double-blind study, with long-term open label extension. *Urology*. 1999;54:473–478.

Pyrroloquinoline quinone (PQQ)

Michael T. Murray, ND

OUTLINE

GENERAL DESCRIPTION

Pyrroloquinoline quinone (PQQ) is a novel vitamin-like compound that acts as a necessary active factor in the functioning of mitochondria (Fig. 108.1). PQQ plays an essential role in human nutrition and will likely be recognized as an essential vitamin in the future.[1,2]

CHEMICAL COMPOSITION

PQQ is a redox active o-quinone that can be reversibly reduced to pyrroloquinoline quinol.[3]

HISTORY AND FOLK USE

Pyrroloquinoline quinone was discovered as the third coenzyme for oxidoreductases, along with nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), in 1979. PQQ appears to be an essential nutrient for mammals.[4] When PQQ is omitted from chemically defined diets in mammals, it leads to growth impairment, compromised immune status, and abnormal reproductive function.[5] The nutritional requirements for PQQ are probably in line with folic acid and biotin in terms of micrograms per day versus milligrams per day. As with essential nutrients, the immune system seems particularly sensitive to low levels of PQQ. With PQQ deprivation, there are multiple defects in immune function and loss of white blood cells to respond properly.[1] PQQ shows a wide range of clinical benefits to brain and body function based on preclinical studies and initial clinical evaluation.[1,2]

There is no known biosynthesis of PQQ in higher organisms. Hence, the major source of PQQ in both plants and animals is believed to be derived from microorganisms. However, common strains of bacteria in the human intestinal tract appear to synthesize little PQQ. The major source for humans is the diet. PQQ has been found in all plant foods analyzed to date.[1] PPQ-rich foods include parsley, green peppers, kiwi fruit, papaya, and tofu.[6] These foods contain about 2 to 3 mcg per 100 grams. Green tea provides about the same amount per 4 oz serving. It is estimated that humans consume 0.1 to 1.0 mg PQQ and its derivatives per day.

PHARMACOLOGY

PQQ stimulates growth and serves as a cofactor for a special class of enzymes involved in cellular function, including cellular growth, development, differentiation, and survival.[1] One key action of PQQ involves a direct action on key enzymes in mitochondria. As a result, PQQ improves energy production.[1,2,7] PQQ is also an extremely powerful antioxidant capable of catalyzing continuous cycling (i.e., the ability to perform repeated oxidation and reduction reactions) to a much greater degree compared with other antioxidants. For example, PQQ can carry out 20,000 catalytic conversions compared with only 4 for vitamin C.[1,2]

In addition, PQQ's antioxidant effect protects against mitochondrial damage and promotes the spontaneous generation of new mitochondria within aging cells, a process known as mitochondrial biogenesis or mitochondriogenesis.[1,2,8,9] This effect is a "fountain of youth" for mitochondrial function.

Given the nutritional importance and tremendous span of physiological effects of PQQ, there are considerable benefits in conditions that revolve around low mitochondrial function, such as aging, brain

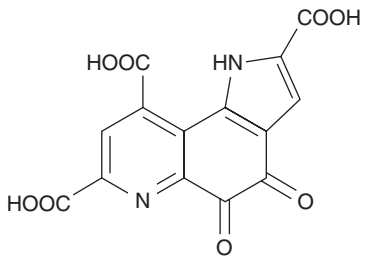


Fig. 108.1 Structure of pyrroloquinoline quinone. (From ResearchGate. Discovery of a eukaryotic pyrroloquinoline quinone-dependent oxidoreductase belonging to a new auxiliary activity family in the database of carbohydrate-active enzymes. <https://www.researchgate.net/Chemical-structure-of-PQQ-doi101371-journalpone0104851g001_fig1_264799310>. Accessed October 1, 2018.)

and neurological disease (e.g., Alzheimer's and Parkinson's disease), and several chronic degenerative diseases. Current research has primarily focused on its ability to protect memory and cognition in both aging animals and humans. In animal studies, PQQ was found to have the following benefits:

- Reverses cognitive impairment caused by chronic oxidative stress and improves performance on memory tests in animal models[1,2,10]
- Stimulates the production and release of nerve growth factor[1,2,11]
- Protects against the self-oxidation of the DJ-1 gene, an early step in the onset of Parkinson's disease[1,12]
- Protects brain cells against oxidative damage in models of strokes[1,2,13]
- Blocks the formation of inducible nitric oxide synthase (iNOS), a major source of reactive nitrogen species (RNS) that are so damaging to brain cells[1,2,14]
- Protects against the likelihood of severe stroke in an experimental animal model for stroke[1,2,15]
- Protects the brain against neurotoxicity induced by other powerful toxins, including mercury, glutamate, and oxidopamine (a potent neurotoxin used by scientists to induce parkinsonism in laboratory animals)[1,2,16,17]
- Prevents development of alpha-synuclein, a protein associated with Parkinson's disease[1,2,18]
- Protects nerve cells from the damaging effects of the beta-amyloid-protein linked with Alzheimer's disease[1,2,19]
- Lowers low-density lipoprotein (LDL) cholesterol, presumably by activating AMP-kinase (AMPk).[2,19]

CLINICAL APPLICATIONS

PQQ activates AMPk, an enzyme that is found inside living cells and serves as a "master regulating switch" in energy metabolism. Low levels of AMPk activity are associated with the following:

- Accelerated aging
- Chronic inflammation
- High blood cholesterol and triglycerides
- Increased visceral "belly" fat
- Insulin resistance
- Mitochondrial insufficiency and dysfunction
- Neurodegeneration
- Obesity
- Poor blood sugar control

Because PQQ increases mitochondrial function and activates AMPk, researchers believe that clinical data will soon produce evidence showing that PQQ is helpful for a long list of health challenges.[1,2]

The ability to enhance mitochondrial energy production is supported by numerous animal studies.[1,2,7,21] In humans, PQQ was administered as a single agent in 10 subjects (5 females, 5 males) between the ages of 21 and 34 years. The subjects were given PQQ (BioPQQ, a form of PQQ produced through a natural fermentation process) in a single dose (0.2 mg PQQ/kg), after which multiple measurements of plasma and urine PQQ levels and changes in antioxidant potential were examined over a 48-hour period.[21] Results indicated a significant increase in antioxidant potential even after one dosage. The same subjects were also given a daily dose of 0.3 mg PQQ/kg and had their blood measured for markers of inflammation (plasma C-reactive protein and interleukin [IL]-6 levels) and urinary metabolites related to energy metabolism before PQQ administration and 72 hours later. PQQ supplementation resulted in significant decreases in the levels of the inflammatory markers of plasma C-reactive protein and IL-6. Furthermore, the changes in urinary metabolites were consistent with enhanced mitochondria-related functions. The data are among the first to link the systemic effects of PQQ in animals to corresponding effects in humans.[21]

In a study with BioPQQ test subjects with an initial level of LDL cholesterol greater than 140 mg/dL, 6 weeks of PQQ supplementation produced a statistically significant decrease in total cholesterol (from an average of 247–216 mg/dL) and LDL cholesterol (from an average of 156–132 mg/dL).[22] Results persisted at 12 weeks and are believed to be caused by PQQ activating AMPk.

Insulin Resistance

PQQ holds great promise in the treatment of insulin resistance, type 2 diabetes, and as an antiobesity agent. There are several additional mechanisms of action that support these clinical applications. It is now well established that type 2 diabetes can be characterized as a mitochondrial disorder.[23] It is also well established that the mitochondrial dysfunction of diabetic subjects is closely related to the degree of hyperglycemia, diet, physical activity, sleep, stress, and other lifestyle factors.[24] As described previously, PQQ supplementation has been revealed to enhance mitochondrial function and biogenesis.

PQQ deficiency increased the plasma glucose level, reduced hepatic mitochondrial content by 20% to 30%, and elevated plasma lipid levels, whereas PQQ supplementation reversed mitochondrial alterations and metabolic impairment and significantly improved the lipid profile in diabetic rats.[20] The mechanisms responsible for the increased mitochondrial biogenesis and function by PQQ are stimulated by the transcriptional coactivator, peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), through activation of the nuclear respiratory factor (NRF-1 and NRF-2). cAMP-responsive element-binding protein (CREB) increases transcription of PGC-1α via a conserved CREB-binding site in the proximal promoter and is activated by exercise or fasting. PQQ elevates PGC-1α promoter activity by the same mechanism. PQQ exposure also increases the levels of NRF-1 and NRF-2, resulting in the upregulation of the mitochondrial transcription factor A and mitochondrial gene expression.[25]

PQQ exerts additional mechanisms on insulin signaling and enhances glucose uptake through the translocation of glucose transporters. Therefore PQQ can be useful in insulin resistance and type 2 diabetes.[2]

Enhancing Cognitive Function

The positive effects of PQQ on the learning and memory function have been demonstrated in animal models.[1,2] Although PQQ was effective on its own in some of these studies, when combined with coenzyme Q10 (CoQ10), better results were observed. This synergistic effect was further demonstrated in a human double-blind, placebo-controlled clinical trial conducted in Japan in 2007.[26] In this study of 71 middle-aged and elderly people aged between 40 and 70, supplementation with 20 mg per day of PQQ (as BioPQQ) resulted in improvements on tests of higher cognitive function using the Stroop test compared with the placebo group. However, in the group receiving 20 mg of PQQ along with 300 mg of CoQ10, the results were even more dramatic. PQQ and CoQ10 are both involved in mitochondrial energy production, which may explain the enhanced results.

Another double-blind study used the repeatable battery for the assessment of neuropsychological status (RBANS) in 65 subjects between 50 and 70 years old who presented with self-identified forgetfulness or forgetfulness identified by a family member, colleague, or acquaintance.[27] The neuropsychological battery questions allow repeated and quick evaluation of disorders in higher-brain function with a variety of brain disease complications. The RBANS consists of five subtests of neurocognitive test paradigms: immediate memory, visuospatial/constructional, language, attention, and delayed memory.

The three arms of the study were PQQ (20 mg/day), PQQ (20 mg/day) + CoQ10 (300 mg/day), and placebo. All three groups showed significantly better total score over time, but the improvement in immediate memory scores at week 8 were significantly better in the PQQ + CoQ10 group than in the placebo group. For analysis of immediate memory, subjects were stratified into two subgroups according to baseline total scores. Although no significant difference was present between groups in the high-scoring subgroup, the PQQ + CoQ10 group in the low-scoring subgroup showed a significantly better score at week 8 and week 16 than the placebo group. This finding shows that individuals with lower RBANS scores may achieve a better degree of improvement in response to PQQ + CoQ10 supplementation than individuals with higher scores.

For patients under 50 years of age, however, there may not be a need for simultaneous use of PQQ and CoQ10 unless the person is taking drugs, such as cholesterol-lowering statins, that interfere with CoQ10 manufacture. Double-blind studies in elderly subjects with PQQ alone show less impressive results than studies when PQQ is combined with CoQ10. In one of these studies, 41 healthy elderly subjects were administered 20 mg of PQQ per day or a placebo for 12 weeks. No significant improvements were noted in the Stroop test or reverse Stroop test, but improvements with PQQ were noted in the assessment of visual-spatial cognitive function via tablet computer.[28]

In another trial relating to cognitive function, 17 healthy middle-aged and elderly subjects ingested 20 mg of PQQ Na2 daily for 8 weeks.[29] The results in the Profile of Mood States–Short Form showed that all six measures of vigor, fatigue, tension/anxiety, depression, anger/hostility, and confusion significantly improved after PQQ supplementation compared with baseline scores. Improvements were also noted for drowsiness at awakening, sleep onset and maintenance, and sleep duration. These improvements correlated with changes in the cortisol awakening response.

PQQ also enhances cerebral blood flow and oxygen utilization in the prefrontal cortex. In a study of 20 healthy subjects between 50 and 70 years of age, subjects were administered 20 mg of PQQ (as BioPQQ) or placebo orally once daily for 12 weeks.[30] Using transcranial near-infrared stimulation (tNIRS), PQQ was shown to increase hemoglobin concentration and reduce absolute tissue oxygen saturation in the right prefrontal cortex, resulting in enhanced cognitive function.

DOSAGE

Although the nutritional requirement for PQQ is likely less than 500 mcg daily, a measured response in mitochondrial function may require increased amounts. The current dosage recommendation of 10 to 20 mg of PQQ daily in humans is based on the equivalent dose in animals that has consistently improved various mitochondrial functions. There are also some clinical and observational studies that justify the dosage.

TOXICITY

There are no known side effects or toxicity with PQQ at recommended levels. In a double-blind safety study, human volunteers were given PQQ at a dosage of either 20 or 60 mg/day, or a placebo, for 4 weeks.[2] No adverse effects were observed in any group. The urinary concentration of N-acetyl-β-d-glucosaminidase (NAG) was also determined in the study. NAG is a sensitive biomarker for renal tubular damage. Levels did not change in any group.

Toxicology studies in animals show an excellent safety profile, with the LD_{50} being 1000 to 2000 mg PQQ/kg body weight in male and 500 to 1000 mg PQQ/kg body weight in female rats. The no-observed-adverse-effect level (NOAEL) in rats is at a dosage of 400 mg PQQ/kg body weight.[31] PQQ exerts no genotoxicity as well.[32]

DRUG INTERACTIONS

There are no known drug interactions with PQQ at recommended levels.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rucker R, Chowanadisai W, Nakano M. Potential physiological importance of pyrroloquinoline quinone. *Altern Med Rev*. 2009;14(3):268–277.
2. Akagawa M, Nakano M, Ikemoto K. Recent progress in studies on the health benefits of pyrroloquinoline quinone. *Biosci Biotechnol Biochem*. 2016;80(1):13–22.
3. Paz MA, Martin P, Fluckiger R, et al. The catalysis of redox cycling by pyrroloquinoline quinone (PQQ), PQQ derivatives, and isomers and the specificity of inhibitors. *Anal Biochem*. 1996;238:145–149.
4. Kasahara T, Kato T. Nutritional biochemistry: a new redox-cofactor vitamin for mammals. *Nature*. 2003;422:832.
5. Steinberg F, Stites TE, Anderson P, et al. Pyrroloquinoline quinone improves growth and reproductive performance in mice fed chemically defined diets. *Exp Biol Med (Maywood)*. 2003;228:160–166.
6. Kumazawa T, Sato K, Seno H, et al. Levels of pyrroloquinoline quinone in various foods. *Biochem J*. 1995;307:331–333.
7. Chowanadisai W, Bauerly KA, Tchaparian E, et al. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. *J Biol Chem*. 2010;285(1):142–152.
8. Stites T, Storms D, Bauerly K, et al. Pyrroloquinoline quinone modulates mitochondrial quantity and function in mice. *J Nutr*. 2006;136:390–396.
9. Chowanadisai W, Bauerly K, Tchaparian E, Rucker RB. Pyrroloquinoline quinone (PQQ) stimulates mitochondrial biogenesis. *FASEB J*. 2007;21:854.
10. Ohwada K, Takeda H, Yamazaki M, et al. Pyrroloquinoline quinone (PQQ) prevents cognitive deficit caused by oxidative stress in rats. *J Clin Biochem Nutr*. 2008;42:29–34.
11. Yamaguchi K, Sasano A, Urakami T, Tsuji T, Kondo K. Stimulation of nerve growth factor production by pyrroloquinoline quinone and its derivatives in vitro and in vivo. *Biosci Biotechnol Biochem*. 1993;57(7):1231–1233.
12. Nunome K, Miyazaki S, Nakano M, Iguchi-Ariga S, Ariga H. Pyrroloquinoline quinone prevents oxidative stress-induced neuronal death probably through changes in oxidative status of DJ-1. *Biol Pharm Bull*. 2008;31(7):1321–1326.
13. Zhang Y, Feustel PJ, Kimelberg HK. Neuroprotection by pyrroloquinoline quinone (PQQ) in reversible middle cerebral artery occlusion in the adult rat. *Brain Res*. 2006;1094:200–206.
14. Hirakawa A, Shimizu K, Fukumitsu H, Furukawa S. Pyrroloquinoline quinone attenuates iNOS gene expression in the injured spinal cord. *Biochem Biophys Res Commun*. 2009;378:308–312.
15. Jensen FE, Gardner GJ, Williams AP, et al. The putative essential nutrient pyrroloquinoline quinone is neuroprotective in a rodent model of hypoxic/ischemic brain injury. *Neuroscience*. 1994;62:399–406.
16. Zhang P, Xu Y, Sun J, et al. Protection of pyrroloquinoline quinone against methylmercury- induced neurotoxicity via reducing oxidative stress. *Free Radic Res*. 2009;43:224–233.
17. Zhang Q, Shen M, Ding M, Shen D, Ding F. The neuroprotective action of pyrroloquinoline quinone against glutamate-induced apoptosis in hippocampal neurons is mediated through the activation of PI3K/Akt pathway. *Toxicol Appl Pharmacol*. 2011;252(1):62–72.
18. Kim J, Harada R, Kobayashi M, Kobayashi N, Sode K. The inhibitory effect of pyrroloquinoline quinone on the amyloid formation and cytotoxicity of truncated alpha-synuclein. *Mol Neurodegener*. 2010;5:20.
19. Kim J, Kobayashi M, Fukuda M, et al. Pyrroloquinoline quinone inhibits the fibrillation of amyloid proteins. *Prion*. 2010;4(1):26–31.
20. Bauerly K, Harris C, Chowanadisai W, et al. Altering pyrroloquinoline quinone nutritional status modulates mitochondrial, lipid, and energy metabolism in rats. *PLoS One*. 2011;6:e21779. https://doi.org/10.1371/journal.pone.0021779.
21. Harris CB, Chowanadisai W, Mishchuk DO, et al. Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects. *J Nutr Biochem*. 2013;24(12):2076–2084.
22. Nakano M, Kawasaki Y, Suzuki N, Takara T. Effects of pyrroloquinoline quinone disodium salt intake on the serum cholesterol levels of healthy japanese adults. *J Nutr Sci Vitaminol*. 2015;61(3):233–240.
23. Gonzalez-Franquesa A, Patti ME. Insulin resistance and mitochondrial dysfunction. *Adv Exp Med Biol*. 2017;982:465–520.
24. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies. *Biochim Biophys Acta*. 2017;1863(5):1066–1077.
25. Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1α expression. *J Biol Chem*. 2010;285:142–152. https://doi.org/10.1074/jbc.M109.030130.
26. Nakano M, Ubukata K, Yamamoto T, Yamaguchi H. Effect of pyrroloquinoline quinone (PQQ) on mental status of middle-aged and elderly persons. *Food Style*. 2009. 21:13(7):50–53.
27. Koikeda T, Nakano M, Masuda K. Pyrroloquinoline quinone disodium salt improves higher brain function. *Med Consult New Remedies*. 2011;48:519–527.
28. Itoh Y, Hine K, Miura H, et al. Effect of the antioxidant supplement pyrroloquinoline quinone disodium salt (BioPQQ™) on cognitive functions. *Adv Exp Med Biol*. 2016;876:319–325.
29. Nakano M, Yamamoto T, Okumura H, Tsuda A, Kowatari Y. Effects of oral supplementation with pyrroloquinoline quinone on stress, fatigue, and sleep. *Funct Foods Health Dis*. 2012;2:307–324.
30. Nakano M, Murayama Y, Hu L, et al. Effects of antioxidant supplements (BioPQQ) on cerebral blood flow and oxygen metabolism in the prefrontal cortex. *Adv Exp Med Biol*. 2016;923:215–222.
31. Liang C, Zhang X, Wang W, Song Y, Jia X. A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt in rats. *Food Chem Toxicol*. 2015;75:146–150.
32. Nakano M, Suzuki H, Imamura T, Lau A, Lynch B. Genotoxicity of pyrroloquinoline quinone (PQQ) disodium salt (BioPQQ). *Regul Toxicol Pharmacol*. 2013;67:189–197.

109

Ruscus aculeatus (Butcher's Broom)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Ruscus aculeatus (family: *Liliaceae*)
Common names: butcher's broom, box holly, knee holly, kneeholy, kneeholm, Jew's myrtle, sweet broom, pettigree, Maeusedornwurzelstock (German)

GENERAL DESCRIPTION

Ruscus aculeatus (butcher's broom) is an evergreen shrub native to Mediterranean Europe and Africa.[1] It has rigid leaves that terminate in a single sharp spine. Its berries remain attached to the plant throughout the winter. The root, the part used medicinally, is thick, typically 2 to 4 inches long, with many woody rootlets growing on its lower surface.

CHEMICAL COMPOSITION

The primary active ingredients of butcher's broom are typically considered to be the steroidal saponin aglycones ruscogenin and neoruscogenin (Fig. 109.1). Recent data, however, suggests that the plant's saponin glycosides ruscin and deglucoruscin and its coumarin glycoside esculin may have greater activity (Figs. 121.2–121.4).[2] The plant also contains fatty acids, sterols, flavonoids, other coumarins, sparteine, tyramine, and glycolic acid.[1,3–6]

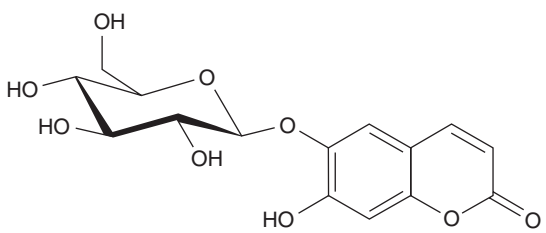


Fig. 109.1 Ruscogenin.

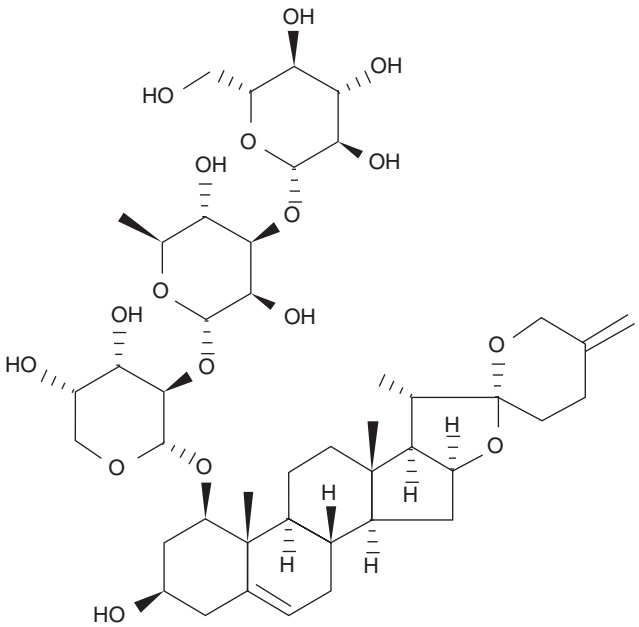


Fig. 109.2 Ruscin.

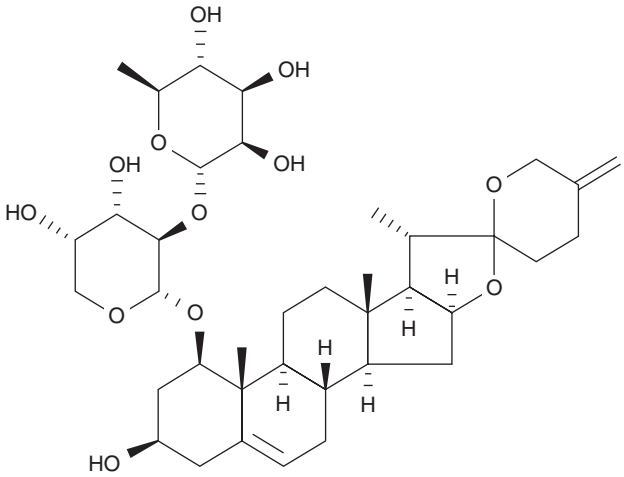


Fig. 109.3 Deglucoruscin.

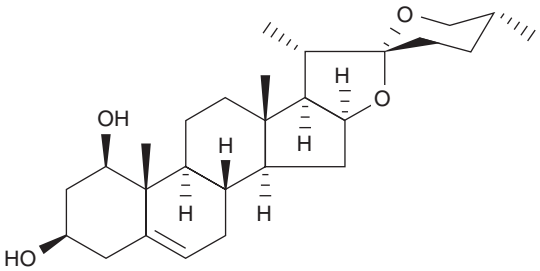


Fig. 109.4 Esculin.

HISTORY AND FOLK USE

In ancient Greece, butcher's broom was used as a laxative and to treat kidney stones.[7] In Europe, a wine decoction of the root was used as a diuretic to treat urinary obstructions, kidney stones, and gravel. It was also used to regulate menses, ameliorate jaundice, and treat headaches. In South America, the roasted root was made into a beverage for prostate tumors.[8]

PHARMACOLOGY

Anticancer Effect

Butcher's broom saponins have a cytostatic effect on leukemia HL-60 cells in vitro.[9]

Antielastase Effect

Ruscinogens isolated from butcher's broom have a remarkable antielastase effect in vitro.[10]

Antihypoxic Effect

Hypoxia-induced activation of endothelial cells may be a cause of venous disease. A proprietary extract of butcher's broom known as Cyclo 3 Fort dose-dependently protected human endothelial cells from induced hypoxia in vitro.[10]

Anti-inflammatory Effect

Butcher's broom extract and its isolated steroidal saponins were anti-inflammatory in several animal models.[11] Injected intravenously, it inhibits the permeability-increasing effects of bradykinin, leukotriene B4, and histamine in hamster cheek pouch preparations.[12]

Antimicrobial Effect

Butcher's broom showed a very low activity against *Candida albicans*.[13]

FIBRINOLYTIC EFFECT

Thrombosis in chronic venous insufficiency (CVI) is associated with reduced levels of tissue plasminogen activator in varicose veins. In a small study, 20 patients scheduled for vein stripping surgery took butcher's broom (150 mg) and methyl hesperidin chalcone (150 mg) or a placebo three times daily for 14 days before surgery.[14] Segments of the great saphenous vein removed from patients in the active group had enhanced levels of tissue plasminogen activator and enhanced fibrinolytic activity compared with the placebo.

Lymphatic Flow Effect

Butcher's broom dose-dependently enhanced the duration and flow intensity of the lymphatic vessels in dogs.[15] This effect did not change when the calcium channel antagonist nifedipine was injected, indicating that butcher's broom's lymphatic effect does not involve the opening of calcium channels but appears to act directly on peripheral lymphatics.

Vasoconstrictive Effect

Topical administration of butcher's broom extract in a hamster cheek preparation dose-dependently inhibited histamine-induced increases in permeability.[16] Research suggested that butcher's broom's vasoconstrictive effect is mediated by calcium and α-1 adrenergic receptors. Heat increases, and cold decreases, butcher's broom's ability to increase the release of norepinephrine from the adrenoreceptors.[17]

CLINICAL APPLICATIONS

There are few clinical studies on the effect of butcher's broom alone. Instead, most of the clinical studies investigated a proprietary combination formula known as Cyclo 3 Fort by Pierre Fabre Medicament of Paris, France, or Phlebodril by Pierre Fabre Pharma GmbH of Freiburg, Germany. These formulas combine butcher's broom with trimethylhesperidin chalcone (a flavonoid precursor) and ascorbic acid. Butcher's broom has action independent of the other compounds in the formula, and some studies indicated that butcher's broom used alone may have a stronger effect. However, other studies indicated that the combination may have a synergistic effect. All discussions of butcher's broom trials in the following content therefore include the possibility that it was not butcher's broom by itself that was active.

Butcher's broom is also paired with *Melilotus* spp. (sweet clover) in some topical preparations. There are no studies indicating whether butcher's broom alone would achieve the same results.

Chronic Venous Insufficiency

Butcher's broom is most commonly used to alleviate the symptoms of CVI, including ankle edema, itching, tension, and cramping of the legs. Both animal and clinical studies support its effectiveness in CVI, and the German Commission E approved butcher's broom as an adjunct treatment for this condition.[1] A meta-analysis evaluated 20 placebo-controlled, double-blind studies and 5 randomized trials against a comparator drug along with 6 single-arm studies without a placebo. The review included information on 10,246 subjects. Although data quality varied, Cyclo 3 Fort significantly reduced CVI symptoms compared with placebos.[17]

Ten of these studies are summarized in Table 109.1.[18–26] All but two were randomized, double-blind trials. In one large multicenter

TABLE 109.1 Studies on Butcher's Broom in Chronic Vascular Insufficiency (CVI)

Study	No. of participants	Type	Product	Focus	Result	Duration
Cappelli et al.[18]	40	Double-blind, crossover placebo-controlled, prospective	2 capsules (16.5 mg ruscinogens, 75 mg hesperidin, 50 mg ascorbic acid) 3 times a day	CVI, varicosities	Itching, edema, and paresthesia improved greatly	2 mo with 15-d washout and 2-mo crossover
Rudofsky[19]	141 + 20 healthy volunteers	Randomized, double-blind, multicenter	2 capsules (450 mg *Ruscus* extract) 3 times daily for 4 wk, then 2 capsules 2 times daily for 8 wk	CVI	Continuous decrease in foot and ankle volume, decrease in leg swelling, improved venous pumping	2-wk washout followed by 12-wk treatment
Haas et al.[20]	20	Placebo-controlled, double-blind	1 capsule (150 mg *Ruscus,* 150 mg methyl hesperidin chalcone) 3 times a day	CVI stage I and II, scheduled for surgery	Significantly increased fibrinolytic activity of removed great saphenous vein	14 d
Kiesewetter et al.[21]	30	Random selection, uncontrolled	3 *Ruscus* capsules twice daily for 5 wk, then 2 capsules twice daily; amount of *Ruscus* in capsules not stated	CVI	Reduced circumferences of lower legs and malleoli, subjective complaints; greater rheologic improvement in patients with advanced stages of CVI	5 mo
Beltramino et al.[22]	80	Open-label, randomized, multicenter	2 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) daily: control 2 tablets of 500 mg hydroxyethyl rutoside daily	CVI (heavy, tired, swollen, or painful legs)	Significant improvement in symptoms, reduction in limb circumference; physicians and patients had a more favorable opinion of *Ruscus* than of rutoside	90 d
Le Devehat et al.[23]	60 + 7 healthy volunteers	Randomized, double-blind, placebo-controlled	2 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) daily	CVI; blood samples drawn from foot before and after provoked venous stasis	Improved blood viscosity disturbances caused by venous stasis	4 wk
Seydewitz et al.[24]	36	Randomized, double-blind, placebo-controlled	3 capsules (150 mg *Ruscus,* 150 mg trimethyl hesperidin chalcone) daily	Stage IV varicosities with stage I or stage II CVI, scheduled for vein stripping	Increase in enzyme activity in the proximal segment of the vein, distinctly higher incidence of subjective improvement of symptoms	4 wk
Guex JJ et al.[25]	917	Observational, multicenter, prospective	150 mg Ruscus aculeatus and 100 mg ascorbic acid	CVI (symptoms, ankle swelling)	Significant improvement in quality of life and symptoms	12 wk
Vanscheidt et al.[26]	148 women	Multicenter, double-blind, randomized, placebo-controlled	36–37 mg butcher's broom extract twice daily	CVI	Significant, positive changes in CVI symptoms	12 wk
Guex JJ et al.[25]	918	Observational, multicenter, prospective	2 capsules (150 mg *Ruscus,* 150 mg trimethyl hesperidin chalcone) daily	CVI, CEAP C0s–C3	All symptoms and quality of life improved significantly	12 wk

CVI, Chronic venous insufficiency; *CEAP,* a clinical venous classification system.

trial, 81.6% of treating physicians rated its efficacy as excellent, and the remaining physicians rated it as good.[22] Butcher's broom acts most strongly when circulation is impaired, but some researchers caution that it is less effective when the disease has compromised the activity of venous wall receptors.[20] Finally, a meta-analysis of randomized, double-blind, placebo-controlled trials of butcher's broom extracts concluded that high-quality evidence exists showing that these extracts are very effective in reducing symptoms and edema in patients with CVI. [27]

Edema and Varicosities

Butcher's broom shows promise as a treatment for a wide variety of edemas and circulatory disturbances of the venous system. The results of these clinical trials are summarized in Table 109.2.[28–37] These trials were also on combination extracts with butcher's broom as a major component. Many of the studies are small, and some are poorly designed, but the overall picture supports the ability of butcher's broom to reduce the swelling and discomfort associated with primary and secondary edema. It has positive effects in pregnancy-related venous insufficiency, varicosities, lymphedema, symptoms of swelling of ankles and breast tenderness in premenstrual syndrome (PMS), edema secondary to calcium channel antagonist therapy for hypertension, and swelling secondary to soft tissue injury.

Hemorrhoids

The German Commission E approved butcher's broom as a treatment for hemorrhoids.[1] In an open-label multicenter study of 124 patients with hemorrhoids, 69% of patients and 75% of the treating physicians rated the butcher's broom combination extract Cyclo 3 Fort as having good or excellent efficacy.[38] Ninety-two percent of physicians rated the treatment as safe and well tolerated. Significant positive effects were observed after 7 days of treatment. Although controlled trials are warranted, butcher's broom may be a useful treatment for patients with hemorrhoids.

Diabetic Retinopathy

Diabetic retinopathy can abruptly cause severe deterioration of vision, and it is estimated that the incidence of retinopathy may be as high as 80% in patients with long-standing, poorly controlled diabetes. In a study of 60 patients with non–insulin-dependent diabetes, butcher's broom compared favorably with troxerutin.[39] Butcher's broom produced a 23% regression of negative changes in the fundus of the eye, with no sign of progression. Troxerutin produced a somewhat larger regression (27.8%) but also had a greater (5.8%) progression rate. Butcher's broom also decreased blood glucose and cholesterol levels and significantly increased high-density lipoproteins in this preliminary study.

Premenstrual Syndrome

Butcher's broom significantly reduced symptoms of breast tenderness in women with diagnosed PMS in a randomized, placebo-controlled, double-blind study of 40 women with PMS of an average duration of 67 months.[34] The amount of butcher's broom administered for 90 days was not stated. Eighty percent of the women in the active group reported a complete resolution of PMS symptoms of mastalgia and breast tension. Unexpectedly, butcher's broom also had a dramatic effect on some of the psychological symptoms associated with PMS (e.g., complete resolution in 85% of the subjects of symptoms such as irritableness, "hair-trigger temper," and "temporary alienation"). In vitro research indicated that butcher's broom acts more efficiently in the presence of progesterone; butcher's broom may be most effective during the luteal phase of a woman's menstrual cycle.

Soft Tissue Injuries

In a small study, butcher's broom applied topically reduced the swelling and pain caused by soft tissue sports injuries.[27] Several in vitro studies showed that butcher's broom's action is enhanced by heat, suggesting that it may be advantageous to apply heat to the site of the injury when using the herb.

DOSAGE

The dosage of butcher's broom for therapeutic use should be based on the ruscogenin content in the range of 16.5 to 33 mg of ruscogenins two to three times daily. Standardized extracts are preferred for therapeutic use because they allow for more accurate dosages. Recommendations for other forms of butcher's broom are as follows:

Dried rootstalk in capsules, tablets or tea: 500 to 1000 mg three times a day
Tincture (1:5): 2 to 4 mL three times a day
Fluid extract (1:1): 1 to 2 mL three times a day

TOXICOLOGY

Butcher's broom is generally considered very safe. It occasionally causes gastrointestinal distress, and in rare instances, it causes lymphatic colitis.[1,40,41] Thus butcher's broom should probably not be combined with nonsteroidal anti-inflammatory drugs or other drugs linked with lymphatic colitis, and use should be discontinued if negative gastrointestinal symptoms are severe or persist.[40] One author cautioned against the use of butcher's broom in pregnancy based on a lack of proof of safety.[3] Although not definitive, preliminary animal and clinical studies cited previously indicated that butcher's broom does not have negative effects when used during pregnancy. Once case report claims that a butcher's broom extract combined with metformin precipitated diabetic ketoacidosis in a 39-year-old woman with a history of poor blood sugar control, although various other complications in her health picture (e.g., recent vomiting and diarrhea) could have been a factor.[42]

DRUG INTERACTIONS

In an in vitro study, adenosine, cocaine, phentolamine, prazosin, and verapamil reduced butcher's broom's vasoconstrictive effect.[43] Acetylcholine and rauwolscine potentiated its vasoconstrictive action. No clinical studies have been conducted to determine whether butcher's broom interferes with or affects the action of the aforementioned, or any other, drugs.

REFERENCES

See www.expertconsult.com for a complete list of references.

TABLE 109.2 Clinical Studies on Butcher's Broom in Edema and Varicosities

Study	No. of participants	Type	Product	Focus	Result	Length
Berg[28]	18	Placebo- controlled	Melilot and *Ruscus* cream (4–6 g cream/d)	Effect of cream on size of femoral veins in healthy volunteers	Change in size of femoral vein	1 d
Baudet et al.[29]	20	Uncontrolled	2 capsules of *Ruscus* extract/day (amount not stated) from wk 21–24 of pregnancy	Pregnancy-related symptoms of venolymphatic insufficiency	Improved symptoms based on subjective criteria; "absolute harmlessness" to infant	Women monitored from early pregnancy; infants studied before and after birth
Berg[28]	9	Placebo- controlled	Compression stocking, kinesitherapy, and melilot and *Ruscus* cream (4–6 g cream/d)	Pregnant women with painful pregnancy-related varicosity of one limb	Statistically significant reduction in size of femoral vein and pain	Treatment beginning in second trimester
Bohmer[30]	48	Placebo- controlled	4 g *Ruscus* cream (1.6 g *Ruscus* and 1.6 g melilot/100 g) 3 times daily	Sports injuries, patients with sprain or contusions of lower limb or ankle	Increased muscle strength, reduced swelling, decreased temperature, and resolved pain more quickly	14 d
Cluzan et al.[31]	57	Placebo- controlled, double-blind	3 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl-chalcone, 100 mg ascorbic acid) 3 times daily plus physiotherapy	Lymphedema after mastectomy	Reduced edema by second month, positive subjective improvement in symptoms	90 d
Cossio et al.[32]	12	Uncontrolled	Drinkable ampules; week 1: 180 mg *Ruscus* and 900 mg hesperidin methyl chalcone per day; weeks 2–8: 120 mg *Ruscus* and 600 mg hesperidin methyl chalcone per day	Lymphedema of extremities	Significant improvement in symptoms, reduction of edema in 81% of participants	2 mo
Jager et al.[33]	12	Open, uncontrolled	3 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) per day	Primary varicosity of greater saphenous vein	Significant changes in some venous parameters	7 ± 1 d
Weindorf et al.[34]	50	Double-blind, placebo-controlled	450 mg *Ruscus,* 450 mg hesperidin, 300 mg ascorbic acid daily	Trunk or branch varicose veins	Venous capacity and improved at rest; expelled blood volume during exercise improved	2 wk
Lagrue et al.[35]	11	Open, uncontrolled	3 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) daily	Edema secondary to calcium antagonist treatment of hypertension	4/9 showed complete improvement; 3/9 showed partial improvement; 2/9 showed no change in edema	6–8 wk
Le Devehat et al.[36]	25 + 20 healthy controls	Double-blind, placebo- controlled	2 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) daily	Venous insufficiency characterized by varicose veins; blood samples drawn before and after provoked venous stasis	Significant improvement in several microrheologic factors of blood viscosity	Not found
Monteil-Seurin and Ladure[37]	40	Randomized, double-blind, placebo- controlled	2 capsules (150 mg *Ruscus,* 150 mg hesperidin methyl chalcone, 100 mg ascorbic acid) daily	Premenstrual syndrome symptoms for at least 60 months (breast tension, pelvic heaviness, "menstrual psychopathic disorder," ankle edema)	Significantly reduced breast tension, pelvic heaviness, and menstrual "psychopathic disorder"	90 d

From Guex JJ, Enriquez Vega DME, Avril L, et al. Assessment of quality of life in Mexican patients suffering from chronic venous disorder—impact of oral *Ruscus aculeatus*-hesperidin-methyl-chalcone-ascorbic acid treatment—"quality study." Phlebol. 2009; 24:157–165.

REFERENCES

1. In Blumenthal M, Goldberg A, Brinckmann J, eds. *Herbal Medicine: Expanded Commission E Monographs*. Newton, MA: Integrative Medicine Communications; Butcher's Broom, 40–43.
2. Barbic M, William EA, Rothenhofer M, et al. Spirostanol saponins and esculin from Rusci rhizome reduce the thrombin-induced heperpermeability of endothelial cells. *Phytochem*. 2013;90:106–113.
3. Duke JA. *Handbook of Medicinal Herbs*. 2nd ed. Boca Raton, FL: CRC Press; 1985.
4. Fetrow CW, Avila JR. *Professional's Handbook of Complementary and Alternative Medicines*. Springhouse, PA: Springhouse Corp; 2001.
5. Rauwald HW, Grunwidl J. *Ruscus aculeatus* extract: unambiguous proof of the absorption of spirostanol glycosides in human plasma after oral administration. *Planta Med*. 1991;57:A75–A76.
6. Dunouau C, Belle R, Oulad-Ali A, et al. Triterpenes and sterols from *Ruscus aculeatus*. *Planta Med*. 1996;62:189–190.
7. Potterton D. *Culpeper's Color Herbal*. New York: Sterling Publishing; 2007.
8. Hartwell JL. *Plants Used Against Cancer*. Lawrence, MA: Quarterman Publications; 1982.
9. Mimaki Y, Kuroda M, Kameyama A, et al. New steroidal constituents of the underground parts of *Ruscus aculeatus* and their cytostatic activity on HL-60 cells. *Chem Pharm Bull (Tokyo)*. 1998;46:298–303.
10. Baurain R, Dom G, Trouet A. Protecting effect of Cyclo 3 Fort and its constituents for human endothelial cells under hypoxia. *Clin Hemorheol*. 1994;14:S15.
11. Mimaki Y, Kuroda M, Kameyama A, et al. Steroidal saponins from the underground parts of *Ruscus aculeatus* and their cytostatic activity on HL-60 cells. *Phytochemistry*. 1998;48:485–493.
12. Bouskela E, Cyrino FZ, Marcelon G. Inhibitory effect of the *Ruscus* extract and of the flavonoid hesperidine methylchalcone on increased microvascular permeability induced by various agents in the hamster cheek pouch. *J Cardiovasc Pharmacol*. 1993;22:225–230.
13. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, et al. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *J Ethnopharmacol*. 1998;60:265–271.
14. Facino RM, Carini M, Stefani R, et al. Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from *Hedera helix, Aesculus hippocastanum,* and *Ruscus aculeatus*: factors contributing to their efficacy in the treatment of venous insufficiency. *Arch Pharm*. 1995;328:720–724.
15. Pouget G, Ducros L, Marcelon G. Effect of *Ruscus* extract on peripheral lymphatic vessel pressure and flow. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libby Eurotext; 1991:89–95.
16. Bouskela E, Cyrino FZ, Marcelon G. Effects of *Ruscus* extract on the internal diameter of arterioles and venules of the hamster cheek pouch microcirculation. *J Cardiovasc Pharmacol*. 1993;22:221–224.
17. Boyle P, Diehm C, Robertson C. Meta-analysis of clinical trials of Cyclo 3 Fort in the treatment of chronic venous insufficiency. *Int Angiol*. 2003;22:250–262.
18. Cappelli R, Nicora M, Di Perri T. Use of extract of *Ruscus aculeatus* in venous disease of the lower limbs. *Drugs Exp Clin Res*. 1988;14:277–283.
19. Rudofsky G. Efficacy of *Ruscus* extract in venolymphatic edema using foot volumetry. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:121–130.
20. Haas S, Lill G, Stiller A, et al. Influence of *Ruscus* extract and methylhesperidine chalcone on the fibrinolytic activity of the vein wall. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:157–162.
21. Kiesewetter H, Scheffler P, Jung F, et al. Effect of *Ruscus* extract in chronic venous insufficiency stage I, II, and III. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:163–169.
22. Beltramino R, Penenory A, Buceta AM. An open-label, randomized multicenter study comparing the efficacy and safety of Cyclo 3 Fort versus hydroxyethyl rutoside in chronic venous lymphatic insufficiency. *Angiology*. 2000;51:535–544.
23. Le Devehat C, Khodabandehlou T, Dougny M. The effects of Cyclo 3 Fort treatment on hemorheological disturbances during a provoked venous stasis in patients with chronic venous insufficiency. *Clin Hemorh*. 1994;14(suppl 1):S53–S63.
24. Seydewitz V, Berg D, Welbers P, et al. Biochemical investigations on the action of *Ruscus* extract and trimethylhesperidinchalcon (TMHC). In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:151–156.
25. Guex JJ, Enrique Vega DME, Avril L, et al. Assessment of quality of life in Mexican patients suffering from chronic venous disorder—impact of oral *Ruscus aculeatus*-hesperadin-methyl-chalcone-ascorbic acid treatment—'quality study. *Phlebol.* 2009;24:157–165.
26. Vanscheidt W, Jost V, Wolna P, et al. Efficacy and safety of a butcher's broom preparation (*Ruscus aculeatus* L. extract) compared to placebo in patients suffering from chronic venous insufficiency. *Arzneimittel-Forsch/Drug Res.* 2002;52:243–250.
27. Kakkos SK, Allaert FA. Efficacy of *Ruscus* extract, HMC and vitamin C, constituents of Cyclo 3 Fort, on improving individual venous symptoms and edema: a systematic review and meta-analysis of randomized double-blind placebo-controlled trials. *Int Angiol*. 2017;36:93–106.
28. Berg D. First results with *Ruscus* extract in the treatment of pregnancy related varicose veins. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:55–61.
29. Baudet JH, Collet D, Aubard Y, et al. Therapeutic test of *Ruscus* extract in pregnant women: evaluation of the fetal tolerance applying the pulse Doppler's method of the cord. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:63–71.
30. Bohmer D. Action of *Ruscus* extract cream in the treatment of acute sport injuries. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:171–179.
31. Cluzan RV, Alliot F, Ghabboun S, et al. Treatment of secondary lymphedema of the upper limb with Cyclo 3 Fort. *Lymphology*. 1996;29:29–35.
32. Jimenez Cossio JA, Magallon Ortin PJ, Capilla Montes MT, et al. Therapeutic effect of *Ruscus* extract in lymphedemas of the extremities. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:111–119.
33. Jager K, Eichlisberger R, Jeanneret CH, et al. Pharmacodynamic effects of *Ruscus* extract (Cyclo 3 Fort) on superficial and deep veins in patients with primary varicose veins assessment by duplex sonography. *Clin Drug Invest*. 1999;17:265–273.
34. Weindorf N, Schultz-Ehrenburg U. Kontrollierte studie zur oralen venentonisierung der primaeren varikosis mit *Ruscus aculeatus* und trimethylhesperdinechalckon. *Z Hautkr*. 1987;62:28–38.
35. Lagrue G, Behar A, Chaabane A, et al. Edema induced by calcium antagonists: effects of Ruscus extract on clinical and biological parameters. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:105–109.
36. Le Devehat C, Khodabandehlou T, Vimeux M, et al. Hemorheological concepts in venous insufficiency and implications for treatment with *Ruscus* extract. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:225–236.
37. Monteil-Seurin J, Ladure PH. Efficacy of *Ruscus* extract in the treatment of the premenstrual syndrome. In: Vanhoutte PM, ed. *Return Circulation and Norepinephrine*. Paris: John Libbey Eurotext; 1991:43–53.
38. Bennani A, Biadillah MC, Cherkaoui A, et al. Acute attack of hemorrhoids: Efficacy of Cyclo 3 Fort based on results in 124 cases reported by specialists. *Phlebologie*. 1999;52:89–93. [French].
39. Archimowicz-Cyrylowska B, Adamek B, Drozdzik M, et al. Clinical effect of buckwheat herb, *Ruscus* extract and troxerutin on retinopathy and lipids in diabetic patients. *Phytother Res*. 1996;10:659–662.
40. Dharancy S, Dapvril V, Dupont-Evrard F, et al. Cyclo 3 Fort-induced lymphocytic colitis associated with ileal villous atrophy. *Gastroenterol Clin Biol*. 2000;24:134–135. [French].
41. Mennecier D, Thiolet C, Bredin C, et al. Lymphocytic colitis after ingestion of *Rustacea* flavonoid extract. *Presse Med*. 2001;30:1063.
42. Sadarmin PP, Timperley J. An unusual case of butcher's broom precipitating diabetic ketoacidosis. *J Emerg Med*. 2013;45:e63–e65.
43. Rubanyi G, Marcelon G, Vanhoutte PM, et al. Effect of temperature on the responsiveness of cutaneous veins to the extract of *Ruscus aculeatus*. *Gen Pharmacol*. 1984;15:431–434.

SAMe (S-Adenosylmethionine)

Michael T. Murray, ND

OUTLINE

INTRODUCTION

S-Adenosylmethionine (SAMe) is an important physiological agent formed in the body through combination of the essential amino acid methionine with adenosine triphosphate (ATP) (Fig. 110.1). SAMe was discovered in Italy in 1952. Not surprisingly, most of the research on SAMe has been conducted in the country of its discovery.

Because SAMe is manufactured from methionine, one would think that dietary sources of methionine would provide the same benefits as SAMe. However, high doses of methionine have not been shown to raise levels of SAMe, nor do they provide the same pharmacological activity as SAMe and increase homocysteine levels. High doses of methionine are associated with some degree of toxicity.[1]

Normally the body manufactures all the SAMe it needs from the amino acid methionine. However, a deficiency of methionine, vitamin B_{12}, or folic acid can result in decreased SAMe synthesis. In addition, tissue levels of SAMe are typically low in the elderly and in patients with osteoarthritis, depression, and various liver disorders.

PHARMACOLOGY

SAMe is involved in more than 40 biochemical reactions in the body. It functions closely with folic acid and vitamin B_{12} in methylation reactions. Methylation is the process of adding a single carbon unit (a methyl group) to another molecule. SAMe is many times more effective in transferring methyl groups than are other methyl donors. Methylation reactions are critical in the manufacture of many body components, especially brain chemicals, as well as in detoxification reactions.

SAMe is also required in the manufacture of sulfur-containing compounds in the human body, including glutathione (discussed later) and various sulfur-containing cartilage components.

The beneficial effects of SAMe supplementation are far-reaching owing to this agent's central role in so many metabolic processes.

Available Forms

SAMe has been available commercially in Europe since 1975 and in the United States since 1999. The commercial form of SAMe is a stabilized salt produced under US patents no. 3,954,726 (1976) and no. 4,057,686 (1977).

CLINICAL INDICATIONS

The five principal uses of SAMe are for depression, osteoarthritis, fibromyalgia, liver disorders, and migraine headaches.

Depression

SAMe is necessary in the manufacture of important brain compounds, such as neurotransmitters and phospholipids like phosphatidylcholine and phosphatidylserine. Adding SAMe to the diet of depressed patients raises levels of serotonin, dopamine, and phosphatidylserine and improves binding of neurotransmitters to receptor sites, resulting in increased serotonin and dopamine activity and better brain-cell membrane fluidity, leading to significant clinical improvement.[2-4]

The antidepressant effects of folic acid (see Chapter 142) are mild compared with the effects noted in clinical trials of SAMe. In these clinical studies SAMe has shown promise as an antidepressant agent.[4-6] One study, however, indicates that there may be significant gender differences in the response to SAMe. Data from a 12-week double-blind trial assessed the efficacy of SAMe versus placebo and a comparator selective serotonin reuptake inhibitor (escitalopram) included a post hoc analysis to evaluate effects of patient gender on treatment response. SAMe was found superior to placebo among males (n = 51), but not among females (n = 62).[7] Men have shown a significantly lower SAMe to S-adenosylhomocysteine (SAH) ratio than women, suggesting SAMe supplementation may affect men's biochemistry more significantly. A 6-week double-blind study of SAMe augmentation for selective serotonin reuptake inhibitors (SSRI)/serotonin norepinephrine reuptake inhibitor nonresponders revealed that males treated with adjunctive SAMe demonstrated significantly improved sexual arousal compared with the adjunctive placebo group.[8] It was suggested that because SAMe is a regulator of cystathionine beta-synthase, it may result in improved nitric oxide-mediated vasodilation of penile tissues. SAMe may also enhance dopamine's role in enhancing sexual function in men. Not all of the research shows a better response in men. For

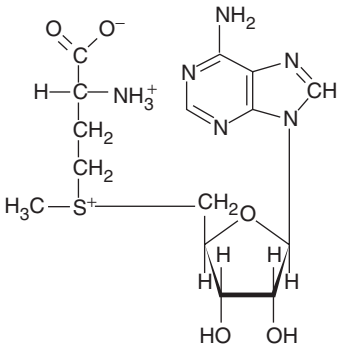


Fig. 110.1 S-Adenosylmethionine.

example, a small, 8-week double-blind study in patients with chronic schizophrenia given either SAMe 800 mg or placebo found improvement of depressive symptoms only in females.[9]

Although most of the early studies used injectable SAMe, most recent studies have used an oral dose of 400 mg four times daily (1600 mg total) and have demonstrated that SAMe is just as effective given orally as when it is given intravenously.[4,10-15] That is not surprising, because there are no significant differences in the pharmacokinetic parameters of SAMe 1000 mg administered orally or intravenously.[16]

Several studies have compared SAMe with tricyclic antidepressants. Before the rise of SSRIs, tricyclics were the mainstay pharmaceutical treatment for depression. A 2002 review concluded that, "at doses of 200 to 1600 mg/day, SAMe is superior to placebo and is as effective as tricyclic antidepressants in alleviating depression, although some individuals may require higher doses."[10] The reviewers found six of eight placebo-controlled studies, featuring anywhere from 40 to 100 participants, demonstrating that SAMe was superior to placebo; moreover, six of eight comparison studies showed that SAMe was just as effective as tricyclic antidepressants. These studies have shown that SAMe is better tolerated than the tricyclics and has a quicker onset of antidepressant action.[11]

In one study comparing SAMe with desipramine, in addition to determining the clinical response, the blood level of SAMe was determined in both groups. At the end of the 4-week trial, 62% of the patients treated with SAMe and 50% of those treated with desipramine had significantly improved. Regardless of the type of treatment, patients with a 50% decrease in scores on the Hamilton Depression Scale showed a significant rise in plasma SAMe concentration. These results suggest that one of the ways in which tricyclic antidepressant agents exert their antidepressant effects is by raising SAMe levels.[17]

In two separate double-blind studies, SAMe was given orally (1600 mg daily) or intramuscularly (400 mg daily) and compared with 150 mg of imipramine given orally daily. In both studies, the results of SAMe and imipramine treatment did not differ significantly for any efficacy measure. However, significantly fewer adverse events were observed in the patients treated with SAMe. The researchers concluded that the antidepressant efficacy of 1600 mg SAMe orally and 400 mg SAMe intramuscularly is comparable with that of 150 mg of imipramine but that SAMe is significantly better tolerated.[18]

Several studies have compared SAMe with SSRIs. In one double-blind study, 144 subjects with major depressive disorder were given SAMe (1600–3200 mg/daily), escitalopram (10–20 mg/daily), or matching placebo for 12 weeks.[19] Using the Hamilton Depression Rating Scale (HAMD-17), SAMe showed a moderate to large effect size from baseline to the end of 12 weeks. Response rates (HAMD-17 ≥50% reduction) at endpoint were 45%, 31%, and 26% for SAMe, escitalopram, and placebo, respectively; whereas remission rates (HAM-D ≤7) were 34% for SAMe, 23% for escitalopram, and 6% for placebo.

A similar study did not show nearly the same advantage, as neither SAMe nor escitalopram showed a better effect than a placebo.[20]

SAMe can be used safely with SSRIs and may have a synergistic effect. In a double-blind, randomized clinical trial lasting 6 weeks, 73 depressed patients who were unresponsive to SSRI medications alone were given 800 mg of SAMe or placebo twice daily along with their SSRIs.[21] The response and remission rates according to the Hamilton Depression Scale were higher for patients treated with adjunctive SAMe (36.1% and 25.8%, respectively) than adjunctive placebo (17.6% vs. 11.7%, respectively). These results indicate that SAMe can be used safely with SSRIs and may have a synergistic effect.

In a study on 20 HIV-seropositive individuals with depression, SAMe as a sole antidepressant medication showed significant effect. There was a significant reduction in acute depressive symptomatology, reaching the defined clinical treatment response threshold of a greater than 50% reduction in symptom scores. The researchers also noted that SAMe had a rapid effect as early as after only 1 week, with progressive decreases in symptom scores throughout the study.[22]

SAMe has also been shown to produce significant effects in other conditions associated with depression, such as during the postpartum period and in drug rehabilitation. SAMe's benefits in these conditions are probably due to a combination of its effects on brain chemistry and liver function (discussed later). In the study in postpartum depression, the administration of SAMe (1600 mg/day) was associated with significantly better mood scores compared with placebo.[23] In another study, SAMe (1200 mg/day) was shown to significantly reduce psychological distress (chiefly anxiety and depression) in the detoxification and rehabilitation of opiate abusers.[24]

SAMe may also be helpful in managing the symptomatology of schizophrenia. In one double-blind study, 18 patients with chronic schizophrenia were randomly assigned to receive either SAMe (800 mg) or placebo for 8 weeks. Results indicated some reduction in aggressive behavior and improved quality of life after SAMe administration. Female patients showed improvement of depressive symptoms, whereas male patients showed no change compared with placebo. Blood dopamine levels increased in women but decreased in men receiving SAMe. Clinical improvement did not correlate with serum SAMe levels. Two patients receiving SAMe exhibited some exacerbation of irritability; therefore, it should be used with caution in these patients.[9]

Detailed clinical evaluations using electroencephalography, event-related potentials, and low-resolution brain electromagnetic tomography have clearly indicated a central nervous system antidepressant action of SAMe.[18]

Osteoarthritis

SAMe has also demonstrated impressive results in the treatment of osteoarthritis.[25] A deficiency of SAMe in the joint tissue, just like a deficiency of glucosamine, leads to loss of the gel-like nature and shock-absorbing qualities of cartilage. As a result, osteoarthritis can develop.

In vitro studies have shown that SAMe exerts a number of effects that appear to be highly relevant in the treatment of osteoarthritis.[26] First, the agent has been shown to be important in the manufacture of cartilage components. This effect has been demonstrated very well in humans. In one double-blind study conducted in Germany, the 14 patients with osteoarthritis of the hands who were given SAMe showed greater cartilage formation on magnetic resonance imaging.[27] These results indicate that SAMe is capable of improving the structure and function of cartilage in joints affected by osteoarthritis. Second, SAMe has shown some mild pain-relieving and anti-inflammatory effects in animal studies.[1] All of these effects combine to produce clinical benefits.

In double-blind trials, SAMe has demonstrated similar reductions in pain scores and clinical symptoms to those produced by nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, indomethacin, naproxen, nabumetone, celecoxib, and piroxicam.[25] In one double-blind study, SAMe was compared with ibuprofen.[28] The 36 subjects with osteoarthritis of the knee, hip, and/or spine received a daily oral dose of 1200 mg of SAMe or 1200 mg of ibuprofen for 4 weeks. Morning stiffness, pain at rest, pain on motion, crepitation (crackling noise upon movement of a joint), swelling, and limitation of motion of the affected joints were assessed before and after treatment. The total score obtained by evaluating all the individual clinical parameters improved to the same extent in patients treated with SAMe and in those treated with ibuprofen. In two other studies, SAMe was actually shown to produce slightly better results.[29,30]

SAMe has also been compared with naproxen (Naprosyn). In one double-blind study, 20 patients with osteoarthritis of the knee were given either SAMe or naproxen for 6 weeks.[31] During the first week, SAMe was administered at a dose of 400 mg three times daily and afterward at a dose of 400 mg twice daily, whereas the dose of naproxen during the first week was 250 mg three times daily and subsequently 250 mg twice daily. During the first 2 weeks, the patients were allowed to take acetaminophen as an additional analgesic if the pain was severe. The patients were examined at the beginning of the study and after 2, 4, and 6 weeks. The parameters tested were pain, crepitation, joint swelling, circumference of joint, extent of motility, and 10-meter walking time. At the end of the sixth week, no statistically significant difference between the two patient groups was found; both groups exhibited a marked improvement on all parameters.

Another double-blind study compared SAMe with both naproxen and placebo in the treatment of osteoarthritis of the hip, knee, spine, and hand. The study involved 33 rheumatologic and orthopedic medical centers and a total of 734 subjects. SAMe administered orally at a dose of 1200 mg daily was shown to exert the same analgesic (pain-relieving) activity as naproxen at a dose of 750 mg daily. However, SAMe was found to be significantly more effective than naproxen, both in terms of physicians' and patients' judgments and in terms of the number of patients with side effects. In fact, SAMe was better tolerated than placebo. A total of 10 patients in the SAMe group and 13 in the placebo group withdrew from the study because of intolerance to the intervention.[32] Other double-blind studies have shown SAMe to offer pain-relieving and anti-inflammatory benefits similar to those of drugs like indomethacin and piroxicam while also being generally much better tolerated than these potent NSAIDs.[33,34]

Perhaps the most meaningful study of SAMe in the treatment of osteoarthritis was a long-term multicenter open 2-year trial involving 97 patients with osteoarthritis of the knee, hip, and spine.[35] The patients received 600 mg of SAMe daily for the first 2 weeks and thereafter 400 mg daily until the end of the 24th month of treatment. Separate evaluations were made for osteoarthritis of the knee, hip, cervical spine, and dorsal-lumbar spine. The severity of the clinical symptoms (morning stiffness, pain at rest, and pain on movement) was assessed with scoring before the start of the treatment, at the end of the first and second weeks of treatment, and then monthly until the end of the 24-month period. SAMe showed good clinical effectiveness and was well tolerated. Improvement in the clinical symptoms during therapy with SAMe was already evident after the first weeks and continued to the end of treatment. Nonspecific side effects occurred in 20 patients, but in no case did therapy have to be discontinued. Most side effects disappeared during the course of therapy. Moreover, during the last 6 months of treatment, no adverse effect was recorded. Detailed laboratory tests carried out at the start and after 6, 12, 18, and 24 months of treatment showed no pathological changes. SAMe administration also improved the depressive feelings often associated with osteoarthritis.

One study compared the efficacy and tolerability of SAMe (1200 mg/day) and nabumetone (Relafene, 1000 mg/day) in 134 patients with osteoarthritis of the knee for 8 weeks.[36] An analysis of changes in pain intensity between weeks 0 and 8 found that both SAMe and nabumetone effectively reduced pain intensity from baseline in each group and that the degree of decrease in pain intensity was not significantly different between the groups. The patients' global assessments of disease status, physician's global assessment of response to therapy, and Western Ontario and McMaster Universities Arthritis Index (WOMAC) index scores were not significantly different between the groups.

A randomized, double-blind, crossover study comparing SAMe (1200 mg) with celecoxib (Celebrex 200 mg) for 16 weeks to reduce pain associated with osteoarthritis of the knee in 56 subjects showed that SAMe has a slower onset of action but is as effective as celecoxib in the management of symptoms of knee osteoarthritis.[37]

Finally, in the largest study, 20,641 patients with osteoarthritis of the knee, hip, and spine and also with osteoarthritic polyarthritis of the fingers were studied over 8 weeks.[38] The patients were given 400 mg of SAMe three times daily for the first week, 400 mg twice daily for the second week, and 200 mg twice daily from the third week onward. No additional analgesic or anti-inflammatory treatment was allowed. The efficacy of SAMe was comparable with results achieved with NSAIDs. Efficacy was described as very good or good in 71% of cases, moderate in 21%, and poor in 9%; tolerance of the agent was assessed as very good or good in 87%, moderate in 8%, and poor in 5%.

What all of these studies indicate is that SAMe appears to offer significant advantages over NSAIDs. The latter drugs are associated with significant risks of toxicity, side effects, and actual promotion of the disease process in osteoarthritis, but SAMe offers similar benefits without risks or side effects.

Fibromyalgia

SAMe has been shown in four double-blind clinical studies to produce excellent benefits in patients suffering from fibromyalgia.[39-42] In two of the studies, injectable SAMe (200 mg daily) was used. During treatment, subjects demonstrated significant reductions in the number of trigger points and painful areas as well as improvements in mood.[39,40]

In one of the studies, oral SAMe (800 mg/day) was compared with a placebo for 6 weeks in 44 patients with fibromyalgia.[41] Tender-point score, muscle strength, disease activity, subjective symptoms, mood parameters, and side effects were evaluated. Patients given SAMe demonstrated improvements in clinical disease activity, pain experienced during the last week, fatigue, morning stiffness, and mood; however, the tender-point score and muscle strength did not differ in the two treatment groups. SAMe was without side effects.

In one of the studies, SAMe was compared with transcutaneous electrical nerve stimulation—a popular treatment for fibromyalgia—in 30 patients with this disorder.[43] Patients receiving SAMe (200 mg by injection and 400 mg/day orally) experienced significantly greater clinical benefit as shown by a decreased number of tender points, diminished subjective feelings of pain and fatigue, and improved mood. Transcutaneous electrical nerve stimulation offered little benefit for most symptoms, whereas SAMe was deemed "effective in relieving the signs and symptoms of primary fibromyalgia."[42]

Liver Disorders

SAMe has been shown to be beneficial in several liver disorders, including cirrhosis, Gilbert syndrome, and oral contraceptive–induced liver damage. Its benefits are related to its function as the major methyl donor in the liver as well as restoring hepatic glutathione concentrations, thereby reducing liver injury. It also possesses lipotropic activity.

One of the leading contributors to impaired liver function is diminished bile flow, or cholestasis. SAMe is beneficial for a variety of liver disorders because of its lipotropic ability to promote bile flow and relieve cholestasis.[43,44]

Another key function of SAMe in the liver is the inactivation of estrogens. Clinical studies have shown that SAMe is useful in protecting the liver from damage and improving liver function in conditions associated with estrogen excess—namely, oral contraceptive use, pregnancy, and premenstrual syndrome.[45-48] SAMe has shown benefit in the treatment of intrahepatic cholestasis of pregnancy, a special complication of pregnancy characterized by skin pruritus, abnormal liver function tests, and bile acids. Alhtough helpful, SAMe is not as effective as a monotherapy as ursodeoxycholic acid or the two agents combined.[47,48]

Another key indication for the use of SAMe is in the treatment of Gilbert syndrome, a common disorder characterized by a chronically elevated serum bilirubin level (1.2–3.0 mg/dL) typically due to underactivity of uridine disphosphate-glucuronosyltransferase-1A1. Previously considered rare, this disorder is now known to affect as many as 5% of the general population. The condition is usually without symptoms, although some patients complain of loss of appetite, malaise, and fatigue (typical symptoms of impaired liver function). Also to be considered is the decreased ability to detoxify environmental toxins normally neutralized by this pathway. SAMe at a dosage of 400 mg three times daily has resulted in a significant decrease in serum bilirubin in patients with Gilbert syndrome.[49]

In addition to these relatively minor disturbances in liver function, SAMe offers some benefits in the treatment of chronic liver disorders, including cirrhosis.[50] Its effect in cirrhosis appears to be due to an ability to overcome the SAMe depletion characterized by the disorder. Because SAMe is involved in so many liver processes, the depletion of levels of SAMe within the liver has serious consequences. Supplementation with SAMe in patients with liver cirrhosis not only improves bile flow and clinical signs and symptoms but also promotes membrane function and increases levels of glutathione.[51-54] Glutathione assumes a critical role in detoxification as well as in the defense against a variety of injurious agents by combining directly with these toxic substances to eventually form water-soluble compounds. Because many of the toxic compounds are lipid (fat) soluble, conversion to water-soluble compounds results in more efficient excretion via the kidneys. When higher levels of toxic compounds are present or liver function is impaired, higher glutathione levels are required.

One of the greatest risks of chronic liver diseases such as chronic hepatitis is liver cancer. Supplementation with SAMe appears to be strongly indicated in patients with such diseases in the attempt to reduce the risk of liver cancer. Animal studies have shown a significant protective effect of supplemental SAMe against liver cancer in animals exposed to liver carcinogens.[55]

Migraine

SAMe has also been shown to be of benefit in the treatment of migraine headaches. The benefit arises gradually and long-term treatment is required for therapeutic effectiveness.[56]

DOSAGE

In general, the longer SAMe is used, the more beneficial the results. This agent is perfectly suited for long-term use because of its excellent safety profile. Although some studies have used intravenous dosages, there were no significant differences in the pharmacokinetic parameters of SAMe between single- and multiple-dose administration of a standard 1000-mg dose administered orally or intravenously. No evidence of accumulation of SAMe in plasma was found on multiple dosing. The dosage ranges for the various clinical indications are as follows:

Depression: 400 to 1600 mg daily. Because SAMe can cause nausea and gastrointestinal disturbances in some people, it is recommended that it be started at a dosage of 200 mg twice daily for the first day, increased to 400 mg twice daily on day 3, and finally raised to the full dosage of 800 mg twice daily after 10 days if needed

Osteoarthritis: The dosage is best started out as mentioned previously for depression. After 21 days at a dosage of 1600 mg daily, the maintenance dosage is reduced to 200 mg twice a day

Fibromyalgia: 200 to 400 mg two times a day

Liver disorders: 200 to 400 mg two to three times a day

Migraine headaches: 200 to 400 mg two times a day

TOXICOLOGY

No significant side effects have been reported with oral SAMe other than occasional nausea and gastrointestinal disturbances. However, individuals with bipolar (manic) depression should not take SAMe unless they are under strict medical supervision, because SAMe's antidepressant activity may lead to the manic phase in these individuals; this effect is seen only in some people with bipolar depression.

INTERACTIONS AND CONTRAINDICATIONS

SAMe functions very closely with vitamin B_{12}, folic acid, vitamin B_6, and choline in methylation reactions. Because of SAMe's effects on the liver, it may enhance the elimination of various drugs from the body.[57] The clinical significance of this effect has not been fully determined.

It has been cautioned that SAMe should be avoided in patients with Parkinson's disease. Animal studies indicate that excessive methylation is associated with Parkinson's disease, and SAMe excess has caused Parkinson's disease–like effects in animal studies.[57] In addition, both animal and human studies indicate that increased methylation can cause the depletion of dopamine and block the effects of L-dopa.[53,58,59] This line of research is somewhat contradicted, however, by preliminary evidence that SAMe may improve the emotional depression and impaired mental function often associated with this disorder.[60] Nonetheless, it is recommended that patients with Parkinson's disease avoid supplementing with SAMe until more is known.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Stramentinoli G. Pharmacologic aspects of S-adenosylmethionine: pharmacokinetics and pharmacodynamics. *Am J Med*. 1987;83:35–42.
2. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademetionine (S-adenosylmethionine) in neurological disorders. *Drugs*. 1994;48:137–152.
3. Fava M, Rosenbaum JF, MacLaughlin R, et al. Neuroendocrine effects of S-adenosyl-l-methionine, a novel putative antidepressant. *J Psychiatr Res*. 1990;24:177–184.
4. Papakostas GI. Evidence for S-adenosyl-L-methionine (SAM-e) for the treatment of major depressive disorder. *J Clin Psychiatry*. 2009;70(suppl 5):18–22.
5. Sharma A, Gerbarg P, Bottiglieri T, et al. S-Adenosylmethionine (SAMe) for neuropsychiatric disorders: a clinician-oriented review of research. *J Clin Psychiatry*. 2017;78(6):e656–e667.
6. Galizia I, Oldani L, Macritchie K, et al. S-adenosyl methionine (SAMe) for depression in adults. *Cochrane Database Syst Rev*. 2016;10:CD011286.
7. Sarris J, Price LH, Carpenter LL, et al. Is S-adenosyl methionine (SAMe) for depression only effective in males? A re-analysis of data from a randomized clinical trial. *Pharmacopsychiatry*. 2015;48(4–5):141–144.
8. Dording CM, Mischoulon D, Shyu I, Alpert JE, Papakostas GI. SAMe and sexual functioning. *Eur Psychiatry: J Assoc Eur Psychiatr*. 2012;27(6):451–454.
9. Strous RD, Ritsner MS, Adler S, et al. Improvement of aggressive behavior and quality of life impairment following S-adenosyl-methionine (SAM-e) augmentation in schizophrenia. *Eur Neuropsychopharmacol*. 2009;19(1):14–22.
10. Mischoulon D. Fava M.Role of S-adenosyl-L-methionine in the treatment of depression: a review of the evidence. *Am J Clin Nutr*. 2002;76(5):1158S–1161S.
11. Delle Chiaie R, Pancheri P, Scapicchio P. Efficacy and tolerability of oral and intramuscular S-adenosyl-L-methionine 1,4-butanedisulfonate (SAMe) in the treatment of major depression: comparison with imipramine in 2 multicenter studies. *Am J Clin Nutr*. 2002;76(5):1172S–1176S.
12. Kagan BL, Sultzer DL, Rosenlicht N, et al. Oral S-adenosylmethionine in depression: a randomized, double-blind, placebo-controlled trial. *Am J Psychiatry*. 1990;147:591–595.
13. Rosenbaum JF, Fava M, Falk WE, et al. An open-label pilot study of oral S-adenosylmethionine in major depression: interim results. *Psychopharmacol Bull*. 1988;24:189–194.
14. De Vanna M, Rigamonti R. Oral S-adenosyl-l-methionine in depression. *Curr Ther Res*. 1992;52:478–485.
15. Salmaggi P, Bressa GM, Nicchia G, et al. Double-blind, placebo-controlled study of S-adenosyl-l-methionine in depressed postmenopausal women. *Psychother Psychosom*. 1993;59:34–40.
16. Yang J, He Y, Du YX, et al. Pharmacokinetic properties of S-adenosylmethionine after oral and intravenous administration of its tosylate disulfate salt: a multiple-dose, open-label, parallel-group study in healthy Chinese volunteers. *Clin Ther*. 2009;31(2):311–320.
17. Bell KM, Potkin SG, Carreon D, et al. S-adenosylmethionine blood levels in major depression: changes with drug treatment. *Acta Neurol Scand Suppl*. 1994;154:15–18.
18. Delle Chiaie R, Pancheri P, Scapicchio P. Efficacy and tolerability of oral and intramuscular S-adenosyl-l-methionine 1,4-butanedisulfonate (SAMe) in the treatment of major depression: comparison with imipramine in 2 multicenter studies. *Am J Clin Nutr*. 2002;76:1172S–1176S.
19. Sarris J, Papakostas GI, Vitolo O, Fava M, Mischoulon D. S-adenosyl methionine (SAMe) versus escitalopram and placebo in major depression RCT: efficacy and effects of histamine and carnitine as moderators of response. *J Affect Disord*. 2014;164:76–81.
20. D1 M, Price LH, Carpenter LL, et al. A double-blind, randomized, placebo-controlled clinical trial of S-adenosyl-L-methionine (SAMe) versus escitalopram in major depressive disorder. *J Clin Psychiatry*. 2014;75(4):370–376.
21. Papakostas GI, Mischoulon D, Shyu I, et al. S-adenosyl methionine (SAMe) augmentation of serotonin reuptake inhibitors for antidepressant nonresponders with major depressive disorder: a double-blind, randomized clinical trial. *Am J Psychiatry*. 2010;167(8):942–948.
22. Shippy RA, Mendez D, Jones K, et al. S-adenosylmethionine (SAM-e) for the treatment of depression in people living with HIV/AIDS. *BMC Psychiatry*. 2004;4:38.
23. Cerutti R, Sichel MP, Perin M, et al. Psychological distress during puerperium: a novel therapeutic approach using S-adenosylmethionine. *Curr Ther Res*. 1993;53:707–717.
24. Lo Russo A, Monaco M, Pani A, et al. Efficacy of S-adenosyl-l-methionine in relieving psychological distress associated with detoxification in opiate abusers. *Curr Ther Res*. 1994;55:905–913.
25. Soeken KL, Lee WL, Bausell RB, et al. Safety and efficacy of S-adenosylmethionine (SAMe) for osteoarthritis. *J Fam Pract*. 2002;51:425–430.
26. Harmand MF, Vilamitjana J, Maloche E, et al. Effects of S-adenosymethionine on human articular chondrocyte differentiation: an in vitro study. *Am J Med*. 1987;83:48–54.
27. Konig H, Stahl H, Sieper J, et al. Magnetic resonance tomography of finger polyarthritis: morphology and cartilage signals after ademetionine therapy. *Aktuelle Radiol*. 1995;5:36–40.
28. Muller-Fassbender H. Double-blind clinical trial of S-adenosylmethionine versus ibuprofen in the treatment of osteoarthritis. *Am J Med*. 1987;83:81–83.
29. Glorioso S, Todesco S, Mazzi A, et al. Double-blind multicentre study of the activity of S-adenosylmethionine in hip and knee osteoarthritis. *Int J Clin Pharmacol Res*. 1985;5:39–49.
30. Marcolongo R, Giordano N, Colombo B, et al. Double-blind multicentre study of the activity of S-adenosyl-methionine in hip and knee osteoarthritis. *Curr Ther Res*. 1985;37:82–94.
31. Domljan Z, Vrhovac B, Durrigl T, et al. A double-blind trial of ademetionine vs naproxen in activated gonarthrosis. *Int J Clin Pharmacol Ther Toxicol*. 1989;27:329–333.
32. Caruso I, Pietrogrande V. Italian double-blind multicenter study comparing S-adenosylmethionine, naproxen, and placebo in the treatment of degenerative joint disease. *Am J Med*. 1987;83:66–71.
33. Vetter G. Double-blind comparative clinical trial with S-adenosylmethionine and indomethacin in the treatment of osteoarthritis. *Am J Med*. 1987;83:78–80.
34. Maccagno A, Di Giorgio EE, Caston OL, et al. Double-blind controlled clinical trial of oral S-adenosylmethionine versus piroxicam in knee osteoarthritis. *Am J Med*. 1987;83:72–77.
35. Konig BA. Long-term (two years) clinical trial with S-adenosylmethionine for the treatment of osteoarthritis. *Am J Med*. 1987;83:89–94.
36. Kim J, Lee EY, Koh EM, et al. Comparative clinical trial of S-adenosylmethionine versus nabumetone for the treatment of knee osteoarthritis: an 8-week, multicenter, randomized, double-blind, double-dummy, Phase IV study in Korean patients. *Clin Ther*. 2009;31(12):2860–2872.
37. Najm WI, Reinsch S, Hoehler F, et al. Harvey PW.S-adenosyl methionine (SAMe) versus celecoxib for the treatment of osteoarthritis symptoms: a double-blind cross-over trial. *BMC Musculoskelet Disord*. 2004; 26(5): 6.
38. Berger R, Nowak H. A new medical approach to the treatment of osteoarthritis: report of an open phase IV study with ademetionine (Gumbaral). *Am J Med*. 1987;83:84–88.
39. Tavoni A, Vitali C, Bombardieri S, et al. Evaluation of S-adenosylmethionine in primary fibromyalgia: a double-blind crossover study. *Am J Med*. 1987;83:107–110.
40. Volkmann H, Norregaard J, Jacobsen S, et al. Double-blind, placebo-controlled cross-over study of intravenous S-adenosyl-l-methionine in patients with fibromyalgia. *Scand J Rheumatol*. 1997;26:206–211.
41. Jacobsen S, Danneskiold-Samsoe B, Andersen RB. Oral S-adenosylmethionine in primary fibromyalgia: double-blind clinical evaluation. *Scand J Rheumatol*. 1991;20:294–302.
42. Di Benedetto P, Iona LG, Zidarich V. Clinical evaluation of S-adenosyl-l-methionine versus transcutaneous nerve stimulation in primary fibromyalgia. *Curr Ther Res*. 1993;53:222–229.
43. Avila MA, Garcia-Trevijano ER, Martinez-Chantar ML, et al. S-Adenosylmethionine revisited: its essential role in the regulation of liver function. *Alcohol*. 2002;27:163–167.

44. Frezza M, Surrenti C, Manzillo G, et al. Oral S-adenosylmethionine in the symptomatic treatment of intrahepatic cholestasis: a double-blind, placebo-controlled study. *Gastroenterology*. 1990;99:211–215.
45. Di Padova C, Tritapepe R, Di Padova F, et al. S-adenosyl-l-methionine antagonizes oral contraceptive-induced bile cholesterol supersaturation in healthy women: preliminary report of a controlled randomized trial. *Am J Gastroenterol*. 1984;79:941–944.
46. Frezza M, Pozzato G, Pison G, et al. S-adenosylmethionine counteracts oral contraceptive hepatotoxicity in women. *Am J Med Sci*. 1987;293:234–238.
47. Nicastri PL, Diaferia A, Tartagni M, et al. A randomised placebo-controlled trial of ursodeoxycholic acid and S-adenosylmethionine in the treatment of intrahepatic cholestasis of pregnancy. *Br J Obstet Gynaecol*. 1998;105:1205–1207.
48. Zhang L, Liu XH, Qi HB, et al. Ursodeoxycholic acid and S-adenosylmethionine in the treatment of intrahepatic cholestasis of pregnancy: a multi-centered randomized controlled trial. *Eur Rev Med Pharmacol Sci*. 2015;19(19):3770–3776.
49. Bombardieri G, Milani A, Bernardi L, et al. Effects of S-adenosyl-methionine (SAMe) in the treatment of Gilbert's syndrome. *Curr Ther Res*. 1985;37:580–585.
50. Guo T, Chang L, Xiao Y, Liu Q. S-adenosyl-L-methionine for the treatment of chronic liver disease: a systematic review and meta-analysis. *PLoS One*. 2015;10(3):e0122124.
51. Angelico M, Gandin C, Nistri A, et al. Oral S-adenosyl-l-methionine (SAMe) administration enhances bile salt conjugation with taurine in patients with liver cirrhosis. *Scand J Clin Lab Invest*. 1994;54:459–464.
52. Kakimoto H, Kawata S, Imai Y, et al. Changes in lipid composition of erythrocyte membranes with administration of S-adenosyl-l-methionine in chronic liver disease. *Gastroenterol Jpn*. 1992;27:508–513.
53. Loguercio C, Nardi G, Argenzio F, et al. Effect of S-adenosyl-l-methionine administration on red blood cell cysteine and glutathione levels in alcoholic patients with and without liver disease. *Alcohol*. 1994;29:597–604.
54. Mato JM, Camara J, Fernandez de Paz J, et al. S-adenosylmethionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J Hepatol*. 1999;30:1081–1089.
55. Pascale RM, Marras V, Simile MM, et al. Chemoprevention of rat liver carcinogenesis by S-adenosyl-l-methionine: a long-term study. *Cancer Res*. 1992;52:4979–4986.
56. Gatto G, Caleri D, Michelacci S, et al. Analgesizing effect of a methyl donor (S-adenosylmethionine) in migraine: an open clinical trial. *Int J Clin Pharmacol Res*. 1986;6:15–17.
57. Reicks M, Hathcock JN. Effects of methionine and other sulfur compounds on drug conjugations. *Pharmacol Ther*. 1988;37:67–79.
58. Cheng H, Gomes-Trolin C, Aquilonius SM, et al. Levels of l-methionine S-adenosyltransferase activity in erythrocytes and concentrations of S-adenosylmethionine and S-adenosylhomocysteine in whole blood of patients with Parkinson's disease. *Exp Neurol*. 1997;145:580–585.
59. Charlton CG, Crowell Jr B. Parkinson's disease-like effects of S-adenosyl-l-methionine effects of l-dopa. *Pharmacol Biochem Behav*. 1992;43:423–431.
60. Di Rocco A, Rogers JD, Brown R, et al. S-Adenosyl-methionine improves depression in patients with Parkinson's disease in an open-label clinical trial. *Mov Disord*. 2000;15:1225–1229.

Sarsaparilla Species

Michael T. Murray, ND

OUTLINE

Smilax aristolochiifolia (family: Liliaceae)
Synonym: *Smilax medica*
Common name: Mexican sarsaparilla
Smilax chinensis (family: Liliaceae)
Common name: Chinese sarasaparilla
Smilax officinalis (family: Liliaceae)
Synonym: *Smilax regelii*
Common name: Honduras sarsaparilla

GENERAL DESCRIPTION

Sarsaparilla is a tropical American perennial plant. Its long slender root and short thick rhizomes produce a vine that trails on the ground and climbs by means of tendrils growing in pairs from the petioles of the alternate obicular-to-ovate evergreen leaves. The root is the part of the plant used for medicinal purposes.

CHEMICAL COMPOSITION

Sarsaparilla contains 1.8% to 2.4% steroid saponins, including the following:

- Sarsaponin
- Smilasaponin
- Sarsaparilloside and its aglycones, sarsapogenin (Fig. 111.1), smilagenin, and pollinastanol

Other constituents are starch, resins, polyphenols (stilbenes, including resveratrol), flavonoids, and a trace of volatile oil.[1–3]

HISTORY AND FOLK USE

Smilax species are distributed widely in tropical and temperate regions throughout the world and have been used in many different cultures for the same conditions—namely gout, arthritis, fevers, digestive disorders, skin disease, and cancer.[1] From a historic perspective, sarsaparilla's medicinal use has been as a tonic and blood purifier. *Tonics* are defined as agents that "permanently exalt the energies of the body at large, without vitally affecting any one organ in particular. In short, tonics tone the whole system. A blood purifier or depurative [is] an agent that cleanses and purifies the system."[4] Sarsaparilla's reputation in this regard probably stems from its importation from the Caribbean and South America to Europe in the 16th century for the treatment of syphilis.[5]

Historic Use in the Treatment of Syphilis

Nicholas Monardes, a French physician, published a comprehensive account of sarsaparilla and several other "new" drugs for the treatment of syphilis in 1574. Many Europeans at the time believed that syphilis had come to Europe from the West Indies, with Columbus' sailors. Because there was a general belief that whatever disease was native to a country might be cured by the medicinal herbs grown in that region, it was only natural for sarsaparilla to become a popular remedy. Furthermore, use of the standard treatment for syphilis, mercury, often resulted in greater morbidity and mortality than the disease itself.

Sarsaparilla was a welcome alternative, but despite initial excitement, Monardes' sarsaparilla cure fell out of favor. This was probably because of other aspects of the cure, which involved confinement to a warm room for 30 days, followed by 40 days of abstinence from both wine and sexual intercourse.[5]

However, sarsaparilla continued to be used in the treatment of syphilis. During military operations in Portugal in 1812, a British inspector general of hospitals noted that the Portuguese soldiers suffering from syphilis who used sarsaparilla recovered much faster and more completely than their British counterparts, who were treated with mercury.[5]

Sarsaparilla was also used by the Chinese in the treatment of syphilis. Clinical observations in China show that sarsaparilla was effective, according to blood tests, in about 90% of acute cases and 50% of chronic cases.[1,6]

Although sarsaparilla was clearly more beneficial than mercury in the treatment of syphilis, it was mercury that established itself as the standard treatment for more than four and a half centuries. It has been stated that "the use of mercury in the treatment of syphilis may have been the most colossal hoax ever perpetrated" in the history of medicine.[5] Mercury represented a new kind of medicine, one formulated and prepared in a laboratory using the new techniques of chemistry. It helped prepare the way for the future use of drugs rather than herbal medicines.

PHARMACOLOGY

Various steroidal saponins, polyphenols, and flavonoids from *Smilax* species have shown anti-inflammatory and cytotoxic effects against

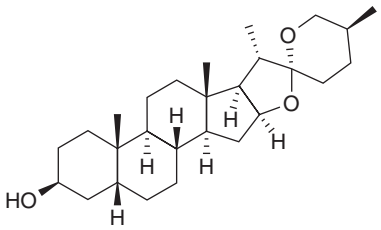


Fig. 111.1 Sarsasapogenin.

several tumor cell lines.[7–10] Yet, sarsaparilla's mechanism of action is largely unknown. Clinically, it has been shown to be effective in the treatment of psoriasis.[1,11,12] This evidence points to a possible effect on the binding of cholesterol and bacterial toxins in the intestines. Sarsaparilla has also demonstrated hepatoprotective effects[13] and an ability to protect against lead-induced oxidative stress in an animal model.[14]

Endotoxin Binding

Evidence seems to support sarsaparilla as an endotoxin binder. Endotoxins are cell wall constituents of bacteria absorbed from the gut. Normally, the liver plays a vital role by filtering these and other gut-derived compounds before they reach the general circulation. If the amount of endotoxin absorbed is excessive or the liver is not functioning adequately, it can become overwhelmed, and endotoxins then spill into the blood.

If endotoxins are allowed to circulate, activation of the alternate complement system occurs. This system plays a critical role in aggravating inflammatory processes, and activation of complement is responsible for much of the inflammation and cell damage seen in many diseases, including gout, arthritis, and psoriasis. Historically, these conditions have been treated with sarsaparilla.

In further support of sarsaparilla's effect as a binder of endotoxin is its historic use in the treatment of fever, because absorbed endotoxins produce fever. Sarsaparilla also exhibits some immune enhancing activity. *Smilax glabra* root contains a heteropolysaccharide that promotes phagocytosis and increased macrophage-derived biological factors including nitric oxide, interleukin-6, tumor necrosis factor-α, and interleukin-1β. This activation of macrophages enhances systemic host immune system function.[1]

CLINICAL APPLICATION

Sarsaparilla's medicinal action appears to be a result of its binding of bacterial endotoxins in the gut, which makes them unabsorbable. This action greatly reduces the stress on the liver and other organs and is probably responsible for sarsaparilla's historic use as a tonic and blood purifier. The ability to bind endotoxins is also probably the reason that sarsaparilla is effective in many cases of psoriasis, gout, and arthritis.

Psoriasis

Individuals with psoriasis have been shown to have high levels of circulating endotoxins. Binding of endotoxin in the gut is associated with clinical improvement in these individuals. In a controlled study of 92 patients, an endotoxin-binding saponin (sarsaponin) from sarsaparilla greatly improved the psoriasis in 62% of the patients and resulted in complete clearance in 18%.[12]

DOSAGE

- Dried root: 1 to 4 g or by decoction three times a day
- Liquid extract (1:1): 2 to 4 mL three times a day
- Solid extract (4:1): 250 to 500 mg three times a day

TOXICOLOGY

Although no adverse effects have been reported for sarsaparilla, it is possible that problems can arise if large doses are used over a long time.

DRUG INTERACTIONS

No drug interactions have been reported for sarsaparilla. However, it has been theorized that sarsaparilla may increase the absorption of certain drugs, notably digitalis and bismuth, thereby potentially increasing the chance of drug toxicity.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wang M, Yang XB, Zhao JW, Lu CJ, Zhu W. Structural characterization and macrophage immunomodulatory activity of a novel polysaccharide from Smilax glabra Roxb. *Carbohydr Polym*. 2017;156:390–402.
2. Shao B, Guo HZ, Cui YJ, et al. Simultaneous determination of six major stilbenes and flavonoids in Smilax China by high performance liquid chromatography. *J Pharm Biomed Anal*. 2007;44(3):737–742. Epub 2007 Mar 14.
3. Huang HL, Zhang JQ, Chena GT, et al. Simultaneous determination of oxyresveratrol and resveratrol in rat bile and urine by HPLC after oral administration of Smilax China extract. *Nat Prod Commun*. 2009;4(6):825–830.
4. Felter HW. *The Eclectic Materia Medica, Pharmacology and Therapeutics.* Portland, OR: Eclectic Medical Publications
5. Van der Zee B, Griggs B. *Green Pharmacy: a History of Herbal Medicine.* London: Jill Norman & Hobhouse
6. Bensky D, Gamble A. *Chinese Herbal Medicine: Materia Medica.* Seattle, WA: Eastland Press.
7. Shao B, Guo H, Cui Y, et al. Steroidal saponins from Smilax China and their anti-inflammatory activities. *Phytochemistry*. 2007;68(5):623–630.
8. Wu LS, Wang XJ, Wang H, et al. Cytotoxic polyphenols against breast tumor cell in smilax China L. *J Ethnopharmacol*. 2010;130(3):460–464.
9. Sa F, Gao JL, Fung KP, et al. Anti-proliferative and pro-apoptotic effect of Smilax glabra Roxb. extract on hepatoma cell lines. *Chem Biol Interact*. 2008;171(1):1–14.
10. Li YL, Gan GP, Zhang HZ, et al. A flavonoid glycoside isolated from Smilax China L. rhizome in vitro anticancer effects on human cancer cell lines. *J Ethnopharmacol*. 2007;113(1):115–124.
11. Duke JA. *CRC Handbook of Medicinal Herbs.* Boca Raton, FL: CRC Press.
12. Thurmon FM. The treatment of psoriasis with sarsaparilla compound. *N Engl J Med*. 1942;337:128–133.
13. Rafatullah S, Mossa JS, Ageel AM, et al. Hepatoprotective and safety evaluation studies on sarsaparilla. *Int J Pharmacognosy*. 1991;29:296–301.
14. Xia D, Yu X, Liao S, et al. Protective effect of Smilax glabra extract against lead-induced oxidative stress in rats. *J Ethnopharmacol*. 2010;130(2):414–420.

Serenoa repens (Saw Palmetto)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

Serenoa repens (family: *Arecaceae*)
Common names: saw palmetto, palmetto scrub, Sabal serrulata

GENERAL DESCRIPTION

Serenoa repens is a small palm tree native to the West Indies and southeastern United States, particularly Florida (Fig. 112.1). The deep red-brown to black fruits, the medicinal parts, are wrinkled and oblong, measuring 0.5 to 1 in. in length with a diameter of 0.5 in.[1] They are technically considered drupes (Fig. 112.2).

CHEMICAL COMPOSITION

Saw palmetto drupes contain about 1.5% of a fruity-smelling oil containing saturated and unsaturated fatty acids and sterols.[1] About 63% of this oil is composed of free fatty acids, including capric, caprylic, caproic, lauric, palmitic, and oleic acids. The remaining portion is composed of ethyl esters of these fatty acids and sterols, including beta-sitosterol and its glucoside. The lipid-soluble compounds are thought to be the major pharmacological components. Other components of the fruit are proanthocyanidins, carotenes, lipase, tannins, and sugars.

The purified fat-soluble extract, the most researched medicinal product, contains 85% to 95% fatty acids and sterols. It is made up predominantly of a complex mixture of saturated and unsaturated free fatty acids, their methyl- and ethyl-esters (approximately 7%), long-chain alcohols in free and esterified forms, and various free and esterified sterol derivatives.

HISTORY AND FOLK USE

The American Indians, and later Eclectic and naturopathic physicians, used saw palmetto drupes in the treatment of genitourinary tract disturbances and as a tonic to support the body nutritionally.[2,3] This substance was strongly recommended as a remedy for symptoms of benign prostatic hyperplasia as early as 1919.[4] It was used in men to increase the function of the testicles and relieve irritation in mucous membranes, particularly those of the genitourinary tract and prostate. Saw palmetto has been used in women with disorders of the mammary glands; long-term use was reputed to cause the breasts to enlarge slowly.[2] Many herbalists have regarded saw palmetto as an aphrodisiac.[1]

Fig. 112.1 Serenoa repens.

PHARMACOLOGY

A standardized liposterolic (fat-soluble), saw palmetto drupe extract has demonstrated numerous pharmacological effects relating to its primary clinical application in the treatment of benign prostatic hyperplasia (BPH), a common disorder of the prostate gland (Fig. 112.3). Saw palmetto extract affects BPH through multiple mechanisms, including inhibition of the intraprostatic conversion of testosterone to DHT and of its intracellular binding and transport, antiestrogenic, and receptor site–binding effects.[5–7]

Estrogen may contribute to BPH because it inhibits the hydroxylation and subsequent elimination of DHT among other mechanisms. *Serenoa* inhibits the activity of estrogen in the prostate. In one double-blind study, 35 men with BPH were randomized to saw palmetto extract 160 mg twice daily or placebo for 90 days.[5] Men receiving the saw palmetto extract had significantly lower cytosol and receptor values for estrogen and progesterone than the placebo group. The results imply that at least part of the efficacy of the saw palmetto extract is because of its antiestrogenic effect.

There was no change in the number of cytosol androgen receptors, but the number of nuclear androgen receptors was significantly lower in the saw palmetto group (60% of the placebo group tested positive for the nuclear receptor compared with 10% of the saw palmetto group). These results indicate that the saw palmetto extract probably competitively blocks the translocation of the cytosol androgen receptor to the nucleus.

Preliminary analysis of the extract demonstrates that separate fractions are responsible for the antiandrogenic and antiestrogenic effects. Researchers in this study said, "It cannot be excluded, however, that the primary effect is antiestrogenic and that the inactivation of androgen receptors and progesterone receptors and of the 5-α-reductase activity is secondary to the estrogen receptor blockade."[7]

Serenoa standardized extracts do not affect systemic levels of androgens, follicle-stimulating hormone, or luteinizing hormone in men with BPH.[8] This may help explain the relatively low incidence of adverse effects of this substance in clinical trials. These findings do not, however, rule out localized effects of saw palmetto on androgen or estrogen effects in other tissues of the body.

Various locally produced growth factors also play a role in the pathogenesis of BPH, and liposterolic extracts of *Serenoa* block the ability of one of them, basic fibroblast growth factor, to induce prostatic hyperplasia in vitro.[9] High prolactin levels may also stimulate prostate hyperplasia; *Serenoa* extracts interfere with this process in rats, unlike finasteride.[10]

Saw palmetto extracts exert antispasmodic effects on smooth muscle. Rat smooth muscle was originally shown to be inhibited by two *Serenoa* extracts owing to inhibition of calcium ion influx.[11] A later study found that *Serenoa* extract but not pumpkin seed extract, stinging nettle root extract, or β-sitosterol consistently inhibited human α_1-adrenergic receptors in vitro.[12] Whether this effect is relevant clinically is still unknown.

The standardized extract has demonstrated anti-inflammatory effects, and the polysaccharide components have been shown to have immunostimulatory effects.[13–16] *Serenoa* extract and myristoleic acid–induced apoptosis and necrosis in an androgen-sensitive human prostate cancer cell line in vitro.[17]

Fig. 112.2 Dried fruit (drupes). (From *Serenoa repens*.)

CLINICAL APPLICATIONS

The primary clinical application of saw palmetto drupes (specifically the fat-soluble extract) is in the treatment of men with BPH. There

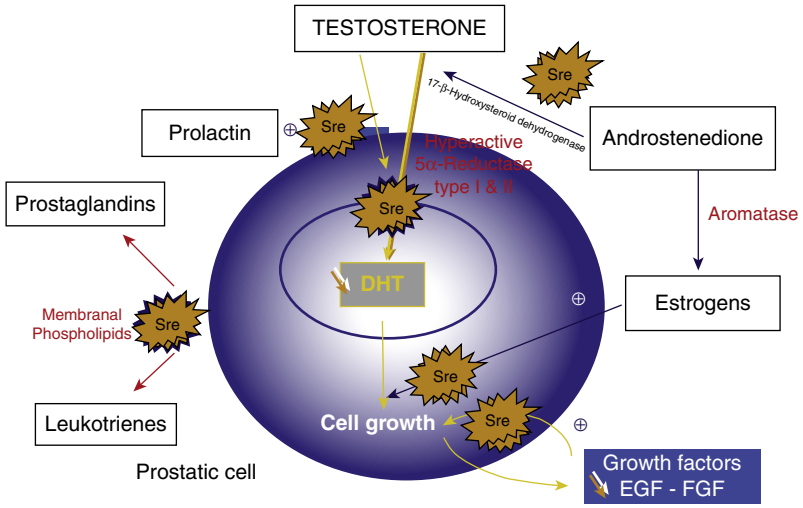


Fig. 112.3 Some of the *Serenoa repens* (Sre) extract targets at the level of the prostate cell. *DHT,* dihydrotestosterone; *EGF,* epidermal growth factor; *FGF,* fibroblast growth factor. (From Habib FK. *Serenoa repens*: the scientific basis for the treatment of benign prostatic hyperplasia. *European Urology Supplements.* 2009;8[13]:887–893.)

is also preliminary evidence that saw palmetto may also help counter male-pattern baldness and chronic prostatitis. On the basis of its pharmacology, this extract may also be of benefit in conditions of androgen excess in women, such as hirsutism and polycystic ovary disease.

Benign Prostatic Hyperplasia

The major symptoms of BPH (increased urinary frequency, nighttime awakening to empty the bladder, and reduced force and caliber of urination; see Chapter 154), have been shown to be significantly improved by saw palmetto extract in more than a dozen double-blind placebo-controlled clinical trials (summarized in Table 112.1).[18–31] At least four meta-analyses have combined data from these and other trials and concluded that, despite some limitations in trial design, *Serenoa* extracts have been shown to reduce symptoms of BPH and increase urine flow in comparison with placebo without affecting prostate volume.[32–36] One of these analyses also concluded that *Serenoa* extracts are as effective as the 5-α-reductase–inhibiting drug finasteride (Proscar), with significantly fewer adverse effects.[37] In another meta-analysis, it was concluded that saw palmetto extracts were not superior to a placebo for symptoms of BPH but were just as effective as finasteride and tamsulosin.[35] However, this analysis was strongly affected by a single negative trial involving 225 men with moderate to severe BPH randomized to receive *Serenoa* extract 160 mg twice daily or a placebo.[19] After 1 year of treatment, there was no significant difference in the scores on the American Urological Association Symptom Index and the maximal urinary flow rate in men receiving 320 mg per day of the liposterolic *Serenoa* extract or the placebo. Secondary outcome measures, including changes in prostate size, residual urinary volume after voiding, quality of life, laboratory values, and the rate of reported adverse effects also did not differ between the two groups.

TABLE 112.1 Clinical Studies Evaluating the Efficacy of *Serenoa repens* Extract[a] for Benign Prostatic Hyperplasia

Study	Type of Study	No. of Patients	Length of Study	Results
Controlled Trials				
Boeri et al.[55]	Double-blind, silodosin control	189	1 yr	Silodosin + *Serenoa* superior to silodosin
Barry et al.[38]	Double-blind, placebo-controlled	369	72 wk	Equal to placebo
Shi et al.[18]	Double-blind, placebo-controlled	92	12 wk	Significant for flow rate
Hizli and Uygur[42]	Double-blind, tamsulosin control	60	24 wk	Equal to tamsulosin
Engelmann et al.[54]	Double-blind, tamsulosin control	140	60 wk	Equal to tamsulosin
Bent et al.[19]	Double-blind, placebo-controlled	225	1 yr	Equal to placebo
Willetts et al.[20]	Double-blind	91	12 wk	Equal to placebo
Debruyne et al.[39]	Double-blind, tamsulosin control	542	1 yr	Equal to tamsulosin
Glemain et al.[41]	Double-blind, tamsulosin control	329	1 yr	Tamsulosin superior to *Serenoa*
Carraro et al.[37]	Double-blind, finasteride control	1,069	26 wk	Equal to finasteride for symptoms; only finasteride shrank prostate
Boccafoschi and Annoscia[21]	Double-blind, placebo-controlled	22	60 d	Significant difference for volume voided, maximum flow, mean flow, dysuria, nocturia
Emili et al.[22]	Double-blind, placebo-controlled	30	30 d	Significant difference for number of voided, strangury, maximum and mean urine flow, residual urine
Duvia et al.[23]	Controlled	30	30 d	Significant difference for voiding rate vs. *Pygeum africanum*
Tasca et al.[24]	Double-blind, placebo-controlled	30	31–90 d	Significant difference for frequency, urine flow measurement
Cukier et al.[25]	Double-blind, placebo-controlled	168	60–90 d	Significant difference for dysuria, frequency, residual urine
Champault et al.[27]	Double-blind, placebo-controlled	168	60–90 d	Significant difference for objective and subjective parameters
Champault et al.[27]	Double-blind, placebo-controlled	110	28 d	Significant difference for dysuria, nocturia, flow measurement, residual urine
Mattei et al.[28]	Double-blind, placebo-controlled	40	3 mo	Significant difference for dysuria, nocturia, residual urine
Open Long-Term Trials				
Djavan et al.[29]	Open, watchful waiting control	189	2 yr	Significant for symptom relief, reduction in surgical rates compared with control
Pytel et al.[30]	Open	255	2 yr	Significant for symptom relief
Pierre Fabre[31]	Open	154	2 yr	Significant for symptom relief

[a]Extract contained 85% to 95% fatty acids and sterols at a dosage of 320 mg/day.

These results may simply indicate that treatment of moderate to severe BPH is likely unresponsive to *Serenoa* therapy. A large negative clinical trial of *Serenoa* in 369 men with mild to moderate BPH failed to show a benefit for up to 960 mg/day of standardized extracts compared with the placebo.[38]

The single largest (n = 1069) clinical trial of *Serenoa* extract compared 160 mg of *Serenoa* twice daily with finasteride 5 mg daily for 26 weeks.[37] The two agents were equally effective at improving symptoms (by 37%–39% on average) and increasing peak urine flow (by 38%–41% on average). Although finasteride decreased prostate volume and levels of prostate-specific antigen (PSA) significantly, *Serenoa* extract had no such effect. Finasteride caused significantly more sexual side effects than saw palmetto.

In a double-blind comparison trial, 542 men with BPH symptoms were randomly assigned to receive either the α blocker tamsulosin or *Serenoa* extract for 1 year.[39] The two groups showed identical levels of improvement in symptoms. Tamsulosin was more frequently associated with ejaculatory disorders than *Serenoa* extract. In the subset of patients with the most severe BPH in this trial, *Serenoa* extract was actually statistically significantly superior to tamsulosin at relieving symptoms.[40] In a separate double-blind trial involving 329 men with BPH symptoms, tamsulosin combined with placebo was just as effective as tamsulosin combined with *Serenoa* extract at relieving symptoms.[41] Adding *Serenoa* extract to tamsulosin caused no alteration in adverse effects. A 6-month double-blind trial of *Serenoa* extract with 60 men found results very similar to those of the prior two studies, although it also demonstrated an equivalent improvement in maximum flow rate between the groups.[42]

Serenoa may work most effectively in combination with *Urtica urens* root extract. In a long-term study, the efficacy and tolerability of this combination were investigated in a prospective multicenter trial comprising elderly male patients suffering from lower urinary tract symptoms (LUTS) caused by BPH. A total of 257 patients were randomized to treatment with the combination (320 mg of *Serenoa* and 240 mg of *U. urens* extracts per day) or placebo. After a single-blind placebo run-in phase of 2 weeks, the patients received either the study medication or placebo under double-blind conditions over 24 weeks. The double-blind treatment was followed by an open control period of 24 weeks during which all patients were given the *Serenoa* and *U. urens* combination. Outcome measures for treatment efficacy included the assessment of the patients' LUTS by means of a self-rating questionnaire and a quality-of-life index in addition to uroflow and sonographic parameters. Using the International Prostate Symptom Score (I-PSS), patients treated with the *Serenoa* and *U. urens* combination exhibited a substantially higher total score reduction after 24 weeks of double-blind treatment than patients of the placebo group (6 points vs. 4 points; $P = 0.003$), with a tendency in the same direction after 16 weeks. This applied to obstructive and to irritative symptoms and to patients with moderate or severe symptoms at baseline. Patients randomized to placebo showed a marked improvement in LUTS (as measured by the I-PSS) after being switched to the *Serenoa* and *U. urens* combination during the control period.[43]

Chronic Prostatitis

Several clinical trials indicate that *S. repens* may have clinical benefit in chronic prostatitis or pelvic pain. One double-blind, randomized trial compared *S. repens* extract alone with a combination of *S. repens,* selenium, and lycopene in men with nonbacterial chronic pelvic pain.[44] After 8 weeks, the two groups had equivalent improvements in symptoms, whereas only the combination product lowered PSA and levels of white blood cell in urine.

One study used a combination of *S. repens* extract 160 mg, *Urtica dioica* root extract 120 mg, quercetin 100 mg, and curcumin 200 mg once daily with the antibiotic prufloxacin 600 mg once daily in men with bacterial prostatitis.[45] One fourth of the 143 subjects were randomly assigned to take prufloxacin alone. Treatment lasted 14 days. One month after treatment, patients in the combination group were dramatically more likely (88% vs. 27%) to be symptom-free than the antibiotic-only group. Subsequent randomized trials confirm that saw palmetto extract by itself and in combination with selenium, lycopene, bromelain, and methylsulfonylmethane or arbutin and *Lactobacillus sporogenes* enhance the efficacy of antibiotics in patients with chronic prostatitis.[46–48]

Male-Pattern Baldness

A combination of 400 mg *S. repens* extract and 100 mg beta-sitosterol daily was found effective for male-pattern baldness in a pilot double-blind, placebo-controlled trial.[49] The 5-month trial involved 19 men with mild to moderate baldness. Sixty percent (6 of 10) of the men in the treatment group were rated as having improved by blinded observers, whereas only 11% (1 of 9) of men in the placebo group were rated as improved. Thirty-three percent (3 of 9) of the men in the placebo group showed deterioration compared with none in the treatment group. There were no major adverse effects. A comparative trial in 100 men over 2 years found that finasteride 1 mg was superior to *S. repens* extract 320 mg for improving baldness, but both extracts did lead to significant increases in hair growth compared with baseline.[50] An open trial in 50 men found that a topical *S. repens* extract applied for 4 weeks effectively improved hair growth lasting 24 weeks.[51]

Dosage

The dosage for the liposterolic extract of saw palmetto fruit (containing 85%–95% fatty acids and sterols) is 160 mg twice daily or 320 mg daily; the two regimens have been shown to be equally effective.[52] A similar dosage using fluid extracts and tinctures would require extremely large quantities of alcohol if the liposterolic components were the primary active constituents. The Eclectics used comparatively small dosages of saw palmetto in crude extracts effectively for BPH with apparent clinical success. Ellingwood[4] lists a dose as 10 drops to 1 dram of specific *S. repens* (meaning basically a fresh drupe tincture). We are unaware of any published clinical study of a crude extract.

Dosages are as follows:

- Crude drupes: 10 g twice a day
- Liposterolic extract (standardized at 85%–95% fatty acids and sterols): 160 mg twice a day or 320 mg once a day

The dosage of fluid extracts or tinctures is typically 3 to 5 mL two to three times per day, although there is a lack of research on this dose form.

TOXICOLOGY

No significant adverse effects have been reported in clinical trials of the saw palmetto fruit extract or for saw palmetto drupe ingestion; rates of mild adverse effects are no different than those seen with placebo treatment.[53] Mild gastrointestinal upset and erectile dysfunction (in approximately 1% of users in clinical trials)[3] occasionally occur.

DRUG INTERACTIONS

No drug–herb interactions had been reported as of 2019. One clinical trial found no effect of saw palmetto liposterolic extract on CYP3A4 or 2D6 in healthy adults.[56]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Duke JA. *CRC Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1992.
2. Felter HW, Lloyd JU. *King's American Dispensatory (1898)*. Portland, OR: Eclectic Medical Publications (reprint); 1983.
3. Kuts-Cheraux AW. *Naturae Medicina and Naturopathic Dispensatory*. Yellow Springs, OH: Antioch Press; 1953.
4. Ellingwood F. *American Materia Medica, Therapeutics and Pharmacognosy*. Sandy, OR: Eclectic Medical Publications; 1919, reprinted.
5. Carilla E, Briley M, Fauran F, et al. Binding of permixon, a new treatment for prostatic benign hyperplasia, to the cytosolic androgen receptor in the rat prostate. *J Steroid Biochem*. 1984;20:521–523.
6. Sultan C, Terraza A, Devillier C, et al. Inhibition of androgen metabolism and binding by a liposterolic extract of "*Serenoa repens* B" in human foreskin fibroblasts. *J Steroid Biochem*. 1984;20:515–519.
7. Di Silverio F, D'Eramo G, Lubrano C, et al. Evidence that *Serenoa repens* extract displays antiestrogenic activity in prostatic tissue of benign prostatic hypertrophy. *Eur Urol*. 1992;21:309–314.
8. Casarosa C, Cosci di Coscio M, Fratta M. Lack of effects of a liposterolic extract of *Serenoa repens* on plasma levels of testosterone, follicle-stimulating hormone, and luteinizing hormone. *Clin Ther*. 1988;10:585–588.
9. Paubert-Braquet M, Cousse H, Raynaud JP, et al. Effect of the lipidosterolic extract of *Serenoa repens* (Permixon) and its major components on basic fibroblast growth factor-induced proliferation of cultures of human prostate biopsies. *Eur Urol*. 1998;33:340–347.
10. Van Coppenolle F, Le Bourhis X, Carpentier F, et al. Pharmacological effect of the lipidosterolic extract of *Serenoa repens* (Permixon) on rat prostate hyperplasia induced by hyperprolactinemia: comparison with finasteride. *Prostate*. 2000;43:49–58.
11. Gutierrez M, García de Boto J, Cantabrana B, et al. Mechanisms involved in the spasmolytic effect of extracts from Sabal serrulata fruit on smooth muscle. *Gen Pharmacol*. 1996;27:171–176.
12. Goepel M, Hecker U, Krege S, et al. Saw palmetto extracts potently and noncompetitively inhibit human alpha-1-adrenoceptors in vitro. *Prostate*. 1999;38:208–215.
13. Tarayre JP, Delhon A, Lauressergues H, et al. Anti-edematous action of a hexane extract of the stone fruit of *Serenoa repens* Bartr. *Ann Pharm Fr*. 1983;41:559–570.
14. Latil A, Pétrissans MT, Rouquet J, et al. Effects of hexanic extract of *Serenoa repens* (Permixon 160 mg) on inflammation biomarkers in the treatment of lower urinary tract symptoms related to benign prostatic hyperplasia. *Prostate*. 2015;75(16):1857–1867.
15. Morgia G, Cimino S, Favilla V, et al. Effects of *Serenoa repens,* selenium and lycopene (Profluss) on chronic inflammation associated with benign prostatic hyperplasia: results of "FLOG" (Flogosis and Profluss in Prostatic and Genital Disease), a multicentre Italian study. *Int Braz J Urol*. 2013;39(2):214–221.
16. Wagner H, Proksch A. Immunostimulatory drugs of fungi and higher plants. *Econ Med Plant Res*. 1985;1:113–153.
17. Iguchi K, Okumura N, Usui S, et al. Myristoleic acid, a cytotoxic component in the extract from *Serenoa repens,* induces apoptosis and necrosis in human prostatic LNCaP cells. *Prostate*. 2001;47:59–65.
18. Shi R, Xie QW, Gang X, et al. Effect of saw palmetto soft gel capsule on lower urinary tract symptoms associated with benign prostatic hyperplasia: a randomized trial in Shanghai, China. *J Urology*. 2008;179:610–615.
19. Bent S, Kane C, Shinohara K, et al. Saw palmetto for benign prostatic hyperplasia. *N Engl J Med*. 2006;354(6):557–566.
20. Willetts KE, Clements MS, Champion S, et al. *Serenoa repens* extract for benign prostatic hyperplasia: a randomized clinical trial. *BJU Int*. 2003;92:267–270.
21. Boccafoschi C, Annoscia S. Comparison of *Serenoa repens* extract with placebo by controlled clinical trial in patients with prostatic adenomatosis. *Urologia*. 1983;50:1257–1268.
22. Emili E, Lo Cigno M, Petrone U. Clinical trial of a new drug for treating hypertrophy of the prostate (Permixon). *Urologia*. 1983;50:1042–1048.
23. Duvia R, Radice GP, Galdini R. Advances in the phytotherapy of prostatic hypertrophy. *Med Praxis*. 1983;4:143–148.
24. Tasca A, Barulli M, Cavazzana A, et al. Treatment of obstructive symptomatology caused by prostatic adenoma with an extract of *Serenoa repens*: double-blind clinical study vs. placebo. *Minerva Urol Nefrol*. 1985;37:87–91.
25. Cukier J, Ducassou J, Le Guillou M, et al. Permixon versus placebo. *C R Ther Pharmacol Clin*. 1985;4:15–21.
26. Champault G, Patel JC, Bonnard AM. A double-blind trial of an extract of the plant *Serenoa repens* in benign prostatic hyperplasia. *Br J Clin Pharmacol*. 1984;18:461–462.
27. Champault G, Bonnard AM, Cauquil J, et al. Medical treatment of prostatic adenoma. Controlled trial. PA 109 vs placebo in 110 patients. *Ann Urol (Paris)*. 1984;18:407–410.
28. Mattei FM, Capone M, Acconcia A. *Serenoa repens* extract in the medical treatment of benign prostatic hypertrophy. *Urologia*. 1988;55:547–552.
29. Djavan B, Fong YK, Chaudry A, et al. Progression delay in men with mild symptoms of bladder outlet obstruction: a comparative study of phytotherapy and watchful waiting. *World J Urol*. 2005;23:253–256.
30. Pytel YA, Vinarov A, Lopatkin N, et al. Long-term clinical and biologic effects of the lipidosterolic extract of *Serenoa repens* in patients with symptomatic benign prostatic hyperplasia. *Adv Ther*. 2002;19(6):297–306.
31. Pierre Fabre. *Study 95GP401*. 2002. (unpublished study).
32. Boyle P, Robertson C, Lowe F, et al. Meta-analysis of clinical trials of permixon in the treatment of symptomatic benign prostatic hyperplasia. *Urology*. 2000;55:533–539.
33. Wilt TJ, Ishani A, Stark G, et al. Saw palmetto extracts for treatment of benign prostatic hyperplasia: a systematic review. *JAMA*. 1998;280:1605–1609.
34. Boyle P, Robertson C, Lowe F, et al. Updated meta-analysis of clinical trials of *Serenoa repens* extract in the treatment of symptomatic benign prostatic hyperplasia. *BJU Int*. 2004;93(6):751–756.
35. Tacklind J, MacDonald R, Rutks I, et al. *Serenoa repens* for benign prostatic hyperplasia. *Cochrane Database Syst Rev*. 2009;(2):CD001423.
36. Vela-Navarrete R, Alcaraz A, Rodríguez-Antolín A, et al. Efficacy and safety of a hexanic extract of *Serenoa repens* (Permixon) for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia (LUTS/BPH): systematic review and meta-analysis of randomized controlled trials and observational studies. *BJU Int*. 2018. https://doi.org/10.1111/bju.14362. [Epub ahead of print].
37. Carraro J-C, Raynaud J-P, Koch G, et al. Comparison of phytotherapy (Permixon (R)) with finasteride in the treatment of benign prostate hyperplasia: a randomized international study of 1,098 patients. *Prostate*. 1996;29(4):231–240.
38. Barry MJ, Meleth S, Lee JY, et al. Effect of increasing doses of saw palmetto extract on lower urinary tract symptoms: a randomized trial. *JAMA*. 2011;306(12):1344–1351.
39. Debruyne F, Koch G, Boyle P, et al. Comparison of a phytotherapeutic agent (Permixon) with an alpha-blocker (Tamsulosin) in the treatment of benign prostatic hyperplasia: a 1-year randomized international study. *Eur Urol*. 2002;41:497–506.
40. Debruyne F, Boyle P, Calais Da Silva F, et al. Evaluation of the clinical benefit of permixon an tamsulosin in severe BPH patients–PERMAL study subset analysis. *Eur Urol*. 2004;45:773–780.
41. Glemain P, Coulange C, Billebaud T, et al. Tamsulosin with or without *Serenoa repens* in benign prostatic hyperplasia: the OCOS trial. *Prog Urol*. 2002;12:395–403.
42. Hizli F, Uygur C. A prospective study of the efficacy of *Serenoa repens,* tamsulosin, and Serenoa repens plus tamsulosin treatment for patients with benign prostate hyperplasia. *Int Urol Nephrol*. 2007;39:879–886.
43. Lopatkin N, Sivkov A, Walther C, et al. Long-term efficacy and safety of a combination of sabal and urtica extract for lower urinary tract symptoms: a placebo-controlled, double-blind, multicenter trial. *World J Urol*. 2005;23:139–141.
44. Morgia G, Mucciardi G, Galì A, et al. Treatment of chronic prostatitis/chronic pelvic pain syndrome category IIIA with *Serenoa repens* plus selenium and lycopene (Profluss) versus *S. repens* alone: an Italian randomized multicenter-controlled study. *Urol Int*. 2010;84(4):400–406.
45. Cai T, Mazzoli S, Bechi A, et al. *Serenoa repens* associated with Urtica dioica (ProstaMEV) and curcumin and quercitin (FlogMEV) extracts are able to improve the efficacy of prulifloxacin in bacterial prostatitis patients: results from a prospective randomised study. *Int J Antimicr Agents*. 2009;33:549–533.

46. Stamatiou K, Pierris N. *Serenoa repens* extract additionally to quinolones in the treatment of chronic bacterial prostatitis. The preliminary results of a long term observational study. *Arch Ital Urol Androl*. 2013;85(4):190–196.
47. Cai T, Tiscione D, Gallelli L, et al. *Serenoa repens* associated with selenium and lycopene extract and bromelain and methylsulfonylmethane extract are able to improve the efficacy of levofloxacin in chronic bacterial prostatitis patients. *Arch Ital Urol Androl*. 2016;88(3):177–182.
48. Busetto GM, Giovannone R, Ferro M, et al. Chronic bacterial prostatitis: efficacy of short-lasting antibiotic therapy with prulifloxacin (Unidrox) in association with saw palmetto extract, lactobacillus sporogens and arbutin (Lactorepens). *BMC Urol*. 2014;14:53.
49. Prager N, Bickett K, French N, et al. A randomized, double-blind, placebo-controlled trial to determine in the effectiveness of botanically derived inhibitors of 5-alpha-reductase in the treatment of androgenetic alopecia. *J Altern Complement Med*. 2002;8:143–152.
50. Rossi A, Mari E, Scarno M, et al. Comparative effectiveness of finasteride vs *Serenoa repens* in male androgenetic alopecia: a two-year study. *Int J Immunopathol Pharmacol*. 2012;25(4):1167–1173.
51. Wessagowit V, Tangjaturonrusamee C, Kootiratrakarn T, et al. Treatment of male androgenetic alopecia with topical products containing *Serenoa repens* extract. *Australas J Dermatol*. 2016;57(3):e76–e82.
52. Braeckman J, Bruhwyler J, Vandekerckhove K, et al. Efficacy and safety of the extract of *Serenoa repens* in the treatment of benign prostatic hyperplasia: therapeutic equivalence between twice and once daily dosage forms. *Phytother Res*. 1997;11:558–563.
53. Agbabiaka TB, Pittler MH, Wilder B, et al. *Serenoa repens* (saw palmetto): a systematic review of adverse events. *Drug Saf*. 2009;32(8):637–647.
54. Engelmann U, Walther C, Bondarenko B, et al. Efficacy and safety of a combination of sabal and urtica extract in lower urinary tract symptoms. A randomized, double-blind study versus tamsulosin. *Arzneim Forsch*. 2006;56(3):222–229.
55. Boeri L, Capogrosso P, Ventimiglia E, et al. Clinically meaningful improvements in LUTS/BPH severity in men treated with silodosin plus hexanic extract of *Serenoa repens* or silodosin alone. *Sci Rep*. 2017;7(1):15179.
56. Markowitz JS, Donovan JL, DeVane CL, et al. Multiple doses of saw palmetto (Serenoa repens) did not alter cytochrome P450 2D6 and 3A4 activity in normal volunteers. *Clin Pharmacol Ther*. 2003;74:536–549.

Silybum marianum (Milk Thistle)

Michael T. Murray, ND

OUTLINE

Silybum marianum (family: *Compositae*)
Synonym: *Carduus marianus*
Common names: milk thistle, marian thistle, St. Mary's thistle

GENERAL DESCRIPTION

Silybum marianum is a stout annual or biennial plant found in dry, rocky soils in southern and western Europe and some parts of the United States. The branched stem grows 1 to 3 ft high and bears alternate dark green, shiny leaves with spiny, scalloped edges that are markedly streaked with white along the veins (Fig. 113.1). The solitary flower heads are reddish purple with bracts ending in sharp spines. The flowering season is from June to August. The seeds, fruit, and leaves are used for medicinal purposes.

CHEMICAL COMPOSITION

S. marianum contains *silymarin,* a mixture of flavanolignans, consisting chiefly of silybin, silydianin, and silychristine. The concentration of silymarin is highest in the fruit, but it is also found in the seeds and leaves. Other flavanolignans contained in *Silybum* include silandrin, silyhermin, silymonin, and neosilyhermin. Silybin is the silymarin component with the greatest degree of biological activity.[1]

HISTORY AND FOLK USE

Perhaps the most widespread folk use of this plant has been in assisting the nursing mother in the production of milk. It was also used in Germany for curing jaundice and biliary derangements. It is interesting to note that the discovery of the liver-protecting flavanolignans in *S. marianum* was the result not of systemic pharmacological screening but rather of investigation of *Silybum*'s empiric effects in liver disorders.

PHARMACOLOGY

Currently, *S. marianum* extracts (usually standardized to contain 70% silymarin) are widely used in supporting liver health and for hepatic disorders. Silymarin is one of the most potent liver-protecting substances known.[1,2] *S. marianum* extracts exert beneficial properties in a wide variety of other disorders, such as renal protection, hypolipidemic and antiatherosclerosis activities, cardiovascular protection, and prevention of insulin resistance, especially in cirrhotic patients, cancer, and Alzheimer's prevention.[3]

Hepatoprotection Effects

Free-Radical Scavenging

Silybum's ability to prevent liver destruction and enhance liver function is due largely to silymarin's inhibition of the factors responsible for hepatic damage—free radicals and leukotrienes—coupled with its ability to stimulate liver protein synthesis (Fig. 113.2). *Silybum* components prevent free-radical damage by acting as antioxidants.[1–3] Silymarin is many times more potent in antioxidant activity than vitamin E.

Effects on Hepatic Glutathione

Silymarin prevents the depletion of glutathione (GSH) induced by alcohol and other liver toxins. Even in normal people, silymarin has been shown to raise the basal GSH level in the liver by 35%.

Protection from Liver-Damaging Chemicals and Drugs

The protective effect of *Silybum* against liver damage has been demonstrated in several experimental and clinical studies. Experimental liver damage in animals can be produced by such diverse toxic chemicals as carbon tetrachloride, galactosamine, ethanol, and praseodymium nitrate. Silymarin has been shown to protect the liver from all these toxins.

Perhaps the most impressive of silymarin's protective effects is against the severe poisoning by *Amanita phalloides* (the "death cap" or toadstool mushroom), an effect that has long been recognized in folk medicine. Ingestion of *A. phalloides* or its toxins causes severe poisoning and, in approximately 30% of victims, death.

Among the experimental models for measuring protection against liver damage, those based on amanitin or phalloidin toxicity are the most important because these two peptides from *A. phalloides* are the most powerful liver-damaging substances known. Silymarin has demonstrated impressive results in these experimental models. When silymarin was administered before amanita toxin poisoning, it was 100% effective in preventing toxicity.[4] Even if given 10 minutes after the amanita toxin, it completely counteracted the toxic effects.

In two cases reported in the literature, silymarin prevented death and greatly reduced the amount of liver damage up to 24 hours after ingestion of *A. phalloides*.[5] This study reported on a husband and wife who ate toxic mushrooms and experienced gastrointestinal symptoms 18 hours later. Despite initial conventional treatment with gastric emptying, intravenous fluids, activated charcoal, and a duodenal tube, both patients' laboratory parameters showed deteriorating liver and renal function. Mild hepatic encephalopathy developed in one of the patients. Treatment with intravenous silybinin at a dose of 20 mg/kg body weight, penicillin, and glucose for 3 days resulted in a reversal of both the organ failures and encephalopathy.

Silymarin may also be of great value as an adjunct in patients receiving long-term drug therapy. A very interesting study found that silymarin, in an unusually high dose of 800 mg/day, given to psychiatric patients receiving phenothiazines or butyrophenones resulted in significant protection of the liver as measured by malondialdehyde serum liver enzyme levels.[6] The silymarin did not interfere with the efficacy of the antidepressants.

Fig. 113.1 *Silybum marianum*. (From iStock.)

Stimulation of Hepatic Protein Synthesis

Perhaps the most interesting effect of *Silybum* components on the liver is their ability to stimulate protein synthesis. This results in an increase in the production of new liver cells to replace the damaged old ones. Sonnenbichler and Zetl[7] have suggested that "silybinin imitates in some way a physiological regulator in animal cells, so that the structure fits into a specific binding site on the polymerase and in such a way causes the observed effects on rRNA synthesis making the drug from *Silybum marianum* indeed interesting for liver therapy." Interestingly, silybinin does not have a stimulatory effect on malignant hepatic tissue.[8]

Anti-Inflammatory Effects

Leukotrienes, key chemical mediators of inflammation produced by the transfer of oxygen to polyunsaturated fatty acids (a reaction catalyzed

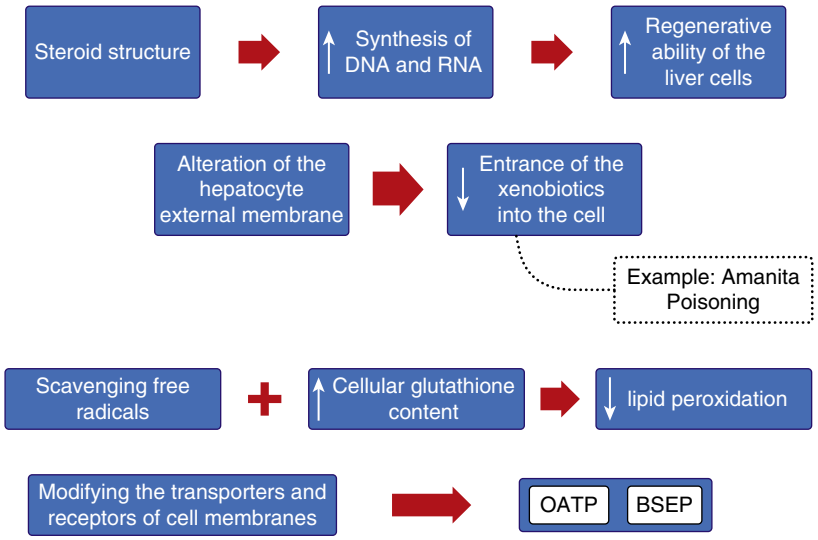


Fig. 113.2 Different mechanisms of action of silymarin. Mechanisms include increasing the regenerative ability of the liver cells by enhancing the synthesis of DNA and RNA, as silymarin has a steroid structure; altering the structure of the hepatocyte external membrane, which prevents entrance of the xenobiotics into the cell (poisoning with *Amanita* mushroom is a noteworthy example of such mechanism); and scavenging free radicals and increasing the cellular content of glutathione, which leads to the inhibition of lipid peroxidation. Another mechanism of action of silymarin is modifying the transporters and receptors of cell membranes such as ABC transporters (P-gp), organic anion uptake transporter peptides (OATPs), bile salt export pump, and tumor necrosis factor-α–dependent transporters. (From Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. "Silymarin," a promising pharmacological agent for treatment of diseases. *Iran J Basic Med Sci.* 2011;14[4]:308–317. PubMed PMID: 23492971.)

by the enzyme lipoxygenase), can also damage the liver. Silymarin has been shown to be a potent inhibitor of this enzyme, thereby inhibiting the formation of damaging leukotrienes.

Silymarin has also been demonstrated to inhibit prostaglandin synthesis during inflammation. Free-radical damage to membrane structures due to organic disease or intoxication results in an increased release, through lipolysis, of fatty acids. This leads, among other things, to greater prostaglandin and leukotriene synthesis. Silymarin counteracts this deleterious process by suppressing the pathological decomposition of membrane lipids and inhibiting prostaglandin formation. Leukotrienes and inflammatory prostaglandins are also involved in damage of the liver by toxins, so their neutralization by silybin is another mechanism for its protection of the liver.

Anticancer Effects

Silymarin has shown significant anticancer effects in both in vivo and in vitro cancer models—including skin, breast, lung, colon, bladder, prostate, and kidney carcinomas—as well as adjunctive actions when combined with standard cancer therapies.[9] Some of the effects noted include that it:

- Prevents the expression of genes and enzymes pivotal in cancer development
- Modulates imbalance between cell survival and apoptosis through interference with the expression of cell-cycle regulators and proteins involved in apoptosis
- Exerts significant antimetastatic effects
- Has a synergistic effect when combined with conventional chemotherapy agents, including growth inhibition, reversal of chemoresistance, apoptosis induction, and reduced chemotherapy side effects in a variety of models

These preliminary studies suggest a clinical application in cancer patients as an adjunct to established therapies to prevent or reduce chemotherapy as well as radiotherapy-induced toxicity.[6] Considering the significant problem of serious nephrotoxicity from cisplatin and other chemotherapeutic agents, silybinin may be of great value as an adjunct in the treatment of cancer.

Other Pharmacological Actions

Silymarin shows antiviral effects against hepatitis C virus (HCV) cell culture infection, including inhibition of virus entry, RNA and protein expression, and infectious virus production.[10]

In animal studies, silymarin has been shown to increase the proliferation of lymphocytes as well as levels of interferon-gamma, interleukin (IL)-4, and IL-10 cytokines in a dose-dependent manner.[11]

Silymarin is a strong inhibitor of cyclic adenosine monophosphate (cAMP) phosphodiesterase, being 13 to 50 times more active than theophylline and 1 to 3 times more active than papaverine.[11] Silymarin has also been shown to prevent the toxic effects of a variety of compounds, such as hemolysis induced by phenylhydrazine, damage from x-irradiation, and brain edema induced by triethyltinsulfate. Presumably these effects are related to silymarin's significant membrane-stabilizing and antioxidant actions. Its action in increasing the osmotic resistance of red blood cells is also quite significant.

S. marianum extracts are particularly indicated for metabolic syndrome because they have been found to exhibit antioxidant, lipid-lowering, antihypertensive, antidiabetic, antiatherosclerotic, antiobesity, and hepatoprotective effects.[12]

Silymarin exerts significant immunomodulatory effects. As an immunomodulator agent, silymarin inhibits T-lymphocyte function at low doses, whereas it stimulates inflammatory processes at high doses. Studies have shown that silymarin has attenuated autoimmune, allergic, preeclampsia, cancer, and immune-mediated liver diseases and also has suppressed oxidative and nitrosative immunotoxicity. Silymarin also has indicated dual effects on proliferation and apoptosis of different cells.[13]

In patients with hemochromatosis, silybin administration at meals was shown to reduce iron absorption.[14]

CLINICAL APPLICATIONS

Silymarin's primary use is as an aid to the liver, although additional clinical applications are regularly being discovered. This substance can be used to support detoxification reactions or in the treatment of more severe liver disease. In numerous clinical studies, silymarin has been shown to have positive effects in treating several types of liver disease, including the following[2]:

- Cirrhosis
- Chronic hepatitis
- Chemically induced liver damage
- Alcohol-induced fatty liver
- Nonalcoholic hepatosteatosis
- Subclinical cholestasis of pregnancy
- Cholangitis and pericholangitis

The therapeutic effect of silymarin in these disorders has been confirmed by histological, clinical, and laboratory data. Silymarin may also be useful in improving the solubility of the bile in the treatment of gallstones as well as in psoriasis and as a galactagogue.

Alcohol- and Chemically Induced Liver Damage

In one of the first extensive double-blind clinical trials investigating silymarin's therapeutic effect in liver disorders, the substance demonstrated impressive results in 129 patients with toxic metabolic liver damage, fatty degeneration of the liver of various origins, or chronic hepatitis who were compared with a control group of 56 patients. The results might have been even more impressive if the study had lasted longer than 35 days.[15]

A follow-up study of patients with liver damage due to alcohol, diabetes, viruses, or toxic exposure yielded even more striking results. Patients were monitored for 7 weeks. Not only were clinical findings markedly improved in the silymarin-treated groups, but laboratory and liver biopsy data improved as well. Highly significant results were obtained in bromsulphalein retention as well as in measurements of alanine transaminase (ALT), iron, and cholesterol levels. Biopsy showed remarkable tissue-restorative effects. Upon completion of silymarin therapy, restitution of normal cell structure was found even in severely damaged livers. These effects on the tissue level correlated well with improvements in blood chemistry values.[16]

Another study highlighted the benefit of silymarin in individuals exposed to toxic chemicals. In this study, abnormal results of liver function tests (elevated levels of aspartate aminotransferase [AST] and ALT activity) and/or abnormal hematological values (low platelet counts, increased white blood cell [WBC] counts, and a relative increase of lymphocytes compared with other WBCs) were observed in 49 of 200 workers exposed to toxic toluene and/or xylene vapors for 5 to 20 years.[17] Thirty of the affected workers were treated with silymarin, and the remaining 19 were left without treatment. Under the influence of silymarin, liver function parameters and platelet counts significantly improved. The WBC counts also showed a tendency toward improvement.

Pooled data from case record studies involving 452 patients with *A. phalloides* poisoning show a highly significant difference in mortality in favor of silymarin therapy. The mortality rate for silymarin therapy is 9.8%, compared with 18.3% for standard treatment.[18]

Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatosis

Silymarin is also helpful in nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatosis (NASH). In a randomized, double-blind, placebo-controlled trial of consecutive adults with biopsy-proven NASH and an NAFLD activity score (NAS) of 4 or more, patients were randomly assigned to groups given silymarin (700 mg) or placebo ($n = 50$ patients) three times daily for 48 weeks. After this 48-week period, liver biopsies were repeated. Although there was no effect on the primary efficacy outcome of a decrease of 30% or more in NAS, the findings from 48-week liver biopsies showed that a significantly higher proportion of patients in the silymarin group had reductions in fibrosis based on histology, and based on liver stiffness measurements, than did the placebo group. The silymarin group also had significant reductions in mean ratio of aspartate aminotransferase to platelet index, Fibrosis-4 score, and NAFLD fibrosis score; these changes were not observed in the placebo group.[19]

Cirrhosis

As previously described, silymarin is quite effective in treating alcohol-related liver disease. There is a tremendous range in the severity of alcohol-related liver disease, from relatively mild to serious damage, such as cirrhosis. Even in this severe state, silymarin has shown benefit. Perhaps the most significant benefit is in extending the life spans of patients with these disorders.

In one study, 87 patients with cirrhosis (46 with alcoholic-related cirrhosis) received silymarin, whereas 83 patients (45 with alcoholic cirrhosis) received a placebo.[20] The mean observation period was 41 months. In the treatment group, there were 24 deaths, 18 related to liver disease; in the control group, there were 37 deaths, 31 related to liver disease. The 4-year survival rate was 58% in the treatment group compared with 39% in the controls.

Analysis of the results of five trials involving a total of 602 patients with liver cirrhosis demonstrated that although silymarin produced a statistically insignificant reduction of total mortality by 4.2% in comparison with placebo, it did lead to a statistically significant reduction in liver-related mortality of 7%.[21] Silymarin can also improve immune function in patients with cirrhosis.[2]

Viral Hepatitis

Silymarin is useful in helping reverse virally induced liver damage. It is effective in both acute and chronic viral hepatitis. In one study of acute viral hepatitis, 29 patients treated with silymarin showed a definite therapeutic influence on the characteristic increased serum levels of bilirubin and liver enzymes compared with a placebo group.[22] The laboratory parameters had regressed more in the silymarin group than in the placebo group by the fifth day of treatment. The number of patients attaining normal liver values after 3 weeks of treatment was significantly higher in the silymarin group than in the placebo group.

In a double-blind study of various causes of acute hepatitis, patients were given either a standard recommended dose of 140 mg of silymarin or a placebo three times daily for 4 weeks. Patients randomized to the silymarin group had a quicker resolution of symptoms related to biliary retention: dark urine, jaundice, and scleral icterus. There was a reduction in indirect bilirubin among those assigned to silymarin, but other variables, including direct bilirubin, ALT, and AST, were not significantly reduced.[23]

In a study of chronic viral hepatitis, silymarin was shown to result in dramatic improvement. Used at a high dose (420 mg) for periods of 3 to 12 months, silymarin resulted in a reversal of liver cell damage (as noted on biopsy), a rise in protein level in the blood, and a lowering of liver enzyme values. Common symptoms of hepatitis (e.g., abdominal discomfort, decreased appetite, and fatigue) were all improved.[24]

Silymarin has been studied in hepatitis C, with little evidence of efficacy. In a meta-analysis of five double-blind, placebo-controlled trials, 389 patients were randomly treated with silymarin or placebo. Silymarin was well tolerated in chronic HCV-infected patients, but there was no evidence of positive effects for oral silymarin, although intravenous silybin did show some benefits.[25]

Gallstones

Silymarin may help prevent or treat gallstones through its ability to increase the solubility of the bile. In one study, the composition of the bile was assayed in 19 patients with a history of gallstones (4 patients) or removal of the gallbladder due to gallstones (15) before and after silymarin (420 mg/day for 30 days) or placebo. Silymarin treatment led to a significant reduction in the biliary cholesterol concentration and bile saturation index.[26]

Psoriasis

Correction of abnormal liver function is indicated in the treatment of psoriasis. Silymarin has been reported to be of value in the treatment of psoriasis; this effect may be due to its ability to inhibit the synthesis of leukotrienes and improve liver function.[27]

The connection between the liver and psoriasis relates to one of the liver's basic tasks, filtering of the blood. Psoriasis has been shown to be linked to high levels of circulating endotoxins, such as those found in the cell walls of gut bacteria. If the liver is overwhelmed by an increased number of endotoxins or chemical toxins or if the liver's functional ability to filter and detoxify is decreased, the psoriasis is aggravated. Another factor in psoriasis is excessive production of leukotrienes. Silymarin has been shown to reduce leukotriene formation by inhibiting lipoxygenase. Therefore silymarin would inhibit one of the causes of the excessive cellular replication.

Silymarin has other effects of value in patients with psoriasis. Most of them revolve around correcting the abnormal ratio of cAMP to cyclic guanosine monophosphate (cGMP) observed in the skin of patients with psoriasis. The ratio of these two cellular control agents governs cellular replication. In psoriasis, cGMP levels are high relative to cAMP levels. Silymarin works to lower cGMP levels and raise cAMP levels.

Silymarin as a Galactagogue

A micronized form of silymarin has been shown to exert clear galactagogue effects. In a double-blind study in 50 healthy lactating women, micronized silymarin (420 mg/day) increased daily milk production by 86%, compared with 32% for the placebo. Milk quality was unaffected. The galactagogue effect may be the result of increased circulating prolactin levels.[28]

Type 2 Diabetes and Metabolic Syndrome

Given the liver's involvement in type 2 diabetes (T2D) and metabolic syndrome, silymarin is almost assuredly of benefit in these conditions. Several clinical trials have been conducted. In the most recent, 40 patients with T2D were randomly assigned to the silymarin (140 mg three times daily) or placebo groups for 45 days. Silymarin supplementation led to a significant reduction in fasting blood sugar (–11), serum insulin (–14%), the homeostatic model assessment for insulin resistance (–26%), serum triglycerides (–24%), and the ratio of triglycerides to high-density lipoprotein cholesterol (–28%) compared with the placebo. There was a significant increase in high-density lipoprotein cholesterol levels and the quantitative insulin sensitivity check index in the silymarin group compared with the placebo group, by 6.88% and 5.64% respectively. Total cholesterol and low-density lipoprotein cholesterol concentrations significantly decreased in the silymarin group

compared with the baseline, by 7.93% and 7.15%, respectively.[29] In the same study, silymarin supplementation also improved some antioxidant indices (SOD, GPX, and TAC) and decreased high-sensitive C-reactive protein (hs-CRP) levels.[30]

Silymarin may also help reduce the complications of diabetes, including nephropathy, neuropathy, healing delays, oxidative stress, hepatotoxicity, and cardiomyopathy.[31]

Silybin Bound to Phosphatidylcholine

Given the poor relative bioavailability of silymarin components, methods are being developed to increase their bioavailability to improve clinical effects. One enhanced form of silymarin binds silybin to phosphatidylcholine. Research indicates that phosphatidylcholine-bound silybin is better absorbed and produces better clinical results than other forms.

Absorption Studies

Several human and animal studies have shown that phosphatidylcholine-bound silybin is better absorbed. In one study, the excretion of silybin, the major component of silymarin, in the bile was evaluated in patients undergoing gallbladder removal (cholecystectomy). A drainage tube, the T-tube, was used to sample the bile. Patients were given either a single oral dose of the silybin-phosphatidylcholine complex or silymarin. The amount of silybin recovered in the bile in free and conjugated form within 48 hours was 11% for the silybin-phosphatidylcholine group and 3% for unmodified silybin group.[32]

One of the significant features of this study is the fact that silymarin has been shown to improve the solubility of the bile. Because more silybin is being delivered to the liver and gallbladder when the phosphatidylcholine-bound silybin is used, this form is ideal for individuals with gallstones or fatty infiltration of the liver, two conditions characterized by decreased bile solubility.

In another study, plasma silybin levels were determined after administration of single oral doses of silybin-phosphatidylcholine complex and a similar amount of silymarin to nine healthy volunteers. Although absorption was rapid with both preparations, the bioavailability of the silybin-phosphatidylcholine complex was much greater than that of silymarin, as indicated by higher plasma silybin levels at all sampling times after intake of the complex. The researchers in this study concluded that complexation with phosphatidylcholine greatly increases the oral bioavailability of silybin, probably by facilitating its passage across the gastrointestinal mucosa.[33]

Clinical Studies

Several clinical studies have shown phosphatidylcholine-bound silybin to be more effective than silymarin. In one study, eight patients with chronic viral hepatitis (three with hepatitis B, three with both hepatitis B and hepatitis C, and two with hepatitis C) were given one capsule of phosphatidylcholine-bound silybin (equivalent to 140 mg silymarin) between meals for 2 months.[34] After treatment, serum malondialdehyde levels (an indicator of lipid peroxidation) decreased by 36%, and the quantitative liver function evaluation as measured by galactose elimination capacity increased by 15%. A statistically significant reduction of liver enzymes was also seen: AST diminished by 17%, and ALT rose by 16%.

In another study designed primarily to evaluate the dose–response relationship of phosphatidylcholine-bound silybin, positive effects were again displayed.[35] In this study, patients with chronic hepatitis due to either a virus or alcohol were given different doses for 2 weeks: 20 patients received 80 mg twice daily, 20 received 120 mg twice daily, and 20 received 120 mg three times daily. At all tested doses, phosphatidylcholine-bound silybin produced remarkable and statistically significant decreases in mean serum and total bilirubin levels. When used at the dose of 240 or 360 mg/day, it also resulted in remarkable and statistically significant decreases in the liver enzymes ALT and gamma-glutamyl-transpeptidase. These results indicate that even short-term treatment of viral- or alcohol-induced hepatitis with relatively low doses of phosphatidylcholine-bound silybin can be effective; for the best results, however, higher doses are needed.

Treatment with phosphatidylcholine-bound silybin is associated with reduced body iron stores, especially among patients with the advanced fibrosis stage of chronic viral hepatitis.[36]

DOSAGE

The standard dose of milk thistle is based on its silymarin content (70–210 mg three times daily). For this reason, standardized extracts are preferred. The best results are achieved at higher dosages—210 to 700 mg of silymarin three times daily.

The dosage for silybin bound to phosphatidylcholine is 120 to 240 mg twice daily.

Alcohol-based extracts are virtually always contraindicated in liver disease because a relatively large amount of alcohol is administered to obtain an adequate dose of silymarin in this form.

TOXICITY

Silymarin preparations are widely used, with a considerable body of evidence pointing to very low toxicity. When used at high doses for short periods, silymarin given by various routes to mice, rats, rabbits, and dogs has shown no toxic effects. Studies in rats receiving silymarin for protracted periods have also demonstrated a complete lack of toxicity.[1]

Because silymarin possesses choleretic activity, it may produce a looser stool as a result of greater bile flow and secretion. If higher doses are used, it may be appropriate to use bile-sequestering fiber compounds (e.g., guar gum, pectin, psyllium, oat bran) to prevent mucosal irritation and loose stools. Because of silymarin's lack of toxicity, its long-term use is feasible when necessary.

DRUG INTERACTIONS

Although silymarin components have been shown to interact with drug-metabolizing cytochrome P_{450} enzymes in in vitro studies, the concentrations showing inhibitory effects are not achieved with normal dosage recommendations.[37] Clinical studies have shown that coadministration of silymarin with drugs metabolized by P_{450} enzymes (e.g., indinavir) causes no adverse interactions.[38,39]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bijak M. Silybin, a major bioactive component of milk thistle (*Silybum marianum* L. Gaernt.)—chemistry, bioavailability, and metabolism. *Molecules*. 2017;22(11). pii :E1942. https://doi.org/10.3390/molecules22111942.
2. Federico A, Dallio M, Loguercio C. Silymarin/Silybin and chronic liver disease: a marriage of many years. *Molecules*. 2017;22(2). pii :E191. https://doi.org/10.3390/molecules22020191.
3. Bahmani M1, Shirzad H, Rafieian S, Rafieian-Kopaei M. *Silybum marianum*: beyond hepatoprotection. *J Evid Based Complementary Altern Med*. 2015;20(4):292–301.
4. Vogel G, Tuchweber B, Trost W, et al. Protection against *Amanita phalloides* intoxication in beagles. *Toxicol Appl Pharm*. 1984;73:355–362.
5. Serne EH, Toorians AWF, Gietema JA, et al. *Amanita phalloides*, a potentially lethal mushroom: its clinical presentation and therapeutic options. *Neth J Med*. 1996;49:19–23.
6. Ting H, Deep G, Agarwal R. Molecular mechanisms of silibinin-mediated cancer chemoprevention with major emphasis on prostate cancer. *AAPS J*. 2013;15(3):707–716. https://doi.org/10.1208/s12248-013-9486-2.
7. Sonnenbichler J, Zetl I. Biochemical effects of the flavanolignane silybinin on RNA, protein and DNA synthesis in rat livers. In: Cody V, Middleton E, Harbourne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships*. New York: Liss; 1986:319–331.
8. Sonnenbichler J, Goldberg M, Hane L, et al. Stimulatory effect of silybinin on the DNA synthesis in partially hepatectomized rat livers: non-response in hepatoma and other malignant cell lines. *Biochem Pharmacol*. 1986;35:538–541.
9. Greenlee H, Abascal K, Yarnell E, et al. Clinical applications of *Silybum marianum* in oncology. *Integr Cancer Ther*. 2007;6(2):158–165.
10. Wagoner J, Negash A, Kane OJ, et al. Multiple effects of silymarin on the hepatitis C virus lifecycle. *Hepatology*. 2010;51(6):1912–1921.
11. Kock HP, Bachner J, Loffler E. Silymarin: potent inhibitor of cyclic AMP phosphodiesterase. *Methods Find Exptl Clin Pharmacol*. 1985;7:409–413.
12. Tajmohammadi A, Razavi BM, Hosseinzadeh H. *Silybum marianum* (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: a review. *Phytother Res*. 2018. https://doi.org/10.1002/ptr.6153.
13. Esmaeil N, Anaraki SB, Gharagozloo M, Moayedi B. Silymarin impacts on immune system as an immunomodulator: one key for many locks. *Int Immunopharmacol*. 2017;50:194–201. https://doi.org/10.1016/j.intimp.2017.06.030. Epub 2017 Jun 30.
14. Hutchinson C, Bomford A, Geissler CA. The iron-chelating potential of silybin in patients with hereditary haemochromatosis. *Eur J Clin Nutr*. 2010;64(10):1239–1241.
15. Schopen RD, Lange OK, Panne C, et al. Searching for a new therapeutic principle: experience with hepatic therapeutic agent Legalon. *Med Welt*. 1969;20:888–893.
16. Schopen RD, Lange OK. Therapy of hepatoses: therapeutic use of silymarin. *Med Welt*. 1970;21:691–698.
17. Szilard S, Szentgyorgyi D, Demeter I. Protective effect of Legalon in workers exposed to organic solvents. *Acta Med Hung*. 1988;45:249–256.
18. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs*. 2001;61:2035–2063.
19. Wah Kheong C, Nik Mustapha NR, Mahadeva S. A Randomized trial of silymarin for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2017;15(12). https://doi.org/10.1016/j.cgh.2017.04.016. 1940–1949.e8.
20. Ferenci P, Dragosic SB, Dittrich H, et al. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. *J Hepatol*. 1989;9:105–113.
21. Saller R, Brignoli R, Melzer J, Meier R. An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Forsch Komplementmed*. 2008;15(1):9–20. https://doi.org/10.1159/000113648.
22. Magliulo E, Gagliardi B, Fiori GP. Results of a double blind study on the effect of silymarin in the treatment of acute viral hepatitis, carried out at two medical centres. *Med Klin*. 1978;73:1060–1065.
23. El-Kamary SS, Shardell MD, Abdel-Hamid M, et al. A randomized controlled trial to assess the safety and efficacy of silymarin on symptoms, signs and biomarkers of acute hepatitis. *Phytomedicine*. 2009;16(5):391–400.
24. Berenguer J, Carrasco D. Double-blind trial of silymarin versus placebo in the treatment of chronic hepatitis. *Munch Med Wochenschr*. 1977;119:240–260.
25. Yang Z, Zhuang L, Lu Y, Xu Q, Chen X. Effects and tolerance of silymarin (milk thistle) in chronic hepatitis C virus infection patients: a meta-analysis of randomized controlled trials. *Biomed Res Int*. 2014;2014:941085. https://doi.org/10.1155/2014/941085. Epub 2014 Aug 27.
26. Nassauto G, Iemmolo RM, Strazzabosco M, et al. Effect of silybinin on biliary lipid composition: experimental and clinical study. *J Hepatol*. 1991;12:290–295.
27. Weber G, Galle K. The liver: a therapeutic target in dermatoses. *Med Welt*. 1983;34:108–111.
28. Di Pierro F, Callegari A, Carotenuto D, et al. Clinical efficacy, safety and tolerability of BIO-C (micronized silymarin) as a galactagogue. *Acta Biomed*. 2008;79(3):205–210.
29. Ebrahimpour-Koujan S, Gargari BP, Mobasseri M, Valizadeh H, Asghari-Jafarabadi M. Lower glycemic indices and lipid profile among type 2 diabetes mellitus patients who received novel dose of *Silybum marianum (L.) Gaertn*. (silymarin) extract supplement: a triple-blinded randomized controlled clinical trial. *Phytomedicine*. 2018;44:39–44. https://doi.org/10.1016/j.phymed.2018.03.050. Epub 2018 Mar 19.
30. Ebrahimpour KS, Gargari BP, Mobasseri M, Valizadeh H, Asghari-Jafarabadi M. Effects of *Silybum marianum (L.) Gaertn*. (silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: a randomized, triple-blind, placebo-controlled clinical trial. *Phytomedicine*. 2015;22(2):290–296. https://doi.org/10.1016/j.phymed.2014.12.010. Epub 2015 Jan 19.
31. Stolf AM, Cardoso CC, Acco A. Effects of silymarin on diabetes mellitus complications: a review. *Phytother Res*. 2017;31(3):366–374. https://doi.org/10.1002/ptr.5768. Epub 2017 Jan 25.
32. Schandalik R, Gatti G, Perucca E. Pharmacokinetics of silybin in bile following administration of silipide and silymarin in cholecystectomy patients. *Arzneimittelforschung*. 1992;42:964–968.
33. Barzaghi N, Crema F, Gatti G, et al. Pharmacokinetic studies on IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. *Eur J Drug Metab Pharmacokinet*. 1990;15:333–338.
34. Moscarella S, Giusti A, Marra F, et al. Therapeutic and antilipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease: preliminary results. *Curr Ther Res*. 1993;53:98–102.
35. Vailati A, Aristia L, Sozze E, et al. Randomized open study of the dose-effect relationship of a short course of IdB 1016 in patients with viral or alcoholic hepatitis. *Fitoterapia*. 1993;64:219–228.
36. Bares JM, Berger J, Nelson JE, et al. Silybin treatment is associated with reduction in serum ferritin in patients with chronic hepatitis C. *J Clin Gastroenterol*. 2008;42(8):937–944.
37. Zuber R, Modriansky M, Dvorak Z, et al. Effect of silybin and its congeners on human liver microsomal cytochrome P450 activities. *Phytother Res*. 2002;16:632–638.
38. DiCenzo R, Shelton M, Jordan K, et al. Coadministration of milk thistle and indinavir in healthy subjects. *Pharmacotherapy*. 2003;23:866–870.
39. Piscitelli SC, Formentini E, Burstein AH, et al. Effect of milk thistle on the pharmacokinetics of indinavir in healthy volunteers. *Pharmacotherapy*. 2002;22:551–556.

114

Soy Isoflavones and Other Constituents

Cheryl Kos, ND

OUTLINE

INTRODUCTION

Soybeans have a history of human food use spanning millennia, particularly in Asia, where they are prepared as foods, beverages, and medicines. Epidemiological data indicating that people from Asian cultures have lower rates of breast, prostate, and colon cancer have sparked an interest in soy foods as a contributing factor. Greater dietary utilization of both soy foods and isolated soy phytonutrients has drawn attention to their benefits as well as to potential health risks for subpopulations that may be sensitive to their effects. Recent research has investigated not only individual soy constituents for their influences on hormonal and cardiometabolic parameters but is also increasingly considering the effect of whole soy foods on the human microbiome, which may have a significant effect on their biological activities.

Thousands of scientific papers have been published on soy and its components, and the National Library of Medicine's online clinical trials database typically lists hundreds of ongoing or planned studies involving soy. Although many soy constituents have been investigated, those that seem to hold the greatest therapeutic promise are the isoflavones genistein and daidzein. There is also increasing interest in equol, a human metabolite of daidzein, and the possibility that the ability to produce equol may mediate some of the effects of soy.

Research methods have evolved tremendously during the decades of research on soy, and a 2010 guidance paper from a National Institutes of Health (NIH) workshop on designing and reporting on soy intervention trials meticulously outlines methods for conducting studies that can take into account crucial factors such as differences among soy preparations and individual variations in the human response to soy as well as recommending ways to better standardize the evaluation and interpretation of results.[1] This guidance will facilitate the generation of higher-quality data to help answer remaining questions about the health effects and safety considerations of consuming soy and its bioactive constituents.

CHEMICAL COMPOSITION OF SOY

Hundreds of nutrients and phytochemicals have been identified in the soy plant, including proteins, fibers, fatty acids, isoflavones and other polyphenolic substances, phytosterols, saponins, coumestans (including coumestrol), choline, chlorophyll, resistant starch, sugars and saccharides, enzymes, enzyme inhibitors, nucleotides, minerals, vitamins and provitamins, oxalate, phytate, all essential amino acids, arginine, and many others.[2] The principal isoflavones in soy are genistein (4′,5,7-trihydroxyisoflavone) and daidzein (4′,7-dihydroxyisoflavone) (Fig. 114.1).

Isoflavones are the most abundant subcategory of isoflavonoids, which are in turn members of the greater family of flavonoids, themselves a subset of the large group of plant polyphenols (Fig. 114.2). Approximately 600 isoflavonoids have been identified and categorized. Soybeans generally provide around 1 to 3 mg of isoflavones

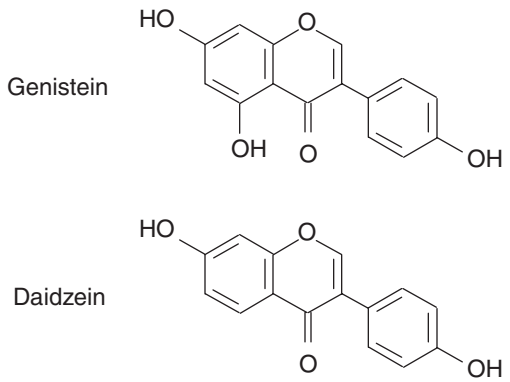


Fig. 114.1 Chemical structure of genistein and daidzein.

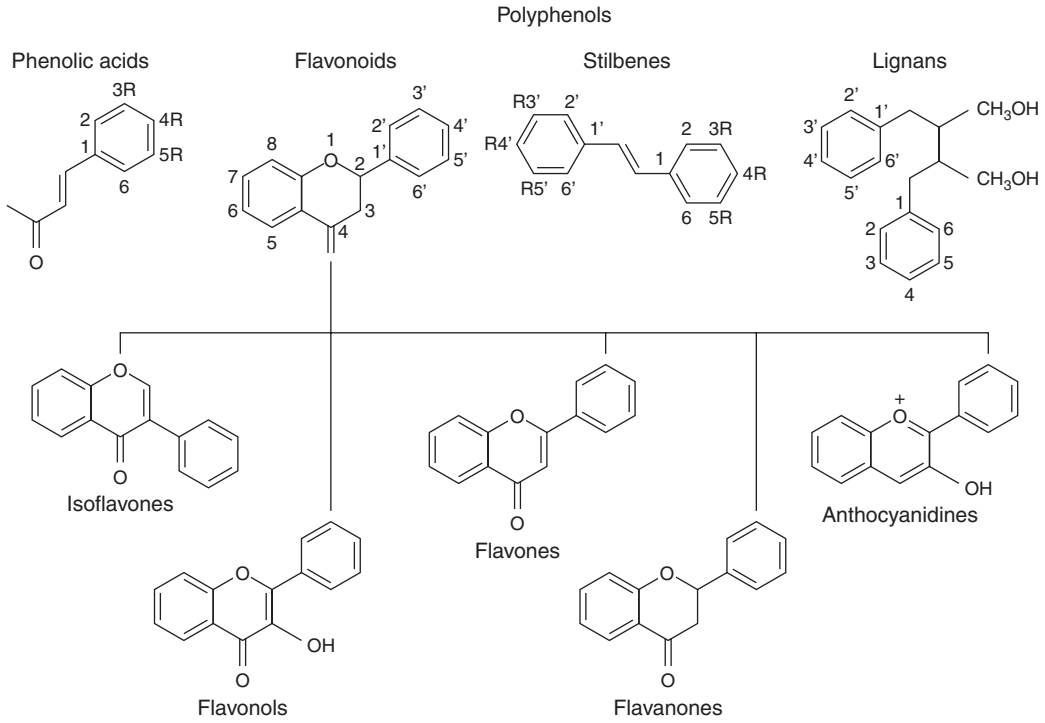


Fig. 114.2 Chemical structure of polyphenols, with emphasis on flavonoids.

TABLE 114.1 Percentage and Ratio of Principle Isoflavones in Common Soy Products

Soy Product	Isoflavone Content	Genistein:Daidzein
Whole soybeans	0.1%–0.2%	1:1
Soy flour (defatted)	0.07%–0.3%	1:1
Soy germ	5%	1:4
Synthetic genistein	100%	1:0
Soy protein isolate	0.01%–0.3%	1:1
Tofu	0.02%–0.07%	1:0.7
Soy milk	0.001%–0.02%	1:1
Miso (dry)	0.02%–0.09%	1:0.9

Data from Empie MW. Compositional specificity in soy isoflavone supplements. Soy/protein/isoflavone research: challenges in designing and evaluating intervention studies. NIH Workshop, Bethesda, MD, July 28–29, 2009.

(the aglycones genistein, daidzein, and glycitein, and their glycoside forms genistin, daidzin, and glycitin) per gram of protein, and considering all forms of each individual isoflavone, the breakdown of genistein, daidzein, and glycitein supplied by soy foods is approximately 50%, 40%, and 10%, respectively.[3] Commonly consumed soy foods are generally considered rich sources of dietary isoflavones.[4] In fermented soy foods like tempeh, miso, soy sauce, and natto, aglycone forms generally dominate, whereas glycosides are the major forms in nonfermented foods such as soy milk, soy flour, protein powders, edamame, soy nuts, and tofu, as well as in most dietary supplements.[5]

Isoflavone contents and proportions in soy foods vary according to plant parts and genetics; growing and harvesting conditions; and storage, extraction, and processing methods. The typical isoflavone contents of several soy products and their relative provisions of genistein and daidzein are given in Table 114.1. For those interested in learning the isoflavone contents of hundreds of foods and beverages, the most recent version of the U.S. Department of Agriculture (USDA) Database on the Isoflavone Content of Selected Foods is available online at https://www.ars.usda.gov/nutrientdata, under Flavonoid Databases.

The average daily intake of isoflavones among Japanese women is 20 to 80 mg, whereas that of American women is about 1 to 3 mg,[6] a manifold difference. Isoflavone intakes among various Chinese populations are estimated to range from around 10 to 40 mg daily[7] but are quite low among Europeans and Canadians, averaging less than 1 mg daily.[8,9] Among Westerners, many health-conscious consumers are eating soy foods more frequently, and in one British study, vegans and vegetarians showed the highest intakes, receiving up to 30 g soy protein and 130 mg soy isoflavones daily.[10] Patients with breast cancer at one U.S. hospital were found to have mean daily genistein and daidzein intakes of 11.6 mg and 7.4 mg, respectively—much higher than the American average.[11] Americans consume less soy than the Chinese and Japanese but tend to ingest more in dietary supplements and in processed food forms such as texturized vegetable protein and isolated soy protein, which are added to meat substitutes, breads, processed meats, and other foods. Up to 25% of formula-fed infants consume soy-based formula, and these infants can show higher isoflavone concentrations on a body-weight basis than those of adults with high soy intakes.[12]

Isolated soy protein contains all essential amino acids and has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.0, the highest score possible, although other soy protein preparations may display PDCAAS scores of as low as 0.9.[13–15] Soy is considered one of the eight major food allergens, with albumin, globulin, and prolamin proteins being its primary allergenic constituents in sensitive individuals.

Other soy constituents of interest include phytosterols, saponins, protease inhibitors, and phytates. Phytosterols bind cholesterol in the gut, and although the U.S. Food and Drug Administration (FDA),[16] Health Canada,[17] and the European Union's European Food Safety Authority (EFSA)[18] have approved health claims regarding the cholesterol-lowering effects of plant sterols added to foods, naturally occurring levels in soy may be insufficient to derive such benefits. Soy

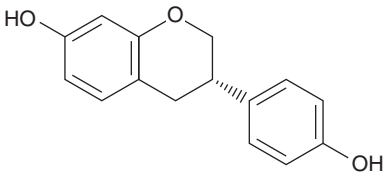


Fig. 114.3 Chemical structure of equol.

saponins may also contribute to the influence of soy foods through their anticholesterol effects.[19] Raw soybeans contain several protease inhibitors, but their inactivation during processing into soy food products makes it unlikely that they have much effect in humans. Phytates have been shown to interfere with the absorption of some minerals, yet they also display strong antioxidant and antitumor properties.[20,21]

Absorption, Metabolism, and Excretion of Isoflavones

Primary human exposure to isoflavones comes from the diet, and although other plants such as red clover (*Trifolium pratense*) and kudzu (*Pueraria lobata*) contain isoflavones, only soy foods supply physiologically relevant amounts of isoflavones in the human diet. Much evidence indicates wide individual variation in absorption and metabolism of isoflavones, which is influenced by the composition of the intestinal microbiome, liver metabolism, enterohepatic circulation, and habitual intakes of soy foods, and coingestion of prebiotic and probiotic foods may also have effect.[22,23]

Dietary soy isoflavones occur bound to sugars that are not readily absorbed and may be modified extensively in the gastrointestinal tract, although the degree of metabolism and absorption varies considerably among individuals.[24,25] Intestinal and bacterial glucosidases release genistein and daidzein from genistin and daidzin, after which they may be conjugated through sulfation or glucuronidation, dehydroxylated, reduced, or demethylated to give rise to metabolites of varying biological activity, including S-(-)equol, 5-hydroxyequol, O-desmethylangolensin, dihydrogenistein, and others. Genistein and daidzein may also circulate freely or be bound to plasma proteins and are distributed widely in body tissues.[26] Isoflavones attain maximal plasma levels 4 to 8 hours after ingestion and are excreted within 24 hours, mainly in urine but also in feces.[27] Metabolites of soy isoflavones include compounds with molecular structures similar to those of reproductive steroid hormones (Fig. 114.3).

The relative bioavailability of isoflavone glycoside and aglycone forms is debated, and numerous articles have weighed in on both sides of this question. Aglycones from soy products were absorbed more quickly and completely than glycosides in several studies,[28–31] although others have shown the converse or no difference.[32–34] Further research and survey of the evidence may reveal differences among individuals or populations that account for these seemingly conflicting findings.

A number of clinical trials in both genders in various disease states have hinted that the ability to produce the human metabolite S-(-) equol from its isoflavone precursor daidzein (through the intermediate dihydroequol) may hold unique health benefits. Franke et al. cite studies showing that about 60% of vegetarians and Asians produce equol, whereas only about 30% to 35% of omnivores do so.[35] Infants and children aged 6 to 36 months showed gradually increasing equol production in one study, particularly those receiving soy milk– or cow's milk–based formula and those not receiving antibiotics.[36] It may be useful to note that the R-(+)equol isomer is not an endogenous human form.

Setchell et al. found that S-(-)equol is a selective estrogen receptor-β agonist (with about one-fifth the affinity of estradiol)[37] and yet also noted, with interest, that it is an androgen antagonist.[38] Lampe reports that although research findings are mixed, equol production has been associated with lower breast density in response to weekly soy consumption as well as better bone protection with isoflavone intake and greater improvements in cholesterol levels and brachial artery flow-mediated vasodilation with soy germ supplementation in hypercholesterolemic subjects. Equol producers among postmenopausal women in Hong Kong had significantly higher fat-free body mass and significantly lower systolic and diastolic blood pressures, as well as lower levels of triglycerides, high-sensitivity C-reactive protein (hsCRP), and free fatty acids compared with nonproducers.[39] Miller et al. did not find a significant association between equol production status and body mass index (BMI) or obesity in peri- and postmenopausal women, although BMI and obesity related to the inability to convert daidzein into a different metabolite, O-desmethylangolensin.[40] Although genetic, dietary, and lifestyle factors relating to equol production status are still unclear, Lampe has suggested that it may correlate with lactose malabsorption.[41]

Several research groups have noted that adults also may change from nonproducer to producer status after heightened soy consumption, although not all studies concur. Franke et al. found that both post- and premenopausal women may begin to produce equol with increased isoflavone exposure.[42,43] Healthy Caucasian men with isoflavone intakes above 30 mg daily for at least 2 years were over five times likelier to produce equol than those consuming 5 mg or less daily.[44] Tanaka et al. found that after 3 months of isoflavone supplementation, healthy Japanese male nonproducers showed detectable serum equol levels.[45] Development of a recognized, validated testing method and threshold equol value or equol-to-daidzein ratio will aid future interpretation of evidence.

Early in vitro research suggests that soy isoflavones may constitute an energy substrate for the proliferation of equol-producing intestinal microbes and enhance the production of short-chain fatty acids (SCFAs); both equol and SCFAs are increasingly considered pivotal in delivering isoflavone benefits.[46] Nakatsu et al. found significant increases in fecal *Bifidobacteria* in postmenopausal women after just 1 week of consuming bars containing 160 mg soy isoflavones and 1 g saponin, particularly among equol producers; this team noted associations of *Bifidobacteria, Collinsella,* and *Lactococcus* with equol or other isoflavone metabolites, although the significance of these relationships is not yet fully understood.[47] However, consistent global changes in gut populations of major bacterial groups were not seen in menopausal equol producers or nonproducers given 80 mg isoflavones for 6 months, although equol producers showed increased counts for total microbial fecal populations, whereas overall counts in nonproducers decreased significantly. In some women, growth of phytoestrogen-metabolizing microorganisms such as *Bifidobacteria, Faecalibacterium prausnitzii,* and *Collinsella* may have been facilitated by increased exposure to isoflavones and/or their metabolites, although in several cases this appeared to depend more on the individual microbiome than metabolite producer status.[48]

PHARMACOLOGY

Soy isoflavones (and their metabolites) and other soy constituents have shown varied activities in humans, animals, tissues, and cells (Table 114.2).

Hormonal Effects of Soy

The degree to which soy foods modify endogenous hormone influences in both genders is of prime importance in evaluating their benefits, and long-term observation of populations that consume considerable amounts of soy foods may hold the most complete answers

TABLE 114.2 Activities of Soy Constituents and Metabolites

Protein Isolate	Activation of mTOR[303]
Protein with isoflavones	Cholesterol metabolism modulation
	Progesterone receptor expression modulation[304]
	Possible adiponectin raising[305]
	Possible IL-6 lowering[306]
Isoflavones	Estrogenic and antiestrogenic
	Possible FSH and LH lowering (postmenopausal)[307]
	Possible 2-hydroxyestrone raising[308]
	Estrogen receptor-β agonism[309]
	Angiogenesis inhibition[310]
	5α-reductase inhibition[311]
	Stimulation of mitochondrial biogenesis[312]
	Possible IL-6 and TNF-α lowering (perimenopausal)[313]
	Inhibit bone resorption and stimulate bone formation[314,315]
	Modulation of bone-related gene expression[316]
	Possible improved endothelial function[317]
	Upregulation of PPARα/γ, LXR, and PGC-1α expression[318–320]
	Modification of DNA methylation and other epigenetic events[321]
	Inhibition of matrix metalloproteinase production in cancer cells[322]
	Modulation of thyroid hormone production
	Antioxidant
Genistein	Progesterone receptor upregulation[323]
	Inhibition of LDL-c oxidation[324]
	COX inhibition[325]
	Antithyroglobulin (in iodine insufficiency)[326]
	Pancreatic beta cell protection[327]
	Human sirtuin 1 activation[328]
	Inhibition of cancer cell detachment and metastasis[329]
	Upregulation of *BRCA1* expression[330]
	Modulation of activities of kinases related to cancer cell cycle and apoptosis[331,332]
	Possible chemo-, radio-, and phototherapeutic synergist[333]
Daidzein	Modulation of cholesterol-related gene expression[334]
	Aldehyde dehydrogenase inhibition[335]
Equol	Estrogen receptor-β agonism and androgen antagonism[336]
	Dihydrotestosterone binding and sequestration[337]
	Inhibition of PSA expression[338]
	Substrate for SCFA production[339]
	Possible bifidogenic effects[340]
Phytosterols	Cholesterol binding[341]
	5α-reductase inhibition[342]
	Antiproliferative[343]
Coumestrol	Estrogenic/antiestrogenic[344]
Saponins	Anticholesterol[345]
Protease inhibitors	Anticarcinogenic[346]
Phytates	Possible anti–colon cancer effect[347]
	Iron chelation, antioxidant[348]

COX, Cyclooxygenase; *FSH,* follicle-stimulating hormone; *IL-6,* interleukin-6; *LDL-c,* low-density lipoprotein cholesterol; *LH,* luteinizing hormone; *LXR,* liver X receptor; *PPARα/γ,* peroxisome proliferator-activated receptor-α/γ; *PGC-1α,* peroxisome proliferator-activated receptor γ coactivator 1α; *PSA,* prostrate-specific antigen; *SCFA,* short-chain fatty acid; *TNF-α,* tumor necrosis factor-α.

to this question. The possibility that long-term or lifelong soy food use may alter the composition of the gut microbiome to induce equol production points toward greater impacts from lifestyles rather than simply "grafting" single foods like soybeans into a Westernized diet. Research is also increasingly considering genetic characteristics that interact with diet and lifestyle to change disease risk for certain subpopulations. Much evidence suggests that soy foods generally exert a mild to moderate inhibitory effect on reproductive hormones and their binding proteins in most populations and that this may explain the reduced risk for breast and prostate cancers enjoyed by people in countries consuming higher levels of soy foods.

Infants

Soy protein–based infant formula has been employed for almost a century and may comprise up to 25% of the U.S. formula market, and soy-based infant formula preparations have been approved by both

the FDA and EFSA for safe use.[49,50] However, the potential for estrogenic effects in infants given soy formula has raised long-term safety concerns.

The relative proportions of genistein, daidzein, and glycitein in soy formula are about 58% to 70%, 22% to 44%, and 4% to 16%, respectively. Infants fed soy formula show higher levels of genistein and daidzein compared with most other populations, although Asian infants consuming traditional diets and vegan infants also have relatively high levels.[51,52] A safety review and meta-analysis by Vandenplas et al. concluded that children given modern soy infant formula show growth and development patterns similar to those seen in infants fed cow's milk or breastmilk in regard to immune, neurological, reproductive, endocrine, bone, and metabolic parameters,[53] and the Arkansas Children's Nutrition Center 5-year study of the same forms of infant nutrition reached a similar conclusion.[54] A 2008 statement updating the American Academy of Pediatrics' 1998 review of soy-based infant formula found no conclusive evidence that soy formula adversely affects human development or endocrine (including thyroid) or reproductive functions but noted the following:

1. Soy formula is not recommended for use in preterm infants.
2. Soy formula is contraindicated for use in infants with hereditary fructose intolerance or sucrase-isomaltase deficiency (although it is safe in infants with galactosemia or primary lactase deficiency).
3. Soy formula is not needed by infants with lactose intolerance (as opposed to overt lactase deficiency).
4. Soy formula should not be given to infants with cow's milk enteropathy or enterocolitis due to the likelihood of cross-sensitivity.
5. The use of an extensively hydrolyzed soy protein–based formula (although not nonhydrolyzed) can be considered for infants with immunoglobulin E–mediated cow's milk allergy.[55]

Andres et al. found that 1-year-old infants given cow's milk– or soy-based formula showed no differences in mental, motor, or language development, and although breastfed infants showed slightly higher scores in some areas, all three groups scored within normal ranges.[56] Portman et al. identified a significantly increased risk for Kawasaki disease in ethnically diverse U.S. children with high intakes of total isoflavones and of genistein compared with those with low intake levels, whereas maternal intakes during pregnancy did not show a relationship with the incidence of the disease in offspring.[57]

Women

Findings among women vary considerably by life stage, lifestyle, and genetic individuality. A 2009 meta-analysis by Hooper et al. of soy's effects in 579 premenopausal, 69 perimenopausal, and 1165 postmenopausal women concluded that soy isoflavones may lower follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and extend the menstrual cycle by about 1 day premenopausally and may nonsignificantly increase total (but not free) estradiol levels postmenopausally.[58] A randomized controlled trial (RCT) in more than 200 postmenopausal women given 80 or 120 mg isoflavones over 3 years showed no evidence of changes in endometrial thickness, estradiol or estrone sulfate levels, thyroid-stimulating hormone (TSH) levels, or incidence of adverse events compared with placebo.[59] Fuhrman et al. showed in 2009 that regular soy intake in Asian American women aged 20 to 55 is associated with increased formation of the weakly estrogenic 2-hydroxyestrone (2-OHE1) and decreased production of the more potent, more persistent, and more genotoxic 16α-hydroxyestrone (16α-OHE1) for an overall significant increase in the 2/16α-OHE1ratio.[60] Nettleton et al. found that breast cancer survivors consuming soy protein isolate with isoflavones show increased 2-OHE1 and 16α-OHE1 levels without a change in ratio, although subjects with high plasma equol levels also showed a significant increase in the 2/16α-OHE1 ratio; an interesting finding was that breast cancer survivors generally demonstrated lower baseline 2/16α-OHE1 ratios compared with controls.[61] Postmenopausal Thai women given a fermented soybean preparation providing about 60 mg isoflavones showed no change in estradiol levels but a significant increase in progesterone levels and a significant decrease in total cholesterol, and the researchers suggested that soy supplementation may induce progesterone receptor expression.[62]

A 2010 meta-analysis of eight RCTs found no effect of dietary isoflavones on breast density in women in general and postmenopausally but showed a possible modest increase in breast density (a mean of 1.83%) in premenopausal women with higher intakes,[63] although a review of 14 observational studies by Maskarinec et al. did not find an association between soy intake and mammographic density.[64]

Men

Noted soy researcher Mark Messina in 2010 found no evidence that isoflavone supplements or isoflavone-rich soy foods affect levels of estrogens or total or free testosterone in men, and that they have no effect on sperm, semen, or erectile function in men, even considering intake levels above those commonly consumed by male Asian populations.[65] Dillingham et al. observed that healthy young men supplemented with soy protein isolate with either low or high levels of isoflavones showed significantly decreased dihydrotestosterone (DHT) levels as well as DHT/testosterone ratios compared with those given milk protein isolate, and the low-isoflavone soy group also showed significantly increased dehydroepiandrosterone sulfate levels as well as smaller increases in estradiol and estrone sulfate relative to the milk protein isolate group.[66] A 2010 meta-analysis of clinical studies on the effects of soy on testosterone-related values in men found no significant influence of either soy protein or isoflavones on free androgen index or testosterone, free testosterone, or sex hormone–binding globulin (SHBG) levels, although inclusion of small and/or short-term trials may have limited the findings.[67] However, in healthy Japanese males aged 30 to 59 given 60 mg soy isoflavones daily for 3 months, levels of DHT and free testosterone decreased, whereas SHBG levels increased significantly in equol producers but not nonproducers; an intriguing finding was that serum equol levels in 2 of 10 previous nonproducers became detectable by the end of this trial.[68]

CLINICAL APPLICATIONS

Cardiovascular Disease

Soy foods are considered heart-healthy overall due to their generous contents of fiber, polyunsaturated fats, vitamins, minerals, and phytonutrients as well as their low saturated fat levels.[70] Interest in the potential role of soy protein and isoflavones to reduce risk factors associated with cardiovascular and related metabolic diseases stems from decades of human and preclinical research that formed the basis for the 1999 FDA-approved health claim for soy foods and coronary heart disease, stating that "25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."[71] In 2008 the FDA received a petition to revoke this health claim, but despite denying that petition, the FDA decided to reconsider its 1999 position and to again review the scientific evidence. Late in 2017 the FDA announced a proposal to revoke the claim, mainly due to difficulty in achieving "significant scientific agreement" among the mixed results seen among heterogeneously designed intervention studies regarding soy intake and cardiovascular outcomes.[72] The American Heart Association (AHA) has acknowledged that soy foods are beneficial for cardiovascular and overall health but concluded that the direct cholesterol-lowering effects of soy protein were of relatively little

significance.[73] On the other hand, a 2017 study of cost-effectiveness of plant sterol- or stanol-enriched foods determined that encouraging and subsidizing preventive use of such products would likely bring cost savings to healthcare systems and improve cardiovascular disease (CVD) outcomes, particularly among men and older age groups; it estimated that at only 50% compliance, 69 CVD events could be avoided per 10,000 men over 20 years.[74]

Beneficial cardiovascular effects of consuming soy foods seen in many human studies appear to be stronger when they replace (rather than merely supplement) common dietary proteins with higher saturated fat contents. Epidemiological studies in populations that commonly consume soy foods have shown that higher intakes may be inversely related to incidences of coronary events such as nonfatal myocardial infarction[75,76] and heart disease mortality,[77] although some suggest that results may be gender dependent.

In 2011 Hodis et al. found after a 3-year clinical trial that 25 g soy protein enriched with 99 mg isoflavones nonsignificantly reduced the progression of atherosclerosis (via carotid intima media thickness) in postmenopausal women and proposed that a longer exposure period may have resulted in more significant differences because changes observed between the treatment groups increased as the study progressed.[78] In two studies, higher soy intake was found to significantly correlate to reduced incidence of heart disease,[79,80] and another concluded that consumption of soy phytoestrogens might prevent the progression of coronary artery atherosclerosis in postmenopausal women.[81] A Japanese study of more than 40,000 adults aged 40 to 59 found that women consuming soy foods at least five times weekly had a relative risk for CVD mortality of about 0.31 times that of women eating soy foods two or fewer times per week; women (especially postmenopausal) with higher soy food consumption also showed significantly decreased risks for myocardial and cerebral infarction in this study.[82] However, a 2015 nested case-control study in postmenopausal Chinese women aged 40 to 70 who had never used hormone replacement therapy found that a high intake of soy isoflavones was modestly yet significantly associated with increased risk for ischemic stroke; from the lowest to highest consumption levels, the relative risk ranged from 1.0 to 1.24 compared with baseline, yet no relation was found between urinary isoflavone metabolite excretion and risk.[83] A 2014 study in Singaporean Chinese adults did not find significant associations between soy protein intake and cardiovascular mortality or isoflavone intake and death from coronary heart disease.[84]

Cholesterol Metabolism

An enormous amount of research has examined the influences of soy on cholesterol metabolism. Despite broad variety in soy preparations and great heterogeneity among study designs, a fairly consistent result has been that regular intake of soy foods, especially in place of more common animal protein sources, leads to modest yet meaningful cholesterol reductions in hypercholesterolemic individuals. Soy's main mechanisms of action for this appear to be interfering with cholesterol absorption, increasing fecal bile acid excretion, and upregulating low-density lipoprotein (LDL) receptor expression.

A 2008 meta-analysis evaluating the provision of 25 grams of soy protein daily concluded that it resulted in a small but significant 6% decrease in total and LDL cholesterol levels in normocholesterolemic and hypercholesterolemic adults.[85] A large meta-analysis by Anderson et al. comparing soy and animal protein diets found a significant 12.9% decrease in serum lipids with soy protein, especially in subjects with hypercholesterolemia.[86] More recent meta-analyses show consistent LDL lowering with soy protein but at a more modest average reduction of 3% at a daily intake of about 50 grams.[87,88] A 2007 meta-analysis of soy isoflavones by Taku et al. found that they significantly reduced total and LDL cholesterol levels, with larger changes in hypercholesterolemic subjects,[89] and a meta-analysis of soy protein containing isoflavones by Zhan et al. arrived at a similar conclusion.[90]

Individual studies can provide detailed insights. Jenkins et al. found that hyperlipidemic men and women receiving soy protein with isoflavones in addition to National Cholesterol Education Program (NCEP) Step II diets showed significantly lower total cholesterol, estimated coronary artery disease (CAD) risk, total/high-density lipoprotein (HDL) ratio, LDL/HDL ratio, and apolipoprotein B/A-1 ratio than those using the Step II diet alone; LDL cholesterol, apolipoprotein B, homocysteine, and oxidized LDL levels were also lower with soy than during the Step II diet alone. This study also demonstrated that men receiving soy showed a significant decrease in systolic blood pressure compared with the Step II diet alone and that during high-isoflavone intakes, LDL cholesterol and apolipoprotein B levels were lower than during the Step II diet alone.[91] In a study of a similar design by Baum et al., hypercholesterolemic postmenopausal women receiving soy protein and isoflavones in addition to NCEP Step I diets showed a greater decrease in non-HDL cholesterol than those receiving Step I diets alone, and those receiving soy protein and isoflavones also showed increases in HDL cholesterol as well as in LDL receptor RNA levels compared with decreases in both measures in those receiving Step I diets alone.[92] Those with type 2 diabetes receiving soy protein isolate showed reduced LDL cholesterol, LDL/HDL cholesterol ratio, and apolipoprotein B/A-1 ratio compared with those receiving milk protein isolate.[93] In 2012, Wong et al. found that although soy foods reduced LDL cholesterol levels equally in hyperlipidemic equol producers and nonproducers, equol producers maintained higher HDL levels than nonproducers.[94] In postmenopausal women, Allen et al. found that those receiving soy protein containing isoflavones rather than casein showed significant reductions in total and LDL cholesterol levels as well as in LDL particle number.[95] Puska et al. found that hypercholesterolemic subjects receiving soy protein plus isoflavones and fiber showed significant decreases in total and LDL cholesterol levels compared with controls, and the soy group also showed a significant decrease in homocysteine levels, whereas controls showed an increase.[96] In a study of healthy U.S. adults, those receiving soy protein showed significantly better improvement in HDL cholesterol levels and HDL/total cholesterol ratios compared with subjects given milk protein.[97] Chinese equol-producing postmenopausal women receiving 40 g soy flour showed significant reductions in LDL cholesterol and hs-CRP compared with subjects receiving either milk powder plus 63 mg daidzein or milk powder alone.[98]

The results of a 2010 study by Wong et al. suggest that soy foods' potential benefits to lipid profiles may be significantly improved by combination with a prebiotic like inulin. Hyperlipidemic men and women given 30 g soy protein, 61 mg soy isoflavones, and 10 g oligofructose-enriched inulin showed significant improvements in measures of LDL and HDL cholesterol compared with either soy or prebiotic alone.[99]

Although the Japanese Ministry of Health, Labor and Welfare has approved the use of cholesterol-lowering claims on certain soy products in Japan, the EFSA does not allow such claims on EU food products.

Blood Pressure

Soy protein and/or isoflavones may also have modest effects on blood pressure (BP), although research results have been mixed. Considerable heterogeneity in study design, as well as dosage and form of soy used, may interfere with the interpretation of overall study results, and adequately powered studies comparing the effects of different soy preparations could provide further clarification.

Healthy 50- to 75-year-old men and postmenopausal women receiving 40 g soy protein with 118 mg isoflavones versus casein showed significant reductions in BP.[100] Soy milk consumption for 3 months has also lowered mean, systolic, and diastolic BP values in hypertensive men and women compared with those receiving cow's milk; urinary genistein levels correlated significantly with decreases in BP.[101] Sagara et al. found that middle-aged men with elevated total cholesterol and/or BP levels receiving soy protein plus soy isoflavones showed significant reductions in systolic and diastolic BP, total cholesterol, and non-HDL cholesterol levels compared with active placebo, and they also showed significant increases in HDL-cholesterol.[102] Soy protein has been shown to lower BP in perimenopausal women, and soy protein with dietary fiber may reduce BP in both men and women.[103,104] Not all studies agree, though, and both the Agency for Health Research and Quality (which evaluates evidence presented for FDA health claims) and the AHA have concluded that neither soy protein nor isoflavones have hypotensive effects.[105,106] However, an interesting 2017 study of Japanese adults aged 40 to 69 found that fermented (but not unfermented) soy product intake was inversely associated with the development of hypertension, with an odds ratio of 0.72 in the highest tertile of intake.[107] A 12-month RCT in postmenopausal women aged 60 to 75 found that among those receiving soy protein containing 99 mg isoflavones, systolic and diastolic BP decreased in equol producers and increased in nonproducers compared with subjects receiving milk protein.[108]

Cardiometabolic Disease Factors

Studies exploring soy foods and cardiometabolic outcomes are increasingly examining soy constituents' mechanisms of action beyond cholesterol-lowering, looking more closely at their influences on macronutrient metabolism, endothelial function, and inflammatory markers.

A 2017 review by Ramdath et al. found that soy isoflavones and their metabolites appear to improve blood pressure, glycemic control, obesity, and inflammation and may act in synergy with soy protein and other constituents to modulate plasma lipids as well as satiety.[109] Another 2017 systematic review by Chalvon-Demersay et al. found evidence that consuming soy protein associated with isoflavones in place of more common animal proteins may help prevent the onset of risk factors associated with cardiovascular conditions and metabolic syndrome, such as hypertension and hypercholesterolemia.[110] A 2010 meta-analysis of isoflavones in postmenopausal women found that daily intake levels of about 50 to 100 mg markedly improved endothelial function in subjects with impairment,[111] and a 2015 crossover RCT showed that adults at cardiometabolic risk consuming soy nuts providing 101 mg total isoflavones daily for 1 month displayed significantly improved arterial stiffness as measured by peripheral arterial tonometry.[112] In middle-aged and older Chinese women, higher soy food intake is inversely associated with levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6, proinflammatory markers for metabolic and cardiovascular risk.[113]

Body Composition, Weight, and Glucose Metabolism

In studies relating to energy metabolism and weight, soy protein and isoflavones have displayed a number of intriguing properties that could potentially aid in the maintenance of glycemic control, weight, and body composition, perhaps especially during aging. This growing evidence represents an exciting opportunity for populations challenged by dysglycemia, weight concerns, and excess adiposity. As detailed in this section, soy has shown effects on peroxisome proliferator-activated receptors (PPARs), sirtuins, mTOR, and other cellular targets, which suggests complementary and even synergistic effects of soy on these energy-sensing and metabolism-altering pathways.

A review of clinical and preclinical evidence found that higher intakes of soy isoflavones may inhibit enlargement of adipose tissue through modifying signaling in pathways relating to fatty-acid and glucose metabolism, although some of these effects may be mediated by equol production.[114] Soy protein has been shown to spare muscle mass in overweight and obese subjects losing weight,[115] and subjects receiving a diet high in soy protein demonstrated improved glycemic control and lipid profiles.[116] A randomized, controlled weight-loss study of soy milk–based meal replacements (MRs) versus cow's milk–based MRs found that soy milk MRs resulted in slightly greater weight loss and significant reductions in serum triglyceride levels compared with cow's milk MRs.[117] A 12-week study conducted by Charles et al. in healthy postmenopausal women found that daily consumption of 20 grams of soy protein containing 160 mg total soy isoflavones significantly increased levels of adiponectin (an adipokine hormone considered to have anti-inflammatory and antiatherogenic properties), whereas those receiving soy protein without isoflavones showed significant improvements in levels of the proinflammatory marker TNF-α.[118] Another study using the same 20 g soy protein plus 160 mg isoflavones daily dosing versus casein protein in normal or overweight postmenopausal women found that the soy combination reduced total and subcutaneous abdominal fat, whereas casein increased both measures, with a significant difference between treatments.[119] A third study, again using the same dosing, in obese African American and white postmenopausal women found that compared with casein, soy reduced total or abdominal fat (in African American and white women, respectively) and significantly lowered levels of IL-6, increasingly considered a biomarker for cardiovascular risk.[120] Overweight Chinese adults receiving a low-calorie diet based on soy protein showed significantly greater reductions in body fat, total cholesterol, and LDL cholesterol and on liver function tests compared with subjects receiving a low-calorie diet based on traditional protein sources; both groups experienced significant drops in weight, waist circumference, and body mass index (BMI).[121] A 12-week trial of soy nuts or texturized soy protein in older women with metabolic syndrome found that either type of soy food mildly improved overall body composition and lipid profile compared with controls.[122,123] Perimenopausal women receiving 40 g soy protein plus 80 mg isoflavones showed significantly increased hip lean tissue and reduced thigh fat over 24 weeks compared with those receiving low-isoflavone soy protein and whey protein.[124] In postmenopausal women, genistein supplementation significantly decreased fasting glucose, fasting insulin, and fibrinogen levels as well as HOMA-IR values.[125] Women with polycystic ovary syndrome (PCOS) given 50 mg isoflavones daily for 12 weeks showed significant improvements in markers of insulin resistance and oxidative stress as well as significant reductions in free androgen index and triglyceride levels compared with controls.[126]

It is worthwhile to note that soybeans have a low glycemic index of about 14 to 20 (against glucose) or 20 to 29 (against white bread) as well as a low glycemic load.[127] Asian women consuming higher levels of soy foods display lower risks for type 2 diabetes,[128,129] and a 2011 meta-analysis by Liu et al. found that consumption of whole soy foods was associated with significantly reduced fasting glucose concentrations.[130] Soy protein with high isoflavone content lowers fasting insulin, hemoglobin A1c, insulin resistance, and LDL cholesterol levels in postmenopausal women with type 2 diabetes, and postmenopausal women with higher intakes of genistein seem to have lower BMIs, waist circumferences, and fasting insulin levels than those with lower intakes.[131,132]

Proposed mechanisms for soy's effects on weight, body composition, and glycemic control include increased beta-oxidation of fatty acids; slowing gastric emptying; activation of mTOR to aid skeletal

muscle anabolic processes[133]; improving glucose transport; increasing tissue insulin sensitivity; improving insulin receptor affinity; upregulation of PPARα,[134] PPARγ/PGC-1α,[135] and LXR expression[136]; stimulation of adiponectin; and inhibition of hepatic fatty acid synthesis, among others.[137] In animals fed a high-fat Western diet, those given soy protein isolate developed less insulin resistance, hypercholesterolemia, and hepatosteatosis compared with those given casein.[138] In rats fed a high-sucrose diet, soy protein reversed the resulting hepatosteatosis, normalized glucose homeostasis and skeletal muscle glucose oxidation, improved insulin sensitivity, and limited weight and visceral fat gain.[139] Genistein has displayed protective effects on pancreatic beta cells, improvement of insulin and glucose metabolism, improvement of endothelial function, and reduction of leptin levels, and it may also potentiate insulin secretion; these effects may account for some of the reduced risk for type 2 diabetes and beneficial effects on insulin and glucose metabolism observed in humans consuming soy or soy components.[140–144]

Early research suggests that soy or its components may indirectly and/or directly activate or upregulate human sirtuin 1, although the significance of these findings is not yet known.[145–147] Sirtuins are NAD-dependent enzymes involved in genomic stability, cell cycles, gene transcription, and glucose and lipid metabolism[148] and may be implicated in the benefits of full-nutrition caloric restriction.

Cancer

The first clue that soy might convey protection from cancer came from epidemiological studies of Asian cultures with high soy food intakes showing lower rates of several types of cancers. In an important 1994 review, Messina et al. identified 10 studies demonstrating that nonfermented soy products were associated with decreased risks for breast, prostate, lung, stomach, colon, and rectal cancers, although results were mixed for fermented soy foods, which showed positive relationships to esophageal, stomach, pancreatic, and colorectal cancers in some studies.[149]

Breast Cancer

Breast cancer occurs in Chinese women at one-third to one-half the rate seen in Caucasian women, and evidence suggests that dietary and lifestyle factors, including lifelong soy intakes, outweigh genetic factors in this difference.[150] Because breast tumor tissue may elaborate much higher concentrations of 17β-estradiol, especially in postmenopausal women,[151] and the estrogen-modulating effects of soy isoflavones may be useful for them, thorough evaluation of individual variations in metabolism, genetics, and receptor sensitivity is advisable.

In a 2011 meta-analysis of prospective studies on soy isoflavone intake and breast cancer risk, Dong et al. found a protective effect of soy for both the incidence and recurrence of breast cancer in Asian populations.[152] Trock et al. conducted a meta-analysis of soy protein intake and breast cancer risk and found a modest but significant risk reduction (odds ratio of 0.86) at high intake levels, with stronger effects in premenopausal women.[153] Examining a wider range of studies, Qin et al. also found a relative breast cancer risk of 0.75 among those with higher soy food intakes, with isoflavone intakes contributing to this effect.[154] A 2013 study of Japanese women showed lower breast cancer risk among postmenopausal (although not premenopausal) women with moderate or higher soy intakes,[155] and a 2014 systematic review of studies in Japanese women reached an analogous conclusion.[156] A 2011 study among Chinese women found that soy isoflavone and protein intakes decreased overall breast cancer risk, with the strongest associations in postmenopausal women and for estrogen- and progesterone-receptor positive breast cancer,[157] and a 2010 study among Korean women had similar findings.[158] In a multiethnic U.S. population with considerably lower isoflavone intakes than in Asian populations, no significant relationships were seen between isoflavones and overall breast cancer risk, although the data suggested that subpopulations with higher intakes may enjoy greater protection[159]; among those later diagnosed, prediagnostic soy intake was not related to breast cancer mortality.[160] In an RCT of U.S. women with increased breast cancer risk, Khan et al. found that soy isoflavones altered the activities of genes related to proliferation, apoptosis, and estrogenic effect, yet the researchers did not observe either positive or negative effects on breast epithelial cell proliferation over 6 months.[161] A 2013 systematic review of isoflavones concluded that soy intakes comparable to those among the Japanese (providing ~25–50 mg isoflavones daily) may protect against the incidence and recurrence of breast cancer as well as mortality.[162] Although most studies have found more postmenopausal benefits relating to soy and breast cancer, studies in Shanghai and Guangzhou have also uncovered evidence for reduced premenopausal breast cancer risk at higher intakes of soy isoflavones and protein; the Guangzhou study also noted protective effects for all subtypes of estrogen- and progesterone-receptor status.[163,164] A few studies also suggest an inverse relationship between exposure to soy and/or its constituents during childhood or adolescence and future breast cancer risk.[165–167]

A 2012 pooled analysis of 9514 breast cancer survivors in the United States and China found that intakes of at least 10 mg isoflavones daily were associated with significantly reduced risk of breast cancer recurrence and nonsignificantly reduced risks for breast cancer–specific and all-cause mortalities.[168] Two studies included in this analysis found especially strong protection among tamoxifen users, thus contributing to the evidence that isoflavones may not interfere with this important therapy.[169,170] Women with invasive breast cancer or ductal carcinoma in situ aged 30 to 75 receiving 50 mg soy isoflavones for 12 months showed no significant changes in breast mammographic density or magnetic resonance imaging (MRI) fibroglandular tissue density.[171] Kang et al. found that postmenopausal patients with breast cancer with estrogen- and progesterone-receptor–positive disease and high isoflavone intakes receiving tamoxifen or anastrozole had a significantly reduced risk for recurrence compared with those with lower intakes, although such associations were not seen in premenopausal patients.[172] A U.S. study of postmenopausal women with abnormal mammograms found no association between equol production and breast pathology but also noted that isoflavone intakes among these women were quite low (mean value 0.3 mg), which likely precluded findings.[173]

The intestinal milieu may play an important role in the effects of soy foods on breast cancer risk. In a very interesting 2013 study conducted by Toi et al., regular consumption of both soy isoflavones and the probiotic *Lactobacillus casei* Shirota showed a significant dose-dependent reduction in breast cancer risk, with relative risk decreasing from 0.76 to 0.48 through the second to fourth quartiles of intake[174]; this probiotic strain may help convert soy isoflavone glycosides into their aglycone forms and beneficially influence the microbiota in which isoflavone metabolism takes place.[175]

Soy may also modulate breast cancer recurrence or mortality. Among Korean women with breast cancer, Woo et al. noted in 2012 that although isoflavone intake was inversely associated with recurrence in HER2-negative patients, among HER2-positive patients, high isoflavone intake increased recurrence risk.[176] Single-nucleotide polymorphisms (SNPs) in genes coding for the growth hormone insulin-like growth factor (IGF) and its binding proteins may affect the risk for breast cancer. In 2016 Wang et al. found that one such polymorphism combined with being either overweight or underweight and having a low isoflavone intake could virtually double breast cancer risk in general Chinese female populations as well as postmenopausal women.[177] Another Chinese study also found that higher soy intake interacted

with another IGF-related polymorphism to significantly reduce IGF-1 levels, which, when elevated, increase breast cancer risk.[178] Soy intake may also interact with genetic variation in SNPs for PPARγ; Lee et al. found one that, combined with high soy consumption, was associated with reduced mammographic density compared with low soy consumption, with a decrease of almost 4% in density per copy of the allele in the highest quartile of intake.[179] Other relationships among breast cancer risk, soy intake, and SNPs are being discovered as well.[180,181]

Prostate Cancer

The incidence of prostate cancer is much lower in Japan than in Western nations; however, among Japanese migrants to areas where a Western diet is consumed, prostate cancer rates are six times higher than in their native population.[183] High soy intake among Japanese men is thought to contribute to these findings.

A comprehensive 2018 meta-analysis of 30 studies examining the relationship between soy and prostate cancer concluded that total soy food intake, as well as genistein, daidzein, and unfermented soy food intakes, are associated with a significantly reduced risk for prostate cancer; it further clarified that circulating isoflavone levels were not related to advanced prostate cancer risk.[182]

A 2014 meta-analysis by van Die et al. found modest support for soy and soy isoflavones in reducing prostate cancer risk, and although the researchers concluded that soy and isoflavone supplementation displayed a good safety profile, they noted that soy's effects on prostate-specific antigen (PSA), testosterone, and SHBG levels were not clear.[184] Two 2009 meta-analyses also cautiously concluded that higher soy food intakes reduced prostate cancer risk.[185,186] In 2012 Miyanaga et al. found that Japanese men aged 50 to 75 with PSA levels of 2.5 to 10 ng/mL receiving 60 mg soy isoflavones daily showed a nonsignificant reduction in the risk of prostate cancer compared with placebo, but significantly reduced risk was seen in subjects aged 65 or older; the relatively low incidence of prostate cancer among Japanese men may have precluded significant overall results.[187] In 2015 Wu et al. found that patients with prostate cancer in China had significantly lower median genistein levels in plasma compared with nonprostate cancer patients, suggesting that genistein intake may potentially contribute to the low incidence of prostate cancer in China.[188] In a 2008 Japanese nested case-control study by Kurahashi et al. of men at least 40 years old, higher levels of genistein and equol were dose-dependently associated with significantly reduced risk for total and localized prostate cancer,[189] and similar results were seen in an earlier study by Ozasa et al.[190] In examining diet and lifestyle effects on prostate cancer risk, Leitzmann and Rohrmann concluded that the lack of risk reduction seen in Western countries was due to Western soy intakes not being high enough to produce comparable inverse associations.[191] The 2009 European Prospective Investigation into Cancer and Nutrition (EPIC) study did, however, find a significant inverse relationship between plasma genistein concentrations and prostate cancer risk.[192] A 2013 systematic review suggests that equol producers may have significantly less risk for developing prostate cancer[193]; in rats, equol binds dihydrotestosterone and prevents it from interacting with the androgen receptor.[194]

A 2004 Australian study in men with prostate cancer given bread made with soy grits found that the soy bread lowered total PSA levels and increased the free/total PSA ratio compared with wheat bread.[195] A 2013 multicenter RCT of the effects of 20 g soy protein with 160 mg isoflavones on hot flashes in men with prostate cancer found no effect, although several quality-of-life scores significantly improved over the 12-week study.[196]

Soy intake may also interact with SNPs relating to estrogen and aromatic hydrocarbon metabolism to modify prostate cancer risk. Sonoda et al. discovered a CYP1A1 polymorphism associated with reduced prostate cancer risk despite soy isoflavone intake under 60 mg daily, but a different polymorphism at the same location in combination with an estrogen-receptor SNP may connote high prostate cancer risk even at higher isoflavone intakes.[197]

Other Cancers

Dietary intakes of soy and its components have also generally been associated with reduced risk for other cancers, although the potential for estrogen-modulating effects to contribute to hepatocellular carcinoma risk in women should be noted.

A 2016 meta-analysis of 22 case-control and 18 cohort studies relating soy intakes and gastrointestinal (GI) cancers found small reductions in combined GI cancers as well as colon and colorectal cancers in pooled analysis, whereas subgroup analysis for isoflavones found stronger protective associations with higher intake levels.[198] A case-control study by Shin et al. concluded that compared with lower isoflavone intakes, Korean men and women in the highest quartile showed a reduced risk for colorectal cancer. Stronger relations between soy food intake were found for postmenopausal women, distal colon cancer in men, and rectal cancer in women, but high intake of fermented soy paste was associated with an elevated risk of colorectal cancer in men.[199] In a Chinese population, data trended toward protective effects of nonfermented soy foods on gastric cancer, although overall findings did not reach significance,[200] but an earlier study found that soy foods may reduce colorectal cancer risk in postmenopausal women.[201] A study in a Japanese population found that high intake of soy, especially nonfermented forms, was associated with significantly reduced risk for stomach cancer, which occurs with a higher incidence in Japan than in Western regions.[202] Although an earlier Japanese study in an older population did not reach this same conclusion, it uncovered a trend toward increased gastric cancer risk among women with high isoflavone intakes taking exogenous female hormones.[203] A 2008 study of Japanese men and women aged 45 to 74 found no substantial effect of soy foods and isoflavones on overall colorectal cancer risk, although proximal colon cancer risk in men was inversely related to soy and isoflavone intakes.[204] Among Dutch men aged 40 to 75 at increased risk for colorectal cancer, Vrieling et al. noted that equol producers receiving 84 mg of isoflavones showed an inverse association between serum equol level and that of IGF-1,[203] which is positively linked to increased risk for colorectal and other cancers.

In a multiethnic population of postmenopausal U.S. women aged 45 to 75, higher isoflavone intakes were associated with reduced risk for endometrial cancer,[206] although analogous findings for isoflavones from traditional soy foods were not seen in a Japanese study of women of similar age, which may be partially attributable to the low incidence of this disease in Japan.[207] A 3-year RCT of soy protein with isoflavones in postmenopausal women in the United States found no significant influence on endometrial thickness or incidences of endometrial cancer or hyperplasia,[208] although results of an earlier case-control study suggest that total isoflavone intake may reduce endometrial cancer risk among lean women.[209] A 2014 meta-analysis and a 2011 case-control study have found some evidence that soy foods or isoflavones may reduce the risk for ovarian cancer,[210,211] but a prospective Swedish study showed no significant relationship between soy foods and ovarian cancer risk at any age.[212]

A 2013 meta-analysis showed a borderline inverse association between soy protein intake and the risk for lung cancer, which was significant in nonsmokers.[213] Yang et al. found a significant inverse relationship between soy food intake and the risk for lung cancer (particularly aggressive forms) in Chinese women (especially nonsmokers),[214] and the same research team also demonstrated significant soy

food and isoflavone protection from lung cancer mortality in women with lung cancer, which was stronger among never-smokers.[215] Other studies have shown that estrogen therapy increased the incidence of and mortality from lung cancer,[216] whereas tamoxifen reduced lung cancer deaths in patients with breast cancer,[217] supporting an estrogenic link to lung cancer etiology.

In 2009 Kurahashi et al. identified a dose-dependent positive association between genistein and daidzein intakes in women (but not in men) and the risk for hepatocellular carcinoma (HCC). Natto and tofu consumption showed the strongest associations among soy food types, and the relationship was also seen in women positive for either or both hepatitis virus B (HBV) and hepatitis virus C (HCV); the authors suggested, based on previous research in Asian populations, that estrogen may play a protective role against HCC.[218]

A distinctive 2003 study by Haselkorn et al. showed an inverse relationship between soy isoflavone intake and the risk for thyroid cancer in Southeast Asian women living in the United States, although contributions from other factors were also important.[219] Genistein has shown in vitro synergy with photodynamic therapy used to induce apoptosis in thyroid cancer cells.[220]

One accepted mechanism by which soy isoflavones modulate the risk for hormone-related conditions is by competing with endogenous estrogens for estrogen receptor binding while providing a fraction of the hormonal influence of estrogen, thereby modulating estrogen's stronger influences on cell growth and proliferation; isoflavones are estimated to have about 1/1000th the potency of estradiol activation at estrogen receptor (ER)-α and around one third of estrogen's potency at ERβ.[221] Direct chemopreventive and antiproliferative mechanisms of action for soy components and metabolites have also been investigated, including inhibition of angiogenesis[222]; antagonism of steroid hormone and growth factor signaling pathways; modulation of prostaglandin metabolism; dihydrotestosterone binding and sequestration[223]; modification of DNA methylation and other epigenetic events[224,225]; encouraging cell differentiation; inhibiting matrix metalloprotease (MMP) production and related cancer cell detachment, invasion, and metastasis[226,227]; DNA repair; upregulation of *BRCA1* expression[228]; inhibiting androgen receptor and PSA expression[229]; inhibition of nuclear factor (NF)-κB pathway activation, DNA topoisomerase,[230] and 5α-reductase[231]; antioxidative effects; and influencing a variety of kinases related to cell cycles, cellular energy production, and apoptosis.[232–234] Fan et al. found that genistein may inhibit breast cancer cell proliferation and encourage apoptosis in these cells by inhibiting stem cell activation and early tissue development.[235] Genistein, soy isoflavones, and equol have been shown to modulate aromatase activity both positively and negatively in laboratory work, and although there is little clinical evidence to confirm how soy influences aromatase in humans, genistein could potentially interact with drugs used to inhibit aromatase.[236–240] Genistein may also enhance the activities of other chemo-, photo-, and radiotherapies, although the specificity and extent of these effects are not fully understood.[241]

Menopausal Symptoms

The observation that Japanese women experience around one-tenth the incidence of vasomotor symptoms in menopause compared with women consuming Western diets led to great interest in causative factors, including phytoestrogens such as those in soy.[242] More recent research has focused on soy isoflavones and on the daidzein metabolite equol. Considering the risks associated with hormone replacement, soy foods and their ingredients may present a reasonable alternative to many women.

In a 2016 crossover RCT by Tranche et al., postmenopausal women with menopausal symptoms receiving 15 g soy protein with 50 mg isoflavones showed significant reductions in climacteric and urogenital symptoms and significantly improved health-related quality-of-life scores on the Menopause Rating Scale compared with controls.[243] A 2012 meta-analysis by Taku et al. found that soy isoflavone supplementation (generally more than 50 mg daily) decreased the frequency and severity of menopausal hot flashes by 20.62% and 20.19% over placebo, respectively,[244] and a 2014 review of clinical trials of isoflavones for menopausal symptoms came to a similar conclusion.[245] Postmenopausal Brazilian women receiving 60 mg soy isoflavones daily showed significant reductions in hot flashes compared with placebo as well as increased estradiol and HDL levels, reduced LDL levels, and no changes in vaginal cytology.[246]

S-Equol has been commercially produced using a human strain of *Lactococcus* on soy germ, and Japanese nonequol producers with menopausal symptoms receiving 10 mg of this S-equol preparation experienced fewer and less severe hot flashes and less neck and shoulder stiffness and showed trends toward the improvement of other symptoms as well.[247] A 2015 study of perimenopausal women found that about 35% produced equol, and that among equol producers, those in the highest quartile of daidzein intake were 76% less likely to have vasomotor symptoms than those in the lowest quartile, whereas there was no association between daidzein and vasomotor symptoms in nonproducers.[248] An earlier study employing S-equol for menopausal symptoms found that in nonproducers, supplementing 30 mg S-equol significantly decreased all symptom scores except for depression, whereas overall results in producers and nonproducers showed significant improvements in measures of depression, tension/anxiety, fatigue, and vigor.[249]

A 2017 cross-sectional study of breast cancer survivors found that the highest soy food intake correlated with halving of menopausal symptoms and fatigue[250]; however, in a study of Chinese women aged 20 to 75 being treated for breast cancer, higher soy food intake was not found to reduce menopausal symptoms and was associated with an increased number of hot flashes in premenopausal patients.[251]

Osteoporosis

A considerable body of research has been devoted to investigating the impact of isoflavones with or without soy protein on measures of bone mineral density (BMD), bone formation, and bone resorption.

Several meta-analyses and RCTs in peri- and postmenopausal women have concluded that soy isoflavones (with or without soy protein) can attenuate spinal bone loss (especially lumbar), decrease levels of bone resorption markers, and/or increase levels of bone formation in these at-risk populations.[252–258] A 2010 meta-analysis of isoflavones including 1240 menopausal women indicated that an average intake of 82 mg soy isoflavones significantly increased spinal BMD by 22.25 mg/cm (2.38%) over 6 to 12 months compared with controls, although similar results were not found at the hip.[259] A 2009 randomized crossover trial in postmenopausal women found that isoflavones from soy suppressed net bone resorption, although to a lesser extent than 5 mg risedronate or 1 mg estradiol plus 2.5 mg medroxyprogesterone. The researchers noted that serum genistein levels reflected antiresorptive effects, whereas those of daidzein, equol, and O-desmethylangolensin did not.[260] A 2-year multicenter trial of 120 mg soy isoflavones in menopausal women found reduced whole-body bone loss yet not at the hip or lumbar spine; other evidence suggested that higher genistein delivery may have contributed to isoflavone effects previously seen in the lumbar spine.[261] This contrasts the 2015 conclusion of Pawlowski et al. that mixed isoflavones were more effective than a genistein-rich isoflavone preparation in increasing bone calcium retention in postmenopausal women. In this randomized trial, equol production status did not alter the effects.[262] A review of 15 clinical trials by Messina

et al. reported that although clinical data indicate daily intake of approximately 80 mg isoflavones is needed to derive skeletal benefits, epidemiological evidence suggests that lower amounts may also be efficacious.[263] Sathyapalan et al. showed that women experiencing early menopause and receiving 15 g soy protein with 66 mg isoflavones daily for 6 months showed significant reductions in a marker of bone resorption as well as reductions in fasting insulin and glucose, insulin resistance, and systolic blood pressure, whereas no changes in insulin resistance or blood pressure were seen in women receiving soy protein alone.[264] The same research team provided this treatment to men with type 2 diabetes with hypogonadism and found that it also significantly reduced the same marker of bone resorption in this population and that the drop correlated positively with reductions in Hb A_{1c} levels and HOMA-IR scores.[265] In pre- or early menopausal Chinese women, Chi et al. noted that 90 mg isoflavones daily increased tibial bone density, reduced menopause-related symptom scores, and lowered levels of alkaline phosphatase, IL-6, and TNF-α.[266] In Taiwanese women in early postmenopause (a particularly susceptible period), 100 mg isoflavones significantly increased lumbar and femoral neck BMD after 1 year of supplementation.[267] In breast cancer survivors, however, soy isoflavone intakes over ~62 mg daily have been associated with lower proximal forearm BMD, especially early in menopause.[268]

The evidence thus points to isoflavones as the soy component responsible for the beneficial bone effects observed.[269] They may act by binding estrogen receptors, regulating estrogen receptor gene expression, increasing serum osteocalcin levels, influencing alkaline phosphatase activity, increasing insulin-like growth factor transcription in bone, decreasing TNF-α levels, and modulating gene expression of bone-related genes, among other mechanisms.[270] In vitro research by Tadaishi et al. suggests that carotenoids such as beta-carotene, lutein, and zeaxanthin may cooperate with soy isoflavones in suppressing the formation of osteoclasts and thereby reducing bone resorption.[271] It may be interesting to note that although soy milk contains oxalate and phytate, which are known to inhibit calcium absorption, these compounds did not negatively affect the uptake of this mineral from calcium-fortified soy milk in women.[272]

Cognitive Function

Despite modest positive trial results in the early 2000s,[273–275] more recent direct evidence for cognitive benefit from soy and isoflavones is lacking. However, the indirect effects of soy constituents on estrogen and energy metabolism, cardiovascular function, and redox homeostasis may contribute to overall cognitive health.

A 2015 meta-analysis of lifestyle contributions to cognitive decline concluded that soy isoflavones may improve memory without affecting global cognition.[276] The largest and longest trial in this analysis found improvements in measures of visual memory,[277] although further analysis found an inverse association between urinary isoflavonoids and general intelligence test scores.[278] Healthy men receiving 116 mg isoflavones daily showed improvements in measures of spatial working memory.[279] Postmenopausal women in the United States receiving 20 g soy protein with 160 mg isoflavones for 12 weeks showed no significant change in cognition.[280] Follow-up analyses on data collected in the Women's Health Initiative and its Memory Study found evidence of a positive effect on cognitive function from isoflavones, although not all studies concurred.[281] Two 2007 studies found no cognitive changes in postmenopausal women after isoflavone supplementation.[282,283] A notable recent finding is that, among older Japanese, equol production has been identified as an independent risk factor for mild cognitive impairment (MCI).[284] Men and women with Alzheimer's disease receiving 100 mg isoflavones daily for 6 months showed no changes in cognition compared with placebo, although plasma equol levels correlated positively with measures of speeded dexterity and verbal fluency.[285]

Fatty Liver Disease

Limited research suggests that soy consumption may hold benefits for fatty liver disease. A 2014 study in patients with nonalcoholic fatty liver disease (NAFLD) compared the effects of a low-calorie diet (LCD), a low-carbohydrate LCD, and low-carbohydrate LCD containing soy found that the soy-containing diet produced greater reductions in alanine and aspartate aminotransferases (ALT and AST), fibrinogen, and malondialdehyde compared with the other diets.[286] A 2012 review of the mechanisms of NAFLD concluded that soy constituents may prevent or even reduce fatty accumulation in the liver by modifying lipid metabolism and the genetic expression of sterol regulatory-element binding proteins and PPARα.[287] In patients with hepatitis C given 32 g soy protein daily, significant reductions in ALT levels were seen, and in a subgroup (34.7%) with steatosis, it significantly improved associated measures, including gamma-glutamyltransferase (GGT) levels.[288]

Soy Isoflavone Safety

(Please also refer to Pharmacology and Clinical Applications sections.)

Soy products are generally well tolerated, and the most common adverse effects are constipation, bloating, and nausea, although insomnia, migraines, rash, itching, and hypersensitivity reactions have also been reported.

In perhaps the most comprehensive review of soy isoflavone safety in relation to menopausal health in women, the EFSA in 2015 found no effects on endometrial thickness and no histopathological uterine changes considering intakes of up to 150 mg soy isoflavones per day for up to 30 months. Additionally, the EFSA found no support for the hypothesis that isoflavone supplementation increases the risk for breast cancer or alters mammographic density or thyroid hormone levels. The EFSA concluded that dosages of soy isoflavones used in intervention studies (ranging from around 40 to 100 mg isoflavones daily) could guide consumers as to daily supplementation levels.[289]

Recent clinical and preclinical studies suggest that isoflavones can influence the production and regulation of thyroid hormones both positively and negatively.[290–295] Although not frequently seen in humans, it is important to respect the potential for high soy intake to precipitate or exacerbate thyroid dysfunction, especially among women, children, and iodine-deficient individuals. In those with low-iodine status, soy products may increase TSH levels, and TSH may need to be monitored. A 2006 review of 14 clinical trials concluded that in euthyroid individuals receiving sufficient iodine, there was no evidence that isoflavones affect thyroid function.[296] Women experiencing early menopause and given 15 g soy protein with 66 mg isoflavones showed significant increases in TSH as well as a reduction in free thyroxine levels, although no hypothyroid symptoms or changes in free T3 levels were seen.[297] High soy intake may lower parathyroid hormone levels in postmenopausal women.[298]

DRUG–NUTRIENT INTERACTIONS

Soy in various forms has been reported to interact with several types of drugs, and some soy constituents' mechanisms of action may have additive effects with some drugs. Soy may have additive effects with antidiabetic drugs and may increase the risk for hypoglycemia, and drug dosages may need to be adjusted. Soy protein may have additive effects with hypotensive drugs and may increase the risk for hypotension, and drug dosages may need to be adjusted. Soy products may competitively inhibit estrogens and may have additive effects with antiestrogenic drugs, and drug dosages may need to be adjusted. Some

soy products may inhibit platelet aggregation and may interact with blood clotting agents; blood clotting function may need to be monitored and drug dosages adjusted.[299] Soy protein may decrease the absorption of oral levothyroxine, and these substances should be used at separate times.[300] Those with cancer or other hormone-sensitive health conditions should discuss soy with their physicians because soy components may interact with hormone therapies. Very high intake of soy may increase the risk of bladder cancer. Women testing positive for HBV or HCV may be advised to avoid isoflavone supplementation.

Cadmium Content

Chronic, low-level cadmium exposure has been associated with an increased risk of several conditions, including osteoporosis, kidney damage, hypertension, insulin resistance, cancer, diabetes, uterine fibroids, and increased rates of miscarriage. A study from the Fred Hutchinson Cancer Research Center in Seattle, Washington, discovered that the greatest dietary contributor of cadmium was tofu.[69] This study included several other food sources of soy, such as tempeh, tofu hot dogs, tofu cheese, and soy burgers. The data revealed that once-weekly consumption of tofu increased urinary cadmium by 0.11 μg/g, only 0.02 μg/g less than the increase attributed to smoking. In women who ingested any of the listed tofu products twice weekly or more, the urinary cadmium burden increased by 0.30 μg/g, more than twice the increase found from smoking. Presumably (the research is inconsistent) this was due to the soy being grown conventionally with high-phosphate fertilizers that are commonly contaminated with cadmium. Those consuming soy foods are recommended to select soy products from organically grown soybeans or products that have been shown to have no cadmium contamination.

CONCLUSION

From early interest in soy as an alternative protein source to curiosity about whether or not one can be converted into a producer of beneficial soy metabolites, studies conducted in humans, animals, test tubes, and in silico are increasingly reassuring about soy's benefits for most populations while also clarifying the characteristics of individuals who may be at risk for its uncommon hazards. Science is additionally seeking ways to increase soy foods' palatability as well as the bioavailability of their isoflavones.[301]

Unlocking the benefits of soy foods is relatively easy for the majority with no sensitivity to soy: enjoy more of the broad variety of food choices, from creamy yuba (skimmed from tofu-making) and fermented soy milk to high-fiber soy hulls and chewy texturized vegetable protein used in meat-mimicking foods and snacks. Longtime soy researcher Mark Messina has recommended the following optimal intakes for soy and its components for women: 15 to 20 g soy protein (more if cholesterol reduction is the goal) and 50 to 90 mg soy isoflavones daily,[302] which corresponds to a few servings of soy foods.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Klein MA, Nahin RL, Messina MJ, et al. Guidance from an NIH workshop on designing, implementing, and reporting clinical studies of soy interventions. *J Nutr*. 2010;140:1192S–1204S.
2. Duke's D. Phytochemical and Ethnobotanical databases. Available at: https://phytochem.nal.usda.gov.
3. Murphy PA, Barua K, Hauck CC. Solvent extraction selection in the determination of isoflavones in soy foods. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2002;777:129–138.
4. Xiao CW. Health effects of soy protein and isoflavones in humans. *J Nutr*. 2008;138:1244S–1249S.
5. Coward L, Barnes NC, Setchell KD, Barnes S. Genistein, daidzein, and their beta-glycoside conjugates: antitumor isoflavones in soybean foods from american and asian diets. *J Agric Food Chem*. 1993;41:1961–1967.
6. Barnes S, Peterson TG, Coward L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *J Cell Biochem*. 1995;22(suppl):181–187.
7. Messina M, Nagata C, Wu AH. Estimated asian adult soy protein and isoflavone intakes. *Nutr Cancer*. 2006;55:1–12.
8. Zamora-Ros R, Knaze V, Lujan-Barroso L, et al. Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. *Eur J Clin Nutr*. 2012;66:932–941.
9. Cotterchio M, Boucher BA, Kreiger N, et al. Dietary phytoestrogen intake—lignans and isoflavones—and breast cancer risk (Canada). *Cancer Causes Control*. 2008;19:259–272.
10. Rizzo G, Baroni L. Soy, Soy Foods and Their Role in Vegetarian Diets. *Nutrients*. 2018;10(1). pii: E43. https://doi.org/10.3390/nu10010043.
11. Lammersfeld CA, King J, Walker S, et al. Prevalence, sources, and predictors of soy consumption in breast cancer. *Nutr J*. 2009;8:2.
12. Setchell KD, Zimmer-Nechemias L, Cai J, et al. Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet*. 1997;350:23–27.
13. Hughes GJ, Ryan DJ, Mukherjea R, Schasteen CS. Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) for soy protein isolates and concentrate: criteria for evaluation. *J Agric Food Chem*. 2011;59:12707–12712.
14. Henley EC, Kuster JM. Protein quality evaluation by protein digestibility-corrected amino acid scoring. *Food Technology*. 1994;48:74–77.
15. Rand WM, Pellett PL, Young VR. Meta-analysis of nitrogen balances studies for estimating protein requirements in healthy adults. *Am J Clin Nutr*. 2003;77:109–127.
16. Code of Federal Regulations Title 21, Subchapter B, Part 101, Subpart E, §101.83. Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD). 65 FR 54717, Sept. 8, 2000; 65 FR 70466, Nov. 24, 2000, as amended at 66 FR 66742, Dec. 27, 2001; 68 FR 15355, Mar. 31, 2003; 70 FR 41958, July 21, 2005.
17. Health Canada. Abbreviated Labelling Standards. *Free Plant Sterols*. Available at: http://webprod.hc-sc.gc.ca. Accessed 8 Sept 2019.
18. EFSA. Opinion on the substantiation of health claims related to plant sterols and plant stanols and maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140), and maintenance of normal prostate size and normal urination (ID 714, 1467, 1635) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA J*. 2010;8(10):1813
19. Oakenfull D. Soy protein, saponins and plasma cholesterol. *J Nutr*. 2001;131:2971–2972.
20. Graf E, Eaton JW. Dietary suppression of colonic cancer: fiber or phytate? *Cancer*. 1985;56:717–718.
21. Vucenik I, Tomazic VJ, Fabian D, et al. Antitumor activity of phytic acid (inositol hexaphosphate) in murine transplanted and metastatic fibrosarcoma, a pilot study. *Cancer Lett*. 1992;65:9–13.
22. Takagi A, Kano M, Kaga C. Possibility of breast cancer prevention: use of soy isoflavones and fermented soy beverage produced using probiotics. *Int J Mol Sci*. 2015;16:10907–10920.
23. Wong JM, Kendall CW, de Souza R, et al. The effect on the blood lipid profile of soy foods combined with a prebiotic: a randomized controlled trial. *Metab Clin Exp*. 2010;59(9):1331–1340.
24. Xu X, Harris KS, Wang HJ, et al. Bioavailability of soybean isoflavones depends on gut microflora in women. *J Nutr*. 1994;124:2307–2315.
25. Reverri EJ, Slupsky CM, Mishchuk DO, Steinberg FM. Metabolomics reveals differences between three daidzein metabolizing phenotypes in adults with cardiometabolic risk factors. *Mol Nutr Food Res*. 2017;61(1):1800132.
26. Setchell KD, Brown NM, Desai P, et al. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J Nutr*. 2001;131:1362S–1375S.
27. Setchell KD. Absorption and metabolism of soy isoflavones-from food to dietary supplements and adults to infants. *J Nutr*. 2000;130:654S–6545S.
28. Kano M, Takayanagi T, Harada K, et al. Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *J Nutr*. 2006;136:2291–2296.
29. Okabe Y, Shimazu T, Tanimoto H. Higher bioavailability of isoflavones after a single ingestion of aglycone-rich fermented soybeans compared with glucoside-rich non-fermented soybeans in Japanese postmenopausal women. *J Sci Food Agric*. 2011;91(4):658–663.
30. Hutchins AM, Slavin JL, Lampe JW. Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J Am Diet Assoc*. 1995;95:545–551.
31. Izumi T, Piskula MK, Osawa S, et al. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr*. 2000;130:1695–1699.
32. Rufer CE, Bub A, Moseneder J, et al. Pharmacokinetics of the soybean isoflavone daidzein in its aglycone and glucoside form: a randomized, double-blind, crossover study. *Am J Clin Nutr*. 2008;87(5):1314–1323.
33. Zubik L, Meydani M. Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *Am J Clin Nutr*. 2003;77(6):1459–1465.
34. Manach C, Williamson G, Morand C, et al. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(suppl):230S–242S.
35. Franke AA, Lai JF, Halm BM. Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake. *Arch Biochem Biophys*. 2014;559:24–28.
36. Brown NM, Galandi SL, Summer SS, et al. S-(-)equol production is developmentally regulated and related to early diet composition. *Nutr Res*. 2014;34(5):401–409.
37. Setchell KD, Clerici C, Lephart ED, et al. S-Equol, A potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by the human intestinal bacterial flora. *Am J Clin Nutr*. 2005;81:1072–1079.
38. Lund TD, Munson DJ, Haldy ME, et al. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod*. 2004;70(4):1188–1195.
39. Liu Z, Ho SC, Chen Y, et al. Cardiovascular risks in relation to daidzein metabolizing phenotypes among Chinese postmenopausal women. *PLoS One*. 2014;9(2):e87861.
40. Miller LM, Lampe JW, Newton KM, et al. Being overweight or obese is associated with harboring a gut microbial community not capable of metabolizing the soy isoflavone daidzein to O-desmethylangolensin in peri- and post-menopausal women. *Maturitas*. 2017;99:37–42.
41. Lampe JW. Is equol the key to the efficacy of soy foods? *Am J Clin Nutr*. 2009;89(suppl):1664S–1667S.
42. Franke AA, Lai JF, Halm BM, et al. Equol production changes over time in postmenopausal women. *J Nutr Biochem*. 2012;23(6):573–579.
43. Franke AA, Lai JF, Pagano I, et al. Equol production changes over time in premenopausal women. *Br J Nutr*. 2012;107(8):1201–1206.
44. Hedlund TE, Maroni PD, Ferucci PG, et al. Long-term dietary habits affect soy isoflavone metabolism and accumulation in prostatic fluid in caucasian men. *J Nutr*. 2005;135:1400–1406.
45. Tanaka M, Fujimoto K, Chihara Y, et al. Isoflavone supplements stimulated the production of serum equol and decreased the serum dihydrotestosterone levels in healthy male volunteers. *Prostate Cancer Prostatic Dis*. 2009;12(3):247–252.
46. Guadamuro L, Dohrmann AB, Tebbe CC, Mayo B, Delgado S. Bacterial communities and metabolic activity of faecal cultures from equol pro-

ducer and non-producer menopausal women under treatment with soy isoflavones. *BMC Microbiol*. 2017;17:93.
47. Nakatsu CH, Armstrong A, Clavijo AP, et al. Fecal bacterial community changes associated with isoflavone metabolites in postmenopausal women after soy bar consumption. *PLoS One*. 2014;9(10):e108924.
48. Guadamuro L, Delgado S, Redruello B, et al. Equol status and changes in fecal microbiota in menopausal women receiving long-term treatment for menopause symptoms with a soy-isoflavone concentrate. *Front Microbiol*. 2015;6:777.
49. Code of Federal Regulations, 21 CFR Part 107. Rules and regulations: nutrient requirements for infant formulas. *Fed Regist*. 1985;50:45106–45108.
50. EFSA. Scientific Opinion on the essential composition of infant and follow-on formulae. *EFSA J*. 2014;12(7):3760.
51. National toxicology program/center for the evaluation of risks to human reproduction. NTP-CERHR Monograph on soy infant formula. *NIH Publication No. 10-5995*. September 2010.
52. Cao YA, Calafat AM, Doerge DR, et al. Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula. *J Espo Sci Environ Epidemiol*. 2009;19:223–234.
53. Vandenplas Y, Castrellon PG, Rivas R, et al. Safety of soya-based infant formulas in children. *Br J Nutr*. 2014;111(8):1340–1360.
54. Badger TM, Gilchrist JM, Pivik RT, et al. The health implications of soy infant formula. *Am J Clin Nutr*. 2009;89(suppl):1668S–1672S.
55. Bhatia J, Greer F, the Committee on Nutrition. Use of soy protein-based formulas in infant feeding. *Pediatrics*. 2008;121(5):1062–1068.
56. Andres A, Cleves MA, Bellando JB, et al. Developmental status of 1-year-old infants fed breast milk, cow's milk formula, or soy formula. *Pediatrics*. 2012;129(6):1134–1140.
57. Portman MA, Navarro SL, Bruce ME, Lampe JW. Soy isoflavone intake is associated with risk of Kawasaki disease. *Nutr Res*. 2016;36(8):827–834.
58. Hooper L, Ryder JJ, Kurzer MS, et al. Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis. *Hum Reprod Update*. 2009;15(4):423–440.
59. Alekel DL, Genschel U, Koehler KJ, et al. Soy Isoflavones for Reducing Bone Loss (SIRBL) Study: effect of a three-year trial on hormones, adverse events, and endometrial thickness in postmenopausal women. *Menopause*. 2015;22(2):185–197.
60. Fuhrman BJ, Pfeiffer R, Xu X, et al. Soy intake is associated with increased 2-hydroxylation and decreased 16alpha-hydroxylation of estrogens in Asian-American women. *Cancer Epidemiol Biomarkers Prev*. 2009;18(10):2753–2760.
61. Nettleton JA, Greany KA, Thomas W, et al. The effect of soy consumption on the urinary 2:16-hydroxyestrone ratio in postmenopausal women depends on equol production status but is not influenced by probiotic consumption. *J Nutr*. 2005;135:603–608.
62. Sapbamrer R, Visavarungroj N, Suttajit M. Effects of dietary traditional fermented soybean on reproductive hormones, lipids, and glucose among postmenopausal women in northern Thailand. *Asia Pac J Clin Nutr*. 2013;22(2):222–228.
63. Hooper L, Madhavan G, Tice JA, et al. Effects of isoflavones on breast density in pre- and post-menopausal women: a systematic review and meta-analysis of randomized controlled trials. *Hum Reprod Update*. 2010;16(6):748–760.
64. Maskarinec G, Verheus M, Tice JA. Epidemiologic studies of isoflavones & mammographic density. *Nutrients*. 2010;2:35–48.
65. Messina M. Soybean isoflavone exposure does not have feminizing effects on men: a critical examination of the clinical evidence. *Fertil Steril*. 2010;93(7):2095–2104.
66. Dillingham BL, McVeigh BL, Lampe JW, Duncan AM. Soy protein isolates of varying isoflavone content exert minor effects on serum reproductive hormones in healthy young men. *J Nutr*. 2005;135:584–591.
67. Hamilton-Reeves JM, Vazquez G, Duval SJ, et al. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. *Fertil Steril*. 2010;94(3):997–1007.
68. Tanaka M, Fujimoto K, Chihara Y, et al. Isoflavone supplements stimulated the production of serum equol and decreased the serum dihydrotestosterone levels in healthy male volunteers. *Prostate Cancer Prostatic Dis*. 2009;12(3):247–252.
69. Adams SV, Newcomb PA, Shafer MM, et al. Sources of cadmium exposure among healthy premenopausal women. *Sci Total Environ*. 2011;409(9):1632–1637. PubMed PMID: 21333327.
70. Sacks FM, Lichtenstein A, Van Horn L, et al. Soy protein, isoflavones, and cardiovascular health: an americal heart association science advisory for professionals from the nutrition committee. *Circulation*. 2006;113:1034–1044.
71. Code of Federal regulations. 21 CFR Part 101. Food Labeling: health claims, soy protein and coronary heart disease. *Federal Register*. 1999;64:57699–57733.
72. Code of Federal Regulations. 21 CFR Part 101. Food Labeling: Health Claims, Soy Protein and Coronary Heart Disease. *Proposed Rule*. Docket No. FDA-2017-N-0763. Posted October 30, 2017.
73. Sacks FM, Lichtenstein A, Van Horn L, et al. Soy protein, isoflavones, and cardiovascular health: an americal heart association science advisory for professionals from the nutrition committee. *Circulation*. 2006;113:1034–1044.
74. Yang W, Gage H, Jackson D, Raats M. The effectiveness and cost-effectiveness of plant sterol or stanol-enriched functional foods as a primary prevention strategy for people with cardiovascular disease risk in England: a modeling study. *Eur J Health Econ*. 2017. https://doi.org/10.1007/s10198-017-0934-2.
75. Zhang X, Shu XO, GAo YT, et al. Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. *J Nutr*. 2003;133:2874–2878.
76. Sasazuki S, Fukuoka Heart Study Group. Case-control study of nonfatal myocardial infarction in relation to selected food in Japanese men and women. *Jpn Circ J*. 2001;65:200–206.
77. Nagata C. Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan. *Int J Epidemiol*. 2000;29:832–836.
78. Hodis HN, Mack WJ, Kono N, et al. Isoflavone soy protein supplementation and atherosclerosis progression in healthy postmenopausal women: a randomized controlled trial. *Stroke*. 2011;42:3168–3175.
79. Barnes S. Evolution of the health benefits of soy isoflavones. *Proc Soc Exp Biol Med*. 1998;217:386–392.
80. Kurzer MS, Xu X. Dietary phytoestrogens. *Annu Rev Nutr*. 1997;17:353–381.
81. Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. *J Clin Endocrinol Metab*. 2001;86:41–47.
82. Kokubo Y, Iso H, Ishihara J, et al. Association of dietary intake of soy, beans, and isoflavones with risk of cerebral and myocardial infarctions in Japanese populations. *Circulation*. 2007;116:2553–2562.
83. Yu D, Shu XO, Li H, et al. Dietary isoflavones, urinary isoflavonoids, and risk of ischemic stroke in women. *Am J Clin Nutr*. 2015;102:680–686.
84. Talaei M, Koh WP, van Dam RM, et al. Dietary soy intake is not associated with risk of cardiovascular disease mortality in Singapore Chinese adults. *J Nutr*. 2014;144:921–928.
85. Harland JI, Haffner TA. Systematic review, meta-analysis and regression of randomised controlled trials reporting an association between an intake of circa 25 g soy protein per day and blood cholesterol. *Atherosclerosis*. 2008;200:13–27.
86. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med*. 1995;333:276–282.
87. Sacks FM, Lichtenstein A, Van Horn L, et al. Soy protein, isoflavones, and cardiovascular health: an American heart association science advisory for professionals from the nutrition committee. *Circulation*. 2006;113:1034–1044.
88. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr*. 2005;81:397–408.

89. Taku K, Umegaki K, Sato Y, et al. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. *Am J Clin Nutr*. 2007;85:1148–1156.
90. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr*. 2005;81:397–408.
91. Jenkins DJ, Kendall CW, Jackson CJ, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr*. 2002;76:365–372.
92. Baum J, Teng H, Erdman JW, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *Am J Clin Nutr*. 1998;68:545–551.
93. Pipe EA, Gobert CP, Capes SE, et al. Soy protein reduces serum LDL cholesterol and the LDL cholesterol:HDL cholesterol and apolipoprotein B:apolipoprotein A-I ratios in adults with type 2 diabetes. *J Nutr*. 2009;139:1700–1706.
94. Wong JM, Kendall CS, Marchie A, et al. Equol status and blood lipid profile in hyperlipidemia after consumption of diets containing soy foods. *Am J Clin Nutr*. 2012;95:564–571.
95. Allen JK, Becker DM, Kwiterovich PO, et al. Effect of soy protein-containing isoflavones on lipoproteins in postmenopausal women. *Menopause*. 2007;14(1):106–114.
96. Puska P, Korpelainen V, Hoie LH, et al. Soy in hypercholesterolaemia: a double-blind, placebo-controlled trial. *Eur J Clin Nutr*. 2002;56(4):352–357.
97. Wofford MR, Rebholz CM, Reynolds K, et al. Effect of soy and milk protein supplementation on serum lipid levels: a randomized controlled trial. *Eur J Clin Nutr*. 2012;66(4):419–425.
98. Liu ZM, Ho SC, Chen YM, et al. Whole soy, but not purified daidzein, had a favorable effect on improvement of cardiovascular risks: a 6-month randomized, double-blind, and placebo-controlled trial in equol-producing postmenopausal women. *Mol Nutr Food Res*. 2014;58(4):709–717.
99. Wong JM, Kendall CW, de Souza R, et al. The effect on the blood lipid profile of soy foods combined with a prebiotic: a randomized controlled trial. *Metab Clin Exp*. 2010;59(9):1331–1340.
100. Teede HJ, Dalais FS, Kotsopolous D, et al. Dietary soy has both beneficial and potentially adverse cardiovascular effects: a placebo-controlled study in men and postmenopausal women. *J Clin Endocrinol Metab*. 2001;86:3053–3060.
101. Rivas M, Garay RP, Escanero JF, et al. Soy milk lowers blood pressure in men and women with mild to moderate essential hypertension. *J Nutr*. 2002;132:1900–1902.
102. Sagara M, Kanda T, NJelekera M, et al. Effects of dietary intake of soy protein and isoflavones on cardiovascular disease risk factors in high risk, middle-aged men in Scotland. *J Am Coll Nutr*. 2003;23(1):86–91.
103. Washburn S, Burke GL, Morgan T, et al. Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in premenopausal women. *Menopause*. 1999;6:7–13.
104. Burke GL, Hodgson JM, Beilin LJ, et al. Dietary protein and soluble fiber reduce ambulatory blood pressure in treated hypertensives. *Hypertension*. 2001;38:821–826.
105. Sacks FM, Lichtenstein A, Van Horn L, et al. Soy protein, isoflavones, and cardiovascular health: an American heart association science advisory for professionals from the nutrition committee. *Circulation*. 2006;113:1034–1044.
106. Balk E, Chung M, Chew P, et al. *Effects of Soy on Health Outcomes. Evidence Report/Technology Assessment No. 126*. AHRQ Publication No. 05-E024-2; 2005.
107. Nozue M, Shimazu T, Sasazuki S, et al. Fermented soy product intake is inversely associated with the development of high blood pressure: the Japan public health center-based prospective study. *J Nutr*. 2017;147(9):1749–1756.
108. Kreijkamp-Kaspers S, Kok L, Bots ML, et al. Randomized controlled trial of the effects of soy protein containing isoflavones on vascular function in postmenopausal women. *Am J Clin Nutr*. 2005;81:189–195.
109. Ramdath DD, Padhi EM, Sarfaraz S, et al. Beyond the cholesterol-lowering effect of soy protein: a review of the effects of dietary soy and its constituents on risk factors for cardiovascular disease. *Nutrients*. 2017;9:324.
110. Chalvon-Demersay T, Azzout-Marniche D, Arfsten J, et al. A systematic review of the effects of plant compared with animal protein sources on features of metabolic syndrome. *J Nutr*. 2017;147:281–292.
111. Li SH, Liu XX, Bai YY, et al. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. *Am J Clin Nutr*. 2010;91:480–486.
112. Reverri EJ, LaSalle CD, Franke AA, Steinberg FM. Soy provides modest benefits on endothelial function without affecting inflammatory biomarkers in adults at cardiometabolic risk. *Mol Nutr Food Res*. 2015;59(2):323–333.
113. Wu SH, Shu XO, Chow WH, et al. Soy food intake and circulating levels of inflammatory markers in Chinese women. *J Acad Nutr Diet*. 2012;112(7):996–1004.
114. Orgaard A, Jensen L. The effects of soy isoflavones on obesity. *Exp Biol Med*. 2008;233:1066–1080.
115. Liao FH, Shieh MJ, Yang SC, et al. Effectiveness of a soy-based compared with a traditional low-calorie diet on weight loss and lipid levels in overweight adults. *Nutrition*. 2007;23(7–8):551–556.
116. Deibert P, Konig D, Schmist-Trucksaess A, et al. Weight loss without losing muscle mass in pre-obese and obese subjects induced by a high-soy-protein diet. *Int J Obes*. 2004;28:1349–1352.
117. Anderson JW, Hoie LH. Weight loss and lipid changes with low-energy diets: comparator study of milk-based versus soy-based liquid meal replacement interventions. *J Am Coll Nutr*. 2005;24(3):210–216.
118. Charles C, Yuskavage J, Carlson O, et al. Effects of high-dose isoflavones on metabolic and inflammatory markers in healthy postmenopausal women. *Menopause*. 2009;16(2):395–400.
119. Sites CK, Cooper BC, Toth MJ, et al. Effect of a daily supplement of soy protein on body composition and insulin secretion in postmenopausal women. *Fertil Steril*. 2007;88(6):1609–1617.
120. Christie DR, Grant J, Darnell BE, et al. Metabolic effects of soy supplementation in postmenopausal white and African American women: a randomized, placebo-controlled trial. *Am J Obstet Gynecol*. 2010;203(2):153.e1–153.e9.
121. Liao FH, Shieh MJ, Yang SC, et al. Effectiveness of a soy-based compared with a traditional low-calorie diet on weight loss and lipid levels in overweight adults. *Nutr*. 2007;23:551–556.
122. Bakhtiari A, Yassin Z, Hanachi P, et al. Effects of soy on body composition: a 12-week randomized controlled trial among Iranian elderly women with metabolic syndrome. *Iran J Publ Health*. 2012;41(4):9–18.
123. Bakhtiary A, Yassin Z, Hanachi P, et al. Effects of soy on metabolic biomarkers of cardiovascular disease in elderly women with metabolic syndrome. *Arch Iran Med*. 2012;15(8):462–468.
124. Moeller LE, Peterson CT, Hanson KB, et al. Isoflavone-rich soy protein prevents loss of hip lean mass but does not prevent the shift in regional fat distribution in perimenopausal women. *Menopause*. 2003;10(4):322–331.
125. Crisafulli A, Altavilla D, Marini H, et al. Effects of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women. *Menopause*. 2005;12(2):186–192.
126. Jamilian M, Asemi Z. The effects of soy isoflavones on metabolic status of patients with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2016;101(9):3386–3394.
127. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr*. 2002;76:5–56.
128. Nanri A, Mizoue T, Takahashi Y, et al. Soy product and isoflavone intakes are associated with a lower risk of type 2 diabetes in overweight Japanese women. *J Nutr*. 2010;140:580–586.
129. Villegas R, Gao YT, Yang G, et al. Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *Am J Clin Nutr*. 2008;87:162–167.
130. Liu A, Chen Y, Ho SC. Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2011;93:1092–1101.

131. Jayagopal V, Albertazzi P, Kilpatrick ES, et al. Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. *Diabetes Care*. 2002;25:1709–1714.
132. Goodman-Gruen D, Kritz-Silverstein D. Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. *J Clin Endocrinol Metab*. 2001;86:3045–3052.
133. Anthony TG, McDaniel BJ, Knoll P, et al. Feeding meals containing soy or whey protein after exercise stimulates protein synthesis and translation initiation in the skeletal muscle of male rats. *J Nutr*. 2007;137:357–362.
134. Mezei O, Li Y, Mullen E, et al. Dietary isoflavone supplementation modulates lipid metabolism via PPARα-dependent and -independent mechanisms. *Physiol Genomics*. 2006;26:8–14.
135. Cederroth CR, Vinciguerra M, Gjinovci A, et al. Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*. 2008;67:1176–1185.
136. Oliveira LPM, de Jesus RP, Freire TO, et al. Possible molecular mechanisms soy-mediated in preventing and treating nonalcoholic fatty liver disease. *Nutr Hosp*. 2012;27(4):991–998.
137. Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. *Int J Med*. 2007;4:72–82.
138. Ronis MJ, Chen Y, Badeaux J, Badger TM. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. *J Nutr*. 2009;139:1431–1438.
139. Oliva ME, Chicco AG, Lombardo YB. Soya protein reverses dyslipidaemia and the altered capacity of insulin-stimulated glucose utilization in the skeletal muscle of sucrose-rich diet-fed rats. *Br J Nutr*. 2009;102:60–68.
140. Gilbert ER, Liu D. Anti-diabetic functions of soy isoflavone genistein: mechanism underlying effects on pancreatic β-cell function. *Food Funct*. 2013;4(2):200–212.
141. Bitto A, Altavilla D, Bonaiuto A, et al. Effects of aglycone genistein in a rat experimental model of postmenopausal metabolic syndrome. *J Endocrinol*. 2009;200:367–376.
142. Fu Z, Zhang W, Zhen W, et al. Genistein induces pancreatic β-cell proliferation through activation of multiple signaling pathways and prevents insulin-deficient diabetes in mice. *Endocrinol*. 2010;151(7):3026–3037.
143. Liu D, Zhen W, Yang Z, et al. Genistein acutely stimulates insulin secretion in pancreatic β-cells through a cAMP-dependent protein kinase pathway. *Diabetes*. 2006;55:1043–1050.
144. Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. *Int J Med Sci*. 2007;4(2):72–82.
145. Rasbach KA, Schnellmann RG. Isoflavones promote mitochondrial biogenesis. *J Pharmacol Exp Ther*. 2008;325(2):536–543.
146. Hirasaka K, Maeda T, Ikeda C, et al. Isoflavones derived from soy beans prevent MuRF1-mediated muscle atrophy in C2C12 myotubes through SIRT1 activation. *J Nutr Sci Vitaminol*. 2013;59:317–324.
147. Chen JR, Lazarenko OP, Blackburn ML, et al. Soy protein isolate inhibits high-fat diet-induced senescence pathways in osteoblasts to maintain bone acquisition in male rats. *Endocrinol*. 2015;156:475–487.
148. Yu J, Auwerx J. The role of sirtuins in the control of metabolic homeostasis. *Ann NY Acad Sci*. 2009;1173(suppl 1):E10–E19.
149. Messina MJ, Persky V, Setchell KD, et al. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr Cancer*. 1994;21:113–131.
150. Shu XO, Jin F, Dai Q, et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. *Cancer Epidemiol Biomarkers Prev*. 2001;10:483–488.
151. Nakata T, Takashima S, Shiotsu Y, et al. Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients. *J Steroid Biochem Mol Biol*. 2003;86(3–5):455–460.
152. Dong JY, Qin LQ. Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. *Breast Cancer Res Treat*. 2011;125(2):315–323.
153. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. *J Natl Cancer Inst*. 2006;98(7):459–471.
154. Qin LG, Xu JY, Wang PY, Hoshi K. Soyfood intake in the prevention of breast cancer risk in women: a meta-analysis of observational epidemiological studies. *J Nutr Sci Vitaminol*. 2006;52:428–436.
155. Wada K, Nakamura K, Tamai Y, et al. Soy isoflavone intake and breast cancer risk in Japan: from the Takayama study. *Int J Cancer*. 2013;133:952–960.
156. Nagata C, Mizoue T, Tanaka K, et al. Soy intake and breast cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol*. 2014;44(3):282–295.
157. Zhu YY, Zhou L, Jiao SC, Xu LZ. Relationship between soy food intake and breast cancer in China. *Asian Pac J Cancer Prev*. 2011;12(11):2837–2840.
158. Cho YA, Kim J, Park KS, et al. Effect of dietary soy intake on breast cancer risk according to menopause and hormone receptor status. *Eur J Clin Nutr*. 2010;64(9):924–932.
159. Morimoto Y, Maskarinec G, Park SY, et al. Dietary isoflavone intake is not statistically significantly associated with breast cancer risk in the Multiethnic Cohort. *Br J Nutr*. 2014;112(6):976–983.
160. Conroy SM, Maskarinec G, Park SY, et al. The effects of soy consumption before diagnosis on breast cancer survival: the Multiethnic Cohort Study. *Nutr Cancer*. 2013;65(4):527–537.
161. Khan SA, Chatterton RT, Michel N, et al. Soy isoflavone supplementation for breast cancer risk reduction: a randomized phase II trial. *Cancer Prev Res*. 2012;5(2):309–319.
162. Fritz H, Seely D, Flower G, et al. Soy, red clover, and isoflavones and breast cancer: a systematic review. *PLoS One*. 2013;8(11):e81968.
163. Lee SA, Shu XO, Li H, et al. Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *Am J Clin Nutr*. 2009;89:1920–1926.
164. Zhang C, Ho SC, Lin F, et al. Soy product and isoflavone intake and breast cancer risk defined by hormone receptor status. *Cancer Sci*. 2010;101(2):501–507.
165. Lee SA, Shu XO, Li H, et al. Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *Am J Clin Nutr*. 2009;89:1920–1926.
166. Thanos J, Cotterchio M, Boucher BA, et al. Adolescent dietary phytoestrogen intake and breast cancer risk (Canada). *Cancer Causes Control*. 2006;17(10):1253–1261.
167. Korde LA, Wu AH, Fears T, et al. Childhood soy intake and breast cancer risk in Asian American women. *Cancer Epidemiol Biomarkers*. 2009;18(4):1050–1059.
168. Nechuta SJ, Caan BJ, Chen WY, et al. Soy food intake after diagnosis of breast cancer and survival: an in-depth analysis of combined evidence from cohort studies of US and Chinese women. *Am J Clin Nutr*. 2012;96:123–132.
169. Caan BJ, Natarajan L, Parker B, et al. Soy food consumption and breast cancer prognosis. *Cancer Epidemiol Biomarkers Prev*. 2011;20(5):854–858.
170. Guha N, Kwan ML, Quesenberry CP, et al. Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: life after cancer epidemiology (lace) study. *Breast Cancer Res Treat*. 2009;118(2):395–405.
171. Wu AH, Spicer D, Garcia A, et al. Double-blind randomized 12-month soy intervention had no effects on breast MRI fibroglandular tissue density or mammographic density. *Cancer Prev Res*. 2015;8(10):942–951.
172. Kang X, Zhang Q, Wang S, et al. Effect of soy isoflavones on breast cancer recurrence and death for patients receiving adjuvant endocrine therapy. *CMAJ*. 2010;182(17):1857–1862.
173. Virk-Baker MK, Barnes S, Krontiras H, Nagy TR. S-(-)equol producing status not associated with breast cancer risk among low isoflavone consuming US postmenopausal women undergoing a physician recommended breast biopsy. *Nutr Res*. 2014;34(2):116–125.
174. Toi M, Hirota S, Tomotaki A, et al. Probiotic beverage with soy isoflavone consumption for breast cancer prevention: a case-control study. *Curr Nutr Food Sci*. 2013;9:194–200.
175. Takagi A, Kano M, Kaga C. Possibility of breast cancer prevention: use of soy isoflavones and fermented soy beverage produced using probiotics. *Int J Mol Sci*. 2015;16:10907–10920.
176. Woo HD, Park KS, Ro J, Kim J. Differential influence of dietary soy intake on the risk of breast cancer recurrence related to HER2 status. *Nutr Cancer*. 2012;64(2):198–205.
177. Wang Q, Liu L, Li H, et al. Effects of high-order interactions among IGFBP-3 genetic polymorphisms, body mass index and soy isoflavone intake on breast cancer susceptibility. *PLoS One*. 2016;11(9):e0162970.

178. Wang Q, Liu L, Li H, et al. Genetic and dietary determinants of insulin-like growth factor (IGF-1) and IGF binding protein (BP)-3 levels among Chinese women. *PLoS One*. 2014;9(10):e108934.
179. Lee E, Hsu C, Van den Berg D, et al. Genetic variation in peroxisome proliferator-activated receptor gamma, soy, and mammographic density in Singapore Chinese women. *Cancer Epidemiol Biomarkers Prev*. 2012;21(4):635–644.
180. Wang Q, Li H, Tao P, et al. Soy isoflavones, CYP1A1, CYP1B1, and COMT polymorphisms, and breast cancer: a case-control study in Southwestern China. *DNA Cell Biol*. 2011;30(8):585–595.
181. Wang Q, Wang YP, Li JY, et al. Polymorphic catechol-O-methyltransferase gene, soy isoflavone intake and breast cancer in postmenopausal women: a case-control study. *Chin J Cancer*. 2010;29(7):683–688.
182. Applegate CC, Rowles JL, Ranard KM, et al. Soy Consumption and the Risk of Prostate Cancer: An Updated Systematic Review and Meta-Analysis. *Nutrients*. 2018;10(1). pii: E40. https://doi:10.3390/nu10010040.
183. Nagata Y, Sonoda T, Mori M, et al. Dietary isoflavones may protect against prostate cancer in Japanese men. *J Nutr*. 2007;137(8):1974–1979.
184. van Die MD, Bone KM, Williams SG, Pirotta M. Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials. *GJU Int*. 2014;113:E119–E130.
185. Hwang YW, Kim SY, Jee SH, et al. Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. *Nutr Cancer*. 2009;61(5):598–606.
186. Yan L, Spitznagel EL. Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am J Clin Nutr*. 2009;89:1155–1163.
187. Miyanaga N, Akaza H, Hinotsu S, et al. Prostate cancer chemoprevention study: an investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen. *Cancer Sci*. 2012;103(1):125–130.
188. Wu Y, Zhang L, Na R, et al. Plasma genistein and risk of prostate cancer in Chinese population. *Int Urol Nephrol*. 2015;47:965–970.
189. Kurahashi N, Iwasaki M, Inoue M, et al. Plasma isoflavones and subsequent risk of prostate cancer in a nested case-control study: the Japan public health center. *J Clin Oncol*. 2008;26:5923–5929.
190. Ozasa K, Nakao M, Watanabe Y, et al. Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men. *Cancer Sci*. 2004;95(1):65–71.
191. Leitzmann MF, Rohrmann S. Risk factors for the onset of prostatic cancer: age, location, and behavioral correlates. *Clin Epidemiol*. 2012;4:111.
192. Travis RC, Spencer EA, Allen NE, et al. Plasma phyto-oestrogens and prostate cancer in the European prospective investigation into cancer and nutrition. *Br J Cancer*. 2009;100:1817–1823.
193. Sugiyama Y, Masumori N, Fukuta F, et al. Influence of isoflavone intake and equol-producing intestinal flora on prostate cancer risk. *Asian Pac J Cancer Prev*. 2013;14(1):1–4.
194. Lund TD, Munson DJ, Haldy ME, et al. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod*. 2004;70(4):1188–1195.
195. Dalais FS, Meliala A, Wattanapenpaiboon N, et al. Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. *Urology*. 2004;64:510–515.
196. Vitolins M, Griffin L, Tomlinson WV, et al. Randomized trial to assess the impact of venlafaxine and soy protein on hot flashes and quality of life in men with prostate cancer. *J Clin Oncol*. 2013;31(32):4092–4098.
197. Sonoda T, Suzuki H, Mori M, et al. Polymorphisms in estrogen related genes may modify the protective effect of isoflavones against prostate cancer risk in Japanese men. *Eur J Cancer Prev*. 2010;19(2):131–137.
198. Tse G, Eslick GD. Soy and isoflavone consumption and risk of gastrointestinal cancer: a systematic review and meta-analysis. *Eur J Nutr*. 2016;55(1):63–73.
199. Shin A, Lee J, Lee J, et al. Isoflavone and soyfood intake and colorectal cancer risk: a case-control study in Korea. *PLoS One*. 2015;10(11):e0143228.
200. Kweon SS, Shu XO, Xiang Y, et al. Intake of specific nonfermented soy foods may be inversely associated with risk of distal gastric cancer in a Chinese population. *J Nutr*. 2013;143:1736–1742.
201. Yang G, Shu XO, Li H, et al. Prospective cohort study of soy food intake and colorectal cancer risk in women. *Am J Clin Nutr*. 2009;89:577–583.
202. Wada K, Tsuji M, Tamura T, et al. Soy isoflavone intake and stomach cancer risk in Japan: from the Takayama study. *Int J Cancer*. 2015;137:885892.
203. Hara A, Sasazuki S, Inoue M, et al. Isoflavone intake and risk of gastric cancer: a population-based prospective cohort study in Japan. *Am J Clin Nutr*. 2012;95:147–154.
204. Akhter M, Inouse M, Kurahashi N, et al. Dietary soy and isoflavone intake and risk of colorectal cancer in the Japan Public Health Center-Based Prospective Study. *Cancer Epidemiol Biomarkers Prev*. 2008;17(8):2128–2135.
205. Vrieling A, Rookus MA, Kampman E, et al. Isolated isoflavones do not affect the circulating insulin-like growth factor system in men at increased colorectal cancer risk. *J Nutr*. 2007;137:379–383.
206. Ollberding NJ, Lim U, Wilkens LR, et al. Legume, soy, tofu, and isoflavone intake and endometrial cancer risk on postmenopausal women in the Multiethnic Cohort Study. *J Natl Cancer Inst*. 2014;104:67–76.
207. Budhathoki S, Iwasaki M, Sawada N, et al. Soy food and isoflavone intake and endometrial cancer risk: the Japan Public Health Center-based prospective study. *BJOG*. 2015;122(3):304–311.
208. Quaas AM, Kono N, Mack WJ, et al. The effect of isoflavone soy protein supplementation on endometrial thickness, hyperplasia and endometrial cancer risk in postmenopausal women: a randomized controlled trial. *Menopause*. 2013;20(8):840–844.
209. Bandera EV, Williams MG, Sima C, et al. Phytoestrogen consumption and endometrial cancer risk: a population-based case-control study in New Jersey. *Cancer Causes Control*. 2009;20(7):1117–1127.
210. Bandera EV, King M, Chandran U, et al. Phytoestrogen consumption from foods and supplements and epithelial ovarian cancer risk: a population-based case control study. *BMC Womens Health*. 2011;11:40.
211. Qu XL, Fang Y, Zhang M, Zhang YZ. Phytoestrogen intake and risk of ovarian cancer: a meta-analysis of 10 observational studies. *Asian Pac J Cancer Prev*. 2014;15(21):9085–9091.
212. Hedelin M, Lot M, Andersson TM, et al. Dietary phytoestrogens and the risk of ovarian cancer in the Women's lifestyle and health cohort study. *Cancer Epidemiol Biomarkers Prev*. 2011;20(2):307–317.
213. Wu SH, Liu Z. Soy food consumption and lung cancer risk: a meta-analysis using a common measure across studies. *Nutr Cancer*. 2013;65(5):625–632.
214. Yang G, Shu XO, Chow WH, et al. Soy food intake and risk of lung cancer: evidence from the Shanghai Women's Health Study and a meta-analysis. *Am J Epidemiol*. 2012;176(10):846–855.
215. Yang G, Shu XO, Li HL, et al. Prediagnosis soy food consumption and lung cancer survival in women. *J Clin Oncol*. 2013;31:1548–1553.
216. Chlebowski RT, Schwartz AG, Wakelee H, et al. Oestrogen plus progestin and lung cancer in postmenopausal women (Women's Health Initiative trial): a post-hoc analysis of a randomised controlled trial. *Lancet*. 2009;374:1243–1251.
217. Bouchardy C, Benhamou S, Schaffer R, et al. Lung cancer mortality risk among breast cancer patients treated with anti-oestrogens. *Cancer*. 2011;117:1288–1295.
218. Kurahashi N, Inoue M, Iwasaki M, et al. Isoflavone consumption and subsequent risk of hepatocellular carcinoma in a population-based prospective cohort of Japanese men and women. *Int J Cancer*. 2009;124:1644–1649.
219. Haselkorn T, Stewart SL, Horn-Ross PL. Why are thyroid cancer rates so high in Southeast Asian women living in the United States? The Bay Area Thyroid Cancer Study. *Cancer Epidemiol Biomarkers Prev*. 2003;12:144–150.
220. Ahn JC, Biswas R, Chung PS. Combination with genistein enhances the efficacy of photodynamic therapy against human anaplastic thyroid cancer cells. *Lasers Surg Med*. 2012;44(10):840–849.
221. Sirtori CR. Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symtoms and osteoporosis. *Drug Saf*. 2001;24:665–682.
222. Varinska L, Gal P, Mojzisova G, et al. Soy and breast cancer: focus on angiogenesis. *Int J Mol Sci*. 2015;16:11728–11749.
223. Lund TD, Munson DJ, Haldy ME, et al. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod*. 2004;70(4):1188–1195.

224. Vardi A, Bosviel R, Rabiau N, et al. Soy phytoestrogens modify DNA methylation of GSTP1, RASSF1A, EPH2 and BRCA1 promoter in prostate cancer cells. *In Vivo*. 2010;24(4):393–400.
225. Adjakly M, Ngollo M, Boiteux JP, et al. Genistein and daidzein: different molecular effects on prostate cancer. *Anticancer Res*. 2013;33:39–44.
226. Mahmoud A, Yang W, Bosland MC. Soy isoflavones and prostate cancer: a review of molecular mechanisms. *J Steroid Biochem Mol Biol*. 2014;140:116–132.
227. Pavese JM, Krisha SN, Bergan RC. Genistein inhibits prostate cancer cell detachment, invasion, and metastasis. *Am J Clin Nutr*. 2014;100(suppl):431S–436S.
228. Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L. The role of early life genistein exposures in modifying breast cancer risk. *Br J Cancer*. 2008;98:1485–1493.
229. Itsumi M, Shiota M, Takeuchi A, et al. Equol inhibits prostate cancer growth through degradation of androgen receptor by S-phase kinase-associated protein 2. *Cancer Sci*. 2016;107:1022–1028.
230. Mizushina Y, Shiomi K, Kuriyama I, et al. Inhibitory effects of a major soy isoflavone, genistein, on human DNA topoisomerase II activity and cancer cell proliferation. *Int J Oncol*. 2013;43:1117–1124.
231. Bae M, Woo M, Kusuma IW, et al. Inhibitory effects of isoflavonoids on rat prostate testosterone 5alpha-reductase. *J Acupunct Meridian Stud*. 2012;5(6):319–322.
232. Uifalean A, Schneider S, Ionescu C, et al. Soy isoflavones and breast cancer cell lines: molecular mechanisms and future perspectives. *Molecules*. 2015;21(1):E13.
233. Zhao Q, Zhao M, Parris AB, et al. Genistein targets the cancerous inhibitor of PP2A to induce growth inhibition and apoptosis in breast cancer cells. *Int J Oncol*. 2016;49:1203–1210.
234. Liu J, Viswanadhapalli S, Garcia L, et al. Therapeutic utility of natural estrogen receptor beta agonists on ovarian cancer. *Oncotarget*. 2017;8(30):50002–50014.
235. Fan P, Fan S, Wang H, et al. Genistein decreases the breast cancer stem-like cell population through Hedgehog pathway. *Stem Cell Res Ther*. 2013;4:146.
236. Amaral C, Toloi MR, Vasconcelos LD, et al. The role of soybean extracts and isoflavones in hormone-dependent breast cancer: aromatase activity and biological effects. *Food Funct*. 2017;8(9):3064–3074.
237. Christensen MJ, Quiner TE, Nakken HL, et al. Combination effects of dietary soy and methylselenocysteine in a mouse model of prostate cancer. *Prostate*. 2013;73(9). https://doi.org/10.1002/pros.22646.
238. Mahalingam S, Gao L, Gonnering M, et al. Equol inhibits growth, induces atresia, and inhibits steroidogenesis of mouse antral follicles in vitro. *Toxicol Appl Pharmacol*. 2016;295:47–55.
239. Ju YH, Doerge DR, Woodling KA, et al. Dietary genistein negates the inhibitory effect of letrozole on the growth of aromatase-expressing estrogen-dependent human breast cancer cells (MCF-7Ca) in vivo. *Carcinogenesis*. 2008;29(11):2162–2168.
240. van Duursen MB, Nijmeijer SM, de Morree ES, et al. Genistein induces breast cancer-associated aromatase and stimulates estrogen-dependent tumor cell growth in in vitro breast cancer model. *Toxicology*. 2011;289(2–3):67–73.
241. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. *Cancer Lett*. 2008;269(2):226–242.
242. Brzezinski A, Adlercreutz H, Shaoul R, et al. Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause*. 1997;4:89–94.
243. Tranche S, Brotons C, Pascual de la Pisa B, et al. Impact of a soy drink on climacteric symptoms: an open-label, crossover, randomized clinical trial. *Gynecol Endocrinol*. 2016;32(6):477–482.
244. Taku K, Melby MK, Kronenberg F, et al. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. *Menopause*. 2012;19:776–790.
245. Thomas A, Ismail R, Taylor-Swanson L, et al. Effects of isoflavones and amino acid therapies for hot flashes and co-occurring symptoms during the menopausal transition and early post menopause: a systematic review. *Maturitas*. 2014;78(4):263–276.
246. Petri Nahas E, Nahas Neto J, De Luca L, et al. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. *Maturitas*. 2004;48:372–380.
247. Aso T. Equol improves menopausal symptoms in Japanese women. *J Nutr*. 2010;140:1386S–1389S.
248. Newton KM, Reed SD, Uchiyama S, et al. A cross-sectional study of equol producer status and self-reported vasomotor symptoms. *Menopause*. 2015;22(5):489–495.
249. Ishiwata N, Melby MK, Mizuno S, Watanabe S. New equol supplement for relieving menopausal symptoms: randomized, placebo-controlled trial of Japanese women. *Menopause*. 2009;16(1):141–148.
250. Nomura SJ, Hwang YT, Gomez SL, et al. Dietary intake of soy and cruciferous vegetables and treatment-related symptoms in Chinese-American and non-Hispanic White breast cancer survivors. *Breast Cancer Res Treat*. 2017. https://doi:101007/s10549-017-4578-9.
251. Dorjgochoo T, Gu K, Zheng Y, et al. Soy intake in association with menopausal symptoms during the first 6 and 36 months after breast cancer diagnosis. *Breast Cancer Res Treat*. 2011;130(3):879–889.
252. Lee H, Choue R, Lim H. Effect of soy isoflavones supplement on climacteric symptoms, bone biomarkers, and quality of life in Korean postmenopausal women: a randomized clinical trial. *Nutr Res Pract*. 2017;11(3):223–231.
253. Wei P, Liu M, Chen Y, Chen DC. Systematic review of soy isoflavone supplements on osteoporosis in women. *Asian Pac J Trop Med*. 2012;5(3):243–248.
254. Ma DF, Qin LQ, Wang PY, et al. Soy isoflavone intake increases bone mineral density in the spine of menopausal women: meta-analysis of randomized controlled trials. *Clin Nutr*. 2008;27:57–64.
255. Marini H, Minutoli L, Polito P, et al. Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Ann Intern Med*. 2007;146:839–847.
256. Setchell KD, Lydeking-Olsen E. Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. *Am J Clin Nutr*. 2003;78:593S–609S.
257. Alekel DL, St Germain A, Peterson CT, et al. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *Am J Clin Nutr*. 2000;72:844–852.
258. Potter SM, Baum JA, Teng H, et al. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr*. 1998;68:1375S–1379S.
259. Taku K, Melby MK, Takebayashi J, et al. Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. *Asia Pac J Clin Nutr*. 2010;19:33–42.
260. Weaver CM, Martin BR, Jackson GS, et al. Antiresorptive effects of phytoestrogen supplements compared with estradiol or risedronate in postmenopausal women using 41Ca methodology. *J Clin Endocrinol Metab*. 2009;94(10):3798–3805.
261. Wong WW, Lewis RD, Steinberg FM, et al. Soy isoflavone supplementation and bone mineral density in menopausal women: a 2-y multicenter clinical trial. *Am J Clin Nutr*. 2009;90:1433–1439.
262. Pawlowski JW, Martin BR, McCabe GP, et al. Impact of equol-producing capacity and soy-isoflavone profiles of supplements on bone calcium retention in postmenopausal women: a randomized crossover trial. *Am J Clin Nutr*. 2015;102:695–703.
263. Messina M, Ho S, Alekel DL. Skeletal benefits of soy isoflavones: a review of the clinical trial and epidemiologic data. *Curr Opin Clin Nutr Metab Care*. 2004;7:649–658.
264. Sathyapalan T, Aye M, Rigby AS, et al. Soy reduces bone turnover markers in women during early menopause: a randomized controlled trial. *J Am Soc Bone Miner Res*. 2017;32(1):157–164.
265. Sathyapalan T, Aye M, Rigby AS, et al. Effects of soy on bone turn-over markers in men with type 2 diabetes and hypogonadism—a randomised controlled study. *Sci Rep*. 2017;7(1):15366.
266. Chi XX, Zhang T. The effects of soy isoflavone on bone density in north region of climacteric Chinese women. *J Clin Biochem Nutr*. 2013;53(2):102–107.

267. Huang HY, Yang HP, Yang HT, et al. One-year soy isoflavone supplementation prevents early postmenopausal bone loss but without a dose-dependent effect. *J Nutr Biochem*. 2006;17:509–517.
268. Baglia ML, Gu K, Zhang X, et al. Soy isoflavone intake and bone mineral density in breast cancer survivors. *Cancer Causes Control*. 2015;26(4):571–580.
269. Xiao CW. Health effects of soy protein and isoflavones in humans. *J Nutr*. 2008;138:1244S–1249S.
270. Zheng X, Lee SK, Chun OK. Soy isoflavones and osteoporotic bone loss: a review with an emphasis on modulation of bone remodeling. *J Med Food*. 2016;19(1):1–14.
271. Tadaishi M, Nishide Y, Tousen Y, et al. Cooperative effects of soy isoflavones and carotenoids on osteoclast formation. *J Clin Biochem Nutr*. 2014;54(2):109–115.
272. Zhao Y, Martin BR, Weaver CM. Calcium bioavailability of calcium carbonate fortified soymilk is equivalent to cow's milk in young women. *J Nutr*. 2005;135:2379–2382.
273. File SE, Hartley DE, Elsabagh S, et al. Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause*. 2005;12:193–201.
274. Kritz-Silverstein D, Von Huhlen D, Barrett-Connor E, Bressel MA. Isoflavones and cognitive function in older women: the Soy and Postmenopausal Health in Aging (Sophia) Study. *Menopause*. 2003;10:196–202.
275. Duffy R, Wiseman H, File SE. Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacol Biochem Behav*. 2003;75:721–729.
276. Lehert P, Villaseca P, Hogervorst E, et al. Individually modifiable risk factors to ameliorate cognitive aging: a systematic review and meta-analysis. *Climacteric*. 2015;18(5):678–689.
277. Henderson VW, St John JA, Hodis HN, et al. Long-term soy isoflavone supplementation and cognition in women. *Neurology*. 2012;78:1841–1848.
278. John JA S, Henderson VW, Hodis HN, et al. Associations of urine excretion of isoflavonoids with cognition in postmenopausal women in the Women's Isoflavone Soy Health Clinical Trial. *J Am Geriatr Soc*. 2014;62(4):629–635.
279. Thorp AA, Sinn N, Buckley JD, et al. Soya isoflavone supplementation enhances spatial working memory in men. *Br J Nutr*. 2009;102(9):1348–1354.
280. Basaria S, Wisniewski A, Dupree K, et al. Effect of high-dose isoflavones on cognition, quality of life, androgens, and lipoprotein in post-menopausal women. *J Endocrinol Invest*. 2009;32(2):150–155.
281. Zhao L, Brinton RD. WHI and WHIMS follow-up and human studies of soy isoflavones on cognition. *Expert Rev Neurother*. 2007;7(11):1549–1564.
282. Fournier LR, Ryan Borchers TA, Robison LM, et al. The effects of soy milk and isoflavone supplements on cognitive performance in healthy, postmenopausal women. *J Nutr Health Aging*. 2007;11(2):155–164.
283. Ho SC, Chan AS, Ho YP, et al. Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. *Menopause*. 2007;14(3 Pt 1):489–499.
284. Igase M, Igase K, Tabara Y, et al. Cross-sectional study of equol producer status and cognitive impairment in older adults. *Geratr Gerontol Int*. 2017. https://doi.org/10.1111/ggi.13029.
285. Gleason CE, Fischer BL, Dowling NM, et al. Cognitive effects of soy isoflavones in patients with Alzheimer's disease. *J Alzheimers Dis*. 2015;47(4):1009–1019.
286. Kani AH, Alavian SM, Esmaillzadeh A, et al. Effects of a novel therapeutic diet on liver enzymes and coagulating factors in patients with non-alcoholic fatty liver disease: a parallel randomized trial. *Nutrition*. 2014;30(7–8):814–821.
287. Oliveira LP, de Jesus RP, Freire TO, et al. Possible molecular mechanisms soy-mediated in preventing and treating nonalcoholic fatty liver disease. *Nutr Hosp*. 2012;27(4):991–998.
288. Oliveira LP, de Jesus RP, Boulhosa RS, et al. Effect of soy protein supplementation in patients with chronic hepatitis C: a randomized clinical trial. *World J Gastroenterol*. 2012;18(18):2203–2211.
289. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Risk assessment for peri- and post-menopausal women taking food supplements containing isolated isoflavones. *EFSA J*. 2015. https://doi.org/10.2903/j.efsa.2015.4246.
290. Tonstad S, Jaceldo-Siegl K, Messina M, et al. The association between soya consumption and serum thyroid-stimulating hormone concentrations in the Adventist Health Study-2. *Public Health Nutr*. 2015;19(8):1465–1470.
291. Colucci R, Lotti F, Arunachalam M, et al. Correlation of serum thyroid hormones autoantibodies with self-reported exposure to thyroid disruptors in a group of nonsegmental vitiligo patients. *Arch Environ Contam Toxicol*. 2015;69(2):181–190.
292. Zhang K, Wang Y, Ma W, et al. Genistein improves thyroid function in Hashimoto's thyroiditis patients through regulating Th1 cytokines. *Immunobiology*. 2017;222(2):183–187.
293. Sosvorova L, Miksatkova P, Bicikova M, et al. The presence of monoiodinated derivates of daidzein and genistein in human urine and its effect on thyroid gland function. *Food Chem Toxicol*. 2012;50(8):2774–2779.
294. Renko K, Schache S, Hoefig CS, et al. An improved nonradioactive screening method identifies genistein and xanthohumol as potent inhibitors of iodothyronine deiodinases. *Thyroid*. 2015;25(8):962–968.
295. Sosic-Jurjevic B, Filipovic B, Wirth EK, et al. Soy isoflavones interfere with thyroid hormone homeostasis in orchidectomized middle-aged rats. *Toxicol Appl Pharmacol*. 2014;278(2):124–134.
296. Messina M, Redmond G. Effects of soy protein and soybean isoflavones on thyroid function in healthy adults and hypothyroid patients: a review of the relevant literature. *Thyroid*. 2006;16:249–258.
297. Sathyapalan T, Aye M, Rigby AS, et al. Soy reduces bone turnover markers in women during early menopause: a randomized controlled trial. *J Am Soc Bone Miner Res*. 2017;32(1):157–164.
298. Mei J, Yeung SS, Kung AW. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. *J Clin Endocrinol Metab*. 2001;86:5217–5221.
299. Natural Medicines Comprehensive Database. *Soy*. Available at: www.naturaldatabase.com. Accessed 8 Sept 2019.
300. Bell DS, Ovalle F. Use of soy protein supplement and resultant need for increased dose of levothyroxine. *Endocr Pract*. 2001;7:193–194.
301. Takagi A, Kano M, Kaga C. Possibility of breast cancer prevention: use of soy isoflavones and fermented soy beverage produced using probiotics. *Int J Mol Sci*. 2015;16:10907–10920.
302. Messina M. Investigating the optimal soy protein and isoflavone intakes for women: a perspective. *Women's Health*. 2008;4(4):337–356.
303. Anthony TG, McDaniel BJ, Knoll P, et al. Feeding meals containing soy or whey protein after exercise stimulates protein synthesis and translation initiation in the skeletal muscle of male rats. *J Nutr*. 2007;137:357–362.
304. Dewi FN, Wood CE, Lees CJ, et al. Dietary soy effects on mammary gland development during the pubertal transition in nonhuman primates. *Cancer Prev Res*. 2013;6(8):832–842.
305. Charles C, Yuskavage J, Carlson O, et al. Effects of high-dose isoflavones on metabolic and inflammatory markers in healthy postmenopausal women. *Menopause*. 2009;16(2):395–400.
306. Christie DR, Grant J, Darnell BE, et al. Metabolic effects of soy supplementation in postmenopausal white and African American women: a randomized, placebo-controlled trial. *Am J Obstet Gynecol*. 2010;203(2):153.e1–153.e9.
307. Hooper L, Ryder JJ, Kurzer MS, et al. Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis. *Hum Reprod Update*. 2009;15(4):423–440.
308. Fuhrman BJ, Pfeiffer R, Xu X, et al. Soy intake is associated with increased 2-hydroxylation and decreased 16alpha-hydroxylation of estrogens in Asian-American women. *Cancer Epidemiol Biomarkers Prev*. 2009;18(10):2753–2760.

309. Barnes S, Kim H, Darley-Usmar V, et al. Beyond ERalpha and ERbeta: estrogen receptor binding is only part of the isoflavone story. *J Nutr*. 2000;130:656S–6567S.
310. Magee PJ, Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. *Br J Nutr*. 2004;91:513–531.
311. Bae M, Woo M, Kusuma IW, et al. Inhibitory effects of isoflavonoids on rat prostate testosterone 5alpha-reductase. *J Acupunct Meridian Stud*. 2012;5(6):319–322.
312. Rasbach KA, Schnellmann RG. Isoflavones promote mitochondrial biogenesis. *J Pharmacol Exp Ther*. 2008;325(2):536–543.
313. Chi XX, Zhang T. The effects of soy isoflavone on bone density in north region of climacteric Chinese women. *J Clin Biochem Nutr*. 2013;53(2):102–107.
314. Lee H, Choue R, Lim H. Effect of soy isoflavones supplement on climacteric symptoms, bone biomarkers, and quality of life in Korean postmenopausal women: a randomized clinical trial. *Nutr Res Pract*. 2017;11(3):223–231.
315. Wei P, Liu M, Chen Y, Chen DC. Systematic review of soy isoflavone supplements on osteoporosis in women. *Asian Pac J Trop Med*. 2012;5(3):243–248.
316. Zheng X, Lee SK, Chun OK. Soy isoflavones and osteoporotic bone loss: a review with an emphasis on modulation of bone remodeling. *J Med Food*. 2016;19(1):1–14.
317. Li SH, Liu XX, Bai YY, et al. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. *Am J Clin Nutr*. 2010;91:480–486.
318. Mezei O, Li Y, Mullen E, et al. Dietary isoflavone supplementation modulates lipid metabolism via PPARα-dependent and -independent mechanisms. *Physiol Genomics*. 2006;26:8–14.
319. Cederroth CR, Vinciguerra M, Gjinovci A, et al. Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*. 2008;67:1176–1185.
320. Oliveira LPM, de Jesus RP, Freire TO, et al. Possible molecular mechanisms soy-mediated in preventing and treating nonalcoholic fatty liver disease. *Nutr Hosp*. 2012;27(4):991–998.
321. Vardi A, Bosviel R, Rabiau N, et al. Soy phytoestrogens modify DNA methylation of GSTP1, RASSF1A, EPH2 and BRCA1 promoter in prostate cancer cells. *Vivo*. 2010;24(4):393–400.
322. Mahmoud A, Yang W, Bosland MC. Soy isoflavones and prostate cancer: a review of molecular mechanisms. *J Steroid Biochem Mol Biol*. 2014;140:116–132.
323. Overk CR, Yao P, Chadwick LR, et al. Comparison of the in vitro estrogenic activities of compounds from hops *(Humulus lupulus)* and red clover *(Trifolium pratense)*. *J Agric Food Chem*. 2005;53(16):6246–6253.
324. Wiseman H, O'Reilly JD, Adlercreutz H, et al. Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. *Am J Clin Nutr*. 2000;72:395–400.
325. Gardner-Thorpe D, O'Hagen C, Young I, Lewis SJ, Dietary supplements of soya flour lower serum testosterone concentrations and improve markers of oxidative stress in men. *Eur J Clin Nutr*. 2003;57:100–106.
326. Divi RL, Chang HC, Doerge DR. Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. *Biochem Pharmacol*. 1997;54:1087–1096.
327. Gilbert ER, Liu D. Anti-diabetic functions of soy isoflavone genistein: mechanism underlying effects on pancreatic β-cell function. *Food Funct*. 2013;4(2):200–212.
328. Hirasaka K, Maeda T, Ikeda C, et al. Isoflavones derived from soy beans prevent MuRF1-mediated muscle atrophy in C2C12 myotubes through SIRT1 activation. *J Nutr Sci Vitaminol*. 2013;59:317–324.
329. Pavese JM, Krisha SN, Bergan RC. Genistein inhibits prostate cancer cell detachment, invasion, and metastasis. *Am J Clin Nutr*. 2014;100(suppl):431S–436S.
330. Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L. The role of early life genistein exposures in modifying breast cancer risk. *Br J Cancer*. 2008;98:1485–1493.
331. Zhao Q, Zhao M, Parris AB, et al. Genistein targets the cancerous inhibitor of PP2A to induce growth inhibition and apoptosis in breast cancer cells. *Int J Oncol*. 2016;49:1203–1210.
332. Fan P, Fan S, Wang H, et al. Genistein decreases the breast cancer stem-like cell population through Hedgehog pathway. *Stem Cell Res Ther*. 2013;4:146.
333. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. *Cancer Lett*. 2008;269(2):226–242.
334. Kim E, Woo MS, Qin L, et al. Daidzein augments cholesterol homeostasis via ApoE to promote functional recovery in chronic stroke. *J Neurosci*. 2015;35(45). 15113–1126.
335. Keung WM, Klyosov AA, Vallee BL. Daidzin inhibits mitochondrial aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. *Proc Natl Acad Sci USA*. 1997;94(5):1675–1679.
336. Setchell KD, Clerici C, Lephart ED, et al. S–Equol, A potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by the human intestinal bacterial flora. *Am J Clin Nutr*. 2005;81:1072–1079.
337. Sugiyama Y, Masumori N, Fukuta F, et al. Influence of isoflavone intake and equol-producing intestinal flora on prostate cancer risk. *Asian Pac J Cancer Prev*. 2013;14(1):1–4.
338. Itsumi M, Shiota M, Takeuchi A, et al. Equol inhibits prostate cancer growth through degradation of androgen receptor by S-phase kinase-associated protein 2. *Cancer Sci*. 2016;107:1022–1028.
339. Guadamuro L, Dohrmann AB, Tebbe CC, Mayo B, Delgado S. Bacterial communities and metabolic activity of faecal cultures from equol producer and non-producer menopausal women under treatment with soy isoflavones. *BMC Microbiol*. 2017;17:93.
340. Nakatsu CH, Armstrong A, Clavijo AP, et al. Fecal bacterial community changes associated with isoflavone metabolites in postmenopausal women after soy bar consumption. *PLoS One*. 2014;9(10):e108924.
341. Racette SB, Lin X, Lefevre M, et al. Dose effects of dietary phytosterols on cholesterol metabolism: a controlled feeding study. *Am J Clin Nutr*. 2010;91:32–38.
342. Cabeza M, Bratoeff E, Heuze I, et al. Effect of beta-sitosterol as inhibitor of 5 alpha-reductase in hamster prostate. *Proc West Pharmacol Soc*. 2003;46:153–155.
343. Kassen A, Berges R, Senge T, et al. Effect of beta-sitosterol on transforming growth factor-beta-1 expression and translocation protein kinase C alpha in human prostate stromal cells in vitro. *Eur Urol*. 2000;37:735–741.
344. Lecomte S, Lelong M, Bourgine G, et al. Assessment of the potential activity of major dietary compounds as selective estrogen receptor modulators in two distinct cell models for proliferation and differentiation. *Toxicol Appl Pharmacol*. 2017;325:61–70.
345. Lin CY, Tsai CY, Lin SH. Effects of soy components on blood and liver lipids in rats fed high-cholesterol diets. *World J Gastroenterol*. 2005;11(35):5549–5552.
346. Messadi DV, Billings P, Shklar G, Kennedy AR. Inhibition of oral carcinogenesis by a protease inhibitor. *J Natl Cancer Inst*. 1986;76:447–452.
347. Graf E, Eaton JW. Suppression of colonic cancer by dietary phytic acid. *Nutr Cancer*. 1993;19(1):11–19.
348. Graf E, Eaton JW. Antioxidant functions of phytic acid. *Free Radic Biol Med*. 1990;8(1):61–69.

115

Tabebuia avellanedae (syn. *T. Impetiginosa,* Lapacho, Pau d'Arco, Ipe Roxo)

Terry Willard, CIH, PhD, and *Michael T. Murray*, ND*

OUTLINE

INTRODUCTION

The taxonomic division of plants with medicinal uses in the *Bignoniaceae* family is confusing. The literature often interchanges the genera of *Tecoma* and *Tabebuia.* Some are now using the name *Tabebuia impetiginosa* as a synonym. At least four species have been called lapacho:

- *Tecoma ochracea*
- *Tecoma ipe*
- *Tabebuia cassinoides*
- *Tabebuia avellanedae* syn. *Tabebuia impetiginosa*

This chapter considers the *Tabebuia* genus.

GENERAL DESCRIPTION

The tree from which lapacho is obtained is a member of the *Bignoniaceae* family known as *T. avellanedae, T. impetiginosa,* or *T. ipe.* There are about 100 species of these evergreen trees or shrubs, native to tropical America. They can grow up to 125 ft tall and have very showy rose- to violet-colored flowers that bloom in profusion just before the new leaves appear.

Because the taxonomy of these plants is so difficult, it is quite possible that there is confusion even among trained gatherers.

CHEMICAL COMPOSITION

Many of the studies and chemical analyses on *Tabebuia* species have been performed on the heartwood, but the bark and inner bark are the products available in the marketplace and the parts used in folklore. The major components of *T. avellanedae* are 16 quinones (mostly with C_{15} skeleton) containing both naphthoquinones (seven, C_{10}–C_5) and anthraquinones (nine, C_{14}–C_1). Both of these groups of quinones rarely occur in the same plant. The lapachol content is usually 2% to 7%. The quinones are listed in Box 115.1. Other compounds found are lapachenole, quercetin, and *o*- and *p*-hydroxybenzoic acids.[1] The action of lapacho comes from an array of quinones and other constituents, not just one or two.

HISTORY AND FOLK USE

The indigenous peoples of Brazil also refer to this tree as pau d'arco or ipe roxo. The inner bark has been used for medicinal purposes for centuries as a folk remedy for a wide variety of afflictions, including the following[2–5]:

- Boils
- Chlorosis
- Colitis
- Diarrhea
- Dysentery
- Enuresis
- Fever
- Pharyngitis
- Snakebite
- Syphilis
- Wounds
- Ulcers
- Respiratory problems
- Arthritis
- Cystitis
- Constipation
- Prostatitis
- Poor circulation
- Constipation

* Previous edition contributor

BOX 115.1 Quinones in *Tabebuia avellanedae*

Naphthoquinones

- Lapachol
- Menaquinone-I
- Deoxylapachol
- Beta-lapachone
- Alpha-lapachone
- Dehydro-α-lapachone

Anthraquinones

- 2-Methylanthraquinone
- 2-Hydroxymethylanthraquinone
- 2-Acetoxymethylanthraquinone
- Anthraquinone-2-aldehyde
- 1-Hydroxyanthraquinone
- 1-Methoxyanthraquinone
- 2-Hydroxy-3-methylquinone
- Tabebuin (a newly discovered compound)

Fig. 115.1 *Tabebuia avellanedae.*

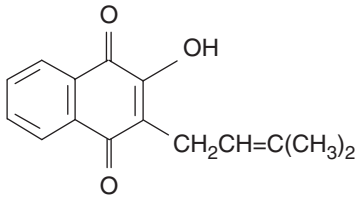


Fig. 115.2 Lapachol.

- Cancers of the esophagus, head, intestine, lung, prostate, and tongue

Lapacho is reported to be alexiteric, analgesic, anodyne, antidotal, antimicrobial, diuretic, and fungicidal.[2]

PHARMACOLOGY

During the 19th century, lapacho was subjected to scientific scrutiny, with the first active constituent, lapachol, isolated by Paterno in 1882 (Fig. 115.1 and Fig 115.2).

Interestingly, many of the scientific studies have found lapacho and lapachol to be more effective in treating malaria and cancerous tumors through oral ingestion rather than intravenous or intramuscular injection.[6]

An herbalist's interpretation would be that the body's recuperative powers are more effectively stimulated by the natural route of nutrient plant material absorption through the digestive tract. Although most of the studies have been of individual chemicals, some show significantly better results with the whole extract and diminishing effectiveness as the extracts are refined or individual chemicals are tested.[6,7]

Antimicrobial Activity

Antibacterial Activity

Lapachol isolated from the *T. avellanedae* tree has exhibited antimicrobial activity against gram-positive and acid-fast bacteria and *Brucella* species as well as fungistatic behavior.[8,9] It is important to note that the research team found that progressive purification reduced the antimicrobial activity of the extract. This finding led to the conclusion that more than one active substance was present in the original extract.

It was eventually found that along with lapachol, the extract of the *Tabebuia* tree contained α-lapachone, β-lapachone, and a newly discovered quinone called xyloidone.

Several of the naphthoquinones[1] exhibit strong microbicidal and fungicidal activities (Table 115.1).

Lapachol has been shown to have both antimicrobial and antiviral activity.[8,10,11] Beta-lapachone shows diversified antiparasitic activity as well as antiviral action.[12,13] Alpha-lapachone is also active against certain parasites, and xyloidone is active against numerous bacteria and fungi.[14,15] Another lapacho component, the flavonoid quercetin, is cytotoxic for certain parasites.[16]

Xyloidone is effective against a wide array of organisms, including *Staphylococcus aureus* and the *Brucella* species. This component also inhibits the causative agents of tuberculosis, dysentery, and anthrax.

Mechanism of action. Lapachol, like many naphthoquinones, acts as a respiratory poison by interfering with electron transport in microbes.[17,18] At concentrations of 100 mg/L, lapachol was found to inhibit O_2 uptake by *Plasmodium knowles* by 74% and succinate oxidase systems by 26% and thus to exhibit antimalarial activity.[19,20] Mitochondrial respiration is inhibited by 50% at a lapachol concentration of less than 110 μmol/L.[21] Increasing doses of lapachol produced progressive respiratory inhibition in tumor cells isolated from animals. Oxygen consumption and oxygen metabolite production are inhibited in neutrophils on the administration of lapachol.[22]

Lapachol has now been shown to act as an uncoupler of oxidative phosphorylation. Lapachol prevents the synthesis of adenosine triphosphate by stimulating respiration in the absence of a phosphate acceptor. This effect is most pronounced at a high pH, at which lipid solubility is the lowest.[23]

Lapachol inhibits certain enzymes; in particular, it is a competitive inhibitor of glycolase I in erythrocytes. Lapachol also demonstrates noncompetitive inhibition of α-keto-aldehyde dehydrogenase, leading to the accumulation of toxic α-ketoaldehydes.[24] Lapachol demonstrated 64% inhibition of 3-α-hydroxysteroid–mediated transhydrogenase at a concentration of 10^{-5}M (well within the dosage range).[25]

Antiviral Activity

Lapachol has proved to be active against certain viral strains, including herpesvirus hominis types I and II.[25] Hydroxynaphthoquinone has been shown to effectively inhibit four influenza viruses. Lapachol also significantly inhibits poliovirus and vesicular stomatitis virus.[10]

Beta-lapachone's antiviral activity has been demonstrated by its ability to inhibit certain enzymes, such as DNA and RNA polymerases.[26] Beta-lapachone was tested against avian myeloblastosis virus and Rauscho murine leukemia virus and found to inhibit retrovirus reverse transcriptase.[27] In the presence of dithioreitol, β-lapachone inhibits eukaryotic DNA polymerase-alpha activity. Although

TABLE 115.1 Antimicrobial Activity of *Tabebuia avellanedae*[a]

Microorganism	Lapachol	Chorohidro-Lapachol	Alpha-Lapachone	Beta-Lapachone	Xyloidone
B. subtilis	60.0–80.0	8.0–10.0	40.0–50.0	1.0–2.0	4.0–6.0
B. mycoides	40.0–60.0	10.0–15.0	40.0–50.0	5.0–8.0	20.0–30.0
B. anthracis	40.0–60.0	20.0–30.0	40.0–50.0	4.0–6.0	20.0–30.0
S. aureus	60.0–80.0	30.0–40.0	30.0–40.0	2.0–4.0	15.0–20.0
Sar. lutea	40.0–60.0	15.0–20.0	30.0–40.0	4.0–6.0	20.0–30.0
S. hemolyticus	>100.0	60.0–80.0	60.0–80.0	10.0–15.0	>50.0
M. tuberculosis	80.0–100.0	40.0–60.0	30.0–50.0	10.0–15.0	10.0–15.0
M. smegmatis	80.0–100.0	60.0–80.0	30.0–50.0	15.0–20.0	15.0–20.0
N. asteroids	>100	40.0–60.0	60.0–80.0	10.0–15.0	20.0–30.0
N. catarrhalis	40.0–60.0	30.0–50.0	80.0–100.0	10.0–15.0	<20.0
E. coli	>100.0	>100.0	>100.0	>100.0	>100.0
K. pneumoniae	>100.0	>100.0	>100.0	>100.0	>100.0
S. typhosa	>100.0	>100.0	>100.0	>100.0	>100.0
Br. suis	15.0–20.0	2.0–4.0	20.0–30.0	0.6–1.0	0.8–1.0
Br. abortus	15.0–20.0	2.0–4.0	30.0–40.0	1.0–2.0	1.5–2.0
Br. melitensis	10.0–15.0	2.0–4.0	30.0–40.0	1.0–2.0	1.5–2.0
C. albicans	>100.0	40.0–60.0	80.0–100.0	80.0–100.0	30.0–50.0
C. krusei	>100.0	40.0–60.0	80.0–100.0	80.0–100.0	50.0–60.0
C. neoformans	>100.0	40.0–60.0	50.0–80.0	30.0–50.0	40.0–60.0

[a]Minimum inhibitory concentration (mcg/mL).

the mechanism of action for enzyme inhibition is complex, it may be related to superoxide production.[26] This issue has great significance for possible treatment of both human immunodeficiency virus and Epstein–Barr virus (the implicated viruses in acquired immunodeficiency syndrome and Epstein–Barr syndrome, respectively).

Beta-lapachone also inhibits Friend virus and was the only substance among a number tested that prolonged the survival time of chickens infected intraperitoneally with Rous sarcoma virus.[28]

Antiparasitic Activity

The trematode *Schistosoma mansoni* is the causative agent of the common tropical disease schistosomiasis. The cercariae of this blood fluke live in water and enter the host by penetrating the skin. This debilitating disease, a serious problem in many tropical areas, causes weakening of the host and increases susceptibility to a variety of other pathogens, some of which may be fatal.

Lapachol has been tested as a topical barrier to the cercariae and has been found to be highly effective at preventing its penetration.[26,29] Oral lapachol was also tested and found to significantly reduce penetration. After being consumed, the lapachol was secreted onto the skin, apparently by the sebaceous glands, where it again acted as a topical barrier. The cercariae seek to penetrate the host through or near the sebaceous glands, suggesting that dietary administration of lapachol would be an efficient means of protecting against infection. Alpha-lapachone and β-lapachone, also components of lapacho, both exhibited activity against *S. mansoni.*[8] Beta-lapachone is notably effective against *Trypanosoma cruzi,* a zoomastigote responsible for trypanosomiasis, or Chagas' disease, which occurs in both acute and chronic forms and has no known cure.

Beta-lapachone causes complete inhibition of *T. cruzi* at concentrations of 0.8 to 5.0 μg/mL and progressively inhibits motility with increasing concentrations. When *T. cruzi* epimastigotes were incubated with the quinone, they were subjected to nuclear, mitochondrial, endoplasmic reticular, and cytoplasmic membrane damage and underwent alterations in the chromatin distribution. Respiration rates were lowered, the mitochondria became swollen, and glucose and pyruvate oxidation was inhibited. Lipid peroxidation was stimulated, leading to decreased cell viability.

In addition, in vitro testing resulted in the rapid decay of DNA, RNA, and protein as well as DNA breakage in *T. cruzi.* This was accompanied by inhibition of DNA, RNA, and protein synthesis and the instigation of "unscheduled" DNA synthesis.[30,31]

It is thought that this toxic action against parasites is at least partly due to superoxide production.[32] Both O_2^- and H_2O_2 are intermediates of oxygen reduction, and both are toxic to living organisms. When β-lapachone is introduced to *T. cruzi,* it rapidly enters the epimastigote and is reduced to its semiquinone form in the mitochondria and microsomes of the pathogen.[33] Superoxide is produced by the reduction of molecular oxygen, which is facilitated by autooxidation of the semiquinone free radical. Superoxide is then converted to hydrogen peroxide via superoxide dismutase. Stimulation of lipid peroxidation follows, and the cell degenerates.

Antifungal Activity

In addition to its activity against a variety of bacteria, components of lapacho are known to possess potent antifungal capabilities.[11,34] The quinone xyloidone inhibits several species of fungus (including *Candida albicans, Candida krusei,* and *Cryptococcus neoformans*).[11] The antifungal activity of aqueous, dichloromethane, and methanol extracts from *T. avellanedae* were also compared, and it was found that the aqueous and methanol extracts showed the highest antifungal activity.[35] *T. avellanedae* bark was found to be active against *Aspergillus fumigatus, C. neoformans, Microsporum gypseum, Penicillium purpurogenum, Saccharomyces cerevisiae,* and *Trichophyton mentagrophytes.*[35]

Antineoplastic Effects

Because of the folklore information surrounding the tumor-reducing qualities of the herb lapacho, it underwent extensive study by the National Cancer Institute (NCI). After the initial positive results, lapachol was judged to be the most active antineoplastic agent. Lapachol

entered Phase I clinical trials at the NCI in 1968 on the basis of its activity against Walker 256 tumors (with a confidence rate exceeding 90%). During these trials, it was difficult to obtain therapeutic blood levels of lapachol without some mild toxic side effects, such as nausea, vomiting, and anti–vitamin K activity. This occurrence is quite difficult to understand because later studies found the toxicity to be very low.[2] The investigative new drug (IND) status for the drug was closed in 1970 due to these toxicity issues.[36]

It has been shown, however, that some of the anthraquinones in lapacho have vitamin K activity; therefore use of the whole herb would compensate for lapachol's effect on vitamin K.[37]

The approach just described indicates a flaw in the underlying philosophy of the pharmaceutical sciences and the NCI program. Because the initial studies came from a whole plant, the detailed studies should have been undertaken on the whole plant. Some of the other quinones have also been shown to have antineoplastic activities. Was it too complex to consider the chemical reactions of the more than 20 components found in lapacho? Or was the standard economic/political incentive for patenting an analog an impediment to the investigation of a plant species?

Lapachol is rapidly absorbed through the gastrointestinal tract after oral administration to rats bearing Walker 256 tumors. It is taken up by all tissues except the brain and blood cells. A significant amount appears in the tumor after 6 hours, with most of the drug disappearing from the other body tissues. The half-life of intravenous lapachol is 33 minutes in mice (75 minutes in dogs). Lapachol is extensively metabolized and excreted mostly in the feces.[38] Beta-lapachone has been shown to inhibit the growth of a large variety of tumor cells, including epidermoid laryngeal cancer; prostate, colon, ovary, and breast cancers; and also different types of leukemia cells.[39]

The theories on how lapachol works as an antineoplastic agent vary considerably. One of the most prominent involves reduction-oxidation (redox) cycle capabilities. Lapachol was found to be an in vivo inhibitor of respiration at chemotherapeutic doses.[5] Later, lapachol was shown to augment the flow of electrons from reduced nicotinamide adenine dinucleotide phosphate to form oxygen-related free radicals. These seem to be site-specific free radicals that bind to the cancerous DNA or RNA, producing either superoxides or free hydroxyl radicals.[40,41]

There seems to be a redox potential that is important for the inhibition of electron transfer in coenzyme Q_{10}.[42] Bennett et al.[43] argued that this respiration poisoning is not the mechanism of antitumor activity. In their study, lapachol was shown to significantly reduce the pool of uridine triphosphate, the largest pool of the pyrimidine nucleotides (exposure time was very short, 2–4 hours).

Lapachol is theorized to be like dichloroallyl lawsone in that it blocks pyrimidine biosynthesis through inhibition of dihydroorotate dehydrogenase.[17] It is also believed that the antineoplastic activities of lapachol might stem from its interaction with nucleic acids,[41] and it has been proposed that interaction of the naphthoquinone moiety between base pairs of the DNA helix occurs with subsequent inhibition of DNA replication and/or RNA synthesis. Free amino groups in the sugar moiety are necessary for DNA binding.[44]

Beta-lapachone has an effect on the modulation of cell growth and apoptosis in human colon carcinoma tumor (HCT-116) cell lines. Exposure of HCT-116 cells to β-lapachone resulted in growth inhibition and induction of apoptosis in a dose-dependent manner. The researchers suggest therefore that β-lapachone–induced apoptosis may be partly regulated through the inactivation of the second messenger nuclear factor (NF)-κβ system.[45]

Beta-lapachone was also shown to decrease the viability of sarcoma cells by stimulating lipid peroxidation. This was accomplished through the following activities[32]:

- Reduction of lapachone at the mitochondrial and microsomal membranes with the generation of the semiquinone form
- Autooxidation to produce O_2
- Production of H_2O_2 via superoxide dismutase

More than one mechanism has been shown to underlie the antineoplastic effect.[7] There is ongoing positive research on cancers of the liver,[46,47] bladder,[48] prostate,[49,50] lung,[51] colon,[45] and breast (estrogen receptor site inhibition).[52]

Anti-Inflammatory Activity

Extracts of the bark from *T. avellanedae* demonstrate clear anti-inflammatory activity with low toxicity.[53] Pau d'arco douching and the use of tampons soaked in an alcoholic extract of lapacho have been shown to be very successful against a wide range of inflammations, such as vaginitis, cervicitis, and cervicovaginitis.[1,27,34]

An aqueous extract of the inner bark of *T. avellanedae* has demonstrated antinociceptive and antiedematogenic activities in mouse models, with a possible antinociceptive effect correlated with the adenosine system.[54] Adenosine itself is posited to interact with receptors coupled with G protein for this effect.[53,55] Two animal studies using carrageenan-induced subplantar edema have also exhibited the anti-inflammatory abilities of lapachol.[54,56] In one study, the second phase of inflammation, which is correlated with the appearance of prostaglandins and kinins, was where lapachol seemed to exert the greatest anti-inflammatory effect.[56] Lapacho compounds have also demonstrated potent activity against the growth of human keratinocytes and appear to be promising as effective antipsoriatic agents; β-lapachone displayed activity comparable with that of the antipsoriatic drug anthralin. It has been shown that the decoction similar to ethnobotanical use suppresses PGE_2.[57] Beta-lapachone has been found effective in wound healing by inducing cell proliferation, including keratinocytes, fibroblasts, and endothelial cells.[58]

Other Studied Actions

T. avellanedae has been investigated as a possible antidepressant[59] and is also active against ulcers (as well as *Heliobacter pylori*),[60,61] immune modulation,[62] platelet aggregation,[63] and antioxidant volatile fractions.[64]

Quercetin

Quercetin is a highly active flavonoid that inhibits a wide range of enzymes and suppresses the synthesis of DNA, RNA, and proteins.[65] Quercetin's cytotoxicity may be due to the fact that it inhibits mitochondrial electron transport.[66] Hodnick et al.[66] have noted that quercetin produced a substrate-independent potassium cyanide–insensitive respiratory burst in mitochondria. Other flavonoids that have demonstrated this activity produced O_{2^-} and H_2O_2, suggesting that quercetin may also generate these cytotoxic chemicals.

The following are among the enzymes inhibited by quercetin[30,32,67]:

- Nicotinamide adenine dinucleotide (NADH) oxidase
- Phosphodiesterase
- Cyclic adenosine monophosphate (cAMP)–independent protein kinases
- $Ca2^+$ phospholipid–dependent protein kinase
- Tyrosine-protein kinases

Shapiro et al.[16] suggested that the cytotoxicity of quercetin may be due to its chelating abilities. This agent is trypanocidal to *Trypanosoma brucei,* a livestock parasite belonging to the same genus as *T. cruzi.* Soon after the parasite enters the host, the host's unsaturated iron-building proteins remove iron from the hemoflagellate. Because the parasite is unable to synthesize heme, it encounters a shortage of iron. By chelating the host's iron, quercetin blocks utilization of iron by the parasite

yet does not adversely affect the host. This flavonoid is also cytotoxic against *Crithidia fasciculata.* The fact that quercetin is small and lithophilic seems to be important to its activity.

Like lapachol, quercetin inhibited O_2 consumption and H_2O_2 production in neutrophils.[68] Many flavonoids exhibit antiviral activity. When mice that had been intracerebrally infected with attenuated viruses, including rabies, were given quercetin in their diet, a prophylactic effect was observed.[69] Quercetin inactivates the following viruses (*inactivation* is defined as reducing viral infectivity tenfold):

- Herpes simplex type 1
- Respiratory syncytial virus
- Pseudorabies/Aujeszky's virus
- Poliovirus type 1
- Parainfluenza type 3
- Sindbis virus
- Potato virus x

CLINICAL APPLICATIONS

The spectrum of clinical applications of *T. avellanedae* is quite broad. Current use has focused on lapacho's antineoplastic and antimicrobial activity. Its use is extremely popular in the treatment (both topical and internal) of intestinal candidiasis and vaginal candidiasis. Research has also focused on antipsoriatic and antiedema properties. There are also many anecdotal reports of remission of different forms of cancer with the use of this botanical.[68]

Unfortunately, because of the lack of quality control and confusion about the portion of the plant to use, it is highly likely that many practitioners are not using effective materials. This likelihood could explain varying clinical results.

Cancer

Only one recent clinical study appears to be indexed in PubMed. In this Phase II trial, *T. avellanedae* was used an adjunct to conventional cancer treatment. It was shown to be safe and effective in preventing and/or reducing oral mucositis during radiotherapy for head and neck cancer.[70]

Dosage

The usual form of administration of lapacho is as a decoction, with the standard dose being 1 cup of decocted bark two to eight times per day. The decoction is made by boiling 1 teaspoon of lapacho for each cup of water for 5 to 15 minutes.

A more precise dosage based on a lapachol content of 2% to 4% would be 15 to 20 g of bark boiled in 500 mL or 1 pint of water for 5 to 15 minutes three to four times daily.

Dosages of other forms (aqueous extract, fluid extract, solid extract) should be based on lapachol content, providing a daily lapachol intake of 1.5 to 2 g/day.

A tampon that has been soaked in the decoction or fluid extract is used in the treatment of vaginitis and cervicitis. The tampon is inserted vaginally and changed every 24 hours until resolution.

TOXICOLOGY

Although anti–vitamin K activity has been reported for lapachol, the presence of several vitamin K–like substances in the whole plant suggests that this is not a problem.

Long-term administration of lapachol at a dose of 0.0625 to 0.25 g/kg per day to monkeys was found to produce moderate to severe anemia. The anemia was most pronounced during the first 2 weeks of treatment. Death occurred in monkeys after six doses of lapachol at 0.5 g/kg per day and after five doses of 1 g/kg per day.[6]

There have been no reports in the literature of human toxicity when the whole bark is used as a decoction. Long-term use of large doses of the decoction by indigenous peoples has shown no adverse reactions. Because low doses have been reported to cause nausea and vomiting,[71] only a qualified naturopathic physician, herbalist, or other practitioner experienced in its use should attempt to use this botanical medicine.

DRUG INTERACTIONS

No interactions with pharmaceutical drugs have been reported, but because of its known anti–vitamin K–like activity, there may be an interaction with anticoagulant medications, such as warfarin, that could theoretically result in increased bleeding tendencies. This effect would probably be minimized if treatment involved the whole herb rather than specific components. In any case, caution is warranted.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Burnett AR, Thomson RH. Naturally occurring quinones. Part X. the quinonoid constituents of *Tabebuia avellanedae (Bignoniaceae)*. *J Chem Soc*. 1967;C:2100–2104.
2. Canadian Health Protection Branch. Herbs and botanical preparations. *Information Letter*. 1987;13:726.
3. Duke JA. *CRC Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1985.
4. Bernardes A. *A Pocket Book of Brazilian Herbs (Folklore—History—Uses)*. Rio de Janeiro: Shogun Editora; 1984.
5. Hartwell JL. *Plants Used Against Cancer: A Survey*. Lawrence, MA: Quarterman Publications; 1982.
6. Morrison RK, Brown DE, Oleson JJ, et al. Oral toxicology studies with lapachol. *Toxicol Appl Pharmacol*. 1970;17:1–11.
7. Queiroz ML, Valadares MC, Torello CO, et al. Comparative studies of the effects of *Tabebuia avellanedae* bark extract and beta-lapachone on the hematopoietic response of tumour-bearing mice. *J Ethnopharmacol*. 2008;117(2):228–235. Epub 2008 Feb 8.
8. de Lima OG, d'Albuquerque IL, Machado MP, et al. Primeiras observacoes sobre a acao antimicrobiana do lapachol. *Anais da Sociedade de biologica de pernambuco*. 1956;XIV:129–135.
9. de Lima OG, d'Albuquerque IL, Machado MP, et al. Uma nova substancia antibiotica isolada do "Pau D'Arco," *Tabebuia* sp. *Anais da Sociedade de biologica de pernambuco*. 1956;XIV:136–140.
10. Lagrota M, et al. Antiviral activity of lapachol. *Rev Microbiol*. 1983;14:21–26.
11. Guiraud P, Steiman R, Campos-Takaki GM, et al. Comparison of antibacterial and antifungal activities of lapachol and beta-lapachone. *Planta Med*. 1994;60:373–374.
12. Lopes JN, Cruz FS, Docampo R, et al. In vitro and in vivo evaluation of the toxicity of 1,4-naphthoquinone and 1,2-naphthoquinone derivatives against *Trypanosoma cruzi*. *Ann Trop Med Parasitol*. 1978;72:523–531.
13. Schuerch AR, Wehrli W. Beta-lapachone, an inhibitor of oncornavirus reverse transcriptase and eukaryotic DNA polymerase-alpha: inhibitory effect, thiol dependence and specificity. *Eur J Biochem*. 1978;84:197–205.
14. Pinto AV, Pinto MD, Gilbert B, et al. Schistosomiasis mansoni: blockage of cercarial skin penetration by chemical agents. I. Naphthoquinones and derivatives. *Trans R Soc Trop Med Hyg*. 1977;71:133–135.
15. de Lima OG, d'Albuquerqul IL, de Lima CG, et al. Antividade antimicrobiana de alguns derivados do lapachol em comparacao com a xiloidona, Nova ortonaftoquinona natural isolada de extractos do cerne do "Pau d'Arco" roxo, *Tabebuia avellanedae* Lor. ex. Griseb. Substancias antimicrobianas de plantas superiores. *Revista do Instituto de Antibioticos Recife*. 1962;4:20.
16. Shapiro A, Nathan HC, Hutner SH, et al. In vivo and in vitro activity by diverse chelators against *Trypanosoma brucei brucei*. *J Protozool*. 1982;29:85–90.
17. Howland JL. Uncoupling and inhibition of oxidative phosphorylation by 2-hydroxy-3-alkyl-1,4-naphthoquinones. *Biochem Biophys Acta*. 1963;77:659–662.
18. Wendel WB. The influence of naphthoquinones upon the respiratory and carbohydrate metabolism of malarial parasites. *Fed Proc*. 1946;5:406–407.
19. Ball EG, Anfinsen CB, Cooper O. The inhibitory action of naphthoquinones on respiratory processes. *J Biol Chem*. 1947;168:257–270.
20. Fieser LF, Richardson AP. Naphthoquinone antimalarials. II. Correlation of structure and activity against *Plasmodium lophurae* in ducks. *J Am Chem Soc*. 1948;70:3156–3165.
21. Gosalvez M, Garcia-Canero R, Blanco M, et al. Effects and specificity of anticancer agents on the respiration and energy metabolism of tumor cells. *Cancer Treat Rep*. 1976;60:1–8.
22. Crawford DR, Schneider DL. Identification of ubiquinone-50 in human neutrophils and its role in microbicidal events. *J Biol Chem*. 1982;257:6662–6668.
23. Howland JL. Influence of alkylhydroxynaphthoquinones on the mitochondrial oxidation of tetramethyl-p-phenylenediamine. *Biochim Biophys Acta*. 1967;131:247–254.
24. Hadler HI, Moreau TL. The induction of ATP energized mitochondrial volume changes by the combination of the two antitumour age agents showdomycin and lapachol. *J Antibiot (Tokyo)*. 1969;22:513–520.
25. Koide SS. Inhibition of 3alpha-hydroxysteroid-mediated transhydrogenase of rat liver by various quinones and flavonoids. *Biochem Biophys Acta*. 1962;59:708–710.
26. Austin FG. *Schistosoma mansoni* chemoprophylaxis with dietary lapachol. *Am J Trop Med Hyg*. 1974;23:412–419.
27. Wanick MC, Bandeira JA, Fernandes RV. Acao antiinflamatoria e cicatrizante do extrato hidroalcoolico do liber do pau d'arco roxo *(Tabebuia avellanedae),* em pacientes portadoras de cervicites e cervico-vaginites. *Separata da Revista do Instituto de Antibioticos*. 1970;10:41–46.
28. Schaffner-Sabba K, Schmidt-Ruppin KH, Wehrli W, et al. Beta-lapachone: synthesis of derivatives and activities in tumor models. *J Med Chem*. 1984;27:990–994.
29. Gilbert B, de Souza JP, Fascio M, et al. Schistosomiasis: protection against infection by terpenoids. *An Acad Bras Cienc*. 1970;42:397–400.
30. Goijman SG, Stoppani AO. Oxygen radicals and macromolecule turnover in *Trypanosoma cruzi*. *Life Chem Rep*. 1984;2:216–221.
31. Menna-Barreto RF, Henriques-Pons A, Pinto AV, et al. Effect of a beta-lapachone-derived naphthoimidazole on *Trypanosoma cruzi*: identification of target organelles. *J Antimicrob Chemother*. 2005;56(6):1034–1041. Epub 2005 Nov 3.
32. Docampo R, Cruz FS, Boveris A, et al. Beta-lapachone enhancement of lipid peroxidation and superoxide anion and hydrogen peroxide formation by sarcoma 180 ascites tumor cells. *Biochem Pharmacol*. 1979;28:723–728.
33. Boveris A, Stoppani AO, Docampo R, et al. Superoxide anion production and trypanocidal action of naphthoquinones on *Trypanosoma cruzi*. *Comp Biochem Physiol C*. 1978;61:327–329.
34. Genet J. Natural remedies for vaginal infections. *Sidahora*. 1995:40–41. Winter.
35. Portillo A, Vila R, Freixa B, et al. Antifungal activity of Paraguayan plants used in traditional medicine. *J Ethnopharmacol*. 2001;76:93–98.
36. Block JB, Serpick AA, Miller W, et al. Early clinical studies with lapachol (NSC-11905). *Cancer Chemother Rep*. 1974;2(4):27–28.
37. Preusch PC, Suttie JW. Lapachol inhibition of vitamin K epoxide reductase and vitamin K quinone reductase. *Arch Biochem Biophys*. 1984;234:405–412.
38. Guiraud P, Steiman R, Campos-Takaki GM, et al. Comparison of antibacterial and antifungal activities of lapachol and beta-lapachone. *Planta Med*. 1994;60:373–374.
39. Dubin M, Fernandez Villamil SH, Stoppani AO. Cytotoxicity of beta-lapachone, an naphthoquinone with possible therapeutic use. *Medicina (B Aires)*. 2001;61:343–350.
40. Bachur NR, Gordon SL, Gee MV, et al. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc Natl Acad Sci USA*. 1979;76:954–957.
41. Bachur NR, Gordon SL, Gee MV. A general mechanism for microsomal activation of quinone anticancer agents to free radicals. *Cancer Res*. 1978;38:1745–1750.
42. Iwamoto Y, Hansen IL, Porter TH, et al. Inhibition of coenzyme Q_{10}-enzymes, succinoxidase and NADH-oxidase, by Adriamycin and other quinones having antitumor activity. *Biochem Biophys Res Commun*. 1974;58:633–638.
43. Bennett Jr LL, Smithers D, Rose LM, et al. Inhibition of synthesis of pyrimidine nucleotides by 2-hydroxy-3-(3,3-dichloroallyl)-1,4-naphthoquinone. *Cancer Res*. 1979;39:4868–4874.
44. Lee SH, Sutherland TO, Deves R, et al. Restoration of active transport of solutes and oxidative phosphorylation by naphthoquinones in irradiated membrane vesicles from *Mycobacterium phlei*. *Proc Natl Acad Sci U S A*. 1980;77:102–106.
45. Choi BT, Cheong J, Choi YH. Beta-lapachone-induced apoptosis is associated with activation of caspase-3 and inactivation of NF-kappaB in human colon cancer HCT-116 cells. *Anticancer Drugs*. 2003;14:845–850.
46. Kim SO, Kwon JI, Jeong YK, et al. Induction of Egr-1 is associated with anti-metastatic and anti-invasive ability of beta-lapachone in human hepa-

tocarcinoma cells. *Biosci Biotechnol Biochem*. 2007;71(9):2169–2176. Epub 2007 Sep 7.
47. Kim SO, Kwon JI, Jeong YK, et al. Induction of Egr-1 is associated with anti-metastatic and anti-invasive ability of beta-lapachone in human hepatocarcinoma cells. *Biosci Biotechnol Biochem*. 2007;71(9):2169–2176. Epub 2007 Sep 7.
48. Lee JI, Choi DY, Chung HS, et al. Beta-lapachone induces growth inhibition and apoptosis in bladder cancer cells by modulation of Bcl-2 family and activation of caspases. *Exp Oncol*. 2006;28(1):30–35.
49. Lee JH, Cheong J, Park YM, et al. Down-regulation of cyclooxygenase-2 and telomerase activity by beta-lapachone in human prostate carcinoma cells. *Pharmacol Res*. 2005;51(6):553–560.
50. Choi YH, Kang HS, Yoo MA. Suppression of human prostate cancer cell growth by beta-lapachone via down-regulation of pRB phosphorylation and induction of Cdk inhibitor p21(WAF1/CIP1). *J Biochem Mol Biol*. 2003;36(2):223–229.
51. Woo HJ, Choi YH. Growth inhibition of A549 human lung carcinoma cells by beta-lapachone through induction of apoptosis and inhibition of telomerase activity. *Int J Oncol*. 2005;26(4):1017–1023.
52. Mukherjee B, Telang N, Wong GY. Growth inhibition of estrogen receptor positive human breast cancer cells by Taheebo from the inner bark of *Tabebuia avellandae* tree. *Int J Mol Med*. 2009;24(2):253–260.
53. Fredholm BB, Gustafsson LE, Hedquist P, et al. Adenosine in the regulation of neurotransmitter release in the peripheral nervous system. In: Berne RM, Rall TW, Rubio R, eds. *Regulatory Function of Adenosine: Proceedings of the International Symposium on Adenosine, Charlottesville, Virginia, June 7–11, 1982*. Boston: M. Nijhoff Publishers; 1983:479–493.
54. de Miranda FG, Vilar JC, Alves IA, et al. Antinociceptive and antiedematogenic properties and acute toxicity of *Tabebuia avellanedae* Lor. ex Griseb. inner bark aqueous extract. *BMC Pharmacol*. 2001;1:6.
55. Daval JL, Nehlig A, Nicolas F. Physiological and pharmacological properties of adenosine: therapeutic implications. *Life Sci*. 1991;49:1435–1453.
56. de Almeida ER, da Silva Filho AA, dos Santos ER, et al. Antiinflammatory action of lapachol. *J Ethnopharmacol*. 1990;29:239–241.
57. Byeon SE, Chung JY, Lee YG, et al. In vitro and in vivo anti-inflammatory effects of taheebo, a water extract from the inner bark of *Tabebuia avellanedae*. *J Ethnopharmacol*. 2008;119(1):145–152. Epub 2008 Jun 27.
58. Kung HN, Yang MJ, Chang CF, et al. In vitro and in vivo wound healing-promoting activities of beta-lapachone. *Am J Physiol Cell Physiol*. 2008;295(4):C931–C943. Epub 2008 Jul 23.
59. Freitas AE, Budni J, Lobato KR, et al. Antidepressant-like action of the ethanolic extract from *Tabebuia avellanedae* in mice: evidence for the involvement of the monoaminergic system. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;34(2):335–343. Epub 2009 Dec 22.
60. Twardowschy A, Freitas CS, Baggio CH, et al. Antiulcerogenic activity of bark extract of *Tabebuia avellanedae,* Lorentz ex Griseb. *J Ethnopharmacol*. 2008;118(3):455–459. Epub 2008 May 18.
61. Park BS, Lee HK, Lee SE, et al. Antibacterial activity of *Tabebuia impetiginosa Martius* ex DC (Taheebo) against *Helicobacter pylori*. *J Ethnopharmacol*. 2006;105(1–2):255–262. Epub 2005 Dec 15.
62. Böhler T, Nolting J, Gurragchaa P, et al. *Tabebuia avellanedae* extracts inhibit IL-2-independent T-lymphocyte activation and proliferation. *Transpl Immunol*. 2008;18(4):319–323. Epub 2007 Sep 4.
63. Son DJ, Lim Y, Park YH, et al. Inhibitory effects of *Tabebuia impetiginosa* inner bark extract on platelet aggregation and vascular smooth muscle cell proliferation through suppressions of arachidonic acid liberation and ERK1/2 MAPK activation. *J Ethnopharmacol*. 2006;108(1):148–151.
64. Park BS, Lee KG, Shibamoto T, et al. Antioxidant activity and characterization of volatile constituents of taheebo (*Tabebuia impetiginosa martius* ex DC). *J Agric Food Chem*. 2003;51(1):295–300.
65. Graziani Y. The effect of quercetin of $pp60^{v\text{-}src}$ kinase activities. In: Cody V, Middleton E Jr, Harborne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships: Proceedings of a Symposium Held in Buffalo, New York, July 22–26, 1985.* New York: Liss; 1986:301–313.
66. Hodnick WF, Roettger WJ, Kung FS. Inhibition of mitochondrial respiration and production of superoxide and hydrogen peroxide by flavonoids: a structure activity study. In: Cody V, Middleton E. Jr, Harborne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships: Proceedings of a Symposium Held in BUFFALO, New York, July 22–26, 1985.* New York: Liss; 1986:249–252.
67. Srivastava AK, Chiasson JL. Effect of quercetin on serine/threonine and tyrosine protein kinases. In: Cody V, Middleton E. Jr, Harborne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships: Proceedings of a Symposium Held in Buffalo, New York, July 22–26, 1985.* New York: Liss; 1986:315–318.
68. Weed B. *Second Opinion: Lapacho Fight Against Cancer*. Vancouver: Rostrum Communication; 1984.
69. Selway JWT. Antiviral activity of flavones and flavins. In: Cody V, Middleton E. Jr, Harborne JB, eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships: Proceedings of a Symposium Held in Buffalo, New York, July 22–26, 1985.* New York: Liss; 1986:521–536.
70. Giacomelli I, Scartoni D, Fiammetta M, et al. Oral lapacho-based medication: an easy, safe, and feasible support to prevent and/or reduce oral mucositis during radiotherapy for head and neck cancer. *Nutr Cancer*. 2015;67(8):1247–1253.
71. Questionable methods of cancer management: "nutritional" therapies. *CA Cancer J Clin*. 1993;43:309–319.

116

Tanacetum parthenium (Feverfew)

Kathy Abascal, BS, JD, RH(AHG), and *Eric L. Yarnell, ND, RH(AHG)*

OUTLINE

GENERAL DESCRIPTION

Fever few *(Tanacetum parthenium)* is a composite plant cultivated in flower gardens throughout Europe and the United States (Figs. 116.1 and 116.2).

CHEMICAL COMPOSITION

The major active chemicals in the plant have been hypothesized to be sesquiterpene lactones, principally parthenolide. However, many investigators have found evidence of other active constituents, including both lipophilic compounds (e.g., sesquiterpene lactones) and hydrophilic compounds, although their exact identity has not been determined.[1–3] The flowering herb also contains 0.02% to 0.07% volatile oils (L-camphor, L-borneol, terpenes, and miscellaneous esters).[4,5] Various compounds in the volatile oil have shown pharmacological activity (Fig. 116.3).[6]

Fig. 116.1 *Tanacetum parthenium* (family: *Asteraceae*); synonym: *Chrysanthemum parthenium*; common names: feverfew, featherfew. (Image from Eric Yarnell.)

HISTORY AND FOLK USE

Feverfew has been used for centuries as a febrifuge and for the treatment of migraines and arthritis. Other historical uses of feverfew have been in the treatment of anemia, earache, dysmenorrhea, dyspepsia, trauma, and intestinal parasites.[4] It has also been used as an abortifacient and in gardens to control noxious pests (its pyrethrin component is an effective insecticide and herbicide).

PHARMACOLOGY

Feverfew has demonstrated some remarkable pharmacological effects in experimental studies. Extracts of feverfew have been shown to inhibit the synthesis of compounds that promote inflammation, including inflammatory prostaglandins, leukotrienes, and thromboxanes. No adverse effects reported for feverfew mimic those of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs), suggesting that the effects of feverfew are in some way distinct from those of the drugs. Inhibition of phospholipase A_2 is more like the effects of corticosteroids.[7] Many other inflammation-modulating effects of feverfew and parthenolide have been shown over the years.[8,9]

Feverfew also has favorable effects on the behavior of blood platelets in vitro,[7,10] including inhibition of platelet aggregation and the secretion of inflammatory and allergic mediators like histamine and serotonin. Parthenolide components also exert a tonic effect on vascular smooth muscle.[11]

A growing body of preclinical research also supports that feverfew extracts and parthenolide have significant anticancer effects. This includes induction of apoptosis in and inhibition of growth of cancer cells as well as decreasing angiogenesis.[12–16]

Fig. 116.2 Feverfew whole plant (A), flower (B), and feathery leaves (C). (From https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3210009/.)

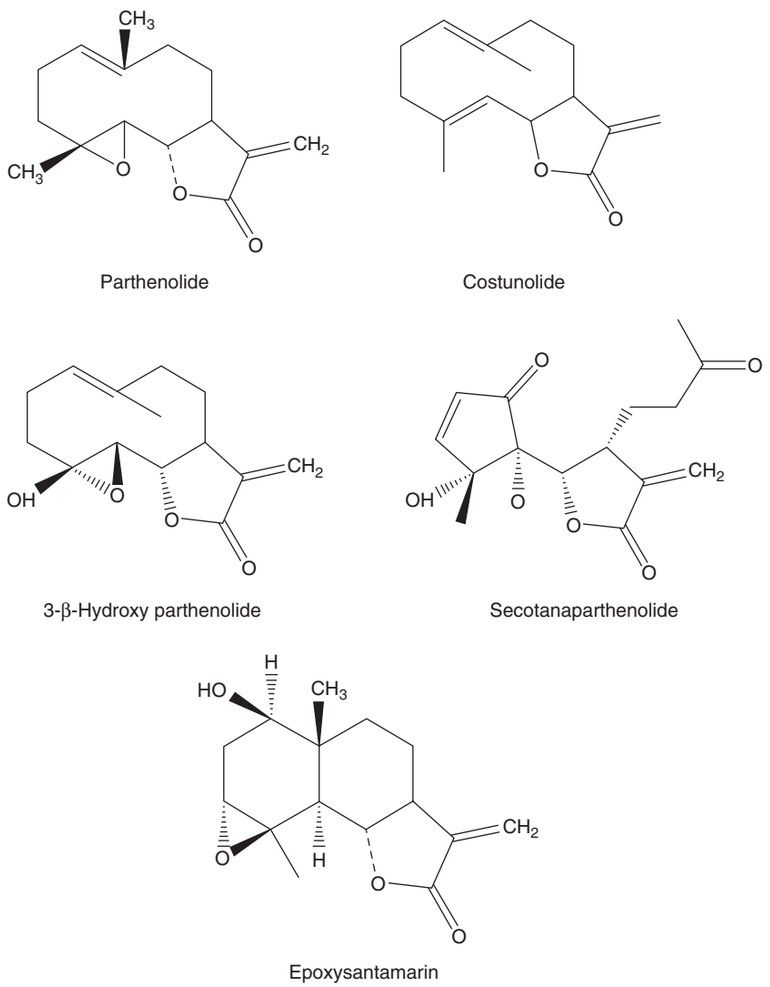


Fig. 116.3 Sesquiterpene lactones of *Tanacetum parthenium*. (From https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3210009/.)

CLINICAL APPLICATIONS

Feverfew has been used for centuries to relieve fever, migraines, and arthritis. The only condition with confirmed scientific documentation at present is in the prevention and treatment of migraine headache.

MIGRAINE HEADACHE

Physician John Hill, in his book *The Family Herbal* (1772), noted, "In the worst headache this herb exceeds whatever else is known." A 1983 survey found that 70% of 270 migraine sufferers who had eaten feverfew daily for prolonged periods claimed that the herb decreased the frequency and/or intensity of their attacks.[17] Many of these patients had shown no response to orthodox medicines.

Numerous double-blind trials have been conducted on the efficacy of feverfew in patients with migraine,[17–22] the results of which have been assessed in three meta-analyses.[23–25] The first two of these concluded that the majority of studies show that feverfew extracts are superior to placebo for decreasing the frequency and severity of migraine headaches, although most of the studies were of relatively

low methodological quality, and efficacy was not proved beyond a reasonable doubt.[21,24] The third meta-analysis concluded that feverfew extracts have not been proved in controlled trials to prevent migraine better than placebo.[25]

The highest-quality study among feverfew clinical trials used a granulated ethanol extract of feverfew and found it ineffective compared with placebo, whereas all the other trials used unextracted powdered feverfew. As a result, careful attention must be paid in future studies to the types of products used, and the results of trials using different products should probably not be combined in meta-analyses.

One double-blind, randomized clinical trial compared the effects of a combination of a feverfew extract (100 mg) with riboflavin 400 mg and magnesium 300 mg taken daily with riboflavin 25 mg.[26] The control, riboflavin 25 mg, appeared to be just as active as the combination formula, and the trial showed that combining feverfew and magnesium with riboflavin adds no additional benefits for preventing migraine headaches.

In an open trial, feverfew combined with *Salix alba* (white willow) extracts, 300 mg of each twice a day, has been shown effective at preventing and reducing the severity of migraine without aura.[27] A combination of homeopathic dilutions of *Zingiber officinale* (ginger) and feverfew was effective in one open and one double-blind, randomized, placebo-controlled trial for relieving acute migraine pain.[28,29] It is not known if either homeopathic is superior to feverfew alone or how they would compare with botanical extracts in a head-to-head comparative trial.

RHEUMATOID ARTHRITIS

Inflammatory compounds released by white blood cells and platelets contribute greatly to the inflammation and cellular damage found in rheumatoid arthritis. Inhibition of the release of inflammatory particles by feverfew is much greater than that achieved by NSAIDs like aspirin.[10] This finding, coupled with many of feverfew's other effects, indicates that feverfew can reduce inflammation in rheumatoid arthritis.

Although a double-blind, placebo-controlled study demonstrated no apparent benefit from oral feverfew in rheumatoid arthritis, the dosage used was small (76 mg dried powdered feverfew leaf, corresponding to two medium-sized leaves), the level of parthenolide was not determined in the product, and patients continued to take NSAIDs, a practice that has been suggested to reduce the efficacy of feverfew.[30] Therefore the benefit of feverfew in rheumatoid arthritis has not yet been determined.

DOSAGE

The effectiveness of feverfew was thought to depend on adequate levels of parthenolide, although multiple studies confirm that there are multiple active compounds in feverfew and that parthenolide-free extracts are still active.[31,32] It is difficult to determine the relevance of measuring parthenolide in extracts; assessments of activity of extracts would perhaps be superior, although more expensive.

The preparations used in some successful clinical trials had a parthenolide content of 0.4% to 0.66%. The dosage of feverfew used in the London Migraine Clinic study was one capsule containing 25 mg of the freeze-dried pulverized leaves given twice a day.[6] In the Nottingham study it was one capsule containing 82 mg of dried powdered leaves given once a day.[7] Therefore the daily dosage of parthenolide that may be effective in preventing a migraine headache is roughly 0.25 to 0.5 mg.

Although these low dosages may be effective in preventing an attack, a higher dose (1–2 g) is necessary during an acute attack: dried pulverized leaves 25 to 50 mg given twice a day.

TOXICITY

There were no reports of toxic reactions in patients taking feverfew in the 6-month migraine study. Feverfew has been used by large numbers of people for many years without reports of toxicity. Chewing the leaves, however, may result in aphthous ulcerations, and some sensitive persons experience an exudative dermatitis from external contact.[33] In clinical trials, neither mouth ulcers nor dermatitis was more common in the feverfew groups than the placebo groups; in one trial, aphthous ulcers were actually more common in the placebo group.[19]

DRUG INTERACTIONS

Because feverfew affects the activity of several of the CYP liver enzymes involved in drug metabolism, a number of interactions are theoretically possible.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Brown AMG, Edwards CM, Davey MR, et al. Pharmacological activity of feverfew *(Tanacetum parthenium (L) Schultz-Bip.)*: assessment by inhibition of human polymorphonuclear leukocyte chemiluminescence in-vitro. *J Pharm Pharmacol*. 1997;49:558–561.
2. Sumner H, Salan U, Knight DW, et al. Inhibition of 5-lipoxygenase and cyclo-oxygenase in leukocytes by feverfew: involvement of sesquiterpene lactones and other components. *Biochem Pharmacol*. 1992;43:2313–2320.
3. Marles RJ, Kaminski J, Arnason JT, et al. A bioassay for inhibition of serotonin release from bovine platelets. *J Nat Prod*. 1992;55:1044–1056.
4. Duke JA. *CRC Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1985.
5. Bohlmann F, Zdero C. Sesquiterpene lactones and other constituents from Tanacetum parthenium. *Phytochemistry*. 1982;21:2543–2549.
6. Pugh WJ, Sambo K. Prostaglandin synthetase inhibitors in feverfew. *J Pharm Pharmacol*. 1988;40:743–745.
7. Makheja AN, Bailey JM. A platelet phospholipase inhibitor from the medicinal herb feverfew *(Tanacetum parthenium)*. *Prostaglandins Leukot Med*. 1982;8:653–660.
8. Arıkan-Ayyıldız Z, Karaman M, Özbal S, et al. Efficacy of parthenolide on lung histopathology in a murine model of asthma. *Allergol Immunopathol (Madr)*. 2017;45:63–68.
9. de Carvalho LSA, Fontes LBA, Gazolla MC, et al. Parthenolide modulates immune response in cells from C57BL/6 mice induced with experimental autoimmune encephalomyelitis. *Planta Med*. 2017;83:693–700.
10. Heptinstall S, White A, Williamson L, et al. Extracts of feverfew inhibit granule secretion in blood platelets and polymorphonuclear leukocytes. *Lancet*. 1985;1:1071–1074.
11. Barsby RW, Salan U, Knight DW, et al. Feverfew and vascular smooth muscle: extracts from fresh and dried plants show opposing pharmacological profiles, dependent upon sesquiterpene lactone content. *Planta Med*. 1993;59:20–25.
12. Jafari N, Nazeri S, Enferadi ST. Parthenolide reduces metastasis by inhibition of vimentin expression and induces apoptosis by suppression elongation factor α-1 expression. *Phytomedicine*. 2018;41:67–73.
13. Lin M, Bi H, Yan Y, et al. Parthenolide suppresses non-small cell lung cancer GLC-82 cells growth via B-Raf/MAPK/Erk pathway. *Oncotarget*. 2017;8:23436–23447.
14. Duan D, Zhang J, Yao J, et al. Targeting thioredoxin reductase by parthenolide contributes to inducing apoptosis of HeLa cells. *J Biol Chem*. 2016;291:10021–10031.
15. Al-Fatlawi AA, Al-Fatlawi AA, Irshad M, et al. Effect of parthenolide on growth and apoptosis regulatory genes of human cancer cell lines. *Pharm Biol*. 2015;53:104–109.
16. Kim SL, Lee ST, Trang KT, et al. Parthenolide exerts inhibitory effects on angiogenesis through the downregulation of VEGF/VEGFRs in colorectal cancer. *Int J Mol Med*. 2014;33:1261–1267.
17. Johnson ES, Kadam NP, Hylands DM, et al. Efficacy of feverfew as prophylactic treatment of migraine. *Br Med J (Clin Res Ed)*. 1985;291:569–573.
18. Diener HC, Pfaffenrath V, Schnitker J, et al. Efficacy and safety of 6.25 mg t.i.d. feverfew CO_2-extract (MIG-99) in migraine prevention—a randomized, double-blind, multicentre, placebo-controlled study. *Cephalalgia*. 2005;25(11):1031–1041.
19. Murphy JJ, Heptinstall S, Mitchell JRA. Randomized double-blind placebo-controlled trial of feverfew in migraine prevention. *Lancet*. 1988;2:189–192.
20. Palevitch D, Earon G, Carasso R. Feverfew *(Tanacetum parthenium)* as a prophylactic treatment for migraine: a double-blind placebo-controlled study. *Phytother Res*. 1997;11:508–511.
21. De Weerdt CJ, Bootsma HPR, Hendriks H. Herbal medicine in migraine prevention. Randomized double-blind placebo-controlled crossover trial of a feverfew preparation. *Phytomedicine*. 1996;3:225–230.
22. Kuritzky A, Elhacham Y, Yerushalmi Z, et al. Feverfew in the treatment of migraine: its effect on serotonin uptake and platelet activity. *Neurology*. 1994;44(suppl 2):293P.
23. Pittler MH, Vogler BK, Ernst E. Feverfew for preventing migraine. *Cochrane Database Syst Rev*. 2000:CD002286.
24. Vogler BK, Pittler MH, Ernst E. Feverfew as a preventive treatment for migraine: a systematic review. *Cephalalgia*. 1998;18:704–708.
25. Pittler MH, Ernst E. Feverfew for preventing migraine. *Cochrane Database Syst Rev*. 2004;1:CD002286.
26. Maizels M, Blumenfeld A, Burchette R. A combination of riboflavin, magnesium, and feverfew for migraine prophylaxis: a randomized trial. *Headache*. 2004;44:885–890.
27. Shrivastava R, Pechadre JC, John GW. *Tanacetum parthenium* and *Salix alba* (Mig-RL) combination in migraine prophylaxis: a prospective, open-label study. *Clin Drug Invest*. 2006;26(5):287–296.
28. Cady RK, Schreiber CP, Beach ME, et al. Gelstat Migraine (sublingually administered feverfew and ginger compound) for acute treatment of migraine when administered during the mild pain phase. *Med Sci Monit*. 2005;11(9):PI65–PI69.
29. Cady RK, Goldstein J, Nett R, et al. A double-blind placebo-controlled pilot study of sublingual feverfew and ginger (LipiGesic™ M) in the treatment of migraine. *Headache*. 2011;51:1078–1086.
30. Pattrick M, Heptinstall S, Doherty M. Feverfew in rheumatoid arthritis: a double blind, placebo controlled study. *Ann Rheum Dis*. 1989;48:547–549.
31. Sur R, Martin K, Liebel F, et al. Anti-inflammatory activity of parthenolide-depleted feverfew *(Tanacetum parthenium)*. *Inflammopharmacology*. 2009;17(1):42–49.
32. Sumner H, Salan U, Knight DW, et al. Inhibition of 5-lipoxygenase and cyclo-oxygenase in leukocytes by feverfew. Involvement of sesquiterpene lactones and other components. *Biochem Pharmacol*. 1992;43(11):2313–2320.
33. Awang DVC. Feverfew. *Can Pharm J*. 1989;122:266–270.

117

Taraxacum officinale (Dandelion)

Michael T. Murray, ND

OUTLINE

Taraxacum officinale (family: *Compositae*)
Common names:

- English: dandelion, wet-a-bed, lion's tooth
- French: *dent-de-lion, pissenlit*
- German: *Lowenzahn, Pfaffenrohrlein*
- Spanish: *diente de leon*
- Italian: *tarassaco*
- Chinese: *p'u kung ying, Ching p'o po, chiang-nou-ts'ao, huang-hua-tii-ting*

GENERAL DESCRIPTION

Dandelion *(Taraxacum officinale)* is a member of the *Compositae* family and is closely related to chicory. Several origins have been attributed to the name *Taraxacum,* the most likely being from the Greek *taraxo* ("disorder," "disturbance"), *akos* ("remedy"), and *akeomai* ("I heal") and from *tharakhcharkon,* possibly a derivative of a Persian-Arabic word for "edible" and the name by which the plant is referred to in a 13th-century Arabian botanical work.[1]

Taraxacum is known around the world by a variety of names. In English-speaking countries, dandelion (from the French *dent-de-lion,* referring to the plant's lion's tooth leaves) is its most common name. It is also known as wet-a-bed (after its diuretic action), lion's tooth, fairy clock, priest's crown, swine's snout, blowball, milk gowan, wild endive, white endive, cankerwort, puffball, and Irish daisy.

Dandelion is a variable perennial growing to a height of 12 in. Its spatula-like leaves are deeply toothed, shiny, and hairless and are arranged in a ground-level rosette. The yellow flowers bloom for most of the year and are sensitive to light and weather—opening at daybreak and closing at nightfall and opening in dry weather and closing in wet weather (a closed dandelion flower signals rain). When the flower matures, it closes up, the petals wither, and it forms into a puffball containing seeds that are dispersed by the breeze.

The rosette formation of grooved leaves channels rainwater into the center and down to a taproot, which is thick and dark brown, almost black on the outside. The root is cylindrical, tapering, and somewhat branched. It has a slight odor and sweetish taste. The inside of dried dandelion root is yellowish, very porous, and without pith. It is believed that the plant originated in Central Asia and spread throughout most of the world, preferring the cooler climates. Although *Taraxacum* is very adaptable, it prefers moist, nitrogen-rich soils at altitudes less than 6000 ft. Most species occur in the temperate zones of the northern hemisphere, with the greatest concentration in northwest Europe.

The portion of the plant that is most commonly used is the root; however, the leaves and whole plant can also be used. In addition to its medicinal use, dandelion is a nutritious food and beverage. Tender leaves are used raw in salads and sandwiches or lightly cooked as a vegetable. Tea is made from the leaves, coffee substitute from the roots, and wine and schnapps from the flowers.

CHEMICAL COMPOSITION

Dandelion root's calorie count is exceptionally low—a cup is only 25 calories—and its nutrient content is exceptionally high. In fact, dandelion root has greater nutritional value than many other vegetables. It is particularly high in vitamins and minerals, protein, choline, inulin, and pectin. Its carotenoid content is extremely high, as reflected by the fact that it has a higher vitamin A content (as beta-carotene) than carrots (dandelion has 14,000 IU of vitamin A per 100 grams, compared with 11,000 IU for carrots). Dandelion greens are an outstanding source of vitamin A and an excellent source of vitamin C, riboflavin, vitamin B_6, and thiamine as well as calcium, copper, manganese, and iron.

The primary therapeutic actions of dandelion are believed to be due to the bitter principle of taraxacin, various terpenoids, inulin, and its high concentration of nutrients, especially choline. Other constituents of dandelion that may contribute to its pharmacological effects are resins, pectin, taraxanthin (a carotenoid pigment in the flowers), fatty acids, and flavonoids.[2,3]

The roots of *T. officinale* contain the triterpenes b-amyrin, taraxasterol, and taraxerol and the sterols sitosterin, stigmasterin, and phytosterin (Figs. 117.1 and 117.2).[4]

Later research has elicited several compounds that are likely to be clinically significant. Three flavonoid glycosides—luteolin 7-glucoside and two luteolin 7-diglucosides—have been isolated from dandelion flowers and leaves, together with free luteolin and chrysoeriol

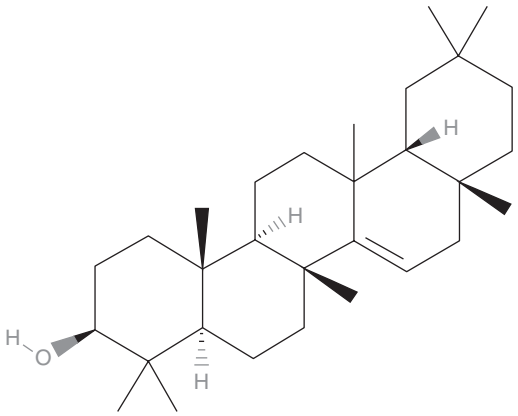


Fig. 117.1 Taraxerol.

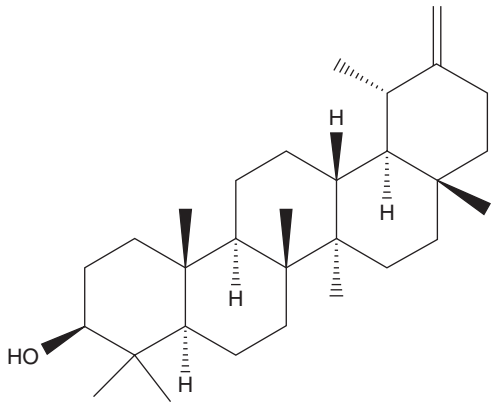


Fig. 117.2 Taraxasterol.

in the flower tissue. Three hydroxycinnamic acids—chicoric acid, monocaffeyltartaric acid, and chlorogenic acid—have been found throughout the plant, and the coumarins cichoriin and aesculin have been identified in leaf extracts. Chicoric acid and the related monocaffeyltartaric acid were found to be the major phenolic constituents in dandelion flowers, roots, leaves, and involucral bracts and also in the medicinal preparations tested.[5]

HISTORY AND FOLK USE

Although many individuals may consider the common dandelion an unwanted weed, herbalists all over the world have revered this valuable herb. Generally regarded as a liver remedy, dandelion has a long history of folk use throughout the world. In Europe, dandelion has been used in the treatment of the following conditions:

- Fevers
- Boils
- Eye problems
- Diarrhea
- Fluid retention
- Liver congestion
- Heartburn
- Various skin problems

In China, dandelion has been used to treat the following disorders:

- Breast problems (cancer, inflammation, lack of milk flow, etc.)
- Liver diseases
- Appendicitis
- Digestive ailments

Dandelion's use in India, Russia, and most other parts of the world has revolved primarily around its action on the liver.

PHARMACOLOGY

The primary pharmacological actions of dandelion relate to digestion, liver function, and diuresis.

Digestive Effects

Bitter herbs like dandelion are used to aid digestion on the basis of the belief that bitter principles stimulate the initial phase of digestion, including the secretion of salivary and gastric juices. Dandelion goes beyond this initial phase by stimulating the release of bile by the liver and gallbladder.

The digestive tonic properties attributed to dandelion are now believed to be due to a bitter principle researchers have named *taraxacin* and have identified as belonging to a class of active substances called guaianolides, which have intestinal antiseptic, germicidal, and expectorant effects.[6]

Dandelion root contains a very high concentration (up to 40%) of inulin, which serves as a food source for the "friendly" colonic bacteria species *Bifidobacterium* and *Lactobacillus* and thus promotes their growth. Aqueous root extracts of *Taraxacum officinale* were tested for their activity in stimulating the growth of 14 different strains of bifidobacteria. The growth of six strains (*B. adolescentis* 1 and 2, *B. bifidum* 1, *B. catenulatum,* and *B. longum* 2) was significantly enhanced; two strains were slightly enhanced, whereas the remaining six strains exhibited no enhancement.[7]

Blood Sugar Control

A number of components in dandelion have demonstrated antidiabetic effects.[8] Foremost are inulin and taxasterol, but also the sesquiterpene lactones, phenols, flavonoids, and phenolic acids. Inulin is also helpful in improving blood sugar control and diabetes.[9] Studies on dandelion extracts showed an ability to stimulate the release of insulin in pancreatic β-cells as well as improve blood sugar control in a number of different animal models.[8]

Liver Tonic

Studies in humans and laboratory animals have shown that dandelion root enhances the flow of bile, improving such conditions as liver congestion, bile duct inflammation, hepatitis, gallstones, and jaundice.[1,2,10,11] Dandelion's action in increasing bile flow is twofold: it has a direct effect on the liver, causing rises in bile production and flow to the gallbladder (choleretic effect), and a direct effect on the gallbladder, causing its contraction and the release of stored bile (cholagogue effect). The high choline content of the root may be a major factor in dandelion's ability to act as a "tonic" to the liver. Historically, dandelion's positive effect on such a wide variety of conditions is probably closely related to its ability to improve liver function.

In one animal study, dandelion significantly improved the liver's ability to clear toxins by 244%. Dandelion's effectiveness in improving the liver's ability to clear potentially toxic agents was also demonstrated in a study in which rats were given the antimicrobial drug ciprofloxacin (Cipro). In those rats that also received dandelion, levels of the drug were rapidly and significantly lowered by 73% compared with the rats that did not receive the dandelion.[12] Several studies have shown that the aqueous extract of *T. officinale* root has protective action against alcohol or chemically induced toxicity in the liver by elevating antioxidative potentials and decreasing lipid peroxidation.[13,14] In carbon tetrachloride–induced liver fibrosis, *Taraxacum* extract also inactivated the hepatic stellate cells responsible for fibrosis and enhanced hepatic regenerative capabilities.[15,16]

Diuretic and Weight Loss Effects

The leaves of dandelion have confirmed diuretic activity. In one study in mice, dandelion exerted a diuretic effect comparable with that of furosemide (Lasix).[17] Because dandelion replaces potassium lost through diuresis, it does not have the potential side effects of furosemide, such as hepatic coma and circulatory collapse. The dose given was 8 mL/kg body weight of the aqueous fluid extract of the leaves. This dose produced a 30% loss of body weight in mice and rats in a 30-day period. Much of the weight loss was attributed to the significant diuretic effect.

Anticancer and Immune-Enhancing Effects

Various dandelion extracts and components have demonstrated antitumor properties in animal experiments.[18–20] Evidence also indicates that dandelion enhances cell-mediated, humoral, and nonspecific immunity; induces the secretion of tumor necrosis factor-alpha; and influences the production of nitric oxide.[21,22] In one study, three aqueous extracts were prepared from the mature leaves, flowers, and roots and tested for their effects on tumor progression–related processes such as proliferation and invasion.[23] Dandelion leaf extract decreased the growth of breast cancer cells, whereas the aqueous extracts of dandelion flower and root had no effect on such growth. Dandelion root extract was found to block the invasion of breast cancer cells, and the leaf extract blocked the invasion of prostate cancer cells.

CLINICAL APPLICATIONS

Dandelion's specific action is on the liver, but as an alterative or tonic, it benefits the body as a whole. It is often used as follows:

- As a diuretic
- As a laxative
- As a cholagogue
- As a general stimulant for the urinary system
- As a choleretic
- As a depurative (purifier)
- As a hypoglycemic agent
- As an antitumor agent

Two human studies have demonstrated the liver-healing properties of dandelion. In a 1938 study in Italy, 12 patients with severe liver imbalances—many exhibiting classic symptoms such as loss of appetite, low energy, and jaundice—were treated with dandelion extract (one 5-mL injection daily for 20 days).[1] Of the 12 patients, 11 showed a considerable drop in blood cholesterol. In the other study, dandelion extract was shown to successfully treat hepatitis, swelling of the liver, jaundice, and dyspepsia with deficient bile secretion.[9]

In a pilot study to assess its diuretic activity in humans, dandelion leaf extract at a dosage of 8 mL three times a day produced a significant increase in the frequency of urination in the 5-hour period after the first dose, a significant increase in the excretion ratio in the 5-hour period after the second dose, and no effect after the third dose.[24] These results indicate that dandelion produces an acute diuretic effect.

DOSAGE

As a general tonic and mild liver remedy, dandelion root can be used at the following dosages three times per day:

- Dried root: 2 to 8 g by infusion or decoction
- Fluid extract (1:1): 4 to 8 mL (1–2 tsp)
- Tincture: Alcohol-based tinctures of dandelion are not recommended because of the extremely high dosage required.
- Juice of fresh root: 4 to 8 mL (1–2 tsp)
- Powdered solid extract (4:1): 250 to 500 mg

Preparations of the leaves can be used as a mild diuretic and weight-loss agent at the following dosages three times a day:

- Dried leaf: 4 to 10 g by infusion
- Fluid extract (1:1): 4 to 8 mL (1–2 tsp)

TOXICITY

No toxic or adverse effects have been reported for either the external or internal use of dandelion. It is considered safe to use even in large amounts, with virtually no documented side effects.[1] There has been one case report of an atopic individual experiencing an anaphylactic reaction after the oral ingestion of an herbal combination containing *T. officinale*. In this case the herbal compound was found to have trace amounts of pollen from *T. officinale* and several other medicinal plants, which resulted in this systemic reaction.[25] This single case report indicates a possible need for caution in individuals with pollen allergy to other plants of the *Compositae* family.

The carcinogenicity of *T. officinale* has been investigated in an animal model. No carcinogenic activity was observed after 120 days of administration.[26]

DRUG INTERACTIONS

In an animal study, administration of an extract of the whole dandelion plant concomitantly with ciprofloxacin decreased absorption of the drug.[27] The investigators found that this effect was due to the high mineral content of the dandelion herb. Until further information is available, ciprofloxacin should not be taken within 2 hours of any dandelion supplement, including tea.

In general, it is often recommended that botanical medicines having a diuretic effect be avoided by anyone taking diuretic medications because the former may enhance the effect of the latter and lead to possible cardiovascular side effects.

Dandelion ingestion may increase the hypoglycemic effect of insulin. A single case report exists describing a 58-year-old-woman with type 2 diabetes mellitus on insulin therapy presenting to an emergency department with a hypoglycemic episode after 2 weeks of daily ingestion of fresh dandelion leaves in salad.[28]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Schütz K, Carle R, Schieber A. *Taraxacum:* a review on its phytochemical and pharmacological profile. *J Ethnopharmacol*. 2006;107(3):313–323.
2. Martinez M, Poirrier P, Chamy R, et al. *Taraxacum officinale* and related species-An ethnopharmacological review and its potential as a commercial medicinal plant. *J Ethnopharmacol*. 2015;169:244–262.
3. Broda B, Andrzejewska E. Choline content in some medicinal plants. *Farm Pol*. 1966;22:181–184.
4. Devys M. Triterpene alcohols from dandelion (*T. dens-leonis*) pollen. *CR Acad Sci Ser D*. 1969;269:798–801.
5. Williams CA, Goldstone F, Greenham J. Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of *Taraxacum officinale*. *Phytochemistry*. 1996;42:121–127.
6. Ahmad VU, Yasmeen S, Ali Z, et al. Taraxacin, a new guaianolide from *Taraxacum wallichii*. *J Nat Prod*. 2000;63:1010–1011.
7. Trojanová V, Rada L, Kokoška, et al. The bifidogenic effect of *Taraxacum officinale root*. *Fitoterapia*. 2004;75:760–763.
8. Wirngo FE, Lambert MN, Jeppesen PB. The physiological effects of dandelion (*Taraxacum Officinale*) in type 2 diabetes. *Rev Diabet Stud*. 2016 Summe r-Fall;13(2–3):113–131.
9. Yamashita K, Kawai K, Itakura M. Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr Res*. 1984;4:961–966.
10. Susnik F. Present state of knowledge of the medicinal plant *Taraxacum officinale* Weber. *Med Razgl*. 1982;21:323–328.
11. Bohm K. Choleretic action of various plant drugs. *Arzneimittelforschung*. 1959;9:376–378.
12. Cho SY, Park JY, Park EM, et al. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. *Clin Chim Acta*. 2002;317:109–117.
13. You Y, Yoo S, Yoon HG, et al. In vitro and in vivo hepatoprotective effects of the aqueous extract from *Taraxacum officinale* (dandelion) root against alcohol-induced oxidative stress. *Food Chem Toxicol*. 2010;48(6):1632–1637.
14. Choi UK, Lee OH, Yim JH, et al. Hypolipidemic and antioxidant effects of dandelion (*Taraxacum officinale*) root and leaf on cholesterol-fed rabbits. *Int J Mol Sci*. 2010;11(1):67–78.
15. Domitrović R, Jakovac H, Romić Z, et al. Antifibrotic activity of *Taraxacum officinale* root in carbon tetrachloride-induced liver damage in mice. *J Ethnopharmacol*. 2010;130(3):569–577.
16. Mahesh A, Jeyachandran R, Cindrella L, et al. Hepatocurative potential of sesquiterpene lactones of *Taraxacum officinale* on carbon tetrachloride induced liver toxicity in mice. *Acta Biol Hung*. 2010;61(2):175–190.
17. Racz-Kotilla E, Racz G, Solomon A. The action of *Taraxacum officinale* extracts on the body weight and diuresis of laboratory animals. *Planta Med*. 1974;26:212–217.
18. Sieyaku KK. Taraxacum extracts as antitumour agents. *Chem Abstr*. 1981;94:374.
19. Takasaki M, Konoshima T, Tokuda H, et al. Anti-carcinogenic activity of *Taraxacum* plant. II. *Biol Pharm Bull*. 1999;22:606–610.
20. Takasaki M, Konoshima T, Tokuda H, et al. Anti-carcinogenic activity of *Taraxacum* plant. I. . *Biol Pharm Bull*. 1999;22:602–605.
21. Kim HM, Shin HY, Lim KH, et al. *Taraxacum officinale* inhibits tumor necrosis factor-alpha production from rat astrocytes. *Immunopharmacol Immunotoxicol*. 2000;22:519–530.
22. Kim HM, Lee EH, Shin TY, et al. *Taraxacum officinale* restores inhibition of nitric oxide production by cadmium in mouse peritoneal macrophages. *Immunopharmacol Immunotoxicol*. 1998;20:283–297.
23. Sigstedt SC, Hooten CJ, Callewaert MC, et al. Evaluation of aqueous extracts of *Taraxacum officinale* on growth and invasion of breast and prostate cancer cells. *Int J Oncol*. 2008;32(5):1085–1090.
24. Clare BA, Conroy RS, Spelman K. The diuretic effect in human subjects of an extract of *Taraxacum officinale* folium over a single day. *J Altern Complement Med*. 2009;15(8):929–934.
25. Lovell CR, Rowan M. Dandelion dermatitis. *Contact Dermatitis*. 1991;25:185–188.
26. Hirono I, Mori H, Kato K, et al. Safety examination of some edible plants, Part 2. *J Environ Pathol Toxicol*. 1978;1:71–74.
27. Zhu M, Wong PY, Li RC. Effects of *Taraxacum mongolicum* on the bioavailability and disposition of ciprofloxacin in rats. *J Pharm Sci*. 1999;88:632–634.
28. Goksu E, Eken C, Karadeniz O, et al. First report of hypoglycemia secondary to dandelion (*Taraxacum officinale*) ingestion. *Am J Emerg Med*. 2010;28(1):111.e1–111.e2.

Taxus brevifolia (Pacific Yew)

Eric L. Yarnell, ND, RH(AHG)

OUTLINE

Taxus brevifolia (family: *Taxaceae*)
Common names: Pacific yew

GENERAL DESCRIPTION

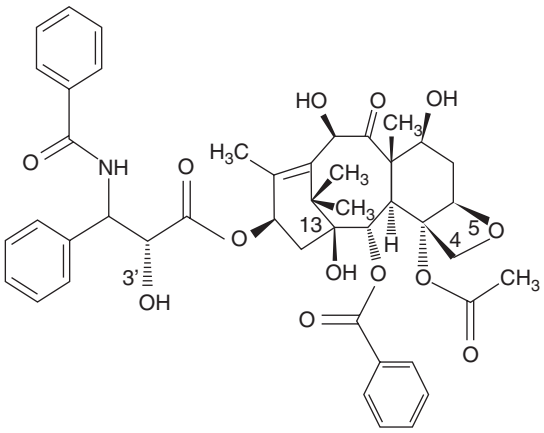
The Pacific yew is now well known as the original source of the chemotherapy agent paclitaxel. Because of the low yield, cost of extraction, and environmental concerns, alternative sources were developed. Nonetheless, the success of paclitaxel provides strong support that mining nature for new medicines is an extremely viable approach to drug development.

Yews are evergreen trees or shrubs that were difficult to classify taxonomically because they look like conifers, but because of the absence of cones and resin ducts, they were placed in a separate order (Fig. 118.1). Although the yew appears to be tenacious, it is slow to reproduce. The species is dioecious, with female trees producing relatively scarce fruits. Seedlings are rare. Most often, new yew trees come from offshoots of a "mother tree," which is why they are usually found in clusters. They grow best on deep, moist, rich rocky or gravelly soils. The largest known living Pacific yew is in Lewis County, in Washington State, near Mount Rainier. It stands 21.3 m tall, has a girth of 4.5 m, and is estimated to be 1000 years old.

Paclitaxel (Taxol) and complex diterpenoid taxanes are compounds found in *Taxus brevifolia.* Docetaxel (Taxotere) is a semisynthetic agent similar in action to paclitaxel derived from 10-deacetylbaccatin III, a taxane isolated from the needles of *Taxus baccata,* the English yew. Before their formal drug development, these compounds were cited as among the most promising plant compounds tested for anticancer properties in 1990.[1] The Pacific yew was first collected in 1962 by a U.S. Department of Agriculture team in Washington State as part of the large natural products screening program of the U.S. National Cancer Institute. Confirmed activity by a bark extract against the KB cell line in tissue culture was reported in 1964. Isolation studies began in 1965, and by 1971, Wall et al.[2] at the Research Triangle Institute (Durham, NC) had identified paclitaxel as the active constituent.[2] Paclitaxel became big news in 1989 when investigators at the Johns Hopkins Oncology Center in Baltimore reported a 30% response rate in cases of refractory ovarian cancer, a remarkable rate for this type of cancer.[3] The development of docetaxel began in 1981 in France. Taxanes used in conjunction with chemotherapy and irradiation have demonstrated improved results compared with either of these therapies alone and better tolerance of these therapies.[4]

From 1967 to 1993, almost all paclitaxel produced was derived from the bark of the Pacific yew, the harvesting of which kills the tree. Currently, all paclitaxel production uses plant cell fermentation technology. This starts from a specific *Taxus* cell line propagated in an aqueous medium in large fermentation tanks. Paclitaxel is then extracted directly, purified by chromatography, and isolated by crystallization.

Fig. 118.1 Taxus brevifolia. (From <https://www.istockphoto.com/photo/yew-tree-branches-with-red-berries-gm488999880-74478915>. Accessed October 2, 2018.)



Empirical formula: $C_{47}H_{51}NO_{14}$
Molecular weight: 853.9 g/mol
Average mass: 853.9 Da
Systemic name: (2α, 5β, 7β, 10β, 13α)-4,10-Diacetoxy-13-{[(2R, 3S)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate

Fig. 118.2 Structure of paclitaxel. (From ResearchGate. Paclitaxel and its evolving role in the management of ovarian cancer. <https://www.researchgate.net/Chemical-structure-of-paclitaxel-Paclitaxel-consist-of-taxane-ring-with-a-four-membered_fig3_279153612>. Accessed October 2, 2018.)

CHEMICAL COMPOSITION

The Pacific yew is poisonous because it contains at least 11 diterpene alkaloids, known collectively as *taxines*. The structure of only two of the alkaloid constituents is known: taxine A, which accounts for 30%, and taxine B, which accounts for 2%. Paclitaxel (Fig. 118.2), like all taxines, is a pseudoalkaloid because its nitrogen is a pseudoalkaloid but not a constituent of taxine because its nitrogen is acylated with benzoic acid and has no basic principle. The concentration of paclitaxel in yew bark is low, only 0.01%. Pacific yew needles contain a similar concentration of paclitaxel.[4a]

HISTORY AND FOLK USE

Historically, the yew has been highly valued for its dense, resilient, decay-resistant, tight-grained wood and its medicinal properties. The Greeks named the yew *toxus* in reference to its use for making a strong bow *(toxon)*, and its poisonous nature *(toxikon)*.[2] It was used as an animal and fish poison by primitive cultures and also for murder and suicide. In the 1st century AD, Claudius suggested its use as an antidote for viper bites. Europeans used it as an abortifacient and to treat heart ailments and hydrophobia.[2] Native Americans used the yew for many ailments including the following:

- Rheumatism
- Lung ailments
- Colds and fever
- Pain and numbness
- Paralysis
- Scurvy
- Stomachache
- Bowel ailments
- Dysmenorrhea
- Gonorrhea

Women ate yew arils to prevent conception. Youths rubbed smooth sticks of yew on their developing bodies to gain its strength. Both the bark and the leaves have been brewed for tea, and powders have been made from the bark alone. The fleshy red aril that surrounds the seed is not poisonous, although the seed itself is.[5]

There is a lot of folklore about the yew's supernatural powers. Because it is a slow-growing, long-lived tree, it was associated with immortality and used in spells to raise the dead.[6] Because it was regarded as among the most potent of trees for protection against evil, it was considered unlucky to cut down or damage a yew tree. Many were planted in churchyards/graveyards and alongside homes for protection. Specimens survive today despite main trunks being hollowed out from decay after hundreds of years of existence.

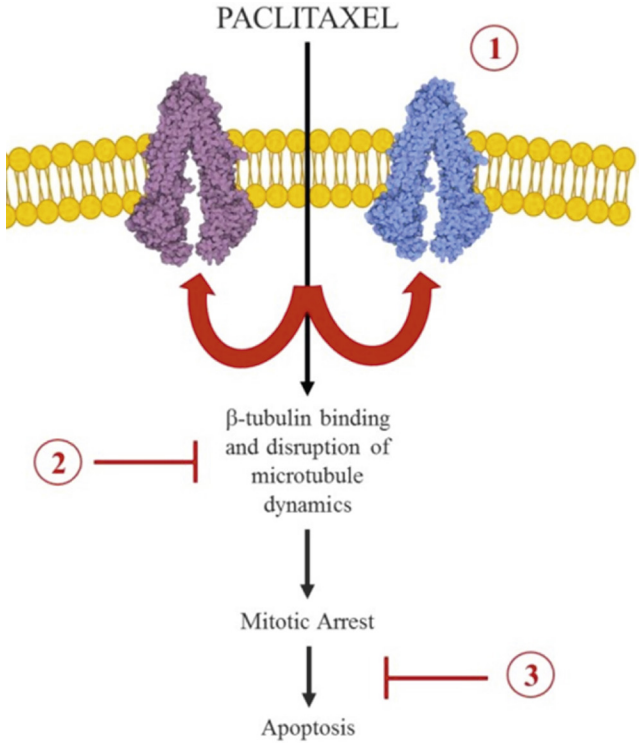


Fig. 118.3 The cellular mechanism of action by which paclitaxel serves as an anticancer drug. (From Barbuti AM, Chen ZS. Paclitaxel through the ages of anticancer therapy: exploring its role in chemoresistance and radiation therapy. *Cancers (Basel)*. 2015;7[4]:2360–2371. PubMed PMID: 26633515. <https://openi.nlm.nih.gov/detailedresult.php?img=PMC4695897_cancers-07-00897-g002&query=paclitaxel&req=4&npos=484>. Accessed October 2, 2018.)

PHARMACOLOGY

Paclitaxel's anticancer action is unique in that it inhibits cell division by promoting the formation of microtubules, the rod-like structures that function as a cell skeleton, making cells more stable and resistant to depolymerization (Fig. 118.3). In contrast, other anticancer phytoagents (e.g., colchicine and vinca alkaloids) induce the polymerization

of microtubules. In addition, under the influence of paclitaxel, the microtubules polymerize independently of the microtubule-organizing center, which is in a perinuclear area, and instead localize predominantly in the cell's periphery.[7] This interferes with the mitotic spindle and selectively blocks cells in the G_2 and M phases of the cell cycle, the most radiosensitive phases. Additionally, paclitaxel induces the formation of abnormal spindle asters that do not require centrioles for enucleation and are reversible after treatment.[8–11] Furthermore, in vivo, paclitaxel has demonstrated an ability to activate the local release of an apoptosis-inducing cytokine.[12] Studies on cancer cell lines induced with *gml,* a novel gene, have demonstrated a marked increase in sensitivity to paclitaxel via apoptosis. An assay for *gml* expression could serve as a clinically useful predictor of chemotherapeutic sensitivity.[13]

CLINICAL APPLICATIONS

Paclitaxel has demonstrated a broad spectrum of antitumor activity. Phase I trials, begun in 1983, demonstrated paclitaxel's antineoplastic activity against several tumor types, such as the following[14–21]:

- Melanoma
- Adenocarcinoma of unknown origin
- Refractory ovarian carcinoma
- Small-cell and non–small-cell lung carcinoma
- Gastric, colon, prostate, breast, and head and neck carcinomas
- Lymphoblastic and myeloblastic leukemias

In early studies, impressive results were seen using paclitaxel in combination with other antineoplastic agents, such as cisplatin.[22] Having treated patients who were previously resistant to cisplatin with the combination of cisplatin and paclitaxel, the Gynecology Oncology Group reported a 33% response rate.[23] Trials were quickly conducted on a number of solid tumor types, including small-cell and non–small-cell lung, renal, gastric, breast, advanced ovarian, colon, and cervical carcinomas; head and neck cancers; small-cell prostate carcinomas; and low-grade non-Hodgkin lymphoma.[24] Paclitaxel also has activity in other malignancies that are refractory to conventional chemotherapy, including previously treated lymphoma; small-cell lung cancers; and esophageal, gastric, endometrial, bladder, and germ-cell tumors. Paclitaxel is also active against AIDS-associated Kaposi's sarcoma. Although paclitaxel is a well-accepted treatment option for these cancers and others, significant toxicities, such as myelosuppression and peripheral neuropathy, limit the effectiveness of paclitaxel-based treatment regimens.[25,26] These toxicities will be discussed further.

TOXICITY

Human poisoning from the deliberate consumption of yew leaves or seeds is now rare.[27] Published cases involving psychiatric patients and prisoners describe the first symptoms of intoxication as appearing 1 hour after ingestion. The manifestations include mydriasis, nausea, vomiting, abdominal cramping, and arrhythmia. Death occurs from cardiac arrest 3 to 24 hours after ingestion.[28] The lethal dose in humans is approximately four or five handfuls of leaves, corresponding to 150 needles. No specific antidote is known.

Paclitaxel binds 95% to 98% with plasma proteins yet is readily eliminated through hepatic metabolism, biliary excretion, and/or extensive tissue binding. Total urinary excretion has been insignificant, indicating that renal clearance contributes minimally to systemic clearance.[21,29–31] Hepatic metabolism via cytochrome P450 (CYP) is involved for both paclitaxel and docetaxel. However, the former is hydroxylated by CYP2C8, whereas the latter is hydroxylated by CYP3A4.[32]

The role of whole yew preparations in clinical practice today is uncertain. While tincture is being used empirically for cancer patients, there is no clinical research regarding efficacy or safety. Non-paclitaxel taxanes have been shown to reverse resistance to paclitaxel in cancer cells in vitro, giving an experimental basis for the potential importance of whole-plant (at very low doses) compared to using just the isolated constituents.[31a] Non-paclitaxel taxanes enhance the absorption of paclitaxel experimentally, another reason to research whole plant extracts for clinical use.[31b]

Toxicity manifests as follows:

- Bone marrow suppression
- Hypersensitivity
- Cardiovascular abnormalities
- Neurotoxicity
- Arthralgias and myalgias
- Alopecia
- Gastrointestinal upset

Junctional tachycardia via conduction block rather than direct primary toxicity on myocytes has been suggested.[33] Hypersensitivity, skin reactions, and accumulated fluid retention syndrome are minimized with a 3- to 5-day regimen of corticosteroids before paclitaxel infusion.[34]

Patients with leukemia who are treated with high doses of paclitaxel exhibited mucositis, which has also appeared in response to lower, cumulative dosing. An accumulation of epidermal cells with abnormal paclitaxel-induced spindle asters has been evident in ulcerated mucosa, indicating that the cell cycle was arrested in mitosis.[35]

Paclitaxel has been known to inhibit neurite growth and induce prominent morphological effects, such as microtubule bundles in neurons, satellite cells, and Schwann cells in organotypic dorsal root ganglion cultures.[35–41] Clinically, the most common symptoms have been glove-and-stocking paresthesias and perioral numbness. Distal sensory loss to large-fiber (proprioception, vibration) and small-fiber (pinprick, temperature) modalities and loss or decrease of distal deep tendon reflexes have been noted, although motor nerves seem to be spared. In general, the clinical incidence and severity of peripheral neurotoxicity have been dose-related. Patients with a history of substantial alcohol use have appeared to be more predisposed to the development of the neurosensory toxic effects of cisplatin and paclitaxel.[14–17,42–44]

The development of a suitable clinical formulation has been hampered by paclitaxel's poor aqueous solubility. Cremophor is being used as the vehicle for administration and has been implicated in side effects, such as type 1 hypersensitivity reactions.[45]

Neutropenia, the principal dose-limiting toxic effect of paclitaxel, resolves rapidly (in 15–21 days) after treatment is stopped.[42] The major clinical risk factor for neutropenia seems to be the extent of prior myelotoxic chemotherapy and/or irradiation.

Bradyarrhythmias, which have been noted as transient and asymptomatic, have been reported during paclitaxel infusion in at least 29% of patients with ovarian cancer.[42] This development appears to be related more to paclitaxel because other agents formulated with Cremophor have not been associated with similar arrhythmias. Atypical chest pains during paclitaxel infusion have been observed, but they are believed to be a manifestation of a hypersensitivity reaction.[27,43]

Other paclitaxel- or Cremophor-related side effects are sudden and complete alopecia, often occurring in a single day; local venous toxic effects such as erythema, tenderness, and cellulitis in areas of dermal extravasation; and fatigue, headaches, taste perversions, significant elevations in serum triglycerides, and minor rises in hepatic and renal function values.[21]

Medical problems that may affect the use of paclitaxel are listed in Box 118.2. Studies in rats and rabbits have shown that paclitaxel causes miscarriages and fetal deaths. Breastfeeding is contraindicated during paclitaxel therapy. Paclitaxel bound to albumin rather than using a solvent results in fewer and less severe side effects, with much-reduced allergic reactions.[46]

BOX 118.1 Medications That Should Be Avoided With Paclitaxel

- Deferiprone
- Dexrazoxane
- Ganciclovir
- Palifermin
- Primaquine
- Radium Ra 223 dichloride
- Sipuleucel-T
- Thiotepa
- Valganciclovir

BOX 118.2 Medical Problems That May Affect the Use of Paclitaxel

- Chickenpox
- Herpes zoster
- Cardiac arrhythmias
- Infection

DRUG INTERACTIONS

There are conflicting reports regarding drug interactions of *Taxus brevifolia*. Considering paclitaxel is metabolized by CYP450 enzymes, administration with other drugs can alter metabolism depending on the target molecule, schedule, and sequence of administration. The pharmaceutical drug, Taxol, interacts with more than 200 different medications. Because of enhanced toxicity, paclitaxel should be administered 24 hours after other chemotherapeutic agents, such as doxorubicin and epirubicin.[47] Vaccinations that include live viruses are contraindicated because the risk of disseminated infection is increased, resulting from the immunosuppressive effects of paclitaxel. Box 118.1 includes a list of medications that should also be avoided with concurrent use of paclitaxel.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bolsinger C, Jaramillo AE. Taxus brevifolia Nutt. Pacific Yew. In: Burns RM, Honkala BH, eds. *Silvics of forest trees of North America*. Portland: OR: Pacific Northwest Research Station, USDA Forest Service; 1990:17.
2. Hartzell H. *The yew Tree: a Thousand Whispers*. Eugene: OR: Hulogosi; 1991.
3. Rowinsky EK, Donehower RC. Taxol: twenty years later, the story unfolds. *J Natl Cancer Inst*. 1991;83:1778–1781.
4. Cragg GM. Paclitaxel (Taxol): a success story with valuable lessons for natural product drug discovery and development. *Med Res Rev*. 1998;18(5):315–331.

4a. Witherup KM, Look SA, Stasko MW, et al. Taxus spp. needles contain amounts of taxol comparable to the bark of Taxus brevifolia: analysis and isolation. *J Nat Prod*. 1990;53(5):1249–1255.

5. Duke J. *Handbook of Northeastern Indian Medicinal Plants*. Lincoln, MA: Quarterman; 1986.
6. Cunningham S. *Encyclopedia of Magical Herbs*. St. Paul, MN: Llewellyn Publications; 1985.
7. Wehland J, Henkart M, Klausner R, et al. Role of microtubules in the distribution of the Golgi apparatus: effect of Taxol and microinjected anti-alpha-tubulin antibodies. *Proc Natl Acad Sci U S A*. 1983;80:4286–4290.
8. Rowinsky EK, Donehower RC, Jones RJ, et al. Microtubule changes and cytotoxicity in leukemic cell lines created with Taxol. *Cancer Res*. 1988;48:4093–4100.
9. De Brabander M, Geuens G, Nuydens R, et al. Taxol induces the assembly of free microtubules in living cells and blocks the organizing capacity of the centrosome and kinetochores. *Proc Natl Acad Sci U S A*. 1981;78:5608–5612.
10. Brasch RC, Rockoff SD, Kuhn C, et al. Contrast media as histamine liberators. II. Histamine release into venous plasma during intravenous urography in man. *Invest Radiol*. 1970;5:510–513.
11. Shedadi WH. Adverse reactions to intravenous administration of contrast media: a comparative study based on a prospective study. *Am J Roentgenol*. 1975;124:145–151.
12. Lanni JS, Lowe SW, Licitra EJ, et al. p53-independent apoptosis induced by paclitaxel through an indirect mechanism. *Proc Natl Acad Sci U S A*. 1997;94:9679–9683.
13. Kimura Y, Furuhata T, Shiratsuchi T, et al. GML sensitizes cancer cells to Taxol by induction of apoptosis. *Oncogene*. 1997;15:1369–1374.
14. Koeller J, Brown T, Havlin K, et al. A phase I/pharmacokinetic study of Taxol given by prolonged infusion without premedication. *Proc ASCO*. 1989;8:82.
15. Donehower RC, Rowinsky EK, Grochow LB, et al. Phase I trial of Taxol in patients with advanced cancer. *Cancer Treat Rep*. 1987;71:1171–1177.
16. Wiernik PH, Schwartz EL, Strauman JJ, et al. Phase I clinical and pharmacokinetic study of Taxol. *Cancer Res*. 1987;47:2486–2493.
17. Wiernik PH, Schwartz EL, Einzig A, et al. Phase I trial of Taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. *J Clin Oncol*. 1987;5:1232–1239.
18. Legha SS, Tenney DM, Krakoff IR. Phase I study of Taxol using a 5-day intermittent schedule. *J Clin Oncol*. 1986;4:762–766.
19. Grem JL, Tutsch KD, Simon KJ, et al. Phase I study of Taxol administered as a short i.v. infusion daily for 5 days. *Cancer Treat Rep*. 1987;71:1179–1184.
20. Ohnuma T, Zimet AS, Coffey VA, et al. Phase I study of Taxol in a 24-hour infusion schedule. *Proc Am Assoc Cancer Res*. 1985;26:662.
21. Kris MG, O'Connell JP, Gralla RJ, et al. Phase I trial of Taxol given as a 3-hour infusion every 21 days. *Cancer Treat Rep*. 1986;70:605–607.
22. Belani C, TAX 326 Study Group. Phase III randomized trial of docetaxel in combination with cisplatin or carboplatin or vinorelbine plus cisplatin in advanced non–small cell lung cancer: interim analysis. *Semin Oncol*. 2001;28(3 suppl 9):10–14.
23. Thigpen JT, Blessing JA, Ball H, et al. Phase II trial of Taxol as second-line therapy for ovarian carcinoma: a gynecologic oncology group study. *Proc Am Soc Clin Oncol*. 1990;9:604.
24. Rowinsky EK. Paclitaxel pharmacology and other tumor types. *Semin Oncol*. 1997;24(6 suppl 19):S19-1–S19-12.
25. Marupudi NI, Han JE, Li KW, et al. Paclitaxel: a review of adverse toxicities and novel delivery strategies. *Expert Opin Drug Saf*. 2007;6(5):609–621. Review.
26. Mekhail TM, Markman M. Paclitaxel in cancer therapy. *Expert Opin Pharmacother*. 2002;3(6):755–766.
27. Pilija V, Djurendic-Brenesel M, Miletic S. Fatal poisoning by ingestion of *Taxus baccata* leaves. *Forensic Sci Int*. 2018;24. https://doi.org/10.1016/j.forsciint.2018.07.017. Epub ahead of print.
28. Appendino G. Taxol (paclitaxel): historical and ecological aspects. *Fitoterapia*. 1993;64:5–25.
29. Jacrot M, Riondel J, Picot F, et al. Action of Taxol on human tumors transplanted in athymic mice. *C R Seances Acad Sci III*. 1983;297:597–600.
30. Riondel J, Jacrot M, Nissou MF, et al. Antineoplastic activity of two Taxol derivatives on an ovarian tumor xenografted into nude mice. *Anticancer Res*. 1988;8:387–390.
31. Sternberg CN, Sordillo PP, Cheng E, et al. Evaluation of new anticancer agents against human pancreatic carcinomas in nude mice. *Am J Clin Oncol*. 1987;10:219–221.

31a. Ojima I, Bounaud PY, Takeuchi C, et al. New taxanes as highly efficient reversal agents for multidrug resistance in cancer cells. *Bioorg Med Chem Lett*. 1998;8(2):189–194.

31b. Liu ZH, Xiao ZG, Lv JJ, et al. Pharmacokinetic synergy from the taxane extract of Taxus chinensis improves the bioavailability of paclitaxel. *Phytomedicine*. 2015;22:573–578.

32. Dorr RT. Pharmacology of the taxanes. *Pharmacotherapy*. 1997;17:S96–S104.
33. Faivre S, Goldwasser F, Soulie P, et al. Paclitaxel (Taxol)-associated junctional tachycardia. *Anticancer Drugs*. 1997;8:714–716.
34. Von Hoff DD. The taxoids: same roots, different drugs. *Semin Oncol*. 1997;24:S3–S10.
35. Hruban RH, Yardley JH, Donehower RC, et al. Taxol toxicity: epithelial necrosis in the gastrointestinal tract associated with polymerized microtubule accumulation and mitotic arrest. *Cancer*. 1989;63:1944–1950.
36. Masurovsky EB, Peterson ER, Crain SM, et al. Microtubule arrays in Taxol-treated mouse dorsal root ganglion-spinal cord cultures. *Brain Res*. 1981;217:392–398.
37. Masurovsky EB, Peterson ER, Crain SM, et al. Morphological alterations in satellite and schwann after exposure of fetal mouse dorsal root ganglia-spinal cord culture to Taxol. *IRCS Med Sci Libr Compend*. 1981;9:968–969.
38. Masurovsky EB, Peterson ER, Crain SM, et al. Morphological alterations in dorsal root ganglion neurons and supporting cells of organotypic mouse spinal cord-ganglion cultures exposed to Taxol. *Neuroscience*. 1983;10:491–509.
39. Letourneau PC, Ressler AH. Inhibition of neurite initiation and growth by Taxol. *J Cell Biol*. 1984;98:1355–1362.
40. Letourneau PC, Shattuck TA, Ressler AH. Branching of sensory and sympathetic neuritis in vitro is inhibited by treatment with Taxol. *J Neurosci*. 1986;6:1912–1917.
41. Roytta M, Horwitz SB, Raine CS. Taxol-induced neuropathy: short-term effects of local injection. *J Neurocytol*. 1984;13:685–701.
42. McGuire WP, Rowinsky EK, Rosenshein NB, et al. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Ann Intern Med*. 1989;111:273–279.
43. Rowinsky EK, Burke PJ, Karp JE, et al. Phase I and pharmacodynamic study of Taxol in refractory acute leukemias. *Cancer Res*. 1989;49:4640–4647.
44. Burgoyne RD, Cumming R. Taxol stabilizes synaptosomal microtubules without inhibiting acetylcholine release. *Brain Res*. 1983;280:190–193.
45. Laussus M, Scott D, Leyland-Jones B. Allergic reactions (ar) associated cremophor (c) containing antineoplastic (anp). *Proc Am Soc Clin Oncol*. 1985;4:1042.
46. Montana M, Ducros C, Verhaeghe P, et al. Albumin-bound paclitaxel: the benefit of this new formulation in the treatment of various cancers. *J Chemother*. 2011;23(2):59–66.
47. Jauhari S, Singh S, Dash AK. Chapter 7 Paclitaxel. *Profiles of Drug Substances, Excipients, and Related Methodology*. 2009;34:299–344. PubMed PMID: 22469177.

Urtica dioica (Stinging Nettle)

Eric L. Yarnell, ND, RH(AHG) and *Kathy Abascal, BS, JD, RH(AHG)*

OUTLINE

Urtica dioica ssp. *dioica* (family: *Urticaceae*)
Synonym: *Urtica dioica*
Common names: stinging nettle, nettle, great nettle

GENERAL DESCRIPTION

Urtica dioica is a prolific Eurasian perennial that has become naturalized around the globe. Two major subspecies are recognized as important in medicine in the West: *dioica,* the standard stinging nettle; and *gracilis,* the California nettle. *Urtica urens,* the dwarf or dog nettle, is also used interchangeably. The leaves, fruit (often inappropriately called seeds), and root are all used, each somewhat differently (Fig. 119.1).

CHEMICAL COMPOSITION

Nettle leaves are known to contain flavonoid glycosides (including quercetin and kaempferol), phenylpropanoids (including caffeic malic acid), sterols, carotenoids, vitamins, and minerals.[1] The stinging hairs contain formic acid, histamine, serotonin, and choline, among other irritants. Very little is known about the chemistry of the fruits except that they contain protein, carbohydrate, and 30% fatty acids.[1]

The chemistry of the roots of stinging nettle has been the most thoroughly examined.[2] Acidic polysaccharides, lectins, sterols (including β-sitosterol), triterpenoids, the coumarin-like compound scopoletin, phenylpropanoids, ceramides, and lignans are all potentially related to the physiological effects of the roots (Figs. 119.2 and 119.3).[3] Lignans of potential importance include neoolivil and secoisolariciresinol, which are metabolized by human gut flora to enterodiol and enterolactone, well-characterized phytoestrogenic compounds.[4]

HISTORY AND FOLK USE

Urtica species have long been employed as medicine in Europe and Asia. The steamed or dried leaves were eaten as a vegetable or seasoning and often still are.[4] Nettle fiber was formerly processed into thread used to make clothing, rope, and sailcloth, although it was ultimately replaced by flax. The source of the fiber known as "ramie" comes from the plant *Boehmeria nivea,* which is also in the *Urticaceae* family. The word *nettle* appears to be derived from the old Anglo-Saxon *noedl,* "needle," and even before that to the common Indo-European verb *ne,* "to spin/sew." There is little record of the use of the roots in traditional cultures except as a source of yellow dye.[4]

Medicinally, stinging nettle leaves were once considered a spring tonic, taken each year in particular by patients with chronic skin or rheumatic conditions.[5] The leaves' diuretic (also ascribed to the fruits), tonifying, lactagogue, antiasthmatic, antihemorrhagic, antidiabetic, and "blood-building" properties are consistently referenced by European herbalists.[6,7] Up to modern times, there are descriptions of applications of fresh leaves to aching joints, intentionally causing stings to relieve symptoms. Stinging nettle leaves are also frequently said to promote hair growth.

PHARMACOLOGY

The two principal effects of stinging nettle leaves are inhibition of inflammation and promotion of diuresis. Most research has involved a proprietary 50% ethanol 10:1 extract of the leaves known as IDS 23 (Strathmann AG & Co, Hamburg, Germany). In vitro, IDS 23 extract and caffeic malic acid inhibit 5-lipoxygenase and cyclooxygenase (isotype not determined); the whole extracts are more potent than caffeic malic acid alone.[8] IDS 23 also inhibits the secretion of tumor necrosis factor-α and interleukin-1-β induced by lipopolysaccharide in human blood; phenylpropanoids and flavonoids isolated from the leaves were not effective in this system.[9] Perhaps even more important, IDS 23 has been shown to inhibit activation of nuclear factor kappa B (NFκB) by preventing degradation of its inhibitory subunit, IκB, in vitro.[10]

In vitro, IDS 23 stimulated the production of interleukin (IL)-4 by monocytes and inhibited the production of IL-2 and interferon-γ (IFN-γ).[11] IL-4 production promotes the formation of type 2 helper T cells, which are associated with much less inflammation than type 1 helper T cells. Preliminary in vitro research also shows that nettle leaves are strong antioxidants.[12] The multiple inflammation-modulating,

Fig. 119.1 *Urtica dioica*. (From JanHerodes/iStock.com)

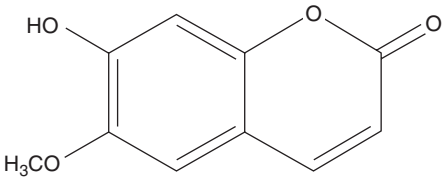


Fig. 119.2 Structure of Scopoletin. (From Scopoletin inhibits rat aldose reductase activity and cataractogenesis in galactose-fed rats. ResearchGate. https://www.researchgate.net/Chemical-structure-of-scopoletin_fig1_257531542. Accessed October 10, 2018.)

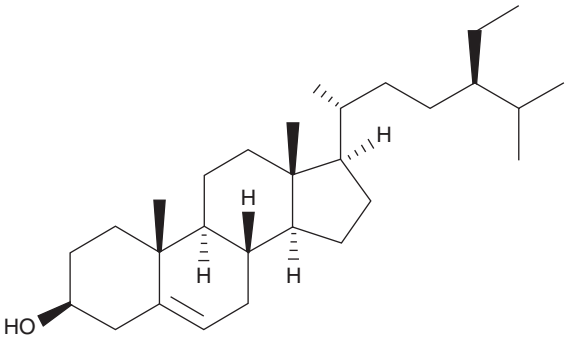


Fig. 119.3 Structure of beta-sitosterol.

immunomodulating, and antioxidant effects of stinging nettle leaves help explain how they could be helpful for so many inflammatory diseases.

A continuous intravenous infusion of an aqueous stinging nettle leaf extract increased natriuresis and urine volume in rats, leading to a reduction in blood pressure.[13] In a small uncontrolled trial of patients with congestive heart failure, stinging nettle leaf juice showed a diuretic effect.[14] Although much more work is needed, existing data support the traditional belief that stinging nettle leaves are a mild diuretic. An extract combining stinging nettle root and *Prunus africanum* (pygeum) bark weakly inhibited 5-α-reductase and moderately inhibited aromatase in prostate tissue.[15] A separate study found that stinging nettle root extract inhibited aromatase in vitro, although much less potently than *Serenoa repens* (saw palmetto) extract.[16] The two combined showed some additive effects. No effect was noted for another stinging nettle root extract on prostatic α_1-adrenergic receptors.[17]

A double-blind placebo-controlled trial confirms that stinging nettle root extracts inhibit or reduce sex hormone–binding globulin (SHBG) levels in men with benign prostatic hyperplasia (BPH).[18] In vitro, ethanolic extracts were shown to decrease the binding of dihydrotestosterone by SHBG.[19,20] Lignans and trihydroxyoctadecenoic acids but not triterpenoids were active in this study. Stinging nettle root extracts have also been repeatedly shown to interfere with the binding of epidermal growth factor, a major stimulus of BPH, to its receptor on prostate cells.[4,21] Lectins appeared to be the most active compounds isolated in these studies.

CLINICAL APPLICATIONS

The major uses of stinging nettle, organized according to the part being used and the route of administration, are shown in Table 119.1.[22]

Benign Prostatic Hyperplasia

Extracts of stinging nettle root have been the subject of several clinical trials showing its benefit for the symptoms of BPH (Table 119.2). Many of these are small, older, short trials, although longer, modern trials have also been conducted, and all show at least some benefit over placebo. The three most rigorous trials are reviewed in more depth here.

In one double-blind trial, 50 men with stage I or II (mild to moderate) BPH symptoms were randomly assigned to receive either placebo or 300 mg of an extract of stinging nettle root standardized to an unspecified amount of an unspecified steroid glycoside twice a day.[23] SHBG levels were significantly lower and urine volume and maximum flow (but not average flow) significantly higher in the stinging nettle group than in the placebo group. Residual urine volume rose slightly and nonsignificantly in both groups. No adverse effects were reported.

The most rigorous trial to date was a 1-year trial involving 246 men in which 459 mg of a stinging nettle root extract was significantly more effective than placebo at improving BPH symptoms.[24] Urine flow and residual volume were minimally affected, with no statistical difference between the nettle extract and placebo. Adverse effect rates did not differ between the treatment groups.

In a large double-blind trial, 558 men with BPH were randomized to take either 120 mg of a 20:1 diethyl ether extract in 18 drops of liquid three times a day or placebo for 1 year.[25] Symptoms, maximum flow rate, and postvoid residual urine amounts improved significantly in the nettle group compared with placebo. There was also a small decrease in prostate volume (approximately 3 mL) versus none in the placebo group, a significant difference. There were no adverse effects in either group.

No clinical trial has compared nettle root alone with available drugs for the treatment of BPH, although combination studies have done so. Stinging nettle combined with saw palmetto was as effective as finasteride (Proscar) and had significantly fewer adverse effects in a double-blind trial.[26] This trial ran for 1 year and involved 543 men with symptomatic BPH. An uncontrolled postmarketing study that monitored 2080 patients with mildly symptomatic BPH who took the same saw palmetto and stinging nettle extract documented only 15 mild adverse events.[27] The extract combination was very helpful in relieving symptoms. Other double-blind trials confirm that a combination of saw palmetto and nettle root extracts are superior to placebo at relieving the symptoms of BPH, with minimal adverse effects.[28,29]

In summary, these trials support the notion that stinging nettle root has some benefit in men with symptomatic BPH, although higher-quality clinical trials are needed to confirm this benefit.

It should also be noted that a combination of nettle root and saw palmetto extracts, quercetin, and curcumin along with the

TABLE 119.1 Major Uses of *Urtica dioica* by Plant Part

Plant Part	Route of Administration	Major Action	Main Indications	Level of Clinical Support[a]	Approved by Commission E[b]
Leaf	Internal	Anti-inflammatory, diuretic	Urinary tract Infection	Absent	Yes
			Allergic rhinitis	Poor	No
			Arthritis	Poor	Yes
			Kidney stone prevention	Absent	Yes
Leaf	Topical	Anti-inflammatory, counterirritant	Arthritis	Poor	No
Seed	Internal	Nephroprotective	Chronic renal failure	Absent	No
Root	Internal	Interference with sex hormone–binding globulin and growth factors, aromatase inhibition	Benign prostatic hyperplasia	Good	Yes

[a]Level of clinical support refers to the number of published clinical trials with positive results. Poor = one or two trials and mostly of low quality; good = more than two trials of good or high quality.
[b]Data from Blumenthal M, Busse WR, Goldberg A, et al., eds. *The complete German Commission E monographs: therapeutic guide to herbal medicines*. Boston: Integrative Medicine Communications; 1998: 216–217.

TABLE 119.2 Randomized Controlled Trials of Nettle Root for Benign Prostatic Hyperplasia

Trial	Subjects (*n*, on nettle; *p*, on placebo)	Extract and Dose	Duration	Outcome
Vontobel et al.[23]	*n* = 25, *p* = 25	300 mg bid (5:1 methanol extract)	9 wk	Nettle improves maximum flow better than placebo
Dathe and Schmid[47]	*n* = 35, *p* = 37	300 mg bid (5:1 methanol extract)	6–8 wk	Nettle not superior to placebo
Fischer and Wilbert[18]	*n* = 20, *p* = 20	300 mg bid (5:1 methanol extract)	7 mo	Nettle superior to placebo for symptoms, mean flow improvement
Engelmann[48]	n = 47, p = 47	459 mg qd of a 7–14:1 methanol extract	6 mo	Nettle superior to placebo for IPSS[a] > and maximum flow
Schneider and Rübben[24]	*n* = 124, *p* = 122	459 mg qd of a 7–14:1 methanol extract	12 mo	Nettle superior to placebo for IPSS reduction
Safarinejad[25]	*n* = 287, *p* = 271	120 mg tid of 20:1 diethyl ether extract	6 mo	Nettle superior to placebo for IPSS and maximum flow

[a]*IPSS,* International Prostate Symptom Score, a standardized seven-item questionnaire used to monitor symptoms of BPH.

fluoroquinolone antibiotic prulifloxacin was significantly more likely to result in resolution of chronic prostatitis symptoms than prulifloxacin alone in one randomized trial.[30] The combination group was also significantly less likely to have recurrent symptoms 6 months later. More research is needed to determine to what extent nettle root has a beneficial role in chronic prostatitis.

Arthritis

Stinging nettle leaves have been used internally and topically to treat arthritis. An initial uncontrolled clinical trial found that 1340 mg a day of a nettle leaf extract (6.4:1–8.1:1) reduced the need for nonsteroidal anti-inflammatory drugs (NSAIDs) by 50% in patients with osteoarthritis of the knee.[31]

A subsequent open trial involving patients with acute exacerbations of various forms of arthritis compared the use of a standard 200-mg dose of the NSAID diclofenac (Cambia, Cataflam, Voltaren, Voltarol, Zipsor) with a subtherapeutic 50-mg dose of diclofenac combined with 50-g of freeze-dried nettle once a day for 2 weeks.[32] The two groups had similar levels of improvement (no significant differences between the groups) compared with baseline on various pain rating scales. There were no serious adverse events. Double-blind trials to confirm these results are warranted.

Two case studies published in 1994 heralded the first mention in the modern medical literature of topical stinging nettles as potentially effective pain relievers for patients with arthritis.[33] The practitioner who noted these cases went on to publish a case series, again showing that many patients treating themselves with topical nettle stings were experiencing noticeable pain relief, allowing for reductions in NSAID dosage.[34]

A double-blind trial has been conducted evaluating the effects of nettles in patients with osteoarthritis of the thumb or index finger.[35] The placebo chosen was *Lamium album* (white deadnettle) leaf, a stingless mimic of *U. dioica.* Each patient used the leaf of the plant he or she was being treated with once a day, applying it to three different spots for 30 seconds each. After 1 week of treatment, there was a 5-week washout period, followed by a 1-week crossover treatment with the other plant. When patients were treated with stinging nettle, their pain and disability scores were significantly better than during deadnettle treatment. Although the use of a nonstinging placebo may have compromised this trial, it does provide support for the folk use of topical application of nettles to reduce arthritis symptoms.

A similar single-blind, randomized trial using nonstinging *Urtica galeopsifolia* as a control did not find that stinging nettle leaves were more effective for patients with knee osteoarthritis, although treatment was considered tolerable.[36] A formula combining nettle leaf extract, rose hips concentrate, and *Harpagophytum procumbens/H. zeyheri* (devil's claw) was tested against placebo in a double-blind, randomized trial in patients with knee osteoarthritis.[37] After 3 months, the herbal combination significantly reduced symptoms compared with the placebo with minimal adverse effects.

Diabetes Mellitus

Numerous studies have investigated the traditional use of nettle leaf for people with diabetes. A double-blind, randomized trial of 500 mg of nettle leaf extract three times a day for 3 months found that it significantly improved glycemic control in patients with type 2 diabetes compared with placebo.[38] It was safely combined with a range of oral hypoglycemic medications in this trial. In another double-blind, randomized trial, a formula combining nettle leaf, *Silybum marianum* (milk thistle), and *Boswellia serrata* (frankincense) was similarly found to improve glycemic control, while also lowering triglyceride levels, compared with placebo.[39] Other double-blind trials in patients with type 2 diabetes have found that nettle leaf extracts can reduce inflammatory markers, reduce excessive oxidation, improve blood lipid parameters, lower serum transaminases, and lower blood glucose levels significantly compared with placebo.[40–42] Hypoglycemia did not occur with nettle leaf.

Allergic Rhinitis

One double-blind trial reported on the efficacy of stinging nettle leaves for allergic rhinitis.[43] A total of 69 patients with acute onset of allergic rhinitis symptoms took 600 mg of freeze-dried stinging nettle or placebo at the onset of symptoms, then recorded their responses 1 hour later. The total number of doses and general responses were recorded over the remaining week. Overall assessment of the efficacy of stinging nettle was better than that of a placebo, although differences in daily symptom logs did not show a strong difference. No statistical analysis was provided. Two patients in the nettle group dropped out owing to an intensification of symptoms, but no other significant adverse effects were noted. A second double-blind, randomized trial of 40 patients found no difference between a nettle root extract and placebo for allergic rhinitis symptoms after 1 month.[44] There was a significant reduction in interferon-gamma levels with nettle root over placebo, suggesting a longer or larger trial (or using a different dose or extract) might ultimately show benefit.

Chronic Renal Failure

The seed of nettle may have the ability to nonspecifically protect nephrons from multiple insults and thus delay the progression of chronic renal failure. Two case studies have been published, one patient having lupus nephritis and a kidney transplant and another who underwent nephrectomy for cancer and whose serum creatinine levels declined during treatment with a nettle seed tincture.[45] The dosage used was 5 mL three times a day.

Galactagogue

An herbal tea containing nettle leaf, *Melissa officinalis* (lemon balm), *Carum carvi* (caraway), *Pimpinella anisum* (anise), *Foeniculum vulgare* (fennel), *Galega officinalis* (goat's rue), and *Cymbopogon citratus* (lemongrass) was compared with a tea containing hibiscus and rose hips or supportive measures only in 95 women with preterm infants in a randomized open trial.[46] Breast milk production increased by 80% with the herbal tea, compared with 30% to 34% in the control groups. Infant weight gain did not vary between groups because mothers who were not able to produce enough milk supplemented with formula.

TABLE 119.3 Dosage Forms of Stinging Nettle

Dosage Form	Leaf	Root
Tea	Infuse 4 g in 150 mL water three times a day[49]	Decoct 4–6 g in 150 mL water once a day[49]
Freeze-dried capsules	300 mg twice a day, up to 50 g once a day[32,43]	Not available
Concentrated extracts	Not available	5:1–10:1 extract 500 mg three times a day[31]
Tincture	30% ethanol, 1:2–1:5 weight to volume, 3–14 mL three times a day[50]	50% ethanol, 1:2–1:5, 4–9 mL three times a day[50]

DOSAGE

Stinging nettle leaf and root are available in several dosage forms, as summarized in Table 119.3. Fresh leaves are applied one to two times a day to every few days depending on symptoms, so treatment obviously requires ongoing access to growing plants.

TOXICITY

Oral use of stinging nettle leaf, fruit, or root is not associated with any significant adverse effects. Topical application of fresh leaf raises mildly painful, inflamed wheals because of the stinging hairs. As previously noted, this effect is sometimes exploited therapeutically. The welts usually clear completely within 2 hours, with lessening of symptoms after 30 minutes. As with all herbal medicines, it is possible for patients to be allergic to stinging nettle, although this reaction is exceedingly rare.

DRUG INTERACTIONS

There are no documented negative interactions between stinging nettle and any other medication. Some open trials (described previously) suggest that stinging nettle leaves potentiate NSAIDs, allowing for dose reductions of these agents.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bisset NG, Wichtl M, eds. *Herbal Drugs and Phytopharmaceuticals*. Stuttgart: Medpharm Scientific Publishers; 1994.
2. Upton R, Axentiev P, Soria T, eds. *Stinging Nettle Root, Urtica Dioica L. Standards of Analysis, Quality Control, and Therapeutics*. Scotts Valley, CA: American Herbal Pharmacopoeia and Therapeutic Compendium; 2010.
3. Wagner H, Willer F, Samtleben R, et al. Search for the antiprostatic principle of stinging nettle (Urtica dioica) roots. *Phytomedicine*. 1994;1:213–224.
4. Schöttner M, Gansser D, Spiteller G. Lignans from the roots of Urtica dioica and their metabolites bind to human sex hormone–binding globulin (SHBG). *Planta Med*. 1997;63:529–532.
5. Foster S. *Herbal Renaissance: Growing, Using & Understanding Herbs in the Modern World*. Salt Lake City, UT: Gibbs-Smith; 1993.
6. Grieve M. *A Modern Herbal*. Vol. 2. New York: Dover Publications; 1971.
7. Asadi-Samani M, Moradi MT, Mahmoodnia L, et al. Traditional uses of medicinal plants to prevent and treat diabetes. an updated review of ethnobotanical studies in Iran. *J Nephropathol*. 2017;6(3):118–125.
8. Obertreis B, Giller K, Teucher T, et al. Anti-inflammatory effects of *Urtica dioica* folia extract in comparison to caffeic malic acid. *Arzneimittelforschung*. 1996;46:52–56.
9. Obertreis B, Ruttkowski T, Teucher T, et al. Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum *Urticae dioicae* foliorum. *Arzneimittelforschung*. 1996;46:389–394.
10. Riehemann K, Behnke B, Schulze-Osthoff K. Plant extracts from stinging nettle *(Urtica dioica)*, an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. *FEBS Lett*. 1999;442:89–94.
11. Klingelhoefer S, Obertreis B, Quast S, et al. Antirheumatic effect of IDS 23, a stinging nettle leaf extract, on in vitro expression of T helper cytokines. *J Rheumatol*. 1999;26:2517–2522.
12. Pieroni A, Janiak V, Durr CM, et al. In vitro antioxidant activity of non-cultivated vegetables of ethnic Albanians in southern Italy. *Phytother Res*. 2002;16:467–473.
13. Tahri A, Yamani S, Legssyer A, et al. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of *Urtica dioica* in the rat. *J Ethnopharmacol*. 2000;73:95–100.
14. Kirchhoff HW. Nettle juice as a diuretic. *Z Phytother*. 1983;4:621–626.
15. Hartmann RW, Mark M, Soldati F. Inhibition of 5 alpha-reductase and aromatase by PHL-00801 (Prostatonin), a combination of PY 102 *(Pygeum africanum)* and UR 102 *(Urtica dioica)* extracts. *Phytomedicine*. 1996;3:121–128.
16. Koch E, Biber A. Pharmacological effects of *Sabal* and *Urtica* extracts as basis for a rational medication of benign prostatic hyperplasia. *Urologe*. 1994;334:90–95.
17. Goepel M, Hecker U, Krege S, et al. Saw palmetto extracts potently and noncompetitively inhibit human alpha-1-adrenoceptors in vitro. *Prostate*. 1999;38:208–215.
18. Fischer M, Wilbert D. Test of a phytomedicine for treatment of benign prostatic hyperplasia (BPH). In: Rutishauser G, ed. *Benigne Prostatahyperplasie III: Klinische und experimentelle Urologie*. Vol. 22. New York: W Zuckswerdt; 1992:79–84.
19. Gansser D, Spiteller G. Plant constituents interfering with human sex hormone-binding globulin evaluation of a test method and its application to *Urtica dioica* root extracts. *Z Naturforsch*. 1995;50:98–104.
20. Hryb DJ, Khan MS, Romas NA, et al. The effect of extracts of the roots of the stinging nettle *(Urtica dioica)* on the interaction of SHBG with its receptor on human prostatic membranes. *Planta Med*. 1995;61:31–32.
21. Wagner H, Geiger WN, Boos G, et al. Studies on the binding of *Urtica dioica* agglutinin (UDA) and other lectins in an in vitro epidermal growth factor receptor test. *Phytomedicine*. 1995;2:287–290.
22. Blumenthal M, Busse WR, Goldberg A, et al., eds. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*. Boston: Integrative Medicine Communications; 1998:216–217.
23. Vontobel HP, Herzog R, Rutishauser G, et al. Results of a double-blind study on the effectiveness of ERU (extractum radicis urticae) capsules in conservative treatment of benign prostatic hyperplasia. *Urologe A*. 1985;24:49–51.
24. Schneider T, Rübben H. Stinging nettle root extract (Bazoton-uno) in long term treatment of benign prostatic syndrome (BPS) results of a randomized, double-blind, placebo controlled multicenter study after 12 months. *Urologe A*. 2004;43:302–306.
25. Safarinejad MR. Urtica dioica for treatment of benign prostatic hyperplasia: a prospective, randomized, double-blind, placebo-controlled, crossover study. *J Herb Pharmacother*. 2005;5:1–11.
26. Sökeland J, Albrecht J. Combination of *Sabal* and *Urtica* extract vs. finasteride in benign prostatic hyperplasia (Aiken stages I to II). Comparison of therapeutic effectiveness in a one year double-blind study. *Urologe A*. 1997;36:327–333.
27. Schneider HJ, Honold E, Masuhr T. Treatment of benign prostatic hyperplasia results of a treatment study with the phytogenic combination of *Sabal* extract WS 1473 and *Urtica* extract WS 1031 in urologic specialty practices. *Fortschr Med*. 1995;113:37–40.
28. Lopatkin N, Sivkov A, Walther C, et al. Long-term efficacy and safety of a combination of *Sabal* and *Urtica* extract for lower urinary tract symptoms–a placebo-controlled, double-blind, multicenter trial. *World J Urol*. 2005;23(2):139–146.
29. Popa G, Hägele-Kaddour H, Walther C. Efficacy of a combined *Sabal-Urtica* preparation in the symptomatic treatment of benign prostatic hyperplasia. Results of a placebo-controlled double-blind study. *MMW Fortschr Med*. 2005;147(suppl 3):103–108 ([in German]).
30. Cai T, Mazzoli S, Bechi A, et al. Serenoa repens associated with *Urtica dioica* (ProstaMEV) and curcumin and quercetin (FlogMEV) extracts are able to improve the efficacy of prulifloxacin in bacterial prostatitis patients: results from a prospective randomized study. *Int J Antimicrob Agents*. 2009;33(6):549–553.
31. Ramm S, Hansen C. Nettle leaf extract for arthrosis and rheumatoid arthritis. *Therapiewoche*. 1996;28:3–6.
32. Chrubasik S, Enderlein W, Bauer R, et al. Evidence for antirheumatic effectiveness of herba *Urticae dioicae* in acute arthritis: a pilot study. *Phytomedicine*. 1997;4:105–108.
33. Randall CF. Stinging nettles for osteoarthritis pain of the hip. *Br J Gen Pract*. 1994;44:533–534.
34. Randall C, Meethan K, Randall H, et al. Nettle sting of *Urtica dioica* for joint pain: an exploratory study of this complementary therapy. *Complement Ther Med*. 1999;7:126–131.
35. Randall C, Randall H, Dobbs F, et al. Randomized controlled trial of nettle sting for treatment of base-of-thumb pain. *J R Soc Med*. 2000;93:305–309.
36. Randall C, Dickens A, White A, et al. Nettle sting for chronic knee pain: a randomised controlled pilot study. *Complement Ther Med*. 2008;16(2):66–72.
37. Moré M, Gruenwald J, Pohl U, Uebelhack R. A Rosa canina—*Urtica dioica*—Harpagophytum procumbens/zeyheri combination significantly reduces gonarthritis symptoms in a randomized, placebo-controlled double-blind study. *Planta Med*. 2017;83(18):1384–1391.
38. Kianbakht S, Khalighi-Sigaroodi F, Dabaghian FH. Improved glycemic control in patients with advanced type 2 diabetes mellitus taking *Urtica dioica* leaf extract: a randomized double-blind placebo-controlled clinical trial. *Clin Lab*. 2013;59(9–10):1071–1076.
39. Khalili N, Fereydoonzadeh R, Mohtashami R, et al. Silymarin, olibanum, and nettle, a mixed herbal formulation in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *J Evid Based Complementary Altern Med*. 2017;22(4):603–608.
40. Namazi N, Tarighat A, Bahrami A. The effect of hydro alcoholic nettle *(Urtica dioica)* extract on oxidative stress in patients with type 2 diabetes: a randomized double-blind clinical trial. *Pak J Biol Sci*. 2012;15(2):98–102.
41. Namazi N, Esfanjani AT, Heshmati J, Bahrami A. The effect of hydro alcoholic nettle *(Urtica dioica)* extracts on insulin sensitivity and some inflammatory indicators in patients with type 2 diabetes: a randomized double-blind control trial. *Pak J Biol Sci*. 2011;14(15):775–779.
42. Amiri Behzadi A, Kalalian-Moghaddam H, Ahmadi AH. Effects of *Urtica dioica* supplementation on blood lipids, hepatic enzymes and nitric oxide levels in type 2 diabetic patients: a double blind, randomized clinical trial. *Avicenna J Phytomed*. 2016;6(6):686–695.
43. Mittman P. Randomized, double-blind study of freeze-dried *Urtica dioica* in the treatment of allergic rhinitis. *Planta Med*. 1990;56:44–47.

44. Bakhshaee M, Mohammad Pour AH, Esmaeili M, et al. Efficacy of supportive therapy of allergic rhinitis by stinging nettle *(Urtica dioica)* root extract: a randomized, double-blind, placebo- controlled, clinical trial. *Iran J Pharm Res*. 2017;16(suppl):112–118.
45. Treasure J. Urtica semen reduces serum creatinine levels. *J Am Herbal Guild*. 2003;4(2):22–25.
46. Özalkaya E, Aslandoğdu Z, Özkoral A, et al. Effect of a galactagogue herbal tea on breast milk production and prolactin secretion by mothers of preterm babies. *Niger J Clin Pract*. 2018;21(1):38–42.
47. Dathe G, Schmid H. Phytother for benign prostatic hyperplasia (BPH). *Urologe B*. 1987;27:223–226.
48. Engelmann U. *A Prospective, Randomized, Parallel-Group Comparison, Placebo-Controlled, Double-BLIND, Multicenter, Phase 4 Clinical Trial to Consider the Effectiveness and Tolerability of BU08-Film-Coated Tablets (Extract of Nettle Root) for the Treatment of Urinary Difficulties Due to Alken Stage II Prostatic Adenoma*. Cologne, Germany: Boots Pharma GmbH, Abschlussbericht; 1996.
49. Tyler V. *Herbs of Choice: The Therapeutic Use of Phytomedicinals*. New York: Pharmaceutical Products Press; 1994.
50. Mills S, Bone K. *Principles and Practice of Phytotherapy: Modern Herbal Medicine*. Edinburgh: Churchill Livingstone; 2000.

Uva ursi (Bearberry)

Michael T. Murray, ND

OUTLINE

Arctostaphylos uva ursi (family: *Ericaceae*)
Common names: bearberry, upland cranberry

GENERAL DESCRIPTION

Arctostaphylos uva ursi is a small evergreen shrub found in the northern United States and in Europe. A single long, fibrous main root sends out several prostrate or buried stems 4 to 6 inches long. The bark is dark brown, the leaves are obovate to spatulate and 0.5 to 1 inch long, the flowers are pink or white and grow in sparse terminal clusters, and the fruit is a bright red or pink (Figs. 120.1A and B).

CHEMICAL COMPOSITION

The most active ingredient of *uva ursi* is arbutin, which typically composes 7% to 9% of the leaves (Fig. 120.2). Other constituents are as follows[1–4]:

- Tannins (6%–7%)
- Flavonoids (quercetin)
- Allantoin
- Gallic and ellagic acids
- Volatile oils
- A resin (urvone)

HISTORY AND FOLK USE

This plant has a long history of use for its diuretic and astringent properties. Conditions for which *uva ursi* was used include chronic cystitis, nephritis, kidney stones, and bronchitis.[1]

PHARMACOLOGY

Antimicrobial Effects

Although pharmacological research has focused primarily on arbutin, the pharmacology of the whole plant is different from that of arbutin alone. The crude plant extracts are much more effective medicinally than the isolated constituent arbutin.[5] This fact appears to be related to the activity of gallic acid, which prevents the splitting of arbutin by such enzymes as β-glucosidase contained in gut bacteria.[2] Arbutin undergoes hydrolysis in the stomach or intestinal tract to produce hydroquinone, its aglycone, which has urinary antiseptic properties.[6] The hydrolysis of arbutin is responsible for much of the therapeutic effect of *uva ursi*.[1,6] By preventing the splitting of arbutin, the flavonoid components allow more arbutin to be hydrolyzed and absorbed than when arbutin is administered as an isolated component. Approximately 65% of an arbutin dosage is excreted in the urine as hydroquinone glucuronide or sulfate.[7,8]

Arbutin alone has been reported to be an effective urinary antibiotic, but only if taken in large doses and if the urine is alkaline (once again documenting the value of whole-plant medicines).[1] It is reported to be active against *Candida albicans* and *Staphylococcus aureus* and especially against *Escherichia coli*.[5,9] *Uva ursi* also has diuretic properties.[1]

Anti-Inflammatory Effects

Some early animal research is now showing that arbutin, and possibly other constituents of *uva ursi,* potentiate the activity of commonly prescribed anti-inflammatory drugs. One study found that an aqueous extract increased the inhibitory activity of dexamethasone in allergic and inflammatory models without increasing any of the side effects.[10] Similar results have been demonstrated with isolated arbutin combined with indomethacin.[11]

Inhibition of Melanin Synthesis

Uva ursi extract has been shown to inhibit the enzyme tyrosinase.[12] This effect impairs melanin synthesis, which indicates that *uva ursi* extract may be effective as a whitening agent for the skin. In fact, hydroquinone is approved by the U.S. Food and Drug Administration as an agent in over-the-counter skin products for bleaching age spots and hyperpigmentation.

CLINICAL APPLICATIONS

Crude extracts are widely used in Europe as components in certain diuretic and laxative products, but the major use of *uva ursi* is as a urinary disinfectant in cases of urinary tract infection.[1]

Fig. 120.1 (A) *Arctostaphylos uva-ursi* flower leaf. (B) *A. uva-ursi* habit.

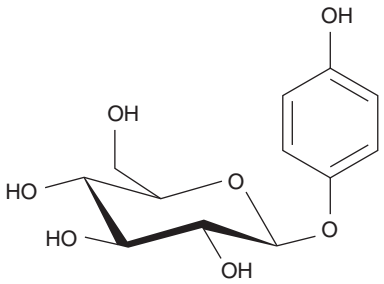


Fig. 120.2 Arbutin.

Urinary Tract Infections

Uva ursi is reported to be especially active against *E. coli*.[6] As well as exerting direct antibiotic effects, *uva ursi* extract promotes the aggregation of *E. coli* in vitro.[9] These combined effects indicate that *uva ursi* can be used both in the immediate treatment and in the prevention of recurrent cystitis. In one double-blind study, the prophylactic effects of a standardized *uva ursi* extract and a placebo on recurrent cystitis were evaluated in 57 women.[13] At the end of 1 year, 5 of 27 women in the placebo group had a recurrence, whereas none of the 30 women receiving *uva ursi* extract had a recurrence. No side effects were reported in either group. These impressive results indicate that regular use of *uva ursi* is a safe and effective measure to prevent recurrent cystitis. In a study of normal subjects, consuming *uva ursi* tea was shown to have bacteriostatic effects greater than normal urine.[6] The effectiveness of the tea in inhibiting bacterial growth (*S. aureus* and *E. coli*) depended on adequate excretion products of arbutin (hydroquinone paired with glucuronate or sulfur) and a pH above 8. Alkalinization of the urine with bicarbonate or citrate salts in conjunction with *uva ursi* may enhance the therapeutic effects in acute urinary tract infections.

DOSAGES

The dosages for *uva ursi* are as follows:

- Dried leaves or as an infusion: 1.5 to 4.0 g (1–2 tsp) three times daily
- Freeze-dried leaves: 500 to 1000 mg three times daily
- Tincture (1:5): 4 to 68 mL (1–2 tsp) three times daily
- Fluid extract (1:1): 2 to 4 mL (1/2–1 tsp) three times daily
- Powdered solid extract (10% arbutin): 250 to 500 mg three times daily

TOXICOLOGY

The toxicological concern with *uva ursi* is related to the conversion of arbutin to hydroquinone, but toxicity is extremely unlikely with the consumption of commercial preparations.[7,14] In the most recent evaluation, *uva ursi* was judged to be very safe in the treatment of urinary tract infections.[15] Hydroquinone is metabolized in the liver by Phase II enzymes into hydroquinone glucuronate or sulfate and then excreted in the urine. At therapeutic levels of *uva ursi* leaf extract, the high range may provide 420 mg hydroquinone derivatives calculated as anhydrous arbutin. The calculation is that which would liberate free hydroquinone in the urine at a maximum exposure level of 11 μg/kg body weight. In contrast, in various laboratory animals, the oral LD_{50} of hydroquinone in 2% aqueous solution is between 320 and 550 mg/kg body weight.

Signs and symptoms of *uva ursi* toxicity include the following:

- Tinnitus
- Nausea
- Vomiting
- Sense of suffocation
- Shortness of breath
- Cyanosis
- Convulsions
- Delirium
- Collapse

The only significant side effect reported in the medical literature with *uva ursi* consumption was that of a 56-year-old woman who ingested *uva ursi* for 3 years and presented with vision loss.[16] Diagnosis indicated bilateral bull's-eye maculopathy, presumably due to inhibition of retinal melanin synthesis. This report raises the question of the appropriateness of long-term therapy with *uva ursi*.

DRUG INTERACTIONS

An extract of *uva ursi* has been found to markedly potentiate the effects of beta-lactam antibiotics, such as oxacillin (Bactocil) and cefmetazole (Zefazone), against methicillin-resistant *S. aureus.*[17] The effective compound was identified as the polyphenol corilagin. Corilagin reduced the mean inhibitory concentrations (MICs) of various beta-lactams 100- to 2000-fold but not the MICs of other antimicrobial agents tested. The effect of corilagin and oxacillin was synergistic. Corilagin showed a bactericidal action when added to the growth medium in combination with oxacillin.[18]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Leung AY, Foster S. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. New York: John Wiley; 1996.
2. Budvari S, ed. *Merck index: An Encyclopedia of Chemicals, Drugs, and Biologicals*. Rahway, NJ: Merck; 1996:131.
3. Parejo I, Viladomat F, Bastida J, et al. Single extraction step in the quantitative analysis of arbutin in bearberry *(Arctostaphylos uva-ursi)* leaves by high-performance liquid chromatography. *Phytochem Anal*. 2001;12:336–339.
4. Veit M, Van Rensen I, Kirch J, et al. HPLC analysis of phenolics and flavonoids in Arctostaphylos uvae-ursi. *Planta Med*. 1992;58:A687–A688.
5. Jahodár L, Jílek P, Páktová M, et al. Antimicrobial effect of arbutin and an extract of the leaves of *Arctostaphylos uva-ursi* in vitro. *Cesk Farm*. 1985;34:174–178.
6. Frohne D. The urinary disinfectant effect of extract from leaves of uva ursi. *Planta Medica*. 1970;18:1–25.
7. Quintus J, Kovar KA, Link P, et al. Urinary excretion of arbutin metabolites after oral administration of bearberry leaf extracts. *Planta Med*. 2005;71(2):147–152.
8. Schindler G, Patzak U, Brinkhaus B, et al. Urinary excretion and metabolism of arbutin after oral administration of *Arctostaphylos uvae ursi* extract as film-coated tablets and aqueous solution in healthy humans. *J Clin Pharmacol*. 2002;42(8):920–927.
9. Turi E, Turi M, Annuk H, et al. Action of aqueous extracts of bearberry and cowberry leaves and wild camomile and pineapple-weed flowers on *Escherichia coli* surface structures. *Pharmaceut Biol*. 1999;37:127–133.
10. Matsuda H, Nakamura S, Tanaka T, et al. Pharmacological studies on leaf of *Arctostaphylos uva-ursi (L.) Spreng. V*. Effect of water extract from *Arctostaphylos uva-ursi (L.)* Spreng. (bearberry leaf) on the antiallergic and anti-inflammatory activities of dexamethasone ointment. *Yakugaku Zasshi*. 1992;112:673–677.
11. Matsuda H, Tanaka T, Kubo M. Pharmacological studies on leaf of *Arctostaphylos uva-ursi (L.) Spreng. III*. Combined effect of arbutin and indomethacin on immuno-inflammation. *Yakugaku Zasshi*. 1991;111:253–258.
12. Matsuda H, Higashino M, Nakai Y, et al. Studies of cutical drugs from natural sources. IV. Inhibitory effects of Arctostaphylos plants on melanin biosynthesis. *Biol Pharm Bull*. 1996;19:153–156.
13. Larsson B, Jonasson A, Fianu S. Prophylactic effect of UVA-E in women with recurrent cystitis: a preliminary report. *Curr Ther Res*. 1993;53:441–443.
14. Paper DH, Koehler J, Franz G. Bioavailability of drug preparations containing a leaf extract of *Arctostaphylos uva-ursi (Uvae ursi folium)*. *Planta Med*. 1993;59:A589.
15. de Arriba SG, Naser B, Nolte KU. Risk assessment of free hydroquinone derived from *Arctostaphylos Uva-ursi* folium herbal preparations. *Int J Toxicol*. 2013;32(6):442–453.
16. Wang L, Del Priore LV. Bull's-eye maculopathy secondary to herbal toxicity from uva ursi. *Am J Ophthalmol*. 2004;137(6):1135–1137.
17. Shimizu M, Shiota S, Mizushima T, et al. Marked potentiation of activity of beta-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. *Antimicrob Agents Chemother*. 2001;45:3198–3201.
18. Shiota S, Shimizu M, Sugiyama J, et al. Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Microbiol Immunol*. 2004;48(1):67–73.

Vaccinium macrocarpon (Cranberry)

Sherry Torkos, BSc, Phm, RPh, Rhonda Dorren, BScPharm,* and *Michael T. Murray*, ND*

OUTLINE

GENERAL DESCRIPTION

The shiny scarlet cranberry, like its cousin the blueberry, belongs to the genus *Vaccinium* and is found growing in the wild in northern Europe, northern Asia, and North America. The American cranberry *(Vaccinium macrocarpon)* is the species associated with the majority of the scientific research on health benefits. Cultivated cranberries are grown in huge sandy bogs on low, trailing vines. Cranberries have also been called "bounce berries," because ripe berries bounce, and "crane berries," a reference to the cranberry shrub's pale pink blossoms, which resemble the heads of the cranes that frequent cranberry bogs.

Cranberry has garnered a lot of attention as a possible alternative to antibiotics in the prevention of urinary tract infections (UTIs). Low-dose antibiotics are often prescribed for prevention of infections, but this regime leads to increases in antibiotic resistance as well as side effects and treatment complications for the patient. The emergence of multiple antibiotic–resistant organisms in the general community is a potentially serious threat to public health.[1] A paradigm shift in the prevention and treatment of infectious disease, particularly chronic infections, may be required to prevent antibiotics from becoming obsolete; where appropriate, alternatives to antibiotics require consideration.[2] As a result, for prevention of infections, there has been an increase in the use of natural products as well as the consumption of cranberries (processed and fresh) in North America (Fig. 121.1).

The majority of research on cranberry involves urinary tract health. However, the historical bibliography of investigations on cranberry also includes but is not limited to type 2 diabetes; prevention of blockage in urinary catheters; deodorizing of offensive urine; healing skin around urostomy stomas; and as an anticarcinogen, antifungal, and antioxidant agent.[3–9]

CHEMICAL COMPOSITION

In addition to ascorbic acid, cranberry juice contains a variety of substances that may be biologically active. Cranberries contain flavonoids and other phenolic compounds.[10] The quantity of flavonoids in cranberry juice (approximately 31.9 mg per serving) is higher than in red wine (approximately 22 mg) but lower than in dark chocolate containing high flavanol levels (approximately 165 mg) and apples (approximately 147 mg). In a sample of freshly squeezed cranberry juice, one study found 400 mg of total flavonoids and phenolic compounds per liter of sample, which was distributed as about 44% of phenolic acids and 56% of flavonoids. Benzoic acid was the major phenolic compound, and the major flavonols were quercetin and myricetin. Anthocyanins are also found in cranberry and are responsible for the red pigmentation of the fruit. They are abundant in the form of cyanidin glycosides (sugar groups attached).[11,12] Cranberries also contain organic acids, including quinic, malic, and citric acids.[13]

Cranberries contain proanthocyanidins (also referred to as condensed tannins), which are composed of epicatechins linked together by unusual A-type bonds, not found in most other tannin-containing foods. These different forms of polyphenols are often mentioned in the scientific literature on cranberry.

HISTORY AND FOLK USE

Native Americans used cranberry both as food and for the treatment of bladder and kidney diseases. The Pilgrims learned about cranberry from local tribes for a condition referred to as "bladder gravel" (small bladder stones) and to remove blood toxins. Cranberries were served to ships' crews as a source of vitamin C to prevent scurvy. Other documented medical applications in the 17th century include the relief of stomach ailments, liver problems, blood disorders, and cancer. The boiled berry and seal oil were used to reduce the severity of gallbladder attacks.[14]

Cranberry's beneficial effects on urinary tract health were initially thought to be due to acidification of the urine. Direct antibacterial and antifungal effects of cranberry juice have been shown.[15–17] Hippuric acid, converted from the quinic and benzoic acids in cranberry, is a strong bacteriostatic agent, and its potential to acidify urine has been investigated.[5,18–20] In brief, these early studies focused on the ability of cranberry juice to lower urinary pH, on the basis of its apparent ability to suppress infections and enhance antibiotic activity.[21–24] Yet

*Previous edition contributor

Fig. 121.1 *Vaccinium macrocarpon* Fruit.

clinically, the concentration of hippuric acid in cranberry does not routinely reach levels adequate for antibacterial action.

Further research failed to validate the proposed theory that cranberry reduced urinary pH. Cranberry continued to be a popular remedy for urinary health, and only in the past decade has its true mechanism of action been elucidated. Current evidence is strongest for its effectiveness in promoting urinary tract health and reducing the risk of UTIs while sparing the patient the experience of unwanted side effects and the continued use of prophylactic low-dose prescription drugs.[25]

PHARMACOLOGY

The primary application of cranberry is in the prevention of UTIs. Cranberry components interfere with bacterial adherence to mucosal cells—an important step in the development of infection. For bacteria to successfully attach to the host, they must identify and attach to epithelial cell receptor sites. Proteinaceous fibers on the cell wall of uropathogenic *Escherichia coli,* called fimbriae, produce adhesion molecules that attach to these monosaccharide or oligosaccharide receptors on uroepithelial cells. Two different fimbrial types are most important for initiating UTI: type 1 fimbriae, which adhere to mannose-specific receptors; and P fimbriae, which adhere to disaccharide α-D-Gal(1,4)-β-D-Gal (Gal-Gal) receptors.

Modern research has revealed that the proanthocyanidins (PACs) in cranberries can inhibit the adhesion of uropathogenic P-fimbriated strains of *E. coli* to bladder cells.[26–28] Cranberry contains two different inhibitors: fructose, effective for inhibiting the type 1 fimbriae; and a second, unidentified inhibitor that is effective for obstructing the Gal-Gal–specific fimbriae.[29] However, fructose is fully metabolized after consumption and does not reach the urine. Therefore it does not influence bacterial adhesion in the body. In addition, scientific evidence suggests that cranberry acts on the cell wall not only by preventing attachment of the fimbrial subunits but also by serving as a genetic control, preventing the expression of normal fimbrial subunits, or both.[30–33]

In animal models, investigations of approximately 77 clinical isolates of *E. coli* demonstrated that cranberry juice inhibited bacterial adhesion to the bladder by more than 75% for more than 60% of the strains of bacteria.[34] Further research demonstrated that cranberry significantly reduced the adherence of pathogens to urinary epithelial cells isolated from patients with UTIs, including *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter* species, and *Pseudomonas aeruginosa.*[35,36]

The cumulative effects of cranberry have now been demonstrated as successfully acting on cell walls to prevent the proper attachment of the fimbrial subunits, thus inhibiting bacterial adherence to uroepithelial cells, and cellular elongation.[37]

The role of PACs in UTIs has been debated because of their size, poor bioavailability, and extensive metabolism and limited knowledge about their urinary excretion.[38]

In vivo research has demonstrated that consumption of cranberry PACs results in an antiadhesion response in the urine to provide protective effects from bacteria that migrate from the perineum and vagina.[39] Research continues to investigate whether the PACs induce a direct urinary response or are broken down to smaller bioactive metabolites that reach the urine.

Larger PACs that are eliminated through the colon may bind to rectal uropathogenic bacterial isolates in the gut, thus decreasing the virulence of these microbes if they come in contact with the uroepithelium. Researchers in Finland who studied the UTI preventive effects of a commercially available cranberry-lingonberry juice concentrate stated that their findings support bacterial selection in the stool because subjects in the cranberry group had no increase in UTI recurrence during the 6 months after they had stopped the cranberry prophylaxis.[40]

More recently, a study was undertaken to determine whether the polyphenols follow a dose response in vivo at different levels of intake. A double-blind, randomized controlled trial in 10 healthy men with cranberry juice containing 409, 787, 1238, 1534, and 1910 mg total polyphenols was performed. The results demonstrated that plasma polyphenol metabolites are linearly related to the amount of polyphenols consumed and that individual variations may occur due to the gut microbiome.[41]

Emerging concepts regarding preventive considerations for the management of recurrent UTIs (RUTIs) suggest taking cranberry supplements as part of the protocol.[42]

Numerous investigations have been completed on cranberry. The following discussion provides an overview of some of the recent data, including a review of studies on cranberry and urinary tract health.

CLINICAL APPLICATIONS

Urinary Tract Applications

Reduction of Bacteriuria and Pyuria After Ingestion of Cranberry Juice

An early study conducted by Avorn et al.[43] evaluated the effects of cranberry on bacteriuria in 153 elderly women. Participants consumed 300 mL/day of either a commercially available cranberry beverage or a placebo drink that was similar in taste and contained vitamin C but no cranberry content. After 1 month of treatment, 15% of the urine samples of those who had been drinking cranberry juice had bacteriuria with pyuria, compared with 28% of those in the placebo group. Most patients with bacteriuria were asymptomatic. Although statistical significance was not achieved, there was a trend toward less antibiotic use for the treatment of UTIs in the cranberry group (1.7 vs. 3.2 antibiotics per 100 patient-months). A criticism of this trial is that the control group had a higher rate of urinary infection before the study, suggesting there may have been sampling bias, and the main outcome, identified as asymptomatic bacteriuria, does not generally require treatment.[44] Although there was a trend toward fewer clinically relevant urinary infections, the study was not designed to demonstrate that effect. This trial, like others, had a high dropout rate, which casts doubt on the long-term tolerability of cranberry juice.[45,46]

Urinary Tract Infections and Sexual Activity

Foxman et al.[47] randomly sampled 288 control subjects (college students), studying first-time UTIs in young unmarried women. Participants were recruited from a university health service, and all had engaged in sexual intercourse at least once. Compared with the general

population of students, intercourse significantly increased the risk of UTIs (43%), as did drinking carbonated soft drinks (237%), but it was found that drinking cranberry juice reduced the risk (52%).

A study in France investigated the effect of consuming a single postcoital dose of cranberry on the development of UTIs. This double-blind study evaluated 120 female patients with recurrent UTI (six or more in the previous 12 months). Patients were randomized to one of three groups and received either one capsule of infused cranberry powder made by a patented procedure (Bio-Shield, Cran-Max, known commercially in France as GynDelta), one capsule of dry cranberry extract containing 36 mg of PAC, or one capsule containing a placebo. Patients were instructed to take their test product within 6 hours after intercourse. Over the course of the study period of 45 days, 10.8% of patients in the GynDelta group suffered a UTI, compared with 18.9% in the cranberry PAC group and 43.2% in the placebo group. The results for the GynDelta were statistically significant ($P = 0.005$).[48]

Cranberry Juice and the Adhesion of Antibiotic-Resistant Uropathogens

Howell et al.[49] carried out an ex vivo study verifying cranberry's ability to prevent the adhesion of certain *E. coli* strains that are resistant to trimethoprim-sulfamethoxazole.[49] The researchers obtained 39 uropathogenic P-fimbriated *E. coli* isolates, 24 of which were resistant, from women with culture-confirmed UTIs. Incubation of the bacteria in the urine collected from participants who consumed 240 mL of cranberry juice cocktail prevented adhesion in 31 of 39 isolates, including 19 of 24 resistant isolates; antiadhesion activity began within 2 hours of exposure and persisted for up to 10 hours. Because the mechanism of action is not bactericidal, the use of cranberry does not increase the selection for antibiotic-resistant bacterial strains. A reduction in the frequency of infections may decrease the frequency of antibiotic use, saving first-line antibiotics for other infections.[50]

Trials Involving Catheterization, Neurogenic Bladders, and Spinal Cord–Injured Patients

Rates of bacteriuria and symptomatic UTIs in individuals who receive clean intermittent catheterization are an enduring concern. Schlager et al.,[51] comparing cranberry with placebo, completed a 3-month double-blind, placebo-controlled crossover trial of children ($n = 15$) with neurogenic bladders treated with intermittent catheterization. The researchers reported that 75% of 151 cultures indicated bacteriuria at both sample intervals. In this case, there was no difference between the children drinking cranberry concentrate and those receiving a placebo. Reid et al.[52] examined 15 spinal cord–injured (SCI) patients to ascertain whether cranberry juice altered bacterial biofilm load in the bladder. They found that cranberry juice significantly diminished biofilm load compared with baseline, with a reduction in the adhesion of both gram-positive and gram-negative bacteria to cells. Water intake did not affect bacterial adhesion.

Linsenmeyer et al.[53] completed a prospective, double-blind, placebo-controlled crossover study of 21 individuals with neurogenic bladders secondary to SCI who were randomly assigned to receive either 400-mg cranberry tablets or placebo three times a day for 4 weeks.[53] Cranberry tablets did not modify urinary pH or reduce bacterial counts, urinary white blood cell counts, or the number of UTIs in individuals with neurogenic bladder.

The most recent study evaluated the effect of cranberry tablets for the prevention of UTI in SCI patients with neurogenic bladder. Patients were randomized to receive 6 months of cranberry tablets (Cran-Max) or placebo, followed by the alternate preparation for an additional 6 months. Forty-seven subjects completed the trial. The researchers found a reduction in the likelihood of UTI and symptoms for any month in patients receiving the cranberry tablet ($P < 0.05$ for all). During the cranberry period, 6 subjects had 7 UTIs, compared with 16 subjects and 21 UTIs in the placebo period ($P < 0.05$ for both number of subjects and incidence). The frequency of UTI was reduced to 0.3 UTIs per year versus 1 UTI per year in those receiving placebo. Subjects with a glomerular filtration rate (GFR) greater than 75 mL/min received the most benefit.[54]

Further long-term studies evaluating specific types of bladder management and UTI will help determine the role of cranberries in individuals who have neurogenic bladder, require catheterization, or have a spinal cord injury.

Comparative Trial Subsequent to Antibiotic Therapy

The use of cranberry supplements in women with UTI after antibiotic treatment may reduce the recurrence of UTI. In an open-label trial in Finland, Kontiokari et al.[55] treated 150 women with UTI due to *E. coli* with a standard antimicrobial regimen; then the participants were randomly assigned to receive 50 mL of cranberry-lingonberry juice concentrate daily for 6 months, 100 mL of *Lactobacillus* drink 5 days a week for 1 year, or no intervention. At 6 months, 16% of the women in the cranberry group, 39% in the *Lactobacillus* group, and 36% in the control group had UTIs. This finding demonstrates that a regular intake of cranberry juice but not *Lactobacillus* seems to reduce the recurrence of UTI.

Comparative Trials—Antibiotic Versus Cranberry for UTI Prevention

McMurdo et al.[56] compared the effectiveness of cranberry extract or low-dose trimethoprim in the prevention of recurrent UTIs in a group of 137 women with two or more antibiotic-treated UTIs in the previous 12 months. Subjects were randomized to receive either 500 mg of cranberry (Cran-Max) or 100 mg of trimethoprim for 6 months.

Thirty-nine of 137 participants (28%) had an antibiotic-treated UTI (25 in the cranberry group and 14 in the trimethoprim group; difference in proportions relative risk 1.616 [95% confidence interval: 0.93, 2.79], $P = 0.084$). The time to the first recurrence of UTI was not significantly different between the groups ($P = 0.100$). The median time to recurrence of UTI was 84.5 days for the cranberry group and 91 days for the trimethoprim group ($U = 166$, $P = 0.479$).

The researchers concluded that trimethoprim had a very limited advantage over cranberry extract in the prevention of recurrent UTIs in older women and that it had more adverse effects.[56]

Beerepoot et al.[57] conducted a double-blind study of 221 premenopausal women with recurrent UTIs who were randomized to 12-month prophylaxis use of trimethoprim-sulfamethoxazole (TMP-SMX), 480 mg once a day, or cranberry capsules (Cran-Max), 500 mg twice a day. Primary end points were the mean number of symptomatic UTIs over 12 months, the proportion of patients with at least 1 symptomatic UTI, the median time to first UTI, and development of antibiotic resistance in indigenous *E. coli*.

After 12 months, the mean number of patients with at least one symptomatic UTI was higher in the cranberry than in the TMP-SMX group (4.0 vs. 1.8; $P = .02$), and the proportion of patients with at least one symptomatic UTI was higher in the cranberry than in the TMP-SMX group (78.2% vs. 71.1%). Median time to the first symptomatic UTI was 4 months for the cranberry and 8 months for the TMP-SMX group. Although the antibiotic was slightly more effective in preventing UTI, the subjects receiving antibiotics developed significant resistance to several antibiotics primarily used to prevent and treat UTIs.

After 1 month, in the TMP-SMX group, 86.3% of fecal and 90.5% of asymptomatic bacteriuria *E. coli* isolates were TMP-SMX resistant. There were also significantly increased resistance rates for

trimethoprim alone, amoxicillin, and ciprofloxacin in these *E. coli* isolates after 1 month in the TMP-SMX group. Antibiotic resistance did not increase in the cranberry group. The cranberry product and TMP-SMX were equally well tolerated.

Effectiveness as a Prophylactic Against Urinary Tract Infection

Stothers[58] executed a randomized, placebo-controlled trial that evaluated, from a societal perspective, the comparative effectiveness and cost-effectiveness of concentrated cranberry tablets, cranberry juice, and placebo for prophylaxis against lower UTI in adult women. Sexually active women (n = 150) aged 21 to 72 years were randomly assigned for 1 year to one of the following three groups:

- Placebo juice + placebo tablets
- Placebo juice + cranberry tablets
- Cranberry juice + placebo tablets

The tablets were a proprietary extract of cranberry (30:1), given twice a day. The juice was a pure unsweetened cranberry juice (not cranberry cocktail) given in doses of 250 mL three times a day. Outcome measures demonstrated greater than 50% decreases in both symptomatic UTIs per year and annual antibiotic consumption. Both cranberry juice and cranberry tablets, in a statistically significant manner, decreased the number of patients experiencing at least one symptomatic UTI per year (20% and 18%, respectively) compared with placebo (32%). Total annual antibiotic consumption was lower in both treatment groups than in the placebo group. Cost-effectiveness ratios showed that cranberry tablets were twice as cost-effective as juice for the prevention of UTIs.

Ledda et al. investigated the prophylactic effects of a standardized cranberry extract in young subjects (age 12–18 years) with a previous history of recurrent UTIs, over a 2-month follow-up. Subjects received either standard management (SM) (control group, n = 17) or SM with daily oral supplementation (supplementation group, n = 19). Oral supplementation consisted of one capsule containing 120 mg of cranberry extract (Anthocran), standardized to 36 mg proanthocyanidins, for 60 days. The effectiveness in the prevention of UTIs was determined by the number of UTIs evaluated 2 months before the inclusion in the registry and during the supplementation period and the number of symptom-free subjects during the registry period. Safety considerations were included, and measurement of adherence to treatment was also performed.[59]

The mean number of UTIs observed during the registry in the supplemented group (0.31 ± 0.2) was significantly lower compared with the control group (2.3 ± 1.3) and with the mean number of UTIs assessed before inclusion (1.74 ± 1.1) (p-value = 0.0001 for both). Moreover, 63.1% of supplemented subjects were symptom-free during the registry period, whereas 23.5% subjects were asymptomatic in the control group (p-value < 0.05). This study provides encouraging evidence on the efficacy of cranberry for prevention in young subjects experiencing recurrent UTIs.

A Cochrane review of clinical trials on cranberry for preventing UTIs was conducted in 2008. Ten studies (n = 1049) met the inclusion criteria (five crossover, five parallel-group). Cranberry/cranberry-lingonberry juice versus placebo, juice, or water was evaluated in seven studies, and cranberry tablets versus placebo in four studies (one study evaluated both juice and tablets). Cranberry products significantly reduced the incidence of UTIs at 12 months (risk ratio [RR] 0.65, 95% confidence interval [CI] 0.46–0.90) compared with placebo. Cranberry products were more effective in reducing the incidence of UTIs in women with recurrent UTIs compared with elderly men and women or people requiring catheterization. Side effects and dropouts or withdrawals were common in all studies and were high in several of the studies. The authors concluded, "there is some evidence that cranberry juice may decrease the number of symptomatic UTIs over a 12-month period, particularly for women with recurrent UTIs. Its effectiveness for other groups is less certain. The large number of drop-outs/withdrawals indicates that cranberry juice may not be acceptable over long periods of time. It is not clear what the optimum dosage or method of administration (e.g., juice, tablets, or capsules) is. Further properly designed studies with relevant outcomes are needed."[60] A more recent 2017 Cochrane review reported similar benefit in reducing UTIs (weighted RR 0.67, $p < 0.0001$), with the best results seen in those who, for whatever reason, were more susceptible to UTIs.[61]

Subsequent to Cochrane's earlier review, further studies have evaluated the effect of cranberry supplements, including two studies that compared cranberry (Cran-Max) to antibiotics (as previously discussed) for reducing UTI recurrence.

Sengupta et al.[62] evaluated the effects of a standardized cranberry product (PACran) on reducing the recurrence of symptomatic UTI in culture-positive subjects. This 90-day randomized clinical trial involved 60 female subjects aged 18 to 40 years who were randomly assigned to three groups: an untreated control group (n = 16), a low-dose (500 mg daily, n = 21) treatment group, and a high-dose (1000 mg daily, n = 23) treatment group. The safety of the cranberry product was assessed by evaluation of biochemical and hematological parameters on days 10, 30, 60, and 90, comparing the values with those at the baseline. The occurrence of UTI at baseline and during the follow-up period was characterized by the presence of symptoms and *E. coli* in the culture of urine samples. At the end of the study, the change in the presence of *E. coli* in the untreated control group was not significant, whereas there was significant reduction ($P < 0.05$) in the subjects positive for *E. coli* in both the high- and low-dose treatment groups, compared with baseline evaluation. However, there were no significant differences in bacteriuria in the urine between in the cranberry groups and the untreated control group at any time point after the study commenced. Symptomatic relief was also reported in the low- and high-dose treatment groups, whereas none was reported by subjects in the untreated control group.[62]

Prophylaxis Urinary Tract Infections in Men With Prostate Disease

Lower urinary tract symptoms (LUTSs) are a common condition in older men, particularly among those with benign prostatic hyperplasia (BPH) and chronic prostatitis (CP). It is postulated that cranberry may offer benefits for men with prostate disease above and beyond its ability to inhibit uroepithelial bacterial adhesion and reduce UTI occurrence. Among the recently reported effects of cranberry are its anti-inflammatory action through reduced cyclooxygenase-2 expression, suppression of IkBa degradation in human colon cancer cells,[63] and inhibition of the growth and proliferation of several types of tumor cells, including prostate cancer cells.[64]

A study was conducted to evaluate the effect of a 6-month daily consumption of 1500 mg cranberry fruit powder (PACran) on urinary tract function in men with LUTSs based on the International Prostate Symptom Score (IPSS), elevated prostate-specific antigen (PSA), BPH, and histopathologically confirmed nonbacterial CP. In contrast to the control group, patients in the cranberry group had statistically significant improvement in the IPSS; quality-of-life score; urination parameters, including voiding parameters (rate of urine flow, average flow, total volume, and postvoid residual urine volume); and lower total PSA level on day 180 of the study. There was no statistically significant improvement in the control group. The results of this preliminary trial are the first firm evidence that cranberries may ameliorate LUTSs, independent of BPH or C-reactive protein level.[65]

Reducing Asymptomatic Bacteriuria in Ileal Enterocystoplasty

Bacteriuria is a usual complication of enterocystoplasty after cystectomy. Botto et al.[66] evaluated the effectiveness of treatment with a cranberry supplement in the prevention of repeated bacteriuria in patients with an ileal enterocystoplasty. This open study included 15 patients with a history of repeated urinary infection and/or bacteriuria during the pretreatment phase. During the treatment phase, patients received a cranberry preparation with 36 mg PACs (Urell), one capsule a day. The primary end point was the absence of bacteria in urine culture. The secondary end points were the presence or absence of symptoms (pain, fever), continence status, and upper excretory tract enlargement. There was a significant decrease in the number of positive urine cultures during cranberry compound treatment. These results need to be validated by further double-blind, randomized studies.

Reduction in Urinary Odor

Urinary odor, common in elderly people, may be reduced after the administration of cranberry.[4,67,68] Ammonia odor may be reduced by cranberry because the bacterial populations are decreased, and they produce less ammonia as a by-product of metabolism.

Other Clinical Applications

Although much of the focus on the benefits of cranberry has been on UTIs, cranberry may offer other health benefits, some of which are described in the following subsections.

Antioxidant

Oxidation, or the production of free radicals, is involved in the progression of many bodily processes that can lead to disease and afflictions, especially as we age. Cranberry contains antioxidants that can scavenge free radicals. Of the berries, blackberries, blueberries, cranberries, and raspberries have the highest levels of antioxidants.[69–71] Pedersen et al.[72] studied the effects of blueberry and cranberry consumption on the plasma antioxidant capacity of healthy female volunteers.[72] Daily ingestion of 500 mL of cranberry juice increased plasma phenolic content and antioxidant capacity. Consumption of a similar amount of blueberry did not have this effect.

Cancer

Cranberries contain salicylic acid, the active metabolite of acetylsalicylic acid (aspirin), which has been used historically to treat pain, inflammation, and fever.

More recently, researchers have shown that regular intake of salicylic acid may be associated with a reduced risk of certain types of cancer, particularly colon cancer. Although the exact mechanism of salicylic acid is unknown, research has shown that it inhibits the expression of cyclooxygenase 2 (COX-2), thereby decreasing the synthesis of proinflammatory prostaglandins.

A study was conducted to measure the bioavailability of these anti-inflammatory compounds in cranberry juice. Two groups of healthy female subjects consumed either 250 mL of cranberry juice or a placebo solution three times a day for 2 weeks. Blood and urine samples were collected from the subjects and measured for salicylic acid and its urinary metabolite, salicyluric acid. After 1 week, researchers saw a marked increase in urinary salicylic acid and salicyluric acid in those consuming cranberry juice over those consuming placebo solution. Concentrations of the urinary metabolite were significantly greater than salicylic acid, which is a good indicator of bioavailability. The increase in plasma concentrations of the cranberry group was less extreme but still statistically significant after 2 weeks of consumption.[73]

Researchers have questioned whether sufficient amounts of salicylic acid can be obtained from the diet to exert anti-inflammatory effects similar to those seen from aspirin. Salicylic acid is present in many plant-based foods in varying amounts, and little is known about the bioavailability of these active compounds when obtained from food sources. However, serum and urinary concentrations of salicylic and salicyluric acids are greater in vegetarians than in nonvegetarians and overlap with those in individuals taking up to 150 mg of aspirin per day (a baby aspirin contains 81 mg). This suggests that potentially therapeutic levels of salicylic acid could be achieved by consuming salicylic acid–rich foods, such as cranberries.

Antiangiogenic approaches to prevent and treat cancer represent a priority area in investigative tumor biology. Vascular endothelial growth factor plays a crucial role in the vascularization of tumors. In vitro research on human dermal microvascular endothelial cells has shown that edible berries impair angiogenesis.[74] Several in vitro studies on cranberry have reported similar promising outcomes.

Researchers at the University of Western Ontario discovered that mice receiving cranberry juice and cranberry products had a significantly lower incidence of breast cancer than the control group, and the development of tumors was delayed in the cranberry-fed group. The proliferation of tumors to the lungs and lymph nodes was markedly reduced in the cranberry-fed group. A greater effect was demonstrated by the cranberry solids than the juice. Ongoing research is examining whether the activity of the berry solids traces largely to one component or to several potentially acting in synergy.[75]

A PAC fraction, an extract of concentrated flavonoids, and triterpenoid esters of cranberry were evaluated in an in vitro cell culture model and exhibited potential anticarcinogenic activity, demonstrating an ability to inhibit tumor cell growth.[76–78] A synergistic or additive antiproliferative interaction of the anthocyanins, PACs, and flavonol glycosides within cranberry extract has also been demonstrated by in vitro research on human tumor cells.[79]

A combination of six berry extracts (wild blueberry, bilberry, cranberry, elderberry, raspberry seeds, strawberry) was studied for antioxidant efficacy, cytotoxic potential, cellular uptake, and antiangiogenic properties. The combination of anthocyanin-rich berries was reported to show antiangiogenic, antioxidant, and anticarcinogenic potentials.[80]

Vascular Disease

Research indicates that the consumption of flavonoids in foods and beverages may reduce the risk of atherosclerosis.[81] In vitro and in vivo experiments with cranberry flavonoids demonstrate that they inhibit the oxidation of low-density lipoprotein cholesterol (LDL-c), platelet aggregation and adhesion, and enzymes involved in lipid/lipoprotein metabolism (which affects the immune response to oxidized LDL-c and its uptake by endothelial macrophages). It may also induce endothelium-dependent vasorelaxation and increase reverse cholesterol transport, thereby decreasing total cholesterol and LDL-c values. Wilson et al.[82] showed in vitro that cranberry extracts reduce oxidation of LDL-c.[82] A review by Reed[83] examined the effects of flavonoids on atherosclerosis, with an emphasis on the potential positive effects of the flavonols and PACs in cranberries.[83]

Youdim et al.[84] investigated the putative antioxidant and anti-inflammatory effects of blueberry and cranberry anthocyanins and hydroxycinnamic acids. The polyphenols isolated from both berries afforded protection for endothelial cells against stressor-induced upregulation of oxidative and inflammatory insults. This may have beneficial actions against the initiation and development of vascular diseases and may be a contributing factor in the reduction of age-related deficits in neurological impairments.[84]

Ruel et al.[85] examined the effect of daily consumption of a low-calorie cranberry juice cocktail on plasma oxidized LDL (oxLDL), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion

molecule-1 (VCAM-1), and E-selectin concentrations in men. Thirty men (mean age 51 years) were recruited and asked to consume increasing daily doses of cranberry juice cocktail (125, 250, and 500 mL/d) over three successive periods of 4 weeks. Plasma oxLDL and adhesion molecule concentrations were measured by enzyme-linked immunosorbent assay (ELISA) before and after each phase. The researchers noted a statistically significant decrease in plasma oxLDL, ICAM-1, and VCAM-1.

Glycemic Control and Insulin Resistance

There is mounting evidence from animal studies supporting the benefits of dietary polyphenols for the prevention and management of type 2 diabetes by improving glycemic control and reducing postprandial hyperglycemia. The benefits may be due to inhibition of carbohydrate digestion by inhibiting salivary and pancreatic α-amylase and α-glucosidase in the small intestinal brush border, inhibition of glucose absorption, and stimulation of insulin secretion and protection of pancreatic β-cells against glucotoxicity. Polyphenols may suppress glucose release from the liver and improve glucose uptake in peripheral tissues by modulating intracellular signaling. Polyphenols have antioxidant activity and can inhibit advanced glycation endproduct (AGE) formation.[86,87]

A study by Paquette et al. evaluated the effects of strawberry and cranberry polyphenols (SCPs) on insulin sensitivity, glucose tolerance, insulin secretion, lipid profile, inflammation, and oxidative stress markers in free-living insulin-resistant overweight or obese human subjects (n = 41) in a parallel, double-blind, controlled, randomized clinical trial. The experimental group consumed an SCP beverage (333 mg SCP) daily for 6 weeks, whereas the control group received a flavor-matched control beverage that contained 0 mg SCP. At the beginning and end of the experimental period, insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp, and glucose tolerance and insulin secretion were assessed by a 2-h oral glucose tolerance test (OGTT). Insulin sensitivity increased in the SCP group compared with the control group ($+0.9$ [sem 0.5] $\times 10^{-3}$ vs. -0.5 [sem 0.5] $\times 10^{-3}$ mg/kg per min per pmol, respectively, $P = 0.03$). Compared with the control group, the SCP group had a lower first-phase insulin secretion response as measured by C-peptide levels during the first 30 min of the OGTT ($P = 0.002$). No differences were detected between the two groups for lipids and markers of inflammation and oxidative stress. The authors concluded that a 6-week dietary intervention with 333 mg of polyphenols from strawberries and cranberries improved insulin sensitivity in overweight and obese nondiabetic, insulin-resistant human subjects but was not effective in improving other cardiometabolic risk factors.[88]

Helicobacter Pylori

Burger et al.[89] argue that because cranberry has been shown to inhibit bacterial adhesion to uroepithelial cells, it may also be useful as antiadhesion therapy in *H. pylori* infections. In a number of small in vitro studies, the investigators demonstrated that a high-molecular-weight (HMW) constituent of cranberry inhibits sialyllactose-specific adhesion of *Heliobacter pylori* to a human gastric cell line and mucus as well as human erythrocytes.[89] This research warrants further investigation into the use of cranberry as it relates to *H. pylori.*

Oral Bacteria

The effect of cranberry on the coaggregation (massing together) of oral bacteria has been tested. Weiss et al.[90] discovered an HMW nondialyzable material, composed mainly of PACs isolated from cranberry juice. Although the molecular structure of this material and its mechanism of action are not known, in vitro studies showed that coaggregation of pairs of bacteria was inhibited, and pilot in vivo studies showed that coaggregation of a large number of different pairs was reversed.[91]

Studies using mouthwash demonstrated positive results and warrant additional research. Weiss[92] conducted a small pilot study of individuals (n = 59) using a standard mouthwash that contained this HMW constituent.[92] After 6 weeks of daily use, results showed a reduction in colony-forming units of bacteria in saliva compared with placebo. These data and those of more recent studies provide evidence that inhibition of oral streptococci colonization and attachment to the tooth surface in vivo is due to the antiadhesion activity of the cranberry constituent.[93] Another study hypothesized the inhibition of extracellular polysaccharide synthesis, which promotes the sucrose-dependent adhesion of oral bacteria.[94,95]

Peristomal Problems

Tsukada et al.[6] conducted a small study on the incidence of peristomal problems in 13 patients who had undergone urostomy. Patients drank 160 to 320 g/day of cranberry juice for an average of 6 months. Four of six patients who initially had erythema, maceration, or pseudoepithelial hyperplasia at the stoma site showed improvement. This apparent protective effect for the skin against urine may be relevant for patients who are immobile and incontinent and suffer skin damage as a result. Additional studies to confirm this finding are warranted.[28]

Managing Calcium Oxalate Urolithiasis

Investigations of the potential influence of cranberry juice on urinary biochemical and physicochemical risk factors associated with the formation of calcium oxalate kidney stones have been reported. Older research with renal stones reported a reduction in urinary calcium excretion after the studied patients drank cranberry juice, suggesting that it may be useful in the treatment of recurrent renal stones.[96]

Urinary variables were assessed by McHarg et al.[97] in a randomized crossover trial (n = 20) in participants with no previous history of kidney stones. The ingestion of cranberry juice significantly and uniquely altered three key urinary risk factors. Oxalate and phosphate excretion decreased, whereas citrate excretion increased. In addition, there was a reduction in the relative supersaturation of calcium oxalate, which tended to be significantly lower than that induced by water alone. Each of these outcomes is desirable in patients at risk for nephrolithiasis (urolithiasis). The investigators concluded that cranberry juice has antilithogenic properties and deserves consideration as a conservative therapeutic protocol in managing calcium oxalate urolithiasis. The use of cranberry may be warranted in some patients with recurrent renal stones, but supplementary research is needed.[28]

DOSAGE

There are a variety of cranberry products to choose from that vary greatly in quality, potency, and activity. Differences exist between the level of bioactives (PACs) and formulation in commercially available tablets, capsules, and juices. Many products on the market are not standardized, being either dried whole-fruit powders, juice powders, or mixed juices with undefined amounts of cranberry juice.

Although drinking cranberry juice is one way to get the health benefits of this berry, it may not be ideal for all people. Most commercial cranberry juice cocktails contain only 27% to 33% pure cranberry juice, with sugar and water making up the rest of the volume. Such drinks are often high in calories. Encapsulated cranberry powders can be a convenient alternative to cranberry juice cocktail, especially for people who do not like the taste of cranberry; those who are traveling; or those concerned about the calories, sugar, or other sweeteners.

Healthcare providers directing their patients in selecting effective products should ensure that they find the most palatable product and should encourage long-term adherence. In the case of UTI prevention,

this strategy may reduce an individual's potential for the development of the disorder while also decreasing the need for antibiotics.

On the basis of the clinical studies, the amount of cranberry juice recommended for the prevention of UTIs is 1 to 10 oz a day; however, the ideal dosage has not yet been determined.[43,59,98] For use as a urinary deodorizer for incontinent patients, 3 to 6 oz (90–180 mL) per day of cranberry juice has been used.[63]

Tablets and capsules are often taken in doses of 500 mg once or twice a day, depending on the concentration of cranberry. Approximately 1500 g of fresh fruit produces 1 L of juice.[14,98]

ADVERSE EFFECTS

Taken orally, cranberry is generally well tolerated. No significant adverse effects have thus far been reported. However, cranberry juice at high doses of 3 to 4 L per day may cause gastrointestinal upset and diarrhea.[5]

Patients with diabetes should minimize cranberry juice sweetened with sugar or fruit juice.[98]

In theory, regular cranberry juice intake may precipitate the formation of urinary stones. However, urinary oxalate excretion does not rise after the drinking of cranberry juice, and no studies have reported stone formation as a complication.

Cranberry contains salicylic acid, which is structurally similar to acetylsalicylic acid (aspirin) and thus should be used cautiously in those with an allergy to aspirin.

DRUG INTERACTIONS

Cranberry contains flavonoids, which are known to inhibit cytochrome P450 enzymes; thus various drug interactions have been theorized.

One recent study investigated the effect of cranberry on the cytochrome P450 3A4 enzymes. Human CYP3A4 is the most important human CYP isozyme because it is involved in the metabolism of approximately 50% of drugs and some environmental toxins. Several brands of cranberry supplements were included in this study. The researchers found that cranberry had no significant inhibitory effect on human CYP3A4, and thus it is unlikely to cause significant interactions with drugs metabolized by CYP3A4, such as felodipine, nifedipine, midazolam, cyclosporine, and atorvastatin.

A few case reports have suggested an interaction between cranberry and warfarin, affecting international normalized ratio (INR) values and increasing the risk of bruising and bleeding. Warfarin is metabolized by the cytochrome P450 2C9 enzymes. These reports were poorly documented, and confounding factors may have been involved.[99–101]

Recently, several researchers have examined the potential interaction between cranberry and warfarin. Seven separate interaction studies assessed valid and accepted pharmacodynamic (PD) and/or pharmacokinetic end points, examining a total exposure of 75 patients and healthy volunteers. Six of these studies concluded that a cranberry juice–warfarin interaction is unlikely.[102–107] One study claimed a potential PD interaction on the basis of the assessment of an inappropriate and unconventional AUC-based PD parameter and the use of a single, very high dose (25 mg) of warfarin in healthy volunteers.[108]

A review of the body of research on the potential interaction between cranberry and warfarin concluded that there is no evidence of the risk of a clinically relevant interaction between warfarin and cranberry products from peer-reviewed interaction studies when cranberry juice is consumed in moderation. One cannot exclude the possibility of an interaction with the consumption of excessive quantities of cranberry products; however, it does not appear necessary to avoid normal levels of consumption of cranberry products (two 8-ounce glasses a day).[109]

Cranberry is often recommended along with low-dose oral antibiotics for prophylaxis for recurrent UTIs. One recent study evaluated the potential risk of pharmacokinetic interactions between cranberry juice and the β-lactams amoxicillin (amoxicillin) and cefaclor and determined that concurrent use of cranberry juice had no significant effect on the extent of oral absorption or the renal clearance of amoxicillin and cefaclor.[110]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Cunha BA. Strategies to control antibiotic resistance. *Semin Respir Infect*. 2002;17:250–258.
2. Carson CF, Riley TV. Non-antibiotic therapies for infectious diseases. *Commun Dis Intell*. 2003;27(suppl):S143–S146.
3. Chambers BK, Camire ME. Can cranberry supplementation benefit adults with type 2 diabetes? *Diabetes Care*. 2003;26:2695–2696.
4. Rogers J. Pass the cranberry juice. *Nurs Times*. 1991;87:36–37.
5. Jackson B, Hicks LE. Effect of cranberry juice on urinary pH in older adults. *Home Health Nurse*. 1997;15:198–202.
6. Tsukada K, Tokunaga K, Iwama T, et al. Cranberry juice and its impact on peristomal skin conditions for urostomy patients. *Ostomy Wound Manage*. 1994;40:60–62. 64,66–68.
7. Bomser J, Madhavi DL, Singletary K, et al. In vitro anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med*. 1996;62:212–216.
8. Swartz JH, Medrek TF. Antifungal properties of cranberry juice. *Appl Microbiol*. 1968;16:1524–1527.
9. Wilson T, Porcari JP, Harbin D. Cranberry extract inhibits low density lipoprotein oxidation. *Life Sci*. 1998;62:PL381–PL386.
10. Chen H, Zuo Y, Deng Y. Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. *J Chromatogr A*. 2001;913:387–395.
11. Flavonoids. *In The phytochemistry of herbs*. www.herbalchem.net. Accessed 6/4/04.
12. Manach C, Scalbert A, Morand C, et al. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79:727–747.
13. Coppola ED, Conrad EC, Cotter R. High pressure liquid chromatographic determination of major organic acids in cranberry juice. *J Assoc Off Anal Chem*. 1978;61:1490–1492.
14. Consumer Lab. *Natural products encyclopedia: cranberry monograph*. www.consumerlab.com. Accessed 6/4/04.
15. Swartz JH, Medrek TF. Antifungal properties of cranberry juice. *Appl Microbiol*. 1968;16:1524–1527.
16. Borukh IF, Kirbaba VI, Senchuk GV. Antimicrobial properties of cranberry. *Vopr Pitan*. 1972;31:82.
17. Ibragimov DI, Kazanskaia GB. Antimicrobial action of cranberry bush, common yarrow and *Achillea biebersteinii*. *Antibiotiki*. 1981;26:108–109.
18. Kinney AB, Blount M. Effect of cranberry juice on urinary pH. *Nurs Res*. 1979;28:287–290.
19. Kahn HD, Panariello VA, Saeli J, et al. Effect of cranberry juice on urine. *J Am Diet Assoc*. 1967;51:251–254.
20. Bodel PT, Cotran R, Kass EH. Cranberry juice and the antibacterial action of hippuric acid. *J Lab Clin Med*. 1959;54:881–888.
21. Nahata MC, Cummins BA, McLeod DC, et al. Effect of urinary acidifiers on formaldehyde concentration and efficacy with methenamine therapy. *Eur J Clin Pharmacol*. 1982;22:281–284.
22. Simpson GM, Khajawall AM. Urinary acidifiers in phencyclidine detoxification. *Hillside J Clin Psychiatry*. 1983;5:161–168.
23. Walsh BA. Urostomy and urinary pH. *J ET Nurs*. 1992;19:110–113.
24. Chernomordik AB, Vasilenko EG. Increase in novobiocin activity and an expansion of its antimicrobial action spectrum. *Antibiotiki*. 1981;26:456–460.
25. Jones AH. The next step in infectious disease: taming bacteria. *Med Hypotheses*. 2003;60:171–174.
26. Ofek I, Goldhar J, Zafiri D, et al. Anti-escherichia adhesin activity of cranberry and blueberry juices. *N Engl J Med*. 1991;324(22):1599.
27. Howell AB, Vorsa N, Der Marderosian A, et al. Inhibition of adherence of P-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. *N Engl J Med*. 1998;339:1085–1086.
28. Sobota AE. Inhibition of bacterial adherence by cranberry juice: potential use for the treatment of urinary tract infections. *J Urol*. 1984;131:1013–1016.
29. Zafriri D, Ofek I, Adar R, et al. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. *Antimicrob Agents Chemother*. 1989;33:92–98.
30. Ahuja S, Kaack B, Roberts J. Loss of fimbrial adhesion with the addition of Vaccinum macrocarpon to the growth medium of P-fimbriated *Escherichia coli*. *J Urol*. 1998;159:559–562.
31. Foo LY, Lu Y, Howell AB, et al. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated Escherichia coli in vitro. *Phytochemistry*. 2000;54:173–181.
32. Foo LY, Lu Y, Howell AB, et al. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. *J Nat Prod*. 2000;63:1225–1228.
33. Reid G. The role of cranberry and probiotics in intestinal and urogenital tract health. *Crit Rev Food Sci Nutr*. 2002;42(suppl):293–300.
34. Sobota AE. Inhibition of bacterial adherence by cranberry juice: potential use for the treatment of urinary tract infections. *J Urol*. 1984;131:1013–1016.
35. Schmidt DR, Sobota AE. An examination of the anti-adherence activity of cranberry juice on urinary and nonurinary bacterial isolates. *Microbios*. 1988;55:173–181.
36. Habash MB, Van der Mei HC, Busscher HJ, et al. The effect of water, ascorbic acid, and cranberry derived supplementation on human urine and uropathogen adhesion to silicone rubber. *Can J Microbiol*. 1999;45:691–694.
37. Sharon N, Ofek I. Fighting infectious diseases with inhibitors of microbial adhesion to host tissues. *Crit Rev Food Sci Nutr*. 2002;42(suppl):267–272.
38. Peron G, Pellizzaro A, Brun P, et al. Antiadhesive Activity and Metabolomics Analysis of Rat urine after cranberry (*Vaccinium macrocarpon Aiton*) administration. *J Agric Food Chem*. 65(28):5657–5667.
39. Howell AB, Leahy M, Kurowska E, et al. In vivo evidence that cranberry proanthocyanidins inhibit adherence of p-fimbriated E. coli bacteria to uroepithelial cells. *FASEB J*. 2001;15:A284.
40. Kontiokari T, Sundqvist K, Nuutinen M, et al. Randomised trial of cranberry-lingonberry juice and *Lactobacillus* GG drink for the prevention of urinary tract infections in women. *British Med J*. 2001;322:1571–1573.
41. Feliciano RP, Mills CE, Istas G, et al. Absorption, metabolism and excretion of cranberry (Poly)Phenols in humans: a dose response study and assessment of Inter-individual Variability. *Nutrients*. 2017;9(3):pii: E268.
42. Gormley EA. Recurrent urinary tract infection in women: emerging concepts regarding etiology and treatment considerations. *Curr Urol Rep*. 2003;4:399–403.
43. Avorn J, Monane M, Gurwitz JH, et al. Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *JAMA*. 1994;271:751–754.
44. Nicolle LE, Mayhew WJ, Bryan L. Prospective randomized comparison of therapy and no therapy for asymptomatic bacteriuria in institutionalized elderly women. *Am J Med*. 1987;83:27–33.
45. High KP. Nutritional strategies to boost immunity and prevent infection in elderly individuals. *Clin Infect Dis*. 2001;33:1892–1900.
46. Fleet JC. New support for a folk remedy: cranberry juice reduces bacteriuria and pyuria in elderly women. *Nutr Rev*. 1994;52:168–170.
47. Foxman B, Geiger AM, Palin K, et al. First-time urinary tract infection and sexual behavior. *Epidemiology*. 1995;6:162–168.
48. Bohbot JM. Results of a randomized, double-blind study on the prevention of recurrent cystitis with GynDelta. *Gynaecologist's Obstetrician's J*. 2007. January, Special Issue.
49. Howell AB, Foxman B. Cranberry juice and adhesion of antibiotic-resistant uropathogens. *JAMA*. 2002;287:3082–3083.
50. Walsh N. Growing evidence: cranberry juice tied to lower UTI risk—NIH undertakes large study. *Ob Gyn News*. 2003;38:18.
51. Schlager TA, Anderson S, Trudell J, et al. Effect of cranberry juice on bacteriuria in children with neurogenic bladder receiving intermittent catheterization. *J Pediatr*. 1999;153:698–702.
52. Reid G, Hsiehl J, Potter P, et al. Cranberry juice consumption may reduce biofilms on uroepithelial cells: pilot study in spinal cord injured patients. *Spinal Cord*. 2001;39:26–30.
53. Linsenmeyer TA, Harrison B, Oakley A, et al. Evaluation of cranberry supplement for reduction of urinary tract infections in individuals with neurogenic bladders secondary to spinal cord injury: a prospective, double-blinded, placebo-controlled, crossover study. *J Spinal Cord Med*. 2004;27:29–34.
54. Hess MJ, Hess PE, Sullivan MR, et al. Evaluation of cranberry tablets for the prevention of urinary tract infections in spinal cord injured patients with neurogenic bladder. *Spinal Cord*. 2008;46(9):622–626.

55. Kontiokari T, Sundqvist K, Nuutinen M, et al. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. *BMJ*. 2001;322:1571.
56. McMurdo M, Argo I, Phillips G, et al. Cranberry or trimethoprim for the prevention of recurrent urinary tract infections? A randomized controlled trial in older women. *J Antimicrob Chemother*. 2009;63(2):389–395.
57. Beerepoot MAJ, ter Riet G, Nys S, et al. Cranberries vs antibiotics to prevent urinary tract infections. *Arch Intern Med*. 2011;171(14):1270–1278.
58. Stothers L. A randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women. *Can J Urol*. 2002;9:1558–1562.
59. Ledda A, Belcaro G, Dugall M, et al. Highly standardized cranberry extract supplementation (Anthocran®) as prophylaxis in young healthy subjects with recurrent urinary tract infections. *Eur Rev Med Pharmacol Sci*. 2017;21(2):389–393.
60. Jepson RG, Craig JC. Cranberries for preventing urinary tract infections. *Cochrane Database Syst Rev*. 2008;1:CD001321.
61. Luis A, Domingues F, Pereira L. Can Cranberries Contribute to reduce the incidence of urinary tract infections? A Systematic review with Meta-Analysis and trial Sequential Analysis of clinical trials. *J Urol*. https://doi.org/10.1016/j.juro.2017.03.078.
62. Sengupta K, Alluri KV, Golakoti, et al. A randomized, double blind, controlled, dose dependent clinical trial to evaluate the efficacy of a proanthocyanidin standardized whole cranberry (*Vaccinium macrocarpon*) powder on infections of the urinary tract. *Curr Bioact Compounds*. 2011;7:39–46.
63. Narayansingh R, Hurta RAR. Cranberry extract and quercetin modulate the expression of cyclooxygenase-2 (COX-2) and IkBa in human colon cancer cells. *J Sci Food Agric*. 2008;89:542–547.
64. Neto CC, Amoroso JW, Liberty AM. Anticancer activities of cranberry phytochemicals: an update. *Mol Nutr Food Res*. 2008;52:S18–S27.
65. Vidlar A, Vostalova J, Ulrichova J, et al. The effectiveness of dried cranberries (*Vaccinium macrocarpon*) in men with lower urinary tract symptoms. *Brit J Nutr*. 2010;104:1181–1189.
66. Botto H, Neuzillet Y. Effectiveness of a cranberry (*Vaccinium macrocarpon*) preparation in reducing asymptomatic bacteriuria in patients with an ileal enterocystoplasty. *Scand J Urol Nephrol*. 2010;44(3):165–168.
67. Kraemer RJ. Cranberry Juice and the reduction of ammoniacal odor of urine. *Southwest Med*. 1964;45:211–212.
68. DuGan CR, Cardaciotto PS. Reduction of ammoniacal urinary odors by the sustained feeding of cranberry juice. *J Psychiatr Nurs*. 1966;8:467–470.
69. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J Agric Food Chem*. 2000;48:5677–5684.
70. Kahkonen MP, Hopia AI, Heinonen M. Berry phenolics and their antioxidant activity. *J Agric Food Chem*. 2001;49:4076–4082.
71. Zheng W, Wang SY. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem*. 2003;51:502–509.
72. Pedersen CB, Kyle J, Jenkinson AM, et al. Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers. *Eur J Clin Nutr*. 2000;54:405–408.
73. Duthie GG, Kyle J, McE A, et al. Increased salicylate concentrations in urine of human volunteers after consumption of cranberry juice. *J Agric Food Chem*. 2005;53:2897–2900.
74. Roy S, Khanna S, Alessio HM, et al. Anti-angiogenic property of edible berries. *Free Radic Res*. 2002;36:1023–1031.
75. Guthrie N. Effect of cranberry juice and products on human breast cancer cell growth (abstract 531.13). *FASEB J*. 2000;14.
76. Ferguson PJ, Kurowska E, Freeman DJ, et al. A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. *J Nutr*. 2004;134:1529–1535.
77. Bomser J, Madhavi DL, Singletary K, et al. In vitro anticancer activity of fruit extracts from Vaccinium species. *Planta Med*. 1996;62:212–216.
78. Murphy BT, MacKinnon SL, Yan X, et al. Identification of triterpene hydroxycinnamates with in vitro antitumor activity from whole cranberry fruit (*Vaccinium macrocarpon*). *J Agric Food Chem*. 2003;51:3541–3545.
79. Seeram NP, Adams LS, Hardy ML, et al. Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *J Agric Food Chem*. 2004;52:2512–2517.
80. Bagchi D, Sen CK, Bagchi M, et al. Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. *Biochemistry (Mosc)*. 2004;69:75–80.
81. Yan X, Murphy BT, Hammond GB, et al. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J Agric Food Chem*. 2002;50:5844–5849.
82. Wilson T, Porcari JP, Harbin D. Cranberry extract inhibits low density lipoprotein oxidation. *Life Sci*. 1998;62:PL381–PL386.
83. Reed J. Cranberry flavonoids, atherosclerosis and cardiovascular health. *Crit Rev Food Sci Nutr*. 2002;42(suppl):301–316.
84. Youdim KA, McDonald J, Kalt W, et al. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J Nutr Biochem*. 2002;13:282–288.
85. Ruel G, Pomerleau S, Couture P, et al. Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. *Br J Nutr*. 2008;99:352–359.
86. Hanhineva K, Torronen R, Bondia-Pons I, et al. Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci*. 2010;11:1365–1402.
87. Xiao JB, Hogger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. *Curr. Med. Chem*. 2015;22:23–38.
88. Paquette M, Medina Larqué AS, Weisnagel SJ, et al. Strawberry and cranberry polyphenols improve insulin sensitivity in insulin-resistant, Non-Diabetic adults: a parallel, double-blind, controlled and Randomised clinical trial. *Br J Nutr*. 117(4):519–531.
89. Burger O, Weiss E, Sharon N, et al. Inhibition of Helicobacter pylori adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. *Crit Rev Food Sci Nutr*. 2002;42(suppl):279–284.
90. Weiss EI, Lev-Dor R, Kashamn Y, et al. Inhibiting interspecies coaggregation of plaque bacteria with a cranberry juice constituent [errata J Am Dent Assoc 1999;130:36 and 1999;130:332]. *J Am Dent Assoc*. 1998;129:1719–1723.
91. Weiss EL, Lev-Dor R, Sharon N, et al. Inhibitory effect of a high-molecular-weight constituent of cranberry on adhesion of oral bacteria. *Crit Rev Food Sci Nutr*. 2002;42(suppl):285–292.
92. Weiss EI, Kozlovsky A, Steinberg D, et al. A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits in vitro adhesion to hydroxyapatite. *FEMS Microbiol Lett*. 2004;232:89–92.
93. Yamanaka A, Kimizuka R, Kato T, et al. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. *Oral Microbiol Immunol*. 2004;19:150–154.
94. Steinberg D, Feldman M, Ofek I, et al. Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm. *J Antimicrob Chemother*. 2004;54:86–89.
95. Neto CC, Penndorf KA, Feldman M, et al. Characterization of Non-Dialyzable constituents from cranberry juice that inhibit adhesion, Co-aggregation and biofilm formation by oral bacteria. *Food Function*. 1965;8(5):1955.
96. Light I, Gursel E, Zinnser HH. Urinary ionized calcium in urolithiasis: effect of cranberry juice. *Urology*. 1973;1:67–70.
97. McHarg T, Rodgers A, Charlton K. Influence of cranberry juice on the urinary risk factors for calcium oxalate kidney stone formation. *BJU Int*. 2003;92:765–768.
98. Wanwimolruk S, Prachayasittikul A, Prachayasittikul V, et al. Effect of cranberry dietary supplements with different brands on human CYP3A4 enzyme. *EXCLI J*. 2012;11:108–115.
99. Hodek P, Trefil P, Stiborova M. Flavonoids: potent and versatile biologically active compounds interacting with cytochromes P450. *Chem Biol Interact*. 2002;139:1–21.
100. Rettie AE, Korzekwa KR, Kunze KL, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. *Chem Res Toxicol*. 1992;5:54–59.
101. Grant P. Warfarin and cranberry juice: an interaction? *J Heart Valve Dis*. 2004;13:25–26.

102. Li Z, Seeram NP, Carpenter CL, et al. Cranberry does not affect prothrombin time in male subjects on warfarin. *J Amer Diet Assoc*. 2006;106(12):2057–2061.
103. Lilja JJ, Backman JT, Neuvonen PJ. Effects of daily ingestion of cranberry juice on the pharmacokinetics of warfarin, tizanidine, and midazolam probes of CYP2C9, CYP1A2, and CYP3A4. *Clin Pharmacol Ther*. 2007;81(6):833–839.
104. Mellen CK, Ford M, Rindone JP. Effect of high dose cranberry juice on the pharmacodynamics of warfarin in patients. *Br J Clin Pharmacol*. 2010;70(1):139–142.
105. Greenblatt DJ, von Moltke LL, Perloff ES, et al. Interaction of flurbiprofen with cranberry juice, grape juice, tea and fluconazole: in vitro and clinical studies. *Clin Pharmacol Ther*. 2006;79:125–133.
106. Ansell J, McDonough M, Zhao Y, et al. The absence of an interaction between warfarin and cranberry juice: a randomized double blind trial. *J Clin Pharmacol*. 2009;49:824–830.
107. Ushijima K, Tsuruoka S, Tsuda H, et al. Cranberry juice suppressed the diclofenac metabolism by human liver microsomes, but not in healthy human subjects. *Br J Clin Pharmacol*. 2009;68(2):194–200.
108. Abdul MI, Jiang X, Williams KM, et al. Pharmacodynamic interaction of warfarin with cranberry juice but not garlic in healthy subjects. *Br J Pharmacol*. 2008;154:1691–1700.
109. Ansell J. Cranberry juice is safe to consume with warfarin. *The Anticoagulation Forum*. Spring 2011. Special Edition.
110. Li M, Andrew MA, Wang J, et al. Effects of cranberry juice on pharmacokinetics of β-Lactam antibiotics following oral administration. *Antimicrob Agents Chemother*. 2009;53(7):2725–2732.

Vaccinium myrtillus (Bilberry)

Michael T. Murray, ND

OUTLINE

Vaccinium myrtillus (family: *Ericaceae*)
Common names: bilberry, huckleberry, European blueberry, whortleberry, blueberry

GENERAL DESCRIPTION

The genus *Vaccinium* in the family *Ericaceae* comprises nearly 200 species, most of which are found in the northern hemisphere. This chapter focuses on *Vaccinium myrtillus* and the medicinal use of extracts of its fruit.

V. myrtillus, or bilberry, is a shrubby perennial plant that grows in the sandy areas of the northern United States and in the woods and forest meadows of Europe (Fig. 122.1). The angular, green, branched stem grows from a creeping rootstock to a height of 1 to 1.5 ft. The 0.5- to 1-in-long leaves are oval, slightly dentate, and bright green, whereas the flowers are reddish- or greenish-pink and bell-shaped. The bilberry's flowering season is April through June. The fruit is a blue-black berry.[1]

CHEMICAL COMPOSITION

The pharmacologically active constituents of bilberries are its flavonoid components, specifically its anthocyanosides. An anthocyanoside is composed of an aglycone (e.g., anthocyanidin) bound to one of three glycosides (arabinoside, glucoside, or galactoside). More than 15 different anthocyanosides originate from the five aglycones found in *V. myrtillus*.[2] Other members of the genus *Vaccinium,* as well as *Ribes nigrum* (black currant) and *Vitis vinifera* (grape), contain similar anthocyanosides.[3] Extracts of these fruits are also used for medicinal purposes in Europe.

The concentration of anthocyanosides in the fresh fruit is approximately 0.1% to 0.25%, whereas concentrated extracts of *V. myrtillus* are produced that yield an anthocyanidin content of 25%.[2] An extract with an anthocyanidin content of 25% contains about 37% anthocyanosides owing to the conjugation of the anthocyanidin with a glycoside. (For analytic purposes, the anthocyanosides content should always be expressed in terms of anthocyanidin.) Only very small amounts of free anthocyanidins exist in nature and in *V. myrtillus* extracts.

Bilberries are also good sources of bioavailable resveratrol[4] and quercetin,[5] although the levels of these compounds are lower than those found in other foods (e.g., the level of resveratrol in bilberry is less than 10% of that reported for grapes). Nonetheless, consumption of bilberries may lead to significant elevations of these compounds in the serum because they are more bioavailable. In one study, 40 healthy men consumed 100 g/day of berries (black currants, lingonberries, and bilberries) for 8 weeks.[5] Twenty subjects consuming their habitual diets served as controls. Blood levels of quercetin were obtained at baseline and at 2, 4, and 8 weeks. During the berry-consumption period, mean serum concentrations of quercetin ranged between 21.4 and 25.3 mg/L in the berry group, which translated into 32% to 51% higher than in the control group—indicating that the berries used in this study were a good source of bioavailable quercetin.

HISTORY AND FOLK USE

Bilberries have, of course, been used as food and for their high nutritive value. Medicinally, they have been used in the treatment of scurvy and urinary complaints (including infection and stones).[1] The dried berries have been used primarily for their astringent qualities in the treatment of diarrhea and dysentery. Decoctions of the leaves have been used in the treatment of diabetes.[1]

PHARMACOLOGY

The pharmacology of *V. myrtillus* is discussed almost entirely in relation to its anthocyanoside content because research has focused primarily on the anthocyanosides.

Collagen-Stabilizing Action

Anthocyanosides possess significant collagen-stabilizing action.[6–12] Collagen, the most abundant protein of the body, is responsible for maintaining the integrity of "ground substance" as well as tendons,

ligaments, and cartilage. Collagen is destroyed during the inflammatory processes that occur in rheumatoid arthritis, periodontal disease, and other inflammatory conditions involving bones, joints, cartilage, and other connective tissue.

Anthocyanidins, proanthocyanidins, and other flavonoids are remarkable in their ability to prevent collagen destruction. The anthocyanidins in *V. myrtillus* extracts have been shown to affect collagen metabolism in the following ways:

- Anthocyanosides cross-link collagen fibers, resulting in strengthening of the natural cross-linking of the collagen that forms the collagen matrix of connective tissue (ground substance, cartilage, tendon, etc.).[6–10]
- Anthocyanosides, with their potent antioxidant and free-radical–scavenging action, prevent free-radical damage.[6–9,11,60]
- Anthocyanosides inhibit enzymatic cleavage of collagen by enzymes secreted by leukocytes during inflammation.[6–8,10–12]
- Anthocyanosides and other flavonoid components of *V. myrtillus* prevent the release and synthesis of compounds that promote inflammation, such as histamine, serine proteases, prostaglandins, and leukotrienes.[6–8,13,14]
- Anthocyanosides promote mucopolysaccharide and collagen biosynthesis and stimulate reticulation of collagen fibrils.[15–17]

Normalization of Capillary Permeability

Anthocyanosides have strong "vitamin P" activity.[6] Included in their effects is the ability to raise intracellular vitamin C levels and reduce capillary permeability and fragility.[6–8] Their effect in reducing capillary fragility and permeability is roughly twice that of rutin in both intensity and duration of action.[18]

Fig. 122.1 *Vaccinium myrtillus.* (silkfactory/iStock.com)

V. myrtillus extracts have been widely used in Europe in the treatment of various arterial, venous, and capillary disorders. Clinical studies have demonstrated a positive effect in the treatment of the following conditions[19–24]:

- Capillary fragility
- Blood purpuras
- Various encephalic circulation disturbances (similar to *Ginkgo biloba*)
- Venous insufficiency
- Varicose veins
- Microscopical hematuria caused by diffuse and kidney capillary fragility

V. myrtillus's efficacy in the treatment of a variety of venous disorders relates to the ability of anthocyanosides to protect altered veins (postphlebitic veins as well as varicose veins) via two mechanisms[17]: (1) increasing the endothelial barrier effect by stabilizing the membrane phospholipids and (2) increasing the biosynthesis of the acid mucopolysaccharides of the connective ground substance, thus restoring the altered mucopolysaccharide pericapillary sheath. This latter effect leads to a marked increase in newly formed capillaries and collagen fibrils.

One interesting effect of the normalization of collagen structures and capillaries is the demonstration that anthocyanosides from *V. myrtillus* reduce the permeability of the blood–brain barrier.[10,25] Greater blood–brain permeability has been linked to autoimmune diseases of the central nervous system (CNS), schizophrenia, "cerebral allergies," and a variety of other CNS disorders. Presumably, the anthocyanosides inhibit both enzymatic and nonenzymatic degradation of the basement membrane collagen of brain capillaries, thus helping maintain or restore the brain's protection from drugs, pollutants, naturally occurring degradation products, and other cerebral toxins.[9–12,25]

Another study further demonstrated the remarkable efficacy of bilberry in protecting and strengthening the capillaries and microcirculation. In this study, the effects of anthocyanidins on hamster cheek microcirculation after ischemia and reperfusion were investigated. The treated group had diminished adherence of leukocytes to the venules after reperfusion, resulting in prevention of the markedly increased capillary permeability seen in the placebo group.[26]

Antiaggregation Effect on Platelets

Anthocyanosides, like many other flavonoids, have been shown to have significant antiaggregation effects on platelets.[27–29] Their action in vivo appears to be direct antiaggregation effects on the platelets and an indirect effect via prostacyclin-like action.[27–29] Prostacyclin is a potent stimulator of adenyl cyclase, the enzyme that catalyzes the production of cyclic adenosine monophosphate from adenosine triphosphate. Cyclic adenosine monophosphate prevents platelets from aggregating and adhering to the endothelial surface.

Smooth Muscle–Relaxing Activity

Anthocyanoside extracts have demonstrated significant vascular smooth muscle–relaxing effects in a variety of experimental models.[30–32] The clinical application of this research may be in the treatment of dysmenorrhea, for which a preliminary study demonstrated positive effects.[33]

Effects on Cancer Cells

An in vitro study demonstrated that among ethanol extracts of 10 edible berries, bilberry extract was the most effective at inhibiting the growth of human leukemia cells and colon carcinoma cells.[34] Bilberry extract was shown to possess dual action in this regard through the induction of apoptosis (programmed cell death) and promotion of nucleosomal DNA fragmentation (Fig. 122.2). Bilberry anthocyanidins have also been shown to inhibit angiogenesis.[35] Bilberry extract was the most potent of the extracts tested because it contained the largest amounts of phenolic compounds, including anthocyanins, and showed the greatest free-radical–scavenging activity. Pure delphinidin and malvidin, like the glycosides isolated from the bilberry extract, induced apoptosis in the leukemia cells. However, only pure delphinidin and the glycoside isolated from the bilberry extract, but not malvidin and the glycoside, inhibited the growth of human colon cancer cells. Twenty-five patients with colorectal cancer scheduled to undergo resection of primary tumors or liver metastases received 1.4, 2.8, or 5.6 g

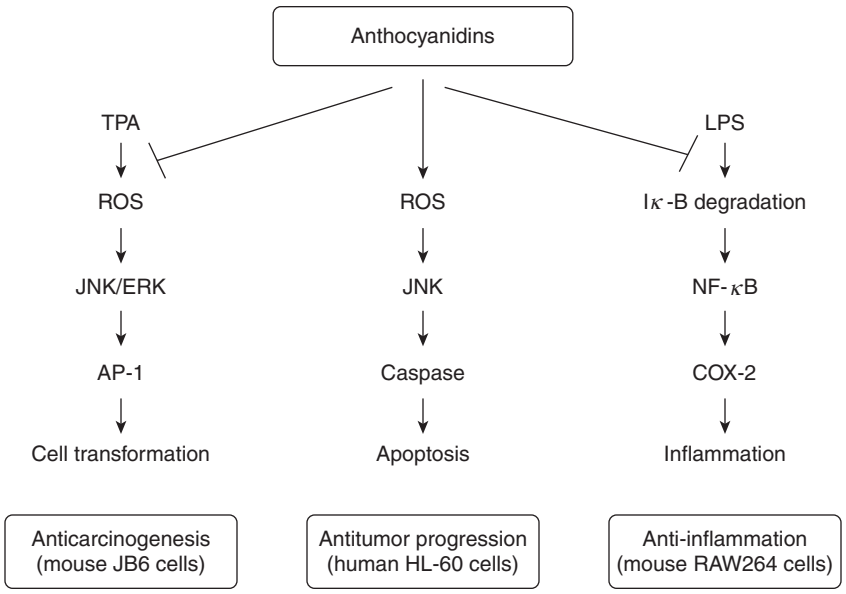


Fig. 122.2 A schematic molecular view of cancer chemoprevention by anthocyanidins. Anthocyanidins may contribute to cancer chemoprevention by targeting three different signal transduction pathways and downstream genes. *AP-1,* activator protein-1; *ERK,* extracellular signal-regulated kinase; *JNK,* c-Jun NH2-terminal kinase; *LPS,* lipopolysaccharide; *NF-κB,* nuclear factor κB; *ROS,* reactive oxygen species; *TPA,*12-O-tetradecanoylphorbol-13-acetate. (From Hou DX, Fujii M, Terahara N, Yoshimoto M. Molecular mechanisms behind the chemopreventive effects of anthocyanidins. *J Biomed Biotech.* 2004[5]:321–325. PubMed PMID: 15577196.)

(containing 0.5–2.0 g of anthocyanins) daily for 7 days before surgery. In tumor tissue from all patients, proliferation was decreased by 7% compared with preintervention values.[36]

CLINICAL APPLICATIONS

The primary clinical application of bilberry extracts has been in the prevention and treatment of a diverse group of disorders of the eye and vision. However, the same mechanisms of action that benefit the eye are of value for other health problems, especially those involving capillary dysfunction or inflammation.

Ophthalmological Applications

Night Vision and Eye Fatigue

Perhaps the most significant clinical applications for *V. myrtillus* extracts are in the field of ophthalmology. Interest in *V. myrtillus* anthocyanosides was first aroused when it was observed that the administration of bilberry extracts to healthy subjects resulted in improved nighttime visual acuity, quicker adjustment to darkness, and faster restoration of visual acuity after exposure to glare.[37,38] Further studies confirmed these results,[39–42] which were most impressive in individuals with pigmentary retinitis and hemeralopia. (*Hemeralopia* refers to "day blindness," or an inability to see as distinctly in bright light as in dim light.)

It appears that in addition to their effect on capillaries, *V. myrtillus* anthocyanosides have an affinity for the pigmented epithelium of the retina, which makes up the optical or functional part of the retina.[43] This feature is consistent with several of the clinical effects observed.

Anthocyanoside extracts of *V. myrtillus* appear to be of great value in both poor night vision and poor day vision. In a double-blind study in patients with eye strain and clinical symptoms of low to moderate myopia, 30 subjects were given either a purified high-dose anthocyanoside oligomer (a 100-mg tablet comprising 85% anthocyanoside oligomer) or a placebo tablet twice daily for 4 weeks. Before the treatment, the placebo and anthocyanoside groups were similar in terms of age and contrast sensitivity. Before and after treatment, subjects completed a questionnaire to determine their clinical symptoms and were also assessed for nocturnal visual function using contrast sensitivity testing. Questionnaire data analysis showed that after treatment, 22 (73.3%) anthocyanoside subjects showed improved symptoms, whereas only 1 placebo subject showed an improvement. Contrast sensitivity levels significantly improved in the anthocyanoside group and remained stable in the placebo group. The mean change in contrast sensitivity in the anthocyanoside group was 2.41, compared with −0.66 decibels (dB) for the placebo group. At all cycle-per-degree levels, there were greater improvements in contrast sensitivity in the anthocyanoside group than in the placebo group. This very detailed clinical assessment demonstrated that the administration of anthocyanoside oligomer improves both subjective symptoms and objective contrast sensitivity in myopia subjects with asthenopia.[44]

One of the more practical applications of the beneficial effects of anthocyanosides and eye health is reducing eye fatigue from looking at a computer screen. In one double-blind trial, 88 office workers aged 20 to 40 years who used computer terminals and displayed low critical flicker fusion (CFF) and near-point accommodation (NPA) were randomized to either a bilberry extract (480 mg/day) or placebo (vehicle) group for 8 weeks. The reduction in CFF was alleviated after 8 weeks of bilberry extract supplementation in contrast to placebo supplementation, whereas NPA variation was not. Of the subjective symptoms of eye fatigue, computer screen load–induced ocular fatigue sensation, ocular pain, eye heaviness, uncomfortable sensation, and foreign-body sensation were mitigated more in the bilberry extract group than in the control group.[45]

Glaucoma

V. myrtillus may play a significant role in the prevention and treatment of glaucoma via its effect on ocular collagen structures. In the eye,

collagen provides tensile strength and integrity to the tissues—cornea, sclera, lamina cribrosa, trabecular meshwork, and vitreous.

Morphological changes in the collagen of the eye precede clinically detectable abnormalities. These changes may result in elevated intraocular pressure (IOP) readings or, perhaps more significantly, the progression of peripheral vision loss. Changes in collagen structure would explain the following observations:

- Similar peripheral vision loss in patients with normal and elevated IOP
- Cupping of the optic disc even at low IOP levels
- No apparent anatomical reason for decreased aqueous outflow (see Chapter 175 for complete discussion and references)

Therefore primary prevention of glaucoma involves maintaining the integrity of the ground substance and collagen framework. It appears that preventing the breakdown of the collagen matrix is as important here as it is in other conditions involving collagen abnormalities, such as atherosclerosis, rheumatoid arthritis, and periodontal disease.

Consumption of *V. myrtillus* may offer significant protection against the development of glaucoma because of its collagen-enhancing actions. In addition, anthocyanosides may be of benefit in the treatment of chronic glaucoma because rutin has been demonstrated to lower IOP when used as an adjunct in patients who are unresponsive to miotics alone.[46] *V. myrtillus* anthocyanosides are generally much more biologically active than rutin.[18]

Cataracts and Retinal Degeneration

V. myrtillus anthocyanosides may offer significant protection against the development of retinal (macular) degeneration and cataracts, particularly diabetic retinopathy and cataracts.[47] Both the rate of retinal degeneration and the occurrence of cataracts in rats can be retarded by changing their diet from a commercial laboratory chow to a "well-defined diet."[48,49] Preliminary research suggests that flavonoid components in the well-defined diet may be responsible for the protective effects against cataracts and retinal degeneration.[50] Limited research has shown that when combined with vitamin E, bilberry significantly slows the progression of senile cataracts in humans.[51]

V. myrtillus anthocyanoside extracts are widely used in Europe for the prevention of diabetic retinopathy.[51–53] The positive effects noted in clinical trials may be due to improved capillary integrity as well as inhibition of sorbitol production (see Chapter 165). Flavonoids have been shown to be potent in vitro and in vivo inhibitors of sorbitol accumulation. In laboratory experiments, they have been found capable of inhibiting the development of diabetic cataracts.[54–56]

Other Clinical Applications

Diabetes Mellitus

A decoction of bilberry leaves has a long history of folk use in the treatment of diabetes. This use is supported by research, which has shown that oral administration reduces hyperglycemia in normal and depancreatized dogs even when glucose is concurrently injected intravenously.[53,57]

Bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of adenosine monophosphate (AMP)–activated protein kinase.[58] The anthocyanoside myrtillin (3-glucoside of delphinidin) is apparently the most active hypoglycemic component of *V. myrtillus.* Upon injection, it is somewhat weaker than insulin, but it is also less toxic, even at 50 times the therapeutic dose of 1 g/day. It is of interest to note that a single dose can have beneficial effects lasting for several weeks.[53]

However, the most important benefits from the use of anthocyanosides in the treatment of diabetes relate to their ability to improve collagen integrity and capillary permeability. Benefit also possibly derives from their ability to inhibit sorbitol accumulation, thus providing protection from the serious vascular and neurological sequelae of diabetes.

V. myrtillus anthocyanosides have also been shown to have a protective effect on capillary fragility in diabetes and to reduce serum cholesterol and triglyceride levels in primary dyslipidemia.[59]

In animal studies, anthocyanosides significantly decrease the intimal proliferation, extracellular matrix production, and calcium and lipid deposition found in the aorta of untreated atherosclerotic rabbits. Presumably, this decrease is a result of greater collagen cross-linking, which thus diminishes the permeability in small and large blood vessels.[60]

Atherosclerosis and Metabolic Syndrome

Consumption of bilberries (like blueberries) or anthocyanoside extracts has been shown to protect against atherosclerosis through a number of mechanisms, including by decreasing total cholesterol, glucose, C-reactive protein, and platelet aggregation.[61–64] The ability to reduce C-reactive protein and other markers of low-grade inflammation was confirmed in patients with metabolic syndrome.[63]

Another very novel effect noted in the protection against vascular disease is improved endothelial function. Individuals given oral anthocyanins (320 mg) for 12 weeks showed significant improvements in brachial artery flow-mediated dilation (FMD) and cyclic guanosine monophosphate (cGMP).[65] Vascular dilation is directly stimulated by nitric oxide (NO) released from the vascular endothelial cells. The vasodilator activity of NO is attributed to its diffusion to the vascular smooth muscle cells and the production of cGMP and cGMP-mediated vasodilation. Because the half-life of NO is extremely short, the circulating cGMP concentration is used as the index of NO activity and thus as an indirect marker of endothelium-dependent vasodilation. Compared with the control group, the treatment group showed significant increases in FMD (28.4% vs. 2.2%), cGMP (12.6% vs. −1.2%), and high-density lipoprotein (HDL)-cholesterol concentrations but decreases in the serum soluble vascular adhesion molecule-1 and low-density lipoprotein (LDL)-cholesterol levels. Another piece of evidence indicating that anthocyanins increase NO vasodilation is that in the presence of inhibitors of NO-cGMP vasodilators, the effects of anthocyanin on endothelial function were not seen.

Inflammatory Joint Disease

The effects of anthocyanosides on collagen structures and their potent antioxidant activity make *V. myrtillus* anthocyanoside extracts very useful in the treatment of a wide variety of inflammatory conditions, most notably rheumatoid arthritis. Bioflavonoids have been found to increase collagen synthesis and inhibit collagen catabolism in rats with adjuvant-induced arthritis (a chronic progressive polyarthritis with some similarities to rheumatoid arthritis).[15]

Bilberries, like cherries, are particularly indicated in the treatment of gout because their flavonoid components can reduce both uric acid levels and tissue destruction (see Chapter 191).

Inflammatory Bowel Disease

Bilberries or anthocyanin-rich extracts may have benefits in patients with inflammatory bowel disease. In an open pilot trial with a total follow-up of 9 weeks, the effect of a daily standardized anthocyanin-rich bilberry extract was tested in 13 patients with mild to moderate ulcerative colitis (UC).[66] At the end of the 6-week treatment interval, 63.4% of patients achieved remission, and 90.9% of patients showed a response. In all patients, a decrease in total Mayo score was detected (mean: 6.5 and 3.6 at screening and week 7, respectively; $p < 0.001$).

Levels of fecal calprotectin, a marker of intestinal inflammation, significantly decreased during the treatment phase (baseline: mean 778 µg/g; end of treatment: mean 305 µg/g), including four patients achieving undetectable levels at the end of treatment. Bilberry anthocyanins inhibit interferon-induced signaling and downstream effects in human macrophages cells as well as modulate intestinal cytokine activity.[67]

Microscopical Hematuria

The effect of *V. myrtillus* in the reduction of microscopical hematuria may reflect its tissue distribution. Pharmacokinetic studies in rats have demonstrated an affinity for the skin and kidneys.[68] Anthocyanosides' affinity for these tissues reflects the high concentration of collagen and mucopolysaccharides in the skin and kidneys and the fact that they are excreted via the kidneys.

Other Effects

As mentioned previously, the smooth muscle–relaxing effects of bilberry extract have been put to good use in the treatment of dysmenorrhea.[33] A significant improvement in pelvic-lumbosacral pain, mammary tension, nausea, and lower-limb heaviness was noted when women with dysmenorrhea were given bilberry extract (115 mg anthocyanosides per day) for 3 days before and during menstruation.

Bilberry extracts have demonstrated antiulcer effects in various experimental models and have been promoted as an ulcer medication in Italy.[69]

Summary of Clinical Applications

V. myrtillus anthocyanosides exhibit significant pharmacological activity, particularly on collagen structures. Research has demonstrated a positive effect in the treatment of the following problems:

- Capillary fragility
- Blood purpuras
- Various encephalic circulation disturbances
- Venous insufficiency
- Varicose veins
- Microscopical hematuria caused by diffuse and kidney capillary fragility
- Poor night vision
- Hemeralopia
- Diabetic retinopathy

Experimental studies indicate that anthocyanosides should also be useful in most inflammatory or degenerative conditions involving connective tissues (e.g., osteoarthritis, gout, rheumatoid arthritis, periodontal disease) as well as in glaucoma, diabetes, cataracts, retinal degeneration, and schizophrenia.

DOSAGE

The standard dose for *V. myrtillus* is based on its anthocyanoside content, as calculated from its anthocyanidin percentage. Widely used pharmaceutical preparations in Europe are typically standardized for a 25% anthocyanidin content. Dosages are as follows:

- Anthocyanosides (calculated as anthocyanidin): 20 to 40 mg three times a day
- *V. myrtillus* extract (25% anthocyanidin content): 80 to 160 mg three times a day
- Fresh berries: 55 to 115 g three times a day

TOXICOLOGY

Extensive toxicological investigation has demonstrated that *V. myrtillus* anthocyanoside extracts are devoid of toxic effects. Administration to rats of dosages as high as 400 mg/kg has no apparent side effects, and excess levels are quickly excreted through the urine and bile.[18,26]

DRUG INTERACTIONS

No drug interactions have been reported for *V. myrtillus*.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Grieve M. *A Modern Herbal*. vol. 1. New York: Dover Publications; 1971.
2. Baj A, Bombardelli E, Gabetta B, et al. Qualitative and quantitative evaluation of *Vaccinium myrtillus* anthocyanins by high-resolution gas chromatography and high-performance liquid chromatography. *J Chromatogr*. 1983;279:365–372.
3. Andersen OM. Anthocyanins in fruits of *Vaccinium uliginosum L.* (bog whortleberry). *J Food Sci*. 1987;52:665–656, 680.
4. Lyons MM, Yu C, Toma RB, et al. Resveratrol in raw and baked blueberries and bilberries. *J Agric Food Chem*. 2003;51:5867–5870.
5. Erlund I, Marniemi J, Hakala P, et al. Consumption of black currants, lingonberries and bilberries increases serum quercetin concentrations. *Eur J Clin Nutr*. 2003;57:37–42.
6. Kuhnau J. The flavonoids: a class of semi-essential food components. Their role in human nutrition. *World Rev Nutr Diet*. 1976;24:117–191.
7. Gabor M. Pharmacologic effects of flavonoids on blood vessels. *Angiologica*. 1972;9:355–374.
8. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol*. 1983;32:1141–1148.
9. Monboisse JC, Braquet P, Randoux A, et al. Non-enzymatic degradation of acid-soluble calf skin collagen by superoxide ion protective effect of flavonoids. *Biochem Pharmacol*. 1983;32:53–58.
10. Detre A, Jellinek H, Miskulin M, et al. Studies on vascular permeability in hypertension: action of anthocyanosides. *Clin Physiol Biochem*. 1986;4:143–149.
11. Faria A, Oliveira J, Neves P, et al. Antioxidant properties of prepared blueberry *(Vaccinium myrtillus)* extracts. *J Agric Food Chem*. 2005; 53(17):6896–6902.
12. Jonadet M, Meunier MT, Bastide J, et al. Anthocyanosides extracted from *Vitis vinifera, Vaccinium myrtillus* and *Pinus maritimus*. I. Elastase-inhibiting activities in vitro. II. Compared angioprotective activities in vivo. *J Pharm Belg*. 1983;38:41–46.
13. Middleton E. The flavonoids. *Trends Pharm Sci*. 1984;5:335–358.
14. Amella M, Bronner C, Briancon F, et al. Inhibition of mast cell histamine release by flavonoids and bioflavonoids. *Planta Med*. 1985;51:16–20.
15. Rao CN, Rao VH, Steinman B. Influence of bioflavonoids on the collagen metabolism in rats with adjuvant induced arthritis. *Ital J Biochem*. 1981;30:54–62.
16. Ronziere MC, Herbage D, Garrone R, et al. Influence of some flavonoids on reticulation of collagen fibrils in vitro. *Biochem Pharmacol*. 1981;30:1771–1776.
17. Mian E, Curri SB, Lietti A, et al. Anthocyanosides and the walls of the microvessels: further aspects of the mechanism of action of their protective effect in syndromes due to abnormal capillary fragility. *Minerva Med*. 1977;68:3565–3581.
18. Lietti A, Cristoni A, Picci M. Studies on *Vaccinium myrtillus* anthocyanosides. I. Vasoprotective and anti-inflammatory activity. *Arzneim Forsch*. 1976;26:829–832.
19. Ghiringhelli C, Gregoratti L, Marastoni F. Capillarotropic action of anthocyanosides in high doses in phlebopathic statis. *Minerva Cardioangiol*. 1978;26:255–276.
20. Treviso A. Therapeutic value of the association of anthocyanin glucosides with glutamine and phosphorylserine in the treatment of learning disturbances at different ages. *Gazz Med Ital*. 1979;138:217–232.
21. Grismondi GL. Treatment of phlebopathies caused by stasis in pregnancy. *Minerva Ginecol*. 1981;33:221–230.
22. Piovella F, Almasio P, Ricetti MM, et al. Results with anthocyanidins in the treatment of haemorrhagic diathesis due to defective primary haemostasis. *Gazz Med Ital*. 1981;140:445–449.
23. Pennarola R, Roco P, Matarazzo G, et al. The therapeutic action of the anthocyanosides in microcirculatory changes due to adhesive-induced polyneuritis. *Gazz Med Ital*. 1980;139:485–491.
24. Amouretti M. Therapeutic value of *Vaccinium myrtillus* anthocyanosides in an internal medicine department. *Therapeutique*. 1972;48:579–581.
25. Robert AM, Godeau G, Moati F, et al. Action of the anthocyanosides of *Vaccinium myrtillus* on the permeability of the blood brain barrier. *J Med*. 1977;8:321–332.
26. Bertuglia S, Malandrino S, Colantuoni A. Effect of *Vaccinium myrtillus* anthocyanoside on ischemia reperfusion injury in hamster cheek pouch microcirculation. *Pharmacol Res*. 1995;31:183–187.
27. Zaragoza F, Iglesias I, Benedi J. Comparative study of the anti-aggregation effects of anthocyanosides and other agents. *Arch Pharmacol Toxicol*. 1985;11:183–188.
28. Morazzoni P, Magistretti MJ. Effects of *Vaccinium myrtillus* anthocyanosides on prostacyclin like activity in rat arterial tissue. *Fitoterapia*. 1986;57:11–14.
29. Bottecchia D, Bettini V, Martino R, et al. Preliminary report on the inhibitory effect of *Vaccinium myrtillus* anthocyanosides on platelet aggregation and clot retraction. *Fitoterapia*. 1987;48:3–8.
30. Bettini V, Mavellaro F, Ton P, et al. Effects of *Vaccinium myrtillus* anthocyanosides on vascular smooth muscle. *Fitoterapia*. 1984;55:265–272.
31. Bettini V, Mavellaro F, Patron E, et al. Inhibition by *Vaccinium myrtillus* anthocyanosides of barium-induced contractions in segments of internal thoracic vein. *Fitoterapia*. 1984;55:323–327.
32. Bettini V, Mayellaro F, Pilla I, et al. Mechanical responses of isolated coronary arteries to barium in the presence of *Vaccinium myrtillus* anthocyanosides. *Fitoterapia*. 1985;56:3–10.
33. Colombo D, Vescovini R. Controlled clinical trial of anthocyanosides from *Vaccinium myrtillus* in primary dysmenorrhea. *G Ital Obstet Ginecol*. 1985;7:1033–1038.
34. Katsube N, Iwashita K, Tsushida T, et al. Induction of apoptosis in cancer cells by bilberry *(Vaccinium myrtillus)* and the anthocyanins. *J Agric Food Chem*. 2003;51:68–75.
35. Matsunaga N, Tsuruma K, Shimazawa M, et al. Inhibitory actions of bilberry anthocyanidins on angiogenesis. *Phytother Res*. 2010;24(suppl 1):S42–S47.
36. Thomasset S, Berry DP, Cai H, et al. Pilot study of oral anthocyanins for colorectal cancer chemoprevention. *Cancer Prev Res (Phila)*. 2009;2(7):625–633.
37. Jayle GE, Aubert L. Action of the anthocyanin glycosides on the scotopic and mesopic vision of the normal subject (des glucosides d'anthocyanes sur la vision scotopique et mésopique du sujet normal). *Therapie*. 1964;19:171–185.
38. Terrasse J, Moinade S. Premiers resultats obtenus avec un nouveau facteur vitamininique P 'les anthocyanosides' extraits du *Vaccinium myrtillus*. *Presse Med*. 1964;72:397–400.
39. Sala D, Rolando M, Rossi PL, et al. Effect of anthocyanosides on visual performance at low illumination. *Minerva Oftalmol*. 1979;21:283–285.
40. Gloria E, Peria A. Effect of anthocyanosides on the absolute visual threshold. *Ann Ottalmol Clin Ocul*. 1966;92:595–607.
41. Junemann G. On the effect of anthocyanosides on hemeralopia following quinine poisoning. *Klin Monatsbl Augenheilkd*. 1967;151:891–896.
42. Caselli L. Clinical and electroretinographic study on activity of anthocyanosides. *Arch Med Intern*. 1985;37:29–35.
43. Wegmann R, Maeda K, Tronche P, et al. Effects of anthocyanosides on photoreceptors: cytoenzymatic aspects. *Ann Histochim*. 1969;14:237–256.
44. Lee J, Lee HK, Kim CY, et al. Purified high-dose anthocyanoside oligomer administration improves nocturnal vision and clinical symptoms in myopia subjects. *Br J Nutr*. 2005;93(6):895–899.
45. Ozawa Y1, Kawashima M, Inoue S, et al. Bilberry extract supplementation for preventing eye fatigue in video display terminal workers. *J Nutr Health Aging*. 2015;19(5):548–554.
46. Stocker F. New ways of influencing the intraocular pressure. *N Y State J Med*. 1949;49:58–63.
47. Matsunaga N, Imai S, Inokuchi Y, et al. Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage in vitro and in vivo. *Mol Nutr Food Res*. 2009;53(7):869–877.
48. Pautler EL, Ennis SR. The effect of diet on inherited retinal dystrophy in the rat. *Curr Eye Res*. 1984;3:1221–1224.
49. Hess H, Knapka JJ, Newsome DA, et al. Dietary prevention of cataracts in the pink-eyed RCS rat. *Lag Anim Sci*. 1985;35:47–53.
50. Pautler EL, Maga JA, Tengerdy C. A pharmacologically potent natural product in the bovine retina. *Exp Eye Res*. 1986;42:285–288.

51. Bravetti G. Preventive medical treatment of senile cataracts with vitamin E and anthocyanosides. Clinical evaluation. *Ann Ottalmol Clin Ocul*. 1989;115:109.
52. Scharrer A, Ober M. Anthocyanosides in the treatment of retinopathies. *Klin Monatsbl Augenheilkd*. 1981;178:386–389.
53. Bever B, Zahnd G. Plants with oral hypoglycemic action. *Q J Crude Drug Res*. 1979;17:139–196.
54. Chaundry PS, Cambera J, Juliana HR, et al. Inhibition of human lens aldose reductase by flavonoids, sulindac and indomethacin. *Biochem Pharmacol*. 1983;32:1995–1998.
55. Varma SD, Mizuno A, Kinoshita JH. Diabetic cataracts and flavonoids. *Science*. 1977;195:87–89.
56. Varma SD, El-aguizy HK, Richards RD. Refractive change in alloxan diabetic rabbits: control by flavonoids I. *Acta Ophthalmol*. 1980;58:748–759.
57. Allen FM. Blueberry leaf extract. Physiologic and clinical properties in relation to carbohydrate metabolism. *JAMA*. 1927;89:1577–1581.
58. Takikawa M, Inoue S, Horio F, et al. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *J Nutr*. 2010;140(3):527–533.
59. Passariello N, Bisesti V, Sgambato S. Influence of anthocyanosides on the microcirculation and lipid picture in diabetic and dyslipidic subjects. *Gazz Med Ital*. 1979;138:563–566.
60. Kadar A, Robert L, Miskulin M, et al. Influence of anthocyanoside treatment on the cholesterol-induced atherosclerosis in the rabbit. *Paroi Arterielle*. 1979;5:187–206.
61. Qin Y, Xia M, Ma J, et al., eds. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am J Clin Nutr*. 2009;90:485–492.
62. Habanova M, Saraiva J, Haban M, et al. Intake of bilberries *(Vaccinium myrtillus L.)* reduced risk factors for cardiovascular disease by inducing favorable changes in lipoprotein profiles. *Nutr Res*. 2016;36(12):1415–1422.
63. Kolehmainen M, Mykkänen O, Kirjavainen PV, et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Mol Nutr Food Res*. 2012;56(10):1501–1510.
64. Erlund I, Kol R, Alfthan G, et al., eds. Favourable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *Am J Clin Nutr*. 2008;87:323–331.
65. Zhu Y, Xia M, Yang Y, et al. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clin Chem*. 2011;57(11):1524–1533.
66. Biedermann L, Mwinyi J, Scharl M, et al. Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis—an open pilot study. *J Crohns Colitis*. 2013;7(4):271–279.
67. Roth S, Spalinger MR, Gottier C, et al. Bilberry-derived anthocyanins modulate cytokine expression in the intestine of patients with ulcerative colitis. *PLoS One*. 2016;11(5):e0154817.
68. Lietti A, Forni G. Studies on *Vaccinium myrtillus* anthocyanosides. II. Aspects of anthocyanins pharmacokinetics in the rat. *Arzneimittelforschung*. 1976;26:832–835.
69. Magistretti MJ, Conti M, Cristoni A. Antiulcer activity of an anthocyanidin from *Vaccinium myrtillus*. *Arzneimitteforschung*. 1988;38:686–690.

Valeriana officinalis (Valerian)

Michael T. Murray, ND

OUTLINE

Valeriana officinalis (family: *Valerianaceae*)
Common names: valerian, all heal

GENERAL DESCRIPTION

Valerian is a perennial plant native to North America and Europe. The yellow-brown tuberous rootstock produces a flowering stem 2 to 4 ft high. The stem is round but grooved and hollow, with leaves arranged in pairs. The small rose-colored flowers are in bloom from June to September. The rootstock is the portion used medicinally (Fig. 123.1A and B).

CHEMICAL COMPOSITION

The important active compounds of valerian are the valepotriates (iridoid molecules) (Fig. 123.2) and valerenic acid. These compounds are found exclusively in valerian. Originally it was thought that just the valepotriates were responsible for valerian's sedative effects, but an aqueous extract of valerian has also been shown to have a sedative effect. Because the valepotriates are not soluble in water, it was concluded that valerenic acid also possesses sedative action and is the chemical factor responsible for the sedative effect noted in human clinical trials with aqueous extracts of valerian root (see later discussion).

Moreover, because the safety of valepotriates was questioned after studies demonstrated mutagenicity, most commercial extracts feature water-soluble extracts standardized for valerenic acids.[1–3]

Other components of valerian are a volatile oil (0.5%–2%), choline (3%), flavonoids, sterols, and several alkaloids (actinidine, valerianine, valerine, and chatinine).[1]

HISTORY AND FOLK USE

Valerian's primary traditional use has been as a sedative for the relief of insomnia, anxiety, and conditions associated with pain. Specific conditions for which it has been used are migraine, insomnia, hysteria, fatigue, intestinal cramps, and other nervous conditions.

PHARMACOLOGY

Valerian has demonstrated a number of pharmacological effects, such as the following[4–7]:

- Normalizing of the central nervous system (it acts as a sedative in states of agitation and as a stimulant in cases of extreme fatigue)
- Lowering of blood pressure
- Enhancement of the flow of bile (choleretic effect)
- Relaxing the intestinal muscles
- Antitumor and antibiotic activity

Its prime pharmacological effect, however, is consistent with its historical use as a sedative. Pharmacological studies indicated that both valepotriates and valerenic acid are capable of binding to gamma-aminobutyric acid (GABA) receptors, much like the benzodiazepines.[8] However, valerian does not appear to act in a similar fashion in that side effects such as impaired mental function, morning hangover, and dependency have not been reported with valerian. In addition, valerian compounds that do not bind to GABA receptors have also been shown to produce sedative effects. Therefore other mechanisms may be more important, including valerenic acids modulating GABA receptors or promoting the release of GABA.[9]

In a double-blind, randomized, crossover, placebo-controlled study, a dosage of 900 mg of valerian extract (valerenic acid 0.8%) was shown to significantly reduce intracortical facilitation induced by transcranial magnetic stimulation (TMS), thereby indicating reduced cortical excitability. This effect may be a key mechanism for valerian's effects on sleep and anxiety.[10]

Another key mechanism of action of valerian components is acting as an agonist to adenosine receptors. Adenosine is not a typical hormone or neurotransmitter but is probably the most important neuromodulator in the central and peripheral nervous systems. A neuromodulator is a compound that has a modulatory effect on neuronal activity, increasing or decreasing the rate at which a nerve cell fires.

Neuromodulators are distinct from neurotransmitters (e.g., glutamate), which are typically stored in the presynaptic terminal, are released into the synaptic cleft, and then interact with postsynaptic

Fig. 123.1 (A) *Valeriana officinalis* inflorescence. (B) *V. officinalis* leaf.

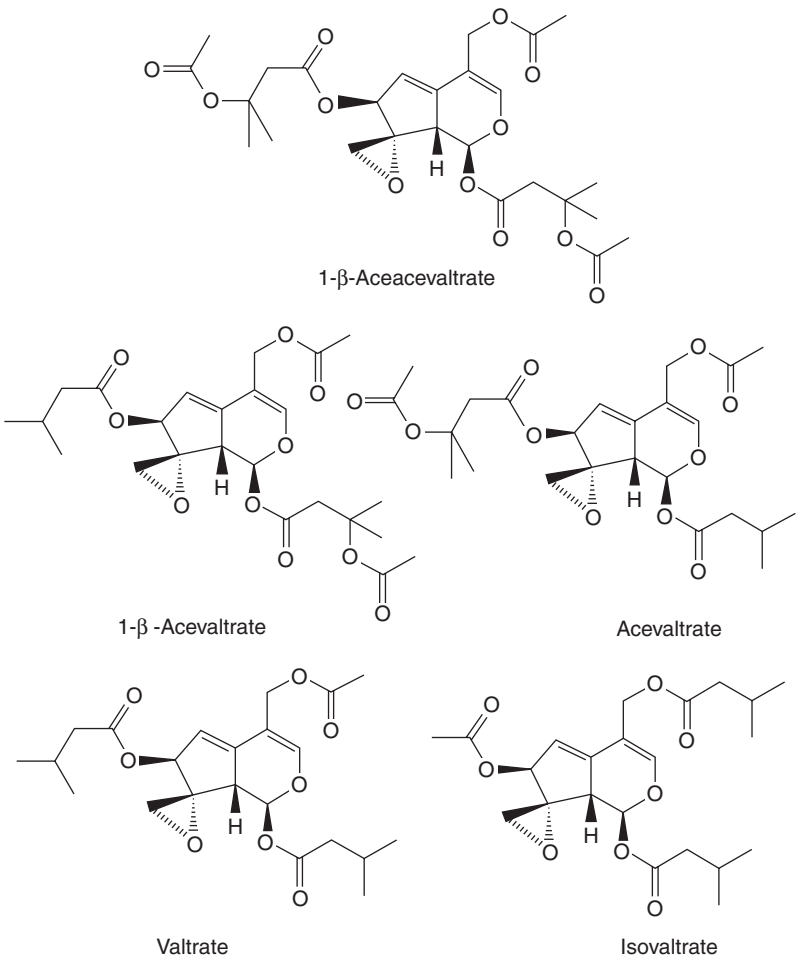


Fig. 123.2 Valepotriates in *Valeriana officinalis*.

receptors and are either taken up or metabolized. A neuromodulator such as adenosine is more likely to be either constitutively released or released at times of high or low metabolic activity. Neuromodulators may act presynaptically or postsynaptically and may then be either taken up or metabolized. The stimulant properties of the methylxanthines (caffeine, theophylline, theobromine, etc.) are partially explained by their antagonism of adenosine receptors. In contrast, valerian has been shown to act as an adenosine agonist.[11]

CLINICAL APPLICATIONS

The primary clinical application for valerian is as a sedative in the treatment of insomnia. It can also be used in the treatment of stress and anxiety.

Insomnia

More than 20 double-blind clinical studies have now substantiated valerian's ability to improve sleep quality and relieve insomnia.[12–23] Most of these studies have shown positive results.[24] Several recent double-blind studies have shown no significant benefit with valerian in improving sleep parameters in insomnia. In one study, a dosage of 300 mg valerian extract failed to show any statistically significant difference versus a placebo after a single dose or after 2 weeks of nightly dosing on any measure of sleep latency, wake after sleep onset, sleep efficiency, and self-rated sleep quality.[25] In another very detailed clinical study, standardized sleep electroencephalographic (EEG) and psychometric tests were used to evaluate the clinical efficacy of valerian extract 300 mg, valerian extract 600 mg, or placebo.[26] Results showed no significant effect between valerian 300 mg, valerian 600 mg, or placebo on any EEG parameter or psychometric measure. Nonetheless, these results are not viewed as being conclusive, given the results of other trials and valerian's long history of use.[24]

The initial studies were performed on subjects who did not have insomnia. These studies showed quite clearly that extracts of valerian root improved subjective ratings of sleep quality and sleep latency (the time required to go to sleep) but left no "hangover" the next morning.[12] In one early study, the effects of valerian on sleep were studied in two groups of healthy young subjects.[13] One group slept at home and the other in a sleep laboratory. Sleep was evaluated on the basis of questionnaires, self-rating scales, and nighttime motor activity. Under home conditions, both doses of an aqueous valerian extract (450 and 900 mg) reduced perceived sleep latency and wake time after sleep onset. Nighttime motor activity was enhanced in the middle third of the night and reduced in the last third. The data suggest a dose-dependent effect. In the sleep laboratory, where only the higher dose of valerian was tested, no significant differences from placebo were obtained. However, the directions of the changes in the subjective and objective measures of sleep latency and wake time after sleep onset, as well as in nighttime motor activity, corresponded to those observed under home conditions. There was no evidence of a change in sleep stages or EEG spectra. The results indicate that the aqueous valerian extract exerts a mild sedative effect.

Although the initial studies demonstrated that valerian could improve sleep quality in normal subjects, they failed to answer the question of whether valerian can improve sleep patterns in people suffering from insomnia. In a follow-up to these two preliminary studies, in more than 15 double-blind studies, valerian extract has been shown to significantly reduce sleep latency, improve sleep quality, and reduce nighttime awakenings in sufferers of insomnia.[14] These studies were usually performed under strict laboratory conditions and demonstrated quite clearly that valerian is as effective in reducing sleep latency as are small doses of barbiturates or benzodiazepines. However, although the latter compounds also increase morning sleepiness, valerian usually reduces morning sleepiness.

In one of the better-designed double-blind trials, patients 18 to 73 years of age diagnosed with nonorganic insomnia, according to the *International Classification of Diseases,* 10th edition, were treated at night with either 600 mg of valerian extract or 10 mg of oxazepam (Serax) for 6 weeks.[17] A total of 202 outpatients with a mean duration of insomnia of 3.5 months at baseline were involved in the study. Sleep quality after 6 weeks measured by Sleep Questionnaire B showed that the 600-mg dose of valerian extract was at least as efficacious as a treatment with 10 mg of oxazepam. Both treatments markedly increased sleep quality compared with baseline ($P < 0.01$). The other sleep function subscales—feeling of refreshment after sleep, psychic stability in the evening, psychic exhaustion in the evening, psychosomatic symptoms in the sleep phase, dream recall, and duration of sleep—confirmed similar effects of both treatments. The Clinical Global Impressions scale and Global Assessment of Efficacy completed by investigators and patients again showed similar effects of both treatments. No significant side effects or serious adverse reactions were reported in either group. Most patients assessed their respective treatment as very good (82.8% in the valerian group, 73.4% in the oxazepam group). These results show that valerian extract displays a comparable efficacy with that of 10 mg of oxazepam in the therapy of nonorganic insomnia.

In another study comparing the effects of valerian and oxazepam, 75 patients with nonorganic and nonpsychiatric insomnia between 18 and 70 years of age were randomly allocated to receive either 600 mg of valerian extract or 10 mg of oxazepam every day 30 minutes before going to bed over a period of 28 days. The results of this study were consistent with those of the other in that both groups' measures of sleep quality improved significantly, but there was no statistically significant difference between groups.[22]

Valerian extract has also been shown to help improve sleep and relieve insomnia in people withdrawing from chronic benzodiazepine use.[18] The study consisted of patients with insomnia who complained of poor sleep despite long-term use of benzodiazepines. These patients had taken benzodiazepines nightly for an average of 7 years. The sleep electroencephalograms of the patients were analyzed with period amplitude analysis and associated algorithms during chronic benzodiazepine use (night 1) and then after 15 days of a valerian-placebo trial (initiated after washout of benzodiazepines; night 2). The subjects given valerian extract reported significantly better subjective sleep quality than those given placebo. The EEG results also showed significant less wake time after sleep onset in valerian subjects than in placebo subjects, and the valerian-treated patients experienced longer sleep latency and increased alpha counts in slow-wave sleep. Taken together, these results indicate that valerian extract has a positive effect on withdrawal from benzodiazepine use.

Valerian may be helpful in patients with restless legs syndrome (RLS). In one study,[27] patients with RLS were randomly assigned to receive 800 mg of valerian extract or placebo for 8 weeks. The results indicated that valerian supplementation for 8 weeks improved symptoms of RLS and decreased daytime sleepiness.[28]

Valerian extract also appears to be useful in children. Serious sleep disturbances are particularly problematic for children with intellectual deficits and are often the source of much distress for both the children and caregivers. Conventional drugs are not suitable for long-term treatment, making valerian an attractive alternative. In one small double-blind study in five children with varying intellectual deficits and different primary sleep problems, treatment with valerian extract led to significant reductions in sleep latencies and nocturnal time awake, lengthened total sleep time, and improved sleep quality.[19] The treatment was apparently most effective in children with deficits that

involved hyperactivity. Although the findings were preliminary and are in need of replication, the results imply that valerian may be useful in the safe and effective long-term treatment of sleep difficulties in children with intellectual deficits.

One of the major advantages of valerian extract is that it rarely has a negative effect on reaction time, alertness, or concentration the morning after intake. In one double-blind trial, 102 male and female volunteers were studied to determine whether reaction time, alertness, and concentration might be impaired by treatment with a valerian root extract.[29] The effect was first examined the morning after a single evening dose of either valerian extract (600 mg), flunitrazepam (Rohypnol) (1 mg), or a placebo and then after 2 weeks of evening administration of valerian extract (600 mg) or a placebo. Evaluation of the primary criterion (the median of reaction time measured with the Vienna Determination Test) and secondary criteria (alertness test, tracking test, sleep quality, additional Vienna Determination Test parameters, and safety criteria) all demonstrated that neither a single administration nor 14 days of valerian extract treatment produced an impairment in reaction abilities, concentration, or coordination.

Anxiety and/or Depression

Valerian extracts, based on preliminary studies, have shown some evidence of usefulness in the treatment of generalized anxiety and depression. For example, in one study involving 36 outpatients with generalized anxiety disorder, patients underwent a 2-week washout period and then were randomly assigned to one of the following three treatments for 4 weeks: valepotriates (mean daily dose: 81.3 mg), diazepam (Valium) (mean daily dose: 6.5 mg), and placebo.[30] Although no significant difference was observed among the three groups at baseline or in the change from baseline on the Hamilton Anxiety Scale—probably because of the small sample size in each group—there was some evidence of benefit nonetheless. Specifically, only the diazepam and valepotriates groups showed a significant reduction in the psychic factor of the Hamilton Anxiety Scale.

In one study, valerian extract was shown to be effective in reducing the physiological effects of psychological stress induced under laboratory conditions in a group of healthy volunteers.[31] Valerian extract produced a significant decrease in systolic blood pressure responsivity, no significant reductions in diastolic blood pressure, and a decrease in the heart-rate reaction to mental stress.

Valerian may prove useful in relieving anxiety in patients with comorbid depression. In an open clinical trial, the combination of valerian with St. John's wort was found to improve symptoms more quickly than did St. John's wort monotherapy.[32] The combination therapy was well tolerated, and no significant side effects occurred.

Valerian was also shown to be effective in reducing anxiety in a double-blind clinical trial in 64 infertile women undergoing hysterosalpingography, indicating that valerian can be effective in situational anxiety.[33]

Menopausal Symptoms

Valerian may be helpful during menopause to help with both insomnia and hot flashes. In regard to the later, two controlled clinical trials have shown benefit.[34,35] In the first double-blind clinical trial, 76 menopausal women with the chief symptom of hot flashes were enrolled, with the treatment group receiving a 225-mg valerian capsule three times per day and the other group receiving a placebo, both for 8 weeks. After 4 and 8 weeks of treatment, the evaluation of results showed a meaningful difference between the valerian group and the placebo group. At week 4 and week 8 posttreatment, the valerian group had significantly less severe hot flashes (5.23 after 8 weeks) compared with the placebo group (9.86 after 8 weeks) as well as significantly fewer hot flashes compared with the placebo group, 4.83 versus 7.75, respectively.[33]

DOSAGE

As a mild sedative, valerian may be taken 30 to 45 minutes before bedtime. This timing of dosage has been verified by pharmacokinetic studies showing that the peak blood levels of valerenic acid occur within 1 to 2 hours after ingestion. Dosage recommendations are as follows:

- Dried root (or as tea): 1.5 to 3 g
- Tincture (1:5): 4 to 8 mL (1–2 tsp)
- Fluid extract (1:1): 2 to 4 mL (1/2–1 tsp)
- Solid (dry powdered) extract (4:1): 400 to 800 mg
- Valerian extract (1.0%–1.5% valtrate or 0.5% valerenic acid): 300 to 600 mg

For the rare patient with increased morning sleepiness, reducing the dosage will eliminate the problem. For the best results, dietary factors that disrupt sleep, such as caffeine and alcohol (see Chapter 187), should be eliminated.

TOXICITY

Valerian is generally regarded as safe and is approved for food use by the U.S. Food and Drug Administration. A major concern for any sedative or antianxiety medication is its potential to affect a person's ability to drive or operate potentially dangerous machinery. Valerian at a one-time 1600-mg dose did not affect standardized field sobriety testing (SFST) and driving simulator performance parameters in a double-blind study.[36] Another double-blind study evaluated the effect of a valerian–lemon balm preparation on psychomotor and mental performance tests.[37] No effect was found on reaction time, concentration, or attentiveness.

One case of valerian overdose has been reported in the literature.[27] The patient presented with mild symptoms, all of which disappeared within 24 hours after the patient had taken an overdose—approximately 20 times the recommended therapeutic dose of valerian. Another case history reports valerian-induced hepatotoxicity.[38]

DRUG INTERACTIONS

Although in vitro studies have demonstrated that valerian inhibits drug-metabolizing enzymes, at typical doses it is unlikely to produce clinically significant effects on the disposition of medications dependent on the CYP2D6 or CYP3A4 pathways of metabolism.[39] There is some concern that valerian may prolong thiopental- and pentobarbital-induced sleep—a concern based on animal data and indicating the need to avoid concurrent use of valerian and barbiturates.

Valerian was shown to significantly improve sleep and anxiety in HIV-positive patients taking the antiviral drug efavirenz.[40]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Houghton PJ. The biological activity of valerian and related plants. *J Ethnopharmacol*. 1988;22:121–142.
2. von der Hude W, Scheutwinkel-Reich M, Braun R. Bacterial mutagenicity of the tranquilizing constituents of Valerianaceae roots. *Mutat Res*. 1986;169:23–27.
3. von der Hude W, Scheutwinkel-Reich M, Braun R. In vitro mutagenicity of valepotriates. *Arch Toxicol*. 1985;56:267–271.
4. Takeda S, Endo T, Aburada M. Pharmacological studies on iridoid compounds. III. The choleretic mechanism of iridoid compounds. *J Pharmacobiodyn*. 1981;4:612–623.
5. Hendriks H, Bos R, Allersma DP, et al. Pharmacological screening of valerenal and some other components of essential oil of *Valeriana officinalis*. *Planta Med*. 1981;42:62–68.
6. Hazelhoff B, Malingre TM, Meijer DK. Antispasmodic effects of *Valeriana* compounds: an in-vivo and in-vitro study on the Guinea pig ileum. *Arch Int Pharmacodyn*. 1982;257:274–287.
7. Bounthanh C, Bergmann C, Beck JP, et al. Valepotriates, a new class of cytotoxic and antitumor agents. *Planta Med*. 1981;41:21–28.
8. Mennini T, Bernasconi P, Bombardelli E, et al. In vitro study on the interaction of extracts and pure compounds from *Valeriana officinalis* roots with GABA, benzodiazepine and barbiturate receptors in rat brain. *Fitoterapia*. 1993;64:291–300.
9. Trauner G, Khom S, Baburin I, et al. Modulation of GABAA receptors by valerian extracts is related to the content of valerenic acid. *Planta Med*. 2008;74(1):19–24.
10. Mineo L, Concerto C, Patel D, et al. Valeriana officinalis root extract modulates cortical excitatory circuits in humans. *Neuropsychobiology*. 2017;75(1):46–51.
11. Muller CE, Schumacher B, Brattstrom A, et al. Interactions of valerian extracts and a fixed valerian-hop extract combination with adenosine receptors. *Life Sci*. 2002;71:1939–1949.
12. Leathwood P, Chauffard F, Heck E, et al. Aqueous extract of valerian root (*Valeriana officinalis* L.) improves sleep quality in man. *Pharmacol Biochem Behav*. 1982;17:65–71.
13. Balderer G, Borbely AA. Effect of valerian on human sleep. *Psychopharmacology (Berl)*. 1985;87:406–409.
14. Leathwood PD, Chauffard F. Aqueous extract of valerian reduces latency to fall asleep in man. *Planta Med*. 1985;54:144–148.
15. Lindahl O, Lindwall L. Double blind study of a valerian preparation. *Pharmacol Biochem Behav*. 1989;32:1065–1066.
16. Dressing H, Riemann D, Low H. Insomnia: are valerian/Melissa combinations of equal value to benzodiazepine? *Therapiewoche*. 1992;42:726–736.
17. Ziegler G, Ploch M, Miettinen-Baumann A, et al. Efficacy and tolerability of valerian extract LI 156 compared with oxazepam in the treatment of non-organic insomnia—a randomized, double-blind, comparative clinical study. *Eur J Med Res*. 2002;7:480–486.
18. Poyares DR, Guilleminault C, Ohayon MM, et al. Can valerian improve the sleep of insomniacs after benzodiazepine withdrawal? *Prog Neuropsychopharmacol Biol Psychiatry*. 2002;26:539–545.
19. Francis AJ, Dempster RJ. Effect of valerian, Valeriana edulis, on sleep difficulties in children with intellectual deficits: randomised trial. *Phytomedicine*. 2002;9:273–279.
20. Herrera-Arellano A, Luna-Villegas G, Cuevas-Uriostegui ML, et al. Polysomnographic evaluation of the hypnotic effect of *Valeriana edulis* standardized extract in patients suffering from insomnia. *Planta Med*. 2001;67:695–699.
21. Wheatley D. Kava and valerian in the treatment of stress-induced insomnia. *Phytother Res*. 2001;15:549–551.
22. Dorn M. Efficacy and tolerability of Baldrian versus oxazepam in non-organic and non-psychiatric insomniacs: a randomised, double-blind, clinical, comparative study. *Forsch Komplementarmed Klass Naturheilkd*. 2000;7:79–84.
23. Donath F, Quispe S, Diefenbach K, et al. Critical evaluation of the effect of valerian extract on sleep structure and sleep quality. *Pharmacopsychiatry*. 2000;33:47–53.
24. Fernández-San-Martín MI, Masa-Font R, Palacios-Soler L, et al. Effectiveness of valerian on insomnia: a meta-analysis of randomized placebo-controlled trials. *Sleep Med*. 2010;11(6):505–511.
25. Taibi DM, Vitiello MV, Barsness S, et al. A randomized clinical trial of valerian fails to improve self-reported, polysomnographic, and actigraphic sleep in older women with insomnia. *Sleep Med*. 2009;10(3):319–328.
26. Diaper A, Hindmarch I. A double-blind, placebo-controlled investigation of the effects of two doses of a valerian preparation on the sleep, cognitive and psychomotor function of sleep-disturbed older adults. *Phytother Res*. 2004;18(10):831–836.
27. Wiley LB, Mady SP, Cobaugh DJ, et al. Valerian overdose: a case report. *Vet Hum Toxicol*. 1995;37:364–365.
28. Cuellar NG, Ratcliffe SJ. Does valerian improve sleepiness and symptom severity in people with restless legs syndrome? *Altern Ther Health Med*. 2009;15(2):22–28.
29. Kuhlmann J, Berger W, Podzuweit H, et al. The influence of valerian treatment on "reaction time, alertness and concentration" in volunteers. *Pharmacopsychiatry*. 1999;32:235–241.
30. Andreatini R, Sartori VA, Seabra ML, et al. Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. *Phytother Res*. 2002;16:650–654.
31. Cropley M, Cave Z, Ellis J, et al. Effect of kava and valerian on human physiological and psychological responses to mental stress assessed under laboratory conditions. *Phytother Res*. 2002;16:23–27.
32. Muller D, Pfeil T, von den Driesch V. Treating depression comorbid with anxiety: results of an open, practice-oriented study with St John's wort WS 5572 and valerian extract in high doses. *Phytomedicine*. 2003;10(suppl 4):25–30.
33. Gharib M, Samani LN, Panah ZE, et al. The effect of valeric on anxiety severity in women undergoing hysterosalpingography. *Glob J Health Sci*. 2015;7(3):358–363.
34. Mirabi P, Mojab F. The effects of valerian root on hot flashes in menopausal women. *Iran J Pharm Res*. 2013;12(1):217–222.
35. Jenabi E, Shobeiri F, Hazavehei SMM, Roshanaei G. The effect of valerian on the severity and frequency of hot flashes: a triple-blind randomized clinical trial. *Women Health*. 2018;58(3):297–304.
36. Thomas K, Canedo J, Perry PJ, et al. Effects of valerian on subjective sedation, field sobriety testing and driving simulator performance. *Accid Anal Prev*. 2016;92:240–244.
37. Albrecht M, Berger W, et al. Psychopharmaceuticals and safety in traffic. *Zeits Allegmeinmed*. 1995;71:1215–1221.
38. Vassiliadis T, Anagnostis P, Patsiaoura K, et al. *Valeriana* hepatotoxicity. *Sleep Med*. 2009;10(8):935.
39. Donovan JL, DeVane CL, Chavin KD, et al. Multiple night-time doses of valerian (Valeriana officinalis) had minimal effects on CYP3A4 activity and no effect on CYP2D6 activity in healthy volunteers. *Drug Metab Dispos*. 2004;32(12):1333–1336.
40. Anderson GD, Elmer GW, Kantor ED, et al. Pharmacokinetics of valerenic acid after administration of valerian in healthy subjects. *Phytother Res*. 2005;19(9):801–803.

124

Viscum album (European Mistletoe)

Michael T. Murray, ND

OUTLINE

Viscum album L. (family: *Loranthaceae*)
Common names: European mistletoe, all-heal, birdlime, devil's fuge

GENERAL DESCRIPTION

Viscum album, or European mistletoe, is an evergreen semiparasitic plant found on the branches of deciduous trees in Europe and northern Asia. The roots of the plant penetrate through the bark into the wood of the host tree. The green branches are 1 to 2 ft long and form pendent bushes with leaves that are opposite, leathery, yellow-green, and narrowly obovate. Inconspicuous pale yellow or green flowers appear from March to May, the female developing into sticky white berries that ripen from September to November.[1]

Viscum is most commonly seen on old apple, ash, and hawthorn trees. Traditionally, mistletoe from oak has been the most widely used, although it does not grow as well on oak as on the previously mentioned trees (Fig. 124.1).

CHEMICAL COMPOSITION

V. album contains a variety of pharmacologically active substances, including alkaloids, polysaccharides, phenylpropanes, lignins, lectins, and viscotoxins.[2–10] Some specific compounds found in *Viscum* are as follows:

- A wide range of carbohydrates, including simple sugars as well as polysaccharides
- Phenolic compounds such as flavonoids, caffeic acid, syringin, and eleutherosides
- Sterols, including beta-sitosterol, stigmasterol, and triterpenes
- Various amino acids as well as vasoactive amines, including tyramine, phenylethylamine, and histamine
- Fatty acids such as linoleic, palmitic, and oleic acids

The alkaloids isolated from *Viscum* appear to be related to those found in the host plant.[3,4] For example, mistletoes growing on Solanaceae shrubs contain nicotine alkaloids like hyoscine, anabasine, and isopelletierine. Cardiac glycosides have been found in mistletoe growing on *Nerium oleander,* strychnine has been found in mistletoe growing on *Strychnos* species, and caffeine has been found in mistletoe growing on coffee plants.

Because pharmacologically active compounds appear to be concentrated within the mistletoe, different host trees providing diverse chemical constituents could be used for different therapeutic actions. Also of importance is the fact that the proteins/lectins are present only in aqueous extracts, indicating that their therapeutic activity would differ from that of the alcoholic/aqueous extracts. The alcoholic/aqueous extracts also demonstrate considerably less toxicity.

HISTORY AND FOLK USE

Mistletoe was held in great reverence by the Druids who, dressed in white robes, would search for the sacred plant. When some was discovered, a great ceremony would ensue, culminating in the mistletoe's separation from the oak tree with a golden knife. The Druids believed that mistletoe protected them from all evil and that the oaks on which it was seen growing were to be respected because of the wonderful cures that the priests were able to produce with it.[1]

Mistletoe's use has been recorded in the Middle East, Africa, India, and Japan, and it was mentioned as an anticancer drug by Pliny, Dioscorides, and Galen.[3] In 1720 an English physician, Sir John Colbatch, extolled the virtues of *Viscum* in a pamphlet titled *The Treatment of Epilepsy by Mistletoe.* For many years, mistletoe was used in the treatment of a variety of nervous system disorders, including convulsions, delirium, hysteria, neuralgia, and nervous debility.[1,3] It has been used in naturopathic medicine in the treatment of hypertension and vascular disorders of the uterus, bladder, and intestines.

Probably because of *Viscum's* potential toxicity, its use appears to have fallen into some disrepute shortly after Colbatch's work. For many years it was used only in external preparations for the treatment of dermatitis. Then, in 1906, a study demonstrating *Viscum*'s hypotensive effect in animals and humans was published. This paper appears to have restored its medical prestige, initially in France and eventually throughout Europe.[3]

Fig. 124.1 Viscum album habit.

PHARMACOLOGY

V. album exhibits diverse pharmacological actions. The herb and various extracts have demonstrated that it serves as follows:

- As a hypotensive
- As a vasodilator
- As a cardiac depressant
- As a sedative
- As an antispasmodic
- As an immunostimulator
- As an antineoplastic agent

Purified mistletoe lectins are, in general, not as active in experimental studies as are crude preparations.[11,12] Presumably, the crude preparations contain a number of compounds acting synergistically. It has also been proposed that alkaloid components are responsible for the maintenance of lectin structure and activity.[5] During isolation and purification procedures, alkaloid linkages are cleaved from the lectins, resulting in a loss of specificity for target molecules. Unfermented *Viscum* preparations typically demonstrate greater direct cytotoxicity to tumor cells owing to higher concentrations of the viscotoxin mistletoe lectin I (ML I).[13–15]

Cardiovascular Effects

Viscum has exhibited a variety of effects on the cardiovascular system.[3,16] In particular, *Viscum* has repeatedly demonstrated hypotensive action in animal studies. The mechanism of action for its hypotensive effect is still not entirely clear, and no recent investigations have been published. *Viscum* has been shown to inhibit the excitability of the vasomotor center in the medulla oblongata and to possess cholinomimetic activity.[16]

Viscum's hypotensive activity may depend on the form in which the mistletoe is administered and the host tree from which it was collected. Studies indicate that aqueous extracts are more effective; the highest hypotensive activity was demonstrated by a macerate of leaves of mistletoe parasitizing on willow and gathered in January.[16]

Viscum's nonprotein components—flavonoids, phenol carboxylic acids, phenylpropanes, and lignins—have been shown to possess hypotensive action. Alcoholic solutions (tinctures and fluid extracts) contain these compounds but not viscotoxins or lectins. As previously stated, however, aqueous extracts appear to be more effective.

Several *Viscum* preparations for hypertension are currently available in Europe. In fact, in Great Britain alone, more than 150 different mistletoe preparations are available.[3] These preparations typically have small amounts of *Viscum* in combination with other botanicals with hypotensive action, such as garlic, *Crataegus oxyacantha,* and *Tilia platyphyllos.*

Antineoplastic and Immunostimulatory Effects

Viscum preparations have been used clinically in Europe for the treatment of cancer since 1926, when Iscador, a fermented product made from the crude pressed juice, was introduced as an immunotherapeutic agent for cancer. This work was carried out under the direction of Rudolph Steiner. Since that time, numerous studies have shown that Iscador and other *Viscum* preparations and components are effective antineoplastic and immunostimulatory agents.[11–15,17–31]

Iscador and other fermented *Viscum* preparations differ from nonfermented extracts in their greater effectiveness and their lower toxicity.[17] Specifically, the major viscotoxin, ML I, is not found in Iscador.[18] It is thought that fermentation transforms ML I to its A and B chains, which have important immunological properties.[13] The A chain has mitogenic effects, and the B chain stimulates macrophages and the release of lymphokines. In addition, there is a rapid decrease of lectins during fermentation.

The pharmacological activity of Iscador has been shown to be due to its *Viscum* components rather than to other constituents, such as lactobacilli, which possess adjuvant activity. The lectins have clearly been demonstrated to be the *Viscum* components largely responsible for Iscador's adjuvant activity. Although unfermented plant juice has demonstrated tenfold-greater cytotoxicity to tumor cells than Iscador in vitro, fermented Iscador contains a great number of substances that may act synergistically. In vivo studies in mice have demonstrated Iscador to have greater adjuvant activity than purified mistletoe lectin and to be without secondary toxic effects.[19,20]

Viscum's adjuvant activity is demonstrable in both delayed-type hypersensitivity and antibody responses of mice to sheep red blood cells.[19,20] Like other adjuvants (e.g., bacille Calmette-Guérin, levamisole, muramyl dipeptide, bacterial and yeast components, etc.), Iscador is most effective when it is administered near the tumor, although systemic administration has also yielded positive results. Upon local administration, an inflammatory process ensues that promotes white blood cell (WBC) infiltration and encapsulation of the tumor.

The nonspecific host defense factors stimulated by *Viscum* include the following:

- Enhanced macrophage phagocytic and cytotoxic activity[17,20]
- Increased neutrophil production[17]
- Increased thymic weight and enhanced cortical thymocyte activity and proliferation[21,23]
- Enhanced natural killer (NK) cell activity[17,22,24,32]
- Increased antibody-dependent cell-mediated cytotoxicity[17,22]
- Inhibition of angiogenesis[33]

Iscador's effects on these immunological parameters have been confirmed in patients with cancer.[17,22]

Iscador's effect in stimulating the thymus gland has been demonstrated in several studies.[21,23] Its ability to induce hyperplasia of the thymic cortex and accelerate the regeneration of hematopoietic cells after x-irradiation is much greater than that of any other agent reported to date.[21] In addition, thymic lymphocytes became 29 times more responsive to concanavalin A as a result of Iscador administration.

In summary, *V. album* exhibits numerous cytotoxic, adjuvant, and immunostimulatory effects that indicate a therapeutic effect in human

cancer. These effects have been confirmed in vivo against murine tumors, Lewis lung carcinoma, colon adenocarcinoma 38, and C3H mammary adenocarcinoma 16/C.[4]

CLINICAL APPLICATIONS

Cancer

The clinical application with the most significant scientific documentation is the use of *Viscum* preparations as adjuncts in cancer therapy. In published studies, the route of administration of *Viscum* preparations used has been subcutaneous or intravenous. Therefore it is not known to what degree (if any) the effects noted for Iscador as well as other preparations and *Viscum* compounds can be achieved with oral administration, although one study has shown that an enteric-coated, specially prepared *Viscum* preparation led to the absorption of intact lectins.[34]

Early clinical investigations of Iscador were not well documented. Owing to the shortage of acceptable controlled clinical trials, the use of Iscador and other *Viscum* preparations for the treatment of cancer in Europe has remained controversial, although positive effects in the postoperative treatment of lung, breast, colon, and cervical carcinomas have been shown in several controlled studies.[25] The problem is that the methodological criteria in these studies are poor. Until their benefits are better documented, mistletoe preparations appear most useful as adjuncts to standard therapy.

The results of a prospective study indicate that Iscador is helpful in increasing the survival rate for various cancers.[35] The prospective long-term epidemiological study sought to determine whether Iscador treatment prolongs the survival time of patients with carcinoma of the colon, rectum, or stomach; breast carcinoma with or without axillary or remote metastases; or small cell or non–small cell bronchogenic carcinoma. A total of 10,226 patients with cancer were evaluated. Of these patients, 1668 had been treated with Iscador, and 8475 had taken neither Iscador nor any other mistletoe product (control patients). In the nonrandomized matched-pair study, the survival time of patients treated with Iscador was longer for all types of cancer studied. In the pool of 396 matched pairs, mean survival time in the Iscador groups (4.23 years) was roughly 40% longer than in the control groups (3.05 years).

Perhaps even more beneficial than Iscador is the new generation of mistletoe preparations standardized on ML I (e.g., Eurixor, Lektinol). This greater standardization offers significant advantages. ML I is a potent inducer of cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor. Its cytotoxic effects are also related to its ability to induce apoptosis (programmed cell death).[26]

In one study, the effect of Eurixor was examined in 40 patients with advanced carcinoma of the breast. Along with standard chemotherapy (vinblastine, epidoxorubicin, and cisplatin [VEC] regimen), 21 patients were assigned to receive mistletoe (treatment group), and 19 were given a placebo (control group).[27] After the fourth cycle of chemotherapy, the treatment group had statistically significantly higher leukocyte levels ($P < 0.001$) than the control group; the treatment group had an average WBC count of 3000/mm^3, and the control group had an average WBC count of 1000/mm^3. Furthermore, the parameters of quality of life and anxiety revealed significantly better values in the treatment group than in the control group. These results show that adjuvant treatment with mistletoe extracts, in this case Eurixor, is a valuable addition to standard chemotherapy for patients with advanced breast cancer. These results are important because advanced breast cancer carries a very poor prognosis. Similar results in better-designed studies have been shown with various *Viscum* preparations in advanced pancreatic, colon, and liver cancers.[28,36–41]

Detailed reviews of published studies with *Viscum* preparations indicate significant improvements in various quality-of-life parameters, such as coping, fatigue, sleep, exhaustion, energy, nausea, vomiting, appetite, depression, anxiety, ability to work, and emotional and functional well-being in general and, less consistently, in regard to pain, diarrhea, general performance, and side effects of conventional treatments.[42,43]

A study in ovarian cancer consisted of two randomized matched-pair substudies: The first substudy comprised 42 patients with ovarian cancer without distant metastases (Ovar) and 40 ovarian cancer patients with distant metastases (OvarRand). Patients were paired in random allocation to either Iscador or control. The second substudy comprised 150 patients with ovarian cancer without distant metastases (Ovar) and 124 ovarian cancer patients with distant metastases (OvarRand). Patients who had already received therapy with Iscador were matched by the same criteria to control patients. Increased survival rates with Iscador were significant in OvarRand but not in Ovar in both studies.[44]

One study in 220 patients with late-stage pancreatic cancer compared the overall survival of patients receiving an extract of *V. album* or no antineoplastic therapy. Patients with locally advanced or metastatic cancer of the pancreas were stratified according to a binary prognosis index, composed of tumor stage, age, and performance status, and were evenly randomized to subcutaneous injections of *V. album* extract or no antineoplastic therapy. The extract was administered in a dose-escalating manner from 0.01 mg up to 10 mg three times per week. Median overall survival was 4.8 for the group receiving the *Viscum* extract and 2.7 months for control patients (prognosis-adjusted hazard ratio [HR] = 0.49; $p < 0.0001$). Within the "good" prognosis subgroup, median overall survival was 6.6 versus 3.2 months, within the "poor" prognosis subgroup, it was 3.4 versus 2.0 months, respectively. These results showed a significant and clinically relevant prolongation of overall survival with the administration of *V. album* extract.[45]

In a study in 32 operated gastric cancer patients (stage Ib or II) treated with either 5-FU prodrug doxifluridine alone or in combination with *V. album* extract, those in the *Viscum* group demonstrated improvement in white blood counts, reduced reported bouts of diarrhea (7% vs. 50%), and improved quality-of-life scores.[46]

Presurgical treatment with *Viscum* preparations may help prevent the decreased NK cell activity associated with surgery. In a study of patients with colorectal cancer undergoing open tumor resection, patients were randomly assigned to either mistletoe infusion or no additional therapy. The NK cell activity of patients treated with mistletoe extract did not change significantly during the course of the study, whereas the control patients' NK cell activity decreased significantly after surgery (–44.4% at 24 hours).[47]

Immune Support

The immune-enhancing effects of *Viscum* preparations may extend to conditions beyond cancer. For example, Iscador was shown to reduce the rate of recurrent respiratory infections (RRIs) in children 5 to 14 years old living in areas exposed to the radioactive fallout from the Chernobyl nuclear reactor accident.[48] The dosage was two subcutaneous injections a week for 5 weeks. One year after a single-treatment course, the frequency of RRI relapses decreased by 75%. Furthermore, *V. album* therapy resulted in normalization of initial immune indices that were either below or above the normal ranges. This immunomodulatory effect was assessed by investigation of lymphocyte subsets, NK cell activity, phagocytic and oxidative activity of polymorphonuclear leukocytes, and antiviral activity of serum before and 1 week after treatment.

Clearly, additional investigations into the pharmacology of *V. album* are needed. Specifically, it must be determined whether the

effects noted both in vitro and in vivo in animals as well as in patients receiving injectable *Viscum* preparations can be attained with oral administration. In addition, greater clarification is needed to determine optimal *Viscum* preparations. What host tree should be selected for which condition? What is the optimal harvesting time? In what form should the *Viscum* be administered—crude herb, aqueous or alcoholic extract, fermented or nonfermented?

Viscum is undoubtedly one of the most complex botanicals; current data suggest that the future medicinal use of *V. album* looks promising.

DOSAGE

The standard historical dose of *V. album,* based on the *British Herbal Pharmacopoeia,*[49] is as follows:

- Dried leaves: 2 to 6 g (or by infusion) three times a day
- Tincture 1:5 (45% alcohol): 1 to 3 mL three times a day
- Fluid extract 1:1 (25% alcohol): 0.5 mL three times a day
- Dried aqueous extract 4:1: 100 to 250 mg three times a day

TOXICITY

V. album possesses significant toxicity. Historically, the berries have been regarded as being considerably more toxic than the leaves and stems despite the fact that they all contain similar toxic compounds. The berries are considered to be more toxic, probably because of the fatal poisonings of children who ingest the berries.

Lethal doses of *Viscum* lectins administered by various routes to mice produce the following two types of toxicity: (1) a typical type characterized by death after 3 to 4 days with marasmus-like symptoms and (2) an atypical type characterized by immediate death from respiratory paralysis.[23] In mice, the dose that is fatal to 50% of the subjects (LD_{50}) is 32 mg (dry weight) per kilogram of body weight of the plant juice administered intraperitoneally.[29]

The LD_{50} values of *V. album,* either orally administered or in extract form, have not yet been determined. As stated earlier, alcohol-based extracts contain virtually no *Viscum* proteins. This fact would imply significantly less toxicity with these preparations; however, it would also imply loss of activity because much of the pharmacology of *Viscum* relates to its protein content, especially its immune-enhancing activity.

The toxicity of Korean mistletoe, *Viscum album coloratum,* appears to be lower than that of European mistletoe.[30,31] This other species has also demonstrated anticancer effects, but they appear to be due to highly cytotoxic alkaloids rather than to lectins.[5,30] Studies comparing Korean *Viscum* extracts with European extracts as well as their alkaloid components have demonstrated that the Korean mistletoe has greater activity in inhibiting cancer cells. In addition, fresh Korean mistletoe extracts exhibited greater activity than fermented extracts.[5,30] Korean mistletoe may ultimately prove to be superior to European mistletoe.

DRUG INTERACTIONS

There is some theoretical concern that mistletoe's hypotensive effects might be additive with conventional antihypertensive drugs and that its immunostimulant effects could counter immunosuppressant drugs. However, there appear to be no clinical reports of such effects.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Becker H. Botany of European mistletoe *Viscum album (L.)*. *Oncology*. 1986;43:2–7.
2. Nazaruk J, Orlikowski P. Phytochemical profile and therapeutic potential of *Viscum album L. Nat Prod Res*. 2016;30(4):373–385.
3. Anderson LA, Phillipson JD. Mistletoe: the magic herb. *Pharm J*. 1982;229:437–439.
4. Khwaja TA, Dias CB, Pentecost S. Recent studies on the anticancer activities of mistletoe (*Viscum album*) and its alkaloids. *Oncology*. 1986;43:42–50.
5. Jordan E, Wagner H. Structure and properties of polysaccharides from *Viscum album (L.)*. *Oncology*. 1986;43:8–15.
6. Wagner H, Jordan E, Feil B. Studies on the standardization of mistletoe preparations. *Oncology*. 1986;43:16–22.
7. Franz H, Ziska P, Kindt A. Isolation and properties of three lectins from mistletoe *Viscum album (L.)*. *Biochem J*. 1981;195:481–484.
8. Olsnes S, Stirpe F, Sandvig K, et al. Isolation and characterization of viscumin, a toxic lectin from *Viscum album L.* (mistletoe). *J Biol Chem*. 1982;257(13):263–270.
9. Petricic J, Kalogjera Z. Isolation of glucosides from mistletoe leaves *Viscum album (L.)*. *Acta Pharm Jugosl*. 1980;30:163.
10. Wagner H, Feil B, Kalogjera Z, et al. Phenylpropanes and lignins of *Viscum album* cardioactive drugs. *Planta Medica*. 1986;2:102.
11. Evans MR, Preece AW. *Viscum album*: a possible treatment for cancer? *Bristol Med Chir J*. 1973;88:17–20.
12. Klamerth O, Vester F, Kellner G. Inhibitory effects of a protein complex from *Viscum album* on fibroblasts and HeLa cells. *Hoppe Seylers Z Physiol Chem*. 1968;349:863–864.
13. Ribereau-Gayon G, Jung ML, Di Scala D, et al. Comparison of the effects of fermented and unfermented mistletoe preparations on cultured tumor cells. *Oncology*. 1986;43:35–41.
14. Hulsen H, Doser C, Mechelke F. Differences in the in vitro effectiveness of preparations produced from mistletoes of various host trees. *Arzneimitteforschung*. 1986;36:433–436.
15. Hulsen H, Mechelke F. The influence of a mistletoe preparation on suspension cell cultures of human leukemia and human myeloma cells. *Arzneimitteforschung*. 1982;32:1126–1127.
16. Petkov V. Plants with hypotensive, antiatheromatous and coronarodilatating action. *Am J Chin Med*. 1979;7:197–236.
17. Hajto T. Immunomodulatory effects of Iscador: a *Viscum album* preparation. *Oncology*. 1986;43:51–65.
18. Jordan E, Wagner H. Detection and quantitative determination of lectins and viscotoxins in mistletoe preparations. *Arzneimitteforschung*. 1986;36:428–433.
19. Bloksma N, Dijk HV, Korst P, et al. Cellular and humoral adjuvant activity of a mistletoe extract. *Immunobiology*. 1979;156:309–319.
20. Bloksma N, Schmiermann P, deReuver MD, et al. Stimulation of humoral and cellular immunity by viscum preparations. *Planta Med*. 1982;46:221–227.
21. Rentea R, Lyon E, Hunter R. Biological properties of Iscador. A *Viscum album* preparation I. Hyperplasia of the thymic cortex and accelerated regeneration of hematopoietic cells following X-irradiation. *Lab Invest*. 1981;44:43–48.
22. Hajto T, Lanzrein C. Natural killer and antibody-dependent cell-mediated cytotoxicity activities and large granular lymphocyte frequencies in *Viscum album*-treated breast cancer patients. *Oncology*. 1986;43:93–97.
23. Nienhaus J, Stoll M, Vester F. Thymus stimulation and cancer prophylaxis by *Viscum* proteins. *Experientia*. 1970;26:523–525.
24. Hamprecht K, Handretinger R, Voetsch W, et al. Mediation of human NK-activity by components in extracts of *Viscum album*. *Int J Immunopharmacol*. 1987;9:199–209.
25. Kleijnen J, Knipschild P. Mistletoe treatment for cancer: review of controlled trials in humans. *Phytomedicine*. 1994;1:255–260.
26. Janssen O, Scheffler A, Kabelitz D. In vitro effects of mistletoe extracts and mistletoe lectins: cytotoxicity towards tumor cells due to the induction of programmed cell death (apoptosis). *Arzneimitteforschung*. 1993;43:1221–1225.
27. Heiny BM. Adjuvant treatment with standardized mistletoe extract reduces leukopenia and improves the quality of life of patients with advanced carcinoma of the breast getting palliative chemotherapy (VEC regimen). *Krebsmedizin*. 1991;12:3–14.
28. Friess H, Beger HG, Kunz J, et al. Treatment of advanced pancreatic cancer with mistletoe: results of a pilot trial. *Anticancer Res*. 1996;16:915–920.
29. Duke JA. *Handbook of Medicinal Herbs*. Boca Raton, FL: CRC Press; 1986.
30. Khwaja TA, Varven JC, Pentecost S, et al. Isolation of biologically active alkaloids from Korean mistletoe *Viscum album*, coloratum. *Experientia*. 1980;36:599–600.
31. Manjikian S, Pentecost S, Khwaja TA. Isolation of cytotoxic proteins from *Viscum album,* coloratum. *Proc Am Assoc Cancer Res*. 1986;27:266.
32. Braedel-Ruoff S. Immunomodulatory effects of *Viscum album* extracts on natural killer cells: review of clinical trials. *Forsch Komplementmed*. 2010;17(2):63–73.
33. Elluru SR, Duong Van Huyen JP, et al. Antiangiogenic properties of *Viscum album* extracts are associated with endothelial cytotoxicity. *Anticancer Res*. 2009;29(8):294529–294550.
34. Lyu SY, Kwon YJ, Joo HJ, et al. Preparation of alginate/chitosan microcapsules and enteric coated granules of mistletoe lectin. *Arch Pharm Res*. 2004;27:118–126.
35. Grossarth-Maticek R, Kiene H, Baumgartner SM, et al. Use of Iscador, an extract of European mistletoe *(Viscum album)*, in cancer treatment: prospective nonrandomized and randomized matched-pair studies nested within a cohort study. *Altern Ther Health Med*. 2001;7:57–66, 68–72, 74–76.
36. Cazacu M, Oniu T, Lungoci C, et al. The influence of isorel on the advanced colorectal cancer. *Cancer Biother Radiopharm*. 2003;18:27–34.
37. Mabed M, El-Helw L, Shamaa S. Phase II study of viscum fraxini-2 in patients with advanced hepatocellular carcinoma. *Br J Cancer*. 2004;90:65–69.
38. Kovacs E, Kuehn JJ. Measurements of IL-6, soluble IL-6 receptor and soluble gp130 in sera of B-cell lymphoma patients: does viscum album treatment affect these parameters? *Biomed Pharmacother*. 2002;56:152–158.
39. Semiglasov VF, Stepula VV, Dudov A, et al. The standardised mistletoe extract PS76A2 improves QoL in patients with breast cancer receiving adjuvant CMF chemotherapy: a randomised, placebo-controlled, double-blind, multicentre clinical trial. *Anticancer Res*. 2004;24:1293–1302.
40. Bock PR, Friedel WE, Hanisch J, et al. [Efficacy and safety of long-term complementary treatment with standardized European mistletoe extract *(Viscum album L.)* in addition to the conventional adjuvant oncologic therapy in patients with primary non-metastasized mammary carcinoma. Results of a multi-center, comparative, epidemiological cohort study in Germany and Switzerland. *Arzneimittelforschung*. 2004;54:456–466.
41. Piao BK, Wang YX, Xie GR, et al. Impact of complementary mistletoe extract treatment on quality of life in breast, ovarian and non-small cell lung cancer patients: a prospective randomized controlled clinical trial. *Anticancer Res*. 2004;24:303–309.
42. Attar R, Tabassum S, Fayyaz S, et al. Natural products are the future of anticancer therapy: Preclinical and clinical advancements of Viscum album phytometabolites. *Cell Mol Biol (Noisy-le-grand)*. 2015;61(6):62–68.
43. Evans M, Bryant S, Huntley AL, Feder G. Cancer patients' Experiences of using mistletoe *(Viscum album)*: a qualitative systematic review and synthesis. *J Altern Complement Med*. 2016;22(2):134–144.
44. Grossarth-Maticek R, Ziegler R. Prospective controlled cohort studies on long-term therapy of ovairian cancer patients with mistletoe (*Viscum album L.)* extracts iscador. *Arzneimittelforschung*. 2007;57(10):665–678.
45. Tröger W, Galun D, Reif M, et al. *Viscum album [L.]* extract therapy in patients with locally advanced or metastatic pancreatic cancer: a randomised clinical trial on overall survival. *Eur J Cancer*. 2013;49(18):3788–3797.
46. Kim KC, Yook JH, Eisenbraun J, Kim BS, Huber R. Quality of life, immunomodulation and safety of adjuvant mistletoe treatment in patients with gastric carcinoma—a randomized, controlled pilot study. *BMC Complement Altern Med*. 2012;12:172.
47. Schink M, Tröger W, Dabidian A, et al. Mistletoe extract reduces the surgical suppression of natural killer cell activity in cancer patients: a randomized phase III trial. *Forsch Komplementmed*. 2007;14(1):9–17.
48. Chernyshov VP, Heusser P, Omelchenko LI, et al. Immunomodulatory and clinical effects of Viscum album (Iscador M and Iscador P) in children with recurrent respiratory infections as a result of the Chernobyl nuclear accident. *Am J Ther*. 2000;7:195–203.
49. British Herbal Medicine Association, Scientific Committee. British herbal pharmacopoeia. Cowling. *Br Herb Med Associa*. 1983:235–236.

Vitamin A

Joseph Katzinger, ND, and *Michael T. Murray*, ND*

OUTLINE

INTRODUCTION

Vitamin A was the first fat-soluble vitamin to be recognized. Although identified as a necessary growth factor in 1913, it was not characterized chemically until 1930. The initial discovery of vitamin A was made almost simultaneously by two groups of research workers, McCollum and Davis at the University of Wisconsin and Osborne and Mendel at Yale University. They found that young animals fed a diet deficient in natural fats became very unhealthy, as evidenced by the inability to grow and poor immune function. These researchers also noted that the animals' eyes would become severely inflamed and infected with the restricted diet and that this condition could be quickly relieved by the addition to the diet of either butterfat or cod liver oil. Vitamin A was once known as the "antiinfective vitamin," and vitamin A status remains a major determinant of immune status.

Nomenclature

When isolated in its pure form, vitamin A is a pure, lipid-soluble yellow crystal with a condensed formula $C_{20}H_{29}OH$. Vitamin A is termed retinol, signifying that it is an alcohol that is intricately involved in the function of the retina of the eye. All-trans retinol is found in nature primarily as long-chain retinyl esters. The aldehyde form of all-trans retinol is commonly designated retinaldehyde or retinal, whereas the acidic form is termed retinoic acid. It has been suggested that retinol serves only as a precursor to these two active forms of vitamin A—retinal being primarily involved with vision and reproduction and retinoic acid being important in other somatic functions, such as growth and differentiation (Fig. 125.1).[1]

Synthetic derivatives of retinoic acid have been developed to treat many dermatological conditions and, more recently, certain forms of cancer. These compounds, however, are not without side effects.

Recommended Dietary Allowance

Vitamin A was originally measured in international units (IUs), with 1 IU being defined as 0.3 mg of crystalline all-trans retinol or 0.6 mg β-carotene. In 1967, an Expert Committee of the United Nations Food and Agriculture Organization and World Health Organization recommended that vitamin A activity be referred to in terms of retinol equivalents rather than in IU, with 1 mg of retinol being equivalent to 1 retinol equivalent (RE). The amount of β-carotene required for 1 RE is 6 mg, whereas the amount required for other provitamin A carotenoids is

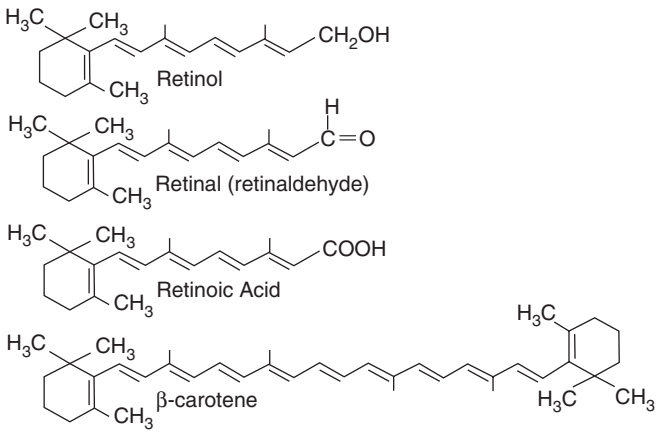


Fig. 125.1 Forms of vitamin A.

*Previous edition contributor

12 mg. In 1980, the Food and Nutrition Board of the National Research Council/National Academy of Sciences adopted this recommendation, and the 1980 recommended dietary allowance (RDA) for vitamin A is stated in milligrams and retinol equivalents; in 2001 the RDAs were revised to today's values (Table 125.1), which use μg RAE (retinol activity equivalent) instead of RE as the standard unit, with 1 μg RAE equivalent to 1 μg all-trans retinol. A distinction between dietary β-carotene and supplemental β-carotene was also made because the latter has much greater absorption as an oil. Thus the current accepted conversion is 1 μg RAE = 1 μg of all-trans retinol = 2 μg of supplemental all-trans β-carotene = 12 μg of dietary all-trans β-carotene = 24 μg of other dietary provitamin A carotenoids (see Fig. 125.2).[2]

Dietary Sources

The most concentrated sources of preformed vitamin A are liver, kidney, butter, whole milk, and fortified skim milk, whereas the leading sources of provitamin A are dark-green leafy vegetables (collards and spinach) and yellow-orange vegetables (carrots, sweet potatoes, yams, and squash) (Table 125.2). Ingestion of excessive amounts of liver—2.7 to 11 kg/week—has been reported to cause hypervitaminosis A.[4]

DEFICIENCY

Vitamin A deficiency may be due to inadequate dietary intake (primary deficiency) or to some secondary factor that interferes with the absorption, storage, or transportation of vitamin A. Some factors known to induce a vitamin A deficiency are as follows:

- Malabsorption due to bile acid or pancreatic insufficiency
- Protein-energy malnutrition
- Liver disease
- Zinc deficiency
- Abetalipoproteinemia
- Poor conversion of β-carotene to vitamin A

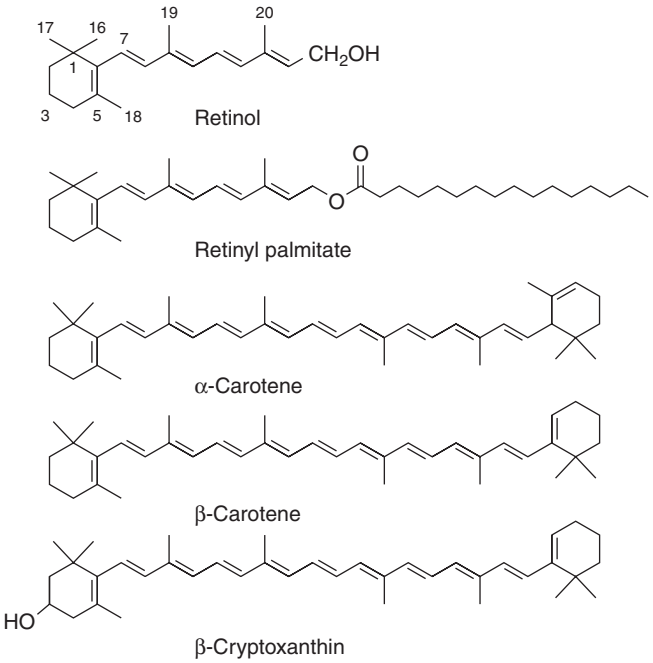


Fig. 125.2 Major forms of vitamin A. Preformed retinol is primarily found as retinyl palmitate in most animal livers, fortificants, and supplements. Of the approximately 50 provitamin A carotenoids in plants, the common ones include α-carotene, β-carotene, and β-cryptoxanthin. These forms of provitamin A carotenoids are often quantified in human serum. (From Tanumihardjo SA, Russell RM, Stephensen CB, et al. Biomarkers of nutrition for development (bond)-vitamin A review. J. Nutr. 2016;146:1816S–1848S. PMID: 27511929. Reproduced from a free access article, distributed under terms [http://www.nutrition.org/publications/guidelines-and-policies/license/].)

TABLE 125.1 Recommended Dietary Allowances

Life Stage Group	Retinol MCG/Day (Retinol Equivalents)	International Units (IU)
Infants (adequate intakes)		
0–6 months	400	1320
7–12 months	500	1500
Children		
1–3 years	300	1000
4–8 years	400	1320
Males		
9–13 years	600	2000
14–18 years	900	3000
19 to >70 years	900	3000
Females		
9–13 years	600	2000
14–18 years	700	2310
19 to >70 years	700	2310
Pregnancy		
<19 years	750	3750
19 to >50 years	770	3950
Lactation		
<19 years	1200	4000
19 to >50 years	1300	4300

TABLE 125.2 Food Sources of Vitamin A

Food	Portion Size	IU/Portion
Meats		
Beef liver, fried	100 g	50,375
Calf liver, cooked	100 g	26,872
Chicken liver, cooked	2 livers	25,760
Vegetables		
Sweet potatoes, baked	1 medium	14,600
Carrots, raw	1 large	11,000
Spinach, raw	100 g	8100
Carrots, cooked	½ cup	8000
Pumpkin, cooked	½ cup	8000
Spinach, cooked	½ cup	7300
Collard greens	½ cup	5400
Broccoli, cooked	½ cup	1900
Fruits		
Watermelon	1/16 melon	5310
Cantaloupe	¼ melon	3400
Apricots, dried	4 halves	2275
Apricots, raw	2–3 medium	2700
Nectarines, raw	1 medium	1650

Data from Selhorst JB, Waybright EA, Jennings S, et al. Liver lover's headache: pseudotumor cerebri and vitamin A intoxication. *JAMA*. 1984;252:3365.

Immune System Effects

Immune system abnormalities associated with a vitamin A deficiency include impaired ability to mount an effective antibody response, decreased levels of helper T cells, and alterations in the mucosal linings of the respiratory and gastrointestinal tracts. Vitamin A–deficient individuals are more susceptible to infectious diseases and have higher mortality rates. It appears that although a vitamin A deficiency may predispose an individual to an infection, vitamin A stores are also severely depleted during the course of an infection. Thus a vicious cycle ensues. Infectious conditions associated with vitamin A deficiency include measles, chickenpox, respiratory syncytial virus, acquired immunodeficiency syndrome, and pneumonia. Lower vitamin A levels have also been linked to asthma, at least in children, and may parallel pulmonary function in this population. Despite this association, supplementation has not yet shown to be of benefit.[5–7]

Other Effects

Prolonged vitamin A deficiency results in the characteristic signs of follicular hyperkeratosis (buildup of cellular debris in the hair follicles, giving the skin a goose-bump appearance, which occurs most often on the back of the upper arm), night blindness, and a higher rate of infection. As the condition worsens, the mucous membranes of the respiratory tract, gastrointestinal tract, and genitourinary tract also become affected, and the classic eye disease known as xerophthalmia due to vitamin A deficiency ensues. Even a mild vitamin A deficiency is associated with a significant rise in mortality. This association is extremely significant because vitamin A deficiency is particularly widespread in developing countries, especially in Asia, where as many as 10 million children are found to have xerophthalmia each year.[8] In 2009, the World Health Organization estimated that as many as 190 million preschool children and over 19 million pregnant women have a vitamin A deficiency, using serum retinol as a criterion.[9] The prevalence of vitamin A deficiency in developed countries is less recognized but may be more common than appreciated. For example, data from the U.S. National Health and Nutrition Examination Survey (NHANES) from 2003 to 2008 suggest that more than 75% of those age 19 to 50 have an intake below guidelines.[10]

Xerophthalmia

The term *xerophthalmia* is generally used to cover all the ocular manifestations of vitamin A deficiency. Blindness is one of the most serious consequences of vitamin A deficiency. Although it rarely occurs in the United States, it is the major preventable cause of blindness in Asia. The xerophthalmia of vitamin A deficiency is staged as shown in Table 125.3.

TABLE 125.3 Staging of the Xerophthalmias

Stage	Diagnosis	Signs and Symptoms
X0	Night blindness	Poor dark adaptation
X1A	Xerosis of conjunctiva	Dryness with "lackluster" appearance, thickening, wrinkling, and diffuse pigmentation of conjunctiva
X1B	Biot spots	Usually triangular collections of desquamated, keratinized epithelial cells and mucus
X2	Xerosis of cornea	Dryness of cornea, leading to keratinization and a hazy, milky appearance
X3	Keratomalacia	Ulceration, distortion, and softening of the cornea with eventual perforation and prolapse and infection of the iris

In an effort to prevent vitamin A deficiency in underdeveloped countries, large prophylactic doses (200,000 IU) are given by the World Health Organization to children every 6 months.

Determination of Deficiency

The rapid dark adaptation test is perhaps the most sensitive of the currently available tests designed to determine vitamin A deficiency. Measurement of serum retinol levels is usually not useful because they may not drop until marked deficiency occurs. Retinol isotope dilution is the most sensitive test to measure vitamin A status, and although technically difficult to perform, it measures total body stores ranging from deficiency to hypervitaminosis. The MRDR test is less demanding to perform, and although more accurate than serum retinol levels, it cannot define vitamin A status above adequacy.[3] Deficiency in the United States and other developed countries is usually secondary to malabsorption, liver disease, or proteinuria.

METABOLISM

Absorption

A variety of factors are known to influence the absorptive efficiency of vitamin A and carotenoids. Although retinol does not require bile acids to facilitate absorption, carotenoids do. Other factors that affect vitamin A and carotenoid absorption are as follows:

- The presence of fat, protein, and antioxidants in food
- The presence of bile and a normal complement of pancreatic enzymes in the intestinal lumen
- The integrity of the mucosal cells

The absorptive efficiency of dietary vitamin A is usually quite high (80%–90%), with only a slight reduction in efficiency at high doses. In contrast, dietary β-carotene's absorptive efficiency is much lower (approximately 10%), and it diminishes rapidly with increasing dosage. Carotene supplements are better absorbed than the carotenes from foods (β-carotene has a roughly sixfold higher absorption as a supplement vs. dietary source.)[11] Both retinyl palmitate and β-carotene are transferred to lipid droplets and mixed micelles; although β-carotene is unaltered at this stage, retinyl palmitate is hydrolyzed to retinol by enzymes that include pancreatic lipase (encoded by PNLIP; Fig. 125.3).

Transformation in the Intestinal Mucosa

The majority of absorbed retinol is esterified with palmitic acid or another free fatty acid within the intestinal mucosal cells. To cross into the cytoplasm of the enterocyte, it is currently speculated that β-carotene's uptake is facilitated by scavenger-receptor class B-type I (SR-BI) and CD36 molecule, although this mechanism is not well established. SR-BI also facilitates cholesterol, vitamin E, and vitamin K uptake.

A number of additional enzymes are thought to be involved in the metabolism, transport, and ultimately the incorporation (along with triglycerides, phospholipids, and cholesterol esters) into chylomicra, including cellular retinol-binding protein type II (CRBPII) and β-carotene oxygenase 1 (BCO1), the primary enzyme involved in β-carotene cleavage (Fig. 125.4). Each chylomicron is transported through the lymphatic channels into the general circulation and eventually removed from the circulation by the liver.

Transport, Storage, and Excretion

Upon reaching the liver, vitamin A is stored primarily in special perisinusoidal lipocytes (Ito cells), which store 70% to 90% of liver vitamin A; the hepatocytes contain only a minor fraction of the total vitamin A stored in the liver. Although small amounts of vitamin A are found in most tissues (Table 125.4), more than 70% of the total body vitamin A content is stored in the liver.[12] It is stored as a lipoglycoprotein complex

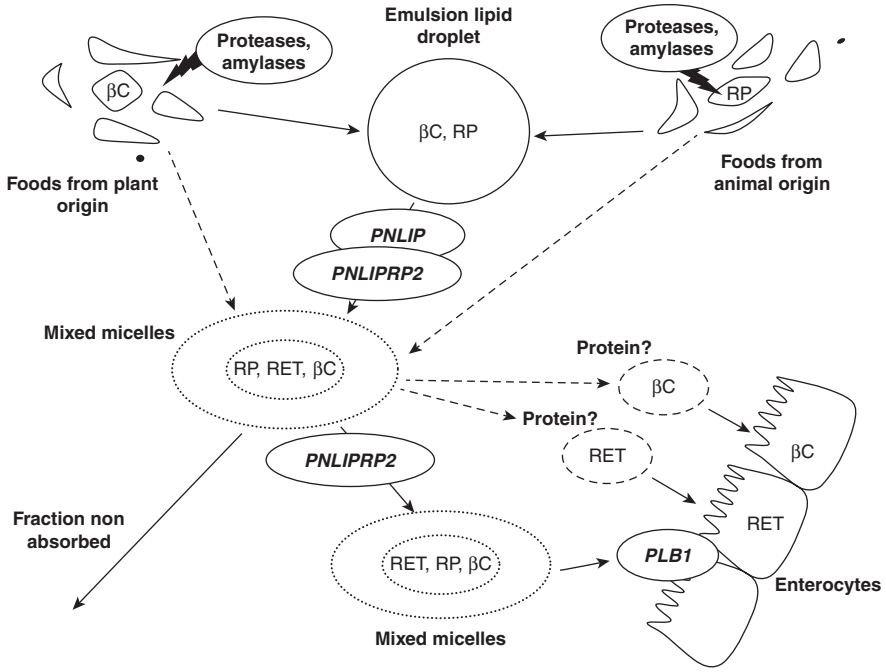


Fig. 125.3 Vitamin A metabolism in upper gastrointestinal tract. Proteins are involved, or hypothesized to be involved, in vitamin A (VA) metabolism within the lumen of the upper gastrointestinal tract. *βC,* β-carotene and all other provitamin A carotenoids; *PLB1,* phospholipase B; *PNLIP,* pancreatic lipase; *PNLIPRP2,* pancreatic lipase-related protein 2; *RET,* retinol; *RP,* retinyl palmitate and all other retinyl esters. Proteins followed by a question mark have been hypothesized to be involved because RET and βC are not soluble in water, and thus nonmicellarized VA is assumed to be associated with proteins. A dotted arrow means the pathway is suspected to exist, but there is no evidence thereof yet.[13] (From Borel P, Desmarchelier C. Genetic variations associated with vitamin A status and vitamin A Bioavailability. *Nutrients.* 2017;9(3):E246. PMID: 28282870 Reproduced from an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license [http://creativecommons.org/licenses/by/4.0/].)

consisting of 96% retinyl esters and 4% unesterified retinol. At least two lipases are necessary for the mobilization of retinyl ester stores, adipose triglyceride lipase (ATGL) and patatin-like phospholipase domain-containing 3 (PNPLA3) protein. The retinyl esters are hydrolyzed by a tightly bound retinyl ester hydrolase, which transfers the released all-trans retinol to intracellular retinol-binding protein (RBP), which in turn binds to transthyretin (TTR) (Fig. 125.5).[13] The bound retinol is then processed through the Golgi apparatus and secreted into the plasma, where it forms a reversible 1:1 molar complex with prealbumin.[1,14]

It is worth mentioning that a polymorphism in one of the lipases just mentioned, PNPLA3, has emerged as a robust link between an apparent vitamin A deficiency and nonalcoholic fatty liver disease (NAFLD). Although many details regarding the precise role of this enzyme are still unknown, it appears that the I148M variant (rs738409, G allele) has limited lipase activity, leading to lower circulating retinol levels, particularly among overweight individuals. Despite an apparent vitamin A deficiency (as measured by serum retinol), hepatic stellate cell retention of retinol is increased, and along with increased production of triglycerides, this appears to drive the pathogenesis of NAFLD, as well as progression to steatohepatitis (NASH) and cancer.[15] Furthermore, the at-risk allele has been linked to both NASH as well as low bone mineral density among adolescents and may be a causal factor.[16]

Adequate dietary protein, iron, and zinc are necessary for proper retinol mobilization. The half-lives of RBP and prealbumin are less than 12 hours, making them particularly likely to be deficient during protein-calorie malnutrition or other situations in which protein metabolism is abnormal. A zinc, iron, or vitamin E deficiency also severely impairs vitamin A metabolism because these nutrients function synergistically in many physiological processes of vitamin A metabolism (absorption, transport, and mobilization in particular).[17,18]

Retinol is transferred into the cell after RBP binds to a cell-surface receptor. The retinol is then quickly bound by cellular retinol-binding protein (CRBP) in the cell cytosol.

Retinoic acid is metabolized differently from retinol. It is absorbed through the portal system and transported in the plasma bound to albumin. It does not accumulate in the liver or other tissues in any appreciable amounts. It is metabolized quite rapidly to more polar oxygenated compounds. Intracellularly, it is bound to the cellular retinoic acid–binding protein.

Vitamin A metabolites are excreted mainly through the feces (via the bile) and the urine. During periods of deficiency, there appears to be an adaptation in utilization, as evidenced by a reduction in the rate of vitamin A catabolism.[1,14] Indeed, blood levels of retinol are tightly regulated, with fasting concentrations ranging from 2 to 4 μM in adults).[12]

Conversion of Beta-Carotene to Vitamin A

Emerging research is revealing genomic variations that result in impaired conversion of β-carotene to vitamin A are surprisingly common. Single-nucleotide polymorphisms have been identified that result in decreased activity of 15,15′-monooxygenase, the key enzyme responsible for the conversion of β-carotene to retinol. The R267S (rs12934922) and A379V (rs7501331) variants are common, with frequencies in the population of 42% and 24%, respectively. Of these two variants, the latter appears to have a greater effect; those with at least one copy of the less common allele have a 32% drop in activity (presumably more among homozygotes). Those with both 267S and 379V have a 57% reduction in

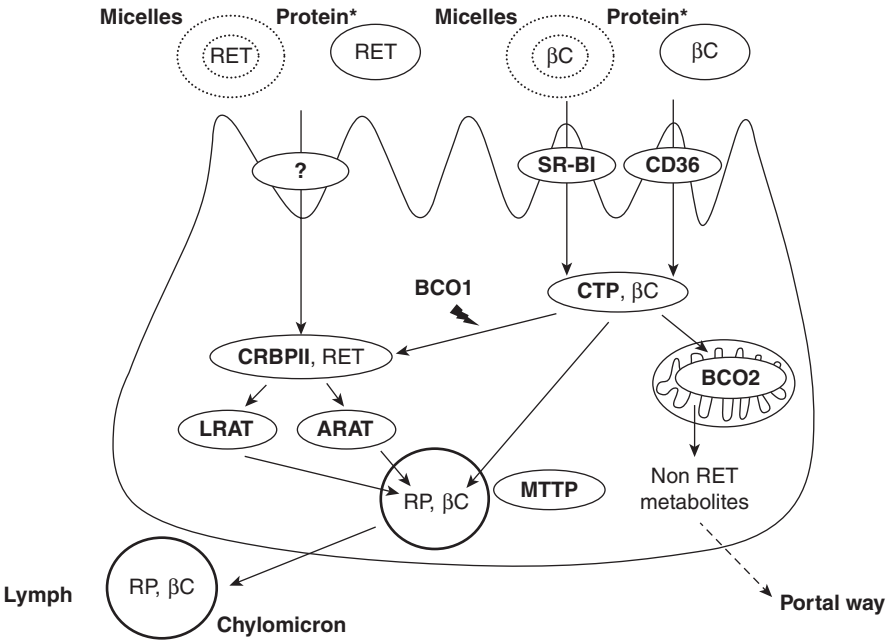


Fig. 125.4 Enterocyte metabolism of vitamin A. Proteins involved in vitamin A metabolism within the enterocyte. *ARAT,* acyl-CoA:retinol acyltransferases; *βC,* β-carotene and all other provitamin A carotenoids; *BCO1,* β-carotene oxygenase 1; *BCO2,* β-carotene oxygenase 2; *CD36,* cluster determinant 36; *CRBPII,* cellular retinol binding protein II; *CTP,* cellular transport protein (BCO1 and L-FABP are candidates); *LRAT,* lecithin retinol acyltransferase; *MTTP,* microsomal triglyceride transfer protein; *RET,* retinol; *RP,* retinyl palmitate and all other retinyl esters; *SR-BI,* scavenger receptor class B type I. It is assumed that there is an apical transporter of RET, but because it has not been identified, a question mark has been added.[13] (From Borel P, Desmarchelier C. Genetic variations associated with vitamin A status and vitamin A Bioavailability. *Nutrients*. 2017;9(3):E246. PMID: 28282870 Reproduced from an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license [http://creativecommons.org/licenses/by/4.0/].)

TABLE 125.4 Distribution of Vitamin A in Some Human Tissues (mcg/kg)

Tissue	Vitamin A
Adrenal gland	10.4 ± 7.1
Liver	149 ± 132
Testis	1.14 ± 1.23
Fat	1.46 ± 1.55
Pancreas	0.52 ± 0.28
Spleen	0.89 ± 0.88
Lung	0.91 ± 1.89
Thyroid	0.43 ± 0.33

conversion rate. Those with these variants have increased blood levels of β-carotene and decreased blood levels of vitamin A.[19]

This may be especially concerning among groups with a low intake of preformed vitamin A, such as vegans. A review of dietary intake among Western industrialized nations found that approximately 35% of vitamin A intake is via carotenoids, although individuals who consume only one component (preformed vitamin A, or carotenoids alone) are unlikely to reach a recommended intake, a concern exacerbated by poor conversion.[20]

PHYSIOLOGICAL ROLES OF VITAMIN A

Vision

The best-understood physiological role of vitamin A is its effects on the visual system. The human retina has four kinds of vitamin A–containing photopigments. Rhodopsin, maximum absorption at 498 nm, is present in the rods. The three iodopsins, as follows, are in the cones:

- Blue cones (maximum absorption 420 nm)
- Green cones (maximum absorption 534 nm)
- Red cones (maximum absorption 563 nm)

The vitamin A form found in these pigments is the 11-cis isomer of vitamin A aldehyde (retinal). When a photon of light strikes the dark-adapted retina, the 11-cis configuration is converted to the all-trans form of the retinaldehyde and split from the rhodopsin molecule to yield opsin and all-trans retinol. This leads to a change in membrane potential and subsequent visual excitation. During light adaptation, because the visual processes largely depend on the cone cells, the released all-trans retinal or retinol from the rod cells is transported to pigment epithelial cells and stored as retinyl palmitate. During dark adaptation, these processes are reversed, and the retinal is isomerized to the 11-cis form. The rod cells are particularly sensitive to vitamin A deficiency, so night blindness or poor dark adaptation is an early consequence of vitamin A deficiency. This sensitivity may have clinical significance in older adults with normal retinal health or early age-related maculopathy (ARM). In a double-blind, placebo-controlled experiment, 104 adults 50 years of age or older whose fundic photographs for the eye fell within steps 1 to 9 of the Age-Related Eye Disease Study Grading System were randomly assigned to a 30-day course of 50,000 IU oral retinol or a placebo. At 30 days, the retinol intervention group had significantly larger (i.e., steeper) rod slopes, indicating faster recovery of sensitivity, than did the placebo group. It is believed that the structural changes in the retinal pigment epithelium and Bruch's membrane in aging and early ARM cause a localized vitamin A deficiency.[14] Despite this finding, the link between intake of vitamin A (as well as β-carotene) and macular drusen (a

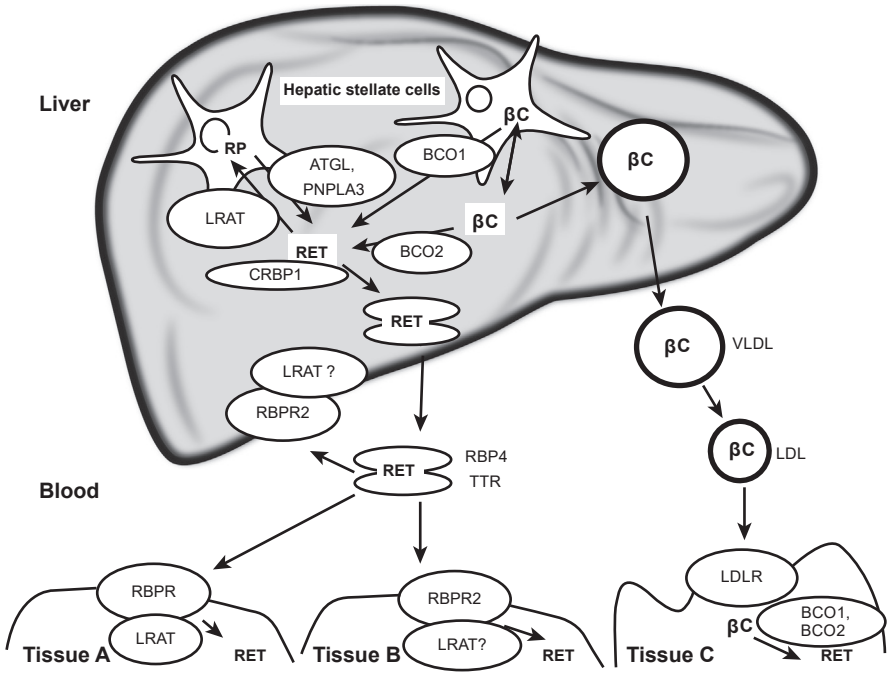


Fig. 125.5 Proteins involved in the liver metabolism of vitamin A. *ATGL,* adipose triglyceride lipase; *βC,* β-carotene and all other provitamin A carotenoids; *BCO1,* β-carotene oxygenase 1; *BCO2,* β-carotene oxygenase 2; *CD36,* cluster determinant 36; *CRBPI,* cellular retinol binding protein I; *LDLR,* LDL receptor; *LRAT,* lecithin retinol acyltransferase; *PNPLA3,* patatin-like phospholipase domain-containing 3; *RBPR2,* RBP4 receptor-2; *RBP4,* serum retinol-binding protein; *RBPR,* RBP receptor (encoded by STRA6); *RET,* retinol; *RP,* retinyl palmitate and all other retinyl esters; *TTR,* transthyretin. The liver is the hub of vitamin A metabolism: it is the main organ that stores VA and distributes it to the peripheral tissues. VA reaches the liver mainly as retinyl esters, mainly RP, and provitamin A carotenoids, mainly βC, incorporated in chylomicrons after VA absorption. VA is then mostly stored in hepatic stellate cells. This figure shows the main proteins involved in the mobilization of the liver stores of VA and in the distribution of liver VA to peripheral tissues.[13] (From Borel P, Desmarchelier C. Genetic variations associated with vitamin A status and vitamin A Bioavailability. *Nutrients.* 2017;9(3):E246. PMID: 28282870 Reproduced from an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license [http://creativecommons.org/licenses/by/4.0/].)

precursor for degeneration) is still in question. Recent studies have pointed to an increase in risk for drusen with a higher dietary intake of preformed vitamin A in those with a genetic predisposition, possibly due to an increase in retinol-based toxic waste products and/or lipofuscin.[21]

Growth and Development

Vitamin A is believed to affect growth and development through its necessary role in the synthesis of many glycoproteins (e.g., mucus), some of which may control cellular differentiation, and by its function as CRBP in controlling gene expression.[1]

The adhesion between cells is apparently related to glycoprotein synthesis, which is markedly depressed in vitamin A deficiency. Consequently, there is a loss of normal stimuli for cellular growth and differentiation during deficiency. CRBP is transferred directly into the nucleus and may function much like some of the steroid hormones. The effects of a vitamin A deficiency most readily seen at the cellular level are in those differentiating tissues that have a rapid turnover rate—epithelial cells of the oral cavity, respiratory tract, urinary tract, and ducts of the secretory glands.[1]

Development and Maintenance of Epithelial Tissue

The role of vitamin A and carotenoids in the development and maintenance of epithelial tissue cannot be overstated. Vitamin A status determines whether mucin or keratin is synthesized in epidermal cells—the presence of adequate vitamin A results in mucin production, whereas a lack leads to hyperkeratinization of the skin, cornea, upper respiratory tract, and genitourinary tract. Mucopolysaccharide synthesis also appears to depend on adequate vitamin A status.[1,22]

Reproduction

The requirement of vitamin A for reproductive functions in higher animals has been known since 1922. Beta-carotene has also been reported to have a specific effect in fertility distinct from its role as a precursor to vitamin A.[23–25] In bovine nutritional studies, cows fed β-carotene–deficient diets exhibited delayed ovulation and an increase in the number of follicular and luteal cysts.[23,25] The corpus luteum has the highest concentration of β-carotene of any organ measured.[25] The carotene cleavage activity changes with the ovulation cycle, with the highest activity occurring during the midovulation stage. It has been speculated that a proper ratio of carotene to retinol must be maintained to ensure proper functioning of the corpus luteum.

The corpus luteum produces progesterone; therefore inadequate corpus luteum function could have significant deleterious effects. Inadequate corpus luteum secretory function is one of the characteristic features of infertile and/or irregular menstrual cycles.[26] Furthermore, an increased estrogen/progesterone ratio has been implicated in a variety of clinical conditions, including ovarian cysts, premenstrual tension syndrome, fibrocystic breast disease, and breast cancer.[27] Because supplemental β-carotene given to cows significantly reduced

the incidence of ovarian cysts (42% in the control group vs. 3% in the β-carotene group), it may have a similar effect in humans.[24,25] Another bovine condition that has benefited from higher levels of dietary β-carotene is cystic mastitis.[26] It appears that farmers have a greater appreciation of β-carotene than many nutritionists. Of course, there are significant financial reasons for this because the annual monetary loss from bovine mastitis in the United States has been estimated to be at least $1.5 to $2 billion, and ovarian cysts represent the major cause of infertility in cattle.

Immune System

Vitamin A is absolutely essential to proper immune function. Vitamin A plays an essential role in maintaining the epithelial and mucosal surfaces and their secretions. These parts of the immune system constitute a primary nonspecific host defense mechanism. Furthermore, vitamin A has been shown to stimulate and/or enhance numerous immune processes, including induction of antitumor activity, enhancement of white blood cell function, and an increased antibody response.[28] These effects are not due simply to a reversal of vitamin A deficiency because many are further enhanced by (supposedly) excessive amounts of vitamin A. Retinol has also demonstrated significant antiviral activity and has prevented the immunosuppression induced by glucocorticoids, severe burns, and surgery. Some of these effects are probably related to vitamin A's ability to prevent stress-induced thymic involution and promote thymic growth. The carotenes, which are better antioxidants, may turn out to be even more effective in protecting the thymus gland than vitamin A because the thymus gland is particularly susceptible to free radical and oxidative damage.

CLINICAL APPLICATIONS

Adequate tissue vitamin A levels are vital for optimal health. In addition, this nutrient can be used beyond its "physiological" role in the treatment of various conditions. Supplemental vitamin A is used primarily to enhance the immune system in viral illnesses and to treat numerous skin disorders.

Natural vitamin A is available as either retinol or retinyl-palmitate. Absorption may be improved via micellization or emulsification. Micellization is the process of making the fat-soluble vitamin A into very small droplets (micelles) so that the material is dispersed in water. Emulsification is the process of suspending vitamin A in a colloidal mixture with another chemical (e.g., lecithin) so that it can mix with water. Despite manufacturers' claims, it is important to remember that regular vitamin A is absorbed at a rate of 80% to 90%.

Infections

As discussed previously, vitamin A is absolutely critical to a healthy, functioning immune system. Vitamin A–deficient individuals are more susceptible to infectious diseases in general but especially parasitical and viral infections. Although vitamin A deficiency may predispose an individual to an infection, vitamin A stores are severely depleted during an infection.

Parasitical Infections

Vitamin A is critical not only to immune function but also to the integrity and function of the intestinal barrier. Hence vitamin A deficiency may be a predisposing factor for intestinal parasitical infections. In one study evaluating the effects of retinol on intestinal barrier function, growth, total parasites, and *Giardia* species infections in children in northeastern Brazil, 79 children received vitamin A 100,000 to 200,000 IU ($n = 39$) or placebo ($n = 40$) at enrollment and then again at 4 and 8 months; they were followed for 36 months. Total intestinal parasitical, specifically new, infections were significantly lower in the vitamin A group compared with the control group; this outcome was accounted for entirely by significantly fewer new *Giardia* infections in the vitamin A group, indicating that adequate vitamin A stores may help prevent *Giardia* infections.[29]

In another study, the effect of vitamin A supplementation on the overall incidence of diarrheal disease and respiratory tract infections in children was evaluated in 188 children, aged 6 to 15 months, from periurban marginalized communities of Mexico City. The children were assigned to receive vitamin A (below 12 months of age, 20,000 IU retinol; at or above 12 months, 45,000 IU retinol) or a placebo every 2 months and were followed for up to 15 months. Vitamin A supplementation had no significant effect on the risk of overall diarrheal disease but reduced mild watery diarrhea (incidence rate ratio [RR] 0.69) and cough with fever (RR, 0.69). Vitamin A supplementation also decreased diarrheal disease during the summer (RR, 0.74), among nonstunted children (RR, 0.69) and among children from higher socioeconomic level households.[30]

Measles

Vitamin A deficiency is a major problem in many developing countries. A number of well-designed studies have confirmed an effect first noted in 1932: vitamin A supplementation can significantly reduce infant mortality due to measles by at least 50%. Typically, the dosage of vitamin A in double-blind studies has been 200,000 to 400,000 IU administered only once or twice to replenish body stores.[31,32] However, the benefit of vitamin A supplementation in the treatment of measles is not limited to developing countries. A study of well-nourished children in Long Beach, California, suffering from measles indicated that 50% were deficient in vitamin A.[33] This finding supports the use of vitamin A supplementation even in the United States.

Infants With Respiratory Syncytial Virus Infections

Wide-scale immunization programs have reduced the risk of measles in children. However, vitamin A therapy appears appropriate for other childhood viral illnesses as well. One of the more common viruses is respiratory syncytial virus (RSV), a common cause of severe respiratory disease in young children. Studies have shown that children with RSV have low serum levels of vitamin A. Furthermore, the lower the vitamin A level, the greater the severity of the disease, similar to the relation shown in measles. Because vitamin A supplementation diminishes the morbidity and death caused by measles, a group of researchers decided to determine vitamin A's safety and absorption pattern in RSV as a first step in assessing its therapeutic effectiveness.[34]

Twenty-one children with a mean age of 2.3 months (range 1–6 months) with mild RSV infection were treated with 12,500 to 25,000 IU of oral micellized vitamin A. Baseline vitamin A levels were shown to be low; however, within 6 hours of administration of 25,000 IU but not 12,500 IU of vitamin A, normal levels were reestablished. Despite their young age, none of the children experienced any obvious signs or symptoms of vitamin A toxicity. Although the study was not designed as a therapeutic trial, the subjects receiving vitamin A had shorter hospital stays than children with a similar severity of illness who were not enrolled in the study.

Although vitamin A supplementation is an attractive treatment of RSV infections because of its low cost, wide availability, and ease of administration, placebo-controlled trials have suggested that it may be of value only in the most severe cases. A placebo-controlled study of 180 children in Chile with RSV provided 50,000 to 200,000 IU of retinyl palmitate (according to age) within 2 days of admission.[35] Supplementation resulted in no significant benefit except for those

children suffering from hypoxemia. These children experienced substantial benefit, consisting of a more rapid resolution of tachypnea and shortening of their hospital stay from 9.3 to 5.5 days. Similar results were seen in a study involving hospitalized pediatric patients with nonspecific upper respiratory infections.[36]

Acquired Immunodeficiency Syndrome

Patients with acquired immunodeficiency syndrome (AIDS) may also benefit from vitamin A supplementation. One study showed that a vitamin A deficiency is quite common during human immunodeficiency virus (HIV) infection and that vitamin A deficiency is clearly associated with a decreased level of circulating helper T cells, one of the hallmarks of AIDS.[37]

Analysis of vitamin A levels, helper T cells, and other blood parameters in individuals with HIV indicated that more than 15% had low serum vitamin A levels. Helper T-cell levels were much lower in patients with HIV infection whose vitamin A levels were low than in those who had normal levels of vitamin A. Vitamin A deficiency was also shown to be associated with a higher rate of mortality due to HIV.

Increasing β-carotene may be the preferred form of vitamin A for supplementation in patients with HIV because retinoic acid, the active form of vitamin A, may actually increase HIV replication in humans. Low β-carotene levels are common in AIDS, presumably as a result of fat malabsorption. Low β-carotene levels are associated with greater impairment of immune function.[38]

Skin Disorders

The use of high-dose vitamin A therapy for acne and other skin disorders was introduced in dermatology in the late 1930s. It is still used by a few dermatologists, although this type of therapy is not as popular as before the advent of synthetic retinoids. Vitamin A therapy has been shown to be quite effective in treating skin conditions associated with excessive formation of keratin (hyperkeratosis), a skin protein that can clog the pores of the skin to produce a "goose bump" effect. Examples of some skin conditions associated with hyperkeratosis include acne, psoriasis, ichthyosis, lichen planus, Darier's disease, palmoplantar keratoderma, and pityriasis rubra pilaris. The dosages of vitamin A used to treat these conditions have typically been quite high (300,000–500,000 IU/day for 5–6 months in the treatment of acne; 1–3.5 million IU/day for 1–2 weeks for the other conditions).[39–42] The use of these high dosages usually results in the development of significant toxicity (see later discussion). Although there is some evidence that carotenes may be more useful and less toxic in some of these conditions, the pharmacological activity responsible for the effects of vitamin A in hyperkeratosis is believed to occur when serum retinol levels exceed serum RBP capacity, causing destabilization of membranes and destruction of the keratin-producing cells.[38]

In monitoring for vitamin A toxicity, laboratory tests appear unreliable until obvious toxicity symptoms are apparent. The first significant toxic symptom is usually headache, followed by fatigue, emotional instability, and muscle and joint pain. Chapped lips (cheilitis) and dry skin (xerosis) generally occur in the majority of patients, particularly in dry weather. Because high doses of vitamin A during pregnancy can cause birth defects, women of childbearing age should use effective birth control during vitamin A treatment and for at least 1 month after discontinuation.

High doses of vitamin A may not be necessary if other nutritional factors, like zinc and vitamin E, are included. These nutrients work with vitamin A in promoting healthy skin. A safe and effective recommendation for vitamin A in the treatment of acne is less than 25,000 IU/day.

Dry Eyes

Dry-eye disorders are a complex group of diseases characterized by a localized water deficiency in the tear ducts, a mucin deficiency, or a combination of the two. Despite the diversity of underlying causes, the changes in the conjunctiva of the eye are similar in all cases—loss of goblet cells (mucin-producing cells); abnormal enlargement of nongoblet epithelial cells; and increases in cellular layers, keratin deposition and stratification, and keratinization.

Apart from topical vitamin A therapy, all other nonsurgical therapies for dry eye (i.e., the frequent application of artificial tears, lubricants, or slow-releasing polymers and the therapeutic use of soft contact lenses) are not directed toward reversing the underlying process but, rather, toward alleviating the symptoms.

The hypothesis that a localized vitamin A deficiency in the lining of the outer eye may be responsible for dry eye, based on vitamin A's vital role in epithelial tissue, seems obvious. Clinical studies featuring commercial vitamin A eyedrops (Viva-Drops, Vision Pharmaceuticals, vivadrops.com, Mitchell, South Dakota) have yielded impressive clinical results in the treatment of dry eyes.[43,44]

Emerging Potential Applications

It has long been recognized that both iodine and vitamin A deficiencies contribute to hypothyroidism, and their combined use may be more effective than either alone.[45] A recent clinical trial conducted among premenopausal women suggests that retinyl palmitate (25,000 IU/day) may reduce the risk of subclinical hypothyroidism—significant increases in T3 and decreases in thyroid-stimulating hormone (TSH) were found compared with placebo. More clinical data are needed before broad recommendations can be made, but this suggests a potential for supplementation even without documented deficiency.

Neurodegenerative diseases, including Alzheimer's disease and multiple sclerosis, may prove to be valid applications of vitamin A therapy. One recent study found that a marginal vitamin A deficiency is more than twice as common as a vitamin A deficiency among the elderly, with lower levels associated with greater cognitive decline, possibly by stimulating Aβ production and neuritic plaque formation.[46] Evidence also indicates that patients with multiple sclerosis have lower plasma levels of vitamin A, which may lead to impaired immune tolerance. Clinical trials (25,000 IU per day) with retinyl palmitate have shown improvement in the balance of Th1 and Th2 cells, as well as improvements in fatigue and depression.[47,48]

DOSAGE

Dosage ranges for vitamin A reflect the intent of use. For general health purposes, a dosage of 5000 IU for men and 2500 IU for women appears reasonable. During an acute viral infection, a single oral dosage of 50,000 IU for 1 or 2 days appears to be safe even in infants (note that women who might be pregnant must not use vitamin A supplements; β-carotene, however, is fine). For the treatment of acne and hyperkeratotic skin disorders, high-dose therapy may be useful but should be monitored closely by a physician.

TOXICITY

Vitamin A supplementation must be avoided during pregnancy because large doses have been shown to be teratogenic. Unfortunately, safe dosages for pregnant women have not yet been determined, although combined human and animal data suggest that 30,000 IU/day should be considered safe.[49] Nonetheless, women who may become pregnant should keep their supplemental vitamin A levels below 5000 IU or, better yet, switch to carotenes.[50] (Emerging

research is showing that 20%–30% of the population is unable to convert β-carotene to vitamin A. The only way to know is by measuring blood levels.) Excess dietary intake of vitamin A has been associated with birth defects in humans in fewer than 20 reported cases over the past 30 years.[51] Acute toxicity with vitamin A, which is most often seen in children as a result of the accidental ingestion of a single large dose of vitamin A (100,000–300,000 IU), manifests as follows:

- Raised intracranial pressure with vomiting
- Headache
- Joint pain
- Stupor
- Occasional papilledema

Symptoms rapidly subside upon withdrawal of the vitamin, and complete recovery always ensues.

Vitamin A toxicity may occur in adults who have taken more than 50,000 IU/day for several years. Smaller daily doses may produce toxicity symptoms if there are defects in the storage and transport of vitamin A, as occurs in cirrhosis of the liver, hepatitis, or protein-calorie malnutrition as well as in children and adolescents.[52,53] Signs of vitamin A toxicity generally include the following:

- Dry, fissured skin
- Brittle nails
- Alopecia
- Gingivitis
- Cheilosis
- Anorexia
- Irritability
- Fatigue
- Nausea

Serum levels of vitamin A between 250 and 6600 IU/dL are typical of toxicity. Prolonged, severe hypervitaminosis A leads to bone fragility and thickening of the long bones.

Toxicity is typically encountered during high-dose vitamin A therapy for various skin conditions. Although dosages smaller than 300,000 IU/day for a few months rarely cause toxicity symptoms, early recognition is still very important. Cheilitis (chapped lips) and xerosis (dry skin) generally appear in the majority of patients, particularly in dry weather. The first significant toxicity symptom is usually headache, followed by fatigue, emotional lability, and muscle/joint pain. Laboratory tests are of little value in monitoring toxicity because serum vitamin A levels correlate poorly with toxicity, and values of serum glutamic-oxaloacetic transaminase and serum glutamate pyruvate transaminase are elevated only in symptomatic patients.[38]

Prostate cancer risk may also be an area for toxicity concern. Serum retinol was recently associated with both total (odds ratio [OR] 1.3) and high-grade prostate cancer risk (OR 1.74) in the large nested case-control Prostate Cancer Prevention Trial. The increase in risk appeared to be limited to men with a for-cause biopsy and those who reported supplement use, suggesting that supplemental vitamin A may be more of a risk factor than dietary sources.[54] This is in agreement with one of the largest prospective studies to date, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, as well as a large pooled analysis of nearly 11,000 cases, both of which found associations between serum retinol and prostate cancer risk, with either no effect or a protective association with carotenoids such as lycopene and β-carotene.[55,56]

DRUG INTERACTIONS

Vitamin E and zinc are particularly important to the proper function of vitamin A. A deficiency of zinc, vitamin C, protein, or thyroid hormone will impair the conversion of provitamin A carotenes to vitamin A.

Excessive vitamin A intake can inhibit vitamin D–mediated calcium absorption, accelerate bone loss, and inhibit the formation of new bone.[57,58] All of these actions may raise the risk of osteoporosis and hip fracture. In one prospective study, a vitamin A intake greater than 5000 IU/day, compared with a lower intake, was associated with a reduction in bone mineral density that approximately doubled the risk of hip fracture.[59] However, a recent long-term study that enrolled 663 men and 335 women taking either retinol (7.5 mg RE/d) or beta-carotene (30 mg/d) found no increase in risk for osteoporotic fracture, suggesting this relationship needs more data, although compelling evidence suggests that only in the context of vitamin D deficiency does vitamin A pose a risk for osteoporosis.[60,61]

Studies have demonstrated a link between exposure to toxic chemicals and vitamin A nutrition. Administration of compounds such as polybrominated biphenyls, dioxin, and other toxic chemicals to rats leads to a decrease in the hepatic content of vitamin A. Concurrent administration of vitamin A and the xenobiotics partially prevents the symptoms of toxicity. Exposure to these compounds results in an increased vitamin A requirement because of the enhanced degradation of vitamin A in the liver.[62,63]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gerster H. Vitamin A: functions, dietary requirements and safety in humans. *Int J Vitam Nutr Res*. 1997;67(2):71–90.
2. Vitamin A of Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, Food and Nutrition Board of the Institute of Medicine, pages 82–161. 2001. Accessed from https://www.nap.edu/read/10026/chapter/6#. Accessed January 2019.
3. Tanumihardjo SA, Russell RM, Stephensen CB, et al. Biomarkers of nutrition for development (bond)-vitamin A review. *J Nutr*. 2016;146:1816S–1848S. PMID: 27511929.
4. Selhorst JB, Waybright EA, Jennings S, et al. Liver lover's headache: pseudotumor cerebri and vitamin A intoxication. *JAMA*. 1984;252:3365.
5. Bai YJ1, Dai RJ2. Serum levels of vitamin A and 25-hydroxyvitamin D3 (25OHD3) as reflectors of pulmonary function and quality of life (QOL) in children with stable asthma: a case-control study. *Medicine (Baltimore)*. 2018;97(7):e9830. PMID: 29443744.
6. Al Senaidy AM1. Serum vitamin A and beta-carotene levels in children with asthma. *J Asthma*. 2009;46(7):699–702. PMID: 19728208.
7. Checkley W1, West Jr KP, Wise RA, et al. Supplementation with vitamin A early in life and subsequent risk of asthma. *Eur Respir J*. 2011;38(6):1310–1319. PMID: 21700611.
8. Sommer A. Vitamin a deficiency and clinical disease: an historical overview. *J Nutr*. 2008;138(10):1835–1839.
9. WHO. Global prevalence of vitamin A deficiency in populations at risk: 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva (Switzerland): WHO; 2009. Accessed https://www.who.int/vmnis/database/vitamina/x/en/. Accessed January 2019.
10. Troesch B, Hoeft B, McBurney M, et al. Dietary surveys indicate vitamin intakes below recommendations are common in representative Western countries. *Br J Nutr*. 2012;108(4):692–698. PMID: 22691229.
11. Brown ED, Micozzi MS, Craft NE, et al. Plasma carotenoids in normal men after a single ingestion of vegetables or purified beta-carotene. *Am J Clin Nutr*. 1989;49:1258–1265.
12. O'Byrne SM, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. *J Lipid Res*. 2013;54:1731–1743. PMID: 23625372.
13. Borel P, Desmarchelier C. Genetic variations associated with vitamin A status and vitamin A Bioavailability. *Nutrients*. 2017;9(3):E246. PMID: 28282870 Reproduced from an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license http://creativecommons.org/licenses/by/4.0/. Accessed October 25 2019.
14. Owsley C, McGwin G, Jackson GR, et al. Effect of short-term, high-dose retinol on dark adaptation in aging and early age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2006;47(4):1310–1318.
15. Mondul A, Mancina RM, Merlo A, et al. PNPLA3 I148M variant influences circulating retinol in adults with nonalcoholic fatty liver disease or Obesity. *J Nutr*. 2015;145(8):1687–1691. PMID: 2613658752.
16. Mosca A, Fintini D, Scorletti E, et al. Relationship between non-alcoholic steatohepatitis, PNPLA3 I148M genotype and bone mineral density in adolescents. *Liver Int*. 2018;38(12):2301–2308. PMID: 30176114.
17. Rahman MM, Wahed MA, Fuchs GJ, et al. Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. *Am J Clin Nutr*. 2002;75:92–98.
18. Muñoz EC, Rosado JL, Lopez P, et al. Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers. *Am J Clin Nutr*. 2000;71:789–794.
19. Leung WC, Hessel S, Méplan C, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15′-monooxygenase alter beta-carotene metabolism in female volunteers. *FASEB J*. 2009;23(4):1041–1053.
20. Weber D, Grune T. The contribution of β-carotene to vitamin A supply of humans. *Mol Nutr Food Res*. 2012;56(2):251–258. PMID: 21957049.
21. Munch IC1,2, Toft U3, Linneberg A, et al. Precursors of age-related macular degeneration: associations with vitamin A and interaction with CFHY402H in the Inter99 Eye Study. *Acta Ophthalmol*. 2016;94(7):657–662. PMID: 27502478.
22. Zile MH, Cullum ME. The function of vitamin A: current concepts. *Proc Soc Exp Biol Med*. 1983;172:139–152.
23. Folman Y, Rosenberg M, Ascarelli I, et al. The effect of dietary and climatic factors on fertility, and on plasma progesterone and oestradiol-17B levels in dairy cows. *J Steroid Biochem*. 1983;19:863–868.
24. Metabolism of beta-carotene by the bovine corpus luteum. *Nutr Rev*. 1983;41:357–358.
25. Lotthammer KH. Importance of beta-carotene for the fertility of dairy cattle. *Feedstuffs*. 1979;51:16–19.
26. O'Fallon JV, Chew BP. The subcellular distribution of beta-carotene in bovine corpus luteum. *Proc Soc Exp Biol Med*. 1984;177:406–411.
27. Sherman BM, Korenman SG. Inadequate corpus luteum function: a pathophysiological interpretation of human breast cancer epidemiology. *Cancer*. 1974;33:1306–1312.
28. Semba RD. Vitamin A, immunity, and infection. *Clin Infect Dis*. 1994;19:489–499.
29. Lima AA, Soares AM, Lima NL, et al. Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific Giardia spp infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial. *J Pediatr Gastroenterol Nutr*. 2010;50(3):309–315.
30. Long KZ, Rosado JL, DuPont HL, et al. Supplementation with vitamin A reduces watery diarrhoea and respiratory infections in Mexican children. *Br J Nutr*. 2007;97(2):337–343.
31. Huiming Y, Chaomin W, Meng M. Vitamin A for treating measles in children. *Cochrane Database Syst Rev*. 2005;19(4):CD001479.
32. D'Souza RM, D'Souza R. Vitamin A for the treatment of children with measles: a systematic review. *J Trop Pediatr*. 2002;48(6):323–327.
33. Arrieta AC, Zaleska M, Stutman HR, et al. Vitamin A levels in children with measles in Long Beach, California. *J Pediatr*. 1992;121:75–78.
34. Neuzil KM, Gruber SC, Chytil F, et al. Safety and pharmacokinetics of vitamin A therapy for infants with respiratory syncytial infections. *Antimicrob Agents Chemother*. 1995;39:1191–1193.
35. Dowell SF, Papic Z, Bressee JS, et al. Treatment of respiratory syncytial virus infection with vitamin A: a randomized, placebo-controlled trial in Santiago. Chile. *Pediatr Infect Dis J*. 1996;15:782–786.
36. Julien MR, Gomes A, Varandas L, et al. A randomized, double-blind, placebo-controlled clinical trial of vitamin A in Mozambican children hospitalized with nonmeasles acute lower respiratory tract infections. *Trop Med Int Health*. 1999;4:794–800.
37. Semba RD, Graham NM, Caiaffa WT, et al. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. *Arch Intern Med*. 1993;153:2149–2154.
38. Ullrich R, Schneider T, Heise W, et al. Serum carotene deficiency in HIV-infected patients. *AIDS*. 1994;8:661–665.
39. Kligman AM, Mills OH, Leyden JJ, et al. Oral vitamin A in acne vulgaris: preliminary report. *Int J Dermatol*. 1981;20:278–285.
40. Thomas 3rd JR, Cooke JP, Winkelmann RK. High-dose vitamin A in Darier's disease. *Arch Dermatol*. 1982;118:891–894.
41. Randle HW, Diaz-Perez JL, Winkelmann RK. Toxic doses of vitamin A for pityriasis rubra pilaris. *Arch Dermatol*. 1980;116:888–892.
42. Winkelmann RK, Thomas 3rd JR, Randle HW. Further experience with toxic vitamin A therapy in pityriasis rubra pilaris. *Cutis*. 1983;31:621–632.
43. Rengstorff RH. Topical treatment of external eye disorders with preparations containing vitamin A. *Practical Optometry*. 1993;4:163–165.
44. Westerhout D. Treatment of dry eyes with aqueous antioxidant eye drops. *Contact Lens J*. 1989;19:165–173.
45. Zimmermann MB1, Jooste PL, Mabapa NS, et al. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. *Am J Clin Nutr*. 2007;86(4):1040–1044. PMID: 17921382.
46. Zeng J, Chen L, Wang Z, et al. Marginal vitamin A deficiency facilitates Alzheimer's pathogenesis. *Acta Neuropathol*. 2017;133(6):967–982. PMID: 28130638.
47. Mohammadzadeh Honarvar N, Harirchian MH, et al. Retinyl palmitate supplementation Modulates T-bet and Interferon Gamma gene expression in multiple sclerosis patients. *J Mol Neurosci*. 2016;59(3):360–365. PMID: 27122150.

48. Bitarafan S, Saboor-Yaraghi A, Sahraian MA, et al. Effect of vitamin A supplementation on fatigue and depression in multiple sclerosis patients: a double-blind placebo-controlled clinical trial. *Iran J Allergy Asthma Immunol*. 2016;15(1):13–19. PMID: 26996107.
49. Wiegand UW, Hartmann S, Hummler H. Safety of vitamin A: recent results. *Int J Vitam Nutr Res*. 1998;68:411–416.
50. Rothman KJ, Moore LL, Singer MR, et al. Teratogenicity of high vitamin A intake. *N Engl J Med*. 1995;333:1369–1373.
51. Azais-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits. *Am J Clin Nutr*. 2000;71(suppl):1325S–1333S.
52. Hatoff DE, Gertler SL, Miyai K, et al. Hypervitaminosis A unmasked by acute viral hepatitis. *Gastroenterology*. 1982;82:124–128.
53. Farris WA, Erdman Jr JW. Protracted hypervitaminosis: a following long-term, low level intake. *JAMA*. 1982;247:1317.
54. Nash SH1, Till C2, Song X, et al. Serum retinol and carotenoid concentrations and prostate cancer risk: results from the prostate cancer prevention trial. *Cancer Epidemiol Biomarkers Prev*. 2015;24(10):1507–1515. PMID: 26269564.
55. Mondul AM, Watters JL, Mannisto S, et al. Serum retinol and risk of prostate cancer. *Am J Epidemiol*. 2011;173:813–821. PMID: 21389041.
56. Key TJ, Appleby PN, Travis RC, et al. Serum retinol and carotenoid concentrations and prostate cancer risk: results from the prostate cancer prevention trial. *Cancer Epidemiol Biomarkers Prev*. 2015;24(10):1507–1515.
57. Genaro Pde S, Martini LA. Vitamin A supplementation and risk of skeletal fracture. *Nutr Rev*. 2004;62:65–67.
58. Johansson S, Melhus H. Vitamin A antagonizes calcium response to vitamin D in man. *J Bone Miner Res*. 2001;16:1899–1905.
59. Melhus H, Michaelsson K, Kindmark A, et al. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med*. 1998;129:770–778.
60. Ambrosini GL, Alfonso H, Reid A, et al. Plasma retinol and total carotenes and fracture risk after long-term supplementation with high doses of retinol. *Nutrition*. 2014;30(5):551–556. PMID: 24698346.
61. Joo NS, Yang SW, Song BC, et al. Vitamin A intake, serum vitamin D and bone mineral density: analysis of the Korea national health and nutrition examination survey (KNHANES, 2008-2011). *Nutrients*. 2015;7(3):1716–1727. PMID: 25763530.
62. Cullum ME, Zile MH. Acute polybrominated biphenyl toxicosis alters vitamin A homeostasis and enhances degradation of vitamin A. *Toxicol Appl Pharmacol*. 1985;81:177–181.
63. Thunberg T, Ahlborg U, Wahlstrom B. Comparison of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and six other compounds on vitamin A storage, the UDP-gluconosyltransferase and aryl hydrocarbon hydroxylase activity in the rat liver. *Arch Toxicol*. 1984;55:16–19.

Vitamin K

Cristiana I. Paul, MS Nutrition

OUTLINE

INTRODUCTION

When vitamin K_1 was discovered, it was believed to have only the one role of supporting coagulation, hence the name K, from the German word *Koagulation*. Research from more than two decades has uncovered numerous functions for vitamin K beyond that of clotting. We have also become aware of different forms of vitamin K, while the vitamin K_2 family emerges with distinct cellular effects from vitamin K_1. It seems that nature has evolved a very complex system of vitamin K forms that work together to support various physiological functions for successful survival and reproduction even when its food sources are scarce.

Most importantly, current knowledge of the newly discovered functions of vitamin K is causing a paradigm shift in defining optimal vitamin K intake for supporting many critical aspects of health. In addition, vitamin K's roles in human physiology opens exciting opportunities to intervene with the therapeutic vitamin K doses for the management and potentially reversal of many common health conditions.

FORMS, SOURCES, ABSORPTION, AND METABOLISM

The currently known forms of vitamin K can be grouped in three categories: vitamin K_1 (one type), vitamin K_2 (14 types), and vitamin K_3 (one type) (Fig. 126.1). Vitamins K_1 and K_2 are fat-soluble, whereas vitamin K_3 is water-soluble.[1] Based on current Reference Daily Intake (RDI), only vitamin K_1 is recognized as a true vitamin, essential for human and animal well-being. Vitamins K_2 and K_3 can be derived as metabolites of vitamin K_1 in various tissues or the gastrointestinal tract in animals or humans. However, there may be many clinical situations that can benefit from supplementation with vitamin K_2 (from diet and/or supplements) or from the intravenous administration of vitamin K_3. Oral vitamin K_3 is used only in animal nutrition; for humans, it is currently allowed only for research purposes.[2]

The structures of vitamins K_1, K_2, and K_3 are similar in regard to their core molecule, called menadione (or 2-methyl-1,4-naphtoquinone). Vitamin K_3 is simply composed of the core molecule menadione (see Fig. 126.1C), whereas vitamins K_1 and K_2 contain the core molecule menadione plus specific side chains that distinguish them as follows[3]:

- In addition to the menadione core, vitamin K_1 contains a side chain of one unsaturated isopentenyl unit and three saturated isopentyl units (see Fig. 126.1A).
- In addition to the menadione core, each member of the vitamin K_2 family contains a side chain of specific length, anywhere from 1 to 14 repeats of unsaturated isopentenyl units (see Fig. 126.1B). The length of the side chain determines the name of each type of vitamin K_2 as follows: MK-1 (with 1 unit), MK-2 (with 2 units), and so on, through MK-14 (with 14 units). In this review, a K_2 designation is added in front of their names, as follows: K_2 (MK-1), K_2 (MK-2 through MK-14) to avoid confusion with vitamins K_1 and K_3.

On average, it is estimated that 80% to 90% of dietary vitamin K intake is typically derived from vitamin K_1, with the rest coming from various types of vitamin K_2.[4]

Vitamin K_1

Vitamin K_1, also called phylloquinone or phytomenadione, is synthesized only by plants or algae, where it is bound in the thykaloid membranes of chloroplasts and participates in the electron transport chain of photosynthesis. Vitamin K_1 occurs in significant amounts (50–800

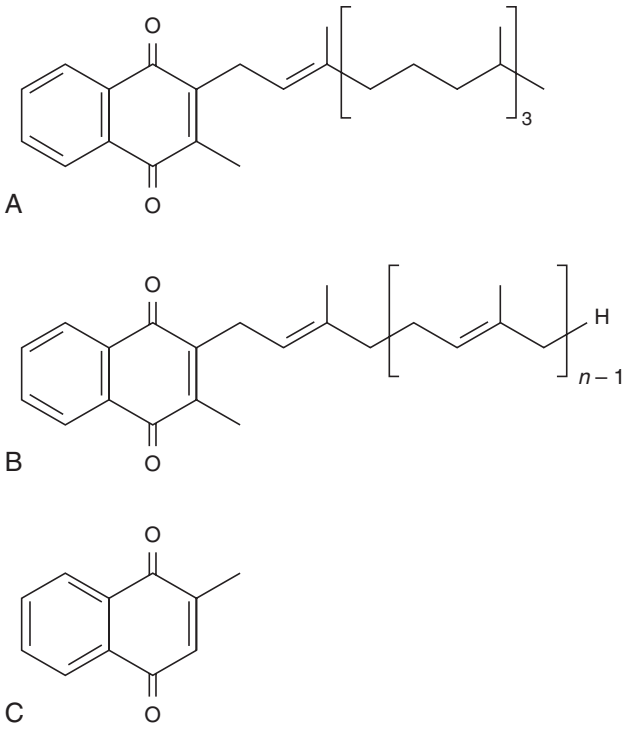


Fig. 126.1 (A) Vitamin K_1, also called phylloquinone or phytomenadione. (B) Vitamin K_2 family, K_2 (MKn) n = 1 to 14, also called menaquinones. Menaquinones have side chains composed of a varying number of isoprenoid residues. (C) Vitamin K_3, menadione.

mcg/100 g food) in certain vegetables, vegetable oils, seeds, algae, and in small amounts (<50 mcg/100 g food) in the rest of the vegetable and animal foods, as listed in Fig. 126.2. Table 126.1 lists the typical yield of vitamin K_1 in common measures of select vegetables, which are the most significant sources of vitamin K_1 of all vegetable foods.

Vitamin K_1 is also available for oral or intramuscular injection to correct clotting deficiency in newborns or to compensate for anticoagulant overdose in adults. Studies are conflicting in regard to the concern that vitamin K_1 injection may increase the risk of various cancers, including leukemia in infants. Recent research seems to have established that the risk was related to the route of administration, specifically to injection and not via the oral route. Also, the safest and most reliable way to increase vitamin K_1 in the unborn fetus and reduce the risk of bleeding after birth seems to be by increasing the mother's vitamin K status, as some studies suggest.[5]

Vitamin K_1 Absorption

Absorption of vitamin K_1 varies considerably depending on its source. When it is delivered in a vegetable, its bioavailability is low (3%–10%) because it is trapped in the chloroplast.[6] Cooking and/or mechanical processing (blending, juicing) of the vegetables or pressing the oil out of the vegetable seeds (such as sunflower seeds) increases the vitamin's bioavailability and absorption up to as much as 50% compared with the raw vegetable.[7]

By adding dietary fat to the vitamin K_1-containing food, absorption of the vitamin can be doubled; it may then reach 10% to 15%.[8] Following is a summary of estimated vitamin K_1 absorption values, obtained by comparing a number of studies[9–17]:

- Absorption from raw vegetables (such as spinach or broccoli): 3% to 5%; some suggest as high as 10%
- Absorption from cooked vegetables without fat: some have found no difference compared with raw vegetables
- Absorption from cooked vegetables with fat: approximately 2.5 times the absorption from raw vegetables
- Absorption from vitamin K_1 dissolved in vegetable oil: approximately 50% to 80%
- Absorption from a synthetic vitamin K_1 supplement: approximately 10% (if mixed in a capsule with 85% cellulose) and approximately 80% (if mixed in an emulsion containing lecithin and a bile acid)

Exposure to ultraviolet (UV) light inactivates vitamin K. When oils containing vitamin K_1 are hydrogenated (during industrial processing, cooking, frying, or baking), a big percentage (40%–50%) of the vitamin K_1 content is also hydrogenated. Studies have shown that hydrogenated vitamin K_1 has very little biological activity.[18] It is important to note that a large section of the population derives a majority of its vitamin K_1 from vegetable oils as opposed to vegetables, so the effect of vitamin K inactivation by hydrogenation may have an important effect on common deficiencies of vitamin K, which may be higher than estimated from just vitamin K_1 intake surveys.

Vitamin K_1 Transport, Metabolism, and Distribution in Human Tissues

Once ingested, vitamin K_1 can be metabolized through various routes in the body (see Fig. 126.2).

Vitamin K_1 is transported initially in the chylomicrons through lymph and blood to the liver and other tissues. Once taken up by various tissues, vitamin K_1 may be stored and utilized unchanged, while part of it may be converted to K_2 (MK-4). For unclear reasons, the steady-state ratio of K_1/K_2 (MK-4) stored in each tissue varies based on tissue type and from individual to individual. See Fig. 126.2 for a list of these ratios in various tissues and organs derived from an animal study.[19]

One study estimates that 5% to 25% of the absorbed vitamin K_1, the same as various K_2 forms, is converted to K_3 in the intestinal cells.[20] Various cells in the body may take up vitamin K_3 and convert it to vitamin K_2 (MK-4). A portion of vitamin K_3 is converted to K_2 (MK-4) in each tissue.[21]

The unabsorbed fraction of vitamin K_1 is metabolized by certain types of gastrointestinal bacteria (*Bacteroides, Escherichia coli,* and *Propionibacterium*) and converted into vitamin K_3 or into vitamin K_2 (MK-n, n = 1–14), the most predominant forms being the longer-chains MK-7, 8, 9, 10, and 11.

As a result of uptake in all the metabolic pathways described previously, vitamin K_1 intake can lead to an increase of various forms of vitamin K in the body, as follows: vitamin K_1 in serum and tissues/organs, vitamin K_3 especially in the serum, vitamin K_2 (MK-4) in tissues/organs, and long-chain K_2s are typically stored in the liver.

One study looked at the vitamin K stored in the rat brain in response to supplementation with either vitamin K_1 or vitamin K_2 (MK-4).[20] Contrary to what was expected, the results showed that supplementation with vitamin K_1 was able to increase the brain content of K_2 (MK-4) more than direct supplementation with vitamin K_2 (MK-4).

Vitamin K_2 (Menaquinones K_2 (MK-1) Through K_2 (MK-14)

Food sources, metabolism. Vitamin K_2 is not considered a vitamin in the true sense because theoretically it is not an essential nutrient. This is because animal and human tissues synthesize vitamin $K_{2\ (MK-4)}$ from the vitamin K_1 ingested, and intestinal bacteria can synthesize various long-chain K_2s from it as well. Although there is an overlap in their carboxylating function, it is not clear whether any of the vitamin K_2 forms can perform all the functions that vitamin K_1 exerts, and there is no proof that they can convert into vitamin K_1.

Vitamin K_2 can be viewed as a metabolite of vitamin K_1 inside human/animal tissues, but some researchers are calling it "the active form of vitamin K_1." Consequently, vitamin K_2 may be conditionally essential for certain individuals owing to their genotypes, aging, and metabolic or disease conditions where the conversion of vitamin K_1 to K_2 is inadequate.

Like the essential role of vitamin K_1 in the energy production (photosynthesis) of plants or algae, vitamin K_2 is an obligatory component of the

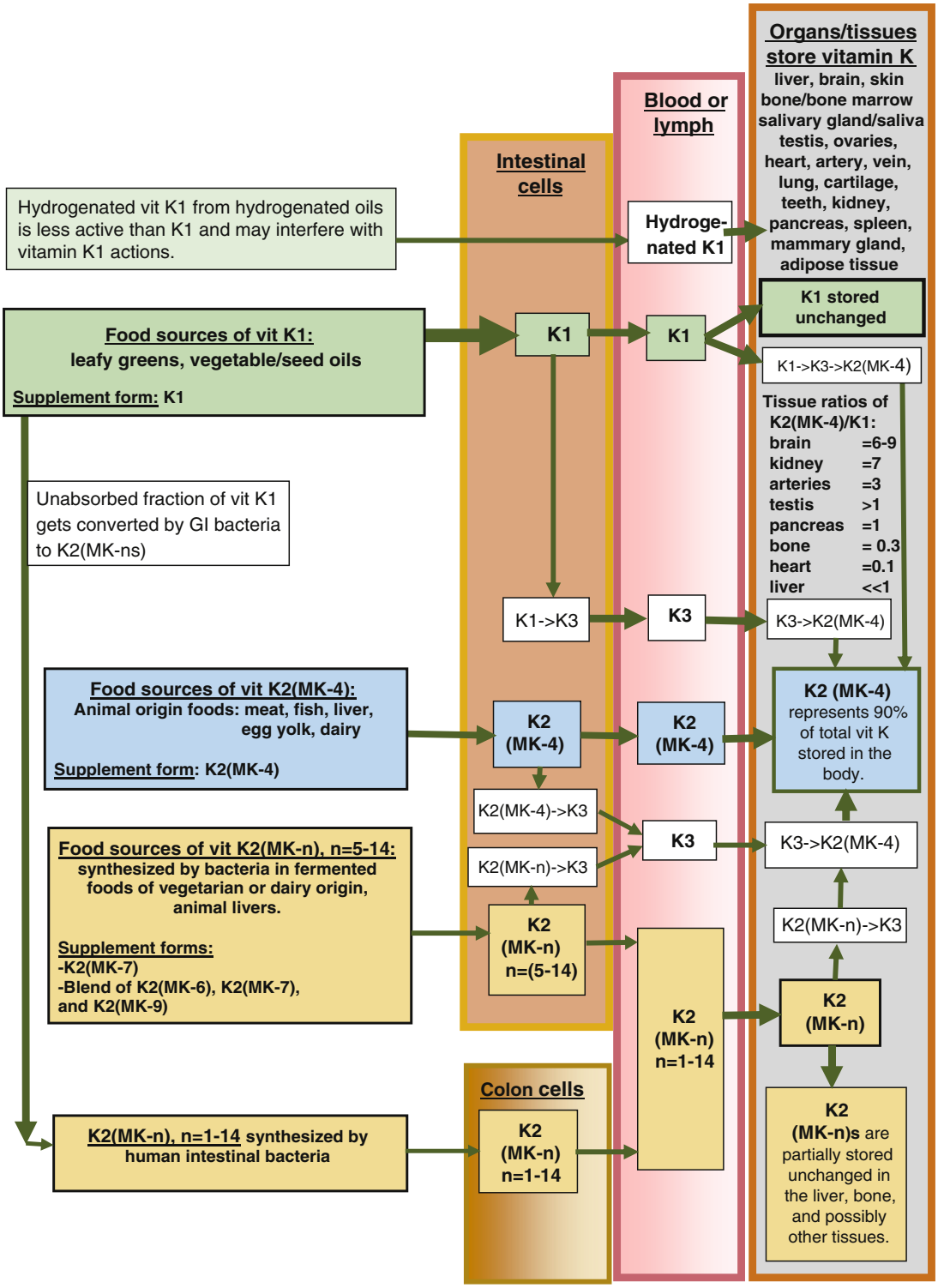


Fig. 126.2 Vitamin K forms, sources, absorption, conversions, transport, and distribution in the blood and organs.

electron transfer pathway in the mitochondria of certain bacteria, analogous to the role of the quinone coenzyme Q10 in animal mitochondria.[22]

Vitamin K_1 Conversion to Vitamin K_2 (MK-4): Tissue Distribution of Vitamin K_1 Versus Vitamin K_2 (MK-n), n = 1 to 14

Vitamin K_2 (MK-4) is the most prevalent form of vitamin K in human and animal tissues, at about 90% of total vitamin K stored, with the remainder as mostly vitamin K_1 and small amounts of long-chain K_2 (MK-n)s (n = 7–11).

The distribution of K_1 versus K_2 in tissues/organs was found to vary widely in an animal study, as shown in Fig. 126.2. The ratio of K_1/K_2 (MK-4) stored varies based on relative and absolute intakes of various forms of vitamin K.

Many studies have shown that various long-chain K_2 (MK-n)s are converted to K_2 (MK-4) in all tissues with the exception of the liver. For example, dietary supplementation of rats with a dose of K_2 (MK-7), equivalent to approximately 90 mcg in humans, was shown to result in a significant increase of K_2 (MK-4) in bone, whereas no

TABLE 126.1 Foods With High Vitamin K_1 Content

Food	Weight (g)	Measure	Amount of K_1 (mcg)
Kale, cooked and drained	130	1 cup	1062
Collards, cooked and drained	170	1 cup	1059
Spinach, cooked and drained	180	1 cup	889
Beets, cooked and drained	144	1 cup	697
Broccoli, cooked drained	156	1 cup	220
Brussels, cooked and drained	156	1 cup	219
Onions, raw	100	1 cup	207
Parsley, raw	10	10 sprigs	164
Cabbage, cooked drained	150	1 cup	163
Spinach, raw	30	1 cup	145
Asparagus, cooked and drained	180	1 cup	144
Lettuce, iceberg	129	1 head	129
Broccoli, raw	88	1 cup	89
Cabbage, raw	70	2 cups	53
Cucumber, raw	301	1 large	49

Data from USDA National Nutrient Database for Standard Reference, Release 17. Content of Selected Foods per Common Vitamin K (phylloquinone) Measure, sorted by nutrient content, http://www.nal.usda.gov/fnic/foodcomp/Data/SR17/wtrank/sr17w430.pdf. Accessed February 13, 2012.

MK-7 was detected in the bone.[23] However, another animal study reported that a fraction of supplemented K_2 (MK-7) is deposited as such in bone. However, this rat study used a dose equivalent to a human dose of 85 mg of K_2 (MK-7), which does not translate into practical applications.[86]

A substantial amount of long-chain K_2 [MK-n]s, n = 7 to 11 is stored in the liver unchanged, and this organ has a distinct distribution of vitamin K compared with all other tissues: that is, 90% long-chain menaquinones (K_2 [MK-n]s, n = 8–11) and 10% vitamin K_1. This may be because the liver is the first organ to have access to the vitamin K_2 synthesized by the intestinal bacteria, which is absorbed through the lower ileum. This preferential sequestering of MK-ns in the liver is hypothesized to be an evolutionary adaptation for securing a low-turnover vitamin K pool to support clotting needs and protect against the dietary variability of vitamin K_1 or K_2 (MK-4).

Dietary Sources of Various Forms of Vitamin K_2

Dietary sources of various forms of vitamin K_2 (various menaquinones) include animal meats and dairy as well as fermented foods of animal or vegetable origin, as follows:

Animal foods. Meats (2–3 mcg/100 g), egg yolk (10 mcg/one egg), and milk. These sources preferentially provide the K_2 (MK-4) form along with similar amounts of vitamin K_1. Table 126.2 contains a listing of the vitamin K_2 content of various foods. Table 126.2 lists the vitamin K_2 content of select foods from three studies in the United States, Holland, and Japan.[25]

The content of vitamins K_1 and K_2 in human milk depends on the mother's intake of these vitamins.[26] The same may be true for cow's milk and other animal milks. Similarly, the vitamin K content of animal tissues may be lower for animals fed mostly corn versus those that

TABLE 126.2 Significant Food Sources of Vitamin K_2

	VITAMIN K FORMS (mcg/100 g OR mcg/100 mL)						
Food	K_1	K_2 (MK-4)	K_2 (MK-5)	K_2 (MK-6)	K_2 (MK-7)	K_2 (MK-8)	K_2 (MK-9)
Meats							
Beef	0.6	1.1	–	–	–	–	–
Beef chuck	0.6	15.0	–	–	–	–	–
Beef liver	3.1	0.4	–	–	–	–	–
Chicken breast	–	8.9	–	–	–	–	–
Chicken leg	–	8.5	–	–	–	–	–
Chicken liver	4.0	14.0	–	–	–	–	–
Chicken thigh	–	27.0	–	–	–	–	–
Deer back	2.0	0.7	–	–	–	–	–
Duck breast	1.9	3.6	–	–	–	–	–
Goose leg	4.1	31.0	–	–	–	–	–
Goose liver paté	10.9	369.0	–	–	–	–	–
Ground beef (medium fat)	1.3	8.1	–	–	–	–	–
Hare leg	4.8	0.1	–	–	–	–	–
Pork liver	0.2	0.3	–	–	–	–	–
Pork steak	0.3	2.1	–	–	0.5	1.1	–
Pork thigh	–	6.0	–	–	–	–	–
Salami	2.3	9.0	–	–	–	–	–
Fish							
Plaice	–	0.2	–	0.3	0.1	1.6	–
Salmon	0.1	0.5	–	–	–	–	–
Eel	0.3	1.7	–	0.1	0.4	–	–

TABLE 126.2 Significant Food Sources of Vitamin K_2—cont'd

	VITAMIN K FORMS (mcg/100 g OR mcg/100 mL)						
Food	**K_1**	**K_2 (MK-4)**	**K_2 (MK-5)**	**K_2 (MK-6)**	**K_2 (MK-7)**	**K_2 (MK-8)**	**K_2 (MK-9)**
Vegetables							
Sauerkraut	25.0	0.4	0.8	1.5	0.2	0.8	1.1
Boiled cabbage	180.0	0.4	–	–	–	–	–
Natto	34.0	–	7.5	13.8	998.0	84.0	–
Dairy Products							
Whole milk	0.5	0.8	0.1	–	–	–	–
Buttermilk	–	0.2	0.1	0.1	0.1	0.6	1.4
Whole yogurt	0.4	0.6	0.1	–	–	0.2	–
Skimmed yogurt	–	–	–	–	–	0.1	–
Whipping cream	5.1	5.4	–	–	–		–
Hard cheese	10.4	4.7	1.5	0.8	1.3	16.9	51.1
Soft cheese	2.6	3.7	0.3	0.5	1.0	11.4	39.6
Curd cheese	0.3	0.4	0.1	0.2	0.3	5.1	18.7
Egg yolk	2.1	31.4	–	0.7	–	–	–
Egg white	–	0.9	–	–	–	–	–
Fats and Oils							
Butter	2.0	21.0	–	–	–	–	–
Mayonnaise (with egg)	189.0	38.0	–	–	–	–	–

Data from Kamao M, Suhara Y, Tsugawa N, et al. Vitamin K content of foods and dietary vitamin K intake in Japanese young women. *J Nutr Sci Vitaminol (Tokyo).* 2007;53(6):464–470; Elder SJ, Haytowitz DB, Howe J, Peterson JW, Booth SL. Vitamin K contents of meat, dairy, and fast food in the U.S. diet. *J Agric Food Chem.* 2006;54(2):463–467; Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis.* 2000;30(6):298–307.

are exclusively grass-fed, although no studies have measured this. The vitamin K_2 content of animal-derived food may be higher when they are supplemented with vitamin K_3 (a common practice). Because vitamin K_3 supplementation has proved detrimental for humans, it may be of questionable safety for animals. Also, continuous antibiotic treatment of the animals (to enhance growth) may impair their intestinal bacterial production of vitamin K_2.

Fermented foods (animal or vegetable). Various forms of vitamin K_2 are produced by certain bacteria, ranging from K_2 (MK-4) to K_2 (MK-12) (see Table 126.2):

1. Animal origin: cheese and other fermented milk products (yogurt, kefir, buttermilk, sour cream) provide vitamin K_2 forms mostly as K_2 (MK-8) and K_2 (MK-9).
2. Vegetable origin: natto, sauerkraut, kimchi. For example, the bacteria specific to natto (a fermented mix of soybeans and rice) will provide vitamin K_2 mostly in the K_2 (MK-7) form; bacteria in foods like sauerkraut (pickled cabbage) provide vitamin K_2 in a wide variety of forms, such as K_2 (MK-5) through K_2 (MK-9).

Vitamin K_2 Produced by Human Intestinal Bacteria

Certain bacteria residing in the human intestinal tract convert the ingested vitamin K_1 or other dietary components (such as alpha-ketoglutarate) to various forms of vitamin K_2 (MK-n). The types of bacteria that have this property are mostly gram-negative, such as *Bacteroides fragilis, Propionibacterium,* and particular strains of *E. coli* (normal gastrointestinal resident strain, not necessarily pathogenic). Certain strains of *Lactobacillus* were also found to produce vitamin K_2, although these are not the commonly known strains of *Lactobacillus* or probiotics used in supplements or found in yogurt. Vitamin K produced by the gut bacteria may represent a significant portion of the total vitamin K_2 acquired by some people if their vitamin K status is marginal.[27]

Vitamin K_2 Absorption and Transport

Very few studies have evaluated the absorption of various forms of vitamin K_2.[28] One study found that absorption of K_2 (MK-4) was 16%, 21%, 44%, and 44% with meals containing 8 g, 20 g, 35 g, and 45 g fat, respectively. One study administered K_2 (MK-7) from a natto extract and claimed to have obtained "almost complete" absorption, whereas the absorption of K_2 (MK-7) derived from a supplement was 78% of that from natto.

Vitamins K_2 (MK-4) and K_2 (MK-7) are believed to be partially cleaved (around 5%–25%) in the intestinal wall to form vitamin K_3, which was found in the urine 2 to 3 hours after ingestion of these vitamers.

Plasma half-life of a one-time administration of K_2 (MK-4) is around 1 to 2 hours, whereas that of K_2 (MK-7) is around 2 days. Some hypothesize that K_2 (MK-4) is taken up by the tissues a lot faster because it is the preferred storage form of vitamin K_2. However, after 4 weeks of supplementation with 1.5 mg K_2 (MK-4), for example, fasting plasma levels of K_2 (MK-4) increased from 0.2 ng/mL to 1.5 ng/mL.[184]

Table 126.3 shows the average intakes of various forms of vitamin K from an epidemiological study in a Dutch population. The particularly high contributions from MK-9 and MK-8 are known to be a result of cheese consumption, whereas MK-4 is derived mostly from meat and eggs.

Supplement Forms and Doses Available

Vitamin K_2 is currently available as a supplement in three forms:

1. Vitamin K_2 (MK-4) (menaquinone-4 or menatrenone), which is provided in a synthetic version. It is typically available in doses in

TABLE 126.3 Average Intake of Various Forms of Vitamin K in a Dutch Population (All Values in µg/day)

			VITAMIN K_2 FORMS					
	K_1	Total K_2	MK-9	MK-4	MK-8	MK-7	MK-5	MK-6
Mean	211.7	29.1	14.7	7.1	6.0	0.4	0.3	0.3
Standard deviation	±100.3	±12.8	±8.1	±2.1	±3.4	±0.3	±0.2	±0.2
Lower end	9.1	0.9	0	0.5	0	0	0	0
Upper end	991.1	128	81.9	28.2	32.8	2.2	2.1	1.5

Data from Gast GC, de Roos NM, Sluijs I, et al. A high menaquinone intake reduces the incidence of coronary heart disease. *Nutr Metab Cardiovasc Dis*. 2009;19(7):504–510. Epub 2009 Jan 28.

the range of 40 to 50 mcg, 1 mg, 15 mg, or 45 mg in stand-alone vitamin K formulas, vitamin D combination with vitamin K formulas, or bone-specific formulas. It is also available as a prescription medication in Japan, called Glakay, in 15-mg doses (with the indication of three times daily).[29]

2. Vitamin K_2 (menaquinone-7, MK-7) is available either in a synthetic version or as a product of fermentation. It can, of course, be ingested with the natto food itself, which provides an average of 1000 mcg/100 g serving. The average vitamin K_2 intake found in young Japanese women was 61.7 mcg. From this, K_2 (MK-7) typically represents 57.4 mcg owing to the common consumption of natto.
3. A blend of three menaquinones (MK-6, MK-7, MK-9), a product of fermentation. This particular mix is meant to better mimic a natural dietary mix of menaquinones.

Vitamin K_3 (Menadione)

Occurrence, Properties, and Special Considerations for Animal and Human Consumption

Vitamin K_3 (menadione) has not been observed to occur in natural foods in any significant amount. It is believed to be formed inside intestinal cells and possibly other tissues and organs of humans and animals and can be found in the plasma and urine 2 to 3 hours after ingestion of either vitamin K_1, K_2 (MK-4), or K_2 (MK-7). Certain intestinal bacteria can convert the ingested vitamin K_1 to K_3 or de novo synthesize vitamin K_3 from compounds such as alpha-keto-glutarate.

Vitamin K_3 is not a vitamin in the true sense; initially it was synthesized to be used as an oral provitamin K compound, most likely because of its conversion to vitamin K_2 (MK-4) in human and animal tissues.

Vitamin K_3 may have been chosen as a supplement because it is a much simpler molecule to synthesize than vitamins K_1 or K_2; thus it was less expensive to produce. However, oral consumption of vitamin K_3 was banned in humans when various side effects were noticed, such as increased red blood cell lysis, excessive oxidative stress, and nonspecific binding of thiols from large and small molecules in the blood.

However, the cytotoxic effects of vitamin K_3 were recognized as useful in support of cancer treatment, alone or in addition to chemotherapy, and K_3 has been used in the oral and intravenous forms (discussed later) in research studies involving humans. Vitamin K_3 is not currently available as a drug or supplement for human use.

It is important to keep in mind that serum vitamin K_3 can rise significantly as a result of ingesting supraphysiological doses of vitamins K_1 or K_2 (above 1 mg), especially if given in one bolus. (This concern is discussed under "Potential Side Effects of High Doses of Vitamins K_1, K_2, and K_3").

Animal nutrition. Certain animal feeding practices include synthetic vitamin K_3 as a supplement, probably because animals fed corn instead of grass receive little natural vitamin K_1. Vitamin K_3 is chosen because it costs significantly less than it would to add vitamin K_1 or incorporate foods naturally high in vitamin K_1, such as grass. No studies have reported the content of vitamin K_3 in animal foods from animals supplemented with vitamin K_3, and very few have reported the tissue content of vitamin K_2 (MK-4) in these animals. There is at least a theoretical concern that these foods may contain abnormally high levels of vitamin K_3 owing to incomplete conversion to the normally occurring K_2 (MK-4). It is not clear whether organic meats come from animals fed vitamin K_3, because they get plenty of vitamin K_1 from the grass.

PHYSIOLOGICAL ROLES OF VITAMIN K: EFFECTS OF NORMAL TO SUPRAPHYSIOLOGICAL DOSES

Mechanisms of Action of Vitamins K_1 and K_2

Aside from blood clotting support, new research is revealing numerous roles for vitamin K in many tissues and organs through a variety of mechanisms.

Vitamin K's actions in human physiology are very complex:

1. Posttranslational modification (through carboxylation) of a number of functional proteins, called vitamin K-dependent proteins (VDKs)
2. Modulation of inflammation through downregulation of cytokine IL-6 and prostaglandin E2 (PGE_2) (by inhibiting COX-2)
3. Downregulation of cell signaling proteins (produced downstream of mevalonate and 3-hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA]), which affect cell differentiation, proliferation, or apoptosis
4. Neurological effects through modulation of galactocerebroside sulfotransferase enzyme
5. Genetic expression modulation. K_2 (MK-4) stimulates the steroid and xenobiotic receptor, which upregulates expression of bone blastic proteins and collagen.
6. Anticancer effects

Vitamin K activates a variety of VKDs throughout the body by carboxylating a specific number of the glutamatic acid residues they contain, which become, as a result, gamma-carboxyglutamate residues, also called Gla. One molecule of vitamin K_1 or K_2 can be recycled many times, so it is used multiple times to produce Gla residues before it is metabolized for elimination (Fig. 126.3).

Once carboxylated, these residues can bind calcium or interact with cell membranes more efficiently. The more glutamate residues are carboxylated on a VKD protein, the better its functionality. Ultimately, the more vitamin K is available in the body, the higher the percentage of fully carboxylated VKDs will be.

Until now, the AI (adequate intake) for vitamin K has been defined as the amount needed to completely carboxylate prothrombin. With the discovery of new roles for various VKDs throughout the body, many researchers are now proposing redefining the AI at a level where all VKDs in the body are 100% carboxylated. A review by

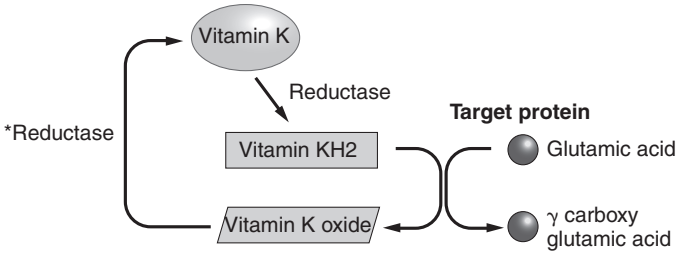


Fig. 126.3 Vitamin K recycling and carboxylation reactions. (From Bowen RA. Vitamin K. http://www.vivo.colostate.edu/hbooks/pathphys/misc_topics/vitamink.html. Accessed May 29, 2012.)

McCann and Ames points out that vitamin K affinity varies greatly for each type of VKD, which may influence the order in which VKDs are carboxylated. Most likely, this is done in an order of physiological priority, selected by evolutionary pressure for survival.[30] For example, clotting may have the highest priority in utilizing most of the vitamin K ingested when intake is around or below 100 mcg/day. In this situation, most of the other VKDs in the body will tend to be inadequately carboxylated and so have poor functionality. Unfortunately, there are no tests to verify the carboxylation of all the known VKDs except for the three that will be discussed (protein induced by vitamin K antagonist-II [PIVKA II], osteocalcin, and matrix GLA protein [MGP]). Important cofactors affecting the rate of carboxylation of VKDs are manganese and vitamin B_6, specifically pyridoxal-5-phosphate. Deficiency of these cofactors, as well as that of estrogen and thyroid hormone may downregulate carboxylation, whereas supplementation with pyridoxal-5-phosphate was shown in vitro to upregulate it twofold.

Osteocalcin is a protein produced by osteoblasts (bone-building cells) that transports calcium inside the bone for fixating it in the hydroxyapatite structure, thus supporting bone mineralization. Vitamin K has been shown to stimulate osteoblasts to increase their production of osteocalcin and collagen type I, both important components of the bone structure. Increased levels of osteocalcin are able to better support bone remodeling and thus improve bone architecture, which translates into better bone elasticity and thus reduced risk of fracture.[31–33]

Osteodentin (tooth dentin) is responsible for tooth mineralization, similar to osteocalcin's role in the bone.[34] Note that the salivary gland concentrates a significant amount of vitamin K, which may have a role in supporting oral health. Vitamin K is known to affect certain bacteria by participating as a cofactor in their energy production (similar to coenzyme Q10 in animal mitochondria). If it is in fact proved that salivary glands secrete vitamin K, this would affect oral bacteria; one researcher has stated that excess vitamin K may exacerbate periodontal disease.[35]

Nephrocalcin is found in kidney tissue and is believed to support calcium ion solubilization and inhibit crystal nucleation, thus reducing the risk of kidney stones.

Matrix Gla protein, or MGP, is found in many soft tissues, where it has at least one identified role, that of rejecting calcium deposition. Specifically, it is found in the skin and in the walls of arteries and veins, where it prevents the calcification of elastin fibers, thus reducing the risk of elasticity loss in these tissues. It is also found incorporated with underlying ground substance of the skin and cartilage (associated with chondrocytes), where it seems to play a role in the synthesis of glycosaminoglycans.[36] MGPs have also been identified in testes and sperm; their role there is not clear, but vitamin K deficiency has been shown to reduce testosterone production and sperm motility. One study that looked at primary prostate cancer cells versus metastatic cells found that "loss of MGP expression may be associated with tumor progression and metastasis."[37]

Note here the important roles of vitamins D and A in some of these VKDs:

- The active form of vitamin D (1,25[OH]D3) stimulates the synthesis of osteocalcin and MGP.[38]
- Vitamin A (retinoic acid) stimulates the synthesis of osteocalcin but inhibits that of MGP.[39]

Consequently, vitamins K, D, and A work in concert and have interdependent actions. Thus for vitamin K to fully realize its potential benefits, the status of vitamins D and A must be optimized so that VKDs are expressed adequately. It has also been established that the genetic effects of 1,25(OH)D3 are mediated by vitamin A through its nuclear receptor, which exerts a permissive activation on the vitamin D nuclear receptor.[40]

GAS-6 protein (growth arrest-specific gene 6) is believed to play a role in platelet aggregation, cell growth, and apoptosis and has been shown to support the survival of cells in tissues such as arterial muscle, eye lens epithelium, brain, and possibly others. It is also involved in supporting adequate innate immunity (natural killer [NK] cells) and reducing the risk or severity of autoimmune cellular events.[41]

Modulation of Inflammation by Downregulation of Inflammatory Cytokines

Vitamin K may reduce inflammation by downregulating of interleukin-6 (IL-6) and PGE_2 as follows:

- Metabolites of vitamin K, the 5- and 7-carbon carboxylic acid catabolites of vitamins K_1 or K_2 (MK-4 or MK-7), inhibit the release of IL-6. This was shown specifically for osteoblasts.[42]
- Vitamin K_2 (MK-4) has been shown to reduce the production of PGE_2 by direct inhibition of the COX-2 enzyme.[43]

These actions of vitamin K may reduce the activity of any inflammatory/autoimmune diseases that are exacerbated by PGE_2 and IL-6.[44] In osteoporosis, IL-6 and PGE_2 have been shown to enhance bone resorption through activation of the RANKL receptor, which in turn upregulates osteoclast differentiation.[45] This mechanism is considered one of the targets of treatment with high doses of K_2 (MK-4) in reducing and reversing bone loss. Vitamin K_2 has also been shown to reduce the activity of rheumatoid arthritis by inhibiting the proliferation of the rheumatoid synovial cells.[46] A correlation has been found between vitamin K status (plasma vitamin K_1) and osteoarthritic disease activity in the hands and knees.[47] In the Framingham study, plasma vitamin K_1 was shown to inversely correlate with C-reactive protein (CRP), a marker of inflammation.

An elevated PGE_2 should also be corrected by addressing the arachidonic acid/eicosapentaenoic acid (AA/EPA) ratio. This, in effect, modulates the substrates of the COX2 enzyme and would have an additional effect on PGE_2 production when added to the effect of vitamin K_2 (MK-4) of inhibiting COX2. One study states the effect of the AA/EPA ratio on another inflammatory intermediate as follows: "nuclear factor κB is activated by arachidonic acid but not by eicosapentaenoic acid."[48]

Downregulation of Cell-Signaling Proteins (Produced Downstream of Mevalonate and HMG-CoA) Affecting Cell Differentiation and Apoptosis

Vitamin K_2 (MK-4) is believed to have an inhibitory effect in pathways downstream of mevalonate, the same pathways where statins and bisphophonates are believed to inhibit key enzymes (HMG-CoA reductase and protein farnesyl transferase, respectively). However, the actions of K_2 (MK-4) are not the same as those of pharmaceutical

drugs, which have a direct enzyme inhibitory action. Instead, K_2 (MK-4) seems to work through a feedback inhibition effect on the mevalonate pathway by "mimicking" one of its downstream metabolites, the molecule geranylgeranyl pyrophosphate.[49,50] This mimicry occurs by means of the side chain of vitamin K_2 (MK-4), which is the molecule. This may be why K_2 (MK-4) has distinct effects compared with those of other forms of vitamins K_2 or K_1.

The inhibition of the mevalonate pathway has multiple effects on human physiology besides cholesterol inhibition because it also downregulates the synthesis of various geranylgeranylated proteins (rho, rab, rac) involved in cell proliferation, cell death, and survival, inflammation, and immune response.[51] Consequently when vitamin K_2 (MK-4) is supplied orally in large doses (many studies have used 45 mg and even 90 mg,[52] which are in the supraphysiological range), it exerts a "mass action" characterized by some researchers as "pharmacological" in nature.

K_2 (MK-4) has been used as a drug in Japan and as a therapeutic nutrient in the United States for the prevention or reversal of osteoporosis, prevention or reversal of cardiovascular calcification, cancer prevention, and treatment support.

In conclusion, vitamin K as K_1 or K_2 has a plethora of actions and specific benefits for health maintenance and disease prevention or treatment.

Bone Health and Therapeutic Interventions for Osteoporosis

The composition of bone is relevant for determining the nutrients needed to maintain its physical structure and those required for the biochemical processes that support this structure: 70% of bone mass is represented by minerals (mostly calcium and phosphorus along with magnesium and silicon) with the rest being composed of 20% organic matter and 10% water. Organic matter is in turn composed of cells and proteins, of which collagen represents 80% to 90%.[53] Collagen fibrils connect bone mineral crystals of calcium and phosphate (Fig. 126.4) and contribute to bone elasticity, strength, and overall quality, which are important determinants of fracture risk (FR).[54]

Bones undergo continuous remodeling by two opposing processes: (1) bone breakdown (or bone resorption) by osteoclast cells and (2) bone building by osteoblast cells. Vitamins K_1 and/or K_2 are stored in bone, partially in the same form as ingested, and partially after being converted to K_2 (MK-4). One animal study revealed a proportion of 10% K_1 and 90% K_2 (MK-4) stored in bone when only vitamin K_1 was ingested.[24] Regarding MK-7, reports are conflicting, with some stating that no MK-7 is detected in bone after MK-7 supplementation[23] probably due to conversion to MK-4, whereas others state that some of it ends up as such in bone. It is not clear what determines the relative distribution of vitamin K forms stored, but it is likely influenced by the absolute and relative amounts of vitamin K forms ingested and the duration of this exposure. The type and amount of vitamin K stored in bones is likely an important determinant of its clinical effects and should factor in choosing various vitamin K forms, alone or in combination, for clinical applications in future studies.

Vitamins K_1 and K_2 modulate bone metabolism as follows:

1. Vitamins K_1 and K_2 participate as cofactors in the activation (by carboxylation) of osteocalcin, which enables binding of calcium and its proper deposition in the bone matrix. Other VKDs have been detected in bone and are believed to have functional roles, not well clarified yet.

Intervention studies and large population assessments revealed that a significant percentage of osteocalcin is uncarboxylated (uncarboxylated osteocalcin, or ucOc) in humans of all ages. See Tables 126.4 to 126.7, which list baseline percentage ucOC in study subjects in the range of 2.6% to 65%.

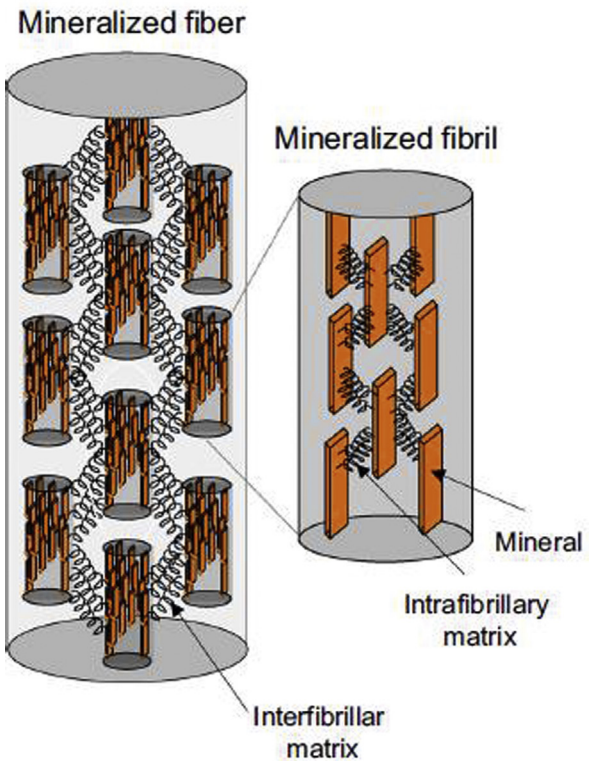


Fig. 126.4 Bone components: collagen fibrils connected to minerals providing a structural scaffold with resilience and elastic properties.

2. Vitamin K_2 (MK-4) is unique among the vitamin K_2 family of compounds because the ligand it contains, geranylgeranyl (GG), is similar to one intermediate of the cholesterol synthesis pathway, geranylgeranyl diphosphate (GGDP). GG it is believed to have inhibitory actions downstream of mevalonate and HMG-CoA, on various metabolites, including synthesis of cholesterol, inflammatory and proproliferatory compounds, thus reducing osteoclast proliferation.[49,55,56]
3. Vitamin K_2 (MK-4) stimulates synthesis of the bone blastic proteins osteoprotegerin and osteopontin.[57]
4. In vitro studies with vitamin K_2 (MK-4) have shown it to increase osteoblast production of osteocalcin and collagen type I, via steroid and xenobiotic receptor activation.[58,59] This increase in collagen production may, at least partially, explain why vitamin K_2 improves bone strength and FR even when BMD is not improved, because collagen represents 80% to 90% of the organic mass of bone.[60–62]

Although MK-7 was also shown to activate the steroid and xenobiotic receptor (SXR) in vitro, there is no exact overlap of its actions with those of MK-4, as a result of distinct effects on other genes.[63] Because MK-7 converts to MK-4 (partially or entirely) in human physiology, it is not clear what the difference would be between the genetic effects of oral administration of these two different forms of vitamin K_2, as no studies have investigated them side by side.

5. Vitamin K_2 (MK-4) was shown to reduce osteoblast apoptosis in in vitro studies.[64]
6. Vitamin K_2, as MK-4 and MK-7, demonstrated an inhibitory effect on RANKL-induced osteoclast differentiation and bone resorption in in vitro studies, whereas MK-4 exhibited a significantly greater efficiency compared with MK-7.[65]
7. Vitamin K_2 (MK-4) reduces osteoclast production from bone marrow cells[66] and induces osteoclast apoptosis, which in effect reduces the total number of osteoclasts. These metabolic effects are achieved

through vitamin K_2's ability to downregulate inflammation (IL-6, PGE_2) and cell proliferation while increasing apoptosis (through the geranylated proteins produced in the mevalonate pathway).[67] This is especially beneficial when osteoclastic activity is elevated, as is often the case in menopause/andropause, likely as a result of estrogen or testosterone insufficiencies.

In conclusion, both K_1 and K_2 (as MK-4 or MK-7) forms can directly carboxylate osteocalcin as such. MK-4 (whether supplemented as such or derived from K_1 or MK-7) induces mechanisms that reduce bone resorption and increase collagen and osteocalcin content of bone. The MK-7 form exerts some but not all the effects of MK-4. This may be because of the unique ligand, GG, contained in MK-4, and its pharmacokinetics, which may explain its unique benefits.[49,55]

Optimal Dose of Vitamin K_1 and/or K_2 for Complete Activation (Carboxylation) of Osteocalcin

Table 126.4 lists studies that investigated the relationship between the level of vitamin K_1 and/or K_2 ingested from a supplement plus from the diet and the level achieved for percentage of ucOc. Tables 126.5 to 126.7 list additional studies that show the change in percentage of ucOc from various interventions with vitamin K_1 or K_2, aimed at evaluating effects on bone density, bone strength, FR, and bone turnover markers. The combined evidence from these studies shows the following:

- With respect to vitamin K_1 interventions, a dose as high as 1 mg is necessary to maximize carboxylation of Oc, which is reflected by minimizing the percentage of ucOc (% ucOc = ucOc/Total Oc) to values such as: 1.9%[25] or 0.54% (close to the detection limit).[68] A dose of 0.5 mg K_1 was able to lower ucOc down to 2.5% in one study,[25] but others showed less lowering, such as 21.1%[69] or 17.4% (males) and 24.1% (females).[70] The discrepancy between studies may be a result of testing methods, and possibly other characteristics, such as different baseline percentage ucOC.
- With respect to vitamin K_2 (MK-4), a dose of 45 mg lowered % ucOc to approximately 4.5%[65] or 5%,[71] whereas a 1.5 mg dose of MK-4 lowered it only to 22% or 14.3%.[72]
- With respect to vitamin K_2 (MK-7), doses of 0.36 mg or 0.375 mg lowered % ucOc to 10%[73,74] or 9%,[75] respectively. A dose of 0.18 mg MK-7 lowered % ucOc down to 24%,[76] whereas a dose of 0.045 mg of K_2 (MK-7) reported a drop in % ucOc from 48% to 38%.[77]

The subjects in the intervention studies that used K_1 or K_2 (MK-4 or MK-7) as a supplement were also ingesting K_1 from their regular diets. These values are specified in Tables 126.4 to 126.7, based on what was reported in each study or an average intake of K_1 from the country where the study took place.

Discussion of results from interventions with various forms of vitamin K are grouped as follows by vitamin K_1, K_2 (MK-4), and K_2 (MK-7) forms because they have different pharmacokinetics and tissue deposition.

Interventions With Vitamin K_1 Alone or in Comparison With K_2 (MK-4 and MK-7)

Table 126.5 lists the most relevant interventions with vitamin K_1 to date, with the majority assessing bone mineral density (BMD), one evaluating FR, and a few of them looking at markers of bone formation and breakdown. The treatment doses of vitamin K_1 used in these studies range from 0.1 mg to 1.0 mg. Some authors claimed to have chosen the K_1 dose from a range of 0.1 mg to 0.5 mg, determined to be equivalent to what may be derived from dietary intake. However, an intake of 1 mg/day is achievable if one ingests a considerable amount of leafy greens or possibly their juices. Within a dietary range of 0.1 mg to 1.0 mg of K_1 intake, this vitamin is expected to exert its effects within normal physiological function based on evolutionary adaptation[78]; thus we will refer to these as "physiological" doses, with higher ones considered "supraphysiological." It is not clear why K_1 doses lower than 1 mg were chosen in any of these studies published after 2002, because at that time, a study demonstrated this to be the dose needed for complete carboxylation of osteocalcin.[79] This likely maximizes the bone building effects of treatments with vitamin K_1 combined with calcium and vitamin D. In the following section is a discussion of results obtained from the studies presented in Table 126.5, which used doses of vitamin K_1 in a physiological range (0.1–1 mg) for women in menopause.

Effects of Vitamin K_1 Supplementation in the Range of 0.1 mg to 1 mg on Bone Mineral Density

Only one study reported increases in whole body BMD of 1.3% and lumbar spine BMD of 1.6% in the group treated with 0.1 mg K_1 (plus 0.11 mg K_1 from the diet), which is the lowest dose used among all listed studies.[80] These interventions included approximately 1000 mg of calcium (from diet + supplementation) and 400 mg magnesium (from diet), highest among all listed studies. The study setting was in Greece, where the diet was most likely of a Mediterranean type. It was the only study that imposed a certain amount of daily physical activity, which was monitored by a pedometer. Bioavailability of K_1 may have been enhanced by delivery in dairy products (milk or yogurt). Only one other study, using a 0.2 mg dose of vitamin K_1, showed an increase in BMD, and only at the ultradistal radius, of 6.2 mg/cm,[81] whereas the control group showed a decline in BMD. This study also included a high calcium intake of 1000 mg, similar to the study discussed previously. Other studies listed in Table 126.5 did not achieve increases in BMD despite using higher doses of vitamin K_1 (0.5–1 mg). They reported either a slower or similar BMD decline compared with the control groups. One explanation could be that all these studies incorporated insufficient calcium intake, in the range of 500 to 700 mg. In addition, for all studies listed, the vitamin D status, as reported by serum 25(OH)D3, was typically near the lower limit of the normal reference range. This was not optimal, by US standards, but not surprising because the amounts of vitamin D given in supplement form were: 0, 320, 400, and 600 IUs, with no significant sun exposure.

Effects of Supplementation With 0.1 mg to 1 mg Vitamin K_1 on Markers of Bone Metabolism

Urinary D-Pyd decreased by 22% in the group treated with 0.1 mg K_1 for 1 year.[80] The other study that measured markers of bone breakdown found no significant changes.[82] Unfortunately, none of the vitamin K_1 studies that supplemented with physiological doses reported FR.

Effects of Supplementation With 5 mg and 10 mg Vitamin K_1 on Bone Mineral Density, Fracture Rate, and Markers of Bone Metabolism

Table 126.5 lists a few studies that used supraphysiological doses of vitamin K_1 at 5 mg and 10 mg. The rationale for these doses was not explained by the authors, but it was possibly under the assumption that they would have more pronounced effects on bone metabolism, possibly by increasing conversion of vitamin K_1 to K_2 (MK-4) and their stimulatory effects on bone metabolic pathways. The resulting increase in vitamin K_2 (MK-4) stored in bone would be expected to reduce inflammation and osteoclast activity and cause osteoclast apoptosis, possibly similar to supplementing directly with high doses of K_2 (MK-4). The study that used a dose of 5 mg in conjunction with 1500 mg calcium for postmenopausal women found a 50% reduction in FR after 2 years, whereas the BMD decline was the same as in the control group at −1.28%. Two studies used a dose of 10 mg K_1 in young athletes, some with amenorrhea, who had similar hormone levels to those

TABLE 126.4 Summary of Studies Using Interventions With K_1, MK-4, or MK-7 to Assess Changes in Osteocalcin Carboxylation

Reference	Length	Age, Gender	Vit K Supplement	Vit K from Diet	Baseline % ucOc	Final% ucOc	Final UcOc	Comments
Binkley[79] 2002, USA	2 wks	25 yrs, females	0.25 mg K_1	0.08 mg K_1	7.9%	ns.[a]		Maximal carboxylation of Oc may be achieved with a supplement of ≥1 mg K_1 BMI = 26
			0.37 mg K_1	0.12 mg K_1	6.5%	ns.[ab]		
			0.5 mg K_1	0.09 mg K_1	8.7%	2.5%		
			1 mg K_1	0.08 mg K_1	8.3%	1.9%[b]		
Koitaya[184] 2009, Japan	4 wks	53–65 yrs, females in menopause	1.5 mg MK-4	0.22 mg K_1	Approx. 43%	Approx. 22%		Plasma MK-4 raised from 0.2 to 1.5 ng/mL
			Placebo	0.31 mg K_1	No significant change			
Nakamura[72]2014, Japan	1 wk	25 yrs males	Placebo	0.07 mg K_1	21%	21%	-	A dose ≥ 0.6 mg MK-4 dose is needed to reduce total ucOc level below the cut-off value of 4.5 ng/mL, which was assessed to be relevant for increased fracture risk.[72] Average PT-INR was maintained in the normal range. Plasma MK-4 raised from 0.14 to 0.58 ng/mL
			0.3 mg MK-4	0.09 mg K_1	21%	24%	6.8 ng/mL	
			0.6 mg MK-4	0.05 mg K_1	21%	15.9%	4.8 ng/mL	
			0.9 mg MK-4	0.02 mg K_1	21%	11.5%	2.9 ng/mL	
			1.5 mg MK-4	0.02 mg K_1	21%	14.3%	3.9 ng/mL	
Dalmeijer[189]2012, Holland	12 wks, n = 60	40–65 yrs	0.18 mg MK-7	0.18 mg K_1, 0.02 mg K_2	32%	13%		dp-ucMGP was reduced from 401 to 276 pmol/L, by 31%
			0.36 mg MK-7	0.19, mg K_1 0.02 mg K_2	30%	10%		dp-ucMGP was reduced from 391 to 209 pmol/L, by 46%

[a]There was no statistical difference from baseline
[b]This value is said to be close to the detection limit.

TABLE 126.5 Summary of Studies Using Interventions With K_1 or K_1 Versus K_2

Reference	Length	Age, Gender	Vit K Supplement	Vit K From Diet	Vit D	Minerals (Ca, Mg, Zn)	Baseline %ucOc	Final %ucOc	BMD, BMC, Fracture Rate, Bone Strength, Markers of Bone Turnover	Comments
Braam[68] 2003, Holland	3 yrs	50–60 yrs, fem	1 mg K_1	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	320 IU	500 mg Ca, 150 mg Mg, 10 mg Zn	3%	0.54%	ΔBMD = –3.8%	Study sponsored by Novartis, which is a maker of a bisphosphonate, thus presenting a potential conflict of interest. The study concludes: nutritional interventions are not as effective at increasing or preventing BMD decline as bisphosphonates do.
			Placebo		320 IU	500 mg Ca, 150 mg Mg, 10 mg Zn	2.6%	2.5%	ΔBMD = –5.1%	
			Placebo		-	-	2.8%	2.8%	ΔBMD = –5.5%	
Bolton-Smith[81] 2007, UK	2 yrs	67 yrs, fem	0.2 mg K_1	0.08 mg K_1	600 IU	1000 mg Ca	50.0%	23.0%	ΔBMD (ultra distal radius) = +6.2 mg/cm ΔBMD (mid distal radius) = –16.9 mg/cm,	Serum vitamin K_1 increased from 0.22 to 0.55 μg/L. Plasma 25(OH)D3 range = 24.7–28.3 μg/L. It took 6 mo for ucOc to attain at the final lower value of ucOc of 23%. BMI = 26.
			Placebo		600 IU	1000 mg Ca			ΔBMD (mid distal radius) = –16.6 mg/cm	
			0.2 mg K_1		-	-	48.0%	25.2%	ΔBMD (mid distal radius) = –18.6 mg/cm	
			Placebo	-	-	-			ΔBMD (mid distal radius) = –20.4 mg/cm	
Cheung[129] 2008, Canada	2 yrs	59 yrs, fem	5 mg K_1	Approx. 0.12 mg[a] K_1	800 IU	1500 mg Ca	44.8%	23.8%	RR of fracture was reduced by 50%. ΔBMD = –1.28%, and not statistically different from placebo.	The K_1 dose of 5 mg did not lower % ucOc similar to other studies to less than 3%. Serum vitamin K_1 increased approx. 10 times from 1.8 nmol/L to 22.6 nmol/L.
			Placebo		-	-	46.8%	No change	ΔBMD = –1.22%	
Ned[82] 2007, Holland	6 wks	62 yrs, fem	0.2 K_1	0.18 mg K_1, 0.03 mg[a] K_2	400 IU	700 mg Ca (from diet)	Not reported	24%	No significant change in bone turnover marker serum bone alkaline phosphatase or urinary markers of bone resorption: NTX (N-telopeptide), pyridinoline and deoxypyridinoline.	Final serum vitamin K_1 attained the following levels: 2.4 nmol/L, 3 nmol/L, 4.7 nmol/L, I for K_1 intake of 0.18 mg, 0.38 mg, 0.67 mg, respectively. Average dietary phosphorus was 961 mg/day. BMI = 26.
			0.5 K_1		400 IU			13%		
			Placebo		400 IU			43%		
Booth[70] 2008, US	3 yrs	60–80 yrs, fem	0.5 mg K_1, effervescent tablet	Approx. 0.12 mg[a] K_1	400 IU	600 mg Ca	35.9% (M) 42.8% (F)	17.4% (M) 24.1% (F)	ΔBMD = –0.9% in males, ΔBMD = –1.8% in females	Serum K_1 (nmol/L) increased from 1.4 (M), 1.1 (F) to 1.4 (M), 2.3 (F), which is not as high as in other studies after supplementation with vit K_1 = 0.5 mg. Plasma 25(OH)D3 range: 20.8–24.3 and 25–26.5 (μg/L).
			Placebo				39.1% (M) 41.6% (F)	40.7% (M) 44.7% (F)	BMD loss was not statistically different compared with the vit K_1 group	
Shea[69] 2008, US	3 yrs	60–81 yrs, fem	0.5 mg K_1	Approx. 0.12 mg[a] K_1	400 IU	600 mg Ca plus a multivitamin	40.1%	21.1%	No change in BMD in both groups. No BMD decline in the placebo group is an unexpected finding. No change in inflammatory cytokines.	36% of subjects were on statins, which are known to impair conversion of vitamin K_1 to K_2, but may help reduce bone loss. Plasma 25(OH)D3 was not assessed. BMI = 27.2–28.4
			Placebo				40.8%	42.8%		

Continued

TABLE 126.5 Summary of Studies Using Interventions With K_1 or K_1 Versus K_2—cont'd

Reference	Length	Age, Gender	Vit K Supplement	Vit K From Diet	Vit D	Minerals (Ca, Mg, Zn)	Baseline %ucOc	Final %ucOc	BMD, BMC, Fracture Rate, Bone Strength, Markers of Bone Turnover	Comments
Binkley[92] 2009, US	1 yr, n = 381	62 yrs, post-menopausal fem	1 mg K_1	Approx. 0.12 mg[a] K_1	400 IU	630 mg Ca citrate	13%	5%	No BMD changes in any of the groups. No bone loss in the placebo group during menopause is an unexpected finding	Study supported by Eisai, the maker of the pharmaceutical version of 15 mg MK-4. At baseline, females were osteopenic but not osteoporotic.
			45 mg MK-4 (15 mg 3×/day)				11.60%	4.55%		
			Placebo				12%	?		
Moschonis[185] 2011, Greece	1 yr, n = 173	60–62 yrs	0.1 mg K_1	0.11 mg K_1	Approx. 400 IU	1068 mg Ca 393 mg Mg	40.8%	27.5%	Total BMD increased by 1.3%, BMD BMD (lumbar spine) increased by 1.6% urinary D-Pyd decreased by 22%	Total calcium intake was derived from a supplement of 800 mg Ca, and the rest from the diet. Magnesium was derived from diet only. Plasma 25(OH)D3 range: 21.5–25.7 ng/mL Funded by a producer of dairy (Friedland Campina). Vitamin K supplements were administered in milk or yogurt, which may have increased their bioavailability and compliance. The Greek diet (Mediterranean) may be more supportive of bone health compared with diets of subjects in other studies (US, Northern Europe) due to its alkalinity and anti-inflammatory components. Imposed exercise assessed by pedometer, 8000–9000 steps/day. Ave BMI = 30.
			0.1 mg MK-7	0.14 mg K_1	Approx. 400 IU	1077 mg Ca 363 mg Mg	47.0%	23.4%	Total BMD increased by 1.3%, BMD BMD (lumbar spine) increased by 0.6%, urinary D-Pyd decreased by 21%	
			Placebo	0.12 mg K_1	Approx. 400 IU	1188 mg Ca 410 mg Mg	64.50%	77.30%	Total BMD increased by 2.4%, BMD BMD (lumbar spine) did not change. Authors comment on the higher increase in BMD possibly resulting from calcium deposits in soft tissues other than bone, due to low vit K intake.	
			Placebo	0.11 mg K_1	Placebo	781 mg Ca from diet 410 mg Mg	65.10%	93.80%	No significant changes in BMD	
Craciun[84] 1998, Holland	1 mo	Female athletes	10 mg K_1	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	-	Stated as deficient	%ucOc was improved but values were not reported		Improved markers of bone formation (bone alkaline phosphatase) and bone resorption hydroxyproline, deoxypyridinoline)	Only 8 subjects, single-arm observational study. Female athletes with low estradiol levels (similar to menopausal women). 4 out of 8 were amenorrhoeic.

TABLE 126.5 Summary of Studies Using Interventions With K_1 or K_1 Versus K_2—cont'd

Reference	Length	Age, Gender	Vit K Supplement	Vit K From Diet	Vit D	Minerals (Ca, Mg, Zn)	Baseline %ucOc	Final %ucOc	BMD, BMC, Fracture Rate, Bone Strength, Markers of Bone Turnover	Comments
Braam[83] Holland	2 yrs	Female athletes	10 mg K_1, eumenorrheic	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	-	750 mg Ca	Not reported		ΔBMD (femoral neck) = –3.2%. BMD (lumbar spine) did not change	Vit K_1 did not affect the rate of BMD loss in any of the three types of groups. 31% of participants discontinued participation for personal or professional reasons, which may have affected outcomes.
			Placebo, eumenorrheic		-		Not reported			
			10 mg K_1 estrogen supp.		-		Not reported		ΔBMD (femoral neck) = –3.9% BMD (lumbar spine) did not change	
			Placebo, estrogen supp.		-		Not reported			
			10 mg K_1, amenorrhoeic		-		Not reported		ΔBMD (femoral neck) = –6.5% BMD (lumbar spine) did not change	
			Placebo, amenorrhoeic		-		Not reported			

TABLE 126.6 Summary of Studies Using Interventions With MK-7

Reference	Length	Age, Gender	Vit K Supplement	Vit K From Diet	Vit D	Minerals (Ca, Mg, Zn)	Baseline %ucOc	Final %ucOc	BMD, BMC, Fracture Rate, Bone Strength, Markers of Bone Turnover	Comments
Emaus[74] 2010, Norway	1 yr, n = 354	50–60 yrs, fem meno-pausal	0.36 mg MK-7 placebo	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	- -	- -	23% 24%	10% 20%	No significant difference in BMD decline between treatment and placebo groups.	The subjects were an avg. of 3.6 yrs post menopause onset. Some authors claim that interventions to reverse bone loss are likely more successful when implemented before significant drops in estrogen/progesterone levels, due to loss of trabecular networks. BMI = 24.5.
Knapen[76] 2013, Holland	3 yrs, n = 244	55–65 yrs, fem meno-pausal	0.18 mg MK-7 Placebo	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	- -	- -	40% 39%	24% 39%	BMD and BMC declined less in the MK-7 group compared with the placebo group at the spine and femoral neck but not at the hip. The "impact strength index," declined less in the MK-7 group compared with placebo, while there was not statistical difference for the other two bone strength indices between the two groups. Vertebral height (in the lower thoracic region) was reduced less in the treatment group.	Study supported and vitamin K_2 provided by Nattopharma. BMI >30. Plasma 25(OH)D3 range: 29.8–30.8 (ng/mL). One vertebral fracture occurred in the treatment group and six in the placebo group, but these results could not be given statistical significance.
Ronn[85] 2017, Den-mark	1 yr, n = 149	60–80 yrs, fem meno-pausal	0.375 mg MK-7	Approx. 0.21 mg[a] K_1 0.03 mg[a] K_2	1520 IU	800 mg	25%	9%	The BMD decline was small but with no significant difference between groups. In the tibia, markers of bone quality/ microarchitecture (trabecular numbers, spaces and thickness) were unchanged in the MK-7 group while changing unfavorably in the placebo group. There was a small significant increase in bone formation marker BAP (Bone Alkaline Phosphatase)	Study supported by Orkla Health, which provided the vit K_2, Ca, and vit D. T-score at baseline > –2.5 and < –1, 25(OH)D3 range: 88–86 nmol/L, 16.9–17.5 years since menopause.

[a]Based on average intake of K_1 in the geographical region where the study took place.
Reference range by a US commercial laboratory for serum K_1: 0.08–1.16 µg/L or 0.17–2.6 nmol/L. It is influenced by plasma triglycerides and various genetic polymorphisms.
Reference range by a US commercial laboratory for serum 25(OH)D3: 30–100 µg/L (ng/mL) or 74.9–249 nmol/L. It is influenced by various genetic polymorphisms.

TABLE 126.7 Summary of a Few Selected Studies Using Interventions With MK-4

Reference	Length	Age, Gender	Vit K Supplement	Vit K From Diet	Vit D	Minerals (Ca, Mg, Zn)	Baseline %ucOc	Final %ucOc	BMD, BMC, Fracture Rate, Bone Strength, Markers of Bone Turnover	Comments
Koitaya[186] 2014, Japan	1 yr, n = 50	50–60 yrs, fem meno.	1.5 mg MK-4	0.17 mg K_1, 0.06 mg[a] K_2	Approx. 312 IU	Approx. 467 mg Ca, 272 mg Mg	24%	14%	No decline in forearm BMD	Plasma pentosidine (a marker of non-enzymatic collagen cross-linking) decreased significantly. This may be attributed to antioxidant activity of MK-4. Plasma MK-4 increased from 0.1 to 0.29 ng/mL. Plasma 25(OH)D3 range: 19.4–20.5 ng/mL
			Placebo	0.18 mg K_1, 0.06 mg[a] K_2	Approx. 336 IU	Approx. 508 mg Ca, 266 mg Mg	22%	24%	Decline in forearm BMD was –2.4%	
Knape[71]n 2007, Holland	3 yrs	55–75 yrs, meno.	45 mg MK-4 (15 mg 3x/day)	Approx. 0.21 mg[a] K_1	-	Approx. 870 mg from diet	25%	5%	Femoral neck width and its BMC increased in the MK-4 group relative to placebo. The overall BMC declined, but at a lower rate than placebo. BMD of femoral neck and of total hip declined the same as in placebo group. One of three indices of hip bone strength was maintained in the MK-4 group but declined in placebo group, whereas the other two declined much less than in the placebo group.	Study was sponsored by Eisai, a pharmaceutical manufacturer of Glakay (15 mg MK-4). Plasma 25(OH)D3 range: 67–72.3 [nmol/l].
			Placebo	0.03 mg[a] K_2	-	Approx. 811 mg from diet	30%	Not statistically different from baseline		
Ushiro-yama[90] 2002, Japan	2 yrs	Meno.	45 mg MK-4	Approx. 0.15 mg[a] K_1	1 μg 1-α OH-D3	Guidelines to eat dairy	Not reported		ΔBMD = +4.92%	1 μg 1-α OH-D3 is the active form of vitamin D_3, a prescription medication in Japan. Authors state that vitamin D_3 supplements were not available in Japan at the time when many studies with Glakay (45 mg MK-4) were performed.
			45 mg MK-4	0.06 mg[a] K_2	-		Not reported		No significant decline in BMD	
			Placebo		1 μg 1-α OH-D3		Not reported		No significant decline in BMD	
			Placebo		-		Not reported		BMD declined	

of postmenopausal women, as a result of intense training and/or low body fat. One of the studies found no effect on BMD in the groups supplemented with 10 mg vitamin K_1 compared with controls,[83] whereas the other study reported improved markers of bone formation and resorption after 1 month of supplementing with 10 mg vitamin K_1.[84]

Interventions With Vitamin K_2 (MK-7)

Table 126.6 lists three of the only four human trials with K_2 (MK-7) interventions available, which used doses of 0.18 mg, 0.36 mg, and 0.375 mg. The fourth study used a dose of 0.1 mg K_2 (MK-7) in a side-by-side comparison with 0.1 mg K_1, see details in Table 126.5.[80]

These K_2 (MK-7) doses can be considered nutritionally equivalent to a fraction of a serving of natto, which provides approximately 400 mcg K_2 (MK-7)/serving. These doses are higher than average consumption of K_2 (MK-7) in Japanese and Western diets, reported at 57.4 mcg or 7 mcg MK-7, respectively. To put this in perspective, total consumption of all forms of K_2 averages 61.7 mcg and 29.1 mcg in Japanese and Western diets, respectively.

All three listed studies were done in Northern Europe, where average vitamin K_1 intake was reported at 0.21 mg, which counts toward the total vitamin K intervention. Vitamin D and calcium were supplemented in only two of these studies.[80,85]

Effects of MK-7 on Bone Mineral Density and Bone Mineral Content

Studies which used supplemental doses of 0.36 mg[74] and 0.375 mg[85] K_2 (MK-7) reported no difference in BMD decline compared with placebo, whereas the study, using the dose of 0.18 mg K_2 (MK-7), reported a slower decline in total BMD and bone mineral content (BMC) compared with control at the spine and femoral neck but not at total hip.[76] The study that used 0.1 mg K_2 (MK-7) (plus 0.14 mg K_1 from diet) noted an increase in total BMD of 1.3%, and of 0.6% for BMD of the lumbar spine.[80] This study reported the same benefit on total BMD for the vitamin K_1 intervention with 0.1 mg (plus 0.14 mg K_1 from diet) but better improvement of BMD at the lumber spine of 1.6% with the vitamin K_1 intervention. The reason why this study achieved better improvement in BMD compared to all other interventions with K_1 or K_2 (MK-7), may be a result of better bioavailability of vitamin K_2 (MK-7) and that of vitamin K_1 administered in dairy; higher calcium, magnesium, and vitamin D intake; enforcement of exercise; and a Mediterranean diet.

Effects of MK-7 on Bone Strength, Bone Quality

The study that used the 0.18 mg K_2 (MK-7) dose for 3 years reported that one out of three bone strength indices declined less in the treatment compared with placebo group, whereas the other two declined to the same degree as placebo. Also, loss of height for a few lower thoracic region vertebrae was less pronounced in the 0.18 g MK-7 group.[76] The study that used the 0.375 mg K_2 (MK-7) dose for 1 year reported that markers of bone quality (such as trabecular numbers, average thickness and spaces) were unchanged in the treatment group, while being changed unfavorably in the control group.[85]

Effects of MK-7 on Markers of Bone Metabolism

A small but significant increase in the bone formation marker bone alkaline phosphatase was reported for the treatment group with 0.375 mg K_2 (MK-7), after 1 year.[85] Urinary D-Pyd decreased by 21% in the group treated with 0.1 mg K_2 (MK-7) for 1 year.[80]

Effects of MK-7 on Raising Bone Content of Vitamin K

One animal study compared the effects of supplementing ovariectomized rats with equimolar doses of K_2 (MK-7) and K_2 (MK-4) for 6 weeks, on changes in bone content of various forms of vitamin K.[86] This study showed that supplementation with an equivalent human dose of 57 mg of K_2 (MK-4) was more efficient than an equimolar human dose of 85 mg of K_2 (MK-7) at raising bone content of vitamin K, as illustrated by the following posttreatment levels:

- After the K_2 (MK-4) intervention, bone levels were: 62.4 nmol/L MK-4
- After the K_2 (MK-7) intervention, bone levels were: 16.7 nmol/L MK-7+1 nmol/L MK-4

Interventions With Vitamin K_2 (MK-4) With Doses in the Supraphysiological Range

Average dietary consumption of K_2 (MK-4) in Western diets was estimated at 7.1 mcg (2.1–28 mcg), whereas that of all forms of K_2 was 29.1 mcg (0.1–128 mcg). Knowing that a large proportion of vitamin K_1 converts to K_2 (MK-4) in human physiology, a dose as high as 1 mg MK-4 may still be considered within physiological range. Such doses were investigated only for their effect on carboxylation of Oc[72] (see Table 126.4). The results show that MK-4 is not as efficient at caboxylating Oc as K_1, because a dose of MK-4 of 0.9 mg was only able to lower it to 11.9% ucOc. On the other hand, all interventions designed with bone health outcomes documented so far used supraphysiological doses of K_2 (MK-4), (e.g., 45 mg). Most of these studies were done in Japan in the form of a pharmaceutical drug called Glakay, intended for menopausal or cortisone-induced osteoporosis. It is claimed that this dose was selected after lower doses of K_2 (MK-4) were studied (unpublished data) and did not perform as well. This dose is likely intended to enhance vitamin K_2 (MK-4) storage in bone and exert intense stimulation on bone biochemical pathways (Fig. 126.5).

A recent study investigated a much lower dose of K_2 (MK-4) of 1.5 mg/day in menopausal women and reported no change in forearm BDM, whereas in the control group there was a 2.4% decline in BMD (see data in Table 126.7).

Treatment with 45 mg MK-4 (15 mg 3x/day) has been reported in many studies to reduce FR significantly, even when BMD was not affected.[32,87] Examples of such FR reduction are: 77% for hip, 60% for vertebral, and 81% for nonvertebral types.[32]

A meta-analysis study concludes: "high dose vitamin K_2 (MK-4) improved indices of bone strength in the femoral neck and reduced incidence of clinical fractures," and "bone strength is a reflection of both bone mass and bone quality independent of the BMD."[87] Two meta-analysis studies concluded that the overall effect of vitamin K_2 (MK-4) on BMD was modest and only at the lumbar spine and not femoral neck.[88,89] However, it is important to note that only a few of these studies used the pharmaceutical form of vitamin D 1,25 (OH) D3, which may have had some of the benefits seen when raising vitamin D status with 25(OH)D3. The data from one of these studies are presented in Table 126.7 and show that treatment of menopausal women with 1,25 (OH)D3 alone, or K_2 (MK-4) alone, could only stall the BMD decline, but the combination of these treatments was able to produce an increase in BMD of 4.92% after 2 years.[90]

A Glakay 1996 postmarketing population survey done in Japan of 4000 people taking the same dosage of 45 mg/day of K_2 (MK-4) did not measure BMD and showed no effect on overall FR; however, a subgroup that had at least five fractures at baseline did show a fracture reduction of about 40%.[91] These disappointing results may be due to a likely suboptimal vitamin D status in the Japanese patients taking Glakay and likely lack of guidelines to supplement with calcium and vitamin D, or to increase sun exposure.

It is interesting that two studies conducted in Holland and the United States, where the investigators used the same K_2 (MK-4) material from Japan (Glakay), obtained no effect on BMD (see data in Tables 126.7[71] and 126.5[92]), but this may also be explained by low vitamin D status.

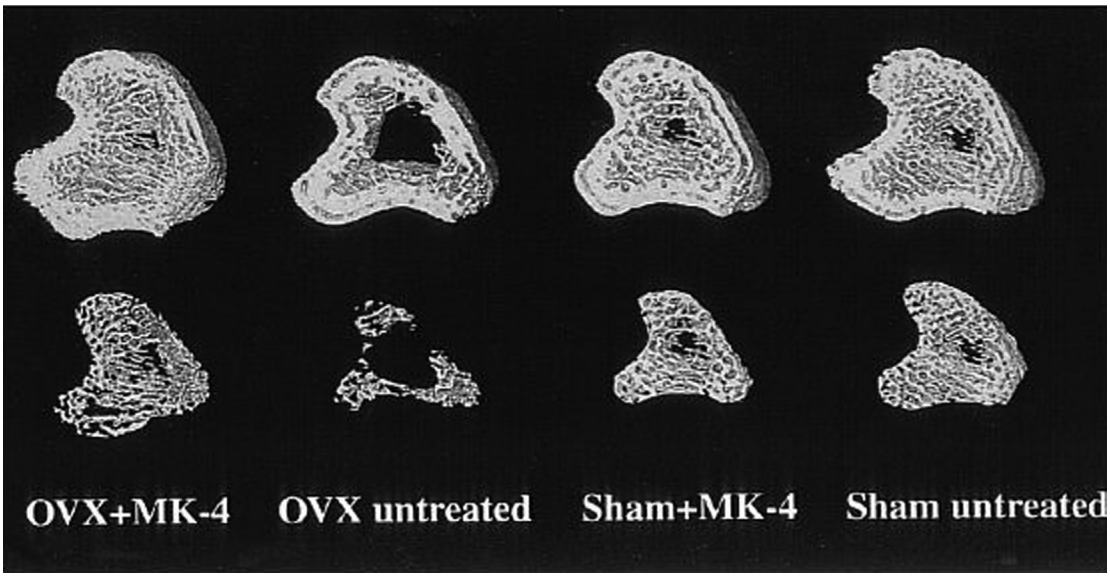


Fig. 126.5 Rat bone specimens after the removal of the cortex. Treatment with 30 mg/kg body weight of vitamin K_2 (MK-4), for 8 weeks, prevented OVX-induced cancellous bone loss. *OVX*, Ovariectomy; *Sham*, sham operation; *MK-4*, vitamin K_2 (MK-4).[183]

However, the Dutch study found other positive changes in markers of bone quality, such as increased femoral neck width and BMC, even though BMD of the femoral neck and of total hip declined the same as with placebo. One of three indices of hip bone strength was maintained in the K_2 (MK-4) group but declined in the placebo group, whereas the other two indices declined less than in the placebo group.

Interventions for osteoporosis are especially needed for women with insufficient estrogen resulting from menopause, ovariectomy,[93] anorexia, or excess exercise. When estrogen levels drop dramatically, it promotes the following changes: (1) elevated osteoclast cell activity, (2) higher ucOc levels (probably caused by reduced carboxylation efficiency needed for Oc carboxylation), (3) increased cortisol levels,[94] and (4) decreased whole body collagen synthesis, which is relevant for bone collagen.[95]

One Japanese study has compared the bone loss in three groups of menopausal women with that of women on placebo: group 1 took 45 mg of K_2 (MK-4) alone, group 2 took vitamin D in a drug form (active vitamin 1,25(OH)D3) alone, and group 3 took hormone replacement therapy (HRT) in the form of conjugated equine estrogen mix (CEE) 0.625 mg/day together with progestin (2.5 mg/day).[96] After a year, the groups had the following changes in BMD: +0.23% for group 1 (with K_2(MK-4)), −0.47% for group 2 (with D3), +4% for group 3 (with HRT), and −2.87 for placebo. Only the result for group 3 was statistically significant in differing from placebo. The results of this study suggest that vitamin K_2 (MK-4) at 45 mg/day may at least forestall the bone loss typical of menopause. Had these investigators combined vitamin K treatment with adequate vitamin D and minerals, the results might have become significant, with a potentially larger increase in BMD. Also, it should be noted that this study used equine HRT. No studies have looked at the effects of vitamin K_2 treatment in comparison with—or ideally, in conjunction with—bioidentical HRT (BHRT), which for numerous reasons is superior, safer, and more effective than equine-based HRT. Combining vitamin K_2 and BHRT treatment is likely to produce better results, not only in terms of bone, but in effects on overall health.

Similarly, one study showed that for men with low testosterone due to leuprolide treatment (for prostate cancer), a daily treatment of 45 mg of vitamin K_2 (MK-4) was able to reduce the expected decrease in BMD.[43] It is conceivable that the same treatment may be helpful for other low testosterone states as well.

Corticosteroids have been shown to downregulate Oc expression, but vitamin K_2 (MK-4) supplementation at 45 mg was able to partially mitigate this effect.

How Does K_2 (MK-4) Compare With Bisphosphonate Actions and Does It Make Sense to Combine Them?

One Japanese study showed that combining K_2 (MK-4) supplementation with bisphosphonate (BP) treatment showed an additive and synergistic effect, probably because of the complementary actions exerted by vitamin K_2 (MK-4) in addition to osteoclast apoptosis, which is the sole mechanism of typical BPs.[97,98] Another study showed that vitamin K_2 (MK-4) improved the effect of the BP in reducing FR significantly (slightly more than 50%) but had only a modest effect in improving BMD. Overall, BPs seem to have a much stronger effect in increasing BMD than K_2 (MK-4). However, some BPs seem to produce an increase in certain types of FR after long-term use.[99] Also, as discussed in Chapter 203, Osteoporosis, although bisphosphonates increase BMD short term, such bones are less remodeled and seem to be less elastic and more brittle, and therefore, with time, tend to become more vulnerable to fracture. Another reason to add K_2 (MK-4) to BP treatment is because of the fact that BPs, like statins, inhibit formation of metabolites of mevalonate-derived pathways (downstream from HMG-CoA). One of these metabolites is geranylgeranyl, which is used in the conversion of all forms of vitamin K to K_2 (MK-4). Thus because some of the benefits of taking K_1 and K_2 (MK-7) are derived from their partial conversion to K_2 (MK-4), it is especially important to incorporate K_2 (MK-4) with vitamin K supplementation for patients taking BPs or statins.

The Potential Reasons for Some of the Disappointing and Conflicting Results Among Vitamin K Studies May Be Explained by Some of the Following:

1. Duration of some studies was not long enough, such as 6 weeks or 1 year, to allow for statistically significant changes to occur.
2. Insufficient doses of vitamin K_1 and/or K_2, derived from diet plus supplementation, in most intervention studies compared with levels that would likely achieve complete carboxylation (activation) of Oc.

3. Insufficient doses of the nutrients essential to the specific actions of vitamin K on bone (such as vitamin D and calcium) as well as those addressing other aspects of bone metabolism, such as magnesium, zinc, manganese, vitamin B_6, vitamin A, and omega-3 fatty acids.
4. Vitamin K is a fat-soluble ingredient that is best delivered with fat and emulsifiers. The formulations given in different studies may have had different absorption rates as a result of delivery medium (capsule vs. soft gel, emulsifiers, mixed in dairy or delivered as an effervescent tablet to be dissolved in water, etc.).[100] Also, different forms of calcium have vastly different absorption rates.
5. The common diagnostic technique to measure BMD used in studies of vitamin K is not as sensitive in detecting the specific actions of vitamin K on bone as are other types of evaluations of bone health and bone quality, such as BU (bone ultrasound test of the heel), BMC, BG (bone geometry or size of various bone components, including height changes), or BS (bone strength, calculated from BMD, BG, height, and weight). By the nature of the measurement, BMD is thought to be unable to "give the complete picture" of bone status because it cannot distinguish architectural aspects of the bone quality, which involve its collagen scaffold.[101] This in turn determines its elasticity and strength, which are all important predictors of FR. This explains why vitamin K_1 and K_2 supplementation was shown to improve bone quality even in studies where BMD did not change as expected.[60,71,102,103]

Researchers estimate that vitamin K status, as assessed by plasma undercarboxylated Oc (ucOc), may correlate better with BU and FR than BMD.[104] Bone heel BU was found to have an accuracy and ability to discriminate osteoporotic patients similar to lumbar spine BMD (by dual-emission x-ray absorptiometry, or DEXA), with potentially better prediction for FR. Consequently, it may be useful to do a BU measurement (in addition to or instead of DEXA) in assessing the effects of vitamin K interventions in clinical practice. One study found that "ultrasound parameters were still significant independent predictors of vertebral fracture even after adjusting for BMD."[105] The BU test may give additional information to the BMD test, especially for patients who, for example, have increased their BMD after many years of bisphosphonate therapy (which reduces bone remodeling severely owing to its main mechanism of action of inducing osteoclast apoptosis).

Potential Synergistic Action Between Vitamin K_2 (MK-4) and Supplemented Hydrolyzed Collagen Protein

As mentioned previously, vitamin K_2 (MK-4) was demonstrated to stimulate the synthesis of bone collagen, which represents the scaffold that holds the mineral crystals in an elastic, resilient architecture. Dietary collagen protein has a very similar amino acid composition to human collagens, which represent 25% to 30% of total body protein, and 16% to 18% of bone mass. On the other hand, dietary collagen protein is unique, with a very distinct amino acid composition compared with all other food proteins, as a result of the very high proportion of glycine, proline, and hydroxyproline.

A few recent human studies have shown that supplementing with 5 g or 10 g of hydrolyzed collagen in postmenopausal women for 1 year has resulted in increases in BMD of 6.4% in the femoral neck and 5.5% in the spine[106] or 79% reduction of FR.[107] In vitro studies have demonstrated that collagen peptides stimulate bone collagen synthesis while also providing collagen amino acids inside cells,[108] thus we hypothesize on a potential synergy between vitamin K_2 (MK-4) and hydrolyzed collagen supplementation.

The Synergy and Necessity of Combining Vitamins K and D

Vitamin D is known to enhance Oc expression, which many be synergistic with the same action by vitamin K_2. Vitamin D increases calcium absorption in the gut, whereas vitamin K was shown to enhance calcium reabsorption in the kidneys. In vitro studies show that the most active form of vitamin D (1,25[OH]D3) enhances the effect of vitamin K_2 on Oc.[109] Thus vitamin D in conjunction with adequate calcium intake has potential to magnify the effects of vitamin K on calcium metabolism.[110] As discussed earlier, none of the studies employing vitamin K_1 or K_2 supplementation optimized the vitamin D status (as assessed by plasma 25[OH]D3) with sun exposure or vitamin D supplementation, compared with midrange values within laboratory reference ranges in the United States. Unfortunately, the doses necessary to achieve this, such as 2000 to 5000 IU of vitamin D_3 per day (depending on sun exposure), are still considered above the commonly accepted UL (upper tolerable limit). Plasma levels of 25(OH) D3 in vitamin K studies discussed here were close to the bottom of the reference range as a result of use of doses of vitamin D in the range of 200 to 1500 IU. Interestingly, the studies that administered 45 mg K_2 (MK-4) in conjunction with the active form of vitamin D (1,25(OH) D3) reported some of the best benefits on BMD among similar interventions with vitamin K.

Important Complementary Interventions to Increase Vitamin K Supplementation Efficacy

Adequate vitamin K intake from food and/or supplements or therapeutic high-dose supplementation may achieve the best results if it is coordinated with:

- Optimal vitamin D and vitamin A status.[111] Hypervitaminosis A has been shown to cause an excessive increase in bone turnover and to reduce bone quality.[112]
- Adequate intake of well absorbed minerals (Ca, Mg, Zn, boron), vitamin B_6, and vitamin C[113] and consider supplementation with 5 to 10 g of optimally hydrolyzed collagen protein.
- Optimal omega-3/omega-6 and EPA/AA (arachidonic acid) balance (to prevent excessive PGE_2 formation)[114]
- Proper acid/alkaline balance (possibly by monitoring urine pH) through dietary balance, optimal minerals, and potassium intake[115]
- Optimal levels of hormones (estrogen, progesterone, testosterone, DHEA, growth hormone, cortisol, thyroid)[116] as appropriate based on age and risk/benefit assessment.

Conclusions: What Is the Best Approach to Optimizing Status of Vitamin K in Support of Bone Health?

Some of the intervention studies discussed here, for each form of vitamin K, show promising results, although few proved to completely reverse all aspects of osteoporosis progression. Most studies did not report a restoration to healthy levels for BMD, BMC, or other indices of bone strength or bone quality, nor did they report normalization in markers of bone turnover. At best, many studies showed a slower decline or no decline in the previously-mentioned markers in menopausal women with established osteoporosis, whereas some reported significant reduction in FR for the high-dose treatment with 45 mg K_2 (MK-4) or 5 mg K_1. However, it seems that there is great potential in a therapy that combines all known bone beneficial nutrients, in bioavailable forms and adequate doses (from diet, supplements, and sun), along with optimizing vitamin K status. All forms of vitamin K seemed to show some benefit, whether at physiological or supraphysiological doses and the question arises, when is supplementation warranted in addition to diet, with what doses, which forms, and alone or in combination? Nutritional bone support should be tailored based on age and gender, but the available data are mostly from studies involving women in menopause.

A foundational recommendation for any age or gender is to derive as much vitamin K_1 from an abundant intake of leafy greens

or their juices (which are also alkalinizing) and healthy oils, such as olive oil. Human physiology is evolutionarily adapted to an intake of vitamin K_1 as high as 1 mg/day.[78] This level of intake happens to also support almost complete carboxylation of Oc, as discussed earlier. However, it is not practical to believe that most individuals will achieve this goal by adjusting their diet, because average intakes have been reported at 0.12 mg in the United States and 0.21 mg in Europe. Thus supplementation with K_1 and/or K_2 may be warranted for a large proportion of these populations. The question is, which forms and doses of vitamin K should be supplemented based on bone health goals and clinical status? A practical approach takes into consideration efficacy proven in clinical trials, pharmacokinetics, and the relative cost of the forms, given that on a per milligram basis the MK-7 ingredient is 10 times more expensive than MK-4, which in turn is 10 times more expensive than K_1. If cost is the primary concern, the actual clinical dosages prescribed should be used to determine actual relative costs.

Recommendation for Young Individuals

Optimizing vitamin K status with vitamin K_1 from diet plus vitamin K_1 supplements may be sufficient for men and women during development and reproductive age, with the goal of achieving peak bone mass and maintaining it until significant age-related decline in hormones occurs. In this case supplementing with K_1, at the level of 0.5 mg to 1 mg, depending on one's diet, may support complete carboxylation of Oc (possibly also that of MGP, discussed in the next section). At this level, a portion of vitamin K_1 is expected to convert to K_2 (MK-4) before, as well as after it is deposited in bone and other tissues. For individuals that take statins or bisphosphonates, it makes sense to supplement with additional K_2 (MK-4) above this amount, because these pharmaceuticals impair conversion of K_1 to K_2 (MK-4).

Recommendation for Older Individuals, Especially Women in Peri- and Established Menopause or Men With Lower Than Optimal Testosterone Levels

Additional vitamin K_2 may be needed to possibly compensate for poor vitamin K_1 to K_2 (MK-4) conversion and take advantage of intensive metabolic signaling that only vitamin K_2 can exert. The question is which form and doses of vitamin K_2 should be chosen to reverse bone loss or stall its decline, if peak bone mass was optimal. The best evidence currently available is for the high-dose K_2 (MK-4) at 45 mg/day (15 mg 3x/day) to reduce FR and increase BMD in menopausal women or men with low testosterone, especially in studies where vitamin D status was improved. This author believes current evidence does not support recommending supplementation with the MK-7 form of vitamin K_2 over the MK-4 for the following reasons.

Studies with nutritional doses of MK-7 are few, and although they have shown some benefits, the majority did not prove the ability to stall or reverse a decline in BMD or all markers of bone strength in menopausal subjects. None of the studies obtained a statistically significant effect on FR. The one study that reported a benefit for BMD, at a dose of 0.1 mg K_2 (MK-7), showed that the same result was obtained from an equivalent dose of K_1. One animal study showed that supplementation with K_2 (MK-4) was more efficient than with K_2 (MK-7) on raising bone content of vitamin K_2 (MK-4) and total vitamin K_2.[86] Unfortunately, there are no human studies comparing the effects on bone between K_2 (MK-7) and K_2 (MK-4).

In conclusion, for older individuals that need intensive bone support, it makes sense to supplement with vitamin K_1 at the level of 0.5 to 1.0 mg, or as high as 5 mg K_1, depending on one's diet, and an additional 45 mg K_2 (MK-4) (15 mg 3x/day). It is conceivable that less MK-4 may be just as effective in a setting where at least 1 mg K_1 is provided from diet plus supplements.

Dental Bone Support

Vitamin K status may be essential for oral health because it supports the activation of Oc and osteodentin, two functional proteins involved in tooth mineralization and dental bone metabolism. In addition, salivary glands have a high content of vitamin K, which suggests additional roles for it in oral health.

Cardiovascular Health

Vitamin K influences many aspects of cardiovascular physiology: blood clotting, calcification of tissues critical to cardiovascular function, arterial elasticity, inflammation, and oxidative stress.

Vitamin K is an important cofactor necessary for the physiological process of inhibiting inappropriate calcification of soft tissues that are not normally calcified, such as arterial/venous vessel walls, heart valves, skin (elastin/collagen fibers), cartilage (other than the normal growth-related mineralization), and kidney tissues.[117] Vitamin K mediates this defensive action by carboxylation of various VKDs, such as MGP and GAS-6, which enhances their ability to bind calcium and prevent deposition in tissues where it has pathological effects.

MGP, which contains five GLA residues, must be completely carboxylated and phosphorylated to maximize calcium binding. The levels of plasma dephosphorylated-uncarboxylated MGP (dp-ucMGP), a fraction of MGP, have been found to be a marker of arterial or other types of soft tissue calcification.[118,119]

The types of calcification that occur in the arterial wall can be grouped based on their location, but vitamin K status and vitamin K interventions may affect each type of calcification differently. Data in Table 126.8 summarize results from relevant interventions with various forms of vitamin K for cardiovascular pathologies.

Intimal Calcification

Intimal calcification occurs within the atherosclerotic plaque, adjacent to the intima layer of arteries, and is always associated with atherosclerotic disease. The question is whether optimizing vitamin K status or using high-dose vitamin K interventions may help prevent or reduce the rate of intimal plaque formation and calcification, in conjunction with established interventions that address classical cardiovascular risk factors.

One animal study, with rabbits, showed that supplementation with very high doses of vitamin K_2 (MK-4) (1, 10, or 100 mg/kg body weight) suppressed progression of arterial plaque, reduced intimal thickening, pulmonary atherosclerosis, total cholesterol, and lipid peroxidation while not promoting coagulative tendencies.[120] In vitro studies with vitamin K have revealed various mechanisms that may mitigate processes involved in atherosclerotic plaque formation and rupture: enhanced mitochondrial function, vitamin K_2 (MK-4) reduces inflammation, as evidenced by lowering PGE_2, and both vitamins K_1 and K_2 (MK-4) were shown to have antioxidant activity.[121]

Unfortunately, no human studies have been done with vitamin K to investigate the previously mentioned CVD-related outcomes.

Medial Calcification (or Monckeberg's Arteriosclerosis)

Medial calcification occurs within the middle layer of the arterial wall, surrounding elastin/collagen fibers, and within transformed vascular smooth muscle cells, as well as within the heart valves and vein walls. Unlike intimal calcification, this type of calcification is not necessarily associated with cardiovascular plaque pathology. It seems so common that one study found it present in 95% of men and women

TABLE 126.8 Results of Intervention With Three Vitamin K Forms on Markers of Cardiovascular Health

	Study Results With K_1	Study Results With K_2 (MK-4)	Study Results With K_2 (MK-7)
Effects on Arterial Stiffness or Other CVD Health Markers	(1 mg K_1 or placebo) + (500 mg Ca, 320 IU D3, 10 mg Zn, 150 mg Mg) and placebo alone (3 yrs, women age 50–60). Results: the placebo and 1 mg + minerals group had no changes in carotid elasticity. The placebo and "minerals only" group had 6.3% and 13% increases in pulse pressure and young modulus and 8.8% and 8.6% decreases in arterial compliance coefficient and distensibility coefficient[187]	(45 mg K_2 (MK-4) and no placebo) (1 yr, males/fem, avg. age = 69 yrs). Results: reduced arterial stiffness by 16% in subjects deficient in vitamin K. Statin use: 58%. Vit D and Ca, which contribute to medial calcification and stiffness, were not supplemented or assessed in this study[142]	(0.18 mg K_2 (MK-7) or placebo) (3 yrs, postmenopausal, avg. age 59 yrs). Results: reduced index of arterial stiffness (AT) by 6% in a subgroup with high baseline AT. Statin use: 11%. Vit D and Ca, which contribute to CAC and stiffness, were not supplemented or assessed[143]
		(1, 10, 100 mg K_2 (MK-4)/kg BW or control), (rabbits, 10 wks). Results: all interventions reduced cholesterol deposition in aorta, lipid peroxidation and total cholesterol[120]	(0.1 mg K_2 (MK-7) or placebo), (6 mo., males/fem, avg. age = 76 yrs). Results: No changes in endothelial function, vascular elasticity, or other CVD health markers. Statin use: 26%[144]
Effects on Coronary Artery Calcification (CAC)	(2 mg K_1 or placebo) (1 yrs, males, females, avg. age 69.3 yrs). Results: reduced progression of CAC by 45%. Statin use: 71%[139]	45 mg K_2 (MK-4) and no placebo) (1 yr, males/fem, avg. age 69 yrs). Results: treatment did not reduce CAC. Vit D and Ca, which contribute to medial calcification and stiffness, were not supplemented or assessed in this study[142]	Effects of K_2 (MK-7) on CAC in humans or animals with chronic kidney disease (CKD) are not included in this table, because the physiology of CKD is altered significantly from normal physiology
	(0.5 mg K_1 or placebo) + 600 mg Ca + 400 IU vit D multivitamin (3 yrs, men/women, avg. age = 56–62). Results: 6% less progression of CAC than in the control group. Statin use: 25%[139]		
	0.1 mg or 2 mg K_1/day (in rats, 6 wks). Results: the dose of 2 mg K_1/day, equivalent to 8 mg/kg body weight in rats,[a] reduced CAC by 50% and improved arterial distensibility. CAC resulted from treatment with anticoagulant warfarin[141]	0.1 mg or 2 mg K_2 (MK-4)/day (in rats, 6 wks). Results: the dose of 2 mg K_2 (MK-4)/day, equivalent to 8 mg/kg body weight in rats,[a] reduced CAC by 50% and improved arterial distensibility. CAC resulted from treatment with anticoagulant warfarin[141]	(0.36 mg K_2(MK-7) or placebo) (6 mo., 69 yrs, male/fem with type 2 diabetes, CVD). Results: compared to placebo, treatment with K_2(MK-7) did not slow down the progression of arterial calcification, which measured 10% higher after 6 mo. compared to baseline. dp-ucMGP decreased by –33% (from 613 to 408 pmol/L).[190]
Effects on dp-ucMGP Changes (Human Studies)	0.5 mg K_1 decreased dp-ucMGP by –80%, from 485 to 97 pmol/L. dp-ucMGP levels were not associated with CAC[188]	No studies available for effects of K_2 (MK-4) on dp-ucMGP	0.1 mg K_2 (MK-7) decreased dp-ucMGP by –16%, 789 down to 668 pmol/L[144]
			0.18 mg K_2 (MK-7) decreased dp-ucMGP by –31%, 401 down to 294 pmol/L[189] –50%, to 256 pmol/L[143]
	2 mg K_1 decreased dp-ucMGP by –45%, from 432 to 243 pmol/L[140]		0.36 mg K_2 (MK-7) decreased dp-ucMGP by –46%, 391 down to 209 pmol/L[189] –56%, 389 down to 171 pmol/L[145]

dp-ucMGP; Desphospho-uncarboxylated matrix Gla-protein.
[a]The human equivalent dose of the rat dose of 8 mg/kg body weight can be calculated as follows: 8 mg/kg body weight of the rat × 0.162 = 1.296 mg/kg body weight of the human. This translates into 64.8 mg for a 50 kg human.

at autopsy.[122] It seems to progress slowly with aging and may be an important component of the age-associated increase in coronary artery calcification (CAC).

Suboptimal vitamin K status cannot support maximal carboxylation of MGP, leaving it incompletely functional, and results in the following pathological phenomena:

1. Calcium deposition around the elastin/collagen fibers inside the arterial wall, which causes them to lose elasticity. This, in turn, will lead to reduced arterial compliance and increased risk of essential hypertension. Incidentally, the same phenomenon occurs in the skin elastin/collagen fibers, which may be one reason why skin elasticity decreases with aging.
2. A fraction of vascular smooth muscle cells belonging to the medial arterial wall change phenotypically to bone marrow type cells, which express osteocalcin. This in turn promotes calcium retention in the arteries.[123–125]
3. Calcium deposition in the walls and valves of veins and heart valves leads to dysfunctional changes in these cardiovascular tissues and impaired return circulation to and from the heart.

In vitro studies have shown that vitamin K_2 was shown to stimulate collagen and elastin synthesis.[58,59] This implies that vitamin K deficiency may reduce collagen and elastin turnover and renewal, which may contribute to loss of elasticity in many tissues that rely on it for their function.

Evidence From Epidemiological Studies for Relationships Between Vitamin K_1 and/or K_2 Intake and Cardiovascular Disease

Some epidemiological studies found correlations between intake of vitamin K_1 or K_2 and cardiovascular disease (CVD)-related outcomes. The study performed in the United States (Nurses' Health Study) found that the highest (300 mcg) versus lowest (87 mcg) quintile of vitamin K_1 intake had a multivariate relative risk (RR) of CVD events of 0.84, after data were adjusted for a number of dietary factors relevant to CVD.[82] The PREDIMED (Prevención con Dieta Mediterránea) study found that baseline vitamin K_1 intake was inversely associated with all-cause mortality (hazard ratio [HR] = 0.64), while individuals who increased their intake of dietary vitamin K_1, over the study period, had a RR = 0.52 of cardiovascular mortality, while no association was observed between changes in vitamin K_2 intake and CVD mortality.[126]

One study with postmenopausal women found a significant difference for total vitamin K intake between subjects with CAC (243.6 mcg) versus those without CAC (189.9 mcg).[127]

In contrast, an epidemiological study performed in Dutch subjects (the Rotterdam study) found no correlation between CVD mortality or CAC with vitamin K_1 intake. Instead, this study reported a RR of CVD mortality of 0.73 for the upper tertile of dietary vitamin K_2 intake (average of 40.9 mcg) compared with the lower tertile (average 15.1 mcg).[73] Dietary vitamin K_2 composition was represented in majority by MK-9 (14.7 mcg) and MK-8 (6 mcg), both derived mostly from cheese, and MK-4 (7.1 mcg), derived mostly from meats and eggs. MK-7 content was only 0.4 mcg. However, it is unlikely that an intake of 40.9 mcg K_2 explains the risk reduction of CVD mortality and arterial calcification, because much higher levels of vitamin K (360–500 mcg) are necessary to achieve significant carboxylation of MGP (see data in Table 126.8). No study to date has shown that supplementation with small doses of vitamin K_2 can produce such benefits. For example, a study that supplemented subjects with 100 mcg K_2 (MK-7) for 6 months did not report any improvements in CVD-related outcomes. In addition, the effects of vitamin K_2 intake cannot be interpreted in isolation from concurrent vitamin K_1 intake because their effects are additive for carboxylation of MGP and other VKDs. In the Rotterdam study, the upper tertile of vitamin K_1 intake was 311 mcg. It would have been useful if the authors had investigated the correlations involving total vitamin K intake (K_1+K_2).

The conflicting reports on correlations with vitamin K_1 and/or K_2 may be caused by associated patterns of food intake that have CVD benefits.[70,128] For example, vitamin K_1 is associated with intake of leafy greens and vegetable oils, and long-chain menaquinones are primarily provided by fermented foods (dairy or vegetarian origin), which may also be a source of probiotics. Thus the relationship between vitamin K and CVD pathologies is not likely to be revealed in correlation studies because average intakes of K_1 are in the range of 100 to 200 mcg and for K_2 around 29 mcg in Western countries, which are too low to support adequate carboxylation of MGP (as depicted in Table 126.8) or have other therapeutic effects specific to vitamin K_2. Intervention studies have used much higher doses of vitamin K_1 or K_2 to test CVD outcomes, as discussed later.

Vitamin K Interactions With Vitamins/Hormones Affecting Matrix Gla Protein Expression and Carboxylation

A 68% reduction in MGP expression was associated with a 33% increase in arterial calcium content.[129] Vitamin A and thyroid supplements are both modulators of MGP expression. Excess vitamin A has been shown to increase the risk of arterial calcification, which may be related to the fact that it downregulates MGP expression. Similarly, the thyroid hormone T3 is essential in supporting adequate expression of MGP, and it has been shown that MGP expression is reduced in hypothyroidism. Estrogen deficiency reduces carboxylation efficiency of VKD proteins. Vitamin B_6 and manganese are cofactors for the carboxylation reaction, whereas excess vitamin E (specifically alpha-tocopherol) has been shown to interfere with vitamin K recycling (similar to warfarin), thus reducing its ability to carboxylate VKDs.[130] Other interactions between vitamin E and K are discussed under "Vitamin K Interactions."

In the setting of vitamin K deficiency, adequate intake of vitamin D and calcium promotes calcification of soft tissues and, as a result, an increase in arterial stiffness. Fortunately, one human study showed that the addition of 1 mg K_1 to the supplementation of calcium and vitamin D (plus zinc and magnesium) completely prevented an arterial stiffness increase, as observed in the control group that was supplemented only with calcium and vitamin D (plus zinc and magnesium), see detailed data in Table 126.8.[131] [JP. Yet another example of the problem with researching nutrients using an isolation study like a drug, rather than as part of a complex matrix requiring multiple nutritional factors to function properly.]

What Is the Potential of Preventing or Reversing Arterial Calcification With Vitamin K Supplementation?

Table 126.8 presents results from intervention studies aimed at either evaluating effects on CAC or arterial stiffness. The CAC scores represent the sum of intimal and medial calcium content.

An animal study showed that upon vitamin K_1 supplementation, the conversion to K_2 (MK-4) resulted in an arterial tissue deposition pattern of 75% K_2 (MK-4) with the rest of 25% as vitamin K_1, suggesting extensive systemic and local conversion of K_1 to K_2. Thus K_2 (MK-4) seems to be the preferred form in this tissue.

Vitamin K_2 (MK-4) is unique among the vitamin K_2 family of compounds because the ligand it contains, geranylgeranyl (GG), is similar to one intermediate of the cholesterol synthesis pathway (downstream of mevalonate and HMG-CoA). There, GG is believed to have inhibitory actions on various metabolites, including cholesterol, inflammatory compounds and, as mentioned earlier, on osteoclast proliferation.[49,55,56]

The conversion of vitamin K_1 to K_2 (MK-4) seems crucial for vitamin K to exert its role in preventing arterial calcification. This was demonstrated by one study that gave warfarin plus either K_1 (WK_1) or K_2 (WK_2) to rats, knowing that warfarin blocks K_1 to K_2 (MK-4) conversion.[55] The doses of vitamin K_1 and K_2 were 1.5 mg/g diet, devised to be high enough to compensate for the arterial calcification effect of warfarin at 3 mg/g diet. Vitamin K_2 prevented calcification in the WK_2 group, whereas an equal dose of K_1 did not. This study was not designed to explore the possible benefits of giving vitamin K along with warfarin because the dose used canceled any anticoagulant effects from warfarin, but it proved the point that vitamin K_1 must be converted to vitamin K_2 to prevent arterial calcification.[132] This is why it is important to keep in mind that statins impair the conversion of vitamin K_1 to K_2 (MK-4) by reducing the availability of GG ligand necessary to form K_2 (MK-4) from the vitamin K_3 intermediate (generated from K_1). The conversion of other menaquinones, such as K_2 (MK-7) to K_2 (MK-4), may be impaired as well, because they too convert to the vitamin K_3 intermediate.[133] In addition, some studies claim that a fraction of K_2 (MK-4) converts to K_3 during gut absorption and then is converted back to K_2 (MK-4) inside various tissues.[24,134–138]

For this reason, the percentage of subjects using statins has been noted in Table 126.8, to emphasize the potential influence of impaired conversions of various vitamin K forms to K_2 (MK-4) on the reported results.

Statins have shown some potential in retarding intimal and/or medial calcification, possibly by reducing inflammation and low-density lipoprotein load, but no studies have shown potential to reverse established calcifications.

Two human studies showed that supplementation with 0.5 mg or 2 mg of vitamin K_1 (for 3 or 2 years, respectively) could slow the CAC progression by 6%[139] or 45%,[140] respectively. The corresponding reductions of dp-ucMGP were −80% and −45%, respectively. It is not clear why the authors chose the 0.5 mg dose of K_1 because doses higher or equal to 1 mg were demonstrated to support almost complete carboxylation of Oc, which were shown to correlate with the degree of MGP carboxylation.[79]

The potential to reverse already established CAC of the medial layer has been demonstrated in a rat study from 2007 and such reversal has yet to be reproduced in humans.[141] In the first part of the study, rats were in a protocol designed to cause them to develop arterial calcification by treatment with warfarin, which causes a state of severe vitamin K deficiency. In the second part of the study, the hypothesis tested was whether a low-dose or high-dose vitamin K_1 or K_2 (MK-4) (0.1 mg or 2 mg/day) could reverse this calcification.

Warfarin was discontinued when the desired calcification level had been achieved. Vitamin K_1 or K_2 (MK-4) supplementation was given right after warfarin treatment was stopped. Both treatments of 6 weeks with 2 mg/day of vitamin K_1 and 2 mg/day or vitamin K_2 (MK-4) were able to reduce the baseline calcification by 37%. The lower dose of 0.1 mg/day of either K_1 or K_2 did not achieve the same; in fact, arterial calcification progressed in this group in spite of the fact that warfarin had been discontinued. The calcification reversal observed in this study is impressive, and the dose of 2 mg/day may be translated into an equivalent human dose as follows: 8 mg/kg body weight(rat)*0.16 = 1.28 mg/kg body weight (human) or 64 mg for a 50 kg person. This dose obviously falls in a supraphysiological range. Unfortunately, the animal study previously discussed did not test any intermediate doses between 2 mg and 0.1 mg/day for the same outcome.

One human study has investigated effects of supraphysiological doses of vitamin K_2 (MK-4) at 45 mg on CAC and arterial stiffness for 1 year.[142] No effect was observed on CAC progression, but arterial stiffness declined by 16% in a subgroup that was vitamin K deficient at baseline, as assessed by PIVKA-2.[142] Unfortunately, this study did not report the change in levels of dp-ucMGP, and no other studies did so either.

Supplementation with vitamin K_2 (MK-7) was investigated for effects on arterial stiffness[143,144] and other markers of CVD health.[144] Supplementation with 0.1 mg K_2 (MK-7), given for 6 months, produced no significant CVD-related improvements,[144] and the reduction obtained in levels of dp-ucMGP was only −16%, which may explain the lack of benefits observed. Supplementation with 0.18 mg vitamin K_2 (MK-7) for 3 years resulted in a 6% reduction of arterial stiffness in a subgroup that had elevated arterial stiffness at baseline, and obtained a 50% reduction in levels of dp-ucMGP.[143] Results from other studies are listed in Table 126.8, where reductions of 46%[143] and 56%[145] were obtained for the highest dose tested of 0.36 mg vitamin K_2 (MK-7).

In conclusion, the best reduction in dp-ucMGP levels was obtained at −80% by the intervention with 0.5 mg vitamin K_1, to a level of 97 pmol/L.[145] It is important to note that although the studies done with 45 mg vitamin K_2 (MK-4) and 0.18 mg vitamin K_2 (MK-7) showed a reduction in arterial stiffness, they did not supplement with calcium and vitamin D, which have been proven necessary for bone health. Thus these results cannot be compared with those obtained with 1 mg vitamin K_1, which showed a prevention of arterial stiffness increase in those supplemented with calcium and vitamin D.

Supplementing humans with 0.36 mg/day of K_2 (MK-7) did not slow down CAC progression (see Table 126.8).[190]

The benefits of all three forms of vitamin K on arterial elasticity may be caused in part by the reduction of arterial calcium deposition, and also by the demonstrated ability of vitamin K_2 to stimulate collagen and elastin synthesis.[58,59] This is especially needed in menopause when collagen and elastin synthesis is reduced as a result of estrogen deficiency.[95]

What May Be the Most Efficacious Intervention With Vitamin K Supplements to Prevent or Benefit Cardiovascular Disease?

A preventive approach to CVD is to maintain *an optimal vitamin K status* throughout life, which would likely prevent/retard age-related increases in arterial medial calcification and stiffness. In fact, the term *age-related calcification* may be replaced with *vitamin K deficiency-related calcification* and treated as a reversible condition.

The question is what would be the most effective doses and forms of vitamin K for therapeutic interventions for those with established CVD and CAC? Current commercially available forms of vitamin K are: K_1, K_2 (MK-4), K_2 (MK-7) and/or a mix of long-chain menaquinones (MK-6,-7,-9), but K_2 (MK-4) and long menaquinones are 10 times and 100 times more expensive than K_1 per microgram, whereas there are no side by side comparisons on relative efficacy for CVD.

As discussed in the previous section, human interventions with vitamin K_1 seem to provide good evidence for reducing CAC progression and maintaining arterial elasticity. In spite of promising results from animal studies that showed that supraphysiological doses of K_1 and K_2 (MK-4) can reverse vitamin K deficiency-induced CAC, one human study with K_2 (MK-4) reported no changes in CAC progression, whereas the rest did not assess it. Interventions with K_2 (MK-4) or K_2 (MK-7) reported reduced arterial stiffness by 16% and 6%, respectively, but it is not clear if they would completely prevent an increase in arterial stiffness when supplemented along with adequate amounts of calcium and vitamin D, as proven by vitamin K_1 supplementation.

Because vitamin K_1 interventions did not completely stall or reverse CAC, and the role of K_2 (MK-4) is established to be critical in this process, it is possible that a combination of vitamin K_1 and K_2 may provide a synergistic and more efficacious intervention than vitamin K_1 alone, especially for older individuals. For patients who take statins, supplementation with large doses of K_2 (MK-4) is warranted, because these medications impair conversion of K_1 to K_2 (MK-4) by reducing synthesis of geranylgeraniol.

As discussed in the osteoporosis section, average intake of vitamin K_1 is low in Western and Asian populations and it is not practical to believe that most individuals would change their diet enough to raise it to a physiologically replete level (close to 1 mg/day). Thus in most cases, it makes sense to suggest supplementing with 1 mg vitamin K_1, as a foundation for optimizing vitamin K status. Human physiology has likely adapted through evolution to an intake close to 1 mg K_1,[78] and relatively small amounts of additional MK-4 and long-chain menaquinones, probably around 0.05 mg (based on average intake in Holland). Supplementing with at least 1 mg K_1 is important for maintaining arterial elasticity, especially females, who need to ingest adequate amounts of calcium (from diet + supplements) and vitamin D.

The question is which form(s) of vitamin K_2 should be added to a foundational amount of K_1, and in what therapeutic doses, to improve the odds of stalling or reversing CAC and arterial stiffness?

There are no side-by-side comparison studies available for treatments with K_2 (MK-4) and K_2 (MK-7) that evaluated CVD outcomes or the types and amounts of vitamin K stored in arterial tissue. The

effects of treatments with 45 mg K_2 (MK-4) on reducing arterial stiffness were stronger than those with 0.18 mg K_2 (MK-7). Thus based on current data, it makes sense to choose supplementation with K_2 (MK-4) over that with K_2 (MK-7), especially because K_2 (MK-7) likely derives all its benefits from its conversion to K_2 (MK-4).

Future studies should investigate the effects of combinations of a foundational amount of K_1 (e.g., 1 mg) plus various amounts and forms of vitamin K_2 on CAC- and CVD-related benefits.

Are There Benefits From Adding Vitamin K_1 or K_2 to Anticoagulant Treatments Such as Warfarin?

Anticoagulant therapy induces an extreme state of vitamin K deficiency, and studies have shown that it may increase arterial calcification by twofold to eightfold as well as increasing aortic pulse pressure and heart valve calcification.[141]

Patients on anticoagulant treatment are told to minimize intake of vitamin K-containing foods, such as green leafy vegetables. Most patients end up not eating enough vegetables in fear of counteracting their anticoagulant therapy. This is unfortunate, because they would be obviously missing important benefits. Some studies have proposed and demonstrated that supplementing with vitamin K_1 doses in the range of 0.1 to 5 mg, during anticoagulant therapy, may help minimize the need for adjusting the dose of the anticoagulant in response to dietary variations of vitamin K.[146,147] One shortcoming of using vitamin K_1 in this scenario is that warfarin blocks the conversion of vitamin K_1 to K_2, which eliminates many of its benefits, especially in the extrahepatic tissues.

This strategy could potentially be implemented with vitamin K_2 (MK-4) in addition to vitamin K_1, although no studies have explored this yet. The advantages of using additional vitamin K_2 would be as follows: (1) it would bypass the warfarin block on vitamin K_1 to K_2 (MK-4) conversion and (2) a fraction of the ingested vitamin K_2 (MK-4) is absorbed intact and can reach arteries (and other peripheral tissue such as bone), where it may partially compensate for the undesirable effects of the anticoagulant, such as tissue calcifications (in arteries, veins, and heart valves).

Will Therapeutic Doses of Vitamin K Increase Clotting and Thrombosis Excessively?

As discussed in detail, in the last section titled "Safety", vitamin K doses higher than those needed to fully carboxylate VKDs involved in clotting have not been shown to further increase clotting and thrombosis tendencies beyond normal physiological function.

Effects on Cancer of Vitamins K_1, K_2, and K_3

Epidemiological, interventional, and in vitro studies provide evidence that vitamin K plays a role in reducing the incidence and progression of cancer. The three different forms of vitamin K each seem to manifest distinct activities in vitro against cancer cells. Vitamin K_3 has been found to have the highest anticancer activity through multiple mechanisms, followed by vitamin K_2, whereas vitamin K_1 had the weakest effect. Vitamin K_3 was shown in vitro and in vivo to induce oxidative stress, generate hydrogen peroxide, and deplete glutathione, unlike vitamins K_1 and K_2. In fact, some studies showed an antioxidant activity for the latter.[148]

Most likely the human body derives a significant amount of vitamin K_3 from cleaving the side chains of vitamin K_1 or K_2, especially when these agents are ingested in supraphysiological doses. One study found that urinary excretion of vitamin K_3 was significant 2 to 3 hours after vitamin K_1 ingestion, but plasma vitamin K_3 was not measured.[20] It was also estimated that the percentages of vitamin K_1 or K_2 (MK-4) or K_2 (MK-7) to K_3 conversion are around 5% to 25%. This is believed to occur in the intestinal cells and other extrahepatic tissues, possibly also in the liver. Any effect of oral vitamin K_1 on cancer could be attributed to its direct actions and its partial conversion to K_3 and K_2. Similarly, any effect on cancer from oral vitamin K_2 could be attributed to its direct actions and its partial conversion to K_3.

Vitamin K_3 is cytotoxic to cancer cells through oxidative effects and various other mechanisms of action. In vitro studies with vitamin K_3 have shown an inhibitory effect against breast, endometrial, urological, and prostate cancer as well as leukemia and squamous cell carcinoma.[2] Unfortunately, K_3 is also toxic to normal cells, especially red blood cells, which is why it is no longer used as a provitamin in humans. This profile would most likely qualify vitamin K_3 as a chemotherapeutic agent, but because it is not a patentable molecule, there are not enough studies to achieve U.S. Food and Drug Administration (FDA) evaluation. Currently, vitamin K_3 is only allowed to be used for research purposes in humans; in animals, it is used as a vitamin K precursor to vitamin K_2.

Many studies have observed a synergistic benefit from combining vitamin K_3 with vitamin C (in the intravenous and oral forms) for reducing the rise of prostate-specific antigen in prostate cancer patients[149] and also with all-transretinoic acid (ATRA) for the treatment of leukemia.[2] Vitamin K_3 has also been shown to inhibit DNA polymerase and angiogenesis[150] and to act as a radiation sensitizer.

Oral and intravenous vitamin K_3 has been used in studies for the treatment of various cancers (leukemia, lung, prostate, and bladder) with and without chemotherapy; such treatment has resulted in improved survival and reduced cancer growth but not complete cures.[2]

One epidemiological study of a Dutch population found a reduced risk of overall cancer incidence and mortality by 14% and 28%, respectively, for the upper quintiles of vitamin K_2 intake, whereas no correlation was found with the intake of vitamin K_1.[151] The risk reduction was most pronounced for lung and prostate cancer. These results are strikingly similar to the correlations found for vitamin K_2 intake and reduced risk of cardiovascular calcification in the Dutch epidemiological studies. Considering that the average intake of vitamin K_2 in the population studied is in the range of 26 mcg (first quintile) to 42 mcg (fourth quintile), the results are surprising and warrant future interventional studies with doses of vitamin K_2 (MK-4) in that range and above.

Vitamin K_1 and Cancer

Very few studies have investigated the effects of oral supplementation with vitamin K_1 on cancer but those that have been done include the following:

1. Oral supplementation with 5 mg of vitamin K_1 for 4 years, along with 800 IU of vitamin D_3 and 1500 mg of calcium, resulting in an approximate 75% reduction in all types of cancer in postmenopausal women. This was a randomized controlled trial designed to observe the effect of vitamin K_1 plus vitamin D and calcium on bone density; the cancer findings were incidental.[104]
2. A number of Phase I/Phase II trials investigated the effect of oral supplementation with 40 mg (20 mg twice daily) of vitamin K_1 in patients with hepatocellular carcinoma. All studies found improved survival rates, temporary disease stabilization, and reduced tumor growth and invasion.[2]
3. A recent study used both an oral dose of 40 mg/day of vitamin K_1 in one group and incremental intravenous doses of vitamin K_1 (from 40 mg to 1000 mg) in small groups of patients with unresectable hepatocellular carcinoma and distant metastases. All doses, oral and intravenous, were tolerated without toxicity. A small percentage of patients, 3 out of the group of 23 who received oral vitamin K_1 and 3 out of the group of 27 who received intravenous vitamin

K_1, had tumor shrinkage, and 16 of each group had tumor stabilization (according to computed tomography). Also, 3 and 6 patients of the oral and intravenous groups, respectively, had a reduction in the tumor marker alfa fetoprotein (AFP).[152] In vitro testing of tumor cells extracted from the subjects in this study has shown that vitamin K_1 induced an inhibition of cell growth through "phosphorylation of JNK and c-Jun and caspase-mediated apoptosis." Even though the authors were aware of the evidence for the efficacy of vitamin K_2 (MK-4) in this type of cancer, they stated that they chose vitamin K_1 instead because "it is the only form of vitamin K available clinically in the US." Also, the study authors stated that the dose of 40 mg was chosen based on "how many tablets of the drug Mephyton (5 mg K_1) were the patients willing to swallow comfortably."

Vitamin K_2 and Cancer

In vitro studies have identified an anticancer activity for vitamin K_2 against liver, colon, leukemia, lung, stomach, lymphocyte, osteosarcoma, epidermoid, glioma, hepatoma, and breast cancer cell lines. Some of the anticancer mechanisms that have been identified for vitamin K_2 are induction of apoptosis, cell cycle arrest (at G1/S transition), cell differentiation, reduction of cell proliferation and angiogenesis (resulting from inhibition of COX2), inhibition of signaling for tumor growth, and activation of the PXR genes. Many of these actions are not shared by vitamin K_1, which may be because of vitamin K_2's specific side chain, the molecule geranylgeranyl.

Interventions with oral doses of 20 to 135 mg (but most frequently with 15 mg three times daily) of vitamin K_2 (MK-4) have led to improved survival rates for hepatocellular carcinoma, with a slower progression and reduced recurrence rates (especially when patients were infected with the hepatitis C virus).[153] The combination of vitamin K_2 (MK-4) treatment with an angiotensin-converting enzyme inhibitor decreased the recurrence of hepatocellular carcinoma.

Because none of the studies employing high doses of vitamin K_1 or K_2 investigated any lower doses of vitamin K, such as 1 mg or any dose in between 1 and 40 mg of vitamin K_1 or 1 and 45 mg of vitamin K_2 (MK-4), the minimum dose that would achieve the same effect is not clear, nor, most importantly, is it known at what point no further benefit can be achieved by increasing the dose.

Some promising responses have been obtained from using high doses of vitamin K_2 (MK-4) (45 mg) for patients with leukemia or myelodysplastic syndrome, with an improvement of anemia and thrombocytopenia and sometimes complete remission.[154] The effect was even more pronounced for the group who were also given vitamin D_3.

Vitamin K status, measured by the percent uncarboxylated osteocalcin in serum, was found to correlate with advanced-stage and high-grade prostate cancer.[155] As discussed later, this biomarker of vitamin K status can be considered a marker not only for bone health but also for risk prediction for other conditions where vitamin K plays an important role.

In using vitamin K supplementation for general health and for cancer therapy, it is important to keep in mind that, in advanced stages of certain cancers, thrombosis is abnormally activated. This is mediated through unclear mechanisms that may be unique to each cancer's strategy to promote its survival and metastasis. Upregulation of clotting factors and other VKDs, as mentioned, may have an important role in this pathological process. McCann et al. point out that in some specific cancer cases, warfarin treatment has been shown to improve survival rates.[30]

In utilizing vitamin K for cancer risk reduction or treatment, the patient should be evaluated first for coagulation/thrombosis status to make sure that he or she is not in a state where the cancer process is upregulating that status.

In addition, for therapeutic effects, the vitamin K_2 (MK-4) form may be a better choice than vitamin K_1 in implementing a therapy with supraphysiological doses. The dose in the vicinity of 45 mg has a good safety record and the most evidence for efficacy. High doses of vitamin K_2 (MK-4) were found not to increase clotting or platelet aggregation while actually reducing thrombotic tendencies. On the contrary, vitamin K_1 was shown to increase thrombotic tendencies at very high doses. This and the fact that the overall efficacy of vitamin K_1 against cancer is weaker compared with K_2 (MK-4) make vitamin K_2 (MK-4) the overall better candidate for therapeutic doses in cancer treatment and prevention in high-risk patients.

Effects on Various Other Conditions: Insulin, Kidney Stones, Skin Elasticity, Cystic Fibrosis, Brain Protection, Hemorrhage, Joint Health, and Inflammation After Laser Therapy

The optimization of vitamin K status with diet and/or supplements, as well as therapeutic doses of these two vitamers, may have many additional benefits to those previously discussed. These additional effects can be inferred from the presence of VKDs in many tissues, even though all their roles have not been precisely identified. In addition, observing vitamin K deficiency states or inducing them in laboratory experiments for various tissues has provided many clues on vitamin K's potential to alleviate various pathological processes.

Vitamin K has a complex role in the clotting processes supporting both the coagulation and anticoagulation/balancing pathways. Its deficiency may lead to increased risk of hemorrhage in various conditions: excessive menstrual, gum, or nose bleeding and increased risk of hemorrhage in infants born and breastfed by vitamin K-deficient mothers.[156,157]

Vitamin K deficiency may have many other consequences:

1. Improper cartilage maturation as a result of excessive growth plate mineralization[158]
2. Accelerated age-related degeneration of the intervertebral discs (increased calcified/uncalcified cartilage ratio)[159]
3. Reduced glucosamine and glycosaminoglycan synthesis, which affects the health of many tissues in the body: joint cartilage, tendons, ligaments, the dermal layer of the skin, the lining of the gastrointestinal tract, as well as the walls of the and blood vessel and veins[36]
4. Suboptimal skin health, elasticity, and overall appearance caused by the following consequences of vitamin K deficiency:
   - Increased skin collagen breakdown
   - Risk of calcification of the elastin fibers, which are a major determinant of skin elasticity
   - Thinning of the skin ground substance layer. MGPs are found in the dermis, and control collagen expression as well as glycosaminoglycan biosynthesis (collagen and glycosaminoglycans are both components of the supporting structure of the dermis).
5. Impaired insulin secretion and increased insulin resistance[160]
6. Increased risk of kidney stone formation[161]
7. In animal studies (rats), vitamin K deficiency was shown to cause suboptimal energy production, a 10% reduction in muscle creatine kinase, and a 20% reduction in the alkaline phosphatase of the intestinal mucosa, which may be caused by structural mitochondrial alterations[162]
8. Inadequate antioxidant protection of the brain
9. Increased severity of cystic fibrosis. Supplementation with 1 mg of vitamin K_1 was shown to be useful in compensating for the carboxylation defects that are characteristic of cystic fibrosis[163]
10. Reduced testosterone production[164]

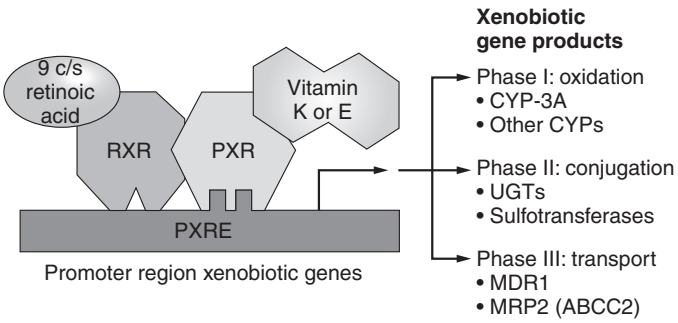


Fig. 126.6 Xenobiotic receptor activation by vitamin E or vitamin K. (From Traber MG, Frei B, Beckman JS. Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr Opin Lipidol.* 2008;19[1]:30–38. Review.)

Other observed benefits: Topical application of vitamin K_1 was shown to reduce laser treatment-induced redness (inflammation).[165] As described, it would seem that a topical cream containing vitamin K might influence skin physiology positively.

INTERACTIONS WITH OTHER VITAMINS

Interactions With Vitamins E, A, and D

These interactions and synergistic effects of vitamin K with vitamin D and vitamin A were discussed previously regarding their influence on the expression of VKDs: osteocalcin and MGP.

Vitamin E May Interfere With Vitamin K Metabolism When Administered in Very High Doses

Supplementation with high doses of vitamin E, such as 800 to 1200 IU of alpha-tocopherol, has been shown to impair blood clotting by interfering with the vitamin K-dependent carboxylation of prothrombin.[130] This is thought to be mediated by a metabolite of oxidized vitamin E called tocopheryl quinone. Another study in rats suggested that high doses of vitamin E may impair the conversion of vitamin K_1 to K_2.[166] This is thought to be a result of the competition that can occur between vitamin K_1 and excess vitamin E for a specific cytochrome P450 enzyme responsible for the first step in the conversion of vitamin K_1 to K_2.

Reviews also point out that excessive doses of vitamin E may upregulate the expression of metabolizing enzymes responsible for both vitamin E and vitamin K clearance.[166] The reverse may be also true when supraphysiological doses of vitamin K are administered, especially when the individual's vitamin E status is marginal. Trauber[167] estimates that 90% of the population does not even meet the recommended daily intake (RDI) of 15 IU/day. High doses of either vitamin E or vitamin K can activate the xenobiotic pathways (via the PXR nuclear receptor) twofold to tenfold. Fig. 126.6 shows the interaction between vitamins A, E, and K in activating the PXR nuclear receptor. Landes et al. comment on this phenomenon as follows: "the organism handles such supranutritive dosages of vitamins like potential harmful xenobiotics."[168] One should keep in mind that the PXR-activated metabolic pathways are also involved in the clearance of many pharmaceutical drugs.

Because vitamin K seems to play an important role in reducing cardiovascular calcification, the interference by excess vitamin E (of various forms) on vitamin K metabolism may be one of the reasons why a few meta-analytic studies have found increased risk of cardiovascular events in populations that supplemented with alpha-tocopherol in excess of 400 IU. The effects of excess vitamin E may be most relevant when vitamin K status is marginal, which is very common. To put things in evolutionary perspective, an analysis by Cordain of the nutritional content of a typical Paleolithic diet found that the average intake of alpha-tocopherol was around 19 IU for a 2200-Kcal diet.[78] However, the average total content of the other forms of vitamin E in that diet (such as beta-, gamma-, or delta-tocopherol) has not been estimated, but it is known that they have many metabolic roles that are distinct from those of alpha-tocopherol. At an intake of alpha-tocopherol above 100 IU, certain metabolic pathways may be pushed beyond the range of "normal physiology."

Interactions With Pharmaceutical Drugs

Anticoagulants Based on Vitamin K Antagonism

Anticoagulants, such as warfarin or coumarin, interfere with vitamin K activation and recycling. Specifically, they inhibit the enzymes epoxide reductase and quinone reductase. It has been estimated that the body reuses the same vitamin K molecule in the range of 100 times before it metabolizes it for excretion through bile or urine. When an anticoagulant is present, vitamin K recycling is greatly reduced. Anticoagulants also interfere with vitamin K activity in extrahepatic tissues, and studies have shown them to increase the risk of osteoporosis and fracture and to delay fracture healing; they may also contribute to arterial or heart valve calcification and hypertension.[169,170] If the total amount of vitamin K from food and supplements is increased by 100 mcg/day, it will most likely require an adjustment in anticoagulant dose. Consequently, vitamin K intake from food and supplements must be closely monitored with this therapy. See the previous discussion about the potential benefits of supplementing with a small dose of vitamin K_2 along with the anticoagulant therapy.

Anticoagulants With Mechanisms of Action Independent of Vitamin K Metabolism

Vitamin K does not interfere with the action of blood thinners such as heparin, antiplatelet agents (clopidrogel, abciximab, tirofiban, and eptifibatide), direct thrombin inhibitors (hirudin, argatroban), or thrombolytic agents (clot-dissolving proteolytic enzymes).[171]

Aspirin and Salycilates Interfere Slightly With Vitamin K Metabolism

Aspirin (acetylsalicylic acid) and salicylates exert most of their blood-thinning effect by reducing platelet aggregation via the inhibition of COX enzymes and potentially other mechanisms. In addition, these compounds have a mild anticoagulant action by inhibiting vitamin K recycling (similar to that of warfarin but a lot milder).[172] Aspirin has been shown to increase bone loss and impair fracture healing, and although the mechanisms have not been elucidated, this could certainly be caused in part by its mild anti–vitamin K effect.

Antibiotics Interfere With Vitamin K Metabolism

Antibiotics may increase the need for vitamin K supplementation because (1) they may kill gut bacteria that normally produce vitamin K_2 in the intestinal tract, (2) they may interfere with vitamin K activation and recycling (similar but milder than anticoagulants like warfarin), and (3) they may inhibit the activation of various proteins (carboxylation) by vitamin K.[173] Examples include broad-spectrum cephalosporins such as cefamandol, moxalactam, and cefoperazone.

Anticonvulsant Drugs

Anticonvulsant drugs induce vitamin K deficiency. Supplementation of rats on phenytoin with very high doses of K_2 (MK-4) (30 mg/kg body weight) prevented BMD reduction.[174]

Butylated Hydroxytoluene Interferes With Vitamin K Metabolism

Butylated hydroxytoluene (BHT), a preservative still used in some packaged foods and topical creams, inhibits vitamin K epoxide

reductase by as much as 20%, which is similar to warfarin's mechanism of action. This may be relevant to the metabolism of vitamin K in the skin when BHT is applied topically, and could, for example, increase the risk of calcification of the elastin fibers, thus decreasing skin elasticity.

VITAMIN K STATUS BIOMARKERS

A big portion of the ingested vitamin K_1 is metabolized and excreted (through bile and urine) within 12 to 24 hours. However as average intake of vitamin K_1 increases, so does fasting plasma level of K_1.[81,82,129] Plasma vitamin K_1 may be useful in monitoring patients on anticoagulants in conjunction with the typical coagulation-related tests such as the international normalized ratio (INR) and prothrombin time.

A complete assessment of vitamin K status should reflect the degree of vitamin K sufficiency for all physiological functions in the body that require vitamin K_1 and/or K_2 for their optimal activity. Unfortunately, such a complete assessment is not available at this time, partly because there are so many VKDs in the body that are carboxylated by vitamin K, and researchers predict that new ones may still be uncovered in future research. Also, many of vitamin K's functions and physiological effects are unrelated to its carboxylation effect.

Unlike other fat-soluble vitamins, vitamins K_1 and K_2 have not been found to be stored in excess anywhere in the body. Even after long-term dosing, such as a few years, supplementation with supraphysiological doses of vitamin K_1 or K_2 (10 or 45 mg, respectively), apparently no toxic storage levels or effects have been observed. Every molecule of vitamin K is reused multiple times through a recycling mechanism before it is excreted, and perhaps that is why there is no need to store large amounts of it in the tissues.

Prolonged intake of a significant dose of vitamin K has been shown to have a cumulative effect in improving functional markers of vitamin K status. For example, it has been shown to cause a progressive increase over many months of the percentage of carboxylated osteocalcin (Oc) and increased levels of total Oc (the sum of carboxylated plus uncarboxylated Oc).

Currently Available Tests for Vitamin K Status

Vitamin K deficiency results in the appearance of undercarboxylated VKDs (ucVKDs) in the plasma and various tissues and organs throughout the body. It is also conceivable, although not yet completely demonstrated, that if the vitamin K intake reaches a high enough level, all VKDs in the body would be close to 100% carboxylated; consequently, all levels of ucVKDs would be close to zero.

Typical biochemical markers of vitamin K status used in studies are plasma levels of ucVKDs or their normalized values. However, one should keep in mind that plasma levels of any one type of ucVKD or carboxylated VKD (cVKD) do not always correlate with their total amounts found throughout the body.

The following vitamin K status tests have been used in studies: plasma undercarboxylated prothrombin (PIVKA II, which stands for proteins induced by vitamin K absence or vitamin K antagonism by anticoagulant drugs), plasma or urine ucOc, and plasma undercarboxylated matrix GLA protein (dp-ucMGP). Of all the tests mentioned, PIVKA II is available from many commercial laboratories in the United States; plasma levels of ucOc are available from only one specialty laboratory in the United States (Genova Diagnostics) and dp-ucMGP is available from Immunodiagnostics Systems Ltd. in the United Kingdom.

It is important to note that as vitamin K_1 and/or K_2 intake increases, clotting-related VKDs (such as prothrombin synthesized in the liver) are carboxylated with higher priority than the extrahepatic VKDs (such as Oc and MGP). This is an example of the "triage theory" presented by McCann and Ames in their review.[30] There they present evidence for the concept that evolutionary pressure may have led to prioritizing nutrient allocation in the order of importance for the "survival of the species." In this case, clotting is obviously more important for survival than bone or arterial health, so the carboxylation of the clotting-related proteins is prioritized.

For example, one study has shown that for patients on a small dose (1 mg) of warfarin, PIVKA II started decreasing when vitamin K_1 intake was around 100 mcg, whereas ucOc plasma levels did not start decreasing until vitamin K_1 intake reached around 300 mcg/day.[147]

Consequently, PIVKA II may decrease significantly and approach zero for vitamin K_1 intakes in the lower range of the average intakes, whereas ucOc levels may not approach zero until vitamin K intake is much higher. One landmark study showed that the percentages of ucOc levels may approach zero when intakes of vitamin K_1 are in the range of 1000 to 2000 mcg.[79] No studies have found an equivalent amount of vitamin K_2 that can achieve the same decrease in percentage of ucOc, which corresponds to an almost complete carboxylation of Oc. Suggestions for possible biomarkers of vitamin K status:

1. PIVKA II is the inactive form of prothrombin, and it is a sensitive marker of vitamin K_1 status at very low intakes (for example, in the vicinity of 100 mcg of vitamin K_1/day).

It is not clear how PIVKA II levels are affected by vitamin K_2 (in various forms) compared with vitamin K_1. One study showed that the INR was lowered similarly by a lower amount of vitamin K_2 (MK-7) compared with vitamin K_1.[175] Consequently, patients taking supplements and foods containing various forms of vitamin K (such as vitamin K_1, MK-4, MK-7) should be very closely monitored for changes in INR.

1. Plasma ucOc level is a test currently available only from one commercial laboratory in the United States specializing in nutrition status-related tests (Metametrix). Some studies have also measured the normalized value of ucOc relative to total osteocalcin (called %ucOc = ucOc/total Oc, or the ratio OCR = ucOc/iOc, iOc with meaning "intact or carboxylated osteocalcin").

The plasma levels of ucOc and its normalized ratios, %ucOc and OCR, have good sensitivity for a wide range of vitamin K_1 and/or K_2 intakes. The upper threshold of sensitivity seems to be achieved for a vitamin K_1 intake in the range of 1000 to 2000 mcg. However, ucOc measurements may not be adequately sensitive for patients on anticoagulant therapy or with very low vitamin K intakes (which represents an alarming 60%–70% of the population).

The measurement of both PIVKA II and ucOc may give a good approximation of vitamin K status with good sensitivity over a wide range of vitamin K intakes even though they reflect only a few facets of the vitamin K status.

It should be noted that even if a patient has a considerably high intake of vitamin K_1 (around 1000–2000 mcg/day) and a corresponding low ucOc level, this does not rule out a deficiency of vitamin K_2 if one were to consider vitamin K_2 a conditionally essential nutrient. This is especially important for certain patients who are older and have genetic polymorphisms or certain severe conditions such as advanced osteoporosis and/or arterial calcification, cancer, multiple sclerosis, or rheumatoid arthritis.

Oc has an established role in bone metabolism and studies have revealed significant direct correlations between ucOc or %ucOc and fracture risk and bone density.[176]

However, the ucOc marker may be useful for the management of diseases besides the ones related to bone because it conceivably correlates with the percentage of carboxylation of other extrahepatic VKDs in the body.

For example, one study found a positive correlation between plasma levels of %uOc and the risk of prostate cancer.[151]

It is important to note that average populations deemed "apparently healthy" were found to have an average of 15% to 50% ucOc. Specifically, one study found teenage girls to have an average %ucOc of 21.9%.[177] This is considered by many researchers a sign of subclinical vitamin K deficiency, which may have various detrimental consequences for bone development, cartilage, brain, and the nervous system, because vitamin K is so critically involved in the physiology of all these tissue and organs. This is just one example why the RDI for vitamin K is outdated.

1. ucMGP is currently assessed only in the United Kingdom but hopefully may be offered in the future by US commercial laboratories because it has good potential in evaluating and devising nutritional interventions for patients at risk of or with existing arterial calcification.

Plasma levels of ucMGP were found to directly correlate with arterial calcium buildup and calcium scores but only for patients with relatively low amounts of arterial calcium and in early stages of cardiovascular disease.[124] Thus the plasma level of ucMGP is thought to be a good indicator of current mild buildup of calcium in the arterial wall and/or of future risk of arterial calcification.

If this test becomes commercially available in the future, it may be helpful in guiding clinicians to devise the appropriate vitamin K intake for each patient so as to maximally reduce the risk of further cardiovascular calcification and possibly even reverse it.

Because the ucMGP measurement is not yet offered in the United States, the best available surrogate marker for it is plasma ucOc and its normalized ratio, which may also predict to a certain extent current or future risk of arterial calcification. However, it is also important to keep in mind that when the ucOc level is close to zero, it may not necessarily mean that the level of ucMGP in the body is also the lowest possible or that vitamin K intake has reached a level high enough to support complete carboxylation of all MGPs in the body and thus is providing significant protection from arterial calcification.

Some researchers point to the fact that osteocalcin contains three GLA residues, whereas MGP contains five; therefore they hypothesize that the carboxylation of MGP may require a higher vitamin K intake than that of Oc. This may be another example of the triage theory presented by McCann and Ames in their review, discussed previously.

In summary, until more vitamin K assessments are available, plasma ucOc is the best biomarker for guiding patients toward optimization of their vitamin K status with vitamin K_1 and/or K_2 from diet and supplements, although it is only a partial marker of vitamin K sufficiency in the body.

Warfarin causes, by design, an impairment in the carboxylation of various clotting proteins, but unfortunately it also affects all other extrahepatic VKDs. For example, one study found that a "minidose" of warfarin of 1 mg/day induced an increase in %ucOc from 8% to around 20%, and it will most likely increase ucMGP as well.[178]

By monitoring ucOc and ucMGP in addition to PIVKA II, along with standard clotting parameters (such as prothrombin time and INR) in these patients, it may be possible to find the appropriate dose of vitamin K_2 for each individual that may reduce to a certain extent the effects of warfarin on extrahepatic tissues. All this would be done while keeping the INR within the therapeutic range.

In interpreting tests that measure the levels of undercarboxylated VKDs, it is important to keep in mind that there are other factors, other than vitamin K, that may affect these results, as follows:

1. Deficiencies of vitamin B_6 and manganese, as well as low sex hormone status (estrogen or testosterone), may reduce the carboxylation rate of VKDs.[3]
2. Factors that can lower the production of osteocalcin or MGP include low vitamin D and K status (including treatment with warfarin), excess or deficiency of vitamin A, corticosteroid therapy, hypothyroid state (defined as suboptimal T3 levels), and possibly high endogenous production of cortisol.[179]
3. Total plasma levels of Oc may be elevated by vitamin D supplementation and in states of increased bone turnover (e.g., during adolescence, after injury, or after menopause).

Because so many factors can influence the levels of osteocalcin, some researchers believe that the normalized values of ucOc (OCR and %ucOc), rather than absolute values of ucOc, may be a better indicator of vitamin K status. However, this is still under debate, and researchers who favor the absolute levels of uOc argue that ucOc may be released more easily from the bone matrix into the blood compared with carboxylated Oc because carboxylation enhances its ability to bind calcium in the bone matrix. Thus the plasma ratios of these two biomarkers may not correlate with their tissue ratios.

1. MGP expression is decreased in hypothyroidism, which will also affect the body's ability to reduce arterial calcification.

Other factors that may increase the need for vitamin K intake and affect the interpretation of the ucVKDs tests include genetic polymorphisms, aging, fat maldigestion and/or malabsorption, pharmaceuticals (antibiotics, antiepileptic drugs, aspirin), or excess vitamin E.

One should keep in mind that oxidative stress may impair the tissues' ability to recycle vitamin K to reuse it for carboxylation multiple times. Thus the vitamin K status should be thought of based not only on vitamin K intake but also how well it increases tissues stores and on how many times each vitamin K molecule can be recycled.

It is also important to note that during active cancer development, various clotting factors may become upregulated, including thrombin.

DOSAGE

Current RDI Versus Expanded Criteria for Defining Optimal Vitamin K Intake

The adequate intake (AI) of vitamin K, specifically for the K_1 form, is 90 mcg for women and 120 mcg for men, or 1 mcg/kg per day.[180] For vitamin K, the AI criterion was initially adequate blood clotting, because that was the only vitamin K role recognized at the time. A new AI may have to be increased significantly to support the function of various tissues and organs affected by vitamin K status.

The average intake of vitamin K_1 in United States was found to be 70 mcg/day for the younger and 120 mcg/day for the older population. Whereas intakes of vitamin K in the United Kingdom are similar to those in the United States, in other European countries, such as Holland, average vitamin K_1 intakes are significantly higher, at 246 mcg.

Surveys have found severe population deficiencies of vitamin K_1, even relative to the current AI.[181] Sixty to seventy percent of the US population does not even meet the current AI (90–120 mcg) for vitamin K, which is already well below what may be necessary for optimal health. Some 5% to 15% of people have been found to have undetectable levels of plasma vitamin K_1.

The majority of vitamin K_1 consumed in the United States is derived from vegetable oils as opposed to vegetables. Owing to the high prevalence of hydrogenated and fried oils and storage in transparent bottles (which allows vitamin K to be destroyed), it is possible that the active amount of vitamin K_1 ingested may be even lower than previously estimated. This widespread deficiency of vitamin K_1 is not surprising, because consumption of vegetables is deficient in the American diet. The well-founded recommendation of consuming five to nine servings of fruits and vegetables per day could provide an amount of vitamin $K_{1\ close}$ to 1000 mcg/day.

Guidelines for Optimal Vitamin K_1 Intake: Preventative Versus Therapeutic Strategies

As discussed, vitamin K_1 has multiple extrahepatic functions. The only function studied with escalating doses of vitamin K_1 was osteocalcin carboxylation. From the results of that study, we can hypothesize that an intake of 1 to 2 mg of vitamin K_1 may be the best preventive dose for supporting bone health, because it supports a virtually complete carboxylation of Oc. However, based on the intake surveys discussed earlier, it is not realistic to assume that individuals would change their diet enough to achieve this level of intake, thus supplementation with 0.9 to 1.0 mg K_1 may be needed in a large proportion of the general population.

One study showed that 5 mg/day of vitamin K_1 may significantly reduce fracture risk and cancer incidence of all types, but it did not include comparison groups taking any other doses between 1 and 5 mg.[104] This dose may be safe to use because there is no UL established for K_1, but one has to keep in mind that this is a supraphysiological dose.

Based on the studies that investigated effects on CVD, vitamin K_1 doses of 0.5 mg or 1.0 mg would likely have a beneficial effect on reducing progression of arterial calcification of the medial layer. It is not clear whether increasing this dose or adding vitamin K_2 may have the potential to completely prevent it. More studies are needed to establish the optimal intake of vitamin K_1 that can minimize the levels of nonfunctional MGP (dp-ucMGP), because carboxylated MGP is a major player in reducing calcification of various cardiovascular tissues (arteries, veins, heart valves). In addition, the 1 mg dose was shown to completely prevent an increase in arterial stiffness caused by supplementation with vitamin D and calcium.

Another line of evidence for optimal vitamin K_1 intake comes from the evolutionary adaptation to its content in the Paleolithic diet. Studies have looked at many nutritional components of this type of diet and preliminary data suggest that Paleolithic humans were ingesting vitamin K_1 in the vicinity of 1.0 mg/day on average.[78] It is likely not a coincidence that the Paleolithic levels of vitamin K_1 intake overlap with the amount of vitamin K_1 needed to completely activate Oc.

It has been established that vitamin K_1 needs to convert to K_2 (MK-4) to reduce arterial calcification. Aging, genetic polymorphisms, various metabolic factors, and treatment with statins (possibly also with bisphosphonates) may reduce K_1 to K_2 (MK-4) conversion, thus the need to consider additional supplementation with K_2 (MK-4).

Guidelines for Optimal Vitamin K_2 Intake: Preventive Versus Therapeutic Strategies

Currently there is no AI set for vitamin K_2, even though blood clotting needs may be satisfied by vitamin K_2 (MK-4) or other long-chain menaquinones at physiologically equivalent doses. However, it is not clear whether any form of vitamin K_2 can satisfy all the extrahepatic functions provided by K_1, simply because not all have been elucidated yet. Vitamin K_2 does not convert to K_1 and many tissues seem to prefer K_1, because they store much higher levels of K_1 compared with those of K_2 (MK-4).

Thus vitamin K_2 may not be used as a substitute for vitamin K_1 needs but may be added to it, based on average evolutionary intake, for basic health support or for therapeutic purposes. The commercially available forms of K_2 are K_2 (MK-4), K_2 (MK-7), or a blend of K_2 (MK-6, MK-7, MK-9).

Evolutionary Intake of Vitamin K_2

The human need for preformed vitamin K_2 may be grounded in an evolutionary perspective of the Paleolithic diet. Its vitamin K_2 content has not been estimated yet, but it was very likely higher than in modern times for several reasons: (a) The modern human's gut flora may be often impaired owing to therapeutic antibiotic use or antibiotics ingested within animal foods and dairy. (2) The gastrointestinal tract of the Paleolithic human was most likely populated with a larger variety of bacteria producing a fair amount of long-chain vitamin K_2 (MK-n)s. (3) Vitamin K_1 intake was higher and a substrate for intestinal bacteria to produce abundant quantities of K_2 (MK-n). (4) Fiber was also more abundant, which is critical in supporting gastrointestinal bacteria.

The only significant dietary sources of long-chain MK-ns for modern humans are cheeses and sauerkraut (in Europe and the United States) or fermented vegetables (kimchi) or soy fermented foods (such as natto, mostly in Asia). The modern content of K_2 (MK-4) in meats may be low because animals are fed mostly corn and not grass. It is unlikely that Paleolithic humans were ingesting any purposely fermented foods, but they may have eaten partially "spoiled" (thus naturally fermented) foods. Dairy foods were probably not often consumed, and neither were grains, legumes, or soy foods.[78]

Average modern intakes of vitamin K_2 (a mix of menaquinoes of various lengths) have been evaluated by a few epidemiological studies in European populations at 29.1 mcg. On the other hand, K_2 (MK-7) has been consumed traditionally in Japan (by approximately 42% of the population) at average levels of 58 mcg/day, with the upper range up to 165 mcg/day. This level of K_2 (MK-7) is typically derived from consuming an average of 400 mcg per serving of natto (one to three times per week).

As discussed earlier, the correlations found between intake of vitamin K (as K_2 and/or K_1) and CVD mortality, overall mortality, or fracture risk are conflicting, with some studies pointing to vitamin K_1 and others to vitamin K_2 to be the form that has more potential to be protective and therapeutic. However, the range of intakes of vitamin K reported by these studies are much lower than intakes able to support complete carboxylation of Oc or lower dp-ucMGP to a large degree. In conclusion, it is not possible to make a recommendation for the amounts and forms of vitamin K_2 that may provide basic health benefits when added to an optimal intake of 1 mg of K_1.

Therapeutic Dosage of Vitamin K_2

Vitamin K_2 may be conditionally essential for certain organs and certain individuals who have difficulty converting vitamin K_1 to vitamin K_2 because of age-related changes, those who need intensive intervention for certain disease states, or have genetic polymorphisms.

If the supplementation goal is to try to compensate for the poor vitamin K_1 to vitamin K_2 (MK-4) conversion, then a dose around 1 mg/day of vitamin K_2 (MK-4) may be appropriate, knowing that 90% of 1 mg vitamin K_1 may convert to vitamin K_2 (MK-4) by the time it is stored inside various tissues.

If the supplementation goal is a major therapeutic intervention for osteopenia, osteoporosis, osteoarthritis, arterial calcification, a high dose may be used, such as 45 mg/day, the same as in the drug form Glakay. This was prescribed in Japan for more than 20 years and was proven effective especially for bone and cardiovascular health. The 45 mg of vitamin K_2 (MK-4) should be administered in 15 mg doses, three times daily for optimal utilization.

Therapeutic doses of vitamin K_2 (MK-7) have not been proven more effective than those with vitamin K_1 or vitamin K_2 (MK-4).

SAFETY

No upper level of toxicity (UL) has been established for vitamin K_1, even though it belongs to the family of fat-soluble vitamins, which all have a UL because of their typical tendency to be stored and accumulated in various organs. This may be because vitamin K_1 is rapidly metabolized out of the plasma and the majority is excreted via bile and urine (50% and 20%, respectively) within a day, with complete excretion of the rest in about 3 days. No evidence has been found so far of toxic accumulation anywhere in the body. A level of 1 mg vitamin K_1 per day may be considered intrinsically safe because it could be normally derived from diets high in leafy greens, seeds, oils, and some organ meats.

Will such very high intakes increase clotting excessively? Some studies suggest that once the liver clotting factors are maximized (by complete carboxylation), no amount of excess vitamin K_1 or K_2 can increase clotting performance any further.[182]

Whenever supplementation with vitamin K is initiated, most people will experience an increase in clotting factors because a large proportion (60%–70%) of the population has a vitamin K intake below the optimal clotting requirement. However, vitamin K also supports the activation of anticlotting proteins that keep overall clotting tendencies in an optimal range (proteins C, Z, S). One study looked at the effects of supplementing postmenopausal women with 45 mg of vitamin K_2 (MK-4) and found that although clotting markers increased, the anticlotting activity increased as well; thus the clotting balance was maintained.[90]

Numerous 2- to 3-year studies have employed doses of 45 mg and even 90 mg of vitamin K_2 (MK-4) for various interventions and none have reported clotting or thrombotic abnormalities.

Regarding vitamin K_1, there are only a few short-term studies that supplemented with vitamin K_1 doses from 500 mcg up to 10 mg, and they have not reported clotting or thrombogenic abnormalities.

The Japanese studies that used 45 to 90 mg of vitamin K_2 (MK-4) have not noted any side effects, which were closely monitored as a result of the drug status of this formulation.

SUMMARY

The current paradigm shift in vitamin K knowledge is very similar to that of vitamin D, for which there is also a big discrepancy between the fast-accumulating new research and the outdated AI and UL or therapeutic guidelines.

Many researchers who performed recent reviews of vitamin K_1 and K_2 call for establishing a higher AI and for more research to be done to further clarify vitamin K physiology and provide more evidence for clinical applications. However, there is no consensus on what the new AI for vitamin K should be and on what doses of vitamin K_1 and/or K_2 may be used effectively and safely for therapeutic purposes.

It certainly makes sense to recommend acquiring as much vitamin K as possible from one's diet in a balance of all forms: K_1, K_2 (MK-4), and some long-chain K_2 (MK-n) from fermented foods. Another aspect to address is the health of the intestinal tract, to allow adequate amounts of K_2 (MK-n) to be derived from intestinal bacterial production. In that respect, avoidance of unnecessary antibiotic treatments and favoring grass-fed animal products would be helpful.

Vitamin K may be supplemented for two major purposes:

1. For complementing the patient's dietary deficiencies and correcting any physiological deficiencies of vitamin K_1, K_2 (MK-4), and various long-chain menaquinones. In most cases, it makes sense to supplement with 1 mg vitamin K_1 as a foundation, and add small amounts of a variety of menaquinones.
2. For therapeutic purposes, the best evidence is available for 1 and 5 mg vitamin K_1 and 45 mg MK-4 (as discussed in the osteoporosis and CVD sections). In this application the patient must be closely monitored for potential side effects and dose adjustments.

Another important aspect to consider is the dose distribution of vitamin K intake throughout the day. For example, a once-a-day high dose of vitamin K_1 or K_2 may not be metabolized properly and may be more likely to have unwanted side effects. One has to keep in mind that for vitamin K_1 supplementation to realize some of its physiological roles in extrahepatic tissues, it may have to be cleaved to form vitamin K_3 by the intestinal cells or convert peripherally to vitamin K_2 in various tissues and organs.

Dietary fat stimulates biliary and pancreatic secretions, so it would be best to incorporate a reasonable amount of fat (for example, 30 g) with the meal ingested at the same time as a vitamin K supplement.

In natural foods, vitamin K_1 is bound in the thykaloid membranes of chloroplasts, whereas mechanical processing, such as blending, juicing, or cooking may further enhance vitamin K_1 bioavailability from food.

The bottom line is that vitamin K_1 or K_2 requirements may vary between individuals based on their particular genetics, health status, dietary habits, and health goals.

Ideally (only theoretical at this time), vitamin K status should be at the level that supports optimal activity for all vitamin K-related functions. Therapeutic supplementation with supraphysiological doses of vitamin K has great potential for the reversal of severe diseases through complex effects. Patients should be monitored for various side effects and interactions with other pharmaceutical drugs, such as statins and bisphosphonates, and through specific vitamin K status tests and other tests relevant to vitamin K action pathways, as discussed previously in the "Status Biomarkers and Commercially Available Assessments" Section.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Suttie JW. *Vitamin K in Health and Disease*. Boca Raton, FL: CRC Press.
2. Lamson DW, Plaza SM. The anticancer effects of vitamin K. *Altern Med Rev*. 2003;8(3):303–318. Review.
3. Olson RE. The function and metabolism of vitamin K. *Annu Rev Nutr*. 1984;4:281–337. Review.
4. Elder SJ, Haytowitz DB, Howe J, et al. VitaminK contents of meat, dairy, and fast food in the U.S. diet. *J Agric Food Chem*. 2006;54(2):463–467.
5. Greer FR, Marshall SP, Foley AL, et al. Improving the vitamin K status of breastfeeding infants with maternal vitamin K supplements. *Pediatrics*. 1997;99(1):88–92.
6. Novotny JA, Kurilich AC, Britz SJ, et al. Vitamin K absorption and kinetics in human subjects after consumption of 13C-labelled phylloquinone from kale. *Br J Nutr*. 2010;104(6):858–862. Epub 2010 Apr 27.
7. Garber AK, Binkley NC, Krueger DC, et al. Comparison of phylloquinone bioavailability from food sources or a supplement in human subjects. *J Nutr*. 1999;129(6):1201–1203.
8. Gijsbers BL, Jie KS, Vermeer C. Effect of food composition on vitamin K absorption in human volunteers. *Br J Nutr*. 1996;76(2):223–229.
9. Booth SL, O'Brien-Morse ME, Dallal GE, et al. Response of vitamin K status to different intakes and sources of phylloquinone-rich foods: comparison of younger and older adults. *Am J Clin Nutr*. 1999;70(3):368–377.
10. Sokoll LJ, Booth SL, O'Brien ME, et al. Changes in serum osteocalcin, plasma phylloquinone, and urinary gamma-carboxyglutamic acid in response to altered intakes of dietary phylloquinone in human subjects. *Am J Clin Nutr*. 1997;65(3):779–784.
11. Booth SL, Lichtenstein AH, Dallal GE. Phylloquinone absorption fro phylloquinone-fortified oil is greater than from a vegetable in younger and older men and women. *J Nutr*. 2002;132(9):2609–2612.
12. Bügel S, Sørensen AD, Hels O, et al. Effect of phylloquinone supplementation on biochemical markers of vitamin K status and bone turnover in postmenopausal women. *Br J Nutr*. 2007;97(2):373–380.
13. Uematsu T, Nagashima S, Niwa M, et al. Effect of dietary fat content on oral bioavailability of menatetrenone in humans. *J Pharm Sci*. 1996;85(9):1012–1016.
14. Shearer MJ, Barkhan P, Webster GR. Absorption and excretion of an oral dose of tritated vitamin K1 in man. *Br J Haematol*. 1970;18(3):297–308.
15. Jones KS, Bluck LJ, Wang LY, et al. A stable isotope method for the simultaneous measurement of vitamin K1 (phylloquinone) kinetics and absorption. *Eur J Clin Nutr*. 2008;62(11):1273–1281.
16. Jones KS, Bluck LJ, Wang LY, et al. The effect of different meals on the absorption of stable isotope-labelled phylloquinone. *Br J Nutr*. 2009;102(8):1195–1202.
17. Shearer MJ, McBurney A, Barkhan P. Studies on the absorption and metabolism of phylloquinone (vitamin K1) in man. *Vitam Horm*. 1974;32:513–542. Review.
18. Booth SL, Lichtenstein AH, O'Brien-Morse M, et al. Effects of a hydrogenated form of vitamin K on bone formation and resorption. *Am J Clin Nutr*. 2001;74(6):783–790.
19. Thijssen HH, Drittij-Reijnders MJ. Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4. *Br J Nutr*. 1996;75(1):121–127.
20. Okano T, Shimomura Y, Yamane M, et al. Conversion of phylloquinone (Vitamin K1) into menaquinone-4 (Vitamin K2) in mice:two possible routes for menaquinone-4 accumulation in cerebra of mice. *J Biol Chem*. 2008;283(17):11270–11279.
21. Thijssen HH, Drittij-Reijnders MJ, Fischer MA. Phylloquinone and menaquinone-4distribution in rats: synthesis rather than uptake determines menaquinone-4 organ concentrations. *J Nutr*. 1996;126(2):537–543.
22. Bentley R, Meganathan R. Biosynthesis of vitamin K (menaquinone) in bacteria. *Microbiol Rev*. 1982;46(3):241–280. Review.
23. Yamaguchi M, Kakuda H, Gao YH, et al. Prolonged intake of fermented soybean (natto) diets containing vitamin K2 (menaquinone-7) prevents bone loss in ovariectomized rats. *J Bone Miner Metab*. 2000;18(2):71–76.
24. Komai M, Shirakawa H. [Vitamin K metabolism. Menaquinone-4 (MK-4) formation from ingested VK analogues and its potent relation to bone function]. *Clin Calcium*. 2007;17(11):1663–1672. Review. Japanese. PubMed PMID: 17982185.
25. Kamao M, Suhara Y, Tsugawa N, et al. Vitamin K content of foods and dietary vitamin K intake in Japanese young women. *J Nutr Sci Vitaminol (Tokyo)*. 2007;53(6):464–470.
26. Thijssen HH, Drittij MJ, Vermeer C, et al. Menaquinone-4 in breast milk is derived from dietary phylloquinone. *Br J Nutr*. 2002;87(3):219–226.
27. Shearer MJ, Newman P. Metabolism and cell biology of vitamin K. *ThrombHaemost*. 2008;100(4):530–547. Review.
28. Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food: effect of food matrix on circulating vitamin K concentrations. *Haemostasis*. 2000;30(6):298–307.
29. Glakay (Capsules 15mg) drug data sheet (MK-4 drug version in Japan), Vitamin K2 Preparation for Treatment of Osteoporosis—by Eisai Co., Revised: November 2009 (9th version) Standard Commodity Classification No. of Japan.
30. McCann JC, Ames BN. Vitamin K an example of triage theory: is micronutrient inadequacy linked to diseases of aging? *Am J Clin Nutr*. 2009;90(4):889–907.
31. Adams J, Pepping J. Vitamin K in the treatment and prevention of osteoporosis and arterial calcification. *Am J Health Syst Pharm*. 2005;62(15):1574–1581. Review.
32. Cockayne S, Adamson J, Lanham-New S, et al. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med*. 2006;166(12):1256–1261. Review.
33. Bügel S. Vitamin K and bone health in adult humans. *Vitam Horm*. 2008;78:393–416. Review.
34. Howe AM, Webster WS. Vitamin K—its essential role in craniofacial development: a review of the literature regarding vitamin K and craniofacial development. *Aust Dent J*. 1994;39(2):88–92. Review.
35. Hojo K, Nagaoka S, Murata S, et al. Reduction of vitamin K concentration by salivary Bifidobacterium strains and their possible nutritional competition with Porphyromonas gingivalis. *J Appl Microbiol*. 2007;103(5):1969–1974.
36. Sharaev PN. The role of vitamin K in the metabolism of connective tissue biopolymers. *Vopr Med Khim*. 1984;30(1):13–17. Review. Russian.
37. Levedakou EN, Strohmeyer TG, Effert PJ, et al. Expression of the matrix Gla protein in urogenital malignancies. *Int J Cancer*. 1992;52(4):534–537.
38. Price PA. Gla-containing proteins of bone. *Connect Tissue Res*. 1989;21(1-4):51–57; discussion 57-60. Review.
39. Cancela ML, Price PA. Retinoic acid induces matrix Gla protein gene expression in human cells. *Endocrinology*. 1992;130(1):102–108.
40. Bettoun DJ, Burris TP, Houck KA, et al. Retinoid X receptor is a nonsilent major contributor to vitamin D receptor-mediated transcriptional activation. *Mol Endocrinol*. 2003;17(11):2320–2328.
41. Bellido-Martín L, de Frutos PG. *Vitam Horm. Vitamin K-dependent actions of Gas6*. 2008;78. Barcelona 08036 Spain: Department of Cell Death and Proliferation, Institute for Biomedical Research of Barcelona, IIBB-CSIC-IDIBAPS; 2008:185–209.
42. Vermeer C, Shearer MJ, Zittermann A, et al. Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. *Eur J Nutr*. 2004;43(6):325–335. Epub 2004 Feb 5. Review.
43. Plaza SM, Lamson DW. Vitamin K2 in bone metabolism and osteoporosis. *Altern Med Rev*. 2005;10(1):24–35. Review.
44. Ohsaki Y, Shirakawa H, Hiwatashi K, et al. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. *Biosci Biotechnol Biochem*. 2006;70(4):926–932.
45. Hiruma Y, Nakahama K, Fujita H, et al. Vitamin K2 and geranylgeraniol, its side chain component, inhibited osteoclast formation in a different manner. *Biochem Biophys Res Commun*. 2004;314(1):24–30. PubMed PMID: 14715241.
46. Okamoto H. Vitamin K and rheumatoid arthritis. *IUBMB Life*. 2008;60(6):355–361. Review.
47. Neogi T, Booth SL, Zhang YQ, et al. Low vitamin K status is associated with osteoarthritis in the hand and knee. *Arthritis Rheum*. 2006 Apr;54(4):1255–1261.

48. Camandola S, Leonarduzzi G, Musso T, et al. Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem Biophys Res Commun*. 1996;229(2):643–647.
49. Hara K, Akiyama Y, Nakamura T, Murota S, Morita I, et al. The inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. *Bone*. 1995;16(2):179–184.
50. Taira H, Fujikawa Y, Kudo O, et al. Menatetrenone (vitamin K2) acts directly on circulating human osteoclast precursors. *Calcif Tissue Int*. 2003;73(1):78–85.
51. Grasser WA, Baumann AP, Petras SF, et al. Regulation of osteoclast differentiation by statins. *J Musculoskelet Neuronal Interact*. 2003;3(1):53–62.
52. Vitamin K_2. Monograph. *Altern Med Rev*. 2009;14(3):284–293. Review.
53. Bala Y, Seeman E. Bone's Material Constituents and their Contribution to Bone Strength in Health, Disease, and Treatment. *Calcif Tissue Int*. 2015;97(3):308–326. https://doi.org/10.1007/s00223-015-9971-y. Epub 2015 Feb 25. Review. PubMed PMID: 25712256.
54. Mackie EJ. Osteoblasts: novel roles in orchestration of skeletal architecture. *Int J Biochem Cell Biol*. 2003;35(9):1301–1305.
55. Spronk HM, Soute BA, Schurgers LJ, et al. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. *J Vasc Res*. 2003;40(6):531–537.
56. Nanke Y, Kotake S, Ninomiya T, Furuya T, Ozawa H, Kamatani N. Geranylgeranylacetone inhibits formation and function of human osteoclasts and prevents bone loss in tail-suspended rats and ovariectomized rats. *Calcif Tissue Int*. 2005 Dec;77(6):376–385. Epub 2005 Dec 8. PubMed PMID: 16362462.
57. Tabb MM, Sun A, Zhou C, et al. Vitamin K_2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J Biol Chem*. 2003;278(45):43919–43927.
58. Ichikawa T, Horie-Inoue K, Ikeda K, Blumberg B, Inoue S. Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J Biol Chem*. 2006;281(25):16927–16934. Epub 2006 Apr 10.
59. Saito M. [Effect of vitamin K on bone material properties]. *Clin Calcium*. 2009;19(12):1797–1804. https://doi.org/CliCa091217971804.
60. Amizuka N, Li M, Guo Y, et al. Biological effects of vitamin K2 on bone quality. *Clin Calcium*. 2009;19(12):1788–1796. Review. Japanese.
61. Stock SR. The Mineral-Collagen Interface in Bone. *Calcif Tissue Int*. 2015;97(3):262–280. https://doi.org/10.1007/s00223-015-9984-6. Epub 2015 Apr 1. Review. PubMed PMID: 25824581; PubMed Central PMCID: PMC4727835.
62. Sroga GE, Vashishth D. Effects of bone matrix proteins on fracture and fragility in osteoporosis. *Curr Osteoporos Rep*. 2012;10(2):141–150. https://doi.org/10.1007/s11914-012-0103-6. Review. PubMed PMID: 22535528; PubMed Central PMCID:PMC3375270.
63. Ichikawa T, Horie-Inoue K, Ikeda K, et al. Vitamin K2 induces phosphorylation of protein kinase A and expression of novel target genes in osteoblastic cells. *J Mol Endocrinol*. 2007;39(4):239–247.
64. Urayama S, Kawakami A, Nakashima T, et al. Effect of vitamin K2 on osteoblast apoptosis: vitamin K2 inhibits apoptotic cell death of human osteoblasts induced by Fas, proteasome inhibitor, etoposide, and staurosporine. *J Lab Clin Med*. 2000;136(3):181–193. PubMed PMID: 10985496.
65. Wu WJ, Kim MS, Ahn BY. The inhibitory effect of vitamin K on RANKL-induced osteoclast differentiation and bone resorption. *Food Funct*. 2015;6(10):3351–3358. https://doi.org/10.1039/c5fo00544b. PubMed PMID: 26267519.
66. Akiyama Y, Hara K, Tajima T, et al. Effect of vitamin K2 (menatetrenone) on osteoclast-like cell formation in mouse bone marrow cultures. *Eur J Pharmacol*. 1994;263(1–2):181–185.
67. Iwamoto J, Takeda T, Sato Y. Effects of vitamin K2 on osteoporosis. *Curr Pharm Des*. 2004;10(21):2557–2576. Review.
68. Braam LA, Knapen MH, Geusens P, et al. Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. *Calcif Tissue Int*. 2003;73(1):21–26.
69. Shea MK, Dallal GE, Dawson-Hughes B, et al. Vitamin K, circulating cytokines, and bone mineral density in older men and women. *Am J Clin Nutr*. 2008;88(2):356–363.
70. Walther B, Karl JP, Booth SL, Boyaval P. Menaquinones, bacteria, and the food supply: the relevance of dairy and fermented food products to vitamin K requirements.
71. Knapen MH, Schurgers LJ, Vermeer C. Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int*. 2007;18(7):963–972.
72. Nakamura E, Aoki M, Watanabe F, Kamimura A. Low-dose menaquinone-4 improves γ-carboxylation of osteocalcin in young males: a non-placebo-controlled dose-response study. *Nutr J*. 2014;13:85. https://doi.org/10.1186/1475-2891-13-85. PubMed Central PMCID: PMC4155127 PubMed PMID: 25163392.
73. Geleijnse JM, Vermeer C, Grobbee DE, et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr*. 2004;134(11):3100–3105. PubMed PMID: 15514282.
74. Emaus N, Gjesdal CG, Almås B, et al. Vitamin K2 (MK-7) supplementation does not influence bone loss in early menopausal women: a randomised double-blind placebo-controlled trial. *Osteoporos Int*. 2010;21(10):1731–1740. https://doi.org/10.1007/s00198-009-1126-4. Epub 2009 Nov 25. PubMed PMID: 19937427.
75. Rønn SH, Harsløf T, Pedersen SB, Langdahl BL. Vitamin K2 (menaquinone-7) prevents age-related deterioration of trabecular bone microarchitecture at the tibia in postmenopausal women. *Eur J Endocrinol*. 2016;175(6):541–549. Epub 2016 Sep 13. PubMed PMID: 27625301.
76. Knapen MH, Drummen NE, Smit E, Vermeer C, Theuwissen E. Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women. *Osteoporos Int*. 2013;24(9):2499–2507. https://doi.org/10.1007/s00198-013-2325-6. Epub 2013 Mar 23. PubMed PMID: 23525894.
77. van Summeren MJ, Braam LA, Lilien MR, et al. The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children. *Br J Nutr*. 2009;102(8):1171–1178.
78. Cordain L. The nutritional characteristics of a contemporary diet based upon Paleolithic food groups. *J Am Neutraceut Assoc*. 2002;5:15–24.
79. Binkley NC, Krueger DC, Kawahara TN, et al. A high phylloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. *Am J Clin Nutr*. 2002;76(5):1055–1060.
80. Harshman SG, Shea MK. The role of vitamin k in chronic aging diseases: inflammation, cardiovascular disease, and osteoarthritis. *Curr Nutr Rep*. 2016;5(2):90–98. Epub 2016 Mar 31. PubMed PMID: 27648390; PubMed Central PMCID: PMC5026413.
81. Harshman SG, Saltzman E, Booth SL. Vitamin K: dietary intake and requirements in different clinical conditions. *Curr Opin Clin Nutr Metab Care*. 2014;17(6):531–538. https://doi.org/10.1097/MCO.0000000000000112. Review. PubMed PMID: 25232640.
82. Erkkilä AT, Booth SL, Hu FB, et al. Phylloquinone intake as a marker for coronary heart disease risk but not stroke in women. *Eur J Clin Nutr*. 2005;59(2):196–204. PubMed PMID: 15454972.
83. Braam LA, Knapen MH, Geusens P, Brouns F, Vermeer C. Factors affecting bone loss in female endurance athletes: a two-year follow-up study. *Am J Sports Med*. 2003;31(6):889–895. PubMed PMID: 14623654.
84. Craciun AM, Wolf J, Knapen MH, Brouns F, Vermeer C. Improved bone metabolism in female elite athletes after vitamin K supplementation. *Int J Sports Med*. 1998;19(7):479–484. PubMed PMID: 9839845.
85. Rønn SH, Harsløf T, Pedersen SB, Langdahl BL. Vitamin K2 (menaquinone-7) prevents age-related deterioration of trabecular bone microarchitecture at the tibia in postmenopausal women. *Eur J Endocrinol*. 2016;175(6):541–549. Epub 2016 Sep 13. PubMed PMID: 27625301.
86. Fu X, Moreines J, Booth SL. Vitamin K supplementation does not prevent bone loss in ovariectomized Norway rats. *Nutr Metab (Lond)*. 2012;9(1):12. https://doi.org/10.1186/1743-7075-9-12. PubMed PMID: 22348311; PubMed Central PMCID: PMC3328282.
87. Iwamoto J. Vitamin K_2 therapy for postmenopausal osteoporosis. *Nutrients*. 2014;6(5):1971–1980. https://doi.org/10.3390/nu6051971. Review. PubMed PMID: 24841104; PubMed Central PMCID: PMC4042573.
88. Iwamoto J, Sato Y, Takeda T, Matsumoto H. High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: a review of the literature. *Nutr Res*. 2009;29(4):221–228. https://doi.org/10.1016/j.nutres.2009.03.012. Review. PubMed PMID: 19410972.

89. Fang Y, Hu C, Tao X, Wan Y, Tao F. Effect of vitamin K on bone mineral density: a meta-analysis of randomized controlled trials. *J Bone Miner Metab*. 2012;30(1):60–68. https://doi.org/10.1007/s00774-011-0287-3. Epub 2011 Jun 15. PubMed PMID: 21674202.
90. Ushiroyama T, Ikeda A, Ueki M. Effect of continuous combined therapy with vitamin K(2) and vitamin D(3) on bone mineral density and coagulofibrinolysis function in postmenopausal women. *Maturitas*. 2002;41(3):211–221.
91. Eisai Announces the Intermediate Analysis of Anti-osteoporosis Treatment Post-marketing Research to Investigate the Benefits of Menatetrenone as Part of the Ministry of Health. Labour and Welfare's Pharmacoepidemiological Drug Review Program. http://www.eisai.co.jp/enews/enews200506.html. Accessed November 12, 2019.
92. Binkley N, Harke J, Krueger D, et al. Vitamin K treatment reduces undercarboxylated osteocalcin but does not alter bone turnover, density, or geometry in healthy postmenopausal North American women. *J Bone Miner Res*. 2009;24(6):983–991.
93. Akiyama Y, Hara K, Ohkawa I, et al. Effects of menatetrenone on bone loss induced by ovariectomy in rats. *Jpn J Pharmacol*. 1993;62(2):145–153.
94. Tsugawa N, Shiraki M, Suhara Y, et al. Vitamin K status of healthy Japanese women: age-related vitamin K requirement for gamma-carboxylation of osteocalcin. *Am J Clin Nutr*. 2006;83(2):380–386.
95. Hall G, Phillips TJ. Estrogen and skin: the effects of estrogen, menopause, and hormone replacement therapy on the skin. *J Am Acad Dermatol*. 2005;53(4):555–568; quiz 569–72. Review. PubMed PMID: 16198774.
96. Iwamoto I, Kosha S, Noguchi S, et al. A longitudinal study of the effect of vitamin K2 on bone mineral density in postmenopausal women a comparative study with vitamin D3 and estrogen-progestin therapy. *Maturitas*. 1999;31(2):161–164.
97. Iwamoto J, Takeda T, Ichimura S. Combined treatment with vitamin K2 and bisphosphonate in postmenopausal women with osteoporosis. *Yonsei Med J*. 2003;44(5):751–756. Review.
98. Morishita M, Nagashima M, Wauke K, et al. Osteoclast inhibitory effects of vitamin K2 alone or in combination with etidronate or risedronate in patients with rheumatoid arthritis: 2-year results. *J Rheumatol*. 2008;35(3):407–413.
99. Shane E, et al. American Society for Bone and Mineral Research. Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res*. 2010;25(11):2267–2294.
100. Patrick L. Comparative absorption of calcium sources and calcium citrate malate for the prevention of osteoporosis. *Altern Med Rev*. 1999;4(2):74–85. Review.
101. Heaney RP. BMD: the problem. *Osteoporos Int*. 2005;16(9):1013–1015. Epub.
102. De Terlizzi F, Battista S, Cavani F, et al. Influence of bone tissue density and elasticity on ultrasound propagation: an in vitro study. *J Bone Miner Res*. 2000;15(12):2458–2466.
103. Cepollaro C, Gonnelli S, Pondrelli C, et al. The combined use of ultrasound and densitometry in the prediction of vertebral fracture. *Br J Radiol*. 1997;70(835):691–696.
104. Cheung AM, Tile L, Lee Y, et al. Vitamin K supplementation in postmenopausal womenwith osteopenia (ECKO trial): a randomized controlled trial. *PLoS Med*. 2008;5(10):e196. Erratum in: *PLoS Med*. 2008;5(12):e247.
105. Gonnelli S, Cepollaro C, Agnusdei D, et al. Diagnostic value of ultrasound analysis and bone densitometry as predictors of vertebral deformity in postmenopausal women. *Osteoporos Int*. 1995;5(6):413–418. Review.
106. König D, Oesser S, Scharla S, Zdzieblik D, Gollhofer A. Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study. *Nutrients*. 2018;10(1): pii: E97. https://doi.org/10.3390/nu10010097. PubMed PMID: 29337906; PubMed Central PMCID: PMC5793325.
107. Adam M, Spacek P, Hulejova H. What is the effect of collagen peptides oral administration in postmenopausal osteoporosis. *Ces Reumatol*. 2002;10:131–137.
108. Guillerminet F, Fabien-Soulé V, Even PC, et al. Hydrolyzed collagen improves bone status and prevents bone loss in ovariectomized C3H/HeN mice. *Osteoporos Int*. 2012;23(7):1909–1919. https://doi.org/10.1007/s00198-011-1788-6. Epub 2011 Sep 17. PubMed PMID: 21927918.
109. Iwamoto J, Takeda T, Ichimura S. Treatment with vitamin D3 and/or vitamin K2 for postmenopausal osteoporosis. *Keio J Med*. 2003;52(3):147–150. Review.
110. Hara K, Akiyama Y, Tomiuga T, et al. Influence of vitamin D3 on inhibitory effect of vitamin K2 on bone loss in ovariectomized rats. *Nippon Yakurigaku Zasshi*. 1994;104(2):101–109. Japanese.
111. Cannell JJ, Hollis BW. Use of vitamin D in clinical practice. *Altern Med Rev*. 2008;13(1):6–20.
112. Barker ME, Blumsohn A. Is vitamin A consumption a risk factor for osteoporotic fracture?. *Proc Nutr Soc*. 2003;62(4):845–850. Review.
113. Amizuka N, Li M, Maeda T. The interplay of magnesium and vitamin K2 on bone mineralization. *Clin Calcium*. 2005;15(7):57–61. Review. Japanese.
114. Maggio M, Artoni A, Lauretani F, et al. The impact of omega-3 fatty acids on osteoporosis. *Curr Pharm Des*. 2009;15(36):4157–4164.
115. Pizzorno J, Frassetto LA. Katzinger Diet-induced acidosis: is it real and clinically relevant? *J Br J Nutr*. 2010;103(8):1185–1194.
116. van Geel TA, Geusens PP, Winkens B, et al. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle mass, muscle strength and bone mineral density in postmenopausal women: a cross-sectional study. *Eur J Endocrinol*. 2009;160(4):681–687.
117. Berkner KL, Runge KW. The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. *J Thromb Haemost*. 2004;2(12):2118–2132. Review.
118. Puzantian H, Akers SR, et al. Circulating dp-ucMGP is associated with kidney dysfunction and arterial stiffness. *Am J Hypertens*. 2018. https://doi.org/10.1093/ajh/hpy079. [Epub ahead of print]. PubMed PMID: 29788226.
119. Dalmeijer GW, van der Schouw YT, Vermeer C, Magdeleyns EJ, Schurgers LJ, Beulens JW. Circulating matrix Gla protein is associated with coronary artery calcification and vitamin K status in healthy women. *J Nutr Biochem*. 2013;24(4):624–628. https://doi.org/10.1016/j.jnutbio.2012.02.012. Epub 2012 Jul 20. PubMed PMID: 22819559.
120. Kawashima H, Nakajima Y, Matubara Y, et al. Effects of vitamin K2 (menatetrenone) on atherosclerosis and blood coagulation in hypercholesterolemic rabbits. *Jpn J Pharmacol*. 1997;75(2):135–143.
121. van Ballegooijen AJ, Beulens JW. The role of vitamin k status in cardiovascular health: evidence from observational and clinical studies. *Curr Nutr Rep*. 2017;6(3):197–205. https://doi.org/10.1007/s13668-017-0208-8. Epub 2017 Jul 10. Review. PubMed PMID: 28944098; PubMed Central PMCID: PMC5585988.
122. Viegas CS, Cavaco S, Neves PL, et al. Gla-rich protein is a novel vitamin K-dependent protein present in serum that accumulates at sites of pathological calcifications. *Am J Pathol*. 2009;175(6):2288–2298.
123. Fodor D, Albu A, Poantă L, et al. Vitamin K and vascular calcifications. *Acta Physiol Hung*. 2010;97(3):256–266.
124. Cranenburg EC, Vermeer C, Koos R, et al. The circulating inactive form of matrix Gla Protein (ucMGP) as a biomarker for cardiovascular calcification. *J Vasc Res*. 2008;45(5):427–436.
125. Proudfoot D, Shanahan CM. Molecular mechanisms mediating vascular calcification: role of matrix Gla protein. *Nephrology (Carlton)*. 2006;11(5):455–461. Review.
126. Juanola-Falgarona M, Salas-Salvadó J,et. al. Dietary intake of vitamin K is inversely associated with mortality risk. *J Nutr*. 2014;144(5):743–750. https://doi.org/10.3945/jn.113.187740. Epub 2014 Mar 19. Erratum in: *J Nutr*. 2016 Mar;146(3):653. PubMed PMID: 24647393.
127. Jie KS, Bots ML, Vermeer C, Witteman JC, Grobbee DE. Vitamin K intake and osteocalcin levels in women with and without aortic atherosclerosis: a population-based study. *Atherosclerosis*. 1995;116(1):117–123. PubMed PMID:7488326.
128. Gast GC, de Roos NM, Sluijs I, et al. A high menaquinone intake reduces the incidence of coronary heart disease. *Nutr Metab Cardiovasc Dis*. 2009;19(7):504–510.

129. Sato Y, Nakamura R, Satoh M, et al. Thyroid hormone targets matrix Gla protein gene associated with vascular smooth muscle calcification. *Circ Res*. 2005;97(6):550–557.
130. Booth SL, Golly I, Sacheck JM, et al. Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. *Am J Clin Nutr*. 2004;80(1):143–148.
131. Braam LA, Hoeks AP, Brouns F, et al. Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. *Thromb Haemost*. 2004;91(2):373–380.
132. Wallin R, Wajih N, Greenwood GT, et al. Arterial calcification: a review of mechanisms, animal models, and the prospects for therapy. *Med Res Rev*. 2001;21(4):274–301. Review.
133. Chen Z, Qureshi AR, Parini P, et al. Does statins promote vascular calcification in chronic kidney disease? *Eur J Clin Invest*. 2017;47(2):137–148. https://doi.org/10.1111/eci.12718. PubMed PMID: 28036114.
134. Hirota Y, Nakagawa K, Sawada N, et al. Functional characterization of the vitamin K2 biosynthetic enzyme UBIAD1. *PLoS One*. 2015;10(4):e0125737. https://doi.org/10.1371/journal.pone.0125737. eCollection 2015. PubMed PMID: 25874989; PubMed Central PMCID: PMC4398444.
135. Al Rajabi A, Booth SL, Peterson JW, et al. Deuterium-labeled phylloquinone has tissue-specific conversion to menaquinone-4 among Fischer 344 male rats. *J Nutr*. 2012;142(5):841–845. https://doi.org/10.3945/jn.111.155804. Epub 2012 Mar 21. PubMed PMID: 22437559; PubMed Central PMCID: PMC3327742.
136. Booth SL, Peterson JW, Smith D, Shea MK, Chamberland J, Crivello N. Age and dietary form of vitamin K affect menaquinone-4 concentrations in male Fischer 344 rats. *J Nutr*. 2008;138(3):492–496. PubMed PMID: 18287355.
137. Harshman SG, Fu X, Karl JP, et al. Tissue concentrations of vitamin K and expression of key enzymes of vitamin K metabolism are influenced by sex and diet but not housing in C57Bl6 mice. *J Nutr*. 2016;146(8):1521–1527. https://doi.org/10.3945/jn.116.233130. Epub 2016 Jul 6. PubMed PMID: 27385762; PubMed Central PMCID: PMC4958292.
138. Al Rajabi A, Peterson J, Choi SW, Suttie J, Barakat S, Booth SL. Measurement of menadione in urine by HPLC. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;878(26):2457–2460. https://doi.org/10.1016/j.jchromb.2010.07.016. Epub 2010 Jul 29. PubMed PMID: 20719580; PubMed Central PMCID: PMC2946828.
139. Shea MK, O'Donnell CJ, Hoffmann U, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr*. 2009;89(6):1799–1807.
140. Brandenburg VM, Reinartz S, Kaesler N, et al. Slower progress of aortic valve calcification with vitamin K supplementation: results from a prospective interventional proof-of-concept study. *Circulation*. 2017;135(21):2081–2083. https://doi.org/10.1161/CIRCULATIONAHA.116.027011. PubMed PMID: 28533322.
141. Schurgers LJ, Spronk HM, Soute BA, et al. Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood*. 2007;109(7):2823–2831.
142. Ikari Y, Torii S, Shioi A, Okano T. Impact of menaquinone-4 supplementation on coronary artery calcification and arterial stiffness: an open label single arm study. *Nutr J*. 2016;15(1):53. https://doi.org/10.1186/s12937-016-0175-8. PubMed PMID: 27175730; PubMed Central PMCID: PMC4866409.
143. Knapen MH, Braam LA, Drummen NE, Bekers O, Hoeks AP, Vermeer C. Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women. A double-blind randomised clinical trial. *Thromb Haemost*. 2015;113(5):1135–1144. https://doi.org/10.1160/TH14-08-0675. Epub 2015 Feb 19. PubMed PMID: 25694037.
144. Fulton RL, McMurdo ME, Hill A, et al. Effect of vitamin K on vascular health and physical function in older people with vascular disease—a randomised controlled trial. *J Nutr Health Aging*. 2016;20(3):325–333. https://doi.org/10.1007/s12603-015-0619-4. PubMed PMID: 26892582.
145. Theuwissen E, Cranenburg EC, Knapen MH, et al. Low-dose menaquinone-7 supplementation improved extra-hepatic vitamin K status, but had no effect on thrombin generation in healthy subjects. *Br J Nutr*. 2012;108(9):1652–1657. https://doi.org/10.1017/S0007114511007185. Epub 2012 Jan 31. PubMed PMID: 22289649.
146. Schurgers LJ, Shearer MJ, Hamulyák K, et al. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. *Blood*. 2004;104(9):2682–2689.
147. Ford SK, Moll S. Vitamin K supplementation to decrease variability of International Normalized Ratio in patients on vitamin K antagonists: a literature review. *Curr Opin Hematol*. 2008;15(5):504–508. Review.
148. Li J, Lin JC, Wang H, et al. Novel role of vitamin K in preventing oxidative injury to developing oligodendrocytes and neurons. *J Neurosci*. 2003;23(13):5816–5826.
149. Verrax J, Cadrobbi J, Delvaux M, et al. The association of vitamins C and K3 kills cancer cells mainly by autoschizis, a novel form of cell death: a basis for their potential use as coadjuvants in anticancer therapy. *Eur J Med Chem*. 2003;38(5):451–457. Review.
150. Matsubara K, Kayashima T, Mori M, et al. Inhibitory effects of vitamin K3 on DNA polymerase and angiogenesis. *Int J Mol Med*. 2008;22(3):381–387.
151. Nimptsch K, Rohrmann S, Linseisen J. Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition. *Am J. Clin Nutr*. 2008;87(4):985–992.
152. Carr BI, Wang Z, Wang M, et al. Differential effects of vitamin K1 on AFP and DCP levels in patients with unresectable HCC and in HCC cell lines. *Dig Dis Sci*. 2010.
153. Mizuta T, Ozaki I, Eguchi Y, et al. The effect of menatetrenone, a vitamin K2 analog, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment: a pilot study. *Cancer*. 2006;106(4):867–872.
154. Abe Y, Muta K, Hirase N, et al. Vitamin K2 therapy for myelodysplastic syndrome. *Rinsho Ketsueki*. 2002;43(2):117–121.
155. Nimptsch K, Rohrmann S, Nieters A, et al. Serum undercarboxylated osteocalcin as biomarker of vitamin K intake and risk of prostate cancer: a nested case-control study in the Heidelberg cohort of the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev*. 2009;18(1):49–56.
156. Dahlbäck B. Blood coagulation. *Lancet*. 2000;355(9215):1627–1632. Review.
157. Autret-Leca E, Jonville-Béra AP. Vitamin K in neonates: how to administer, when and to whom. *Paediatr Drugs*. 2001;3(1):1–8.
158. Yagami K, Suh JY, Enomoto-Iwamoto M, et al. Matrix GLA protein is a developmental regulator of chondrocyte mineralization and, when constitutively expressed, blocks endochondral and intramembranous ossification in the limb. *J Cell Biol*. 1999;147(5):1097–1108.
159. Hyc A, Osiecka-Iwan A, Jóźwiak J, et al. The morphology and selected biological properties of articular cartilage. *Ortop Traumatol Rehabil*. 2001;3(2):151–162.
160. Beulens JW, van der ADL, Grobbee DE, et al. Dietary phylloquinone and menaquinones intakes and risk of type 2 diabetes. *Diabetes Care*. 2010;33(8):1699–1705.
161. Chen J, Liu J, Zhang Y, et al. Decreased renal vitamin K-dependent gamma-glutamyl carboxylase activity in calcium oxalate calculi patients. *Chin Med J (Engl)*. 2003;116(4):569–572.
162. Kolesova GM, Sokol'nikov AA, Kodentsova VM, et al. The effect of vitamin K deficiency in rats on various enzyme systems participating in energy metabolism. *Vopr Med Khim*. 1988;34(4):46–51. Russian.
163. van Hoorn JH, Hendriks JJ, Vermeer C, et al. Vitamin K supplementation in cystic fibrosis. *Arch Dis Child*. 2003;88(11):974–975.
164. Shirakawa H, Ohsaki Y, Minegishi Y, et al. Vitamin K deficiency reduces testosterone production in the testis through down-regulation of the Cyp11a a cholesterol side chain cleavage enzyme in rats. *Biochim Biophys Acta*. 2006;1760(10):1482–1488.
165. Cohen JL, Bhatia AC. The role of topical vitamin K oxide gel in the resolution of postprocedural purpura. *J Drugs Dermatol*. 2009;8(11):1020–1024.
166. Traber MG. Vitamin E and K interactionsa 50-year-old problem. *Nutr Rev*. 2008;66(11):624–629. Review.
167. Traber MG, Frei B, Beckman JS. Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr Opin Lipidol*. 2008;19(1):30–38. Review.

168. Miller 3rd ER, Pastor-Barriuso R, Dalal D, et al. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142(1):37–46.
169. Pearson DA. Bone health and osteoporosis: the role of vitamin K and potential antagonism by anticoagulants. *Nutr Clin Pract*. 2007;22(5):517–544. Review.
170. Krishnan S, Chawla N, Ezekowitz MD, et al. Warfarin therapy and systolic hypertension in men with atrial fibrillation. *Am J Hypertens*. 2005;18(12 Pt 1):1592–1599.
171. Wu KK, Matijevic-Aleksic N. Molecular aspects of thrombosis and antithrombotic drugs. *Crit Rev Clin Lab Sci*. 2005;42(3):249.
172. Di Micco B, Colonna G, Di Micco P, et al. Anti-thrombin action of low-dose acetylsalicylic acid. *Eur J Pharmacol*. 2003;460(1):59–62.
173. Westphal JF, Vetter D, Brogard JM. Hepatic side-effects of antibiotics. *J Antimicrob Chemother*. 1994;33(3):387–401.
174. Onodera K, Takahashi A, Wakabayashi H, et al. Effects of menatetrenone on the bone and serum levels of vitamin K2 (menaquinone derivatives) in osteopenia induced by phenytoin in growing rats. *Nutrition*. 2003;19(5):446–450. PubMed PMID: 12714099.
175. Schurgers LJ, Teunissen KJ, Hamulyák K, et al. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood*. 2007;109(8):3279–3283.
176. Szulc P, Chapuy MC, Meunier PJ, et al. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone*. 1996;18(5):487–488.
177. O'Connor E, Mølgaard C, Michaelsen KF, et al. Serum percentage undercarboxylated osteocalcin, a sensitive measure of vitamin K status, and its relationship to bone health indices in Danish girls. *Br J Nutr*. 2007;97(4):661–666.
178. Bach AU, Anderson SA, Foley AL, et al. Assessment of vitamin K status in human subjects administered "minidose" warfarin. *Am J Clin Nutr*. 1996;64(6):894–902.
179. Yonemura K, Fukasawa H, Fujigaki Y, et al. Protective effect of vitamins K2 and D3 on prednisolone-induced loss of bone mineral density in the lumbar spine. *Am J Kidney Dis*. 2004;43(1):53–60.
180. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press: Washington, DC.
181. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. *J Nutr*. 1998;128(5):785–788. Review.
182. Ronden JE, Groenen-van Dooren MM, Hornstra G, et al. Modulation of arterial thrombosis tendency in rats by vitamin K and its side chains. *Atherosclerosis*. 1997;132(1):61–67.
183. Mawatari T, Miura H, Higaki H, et al. Effect of vitamin K2 on three-dimensional trabecular microarchitecture in ovariectomized rats. *J Bone Miner Res*. 2000;15(9):1810–1817. PubMed PMID: 10977000.
184. Koitaya N, Ezaki J, Nishimuta M, et al. Effect of low dose vitamin K2 (MK-4) supplementation on bio-indices in postmenopausal Japanese women. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(1):15–21.
185. Kanellakis S, Moschonis G, Tenta R, et al. Changes in parameters of bone metabolism in postmenopausal women following a 12-month intervention period using dairy products enriched with calcium, vitamin D, and phylloquinone (vitamin K(1)) or menaquinone-7 (vitamin K (2)): the Postmenopausal Health Study II. *Calcif Tissue Int*. 2012;90(4):251–262. https://doi.org/10.1007/s00223-012-9571-z. Epub 2012 Mar 4. PubMed PMID: 22392526.
186. Koitaya N, Sekiguchi M, Tousen Y, et al. Low-dose (1.5mg) vitamin K2 (MK-4) supplementation for 12 months improves bone metabolism and prevents forearm bone loss in postmenopausal Japanese women. *J Bone Miner Metab*. 2014;32(2):142–150. https://doi.org/10.1007/s00774-013-0472-7. Epub 2013 May 24. PubMed PMID: 23702931.
187. Iwamoto J. Efficacy of combined administration of vitamin D3 and vitamin K2 for primary osteoporosis. *Clin Calcium*. 2002;12(7):955–965. Japanese.
188. Shea MK, O'Donnell CJ, Vermeer C, et al. Circulating uncarboxylated matrix gla protein is associated with vitamin K nutritional status, but not coronary artery calcium, in older adults. *J Nutr*. 2011;141(8):1529–1534. https://doi.org/10.3945/jn.111.139634. Epub 2011 May 31. PubMed PMID: 21628633; PubMed Central PMCID: PMC3138643.
189. Dalmeijer GW, van der Schouw YT, Magdeleyns E, Ahmed N, Vermeer C, Beulens JW. The effect of menaquinone-7 supplementation on circulating species of matrix Gla protein. *Atherosclerosis*. 2012;225(2):397–402. https://doi.org/10.1016/j.atherosclerosis.2012.09.019. Epub 2012 Sep 25. PubMed PMID: 23062766
190. Zwakenberg SR, de Jong PA, Bartstra JW, et al. The effect of menaquinone-7 supplementation on vascular calcification in patients with diabetes: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr*. 2019;110(4):883–890. https://doi.org/10.1093/ajcn/nqz147.

Vitamin Toxicities and Therapeutic Monitoring

Tennille Marx, ND, CFS, and *Joseph E. Pizzorno*, ND*

OUTLINE

INTRODUCTION

Vitamin deficiencies are a much greater problem worldwide than vitamin toxicities. However, when nutrients are being used at high doses for pharmacological effects, physicians must be vigilant in identifying any possible toxicity or adverse effects. In general, vitamin therapy is virtually nontoxic and careful monitoring of the patient can further reduce the small risk of developing any toxicity. Physicians should also be aware of toxicity resulting from self-administered vitamins. At each patient visit, it is important for physicians to inquire and record/update the use of any self-administered vitamins. Specific forms of each vitamin should also be noted, as new innovations have led to preparations that can increase the bioavailability of certain vitamins. For example, micellar applications can enhance the water-solubility of fat-soluble vitamins. The primary signs and symptoms of vitamin toxicity are listed in Tables 127.1 and 127.2, which are complemented by a more detailed discussion of toxicity and guidelines for monitoring select vitamins.

LIPID-SOLUBLE VITAMIN TOXICITY

Vitamin A

Clinical and subclinical toxicities have been associated with excessive intakes of preformed vitamin A. Many cases of hypervitaminosis A involve ingestion of a large quantity at one time by young children who, along with the elderly, are more susceptible to toxicity.[1,2] Acute toxicity is thought to occur when, within a short period of time, adults ingest more than 100 times the recommended daily allowance (RDA) and children ingest more than 20 times the RDA. However, in addition to acute toxicity, chronic intakes of high-dose vitamin A have also been associated with harm.[3]

Adverse reactions to acute toxicity in children can occur with intakes as low as 1500 international units per kilogram daily (IU/kg/day),[4] and they are usually transient. Symptoms of acute hypervitaminosis A in children given 100,000 to 300,000 IU include diarrhea, headache (possibly resulting from elevated intracranial pressure), nausea, vomiting, occasional dizziness, and fever as well as a transient bulging of the fontanelle in infants. In adults, symptoms of toxicity may also include blurred vision and lack of muscular coordination.[5] Chronic vitamin A excesses can precipitate alopecia, arthralgias, anemia, erythema, skin peeling, cheilitis, thickened epithelium, hypertriglyceridemia, hypercholesterolemia and fatty liver as well as heart, kidney, and testicular defects. Other, less commonly reported symptoms include dysphagia caused by vertebral hyperostosis, intrahepatic cholestasis, hepatotoxicity, pseudotumor cerebri syndrome, and renal dysfunction.[6–8] Interestingly, in a small number of case reports of dysphagia, none of the patients reported vitamin A supplementation despite high serum retinol levels, suggesting an impairment of vitamin A metabolism rather than excessive intake.[9] Usually, most of the untoward effects of excess vitamin A intake are resolved with cessation of its use.

Teratogenicity is the most severe and worrisome adverse effect of vitamin A. Fetal effects can include craniofacial, cardiac, thymic, and central nervous system abnormalities.[7] According to one large observational study published in the *New England Journal of Medicine,* women consuming greater than 10,000 IU of vitamin A during pregnancy (specifically during the first 7 weeks after conception) had a 1 in 57 risk for having a child born with a birth defect.[10] Therefore, supplementation above the RDA is not warranted in pregnant or potentially pregnant women. However, vitamin A levels during pregnancy must be carefully assessed because both deficiency and excess can bring about undesirable results. Also to be considered is that this research is in the context of very common vitamin D deficiency. Theoretical considerations suggest that, except at very high dosages, this teratogenicity may be more a result of an imbalance between vitamins A and D.

Another well-recognized result of chronic excessive vitamin A intake is an adverse effect on bone, including bone spurs, calcinosis, and bone resorption resulting in hypercalcemia.[7] Several prospective studies have also indicated that excessive vitamin A can have a lasting detrimental effect on bone by reducing bone mineral density (BMD) and increasing risk of fracture.[11,12] However, the data are mixed and may be confounded by other variables, including vitamins D and K_2 status. After over a decade of research, the association between vitamin A and risk of fracture is still controversial.

*Previous edition contributor

TABLE 127.1 Toxic Dosages and Side Effects of Lipid-Soluble Vitamins

Vitamin	Toxic Dosage	Toxic Signs and Symptoms
Carotenoids	Long-term: none	No apparent toxicity, even at large doses (250 mg/day); synthetic form poses a risk for heavy smokers or asbestos-exposed persons not taking other antioxidants
Vitamin A	Short-term	
	Infants: 75,000–300,000 IU	Anorexia, bulging fontanelle, hyperirritability, vomiting
	Adults: 2–5 million IU	Headache, drowsiness, nausea, vomiting.
	Long-term	
	Infants: 18,000–60,000/day	Premature epiphyseal bone closing, long bone growth retardation
	Adults: 100,000 IU/day	Anorexia, headache, blurred vision, loss of hair, bleeding lips, cracking and peeling skin, muscular stiffness and pain, severe hepatic damage and enlargement, anemia, teratogenesis (craniofacial, cardiac, thymic and central nervous system (CNS) abnormalities)
Vitamin D	Short-term: 1000–3000 IU/kg	Anorexia, nausea, vomiting, diarrhea, headache, polyuria, polydipsia
	Long-term: >40,000 IU/day	Hypercalcemia (unlikely)
Vitamin E	Long-term: >800 IU/day	Severe weakness, fatigue, exacerbation of hypertension, potentiation of anticoagulants. α-tocopherol used alone may increase disease risk
Vitamin K	Long-term: none	Phylloquinone (K_1), unlike menadione (K_3), is not associated with side effects when given orally. Caution is in order with anticoagulant medications

TABLE 127.2 Toxic Dosages and Side Effects of Water-Soluble Vitamins

Vitamin	Toxic Dosage	Toxic Signs and Symptoms
Ascorbic acid	Short-term: usually >10 g	Nausea, diarrhea, flatulence
	Long-term: >3 g/day	Increased urinary oxalate and uric acid levels in rare cases, impaired carotene utilization, chelation and resultant loss of minerals
Biotin	Long-term: >10 mg/day	No reported side effects from oral administration at therapeutic doses
Folic acid	Long-term: 15 mg/day	Abdominal distention, anorexia, nausea, sleep disturbances. May pose increased cancer risk (see text)
Niacin	Short-term: >100 mg	Transient flushing, headache, cramps, nausea, vomiting
	Long-term: 3–7 g/day	Anorexia, abnormal glucose tolerance, increased plasma uric acid levels, gastric ulceration, liver enzyme elevations. In severe cases, elevation of serum bilirubin and ammonia and prolonged prothrombin time
Niacinamide	Long-term: >2000 mg/day	Same as for niacin
Pantothenic acid	Long-term	Occasional diarrhea
Pyridoxine	Short-term	No acute effects have been noted at therapeutic dose
	Long-term: 300 mg/day	Sensory and motor neuropathies
Riboflavin	Long-term	No toxic effects have been noted
Thiamine	Long-term	No toxic effects noted for humans after oral administration
Vitamin B_{12}	Long-term	No side effects from oral administration have been reported

One 9.5-year prospective cohort study involving almost 35,000 postmenopausal women with hip and other fractures found little evidence of an increased risk of fracture with higher intakes of vitamin A or retinol. There was also no evidence of a dose-response relationship in hip fracture risk with increasing amounts of vitamin A or retinol from supplements. Furthermore, the results showed no association between vitamin A ingestion from food and supplements or food only and the risk of fractures of any kind.[13] Similar results were seen in a 2013 intervention study, where no association was found between long-term high retinol intake (25,000 IU retinyl palmitate/day) and risk for any fracture.[14] In another large observational study that included more than 75,000 participants from the Women's Health Initiative, retinol and vitamin A intake were not significantly associated with either hip or total fracture incidence among postmenopausal women. However, women in the highest quintile of retinol and vitamin A intake who also had a low intake of vitamin D did have a modestly (15%–20%) increased total fracture risk.[15] A smaller study that enrolled Spanish postmenopausal women did find an independent risk for osteoporosis among those with the highest vitamin A intake, but this risk was magnified when combined with low vitamin D levels.[16] More recently, an analysis of data from 6481 subjects from the Korea National Health and Nutrition Examination survey revealed that total hip and femoral BMD were positively correlated with dietary vitamin A intake in subjects with serum 25-OH vitamin D levels >75 nmol/L.[17] However, in men with serum 25-OH vitamin D levels <50 nmol/L, both top and bottom tertiles of vitamin A intake were associated with lower BMD. A similar U-shaped relationship between blood retinol levels and hip fractures was noted in 2014 and 2017 meta-analyses, in which both low and high levels of vitamin A had a negative effect on bone.[18,19] The interaction between high vitamin A and low vitamin D levels appears biologically plausible, as vitamin A may antagonize some of vitamin D's actions, including calcium absorption.[20] This may be relevant not only to bone health but possibly to susceptibility to respiratory infection as well.[21]

Interestingly, a 2015 Dutch prospective study in elderly men and women (the Rotterdam study) indicated that dietary vitamin A intake was associated with a higher BMD and lower fracture risk and vitamin D intake or status did not appear to modify these effects.[22] However, the favorable association between vitamin A and BMD disappeared after adjustment for body mass index (BMI). Additionally, when stratified for BMI categories, the positive association between vitamin A intake and fracture risk was only present in overweight subjects and not normal weight subjects.

A 2007 review of the bone effects of vitamin A concluded that the poor sensitivity of laboratory markers and assessment of dietary intake may contribute to the conflicting findings; it suggested that future studies incorporate superior analytic techniques, specifically stable-isotope-dilution methodology.[23] Although serum retinol is often employed to screen for vitamin A toxicity, it is thought to have poor sensitivity because it is subject to homeostatic control over a wide range of intakes as well as hepatic concentrations, and thus does not necessarily represent liver stores.[24] Additionally, many clinical factors interfere with its accuracy. One alternative is the measurement of fasting retinyl ester concentrations. When more than 5% to 10% of circulating vitamin A is in the form of retinyl esters, it may indicate either hepatic storage capacity or the capacity of the retinol-binding protein has been exceeded. Unfortunately, elevated retinyl esters do not necessarily indicate impaired liver function and are not sensitive to subclinical toxicity.[22,25] Although expensive and not widely available, stable isotope dilution techniques appear to correlate well with values determined by liver biopsy and may emerge as the best marker of total vitamin A stores in both deficiency and toxicity. Indeed, variations of this method may be used to determine the intake needed to maintain target body storage levels. In animal models they have shown 100% sensitivity for the diagnosis of hypervitaminosis A.[26,27] Although the deuterated retinol dilution method has been validated in both children and adults to give a quantitative estimate of internal stores, it needs further verification among diverse populations and greater accessibility.[28]

When large doses of vitamin A are being given, careful monitoring is necessary. Rather than sudden ingestion of large doses, a gradual stepwise increase in dosage is indicated, with an evaluation of symptoms made before the dosage is increased. Usually, the first symptom of hypervitaminosis to be recognized is frontal headache. If signs or symptoms appear, supplementation should be discontinued until they disappear. Levels of liver enzymes should be determined periodically to check for hepatic damage. Typically, levels of aspartate transaminase are the first to be affected.[1,2,29,30] Patients whose liver function is compromised by viral hepatitis, protein-energy malnutrition, cirrhosis, or hemodialysis seem to be the most vulnerable to vitamin A toxicity and to require close monitoring.[4]

Finally, as a result of the conflicting results in studies on vitamin A and bone health, anyone consuming large doses of vitamin A longterm should ensure adequate vitamins D and K_2 levels and be monitored for any bone density changes.

Carotenoids

Carotenoids appear to be without toxic effects at the therapeutic doses customarily used. The only effect of large dosages is an apparently benign yellowing of the skin. While carotenoid toxicity is limited, there is concern that some individuals have difficulty converting carotenoids to vitamin A and may be more prone to vitamin A deficiency.[31–33]

Several large, widely publicized therapeutic trials with synthetic beta-carotene have found that it appears to raise the risk of lung cancer in heavy smokers. It may also pose an increased risk for gastric cancer, particularly among smokers and those exposed to asbestos.[34] However, several factors complicate the interpretation of these results. The significance of these trials is fully discussed in Chapter 57.

Vitamin D

Significant advances have been made in understanding the role and importance of vitamin D in human health. The recent implications of vitamin D deficiency in a number of chronic diseases has led to an eightyfold increase in physician requests for serum vitamin D concentration measurements between the years 2000 and 2010.[35] Deficiency is now known to be widespread, with suboptimal levels much more prevalent than toxicity. The use of 25-OH vitamin D is widely accepted as a reliable biomarker, with most indicators suggesting that a level of 75 to 110 nmol/L (or 30–44 ng/mL) is sufficient, although some studies indicate that even higher levels may be optimal.[36,37] Unfortunately, the majority of US adolescents and adults do not sustain vitamin D levels within this range. A 2009 study indicated that only 23% of the population had serum 25-OH vitamin D levels higher than 75 nmol/L.[38] Therefore the evaluation of vitamin D levels should be part of a normal annual preventative checkup. Those in northern latitudes with less sun exposure, as well as the elderly whom have a lower the capacity for vitamin D production, warrant special attention.

The upper limits of 25-OH vitamin D are not clearly established, although levels less than 250 nmol/L are considered safe.[39] Therapeutic strategies should target 25-OH vitamin D levels rather than a specific supplemental dose, as the effect of supplementation on serum levels varies considerably between individuals. Doses between 2000 IU and 4000 IU will bring the majority of individuals within the range of 75 to 110 nmol/L and are without adverse effects.[35,40,41] Nevertheless, some will require higher dosing, and this is also a consideration for individuals with less functional vitamin D receptor polymorphisms. The Endocrine Society, as well as others, suggest levels up to and including 10,000 IU vitamin D (a dose equivalent to full-body sunlight exposure) will maximize physiological benefits and are safe.[42–44]

According to researchers, there are three major hypotheses for vitamin D toxicity: (1) raised plasma 1,25-OH vitamin D concentrations lead to increased intracellular 1,25-OH vitamin D concentrations (not a popular hypothesis as a result of levels of 1,25(OH)D being normal or only marginally elevated in vitamin D toxicity); (2) vitamin D intake raises plasma 25-OH vitamin D levels to concentrations that exceed DBP binding capacity, and "free" 25-OH vitamin D has direct effects on gene expression once it enters target cells; (3) vitamin D intake raises the concentrations of many vitamin D metabolites and these concentrations exceed the DBP binding capacity and release free 1,25-OH vitamin D, which enters target cells.[45,46]

Vitamin D intoxication manifests primarily as hypercalcemia and hypercalciuria as a result of vitamin D's ability to increase calcium absorption in the gastrointestinal tract. Symptoms can include gastrointestinal (nausea, vomiting, constipation), neurological (confusion, lethargy, fatigue), musculoskeletal (muscle weakness and bone pain), cardiovascular (cardiac arrhythmias), and nephrotic (polyuria, polydipsia, and renal calculi) effects. In extreme cases, renal failure can occur.[47] Testing for vitamin D toxicity should include 25-OH vitamin D levels as well as serum calcium levels. It has also been suggested that hypercalciuria may be a more sensitive indicator of vitamin D toxicity than hypercalcemia.[42] A recent randomized, double-blind clinical trial indicated that hypercalciuria and hypercalcemia "episodes" occurred more frequently when participants were consuming 10,000 to 12,000 IU/day of vitamin D_3 compared with 600 to 800 IU/day when coadministered with 2000 mg of calcium. However, these incidences were not associated with kidney stone formation or other adverse effects.[48]

As a result of its wide therapeutic index, vitamin D intoxication is rare and is usually caused by manufacturing errors or incorrect/inappropriate administration.[49,50] In a review of case reports of vitamin D

toxicity spanning over 8 decades, only 13 laboratory-confirmed reports of vitamin D toxicity were found.[46] Of these 13 case studies, doses resulting in toxicity in children under the age of 5 were between 20,000 to 800,000 IU/day. Doses resulting in toxicity in adults ranged from 50,000 to 2,604,000 IU/day. The most common symptoms of toxicity were loss of appetite, vomiting, diarrhea, and altered sensorium. In these cases, serum 25-OH vitamin D concentrations were greater than 150 ng/mL and total calcium levels ranged from 11.1 to 23.08 mg/dL. Another report on data from calls to US poison centers confirmed that serious medical outcomes from vitamin D ingestion were rare (0.02%) and the most common clinical effects were gastrointestinal and mild neurological effects. They did note, however, that there was a 1600% increase in reported exposures in 2005 to 2011 compared with 2000 to 2005.[49]

Doses as high as 40,000 IU per day have not been associated with toxicity.[36] However, very high single doses (500,000 IU in a single annual dose) have been associated with an increased risk for fracture and falls in a temporal pattern, with the highest risk in the period after administration.[51] A 2018 study investigating the effect of monthly doses of 100,000 IU of vitamin D_3 over 3 to 4 years on cardiovascular disease, respiratory infections, and fall and fractures did not find any increased incidences of adverse effects with vitamin D supplementation compared with placebo.[52] However, there was a tendency to report more dizziness in the vitamin D group. Thus lower doses given more frequently (i.e., more physiologically) are preferred. Despite ongoing controversy, vitamin D_3 appears to be more potent and to produce greater storage than D_2.[53]

Oftentimes vitamin D hypersensitivity is mistaken for vitamin D toxicity. For example, granulomatous diseases, such as sarcoidosis, warrant special concern because these individuals are more susceptible to hypercalcemia. Although many have low levels of 25-OH vitamin D (which appears to increase the risk for sarcoidosis), they also have elevated levels of 1,25 dihydroxyvitamin D and thus require careful management.[54]Apparently there is overconversion of 25-OH vitamin D_3 to 1,25(OH)2-vitamin D_3 by macrophages in granulomatous disease.[55] Primary hyperparathyroidism is another example. In this case production of 1,25-OH vitamin D is upregulated by the high parathyroid hormone concentrations.[43] Patients with *Mycobacterium* infections and those treated with thiazide diuretics should also be closely monitored if supplementing with vitamin D because of the potential of these populations being vitamin D sensitive.[42]

Vitamin E

Although for many years observational studies found vitamin E supplementation to be safe, several controlled trials have been published suggesting harm with supplementation. For example, in a large meta-analysis of randomized placebo-controlled trials in which participants were given between 50 and 800 IU natural or synthetic vitamin E per day, supplementation was found to reduce the risk of ischemic stroke by 10% but to increase the risk of hemorrhagic stroke by 22%.[56] Similarly, a meta-analysis published in the *Annals of Internal Medicine* found that supplementation with more than 400 IU vitamin E increased all-cause mortality.[57] A 2012 Cochrane review also concluded that vitamin E seemed to increase all-cause mortality[58]; however, this analysis has been criticized by others for excluding hundreds of studies that had zero mortality.[59] In addition, most vitamin E research is performed with synthetic vitamin E, which is a DL racemic mixture and/or only alpha-tocopherol.

Although the use of synthetic versus natural vitamin E may explain some of the increase in adverse effects, the natural but incomplete form of vitamin E (d-alpha tocopherol) used in many clinical trials is not without risk. For example, supplementation with natural vitamin E at 400 IU per day was associated with an increased risk of heart failure among patients with diabetes or vascular disease.[60] The vitamin E family includes eight isomers. As discussed next, high dosages of a single isomer competes with the absorption of the others.

An explanation that appears more plausible is that despite the physiological benefits of alpha-tocopherol, high-dose supplementation depletes other forms of naturally occurring vitamin E, such as beta- or gamma-tocopherol, or tocotrienols, which have greater physiological significance. For example, in an observational study of elderly patients, higher plasma levels of beta-tocopherol were associated with a reduced risk of developing Alzheimer disease, whereas other forms of vitamin E were only marginally significant.[61] The use of both gamma- and alpha-tocopherol in patients with the metabolic syndrome was shown to be superior to either used alone. Moreover, in vitro and in vivo evidence indicates that alpha-tocopherol not only failed to demonstrate anticancer properties but also blocked the anticancer effects of gamma-tocopherol.[62,63] Gamma-tocopherol is actually more prevalent than the alpha form in the US diet as well as in many plant seeds, although the vast majority of trials and available products use alpha-tocopherol.[64] Tocotrienols have been shown to have superior antioxidant capabilities than alpha-tocopherol.[65,66] Thus it may not be "vitamin E" that has the harmful effects mentioned previously, but rather the isolated use of alpha-tocopherol. As such, researchers have called for manuscripts to be titled with the specific form of the vitamin rather than the umbrella term "vitamin E."[67] Additional factors are likely to have an influence as well, such as age and vitamin C intake.[68] Genetics are also likely to play a role, as diabetic patients with the haptoglobin 2-2 genotype are more likely to receive benefit from supplementation.[69]

Unfortunately, most laboratory evaluations of vitamin E's toxicity are based on alpha-tocopherol plasma or serum levels and thus may not be helpful in determining toxicity. The tolerable upper intake level (UL) established by the Institute of Medicine for vitamin E, which refers to all stereoisomers, is based on the potential risk of bleeding. However, it has been suggested that vitamin E only affects coagulation if vitamin K deficiency is also present.[70,71] Conflicting evidence and a lack of long-term studies adequately analyzing vitamin E's toxicity leaves much to be known about vitamin E and its true safety profile.

Vitamin K

Synthetic water-soluble vitamin K_3, menadione, is thought to interfere with the antioxidant glutathione, resulting in oxidative damage to cell membranes.[72] Large doses of menadione administered to infants may cause liver damage and destruction of red blood cells resulting in hemolytic anemia, hyperbilirubinemia, hepatomegaly, and possibly death. Adults with glucose-6-phosphate dehydrogenase deficiency may show hemolytic reactions.[73] This form vitamin K is no longer used in the United States due to these toxic effects.

The natural vitamin K_1 (phylloquinone) and the menaquinones (MK-4, MK-7) do not appear to cause toxicity when given orally unless huge doses (e.g., 200 mg) are given.[74] In fact, no tolerable upper intake level for vitamin K has been established as a result of the lack of adverse effect data. Dr. Bruce Ames, professor of biochemistry and molecular biology, has provided compelling evidence for the triage theory; that given a suboptimal intake, vitamin K is shunted to basic functions necessary for survival at the expense of less essential functions, which are likely to be associated with aging and chronic disease. Given that many individuals have suboptimal vitamin K intake, supplementation with menaquinones has the potential to reduce the incidence of chronic disease.[75]

All forms of vitamin K can interfere with some anticoagulant medications, such as warfarin (Coumadin), and should be used with

caution. Given that the menaquinones may have greater potency than vitamin K_1, an upper limit of 50 mcg/day (MK-7) has been proposed for those patients on anticoagulant therapy.[76] However, the longer half-life of MK-7 (compared with other forms of vitamin K) may help maintain a more stable international normalized ratio while also protecting these patients from the arterial calcification and osteoporosis for which they are at increased risk.[77]

Water-Soluble Vitamin Toxicity

Ascorbic Acid (Vitamin C)

Vitamin C has been reported to have perhaps the lowest toxicity of all vitamins. Linus Pauling and colleagues began using vitamin C in therapeutic doses—10 g/day intravenously (IV) and orally—in the 1970s and 1980s for cancer patients without toxic effects. Almost a half a decade later, IV vitamin C has been used in Phase I and Phase I/II trials and is widely used in integrative settings both alone and in combination with chemotherapy. A 2014 review of IV vitamin therapy studies that utilized 1 g to more than 200 g ascorbic acid per infusion 2 to 3 times per week confirmed vitamin C's modest, but inconsistent, therapeutic benefit in cancer patients as well as its excellent safety profile.[78] Although one study found IV vitamin C associated with a small increase in urinary oxalate excretion, no related adverse events were noted. The most common adverse events reported were nausea, headache, diarrhea, and flushing. There were a few cases of hypernatremia and hypokalemia and one incident of kidney stone in a patient with a previous history of kidney stones. In a similar 2018 review of 9328 patients using IV vitamin C therapy, the adverse event rate was only 1% and included mild events such as nausea, dizziness, dry mouth, perspiration, and weakness.[79]

Diarrhea, intestinal distention, and gas are the most common complaints when vitamin C is consumed orally at higher dosages. These effects are rare at doses of 2 g/day or less. It has been shown that high doses of vitamin C can:

- Increase the urinary excretion of calcium, iron, and manganese
- Increase the absorption of iron
- Raise urinary oxalate or uric acid levels, but only in a small subgroup of the population (This may vary with the form of vitamin C, as ester-C has been shown to reduce oxalate levels in a crossover study.[80])
- Alter many routine laboratory parameters (e.g., serum vitamin B_{12}, aminotransferases, bilirubin, glucose, stool occult blood)

Clinicians must take these effects into consideration when supplementing with megadoses of vitamin C. Also, the ability of oral vitamin C to increase plasma levels is limited, with IV administration of vitamin C having been shown to raise levels seventyfold higher.[81] Even at the high plasma levels documented with IV therapy, toxicity does not appear to be a significant concern.

One concern with high dosages of vitamin C often cited in the medical literature is the development of calcium oxalate kidney stones. As mentioned previously, various forms of ascorbic acid may negate this effect, as demonstrated with ester-C versus ascorbic acid. Additionally, in vitro oxidation of ascorbic acid to oxalic acid during storage or analysis is thought to be a common confounder in these studies. Nonetheless, 500 mg/day may be a reasonable limit for those prone to stone formation.[82] With regard to IV administration of vitamin C, less than 0.5% of a 100-g dose was converted to oxalic acid in individuals with normal renal function, much less than might be expected from such a large dose.[83]

A second, more theoretical concern about vitamin C relates to its excess use in progressive inflammatory diseases such as rheumatoid arthritis and Crohn's disease. It is theorized that available surplus vitamin C may interact with metal ions, eliciting a prooxidant consequence.[84] Alternatively, more recent data suggest that vitamin C may chelate metal ions, actually reducing their ability to generate reactive oxygen species, and animal models suggest that megadosing may reduce inflammation.[85,86] Although no clinical information is available to clarify this concern, it may be prudent to consider limiting the megadosing of vitamin C in patients with unresponsive or worsening chronic inflammatory conditions and to be cautious about giving large doses of vitamin C to patients with known conditions of iron or copper excess. This may also be relevant to the dietary intake of iron. For example, high dietary heme intake among women taking more than 500 mg vitamin C per day was found to increase the risk of lung cancer, whereas high zinc intake reduced the risk.[87]

There have been reports that abrupt cessation of high-dose vitamin C intake leads to rebound scurvy, which has also been reported to occur after birth in the babies of pregnant women who have been taking high doses. However, other studies do not support the existence of rebound scurvy under these conditions. Although some experts question the existence of rebound scurvy, it is better to err on the side of caution. At this time a safe recommendation to pregnant women would be a daily dosage of 500 mg vitamin C.

Folic Acid (Vitamin B_9)

Although generally considered safe, caution should be exercised in supplementing with folic acid in the presence of a vitamin B_{12} deficiency. Although folic acid will correct a macrocytosis, it will not correct the underlying neurological degeneration caused by vitamin B_{12} deficiency. Additionally, high folic acid levels appear to accentuate the toxicity of low vitamin B_{12}.[88,89]

High-dose folic acid (15 mg/day) has been used without adverse effects in several studies. For example, when given as 5-methyltetrahydrofolate to postmenopausal women, it significantly reduced their blood pressure and homocysteine levels and improved their insulin sensitivity.[90] Folic acid given at 5 mg/day with vitamins B_{12} and B_6 was found to reduce subclinical atherosclerosis among individuals with a fasting homocysteine level greater than 9.0 μmol/L when given over 3 years. This was a randomized placebo-controlled trial with more than 500 participants, and no difference in adverse effects between groups was cited.[91] A meta-analysis also found that folic acid supplementation reduced the risk of stroke in primary prevention, especially when continued for a longer period of time.[92]

A very important placebo-controlled randomized trial, published in 2008, found that folic acid given at 5 mg/day over 3 years reduced the recurrence of colorectal adenomas; the recurrence rate in the placebo group was twice as high as in the folic acid group. Additionally, none of the patients who received folic acid were found to have histologically aggressive adenomas or carcinoma at their final endoscopy.[93] This study is particularly relevant because concerns have been raised that both low and high folate levels may increase the risk of cancer.[94,95] For example, in two previous trials, folic acid given at 1 mg/day was associated with more advanced colorectal lesions as well as an increased risk of prostate cancer.[96,97] It is possible that the high folic acid content of fortified foods can be a confounding variable when a low dose of 1 mg/day is used. Additionally, there is most likely a difference between synthetic folic acid and the more natural reduced and methylated forms, primarily 5-methyl tetrahydrofolate.

In 2015 the US National Toxicology Program (NTP) partnered with the NIH Office of Dietary Supplements and convened an expert panel to evaluate the current state of science surrounding the safe use of folic acid at levels above the RDA. They prioritized four general health categories that had areas of uncertainty when considering the safe use of folic acid: cancer, cognition in conjunction with vitamin B_{12} deficiency, hypersensitivity-related outcomes, and thyroid and

diabetes-related disorders. The panel did not identify conclusive evidence of adverse effects of folic acid; however, they did conclude that more research could be done on all of the topics. With respect to folic acid and cancer, the expert panel indicated there was a "consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental folic acid to justify further research."[98]

Until a more definitive conclusion exists on folic acid supplementation and the risk of cancer, it seems prudent to limit folic acid supplementation to the RDA unless the benefits outweigh the risks. It is important to point out that folate from food is not a concern.

Finally, a serum folate level greater than 45.3 nmol/L is often used to define elevated levels, but it is arbitrarily chosen because of technical difficulties in analysis rather than a functional toxicity.[99]

Niacin (Vitamin B_3)

The acute side effects of niacin (nicotinic acid) are well known. The most common and bothersome is the skin flushing that typically occurs 20 to 30 minutes after the niacin is taken. A gradual increase in daily dose and/or administration of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) 30 minutes to 1 hour before taking niacin can help reduce the flushing effect of niacin.[100,101] Long-term consequences of niacin therapy include gastric irritation, nausea, jaundice, and liver damage. Other rare complications include cardiovascular (palpitations, tachycardia) and ocular (blurred vision, cystoid macular edema) effects.[100]

In an attempt to combat the acute reaction of skin flushing, several manufacturers began marketing "sustained-release," "timed-release," and "slow-release" niacin products. These formulations allow the niacin to be absorbed gradually, thereby reducing the flushing reaction. However, although these forms of niacin reduce skin flushing, they have actually proved to be more toxic to the liver, particularly the slow-release products. One study strongly recommended that the use of slow-release niacin be restricted because of the high percentage (78%) of patient withdrawals from the study because of side effects; 52% of the patients taking the sustained-release niacin had liver damage compared with none of the patients taking immediate-release niacin.[102] "Extended-release (ER)" niacin (Niaspan) appears to have similar toxicity as immediate-release niacin, with a 2008 review documenting that significant increases in liver enzymes with either extended- or immediate-release niacin are rare and that elevations leading to severe hepatotoxicity occur rarely, if at all.[103] A 2014 review of the safety profile of Niaspan in the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial indicated that there were no significant differences in number of serious adverse events between the extended-release niacin and placebo and the rate of serious hemorrhagic events were low.[104]

Niacin has also been shown to cause a 4% to 5% increase in fasting glucose and a 20% to 28% reduction in insulin sensitivity. For this reason it should be used with caution in diabetic patients and those at risk for diabetes. However, its cardiovascular benefit often significantly outweighs the risk, even in these patients. In a 2010 meta-analysis published in *Atherosclerosis,* nicotinic acid given alone or in combination was associated with a reduction in the risk of coronary events and stroke (approximately by 75%) as well as positive effects on the evolution of atherosclerosis when used at 1 to 3 g/day. The authors of this analysis also point out that it reduces low-density-lipoprotein cholesterol to a similar degree as statin medications, and is the only effective therapy for reducing lipoprotein (a).[105] When extended-release niacin was given to diabetic patients at 1000 to 1500 mg/day, it was associated with only a 0.3% increase in hemoglobin A_{1c} compared with placebo, with a dose-dependent improvement in levels of triglycerides and high-density-lipoprotein cholesterol. However, increases in diabetes medications may have prevented a larger increase in hemoglobin A_{1c}.[106] Given that improving high-density-lipoprotein cholesterol is a primary goal in diabetes, the use of nicotinic acid is often indicated for these patients.[107]

Side effects can occur with any form of niacin, including niacinamide. Although niacinamide does not cause the acute flushing of the skin, it can also cause liver damage and has not demonstrated the same benefit to lipid profiles as nicotinamide. Inositol hexaniacinate is an alternative form of niacin that may have very few side effects, but large, well-designed studies to document its benefit are lacking.[108]

Regardless of the form of niacin being used, periodic checking (at least every 3 months) of liver function is indicated when high-dose (i.e., 2–6 g/day) niacin, inositol hexaniacinate, or niacinamide therapy is being used. Niacin should be used with caution in patients with preexisting liver disease or elevation in liver enzyme values, gout, or peptic ulcers. Physicians should also be aware of the promotion and use of niacin as an agent to influence urine drug screens. Several case reports have been published on severe niacin toxicity caused by large doses being consumed for this purpose.[109,110]

Pyridoxine (Vitamin B_6)

Vitamin B_6 is one of the few water-soluble vitamins that is associated with toxicity when taken in large doses or in moderate dosages for long periods, and it is known to cause a transient sensory neuropathy. Large doses of vitamin B_6 are currently being used for a wide variety of conditions.

Doses greater than 1000 mg/day can produce symptoms of nerve toxicity (tingling sensations in the feet, loss of muscle coordination, and degeneration of nerve tissue) in some individuals, with ataxia being the clinical hallmark of vitamin B_6 hypervitaminosis.[111] Long-term intake of dosages greater than 500 mg/day can be toxic if taken daily for several months.[112] There are also a few rare reports of toxicity occurring at chronic long-term dosages as low as 150 mg/day.[113–115] One animal study reports the increased possibility of nerve toxicity in individuals with renal failure who have uremia, owing to decreased pyridoxine excretion, which induces an increase in susceptibility to pyridoxine-induced neuropathy.[116] Because patients with renal failure are commonly given long-term pyridoxine therapy, caution is advised, and it is prudent to look for neuropathic signs in pyridoxine-supplemented uremic patients with renal failure. Although rare, it has also been reported that pyridoxal-5-phosphate (PLP) therapy can exacerbate blood clotting disorders, such as hemophilia, so it is important that a clinician be prudent in using vitamin B_6 therapy in those patients.[117]

Toxicity is thought to occur when supplemental pyridoxine overwhelms the liver's ability to add a phosphate group to produce the active form of vitamin B_6 (PLP). As a result, it is speculated that pyridoxine is either toxic to the nerve cells or that it actually acts as an antimetabolite by binding to PLP receptors, thereby creating a relative deficiency of vitamin B_6. A 2017 in vitro study seemed to confirm both of these hypotheses, demonstrating that pyridoxine significantly increased neuronal cell death whereas other B vitamers did not.[118] When PLP was added to neuronal cells before the addition of pyridoxine, it was able to prevent pyridoxine-induced cell death. Further, it was demonstrated that pyridoxine was able to competitively inhibit PLP-dependent enzymes tyrosine decarboxylase and alanine aminotransferase. Some controversy exists suggesting that vitamin B_6 analog contaminants produced during synthesis may be the actual culprits.

Doses of pyridoxine should therefore be limited to 50 mg. If more than 50 mg is desired, then the doses should be spread throughout the day or the active form (PLP) could be used.

TABLE 127.3 Laboratory Tests for Vitamin Toxicity

Vitamin	Laboratory Measurement
Vitamin A	AST, serum retinol, serum free retinyl esters, stable isotope dilution (preferred)
Vitamin D	Serum calcium, 25(OH) vitamin D
Niacin	AST, ALT
Vitamin C	Urinary oxalate and uric acid

ALT, Alanine transaminase; *AST*, aspartate aminotransferase.

Vitamin B_{12} (Cobalamins)

Vitamin B_{12}, a generic term for a group of cobalt-containing corrinoids, has no appreciable toxicity. Vitamin B_{12} has a long history of safe, long-term use even at high therapeutic doses (1000 mcg/day for years; 100,000 mcg one time dose).[119] In a 1986 study in dialysis patients who could not excrete vitamin B_{12} normally, IV doses of 2.5 mg resulted in a fourfold elevation of plasma vitamin B_{12}. These levels were not associated with any toxic effects.[120] Rare allergic/immune reactions have been reported with IV administrations.[121]

LABORATORY TESTS FOR VITAMIN TOXICITY

Only a limited number of routine laboratory tests are available for detecting vitamin toxicity. These are presented in Table 127.3.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Miloslav R. CRC handbook series in nutrition and food, section E: nutritional disorders. In: *CRC Handbook Series in Nutrition and Food, Section E: Nutritional Disorders*. West Palm Beach, FL: CRC Press; 1.
2. Omaye S. Safety of megavitamin therapy. *Adv Exp Med Biol*. 1984;177:169–203.
3. Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr*. 2006;83(2):191–201.
4. Perrotta S, Nobili B, Rossi F, et al. Vitamin A and infancy: biochemical, functional, and clinical aspects. *Vitam Horm*. 2003;66:457–591.
5. Allen LH, Haskell M. Estimating the potential for vitamin A toxicity in women and young children. *J Nutr*. 2002;132(suppl 9):2907S–2919S.
6. Ramanathan VS, Hensley G, et al. Hypervitaminosis A inducing intra-hepatic cholestasis: a rare case report. *Exp Mol Pathol*. 2010;88(2):324–325.
7. Olson M, Shah NA. Vitamin A toxicity. In: *StatPearls.* Treasure Island, FL: StatPearls Publishing LLC; 2018.
8. Brown AC. Liver toxicity related to herbs and dietary supplements: online table of case reports, part 2 of 5 series. *Food Chem Toxicol*. 2017;107(Pt A):472–501.
9. Wendling D, Hafsaoui C, Laurain JM, et al. Dysphagia and hypervitaminosis A: cervical hyperostosis. *Joint Bone Spine*. 2009;76(4):409–411.
10. Rothman KJ, Moore LL, Singer MR, et al. Teratogenicity of high vitamin A intake. *N Engl J Med*. 1995;333(21):1369–1373.
11. Feskanich D, Singh V, Willett WC, et al. Vitamin a intake and hip fractures among postmenopausal women. *JAMA*. 2002;287:47–54.
12. Melhus H, Michaelsson K, Kindmark A, et al. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med*. 1998;129:770–778.
13. Lim LS, Harnack LJ, Lazovich D, et al. Vitamin A intake and the risk of hip fracture in postmenopausal women: the Iowa women's health study. *Osteoporos Int*. 2004;15:552–559.
14. Ambrosini GL, Bremner AP, Reid A, et al. No dose-dependent increase in fracture risk after long-term exposure to high doses of retinol or beta-carotene. *Osteoporos Int*. 2013;24(4):1285–1293.
15. Caire-Juvera G, Ritenbaugh C, Wactawski-Wende J, et al. Vitamin A and retinol intakes and the risk of fractures among participants of the women's Health Initiative Observational Study. *Am J Clin Nutr*. 2009;89(1):323–330.
16. Mata-Granados JM, Cuenca-Acevedo R, Luque de Castro MD, et al. Vitamin D deficiency and high serum levels of vitamin A increase the risk of osteoporosis evaluated by quantitative ultrasound measurements (QUS) in postmenopausal spanish women. *Clin Biochem*. 2010;43(13-14):1064–1068.
17. Joo N, Yang S, Song BC, Yeum K. Vitamin A intake, serum vitamin D and bone mineral density: analysis of the Korea national health and nutrition examination survey (KNHANES, 2008-2011). *Nutrients*. 2015;7(3). 1716–177.
18. Wu AM, Huang CQ, Lin ZK, et al. The relationship between vitamin A and risk of fracture: meta-analysis of prospective studies. *J Bone Miner Res*. 2014;29(9):2032–2039.
19. Zhang X, Zhang R, Moore JB, et al. The effect of vitamin A on fracture risk: a meta-analysis of cohort studies. *Int J Environ Res Public Health*. 2017;14(9): pii:E1043.
20. Johansson S, Melhus H. Vitamin A antagonizes calcium response to vitamin d in man. *J Bone Miner Res*. 2001;16(10):1899–1905.
21. Cannell JJ, Vieth R, Willett W, et al. Cod liver oil, vitamin A toxicity, frequent respiratory infections, and the vitamin D deficiency epidemic. *Ann Otol Rhinol Laryngol*. 2008;117(11):864–870.
22. de Jonge EAL, Jong JCK, Campos-Obando N, et al. Dietary vitamin A intake and bone health in the elderly: the Rotterdam study. *Eur J Clin Nutr*. 2015;69(15):1360–1368.
23. Ribaya-Mercado JD, Blumberg JB. Vitamin A: is it a risk factor for osteoporosis and bone fracture? *Nutr Rev*. 2007;65(10):425–438.
24. Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *J Natl Cancer Inst*. 1984;74:1439–1444.
25. Ballew C, Bowman BA, Russell RM. Serum retinyl esters are not associated with biochemical markers of liver dysfunction in adult participants in the third national health and nutrition examination survey (NHANES III), 1988–1994. *Am J Clin Nutr*. 2001;73(5):934–940.
26. Haskell MJ, Jamil KM, Peerson JM, et al. The paired deuterated retinol dilution technique can be used to estimate the daily vitamin A intake required to maintain a targeted whole body vitamin a pool size in men. *J Nutr*. 2011;141(3):428–432.
27. Escaron AL, Green MH, Howe JA, et al. Mathematical modeling of serum 13C-retinol in captive rhesus monkeys provides new insights on hypervitaminosis A. *J Nutr*. 2009;139(10):2000–2006.
28. Ribaya-Mercado JD, Maramag CC, et al. Carotene-rich plant foods ingested with minimal dietary fat enhance the total-body vitamin A pool size in filipino schoolchildren as assessed by stable-isotope-dilution methodology. *Am J Clin Nutr*. 2007;85(4):1041–1049.
29. DiPalma J, Ritchie D. Vitamin toxicity. *Annu Rev Pharmacol Toxicol*. 1977;17:133–148.
30. Buist R. Vitamin toxicities, side effects and contraindications. *Int Clin Nutr Rev*. 1984;4:159–171.
31. Tang G. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *Am J Clin Nutr*. 2010;91(5):1468S–1473S.
32. Ferrucci L, Perry JR, Matteini A, et al. Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. *Am J Hum Genet*. 2009;84(2):123–133.
33. Leung WC, Hessel S, Méplan C, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. *FASEB J*. 2009;23(4):1041–1053.
34. Druesne-Pecollo N, Latino-Martel P, Norat T, et al. Beta-carotene supplementation and cancer risk: a systematic review and meta-analysis of randomized controlled trials. *Int J Cancer*. 2010;127(1):172–184.
35. Shahangian S, Alspach TD, Astles JR, et al. Trends in laboratory test volumes for medicare part B reimbursements, 2000–2010. *Arch Pathol Lab Med*. 2014;138(2):189–203.
36. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, et al. Benefit-risk assessment of vitamin D supplementation. *Osteoporos Int*. 2010;21(7):1121–1132.
37. Garland CF, French CB, Baggerly LL, et al. Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. *Anticancer Res*. 2011;3(2):607–611.
38. Ginde AA, Liu MC, Camargo Jr CA. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. *Arch Inter Med*. 2009;169(6):626–632.
39. Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmun Rev*. 2010;9(11):709–715.
40. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr*. 2001;73(2):288–294.
41. Belenchia AM, Tosh AK, Hillman LS, Peterson CA. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: a randomized controlled trial. *Am J Clin Nutr*. 2013;97(4):774–781.
42. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911–1930.
43. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr*. 2007;85(1):6–18.
44. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr*. 1999;69(5):842–856.
45. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. *Nutrients*. 2013;5(9):3605–3616.
46. Jones G. Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr*. 2008;88(2):582s–586s.
47. Galior K, Grebe S, Singh R. Development of vitamin D toxicity from overcorrection of vitamin D deficiency: a review of case reports. *Nutrients*. 2018;10(8):E953.

48. Aloia JF, Katumuluwa S, Stolberg A, et al. Safety of calcium and vitamin D supplements, a randomized controlled trial. *Clin Endocrinol (Oxf)*. 2018;89(6):742–749.
49. Lee JP, Tansey M, Jetton JG, Krasowski MD. Vitamin D toxicity: a 16-Year retrospective study at an academic medical center. *Lab Med*. 2018;49(2):123–129.
50. Spiller HA, Good TF, Spiller NE, Aleguas A. Vitamin D exposures reported to US poison centers 2000–2014: Temporal trends and outcomes. *Hum Exp Toxicol*. 2016;35(5):457–461.
51. Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA*. 2010;303(18):1815–1822.
52. Malihi Z, Lawes CMM, Wu Z, et al. Monthly high-dose vitamin D3 supplementation and self-reported adverse events in a 4-year randomized controlled trial. *Clin Nutr*. 2018;4:pii: S0261-5614(18)31248-2.
53. Heaney RP, Recker RR, et al. Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab*. 2011;96(3):E447–E452.
54. Burke RR, Rybicki BA, Rao DS. Calcium and vitamin D in sarcoidosis: how to assess and manage. *Semin Respir Crit Care Med*. 2010;31(4):474–484.
55. Adams JS, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. *J Clin Invest*. 1983;72:1856–1860.
56. Schürks M, Glynn RJ, Rist PM, et al. Effects of vitamin E on stroke subtypes: meta-analysis of randomized controlled trials. *BMJ*. 2010;341:c5702.
57. Miller 3rd ER, Pastor-Barriuso R, Dalal D, et al. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142(1):37–46.
58. Bjelakovic G, Nikolova D, Gluud LL, et al. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev*. 2012;14(3):Cd007176.
59. Oliver CJ, Myers SP. Validity of a cochrane systematic review and meta-analysis for determining the safety of vitamin E. *BMC Complement Altern Med*. 2017;17(1):408.
60. Lonn E, Bosch J, Yusuf S, et al. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *JAMA*. 2005;293(11):1338–1347.
61. Mangialasche F, Kivipelto M, Mecocci P, et al. High plasma levels of vitamin E forms and reduced Alzheimer's disease risk in advanced age. *J Alzheimers Dis*. 2010;20(4):1029–1037.
62. Devaraj S, Leonard S, Traber MG, et al. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med*. 2008;44(6):1203–1208.
63. Yu W, Jia L, Park SK, et al. Anticancer actions of natural and synthetic vitamin E forms: RRR-alpha-tocopherol blocks the anticancer actions of gamma-tocopherol. *Mol Nutr Food Res*. 2009;53(12):1573–1581.
64. Wagner KH, Kamal-Eldin A, Elmadfa I. Gamma-tocopherol: an underestimated vitamin? *Ann Nutr Metab*. 2004;48(3):169–188.
65. Serbinova E, Kagan V, Han D, Packer L. Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radic Biol Med*. 1991;10:263–275.
66. Serbinova E, Maitra I, Packer L. The synergy between vitamin E and alpha-lipoic acid—possible relationship in protection against oxidative stress in vivo. *Life Chem Rep*. 1994;12:17–21.
67. Sen CK, Khanna S, Roy S. Tocotrienols: vitamin E beyond tocopherols. *Life Sci*. 2006;27;78(18):2088–2098.
68. Hemilä H, Kaprio J. Modification of the effect of vitamin E supplementation on the mortality of male smokers by age and dietary vitamin C. *Am J Epidemiol*. 2009;169(8):946–953.
69. Asleh R, Blum S, Kalet-Litman S, et al. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2-2 genotype. *Diabetes*. 2008;57(10):2794–2800.
70. Diplock AT. Safety of antioxidant vitamins and beta-carotene. *Am J Clin Nutr*. 1995;62(62):1510s–1516s.
71. Rogovik AL, Vohra S, Goldman RD. Safety considerations and potential interactions of vitamins: should vitamins be considered drugs? *Ann Pharmacother*. 2010;44(2):311–324.
72. Higdon Drake V. Vitamin K. In: *Micronutrient Information Center*. The Linus Pauling Institute at Oregon State University; 2011.
73. Krupp MA, Chatton MJ. Current medical diagnosis and treatment. Los Altos, CA: Lange Medical.
74. Bicknell F, Prescott F. *The vitamins in medicine*. 3rd ed. New York: Grune & Stratton.
75. McCann JC, Ames BN. Vitamin K, an example of triage theory is micronutrient inadequacy linked to diseases of aging? *Am J Clin Nutr*. 2009;90(4):889–907.
76. Schurgers LJ, Teunissen KJ, Hamulyák K, et al. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood*. 2007;109(8):3279–3283.
77. Ford SK, Moll S. Vitamin K supplementation to decrease variability of International Normalized Ratio in patients on vitamin K antagonists: a literature review. *Curr Opin Hematol*. 2008;15(5):504–508.
78. Fritz H, Flower G, Weeks L. Intravenous vitamin C and cancer: a systematic review. *Integr Cancer Ther*. 2014;13(4):280–300.
79. Klimant E, Wright H, Rubin D. Intravenous vitamin C in the supportive care of cancer patients: a review and rational approach. *Curr Oncol*. 2018;25(2):139–148.
80. Moyad MA, Combs MA, Crowley DC, et al. Vitamin C with metabolites reduce oxalate levels compared to ascorbic acid: a preliminary and novel clinical urologic finding. *Urol Nurs*. 2009;29(2):95–102.
81. Padayatty SJ, Sun H, Wang Y. Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med*. 2004;140(7):533–537.
82. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate increases human oxaluria and kidney stone risk. *J Nutr*. 2005;135(7):1673–1677.
83. Robitaille L, Mamer OA, Miller Jr WH, et al. Oxalic acid excretion after intravenous ascorbic acid administration. *Metabolism*. 2009;58(2):263–269.
84. Fisher AE, Naughton DP. Vitamin C contributes to inflammation via radical generating mechanisms: a cautionary note. *Med Hypotheses*. 2003;61:657–660.
85. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology*. 2011;283(2-3):65–87.
86. Jeong YJ, Kim JH, Kang JS, et al. Mega-dose vitamin C attenuated lung inflammation in mouse asthma model. *Anat Cell Biol*. 2010;43(4):294–302.
87. Lee DH, Jacobs Jr DR. Interaction among heme iron, zinc, and supplemental vitamin C intake on the risk of lung cancer: iowa women's health study. *Nutr Cancer*. 2005;52(2):130–137.
88. Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *Proc Natl Acad Sci U S A*. 2007;104(50):19995–20000.
89. Selhub J, Morris MS, Jacques PF, et al. Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. *Am J Clin Nutr*. 2009;89(2):702S–706S.
90. Cagnacci A, Cannoletta M, Volpe A. High-dose short-term folate administration modifies ambulatory blood pressure in postmenopausal women: a placebo-controlled study. *Eur J Clin Nutr*. 2009;63(10):1266–1268.
91. Hodis HN, Mack WJ, Dustin L, et al. High-dose B vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. *Stroke*. 2009;40(3):730–736.
92. Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet*. 2007;369(9576):1876–1882.
93. Jaszewski R, Misra S, Tobi M, et al. Folic acid supplementation inhibits recurrence of colorectal adenomas: a randomized chemoprevention trial. *World J Gastroenterol*. 2008;14(28):4492–4498.
94. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inherit Metab Dis*. 2011;34(1):101–109.
95. Sauer J, Mason JB, Choi SW. Too much folate: a risk factor for cancer and cardiovascular disease? *Curr Opin Clin Nutr Metab Care*. 2009;12(1):30–36.
96. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA*. 2007;297(21):2351–2359.

97. Figueiredo JC, Grau MV, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst*. 2009;101(6):432435.
98. Boyles AL, Yetley EA, Thayer KA, Coates PM. Safe use of high intakes of folic acid: research challenges and paths forward. *Nutr Rev*. 2016;74(7):469–474.
99. Pfeiffer CM, Caudill SP, Gunter EW, et al. Biochemical indicators of B vitamin status in the U.S. population after folic acid fortification: results from the national health and nutrition examination survey 1999–2000. *Am J Clin Nutr*. 2005;82(2):442–450.
100. Guyton JR, Bays HE. Safety considerations with niacin therapy. *Am J Cardiol*. 2007;99(6A):22c–31c.
101. Rader JI, Calvert RJ, Hathcock JN. Hepatic toxicity of unmodified and time-release preparations of niacin. *Am J Med*. 1992;92(1):77–81.
102. McKenney JM, Proctor JD, Harris S. A comparison of the efficacy and toxic effects of sustained- vs immediate-release niacin in hypercholesterolemic patients. *JAMA*. 1994;271:672–677.
103. Bays H. Safety of niacin and simvastatin combination therapy. *Am J Cardiol*. 2008;101(8A):3B–8B.
104. Anderson TJ, Boden WE, Desvigne-Nickens P, et al. Safety profile of extended-release niacin in the aim-high trial. *N Engl J Med*. 2014;371(3):288–290.
105. Bruckert E, Labreuche J, Amarenco P. Meta-analysis of the effect of nicotinic acid alone or in combination on cardiovascular events and atherosclerosis. *Atherosclerosis*. 2010;210(2):353–361.
106. Grundy SM, Vega GL, McGovern ME, et al. Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of niaspan trial. *Arch Intern Med*. 2002;162(14):1568–1576.
107. Bell DS, Al Badarin F, O'Keefe Jr JH. Therapies for diabetic dyslipidemia. *Diabetes Obes Metab*. 2011;13(4):313–325.
108. Dib JG, Dedeyan S. Purported benefits of inositol niacinate. *Am J Health Syst Pharm*. 2004;61(3):307–308.
109. Mittal MK, Florin T, Perrone J, et al. Toxicity from the use of niacin to beat urine drug screening. *Ann Emerg Med*. 2007;50:587–590.
110. Daul AM, Beuhler MC. Niacin toxicity resulting from urine drug test evasion. *The Journal of emergency medicine*. 2011;41:e65–68.
111. Gdynia HJ, Müller T, Sperfeld AD, et al. Severe sensorimotor neuropathy after intake of highest dosages of vitamin B6. *Neuromuscul Disord*. 2008;18(2):156–158.
112. Cohen M, Bendich A. Safety of pyridoxine: a review of human and animal studies. *Toxicol Lett*. 1986;34:129–139.
113. Parry GJ, Bredesen DE. Sensory neuropathy with low-dose pyridoxine. *Neurology*. 1985;35:1466–1468.
114. Waterston JA, Gilligan BS. Pyridoxine neuropathy. *Med J Aust*. 1987;146:640–642.
115. Dalton K, Dalton MJ. Characteristics of pyridoxine overdose neuropathy syndrome. *Acta Neurol Scand*. 1987:76 8–7611.
116. Levine S, Saltzman A. Pyridoxine (vitamin B6) toxicity: enhancement by uremia in rats. *Food Chem Toxicol*. 2002;40:1449–1451.
117. Borst AJ, Tchapyjnikov D. B6 and bleeding: a case report of a novel vitamin toxicity. *Pediatrics*. 2018;141:S430–s433.
118. Vrolijk MF, Opperhuizen A, Jansen E, et al. The vitamin B6 paradox: supplementation with high concentrations of pyridoxine leads to decreased vitamin B6 function. *Toxicol In Vitro*. 2017;44:206–212.
119. Gimsing P, Hippe E, Helleberg-Rasmussen I, et al. Cobalamin forms in plasma and tissue during treatment of vitamin B12 deficiency. *Scand J Haematol*. 1982;29(4):311–318.
120. Mangiarotti G, Canavese C, Salomone M, et al. Hypervitaminosis B12 in maintenance hemodialysis patients receiving massive supplementation of vitamin B12. *Int J Artif Organs*. 1986;9:417–420.
121. Carmel R. Efficacy and safety of fortification and supplementation with vitamin B12: biochemical and physiological effects. *Food Nutr Bull*. 2008;29:S177–187.

Vitex agnus castus (Chaste Tree)

Michael T. Murray, ND

OUTLINE

Vitex agnus castus (family: Verbenaceae)
Common name: chaste tree

GENERAL DESCRIPTION

Vitex agnus castus, also known as chaste tree, is a shrub with finger-shaped leaves and slender violet flowers. It grows in creek beds and on riverbanks in valleys and lower foothills in the Mediterranean and Central Asia (Fig. 128.1 A–B). The plant blooms in high summer and, after pollination, develops dark-brown to black fruit the size of peppercorns. The fruit possesses a pepper-like aroma and flavor. The ripe, dried fruit of *V. agnus castus* is the part of the plant used in medicinal preparations today.[1]

CHEMICAL COMPOSITION

The fruit of *Vitex* contains essential oils, iridoid glycosides, and flavonoids.[2] The essential oils include limonene, 1,8-cineole, and sabinene.[3] The primary flavonoids are castican, orientin, and isovitexin. The two isolated iridoid glycosides are agnuside and aucubin (Figs. 128.2 and 128.3).[4] Agnuside serves as a reference material for quality control in the manufacture of *Vitex* extracts, although the flavonoid casticin appears to be the most active component.

HISTORY AND FOLK USE

The genus name *Vitex* is derived from the word *vitilium,* which means "plaiting." The flexible but tough and hard branches were used to construct fences. Plinius, in the first century AD, made the earliest reference to the plant as *Vitex.* The species name *agnus castus* originates from the Latin *castitas* ("chastity") and the equating of the Greek *agnos* with the Latin *agnus* ("lamb").

The English name for *V. agnus castus,* "chaste tree," is derived from the belief that the plant would suppress libido in women who took it. In Greek cities, festivals in the honor of Demeter included a vow of chastity by the local women. The Roman Catholic Church in Europe developed a variation on this theme by placing the blossoms of the plant in the clothing of novice monks to supposedly suppress libido. Interestingly, another common name for *V. agnus castus,* "monk's pepper," derived from the fact that monks in southern Europe commonly used the fruit as a spice in their cooking.

PHARMACOLOGY

Vitex acts on the hypothalamic-pituitary axis. One observed effect is that it increases the production of luteinizing hormone (LH) (Fig. 128.4), resulting in a corpus luteum–like hormonal effect that shifts the estrogen/progesterone ratio in favor of progesterone.[5] The ability of *Vitex* to increase or modulate the body's progesterone levels is therefore an indirect effect and not a direct hormonal action.[6]

Vitex also modulates the secretion of prolactin from the pituitary gland. In studies with rats, it was shown to inhibit prolactin release by the pituitary gland, particularly in conditions of stress. The mechanism of action appears to involve the ability of *Vitex* to directly bind dopamine receptors and subsequently inhibit prolactin release in the pituitary.[7,8] The flavonoid casticin appears to be responsible for this antihyperprolactinemia effect.[9] *Vitex* also possesses significant antioxidant effects.[10,11]

CLINICAL APPLICATIONS

The causes of menstrual disorders are multifaceted and can vary greatly in their manifestations. Frequently, therapeutic interventions must be used on a trial-and-error basis over a number of menstrual cycles to determine their efficacy. Nutritional interventions like vitamin B_6, magnesium, and vitamin E, as well as evening primrose oil for cyclic mastalgia, have all shown greater efficacy when used over several months. This characteristic reflects the gradual balancing effect that many of these interventions have on the female hormonal system. *Vitex* certainly fits this mold.

The majority of earlier clinical studies completed with *Vitex* were uncontrolled studies with large populations of female patients in European gynecology practices. *Vitex,* which has a Commission E Monograph in Germany, is commonly used in these practices as an initial intervention in a number of menstrual disorders, as follows:

- Anovulatory cycles

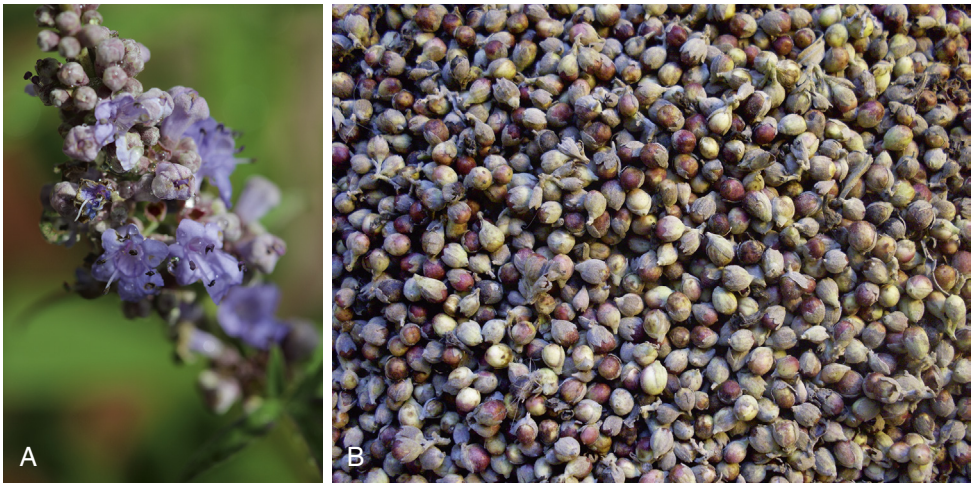


Fig. 128.1 (A) *Vitex agnus castus* flower. (B) *Vitex agnus castus* fruit.

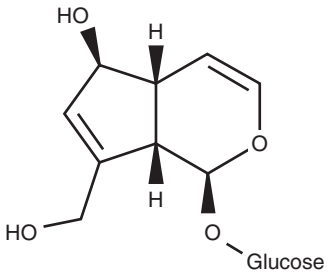


Fig. 128.2 Aucubin.

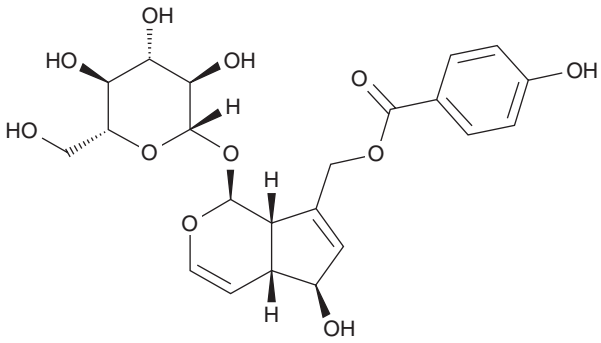


Fig. 128.3 Agnuside.

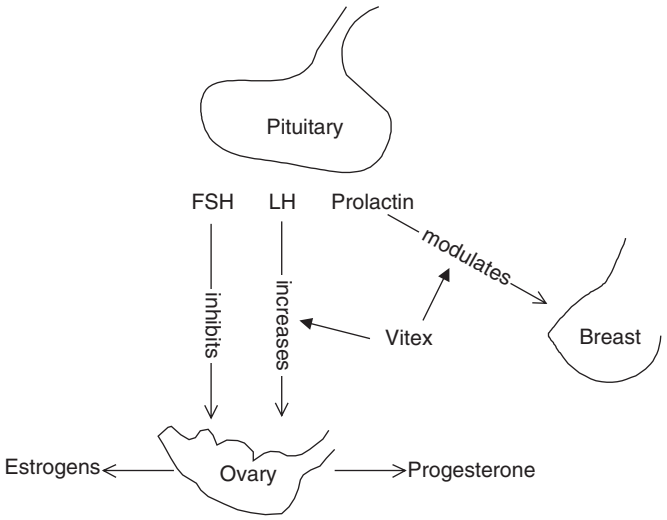


Fig. 128.4 Effect of *Vitex* on pituitary hormone secretion.

- Hypermenorrhea
- Hyperprolactinemia
- Infertility
- Mastalgia
- Polymenorrhea
- Premenstrual syndrome
- Secondary amenorrhea

Many of these conditions have been linked to corpus luteum insufficiency.

Corpus Luteum Insufficiency

Corpus luteum insufficiency (also referred to as luteal phase defect) is a manifestation of suboptimal ovarian function. In laboratory terms, corpus luteum insufficiency is usually defined as an abnormally low progesterone level 3 weeks after the onset of menstruation (serum progesterone below 10–12 ng/mL). This state is normal during puberty and at menopause; however, it is usually considered abnormal in women between the ages of 20 and 40 years.[12]

Corpus luteum insufficiency points to abnormal formation of ovarian follicles, an abnormality that may be so pronounced that no secondary or tertiary follicles are produced, with a resulting lack of ovulation (anovulation). Corpus luteum insufficiency also leads to a relative deficiency of progesterone. Insufficient levels of progesterone may also result in the formation of ovarian cysts.

Corpus luteum insufficiency may give rise to a myriad of menstrual abnormalities. Table 128.1 lists the most common clinical conditions in 1592 women diagnosed with corpus luteum insufficiency. Foremost are hypermenorrhea (heavy periods), polymenorrhea (abnormally frequent periods), and persistent anovulatory bleeding. Secondary amenorrhea (lack of a period) may sometimes be observed in women with corpus luteum insufficiency.

Disturbances of other hormones may also be associated with corpus luteum insufficiency. One study found hyperprolactinemia in 70% of cases.[13] Also noted is an exaggerated response to the thyroid-releasing hormone test, which is associated with manifest or latent hypothyroidism.

Premenstrual Syndrome and Cyclic Mastalgia

Premenstrual syndrome (PMS) is one of the most common complaints found in gynecology practices. Table 128.2 lists the different subgroups of PMS and the symptoms associated with them. Nearly 20 double-blind, placebo-controlled studies have been conducted with *Vitex* extract in PMS. A meta-analysis of the top eight studies in terms of quality concluded that *Vitex* extract is a safe and effective treatment

TABLE 128.1 The Most Common Clinical Conditions in 1592 Women Diagnosed With Corpus Luteum Insufficiency

Diagnosis	No. of Patients	Percentage of Total (%)
Hypermenorrhea	418	26.3
Polymenorrhea	369	23.2
Persistent anovulatory bleeding	216	13.6
Secondary amenorrhea	202	12.7
Dysmenorrhea	186	11.7
Anovulatory cycles	175	11.0
Involuntary sterility	145	9.1
Oligomenorrhea	69	4.3
Menorrhagia/metrorrhagia	66	4.1
Irregular menstrual cycles	32	2.0
Primary amenorrhea	1	0.1

TABLE 128.2 Subgroups of Premenstrual Syndrome (PMS)

Subgroup	Symptoms	Prevalence (%)
PSM-S	Anxiety Nervous tension	75–80
PMS-H	Irritability Fluid retention Weight gain Swollen extremities Abdominal bloating Breast tenderness	60–70
PMS-C	Increased appetite Craving for sweets Headache Fatigue Fainting spells	35–40
PSM-D	Depression Insomnia Forgetfulness	30–35

for PMS and premenstrual dysphoric disorder (PMDD). Three different preparations of *Vitex* extract were tested, and there was significant variability in the measurement of treatment outcomes between the studies. Nevertheless, all eight studies were positive for *Vitex* extract in the treatment of PMS or PMDD and *Vitex* extract was well tolerated.

In a double-blind study, 170 women (mean age, 36 years) with a diagnosis of PMS were randomly assigned to receive either 20 mg of a *Vitex* extract (dried ethanolic extract [6–12:1]) or placebo once a day for three menstrual cycles. Twenty-three women were taking oral contraceptives (11 in the *Vitex* group and 12 in the placebo group) during the study period. At the end of the treatment period, women taking the *Vitex* extract had a significantly greater reduction in overall PMS symptom score than those taking placebo ($P < 0.001$). Five of the six self-assessment items indicated a significantly greater reduction for the *Vitex* group with the exception of the item listed "others and bloating," which was measured as unaffected by treatment with *Vitex*. The Clinical Global Impressions (physician's assessment) also showed a significantly greater reduction in PMS symptoms in the *Vitex* group than in the placebo group ($P < 0.001$). The overall response rate was 52% for the *Vitex* group compared with 24% for the placebo group.[15]

TABLE 128.3 Efficacy of *Vitex*

Outcome	Percentage of Patients
Patient's Assessment	
Improved	57
Relieved	33
No change	4
No data	5
Physician's Assessment	
Very good/good	71
Satisfactory	21
Unsatisfactory	4
No data	3

One study compared the efficacy of *Vitex* (3.5–4.2 mg/day of dried fruit extract) with vitamin B_6 (200 mg/day) in 175 women with premenstrual tension syndrome.[16] Although both agents were effective (the symptom scale decreased from 15.2 to 5.1 in the *Vitex* group and from 11.9 to 5.1 in the vitamin B_6 group), 24.5% of subjects reported excellent results for *Vitex*, compared with only 12.1% for B_6. However, more than twice as many women (12) reported side effects from *Vitex* than from vitamin B_6 (5).

Two double-blind placebo-controlled studies have been conducted in Chinese women. In one double trial in 67 women, *Vitex* reduced the premenstrual syndrome diary (PMSD) sum score from 29.38 at baseline to 4.28 at the third cycle in the treatment group.[17] In the other double-blind study comprising 208 women in the *Vitex* group, the mean total PMSD score decreased from 29.23 at baseline to 6.41 at termination (third cycle) for the treatment group and from 28.14 at baseline to 12.64 at termination (third cycle) for the placebo group.[18] A placebo effect near 50% was found in both studies.

Other studies with *Vitex* for PMS have been uncontrolled or clinical monitoring trials. A monitoring survey of gynecology practices in Germany examined the effect of *Vitex* on 1542 women with a diagnosis of PMS.[19] The patients' mean age was 34.7 years, with a range of 13 to 62 years. Additional diagnoses noted in these patients included corpus luteum insufficiency ($n = 1016$) and uterine fibroids ($n = 170$). Patients were instructed to take 40 drops per day of a *Vitex* liquid extract. The average duration of treatment was 166 days. The efficacy of treatment was assessed by both patients and their physicians. These assessments are listed in Table 128.3.

In more than 90% of the patients, symptoms were completely relieved, with a report of side effects in only 2% (listed in Table 128.3). Only 17 of the 1542 women studied had to stop treatment because of side effects. Improvement in symptoms began after an average treatment duration of 25.3 days. After completion of the monitoring period, 562 patients continued taking *Vitex*. Similar results have been reported in three uncontrolled studies.[20–23]

Vitex has been shown to reduce cyclic breast pain (mastalgia) in many of the PMS studies. In a detailed review of data from randomized and nonrandomized studies regarding the efficacy and safety of *Vitex*, the authors concluded that *Vitex* can be considered a safe and effective agent in the treatment of mastalgia.[24] In one double-blind study of 97 patients suffering from cyclic mastalgia, the intensity of mastalgia, as measured by visual analog score, in patients treated with a liquid product combining *Vitex* with several homeopathic ingredients

significantly decreased after one or two treatment cycles and remained reduced after the third cycle.[25] Not only the intensity but also the duration of pain improved on *Vitex* treatment. In the *Vitex* group, after two cycles 50% of patients did not have severe pain at all during menstrual cycle. Clinicians should expect it to take three or four cycles for *Vitex* to work for mastalgia as well.

Hyperprolactinemia

As mentioned previously, *Vitex* has shown a modulating effect on prolactin and is regarded as a useful agent for the treatment of hyperprolactinemia.[26] A double-blind, placebo-controlled study examined the effect of *Vitex* on 52 women with luteal phase defects caused by latent hyperprolactinemia. The daily dose of the *Vitex* extract was 20 mg and the study lasted for 3 months. Hormonal analysis was performed at days 5 through 8 and day 20 of the menstrual cycle before and after 3 months of therapy. After 3 months of therapy, 37 cases were available for analysis (20 placebo and 17 *Vitex*). Prolactin release was significantly reduced in the *Vitex* group. Both shortened luteal phases and deficits in progesterone production were normalized. No side effects were noted, and two women in the *Vitex* group became pregnant.[27]

Infertility and Secondary Amenorrhea

Twenty patients with secondary amenorrhea were enrolled in a 6-month study using *Vitex* liquid extract at 40 drops daily. Laboratory monitoring of progesterone, follicle-stimulating hormone (FSH), and LH values as well as Papanicolaou smears were performed before the study and at 3 and 6 months. At the end of the study, data were available in 15 patients. The onset of menstrual cycles was observed with *Vitex* treatment in 10 of the 15 patients. The hormone values showed increased values for progesterone and LH, but FSH values either did not change or dropped slightly.[28]

A nonblinded uncontrolled trial studied the effect of *Vitex* on corpus luteum function in 48 infertile women between 23 and 39 years of age. The inclusion criteria were normal prolactin levels (<20 ng/mL), normal results in the prolactin and thyroid-stimulating hormone tests, and an abnormally low serum progesterone levels (<12.0 ng/mL on the 20th day of the cycle). Treatment consisted of *Vitex* liquid extract 40 drops daily without any other medication for 3 months. Forty-five women completed the trial (3 were excluded because of concurrent hormone use). The outcome of therapy was assessed through evaluation for normalization of the midluteal progesterone level and correction (lengthening) of any preexisting shortening of the phases of the cycle. Treatment was deemed successful in 39 of the 45 patients. Seven women became pregnant, serum progesterone was restored to normal (>12 ng/mL) in 25 patients, and there was a trend toward normalization of progesterone levels in 7 patients.[29]

A double-blind, placebo-controlled study involving 89 women with infertility, luteal phase defect, or secondary amenorrhea looked at the effect off a liquid *Vitex* extract (32.4 mg) with homeopathic ingredients on progesterone, LH, and prolactin levels and conception rates over a 3-month span. Of the 66 women evaluated at the end of the study, 31 had normal hormone values. More importantly, 15 women (7 with amenorrhea, 4 with infertility, and 7 with luteal phase defect) conceived during the study.[30]

DOSAGE

The recommended daily dose of *Vitex* is a dried or liquid extract delivering the equivalent of 30 to 40 mg of the dried fruit a day.[31] *Vitex* is typically taken once in the morning before breakfast; it should be taken for at least three menstrual cycles to evaluate efficacy.

TABLE 128.4 Side Effects from *Vitex* in 1542 Women

Side Effect	No. of Patients Reporting
No information	7
Nausea	5
Gastric complaints	3
Acne	3
Changes in menstrual rhythm	2
Diarrhea	2
Erythema	2
Allergy	1
Weight gain	1
Giddiness	1
Heartburn	1
Hypermenorrhea	1
Pruritus	1
Alopecia	1
Cardiac palpitations	1

TOXICOLOGY

Human and animal studies have determined *Vitex* to be safe for most women of childbearing age. *Vitex* is not recommended for use during pregnancy,[32] and its use during lactation is being questioned owing to its possible dopaminergic actions, which might lead to a suppression of lactation. The safety of *Vitex* in children has also not been determined.

Data from clinical trials, postmarketing surveillance studies, surveys, spontaneous reporting schemes, manufacturers, and herbalist organizations indicate that the adverse events after *Vitex* treatment are mild and reversible.[33,34] The most frequent adverse events are nausea, headache, gastrointestinal disturbances, menstrual disorders, acne, pruritus, and erythematous rash. Side effects noted in one large population study are listed in Table 128.4.

DRUG INTERACTIONS

Because of theoretical interactions, some authorities suggest that *Vitex* be avoided by women taking oral contraceptives or on hormone therapy.[35] However, two clinical studies have found no adverse interactions in women taking oral contraceptives concomitantly with *Vitex*.[15,23] The potential dopaminergic action of *Vitex* also suggests that it should not be used concomitantly with dopamine antagonist drugs, such as haloperidol and metoclopramide.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Roemheld-Hamm B. Chasteberry. *Am Fam Physician*. 2005;72(5):821–824.
2. Wuttke W, Jarry H, Christoffel V, et al. Chaste tree (Vitex agnus-castus): pharmacology and clinical indications. *Phytomedicine*. 2003;10(4):348–357.
3. Kustrak D, Kuftinec J, Balzevic N. The composition of the essential oil of *Vitex agnus castus*. *Planta Med*. 1992;58:A681.
4. Gomaa CS. Flavonoids and iridoids from *vitex agnus castus*. *Planta Med*. 1978;33:277.
5. Weiss RF. *Herbal Medicine*. Gothenburg, Sweden: Ab Arcanum.
6. Amann W. Elimination of ostipation with agnolyt. *Ther Ggw*. 1965;104:1263–1265.
7. Sliutz G, Speiser P, Schultz AM, et al. *Agnus castus* extracts inhibit prolactin secretion of rat pituitary cells. *Horm Metab Res*. 1993;25:253–255.
8. Jarry H, Leonhardt S, Wuttke W. *Agnus castus* as dopaminergous effective principle in mastodynon N. *Zeitschrift Phytother*. 1991;12:77–82.
9. Hu Y, Xin HL, Zhang QY, et al. Anti-nociceptive and anti-hyperprolactinemia activities of fructus viticis and its effective fractions and chemical constituents. *Phytomedicine*. 2007;14(10):668–674.
10. Sağlam H, Pabuçcuoğlu A, Kivçak B. Antioxidant activity of vitex agnus-castus l. extracts. *Phytother Res*. 2007;21(11):1059–1060.
11. Hajdú Z, Hohmann J, Forgo P, et al. Diterpenoids and flavonoids from the fruits of vitex agnus-castus and antioxidant activity of the fruit extracts and their constituents. *Phytother Res*. 2007;21(4):391–394.
12. Propping D, Katzorke T, Belkien L. Diagnosis and therapy of corpus luteum deficiency in general practice. *Therapiewoche*. 1988;38:2992–3001.
13. Muhlenstedt D, Wutke W, Schneider HPG. Short luteal phase and prolactin. *Int J Fertil*. 1978;23:213–217.
14. Cerqueira RO, Frey BN, Leclerc E, Brietzke E. Vitex agnus castus for premenstrual syndrome and premenstrual dysphoric disorder: a systematic review. *Arch Womens Ment Health*. 2017;20(6):713–719.
15. Schellenberg R. Treatment of the premenstrual syndrome with agnus castus fruit extract: prospective, randomised, placebo controlled study. *BMJ*. 2001;322:134–137.
16. Lauritzen C, Reuter HD, Repges R, et al. Treatment of premenstrual tension syndrome with *Vitex agnus castus*: controlled, double-blind study versus pyridoxine. *Phytomedicine*. 1997;4:183–189.
17. Ma L, Lin S, Chen R, et al. Treatment of moderate to severe premenstrual syndrome with *vitex agnus castus* (BNO 1095) in chinese women. *Gynecol Endocrinol*. 2010;26(8):612–616.
18. He Z, Chen R, Zhou Y, et al. Treatment for premenstrual syndrome with vitex agnus castus: a prospective, randomized, multi-center placebo controlled study in china. *Maturitas*. 2009;63(1):99–103.
19. Dittmar FW, Bohnert KJ. Premenstrual syndrome: treatment with a phytopharmaceutical. *Therapiewoche Gynakol*. 1992;5:60–68.
20. Coeugniet E, Elek E, Kuhnast R. Premenstrual syndrome (PMS) and its treatment. *Arztezeitchr Naturheilverf*. 1986;27:619–622.
21. Peteres-Welt C, Albrecht M. Menstrual abnormalities and PMS: vitex agnus-castus. *Ther Gynäkol*. 1994;7:49–52.
22. Loch EG, Selle H, Boblitz N. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *vitex agnus castus*. *J Women's Health Gend Based Med*. 2000;9:315–320.
23. Berger D, Schaffner W, Schrader E, et al. Efficacy of *vitex agnus castus* l. extract ZE 440 in patients with premenstrual syndrome (PMS). *Arch Gynecol Obstet*. 2000;264:150–153.
24. Carmichael AR. Can Vitex agnus castus be used for the treatment of mastalgia? what is the current evidence? *Evid Based Complement Alternat Med*. 2008;5(3):247–250.
25. Halaska M, Beles P, Gorkow C, et al. Treatment of cyclical mastalgia with a solution containing *vitex agnus castus* extract: results of a placebo-controlled double-blind study. *Breast J*. 1999;8:175–181.
26. Tamagno G, Burlacu MC, Daly AF, et al. Vitex agnus castus might enrich the pharmacological armamentarium for medical treatment of prolactinoma. *Eur J Obstet Gynecol Reprod Biol*. 2007;135(1):139–140.
27. Milewicz A, Gejdel E, Sworen H, et al. *Vitex agnus castus* extract in the treatment of luteal phase defects due to hyperprolactinemia: results of a randomized placebo-controlled double-blind study. *Arzneimittelforschung*. 1993;43:752–756.
28. Losh EG, Kayser E. Diagnosis and treatment of dyshormonal menstrual periods in the general practice. *Gynakol Prax*. 1990;14:489–495.
29. Propping D, Katzorke T. Treatment of corpus luteum insufficiency. *Z Allgemeinmed Zeits Allgemeinmedizin*. 1987;63:932–933.
30. Gerhard I, Patek A, Monga B, et al. Mastodynon for female infertility. *Forsch Komplementarmed*. 1998;5:272–278.
31. Blumenthal M, Busse WR, Goldberg A, et al., eds. *The Complete Commission E Monographs*. Boston: Integrative Medicine Communications: 108.
32. Blumenthal M, Goldberg A, Brinkmann J, eds. *Herbal medicine: Expanded Commission E Monographs*. Newton, MA: Integrative Medicine Communications: 62–64.
33. Dugoua JJ, Seely D, Perri D, et al. Safety and efficacy of chastetree (Vitex agnus-castus) during pregnancy and lactation. *Can J Clin Pharmacol*. 2008;15(1):e74–e79.
34. Daniele C, Thompson Coon J, Pittler MH, et al. Vitex agnus castus: a systematic review of adverse events. *Drug Saf*. 2005;28(4):319–332.
35. Böhnert KJ. The use of Vitex agnus castus for hyperprolactinemia. *Q Rev Natural Med*. 1997;Spring:19–21.

Water: The Most Basic Nutrient and Therapeutic Agent

Joseph Katzinger, ND, and *Herb Joiner-Bey*, ND*

OUTLINE

Pure water has been undervalued as a therapeutic tool because of a number of false assumptions and beliefs, according to Dr. Fereydoon Batmanghelidj. Conceptual fallacies in regard to the body's need for and use of water are as follows:

- Dry mouth is the only sign of water need.
- Water is merely a passive solvent with no chemical properties of its own.
- The human body can never become depleted of water because water is free and readily available.
- The water needs of the body can easily be met by any commercially prepared fluids.[1]

WATER AND THE HUMAN BODY

Water is the predominant compound in the human body. At birth, a baby's body is 78% water by weight. The average young adult is 70% water, and although the body's water content declines with age, water content remains at no less than 50% even in the geriatric individual. During youth, most cells with the exception of adipose cells are 90% water. In adulthood, two thirds of body water resides within cells; the remainder resides in the extracellular spaces.[2] Even bone tissue is 22% water.

Water has numerous roles to play in human physiology[3]:

- Building material. Water molecules facilitate the folding of amino acid chains assembled on the basis of DNA genetic sequences and reinforce the structural integrity of proteins. Water molecules fill every space not occupied by other molecules in every nook and cranny of every cell.
- Solvent. The ionic nature of water molecules makes water an excellent solvent of ionic compounds: salts, glucose, amino acids, etc.[4] The tendency of water molecules to surround ions and molecules of solutes allows chemical agents to move freely to wherever they are needed.
- Reaction medium and reactant. The biochemical reactions that sustain life occur within the medium of water. Yet water is not just the medium in the background; it is also an active participant—a reactant in the hydrolysis of macromolecules such as proteins, carbohydrates, and fats. Water is also a by-product of the metabolism of food energy molecules that contain hydrogen: carbohydrates, proteins, and fats.
- Carrier for nutrients and waste products. Water is the transport medium that moves nutrients into cells and waste products out of cells and then out of the body. Water is also the most basic constituent of blood, indispensable to the function of the cardiovascular, respiratory, urinary, and nervous systems.[5]
- Thermoregulation. The electromagnetic bonds among water molecules give water a great capacity to absorb heat, hold heat, and resist temperature changes. This property allows water to support homeostasis by helping maintain body temperature. In addition, the evaporation of water from the skin as perspiration releases heat sufficiently to maintain body temperature even when the surrounding atmospheric temperature is higher than body temperature.

*Previous edition contributor

- Lubricant. Water combines with molecules, forming viscous substances that lubricate and protect tissue functions. Examples include the mucus of the respiratory and gastrointestinal mucous membranes, synovial fluid in joints, saliva, tears, etc.
- Shock absorber. Within the cell, water takes the structural configuration of a gel that maintains cellular shape and form. This property cushions tissues against the shock of movement and mild trauma. Water molecules have an affinity for the electronegative molecules of connective tissue matrix (e.g., chondroitin, keratin, etc.), including the nucleus pulposus of the vertebral discs. It is the water molecules surrounding the compounds of the connective tissue matrix that provide the cushioning effect.

Causes of Dehydration

The most common are:
- Vomiting
- Diarrhea
- Blood loss
- Malnutrition
- Failure to replenish liquids lost from sweating and urination
- Among older adults in which dehydration is much more common, lower muscle mass, reduced kidney function, physical and cognitive disabilities, blunted thirst, and polypharmacy may all contribute.[6]
- Other causes include diabetic ketoacidosis, diabetes insipidus, burns, medications, and many others.

THIRST: THE SENSE THAT CANNOT BE TRUSTED?

The osmoreceptors in the forebrain are sensitive to only certain solutes in the blood (e.g., sodium) but insensitive to others (e.g., urea).[7] Thus the osmoreceptors are not accurate gauges of all blood solute concentrations but directly detect changes to the osmolality of the blood. We now know that these changes are detected by two small structures in the forebrain, outside of the blood–brain barrier, known as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis. The precise mechanism remains unknown but has been speculated to be mediated via stretch-sensitive ion channels embedded in the plasma membranes of specialized neurons in these locations.[8]

Originally thought to be a limiting factor for optimal hydration, the sense of thirst in the mouth (dryness) is quickly and easily satiated by small amounts of water, stimulating the moisture receptors within the mucous membranes of the mouth, throat, and upper gastrointestinal tract. Now it is understood that thirst is simply not quenched by the same process that initiated it. "Instead, the brain terminates thirst by using sensory cues from the oropharynx to track ongoing water consumption and then estimate how this water intake will alter fluid balance in the future, after the water has been absorbed."[8] Thus thirst is quenched in anticipation of water absorption, likely as a preventative factor for overconsuming water and creating dangerous imbalances.

Signs and Symptoms of Dehydration

Clinical dehydration is a serious medical condition that must be treated immediately. Here are the indications to look for, though unfortunately most are low reliability indicators, especially in an older population[9]:
- Fatigue and weakness
- Headache
- Rough, dry skin or tenting of the skin
- Dry mucous membranes in the nose, mouth, or throat
- Nosebleeds (especially in dry interior air during winter)
- Dark, concentrated, strong-smelling urine produced in small quantities
- Irritability, irrational behavior (altered mental status)
- Constipation
- Nausea
- Intestinal cramps
- Weak, irregular pulse
- Low blood pressure
- Shallow, rapid breathing
- Increased capillary refill time
- Increased blood urea nitrogen, hematocrit, or serum sodium

Sources of Water for the Body

Human beings derive their water intake from these basic sources:
- Beverages (70%–80%)
- Foods with high water content (20%–30%)
- Liberation of water molecules during oxidative metabolism of food sources[10]

Water Losses From the Body

Water exits the body via several portals:
- Skin—insensible perspiration (450 mL daily in temperate climates). Of course, elevations in body temperature can lead to massive increases in water losses in the form of sweat.
- Kidneys—1 to 2 L of urine daily. Water loss via urine increases during hyperglycemia (diabetes mellitus) as well as in disorders involving antidiuretic hormone (vasopressin).
- Respiratory mucous membranes—breath (250–350 mL daily). Additional water loss occurs during respiratory infections, which trigger increased discharge of mucous secretions.
- Digestive system, including feces (200 mL daily). Dramatic water losses arise from disorders whose symptoms include vomiting and/or diarrhea.

Perspiration is hypotonic; that is, it has lower electrolyte content than plasma or extracellular fluid. This phenomenon means that sweating leads to more water loss than electrolyte loss. As the electrolyte concentration in the extracellular fluid rises, it draws water out of cells (intracellular fluid), leading to cellular dehydration. This state of hypertonic dehydration demands replenishment with hypotonic beverages, such as pure water. Water need supersedes salt need during endurance exercise.[11]

Factors That Influence Water Need

Water intake requirements vary according to individual needs and circumstances. Some of the factors that affect water need are:
- Physical activity/exercise
- Metabolism
- Diet
- Ambient atmospheric temperature
- Humidity
- Health status

Ideal Water Intake

In units of liters per day, the water intake levels recommended by the US Food and Nutrition Board, based on age and gender, are as follows[12]:
- Infants, 0.7 to 0.8 L
- Children
  - 1 to 3 years, 1.3 L
  - 4 to 8 years, 1.7 L
- Males
  - 9 to 13 years, 2.4 L

  - 14 to 18 years, 3.3 L
  - 19+ years, 3.7 L
- Females
  - 9 to 13 years, 2.1 L
  - 14 to 18 years, 2.3 L
  - 19+ years, 2.7 L
  - During pregnancy, 3.0 L
  - During lactation, 3.8 L

Hydration for Athletes and Laborers

The National Athletic Trainers' Association has published guidelines for athletic hydration. Here are some highlights of their recommendations that apply equally to "weekend warriors" and those involved in heavy physical labor[13]:

- Establish hydration protocols, accommodating sweat rate, rest breaks, fluid access, environmental factors, acclimatization state, exercise duration and intensity, and personal preferences.
- Make fluid-replacement beverages easily accessible in individual containers to permit easier monitoring of fluid intake.
- Ensure that athletic activity begins in a well-hydrated state. Preexercise hydration entails consuming 500 to 600 mL (17–20 fl oz) of water 2 to 3 hours before exercise and 200 to 300 mL (7–10 fl oz) of water 10 to 20 minutes before exercise.
- Replace fluids sufficiently to meet sweat and urine losses and maintain hydration by preventing at less than 2% body weight reduction. This need requires 200 to 300 mL (7–10 fl oz) every 10 to 20 minutes.
- Hydrate postexercise to correct any fluid loss, ideally within 2 hours after exercise. When rehydration must be rapid, compensate for urine losses during rehydration by drinking 25% to 50% more than sweat losses to assure optimal rehydration 4 to 6 hours after exercise.
- Be alert for signs and symptoms of dehydration—thirst, irritability, general discomfort, followed by headache, weakness, dizziness, cramps, chills, vomiting, nausea, head or neck heat sensations, and decreased performance.

In 2017 this position statement was updated, with a number of changes that recognize the danger of both hypohydration as well as hyperhydration, and that encourage all athletes to develop individualized plans to accommodate unique differences in "sweat rate, environment, acclimatization state, body size, exercise duration, exercise intensity, and individual fluid preferences and tolerance."[14]

PURE WATER VERSUS OTHER BEVERAGES

By definition, *osmosis* is the diffusion of water molecules from a place of relatively high concentration across a semipermeable membrane to a place of relatively low concentration. The amount of pressure that must be applied against this osmotic movement of water is called *osmotic pressure*. It is a measure of how vigorously water is attempting to cross the membrane. The differences in water concentration across membranes define the "water concentration gradient" across that membrane. Thus the osmotic pressure is proportional to the concentration gradient. The higher the water concentration on one side of the membrane relative to the other side, the higher the osmotic pressure and the greater the vigor of movement of water molecules across the membrane. Any solutes dissolved in water outside the membrane decrease the water concentration gradient across the membrane and the vigor of water movement. If the concentration of solutes in the water outside the cell is less than the concentration of solutes within the cell, the concentration of water molecules will be greater outside the cell than within it. Therefore water molecules will move by osmosis down their concentration gradient from the exterior to the interior and the cell will be hydrated.[15]

Conversely, if the concentration of solutes is higher in the exterior than the interior, water molecules are in higher concentration within the cell. Therefore water molecules will move down their concentration gradient from the interior to the exterior; thus the cell becomes dehydrated.

Obviously, the beverage that has the least amount of solutes and therefore the highest osmotic drive to hydrate the body down to the cellular level is pure water. If anything is dissolved in water (coffee, tea, sugar, flavorings, colors, protein, etc.), osmosis is reduced. Body and cell hydration are therefore inhibited by the consumption of beverages containing solutes. Thus the basic principles of physiology indicate that pure water is the beverage of choice for optimal hydration of the body.[16] Exceptions to this may be made for athletes participating in events lasting longer than 1 hour (e.g., marathon runners) or those with high sodium sweat concentrations (>60 mEq/L), which may benefit from replacement sodium only, not beyond the amount lost.[17]

WATER TEMPERATURE

According to the principles of traditional Chinese medicine, the consumption of cold food and drink is a bane of optimal health and wellness among Western people. The interior temperature of the body is higher than oral, axillary, or rectal temperature. For example, the interior temperature of the stomach is about 102°F and that of the heart is approximately 106°F. These warm temperatures are appropriate for the optimal physiological functions and biochemical processes that sustain life. The ingestion of large amounts of cold food and beverages tends to inhibit these processes. Interestingly, cool water inhibits subfornical organ thirst neurons more effectively than warm, possibly because of a learned association between cooling of the oropharynx and water consumption.

For this reason, it is prudent to ensure that water consumed is at room temperature or warmer.

SPECIAL NEEDS

Particular care must be taken to adequately hydrate people who, because of special circumstances, may need an even greater intake of water than recommended earlier. These persons include the following:

- Infants fed high-protein formulas (with caution not to overhydrate infants)
- Persons eating high-protein diets
- Patients suffering from disorders whose symptoms include fever, vomiting, diarrhea, respiratory discharges, and other types of water loss
- Patients taking diuretics
- People living in environments with high atmospheric temperatures

PHYSIOLOGY OF WATER METABOLISM

Oxygen Transport

In the alveoli and in tissue capillaries and connective tissue, water is the medium in which gas exchange occurs. Without the ready solubility of oxygen and carbon dioxide in water, gas exchange in living systems would not be possible. Oxygen diffuses from atmospheric air into the water of alveolar tissues and plasma before it associates with hemoglobin in red blood cells. After being carried to peripheral tissue capillaries, oxygen dissociates from hemoglobin into the water of plasma and connective tissue. Water carries oxygen into the cell through membrane aquaporins.[18,19]

Role of Water in Protein Structure and Function

The function of a protein is determined by its form—its three-dimensional shape. The unique sequence of amino acids, coded by DNA, determines the shape or form of a protein. That sequence is determined by the sequence of the coding genes on chromosomal DNA that initiate the synthesis of that protein.[20]

Water molecules provide the environment that not only allows proteins to function but also supports their endeavors in unrecognized ways. To fulfill their respective missions, the amino acid strands of proteins must bend, twist, and contort their primary linear structure into secondary, tertiary, and quaternary forms. The hydrophobic and hydrophilic interactions between water and amino acid strands drive the conformational changes needed for proteins to realize their ultimate functional shapes. The final shape of a protein is determined by the manner in which water and amino acid strands are bonded to each other. As a lubricating agent, water facilitates the breaking and reestablishing of hydrogen bonds and other links between the various parts of protein. Once the appropriate bonding is in place, water molecules are critical to the continued integrity and stability of protein structure.[21] It can therefore be suggested that the DNA genetic code is arranged in a fashion that specifically anticipates the conformational interaction of water molecules required to finish protein-generating tasks.[22,23]

Cell Hydration and Cell Behavior

Because of the lack of routine techniques for measuring cell hydration and volume in patients, naturopaths and conventional practitioners alike often fail to consider cell hydration state during clinical assessment. This is an unfortunate oversight because of the effect of cell hydration state on the behavior of cells.

There is evidence that cell hydration state is an important determinant of protein and RNA turnover—anabolism and catabolism. It appears that hormones and amino acids modify protein turnover, partly by altering the cell hydration state, which alters cell volume. Changes in cell volume can serve as signals mediating hormone and amino acid effects, which control protein turnover. Increases in cell volume (swelling) inhibit protein catabolism and RNA degradation while stimulating the synthesis of protein, DNA, and RNA. Loss of cell volume (shrinkage) has the opposite effect on RNA, DNA, and protein. Consequently, cell swelling stimulates anabolic proliferative metabolism, whereas cell shrinkage has a catabolic and antiproliferative influence.[24,25]

In view of the protein catabolism that arises in disease states, researchers have proposed that cell shrinkage or loss of cellular volume caused by a loss of intracellular water volume, as is often seen in skeletal muscle and liver tissue, may be a common endpoint triggering protein catabolism in a variety of disease states.

CLINICAL APPLICATIONS

Hydration and the Elderly

Dehydration is one of the most common causes of hospitalization among persons over the age of 65. It has been estimated that one half of those admitted for clinical dehydration die within a year of admission.

There are many factors leading to clinical and subclinical dehydration among seniors:

- Lower body water percentage
- Lack of awareness of hydration needs
- Lack of mental clarity and attentiveness to personal needs
- Illnesses that accelerate water loss by inducing vomiting, fever, and/or diarrhea
- Decline of thirst sensation with age
- Higher likelihood of multiple medication usage, including diuretics, which increase fluid loss, and or sedating medications, which reduce the desire for fluid intake[26]

Gradual total body dehydration is a hallmark of the aging process, and occurs in 20% to 30% of older adults.[27] In addition, the preponderance of body water shifts gradually with age from cell interiors to the exterior connective tissue, and a decline in fat-free mass and muscle reduce the fluid reserve from these tissues. Kidney function also declines, limiting the ability to concentrate urine and retain fluid. Even death itself is frequently linked to dehydration. Long-term chronic disease eventually wears the body down to the point where patients can no longer ingest water and food, ultimately succumbing to dehydration.

To make matters worse, our sense of thirst declines with age, though the reaction to thirst does not. Researchers have concluded that after water deprivation, "there is a deficit in thirst and water intake in healthy elderly men, compared with younger men." This is true despite the fact that the antidiuretic hormone response is maintained.[28]

Chronic "subclinical" dehydration (or "hypohydration") contributes to the aging process. In its struggle for survival, the body shifts precious resources away from processes that increase longevity toward those needed for short-term survival.

Winter Hydration

The human body loses water in many ways during winter. Some are obvious; others are not:

- Exercising in cold weather mutes the experience of sweating in many people, deceiving them into thinking that their bodies are not losing much water.
- Cold air cannot hold as much moisture as warm air. Therefore winter air is drier than summer air. The drier air draws more water from the lungs as we breathe, so we exhale more moisture during winter months.
- Interior environments are usually very dry in the winter because of the use of dehumidifying heating methods. The decreased interior humidity increases water loss from the lungs and skin.
- When the body gets chilled, blood is shifted away from the periphery toward the interior organs to preserve vital heat. The shunting of blood to the interior increases renal arterial flow, glomerular filtration rate, and urine output. This effect is called cold diuresis.
- Cold weather increases body metabolism and the associated water needs required to maintain healthy body temperature.
- Respiratory illnesses of winter cause the body to generate large amounts of mucus to dispose of pathogens. The water in these discharges must be replaced.
- Intestinal influenza, with diarrhea and vomiting, requires additional water and perhaps electrolyte replacement.

Dehydration and Jet Lag

The cabin atmospheric pressure of modern passenger jets is equivalent to that found on land at an elevation of 8000 ft above sea level. Although the relative humidity of the Sahara Desert is typically 20% to 25%, the humidity of jet cabin air can be as dry as 1% to 10%. For these reasons, dehydration is a major factor in the experience of jet lag. People should keep thoroughly hydrated with pure water and avoid the other beverages offered by flight attendants. It is prudent to drink at least 8 ounces of pure water per hour.

Effect of Hydration State on Blood Flow

Whole-blood viscosity has been identified as an independent risk factor for atherosclerosis and cardiovascular events. The most dangerous time of day for heart attack is the last period of sleep and the first few hours in the morning after awakening. A number of factors may play

a role in this trend; hydration state may be one of them. Overnight fasting from fluid intake increases blood viscosity and adversely affects blood flow (rheology) to major organs. Consumption of 200 mL of water under these circumstances helps normalize blood flow to major organs.[29]

Cognitive-Motor Function and Hydration State

In young adults, neither cognitive-motor function nor neurophysiological function differs between people under water deprivation and controls. On the other hand, subjective ratings of mental performance during water deprivation point to increased fatigue as well as impaired alertness and the concentration needed to complete tasks. Healthy people tend to employ compensating mechanisms for increased fatigue and reduced alertness. Test of reaction time reveal significant gender differences in the response to dehydration. Water deprivation induces a prolonged reaction time in women but a shortened one in men.[30]

Multiple-Organ-System Effects of Chronic Subclinical Dehydration

The insidious effects of chronic hypohydration are wide ranging and staggering. Consider the following effects:

- Disintegration of cellular structure
- Impaired flow of nutrients into the cell as a result of compromised membrane protein channels and insufficient carrier solvent (water)
- Local tissue resistance to endocrine hormones caused by faulty integrity and responsiveness of membrane receptors
- Chronic fatigue resulting from lack of enzyme-catalyzed energy production[31]
- Free radical damage to cell structures, including DNA, as a result of reduced enzymatic free radical scavenging
- Inadequate repair of nuclear DNA damage resulting from faulty enzyme repair activity
- Reduced production of key bioactive compounds, such as hormones, digestive enzymes, neurotransmitters, etc.
- Accumulation of toxins within cells
- Increased synthesis of histamine within the central nervous system[32]
- Chronically elevated levels of antidiuretic hormone (vasopressin)
- Chronically elevated levels of cortisol, with adverse effects on bone integrity, muscle mass, connective tissue, blood pressure, and immunity[2]
- Development of chronic kidney disease, exacerbated by increased vasopressin release, cortical aldose reductase activation (which leads to uric acid production and oxidative stress), and hyperuricemia[33]

Subclinical Dehydration (Hypohydration): The Missing Diagnosis

Water cures nothing except dehydration. However, once dehydration has been corrected at the cellular level, healing is possible. This is because hypohydration at the cellular level is a major contributing factor to many ailments. Based on years of research with inmates of an Iranian prison, Dr. Fereydoon Batmanghelidj has proposed a new paradigm of disease and healing. It states that the suffering associated with many disorders is triggered or worsened by dehydration.

The reason for this effect is that, under the stress of dehydration, the body takes desperate measures to conserve water. Part of this effort involves the synthesis and release of histamine. It appears that histamine release activates other systems designed to save body water. Antidiuretic hormone decreases water loss in urine. Histamine and kinin compounds influence the escape of water from capillaries into connective tissue. Decreasing blood water content decreases blood volume and increases plasma sodium concentration, activating the renin-angiotensin system and vasopressin to elevate blood pressure.[4,5,34]

A key concept of Dr. Batmanghelidj's new paradigm is based on his years of clinical observation and practice with only the simplest crude healing tools at his disposal. He writes, "Chronic cellular dehydration painfully and prematurely kills. Its initial outward manifestations have until now been labeled as diseases of unknown origin."

Recurrent noninfectious conditions associated with pain and discomfort in various parts of the body, which cannot be explained by other identifiable causes, can be interpreted as expressions of water deficits at the sites of the tissue manifesting symptoms.[4,35]

COMMON CONDITIONS IMPROVED BY WATER

On the basis of empirical experience, the scope of application of water as a therapeutic tool is immense. The only caveat is that kidney filtration must be intact and healthy to allow the excretion of water and accompanying toxins. Additionally, given the vast number of pollutants found in most water sources, an effort to provide pure water must be made.[36,37] The following is a list of disorders in which water therapy is indicated:

- Coronary heart disease
- Peptic ulcers
- Arthritis
- Hypertension
- Low back pain
- Intermittent claudication
- Fibromyalgia
- Migraine headache
- Hangover headache
- Constipation
- Colitis
- Obesity
- Edema of unknown origin
- Chronic fatigue syndrome

A few of these are considered in more detail later.

Coronary Heart Disease and Fatal Myocardial Infarction

It has been known for some time that high blood and plasma viscosity, high hematocrit, and high blood concentrations of fibrinogen are correlated with coronary heart disease and atherosclerosis. Remarkably, even high "normal" levels of these parameters are considered independent risk factors.[21,31,34,35,38–43] These factors have also been linked to intermittent claudication.[41] High hematocrit has been associated with tachycardia, the magnitude of heart tissue damage from myocardial infarction, reduced oxygen transport, and reduced blood supply to heart tissue.

Based on this evidence, researchers at the Heart Institute of Loma Linda University analyzed lifestyle data to determine what influence the consumption of pure water and other beverages would have on the risk of fatal heart attack.

Compared with those who drank two or fewer glasses of pure water daily, men who drank five or more had only 46% of the risk of having a fatal heart attack and women had only 59% of the risk. Even more remarkable, compared with those who drank two or fewer glasses of fluids other than pure water (e.g., tea, soft drink, juice, etc.), women who drank five or more had 147% greater risk and men had 46% greater risk. Moreover, these relative risk relationships held regardless of adjustments for any other factors. In essence, the consumption of pure water decreases the risk of fatal heart attack; consumption of other beverages increases the risk.[32]

According to the researchers, failing to drink enough water can be as harmful to heart health as smoking. Just by increasing pure water intake, one can reduce the risk of death from heart attack by one half. This amount of benefit is greater than that gained by ceasing smoking, reducing cholesterol, exercising, or maintaining ideal body weight. Thus increasing one's intake of pure water could be the cheapest and simplest imaginable method of reducing fatal heart attack risk.

Peptic Ulcers and Dyspepsia

These conditions were the first that brought hidden, subclinical dehydration to the attention of Dr. Batmanghelidj in the Iranian prison. Thousands of cases successfully treated with water therapy lend credence to this modality. Chronic inflammation of the stomach and duodenum, with heartburn and acid reflux, are prime indications for water intervention. The concept here is not farfetched when the function of the intestinal organs is understood. The gastrointestinal mucous membranes must produce sufficient mucus to protect themselves from damage from digestive acid and enzymes. All of these secretions are water-based. Hypohydration inhibits both digestion and membrane protection. In addition, water deficits reduce the volume of bicarbonate-containing fluids released from the pancreas. This deficit results in a failure to properly neutralize stomach acid, leading to duodenal ulcers.[44]

Asthma and Allergies

Histamine is a prime mediator of allergy and asthma. It is generated in the central nervous system when the body is dehydrated. It is also released by mast cells located on the mucous membranes of the respiratory and gastrointestinal tracts. Histamine works with the immune system, facilitating the movement of white blood cells to sites of microbial invasion.

Of particular note is the new scientific light being shed on exercise-induced asthma. Studies indicate that dehydration during exercise can increase the intensity of asthma symptoms in persons subject to asthmatic attacks. Dehydration can increase spasms of the bronchial smooth muscle because of overly dry airway membranes. Dehydration of mucous membranes occurs before the asthmatic athlete even begins training. Dehydrated asthmatics begin exercise with reduced hydration capacity; therefore a pathological respiratory state occurs more rapidly. Researchers have concluded that, "Exercise induced asthma is an exaggerated airway response to airway dehydration." Airway narrowing from exercise in elite athletes and otherwise healthy subjects is now considered a physiological response to pathological changes in airway cells resulting from "dehydration injury."[45] These changes also occur in healthy subjects exercising intensely for long periods and breathing cold air, dry air, or both.[46]

To minimize the risk of asthmatic attacks during exercise, it is imperative for the athlete to be in a state of optimal hydration before training begins and for good hydration status to be maintained throughout the exercise and during recovery. Pure water can be a very dear friend to an asthmatic.

Migraine Headache

As with allergies and asthma, histamine is believed to be a contributing factor in migraine headache. (See Chapter 198, Migraine, for more complete discussion.) Hypohydration induces the central nervous system to increase its synthesis of histamine. Thus optimal hydration should minimize histamine synthesis as well as associated migraine headache. Additionally, hydration status may also affect other variables known to trigger migraines. For example, changes in physical exercise and weather may be related to water loss, and a drop in osmolality during the luteal phase of the menstrual cycle may have an effect.[47] Anecdotal clinical evidence is mounting in support of pure water as a helpful adjunct to other natural approaches.

Weight Loss

A higher water intake has been associated with more significant weight and body fat loss over time, independently of other variables.[48] Additionally, in a randomized controlled trial, water preloading before a meal was also associated with additional weight loss.[49] This is likely mediated via a reduced energy intake with the subsequent meal.[50]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Meyerowitz S. *Water: The Ultimate Cure*. Summertown, TN: Book Publishing Co.
2. Wang Z, Deurenberg P, Wang W, et al. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr*. 1999 May;69(5):833–841.
3. Jéquier E, Constant F. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr*. 2010;64(2):115–123. Epub 2009 Sep 2.
4. Häussinger D. The role of cellular hydration in the regulation of cell function. *Biochem J*. 1996;313(Pt 3):697–710.
5. Szinnai G, Schachinger H, Arnaud MJ, et al. Effect of water deprivation on cognitive-motor performance in healthy men and women. *Am J Physiol Regul Integr Comp Physiol*. 2005;289(1):R275–R280. Epub 2005 Apr 21.
6. Hooper L, Bunn D, Jimoh FO, et al. Water-loss dehydration and aging. *Mech Ageing Dev*. 2014;136–137:50–58. PMID:24333321.
7. Berne RM, Levy MN. In: *Principles of Physiology*. 3rd ed. St. Louis: Mosby, Inc; 2000:4-18:434–454.
8. Leib DE, Zimmerman CA, Knight ZA. Thirst. *Curr Biol*. 2016;26(24):R1260–R1265. PMID:27997832.
9. Hooper L, Abdelhamid A, Attreed NJ, et al. Clinical symptoms, signs and tests for identification of impending and current water-loss dehydration in older people. *Cochrane Database Syst Rev*. 2015;4:CD009647. PMID:25924806.
10. Draft Dietary reference values for water. Scientific opinion of the panel on dietetic products, nutrition and allergies. *Eur Food Safety J*. 2008:1–49.
11. Sawka MN, Cheuvront SN, Carter III R. Human water needs. *Nutr Rev*. 2005;63(6 Pt 2):S30–S39.
12. Stallings VA, Harrison M, Oria M. *Dietary Reference Intakes: Electrolytes and water. DRI table for sodium, chloride, potassium, inorganic sulfate and water. National Academy of Sciences. Institute of Medicine*. Washington, DC: Food and Nutrition Board; 2019.
13. Case DJ, et al. National Athletic Trainers' Association position statement: fluid replacement for athletes. *J Athl Train*. 2000;35(2):212–224.
14. McDermott BP, Anderson SA, Armstrong LE, et al. National athletic trainers' association position statement: fluid replacement for the physically active. *J Athl Train*. 2017;52(9):877–895. PMID:28985128.
15. Berne RM, Levy MN. In: *Principles of Physiology*. 3rd ed. St. Louis: Mosby, Inc; 200:10.
16. Garrow JS, et al. *Human Nutrition and Dietetics*. London: Churchill Livingstone;149.
17. Montain SJ, Cheuvront SN, Sawka MN. Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. *Br J Sports Med*. 2006;40(2):98–105. PMID:16431994.
18. Berne RM, Levy MN. In: *Principles of Physiology*. 3rd ed. St. Louis: Mosby, Inc; 2000:334.
19. Borgnia M, et al. Cellular and molecular biology of the aquaporin water channels. *Annu Rev Biochem*. 1999;68:425–458.
20. Champe P, Harvey R. In: *Lippincott's Illustrated Reviews: Biochemistry*. 2nd ed. Philadelphia: J. B. Lippincott Company; 1994:13.
21. de Simone G, et al. Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. *Circulation*. 1990;81(1):107–117.
22. Chaplin M. *School of Applied Sciences*. South Bank University, UK. www.sbu.ac.uk/water. Accessed 25 Oct 2019.
23. Watterson JG. The role of water in cell architecture. *Mol Cell Biochem*. 1988;79(2):101–105.
24. Häussinger D. Control of protein turnover by the cellular hydration state. *Ital J Gastroenterol*. 1993;25(1):42–48.
25. Häussinger D, Roth E, Lang F, et al. Cellular hydration state: an important determinant of protein catabolism in health and disease. *Lancet*. 1993;341(8856):1330–1332.
26. Collins M, Claros E. Recognizing the face of dehydration. *Nursing*. 2011;41(8):26–31. PMID:21750468.
27. Miller HJ. Dehydration in the Older Adult. *J Gerontol Nurs*. 2015;41(9):8–13. PMID:26375144.
28. Phillips PA, et al. Reduced thirst after water deprivation in healthy elderly men. *N Engl J Med*. 1984;311(12):753–759.
29. Vlastos GA, Tangney CC, Rosenson RS. Effects of hydration on blood rheology. *Clin Hemorheol Microcirc*. 2003;28(1):41–49.
30. Szinnai G, Schachinger H, Arnaud MJ, et al. Effect of water deprivation on cognitive-motor performance in healthy men and women. *Am J Physiol Regul Integr Comp Physiol*. 2005;289(1):R275–R280.
31. Kannel WB, McGee DL. Update on some epidemiologic features of intermittent claudication: the Framingham study. *J Am Geriatr Soc*. 1985;33(1):13–18.
32. Chan J, et al. Water, other fluids, and fatal coronary heart disease: the adventist health study. *Am J Epidemiol*. 2002;155(9):827–833.
33. Roncal-Jimenez C1, Lanaspa MA, et al. Mechanisms by which dehydration may lead to chronic kidney disease. *Ann Nutr Metab*. 2015;66(suppl 3):10–13. PMID:26088040.
34. Kjaer A, et al. Dehydration stimulates hypothalamic gene expression of histamine synthesis enzyme: importance for neuroendocrine regulation of vasopressin and oxytocin secretion. *Endocrinology*. 1995;136(5):2189–2197.
35. Batmanghelidj F. Pain: a need for paradigm change. *Anticancer Res*. 1987;7(5B):971–989.
36. Roh T, Lynch CF, Weyer P, et al. Low-level arsenic exposure from drinking water is associated with prostate cancer in Iowa. *Environ Res*. 2017;159:338–343. PMID:28841521.
37. Mendez WM Jr, Eftim S, Cohen J, et al. Relationships between arsenic concentrations in drinking water and lung and bladder cancer incidence in U.S. counties. *J Expo Sci Environ Epidemiol*. 2017;27(3):235–243. PMID:27901016.
38. Lowe GD, et al. Blood viscosity and risk of cardiovascular events: the Edinburgh artery study. *Br J Haematol*. 1997;96(1):168–173.
39. Lee AJ, et al. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh artery study. *Circulation*. 1998;97(15):1467–1473.
40. Lowe GD, et al. Relation between extent of coronary artery disease and blood viscosity. *Br Med J*. 1980;280(6215):673–674.
41. Koenig W, Ernst E. The possible role of hemorheology in atherothrombogenesis. *Atherosclerosis*. 1992;94(2-3):93–107.
42. Erikssen G, et al. Haematocrit: a predictor of cardiovascular mortality? *J Intern Med*. 1993;234(5):493–499.
43. Resch KL, et al. Can rheologic variables be of prognostic relevance in arteriosclerotic diseases? *Angiology*. 1991;42(12):963–970.
44. Batmanghelidj F. A new and natural method of treatment of peptic ulcer disease. *J Clin Gastroenterol*. 1983;5(3):203–205.
45. Medrala W, et al. Pathogenesis of exercise induced asthma. *Pol Merkuriusz Lek*. 2001;11(63):203–205.
46. Anderson SD, Holzer K. Exercise-induced asthma: is it the right diagnosis in elite athletes? *J Allergy Clin Immunol*. 2000;106(3):419–428.
47. Martins IP, Gouveia RG. More on water and migraine. *Cephalalgia*. 2007;27(4):372–374. PMID:17376115.
48. Stookey JD, Constant F, Popkin BM, et al. Drinking water is associated with weight loss in overweight dieting women independent of diet and activity. *Obesity (Silver Spring)*. 2008;16(11):2481–2488. PMID:18787524.
49. Parretti HM, Aveyard P, Blannin A, et al. Efficacy of water preloading before main meals as a strategy for weight loss in primary care patients with obesity: RCT. *Obesity (Silver Spring)*. 2015;23(9):1785–1791. PMID:26237305.
50. Dennis EA, Dengo AL, Comber DL, et al. Water consumption increases weight loss during a hypocaloric diet intervention in middle-aged and older adults. *Obesity (Silver Spring)*. 2010;18(2):300–307. PMID:19661958.

Zingiber officinale (Ginger)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

Zingiber officinale (family: *Zingiberaceae*)
Common name: ginger

GENERAL DESCRIPTION

Ginger is an erect perennial herb with thick tuberous rhizomes (underground stems) from which the aerial stem grows to a height of 2 to 4 ft. Grasslike alternate leaves 6 to 12 in. long and 0.75 in. wide shoot off from the aerial stem. Wild ginger produces a beautiful flower, but cultivated ginger rarely flowers.

Although ginger is native to southern Asia, it is now extensively cultivated throughout the tropics (e.g., India, China, Jamaica, Haiti, and Nigeria). Jamaica is the major producer, with exports to all parts of the world amounting to more than 2 million pounds annually.

The knotted and branched rhizome, commonly called the root, is the portion of ginger used for culinary and medicinal purposes (Fig. 130.1A and B). Extracts and the oleoresin are produced from dried unpeeled ginger because peeled ginger loses much of its essential oil content.[1,2] Ginger oil is produced from the fresh ginger via steam distillation.

CHEMICAL COMPOSITION

The following compounds have been isolated from ginger[1,2]:

- Starch (up to 50%)
- Protein (about 9%)
- Lipids (6%–8%) composed of triglycerides, phosphatidic acid, lecithins, and free fatty acids
- A protease (2%)
- Volatile oils (1%–3%), the principal components of which are sesquiterpenes (bisabolene, zingiberene, and zingiberol) and various "pungent" principles, aromatic ketones, known collectively as gingerols vitamins (especially niacin and vitamin A)
- Resins

The pungent principles are thought to be the most pharmacologically active components of ginger. Gingerol and its derivatives can be found in concentrations as high as 33% in ginger oleoresin (Fig. 130.2). The fresh oleoresin will have a higher percentage of the more pungent gingerol because gingerol can be dehydrated during storage to form shogaol or have its fatty-acid moiety cleaved to form zingerone (Figs. 130.3 and 130.4). The oleoresin is made by extracting the oily and resinous materials with the aid of a solvent (alcohol, hexane, or acetone). Pharmacokinetic studies in humans show that the major pungent principles are absorbed after oral dosing and can be detected as glucuronide and sulfate conjugates in the blood.[3]

HISTORY AND FOLK USE

Ginger has been used for thousands of years in China for medicinal purposes. Chinese records dating from the 4th century BC indicate that it was used to treat the following conditions[1]:

- Stomachache
- Diarrhea
- Nausea
- Cholera
- Hemorrhage
- Rheumatism
- Toothache

It was used by eclectic physicians in the United States in the late 1800s as a carminative, diaphoretic, appetite stimulant, and local counterirritant.[4]

Ginger is widely used as a spice, especially in Asian and Indian cuisine. It is also used in many baked goods, beverages (ginger ale), candy, liqueurs, and cosmetic products (perfumes, soaps, creams, etc.).

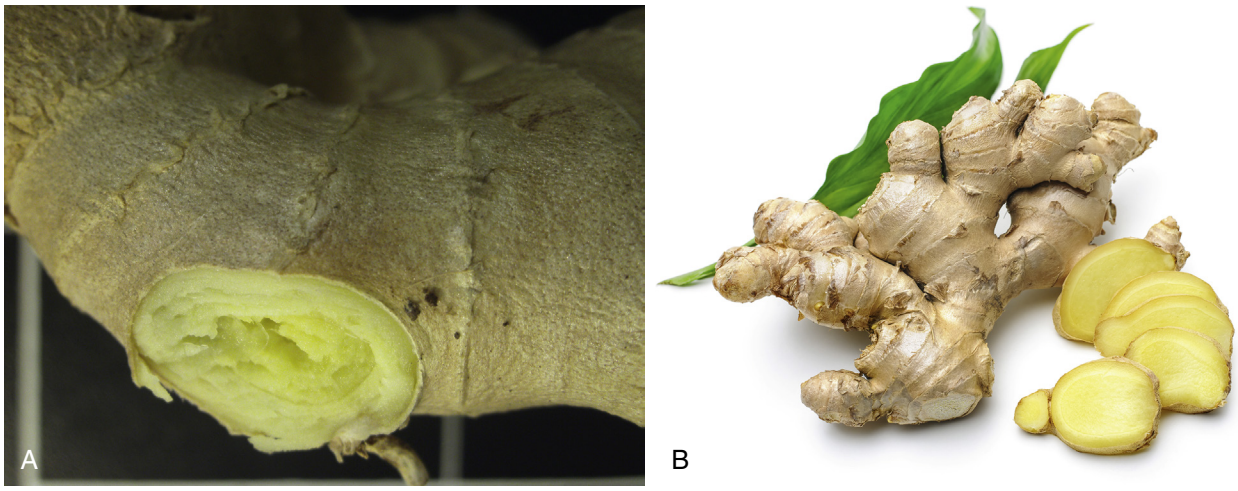


Fig. 130.1 (A) *Zingiber officinale* root. (B) *Z. officinale* rhizome. (B, From ValentynVolkov/iStock.com.)

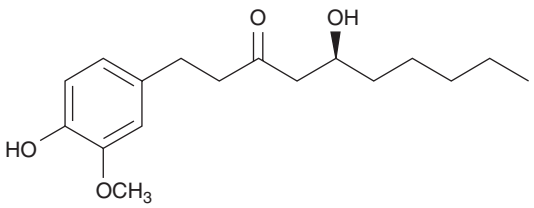


Fig. 130.2 Gingerol.

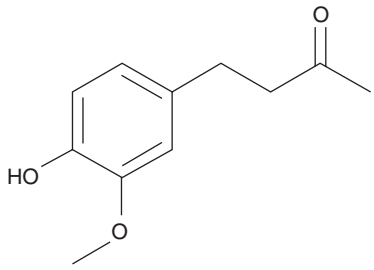


Fig. 130.3 Zingerone.

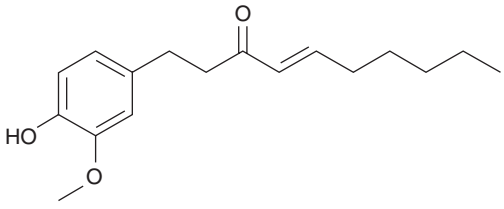


Fig. 130.4 Shogaol.

PHARMACOLOGY

Ginger possesses numerous pharmacological properties; the following are the most relevant:

- Antioxidant effects
- Inhibition of prostaglandin, thromboxane, and leukotriene synthesis
- Inhibition of platelet aggregation
- Cholesterol-lowering actions
- Choleretic effects
- Cardiotonic effects
- Gastrointestinal actions
- Thermogenic properties
- Antibiotic activities

Antioxidant Effects

Ginger has shown antioxidant effects in experimental studies.[5] In a study in rats, ginger significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase. The blood glutathione content was significantly increased in ginger-fed rats. To achieve a similar effect with vitamin C, the dosage required was 100 mg/kg body weight.[6]

Ginger's strong antioxidant properties have led to its being investigated for preventing the development of rancidity in meat products.[7] Ginger has been shown to prolong the shelf life of fresh, frozen, and precooked pork patties. Because the use of many synthetic antioxidants is prohibited by law, ginger may one day be used commercially to extend the shelf lives of meats and other foods.

Effects on Prostaglandin and Leukotriene Metabolism

Numerous constituents in ginger have been shown to be potent inhibitors of prostaglandin and leukotriene synthesis through blocking of the cyclooxygenase (COX) enzymes.[8–13] The most potent components appear to be the pungent principles, although the aqueous extract has also demonstrated inhibition. Inhibition of prostaglandin and leukotriene formation could explain some of ginger's historical use as an anti-inflammatory agent. However, ginger and its extracts also have strong antioxidant activities, and fresh ginger contains a protease with action that may be similar to that of other plant proteases (e.g., bromelain, ficin, papain) on inflammation.[1] Repeated ginger administration to mice augmented corticosteroid secretion, indicating that chronic ingestion may produce an anti-inflammatory effect via this mechanism as well.[14]

Effects on Platelets and Fibrinolysis

Ginger, like garlic and onions, is an inhibitor of platelet aggregation.[11] However, ginger's effects may be far more powerful. In a comparison, an aqueous extract of ginger was shown to exert greater inhibitory effects on platelet aggregation than aqueous garlic and onion extracts.[15] Ginger was shown to produce a greater inhibition of thromboxane formation and proaggregatory prostaglandins. Ginger, but not onion or garlic, also significantly reduced platelet lipid peroxide formation. In another model, gingerol compounds and their derivatives were more potent antiplatelet agents than aspirin.[11]

The superiority of ginger over onions was also demonstrated in a controlled study.[16] Female volunteers given either 70 g raw onion or 5 g raw ginger demonstrated that ginger has a pronounced effect in lowering platelet thromboxane production, whereas onion actually produced a mild elevation (pooled results).

In addition to acting on platelets, ginger promotes fibrinolysis. In one study, administration of 50 g of fat to 30 healthy adult volunteers decreased fibrinolytic activity from a mean of 64.20 to 52.10 U.[17] Supplementation of 5 g of ginger powder with the fatty meal not only prevented the drop in fibrinolytic activity but actually increased the activity significantly.

Cholesterol-Lowering and Hepatic Effects

Ginger has been shown to significantly reduce serum and hepatic cholesterol levels in cholesterol-fed rats by impairing cholesterol absorption as well as stimulating cholesterol-7-alpha-hydroxylase, the rate-limiting enzyme of bile acid synthesis.[18–20] In addition, ginger has been shown to increase bile secretion.[21] Therefore ginger works to lower cholesterol by promoting excretion and impairing absorption.

Cardiotonic and Hypotensive Properties

Gingerol has shown potent cardiotonic activity (positive inotropic and chronotropic effects) on isolated guinea pig left atria.[22,23] These effects are a result of the acceleration of calcium uptake by the sarcoplasmic reticulum. Gingerol was the first substance shown to produce these effects.

Individuals with heart problems or high blood pressure may benefit more from using fresh ginger rather than a dried preparation. This recommendation is based not only on the fact that gingerol is the more potent cardiotonic but also because animal studies demonstrate that shogaol has a blood pressure–elevating effect.[24] Gingerol is found predominantly in fresh ginger, whereas shogaol is rarely found in dried ginger.

Analgesic Effects

Ginger has demonstrated analgesic effects in experimental studies in animals.[25] This effect is thought to be a result of inhibition by shogaol of the release of substance P, much like that by capsaicin, the pungent principle of red pepper *(Capsicum frutescens)*.

Gastrointestinal Smooth Muscle Effects

Another aspect of ginger is its ability to simultaneously improve gastric motility and exert antispasmodic effects. This action is consistent with its use as a gastrointestinal tonic. A lipophilic ginger extract was shown in one study to enhance gastric motility, as evidenced by increased intestinal transport of a charcoal meal fed to rats,[26] and various fat-soluble components of ginger, such as galanolactone, demonstrated antagonism of serotonin receptor sites.[27] This latter mechanism may be responsible for ginger's antispasmodic effects on visceral and vascular smooth muscle.

In a human study, 1000 mg of dried ginger did not affect lower esophageal sphincter (LES) pressure at rest or esophageal contractile amplitude and duration when swallowing but caused more relaxation of the LES (after 90, 150, and 180 minutes, when swallowing) and decreased the esophageal contraction velocity, which may produce the expulsion of gastric gas or have an antiflatulent effect.[28] Ginger has also been shown to inhibit serotonin-induced diarrhea and exert antiemetic effects in experimental models.[29,30]

Via inhibition of prostaglandin production, ginger also prevents the slow-wave dysrhythmias produced by acute gastrointestinal hyperglycemia.[31]

Ginger accelerates gastric emptying and stimulates antral contractions in healthy volunteers.[32] Oral ginger extract was also shown to improve gastroduodenal motility in the fasting state and after a standard test meal in healthy human volunteers.[33]

Antiulcer Effects

Ginger has demonstrated significant antiulcer effects in a variety of animal models.[34–36] Ginger prevents ulcer formation from ethanol, indomethacin, aspirin, and other common ulcerogenic compounds. The pungent principles appear to be responsible for this effect. In one study, roasted ginger inhibited ulcer formation in three gastric ulcer models, but dry ginger had no such effect.[37]

A methanol extract of the dried powdered ginger rhizome, fractions of the extract, and the isolated constituents gingerol and shogaol were tested against 19 strains of *Helicobacter pylori*—a bacterium associated with peptic ulcers and gastric cancer. The methanol extract of ginger rhizome inhibited the growth of all 19 strains in vitro, with a minimum inhibitory concentration range of 6.25 to 50 mg/mL. One fraction of the crude extract, containing the gingerols, was active and inhibited the growth of all strains with a minimum inhibitory concentration range of 0.78 to 12.5 μg/mL.[38]

Thermogenic Properties

Ginger is noted for its ability to subjectively warm the body and has historically been used as a diaphoretic. In animal studies, ginger has been shown to help maintain body temperature and to inhibit serotonin-induced hypothermia.[29,39]

Crude extracts and the pungent components of ginger have been shown to increase oxygen consumption, perfusion pressure, and lactate production in the perfused rat hind limb.[40] These effects signify increased thermogenesis. Gingerol is the most potent thermogenic component of ginger. A human study demonstrated that consuming a ginger sauce (containing unspecified amounts of ginger principles) with a meal had no significant effect on metabolic rate.[41] However, there were two problems with this study: (1) the concentration of gingerol in the preparation used was probably low or zero, and (2) the effective concentration range of gingerol for its thermogenic effects is quite narrow.

Given ginger's historical use as a "warming" substance, these scientific investigations appear to support its use as a diaphoretic and thermogenic aid, although confirmation in humans is still lacking.

Antibiotic Activity

Ginger, shogaol, and zingerone have been shown to be strongly inhibitory against *Salmonella typhi, Vibrio cholerae,* and *Trichophyton violaceum,* whereas aqueous extracts at 2.5%, 5%, and 25% concentrations have been shown to be effective against *Trichomonas vaginalis.*[42] Ginger and its pungent principles were also demonstrated to possess significant antifungal activity against pathogenic yeast.[43]

Anticancer Effects

Ginger extracts and some pungent constituents present in ginger have exhibited antitumor activity in experimental models of carcinogenesis.[44] In 2018 a comprehensive review examined the literature pertaining to the use of ginger extract and [6]-gingerol against tumorigenic and oxidative and inflammatory processes associated with cancer, along with the underlying mechanisms of action involved in signaling pathways.[45] Data collected from in vitro or in vivo experiments and clinical studies indicate that ginger extract and [6]-gingerol exert their action through important mediators and pathways of cell signaling, including Bax/Bcl2, p38/MAPK, Nrf2, p65/nuclear factor (NF)-κB, tumor necrosis factor-α (TNF)-α, ERK ½, SAPK/JNK, ROS/NF-κB/COX-2,

caspase-3 and caspase-9, and p53. This suggests that ginger derivatives (extract or isolated compounds) exhibit relevant antiproliferative, antitumor, invasive, and anti-inflammatory activities.

Effects on Insulin and Blood Glucose

Patients with type 2 diabetes mellitus (T2DM) or metabolic syndrome (MetS) share common characteristics of raised blood sugar, decreased insulin sensitivity, obesity, dyslipidemia, and hypertension, which often appear simultaneously rather than alone. Ginger has been documented to ameliorate hyperlipidemia, hyperglycemia, oxidative stress, and inflammation and may therefore be a promising therapy for T2DM and MetS mediated by transcription factors, such as peroxisome proliferator-activated receptors, adenosine monophosphate-activated protein kinase, and NF-κB.[46] Additional proposed mechanisms of ginger include the inhibition of hepatic phosphorylase preventing glycogenolysis in hepatic cells, inhibition of hepatic glucose-6-phosphatase activity, and increasing glucose transporter type 4 (GLUT-4) to promote glucose uptake in adipocytes and skeletal muscle cells.

A thorough review and meta-analysis of randomized controlled trials revealed that ginger significantly reduces fasting blood glucose and HbA_{1c}, significantly improves fasting insulin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and ameliorates most of the MetS risk factors.[47] As an example, one randomized, double-blind, placebo-controlled trial of 88 patients with T2DM investigated the effect of ginger supplementation on insulin resistance and glycemic indices.[48] The cohort was randomly assigned to ginger or placebo groups. The ginger group consumed three 1-g capsules containing ginger powder daily, whereas the placebo group received capsules of the same color and number but containing cellulose microcrystalline. After 8 weeks, fasting blood sugar mean showed a decrease of 10.5% in the ginger group, whereas the mean had an increase of 21% in the placebo group. Variations in HbA_{1c} mean correlated with that of fasting blood sugar. A statistical difference was also found before and after the intervention in the median of fasting insulin level, insulin sensitivity, and HOMA-IR.

Two additional randomized, double-blind, placebo-controlled trials evaluated the effects of ginger on glycemic indices in patients with T2DM. The first study involved 64 patients with T2DM randomly assigned to either ginger (2 g/day) or placebo (2 g/day) groups.[49] After 2 months of intervention, ginger supplementation significantly lowered the levels of insulin, low-density lipoprotein (LDL) cholesterol, triglycerides, and the HOMA-IR. However, no significant changes were observed in fasting blood glucose, total cholesterol, high-density lipoprotein (HDL), and HbA_{1c}. In the second study, 20- to 60-year-old patients with T2DM who did not receive insulin were given 3 g of powdered ginger (intervention group) or 3 g of lactose (placebo group) daily for 3 months.[50] In addition to glycemic indices, the researchers also examined the effects of ginger on total antioxidant capacity (TAC), malondialdehyde (MDA), C-reactive protein (CRP), and serum paraoxonase (PON-1). At the end of the study, compared with placebo, the intervention (ginger) group had statistically significant reductions in serum glucose, HbA_{1c} percentage, insulin, insulin resistance, high-sensitive CRP, and MDA as well as statistically increased TAC and PON-1 activity. Although promising, further high-quality studies with larger sample sizes and longer duration of treatment are needed to examine these findings and evaluate discrepancies.

CLINICAL APPLICATIONS

Ginger is widely used as a condiment for its unique flavors, but from the previously described pharmacology, it obviously has important medicinal effects as well. In general, like many other culinary herbs and spices, such as garlic and onions, ginger provides many health-promoting effects. Specifically, ginger provides benefit to many body systems, including the digestive, hepatobiliary, and cardiovascular systems.

Historically, the majority of complaints for which ginger was used concerned the gastrointestinal system. A clue to ginger's efficacy in alleviating gastrointestinal distress is offered in several double-blind studies on motion sickness, hyperemesis gravidum, and postoperative nausea and vomiting. Human studies have also shown a positive effect in arthritis and migraine headaches.

Motion Sickness

Ginger was first shown to be effective in treating motion sickness by Mowrey and Clayson in 1982.[51] In their study, ginger (940 mg) was shown to be far superior to dimenhydrinate (Dramamine) 100 mg in relieving symptoms of nausea and vomiting. Since this initial study, several better-designed follow-up studies have evaluated the effectiveness of ginger as a motion sickness medication.

The appearance of motion sickness trials using ginger prompted an interest in ginger by the National Aeronautics and Space Administration, which subsequently funded a study at Louisiana State University. This study compared ginger, both fresh and dried powdered, with scopolamine by measuring the number of head movements experimental subjects could make in a rotating chair until they reached an end point defined as motion sickness short of vomiting. Ginger was not shown to produce any protection against motion sickness in this model or in two additional protocols (vestibular stimulation only and combined vestibular-visual stimulation).[52] However, in a double-blind trial, perhaps a more "real-life" test, ginger (1 g) given to naval cadets unaccustomed to sailing in heavy seas was shown to reduce the tendency toward vomiting and cold sweating in comparison with a placebo.[53]

Mowrey and Clayson[51] proposed that the anti–motion sickness effects of ginger were due to local gastrointestinal tract effects rather than to central nervous system effects. Although ginger's mechanism of action in alleviating gastrointestinal distress has yet to be fully elucidated, there is evidence to support this hypothesis. Ginger has been shown to partially inhibit the excessive gastric motility characteristic of motion sickness.[52] To further support a gastric versus a central nervous system mechanism of action, one study clearly demonstrated that neither the vestibular system nor the oculomotor system, both of which are critical in the occurrence of motion sickness, was influenced by ginger (1 g).[54] However, in a double-blind, crossover, placebo-controlled study, ginger (1 g) was shown to significantly reduce induced vertigo but not nystagmus.[55]

It has been hypothesized by others that ginger ameliorates the nausea associated with motion sickness by preventing the development of gastric dysrhythmias and the elevation of plasma vasopressin. To test this hypothesis, 13 volunteers with a history of motion sickness underwent circular vection, during which nausea (scored 0–3, i.e., none to severe), electrogastrographic recordings, and plasma vasopressin levels were assessed with or without ginger pretreatment in a crossover, double-blind, randomized, placebo-controlled study.[56] Circular vection induced a maximal nausea score of 2.5 and increased tachygastric activity and plasma vasopressin. Pretreatment with ginger (1000 and 2000 mg) effectively reduced nausea, tachygastric activity, and vasopressin release induced by circular vection.

The overall effectiveness of ginger in motion sickness has yet to be definitively determined. Issues that the studies have raised include the variability in the quality of commercial ginger preparations and the time required for ginger to produce its effects. Commercial preparations vary widely in chemical composition and often contain adulterants, and in the ginger study conducted at sea, ginger reduced

symptoms of cold sweating and vomiting only at the end of 4 hours. In other words, it appears that ginger may prove to be more effective when well-defined preparations are given at least 4 hours before motion is experienced.

Nausea and Vomiting

The mechanisms of action underlying ginger's efficiency in reducing nausea and vomiting have been investigated, and dual antiemetic actions have been highlighted: (1) gingerols and shogaols act as antagonists of cholinergic M3 and serotonin 5-HT3 receptors of the central nervous system; and (2) ginger's constituents improve the gastric tonus, motility, and emptying due to peripheral anticholinergic and antiserotonergic actions.[57] Ginger's antiemetic action has been studied in hyperemesis gravidum, the most severe form of pregnancy-related nausea and vomiting. This condition usually requires hospitalization. In a double-blind, randomized, crossover trial, ginger root powder at a dose of 250 mg four times a day brought about a significant reduction in both the severity of the nausea and the number of attacks of vomiting in 19 of 27 patients in the early stages of pregnancy (<20 weeks).[58]

Another natural approach to nausea and vomiting of pregnancy is vitamin B_6. In one double-blind study, 138 women were given either 500 mg of ginger or 10 mg of vitamin B_6 three times daily for 3 days.[59] Subjects graded the severity of their nausea using visual analog scales before treatment and recorded the number of vomiting episodes in the previous 24 hours and again during 3 consecutive days of treatment. The ginger and vitamin B_6 significantly reduced the nausea scores from 5 to 3.6 and 5.3 to 3.3, respectively, and the number of vomiting episodes from 1.9 to 1.2 and 1.7 to 1.2, respectively. There was no significant difference between ginger and vitamin B_6 for the treatment of nausea and vomiting during pregnancy. However, in another study, ginger was shown to be more effective.[60] In that study, 70 women were randomized to receive either ginger 1 g/day or vitamin B_6 40 mg/day for 4 days. Subjects graded the severity of their nausea using a visual analog scale and recorded the number of vomiting episodes in the 24 hours before treatment and during 4 consecutive days while taking treatment. Compared with baseline, the decrease in scores of posttherapy nausea in the ginger group was significantly greater than that for the vitamin B_6 group. In the ginger group, 29 of 35 women reported an improvement in nausea symptoms compared with 23 of 34 women in the vitamin B_6 group. These results indicate that ginger is more effective than vitamin B_6 for relieving the severity of nausea and is equally effective for decreasing the number of vomiting episodes in early pregnancy.

In a placebo-controlled study, 70 women with nausea and vomiting of pregnancy were randomly assigned to receive either oral ginger 1 g/day or an identical placebo for 4 days.[61] Subjects graded the severity of their nausea using visual analog scales and recorded the number of vomiting episodes in the 24 hours before treatment and again during 4 consecutive days during treatment. At a follow-up visit 7 days later, the number of vomiting episodes had decreased significantly in the ginger group; 28 of 32 in the ginger group had an improvement in nausea symptoms, compared with 10 of 35 in the placebo group.

In a double-blind study comparing ginger with dimenhydrinate, 170 pregnant women with the symptoms of nausea and vomiting in pregnancy were randomly allocated to take one capsule of ginger twice daily (one capsule contained 0.5 g of ginger powder), whereas the patients in group B received an identical capsule of 50 mg dimenhydrinate twice daily.[62] The results showed that ginger is as effective as dimenhydrinate in the treatment of nausea and vomiting during pregnancy and has fewer side effects. Specifically, there was a statistically significant difference in the side effect of drowsiness after treatment in diphenhydramine group compared with the ginger group (78% vs. 6%, respectively).

These clinical results and those reported by others,[63–67] along with the safety and the relatively small dose of ginger required and the problems (e.g., teratogenicity) with antiemetic drugs in pregnancy, support the use of ginger for nausea and vomiting in pregnancy. This recommendation is becoming a well-accepted prescription even in orthodox obstetrical practices.

Ginger appears to be very safe for use during pregnancy. A systematic review evaluating the efficacy and safety of ginger for nausea and vomiting of pregnancy did not identify any major toxicities from in vivo studies, and after observations of clinical studies, the use of ginger showed a significant decrease in nausea and vomiting and no risk for the mother or her future baby.[68]

The antiemetic action of ginger has also been observed in women who had undergone major gynecological surgery. In four double-blind studies, 1000 to 1500 mg of dried powdered ginger root per day was shown to significantly reduce the incidence of nausea compared with placebo in a manner similar to the drug metoclopramide.[69–72]

Ginger also has shown good results in alleviating chemotherapy-induced nausea and vomiting. In one study, 60 chemotherapy cycles of cisplatin/doxorubicin in patients with bone sarcoma were randomized to ginger root powder capsules or placebo capsules as an additional antiemetic to ondansetron and dexamethasone in a double-blind design.[73] Acute moderate to severe nausea was observed in 28 of 30 (93.3%) cycles in the control group compared with 15 of 27 (55.6%) cycles in the ginger group. In a study in women receiving cisplatin for gynecological cancers, the addition of ginger to a standard antiemetic regimen had no advantage in reducing nausea or vomiting in the acute phase of cisplatin-induced emesis.[74] However, after the initial 24 hours after cisplatin, ginger was shown to be equal to metoclopramide. In still another study, ginger provided no additional benefit in reducing the prevalence or severity of acute or delayed chemotherapy-induced nausea and vomiting when given with 5-HT3 receptor antagonists and/or aprepitant (Emend).[75]

Inflammatory Conditions

Ginger's ability to inhibit the formation of inflammatory prostaglandins, thromboxanes, and leukotrienes and its strong antioxidant activities and protease component suggest a possible benefit in inflammatory conditions. To test this hypothesis, a preliminary clinical study was conducted in seven patients with rheumatoid arthritis, in whom conventional drugs had provided only temporary or partial relief.[76] One patient took 50 g/day of lightly cooked ginger, and the other six took either 5 g of fresh or 0.1 to 1 g of powdered ginger daily. All patients reported substantial improvement, including relief of pain, greater joint mobility, and decreased swelling and morning stiffness.

In the follow-up to this study, 28 patients with rheumatoid arthritis, 18 with osteoarthritis, and 10 with muscular discomfort who had been taking powdered ginger for periods ranging from 3 months to 2.5 years were evaluated. On the basis of their clinical observations, Srivastava and Mustafa[77] reported that 75% of the patients with arthritis and 100% of the patients with muscular discomfort experienced relief in pain or swelling. The recommended dosage was 500 to 1000 mg/day, but many patients took three to four times this amount. Patients taking the higher dosages also reported quicker and better relief.

Three double-blind studies with standardized and highly concentrated extracts of ginger provide additional support for the usefulness of ginger in osteoarthritis, although in one study ginger was effective only after 3 months of use.[78–80] In the largest of the three studies, 261 patients with osteoarthritis of the knee were given either ginger extract or placebo twice daily, with acetaminophen allowed as rescue medication.[79] The primary efficacy variable was the proportion of responders experiencing a reduction in "knee pain on standing," with the use of an intent-to-treat

analysis. The percentage of responders experiencing a reduction in knee pain on standing was superior in the ginger extract group to that in the control group (63% vs. 50%). Analysis of the secondary efficacy variables revealed a consistently greater response in the ginger extract group compared with the control group in the following mean values: reduction in knee pain on standing (24.5 vs. 16.4 mm), reduction in knee pain after walking 50 ft (15.1 vs. 8.7 mm), and reduction in the Western Ontario and McMaster Universities' Osteoarthritis Composite Index (12.9 vs. 9 mm). Change in global status and reduction in intake of rescue medication were also numerically greater in the ginger extract group.

The effect of ginger on proinflammatory cytokines in patients with osteoarthritis was examined in a randomized, double-blind, placebo-controlled, 3-month clinical trial.[81] 120 participants were assigned to one of two groups: the ginger group (500 mg ginger powder) or the placebo group (500 mg starch). Although proinflammatory cytokine levels did not differ by group at baseline, at the end of the trial, serum levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) decreased in the ginger group relative to placebo.

One evaluation looking at ginger's ability to reduce muscle pain consisted of two identical double-blind, placebo-controlled randomized studies with participants consuming either 2 g of raw (study 1) or heated (study 2) ginger or placebo for 11 consecutive days.[82] Participants performed 18 eccentric actions of the elbow flexors to induce pain and inflammation. Pain intensity, perceived effort, plasma prostaglandin E(2), arm volume, range of motion, and isometric strength were assessed before and for 3 days after exercise. Raw and heat-treated ginger resulted in similar pain reductions 24 hours after eccentric exercise compared with placebo (25% and 23% reduction, respectively). In another study, a single 2-g dose of ginger did not attenuate eccentric exercise-induced muscle pain, inflammation, or dysfunction 45 minutes after ingestion, indicating that ginger's effects are likely more cumulative in reducing the day-to-day progression of muscle pain.[83]

It has also been reported that ginger is beneficial in migraine headache.[84] A double-blind, placebo-controlled, randomized clinical trial evaluated the potential of ginger to improve acute migraine as an add-on strategy to standard treatment.[85] Sixty participants were randomized into two groups in which they received 400 mg of ginger extract (5% active ingredient) or placebo (cellulose), in addition to an intravenous drug (100 mg of ketoprofen) to treat the migraine attack. Patients treated with ginger showed a significantly better clinical response after 1 hour, 1.5 hours, and 2 hours. Furthermore, ginger treatment promoted a reduction in pain and improvement in functional status at all times assessed. Given ginger's effects on platelets, eicosanoids, and serotonin inhibition, this recommendation makes sense.

DOSAGE

Many questions remain concerning the best form of ginger and the proper dosage. Most research studies have used 1 g of dried powdered ginger root. Practically speaking, this is a small dose. For example, ginger is commonly consumed in India at a daily dose of 8 to 10 g. Furthermore, although most studies have used powdered ginger root, fresh (or possibly freeze-dried) ginger root or extracts (concentrated for gingerol) at an equivalent dosage may yield even better results because they may deliver higher levels of gingerol as well as the active protease.

In the treatment of nausea and vomiting due to motion sickness or pregnancy or after surgery, a dosage of 1 to 2 g of dried powdered ginger may be effective. This would be equivalent to approximately 10 g or 1/3 oz of fresh ginger root, roughly a ¼-in. slice. For inflammatory conditions like rheumatoid arthritis, the dosage should be double this amount.

For ginger extracts standardized to contain 20% gingerol and shogaol, an equivalent dosage in treating motion sickness or nausea and vomiting would be 100 to 200 mg. For other applications, the dosage is 100 to 200 mg three times daily.

TOXICOLOGY

Ginger does not appear to produce any toxicity problems when used at normal dosages. Although ginger extracts and several components in ginger have been shown to possess potent mutagenic activity, ginger also contains several equally potent antimutagenic substances.[86,87] The significance of this mutagenicity (the study was conducted in *Escherichia coli* and did not use the Ames test) has not been entirely determined, but the long history of ginger's use and the lack of carcinogenic or toxic effect in animals suggest that toxicity is not a problem.

In acute toxicity tests in mice, ginger extract administered as a lavage was tolerated up to 2.5 g/kg with no mortality or side effects during a 7-day trial period.[88] Increasing the dosage to 3 to 3.5 g/kg resulted in a 10% to 30% mortality rate. In comparison, 0.6 g/kg of aspirin produced mortality in 25%, stomach ulcers in 40%, and hypothermia in 60% of subjects.

Some individuals consuming high doses—more than the equivalent of 6 g of dried powdered ginger—alone on an empty stomach may experience some gastrointestinal discomfort. Administration of 6 g of dried powdered ginger has been shown to increase the exfoliation of gastric surface epithelial cells in human subjects.[89] This effect may cause some gastric distress and ultimately could lead to ulcer formation. Therefore it is recommended that dosages consumed on an empty stomach be less than 6 g.

DRUG INTERACTIONS

Ginger may potentiate antiplatelet therapy.[90] However, ginger administration had no such effect in 12 healthy male subjects who received coumadin alone or with ginger. No changes in platelet aggregation, the international normalized ratio of prothrombin time, warfarin enantiomer protein binding, warfarin enantiomer concentrations in plasma, or S-7-hydroxywarfarin concentration in urine were seen.[91]

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Afzal M, Al-Hadidi D, Menon M, et al. Ginger: an ethnomedical, chemical and pharmacological review. *Drug Metab Drug Interact*. 2001;18:159–190.
2. Tyler V, Brady L, Robbers J In: *Pharmacognosy*. Philadelphia: Lea & Febiger; 1981:156–157.
3. Zick SM, Djuric Z, Ruffin MT, et al. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev*. 2008;17(8):1930–1936.
4. Felter H. *The eclectic Materia Medica, Pharmacology and Therapeutics*. Portland, OR: Eclectic Medical Publications; 1983.
5. Reddy AC, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem*. 1992;111:117–124.
6. Ahmed RS, Seth V, Banerjee BD. Influence of dietary ginger (*Zingiber officinale* Rosc) on antioxidant defense system in rat: comparison with ascorbic acid. *Indian J Exp Biol*. 2000;38:604–606.
7. Lee YB, Kim YS, Ashmore CR. Antioxidant property in ginger rhizome and its application to meat products. *J Food Sci*. 1986;51:20–23.
8. Kiuchi F, Iwakami S, Shibuya M, et al. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. *Chem Pharm Bull*. 1992;40:387–391.
9. Kiuchi F, Shibuyu M, Sankawa U. Inhibitors of prostaglandin biosynthesis from ginger. *Chem Pharm Bull*. 1982;30:754–757.
10. Srivastava KC. Isolation and effects of some ginger components of platelet aggregation and eicosanoid biosynthesis. *Prostaglandins Leukot Med*. 1986;25:187–198.
11. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, et al. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. *Thromb Res*. 2003;111:259–265.
12. Thomson M, Al-Qattan KK, Al-Sawan SM, et al. The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:475–478.
13. Tjendraputra E, Tran VH, Liu-Brennan D, et al. Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorg Chem*. 2001;29:156–163.
14. Ueda H, Ippoushi K, Takeuchi A. Repeated oral administration of a squeezed ginger (*Zingiber officinale*) extract augmented the serum corticosterone level and had anti-inflammatory properties. *Biosci Biotechnol Biochem*. 2010;74(11):2248–2252.
15. Srivastava K. Effects of aqueous extracts of onion, garlic and ginger on the platelet aggregation and metabolism of arachidonic acid in the blood vascular system. In vitro study. *Prostaglandins Leukot Med*. 1984;13:227–235.
16. Srivastawa KC. Effect of onion and ginger consumption on platelet thromboxane production in humans. *Prostaglandins Leukot Essent Fatty Acids*. 1989;35:183–185.
17. Verma SK, Bordia A. Ginger, fat and fibrinolysis. *Indian J Med Sci*. 2001;55:83–86.
18. Gujral S, Bhumra H, Swaroop M. Effect of ginger (*Zingiber officinale* Roscoe) oleoresin on serum and hepatic cholesterol levels in cholesterol fed rats. *Nutr Rep Int*. 1978;17:183–189.
19. el-Elrokh SM, Yassin NA, El-Shenawy SM, et al. Antihypercholesterolaemic effect of ginger rhizome (*Zingiber officinale*) in rats. *Inflammopharmacol*. 2010;18(6):309–315.
20. Srinivasan K, Sambaiah K. The effect of spices on cholesterol 7 alpha-hydroxylase activity and on serum and hepatic cholesterol levels in the rat. *Int J Vitam Nutr Res*. 1991;61:364–369.
21. Yamahara J, Miki K, Chisaka T, et al. Cholagogic effect of ginger and its active constituents. *J Ethnopharmacol*. 1985;13:217–225.
22. Shoji N, Iwasa A, Takemoto T, et al. Cardiotonic principles of ginger (*Zingiber officinale* Roscoe). *J Pharm Sci*. 1982;10:1174–1175.
23. Kobayashi M, Ishida Y, Shoji N, et al. Cardiotonic action of [8]-gingerol, an activator of the Ca^{+}+-pumping adenosine triphosphatase of sarcoplasmic reticulum, in Guinea pig arterial muscle. *J Pharmacol Exp Ther*. 1988;246:667–673.
24. Suekawa M, Aburada M, Hosoya E. Pharmacological studies on ginger. II. Pressor action of (6)-shogaol in anesthetized rats, or hindquarters, tail and mesenteric vascular beds of rats. *J Pharmacobiol*. 1986;9:842–860.
25. Onogi T, Minami M, Kuraishi Y, et al. Capsaicin-like effect of (6)-shogaol on substance P-containing primary afferents of rats: a possible mechanism of its analgesic action. *Neuropharmacol*. 1992;31:1165–1169.
26. Yamahara J, Huang QR, Li YH, et al. Gastrointestinal motility enhancing effect of ginger and its active constituents. *Chem Pharm Bull*. 1990;38:430–431.
27. Huang QR, Iwamoto M, Aoki S, et al. Anti-5-hydroxytryptamine effect of galanolactone, diterpinoid isolated from ginger. *Chem Pharm Bull*. 1991;39:397–399.
28. Lohsiriwat S, Rukkiat M, Chaikomin R, et al. Effect of ginger on lower esophageal sphincter pressure. *J Med Assoc Thai*. 2010;93(3):366–372.
29. Huang Q, Matsuda H, Sakai K, et al. The effect of ginger on serotonin induced hypothermia and diarrhea. *Yakugaku Zasshi*. 1990;110:936–942.
30. Vishwakarma SL, Pal SC, Kasture VS, et al. Anxiolytic and antiemetic activity of *Zingiber officinale*. *Phytother Res*. 2002;16:621–626.
31. Gonlachanvit S, Chen YH, Hasler WL, et al. Ginger reduces hyperglycemia-evoked gastric dysrhythmias in healthy humans: possible role of endogenous prostaglandins. *J Pharmacol Exp Ther*. 2003;307:1098–1103.
32. Wu KL, Rayner CK, Chuah SK, et al. Effects of ginger on gastric emptying and motility in healthy humans. *Eur J Gastroenterol Hepatol*. 2008;20(5):436–440.
33. Micklefield GH, Redeker Y, Meister V, et al. Effects of ginger on gastroduodenal motility. *Int J Clin Pharmacol Ther*. 1999;37:341–346.
34. al, Yahya MA, Rafatullah S, Mossa JS, Ageel AM, Parmar NS, Tariq M. Gastroprotective activity of ginger, *Zingiber officinale* Rosc., in albino rats. *Am J Chin Med*. 1989;17:51–56.
35. Yamahara J, Mochizuki M, Rong HQ, et al. The anti-ulcer effect in rats of ginger constituents. *J Ethnopharmacol*. 1988;23:299–304.
36. Yamahara J, Hatakeyama S, Taniguschi K, et al. Stomachic principles in ginger. II. Pungent and anti-ulcer effects of low polar constituents isolated from ginger, the dried rhizome of *Zingiber officinale* Roscoe cultivated in Taiwan: the absolute stereostructure of a new diarylheptanoid. *J Pharm Soc Japan*. 1992;112:645–655.
37. Wu H, Ye D, Bai Y, et al. Effect of dry ginger and roasted ginger on experimental gastric ulcers in rats. *China J Chinese Materia Medica*. 1990;5:278–280.
38. Mahady GB, Pendland SL, Yun GS, et al. Ginger (*Zingiber officinale* Roscoe) and the gingerols inhibit the growth of Cag A+ strains of *Helicobacter pylori*. *Anticancer Res*. 2003;23:3699–3702.
39. Kano Y, Zong QN, Komatsu K. Pharmacological properties of galenical preparation. XIV. Body temperature retaining effect of the Chinese traditional medicine, "goshuyu-to" and component crude drugs. *Chem Pharm Bull*. 1991;39:690–692.
40. Elderhsaw TP, Colquhoun EQ, Dora KA, et al. Pungent principles of ginger (*Zingiber officinale*) are thermogenic in the perfused rat hind limb. *Int J Obes Relat Metab Disord*. 1992;16:755–763.
41. Henry CJ, Piggott SM. Effect of ginger on metabolic rate. *Human Nutr Clin Nutr*. 1987;41:89–92.
42. Chang HM, But PPH. *Pharmacology and Applications of Chinese Materia Medica*. vol. 1. Philadelphia: World Scientific; 1986.
43. Ficker C, Smith ML, Akpagana K, et al. Bioassay-guided isolation and identification of antifungal compounds from ginger. *Phytother Res*. 2003;17:897–902.
44. Kundu JK, Na HK, Surh YJ. Ginger-derived phenolic substances with cancer preventive and therapeutic potential. *Forum Nutr*. 2009;61:182–192.
45. de Lima RMT, Dos Reis AC, de Menezed APM, et al. Protective and therapeutic potential of ginger (*Zingiber officinale*) extract and [6]-gingerol in cancer: a comprehensive review. *Phytother Res*. 2018. https://doi.org/10.1002/ptr.6134. [Epub ahead of print]. PubMed PMID: 30009484.
46. Wang J, Ke W, Bao R, Hu X, Chen F. Beneficial effects of ginger *Zingiber officinale* Roscoe on obesity and metabolic syndrome: a review. *Ann N Y Acad Sci*. 2017;1398(1):83–98. PubMed PMID: 28505392.
47. Zhu J, Chen H, Song Z, Wang X, Sun Z. Effects of ginger (*Zingiber Officinale* Roscoe) on type 2 diabetes mellitus and components of the metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Complement Alternat Med*. 2018:5692962. PubMed PMID: 29541142.
48. Mozaffari-Khosravi H, Talaei B, Jalali BA, Najarzadeh A, Mozayan MR. The effect of ginger powder supplementation on insulin resistance and glycemic indices in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Complement Ther Med*. 2014;22(1):9–16. PubMed PMID: 24559810.

49. Mahluji S, Attari VE, Mobasseri M, Payahoo L, Ostadrahimi A, Golzari SE. Effects of ginger (*Zingiber officinale*) on plasma glucose level, HbA1c and insulin sensitivity in type 2 diabetic patients. *Inter J Food Sci Nutri*. 2013;64(6):682–686. PubMed PMID: 23496212.
50. Shidfar F, Rajab A, Rahideh T, Khandouzi N, Hosseini S, Shidfar S. The effect of ginger (*Zingiber officinale*) on glycemic markers in patients with type 2 diabetes. *J Complement Integ Med*. 2015;12(2):165–170. PubMed PMID: 25719344.
51. Mowrey DB, Clayson DE. Motion sickness, ginger, and psychophysics. *Lancet*. 1982;I:655–657.
52. Stewart JJ, Wood MJ, Wood CD, et al. Effects of ginger on motion sickness susceptibility and gastric function. *Pharmacol*. 1991;42:111–120.
53. Grontved A, Brask T, Kamskard J, et al. Ginger root against seasickness. A controlled trial on the open sea. *Acta Otolaryngol*. 1988;105:45–49.
54. Holtman S, Clarke AH, Scherer H, et al. The anti-motion sickness mechanism of ginger: a comparative study with placebo and dimenhydrinate. *Acta Otolaryngol*. 1989;108:168–174.
55. Grontved A, Hentzer E. Vertigo-reducing effect of ginger root: a controlled clinical study. *ORL J Otorhinolaryngol Relat Spec*. 1986;48:282–286.
56. Lien HC, Sun WM, Chen YH, et al. Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. *Am J Physiol Gastrointest Liver Physiol*. 2003;284:G481–G489.
57. Lete I, allue J. The effectiveness of ginger in the prevention of nausea and vomiting during pregnancy and chemotherapy. *Integr Med Insights*. 2016;11:11–17. PubMed PMID: 27053918.
58. Fischer-Rasmussen W, Kjaer SK, Dahl C, et al. Ginger treatment of hyperemesis gravidarum. *Eur J Obstet Gynecol Reprod Biol*. 1990;38: 19–24.
59. Sripramote M, Lekhyananda N. A randomized comparison of ginger and vitamin B6 in the treatment of nausea and vomiting of pregnancy. *J Med Assoc Thai*. 2003;86:846–853.
60. Ensiyeh J, Sakineh MA. Comparing ginger and vitamin B6 for the treatment of nausea and vomiting in pregnancy: a randomised controlled trial. *Midwifery*. 2009;25(6):649–653.
61. Portnoi G, Chng LA, Karimi-Tabesh L, et al. Prospective comparative study of the safety and effectiveness of ginger for the treatment of nausea and vomiting in pregnancy. *Am J Obstet Gynecol*. 2003;189: 1374–1377.
62. Pongrojpaw D, Somprasit C, Chanthasenanont A. A randomized comparison of ginger and dimenhydrinate in the treatment of nausea and vomiting in pregnancy. *J Med Assoc Thai*. 2007;90(9):1703–1709.
63. Vutyavanich T, Kraisarin T, Ruangsri R. Ginger for nausea and vomiting in pregnancy: randomized, double-masked, placebo-controlled trial. *Obstet Gynecol*. 2001;97:577–582.
64. Keating A, Chez RA. Ginger syrup as an antiemetic in early pregnancy. *Altern Ther Health Med*. 2002;8:89–91.
65. Smith C, Crowther C, Willson K, et al. A randomized controlled trial of ginger to treat nausea and vomiting in pregnancy. *Obstet Gynecol*. 2004;103(4):639–645.
66. Willetts KE, Ekangaki A, Eden JA. Effect of a ginger extract on pregnancy-induced nausea: a randomised controlled trial. *Aust N Z J Obstet Gynaecol*. 2003;43(2):139–144.
67. Ozgoli G, Goli M, Simbar M. Effects of ginger capsules on pregnancy, nausea, and vomiting. *J Altern Complement Med*. 2009;15(3):243–146.
68. Stanisiere J, Mousset PY, Lafay S. How safe is ginger rhizome for decreasing nausea and vomiting in women during early pregnancy? *Foods*. 2018;7(4):E50. PubMed PMID: 29614764.
69. Bone ME, Wilkinson DJ, Young JR, et al. Ginger root—a new antiemetic. The effect of ginger root on postoperative nausea and vomiting after major gynecological surgery. *Anaesthesia*. 1990;45:669–671.
70. Pongrojpaw D, Chiamchanya C. The efficacy of ginger in prevention of post-operative nausea and vomiting after outpatient gynecological laparoscopy. *J Med Assoc Thai*. 2003;86:244–250.
71. Nanthakomon T, Pongrojpaw D. The efficacy of ginger in prevention of postoperative nausea and vomiting after major gynecologic surgery. *J Med Assoc Thai*. 2006;89(suppl 4):S130–S136.
72. Apariman S, Ratchanon S, Wiriyasirivej B. Effectiveness of ginger for prevention of nausea and vomiting after gynecological laparoscopy. *J Med Assoc Thai*. 2006;89(12):2003–2009.
73. Pillai AK, Sharma KK, Gupta YK, et al. Anti-emetic effect of ginger powder versus placebo as an add-on therapy in children and young adults receiving high emetogenic chemotherapy. *Pediatr Blood Cancer*. 2011;56(2):234–238.
74. Zick SM, Ruffin MT, Lee J, et al. Phase II trial of encapsulated ginger as a treatment for chemotherapy-induced nausea and vomiting. *Support Care Cancer*. 2009;17(5):563–572.
75. Manusirivithaya S, Sripramote M, Tangjitgamol S, et al. Antiemetic effect of ginger in gynecologic oncology patients receiving cisplatin. *Int J Gynecol Cancer*. 2004;14(6):1063–1069.
76. Srivastava KC, Mustafa T. Ginger (*Zingiber officinale*) and rheumatic disorders. *Med Hypotheses*. 1989;29:25–28.
77. Srivastava KC, Mustafa T. Ginger (*Zingiber officinale*) in rheumatism and musculoskeletal disorders. *Med Hypotheses*. 1992;39:342–348.
78. Altman RD, Marcussen KC. Effects of a ginger extract on knee pain in patients with osteoarthritis. *Arthritis Rheum*. 2001;44. 2531–2438.
79. Wigler I, Grotto I, Caspi D, et al. The effects of Zintona EC (a ginger extract) on symptomatic gonarthritis. *Osteoarthritis Cartilage*. 2003;11:783–789.
80. Bliddal H, Rosetzsky A, Schlichting P, et al. A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. *Osteoarthritis Cartilage*. 2000;8:9–12.
81. Mozaffari-Khosravi H, Naderi Z, Dehghan A, Nadjarzadeh A, Fallah Huseini H. Effect of ginger supplementation on proinflammatory cytokines in older patients with osteoarthritis: outcomes of a randomized controlled clinical trial. *J Nutri Gerontolgy Geriatrics*. 2016;35(3):209–218. PubMed PMID: 27559855.
82. Black CD, Herring MP, Hurley DJ, et al. Ginger (*Zingiber officinale*) reduces muscle pain caused by eccentric exercise. *J Pain*. 2010;11(9):894–903.
83. Black CD, O'Connor PJ. Acute effects of dietary ginger on muscle pain induced by eccentric exercise. *Phytother Res*. 2010;24(11):1620–1626.
84. Mustafa T, Srivastava KC. Ginger (*Zingiber officinale*) in migraine headaches. *J Ethnopharmacol*. 1990;29:267–273.
85. Martins LB, Rodrigues AMDS, Rodrigues DF, Dos Santos LC, Teixeira AL, Ferreira AVM. Double-blind placebo-controlled randomized clinical trial of ginger (*Zingiber officinale* Rosc.) addition in migraine acute treatment. *Cephalalgia*. 2018. https://doi.org/10.1177/0333102418776016. PubMed PMID: 29768938.
86. Nakamura H, Yamamoto T. Mutagen and antimutagen in ginger. *Zingiber Officinale Mutation Res*. 1982;103:119–126.
87. Nagabhushan M, Amonkar AJ, Bhide SV. Mutagenicity of gingerol and shogaol and antimutagenicity of zingerone in salmonella/microsome assay. *Cancer Lett*. 1987;36:221–233.
88. Macolo N, Jain R, Jain SC, et al. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol*. 1989;27:129–140.
89. Desai HG, Kalro RH, Choksi AP. Effect of ginger and garlic on DNA content of gastric aspirate. *Indian J Med Res*. 1990;92:139–141.
90. Young HY, Liao JC, Chang YS, et al. Synergistic effect of ginger and nifedipine on human platelet aggregation: a study in hypertensive patients and normal volunteers. *Am J Chin Med*. 2006;34(4):545–551.
91. Jiang X, Williams KM, Liauw WS, et al. Effect of ginkgo and ginger on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. *Br J Clin Pharmacol*. 2005;59(4):425–432.

SECTION 5

Syndromes and Special Topics[1]

Careful study of the various diseases to which humans are heir indicates that a limited number of underlying problems either cause or significantly contribute to most diseases. The reductionist philosophy underlying most of conventional medical research and practice results in more accurate diagnosis and more specific therapies for a given disease but makes recognition of broad patterns of disease causation progressively more difficult.

We believe the reader will find the syndromes discussed in this section interesting, relevant, and a compelling inducement for the development of a more holistic approach to patients. Old-fashioned ideas such as "bowel toxemia" and "liver toxicity" not only are intuitively reasonable but now have solid scientific evidence to support their validity. As one reads the chapters in this section, a compelling concept of medicine emerges:

Poor diet + unhealthy lifestyle + toxin exposure
↓
Nutritional deficiencies + toxicity
↓
(In the context of genetic susceptibility)
Metabolic dysfunction + subacute disease
↓
Clinically recognizable diseases that are apparently unrelated to the underlying causes

[1] Users of a previous edition of the TBNM will notice that this section moved from IV to V. This was done to improve the flow of the sections and to more logically combine Sections V and VI into the second volume.

131

Lifestyle Factors in Cancer

Lise Alschuler, ND

OUTLINE

INTRODUCTION

The mainstays of conventional cancer treatment—namely, surgery, chemotherapy, and radiation therapy—although preserving and prolonging the lives of many, present toleration challenges and often lack the desired effectiveness. An integrative naturopathic approach offers an adjunctive strategy that can improve both the toleration and efficacy of conventional cancer treatment. Fundamental to the naturopathic approach are diet, exercise, and stress management. Diet can be used to improve tolerance to radiation and chemotherapy, and specific dietary nutrients offer synergistic antineoplastic actions. Exercise is perhaps one of the most effective strategies to improve both tolerance and outcome. Finally, the effect of stress on prognosis is proving to be significant, pointing to the important role of stress management during any form of cancer treatment.

NUTRITION

Diet

There are several data points demonstrating a protective role of diet in cancer prevention and cancer control. Further, diet has considerable potential to optimize tolerance to conventional cancer therapies. Notably, there is evidence to suggest that patients who undergo conventional treatments without receiving nutritional support have higher complication rates.[1] Diet can be used to support optimal weight, specifically to prevent weight loss during treatment, to support bowel regularity, and to reduce localized areas of inflammation and pain (e.g., headaches, arthralgia, and mucositis).[2] Although the full arsenal of dietary interventions is beyond the scope of this chapter, several dietary approaches will be highlighted.

DIETARY PATTERNS

There are several dietary patterns and specific nutrients associated with improved survival in cancer treatment. However, when one looks across at the body of data as a whole, it is evident that the common denominator is a plant-based diet. Fruit, vegetables, and certain components of plant foods, such as fiber and polyphenols, have a large body of data supporting a protective effect against cancer.[3] Adherence to a Mediterranean diet (a diet high in plant foods, as well as olive oil, fish, and moderate wine) was found to be associated with a reduced risk of cancer incidence in the Greek EPIC trial, which comprised 25,623 participants. Adherence to the diet was assessed using a 10-point scale, and every 2-point increase in the score corresponded to a 12% reduction in cancer incidence (adjusted hazard ratio [HR], 0.88 [95% confidence interval (CI) 0.80, 0.95]).[4] The effect of this general dietary pattern of a plant-based diet has also been studied in specific populations of cancer patients.

In women diagnosed with breast cancer and treated with chemotherapy, self-report of hot flashes (HFs) after treatment for early-stage breast cancer has been associated with an approximately 25% to 30% decreased risk for additional breast cancer events, independent of the subsequent type of antiestrogen therapy. The HFs are due, in part, to lowered levels of circulating estrogen. With this in mind, the protective effect of a whole-foods, vegetable-rich diet might be especially relevant to women without HFs—essentially women with potentially higher circulating estradiol levels and worse prognosis.[5] Specifically, changes in dietary patterns to either decrease energy from fat or to increase fiber intake can alter the enterohepatic recirculation of estrogens, leading to lower circulating estrogen concentrations. A low-fat/high-fiber diet can be expected to reduce serum estradiol by an average of 7.5%,[6] an effect of particular importance to women diagnosed with estrogen receptor–positive (ER+) breast cancer. Although this effect is modest, if it persists over years, this would have biological significance. A secondary analysis of the Women's Healthy Eating and Living (WHEL) Randomized Trial[7] was conducted to determine whether HF-negative women gained specific benefit from the study diet, which consisted of the following: 5 vegetable servings plus 16 oz of vegetable juice, 3 fruit servings, 30 g of fiber, and limited energy intake from fat of 15% to 20% of total caloric intake.[8] Among women who reported no HFs (therefore presumably with higher estradiol levels and at greater risk) at baseline, there was a 31% lower recurrence rate in the group of women following these dietary recommendations than in the HF-negative women in the comparison group (no dietary intervention) over 7.3 years of follow-up. Among HF-negative postmenopausal women, the intervention effect was more significant, with a 47% reduction in risk compared with HF- women assigned to the comparison group.

The beneficial effect of fiber specifically has been noted in other trials. For instance, women diagnosed with breast cancer who, within 12

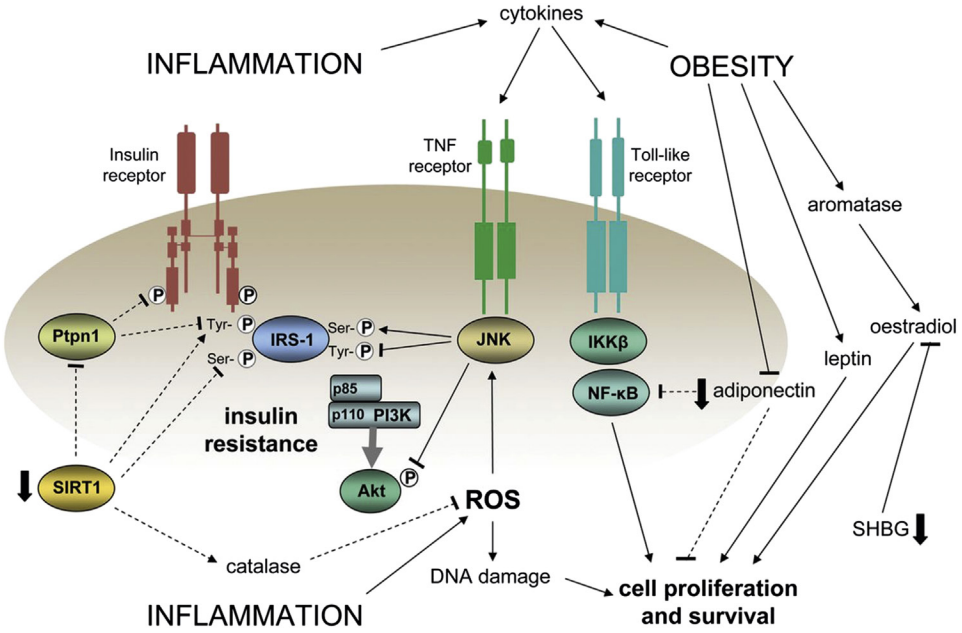


Fig. 131.1 Coincident promotion of insulin resistance and cell proliferation and survival in inflammation.[28] Activated cells of the immune system release cytokines and ROS, which induce insulin resistance via JNK and cell proliferation and survival via NF-κB. Positive influences are shown by lines ending in arrows and inhibitory influences by lines ending in bars. (Source: Godsland Ian F. Insulin resistance and hyperinsulinemia in the development and progression of cancer. *Clinical Science.* 118(5):315–332.)

months of their diagnosis, consume significant fiber (average consumption of 15.5 g/day of insoluble dietary fiber) experience a 49% reduction in the likelihood of having elevated C-reactive protein (CRP) concentrations (odds ratio [OR], 0.51; 95% CI, 0.27, 0.95) compared with those who consumed an average of 5.4 g/day ($P = 0.053$). This suggests an anti-inflammatory effect of fiber consumption, which in turn improves treatment toleration and is associated with improved survival.[9]

A dietary pattern characterized by a significant reduction in the consumption of saturated fat, increased consumption of vegetable proteins, and reductions in animal proteins and dairy products has been shown to significantly increase the doubling time of prostate-specific antigen (PSA) in men with prostate cancer.[10] The slowed PSA doubling time reflects decreased prostate cancer progression.

Colon cancer development and progression are also influenced by diet. Frequent consumption of red meat, refined carbohydrates, dairy, and eggs is associated with an increased risk for the development of colorectal cancer compared with infrequent consumption.[11] There is also a significant inverse relationship between total fiber intake and the risk of colorectal cancer (OR 0.57, 95% CI 0.47–0.68). Vegetable fiber appears to be more protective than either fruit or grain fiber.[12] In patients with diagnosed colon cancer, a dietary pattern that emphasizes plant foods and minimizes animal sources of protein would be expected to exert a beneficial effect on the colon, perhaps influencing progression risk.

Obesity

It is now estimated that 2.4% to 3.9% of cancer deaths can be attributed to obesity.[13] In an analysis of 70 clinical trials comprising 80,000 patients with early-stage breast cancer, the relative risk of dying from breast cancer was increased by 34% in obese (body mass index [BMI] > 30) premenopausal women (younger than age 55 y) with ER+ tumors.[14] In other words, the absolute 10-year breast cancer mortality for premenopausal women with ER+ disease was 21.5% for obese women compared with 16.6% for nonobese women. Postmenopausal obese women with ER+ disease had a 6% increased risk of dying from breast cancer. There was no association between obesity and breast cancer death in women with estrogen receptor–negative (ER–) tumors. Genetic analysis of pretreatment tumor biopsies has identified 121 genes with statistically significant changes in expression between obese and nonobese women.[15] In addition, obesity is characterized by hyperinsulinemia, estrogen signaling, and inflammation—all of which play important roles in obesity-accelerated breast cancer aggressiveness.

Obesity is also associated with unfavorable outcomes for patients with prostate cancer. A higher BMI (consistent with being overweight and obese) is predictive of a greater likelihood of rising PSA after surgery, indicating prostate cancer recurrence.[16] Furthermore, overweight and obese men experience shorter times to biochemical recurrence after surgery than normal-weight men.

Obesity is a known risk factor for the development of colorectal cancer, as well as its progression. Obesity-related dyslipidemias, increased adipokines, and elevated insulin and insulin-like growth factor-1 (IGF-1) are collectively associated with both increased colorectal cancer incidence and increased mortality in both men and women (see Fig. 131.1).[17] Obesity also negatively affects the effectiveness of conventional treatment with a mainstay of colorectal cancer treatment, bevacizumab. Bevacizumab is the main targeted therapy for inhibiting tumor angiogenesis by blocking the vascular endothelial growth factor (VEGF)/VEGF-receptor pathway. Obesity is associated with increased levels of VEGF, which could lead to resistance to anti-VEGF bevacizumab therapy. In fact, a prospective clinical trial demonstrated that in patients with metastatic colorectal cancer who were treated with bevacizumab, those who were overweight (BMI > 25 kg/m^2) experienced a significantly shorter time to progression ($p = 0.01$; HR: 4.37).[18]

Insulin Resistance

An important driver of malignant behavior in many cancer cell types is the significant expression of insulin and IGF-1 receptors.[19] As noted previously, insulin and IGF-1 are direct growth factors in these cancer cells.[20] Insulin and IGF-1 stimulate cellular proliferation in malignant cells via the constitutively "turned on" insulin receptor (IR) and IGF-1 receptors

(IGF-1R), culminating in mTOR activation. Activated mTOR drives proliferation, alters mitochondrial metabolism toward anabolism (aerobic glycolysis), and decreases apoptosis.[21] Interestingly, some cancers rely exclusively on insulin and IGF-1 for their growth, including an estimated 27% of breast cancers. Approximately 8% of these cases have upregulation of the PIK3/Akt pathway.[22] Additionally, IGF-IR is autophosphorylated in breast cancer cells with a predilection for metastasis to the brain. In vivo models demonstrate that experimental deactivation of IGF-IR attenuates the invasive and metastatic potential of these breast cancer cells, thereby delaying the development of brain metastases and prolonging survival. These preclinical findings are corroborated by the fact that 25% to 40% of patients with Her2+ and those with triple-negative breast cancer have a significantly increased risk of brain metastasis. This clinical finding correlates with increased IGF-IR signaling in these breast cancer subtypes.[23]

This concept has clinical application in the dietary advice given to patients. A trial followed 87 women with metastatic breast cancer receiving first-line liposomal doxorubicin and cyclophosphamide chemotherapy for a median of 15 months.[24] Of the subjects, 87% had hormone receptor–positive disease, and 48% were insulin resistant, with insulin resistance defined as a Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) score of greater than 2.5. (Of note, the HOMA-IR score can be calculated as serum glucose [mg/dL] × plasma insulin [uU/mL]/405, with a value greater than 2.5 indicative of insulin resistance.) Even after adjusting for other prognostic factors, such as patient age, the endocrine status of the tumor, visceral disease, and BMI, patients with advanced breast cancer and insulin resistance had a statistically significant higher risk of disease progression ($P = .035$). The median progression-free survival was 8 months in women with insulin resistance, compared with 14 months for those who did not have insulin resistance ($P = .04$).

A prospective, observational study of 1011 patients with stage III colon cancer reported their dietary intake during and for 6 months after conventional treatment.[25] The median follow-up from the time of completion of adjuvant therapy was 7.3 years. A higher dietary glycemic load was associated with statistically significant worse disease-free, recurrence-free, and overall survival. Specifically, patients with stage III colon cancer who were in the highest quintile of dietary glycemic load experienced an adjusted HR for disease recurrence of 1.79 (95% CI = 1.29–2.48) compared with those in the lowest quintile (HR = 1) (P trend across quintiles < .001). Increased glycemic load was associated with decreased overall survival (P trend across quintiles < .001). These associations were strongest for overweight patients (BMI > 25; HR 2.26). These data points support the use of a low-glycemic, nutrient-dense diet in people diagnosed with cancer.

Anti-Inflammatory Nutrients

Plant foods and spices concentrate polyphenols, which possess uniquely potent anti-inflammatory effects. The anti-inflammatory effects of polyphenols are illustrated, for instance, in a parallel-designed, placebo-controlled clinical trial of 120 men and women aged 40 to 74 years that compared the effect of 300 mg of an anthocyanin-rich drink isolated from bilberries and black currants to placebo over a 3-week period.[26] Consumption of the proanthocyanin-containing beverage was found to decrease nuclear factor kappa B (NF-κB)–controlled proinflammatory chemokines and interferon (IFN)-alpha (an inducer of NF-κB activation) by 45% and 40%, respectively, versus 20% and 15% in the placebo group ($P < 0.050$). Another trial assessed the effect of 30 grams of freeze-dried vegetable soup added to the daily diet of five patients with stage I non–small-cell lung cancer (NSCLC) in a toxicity study group and 6 patients with stage III and IV NSCLC in a treatment group for up to 24 months.[27] These patients were matched to 13 patients with stage III and IV NSCLC in the control group. The vegetable soup was a freeze-dried medicinal vegetable soup that included soybean, shiitake mushroom, mung bean, red date, scallion, garlic, lentil bean, leek, hawthorn fruit, onion, ginseng, angelica root, licorice, dandelion root, senegal root, ginger, olive, sesame seed, and parsley. All patients were treated with conventional therapies, including radiation, surgery, and/or chemotherapy. Those patients eating the vegetable soup had a median survival time of 15.5 months compared with a median survival time of 4.5 months in the control group ($P < 0.01$). There was no adverse toxicity in the vegetable group.[28]

In a controlled trial, 87 patients, 36 with resected colon cancer and 51 patients after polypectomy, were divided into two groups.[29] One group of 31 patients was treated daily with a flavonoid mixture of 20 mg apigenin and 20 mg epigallocatechin-gallate and compared with a matched control group of 56 patients. Both groups were observed for 3 to 4 years by surveillance colonoscopy and by questionnaire. Among the 14 patients with resected colon cancer and treated with the flavonoid mixture, there was no cancer recurrence, and only one adenoma developed. The cancer recurrence rate of the 15 matched untreated controls was 20% (3 of 15), and adenomas evolved in 4 of those patients (27%). The combined recurrence rate for neoplasia was 7% (1 of 14) in the treated patients and 47% (7 of 15) in the controls ($P = 0.027$).

In a trial of 26 men with newly diagnosed localized prostate cancer, the subjects were randomized to either 30 mg lycopene or no supplement before radical prostatectomy.[30] In the lycopene group, at surgery, 84% had tumors less than 4 mL, versus 45% in the control group. Additionally, 73% of the lycopene group and only 18% of the control group had clean margins. Prostate intraepithelial neoplasia was present in 67% of the lycopene group compared with 100% of the control group. Finally, PSA decreased by 18% in the lycopene group versus an increase of 14% in the control group.

A pooled analysis of three large prospective trials—the Shanghai Breast Cancer Survival Study (SBCSS), the Life After Cancer Epidemiology (LACE) Study, and the Women's Healthy Eating & Living (WHEL) Study—collectively representing 9514 breast cancer survivors with a mean follow-up of 7.4 years, assessed the effect of soy isoflavone.[31] Consumption of more than 10 mg isoflavones per day was associated with a 25% reduced risk of recurrence. This inverse association was seen in tamoxifen users, ER– women, and ER+ women.

The polyphenols found in plant foods both down-regulate inflammatory NF-κB and upregulate the transcription factor Nrf2. Nrf2 is normally sequestered in the cytoplasm as an inactive complex with its cytosolic repressor Keap-1. Phytochemicals, specifically polyphenolic flavonoids, activate diverse upstream kinases, which in turn stimulate dissociation of Nrf2 from Keap-1. Once released from Keap-1 repression, Nrf2 translocates to the nucleus, forms a heterodimer with small Maf protein, and binds to ARE/EpRE sequences located in the promoter region of genes encoding antioxidant and detoxifying enzymes.[32] This effect is synergistic with chemotherapy insofar as intracellular antioxidants are required to preserve the apoptotic (cell death) cascade initiated by chemotherapy. Additionally, polyphenols directly upregulate apoptosis, the ultimate step in removing aberrant cells. There are many examples of these proapoptotic polyphenols, such as transresveratrol[33] from grapes, peanuts, berries, and red wine. Genistein[34] from soy and curcumin[35] from turmeric activate apoptosis.

Fasting

A promising approach to improve patient tolerance of chemotherapy is concurrent fasting during chemotherapy. This approach has gained significant momentum from the research of Valter Longo, PhD. The premise of short-term starvation (STS) in an oncology context is twofold. First, when energy is scarce, cells will use energy preferentially for maintenance functions at the price of growth. Furthermore, IGF-1 levels decrease dramatically in response to short-term (36–120 hours)

of starvation. Cells throughout the body use IGF-1 to signal their growth. Thus fasting results in growth arrest of normal cells. However, most tumor cells have mutations in pTEN, p53, and the PI3K/Akt/mTOR pathway, leading to constitutive upregulation of insulin and the IGF-1–initiated proliferation pathway.[36] Thus, in malignant cells, STS and the resultant decrease in IGF-1 do not downregulate the PI3K/Akt/mTOR pathway and therefore do not arrest growth in cancer cells. This differential effect can be used concurrently with chemotherapy to preferentially protect healthy cells that will be in a dormant, maintenance, and nonproliferative state. This state renders these cells somewhat immune to the effects of chemotherapy. At the same time, the malignant cells retain their proliferation during STS and so remain susceptible to chemotherapy.

The effects of STS were demonstrated in a case-series report of 10 patients (7 females and 3 males) with cancer (4 with stage IIA breast cancer, 2 with prostate cancer [stage II and stage IV], 1 with stage IA ovarian cancer, 1 with stage IV endometrial cancer, 1 with stage IV NSCLC, and 1 with stage IVB esophageal cancer). [37] All patients received chemotherapy and underwent a water-only fast for 48 to 140 hours pre-chemotherapy and continued for 5 to 56 hours post-chemotherapy. Patients served as their own controls, and during fasted cycles, they experienced less toxicity even after nonfasted accumulation of toxicity. Patients received an average of our cycles of various chemotherapy drugs, including docetaxel/cyclophosphamide, docetaxel/carboplatin/±5-FU, carboplatin/paclitaxel, gemcitabine/docetaxel, docetaxel, and doxorubicin/cyclophosphamide. Specifically, the chemotherapy received during the water fast resulted in less fatigue, weakness, and gastrointestinal side effects.

Although the benefit and safety of this approach, specifically the effect of fasting on treatment response and survival, are still under clinical investigation, it could be considered empirically in patients who experience significant chemotherapy-induced toxicity to a level that is threatening their ability to complete treatment. Of note, preclinical research has indicated that fasting may reduce multidrug resistance in malignant cells[38]; however, this needs to be confirmed in human clinical trials. The exact protocol to optimize fasting regimens is under investigation. One fasting protocol being studied includes 24-, 36-, or 48-hour fasts before chemotherapy.[39] Another active clinical trial of women with gynecological cancers is studying the effect of modified fasting with daily caloric intake of < 400 kcal by juices starting 36 to 48 hours before beginning chemotherapy and lasting to 24 hours after ending each chemotherapy.[40]

Although not clinically evaluated, a variation of STS can also be considered between chemotherapy treatments and as a follow-up to conventional treatment. In the absence of active treatment, diet can be used to influence the same constitutively overactive IGF-1 and insulin-stimulated PI3K/Akt/mTOR pathway in malignant cells. This proliferation pathway's activity is enhanced in the presence of IGF-1 and insulin, both of which are reduced during caloric and carbohydrate restriction. Furthermore, dietary caloric restriction stimulates AMPk, which directly blocks mTOR activation (Fig. 131.2). The result of downregulating mTOR is reduced proliferation. Despite the promising theoretical basis for this approach, the clinical data on the effect of caloric and carbohydrate restriction on overall survival and recurrence risk in humans is yet to be determined.

Cachexia

Of note, this approach should not be considered for any patient at risk for cachexia, a condition of significant weakness and wasting caused by inflammatory cytokines released by malignant tissue. Certain cancers, such as lung cancer, pancreatic cancer, and many

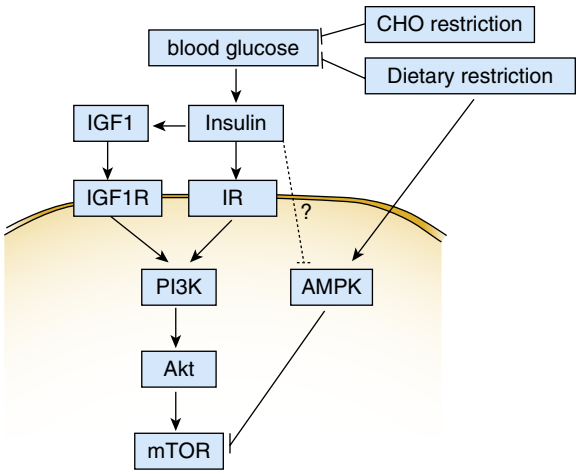


Fig. 131.2 Dietary Restriction and mTOR.

advanced cancers, carry a high risk of cachexia. The consumption of protein and essential fatty acids is a clinically validated way to both prevent and delay cachexia.[41] Protein requirements may exceed 80 gm/day in people at risk for cachexia. Typically, at least 0.45 to 0. 9g protein/2 kg body weight is needed to prevent and manage cachexia. Omega-3 fatty acids, especially eicosapentaenoic acid (EPA) at 2 to 3 g daily, is associated with weight gain and improved quality of life. Feeding (increased caloric intake) has not proven to control cachexia.

EXERCISE

Exercise is a critical component of a lifestyle-based support program during cancer treatment. Data collected over a median of 23 months postdiagnosis (interquartile range 18–32 months) were pooled in the After Breast Cancer Pooling Project (n = 13,302).[42] The study found that 2.5 h (10 metabolic-equivalent tasks [MET]-hours/week) of moderate-intensity physical activity per week was associated with a 27% reduction in all-cause mortality and a 25% reduction in breast cancer mortality compared with women who did not meet the physical activity guidelines (<10 MET-hours/week). In another study, women who engaged in the equivalent of at least 2 to 3 hours of brisk walking each week in the year before they were diagnosed with breast cancer were 31% less likely to die of the disease than women who were sedentary before their diagnosis (HR = 0.69 [95% CI, 0.45–1.06; P = .045]).[43] Women who increased physical activity after diagnosis had a 45% lower risk of death (HR = 0.55; 95% CI, 0.22–1.38) compared with women who were inactive both before and after diagnosis. Conversely, women who decreased physical activity after diagnosis had a fourfold greater risk of death (HR = 3.95; 95% CI, 1.45–10.50).

From a cohort of 184,194 adults without colorectal cancer at baseline in 1992 to 1993, 2293 participants were diagnosed with invasive, nonmetastatic colorectal cancer up to mid-2007.[44] The mean follow-up time from diagnosis to death or the end of the study was 6.8 years. Participants completed detailed questionnaires that included information concerning recreational physical activity and leisure time spent sitting at baseline, before their cancer diagnosis, and again after their cancer diagnosis. The highest prediagnosis recreational physical activity category (8.75 or more MET-hours per week, which is the equivalent of greater than 150 minutes/week) compared with the lowest category (3.5 MET-hours per week) was associated with a 28% lower risk of all-cause mortality. The same comparison for postdiagnosis recreational physical activity resulted in a 42% reduced risk of

mortality. Additionally, leisure time spent sitting 6 or more hours per day on the prediagnosis survey was associated with a statistically significant 36% higher risk of all-cause mortality. Postdiagnosis sitting time was associated with a statistically significant 62% higher risk of colorectal cancer–specific mortality. These studies support recommendations for recreational physical activity and the avoidance of sedentary time among people diagnosed with cancer—throughout the continuum of care.

STRESS MANAGEMENT

A third foundational component of lifestyle-based support of people undergoing cancer treatment is stress management. Elevated and prolonged stress hormones, namely cortisol, epinephrine, and norepinephrine, are associated with the carcinogenic process and shortened survival. The effects of stress on survival was elegantly demonstrated in a prospective trial of 217 participants with newly diagnosed metastatic renal cell cancer, all with a life expectancy of greater than 4 months, with good performance status and no major concurrent diseases.[45] All participants completed depression questionnaires, had salivary cortisol levels assessed, and provided blood samples for genomic analysis at baseline and at 4 months. The following factors were associated with decreased survival time: depression, poor quality of life, and flattened diurnal cortisol slope (with elevation of average cortisol). Genomic analyses identified upregulation of genes involved in inflammation and immune response and downregulation of genes that activate programmed cell death (all $p < .0001$) as well as genes involved in cell trafficking, adhesion, oxygen transport, and hemostasis (all $p < .05$).

Based on rodent models of triple-negative breast cancer, social isolation causes a heightened stress response that, in turn, increases expression of genes in adipocytes that increase glucose metabolism, lipid synthesis, and leptin secretion. These metabolic changes increase the conversion of mammary carcinoma in situ to invasive carcinoma. Mammary fat, in particular, has heightened sensitivity to stress hormones over visceral fat, making breast tissue especially vulnerable to stress.[46] Although the clinical evidence for the negative effects of stress is still developing, early evidence indicates the benefit of stress management on prognosis in people being treated for cancer. Furthermore, a more robust body of data demonstrates the improvement in quality of life that people diagnosed with cancer experience after active stress management.[47–49]

Mindfulness-based stress reduction (MBSR) is a particularly well-researched stress management behavior. A meta-analysis of 10 studies showed a significant improvement in psychological and physical quality of life with the practice of MBSR.[50] MBSR has been shown to reduce depression and fear of recurrence in women diagnosed with breast cancer.[51] MBSR lowers cortisol, reduces interleukin-6 (IL-6), lowers systolic blood pressure, and improves natural killer (NK) cell activity—each of which is correlated with a higher quality of life and a better prognosis.[52]

CONCLUSION

There is ample evidence to support the inclusion of a lifestyle-based approach in people diagnosed with cancer. A plant-based, Mediterranean-style diet is the foundation of such an approach. Intermittent or continuous caloric restriction may have unique benefits to the improved toleration of treatment and overall survival. Exercise remains a potent strategy to increase overall and cancer-specific survival. Finally, stress management has a direct effect on reducing the risk of recurrence and in optimizing the quality of daily living.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jie B, Jiang Z, Nolan M, et al. Impact of nutritional support on clinical outcome in patients at nutritional risk: a multicenter, prospective cohort study in baltimore and beijing teaching hospitals. *Nutrition*. 2010;26(11–12):1088–1093.
2. Marín Caro MM, Laviano A, Pichard C. Nutritional intervention and quality of life in adult oncology patients. *Clin Nutr*. 2007;26(3):289–301.
3. Bradbury KE, Appleby PN, Key TJ. Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the european prospective investigation into cancer and nutrition (EPIC). *Am J Clin Nutr*. 2014;100(suppl):394S– 398S.
4. Benetou V, Trichopoulou A, Orfanos P, et al. Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. *Br J Cancer*. 2008;99(1):191.
5. Gold EB, Pierce JP, Natarajan L, et al. Dietary pattern influences breast cancer prognosis in women without hot flashes: the women's healthy eating and living trial. *J Clin Oncol*. 2008;15. [Epub ahead of print].
6. Gann PH, Chatterton RT, Gapstur SM, et al. The effects of a low-fat/high-fiber diet on sex hormone levels and menstrual cycling in premenopausal women: a 12-month randomized trial (the diet and hormone study). *Cancer*. 2003;98(9):1870–1879.
7. Pierce JP, Natarajan L, Caan BJ, et al. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the women's healthy eating and living (WHEL) randomized trial. *JAMA*. 2007;298(3):289–298.
8. Gold EB, Pierce JP, Natarajan L, Stefanick ML, et al. Dietary pattern influences breast cancer prognosis in women without hot flashes: the women's healthy eating and living trial. *J Clin Oncol*. 2009;27(3):352–359.
9. Villasenor A, Ambs A, Ballard-Barbash R, et al. Dietary fiber is associated with circulating concentrations of C-reactive protein in breast cancer survivors: the HEAL study. *Breast Cancer Res Treat*. 2011;1. [Epub ahead of print].
10. Carmody J, Olendzki B, Reed G, et al. A dietary intervention for recurrent prostate cancer after definitive primary treatment: results of a randomized pilot trial. *Urology*. 2008;72(6):1324–1328.
11. Bidoli E, Franceschi S, Talamini R, et al. Food consumption and cancer of the colon and rectum in northeastern Italy. *Int J Cancer*. 1992;50:223–229.
12. Levi F, Pasche C, Lucchini F, La Vecchia C. Dietary fibre and the risk of colorectal cancer. *Eur J Cancer*. 2001;37:2091–2096.
13. Faeh D1, Braun J, Tarnutzer S, Bopp M. Obesity but not overweight is associated with increased mortality risk. *Eur J Epidemiol*. 2011;26(8):647–655.
14. Pan H, et al. *Early Breast Cancer Trialists' Collaborative Group (EBCTCG) study*. Presented at ASCO; 2014.
15. Fuentes-Mattei E, Velazquez-Torres G, Phan L, et al. Effects of obesity on transcriptomic changes and cancer hallmarks in estrogen receptor-positive breast cancer. *J Natl Cancer Inst*. 2014;23:106–107.
16. Magheli A, Rais-Bahrami S, Trock B, et al. Impact of body mass index on biochemical recurrence rates after radical prostatectomy: an analysis utilizing propensity score matching. *Urology*. 2008;72(6):1246–1251.
17. Muc-Wierzgoń M, Nowakowska-Zajdel E, Dzięgielewska-Gęsiak S, et al. Specific metabolic biomarkers as risk and prognostic factors in colorectal cancer. *World J Gastroenterol*. 2014;20(29):9759–9774.
18. Faruk Aykan N1, Yildiz I, Sen F, et al. Effect of increased body mass index (BMI) on time to tumour progression (TTP) in unresectable metastatic colorectal cancer (mCRC) patients treated with bevacizumab-based therapy. *Med Oncol*. 2013;30(3):679.
19. Frasca F, Pandini G, Sciacca L, et al. The role of insulin receptors and IGF-I receptors in cancer and other diseases. *Arch Physiol Biochem*. 2008;114(1):23–37.
20. Ulanet DB, Ludwig DL, Kahn CR, Hanahan D. Insulin receptor functionally enhances multistage tumor progression and conveys intrinsic resistance to IGF-1R targeted therapy. *Proc Natl Acad Sci U S A*. 2010;107(24):10791–10798.
21. Smith J, Axelrod D, Singh B, Kleinberg D. Prevention of breast cancer: the case for studying inhibition of IGF-1 actions. *Ann Oncol*. 2011;22(suppl 1):i50–i52.
22. Samuels Y1, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science*. 2004;304(5670):554.
23. Saldana SM, Lee HH, Lowery FJ, et al. Inhibition of type I insulin-like growth factor receptor signaling attenuates the development of breast cancer brain metastasis. *PLoS One*. 2013;8(9):e73406.
24. Gennari A et al. Abstract #514, presented at ASCO, May 30–Jun 2, Chicago, IL.
25. Meyerhardt J, Sato K, Niedzwiecki D, et al. Dietary glycemic load and cancer recurrence and survival in patients with stage III colon cancer: findings from CALGB 89803. *J Natl Cancer Inst*. 2012;104(22):1702–1711.
26. Karlsen A, Retterstøl L, Laake P, et al. Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *J Nutr*. 2007;137(8):1951–1954.
27. Sun A, Ostadal O, Ryznar V, et al. Phase I/II study of stage III and IV non-small cell lung cancer patients taking a specific dietary supplement. *Nutri Cancer*. 1999;34(1):62–69.
28. Dayem AA, Choi HY, Yang GM, et al. The anti-cancer effect of polyphenols against breast cancer and cancer stem cells: molecular mechanisms. *Nutrients*. 2016;8(9):581. https://doi.org/10.3390/nu8090581.
29. Hoensch H, Groh B, Edler L, Kirch W. Prospective cohort comparison of flavonoid treatment in patients with resected colorectal cancer to prevent recurrence. *World J Gastroenterol*. 2008;14(14):2187–2193.
30. Kucuk O, Sarkar FH, Djuric Z, et al. Effects of lycopene supplementation in patients with localized prostate cancer. *Exp Biol Med (Maywood)*. 2002;227(10):881–885.
31. Nechuta SJ, Caan BJ, Chen WY, et al. Soy food intake after diagnosis of breast cancer and survival: an in-depth analysis of combined evidence from cohort studies of US and Chinese women. *Am J Clin Nutri*. 2012;96(1):123–132.
32. Surh YJ, Kundu JK, Na HK, Lee JS. Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. *J Nutr*. 2005;135:2993S–3001S.
33. Alkhalaf M. Resveratrol-induced apoptosis is associated with activation of p53 and inhibition of protein translation in T47D human breast cancer cells. *Pharmacology*. 2007;80(2-3):134–143.
34. Vauzour D1, Vafeiadou K, Rice-Evans C, et al. Inhibition of cellular proliferation by the genistein metabolite 5,7,3',4'-tetrahydroxyisoflavone is mediated by DNA damage and activation of the ATR signalling pathway. *Arch Biochem Biophys*. 2007;468(2):159–166.
35. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. *Biochem Pharmacol*. 2008;75(4):787–809.
36. Abraham AG, O'Neill E. PI3K/Akt-mediated regulation of p53 in cancer. *Biochem Soc Trans*. 2014;42(4):798–803.
37. Safdie FM, Dorff T, Quinn D, et al. Fasting and cancer treatment in humans: a case series report. *Aging (Albany NY)*. 2009;1(12):988–1007.
38. Lee C, Raffaghello L, Longo VD. Starvation, detoxification, and multidrug resistance in cancer therapy. *Drug Resist Updat*. 2012;15(1-2):114–122.
39. www.Clinicaltrials.gov Identifier: NCT01175837. Accessed October 12, 2014.
40. www.clinicaltrials.gov Identifier: NCT01954836. Accessed October 12, 2014.
41. Fearon K, von Meyenfeldt M, Moses A, et al. Effect of a protein and energy dense n-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomized double blind trial. *Gut*. 2003;52:1479–1486.
42. Beasley JM, Kwan ML, Chen WY, et al. Meeting the physical activity guidelines and survival after breast cancer: findings from the after breast cancer pooling project. *Breast Cancer Res Treat*. 2012;131(2):637–643.
43. Irwin ML, Smith AW, McTiernan A, et al. Influence of pre- and postdiagnosis physical activity on mortality in breast cancer survivors: the health, eating, activity, and lifestyle study. *J Clin Oncol*. 2008;26(24):3958–3964.
44. Campbell P, Patel AV, Newton CC, et al. Associations of recreational physical activity and leisure time spent sitting with colorectal cancer survival. *J Clin Oncol*. 2013;31(7):876–885.
45. Cohen L, Cole SW, Sood AK, et al. Depressive symptoms and cortisol rhythmicity predict survival in patients with renal cell carcinoma: role of inflammatory signaling. *PLoS One*. 2012;7(8):e42324.

46. Volden P, Wonder EL, Skor MN, et al. Chronic social isolation is associated with metabolic gene expression changes specific to mammary adipose tissue. *Cancer Prev Res*. 2013;6(7):634–645.
47. Fish JA, Ettridge K, Sharplin GR, et al. Mindfulness-based cancer stress management: impact of a mindfulness-based programme on psychological distress and quality of life. *Eur J Cancer Care (Engl)*. 2014;23(3):413–421.
48. Faul LA, Jim HS, Williams C, et al. Relationship of stress management skill to psychological distress and quality of life in adults with cancer. *Psychooncology*. 2010;19(1):102–109.
49. Penedo FJ, Molton I, Dahn JR, et al. A randomized clinical trial of group-based cognitive-behavioral stress management in localized prostate cancer: development of stress management skills improves quality of life and benefit finding. *Ann Behav Med*. 2006;31(3):261–270.
50. Ledesma D, Kumano H. Mindfulness-based stress reduction and cancer: a meta-analysis. *Psychooncology*. 2009;18:571.
51. Lengacher CA, Johnson-Mallard V, Post-White J, et al. Randomized controlled trial of mindfulness-based stress reduction (MBSR) for survivors of breast cancer. *Psychooncology*. 2009;18:1261.
52. Witek-Janusek L, Albuquerque K, Chroniak KR, et al. Effect of mindfulness based stress reduction on immune function, quality of life and coping in women newly diagnosed with early stage breast cancer. *Brain Behav Immun*. 2008;22:969.

Dietary Fiber

John Nowicki, ND

OUTLINE

INTRODUCTION

Anthropology

Early humans obtained fiber principally in the form of soluble fiber from fruits, vegetables, and root vegetables. With the advent of grain cultivation, the human diet shifted more toward insoluble fiber, which has less beneficial metabolic effects, particularly in the context of a higher carbohydrate intake. Although the original Inuit diet of the far north was, calorically speaking, very high in animal fat and, to a lesser extent, animal protein, they supplemented their diet extensively with seasonal berries, roots, various plants, and seaweeds; thus they consumed relatively large amounts of fiber. Further south, aboriginal peoples made extensive use of numerous plant foods that contributed to a large intake of dietary fiber.[1] Around the world, there are few examples of indigenous peoples whose traditional diet did not provide very high quantities of fiber. Only with the advent of commercial food processing and refining has the human diet been so characteristically meager in dietary fiber.

History

Although there is a contemporary revival in medical interest surrounding fiber, references to dietary fiber have long existed in the medical literature in the writings of such historical figures as Hippocrates, the 9th-century Persian physician Hakim, and the Kellogg brothers of the United States.[2] However, the contemporary interest in fiber among physicians, as well as the widespread understanding of fiber's importance among the general public, began with a zealous campaign instituted by the missionary surgeon Denis Burkitt (also the discoverer of Burkitt's lymphoma), beginning in the early 1970s. Over his 20-year career in Africa, Dr. Burkitt observed a dramatic difference in the incidence of many diseases, such as coronary heart disease, diabetes, diverticulosis, and gallstones, among the local tribesmen. He noted that the largely agrarian Africans consumed very large amounts of dietary fiber, often more than 100 g/day. He determined that as a result of this diet, their stool transit time was far more rapid than that of Westerners, and their stools were more frequent and bulky. On the basis of his research, he launched a worldwide campaign to raise awareness of the importance of dietary fiber among medical professionals and the general public. Without a doubt, Dr. Burkitt's zeal was an important factor that catalyzed a veritable revolution in research surrounding dietary fiber.[3] During Burkitt's campaign, contemporaries published the first studies on fiber-related concepts, such as the glycemic index.[4] The complex mechanisms by which fiber exerts its many health effects are now recognized beyond just its effect on laxation.

FIBER

Classification

In the simplest definition, dietary fiber is a nonstarch polysaccharide in (mostly) plant food that is poorly digested by humans. Based on a government consensus report, fiber can exist as dietary fiber (naturally occurring in food) or functional fiber (added during the processing or preparation of food).[5] Fiber can be either insoluble or soluble in water. Insoluble fibers include cellulose, hemicellulose, and lignins, whereas soluble fibers include various gums, pectins, β-glucans, oligosaccharides, resistant dextrans, and resistant starches. Chitin and chitosan are indigestible amino-polysaccharides that are found in or are derived from the exoskeletons of arthropods, such as crabs and lobster, as well as the cell walls of most fungi. Although they could functionally be regarded as fiber, most regulatory authorities do not recognize them as fiber. The distinction between soluble and insoluble fibers is due to the chemical properties of the fiber, resulting in its tendency to absorb water. Various physicochemical properties of fiber (viscosity, water-holding capacity, cation-exchange capacity, adsorption of organic materials, and fermentability) are thought to be fundamental to its beneficial physiological effects. The Institute of Medicine has proposed a new definition of dietary fiber that encompasses both its physical characteristics and its physiological effects in humans.[6] Viscosity, water-holding capacity, and fermentability are the chief determinates of fiber's physiological effects.

The regulatory classification of fiber varies considerably in different countries. In the United States, dietary fiber is defined based on analytic methods rather than its physical or physiological characteristics.

Sources

The typical Western diet is largely devoid of dietary fiber, being composed principally of refined grains and other highly digestible sources of starch, sugar, various fats, and animal products. Children, in particular, are commonly fiber deficient, with daily intakes often under 5 g and with nearly no soluble fiber. Likewise, many adults in Western society consume 5 to 10 g of fiber daily as opposed to the 35 to 50 g that is considered desirable or at least minimal for optimal health. Moreover, because most fiber in the Western diet is derived from cereal grains, the intake of viscous soluble fiber is typically highly inadequate.

A whole-foods-based diet focusing on a large intake of vegetables and fruits as well as unrefined whole grains and legumes should be the foundation of a healthy lifestyle. With a whole-foods-based diet, it is certain that dietary fiber intake will substantially increase.[7] Efforts continue to fortify the Western diet with dietary fiber through the use of various fibers as food additives or ingredients as well as the use of readily accepted fiber supplements.

Health Effects

There are compelling epidemiological and experimental data associating numerous disorders, at least in part, with a lack of dietary fiber. Ischemic heart disease, stroke, atherosclerosis, type 2 diabetes, overweight and obesity, insulin resistance, hypertension, and dyslipidemia, as well as gastrointestinal disorders such as diverticulosis, irritable bowel disease, colon cancer, and cholelithiasis are just a few of the many conditions that seem to be influenced by the adequacy of dietary fiber intake.[8]

Numerous studies have demonstrated that certain fibers decrease the glycemic response to food, promote satiety, lower serum cholesterol, promote bowel regularity, positively influence colonic microflora, provide nutritional substrates for colonic mucosal cells, improve mucosal barrier function, and sequester and eliminate toxic and carcinogenic dietary and environmental compounds.

Viscous dietary fibers are clearly correlated with moderation in blood glucose and cholesterol concentrations, prolonged gastric emptying, and slower transit time through the small intestine.[9] Among viscous fibers, fermentability is mostly associated with large bowel function. Rapidly fermented fiber sources provide substrates for short-chain fatty acid (SCFA) production by microflora in the large bowel, whereas slowly or incompletely fermented fiber sources improve bowel health by promoting laxation, reducing colonic transit time, and increasing stool weight.[10]

MECHANISMS OF ACTION OF DIETARY FIBER

Dietary fiber exerts its effects through an interaction between the physical properties it imparts to food and a complex array of microbiological, biochemical, and neurohormonal influences. Dietary fiber has a strong influence on the palatability of food, thus influencing ingestive behavior. In the stomach, fiber affects the volume and viscosity of food, which has a significant effect on satiety. This "volumetric" effect on food promotes a sense of fullness and a delay in gastric emptying, which tends to naturally result in a decrease in caloric intake. Various fibers differ dramatically in their ability to impart volume and viscosity to foods. Becoming familiar with the use of highly viscous soluble fibers enables healthcare providers to teach patients to choose an eating strategy that promotes and maintains satiety while consuming a diet that is lower in caloric density.

Viscosity of Fiber

Viscosity as related to dietary fiber refers to the ability of some polysaccharides to thicken or form gels when mixed with fluids, resulting from physical entanglements and hydrophilic interactions among the polysaccharide constituents within the fluid or solution.[11] Gums, pectins, and β-glucans make up the majority of viscous dietary fibers. The viscosity that a fiber imparts to the gastric and small intestinal contents is directly correlated with the ability of the fiber to reduce postprandial glycemic response, promote satiety, decrease serum cholesterol, and decrease serum uric acid.[12] The viscosity of fiber is also thought to play an important role in the augmentation of gut mucosal protection through the stimulation of enteral mucus production and goblet-cell hypertrophy and replication.[13] Additionally, those viscous fibers that are largely fermented by colonic microflora exert a wide array of physiological effects through the production of SCFAs (the principle energy substrates of colonocytes), the promotion of beneficial colonic microbial populations, and the augmentation of important gut-derived peptide hormones.

The viscosity of fiber is best measured by methods that quantify a hydrated fiber's internal friction and its ability to resist flow. Viscosity is usually expressed in units of millipascal seconds or centipoise.[14] Other factors, such as shear stress (e.g., mastication, peristalsis), acid pH, dilution, and chemical components of food, determine the real viscosity that a fiber will impart to food rather than just its in vitro viscosity.

The concept of the glycemic index came about through the work of Jenkins et al. as they examined the effect of viscous fiber ingestion on glucose tolerance.[15] The glycemic index of a carbohydrate-containing food is directly correlated to the viscosity of that food after ingestion.

One research group was involved in several studies looking at the effects of adding a novel, highly viscous functional fiber (Polyglycoplex [PGX]) to various foods on the glycemic index, serum cholesterol, hunger, and satiety in healthy humans. The novelty of this fiber relates to its viscosity, which is higher than that of most other forms studied. Unlike some viscous fibers, such as psyllium, which are not fermented, PGX is highly fermentable and prebiotic.[16] PGX, made from glucomannan, sodium alginate, and xanthan gum, is taken through several steps of processing, and the resultant modified fiber is approximately three times the viscosity of glucomannan, a naturally occurring fiber from the Konjac root that may possess the highest viscosity of any naturally occurring fiber. In addition, it was demonstrated that PGX is a novel and stable molecular entity with viscosity that is severalfold higher than its constituent ingredients, through a wide range of pH and shear-stress conditions, and when added to a variety of foods and beverages.[17,18] In addition, this fiber is tasteless and disperses readily when added to food or mixed with beverages. Its viscosity evolves several minutes after initial hydration, making it very easy to consume the small amounts needed to create a highly viscous gastric milieu, leading to its resultant physiological effects.

Substantial reductions in postprandial glycemia along with reduced hunger and increased satiety can be achieved with a very modest quantity of this highly viscous fiber. These findings support the contention that the viscosity that a fiber imparts to the gastric contents is more important than simply the grams of the fiber consumed.[19–23] In a study of Zucker Diabetic Fatty (ZDF) rats, the effects of diets supplemented with nonviscous, insoluble fiber (cellulose); nonviscous, prebiotic fiber (inulin, oligofructose); or a highly viscous, prebiotic fiber (PGX) were compared. Results showed that only the diet supplemented with highly viscous fiber substantially decreased postprandial blood glucose and insulin secretion, decreased hepatic fatty infiltration, and preserved pancreatic β-cell mass. These effects were accompanied by an increase in the production of the important glucoregulatory incretin hormone glucagon-like peptide-1 (GLP-1) and, in a human clinical trial, the appetite-reducing hormone peptide YY (PYY).[24] The substantial glycemic-index–modifying effects have been well confirmed in human studies.[18–20]

Water-Holding Capacity

The ability of a fiber to absorb and hold on to water as it transits the gut is another factor that contributes to its functional effects. Soluble fibers have the capacity to create a stable gel that results in stomach volume being occupied and a sense of satiety being created without the addition of significant calories. The "volumetric" properties of a food consist of a food's volume and viscosity per net quantity of calories. Those who work in the field of obesity management believe that teaching patients strategies that promote a high consumption of low-calorie-density, highly volumetric foods is a mainstay in the dietary management of overweight and obesity.[25–27]

Laxation

The most common clinical application of fiber is in the maintenance of bowel regularity. Insoluble fiber, perhaps in combination with soluble fiber, is most effective in increasing stool bulk, reducing gut transit time, and increasing the frequency of defecation.[28] The health-promoting effects of insoluble fiber from the bran of grains is well documented and certainly extends past its laxative effects.[29] However, the benefits of reduced stool transit time and increased fecal frequency may have more profound long-term benefits beyond the comfort that accompanies bowel regularity.[30–32]

Prebiotics

It is recognized that certain forms of fiber are fermentable, providing an important substrate in the metabolism of gut flora. By definition, humans lack the enzymes specific to digest fiber. In the case of insoluble fiber, most gut microbes cannot use it as an energy source, and so it is typically excreted without any molecular alteration other than by forming a surface for the adsorption of organic matter and cations. Soluble fibers vary in their fermentability and in the specific microbes that can use them as substrates. The term "prebiotic" was coined by Gibson and Roberfroid in 1995 and was defined as an indigestible carbohydrate that is fermentable in the lower gastrointestinal tract that selectively promotes the growth of desirable (prebiotic) microflora and is associated with a positive health outcome.[33,34] Although there may be exceptions, prebiotics tend to reduce populations of potentially pathogenic flora while promoting desirable commensals such as *Bifidobacteria*.

Evidence suggests that this effect might play an important role in both the reduction in adiposity and in a reduction in the contribution of adipocytes to inflammation. It was demonstrated that prebiotic supplementation might reduce the development of large adipocytes (those that predominate in the visceral compartment), which are highly involved in the overactivation of a wide variety of inflammatory processes that may contribute to overeating, obesity, diabetes, cardiovascular disease, and pain.[35,36]

Pathways of inflammation associated with atopic disease are also intimately associated with gut flora. Both prebiotics and probiotics are believed to hold significant promise in the prevention and treatment of allergies and atopic disorders, such as eczema.[37–40]

The lack of sufficient intake of prebiotics early in life has lasting ill-effects on gluco-regulation, which may result in a substantial predilection toward obesity, diabetes, and cardiovascular disease later in life.[41] Decreased intake of fermentable soluble fiber may have serious consequences and may be one of the reasons for the obesity epidemic. The food industry has responded to this with the introduction of prebiotic-fortified infant formulas. Because breast milk contains significant quantities of prebiotic oligosaccharides, adding analogous agents to infant formulas would help make these more closely mimic the natural diet. This strategy results in a gut flora predominated by *Bifidobacteria* rather than potential pathogens such as *Clostridia* and *Enterobacter* that tend to predominate in formula-fed infants.[42]

Prebiotics, namely lactulose, have been used for many years in the management of hepatic encephalopathy.[43] It has long been a marvel for modifying the colonic flora, and giving lactulose can restore sanity to a delirious patient with liver failure. It is recognized that the prebiotic effects of this treatment result in a reduction in neurotoxic bacterial metabolites that can normally be eliminated by the healthy liver. Similarly, prebiotics have found their place in the treatment of patients with HIV[44] and in allergy prevention and treatment.[45]

Prebiotics may play an important role in the modification of carcinogens and in the promotion of substances that reduce cancer risk, at least in the gastrointestinal tract.[46] Dietary fiber has the capacity to sequester and increase the elimination of a wide range of organic molecules as well as metal ions. This effect, along with the resultant decrease in colonic transit time, results in reduced colonocyte exposure to potentially harmful xenobiotics and endogenously produced, but potentially harmful, compounds. Fermentable soluble fiber is metabolized anaerobically into SCFAs such as butyrate, acetate, and propionate. These simple molecules are the principal energy substrates of colonocytes, and an adequate and ongoing supply of these may reduce the risk of cancer, metabolic disorders, and other gastrointestinal disorders, such as diverticulosis and inflammatory bowel disease.[47,48]

Neuroendocrine Effects of Fiber

Perhaps the most intriguing discovery is related to the role played by dietary fiber in the modulation of important neuroendocrine physiology, which may be fundamentally related to the etiology of several serious disorders. Of particular interest is the effect of fiber on the density and activity of a specialized enteroendocrine cell known as the L-cell.[49] The L-cell is located throughout the terminal ileum and colon, and it is responsible for the secretion of the peptide hormones GLP-1, PYY, and oxyntomodulin.[50] After a meal, oxyntomodulin and PYY are released synchronously; both act as potent anorexigens. The rapid rise of circulating hormone levels signals a change in energy status to the brain and also acts locally to enhance digestive processes. GLP-1 is an incretin hormone that is also secreted by the L-cell and, as well as being an anorexigen, it plays a pivotal role in glucoregulation through the stimulation of accurately timed insulin secretion and suppression of glucagon secretion from the pancreas. It has been established that diminished GLP-1 production plays a central role in the etiology of diabetes, a discovery that has led to the development of the most important class of diabetes drugs (the incretin analogs) since the discovery of insulin. A randomized clinical study demonstrated that select SCFA-producing gut bacteria were promoted by dietary fibers, improving hemoglobin A1c and alleviating type 2 diabetes, partly via increased GLP-1 production.[51] Studies demonstrate that (bariatric) gastric bypass surgery results in rapid amelioration of diabetes that is out of proportion with and precedes significant weight loss.[52] Evidence points to a rapid and sustained increase in circulating GLP-1 and PYY after this procedure, which has profound effects on appetite and glucoregulation. It is thought that the malabsorption of carbohydrates and rapid gastrointestinal transit after gastric bypass results in increased delivery of carbohydrate fermentation products (SCFAs) and bile acids, both of which activate L-cells via free fatty acid (FFA) receptors and bile acid receptors.[53–57]

It is most interesting that fermentable soluble fiber has the potential to generate a significant supply of SCFAs that might mimic, on a lesser scale, the mechanism of gastric bypass surgery through the stimulation of FFA receptors, with a resultant increase in GLP-1 and PYY.[58] Because viscous soluble fiber also effectively sequesters bile acids, reducing their usual absorption through the jejunum and delivering them to the same L-cells, viscous soluble fiber that is also fermentable

may exert an appetite-reducing and glucoregulating effect through L-cell activation via both FFA receptors and bile acid receptors as well as by suppression of the orexigenic (appetite stimulating) hormone ghrelin.[59,60]

Sequestration of bile acids by viscous soluble fiber is also known to be a principal mechanism by which viscous fiber lowers serum cholesterol because sequestration of bile acids decreases the enterohepatic recycling of bile acids, a major cholesterol reservoir for the human.[61]

CLINICAL APPLICATIONS

With the abundant and ever-growing evidence that has elevated the role of fiber well beyond its place in the promotion of bowel regularity, dietary fiber should be respected by healthcare providers as an important prophylactic and therapeutic agent. The importance of viscous, fermentable soluble fiber in the prevention and management of obesity and diabetes is of particular importance to clinicians who are on the front lines facing the obesity epidemic.

It is clear that fiber, especially viscous and (prebiotic) fermentable soluble fiber, plays a key role in the risk of obesity, diabetes, cardiovascular disease, cancers, atopic illness, and a wide range of gastrointestinal disorders. It is also clear that the lack of adequate dietary fiber, particularly prebiotic and viscous fibers in childhood and infancy, may set the stage for a higher risk of the same array of disorders, and that some of that increased risk may linger for the remainder of life. Clinicians must develop effective strategies to educate their patients and assist them in the incorporation of much higher levels of fiber in their daily diet. Because the greatest benefits from fiber are derived from highly viscous, prebiotic fibers, clinicians should become especially knowledgeable in the effective use of these agents, particularly when treating patients who are obese or exhibit dysglycemia.

In 1968, polychlorinated biphenyl (PCB)–contaminated rice-bran cooking oil poisoned a great number of Japanese individuals in what came to be called "Yusho disease" (oil poisoning). Rice-bran fiber (RBF) has demonstrated the ability to bind PCBs and other toxicants, including the combustion by-product benzo(a)pyrene. When measured against other fibers, RBF dramatically reduced the reabsorption of PCBs from the intestines in animals, and in PCB-exposed animals, RBF increased fecal PCB excretion by 6.6 times and spinach fiber by 4.1 times.[62] Yusho patients who consumed 7 to 10 g of fermented RBF three times daily (after each meal) for a year enjoyed 81% greater elimination of furans and 74% greater elimination of dioxins compared with those not consuming the fiber.[63]

DOSAGE AND ADMINISTRATION

The dietary fiber intake for typical Americans is usually less than desirable, with typical intakes averaging only 14 to 15 g/day and children consuming less than 5 g/day.[64] The American Dietetic Association currently recommends that healthy adults should consume 20 to 35 g of fiber per day, and children should consume at least 5 g/day plus 1 g for every year of their age.[65] The American Dietetic Association points out that insoluble, nonfermentable, and low-viscosity fiber is principally consumed to promote laxation and other aspects of colon health, whereas viscous soluble fibers are necessary to reduce serum cholesterol, blunt postprandial glycemic response, and promote satiety. A fiber-rich meal, particularly a meal high in viscous, soluble fiber, is processed more slowly, promoting earlier satiety, and is frequently less calorically dense and lower in fat and added sugars. All of these characteristics are typical of a dietary profile optimized to treat and prevent obesity. Unfortunately, viscous soluble fiber is not easily incorporated into the regular diet, but fiber supplements may provide a practical means to ensure that most meals contain optimal quantities of viscous fiber. Pal et al.[66] at Curtin University in Australia demonstrated the utility of high-dose psyllium given as a premeal supplement as an adjunct to a calorie-reduced weight loss program. However, the dosage of psyllium used in this application was very large (12 g with each meal) and might pose a compliance problem for many subjects. Using very high-viscosity fiber (such as PGX) may overcome this limitation because the dosages required to achieve a reduction in postprandial glycemia and an increase in satiety may be much less than those of fibers with lesser viscosity.[19,67]

DRUG INTERACTIONS

Clinicians prescribing higher doses of supplementary fibers should be aware of the potential drug–food interactions in those patients requiring pharmaceuticals. Most fiber, especially viscous fibers, has the potential to modify the rate or total absorption of various drugs.[68] In most cases, taking medications 1 hour before or 2 hours after consumption of the supplementary fiber will reduce the effect of this phenomenon.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Kuhnlein HV, Taylor NK. *Traditional Plant Foods of Canadian Indigenous Peoples: Nutrition, Botany, and Use (Food and Nutrition in History and Anthropology)*. Washington, DC: Taylor & Francis; 1991:26–29.
2. Kritchevsky D. Dietary fiber. *Annu Rev Nutr*. 1988;8:301–328.
3. Burkett DP, Trowell HC. *Refined Carbohydrate Foods and Disease: Some Implications of Dietary Fiber*. London: Academic Press; 1975.
4. Jenkins DJ, Ghafari H, Wolever TM, et al. Relationship between rate of digestion of foods and post-prandial glycaemia. *Diabetologia*. 1982;22:450–455.
5. Panel on the definition of dietary fiber, standing committee on the scientific evaluation of dietary reference intakes, food and nutrition board. Chapter 7. Dietary, functional and total fiber. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: US Department of Agriculture, National Agricultural Library and National Academy of Sciences, Institute of Medicine, Food and Nutrition Board; 2005:321–439.
6. *Panel on the Definition of Dietary Fiber, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Dietary Reference Intakes: Proposed Definitions of Dietary Fiber*. Washington, DC: National Academy Press; 2001.
7. Lindberg S. *Food and Western Disease: Health and Nutrition from an Evolutionary Perspective*. Hoboken, NJ: John Wiley and Sons; 2010.
8. Timm D, Slavin J. Dietary fiber and the relationship to chronic diseases. *Am J Lifestyle Med*. 2008;2:233–240.
9. Mälkki A. Physical properties of dietary fiber as keys to physiological functions. *Cereal Foods World*. 2001;46:196–199.
10. Edwards CA. The physiological effects of dietary fiber. In: Kritchevsky D, Bonfield C, eds. *Dietary Fiber in Health and Disease*. St. Paul, MN: Eagan Press; 1995:58–71.
11. Dikeman C, Fahey G. Viscosity as related to dietary fiber: a review. *Crit Rev Food Sci Nutr*. 2006;46:649–663.
12. Koguchi T, Nakajima H, Koguchi H, et al. Suppressive effect of viscous dietary fiber on elevations of uric acid in serum and urine induced by dietary RNA in rats is associated with strength of viscosity. *Int J Vitam Nutr Res*. 2003;73:369–376.
13. Ito H, Satsukawa M, Arai E, et al. Soluble fiber viscosity affects both goblet cell number and small intestine mucin secretion in rats. *J Nutr*. 2009;139:1640–1647.
14. Bourne M. *Food Texture and Viscosity: Concept and Measurement*. 2nd ed. San Diego, CA: Academic Press; 2002.
15. Jenkins DJ, Wolever TM, Leeds AR, et al. Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *BMJ*. 1978;1:1392–1394.
16. Reimer RA, Pelletier X, Carabin IG, et al. Faecal short chain fatty acids in healthy subjects participating in a randomised controlled trial examining a soluble highly viscous polysaccharide versus control. *J Hum Nutr Diet*. 2012 Feb 9. https://doi.org/10.1111/j.1365-277X.2012.01230.x.
17. Harding SE, Smith IH, Lawson CJ, et al. Studies on macromolecular interactions in ternary mixtures of konjac glucomannan, xanthan gum and sodium alginate. *Carbohydr Polymers*. 2011;83:329–338.
18. Abdelhameed AS, Ang S, Morris GA, et al. An analytical centrifuge study on ternary mixtures of konjac glucomann supplemented with sodium alginate and xanthan gum. *Carbohydr Polymers*. 2010;81:145–148.
19. Brand-Miller JC, Atkinson FS, Gahler RJ, et al. Effects of PGX, a novel functional fibre, on acute and delayed postprandial glycaemia. *Eur J Clin Nutr*. 2010;64:1488–1493.
20. Jenkins AL, Kacinik V, Lyon M, et al. Effect of adding the novel fiber, PGX, to commonly consumed foods on glycemic response, glycemic index and GRIP: a simple and effective strategy for reducing post prandial blood glucose levels–a randomized, controlled trial. *Nutr J*. 2010;9:58. https://doi.org/10.1186/1475-2891-9-58.
21. Jenkins AL, Kacinik V, Lyon M, et al. Reduction of postprandial glycemia by the novel viscous polysaccharide PGX, in a dose-dependent manner, independent of food form. *J Am Coll Nutr*. 2010;29:92–98.
22. Kacinik V, Lyon M, Purnama M, et al. Effect of PGX, a novel functional fibre supplement, on subjective ratings of appetite in overweight and obese women consuming a structured, low calorie diet. A double blind, placebo controlled trial. *Nutrition and Diabetes*. 2011;1:e22. https://doi.org/10.1038/nutd.2011.18.
23. Vuksan V, Panahi S, Lyon M, et al. Viscosity of fiber preloads affects food intake in adolescents. *Nutr Metab Cardiovasc Dis*. 2009;19:498–503.
24. Pelletier X, Carabin IG, Lyon M, et al. Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur J Clin Nutr*. 2010;64:1186–1191.
25. Rolls BJ. Plenary Lecture 1: dietary strategies for the prevention and treatment of obesity. *Proc Nutr Soc*. 2010;69:70–79.
26. Ello-Martin JA, Roe LS, Ledikwe JH, et al. Dietary energy density in the treatment of obesity: a year-long trial comparing 2 weight-loss diets. *Am J Clin Nutr*. 2007;85:1465–1477.
27. Rolls BJ. The relationship between dietary energy density and energy intake. *Physiol Behav*. 2009;97:609–615.
28. Vuksan V, Jenkins AL, Jenkins DJ, et al. Using cereal to increase dietary fiber intake to the recommended level and the effect of fiber on bowel function in healthy persons consuming North American diets. *Am J Clin Nutr*. 2008;88:1256–1262.
29. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? (review). *Nutr Res Rev*. 2010;23:65–134.
30. McDonald J, Pirhonen D, Rangam MA. High fiber diets: their role in gastrointestinal disorders. *Can Fam Phys*. 1983;29:1632–1638.
31. Uccheddu A, Murgia C, Licheri S, et al. The incidence of 1,2-dimethylhydrazine-induced colonic neoplasms in the rat: the effect of constipation. *Giornale di Chirurgia*. 1991;12:572–574. [Italian].
32. Huang YG, Ji JD, Hou QN. A study on carcinogenesis of endogenous nitrite and nitrosamine, and prevention of cancer. *Mutat Res*. 1996;358:7–14.
33. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*. 1995;125:1401–1412.
34. Roberfroid M. Prebiotics: concept, definition, criteria, methodologies, and products. In: Gibson GR, Roberfroid M, eds. *Handbook of Prebiotics*. Boca Raton, FL: CRC Press; 2008:39–69.
35. Dewulf EM, Cani PD, Neyrink AM, et al. Inulin-type fructans with prebiotic properties counteract GPR43 overexpression and PPARgamma-related adipogenesis in the white adipose tissue of high-fat-diet-fed mice. *J Nutr Biochem*. 2011;22:712–722.
36. Delzenne NM, Cani PD. Interaction between obesity and the gut microbiota: relevance in nutrition. *Annu Rev Nutr*. 2011;31:15–31.
37. Gruber C. Prevention of allergy by pro- and prebiotics. *Exp Rev Clin Immunol*. 2009;5:1–3.
38. Eiwegger T, Stahl B, Haidl P. Prebiotic oligosaccharides: in vitro evidence for gastrointestinal epithelial transfer and immunomodulatory properties. *Pediatr Allergy Immunol*. 2010;21:1179–1188.
39. Van der Aa LB, van Aalderen WM, Heymans HS, et al. Synbad Study Group. Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis. *Allergy*. 2011;66:170–177.
40. Gruber C, van Stuijvenberg M, Mosca F, et al. MIPS 1 Working Group. Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *J Allergy Clin Immunol*. 2010;126:791–797.
41. Maurer AD, Eller LK, Hallam MC, et al. Consumption of diets high in prebiotic fiber or protein during growth influences the response to a high fat and sucrose diet in adulthood in rats. *Nutr Metab*. 2010;7:77. https://doi.org/10.1186/1743-7075-7-77.
42. Veereman-Wauters G, Staelens S, Van de Broek H, et al. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. *J Pediatr Gastroenterol Nutr*. 2011;52:763–771.
43. Shuka S, Shukla A, Mehboob S, et al. Meta-analysis: the effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy. *Alimen Pharmacol Ther*. 2011;33:662–671.
44. Hummelen R, Vos AP, van't Land B, et al. Altered host-microbe interaction in HIV: a target for intervention with pro- and prebiotics. *Int Rev Immunol*. 2010;29:485–513.
45. Tang ML, Lahtinen SJ, Boyle RJ. Probiotics and prebiotics: clinical effects in allergic disease (review). *Curr Opin Pediatr*. 2010;22:626–634.
46. Komiyama Y, Mitsuyama K, Masuda J, et al. Prebiotic treatment in experimental colitis reduces the risk of colitic cancer. *J Gastroenterol Hepatol*. 2011;26:1298–1308.

47. Liong MT. Roles of probiotics and prebiotics in colon cancer prevention: postulated mechanisms and in-vivo evidence. *Int J Mol Sci*. 2008;9:854–863.
48. Jouet P, Sabate JM, Coffin B, et al. Fermentation of starch stimulates propagated contractions in the human colon. *Neurogastroenterol Motil*. 2011;23:450–456.
49. Kaji I, Karaki S, Tanaka R, et al. Density distribution of free fatty acid receptor 2 (FFA2)-expressing and GLP-1-producing enteroendocrine L cells in human and rat lower intestine, and increased cell numbers after ingestion of fructo-oligosaccharide. *J Molecular Histol*. 2011;42:27–38.
50. Strader AD, Woods SC. Gastrointestinal hormones and food intake. *Gastroenterol*. 2005;128:175–191.
51. Zhao L, Zhang F, Ding X, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*. 2018;359(6380):1151–1156. PubMed PMID: 29590046.
52. Cummings DE. Endocrine mechanisms mediating remission of diabetes after gastric bypass surgery. *Int J Obes*. 2009;33(suppl 1):S33–S40.
53. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun*. 2005;329:386–390.
54. Reimann F, Ward PS, Gribble FM. Signalling mechanisms underlying the release of glucagon-like peptide-1. *Diabetes*. 2006;55(suppl 2):S78–S85.
55. Hirasawa A, Tsumaya K, Awaji T, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med*. 2005;11:90–94.
56. Tolhurst G, Reimann F, Gribble FM. Nutritional regulation of glucagon-like peptide-1 secretion. *J Physiol*. 2009;587:27–32.
57. Patti ME, Houten SM, Bianco AC, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obesity*. 2009;17:1671–1677.
58. Ichimura A, Hirasawa A, Hara T, et al. Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. *Prostaglandins Other Lipid Mediat*. 2009;89:82–88.
59. Roberts RE, Glicksman C, Alaghband-Zadeh J, et al. The relationship between postprandial bile acid concentration, GLP-1, PYY and ghrelin. *Clin Endocrinol*. 2011;74:67–72.
60. Knop FK. Bile-induced secretion of glucagon-like peptide-1: pathophysiological implications in type 2 diabetes? *Am J Physiol—Endocrinol Metab*. 2011;299:E10–E13.
61. Jones PJ. Dietary agents that target gastrointestinal and hepatic handling of bile acids and cholesterol. *J Clin Lipid*. 2008;2:S4–S10.
62. Morita K, Hamamura K, Iida T. Binding of PCB by several types of dietary fiber in vivo and in vitro. *Fukuoka Igaku Zasshi*. 1995;86:212–217. PubMed PMID: 7628811.
63. Nagayama J, Takasuga T, Tsuji H, et al. Active elimination of causative PCDFs/DDs congeners of Yusho by one year intake of FEBRA in Japanese people. *Fukuoka Igaku Zasshi*. 2003;94:118–125.
64. Alaimo K, McDowell MA, Briefel RR, et al. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the united states: third national health and nutrition examination survey, phase 1, 1988–91. *Adv Data*. 1994;258:1–28.
65. Position of the American Dietetic Association. Health implications of dietary fiber. *J Am Diet Assoc*. 2002;102:993–1000.
66. Pal S, Khossousi A, Binns C, et al. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. *Br J Nutr*. 2011;105:90–100.
67. Lyon M, Wood S, Pelletier X, et al. Effects of a 3-month supplementation with a novel soluble highly viscous polysaccharide on anthropometry and blood lipids in nondieting overweight or obese adults. *J Human Nutr Diet*. 2011;24:351–359.
68. González Canga A, Fernández Martínez N, Sahagún Prieto AM, et al. Dietary fiber and its interaction with drugs. *Nutr Hosp*. 2010;25:535–539.

Digestive Support

Michael T. Murray, ND

OUTLINE

INTRODUCTION

Proper digestion, absorption, and elimination are necessary to gain the nutritional benefits from foods. Any disruption of these processes causes substantial, and usually progressive, health problems throughout the body. This chapter provides some overview of digestive dysfunction and ways to improve digestion. For specific digestive tract disorders (e.g., irritable bowel syndrome, peptic ulcers, inflammatory bowel disease, celiac disease, gastroesophageal reflux disease [GERD]), see Section 6. For information on the various laboratory procedures for evaluation of digestive function (e.g., comprehensive digestive stool analysis, intestinal permeability assessment, small intestinal bacterial overgrowth breath test), see Section 2.

The recommendations in this chapter are designed to support digestive function in those patients with *true* functional gastrointestinal disorders (FGIDs). The emphasis on *true* FGID is to signify that with detailed clinical evaluation, the cause of the patient's gastrointestinal (GI) symptoms can often be identified. Small intestinal bacterial overgrowth (SIBO), dysbiosis, and intestinal barrier dysfunction likely account for the underlying cause in most of these patients.[1] Also of importance are hypochlorhydria and pancreatic enzyme deficiency. The latter is common in type 1 diabetes and, to a lesser extent, type 2 diabetes.

Classically defined, FGIDs are common disorders that are characterized by persistent and recurring GI symptoms due to abnormal functioning of the GI tract. More than 20 functional GI disorders have been identified, with irritable bowel syndrome (IBS) being the most common. Approximately 25 million Americans have functional GI disorders, and 50% to 80% of people with FGID symptoms do not consult a physician.

Conventional medicine focuses on three primary features of FGIDs: motility, sensation, and brain–gut dysfunction. More recent studies have also shown that psychological distress may alter the systemic and gut immunity, which is increasingly recognized as a pathophysiological feature of FGID (Fig. 133.1). The reason is likely the focus on a pharmacological approach in dealing with these three areas.

This chapter, instead, focuses on some considerations in aiding general digestion when no underlying disturbance can be found. This is becoming an infrequent occurrence due to the diagnostic tools physicians have access to as well as to the various effective dietary and natural approaches that appear to improve digestive function.

THERAPEUTIC CONSIDERATIONS

In a survey of more than 20,000 adults in the United States, the overall prevalence of at least one upper GI symptom was reported in 45% of those surveyed (based on the preceding 3-month period); symptoms included heartburn, early satiety, loss of appetite, and postprandial fullness (i.e., bloating).[2] These symptoms of indigestion can be attributed to several causes.

Patients use common terms to describe their digestive issues, such as *indigestion, gas,* and *bloating.* So, it is important to help them define the nature and timing of their symptoms because they can provide valuable clues. It is also important to address some of the basics of digestion with patients. For example, proper digestion requires the body to be in a parasympathetic-dominant state. Unfortunately, many patients with digestive issues eat on the run or in a state of stress, and they often also fail to chew their food thoroughly. Teaching mindfulness and diaphragmatic breathing has been shown to help with common FGIDs like IBS and GERD. There are good reasons for this response. The diaphragm muscle is closely linked to the vagus nerve, which is a mixed nerve consisting of 20% efferent cholinergic descendent fibers and 80% afferent ascending fibers. In patients affected by IBS, GERD, and other GI disorders, the diaphragmatic muscles and corresponding activation of the vagus nerve and other accommodation mechanisms do not function properly. Patients often experience a paradoxical effect consisting of a contraction of the diaphragm muscle and a relaxation of the upper portion of the abdominal wall, whereas in healthy subjects, concomitant relaxation of the diaphragm muscle and activation of the rectus abdominis (the upper part) and external oblique usually happen. This phenomenon can lead to dyspepsia and feelings of bloatedness. The technical name for this occurrence is "abdomino-phrenic dyssynergia." The solution is teaching mindfulness and diaphragmatic breathing to patients. This intervention is efficacious in relieving the symptoms of IBS and GERD.[3,4]

Many patients with minor digestive disorders treat themselves with over-the-counter medications and dietary supplements such as antacids, acid-blocking drugs, or probiotics. Sometimes this results in effective relief of symptoms. However, the use of antacids and acid-blocking drugs can lead to deterioration of digestive functions.

Antacids and acid-blocking drugs typically raise the gastric pH above 3.5, effectively inhibiting the action of pepsin, the enzyme involved in protein digestion that can be irritating to the stomach. Although raising the pH can reduce symptoms, it also substantially

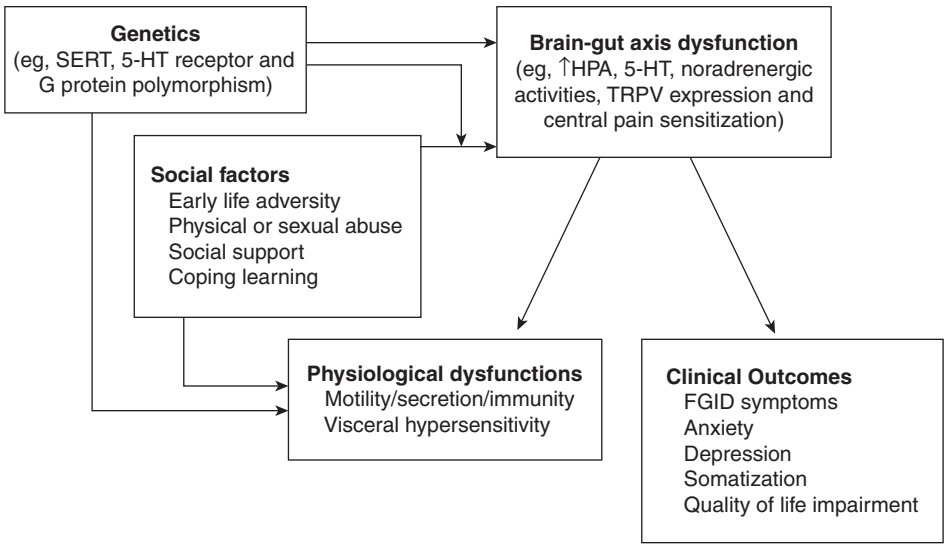


Fig. 133.1 Schematic diagram illustrating the pathophysiological links between functional gastrointestinal disorder and psychiatric disorders. *SERT,* serotonin reuptake transporter; *5-HT,* serotonin; *HPA,* hypothalamic-pituitary-adrenal; *TRPV,* transient receptor potential vanilloid; *FGID,* functional gastrointestinal disorder. (From Wu JC. Psychological co-morbidity in functional gastrointestinal disorders: epidemiology, mechanisms and management. *J Neurogastroent. Motil.* 2012;18[1]:13–18. PubMed PMID: 22323984.)

impairs protein digestion and mineral disassociation. In addition, the change in pH can adversely affect gut microbial flora, including the promotion of an overgrowth of *Helicobacter pylori.* Finally, most nutrition-oriented physicians believe that lack of acid, not excess, is the true culprit in most patients with indigestion.

Most people use these drugs because they self-diagnose hyperacidity. However, in most cases, the symptoms of reflux esophagitis are most often caused by overeating or eating foods that promote gastric reflux, not excessive acid production. See the chapter on GERD for a more complete discussion. Other common causes of heartburn or indigestion are as follows:

- Obesity
- Cigarette smoking
- Chocolate
- Fried foods
- Carbonated beverages
- Alcohol
- Coffee

These factors most often either increase intraabdominal pressure or decrease the tone of the esophageal sphincter.

Another important dietary consideration is the glycemic load of a meal. In the Study on the Epidemiology of Psychological, Alimentary Health and Nutrition (SEPAHAN), a high-glycemic-load meal or habitual diet was significantly associated with heartburn/dyspepsia, especially in men.[5]

Complaints of indigestion and heartburn may also be a sign of a hiatal hernia. Although 50% of people older than 50 years have hiatal hernias, only 5% of patients with hiatal hernias experience reflux esophagitis and/or symptoms of GERD. Perhaps the most effective treatment of chronic reflux esophagitis and symptomatic hiatal hernias is to use gravity. The standard recommendation is to place 4-inch blocks under the bedposts at the head of the patient's bed. This elevation of the head is very effective in many cases. Another recommendation is for the patient to drink a glass of alkaline water (pH > 8.8) or lemon water after a meal. This practice was compared with proton pump inhibitors in patients with laryngopharyngeal reflux and was found to produce better results based on changes in Reflux Symptom Index (RSI).[6]

BOX 133.1 Common Signs and Symptoms of Low Gastric Acidity

Bloating, belching, burning, and flatulence immediately after meals
A sense of "fullness" after eating
Indigestion, diarrhea, or constipation
Multiple food allergies
Nausea after taking supplements
Itching around the rectum
Weak, peeling, and cracked fingernails
Dilated blood vessels in the cheeks and nose
Acne
Iron deficiency
Chronic intestinal parasites or abnormal flora
Undigested food in stool
Chronic *Candida* infections
Upper digestive tract gassiness

Hypochlorhydria

In the patient with chronic indigestion, rather than focusing on blocking the digestive process with antacids, the natural approach focuses on aiding digestion. Although much is said about hyperacidity conditions, a more common cause of indigestion is a lack of gastric acid secretion. Many symptoms and signs suggest impaired gastric acid secretion, and several specific diseases have been associated with insufficient gastric acid output.[7–17] They are listed in Boxes 133.1 and 133.2.

Several studies have shown that the ability to secrete gastric acid decreases with age.[18–20] Some studies found low stomach acidity in more than half of the subjects older than 60 years. The best method of diagnosing a lack of gastric acid is the Heidelberg gastric analysis (see Chapter 17).[21] Wright[22] suggested that the response to a bicarbonate challenge during Heidelberg gastric analysis, not simply resting pH, was the true test of the functional ability of the stomach to secrete acid.

Because the Heidelberg gastric acid analysis is not widely available, a clinical trial of hydrochloric acid (HCl) supplements can be used, as described in Appendix 8.

BOX 133.2 Diseases Associated With Low Gastric Acidity

- Addison's disease
- Asthma
- Celiac disease
- Dermatitis herpetiformis
- Diabetes mellitus
- Eczema
- Gallbladder disease
- Graves' disease
- Chronic autoimmune disorders
- Hepatitis
- Hyperthyroidism/hypothyroidism
- Hives (chronic)
- Myasthenia gravis
- Osteoporosis
- Pernicious anemia
- Psoriasis
- Rheumatoid arthritis
- Rosacea
- Sjögren's syndrome
- Systemic lupus erythematosus
- Thyrotoxicosis
- Vitiligo

Etiology of Hypochlorhydria

Like peptic ulcer disease, achlorhydria and hypochlorhydria have been linked to the overgrowth of the bacteria *H. pylori.* Approximately 90% to 100% of patients with duodenal ulcers, 70% of patients with gastric ulcers, and about 50% of people older than 50 years test positive for *H. pylori.* The presence of *H. pylori* is determined by measuring the level of antibodies to *H. pylori* in the blood or saliva or by culturing material collected during an endoscopy as well as measuring the breath for urea. A breath test is also available for assessment of current *H. pylori* activity.

Low gastric output is thought to predispose to *H. pylori* colonization, and *H. pylori* colonization increases gastric pH, thereby setting up a positive-feedback scenario and increasing the likelihood of colonization of the stomach and duodenum by other organisms. Not surprisingly, HCl antisecretory drugs (H_2 receptor antagonists and proton pump inhibitors) may promote *H. pylori* overgrowth. Patients with *H. pylori* experience an exaggerated response in elevations of pH with antisecretory therapy. Eradication of *H. pylori* is associated with a return to normal gastric acidity and pepsinogen ratio.[23]

Although the typical conventional medicine approach is to focus only on the infective agent, the usual host defense factors are equally important. Unfortunately, research has focused on eradicating the organism, and there is little information on protective factors against infection. Proposed protective factors against *H. pylori*–induced intestinal damage include maintaining a low pH and ensuring adequate antioxidant defense mechanisms. Low levels of vitamin C and vitamin E and other antioxidant factors in the gastric juice appear to not only lead to the progression of *H. pylori* colonization but also to contribute to ulcer formation because the mechanism by which *H. pylori* damages the stomach and intestinal mucosa is oxidative.[24] Furthermore, antioxidant status and gastric acid output appear to explain the observation that most people infected with *H. pylori* do not experience peptic ulcer disease or gastric cancer. For more information on natural approaches for eradicating *H. pylori,* see Chapter 207.

One natural medicine that may be useful against *H. pylori* is bovine lactoferrin. Lactoferrin exerts broad-spectrum antimicrobial action because it has been shown to be effective in inhibiting the growth of disease-causing protozoa, yeast, bacteria, and viruses. More important than its ability to kill organisms is the discovery that lactoferrin prevents the attachment of disease-causing organisms to cells that line the mouth and entire gastrointestinal tract. At the same time, lactoferrin is a powerful booster of health-promoting bacteria like *Bifidobacteria* and *Lactobacillus* species. By preventing the growth of harmful bacteria while promoting the growth of beneficial *Bifidobacteria,* lactoferrin assists in the development of proper intestinal flora.

The standard medical treatment of *H. pylori* infection is a 1- or 2-week course of triple-drug therapy. It involves taking two antibiotics to kill the bacteria and an acid-suppressor drug. Based on the results of clinical trials, lactoferrin alone or in combination with triple therapy may be an excellent treatment option. In one study, 151 patients testing positive for *H. pylori* with indigestion symptoms were given either triple therapy alone or with lactoferrin. *H. pylori* status assessed 8 weeks after the end of the treatment indicated a 95.9% eradication rate for the group receiving the lactoferrin; the other group had only a 72.5% eradication rate.[25] The effective dose of lactoferrin in this application is 300 mg/day.

Pancreatic Insufficiency

The most severe level of exocrine pancreatic insufficiency (EPI) is seen in cystic fibrosis. Next in severity is the EPI associated with the late stages of pancreatitis. These more severe causes of EPI are most often easily recognized, but causes of mild EPI are more insidious and difficult to diagnose. Patients with type 1 diabetes not only have damaged beta cells, but the enzyme-producing alpha cells are damaged as well.

Both physical symptoms and laboratory tests can be used to assess pancreatic function in patients in whom mild EPI is suspected. Common symptoms of EPI are abdominal bloating and discomfort, gas, indigestion, and the passing of undigested food in the stool. For laboratory diagnosis, a comprehensive stool and digestive analysis (discussed in Chapter 28) is quite useful. In addition, the measurement of fecal elastase 1 concentrations using an enzyme-linked immunosorbent assay is an accepted indirect test of the exocrine pancreatic function. It shows higher sensitivity and specificity for EPI than does fecal chymotrypsin determination and is comparable to oral pancreatic function tests, such as the pancreolauryl test.[26] However, the test may not show levels of active secreted enzymes. Each pancreatic enzyme has a very narrow range of pH activity as well as being inhibited by several foods, microbial compounds, persistent organic pollutants, heavy metals, and many other factors. Even so, the measurement of fecal elastase has shown that pancreatic insufficiency is associated with normal aging in healthy populations, especially after age 70 years.[27] Patients may have few symptoms associated with EPI, for example, steatorrhea, diarrhea, abdominal pain, and weight loss, but they may experience gas, bloating, and indigestion.

Digestive Enzyme Supplements

Pancreatic enzyme products are an effective treatment for EPI and are widely used. Most commercial preparations are prepared from fresh hog pancreas (i.e., pancreatin) (see Chapter 100 for a full discussion). Enzyme products are often enteric coated, primarily to protect the more fragile porcine lipase. However, some studies have shown that non–enteric-coated enzyme preparations outperform enteric-coated products if they are given before a meal (for digestive purposes) or on an empty stomach (for anti-inflammatory effects). In general, there is no significant difference in clinical response to various delivery forms of pancreatin.[28]

Alternatives to porcine pancreatin include plants (e.g., bromelain, papain, etc.) and enzymes extracted from various microbes or

yeast (e.g., *Aspergillus oryzae*). These enzymes are more resistant to digestive secretions and have a broader range of activity, including pH range (see Chapter 59, Bromelain, and Chapter 95, Microbial Enzyme Therapy). One double-blind crossover trial in 17 patients with severe EPI and steatorrhea compared the effects of a non–enteric-coated pancreatic enzyme preparation (360,000 lipase U/day), an enteric-coated pancreatic enzyme preparation (100,000 lipase U/day), and a fungal enzyme preparation (75,000 lipase U/ day).[25] All three treatment preparations in both groups yielded a significant reduction in total daily stool weight and total daily fecal fat excretion compared with controls. It is interesting to point out, however, that the fungal enzyme preparation produced similar benefit at three-fourths the dose of the enteric-coated pancreatic enzyme and one-fifth the dose of the non–enteric-coated pancreatic enzyme preparation.

PROBIOTIC SUPPLEMENTS

Probiotic supplementation may be useful in improving digestion. However, it is important to point out that although probiotics may play a role in general digestive health, immune function, and other aspects of the microbiome, probiotics do not directly aid digestion in terms of breaking down food into smaller units.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Marlicz W, Yung DE, Skonieczna-Żydecka K, et al. From clinical uncertainties to precision medicine: the emerging role of the gut barrier and microbiome in small bowel functional diseases. *Expert Rev Gastroenterol Hepatol*. 2017;11(10):961–978.
2. Camilleri M, Dubois D, Coulie B, et al. Prevalence and socioeconomic impact of upper gastrointestinal disorders in the United States: results of the US Upper Gastrointestinal Study. *Clin Gastroenterol Hepatol*. 2005;3:543–552.
3. Bordoni B, Morabito B. Symptomatology correlations between the diaphragm and irritable bowel syndrome. *Cureus*. 2018;10(7):e3036.
4. Ong AM, Chua LT, Khor CJ, et al. Diaphragmatic breathing reduces belching and proton pump inhibitor refractory gastroesophageal reflux symptoms. *Clin Gastroenterol Hepatol*. 2018;16(3):407–416.
5. Keshteli AH, Haghighatdoost F, Azadbakht L, et al. Dietary glycaemic index and glycaemic load and upper gastrointestinal disorders: results from the SEPAHAN study. *J Hum Nutr Diet*. 2017;30(6):714–723.
6. Zalvan CH, Hu S, Greenberg B, Geliebter J. A comparison of alkaline water and Mediterranean diet vs proton pump inhibition for treatment of laryngopharyngeal reflux. *JAMA Otolaryngol Head Neck Surg*. 2017;143(10):1023–1029.
7. Bray GW. The hypochlorhydria of asthma in childhood. *Q J Med*. 1931;24:181–197.
8. Rabinowitch IM. Achlorhydria and its clinical significance in diabetes mellitus. *Am J Dig Dis*. 1949;18:322–333.
9. Capper WM, Butler TJ, Kilby JO, et al. Gallstones, gastric secretion, and flatulent dyspepsia. *Lancet*. 1967;1:413–415.
10. Rawls WB, Ancona VC. Chronic urticaria associated with hypochlorhydria or achlorhydria. *Rev Gastroenterol*. 1951;18:267–271.
11. Giannella RA, Broitman SA, Zamcheck N. Influence of gastric acidity on bacterial and parasitic enteric infections: a perspective. *Ann Intern Med*. 1973;78:271–276.
12. De Witte TJ, Geerdink PJ, Lamers CB, et al. Hypochlorhydria and hypergastrinaemia in rheumatoid arthritis. *Ann Rheum Dis*. 1979;38:14–17.
13. Ryle JA, Barber HW. Gastric analysis in acne rosacea. *Lancet*. 1920;2:1195–1196.
14. Ayres S. Gastric secretion in psoriasis, eczema and dermatitis herpetiformis. *Arch Dermatol*. 1929:854–859.
15. Dotevall G, Walan A. Gastric secretion of acid and intrinsic factor in patients with hyper and hypothyroidism. *Acta Med Scand*. 1969;186:529–533.
16. Howitz J, Schwartz M. Vitiligo, achlorhydria, and pernicious anemia. *Lancet*. 1971;1:1331–1334.
17. Howden CW, Hunt RH. Relationship between gastric secretion and infection. *Gut*. 1987;28:96–107.
18. Rafsky HA, Weingarten M. A study of the gastric secretory response in the aged. *Gastroenterol*. 1947:348–352.
19. Davies D, James TG. An investigation into the gastric secretion of a hundred normal persons over the age of sixty. *BJM*. 1930;i:1–14.
20. Baron JH. Studies of basal and peak acid output with an augmented histamine test. *Gut*. 1963;4:136–144.
21. Mojaverian P, Ferguson RK, Vlasses PH, et al. Estimation of gastric residence time of the Heidelberg capsule in humans: effect of varying food composition. *Gastroenterol*. 1985;89:392–397.
22. Wright J. A proposal for standardized challenge testing of gastric acid secretory capacity using the Heidelberg capsule radiotelemetry system. *J John Bastyr Col Nat Med*. 1979;1:3–11.
23. Kato S, Matsukura N, Matsuda N, et al. Normalization of pH level and gastric mucosa after eradication of H. pylori in the remnant stomach. *J Gastroenterol Hepatol*. 2008;23(suppl 2):S258–S261.
24. Baik SC, Youn HS, Chung MH, et al. Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. *Cancer Res*. 1996;56:1279–1282.
25. Schneider MU, Knoll-Ruzicka ML, Domschke S, et al. Pancreatic enzyme replacement therapy: comparative effects of conventional and enteric-coated microspheric pancreatin and acid-stable fungal enzyme preparations on steatorrhoea in chronic pancreatitis. *Hepatogastroenterol*. 1985;32:97–102.
26. Dominici R, Franzini C. Fecal elastase-1 as a test for pancreatic function: a review. *Clin Chem Lab Med*. 2002;40:325–332.
27. Löhr JM, Panic N, Vujasinovic M, Verbeke CS. The ageing pancreas: a systematic review of the evidence and analysis of the consequences. *J Intern Med*. 2018;283(5):446–460.
28. Taylor JR, Gardner TB, Waljee AK, et al. Systematic review: efficacy and safety of pancreatic enzyme supplements for exocrine pancreatic insufficiency. *Aliment Pharmacol Ther*. 2010;31:57–72.

134

Homocysteine Metabolism: Nutritional Modulation and Effect on Health and Disease

Jessica Tran, ND, MBA, Gregory S. Kelly, ND,* and *Alan L. Miller*, ND*

OUTLINE

INTRODUCTION

Homocysteine is an intermediate in the conversion of the amino acid methionine to cysteine. Elevated homocysteine levels are an independent risk factor for coronary heart disease, stroke, and other vascular conditions. Homocysteine and its relationship to cardiovascular disease emerged in the late 1960s, when Kilmer McCully, MD, encountered two children with homocystinuria (a rare autosomal-recessive condition) who had advanced atherosclerosis, although the coronary plaques contained no lipids. Increased homocysteine levels have been implicated in several other clinical conditions, including neural tube defects, spontaneous abortion, placental abruption, renal failure, non–insulin-dependent diabetes and complications of diabetes, rheumatoid arthritis, alcoholism, osteoporosis, and neuropsychiatric disorders.

HOMOCYSTEINE METABOLISM

Homocysteine is metabolized along two pathways: remethylation to methionine (which requires methionine synthase along with vitamin B_{12} and folate or betaine) or transsulfuration to cysteine (which requires vitamin B_6). A defect in either of these pathways leads to the accumulation of homocysteine. Insufficient dietary intake of vitamin B_6, vitamin B_{12}, and folate can lead to increased homocysteine levels.

Gender and Genetics

Studies of healthy men and women indicate that certain acquired and genetic determinants may affect total plasma homocysteine. Women tend to have lower basal levels than men,[1] and neither contraceptives nor hormone replacement therapy seem to alter their levels significantly.[2] However, in postmenopausal women, hormone replacement therapy might slightly decrease elevated homocysteine concentrations. No significant lowering effect was observed in women with low homocysteine levels.[3] Generally, homocysteine concentrations are significantly higher in postmenopausal women than in premenopausal women; however, the previously mentioned sex differences in homocysteine concentrations persist even in elderly populations.[4–6] The antiestrogen drug tamoxifen, used in the long-term treatment of breast cancer patients, was reported to decrease homocysteine levels in postmenopausal women with breast cancer.[7]

A number of reports also demonstrated that elevated plasma total homocysteine in children was correlated with either cardiovascular disease or death in their parents or close relatives. This relationship

*Previous edition contributor

was observed in both white and black children and in white children with hypercholesterolemia. In the latter study group, the 5,10-methylenetetrahydrofolate reductase (MTHFR) *TT* genotype tended to be most frequent in children with a parental history of cardiac disease.[8] Epidemiological evidence showed homocysteine levels to be more than 45% lower in Westernized adult black Africans than in age-matched white adults, revealing racial genetic differences in homocysteine metabolism.[9]

The *MTHFR* gene has two different alleles, where the "T" allele is associated with decreased enzyme activity, hyperhomocysteinemia, and increased risk for thromboembolism in coronary heart disease. The presence of the "C" allele is correlated to lower homocysteine levels and, not surprisingly, may even provide protection against occlusion of coronary arteries. One Hungarian study concluded that the carriers of the T allele with coronary heart disease died earlier due to myocardial infarction.[10]

Lifestyle

An association between coffee and black tea consumption and the concentration of total homocysteine in plasma has been reported.[11] A marked positive dose–response relation between coffee consumption and plasma homocysteine levels was observed. The relationship was most marked in males and females consuming more than eight cups of coffee per day. The combination of cigarette smoking and high coffee intake was associated with particularly high homocysteine concentrations.[12] Long-term ingestion of alcohol has also been associated with increased homocysteine levels.[13,14] Plasma total homocysteine is inversely related to exercise.[15]

Nutritional Relationships

Nutrition affects homocysteine concentrations in both men and women. For example, individuals in the lowest quartiles for serum folate and vitamin B_{12} have significantly higher concentrations of homocysteine, and men in the lowest quartile of serum pyridoxal 5′-phosphate (P^5P; the bioactive form of vitamin B_6) also have increased homocysteine concentrations.[2]

Methionine

Metabolism of the amino acid methionine, a limiting amino acid in the synthesis of many proteins, affects several biochemical pathways involving the production of nutrients that are essential to the optimal functioning of the cardiovascular, skeletal, and nervous systems.

Homocysteine is an intermediate product of methionine metabolism and is metabolized by two pathways: the remethylation pathway, which regenerates methionine; and the transsulfuration pathway, which degrades homocysteine into cysteine and then taurine. In essence, the intermediate metabolite homocysteine is located at a metabolic crossroads, so it directly and indirectly affects all methyl and sulfur group metabolism occurring in the body. Experiments demonstrated that high levels of L-homocysteine and adenosine in the cell inhibit all methylation reactions.[16]

The remethylation pathway (Fig. 134.1) comprises two intersecting biochemical pathways and results in the transfer of a methyl group (CH_3) to homocysteine by either methylcobalamin or betaine (trimethylglycine). Methylcobalamin originally receives its methyl group from S-adenosylmethionine (SAMe) or 5-methyltetrahydrofolate (5-methylTHF). After remethylation, methionine can be reused

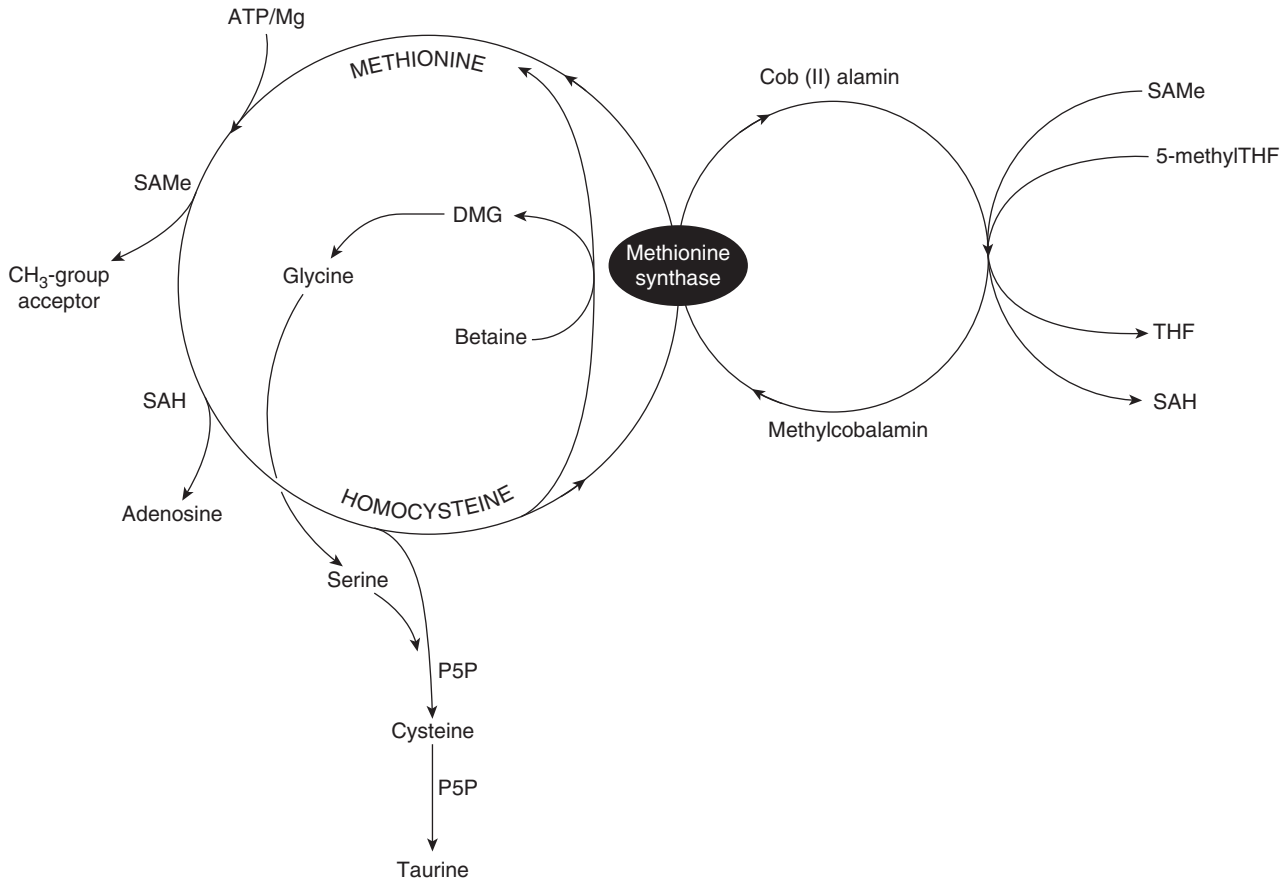


Fig. 134.1 Homocysteine metabolism. *DMG,* dimethylglycine; *5-methylTHF,* 5-methyltetrahydrofolate; *P5P,* pyridoxal 5′-phosphate (vitamin B_6); *SAH,* S-adenosylhomocysteine; *SAMe,* S-adenosylmethionine; *THF,* tetrahydrofolate.

to produce SAMe, the body's "universal methyl donor," which participates in several key metabolic pathways, including methylation of DNA and myelin, synthesis of carnitine, coenzyme Q_{10} (CoQ_{10}), creatine, epinephrine, melatonin, methylcobalamin, and phosphatidylcholine (PC), as well as Phase II methylation detoxification reactions.

The transsulfuration pathway of methionine/homocysteine degradation (see Fig. 134.1) produces the amino acids cysteine and taurine, which are important nutrients for cardiac health, hepatic detoxification, cholesterol excretion, bile salts formation, and glutathione (GSH) production. This pathway depends on adequate dietary intake and hepatic conversion of vitamin B_6 into its active form, P^5P. The amino acid serine, a down-line metabolite generated from betaine via the homocysteine remethylation pathway, is also necessary.

In addition to 5-methylTHF, methylcobalamin, betaine, and P^5P, *N*-acetylcysteine (NAC) has been reported to significantly lower homocysteine levels.[17]

S-Adenosylhomocysteine

The metabolic precursor of homocysteine in all tissues is S-adenosylhomocysteine, which is more difficult to measure than homocysteine but has recently been indicated as a more sensitive marker for cardiovascular disease and Alzheimer's disease. S-adenosylhomocysteine is formed by the demethylation of SAMe (Fig. 134.2). S-adenosylhomocysteine hydrolase catalyzes the hydrolysis of S-adenosylhomocysteine to adenosine and homocysteine. S-adenosylhomocysteine hydrolase deficiency is a genetic disorder of methionine metabolism that results in slow psychomotor development.

Increased levels of S-adenosylhomocysteine may occur when the intracellular concentration of homocysteine increases.[18–20]

Methyltetrahydrofolate

Folates function as carbon donors in the synthesis of serine from glycine, directly in the synthesis of purines and pyrimidine bases, and indirectly in the synthesis of transfer RNA. Folates also function as methyl donors to create methylcobalamin, which is used for remethylation of homocysteine to methionine.

Folic acid is the synthetic form of folate and requires several metabolic steps within the cell to be converted into the metabolically active tetrahydrofolate. It is an oxidized synthetic water-soluble member of the vitamin-B complex family and is found in fortified foods, supplements, and pharmaceuticals. L-5-methyl-tetrahydrofolate (L-5-methyl-THF) is the predominant form of dietary folate that is transported into peripheral tissues to be used for cellular metabolism.

Synthesis of the active forms of folate is a complex process requiring several enzymes, as well as adequate supplies of niacin, P^5P, and serine as cofactors (Fig. 134.3). In plants, folate is formed from a hetero-bicyclic pteridine ring, para-aminobenzoic acid, and glutamic acid. Folate is initially deconjugated in the cells of the intestinal wall to the monoglutamate form. This is then reduced to dihydrofolate and then to tetrahydrofolate (THF) via folate and dihydrofolate reductase. Both enzymes require reduced nicotinamide adenine dinucleotide phosphate (niacin dependent) as a cofactor. Serine combines with P^5P to transfer a hydroxymethyl group to THF. This results in the formation of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) and glycine. This molecule is of central importance, being the precursor of the metabolically active 5-methylTHF, which is involved in homocysteine metabolism, and 10-formyltetrahydrofolate (involved in purine synthesis), as well as functioning on its own in the generation of thymine side chains for incorporation into DNA.

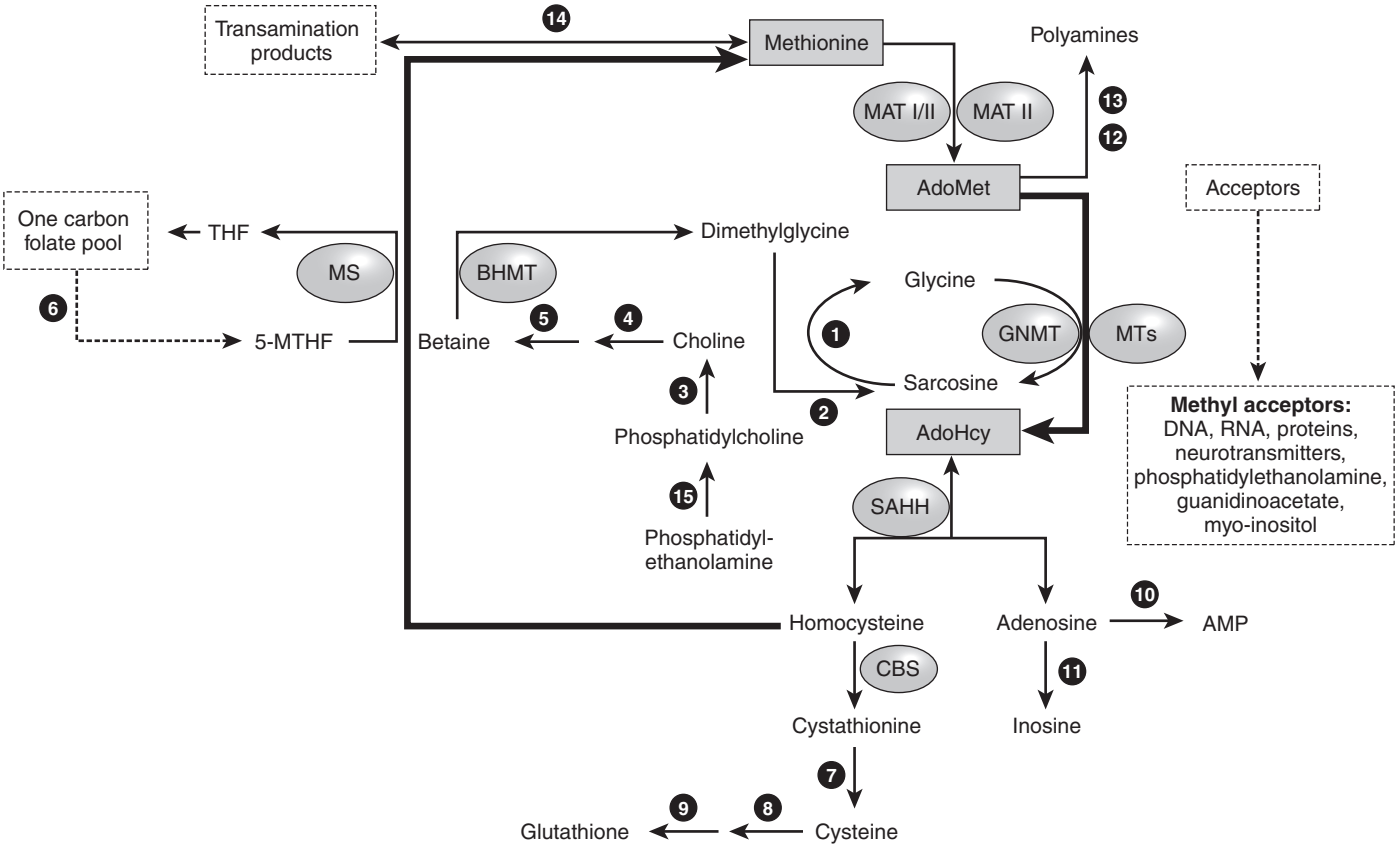


Fig. 134.2 Methionine, AdoMet (S-adenosylmethionine), and AdoHcy metabolism. (From Baric I, Fumić K, Glenn B, et al. S-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. *Proc Natl Acad Sci.* 2004;101:4234–4239.)

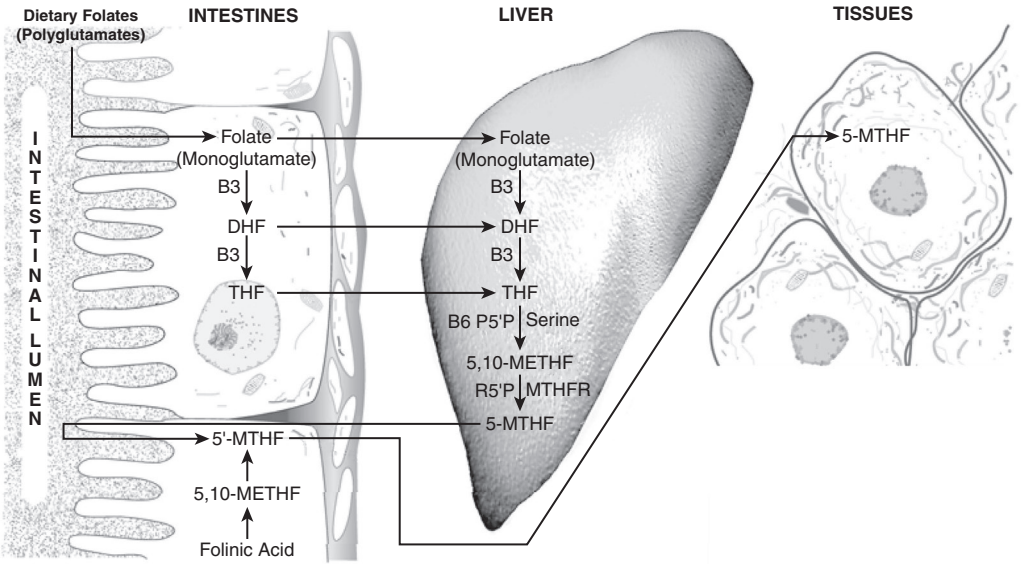


Fig. 134.3 Absorption and activation of folic acid. *DHF,* dihydrofolate; *5,10-METHF,* 5,10-methylenetetrahydrofolate; *5-MTHF,* 5-methyltetrahydrofolate; *MTHFR,* methylenetetrahydrofolate reductase; *P5′-P,* pyridoxal 5′-phosphate; *R5′-P,* riboflavin 5′-phosphate; *THF,* tetrahydrofolate.

The following may contribute to a deficiency of folate:

- A deficient food supply
- A defect in utilization, as in alcoholics
- Malabsorption
- Increased needs in pregnant women and cancer patients
- Metabolic interference by drugs
- Folate losses in hemodialysis
- Enzyme or cofactor deficiency necessary for the generation of folate

Individuals using supplements or consuming either breakfast cereals or green leafy vegetables have significantly greater plasma folate and lower homocysteine levels than those who do not.[1]

Folinic acid (5-formylTHF), available supplementally as calcium folinate (also known as leucovorin calcium), is an immediate precursor to 5,10-methyleneTHF and 5-methylTHF. Folinic acid can correct deficiencies of the active forms of folate, is more stable than folic acid, and has a longer half-life in the body. Folinic acid also readily crosses the blood–brain barrier and is slowly cleared, compared with folic acid, which is poorly transported into the brain and, once in the central nervous system (CNS), is rapidly cleared.[21]

Methylcobalamin

The coenzyme form of vitamin B_{12} is a complex molecule containing cobalt bound to five nitrogens and one carbon. The metal–carbon bond found on this coenzyme is the only known biological example of this type of linkage. The use of cobalt in the two biologically active forms of cobalamin, adenosylcobalamin and methylcobalamin, is the only known function of this metal in biological systems.

In humans, the cobalt in cobalamin exists in a univalent oxidation state, designated as cob(I)alamin. The compound commonly referred to as *vitamin B_{12}* has a cyanide molecule at the metal–carbon position, and the oxidation state of the cobalt is +3 instead of the biologically active +1. To be used in the body, the cyanide molecule must be removed. It is thought that the compound GSH may perform this function. Other available forms of vitamin B_{12} include hydroxocobalamin and the two active forms, adenosylcobalamin (cobamamide) and methylcobalamin.

The absorption of dietary cobalamin requires the formation of a complex between dietary vitamin B_{12} and R-proteins and the secretion, by the stomach mucosa, of intrinsic factor. The vitamin B_{12} complex is split by pancreatic proteases, and the released vitamin B_{12} attaches to intrinsic factor and is absorbed in the distal ileum. The amount of cobalamin required in the diet is low, and even people with pernicious anemia can generally absorb sufficient amounts if the coenzyme is supplemented at a high enough dosage.

Although the basic cobalamin molecule is only synthesized by microorganisms, all mammalian cells can convert this into the coenzymes adenosylcobalamin and methylcobalamin. Adenosylcobalamin is the major form in cellular tissues, where it is retained in the mitochondria. Methylcobalamin predominates in blood plasma and certain other body fluids and in cells found in the cytosol.

Adenosylcobalamin functions in reactions in which hydrogen groups and organic groups exchange places. In humans, adenosylcobalamin is required in only two reactions: the catabolic isomerization of methylmalonyl coenzyme A (CoA) to succinyl-CoA, and interconversion of α- and β-leucine. After its formation from methylmalonyl-CoA, succinyl-CoA is either involved in the synthesis of porphyrin molecules (along with glycine) or it transfers its CoA to form acetyl-CoA. The latter reaction is magnesium dependent, and the remaining succinate is fed into the citric acid cycle. Deficiencies in this coenzyme form of vitamin B_{12} result in increased amounts of methylmalonyl-CoA and generally an increase in glycine levels.

Methylcobalamin's only known biological function in humans is in the remethylation of homocysteine to methionine via the enzyme methionine synthase, also known as 5-methyltetrahydrofolate-homocysteine methyltransferase. To originally form methylcobalamin from cyanocobalamin or other Cob(III)alamin or Cob(II)alamin precursors, SAMe must be available to supply a methyl group. Once methylcobalamin is formed, it functions in the regeneration of methionine by transferring its methyl group to homocysteine. Methyl-cobalamin can then be regenerated by 5-methylTHF (Fig. 134.4). The cell's ability to methylate important compounds such as proteins, lipids, and myelin is compromised by a deficiency of either folate or vitamin B_{12}.[22] Shortages of folate, SAMe, or a dietary deficiency of cobalamin lead to a decrease in the generation of methylcobalamin and a subsequent impairment of homocysteine metabolism. Because lack of methylcobalamin leads to depressed DNA synthesis, rapidly dividing cells in the brain and elsewhere are affected.

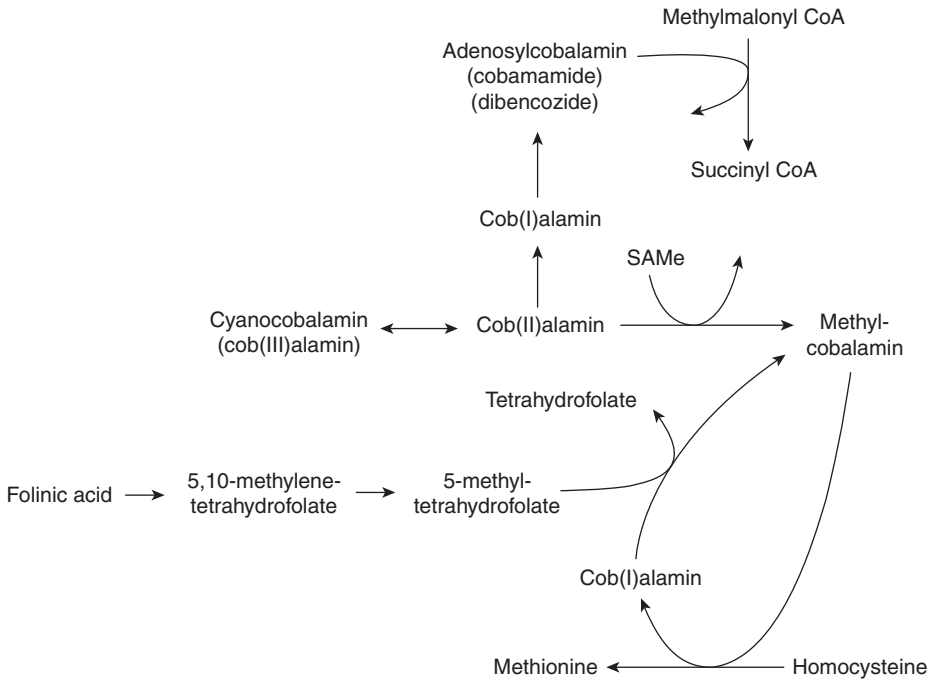


Fig. 134.4 Cobalamin metabolism.

At least 12 different inherited inborn errors of metabolism related to cobalamin are known. Abnormalities are detectable by urine and plasma assays of methylmalonic acid and homocysteine and by plasma and erythrocyte analysis of cobalamin coenzymes, which can reveal deficiencies of methylcobalamin or adenosylcobalamin.[23]

Betaine

The metabolic pathways of betaine, methionine, methylcobalamin, and methylTHF are interrelated, intersecting at the regeneration of methionine from homocysteine. This regeneration is accomplished in one of two ways. One involves the generation in the cytosol of 5-methylTHF from methylene THF and the transfer of its methyl group to regenerate methylcobalamin, which then acts as a coenzyme in the regeneration of methionine. Because THF and its derivatives can only cross the mitochondrial membrane slowly, inside, the mitochondria regeneration of methionine relies on recovery of a methyl group from betaine.

Betaine donates one of its three methyl groups via the enzyme betaine-homocysteine methyltransferase to homocysteine, resulting in the regeneration of methionine. After the donation of the methyl group, one molecule of dimethylglycine remains. This molecule is oxidized to glycine and to two molecules of formaldehyde by riboflavin-dependent enzymes. The formaldehyde can combine with THF within the mitochondria to generate methylenetetrahydrofolate, which can be converted to 5-methylTHF and subsequently used as a methyl donor (Fig. 134.5).

In animal studies, a disturbance in the metabolism of either of the two methyl-donor pathways, due to limited availability of either betaine or folates and vitamin B_{12}, affects levels of nutrients in the coexisting pathway, because more of a drain is placed on the other pathway as a source of methyl groups. Rats fed diets deficient in choline and methionine had hepatic folate concentrations half that of controls after 5 weeks.[24] During choline deficiency, hepatic SAMe concentrations were also shown to decrease by as much as 50%.[25] Similarly, THF deficiency resulted in decreased hepatic total choline levels.[26]

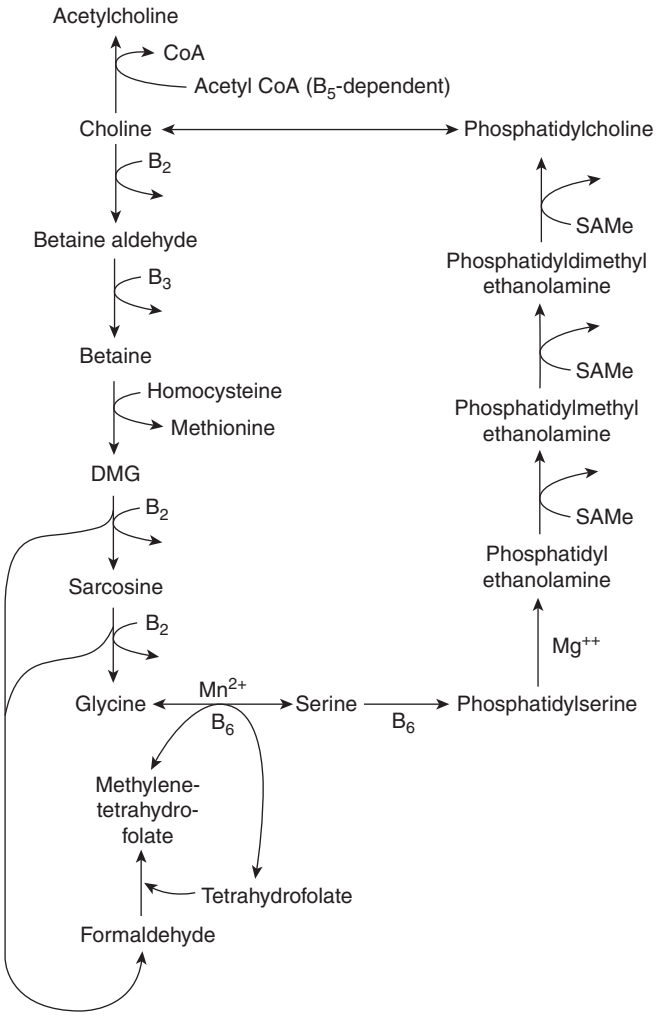


Fig. 134.5 Phosphatidylcholine metabolism.

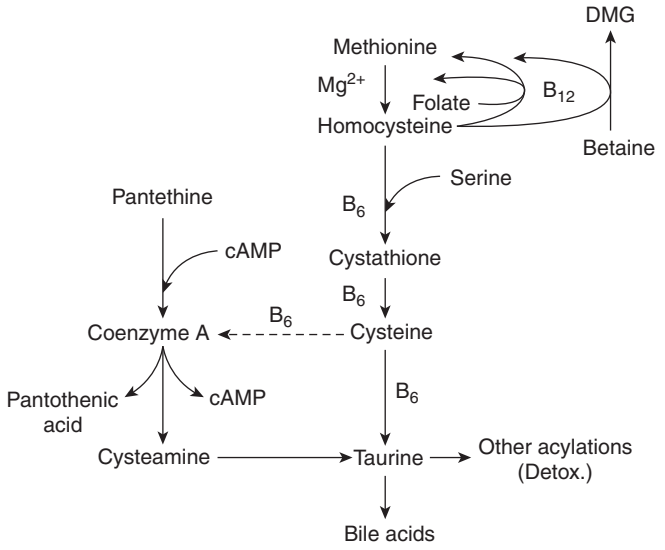


Fig. 134.6 Synthesis of taurine.

Patients with a congenital deficiency of the enzyme MTHFR, which is needed for the formation of 5-methylTHF, have reduced levels of both methionine and SAMe in the cerebrospinal fluid and show demyelination in the brain and degeneration of the spinal cord. Methionine is effective in the treatment of some of these patients; however, betaine was shown to restore cerebrospinal fluid SAMe levels to normal and to prevent the progression of neurological symptoms in all patients in whom it was tried.[27]

Betaine supplementation has been shown to reduce homocysteine levels[28] while resulting in modest increases of plasma serine and cysteine levels.[29] Stimulation of betaine-dependent homocysteine remethylation causes a commensurate decrease in plasma homocysteine that can be maintained as long as supplemental betaine is taken.[30]

Serine levels are depressed in some individuals with excess homocysteine who are treated with folic acid, cobalamin, and vitamin B_6.[31] Because serine is required (1) for the conversion of folic acid to its active form, (2) as a shuttle for methyl groups between the cytosol and the mitochondria, and (3) as a cofactor in the transsulfuration pathway of methionine/homocysteine metabolism, supplementation with betaine should be included with folic acid, cobalamin, and P^5P to optimize the interrelated pathways of homocysteine metabolism.

Pyridoxal 5´-Phosphate

P^5P is the active coenzyme form of vitamin B_6. This cofactor is involved in myriad biological processes, including the transsulfuration pathway of homocysteine. This degradation pathway involves a two-step process, resulting in the formation of cystathionine and its subsequent cleavage to cysteine. Cystathionine synthase and cystathionase require P^5P as a cofactor, and the committed first step in the degradation of homocysteine, cystathionine synthase, also requires serine, a downstream metabolite of betaine (Fig. 134.6). Some studies suggest that cystathionine could be a useful marker to assess the effect of vitamin B_6 and should be monitored with homocysteine to better elucidate clinical outcomes.[32]

Once cysteine is generated, it can be directed into several different pathways, including synthesis of GSH, acetyl-CoA, and taurine. Three pathways from cysteine to taurine are known; all require P^5P.

RENAL FUNCTION

Impaired kidney function appears to be a factor in raised homocysteine concentrations.[33] It has been shown that a decreased glomerular filtration rate may be a contributing factor, and some researchers suggest that hyperhomocysteinemia may actually reflect early nephrosclerosis,[8] although other studies demonstrate only a minor renal influence.[34] Fenofibrate treatment, known to increase homocysteine levels, is also known to promote elevations in serum creatinine (see "Pharmaceutic Drug Effects on Homocysteine").[35] Cystatin C, a creatinine-independent indicator of glomerular filtration rate, has also been shown to be well associated with homocysteine levels in kidney transplantation patients, as well as those with coronary disease.[35,36]

PHARMACEUTIC DRUG EFFECTS ON HOMOCYSTEINE

Fibrates such as gemfibrozil are a class of drugs used to lower triglycerides and raise high-density lipoprotein levels. In a paradoxical effect working against a safer cardiac risk profile, these drugs have been observed to raise homocysteine levels up to 40%.[35,37,38] The mechanism of this observation is unknown, although it seems that renal mechanisms may be involved (see "Renal Function").

Thiazides and angiotensin-converting enzyme inhibitors are often a first-line conventional treatment to lower elevated blood pressure. One preliminary, randomized, prospective treatment study investigated 40 patients with hypertension after treatment with hydrochlorothiazide or captopril. In this study, vitamins B_6, B_{12}, folic acid, creatinine, and cystatin C were used as parameters of renal function. For 31 and 29 days, respectively, 21 patients were prescribed hydrochlorothiazide, and 19 were prescribed captopril. It was found that hydrochlorothiazide, but not captopril, significantly raised homocysteine by 16%, as well as creatinine and cystatin C.[39]

EFFECT OF HOMOCYSTEINE ON KEY NUTRIENTS

Because of its central role in sulfur- and methyl-group metabolism, elevated levels of homocysteine would be expected to negatively affect the biosynthesis of all of the following: SAMe, carnitine, chondroitin sulfates, CoA, coenzyme Q10 (CoQ_{10}), creatine, cysteine, dimethylglycine, epinephrine, glucosamine sulfate, glutathione (GSH), glycine, melatonin, pantethine, phosphatidylcholine, phosphatidylserine, serine, and taurine.

S-Adenosylmethionine

SAMe is formed by the transfer of an adenosyl group from adenosine triphosphate to the sulfur atom of methionine. This reaction requires magnesium as a cofactor. When methyl groups are transferred from SAMe, S-adenosylhomocysteine is formed. This is then hydrolyzed to release the adenosine and results in the formation of homocysteine.

SAMe is known to be used in the synthesis of the following compounds: carnitine, CoQ_{10}, creatine, methylcobalamin from cob(III) alamin, 1-methylnicotinamide, *N*-methyltryptamine, PC, and polyamines. It is also used in methylation reactions as part of hepatic Phase II detoxification.

Carnitine

A trimethylated amino acid roughly similar in structure to choline, carnitine is a cofactor for the transformation of free long-chain fatty acids into acyl-carnitines and their transport into a mitochondrial matrix, where they undergo β-oxidation for cellular energy production. Synthesis of carnitine begins with the methylation of the amino acid L-lysine by SAMe. Methionine, magnesium, vitamin C, iron, P^5P, and niacin, along with the cofactors responsible for regenerating SAMe from homocysteine (5-methylTHF, methylcobalamin, and betaine), are required for optimal carnitine synthesis (Fig. 134.7).

A pivotal enzyme in carnitine synthesis, betaine aldehyde dehydrogenase, is the same enzyme responsible for the synthesis of betaine from choline. Studies suggest this enzyme has a preference for the choline–betaine conversion and that choline supplementation may decrease carnitine synthesis; therefore it may be of greater benefit to supplement with betaine rather than its precursor, choline.[40,41]

Chondroitin Sulfates, Glucosamine Sulfate, and Other Sulfated Proteoglycans

Proteoglycans are amino sugars found in all tissues, the highest being in cartilage, tendons, ligaments, synovial fluid, skin, fingers, toenails, heart valves, and the basement membrane of all blood vessels. Perhaps the most widely known of the amino sugars are the chondroitin sulfates and glucosamine sulfate (see Chapter 83 for further discussion).

Chondroitin sulfates are primarily composed of alternating residues of *N*-acetyl-D-galactosamine and D-glucuronate. Sulfate residues are present on C-4 of the galactosamine residues in one type of chondroitin and on C-6 in another. Glucosamine sulfate is a simple molecule composed of glucose, the amino acid glutamine, and a sulfate group. Other sulfated proteoglycans include dermatan sulfates, keratan sulfates, and heparan sulfates.

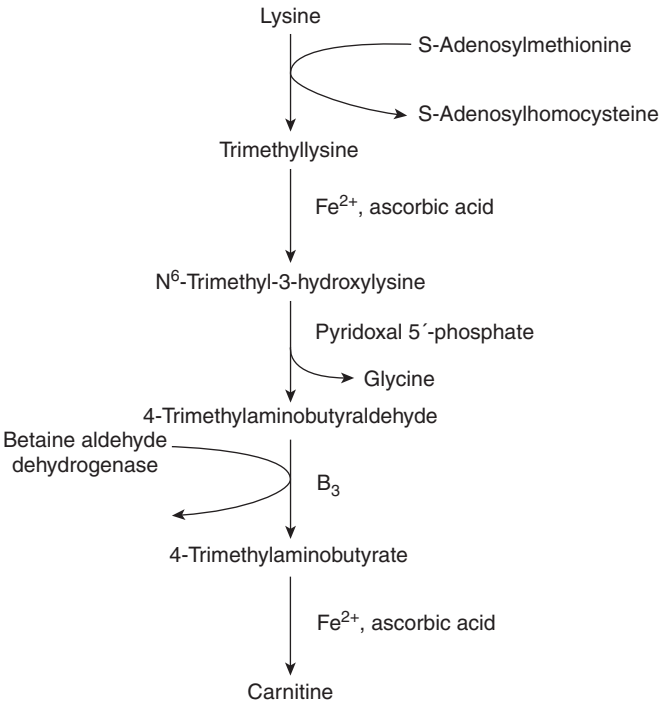


Fig. 134.7 Synthesis of carnitine.

High levels of homocysteine are likely to negatively affect the formation of the sulfated amino sugars because although some sulfates are present in the diet, the sulfoxidation of cysteine is an important source of sulfate molecules. The sulfoxidation pathway proceeds through the toxic intermediate sulfite and requires molybdenum as a cofactor.

Coenzyme A

CoA consists of an adenine nucleotide and phosphopantetheine. Contained within the structure of this coenzyme is pantothenic acid; however, the reactive component of the molecule is a sulfhydryl group that is not contained within the vitamin. To form the sulfhydryl-containing molecule (pantetheine), pantothenic acid must combine with cysteamine. Cysteamine is formed through conjugation and decarboxylation reactions of cysteine. The disulfate form of pantetheine, known as pantethine, as opposed to pantothenic acid, bypasses cysteine conjugation and decarboxylation. This might account for some of the clinical benefits seen with pantethine supplementation that have not been reproduced with the supplementation of pantothenic acid. See Fig. 134.8 A to D for the chemical structures of pantetheine, pantethine, pantothenic acid, and cysteamine.

Coenzyme Q_{10}

CoQ_{10} is a fat-soluble quinone occurring in the mitochondria of each cell (see Chapter 68 for a full discussion). The primary biochemical action of CoQ_{10} is as a cofactor in the electron transport chain, the biochemical pathway that generates adenosine triphosphate. Because most cellular functions depend on an adequate supply of adenosine triphosphate, CoQ_{10} is essential for the health of virtually all human tissues and organs. CoQ_{10} also functions as an antioxidant, assisting in the recycling of vitamin E.[42,43]

Biosynthesis of CoQ_{10} begins with the amino acid tyrosine. Pantothenic acid, P^5P, and vitamin C are all required for the initial steps in its synthesis. An isoprenyl side chain from farnesyl diphosphate, an intermediate in cholesterol synthesis between 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and squalene, is then added. An inadequate supply of this intermediate, which can be caused by HMG-CoA reductase inhibitors (cholesterol-lowering drugs of the statin family), results in decreased levels of CoQ_{10}.[44]

In two of the final steps in the synthesis of CoQ_{10}, methyl groups are provided by SAMe (Fig. 134.9). Adequate dietary methionine and a sufficient supply of the nutrients required for the remethylation of homocysteine to methionine (5-methylTHF, methylcobalamin, and betaine) are required to generate sufficient SAMe. Suboptimal amounts of SAMe may negatively affect the body's ability to synthesize sufficient CoQ_{10}. This relationship between SAMe and CoQ_{10} has been suggested in various animal studies.[45,46]

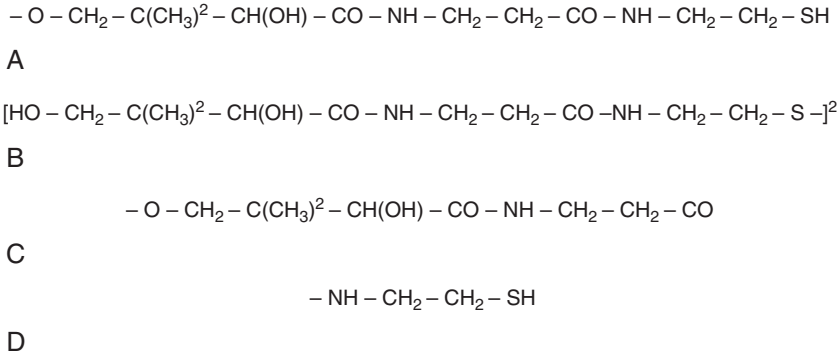


Fig. 134.8 (A) Pantetheine. (B) Pantethine. (C) Pantothenic acid. (D) Cysteamine.

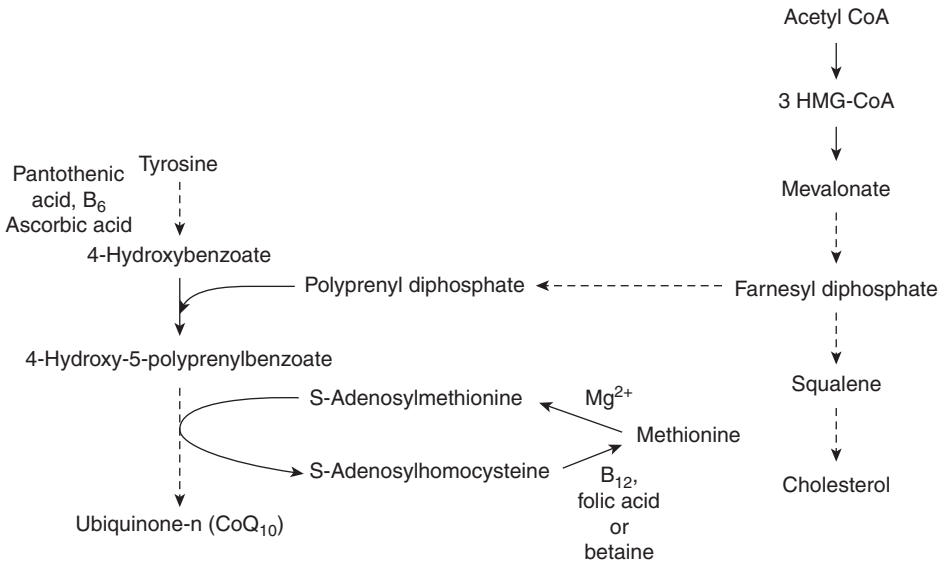


Fig. 134.9 Synthesis of coenzyme Q_{10}.

Creatine

In humans, more than 95% of the total creatine content is located in skeletal muscle, of which approximately one third is in its free form as creatine, also known as methyl-guanidinoacetic acid, whereas the remainder is present in a phosphorylated form as creatine phosphate (also called phosphocreatine). Creatine phosphate is used within skeletal muscle as a means of storing high-energy phosphate bonds.

Creatine is formed in the liver, kidney, and pancreas, beginning with the combination of arginine and glycine to produce guanidinoacetate. A methyl group from SAMe is then transferred, resulting in the formation of creatine. The by-product of this reaction, S-adenosylhomocysteine, is subsequently hydrolyzed into homocysteine and adenosine. To optimize the endogenous production of creatine, the amino acids arginine, glycine, and methionine must be available as substrates. Additionally, cofactors needed to optimize remethylation of homocysteine to form methionine are required to recycle the homocysteine to methionine for reuse as SAMe (Fig. 134.10). Serum creatine levels have been positively correlated with plasma homocysteine levels (i.e., as creatine levels rise, so do homocysteine levels).[47]

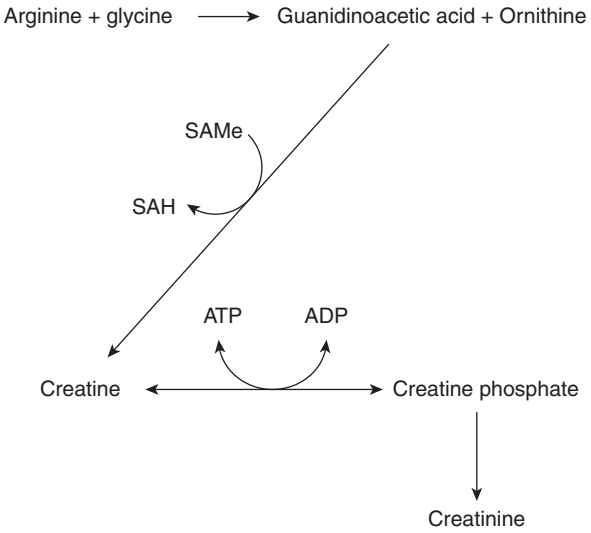


Fig. 134.10 Synthesis of creatine, creatine phosphate, and creatinine.

Epinephrine and Melatonin

Derivatives of the aromatic amino acids, L-tyrosine and L-tryptophan, require methylation for the biosynthesis of their down-line metabolites.

The biosynthesis of catecholamines begins with the amino acid L-tyrosine and proceeds through dopa and dopamine, resulting in the formation of norepinephrine, the neurotransmitter substance found in most sympathetic nerve terminals, as well as in some synapses of the CNS. In the chromaffin cells of the adrenal medulla, a methyl group is provided by SAMe, resulting in the formation of epinephrine from norepinephrine. A number of metabolites are formed from the degradation of both norepinephrine and epinephrine. Catecholamine degradation proceeds independently, and in conjunction with monoamine oxidase, by catechol-*O*-methyltransferase. This enzyme catalyzes the transfer of a methyl group donated by SAMe and, depending on the substrate, results in the formation of homovanillic acid, normetanephrine, and metanephrine.

The formation of melatonin from L-tryptophan proceeds through 5-hydroxytryptophan, serotonin, and *N*-acetylserotonin. Melatonin is then formed in the pineal gland by the donation of a methyl group. 5-Methoxytryptamine, an alternate metabolite of serotonin, also requires the addition of a methyl group.

Phosphatidylcholine

Phosphatidylcholine (PC) is a primary component of lecithin. It is the most frequently encountered phospholipid in animals and is structurally related to phosphatidylserine and phosphatidylethanolamine. PC consists of a glycerol backbone that is esterified with fatty acids on carbon atoms 1 and 2, with a phosphoric acid/choline complex in position 3. Although PC is usually referred to as if it were a single compound, it is actually a group of related compounds that vary depending on the fatty acid composition at positions C-1 and C-2.

Dietary choline is derived primarily from PC, which, after absorption by the intestinal mucosa, is metabolized to choline in the liver by the enzyme phospholipase D. Most choline is rephosphorylated to PC; however, a small amount is carried to the brain via the bloodstream, where it is converted to the neurotransmitter acetylcholine. If PC or choline is lacking in the diet, it can be synthesized from phosphatidylserine and phosphatidylethanolamine (see Fig. 134.5). Synthesis of PC depends on the availability of SAMe as a methyl donor because synthesis involves the transfer of methyl groups from three SAMe molecules to phosphatidylethanolamine to generate one molecule of PC.

The metabolic pathways of PC, methionine, methylcobalamin, and 5-methylTHF are interrelated, intersecting at the regeneration of methionine from homocysteine by betaine (see Fig. 134.1). The use of choline molecules as methyl donors in this process is probably the main factor that determines how rapidly a diet deficient in choline induces pathological changes.[25]

Taurine

Taurine is a unique amino acid because it carries a sulfonic acid group ($-SO_3H$) instead of a carboxyl group ($-CO_2H$). Taurine is biosynthesized from methionine or from cysteine via the transsulfuration pathway (see Fig. 134.6). Once cysteine is generated, it can be directed into several different pathways, including synthesis of GSH, acetyl CoA, 3′-phosphate 5′-phosphosulfate (PAPS), and taurine. The committed first step in the degradation of homocysteine requires cystathionine synthase, P^5P, and serine. P^5P is also required as a cofactor in the cleavage of cystathionine to cysteine. Homocystinuria, resulting from an absence of cystathionine synthase, can lead to mental retardation. Low levels of cystathionine synthase can also lead to abnormally high levels of homocysteine, especially when remethylation cofactors are also deficient.

CLINICAL APPLICATIONS

Phase II Detoxification

Because homocysteine is a critical intermediate in both methyl- and sulfur-group metabolism, elevated levels could indicate nutrient deficiencies that might compromise function in several of the hepatic Phase II detoxification reactions.

Amino acid conjugation reactions require either glycine, glutamine, or taurine. Glycine functions in the conjugation of aromatic acids (e.g., benzoic acid to hippuric acid). Elevated levels of homocysteine might indicate reduced nutritional levels of betaine and subsequently its down-line metabolite, glycine. Taurine functions in acylations (e.g., bile conjugation).

Sulfur conjugation requires NAC, GSH, PAPS, or methionine/cysteine. NAC is used for mercapturic acid synthesis and is involved in detoxification of a wide variety of compounds, including aromatic hydrocarbons, some phenols, halides, esters, epoxides, and caffeine. GSH is involved in dismutation reactions of organic nitrates (e.g., nitroglycerin). PAPS is used in sulfate ester synthesis, mostly with phenols, and some aliphatic alcohols (e.g., ethanol) and aromatic amines. Methionine and cysteine are used in cyanide-thiocyanate detoxification. A portion of the inorganic sulfur needed for the formation of all of these compounds passes through the homocysteine cycle.

Alkylation reactions require SAMe, methylcobalamin, or 5-methylTHF. These compounds provide methyl groups to detoxify compounds containing hydroxide, SH, or NH_2 groups. Examples of these reactions include norepinephrine to epinephrine, epinephrine to metanephrine, guanidoacetic acid to creatine, and *N*-acetylserotonin to melatonin.

Other Phase II detoxification reactions that might be affected by elevated homocysteine as a biological marker of reduced nutrient formation include acetylation by acetyl-CoA, which requires cysteine as a source of its cysteamine component, and the use of carnitine for the conversion of valproic acid to valpropylcarnitine.

Heart Disease

Increased blood levels of homocysteine are correlated with significantly increased risk of coronary artery disease (CAD),[48–54] myocardial infarction,[55,56] peripheral occlusive disease,[57–60] cerebral occlusive disease,[57,60] and retinal vascular occlusion (Box 134.1).[61]

BOX 134.1 What Homocysteine and Homocysteine Thiolactone Do to Arteries

- Generate superoxide and hydrogen peroxide, which have been linked to damage to arterial endothelium
- Change coagulation factor levels so as to encourage clot formation
- Prevent small arteries from dilating, so they are more vulnerable to obstruction
- Cause smooth muscle cells in the arterial wall to multiply
- Interact with low-density lipoproteins, causing them to precipitate and damage endothelial tissue
- Cause platelets to aggregate

Inborn errors of homocysteine metabolism result in high levels of homocysteine in the blood and severe atherosclerotic disease; even within the range considered normal (4–16 μmol/L), there is a graded increase in risk for CAD. In a study of 304 patients with CAD versus controls, the odds ratio for CAD increased as plasma homocysteine increased, even within the normal range. A 5 μmol/L increase in plasma homocysteine was correlated with an increase in the odds ratio of 2.4 ($P < 0.001$), with no "threshold effect."[51]

A review of numerous studies found that mild hyperhomocysteinemia after a methionine load test occurred in 21%, 24%, and 32% of patients with CAD, cerebrovascular disease, and peripheral vascular disease, respectively.[46] Another group of researchers found a 29.3% incidence of hyperhomocysteinemia (>14 μmol/L by their definition) in a group of 1160 elderly (ages 67–96 years) individuals in the Framingham Heart Study. The study also indicated that plasma homocysteine levels increased with age.[57]

A number of interrelated atherogenic mechanisms are thought to be involved with hyperhomocysteinemia. These include advanced thickening and smooth muscle cell proliferation of endothelial vessel wall intima, enhanced lipid deposition in the vessel wall, forced detachment of endothelial cells, activation of leukocytes and thrombocytes, increased low-density lipoprotein oxidation, initiation of platelet thromboxane synthesis, enhanced oxidative stress induced by peroxide formation during homocysteine oxidation, and prothrombotic coagulation interference.[62,63] One way homocysteine assists this process is by the generation of hydrogen peroxide.[64] By creating oxidative damage to low-density lipoprotein cholesterol and endothelial cell membranes, hydrogen peroxide can then catalyze injury to vascular endothelium.[64,65]

Nitric oxide and other oxides of nitrogen released by endothelial cells (also known as endothelium-derived relaxing factor) protect endothelial cells from damage by reacting with homocysteine, forming S-nitrosohomocysteine, which inhibits hydrogen peroxide formation. However, as homocysteine levels increase, this protective mechanism can become overloaded, allowing damage to endothelial cells to occur.[65–67] Because of the role of sulfate compounds in the formation of amino sugars needed to form the basement membrane of blood vessels, high levels of homocysteine are likely to contribute to the formation of blood vessels that are more susceptible to oxidative stress.[67] The end result of the combination of oxidative damage and endothelial collagen instability can be the formation of atherosclerotic plaques.

Decreased plasma folate levels are correlated with increased levels of homocysteine, as well as a subsequent increased incidence of CAD. In a 15-year Canadian study of CAD mortality in 5056 men and women ages 35 to 79 years, lower serum folate levels were correlated with a significantly increased risk of fatal CAD.[68] In a cohort from the Framingham Heart Study, concentrations of folate and P^5P were inversely correlated with homocysteine levels and the risk of

extracranial carotid artery stenosis.[57] Low P5P and low vitamin B_{12} have also been linked with hyperhomocysteinemia and a significantly increased risk of CAD.[51] Another study of 160 cardiac transplantation patients followed for an average of 28 days found that the high homocysteine levels seen in 99 of these patients surprisingly demonstrated no causal role in the atherothrombotic vascular complications of transplantation. Vitamin B_6 deficiency was seen in 21% of recipients with, and in 9% without, cardiovascular complications or death, or both. Compared with patients with normal B_6 levels, there was a 2.7-fold increase in untoward cardiac events for those patients with B_6 levels less than or equal to 20 nmol/L.[69]

Remethylation of homocysteine and the subsequent formation of SAMe are critical for biosynthesis of L-carnitine, CoQ_{10}, and creatine. Similarly, the transsulfuration pathway must be functioning properly for optimal biosynthesis of cysteine, GSH, pantethine, and taurine. All of these nutrients are used clinically to reduce oxidative stress, improve risk factor markers, or treat heart disease.

Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine.[18]

Peripheral Vascular Disease

Elevated homocysteine levels have been established as an independent risk factor for intermittent claudication and deep vein thrombosis. Elevated homocysteine levels corresponded with an increased incidence of intermittent claudication and decreased serum folate levels in a study of 78 patients with intermittent claudication.[70] A fourfold increase in the risk of peripheral vascular disease was noted in individuals with hyperhomocysteinemia compared with those with normal homocysteine levels.[71] A group of researchers in the Netherlands found high homocysteine levels to be a significant risk factor for deep vein thrombosis, with a stronger relationship among women than men.[72]

An increased risk of peripheral vascular occlusion was noted in women taking oral contraceptives, which might be linked to the significantly increased homocysteine levels in women so affected. It is already known that oral contraceptives can cause declines or deficiencies in vitamins B_6, B_{12}, and folate, nutrients integral to the processing of homocysteine. Laboratory assessment of plasma homocysteine levels may be helpful to detect women who may be predisposed to peripheral vascular occlusion while on oral contraceptives.[73]

Stroke

Stroke patients have significantly elevated homocysteine levels compared with age-matched controls,[74] with a linear relationship between risk of stroke and homocysteine levels[75] and a significant decrease in blood folate concentrations in those with elevated homocysteine.[76] One investigation revealed that people with a dietary intake of at least 300 mcg/day of folic acid reduced their risk of stroke and heart disease by 20% and 13%, respectively, compared with those who consumed less than 136 mcg of folic acid per day.[77]

Pregnancy

Biochemical enzyme defects and nutritional deficiencies can contribute to neural tube defects (NTDs), as well as other negative pregnancy outcomes, including spontaneous abortion, placental abruption (infarct), preterm delivery, and low infant birth weight. Evidence has suggested that derangement of methionine-homocysteine metabolism could be the underlying mechanism of pathogenesis of NTDs and might be the mechanism of prevention observed with supplementation of folic acid.[78,79] It has been firmly established that a low dietary intake of folic acid increases the risk for delivery of a child with an NTD and that periconceptional folic acid supplementation reduces the occurrence of NTDs.[80–86] Research also indicates that supplemental folic acid intake results in increased infant birth weight and improved Apgar scores, along with a concomitant decreased incidence of fetal growth retardation and maternal infections.[87–90]A derangement in methionine-homocysteine metabolism has also been correlated with recurrent miscarriage and placental infarcts (abruption).[91]

The amino acid homocysteine, when elevated, might be a teratogenic agent contributing to congenital defects of the heart and neural tube. Evidence from experimental animals lends support to this belief. When avian embryos were fed homocysteine to raise serum homocysteine to over 150 nmol/mL, dysmorphogenesis of the heart and neural tube, as well as of the ventral wall, was observed.[92]

Because homocysteine metabolism, through the remethylation and transsulfuration pathways, affects several biochemical pathways involving the production of nutrients that are essential to the optimal functioning of the cardiovascular, skeletal, and nervous systems, it is not surprising that these other nutrients have been linked to complications of pregnancy in animal models and humans. Low plasma vitamin B_{12} levels have been shown to be an independent risk factor for NTD.[93,94] Methionine has been shown to reduce the incidence of NTD by 41% in an animal model when administered on days 8 and 9 of pregnancy.[95,96] This evidence indicates that a disturbance in the remethylation pathway with a subsequent decrease in SAMe may be a contributing factor to these complications of pregnancy.

PC, due to its role as a precursor to acetylcholine and choline, is acknowledged as a critical nutrient for brain and nerve development and function.[97–99] Because the metabolic pathways of choline (via betaine), methionine, methylcobalamin, and 5-methylTHF are interrelated, intersecting at the regeneration of methionine from homocysteine, a disturbance in the metabolism of either of these two methyl-donor pathways, due to limited availability of key nutrients or decreased enzyme activity, directly affects the body's ability to optimize levels of SAMe.

Evidence suggests that women with a history of NTD-affected pregnancies have altered folate metabolism.[100–103] Patients with a severe congenital deficiency of the enzyme MTHFR, which is needed for the formation of 5-methylTHF, have reduced levels of both methionine and adenosylmethionine in their cerebrospinal fluid and show demyelination in the brain and degeneration of the spinal cord.[2,104] Because of its direct effect on the activation of folic acid to its methyl derivative, a milder version of this enzyme defect is also strongly suspected to increase the incidence of NTDs.[105]

It is established that high vitamin A intake during the first 2 months of pregnancy is associated with a severalfold higher incidence of birth defects.[106,107] Although the mechanism of action remains to be elicited, in an animal model, the activity of hepatic MTHFR was suppressed with high vitamin A levels, suggesting that its teratogenic effect during early pregnancy might be associated with a subsequent derangement in the remethylation of homocysteine.[108]

Because a more significant correlation has been found between high homocysteine levels in women experiencing placental abruption, infarction, and spontaneous abortion than in control women, and homocysteine and CoQ_{10} synthesis depend on the methionine-SAMe-homocysteine pathway, it is possible that low CoQ_{10} and elevated homocysteine independently found in complicated pregnancy may also be found in related conditions.[109,110]

Nutritional intervention with the cofactors required for optimal metabolism of the methionine-homocysteine pathways offers a new integrated possibility for primary prevention of NTD and several other complications of pregnancy. Supplementation with betaine and the active forms of cobalamin and folic acid, such as methylcobalamin and folinic acid, along with riboflavin-5′-phosphate (because of its role as a cofactor for the MTHFR enzyme), may play a significant role in reducing or preventing these emotionally devastating outcomes.

There is an effect of thyroid hormone on folate and vitamin B_{12}-dependent biochemical processes during early growth and development. Higher thyroid function is associated with higher homocysteine concentration in pregnant women and in neonates.

Neurological and Mental Disorders

In addition to the known effect of homocysteine on the cardiovascular system and micronutrient biochemical pathways, numerous diseases of the nervous system are correlated with high homocysteine levels and alterations in vitamin B_{12}, folate, or vitamin B_6 metabolism, including depression, schizophrenia, multiple sclerosis, Parkinson's disease, Alzheimer's disease (AD), and cognitive decline in the elderly.

Methylation reactions via SAMe, including methylation of DNA and myelin, are vitally important in the CNS. The neurological complications of vitamin B_{12} deficiency are likely due to a reduction of activity of the vitamin B_{12}-dependent enzyme methionine synthase and the subsequent reduction of SAMe production. The CNS lacks the alternate betaine pathway of homocysteine remethylation; therefore if methionine synthase is inactivated, the CNS has a greatly reduced methylation capacity.[111] Other causes of reduced methionine synthase activity include folic acid deficiency and nitrous oxide anesthesia exposure.[112]

Homocysteine has also been found to be a neurotoxin, especially in conditions in which glycine levels are elevated, including head trauma, stroke, and vitamin B_{12} deficiency.[113] Homocysteine interacts with the *N*-methyl-D-aspartate receptor, causing excessive calcium influx and free radical production, resulting in neurotoxicity.[94] The neurotoxic effects of homocysteine or reduced methylation reactions, or both, in the CNS contribute to the mental symptomatology seen in vitamin B_{12} and folate deficiency. Increased homocysteine levels can also be seen in schizophrenics.[114]

Significant deficiencies in vitamin B_{12} and folate are common in the elderly population and can contribute to a decline in cognitive function.[115–117] An investigation of cognitive ability in older men (54–81 years old) found poorer spatial copying skills in those with higher homocysteine levels. Better memory performance was correlated with higher vitamin B_6 levels.[118]

Vitamin B_{12} deficiency and increasing severity of cognitive impairment have been seen in patients with AD compared with controls and patients with other dementias.[119] In a study of 52 AD patients, 50 hospitalized nondemented controls, and 49 elderly subjects living at home, patients with AD were found to have the highest homocysteine levels and the highest methylmalonic acid (an indicator of vitamin B_{12} deficiency) levels.[120]

In another Framingham study cohort with an average of 8 years' follow-up, dementia developed in 111 of 1092 nondementia subjects, including 83 who were diagnosed with AD. The adjusted relative risk of dementia was 1.4 for each increase of 1 standard deviation in the logarithmically adjusted homocysteine value. The relative risk of AD was 1.8 per increase of 1 standard deviation at baseline and 1.6 per increase of 1 standard deviation 8 years before baseline. Additionally, in those with a plasma homocysteine level that was greater than 14 μmol/L, the risk of AD nearly doubled.[121]

In a study of 741 psychogeriatric patients, high plasma homocysteine levels were found in demented and nondemented patients; however, only demented patients also had lower blood folate concentrations compared with controls. Patients with concomitant vascular disease had significantly higher plasma homocysteine than those without diagnosed vascular disease. Significantly higher homocysteine levels, compared with controls, have also been found in Parkinson's disease patients.[122]

Homocysteine's effects on neurotransmitter metabolism, along with its potential reduction of methylation reactions, could be a contributing factor to the etiology of depression. Folate and vitamin B_{12} deficiency can cause neuropsychiatric symptoms, including dementia and depression. SAMe is used therapeutically as an antidepressant in Europe and was the third-most-popular antidepressant treatment in Italy in 1995.[123,124] Long-term treatment of poststroke survivors with folic acid (2 mg), vitamin B_6 (25 mg), and vitamin B_{12} (0.5 mg) was associated with a reduction in major depression in a 563-patient, randomized, double-blind, placebo-controlled trial.[125]

Methylation of myelin basic protein is vital to the maintenance of the myelin sheath. The worst-case scenario of folate and vitamin B_{12} deficiency includes demyelination of the posterior and lateral columns of the spinal cord, a disease process called subacute combined degeneration of the spinal cord (SCD).[111] SCD can also be precipitated by nitrous oxide anesthesia, which causes an irreversible oxidation of the cobalt moiety of the vitamin B_{12} molecule and the subsequent inhibition of methionine synthase activity, a decrease in homocysteine remethylation, and decreased SAMe production.[112] This has been treated using supplemental methionine, which further supports the theory of a nitrous oxide–induced biochemical block at methionine synthase.[126] Particularly at risk for this condition are vitamin B_{12}-deficient individuals who visit their dentist and receive nitrous oxide.[112,127]

Abnormal methylcobalamin metabolism is one of the proposed mechanisms for the pathophysiology of the demyelinating disease multiple sclerosis (MS). Deficiency of vitamin B_{12} has been linked to some cases of MS, and it has been suggested that dietary deficiency, or more likely, a defect in R-protein–mediated absorption or methylation of vitamin B_{12}, might be a significant contributor to the pathogenesis of MS.[128]

Individuals with genetic variation in the 5,10-MTHFR gene are more susceptible to having a psychiatric disorder. The genetic variation MTHFR C677T was significantly associated with schizophrenia, bipolar disorder, and unipolar depressive disorder.[129]

Diabetes Mellitus

Homocysteine levels appear to be lower in individuals with type 1 diabetes mellitus. Forty-one subjects with type 1 diabetes (age 34.8 ± 12 years; duration of illness: 10.7 ± 11.1 years) were compared with 40 age-matched control subjects (age 34.2 ± 9.1 years). After an overnight fast, homocysteine was significantly lower ($P = 0.0001$) in the diabetic group (6.8 ± 2.2) than in the controls (9.5 ± 2.9). This difference was apparent in male and female subgroups.[130] However, increased levels of homocysteine have been reported in type 1 diabetics with proliferative retinopathy[131] and nephropathy.[131,132]

Evidence to date suggests that the metabolism of homocysteine is impaired in patients with non–insulin-dependent diabetes mellitus (NIDDM). After a methionine load, hyperhomocysteinemia occurred with significantly greater frequency in patients with NIDDM (39%) compared with age-matched controls (7%). The area under the curve over 24 hours, reflecting the total period of exposure to increased homocysteine, was also elevated with greater frequency in patients with NIDDM and macrovascular disease (33%) compared with controls (0%). The authors concluded that hyperhomocysteinemia was associated with macrovascular disease in a significant proportion of patients with NIDDM.[133] Other researchers reported a correlation between increased homocysteine levels and the occurrence of macroangiopathy in patients with NIDDM. Intramuscular injection of 1000 μg methylcobalamin daily for 3 weeks reduced the elevated plasma levels of homocysteine in these individuals.[134]

Elevated homocysteine levels appear to be a risk factor for diabetic retinopathy in individuals with NIDDM. This might be due to a point

mutation on the gene for the enzyme MTHFR.[135,136] A significantly higher percentage of diabetics with retinopathy exhibit this mutation.[137] Elevated homocysteine levels cause cell injury to the small vessels, which may contribute to the development of retinopathy, as well as macroangiopathy in the cardiovascular system.[135]

Rheumatoid Arthritis

Elevated total homocysteine levels have been reported in patients with rheumatoid arthritis (RA) and psoriasis. Twenty-eight patients with RA and 20 healthy age-matched control subjects were assessed for homocysteine levels while fasting and in response to a methionine challenge. Fasting levels were 33% higher in RA patients than in controls. Four hours after the methionine challenge, the increase in plasma homocysteine concentration was also higher in patients with RA.[137] Another study found statistically significant increases in homocysteine in RA patients ($P = 0.003$), with 20% of the patients having homocysteine levels above the reference range.[138] A mechanism for this increased homocysteine in RA patients has not been elucidated. Penicillamine, a common sulfhydryl-containing arthritis treatment, has been found to lower elevated homocysteine levels in vivo.[139]

Psoriasis

One study of 30 psoriasis patients and their matched controls were evaluated for blood concentrations of lipids, lipoproteins, acute-phase reactants, homocysteine, and atherothrombotic markers. This study observed that more than 50% of the patients with psoriasis had homocysteine levels that were higher than 15 mmol/L, whereas only 20% of the control individuals were above this cutoff point. It was concluded that the mean levels of serum homocysteine, fibrinogen, fibronectin, intercellular adhesion molecules, plasminogen activator inhibitor, and autoantibodies against oxidized low-density lipoprotein were increased in psoriatic patients, whereas tissue plasminogen factor, vitamin B_{12}, and folate levels were decreased significantly.[62]

Further investigation into both the prevalence of hyperhomocysteinemia and the mechanism of action affecting RA and psoriasis is necessary.

Kidney Failure

Because homocysteine is cleared by the kidneys, chronic renal failure, as well as absolute or relative deficiencies of 5-methylTHF, methylcobalamin, P^5P, or betaine, results in increased homocysteine levels. In 176 patients with end-stage renal disease on peritoneal or hemodialysis, homocysteine concentrations averaged 26.6 ± 1.5 μmol/L in patients with renal failure compared with 10.1 ± 1.7 μmol/L in normal subjects. Abnormal values exceeded the 95th percentile for normal controls in 149 of the patients with renal failure.[140] Data also indicated that plasma homocysteine values represented an independent risk factor for vascular events in patients on peritoneal and hemodialysis. Patients with a homocysteine concentration in the upper two quintiles (>27.8 μmol/L) had an independent odds ratio of 2.9 (confidence interval, 1.4–5.8; $P = 0.007$) of vascular complications. Vitamin B levels were also lower in patients with vascular complications than in those without such complications.[141]

Alcoholism and Ethanol Ingestion

Chronic alcoholism is known to interfere with one-carbon metabolism. Because of this, it is not surprising to find that mean serum homocysteine levels are two times higher in chronic alcoholics than in nondrinkers ($P < 0.001$). Beer consumers have lower concentrations of homocysteine compared with drinkers of wine or spirits ($P = 0.05$). In chronic alcoholics, serum P^5P and red blood cell folate concentrations have been shown to be significantly lower than in control subjects.[13] Plasma homocysteine was significantly higher, compared with controls, in 42 active alcoholics hospitalized for detoxification. In another group of 16 alcoholics who abstained from ethanol ingestion, plasma homocysteine did not deviate from that of controls.[14]

Feeding ethanol to rats produces prompt inhibition of methionine synthase, as well as a subsequent increase in activity of betaine homocysteine methyltransferase. Despite the inhibition of methionine synthase, the enhanced betaine homocysteine methyltransferase pathway uses hepatic betaine pools to maintain levels of SAMe.[142] Results indicate that ethanol feeding produces a significant loss in SAMe in the first week, with a return to normal SAMe levels in the second week. Betaine feeding enhances hepatic betaine pools in control animals, as well as ethanol-fed animals; attenuates the early loss of SAMe in ethanol-fed animals; produces an early increase in betaine homocysteine methyltransferase activity; and generates increased levels of SAMe in both control and ethanol-fed groups.[143] It has been shown that minimal supplemental dietary betaine at the 0.5% level increases SAMe twofold in control animals and fivefold in ethanol-fed rats. Concomitant with the betaine-generated SAMe, ethanol-induced hepatic fatty infiltration was ameliorated.[142] Betaine supplementation also reduces the accumulation of hepatic triglyceride produced after ethanol ingestion.[143]

Gout

Although homocysteine levels have been positively correlated with increased uric acid levels,[2,144,145] no studies have investigated homocysteine levels in gout patients. It is possible the increased uric acid levels in gout are due to decreased SAMe production because of the reduction in homocysteine recycling. The excess adenosine, which reacts with methionine to form SAMe, is degraded to form uric acid as its end product.

Niacin is contraindicated in gout because it competes with uric acid for excretion.[146] Animal studies have shown that increased levels of S-adenosylhomocysteine, and thus homocysteine, cause significant reductions in SAMe-dependent methylation reactions.[16] Therefore because degradation of the niacin-containing coenzyme nicotinamide adenine dinucleotide is dependent on methylation by SAMe, and SAMe activity is severely reduced in hyperhomocysteinemia, niacin levels might be higher in these people, resulting in less uric acid excretion, higher uric acid levels, and increased gout symptoms in susceptible individuals. This possibility and its mechanism need further investigation.

Osteoporosis

Osteoporosis is a common presenting symptom in children with homocystinuria due to cystathionine synthase deficiency, as a result of an autosomal-recessive error of sulfur amino metabolism.[147,148] Individuals with cystathionine synthase deficiency have decreased concentrations of cysteine and its disulfide form, cystine. Because of the role of sulfur compounds in the formation of sulfated amino sugars, disturbed cross-linking of collagen has been proposed as a possible mechanism of action. One group of researchers studying 10 patients with homocystinuria found normal synthesis of collagen but a significant reduction of cross-links.[149]

Because of the correlation between homocystinuria and osteoporosis in children with this amino acidopathy and the increase in homocysteine concentrations in postmenopausal women, several authors have implied that elevated homocysteine levels contribute to postmenopausal osteoporosis. The Framingham study of 825 men and 1174 women, ranging from 59 to 91 years old, affirmed this suspicion by evaluating plasma homocysteine levels and relative incident of hip fracture over a period of 16 to 19 years. The mean plasma total

homocysteine concentration was 13.4 ± 9.1 μmol/L with 41 hip fractures among men and 12.1 ± 5.3 μmol/L and 146 hip fractures among women. It was shown that both men and women in the highest quartile of plasma homocysteine had a greater risk of hip fracture than those in the lowest quartile, where the risk was almost 4 times as high for men and 1.9 times as high for women.[150] Because physical activity is well known to lower homocysteine, as well as prevent falls, it may therefore influence the association between homocysteine levels and the risk of fracture.[15] Therefore it seems prudent to prescribe exercise, as well as appropriate supplemental therapies, for women and men with high homocysteine and osteopenia/osteoporosis.

Autism

Children with autism have higher levels of urinary homocysteine than children without autism, which may reflect nutritional deficiencies of folic acid, vitamin B_6, and vitamin B_{12}.[151] The increase in homocysteine concentration is significantly and directly correlated with the severity of deficit in communication skills but is unrelated to a deficit in socialization or repetitive/restricted behavior.[152]

In addition, autistic children have elevated levels of S-adenosylhomocysteine (SAH) and adenosine, whereas levels of methionine, S-adenosylmethionine (SAM), and the SAM/SAH ratio are significantly decreased compared with age-matched controls.[153]

Nonalcoholic Fatty Liver Disease (NAFLD)

There are studies that have shown an association between increased homocysteine levels and NAFLD.[154–156] One study has determined increased homocysteine levels and increased prevalence of homozygote MTHFR 677C/T and MTHFR 1298A/C mutations in patients with NAFLD compared with healthy controls.[157] A meta-analysis has shown that the T/T genotype of MTHFR C677T polymorphism and C/C genotype of MTHFR A1298C are more likely to be associated with the susceptibility to NAFLD.[158] The increased homocysteine levels could possibly contribute to the development of NAFLD or be a result of impaired hepatic metabolism.[159,160]

Drug-Induced Hyperhomocysteinemia

Because fibrate drugs are known to increase homocysteine levels (see "Pharmaceutic Drug Effects on Homocysteine"), one randomized, double-blind crossover study investigated the effect of using fenofibrate adjunctively with 650 mcg of folic acid, 5 mg of vitamin B_6, and 50 mcg of vitamin B_{12} or just fenofibrate in men with hyperlipidemia. Subjects who received the fenofibrate plus placebo had an average increase in homocysteine concentration of 44%. Subsequent to the fenofibrate-plus-vitamin treatment, it was 13%. In this study, vitamins significantly prevented most of the homocysteine increase seen after fenofibrate plus placebo. The authors of this study suggested that routine use of these nutrients might be beneficial with this pharmaceutical therapy.[35]

DIAGNOSTIC CONSIDERATIONS

Many studies cited herein have used a reference range, with 12 to 16 μmol/L being the upper limit of normal for homocysteine. Researchers found a highly significant increase in relative risk of atherosclerotic cardiovascular disease and other disease processes as homocysteine levels increased, even within the "normal" range. Optimal levels of homocysteine and vitamin B_{12} are needed to maintain the methylation cycle, along with adequate levels of vitamin B_6 to convert to cysteine, which is a precursor to form GSH. A number of clinical laboratories currently perform plasma homocysteine determinations, by themselves or within a cardiovascular panel.

THERAPEUTIC CONSIDERATIONS

Although folic acid or folate supplementation (400 mcg/day) alone can reduce homocysteine levels in many subjects, given the importance of vitamins B_{12} and B_6 to proper homocysteine metabolism, all three should be used together. In one study, the prevalence of suboptimal levels of these nutrients in men with elevated homocysteine levels was 56.8%, 59.1%, and 25% for folic acid, vitamin B_{12}, and vitamin B_6, respectively, indicating that folic acid supplementation alone would not lower homocysteine levels in many cases.[161] In other words, folic acid supplementation will only lower homocysteine levels if there are adequate levels of vitamin B_{12} and B_6. This fact could reduce the effect of folic acid fortification of food. In 1998 the U.S. Food and Drug Administration mandated the fortification of food products with folic acid. Although homocysteine levels have decreased modestly after the fortification of food with folic acid, the effect on mortality has been minor at best.[22] This indicates the importance of more aggressive supplementary measures to reduce homocysteine-associated cardiovascular risk. In one study of 100 men with hyperhomocysteinemia, oral therapy with 650 mcg folic acid, 400 mcg vitamin B_{12}, 10 mg vitamin B_6, or a combination of the three nutrients was given daily for 6 weeks. Plasma homocysteine was reduced by 41.7% during folate therapy and 14.8% during vitamin B_{12} therapy, whereas 10 mg of vitamin B_6 did not reduce plasma homocysteine significantly. The combination worked synergistically to reduce homocysteine levels by 49.8%.[162]

Several studies using folic acid, vitamin B_6, vitamin B_{12}, and betaine, either alone or in combination, demonstrated the ability of these nutrients to normalize homocysteine levels.[29,32,58,60,163,179] Other studies confirmed that oral folate supplementation alone would almost always lower high homocysteine, whereas B_6 and B_{12} would lower homocysteine only in those with a genetic metabolic defect or dietary deficiency in those nutrients, or both.[163,164] Some studies used high-dosage oral folate therapy (2.5–5 mg) to effectively reduce hyperhomocysteinemia in patients with peripheral atherosclerotic vascular disease, 50% of whom had abnormally high fasting plasma homocysteine levels, whereas 100% had abnormal plasma homocysteine after a methionine load.

Despite the demonstrated efficacy of individual studies lowering homocysteine levels with folic acid and B vitamins, a meta-analysis of eight randomized trials of folic acid and vitamin B supplementation to lower homocysteine levels was found to have no significant effects within 5 years on cardiovascular events, cancer, or mortality.[165]

If a dietary deficiency or an increased demand resulting from genetic biochemical individuality exists for 5-methylTHF, methylcobalamin, P^5P, or betaine, treatment with these active forms of micronutrients should reduce homocysteine levels better than the nonactive forms (e.g., folic acid, cyanocobalamin, pyridoxine, betaine hydrochloride). In addition, betaine is important when there is a deficiency of the P^5P-dependent enzyme cystathionine synthase—the most common genetic abnormality affecting the transsulfuration pathway of homocysteine breakdown. Betaine supplementation in combination with vitamin B_6 corrects the hyperhomocysteinemia in these individuals.[28,163]

There is speculation that the fortification of foods and supplementation of folic acid, the synthetic form of folate, have contributed increased risk of carcinogenesis.[166] Although some studies have shown that moderate doses of folic acid reduce the risk of carcinogenesis, other studies suggest that larger doses or supplementation after a neoplastic lesion enhances the risk of carcinogenesis.[167–171] The genetic variants in folate metabolism may also be a factor in the risk of colorectal carcinoma and adenoma. The homozygous C677T variant of methylenetetrahydrofolate reductase (MTHFR) may modify the association of folate status with risk of colorectal carcinoma and adenoma.[172–174] Naturally occurring folates have a distinct metabolism that differs from folic acid.

Folic acid is inactive until it is metabolized into the natural reduced forms, including 5-methlytetrahydrofolate. There are studies that have demonstrated that the unmetabolized folic acid in the plasma reduces natural killer cell cytotoxicity in vitro.[175,176] Elevated levels of plasma methylated folates (the sum of 5-methyl-tetrahydrofolate and 4-alfa-hydroxy-5methyl-THF) are positively associated with increased risk of advanced or multiple colorectal adenomas. Therefore careful consideration should be given to the benefit and risk of folic acid and methylated folate supplementation.[177] There are advantages to using 5-methylTHF over folic acid, such as the potential negative effects of unmetabolized folic acid in the peripheral circulation. In addition, using 5-methylTHF instead of folic acid overcomes metabolic defects caused by methylenetetrahydrofolate reductase polymorphism, reduces interactions with drugs that inhibit dihydrofolate reductase, and reduces the potential for masking hematological symptoms of vitamin B_{12} deficiency.

Therapeutic Approach

Supplements

- Vitamin B_{12}: 800 to 5000 mcg/day
- Pyridoxine: 25 to 50 mg/day
- 5-MethylTHF: 400 to 1000 mcg/day

If unresponsive to the previous dosages, patients may take the following:

- Folinic acid: 800 mcg/day
- Methylcobalamin: 1000 to 5000 mcg/day
- P^5P: 20 mg/day
- Betaine (trimethylglycine): 1200 mg/day
- NAC: 500 to 1800 mg/day
- 5-MethylTHF: 1 to 15 mg/day

Injectable B_{12}

- Hydroxocobalamin: 1000 mcg weekly
- Methylcobalamin 25 mg/mL: 0.1 mL three times a week

For children with autism spectrum disorder (ASD) to improve behavioral symptoms and metabolic markers of abnormal redox and methylation metabolism[178]:

- 75 mcg/kg methylcobalamin subcutaneous injection in fatty tissue of the gluteal region every third day, plus oral supplementation with folinic acid (400 mcg twice daily) for 3 months

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Tucker KL, Selhub J, Wilson PW, et al. Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. *J Nutr*. 1996;126:3025–3031.
2. Lussier-Cacan S, Xhignesse M, Piolot A, et al. Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. *Am J Clin Nutr*. 1996;64:587–593.
3. van der Mooren MJ, Wouters MG, Blom HJ, et al. Hormone replacement therapy may reduce high serum homocysteine in postmenopausal women. *Eur J Clin Invest*. 1994;24:733–736.
4. Wouters MG, Moorrees MT, van der Mooren MJ, et al. Plasma homocysteine and menopausal status. *Eur J Clin Invest*. 1995;25:801–805.
5. Brattstrom L, Lindgren A, Israelsson B, et al. Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. *J Intern Med*. 1994;236:633–641.
6. Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA*. 1995;274:1526–1533.
7. Anker G, Lonning PE, Ueland PM, et al. Plasma levels of the atherogenic amino acid homocysteine in post-menopausal women with breast cancer treated with tamoxifen. *Int J Cancer*. 1995;60:365–368.
8. Ueland PM, Refsum H, Beresford SA, et al. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr*. 2000;72:324–332.
9. Vermaak WJ, Ubbink JB, Delport R, et al. Ethnic immunity to coronary heart disease? *Atherosclerosis*. 1991;89:155–162.
10. Kalina Á, Czeizel AE. The methylenetetrahydrofolate reductase gene polymorphism (C677T) is associated with increased cardiovascular mortality in Hungary. *Int J Cardiol*. 2004;97:333–334.
11. Olthof MR, Hollman PC, Zock PL, et al. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am J Clin Nutr*. 2001;73: 532–538.
12. Nygard O, Refsum H, Ueland PM, et al. Coffee consumption and plasma total homocysteine. The Hordaland Homocysteine Study. *Am J Clin Nutr*. 1997;65:136–143.
13. Cravo ML, Gloria LM, Selhub J, et al. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. *Am J Clin Nutr*. 1996;63:220–224.
14. Hultberg B, Berglund M, Andersson A, et al. Elevated plasma homocysteine in alcoholics. *Alcohol Clin Exp Res*. 1993;17:687–689.
15. Auer J, Lamm G, Eber B, et al. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med*. 2004;351:1027–1030.
16. Duerre JA, Briske-Anderson M. Effect of adenosine metabolites on methyltransferase reactions in isolated rat livers. *Biochim Biophys Acta*. 1981;678:275–282.
17. Wiklund O, Fager G, Andersson A, et al. N-acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein(a) levels. *Atherosclerosis*. 1996;119:99–106.
18. Kerins DM, Koury MJ, Capdevila A, et al. Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine. *Am J Clin Nutr*. 2001;74:723–729.
19. Popp J, Lewczuk P, Linnebank M, et al. Homocysteine metabolism and cerebrospinal fluid markers for Alzheimer's disease. *J Alzheimers Dis*. 2009;18:819–828.
20. Wagner C, Koury MJ, S-adenosylhomocysteine. A better indicator of vascular disease than homocysteine? *Am J Clin Nutr*. 2007;86:1581–1585.
21. Spector R. Cerebrospinal fluid folate and the blood-brain barrier. In: Botez MI, Reynolds EH, eds. *Folic Acid in Neurology, Psychiatry, and Internal Medicine*. New York: Raven Press; 1979:187.
22. Scott JM, Weir DG, Molloy A, et al. Folic acid metabolism and mechanisms of neural tube defects. *Ciba Found Symp*. 1994;181:180–187.
23. Linnell JC, Bhatt HR. Inherited errors of cobalamin metabolism and their management. *Baillière's Clin Haematol*. 1995;8:567–601.
24. Horne DW, Cook RJ, Wagner C. Effect of dietary methyl group deficiency on folate metabolism in rats. *J Nutr*. 1989;119:618–621.
25. Zeisel SH, Zola T, daCosta KA, et al. Effect of choline deficiency on S-adenosylmethionine and methionine concentrations in rat liver. *Biochem J*. 1989;259:725–729.
26. Zeisel SH, Epstein MF, Wurtman RJ. Elevated choline concentration in neonatal plasma. *Life Sci*. 1980;26:1827–1831.
27. Hyland K, Smith I, Bottiglieri T, et al. Demyelination and decreased S-adenosylmethionine in 5, 10-methylenetetrahydrofolate reductase deficiency. *Neurology*. 1988;38:459–462.
28. Olthof MR, van Vliet T, Boelsma E, et al. Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. *J Nutr*. 2003;133:4135–4138.
29. Wilcken DE, Dudman NP, Tyrrell PA. Homocystinuria due to cystathionine beta-synthase deficiency—the effects of betaine treatment in pyridoxine-responsive patients. *Metabolism*. 1985;34:1115–1121.
30. Dudman NP, Guo XW, Gordon RB, et al. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. *J Nutr*. 1996;126:1295S–1300S.
31. Dudman NP, Tyrrell PA, Wilcken DE. Homocysteinemia: depressed plasma serine levels. *Metabolism*. 1987;36:198–201.
32. Bleie O, Refsum H, Ueland PM, et al. Changes in basal and postmethionine load concentrations of total homocysteine and cystathionine after B vitamin intervention. *Am J Clin Nutr*. 2004;80:641–648.
33. Nilsson K, Gustafson L, Hultberg B. Role of impaired renal function as a cause of elevated plasma homocysteine concentration in psychogeriatric patients. *Scand J Clin Lab Invest*. 2002;62:385–389.
34. Jonasson T, Ohlin H, Andersson A, et al. Renal function exerts only a minor influence on high plasma homocysteine concentrations in patients with acute coronary syndromes. *Clin Chem Lab Med*. 2002;40:137–142.
35. Dierkes J, Westphal S, Kunstmann S, et al. Vitamin supplementation can markedly reduce the homocysteine elevation induced by fenofibrate. *Atherosclerosis*. 2001;158:161–164.
36. Bostom AG, Bausserman L, Jacques PF, et al. Cystatin C as a determinant of fasting plasma total homocysteine levels in coronary artery disease patients with normal serum creatinine. *Arterioscler Thromb Vasc Biol*. 1999;19:2241–2244.
37. Dierkes J, Westphal S, Luley C. Serum homocysteine increases after therapy with fenofibrate or bezafibrate. *Lancet*. 1999;354:219–220.
38. Jonkers IJ, de Man FH, Onkenhout W, et al. Implication for fibrate therapy for homocysteine. *Lancet*. 1999;354:1208.
39. Westphal S, Rading A, Luley C, et al. Antihypertensive treatment and homocysteine concentrations. *Metabolism*. 2003;52:261–263.
40. Daily III JW, Sachan DS. Choline supplementation alters carnitine homeostasis in humans and Guinea pigs. *J Nutr*. 1995;125:1938–1944.
41. Dodson WL, Sachan DS. Choline supplementation reduces urinary carnitine excretion in humans. *Am J Clin Nutr*. 1996;63:904–910.
42. Thomas S, Neuzil J, Stocker R. Cosupplementation with coenzyme Q prevents the prooxidant effect of alpha-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. *Arterioscler Thromb Vasc Biol*. 1996;16:687–696.
43. Weber C, Sejersgard Jakobsen T, Mortensen S, et al. Antioxidative effect of dietary coenzyme Q10 in human blood plasma. *Int J Vitam Nutr Res*. 1994;64:311–315.
44. Bargossi AM, Grossi G, Fiorella PL, et al. Exogenous CoQ_{10} supplementation prevents plasma ubiquinone reduction induced by HMG-CoA reductase inhibitors. *Mol Aspects Med*. 1994;15:S187–S193.
45. Kang D, Fujiwara T, Takeshige K. Ubiquinone biosynthesis by mitochondria, sonicated mitochondria, and mitoplasts of rat liver. *J Biochem (Tokyo)*. 1992;111:371–375.
46. Donchenko GV, Kruglikova AA, Shavchko LP, et al. The role of vitamin E in the biosynthesis of ubiquinone (Q) and ubichromenol (QC) in rat liver. *Biokhimiia*. 1991;56:354–360. [Russian].
47. Silberberg J, Crooks R, Fryer J, et al. Fasting and post-methionine homocyst(e)ine levels in a healthy Australian population. *Aust N Z J Med*. 1997;27:35–39.
48. Hopkins P, Wu L, Wu J, et al. Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1995;15:1314–1320.
49. Loehrer F, Angst C, Haefeli W, et al. Low whole-blood S-adenosylmethionine and correlation between 5-methyltetrahydrofolate and

homocysteine in coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1996;16:727–733.
50. Boushey C, Beresford S, Omenn G, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA*. 1995;274:1049–1057.
51. Robinson K, Mayer E, Miller D, et al. Hyperhomocysteinemia and low pyridoxal phosphate. Common and independent reversible risk factors for coronary artery disease. *Circulation*. 1995;92:2825–2830.
52. Refsum H, Ueland PM, Nygård O, et al. Homocysteine and cardiovascular disease. *Annu Rev Med*. 1998;49:31–62.
53. Gauthier GM, Keevil JG, McBride PE. The association of homocysteine and coronary artery disease. *Clin Cardiol*. 2003;26:563–568.
54. Bozkurt E, Keles S, Acikel M, et al. Plasma homocysteine level and the angiographic extent of coronary artery disease. *Angiology*. 2004;55:265–270.
55. Landgren F, Israelsson B, Lindgren A, et al. Plasma homocysteine in acute myocardial infarction: homocysteine-lowering effect of folic acid. *J Intern Med*. 1995;237:381–388.
56. Chasan-Taber L, Selhub J, Rosenberg I, et al. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr*. 1996;15:136–143.
57. Selhub J, Jacques P, Bostom A, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med*. 1995;332:286–291.
58. Van den Berg M, Boers G, Franken D, et al. Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest*. 1995;25:176–181.
59. Van den Berg M, Stehouwer C, Bierdrager E, et al. Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. *Arterioscler Thromb Vasc Biol*. 1996;16:165–171.
60. Franken D, Boers G, Blom H, et al. Treatment of mild hyperhomocysteinemia in vascular disease patients. *Arterioscler Thromb*. 1994;14:465–470.
61. Wenzler E, Rademakers A, Boers G, et al. Hyperhomocysteinemia in retinal artery and retinal vein occlusion. *Am J Ophthalmol*. 1993;115:162–167.
62. Vanizor Kural B, Orem A, Cimsit G, et al. Plasma homocysteine and its relationships with atherothrombotic markers in psoriatic patients. *Clin Chim Acta*. 2003;332:23–30.
63. Herrmann W, Quast S, Ullrich M, et al. Hyperhomocysteinemia in high-aged subjects: relation of B-vitamins, folic acid, renal function and the methylenetetrahydrofolate reductase mutation. *Atherosclerosis*. 1999;144:91–101.
64. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest*. 1986;77:1370–1376.
65. Stamler J, Osborne J, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest*. 1993;91:308–318.
66. Stamler J, Loscalzo J. Endothelium-derived relaxing factor modulates the atherothromogenic effects of homocysteine. *J Cardiovasc Pharmacol*. 1992;20(suppl 12):S202–S204.
67. Stamler J, Slivka A. Biological chemistry of thiols in the vasculature and in vasculature-related disease. *Nutr Rev*. 1996;54:1–30.
68. Morrison H, Schaubel D, Desmeules M, et al. Serum folate and risk of fatal coronary heart disease. *JAMA*. 1996;275:1893–1896.
69. Nahlawi M, Seshadri N, Boparai N, et al. Usefulness of plasma vitamin B(6), B(12), folate, homocysteine, and creatinine in predicting outcomes in heart transplant recipients. *Am J Cardiol*. 2002;89:834–837.
70. Molgaard J, Malinow MR, Lassvik C, et al. Hyperhomocysteinaemia: an independent risk factor for intermittent claudication. *J Intern Med*. 1992;231:273–279.
71. Cheng SW, Ting AC, Wong J. Fasting total plasma homocysteine and atherosclerotic peripheral vascular disease. *Ann Vasc Surg*. 1997;11:217–223.
72. den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med*. 1996;334:759–762.
73. Beaumont V, Malinow MR, Sexton G, et al. Hyperhomocysteinemia, anti-estrogen antibodies and other risk factors for thrombosis in women on oral contraceptives. *Atherosclerosis*. 1992;94:147–152.
74. Brattstrom L, Lindgren A, Israelsson B, et al. Hyperhomocysteinaemia in stroke: prevalence, cause, and relationships to type of stroke and stroke risk factors. *Eur J Clin Invest*. 1992;22:214–221.
75. Perry IJ, Refsum H, Morris RW, et al. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet*. 1995;346:1395–1398.
76. Hultberg B, Andersson A, Lindgren A. Marginal folate deficiency as a possible cause of hyperhomocystinaemia in stroke patients. *Eur J Clin Chem Clin Biochem*. 1997;35:25–28.
77. Bazzano LA, He J, Ogden LG, et al. Dietary intake of folate and risk of stroke in US men and women: NHANES I EPIDEMIOLOGIC follow-up study. National health and nutrition Examination survey. *Stroke*. 2002;33:1183–1188.
78. Eskes TK. Possible basis for primary prevention of birth defects with folic acid. *Fetal Diagn Ther*. 1994;9:149–154.
79. Steegers-Theunissen R, Boers G, Trijbels FJ, et al. Neural-tube defects and derangement of homocysteine metabolism. *N Engl J Med*. 1991;324:199–200.
80. MRC Vitamin Study Research Group. Prevention of neural tube defects. Results of the medical research council vitamin study. *Lancet*. 1991;338:131–137.
81. Vergel RG, Sanchez LR, Heredero BL, et al. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. *Prenat Diagn*. 1990;10:149–152.
82. Milunsky A, Jick H, Jick SS, et al. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA*. 1989;262:2847–2852.
83. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. 1992;327:1832–1835.
84. Bower C, Stanley FJ. Dietary folate as a risk factor for neural tube defects: evidence from a case-control study in Western Australia. *Med J Aust*. 1989;150:613–619.
85. Werler MM, Shapiro S, Mitchell AA. Periconceptional folic acid exposure and risk of occurrent neural tube defects. *JAMA*. 1993;269:257–261.
86. Shaw GM, Schaffer D, Velie EM, et al. Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology*. 1995;6:219–226.
87. Tamura T, Goldenberg R, Freeberg L, et al. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. *Am J Clin Nutr*. 1992;56:365–370.
88. Scholl TO, Hediger ML, Schall JI, et al. Dietary and serum folate: their influence on the outcome of pregnancy. *Am J Clin Nutr*. 1996;63:520–525.
89. Frelut ML, de Coucy GP, Christides JP, et al. Relationship between maternal folate status and foetal hypotrophy in a population with a good socio-economical level. *Int J Vitam Nutr Res*. 1995;65:267–271.
90. Goldenberg RL, Tamura T, Cliver SP, et al. Serum folate and fetal growth retardation: a matter of compliance? *Obstet Gynecol*. 1992;79:719–722.
91. Goddijn-Wessel TA, Wouters MG, van de Molen EF, et al. Hyperhomocysteinemia: a risk factor for placental abruption or infarction. *Eur J Obstet Gynecol Reprod Biol*. 1996;66:23–29.
92. Rosenquist TH, Ratashak SA, Selhub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. *Proc Natl Acad Sci U S A*. 1996;93:15227–15232.
93. Kirke PN, Molloy AM, Daly LE, et al. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Q J Med*. 1993;86:703–708.
94. Mills JL, Scott JM, Kirke PN, et al. Homocysteine and neural tube defects. *J Nutr*. 1996;126:756S–760S.
95. Essien FB, Wannberg SL. Methionine but not folinic acid or vitamin B-12 alters the frequency of neural tube defects in Axd mutant mice. *J Nutr*. 1993;123:973–974.

96. Potier de Courcy G, Bujoli J. Effects of diets with or without folic acid, with or without methionine, on fetus development, folate stores and folic acid-dependent enzyme activities in the rat. *Biol Neonate*. 1981;39:132–140.
97. Zeisel SH, Blusztajn JK. Choline and human nutrition. *Annu Rev Nutr*. 1994;14:269–296.
98. Garner SC, Mar MH, Zeisel SH. Choline distribution and metabolism in pregnant rats and fetuses are influenced by the choline content of the maternal diet. *J Nutr*. 1995;125:2851–2858.
99. Meck WH, Smith RA, Williams CL. Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Dev Psychobiol*. 1988;21:339–353.
100. Wild J, Seller MJ, Schorah CJ, et al. Investigation of folate intake and metabolism in women who have had two pregnancies complicated by neural tube defects. *Br J Obstet Gynaecol*. 1994;101:197–202.
101. Wild J, Schorah CJ, Sheldon TA, et al. Investigation of factors influencing folate status in women who have had a neural tube defect-affected infant. *Br J Obstet Gynaecol*. 1993;100:546–549.
102. Yates JR, Ferguson-Smith MA, Shenkin A, et al. Is disordered folate metabolism the basis for the genetic predisposition to neural tube defects? *Clin Genet*. 1987;31:279–287.
103. Lucock MD, Wild J, Schorah CJ, et al. The methylfolate axis in neural tube defects: in vitro characterisation and clinical investigation. *Biochem Med Metab Biol*. 1994;52:101–114.
104. Kluijtmans LA, Van den Heuvel LP, Boers GH, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor in cardiovascular disease. *Am J Hum Genet*. 1996;58:35–41.
105. Whitehead AS, Gallagher P, Mills JL, et al. A genetic defect in 5,10 methylenetetrahydrofolate reductase in neural tube defects. *QJM*. 1995;88:763–766.
106. Kubler W. Nutritional deficiencies in pregnancy. *Bibl Nutr Dieta*. 1981;30:17–29.
107. Martinez-Frias ML, Salvador J. Epidemiological aspects of prenatal exposure to high doses of vitamin A in Spain. *Eur J Epidemiol*. 1990;6:118–123.
108. Fell D, Steele RD. Modification of hepatic folate metabolism in rats fed excess retinol. *Life Sci*. 1986;38:1959–1965.
109. Noia G, Littarru GP, De Santis M, et al. Coenzyme Q_{10} in pregnancy. *Fetal Diagn Ther*. 1996;11:264–270.
110. Noia G, Lippa S, Di Maio A, et al. Blood levels of coenzyme Q_{10} in early phase of normal or complicated pregnancies. In: Folkers K, Yamamura Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q*. Amsterdam: Elsevier; 1996:209–213.
111. Weir DG, Scott JM. The biochemical basis of the neuropathy in cobalamin deficiency. *Baillière's Clin Haematol*. 1995;8:479–497.
112. Flippo TS, Holder Jr WD. Neurologic degeneration associated with nitrous oxide anesthesia in patients with vitamin B12 deficiency. *Arch Surg*. 1993;128:1391–1395.
113. Lipton SA, Kim WK, Choi YB, et al. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A*. 1997;94:5923–5928.
114. Regland B, Johansson BV, Grenfeldt B, et al. Homocysteinemia is a common feature of schizophrenia. *J Neural Transm Gen Sect*. 1995;100:165–169.
115. Metz J, Bell AH, Flicker L, et al. The significance of subnormal serum vitamin B12 concentration in older people: a case control study. *J Am Geriatr Soc*. 1996;44:1355–1361.
116. Quinn K, Basu TK. Folate and vitamin B12 status of the elderly. *Eur J Clin Nutr*. 1996;50:340–342.
117. Fine EJ, Soria ED. Myths about vitamin B12 deficiency. *South Med J*. 1991;84:1475–1481.
118. Riggs KM, Spiro III A, Tucker K, et al. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr*. 1996;63:306–314.
119. Levitt AJ, Karlinsky H. Folate, vitamin B12 and cognitive impairment in patients with Alzheimer's disease. *Acta Psychiatr Scand*. 1992;86:301–305.
120. Joosten E, Lesaffre E, Riezler R, et al. Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? *J Gerontol A Biol Sci Med Sci*. 1997;52:M76–M79.
121. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002;346:476–483.
122. Allain P, Le Bouil A, Cordillet E, et al. Sulfate and cysteine levels in the plasma of patients with Parkinson's disease. *Neurotoxicol*. 1995;16:527–529.
123. Reynolds EH, Carney MW, Toone BK. Methylation and mood. *Lancet*. 1984;2:196–198.
124. Arpino C, Da Cas R, Donini G, et al. Use and misuse of antidepressant drugs in a random sample of the population of Rome, Italy. *Acta Psychiatr Scand*. 1995;92:7–9.
125. Osvaldo P, Marsh K, Helman A, et al. B-vitamins reduce the long-term risk of depression after stroke: the VITATOPS-DEP trial. *Ann Neurol*. 2010;68(4):503–510.
126. Stacy CB, Di Rocco A, Gould RJ. Methionine in the treatment of nitrous-oxide-induced neuropathy and myeloneuropathy. *J Neurol*. 1992;239:401–403.
127. Schilling RF. Is nitrous oxide a dangerous anesthetic for vitamin B12-deficient subjects? *JAMA*. 1986;28(255):1605–1606.
128. Reynolds EH, Bottiglieri T, Laundy M, et al. Vitamin B12 metabolism in multiple sclerosis. *Arch Neurol*. 1992;49:649–652.
129. Peerbooms OL, van Os J, Drukker M, et al. Meta-analysis of MTHFR gene variants in schizophrenia, bipolar disorder and unipolar depressive disorder: evidence for a common genetic vulnerability? *Brain Behav Immunol*. 2010;25(8):1530–1543.
130. Robillon JF, Canivet B, Candito M, et al. Type 1 diabetes mellitus and homocysteine. *Diabetes Metab*. 1994;20:494–496.
131. Hultberg B, Agardh E, Andersson A, et al. Increased levels of plasma homocysteine are associated with nephropathy, but not severe retinopathy in type 1 diabetes mellitus. *Scand J Clin Lab Invest*. 1991;51:277–282.
132. Agardh CD, Agardh E, Andersson A, et al. Lack of association between plasma homocysteine levels and microangiopathy in type 1 diabetes mellitus. *Scand J Clin Lab Invest*. 1994;54:637–641.
133. Munshi MN, Stone A, Fink L, et al. Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. *Metabolism*. 1996;45:133–135.
134. Araki A, Sako Y, Ito H. Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. *Atherosclerosis*. 1993;103:149–157.
135. Vaccaro O, Ingrosso D, Rivellese A, et al. Moderate hyperhomocysteinaemia and retinopathy in insulin-dependent diabetes. *Lancet*. 1997;349:1102–1103.
136. Neugebauer S, Baba T, Kurokawa K, et al. Defective homocysteine metabolism as a risk factor for diabetic retinopathy. *Lancet*. 1997;349:473–474.
137. Roubenoff R, Dellaripa P, Nadeau MR, et al. Abnormal homocysteine metabolism in rheumatoid arthritis. *Arthritis Rheum*. 1997;40:718–722.
138. Krogh Jensen M, Ekelund S, et al. Folate and homocysteine status and haemolysis in patients treated with sulphasalazine for arthritis. *Scand J Clin Lab Invest*. 1996;56:421–429.
139. Kang SS, Wong PW, Glickman PB, et al. Protein-bound homocyst(e)ine in patients with rheumatoid arthritis undergoing D-penicillamine treatment. *J Clin Pharmacol*. 1986;26:712–715.
140. Dennis VW, Robinson K. Homocysteinemia and vascular disease in end-stage renal disease. *Kidney Int Suppl*. 1996;57:S11–S17.
141. Robinson K, Gupta A, Dennis V, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation*. 1996;94:2743–2748.
142. Barak AJ, Beckenhauer HC, Tuma DJ. Betaine, ethanol, and the liver: a review. *Alcohol*. 1996;13:395–398.
143. Barak AJ, Beckenhauer HC, Tuma DJ. Betaine effects on hepatic methionine metabolism elicited by short-term ethanol feeding. *Alcohol*. 1996;13:483–486.
144. Malinow MR, Levenson J, Giral P, et al. Role of blood pressure, uric acid, and hemorheological parameters on plasma homocysteine concentration. *Atherosclerosis*. 1995;114:175–183.

145. Coull BM, Malinow MR, Beamer N, et al. Elevated plasma homocysteine concentration as a possible independent risk factor for stroke. *Stroke*. 1990;21:572–576.
146. Gershon SL, Fox IH. Pharmacologic effects of nicotinic acid on human purine metabolism. *J Lab Clin Med*. 1974;84:179–186.
147. Tamburrini O, Bartolomeo-De Iuri A, Andria G, et al. Bone changes in homocystinuria in childhood. *Radiol Med (Torino)*. 1984;70:937–942. [Italian].
148. Kaur M, Kabra M, Das GP, et al. Clinical and biochemical studies in homocystinuria. *Indian Pediatr*. 1995;32:1067–1075.
149. Lubec B, Fang-Kircher S, Lubec T, et al. Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. *Biochim Biophys Acta*. 1996;1315:159–162.
150. McLean RR, Jacques PF, Selhub J, et al. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med*. 2004;350:2042–2049.
151. Kałużna-Czaplińska J, Michalska M, Rynkowski J. Homocysteine level in urine of autistic and healthy children. *Acta Biochim Pol*. 2011;58:31–34.
152. Puig-Alcaraz, Carmen et al. Increased homocysteine levels correlate with the communication deficit in children with autism spectrum disorder. *Psychiatry Res.* 229(3):1031–1037.
153. James S, Melnyk S, Jernigan S, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B:947–956.
154. Dai, et al. Association between homocysteine and non-alcoholic fatty liver disease in Chinese adults: a cross-sectional study. *Nutri J*. 2016;15:102.
155. Gulsen, et al. Elevated plasma homocysteine concentrations as a predictor of steatohepatitis in patients with non-alcoholic fatty liver diseases. *J Gastroenterol Hepatol*. 2005;20:1448–1455.
156. Carvalho Sde, et al. Plasmatic higher levels of homocystein in non-alcoholic fatty liver disease (NAFLD). *Nutri J*. 2013;12. https://doi.org/10.1186/1475-2891-12-37.
157. Kasapoglu B, Turkay C, Yalcin KS, Kosar A, Bozkurt A. MTHFR 677C/T and 1298A/C mutations and non-alcoholic fatty liver disease. *Clin Med*. 2015;15(3):248–251. Epub 2015/06/03 PMID: 26031974.
158. Sun MY, Zhang L, Shi SL, Lin JN. Associations between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and NonAlcoholic fatty liver disease (NAFLD) risk: a meta-analysis. *PLoS ONE*. 2016;11(4):e0154337. https://doi.org/10.1371/journal.pone.0154337.
159. Pacana T, Cazanave S, Verdianelli A, et al. Deregulated hepatic methionine metabolism drives homocysteine elevation in diet-induced nonalcoholic fatty liver disease. *PLoS One*. 2015;10:e0136822. https://doi.org/10.1371/journal.pone.0136822. [PMC free article] [PubMed][Cross Ref].
160. Ubbink JB, Vermaak WJ, van der Merwe A, et al. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr*. 1993;57:47–53.
161. Polyzos SA, Kountouras J, Tsoukas MA. Circulating homocysteine in non-alcoholic fatty liver disease. *Eur J Intern Med*. 2015;26(2):152–153.
162. Ubbink J, Vermaak W, van der Merwe A, et al. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. *J Nutr*. 1994;124:1927–1933.
163. Dudman N, Wilcken D, Wang J, et al. Disordered methionine/ homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. *Arterioscler Thromb*. 1993;13:1253–1260.
164. Mason JB, Miller JW. The effects of vitamins B12, B6, and folate on blood homocysteine levels. *Ann N Y Acad Sci*. 1992;669:197–203. Portions reprinted with permission from Alternative Medicine Review. 1997 2:234-254.
165. Clarke R, Halsey J, Lewington S, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med*. 2010;170:1622–1631.
166. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA*. 2007;297:2351–2359. PMID: 17551129.
167. Kim YI. Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. *Environ Mol Mutagen*. 2004;44:10–25. PMID: 15199543.
168. Melnyk S, Pogribna M, Miller BJ, Basnakian AG, Pogribny IP, James SJ. Uracil misincorporation, DNA strand breaks, and gene amplification are associated with tumorigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. *Cancer Lett*. 1999;146:35–44. PMID: 10656607.
169. Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. *Cancer Res*. 2000;60:5434–5440. PMID: 11034085.
170. Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. *Cancer Res*. 2000;60:3191–3199. PMID: 10866310.
171. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. *J Nutr*. 2000;130:129–132. PMID: 10720158.
172. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*. 1996;56:4862–4864. [PubMed: 8895734].
173. Ulrich CM, Kampman E, Bigler J, et al. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? *Cancer Epidemiol Biomarkers Prev*. 1999;8:659–668. PMID: 10744125.
174. Kim DH. The interactive effect of methyl-group diet and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer. *Mutation Res*. 2007;622:14–18. PMID: 17602711.
175. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr*. 2006;136:189–194. PMID: 16365081.
176. Sawaengsri H, Wang J, Reginaldo C, et al. High folic acid intake reduces natural killer cell cytotoxicity in aged mice. *J Nutr Biochem*. 2016;30:102–107. PMID: 27012626.
177. Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. *Xenobiotica*. 2014;44(5):480–488.
178. Frye RE, et al. Effectiveness of methylcobalamin and folinic acid treatment on adaptive behavior in children with autistic disorder is related to glutathione redox status. *Autism Res Treat*. 2013. https://doi.org/10.1155/2013/609705.
179. Wilcken DE, Wilcken B, Dudman NP, et al. Homocystinuria–the effects of betaine in the treatment of patients not responsive to pyridoxine. *N Engl J Med*. 1983;309:448–453.

135

Hyperventilation Syndrome/ Breathing Pattern Disorders

Angela Sadlon, ND, and *Leon Chaitow*[*]*, ND, DO*

OUTLINE

INTRODUCTION

Hyperventilation Syndrome/Breathing Pattern Disorders Defined

Hyperventilation syndrome/breathing pattern disorders (HVS/BPDs) are described as follows:

- Hyperventilation is a pattern of overbreathing in which the depth and rate exceed the metabolic needs of the body at that time. This is usually seen at 30 breaths/min or more.
- Breathlessness usually occurs at rest or with only mild exercise.
- Physical, environmental, or psychological stimuli override the automatic activity of the respiratory centers, which are tuned to maintain arterial carbon dioxide ($Paco_2$) levels within a narrow range.
- Although at any given time the body's carbon dioxide (CO_2) production is set at a certain level, the exaggerated breathing depth and rate associated with HVS/BPDs eliminates CO_2 at a faster pace, resulting in arterial hypocapnia (low CO_2 in blood).
- The arterial hydrogen ion (pH) (acid/alkaline balance) rises into the alkaline region, thus inducing respiratory alkalosis.[1]

As a direct result of HVS/BPDs, many patients present with multiple symptoms, some of which mimic serious disease. However, blood tests, electrocardiograms (ECGs), and thorough physical examinations may reveal nothing out of the ordinary. Up to 10% of patients in general internal medicine practice reportedly experience HVS/BPDs as their primary diagnosis.[2] Many individuals with HVS/BPDs experience severe and genuinely distressing symptoms, and considerable medical expenses are incurred in excluding more serious pathology.

Gender

More females than males have HVS/BPDs, ranging from a ratio of 2:1 to 7:1. The peak age of incidence is 15 to 55 years, although other ages can be affected.[2] Women may be more at risk because of hormonal influences, because progesterone stimulates respiratory rate, and in the luteal (postovulation/premenstrual) phase, CO_2 levels drop 25% on average. Additional stress can then "increase ventilation at a time when CO_2 levels are already low."[3] A case report linked progesterone (medroxyprogesterone) therapy as a cause of hyperventilation in a 52-year-old menopausal woman.[4]

Normal Breathing Pattern

To recognize HVS/BPDs, one must be aware of the characteristics of a normal breathing pattern:

- The breathing rate should be 10 to 14 breaths/min, moving 3 to 5 L of air per minute through the airways of the chest.
- During the active inhalation phase, air flows in through the nose, where it is warmed, filtered, and humidified before being drawn into the lungs by the downward movement of the diaphragm and the outward movement of the abdominal wall and lower thoracic structures.

[*]Previous edition contributor

- The upper chest and accessory breathing muscles should remain relaxed.
- The expiratory phase is ideally effortless as the abdominal wall and lower intercostals relax downward and the diaphragm ascends back to its original domed position, aided by the elastic recoil of the lung.
- A relaxed pause at the end of exhalation releases the diaphragm briefly from the negative and positive pressures exerted across it during breathing.
- Under normal circumstances, individuals are quite unaware of their breathing.
- Breathing rates and volumes increase or fluctuate in response to physical or emotional demands, but in normal subjects, they return to relaxed, low-chest patterns after the stimuli cease.

Patients, especially those whose symptom presentation includes chronic fatigue or anxiety, or both, and who display or report a number of the following signs or symptoms, might be considered suitable candidates for respiratory treatment (see also "Diagnostic Considerations").

Benefits of Normal Respiratory Function

The exchange of gases involving the acquisition of oxygen (O_2) and elimination of CO_2 leads to enhanced cellular function and facilitates the following:

- Normal performance of the brain, organs, and tissues of the body
- Normal speech and human nonverbal expression (e.g., sighing)
- Fluid movement (lymph, blood)
- Spinal mobility through regular, mobilizing, thoracic cage movement
- Digestive function via rhythmic positive and negative pressure fluctuations, via normal diaphragmatic function

Any chronic alteration in breathing function automatically modifies these functions negatively.

The effect of habitual BPDs, such as hyperventilation, on an individual's physiology can be profound, potentially resulting in or exacerbating a wide range of health problems, sometimes severely, ranging from anxiety and panic attacks to fatigue and chronic pain.

The Carbon Dioxide–Oxygen Balancing Act

Chaitow et al.[5] addressed the misconception that oxygen is "good" and carbon dioxide is "bad" by stating:

> *Yet if one considers that life-giving oxygen can also be corrosive and toxic, and that a deficiency of the waste gas carbon dioxide (CO_2) can cause fainting, seizures, or death, then the "good/bad" distinction must be restated as "good within certain limits" and "bad within certain limits." Maintaining these two gases within those limits is a complex task for the body, even more so because the supply of each gas fluctuates with each breath.*

Respiratory Homeostasis

Jennett[6] described the delicate homeostatic balancing act in which pH and CO_2 are key features:

> *When there are not other overriding drives affecting breathing, the neural control system acts to maintain a constant arterial Pco_2. This must mean that the volume of CO_2 expired continually balances the volume produced by tissue metabolism. Measurements show that alveolar and arterial concentrations of CO_2 stay constant, which means that the volume of gas breathed out from the functional (alveolar) volume of the lungs must vary precisely with the rate of metabolic CO_2 production. In rest and activity, when the system is left to itself, it is so efficient that the matching occurs virtually breath-by-breath, even when metabolic activity is continually changing.*

Pathophysiology

Physiological and Pathophysiological Causes of Altered Patterns of Breathing

Hyperventilation can be an appropriate physiological response to the body's metabolic needs; for example, tachypnea (rapid breathing) or hyperpnea (increase in respiratory rate proportional to increase in metabolism) may result as the respiratory centers respond automatically and appropriately to rising CO_2 production due to exercise or organic disease that may be creating acidosis. It is therefore important to exclude organic causes that diminish PaO_2 or elevate $Paco_2$ levels.[7]

Organic causes of HVS/BPDs that should be excluded and/or identified before breathing rehabilitation is initiated include the following:

- Respiratory: asthma, chronic obstructive respiratory disease, pneumonia, pulmonary embolus, pneumothorax, and pleural effusion.
- A case of carbon monoxide poisoning presenting as HVS has been reported.[8]
- Cardiovascular: acute and chronic left heart failure, right heart failure, tachyarrhythmias
- Hematopoietic: anemia
- Renal: nephrotic syndrome, acute and chronic kidney failure
- Endocrine: diabetes with ketoacidosis, pregnancy
- Metabolic: liver failure
- Pharmaceutic: aspirin, caffeine, amphetamine, nicotine, progesterone therapy

BPDs may also emerge from a background of established pathology (e.g., asthma, cardiovascular disease, kidney failure, chronic pain). Even tumor infiltrates into brain respiratory centers and central chemoreceptors have caused hyperventilation.[9] Where this is the case, the aim of this chapter is not to explore these states because they are discussed elsewhere in this textbook.

Fluctuating blood glucose levels may trigger HVS/BPD symptoms in patients with high-carbohydrate diets, which produce rapid rises followed by sharp falls to fasting levels or below.[7,10]

Chaitow et al.[5] noted that the following factors could lead to altered breathing patterns through pH shifts:

- Ketoacidosis promotes deeper, faster breathing because the breathing centers respond to the higher CO_2 content.
- Diarrhea results in the loss of alkaline plasma bicarbonate ions, which if prolonged, leads to acidosis. This stimulates corrective overbreathing to remove CO_2 (as carbonic acid [H_2CO_3]) and normalizes the pH.
- Excessive vomiting causes loss of hydrochloric acid, shifting the body toward alkalosis, slowing breathing to allow CO_2 to build up and restore pH. Hypoventilation is the result.
- Use of steroids and diuretics can lead to alkalosis.

Categorization of Causes

It is possible to place the common etiological features of HVS/BPD into biomechanical, biochemical, psychological, environmental, pathological, and habitual categories.

The reasons for an individual breathing inappropriately can derive directly from structural, biomechanical causes, such as a restricted thoracic spine, rib immobility, or shortness of key primary and accessory respiratory muscles.

Causes of breathing dysfunction can also have a more biochemical etiology, possibly involving allergy or infection, which triggers narrowing of breathing passages and subsequent asthmatic-type responses. Acidosis, resulting from conditions such as kidney failure, also directly alters breathing function as the body attempts to reduce acid levels via elimination of CO_2 through hyperventilation.

The link between psychological distress and breathing makes this another primary cause of many manifestations of dysfunctional respiration. Examining a person with anxiety or depression without breathing dysfunction being noted is difficult to imagine.

Other catalysts that may affect breathing function include environmental factors (e.g., altitude, humidity).[11]

The etiology of HVS/BPD may involve combinations of the factors listed previously; however, in most instances, altered breathing patterns, whatever their origins, seem to be maintained by nothing more sinister than pure habit.[7,12]

The Hydrogen Ion Factor

Chaitow et al.[5] stated the following:

> *The story [of HVS/BPD] might best begin with pH, because this factor influences every organ of the body. The variable of relative acidity facilitates many metabolic exchanges and it must be kept in careful balance. Since pH describes proportion of hydrogen ions available for combination, and the pH is on a log scale, a small change in pH, such as 7.4 to 7.2, means roughly a doubling of the number of hydrogen ions present. The binding of hydrogen to negative sites helps to regulate enzymatic action, endocrine secretion, integrity of protein molecules, and cellular metabolism, including oxygen absorption and release. A pH of 7.2 would seriously compromise many physiologic functions. The physiologic normal pH in the arterial blood is around 7.4, with an acceptable range from 7.35 to 7.45. Outside these limits lie ill effects of many kinds. The body will sacrifice many other things in order to maintain proper pH.*

The Carbon Dioxide–Hydrogen Ion Connection

- The acidity of the blood is determined mainly by CO_2.
- CO_2 is the end-product of aerobic metabolism, deriving mainly from the mitochondria. CO_2 is odorless, heavier than air, and, if inhaled in its pure form, causes suffocation.
- CO_2 is present in the atmosphere at a concentration of around two-hundredths of 1% and is harmless to humans but adequate to sustain plant life.
- Transportation of CO_2 occurs from the tissues into the blood and then to the lungs for exhalation. The body converts CO_2 to H_2CO_3, of which there is a perpetual surplus.
- The lungs exhale around 12,000 mEq of H_2CO_3 per day, compared with less than 100 mEq of fixed acids excreted by the kidneys. The normal range of end-tidal CO_2 pressure is 35 to 45 mm Hg.[13]
- Any increase in bodily activity produces CO_2, acidifying the blood, unless more CO_2 is excreted and/or exhaled.
- It is therefore obvious that changes in breathing volume relative to CO_2 production regulate the moment-to-moment concentration of pH of the bloodstream (longer-term regulation of pH is shared with the kidneys).
- It is the concentration of CO_2 in the blood, not oxygen, that is the major regulator of breathing drive.
- Higher CO_2 levels immediately stimulate more breathing, apparently on the assumption that abundance of CO_2 means oxygen-poor air is being breathed, breathing has stopped, or something else is happening that is an antecedent to suffocation.

The Bicarbonate Buffer

Chaitow et al.[5] explained the following:

> *There exists a further balancing mechanism, the bicarbonate buffer. The bicarbonate ion (HCO_3–) is derived from CO_2 during its ride in the bloodstream; CO_2 dissociates into hydrogen ions (H+) and (HCO_3–). This bicarbonate reserve is adjustable as needed, up to a point, and constitutes a major alkaline buffer system which opposes rises in acidity. The kidneys regulate the regulators by adjusting the amount of bicarbonate returned to the bloodstream. If the kidneys detect excess acidity (a surplus of positively-charged hydrogen ions) they will try to retain more bicarbonate to balance the acid, but this is not a fast process; adjusting their filtration characteristics takes hours to days. In the short run, meanwhile, if bicarbonate buffering of excess acid is not sufficient, or if the bicarbonate is depleted, a faster back-up buffering system is available: hyperventilation. Excessive breathing exhales more CO_2 (acid), thereby bringing pH closer to normal.*

Oxygen Delivery and Smooth Muscle Constriction

The blood carries oxygen mainly in molecules of hemoglobin, which are contained in red blood cells. In an appropriate environment, hemoglobin combines readily with oxygen (to form oxyhemoglobin). This process varies according to local pH, as well as Po_2. This ability to combine is important for both absorbing oxygen through the alveoli and also for releasing oxygen through the capillary walls, where oxygen diffuses into the tissues.

These two properties are largely determined by local conditions, so when pH is low (i.e., the blood is more acidic), hemoglobin in that area is stimulated to release additional oxygen. This is true of metabolically active tissues in general but especially of muscles. An exercising muscle needs all the oxygen it can get, and this is assisted by its chemical nature, explained by West as follows:

> *An exercising muscle is acid, hypercarbic, and hot, and it benefits from increased unloading of oxygen from its capillaries.*[11]

The effect of pH on oxyhemoglobin dissociation is called the Bohr effect.

In the lungs, the need is to bind oxygen to hemoglobin, not release it. Not surprisingly, the lungs have a more alkaline environment.

The fact that a shift of the blood toward acidity promotes dissociation and release of oxygen from the hemoglobin is particularly important when considering hyperventilation because the resulting alkalinity causes the hemoglobin molecule to retain more oxygen than usual. With increased alkalinity encouraging smooth muscle contraction and therefore diminished diameter of blood vessels, as well as the reluctance of hemoglobin to release its oxygen, a relative oxygen deficit is likely in tissues and the brain, leading to symptoms such as fatigue, aching, cramping, and cognitive problems.

Psychology and Hyperventilation Syndrome/Breathing Pattern Disorders

On a psychological level, Bradley[14] described a "cascade of symptoms" (Fig. 135.1) in which an original cause (emotional or physical) leads to tension and anxiety that result in hyperventilation, possibly an acute hyperventilation attack, which (with repetition), over time, results in anticipation, anxiety, and avoidance behaviors or phobias, or both.

Chaitow et al. and others describe similar aspects of the influence of emotion on breathing[5,15–17]:

> *Increased variability from breath to breath is known to correlate with anxiety states. Low Pco_2 and increased frequency of sighing are typical of those with panic disorder even when not panicking. These generalizations miss individuals who do not have a chronic BPD, but who do experience disrupted breathing under particular conditions. These "conditions" can provoke either an amygdala "alarm" discharge or some specific, learned breathing response, as well as occasional panic.*

Conway et al.[18] used hypnosis to investigate the sources of hyperventilation episodes and found emotional events such as loss, separation, and impotent anger were common precipitating factors that

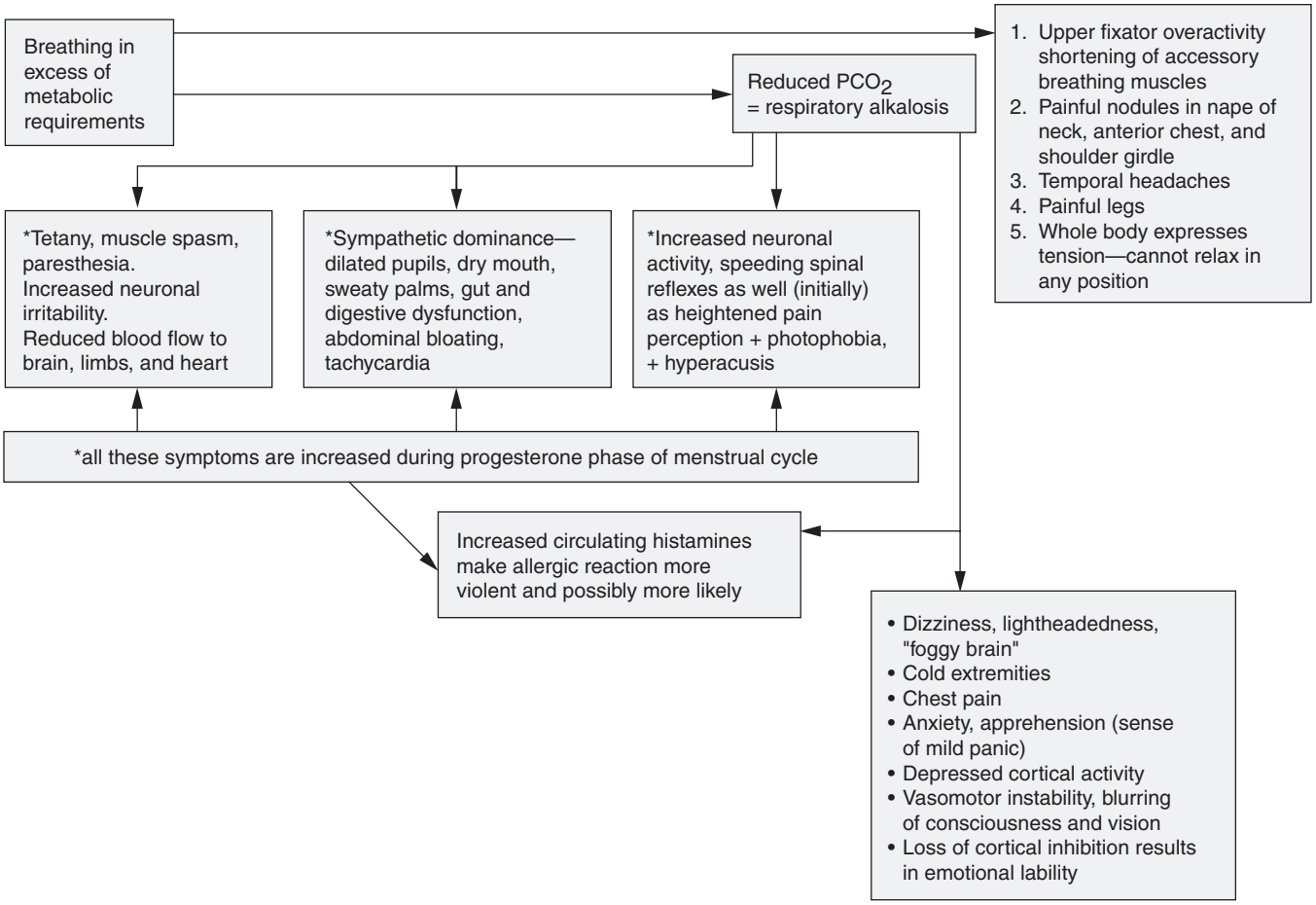


Fig. 135.1 Negative health influences of a dysfunctional breathing pattern such as hyperventilation. (From Chaitow L, Bradley D, Gilbert C. *Multidisciplinary Approaches to Breathing Pattern Disorders*. London: Churchill Livingstone; 2002: 90.)

began the hyperventilation trend. They concluded that hypnosis might be helpful in discovering the underlying cause of hyperventilation.

Freeman et al.[19] also showed that individuals who reported several symptoms indicating hyperventilation (including chest pain/palpitations and dizziness—not exclusively respiratory symptoms) displayed rather strong hyperventilation in response to recalling emotionally disturbing events, whereas the control subjects did not. Bereavement, loss of control, grief, and anger were common topics associated with the symptoms.

Chaitow et al.[5] concluded (Fig. 135.2):

> *Aside from medical problems, there are still many factors in the psychological and behavioral realms competing for control of breathing. Successful regulation must take all factors into account, with special consideration for priorities of survival. The human brain adds a layer of complication with its power to imagine, project, and recall, often stimulating breathing reflexes without apparent reason.*

DIAGNOSTIC CONSIDERATIONS

Symptoms

Acute hyperventilation represents approximately 1% of all cases of hyperventilation, which is well outnumbered by chronic hyperventilation.[20] The symptoms and signs of HVS are extremely variable, and none is absolutely diagnostic. The following symptoms are indications of possible breathing pattern dysfunction:

- A feeling of constriction in the chest
- Shortness of breath
- Accelerated or deepened breathing
- Inability to breathe deeply
- Feeling tense (the Nijmegen questionnaire avoids the use of the word *anxiety*)
- Tightness around the mouth
- Stiffness in the fingers or arms
- Cold hands or feet
- Tingling fingers
- Bloated abdominal sensation
- Dizzy spells
- Blurred vision
- Feeling of confusion or losing touch with environment

Table 135.1 is not fully comprehensive but presents the most common symptoms and signs of HVS/BPD. For greater depth, see Timmons and Ley,[7] Gardner,[21] Nixon,[22] and Chaitow et al.[5]

Metabolic Disturbances and Hyperventilation Syndrome

- Two tests of nerve hyperexcitability produced by hypocapnia-induced hypocalcemia are Trousseau's and Chvostek's signs.
- Chest pain associated with HVS/BPDs requires that heart disease is excluded as a diagnosis. Adrenaline-induced electrocardiographic changes can occur in hyperventilation, uncomplicated by coronary heart disease. One study suggested that up to 90% of noncardiac chest pain is thought to be induced by HVS/BPDs.[23]

In older patients, established coronary artery disease can be exacerbated by vasoconstriction arising from hypocapnia, which puts them at risk of coronary occlusion and myocardial damage.

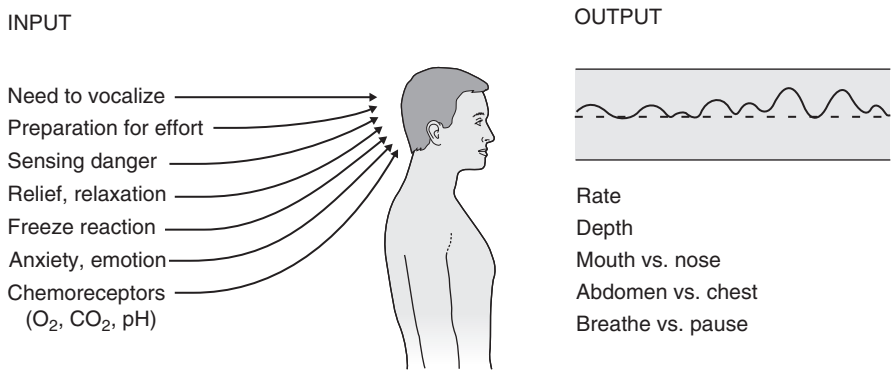


Fig. 135.2 Final modulation of the breathing act includes input from many possible sources: the need for vocalization (a cry for help, a shouted warning, perhaps a growl), preparation for exertion, the need to freeze and become less noticeable, the need to maximize sensory acuity by keeping the body still, and the need to either remain calm or return to a baseline state of calmness. These inputs often conflict with each other, but if there is a hint of a threat to survival, they seem to have priority over all other considerations. (From Chaitow L, Bradley D, Gilbert C. *Multidisciplinary Approaches to Breathing Pattern Disorders.* London: Churchill Livingstone; 2002: 122.)

TABLE 135.1 Most Common Symptoms and Signs of Hyperventilation Syndrome/Breathing Pattern Disorders

System	Symptoms	Suggested Causes
Cardiovascular	Chest pain and angina, palpitation and arrhythmias, tachycardia, lightheadedness and syncope, altered ECG features	Reduced coronary blood flow, altered excitability of SA and AV nodes of cardiac muscle, reduced cardiac output, peripheral vasodilatation
Gastrointestinal	Discomfort in lower chest and epigastric area, esophageal reflux and heartburn, bloating/distension, exacerbation of hiatal hernia symptoms, dry mouth, air swallowing and belching	Aerophagia, increased swallowing rate, mouth breathing
Neurological	Headache; numbness and tingling (mainly involving extremities and perioral); positive Trousseau's and Chvostek's signs; dizziness/giddiness; ataxia and tremor; blurred and tunnel vision; anxiety and panic; phobias; irritability; depersonalization; detachment from reality; impaired concentration, cognition, performance; easy fatigue; insomnia; hallucinations	Cerebrovascular constriction (see notes on smooth muscle contraction); vasoconstriction of vertebral or carotid arteries, or both; reduced oxygen delivery, neuronal excitability resulting from alkalosis; hypocalcemia
Respiratory	Breathlessness; restricted sensation around thorax; sighing/yawning; obvious use of upper chest, accessory breathing muscles (e.g., scalenes) on inhalation; chest tenderness	Overuse of accessory breathing muscles and fatigue of primary respiratory muscles
Muscular	Stiffness and aching, weakness in limbs, cramping, carpopedal spasm, tetany	Hyperexcitability of motor nerves, muscle fatigue, calcium/magnesium imbalance

AV, Atrioventricular; *ECG,* electrocardiogram; *SA,* sinoatrial.

Alternatively, hyperventilation can trigger spasms of normal-caliber coronary arteries.

- Rapid breathing or mouth breathing instigates aerophagia from air gulping, causing bloating, burping, and extreme epigastric discomfort. Irritable bowel syndrome is listed as a common symptom of chronic overbreathing. Fear and anxiety may induce abdominal cramps and diarrhea.[1] The median swallowing rate in healthy, nondyspeptic controls is three or four swallows per 15 minutes. In the absence of food, up to 5 mL of air accompanies saliva into the gastrointestinal tract with each swallow.[24]
- Hyperventilation leading to hypocapnia causes cerebral arterioles to constrict, increasing vascular resistance and reducing cerebral blood flow. This is a natural response to changes in CO_2 to regulate oxygen delivery to the brain.[25]

Acute Hyperventilation Progression

- The patient presenting with an acute hyperventilation episode appears distressed.
- The pattern of respiration is of deep, rapid breaths, using the accessory muscles visible in the neck and the upper chest.
- Wheezing may be heard as a result of bronchospasm triggered by hypocapnia.
- A stressful precipitating event is usually reported.
- Hypocapnia reduces blood flow to the brain (2% decrease in flow per 1 mm Hg reduction in arterial CO_2), causing frightening central nervous system symptoms. The reduced oxygenation of brain and tissues of the body results from the contraction of smooth muscle surrounding blood vessels and a reluctance of the hemoglobin carrier molecule to release oxygen in the increasingly alkaline environment caused by excessive loss of CO_2.
- Poor concentration and memory lapses may occur as a result, with tunnel vision and onset in those susceptible to migraine-type headaches or tinnitus.
- Sympathetic dominance brings on tremors, sweating, clammy hands, palpitations, and autonomic instability of blood vessels, causing labile blood pressures.[26]

- Bilateral perioral and upper extremity paresthesia and numbness may be reported. Unilateral tingling is most often confined to the left side.
- Dizziness, weakness, visual disturbances, tremor, and confusion—sometimes fainting or even seizures—are typical symptoms.
- Spinal reflexes become exaggerated through increased neuronal activity caused by loss of CO_2 ions from the neurons.
- Tetany and cramping may occur in severe bouts.[27]

Chronic Hyperventilation

The diagnosis of chronic and intermittent hyperventilation is more difficult than acute hyperventilation. A careful history and systemic inquiry, checking all other symptoms in the other body systems, usually highlights a suspicious pattern, particularly to the experienced clinician who can think beyond his or her own specialist area. Examination must exclude organic diseases of the brain and nervous system; the heart, particularly angina and heart failure; respiratory disease; and gastrointestinal conditions, especially if there are suspicious symptoms in these systems.

Careful inquiries as to the precipitating causes of attacks help with both the diagnosis and focusing on the choice of treatment. Nixon[22] suggested that there are often attacks where there is no preceding stressful event. For example, in chronic hyperventilators, the respiratory center may have been reset to tolerate a lower than normal partial pressure of $Paco_2$ levels in the blood. In such patients, a single sigh or one deep breath may reduce the $Paco_2$ enough to trigger symptoms.

Laboratory and Office Tests for Hyperventilation Syndrome/Breathing Pattern Disorders

Many possible tests for respiratory function exist. Some are difficult to perform (e.g., airway resistance), and some are invasive (e.g., blood gases).[14] A selection of tests is as follows:

- Preliminary tests should be conducted to exclude respiratory and cardiac disease, including peak expiratory flow rate, chest radiograph, ECG, and an exercise ECG if chest pain is present.
- Palpation and observation can demonstrate a paradoxical breathing pattern in which the abdomen retracts and the upper chest expands on inhalation (as opposed to normal abdominal protrusion and lower thorax expansion).
- The breath-holding time test does not require additional measurements or equipment. The time a hyperventilating patient can hold his or her breath is usually greatly reduced, often not beyond 10 to 12 seconds. Thirty seconds has been used as the approximate dividing line between hyperventilators and normals by some clinicians. It is worth noting that breathless patients without hyperventilation may have equal difficulty in breath holding.[21]
- A peak expiratory flow rate measurement, compared with age, sex, and height tables, provides a simply done, quick exclusion of significant respiratory restriction in the clinic room.
- If a hyperventilation provocation test (HVPT) is performed (during which the patient is asked to voluntarily overbreathe to bring on symptoms), ECG should be monitored (see "Caution" later).
- Elevated erythrocyte carbonic anhydrase (ECA) was recently (2009) suggested as a clinical marker for hyperventilation. The values of ECA were significantly elevated (~31 U/g hemoglobin) in patients who hyperventilated compared with controls (24.7 U/g hemoglobin). However, hyperventilation sensitivity and specificity were only 52.1% and 76.7%, respectively. There are other conditions that might elevate ECA, such as glucose-6-phosphate dehydrogenase deficiency and different anemias (aplastic, iron deficiency, autoimmune hemolytic, β-thalassemia).[28]
- Arterial blood gas determination is invasive and painful (arterial puncture) but appropriate in the emergency department, where the diagnosis of acute hyperventilation is required. For patients in whom chronic hyperventilation is suspected, the pressure of end-tidal carbon dioxide (PET CO_2) can be measured noninvasively from a continuous sampling through nasal prongs or cannula with the mouth occluded, or the tube can be sited in an oral airway for those with nasal obstruction to monitor CO_2 deficits. The PET CO_2 is the level of CO_2 released at the end of expiration.
- Capnography: PET CO_2 can be evaluated after a 4-minute quiet breathing rest period, followed by exercise and recovery, or a HVPT can be conducted in the recovery period. Most patients with chronic hyperventilation have a PET CO_2 at or below 30 mm Hg and a markedly delayed recovery from hypocapnia after overbreathing, sometimes lasting 30 minutes after testing.[29,30] By measuring PET CO_2 or transcutaneous CO_2 levels while a hyperventilation-provoking activity is performed, a potential link can be made between symptoms and CO_2 levels.[31]
- The think test[32] may be initiated 3 to 4 minutes into the recovery period. The patient is asked to recall a painful emotional experience during which symptoms developed. If the PET CO_2 drops 10 mm Hg, the test supports hyperventilation. Bradley[14] noted: "In some patients with hyperventilation the $Paco_2$ and the PET CO_2 may be in the normal range. In those who are asymptomatic at the time of testing, this finding could be accepted. However, a normal level while experiencing symptoms negates hypocapnia as the cause of symptoms. It prompts a search for an alternative explanation."
- Buteyko performed comparative studies with a simple breath-holding technique to test CO_2 levels and found that a simple technique of breath holding after expiration could predict the percent of alveolar CO_2 and therefore the degree of hyperventilation to a high degree of accuracy. According to his calculations, optimal levels of alveolar Pco_2 correlated with postexpiratory breath-holding time of 40 to 60 seconds. Many asthmatics and hyperventilators are found to be able to hold the breath out for less than 10 seconds.[33–35]

Caution

Bradley[14] cautioned the following:

> *The hyperventilation provocation test is best done before explanations of symptoms, to prevent suggestion and bias. Patients need to be warned only of a dry mouth. The patient is asked to concentrate on how they feel during the 1-2 minute period when they are over breathing at the rate of 30-40 per minute. The rate is set by the examiner's hand movements. The operator must stress the importance of the test and the need to continue for as long as they can. An arterial blood gas determination at the end of the test can be of use to establish the depth of hypocapnia. Some clinicians rely on as little as 12 deep breaths which the patient can recover from easily, and subjective symptoms produced are recorded. In both the breath holding and voluntary over-breathing tests, the skills of the clinician are important for maintaining the trust and co-operation of patients.*

The Nijmegen Questionnaire

Bradley[14] pointed out that no "gold standard" exists for chronic HVS, but the Nijmegen Questionnaire is noninvasive, with a high level of sensitivity (up to 91%)[36] and specificity (up to 95%).[37] It is also a way to monitor the progress of treatment by reevaluating symptoms. Bradley noted that the results of this simple test also helped indicate whether the initiating trigger causing the HVS/BPDs resolved, suggesting that the patient had to deal with only the "bad breathing" habit and musculoskeletal and motor pattern changes, or whether the initiating

	Never 0	Rare 1	Sometimes 2	Often 3	Very often 4
Chest pain					
Feeling tense					
Blurred vision					
Dizzy spells					
Feeling confused					
Faster or deeper breathing					
Short of breath					
Tight feelings in chest					
Bloated feeling in stomach					
Tingling fingers					
Unable to breathe deeply					
Stiff fingers or arms					
Tight feelings round mouth					
Cold hands or feet					
Palpitations					
Feelings of anxiety					
			Total:	/64*	

*Nijmegen. Patients mark with a tick how often they suffer from the symptoms listed. A score above 23/64 is diagnostic of hyperventilation syndrome.

Fig. 135.3 Nijmegen questionnaire. (From Chaitow L, Bradley D, Gilbert C. *Multidisciplinary Approaches to Breathing Pattern Disorders.* London: Churchill Livingstone; 2002: 176.)

triggers were ongoing or unresolved and might need further cognitive help (Fig. 135.3).

Warburton and Jack[30] stated that the Nijmegen Questionnaire was neither sensitive nor specific for chronic idiopathic hyperventilation without physiological testing, because many of the symptoms on the questionnaire are common to an organic respiratory disease.

BIOMECHANICAL (STRUCTURAL) CONSIDERATIONS

The Structure–Function Continuum

Nowhere in the body is the axiom of structure governing function more apparent than in its relation to respiration. Just as prolonged changes in patterns of use, such as an inappropriate (hyperventilation) breathing pattern, induce structural changes involving the muscles and joints (e.g., rib) of respiration, so do these changes ultimately prevent normal function.

Ultimately, the self-perpetuating cycle of functional change creating a structural modification, leading to reinforced functional tendencies, can become complete, from whichever direction dysfunction derives. Examples follow:

- Structural adaptations can prevent normal breathing function.
- Abnormal breathing function ensures continued structural adaptational stresses.[38]

The restoration of normal function demands the restoration of adequate structural mobility, whereas maintenance of restored biomechanical flexibility requires that function (how the individual breathes) should be normalized through reeducation and training.

Individually, neither approach is as useful as a combination of restoration of structural integrity, combined with functional improvement. It should be obvious that other underlying etiological features, whether these relate to psychosocial, biochemical, or biomechanical factors, should be addressed as far as possible as a prerequisite of rehabilitation.

The Muscles of Breathing

The muscles associated with breathing function can be grouped as either inspiratory or expiratory. They are either primary in that capacity or provide accessory support.

Because expiration is primarily an elastic response of the lungs, pleura, and "torsion rod" elements of the ribs, all muscles of expiration could be considered accessory muscles because they are recruited only during increased demand. They include internal intercostals, abdominal muscles, transverse thoracis, and subcostales. With increased demand, the iliocostalis lumborum, quadratus lumborum, serratus posterior inferior, and latissimus dorsi may support expiration, including during the high demands of speech, coughing, sneezing, singing, and other special functions associated with breathing.

Space does not permit in-depth discussion of the muscles of respiration, further details of which can be found in Chaitow et al.[5]

Neural Regulation of Breathing

Respiratory centers in the brainstem unconsciously influence and adjust alveolar ventilation to maintain arterial blood oxygen and CO_2 pressures at relatively constant levels to sustain life under varying conditions and requirements.[5]

The three main groups are as follows:

- The dorsal respiratory group, located in the distal portion of the medulla, receives input from peripheral chemoreceptors and other types of receptors via the vagus and glossopharyngeal nerves. These impulses generate inspiratory movements and are responsible for the basic rhythm of breathing.
- The pneumotaxic center in the superior part of the pons transmits inhibitory signals to the dorsal respiratory center, controlling the filling phase of breathing.

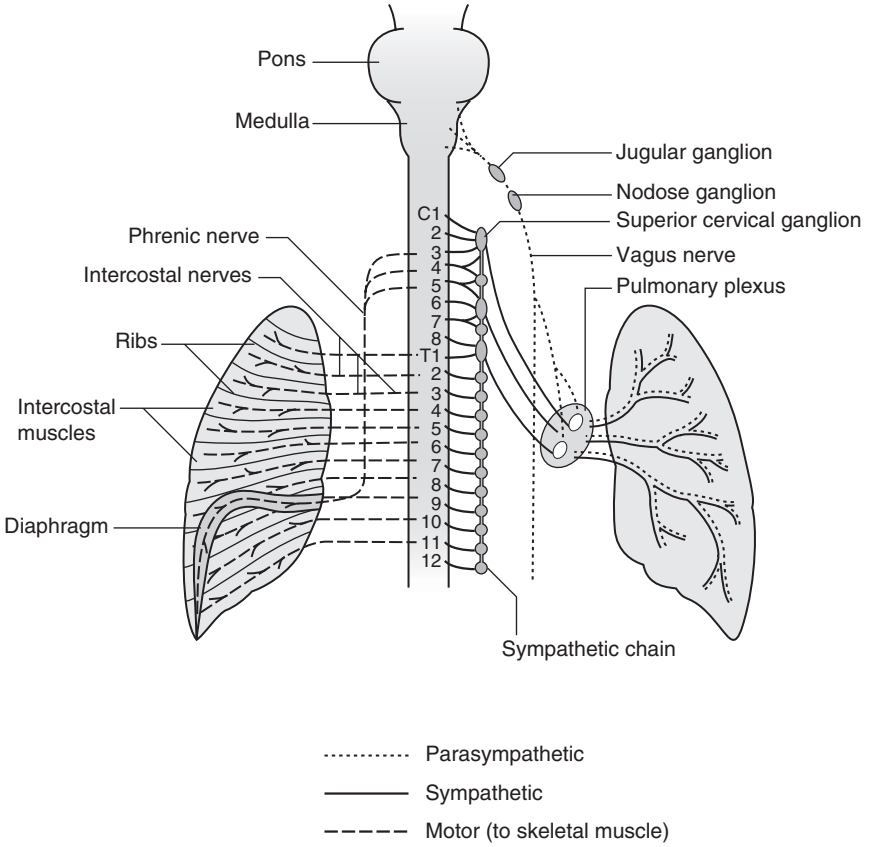


Fig. 135.4 Schema of the autonomic innervation (motor and sensory) of the lung and the somatic (motor) nerve supply to the intercostal muscles and diaphragm. (From Scanlan C, Wilkins R, Stoller J. *Egan's Fundamentals of Respiratory Care*. 7th ed. St. Louis: Mosby; 1999.)

- The ventral respiratory group, located in the medulla, causes either inspiration or expiration. It is inactive in quiet breathing but is important in stimulating abdominal expiratory muscles during levels of high respiratory demand.

The Hering–Breuer reflex prevents overinflation of the lungs and is initiated by nerve receptors in the walls of the bronchi and bronchioles, sending messages to the dorsal respiratory center, via the vagus nerve. The reflex "switches off" excessive inflation during inspiration, as well as excessive deflation during exhalation.

The autonomic nervous system enables the automatic, unconscious maintenance of the internal environment of the body in ideal efficiency and adjusts to the various demands of the external environment, be it sleep with repair and growth, quiet or extreme physical activity, or stress (Fig. 135.4).

A "third" nervous system regulating the airways has been recognized, called the nonadrenergic noncholinergic (NANC) system. Containing inhibitory and stimulatory fibers, nitric oxide has been identified as the NANC neurotransmitter.[39]

- NANC inhibitory nerves cause calcium ions to enter the neuron, mediating smooth muscle relaxation and bronchodilation.
- NANC stimulatory fibers—also called C-fibers—are found in the lung supporting tissue, airways, and pulmonary blood vessels, and appear to be involved in bronchoconstriction after exercise-induced asthma.[40]

Chemical Control of Breathing

The central role of respiration is to maintain balanced concentrations of oxygen and CO_2 in the tissues. Increased levels of CO_2 act on the central chemosensitive areas of the respiratory centers themselves, increasing inspiratory and expiratory signals to the respiratory muscles. In contrast, oxygen acts on peripheral chemoreceptors located in the carotid body (in the bifurcation of the common carotid arteries) via the glossopharyngeal nerves and the aortic body (on the aortic arch), which sends the appropriate messages via the vagus nerves to the dorsal respiratory center.

Voluntary Control of Breathing

Automatic breathing can be overridden by higher cortical conscious input (directly via the spinal neurons, which drive the respiratory muscles) in response to, for instance, fear or sudden surprise. Speaking requires voluntary control to interrupt the normal rhythmicity of breathing, as do singing and wind instrument playing. Evidence indicates that the cerebral cortex and thalamus also supply part of the drive for normal respiratory rhythm during wakefulness. (Cerebral influences on the medullary centers are withdrawn during sleep.) Habitual BPDs and HVS probably originate from some of these higher centers.[7]

THERAPEUTIC CONSIDERATIONS AND THERAPEUTIC APPROACH

Treatment and Rehabilitation of Hyperventilation Syndrome/Breathing Pattern Disorders

Different models of care in managing HVS/BPDs are available.

It is assumed that all organic causes of breathing pattern changes have been excluded and that coexisting problems such as asthma, chronic obstructive airway disease, chronic pain, and hormonal imbalances receive appropriate specialized attention. It is also assumed that manual therapy approaches would be incorporated as an essential element of rehabilitation.

Bradley's[14,41] physical therapy rehabilitation follows stages that are summarized in the acronym BETTER: breathing retraining, esteem/self-image, total body relaxation, talk/breath control, exercise prescription, rest/sleep.

Breathing retraining incorporates a number of elements:

- Awareness of faulty breathing patterns
- Relaxation of the upper chest, shoulders, and accessory muscles
- Abdominal/low-chest breathing pattern retraining
- Awareness of normal breathing rates and rhythms both at rest and during activity

The elements of the physical therapy protocol involving relaxation, exercise, talk/breath control, and sleep are all individualized and are not described here.

An Osteopathic/Naturopathic Protocol for Care of Hyperventilation Syndrome/Breathing Pattern Disorders

- Initial (and continual or periodic) assessment of breathing function based on functional evidence and palpation determines what needs to be done to improve breathing function.
- Education and information are vital for creating motivation and awareness as to why homework is essential in normalizing BPDs.
- The patient must understand clearly that the practitioner or therapist can do no more than create an environment, a possibility, for the restoration of more normal function, but the breathing work itself is up to the patient.
- Treatment of muscles and joints alone, no matter how appropriate, can never restore normal breathing patterns without cooperative effort.
- Conversely, breathing retraining without the freeing of restricted structures is far more difficult to achieve.
- Psychotherapy and counseling are also unlikely to be successful unless retraining is introduced and structural factors are dealt with.
- Manual attention to the upper fixators and/or accessory breathing muscles (upper trapezii, levator scapulae, scalenes, sternocleidomastoid, pectorals, and latissimus dorsi) is usually required.
- The diaphragm area also requires direct attention as a rule (lower anterior intercostals, sternum, costal margin, beneath costal margin, abdominal attachments, quadratus lumborum, and psoas).
- Active trigger points in these muscles may need deactivating manually or via acupuncture.
- Acupuncture being administered for 30 minutes, twice weekly, for 4 weeks showed a reduction in Nijmegen score from 31 to 24. The focus was on reducing anxiety, thereby reducing hyperventilation. The points used were colon 4, liver 3, and stomach 36 bilaterally.[42]
- The thoracic spine and ribs may require mobilization (osteopathic or chiropractic adjustments).
- Osteopathic lymphatic pump methods may be required if there is evidence of stasis.
- Retraining: various breathing exercises should be introduced, individualized to the specific needs of the patient, commonly on the basis of pursed-lip breathing and pranayama yoga methods (Box 135.1).[43–47]
- Relaxation methods, including autogenic training or progressive muscular relaxation, or both, might usefully be introduced. One small placebo-controlled trial showed improvement on anxiety and hyperventilation in HIV-infected patients after four Swedish massage sessions (once weekly for 30 minutes to the back).[48]
- Sleep pattern disturbances might require attention.
- Exercise of aerobic nature should be carefully introduced.
- Dietary advice and counseling should be introduced as appropriate.

BOX 135.1 Breathing Rehabilitation Exercises

1. Pursed lip breathing[44,45]: Pursed lip breathing, combined with diaphragmatic breathing, enhances pulmonary efficiency.
   - The patient is seated or supine with the dominant hand on the abdomen and the other hand on the chest.
   - The patient is asked to breathe in through the nose and out through the mouth, with pursed lips, ensuring diaphragmatic involvement by means of movement of the abdomen against the hand on inhalation.
   - Exhalation through the pursed lips is performed slowly and has been shown to relieve dyspnea, slow the respiratory rate, increase tidal volume, and help restore diaphragmatic function.

   Author's Note: Essentially, blowing firmly and slowly through a narrow aperture, such as pursed lips, effectively tones the diaphragm via eccentric isotonic activity.
2. Antiarousal breathing[43,46]: The patient is asked to sit or recline comfortably and exhale slowly and fully through pursed lips, and the following guidance is given:
   - Imagine a candle flame about 6 inches from your mouth, and blow a thin stream of air at it (pursed-lip breathing).
   - As you exhale, count silently to establish the length of the "out breath."
   - When you have exhaled fully, without strain, pause for a count of "1," then inhale through the nose.
   - Full exhalation creates a "coiled spring," making inhalation easier.
   - Count to yourself to establish how long your "in breath" lasts.
   - Without pausing to hold the breath, exhale slowly and fully, through pursed lips, blowing the air in a thin stream, and then pause for a count of 1.
   - Repeat the inhalation and exhalation for at least 30 cycles twice daily.
   - After some weeks of daily practice, you should achieve an inhalation phase that lasts 2 to 3 seconds and an exhalation phase of 6 to 7 seconds, without strain.
   - Exhalation should always be slow and continuous; there is little value in breathing the air out in 2 seconds and then simply waiting until the count reaches 8 before inhaling again!
   - Feelings of anxiety should reduce with this exercise.
   - Practice twice daily, and repeat the exercise for a few minutes (6 cycles takes about 1 minute) every hour if anxious or when stress increases.
   - Practice on waking, before bedtime, and if possible, before meals.
3. Recitation of mantra or prayer
   - The respiratory (and cardiovascular) effects of rosary prayer ("Ave Maria" in Latin) and recitation of a yoga mantra were assessed.[47]
   - Results were similar, showing a slowing of respiration to approximately 6 counts per minute and synchronization of all cardiovascular rhythms (Traube–Hering–Mayer oscillations), representing blood pressure, heart rate, cardiac contractility, pulmonary blood flow, cerebral blood flow, and movement of cerebrospinal fluid.
   - This influence on autonomic activity, represented by the Traube–Hering–Mayer oscillations, is clearly health enhancing because it slows the respiratory rate to the level considered optimal in breathing rehabilitation.[1,7,12]

Breathing Rehabilitation Exercises

Box 135.1 describes three breathing rehabilitation exercises.

Chronic HVS/BPDs are commonly successfully treated. However, a time frame of 12 to 26 weeks may be required, with active patient participation throughout to break well-established habits.

Lum[1] reported that more than 1000 anxious and phobic patients were treated using breathing retraining, physical therapy, and relaxation. The results indicated the following:

- Symptoms were usually abolished in 1 to 6 months, with some younger patients requiring only a few weeks.
- At 12 months, 75% were free of all symptoms, 20% had only mild symptoms, and about 1 in 20 patients had intractable symptoms.

Breathing rehabilitation therapy was evaluated in patients with HVS; the diagnosis was based on the presence of several stress-related complaints and reproduced by voluntary hyperventilation.[49] Patients with organic diseases were excluded, and most patients met the criteria for an anxiety disorder.

Therapy was conducted in the following sequence:

- Brief, voluntary hyperventilation to reproduce the reported complaints
- Reattribution of the cause of the symptoms to hyperventilation
- Explaining the rationale of therapy involving a reduction of hyperventilation by acquiring an abdominal breathing pattern, with slowing down of expiration
- Breathing retraining for 2 to 3 months working with a physiotherapist

After breathing therapy, the sum scores of the Nijmegen Questionnaire were markedly reduced. A canonical correlation analysis relating the changes of the various complaints to the modifications of breathing variables showed that the improvement of the complaints was correlated mainly with the slowing down of breathing frequency.

SUMMARY

HVS/BPDs are a common disorder affecting up to 10% of patients in a general internal medicine practice. However, it is rarely recognized or appropriately treated. Breathing retraining is effective clinically and economically, as long as enough patience is exercised to actively engage the patient in a program requiring weeks to months of behavioral change. Acupuncture was also shown to improve symptoms of HVS, especially with anxiety as the etiology.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lum LC. Hyperventilation syndromes in medicine and psychiatry: a review. *J R Soc Med*. 1987;80(4):229–231.
2. Kern B, Rosh A, Byrd R, et al. *Hyperventilation Syndrome*; 2016. https://emedicine.medscape.com/article/807277-overview. Accessed January 15, 2018.
3. Damas-Mora J, Davies L, Taylor W, Jenner FA. Menstrual respiratory changes and symptoms. *Br J Psychiatry*. 1980;136:492–497.
4. Farrukh A, Ramsden GH. Hyperventilation with medroxyprogesterone therapy in a postmenopausal woman. *J Obstet Gynaecol*. 2007;27(3):323.
5. Chaitow L, Bradley D, Gilbert C. *Multidisciplinary Approaches to Breathing Pattern Disorders*. Edinburgh: Churchill Livingstone; 2002.
6. Jennett S. Control of breathing and its disorders. In: Timmons BH, Ley R, eds. *Behavioral and Psychological Approaches to Breathing Disorders*. New York: Plenum Press; 1994.
7. Timmons BH, Ley R, eds. *Behavioral and Psychological Approaches to Breathing Disorders*. New York: Plenum Press; 1994.
8. Ong JR, Hou SW, Shu HT, Chen HT, Chong CF. Diagnostic pitfall: carbon monoxide poisoning mimicking hyperventilation syndrome. *Am J Emerg Med*. 2005;23(7):903–904.
9. Tarulli AW, Lim C, Bui JD, Saper CB, Alexander MP. Central neurogenic hyperventilation: a case report and discussion of pathophysiology. *Arch Neurol*. 2005;62(10):1632–1634.
10. Brostoff J. *Complete Guide to Food Allergy*. London: Bloomsbury; 1993.
11. West JB. *Respiratory Physiology: The Essentials*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.
12. Lum LC. Hyperventilation and anxiety state. *J R Soc Med*. 1981;74(1):1–4.
13. Farmand M. Blood gas analysis and the fundamentals of acid-base balance. *Neonatal Netw*. 2009;28(2):125–128.
14. Bradley D. Physiotherapy breathing rehabilitation strategies. In: *Multidisciplinary Approaches to Breathing Pattern Disorders*. Edinburgh: Churchill Livingstone; 2002.
15. Han JN, Stegen K, Simkens K, et al. Unsteadiness of breathing in patients with hyperventilation syndrome and anxiety disorders. *Eur Respir J*. 1997;10(1):167–176.
16. Beck JG, Shipherd JC, Ohtake P. Do panic symptom profiles influence response to a hypoxic challenge in patients with panic disorder? A preliminary report. *Psychosom Med*. 2000;62(5):678–683.
17. Wilhelm FH, Trabert W, Roth WT. Characteristics of sighing in panic disorder. *Biol Psychiatry*. 2001;49(7):606–614.
18. Conway AV, Freeman LJ, Nixon PG. Hypnotic examination of trigger factors in the hyperventilation syndrome. *Am J Clin Hypn*. 1988;30(4):296–304.
19. Freeman LJ, Conway A, Nixon PG. Physiological responses to psychological challenge under hypnosis in patients considered to have the hyperventilation syndrome: implications for diagnosis and therapy. *J R Soc Med*. 1986;79(2):76–83.
20. Lum LC. Hyperventilation: the tip and the iceberg. *J Psychosom Res*. 1975;19(5–6):375–383.
21. Gardner WN. The pathophysiology of hyperventilation disorders. *Chest*. 1996;109(2):516–534.
22. Nixon PG. The grey area of effort syndrome and hyperventilation: from Thomas Lewis to today. *J R Coll Physicians Lond*. 1993;27(4):377–383.
23. DeGuire S, Gevirtz R, Kawahara Y, Maguire W. Hyperventilation syndrome and the assessment of treatment for functional cardiac symptoms. *Am J Cardiol*. 1992;70(6):673–677.
24. Calloway SP, Fonagy P. Aerophagia and irritable bowel syndrome. *Lancet*. 1985;2(8468):1368.
25. Kucewicz JC, Dunmire B, Giardino ND, et al. Tissue pulsatility imaging of cerebral vasoreactivity during hyperventilation. *Ultrasound Med Biol*. 2008;34(8):1200–1208.
26. Magarian GJ. Hyperventilation syndromes: infrequently recognized common expressions of anxiety and stress. *Medicine (Baltimore)*. 1982;61(4):219–236.
27. Fried R, Grimaldi J. The psychology and physiology of breathing. *Behavioral Medicine, Clinical Psychology, and Psychiatry*. New York: Plenum Press; 1993.
28. Teng YH, Tsai HT, Hsieh YS, et al. Elevated erythrocyte carbonic anhydrase activity is a novel clinical marker in hyperventilation syndrome. *Clin Chem Lab Med*. 2009;47(4):441–445.
29. Chambers JB, Kiff PJ, Gardner WN, Jackson G, Bass C. Value of measuring end tidal partial pressure of carbon dioxide as an adjunct to treadmill exercise testing. *Br Med J (Clin Res Ed)*. 1988;296(6632):1281–1285.
30. Warburton CJ, Jack S. Can you diagnose hyperventilation? *Chron Respir Dis*. 2006;3(3):113–115.
31. White KM, Quinn JM, Hagan LL, Johnson TL. Exercise-induced hyperventilation. *Ann Allergy Asthma Immunol*. 2008;100(2):171–172.
32. Nixon PG, Freeman LJ. The "think test": a further technique to elicit hyperventilation. *J R Soc Med*. 1988;81(5):277–279.
33. Courteney R. The Buteyko Method—an osteopathic approach to asthma? In. *Osteopathy Today*. 2002;August:16–19.
34. Bowler SD, Green A, Mitchell CA. Buteyko breathing techniques in asthma: a blinded randomised controlled trial. *Med J Aust*. 1998;169(11–12):575–578.
35. Buteyko K. *Buteyko Method; Experience of Application in Medical Practice*. Moscow: Patriot; 1990.
36. Vansteenkiste J, Rochette F, Demedts M. Diagnostic tests of hyperventilation syndrome. *Eur Respir J*. 1991;4(4):393–399.
37. van Dixhoorn J, Duivenvoorden HJ. Efficacy of Nijmegen Questionnaire in recognition of the hyperventilation syndrome. *J Psychosom Res*. 1985;29(2):199–206.
38. Garland W. *Somatic Changes in Hyperventilating Subject.* Saint Flour, France: International Society for the Advancement of Respiratory Psychophysiology Congress; 1994.
39. Snyder SH. Nitric oxide: first in a new class of neurotransmitters. *Science*. 1992;257(5069):494–496.
40. Beachey W. *Respiratory Care Anatomy and Physiology: Foundations for Clinical Practice*. St. Louis: Mosby; 1998.
41. Bradley D. *Hyperventilation Syndrome/Breathing Pattern Disorders*. Auckland, NZ: Tandem Press; 1998.
42. Gibson D, Bruton A, Lewith GT, Mullee M. Effects of acupuncture as a treatment for hyperventilation syndrome: a pilot, randomized crossover trial. *J Altern Complement Med*. 2007;13(1):39–46.
43. Cappo BM, Holmes DS. The utility of prolonged respiratory exhalation for reducing physiological and psychological arousal in non-threatening and threatening situations. *J Psychosom Res*. 1984;28(4):265–273.
44. Faling L. Controlled breathing techniques and chest physical therapy in chronic obstructive pulmonary disease. In: Casaburi R, ed. *Principles and Practices of Pulmonary Therapy*. Philadelphia: WB Saunders; 1993.
45. Tiep BL, Burns M, Kao D, Madison R, Herrera J. Pursed lips breathing training using ear oximetry. *Chest*. 1986;90(2):218–221.
46. Grossman P, de Swart JC, Defares PB. A controlled study of a breathing therapy for treatment of hyperventilation syndrome. *J Psychosom Res*. 1985;29(1):49–58.
47. Bernardi L, Sleight P, Bandinelli G, et al. Effect of rosary prayer and yoga mantras on autonomic cardiovascular rhythms: comparative study. *BMJ*. 2001;323(7327):1446–1449.
48. Gregory R, Gilles C, Aude A, et al. Effects of massage therapy on anxiety, depression, hyperventilation and quality of life in HIV infected patients: a randomized controlled trial. *Complement Ther Med*. 2017;32:109–114.
49. Han JN, Stegen K, De Valck C, Clément J, Van de Woestijne KP. Influence of breathing therapy on complaints, anxiety and breathing pattern in patients with hyperventilation syndrome and anxiety disorders. *J Psychosom Res*. 1996;41(5):481–493.

136

Immune System Support

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

INTRODUCTION

The immune system is a complex integration of synergistic segments that are continuously barraged by stimuli from both internal and external sources. Immunology continues to be a rapidly developing field in which mechanisms are constantly being conceptualized and revised. For the physician interested in assessing and maintaining a patient's health, the development of a thorough understanding of the clinical aspects of the immune system and the many factors that enhance and/or inhibit normal function is essential. The immune system is truly "holistic," as evidenced by the close association of psychological, neurological, nutritional, environmental, and hormonal factors with immune function.

Supporting the immune system is critical to good health. Conversely, good health is critical to supporting the immune system. The best approach to supporting immune function is a comprehensive plan involving lifestyle, stress management, exercise, diet, nutritional supplementation, and the use of plant-based medicines.

IMMUNE SYSTEM OVERVIEW

The immune system has two fundamental and complementary components: the innate and adaptive immune systems, with the division largely defined by the speed and specificity of the response. The innate branch of the immune system is generally defined by a consistent response; however, when an antigen is encountered, the immune response includes phagocytic cells such as neutrophils, monocytes, and macrophages, as well as the various physical and chemical barriers to infection. The adaptive immune system, however, improves upon repeated exposure to a given infection because these cells develop a memory of past encounters. The adaptive immune system is composed of B (humoral) and T (cell-mediated) cells that work together to create antibodies specific to an invading organism.

Macrophages and dendritic cells, often referred to as antigen-presenting cells (APCs), are critical for the development of naïve T cells into either type 1 helper T (Th1) cells or type 2 helper (Th2) cells. After ingesting the pathogen, the macrophage will display the antigenic signature of the pathogen and send a chemical messenger, interleukin (IL)-1, to notify the T-helper cells (that have CD4 phenotype markings) of the pathogens' presence. From there, they will develop into Th1 and Th2 cells (based on the cytokine they produce). The cytokines produced by T-helper cells modulate other T-helper-type activities (Fig. 136.1). For example, Th1 produces interferon (IFN)-γ, tumor necrosis factor (TNF)-β, IL-2, IL-10, and lymphotoxin, which then stimulates the cell-mediated immune responses against viral and bacterial pathogens and tumor cells through activation of macrophages and cytotoxic T-cells. IFN-γ, among its other actions, activates macrophages and stimulates NK cell and lymphokine-activated killer activities. Th2 produces IL-4, IL-5, IL-10, IL-13, IL-21, and IL-31, which stimulate B cells to produce antibodies immunoglobulin (Ig)M, IgG1, IgA, and IgE. Type 3 helper T (Th3) cells help to maintain homeostasis between Th1 and Th2 cells, and type 17 helper T (Th17) cells appear to downregulate the production of both Th1 and Th2 cytokines.

PSYCHONEUROIMMUNOLOGY

Psychoneuroimmunology is the term used to describe the interactions between the emotional state, nervous system function, and the immune system.[1] Investigations into these interactions have documented that the mind and attitude play a significant role in the functioning of the immune system. However, a complete and detailed account of the many facets of psychoneuroimmunology, or behavioral immunology, is beyond the scope of this chapter. An overview of the role of attitude and emotions in immune function is discussed in Chapter 7, and the importance of stress management to a healthy immune system is discussed in Chapter 140.

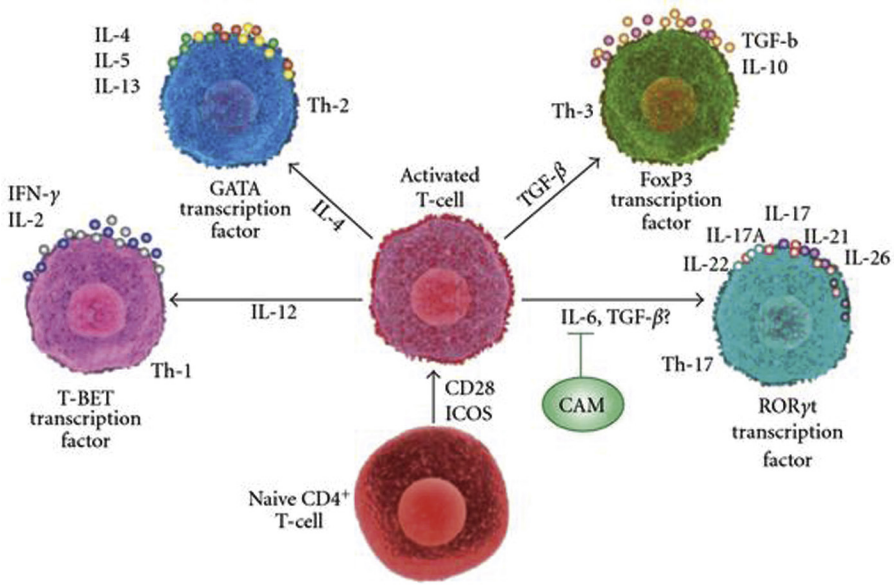


Fig. 136.1 General scheme of differentiation of T-helper cells. Naive CD4+ T cells, after activation by T-cell receptor and costimulatory molecules, such as CD28 and inducible T-cell costimulatory (ICOS), can differentiate into four effector T-helper cells: Th1, Th2, Th3, or Th17 cells. These cells produce different cytokines, which have specialized immunoregulatory functions. Interferon (IFN) produced by Th1 cells is important in the regulation of antigen presentation and cellular immunity. Interleukin (IL)-4, IL-5, and IL-13 produced by Th2 cells regulate B-cell responses, important mediators of allergic diseases. Transforming growth factor (TGF) and IL-10 are produced by Th3 cells to regulate Th1 and Th2 cells. Th17 cells regulate inflammatory responses by expressing IL-17, IL-21, IL-22, and IL-26. Complementary and alternative medicine (CAM) protocols can be implemented to reduce the level of proinflammatory cytokine IL-6, thereby inhibiting the conversion of activated T cells into pathogenic Th17 cells. (From Vojdani A, Lambert J. The role of Th17 in neuroimmune disorders: target for CAM therapy. Part I. *Evid.-Based Complem. Altern. Med.* 2011: 927294.)

Many clinical and experimental studies have clearly demonstrated that stress, personality, attitude, and emotion are etiological or contributory in suppressing the immune system as well as leading to the development of many diseases.[1] Reaction to stressful stimuli is entirely individual, reinforcing the fact that people differ significantly in their perceptions of and responses to various life events. The variations in response help account for the wide diversity of stress-induced illnesses. Stress-induced increases in corticosteroid and catecholamine levels lead to an immunosuppressed state, leaving the host susceptible to infectious and carcinogenic illnesses. This immunosuppression is proportional to the level of stress, and although the effects are numerous, they appear to involve a common mechanism: an increase in glucocorticoids, proinflammatory cytokines, and catecholamines resulting in significant alterations in hypothalamic–pituitary–adrenal (HPA) and the sympathetic-adrenal medullary axes, leukocyte function, thymic involution, and suppressed lymphopoiesis. More than 150 clinical studies have shown that stress can alter immune function and contribute to the development of significant disease and poor health.[1–3]

Lymphocytes, monocytes or macrophages, and granulocytes have receptor sites for the many regulating hormones and neurotransmitters of the HPA and sympathetic-adrenal medullary axes. Alterations in these compounds lead to disruption of cellular trafficking, proliferation, cytokine secretion, antibody production, and cytolytic activity. For example, glucocorticoids inhibit the production of IL-12, IFN-γ, IFN-α, and TNF-α by antigen-presenting cells and Th1 cells, but upregulate the production of IL-4, IL-10, and IL-13 by Th2 cells. This mechanism systemically causes a selective suppression of the Th1–cellular immunity axis and a shift toward Th2-mediated humoral immunity, rather than generalized immunosuppression. During an immune response and inflammation, the activation of the stress system, and thus increased levels of systemic glucocorticoids through induction of a Th2 shift, may protect the organism from systemic "overshooting" with Th1 proinflammatory cytokines and other products of activated macrophages with tissue-damaging potential. However, conditions associated with significant changes of glucocorticoids, such as acute or chronic stress or cessation of chronic stress, severe exercise, pregnancy, and the postpartum state, through modulation of the Th1–Th2 balance, may affect the susceptibility to or the course of infections as well as autoimmune and atopic and/or allergic diseases.[4,5] A study in medical students taking examinations found that psychological stress produced a shift in the cytokine balance toward a Th2 profile.[6] The data showed decreased synthesis of Th1 cytokines, including IFN-γ, and increased production of Th2 cytokines, including IL-10. Therefore a stress-induced decrease of Th1 cytokines results in dysregulation of cell-mediated immune responses.

To help demonstrate causal relations between psychosocial stressors and the development of infectious illness under well-controlled conditions, investigators inoculated subjects with several different types of vaccines to demonstrate clinically relevant alterations in the immunological response to challenge. The chronic stress associated with caring for a spouse with Alzheimer's disease or, for younger people, experiencing stressful life events was associated with a poorer antibody response to an influenza virus vaccine than in well-matched control subjects.[7,8] The premise was that the production of a delayed, weaker, and shorter-lived immune response to a vaccine would be analogous to impaired immune responses to other pathogens. Consistent with this concept, subjects who showed poorer responses to vaccines also experienced higher rates of clinical illness as well as longer-lasting infectious episodes. Healthy infants with a higher cortisol response to pain were shown to have differences in T-cell concentrations and a lower

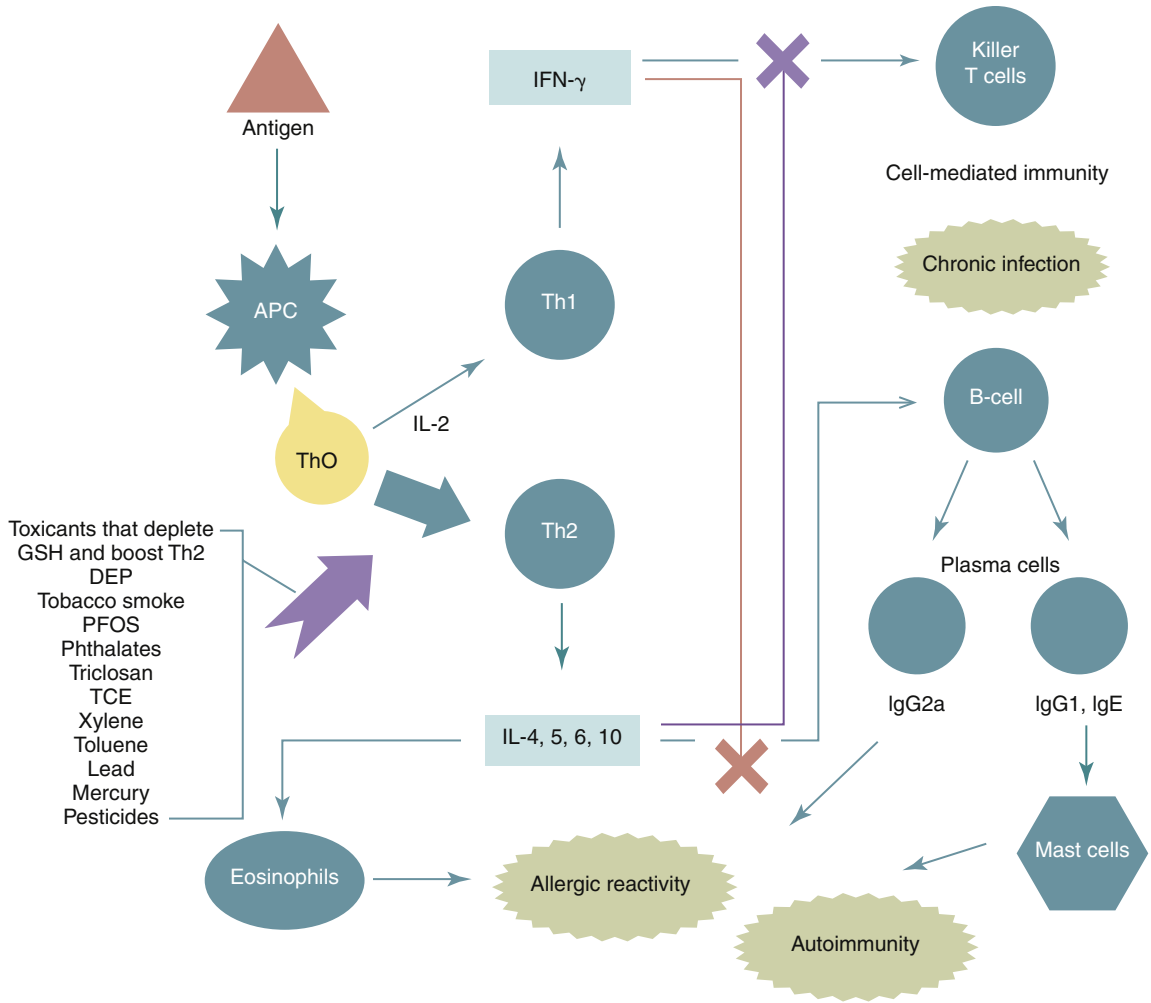


Fig. 136.2 Many immune-toxic pollutants reduce glutathione levels leading to immune system dysregulation increasing the risk for infections, allergies, autoimmunity, and cancer.

delayed-type hypersensitivity to vaccination, with sex differences in the immune system seen as early as 6 weeks of age.[9]

Fortunately, the effects of stress on the immune system can be attenuated or even overcome with positive mood, effective stress reduction techniques, humor, laughter, and guided imagery.[1,10]

ENVIRONMENTAL TOXINS AND IMMUNE FUNCTION

Toxic compounds exert several different effects, usually simultaneously, that cause complex dysfunction to the immune system. Toxins often deplete the glutathione (GSH) content in APCs, thereby affecting whether these cells will be Th1 or Th2 dominant.[11] Low GSH levels lead to an increase in Th2 dominance, whereas higher GSH levels promote Th1 immune response.[12] Many immune-toxic pollutants reduce glutathione levels, including mercury, lead, perfluorocarbons, phthalates, solvents, polychlorinated biphenyl (PCBs), chlorinated pesticides, organophosphate pesticides, particulate matter, tobacco smoke, and diesel exhaust particles (Fig. 136.2).[13] A diminished Th1 response will reduce the ability to respond to bacterial, viral, and fungal invasion, typically resulting in chronic infections.

In an animal model, the consumption of PCB containing fat resulted in diminished mitogen response, decreased phagocytosis, diminished numbers of CD8+ cells, and thymic atrophy.[14–16] Mobile home dwellers exposed to formaldehyde were found to have lower T and B cells and diminished mitogen response to phytohemagglutinin (PHA).[17] Mold exposure has also been shown to reduce mitogen responsiveness in humans.[18]

LIFESTYLE EFFECTS ON IMMUNE FUNCTION

A healthy lifestyle goes a long way in establishing a healthy immune system. This benefit is perhaps most obvious when one looks at the effects of lifestyle on natural killer cell activity.[19–21] Box 136.1 lists the lifestyle practices associated with higher natural killer cell activity. One lifestyle factor that is critical to healthy immune function is adequate sleep. In healthy humans, sleep deprivation has consistently been demonstrated to impair different parameters of immune function and mood. Interestingly, the deterioration of immune function precedes the plummeting of subjective well-being and psychosocial performance in sleep-deprived subjects.[22]

NUTRITIONAL FACTORS AFFECTING IMMUNE FUNCTION

The health of the immune system is greatly affected by a person's nutritional status. Dietary factors that depress immune function include nutrient deficiency, excess consumption of sugar, consumption of allergenic foods, and high cholesterol levels in the blood. Dietary factors that enhance immune function include all essential nutrients, antioxidants, carotenes, and flavonoids.

Consistent with good health, optimal immune function requires a healthy diet that has the following characteristics:

BOX 136.1 Lifestyle Practices Associated With Higher Natural Killer Cell Activity

- Not smoking
- Increased intake of green vegetables
- Regular meals
- Proper body weight
- More than 7 hours of sleep per night
- Regular exercise
- A vegetarian diet

- Is rich in whole, natural foods, such as fruits, vegetables, grains, beans, seeds, and nuts
- Is low in fats and refined sugars
- Contains adequate, but not excessive, amounts of protein

In addition, individuals are encouraged to drink five or six 8-ounce glasses of water (preferably pure) per day. These dietary recommendations, along with a positive mental attitude, a good high-potency multivitamin and mineral supplement, a regular exercise program, deep-breathing and relaxation exercises (meditation, prayer, etc.), and at least 7 hours of sleep daily, will go a long way in helping the immune system function at an optimum level.

Nutrient Deficiency

Nutrient deficiency is the most common cause of a depressed immune system. Although research relating nutritional status to immune function has historically concerned itself with severe malnutrition states (i.e., kwashiorkor and marasmus), attention is shifting toward marginal deficiencies of single or multiple nutrients and the effects of overnutrition. The plethora of clinical and experimental data has concluded that a single nutrient deficiency can profoundly impair the immune system.

Given the widespread problem of subclinical nutrient deficiency in Americans, it is likely that many have impaired immunity amenable to nutritional supplementation. This statement is particularly true in the elderly. Numerous studies have shown that most elderly Americans are deficient in at least one nutrient, and studies show that taking a multivitamin and mineral supplement enhances immune function in elderly subjects (whether they have an overt nutritional deficiency or not).[23–25] These findings have considerable fundamental, clinical, and public health significance.

Protein

The importance of adequate protein intake to proper immune function has been extensively studied.[10] Although all facets of immune function are ultimately affected, the most severe effects of protein–calorie malnutrition (PCM) are on cell-mediated immunity. PCM is associated with multiple nutrient deficiencies, and some immune dysfunctions attributed to PCM are likely the result of these deficits. Partial deficiencies of dietary vitamins produce a comparatively greater depression in the natural and inducible levels of cytotoxic activities than do partial protein deficiencies. Nonetheless, adequate protein is essential for optimal immune function.

Sugar

The oral administration of 100-g portions of carbohydrate as glucose, fructose, sucrose, honey, or orange juice significantly reduces neutrophil phagocytosis, but starch has no effect. As can be seen in Fig. 136.3, effects start within 30 minutes, last for more than 5 hours, and typically show a 50% reduction in phagocytic activity at the peak of inhibition (usually 2 hours after ingestion).[26,27] Because polymorphonuclear leukocytes (PMNs) constitute 60% to 70% of the total white blood cells (WBCs) and are a major portion of the defense mechanism, impairment of phagocytic activity leads to an immune-compromised state. Oral administration of increasing amounts of glucose progressively lowers neutrophil phagocytosis, with maximal inhibition corresponding to maximal blood glucose levels.

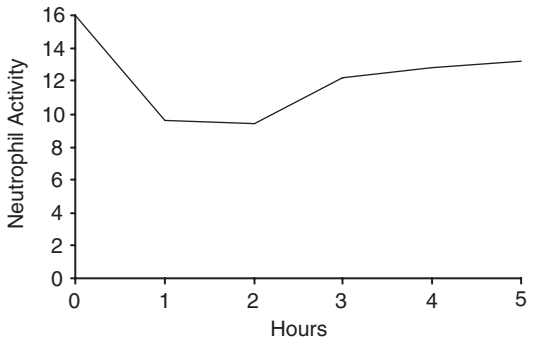


Fig. 136.3 The effects of sugar on white blood cell phagocytic activity.

In addition, oral ingestion of 75 g of glucose has been shown to depress lymphocyte response to mitogens, apparently through the elevation of insulin levels.[28] Other parameters of immune function are also undoubtedly affected by sugar consumption.

It has been hypothesized that the ill effects of high glucose levels result from the elevation of insulin values and competition with vitamin C for membrane transport sites.[29,30] This hypothesis is based on evidence that vitamin C and glucose appear to have opposite effects on immunological function and the fact that both substances require insulin for membrane transport into many tissues.

Considering that the average American consumes 125 g of sucrose, plus 50 g of other refined simple sugars each day, the conclusion that most Americans have chronically depressed immune systems is inescapable. It is clear, particularly during an infection, that the consumption of simple sugars, even in the form of fruit juice, is deleterious to the host's immune status.

Short-term fasting could be encouraged, particularly during the first 24 to 48 hours of an acute infectious illness, because it results in a significant (up to 50%) increase in phagocytic index.[26] The fast should not be continued for a long period; over time, the leukocytes' energy sources will become depleted.

Obesity

Obesity is associated with decreased immune status, as evidenced by reduced bactericidal activity of leukocytes, and higher morbidity and mortality from infections as well as from certain cancers in obese individuals.[31] Obesity also increases the risk of asthma, atopic, and autoimmune diseases by inducing decreased immunological tolerance as a consequence of immunological changes induced by adipokines (e.g., leptin and adiponectin) and cytokines (e.g., IL-6 and TNF-α) secreted by white adipose tissue.[32] In addition, cholesterol and lipid values are usually elevated in obese individuals, which contribute to impaired immune function.

Blood Lipids

Increased blood levels of cholesterol, free fatty acids, triglycerides, and bile acids inhibit various immune functions, including the following[33,34]:

- Lymphoproliferation
- Response to mitogens
- Antibody response
- PMN chemotaxis
- Phagocytosis

Therefore optimal immune function depends on control of these serum components. Interestingly, L-carnitine, even at minimal concentrations, has been shown to neutralize lipid-induced immunosuppression.[35] This effect is probably due to carnitine's role as a rate-limiting factor in the removal of fat emulsion from the blood.[36]

Alcohol

Alcohol increases the susceptibility to experimental infections in animals, and alcoholics are known to be more susceptible to infections, especially pneumonia. Studies of immune function in alcoholism showed a profound depression in most parameters of immunity.[37]

Vitamins

Vitamin A and Carotenes

Vitamin A plays an essential role in maintaining the integrity and secretions of the epithelial and mucosal surfaces. These systems constitute a primary nonspecific host defense mechanism. Vitamin A has been shown to stimulate and/or enhance numerous immune processes, including the following:

- Induction of cell-mediated cytotoxicity against tumors
- Natural killer cell activity
- Lymphocyte blastogenesis
- Mononuclear phagocytosis
- Antibody response

These effects are not simply the result of the reversal of vitamin A deficiency because many of them are further enhanced by the administration of (supposedly) excessive levels of vitamin A.[25,38] In addition, vitamin A prevents and reverses stress-induced thymic involution, and additional vitamin A can promote thymus growth.[39]

Carotenes have also demonstrated several immune-enhancing effects. In addition to being converted into vitamin A, carotenes function as antioxidants. Because the thymus gland is susceptible to damage by free radicals, β-carotene may be more advantageous in enhancing the immune system than retinol. For more information, see Chapter 125.

Vitamin C

Vitamin C (ascorbic acid) plays an important role in the natural approach to immune enhancement. Although vitamin C has been shown to be antiviral and antibacterial, its main effect is via improvement in host resistance. Many immunostimulatory effects have been demonstrated including enhancing lymphoproliferative response to mitogens and lymphotropic activity, as well as increasing INF levels, antibody responses, immunoglobulin levels, secretion of thymic hormones, and integrity of ground substance.[25,40] Vitamin C also has direct biochemical effects similar to those of INF.[41]

Numerous clinical studies support the use of vitamin C in the treatment of infectious conditions. In addition to its effects on the common cold, vitamin C has also been shown to be useful in other infectious conditions.[42] Vitamin C levels are quickly depleted during the stress of an infection as well as in chronic disease.[43]

It is useful to supplement vitamin C concurrently with flavonoids, which raise the concentration of vitamin C in some tissues and potentiate its effects as well as exert their own effects.[44]

Vitamin D

The importance of vitamin D on the regulation of cells of the immune system has gained increased appreciation with the discovery of the vitamin D receptor and key vitamin D metabolizing enzymes expressed by cells of the immune system. Effective in supporting both innate and adaptive immunity,[45–49] vitamin D has been shown to do the following:

- Upregulate antimicrobial peptides, namely cathelicidin, to enhance clearance of bacteria at various barrier sites and in immune cells
- Modulate the adaptive immune system by direct effects on T-cell activation and on the phenotype and function of APCs
- Protect against the development of autoimmune diseases (e.g., Crohn's disease, juvenile diabetes mellitus, multiple sclerosis, asthma, and rheumatoid arthritis)
- Reduce the frequency of viral upper respiratory infections

Vitamin D appears to be especially important in protection against viral or bacterial upper respiratory infection.[50] In Mongolian children with vitamin D deficiency who received milk fortified with 300 IU of vitamin D_3, supplementation significantly reduced the risk of acute respiratory infections in winter.[51] Additionally, 1200 IU/day of vitamin D_3 supplementation during the winter may reduce the incidence of influenza A and enhance innate immunity by upregulating antimicrobial peptides, especially in specific subgroups of schoolchildren.[52] In addition, weekly supplementation with 10,000 IU of vitamin D_3 is preventive for upper respiratory tract infections in young adults.[53] A meta-analysis that considered 25 eligible randomized controlled trials found that vitamin D supplementation is safe and protects against acute respiratory tract infection overall, and patients who are very deficient in vitamin D and those not receiving bolus doses experienced the most benefit.[54]

Vitamin E

Vitamin E enhances both humoral immunity and cell-mediated immunity. Deficiency in vitamin E results in lymphoid atrophy and decreases of lymphoproliferative response to mitogens, splenic plaque-forming colonies, antibody response, and monocyte function. Vitamin E supplementation (30–150 international units [IU]) has been shown to do the following[55]:

- Increase lymphoproliferative response to mitogens
- Prevent free-radical–induced thymus atrophy
- Enhance helper T-cell activity
- Increase splenic plaque-forming colonies, serum immunoglobulins, antibody response, PMN phagocytosis, and reticuloendothelial system activity

Elderly subjects may benefit from even higher dosages of vitamin E. One study sought to determine the effect of vitamin E supplementation at different dosages on immune function in 88 patients older than 65 years.[56] The researchers measured T-cell function by assessing delayed-type hypersensitivity skin response; antibody response to hepatitis B, tetanus, diphtheria, and pneumococcal vaccines; and autoantibodies to DNA and thyroglobulin to determine the effect of vitamin E on immune function. Vitamin E was given at 60, 200, or 800 IU for 235 days. Although the placebo group experienced an 8% increase in delayed-type hypersensitivity, the 60-IU group had a 20% increase, the 200-IU group had a 58% increase, and the 800-IU group had a 65% increase. Regarding antibody production, the best results were observed in the patients who received 200 IU daily. No effect on autoimmune antibodies was noticed. No adverse effects were observed at any of the three dosage schedules of vitamin E.

In another double-blind study of 451 elderly participants in a nursing home, vitamin E supplementation (200 IU/day) demonstrated a protective effect against upper respiratory tract infections, particularly the common cold.[57]

Pyridoxine

A pyridoxine deficiency results in depression of cellular and humoral immunity, lymphoid tissue atrophy, leukopenia, reduction in quantity and quality of antibody production, diminished lymphoproliferative response to mitogens, and decreased thymic hormone activity.[25]

Factors predisposing to deficiency are low dietary pyridoxine intake, excess protein intake, consumption of hydralazine (yellow) dyes, and use of alcohol and oral contraception.

Folic Acid and Vitamin B_{12}

The megaloblastic state induced by a deficiency of vitamin B_{12} and/or folate results in improper WBC production and abnormal lymphocyte responses. Folic acid deficiency (the most common vitamin deficiency in the United States) has been shown to result in lymphoid atrophy and decreased lymphoproliferative response to mitogens, splenic plaque-forming colonies, and antibody production. A B_{12} deficiency, besides producing a deficiency in folate conversion to its active tetrahydrofolate form, leads to impairment of PMN phagocytosis and bactericidal action.[25]

Other B Vitamins

Deficiencies of thiamin, riboflavin, and pantothenic acid lead to reduced antibody response, decreased splenic plaque-forming colonies, and lymphoid atrophy.

Minerals

Iron

Iron deficiency is a commonly encountered isolated nutritional deficiency causing immune dysfunction in large numbers of patients. Marginal iron deficiency, even at levels that do not lower hemoglobin values, can influence the immune system. Lymphoid tissue atrophy, decreased lymphoproliferative response to mitogens, defective macrophage and neutrophil function, and decreased T-cell/B-cell ratios are common experimental and clinical findings.[25]

Iron is an important nutrient for bacteria as well as for humans. During infection, one of the body's nonspecific defense mechanisms to limit bacterial growth is to reduce plasma iron, and in vitro studies have shown that the bacteriostatic effects and some of the bactericidal effects of serum are eliminated by the addition of iron to the serum.[58] As temperature rises, plasma iron levels drop, and when temperature is raised to fever levels, the growth of bacteria is inhibited, but not at high iron concentrations.

These observations suggest that iron supplementation may be contraindicated during acute infection, especially in patients with low transferrin levels. However, in patients with impaired immune function, chronic infections, and subnormal iron levels, adequate supplementation is essential.

Trace Minerals

Trace minerals function primarily as activators of enzyme–metal–substrate complexes in which they are loosely bound cofactors. The role of these elements in metalloenzymes is either structural, in which they influence the reactivity of the protein by stabilizing strained configurations of binding ligands about the metal atom, or catalytic, in which they act as centers of positive charge.

Zinc

Acrodermatitis enteropathica, a hereditary zinc-deficiency disease, offers an excellent model for understanding the role of zinc in immune function. In acrodermatitis enteropathica, the number of T cells is reduced, lymphoproliferative response to mitogens is diminished; thymic hormone levels are lower; delayed cutaneous hypersensitivity is decreased; and PMN phagocytosis, chemotaxis, and cytotoxic activities are impaired. All these effects are reversible upon adequate administration and absorption of zinc.[59]

Zinc serves a vital role in many immune system reactions, For example, it promotes the binding of complement (C1q) to immune complex, acts as a protectant against iron-catalyzed damage by free radicals, acts synergistically with vitamin A, is required for lymphocyte transformation, acts independently on lymphocytes as a mitogen, and is a necessary cofactor in activating serum thymic factor. In vitro, zinc inhibits the growth of several viruses, including rhinoviruses, picornaviruses, togaviruses, herpes simplex virus, and vaccinia virus.[60]

Adequate zinc nutrition is particularly important in the elderly, and zinc supplementation in elderly subjects results in increased numbers of T cells and enhanced cell-mediated immune responses.[60]

Throat lozenges containing zinc became popular in the treatment of the common cold because of a double-blind clinical trial in 1984 demonstrating that zinc-containing lozenges significantly reduced the average duration of common colds by 7 days.[61] The lozenges used in this study contained 23 mg of elemental zinc, which the patients were instructed to dissolve in their mouths every 2 waking hours after an initial double dose. After 7 days, 86% of the 37 zinc-treated subjects were symptom-free compared with 46% of the 28 placebo-treated subjects. Additional studies confirmed these results.[62] Meta-analyses demonstrated that zinc may shorten the duration of colds by approximately 33%, and patients with a common cold may be instructed to try zinc within 24 hours of the onset of symptoms.[63] Zinc gluconate lozenges were found to be as effective as zinc acetate lozenges. No evidence supports that zinc doses more than 100 mg/day might lead to greater efficacy in the treatment of the common cold. Because high doses of zinc can impair immune function, a daily intake higher than 150 mg for longer than 1 week cannot be recommended.

Selenium

Selenium plays a vital role in glutathione peroxidase and affects all components of the immune system, including the development and expression of all WBCs. Selenium deficiency results in depression of immune function, and selenium supplementation results in augmentation and/or restoration of immune functions. Selenium deficiency has been found to inhibit resistance to infection through impairment of WBC and thymus function, whereas selenium supplementation (100–200 mcg/day) has been shown to stimulate WBCs and thymus function.[64–67]

The ability of selenium supplementation to enhance immune function goes beyond simply restoring selenium levels. For example, in one study, selenium supplementation (200 mcg/day) to individuals with normal blood selenium concentrations resulted in a 118% improvement in the ability of lymphocytes to kill tumor cells and an 82.3% rise in the activity of natural killer cells.[65] These effects were related to the ability of selenium to enhance the expression of the immune-enhancing compound IL-2 and, consequently, the rate of WBC proliferation and differentiation into forms capable of killing tumor cells and microorganisms. The supplementation regimen did not produce significant changes in the blood selenium levels of the participants. The results indicated that the immune-enhancing effects of selenium in humans require supplementation above the normal dietary intake.

ENHANCING THYMUS FUNCTION

Perhaps the most effective method of reestablishing a healthy immune system is employing measures to improve thymus function. Promoting optimal thymus gland activity involves the following:

- Prevention of thymic involution or shrinkage by ensuring adequate dietary intake of antioxidant nutrients
- Use of nutrients that are required in the manufacture or action of thymic hormones

Antioxidants

The thymus gland shows maximum development immediately after birth. During the aging process, the thymus gland undergoes a process of shrinkage, or involution. Involution occurs because the thymus gland is extremely susceptible to free-radical and oxidative damage caused by stress, radiation, infection, and chronic illness.

Many patients with impaired immune function as well as conditions associated with impaired immunity (e.g., chronic fatigue syndrome, cancer, acquired immunodeficiency syndrome) experience a state of oxidative imbalance characterized by a greater number of prooxidants than antioxidants. This situation is quite detrimental to thymus function. One primary way that antioxidants affect the immune system, particularly cell-mediated immunity, may be by protecting the thymus gland from damage. The antioxidant nutrients most important for protecting the thymus include the carotenes, vitamin C, vitamin E, zinc, and selenium.

Nutrients

Many nutrients function as important cofactors in the manufacture, secretion, and function of thymic hormones. A deficiency in any one of these nutrients results in decreased thymic hormone action and impaired immune function. Zinc, vitamin B_6, and vitamin C are perhaps the most critical. Supplementation with these nutrients has been shown to improve thymic hormone function and cell-mediated immunity.

Zinc may be the critical mineral involved in thymus gland function and thymus hormone action. Zinc is involved in virtually every aspect of immunity. When zinc levels are low, the number of T cells is reduced, thymic hormone levels are lower, and many WBC functions critical to the immune response are severely lacking. All these effects are reversible with adequate administration and absorption of zinc.[68,69]

BOTANICALS

Many herbs have been shown to have antibacterial, antiviral, and immunostimulatory effects. A complete discussion is outside the scope of this chapter, but several immune-enhancing botanicals (e.g., *Echinacea, Hydrastis canadensis,* and *Panax ginseng*) are discussed in depth in Section 5.

Astragalus membranaceus is a traditional Chinese medicine used for viral infections. As with Echinacea, the polysaccharides contained in the root of *Astragalus membranaceus* contribute to the immune-enhancing effects. Clinical studies in China have shown it to be effective when used prophylactically against the common cold.[70] It has also been shown to reduce the duration and severity of symptoms in the acute treatment of the common cold as well as to raise WBC counts in persons with chronic leukopenia. Research in animals showed that *Astragalus* works by stimulating several factors of the immune system, including enhancing the phagocytic activity of monocytes and macrophages, increasing INF production and natural killer cell activity, improving T-cell activity, and potentiating other antiviral mechanisms.[70,71] *Astragalus* may be particularly useful in cases in which the immune system has been damaged by chemicals or radiation. In immuno-depressed mice, *Astragalus* was found to reverse the T-cell abnormalities caused by cyclophosphamide, radiation, and aging.[72]

Yeast Beta-Glucans and Medicinal Mushrooms

Extracts and preparations of Baker's yeast and medicinal mushrooms like maitake (*Grifola frondosa*), shiitake (*Lentinus edodes*), reishi (*Ganoderma lucidum*), and *Cordyceps sinensis* possess significant immune-enhancing effects. Much of the immune-enhancing activity is due to the presence of β-glucans. Numerous in vitro, in vivo, and clinical studies showed that yeast and fungal β-glucans activate WBCs by binding to receptors like dectin-1 on the outer membranes of neutrophils, macrophages, natural killer cells, and cytotoxic T cells.[73,74] Similar to a key in a lock, the binding of β-glucan to cellular receptors flips WBCs on and triggers a chain reaction leading to increased immune activity. In addition to increasing the ability of neutrophils and macrophages to engulf and destroy microbes, cancer cells, and other foreign cells, the binding stimulates the production of important signaling proteins such as IL-1, IL-2, and lymphokines. These immune activators ramp up defenses by activating immune cells.

One of the best-researched β-glucan sources is Wellmune WGP, a whole glucan particle composed of 1,3-1,6-β-glucan derived from the cell walls of a highly purified, proprietary baker's yeast *(Saccharomyces cerevisiae).* Once absorbed, Wellmune is taken up by macrophages, digested into smaller fragments, and slowly released over several days. The fragments bind to neutrophils, via complement receptor 3, and enhance their activity. Double-blind clinical studies have been conducted with Wellmune WGP, demonstrating positive results in reducing the signs, symptoms, frequency, and duration of upper respiratory infections. In a study involving marathon runners, Wellmune WGP significantly reduced symptoms of upper respiratory tract infection (e.g., sore throat, stuffy nose) in test subjects.[75] Furthermore, the Wellmune group reported 22% higher scores in vigor, a 48% reduction in fatigue, a 38% reduction in tension, and a 38% reduction in confusion over the control groups.

In a double-blind study during the cold and flu season, compared with the placebo group, the Wellmune WGP group reported (1) no incidence of fever compared with 3.5 incidences over a 90-day period; (2) no need to take a "sick day" from work or school, compared with 1.38 days of work/school missed for the placebo group; and (3) an increase in general health, including physical energy and emotional well-being.[76]

In a study of 122 healthy volunteers, participants taking 250 mg of Wellmune WGP daily for 12 weeks reported a 58% reduction in upper respiratory tract infection symptoms compared with individuals taking a placebo.[77] These subjects also experienced improvement in energy levels compared with the placebo group.

Microbiota

Bacterial colonization of the intestine is critical for the normal function of the human immune system. The specific molecules produced by commensal bacteria that contribute to the modulation of the host immune system are largely uncharacterized. However, polysaccharide A (PSA), produced by the human commensal *Bacteroides fragilis,* is a model symbiosis factor. PSA is capable of activating T-cell–dependent immune responses that can affect the development and homeostasis of the host immune system and also has a critical influence on the stimulation of IL-10–producing CD4+ T cells.[78] Consequently, PSA confers benefit to the host with regard to autoimmune, inflammatory, and infectious diseases. The effect of the microbiota composition on systemic immune-mediated diseases is just beginning to be appreciated. However, there is strong evidence for a functional link between the composition of the intestinal microbiota and susceptibility to several systemic immune disorders, such as type 1 diabetes, rheumatoid arthritis, and allergic diseases (Fig. 136.4).[79]

THERAPEUTIC APPROACH

A major challenge to the discerning clinician is determining which of the preceding factors is key to reactivating or supporting a patient's immune system. The regimen presented here is meant as a general approach and must be tailored to the patient's specific needs to maximize the desired effects and limit unnecessary treatment.

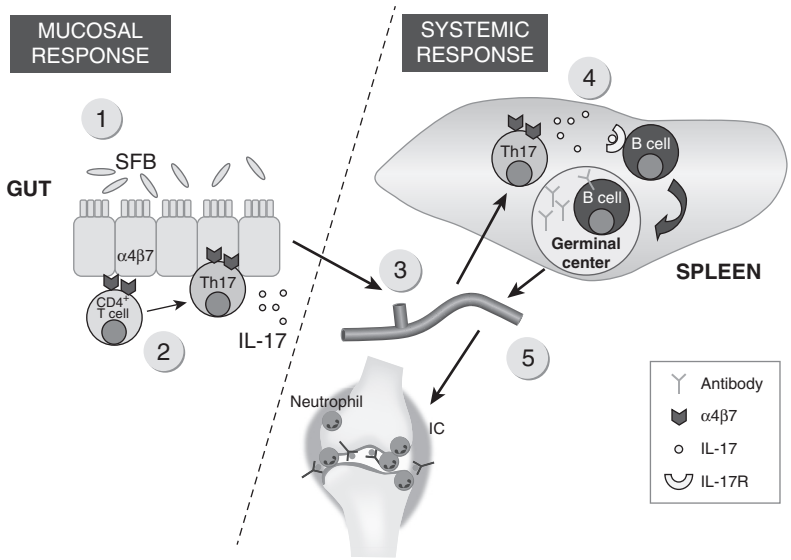


Fig. 136.4 An autoimmune arthritis model that demonstrates the link between gut microbiota and extraintestinal disease. *SFB,* Segmented filamentous bacteria The K/BxN arthritis model was used to demonstrate how the gut microbiota can influence a non–gut-associated disease. K/BxN mice express a transgene-encoded T-cell receptor that reacts to a self-peptide. Colonization of SFB in the gut induces the differentiation of Th17 cells (step 1 and 2), which subsequently exit the gut and migrate into the peripheral lymphoid tissue. The gut origin of Th17 cells can be identified by their expression of the α4β7 receptor, imprinted on these T cells by intestinal-mucosa-associated DCs (step 3). Interleukin (IL)-17, in turn, acts directly on B cells to provide help in the differentiation of germinal center B cells and the production of autoantibody in the spleen (step 4). The autoantibody then circulates into its target organ joints, which ultimately leads to the development of disease. (From Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes.* 2012;3[1]:4–14. PubMed PMID: 22356853.)

General Measures

- Get sufficient rest (bed rest is better).
- Drink a large amount of fluids (preferably diluted vegetable juices, soups, and herb teas).
- Limit simple sugar consumption (including fruit sugars) to less than 50 g/day.

Supplements

- High-potency multivitamin and mineral formula:
  - Vitamin C, 500 to 1000 mg every 2 hours
  - Bioflavonoids, 1000 mg/day
  - Vitamin A, 2500 IU/day (if patient has genetics to convert to vitamin A: β-carotene 25,000 IU/day can be used as an alternative)
  - Vitamin D_3, 2000 IU/day
  - Zinc, 30 mg/day

Botanicals

All doses of botanicals should be given three times per day.

Echinacea spp

- Dried root (or as tea), 0.5 to 1 g
  - Freeze-dried plant, 325 to 650 mg
  - Juice of aerial portion of *Echinacea purpurea* stabilized in 22% ethanol, 2 to 3 mL
  - Tincture (1:5), 2 to 4 mL
  - Fluid extract (1:1), 2 to 4 mL
  - Solid (dry powdered) extract (6.5:1 or 3.5% echinacoside), 150 to 300 mg

Astragalus membranaceus

- Dried root (or as decoction), 1 to 2 g
  - Tincture (1:5), 2 to 4 mL
  - Fluid extract (1:1), 2 to 4 mL
  - Solid (dry powdered) extract (0.5% 4-hydroxy-3-methoxy isoflavone), 100 to 150 mg

Yeast and Medicinal Mushrooms

The dosage for Wellmune WGP used in clinical trials was 250 mg of 1,3-1,6-β-glucan daily. For maitake, the dosage is based on body weight and β-glucan content, stated as MD- or D-fraction (typically 0.5–1.0 mg for every kilogram of body weight per day). The dosage for shiitake and reishi is based on historical dosage levels of the dried mushrooms of 6 to 9 g/day.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Campeau S, Day HE, Helmreich DL, et al. Principles of psychoneuroendocrinology. *Psychiatr Clin North Am*. 1998;21:259–276.
2. Olff M. Stress, depression and immunity: the role of defense and coping styles. *Psychiatry Res*. 1999;85:7–15.
3. Padgett DA, Glaser R. How stress influences the immune response. *Trends Immunol*. 2003;24:444–448.
4. Mulla A, Buckingham JC. Regulation of the hypothalamo-pituitary-adrenal axis by cytokines. *Baillieres Best Pract Res Clin Endocrinol Metab*. 1999;13:503–521.
5. Matalka KZ. Neuroendocrine and cytokines-induced responses to minutes, hours, and days of mental stress. *Neuro Endocrinol Lett*. 2003;24:283–292.
6. Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. *Ann N Y Acad Sci*. 2004;1024:138–146.
7. Kiecolt-Glaser JK, Glaser R, Gravenstein S, et al. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci U S A*. 1996;93:3043–3047.
8. Burns VE, Carroll D, Drayson M, et al. Life events, perceived stress and antibody response to influenza vaccination in young, healthy adults. *J Psychosom Res*. 2003;55:569–572.
9. Huda MN, Ahmad SM, Alam MJ, et al. Infant cortisol stress-response is associated with thymic function and vaccine response. *Stress*. 2018:1–8. https://doi.org/10.1080/10253890.2018.1484445. [Epub ahead of print]. PubMed PMID: 29932814.
10. MacDonald CM. A chuckle a day keeps the doctor away: therapeutic humor and laughter. *J Psychosoc Nurs Ment Health Serv*. 2004;42:18–25.
11. Peterson JD, Herzenberg LA, Vasquez K, Waltenvaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci U S A*. 1998;95:3071–3076. PubMed PMID: 9501217.
12. Fraternale A, Paoletti MF, Dominici S, et al. Modulation of Th1/Th2 immune responses to HIV-1 Tat by new pro-GSH molecules. *Vaccine*. 2011;29(40):6823–6829. PubMed PMID: 21816192.
13. Crinnion WJ, Pizzorno JE. *Clinical Environmental Medicine: Identification and Natural Treatment of Diseases Caused by Common Pollutants*. St. Louis, MO: Elsevier; 2019.
14. Fournier M, Degas V, Colborn T, et al. Immunosuppression in mice fed on diets containing beluga whale blubber from the St. Lawrence Estuary and the Arctic populations. *Toxicol Letter*. 2000;112–113:311–317.
15. Davis D, Safe S. Immunosuppressive activities of polychlorinated biphenyls in C57BL/6N mice: structure-activity relationships as Ah receptor agonist and partial antagonists. *Toxicology*. 1990;63:97–111.
16. Tan Y, Li D, Song R, Lawrence D, Carpenter DO. Ortho-substituted PCBs kill thymocytes. *Toxicol Sci*. 2003;76:328–337.
17. Thrasher JD, Vojdani A, Cheung G, Heuser G. Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile home. *Arch Environ Occup Health*. 1987;42(6):347–351.
18. Gray MR, Thrasher JD, Crago R, et al. Mixed mold mycotoxicosis: immunological changes in humans following exposure in water-damaged buildings. *Arch Environ Occup Health*. 2003;58(7):410–420. PubMed PMID: 15143854.
19. Kusaka Y, Kondou H, Morimoto K. Healthy lifestyles are associated with higher natural killer cell activity. *Prev Med*. 1992;21:602–615.
20. Nakachi K, Imai K. Environmental and physiological influences on human natural killer cell activity in relation to good health practices. *Jpn J Cancer Res*. 1992;83:789–805.
21. Morimoto K, Takeshita T, Inoue-Sakurai C, et al. Lifestyles and mental health status are associated with natural killer cell and lymphokine-activated killer cell activities. *Sci Total Environ*. 2001;270:3–11.
22. Heiser P, Dickhaus B, Opper C, et al. Alterations of host defence system after sleep deprivation are followed by impaired mood and psychosocial functioning. *World J Biol Psychiatry*. 2001;2:89–94.
23. Marcos A, Nova E, Montero A. Changes in the immune system are conditioned by nutrition. *Eur J Clin Nutr*. 2003;57(suppl 1):S66–S69.
24. Chandra RK. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr*. 2002;56(suppl 3):S73–S76.
25. Chandra RK. Impact of nutritional status and nutrient supplements on immune responses and incidence of infection in older individuals. *Ageing Res Rev*. 2004;3:91–104.
26. Sanchez A, Reeser J, Lau H, et al. Role of sugars in human neutrophilic phagocytosis. *Am J Clin Nutr*. 1973;26:1180–1184.
27. Ringsdorf Jr WM, Cheraskin E, Ramsay Jr RR. Sucrose, neutrophilic phagocytosis and resistance to disease. *Dent Surv*. 1976;52:46–48.
28. Nauss K, Bernstein J, Alpert S, et al. Depressed lymphocyte transformation in a whole blood culture system after oral glucose ingestion. *Nutr Res*. 1984;4:819–822.
29. Mann G. Hypothesis: the role of vitamin C in diabetic angiopathy. *Perspect Biol Med*. 1974;17:210–217.
30. Mann G, Newton P. The membrane transport of ascorbic acid. *Ann N Y Acad Sci*. 1975;258:243–252.
31. Martí A, Marcos A, Martínez JA. Obesity and immune function relationships. *Obes Rev*. 2001;2:131–134.
32. Hersoug LG, Linneberg A. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? *Allergy*. 2007;62:1205–1211.
33. Waddell CC, Taunton OD, Twomey JJ. Inhibition of lymphoproliferation by hyperlipoproteinemic plasma. *J Clin Invest*. 1976;58:950–954.
34. Dianzani M, Torrielli M, Canuto R, et al. The influence of enrichment with cholesterol on the phagocytic activity of rat macrophages. *J Pathol*. 1976;118:193–199.
35. De Simone C, Ferrari M, Lozzi A, et al. Vitamins and immunity. II. Influence of l-carnitine on the immune system. *Acta Vitaminol Enzymol*. 1982;4:135–140.
36. De Simone C, Ferrari M, Meli D, et al. Reversibility by l-carnitine of immunosuppression induced by an emulsion of soya bean oil, glycerol and egg lecithin. *Arzneimittelforschung*. 1982;32:1485–1488.
37. Frank J, Witte K, Schrodl W, et al. Chronic alcoholism causes deleterious conditioning of innate immunity. *Alcohol*. 2004;39:386–392.
38. Semba RD. Vitamin A, immunity, and infection. *Clin Infect Dis*. 1994;19:489–499.
39. Seifter E, Rettura G, Seiter J, et al. Thymotrophic action of vitamin A. *Fed Proc*. 1973;32:947.
40. Bendich A. Vitamin C and immune responses. *Food Technol*. 1987;41:112–114.
41. Scott J. On the biochemical similarities of ascorbic acid and interferon. *J Theor Biol*. 1982;98:235–238.
42. Bendich A, Langseth L. The health effects of vitamin C supplementation: a review. *J Am Coll Nutr*. 1995;14:124–136.
43. Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease. *Nutr Clin Care*. 2002;5:66–74.
44. Di Carlo G, Mascolo N, Izzo AA, et al. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci*. 1999;65:337–353.
45. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity (review). *J Mol Med*. 2010;88:441–450.
46. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Endocrinol Metab Clin North Am*. 2010;39:365–379.
47. Hayes CE, Nashold FE, Spach KM, et al. The immunological functions of the vitamin D endocrine system. *Cell Mol Biol (Noisy-le-grand)*. 2003;49:277–300.
48. Baeke F, Takiishi T, Korf H, et al. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol*. 2010;10:482–496.
49. Maruotti N, Cantatore FP. Vitamin D and the immune system. *J Rheumatol*. 2010;37:491–495.
50. Yamshchikov AV, Desai NS, Blumberg HM, et al. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. *Endocr Pract*. 2009;15:438–449.
51. Camargo Jr CA, Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. *Pediatrics*. 2012;130(3):e561–e567. PubMed PMID: 22908115.
52. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clini Nutr*. 2010;91(5):1255–1260. PubMed PMID: 20219962.

53. Goodall EC, Granados AC, Luinstra K, et al. Vitamin D3 and gargling for the prevention of upper respiratory tract infections: a randomized controlled trial. *BMC Infect Dis*. 2014;14:273. PubMed PMID: 24885201.
54. Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ*. 2017;356:i6583. PubMed PMID: 28202713.
55. Kelleher J. Vitamin E and the immune response. *Proc Nutr Soc*. 1991;50:245–249.
56. Meydani SN, Meydani M, Blumberg JB, et al. Vitamin E supplementation and in vivo immune response in healthy elderly subjects: a randomized controlled trial. *JAMA*. 1997;277:1380–1386.
57. Meydani SN, Leka LS, Fine BC, et al. Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA*. 2004;292:828–836.
58. Stockman J. Infections and iron: too much of a good thing? *Am J Dis Child*. 1981;135:18–20.
59. Hadden JW. The treatment of zinc deficiency is an immunotherapy. *Int J Immunopharmacol*. 1995;17:697–701.
60. Walker CF, Black RE. Zinc and the risk for infectious disease. *Annu Rev Nutr*. 2004;24:255–275.
61. Eby GA, Davis DR, Halcomb WW. Reduction in duration of common colds by zinc gluconate lozenges in a double-blind study. *Antimicrob Agents Chemother*. 1984;25:20–24.
62. Mossad SB, Macknin ML, Medendorp SV, et al. Zinc gluconate lozenges for treating the common cold: a randomized, double-blind, placebo-controlled study. *Ann Intern Med*. 1996;125:81–88.
63. Hemila H. Zinc lozenges and the common cold: a meta-analysis comparing zinc acetate and zinc gluconate, and the role of zinc dosage. *JRSM Open*. 2017;8(5):2054270417694291. PubMed PMID: 28515951.
64. Kiremidjian-Schumacher L, Stotzky G. Selenium and immune responses. *Environ Res*. 1987;42:277–303.
65. Kiremidjian-Schumacher L, Roy M, Wishe HI, et al. Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells. *Biol Trace Elem Res*. 1994;41:115–127.
66. Roy M, Kiremidjian-Schumacher L, Wishe HI, et al. Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. *Biol Trace Elem Res*. 1994;41:103–114.
67. Broome CS, McArdle F, Kyle JA, et al. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *Am J Clin Nutr*. 2004;80:154–162.
68. Dardenne M, Pleau JM, Nabarra B, et al. Contribution of zinc and other metals to the biological activity of the serum thymic factor. *Proc Natl Acad Sci U S A*. 1982;9:5370–5373.
69. Bogden JD, Oleske JM, Munves EM, et al. Zinc and immunocompetence in the elderly: baseline data on zinc nutriture and immunity in unsupplemented subjects. *Am J Clin Nutr*. 1987;46:101–109.
70. Chang HM, But PPH. In: *Pharmacology and Applications of Chinese Materia Medica*. Singapore: World Scientific; 1986:1041–1046.
71. Zhao KS, Mancini C, Doria G. Enhancement of the immune response in mice by Astragalus membranaceus extracts. *Immunopharmacology*. 1990;20:225–233.
72. Chu DT, Wong WL, Mavligit GM. Immunotherapy with Chinese medicinal herbs. I. Immune restoration of local xenogeneic graft-versus-host reaction in cancer patients by fractionated Astragalus membranaceus in vitro. *J Clin Lab Immunol*. 1988;25:119–123.
73. Goodridge HS, Wolf AJ, Underhill DM. Beta-glucan recognition by the innate immune system. *Immunol Rev*. 2009;230:38–50.
74. Volman JJ, Ramakers JD, Plat J. Dietary modulation of immune function by beta-glucans. *Physiol Behav*. 2008;94:276–284.
75. Talbott S, Talbott J. Effect of beta 1,3/1,6 glucan on upper respiratory tract infection symptoms and mood state in marathon athletes. *J Sports Sci Med*. 2009;8:509–515.
76. Feldman S, Schwartz H, Kalman D, et al. Randomized phase II clinical trials of Wellmune WGP® for immune support during cold and flu season. *J Appl Res*. 2009;9:20–42.
77. Talbott S, Talbott J. Beta 1,3/1,6 glucan decreases upper respiratory tract infection symptoms and improves psychological well-being in moderate to highly-stressed subjects. *Agro Food Industry Hi-Tech*. 2010;21:21–24.
78. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*. 2008;453(7195):620–625. PubMed PMID: 18509436.
79. Geuking MB, Koller Y, Rupp S, McCoy KD. The interplay between the gut microbiota and the immune system. *Gut Microbes*. 2014;5(3):411–418. PubMed PMID: 24922519.

Let the Data Speak

Sidney MacDonald Baker, MD

OUTLINE

INTRODUCTION

The importance of carefully eliciting and interpreting all possible information about a patient was discussed extensively in Chapter 140, Autism Spectrum Disorders. The autism spectrum is a medical landscape where there are more symptoms per patient than in other chronic illnesses. The collection, review, and analysis of such symptom data provide a rich resource for utilizing such data to understand their implications for the individual, the diagnostic category, and all individuals with chronic illness. The data discussed in this chapter are from Morgan, the patient described in Chapter 140. His mom had done a masterful job of completing a questionnaire's 500-symptom checklist, noting 24 strengths, 75 past symptoms, and 119 current symptoms. Of the past symptoms, 32 (43%) carry implications, as do 65 (54%) of his current symptoms. The breakdown of these indicators for Morgan is as follows:

Number	Code	Implication
24	NMI	Neuromuscular irritability
14	LIT	Loss of immune tolerance
5	LITs	Loss of sensory tolerance[a]
3	Y	Yeast issues
9	Dig	Digestive problems
8	FL	Abnormal microbiome
2	ANS	Dysautonomia

[a]The immune and sensory system join in noticing, remembering, and responding to environmental stimuli. As such, they are functions that are joined in the word "sensitive" that describes individuals in the autism spectrum more so than in other conditions.

These data offered options for therapeutic trials of *Saccharomyces boulardii*, other antifungals, Earth Dragon, and then *Hymenolepis diminuta cysticercoids* that led to his transformation from a child lost on the autism spectrum to one who performed at a very high scholarly and social level. What follows is an evaluation of a system for patients/parents and physicians to develop a comprehensive symptom portrait that captures individuality, reveals implications for actionable options, and offers a low-cost means for clinicians and parents to share a document conveying the meaning of symptoms from the dual perspective of etiology and therapeutic options. Access to an anonymous online interface for recording and reporting symptoms allows patients/parents and physicians to prepare for a thrifty but comprehensive process of prioritizing symptom-based treatment options that may be overlooked when diagnostic labels and laboratory data crowd therapeutic guidance.

GATHERING *ALL* THE DATA

My greeting Morgan with a swing test matches the placement of his strengths at the top of the patient's symptom checklist, making the point that nature's strong impulse toward healing is engaged when strengths are acknowledged and included in the treatment plan. Such treatment plans might include applied behavioral analysis; cognitive-behavioral therapy; developmental individual-difference relationship-based model (DIR)/floor time; discrete trial training; Early Start Denver model therapy; Lego-based therapy; occupational therapy; pivotal response training; a picture-exchange communication system; social scripts, social stories, and comic strip conversations; speech and language; sensory integration; music; and hyperbaric oxygen, where the question of "what are you good at?" may be overlooked. Morgan had a long list of strengths, and the attention called to them by their place in his questionnaire and in the moment of my exchange with him on the swing helped open a conversation with his mom that encouraged her inclusion of play and planned activities in which his strengths were engaged and honored. Our plans for patients of all ages and diagnostic classifications benefit if we embed this principle. I remind parents that when there may come a choice between a child's engagement in a favorite sport versus a therapy session, it is helpful to remember the value to all of us doing something that we are good at.

I created the tables shown in Tables 137.1 and 137.2 in Morgan's record by pasting his mom's symptom checklist from a Microsoft Word document into Excel, where I placed the codes, such as NMI, next to symptoms for which such an assignment was appropriate. The table listing his strengths is presented in Chapter 140. Tables 137.1 and 137.2, sorted by severity for past and present symptoms, show the codes I assigned to denote an implication if such could be recognized

Table 137.1 and 137.2's inventory of Morgan's "codable" symptoms, including those symptoms for which an implication cannot be assigned, provide an overview, the details of which are accessible and offer a picture of him that stands in contrast to the overview presented by his diagnostic label of autism spectrum disorder. The assignment of implications has been an exercise I have done manually in each

TABLE 137.1 Past Symptoms

Code	Symptom	Mild	Moderate	Severe	Occasional	Frequent	Always
NMI	Difficulty falling asleep			x			x
NMI	Insomnia			x			x
NMI	Sleeps less than normal			x			x
NMI	Colic			x			x
	Pica (eating nonedibles)			x			x
	Abnormal food cravings			x			x
	Behavior purposeless			x			x
	Aloof, indifferent, remote			x			x
	No purpose to play			x			x
	Uninterested in live pet			x			x
	Erratic			x			x
	Unable to predict actions			x			x
NMI	Hyperactive			x			x
NMI	Constant movement			x			x
NMI	Climbs to high places			x			x
	Spends time w/ pointless task			x			x
NMI	Toe walking			x			x
LITs	Licking			x			x
	Likes spinning objects			x			x
LITs	Sensitive to loud noise			x			x
LITs	Sounds seem painful			x			x
LITs	Bothered by bright lights			x			x
	Unaware of danger			x			x
	Repetitive play/objects			x			x
LITs	Hates wearing shoes			x			x
	Runs away		x				x
LITs	Covers ears with sounds		x				x
LITs	Insensitive to pain		x				x
	Uses adult's hand for activity						x
	Asks using "you" not "I"						x
	Answers by repeating question						x
	Receptive language poor						x
	Headaches			x		x	
NMI	Awakens screaming/crying		x			x	
	Cold sores on lips, face		x			x	
NMI	Teeth grinding		x			x	
	Poor appetite		X			x	
	Sits for long time staring		x			x	
	Poor sharing		x			x	
	Destructive		x			x	
NMI	Jumps when pleased		x			x	
NMI	Whirls self like a top		x			x	
NMI	Shrieks		x			x	
NMI	Flaps hands		x			x	
NMI	Likes to spin things		x			x	
NMI	Visual stims		x			x	
	Apathy		x			x	
	Blank look		x			x	
	Depression		x			x	
	Detached		x			x	
	Disinterested		x			x	
NMI	Anguish		x			x	
	Discontented		x			x	
NMI	Inconsolable crying		x			x	
NMI	Unhappy		x			x	
	Likes certain sounds		x			x	
	Conjunctivitis		x			x	
Y	Eye crusting		x			x	

TABLE 137.1 Past Symptoms—cont'd

Code	Symptom	Mild	Moderate	Severe	Occasional	Frequent	Always
Y	Lid margin redness		x			x	
	Likes fans		x			x	
	Likes flickering lights		x			x	
	Looks out of corner of eye		x			x	
	Unaware of people's feelings		x			x	
	Likes head burrowed		x			x	
	Likes head under blanket		x			x	
LITs	Very insensitive to pain		x			x	
	Muscle tone low trunk		x			x	
	Lost language @ 12–24 months		x			x	
	Occasional words when excited		x				
	Speech apraxia		x				
Y	Diaper rash	x			x		
NMI	Falls gets hurt running climbing	x			x		
	Stares at own hands	x			x		
	No answers to simple questions						

LITs, Loss of sensory tolerance; *NMI,* neuromuscular irritability; *Y,* yeast issues.

TABLE 137.2 Current Symptoms

		Mild	Moderate	Severe	Occasional	Frequent	Always
	Looks sick			x			x
LIT	Dark circles under eyes			x			x
	Paleness, severe			x			x
	Skin pale			x			x
Dig	Abdominal bloating			x			x
Dig	Abdomen distended			x			x
FI	Stools very stinky			x			x
FI	Stool odor foul			x			x
FI	Stools slimy			x			x
	Fixated on one topic		x				x
	Likes to be held upside down		x				x
	Likes to be swung in the air		x				x
	Clumsiness		x				x
	Coordination		x				x
	Fine motor poor		x				x
	Gross motor poor		x				x
	Sleeps with parent(s)						x
	Says "I"						x
	Says "no"						x
	Says "yes"						x
	Inability to tan		x			x	
LIT	Itchy penis		x			x	
FI	Breath bad		x			x	
Dig	Abdominal pain		x			x	
Dig	Crampy pain with pooping		x			x	
NMI	Constipation		x			x	
FI	Farting—stinky		x			x	
Y	Red ring around anus		x			x	
Dig	Stools with mucous		x			x	
Dig	Stools with undigested food		x			x	
Dig	Stools watery		x			x	
LIT	Casein intolerance		x			x	
LIT	Specific food(s) intolerance		x			x	
LIT	Behavior worse with food		x			x	
	Doesn't do for self		x			x	

Continued

TABLE 137.2 Current Symptoms—cont'd

		Mild	Moderate	Severe	Occasional	Frequent	Always
	Hides skill/knowledge		x			x	
	Lacks initiative		x			x	
	Poor focus, attention		x			x	
	Won't attempt/can't do		x			x	
	Holds hands in strange pose		x			x	
	Imitates others		x			x	
	Eye contact poor		x			x	
NMI	Anxious		x			x	
	Repeats old phrases		x			x	
NMI	Holds bizarre posture		x			x	
	Poor attention, focus		x			x	
NMI	Scripting		x			x	
NMI	Talks to self		x			x	
LIT	Congestion w/change in season		x			x	
LIT	Congestion in the fall		x			x	
LIT	Congestion in the spring		x			x	
LIT	Congestion in the winter		x			x	
LIT	Runny nose		x			x	
NMI	Early waking	x				x	
ANS	Red lips	x				x	
Dig	Burping	x				x	
Mg	Craving for salt	x				x	
NMI	Meltdowns	x				x	
NMI	Chews on things	x				x	
NMI	Agitated	x				x	
	Adopts complicated rituals	x				x	
	Collects particular things	x				x	
LITs	Very sensitive to pain	x				x	
	Slow and sluggish	x				x	
NMI	Rigid behaviors	x				x	
	Poor confidence	x				x	
	Corrects imperfections					x	
	Dry at night					x	
LIT	Itchy eyes		x		x		
Dig	Diarrhea		x		x		
	Looks like in pain		x		x		
NMI	Fearful of unusual events		x		x		
	Timid		x		x		
FI	Odd urinary odor		x		x		
	Expressive language delay		x				
	Expressive language poor		x				
	Does not asks questions		x				
NMI	Awakes at night	x			x		
NMI	Jerks during sleep	x			x		
NMI	Nightmares	x			x		
	Glazed look	x			x		
ANS	Pupils unusually large	x			x		
LIT	Head sweats	x			x		
LIT	Night sweats	x			x		
LIT	Sensitive to insect bites	x			x		
	Bugs love to bite you	x			x		
	Sore throat	x			x		
FI	Fecal belching	x			x		
FI	Flatulence	x			x		
Y	Craving for juice	x			x		
	Unusual play	x			x		
	Extremely cautious	x			x		
	Lost in thought, unreachable	x			x		

TABLE 137.2 Current Symptoms—cont'd

		Mild	Moderate	Severe	Occasional	Frequent	Always
	Rejects help	x			x		
	Curious/gets into things	x			x		
NMI	Tantrums	x			x		
	Insists on what wanted	x			x		
	Tries to control others	x			x		
Y	Silly	x			x		
NMI	Fright without cause	x			x		
NMI	Irritable	x			x		
NMI	Phobias	x			x		
NMI	Restless	x			x		
LITs	Bothered by certain sounds	x			x		
LITs	Hearing acute	x			x		
LITs	Acute sense of smell	x			x		
LITs	Examines by smell	x			x		
NMI	Upset if things change	x			x		
NMI	Upset if things aren't right	x			x		
	Lines objects precisely	x			x		
	Likes head pressed hard	x			x		
NMI	Hyperactivity	x			x		
	Physically awkward	x			x		
NMI	Jaw clenching	x			x		
	Poor auditory processing	x			x		
	Uses one word for another	x			x		
	Bed wetting after age 4	x			x		
	Daytime sleepiness				x		
	Heart murmur	x					

ANS, Dysautonomia; *Dig,* digestive problems; *FL,* abnormal microbiome; *LIT,* loss of immune tolerance; *LITs,* loss of sensory tolerance; *NMI,* neuromuscular irritability; *Y,* yeast issues.

patient's Excel spreadsheet over the past 40 years of my practice. Doing so gave me a feel for the patient, similar to how chefs get a feel of the meal as it passes through their hands in the kitchen. I took a sample of 20,000 symptoms from the questionnaires of many patients, and from that body of data, I reconciled variations in my coding assignments that occurred over the years. Coding the implication of symptoms was facilitated by the use of a system in which the body system, function, and location are used as a basis for establishing the literal meaning of the words we use to name symptoms. Using symptom, function, and location from the conventional medical lexicon expanded to include designations made in the course of patients' narrative, the system scheme grew to include 50 systems, 50 functions, and hundreds of locations, the latter ranging from anatomic to geographic.[1] Table 137.3 provides three examples of NMI symptoms in which the English-language vernacular "uptight" gives a glimpse of a unifying feature that is not found in the commonalities of body system, function, or location.

From those normalized data, I was able to conclude the remarkably small number of implications to be found in a diverse population of patients of all ages and diagnoses. The small number of implications came from letting the data talk and in no way was a list that I had created at the beginning of this project except that I had, in the course of the project, created a mini-checklist for neuromuscular irritability as a teaching tool for patients to understand the functional medicine style of thinking when it comes to the clues to unmet needs for magnesium. Table 137.4 provides the magnesium checklist used for data acquisition with general medical patients.[2]

Table 137.5 shows Morgan's list of past NMI symptoms, and Table 137.6 shows his current symptoms.

As it happens, Morgan has an exceptionally large group of such symptoms. Clinicians may protest that the questionnaire and its harvest are overkill. Parents, on the other hand, benefit from having the details of a story that has so intensely occupied their time, emotions, and curiosity be heard and having sense made of it. The data on which we drive the decision to prescribe magnesium "to bowel tolerance" is soft. I should note that our colleague Derrick Lonsdale, MD, makes the point that unmet needs for vitamin B_1 have a symptom list that overlaps with this one, which reminds us that the data we are gathering and using to drive simple clinical decisions about interventions are essentially free of risk, with good odds for benefit, at low cost, in a setting where the stakes are very high. On the other hand, we have here a situation in which the only accurate way to assess magnesium status is a pair of 24-hour urine measurements with an intramuscular dose of magnesium in each buttock that could easily be wasted into wet pants, bed, or diapers. There is a good chance that all children on the autism spectrum and most individuals with chronic illness have unmet needs for magnesium. Why go to the trouble of asking all these questions if a clinical trial of magnesium to bowel tolerance may do the job? Respect! Respect for the sacred, respectful conversation. Respect for the data; the patient/parent who has harvested it with worry, attention, and love; and our own integrity—threatened as it is by the demands of complex patients and a tight appointment schedule.

TABLE 137.3 "Uptight"

	System	Function	Where
Constipation	Digestive	Decrease	Stool
Anxiety	Emotion	Abnormal	Fear
Calf cramps	Neuromuscular	Increase	Calf

TABLE 137.4 Magnesium Deficiency Symptoms by System

Skeletal Muscle

Muscle cramps, including backache, neck pain, tension headache, temporomandibular joint dysfunction
Muscle twitches
Muscle tension
Muscle soreness
Chest tightness or a peculiar "I can't seem to take a deep breath" or "I have to think about my breathing" that is often interpreted as hysterical. In children, this symptom is often seen as sighing.

Other Muscles

Constipation
Anal spasms—such as awaken people at night
Urinary spasm
Difficulty swallowing or "lump in throat," "globus hystericus"
Difficulty with adjusting to oncoming bright headlights because of spasm of the muscles that fine-tune pupillary diameter
Cold hands and feet due to vasospasm
Loud noise sensitivity due to abnormal tension on the stapedius muscle
Endometriosis due to "constipation" or reverse peristalsis of fallopian tubes
Menstrual cramps
Asthma/wheezing from constriction of bronchial muscles

Central Nervous System

Insomnia
Anxiety
Hyperactivity and restlessness, constant movement
Panic attacks
Agoraphobia
Irritability

Peripheral Nervous System

Numbness
Tingling
Other abnormal sensations, including "zips," "zaps," vibratory and other peculiar sensations

Cardiovascular

Mitral valve prolapse
Palpitations
Arrhythmias
Vasospastic angina
Hypertension

Other

Salt craving
Carbohydrate craving
Carbohydrate intolerance
Kidney stones

For four decades my parents and patients have filled out my questionnaire as a Word document, from which I move the symptoms checklist to Excel, where I tag the symptoms that guide decisions. I move it back to the patient's Word document, where the effort taken to record the data is rewarded in three ways:

- Patients who have previously had doctors ignore records are relieved to see them taken seriously.
- Patients understand that symptoms drive decisions in sometimes very specific ways.
- At a subsequent follow-up visit, patients are less likely to have simply forgotten the very symptoms that had driven treatment decisions.
- The idea and practice that the patient, not the disease, is the target of treatment are honored while crossing the solid bridge between the details of the narrative and a solution aimed at the individual.

Unmet needs for magnesium are rampant in our culture and displayed in all sorts of symptoms of being uptight—chronic stress; dietary sugars; and the agricultural use of nitrogen, phosphorus, and potassium fertilizers that lack the magnesium needed to meet the demands of animals and, needless to say, humans, such as patients stressed by chronic illness.

QUESTIONNAIRE INTERPRETATION

If a patient will bring the questionnaire input to a simple interface on an anonymous website where the data are examined and understood from the same perspective that I have described, then a simple report can be shared with the clinician who made the referral to the website. Done in this way, the process creates a shared responsibility in which the input helps awaken the patient to the value of the narrative and rewards the patient with simple information about steps to be taken in collaboration with the referring clinician. Instead of poring over a long questionnaire and writing up a plan based on its most conspicuous options, the clinician's job of interpreting the implications of the patient's symptoms is simplified by the tailored documentation of the part of the patient's plan that that is based on symptoms and leaves room for interpretation and explanation of laboratory results.

Such a website is pending. You will be able to help the website grow to be a collaborative endeavor in which users may offer pearls and the string that joins them in ways that reflect a practice of medicine in which not the disease but the patient is the target of treatment and the symptoms of the patient provide the most sensitive data that captures the patient's individuality.

NMI Guidance Example

NMI is an abbreviation for neuromuscular irritability.
Neuromuscular means nerves, brain, and/or muscles.
Irritability means an increase in function triggered at a low threshold.

Short Guidance Report

You (patient or parent) report the following symptoms of NMI, or you have been linked here through constipation and/or reference to magnesium.

(NMI symptoms in Tables 137.5 and 137.6.)

PROBLEM: Over the past centuries, people living in modern societies have suffered from changes in soil, food, and stress, giving rise to unmet needs for magnesium.

ANSWER: Supplemental magnesium is safe and convenient. Dietary change is difficult but necessary. Stress reduction requires taking its measure and tailoring to individual sensitivity.

OPTIONS

a. Laboratory testing is only of value if done in a way that is inconvenient but may be critical for some individuals; however, it is not well suited to children.
b. Avoidance of sugar
c. Supplementation with magnesium

EXAMPLES: A boy in his late teens goes away to college and starts to live on pizza, beer, and ice cream. Although a good student, he starts having severe anxiety to the point of panic attacks. He develops an irregular heartbeat called paroxysmal tachycardia. He is constipated, his sleep is poor, and he awakens with leg cramps. Over the winter

TABLE 137.5 Morgan's List of Past NMI Symptoms

Symptom	Mild	Moderate	Severe	Occasional	Frequent	Always
Falls or gets hurt when running, climbing	x			x		
Awakens screaming/crying		x			x	
Teeth grinding		x			x	
Jumps when pleased		x			x	
Whirls self like a top		x			x	
Shrieks		x			x	
Flaps hands		x			x	
Likes to spin things		x			x	
Visual stims		x			x	
Anguish		x			x	
Inconsolable crying		x			x	
Unhappy		x			x	
Difficulty falling asleep			x			x
Insomnia			x			x
Sleeps less than normal			x			x
Colic			x			x
Hyperactive			x			x
Constant movement			x			x
Climbs to high places			x			x
Toe walking			x			x

TABLE 137.6 Morgan's List of Current NMI Symptoms

	Mild	Moderate	Severe	Occasional	Frequent	Always
Craving for salt[a]	X				x	
Early waking	x				x	
Meltdowns	x				x	
Chews on things	x				x	
Agitated	x				x	
Rigid behaviors	x				x	
Awakes at night	x			x		
Jerks during sleep	x			x		
Nightmares	x			x		
Tantrums	x			x		
Fright without cause	x			x		
Irritable	x			x		
Phobias	x			x		
Restless	x			x		
Upset if things change	x			x		
Upset if things aren't right	x			x		
Hyperactivity	x			x		
Jaw clenching	x			x		
Constipation		x			x	
Anxious		x			x	
Holds bizarre posture		x			x	
Scripting		x			x	
Fearful of unusual events		x		x		

[a]Note that the symptom of a craving for salt gets a place in the list because it is a soft sign for unmet needs for magnesium, which is the first clinical takeaway from this group of symptoms.

holiday, his family doctor prescribes a tranquilizer. A second opinion from a doctor who is a neighbor and functional medicine practitioner suggests that he take magnesium citrate capsules 150 mg every evening and morning, increasing daily until he reaches bowel tolerance. Within a week, he reaches a daily dose of 4 to 6 capsules in the evening and 2 to 3 in the morning. His symptoms clear, and he agrees to modify his diet.

For further guidance on this subject, click here: Magnesium EXPANDED GUIDANCE.

Expanded Guidance Report

It tells us something that if we Google "magnesium deficiency", we do not come up with the name of even one "condition" or "disease."

Hypomagnesemia is mentioned, but that just means low blood magnesium.

My friend and colleague Leo Galland, MD, awakened my interest in 1980 after he had met Mildred Seelig, author of *Magnesium Deficiency in the Pathogenesis of Disease*.[3] It says a lot that a medical book would be reprinted in its original text 32 years later. A lot of things in medicine had changed, but not the basics of magnesium, including the indifference of most medical professionals while all sorts of drugs have come along to treat symptoms that would respond promptly to a supplement of magnesium.

Most people have little need for nutritional supplements if they consume a good diet. Vitamins A and D are exceptions, and magnesium is noteworthy for the prevalence of unmet needs for magnesium. There is, moreover, a wide range of effective magnesium doses. A few people have very large needs. They are "magnesium wasters," about whom I published "Magnesium Deficiency in Primary Care and Preventive Medicine: Symptom Profiles in Relation to Magnesium Loading Studies."[4] Let me briefly cover the laboratory test that helps discover such individuals for whom certain symptoms may be clues to their special needs.

The checklist in Table 137.4 helped me alert my patients' attention to the diverse symptoms that indicate an unmet need for magnesium. Such symptoms scatter in questionnaires and checklists of specialists who naturally group symptoms focused in their areas of interest. Here they are grouped to illustrate how the notion of "uptight" is expressed in different ways. Magnesium wasters are distinctive in having low blood pressure, fewer digestive symptoms, and fewer symptoms of inflammation, but more emotional symptoms.

Testing for Magnesium Status

The laboratory test that accurately measures a person's magnesium status is a 24-hour urinary loading test, which takes 2 days before and during which diet and supplements are unchanged. After the first 24 hours, when every drop of urine is collected, a second 24-hour collection is started after injecting 200 mg of elemental magnesium, as the sulfate. It is painful, and thus it is best to add a local anesthetic and give it slowly, deep in the upper outer buttocks, giving half of the 5-cc volume in each cheek (2 mL of 50% MgSO4 plus 0.5 mL of lidocaine). A second 24 hours' worth of urine is then collected, and calculations assess the percentage of the injected magnesium dose that was retained.

- Normal retention is up to 10%.
- Borderline retention is 10% to 20%.
- More than 20% retention is consistent with magnesium deficiency.
- Retention of 50% or more indicates marked deficiency.
- Wasters excrete 15% or more of the injected dose.

Now I tend to use this test only when symptoms suggest that a person may be a magnesium waster or if supplementing magnesium to a dose that is just short of producing frequent or loose bowel movements—what I call bowel tolerance—does not alleviate the symptoms that suggested unmet needs for magnesium.

Beyond that consideration, the magnesium story is quite simple: The benefit is high, the risk is zero, the odds are good, the cost is minimal, and the stakes are quite high given the importance of magnesium in more than 300 places in the body's chemistry.

NMI symptoms predict a positive response to meeting an unmet need for magnesium. Most, but not all, people with unmet needs for magnesium have at least a few of these symptoms, which overlap with those that brought the patient to this insight. Some of the symptoms have a higher value than others in that prediction, and the more symptoms a patient has, the greater becomes the chance of relief with magnesium supplementation.

How Do We Develop Unmet Needs for Magnesium?

Imagine your five-hundredth great-great-great-grandmother. She was a hunter-gatherer heading down to a river to fill her calabash with water for her migrating family. On the way, she encounters a beast and flees, with the beast in pursuit. Good fortune and strong legs bring her to a tree she can climb. There, she hangs on for dear life until the beast finally gets bored and leaves. Descending from the tree, she empties her bladder before heading back to the river. Her urine is rich in magnesium. It had shifted from inside her muscle cells into the bloodstream and into her bladder during her previous running, climbing, and hanging on. That shift was an essential component of her stress response.

Grandma returns to her campsite and replenishes her magnesium losses with a good meal of vegetation and a side order of beast. In other words, stress of the kind Grandma experienced is associated with a natural loss of magnesium, which must then be replenished by eating. Encounters with beasts in Grandma's time were infrequent. The muscular demands for magnesium's shifts for daily activities were modest. Her diet was adequate and free of sugar.

Compare her situation with those of us who live the modern way:

1. Since the 1950s, the soil of the major agricultural areas where our food is grown has been fertilized with nitrogen, potassium, and phosphorus but not with magnesium.
2. The magnesium content of the harvests has fallen, affecting consumers while not impairing the hardiness of the plants.
3. Since the 1950s, the diet of those living in modern industrialized countries has become sugarier and starchier.
4. Finally, the stresses in modern life are more like having a constant beastly presence than rare encounters with beastly attacks.

The combined effects of low magnesium intake and high stress-related magnesium losses have caused a failure to meet the needs of a very substantial proportion of the population.

Uptight

Our language aptly summarizes the way our bodies respond to danger. The theme of the checklist's NMI selections is captured by the word *uptight*: It applies equally to muscles and emotions. That response produces a tendency toward magnesium loss that is not replenished when the diet is low in magnesium. The resulting nervous and/or muscular contraction tension is associated with further magnesium shifts and losses. A vicious cycle, right? The more you become deficient in magnesium, the more you tend toward nervousness and/or muscular tension, which, in turn, tends to cause magnesium losses. Magnesium wasters simply cannot hang on to their magnesium and require especially large doses of magnesium given intravenously to correct their problem.

The Patient Is the Best Laboratory

For research, no test substitutes for magnesium-loading testing. The studies that I published revealing the distinctive symptom profile of magnesium wasters could not have been done without many magnesium-loading tests. On the other hand, the symptoms of magnesium deficit are so common and easy to spot that a trial of magnesium supplementation turns out to be a practical first—and often final—step in documenting magnesium problems. Given orally, magnesium is safe, except for individuals who have poor kidney function. The symptom of a magnesium overdose is diarrhea, so with the escalation of magnesium supplementation, loose bowel movements will reliably indicate an excess well before any other mischief could be produced. Taking magnesium "to bowel tolerance" and observing the effect on symptoms of neuromuscular irritability is the best test for unmet needs for magnesium.

To Bowel Tolerance

To replenish magnesium, start with a teaspoon of CALM—in water—magnesium or one capsule of 150 mg of magnesium citrate in the evening. These doses would be safe for children and adults, but for children who do not swallow capsules and for toddlers, CALM alone would do just fine. A daily increase in dosage by adding a teaspoon of CALM and 1 capsule of magnesium citrate (in a child or adult) will, in a matter of a week or two, produce more frequent and loose bowel movements. The idea is to increase up to the point short of risking a bowel accident. For an adult, this is not rocket science. For children and those with impaired sensory function, this trial requires precision and vigilance to avoid the humiliation of a bowel accident. The dose of magnesium that reliably results in two easy bowel movements daily is valuable knowledge. Knowing the limit of the magnesium dose will be useful if constipation is provoked by interventions, such as activated charcoal and antifungal medication, described in Section 4 and presented in detail in this chapter.

The Pebble in Your Shoe

Establishing a dose of magnesium at bowel tolerance first provides a way of dealing with constipation if it is already a problem. Then it gives a baseline for dealing with constipation should it arise. Most of all, it lets us see improvement in NMI symptoms and allows us to expect the alleviation of other, less noticeable problems that are solved by meeting magnesium needs. Magnesium is a cofactor in dozens of steps in every aspect of body chemistry. Many of these steps are relatively silent compared with those related to tension in the nerves and muscles. Keep in mind therefore that as important as magnesium is in correcting "uptight" symptoms, those symptoms are not the only target of magnesium supplementation. Removing a pebble from your shoe is not just for your foot but also for the journey.

Hirschsprung's Disease

Hirschsprung's disease is a good example of worst-case scenario thinking that must find its proper place in the minds of doctors and parents. Caused by an inborn lack of certain nerve cells in the lower bowel, the resulting intractable constipation calls for management that may overlap with treatments for "ordinary constipation" but demands other specific approaches. These approaches include the need for location and biopsy of the suspected gap in nerve connections. Surgery may also be required. Mild forms of Hirschsprung's may go undetected for years and complicate the lives of individuals whose constipation does not yield to the measures discussed here.

NMI EXPANDED GUIDANCE SUMMARY

1. The target of treatment in chronic illness is the individual, not the disease.
2. Treatment involves addressing unmet special needs to receive, avoid, or be rid of things, which—if addressed—will favor nature's buoyant impulse toward healing.
3. Before a particular intervention becomes a treatment, it is a test to see if it produces a benefit for the individual in question.
4. In many ways, such a test can be the best (or even the exclusive) way of finding what will work for a given individual.
5. Magnesium is a good example of a commonly effective therapeutic agent for children and adults with every sort of chronic illness. When it relieves symptoms of neuromuscular irritability, including constipation, it will likely benefit many areas of biochemistry where it is an important helper.
6. A person may have other symptoms of neuromuscular irritability but no constipation. A trial of magnesium to bowel tolerance is still a good idea because it is the best means of judging the benefits of magnesium and preparing for the constipation that may come with a Herx reaction.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Baker SM. Autism360: an online database with patient-entered data. *Integrat Med*. 2012;11(1).
2. Baker SM. Magnesium deficiency in primary care and preventive medicine. *Magnes Trace Elem*. 1991–1992;10:251–262.
3. Seelig M. *Magnesium Deficiency in the Pathogenesis of Disease*. New York: Plenum; 1980.
4. Baker SM. Magnesium deficiency in primary care and preventive medicine: symptom profiles in relation to magnesium loading studies. *Magnes Trace Eleme*. 1991–1992;10:251–262.

138

Mycotoxin Exposure: Assessment and Treatment

Matt Pratt-Hyatt, PhD

OUTLINE

INTRODUCTION

Over the past several decades, there has been increased research into the role that mycotoxins from mold have in human health. Mycotoxins bind the ribosome, resulting in an inhibition of protein synthesis. They also inhibit the growth of rapidly proliferating cells, such as bone marrow and the gastrointestinal epithelium.[1] Other organs affected include eyes, ears, nasal cavities, nails, skin, respiratory tracts, liver, and kidneys.[2,3] The most common routes of exposure to mycotoxins are ingestion through contaminated food, inhalation of mycotoxins or fungi spores, or skin contact.[4]

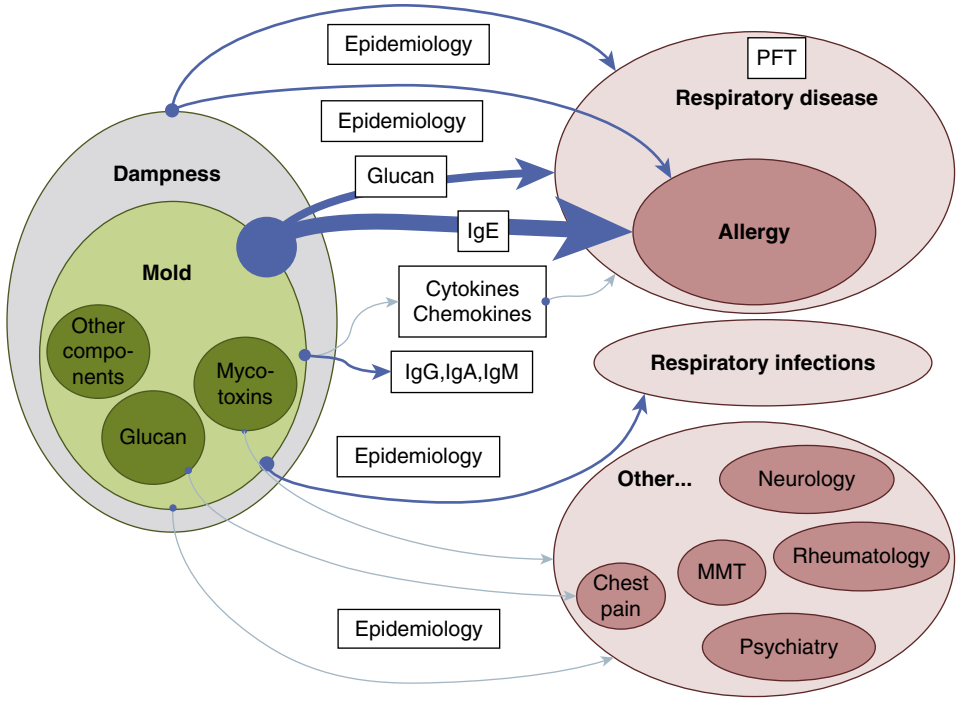
Common symptoms of mycotoxin exposure include cough, irritation of the eyes and skin, joint pain, headaches, and fatigue.[2,5,6] A particularly good example is how research has shown that mold and damp-building exposure are major factors in the asthma epidemic. Studies have shown that dampness or mold in houses causes 21% of asthma in the United States as well as a 30% to 50% increase in asthma and asthma-related health problems.[7] Mold toxicity should be considered in all patients with any chronic respiratory condition, especially those of adult onset (Fig. 138.1). Symptoms such as dyspnea, wheeze, cough, respiratory infections, bronchitis, allergic rhinitis, eczema, vocal cord dysfunction, and upper respiratory tract symptoms should also be included. Once individuals have become sensitized, they become much more reactive to even low to modest exposure. In addition, exposure to mold and dampness increases the risk of allergy to other allergens, such as house dust mites and pollen, and causes epigenetic modulation that upregulates many inflammatory genes.[8]

Mycotoxins are low-molecular-weight molecules and secondary metabolites produced by fungi that evoke a toxic response even in low concentrations.[9,10] There are more than 400 known mycotoxins, of which several are known to affect human health. The molecular masses of these mycotoxins are mostly between 200 and 800 kDa in size.[11] Mycotoxins are also very heat-resistant, being able to withstand temperatures up to 160ºC.[12]

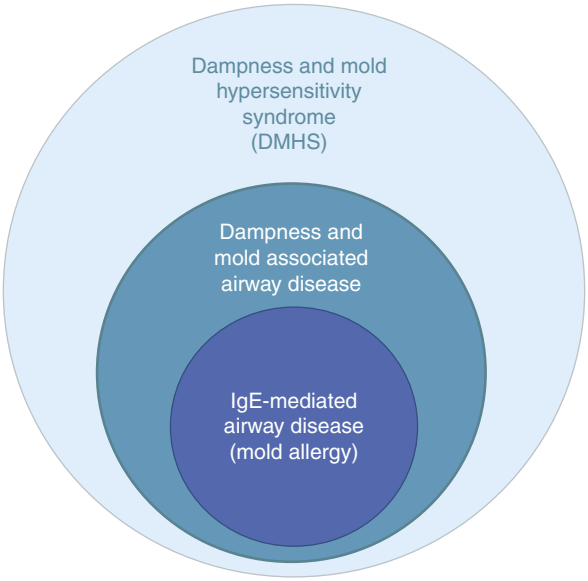
Most mycotoxins are produced by filamentous fungi, which are ubiquitous because of their ability to grow on many different materials and under many different conditions.[13] These fungi use dead matter for energy and are able to produce spores in a variety of different conditions.[14] Many different mycotoxins are produced from species of the genera *Aspergillus, Fusarium, Penicillium,* and *Stachybotrys.* It is common for one species of fungi to produce different mycotoxins, as well as one mycotoxin to be produced by multiple species of fungi (Table 138.1). Mycotoxins can induce disease through several different pathways, which include immunosuppression, cytotoxicity, and DNA damage. Interestingly, many of the clinical effects of mold exposure are very similar to those found with volatile organic compound (VOC) exposure.

Through their mycotoxin metabolites, pathogenic fungi can reduce the efficacy of the immune system. This is accomplished by suppressing multiple different components of the immune system. Mycotoxin metabolites can suppress T- and B-lymphocyte activity, inhibit immunoglobulin production, and reduce antibody production. Some species of fungi release mycotoxins that can interfere with which genes are activated. Some also release polysaccharides that can result in the induction of neutrophil apoptosis. All these processes result in the body's inability to fight off the fungi as well as other infections.[15–17]

Fungi can produce cytotoxicity in human cells through their metabolites, which causes reduced cellular viability.[18] Much of this toxicity in cells comes from the mycotoxins' ability to bind to the 60-S subunit of the ribosome and to the enzyme peptidyl transferase. These interactions inhibit cellular protein biosynthesis, which eventually results in cellular death.[19,20] However, some of these characteristics of mycotoxins are being used for some beneficial purposes. Some species of mycotoxins are being studied for their antitumor capabilities. Cell lines from colon cancer, breast cancer, lung cancer, and prostate cancer have been proven to be sensitive to these mycotoxins.[21]



A



B

Fig. 138.1 A and B, Estimates of evidence for a causal relationship between dampness/mold exposure and disease. The relationship between dampness and mold and respiratory disease is mainly clinical and epidemiological, and besides immunoglobulin E (IgE) as a biomarker of allergy, other biomarkers of exposure or specific to mold-induced disease are beginning to be recognized only now. This explains that visible mold or dampness is frequently assessed as an environmental factor, and in fact, correlation with clinical health effects is frequently higher than when specific exposure markers have been used in distinct settings. *PFT,* Pulmonary function testing; *MMT,* mixed-mold mycotoxicosis. (From Daschner A. An evolutionary-based framework for analyzing mold and dampness-associated symptoms in DMHS. *Front Immunol.* 2017;7:672. PubMed PMID: 28119688.)

Studies have shown that many different types of mycotoxins can be carcinogenic, which seems to stem from three possible factors. First is their ability to cause oxidative stress in cells, and second is their ability to cause DNA adducts.[22] Mycotoxins in cells cause reactive oxygen species (ROS) to increase. These ROS molecules lead to breaks in DNA molecules. However, a more common cause of DNA damage seems to be the mycotoxins' ability to covalently bind to DNA. Reports show that mycotoxins cause adducts on the N-7 position of guanine and the N-3 position of adenine. These adducts can interfere with DNA synthesis and can cause an incorrect substitution of a nucleotide or cause

TABLE 138.1 Mycotoxins Produced by Fungi

Species	Aflatoxin	Gliotoxin	Ochratoxin	Sterigmatocystin	Zearalenone	Roridin E	Verrucarin A	Enniatin B	Mycophenolic Acid	Citrinin
Acremonium sp.				Present						Present
Alternaria		Present								
Aspergillus flavipes				Present						Present
Aspergillus flavus	Present									Present
Aspergillus fumigatus		Present								Present
Aspergillus niger			Present							Present
Aspergillus ochraceus			Present	Present						Present
Aspergillus parasiticus	Present									Present
Aspergillus sydowii				Present						Present
Aspergillus versicolor				Present						Present
Aspergillus viridictum			Present	Present						Present
Aureobasidium				Present						
Chaetomium				Present						
Cladosporium				Present						
Cunninghamella				Present						
Cylindrocarpon						Present				
Dendrodochium						Present	Present			
Exophialo				Present						Present
Fusarium avenaceum				Present	Present			Present		Present
Fusarium cerealis				Present	Present					Present
Fusarium clumonrum				Present	Present					Present
Fusarium equiseti				Present	Present					Present
Fusarium graminearum				Present	Present					Present
Fusarium incarnatum				Present	Present					Present
Fusarium moniliforme				Present	Present			Present		Present
Fusarium solani								Present		
Fusarium verticillioides				Present	Present					Present
Myrothecium roridum						Present				
Myrothecium verrucaria						Present	Present			
Penicillium carbonarius		Present	Present	Present					Present	Present
Penicillium nordicum		Present	Present	Present					Present	Present
Penicillium stoloniferum		Present	Present	Present					Present	Present
Penicillium verrucosum		Present	Present	Present					Present	Present
Phoma sp.				Present						Present
Rhodotorulo				Present						Present
Scopulariopsis				Present						Present
Stachybotrys				Present		Present	Present			Present
Stachybotrys chartarum				Present						
Trichoderma viride		Present		Present						
Ulocaldium										
Verticillium				Present						Present

a deletion to occur.[23] The third possible cause of DNA damage from mycotoxins is inhibition of DNA topoisomerase I and II enzymes. These enzymes are required to untangle DNA during replication, and inhibition of these enzymes could lead to the accumulation of DNA breaks.[24]

MOLD SPECIES

Of the 400 known mycotoxins, there are 10 to 15 that have so far been linked to human disease. These are produced by about 350 different fungi species; however, some species are more prevalent than others.[25] Some of the most common mycotoxin-producing genera are *Aspergillus, Fusarium, Penicillium,* and *Stachybotrys.*[26]

Aspergillus

Aspergillus is the most prevalent mold genus in the environment. The two most common *Aspergillus* mycotoxins are aflatoxin and ochratoxin, and their main target is the liver.[27,28] *Aspergillus* species are commonly associated with indoor air problems.[29] The most common route of transmission for *Aspergillus* is inhalation.[30] Because *Aspergillus* spores are smaller than most other species, 2 to 10 µm, they can reach the lower airways. This can lead to fungal colonization, which results in prolonged mycotoxin exposure and allergic responses. This can lead to allergic fungal rhinosinusitis and asthma.[31]

These toxins have also been found in all major cereal crops, including peanuts, corn, cotton, millet, rice, sorghum, sunflower seeds, wheat, and a variety of spices. They are also found in eggs, milk, and meat from animals fed contaminated grains. This has caused billions of dollars in damage to crops and livestock.[32] There are multiple species of *Aspergillus* that can produce toxic metabolites (see Table 138.1). Some of the more common *Aspergillus* species that can induce diseases are *A. flavus, A. niger, A. fumigatus,* and *A. versicolor.*[33]

A. flavus is one of the more common fungal contaminants in agriculture. It has caused the loss of millions of dollars in crop damage and animal infection. The leading route of exposure to this species is the consumption of contaminated food, such as peanuts, milk, corn, rice, and coffee.[34,35] *A. flavus* infections can lead to cutaneous aspergillosis, keratitis, granulomatous sinusitis, and osteomyelitis.[36]

A. niger is one of the most common mold species because of its ability to grow on many different types of substances, such as food, soil, and indoor material. *A. niger* invades tissues that have been rendered susceptible by bacterial infections or physical injury. It can colonize the ear, nose, or throat, causing chronic infections. Patients who are immune compromised could develop pulmonary aspergillosis, which is characterized by chronic productive cough.[37]

A. fumigatus is the most common mold associated with pulmonary infections. *A. fumigatus* is also able to produce polypeptide allergen molecules that can cause asthma and rhinitis symptoms.[38] *A. fumigatus* mycotoxin products (see Table 138.1) cause immune suppression during hyphal growth in favorable conditions. Recent studies have linked these immune-compromising abilities to other opportunistic infections that occur in patients exposed to this mold.[39]

A. versicolor is commonly found in damp indoor environments and on food products.[40] This mold produces more than 20 allergens that can irritate the nose, eyes, and throat.[41] *A. versicolor* is one of the more invasive species of aspergillosis.[42] This fungus has also been reported to be a major cause of onychomycosis (a fungal infection of the nails).[43]

Fusarium

Fusarium fungi grow best in temperate climate conditions.[44] *Fusarium* is present in both indoor as well as outdoor environments.[45] It also grows worldwide on many different types of grains, including corn and wheat.[46] Most trichothecene mycotoxins are produced from *Fusarium* species. The fungi can infiltrate the body through breaks in the skin or through inhalation. One other source of exposure is ingestion through contaminated foods. *Fusarium* infections are more likely with individuals who are immune compromised. These infections can lead to pneumonia, thrombophlebitis, and sinusitis.[47] Two of the most common species of *Fusarium* are *F. verticilliodes* and *F. solani.*

F. verticilliodes commonly contaminates grains, maize, and rice.[48] *F. verticilliodes* infections are more common in immune-compromised individuals than in healthy individuals. *F. verticilliodes* infections have also been reported in patients who have undergone a major organ transplant. Infections with this fungus have been reported to cause neutropenia, keratomycosis, necrotic lesions on the skin, and fever.[49]

F. solani is one of the most virulent of all of the *Fusarium* species.[50] In patients who are immune compromised, *F. solani* can cause arthritis, cutaneous infections, subcutaneous infections, and sinusitis.[51] Some strains of *F. solani* have been reported to produce a biofilm on soft contact lenses. *F. solani* is also resistant to many antifungal agents but has been shown to be sensitive to amphotericin B and natamycin.

Penicillium

Penicillium is a blue-green mold found on fruits, vegetables, and indoor environments. Many different types of citrus fruits can become contaminated with *Penicillium,* but it can also contaminate seeds and grains. Factors leading to a contamination of *Penicillium* in the home, work, or school environment include inadequate heating and ventilation, water leaks, and low sunlight.[52] Indoor environments where *Penicillium* can be found include wallpaper, carpet, furniture, and fiberglass insulation. Exposure to *Penicillium* can lead to breathing difficulties such as wheezing, persistent cough, and asthma.[53] Besides the mycotoxins that are produced by *Penicillium,* there are several other allergens produced that affect human health. These include alkaline and vacuolar serine proteases, which are referred to as group 13 and group 18 allergens, respectively.[54]

Stachybotrys

Stachybotrys is a greenish-black mold. This mold can grow on materials with a high cellulose and low nitrogen content, such as gypsum board, paper, fiberboard, and ceiling tiles. The humidity requirements for *Stachybotrys* are higher than those for other fungi, around 93%, whereas other fungi grow at approximately 75%, and it often occurs in the presence of other fungi.[6,55] Toxicity caused by *Stachybotrys* is mostly through the toxins and other compounds produced and less so from particle penetration from the spores. *Stachybotrys* spores are between 10 and 60 µm, which is too large to reach the alveoli.[56] Toxin production is dependent on nutrient levels, pH, temperature, and moisture. *Stachybotrys* spores are produced when there is high moisture content, and they become airborne when they dry out and are disturbed or when they are attached to dust particles.[57] In addition to mycotoxins, *Stachybotrys* produces nine phenylspirodrimanes, which inhibit complement activation; spirolactams and spriolactones, which are anticomplement components; cyclosporine, which is a potent immunosuppressor; as well as endothelin receptors antagonists.[58–60]

COMMON MYCOTOXINS

As previously mentioned, there are more than 400 known mycotoxins naturally produced by fungi in nature.[12] These toxins have been observed since the Middle Ages and were recognized to come from fungi in the 17th century.[61] The most common mycotoxins that can cause diseases in humans are aflatoxin, ochratoxin, gliotoxin, sterigmatocystin, and citrinin. Mycotoxins are produced by multiple species

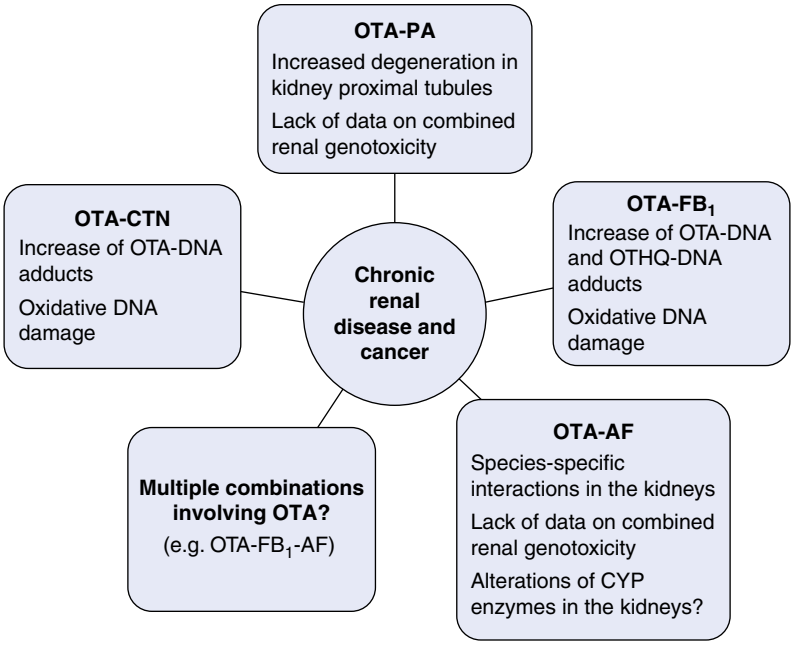


Fig. 138.2 Interactions of mycotoxin combinations involving ochratoxin A (OTA) and their possible role in the development of chronic renal diseases and cancer after chronic exposure to subtoxic concentrations of mycotoxin mixtures. (From Klaric MS, Rasic D, Peraica M. Deleterious effects of mycotoxin combinations involving ochratoxin A. *Toxins (Basel).* 2013;5[11]:196587. PubMed PMID: 24189375.)

of fungi depending on environmental conditions (see Table 138.1). The following summary discusses some of the more common mycotoxins and what organs they target.

Aflatoxin

Aflatoxin is produced by the fungi *Aspergillus* and is one of the most potent hepatocarcinogenic substances known.[62] The production of aflatoxin is dependent on the fungi's access to O_2, CO_2, zinc, and copper.[63] There are four major aflatoxins, called B_1, B_2, G_1, and G_2, of which B_1 is the most toxic.[64] Aflatoxin has been shown to be both toxic and carcinogenic to the human population, and the diseases caused by aflatoxin exposure are labeled aflatoxicoses. The liver is the primary target for aflatoxin. Aflatoxin is activated in the liver by the cytochrome P450s and is then capable of forming adducts to both DNA and proteins.[65] These DNA adducts may result in the activation of oncogenes, inhibition of DNA synthesis, disruption of DNA replication, and improper cellular division, leading to polypoidal cells.[66]

Aflatoxin is removed through the glutathione S-transferase system. This system can conjugate activated aflatoxin with reduced glutathione. This leads to aflatoxin becoming more water soluble, which assists in its excretion. It is theorized that variations in levels of P450s, glutathione transferase, and transporters can account for the variation in the response patients have to aflatoxin exposure.[67]

Ochratoxin

Ochratoxin is produced by species of *Penicillium* and *Aspergillus.* There are three forms of ochratoxin; however, only the A form exerts hazardous effects on humans. Ochratoxin is a common mycotoxin associated with indoor air problems; however, it is also known to contaminate many different types of food, such as cereals, meat, coffee, fruits, wine, and beer.[68,69] Ochratoxin can be distributed to many different tissues throughout the body, but its main targets are the liver and kidney. Ochratoxin is both nephrotoxic and carcinogenic to the kidney (Fig. 138.2).[70] Two of the most important mechanisms involved in ochratoxin toxicity include competition with phenylalanine in the phenylalanyl-tRNA synthase-catalyzed reaction, inhibiting protein synthesis, and induction of oxidative stress by lipid peroxidation.[71,72] Ochratoxin also has immunosuppressive effects. Exposure leads to the inhibition of T-helper-cell function and inhibition of antibody synthesis.[73] Crucial immune organs have also been shown to decrease in size from exposure, such as the thymus, bursa of Fabricius (in birds), the spleen, and lymph nodes.[74] This can lead to the overgrowth of intestinal pathogens such as *Candida* or *Clostridia*.[75]

Gliotoxin

Gliotoxin is produced by species of the fungi *Aspergillus, Penicillium,* and *Alternaria.* Gliotoxin exposure can be the result of contaminated food as well as from water damage. Gliotoxin is secreted by newly established hyphae, and its role is to inhibit the immune system of the host. Gliotoxin suppresses the phagocytic defenses, which is done by interfering with PtdIns (3,4,5) P_3 production. This impairs the macrophages' abilities to recognize, take up, and destroy invading pathogens.[76,77] Another way that gliotoxin promotes immunosuppression is by interfering with the activation of transcription factors that are involved in T-cell activation.[16] Gliotoxin's effects can lead to infections of *Aspergillus,* known as aspergillosis, which typically reside in the pulmonary system, the ears, the eyes, or the nails.

METHODS OF ASSESSMENT

For the past two decades, one of the greatest difficulties involving patients who may have been exposed to mycotoxins was determining whether the patient was exposed. For many years, researchers have been measuring mycotoxins in crops as well as in animals. The agricultural market needed a quick and inexpensive method to determine whether food was contaminated with mycotoxins and if it was fit for consumption. These methods have been attempted to be transferred to the analysis of human exposure; however, there are two barriers that

have prevented an easy transition. The first barrier has been the small quantity of mycotoxin in human samples, often in the parts per trillion (ppt), which can also be labeled ng/L. The U.S. Food and Drug Administration (FDA) action level for aflatoxin in food is 20 ppb (20 μg/L), which is a factor 100 times higher than seen in human samples.[78,79] Sample ranges for many mycotoxins in humans are normally in the range of 60 to 2000 ng/L (.06–2 μg/L).[79] The second barrier for transferring methods used in agriculture to human samples is matrix effects (MEs). MEs are compounds in the sample matrix that are not related to the analyte being measured but can cause a result to be either higher or lower than the actual amount. These MEs can influence any type of test, and protocols must be performed to separate the analyte of interest from these interferences.[80] There are two types of testing used to ascertain whether a patient has been exposed to mycotoxins, enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–mass spectrometry (LC-MS/MS).

ELISA

ELISA has been used for analytical testing since the early 1970s.[81] ELISA can use multiple types of samples, such as (but not limited to) saliva, serum, and urine. It is used to detect the presence of antibodies or antigens in a sample. This technique normally uses 96-well plates, which are analyzed by a spectrometer. Antibodies are produced that can have direct degrees of selectivity to an antigen. The selection of an antibody will determine the specificity for an ELISA test. Most ELISA tests for mycotoxin use the competitive direct ELISA model. There are several steps involved in this model:

1. The primary antibody, which is specific to the analyte that is being measured, is incubated with the sample. During this step, the analyte that is being tested binds with the primary antibody. Any free antibody that is not bound at this step will bind with the antigen that is bound to the wells in step 2.
2. The antibody–antigen complex produced in step 1 is added to 96-well plates. These plates are coated with the same antigen (analyte) being testing. Free antibody that was not bound in step 1 will bind to the antigen coated to the wells. More antigen in the patient sample will lead to less antibody being bound to the plates.
3. After incubation, the plates are washed, which removes the antibodies that have been complexed to the patient's antigens.
4. The next step is to incubate a secondary antibody, which binds to the primary antibody. This secondary antibody has some type of label or enzyme covalently bound, which will cause a color change in the well. There are now three layers bound to the plate. The first layer is the antigen that is bound to the plate, the second layer is the primary antibody, and the third layer is the secondary antibody.
5. The final step is that a substrate is added that interacts with the label bound to the secondary antibody. The plate is then read in a spectrometer, which then gives a readout on the concentration. The result is inverse to the concentration. The more the well changes color, the lower the amount of analyte the patient has in his or her sample.

The benefit of ELISA tests is that it does not take expensive equipment to run the test, which means that the test can be done for a lower cost. An additional benefit is that many different analytes can be measured if specific antibodies can be developed. There are also several drawbacks to ELISA testing. First, there are more opportunities for interferences to influence the results, causing results to be either higher or lower than what is truly in the sample. Second, it is harder to monitor multiple analytes because each analyte requires its own well. Third, the dynamic range for ELISA tests is inherently limited, which causes the test to be more qualitative and less quantitative. This prevents the practitioner from receiving truly quantitative results.

Liquid Chromatography-MS/MS

The introduction of LC-MS/MS has allowed the quantitative measure of many different chemicals. These measurements can be done on compounds either individually or simultaneously if the compounds have similar molecular characteristics. A mass spectrometer is a device that measures the molecular mass of compounds. Many different fields have used this technology, and two of the most common are drug testing and chemical exposure testing, which both need the sensitivity and selectiveness that LC-MS/MS provides.[82] Over the past 10 years, LC-MS/MS has become the gold standard for the measurement of mycotoxins.[83] There are three parts involved in preparation and analysis with LC-MS/MS: extraction of the samples before injection, separation on a column, and fragmentation in the mass spectrometric detector.

Proper sample preparation is a critical step for accurate measurement of most molecules using LC-MS/MS. There are three methods used by laboratory professionals to separate the mycotoxin analytes away from the contaminates that can interfere with the analysis. These methods include liquid-liquid extraction (LLE); solid-liquid extraction (SLE); salting-out liquid-liquid extraction (SALLE); the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method; and the dispersive liquid-liquid micro-extraction method (DLLME). Each of these methods has different benefits; however, the goal of each is to separate the analytes of interest away from matrix interferences and to concentrate the sample.[83]

After the samples are prepped, they must be injected into the LC-MS/MS. The samples are then transported through a separation column by a combination of solvent and water. There are multiple solvents that a laboratory can use, and the ratio of solvent to aqueous can also vary between preparation techniques. Common solvents include acetonitrile, methanol, chloroform, hexane, and ethyl acetate. The columns, which are labeled ultra-performance liquid chromatography (UPLC) columns, can also vary between protocols. Most protocols use C18 reverse-phase columns, which consists of strands of 18 carbon molecules packed into a column, usually between 100 to 150 mm in length. However, there are many different varieties of columns, which vary in their dimensions as well as the packing material. These differences in packing material can alter how quickly the sample will transverse through the column and then enter the mass spectrometer.[84]

Once the analytes enter the mass spectrometer, they are processed in two stages. In the first stage, molecules are ionized by electrospray ionization (ESI).[85] The instrument determines the mass-to-charge (m/z) spectra of the parent ions, which yields the mass of the molecule being analyzed. These molecules are then fragmented in a process called collision-induced dissociation (CID). In CID, an energized molecule collides with a buffer gas, which causes the molecule's bonds to break into predictable patterns. These patterns can be interpreted like a molecular fingerprint.[86] When molecules are fragmented with LC-MS/MS, they give a repeatable pattern, which can be used to properly identify molecules (Fig. 138.3). With this ability to have great specificity in distinguishing between different molecules, mass spectrometry provides both laboratory professionals and health care practitioners great assurance that their data are correct.

TREATMENT

Avoidance and Remediation

Mycotoxin and fungi exposure can lead to many different symptoms, including fatigue, headaches, difficulty breathing, rashes, gastrointestinal issues, joint pain, decreased libido, and more. As previously mentioned, these mycotoxins can come from water-damaged buildings or

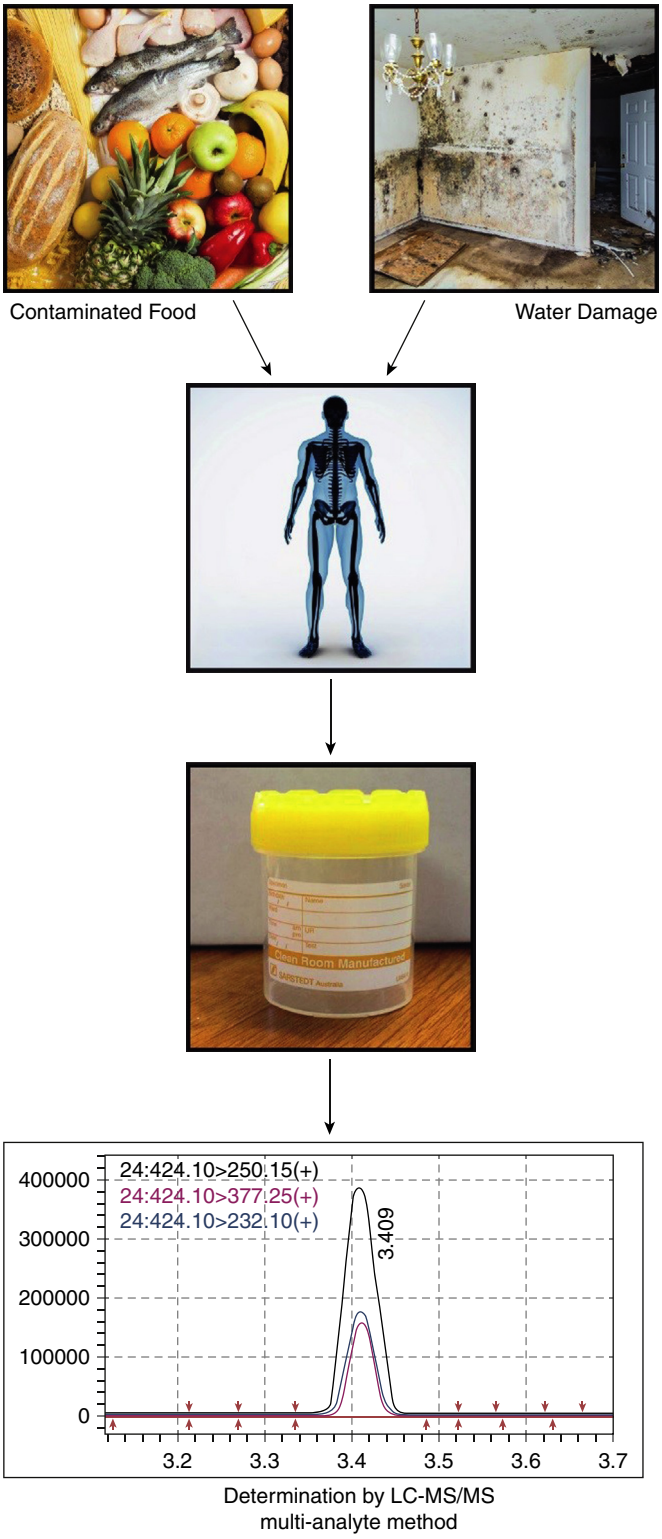


Fig. 138.3 When molecules are fragmented in liquid chromatography with mass spectrometry (LC-MS/MS), they give a repeatable pattern that can be used to properly identify molecules.

from food contamination. The most important element for treatment is the avoidance of further exposure to water-damaged environments as well as contaminated foods. Using a licensed company to inspect and remediate the area is recommended. Remediation of contaminated areas is difficult because heat, ultraviolet (UV) light, bleach, ammonia, and ozone do not completely remove mold from contaminated areas.[87] Air spore counts are frequently done; however, they are usually done over a short period of time and often result in false-negative results. Another problem is that killing the mold does not eliminate the risk because mycotoxins will remain in the environment.

Mycotoxin Excretion and Detoxification

Patients exposed to mycotoxins from mold may have two different conditions to treat. The first is the mycotoxin exposure, which can cause problems in many different tissues mentioned previously, and second is a possible infection and colonization of the fungi. To detoxify the mycotoxins, it is recommended to use a combination of glutathione, sauna, sequestering/binding agents, and antioxidants. Patients may also need to treat fungal infections and colonization.

Detoxification of many mycotoxins is dependent on the endogenous molecule glutathione (GSH). For example, deoxynivalenol (DON) is a mycotoxin produced by certain *Fusarium* species that frequently infect corn, wheat, oats, barley, and rice, which can be detoxified when glutathione-S-transferase forms a conjugant with GSH and DON (Fig. 138.4). GSH is a tripeptide, which is made of glycine, cysteine, and glutamate. Mycotoxin toxicity is intensified in patients with glutathione transferase mutations (GSTP1 and GSTM).[88] GSH can be introduced in several ways, including treating with GSH precursors such as N-acetyl cysteine and whey protein or with an oral liposomal, transdermal, intravenous, nebulized, or intranasal form of GSH. It is recommended to use GSH with sequestering agents because treatment with GSH has been shown to increase the mobilization of mycotoxins in the bloodstream.[89]

Sauna therapy has been used for centuries for the removal of toxins from the body. Some of the most commonly used saunas are dry-heat radiant saunas and far-infrared saunas. Both have been shown to be equally as good at removing toxins from the body. Infrared saunas have the advantage of inducing sweating at lower body temperatures. Sweating is important because many mycotoxins are lipophilic and can bind to lipid proteins, which will allow the mycotoxins to persist in the body for extended periods of time.[90] The use of sauna has two benefits pertaining to detoxification of mycotoxins. First, it breaks the bonds between mycotoxins and lipids in the body, increasing the circulation of mycotoxins; and second, it provides a second excretion route because several mycotoxins have been found in human sweat.[91] One study of 28 individuals who had been exposed to mycotoxins included a regimen of sauna, exercise, and physical therapy. In this study, all 28 individuals experienced significant improvement.[92]

Because both GSH treatment and sauna therapy increase the number of mycotoxins in the bloodstream, it is important to use sequestering agents in the treatment of mycotoxins. Many of these agents have shown efficacy in lowering mycotoxin levels. The most common sequestering agents used are activated charcoal, cholestyramine, clay, and chlorophyll. Activated charcoal has been used for about two decades in the treatment of mycotoxins. Multiple studies have validated the use of activated charcoal because of its high affinity for many different types of mycotoxins.[93–95] Cholestyramine (CSM) is an anion exchange resin that works as a bile-acid–sequestering agent. Multiple studies have demonstrated that CSM treatment leads to a reduction of ochratoxin levels in the plasma and urine and increases the fecal excretion. CSM has also been shown to be safely tolerated in children.[96,97]

Antioxidants

One additional component of treatment that is necessary is the addition of antioxidant support. Mycotoxins exposure induces oxidative stress and cell as cellular DNA damage; however, antioxidants have been shown to decrease these effects. Patients treated with vitamins A,

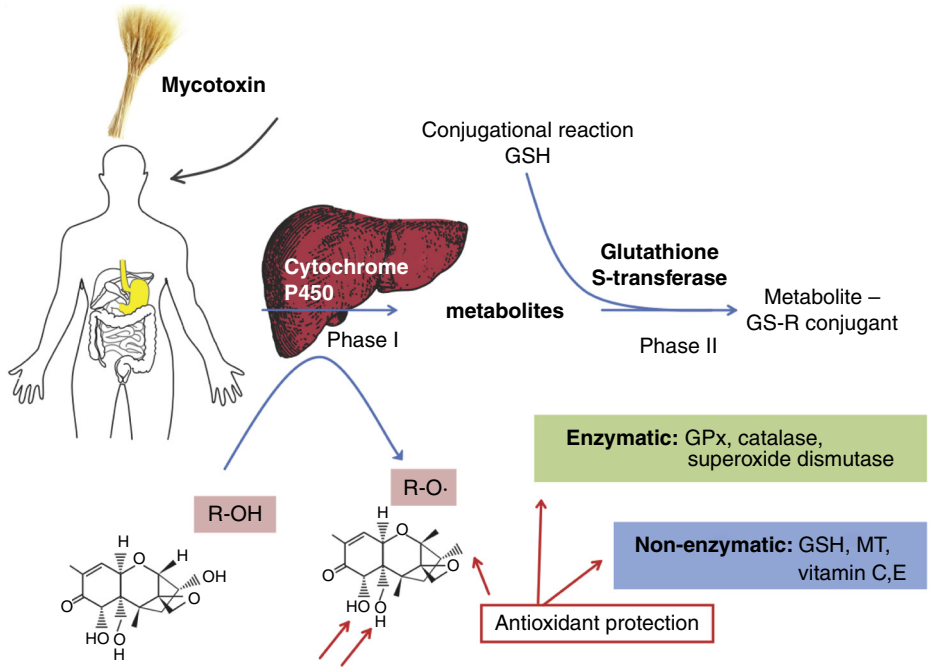


Fig. 138.4 Scheme of the possible manner of deoxynivalenol detoxification. The first—and perhaps the most important—pathway used for the detoxifying of deoxynivalenol (DON) is cytochrome P450, which serves to catalyze the oxidation of organic substances. This pathway, however, can cause free hydroxyl groups of DON to be cleaved, producing DON-radical, which can be more dangerous. The DON-radical can be scavenged by enzymatic (glutathione peroxidase [GPx], catalase, superoxide dismutase) or nonenzymatic (reduced glutathione [GSH], metallothionein [MT], and vitamins) processes. Nevertheless, cytochrome P450 can be followed by Phase II, in which glutathione-S-transferase can form a conjugant with GSH and DON, which results in detoxification of the xenobiotic. (From Sobrova P, Adam V, Vasatkova A, Beklova M, Zeman L, Kizek R. Deoxynivalenol and its toxicity. *Interdisc. Toxicol.* 2010;3[3]:94–99. PubMed PMID: 21217881.)

C, and E were shown to have better outcomes.[98] Another antioxidant that has been shown to cause significant hepatoprotective activity is curcumin. In a rat study, rats that were exposed to aflatoxin B1 and treated with curcumin had significantly lower serum enzyme markers and higher levels of reduced glutathione, catalase, and glutathione peroxidase.[99]

Fungal Treatment

Patients who have high levels of mycotoxins in their bodies may also be suffering from fungal infections and colonization. This is usually caused by direct exposure to elevated levels of mold spores. Fungal infections can occur in many different parts of the body. The most common areas of infection are the sinus cavities, lungs, and intestine. One Mayo Clinic study found fungal growth in 96% of patients with chronic sinusitis. The types of mold found included *Aspergillus, Penicillium, Alternaria, Cladosporium, Fusarium,* and *Cryptococcus.* All of these molds are known to produce mycotoxins and cause significant diseases.[100,101] One study demonstrated that antifungal drug therapies were useful in combating microbial pathogens detected by gas chromatography–mass spectrometry.[102] Another recent study compared organic acid results from patients who had positive results on an LC-MS/MS mycotoxin with control subjects. This study demonstrated that certain markers are more elevated in patients who have been exposed to mycotoxins.[103] The two categories of markers that were most pronounced were fungal markers and mitochondrial markers (Fig. 138.5). The two fungal markers that were significant were 5-hydroxymethyl-2-furioc and Furan-2,5-dicarboxylic, which are both fungal metabolites.[104,105] These data indicate that certain organic acid tests that measure these markers may be helpful in diagnosing fungal infections.

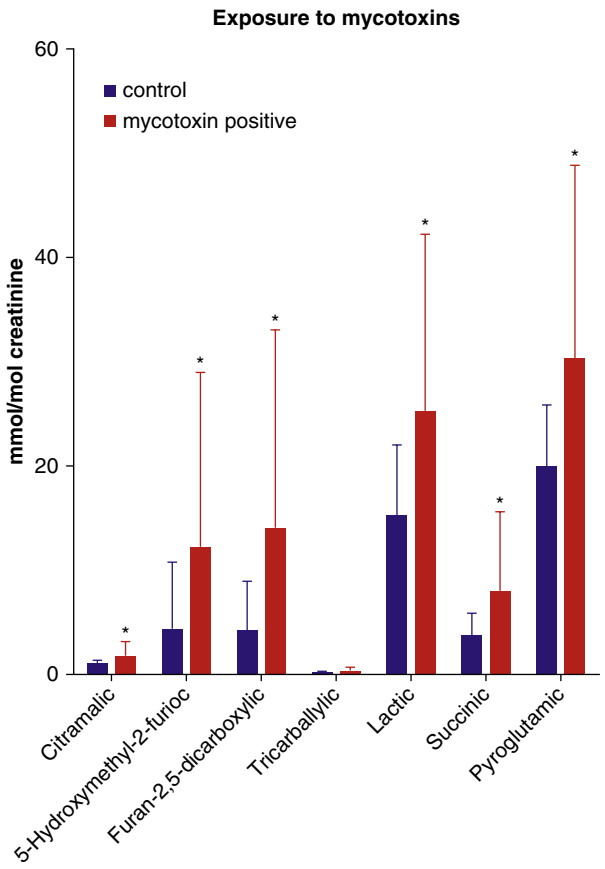


Fig. 138.5 Exposure to mycotoxins.

Treatment for fungal infections may involve several steps. The first step should be the avoidance of elevated spore counts and particulates through proper remediation and air filters. Second, antifungal medications have been shown to be efficacious. Multiple studies have used amphotericin B for the treatment of fungal infections.[106–108] Other antifungals that have been used include ketoconazole, fluconazole, and itraconazole. For intestinal fungal infections, nystatin or liposomal nystatin may be a good alternative.[109,110]

CONCLUSION

Mycotoxin and fungal exposure can lead to many chronic illnesses, including fatigue, headaches, rashes, pulmonary infections, and even cancer. Mycotoxin exposure may also lead to many other coinfections because of the mycotoxins' abilities to impair immune system function. Because the concentrations of mycotoxin metabolites in human patients are quite low, testing has moved away from ELISA to the more sophisticated and sensitive LC-MS/MS technology. Treatment plans for patients exposed to mycotoxins that have been the most efficacious have been multifactorial, including binders, sauna, glutathione, antioxidants, and sometimes antifungal therapies. Treatment plans can take anywhere from a few months to a year to have sufficient removal of the toxins. However, most patients show significant improvements after removal of the mycotoxins.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Shifrin VI, Anderson P. Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. *J Biol Chem*. 1999;274:13985–13992.
2. Howard DH. *Pathogenic Fungi in Humans and Animals*. 2nd ed. New York: Marcel Dekker; 2003.
3. Mullen J, Hodgson MJ, DeGraff CA, Godar T. Case-control study of idiopathic pulmonary fibrosis and environmental exposures. *J Occup Environ Med*. 1998;40:363–367.
4. Bennett JW, Klich M. Mycotoxins. *Clin Microbiol Rev*. 2003;16:497–516.
5. Auger PL, Gourdeau P, Miller JD. Clinical experience with patients suffering from a chronic fatigue-like syndrome and repeated upper respiratory infections in relation to airborne molds. *Am J Ind Med*. 1994;25:41–42.
6. Hodgson MJ, et al. Building-associated pulmonary disease from exposure to Stachybotrys chartarum and Aspergillus versicolor. *J Occup Environ Med*. 1998;40:241–249.
7. Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air*. 2007;17(4):284–296.
8. Miller JD, McMullin DR. Fungal secondary metabolites as harmful indoor air contaminants: 10 years on. *Appl Microbiol Biotechnol*. 2014;98(24):9953–9966.
9. Mudarri DH. Valuing the economic costs of allergic rhinitis, Acute bronchitis, and asthma from exposure to indoor dampness and mold in the US. *J Environ Public Health*. 2016;2016:2386596. https://doi.org/10.1155/2016/2386596.
10. Miller JD. Mycotoxins in small grains and maize: old problems, new challenges. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2008;25:219–230. https://doi.org/10.1080/02652030701744520.
11. Smoragiewicz W, Cossette B, Boutard A, Krzystyniak K. Trichothecene mycotoxins in the dust of ventilation systems in office buildings. *Int Arch Occup Environ Health*. 1993;65:113–117.
12. Bullerman LB, Bianchini A. Stability of mycotoxins during food processing. *Int J Food Microbiol*. 2007;119:140–146. https://doi.org/10.1016/j.ijfoodmicro.2007.07.035.
13. More TT, Yan S, Tyagi RD, Surampalli RY. Potential use of filamentous fungi for wastewater sludge treatment. *Bioresour Technol*. 2010;101:7691–7700. https://doi.org/10.1016/j.biortech.2010.05.033.
14. Hageskal G, Lima N, Skaar I. The study of fungi in drinking water. *Mycol Res*. 2009;113:165–172. https://doi.org/10.1016/j.mycres.2008.10.002.
15. Fontaine T, et al. Galactosaminogalactan, a new immunosuppressive polysaccharide of Aspergillus fumigatus. *PLoS Pathog*. 2011;7:e1002372. https://doi.org/10.1371/journal.ppat.1002372.
16. Pahl HL, et al. The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-kappaB. *J Exp Med*. 1996;183:1829–1840.
17. Corrier DE. Mycotoxicosis: mechanisms of immunosuppression. *Vet Immunol Immunopathol*. 1991;30:73–87.
18. Gniadek A, Macura AB, Gorkiewicz M. Cytotoxicity of *Aspergillus* fungi isolated from hospital environment. *Pol J Microbiol*. 2011;60:59–63.
19. Pace JG, Watts MR, Canterbury WJ. T-2 mycotoxin inhibits mitochondrial protein synthesis. *Toxicon*. 1988;26:77–85.
20. Hassan YI, Watts C, Li XZ, Zhou T. A novel Peptide-binding motifs inference approach to understand deoxynivalenol molecular toxicity. *Toxins (Basel)*. 2015;7:1989–2005. https://doi.org/10.3390/toxins7061989.
21. Chakravarthi BV, Sujay R, Kuriakose GC, Karande AA, Jayabaskaran C. Inhibition of cancer cell proliferation and apoptosis-inducing activity of fungal taxol and its precursor baccatin III purified from endophytic Fusarium solani. *Cancer Cell Int*. 2013;13:105. https://doi.org/10.1186/1475-2867-13-105.
22. Ostry V, Malir F, Toman J, Grosse Y. Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Res*. 2017;33:65–73. https://doi.org/10.1007/s12550-016-0265-7.
23. Cavalieri EL, et al. Catechol ortho-quinones: the electrophilic compounds that form depurinating DNA adducts and could initiate cancer and other diseases. *Carcinogenesis*. 2002;23:1071–1077.
24. Fehr M, et al. Alternariol acts as a topoisomerase poison, preferentially affecting the IIalpha isoform. *Mol Nutr Food Res*. 2009;53:441–451. https://doi.org/10.1002/mnfr.200700379.
25. Tuomi T, et al. Mycotoxins in crude building materials from water-damaged buildings. *Appl Environ Microbiol*. 2000;66:1899–1904.
26. Nikulin M, et al. Comparison of detection methods for trichothecenes produced by Fusarium sporotrichioides on fodder and grains at different air humidities. *Nat Toxins*. 1996;4:117–121.
27. Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ Health Perspect*. 2010;118:818–824. https://doi.org/10.1289/ehp.0901388.
28. Tao Y, et al. Ochratoxin A: toxicity, oxidative stress and metabolism. *Food Chem Toxicol*. 2018;112:320–331. https://doi.org/10.1016/j.fct.2018.01.002.
29. Nielsen KF, et al. Production of mycotoxins on artificially and naturally infested building materials. *Mycopathologia*. 1999;145:43–56.
30. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak settings. *J Hosp Infect*. 2006;63:246–254. https://doi.org/10.1016/j.jhin.2006.02.014.
31. Horner WE, Helbling A, Salvaggio JE, Lehrer SB. Fungal allergens. *Clin Microbiol Rev*. 1995;8:161–179.
32. Vardon PJM C, Nardinelli C. *Potential economic costs of mycotoxins in the United StatesMycotoxins: Risks in Plant, Animal, and Human Systems; Task Force Report*. ; 2003.
33. Galimberti R, Torre AC, Baztan MC, Rodriguez-Chiappetta F. Emerging systemic fungal infections. *Clin Dermatol*. 2012;30:633–650. https://doi.org/10.1016/j.clindermatol.2012.01.011.
34. J.P.F, D M, M.D.AMC. Mycotoxins. *Anim. Feed Sci Technol.* 1997;69.
35. Soliman KM. Incidence, level, and behavior of aflatoxins during coffee bean roasting and decaffeination. *J Agric Food Chem*. 2002;50:7477–7481.
36. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Dennin DW. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology*. 2007;153:1677–1692. https://doi.org/10.1099/mic.0.2007/007641-0.
37. Person AK, Chudgar SM, Norton BL, Tong BC, Stout JE. *Aspergillus Niger:* an unusual cause of invasive pulmonary aspergillosis. *J Med Microbiol*. 2010;59:834–838. https://doi.org/10.1099/jmm.0.018309-0.
38. Kupfahl C, et al. Gliotoxin production by clinical and environmental *Aspergillus fumigatus* strains. *Int J Med Microbiol*. 2008;298:319–327. https://doi.org/10.1016/j.ijmm.2007.04.006.
39. Wartenberg D, et al. Secretome analysis of Aspergillus fumigatus reveals Asp-hemolysin as a major secreted protein. *Int J Med Microbiol*. 2011;301:602–611. https://doi.org/10.1016/j.ijmm.2011.04.016.
40. Engelhart S, et al. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Appl Environ Microbiol*. 2002;68:3886–3890.
41. Benndorf D, et al. Identification of spore allergens from the indoor mould *Aspergillus versicolor*. *Allergy*. 2008;63:454–460. https://doi.org/10.1111/j.1398-9995.2007.01603.x.
42. Charles MP, Noyal MJ, Easow JM, M R. Invasive pulmonary aspergillosis caused by *Aspergillus versicolor* in a patient on mechanical ventilation. *Australas Med J*. 2011;4:632–634. doi:10.4066/AMJ.2011.905.
43. Torres-Rodriguez JM, Madrenys-Brunet N, Siddat M, Lopez-Jodra O, Jimenez T. *Aspergillus versicolor* as cause of onychomycosis: report of 12 cases and susceptibility testing to antifungal drugs. *J Eur Acad Dermatol Venereol*. 1998;11:25–31.
44. Doczi I, Gyetvai T, Kredics L, Nagy E. Involvement of *Fusarium* spp. in fungal keratitis. *Clin Microbiol Infect*. 2004;10:773–776. https://doi.org/10.1111/j.1469-0691.2004.00909.x.
45. Jarvis BB, Lee YW, Comezoglu SN, Yatawara CS. Trichothecenes produced by *Stachybotrys atra* from Eastern Europe. *Appl Environ Microbiol*. 1986;51:915–918.
46. Escobar J, et al. Occurrence and exposure assessment of *Fusarium* mycotoxins in maize germ, refined corn oil and margarine. *Food Chem Toxicol*. 2013;62:514–520. https://doi.org/10.1016/j.fct.2013.09.020.
47. Croft WA, Jastromski BM, Croft AL, Peters HA. Clinical confirmation of trichothecene mycotoxicosis in patient urine. *J Environ Biol*. 2002;23:301–320.

48. Chu FS, Li GY. Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. *Appl Environ Microbiol*. 1994;60:847–852.
49. Cocchi S, et al. Fusarium verticillioides fungemia in a liver transplantation patient: successful treatment with voriconazole. *Diagn Microbiol Infect Dis*. 2011;71:438–441. https://doi.org/10.1016/j.diagmicrobio.2011.08.024.
50. Zhang N, et al. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *J Clin Microbiol*. 2006;44:2186–2190. https://doi.org/10.1128/JCM.00120-06.
51. Summerbell RC, et al. Ecology of fungi in human dwellings. *J Med Vet Mycol*. 1992;30(Suppl 1):279–285.
52. Sharpe R, Thornton CR, Osborne NJ. Modifiable factors governing indoor fungal diversity and risk of asthma. *Clin Exp Allergy*. 2014;44:631–641. https://doi.org/10.1111/cea.12281.
53. Gent JF, et al. Household mold and dust allergens: exposure, sensitization and childhood asthma morbidity. *Environ Res*. 2012;118:86–93. https://doi.org/10.1016/j.envres.2012.07.005.
54. Chou H, et al. Alkaline serine proteinase is a major allergen of *Aspergillus flavus*, a prevalent airborne *Aspergillus* species in the Taipei area. *Int Arch Allergy Immunol*. 1999;119:282–290. https://doi.org/10.1159/000024205.
55. Grant G, Hunter CA, Flannigan B, Bravery AF. The moisture requirements of moulds isolated from domestic buildings. *Int. Biodeterior.* 1989;25:259–284.
56. Giddings MJ, ed. *Canada Health and Welfare*. Ottawa, Canada: Department of National Health and Welfare; 1986.
57. Hintikka EL, Nikulin M. Airborne mycotoxins in agricultural and indoor environments. *Indoor Air Suppl*. 1998;4:66–70.
58. Jarvis BB. Stachybotrys chartarum: a fungus for our time. *Phytochemistry*. 2003;64:53–60.
59. Nakamura M, et al. Stachybocins, novel endothelin receptor antagonists, produced by *Stachybotrys* sp. M6222. I. Taxonomy, fermentation, isolation and characterization. *J Antibiot (Tokyo)*. 1995;48:1389–1395.
60. Miyazaki W, et al. A complement inhibitor produced by *Stachybotrys complementi,* nov. sp. K-76, a new species of fungi imperfecti. *Microbiol Immunol*. 1980;24:1091–1108.
61. Peraica M, Radic B, Lucic A, Pavlovic M. Toxic effects of mycotoxins in humans. *Bull World Health Organ*. 1999;77:754–766.
62. Huynh VL, Gerdes RG, Lloyd AB. Synthesis and degradation of aflatoxins by *Aspergillus parasiticus*. II. Comparative toxicity and mutagenicity of aflatoxin B1 and its autolytic breakdown products. *Aust J Biol Sci*. 1984;37:123–129.
63. Stuart BP, Bedell DM. Mycotoxicosis in swine. *Vet Clin North Am Large Anim Pract*. 1982;4:377–388.
64. Squire RA. Ranking animal carcinogens: a proposed regulatory approach. *Science*. 1981;214:877–880.
65. Kelly JD, Eaton DL, Guengerich FP, Coulombe Jr RA. Aflatoxin B1 activation in human lung. *Toxicol Appl Pharmacol*. 1997;144:88–95. https://doi.org/10.1006/taap.1997.8117.
66. Egner PA, et al. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. *Proc Natl Acad Sci U S A*. 2001;98:14601–14606. https://doi.org/10.1073/pnas.251536898.
67. Raj HG, Prasanna HR, Magee PN, Lotlikar PD. Effect of purified rat and hamster hepatic glutathione S-transferases on the microsome mediated binding of aflatoxin B1 to DNA. *Cancer Lett*. 1986;33:1–9.
68. Krogh P. Mycotoxin tolerances in foodstuffs. *Ann Nutr Aliment*. 1977;31:411–414.
69. Schuster E, Dunn-Coleman N, Frisvad JC, Van Dijck PW. On the safety of *Aspergillus Niger*—a review. *Appl Microbiol Biotechnol*. 2002;59:426–435. https://doi.org/10.1007/s00253-002-1032-6.
70. Ringot D, Chango A, Schneider YJ, Larondelle Y. Toxicokinetics and toxicodynamics of ochratoxin A, an update. *Chem Biol Interact*. 2006;159:18–46. https://doi.org/10.1016/j.cbi.2005.10.106.
71. Sakthivelan SM, Sudhakar Rao GV. Effect of ochratoxin a on body weight, feed intake and feed conversion in broiler chicken. *Vet Med Int*. 2010;2010:590432. https://doi.org/10.4061/2010/590432.
72. Gillman IG, Clark TN, Manderville RA. Oxidation of ochratoxin A by an Fe-porphyrin system: model for enzymatic activation and DNA cleavage. *Chem Res Toxicol*. 1999;12:1066–1076.
73. Lea T, Steien K, Stormer FC. Mechanism of ochratoxin A-induced immunosuppression. *Mycopathologia*. 1989;107:153–159.
74. Boorman GA, et al. Myelotoxicity and macrophage alteration in mice exposed to ochratoxin A. *Toxicol Appl Pharmacol*. 1984;72:304–312.
75. Park SH, Kim D, Kim J, Moon Y. Effects of mycotoxins on mucosal microbial infection and related pathogenesis. *Toxins (Basel)*. 2015;7:4484–4502. https://doi.org/10.3390/toxins7114484.
76. Kupfahl C, Geginat G, Hof H. Gliotoxin-mediated suppression of innate and adaptive immune functions directed against *Listeria monocytogenes*. *Med Mycol*. 2006;44:591–599. https://doi.org/10.1080/13693780600815411.
77. Schlam D, et al. Gliotoxin suppresses macrophage immune function by Subverting Phosphatidylinositol 3,4,5-Trisphosphate Homeostasis. *MBio*. 2016;7:e02242. https://doi.org/10.1128/mBio.02242-15.
78. Mitchell NJ, Bowers E, Hurburgh C, Wu F. Potential economic losses to the US corn industry from aflatoxin contamination. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 2016;33:540–550. https://doi.org/10.1080/19440049.2016.1138545.
79. Solfrizzo M, Gambacorta L, Visconti A. Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. *Toxins (Basel)*. 2014;6:523–538. https://doi.org/10.3390/toxins6020523.
80. Stahnke H, Kittlaus S, Kempe G, Alder L. Reduction of matrix effects in liquid chromatography-electrospray ionization-mass spectrometry by dilution of the sample extracts: how much dilution is needed? *Anal Chem*. 2012;84:1474–1482. https://doi.org/10.1021/ac202661j.
81. Avrameas S, Guilbert B. Enzyme-immunoassay for the measurement of antigens using peroxidase conjugates. *Biochimie*. 1972;54:837–842.
82. JSC T, GL L, Wong RC, Tse HY, eds. *Drug-testing Technologies and Applications*. Humana Press; 2005:29–69.
83. Escriva L, Font G, Manyes L, Berrada H. Studies on the presence of mycotoxins in biological samples: an overview. *Toxins (Basel)*. 2017;9. https://doi.org/10.3390/toxins9080251.
84. Griffiths WJ, Jonsson AP, Liu S, Rai DK, Wang Y. Electrospray and tandem mass spectrometry in biochemistry. *Biochem J*. 2001;355:545–561.
85. Liu WT, Kersten RD, Yang YL, Moore BS, Dorrestein PC. Imaging mass spectrometry and genome mining via short sequence tagging identified the anti-infective agent arylomycin in *Streptomyces roseosporus*. *J Am Chem Soc*. 2011;133:18010–18013. https://doi.org/10.1021/ja2040877.
86. Sleno L, Volmer DA. Ion activation methods for tandem mass spectrometry. *J Mass Spectrom*. 2004;39:1091–1112. https://doi.org/10.1002/jms.703.
87. Peitzch B, Haase M, Larson. *J Environ Monitoring*. 2012;14:908–915.
88. Sun CA, et al. Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. *Carcinogenesis*. 2001;22:1289–1294.
89. Hope J. A review of the mechanism of injury and treatment approaches for illness resulting from exposure to water-damaged buildings, mold, and mycotoxins. *Scientific World J*. 2013;2013:767482. https://doi.org/10.1155/2013/767482.
90. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*. 2010;31:71–82. https://doi.org/10.1093/carcin/bgp264.
91. Genuis S. Personal Communication. 2017.
92. Rea WJ, Pan Y, Griffiths B. The treatment of patients with mycotoxin-induced disease. *Toxicol Ind Health*. 2009;25:711–714. https://doi.org/10.1177/0748233709348281.
93. Diaz DE, et al. Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*. 2004;157:233–241.
94. Gibson NM, Luo TJ, Brenner DW, Shenderova O. Immobilization of mycotoxins on modified nanodiamond substrates. *Biointerphases*. 2011;6:210–217. https://doi.org/10.1116/1.3672489.
95. Avantaggiato G, Havenaar R, Visconti A. Evaluation of the intestinal absorption of deoxynivalenol and nivalenol by an in vitro gastrointestinal model, and the binding efficacy of activated carbon and other adsorbent

materials. *Food Chem Toxicol*. 2004;42:817–824. https://doi.org/10.1016/j.fct.2004.01.004.
96. Kerkadi A, et al. Cholestyramine protection against ochratoxin A toxicity: role of ochratoxin A sorption by the resin and bile acid enterohepatic circulation. *J Food Prot*. 1999;62:1461–1465.
97. Tonstad S, Knudtzon J, Sivertsen M, Refsum H, Ose L. Efficacy and safety of cholestyramine therapy in peripubertal and prepubertal children with familial hypercholesterolemia. *J Pediatr*. 1996;129:42–49.
98. Alpsoy L, Yildirim A, Agar G. The antioxidant effects of vitamin A, C, and E on aflatoxin B1-induced oxidative stress in human lymphocytes. *Toxicol Ind Health*. 2009;25:121–127. https://doi.org/10.1177/0748233709103413.
99. El-Agamy DS. Comparative effects of curcumin and resveratrol on aflatoxin B(1)-induced liver injury in rats. *Arch Toxicol*. 2010;84:389–396. https://doi.org/10.1007/s00204-010-0511-2.
100. Praneenararat S. Fungal infection of the colon. *Clin Exp Gastroenterol*. 2014;7:415–426. https://doi.org/10.2147/CEG.S67776.
101. JU P, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc*. 1999;74:877–884. https://doi.org/10.4065/74.9.877.
102. Shaw W, Kassen E, Chaves E. Assessment of antifungal drug therapy in autism by measurement of suspected microbial metabolites in urine with gas chromatography-mass spectrometry. *Clin Pract Alternat Med*. 2000;1:15–20.
103. *Unpublished Work*. Great Plains Laboratory; 2018.
104. Kimura Y, et al. Nematicidal activity of 5-hydroxymethyl-2-furoic acid against plant-parasitic nematodes. *Z Naturforsch C*. 2007;62:234–238.
105. Karich A, Kleeberg SB, Ullrich R, Hofrichter M. Enzymatic preparation of 2,5-furandicarboxylic acid (FDCA)-A substitute of terephthalic acid-by the joined action of three fungal enzymes. *Microorganisms*. 2018;6. https://doi.org/10.3390/microorganisms6010005.
106. JU P, Sherris DA, Weaver A, Kita H. Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomized, placebo-controlled, double-blind pilot trial. *J Allergy Clin Immunol*. 2005;115:125–131. https://doi.org/10.1016/j.jaci.2004.09.037.
107. Liang KL, et al. Amphotericin B irrigation for the treatment of chronic rhinosinusitis without nasal polyps: a randomized, placebo-controlled, double-blind study. *Am J Rhinol*. 2008;22:52–58. https://doi.org/10.2500/ajr.2008.22.3115.
108. JU P, Sherris DA, Kita H, Kern EB. Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis. *J Allergy Clin Immunol*. 2002;110:862–866.
109. Johnson EM, Ojwang JO, Szekely A, Wallace TL, Warnock DW. Comparison of in vitro antifungal activities of free and liposome-encapsulated nystatin with those of four amphotericin B formulations. *Antimicrob Agents Chemother*. 1998;42:1412–1416.
110. Offner F, et al. Liposomal nystatin in patients with invasive aspergillosis refractory to or intolerant of amphotericin B. *Antimicrob Agents Chemother*. 2004;48:4808–4812. https://doi.org/10.1128/AAC.48.12.4808-4812.2004.

Sports Nutrition

Ralph Esposito, ND, LAc, Jade Teta, ND, and *Keoni Teta, ND*

OUTLINE

Becoming leaner, faster, and stronger, and performing better for longer, all while staying healthy, is the hope of every athlete from the weekend warrior to the elite professional. Sports nutrition is about fueling and enhancing performance, recovering from stress, improving skills quickly, and maintaining optimal body composition for the sport. In this chapter, we will cover the pertinent issues involved in sports nutrition, including supplementation for performance, fitness, muscle maintenance, weight loss, and fat loss.

NUTRITION AND PERFORMANCE

Performance Nutrition and Fat Loss

Before we begin the discussion of sports nutrition, it is useful to cover the topic of body composition. Participating and excelling in sports has much to do with achieving and maintaining an ideal body composition. Athletes seek to optimize the correct ratio of muscle tissue to fat weight that allows the best performance advantage in their sport. More times than not, simply participating in the sport improves body composition. However, when it does not, athletes may, at times, seek to increase muscle mass or decrease their fat weight, or even their overall weight. In addition, the average recreational sport enthusiast, who is not engaged in the competitive side of athletics, will often adopt the eating habits of the elite athletes in the sport they participate in. This practice can be a point of confusion and can lead to undesirable effects.

The "glycogen paradigm" is a way of thinking about sports performance that seeks to maximize muscle sugar storage. Some sports nutritionists believe this effort to enhance muscle sugar storage is the best way to increase training intensity, accelerate recovery, and improve performance. This glycogen dogma has resulted in confusion for athletes and exercise enthusiasts regarding optimal exercise for improving body composition. Health practitioners have known for some time that maximizing glycogen storage increases the performance of endurance athletes. In a 1991 study, Wagenmakers et al.[1] showed this effect conclusively. However, this study also showed a direct negative association between glycogen storage and fat burning. Other studies have shown that exercise done in a glycogen-depleted state may have benefits for increasing fat loss.[2–4]

This information has consequences for athletes and weekend warriors. At times, fat loss is the key aim of an athlete. When this fat loss is the goal, it may be important to tweak the glycogen-centered philosophy of maximizing performance to an approach that instead focuses on fat loss. Health practitioners working with athletes and the general population would be wise to remember the positive association of glycogen with performance and the negative association with fat loss.

Ketogenic Diet and Athletic Performance

Currently, low-carb diets are the latest trendy diets in the weight loss circles. The one that appears to have the most evidence supporting its use for fat loss as well as some disease states is the Ketogenic diet. This diet is best categorized as a high-fat, moderate-protein, and very low-carb meal plan, resulting in significant increase in blood ketone levels, sometimes upwards of 4 mmol/L[5]. Some athletes have implemented the diet to help with athletic performance and weight loss. Although some evidence has been found that the Ketogenic diet can induce

favorable changes in body composition as well as blood lipid/lipoprotein and glucose levels,[6] there is mixed support for its use in improving athletic performance.

Daily carbohydrate intake, and to some extent the protein intake, have to be monitored closely so that the body can keto-adapt, typically requiring carbohydrate intake to less than 50 grams daily. By keto-adapting, ketones become the body's main fuel source over glucose. Once the body keto-adapts or goes into nutritional ketosis, it is forced to start burning fat from the diet and/or from the fat stored in the body via beta-oxidation. High protein intake may make it difficult to put the body into nutritional ketosis, which is due to the gluconeogenic effect of excess protein but also due to the increased supply of oxaloacetate (OAA) from dietary protein, where OAA is the key limiting substrate for the Krebs cycle. Conversely, insufficient protein while in ketosis may affect long-term athletic performance. Furthermore, a keto diet can easily become hypercaloric given the high caloric density of high-fat, low-carbohydrate foods, thus affecting caloric balance and weight loss. This balancing act of hitting the right macronutrient ratio needs to be individualized and carefully monitored; otherwise, it may mean the difference between weight loss and improved performance or fat gain and fatigue.

The paucity of studies on the ketogenic diet in terms of ergogenic effects seem to have mixed results, which may explain the limited research. However, some of the research on ketogenic diet and improving athletic performance is intriguing. From a biochemical standpoint, it seems to make sense, at least for endurance athletes, that a more efficient fuel source would be fat instead of carbohydrates. For years, the thought has been that carbohydrate loading for endurance athletes enhances performance. Indeed, some studies support this approach.[7] However, some of the research on the ketogenic diet contradicts this notion. Thus far, studies of competitive athletes seem to show that this diet does not adversely affect aerobic endurance[8,9] and explosive strength[10] and may help with body composition.[11–13] However, as yet, there is no consensus on their efficacy in improving performance, and some studies show negative effects of very low-carb diets in athletes.[14]

Intermittent Fasting

Another intriguing trend that is becoming more prevalent for losing weight, improving health, and enhancing athletic performance among both athletes and the general public is intermittent fasting (IF). Some fitness enthusiasts think IF is a useful tool for losing excess fat weight while simultaneously helping preserve lean body mass.[15,16] Short-duration IF of 8 to 48 hours enhances fat burning without causing the rebound weight-gain effects of long-term energy restriction (e.g., the "eat less, exercise more" dogma). Because IF is voluntary as opposed to involuntary fasting (i.e., as in famines, starvation, and long-term dieting), the stress of short-term fasting seems to have significant benefit for the athlete and average person who needs to lose excess weight, the main one being improved compliance. Also, IF and the ketogenic diet seem to potentiate one another in regard to ease of implementation and keto-adapting.[17]

IF is one way for the average person to lower elevated insulin levels quickly, thus enabling fat burning.[18,19] The less insulin in the body, the better one is able to burn fat. Just adding more time without eating between meals over time will help improve insulin sensitivity. Also, high insulin levels tend to cause the kidneys to retain sodium, and thus result in water retention. Becoming more insulin sensitive helps the body shed excess water, which is one of the first signs that a person is less resistant to insulin and that fat burning is beginning to happen.

There are many myths about IF that are not borne out in the research, because many of the studies are done on complete fasting (some with individuals who were even foregoing water) and long-term caloric restriction. IF is very different physiologically from starvation or long-term energy deficient states. Some of the more common misconceptions are the following:

- IF causes muscle loss.
- IF causes fatigue, irritability, and low energy.
- IF causes athletic performance to suffer.

Although it may be true that fasting more than 24 hours can cause athletic performance to be compromised in some individuals, and much longer fasts (longer than 72 hours) can cause muscle loss and poor mood, IF for less than 24 hours typically will not have such results and may even improve performance, depending on the sport. In addition, the notion that short-term fasting causes muscle loss or fat gain, or low energy, poor moods, and compromised focus simply is, as of yet, unfounded. In fact, the opposite may be true.[20]

From an ancient ancestral perspective, our species endured numerous periods of feast and famine. If they had not adapted to that forced intermittent fasting, our species, as we know it, would not have survived. Those experiences primed our ancient ancestors and us to preserve our muscle mass and brain function during times of food scarcity so that we would have the energy level and focus to find food. This process occurs because the body has an alternative fuel source like ketones, which result from burning fat. Keto-adaptation (nutritional ketosis) helps preserve muscle mass, energy levels, and brain function so that food can be pursued and found.[21] If the famine continues too long, then starvation ketosis sets in, and muscle mass, energy levels, and eventually focus suffer.

The benefits of IF over conventional weight loss recommendations are that it seems to increase compliance,[22] improve health,[23] and possibly improve performance[24] and mental focus.[25] Much more study is needed, but thus far, IF seems to have many health benefits over long-term energy restriction for fat loss and may improve athletes' performance if done intermittently for short periods of time while ensuring the unique needs of the athlete are met.

Diet Concerns for Athletes

Nutrition for exercise performance involves fueling the body for performance. This process requires optimal energy resources to make up for caloric expenditure during exercise. Athletic activities can use significant resources. High-intensity daily exercise can burn from 600 to 1200 calories/day.[26–28] Elite athletes, such as professional cyclists, can burn as many as 12,000 calories/day, necessitating a large compensatory calorie intake. Caloric needs this high can be extremely difficult to obtain through "real" food alone.[26–31] This difficulty makes supplementation of functional foods and supplements necessary for athletes undergoing heavy training.

Health care providers advising athletes on nutrition must take many factors into account. In addition to the caloric issues just described, there are several other issues. Many athletes undergoing the intense stress of training can experience loss of appetite, especially after intense exercise.[32] Athletes are often also under the constraints of tight schedules of competition and travel requirements, which can prevent or interrupt scheduled meals. Given the already tight training schedules, athletes can quickly become overwhelmed by food considerations. Another potential problem may be lack of variety in the diet, which can lead to lack of enjoyment in eating and loss of appetite.

To address these issues, athletes engaged in heavy training should work to ensure muscle is not lost and weight is maintained, which means eating calorie-dense meals and snacks that are convenient for an athlete's lifestyle. Although real and whole foods should be emphasized, the use of protein bars, meal-replacement shakes, electrolyte beverages, and nutritional vitamin and mineral supplements will often be needed. Ideally, athletes involved in intense training should eat

between four and six meals per day and should eat in consistently timed intervals. Meal and snack timing around training is also an important consideration to replenish energy and aid recovery.[32] Athletes should understand that without calorie balance, many supportive training aids will not provide much advantage. Maintaining caloric needs is the foundation on which all other ergogenic aids should be built.

Macronutrient Ratios

In addition to the caloric considerations of athletes, balancing the protein, fat, and carbohydrate ratio is also important. Recreationally active individuals are usually advised to consume from 45% to 55% carbohydrates, 10% to 15% protein, and 25% to 35% fat.[26–28] The macronutrient needs of athletes can far exceed these numbers. Carbohydrate requirements for athletes can increase up to 10% to replenish and maximize liver and muscle glycogen storage.[27,28] These carbohydrates should come from low-glycemic-index sources that do not cause rapid fluctuations in blood glucose. Because higher levels of complex, low-glycemic carbohydrates can be hard to consume, fruit juices, energy bars, and other convenience foods may be considered.

Research related to carbohydrate intake in athletes shows that athletes may reach what is called a "carbohydrate tipping point."[27] This point is a level of carbohydrate beyond which there ceases to be a performance advantage. Research suggests the body can burn 1 to 1.1 g of carbohydrate per minute, amounting to roughly 60 g of carbohydrate per hour of exercise.[27] Harger-Domitrovich et al.[33] showed approximately 50 g of carbohydrate for a 165-lb individual was the optimal intake of carbohydrates for athletes. This intake amounts to 0.27 g of carbohydrate needed per pound of body weight each hour during exercise.

It is also interesting to note that not all carbohydrates are created equal. Research suggests that sugars composed mainly of glucose (maltose, maltodextrin, and other polysaccharides) are burned at a higher rate in comparison with nonglucose sugar (fructose, galactose, etc.). The combination of these sugars seems to be most advantageous.[27] Evaluating carbohydrate sources on the relative ratios of these sugars may be wise. The glucose/fructose ratio of close to 1:1 seems optimal.

Protein

Protein is an essential nutrient for all humans but especially so for athletes. Recent research on protein intake has shown that athletes require two times or more of the reference daily intake.[27,34–39] It is now recommended that athletes involved in very high-volume training catabolize between 0.7 and 0.9 g of protein per pound of body weight per day. This amounts to between 115 and 150 g of protein per day for a 165-lb athlete. Lean proteins sources such as chicken breast, fish, and lean meats provide approximately 25 g of protein per 3-oz serving; therefore athletes benefit most from consuming approximately 5 to 7 oz of these lean protein sources per day. This amount would be the equivalent of about five to seven 3-oz servings of chicken, fish, or other lean protein per day, with the assumption of fats and carbohydrates to be negligible in lean protein sources. Protein considerations are especially important for endurance athletes, who are more susceptible to protein malnutrition due to the catabolic hormonal environment created by their sport.[39–41]

Like carbohydrates, not all protein is the same. The amino acid content of protein sources varies and has direct bearing on quality. Different types of protein can be described as fast proteins or slow proteins.[42,43] Slow proteins are digested more evenly and take longer to process. Fast proteins are digested more rapidly and allow amino acids to be quickly available to the body. The typical fast- and slow-protein sources are whey and casein, respectively. Health care providers working with athletes may want to look at slow proteins as good meal options, whereas fast protein may be great options to aid performance and recovery by timing them for intake before and after exercise. The best sources of protein are low fat and have a high biological value with optimal amino acid ratios. These sources include skinless chicken, lean beef, fish, egg whites, skim milk, lean pork, and the supplemental milk proteins casein and whey.

The International Society of Sports Nutrition published its position on protein intake in 2017, highlighting the following points[44,45]:

1. Highly active individuals should consume between 1.4 and 2 g of protein per kilogram of body weight.
2. Higher protein intakes (2.3–3.1 g/kg/day) may be needed to maintain lean body mass in resistance-trained athletes following a hypocaloric regime.
3. Concern that protein intake within this range is unhealthy is unfounded.
4. Attempts should be made to get protein from whole foods, but supplemental protein is a safe and viable method of protein intake.
5. Timing protein intake before and after exercise may have benefits, including enhanced recovery and development of muscle mass.
6. Certain amino acids, such as leucine, a branched chain amino acid (BCAA), have been shown to be beneficial for increasing the rate of protein synthesis, decreasing protein breakdown, and increasing recovery from exercise.[46]

Exercising individuals require more protein than their sedentary counterparts require.

Fat

Fat intake for athletes involves several important considerations and depends on an athlete's goals and training state. In general, athletes' fat recommendations should be at or slightly greater than those for their nonathlete sedentary counterparts.[27,34] High-volume athletic training has been shown to lower testosterone concentrations, and decreasing fat intake can exacerbate this effect.[47–49] Most research suggests athletes should keep their dietary fat intake at around 30% of total calories. However, ultraendurance athletes, in particular, may go much higher than this. Athletes undergoing very intense training regimes have been shown to safely tolerate and benefit from diets containing as much as 50% of total calories from fat.[50] As mentioned previously, weight loss is occasionally a concern for athletes. In those cases, a lower-fat diet may be advisable, with research suggesting a diet of 0.25 to 0.5 g of fat per pound per day.[51] Fat quality is also a concern. The polyunsaturated fatty acid ratio of ω-6/ω-3 may be a concern in immune system function and inflammatory responses. Saturated fat intake may also be associated with more optimal testosterone responses.[49] Given these considerations of fat and the emerging understanding of the different function of fats, it is wise for athletes to consume a wide range of dietary fats, with a special focus on balancing the ω-3/ω-6 ratio.

Improving Athletic Performance with Nutrition

International Society of Sports Nutrition position stand: nutrient timing 2010

Prolonged exercise (>60–90 min) of moderate-to high-intensity exercise will deplete the internal stores of energy, & prudent timing of nutrient delivery can help offset these changes.

During intense exercise, regular consumption (10–15 fl oz.) of a carbohydrate/electrolyte solution delivering 6%–8% CHO (6–8 g CHO/100 ml fluid) should be consumed every 15–20 minutes to sustain blood glucose levels.

Glucose, fructose, sucrose, and other high-glycemic CHO sources are easily digested, but fructose consumption should be minimized as it is absorbed at a slower rate and increases the likelihood of gastrointestinal problems.

Continued

Improving Athletic Performance with Nutrition—cont'd

The addition of PRO (0.15–0.25 g PRO/kg/day) to CHO at all time points, especially post-exercise, is well tolerated and may promote greater restoration of muscle glycogen when carbohydrate intakes are suboptimal.

Ingestion of 6–20 gms of essential amino acids and 30–40 gms of high-glycemic CHO within 3 hours after an exercise bout and immediately before exercise has been shown to significantly stimulate muscle PRO synthesis.

Daily post-exercise ingestion of a CHO plus PRO supplement promotes greater increases in strength and improvements in lean tissue and body fat percentage during regular resistance training.

Milk PRO sources (e.g., whey and casein) exhibit different kinetic digestion patterns and may subsequently differ in their support of training adaptations.

Addition of creatine monohydrate to a CHO plus PRO supplement in conjunction with regular resistance training facilitates greater improvements in strength and body composition as compared with when no creatine is consumed.

Dietary focus should center on adequate availability and delivery of CHO and PRO. Including small amounts of fat does not appear to be harmful and may help to control glycemic responses during exercise.

Irrespective of timing, regular ingestion of snacks or meals providing both CHO and PRO (3:1 CHO: PRO ratio) helps to promote recovery and replenishment of muscle glycogen when lesser amounts of CHO are consumed.

CHO, Carbohydrate; *PRO*, Protein.
From Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr.* 2017;14:33. https://doi.org/10.1186/s12970-017-0189-4.

Performance, Recovery, and Nutrient Timing

Athletes and those working with them can benefit greatly from understanding how to time meals for performance and recovery. When it comes to performance nutrition, it is useful to think about first maximizing storage capacity (i.e., filling the liver and muscle glycogen stores) and fueling exercise (i.e., having nutrients more recently consumed to fuel exercise).[26–28,46] Complex carbohydrates take about 4 to 6 hours to be digested, absorbed, and then stored in the liver and muscle as glycogen.[26–28,46,52] Recommendations regarding carbohydrate loading for training and competition should take that time into account. Morning training sessions or competition will benefit from nighttime loading strategies, whereas afternoon competition and training sessions will benefit from morning loading strategies. A light carbohydrate and protein shake roughly 45 minutes (30–60 minutes) before activity has been shown to improve performance toward the end of high-intensity activity. The inclusion of protein also serves to spare muscle tissue by decreasing the need for the body to cannibalize its own muscle tissue.[53,54]

Although exercise sessions of less than an hour require no special nutritional or hydration strategies, exercise sessions lasting longer do. Strategies that manage pre-, post-, and within-workout nutritional requirements can dramatically aid performance and recovery. This is where electrolyte solutions and sports drinks consumed before and during exercise have an important role to play. These beverages can help prevent low blood sugar, optimize hydration, replace lost minerals, and reduce the immune suppression that occurs after intense long-duration exercise.[55,56] A carbohydrate beverage of 6% to 8% with an equal mixture of glucose and fructose taken every 20 minutes during exercise is advised.[27,54,57] In addition, adding protein to carbohydrate intake may result in higher rates of glycogen storage after exercise.[32,55,57,58] Research suggests consumption of approximately 20 g of a rich source of essential amino acids (e.g., whey protein) combined with 40 g of a good glucose source (e.g., grape juice) taken 40 minutes before exercise may be useful and within 3 hours after exercise may improve performance and aid recovery. The addition of a small amount of fat may also be helpful in stabilizing blood glucose levels.[57]

After exercise, there is a unique opportunity to fuel the body for recovery. During this "recovery phase," nutritional strategies should be instituted as soon as possible and at least within the first 30 minutes after the cessation of exercise. A mixed carbohydrate–protein beverage containing close to a 3:1 ratio of carbohydrate to protein within 3 hours after exercise appears to provide greater recovery benefits than do lesser amounts of carbohydrate.[32,55,57–59] Upon completion of this postworkout "snack," another more carbohydrate-heavy postworkout meal should be eaten again within 90 minutes.

Most athletes will taper their training by one third to one half 2 to 5 days before their events. During that time, it may be beneficial to consume 200 to 300 extra grams of carbohydrates daily. This technique has been shown to maximize glycogen storage before the event and improve performance.[57]

Water

Easily the most beneficial ergogenic aid is water. Working to prevent dehydration during exercise is one of the most useful endeavors for improving exercise performance. Intense exercise can result in significant loss of water through sweat. When this fluid is not replaced, athletic performance will suffer. A loss of 2% water through sweat can impair the ability to compete, and a loss of 4% can result in the inability of the body to cool itself during exercise.[56] Athletes can lose 1 to 2 L in sweat through exercise per hour. Unfortunately, athletes cannot rely on thirst perception to regulate fluid balance.[56] There are several objective ways for athletes to measure fluid loss.[56] One way to ensure adequate hydration recovery is for athletes to weigh themselves before and after exercise and drink 3 cups of liquids per pound of weight lost through exercise. During heavy exercise, athletes should consume 1 to 2 L of water or glucose and/or electrolyte beverage per hour.[56,60]

Vitamins and Minerals

It is now recommended by most medical organizations that a low-dose multivitamin be consumed daily to assure optimal vitamin and mineral status.[27,61] Few, if any, vitamins and minerals have been shown conclusively in research to have any performance benefit. However, the nutritional status of an athlete can affect quality of performance, training, and recovery.[27,61–64] When considering vitamin and mineral supplementation for athletes, health care practitioners should view their recommendations in the context of nutrient adequacy. Athletes may be more susceptible to vitamin and mineral inadequacies, given the previously mentioned issues on nutrition and potential caloric deficits. Although there may be no direct benefits to performance, many vitamins and minerals aid recovery and support.[27,61–64]

Vitamin C and zinc in particular have good research suggesting immune enhancement during intense training periods.[27] Vitamins C and E, along with other antioxidant nutrients, may also protect athletes from excessive oxidative damage, which could also lead to immune suppression.[27]

Mineral deficiencies are particularly problematic for athletes. Restoring any mineral deficiencies can aid performance.[27] Certain minerals may also provide benefit for optimizing performance. Calcium is one mineral that can aid athletes. Although there is no evidence that calcium supplementation improves performance, at supplement levels of approximately 1000 mg per day, calcium has been shown to assist athletes susceptible to osteoporosis as well as improve body composition.[65,66] Phosphorus supplementation may have ergogenic effects when supplemented as sodium phosphate but not in other forms (calcium phosphate, potassium phosphate, etc.).[67] Levels of supplementation for phosphate are 4000 mg/day (1 g tribasic

dodecahydrate sodium phosphate 4 times/day) for 3 days for endurance improvement. Sodium intake has benefit for training in the heat and reduces the risk of hyponatremia.[48] With most minerals, as with vitamins, the real benefit to athletes comes when identified deficiencies are corrected. Restoring vitamin and mineral status to optimal levels can aid exercise performance.[7,41-44]

Along these lines, vitamin D is a special consideration. Vitamin D deficiency is now epidemic, and athletes have the same rates of deficiency as the rest of the public have.[68,69] In one study of elite female gymnasts, 77% were found to have vitamin D levels lower than 35 ng/mL, and a full third had levels lower than 10 ng/mL (which is defined as a clinical deficiency).[69] In a group of athletes susceptible to prolonged and constant bone stress (i.e., long-distance runners), this finding was especially troubling and underscores the need for athletes to ensure adequate vitamin D status.

Although vitamin D supplementation in individuals with sufficient vitamin D does not appear to increase performance, vitamin D therapy delivered to deficient athletes may improve performance.[69,70] Vitamin D is known for its role in bone metabolism. However, the fact that skeletal muscle has vitamin D receptors is not commonly known. As far back as the 1930s, there have been numerous reports of the beneficial effects of ultraviolet therapy on athletic performance.[71,72] In addition, some studies suggest the season of training makes a difference. One of these studies showed that training in the summer months creates greater gains than the same volume of training in autumn or winter does, despite the same stimulus.[72]

In both older and younger individuals, adequate vitamin D status affects neuromuscular function and may have a specific relationship to the maintenance of the fast-twitch type 2 muscle fibers.[68,71,73,74] In a study on teenage athletes, vitamin D deficiency lowered muscle power and force.[75] Vitamin D levels are also related to myalgia, fatigue, and reduced motivation to exercise. Studies in older adults showed that levels of vitamin D were correlated with the propensity to fall.[76] A meta-analysis of vitamin D levels showed a 20% reduction in the risk of falling among people with higher levels.[77] This reduction was likely due to vitamin D's ability to improve reaction speed, balance, and neuromuscular performance. Much of this effect may be explained by the ability of vitamin D to help maintain and even build type 2 muscle fibers.

Athletes should be tested for vitamin D levels with a serum 25-hydroxy vitamin D laboratory test with a target between 50 and 100 ng/mL. If they are found to be insufficient, a combination of supplementation and sun exposure is advisable. For quick restoration of adequate vitamin D levels, 50,000 International Units (IUs) of vitamin D per week for 12 weeks has been shown to allay symptoms of vitamin D deficiency. Maintenance doses of between 1000 and 10,000 IU/day, depending on sun exposure and living latitude, should also be considered to prevent subclinical and clinical deficiencies.

ASSESSMENT

Serum Nutrient Testing

Nutrient deficiencies can affect many aspects of physical performance, recovery, and immune function. Athletes, because of the imposed increases in metabolism, can be at an increased risk for nutrient deficiencies. Serum testing is a reliable and useful tool for diagnosis of severe nutrient deficiencies but may be inadequate for certain vitamins and minerals.[78] Therefore those working with athletes will find it useful to have more functional tools for assessment of metabolic and nutritional needs.

Testing blood for nutritional levels can be misleading. Serum nutrient concentrations are closely regulated by the body and can fluctuate based on recent intake. Furthermore, some vitamins and minerals are almost entirely found intracellularly. As stated by Lord and Bralley in their textbook *Laboratory Evaluations for Integrative and Functional Medicine*, "For various reasons specific to each vitamin, it is possible for an individual to have normal serum levels of a vitamin while exhibiting signs of insufficiency for that vitamin owing to a lack of adequate intracellular concentration to meet the metabolic demands of the cells."[78] Given these factors, serum may not always be the best means of determining nutritional status for all nutrients. A range of laboratory analyses that can provide more functional assessments of nutrition status are now available.[78] The ones that may be most beneficial are adrenal hormone profiles (cortisol and dehydroepiandrosterone [DHEA]), organic acid testing, and intracellular nutrient analysis.

Adrenal Hormone Testing

Analyzing the adrenal hormones cortisol and DHEA can give insight into training status and into whether an athlete may be overtraining or overreaching.[79–81] Salivary cortisol and DHEA levels provide an indication of catabolic versus anabolic balance in the body as well as neuroendocrine adaptation to stress. The optimal ratio of salivary cortisol to DHEA is approximately 5:1 to 6:1, which indicates an appropriate state of stress adaptation. The closer this ratio is to 1:1, the less likely it is for the individual to be able to adapt to daily stressors.[82] The test is simple and noninvasive, involving salivary collection at four time points during the day (morning, noon, evening, and night). Depending on the laboratory, the cortisol and DHEA test may also provide markers of immune function, such as immunoglobulin-A. All these measures are important for athletes looking to compete at high levels for prolonged periods.

The body goes through four stages in response to stress. Stage 1 occurs in response to acute stress and involves elevations in cortisol with no changes in DHEA. Stage 2 involves continued stress and is marked by a sustained cortisol peak with a matching elevation of DHEA. At stage 3, when stress continues and becomes chronic, cortisol levels drop, whereas DHEA stays high. Finally, both cortisol and DHEA fall, a stage often referred to as adrenal exhaustion or stage 4.

Testing athletes for levels of adrenal hormones gives important guidance concerning nutritional therapy and supplementation. Cortisol is a highly catabolic agent, and increased cortisol levels with normal to low DHEA indicate excessive stress and potential muscle wasting.[78] This status necessitates increased protein and amino acid intake, especially increased glutamine.[83] High cortisol levels also give an indication of increased need for other nutrients that are easily depleted through increased metabolic activity (e.g., magnesium, zinc, and vitamin B_6).[78] Cortisol-suppressing supplements, such as phosphatidylserine, may also be indicated,[84] as is the potential for DHEA supplementation and/or immune support.[85] Changes in adrenal hormones may occur long before clinical symptoms appear and can be used to monitor training status and recovery.

Organic Acid Testing

Organic acid testing is a useful analysis that looks at metabolic by-products in urine.[78] The test does not measure actual vitamins and minerals or their absolute values but assesses them indirectly by measuring excreted metabolic by-products. Although the test is not diagnostic, it can be used to analyze the metabolic consequences of insufficient nutrient intake. It can also offer insights into toxic exposures, digestive health, amino acid need, and neuroendocrine function.

Fats, proteins, and carbohydrates become carboxylic acids before they are completely burned to carbon dioxide. These acids, formed by the body's metabolic processes, are usually low or nonexistent in urine. When perturbations occur in metabolism due to inefficient enzymes as a result of genetic polymorphisms and/or lack of a vitamin or mineral cofactor, the metabolic by-products can build up in the cell, be

released into the blood, and then begin to show up in the urine. The organic acids that appear can then be traced back to known biochemical pathways, indicating a particular nutrient need.

Given the fact that almost all nutrients become ergogenic when there is a deficiency or increased need, organic acid testing may be useful in understanding which nutrients may be indicated for a particular athlete and whether the supplements they are currently taking are actually doing the job. Using organic acid testing may provide health care practitioners working with athletes a way to pinpoint potential nutrient issues and bolster key metabolic pathways.

Intracellular Nutrient Analysis

Because many nutrients, like magnesium, are not well measured in serum, another useful functional analysis for athletes is intracellular nutrient analysis.[78] This testing involves drawing blood, isolating white blood cells, and growing them in a nutrient-rich medium. Individual nutrient concentrations are then manipulated one by one while measuring cell growth rates. Cellular growth from an athlete with insufficient levels of a nutrient will be slower than from an athlete with sufficient levels.[86] This analysis allows tailored nutrient supplementation to optimize intracellular levels of all nutrients.

Amino Acid Testing

Due to the high turnover of amino acids required in the participation and recovery from intense exercise, amino acid analysis can be useful for athletes.[78,87] When testing athletes for amino acids, it may be useful to measure plasma levels, urine levels, and organic acids. Plasma levels give the best indication of protein utilization and are the most scientifically validated. However, urinary amino acids may give greater insight into protein breakdown from factors interfering with amino acid utilization (i.e., micronutrient deficiency or toxic exposures). These two measures, along with the ability to map biochemical pathways through organic acid testing, can provide an athlete with in-depth information about protein utilization, breakdown, and metabolic needs.

CRITICAL EVALUATION OF ERGOGENIC AIDS

It is important for the health care practitioner to be aware of the large gap that often exists between supplement marketing and research. Given that nutritional supplements are not regulated to the same degree as pharmaceuticals, and little financial incentive exists to research natural compounds, it is important to look for valid and objective resources on nutritional supplements. When evaluating whether a nutraceutical might be useful, it is important to keep several things in mind.[27]

A good first question to ask is, "Does the basic science make sense?" Natural health practitioners need to be good students of biochemistry and physiology. Knowing the compound and its involvement in biochemical pathways is a necessary first step. If the mechanism of action "makes sense" from a biochemical perspective, the supplemental aid may have merit. A good example here is the use of the nutrient L-carnitine. Understanding the biochemistry of this nutrient as key in delivering fat to the mitochondria to be burned for energy allows consideration that it may have fat loss and performance benefits.

The next question must be, "Is it feasible to deliver the nutrient in a form that can actually be absorbed and utilized by the body?" Although a nutrient may seem like a perfect candidate for performance enhancement properties, if it cannot be delivered to the body in a safe, feasible, and usable form, it will not have much use. In the case of L-carnitine, several issues warrant consideration. First, L-carnitine is not well absorbed; therefore understanding the different delivery forms is essential. The next obvious question, "Would L-carnitine bound to tartrate, fumarate, or another molecule be most feasible for delivery?" Also, in the case of amino acids like carnitine, there are two forms, L-carnitine and D-carnitine. L-carnitine is active in the body, whereas D-carnitine is not and can actually block the action of L-carnitine. Confidence would therefore be less on studies using the racemic mixture of this nutrient versus those using pure L-carnitine.

Another important consideration is, "Does any good research exist on the substance?" Health care practitioners should further consider other aspects: Are the studies in vitro or in vivo studies? Were they animal or human studies? Was the sample size big enough? Was the study designed appropriately? Was it well controlled? Human clinical trials with a large sample size that are double-blinded and placebo-controlled are obviously the gold standard and should be weighted heavier. However, these types of studies often do not exist for sports performance supplements, accounting for much of the skepticism regarding sports performance aids. Moreover, these types of studies are cost-prohibitive, especially when considering the diminutive financial gain, if any at all, of doing natural compound studies because there is essentially no patent protection. Much of this skepticism is warranted, although discounting a supplement purely on the basis of lack of these types of trials is probably not wise.

Some final considerations would be whether or not the product is safe, legal, and appropriate. In 2006 the 109th Congress passed the Dietary Supplement and Nonprescription Drug Consumer Act. This law requires supplement companies to disclose all complaints about their products and make them available to the public. For supplement issues that are more serious in nature, the companies must notify the U.S. Food and Drug Administration within a 2-week time frame. There are also consumer organizations that help police the industry. These organizations independently test supplement products against claims of efficacy and issues with contamination, and such groups can be useful resources to those advising supplement use. Finally, there are always completely safe products that may present issues in certain populations. All these considerations should be evaluated when using nutritional supplementation.

GENERAL HEALTH CONCERNS FOR ATHLETES

The American Medical Association has suggested Americans use supplemental vitamins to maintain health; these recommendations seem especially prudent in the athletic world. Although there is no performance benefit to the use of multiple vitamins and minerals, as discussed previously, athletes can be susceptible to deficiencies and/or require more support in areas due to increased vulnerability to stress or injuries encountered during sports. Glucosamine can aid in the healing of damaged cartilage and reduce joint pain.[69] Supplements such as zinc, glutamine, vitamin C, lipoic acid, selenium, and other nutrients may support immune function and antioxidant capacity.[26,27,46,88–90] Amino acids are especially useful. Creatine, whey protein, BCAAs, and L-carnitine tartrate have all been shown to help athletes through the stress of intense training periods.[26,27,46] Finally, ω-3 fats can help balance inflammatory and anti-inflammatory reactions in the body.

NUTRITIONAL SUPPLEMENTS

Obviously, a full treatment of all the nutraceuticals purported to have beneficial effects in exercise and body composition could fill an entire book. We selected the compounds discussed here that demonstrated the most research supporting their use as ergogenic aids. We attempted to avoid looking at compounds that have strong theoretical support for their use but have not yet been studied. We gave special consideration to compounds that have been studied in humans and have been

evaluated under double-blinded and placebo-controlled conditions. Newer compounds that did not meet these criteria may not be covered. In certain cases, we included compounds that are novel in their reported use as ergogenic substances.

Arginine

Arginine has several potential benefits for athletes, including increasing human growth hormone production, blood flow, mitochondrial biogenesis, fat loss, and muscle gain. Many of these effects are related to nitric oxide, for which arginine is a precursor. However, several of these benefits are relatively new discoveries regarding arginine supplementation. Animal studies and human studies show arginine might have particular benefit in improving exercise capacity and body composition.

Arginine may play a role in endurance performance. A 2010 study showed that a proprietary arginine supplement given to elderly male cyclists was able to positively affect performance. The arginine supplement significantly increased the anaerobic threshold within 1 week of beginning supplementation, with the effect lasting throughout the 3-week study.[91] No change in maximal oxygen consumption (VO_2max) among the cyclists was found in this particular study. However, in another study, this time on younger cyclists, oxygen kinetics were sped up in relation to L-arginine supplementation.[92] Another study in 2010 showed arginine's potential role in illness associated with reduced cardiovascular capacity.[93] In this analysis, arginine supplementation improved exercise capacity in heart transplantation patients, resulting in furthering the distance walked and delaying the ventilatory threshold by 1.2 minutes.

In addition to endurance benefits, arginine may also have a role to play in strength training. An April 2010 study showed increased growth hormone and insulin-like growth factor 1 release in response to heavy resistance training.[94] This newer study lends credibility to older reports.

Research on rats, pigs, and humans showed arginine to be a potential antiobesity aid through several unique mechanisms.[95] Supplemental arginine appears to have activity that increases glucose and long-chain fatty acid oxidation while at the same time suppressing gluconeogenesis and lipogenesis. In rats, L-arginine was shown to increase muscle mass by 12% and increase glucose metabolism by 14% without affecting insulin. Most interestingly, arginine was shown to coax white adipose tissue to become brown adipose tissue and, as a result, significantly elevated the metabolic rate. Arginine appears to act partly via its conversion to nitric oxide. It may act via cyclic guanosine monophosphate and cyclic adenosine monophosphate dependent mechanisms. It was found to increase mitochondrial biogenesis, upregulate GLUT-4 receptors, and improve muscle mass.

A 21-day randomized and double-blind, placebo-controlled study of arginine in obese men was conducted in 2006 by Lucotti et al.[96] Thirty-three obese males were put on a low-calorie diet (1000 kcal/day) and an exercise program (45 minutes of exercise twice per day for 5 days/week). They were then randomized to receive 8.3 g per day of arginine or placebo. As expected, the lifestyle intervention resulted in weight loss, waist circumference reduction, lowered fasting glucose, and reduced insulin in both groups. However, the arginine group had statistically better responses in most measures in comparison with the placebo group ($P < 0.0001$). Most interestingly, the arginine group was able to maintain their muscle mass, whereas the placebo group had significant reduction in lean body mass. Forty-three percent of the weight lost in the placebo group was fat in comparison with a full 100% for the arginine group.

Although earlier research shows the athletic performance benefits of L-arginine, more recent analyses suggest the ergogenic benefit is nonexistent to minimal at best. Using various doses of arginine, from 0.04 g/kg, 0.75 g/kg[97] to absolute amounts of 6 g,[98,99] with various forms, including arginine alpha-ketoglutarate to pure L-arginine, creates a situation that makes it difficult to construct a valid comparative analysis. However, among these various studies, arginine showed no change in performance outcomes[97] or only very slight improvements.[99]

Not to be ignored, many of the studies testing the efficacy of L-arginine on athletic performance are studying individuals under unique circumstances, all with significant effect on performance. The variables among these studies are inconsistent with variation in supplement duration, combination of arginine with other agents,[100] varying exercise protocols from aerobic endurance, high-intensity to combative sports,[99] and the fitness level of the athlete (healthy men to highly trained athletes).[97,98] Furthermore, their use of small sample sizes questions the power of the studies. However, this research is what the literature provides, which then requires a thorough analysis of the research as a whole, which must then be applied through the lens of the patient.

Dosage

The appropriate dose of arginine based on the animal and human studies appears to be 80 mg/kg body weight per day, or 3 g three times daily for a 155-lb adult. Results of the National Health and Nutrition Examination Survey indicated arginine intake in the United States averages about 4.4 g per day, with 25% of the population receiving suboptimal levels.[95]

Other Considerations

Both animal and human studies suggest there are no safety concerns related to arginine supplementation when it is taken in appropriate doses and in an appropriate form. There is a theoretical concern that increased arginine supplementation will alter the arginine/lysine ratio and possibly increase herpes virus activity. Arginine is also known to increase the inducible form of nitric oxide synthase, resulting in potential negative cytokine activity and immune consequences. This result is a special concern for those suffering from autoimmunity.

Biochemically, an intricate relationship exists between citrulline and arginine in the synthesis of nitric oxide, as citrulline acts as a precursor to arginine. Several studies have shown citrulline supplementation at doses up to 6 g daily to improve arginine levels and NO levels in the blood superior to arginine alone.[101–103] Although this can simply be attributed to citrulline being a precursor, thus increasing usable substrate, additional research further suggests arginine is catabolized via first-pass metabolism in the gut and liver by arginase. Citrulline does not undergo this process and further acts as an allosteric inhibitor of arginase, resulting in increased peripheral circulation of arginine, thus increasing available arginine as a substrate for NOS.[103]

Beta-Alanine

Beta-alanine is a precursor, along with histidine, of carnosine and is found in high concentration in skeletal muscle. It is widely assumed carnosine has performance benefits due to its ability to resist pH changes in muscle. In one study, oral intake of β-alanine for 4 weeks was shown to increase carnosine levels.[104] Another study showed supplementation with β-alanine for 10 weeks significantly elevated carnosine levels at weeks 4 and 10.[105] Subjects taking β-alanine have been shown to complete a greater number of repetitions during resistance exercise,[81-82] resist fatigue,[105–107] generate greater force with the legs,[108] and even improve body composition.[109] The intention and goals are to determine whether and how β-alanine use translates to actual benefits in athletes and their performance. The aforementioned improvements are assumed to convert into improved performance, which has been confirmed by several studies.[110–112] In athletes, most benefits have been

identified with improvements in intense, short-duration exercises of 0.5 to 10 minutes,[112] such as sprinting and jumping ability.[111] Although the benefits of β-alanine are much more pronounced in untrained athletes, benefits were still evident in trained athletes, which can be explained by improved exercise adaptation in highly trained athletes. However, the enhancements in trained athletes may be worthwhile if used in an applied setting, such as competition, where even a slight advantage may have profound results.[112]

Dosage

Typical doses in studies range between 400 and 800 mg given multiple times throughout the day to achieve between 4 and 8 g. Doses above 800 mg at any given time may cause transient paresthesias. β-alanine appears to have a low toxic profile and is generally considered a safe nutraceutical.

Other Considerations

β-alanine and sodium bicarbonate together, over β-alanine alone,[113–115] have shown synergism in improving performance in highly trained athletes. Gains were most impressive after chronic use for 7 days, with the mechanism theorized to be due to the improved muscular alkalosis.[116]

Beta-Hydroxy Beta-Methylbutyrate

Beta-hydroxy beta-methylbutyrate (HMB) is derived from the BCAA leucine, a known activator of mTORc1 (mechanistic target of rapamycin).[117] HMB use at levels from 1 to 3 g per day while undergoing weight training may increase strength results and have positive effects on lean muscle gains.[38,118,119] The largest and most beneficial responses come from studies on untrained athletes. Older individuals may also enjoy benefits from supplementation. Volkovich et al., in 2001, showed 3 g of HMB daily over 8 weeks given to elderly men and women led to significantly greater loss in fat in comparison with a placebo group.[120] Muscle mass gains usually ranged from 1 to 2.2 lb over 3 to 6 weeks of training for those taking HMB versus placebo.[27]

Until recently, performance benefits in athletes or well-trained individuals were not as convincing as results in untrained subjects. However, the literature has shown significant benefits in young trained and untrained individuals. One quality study by Knitter et al., in 2000, showed HMB might protect against muscle damage from exercise.[121] Well-trained runners were supplemented with 3 g per day over 6 weeks. In comparison with placebo, HMB-supplemented runners had lower markers of muscle damage evident by lower lactate dehydrogenase and creatine phosphokinase. Additional studies in younger trained subjects, showed supplementation of 3 g of HMB improved peak power and 1 rep-max (1RM) leg press,[122] along with increased strength in bench press, squat, and deadlift, vertical jump power, and improved lean body mass.[123,124]

Strikingly, HMB also shows promise as an ergogenic aid via its effect on the endocrine system. Studies in young, resistance-trained men taking HMG along with a resistance training protocol exhibited significant increases in growth hormone (GH), insulin-like growth factor 1 (IGF-1), and attenuation of elevated cortisol.[123–125]

The mechanism is not completely understood. However, it is suspected to induce mTORc1, inhibit the ubiquitin proteasome pathway thus reducing muscle degradation, stimulate mitochondrial biogenesis,[126] and induce synthesis of skeletal muscle satellite cells,[117,127,128] with the result of increased muscle hypertrophy and muscle recovery.

Dosage

The dose of HMB shown to be effective is 1 to 3 g per day.

Other Considerations

HMB supplementation along with creatine may behave in a synergistic fashion.[129] HMB is generally considered safe to use.

Branched Chain Amino Acids

BCAAs are perhaps the most beneficial aspect of increased protein intake. They have been shown to increase protein synthesis, decrease protein breakdown, and speed recovery from exercise.[130–132] Research suggests they may have some acute performance enhancement effects and play a key role in body composition.[133–135]

BCAAs include leucine, isoleucine, and valine. The body cannot synthesize these amino acids, making their dietary intake essential. BCAAs are necessary for production of other amino acids, most notably alanine and glutamine, two of the most important amino acids used in gluconeogenesis. Low-calorie diets and stress can greatly increase the need of amino acid precursors for gluconeogenesis. Because BCAA are mainly metabolized by the muscle in comparison with other amino acids, which are metabolized by the liver,[136] a large pool of BCAAs through dietary intake spares muscle protein breakdown and likely promotes muscle protein synthesis.[137–139]

BCAA supplementation has shown some effect on exercise performance, perceived exertion, and recovery. Matsumoto et al.[135] had subjects drink either a BCAA beverage or a placebo 6 days before performing high-intensity cycling until exhaustion. The trial was a double-blind, crossover, placebo-controlled study with a 1-week washout period between exercise sessions. BCAA increased plasma levels of leucine, isoleucine, and valine during the exercise test. The contribution of fat burned during the exercise session was significantly greater for BCAA in comparison with placebo, as was the oxygen uptake and lactate thresholds at equivalent work levels. VO_2max was also greater in the BCAA supplementation. Taken together, these results suggest BCAA supplementation may improve endurance exercise performance.

Another study by Greer et al.[132] looked at BCAA for endurance exercise improvement. This study did not show an acute performance advantage for BCAA during exercise but did show reduced damage. BCAA supplementation was significantly better than consumption of either a carbohydrate beverage or placebo at reducing markers of muscle damage. An earlier study by the same group showed comparable findings.[134]

One of the limiting factors for athletes is recovery. There are impressive findings on enhanced recovery from exercise using BCAA in response to high-intensity weight lifting and resistance training,[131] with reductions in muscle damage evaluated via creatine kinase, lactate dehydrogenase, myoglobin, delayed-onset muscle soreness, and muscle function alteration.[136] Preexercise supplementation of BCAAs in young trained athletes reduced exercise-induced muscle damage, attenuate the loss of strength and power after exercise,[140] and reduce overall perception of muscle soreness.[140,141] It is suspected that in addition to being a fuel source for myocytes, BCAA may induce mitochondrial biogenesis and scavenge ROS via upregulation of peroxisome proliferator-activated receptor-γ coactivator 1α.[142]

One of the more intriguing aspects of BCAA supplementation may be its role in optimizing body composition. Low-calorie diets and aerobic exercise are both known to reduce lean muscle tissue. BCAA supplementation attenuates this response and helps maintain muscle mass.[133] In addition, BCAAs, specifically leucine, have unique actions as metabolic signaling molecules that may be able to increase energy expenditure,[143] suppress appetite,[144] and lower insulin.[145] Although all these relatively new mechanisms have been shown only in mice, one small pilot human study including BCAA supplementation suggested that the same mechanisms may be at play in humans.[146] Fifty-nine

healthy subjects participated in the study and were divided into a placebo group (fiber) and then given either a high-concentration (12 g per day) or low-concentration (6 g per day) BCAA supplement. Subjects were supplemented for 14 days and were encouraged not to modify either eating behavior or exercise habits.

Degree of weight loss was directly correlated with dose of BCAA given, with the largest weight loss being achieved in those who received the 12 g per day dose. Given the short time of the study, statistical significance was not achieved. The authors noted, "Design of this pilot study has more effects than can be analyzed satisfactorily with available sample sizes. An analysis with "best possible" outcome did show that significance would have been possible with maximum weight loss difference from one group to another." Based on this study, it is not yet possible to make a determination as to BCAA supplementation in weight loss. However, given the new understanding of BCAA signaling, the promising results in animals, and the interesting, although not statistically significant, findings of this study, further evaluation certainly seems warranted.

Dosage

Most research suggests a dose of between 6 and 12 g per day of BCAA.

Other Considerations

Given that the dosages of BCAA are so high, it may be impractical to recommend this supplement in capsule form. BCAA powders are widely available and may provide convenience. Mixing BCAA powder in a small amount of high-glucose fruit juice, such as grape juice, may help increase compliance by masking the bitter taste and increasing absorption and utilization.

Caffeine

Perhaps the most well-known performance-enhancing aid is caffeine. The consensus of studies shows caffeine has value in endurance exercise performance, anaerobic exercise outcomes, exercise recovery, and weight loss.[147–149] In the body, caffeine absorption occurs rapidly and is then metabolized in the liver into theophylline, theobromine, and paraxanthine. Caffeine is lipid soluble, giving it quick access to the brain and other tissues. Caffeine is detectable in blood within 15 minutes of intake, reaching peak levels at 1 hour. Caffeine is cleared from the system in 1 to 12 hours, depending on the individual's activity of CYP1A2.

Caffeine exerts strong influence on the nervous system, affecting both the central nervous system and peripheral nervous system, and several review studies suggest this is the most beneficial action of caffeine related to performance enhancement.[147–149] It acts to increase mental focus and fine motor skills. Beta-endorphin concentrations also rise with caffeine consumption, possibly resulting in decreased pain perception during intense activity.[147] Also, the nervous system activation plays a strong role in sports, requiring bursts of speed and intensity.

Caffeine spares muscle glycogen and shifts metabolism toward the increased use of fat from both subcutaneous and intramuscular compartments.[149–151] Given that fat oxidation is a key component of endurance sports, caffeine can improve outcomes in long-distance events. It also has an interesting effect when dosed along with carbohydrate.[152] A carbohydrate electrolyte beverage (6% glucose) spiked with caffeine (5 mg/kg of body weight) was able to bolster endurance performance by 9%.[151]

In high-intensity exercise, caffeine raises catecholamine production, increasing both lipolysis and glycogenolysis.[147–149] This effect, along with increased activation of the central nervous system, may be the mechanism responsible for better performance in high-intensity endeavors. In 2006 Schneiker et al.[153] compared 6 mg/kg of body weight caffeine ingestion with placebo in amateur male athletes. In this study, either caffeine or placebo was taken 60 minutes before two 36-minute exercise sessions consisting of multiple short sprints separated by 2 minutes of recovery. Caffeine intake increased sprint performance 8.5% and 7.6% over placebo in the first and second bouts, respectively. Caffeine also increased power output in comparison with placebo. Additional studies confirm these ergogenic effects of caffeine in high-intensity and resistance training activities. When comparing caffeine intake and placebo, caffeine showed significant improvements in total weight lifted in comparison with placebo. Interestingly, the same study compared coffee with decaffeinated coffee with added caffeine and found both the coffee and decaffeinated plus caffeine improved power output, thus suggesting the benefits of coffee may be due to the caffeine content.[154] Two well-designed meta-analyses both concluded caffeine to be a beneficial ergogenic aid in improving anaerobic performance, peak power output, and total power output.[155,156] Furthermore, it appears to be most effective in exercises lasting 45 seconds to 8 minutes.[143]

This same combination, carbohydrate and caffeine, may also play a role in recovery from activity. In a study comparing a carbohydrate recovery beverage alone with one spiked with caffeine, glycogen synthesis was increased by 66% in the carbohydrate-plus-caffeine group.[157] This may be mostly due to caffeine's ability to increase the absorption of glucose from the digestive tract into the bloodstream.

Caffeine's activity as a fat-loss aid comes from its epinephrine-inducing effects. Studies as far back as 1990 showed as little as 100 mg of caffeine, the equivalent of a cup of coffee, could increase thermogenesis even in habitual coffee drinkers.[158] Other studies on caffeine showed increased fat oxidation, with one older study showing a 30% greater blood free fatty acid level 2 hours after workout in comparison with placebo.[147–150]

Although the mechanism by which caffeine exerts its benefits is unclear, it is suspected to be due to several factors, including improved catecholamine response to the stress of exercise, improved contractility by reduced intramuscular potassium accumulation,[159] a decreased perception of pain, and increased alertness and arousal.[147]

Dosage

Caffeine intake appears to be physiologically somewhat different from coffee intake. Although the research on pure supplemental caffeine consumption is fairly clear on performance benefits, effects of coffee consumption are less clear. Caffeine consumption shows effects when taken from 15 to 60 minutes before exercise at a dose of 6 mg/kg of body weight. For a 160-lb individual, this amount is the equivalent of about 430 mg of caffeine. An 8-oz cup of coffee has about 150 mg of caffeine on the high end. This amounts to close to 3 cups of drip coffee as the dose for a 160-lb individual.[147–149]

Other Considerations

Concerns about coffee intake and risk of dehydration are unfounded. No good evidence suggests caffeine intake leads to an increased risk of dehydration. Users of caffeine will develop some tolerance. Nonhabitual users experience slightly better benefits from coffee intake in comparison with regular users. Coffee intake can also have a negative effect on digestive function.[147–149] High amounts of caffeine intake can cause negative symptoms, including heart palpitations, insomnia, anxiety, and tachycardia. Coffee intake and caffeine intake can be especially problematic for individuals who are genetically known as "slow metabolizers" of caffeine (CYP1A2*1C). As such, the therapeutic window for enhancing athletic performance may be enhanced in comparison with a "fast metabolizer" of caffeine.

Creatine Monohydrate

The International Society of Sports Nutrition has called creatine "the most effective nutritional supplement available to athletes to increase high-intensity exercise capacity and muscle mass during training."[27] All the performance-enhancing benefits are hypothesized to come down to two factors: increasing the amount of energy available to mitochondria in the form of adenosine triphosphate and increasing cellular hydration.

Creatine has been under investigation for more than 60 years. It is highly concentrated in muscle tissue, with all but 4% to 5% of body creatine residing in the muscle. Most of the creatine in the body is in a phosphorylated form called phosphocreatine. The major function of this supplement is to provide a rapid source of energy by maintaining adenosine triphosphate (ATP) availability.

Although creatine can be made in the body, it requires significant resources, including the amino acids arginine and glycine, magnesium, S-adenosylmethionine, vitamin B_{12}, and folate, among others. Creatine is also present in the food supply and is especially rich in animal protein. The highest concentrations are found in lean cuts of red meat, game meat, and fish, including salmon and tuna. Two pounds of lean red meat contains close to 5 g of creatine. Cooking food may have a negative effect on the availability of creatine.

Creatine Monohydrate (CM)
(The Most Effective Supplement for Enhancing Athletic Performance)

International Society of Sports Nutrition position stand on CM 2010

1. The most effective ergogenic nutritional supplement currently available to athletes in terms of high-intensity exercise capacity (HIEC) and lean body mass during training.
2. It is not only safe, but possibly beneficial in regard to preventing injury and/or management of select medical conditions when taken w/in recommended guidelines.
3. There is no compelling scientific evidence that the short- or long-term use has any detrimental effects on otherwise healthy individuals.
4. If proper precautions and supervision are provided, supplementation in young athletes is acceptable and may provide a nutritional alternative to potentially dangerous anabolic drugs.
5. CM is the most extensively studied and clinically effective form of creatine for use in nutritional supplements in terms of muscle uptake and ability to HIEC.
6. The addition of carbohydrate or carbohydrate and protein to a creatine supplement appears to t muscular retention of creatine, although the effect on performance measures may not be greater than using creatine monohydrate alone.
7. The quickest method of t muscle creatine stores appears to be to consume ~0.3 grams/kg/d of CM for at least 3 days followed by 3–5 g/d thereafter to maintain elevated stores. Ingesting smaller amounts of CM (e.g., 2–3 g/d) will T muscle creatine stores over a 3–4 wk period, however, the performance effects of this method of supplementation are less supported.
8. CM has been reported to have a number of potentially beneficial uses in several clinical populations.

(From Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr.* 2017;14:33. https://doi.org/10.1186/s12970-017-0189-4.)

Phosphocreatine can supply ATP for the energy required for quick bursts of activity lasting around 10 seconds. Once these high-energy phosphates are depleted, the body relies more heavily on anaerobic metabolism. Supplementation of creatine is thought to "supersaturate muscle" and allow for greater performance of short, explosive exercise.

Another benefit of creatine that may be key is cellular hydration. Creatine storage in the muscle is thought to set up a hyperhydration muscle cellular environment, which then improves the thermoregulatory response of the cell, allowing for improved adaptation to heat stress, thus better helping the cell respond to the stressors of exercise.[148,160,161] Research has shown hydrated muscle tissue may be better able to both synthesize new muscle tissue and resist protein breakdown.[26–28,162]

Studies on weight training and high-intensity interval exercise showed creatine as a reliable and beneficial training aid. Rawson et al.[163] in an April 2011 study showed that, in comparison with placebo, a low-dose creatine supplement (approximately 2 g daily) taken for 6 weeks significantly enhances the ability of muscle to resist fatigue.[117] Kerksick et al.[164] showed 4 weeks of creatine supplementation significantly improved both strength and lean body mass when loaded at 20 g per day for 5 days followed by 5 g per day. In another study on creatine, 23 young males were divided into a creatine group and a placebo group.[165] The creatine dose was given 5 g four times daily for 6 days. Both before and 7 days after supplementation, participants did 30 seconds of maximal sprinting on a bicycle ergometer. This regimen was repeated five times with 2 minutes rest in between. The creatine group enjoyed greater power output and less fatigue in comparison with the placebo group. Researchers found 7.6% increased power in the creatine group from the first to the second test, whereas the placebo group saw no improvement. Compounded lower body movements are key exercises in athletic training and performance. Creatine supplementation was shown to significantly improve maximal weight lifted in squats and leg presses by 8% and 3%, respectively.[166]

The ergogenic benefits of creatine are very impressive, with several large meta-analyses suggesting creatine as a significant athletic performance aid, especially in exercises requiring athletes to perform more series of sets or sprints. By increasing gains in athletic training such as increased strength, muscle mass, and endurance, supplementation with creatine allows athletes to achieve the most benefit out of their training, thus resulting in improved real-life athletic performance.[167]

In addition to improving performance, creatine may also increase recovery from intense exercise and increase lean body mass.[144–146,168] Lean body mass gains can be significantly greater with creatine than with controls while weight training. A review by Krieder et al.[168,169] determined creatine supplementation could generate a 2- to 5-lb greater muscle gain over 4 to 12 weeks of training in comparison with nonsupplementation. Recovery is often overlooked in athletes, with the mentality that more is better; however, overtraining is very common and certainly is a detriment to overall performance. The muscle-sparing and enhanced-recovery effects of creatine are evident in several studies that found anti-inflammatory effects of creatine supplementation, with reductions in prostaglandin E2 (PGE2), tumor necrosis factor alpha (TNF-alpha), lactate dehydrogenase (LDH), creatine kinase (CK), and C-reactive protein (CRP),[170,171] and additionally with sparing muscle glycogen.[172] Those studies strongly suggest that creatine establishes a biochemical environment allowing athletes to tolerated increased exercise volume and intensity.

Dosage

Creatine monohydrate is the most-studied form of creatine. Dosage of creatine is usually prescribed in two ways: a loading dose "fast dose" or a maintenance dose "slow dose." Creatine loading, or "fast dosing," is the most-studied protocol and reliably saturates muscle creatine stores. This dose entails 0.3 mg/kg of body weight daily taken in divided doses every 2 to 4 hours over 3 to 5 days. This dose essentially amounts typically to 20 to 30 g of creatine monohydrate daily for a 160- and 220-lb individual, respectively. Thereafter, the maintenance dose is used and consists of 3 to 5 g of creatine daily. Dosing the maintenance dose from the beginning, or "slow dosing," involves consuming 2 to 5 g per day over 3 to 4 weeks. Consuming creatine with a small amount of

glucose-rich juice, such as grape juice, can improve muscle saturation but has not been shown to increase performance benefits.[27,162]

Other Considerations

Creatine has now been studied extensively in several reviews, and long-term studies on safety have shown it to be safe in adults and adolescents. The only potential drawback to creatine use may be in those predisposed to fat gain.[162,173–175] There also may be implications for testosterone metabolism with creatine, because one study showed increased testosterone-to-dihydrotestosterone conversion with creatine intake.[176]

Green Tea Extract

Tea catechins from green tea have been consumed in Asian countries for centuries. Habitual green tea consumption has been shown to be correlated with lower body weights and less body fat.[177] Studies have shown extracts of catechins from green tea can increase oxidation of fat both at rest[126] and while exercising.[178]

Dulloo et al.[179] showed green tea intake elevated 24-hour resting energy expenditure with increased fatty acid oxidation in comparison with an equal amount of caffeine taken alone. A 90-day clinical trial combining green tea extract (GTE) with a low-calorie diet resulted in significantly greater weight loss in comparison with weight loss in a nontreatment group (30 vs. 11 lbs).[177] Interestingly, the activity of GTE may have particular use against abdominal fat.[180]

Ichnose et al.[181] conducted a double-blind, placebo-controlled trial of GTE intake over a 10-week period. Exercise testing was done before and after the 10-week supplementation. Subjects received either a placebo or GTE (573 mg total of green tea catechins). Both the placebo and GTE contained equal caffeine content. In addition to the supplement, both groups exercised by cycling at 60% VO_2max 60 min/day, 3 days/week. At the conclusion of 10 weeks, no changes in performance parameters were noted between groups. However, there was a statistically significant increase in fat utilization for the GTE group but no such change in the placebo group. The pre-exercise use of GTE was tested in a small human trial and showed induction of GLUT-4 receptors and improved fat oxidation. This finding further supports the use of GTE in improving body composition.[182] Animal studies have shown GTE to improve endurance and reduce fatigue,[183,184] and one human trial showed significant improvement in power and endurance in nontrained healthy males.[185]

Dosage

GTE dosage is usually 300 to 500 mg of a standardized extract daily.

Other Considerations

GTE can cause nausea in susceptible populations. It is also frequently combined with caffeine.

Quercetin

Quercetin is a bioflavonoid present in a variety of foods, including black and green tea, capers, apples, onions (giving them their yellow color), grapes, citrus fruit, tomato, broccoli, leafy green vegetables, and a number of berries. Davis et al.[186] conducted a small double-blind, placebo-controlled crossover trial on quercetin's effect on endurance capacity. Baseline VO_2max and time to exhaustion were determined for 12 participants before 7 days of supplementation with either quercetin (500 mg twice daily) or placebo. On follow-up testing, the quercetin group saw statistically significant increases in time to fatigue (13.2%) and VO_2max (3.9%) in comparison with placebo. Similar but more modest results were seen by Nieman et al.[187] in untrained subjects. Dumke et al.[188] showed no effect of quercetin on trained subjects.

Quercetin's performance-enhancing effects are often attributed to its being a polyphenol and a potent antioxidant. Given that in nature quercetin is present among other polyphenols, the benefits of polyphenols on exercise performance cannot be ignored. Although quercetin, among the many polyphenols, appears to be the most predominant ergogenic polyphenol, polyphenols as a whole appear to moderately improve athletic performance.[189] Although the goal is to be as precise as possible in deciphering which polyphenol and which dose produces beneficial outcomes, there is something to say about the synergism of multiple polyphenols that mimics their occurrence in nature. The synergism of multiple polyphenols is evident in the research showing benefit overall from polyphenol intake up to 688 mg daily, which is certainly feasible through diet.[189]

Dosage

The dosage of quercetin based on the previously mentioned studies is 1000 mg per day in divided doses.

Rhodiola

Rhodiola rosea is widely regarded as an "adaptogen." Adaptogens are defined as substances that aid the physiology in adaptation to stress, increasing function when it may be low and lowering it when it may be high. Anecdotal, animal, and some human research has hinted that *Rhodiola* may have ergogenic properties.[190–193] Several studies on *Rhodiola* suggest it may have benefit for performance, but the consensus leans more toward increased ability to adapt and recover.[191–193]

A well-designed study on *Rhodiola* was completed by Parisi et al.,[194] in which 14 competitively active males between the ages of 20 and 35 were given supplements of 170 mg of *Rhodiola rosea* extract daily for 4 weeks. All subjects completed VO_2max testing before supplementation. At the conclusion of 4 weeks, 30 minutes of cycling at 75% of VO_2max was completed. The subjects then had a 2-week washout period when no supplementation was taken. This process was followed by 4 more weeks of supplementation of placebo. At that point, the testing was repeated.

In this study on *Rhodiola* and placebo supplementation, participants' blood was drawn before testing, during supplementation, and after testing. Their rating of exertion was also monitored via the Borg exertion scale. There were no differences in any performance parameters, including perceived exertion. Both lactate and creatine kinase levels were reduced postexercise in the *Rhodiola* group. The *Rhodiola* group also showed increased levels of total antioxidants in comparison with placebo.

Dosage

Typical dose ranges are between 100 and 600 mg per day of *R. rosea* extract standardized to 3% rosavin + 1% salidroside. *Rhodiola* appears to have very low occurrences of side effects, and clinical evidence suggests it has a low toxicity.

Sodium Bicarbonate

Due to the fast accumulation of protons (hydrogen [H^+]) and carbon dioxide (CO_2) during high-intensity anaerobic activity, buffering capacity in muscle and blood is essential. The chief buffering compound is the bicarbonate ion (HCO_3^-). HCO_3^- buffers by the following reaction:

$H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \longleftrightarrow H_2O + CO_2$. The CO_2 can then be offloaded in the lungs.

Sodium bicarbonate provides a reservoir of HCO_3^-, theoretically giving greater capacity to resist fatigue-induced pH changes at the muscle cell. Several studies have shown supplemental sodium bicarbonate can stabilize pH changes for exercise lasting from 60 seconds to several minutes and improve performance.[195–198] This finding explains the significant benefits seen with the use of sodium bicarbonate in high-intensity short duration exercises such as sprinting.[199] An additional study assessed the benefits of sodium bicarbonate on a basketball

simulation protocol, resulting in significant improvements in sprint time and muscle fatigue attenuation.[200] Benefits in endurance racing were also seen in athletes, with improvements in maximum running speed, muscle fatigue, and increased time to exhaustion.[201,202]

Dosage

Sodium bicarbonate can be taken in a performance dose before exercise or a loading dose several days before an event. The pre-exercise dose is 0.3 g/kg body weight of sodium bicarbonate taken in water between 60 and 90 minutes before an exercise bout. The loading dose of bicarbonate is taken over a 5-day period and consists of 5 g taken twice a day for 5 days.

Other Considerations

No safety issues appear to arise with sodium bicarbonate intake aside from digestive upset at higher doses. However, because the average person consumes excessive amounts of sodium, this intervention should be accompanied by recommendations to decrease other sources. The primarily source is salt, but because sodium is the cation for several chemical food additives, all prepared foods must be considered.

Spirulina

The consumption of the well-known algae *Spirulina* has been a practice among athletes from diverse cultures such as India, China, and Cuba.[203] *Spirulina* is considered a member of the "blue-green algae family" along with another popular algae, *Chlorella*. *Spirulina* differs from *Chlorella* because it does not have a cell wall, rendering it more digestible. Little research has been reported on this compound except the anecdotal reports of use and benefit from athletes in the countries mentioned and some positive outcomes in mice.[204] Although there is very little supportive evidence for its use, we include it here in this chapter as a novel compound that shows some promise.

A 2006 study by Lu et al.[203] followed up on anecdotal reports from athletes and positive outcomes in animals that *Spirulina* may aid in decreasing oxidative stress from exercise. Sixteen student volunteers were divided into two groups. One group added approximately 7 g *Spirulina* to their regular diet, whereas the control group was given the same amount of soy protein. The supplementation lasted for 3 weeks. Blood was collected before and after supplementation in both groups. Both groups also participated in a treadmill run to exhaustion the day before and day after supplementation. After 3 weeks, the *Spirulina* group saw positive changes in blood chemistry, such as reductions in malondialdehyde, and increases in superoxide dismutase (SOD) and lactate, as well as significant improvement in treadmill running time to exhaustion (713 vs. 765 seconds).[203] No significant change was seen in the soy group. These findings suggest that *Spirulina* can reduce oxidative damage induced by exercise while still improving performance.

A 2010 study by Kalafati et al.[205] looked at *Spirulina* supplementation in a small double-blind, placebo-controlled and counterbalanced trial. Each subject received 6 g *Spirulina* or placebo for 4 weeks and then underwent exercise testing. The exercise challenge involved a moderate-intensity run at 70% to 75% of VO_2max for 120 minutes followed by a 95% of VO_2max run until exhaustion. *Spirulina* supplementation decreased carbohydrate oxidation by 10.3% while improving fat burning by 10.9% during the 2-hour run. Time to fatigue was also significantly improved in the *Spirulina* group. Similar to the findings by Lu et al. and others,[206] these performance enhancements were also complemented by multiple positive changes in exercise antioxidant status, such as increased glutathione and reduced lipid peroxidation, even up to 24 hours after exercise.

Although exercise is a physical activity, a mental endurance and focus is essential in athletic performance. Spirulina may affect the physical and mental aspect of training; one study showed 3 g daily of *Spirulina* significantly improved mathematical-based mental fatigue acutely and after 8 weeks, and improved short-term endurance exercise.[207]

Mitochondrial biogenesis	*Cell Metab.* 2010 Oct 6;12(4):362-72. *Aging (Albany NY).* 2011 May;3(5):464-78.
Bone growth	*Biosci Biotechnol Biochem.* 2001 Apr;65(4);913-8.
Muscle support	*Nutr Res.* 2008 Oct;28(10):651-8. *J Nutr.* 2002 Oct;132(10):3228S-33S.
Hypoglycemic effect	*J Nutr.* 2011 Apr 1;141(4):582-7. *Am J Clin Nutr.* 2010 Jan;91(1): 98-105.
Anticancer defense	*Curr Pharm Des.* 2007;13(8):813-28. *Mutat Res.* 2000 Apr;462(2-3):227-33.
Antioxidant enhancement	*Clin Invest Med.* 1989 Dec; 12(6):34-9. *Lipids Health Dis.* 2011 Apr 13;10:57.
Anti-inflammatory defense	*Int J Biochem Cell Biol.* 2013 Aug;45(8):1730-47. *Respir Med.* 2012 Nov:106(11):1526-34.
Anti-hypertensive effect	*Mol Nutr Food Res.* 2012 Feb;56(2):316-24. *Ann Med.* 2013 Feb;45(1):51-6. http://www.innovatewithdairy.com/Publications/Cardio-Health_English.pdf.
Anti-microbial activity	*Virology.* 2008 Sep 15;379(1):143-51. http://www.innovatewithdairy.com/SiteCollection-Documents/Mono_Immunity_0304.pdf.
Reduces cholesterol	*ScientificWorldJournal.* 2012;2012:269476. http://usdec.files.cms-plus.com/Publications/Cardio-Health_English.pdf.
Balances mood	*Am J Clin Nutr.* 2000 Jun;71(6):1536-44. *Psychopharmacology (Berl).* 2008 Nov;201(1):107-14.
Enhances cognitive function	*Am J Clin Nutr.* 2002 Jun;75(6):1051-6. *Am J Clin Nutr.* 2005 May;81(5):1026-33.
Reduced sarcopenia	*Nutr J.* 2012 Dec 11;11:105. *Curr Opin Clin Nutr Metab Care.* 2011 Nov;14(6):569-80.

(From Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr.* 2017;14:33. https://doi.org/10.1186/s12970-017-0189-4.)

Dosage

Spirulina dose, based on these limited studies, appears to be 6 to 7 g per day.

Other Considerations

Quality of algae in products is a concern, because these compounds can easily accumulate heavy metals present in the environment. Clean sources of *Spirulina* should be sought.

Whey Protein

Whey protein is one of the most popular meal replacements and protein sources for athletes. The protein present in whey mimics many of the proteins present in human breast milk. Whey is very rich in essential amino acids and has an exceptionally high biological value of 104. It is a rich source of sulfur-containing amino acids (methionine and cysteine), is high in glutamine, and contains large amounts of the BCAAs leucine, isoleucine, and valine. The large amount of BCAA present in whey protein may be one of its key benefits (see section on "Branched Chain Amino Acids").[208]

Whey moves through the stomach rapidly and is absorbed quickly. For this reason, it has been designated as a "fast protein." Casein, on the other hand, transits the stomach slowly and is referred to as a "slow protein."[209] The different speed of absorption between whey and casein protein fractions can be harnessed when used in muscle-building protocols as described in the "Protein" section.

No studies exist comparing the effects of different protein sources on nitrogen balance, body composition, or performance in trained athletes. However, whey has been shown to promote growth and enhance nitrogen balance in experimental animals and burn victims.[210,211]

Whey protein has one of the highest concentrations of cysteine, the rate-limiting molecule in the de novo synthesis of glutathione.[212] Any supplement that can aid the body's endogenous production of key antioxidants like glutathione may play a role in lessening damage and speeding recovery from exercise. Although free-radical generation during exercise has been shown to be a normal consequence,[213] and training, in and of itself, increases antioxidant systems in the body, supplementation can further aid endogenous antioxidant systems. Although this supplement may help with recovery,[214,215] it has not been shown to directly enhance performance.[216]

Glutathione levels are reduced during exercise.[217,218] Ultraendurance events, such as marathons and triathlons, can increase oxidized glutathione levels by 189%.[219] Because whey protein provides factors that increase glutathione production, a higher intake could play a role in protecting against damage during exercise and recovery after exercise.[220]

Another benefit of whey protein may be its ability to bolster the content of other important amino acids. Whey enhances body levels of cysteine, taurine, glutamine, and BCAAs, among others.[159] This enhancement could have consequences in several areas, including immune function.[221,222] Nothing interrupts training more than illness.

Whey protein also has merit as a fat loss and muscle-building aid. Twenty grams of whey protein taken 20 minutes before exercise increased the number of calories burned after exercise by 100 compared with no supplementation.[223] This increase may make it a useful preworkout meal compared with carbohydrate, which may slow fat loss.[1] More recent studies suggest that the benefit of whey protein in inducing muscle protein synthesis may relate more to the dose rather than the timing.[224] Young resistance-trained males were given 20 g and 40 g of whey protein after training, when the latter group have greater muscle protein synthesis. The exercise stimulus cannot be ignored because these participants trained 3 days a week with total-body exercises, which may increase the demand of amino acids given more muscle groups are exercised. This finding then suggests that perhaps more strenuous and complete total-body exercises may require more protein.[224] With improvements in muscle mass, subsequent increases in basal metabolic rate and improved body composition are expected and confirmed in both male and female athlete and nonathletes.[225–228]

Whey protein also shows a powerful ability to regulate hunger and binge eating for weight control. Pal et al.[229] carried out a randomized crossover study on 22 males given equivalent gram doses of four liquid protein meals. The four protein meals were whey, tuna, turkey, and egg albumin. Four hours after the liquid protein meals, participants were given access to an all-you-can-eat buffet-style meal. The whey protein group had much greater satiety response and consumed significantly less food than any other group reported, showing whey protein as having a potential role in appetite regulation. A similar study by Akhavan et al.[230] showed whey protein taken before an all-you-can-eat pizza meal significantly lowered food intake, postmeal glucose, and insulin levels in response to the meal.

Dosage

As discussed in this section as well as in the sections on protein and meal timing, whey protein can provide benefit when timed appropriately with exercise. Consuming whey protein postworkout may be one of the most beneficial things an athlete can do to maximize lean body mass. Whey protein is low glycemic but insulinogenic at the same time.[222,229] Immediately after a workout until about 2 hours after the workout, the body is uniquely sensitized to insulin. This sensitization may create a unique opportunity for muscle growth without fat gain, especially in a postworkout setting.[222]

The addition of a small amount of carbohydrate may be more beneficial in terms of muscle gain. In studies analyzing postworkout intake of protein alone, carbohydrate alone, or a combination of both, protein and carbohydrate together generated the greatest insulin response.[178,180,181,231] This combination enhances muscle glycogen synthesis and storage and has benefit for athletes in terms of recovery.[178,180] Adding fat to the mix is not beneficial and may blunt this response.[232,233]

Combining whey protein with creatine may have synergistic benefit as well. Men supplementing their diets with a combination of whey protein and creatine together (1.2 g/kg per day of whey with 0.1 g/kg per day creatine) enjoyed greater improvements in strength and muscle mass in comparison with placebo.[234]

Other Considerations

Although many individuals are dairy intolerant, whey protein seems to be handled well by most people, probably because pure whey protein has low levels of lactose and is easily digested. However, people with known dairy sensitivities or allergies should be cautious with their intake of whey.

Bovine serum albumin (BSA) protein in whey has been suggested as a potential cause or possible contributing factor to insulin-dependent diabetes mellitus.[235,236]Anti-BSA antibodies have been found in people with insulin-dependent diabetes mellitus. Proteins on the β-cells of the pancreas may "mimic" the BSA proteins in whey, causing immune reactivity against both the whey protein and the β-cells.[235] Theoretically, this is "protein mimicry" at work and an ongoing debate that has not yet been resolved. Although the research is inconclusive,[237] it should not be completely ruled out as a possible environmental etiology.

Magnesium

From the basic understanding of biochemistry and physiology, the role of magnesium in nerve conduction, skeletal muscle contraction, and cardiovascular physiology is often overlooked as involving an essential mineral. In fact, most Americans are deficient in magnesium; approximately 60% receive less than the recommended 330 to 350 mg per day for men and 255 to 265 mg per day for women. Although we may not see an epidemic of magnesium deficiency, we are seeing a significant increase in chronic inflammatory diseases, which has been connected with subclinical magnesium deficiency.[238–240] Increased levels of inflammatory markers such as CRP, IL-1, fibrinogen, and VCAM-1 are correlated with magnesium deficiency,[238] raise concerns that perhaps magnesium-deficient athletes, given the metabolic stress brought on by rigorous training, may further be affected by inflammation potentially impairing performance and recovery.

Studies assessing magnesium intake in athletes confirms that athletes are not unique, and they too are deficient due to inadequate consumption of dietary magnesium similar to that of the general population,[241–243] with some studies showing consumption as low as 75% of the RDA.[244] Athletes cannot be assumed to be immune to the adverse effects of magnesium deficiency. Athletes with adequate magnesium intake were associated with improved endogenous antioxidants, such as catalase and glutathione.[245]

This finding would lead to the assumption that magnesium is an ergogenic aid. Many of the studies done on athletes assessed

magnesium status via a dietary intake, whereas others showed differences in magnesium status via erythrocyte or plasma magnesium concentrations. Surprisingly, several studies did not show improved performance from magnesium supplementation. However, one study assessing male volleyball athletes did show a small but significant improvement in plyometric countermovement exercise.[246] An additional study in elderly women suggests magnesium supplementation may improve age-related decline in physical performance as evident in functional movements, such as chair-stand time and walking speed.[247]

Taking a holistic, functional approach and given the common deficiency of magnesium among various populations, it would be prudent for athletes to consume at minimum the 400 to 500 mg of magnesium daily, preferably in more bioavailable forms such as citrate and glycinate. Although the literature does not strongly correlate magnesium with athletic performance, supplementation would provide optimal neurological, muscular, and cardiovascular function.

Dosage

Based on the RDA, a minimum of 400 to 500 mg of magnesium supplementation daily would at minimum provide the necessary requirements for muscle function. Loose stools may occur in bolus doses, which may require slow titration up to 500 mg or bowel tolerance.

Cold Water Immersion

Much anecdotal evidence exists asserting cold water immersion (CWI), a form of hydrotherapy or cryotherapy, as helping with recovery. Athletes from high school to the professional level have been using it for years to help with delayed-onset muscle soreness (DOMS) and fatigue. If recovery time can be shortened, then athletic performance can at least be maintained or improved, allowing athletes improved competitive performance and more time to train. This practice can allow for enhanced training loads, resulting in improved muscle function. Currently, the research tends to suggest there may be some benefit[248–250] to using CWI, with improvements in jump performance[250] and improved ability to complete more work subsequent to training sessions.[249] However, blood biochemistries showed no significant variations after cold water immersions.[250] A meta-analysis published in *The Journal of Strength and Conditional Research* by Higgins et al. in general showed benefits from CWI in terms of neuromuscular recovery and the perception of fatigue but not necessarily perception of muscle soreness.[251]

The most benefit from CWI in reducing muscle soreness was noticed at durations of 11 to 15 minutes at 11ºC to 15ºC.[252] Interestingly, this time and temperature range resulted in reduced muscle soreness up to 24 hours postexercise and in some after 24 hours. Some of the acute benefit may be attributed to the analgesic effect of cold on the pain-spasm cycle,[253] but the prolonged benefit may be attributed to the reduction and slowing of the inflammatory response.[254] However, more study is needed in this area to flesh out the details of the mechanics behind CWI.

Considering that CWI tends to be a safe and cost-efficient way of possibly improving performance, an athlete, trainer, or coach looking for a competitive edge may benefit from putting it to use. Caution must be taken with longer emersions and lower temperatures, when CWI may excessively depress sensory and motor neurons, possibly leading to temporary nerve damage.[255] CWI should not be used before exercise or activity, because it reduces nerve conduction velocity, thus affecting the neuromuscular response.[255]

Dosage

Optimal cold immersion appears to be 11 to 15 minutes at 11ºC to 15ºC.

Ribose

The amount of ATP stored in the muscle available for immediate use is limited, and once used, must be resynthesized in the muscle. Ribose, a naturally occurring pentose sugar, is attached to the adenine that is necessary to produce adenosine. Some studies show benefit of ribose supporting and replenishing ATP levels,[256] and although this makes sense biochemically, realistically it has not translated into improved athletic performance. Studies assessing the by-product of adenine nucleotide after ribose ingestion showed no significant changes.[257] There is no convincing evidence suggesting ribose is a limiting factor in muscle ATP synthesis, nor does it improve athletic performance.[257–260]

The literature suggests ribose supplementation may be most beneficial for the elderly population, in comparison with younger individuals. Ribose has a rejuvenating effect on the heart, as it improves well-being and quality of life. As part of the aging process, the ability of mitochondria to synthesize ATP may become less efficient, thus affecting skeletal and cardiac muscle function. Therefore ribose supplementation in older athletes may provide an ergogenic effect in terms of workout recovery and endurance.[261–263]

Although the safety profile of ribose is excellent, it needs much more study before classifying it as an ergogenic aid.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wagenmakers AJ, Brouns F, Saris WH, Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. *J Appl Physiol*. 1993;75(6):2774–2780. https://doi.org/10.1152/jappl.1993.75.6.2774.
2. Bahadori B, McCarty MF, Barroso-Aranda J, Gustin JC, Contreras F. A "mini-fast with exercise" protocol for fat loss. *Med Hypotheses*. 2009;73(4):619–622. https://doi.org/10.1016/j.mehy.2008.09.063.
3. McCarty MF. Optimizing exercise for fat loss. *Med Hypotheses*. 1995;44(5):325–330.
4. Sartor F, de Morree HM, Matschke V, et al. High-intensity exercise and carbohydrate-reduced energy-restricted diet in obese individuals. *Eur J Appl Physiol*. 2010;110(5):893–903. https://doi.org/10.1007/s00421-010-1571-y.
5. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. *Prostaglandins, Leukot Essent Fat Acids*. 2004;70(3):243–251. https://doi.org/10.1016/j.plefa.2003.11.001.
6. Paoli A, Rubini A, Volek JS, Grimaldi KA. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *Eur J Clin Nutr*. 2013;67(8):789–796. https://doi.org/10.1038/ejcn.2013.116.
7. Burke LM, Ross ML, Garvican-Lewis LA, et al. Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J Physiol*. 2017;595(9):2785–2807. https://doi.org/10.1113/JP273230.
8. Lambert EV, Speechly DP, Dennis SC, Noakes TD. Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. *Eur J Appl Physiol Occup Physiol*. 1994;69(4):287–293.
9. Phinney SD, Bistrian BR, Evans WJ, Gervino E, Blackburn GL. The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism*. 1983;32(8):769–776.
10. Paoli A, Grimaldi K, D'Agostino D, et al. Ketogenic diet does not affect strength performance in elite artistic gymnasts. *J Int Soc Sports Nutr*. 2012;9(1):34. https://doi.org/10.1186/1550-2783-9-34.
11. Burke LM. Re-examining high-fat diets for sports performance: did we call the "nail in the coffin" too soon? *Sports Med*. 2015;45(suppl 1):S33–S49. https://doi.org/10.1007/s40279-015-0393-9.
12. McSwiney FT, Wardrop B, Hyde PN, Lafountain RA, Volek JS, Doyle L. Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes. *Metabolism*. 2018;81:25–34. https://doi.org/10.1016/j.metabol.2017.10.010.
13. Zinn C, Wood M, Williden M, Chatterton S, Maunder E. Ketogenic diet benefits body composition and well-being but not performance in a pilot case study of New Zealand endurance athletes. *J Int Soc Sports Nutr*. 2017;14:22. https://doi.org/10.1186/s12970-017-0180-0.
14. Trachtenberg BH, Hare JM. Inflammatory cardiomyopathic syndromes. *Circ Res*. 2017;121(7):803–818. https://doi.org/10.1161/CIRCRESAHA.117.310221.
15. Moro T, Tinsley G, Bianco A, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *J Transl Med*. 2016;14(1):290. https://doi.org/10.1186/s12967-016-1044-0.
16. Tinsley GM, La Bounty PM. Effects of intermittent fasting on body composition and clinical health markers in humans. *Nutr Rev*. 2015;73(10):661–674. https://doi.org/10.1093/nutrit/nuv041.
17. Gershuni VM, Yan SL, Medici V. Nutritional ketosis for weight management and reversal of metabolic syndrome. *Curr Nutr Rep*. 2018;7(3):97–106. https://doi.org/10.1007/s13668-018-0235-0.
18. Chung H, Chou W, Sears DD, Patterson RE, Webster NJG, Ellies LG. Time-restricted feeding improves insulin resistance and hepatic steatosis in a mouse model of postmenopausal obesity. *Metabolism*. 2016;65(12):1743–1754. https://doi.org/10.1016/j.metabol.2016.09.006.
19. Woodie LN, Luo Y, Wayne MJ, et al. Restricted feeding for 9 h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice. *Metabolism*. 2018;82:1–13. https://doi.org/10.1016/j.metabol.2017.12.004.
20. Fond G, Macgregor A, Leboyer M, Michalsen A. Fasting in mood disorders: neurobiology and effectiveness. A review of the literature. *Psychiatry Res*. 2013;209(3):253–258. https://doi.org/10.1016/j.psychres.2012.12.018.
21. Mattson MP, Moehl K, Ghena N, Schmaedick M, Cheng A. Intermittent metabolic switching, neuroplasticity and brain health. *Nat Rev Neurosci*. 2018;19(2):63–80. https://doi.org/10.1038/nrn.2017.156.
22. Snyder KA, Donnelly JE, Jabobsen DJ, Hertner G, Jakicic JM. The effects of long-term, moderate intensity, intermittent exercise on aerobic capacity, body composition, blood lipids, insulin and glucose in overweight females. *Int J Obes Relat Metab Disord*. 1997;21(12):1180–1189.
23. Aksungar FB, Sarikaya M, Coskun A, Serteser M, Unsal I. Comparison of intermittent fasting versus caloric restriction in obese subjects: a two year follow-up. *J Nutr Health Aging*. 2017;21(6):681–685. https://doi.org/10.1007/s12603-016-0786-y.
24. Aird TP, Davies RW, Carson BP. Effects of fasted vs fed-state exercise on performance and post-exercise metabolism: a systematic review and meta-analysis. *Scand J Med Sci Sports*. 2018;28(5):1476–1493. https://doi.org/10.1111/sms.13054.
25. Anton SD, Moehl K, Donahoo WT, et al. Flipping the metabolic switch: understanding and applying the health benefits of fasting. *Obesity*. 2018;26(2):254–268. https://doi.org/10.1002/oby.22065.
26. McArdle WD, Katch FI, Katch VL. *Exercise Physiology: Energy, Nutrition & Human Performance*. 8th ed. Baltimore, MD: LWW; 2014.
27. Kreider RB, Wilborn CD, Taylor L, et al. ISSN exercise sport nutrition review: research; recommendations. *J Int Soc Sports Nutr*. 2010;7(1):7. https://doi.org/10.1186/1550-2783-7-7.
28. Kreider R, Fry A, O'Toole M. *Overtraining in Sport. Human Kinetics*. Champaign, IL: Human Kinetics Publishers; 1997.
29. Kreider RB. Physiological considerations of ultraendurance performance. *Int J Sport Nutr*. 1991;1(1):3–27.
30. Brouns F, Saris WH, Stroecken J, et al. Eating, drinking, and cycling. A controlled Tour de France simulation study, Part II. Effect of diet manipulation. *Int J Sports Med*. 1989;10(s 1):S41–S48. https://doi.org/10.1055/s-2007-1024953. Suppl 1.
31. Brouns F, Saris WH, Beckers E, et al. Metabolic changes induced by sustained exhaustive cycling and diet manipulation. *Int J Sports Med*. 1989;10(suppl 1):S49–S62. https://doi.org/10.1055/s-2007-1024954.
32. Cockburn E, Stevenson E, Hayes PR, Robson-Ansley P, Howatson G. Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage. *Appl Physiol Nutr Metab*. 2010;35(3):270–277. https://doi.org/10.1139/H10-017.
33. Harger-Domitrovich SG, McClaughry AE, Gaskill SE, Ruby BC. Exogenous carbohydrate spares muscle glycogen in men and women during 10 h of exercise. *Med Sci Sport Exerc*. 2007;39(12):2171–2179. https://doi.org/10.1249/mss.0b013e318157a650.
34. Lemon PW, Tarnopolsky MA, MacDougall JD, Atkinson SA. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *J Appl Physiol*. 1992;73(2):767–775. https://doi.org/10.1152/jappl.1992.73.2.767.
35. Tarnopolsky MA, MacDougall JD, Atkinson SA. Influence of protein intake and training status on nitrogen balance and lean body mass. *J Appl Physiol*. 1988;64(1):187–193. https://doi.org/10.1152/jappl.1988.64.1.187.
36. Tarnopolsky MA, Atkinson SA, MacDougall JD, Chesley A, Phillips S, Schwarcz HP. Evaluation of protein requirements for trained strength athletes. *J Appl Physiol*. 1992;73(5):1986–1995. https://doi.org/10.1152/jappl.1992.73.5.1986.
37. Tarnopolsky MA. Protein and physical performance. *Curr Opin Clin Nutr Metab Care*. 1999;2(6):533–537.
38. Slater GJ, Jenkins D. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation and the promotion of muscle growth and strength. *Sports Med*. 2000;30(2):105–116.
39. Driskell JA, Wolinsky I. *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*. Boca Raton, London: CRC Press; 2000.
40. Kreider RB. Effects of protein and amino acid supplementation on athletic performance. *Sport Sci*. 1999;3(1).
41. Kreider R, Kleiner S. Protein supplements for athletes: need vs. convenience. *Your Patient Fit*. 2000;14(6):12–16.

42. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A*. 1997;94(26):14930–14935.
43. Boirie Y, Beaufrère B, Ritz P. Energetic cost of protein turnover in healthy elderly humans. *Int J Obes Relat Metab Disord*. 2001;25(5):601–605. https://doi.org/10.1038/sj.ijo.0801608.
44. Campbell B, Kreider RB, Ziegenfuss T, et al. International Society of Sports Nutrition position stand: protein and exercise. *J Int Soc Sports Nutr*. 2007;4(1):8. https://doi.org/10.1186/1550-2783-4-8.
45. Jäger R, Kerksick CM, Campbell BI, et al. International Society of Sports Nutrition position stand: protein and exercise. *J Int Soc Sports Nutr*. 2017;14(1):20. https://doi.org/10.1186/s12970-017-0177-8.
46. Scott CA. *Primer for the Exercise and Nutrition Sciences*. Gorham, ME: Humana Press; 2008.
47. Hämäläinen EK, Adlercreutz H, Puska P, Pietinen P. Decrease of serum total and free testosterone during a low-fat high-fibre diet. *J Steroid Biochem*. 1983;18(3):369–370.
48. Lambert CP, Frank LL, Evans WJ. Macronutrient considerations for the sport of bodybuilding. *Sports Med*. 2004;34(5):317–327.
49. Fry AC, Kraemer WJ, Ramsey LT. Pituitary-adrenal-gonadal responses to high-intensity resistance exercise overtraining. *J Appl Physiol*. 1998;85(6):2352–2359. https://doi.org/10.1152/jappl.1998.85.6.2352.
50. Venkatraman JT, Leddy J, Pendergast D. Dietary fats and immune status in athletes: clinical implications. *Med Sci Sports Exerc*. 2000;32(7 suppl):S389–S395.
51. Miller WC. Effective diet and exercise treatments for overweight and recommendations for intervention. *Sports Med*. 2001;31(10):717–724.
52. Leutholtz B, Kreider RB. Exercise and sport nutrition. In: *Nutritional Health*. Totowa, NJ: Humana Press; 2001:207–239.
53. Carli G, Bonifazi M, Lodi L, Lupo C, Martelli G, Viti A. Changes in the exercise-induced hormone response to branched chain amino acid administration. *Eur J Appl Physiol Occup Physiol*. 1992;64(3):272–277.
54. Zawadzki KM, Yaspelkis BB, Ivy JL. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. *J Appl Physiol*. 1992;72(5):1854–1859. https://doi.org/10.1152/jappl.1992.72.5.1854.
55. Burke LM. Nutrition for post-exercise recovery. *Aust J Sci Med Sport*. 1997;29(1):3–10.
56. Maughan RJ, Noakes TD. Fluid replacement and exercise stress. A brief review of studies on fluid replacement and some guidelines for the athlete. *Sports Med*. 1991;12(1):16–31.
57. Kerksick C, Harvey T, Stout J, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr*. 2008;5(1):17. https://doi.org/10.1186/1550-2783-5-17.
58. Tarnopolsky MA, Bosman M, Macdonald JR, Vandeputte D, Martin J, Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. *J Appl Physiol*. 1997;83(6):1877–1883. https://doi.org/10.1152/jappl.1997.83.6.1877.
59. Kraemer WJ, Volek JS, Bush JA, Putukian M, Sebastianelli WJ. Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *J Appl Physiol*. 1998;85(4):1544–1555. https://doi.org/10.1152/jappl.1998.85.4.1544.
60. Kovacs EMR, Schmahl RM, Senden JMG, Brouns F. Effect of high and low rates of fluid intake on post-exercise rehydration. *Int J Sport Nutr Exerc Metab*. 2002;12(1):14–23.
61. American Dietetic Association, Dietitians of Canada, American College of Sports Medicine, Rodriguez NR, Di Marco NM, Langley S. Nutrition and athletic performance. *Med Sci Sport Exerc*. 2009;41(3):709–731. https://doi:10.1249/MSS.0b013e31890eb86.
62. Applegate E. Effective nutritional ergogenic aids. *Int J Sport Nutr*. 1999;9(2):229–239.
63. Machefer G, Groussard C, Vincent S, et al. Multivitamin-mineral supplementation prevents lipid peroxidation during "the Marathon des Sables." *J Am Coll Nutr*. 2007;26(2):111–120.
64. Woolf K, Manore MM. B-vitamins and exercise: does exercise alter requirements? *Int J Sport Nutr Exerc Metab*. 2006;16(5):453–484.
65. Zemel MB. Mechanisms of dairy modulation of adiposity. *J Nutr*. 2003;133(1):252S–256S.
66. Zemel MB. Role of dietary calcium and dairy products in modulating adiposity. *Lipids*. 2003;38(2):139–146.
67. Folland JP, Stern R, Brickley G. Sodium phosphate loading improves laboratory cycling time-trial performance in trained cyclists. *J Sci Med Sport*. 2008;11(5):464–468. https://doi.org/10.1016/j.jsams.2007.04.004.
68. Hamilton B, Grantham J, Racinais S, Chalabi H. Vitamin D deficiency is endemic in Middle Eastern sportsmen. *Public Health Nutr*. 2010;13(10):1528–1534. https://doi.org/10.1017/S136898000999320X.
69. Lovell G. Vitamin D status of females in an elite gymnastics program. *Clin J Sport Med*. 2008;18(2):159–161. https://doi.org/10.1097/JSM.0b013e3181650eee.
70. Larson-Meyer DE, Willis KS. Vitamin D and athletes. *Curr Sports Med Rep*. 2010;9(4):220–226. https://doi.org/10.1249/JSR.0b013e3181e7dd45.
71. Cannell JJ, Hollis BW, Sorenson MB, Taft TN, Anderson JJB. Athletic performance and vitamin D. *Med Sci Sport Exerc*. 2009;41(5):1102–1110. https://doi.org/10.1249/MSS.0b013e3181930c2b.
72. Erikssen J, Rodahl K. Seasonal variation in work performance and heart rate response to exercise. A study of 1,835 middle-aged men. *Eur J Appl Physiol Occup Physiol*. 1979;42(2):133–140.
73. Bartoszewska M, Kamboj M, Patel DR. Vitamin D, muscle function, and exercise performance. *Pediatr Clin North Am*. 2010;57(3):849–861. https://doi.org/10.1016/j.pcl.2010.03.008.
74. Sørensen OH, Lund B, Saltin B, et al. Myopathy in bone loss of ageing: improvement by treatment with 1 alpha-hydroxycholecalciferol and calcium. *Clin Sci (Lond)*. 1979;56(2):157–161.
75. Ward KA, Das G, Roberts SA, et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. *J Clin Endocrinol Metab*. 2010;95(10):4643–4651. https://doi.org/10.1210/jc.2009-2725.
76. Dhesi JK, Jackson SHD, Bearne LM, et al. Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing*. 2004;33(6):589–595. https://doi.org/10.1093/ageing/afh209.
77. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, et al. Effect of vitamin D on falls. *JAMA*. 2004;291(16):1999. https://doi.org/10.1001/jama.291.16.1999.
78. Lord R, Bralley J. *Laboratory Evaluations for Integrative and Functional Medicine*. ed 2. Duluth, GA: Metametrix Institute; 2008.
79. Filaire E, Duché P, Lac G. Effects of amount of training on the saliva concentrations of cortisol, dehydroepiandrosterone and on the dehydroepiandrosterone: cortisol concentration ratio in women over 16 weeks of training. *Eur J Appl Physiol Occup Physiol*. 1998;78(5):466–471. https://doi.org/10.1007/s004210050447.
80. Chatard J-C, Atlaoui D, Lac G, et al. Performance and training in elite swimmers. *Int J Sports Med*. 2002;23(7):510–515. https://doi.org/10.1055/s-2002-35073.
81. Bouget M, Rouveix M, Michaux O, Pequignot J-M, Filaire E. Relationships among training stress, mood and dehydroepiandrosterone sulphate/cortisol ratio in female cyclists. *J Sports Sci*. 2006;24(12):1297–1302. https://doi.org/10.1080/02640410500497790.
82. Grisanti R, Weatherby D. Insider's Guide Adrenal stress index interpretation. *Funct Med Univ*. 2016:1–28.
83. Hickson RC, Oehler DT, Byerly RJ, Unterman TG. Protective effect of glutamine from glucocorticoid-induced muscle atrophy occurs without alterations in circulating insulin-like growth factor (IGF)-I and IGF-binding protein levels. *Proc Soc Exp Biol Med*. 1997;216(1):65–71.
84. Monteleone P, Beinat L, Tanzillo C, Maj M, Kemali D. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. *Neuroendocrinology*. 1990;52(3):243–248. https://doi.org/10.1159/000125593.
85. Kroboth PD, Amico JA, Stone RA, et al. Influence of DHEA administration on 24-hour cortisol concentrations. *J Clin Psychopharmacol*. 2003;23(1):96–99.
86. Shive W, Matthews KS. Nutritional requirements for growth of human lymphocytes. *Annu Rev Nutr*. 1988;8(1):81–97. https://doi.org/10.1146/annurev.nu.08.070188.000501.

87. Gropper S, Smith J. *Advanced Nutrition and Human Metabolism*. 6th ed. Belmont, CA: Wadsworth Publishing; 2012.
88. Nieman DC. Exercise immunology: nutritional countermeasures. *Can J Appl Physiol*. 2001;26(suppl):S45–S55.
89. Lowery T. Antioxidants. In: Antonio J, Stout J, eds. *Sports Supplements*. Baltimore, MD: Lippincott Williams & Wilkins; 260–278.
90. Nieman DC. Nutrition, exercise, and immune system function. *Clin Sports Med*. 1999;18(3):537–548.
91. Chen S, Kim W, Henning SM, Carpenter CL, Li Z. Arginine and antioxidant supplement on performance in elderly male cyclists: a randomized controlled trial. *J Int Soc Sports Nutr*. 2010;7(1):13. https://doi.org/10.1186/1550-2783-7-13.
92. McConell GK. Effects of L-arginine supplementation on exercise metabolism. *Curr Opin Clin Nutr Metab Care*. 2007;10(1):46–51. https://doi.org/10.1097/MCO.0b013e32801162fa.
93. Doutreleau S, Rouyer O, Di Marco P, et al. L-arginine supplementation improves exercise capacity after a heart transplant. *Am J Clin Nutr*. 2010;91(5):1261–1267. https://doi.org/10.3945/ajcn.2009.27881.
94. Zajac A, Poprzecki S, Zebrowska A, Chalimoniuk M, Langfort J. Arginine and ornithine supplementation increases growth hormone and insulin-like growth factor-1 serum levels after heavy-resistance exercise in strength-trained athletes. *J Strength Cond Res*. 2010;24(4):1082–1090. https://doi.org/10.1519/JSC.0b013e3181d321ff.
95. McKnight JR, Satterfield MC, Jobgen WS, et al. Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. *Amino Acids*. 2010;39(2):349–357. https://doi.org/10.1007/s00726-010-0598-z.
96. Lucotti P, Setola E, Monti LD, et al. Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. *Am J Physiol Endocrinol Metab*. 2006;291(5):E906–E912. https://doi.org/10.1152/ajpendo.00002.2006.
97. Forbes SC, Harber V, Bell GJ. The acute effects of L-arginine on hormonal and metabolic responses during submaximal exercise in trained cyclists. *Int J Sport Nutr Exerc Metab*. 2013;23(4):369–377.
98. Sandbakk SB, Sandbakk Ø, Peacock O, et al. Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes. *Nitric Oxide - Biol Chem*. 2015;48:10–15. https://doi.org/10.1016/j.niox.2014.10.006.
99. Yavuz H, Turnagol H, Demirel H. Pre-exercise arginine supplementation increases time to exhaustion in elite male wrestlers. *Biol Sport*. 2014;31(3):187–191. https://doi.org/10.5604/20831862.1111436.
100. Chang C-K, Chang Chien K-M, Chang J-H, Huang M-H, Liang Y-C, Liu T-H. Branched-chain amino acids and arginine improve performance in two consecutive days of simulated handball games in male and female athletes: a randomized trial. Tauler P, ed. *PLoS One*. 2015;10(3):e0121866. https://doi.org/10.1371/journal.pone.0121866.
101. Suzuki T, Morita M, Kobayashi Y, Kamimura A. Oral L-citrulline supplementation enhances cycling time trial performance in healthy trained men: double-blind randomized placebo-controlled 2-way crossover study. *J Int Soc Sports Nutr*. 2016;13(1):6. https://doi.org/10.1186/s12970-016-0117-z.
102. Bailey SJ, Blackwell JR, Lord T, Vanhatalo A, Winyard PG, Jones AM. L-Citrulline supplementation improves O2 uptake kinetics and high-intensity exercise performance in humans. *J Appl Physiol*. 2015;119(4):385–395. https://doi.org/10.1152/japplphysiol.00192.2014.
103. Morita M, Hayashi T, Ochiai M, et al. Oral supplementation with a combination of L-citrulline and L-arginine rapidly increases plasma L-arginine concentration and enhances NO bioavailability. *Biochem Biophys Res Commun*. 2014;454(1):53–57. https://doi.org/10.1016/j.bbrc.2014.10.029.
104. Hill CA, Harris RC, Kim HJ, et al. Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids*. 2007;32(2):225–233. https://doi.org/10.1007/s00726-006-0364-4.
105. Derave W, Ozdemir MS, Harris RC, et al. beta-alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *J Appl Physiol*. 2007;103(5):1736–1743. https://doi.org/10.1152/japplphysiol.00397.2007.
106. Hoffman JR, Ratamess NA, Faigenbaum AD, et al. Short-duration beta-alanine supplementation increases training volume and reduces subjective feelings of fatigue in college football players. *Nutr Res*. 2008;28(1):31–35. https://doi.org/10.1016/j.nutres.2007.11.004.
107. Stout JR, Cramer JT, Mielke M, O'Kroy J, Torok DJ, Zoeller RF. Effects of twenty-eight days of beta-alanine and creatine monohydrate supplementation on the physical working capacity at neuromuscular fatigue threshold. *J Strength Cond Res*. 2006;20(4):928. https://doi.org/10.1519/R-19655.1.
108. Hoffman J, Ratamess N, Kang J, Mangine G, Faigenbaum A, Stout J. Effect of creatine and ß-alanine supplementation on performance and endocrine responses in strength/power athletes. *Int J Sport Nutr Exerc Metab*. 2006;16:430–446.
109. Smith AE, Walter AA, Graef JL, et al. Effects of beta-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. *J Int Soc Sports Nutr*. 2009;6(1):5. https://doi.org/10.1186/1550-2783-6-5.
110. Varanoske AN, Hoffman JR, Church DD, et al. β-alanine supplementation elevates intramuscular carnosine content and attenuates fatigue in men and women similarly but does not change muscle l -histidine content. *Nutr Res*. 2017;48:16–25. https://doi.org/10.1016/j.nutres.2017.10.002.
111. Rosas F, Ramírez-Campillo R, Martínez C, et al. Effects of plyometric training and beta-alanine supplementation on maximal-intensity exercise and endurance in female soccer players. *J Hum Kinet*. 2017;58(1):99–109. https://doi.org/10.1515/hukin-2017-0072.
112. Saunders B, Elliott-Sale K, Artioli GG, et al. β-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med*. 2017;51(8):658–669. https://doi.org/10.1136/bjsports-2016-096396.
113. de Salles Painelli V, Roschel H, de Jesus F, et al. The ergogenic effect of beta-alanine combined with sodium bicarbonate on high-intensity swimming performance. *Appl Physiol Nutr Metab*. 2013;38(5):525–532. https://doi.org/10.1139/apnm-2012-0286.
114. Sale C, Saunders B, Hudson S, Wise JA, Harris RC, Sunderland CD. Effect of β-alanine plus sodium bicarbonate on high-intensity cycling capacity. *Med Sci Sport Exerc*. 2011;43(10):1. https://doi.org/10.1249/MSS.0b013e3182188501.
115. Bellinger PM, Howe ST, Shing CM, Fell JW. Effect of combined β-alanine and sodiumbicarbonate supplementation on cycling performance. *Med Sci Sport Exerc*. 2012;44(8):1545–1551. https://doi.org/10.1249/MSS.0b013e31824cc08d.
116. Tobias G, Benatti FB, de Salles Painelli V, et al. Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. *Amino Acids*. 2013;45(2):309–317. https://doi.org/10.1007/s00726-013-1495-z.
117. Silva VR, Belozo FL, Micheletti TO, et al. β-hydroxy-β-methylbutyrate free acid supplementation may improve recovery and muscle adaptations after resistance training: a systematic review. *Nutr Res*. 2017;45:1–9. https://doi.org/10.1016/j.nutres.2017.07.008.
118. Panton LB, Rathmacher JA, Baier S, Nissen S. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) during resistance training. *Nutrition*. 2000;16(9):734–739.
119. Nissen S, Sharp R, Ray M, et al. Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. *J Appl Physiol*. 1996;81(5):2095–2104. https://doi.org/10.1152/jappl.1996.81.5.2095.
120. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. *J Nutr*. 2001;131(7):2049–2052.
121. Knitter AE, Panton L, Rathmacher JA, Petersen A, Sharp R. Effects of beta-hydroxy-beta-methylbutyrate on muscle damage after a prolonged run. *J Appl Physiol*. 2000;89(4):1340–1344. https://doi.org/10.1152/jappl.2000.89.4.1340.
122. Asadi A, Arazi H, Suzuki K. Effects of β-hydroxy-β-methylbutyrate-free acid supplementation on strength, power and hormonal adaptations

following resistance training. *Nutrients*. 2017;9(12):1316. https://doi.org/10.3390/nu9121316.
123. Wilson JM, Lowery RP, Joy JM, et al. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance-trained individuals: a randomized, double-blind, placebo-controlled study. *Eur J Appl Physiol*. 2014;114(6):1217–1227. https://doi.org/10.1007/s00421-014-2854-5.
124. Lowery RP, Joy JM, Rathmacher JA, et al. Interaction of beta-hydroxy-beta-methylbutyrate free acid and adenosine triphosphate on muscle mass, strength, and power in resistance trained individuals. *J Strength Cond Res*. 2016;30(7):1843–1854. https://doi.org/10.1519/JSC.0000000000000482.
125. Townsend JR, Hoffman JR, Gonzalez AM, et al. Effects of β-hydroxy-β-methylbutyrate free acid ingestion and resistance exercise on the acute endocrine response. *Int J Endocrinol*. 2015;2015:1–7. https://doi.org/10.1155/2015/856708.
126. Robinson EH, Stout JR, Miramonti AA, et al. High-intensity interval training and β-hydroxy-β-methylbutyric free acid improves aerobic power and metabolic thresholds. *J Int Soc Sports Nutr*. 2014;11(1):16. https://doi.org/10.1186/1550-2783-11-16.
127. Wilkinson DJ, Hossain T, Hill DS, et al. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. *J Physiol*. 2013;591(11):2911–2923. https://doi.org/10.1113/jphysiol.2013.253203.
128. Pimentel GD, Rosa JC, Lira FS, et al. β-Hydroxy-β-methylbutyrate (HMβ) supplementation stimulates skeletal muscle hypertrophy in rats via the mTOR pathway. *Nutr Metab (Lond)*. 2011;8(1):11. https://doi.org/10.1186/1743-7075-8-11.
129. O'Connor DM, Crowe MJ. Effects of beta-hydroxy-beta-methylbutyrate and creatine monohydrate supplementation on the aerobic and anaerobic capacity of highly trained athletes. *J Sports Med Phys Fitness*. 2003;43(1):64–68.
130. Nygren J, Nair KS. Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. *Diabetes*. 2003;52(6):1377–1385.
131. Sharp CPM, Pearson DR. Amino acid supplements and recovery from high-intensity resistance training. *J Strength Cond Res*. 2010;24(4):1125–1130. https://doi.org/10.1519/JSC.0b013e3181c7c655.
132. Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM. Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. *Int J Sport Nutr Exerc Metab*. 2007;17(6):595–607.
133. Jitomir J, Willoughby DS. Leucine for retention of lean mass on a hypocaloric diet. *J Med Food*. 2008;11(4):606–609. https://doi.org/10.1089/jmf.2008.0058.
134. Greer BK, White JP, Arguello EM, Haymes EM. Branched-chain amino acid supplementation lowers perceived exertion but does not affect performance in untrained males. *J Strength Cond Res*. 2011;25(2):539–544. https://doi.org/10.1519/JSC.0b013e3181bf443a.
135. Matsumoto K, Koba T, Hamada K, Tsujimoto H, Mitsuzono R. Branched-chain amino acid supplementation increases the lactate threshold during an incremental exercise test in trained individuals. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(1):52–58.
136. Fouré A, Bendahan D. Is branched-chain amino acids supplementation an efficient nutritional strategy to alleviate skeletal muscle damage? A systematic review. *Nutrients*. 2017;9(10):1047. https://doi.org/10.3390/nu9101047.
137. She P, Reid TM, Bronson SK, et al. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. *Cell Metab*. 2007;6(3):181–194. https://doi.org/10.1016/j.cmet.2007.08.003.
138. Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ, Yu Y-H. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. *Diabetes*. 2007;56(6):1647–1654. https://doi.org/10.2337/db07-0123.
139. May ME, Buse MG. Effects of branched-chain amino acids on protein turnover. *Diabetes Metab Rev*. 1989;5(3):227–245.
140. Ra S-G, Miyazaki T, Kojima R, et al. Effect of BCAA supplement timing on exercise-induced muscle soreness and damage: a pilot placebo-controlled double-blind study. *J Sports Med Phys Fitness*. 2017. https://doi.org/10.23736/S0022-4707.17.07638-1.
141. Waldron M, Whelan K, Jeffries O, Burt D, Howe L, Patterson SD. The effects of acute branched-chain amino acid supplementation on recovery from a single bout of hypertrophy exercise in resistance-trained athletes. *Appl Physiol Nutr Metab*. 2017;42(6):630–636. https://doi.org/10.1139/apnm-2016-0569.
142. Valerio A, D'Antona G, Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: an evolutionary perspective. *Aging (Albany NY)*. 2011;3(5):464–478. https://doi.org/10.18632/aging.100322.
143. Gualano AB, Bozza T, Lopes De Campos P, et al. Branched-chain amino acids supplementation enhances exercise capacity and lipid oxidation during endurance exercise after muscle glycogen depletion. *J Sports Med Phys Fitness*. 2011;51(1):82–88.
144. Cota D, Proulx K, Smith KAB, et al. Hypothalamic mTOR signaling regulates food intake. *Science*. 2006;312(5775):927–930. https://doi.org/10.1126/science.1124147.
145. Harris RC, Tallon MJ, Dunnett M, et al. The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids*. 2006;30(3):279–289. https://doi.org/10.1007/s00726-006-0299-9.
146. Ordman AB. Pilot study for an age- and gender-based nutrient signaling system for weight control. *Age (Omaha)*. 2008;30(4):201–208. https://doi.org/10.1007/s11357-008-9049-0.
147. Davis JK, Green JM. Caffeine and anaerobic performance. *Sport Med*. 2009;39(10):813–832. https://doi.org/10.2165/11317770-000000000-00000.
148. Lopez RM, Casa DJ, McDermott BP, Ganio MS, Armstrong LE, Maresh CM. Does creatine supplementation hinder exercise heat tolerance or hydration status? A systematic review with meta-analyses. *J Athl Train*. 2009;44(2):215–223. https://doi.org/10.4085/1062-6050-44.2.215.
149. Goldstein ER, Ziegenfuss T, Kalman D, et al. International society of sports nutrition position stand: caffeine and performance. *J Int Soc Sports Nutr*. 2010;7(1):5. https://doi.org/10.1186/1550-2783-7-5.
150. Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. *Med Sci Sports*. 1979;11(1):6–11.
151. Yeo SE, Jentjens RLPG, Wallis GA, Jeukendrup AE. Caffeine increases exogenous carbohydrate oxidation during exercise. *J Appl Physiol*. 2005;99(3):844–850. https://doi.org/10.1152/japplphysiol.00170.2005.
152. Kerksick CM, Arent S, Schoenfeld BJ, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr*. 2017;14(1):33. https://doi.org/10.1186/s12970-017-0189-4.
153. Schneiker KT, Bishop D, Dawson B, Hackett LP. Effects of caffeine on prolonged intermittent-sprint ability in team-sport athletes. *Med Sci Sports Exerc*. 2006;38(3):578–585. https://doi.org/10.1249/01.mss.0000188449.18968.62.
154. Richardson DL, Clarke ND. Effect of coffee and caffeine ingestion on resistance exercise performance. *J Strength Cond Res*. 2016;30(10):2892–2900. https://doi.org/10.1519/JSC.0000000000001382.
155. Grgic J. Caffeine ingestion enhances Wingate performance: a meta-analysis. *Eur J Sport Sci*. 2017:1–7. https://doi.org/10.1080/17461391.2017.1394371.
156. Christensen PM, Shirai Y, Ritz C, Nordsborg NB. Caffeine and bicarbonate for speed. A meta-analysis of legal supplements potential for improving intense endurance exercise performance. *Front Physiol*. 2017;8:240. https://doi.org/10.3389/fphys.2017.00240.
157. Pedersen DJ, Lessard SJ, Coffey VG, et al. High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine. *J Appl Physiol*. 2008;105(1):7–13. https://doi.org/10.1152/japplphysiol.01121.2007.
158. Astrup A, Toubro S, Cannon S, Hein P, Breum L, Madsen J. Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *Am J Clin Nutr*. 1990;51(5):759–767.

159. Mohr M, Nielsen JJ, Bangsbo J. Caffeine intake improves intense intermittent exercise performance and reduces muscle interstitial potassium accumulation. *J Appl Physiol*. 2011;111(5):1372–1379. https://doi.org/10.1152/japplphysiol.01028.2010.
160. Kilduff LP, Georgiades E, James N, et al. The effects of creatine supplementation on cardiovascular, metabolic, and thermoregulatory responses during exercise in the heat in endurance-trained humans. *Int J Sport Nutr Exerc Metab*. 2004;14(4):443–460.
161. Polyviou TP, Pitsiladis YP, Celis-Morales C, Brown B, Speakman JR, Malkova D. The effects of hyperhydrating supplements containing creatine and glucose on plasma lipids and insulin sensitivity in endurance-trained athletes. *J Amino Acids*. 2015;2015:1–8. https://doi.org/10.1155/2015/352458.
162. Godard MP, Johnson BA, Richmond SR. Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. *Obes Res*. 2005;13(8):1335–1343. https://doi.org/10.1038/oby.2005.162.
163. Rawson ES, Stec MJ, Frederickson SJ, Miles MP. Low-dose creatine supplementation enhances fatigue resistance in the absence of weight gain. *Nutrition*. 2011;27(4):451–455. https://doi.org/10.1016/j.nut.2010.04.001.
164. Kerksick CM, Wilborn CD, Campbell WI, et al. The effects of creatine monohydrate supplementation with and without D-pinitol on resistance training adaptations. *J Strength Cond Res*. 2009;23(9):2673–2682. https://doi.org/10.1519/JSC.0b013e3181b3e0de.
165. Okudan N, Gokbel H. The effects of creatine supplementation on performance during the repeated bouts of supramaximal exercise. *J Sports Med Phys Fitness*. 2005;45(4):507–511.
166. Lanhers C, Pereira B, Naughton G, Trousselard M, Lesage F-X, Dutheil F. Creatine supplementation and lower limb strength performance: a systematic review and meta-analyses. *Sports Med*. 2015;45(9):1285–1294. https://doi.org/10.1007/s40279-015-0337-4.
167. Kreider RB, Kalman DS, Antonio J, et al. International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. *J Int Soc Sports Nutr*. 2017;14:18. https://doi.org/10.1186/s12970-017-0173-z.
168. Volek JS, Duncan ND, Mazzetti SA, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc*. 1999;31(8):1147–1156.
169. Kreider RB. Effects of creatine supplementation on performance and training adaptations. *Mol Cell Biochem*. 2003;244(1–2):89–94.
170. Deminice R, Rosa FT, Franco GS, Jordao AA, de Freitas EC. Effects of creatine supplementation on oxidative stress and inflammatory markers after repeated-sprint exercise in humans. *Nutrition*. 2013;29(9):1127–1132. https://doi.org/10.1016/j.nut.2013.03.003.
171. Santos RV, Bassit R, Caperuto E, Costa Rosa LFB. The effect of creatine supplementation upon inflammatory and muscle soreness markers after a 30km race. *Life Sci*. 2004;75(16):1917–1924. https://doi.org/10.1016/j.lfs.2003.11.036.
172. Nelson AG, Arnall DA, Kokkonen J, Day R, Evans J. Muscle glycogen supercompensation is enhanced by prior creatine supplementation. *Med Sci Sports Exerc*. 2001;33(7):1096–1100.
173. Taes YEC, Delanghe JR, Wuyts B, van de Voorde J, Lameire NH. Creatine supplementation does not affect kidney function in an animal model with pre-existing renal failure. *Nephrol Dial Transplant*. 2003;18(2):258–264.
174. Schilling BK, Stone MH, Utter A, et al. Creatine supplementation and health variables: a retrospective study. *Med Sci Sports Exerc*. 2001;33(2):183–188.
175. Kreider RB, Melton C, Rasmussen CJ, et al. Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. *Mol Cell Biochem*. 2003;244(1–2):95–104.
176. van der Merwe J, Brooks NE, Myburgh KH. Three weeks of creatine monohydrate supplementation affects dihydrotestosterone to testosterone ratio in college-aged rugby players. *Clin J Sport Med*. 2009;19(5):399–404. https://doi.org/10.1097/JSM.0b013e3181b8b52f.
177. Di Pierro F, Menghi AB, Barreca A, Lucarelli M, Calandrelli A. Greenselect phytosome as an adjunct to a low-calorie diet for treatment of obesity: a clinical trial. *Altern Med Rev*. 2009;14(2):154–160.
178. Venables MC, Hulston CJ, Cox HR, Jeukendrup AE. Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. *Am J Clin Nutr*. 2008;87(3):778–784.
179. Dulloo AG, Duret C, Rohrer D, et al. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr*. 1999;70(6):1040–1045.
180. Maki KC, Reeves MS, Farmer M, et al. Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. *J Nutr*. 2009;139(2):264–270. https://doi.org/10.3945/jn.108.098293.
181. Ichinose T, Nomura S, Someya Y, Akimoto S, Tachiyashiki K, Imaizumi K. Effect of endurance training supplemented with green tea extract on substrate metabolism during exercise in humans. *Scand J Med Sci Sports*. 2011;21(4):598–605. https://doi.org/10.1111/j.1600-0838.2009.01077.x.
182. Tsai T-W, Chang C-C, Liao S-F, et al. Effect of green tea extract supplementation on glycogen replenishment in exercised human skeletal muscle. *Br J Nutr*. 2017;117(10):1343–1350. https://doi.org/10.1017/S0007114517001374.
183. Teng Y-S, Wu D. Anti-fatigue effect of green tea polyphenols (-)-epigallocatechin-3-gallate (EGCG). *Pharmacogn Mag*. 2017;13(50):326–331. https://doi.org/10.4103/0973-1296.204546.
184. Korf EA, Kubasov IV, Vonsky MS, et al. Green tea extract increases the expression of genes responsible for regulation of calcium balance in rat slow-twitch muscles under conditions of exhausting exercise. *Bull Exp Biol Med*. 2017;164(1):6–9. https://doi.org/10.1007/s10517-017-3913-9.
185. Roberts JD, Roberts MG, Tarpey MD, Weekes JC, Thomas CH. The effect of a decaffeinated green tea extract formula on fat oxidation, body composition and exercise performance. *J Int Soc Sports Nutr*. 2015;12(1):1. https://doi.org/10.1186/s12970-014-0062-7.
186. Davis JM, Carlstedt CJ, Chen S, Carmichael MD, Murphy EA. The dietary flavonoid quercetin increases VO(2max) and endurance capacity. *Int J Sport Nutr Exerc Metab*. 2010;20(1):56–62.
187. Nieman DC, Williams AS, Shanely RA, et al. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. *Med Sci Sports Exerc*. 2010;42(2):338–345. https://doi.org/10.1249/MSS.0b013e3181b18fa3.
188. Dumke CL, Nieman DC, Utter AC, et al. Quercetin's effect on cycling efficiency and substrate utilization. *Appl Physiol Nutr Metab*. 2009;34(6):993–1000. https://doi.org/10.1139/H09-099.
189. Somerville V, Bringans C, Braakhuis A. Polyphenols and performance: a systematic review and meta-analysis. *Sports Med*. 2017;47(8):1589–1599. https://doi.org/10.1007/s40279-017-0675-5.
190. Walker TB, Robergs RA. Does Rhodiola rosea possess ergogenic properties? *Int J Sport Nutr Exerc Metab*. 2006;16(3):305–315.
191. Abidov M, Crendal F, Grachev S, Seifulla R, Ziegenfuss T. Effect of extracts from Rhodiola rosea and Rhodiola crenulata (Crassulaceae) roots on ATP content in mitochondria of skeletal muscles. *Bull Exp Biol Med*. 2003;136(6):585–587.
192. De Bock K, Eijnde BO, Ramaekers M, Hespel P. Acute Rhodiola rosea intake can improve endurance exercise performance. *Int J Sport Nutr Exerc Metab*. 2004;14(3):298–307.
193. Olsson E, von Schéele B, Panossian A. A Randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract SHR-5 of the roots of *Rhodiola rosea* in the treatment of subjects with stress-related fatigue. *Planta Med*. 2009;75(02):105–112. https://doi.org/10.1055/s-0028-1088346.
194. Parisi A, Tranchita E, Duranti G, et al. Effects of chronic Rhodiola rosea supplementation on sport performance and antioxidant capacity in trained male: preliminary results. *J Sports Med Phys Fitness*. 2010;50(1):57–63.
195. McNaughton L, Backx K, Palmer G, Strange N. Effects of chronic bicarbonate ingestion on the performance of high-intensity work. *Eur J Appl Physiol Occup Physiol*. 1999;80(4):333–336. https://doi.org/10.1007/s004210050600.

196. Kraemer WJ, Gordon SE, Lynch JM, Pop ME, Clark KL. Effects of multi-buffer supplementation on acid-base balance and 2,3-diphosphoglycerate following repetitive anaerobic exercise. *Int J Sport Nutr*. 1995;5(4):300–314.
197. Matson LG, Tran ZV. Effects of sodium bicarbonate ingestion on anaerobic performance: a meta-analytic review. *Int J Sport Nutr*. 1993;3(1):2–28.
198. Lindh A, Peyrebrune M, Ingham S, Bailey D, Folland J. Sodium bicarbonate improves swimming performance. *Int J Sports Med*. 2008;29(6):519–523. https://doi.org/10.1055/s-2007-989228.
199. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance. *Sport Med*. 2011;41(10):801–814. https://doi.org/10.2165/11591440-000000000-00000.
200. Ansdell P, Dekerle J. Sodium bicarbonate supplementation delays neuromuscular fatigue without changes in performance outcomes during a basketball match simulation protocol. *J Strength Cond Res*. 2017:1. https://doi.org/10.1519/JSC.0000000000002233.
201. Freis T, Hecksteden A, Such U, Meyer T. Effect of sodium bicarbonate on prolonged running performance: a randomized, double-blind, cross-over study. Earnest CP, ed. *PLoS One*. 2017;12(8):e0182158. https://doi.org/10.1371/journal.pone.0182158.
202. Egger F, Meyer T, Such U, Hecksteden A. Effects of sodium bicarbonate on high-intensity endurance performance in cyclists: a double-blind, randomized cross-over trial. Earnest CP, ed. *PLoS One*. 2014;9(12):e114729. https://doi.org/10.1371/journal.pone.0114729.
203. Lu H-K, Hsieh C-C, Hsu J-J, Yang Y-K, Chou H-N. Preventive effects of Spirulina platensis on skeletal muscle damage under exercise-induced oxidative stress. *Eur J Appl Physiol*. 2006;98(2):220–226. https://doi.org/10.1007/s00421-006-0263-0.
204. Li-xin H, Guangxi Institute of Sports Science. Effects of Spirulina platensis on exercise-induced muscle damage in rats. *Sport Sci*. 2000;20:58–59.
205. Kalafati M, Jamurtas AZ, Nikolaidis MG, et al. Ergogenic and antioxidant effects of spirulina supplementation in humans. *Med Sci Sport Exerc*. 2010;42(1):142–151. https://doi.org/10.1249/MSS.0b013e3181ac7a45.
206. Wu Q, Liu L, Miron A, Klímová B, Wan D, Kuča K. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. *Arch Toxicol*. 2016;90(8):1817–1840. https://doi.org/10.1007/s00204-016-1744-5.
207. Johnson M, Hassinger L, Davis J, Devor ST, DiSilvestro RA. A randomized, double blind, placebo controlled study of spirulina supplementation on indices of mental and physical fatigue in men. *Int J Food Sci Nutr*. 2016;67(2):203–206. https://doi.org/10.3109/09637486.2016.1144719.
208. Morifuji M, Koga J, Kawanaka K, Higuchi M. Branched-chain amino acid-containing dipeptides, identified from whey protein hydrolysates, stimulate glucose uptake rate in L6 myotubes and isolated skeletal muscles. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(1):81–86.
209. Mahé S, Roos N, Benamouzig R, et al. Gastrojejunal kinetics and the digestion of [15N]beta-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein. *Am J Clin Nutr*. 1996;63(4):546–552.
210. Mahan DC. Efficacy of dried whey and its lactalbumin and lactose components at two dietary lysine levels on postweaning pig performance and nitrogen balance. *J Anim Sci*. 1992;70(7):2182–2187.
211. Alexander JW, Gottschlich MM. Nutritional immunomodulation in burn patients. *Crit Care Med*. 1990;18(suppl 2):S149–S153.
212. Bounous G, Gervais F, Amer V, Batist G, Gold P. The influence of dietary whey protein on tissue glutathione and the diseases of aging. *Clin Invest Med*. 1989;12(6):343–349.
213. Kanter MM. Free radicals, exercise, and antioxidant supplementation. *Int J Sport Nutr*. 1994;4(3):205–220.
214. Junior PS, Ribeiro AS, Nabuco HCG, et al. Effects of whey protein supplementation associated with resistance training on muscular strength, hypertrophy and muscle quality in pre-conditioned older women. *Int J Sport Nutr Exerc Metab. December*. 2017:1–27. https://doi.org/10.1123/ijsnem.2017-0253.
215. Brown MA, Stevenson EJ, Howatson G. Whey protein hydrolysate supplementation accelerates recovery from exercise-induced muscle damage in females. *Appl Physiol Nutr Metab*. 2017. https://doi.org/10.1139/apnm-2017-0412. apnm-2017-0412.
216. Clarkson PM. Antioxidants and physical performance. *Crit Rev Food Sci Nutr*. 1995;35(1–2):131–141. https://doi.org/10.1080/10408399509527692.
217. Leeuwenburgh C, Leichtweis S, Hollander J, Fiebig R, Gore M, Ji LL. Effect of acute exercise on glutathione deficient heart. *Mol Cell Biochem*. 1996;156(1):17–24.
218. Shephard RJ, Shek PN. Heavy exercise, nutrition and immune function: is there a connection? *Int J Sports Med*. 1995;16(8):491–497. https://doi.org/10.1055/s-2007-973043.
219. Cooper MB, Jones DA, Edwards RH, Corbucci GC, Montanari G, Trevisani C. The effect of marathon running on carnitine metabolism and on some aspects of muscle mitochondrial activities and antioxidant mechanisms. *J Sports Sci*. 1986;4(2):79–87. https://doi.org/10.1080/02640418608732103.
220. Bounous G, Batist G, Gold P. Immunoenhancing property of dietary whey protein in mice: role of glutathione. *Clin Invest Med*. 1989;12(3):154–161.
221. Bounous G, Baruchel S, Falutz J, Gold P. Whey proteins as a food supplement in HIV-seropositive individuals. *Clin Invest Med*. 1993;16(3):204–209.
222. Wong CW, Watson DL. Immunomodulatory effects of dietary whey proteins in mice. *J Dairy Res*. 1995;62(2):359–368.
223. Hackney KJ, Bruenger AJ, Lemmer JT. Timing protein intake increases energy expenditure 24 h after resistance training. *Med Sci Sport Exerc*. 2010;42(5):998–1003. https://doi.org/10.1249/MSS.0b013e3181c12976.
224. Macnaughton LS, Wardle SL, Witard OC, et al. The response of muscle protein synthesis following whole–body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep*. 2016;4(15):e12893. https://doi.org/10.14814/phy2.12893.
225. Taylor LW, Wilborn C, Roberts MD, White A, Dugan K. Eight weeks of pre- and postexercise whey protein supplementation increases lean body mass and improves performance in Division III collegiate female basketball players. *Appl Physiol Nutr Metab*. 2016;41(3):249–254. https://doi.org/10.1139/apnm-2015-0463.
226. Lockwood CM, Roberts MD, Dalbo VJ, et al. Effects of hydrolyzed whey versus other whey protein supplements on the physiological response to 8 weeks of resistance exercise in college-aged males. *J Am Coll Nutr*. 2017;36(1):16–27. https://doi.org/10.1080/07315724.2016.1140094.
227. Dudgeon WD, Kelley EP, Scheett TP. Effect of whey protein in conjunction with a caloric-restricted diet and resistance training. *J Strength Cond Res*. 2017;31(5):1353–1361. https://doi.org/10.1519/JSC.0000000000001196.
228. Zapata RC, Singh A, Pezeshki A, Nibber T, Chelikani PK. Whey protein components—lactalbumin and lactoferrin—improve energy balance and metabolism. *Sci Rep*. 2017;7(1):9917. https://doi.org/10.1038/s41598-017-09781-2.
229. Pal S, Ellis V. The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. *Br J Nutr*. 2010;104(8):1241–1248. https://doi.org/10.1017/S0007114510001911.
230. Akhavan T, Luhovyy BL, Brown PH, Cho CE, Anderson GH. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. *Am J Clin Nutr*. 2010;91(4):966–975. https://doi.org/10.3945/ajcn.2009.28406.
231. Dale RB, Leaver-Dunn D, Bishop P. A Compositional analysis of a common acetic acid solution with practical implications for ingestion. *J Athl Train*. 2003;38(1):57–61.
232. Ivy JL, Goforth HW, Damon BM, McCauley TR, Parsons EC, Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J Appl Physiol*. 2002;93(4):1337–1344. https://doi.org/10.1152/japplphysiol.00394.2002.

233. Roy BD, Tarnopolsky MA. Influence of differing macronutrient intakes on muscle glycogen resynthesis after resistance exercise. *J Appl Physiol*. 1998;84(3):890–896. https://doi.org/10.1152/jappl.1998.84.3.890.
234. Burke DG, Chilibeck PD, Davidson KS, Candow DG, Farthing J, Smith-Palmer T. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *Int J Sport Nutr Exerc Metab*. 2001;11(3):349–364.
235. Karjalainen J, Martin JM, Knip M, et al. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med*. 1992;327(5):302–307. https://doi.org/10.1056/NEJM199207303270502.
236. Ivarsson SA, Månsson MU, Jakobsson IL. IgG antibodies to bovine serum albumin are not increased in children with IDDM. *Diabetes*. 1995;44(11):1349–1350.
237. Persaud DR, Barranco-Mendoza A. Bovine serum albumin and insulin-dependent diabetes mellitus; is cow's milk still a possible toxicological causative agent of diabetes? *Food Chem Toxicol*. 2004;42(5):707–714. https://doi.org/10.1016/j.fct.2004.01.002.
238. Nielsen FH. Magnesium deficiency and increased inflammation: current perspectives. *J Inflamm Res*. 2018;11:25–34. https://doi.org/10.2147/JIR.S136742.
239. Nielsen FH. Effects of magnesium depletion on inflammation in chronic disease. *Curr Opin Clin Nutr Metab Care*. 2014;17(6):525–530. https://doi.org/10.1097/MCO.0000000000000093.
240. Nielsen FH. Magnesium, inflammation, and obesity in chronic disease. *Nutr Rev*. 2010;68(6):333–340. https://doi.org/10.1111/j.1753-4887.2010.00293.x.
241. Silva MRG, Paiva T. Low energy availability and low body fat of female gymnasts before an international competition. *Eur J Sport Sci*. 2015;15(7):591–599. https://doi.org/10.1080/17461391.2014.969323.
242. Wierniuk A, Włodarek D. Estimation of energy and nutritional intake of young men practicing aerobic sports. *Rocz Panstw Zakl Hig*. 2013;64(2):143–148.
243. Imamura H, Iide K, Yoshimura Y, et al. Nutrient intake, serum lipids and iron status of colligiate rugby players. *J Int Soc Sports Nutr*. 2013;10(1):9. https://doi.org/10.1186/1550-2783-10-9.
244. Clark M, Reed DB, Crouse SF, Armstrong RB. Pre- and post-season dietary intake, body composition, and performance indices of NCAA division I female soccer players. *Int J Sport Nutr Exerc Metab*. 2003;13(3):303–319.
245. Tong TK, Lin H, Lippi G, Nie J, Tian Y. Serum oxidant and antioxidant status in adolescents undergoing professional endurance sports training. *Oxid Med Cell Longev*. 2012;2012:1–7. https://doi.org/10.1155/2012/741239.
246. Setaro L, Santos-Silva PR, Nakano EY, et al. Magnesium status and the physical performance of volleyball players: effects of magnesium supplementation. *J Sports Sci*. 2014;32(5):438–445. https://doi.org/10.1080/02640414.2013.828847.
247. Veronese N, Berton L, Carraro S, et al. Effect of oral magnesium supplementation on physical performance in healthy elderly women involved in a weekly exercise program: a randomized controlled trial. *Am J Clin Nutr*. 2014;100(3):974–981. https://doi.org/10.3945/ajcn.113.080168.
248. Versey NG, Halson SL, Dawson BT. Water immersion recovery for athletes: effect on exercise performance and practical recommendations. *Sport Med*. 2013;43(11):1101–1130. https://doi.org/10.1007/s40279-013-0063-8.
249. Roberts LA, Nosaka K, Coombes JS, Peake JM. Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. *Am J Physiol Integr Comp Physiol*. 2014;307(8):R998–R1008. https://doi.org/10.1152/ajpregu.00180.2014.
250. Delextrat A, Calleja-González J, Hippocrate A, Clarke ND. Effects of sports massage and intermittent cold-water immersion on recovery from matches by basketball players. *J Sports Sci*. 2013;31(1):11–19. https://doi.org/10.1080/02640414.2012.719241.
251. Higgins TR, Greene DA, Baker MK. Effects of cold water immersion and contrast water therapy for recovery from team sport: a systematic review and meta-analysis. *J Strength Cond Res*. 2017;31(5):1443–1460. https://doi.org/10.1519/JSC.0000000000001559.
252. Machado AF, Ferreira PH, Micheletti JK, et al. Can water temperature and immersion time influence the effect of cold water immersion on muscle soreness? A systematic review and meta-analysis. *Sport Med*. 2016;46(4):503–514. https://doi.org/10.1007/s40279-015-0431-7.
253. Halson SL, Quod MJ, Martin DT, Gardner AS, Ebert TR, Laursen PB. Physiological responses to cold water immersion following cycling in the heat. *Int J Sports Physiol Perform*. 2008;3(3):331–346.
254. Wilcock IM, Cronin JB, Hing WA. Physiological response to water immersion: a method for sport recovery? *Sports Med*. 2006;36(9):747–765.
255. Herrera E, Sandoval MC, Camargo DM, Salvini TF. Motor and sensory nerve conduction are affected differently by ice pack, ice massage, and cold water immersion. *Phys Ther*. 2010;90(4):581–591. https://doi.org/10.2522/ptj.20090131.
256. Dhanoa TS, Housner JA. Ribose: more than a simple sugar? *Curr Sports Med Rep*. 2007;6(4):254–257.
257. Kerksick C, Rasmussen C, Bowden R, et al. Effects of ribose supplementation prior to and during intense exercise on anaerobic capacity and metabolic markers. *Int J Sport Nutr Exerc Metab*. 2005;15(6):653–664.
258. Berardi JM, Ziegenfuss TN. Effects of ribose supplementation on repeated sprint performance in men. *J Strength Cond Res*. 2003;17(1):47–52.
259. Peveler WW, Bishop PA, Whitehorn EJ. Effects of ribose as an ergogenic aid. *J Strength Cond Res*. 2006;20(3):519–522. https://doi.org/10.1519/17355.1.
260. Hellsten Y, Skadhauge L, Bangsbo J. Effect of ribose supplementation on resynthesis of adenine nucleotides after intense intermittent training in humans. *Am J Physiol Regul Integr Comp Physiol*. 2004;286(1):R182–R188. https://doi.org/10.1152/ajpregu.00286.2003.
261. Bayram M, Cyr JA S, Abraham WT. D-ribose aids heart failure patients with preserved ejection fraction and diastolic dysfunction: a pilot study. *Ther Adv Cardiovasc Dis*. 2015;9(3):56–65. https://doi.org/10.1177/1753944715572752.
262. Omran H, Illien S, MacCarter D, St Cyr J, Lüderitz B. D-Ribose improves diastolic function and quality of life in congestive heart failure patients: a prospective feasibility study. *Eur J Heart Fail*. 2003;5(5):615–619.
263. Pauly DF, Pepine CJ. Ischemic heart disease: metabolic approaches to management. *Clin Cardiol*. 2004;27(8):439–441.

140

Stress Management

Michael T. Murray, ND

OUTLINE

INTRODUCTION

Stress is defined as any disturbance (e.g., heat or cold, chemical toxin, microorganisms, physical trauma, strong emotional reaction) that can trigger the "stress response." How an individual handles stress plays a major role in determining his or her level of health. Comprehensive stress management involves a truly holistic approach designed to counteract the everyday stresses of life. Most often, the stress response is so mild, it goes entirely unnoticed. However, if stress is extreme, unusual, or long lasting, the stress response can be overwhelming and quite harmful to virtually any body system.

Before one can discuss methods for helping patients deal effectively with stress, it is important to understand the stress response. Ultimately, the success of any stress management program depends on its ability to improve an individual's immediate and long-term responses to stress.

THE GENERAL ADAPTATION SYNDROME

The stress response is actually part of a larger response known as the *general adaptation syndrome*, a term coined by the pioneering stress researcher Hans Selye. To fully understand how to combat stress, one must understand the general adaptation syndrome. The syndrome is composed of three phases: alarm, resistance, and exhaustion.[1] These phases are largely controlled and regulated by the adrenal glands.

The initial response to stress is the alarm reaction, which is often referred to as the fight-or-flight response. The fight-or-flight response is triggered by activation of the sympathetic nervous system and, ultimately, the hypothalamic–pituitary–adrenal (HPA) axis, which causes the adrenals to secrete adrenaline and other stress-related hormones.

The fight-or-flight response is designed to counteract danger by mobilizing the body's resources for immediate physical activity. As a result, the heart rate and force of contraction of the heart increase to provide blood to areas necessary for response to the stressful situation. Blood is shunted away from the skin and internal organs, except the heart and lungs, whereas the amount of blood supplying required oxygen and glucose to the muscles and brain is increased. The rate of breathing rises to supply necessary oxygen to the heart, brain, and exercising muscle. Sweat production increases to eliminate toxic compounds produced by the body and to lower body temperature. Production of digestive secretions is severely reduced because digestive activity is not critical for counteracting stress. Blood sugar levels rise dramatically as the liver converts stored glycogen into glucose for release into the bloodstream.

Although the alarm phase is usually short-lived, the next phase—the resistance reaction—allows the body to continue fighting a stressor long after the effects of the fight-or-flight response have worn off. Other hormones, such as cortisol and other corticosteroids secreted by the adrenal cortex, are largely responsible for the resistance reaction. For example, these hormones stimulate the conversion of protein to energy so that the body has a large supply of energy long after glucose stores are depleted, and they promote the retention of sodium to keep blood pressure elevated.

As well as providing the necessary energy and circulatory changes required to deal effectively with stress, the resistance reaction provides the changes required to meet emotional crises, perform strenuous tasks, and fight infection. The effects of adrenal cortex hormones are quite necessary when the body is faced with danger, but prolongation of the resistance reaction or continued stress increases the risk of significant disease (including diabetes, high blood pressure, and cancer) and results in the final stage of the general adaptation syndrome, exhaustion.

Exhaustion may manifest as a partial or total collapse of a body function or specific organ. Two of the major causes of exhaustion are loss of potassium ions and depletion of adrenal glucocorticoid hormones, such as cortisone. Loss of potassium results in cellular dysfunction and, if severe, cell death. Depletion of adrenal glucocorticoid stores diminishes glucose control, leading to hypoglycemia.

Another cause of exhaustion is weakening of the organs. Prolonged stress places a tremendous load on many organ systems, especially the heart, blood vessels, adrenals, and immune system, and is associated with many common diseases, as listed in Box 140.1.

STRESS: A HEALTHY VIEW

Hans Selye is considered the father of modern stress research. Having spent many years studying this subject, Selye developed valuable

BOX 140.1 Diseases Strongly Linked to Stress

- Angina
- Asthma
- Autoimmune disease
- Cancer
- Cardiovascular disease
- Common cold
- Depression
- Diabetes (adult onset—type 2)
- Headaches
- Hypertension
- Immune suppression
- Irritable bowel syndrome
- Menstrual irregularities
- Premenstrual tension syndrome
- Rheumatoid arthritis
- Ulcerative colitis
- Ulcers

Data from Benson H. The relaxation response. New York: William Morrow, 1975.

insights into the role of stress in disease. According to Selye, stress in itself should not be viewed in a negative context. It is not the stressor that determines the response; instead, it is the individual's internal reaction that triggers the response. This internal reaction is highly individualized. What one person may experience as stress, the next person may view entirely differently. Selye perhaps summarized his view best in the following passage from his book *The Stress of Life*[2]:

> *No one can live without experiencing some degree of stress all the time. You may think that only serious disease or intensive physical or mental injury can cause stress. This is false. Crossing a busy intersection, exposure to a draft, or even sheer joy is enough to activate the body's stress mechanisms to some extent. Stress is not even necessarily bad for you; it is also the spice of life, for any emotion, any activity causes stress. But, of course, your system must be prepared to take it. The same stress which makes one person sick can be an invigorating experience for another.*

The key statement Selye made may be "your system must be prepared to take it." A significant body of knowledge has been accumulated delineating methodologies for helping patients develop healthful, rather than disease-facilitating, responses to both short- and long-term stress.

DIAGNOSTIC CONSIDERATIONS

Evaluating the effect of stress on a patient's health status requires a complete clinical assessment (e.g., review of systems, medical history, physical examination, sleep history, etc.). Many people who are "stressed out" may not be able to identify exactly what is causing them to feel stressed. Typical presenting symptoms are insomnia, depression, fatigue, headache, upset stomach, digestive disturbances, and irritability.

One useful tool to assess the role that stress may play is the Social Readjustment Rating Scale developed by Holmes and Rahe[3] (Table 140.1). The scale was originally designed to predict the risk of a serious disease due to stress. Various life-changing events are numerically rated according to their potential to cause disease. Notice that even events commonly viewed as positive, such as an outstanding personal achievement, carry stress.

TABLE 140.1 The Social Readjustment Rating Scale

Rank	Life Event	Mean Value
1	Death of spouse	100
2	Divorce	73
3	Marital separation	65
4	Jail term	63
5	Death of a close family member	63
6	Personal injury or illness	53
7	Marriage	50
8	Fired at work	47
9	Marital reconciliation	45
10	Retirement	45
11	Change in health of family member	44
12	Pregnancy	40
13	Sex difficulties	39
14	Gain of a new family member	39
15	Business adjustment	39
16	Change in financial state	38
17	Death of a close friend	37
18	Change to different line of work	36
19	Change in number of arguments with spouse	35
20	Large mortgage	31
21	Foreclosure of mortgage or loan	30
22	Change in responsibilities at work	29
23	Son or daughter leaving home	29
24	Trouble with in-laws	29
25	Outstanding personal achievement	28
26	Spouse begins or stops work	26
27	Beginning or end of school	26
28	Change in living conditions	25
29	Revision of personal habits	24
30	Trouble with boss	23
31	Change in work hours or conditions	20
32	Change in residence	20
33	Change in schools	20
34	Change in recreation	19
35	Change in church activities	19
36	Change in social activities	18
37	Small mortgage	17
38	Change in sleeping habits	16
39	Change in number of family get-togethers	15
40	Change in eating habits	15
41	Vacation	13
42	Christmas	12
43	Minor violations of the law	11

Data from Holmes TH, Rahe RH. The social readjustment rating scale. *J Psychosom Res* 1967;11:213–218.

If a person is under a great deal of immediate stress or has endured a fair amount of stress for a few months or longer, it is appropriate to assess adrenal dysfunction more accurately with laboratory methods.

The standard interpretation of the Social Readjustment Rating Scale is that a total of 200 or more units in 1 year is considered to

BOX 140.2 Negative Coping Patterns

- Dependence on chemicals: legal and illicit drugs, alcohol, smoking
- Overeating
- Too much television
- Emotional outbursts
- Feelings of helplessness
- Overspending
- Excessive behavior

be predictive of a high likelihood of experiencing a serious disease. However, rather than using the scale solely to predict the likelihood of serious disease, the clinician can use the scale to evaluate a patient's level of stressor exposure because everyone reacts differently to stressful events.

Laboratory assessment generally involves evaluating aspects of the HPA axis. The gold standard for diagnosing dysfunction of the HPA axis is the combined dexamethasone/corticotropin-releasing hormone test. This test is often used in research studies to monitor the clinical course of depression. More popular in the clinical setting are assessment tools based on salivary cortisol levels.[4,5] Salivary hormone levels are reproducible, comparable to plasma levels, and easy to assess.

One straightforward test that is a reasonably good predictor of HPA dysregulation is the cortisol-awakening response. Salivary levels generally show a sharp rise upon awakening and during the first hour after waking. Generally, an initially hyperactive HPA response results in elevated cortisol levels, whereas more chronic stress, insomnia, or depression may blunt this effect.[6,7]

Another popular test is measuring salivary cortisol levels at awakening and in the evening, usually along with dehydroepiandrosterone (DHEA). The classic pattern associated with chronic stress is elevated cortisol combined with reduced DHEA, indicating a shift toward glucocorticoid production and away from sex hormone steroid production. This pattern is often associated with anxiety and depression. Adrenal exhaustion is characterized by low cortisol and low DHEA. Adrenal exhaustion is a common side effect of continual high stress as well as steroid drugs, such as prednisone, which are used in the treatment of allergic or inflammatory diseases.

THERAPEUTIC APPROACH

Whether currently aware of it or not, the patient has developed a pattern for coping with stress. Unfortunately, most people have found patterns and methods that ultimately do not support good health. Negative coping patterns must be identified and replaced with positive ways of coping. The clinician should try to identify any negative or destructive coping patterns, listed in Box 140.2, that the patient may have developed and try to replace those patterns with more positive measures for dealing with stress.

Stress management can be substantially improved by assisting the patient in the following six equally important areas:

- Techniques to calm the mind, promote parasympathetic tone, and promote a positive mental attitude
- Lifestyle factors
- Exercise
- A healthful diet designed to nourish the body and support physiological processes
- Dietary and botanical supplements designed to support the body as a whole, but especially the adrenal glands
- Supervised stress management program

BOX 140.3 The Stress Response

- The heart rate and force of contraction of the heart increase to provide blood to areas necessary for response to the stressful situation.
- Blood is shunted away from the skin and internal organs, except the heart and lungs, whereas the amount of blood supplying required oxygen and glucose to the muscles and brain is increased.
- The rate of breathing rises to supply necessary oxygen to the heart, brain, and exercising muscle.
- Sweat production increases to eliminate toxic compounds produced by the body and to lower body temperature.
- Production of digestive secretions is severely reduced because digestive activity is not critical to counteracting stress.
- Blood sugar levels are raised dramatically as the liver dumps stored glucose into the bloodstream.

BOX 140.4 The Relaxation Response

- The heart rate is reduced, the heart beats more effectively, and blood pressure is reduced.
- Blood is shunted toward internal organs, especially those organs involved in digestion.
- The rate of breathing decreases as oxygen demand is reduced during periods of rest.
- Sweat production diminishes because a person who is calm and relaxed does not experience nervous perspiration.
- Production of digestive secretions is increased, greatly improving digestion.
- Blood sugar levels are maintained in the normal physiological range.

Calming the Mind and Body

Learning to calm the mind and body is extremely important in relieving stress. Among the easiest methods for the patient to learn are relaxation exercises. The goal of relaxation techniques is to produce a physiological response known as a relaxation response—a response that is exactly opposite to the stress response that reflects activation of the parasympathetic nervous system. Although an individual may relax by simply sleeping, watching television, or reading a book, relaxation techniques are designed specifically to produce the relaxation response.

The term *relaxation response* was coined by Harvard professor and cardiologist Herbert Benson in the early 1970s to describe a physiological response that is just the opposite of the stress response.[1] With the stress response (Box 140.3), the sympathetic nervous system dominates. With the relaxation response (Box 140.4), the parasympathetic nervous system dominates. The parasympathetic nervous system controls bodily functions such as digestion, breathing, and heart rate during periods of rest, relaxation, visualization, meditation, and sleep. Although the sympathetic nervous system is designed to protect against immediate danger, the parasympathetic system is designed for repair, maintenance, and restoration of the body.

The relaxation response can be achieved through a variety of techniques. The methodology should be determined by patient interest because all techniques have the same physiological effect—a state of deep relaxation. The most popular techniques are meditation, prayer, progressive relaxation, self-hypnosis, and biofeedback. To produce the desired long-term health benefits, the patient should use the relaxation technique for at least 5 to 10 minutes each day.

Breathing

Producing deep relaxation with any technique requires learning how to breathe. One of the most powerful methods of producing less stress and more energy in the body is by breathing with the diaphragm.

BOX 140.5 Instructions to Help Patients Learn Diaphragmatic Breathing

1. Find a comfortable and quiet place to lie down or sit.
2. Place your feet slightly apart. Place one hand on your abdomen near your navel. Place the other hand on your chest.
3. You will be inhaling through your nose and exhaling through your mouth.
4. Concentrate on your breathing. Note which hand is rising and falling with each breath.
5. Gently exhale most of the air in your lungs.
6. Inhale while slowly counting to four. As you inhale, slightly extend your abdomen, causing it to rise about 1 inch. Make sure that you are not moving your chest or shoulders.
7. As you breathe in, imagine the warmed air flowing in. Imagine this warmth flowing to all parts of your body.
8. Pause for 1 second, then slowly exhale to a count of four. As you exhale, your abdomen should move inward.
9. As the air flows out, imagine all your tension and stress leaving your body.
10. Repeat the process until a sense of deep relaxation is achieved.

Diaphragm breathing activates the relaxation centers in the brain and the parasympathetic nervous system. Box 140.5 lists a 10-step technique for teaching diaphragmatic breathing.

Progressive Relaxation

One of the most popular techniques for producing the relaxation response is progressive relaxation. The technique is based on a very simple procedure of comparing tension with relaxation. Many people are not aware of the sensation of relaxation. In progressive relaxation, an individual is taught what it feels like to relax by comparing relaxation with muscle tension.

The basic technique is to have the patient contract a muscle forcefully for a period of 1 to 2 seconds and then give way to a feeling of relaxation in that muscle. The procedure systematically goes through all the muscles of the body, progressively producing a deep state of relaxation. The procedure begins with contracting the muscles of the face and neck, then the upper arms and chest, followed by the lower arms and hands. The process is repeated progressively down the body, from the abdomen through the buttocks, the thighs, and calves to the feet. This whole process is repeated two or three times. This technique is often used in the treatment of anxiety and insomnia.

Progressive relaxation, deep-breathing exercises, or some other stress reduction technique is an important component of a comprehensive stress management program.

Lifestyle Factors

A patient's lifestyle is a major determinant of his or her stress levels. The two primary areas of concern (other than addressing negative coping patterns) are time management and relationship issues.

One of the biggest stressors for most people is time. They simply do not feel they have enough of it. Box 140.6 provides tips on time management for patients.

Another major cause of stress for many people is interpersonal relationships. Interpersonal relationships can be divided into three major categories: marital, family, and job related. The quality of any relationship ultimately comes down to the quality of the communication. Learning to communicate effectively goes a very long way in reducing the stress and occasional (or frequent) conflicts of interpersonal relationships. Box 140.7 lists seven tips for effective communication, regardless of the type of interpersonal relationship.

BOX 140.6 Patient Tips for Improved Time Management

- Set priorities. Realize that you can only accomplish so much in a day. Decide what is important, and limit your efforts to that goal. Organize your day. There are always interruptions and unplanned demands on your time, but create a definite plan for the day on the basis of your priorities. Avoid the pitfall of always letting the "immediate demands" control your life.
- Delegate authority. Delegate as much authority and work as you can. You cannot do everything yourself. Learn to train and depend on others.
- Tackle tough jobs first. Handle the most important tasks first, when your energy levels are high. Leave the busy work or running around for later in the day.
- Minimize meeting time. Schedule meetings to bump up against the lunch hour or quitting time; that way, they cannot last forever.
- Avoid putting things off. Work done under the pressure of an unreasonable deadline often has to be redone. That creates more stress than if it had been done right the first time. Plan ahead.
- Do not be a perfectionist. You can never really achieve perfection anyway. Do your best in a reasonable amount of time, then move on to other important tasks. If you find time, you can always come back later and polish the task some more.

BOX 140.7 Keys to Assist Patients in Improving Communication

- The first key to successful communication is the most important: learn to be a good listener. Allow the person you are communicating with to really share his or her feelings and thoughts uninterrupted. Empathize with the person; put yourself in his or her shoes. If you first seek to understand, you will find yourself being better understood.
- Be an active listener. This means that you must be truly interested in what the other person is communicating. Listen to what he or she is saying instead of thinking about your response. Ask questions to gain more information or clarify what he or she is telling you. Good questions open lines of communication.
- Be a reflective listener. Restate or reflect back to the other person your interpretation of what he or she is telling you. This simple technique shows the other person that you are both listening to and understanding what he or she is saying. Restating what you think is being said may cause some short-term conflict in some situations, but it is certainly worth the risk.
- Wait to speak until the person you want to communicate with is listening. If the person is not ready to listen, your message will not be heard no matter how well you communicate.
- Do not try to talk over somebody. If you find yourself being interrupted, relax; do not try to out-talk the other person. If you are courteous and allow him or her to speak, eventually (unless extremely rude), he or she will respond likewise. If that does not happen, point out that the other person is interrupting the communication process. You can do this only if you have been a good listener. Double standards in relationships seldom work.
- Help the other person become an active listener. This can be done by asking whether he or she has understood what you were communicating. Ask him or her to tell you what he or she heard. If the other person does not seem to be understanding what you are saying, keep trying until he or she does.
- Do not be afraid of long silences. Human communication involves much more than human words. A great deal can be communicated during silences; unfortunately, in many situations, silence can make us feel uncomfortable. Relax. Some people need silence to collect their thoughts and feel safe in communicating. The important thing to remember during silences is that you must remain an active listener.

Exercise

The immediate effect of exercise is stress on the body. However, with a regular exercise program, the body adapts, and exercise becomes an effective stress reduction technique. With regular exercise, the body becomes stronger, functions more efficiently, and has greater endurance. Exercise is a vital component of a comprehensive stress management program and overall good health.

People who exercise regularly are much less likely to experience fatigue and depression. Tension, depression, feelings of inadequacy, and worries diminish greatly with regular exercise. Exercise alone has been demonstrated to have a tremendous effect in terms of improving mood and the ability to handle stressful life situations in both adults of all ages and adolescents as well. Exercise positively affects mood and reduces stress through physiological and biochemical mechanisms, including endorphins, mitochondria, mammalian target of rapamycin, neurotransmitters and the hypothalamic–pituitary–adrenal axis, and even the microbiome.[8,9]

Dietary Guidelines

An individual with stress or anxiety must support the biochemistry of the body by following some important dietary guidelines. Specifically, the patient must do the following:

- Eliminate or restrict the intake of caffeine.
- Eliminate or restrict the intake of alcohol.
- Eliminate refined carbohydrates from the diet.
- Eat a diverse range of whole foods.
- Increase the potassium-to-sodium (K/Na) ratio.
- Eat regular planned meals in a relaxed environment.
- Control food allergies.

According to Selye,[2] whether or not stress is harmful is based on the strength of the system. From a purely physiological perspective, it can be strongly argued that the delivery of high-quality nutrition to the cells of the body is the critical factor in determining the strength of the system.

When the eating habits of Americans are examined as a whole, it is little wonder that so many people have stress, anxiety, and fatigue. Most Americans are not providing their bodies with the high-quality nutrition they need. Instead of eating foods rich in vital nutrients, most Americans focus on refined foods high in calories, sugar, fat, and cholesterol.

Caffeine

The average American consumes 150 to 225 mg of caffeine daily, or roughly the amount of caffeine in two cups of coffee. Although most people can handle this amount, some people are more sensitive to the effects of caffeine than other people, owing to decreased activity of Phase I detoxification enzymes. Even small amounts of caffeine can affect sensitive people, whereas those with normal sensitivity respond to large amounts. Excessive caffeine consumption can produce "caffeinism characterized by symptoms of depression, nervousness, irritability, recurrent headache, heart palpitations, and insomnia." People prone to feeling stress and anxiety tend to be especially sensitive to caffeine.[10,11]

Alcohol

Alcohol produces chemical stress on the body. It also increases adrenal hormone output and interferes with both normal brain chemistry and normal sleep cycles. Although many people believe that alcohol has a calming effect, a study of 90 healthy male volunteers given either a placebo or alcohol demonstrated significant increases in anxiety scores after consumption of alcohol.[10,11]

Refined Carbohydrates and Glycemic Volatility

One of the consequences of the stress response is abdominal fat cell growth and loss of muscle mass, a scenario that obviously leads to insulin resistance and obesity. It is a complex set of events orchestrated by cortisol released as a result of the stress response that is ultimately responsible for the fact that stress promotes weight gain. Cortisol is also a contributing factor in a high degree of glycemic volatility. Rapidly fluctuating blood sugar levels are generally related to some degree of insulin resistance and made worse by more-than-moderate consumption of foods with a high glycemic impact. Refined carbohydrates (e.g., sugar and white flour) are known to contribute to problems in blood sugar control, especially hypoglycemia, as well as glycemic volatility. The association between hypoglycemia and impaired mental function is well known. Numerous studies have shown a high percentage of hypoglycemia in depressed patients.[12,13] Because depression is one of the most common causes of anxiety, this finding provides a link between hypoglycemia and feelings of stress. Simply eliminating refined carbohydrates from the diet is occasionally all that is needed for effective therapy in patients who have depression or anxiety due to hypoglycemia.

Potassium-to-Sodium Ratio

One of the key dietary recommendations to support the adrenal glands is to ensure adequate potassium levels within the body. This can best be done by consuming foods rich in potassium and avoiding foods high in sodium. Most Americans have a dietary K/Na ratio of less than 1:2. In contrast, most researchers recommend a dietary K/Na ratio higher than 5:1 for health. However, even this recommendation may not be optimal. A natural diet rich in fruits and vegetables can produce a K/Na ratio higher than 50:1 because most fruits and vegetables have a K/Na ratio of more than 100:1.

Meal Planning

Mealtimes should be spent in a relaxed environment. As noted previously, digestion is a process largely controlled by the parasympathetic nervous system. Eating in a rushed manner or in a noisy or hurried environment is not conducive to good digestion or good health.

Food Allergies

People with symptoms of anxiety or chronic fatigue must be concerned about food allergies. As far back as 1930, pioneering allergist Albert Rowe began noticing that anxiety and fatigue were key features of food allergies.[14] Originally, Rowe described a syndrome known as "allergic toxemia" to describe a syndrome that included the symptoms anxiety, fatigue, muscle and joint aches, drowsiness, difficulty concentrating, and depression. Around the 1950s, this syndrome began to be referred to as the "allergic tension-fatigue syndrome." Due to the popularity of chronic fatigue syndrome, many physicians and other people are forgetting that food allergies can lead to anxiety as well as chronic fatigue.

NUTRITIONAL AND BOTANICAL SUPPORT

Nutritional and botanical support for the individual experiencing signs and symptoms of stress largely involves supporting the adrenal glands. Long-term stress and corticosteroids cause the adrenal glands to shrink and become dysfunctional, aggravating the stress symptoms of anxiety, depression, and chronic fatigue.

An abnormal adrenal response, either deficient or excessive hormone release, significantly alters an individual's response to stress. Often, the adrenals become "exhausted" as a result of constant demands put on them. An individual with adrenal exhaustion usually experiences chronic fatigue and may complain of feeling "stressed out" or chronically anxious. He or she typically has reduced resistance to allergies and infection.

Nutritional Supplements

The nutrients especially important for supporting adrenal function are vitamin C, vitamin B_6, zinc, magnesium, and pantothenic acid. All of these nutrients play a critical role in the health of the adrenal gland as well as the manufacture of adrenal hormones. During stress, the levels of these nutrients in the adrenals decrease substantially.

For example, during chemical, emotional, psychological, or physiological stress, the urinary excretion of vitamin C is increased. Examples of chemical stressors are cigarette smoke, pollutants, and allergens. Extra vitamin C in the form of supplementation and a higher intake of vitamin C–rich foods is often recommended to keep the immune system working properly during times of stress.

Equally important during high periods of stress or in individuals needing adrenal support is pantothenic acid (vitamin B_5). Pantothenic acid deficiency results in adrenal atrophy, characterized by fatigue, headache, sleep disturbances, nausea, and abdominal discomfort. Pantothenic acid is found in whole grains, legumes, cauliflower, broccoli, salmon, liver, sweet potatoes, and tomatoes. In patients who have chronic stress or a history of corticosteroid (prednisone) use, the typical level of supplementation is 100 to 500 mg/day.

The appropriate daily dose of vitamin B_6 is 50 to 100 mg; of zinc, 20 to 30 mg; and of magnesium, 250 to 500 mg.

Gamma-aminobutyric Acid

Gamma-aminobutyric acid (GABA) is a major neurotransmitter that is abundantly and widely distributed throughout the central nervous system. Low levels or decreased GABA function in the brain are associated with several psychiatric and neurological disorders, but most primarily anxiety, depression, insomnia, and epilepsy. Currently, many popular antianxiety drugs—the sedative-hypnotics—interact primarily with GABA receptors. These drugs include the benzodiazepine drugs like alprazolam (Alprazolam, Xanax) and diazepam (Valium), as well as drugs like flurazepam (Dalmane), quazepam (Doral), temazepam (Restoril), triazolam (Halcion), zolpidem tartrate (Ambien), and baclofen (Kemstro and Lioresal).

Clinical studies with PharmaGABA, a naturally manufactured supplement via a fermentation process that uses *Lactobacillus hilgardii,* have shown it to produce significant antistress effects.[15,16] Specifically, PharmaGABA has been shown to produce relaxation, as evidenced by changes in brain-wave patterns, pupil diameter, and heart rate, as well as reduce markers of stress, including salivary cortisol and chromogranin A levels. These effects are thought to be the result of activation of the parasympathetic nervous system rather than PharmaGABA crossing the blood–brain barrier. The typical dosage is 100 to 200 mg up to three times daily. As a general guideline, it is recommended to take no more than 1000 mg within a 4-hour period and no more than 3000 mg within a 24-hour period.

Botanical Medicines

Several botanical medicines support adrenal function. Most notable are the ginsengs—Chinese ginseng (*Panax ginseng*) and Siberian ginseng (*Eleutherococcus senticosus*)—rhodiola (*Rhodiola rosea*), and ashwagandha (*Withania somnifera*). All of these plants exert beneficial effects on adrenal function, enhance resistance to stress, and are often referred to as "adaptogens." These plants have historically been used as follows:

- To restore vitality in debilitated and feeble individuals
- To increase feelings of energy
- To improve mental and physical performance
- To prevent the negative effects of stress and enhance the body's response to stress

Both Siberian and Chinese ginsengs possess this kind of equilibrating, tonic, antistress action, and so the term *adaptogen* is quite appropriate in describing their general effects.

The ginsengs have been shown to enhance the ability to cope with various stressors, both physical and mental.[17–19] Presumably, this antistress action is mediated by mechanisms that control the adrenal glands. Ginseng delays the onset and reduces the severity of the alarm-phase response of the general adaptation syndrome.

People taking either of the ginsengs typically report an increased sense of well-being. Clinical studies have confirmed that both Siberian and Chinese ginsengs significantly reduce feelings of stress and anxiety. For example, in one double-blind clinical study, nurses who switched from day to night duty rated themselves for competence, mood, and general well-being and were given a test for mental and physical performance along with blood cell counts and blood chemistry evaluation.[20] The group who were given *P. ginseng* demonstrated higher scores in competence, mood parameters, and mental and physical performance compared with those receiving placebos. The nurses taking the ginseng felt more alert yet more tranquil, and were able to perform better than the nurses who were not taking the ginseng.

In addition to these human studies, several animal studies have shown the ginsengs to exert significant antianxiety effects. In several of these studies, the stress-relieving effects were comparable to those of diazepam (Valium); however, diazepam causes behavior changes, sedative effects, and impaired motor activity, but ginseng has none of these negative effects.[21]

On the basis of the clinical and animal studies, ginseng appears to offer significant benefit to people experiencing stress and anxiety. *P. ginseng* is generally regarded as being more potent than *E. senticosus. P. ginseng* is probably better for the patient who has experienced a great deal of stress, is recovering from a long-standing illness, or has taken corticosteroids, such as prednisone, for a long time. For the patient who is under mild to moderate stress and is experiencing less obvious impairment of adrenal function, *E. senticosus* may be the better choice.

Another useful botanical medicine to support stress management is *R. rosea* (artic root), a popular plant in traditional medical systems in Eastern Europe and Asia, where it has traditionally been recommended to help combat fatigue and restore energy. Modern research has confirmed these effects and its qualities as an adaptogen. However, the adaptogenic actions of *R. rosea* are different from those of Chinese and Siberian ginsengs, which act primarily on the HPA axis. *R. rosea* seems to exert its adaptogenic effects by working on neurotransmitters and endorphins. *R. rosea* appears to offer an advantage over other adaptogens in circumstances of acute stress because it produces a greater feeling of relaxation and antianxiety effects. A single dose of *Rhodiola* extract before acute stressful events has been shown to prevent stress-induced disruptions in function and performance, but like the ginsengs, *R. rosea* has also shown positive results with long-term use.[22–25] In one randomized, placebo-controlled trial of 60 patients with stress-related fatigue, *Rhodiola* was found to have an antifatigue effect that increased mental performance, particularly the ability to concentrate, as well as decreased the cortisol response to awakening stress.[26]

On the basis of the results of clinical trials with a standardized *R. rosea* extract, the therapeutic dose varies according to the rosavin content. For a dosage target of 3.6 to 7.2 mg of rosavin, the daily dose would be 360 to 600 mg for an extract standardized for 1% rosavin, 180 to 300 mg for 2% rosavin, and 100 to 200 mg for 3.6% rosavin. When *R. rosea* is used as an adaptogen, long-term administration is normally begun several weeks before a period of expected increased physiological, chemical, or biological strain and continued throughout the duration of the challenging event or activity. When *R. rosea* is used as a single dose for acute

stress (e.g., for an examination or athletic competition), the suggested dose is three times the dose used for long-term supplementation. No side effects have been reported in the clinical trials, but at higher doses, some individuals might experience greater irritability and insomnia.

Lastly, clinical studies with extracts of roots and leaves from *Withania somnifera* (ashwagandha) have shown considerable antistress and adaptogenic effects.[27] In one human double-blinded study, chronically stressed subjects taking a proprietary extract (Sensoril) had significant reductions in a modified Hamilton anxiety scale, serum cortisol, C-reactive protein, pulse rate, and blood pressure and significant increases of serum DHEA-S and hemoglobin compared with the placebo group. In addition, there were dose-dependent responses in lowering fasting blood glucose and improving the serum lipid levels. Dosage was 125 to 250 mg one to two times daily.[28] In another double-blind study, 50 healthy male/female athletic adults were given a proprietary extract (KSM-66) at a dosage of 300 mg twice daily or a placebo for 12 weeks. Not only were quality-of-life scores significantly improved, but the ashwagandha extract also increased VO_{2max} compared with placebo at 12 weeks (5.67 and 1.86, respectively).[29]

Stress Management Programs

Supervised stress management programs are thought to offer greater compliance and better results than unsupervised, patient-directed programs. In one study, stress management experts evaluated six widely used occupational stress management interventions (relaxation, physical fitness, cognitive restructuring, meditation, assertiveness training, and stress inoculation) on the basis of 10 practicality criteria and 7 effectiveness objectives. They found that relaxation was the most practical intervention and that meditation and stress inoculation were the least practical. Physical fitness was chosen to be the most effective intervention, and both meditation and assertiveness training were rated overall as the least effective. What these results imply is that although relaxation training may be the most practical intervention, physical exercise was the most effective intervention.[30]

Meditation was shown to be the least practical and least effective method in this evaluation, but when it is part of a supervised program, it can be very effective. In one trial of 103 adults, 59% and 61% of the meditation and control groups, respectively, completed the study.[31] The intervention program consisted of an 8-week group stress reduction program in which subjects learned, practiced, and applied "mindfulness meditation" to daily life situations. Those in the control group received educational materials and were encouraged to use community resources for stress management. Compared with the control group, intervention subjects reported significant decreases from baseline in effect of daily hassles (24%), psychological distress, (44%), and medical symptoms (46%) that were maintained at the 3-month follow-up.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Benson H. *The Relaxation Response*. New York: William Morrow; 1975.
2. Selye H. *The Stress of Life*. New York: McGraw Hill; 1978.
3. Holmes TH, Rahe RH. The social readjustment rating scale. *J Psychosom Res*. 1967;11:213–218.
4. Törnhage CJ. Salivary cortisol for assessment of hypothalamic-pituitary-adrenal axis function. *Neuroimmunomodulation*. 2009;16:284–289.
5. Lewis JG. Steroid analysis in saliva: an overview. *Clin Biochem Rev*. 2006;27:139–146.
6. Stetler C, Miller GE. Blunted cortisol response to awakening in mild to moderate depression: regulatory influences of sleep patterns and social contacts. *J Abnorm Psychol*. 2005;114:697–705.
7. Backhaus J, Junghanns K, Hohagen F. Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology*. 2004;29:1184–1191.
8. Mikkelsen K, Stojanovska L, Polenakovic M, Bosevski M, Apostolopoulos V. Exercise and mental health. *Maturitas*. 2017;106:48–56.
9. Kvam S, Kleppe CL, Nordhus IH, Hovland A. Exercise as a treatment for depression: a meta-analysis. *J Affect Disord*. 2016;202:67–86.
10. Chou T. Wake up and smell the coffee: caffeine, coffee, and the medical consequences. *West J Med*. 1992;157:544–553.
11. Monteiro MG, Schuckit MA, Irwin M. Subjective feelings of anxiety in young men after ethanol and diazepam infusions. *J Clin Psychiatry*. 1990;51:12–16.
12. Winokur A, Maislin G, Phillips JL, et al. Insulin resistance after oral glucose tolerance testing in patients with major depression. *Am J Psychiatry*. 1988;145:325–330.
13. Wright JH, Jacisin JJ, Radin NS, et al. Glucose metabolism in unipolar depression. *Br J Psychiatry*. 1978;132:386–393.
14. Rowe AH, Rowe Jr A. *Food Allergy: Its Manifestations and Control and the Elimination Diets: a Compendium*. Springfield, IL: CC Thomas; 1972.
15. Abdoua AM, Higashiguchia S, Horiea K, et al. Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *BioFactors*. 2006;26:201–208.
16. Unpublished data provided by Pharma Foods International LTD., Kyoto, Japan.
17. Davydov M, Krikorian AD. *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (*Araliaceae*) as an adaptogen: a closer look. *J Ethnopharmacol*. 2000;72:345–393.
18. Coleman CI, Hebert JH, Reddy P. The effects of Panax ginseng on quality of life. *J Clin Pharm Ther*. 2003;28:5–15.
19. Ong YC, Yong EL. Panax (ginseng)—panacea or placebo? Molecular and cellular basis of its pharmacological activity. *Ann Acad Med Singapore*. 2000;29:42–46.
20. Hallstrom C, Fulder S, Carruthers M. Effect of ginseng on the performance of nurses on night duty. *Comp Med East West*. 1982;6:277–282.
21. Bhattacharya SK, Mitra SK. Anxiolytic activity of Panax ginseng roots: an experimental study. *J Ethnopharmacol*. 1991;34:87–92.
22. Kelly GS. Rhodiola rosea: a possible plant adaptogen. *Altern Med Rev*. 2001;6:293–302.
23. Shevtsov VA, Zholus BI, Shervarly VI, et al. A randomized trial of two different doses of a SHR-5 Rhodiola rosea extract versus placebo and control of capacity for mental work. *Phytomedicine*. 2003;10:95–105.
24. Darbinyan V, Kteyan A, Panossian A, et al. Rhodiola rosea in stress induced fatigue—a double blind cross-over study of a standardized extract SHR-5 with a repeated low-dose regimen on the mental performance of healthy physicians during night duty. *Phytomedicine*. 2000;7:365–371.
25. Spasov AA, Wikman GK, Mandrikov VB, et al. A double-blind, placebo-controlled pilot study of the stimulating and adaptogenic effect of *Rhodiola rosea* SHR-5 extract on the fatigue of students caused by stress during an examination period with a repeated low-dose regimen. *Phytomedicine*. 2000;7:85–89.
26. Olsson EM, von Schéele B, Panossian AG. A randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract shr-5 of the roots of *Rhodiola rosea* in the treatment of subjects with stress-related fatigue. *Planta Med*. 2009;75:105–112.
27. Yenisetti SC, Manjunath MJ, Muralidhara C. Neuropharmacological properties of *Withania somnifera*—Indian ginseng: an overview on experimental evidence with emphasis on clinical trials and patents. *Recent Pat CNS Drug Discov*. 2016;10(2):204–215.
28. Auddy B, Hazra J, Mitra A, et al. A standardized *Withania somnifera* extract significantly reduces stress-related parameters in chronically stressed humans: a double-blind, randomized, placebo-controlled study. *JANA*. 2008;11:50–56.
29. Choudhary B, Shetty A, Langade DG. Efficacy of Ashwagandha (*Withania somnifera* [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. *Ayu*. 2015;36(1):63–68.
30. Bellarosa C, Chen PY. The effectiveness and practicality of occupational stress management interventions: a survey of subject matter expert opinions. *J Occup Health Psychol*. 1997;2:247–262.
31. Williams KA, Kolar MM, Reger BE, et al. Evaluation of a wellness-based mindfulness stress reduction intervention: a controlled trial. *Am J Health Promot*. 2001;15:422–432.

SECTION 6

Diseases

A big challenge in writing *A Textbook of Natural Medicine* is how to address the concept of disease. Although some disease names correlate well with the pathophysiology and are real entities—a good example is trichomoniasis, which is the result of a successful invasion by a pathological organism—most diseases are simply convenient names for grouped patterns of abnormal physiology. Focusing on the disease name implies it is a real entity separate from the natural body. This focus can obfuscate an understanding of what is actually happening with the patient's physiology. A prime example is type 2 diabetes. Although loss of proper blood sugar control is the common denominator, the actual abnormal physiology could be due to a number of different underlying mechanisms, such as cellular insulin insensitivity, loss of pancreatic production of insulin, or hypercortisolemia, because there are multiple separate substantive dysfunctions—all with very different optimal interventions—that result in loss of blood sugar control. Treating the disease by forcing a lowering of blood sugar may appear effective in the short term, but because the foundational underlying causes have not been addressed, the patient suffers progressively worse sequelae. The failure to address these factors is a key reason why (at the time of this publication) diabetes and its complications account for an astounding 20% of healthcare spending in the United States.

Nonetheless, because most clinicians and patients still have a disease diagnosis and treatment perspective, we address disease—but always in the context of the physiological abnormalities that are the actual causes of the patient's ill health and symptoms.

This section is both a therapeutic handbook and a study of the origins and causes of disease. Each disease is thoroughly discussed, with particular emphasis on the biochemistry of the altered physiology leading to the symptomatology and pathology. We also discuss, as appropriate, the aspects of genetics and lifestyle leading to the disorder. Finally, we present a comprehensive therapeutic regimen using the most natural and least invasive therapies available.

Although we believe this to be the most effective way to present the material, we are extremely concerned that it could continue to promote the disease orientation of the current dominant medical system. We can only offer the following suggestion: Always treat the patient, not the disease!

All recommendations made here should be considered in the context of the whole patient. Our purpose is to help our patients to become well, not to simply alleviate symptoms and allow the true cause(s) of their disease to continue unabated. We therefore recommend that readers carefully study Sections I and IV before reading this section and that treatment providers should always consider the psychological and spiritual reasons people may have for being sick.

Organization

In general, each chapter is divided into the following five parts:

- Diagnostic summary—brief list of the key diagnostic elements
- General considerations—discussion of the underlying pathology and altered physiology
- Therapeutic considerations—discussion of the correctable nutrient deficiencies, possible toxin causes, and various natural therapies that may be used to treat the underlying pathophysiology
- Therapeutic approach—a concise therapeutic regimen
- References—thorough documentation through peer-reviewed scientific research of the discussion to aid those who seek further study of our treatment rationale

The section is arranged alphabetically by disease name. Please also use the Index to look for diseases because some are addressed in other disease chapters or within a grouping, and occasionally disease-naming conventions change. For example, depression is discussed in the chapter "Affective Disorders."

To aid us in the further development of this textbook, we welcome comments. In particular, we are greatly interested in well-documented successes, **and failures**, of the use of these procedures. We would also appreciate recommendations for therapeutic and diagnostic approaches not included here that have documented success.

141

Acne Vulgaris and Acne Conglobata

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Open comedones—dilated follicles with central dark, horny plugs (blackheads)
- Closed comedones—small follicular papules with (red papules) or without (whiteheads) inflammatory changes
- Superficial pustules—collections of pus at follicular opening
- Nodules—tender collections of pus deep in dermis
- Cysts—from nodules that fail to discharge contents to the surface
- Large, deep pustules—from nodules that break down adjacent tissue, leading to scars

GENERAL CONSIDERATIONS

Acne vulgaris is characterized as a pilosebaceous disease with comedones, papules, and pustules, whereas cystic acne is a more severe form, with cyst formation and subsequent scarring. Acne lesions occur predominantly on the face and, to a lesser extent, on the back, chest, and shoulders. It is more common in males, and onset is typically at puberty (somewhat later for the cystic form).

Etiology

Acne is the most common of all skin problems. A key factor is genetics. It is inherited in an autosomal-dominant pattern with incomplete penetrance. If both parents had acne, three of four children will have acne. If one parent had acne, then one of four of the children will have acne.[1]

The onset of acne vulgaris usually reflects an increase in pilosebaceous gland size and sebum secretion due to androgenic stimulation. In puberty, changes in the lipid profile of sebum (dys-seborrhea), stress, irritation, dietary composition, and cosmetics can lead to inflammation. Epidermal dysbiosis leads to disruption in the skin barrier. The severity and progression are determined by a complex interaction among hormones, keratinization, sebum, and bacteria.

The lesions begin in the upper portion of the follicular canal, with hyperkeratinization being the first microscopical change. This leads to blockage of the canal, resulting in dilation and thinning. Eventually, a comedone is formed. The formation of open or closed comedones appears to be related to the degree of keratinization and the level of blockage of the duct.

Despite a large amount of purulent exudate in pustular and cystic lesions, the only bacteria commonly cultured are normal skin species. *Propionibacterium acnes* proliferates in the pilosebaceous unit and releases lipases that hydrolyze sebum triglycerides into free fatty-acid lipoperoxides, activating expression of protease-activated receptors (PARs), tumor necrosis factor-alpha, and toll-like receptors and the production of interferon-gamma, interleukins 1, 8, and 12, and matrix metalloproteinases by keratinocytes, thus promoting an inflammatory cascade and hyperkeratinization of the pilosebaceous unit.[2]

Acne-like lesions can occur in response to various compounds: corticosteroids, halogens, isonicotinic acid, diphenylhydantoin, lithium carbonate, anabolic steroids (e.g., testosterone), and immunotherapeutic agents. Exposure to various industrial pollutants can also cause acne: machine oils, coal tar derivatives, and chlorinated hydrocarbons.

Cosmetics, pomades, overwashing, and repetitive rubbing can produce acne as well.

Chloracne is an acneiform eruption of blackheads, cysts, and nodules associated with exposure to halogenated aromatic compounds, including the dioxins. Most cases of chloracne have resulted from agricultural occupational exposures. However, nonoccupational chloracne has occurred from contaminated industrial wastes and contaminated food products and can accumulate in the human and animal food chains. The primary clinical manifestations of chloracne are noninflammatory comedones and straw-colored cysts that appear on the face (particularly the cheeks), neck, trunk, extremities, genitalia, and/or axilla.[3] The presence of chloracne is considered to be a clinical sign of dioxin exposure.

Endocrinological Aspects

Acne is an androgen-dependent condition, and androgen excess, either systemic or local, is associated with more severe forms of the disease. Androgens control sebaceous gland secretion and exacerbate the development of abnormal keratinizing follicular epithelium. Endocrine disorders producing excess androgens are important etiological factors: idiopathic adrenal androgen excess, partial defect in 21-hydroxylase, and polycystic ovary syndrome. Free testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, and low sex-hormone-binding globulin levels have all been implicated.[4–6] The skin of patients with acne shows greater activity of 5-α-reductase, the enzyme that converts testosterone to a more potent androgen, dihydrotestosterone.[7,8] This increased activity is independent of systemic levels of androgens and may explain the poor correlation between systemic levels of androgens and the severity of acne lesions. Receptors for growth hormone and insulin-like growth factor-1 (IGF-1) are present on the sebaceous gland, and these hormones also stimulate sebum production. Conditions of growth-hormone excess (e.g., acromegaly) are associated with increased sebum production and acne. Insulin at high levels can interact with IGF-1 receptors. IGF-1 promotes the expression of enzymes responsible for androgen biosynthesis and conversion. Elevated cortisol due to chronic stress thickens sebum. The stress of acne compounds this problem.

THERAPEUTIC CONSIDERATIONS

Effective treatment of acne is a significant clinical challenge. Success with natural treatments can be obtained only by the rigorous, comprehensive application of dietary, nutritional, and botanical interventions.

Guidelines for the management of acne were published in 2016 by the American Academy of Dermatology.[9] Referring to these guidelines may be instructive in making alternative natural medicine recommendations.

Diet

Although historically there was controversy regarding diet in the etiology of acne, there is now clear evidence of causality. In Western societies, acne vulgaris is a nearly universal skin disease afflicting 79% to 95% of the adolescent population. Among men and women older than 25 years, 40% to 54% have some degree of facial acne, and clinical facial acne persists into middle age in 12% of women and 3% of men. In contrast, epidemiological evidence suggests that acne incidence rates are considerably lower in non-Western societies. For example, researchers found no cases of acne among the Kitavan Islanders of Papua New Guinea and the Ache hunter-gatherers of Paraguay.[10]

The exact diet for acne sufferers to follow has not been evaluated, but foods high in iodine should be eliminated and milk consumption limited.[11] Milk contains estrogens, progesterone, and androgens, as well as glucocorticoids and IGF-1.[12] Trans-fatty acids and saturated fats should also be eliminated because they can increase sebaceous secretions and increase inflammation (Fig. 141.1). The composition of the diet should be in the range of 45% protein, 35% carbohydrate, and 20% fat because such a composition produces substantially less 5-α-reduction of testosterone and enhanced cytochrome P450 hydroxylation of estradiol, both therapeutic goals.[13] A high-carbohydrate diet (10% protein, 70% carbohydrate, and 20% fat) has the opposite effect. A diet that encourages a high insulin response chronically can promote acne by resulting in elevated levels of IGF-1.[14,15]

Sugar, Insulin, and Chromium

Many dermatologists have reported that insulin is effective in the treatment of acne, suggesting that impaired cutaneous glucose tolerance, insulin insensitivity, or both may contribute to acne lesions.[16,17] In these studies, insulin was either given systemically (5–10 units two to three times a week) or injected directly into the lesions.

One study comparing the results of oral glucose tolerance tests in acne patients showed no differences from controls. However, repetitive skin biopsies revealed that the skin glucose tolerance was significantly impaired in patients with acne.[18] One researcher coined the term *skin diabetes* to describe the disorder of acne.[17] Considering the known immunosuppressive effects of sugar (see Chapter 136), all simple carbohydrates should be strictly eliminated.

High-chromium yeast is known to improve glucose tolerance and enhance insulin sensitivity[19] and has been reported in an uncontrolled study to induce rapid improvement in patients with acne.[20]

A review of the role of diet and acne published in 2014 reaffirmed that the contribution of diet in acne is not a myth.[21] Moderate to severe acne is closely associated with the following:

1. Family history of acne in first-degree relatives
2. Obesity
3. High consumption of milk, particularly skim milk, cheese, and yogurt
4. Consumption of sweets/cakes, chocolate
5. Low consumption of fish
6. Limited intake of fruits and vegetables[22]

The higher content of omega-3 fatty acids in Kitavan and Ache' societies may have also played a role in the absence of acne. Omega-3 contributes to maintaining the stratum corneum permeability barrier, maturation and differentiation of the stratum corneum, formation and secretion of lamellar bodies, and inhibition of proinflammatory eicosanoids. A 2014 study showed for the first time that omega-3 fatty acids and gamma-linolenic acid could be used as adjuvant treatments for patients with acne.[23]

NUTRIENTS

Vitamin A

Many studies have demonstrated that retinols, including oral vitamin A, reduce sebum production and the hyperkeratosis of sebaceous follicles. Retinol has been shown to be effective in treating acne when used at high and potentially toxic dosages (i.e., 300,000–400,000 IU/day for 5–6 months).[24]

Although dosages of vitamin A below 300,000 IU/day for a few months rarely cause toxic symptoms,[25] early recognition is

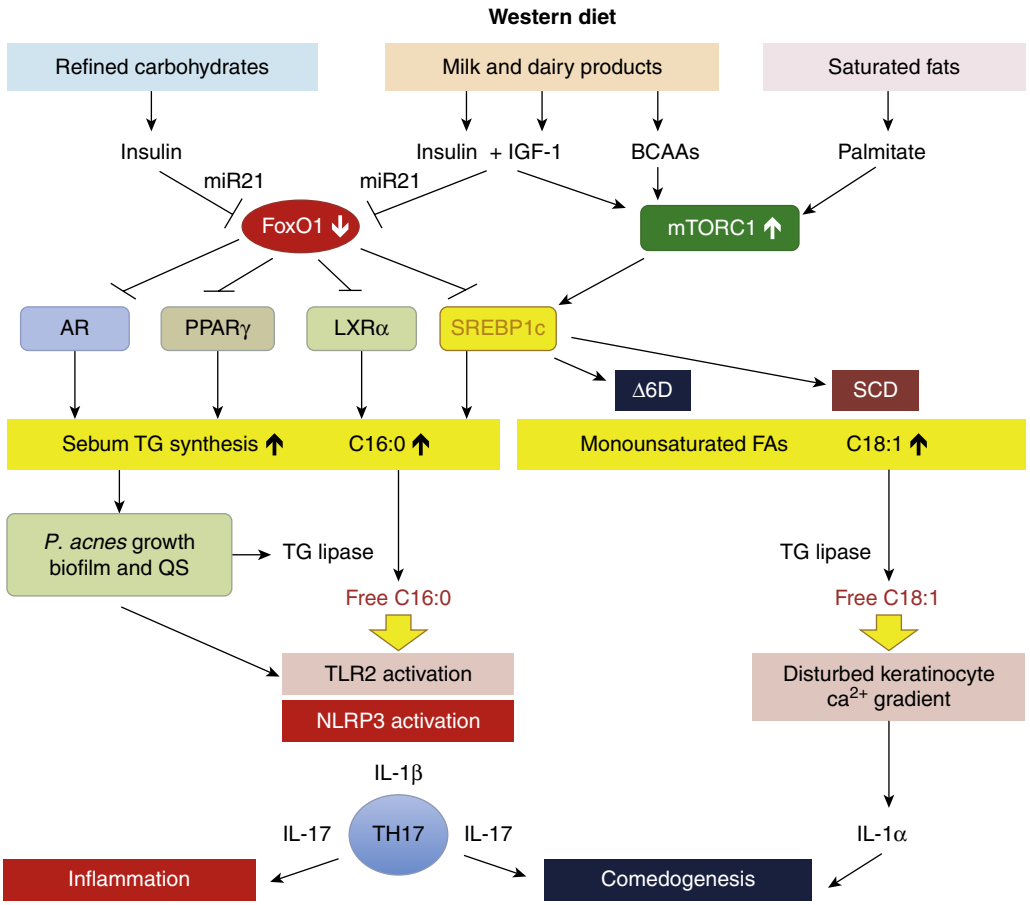


Fig. 141.1 Acne vulgaris, a Western diet–induced sebofollicular inflammasomopathy. *AR,* androgen receptor; *BCAAs,* branched-chain amino acids; *C16:0,* palmitic acid; *C18:1,* oleic acid; *FoxO1,* forkhead box class O1; *IGF-1,* insulin-like growth factor 1; *IL-17,* interleukin-17; *IL-1α,* interleukin-1α*IL-1β,* interleukin-1β; *LXRα,* liver X receptor-α; *miR21,* microRNA-21; *mTORC1,* mechanistic target of rapamycin complex 1; *NLRP3,* Nod-like receptor family, pyrin domain containing 3 inflammasome; *P. acnes, Propionibacterium acnes; PPARγ,* peroxisome proliferator-activated receptor-γ; *QS,* quorum sensing; *SCD,* stearoyl-CoA desaturase; *SREBP1c,* sterol response element-binding protein 1c; *TG,* triglyceride; *Th17,* Th17 T-cell; *TLR2,* toll-like receptor 2; *Δ6D,* Δ6-desaturase. (Michael Traub, ND, DHANP, FABNO & Lavelle Brown, MBS, CMPE, SHRM-SCP).

important. Cheilitis (chapped lips) and xerosis (dry skin) generally occur in most patients on high-dose vitamin A, particularly in dry weather.

The first significant toxic symptom is usually headache, followed by fatigue, emotional lability, and muscle and joint pain. Laboratory tests appear unreliable for monitoring toxicity because serum vitamin A levels correlate poorly with toxicity, and serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase are elevated only in symptomatic patients. Of far greater concern is the teratogenicity of massive dosages of vitamin A. Women of childbearing age must use effective birth control during treatment and for at least 1 month after discontinuation.

Contraception counseling is mandatory, and two negative pregnancy test results are required before the initiation of vitamin A therapy in women of childbearing potential. The baseline laboratory examination should also include cholesterol and triglyceride assessment, hepatic transaminase levels, and a complete blood count. Pregnancy tests and laboratory examinations should be repeated monthly during treatment.

In the author's experience, vitamin A 150,000 IU daily in an emulsified form has proven to be a reliable and safe means of bringing acne under control.

Zinc

Zinc is involved in local hormone activation, retinol-binding protein formation, wound healing, immune system activity, and tissue regeneration.

Zinc supplementation in the treatment of acne has been the subject of much controversy and many double-blind studies. Inconsistent results may be due to the differing absorbability of the various zinc salts used. For example, studies using effervescent zinc sulfate show efficacies similar to those of tetracycline, with fewer side effects from chronic use,[26] whereas those using plain zinc sulfate have shown less beneficial results.[27] The majority of patients required 12 weeks of supplementation before good results were demonstrated, although some showed dramatic improvement immediately.

In another study, 66 patients with inflammatory acne were given zinc gluconate (30 mg elemental zinc) or placebo for 2 months.[28] On

the basis of the number and severity of lesions, an "inflammatory score" was attributed to each patient. In the placebo group, the inflammatory score dropped from 58 to 47 in the 2-month period, whereas in the treatment group, the score dropped from 49 to 27. Physicians rated 24 of 32 patients in the zinc group as responders compared with only 8 of 34 in the placebo group.

At least two additional double-blind studies with zinc gluconate provide additional support,[29,30] but unfortunately, there have been no studies to date using better-absorbed forms of zinc, such as zinc picolinate, citrate, acetate, or monomethionine.

The importance of zinc to normal skin function is well recognized, especially considering the zinc-deficient syndrome acrodermatitis enteropathica. Zinc is essential for retinol-binding protein and thus for normal serum retinol levels.[31] Although low levels of zinc increase 5-α-reduction of testosterone, high concentrations significantly inhibit this reaction.[32] Serum zinc levels are lower in 13- and 14-year-old males than in any other age group.[33]

Vitamin E and Selenium

Serum vitamin A levels in rats on vitamin E–deficient diets remain low regardless of the amount of oral or intravenous vitamin A supplementation given. Serum levels return to normal after vitamin E is restored to the diet. Vitamin E has been shown to regulate retinol levels in humans.

Male patients with acne have significantly decreased levels of erythrocyte glutathione peroxidase, which normalizes with vitamin E and selenium treatment. Acne in both men and women improves with this treatment,[34] likely due to inhibition of lipid peroxide formation, suggesting that the use of other free-radical quenchers may be beneficial.

Pyridoxine

Women with premenstrual aggravation of acne often respond to vitamin B_6 supplementation, reflecting its role in the normal metabolism of steroid hormones.[35] In rats, a vitamin B_6 deficiency appears to cause an increased uptake of and sensitivity to testosterone.[36] However, this observation has never been replicated.

Vitamins A and D

Interleukin-17 is induced by *P. acnes* and expressed in acne lesions. Vitamins A and D inhibit the genetic expression of *P. acnes*–induced Th17 differentiation.[37,38]

TOPICAL TREATMENTS

Various topical gels, ointments, and creams containing natural products are available to treat acne. The goal of these preparations is the same as that of benzoyl peroxide (i.e., to reduce both the bacterial level and inflammation). Although there are many choices, the most popular formulas are those with tea tree oil, azelaic acid, vitamin C, or nicotinamide.

Tea Tree Oil

Melaleuca alternifolia, or "tea tree," is a small tree native to only one area of the world: the northeast coastal region of New South Wales, Australia. The leaves, the portions of the plant that are used medicinally, are the source of a valuable therapeutic oil.

Tea tree oil possesses significant antiseptic properties and is regarded by many as the ideal skin disinfectant. This claim is supported by its efficacy against a wide range of organisms (including 27 of 32 strains of *P. acnes*)[39] and its good penetration and lack of irritation to the skin (although cases of allergic reaction have been reported). The therapeutic uses of tea tree oil are based largely on its antiseptic and antifungal properties.

In a study conducted at the Royal Prince Hospital in New South Wales, a 5% tea tree oil solution demonstrated similar beneficial effects as 5% benzoyl peroxide in acne, but with substantially fewer side effects.[40] However, this 5% tea tree oil solution is probably not strong enough for moderate to severe acne. Stronger solutions (up to 15%) should provide even better results. Numerous studies have shown that tea tree oil is extremely safe for use as a topical antiseptic, but because it can occasionally produce contact dermatitis, a trial dose on a small area should be attempted first to ensure tolerability.

Azelaic Acid

This naturally occurring nine-carbon dicarboxylic acid has exerted much pharmacological activity, including antibiotic activity against *P. acnes.* Clinical studies with 20% azelaic acid cream have shown it to produce results equal to those achieved with benzoyl peroxide, tretinoin, and oral tetracycline.[41] It has been shown to be effective in all forms of acne. To achieve benefits, azelaic acid must be applied to affected areas twice daily for a period of at least 4 weeks. Treatment should be continued for at least 6 months to maintain the benefits produced after the first month.

One review article found a topical cream containing 20% azelaic acid to be as effective as 5% benzoyl peroxide, 4% hydroquinone cream, 0.05% tretinoin, 2% erythromycin, and 0.5 to 1 g/day of oral tetracycline in ameliorating comedonal, papulopustular, and nodulocystic acne but less effective than oral isotretinoin at a dose of 0.5 to 1 mg/kg per day in reducing cystic acne. The authors suggested that the few side effects of topical azelaic acid and lack of overt systemic toxicity made it a better choice for chronic use than other agents. The lower incidence of allergic sensitization, exogenous ochronosis, and residual hypopigmentation offers a clear advantage over conventional drugs.[42]

Vitamin C

Sodium L-ascorbyl-2-phosphate 5% lotion is a stable vitamin C derivative and highly effective antioxidant that has demonstrated statistically significant improvement of acne compared with vehicle in all the parameters measured.[43]

Nicotinamide

Topical nicotinamide inhibits the release of lysosomal enzymes, vasoactive amines, and the activity of *P. acnes* lipase. It is well tolerated, does not induce bacterial resistance, and has shown superior efficacy compared with 1% clindamycin gel for moderate inflammatory acne.[44]

Proprietary Topical Therapy

A randomized, investigator-blinded, split-face 10-day study of an over-the-counter (OTC) botanical spot treatment gel containing 2% salicylic acid, *Rheum palmatum, Portulaca oleracea, Scutellaria baicalensis, Chrysanthemum indicum, Phellodendron amurense, Sanguisorba officinalis,* and *Sapindus mukorossi* conducted in 2017 of 25 patients with mild to moderate acne found that after only five applications (over 36 hours), a dramatic reduction of visible acne, both inflammatory and noninflammatory lesions, was achieved.[45]

New Natural Agents

In 2018 a Korean group of researchers reported their review of new natural products for the topical treatment of acne.[46] Green tea has been found to improve acne by modulating intracellular targets and decreasing *P. acnes.* Lupeol, a constituent of *Solanum melongena,* and cannabdiol demonstrated sebum-reducing, anti-inflammatory, and antimicrobial activities. *Lactobacillus* fermented *Chamaecyparis obtusa* (a species of cypress) showed superiority compared with tea tree oil in a double-blind, randomized, controlled, split-face study. Mangostin, the main component

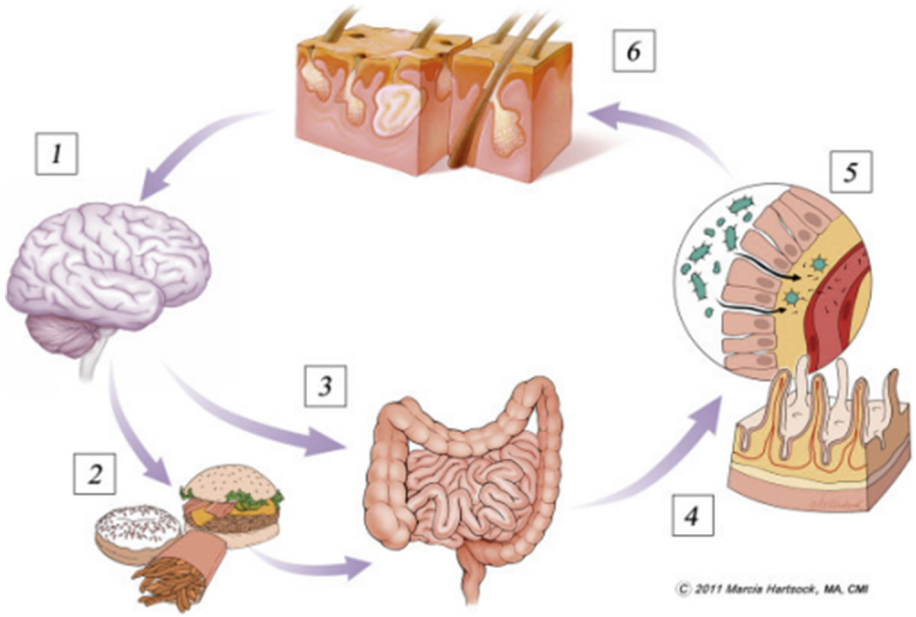


Fig. 141.2 Potential pathways of the gut–brain–skin axis in acne vulgaris. *(1)* Psychological distress alone or in combination with *(2)* high-fat diet and processed comfort foods devoid of fiber cause alterations to *(3)* gut motility and microbiota profile *(4)*. Loss of normal microbial biofilm *(Bifidobacterium in particular)* causes intestinal permeability, and endotoxins gain systemic access *(5)*. Burden of inflammation and oxidative stress is increased, substance P is elevated, and insulin sensitivity is decreased due to endotoxemia *(6)*. In those genetically susceptible to acne vulgaris, this cascade increases the likelihood of excess sebum production, exacerbations in acne, and additional psychological distress. Both probiotics and antimicrobials may play a role in cutting off this cycle at the gut level. (From Bowe WP, Logan AC. Acne vulgaris, probiotics and the gut-brain-skin axis—back to the future? *Gut Pathol.* 2011;3[1]:1. PubMed PMID: 21281494.)

of *Gacinia mangostana,* demonstrated improvement in acne severity score and inflammatory lesion counts when applied in a nanoparticle formulation.

PROCEDURAL TREATMENTS

There is a growing interest in the nonmedical treatment of acne. Traditional acne procedures include comedone extraction, intralesional injections, epidermal exfoliation (cryotherapy, peels, microdermabrasion). More recent procedures include the use of various types of lights and lasers. Blue light may be anti-inflammatory, reducing the cytokine-induced production of IL-1 alpha in keratinocytes.[47]

OTHER CONSIDERATIONS

Psychological support may be necessary because depression is a common occurrence in patients with acne, appearing twofold to threefold more often compared with the general population.[48] In 1948 Sulzberger and Zaidens stated, "There is no single disease which causes more psychic trauma and more maladjustment between parents and children, more general insecurity and feelings of inferiority, and greater sums of psychic assessment than does acne vulgaris."[49]

Acne has always been associated with emotional stress, but it is possible that the emotional stress also plays a role in the disease progression. In the 1940s dermatologists John H. Stokes and Donald M. Pillsbury first proposed a gastrointestinal mechanism for the overlap between depression, anxiety, and skin conditions such as acne.[50] The doctors hypothesized that emotional states might alter the normal intestinal microflora, increase intestinal permeability, and contribute to systemic inflammation. They also noted that as many as 40% of those with acne have hypochlorhydria and hypothesized that less than adequate stomach acid would set the stage for migration of bacteria from the colon toward the distal portions of the small intestine, as well as an alteration of normal intestinal microflora. The remedies these authors discussed to cut off the stress-induced cycle included administration of *Lactobacillus acidophilus* cultures long before they were known as probiotics. Many aspects of this gut–brain–skin unifying theory proposed by Stokes and Pillsbury have been validated (Fig. 141.2). The ability of the gut microflora and oral probiotics to influence systemic inflammation, oxidative stress, glycemic control, tissue lipid content, and even mood itself may have important implications in acne.[51] Probiotic supplementation is often indicated, especially given the common use of oral antibiotics to treat acne.

A trial of 45 females randomized to one of three arms of acne treatment with and without probiotic supplementation found that at 8- and 12-week follow-up, the arm with a probiotic (containing *L. acidophilus, Lactobacillus bulgaricus,* and *Bifidobacterium bifidum*) and minocycline had a significant decrease in total lesion count versus the groups with the probiotic ($p < 0.001$) or minocycline ($p < 0.01$) alone.[52]

Another randomized, double-blind, controlled trial of 20 adults with acne given *Lactobacillus rhamnosus* or placebo for 12 weeks found a 32% reduction ($p < 0.001$) in acne in the probiotic group compared with placebo.[53]

The relationship between hypochlorhydria and acne was investigated in a large study of 13,000 adolescents with acne. An increased prevalence of halitosis, gastroesophageal reflux, bloating, and constipation was observed. Hypochlorhydria is a recognized risk factor in small intestine bacterial overgrowth (SIBO). SIBO has been detected on hydrogen breath testing in 5% of patients on long term proton-pump inhibitor therapy.[54]

THERAPEUTIC APPROACH

Acne is a multifactorial disease requiring an integrated therapeutic approach to avoid toxicity and adverse effects during treatment. Patients should be checked for treatable causes and underlying hormonal abnormalities before specific therapies are initiated.

The most effective acne medication is isotretinoin, a derivative of vitamin A. It is approved only for severe and recalcitrant nodulocystic acne. Concern over the safety and of this drug is widespread. Specifically, reports of intracranial hypertension, depression, and suicidal ideation with isotretinoin use have prompted an examination of its serious and life-threatening potential risk. A warning is attached to its product label for signs of depression and suicidal ideation, and a U.S. Food and Drug Administration–mandated registry exists for all individuals prescribing, dispensing, or taking isotretinoin. This registry aims to further decrease the risk of pregnancy and other potentially dangerous adverse effects during a course of isotretinoin therapy.

Diet

Eliminate all refined and concentrated carbohydrates and limit saturated fats and high-carbohydrate foods. Avoid foods containing trans fatty acids and iodine.

Supplements

- Vitamin A: 150,000 IU/day for 3 months (please note cautions described earlier)
- Vitamin E: 400 IU/day
- Vitamin C: 1000 mg/day
- Zinc: 30 to 45 mg/day (picolinate form may be best)
- Selenium: 200 mcg/day
- Chromium: 200 to 400 mcg daily or brewer's yeast 1 tablespoon twice a day
- Probiotic: 5 to 10 billion live bacteria a day

Physical Medicine

- Sun or ultraviolet lamp
- Fruit acid peels
- Light therapy (blue and red light), intense pulsed light, laser, photodynamic therapy, fractionated light (for acne scars)

Topical Treatments

- Tea tree oil (5%–15%) preparations
- Azelaic acid (20%) preparations
- Nicotinamide gel (4%)
- Thorough daily cleansing with mild cleanser
- Expression of comedones with comedo extractor

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Goulden V, McGeown CH, Cunliffe WJ. The familial risk of adult acne: a comparison between first-degree relatives of affected and unaffected individuals. *Br J Dermatol*. 1999;141(2):297–300.
2. Dreno B. What is new in the pathophysiology of acne, an overview. *J Eur Acad Dermatol Venereol*. 2017;31(Suppl 5):8–12.
3. Passarini B, Infusino SD, Kasapi E. Chloracne: still cause for concern. *Dermatology*. 2010;221(1):63–70. PubMed PMID:20516651.
4. Pochi PE. Acne: endocrinologic aspects. *Cutis*. 1982;30:212–214. 216–217, 219.
5. Schiavone FE, Rietschel RL, Squotas D, et al. Elevated free testosterone levels in women with acne. *Arch Dermatol*. 1983;119:799–802.
6. Darley CR, Moore JW, Besser GM, et al. Androgen status in women with late onset or persistent acne vulgaris. *Clin Exp Dermatol*. 1984;9:28–35.
7. Takayasu S, Wakimoto H, Itami S, et al. Activity of testosterone 5-alpha-reductase in various tissues of human skin. *J Invest Dermatol*. 1980;74: 187–191.
8. Sansone G, Reisner RM. Differential rates of conversion of testosterone to dihydrotestosterone in acne and normal human skin—a possible pathogenic factor in acne. *J Invest Dermatol*. 1971;56:366–372.
9. Zaenglein AL, Pathy AL, Schlosser BJ, et al. Guidelines of care for the management of acne vulgaris. *J Am Acad Dermatol*. 2016;74:945–973.
10. Cordain L, Lindeberg S, Hurtado M, et al. Acne vulgaris: a disease of Western civilization. *Arch Dermatol*. 2002;138:1584–1590.
11. Ayres S, Mihan R. Acne vulgaris: therapy directed at pathophysiologic defects. *Cutis*. 1981;28:41–42.
12. Adebamowo C, Spiegelman D, Danby FW, et al. High school dietary dairy intake and teenage acne. *J Am Acad Dermatol*. 2005;52(2):207–214.
13. Kappas A, Anderson K, Conney A, et al. Nutrition-endocrine interactions: induction of reciprocal changes in the delta 4-5 alpha-reduction of testosterone and the cytochrome P-450-dependent oxidation of estradiol by dietary macronutrients in man. *Proc Natl Acad Sci USA*. 1983;80:7646–7649.
14. Spencer EH, Ferdowsian HR, Barnard ND. Diet and acne: a review of the evidence. *Int J Dermatol*. 2009;48(4):339–334.
15. Berra B, Rizzo AM. Glycemic index, glycemic load: new evidence for a link with acne. *J Am Coll Nutr*. 2009;28(Suppl):450S–454S.
16. Semon H, Herrmann F. Some observations on the sugar metabolism in acne vulgaris, and its treatment by insulin. *Br J Dermatol*. 1940;52:123–128.
17. Grover RW, Arikan N. The effect of intralesional insulin and glucagon in acne vulgaris. *J Invest Dermatol*. 1963;40:259–261.
18. Kader MM, El-Mofty AM, Ismail AA, et al. Glucose tolerance in blood and skin of patients with acne vulgaris. *Ind J Dermatol*. 1977;22:139–149.
19. Cohen JL, Cohen AD. Pustular acne staphyloderma and its treatment with tolbutamide. *Can Med Assoc J*. 1958;80:629–632.
20. Offenbacher EG, Pi-Sunyer FX. Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly patients. *Diabetes*. 1980;29:919–925.
21. McCarty M. High-chromium yeast for acne? *Med Hypotheses*. 1984;14:307–310.
22. Mahmoood SN, Bowe W. Diet and acne update. Carbohydrates emerge as the main culprit. *J Drubs Dermatol*. 2014;1394:428–435.
23. Grossi E, et al. The constellation of dietary factors in adolescent acne: a semantic connectivity map approach. *J Eur Acad Dermatol Venereol*. 2016;30(1):96–100.
24. Joon JY, Kwon HH, et al. Effect of dietary supplementation with omega-3 fatty acid and gamma-linolenic acid on acne vulgaris: a randomised, double-blind, controlled trial. *Acta Derm Venereol*. 2014.
25. Kilgman AM, Mills Jr OH, Leyden JJ, et al. Oral vitamin A in acne vulgaris: a preliminary report. *Int J Dermatol*. 1981;20:278–285.
26. Michaelsson G, Juhlin L, Ljunghall K. A double-blind study of the effect of zinc and oxytetracycline in acne vulgaris. *Br J Dermatol*. 1977;97:561–566.
27. Weimar VM, Puhl SC, Smith WH, et al. Zinc sulphate in acne vulgaris. *Arch Dermatol*. 1978;114:1776–1778.
28. Dreno B, Amblard P, Agache P, et al. Low doses of zinc gluconate for inflammatory acne. *Acta Derm Venereol*. 1989;69:541–543.
29. Meynadier J. Efficacy and safety study of two zinc gluconate regimens in the treatment of inflammatory acne. *Eur J Dermatol*. 2000;10:269–273.
30. Kobayashi H, Aiba S, Tagami H. Successful treatment of dissecting cellulitis and acne conglobata with oral zinc. *Br J Dermatol*. 1999;141:1137–1138.
31. Michaelsson G, Juhlin L, Vahlquist A. Effects of oral zinc and vitamin A in acne. *Arch Dermatol*. 1977;113:31–36.
32. Leake A, Chisholm GD, Habib FK. The effect of zinc on the 5-alpha-reduction of testosterone by the hyperplastic human prostate gland. *J Steroid Biochem*. 1984;20:651–655.
33. Michaelsson G, Vahlquist A, Juhlin L. Serum zinc and retinol-binding protein in acne. *Br J Dermatol*. 1977;96:283–286.
34. Michaelsson G, Edqvist L. Erythrocyte glutathione peroxidase activity in acne vulgaris and the effect of selenium and vitamin E treatment. *Acta Derm Venereol*. 1984;64:9–14.
35. Snider BL, Dieteman DF. Letter: pyridoxine therapy for premenstrual acne flare. *Arch Dermatol*. 1974;110:130–131.
36. Symes EK, Bender DA, Bowen JF, et al. Increased target tissue uptake of, and sensitivity to, testosterone in the vitamin B6 deficient rat. *J Steroid Biochem*. 1984;20:1089–1093.
37. Agak GW. Propionibacterium acnes induces an IL-17 response in acne vulgaris that is regulated by Vitamin A and Vitamin D. *J Invest Dermatol*. 2014;134(2):366–373.
38. Youssef DA, et al. Antimicrobial implications of vitamin D. *Dermatoendocrinol*. 2011;3(4):220–229.
39. Carson CF, Riley TV. The antimicrobial activity of tea tree oil. *Med J Australia*. 1994;160:236.
40. Bassett IB, Pannowitz DL, Barnetson RS. A comparative study of tea-tree oil versus benzoyl peroxide in the treatment of acne. *Med J Aust*. 1990;153:455–458.
41. Nazzaro-Porro M. Azelaic acid. *J Am Acad Dermatol*. 1987;17:1033–1041.
42. Nguyen QH, Bui TP. Azelaic acid. Pharmacokinetic and pharmacodynamic properties and its therapeutic role in hyperpigmentary disorders and acne. *Int J Dermatol*. 1995;34:75–84.
43. Woolery-Lloyd H, Baumann L, Ikeno H. Sodium L-ascorbyl-2-phosphate 5% lotion for the treatment of acne vulgaris: a randomized, double-blind, controlled trial. *J Cosmet Dermatol*. 2010;9(1):22–27.
44. Shalita AR, Smith JG, Parish LC, et al. Topical nicotinamide compared with clindamycin gel in the treatment of inflammatory acne vulgaris. *Int J Dermatol*. 1995;34:434–437.
45. Barak-Shinar D, Draelos ZD. A randomized controlled study of a Novel botanical acne spot treatment. *J Drugs Dermatol*. 2017;16(6):599–603.
46. Shnitkind E, et al. Anti-inflammatory properties of narrow-band blue light. *J Drugs Dermatol*. 2006;5:605–610.
47. Uhlenhake E, Yentzer BA, Feldman SR. Acne vulgaris and depression: a retrospective examination. *J Cosmet Dermatol*. 2010;9(1):59–63.
48. Sulzberger MB, Zaidens SH. Psychogenic factors in dermatologic disorders. *Med Clin North Am*. 1948;32:669–685.
49. Stokes JH, Pillsbury DH. The effect on the skin of emotional and nervous states: theoretical and practical consideration of a gastrointestinal mechanism. *Arch Dermatol Syphilol*. 1930;22:962–993.
50. Bowe WP, Logan AC. Acne vulgaris, probiotics and the gut-brain-skin axis: back to the future? *Gut Pathog*. 2011;3(1):1.
51. Jung GW, et al. Prospective, randomized, open-label trial comparing the safety, efficacy, and tolerability of an acne treatment regimen with and without a probiotic supplement and minocycline in subjects with mild to moderate acne. *J Cutan Med Surg*. 2013;17(2):114–122.
52. Fabbrocini G, et al. Supplementation with Lactobacillus rhamnosus SP1 normalises skin expression of genes implicated in insulin signalling and improves adult acne. *Benef Microbes*. 2016;30:625–630.
53. Zhang H, et al. Risk factors for sebaceous gland diseases and their relationship to gastrointestinal dysfunction in Han adolescents. *J Dermatol*. 2008;35:555–561.
54. Lombardo L, et al. Increased incidence of small intestinal bacterial overgrowth during proton pump inhibitor therapy. *Clin Gastroenterol Hepatol*. 2010;8:504–508.

142

Affective Disorders

Peter B. Bongiorno, ND, MSAc, LAc

OUTLINE

INTRODUCTION

Affective disorders are characterized by a disturbance in a person's emotional state. Although the rubric "mood disorders" refers specifically to depression and bipolarity, affective disorders are broken down into three main types: depression, anxiety, and bipolar disorders.

The burden of affective disorders is profound throughout the world but far outpaced by the burden in Westernized countries. For example, Nigeria has a 0.1% anxiety rate, and New Zealand has a rate of 6.2%. The overall global average prevalence of anxiety disorders rests around 7.3%.[1] In comparison, the U.S. population has prevalence of around 18% to 20%. Furthermore, the United States trumps the world when it comes to low mood, too: the global population of depression in 2015 is approximately 4.4%, but 7% of Americans are depressed.[2,3]

According to 2016 data from the Centers for Disease Control, mood disorders are on the rise, with suicide rates increasing in almost all age groups and states. Since the year 2000, the number of women who have died from suicide has nearly doubled from 6000 to almost 12,000.[4] This increase is despite the fact that diagnoses and prescription medications for mood are at an all-time high. The number of people taking antidepressants increased by 65% between 1999 and 2014.[5] In that same time period, the annual rate of suicide increased from 10.5 to 13 people out of 100,000—a 24% increase, which was the highest in almost three decades.[6] About 16% to 20% of the population take antidepressant or antianxiety drugs.[7]

There isn't much greater parity when it comes to the total spectrum of bipolar disorder, as the United States has a 4.4% lifetime rate, much higher than the 2.4% worldwide average.[8]

DIAGNOSIS

Depression

Characterized by periods of low mood, sadness and anhedonia, depression is broadly grouped into:

- Major depression: Characterized by persistent sad feelings that leave a person unable to functional normally. This is also known as unipolar depression.
- Dysthymia: A persistent chronic form of mild depression that typically allows a person to function at some level, but he or she generally feels somewhat sad and often will still lose interest in activities that would typically be pleasurable.
- Seasonal affective disorder: Low mood or sad feelings that occur during a certain time of year. Typically, this time is during the fall into wintertime, when the hours of daylight decreases.

Anxiety

Anxiety disorders are characterized by extreme nervousness and feelings of fear. Anxiety disorders are further grouped into these types:

- Generalized anxiety disorder, which has symptoms that include 6 or more months of exaggerated fears, worry, and general emotional tension.
- Obsessive compulsive disorder, which is type of anxiety where a person has repetitive images, ideas, and bodily feelings (called obsessions) that drive the person to display behaviors and actions in response (these are the compulsions).
- Panic disorder, which is a type of anxiety in which intense and often sudden anxiety results in a person being overwhelmed by these feelings and unable to function normally.
- Posttraumatic stress disorder, which is anxiety that follows a trauma or major stressor, when a person often has repeated thoughts of an event or subsequent disturbing anxiety that can be triggered by something that reminds the person of the originating event.
- Social anxiety, which involves an anxiety with a main feature in which a person's worry, often regarding other people's judgment or evaluation, leaves him or her unable to function in a social situation.

Bipolar Disorder

Bipolar disorder can include feelings of mania (excessive elation and positivity) and/or feelings of low mood and depression. The types of bipolar disorders are:

- Bipolar I, characterized by manic symptoms that either last 7 days or are so severe the person require some type of hospitalization or restraint for the patient's safety or the safety of others. Depressive episodes are periods of depression that last 2 weeks and may interchange with the symptoms of mania.
- Bipolar II, featuring symptoms of depression along with hypomania, which is a subtler form of mania that usually does not require restraint or hospitalization.
- Cyclothymia, a milder form of bipolar disorder, in which there may be 2-year cyclical occurrences of elevated mood and euphoria (hypomanic symptoms) and depressive symptomology.

Stress → Prefrontal Cortex Activity → Amygdala Activation → HPA Activation → Inflammation

Fig. 142.1 Result of Stress.

CAUSAL CONSIDERATIONS

Stress and Hypothalamic-Pituitary-Adrenal Axis Dysfunction

Although stress is universally recognized as a major player in affective disorder, the definition of stress varies wildly among physicians. Furthermore, there is little agreement on the best ways to handle stress. Hungarian endocrinologist Hans Selye first used the term "stress" when he defined it as a "non-specific strain on the body caused by irregularities in normal body functions. This stress resulted in the release of hormones."[9] Stressors can take many different forms: changes in environmental milieu, weather, social structure, and interpersonal interaction. The stress response can also be initiated as a result of feeling preyed on by onset of new illness, from exposure to toxins, and even by food sensitivity.

Stress results in prefrontal cortex activity sending signals to the amygdala, the fear center of the limbic system. The amygdala plays a key role in emotional processing and will modify activation of the hypothalamic pituitary adrenal (HPA) axis, increasing levels of corticotropin-releasing hormone (CRH); will upregulate inflammation and the cytokine cascade; and will reset downstream glucocorticoid sensitivity.[10] See Fig. 142.1.

Raised levels of CRH are known to have neurotoxic effects.[11] As the final common pathway in the stress response, CRH is released to the hypothalamus and feeds directly to the anterior pituitary and initiates adrenocorticotropin release (ACTH). ACTH travels through the bloodstream and initiates adrenal cortex release of cortisol.[12] CRH and ACTH production is augmented when the hypothalamic paraventricular neurons are chronically activated. Oxytocin counters this effect. Although cortisol will negatively feed back through the action of mineralocorticoid and glucocorticoid receptors to exert negative feedback on the hippocampus, the pituitary, and the hypothalamus,[13] chronic stress encourages glucocorticoid resistance and allows the stress response to continue.

According to McEwen, during a stressor the CNS and the body communicate in a bidirectional fashion via the endocrine, immune, and autonomic systems.[14] In the short term, these responses are beneficial because they serve to promote physiological and psychological survival. In the midterm, regulatory processes, behaviors, and physiology are challenged, but functioning remains relatively normal. In the long term, consistent experience of stress, fear, and/or trauma, and the body's attempts at homeostasis will contribute to the chronic negative effects of chronic stress and behavioral changes that encourage unhealthy lifestyles and illness[15] and support the varied mechanisms of anxiety and mood disorders. Bruce McEwen's research team created the new term "allostatic load" to recognize the parallel healthful and noxious results of the stress response. Although allostatic load works to dynamically rebalance, it can, at the same time, damage tissues, deplete reserves, and encourage development of affective disorders, cardiovascular disease, cancer, and other illnesses.

One notable type of stress is social stress that induces physiological effects that block healing. For example, animals exposed to long-term social subordination will present with altered HPA set points, ineffective hippocampal survival, glucocorticoid resistance, cognitive changes, withdrawal behavior, and lowered immune status in the effort to produce coping mechanisms to the stressor.[16,17] All these precipitate and maintain affective disorders.

This animal research has been duplicated in humans. For example, a San Francisco Federal Reserve study noted that the highest rates of suicide occur among nonaffluent people who live in affluent areas. This study strongly correlated a sense of low worth and utility when individuals' relative income is less than those around them.[18] When social stress creates low self-esteem, affective disorders thrive. Patients under social stress may need significantly more support to help regain mental health.

Disordered Sleep

Adequate sleep is critical for repair, healing, and detoxification. During sleep, inflammation is balanced, mitochondria are recycled and created, neurons regenerate, the hormonal system resets, and the digestive tract cleans and repairs. Without proper sleep, balancing mood is near impossible due to deficiencies and imbalances in all these processes. Poor sleep predicts worse outcomes in depression, anxiety, and bipolar disorder.[19]

Clinicians often find it challenging to ascertain whether sleep disturbances are a precursor to or a sequelae of mood disorder. Sleep is bidirectionally related to mood disturbance, as poor sleep will raise the stress response. Increased stress response will contribute to poor sleep. Research shows us that sleep challenges occur in bipolar stages and will exert a negative effect on disease course, life quality, and treatment outcomes.[20] The clinician should identify and treat any insomnia, REM-sleep concerns, and sleep apnea.

Typical underlying causes for sleep problems include excessive stimulants; psychological stress; high levels of stress hormones (such as cortisol and norepinephrine); disrupted circadian rhythms; low levels of serotonin or melatonin; imbalances in estrogens and progesterone, which affect neurotransmitter production; dyglycemias; and digestive upheaval.

Sunlight, Spending Time in Nature, and Light Therapy

The benefits of light exposure are threefold: to maintain healthy levels of serotonin, support circadian rhythm, and to increase levels of vitamin D in the body.

Vitamin D is produced through exposure to ultraviolet B rays, which transform skin 7-dehydrocholesterol into vitamin D_3. One report suggests a 12-minute exposure of 50% of body skin to noontime sun on a clear day is equivalent to oral intake of 3000 IU of vitamin D_3.[21] Supplemental vitamin D is discussed in a subsequent section of this chapter. Interestingly, a study over a 2.3-year period of 198 patients with multiple sclerosis found that sunlight exposure, and not vitamin D levels, are best correlated with mood and fatigue symptoms.[22] Although ultraviolet B's ability to make vitamin D may be important, sunlight's infrared wavelengths may play a separate and distinct role in mood. Animal research shows the amount of time until an animal will "give up" and become depressed after continuous stress is increased significantly after 4 weeks' exposure to infrared irradiation, suggesting that application of infrared irradiation has an antidepressant effect.[23]

Humans are animals designed to spend time in nature. Natural areas like forests are shown to have calming and balancing effects on the body. The Japanese practice of forest bathing, called *shinrin yoku,* lowers adrenaline levels and improves natural killer cell activity.[24] Studies of postsurgical patients show that those given exposure to a window with a nature view had a better mood, shorter hospital stays, and fewer complications.[25] Patients exposed to houseplants show lowered blood pressures, less pain, and less fatigue and anxiety than individuals in the rooms without plants.[26]

Also known as bright light therapy exposure, phototherapy has been shown to block the negative mood effects of acute tryptophan depletion.[27] Phototherapy, or light-box therapy, is most often used in seasonal affective disorder. Typical light-box therapy involves using a 10,000-lux, full-spectrum white light for at least 30 minutes every morning. Light boxes with smaller power do not seem to provide the same benefit as the 10,000-lux devices. Meta-analysis of light therapy studies showed that enough high-quality studies to confirm benefit are lacking. Nevertheless, in my clinical experience, I see clearly beneficial changes in mood.

Once again, modern research documents the efficacy of foundational naturopathic concepts of health and healing through sun and nature exposure.

Exercise

Known since Hippocrates' time to elevate mood, exercise can help regenerate and protect the brain areas needed for stable temperament and optimal cognition. Movement and exercise assist the brain glial cells to produce brain-derived neurotrophic factor (BDNF), which is responsible for the regeneration and repair of the CNS. Exercise has also been identified to increase hippocampal volume.[28]

Low levels of exercise have been linked to lowered life quality, poor physical function, and mood symptoms.[29] The literature regarding the effect of exercise as an intervention for major depression and bipolar disorder has shown comparable effect sizes.[30] Exercise has been shown to reduce anxiety, counter depression, and improve negative mood, while simultaneously improving self-esteem and even memory.[31] Other research also suggests the efficacy of exercise-augmentation strategies added to cognitive behavioral therapy (CBT) for anxiety disorders.[32]

Often, mood disorders like bipolar and depression present with a strong lack of motivation and interest. These symptoms can make implementing an exercise schedule a challenge. As such, for some patients, it may be best to proffer an exercise protocol that is gentle and does not cause discomfort, especially if patients have concomitant fibromyalgia and chronic fatigue. For fitness, the American College of Sports Medicine recommends 5 days a week of moderate to vigorous exercise for 30 minutes—but clinicians may want to start the patients with mood disorder on a lighter schedule if their energy is not vital or they are new to exercise.[33]

Although not as common, excess exercise may also result in damaging changes to mental health. Some patients become excessively dependent on physical activity and exercise, where excess activity can

cause both disturbances in mood and worsened physical health.[34] Extremely heavy or exhaustive exercise can promote exercise-induced mitochondrial dysfunction, a condition where mitochondria suffer due to nutrient deficiencies, excess reactive oxygen species, and metabolic toxicity, which contribute to neurodegeneration.[35] Heavy exercise may also lead to adrenal fatigue, recognized as an Addison's disease type of overtraining syndrome.[36] In this situation, the adrenal glands cannot competently produce sufficient hormone levels, and the body and mind suffer.

Blood Sugar Dysregulation

Balanced blood sugar is key for mood regulation, whereas dysglycemia (recurrent episodes of either too many lows or highs) will contribute to affective disorders. Research on patients with diabetes suggests good blood sugar control supports healthy mood and good judgment.[37] Hypoglycemia-prone patients are known to be at greater risk for depression. Dysglycemia has been shown to contribute to smaller hippocampal and cortical volumes in bipolar disorder.[38] A study from Johns Hopkins evaluated lung injury patients in an intensive care unit and noticed that subjects who had low blood sugar of under 60 nanograms (normal is 80 to 100 ng) were at a 360% increase risk of depression 3 months later.[39] Associations between anxiety and insulin resistance have been established.

Converse to hypoglycemia, hyperglycemia also contributes to mood disorders. Studies of patients with diabetes are clear that high-blood–sugar episodes will deteriorate mood, instigate cognitive difficultly, and most notably increase sadness and anxiety.[40] People with hyperglycemia or high insulin are also predisposed to depression. A study of young adults in their late 20s and early 30s showed that when blood sugar or insulin was too high, the chances of becoming depressed was 150% to 200% as high.[41] Higher rates of bipolar disorder exist among patients with diabetes as well.[42]

Balancing blood sugar requires quality sleep, lower stress response, and healthy eating. The tenets of blood sugar control include balanced protein intake, healthy fat intake, and consuming healthy carbohydrates while minimizing simple carbohydrates and sugary foods. Small frequent meals can be very helpful for many patients to abate fluctuations that lead to mood changes as noted in the next section. One supplement that can help is chromium, which will be discussed in the mineral section later in this chapter.

Maldigestion

Healthy digestion is critical for mood management. In 1999 Michael Gershon's book, *The Second Brain,* set forth a "new" perspective (almost a century earlier, naturopathic pioneer Otis Carroll, ND, asserted that disease begins in the bowels) regarding the digestive system, seeing it not just as a means for nutrient absorption and elimination but also as a neuroendocrine organ[43] that partners in a bidirectional relationship with the CNS. Evidence shows that bowel disorders are correlated with disruptions in mood. In fact, almost two thirds of patients with anxiety and depression are described to have intestinal function disturbance, such as irritable bowel syndrome (IBS).[44] Almost one third of patients with major depression are thought to have constipation, and patients with IBS are more prone to both anxiety disorders as well as depression than are individuals who do not have IBS.[45] Looking at 136 patients with bipolar disorder versus matched controls, investigators found a strong association between mood and gut symptoms. Interestingly, their study also showed that patients with bipolar disorder with minimal mood symptoms typically do not have more gastrointestinal symptoms versus control subjects.[46] There are also case reports of anxiety and panic attacks in patients before diagnosis of pancreatic cancer.[47] Although the reason is unclear, I have seen very direct clinical associations between pancreatic inflammation, high levels of amylase and lipase, and anxiety and mood disorders.

This bidirectional relationship is in part due to the vitality and composition of the microbiota of the digestive tract, where, researchers suggest, estimates of these bacteria outnumber human cells by an order of 10. The dynamics of the microbiome ultimately affect synthesis of neurotransmitters and metabolites. The microbiome regulates vagus nerve activation and also modulates immunological consequences. Emerging studies in both animals and human subjects suggest perturbations in the microbiome are also key factors in altered gut health. Animal studies have shown alterations create changes in production of BDNF in the hippocampus and amygdala as well as behavioral changes.[48] Furthermore, stress will alter the microbiome, and the microbiome will alter function of the HPA axis, leading to reregulation of the stress response.[49] Evidence suggests that microbiome alterations from antibiotics may also predispose individuals to bipolar disorder, and patients diagnosed with bipolar disorder with more severe symptomology seem to have altered microbiomes that differ from those of healthy controls.[50]

Inflammation

Inflammatory markers such as CRP and IL-6 are typically raised in situations of anxiety,[51] depression,[52] and bipolar disorder.[53] The majority of the body's immune system is housed in digestive tract, where it resides as mucosa-associated lymphoid tissue and gut-associated lymphoid tissue. Stress and poor digestion lead to inflammatory activation. Food sensitivities and food allergens can also trigger inflammation in the body. High-glycemic foods also trigger release of excess insulin. Increased chronic levels of insulin leads to insulin resistance, which is also shown to drive inflammation.[54]

As discussed earlier, imbalances in the microbiome will also upregulate inflammatory cytokines and interleukins. A digestive tract full of *Candida albicans* (yeast), and all the toxins associated with it, may also contribute to mood disorder. Presence of yeast will alter the ability to absorb nutrients and push hypersensitivity reactions of toxin by-products, which translates to inflammation in the body.[55] Inflammation will greatly contribute to depression, anxiety, and poor mental function.

Chronic inflammation in the digestive tract can lead to gut permeability, which is often referred to as "leaky gut." The concept suggests that other structure and repair mechanisms of the digestive tract are compromised over time. With an inflammatory fire partially driven by an imbalance in microbiota, tight junctions deteriorate. When these structures break down, luminal materials gain greater access to the bloodstream. These leaked particles travel through the hepatic portal system and spur upregulation of hepatic Kupffer cells, which can trigger brain inflammation via microglia activation. Inflammation in the brain will result in activation of the HPA axis, lowered serotonin, higher anxiety, and higher glucocorticoid resistance. Over time, higher cortisol levels contribute to brain degeneration and poor mood. The likelihood of this cascade of events is far greater than if the intestines are intact.[56] See Fig. 142.2.[57]

Leaky gut has been a term all but mocked in conventional care circles until relatively recently, where reviews of the literature show connections not only between leaky gut and more direct inflammatory issues like autoimmunity[58] but also with cardiovascular

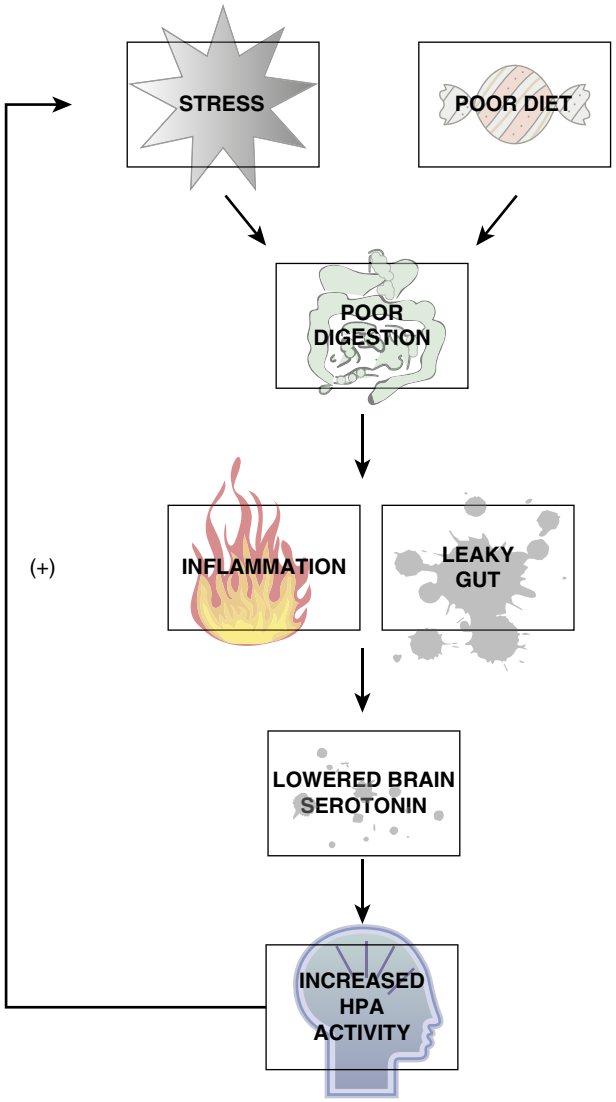


Fig. 142.2 Stress and Diet.

disease,[59] kidney disease,[60] and psychiatric concerns.[61] Out of the purview of this chapter, but covered in Chapter 19 Intestinal Permeability, practitioners should consider that a patient with affective disorder may greatly benefit by testing and treatment of gut permeability.

Hormonal Imbalances

The endocrine system plays a strong role in the signaling and function of the nervous system and inflammatory response. Undoubtedly, it is a contributor to mood disorders.

Thyroid Hormones

Thyroid hormones affect virtually every cell body, and imbalances will contribute to affective disorder. Most notably, hyperthyroidism can contribute to overt feelings of anxiety and mania in bipolar disorder, and low levels will contribute to low mood and depression.[62,63] Regarding the hypothalamic-pituitary-thyroid axis (HPT), high-stress states and allostatic load will also alter the thyroid axis, whereby high levels of ACTH affect levels of thyroid-releasing factor and thyroid-stimulating hormone (TSH). Repeated and chronic stress can lower serum levels of tetraiodothyronine (T4).[64] Glucocorticoid levels will also influence the de-iodinase enzyme that peripherally converts T4 to the three times more active triiodothyronine (T3).[65] T3 augmentation has been shown valuable in treating patients with depression that is resistant to selective serotonin-reuptake inhibitor (SSRI) treatment.[66]

Cortisol

Cortisol is the major glucocorticoid for mediating the stress response. Imbalances in HPA axis function and adrenal production, as seen in treatment-resistant depression, are evidenced by higher cortisol-to-dehydroepiandrosterone (DHEA) ratios.[67] Cortisol and stress catalyze activation of tryptophan oxygenase, resulting in the delivery of less tryptophan to the brain. In addition, cortisol downregulates serotonin receptors in the brain, making them less sensitive to the serotonin that is available. In studies on depression and cortisol levels, particularly the awakening response (CAR), depression itself has also been associated with both increased and lowered or blunted (nonsignificant, nominal change in) CAR. In my experience, cortisol levels throughout the day will be more often dysregulated in patients with affective disorders. This dysregulation can manifest as high levels of cortisol, low levels of cortisol, or varying levels throughout the day, including low early morning and afternoon levels and high nighttime and early morning levels. Assessing cortisol levels via saliva or urine testing can help the practitioner determine an individual patient's adrenal profile.

Dehydroepiandrosterone sulfate

Considered a "neurosteroid," DHEA and dehydroepiandrosterone sulfate (DHEAs) are molecules produced by the adrenal gland. Low DHEA levels are related to symptoms and severity of depression and anxiety.[68] DHEA may protect against the adverse effects of stress, especially with regard to the brain atrophy caused by chronically high levels of cortisol. Like exercise, DHEA can increase neuronal growth in the hippocampus and will protect new nervous tissue from being destroyed by stress hormones. Higher levels are likely present in bipolar disorder.[69] Generally, supplementation when low is helpful. A 2014 literature review looked at 22 studies, finding DHEA supplementation is helpful for depressive symptoms overall.[70] One case in the literature suggested 150 to 200 mg per day of DHEA may have contributed to acute mania onset in a patient with a history of these episodes.[71]

Testosterone

DHEA metabolizes into testosterone. High levels of testosterone are associated aggression, impulsivity, compulsiveness, and paranoia. Testosterone increases monoamine oxidase (MAO) and decreases catechol-o-methyl transferase (COMT), an enzyme responsible for breakdown of both neurotransmitters and estrogens. Low testosterone levels can contribute to fatigue, depression, flat mood, and mood swings, as well as sleep disorders; high levels can also contribute to depression.[72] SSRIs are known to lower levels of testosterone and negatively affect libido. Testosterone administration in 15 females reported reductions in unconscious fears in a placebo-controlled, double-blind crossover trial,[73] suggesting that low levels may participate in difficulty conquering anxiety. Concerning bipolar disorder, testosterone levels are notably lower for male patients in comparison with controls, whereas women with bipolar disorder had significantly

higher testosterone levels versus female controls.[74] Again, assessing individual profiles to help balance levels in each patient is important for optimal care.

Estrogen

As a prime mood-enhancing hormone, estrogen is important to both male and female brain function. The highest density of estrogen receptors are in the amygdala, hippocampus, hypothalamus, and dorsal raphe, where most of the brain serotonin is produced. Estrogens (primarily estradiol) will affect brain estrogen receptor activation, which affects all neurotransmitter levels, with a preference for supporting serotonin and dopamine levels. Estrogen also lowers levels of MAO.[75] Conversely, low estrogen levels increase activation of MAO activity, which will lower levels of neurotransmitters and can contribute to affective disorders. However, research regarding replacement of estrogens as a monotherapy to help mood are equivocal or found it to be even possibly detrimental to cognition.[76] A number of studies and case reports reveal mood benefits, especially in depression. Studies also show estrogen therapy may augment antidepressants' efficacy in treatment-resistant cases.[77] It seems that in the right patient, testing levels and considering natural hormone-replacement therapy might be valuable.

Progesterone

Acting more yin to estrogen's yang, progesterone is generally a more calming hormone that can work by limiting estrogen's ability to support serotonin. Allopregnanolone is a metabolite of progesterone that also affects gamma amino-butyric acid (GABA) activity to drive progesterone's calming effect. HPA axis activation resulting in higher levels of cortisol will lower production of progesterone. A double-blind, placebo-controlled trial of 17 patients documented a clear anxiolytic and lowered irritability effect of progesterone vaginal suppositories (200 mg twice a day) without changes in blood levels of the hormone.[78] Progesterone is also a valuable sleep ally that does not change undisturbed sleep but can restore normal sleep when sleep is disturbed.[79]

Oxytocin

Oxytocin is a hormone that also acts as a neurotransmitter. It is best associated with feelings of love, meaningful touch, cuddling, and combating anxiety. People with higher comfort levels in social situations and who trust are able to release appropriate amounts of oxytocin, whereas people who have social anxiety disorder often have dysregulated oxytocin.

Oxytocin reduces amygdala hyperactivity and blocks the perception of a threatening environment. High levels of oxytocin are consistent with bonding experiences, regular community interaction, and consistent social engagement. Known to be low in people with depression and anxiety,[80] oxytocin may be a reasonable trait marker for bipolar disorder as well.[81] Intranasal administration is shown to enhance social abilities in children with autism[82] and has shown antidepressant properties in rats. No clinical trials for depressed humans have been published as of this writing.[83]

Oxytocin may be one of the reasons pets improve mood; it is released by petting a dog.[84] More oxytocin-raising suggestions are given in the nutrigenomics section of this chapter.

Melatonin

Melatonin is hormone that acts as a powerful antioxidant and is involved with detoxification and immune function. Melatonin facilitates sleep by lowering body temperature and inducing drowsiness. Deficient or delayed melatonin levels may contribute to depression[85,86] and anxiety.[87] Delayed bedtime will encourage the stress response and suppress melatonin. Studies showing supportive effects for melatonin supplementation as a monotherapy for affective disorders, including bipolar, are lacking.[88] Time-released melatonin has shown benefit to support sleep and reduce fatigue in depressed patients.[89] Although melatonin itself has not been shown as successful in alleviating mood disorders on its own, supplemental melatonin has been shown to help improve sleep quality and quantity in depressed patients[90] and will likely help a patient with mood disorder ultimately heal by improving sleep.

Lifestyle Factors: Smoking, Alcohol, and Caffeine

A health-promoting lifestyle and diet are important in the treatment of depression. Particularly important are the cessation of smoking, excessive alcohol consumption, and caffeine.

Smoking

Smoking upregulates cortisol levels, leading to HPA axis and adrenal dysregulation, a feature of affective disorders. Cigarette smoking also leads to a relative vitamin C deficiency. Vitamin C is concentrated in adrenal glands, so adrenal function is likely compromised, which can contribute to depression.[91] Early life smoking neurodevelopmentally encourages anxiety and other mood disorders later due to increases in inflammation, oxidative stress, and mitochondrial dysfunction.[92] Individuals with bipolar disorder are up to 300% more likely to smoke and are far less likely to initiate or maintain smoking cessation.[93]

Alcohol

Alcohol is a potent nervous system depressant that also wreaks havoc with blood sugar regulation, leading to cravings and hypoglycemic episodes (see section on blood sugar imbalances). Interestingly, alcohol seems to stabilize GABA, which may be why alcohol has a temporary calming effect.[94] Chronic alcohol ingestion also leads to multiple vitamin and mineral deficiencies. Patients with alcoholism often have a deficiency in the delta-6 desaturase enzyme, which is responsible for the conversion of linoleic acid to the omega-6 fatty acid gamma-linolenic acid, a precursor to prostaglandin E1 (PGE1). PGE1 helps maintain neurotransmitter levels in the brain to support positive mood and a calm sense. Alcohol may also temporarily stimulate production of PGE1 and help lift mood.[95] If delta-6 desaturase is impaired for any reason, mood can decline—usually more toward depression. Excess sugar intake, high insulin levels, coffee, and trans-fatty acids can all lower inhibit 6 desaturase activity.

Caffeine

Caffeine is the most frequently consumed psychoactive drug in the world. Found in coffee, green tea, and black tea, caffeine may be beneficial or antagonistic to mood disorders. According to a meta-analysis of 11 studies with more than 300,000 people, a range of caffeine intake between 68 mg per day and 509 mg per day (approximately ¾ cup to 6 cups of coffee a day) reduces depression, whereas more can promote depression and suicidal thoughts.[96] In most cases, anxious patients are aggravated by caffeine, although in practice I recognize a subset of anxious patients who can actually benefit from caffeine intake, likely due to it raising low levels of dopamine. Caffeine acts on the neurotransmitter system by antagonizing adenosine receptors, which initiates a cascade that increases glutamate, cortisol, dopamine, and epinephrine. Generally, for anyone who has suspected depleted adrenal function, as evidenced by

low cortisol or DHEA, or for a person with high levels of epinephrine or dopamine, coffee is not recommended—especially if she or he is not sleeping well.

Digital Screen and Social Media Exposure

Although electronics are an important part of our everyday lives, exposure to these large and small screens may be playing a role in the increased levels of affective disorders in our society. Functional MRI studies have shown that short-term use of television may have a relaxing effect on brain insular cortex and amygdala regions,[97] whereas moderate or severe depression level was associated with higher amounts of time spent on TV watching and use of computers for more than 6 hours a day[98] (although this seems like a lot of time, it is not really much considering the millions of students and working people who spend the majority of their time looking at a computer screen—as I do). An analysis of more than 30 years of U.S. national data of 30,000 adults corroborates this, showing that spending time watching television may contribute to viewers' happiness in the short-term moment, but unhappy people watch far more TV than happy people do.[99]

Regarding social media, an online survey of 1730 U.S. adults ages 19 to 32 suggested those who classified themselves as "wired" and "connected" (versus "diffuse dabblers," "concentrated dabblers," and "unplugged") greatly increased the likelihood of anxiety and depression symptoms. Smartphone addiction is correlated with depression.[100] Dependence on media may likely create changes in the dopamine-reward system similar to the changes opioids and other drugs of addiction may cause, as loss of devices such as a smartphone may create feelings of panic, anxiety, or withdrawal. Younger adults seem to be more affected by media and smartphone addiction in terms of anxiety and depression. Besides being causative, use of devices may encourage an underlying trait. For example, someone with social phobia might avoid in-person contact by using the phone, and/or someone with OCD traits may manifest by using his or her phone to check things repeatedly.[101]

It should also be noted that, when used wisely, computers and media can aid those with anxiety and depression. Research shows the effectiveness of Internet-based CBT (ICBT) in treating and controlling psychiatric illnesses, including depression, GAD and social anxiety, panic disorders, phobias, addiction and substance-use disorders, adjustment disorder, bipolar disorder, and OCD, and ICBT may be quite cost effective for many patients.[102]

Environmental Toxicity

Toxic metals (lead, mercury, cadmium, arsenic, nickel, and aluminum) as well as solvents (e.g., cleaning materials, formaldehyde, toluene, and benzene), pesticides, herbicides, and mold toxins all have an affinity for nervous tissue. The oxidative stress from these neurotoxins can substantially contribute to mitochondrial dysfunction (see next section). As a result, various psychological and neurological symptoms can occur, including anxiety and depression as well as headaches, extremity tingling, and abnormal nerve function. For example, social and emotional challenges in children correlate with pre- and postnatal exposure to lead.[103] Methylmercury exposure will produce mitochondrial dysfunctions via oxidative stress and downregulation of tryptophan hydroxylase, the enzyme needed to produce proper amounts of serotonin.[104] Surveys of residents of eight European cities revealed dampness and mold is associated 30% increased depression.[105]

(For a comprehensive discussion of the large body of research on neurotoxins and mood disorders, see Crinnion and Pizzorno, *Environmental Medicine*, Elsevier 2018.)

Mitochondrial Dysfunction

Mitochondria are the considered "the powerhouse of the cell" and are absolutely vital to mental health. These organelles create ATP molecules from glucose molecules needed for maintenance of the sodium and potassium pumps that keep the electrical gradient that is responsible for neuronal depolarization. Mitochondrial dysfunction is responsible for low ATP stores that lead to lack of ATP, which leads to generation of reactive oxygen species, neuroinflammation, and eventual neurodegeneration via apoptosis.[106] Mitochondrial dysfunction is increasingly recognized for its association with neuropsychiatric abnormalities such as dementia, major depression, and bipolar disorder.[107] ATP production is greatly decreased, and mitochondrial DNA deletions are increased in major depression.[108,109] Mitochondrial dysfunction may be most salient when considering natural treatments for bipolar disorder,[110] which seems more correlated with mitochondrial dysfunction than the other affective disorders.

Mitochondria are damaged by poor sleep, insufficient or excessive exercise, excess stress, toxins (heavy metals, solvents, herbicides, and mold), drugs, chronic hyperglycemia, dysinsulinemia, inflammation, and poor nutrition. In a way, this damage encompasses all we discuss in this chapter.

Work on sleep, exercise, and stress balance and removing toxic load is needed for mitochondrial healing. Supplemental strategies include consumption of B vitamins to help Krebs cycle function and fish oil to support mitochondrial dynamics.[111] Foods and supplements that support Phase I and II liver detoxification can help when there's a strong toxicity picture. In addition, direct mitochondrial supports may include carnitine, CoQ10, lipoic acid, glutathione, and nicotinamide. I often prescribe *N*-acetyl cysteine (NAC), because it encourages oxidative phosphorylation, mitochondrial membrane integrity, and homeostasis of the mitochondrial milieu. Selenium deficiency and poor antioxidant status will contribute to mitochondrial dysfunction and should be considered.[112] These supplements are only a partial list, because there are many other evidenced-based supplements I might use, depending on the particulars of a patient's case. I will also recommend hypericum for mitochondria-related depression to help improve mood and promote neuronal mitochondria regeneration.[113]

THERAPEUTIC CONSIDERATIONS

Nowhere more than in the field of mental health is it paramount that the health care practitioner understand the wide breadth and range of factors that can result in an affective disorder. This chapter touches on topics covered throughout the rest of this book. The keen mental health practitioner knows mental health challenges can be symptoms of both psychological and physiological imbalance and should view mood symptoms as an opportunity and a necessity to check in on the multiple body systems and physiology along with a patient's psychological concerns. This section will review those major factors to help best treat the patient.

Conventional Medications

With the number of filled prescriptions for affective disorders soaring, patients are often overmedicated, using pharmaceutical compounds that do not address the underlying causes, and in

many cases create a situation that can inhibit true healing. When used judiciously, however, pharmaceuticals may help patients avoid hurting themselves or someone else in severe and urgent care cases when other, more natural recommendations are not practical.

Meta-analysis has shown that for depression, drugs do not work any better than placebo in mild to moderate cases,[114] the primary reason for millions of prescriptions. Even more startling, these drugs carry with them an increased likelihood of many other problems, with side effects that impair quality of life. For example, antidepressants show a 32% increased risk for all-cause mortality, including a 45% increased risk of stroke[115] in postmenopausal women. A comprehensive review of all the available (published and unpublished) controlled clinical trials of antidepressants in children and adolescents led the U.S. Food and Drug Administration (FDA) to issue a public warning in October 2004 about an increased risk of suicidal thoughts or attempts in children and adolescents treated with SSRI antidepressant medications.

Drugs for anxiety clearly do help relieve symptoms but at a cost. Efficacy of antianxiety medications such as benzodiazepines is high in the short term, but these medications, like their depression counterparts, are fraught with side effects. According to a report in the 2010 *Canadian Journal of Psychiatry,* people who use antianxiety medication have a 36% increased mortality risk.[116] In addition, the long-term efficacy of antianxiety medication has not been shown.[117] Addiction to antianxiety medications is also another problem, as drug-induced changes in brain function will lead to the need for progressively increasing dosages, withdrawal, and increased treatment disability.[118] To sum up, antianxiety medications can help a person feel better in the short term but pose the clear dangers of addiction, withdrawal problems, and increased risk of death.

Medications for bipolar disorder can be lifesaving and allow some patients with this condition who would otherwise be bound to homes or institutions in years past to lead normal lives. These medications unfortunately are also fraught with side effects, including extrapyramidal effects, changes in brain volume, hyperlipidemias, thyroid dysfunction, and kidney burden; via toxicity, they undermine mitochondrial health, which is a key to long-term neurological and emotional well-being (as with mitochondrial function discussed in this chapter). Despite the steady state of psychotic prevalence, antipsychotic medicine use has tripled from 1995 through 2008, with 50% of these medications provided for dubious diagnoses and "off label" use.[119] Especially alarming is the increased use of these medications in children and the elderly.[120]

Given this information, the clinician needs to be prudent and evaluate each patient to help decide the necessity for medication. If there is possible harm to self or others, or if patients cannot function well enough to take care of themselves or their dependents, then medication may be appropriate to help stabilize mood symptoms. Use of such medications during pregnancy and while breastfeeding should be decided on a case-by-case basis. See Fig. 142.3.[121]

Stabilizing mood is a key in naturopathic and integrative care to provide the patient the opportunity to work on the underlying causes such as lifestyle, toxic load, diet, and supplement needs. When a patient presents with affective disorder, the first order of business is safety. Although natural care is powerful and effective, sometimes the dedicated change in lifestyle needed to heal is not possible due to the mental health symptoms. When a person is incapacitated due to mental illness, these changes may not be possible. In this case a drug may be the best lifesaving option if it can create a fast "switch" that affords the patient the ability to start to take care of herself or himself. Once a patient is feeling better with medication, he or she can start working on the underlying factors to heal. As this process takes place, at the right time the practitioner can then slowly help wean the patient off medications. If the patient is not in an urgent care situation and is willing to work with natural care, then clinicians would be prudent to avoid medications entirely.

CHOOSING NATURAL OR CONVENTIONAL THERAPIES

If risk of hurting self or others → use conventional therapies first and natural as an adjunct

If patient cannot take care of self or family → use conventional therapies first and natural as an adjunct

If the above criteria is not met and patient is willing → use natural therapies first

Pregnancy and breast feeding → needs case-by-case evaluation

If patient is on medications → start by working with natural therapies and begin to slowly taper medications after natural treatments begin to take an effect

Fig. 142.3 Choosing Natural and/or Conventional Medicines.

Counseling

A main cause of both stress and disordered sleep is amygdala overactivation. As the fear center of the brain, the amygdala expresses high levels of CRH receptors in chronically stressed individuals, making it more reactive in times of stress. Stress-induced changes in the amygdala are a likely critical step in the pathophysiology of the development of chronic anxiety states. As noted elsewhere in this chapter, chronic stress also encourages maladaptive responses that result in affective disorders.[122]

Cognitive Behavioral Therapy

Therapeutic counseling is of enormous value to help process stress properly. Although many counseling techniques are useful for affective disorders, the one with the best support in the medical literature is CBT. CBT is shown to be especially effective and enduring for depression and anxiety[123] and may be of value for bipolar disorder.[124] Oftentimes, in conventional care, patients taking drugs for mood disorder tend to stay on them for the rest of their lives. That tendency is proven not to be the case with CBT, because the patient is taught new skills with which to deal with depression, and these skills are shown to be at least as enduring in effect as keeping patients on medication.[125] CBT works to help a patient recognize his or her negative automatic thoughts and change these to more empowering, positive thoughts and beliefs.

Mind Body Work

Mindfulness Based Cognitive Therapy (MBCT) is a form of cognitive therapy combined with mindful meditation. Using MBCT, bipolar disorder patients saw improvements in cognitive functioning and emotional regulation, as well as reduction in symptoms of anxiety,

depression, and manic episodes.[126] Studies dating back to the early 1900s have shown that meditation training programs can effectively reduce symptoms of anxiety and panic and help maintain these reductions in patients with generalized anxiety disorder, panic disorder, or panic disorder with agoraphobia.[127]

Yoga and Meditation

Yoga and meditation can both help lower stress hormones and return the body to parasympathetic mode. Numerous studies point to yoga's ability to modulate HPA axis function to effectively reduce depression, anxiety, and stress.[128] Yoga's ability to lower cortisol, modulate hormones, and regulate the activity of both the parasympathetic and sympathetic nervous systems may underlie some of the benefits it offers for both anxiety and stress. Physiologically, yoga has been shown to lower cortisol and decrease inflammatory markers such as C-reactive protein (CRP).

Massage Therapy

Massage therapy can also balance HPA axis dysregulation while helping balance neurotransmitters. A review of 37 randomized, controlled trials on massage found the benefits of massage even rivaled psychotherapy for depression and state anxiety (a temporary anxious change spurred by an outside situation).[129]

Diet

Healthy eating leads to better mental health. Increasingly, research is showing that wholesome, whole food intake helps prevent mood disorders and can help treat them too. Nutrient intake allows for the fundamental building blocks of neurotransmitters, membranes, and neuronal cells. Our food will help maintain neuroplasticity via influence on trophic factors, by creating inflammation balance, and by maintaining a healthful microbiome.

A landmark 5-year study in Spain looked at the lives and eating patterns of 10,000 people. Those people who followed a Mediterranean diet (MD) were 50% less likely to develop anxiety or depression. The study specifically found that intake of fruits, nuts, beans, and olive oil supported mood best.[130] Other studies regarding the MD have also shown that the endothelial linings of the subjects were much healthier, and rates of cardiovascular disease were lower with the MD. Furthermore, additional studies by the same group in Spain found that those people who ate in this healthy way also had higher levels of BDNF. BDNF has been shown to be low in individuals with depression[131] and anxiety.[132] The components of a Mediterranean Diet are listed in Table 142.1.

Mounting research also points to the beneficial effects of a healthy diet on neurotransmitter activity, the inflammasome, oxidative stress, mitochondrial dysfunction, and neurological pathology in patients with bipolar disorder.

Fermented foods contain probiotics, which cultivate many beneficial microorganisms and probiotic strains for gut and brain health. Many of these species and strains are not found in supplementation. A whole foods diet is also replete with nondigestible fiber that is prebiotic, or food for gut microorganisms. The practitioner can tell a patient that even short-term healthy fiber-full changes in diet can improve mood by influencing changes in microorganism species.[134]

Oftentimes, patients and practitioners alike will ask my opinion on the best diet. Although I think these questions are best answered by understanding each patient case and possible food sensitivities, if I do not know the individual or his or her history, I would recommend the Mediterranean diet. Although no one diet is completely perfect for every individual due to possible allergies and sensitivities, there is reason to believe the Mediterranean diet may far surpass the benefits of other choices for people with mood disorders.

TABLE 142.1 Components of a Mediterranean Diet[133]

1. High amounts of mono-unsaturated fats and low amounts of saturated fats
2. High intake of legumes
3. High fish intake
4. High intake of vegetables
5. High intake of fruits and nuts
6. High intake of whole grain cereals and breads
7. Moderate alcohol intake
8. Moderate intake of milk and dairy products
9. Low intake of meat and meat products

Insufficient Protein Intake

Protein breaks down into amino acids, which are used to make neurotransmitters. Inadequate protein intake will contribute to compromised neurotransmitter production. For example, tryptophan depletion studies have shown evidence of a clear decrease in available brain tryptophan as well as low levels of serotonin.[135] Repletion of amino acids to a diet low in protein has been shown in animal studies to correct learning disabilities and cognitive dysfunction.[136] Interestingly, low tryptophan intake, relative to other amino acids, may also contribute to low brain serotonin when other large neutral amino acids (phenylalanine, tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine, and histidine) in the diet compete with tryptophan for transport across the blood–brain barrier. This is why therapeutic doses of amino acids are best taken away from other amino acids and protein sources.

Food Allergies/Sensitivities

Beyond eating healthy, specific food components can provoke the immune system toward proinflammatory pathways that will promote poor mood (see the section on Inflammation). Depression and fatigue have been linked to food allergies for more than 90 years. In 1930 Rowe coined the term "allergic toxemia" to describe a syndrome that included the symptoms of depression, fatigue, muscle and joint aches, drowsiness, difficulty in concentration, and nervousness.[137] Although the term is no longer used, today we know both adults and children dealing with food allergy will show impaired quality of life, a higher level of stress and anxiety, and increased incidence of many diseases.[138] For example, both celiac disease and gluten sensitivities may present with a wide variety of neurological and psychiatric comorbidities.[139] Patients with bipolar disorder show an increase of blood antigliadin deamidated antibodies (IgGs)[140] as well as antibodies to bovine milk caseins.[141] Although beyond the scope of this chapter, helping patients identify and treat food allergies and sensitivities will be of value when helping gut function and balancing immunological activity in most effectively treating affective disorders.

Probiotics

In a number of studies using various animal models, supplemental probiotics have been shown to reverse the effects of stress. Although more studies are welcome, currently a few human clinical trials suggest that supplementation may promote stress resilience or

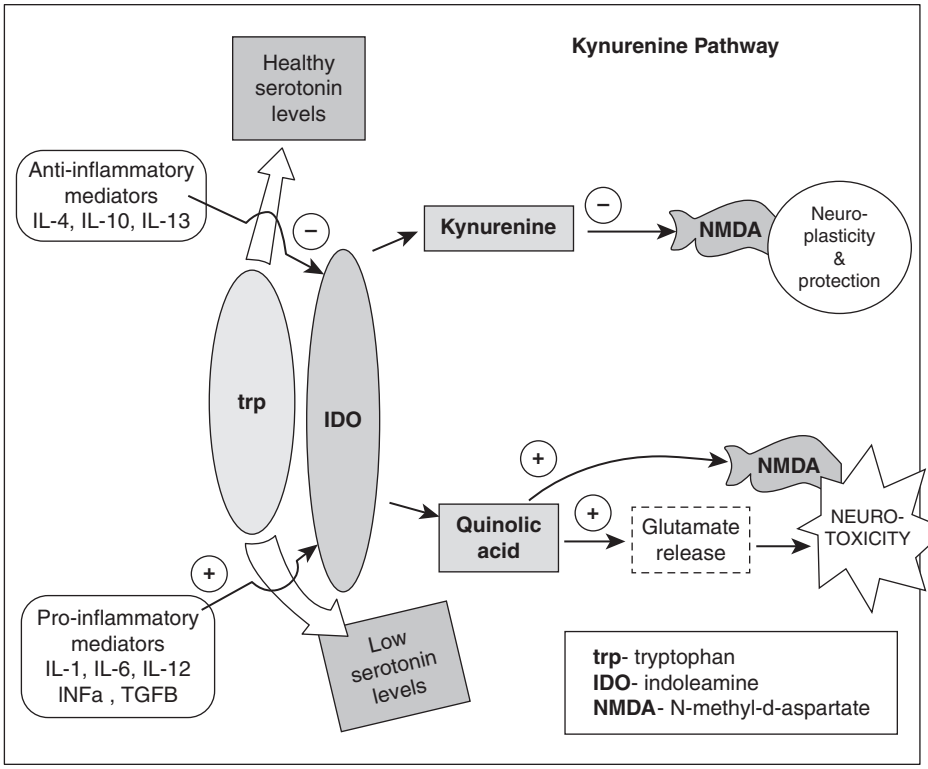


Fig. 142.4 The Inflammation and the Kynurenine Pathway.

reduce stress-induced physical symptoms and cognitive deficits in humans. Clinical evaluations of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 supplementation in rats and a double-blind, placebo-controlled, randomized human trial suggested lowered anxiety and alleviation of psychological distress in subjects after 30 days.[150] In what is probably the first study to look at probiotic supplementation effects on sad mood, a Leiden University evaluation studied 20 patients in a triple-blind study for 4 weeks. These patients were given *B. bifidum, B. lactis, L. acidophilus, L. brevis, L. casei, L. salivarius,* and *L. lactis.* In comparison with controls, the intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts.[151] A meta-analysis of probiotic supplements for mood reviewed five randomized, controlled trials, with a total of about 280 patients during a 4- to 8-week duration. This analysis revealed that probiotics significantly decreased depression scale scores in depressed subjects. Interestingly, there was no effect in patients older than 65 years,[152] which may suggest there are more formidable digestive concerns that need to be addressed among the senior population.

Can probiotic supplements help bipolarity? A Johns Hopkins University School of Medicine study looked at 66 patients hospitalized for mania where patients were randomized after discharge to receive 24 weeks of adjunctive probiotics *L. rhamnosus* strain GG and *B. animalis*. This study found 24 rehospitalizations in the 33 individuals who received placebo and only 8 rehospitalizations in the 33 individuals who received the probiotics. Probiotic treatment also resulted in fewer days actually rehospitalized versus placebo.[153] Even more interesting and as a nice segue to the next section, this study showed us that the positive effects of the probiotic treatment on the prevention of rehospitalization was greater in individuals with elevated levels of systemic inflammation at baseline. This finding suggests to us that the probiotics may have a beneficial effect on lowering inflammation, a known factor in mood disorder.

Amino Acid Therapy

Amino acids are used by the body to synthesize neurotransmitters. Typically taken as supplements, the following amino acids are the most typical ones used to support mood.

Tryptophan and 5-Hydroxytryptophan

Tryptophan is the precursor to hydroxytryptophan (5-HTP), which converts to serotonin, which ultimately makes melatonin. Theoretically, individuals with mood disorders due to low serotonin may benefit by taking supplementation of these serotonin precursors. Despite the widespread use and anecdotal reports, relatively very few studies explored use of tryptophan and 5-HTP for mood disorders. A study of 25 healthy young volunteers showed positive affect scores after subjects consumed a high-tryptophan diet versus a low-tryptophan diet, including fewer depressive symptoms and decreased anxiety.[154] Some research suggests more benefit from tryptophan supplementation in patients with low expression of the 5-hydroxytryptamine transporter-linked promoter region (5-HTTLPR).[155]

5-HTP is the downstream metabolite of tryptophan. Although some published pilot research of small subject groups taking 5-HTP has been completed, clearly more study is needed. For patients with high levels of inflammation, 5-HTP may be a more suitable choice because tryptophan may convert more easily to quinolinic acid via the kynurenine pathway in these patients (see Fig. 142.4). Patients with bipolar disorder tend to shift to the hydroxykynurenine arm of the kynurenine pathway and probably should avoid tryptophan supplementation.[156] In addition, 5-HTP may also be more able to cross the blood–brain barrier; 5-HTP may be contraindicated in depressed patients with already low catecholamine levels and/or as a long-term monotherapy as 5-HTP is recognized to deplete catecholamines, which can participate in depression.[157,158]

One meta-analysis looking at all the depression studies evaluating tryptophan and 5-HTP found that few were well done. Out of 108

studies, only two studies with a total of 64 patients met sufficient quality criteria to be included. However, these studies did suggest 5-HTP and L-tryptophan are better than placebo at alleviating depression.[159]

Two small studies show tryptophan supplementation to be possibly as successful in the treatment of seasonal affective disorder as light therapy. The first study of 16 patients used 3 grams per day for 2 weeks as augmentation after light therapy alone was not sufficient to create a helpful effect. The second 13-patient study used light therapy or tryptophan and showed greater benefits when the supplement was used.[160] There is promising research using tryptophan and 5-HTP, but given the widespread use already, more high-quality research on a larger number of individuals is very welcome.[159]

Phenylalanine and Tyrosine

As tryptophan and 5-hydroxytryptophan are precursors for serotonin, L-phenylalanine and tyrosine are the precursors for neurotransmitters dopamine (DA) and norepinephrine (NE).[162] Phenylalanine is a precursor of brain phenylethylamine (PEA), an amino acid derivative that promotes overall energy and elevation of mood.[163] Phenylalanine also converts to tyrosine, which is in turn converted to dopamine, and subsequently norepinephrine and epinephrine, and is known to mildly stimulate the nervous system, which may have benefit in depression and attention issues.

PEA ← Phenylalanine → tyrosine→ dopamine → norepinephrine → epinephrine

Studies of both phenylalanine-and tyrosine-depleted patients paint a picture of them as less content and more apathetic.[164] However, few clinical studies have examined the effects of supplementing either of these two amino acids for depression. In 1990 a randomized, prospective, double-blind comparison of 65 outpatients with major depression were given oral L-tyrosine or imipramine. Although both treatments trended toward some improvement, imipramine showed greater improvement, with little difference between placebo and tyrosine. The only effect noted to achieve statistical significance was greater dry mouth with imipramine.[165] Researchers in 1978 administered DL-phenylalanine in doses from 75 to 200 mg per day for 20 depressed patients for 20 days. In this trial, 12 patients were able to be discharged without any further treatment due to positive response. Another 4 patients had mildly positive response, and two did not respond. L-phenylalinine is known to raise dopamine. The L version acts more as an analgesic, so if the patient is also suffering from fibromyalgia or other chronic pain, the D-L combination might be most suitable. Neither tyrosine nor phenylamine amino acids are suggested for patients with anxiety or bipolarity. One work suggests increased susceptibility to tardive dyskinesia in neuroleptic-prescribed patients with high blood levels of phenylalanine, so supplementing may be contraindicated in these patients until more is known (Table 142.2).[166]

Acetyl-L-Carnitine

Carnitine is an amino acid that has been shown to help mood, fatigue, and depression in patients with cancer and encephalopathies.[167,168] Acetyl-L-carnitine (ALC) is similar in structure to the neurotransmitter acetylcholine and acts as an acetylating agent and cholinergic neurotransmitter. ALC has also been shown to have epigenetic effects on receptor production in the hippocampus and prefrontal cortex to contribute to a rapid antidepressant effect.[169] MRI studies in geriatric depressed patients found imbalances in the prefrontal cortex were resolved using doses of ALC.[170] As the word "carnitine" comes from the Latin word "carne," which represents meat, it is not surprising that the highest concentrations of this amino acid comes in red meats. Although intraperitoneal administration of carnitine in rats has shown antianxiety benefits, there are no trials showing anxiety benefit in humans.[171]

Glycine

The simplest of the amino acids, glycine is a calming amino acid known to reduce neuronal excitement, optimize GABA levels, and bind to the locus coeruleus to decrease the release of norepinephrine. Two double-blind, placebo-controlled studies in 14 and 16 healthy patients exposed to loud sounds found that high doses of glycine (0.8 g/kg of body weight) calmed the brain's cortex and decreased reaction to the sound by lessening auditory-evoked potentials.[172]

Gamma Amino-Butyric Acid and Phenibut

Gamma amino-butyric acid (GABA) is a natural calming brain neurotransmitter that may act like a natural benzodiazepine. People who suffer with anxiety, insomnia, epilepsy, and other brain disorders often do not manufacture sufficient levels of GABA. Supplemental GABA creates a hypnotic effect by opening chloride channels to allow cell hyperpolarization, thereby inactivating the nerve cell to slow its firing. Also, evidence suggests decreased brain beta waves.[173] Contradicting studies question the ability of this supplement to cross the blood–brain barrier.[174] Very little research has been conducted to evaluate benefit for anxiety. A study of 40 patients with insomnia given 300 mg showed improved sleep latency and efficacy with preservation of slow-wave sleep and REM sleep, which is something sedative hypnotic drugs can impair. Mild abdominal discomfort (2 people), headache (1 person), and drowsiness (1 person) were reported.[175] Given the ability to calm the brain without drowsiness, GABA may be a good choice for anxiety but should be avoided in depression without anxious features. Phenibut is a form of GABA known as beta-phenyl-gamma-amino-butyric acid which acts on γ-aminobutyric acid (GABA) B and A, as well as D β-phenethylamine receptors, to create both anxiolytic and nootropic effects. Used as a prescription drug in Russia, it is available as a supplement in Western countries. Like its GABA counterpart, this supplement has had little in terms of evidenced-based research. I have seen clear anxiolytic properties as a monotherapy. However, for those committed to use and to prescribing only natural products, Phenibut is of concern.

No known toxicity is associated with GABA when used in recommended dosages. Phenibut has had limited research. Some case reports suggest that dependence, tolerance, and withdrawal symptoms are possible,[176] and it is my recommendation to use sparingly, monitor for these concerns, and ensure causes are addressed first.

Taurine

The amino acid–derivative taurine acts by supporting levels of glycine and GABA to help relax the brain and nervous system while keeping levels of toxic glutamate low. It has been shown to block the effects of excess acetylcholine that contributes to bipolar disorder.[177] Taurine is usually dosed about 500 mg up to three times daily.

N-Acetyl-Cysteine

N-Acetyl-cysteine (NAC) is a precursor of the master antioxidant glutathione, a needed molecule for the increase oxidative stress found in bipolar disorder. NAC has been shown to help reverse glutathione depletion, increase both CNS and peripheral glutathione levels, as well as upregulate production of neuronal stem cells. NAC can also promote mitochondrial integrity and the oxidative phosphorylation needed to produce ATP.

A study of 75 patients with bipolar depression in a double-blind study had patients take 1g of NAC twice a day along with standard medications for 20 weeks compared with medication only. The patients taking NAC showed significant improvements based on the Montgomery-Åsberg Depression Rating Scale. Discontinuation of

TABLE 142.2 Studies Using Both Natural Medicines and Antidepressants in Combination

Supplement	Drug	Condition	Study	Outcome	Side effects	Ref
5-HTP—300 mg/day	Clomipramine	Depression	Study of 26 patients	• 5-HTP plus drug patients enjoyed better mood, lower anxiety, and less uncomfortable physical symptoms	No signs of serotonin syndrome or other serious adverse events	
Curcumin (500–1000 mg/day)	Various antidepressants	Depression	12-week, double-blind, placebo-controlled trial, 65 participants	• Much better placebo in improving MADRS scores by 12 and 16 week • Better effect in men than women	None	274
Eicosapentaenoic acid (EPA) 2 g/day	Various antidepressants	Depression	20-patient, 4-week, parallel-group, double-blind study	• Reduction in HAM-D score in patients receiving E-EPA was 12.4 versus 1.6 in patients receiving placebo	None	275
Folic acid 200 mcg / day	Lithium carbonate	Bipolar	Folic acid or matched placebo in a group of 75 patients on lithium therapy	• Highest plasma folate concentrations showed a significant reduction in their affective morbidity	None	276
Folic acid or 5′-methyltetrahydrofolic acid 500 mcg/day	Various antidepressants	Trazodone	Cochrane review of 3 randomized trials of 247 patients	• Two studies showed improved HAM-D scores with folate • One study showed no benefit		277
Inositol	SSRIs	Treatment-resistant depression	Double-blind controlled 4-week trial of 27 patients SSRI plus placebo or SSRI plus inositol	• No benefit with inositol		278
Melatonin 5–10 mg	Various antidepressants	Depression	9 patients with treatment-resistant depression	• 36% decrease insomnia • Half of patients with 50% improvement—significantly lower levels of fatigue	None noted	279
S-adenosyl-methionine (SAMe) 200 mg/day intramuscularly	Imipramine	Depression	63 patients in an 8-week, double-blind study	• Decreased depressive symptoms versus imipramine monotherapy, helped faster onset of drug benefit	None noted	280
Saffron 30 mg/d	Fluoxetine	Depression and sexual side effects from medications	36 men, 4-week randomized double-blind placebo-controlled study	• Significantly greater improvement in erectile function and intercourse satisfaction • No change in orgasmic function, satisfaction, or desire	Same as placebo	281
Saffron 30 mg /d	Fluoxetine	Depression and sexual side effects from medications	38 women, 4-week randomized double-blind placebo-controlled study	• Improved sexual function, arousal, lubrication • No benefit in desire, satisfaction, or orgasm	Same as placebo	282
Tryptophan 2 g daily	Prozac	Depression	One 8-week randomized controlled trial of thirty patients with major depression	• Statistically significant decrease in HAM-D scores • Protective effect on slow-wave sleep	Mild daytime drowsiness reported in the tryptophan group	283
Zinc (25 mg/day)	SSRIs	Tricyclics in depressed patients	12-week, placebo-controlled, double-blind pilot study of versus placebo	• 40% reduction in HAM-D and BDI scores versus placebo treatment	None	284
Zinc (25 mg/day)	SSRIs	Depression	44 patients	• Mean score of Beck Depression Inventory reduced significantly compared with the placebo by end of 6th and end of 12th week	None reported	285

5-HTP, 5-hydroxytryptophan; *BDI, Beck's Depression Inventory*; *E-EPA*, ethyl eicosapentaenoic acid; *HAM-D,* Hamilton Depression Rating Scale; *MADRS,* Montgomery-Åsberg Depression Rating Scale; *SSRIs,* selective serotonin reuptake inhibitors.

the supplement at 24 weeks led to "a dramatic return" of symptoms.[178] A second trial of 149 gave patients already prescribed mood stabilizers 2 g per day NAC as adjunct maintenance treatment with a substantial decrease in symptoms during the 8-week open-label phase.[179]

S-Adenosyl-l-methionine

S-Adenosyl-l-methionine (SAMe) is a naturally occurring molecule derived from the amino acid methionine and adenine triphosphate (ATP). Described as early as 1952, it has a decades-long history of use for depression, especially in Europe. Even the American Psychiatric Association has acknowledged it "might be considered" as an alternative to pharmaceutical medications.[180] Known best as an antidepressant, SAMe serves in many biological reactions by transferring methyl groups to DNA, proteins, fats, and amino acid compounds and supporting production of the monoamine neurotransmitters dopamine, serotonin, and norepinephrine.[181] Via the function of the methionine pathway, deficiency of B vitamins can contribute to homocysteinemia, with subsequent decreases in SAMe. Hyperhomocysteinemia also leads to increased glutamate, a brain toxin linked to mood disorder.

SAMe has been found to be safe and effective in the treatment of mild and moderate depression. According to some accounts, SAMe has a faster onset of action than conventional antidepressants.[182] The most recent meta-analysis suggests promising effects, but evidence is still somewhat limited.[183,184] Anxiety studies have not been conducted, and a few case studies suggest SAMe may exacerbate or precipitate symptoms of mania in bipolar disorder.[185] Its best use may be especially for depression accompanied by low B-vitamin status and hyperhomocysteinemia or known genetic defects in the methionine pathway.

Essential Fatty Acids

The brain neuronal membranes contain a high proportion of omega-3 polyunsaturated fatty acids (PUFAs). Communities who consume high levels through fish are known to have fewer instances of anxiety and depressive illness. Mechanistically, fish oil helps normalizes the membranes of brain tissues and simultaneously supports and calms the adrenal system during anxious and stressful times.[186] Essential fatty acids (EFAs), obtained through both fish oil as well as some vegetable sources in smaller amounts, provide an array of pleiotropic activity, including anti-inflammatory effects, nerve growth factor production, improved glucose metabolism, and calming of the anterior cingulate and prefrontal cortex (areas that are known to be overactive in people with both depression and anxiety).[187,188]

There is conflicting evidence regarding the effective use of omega supplements as a monotherapy for depression. This conflicting evidence may be explained by the presence or absence of essential fat deficiency,[189] intake levels of competing omega-6 fatty acids, and status of the fatty acid desaturase enzyme. (See Nutrigenomics and Single Genetic Nucleotide Polymorphisms section.) For depression, omega supplementation makes sense (unless the patient is allergic) as a nutritive and neuroendocrine support and may be a necessary part of addressing underlying factors. Higher dietary levels of essential fatty acids (EFAs) are inversely associated with anxiety.[190] A meta-analysis reviewing 19 high-quality clinical trials (both placebo and nonplacebo controlled) spanning 2240 participants from 11 countries found significantly reduced anxiety symptoms with treatment of omega-3 PUFA dosage of 2000 mg or more per day compared with controls.[191] In a study of 100 bipolar I patients, either 1000 mg of omega-3 supplement or placebo was given daily for 3 months. Significant improvements were seen in the group with mania.[192]

Vitamin D

Deficiency of the "neurosteroid" vitamin D causes mood changes in several ways. Hypothalamic brain centers are responsive to the presence of vitamin D, where low levels will affect the function of the HPA axis.[193] Vitamin D affects nerve growth factor,[194] which is important for brain and neuronal repair and growth and helps production of serotonin, testosterone, and thyroid hormone.[195] A cross-sectional study of anxiety patients also found significantly lower levels of calcidiol (a vitamin D precursor) in men and women with depression as well as in age-matched patients with anxiety disorders. To be expected, bipolar patients may benefit from proper levels of vitamin D as well. A Dutch study's evaluation of 118 patients with bipolar disorder and 202 patients with schizophrenia or schizoaffective disorder revealed vitamin D deficiency was 4.7 times more common among outpatients with bipolar disorder, schizophrenia, or schizoaffective disorder than among the general population.[196]

A stringent meta-analysis of 15 randomized controlled studies clearly determined statistically significant improvements in depression using vitamin D supplementation greater than 800 IU per day, with an effect size comparable to medications.[197] Although trials are ongoing on vitamin D for bipolar patients, a recent 8-week open trial of 2000 IU did result in improved mood symptoms. Imaging also showed increases in anterior cingulate cortex GABA levels, which may imply a calming effect, too.

Whenever prescribing vitamin D, be sure to consider the status of vitamins A and K_2.

B Vitamins and Folate

B vitamins play an important role in neurotransmitter production, mitochondrial function, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide reduced (NADH) metabolism, prostaglandin formation, and homocysteine regulation, all of which affect mood. An association between B-vitamin deficiencies (especially vitamins B_6, B_{12}, and folate) with higher prevalence of anxiety and depressive symptoms has been described in several epidemiological reports.[198] An evaluation of 3500 adults revealed higher intakes of B_6, B_{12}, and folate, whether through foods or supplementation, were associated with a decreased likelihood of incident depression for up to 12 years of follow-up. For each 10 additional milligram of vitamin B_6 and 10 additional micrograms of vitamin B_{12}, researchers found a 2% decreased risk of depressive symptoms per year.[199]

Vitamin B_3 (niacinamide) increases availability of tryptophan as well as production of 5-HTP. Vitamin B_6 (pyridoxine) is a main cofactor for enzymes that convert L-tryptophan to serotonin. In females with known evidence of serum vitamin B_6 deficiency due to oral contraceptive use, supplementation with 40 mg per day of vitamin B_6 improved both anxiety and depression.[200] Vitamin B_{12} is a key player in the synthesis of serotonin.

Besides being an important factor in serotonin synthesis, folic acid is also needed to support dopamine, norepinephrine, and epinephrine.[201] Generally low folate status seems to be more associated with low mood and depression and but not as much with anxiety.[202] Folic acid has been well implicated in depressive disorders, and folic acid deficiency has been noted among people with depression; some estimates suggest up to 33% of depressed individuals are folate-deficient. Several studies regarding its role in the pathophysiology of depression show that patients with depression often have a functional folate deficiency, and the severity of such deficiency correlates with depression severity.[203] More about methylfolate is discussed in the Nutrigenomics and Genetic Predispositions section.

Inositol

Inositol is an abundant carbohydrate in the body and brain and modulates serotonin receptors and neurotransmitter uptake.[204,205] Low levels of inositol has been reported in the cerebrospinal fluid of

patients with depression. A meta-analysis of seven randomized, controlled trials suggests modest benefit for depression and especial usefulness in patients with premenstrual dysphoric disorder.[206] In one double-blind, controlled, randomized crossover study of 20 subjects with panic attacks, researchers had each participant take 18 grams per day followed by 1 month of fluvoxamine. In the first month, inositol reduced the number of panic attacks per week to 4.0, compared with 2.4 fewer attacks with fluvoxamine.[207]

Triacetyluridine

Uridine is a precursor molecule for ribonucleic acid (RNA). Triacetyluridine (TAU) is a more bioavailable form of this nutrient. Found in high levels in beer, it can be a support for neuronal synthesis. In vitro work shows TAU can inhibit neurotoxic effects by improving mitochondrial recovery. Clinical studies of 18 g per day for 6 weeks showed decreased depressive symptoms in bipolar patients while increasing brain pH.[208] Supplement dosages range from 25 mg up to the study dosage of 18 g per day.

Minerals

Chromium

As increased sugar intake is correlated with depression, the essential trace mineral chromium may be a useful means to balance blood sugar and the brain. Chromium's mechanism of action seems to be via modification of brain serotonin levels[209] as well as by increasing insulin insensitivity.[210] In a 6-month, randomized, double-blind, placebo-controlled trial, subjects received 1000 mcg chromium per day, 600 mcg chromium per day, or placebo. Fasting glucose was significantly reduced in both chromium groups compared with the placebo group; similarly, numerically but not significantly, greater reductions in binge frequency, weight, and symptoms of depression were observed in those treated with chromium versus placebo, although statistical power was limited in this pilot trial. Other research suggests chromium's best mood effect may be in patients with blood sugar regulation (like diabetes) who have concomitant disturbances in dopamine and serotonin function.[211]

Magnesium

Magnesium is a mineral cofactor involved in hundreds of biochemical reactions. Low magnesium intake occurs in almost 70% of the U.S. population.[212] Magnesium deficiency may be a key player in the pathophysiology of affective disorders, as the limbic system and HPA axis function are both sensitive to magnesium status. Lowered magnesium will allow easier damage to nervous tissue, increasing inflammation, as evidenced by increased levels of C- reactive protein.

Iron

Important for myelin-sheath formation, neurotransmitter creation, and energy metabolism, iron is a mineral needed for best mood. Iron deficiency anemia is associated with both depressive mood, anxiety,[213] and gastrointestinal symptoms such as bloating and dyspepsia.[214] Decreased dopamine levels will encourage low iron status, although norepinephrine and glutamate levels tend to increase available iron. Interestingly, levels of GABA are elevated in iron deficiencies, as an overall reduction in neurotransmission rates and brain activity occurs due to insufficient energy.[215]

Excess iron can also be problematic: neuronal damage can be caused by iron overload. Iron levels even in high normal ranges can increase depressive symptoms in young male adults, but not in females,[216] and hemochromatosis has been implicated in treatment-resistant bipolar disorder.[217]

Finally, in this world of heavy contamination of the environment with neurotoxins, the importance of iron for the heme backbone of the cytochromes for their detoxification is critical.

Zinc

Zinc is a mineral that supports hippocampal BDNF production[218] and is a pleiotropic cofactor in multiple reactions involved in energy metabolism and digestive health. Zinc is predominately found in glutamatergic neurons, typically acting as an inhibitory factor at the N-methyl-D-aspartate (NMDA) glutamate receptor.[219] Low zinc status may cause lower levels of GABA, increasing anxiety symptoms. Zinc levels are decreased by inflammation and stress.[220]

Meta-analyses demonstrate clear inverse association between depression severity and serum zinc levels.[221] Zinc supplementation alleviates anger and depression.[222] Patients with anxiety also reveal significantly higher plasma levels of copper and copper-to-zinc ratios as well as lowered absolute zinc levels compared with controls. In treated patients, zinc picolinate daily for 8 weeks restored that balance and improves symptoms.[223] Optimal zinc dosage is 15 to 30 mg per day. It should be taken with food due to the possibility of gastric upset. Top foods sources of zinc include beef, lamb, and turkey.

Lithium

Lithium is a mood-stabilizing mineral with antidepressant, antimanic, and oxytocin-supporting properties. Geographical areas with higher natural lithium concentrations in the drinking water are associated with lower mortality rates from suicide.[224] Most well known in the carbonate form, which is prescribed for patients with bipolar disorder, although now newer drugs are available.

The typical supplement form of lithium is lithium orotate, which is a salt of lithium and orotic acid. Lithium orotate may be the preferred form, because the orotate ion is known to traverse the blood–brain barrier more easily than the drug version, which uses the carbonate ion of lithium. As a result, the orotate form can be used in much lower doses with reasonable results and no side effects.[225] Lithium orotate has not been extensively studied. In one evaluation, alcoholic patients were prescribed 150 mg of lithium orotate (approximately 5 to 7 mg of elemental lithium a day) 4 to 5 times a week. Out of 42 patients, 10 of the patients had no alcoholic relapse for more than 3 and up to 10 years, and 13 patients remained without relapse for 1 to 3 years. The remaining 12 had relapses between 6 to 12 months.[226]

Lithium orotate is commonly dosed at 5 to 20 mg per day of elemental lithium with no toxicity. Its best use seems to be for anxiety and bipolar disorder with a strong manic component. At this time, it is not recommended for depression that does not have a strong anxiety component. There is a case study of lithium orotate overdose in an 18-year-old, with an estimated intake of more than 2000 mg of elemental lithium. This patient had normal vital signs but complained of nausea and vomiting, and she presented with a tremor, which resolved after 90 minutes of IV fluids.[227]

Botanical Medicines

Hypericum perforatum (St. John's Wort)

St. John's wort (SJW) is one of the most studied herbs of all time. The Latin name *Hypericum perforatum* means "above a ghost," and this botanical was originally gathered as a way to ward off evil spirits. Although often thought of as a "natural SSRI," SJW has pleiotropic effects all around the body and brain via multiple mechanisms. The overall benefits of this herb cannot be attributed to a single constituent.

SJW may affect neurotransmitters, acting as a mild monoamine oxidase inhibitor and having serotonin enhancement properties,[228,229] although some literature suggests this effect is too weak to have

therapeutic effect for depression.[230] *Hypericum* also inhibits acetylcholine breakdown,[231] balances norepinephrine and dopamine, and supports GABA production. Besides neurotransmitter actions, it has pleiotropic effects that may also encourages mitochondrial function and oligodendrocyte development.[232] It may have anti-inflammatory effects and nerve protective properties.[233]

As of this writing, the latest meta-analysis of SJW reviewed 27 clinical trials with a total of 3808 patients, which compared the use of St. John's wort with use of SSRI medications. Study durations varied from 4 to 12 weeks and showed St. John's wort has comparable efficacy and safety in comparison with SSRIs as well as a significantly lower patient discontinuation of the herb versus the drugs.[234]

Case reports link St. John's wort to mania, suggesting it may not be a good choice for bipolar disorder treatment.[235] Because hyperforin, one of the chief components of SJW, plays an important role in the induction of cytochrome P450 enzymes and P-glycoprotein transporter,[236] it can affect pharmacokinetics of various drugs and should be used cautiously with pharmaceuticals.

Curcumin

Inflammation is an underlying cause of mood disorder. Baseline levels of high-sensitivity CRP, TNF-α, and IL-6[237] are elevated in patients with major depression.[238] Curcumin, a plant polyphenol with anti-inflammatory, antioxidant, and neuroprotective properties, is considered the main active component of the spice turmeric.

Meta-analysis of six clinical trials showed significant clinical efficacy of curcumin in ameliorating depressive symptoms as well as significant antianxiety effects.[239] Putatively, curcumin may be of benefit for bipolarity due to its influence on known mechanisms of the disorder, including influence on proinflammatory cytokines, growth factors, inflammatory mediators, and antiapoptotic proteins.[240]

Cannabidiol Oil

Cannabidiol (CBD) oil is typically extracted from the hemp plant and is not psychoactive like the tetra hydro cannabinoid (THC) found in marijuana. With known anticonvulsive, anxiolytic, antipsychotic, and neuroprotective effects, this flavonoid does not directly bind with cannabinoid receptors, but it does inhibit degradation of the natural endocannabinoid anandamide.[241] It also affects the adenosine receptor function that controls wakefulness and the vanilloid receptors to regulate inflammation, and it may bind to serotonin receptors as well.[242]

CBD's best use might be for anxiety. CBD was first studied in patients taking marijuana, where it was shown to blunt the anxiety effects of THC.[243] In human trials, CBD has significantly reduced the social anxiety associated with a simulated public speaking tests in healthy subjects where it helped balance amygdala (fear center) and hippocampus (memory and emotion) areas of the limbic system. This effect was comparable to the anxiety medications ipsapirone and diazepam (Valium).[244] Given its mechanisms, it may be beneficial in bipolar disorder. As of this writing, a Brazilian clinical trial is recruiting to study CBD in bipolar patients.[245] More research is needed, but the available research is compelling, and I have seen very good clinical results with CBD.

Passiflora (Passionflower)

Passionflower has a long and rich folklore history of use as a calmative agent because it has anxiolytic properties and is considered an official plant medicine in many countries worldwide. Passionflower works for reducing anxiety in part by binding itself to benzodiazepine receptors in the brain. It has chrysin and other flavonoid-like compounds with confirmed antianxiety, anti-inflammatory activities.[246] A 2007 Cochrane review looked at two studies with a total of 198 participants, which found lack of difference in the efficacy between benzodiazepines and *passiflora*, improved job performance, and lower rate of drowsiness versus the drugs. Although the author concluded that there are too few studies to conclude efficacy for *passiflora*, it seems reasonable to try it given the long history of use for anxiety and minimal side effects.[247] I do not find *passiflora* to be as powerful an anxiolytic as a benzodiazepine might be, but with a full integrative natural medicine plan, it can help create a calming effect to avoid the need for more addictive pharmaceuticals.

Rhodiola rosea

Rhodiola was originally described in the Russian literature as a plant medicine useful to combat physical, biological, and chemical stressors. As an adaptogenic herb, studies show it acts as a neuroprotective, cardioprotective, antifatigue, antidepressive, anxiolytic, nootropic (cognitive enhancing) agent, with life-span–increasing effects and CNS-stimulating activity.[248] Rhodiola is typically standardized for 1% of the molecule rosavin. Longer-term studies of this adaptogen are needed, but what we know so far is that rhodiola is a great choice for patients who are depressed and/or anxious and feeling burnt out.

In an early study pitting an herb directly against an antidepressant, researchers compared 340 mg of a powdered extract of *R. rosea* standardized to a content of 3.7% rosavin, with sertraline HCl (Zoloft) or placebo for 12 weeks. Twenty patients were randomized to the *R. rosea* group, 19 to the sertraline group, and 18 to the placebo group. Interestingly, all treatments had similar findings: nonstatistical reductions in symptom assessments, with more patients suffering adverse reactions with sertraline (63.2%) in comparison with *R. rosea* (30.0%) or placebo (16.7%).[249] These results suggest sertraline had a slightly greater effect versus *R. rosea,* although the botanical medicine had significantly fewer side effects. Strikingly noted is that the benefits of neither the drug nor the herb differed much in comparison with placebo. Remember that meta-analysis has shown that in cases of mild to moderate depression, pharmaceuticals generally do not work any better than placebo,[250] which boasts a 25% to 30% effectiveness rate. This study is consistent with a greater body of research that shows minimal but measurable effects for botanicals in mild and moderate depression, and suggests that given the similar efficacy, ability to heal underlying causes, and lower side effects, these botanicals make a better choice.

Saffron

With serotonergic, anti-inflammatory, neuroendocrine, and neuroprotective properties, the traditional Persian medicine pharmacopeia has long understood the vibrant color and power of saffron. This spice is one of the world's most expensive and healthful. There are a number of clinical trials related to saffron for depression. In 2014 an Australian meta-analysis found large treatment effects, with similar effective rates compared with patient antidepressants.[251]

With a number of studies looking at saffron for depression, a 2018 meta-analysis reviewed 11 clinical studies for qualitative analysis, with 9 studies pooled for statistical analysis, and identified that saffron has a significant effect on the severity of depression.[252] Consistent with past analysis, it seems this herb has at least the same benefits as antidepressant drugs.

What interests many patients is that saffron has also been studied for helping alleviate the sexual side effects of antidepressant medications. Studies have separately looked at the effect on 36 men and 38 women. For the men, greater improvement was apparent in erectile dysfunction and intercourse satisfaction in 9 patients (60%) with saffron versus one patient (7%) in the placebo group who achieved normal erectile function.[253] The women enjoyed significant improvement in arousal, lubrication, and dyspareunia, although desire, satisfaction, and orgasm were not changed.[254] A separate meta-analysis has confirmed saffron's benefit on erectile dysfunction.[255]

Homeopathic Medicines

Studies evaluating homeopathic medicines for psychiatric illness are relatively scant but increasing. One systematic review of studies between 1982 and 2016 found two double-blind placebo-controlled trials looking at using homeopathics in depression. One study was of 91 patients, and another evaluated 133 subjects. Both suggested effects comparable to those of antidepressant medications.[256] A review looking at general practitioner offices found patients with affective disorders who chose to work with doctors prescribing homeopathy reported less use of psychotropic drugs, and they were marginally more likely to experience clinical improvement than patients managed with conventional care.[257] A larger review by the *Journal of Clinical Psychiatry* used very stringent criteria and accepted only 25 studies out of more than 1400 reviewed. Efficacy of homeopathic medicines was certified for fibromyalgia and chronic fatigue syndrome, but benefit was not seen for anxiety or stress. The authors concluded that "Studies in psychiatry are very limited, but results do not preclude the possibility of some benefit."[258] Please see Chapter 39 for more on homeopathy.

Nutrigenomics and Genetic Single Nucleotide Polymorphisms

Although the field of nutrigenomics is still in its infancy, it is progressing rapidly. It may be premature to make dogmatic recommendations regarding the presence of a particular genetic single nucleotide polymorphism (SNP). However, a few SNPs and gene associations are pertinent to affective disorders, and if it is possible to obtain this genetic information, these should be kept in mind and correlated with laboratory testing and clinical symptomology to create an overall holistic plan for the patient with mood disorder. Although this short list of some genetic polymorphisms with reasonable literature to suggest actionable items provides initial information about their properties, much more information is needed to fully use our patient's genetic information.

BDNF (Brain-Derived Neurotrophic Factor) rs6265

The Met-positive polymorphism may confer less ability to manufacture BDNF. Exercise, zinc, and lithium[259] supplementation may be of use to naturally support BDNF production.

COMT (Catechol O-Methyltransferase) rs769224, rs4680, and rs4633

SNPs of this gene can alter catecholamine degradation and may be key in function of the prefrontal cortex's input in personality, behavior, and emotion. Estrogen metabolism may also be altered as well. Knowing this predisposition, correlating with clinical symptoms may help clinicians choose recommendations designed to boost or lower neurotransmitters and modulate estrogens.

CYP2R1 rs10741657 and rs7041

This gene allows for production of the precursor to 25-hydroxy vitamin D_3, which in turn is converted to the more active $1,25(OH)_2$ vitamin D. Certain alleles may result in lower active vitamin D levels. Extra vitamin D may be of value for these patients as correlated with laboratory testing.

DBH (Dopamine Betahyrdoxylase) rs1108580

DBH manufactures the enzyme that breaks down dopamine. Research on personality traits has found that DBH overactivity is associated with impulsiveness traits, and low activity is associated with sensation-seeking.

DRD2 (Dopamine Receptor D2) rs1800497

This gene is responsible for structural components of the dopamine receptor. Gene polymorphisms may be associated with greatly increased risk of addiction and may suggest a poorer result with prescribed SSRIs.

FADS and FADS1 (Fatty Acid Delta-5-Desaturase) rs174537, rs174546, and rs174547

These code for a desaturase enzyme necessary for conversion of plant fats to essential fatty acids in the body. Essential fatty acids may be of special benefit for patients with this genetic SNP who have concomitant affective disorders and low levels of essential fatty acids.

GAD1 (Glutamic Acid Decarboxylase 1) rs2241165

This gene encodes for enzymes needed to convert L-glutamic acid into the calming neurotransmitter GABA. SNPs will contribute to higher levels of anxiety and extremely tight muscles and may contribute to the rare condition known as Stiff Person Syndrome. Patients with this genetic SNP may benefit from more exercise, which can upregulate GAD gene function to enhance GABA production.[260] Extra supplemental vitamin B_6, a cofactor for the glutamic acid decarboxylase enzyme, can help enhance GABA production. Giving supplemental GABA, along with GABA-supportive nutrients such as theanine,[261] lithium orotate, and/or calming botanicals like *passiflora,* may be of value in patients with anxiety who have this polymorphism.

GC (Vitamin D Binding Protein) rs2282679

This gene is responsible for structure of a transporter for vitamin D. Hetero- or homozygous positive genotypes will be at higher risk for lower vitamin D levels, which will predispose for affective disorders. Higher intakes of vitamin D may be required.

IL-6 (Interleukin-6) rs1800795

Meta-analyses indicated that IL-6 is the most consistently elevated cytokine in the blood of patients with major depression.[262] SNP alterations in this gene may predispose to higher levels of inflammation in the CNS and periphery. Recommendations to reduce inflammation may be of use.

MAOA and B (Monoamine Oxidase A and B) Multiple rs Numbers

This gene encodes mitochondrial enzymes that catalyze the oxidative deamination of amines, including dopamine, norepinephrine, and serotonin. Knowing this predisposition may help the practitioner correlate clinical symptoms to choose recommendations to boost or lower monoamines.

MTHFR (Methylene Tetrahydrofolate Reductase) rs1801133 and rs1801131

Polymorphisms predispose to reduced folate conversion to active forms. Patients with this SNP and high homocysteine suggest poor methylation abilities, which will affect neurotransmitter creation. Rs1801131 specifically affects neurotransmitter production by poor cycling of the tetrahydrobiopterin (BH_4) pathway. Concomitant COMT alleles may predispose to further neurotransmitter imbalances. Consider increasing intake of folate-containing foods (green leafy vegetables) and supplementation with methyl-folate, and avoiding synthetic folic acid.

MTR (Methionine Synthase) rs1805087 and MTRR (MTR Reductase) rs1801394

Presence of SNPs in either or both of these might increase requirements for B_{12}, as these support B_{12} recycling. Checking serum B_{12} and methylmalonic acid might be valuable to confirm clinical significance.

OXTR (Oxytocin Receptor) rs53576

Oxytocin was discussed earlier in this chapter. This gene is response for building the oxytocin receptor. The A allele typically predisposes individuals toward poorer stress-management skills, such as the inability to seek help, and these individuals tend to be lonelier.[263] Possibly using natural remedies known to raise oxytocin, such as hugging therapy, massage,[264] or supplemental lithium,[265] would be of value.

SLC39A13 (Solute Carrier Family 39 Member 13)

This gene encodes zinc transporter proteins and may be related to mood disorders.[266] Polymorphisms may suggest additional zinc would be beneficial for the person with affective disorder.

TPH 1 and 2 (Tryptophan Hydroxylase 1 and 2) rs1799913, rs4570625, and rs429027

This gene helps govern the conversion of tryptophan to 5-HTP, and the T allele may affect available serotonin and mood state as a result. Variants affect the circadian-regulating function of suprachiasmatic nuclei, which is a master controller of circadian rhythm.[267] Light therapy, tryptophan, 5-HTP, melatonin, and its cofactor (such as vitamin B_6) might be useful with this genetic SNP.

PRIMARY PREVENTION: THE PRENATAL ENVIRONMENT AND CHILDHOOD EXPERIENCE

Although beyond the scope of this chapter, clinicians need to be aware of the critical effect of the prenatal environment and childhood experiences as dominant factors in the overall tone of the HPA axis. Prenatal and childhood stress will greatly affect CRH and cortisol levels as adults.[268,269] From a holistic standpoint, it should also be noted that besides emotional stressors, dietary imbalances such as poor nourishment and low birth weight, maternal overeating, and prenatal high-fat diets will also contribute to HPA and mood disorder in the next generation. Along with proper nutrition, mind–body modalities in the prenatal and childhood period may hold the greatest promise in preventing affective disorders. Multimodal psychoeducation approaches, yoga, and meditation interventions have shown outcomes like higher birth weight, shorter labor, fewer instrument-assisted births, and reduced perceived stress and anxiety.[270]

PERTINENT LABORATORY TESTING

It will nearly impossible, due to cost and practicality, to order all possible testing. It is important to have an excellent intake with the patient to gather clinical evidence to support the need for looking down various paths with specific tests.

Blood Tests

Blood work can be used to assess multiple factors, including those related to red blood cell, nutrient, inflammation, hormonal and mitochondrial status.

- Blood Sugar: fasting glucose / Serum Insulin / HgbA1C
- Complete Blood Count
- Chemistry Panel
- Iron Panel: ferritin and serum iron
- Lipid Panel
- Homocysteine
- Histamine
- TSH, T3, T4
- DHEA/DHEA sulfate
- Pregnenolone
- Estrogens (E1, E2, E3)
- Progesterone (preferably day 22 of a premenopausal woman's menstrual cycle)
- Free and total testosterone, estrogen, and testosterone
- 25(OH) Vitamin D
- Zinc and Copper
- Vitamin B_{12} and folic acid
- Serum methylmalonic acid
- Serum glutathione
- Serum carnitine
- Serum lactic acid
- Serum CoQ10
- Celiac Panel
- Serum heavy metals: mercury, cadmium, arsenic, lead, aluminum

Food Sensitivity/Food Allergy Testing

Food testing may elucidate which foods are more likely to create a sensitivity or histaminic reaction in the body. See Chapter 14, Food Hypersensitivities, for discussion.

Saliva and Urine Testing

These tests are valuable to took further into HPA-axis dysfunction and can also further elaborate on hormonal status.

Organic Acid Testing

Organic acids look at urine samples to help suggest mold toxicity metabolites. See Chapter 29, Organic Acids Profiling, to learn about this test.

Leaky Gut Tests

Lactulose Mannitol Testing can help identify "gut permeability," which can play a role in altered gut microbiota brain and CNS inflammation.[271] Review Chapter 19, Intestinal Permeability.

Small Intestinal Bowel Overgrowth (SIBO) Test: May help create an effective treatment plan for Irritable Bowel Syndrome, which is highly correlated with anxiety and depression symptoms. See Chapter 9, Bacterial Overgrowth of the Small Intestine Breath Test, for more discussion.

Hair Analysis: Can help discern mineral status as well as heavy metal status. See Chapter 16, Hair Mineral Analysis, for discussion.

Urine Toxic Metal Testing: Both unprovoked and provoked tests are used to evaluate heavy metal status in a patient and need for detoxification techniques, covered in other chapters of this book. Please see Chapter 22, Metal Toxicity: Assessment of Exposure and Retention.

THERAPEUTIC APPROACH

General therapeutic approaches for affective disorders, whether depression, anxiety or bipolarity are as follows.

Diet

Helps address gastrointestinal dysfunction, dysglycemias, nutrient depletions, and inflammation. Focus on whole foods, fiber, and anti-inflammatory diet. Consider a Mediterranean diet, keeping in mind food allergies and sensitivities. Assuring proper

protein for neurotransmitter support, as well intake and small frequent meals, may be ideal to avoid fluctuations in blood sugar. Unsaturated fats (fish, olive oil) are encouraged for optimal fatty acid intake. For a healthy microbiome, incorporate fermented foods to help balance microbiota and fiber foods for prebiotic support.

Sleep

Assure 7 to 9 hours of quality sleep, preferably getting to bed by 10 p.m. Keep the bedroom dark and at an average of 68°F (20°C) or less.

Exercise

Assure a balanced exercise regimen. Five days a week of moderate to vigorous exercise for 30 minutes may be a starting point. For those less vital or motivated, a gentler program might be indicated. See Chapter 36 on The Exercise Prescription.

Lifestyle

Avoid all alcohol and avoid excess caffeine (avoid all caffeine if insomnia is present). Minimize screens and social media.

Psychological Work

Regular cognitive behavioral therapy to work on negative thoughts.

Relaxation Work

- Spending time in nature
- Working with yoga
- Participating in mindfulness-based cognitive therapy (MBCT)

Nature

Patients need to spend ample time outdoors, in green areas when possible.

Environmental Toxicity

Evaluate for toxin load and use detoxification techniques if patient history is suggestive.

Supplementation

Choose supplements most salient to a patient's circumstance and laboratory values.

- Hormone support: supplements, botanicals for supporting the physiology, or actual hormone: thyroid, estrogen, progesterone, melatonin, cortisol, testosterone DHEA
- Probiotics: *Lactobacillus* and *bifidus* spp.
- Essential fatty acids: 1000 to 2000 mg fish oil per day
- Vitamin D: 1000 IU to 5000 IU. Check labs to assure proper dosing. Assess and address status of vitamins A and K_2.
- Vitamin B_6 (pyridoxine 5′ phosphate form): 25 to 50 mg a day
- Vitamin B_{12} (methyl or Adenosylcobalamin form): 1000 up to 10,000 mcg a day
- Methyl folate: 500 mcg up to 5 mg a day
- Magnesium: 125 mg to 500 mg a day
- Iron: 25 mg to 150 mg a day in divided doses, as indicated by laboratory testing
- Zinc: 15 to 30 mg a day
- St. John's wort: 300 mg three times a day. Be mindful of interactions with conventional medications.
- Curcumin: 500 to 1000 mg/day
- Rhodiola: 340 mg up to 1340 mg a day
- Homeopathic medicines: per patient's individual presentation and constitution

SPECIFIC THERAPEUTIC CONSIDERATIONS ACCORDING TO AFFECTIVE DISORDER

Depression

Diet

Depressed patients without insomnia or anxiety may want to try one to two cups of coffee in the morning and early afternoon.

Supplements

- 5-HTP: 50 mg to 100 mg three times a day. May be best avoided in the long term as a monotherapy in patients with depression who are predisposed to be low in catecholamines are not taking catecholamine supports.
- Phenylalanine
- D phenylalanine: for depressed mood, 350 mg per day
- DL phenylalanine: for depression along with a pain picture: 100 to 500 mg per day
- Note: Those with phenylketonuria should not supplement with phenylalanine.
- Tyrosine: 500 to 1000 mg doses two or three times a day
- Acetyl L-carnitine: 1 to 3 grams a day
- Inositol: 12 to 18 grams a day
- Glycine: best avoided in depression
- SAMe: 200 mg twice a day up to 400 mg three times a day; ramp up slowly.

Anxiety

Diet

May be best to avoid all caffeine, except for small percentage of patients with low dopamine status

Supplements

- Glycine: for anxiety only, one to three teaspoons a day. Studies suggest up to 3 tablespoons can be beneficial without side effects.
- GABA: 500 mg three times a day
- Phenibut: 300 mg three times day, do not use more than 2 weeks at a time, with a week in between to avoid reliance. Be aware this is not a natural product, and at the time of this writing the FDA has sent warning letters to manufacturers.
- Lithium orotate: 5 to 20 mg a day
- CBD oil: 15 mg twice a day
- Passiflora: ⅓ tsp of extract in a little warm water three times a day

Bipolar Disorder

Diet

- Gluten-free and casein-free diet

Supplements to Use

- Taurine: 500 mg three times a day
- NAC: 1000 mg twice a day
- Triacetyluridine: 25 mg to 18 grams a day, for mitochondrial support and low symptoms

Supplements to Avoid

- Mucuna
- Creatine
- SAMe
- Tyrosine
- Kava
- Rhodiola
- Ginseng[272]
- D and L phenylalanine

Seasonal Affective Disorder

Light Therapy

10,000-lux full-spectrum white light for at least 30 minutes every morning. This is best initiated in the early fall and discontinued in early spring months.

A NOTE ABOUT INTEGRATIVE USE OF NATURAL MEDICINES AND CONVENTIONAL DRUGS

There are emerging studies looking at the adjunctive use of natural supplementation with pharmaceuticals. A recent Cochrane review suggests many supplements are effective and safe when used in combination with drugs for mood.[273]

It is highly probable that the underlying mechanisms of affective disorders are, in part, due to physiological imbalances and nutrient deficiencies. For example, we know that low serum folic acid and zinc increase the likelihood that antidepressants will not work. Using various supplements with medications, in many cases, may help treatments work best. Table 142.2 provides a selected list of studies using natural medicines along with medications:

Curcumin administration was safe and well tolerated even when combined with antidepressants. Curcumin was more efficacious than placebo in improving MADRS scores, with significant differences between curcumin and placebo emerging at weeks 12 and 16.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Borwin B. Epidemiology of anxiety disorders. *Dialogues Clin Neurosci*. 2015;17(3):327–335.
2. World Health Organization. Depression and Other Mental Health Disorders: Global Estimates; 2017. http://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. Accessed January 5, 2020.
3. National Institute of Mental Health. National Institutes of health. Mental health information. https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml. Accessed July 23, 2018.
4. CDC (Centers for Disease Control and Prevention). National Vital Statistics System, Mortality; 2018.
5. CDC. NCHS Data Brief No. 283; 2017.
6. CDC. NCHS Data Brief No. 241; 2016.
7. U.S. Department. Of health and human Services 2013 medical Expenditure Panel survey (MEPS). https://meps.ahrq.gov/mepsweb/. Accessed August 1, 2018.
8. National Institute of Mental Health. National Institutes of health. Mental health information. https://www.nimh.nih.gov/health/statistics/any-anxiety-disorder.shtml. Accessed July 23, 2018.
9. Iman K, Ansari SA. A study of depression, anxiety and stress among employed and unemployed people. *Natl Multidiscip Res Dev*. 2018;3(2):49–51.
10. Wedenfield J, Ovadia H. Chapter 8: role of the amygdala in regulating the hypothalamic pituitary adrenal Axis. In: *The Amygdala, where Emotions Shape Perceptions, Learning and Memory*. ; 2017. https://www.intechopen.com/books/the-amygdala-where-emotions-shape-perception-learning-and-memories/the-role-of-the-amygdala-in-regulating-the-hypothalamic-pituitary-adrenal-axis. Accessed June 5, 2018.
11. Wong ML, Loddick SA, Bongiorno PB, Gold PW, Rothwell NJ, Licinio J. Focal cerebral ischemia induces CRH mRNA in rat cerebral cortex and amygdala. *Neuroreport*. 1995;6:1785–1788.
12. van Praag HM. Can stress cause depression? *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28:891–907.
13. Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev*. 2005;4:141–194.
14. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338:171–179.
15. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338:171–179.
16. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol Behav*. 2001;73:261–271.
17. Carroll BJ, Curtis GC, Mendels J. Neuroendocrine regulation in depression, I: limbic system-adrenocortical dysfunction. *Arch Gen Psychiat*. 1976;33:1039–1044.
18. Daly MC, Wilson DJ. *Keeping Up with the Joneses and Staying Ahead of the Smiths Evidence from Suicide Data*. Federal Reserve Bank of San Francisco — Working Paper Series. May 2006. https://www.frbsf.org/economic-research/publications/working-papers/2006/wp06-12bk.pdf Accessed September 10, 2018.
19. Erika FH, Saunders MD, Julio Fernandez-Mendoza PD, et al. The effect of poor sleep quality on mood outcome differs between men and women: a longitudinal study of bipolar disorder. *J Affect Disord*. 2015;180:90–96.
20. Gold AK, Sylvia LG. The role of sleep in bipolar disorder. *Nat Sci Sleep*. 2016;8:207–214. https://doi.org/10.2147/NSS.S85754.
21. Garland CF, Gorham ED, Mohr SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol*. 2007;103(3–5):708–711.
22. Knippenberg S, Damoiseaux J, Bol Y, et al. Higher levels of reported sun exposure, and not vitamin D status, are associated with less depressive symptoms and fatigue in multiple sclerosis. *Acta Neurol Scand*. 2013. https://doi.org/10.1111/ane.12155. ([Epub ahead of print]).
23. Tsai JF, Hsiao S, Wang SY. Infrared irradiation has potential antidepressant effect. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31(7):1397–1400.
24. Li Q, Morimoto K, Nakadai A, et al. Forest bathing enhances human natural killer activity and expression of anti-cancer proteins. *Int J Immunopathol Pharmacol*. 2007;20(2 Suppl 2):3–8.
25. Ulrich RS. View through a window may influence recovery from surgery. *Science*. 1984;224:42–421.
26. Park SH, Mattson RH. Ornamental indoor plants in hospital rooms enhanced health outcomes of patients recovering from surgery. *J Altern Complement Med*. 2009;15(9):975–980. https://doi.org/10.1089/acm.2009.0075.
27. aan het Rot M, Benkelfat C, Boivin DB, Young SN. Bright light exposure during acute tryptophan depletion prevents a lowering of mood in mildly seasonal women. *Eur Neuropsychopharmacol*. 2008;18(1):14–23.
28. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A*. 2011;108(7):3017–3022.
29. Sylvia LG, Salcedo S, Bernstein EE, Baek JH, Nierenberg AA, Deckersbach T. Nutrition, exercise, and wellness treatment in bipolar disorder: proof of concept for a consolidated intervention. *Int J Bipolar Disord*. 2013;1(1):24.
30. Hearing CM, Chang WC, Szuhany KL, Deckersbach T, Nierenberg AA. Sylvia LG Physical exercise for treatment of mood disorders: a critical review. *Curr Behav Neurosci Rep*. 2016;3(4):350–359.
31. Coventry PA, Bower P, Keyworth C, et al. The effect of complex interventions on depression and anxiety in chronic obstructive pulmonary disease: systematic review and meta-analysis. *PLoS One*. 2013;8(4):e60532. https://doi.org/10.1371/journal.pone.0060532.
32. Kruisdijk FR, Hendriksen IJ, Tak EC, Beekman AT, Hopman-Rock M. Effect of running therapy on depression (EFFORT-D). Design of a randomised controlled trial in adult patients [ISRCTN 1894]. *BMC Public Health*. 2012;12:50.
33. Garber CE, Blissmer B, Deschenes MR, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Am Colleg Sports Medicine Med Sci Sports Exerc*. 2011;43(7):1334–1359.
34. Raglin JS. Exercise and mental health. Beneficial and detrimental effects. *Sports Med*. 1990;9(6):323–329.
35. Ostojic SM1. Exercise-induced mitochondrial dysfunction: a myth or reality? *Clin Sci (Lond)*. 2016;130(16):1407–1416. https://doi.org/10.1042/CS20160200.
36. Brooks K, Carter J. Overtraining, exercise, and adrenal insufficiency. *J Nov Physiother*. 2013;3(125).
37. Cox DJ, Gonder-Frederick L, Polonsky W, Schlundt D, Kovatchev B, Clarke W. Blood glucose awareness training (BGAT-2): long-term benefits. *Diabetes Care*. 2001;24(4):637–642.
38. Hajek T, Calkin C, Blagdon R. Insulin resistance, diabetes mellitus, and brain structure in bipolar disorders. *Neuropsychopharmacology*. 2014;39:2910–2918.
39. Dowdy DW, Dinglas V, Mendez-Tellez PA, et al. Intensive care unit hypoglycemia predicts depression during early recovery from acute lung injury. *Crit Care Med*. 2008;36(10):2726–2733.
40. Sommerfield AJ, Deary IJ, Frier BM. Acute hyperglycemia alters mood state and impairs cognitive performance in people with type 2 diabetes. *Diabetes Care*. 2004;27(10):2335–2340.
41. Pearson S, Schmidt M, Patton G, et al. Diabetes depression and insulin resistance: cross-sectional associations in young adults. *Care*. 2010;33(5):1128–1133.
42. Ellen Charles F, Christophe Lambert G, Kerner B. Bipolar disorder and diabetes mellitus: evidence for disease-modifying effects and treatment implications. *Int J Bipolar Disord*. 2016;4:13.
43. Gershon M. *The Second Brain*. New York: HarperCollins; 1999.
44. Liu L, Zhu G. Gut–brain axis and mood disorder. *Front Psychiatry*. 2018;9:223. https://doi.org/10.3389/fpsyt.2018.00223.
45. Kabra N, Nadkarni A. Prevalence of depression and anxiety in irritable bowel syndrome: a clinic based study from India. *Indian J Psychiatry*. 2013;55(1):77–80. https://doi.org/10.4103/0019-5545.105520.

46. Karling P, Maripuu M, Wikgren M, Rolf A, Karl-Fredrik Norrback. Association between gastrointestinal symptoms and affectivity in patients with bipolar disorder. *World J Gastroenterol*. 2016;22(38):8540–8548.
47. Passik SD1, Roth AJ. Anxiety symptoms and panic attacks preceding pancreatic cancer diagnosis. *Psycho-Oncology*. 1999;8(3):268–272.
48. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*. 2011;141(2):599–609. 609. e1-3.
49. Foster JA, Rinaman L, Cryan JF. Stress and the gut-brain axis: regulation by the microbiome. *Neurobiol Stress*. 2017;7:124–136.
50. Dickerson F, Severance E, Yolken R. The microbiome, immunity, and schizophrenia and bipolar disorder. *Brain Behav Immun*. 2017;62:46–52.
51. Vogelzangs N, Beekman AT, de Jonge P, Penninx BW. Anxiety disorders and inflammation in a large adult cohort. *Transl Psychiatry*. 2013;3:e249. https://doi.org/10.1038/tp.2013.27.
52. Howren MB, Lamkin DM. Suls Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *J Psychosom Med*. 2009;71(2):171–186.
53. Muneerc A. Bipolar disorder: role of inflammation and the development of disease biomarkers. *Psychiatry Investig*. 2016;13(1):18–33.
54. Steven Shoelson E, Lee J, Allison Goldfine B. Inflammation and insulin resistance. *J Clin Invest*. 2006;116(7):1793–1801.
55. Rucklidge JJ. Could yeast infections impair recovery from mental illness? A case study using micronutrients and olive leaf extract for the treatment of ADHD and depression. *Adv Mind Body Med*. 2013;27(3):14–18.
56. Maes M, Kubera M, Leunis JC. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol Lett*. 2008;29(1):117–124.
57. Bongiorno PB. *Holistic Solutions for Anxiety & Depression: Combining Natural Remedies with Conventional Care a Therapist's Guide*. New York: W.W. Norton; 2015:102.
58. Mu Q, Kirby J, Christopher Reilly M, Xin Luo M. Leaky gut as a danger signal for autoimmune diseases. *Front Immunol*. 2017;8:598.
59. Kasselman LJ, Vernice NA, DeLeon J, Reiss AB. The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity. *Atherosclerosis*. 2018;271:203–213.
60. Meijers B, Farré R, Dejongh S, Vicario M, Evenepoel P. Intestinal barrier function in chronic kidney disease. *Toxins (Basel)*. 2018;10(7).
61. John Kelly R, Paul Kennedy J, John Cryan F, Timothy Dinan G, Clarke G, Niall Hyland P. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci*. 2015;9:392.
62. Hu LY, Shen CC, Hu YW, et al. Hyperthyroidism and risk for bipolar disorders: a nationwide population-based study. *PLoS One*. 2013;8(8):e73057. https://doi.org/10.1371/journal.pone.0073057.
63. Davis JD, Tremont G. Neuropsychiatric aspects of hypothyroidism and treatment reversibility. *Minerva Endocrinol*. 2007;32:953–959.
64. Helmreich DL, Parfitt DB, Lu XY, Akil H, Watson SJ. Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress. *Neuroendocrinology*. 2005;81:183–192.
65. Bianco AC, Nunes MT, Hell NS, Maciel RM. The role of glucocorticoids in the stress-induced reduction of extrathyroidal 3,5,3'-triiodothyronine generation in rats. *Endocrinology*. 1987;120:1033–1038.
66. Touma KTB1, Zoucha AM1, Scarff JR1. Liothyronine for depression: a review and guidance for safety monitoring. *Innov Clin Neurosci*. 2017;14(3–4):24–29.
67. Markopouloua K, Papadopoulosa A, Mario Juruenaa F, Poonb L, Carmine Pariantec M, Anthony Cleare J. The ratio of cortisol/DHEA in treatment resistant depression. *Psychoneuroendocrinology*. 2009;34(1):19–26.
68. Goodyer IM, Herbert J, Tamplin A, Altham PM. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *Br J Psychiatry*. 2000;177:499–504.
69. Gallagher P, Watson S, Smith MS, Young AH, Ferrier IN. Plasma cortisol-dehydroepiandrosterone (DHEA) ratios in schizophrenia and bipolar disorder. *Schizophr Res*. 2007;90(1–3):258–265.
70. Peixoto C, Devicari Cheda JN, Nardi AE, Veras AB, Cardoso A. The effects of dehydroepiandrosterone (DHEA) in the treatment of depression and depressive symptoms in other psychiatric and medical illnesses: a systematic review. *Curr Drug Targets*. 2014;15(9):901–914.
71. Vacheron-Trystram MN, Cheref S, Gauillard J, Plas J. A case report of mania precipitated by use of DHEA]. *Encephale*. 2002;28(6 Pt 1):563–566.
72. Johnson JM, Nachtigall LB, Stern TA. The effect of testosterone levels on mood in men: a review. *Psychosomatics*. 2013;54(6):509–514. https://doi.org/10.1016/j.psym.2013.06.018.
73. van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. *Biol Psychiatry*. 2005;58(3):218–225.
74. Wooderson SC, Gallagher P, Watson S, Allan H, Young. An exploration of testosterone levels in patients with bipolar disorder. *BJ Psych Open*. 2015;1(2):136–138.
75. Carrasco GA, Barker SA, Zhang Y, et al. Estrogen treatment increases the levels of regulator of G protein signaling-Z1 in the hypothalamic paraventricular nucleus: possible role in desensitization of 5-hydroxytryptamine(1A) receptors. *Neuroscience*. 2004;127:261–267.
76. Fischer B, Gleason C, Asthana S. Effects of hormone therapy on cognition and mood. *Fertil Steril*. 2014;101(4):898–904. https://doi.org/10.1016/j.fertnstert.2014.02.025.
77. Rasgon NL, Altshuler LL, Fairbanks LA, et al. Estrogen replacement therapy in the treatment of major depressive disorder in perimenopausal women. *J Clin Psychiatry*. 2002;63(Suppl 7):45–48.
78. Baker ER, Best RG, Manfredi RL, Demers LM, Wolf GC. Efficacy of progesterone vaginal suppositories in alleviation of nervous symptoms in patients with premenstrual syndrome. *J Assist Reprod Genet*. 1995;12(3):205–209.
79. Caufriez A, Leproult R, L'Hermite-Balériaux M, Kerkhofs M, Copinschi G. Progesterone prevents sleep disturbances and modulates GH, TSH, and melatonin secretion in postmenopausal women. *J Clin Endocrinol Metab*. 2011;96(4):E614–E623. https://doi.org/10.1210/jc.2010-2558. Epub 2011 Feb 2.
80. Scantamburlo G, Hansenne M, Fuchs S, et al. Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology*. 2007;32(4):407–410.
81. Turan T, Uysal C, Asdemir A, Kılıç E. May oxytocin be a trait marker for bipolar disorder? *Psychoneuroendocrinology*. 2013;38(12):2890–2896. https://doi.org/10.1016/j.psyneuen.2013.07.017. Epub 2013 Aug 14.
82. Parker KJ, Oztan O, Libove RA, et al. Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism. *Proc Natl Acad Sci U S A*. 2017;114(30):8119–8124.
83. Ji H, Su W, Zhou R, et al. Intranasal oxytocin administration improves depression-like behaviors in adult rats that experienced neonatal maternal deprivation. *Behav Pharmacol*. 2016;27(8):689–696.
84. Handlin L, Hydbring-Sandberg E, Nilsson A, Ejdebäck M, Jansson A, Uvnäs-Moberg K. Short-term interaction between dogs and their owners—effects on oxytocin, cortisol, insulin and heart rate—an exploratory study. *Anthrozoos*. 2011;24:301–315. https://doi.org/10.2752/175303711X13045914865385.
85. Beck-Friis J, von Rosen D, Kjellman BF, LjunggrenJ G. Melatonin in relation to body measures, sex, age, season and the use of drugs in patients with major affective disorders and healthy subjects, Psychoneuroendocrinology 1984;9(3):261 -277.
86. Sundberg I, Ramklint M, Stridsberg M, et al. Salivary melatonin in relation to depressive symptom severity in young adults. *PLoS One*. 2016;11(4):e0152814.
87. Toffol E, Kalleinen N, Haukka J, et al. Melatonin in perimenopausal and postmenopausal women: associations with mood, sleep, climacteric symptoms, and quality of life. *Menopause*. 2014 May;21(5):493–500.
88. De Crescenzo F, Lennox A, Gibson JC, et al. Melatonin as a treatment for mood disorders: a systematic review. *Acta Psychiatr Scand*. 2017;136(6):549 -558.
89. Dalton EJ, Rotondi D, Levitan RD, et al. Use of slow-release melatonin in treatment-resistant depression. *J Psychiatry Neurosci*. 2000;25(1):48-52.
90. Dalton J, Rotondi D, Levitan RD, et al. Use of slow-release melatonin in treatment-resistant depression. *J Psychiatry Neurosci*. 2000;25(1):48–52.

91. Kinsman R, Hood J. Some behavioral effects of ascorbic acid deficiency. *Am J Clin Nutr*. 1971;24:455–464.
92. Moylan S, Jacka FN, Pasco JA, Berk M. How cigarette smoking may increase the risk of anxiety symptoms and anxiety disorders: a critical review of biological pathways. *Brain Behav*. 2013;3(3):302–326.
93. Heffner JL, Strawn JR, DelBello MP, Strakowski SM, Anthenelli RM. The co-occurrence of cigarette smoking and bipolar disorder: phenomenology and treatment considerations. *Bipolar Disord*. 2011;13(0):439–453.
94. Lobo IA, Harris RA. GABA(A) receptors and alcohol. *Pharmacol Biochem Behav*. 2008;90(1):90–94.
95. Horrobin DF. A biochemical basis for alcoholism and alcohol-induced damage including the fetal alcohol syndrome and cirrhosis: interference with essential fatty acid and prostaglandin metabolism. *Med Hypotheses*. 1980;6(9):929–942.
96. Wang L, Shen X, Wu Y. Zhang D Coffee and caffeine consumption and depression: a meta-analysis of observational studies. *Aust N Z J Psychiatry*. 2016;50(3):228–242.
97. Moran JM, Wig GS, Adams Jr RB, Janata P, Kelley WM. Neural correlates of humor detection and appreciation. *Neuroimage*. 2004;21:1055–1060.
98. Madhav KC, Sherchand SP, Sherchan S. Association between screen time and depression among US adults. *Prev Med Rep*. 2017;8:67–71. https://doi.org/10.1016/j.pmedr.2017.08.005.
99. Robinson JP, Martin S. IT and activity displacement: evidence from the general social survey. *Social Indicators Research*. 2008;91:115–139.
100. Alhassan AA, Alqadhib EM, Taha NW, Alahmari RA, Salam M, Almutairi AF. The relationship between addiction to smartphone usage and depression among adults: a cross sectional study. *BMC Psychiatry*. 2018;18(1):148. https://doi.org/10.1186/s12888-018-1745-4.
101. Merlo LJ, Stone AM. Anxiety disorders association of America 28th annual meeting: poster 55. 2007:6–9.
102. Kumar V, Sattar Y, Bseiso A, Khan S, Rutkofsky IH. The effectiveness of internet-based cognitive behavioral therapy in treatment of psychiatric disorders. *Cureus*. 2017;9(8):e1626. https://doi.org/10.7759/cureus.1626.
103. Bellinger D, Leviton A, Allred E, Rabinowitz M. Pre- and postnatal lead exposure and behavior problems in school-aged children. *Environ Res*. 1994;66:12–30.
104. Maximino C, Araujo J, Leao LK, et al. Possible role of serotoninergic system in the neurobehavioral impairment induced by acute methylmercury exposure in zebrafish (Danio rerio) Neurotoxicol Teratol. 2011;33:727–734.
105. Shenassa ED, Daskalakis C, Liebhaber A, Braubach M, Brown M. Dampness and mold in the home and depression: an examination of mold-related illness and perceived control of one's home as possible depression pathways. *Am J Public Health*. 2007;97(10):1893–1899.
106. Schon EA, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest*. 2003;111(3):303–312.
107. Scaglia F. The role of mitochondrial function in psychiatric disease. *Dev Disabil Res Rev*. 2010;16:136–143.
108. Gardner A, Salmaso D, Nardo D, et al. Mitochondrial function is related to alterations at brain SPECT in depressed patients. *CNS Spectr*. 2008;13:805–814.
109. Rezin GT, Gonçalves CL, Daufenbach JF. Acute administration of ketamine reverses the inhibition of mitochondrial respiratory chain induced by chronic mild stress. *Brain Res Bull*. 2009;79:418–421.
110. Pereira C, Chavarria V, Vian J, et al. Mitochondrial agents for bipolar disorder. *Int J Neuropsychopharmacol*. 2018;21(6):550–569.
111. Sun R. Dietary supplementation with fish oil alters the expression levels of proteins governing mitochondrial dynamics and prevents high-fat diet-induced endothelial dysfunction. *Br J Nutr*. 2014;112(2):145–153.
112. Mehta SL, Kumari S, Mendelev N, Li PA. Selenium preserves mitochondrial function, stimulates mitochondrial biogenesis, and reduces infarct volume after focal cerebral ischemia. *BMC Neurosci*. 2012;13:79. https://doi.org/10.1186/1471-2202-13-79.
113. Wang Y, Zhang Y, He J, et al. Hyperforin promotes mitochondrial function and development of oligodendrocytes. *J Neurochem*. 2011;119(3):555–568.
114. Fournier JC, DeRubeis RJ, Hollon SD, et al. Antidepressant drug effects and depression severity: a patient-level meta-analysis. *JAMA*. 2010;303(1):47–53.
115. Smoller JW, Allison M, Cochrane BB, et al. Antidepressant use and risk of incident cardiovascular morbidity and mortality among postmenopausal women in the Women's Health Initiative study. *Arch Intern Med*. 2009;169(22):2128–2139. https://doi.org/10.1001/archinternmed.2009.436.
116. Belleville G. Mortality hazard associated with anxiolytic and hypnotic drug use in the National Population Health Survey. *Can J Psychiatry*. 2010;55(9):558–567.
117. Systematic review of the benzodiazepines. Guidelines for data sheets on diazepam, chlordiazepoxide, medazepam, clorazepate, lorazepam, oxazepam, temazepam, triazolam, nitrazepam, and flurazepam. Committee on the Review of Medicines. *Br Med J*. 1980;280(6218):910–912.
118. Mugunthan K, McGuire T, Glasziou P. Minimal interventions to decrease long-term use of benzodiazepines in primary care: a systematic review and meta-analysis [published correction appears in *Br J Gen Pract*. 2013 Jan;63(606):12]. *Br J Gen Pract*. 2011;61(590):e573-e578.
119. Smith BL. Inappropriate prescribing. *Am Psych Assoc*. 2012;43(6):36. http://www.apa.org/monitor/2012/06/prescribing.aspx. Accessed August 6, 2018.
120. Harrison JN, Cluxton-Keller F, Gross D. Antipsychotic medication prescribing trends in children and adolescents. *J Pediatr Health Care*. 2012;26(2):139–145.
121. Bongiorno PB. *Healing Depression: Integrated Naturopathic and Conventional Treatments*. Toronto: CCNM Press; 2010. p. 29.
122. Shekhar A, Truitt W, Rainnie D, Sajdyk T. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. *Stress*. 2005;8(4):209–219.
123. Hollon SD, Stewart MO, Strunk D. Enduring effects for cognitive behavior therapy in the treatment of depression and anxiety. *Ann Rev Psychol*. 2006;57:285–315.
124. Driessen E, Hollon SD. Cognitive behavioral therapy for mood disorders: efficacy, moderators and mediators. *Psychiatr Clin North Am*. 2010;33(3):537–555.
125. Hollon SD, DeRubeis RJ, Shelton RC, et al. Prevention of relapse following cognitive therapy vs medications in moderate to severe depression. *Arch Gen Psychiatry*. 2005;62(4):417–422.
126. Bojic S, Becerra R. Mindfulness-based treatment for bipolar disorder: a systematic review of the literature. *Eur J Psychol*. 2017;13(3):573–598. https://doi.org/10.5964/ejop.v13i3.1138.
127. Kabat-Zinn J. Mindfulness-based interventions in context: past, present, and future. *Clin Psychol Sci Prac*. 2003;10:144–156.
128. Shohani M, Badfar G, Nasirkandy MP, et al. The effect of yoga on stress, anxiety, and depression in women. *Int J Prev Med*. 2018;9:21. https://doi.org/10.4103/ijpvm.IJPVM_242_16.
129. Moyer Christopher A, Rounds J, Hannum JW, et al. A meta-analysis of massage therapy research. *Psychol Bull*. 2004;130(1):3–18.
130. Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A, et al. Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. *Arch Gen Psychiatry*. 2009;66(10):1090–1098. https://doi.org/10.1001/archgenpsychiatry.2009.129.
131. Yoshimura R, Ikenouchi-Sugita A, Hori H, et al. Blood levels of brain-derived neurotrophic factor (BDNF) in major depressive disorder. *Seishin Shinkeigaku Zasshi*. 2010;112(10):982–985.
132. Suliman S, Hemmings SM, Seedat S. Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis. *Front Integr Neurosci*. 2013;7:55. https://doi.org/10.3389/fnint.2013.00055.
133. Bongiorno PB. *Holistic Solutions for Anxiety & Depression: Combining Natural Remedies with Conventional Care: A Therapist's Guide*. New York: W.W. Norton; 2015:26–27.
134. Bested AC, Logan AC, Selhub EM. Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: part II—contemporary contextual research. *Gut Pathog*. 2013;5(1):3.

135. Young SN. Acute tryptophan depletion in humans: a review of theoretical, practical and ethical aspects. *J Psychiatry Neurosci*. 2013;38(5):294–305.
136. Yokogoshi H, Nomura M. Effect of amino acid supplementation to a low-protein diet on brain neurotransmitters and memory-learning ability of rats. *Physiol Behav*. 1991;50(6):1227–1232.
137. Rowe AH, Rowe A. Jr. *Food Allergy. Its Manifestations and Control and the Elimination Diets: A Compendium*. Springfield, IL: CC Thomas; 1972.
138. Teufel M, Biedermann T, Rapps N, et al. Psychological burden of food allergy. *World J Gastroenterol*. 2007;13(25):3456–3465.
139. Jackson JR, Eaton WW, Cascella NG, Fasano A, Kelly DL. Neurologic and psychiatric manifestations of celiac disease and gluten sensitivity. *Psychiatr Q*. 2012;83(1):91–102.
140. Casella G, Pozzi R, Cigognetti M, et al. Mood disorders and non-celiac gluten sensitivity. *Minerva Gastroenterol Dietol*. 2017;63(1):32–37. https://doi.org/10.23736/S1121-421X.16.02325-4.
141. Severance EG, Dupont D, Dickerson FB, et al. Immune activation by casein dietary antigens in bipolar disorder. *Bipolar Disord*. 2010;12(8):834–842. https://doi.org/10.1111/j.1399-5618.2010.00879.x.
150. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr*. 2010;105(5):755–764.
151. Steenbergen L, Sellaro R, van Hemert S, et al. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain Behav Immun*. 2015. https://doi.org/10.1016/j.bbi.2015.04.003.
152. Huang R, Wang K, Hu J. Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. *Nutrients*. 2016;8(8):483.
153. Dickerson F, Adamos M, Katsafanas E, et al. Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: a randomized controlled trial. *Bipolar Disord*. 2018; 20(7):614–621.
154. Lindseth G, Helland B, Caspers J. The effects of dietary tryptophan on affective disorders. *Arch Psychiatr Nurs*. 2014;29(2):102–107.
155. Gibson EL. Tryptophan supplementation and serotonin function: genetic variations in behavioural effects. *Proc Nutr Soc*. 2018;77(2):174–188.
156. Platzer M, Dalkner N, Fellendorf FT, et al. Tryptophan breakdown and cognition in bipolar disorder. *Psychoneuroendocrinology*. 2017;81:144–150.
157. Hinz M, Stein A, Uncini T. Relative nutritional deficiencies associated with centrally acting monoamines. *Int J Gen Med*. 2012;5:413–430.
158. Bongiorno PB. *Healing Depression: Integrated Naturopathic and Conventional Treatments*. Toronto, Canada: CCNM Press; 2010;94.
159. Shaw K, Turner J, Del Mar C. Are tryptophan and 5-hydroxytryptophan effective treatments for depression? A meta-analysis. *Aust N Z J Psychiatry*. 2002;36(4):488–491.
160. Ghadirian A, Murphy BEP, Gendron MJ. Efficacy of light versus tryptophan therapy in seasonal affective. *J Affect Disord*. 1998;50:23–27.
162. Gelenberg AJ, Gibson CJ. Tyrosine for the treatment of depression. *Nutr Health*. 1984;3(3):163–173.
163. Sabelli HC, Fawcett J, Gusovsky F, et al. Clinical studies on the phenylethylamine hypothesis of affective disorder: urine and blood phenylacetic acid and phenylalanine dietary supplements. *J Clin Psychiatry*. 1986;47(2):66–70.
164. McLean A, Rubinsztein JS, Robbins TW, et al. The effects of tyrosine depletion in normal healthy volunteers: implications for unipolar depression. *Psychopharmacology (Berl)*. 2004 Jan;171(3):286–297.
165. Gelenberg AJ, Wojcik JD, Falk WE, et al. Tyrosine for depression: a double-blind trial. *J Affect Disord*. 1990;19(2):125–132.
166. Gardos G, Cole JO, Matthews JD, Nierenberg AA, Dugan SJ. The acute effects of a loading dose of phenylalanine in unipolar depressed patients with and without tardive dyskinesia. *Neuropsychopharmacology*. 1992;6(4):241–247.
167. Cruciani RA, Dvorkin E, Homel P, et al. Safety, tolerability and symptom outcomes associated with l-carnitine supplementation in patients with cancer, fatigue, and carnitine deficiency: A Phase I/II Study, J Pain Symptom Manage. 2006;32(6):551–559.
168. Malaguarnera M, Bella R, Vacante M, et al. Acetyl-L-carnitine reduces depression and improves quality of life in patients with minimal hepatic encephalopathy. *Scand J Gastroenterol*. 2011;46(6):750–759. https://doi.org/10.3109/00365521.2011.565067.
169. Nasca C, Xenos D, Barone Y, et al. L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. *Proc Natl Acad Sci U S A*. 2013;110(12):4804–4809. https://doi.org/10.1073/pnas.1216100110.
170. Pettegrew JW, Levine J, Gershon S, et al. 31P-MRS study of acetyl-L-carnitine treatment in geriatric depression: preliminary results. *Bipolar Disord*. 2002;4(1):61–66.
171. Levine J, Kaplan Z, Pettegrew JW, et al. Effect of intraperitoneal acetyl-L-carnitine (ALCAR) on anxiety-like behaviours in rats. *Int J Neuropsychopharmacol*. 2005;8(1):65–74.
172. Leung S, Croft RJ, O'Neill BV, Nathan PJ. Acute high-dose glycine attenuates mismatch negativity (MMN) in healthy human controls. *Psychopharmacology (Berl)*. 2008;196(3):451–460.
173. Abdou AM, Higashiguchi S, Horie K, Kim M, Hatta H, Yokogoshi H. Relaxation and immunity enhancement effects of gamma-aminobutyric acid (GABA) administration in humans. *Biofactors*. 2006;26(3):201–208.
174. Boonstra E, de Kleijn R, Colzato LS, Alkemade A, Forstmann BU, Nieuwenhuis S. Neurotransmitters as food supplements: the effects of GABA on brain and behavior. *Front Psychol*. 2015;6:1520. https://doi.org/10.3389/fpsyg.2015.01520.
175. Byun JI, Shin YY, Chung SE, Shin WC. Safety and efficacy of gamma-aminobutyric acid from fermented rice germ in patients with insomnia symptoms: a randomized, double-blind trial. *J Clin Neurol*. 2018;14(3):291–295.
176. Ahuja T, Mgbako O, Katzman C, Grossman A. Phenibut (β-phenyl-γ-aminobutyric acid) dependence and management of withdrawal: emerging nootropics of abuse. *Case Rep Psychiatry*. 2018;2018:9864285. https://doi.org/10.1155/2018/9864285.
177. O'Donnell T, Rotzinger S, Ulrich M, Hanstock CC, Nakashima TT, Silverstone PH. Effects of chronic lithium and sodium valproate on concentrations of brain amino acids. *Eur Neuropsychopharmacol*. 2003;13(4):220–227.
178. Berk M, Copolov DL, Dean O, et al. N-acetyl cysteine for depressive symptoms in bipolar disorder—a double-blind randomized placebo-controlled trial. *Biol Psychiatry*. 2008;64(6):468–475. https://doi.org/10.1016/j.biopsych.2008.04.022.
179. Berk M, Dean OM, Cotton SM, et al. Maintenance N-acetyl cysteine treatment for bipolar disorder: a double-blind randomized placebo controlled trial. *BMC Med*. 2012;10:91. https://doi.org/10.1186/1741-7015-10-91.
180. American Psychiatric Association (APA). *Practice Guideline for the Treatment of Patients with Major Depressive Disorder*, ed 3. Arlington,VA: American Psychiatric Association (APA); 2010:152.
181. Miller A. The methylation, neurotransmitter, and antioxidant connections between folate and depression. *Alt Med Rev*. 2008;13:216–226.
182. Nguyen M, Gregan A. S-adenosylmethionine and depression. *Aust Fam Physician*. 2002;31(4):339–343.
183. Galizia I, Oldani L, Macritchie K, et al. S-adenosyl methionine (SAMe) for depression in adults. *Cochrane Database Syst Rev*. 2016;10:CD011286.
184. Sharma A, Gerbarg P, Bottiglieri T, et al. S-adenosylmethionine (SAMe) for neuropsychiatric disorders: a clinician-oriented review of research. *J Clin Psychiatry*. 2017;78(6):e656–e667.
185. Berigan TR. A case report of a manic episode triggered by S-adenosylmethionine (SAMe). *Prim Care Companion J Clin Psychiatry*. 2002;4(4):159.
186. Delarue J, Matzinger O, Binnert C, Schneiter P, Chiolero R, Tappy L. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. *Diabetes Metab*. 2003;29(3):289–295.
187. Su KP, Matsuoka Y, Pae CU. Omega-3 polyunsaturated fatty acids in prevention of mood and anxiety disorders. *Clin Psychopharmacol Neurosci*. 2015;13(2):129–137.
188. Hashimoto M, Maekawa M, Katakura M, Hamazaki K, Matsuoka Y. Possibility of polyunsaturated fatty acids for the prevention and treatment of neuropsychiatric illnesses. *J Pharmacol Sci*. 2014;124(3):294–300.

189. Wani AL, Bhat SA, Ara A. Omega-3 fatty acids and the treatment of depression: a review of scientific evidence. *Integr Med Res*. 2015;4(3):132–141.
190. Natacci L, Marchioni MD, Goulart CA, et al. Omega 3 consumption and anxiety disorders: a cross-sectional analysis of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). *Nutrients*. 2018;10(6):663. https://doi.org/10.3390/nu10060663.
191. Su KP, Tseng PT, Lin PY, et al. Association of use of omega-3 polyunsaturated fatty acids with changes in severity of anxiety symptoms: a systematic review and meta-analysis. *JAMA Netw Open*. 2018;1(5):e182327. https://doi.org/10.1001/jamanetworkopen.2018.2327.
192. Shakeri J, Khanegi M, Golshani S, et al. Effects of omega-3 supplement in the treatment of patients with bipolar I disorder. *Int J Prev Med*. 2016;7:77. https://doi.org/10.4103/2008-7802.182734.
193. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J Chem Neuroanat*. 2005;29(1):21–30.
194. Wion D, MacGrogan D, Neveu I, Jehan F, Houlgatte R, Brachet P. 1,25-Dihydroxyvitamin D3 is a potent inducer of nerve growth factor synthesis. *J Neurosci Res*. 1991;28(1):110–114.
195. Stumpf WE. Vitamin D sites and mechanisms of action: a histochemical perspective. Reflections on the utility of autoradiography and cytopharmacology for drug targeting. *Histochem Cell Biol*. 1995;104(6):417–427.
196. Boerman R, Cohen D, Schulte PF, Nugter A. Prevalence of vitamin D deficiency in adult outpatients with bipolar disorder or schizophrenia. *J Clin Psychopharmacol*. 2016;36(6):588–592.
197. Spedding S. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. *Nutrients*. 2014;6(4):1501–1518. https://doi.org/10.3390/nu6041501.
198. Sánchez-Villegas A, Doreste J, Schlatter J, Pla J, Bes-Rastrollo M, Martínez-González MA. Association between folate, vitamin B(6) and vitamin B(12) intake and depression in the SUN cohort study. *J Hum Nutr Diet*. 2009;22(2):122–133. https://doi.org/10.1111/j.1365-277X.2008.00931.x.
199. Skarupski KA, Tangney C, Li H, Ouyang B, Evans DA, Morris MC. Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. *Am J Clin Nutr*. 2010;92(2):330–335.
200. Bermond P. Therapy of side effects of oral contraceptive agents with vitamin B6. *Acta Vitaminol Enzymol*. 1982;4(1–2):45–54.
201. Stahl SM. L-methylfolate: a vitamin for your monoamines. *J Clin Psychiatry*. 2008;69(9):1352–1353.
202. Bjelland I1, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. *Arch Gen Psychiatry*. 2003;60(6):618–626.
203. Brocardo PS, Budni J, Lobato KR, Santos AR, Rodrigues AL. Evidence for the involvement of the opioid system in the antidepressant-like effect of folic acid in the mouse forced swimming test. *Behav Brain Res*. 2009;200(1):122–127.
204. Einat H, Clenet F, Shaldubina A, Belmaker RH, Bourin M. The antidepressant activity of inositol in the forced swim test involves 5-HT(2) receptors. *Behav Brain Res*. 2001;118(1):77–83.
205. Brink CB, Viljoen SL, de Kock SE, Stein DJ, Harvey BH. Effects of myo-inositol versus fluoxetine and imipramine pretreatments on serotonin 5HT2A and muscarinic acetylcholine receptors in human neuroblastoma cells. *Metab Brain Dis*. 2004;19(1–2):51–70.
206. Mukai T, Kishi T, Matsuda Y, Iwata N. A meta-analysis of inositol for depression and anxiety disorders. *Hum Psychopharmacol*. 2014;29(1):55–63. https://doi.org/10.1002/hup.2369.
207. Palatnik A, Frolov K, Fux M, Benjamin J. Double-blind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder. *J Clin Psychopharmacol*. 2001;21(3):335–359.
208. Jensen JE, Daniels M, Haws C, et al. Triacetyluridine (TAU) decreases depressive symptoms and increases brain pH in bipolar patients. *Exp Clin Psychopharmacol*. 2008;16(3):199–206.
209. Attenburrow MJ, Odontiadis J, Murray BJ, et al. Chromium treatment decreases the sensitivity of 5HT2A receptors. *Psychopharmacology*. 2002;159:432–436.
210. Anderson RA. Chromium, glucose intolerance and diabetes. *J Am Coll Nutrition*. 1998;17:548–555.
211. Brownley KA, Boettiger CA, Young L, Cefalu WT. Dietary chromium supplementation for targeted treatment of diabetes patients with comorbid depression and binge eating. *Med Hypotheses*. 2015;85(1):45–48.
212. King DE, Mainous 3rd AG, Geesey ME, Woolson RF. Dietary magnesium and C-reactive protein levels. *J Am Coll Nutr*. 2005;24(3):166–171.
213. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. *J Nutr Biochem*. 2014;25(11):1101–1107.
214. Pamuk GE, Uyanik MS, Top MS, Tapan U, Ak R, Uyanik V. Gastrointestinal symptoms are closely associated with depression in iron deficiency anemia: a comparative study. *Ann Saudi Med*. 2015;35(1):31–35.
215. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. *J Nutr Biochem*. 2014;25(11):1101–1107.
216. Richardson AC, Heath AL, Haszard JJ, Polak MA, Houghton LA, Conner TS. Higher body iron is associated with greater depression symptoms among young adult men but not women: observational data from the daily life study. *Nutrients*. 2015;7(8):6055–6072. https://doi.org/10.3390/nu7085270.
217. Serata D, Del Casale A, Rapinesi C, et al. Hemochromatosis-induced bipolar disorder: a case report. *Gen Hosp Psychiatry*. 2012;34(1):101.e1–e3. https://doi.org/10.1016/j.genhosppsych.2011.04.013.
218. Petrilli MA, Kranz TM, Kleinhaus K, et al. The emerging role for zinc in depression and psychosis. *Front Pharmacol*. 2017;8:414. https://doi.org/10.3389/fphar.2017.00414.
219. Szewczyk B, Poleszak E, Sowa-Kućma M, et al. The involvement of NMDA and AMPA receptors in the mechanism of antidepressant-like action of zinc in the forced swim test. *Amino Acids*. 2010;39(1):205–217.
220. Prakash A, Bharti K, Majeed AB. Zinc: indications in brain disorders. *Fundam Clin Pharmacol*. 2015;29(2):131–149.
221. Swardfager W, Herrmann N, Mazereeuw G, Lanctôt KL. Reply to: serum zinc and the risk of depression in men: observations from a 20-year follow-up study. *Biol Psychiatry*. 2015;77(3):e13–e14.
222. Sawada T, Yokoi K. Effect of zinc supplementation on mood states in young women: a pilot study. *Eur J Clin Nutr*. 2010;64(3):331–333.
223. Russo AJ. Decreased zinc and increased copper in individuals with anxiety. *Nutr Metab Insights*. 2011;4:1–5. https://doi.org/10.4137/NMI.S6349.
224. Kapusta ND, Mossaheb N, Etzersdorfer E, et al. Lithium in drinking water and suicide mortality. *Int Clin Psychopharmacol*. 1987;2(1):33–45.
225. Lakhan SE, Vieira KF. Nutritional therapies for mental disorders. *Nutr J*. 2008;21:7. https://doi.org/10.1186/1475-2891-7-2.
226. Sartori SB, Whittle N, Hetzenauer A, Singewald N. Magnesium deficiency induces anxiety and HPA axis dysregulation: modulation by therapeutic drug treatment. *Neuropharmacology*. 2012;62(1):304–312. https://doi.org/10.1016/j.neuropharm.2011.07.027.
227. Pauzé DK, Brooks DE. Lithium toxicity from an Internet dietary supplement. *J Med Toxicol*. 2007;3(2):61–62.
228. Müller WE, Rolli M, Schafer C, Hafner U. Effects of Hypericum extract (LI 160) in biochemical models of antidepressant activity. *Pharmacopsychiatry*. 1997;30(suppl 2):102–107.
229. Morelli V, Zoorob RJ. Alternative therapies: part I. Depression, diabetes, obesity. *Am Fam Physician*. 2000;62(5):1051–1060.
230. Butterweck V. Mechanism of action of St. John's wort in depression. *CNS Drugs*. 2003;17(8):539–562.
231. Re L, Corneli C, Sturani E, et al. Effects of Hypericum extract on the acetylcholine release: a loose patch clamp approach. *Pharmacol Res*. 2003;48(1):55–60.
232. Wang Y, Zhang Y, He J, et al. Hyperforin promotes mitochondrial function and development of oligodendrocytes. *J Neurochem*. 2011;119(3):555–568. https://doi.org/10.1111/j.1471-4159.2011.07433.x. Epub 2011 Sep 21.
233. Wong ML, O'Kirwan F, Hannestad JP, Irizarry KJL, Elashoff D, Licinio J. St John's wort and imipramine-induced gene expression profiles identify cellular functions relevant to antidepressant action and novel pharmacogenetic candidates for the phenotype of antidepressant treatment response. *Mol Psychiatry*. 2004:1–15.
234. Ng QX, Venkatanarayanan N, Ho CY. Clinical use of hypericum perforatum (St John's wort) in depression: a meta-analysis. *J Affect Disord*. 2017;210:211–221. https://doi.org/10.1016/j.jad.2016.12.048. Epub 2017 Jan 3.

235. Güzelcan Y, Scholte WF, Assies J, Becker HE. Mania during the use of a combination preparation with St.John's wort (Hypericum perforatum). *Ned Tijdschr Geneeskd*. 2001;145(40):1943–1945.
236. Chrubasik-Hausmann S, Vlachojannis J, McLachlan AJ. Understanding drug interactions with St John's wort (*Hypericum perforatum L.*): impact of hyperforin content. *J Pharm Pharmacol*. 2019;71(1):129–138. https://doi.org/10.1111/jphp.12858. Epub 2018; Feb 7.
237. Muthuramalingam A, Menon V, Rajkumar RP, Negi VS. Is depression an inflammatory disease? Findings from a cross-sectional study at a tertiary care center. *Indian J Psychol Med*. 2016;38(2):114–119.
238. Glaus J, von Känel R, Lasserre AM, et al. Mood disorders and circulating levels of inflammatory markers in a longitudinal population-based study. *Psychol Med*. 2018;48(6):961–973. https://doi.org/10.1017/S0033291717002744. Epub 2017; Sep 20.
239. Ng QX, Koh SSH, Chan HW, Ho CYX. Clinical use of curcumin in depression: a meta-analysis. *J Am Med Dir Assoc*. 2017;18(6):503–508. https://doi.org/10.1016/j.jamda.2016.12.071. Epub 2017; Feb 22.
240. Brietzke E, Mansur RB, Zugman A, et al. Is there a role for curcumin in the treatment of bipolar disorder? *Med Hypotheses*. 2013;80(5):606–612. https://doi.org/10.1016/j.mehy.2013.02.001. Epub 2013 Feb 26.
241. Leweke FM, Piomelli D, Pahlisch F, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry*. 2012;2:e94.
242. O'Sullivan SE. An update on PPAR activation by cannabinoids. *Br J Pharmacol*. 2016;173(12):1899–1910.
243. Beale C, Broyd SJ, Chye Y, et al. Prolonged cannabidiol treatment effects on hippocampal subfield volumes in current cannabis users. Cannabis Cannabinoid Res. 2018;3, No. 1. Published Online:1.
244. Crippa JA, Derenusson GN, Ferrari TB, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol*. 2011;25(1):121–130.
245. Cannabidiol as an adjunctive treatment for bipolar depression (CBDBD). https://clinicaltrials.gov/ct2/show/NCT03310593. Accessed January 26, 2019.
246. Zhao X, Wang C, Zhang JF, et al. Chronic curcumin treatment normalizes depression-like behaviors in mice with mononeuropathy: involvement of supraspinal serotonergic system and GABAA receptor. *Psychopharmacology (Berl)*. 2014 May;231(10):2171–2187. https://doi.org/10.1007/s00213-013-3368-2. Epub 2013 Dec 3.
247. Miyasaka LS, Atallah AN, Soares BG. Passiflora for anxiety disorder. *Cochrane Database Syst Rev*. 2007;1:CD004518.
248. Panossian A, Wikman G, Sarris J. Rosenroot (*Rhodiola rosea*): traditional use, chemical composition, pharmacology and clinical efficacy. *Phytomedicine*. 2010;17(7):481–493. https://doi.org/10.1016/j.phymed.2010.02.002.
249. Mao JJ, Xie SX, Zee J, et al. Rhodiola rosea versus sertraline for major depressive disorder: a randomized placebo-controlled trial. *Phytomedicine*. 2015;22(3):394–399.
250. Fournier JC, DeRubeis RJ, Hollon SD, et al. Antidepressant drug effects and depression severity: a patient-level meta-analysis. *JAMA*. 2010;303(1):47–53.
251. Lopresti AL, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum Psychopharmacol*. 2014;29(6):517–527. https://doi.org/10.1002/hup.2434.
252. Tóth B, Hegyi P, Lantos T, et al. The efficacy of saffron in the treatment of mild to moderate depression: a meta-analysis. *Planta Med*. 2019;85(1):24–31. https://doi.org/10.1055/a-0660-9565.
253. Modabbernia A, Sohrabi H, Nasehi AA, et al. Effect of saffron on fluoxetine-induced sexual impairment in men: randomized double-blind placebo-controlled trial. *Psychopharmacology (Berl)*. 2012;223(4):381–388.
254. Kashani L, Raisi F, Saroukhani S, et al. Saffron for treatment of fluoxetine-induced sexual dysfunction in women: randomized double-blind placebo-controlled study. *Hum Psychopharmacol*. 2013;28(1):54–60. https://doi.org/10.1002/hup.2282.
255. Maleki-Saghooni N, Mirzaeii K, Hosseinzadeh H, Sadeghi R, Irani M. A systematic review and meta-analysis of clinical trials on saffron (*Crocus sativus*) effectiveness and safety on erectile dysfunction and semen parameters. *Avicenna J Phytomed*. 2018;8(3):198–209.
256. Pilkington K, Kirkwood G, Rampes H, Fisher P, Richardson J. Homeopathy for depression: a systematic review of the research evidence. *Homeopathy*. 2005;94(3):153–163.
257. Grimaldi-Bensouda L, Abenhaim L, Massol J, et al. Homeopathic medical practice for anxiety and depression in primary care: the EPI3 cohort study. *BMC Complement Altern Med*. 2016;16:125. https://doi.org/10.1186/s12906-016-1104-2.
258. Davidson JR, Crawford C, Ives JA, Jonas WB. Homeopathic treatments in psychiatry: a systematic review of randomized placebo-controlled studies. *J Clin Psychiatry*. 2011;72(6):795–805. https://doi.org/10.4088/JCP.10r06580.
259. Dwivedi T, Zhang H. Lithium-induced neuroprotection is associated with epigenetic modification of specific BDNF gene promoter and altered expression of apoptotic-regulatory proteins. *Front Neurosci*. 2015;8:457. https://doi.org/10.3389/fnins.2014.00457.
260. Little HR, Kramer JM, Beatty JA, Waldrop TG. Chronic exercise increases GAD gene expression in the caudal hypothalamus of spontaneously hypertensive rats. *Brain Res Mol Brain Res*. 2001;95(1–2):48–54.
261. Ota M, Wakabayashi C, Sato N, et al. Effect of L-theanine on glutamatergic function in patients with schizophrenia. *Acta Neuropsychiatr*. 2015;27(5):291–296. https://doi.org/10.1017/neu.2015.22.
262. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimaki M. Cumulative meta-analysis of interleukins 6 and 1beta, tumour necrosis factor alpha and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun*. 2015;49:206–215.
263. Gong P, Fan H, Liu J, Yang X, Zhang K, Zhou X. Revisiting the impact of OXTR rs53576 on empathy: a population-based study and a meta-analysis. *Psychoneuroendocrinology*. 2017;80:131–136. https://doi.org/10.1016/j.psyneuen.2017.03.005.
264. Morhenn V, Beavin LE, Zak PJ. Massage increases oxytocin and reduces adrenocorticotropin hormone in humans. *Altern Ther Health Med*. 2012;18(6):11–18.
265. Cui SS, Bowen RC, Gu GB, Hannesson DK, Yu PH, Zhang X. Prevention of cannabinoid withdrawal syndrome by lithium: involvement of oxytocinergic neuronal activation. *J Neurosci*. 2001;21(24):9867–9876.
266. Petrilli MA, Kranz TM, Kleinhaus K, et al. The emerging role for zinc in depression and psychosis. *Front Pharmacol*. 2017;8:414. https://doi.org/10.3389/fphar.2017.00414.
267. Tao S, Chattun MR, Yan R, et al. TPH-2 gene polymorphism in major depressive disorder patients with early-wakening symptom. *Front Neurosci*. 2018;12:827. https://doi.org/10.3389/fnins.2018.00827.
268. Pfeffer CR, Altemus M, Heo M, Jiang H. Salivary cortisol and psychopathology in children bereaved by the September 11, 2001 terror attacks. *Biol Psychiatry*. 2007;61:957–965.
269. Entringer S, Kumsta R, Hellhammer DH, Wadhwa PD, Wüst S. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Horm Behav*. 2009;55(2):292–298.
270. Beddoe AE, Lee KA. Mind-body interventions during pregnancy. *J Obstet Gynecol Neonatal Nurs*. 2008;37(2):165–175. https://doi.org/10.1111/j.1552-6909.2008.00218.x.
271. Clapp M, Aurora N, Herrera L, Bhatia M, Wilen E, Wakefield S. Gut microbiota's effect on mental health: the gut-brain axis. *Clin Pract*. 2017;7(4):987. https://doi.org/10.4081/cp.2017.987.
272. Engelberg D, McCutcheon A, Wiseman S. A case of ginseng-induced mania. *J Clin Psychopharmacol*. 2001;21:535–537.
273. Sarris J, Murphy J, Mischoulon D, et al. Adjunctive nutraceuticals for depression: a systematic review and meta-analyses. *Am J Psychiatry*. 2016;173(6):575–587.
274. Kanchanatawan B, Tangwongchai S, Sughondhabhirom A, et al. Add-on treatment with curcumin has antidepressive effects in Thai patients with major depression: results of a randomized double-blind placebo-controlled study. *Neurotox Res*. 2018;33(3):621–633. https://doi.org/10.1007/s12640-017-9860-4.
275. Nemets B, Stahl Z, Belmaker RH. Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *Am J Psychiatry*. 2002;159(3):477–479.
276. Coppen A, Chaudhry S, Swade C. Folic acid enhances lithium prophylaxis. *J Affect Disord*. 1986;10(1):9–13.

277. Taylor MJ, Carney S, Geddes J, Goodwin G. Folate for depressive disorders. *Cochrane Database Syst Rev*. 2003;4.
278. Levine J, Mishori A, Susnosky M, Martin M, Belmaker RH. Combination of inositol and serotonin reuptake inhibitors in the treatment of depression. *Biol Psychiatry*. 1999;45(3):270–273.
279. Dalton J, Rotondi D, Levitan RD, et al. Use of slow-release melatonin in treatment-resistant depression. *J Psychiatry Neurosci*. 2000;25(1):48–52.
280. Berlanga C, Ortega-Soto H, Ontiveros M, Senties H. Efficacy of S adenosyl-L-methionine in speeding the onset of action of imipramine. *Psychiatry Res*. 1992;44:257–262.
281. Modabbernia A, Sohrabi H, Nasehi AA, et al. Effect of saffron on fluoxetine-induced sexual impairment in men: randomized double-blind placebo-controlled trial. *Psychopharmacology (Berl)*. 2012;223(4):381–388.
282. Kashani L, Raisi F, Saroukhani S, et al. Saffron for treatment of fluoxetine-induced sexual dysfunction in women: randomized double-blind placebo-controlled study. *Hum Psychopharmacol*. 2013;28(1):54–60. https://doi.org/10.1002/hup.2282.
283. Levitan RD, Shen JH, Jindal R, Driver HS, Kennedy SH, Shapiro CM. Preliminary randomized double-blind placebo-controlled trial of tryptophan combined with fluoxetine to treat major depressive disorder: antidepressant and hypnotic effects. *J Psychiatry Neurosci*. 2000;25(4):337–346.
284. Nowak G, Siwek M, Dudek D, et al. Effect of zinc supplementation on antidepressant therapy in unipolar depression: a preliminary placebo-controlled study. *Pol J Pharmacol*. 2003;55(6):1143–1147.
285. Ranjbar E, Kasaei MS, Mohammad-Shirazi M, et al. Effects of zinc supplementation in patients with major depression: a randomized clinical trial. *Iran J Psychiatry*. 2013;8(2):73–79.

143

Alcohol Dependence

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Physical signs of excess alcohol consumption: alcohol odor on breath, flushed face, tremor, ecchymoses
- Alcoholic binges, benders (i.e., 48 or more hours of drinking associated with failure to meet usual obligations), or blackouts
- Evidence of alcohol-induced illnesses: cirrhosis, gastritis, pancreatitis, myopathy, polyneuropathy, cerebellar degeneration
- Psychological/social signs of excess alcohol consumption: depression, loss of friends, arrest for driving while intoxicated, surreptitious drinking, drinking before breakfast, frequent accidents, unexplained work absences
- Alcohol dependence manifested when alcohol is withdrawn: tremulousness, convulsions, hallucinations, delirium

GENERAL CONSIDERATIONS

Alcohol dependence, alcohol-use disorder, or, as it was formerly known, alcoholism is a disabling addictive disorder characterized by alcohol consumption that exceeds acceptable cultural limits or injures health or social relationships. Although alcohol (ethanol) can be directly toxic, with resulting symptoms occurring quickly and transiently, toxicity is dose-dependent, and small amounts may be beneficial. Excess is clearly a serious clinical problem in North America, with a prevalence of lifetime alcohol abuse of 17.8% and a prevalence of 12-month alcohol abuse of 4.7%. In the last edition of the TBNM, estimates were that a prevalence of lifetime and 12-month alcohol dependence in the United States is 12.5% and 3.8%, respectively.[1] Alcohol dependence is significantly more prevalent among men, whites, Native Americans, younger and unmarried adults, and people with lower incomes. Alcohol dependence affects more than 18 million Americans, making it one of the most serious health problems facing physicians today.[1] The total number of Americans affected, either directly or indirectly, is much greater when one considers disruption of family life, automobile accidents, crime, decreased productivity, and mental and physical diseases. With more than 100,000 deaths annually attributed to alcohol misuse, alcohol-related problems are a cause of considerable mortality.[2] As indicated in Box 143.1, the health, social, and economic consequences of alcohol dependence are alarming.

Physicians should consider alcohol dependence when the information provided by the patient and the doctor's own analysis seems to indicate a missing factor. Often, alcohol dependence is a "hidden" disease. The natural consequences of the alcoholic's behavior may be disguised by sympathetic family and friends. This circumstance allows the alcoholic to target other factors as the "real problem," without identifying his or her drinking behavior. Table 143.1 provides an alcohol-dependence–screening questionnaire.

The etiology of alcohol dependence remains obscure. It represents a multifactorial condition involving genetic, physiological, psychological, and social factors, each of which seems to be important. Serious drinking often starts in younger people; approximately 35% of alcoholics develop their first symptoms between 15 and 19 years of age, and more than 80% develop their first symptoms before age 30.[3]

Alcohol dependence is most common in men, but the incidence has been increasing in women. Although the figures were once more disparate, the female-to-male ratio for alcohol dependence has tapered to 1:2.[1,2] Women generally seem to develop disease at a lower level of intake than men do. This difference may be partially because of women's lower volume of distribution for alcohol and may also be related to increased gut permeability to endotoxins.[4]

Genetic Susceptibility

Research indicates that genetic and epigenetic factors may be particularly important (Fig. 143.1).[5] For example, individuals with the inactive ALDH2 allele are poor eliminators of acetaldehyde. The resulting accumulation of acetaldehyde produces unpleasant effects from alcohol (e.g., nausea, vomiting, flushing, increased heart rate), and consequently, these individuals consume little to no alcohol. The finding of a genetic marker for susceptibility to alcohol dependence could result in the diagnosis of the disease in its initial and most reversible stage. Some case–control studies suggest that non–gender-based gene polymorphisms encoding cytokines and other immune modulators may play a role in the predisposition to alcoholism. The gene patterns associated

BOX 143.1 Consequences of Alcohol Dependence

Increased Mortality
- 10- to 12-year decrease in life expectancy
- Double the usual death rate in men, triple in women
- Six times greater suicide rate
- Major factor in the four leading causes of death in men between the ages of 25 and 44: Accidents, homicides, suicides, cirrhosis

Economic Toll (Yearly)
- Lost production: $14.9 billion
- Health care costs: $8.3 billion
- Accident and fire losses: $5 billion
- Cost of violent crime: $1.5 billion
- Total costs (health care, accidents, violence, lost productivity): $136 billion

Health Effects
- Metabolic damage to every cell
- Intoxication
- Abstinence and withdrawal syndromes
- Nutritional diseases
- Cerebellar degeneration
- Cerebral atrophy
- Psychiatric disorders
- Esophagitis, gastritis, ulcer
- Increased cancers of the mouth, pharynx, larynx, and esophagus
- Pancreatitis
- Fatty degeneration and cirrhosis of the liver
- Arrhythmias
- Myocardial degeneration
- Hypertension
- Angina
- Hypoglycemia
- Decreased protein synthesis
- Increased serum and liver triglycerides
- Decreased serum testosterone
- Myopathy
- Neuropathy
- Osteoporosis
- Rosacea, spider veins
- Coagulation disorders

Effects on Fetus
- Growth retardation
- Mental retardation
- Fetal alcohol syndrome
- Teratogenicity

Modified from Hyman SE, Cassem NH. Alcoholism. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American; 1997:III:1-12, 13.

TABLE 143.1 The Brief Michigan Alcohol Dependence Screening Test

1.	Do you feel you are a normal drinker?	Yes (0)	No (2)
2.	Do friends or relatives think you are a normal drinker?	Yes (0)	No (2)
3.	Have you ever attended a meeting of Alcoholics Anonymous (AA)?	Yes (5)	No (0)
4.	Have you ever lost friends or girlfriends or boyfriends because of drinking?	Yes (2)	No (0)
5.	Have you ever gotten into trouble at work because of drinking?	Yes (2)	No (0)
6.	Have you ever neglected your obligations, your family, or your work for 2 or more days in a row because you were drinking?	Yes (2)	No (0)
7.	Have you ever had delirium tremens (DTs), severe shaking, heard voices, or seen things that were not there after heavy drinking?	Yes (2)	No (0)
8.	Have you ever gone to anyone for help about your drinking?	Yes (5)	No (0)
9.	Have you ever been in a hospital because of drinking?	Yes (5)	No (0)
10.	Have you ever been arrested for drunk driving or driving after drinking?	Yes (2)	No (0)

Alcohol dependence is indicated by a score above 5.
Modified from Hyman SE, Cassem NH. Alcoholism. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American, 1997:III, 1-12, 13

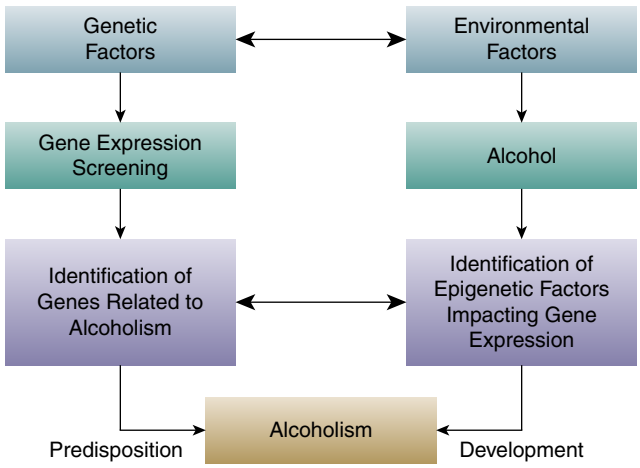


Fig. 143.1 A hypothetical model for the interactions of genetic and environmental factors in the predisposition to and development of alcoholism. (From Starkman BG, Sakharkar AJ, Pandey SC. Epigenetics—beyond the genome in alcoholism. *Alcohol Res*. 2012;34[3]:293-305. PubMed PMID: 23134045.)

with risk reveal that antibody-mediated mechanisms may play a role in disease pathogenesis.[4] The genetic basis of alcohol dependence has also been supported by the following:

- Genealogical studies showing that alcohol dependence is a family condition
- Studies of adopted children of alcoholic parents raised by foster parents demonstrating a continued higher risk of alcohol dependence
- Twin studies showing differences between identical and nonidentical twins
- Association with genetic markers: color vision, nonsecretor ABH, HLA-B_{13}, and low platelet monoamine oxidase (MAO)
- Biochemical studies showing the importance of alcohol dehydrogenase polymorphism in racial susceptibility to alcohol dependence.[5]

Several studies have shown that the incidence of alcohol dependence is four to five times more common in the biological children of alcoholic parents than in biological children of nonalcoholic parents.[5] Although it would be ultimately useful, knowledge of family history suggests that clear evidence of a biological marker may not be necessary for the implementation of a relatively innocuous primary prevention program.

Alcohol Toxicity and Glutathione

The diverse toxic effects of alcohol result not only from the variation in the levels of breakdown products but also from the depletion of glutathione. This depletion results in the upregulation of gamma glutamyl transferase (GGT) to provide more glutathione, likely for Phase II conjugation, as well as to neutralize oxidative stress. Cellular GGT metabolizes extracellular GSH, allowing the precursor cysteine to be reused for de novo synthesis of intracellular GSH.

There are some major limitations in the use of GGT as a measure of excessive alcohol consumption:

1. GGT also elevates by exposure to other chemicals, especially persistent organic pollutants (POPs) and several prescription drugs.
2. Genetics and nutrient availability will affect GGT's sensitivity to alcohol consumption.
3. Some chronic imbibers (upward of 9 drinks per day) have GGT in the "normal" range.

Individuals have great variation in their ability to produce glutathione in response to toxic and oxidative challenge. Nonetheless, GGT is most beneficial when baseline levels are determined on an individual basis. In a uniform population, GGT will increase in direct proportion to alcohol consumption. In a nonuniform population, 40 g of ethanol per day will elevate GGT ~15%, whereas 60 g per day for 5 weeks in young men will almost double GGT from 27 to 52 u/L. However, there is much individual variation. In general, GGT goes back to "normal" after abstinence for 1 month. Early research shows promise in using glutathione transferase (GSTA1) as a measure for advising patients on their alcohol consumption, as GSTA1 has been shown to be a sensitive and reliable marker in ethanol-induced hepatic injury.[6]

INTOXICATION AND WITHDRAWAL

The signs of alcoholic intoxication are typical of a central nervous system depressant: drowsiness, errors of commission, disinhibition, dysarthria, ataxia, and nystagmus. Fifteen milliliters of pure alcohol (the equivalent of 1 oz of whiskey, 4 oz of wine, or 10 oz of beer) raise the blood level of alcohol by 25 mg/dL in a 70-kg person. Table 143.2 shows the effects of varying blood levels of alcohol.

Withdrawal symptoms usually occur 1 to 3 days after the last drink. Animal models, as well as findings obtained in humans, have shed light on the effects that acute and chronic alcohol exposure have on signaling systems involving the neurotransmitters glutamate, gamma-aminobutyric acid (GABA), dopamine, and serotonin, as well as on other signaling molecules, including endogenous opioids and corticotrophin-releasing factor (CRF) (Fig. 143.2).[7] Adaptation to chronic

TABLE 143.2 Effects of Varying Levels of Blood Alcohol

Blood Level (MG/DL)	Effect
<50	No significant motor dysfunction
100	Mild intoxication—decreased inhibitions, slight visual impairment, slight muscular incoordination, slowing of reaction time
	Legally intoxicated in most jurisdictions
150	Ataxia, dysarthria, slurring of speech, nausea and vomiting
350	Marked muscular incoordination, blurred vision, approaching stupor
500	Coma and death

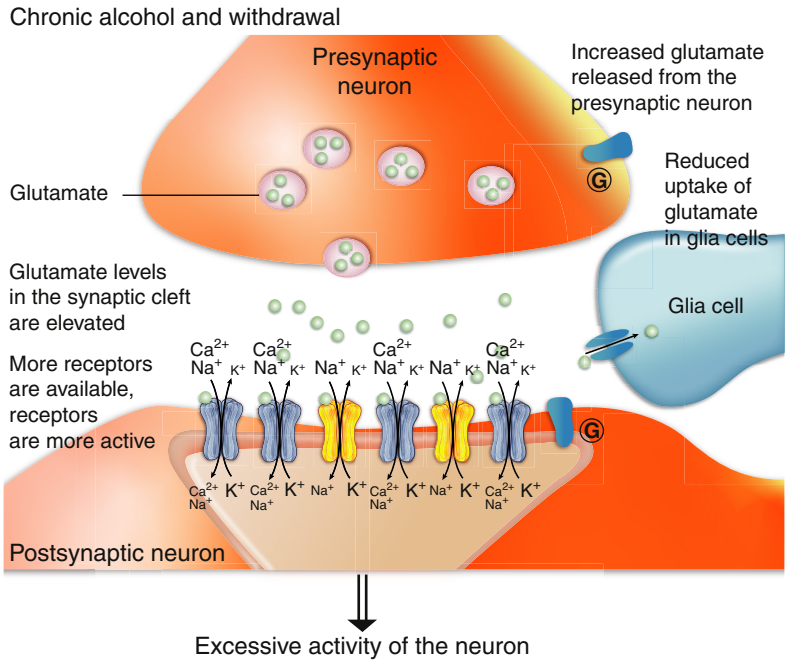


Fig. 143.2 Actions of the brain's glutamate system. After chronic alcohol exposure and during withdrawal, glutamate release at the synapse is enhanced and the number of synaptic N-methyl-d-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) is increased. As a result, glutamate induces excessive activity of the postsynaptic neuron. (From Clapp P, Bhave SV, Hoffman PL. How adaptation of the brain to alcohol leads to dependence: a pharmacological perspective. *Alcohol Res Health*. 2008;31[4]:310-339. PubMed PMID: 20729980.)

alcohol exposure by these systems has been associated with several effects ranging from anxiety and tremulousness to mental confusion, tremor, sensory hyperactivity, visual hallucinations, autonomic hyperactivity, diaphoresis, dehydration, electrolyte disturbances, seizures, and cardiovascular abnormalities.

METABOLIC EFFECTS OF ALCOHOL AND ALCOHOL DEPENDENCE

Ethanol Metabolism

The primary metabolic processes that regulate the rate of alcohol catabolism in normal individuals are[8]:

- The rate of ethanol absorption
- The concentration and activity of liver alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)
- The reduced nicotinamide adenine dinucleotide (NADH)/nicotinamide adenine dinucleotide (NAD)+ ratio in the liver mitochondria.

It is generally accepted that the availability and regeneration of NAD+ are the dominant rate-limiting factors for ethanol oxidation.[9] Ethanol is converted to acetaldehyde by ADH, with NAD+ as a necessary cofactor.

The aldehyde product of ethanol metabolism is believed to be responsible both for many of the harmful effects of alcohol consumption and for the addictive process itself. Acetaldehyde has been shown to promote cancer by interfering with the replication of DNA and by inhibiting the body's ability to repair damaged DNA.[10] Alcohol use increases the risk of developing a variety of cancers, including colon, liver, breast, throat, mouth, and upper respiratory tract cancers. Higher-than-normal blood aldehyde levels have been found in alcoholics and their relatives after alcohol consumption.[9] This finding suggests either increased ADH activity or depressed ALDH activity in people susceptible to alcohol dependence. Acetaldehyde is converted by ALDH to acetate, with little entering the Krebs cycle; most is converted to long-chain fatty acids.[8]

Fatty Liver

All active alcoholics display fatty infiltration of the liver, with the severity roughly proportional to the duration and degree of alcohol excess. Even moderate doses of ethanol may produce both acute and chronic fatty liver infiltrates. The pathogenesis is due to the following[8,11]:

- Increased endogenous fatty acid synthesis
- Diminished triglyceride utilization
- Impaired lipoprotein excretion
- Direct damage to endoplasmic reticulum by free radicals produced by ethanol metabolism
- The high-fat diet of the alcoholic (as is typical of the average American diet).

Leptin is a peptide hormone involved in the regulation of appetite and energy metabolism. It is most likely directly related to liver pathology in alcoholics. High levels of leptin are known to contribute to liver pathology, including increased rates of fibrosis, a known factor in liver steatosis.[12] Research has demonstrated increased circulating leptin levels in a dose-dependent manner in chronic alcohol dependence regardless of nutritional status.[13]

Hypoglycemia

Alcohol induces reactive hypoglycemia. The resultant drop in blood sugar produces a craving for food, particularly foods that quickly elevate blood sugar (e.g., sugar and alcohol). Increased sugar consumption aggravates the reactive hypoglycemia, particularly in the presence of alcohol, owing to alcohol-induced impairment of gluconeogenesis.[8] Hypoglycemia aggravates the mental and emotional problems of the alcoholic and the withdrawing alcoholic with symptoms such as:

- Sweating
- Tremor
- Tachycardia
- Anxiety
- Hunger
- Dizziness
- Headache
- Visual disturbance
- Decreased mental acuity
- Confusion
- Depression

THERAPEUTIC CONSIDERATIONS

Nutrition

Although many of the nutritional problems of alcoholics relate directly to the effects of alcohol, a major contributing factor is that alcoholics tend not to eat, substituting alcohol for food. As a result, the alcoholic has to deal not only with nutritional deficiencies caused by excessive alcohol consumption but also with true nutritional deficiencies due to inadequate intake.

Zinc

One of the key nutrients involved in the metabolic handling of alcohol is zinc, because both ADH and ALDH are zinc-dependent enzymes, with the latter being more sensitive to deficiency.[14] Both acute and chronic alcohol consumption result in zinc deficiency.[14,15] Several factors contribute to the development of zinc deficiency in alcoholics:

- Decreased dietary intake
- Decreased ileal absorption (probably due to interference with the zinc-binding ligand, picolinic acid, and nonspecific mucosal damage)
- Hyperzincuria

On hair analysis, higher zinc and copper values have been observed in male alcoholics versus nonalcoholics.[16] Hair copper was significantly related to the amount of ethanol consumed, whereas hair zinc was higher in consumers of distilled beverages. These data may indicate abnormal metabolism and loss of these minerals in the alcoholic. The clinical symptoms of hypozincemia and copper deficiency in individuals addicted to alcohol often relate to disorders in central nervous system functioning, and they result in decreases in physical and mental quality of life. Low serum zinc levels are associated with impaired alcohol metabolism, a predisposition to cirrhosis, impaired testicular function, and other complications of alcohol abuse.[14,17] Zinc supplementation, particularly when combined with ascorbic acid, greatly increases alcohol detoxification and survival in rats.[18]

Vitamin A

Vitamin A deficiency is also common in alcoholics and appears to work synergistically with zinc deficiency to produce the major complications of alcohol dependence.[11,17] The mechanism has been hypothesized as reduced intestinal absorption of zinc and vitamin A in conjunction with impaired liver function (reduced extraction of zinc, mobilization of retinol binding protein [RBP], and storage of vitamin A), resulting in reduced blood levels of zinc, vitamin A, RBP, and transport proteins as well as a shift to nonprotein ligands. These cause the tissues to have reduced concentrations of zinc and vitamin A, abnormal enzyme activities and glycoprotein synthesis, and impaired DNA/RNA metabolism and cause the kidneys to increasingly lose zinc. These metabolic abnormalities then lead to the common disorders of alcohol dependence[17]:

- Night blindness
- Skin disorders

- Cirrhosis of the liver
- Reduced skin healing
- Decreased testicular function
- Impaired immune function

Vitamin A supplementation inhibits alcohol consumption in female rats, an effect inhibited by testosterone administration.[19] Further research supports the significance of the endocrine system in alcohol preference. Ovariectomized and adrenalectomized rats show a decreased preference for alcohol, whereas corticosterone injections increase alcohol preference.[20]

Vitamin A supplementation in the alcoholic has corrected vitamin A deficiency, as noted by improvements in night blindness and sexual function.[11] Despite the importance and benefits of vitamin A to the alcoholic, great care must be employed in recommending vitamin A supplementation. Excessive amounts of vitamin A are contraindicated owing to the fact that a liver damaged by excessive alcohol consumption significantly loses its ability to store vitamin A. As a result, the alcoholic is at great risk for developing vitamin A toxicity when it is supplemented at dosages above 5000 IU a day.

Antioxidants

Ethanol consumption increases lipid peroxidation in humans, resulting in increased lipoperoxide levels in both the liver and serum. This increase is aggravated, considering that alcoholics are typically deficient in key antioxidant nutrients, particularly vitamin E, selenium, and vitamin C.[21,22] There is a significant correlation between serum lipid peroxide levels, serum glutamic oxaloacetic transaminase (SGOT) activity, and liver cell necrosis.[23] Antioxidant administration, either before or together with ethanol intake, inhibits lipoperoxide formation and prevents fatty infiltration of the liver.[24] Effective antioxidants include vitamins C and E, zinc, selenium, and cysteine.

Carnitine

Although the use of lipotropic agents appears warranted in treating alcoholic fatty liver disease, many commonly used lipotropic agents (e.g., choline, niacin, cysteine) appear to have little value.[25,26] One lipotropic agent, carnitine, significantly inhibits alcohol-induced fatty liver disease. It has been suggested that chronic ethanol consumption results in a functional deficiency in carnitine.[27-29] Because carnitine normally facilitates fatty acid transportation and oxidation in the mitochondria, a high liver carnitine level may be necessary to handle the increased fatty acid load produced by alcohol consumption. Supplemental carnitine reduces serum triglycerides and levels of SGOT while also elevating high-density-lipoprotein cholesterol.[27]

Amino Acids

Amino acid chromatography patterns are aberrant in alcoholics; restoration to normal levels greatly aids the alcoholic patient.[30-33] Because the liver is the primary site of amino acid metabolism, it is not surprising that alcoholics develop abnormal amino acid patterns. Normalization of plasma amino acids is particularly indicated in those patients showing signs or symptoms of hepatic cirrhosis or depression. The branched-chain amino acids—valine, isoleucine, and leucine—inhibit the hepatic encephalopathy and increased protein catabolism that are common sequelae of cirrhosis.[33] Methionine, a known lipotropic, must be activated to S-adenosylmethionine. However, in severe liver disease, the activity of the corresponding enzyme is depressed. In this case supplementation with methionine is not as useful. S-adenosylmethionine would be indicated to ameliorate associated deficiencies and resulting pathology.[34] Derangement of neurotransmitter profiles, particularly due to the low plasma levels of tryptophan typically seen in withdrawing alcoholics, will result if these profiles are not normalized, leading to depression, encephalopathy, and coma.[30,32]

Anomalous amino acid profiles are aggravated by the low-protein diet used as standard therapy for cirrhosis, but this problem can be avoided through the use of free-form amino acids without the risk of hepatic encephalopathy.[30,32] (See "Depression" later for a further discussion of amino acid imbalances.)

Although there are some characteristic amino acid abnormalities in alcoholics, an individual approach is indicated owing to differences in nutritional status, biochemistry, and the amount of liver damage. Correction of the imbalances probably requires amino acid chromatography for best results.

Vitamin C

In one study, a deficiency of ascorbic acid was found in 91% of patients with alcohol-related diseases.[35] Supplemental ascorbic acid helped ameliorate the effects of acute and chronic ethanol toxicity in experimental studies involving humans and guinea pigs, two species unable to synthesize their own ascorbic acid.[18,36] There is a direct correlation between leukocyte ascorbic acid levels (a good index of actual body ascorbic acid status), the rate of ethanol clearance from the blood, and the activity of hepatic ADH.[17] The evidence suggests that ascorbic acid, a strong reducing agent, can function as an electron donor similar to NAD in ethanol metabolism, thereby increasing the conversion of ethanol to acetaldehyde and the further catabolism of acetaldehyde.[18,36]

Selenium

Plasma selenium is clearly lower in alcoholic patients. Serum, erythrocyte, and whole-blood levels of selenium are also decreased in patients with alcohol dependence.[22] Low selenium status encourages depressed mood, whereas high dietary or supplementary selenium has been shown to improve mood.[37] Research has consistently reported that low selenium status was associated with a significantly increased incidence of depression, anxiety, confusion, and hostility.[22] Furthermore, when alcohol dependence and depression occur together, there is an increased risk for suicide.[38]

Given the propensity for low selenium status in alcoholic patients and the relation between selenium levels and mood disorders, selenium supplementation is warranted in an attempt to ameliorate the untoward comorbid psychological and physical profile of patients with alcohol abuse or dependence.

B Vitamins

Alcoholics are classically deficient in most of the B vitamins.[1,11,35] These deficiencies result from various mechanisms:

- Low dietary intake
- Deactivation of the active form
- Impaired conversion to the active form by ethanol or acetaldehyde
- Impaired absorption
- Decreased storage capacity.

Alcohol diminishes thiamine absorption in the intestine and reduces hepatic thiamine storage. It also decreases the phosphorylation of thiamine, leading to a reduction of the active form of thiamine, which also may contribute to development of thiamine deficiency.[39] A thiamine deficiency is both the most common (55% in one study[35]) and the most serious of the B vitamin deficiencies, because a deficiency causes beriberi and the Wernicke-Korsakoff syndrome. In addition, evidence indicates that a thiamine deficiency results in greater intake of alcohol, suggesting that thiamine deficiency is a predisposing factor for alcohol dependence.[40] It should be noted that, once present, Wernicke-Korsakoff syndrome is refractory to oral doses of thiamine; therefore, rapid replacement of depleted brain thiamine levels by repeated parenteral therapy is required.[41]

A functional pyridoxine deficiency is also common in alcoholics, mostly due to impaired conversion to its active form, pyridoxal-5-phosphate, and enhanced degradation.[42] In addition to inhibiting conversion to more active forms, alcohol also decreases the absorption and utilization by the liver and increases the urinary excretion of many B vitamins, especially folic acid.[11,43]

Magnesium

Magnesium deficiency is common in alcoholics. In fact, hypomagnesemia is present in as many as 60% of alcoholics and is strongly linked to delirium tremens.[44] In alcohol-dependent patients, studies suggest measuring ionized magnesium in erythrocytes and plasma as a diagnostic parameter as opposed to total serum magnesium, because this intracellular biologically active form provides a more complex picture of the body's condition.[45] The lower the concentration of ionized magnesium, the worse the quality of life an alcohol-dependent person might experience. It is thought to be the major reason for the increased cardiovascular disease noted in alcoholics.[46] This deficiency is due primarily to a reduced magnesium intake coupled with alcohol-induced renal hyperexcretion of magnesium,[1,44] which continues during withdrawal despite low serum magnesium levels. Alcoholic cardiomyopathy, often associated with thiamine deficiency, may instead be due to a magnesium deficiency.

Essential Fatty Acids

Ethanol has been shown to interfere with essential fatty acid (EFA) metabolism and may produce symptoms of essential fatty acid (EFA) deficiency if consumed in excess.[47] A 5-year study of alcohol-consuming rhesus monkeys linked a marginal EFA diet to alcoholic amblyopia, a rare neuropathy characterized by blurred vision, diminished retinal function, and a reduction in visual acuity.[48] Brain biopsies showed that the fatty acid docosahexaenoic acid (DHA) composition of brain specimens had decreased significantly in comparison with controls. In the retinas of the alcohol-consuming animals at 5 years, there was a similar decrease in DHA. It may be useful to support neurological function by giving EFAs to prevent these ophthalmological sequelae.

Glutamine

Glutamine supplementation (1 g per day) has been shown to reduce voluntary alcohol consumption in uncontrolled human studies and experimental animal studies.[49-51] Although this research occurred more than 50 years ago, there has never been any follow-up to these preliminary studies. The failure to follow up this line of research is unfortunate, because the results were promising and showed the supplement to be safe and relatively inexpensive.

Psychosocial Aspects

Psychological and social measures are critical in the treatment of the alcoholic patient. It is important for the physician to maintain a nonjudgmental but not passive attitude toward the patient. Alcohol dependence should be viewed as a chronic, progressive, addictive, and potentially fatal disease, although many aspects of the alcoholic's behavior may make it difficult to maintain this objectivity.[1]

Social support for both patient and family is important, and success often appears proportional to the involvement of Alcoholics Anonymous (AA), counselors, and other social agencies. Because most physicians have not had adequate training or experience in handling the psychosocial aspects of this problem, it is important to establish a close working relationship with an experienced counselor and AA. Al-Anon and Ala-Teen are useful resources for family members.

Successful initiation of treatment requires the following:

- The patient's agreement that he or she has an alcohol problem
- Education of the patient and family about the physical and psychosocial effects of alcohol dependence
- Immediate involvement of the patient in a treatment program.

Successful programs usually include strict control of drinking and replacement of the alcohol addiction with another addiction that is nonchemical, time consuming, and heavily supported by family, friends, and peers. Although strict abstinence may not be absolutely necessary, at this time it appears the safest and most effective choice.[1]

Depression

Depression is common among alcoholics and is known to lead to their high suicide rate. Many alcoholics are depressed first and later become alcoholic (primary depressives), whereas others become alcoholic first and later develop a depressive condition in the context of their alcohol dependence (secondary depressives). Alterations in the metabolism of serotonin and the availability of its precursor, tryptophan, have been implicated in some forms of depression, whereas other forms have been linked to alterations in catecholamine metabolism and tyrosine availability.

Alcoholics have severely depleted levels of tryptophan, which may explain both the depression and sleep disturbances common in alcohol dependence, because brain serotonin levels depend on circulating tryptophan levels.[52] Ethanol impairs tryptophan transport into the brain. The enzyme tryptophan pyrrolase, considered to be rate-limiting in tryptophan catabolism, is more active in rats during alcohol withdrawal.[31] In one study, 5 of 6 chronic alcoholics had no detectable plasma tryptophan on withdrawal.[30] The tryptophan levels returned to normal after 6 days of treatment and abstinence.

Another factor influencing tryptophan uptake into the brain is competition from amino acids—tyrosine, phenylalanine, valine, leucine, isoleucine, and methionine—that share the same transport; many of these are elevated in malnourished alcoholics.[30,31] Alcoholics have significantly depressed ratios of tryptophan to these amino acids in comparison with normal controls, with depressed alcoholics having the lowest ratios.[31]

The major amino acids, whose elevated levels help lower this ratio, are the catecholamine precursors, tyrosine and phenylalanine. Elevated plasma catecholamine levels are common in alcoholics and may contribute to depression.[53] An interesting finding is that taurine is also low in depressed alcoholics, with the lowest levels being reported in psychotic alcoholics.[30]

Miscellaneous Factors

Intestinal Flora

The intestinal microflora is severely deranged in alcoholics.[54] Clinical and preclinical data suggest that alcohol-related disorders are associated with quantitative and qualitative dysbiotic changes in the intestinal microbiota and may be associated with increased GIT inflammation, intestinal hyperpermeability resulting in endotoxemia, systemic inflammation, and tissue damage/organ pathologies, including ALD (Fig. 143.3).[55] Colonization of the small intestine by endotoxin-producing bacteria may lead to malabsorption of fats, carbohydrates, protein, folic acid, and vitamin B_{12}. This is probably the cause of the abnormalities of the small intestine commonly found in alcoholics. Alcohol ingestion also increases intestinal permeability to endotoxins and macromolecules, allowing increased toxic and antigenic effects.[56] The ensuing allergic reactions and deposition of immune complexes probably contribute to the many complications of alcohol dependence and, considering the addictive tendency of food allergies, may also contribute to alcoholic cravings.

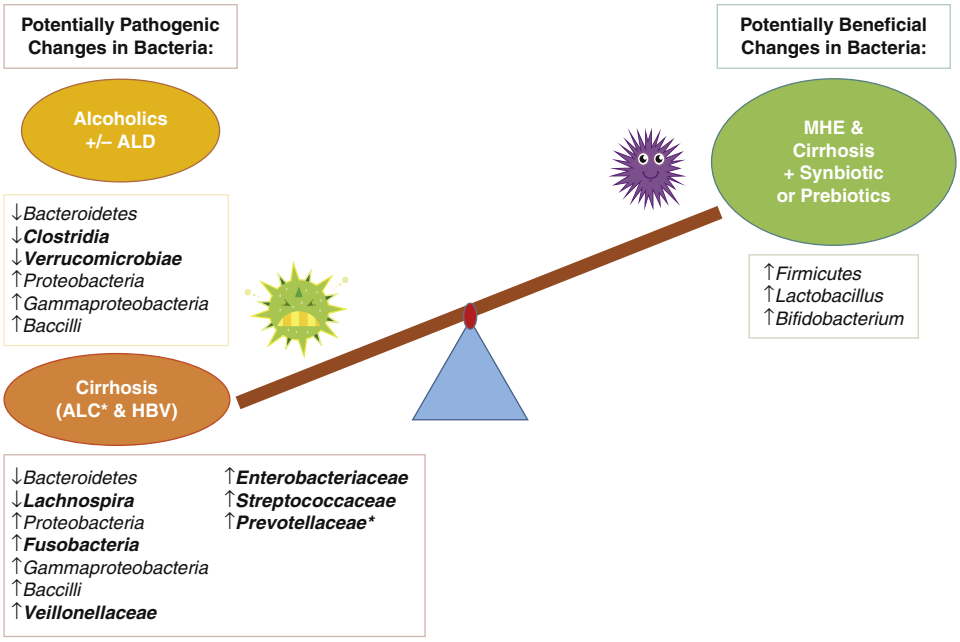


Fig. 143.3 Alcohol-induced imbalances in the microbiome of the gastrointestinal tract (dysbiosis) have been associated with promoting potentially pathogenical changes in bacteria in alcoholics with and without liver disease and in patients with cirrhosis caused by hepatitis B or alcohol. (From Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The gastrointestinal microbiome: alcohol effects on the composition of intestinal microbiota. *Alcohol Res.* 2015;37[2]:223-236. PubMed PMID: 26695747.)

Exercise

Involvement of the alcoholic patient in a graded, individually tailored fitness program has been shown to improve the likelihood of maintaining abstinence.[57] Research has shown that regular exercise is effective in alleviating anxiety and depression and enables individuals to respond better to stress. Improved fitness may allow for more effective responses to emotional upset, thereby reducing the patient's likelihood of resorting to alcohol when he or she is involved in conflict.

Kudzu *(Pueraria lobata)*

Kudzu is one of the earliest medicinal plants used in traditional Chinese medicine. It has many profound pharmacological actions, including antidipsotropic (antialcohol abuse) activity.[58,59] Two of its isoflavones, daidzin and daidzein, account for this effect. It appears to act by inhibiting aldehyde dehydrogenase 2 (ALDH-2).[59] A deficiency of this enzyme reduces the risk of alcohol dependence. Decreased drinking due to ALDH-2 inhibition is attributed to the aversive properties of acetaldehyde accumulated during alcohol consumption. However, daidzin can reduce drinking in some rodents without necessarily increasing acetaldehyde.

In human studies, the results have been mixed. In one study, kudzu treatment resulted in a significant reduction in the number of beers consumed, which was paralleled by an increase in the number of sips and the time to consume each beer and a decrease in the volume of each sip.[60] However, in a double-blind trial, kudzu root extract (1.2 g twice a day) produced no statistically significance difference in craving and sobriety scores in comparison with the placebo group.[61] It may be that kudzu reduces alcohol intake without significantly affecting cravings.

Milk Thistle *(Silybum marianum)*

The flavonoid complex of milk thistle (silymarin) appears to be useful for the alcoholic, especially in the presence of considerable liver involvement or cirrhosis. Silymarin has been shown to be effective in the treatment of the full spectrum of alcohol-related liver disease, from relatively mild to serious cirrhosis. Perhaps the most significant benefit is extending the life span of these patients. In one study, 87 cirrhotics (46 with alcoholic cirrhosis) received silymarin, and 83 cirrhotics (45 with alcoholic cirrhosis) received a placebo.[62] The mean observation period was 41 months. In the treatment group, there were 24 deaths, among which 18 were related to liver disease; among the controls, there were 37 deaths with 31 related to liver disease. The 4-year survival rate was 58% in the treatment group compared with 39% in the controls.

Silymarin can also improve immune function in patients with cirrhosis.[63] Whether this effect is involved in the hepatoprotective action or a result of improved liver function has yet to be determined.

THERAPEUTIC APPROACH

Alcohol dependence is difficult to treat. Although many therapeutic regimens have been attempted, there has been little documented long-term success except for that of AA (and even the overall success of this program is highly controversial). The approach presented here is unique in that we have attempted to develop an integrated, whole-person, stage-oriented program.

The treatment of the alcoholic patient must be optimized for the four stages of alcohol dependence: active alcohol consumption, withdrawal, recovery, and recovered. The recovery stage is defined here as the period between withdrawal and full reestablishment of normal metabolic function. All alcoholics, at whatever stage, benefit the most by employing simultaneous counseling, lifestyle, and metabolism-balancing therapies. The following are the recommended therapies in the usual context, with additional recommendations for each stage. Also, a complete diagnostic workup is necessary owing to the high risk that the alcoholic patient will develop a wide variety of clinical and subclinical diseases.

Recommendations for All Four Stages

Diet

Stabilization of blood sugar levels is critical to successful treatment. Although a strict hypoglycemic diet may not be warranted, most of the dietary guidelines must be followed. These guidelines include elimination of all simple sugars (foods containing added sucrose, fructose, or glucose; fruit juice; dried fruit; and low-fiber fruits such as grapes and citrus fruits); limitation of processed carbohydrates (e.g., white flour, instant potatoes, white rice); and an increase in complex carbohydrates (e.g., whole grains, vegetables, beans).

Supplements

- Vitamin A: 5000 IU a day
- Vitamin B complex: 20 times the recommended daily allowance
- Vitamin C: 1 g two times a day
- Vitamin E: 400 IU a day (d–α-tocopherol)
- Magnesium: 250 mg two times a day
- Selenium: 200 mg a day
- Zinc: 30 mg a day (picolinate)
- Carnitine: 500 mg two times a day (L-carnitine)
- Glutamine: 1 g a day
- *Lactobacillus acidophilus:* 1 tsp a day

Exercise

A graded program should be established, using heart-rate response to determine intensity. The patient should exercise 5 to 7 times a week for 20 to 30 minutes at an intensity sufficient to raise the heart rate to 60% to 80% of maximum for the age group.

Counseling

The physician dealing with alcoholism should have a good working relationship with AA and an experienced counselor who has particular expertise in working with alcoholics.

Additional recommendations for the four stages follow.

Specific Recommendations for Each Stage

Stage I: Active Alcohol Consumption

The physician should work with family, peers, social contacts, and whomever else is involved with the patient to elicit his or her recognition of an alcohol problem and willingness to enter a treatment program.

Additional supplements include the following:

- Pyridoxal-5-phosphate: 20 mg a day
- Riboflavin: 100 mg a day
- Vitamin A: 5000 IU a day
- Zinc: 30 mg a day

Stage II: Withdrawal

The severity of symptomatology varies widely, although it is usually proportional to the degree of physiological dependence and duration of the disease. Milder cases usually start within a few hours after cessation of drinking and typically resolve within 48 hours. More severe cases usually occur only in patients older than 30 years of age and usually develop after about 48 hours of abstinence. These patients should be admitted to an inpatient facility. Some institutions may not allow the use of supplements; this policy should be checked before a patient is admitted.

Additional supplements include the following:

- Tryptophan: 3 g a day
- Riboflavin: 100 mg a day
- Electrolyte replacement as necessary
- Flaxseed oil: 1 tbsp three times a day

Stage III: Recovering

A strong network of caring family, friends, and peers should be established, if possible, to regularly support the patient, who would do well to become involved and busy with intense, people-oriented activities. It is also vital for the patient to recognize that alcohol is no answer to the stresses of life; he or she should be helped to develop more effective ways of handling adversity.

Stage IV: Recovering to Advanced Recovery

The patient's support group must always be maintained. Continued total abstinence is the best policy, although carefully controlled drinking may be possible. After a 6-month abstinence, supplement dosages may be slowly reduced to 25% of the initial dosages.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Hasin DS, Stinson FS, Ogburn E, et al. Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch Gen Psychiatry*. 2007;64(7):830–842.
2. Enoch MA, Goldman D. Problem drinking and alcoholism: diagnosis and treatment. *Am Fam Physician*. 2002;65:441–448.
3. Rohde P, Lewinsohn PM, Kahler CW, et al. Natural course of alcohol use disorders from adolescence to young adulthood. *J Am Acad Child Adolesc Psychiatry*. 2001;40:83–90.
4. Day CP. Who gets alcoholic liver disease: nature or nurture? *J R Coll Physicians Lond*. 2000;34:557–562.
5. Kimura M, Higuchi S. Genetics of alcohol dependence. *Psychiatry Clin Neurosci*. 2011;65(3):213–225.
6. Chang YC, Liu FP, Ma X, Li MM, Li R, et al. Glutathione S-transferase A1 – a sensitive marker of alcoholic injury on primary hepatocytes. *Hum ExpToxicol*. 2016 [Epub ahead of print].
7. Clapp P, Bhave SV, Hoffman PL. How adaptation of the brain to alcohol leads to dependence: a pharmacological perspective. *Alcohol Res Curr Rev*. 2008;31(4):310–339. PubMed PMID: 20729980.
8. Pohorecky LA, Brick J. Pharmacology of ethanol. *Pharmacol Ther*. 1988;36:335–427.
9. Tipton KF, Heneman GTM, McCrodden JM. Metabolic and nutritional aspects of alcohol. *Biochem Soc Trans*. 1983;11:59–61.
10. Seitz HK, Becker P. Alcohol metabolism and cancer risk. *Alcohol Res Health*. 2007;30(1):38–47.
11. Lieber CS. Alcohol, liver, and nutrition. *J Am Coll Nutr*. 1991;10:602–632.
12. Piche T, Vandenbos F, Abakar-Mahamat A, et al. The severity of liver fibrosis is associated with high leptin levels in chronic hepatitis C. *J Viral Hepat*. 2004;11:91–96.
13. Nicolas JM, Fernandez-Sola J, Fatjo F, et al. Increased circulating leptin levels in chronic alcoholism. *Alcohol Clin Exp Res*. 2001;25:83–88.
14. Das I, Burch RE, Hahn HK. Effects of zinc deficiency on ethanol metabolism and alcohol and aldehyde dehydrogenase activities. *J Lab Clin Med*. 1984;104:610–617.
15. Wu CT, Lee JN, Shen WW, et al. Serum zinc, copper, and ceruloplasmin levels in male alcoholics. *Biol Psychiatry*. 1984;19:1333–1338.
16. Gonzalez-Reimers E, Aleman-Valls MR, Barroso-Guerrero F, et al. Hair zinc and copper in chronic alcoholics. *Biol Trace Elem Res*. 2002;85:269–275.
17. Scholmerich J, Lohle E, Kottgen E, et al. Zinc and vitamin A deficiency in liver cirrhosis. *Hepato-Gastroenterol*. 1983;30:119–125.
18. Yunice AA, Lindeman RD. Effect of ascorbic acid and zinc sulphate on ethanol toxicity and metabolism. *Proc Soc Exp Biol Med*. 1977;154:146–150.
19. Messiha FS. Vitamin A, gender and ethanol interactions. *Neuorbehav Toxicol Teratol*. 1983;5:233–236.
20. Morin LP, Forger NG. Endocrine control of ethanol intake by rats or hamsters: relative contributions of the ovaries, adrenals and steroids. *Pharmacol Biochem Behav*. 1982;17:529–537.
21. Lecomte E, Herbeth B, Pirollet P. Effect of alcohol consumption of blood antioxidant nutrients and oxidative stress indicators. *Am J Clin Nutr*. 1994;60:255–261.
22. Sher L. Role of selenium depletion in the etiopathogenesis of depression in patients with alcoholism. *Med Hypotheses*. 2002;59:330–333.
23. Suematsu T, Matsumura T, Sato N, et al. Lipid peroxidation in alcoholic liver disease in humans. *Alcohol Clin Exp Res*. 1981;5:427–430.
24. DiLuzio NR. A mechanism of the acute ethanol-induced fatty liver and the modification of liver injury by antioxidants. *Am J Pharm Sci Support Public Health*. 1966;15:50–63.
25. Stanko RT, Mendelow H, Shinozuka H, et al. Prevention of alcohol-induced fatty liver by natural metabolites and riboflavin. *J Lab Clin Med*. 1978;91:228–235.
26. Hartroft WS, Porta EA, Suzuki M. Effects of choline chloride on hepatic lipids after acute ethanol intoxication. *Q J Stuc Alcohol*. 1964;25:427–437.
27. Sachan DS, Rhew TH, Ruark RA. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. *Am J Clin Nutr*. 1984;39:738–744.
28. Hosein EA, Bexton B. Protective action of carnitine on liver lipid metabolism after ethanol administration to rats. *Biochem Pharmacol*. 1975;24:1859–1863.
29. Sachan DA, Rhew TH. Lipotropic effect of carnitine on alcohol-induced hepatic stenosis. *Nutr Rep Int*. 1983;27:1221–1226.
30. Majumdar SK, Shaw GK, Thomson AD. Changes in plasma amino acid patterns in chronic alcoholic patients during ethanol withdrawal syndrome: their clinical implications. *Med Hypotheses*. 1983;12:239–251.
31. Branchey L, Branchey M, Shaw S, et al. Relationship between changes in plasma amino acids and depression in alcoholic patients. *Am J Psychiatry*. 1984;141:1212–1215.
32. Rosen HM, Yoshimura N, Hodgman JM, et al. Plasma amino acid patterns in hepatic encephalopathy of differing etiology. *Gastroenterology*. 1977;72:483–487.
33. Fischer JE, Rosen HM, Ebeid AM, et al. The effect of normalization of plasma amino acids on hepatic encephalopathy. *Surgery*. 1976;80:77–91.
34. Lieber CS. Hepatic, metabolic, and nutritional disorders of alcoholism: from pathogenesis to therapy. *Crit Rev Clin Lab Sci*. 2000;37:551–584.
35. Baines M. Detection an incidence of B and C vitamin deficiency in alcohol-related illness. *Ann Clin Biochem*. 1978;15:307–312.
36. Yunice AA, Hsu JM, Fahmy A, et al. Ethanol-ascorbate interrelationship in acute and chronic alcoholism in the Guinea pig. *Proc Soc Exp Biol Med*. 1984;177:262–271.
37. Finley JW, Penland JG. Adequacy or deprivation of dietary selenium in healthy men: clinical and psychological findings. *Trace Elem Exp Med*. 1998;11:11–27.
38. Cornelius JR, Salloum IM, Mezzich J, et al. Disproportionate suicidality in patients with comorbid major depression and alcoholism. *Am J Psychiatry*. 1995;152:358–364.
39. Koike H, Mori K, Misu K, et al. Painful alcoholic polyneuropathy with predominant small-fiber loss and normal thiamine status. *Neurology*. 2001;56:1727–1732.
40. Zimatkin SM, Zimatkina TI. Thiamine deficiency as predisposition to, and consequence of, increased alcohol consumption. *Alcohol Alcohol*. 1996;31:421–427.
41. Thomson AD. Mechanisms of vitamin deficiency in chronic alcohol misusers and the development of the Wernicke-Korsakoff syndrome. *Alcohol Alcohol*. 2000;35(suppl 1):2–7.
42. Lumeng L. The role of acetaldehyde in mediating the deleterious effect of ethanol on pyridoxal 5′-phosphate metabolism. *J Clin Invest*. 1978;62:286–293.
43. McMartin KE, Collins TD, Bairnsfather L. Cumulative excess urinary excretion of folate in rats after repeated ethanol treatment. *J Nutr*. 1986;116:1316–1325.
44. Jermain DM, Crismon ML, Nisbet RB. Controversies over the use of magnesium sulfate in delirium tremens. *Ann Pharmacother*. 1992;26:650–652.
45. Ordak M, Maj-Zurawska M, Matsumoto H, et al. Ionized magnesium in plasma and erythrocytes for the assessment of low magnesium status in alcohol dependent patients. *Drug Alcohol Dependence*. 2017;178:271–276. PubMed PMID: 28683422.
46. Abbott L, Nadler J, Rude RK. Magnesium deficiency in alcoholism: possible contribution to osteoporosis and cardiovascular disease in alcoholics. *Alcohol Clin Exp Res*. 1994;18:1076–1082.
47. Horrobin DF. A biochemical basis for alcoholism and alcohol-induced damage including the fetal alcohol syndrome and cirrhosis: interference with essential fatty acid and prostaglandin metabolism. *Med Hypotheses*. 1980;6:929–942.
48. Pawlosky RJ, Bacher J, Salem Jr N. Ethanol consumption alters electroretinograms and depletes neural tissues of docosahexaenoic acid in rhesus monkeys: nutritional consequences of a low n-3 fatty acid diet. *Alcohol Clin Exp Res*. 2001;25:1758–1765.
49. Rogers LL, Pelton RB, Williams RJ. Voluntary alcohol consumption by rats following administration of glutamine. *J Biol Chem*. 1955;214:503–506.
50. Rogers LL, Pelton RB. Glutamine in the treatment of alcoholism. *Q J Stud Alcohol*. 1957;18:581–587.
51. Ravel JM, Felsing B, Lansford Jr EM, et al. Reversal of alcohol toxicity by glutamine. *J Biol Chem*. 1955;214:497–501.

52. Branchey L, Shaw S, Lieber CS. Ethanol impairs tryptophan transport into the brain and depresses serotonin. *Life Sci*. 1981;29:2751–2755.
53. Ireland MA, Vandongen R, Davidson L, et al. Acute effects of moderate alcohol consumption on blood pressure and plasma catecholamines. *Clin Sci (Lond)*. 1984;66:643–648.
54. Bode JC, Bode C, Heidelbach R, et al. Jejunal microflora in patients with chronic alcohol abuse. *Hepatogastroenterology*. 1984;31:30–34.
55. Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The gastrointestinal microbiome: alcohol effects on the composition of intestinal microbiota. *Alcohol Res Curr Rev*. 2015;37(2):223–236. PubMed PMID:26695747.
56. Worthington BS, Meserole L, Syrotuck JA. Effect of daily ethanol ingestion on intestinal permeability to macromolecules. *Am J Dig Dis*. 1978;23: 23–32.
57. Sinyor D, Brown T, Rostant L, et al. The role of a physical fitness program in the treatment of alcoholism. *J Stud Alcohol*. 1982;43:380–386.
58. Keung WM, Vallee BL. Kudzu root: an ancient Chinese source of modern antidipsotropic agents. *Phytochemistry*. 1998;47(4):499–506.
59. Arolfo MP, Overstreet DH, Yao L, et al. Suppression of heavy drinking and alcohol seeking by a selective ALDH-2 inhibitor. *Alcohol Clin Exp Res*. 2009;33(11):1935–1944.
60. Lukas SE, Penetar D, Berko J, et al. An extract of the Chinese herbal root kudzu reduces alcohol drinking by heavy drinkers in a naturalistic setting. *Alcohol Clin Exp Res*. 2005;29(5):756–762.
61. Shebek J, Rindone JP. A pilot study exploring the effect of kudzu root on the drinking habits of patients with chronic alcoholism. *J Altern Complement Med*. 2000;6(1):45–48.
62. Ferenci P, Dragosics B, Dittrich H. Randomized controlled trial of Silymarin treatment in patients with cirrhosis of the liver. *J Hepatol*. 1989;9:105–113.
63. Deak G, Muzes G, Lang I, et al. Immunomodulator effect of silymarin therapy in chronic alcoholic liver diseases. Hungarian. *Orvosi Hetilap*. 1990;131. 1291–1292. 1295-1296.

144

Alzheimer's Disease

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Progressive mental deterioration, loss of memory and cognitive functions, inability to carry out activities of daily life
- Characteristic symmetrical, usually diffuse electroencephalographic (EEG) pattern
- Diagnosis usually made by exclusion
- Definitive diagnosis can be made only by postmortem biopsy of the brain, demonstrating atrophy, senile plaques, and neurofibrillary tangles

GENERAL CONSIDERATIONS

Alzheimer's disease (AD) is a neurodegenerative disorder that clinically manifests itself as a progressive deterioration of memory and cognition or dementia. In the United States the prevalence of AD is now estimated to be 1.6% before the age of 74, with the rate increasing to 19% in the 75- to 84-age group and to 42% in those above 84 years of age. These numbers are striking compared with data from the 1960s, indicating an incidence of only 2% in people over the age of 85 years. The tremendous increase in AD in people over 85 years of age is often referred to as the "Alzheimer's epidemic."

Neuropathology

AD has distinctive neuropathologic features that include[1]:

- Plaque formation
- Amyloid deposition
- Neurofibrillary tangles
- Granulovacuolar degeneration
- The massive loss of telencephalic neurons.

These findings are particularly evident in the cerebral cortex and hippocampal formation.

The clinical features of AD are believed to be related to cholinergic dysfunction due to appreciable reductions in the activity of the enzyme choline acetyltransferase, the enzyme that synthesizes acetylcholine, and the neuronal transfer of choline.

Etiology

The core pathological hallmarks of AD are an accumulation of β-amyloid, neuroinflammation, and the formation of neurofibrillary tangles. *Amyloid* is a general term for protein fragments that the body produces normally. Beta-amyloid is a fragment snipped from an amyloid precursor protein (APP). In a healthy brain, these fragments are broken down and eliminated. In AD, β-amyloid protein fragments accumulate to form hard plaques between neurons, blocking the transmission of messages and leading to the death of brain cells, neurofibrillary tangles, and ultimately, dementia. Tau, a microtubule-associated protein, is the major constituent of neurofibrillary tangles and is produced in soluble hyperphosphorylated form when β-amyloid levels become toxic. Persistent activation of glial cells, a hallmark of neuroinflammation, is now considered a key abnormality in AD.[2]

Genetic factors play a major role and are estimated to account for up to 70% of cases of AD. Several genes have been linked to AD: the APP on chromosome 21 (explaining the close association between Down syndrome and AD); the presenilin genes on chromosomes 14 and 1; and the apolipoprotein E *(ApoE)* gene on chromosome 19. The first two mutations are rare and associated with symptoms developing before the age of 50. The most significant genetic finding is the link with the ApoE gene. *ApoE*, a cholesterol carrier in the brain, works to clear amyloid deposits from the parenchyma of the brain; however, at

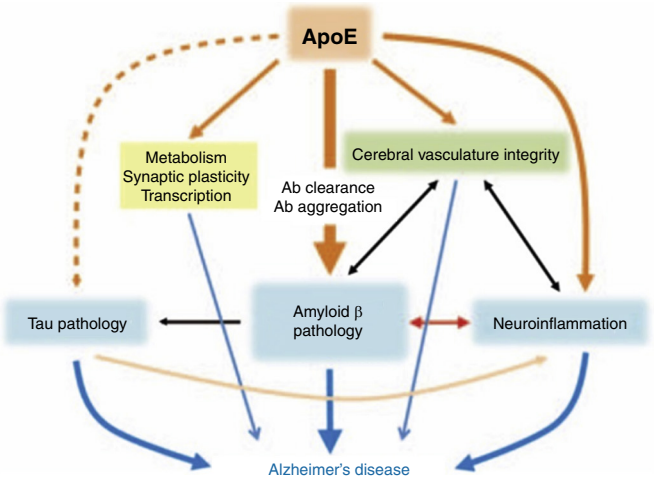


Fig. 144.1 ApoE-mediated pathogenical pathways, leading to Alzheimer's disease. (From Liao F, Yoon H, Kim J. Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's disease. *Curr Opin in Lipidol.* 2017;28[1]:60-67.)

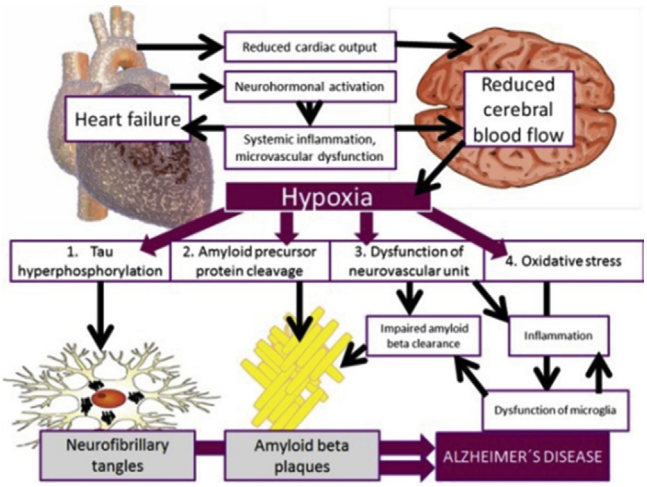


Fig. 144.2 Model of the relationship between heart failure and Alzheimer's disease. (From Carmakova P, Eriksdotter M, Lund LH, Winblad B, Religa P, Religa D. Heart failure and Alzheimer's disease. *J Intern Med.* 2015;277[4]:406-25.)

least 40% of all patients with AD have a genetic variant of APOe4 gene that increases this protein, leading to a significantly greater risk for AD. The e2-type is associated with significant protection. Although the effect of ApoE isoforms on amyloid β metabolism have been considered as its main role in Alzheimer's disease, emerging data suggest that ApoE may also affect AD pathogenesis through amyloid β independent pathways (Fig. 144.1).

Literature is also accumulating regarding the role of genetically linked aberrant immune system regulation of inflammation as a possible contributor to AD. Although innate immune function in the brain is normally modulated to remove plaque in an attempt to maintain health, research is beginning to characterize a chronic and excessive reaction to immune protofibrils of amyloid proteins in the brain that can promote disease.[3] As a result, immunotherapeutic approaches have recently been developed for the treatment of AD. Chief among these strategies is to immunize AD patients with Aβ peptides so that they will generate antibodies that bind to Aβ protein and enhance its clearance.[4] Although preclinical studies were successful, the initial human clinical trial of an active Aβ vaccine was halted owing to the development of meningoencephalitis in approximately 6% of the vaccinated AD patients.

Although genes play a significant role in determining susceptibility to AD, lifestyle and environmental factors also play significant roles. Emerging research reveals that dietary factors are important. Poor-quality diets with excess saturated or trans–fatty acids may predispose neurons to environmental toxicities.[5,6] Some data suggest that abnormal sleep–wake cycles and decreased morning light exposure may play a role in the expression of AD. Traumatic injury to the head; chronic exposure to aluminum, silicon, or both; exposure to neurotoxins from environmental sources; and free radical damage have all been implicated as causative factors as well. As in the pathophysiology of other chronic degenerative diseases, there is considerable evidence that increased oxidative damage plays a central role. Therapies designed to support antioxidant mechanisms may be quite helpful in the prevention of AD.[7]

The tremendous increase in AD parallels the rise in type 2 diabetes and insulin resistance, suggesting a possible connection. It is well established that individuals with type 2 diabetes have a 1.5- to 4-fold higher than normal risk for AD and for vascular dementia. Impaired insulin signaling and insulin resistance in the brain or the decrease in cerebral insulin receptors associated with aging may be another important factor in the pathogenesis of AD. Furthermore, hyperglycemia induces increased peripheral utilization of insulin, resulting in reduced insulin transport into the brain. Insufficient insulin signaling make neurons energy-deficient and vulnerable to oxidizing or other metabolic insults, leading to the destruction of mitochondria and ultimately the neuron. Cerebral hypoinsulinemia (as well as hyperinsulinemia) leads to β-amyloid accumulation and increased tau phosphorylation. Measures to improve glycemic control and improve both peripheral and brain insulin sensitivity appear to be important steps in the prevention of AD.[8,9]

Other known risk factors include family history, hypertension and hypotension, high cholesterol levels, low levels of physical activity, obesity, and heart failure. Reduced cerebral blood flow and dysfunction of the neurovascular unit appear to be the main reasons heart failure contributes to the development of AD (Fig. 144.2).[10] However, multiple cardiovascular conditions often coexist, suggesting that several mechanisms underlying cardiovascular dysfunction may contribute to cognitive decline.

Environmental Toxins

Aluminum

Considerable attention has focused on the association of aluminum concentration in the neurofibrillary tangle (NFT). Whether the aluminum concentration develops in response to AD or whether it initiates the lesions has not yet been determined, but significant evidence shows that it contributes, possibly significantly, to the disease.

Aluminum has a strong affinity for the paired helical filament tau (PHF-t), which is involved in the formation of the NFTs. In fact, studies have demonstrated that aluminum is the cofactor along with this tau filament in the formation of the NFTs.[11] The aluminum selectively binds to PHF-t, induces PHF-t to aggregate, and retards the brain's ability to break down PHF-t. Aluminum's role in AD is further supported by an animal study that evaluated long-term exposure to ecologic doses of aluminum. In this study, researchers found ghostlike neurons with cytoplasmic and nuclear vacuolations, together with aluminum deposits. The hippocampus contained neuritic plaques, and the cerebrovasculature exhibited amyloid deposits. Researchers also found behavioral changes in these rats reminiscent of those observed in AD.[12]

A great deal of circumstantial evidence links chronic aluminum exposure to AD. Increasing aluminum concentrations in the brain could explain why AD increases with increasing age. A study of 356 healthy people showed that serum aluminum concentration increases as people age.[13] Individuals with AD have significantly higher aluminum levels than both healthy people and patients with other types of dementias, such as those due to alcohol, atherosclerosis, and stroke. Efforts to remove the aluminum appear to help somewhat, but such measures are probably too late once the disease is well established. For example, intramuscular injections of deferoxamine (a chelating agent for the removal of iron and aluminum) over a 2-year period showed a significant slowing of the rate of decline in 48 AD patients.[14]

Even in people without mental disease, elevated aluminum levels are associated with poorer mental function. In a study of patients undergoing dialysis, the 13 patients who had a positive aluminum deferoxamine test (a measure of the amount of aluminum in the body) were compared with 13 who had a negative test. Subjecting the entire group to four attention tests and two memory tests revealed that those with higher levels of aluminum had a moderate to considerable disturbance of mental function.[14]

The aluminum appears to come from the water supply, food, antacids, and antiperspirants. The most significant source is drinking water, because the aluminum in water is in a more bioavailable and thus potentially a more toxic form. Researchers measured the aluminum absorption of tap water by adding a small amount of soluble aluminum in a radioactive form to the stomachs of animals. They discovered that the trace amounts of aluminum from this single exposure immediately entered the animals' brain tissue. The frightening news is that aluminum in water occurs not only naturally but is also added (in the form of alum) to treat some water supplies.[15]

Particulate Matter

Air pollution, already shown to begin and fuel neuroinflammation, has also been implicated in increased levels of amyloid deposits and tau protein formation. Particulate matter (PM) can penetrate throughout the body, including the brain, where it is closely linked to inflammation. Making PM particularly toxic is the adsorption of toxic volatile organic compounds (VOCs) to their surface, bypassing normal protective systems. Postmortem assessment of the brains of cognitively intact persons revealed that those who lived in areas of higher vehicular air pollution had greater inflammatory markers and β-amyloid in their frontal cortex and hippocampus (the areas needed for memory function) along with their olfactory lobe.[16]

Children living in the metropolitan area of Mexico City with both pre- and postnatal exposure to high levels of $PM_{2.5}$ have been found to have increased hyperphosphorylation of tau, along with increased levels of β-amyloid.[17] Those children also exhibited decreased cognitive function (attention, memory and IQ) and decreased olfactory sense. The children with APOe4 had greater problems, especially with olfaction.[18]

Pesticides

Pesticide use and exposure has also been strongly associated with higher risks for developing AD. A large study in the southern Spain comprised 17,420 subjects whose hospital records were reviewed between 1998 and 2005. The subjects were classified according to the agricultural pesticide usage in the regions that they lived in. Person living in regions with higher pesticide use were over twice as likely to develop AD and 87% more likely to commit suicide.[19] A study of 1507 elderly French individuals revealed that those who had been occupationally exposed to pesticides in their younger years were 2.39 times more likely to develop AD than were individuals in other occupations.[20]

The largest prospective study to look at the relationship between pesticide use and AD was done in Cache County, Utah.[21] More than 3000 individuals above the age of 65 were enrolled and screened for dementia at baseline and classified according to pesticide-exposure levels. Cognitive function was reassessed after 3, 7, and 10 years. After adjusting for various factors, including APOE4 allele status, it was found that study participants with higher pesticide exposures were 38% more likely to have dementia and 42% more likely to develop AD. When just organophosphates and organochlorine pesticides were singled out, researchers found that chlorinated pesticide exposure increase AD risk by 49%, and organophosphate use increased it by 53%.

DIAGNOSTIC CONSIDERATIONS

A comprehensive diagnostic workup is paramount in the approach to the patient with dementia. It is important to rule out conditions that can mimic dementia. For example, depression, which can mimic dementia in the elderly, is common. Table 144.1 lists other possible causes of dementia. The most common reversible cause of dementia is drug toxicity. Other important causes are metabolic and nutritional disorders, such as hypoglycemia; thyroid disturbances; and vitamin B_{12}, folate, and thiamine deficiencies.

A comprehensive evaluation should include[22]:

- A detailed history
- Neurological and physical examinations
- Psychological evaluation with particular attention to depression
- A general medical evaluation with emphasis on the detection of subtle metabolic, toxic, or cardiopulmonary disorders that can precipitate confusion, especially in the elderly
- A series of standardized neurophysiology tests such as the Mini Mental State Examination or Folstein test to document the type and severity of cognitive impairment.

Table 144.2 lists recommended tests for appropriate laboratory assessment.

The EEG is an important diagnostic tool serving to differentiate types of dementia. Although a normal EEG does not rule out the diagnosis of dementia, particularly in its early stages, it does provide valuable information. AD is associated with a characteristic symmetrical, usually diffuse slowing of the EEG. More important, the EEG differentiates focal (e.g., intracranial mass or vascular disease) from diffuse (e.g., metabolic disorders or normal-pressure hydrocephalus) brain dysfunction.

Computed tomography or magnetic resonance imaging with single photon emission computed tomography (SPECT) or positron emission tomography (PET) can help exclude other cerebral pathology or subtypes of dementia.

A technique known as PiB PET has been developed for directly and clearly imaging amyloid deposits in vivo, using a chemical tracer that binds selectively to the Aβ deposits. This modality shows tremendous promise.

Fingerprint Patterns

Abnormal fingerprint patterns are associated with both AD and Down syndrome.[23] In comparison with the normal population, AD and Down syndrome patients show an increased number of ulnar loops on the fingertips, with a concomitant decrease in whorls, radial loops, and arches. Ulnar loops (pointing toward the ulnar bone, away from the thumb) are frequently found on all 10

TABLE 144.1 Causes of and Mechanisms in the Development of Senile Dementia

Etiology	Pathogenesis
Degenerative etiology	Disturbances of gene expression and thus of protein metabolism
Altered genetic code	Disturbance of the synthesis of specific proteins
Alzheimer's disease	Reduction in acetylcholine synthesis resulting from decreased choline acetyltransferase activity
Huntington's chorea	Disturbance of the GABA-nergic system
Idiopathic dementia	
Localized form	Decline in cognitive function
Parkinson's disease	Reduction in dopamine turnover
Pick's disease	Reduction in cholinergic activity
Loss of neuronal redundancy	Disturbance of cerebral metabolism after infection or trauma
	Reduction in cholinergic activity caused by loss of neurons and synapses
Cerebrovascular disease	
Chronic meningitis	
Tuberculous, mycotic	
Encephalomyelitis	
Encephalopathy after head injury (boxers)	
Epileptic dementia	
Virus encephalopathy	
Nutritive etiology	
Chronic alcoholism	
Diabetes mellitus	
Disturbances of electrolyte metabolism	
Hypoglycemia	Insulin, starvation
Hyponatremia	Diuretics
Hypothyroidism	
Korsakoff's syndrome	Thiamine transketolase deficiency
Nicotinamide deficiency	
Vitamin B deficiency	Disturbances of energy formation
Toxic etiology	
Addiction to barbiturates, psychotropic drugs, etc.	
Chronic carbon monoxide intoxication	
Chronic cesium intoxication	
Mycotoxins	
Renal/hepatic encephalopathy	
Vincristine intoxication	

fingertips. Radial loops (pointing toward the thumb), when they do appear, tend to be shifted away from the index and middle fingers—where they most commonly occur—to the ring and little fingers. In patients with this fingerprint pattern, which is characteristic of AD, it is recommended that an aggressive preventive approach be instituted immediately.

TABLE 144.2 Recommended Laboratory Tests Used in the Diagnosis Dementia

Test	Rationale
CBC	Anemia, infection
VDRL	Syphilis
Electrolytes	Metabolic dysfunction
Liver function tests	Hepatic dysfunction
BUN	Renal dysfunction
TSH, T_4, T_3, T_3U	Thyroid dysfunction
Serum B_{12} and RBC folate	Deficiency
Urinalysis	Renal/hepatic dysfunction
Hair mineral analysis	Heavy metal intoxication
ECG	Heart function
EEG	Focal vs. diffuse
CT scan	Atrophy, intracranial mass

BUN, Blood urea nitrogen; *CBC*, complete blood cell count; *CT*, computed tomography; *ECG*, electrocardiogram; *EEG*, electroencephalogram; *RBC*, red blood count; *TSH*, thyroid-stimulating hormone; *VDRL*, Venereal Disease Research Laboratory.

THERAPEUTIC CONSIDERATIONS

The primary areas of intervention from a natural medicine perspective involve prevention by addressing suspected pathophysiology, avoiding environmental neurotoxins, and using natural measures to improve mental function. In the advanced stages of AD, natural measures provide only limited benefit.

Diet

Dietary factors are clearly important in the etiology of AD. Food choices consistent with the standard American diet are associated with a significant risk for the development of AD. A diet high in saturated fat and trans–fatty acids and low in dietary antioxidants may lead to increased serum and brain concentrations of aluminum and transition metal ions, which are implicated in oxidative stress, potentially leading to the neurological damage characteristic of AD. In addition, poor-quality diets may also increase the prevalence of AD by eliciting cerebral inflammation, which may cause the neurological damage that results in AD.[5,6,14]

Many dietary risk factors for AD are shared with those implicated in atherosclerosis. Likewise, adherence to a Mediterranean diet is associated with decreased cognitive decline, just as it is with a reduced risk for cardiovascular disease. Prospective studies have provided clear evidence that following a Mediterranean diet is associated with slower cognitive decline, reduced risk of progression from mild cognitive impairment (MCI) to AD, reduced risk of AD, and a decreased all-cause mortality in AD patients. These findings suggest a lower risk not only for AD, but also for predementia syndromes and their progression to overt dementia.[24,25]

The key dietary factors from epidemiological data that reduce AD risk are higher fish consumption (and omega-3 fatty acids), monounsaturated fatty acids (primarily from olive oil), light to moderate alcohol use (primarily red wine), and increased nonstarchy vegetable and fruit consumption. It is likely that the combination of all these factors rather than any single one provides the highest degree of protection.[24,25]

Given the ability of the Mediterranean diet to reduce inflammation and improve insulin sensitivity, it is assumed that this action may

be of extreme importance in its ability to reduce AD. However, in a 4-year prospective study, the lower risk of AD demonstrated with the Mediterranean diet did not seem to be mediated by C-reactive protein, fasting insulin, or adiponectin levels.[26] Other aspects of the diet or specific foods are likely responsible, including dietary components that work directly on reducing β-amyloid formation or deposition. For example, polyphenols found in grapes, grape seed extract, and red wine have been shown to prevent β-amyloid formation and promote tau disassembly.[27,28] Pharmacokinetic studies in animals with radiolabeled grape polyphenols show absorption into the brain after oral administration.[29]

Even something as simple as celery (*Apium graveolens*) consumption may offer significant protection against AD. Celery and celery seed extracts contain a unique compound, 3-n-butylphthalide (NBP), that is responsible for both the characteristic odor of celery and its health benefits. In an animal model of AD, NBP treatment significantly improved learning deficits as well as long-term spatial memory. Furthermore, NBP treatment also significantly reduced total cerebral β-amyloid plaque deposition and lowered brain β-amyloid levels. It was also shown that NBP markedly directed amyloid precursor protein processing toward a pathway that precludes β-amyloid formation. The researchers concluded, "NBP shows promising preclinical potential as a multitarget drug for the prevention and/or treatment of AD."[30]

The research on grape polyphenols and NBP stimulates a powerful question. How many other foods contain unique compounds that address the pathophysiology of AD? From preliminary investigations, it appears there are many; especially promising are sources of phenols and polyphenols.[31–35]

Estrogen

Estrogen has been asserted to offer protective and possibly therapeutic benefits in AD. However, the epidemiological and clinical evidence to support the potential benefits of estrogen is contradictory. Sixteen population-based studies have indicated that women on hormone replacement therapy (HRT) had a lower rate of AD.[36] However, the problem with these studies is that the women taking HRT were much healthier before taking the hormones compared with the control group (i.e., women who are prescribed HRT are less likely to have hypertension, diabetes, and history of stroke than nonusers).[36] A randomized, double-blind, placebo-controlled clinical trial evaluated the effects of hormone therapy on amyloid-β deposition in recently postmenopausal women. [37] Participants within 5 to 36 months past menopause in the Kronos Early Estrogen Prevention Study, were randomized to: 1) 0.45mg per day oral conjugated equine estrogens (CEE); 2) 50 μg per day transdermal 17β-estradiol; or 3) placebo pills and patch for 4 years. Oral progesterone (200 mg per day) was given to active treatment groups for 12 days each month. PET imaging was performed in 68 of the 118 participants at the Mayo Clinic approximately 7 years post randomization and 3 years after stopping randomized treatment. Transdermal 17β-estradiol therapy in recently postmenopausal women was associated with a reduced amyloid-β deposition, particularly in apolipoprotein E (APOE)-epsilon4 allele (APOEε4) carriers. Data from the only large randomized controlled trial published to date, the Women's Health Initiative Memory Study, did not confirm these observations and even suggested an increase in dementia risk for women using HRT compared with controls, especially in the cases of women given HRT after menopause.[38] Clinical trials in women with AD have concluded that estrogen therapy does not improve dementia symptoms in women with AD and should definitely be avoided postmenopause.[39–41] Given the cloud of uncertainty about the benefits of HRT, it seems most reasonable to consider that at this point the risks of conventional estrogen therapy to prevent AD outweigh the benefits. See Chapter 196 on menopause for a discussion of the critical differences.

Environmental Toxins

Encouraging the avoidance of all known sources of environmental neurotoxins, especially aluminum and pesticides, is strongly recommended. Eliminating aluminum-containing antacids, using aluminum-containing antiperspirants, cooking in aluminum pots and pans, wrapping food in aluminum foil, and consuming nondairy creamers—certainly seem appropriate. Aluminum is also found in baking powder and table salt to keep them from becoming lumpy. In addition, citric acid, as well as calcium citrate supplements, appear to increase the efficiency of absorption of aluminum (but not lead) from water and food.[42] Aluminum absorption can be decreased by magnesium, because magnesium competes with aluminum for absorption, not only in the intestines but also at the blood-brain barrier.[43] A diet rich in magnesium is recommended, focusing on unprocessed foods, avoiding milk and dairy products, and increasing the consumption of vegetables, whole grains, nuts, and seeds.

The most effective ways to decrease pesticides are to eat organically grown foods and avoid use of pesticides in homes and yards.

NUTRITIONAL CONSIDERATIONS

Nutritional status is directly related to cognitive function in the elderly.[44] Given the frequency of nutrient deficiency in the elderly population, it is likely that many cases of impaired mental function may have a nutritional etiology. As pointed out earlier in this chapter, diet is critically important in the prevention and arrest of AD, with various components working together in a synergistic fashion to address many of the key underlying pathophysiological features of AD.

Antioxidants

Considerable evidence indicates that oxidative damage plays a major role in the development and progression of AD.[7,45,46] Epidemiological evidence suggests that antioxidant nutrients offer significant protection against AD.[5,48] Prospective and clinical studies have primarily focused on vitamins C and E and β-carotene with somewhat favorable results (Table 144.3).[45,48–51] As with other chronic degenerative diseases, better results may be achieved with a broader range of supplemental nutrients. For example, in a French cohort of middle-aged adults, 13 years of daily supplementation of 120 mg of ascorbic acid, 30 mg of vitamin E, 6 mg of β-carotene, 100 mcg of selenium, and 20 mg of zinc compared with placebo was significantly associated with better verbal memory, a cognitive domain that is particularly vulnerable to pathological aging and AD. These results appear to be significantly better than those achieved with vitamins C, E, and β-carotene either alone or in combination.

It is entirely possible (and likely probable) that vitamins E and C, and β-carotene may simply be markers of increased "phytochemical antioxidant" intake rather than playing a significant role on their own. Fruits and vegetables contain an array of antioxidant compounds beyond nutritional antioxidants. Some of these compounds may exert specific effects of considerable benefit against the pathophysiology of AD. Often researchers make the mistake of thinking that the antioxidant activity of a particular fruit or vegetable is due solely to its vitamin C, E, or β-carotene content. However, these nutrient antioxidants often account for only a small fraction of a food's antioxidant effect. For example, these nutrients account for only about 0.5% of the total antioxidant activity of an apple. The overwhelming antioxidant activity of fruits and vegetables comes

TABLE 144.3 Prospective Studies of Antioxidants and the Risk of Alzheimer's Disease

Study	Follow-Up	Findings
Rotterdam Study	6 years	Dietary vitamin E effective (more among current smokers)
Canadian Study of Health and Aging	5 years	Combination of vitamins E and C supplementation and/or multivitamin consumption effective
Chicago Health Aging Study	3.9 years	Dietary vitamin E effective only among non-apoE4 carriers
Washington Heights-Inwood Columbia Aging Project	4 years	No effect of vitamin E (diet or supplement)
Cache County Study	3 years	Vitamin E alone not effective but effective when combined with vitamin C
Honolulu-Asia Aging Study	30 years	Dietary vitamin E not effective
Duke Established Populations for Epidemiological Studies of the Elderly	10 years	No effect of vitamins C and/or E
Group Health Cooperative	5.5 years	No effect of supplemental vitamins E and C alone or in combination

Data from Practico D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal? *Trends Pharmacol Sci*. 2008;29(12):609-615.

from phytochemicals, such as flavonoids, phenols, and other carotenoids.[52] Several phytochemicals are showing tremendous promise in protecting against AD by interfering with the formation and deposition of β-amyloid.[31–33]

Thiamine

Although severe thiamine deficiency is relatively uncommon except in people with alcoholism, many Americans do not consume even the recommended daily allowance (RDA) of 1.5 mg, especially elderly people. In an attempt to gauge the prevalence of thiamine deficiency in the geriatric population, 30 unselected consecutive patients visiting a university outpatient clinic in Tampa, Florida, were tested for thiamine levels. Depending on the thiamine measurement (plasma vs. red blood cell thiamine), low levels (defined as a level below the lowest reference range for younger age groups) were found in 57% and 33%, respectively.[53]

In addition to its role as a nutrient, thiamine also demonstrates some pharmacological effects on the brain. Specifically, it mimics the important neurotransmitter involved in memory—acetylcholine. Thiamine has been shown to potentiate and mimic the effects of acetylcholine in the brain.[54] This effect explains the positive clinical results that have been noted for thiamine, (3 to 8 g per day) in improving mental function in AD and age-related impaired mental function (senility).[55,56] High-dose thiamine supplementation exerts its benefits without side effects.

These results highlight the growing body of evidence that a significant percentage of the geriatric population is deficient in one or more of the B vitamins. Given the essential role of thiamine and other B vitamins in normal human physiology, especially cardiovascular and brain function, routine B-vitamin supplementation appears to be worthwhile in this age group.

Vitamin B_{12}

Vitamin B_{12} deficiency results in impaired nerve function, which can cause numbness, paresthesia, or a burning feeling in the feet as well as impaired mental function, which in the elderly population can mimic AD. Vitamin B_{12} deficiency is thought to be quite common in elders and is a major cause of depression in this age group. Determination of vitamin B_{12} deficiency is best achieved by measuring the level in the blood (serum cobalamin) or the level of methylmalonic acid in the urine. In addition, measurement of the level of plasma homocysteine is an additional method to determine the status of both vitamin B_{12} and folate.

Several investigators have found that the level of vitamin B_{12} declines with age and that vitamin B_{12} deficiency is found in 3% to 42% of persons age 65 and older. Diagnosing cobalamin deficiency early in the elderly population is important because it is easily treatable and, if left untreated, can lead to impaired neurological and cognitive function.[57,58]

In one study among 100 consecutive geriatric outpatients who were seen in office-based settings for various acute and chronic medical illnesses but not for vitamin B_{12} deficiency–related diseases like pernicious anemia, 11 patients had serum cobalamin levels at 148 pmol/L or below; 30 patients had levels between 148 and 295 pmol/L; and 59 patients had levels above 296 pmol/L.[59] After the initial cobalamin determination, the subjects were followed for up to 3 years. The patients with cobalamin levels below 148 pmol/L were treated and not included in the analysis of declining cobalamin levels. The average annual decline in serum cobalamin level was 18 pmol/L for patients who had higher initial serum cobalamin levels (actual range, 224–292 pmol/L). For patients with lower initial cobalamin levels, the average annual serum cobalamin decline was much higher, at 28 pmol/L.

These results indicate that measurement of the level of vitamin B_{12} in the blood (serum cobalamin), the urinary excretion of methylmalonic acid or the level of homocysteine[60] as a screening test for vitamin B_{12} deficiency appears to be indicated in elderly patients, given the positive cost-benefit ratio.[61,62] To test for vitamin B_{12} deficiency, the urinary methylmalonic acid assay is perhaps the best because it is sensitive as well as noninvasive and relatively convenient for the patient. Correction of an underlying vitamin B_{12} deficiency can significantly improve mental function and quality of life in these patients. A growing body of literature demonstrates clear correlations between high levels of serum homocysteine and cardiovascular disease and, more recently, dementia and AD. High homocysteine levels (>14 mmol/L) nearly double the risk of AD.[63]

The importance of a detailed examination in elderly patients with mental symptoms is highlighted by results from a study that analyzed the plasma homocysteine, serum cobalamin, and blood folate in 296 consecutive patients referred to a geriatric psychiatric ward in Sweden for the diagnosis of mental disease.[64] Patients who were deficient in vitamin B_{12} or folic acid or who had elevated levels of homocysteine were given vitamin B_{12} (dosage not specified) or folic acid (10 mg per day), or both. When individuals with low cobalamin levels were supplemented with vitamin B_{12}, significant clinical improvements were noted.

In other studies, supplementation with vitamin B_{12} has shown tremendous benefit in reversing impaired mental function due to low levels of vitamin B_{12}.[57] In one large study, a complete recovery was observed in 61% of similar cases of mental impairment.[65] The fact that 39% did not respond is probably a result of long-term low levels of vitamin B_{12}. Several studies have shown that the best clinical

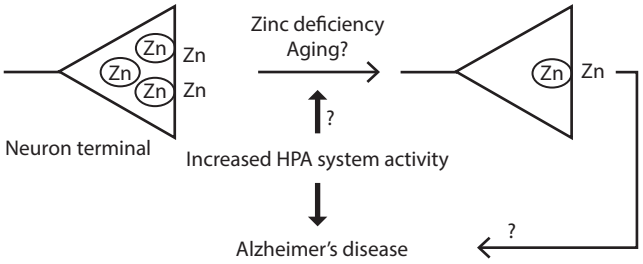


Fig. 144.3 Histochemically reactive zinc level and its relation to the pathogenesis of Alzheimer's disease. (From Takeda A. Insight into glutamate excitotoxicity from synaptic zinc homeostasis. *Int J Alzheimers Dis*, 2011; 491597.)

responders are those who have been showing signs of impaired mental function for less than 6 months.[34] In one study, 18 subjects with low levels of serum cobalamin and evidence of mental impairment were given vitamin B_{12}. Only those patients who had symptoms for less than 1 year showed improvement.[66] The importance of diagnosing and correcting low vitamin B_{12} levels in the elderly population cannot be overstated.

Serum vitamin B_{12} levels are significantly low and vitamin B_{12} deficiency is significantly common in AD patients.[57,67,68] It has been demonstrated that an oral daily dose as low as 50 mcg can significantly increase serum vitamin B_{12} levels in vitamin B_{12}–deficient elderly persons.[69] Supplementation of vitamin B_{12}, folic acid, or both may result in complete reversal in some patients, but generally there is little improvement in patients who have had AD symptoms for more than 6 months. It has been hypothesized that prolonged low levels of vitamin B_{12} may lead to irreversible changes that will not respond to supplementation. Vitamin B_{12} supplementation for elderly subjects with AD will improve hematological parameters but will not usually improve mental function.[70]

Vitamin B_{12} is available in several forms. The most common form is cyanocobalamin; however, vitamin B_{12} is active in the human body in only two forms: methylcobalamin and adenosylcobalamin. Although methylcobalamin and adenosylcobalamin are active immediately on absorption, cyanocobalamin must be converted to either methylcobalamin or adenosylcobalamin by removal of the cyanide molecule and addition of either a methyl or adenosyl group. This conversion may be reduced with aging and may be another factor responsible for the vitamin B_{12} disturbances noted in the elderly population.

Zinc

Zinc deficiency is one of the most common nutrient deficiencies in elderly people and has been suggested to be a major factor in the development of AD.[71] Included in the list of zinc-containing enzymes are most enzymes involved in DNA replication, repair, and transcription. It has been suggested that dementia, possibly because of a long-term zinc deficiency, may represent the long-term cascading effects of error-prone or ineffective DNA-handling enzymes in nerve cells.[72] In addition, zinc is required by many antioxidant enzymes, including superoxide dismutase. The end result could be the destruction of nerve cells and the formation of NFTs and plaques. The levels of zinc in the brain and cerebrospinal fluid in patients with AD are markedly decreased, and there is a strong inverse correlation between serum zinc levels and the senile plaque count.[73]

Dietary zinc deficiency readily decreases serum zinc levels in mice and rats, although it increases serum corticosterone levels through the increased hypothalamic-pituitary-adrenal (HPA) axis activity (Fig. 144.3).[74] Zinc deficiency can reduce histochemically reactive zinc levels, which are estimated to be susceptible to aging. Zinc deficiency, as well as aging, appear to be significant risk factors for AD.

Zinc supplementation has demonstrated good benefits in people with AD. In one study, 10 patients with AD were given 27 mg per day of zinc (as zinc aspartate). Only two patients failed to show improvement in memory, understanding, communication, and social contact. In one 79-year-old patient, the response was labeled "unbelievable" by both medical staff and family.[75] Unfortunately there does not seem to be much interest in the scientific community in following up these impressive results with zinc therapy.

The medical literature acknowledges zinc's apparent duality as "The Zinc Paradox."[76] Fueling this ambivalence is conflicting information that zinc may be problematic for AD patients because, in vitro, it accelerates the formation of insoluble β-amyloid peptide.[77] Although zinc is neurotoxic at high concentrations and accumulates at sites of degeneration, total tissue zinc is markedly reduced in the brains of AD patients. Other research has shown a much higher concentration of copper-zinc superoxide dismutase in and around the damaged brain tissue of AD patients.[78] This finding suggests that the increased concentration of zinc in the damaged areas is due to the body's efforts to neutralize free radicals through the increased local production of dismutases. A possible corollary is that the higher focal levels of zinc result in increased amyloid formation when the free radical scavenging mechanisms have been inadequate.

Phosphatidylcholine

Because dietary phosphatidylcholine can increase acetylcholine levels in the brain in normal patients and AD is characterized by a decrease in cholinergic transmission, it seems reasonable to assume that phosphatidylcholine supplementation would benefit AD patients. However, the basic defect in cholinergic transmission in AD relates to impaired activity of the enzyme acetylcholine transferase. This enzyme combines choline (as provided by phosphatidylcholine) with an acetyl molecule to form acetylcholine, the neurotransmitter. Providing more choline does not necessarily increase the activity of this key enzyme, so phosphatidylcholine supplementation is not beneficial in the majority of patients with AD. Not surprisingly, clinical trials using phosphatidylcholine have largely been disappointing. Studies have shown inconsistent improvements in memory from choline supplementation in both normal and AD patients.[79–82] The studies have been criticized for small sample size, low dose of phosphatidylcholine, and poor design. Furthermore, some researchers are questioning the form of choline used.[83] Other forms, such as phosphatidylserine or choline alphoscerate,[84] may prove more useful in supporting cholinergic transmission.

In a patient with mild to moderate dementia, the use of a high-quality phosphatidylcholine preparation may be worth a try. A dosage of 15 to 25 g per day of phosphatidylcholine is required. If there is no noticeable improvement within 2 weeks, supplementation should be discontinued.

Phosphatidylserine

Phosphatidylserine is the major phospholipid in the brain, where it plays a major role in determining the integrity and fluidity of cell membranes. Normally, the brain can manufacture sufficient levels of phosphatidylserine, but a deficiency of methyl donors like S-adenosylmethionine (SAMe), folic acid, and vitamin B_{12} or essential fatty acids may inhibit the production of sufficient phosphatidylserine. Low levels of phosphatidylserine in the brain are associated with impaired mental function and depression in the elderly.

The primary use of phosphatidylserine is in the treatment of depression, impaired mental function, or both in the elderly, and also

in the treatment of AD. To date, the 11 double-blind published studies have all reported the successful use of phosphatidylserine in the treatment of age-related cognitive decline, AD, or depression.[85–95] In the largest study, a total of 494 elderly patients (between 65 and 93 years of age) with moderate to severe senility were given either phosphatidylserine (100 mg three times a day) or a placebo for 6 months.[95] The patients were assessed for mental performance, behavior, and mood at the beginning and end of the study. Statistically significant ($P < 0.01$) improvements were noted in mental function, mood, and behavior for the phosphatidylserine group.

Acetyl- L-Carnitine

A great amount of research has been conducted over the past decade with acetyl- L-carnitine (LAC) in the treatment of AD, senile depression, and age-related memory defects. LAC is composed of acetic acid and L-carnitine bound together. This reaction occurs naturally in the human brain. Therefore it is not exactly known how much more of an effect is achieved with LAC versus L-carnitine. However, LAC is thought to be substantially more active than other forms of carnitine in conditions involving the brain.[95,96]

The close structural similarity between LAC and acetylcholine led to an interest in using LAC in AD. Research has shown that LAC does mimic acetylcholine and is of benefit, not only in patients with early-stage AD, but also in elderly patients who are depressed or have impaired memory.[96] It has also been shown to act as a powerful antioxidant in the brain cell, to stabilize cell membranes, and to improve energy production in the brain cell, as well as enhance or mimic the function of acetylcholine.[97]

The latest meta-analysis involving studies of LAC in mild cognitive impairment and mild (early) AD is promising in terms of showing both clinical and psychometric improvements. Double-blind, placebo-controlled prospective parallel-group comparison studies of at least 3 months' duration were reviewed. Using a dose range of 1.5 to 3 g per day, treatment time points were assessed at 3, 6, 9, and 12 months. This analysis showed a significant advantage for acetyl-L-carnitine, compared with placebo. The advantage for acetyl-L-carnitine was seen by the time of the first assessment at 3 months and increased over time. Additionally, acetyl-L-carnitine was well tolerated in all studies.[98]

Further studies also show efficacy regarding the use of LAC in situations where AD patients were unresponsive to acetylcholinesterase inhibitors. One study evaluated the effect of LAC, using 2 g per day orally for 3 months in association with donepezil or rivastigmine in 23 patients with mild AD who had not responded to treatment with acetylcholinesterase inhibitors (AChE-Is). Clinical effects were evaluated by assessing cognitive functions, functional status, and behavioral symptoms. The response rate, which was 38% after AChE-I treatment, increased to 50% after the addition of LAC, indicating that the combination of both the pharmaceutical and LAC works better than the pharmacological treatment alone.[99]

The memory impairment need not be as severe as in AD for LAC to demonstrate benefit.[100–102] In one double-blind study of 236 elderly subjects with mild mental deterioration, as evidenced by detailed clinical assessment, the group receiving 1500 mg per day of LAC demonstrated significant improvement in mental function, particularly in memory and constructional thinking.[103]

OTHER THERAPIES

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is the most abundant hormone in the bloodstream and is found in extremely high concentrations in the brain. As DHEA levels decline dramatically with aging, low levels of DHEA in the blood and brain are thought to contribute to many symptoms associated with aging, including impaired mental function.

Although DHEA itself has no known function, it does serve as the source of all other steroid hormones in the body, including sex hormones and corticosteroids. Therefore the function of DHEA seems to lie in supplying the body with what it needs to maintain optimal levels and balance of all the steroid hormones that regulate the body's activities.

Over the past 15 years it has been demonstrated that declining levels of DHEA are linked to conditions such as diabetes, obesity, elevated cholesterol levels, heart disease, arthritis, and other age-related diseases. In addition, DHEA shows promise in enhancing memory and improving cognitive function.[103–105] As of this writing, the only double-blind, placebo-controlled study was a small pilot study (58 subjects) in which 50 mg of DHEA was given twice a day. Although some benefit was reported at 3 months, DHEA did not significantly improve cognitive performance or overall ratings of change in severity in this small-scale pilot study.[106]

The levels of DHEA necessary to improve brainpower in men older than 50 years of age appear to be 25 to 50 mg per day. For women, a dose of 15 to 25 mg appears to be sufficient in most cases. As men and women reach their 70s, they may require higher levels (e.g., 50–100 mg). Excessive dosages of DHEA can cause acne and, in women, menstrual irregularities. Although most elderly people probably need DHEA, laboratory assessment before prescription may help determine whether DHEA would be beneficial in an individual case and to assess the needed doses.

Melatonin

Formed by the body from serotonin and released by the pineal gland, melatonin is considered by many to be "the master hormone" of the body. It is well known for its use in normalizing circadian rhythms and sleep cycles and as a powerful antioxidant for cancer therapies. Medical research is beginning to value its ostensibly commanding role in many physiological processes.

In vitro studies have shown that melatonin protects neuronal cells from heavy metal cobalt damage. Cobalt is a transition metal found in high levels in AD patients. It was found that both the induction of oxidative damage and β-amyloid release were avoided with melatonin treatment. Because cobalt is an essential nutrient, often bound to vitamin B_{12}, melatonin may prove to be an important preventive treatment in AD-susceptible individuals.[107]

One double-blind, placebo-controlled study has studied melatonin in AD patients. Researchers from Nippon Medical School, who previously reported on bright light therapy as a means to improve cognitive function in AD, evaluated the effectiveness of melatonin. Three milligrams of melatonin were given to 11 subjects at 8:30 PM every day for a month, and 9 other subjects were given placebo (mean age of 79.2 years). Based on standard dementia and AD assessment scales, the melatonin group had a significantly increased sleeping time and decreased nighttime activity, with improved levels of cognitive and noncognitive functions. Even though the authors noted that these results were significant and clearly supported the use of melatonin in AD, they acknowledged that morning bright light therapy showed greater improvements in AD patients.[108]

A randomized, double-blind, parallel-group study investigated the effects of add-on prolonged-release melatonin (PRM) (2 mg) to standard therapy on cognitive functioning and sleep in 80 patients diagnosed with mild to moderate AD, with and without insomnia comorbidity, and receiving standard therapy (acetylcholinesterase inhibitors with or without memantine).[109] Patients were treated for

2 weeks with placebo and then randomized (1:1) to receive 2 mg of PRM or placebo nightly for 24 weeks, followed by placebo for 2 weeks. Patients treated with PRM had significantly better cognitive performance, as well as improved sleep efficiency compared with those treated with placebo. The authors concluded that a possible causal link exists between poor sleep and cognitive decline.

Botanical Medicines

Ginkgo Biloba Extract

Ginkgo biloba extract (GBE) standardized to contain 24% ginkgo flavonglycosides exerts many effects beneficial in the prevention and early treatment of AD (see Chapter 82). It must be pointed out that GBE should be taken consistently for at least 12 weeks to determine its effectiveness. Although some people experience benefits within a 2- to 3-week period, most will need to take GBE for a longer time to see any significant changes.

Huperzine A

Huperzine A is an alkaloid isolated from the moss *Huperzia serrata,* long used in China to treat primarily fever and inflammation. Although it has been shown to have no antipyretic or anti-inflammatory properties in experimental models, Huperzine A is a potent inhibitor of acetyl-cholinesterase. In fact, it is significantly more selective and substantially less toxic than the acetylcholinesterase (AChE) inhibitors currently used in conventional medicine (e.g., physostigmine, tacrine, and donepezil). Toxicity with synthetic AChE inhibitors has been a major drawback in their clinical use. In contrast, Huperzine A, purified from *H. serrata,* has been used as a prescription drug in China since the early 1990s and has reportedly been used by more than 100,000 people with no serious adverse effects.[110] Huperzine A may be an effective and well-tolerated agent to both suppress seizures and improve cognition in AD by enhancing cholinergic and GABAergic signaling and mitigating AD-related neurotoxicity (Fig. 144.4). [111]

Initial clinical studies with Huperzine A conducted in China, which first focused on its use in myasthenia gravis, showed considerable benefit in the treatment of dementia. One double-blind clinical study found that Huperzine A at a dose of 200 mcg twice a day produced measurable improvements in memory, cognitive function, and behavioral factors in 58% of AD patients.[112] In contrast, 36% in the placebo group showed improvement.

In a double-blind study, 210 individuals with AD were randomized to receive placebo or Huperzine A (200 mcg or 400 mcg twice a day), for at least 16 weeks. Huperzine A 200 mcg twice a day did not influence any change in the Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-Cog), but this agent at 400 mcg twice a day showed a 2.27-point improvement in ADAS-Cog at 11 weeks versus a 0.29-point decline in the placebo group and a 1.92-point improvement versus a 0.34-point improvement in the placebo arm at week 16. Changes in clinical global impression of change and activities of daily living were not significant at either dose.[113]

Bacopa Monniera

Bacopa monniera (BM) is an Ayurvedic botanical medicine used for memory enhancement, epilepsy, insomnia, and as a mild sedative. Additionally, it has been able to reduce memory dysfunction in rat models of AD. One study evaluated an extract of BM on a culture of purified rat astrocytes exposed to toxins mimicking the effect of excess nitric oxide exposure, a condition shown to be a factor in AD. Results showed that the extract of BM inhibited the formation of reactive species and DNA damage in a dose-dependent manner.

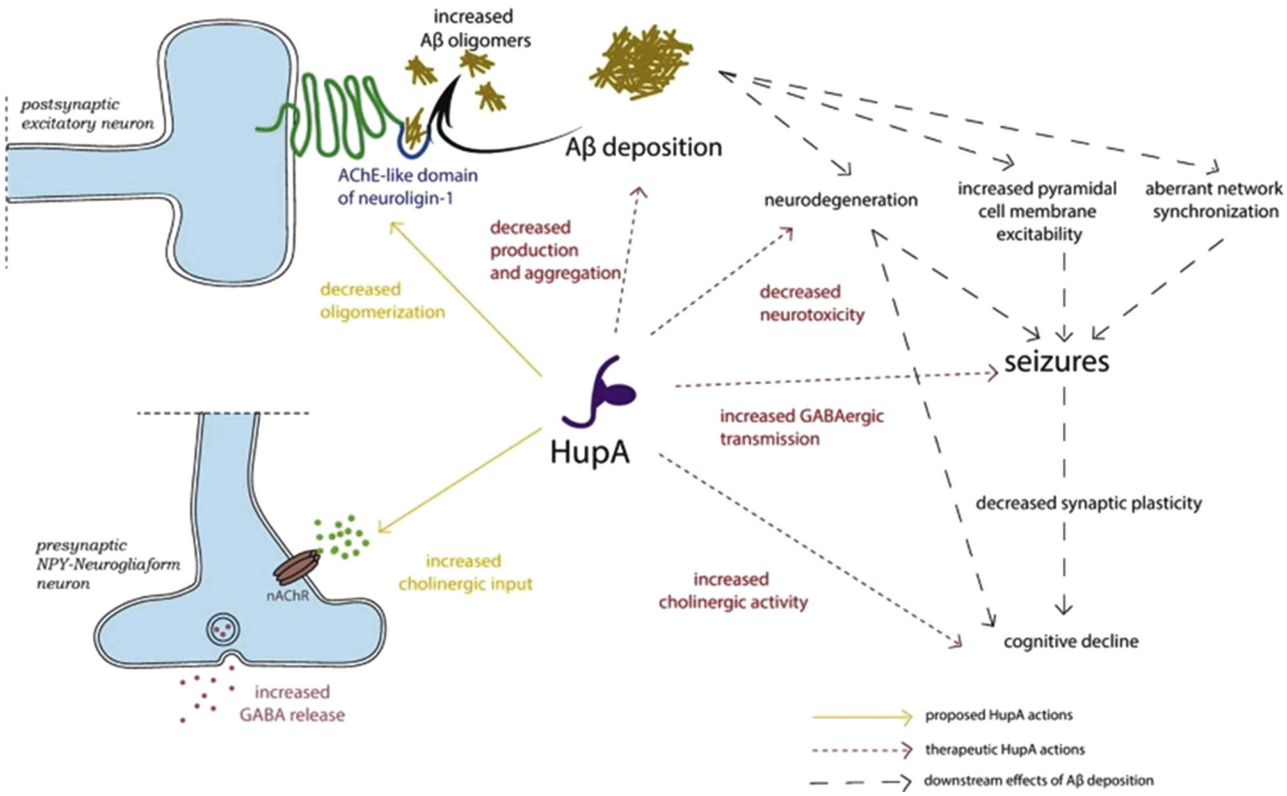


Fig. 144.4 Actions of Huperzine A in seizures and cognitive decline. (From Damar U, Gersner R, Johnstone JT, Scachter S, Rotenberg A, Huperzine A. A promising anticonvulsant, disease modifying, and memory enhancing treatment option in Alzheimer's disease. *Med Hypotheses.* 2017;99:57–62.)

These data support the traditional use of BM and indicate that this medicinal plant has therapeutic potential in the treatment or prevention of AD.[114–116]

Curcuma Longa (Turmeric)

There is considerable experimental evidence that curcumin protects against age-related brain damage and AD in particular. Researchers began exploring this effect after noting that elderly (age 70 to 79) residents of rural India who eat large amounts of turmeric have been shown to have the lowest incidence of AD in the world and 4.4 times lower than that of Americans. In in vitro studies, curcumin has been reported to inhibit amyloid-β-protein (Aβ) aggregation, and Aβ-induced inflammation, as well as the activities of β-secretase and acetylcholinesterase. In in vivo studies, oral administration of curcumin has resulted in the inhibition of Aβ deposition, Aβ oligomerization, and tau phosphorylation in the brains of AD animal models and improvements in behavioral impairment in animal models. Unfortunately, the two clinical trials conducted to date have failed to show any benefit to consuming curcumin.[117] However, the failure to produce positive results may have been due to the poor pharmokinetic profile of the curcumin used in the trials. For more information on curcumin, see Chapter 73.

Studies in stroke models have also suggested a neuroprotective role of curcumin,[9] as well as the ability to reduce plaque burden in models of AD. Curcumin has also been shown to decrease naphthalene and 4-hydroxy-2-nonenal–induced cataract formation on the lens by decreasing the rate of apoptosis and subsequent opacification resistance of the lens.[65,66] The authors postulate that induction of the glutathione S-transferase, which acts to decrease lipid peroxidation, was probably responsible for the cataractogenesis-inhibiting effects.

THERAPEUTIC APPROACH

The primary therapeutic goal is prevention or initiation of therapy as soon as any dementia is noted.

Dietary and Lifestyle Recommendations

- Avoid aluminum (found in many antiperspirants, antacids, and cookware).
- Eat organically grown foods instead of conventional foods contaminated by pesticides
- Follow a generally healthful dietary and lifestyle plan.
- Achieve ideal body weight and take measures to improve insulin sensitivity.
- Increase intake of whole-food products, including fish, cereals, vegetables, and monosaturated fats.
- Employ the principles of the Mediterranean diet.
- Decrease total calories, high-glycemic-load foods, and unhealthy fats.
- Use morning light therapy.

Supplements

- High-potency multivitamin/multimineral supplement
- Vitamin C: 500 to 1000 mg three times a day
- Vitamin E (mixed tocopherols): 400 IU daily
- Thiamin: 3 to 8 g daily
- Fish oils: 1000 to 3000 mg EPA+DHA daily
- Phosphatidylserine: 100 mg three times a day
- L-Acetylcarnitine: 500 mg three times a day
- Methylcobalamin: 1,000-3,000 mcg upon arising
- Melatonin: 3 mg in the evening at least a half hour before bed

Botanical Medicines

- GBE (24% *Ginkgo* flavonglycosides): 240 to 320 mg daily
- Huperzine A: 400 mcg twice a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ballard C, Gauthier S, Corbett A, et al. Alzheimer's disease. *Lancet*. 2011;377(9770):1019–1013.
2. Bronzuoli MR, Iacomino A, Steardo L, Scuderi C. Targeting neuroinflammation in Alzheimer's disease. *J Inflamm Res*. 2016;9:199–208. PubMed PMID: 27843334.
3. Wick G, Berger P, Jansen-Durr P, et al. A Darwinian-evolutionary concept of age-related diseases. *Exp Gerontol*. 2003;38:13–25.
4. Fu HJ, Liu B, Frost JL, et al. Amyloid-beta immunotherapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets*. 2010;9(2):197–206.
5. Solfrizzi V, Panza F, Capurso A. The role of diet in cognitive decline. *J Neural Transm*. 2003;110:95–110.
6. Grant WB, Campbell A, Itzhaki RF, et al. The significance of environmental factors in the etiology of Alzheimer's disease. *J Alzheimers Dis*. 2002;4:179–189.
7. Bonda DJ, Wang X, Perry G, et al. Oxidative stress in Alzheimer disease: a possibility for prevention. *Neuropharmacology*. 2010;59(4–5):290–294.
8. Craft S. Insulin resistance and Alzheimer's disease pathogenesis: potential mechanisms and implications for treatment. *Curr Alzheimer Res*. 2007;4(2):147–152.
9. Luchsinger JA, Small S, Biessels GJ. Should we target insulin resistance to prevent dementia due to Alzheimer disease? *Arch Neurol*. 2011;68(1):17–18.
10. Carmakova P, Eriksdotter M, Lund LH, Winblad B, Religa P, Religa D. Heart failure and Alzheimer's disease. *J Int Med*. 2015;277(4):406–425. PubMed PMID: 25041352.
11. Shin RW. Interaction of aluminum with paired helical filament tau is involved in neurofibrillary pathology of Alzheimer's disease. *Gerontology*. 1997;43(suppl 1):16–23.
12. Miu AC, Andreescu CE, Vasiu R, et al. A behavioral and histological study of the effects of long-term exposure of adult rats to aluminum. *Int J Neurosci*. 2003;113:1197–1211.
13. Zapatero MD, Garcia de Jalon A, Pascual F, et al. Serum aluminum levels in Alzheimer's disease and other senile dementias. *Biol Trace Elem Res*. 1995;47:235–240.
14. Frolich L, Riederer P. Free radical mechanisms in dementia of the Alzheimer's type and the potential for antioxidative treatment. *Arzneimittelforschung*. 1995;45:443–446.
15. Walton J, Tuniz C, Fink D, et al. Uptake of trace amounts of aluminum into the brain from drinking water. *Neurotoxicology*. 1995;16:187–190.
16. Calderón-Garcidueñas L, Reed W, Maronpot RR, et al. Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol Pathol*. 2004;32(6):650–658. PubMed PMID: 15513908.
17. Calderón-Garcidueñas L, Avila-Ramírez J, Calderón-Garcidueñas A, et al. Cerebrospinal Fluid biomarkers in Highly exposed PM2.5 Urbanites: the risk of Alzheimer's and Parkinson's diseases in young Mexico City Residents. *J Alzheimers Dis*. 2016 6;54(2):597–613. PubMed PMID: 27567860.
18. Calderón-Garcidueñas L, Mora-Tiscareño A, Franco-Lira M, et al. Decreases in short term memory, IQ, and altered brain metabolic ratios in urban apolipoprotein ε4 children exposed to air pollution. *J Alzheimers Dis*. 2015;45(3):757–770. https://doi.org/10.3233/JAD-142685. PubMed PMID: 25633678.
19. Parrón T, Requena M, Hernández AF, Alarcón R. Association between environmental exposure to pesticides and neurodegenerative diseases. *Toxicol Appl Pharmacol*. 2011 1;256(3):379–385. PubMed PMID: 21601587.
20. Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues JF, Brochard P. Neurodegenerative diseases and exposure to pesticides in the elderly. *Am J Epidemiol*. 2003;157(5):409–414. PubMed PMID: 12615605.
21. Hayden KM, Norton MC, Darcey D, et al. Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. *Neurol*. 2010;74(19):1524–1530. PubMed PMID: 20458069.
22. Feldman HH, Jacova C, Robillard A, et al. Diagnosis and treatment of dementia: 2. Diagnosis. *CMAJ*. 2008;178(7):825–836.
23. Weinreb HJ. Fingerprint patterns in Alzheimer's disease. *Arch Neurol*. 1985;42:50–54.
24. Solfrizzi V, Panza F, Frisardi V, et al. Diet and Alzheimer's disease risk factors or prevention: the current evidence. *Expert Rev Neurother*. 2011;11(5):677–708.
25. Frisardi V, Panza F, Seripa D, et al. Nutraceutical properties of Mediterranean diet and cognitive decline: possible underlying mechanisms. *J Alzheimers Dis*. 2010;22(3):715–740.
26. Gu Y, Luchsinger JA, Stern Y, et al. Mediterranean diet, inflammatory and metabolic biomarkers, and risk of Alzheimer's disease. *J Alzheimers Dis*. 2010;22(2):483–492.
27. Wang YJ, Thomas P, Zhong JH, et al. Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse. *Neurotox Res*. 2009;15(1):3–14.
28. Wang J, Santa-Maria I, Ho L, et al. Grape derived polyphenols attenuate tau neuropathology in a mouse model of Alzheimer's disease. *J Alzheimers Dis*. 2010;22(2):653–661.
29. Janle EM, Lila MA, Grannan M, et al. Pharmacokinetics and tissue distribution of 14C-labeled grape polyphenols in the periphery and the central nervous system following oral administration. *J Med Food*. 2010;13(4):926–933.
30. Peng Y, Sun J, Hon S, et al. L-3-n-butylphthalide improves cognitive impairment and reduces amyloid-beta in a transgenic model of Alzheimer's disease. *J Neurosci*. 2010;30(24):8180–8189.
31. Kim J, Lee HJ, Lee KW. Naturally occurring phytochemicals for the prevention of Alzheimer's disease. *J Neurochem*. 2010;112(6):1415–1430.
32. Hamaguchi T, Ono K, Murase A, et al. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. *Am J Pathol*. 2009;175(6):2557–2565.
33. Williams P, Sorribas A, Howes MJ. Natural products as a source of Alzheimer's drug leads. *Nat Prod Rep*. 2011;28(1):48–77.
34. Darvesh AS, Carroll RT, Bishayee A, et al. Oxidative stress and Alzheimer's disease: dietary polyphenols as potential therapeutic agents. *Expert Rev Neurother*. 2010;10(5):729–745.
35. Hamaguchi T, Ono K, Murase A, et al. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. *Am J Pathol*. 2009;175(6):2557–2565.
36. Henderson VW. Action of estrogens in the aging brain: dementia and cognitive aging. *Biochim Biophys Acta*. 2010;1800(10):1077–1083.
37. Kantarci K, Lowe VJ, Lesnick TG, et al. Early postmenopausal transdermal 16β–estradiol therapy and amyloid–β deposition. *J Alzheimers Dis*. 53(2):547–556. PubMed PMID: 27163830.
38. Craig MC, Maki PM, Murphy DG. The Women's Health Initiative memory study: findings and implications for treatment. *Lancet Neurol*. 2005;4(3):190–194.
39. Almeida OP, Flicker L. Association between hormone replacement therapy and dementia: is it time to forget? *Int Psychogeriatr*. 2005;17(2):155–164.
40. Craig MC, Murphy DG. Estrogen therapy and Alzheimer's dementia. *Ann N Y Acad Sci*. 2010;1205:245–253.
41. Hogervorst E, Yaffe K, Richards M, et al. Hormone replacement therapy to maintain cognitive function in women with dementia. *Cochrane Database Syst Rev*. 2009;1:CD003799.
42. Nolan CR, DeGoes JJ, Alfrey AC. Aluminum and lead absorption from dietary sources in women ingesting calcium citrate. *South Med J*. 1994;87:894–898.
43. Glick Dementias JL. The role of magnesium deficiency and hypothesis concerning the pathogenesis of Alzheimer's disease. *Med Hypotheses*. 1990;31:211–225.
44. Tucker DM, Penland JG, Sandstead HH, et al. Nutrition status and brain function in aging. *Am J Clin Nutr*. 1990;52:93–102.
45. Praticò D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends Pharmacol Sci*. 2008;29(12):609–615.
46. Gella A, Durany N. Oxidative stress in Alzheimer disease. *Cell Adh Migr*. 2009;3(1):88–93.
47. Jama JW, Launer LJ, Witteman JC, et al. Dietary antioxidants and cognitive function in a population-based sample of older persons. *Am J Epidemiol*. 1996;144:275–280.
48. Luchsinger JA, Tang MX, Shea S, et al. Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol*. 2003;60(2):203–208.
49. Masaki KH, Losonczy KG, Izmirlian G, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology*. 2000;54:1265–1272.

50. Klatte ET, Scharre DW, Nagaraja HN, et al. Combination therapy of donepezil and vitamin E in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2003;17:113–116.
51. Gray SL, Anderson ML, Crane PK, et al. Antioxidant vitamin supplement use and risk of dementia or Alzheimer's disease in older adults. *J Am Geriatr Soc*. 2008;56:291–295.
52. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr*. 2003;78(suppl 3):517S–520S.
53. Chen MF, Chen LT, Gold M, et al. Plasma and erythrocyte thiamin concentration in geriatric outpatients. *J Am Coll Nutr*. 1996;15:231–236.
54. Meador KJ, Nichols ME, Franke P, et al. Evidence for a central cholinergic effect of high dose thiamine. *Ann Neurol*. 1993;34:724–726.
55. Meador K, Loring D, Nichols M, et al. Preliminary findings of high-dose thiamine in dementia of Alzheimer's type. *J Geriatr Psychiatry Neurol*. 1993;6:222–229.
56. Benton D, Fordy J, Haller J. The impact of long-term vitamin supplementation on cognitive functioning. *Psychopharmacol (Berl)*. 1995;117:298–305.
57. van Goor L, Woiski MD, Lagaay AM, et al. Review: cobalamin deficiency and mental impairment in elderly people. *Age Ageing*. 1995;24:536–542.
58. Shevell MI, Rosenblatt DS. The neurology of cobalamin. *Can J Neurol Sci*. 1992;19:472–486.
59. Yao Y, Lu-Yao G, Mesches DN, et al. Decline of serum cobalamin levels with increasing age among geriatric outpatients. *Arch Fam Med*. 1994;3:918–922.
60. Aronow WS. Homocysteine: the association with atherosclerotic vascular disease in older persons. *Geriatrics*. 2003;58:22–24. 27–28.
61. Savage DG, Lindenbaum J, Stabler SP, et al. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin deficiency. *Am J Med*. 1994;96:239–246.
62. Norman EJ, Morrison JA. Screening elderly populations for cobalamin (vitamin B_{12}) deficiency using the urinary methylmalonic acid assay by gas chromatography mass spectrophotometry. *Am J Med*. 1993;94:589–594.
63. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med*. 2002;346:476–483.
64. Nilsson K, Gustafson L, Faldt R, et al. Plasma homocysteine in relation to serum cobalamin and blood folate in a psychogeriatric population. *Eur J Clin Invest*. 1994;24:600–606.
65. Healton EB, Savage DH, Brust JC, et al. Neurologic aspects of cobalamin deficiency. *Med (Baltimore)*. 1991;70:229–245.
66. Martin DC, Francis J, Protetch J, et al. Time dependency of cognitive recovery with cobalamin replacement: a report of a pilot study. *J Am Geriatr Soc*. 1992;40:168–172.
67. Levitt AJ, Karlinsky H. Folate, vitamin B_{12} and cognitive impairment in patients with Alzheimer's disease. *Acta Psychiatr Scand*. 1992;86:301–305.
68. Kristensen MO, Gulmann MC, Christensen JE, et al. Serum cobalamin and methylmalonic acid in Alzheimer dementia. *Acta Neurol Scand*. 1993;87:475–481.
69. Seal EC, Metz J, Flicker L, et al. A randomized, double-blind, placebo-controlled study of oral vitamin B_{12} supplementation in older patients with subnormal or borderline serum vitamin B_{12} concentrations. *J Am Geriatr Soc*. 2002;50:146–151.
70. van Dyck CH, Lyness JM, Rohrbaugh RM, et al. Cognitive and psychiatric effects of vitamin B_{12} replacement in dementia with low serum B_{12} levels: a nursing home study. *Int Psychogeriatr*. 2009;21(1):138–147.
71. Constantinidis J. The hypothesis of zinc deficiency in the pathogenesis of neurofibrillary tangles. *Med Hypoth*. 1991;35:319–323.
72. Burnet FM. A possible role of zinc in the pathology of dementia. *Lancet*. 1981;1:186–188.
73. Tully CL, Snowdon DA, Markesbery WR. Serum zinc, senile plaques, and neurofibrillary tangles: findings from the Nun Study. *Neuroreport*. 1995;6:2105–2108.
74. Takeda A, Tamano H, Kan F, Itoh H, Oku N. Anxiety-like behavior of young rats after 2-week zinc deprivation. *Behav Brain Res*. 2007;177(1):1–6. PubMed PMID: 17166602.
75. Constantinidis J. Treatment of Alzheimer's disease by zinc compounds. *Drug Develop Res*. 1992;27:1–14.
76. Cuajungco MP, Faget KY. Zinc takes the center stage: its paradoxical role in Alzheimer's disease. *Brain Res Brain Res Rev*. 2003;41:44–56.
77. Cuajungco MP, Lees GJ. Zinc and Alzheimer's disease: is there a direct link? *Brain Res Brain Res Rev*. 1997;23:219–236.
78. Furuta A, Price DL, Pardo CA, et al. Localization of superoxide dismutases in Alzheimer's disease and Down's syndrome neocortex and hippocampus. *Am J Pathol*. 1995;146:357–367.
79. Rosenberg G, Davis KL. The use of cholinergic precursors in neuropsychiatric diseases. *Am J Clin Nutr*. 1982;36:709–720.
80. Levy R, Little A, Chuaqui P, et al. Early results from double-blind, placebo controlled trial of high dose phosphatidylcholine in Alzheimer's disease. *Lancet*. 1983;1:987–988.
81. Sitaram N, Weingartner B, Caine ED, et al. Choline: selective enhancement of serial learning and encoding of low imagery words in man. *Life Sci*. 1978;22:1555–1560.
82. Higgins JP, Flicker L. Lecithin for dementia and cognitive impairment. *Cochrane Database Syst Rev*. 2003;3:CD001015.
83. Amenta F, Parnetti L, Gallai V, et al. Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors: ineffective treatments or inappropriate approaches? *Mech Ageing Dev*. 2001;122:2025–2040.
84. Parnetti L, Amenta F, Gallai V. Choline alphoscerate in cognitive decline and in acute cerebrovascular disease: an analysis of published clinical data. *Mech Ageing Dev*. 2001;122:2041–2055.
85. Cenacchi T, Bertoldin T, Farina C, et al. Cognitive decline in the elderly: a double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. *Aging (Milano)*. 1993;5:123–133.
86. Engel RR, Satzger W, Gunther W, et al. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. *Eur Neuropsychopharmacol*. 1992;2:149–155.
87. Crook T, Petri W, Wells C, et al. Effects of phosphatidylserine in Alzheimer's disease. *Psychopharmacol Bull*. 1992;28:61–66.
88. Crook TH, Tinklenberg J, Yeseavage J, et al. Effects of phosphatidylserine in age-associated memory impairment. *Neurology*. 1991;41:644–649.
89. Funfgeld EW, Baggen M, Nedwidek P, et al. Double-blind study with phosphatidylserine (PS) in parkinsonian patients with senile dementia of Alzheimer's type (SDAT). *Prog Clin Biol Res*. 1989;317:1235–1246.
90. Amaducci L. Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. *Psychopharmacol Bull*. 1988;24:1030–1034.
91. Nerozzi D, Aceti F, Melia E, et al. Phosphatidylserine and memory disorders in the aged. *Clin Ter*. 1987;120:399–404.
92. Palmieri G, Palmieri R, Inzoli MR, et al. Double-blind controlled trial of phosphatidylserine in patients with senile mental deterioration. *Clin Trials J*. 1987;24:73–83.
93. Villardita C, Grioli S, Salmeri G, et al. Multicentre clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. *Clin Trials J*. 1987;24:84–93.
94. Delwaide PJ, Gyselynck-Mambourg AM, Hurlet A, et al. Double-blind randomized controlled study of phosphatidylserine in demented patients. *Acta Neurol Scand*. 1986;73:136–140.
95. Bowman B. Acetyl-carnitine and Alzheimer's disease. *Nutr Rev*. 1992;50:142–144.
96. Carta A, Calvani M, Bravi D, et al. Acetyl-L-carnitine and Alzheimer's disease: pharmacological considerations beyond the cholinergic sphere. *Ann N Y Acad Sci*. 1993;695:324–326.
97. Calvani M, Carta A, Caruso G, et al. Action of acetyl-L-carnitine in neurodegeneration and Alzheimer's disease. *Ann N Y Acad Sci*. 1992;663:483–486.
98. Montgomery SA, Thal LJ, Amrein R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo

in the treatment of mild cognitive impairment and mild Alzheimer's disease. *Int Clin Psychopharmacol*. 2003;18:61–71.
99. Bianchetti A, Rozzini R, Trabucchi M. Effects of acetyl-L-carnitine in Alzheimer's disease patients unresponsive to acetylcholinesterase inhibitors. *Curr Med Res Opin*. 2003;19:350–353.
100. Vecchi GP, Chiari G, Cipolli C, et al. Acetyl-L-carnitine treatment of mental impairment in the elderly: evidence from a multicenter study. *Arch Gerontol Geriatr*. 1991;2:159–168.
101. Salvioli G, Neri M. L-acetylcarnitine treatment of mental decline in the elderly. *Drugs Exp Clin Res*. 1994;20:169–176.
102. Cipolli C, Chiari G. Effects of L-acetylcarnitine on mental deterioration in the aged: initial results. *Clin Ter*. 1990;132(suppl 6):479–510.
103. Kalimi M, Regelson W. *The Biologic Role of Dehydroepiandrosterone (DHEA)*. New York, NY: de Gruyter.
104. Yen SS, Morales AJ, Khorram O. Replacement of DHEA in aging men and women: potential remedial effects. *Ann N Y Acad Sci*. 1995;774:128–142.
105. Morales AJ, Nolan JJ, Nelson JC, et al. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J Clin Endocrinol Metab*. 1994;78:1360–1367.
106. Wolkowitz OM, Kramer JH, Reus VI, et al. DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. *Neurology*. 2003;60:1071–1076.
107. Olivieri G, Hess C, Savaskan E, et al. Melatonin protects SHSY5Y neuroblastoma cells from cobalt-induced oxidative stress, neurotoxicity and increased beta-amyloid secretion. *J Pineal Res*. 2001;31:320–325.
108. Asayama K, Yamadera H, Ito T, et al. Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. *J Nippon Med Sch*. 2003;70:334–341.
109. Wade AG, Farmer M, Harari G, et al. Add-on prolonged-release melatonin for cognitive function and sleep in mild to moderate Alzheimer's disease: a 6-month, randomized, placebo-controlled, multicenter trial. *Clin Interv Aging*. 2014;9:947–961. PubMed PMID: 24971004.
110. Skolnick A. Old Chinese herbal medicine used for fever yields possible new Alzheimer disease therapy. *JAMA*. 1997;277:776.
111. Damar U, Gersner R, Johnstone JT, Scachter S, Rotenberg A. Huperzine A: a promising anticonvulsant, disease modifying, and memory enhancing treatment option in Alzheimer's disease. *Med Hypotheses*. 2017;99:57–62. PubMed PMID: 28110700.
112. Xu SS, Gao ZX, Weng Z, et al. Efficacy of tablet huperzine-A on memory, cognition and behavior in Alzheimer's disease. *Zhongguo Yao Li Xue Bao*. 1995;16:391–395.
113. Rafii MS, Walsh S, Little JT, et al. A phase II trial of huperzine A in mild to moderate Alzheimer disease. *Neurology*. 2011;76(16):1389–1394.
114. Colasanti M, Suzuki H. The dual personality of NO. *Trends Pharmacol Sci*. 2000;21:249–252.
115. Russo A, Borrelli F, Campisi A, et al. Nitric oxide-related toxicity in cultured astrocytes: effect of Bacopa monniera. *Life Sci*. 2003;73:1517–1526.
116. Uabundit N, Wattanathorn J, Mucimapura S, et al. Cognitive enhancement and neuroprotective effects of Bacopa monnieri in Alzheimer's disease model. *J Ethnopharmacol*. 2010;127(1):26–31.
117. Hamaguchi T, Ono K, Yamada M. Curcumin and Alzheimer's disease. *CNS Neurosci Ther*. 2010;16(5):285–297.

145

Anemia

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Pallor, weakness, and a tendency to become fatigued easily
- Low volume of blood, low level of total red blood cells, or abnormal size or shape of red blood cells
- Reduction in hemoglobin (less than 13.5 g/dL in men, less than 12.0 g/dL in women) or hematocrit (less than 41.0% in men, less than 36.0% in women)

GENERAL CONSIDERATIONS

Anemia refers to a condition in which the blood is deficient in red blood cell numbers or oxygen carrying capacity. Anemia is not a specific diagnosis, per se, but rather the presentation of an underlying condition. Anemia can be defined as a reduction in hemoglobin (less than 13.5 g/dL in men, less than 12.0 g/dL in women) or hematocrit (less than 41.0% in men, less than 36.0% in women) or red blood cell count. The primary function of the red blood cell (RBC) is to transport oxygen from the lungs to the tissues of the body in exchange for carbon dioxide. More recent experimental evidence indicates that RBCs are important interorgan communication systems with additional functions, including participation in control of systemic nitric oxide metabolism, redox regulation, blood rheology, and viscosity.[1] In anemia, there is a decrease in the oxygen carrying capacity of the blood, leading to tissue hypoxia that adversely affects systemic hemodynamics and myocardial performance. According to the National Cancer Institute, anemia can be graded as the following:

- Mild: hemoglobin 10.0 g/dL to lower limit of normal
- Moderate: hemoglobin 8.0 to 10.0 g/dL
- Severe: hemoglobin 6.5 to 7.9 g/dL
- Life-threatening: hemoglobin < 6.5 g/dL

Anemic conditions can be classified according to the size/morphology of red blood cells (i.e., normocytic, macrocytic, and microcytic; assessed as the mean corpuscular volume [MCV]) and/or the concentration of Hb in RBCs (i.e., normochromic, hypochromic, and hyperchromic; assessed by mean corpuscular hemoglobin [MCH]). In addition, there are three major etiopathologies of anemia: (1) anemia due to excessive blood loss; (2) anemia due to excessive RBC destruction (i.e., hemolysis); and (3) anemia due to deficient RBC or hemoglobin production. Although an exhaustive description of all types of anemia is beyond the scope of this chapter, a few examples of the most common forms of anemia are discussed in more detail elsewhere in this chapter.

TYPES OF ANEMIA

Excessive Blood Loss

Anemia can develop secondary to acute or chronic blood loss. Acute blood loss can be fatal if more than one third of total blood volume is lost (roughly 1.5 L). Because acute blood loss is usually quite apparent, there is little difficulty in diagnosis. Often blood transfusion is required.

Chronic blood loss resulting from conditions such as slow-bleeding peptic ulcer, bleeding hemorrhoids, or excessive menstruation can lead to anemia. In the case of chronic blood loss, identifying the cause through a complete diagnostic workup is essential for proper treatment.

Excessive Red Blood Cell Destruction

Hemolysis is the destruction of old or abnormal RBCs, and when this process exceeds the body's ability to manufacture new RBCs, anemia can result. Old RBCs, as well as abnormal RBCs, are removed from the

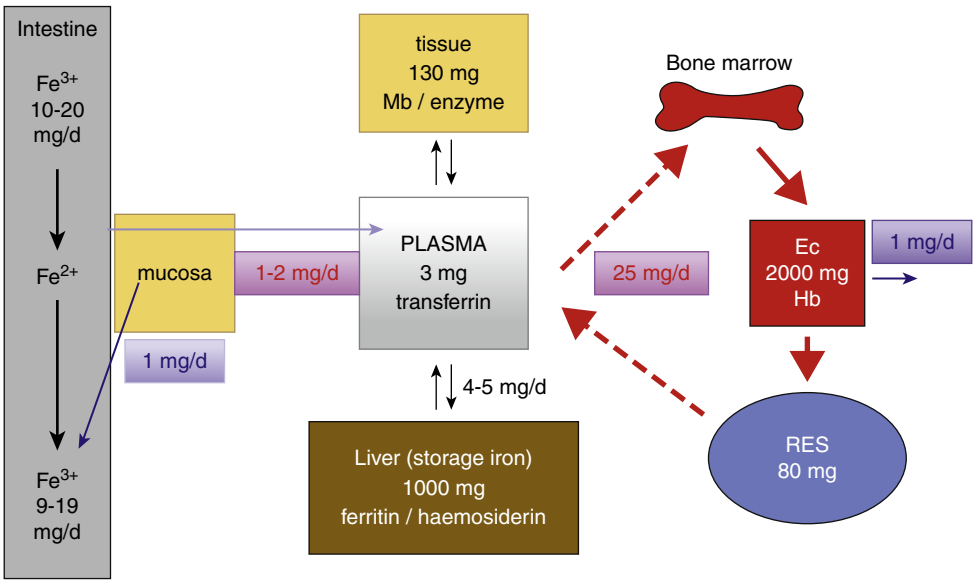


Fig. 145.1 Distribution of iron in the human body (70 kg). (From Rogler G, Vavricka S. Anemia in inflammatory bowel disease: an under-estimated problem? *Front Med (Lausanne).* 2015;1:58.)

circulation primarily by the spleen. The most common cause of excessive destruction of RBCs is abnormal RBC shape. Several processes can lead to increased hemolysis, including: synthesis of defective hemoglobin, as seen in hereditary conditions like sickle-cell anemia; mechanical injury due to trauma or turbulence within arteries; hereditary RBC enzyme defects; presence of antibodies against surface antigens on RBCs, such as antibodies anti-A or anti-B blood group antigens; chronic disease; and chronic inflammation.

Deficient Red Blood Cell or Hemoglobin Production

Impaired erythropoiesis (i.e., insufficient production of RBCs or hemoglobin) is the most common category of anemia, and the most common cause is nutritional deficiency. Although deficiency of several vitamins and minerals can produce anemia, the most common include iron, vitamin B_{12}, and folic acid. Iron-deficiency anemia is characterized as a microcytic anemia, whereas folic acid and B_{12} deficiency anemias are classified as macrocytic.

Iron-Deficiency Anemia

Iron is critical to human life. Less than 10% of the daily iron uptake of around 20 mg is absorbed by the intestinal mucosa.[2] Most of the functional iron in the body is present in the heme proteins, such as hemoglobin of red blood cells, where it functions in transporting oxygen from the lungs to the body's tissues and the transportation of carbon dioxide from the tissues to the lungs (Fig. 145.1). The liver contains the second-highest amount of iron, which is bound to ferritin and hemosiderin as iron storage. Iron is also found in cytochromes, which are involved in mitochondrial electron transfer, in oxygen transport and in the liver Phase I detoxification enzymes. In addition, iron functions in several key enzymes involved with energy production and metabolism including DNA synthesis.

Iron deficiency is the most common mineral deficiency in the United States, and iron-deficiency anemia is one of the most common hematological problems worldwide. The groups at highest risk for iron deficiency are infants under 2 years of age, teenage girls, pregnant women, and elderly persons. Studies have found evidence of iron deficiency in as high as 30% to 50% of people in these groups. Some degree of iron deficiency occurs in 35% to 58% of young, healthy women. During pregnancy, the number is even higher.

Measuring serum ferritin is the best laboratory test for determining body iron stores.[3] However, it must be pointed out that iron deficiency occurs in many stages, and anemia is the last stage of iron deficiency. Iron-dependent enzymes involved in energy production and metabolism are the first to be affected by low iron levels. Patients with a serum ferritin level less than 25 ng/mL have a very high probability of being iron deficient, whereas serum ferritin values greater than 100 ng/mL are associated with a very low likelihood of iron-deficiency anemia.[4] Investigating total iron-binding capacity (TIBC), transferrin saturation, serum iron, and serum transferrin receptor levels may be helpful if the ferritin level is between 46 and 99 ng per mL (Fig. 145.2); bone-marrow biopsy may be necessary in these patients for a definitive diagnosis.[5] A trial of iron is a reasonable approach in children, adolescents, and women of reproductive age if the review of symptoms, history, and physical examination are negative for other causes. However, if there is not a 1 to 2 g per dL (10–20 g per L) increase in the hemoglobin level in a reasonable amount of time, possibilities include malabsorption of oral iron, continued bleeding, or unknown lesion.

Iron deficiency may be caused by an increased iron requirement, decreased dietary intake, diminished iron absorption or utilization, blood loss, or a combination of these factors. Increased requirements for iron occur during the growth spurts of infancy and adolescence as well as during pregnancy and lactation. Most pregnant women are routinely given iron supplements during their pregnancy, as the dramatically increased need for iron during pregnancy cannot usually be met through diet alone. Inadequate intake of iron is common in many parts of the world, especially in areas where people consume primarily a vegetarian diet.

Many standard infant diets in developed countries (high in milk and cereals) are also low in iron. The adolescent who consumes a "standard American diet" is at high risk for iron deficiency. However, the population at greatest risk of dietary iron deficiency is the low-income elderly population; this deficiency is enhanced by impaired iron absorption resulting from decreased hydrochloric acid secretion in the stomach—a common complication in the elderly population.

Other causes of decreased absorption in the general population include chronic diarrhea or malabsorption, the surgical removal of the stomach, and antacid or acid-blocking drug use. Blood loss is the most

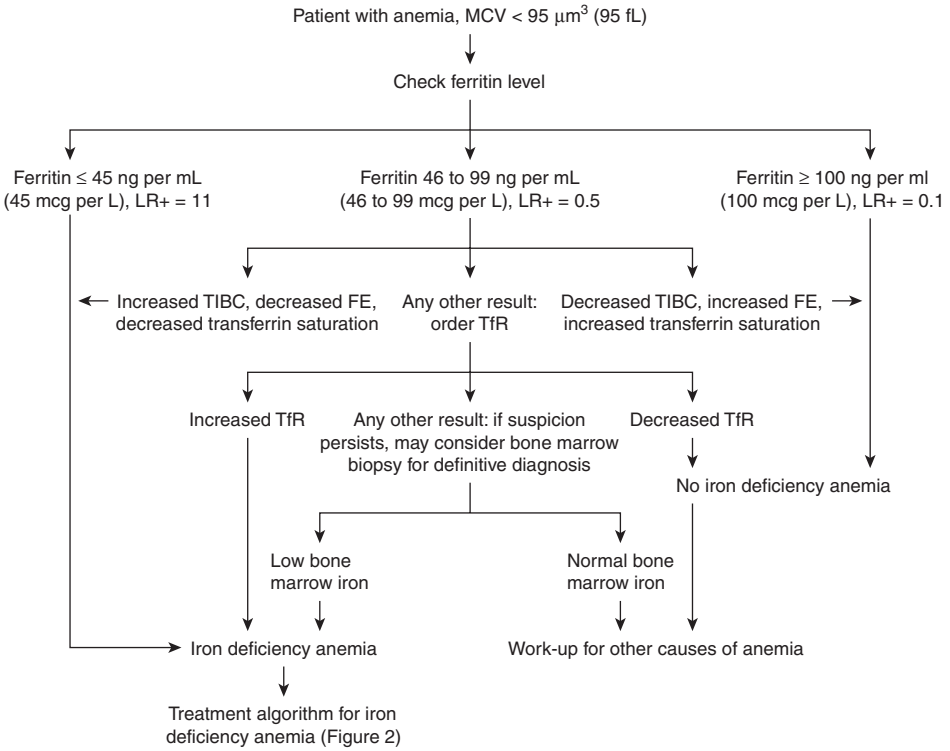


Fig. 145.2 Diagnostic algorithm for iron deficiency anemia. *FE*, Serum iron; *LR+*, positive likelihood ratio; *MCV*, mean corpuscular volume; *TfR*, serum transferrin receptor, *TIBC*, total iron-binding capacity. (From Killip S, Bennett JM, Chambers MD. Iron deficiency anemia. *Am Fam Physician*. 2007;75[5]:671–678.)

common cause of iron deficiency in women of childbearing age, often due to excessive menstrual bleeding. Interestingly, iron deficiency is a common cause of excessive menstrual blood loss, which can create a cycle of cause and effect.[6,7] Other common causes of blood loss include blood donation and bleeding from peptic ulcers and hemorrhoids.

The negative effects of iron deficiency are due largely to the impaired delivery of oxygen to the tissues and the impaired activity of iron-containing enzymes in various tissues. Iron deficiency can lead to anemia, excessive menstrual blood loss, learning disabilities, and impaired immune function, as well as decreased energy levels, detoxification function, and physical performance. Even mild iron-deficiency anemia leads to a reduction in physical work capacity and productivity.

Supplementation with iron has produced rapid improvements in work capacity among iron-deficient individuals. A systematic review of animal and human studies evaluated the causal relationship between iron deficiency and physical work capacity.[8] Iron deficiency was examined along a continuum from severe iron-deficiency anemia (SIDA) to moderate iron-deficiency anemia (MIDA) to iron deficiency without anemia (IDNA). Results demonstrated a strong causal effect of SIDA and MIDA on aerobic capacity in animals and humans, likely a result of reduced oxygen transport associated with anemia. In addition, tissue iron deficiency may play a role through reduced cellular oxidative capacity. The review concluded that energetic efficiency was affected at all levels of iron deficiency in humans, with the reduced work productivity observed in field studies likely due to anemia and reduced oxygen transport. However, impaired physical performance due to iron deficiency is not dependent on anemia because the iron-dependent enzymes involved in energy production and metabolism will be impaired long before anemia occurs.

Vitamin B_{12}–Deficiency Anemia

Vitamin B_{12} (cobalamin) deficiency is most often due to a defect in absorption or cobalamin metabolism rather than a dietary lack of the vitamin. For vitamin B_{12} from food to be absorbed, it must be liberated by hydrochloric acid and bound to intrinsic factor. The B_{12}-intrinsic factor complex is absorbed in the small intestine with the aid of the pancreatic enzyme trypsin.

Intrinsic factor is secreted by the parietal cells of the stomach. These cells are responsible for the secretion of hydrochloric acid. Hence, the secretion of intrinsic factor parallels that of hydrochloric acid. Pernicious anemia (PA) is a consequence of intrinsic factor loss and neutralizing intrinsic factor antibody that impairs cobalamin absorption. The defect is rare before the age of 35. Use of the Schilling test for detection of PA has been mostly supplanted by serological testing for parietal cell and intrinsic factor antibodies. Although PA has been reported in virtually every ethnic group, it is more common in individuals of Scandinavian, English, and Irish descent. The loss of hydrochloric acid leads to iron-deficiency anemia, which often precedes cobalamin-deficient pernicious anemia by 20 years.

A dietary lack of vitamin B_{12} is most often associated with a strict vegetarian or vegan diet. Unlike other water-soluble nutrients, vitamin B_{12} is stored in the liver, kidney, and other body tissues. Because normal body stores of vitamin B_{12} may last an individual 3 to 6 years, signs and symptoms of vitamin B_{12} deficiency arise after years of low dietary intake or inadequate secretion of intrinsic factor. In addition, it appears that a deficiency of vitamin B_{12} also affects the brain and nervous system before the development of pernicious anemia. In other words, waiting for a finding of anemia to diagnose a vitamin B_{12} deficiency may result in significant patient harm.

Symptoms of severe B_{12} deficiency can include pallor, fatigue, shortness of breath, diarrhea, heart palpitations, and a sore, beefy

red, and swollen tongue. Common nervous system symptoms include numbness and tingling of the arms or legs, depression, mental confusion, loss of vibration sense, and loss of deep tendon reflexes. In elderly persons, a vitamin B_{12} deficiency can mimic Alzheimer's disease.

The presence of macrocytosis and characteristic symptoms provide the physician with important clues suggesting B_{12} deficiency. Deficiency of cobalamin, whether nutritional or the result of inborn errors of vitamin B_{12} metabolism, inactivate methionine synthase and mitochondrial methylmalonyl-CoA mutase, leading to the accumulation of homocysteine and methylmalonic acid (Fig. 145.3). Thus homocysteine and methylmalonic acid (MMA) are the preferred serum biomarkers used to determine B_{12} status. Stand-alone serum levels of vitamin B_{12} have limited diagnostic value because low serum levels of vitamin B_{12} do not always represent deficiency, and likewise, severe functional deficiency of the micronutrient has been documented in the presence of normal and even high levels of serum vitamin B_{12}.[9]

FOLATE DEFICIENCY

Folate (or folic acid) deficiency is one of the most common vitamin deficiencies in the world. Unlike vitamin B_{12}, the body does not accumulate a surplus of folate, storing only enough to sustain the body for 1 to 2 months.

Folate deficiency is extremely common among alcoholics, as alcohol consumption impairs folate absorption, disrupts folate metabolism, and causes the body to excrete folate. Folic acid deficiency is also common among pregnant women, resulting from an increased demand. Folate is vital to cell reproduction in the fetus, and a deficiency of folate during development presents an increased risk for the development of birth defects such as neural tube defects. The lowering of folic acid levels by alcohol during pregnancy further increases the risk of fetal alcohol syndrome and/or neural tube defects. In addition to alcohol, several pharmaceuticals can induce a folate deficiency, including anticancer drugs, drugs for epilepsy, and oral contraceptives.

Folic acid deficiency is also common among patients who have chronic diarrhea or malabsorption states such as celiac disease, Crohn's disease, or tropical sprue. Because a deficiency of folate will result in diarrhea and malabsorption, often a vicious circle ensues. The administration of the active form of folate (5-methyltetrahydrofolate) as a preventive measure is warranted in anyone experiencing chronic diarrhea. Often this has a therapeutic effect as well.

Folate deficiency will result in macrocytic anemia similar to that caused by a vitamin B_{12} deficiency. The most sensitive test to assess folic acid deficiency is determining the folate content of the serum and RBC. The reference range for serum folate is 2.5 to 20 ng/mL. In addition to anemia, other symptoms of folic acid deficiency include fatigue, pallor, headaches, palpitations, diarrhea, depression, and a swollen, red tongue.

Note: It is always necessary to supplement vitamin B_{12} along with 5-MTHF to prevent the folate supplement from masking a vitamin B_{12} deficiency. Supplementing with 5-MTHF will correct the anemia of a vitamin B_{12} deficiency, but it cannot overcome the problems that vitamin B_{12} deficiency causes in the brain. Also, a high level of folate will aggravate the problems caused by vitamin B_{12} deficiency.

BONE MARROW AND STEM CELL DYSFUNCTION

Bone marrow and stem cell problems may prevent the body from producing enough red blood cells. If stem cells are too few, defective, or replaced by other cells such as metastatic cancer cells, anemia may result. Anemias resulting from bone marrow or stem cell problems include the following.

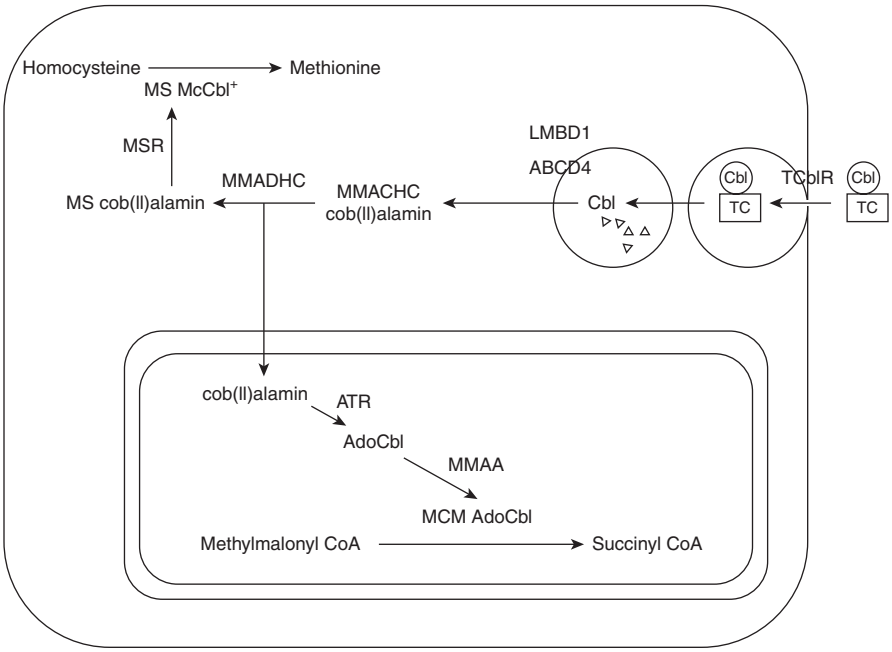


Fig. 145.3 Intracellular processing of cobalamin. *AdoCbl*, Adenosylcobalamin; *Cbl*, cobalamin; *MCM*, methylmalonylCoA mutase; *MeCbl*, methylcobalamin; *MS*, methionine synthase; *MSR*, methionine synthase reductase; *TC*, transcobalamin; *TCblR*, TC receptor. (From Moreno-Garcia MA, Rosenblatt DS, Jerome-Majewska LA. Vitamin B_{12} metabolism during pregnancy and in embryonic mouse models. *Nutrients*. 2013;5[9]:3531–3550.)

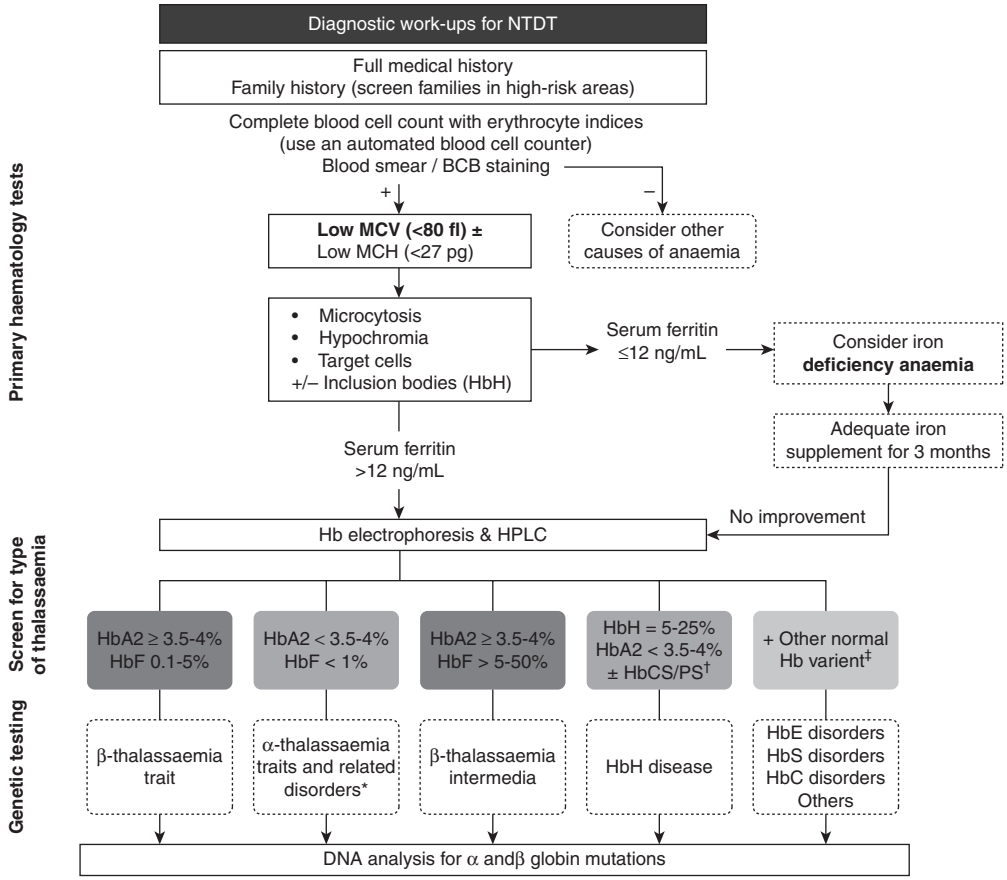


Fig. 145.4 Diagnostic algorithm for nontransfusion-dependent thalassemia (NTDT). *Hb*, hemoglobin; *HPLC*, high-pressure liquid chromatography; *MCH*, mean corpuscular hemoglobin; *MCV*, mean corpuscular volume. (From Viprakasit V, Tyan P, Rodmai S, Taher AT. Identification and key management of non-transfusion-dependent thalassemia patients: not a rare but potentially under-recognised condition. *Orphanet J Rare Dis.* 2014;9:131.)

Aplastic Anemia

Aplastic anemia is a hematological disease characterized by hematopoietic failure of the bone marrow, resulting in a marked reduction in the number of stem cells or the absence of stem cells. It can be inherited, can occur without apparent cause, or can occur when the bone marrow is injured by medications, radiation, environmental toxins, chemotherapy, autoimmune disorders, or infection. Aplastic anemia can be either severe or not severe, and it may occur abruptly or may develop slowly and worsen over time.

Inherited Hemoglobin Disorders

Thalassemia and sickle cell disease are the most common inherited monogenic diseases worldwide characterized by deformed and/or inadequate hemoglobin. This inadequacy results in immature and deformed red blood cells and a decrease in oxygen transport. Several clinical forms of α-thalassemia and β-thalassemia have been described, and severity can range from mild to life threatening. Blood analyses of almost all thalassemias show a reduction in the size and Hb content of mature RBCs, which is evident by a reduced MCV (less than 80 femtoliters/cell) and mean cell MCH (less than 27 picograms/cell) (Fig. 145.4). The most severe form is called Cooley's anemia.

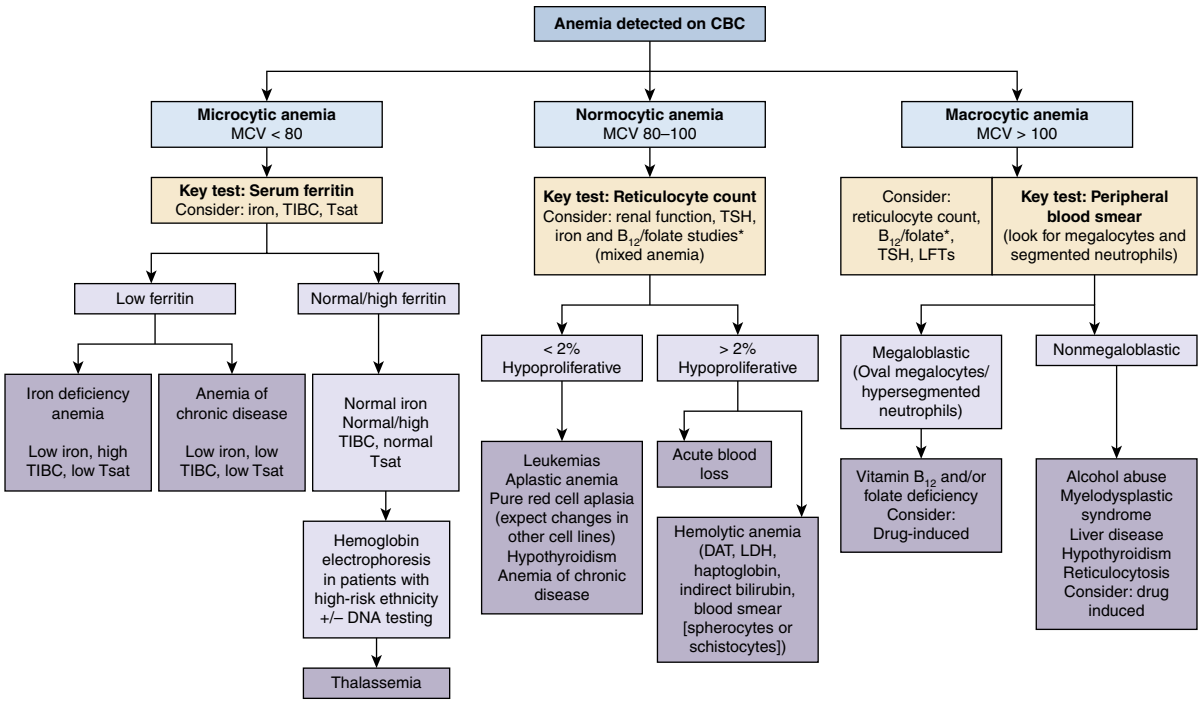
Sickle cell anemia occurs in individuals who are homozygous for a single-nucleotide substitution of the beta-globin gene, often producing a rigid, sicklelike shape of the red blood cells. Several subtypes exist depending on the exact mutation, with clinical manifestations including chronic hemolysis, increased susceptibility to infections, and vaso-occlusive complications.[10] Individuals with a single abnormal copy of the gene often do not have symptoms and are classified as carriers.

Lead Toxicity

Lead is a persistent toxic metal. At least 50% of all lead is stored in the bones, where it has a half-life of years to decades. Lead is readily absorbed through both the respiratory and gastrointestinal tracts. Gastrointestinal absorption of lead in children can be up to five times greater than in adults who are exposed to the same sources. Once absorbed, lead is bound to the red blood cells and moved into soft tissue and bones, with some excretion via the urine. Chronic, low-level lead exposure is associated with several adverse health conditions. In persons with blood lead levels (BLLs) higher than 35 μg/dL, the heme enzyme delta-aminolevulinic acid dehydratase (ALAD) is inhibited, leading to anemia.

DIAGNOSTIC CONSIDERATIONS

Anemia cannot be reliably diagnosed by clinical presentation. Fatigue, the most common presenting symptom, is caused by anemia in approximately 1 out of 52 patients in primary care practice.[11] It is imperative that a comprehensive laboratory analysis of the blood be performed. Fig. 145.5 provides a flow chart of suggested labs as well as potential diagnoses.



DAT = direct antiglobulin test (direct Coombs' test): LFT = liver function tests; LDH = lactate dehydrogenase; MCV = mean corpuscular volume; TIBC = total iron-binding capacity; TSH = thyroid-stimulating hormone. Tsat=transferrin saturation

*Not covered in all provinces.

Sources: 1) BMJ Best Practice. *Assessment of anaemia.* London: BMJ; 2016. **2)** Anemia Review Panel. *Anemia Guidelines for Family Practice. 3rd ed.* Toronto: MUMS Guideline Clearinghouse: 2014.

Fig. 145.5 Algorithm for the assessment of anemia. (Waters, H. Anemia in Adults, Practice-based Small Group Learning Program; *Hamilton.* 2017; 25(10):1-19.)

THERAPEUTIC CONSIDERATIONS

Treatment of anemia is dependent on proper clinical evaluation to determine the origin of the anemia. It is critical that the underlying cause of the anemia be uncovered if the correct therapy is to be employed.

GENERAL NUTRITIONAL SUPPORT FOR ALL TYPES

Increasing iron levels in food may help partially or completely overcome poor iron absorption. Animal proteins are a rich source of heme iron, and dark green leafy vegetables are among the best sources of nonheme iron. Although many individuals shy away from organ meats, liver is an excellent source of iron as well as B vitamins. Lamb and venison also contain significant levels of iron and are leaner and less inflammatory than beef.

In addition to iron, green leafy vegetables contain natural fat-soluble chlorophyll and nutrients such as vitamin C and folic acid. The chlorophyll molecule is similar to the hemoglobin molecule. Fat-soluble (but not water-soluble) chlorophyll products may be beneficial. However, water-soluble chlorophyll is not absorbed from the gastrointestinal tract and therefore has no use in the treatment of anemia.

Because a large percentage of individuals with anemia do not secrete enough hydrochloric acid, it is often important for them to take hydrochloric acid supplements with meals. See Chapter 123, Digestive Support.

Iron-Deficiency Anemia

In iron-deficiency anemia, it is essential to determine the reason for the loss of iron (e.g., chronic blood loss) or the reason for the poor absorption of dietary iron (e.g., low vitamin C or high tannins in diet). Lack of hydrochloric acid is a common reason for impaired iron absorption, especially among elderly persons.

There are two forms of dietary iron: heme iron and nonheme iron. Heme iron is bound to the oxygen-binding proteins hemoglobin and myoglobin. Heme iron is the most efficiently absorbed form of iron. The absorption rate of nonheme iron supplements, such as ferrous sulfate and ferrous fumarate, is 2.9% on an empty stomach and 0.9% with food. This rate is significantly less than the absorption rate of heme iron, which is as high as 35%. In addition, heme iron does not cause the side effects associated with nonheme sources of iron, such as nausea, flatulence, constipation, and diarrhea.

Despite the superiority of heme iron, nonheme iron salts are the most popular iron supplements. Although heme iron is better absorbed, dosing higher quantities of nonheme iron salts results in similar net absorption amounts (i.e., 3 mg of heme iron and 50 mg of nonheme iron will net approximately the same level of absorption).

In adults with iron deficiency anemia, treatment involves the use of 50 to 150 mg elemental iron daily in divided doses. The usual recommendation for any nonheme source is generally up to 60 mg daily in divided doses. For children with iron-deficiency anemia, the dose is 4 to 6 mg/kg per day divided into three doses. High intakes of other minerals, particularly calcium, magnesium, and zinc, can interfere with iron absorption. Therefore, when treating iron deficiency, it is recommended to take iron away from these minerals. In contrast, vitamin C enhances iron absorption.

Ferrous sulfate is the most popular iron supplement, but it is certainly less than ideal, because it often causes constipation or other

TABLE 145.1 Dietary Sources of Iron

Food	Average Serving Size (g)	Iron (mg) Per Serving
Calf or lamb liver	60	9.6
Beef or chicken liver	60	5.2
Beef	90	2.7
Beans cooked	100	2.3
Prunes	100	1.8
Bread (3 slices)	70	1.7
Chicken or turkey	90	1.6
Greens, cooked	75	1.5
Peas	75	1.5
Eggs	50	1.1

TABLE 145.2 Recommended Dietary Allowances (RDA) for Iron

Group	Daily Dose
Infants 7–12 months	11 mg
Children 1–3 years	7 mg
Children 4–8 years	10 mg
Males 9–13 years	8 mg
Males 14–18 years	11 mg
Males ≥ 19 years	8 mg
Females 9–13 years	8 mg
Females 14–18 years	15 mg
Females 19–50 years	18 mg
Females ≥ 51 years	8 mg
Pregnant women	27 mg
Females when lactating between 14–18 years	10 mg
Females when lactating ≥19 years	9 mg

gastrointestinal disturbance. The best forms of nonheme iron are ferrous succinate, glycinate, fumarate, and pyrophosphate. Of these, the preferred form is micronized ferrous pyrophosphate, which is then microencapsulated to allow it to be dispersed and assimilated. Advantages of this form include stability, lack of taste or flavor, and lack of gastrointestinal side effects. In addition, ferrous pyrophosphate provides a sustained-release form of iron (up to 12 hours) with a high relative bioavailability, especially when taken on an empty stomach.[12]

The best dietary source of iron is red meat, as well as liver and fish. Nonmeat sources of iron include beans, molasses, dried fruits, whole-grain and enriched breads, and green leafy vegetables. Nonheme iron absorption is enhanced by vitamin C.

Table 145.1 provides the iron content per serving of some food sources of iron. The table does not factor in absorption. For example, the absorption rate for calf's liver is nearly 30%, whereas the absorption rate for the vegetable sources is approximately 5%. The daily dietary recommended allowance for iron depends on an individual's age and gender (Table 145.2).

Several foods and beverages contain substances that inhibit iron absorption, including tea, coffee, wheat bran, and egg yolk. Antacids and overuse of calcium supplements also decrease iron absorption. These items should be restricted in the diet of individuals who have iron deficiency. Iron supplements can decrease the availability of several medications, including levodopa and levothyroxine, and care should be exercised when simultaneous administration of these substances is indicated.

Cautions and Warnings

Keep all iron supplements out of the reach of children. Acute iron poisoning in infants can result in serious consequences. Severe iron poisoning is characterized by damage to the intestinal lining, liver failure, nausea and vomiting, and shock.

Pernicious Anemia

The usual dietary sources of vitamin B_{12} are animal-derived foods. The richest sources are liver and kidney, followed by eggs, fish, cheese, and meat. Strict vegetarians and vegans have an increased risk of B_{12} deficiency, and therefore it is necessary to identify plant-derived foods that contain high levels of vitamin B_{12}—produced by the bacteria, typically in some kind of fermented form. A survey of naturally occurring and high vitamin B_{12}-containing plant-derived food sources showed that nori is a good vitamin B_{12} source for vegetarians.[13] Consumption of approximately 4 g of dried nori (vitamin B_{12} content: 77.6 μg/100 g dry weight) supplies the RDA of 2.4 μg per day.

Vitamin B_{12} is available in several forms. The most common form is cyanocobalamin. However, vitamin B_{12} is active in only two forms: methylcobalamin and adenosylcobalamin. Both forms are available commercially in tablet form in the United States. Although methylcobalamin is active immediately upon absorption, cyanocobalamin must be converted by the body to either methylcobalamin or adenosylcobalamin by removing the cyanide molecule and adding either a methyl or adenosyl group. Cyanocobalamin is not active in many experimental models, whereas both methylcobalamin and adenosylcobalamin demonstrate high-level activity.

Medical treatment involves injecting vitamin B_{12} at a dose of 1 mg daily for 1 week, until the anemia has resolved. Preventive injections may also be required to prevent deficiency in the future. Oral therapy has been shown to be equally effective.

Oral Versus Injectable B_{12}

Although it is popular to inject vitamin B_{12}, injection is not strictly necessary. Studies show that supplementation with oral vitamin B_{12} is a safe and effective treatment for the B_{12} deficiency state, even in the absence of intrinsic factor. In the United States, oral vitamin B_{12} therapy is rarely used even though it has been shown to be 100% effective in the long-term treatment of pernicious anemia.[14]

Soon after vitamin B_{12} was isolated in 1948, it was introduced in an injectable form, and researchers busily sought an oral alternative. Oral preparations containing intrinsic factor were tried, but some patients developed antibodies against intrinsic factor and therefore would not respond. Studies in the 1950s and 1960s documented that a small but constant proportion of an oral dose of cyanocobalamin at a dosage of 300 mcg to 1000 mcg daily was absorbed even without intrinsic factor through the process of diffusion. Thus by sufficiently increasing the dose, adequate absorption could be attained. A study in 1978 described 64 Swedish patients with pernicious anemia and other vitamin B_{12}-deficiency states who were treated with 1000 mcg of oral cyanocobalamin daily.[15] Complete normalization of serum levels and liver stores for vitamin B_{12}, as well as full clinical remission, was observed in all patients studied over a 3-year period. Since then, several studies have validated the use of oral vitamin B_{12} in the treatment of pernicious anemia.[16]

Despite the research, the use of oral vitamin B_{12} therapy remains uncommon in the United States. In a survey of internists, 91% erroneously believed that vitamin B_{12} could not be absorbed in sufficient quantities without intrinsic factor.[17] Interestingly, 88% of these surveyed doctors also stated that an effective oral vitamin B_{12} therapy would be useful in their practice and further stated that it would be their preferred method of delivery if it were effective. Studies established that the average absorption rate of oral cyanocobalamin by patients with pernicious anemia is 1.2% across a wide range of dosages. Because the daily turnover rate is about 2 mcg, an oral dosage of 200 to 250 mcg daily results in an average absorption of 2.4 to 3 mcg, respectively, which may be adequate for most patients. However, higher doses are often prescribed to ensure effective treatment.

In the treatment of pernicious anemia, the usual intramuscular (IM) dosage recommended by most medical texts is 1000 mcg IM weekly for 8 weeks, then once a month for life. For oral vitamin B_{12}, the recommended dosage is 2000 mcg daily (14,000 mcg weekly) for at least 1 month, followed by a daily intake of 1000 mcg of vitamin B_{12}. Methylcobalamin, the active form of B_{12}, is preferred over cyanocobalamin.

Folic Acid–Deficiency Anemia

Dietary foods high in folic acid include liver, asparagus, dried beans, brewer's yeast, dark green leafy vegetables, and whole grains. Because folic acid is destroyed by heat and light, fruits and vegetables should be eaten fresh or with very little cooking.

To replenish folic acid stores, 800 to 1000 mcg of active folate should be taken every day for up to 1 month. Folic acid is available as folic acid (folate) and folinic acid (5-methyl-tetra-hydrofolate). To utilize folic acid, the body must first convert it to tetrahydrofolate and then add a methyl group to form methyl-tetrahydrofolate (folinic acid). Methyl-tetrahydrofolate is the most active form of folic acid and has been shown to be more efficient at raising body stores than folic acid.[18] Supplying the body with methyl-tetrahydrofolate bypasses these steps and is needed for those with a genetic inability to make the conversion.

THERAPEUTIC APPROACH

General Recommendations

Effective therapy for anemia is dependent on proper classification as to its cause. Blood tests should be performed every 1 to 2 months to determine the efficacy of treatment.

Diet

Perhaps the best food for individuals with anemia is calf's liver. Ingestion of 4 to 6 oz of calf's liver 3 times per week is an option. In addition, the liberal consumption of green leafy vegetables is recommended.

NUTRITIONAL SUPPLEMENTS

Iron-Deficiency Anemia

- Iron: 30 mg, bound to either pyrophosphate, succinate, glycinate, or fumarate, twice per day between meals (if this recommendation results in abdominal discomfort, patients should take 30 mg with meals twice per day)
- Vitamin C: 1 gram 3 times per day with meals

B_{12}-Deficiency Anemia

- Oral vitamin B_{12} (methylcobalamin): 2000 mcg per day sublingually for at least 1 month; adjust dosage according to laboratory results
- 5-MTHF: 800 to 1200 mcg 3 times per day

Folic Acid–Deficiency Anemia

- 5-MTHF: 800 to 1200 mcg 3 times per day
- Vitamin B_{12}: 1000 mcg per day; supplementing vitamin B_{12} with folate prevents the folic acid supplement from masking a vitamin B_{12} deficiency

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Kuhn V, Diederich L, Keller TCS, et al. Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, anemia. *Antioxid Redox Signal*. 2017;26(13):718–742. PubMed PMID: 27889956.
2. Sharp PA. Intestinal iron absorption: regulation by dietary & systemic factors. *Int J Vitam Nutr Res*. 2010;80(4-5):231–242.
3. Killip S, Bennett JM, Chambers MD. Iron deficiency anemia. *Am Fam Physician*. 2007;75(5):671–678.
4. Guyatt GH, Oxman AD, Ali M, Willan A, et al. Laboratory diagnosis of iron-deficiency anemia: an overview [published correction appears in *J Gen Intern Med.* 1992:7, 423]. *J Gen Intern Med.* 1992;7:145–153.
5. Killip S, Bennett JM, Chambers MD. Iron deficiency anemia. *Am Family Physician*. 2007;75(5):671–678. PubMed PMID: 17375513.
6. Arvidsson B, Ekenved G, Rybo G, Sölvell L. Iron prophylaxis in menorrhagia. *Acta Ob Gyn Scand*. 1981;60:157–160.
7. Taymor ML, Sturgis SH, Yahia C. The etiological role of chronic iron deficiency in production of menorrhagia. *JAMA*. 1964;187:323–327.
8. Haas JD, Brownie TIV. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *J Nut*. 2001;131(2S–2):676S–690S. PubMed PMID: 11160598.
9. Hannibal L, Lysne V, Bjørke-Monsen AL, et al. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. *Front Mol Biosci*. 2016;3:27. PubMed PMID: 27446930.
10. Schnog JB, Duits AJ, Muskiet FA, ten Cate H, et al. Sickle cell disease; a general overview. *Netherlands J Med*. 2004;62(10):364–374. PubMed PMID: 15683091.
11. Elnicki DM, Shockcor WT, Brick JE, Beynon D. Evaluating the complaint of fatigue in primary care: diagnoses and outcomes. *Am J Med*. 1992;93:303–306.
12. Fidler MC, Walczyk T, Davidsson L, et al. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. *Br J Nutr*. 2004;91(1):107–112.
13. Watanabe F, Yabuta Y, Bito T, Teng F. Vitamin B_{12}-containing plant food sources for vegetarians. *Nutrients*. 2014;6(5):1861–1873. PubMed PMID: 24803097.
14. Andrès E, Dali-Youcef N, Vogel T, Serraj K, et al. Oral cobalamin (vitamin B12) treatment. An update. *Int J Lab Hematol*. 2009;31(1):1–8.
15. Berlin R, Berlin H, Brante G, Pilbrant A. Vitamin B12 body stores during oral and parenteral treatment of pernicious anaemia. *Acta Med Scand*. 1978;204(1–2):81–84.
16. Oh R, Brown DL. Vitamin B12 deficiency. *Am Family Physician*. 2003;67(5):979–986. PubMed PMID: 12643357.
17. Lederle FA. Oral cobalamin for pernicious anemia: back from the verge of extinction. *J Am Geriatr Society*. 1998;46(9):1125–1127. PubMed PMID: 1125-7.
18. Pietrzik K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet*. 2010;49(8):535–548.

146

Angina

Michael T. Murray, ND,* and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Squeezing or pressure-like pain in the chest appearing immediately after exertion. Other precipitating factors include emotional tension, cold weather, or a large meal. Pain may radiate to the left shoulder blade, left arm, or jaw. The pain typically lasts for only 1 to 20 minutes.
- Stress, anxiety, and high blood pressure are typically present.
- Most people demonstrate an abnormal electrocardiographic reading (transient ST-segment depression) in response to light exercise (stress test).

GENERAL CONSIDERATIONS

Angina pectoris results when the supply of oxygen, and occasionally other nutrients, is inadequate to meet the metabolic needs of the heart muscle. The primary cause is atherosclerosis, although platelet aggregation, coronary artery spasm, nonvascular mechanisms such as hypoglycemia, and increased metabolic need (such as in hyperthyroidism) can also be important.

The primary lesion of atherosclerosis is the atheromatous plaque, which progressively narrows and ultimately blocks the coronary artery, resulting in a decreased supply of blood and oxygen to the heart tissue. Symptoms typically begin to appear after a major coronary artery is blocked by more than 50%.

Blood flow to the heart may also be compromised by transient platelet aggregation (discussed in more detail in Chapter 149) and coronary artery spasm. Prinzmetal's variant angina, the most commonly recognized form of coronary artery spasm, is not due to plaque in the coronary arteries and is more apt to occur at rest or at odd times during the day or night. It is more common in women younger than age 50. Magnesium insufficiency–induced coronary artery spasm, more common in men than women, is now recognized as an important cause of myocardial infarction (MI) and may be of significance in angina pectoris (Fig. 146.1).

*Previous edition contributor

DIAGNOSTIC CONSIDERATIONS

The diagnosis of angina is frequently made by history alone. Clinical evaluation of all patients with angina should include an electrocardiogram (ECG) at rest and a chest radiograph. Because more than one half of patients with typical angina and confirmed coronary atherosclerosis have normal 12-lead ECG readings at rest, diagnosis must often be confirmed using ECG stress testing or 24-hour Holter monitoring (ambulatory ECG).

The most common diagnostic ECG changes associated with angina are evidence of previous MI and ST-segment and T-wave changes that occur *during* attacks of pain. The most characteristic change is displacement of the ST segment with or without T-wave inversion (Fig. 146.2). Complicating diagnosis, however, is the observation that hypoglycemia-induced angina does not manifest with rate or ST-segment abnormalities.[1]

THERAPEUTIC CONSIDERATIONS

Angina is a serious condition that requires careful treatment and monitoring. In the severe case as well as in the initial stages of mild to moderate angina, prescription medications may be necessary. Eventually the condition should be controlled with the help of natural measures. If there is significant blockage of the coronary artery, intravenous ethylenediaminetetraacetic acid (EDTA) chelation therapy, angioplasty, or coronary artery bypass may be appropriate.

From the perspective of natural medicine, there are two primary therapeutic goals in the treatment of angina: improving energy metabolism within the heart and improving blood supply to the heart. These goals are interrelated, as an increased blood flow means improved energy metabolism, and vice versa.

The heart uses fats as its major metabolic fuel. It converts free fatty acids to energy in much the same way as an automobile uses gasoline. Defects in the utilization of fats by the heart greatly increase the risk of atherosclerosis, heart attacks, and anginal pains. Specifically, impaired utilization of fatty acids by the heart results in the accumulation of high concentrations of fatty acids within the heart muscle.

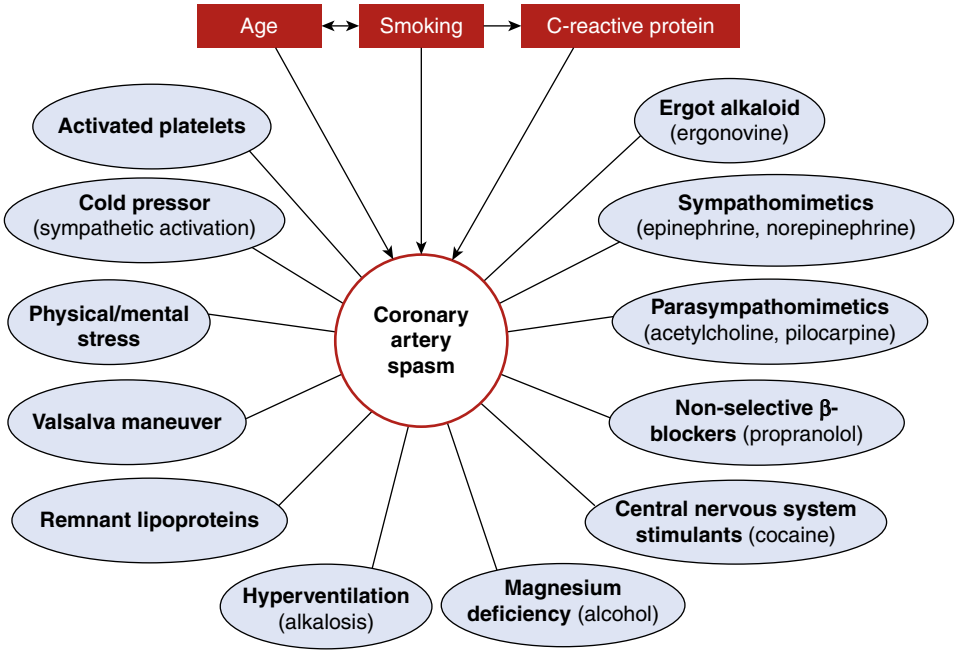
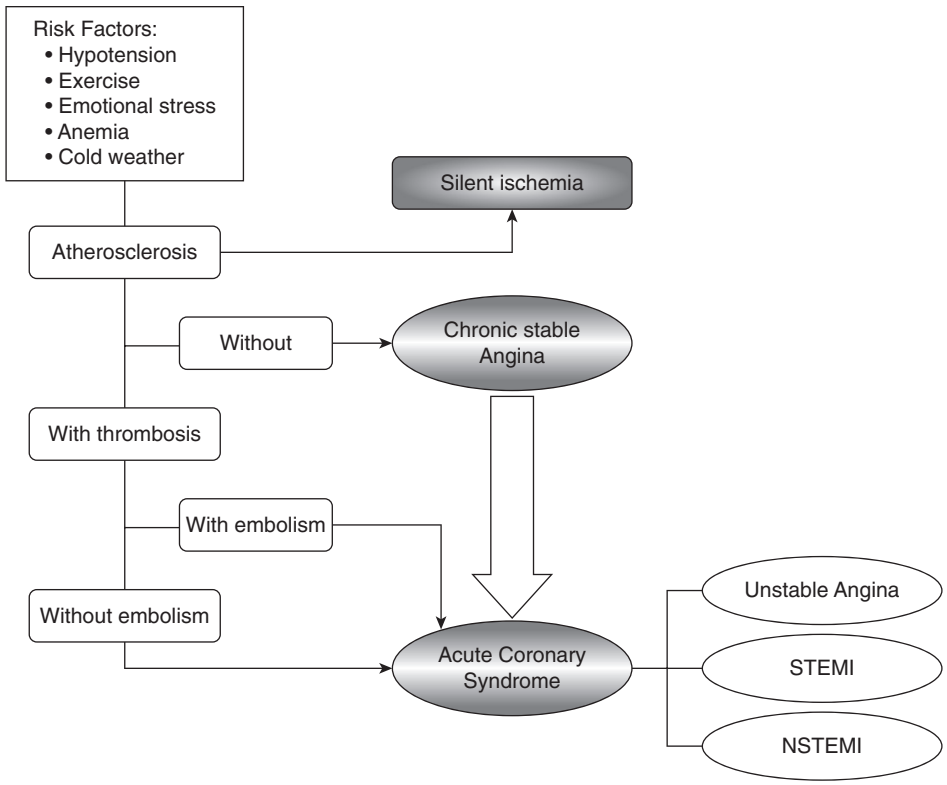


Fig. 146.1 Risk factors and precipitating factors for coronary artery spasm. (From Hung MJ, Hu P, Hung MY. Coronary artery spasm: review and update. *Int J Med Sci.* 2014;11[11],1161-71.)



STEMI = ST-Segment Elevation Myocardial Infarction
NSTEMI = Non-ST Segment Myocardial Infarction

Fig. 146.2 Algorithm of diagnostic in coronary artery disease. (Retrieved from Non-Invasive Imaging in Approaching Ischemic Coronary Artery Disease—Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Algorithm-of-diagnostic-in-coronary-artery-disease-High-risk-of-coronary-artery-disease_fig1_221916523 [accessed May 14, 2019])

This accumulation makes the heart extremely susceptible to cellular damage, which ultimately leads to a heart attack.

Carnitine, pantethine, and coenzyme Q_{10} (CoQ_{10}) are essential compounds in normal fat and energy metabolism and are of extreme benefit to sufferers of angina. These nutrients prevent the accumulation of fatty acids within the heart muscle by improving the conversion of fatty acids and other compounds into energy.

Nutritional Supplements for Angina

The use of antioxidant supplementation in patients with angina is important. In an analysis of normal controls and patients with either stable or unstable angina, the plasma level of antioxidants has been shown to be a more sensitive predictor of unstable angina than the severity of atherosclerosis.[2,3] One group of researchers concluded: "These data are consistent with the hypothesis that the beneficial effects of antioxidants in coronary artery disease (CAD) may result, in part, by an influence on lesion activity rather than a reduction in the overall extent of fixed disease."[2]

Antioxidant nutrients are also important in preventing nitrate tolerance. Oral nitrates are widely used in the conventional treatment of angina, but their continuous administration can result in the rapid development of tolerance. Experimental findings indicate that nitrate tolerance is associated with increased vascular production of superoxide. The superoxide anions generated quickly degrade the nitric oxide formed from the administration of nitroglycerin and result in lower levels of cyclic guanosine monophosphate (an important intracellular regulator that promotes vasorelaxation). Because vitamin C is the main aqueous-phase antioxidant and free radical scavenger of superoxide and vitamin E is the main lipid-phase antioxidant, their importance in preventing nitrate tolerance is obvious. Clinical trials have upheld this connection, showing that high-dose supplementation of vitamins C and E can prevent nitrate tolerance.[4,5]

Carnitine

Carnitine, a vitamin-like compound, stimulates the breakdown of long-chain fatty acids by the energy-producing units in cells—the mitochondria. Carnitine is essential in the transport of fatty acids into the mitochondria. A deficiency in carnitine results in a decrease in fatty-acid concentrations in the mitochondria and reduced energy production.

Normal heart function is critically dependent on adequate concentrations of carnitine. Although the normal heart stores more carnitine than it needs, if the heart does not have a good supply of oxygen, carnitine levels quickly decrease. This decrease leads to decreased energy production in the heart and increased risk for angina and heart disease. Because angina patients have a decreased supply of oxygen, carnitine supplementation makes good sense.

Several clinical trials have demonstrated that carnitine improves angina and heart disease.[6-10] Supplementation with carnitine normalizes heart carnitine levels and allows the heart muscle to use its limited oxygen supply more efficiently. This increased efficiency translates to an improvement in cases of angina. Improvements have been noted in exercise tolerance and heart function and suggest that carnitine is an effective alternative to drugs in cases of angina.

In one study of patients with stable angina, oral administration of 900 mg of L-carnitine increased mean exercise time and the time necessary for abnormalities to occur on a stress test (6.4 minutes in the placebo group compared with 8.8 minutes in the carnitine-treated group).[10] These results indicate that carnitine may be an effective alternative to other antianginal agents such as beta blockers, calcium channel antagonists, and nitrates, especially in patients with chronic stable angina pectoris.

Carnitine, by improving fatty-acid utilization and energy production in the heart muscle, may also prevent the production of toxic fatty-acid metabolites. These compounds are extremely deleterious because they activate various phospholipases and disrupt cellular membrane structures. The changes in the properties of cardiac cell membranes induced by fatty-acid metabolites are thought to contribute to impaired heart muscle contractility and compliance, increased susceptibility to irregular beats, and the eventual death of heart tissue. Supplemental carnitine increases heart carnitine levels and prevents the production of toxic fatty-acid metabolites. This mechanism has been demonstrated clinically, where the early administration of L-carnitine (40 mg/kg per day) in patients having heart attacks was found to considerably reduce heart damage.[11]

Pantethine

Pantethine is the stable form of pantetheine, the active form of pantothenic acid, which is the fundamental component of coenzyme A (CoA). CoA is involved in the transport of fatty acids to and from cells as well as to the mitochondria. The synthetic pathway from pantethine to CoA is much shorter than that of pantothenic acid, making pantetheine the preferred therapeutic substance. In addition, pantetheine has significant lipid-lowering activity, whereas pantothenic acid has very little if any effect in lowering cholesterol and triglyceride levels.

The standard dose for pantethine is 900 mg per day. Like carnitine, pantethine has been shown in clinical trials to significantly reduce serum triglyceride and cholesterol levels and to increase high-density-lipoprotein cholesterol levels.[12-14] Its lipid-lowering effects are most impressive when its toxicity (virtually none) is compared with that of conventional lipid-lowering drugs. Its mechanism of action is due to the inhibition of cholesterol synthesis and acceleration of fatty acid breakdown in the mitochondria.

Pantethine is well indicated in angina. Like carnitine, heart pantethine levels decrease during times of reduced oxygen supply. Demonstrated effects in animals indicate that it would greatly benefit individuals with angina.[15]

Coenzyme Q_{10}

CoQ_{10}, also known as *ubiquinone*, is an essential component of the mitochondria, where it plays a major role in energy production. Like carnitine and pantethine, CoQ_{10} can be synthesized in the body. Nonetheless, deficiency states have been reported. Deficiency can be a result of impaired CoQ_{10} synthesis due to nutritional deficiencies, a genetic or acquired defect in CoQ_{10} synthesis, or increased tissue needs.[16]

Cardiovascular diseases—including angina, hypertension, mitral valve prolapse, and congestive heart failure—are examples of diseases that require increased tissue levels of CoQ_{10}.[16] In addition, many elderly persons may have increased CoQ_{10} requirements; the decline of CoQ_{10} levels that occurs with age may be partly responsible for the age-related deterioration of the immune system.

CoQ_{10} deficiency is common in individuals with heart disease. Heart tissue biopsies in patients with various heart diseases show a CoQ_{10} deficiency in 50% to 75% of cases.[16] One of the most metabolically active tissues in the body, the heart may be unusually susceptible to the effects of CoQ_{10} deficiency. Accordingly, CoQ_{10} has shown great promise in the treatment of heart disease.

In one study, 12 patients with stable angina pectoris were treated with CoQ_{10} (150 mg per day for 4 weeks) in a double-blind crossover trial.[17] In comparison with placebo, CoQ_{10} reduced the frequency of anginal attacks by 53%. In addition, researchers found a significant increase in treadmill exercise–tolerance (time to onset of chest pain and time to development of ECG abnormalities) during CoQ_{10} treatment. The results of this study and others suggest that CoQ_{10} is a safe and effective treatment for angina pectoris.

Carnitine, pantethine, and CoQ_{10} should be considered in all heart disorders, not just angina.

Magnesium

Magnesium deficiency may play a major role in angina, including Prinzmetal's variant. A magnesium deficiency has been shown to produce spasms of the coronary arteries and is thought to be a cause of nonocclusive heart attacks.[18] Furthermore, researchers have observed that men who die suddenly of heart attacks have significantly lower levels of heart magnesium, as well as potassium, compared to matched controls.[19]

Making magnesium the treatment of choice for angina due to coronary artery spasm has been suggested.[19-21] Magnesium administration has also been found to be helpful in the management of arrhythmias and in angina due to atherosclerosis. Its benefit in these situations is presumably via the same mechanisms responsible for its effects in an acute MI.

Since the mid-1980s, eight well-designed studies involving more than 4000 patients have demonstrated that intravenous magnesium supplementation during the first hour of admission to a hospital for an acute MI produces a favorable effect in reducing immediate and long-term complications as well as death rates.[22-24]

The beneficial effects of magnesium in acute MI relate to its ability to:

- Improve energy production within the heart
- Dilate the coronary arteries, resulting in improved delivery of oxygen to the heart
- Reduce peripheral vascular resistance, resulting in reduced demand on the heart
- Inhibit platelets from aggregating and forming blood clots
- Reduce the size of the infarct (blockage)
- Improve heart rate and arrhythmias

Arginine

Arginine supplementation has been shown to be beneficial in several cardiovascular diseases, including angina pectoris. Its benefit is thought to occur via increasing nitric oxide levels, thereby improving blood flow, reducing thrombosis, and improving rheology. The degree of improvement offered by arginine supplementation in angina and other cardiovascular diseases can be quite significant as a result of improved nitric oxide levels. In double-blind studies, it has been shown to be especially effective in increasing exercise tolerance. Typical dosage is 6 g per day in divided dosages.[25-27] In a short-term study, arginine supplementation of 3 g per day for 15 days resulted in increased activity of the free-radical scavenging enzyme superoxide dismutase and increased the levels of total thiols and ascorbic acid, with a concomitant decrease in lipid peroxidation, carbonyl content, serum cholesterol, and the activity of the prooxidant enzyme xanthine oxidase.[28] These beneficial changes point to additional mechanisms for the use of arginine in angina and cardiac ischemia. Use of arginine is cautioned by a report in one study in survivors of MI in whom supplementation with arginine (9 g per day for 6 months) was associated with an increase in mortality compared with the placebo group (8.6% vs. 0%).[29] This effect may have been an aberration or due to the use of higher dosages of arginine.

Botanical Medicines

Crataegus Species

Hawthorn berry and extracts of the flowering tops are widely used in Europe for their cardiovascular activity. They exhibit a combination of effects that are of great value to patients with angina and other heart problems. Numerous experiments and clinical studies have documented the cardiovascular efficacy of the plant through various mechanisms, including positive inotropic and negative chronotropic effects; escalation in coronary blood flow and exercise tolerance; inhibition of the enzymes, such as angiotensinconverting enzyme (ACE) and phosphodiesterase; providing anti-inflammatory and antihyperlipidemic effects; and improving status of antioxidant enzymes, which support its cardioactive efficacy.[30] Studies have demonstrated that hawthorn extracts are effective in reducing anginal attacks as well as in lowering blood pressure and serum cholesterol levels.[31-33]

The beneficial effects in the treatment of angina are due to improvement in the blood and oxygen supply of the heart resulting from dilation of the coronary vessels, as well as improvement of the metabolic processes in the heart.[31-35]

The ability of *Crataegus* species to dilate coronary blood vessels has been repeatedly demonstrated in experimental studies.[31-33] In addition, *Crataegus* extracts have been shown to improve cardiac energy metabolism in human and experimental studies. This combined effect is extremely important in the treatment of angina because it results in improved myocardial function with more efficient use of oxygen. This improvement results not only from increased blood and oxygen supply to the heart muscle but also from hawthorn flavonoids interacting with key enzymes to enhance myocardial contractility.

Because adhesion molecules play an important role in the development and progression of coronary atherosclerosis, one study evaluated the effect of Cratagol herbal tablets, aerobic exercise, and their combination on the serum levels of Intercellular Adhesion Molecule (ICAM)-1 and E-Selectin in patients with stable angina pectoris.[34] Eighty stable angina pectoris patients age 45 to 65 years were randomly divided into four groups including three experimental groups and one control group: aerobic exercise (E), *C. oxyacantha* extract (S), aerobic exercise and *C. oxyacantha* extract (S+E), and control (C). After 12 weeks of treatment, intergroup comparison of the data revealed a significant reduction ($P < 0.01$) in serum levels of ICAM-1 and E-selectin in experimental groups. The authors concluded that aerobic exercise and consuming *C. oxyacantha* extract present an effective complementary strategy to significantly lower the risk of atherosclerosis and heart problems. (See Chapter 71 for a comprehensive discussion of this important botanical.)

Ammi visnaga

Khella is a medicinal plant native to the Mediterranean region, where it has been used in the treatment of angina and other heart ailments since the time of the pharaohs. Several of its components have demonstrated the ability to dilate the coronary arteries. Its mechanism of action appears to be similar to that of the calcium channel blocking drugs.

Since the late 1940s, there have been numerous scientific studies on the clinical effect of khella extracts in the treatment of angina. More specifically, khellin, a derivative of the plant, was shown to be extremely effective in relieving anginal symptoms, improving exercise tolerance, and normalizing ECGs. This finding is evident by the concluding statements in a study by Osher and colleagues[35] in 1951: "The high proportion of favorable results, together with the striking degree of improvement frequently observed, has led us to the conclusion that khellin, properly used, is a safe and effective drug for the treatment of angina pectoris."

At higher doses (120–150 mg per day), pure khellin was associated with mild side effects, such as anorexia, nausea, and dizziness. Although most clinical studies used high dosages, several studies show that as little as 30 mg per day appears to offer as good results with fewer side effects.[36,37]

Rather than using the isolated compound khellin, khella extracts standardized for khellin content (typically 12%) are the preferred form. A daily dose of such an extract would be 250 to 300 mg. Khella appears to work well with hawthorn extracts.

Other Therapies

Acupuncture

Several studies have shown acupuncture to be of benefit in improving angina, specifically in reducing nitroglycerin use, decreasing the number of anginal attacks, and improving exercise tolerance and ECG readings.[38-41]

Relaxation and Breathing Exercises

Relaxation and breathing exercises may be helpful in improving anginal symptoms, especially when anxiety is a significant contributor.[42,43] In one study in patients with cardiac syndrome X, a form of angina in people with otherwise normal coronary arteries, transcendental meditation (20 minutes twice daily of silently chanting a mantra with eyes closed) was found to reduce angina-like chest pain and to normalize ECGs.[42]

Intravenous Ethylenediaminetetraacetic Acid (EDTA) Chelation Therapy

EDTA chelation therapy is an alternative to coronary artery bypass surgery and angioplasty, which may prove to be more effective, safer, and less expensive. EDTA is an amino acid–like molecule that, when slowly infused into the bloodstream, binds with minerals such as calcium, iron, copper, and lead and carries them to the kidneys, where they are excreted. EDTA chelation has been commonly used for lead poisoning, but in the late 1950s and early 1960s it was found to help patients with atherosclerosis.

The discovery of EDTA chelation therapy in the treatment of angina and other conditions associated with atherosclerosis happened accidentally. In 1956 a battery worker whom Norman Clarke was treating with EDTA for lead poisoning noticed that his symptoms of angina disappeared. Clarke and others began using EDTA chelation therapy in patients with angina, cerebral vascular insufficiency, and occlusive peripheral vascular disease.

In a series of 283 patients treated by Clarke and colleagues from 1956 to 1960, a total of 87% showed improvements in their symptoms. Heart patients improved, and patients with blocked arteries in the legs, particularly those with diabetes, avoided amputation.[44,45]

It was originally thought that EDTA opened blocked arteries by chelating out the calcium deposits in the cholesterol plaque. However, the benefit now seems more related to chelating out excess iron and copper, minerals that, in the presence of oxygen, stimulate free radicals. Free radicals damage arterial cells, and this damage is a primary cause of atherosclerosis.

In a review of the progression and regression of atherosclerosis, the authors write that the process of atherosclerosis is "dependent on the presence of some metals (copper and iron) and can be completely inhibited by chelating agents such as EDTA."[46]

Despite obvious benefits to heart patients, EDTA fell out of favor in the mid-1960s. Advocates believe this occurred for two reasons: (1) the lucrative surgical approach to heart and vessel disease was on the rise; and (2) the patent on EDTA that was held by Abbott Laboratories expired, so there was no financial interest for drug companies to fund research.

Fortunately, in 1972 a small group of practicing physicians using EDTA chelation therapy founded an organization now called the American College for the Advancement of Medicine to continue education and research in this important area.

In the early days of EDTA chelation therapy, several serious problems were discovered. Giving too much EDTA or giving it too fast was soon noted to be dangerous. In fact, several deaths attributed to kidney failure were caused by toxicity to EDTA. Fortunately, additional research resulted in more appropriate protocols, and EDTA chelation therapy is now safe. No deaths or significant adverse reactions have occurred in more than 500,000 patients who have undergone EDTA chelation therapy. Because EDTA chelation improves blood flow throughout the body, the "side effects" are usually beneficial and only a few adverse effects are noticed.

A substantial body of scientific evidence exists on the use of EDTA chelation therapy in the treatment of angina, peripheral vascular disease, and cerebral vascular disease.[47-51]

Nonetheless, there is a paucity of well-designed placebo-controlled studies to definitively assess the efficacy of this approach. This shortcoming is unfortunate considering the early successes. The conclusion from a Cochrane Review summarizes the situation well: "At present, there is insufficient evidence to decide on the effectiveness or ineffectiveness of chelation therapy in improving clinical outcomes of patients with atherosclerotic cardiovascular disease." For more information, refer to the American College of Advancement in Medicine (ACAM), 23121 Verdugo Drive, Suite 204, Laguna Hills, CA, 92653; 1-800-532-3688 (outside California) or 1-800-435-6199 (inside California); www.acam.org.

THERAPEUTIC APPROACH

The primary therapy for angina is prevention, because angina is usually secondary to atherosclerosis. Once angina has developed, restoring proper blood supply to the heart and enhancing energy production within the heart are necessary. Particularly important nutrients for accomplishing these results are vitamins C and E, carnitine, pantethine, CoQ_{10}, magnesium, and arginine. Magnesium is of additional benefit because of its ability to relax spastic coronary arteries and improve heart function.

Hawthorn berries or extracts offer several benefits to individuals with angina, including coronary artery dilation and improved heart muscle metabolism.

Patients with unstable angina pectoris (characterized by a progressive increase in the frequency and severity of pain, increased sensitivity to precipitating factors, progression of symptoms over several days, and prolonged coronary pain) should be hospitalized.

Diet

The dietary guidelines given in Chapter 44 are appropriate here. An increase of dietary fiber is recommended, especially the gel-forming or mucilaginous fibers (e.g., flaxseed, oat bran, pectin). Onions and garlic (both raw and cooked), vegetables, and fish should also be increased, and the consumption of saturated fats, cholesterol, sugar, and animal proteins should be reduced. All fried foods and food allergens should be avoided. Patients with reactive hypoglycemia should eat regular meals and carefully avoid simple carbohydrates in all forms (e.g., sugar, honey, dried fruit, and fruit juice).

Lifestyle

The individual with angina should not smoke or drink alcohol or coffee. Stress should be decreased by using stress management techniques such as progressive relaxation, meditation, or guided imagery. A carefully graded progressive aerobic exercise program (30 minutes three times a week) is a necessity. Walking is a good exercise with which to start.

Nutritional Supplements

- Vitamin C: 500 to 1500 mg a day
- Vitamin E: 200 to 400 International Units per day
- CoQ_{10}: 150 to 300 mg a day
- L-Carnitine: 500 mg three times a day

- Pantethine: 300 mg three times a day
- Magnesium, preferably bound to aspartate, citrate, or other Krebs cycle intermediate: 200 to 400 mg three times a day
- Arginine: 1000 to 2000 mg three times a day

Botanical Medicines

- *Crataegus oxyacantha* (three times a day)
  - Berries or flowers (dried): 3 to 5 g or as a tea
  - Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
  - Solid extract (10% procyanidins or 1.8% vitexin-4′-rhamnoside): 100 to 250 mg
- *Ammi visnaga* (three times a day)
  - Dried powdered extract (12% khellin content): 100 mg three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bansal S, Toh SH, LaBresh KA. Chest pain as a presentation of reactive hypoglycemia. *Chest*. 1983;84:641–662.
2. Kostner K, Hornykewycz S, Yang P, et al. Is oxidative stress causally linked to unstable angina pectoris? A study in 100 CAD patients and matched controls. *Cardiovasc Res*. 1997;36:330–336.
3. Vita JA, Keaney Jr JF, Raby KE, et al. Low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome. *J Am Coll Cardiol*. 1998;31:980–986.
4. Watanabe H, Kakihana M, Ohtsuka S, et al. Randomized, double-blind, placebo-controlled study of ascorbate on the preventive effect of nitrate tolerance in patients with congestive heart failure. *Circulation*. 1998;97:886–891.
5. Watanabe H, Kakihana M, Ohtsuka S, et al. Randomized, double-blind, placebo-controlled study of supplemental vitamin E on attenuation of the development of nitrate tolerance. *Circulation*. 1997;96:2545–2550.
6. Lagioia R, Scritinio D, Mangini SG, et al. Propionyl-L-carnitine: a new compound in the metabolic approach to the treatment of effort angina. *Int J Cardiol*. 1992;34:167–172.
7. Bartels GL, Remme WJ, Pillay M, et al. Effects of L-propionylcarnitine on ischemia-induced myocardial dysfunction in men with angina pectoris. *Am J Cardiol*. 1994;74:125–130.
8. Cacciatore L, Cerio R, Ciarimboli M, et al. The therapeutic effect of L-carnitine in patients with exercise-induced stable angina: a controlled study. *Drugs Exp Clin Res*. 1991;17:225–235.
9. Davini P, Bigalli A, Lamanna F. Controlled study on L-carnitine therapeutic efficacy in post-infarction. *Drugs Exptl Clin Res*. 1992;18:355–365.
10. Kamikawa T, Suzuki Y, Kobayashi A, et al. Effects of L-carnitine on exercise tolerance in patients with stable angina pectoris. *Jpn Heart J*. 1984;25:587–597.
11. Rebuzzi AG, Schiavoni G, Amico CM, et al. Beneficial effects of L-carnitine in the reduction of the necrotic area in acute myocardial infarction. *Drugs Exp Clin Res*. 1984;10:219–223.
12. Arsenio L, Bodria P, Magnati G, et al. Effectiveness of long-term treatment with pantethine in patients with dyslipidemias. *Clin Ther*. 1986;8:537–545.
13. Miccoli R, Marchetti P, Sampietro T, et al. Effects of pantethine on lipids and apolipoproteins in hypercholesterolemic diabetic and non-diabetic patients. *Curr Ther Res*. 1984;36:545–549.
14. Gaddi A, Descovich GC, Noseda G, et al. Controlled evaluation of pantethine, a natural hypolipidemic compound, in patients with different forms of hyperlipoproteinemia. *Atherosclerosis*. 1984;50:73–83.
15. Hayashi H, Kobayashi A, Terad H, et al. Effects of pantethine on action potential of canine papillary muscle during hypoxic perfusion. *Jpn Heart J*. 1985;26:289–296.
16. Folkers K, Yamamura Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q: Proceedings of the International Symposium on Coenzyme Q*. Vols 1–4. Amsterdam, Netherlands: Elsevier Scientific.
17. Kamikawa T, Kobayashi A, Yamashita T, et al. Effects of coenzyme Q10 on exercise tolerance in chronic stable angina pectoris. *Am J Cardiol*. 1985;56:247–251.
18. Turlapaty PD, Altura BM. Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. *Science*. 1980;208:198–200.
19. Altura BM. Ischemic heart disease and magnesium. *Magnesium*. 1988;7:57–67.
20. McLean RM. Magnesium and its therapeutic uses: a review. *Am J Med*. 1994;96:63–76.
21. Purvis JR, Movahed A. Magnesium disorders and cardiovascular disease. *Clin Cardiol*. 1992;15:556–568.
22. Hampton EM, Whang DD, Whang R. Intravenous magnesium therapy in acute myocardial infarction. *Ann Pharmacothe*. 1994;28:220–226.
23. Teo KK, Yusuf S. Role of magnesium in reducing mortality in acute myocardial infarction: a review of the evidence. *Drugs*. 1993;46:347–359.
24. Schecter M, Kaplinsky E, Rabinowitz B. The rationale of magnesium supplementation in acute myocardial infarction: a review of the literature. *Arch Intern Med*. 1992;152:2189–2196.
25. Bednarz B, Wolk R, Chamiec T, et al. Effects of oral L-arginine supplementation on exercise-induced QT dispersion and exercise tolerance in stable angina pectoris. *Int J Cardiol*. 2000;75(2-3):205–210.
26. Kobayashi N, Nakamura M, Hiramori K. Effects of infusion of L-arginine on exercise-induced myocardial ischemic ST-segment changes and capacity to exercise of patients with stable angina pectoris. *Coron Artery Dis*. 1999;10(5):321–326.
27. Ceremuzyński L, Chamiec T, Herbaczyńska-Cedro K. Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. *Am J Cardiol*. 1997;80(3):331–333.
28. Tripathi P, Chandra M, Misra MK. Oral administration of L-arginine in patients with angina or following myocardial infarction may be protective by increasing plasma superoxide dismutase and total thiols with reduction in serum cholesterol and xanthine oxidase. *Oxid Med Cell Longev*. 2009;2(4):231–237.
29. Schulman SP, Becker LC, Kass DA, et al. L-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA*. 2006;295(1):58–64.
30. Orhan IE. Phytochemical and Pharmacological Activity Profile of Crataegus oxyacantha L. (Hawthorn) - A Cardiotonic Herb. *Curr Med Chem*. 2018;25(37):4854–4865. PubMed PMID: 27655074.
31. Ammon HPT, Handel M. Crataegus, toxicology and pharmacology. Part I: Toxicity. *Planta Med*. 1981;43:105–120. Part II: Pharmacodynamics. *Planta Med*. 1981;43:209-239; Part III: Pharmacodynamics and pharmacokinetics. *Planta Med*. 1981;43: 313-322.
32. Blesken R. Crataegus in cardiology. *Fortschr Med*. 1992;110:290–292.
33. Nasa Y, Hashizume H, Hoque AN, et al. Protective effect of Crataegus extract on the cardiac mechanical dysfunction in isolated perfused working rat heart. *Arzneimittelforschung*. 1993;43:945–949.
34. Jalaly L, Sharifi G, Faramarzi M. Comparison of the effects of Crataegus oxyacantha extract, aerobic exercise and their combination on the serum levels of ICAM-1 and E-Selectin in patients with stable angina pectoris. *Daru*. 2015;23:54. PubMed PMID: 26687477.
35. Osher HL, Katz KH, Wagner DJ. Khellin in the treatment of angina pectoris. *N Engl J Med*. 1951;244:315–321.
36. Anrep GV, Kenawy MR, Barsoum GS. Coronary vasodilator action of khellin. *Am Heart J*. 1949;37:531–542.
37. Conn JJ, Kissane RW, Koons RA, et al. Treatment of angina pectoris with khellin. *Ann Intern Med*. 1952;36:1173–1178.
38. Ballegaard S, Karpatschoff B, Holck JA, et al. Acupuncture in angina pectoris: do psychosocial and neurophysiological factors relate to the effect? *Acupunct Electrother Res*. 1995;20:101–116.
39. Meng J. The effects of acupuncture in treatment of coronary heart diseases. *J Tradit Chin Med*. 2004;24:16–19.
40. Ballegaard S, Jensen G, Pedersen F, et al. Acupuncture in severe, stable angina pectoris: a randomized trial. *Acta Med Scand*. 1986;220:307–313.
41. Richter A, Herlitz J, Hjalmarson A. Effect of acupuncture in patients with angina pectoris. *Eur Heart J*. 1991;12:175–178.
42. Cunningham C, Brown S, Kaski JC. Effects of transcendental meditation on symptoms and electrocardiographic changes in patients with Cardiac Syndrome X. *Am J Cardiol*. 2000;85:653–655. A10.
43. Gilbert C. Clinical applications of breathing regulation: beyond anxiety management. *Behav Modif*. 2003;27:692–709.
44. Clarke CN, Clarke NE, Mosher RE. Treatment of angina pectoris with disodium ethylene diamine tetraacetic acid. *Am J Med Sci*. 1956;232:654–666.
45. Sr Clarke NE. Atherosclerosis, occlusive vascular disease and EDTA. *Am J Cardiol*. 1960;6:233–236.
46. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med*. 1989;320:915–924.
47. Cranton EM, Frackelton JP. Current status of EDTA chelation therapy in occlusive arterial disease. *J Adv Med*. 1989;2:107–119.
48. Olszewer E, Carter JP. EDTA chelation therapy: a retrospective study of 2,870 patients. *J Adv Med*. 1989;2:197–211.
49. Olszewer E, Sabbag FC, Carter JP. A pilot double-blind study of sodium-magnesium EDTA in peripheral vascular disease. *J Nat Med Assoc*. 1990;82:173–177.
50. Olszewer E, Carter JP. EDTA chelation therapy in chronic degenerative disease. *Med Hypotheses*. 1988;27:41–49.
51. Casdorph HR. EDTA chelation therapy, efficacy in arteriosclerotic heart disease. *J Holistic Med*. 1981;3:53–59.

147

Aphthous Stomatitis

Michael Traub, ND, DHANP, FABNO, and *Michael T. Murray*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Single or several discrete, shallow, painful ulcers found anywhere on the oral mucosa: labial and buccal mucosa, maxillary and mandibular sulci, gingiva, soft palate, tonsillar fauces, floor of the mouth, ventral surface of the tongue.
- 75% to 90% of recurrent aphthous ulcers (RAU) are 5 to 10 mm in size, shallow, and classified as minor aphthae
- 10% to 15% of RAU are greater than 10 mm in size, deep, and classified as major aphthae.
- Lesions have fairly even borders, are surrounded by an erythematous halo, and eventually are covered by a fibrous pseudomembrane.
- Lesions usually resolve in 7 to 10 days but are often recurrent; larger ulcers may last several weeks to months and can leave a temporary scar.
- It is often difficult to eat due to the discomfort of chewing food and the ensuing pain from scraping the food against the lesion. When located near the soft palate or esophagus, it becomes difficult to swallow due to odynophagia. Pain from aphthae on the lip or tongue can also cause patients to limit their speech.

GENERAL CONSIDERATIONS

Aphthous stomatitis, recurrent aphthous ulcers (RAUs), or canker sores is a common condition, affecting 1% to 78% of the population, depending on country and on data from clinical examination or information from the patient's history.[1] RAU is an idiopathic multifactorial disorder that can cause significant morbidity (Fig. 147.1). Although usually self-limited, in some individuals recurrence can appear continuous.

Inflammatory bowel disease (IBS), celiac disease, systemic lupus erythematosus, AIDS, and Behçet's syndrome are systemic disorders associated with RAUs. Many people mistakenly identify RAUs as herpes simplex, although there is an uncommon form, known as herpetiform RAU, consisting of clusters of aphthae less than 1 mm in diameter (Fig. 147.2). Table 147.1 lists several conditions included in the differential diagnosis of RAU.[2]

The etiology, based on studies of initiating factors, appears to be related to genetics, food sensitivities (especially gluten sensitivity), nutrient deficiency, and hormonal changes.[3] These factors unify the key underlying feature of RAU—genetic predisposition and dysregulation of the immune system in the oral mucosa. Family history is positive in up to 40% of patients.[4] Genetic risk factors that modify individual susceptibility to RAU include HLA alleles and DNA polymorphisms, especially those related to alterations in the metabolism of interleukins, interferon (IFN)-c and tumor necrosis factor (TNF)-a.[5]

Histologically, RAU consists of mucosal ulcerations with mixed inflammatory cell infiltrates. T-helper cells predominate in the preulcerative and healing phases, whereas T-suppressor cells predominate in the ulcerative phase. Other findings associated with immune dysregulation include:

- Lymphomononuclear infiltrate and hemagglutination antibodies against oral mucosa
- Reduced response of lymphocytes to mitogens
- Circulating immune complexes
- Alterations in natural killer (NK) cell activity
- Increased adherence of neutrophils
- Release of tumor necrosis factor-alpha (TNF-α)
- Significant involvement of mast cells in the pathogenesis of RAU
- Reduced levels and function of regulatory T cells in aphthae
- Reduced expression of heat shock protein 27 and interleukin 10 in lesions[6]
- Elevated salivary and serum cortisol[7]
- Elevated Toll-like receptor activity[8]
- Oxidative stress as measured by glutathione and malonidialdehyde[9]

THERAPEUTIC CONSIDERATIONS

Allergies and Environmental Factors

The oral cavity is obviously the first site of contact for ingested and many inhaled allergens. The histological appearance of the lesions and the association of RAU with increased serum antibodies to food antigens and atopy suggest that an allergic reaction may be involved.[10]

*Previous edition contributor

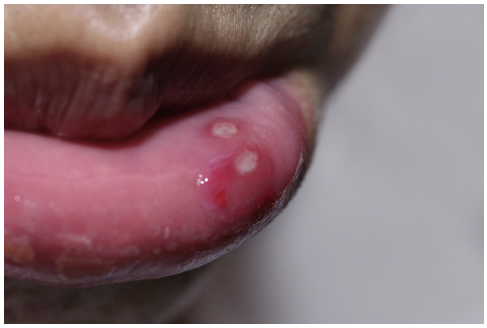


Fig. 147.1 Aphthous stomatitis. (Retrieved from https://www.istockphoto.com/photo/lip-with-aphthous-stomatitis-gm472584972-63480929)

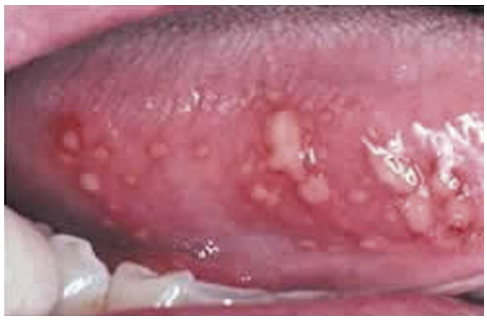


Fig. 147.2 Herpetiform recurrent aphthous stomatitis.

TABLE 147.1 Differential Diagnosis for Recurrent Aphthous Stomatitis

- Herpes zoster
- Pemphigus vulgaris
- Candidiasis
- Acute cutaneous lupus erythematosus
- Allergic contact dermatitis
- Cancers of the oral mucosa
- Cicatricial pemphigoid
- Erythema multiform
- Irritant contact dermatitis
- Langerhans cell histiocytosis
- Lichen planus
- Linear IgA dermatosis
- Paraneoplastic pemphigus
- Sweet syndrome
- Reactive arthritis
- Contact stomatitis
- Oral mucosal manifestations of GI disease
- Oral mucosal manifestations of hand-foot-mouth disease
- Oral manifestations of hematological disease
- Drug-induced bullous disorders
- Drug-induced lupus erythematosus
- Drug-induced pemphigus
- IgA pemphigus
- Sarcoidosis
- Pediatric syphilis
- Morsicatio buccalis
- Traumatic eosinophilic ulcer
- Steven-Johnson syndrome

GI, Gastrointestinal; *IgA*, immunoglobulin A.

Furthermore, immunoglobulin E–bearing lymphocytes are significantly increased in aphthous lesions, and mast cells are increased in tissue sections from prodromal stages of recurrent ulcers.[11] Mast cell degranulation plays an important role in the production of the aphthous lesion.[12] An elimination diet has been shown to have good therapeutic results.[13]

The allergen is not necessarily a food. Additional allergens inducing RAU are[14]:

- Benzoic acid
- Cinnamaldehyde
- Nickel
- Parabens
- Dichromate
- Sorbic acid

Elimination of allergens usually brings significant improvement and in many cases complete resolution.[15]

Local, chemical, or physical trauma often initiates aphthae in susceptible individuals (pathergy). Conclusive evidence is lacking to support the role of an infectious etiology, including herpes simplex virus, human herpes virus, varicella zoster virus, cytomegalovirus, and *Helicobacter pylori*.[16,17] Approximately 66% of HIV-positive individuals have herpetiform ulcers and RAUs, which must be differentiated from those caused by antiretroviral medications and bacterial, viral, or fungal infections.[18] RAU can result from a T cell–mediated response to antigens of *Streptococcus sanguis* that cross-react with heat-shock proteins. One controlled study has shown a statistically significant increase in RAU in subjects taking nonsteroidal anti-inflammatory drugs (NSAIDs).[19] Oral hygiene products, chewing gum, candy, acidic foods, and walnuts have been associated with RAU. The avoidance of toothpaste containing sodium lauryl sulfate may be helpful.[20,21] Two studies of traditional Chinese herbal formulas added to toothpastes have shown significant benefit for RAU patients.[22,23]

Gluten Sensitivity

Considerable evidence indicates that sensitivity to gluten is associated with RAU. The incidence of RAU is increased in patients with celiac disease.[24-27] Jejunal biopsy of 33 patients with RAU showed 8 to have the villous atrophy typical of celiac disease, along with histological signs of immunological reactions to food antigens.[24] The remaining patients also exhibited these types of signs but to a lesser degree. Although villous atrophy is a prerequisite for the diagnosis of celiac disease, gluten sensitivity may take other forms: Gluten may act directly on the oral mucosa or produce functional changes in the small intestine by immunological or other mechanisms that are distinct from those causing the characteristic abnormalities of celiac disease.[26] An underlying gluten-sensitive enteropathy could also contribute to nutritional deficiencies. Withdrawing gluten from the diet results in complete remission of RAU in patients with celiac disease and some improvement in others.[24-27]

Even in the absence of villous atrophy, gluten sensitivity can produce RAU. For example, in one small study, three of four gluten-sensitive patients identified by positive antibodies to alpha-gliadin but with normal small intestinal biopsy responded dramatically to a gluten-free diet.[28]

We recommend ruling out celiac disease by measuring tissue transglutaminase and reticulin antibodies in any patient presenting with RAU.

Dietary Factors

A study of students at Beijing University of Chinese Medicine used a questionnaire to investigate the occurrence of RAU.[29] Researchers found that bedtime later than 11 PM, dry mouth, constant thirst, and frequent intake of carbonated beverages were independent risk factors of RAU. The data also supported avoidance of fried food, sweet drinks, pineapple, and spicy foods, moderate intake of nuts, and cautioned intake of fruits.

Mechanical Injuries

In many RAU patients, lesions appear on the oral mucosa shortly after mechanical trauma to the area.[30] Accidental bites of the mucosal tissue, hard brushing of the mucosal tissue, or injuries from rigid solid foods can lead to posttraumatic aphthae.

Hormonal Changes

RAU may occur or increase in severity during the luteal phase of the menstrual cycle. A case which responded to implants of low doses of testosterone was published in the *British Medical Journal* in 1981.[31]

Stress

Stress is often a precipitating factor in RAU, suggesting a breakdown in normal host protective factors.[32] Elevated urinary and salivary cortisol levels demonstrate a significant correlation as markers for stress in patients with RAU.[33]

Nutrient Deficiency

The oral cavity is often the first place that nutritional deficiency becomes visible to the physician because of the high turnover rate of the mucosal epithelium. Although several nutrient deficiencies can lead to aphthous stomatitis, thiamin appears to be the most significant. In one study seeking to examine whether thiamin deficiency is associated with RAU, the level of transketolase (a thiamin-dependent enzyme) was determined in 70 patients with RAU and 50 patients from a control group.[34] Low levels of transketolase were found in 49 of 70 patients with RAS, compared with only 2 of 50 among the controls. These results clearly demonstrate an association between low levels of thiamin and RAU.

Several other studies show that nutrient deficiencies are much more common in RAU sufferers than in others. For example, a study of 330 patients with recurrent aphthous stomatitis (RAS) found that 47 (14.2%) were deficient in iron, folate, vitamin B_{12}, or a combination of those nutrients.[35] In another study of 60 patients, 28.2% were deficient in thiamin, riboflavin, or pyridoxine.[36] When the patients' deficiencies were corrected, the majority had complete remission. Lower dietary intake of folate and vitamin B_{12} is common in persons with RAU, and treatment with 1000 mcg per day has shown benefit regardless of serum vitamin B_{12} levels.[37] Additional studies have shown similar deficiency rates for the same nutrients and equally positive responses to supplementation.[38]

Zinc supplementation has also been shown to be helpful. A pilot study showed a significant association between zinc deficiency and RAU. In the study, 28% of 25 subjects with RAU had zinc deficiency compared with 4% of 25 healthy controls.[39] In one double-blind study, 40 patients with RAU were given either zinc sulfate (220 mg providing 50 mg of elemental zinc) or a placebo once daily for 1 month.[40] Results showed that the levels of serum zinc before treatment were below the normal value in 42.5% of the patients with RAU. After 1 month of zinc therapy, the aphthae reduced and did not reappear for 3 months.

Low nutrient status may explain why patients with RAU have an increased oxidant/antioxidant ratio in comparison with healthy controls. A small study of adolescents showed a reduction in the incidence of RAU and associated pain from 2000 mg per day of ascorbate.[41] In another study, superoxide dismutase, glutathione peroxidase (GSHPx), and catalase (CAT) activities as well as and malondialdehyde (MDA) and antioxidant potential (AOP) levels were measured in plasma and erythrocytes from 22 patients with RAS and 23 controls.[42] Researchers found decreased CAT and GSHPx activities and AOP levels in the erythrocytes as well as decreased AOP and increased MDA plasma levels in patients with RAU in comparison with control subjects. This study clearly demonstrated that enzymatic and nonenzymatic antioxidant defense systems are impaired in patients with RAU.

In an Italian study, a multivitamin, a proprietary topical drug, and the long-lasting anesthetic ropivacaine were used separately and in combination for patients with RAU.[43] Researchers determined the combination of the three agents was a reliable therapy for patients with oral aphthosis, providing significant reduction of pain and length of healing time.

Oral microbiome research suggests that an imbalance of the oral mucosal microbiome, rather than individual infectious pathogens, may play a role in initiating RAU. The findings raise the question of whether the presence of a lesion alters the microbiota of the oral cavity or a change in microbiota triggers the development of aphthosis. There is likely interplay between the two. One study compared the buccal microbiota of patients with RAU with control subjects and concluded that differences in bacteria were related to the presence of lesions during sampling. Bacterial diversity in the oral microbiota were similar in patients with RAU and controls.[44] Another study found that RAU is associated with dysbiosis of the mucosal and salivary microbiota and identified associations between RAU risk and decreased *Streptococcus salivarius* and increased *Acinetobacter johnsonii*.[45]

Quercetin

Quercetin is known to inhibit mast cell degranulation, basophil histamine release, and the formation of other mediators of inflammation.[46] The antiallergy drug disodium cromoglycate, a compound similar in structure and function to quercetin, has been shown to be effective in the treatment of RAU, resulting in more ulcer-free days and mild symptomatic relief.[47] Other flavonoids (acacetin, apigenin, chrysin, and phloretin but not catechin, flavone, morin, rutin, or taxifolin) have also shown antiallergy effects similar to those of disodium cromoglycate.[46]

Deglycyrrhizinated Licorice

Deglycyrrhizinated licorice (DGL) may be effective in promoting the healing of RAU. In one study, 20 patients were instructed to use a solution of DGL as a mouthwash (200 mg of powdered DGL dissolved in 200 mL of warm water) four times daily.[48] Of the 20 patients, 15 (75%) experienced 50% to 75% improvement within 1 day, followed by complete healing of the ulcers by the third day. DGL in tablet form may be more convenient and effective. Topical preparations containing the solid extract of licorice root are also available (e.g., Canker Cover made by Quantum Health).

A randomized controlled trial showed significant efficacy of a paste containing *Myrtus communis* (myrtle) for RAU when applied four times daily for 6 days.[49]

THERAPEUTIC APPROACH

Data suggests that no single factor is solely responsible for the initiation of aphthous lesions. However, an underlying genetic tendency may be present, the expression of which is facilitated by multiple factors. Considerable evidence suggests that gluten sensitivity may be a contributing factor in some patients. In addition, nutrient deficiencies must be corrected and anti-inflammatory nutrients prescribed. A randomized controlled trial showed significant benefit from individualized homeopathic treatment, when the medicine was administered in 6 c potency as oral liquid for two doses only.[50]

Diet

The patient's diet should be free of known allergens and all gluten sources if gluten sensitivity is present. Fried foods, carbonated sodas, and sweet drinks should be avoided. Acidic foods (tomatoes, citrus, pineapple) should also be avoided if found to trigger aphthae.

Nutritional Supplements

- High-potency multivitamin and mineral formula
- Calcium ascorbate: 2000 mg per day
- Sublingual vitamin B_{12}: 1000 mcg per day
- Zinc lozenges with Echinacea and Vitamin C (available OTC, containing 23 mg of zinc citrate/gluconate)
- "Kanka" liquid treatment for canker sores (an OTC product containing zinc oxide and benzocaine)

Botanical Medicines

- Deglycyrrhizinated licorice (DGL): one to two 380-mg chewable tablets 20 minutes before meals
- Topical licorice root preparations applied as needed

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Safadi RA. Prevalene of recurrent aphthous ulceration in Jordanian dental patients. *BMC Oral Health*. 2009;9:31.
2. Femiano F, Lanza A, Buonaiuto C, et al. Guidelines for diagnosis and management of aphthous stomatitis. *Pediatr Infectious Dis J*. 2007;26(8):728–732.
3. Slebioda Z, Szponar E, Kowalska A. Etiopathogenesis of recurrent aphthous stomatitis and the role of immunologic aspects: literature review. *Arch Immunol Ther Exp (Warsz)*. 2014;62(3):205–215.
4. Chavan M, Jain H, Diwan N, et al. Recurrent aphthous stomatitis: a review. *J Oral Pathol Med*. 2012;41(8):577–583.
5. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: a consensus approach. *J Am Dent Assoc*. 2003;134:200–207.
6. Miyamoto Jr NT, Borra RC, Abreu M, et al. Immune-expression of HSP27 and IL-10 in recurrent aphthous ulceration. *J Oral Pathol Med*. 2008;37(8):462–467.
7. Albanidou-Farmaki E, Poulopoulos AK, Epivatianos A, et al. Increased anxiety level and high salivary and serum cortisol concentrations in patients with recurrent aphthous stomatitis. *Tohoku J Exp Med*. 2008;214(4):291–296.
8. Borra RC, de Mesquita Barros F, de Andrade Lotufo M, et al. Toll-like receptor activity in recurrent aphthous ulceration. *J Oral Pathol Med. Mar*. 2009;38(3):289–298.
9. Arikan S, Durusoy C, Akalin N, et al. Oxidant/antioxidant status in recurrent aphthous stomatitis. *Oral Dis*. 2009;15(7):512–515.
10. Wilson CWM. Food sensitivities, taste changes, aphthous ulcers and atopic symptoms in allergic disease. *Ann Allergy*. 1980;44:302–307.
11. Bays RA, Hamerlinck F, Cormane RH. Immunoglobulin-bearing lymphocytes and polymorphonuclear leukocytes in recurrent aphthous ulcers in man. *Arch Oral Biol*. 1977;22:147–153.
12. Wray D, Vlagopoulos TP, Siraganian RP. Food allergens and basophil histamine release in recurrent aphthous stomatitis. *Oral Surg Oral Med Oral Pathol*. 1982;54:388–395.
13. Hay KD, Reade PC. The use of an elimination diet in the treatment of recurrent aphthous ulceration of the oral cavity. *Oral Surg Oral Med Oral Pathol*. 1984;57:504–507.
14. Nolan A, Lamey PJ, Milligan KA. Recurrent aphthous ulceration and food sensitivity. *J Oral Pathol Med*. 1991;20:473–475.
15. Wright A, Ryan FP, Willingham SE, et al. Food allergy or intolerance in severe recurrent aphthous ulceration of the mouth. *Br Med J (Clin Res Ed)*. 1986;292(6530):1237–1238.
16. Ghodratnama F, Wray D, Bagg J. Detection of serum antibodies against cytomegalovirus, varicella zoster virus and human herpesvirus 6 in patients with recurrent aphthous stomatitis. *J Oral Pathol Med*. 1999;28(1):12–15.
17. Mansour-Ghanaei F, Asmar M, Bagherzadeh AH, et al. Helicobacter pylori infection in oral lesions of patients with recurrent aphthous stomatitis. *Med Sci Monit. Dec*. 2005;11(12):CR576–CR579.
18. MacPhail LA, Greenspan D, Feigal DW, et al. Recurrent aphthous ulcers in association with HIV infection: description of ulcer types and analysis of T-lymphocyte subsets. *Oral Surg Oral Med Oral Pathol*. 1991;71(6):678–683.
19. Hasan AA, Ciancio S. Association between ingestion of nonsteroidal anti-inflammatory drugs and the emergence of aphthous-like ulcers. *J Int Acad Periodontol*. 2009;11(1):155–159.
20. Shim YJ, Choi JH, Ahn HJ, Kwon JS. Effect of sodium lauryl sulfate on recurrent aphthous stomatitis: a randomized controlled clinical trial. *Oral Dis*. 2012;18(7):655–660.
21. Altenburg A, El-Haj N, Micheli C, et al. The treatment of chronic recurrent oral aphthous ulcers. *Dtsch Arztebl Int*. 2014;111(40):665–673.
22. Yang Y, Zhang T, Dong Z, et al. Short-term efficacy of Pudilan Keyanning toothpaste in treatment of minor recurrent aphthous ulcers. *Evid Based Complement Alternat Med*. 2016;2016:9125327.
23. Liu XS, Guan XB, Chen RY, et al. Repurposing of Yunnan Baiyao as an alternative therapy for minor recurrent aphthous stomatitis. *Evid Based Complement Alternat Med*. 2012;2012:6. https://doi.org/10.1155/2012/284620.284620.
24. Ferguson R, Basu MK, Asquith P, et al. Jejunal mucosal abnormalities in patients with recurrent aphthous ulceration. *Br Med J*. 1976;1:11–13.
25. Ferguson MM, Wray D, Carmichael HA, et al. Celiac disease associated with recurrent aphthae. *Gut*. 1980;21:223–226.
26. Wray D. Gluten-sensitive recurrent aphthous stomatitis. *Dig Dis Sci*. 1981;26:737–740.
27. Besu I, Jankovic L, Magdu IU, et al. Humoral immunity to cow's milk proteins and gliadin within the etiology of recurrent aphthous ulcers? *Oral Dis*. 2009;15(8):560–564.
28. O'Farrelly C, O'Mahony D, Graeme-Cook F, et al. Gliadin antibodies identify gluten-sensitive oral ulceration in the absences of villous atrophy. *J Oral Pathol Med*. 1991;20:476–478.
29. Shi L, Wan K, Tan M, et al. Risk factors of recurrent aphthous ulceration among university students. *Int J Clin Exp Med*. 2015;8(4):6218–6223.
30. Polanska B, Niemczuk M, Augustyniak D, et al. Plasma neutrophil elastase in children with recurrent aphthous stomatitis. *Centr Eur J Immunol*. 2006;31:15–17.
31. Misra R, Anderson DC. Treatment of recurrent premenstrual orogenital aphthae with implants of low doses of testosterone. *BMJ*. 1989;299(6703):834.
32. Ship II , Merritt AD, Stanley HR. Recurrent aphthous ulcers. *Am J Med*. 1962;32:32–43.
33. Karthikeyan P, Aswath N. Stress as an etiologic co-factor in recurrent aphthous ulcers and oral lichen planus. *J Oral Sci*. 2016;58(2):237–240.
34. Haisraeli-Shalish M, Livneh A, Katz J. Recurrent aphthous stomatitis and thiamine deficiency. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1996;82:634–636.
35. Wray D, Ferguson MM, Hutcheon AW, et al. Nutritional deficiencies in recurrent aphthae. *J Oral Path*. 1978;7:418–423.
36. Nolan A, McIntosh WB, Allam BF, et al. Recurrent aphthous ulceration. Vitamin B_1, B_2, and B_6 status and response to replacement therapy. *J Oral Pathol Med*. 1991;20:389–391.
37. Carrozzo M. Vitamin B12 for the treatment of recurrent aphthous stomatitis. *Evid Based Dent*. 2009;10(4):114–115.
38. Wray D, Ferguson MM, Mason DK, et al. Recurrent aphthae: treatment with vitamin B_{12}, folic acid, and iron. *Br Med J*. 1975;2:490–493.
39. Ozler GS. Zinc deficiency in patients with recurrent aphthous stomatitis: a pilot study. *J Laryngol Otol*. 2014;128(6):531–533.
40. Orbak R, Cicek Y, Tezel A, et al. Effects of zinc treatment in patients with recurrent aphthous stomatitis. *Dent Mater J*. 2003;22:21–29.
41. Yasui K, Kurata T, Yashiro M, et al. The effect of ascorbate on minor recurrent aphthous stomatitis. *Acta Paediatr. Dec*. 2009;10:442–445.
42. Cimen MY, Kaya TI, Eskandari G, et al. Oxidant/antioxidant status in patients with recurrent aphthous stomatitis. *Clin Exp Dermatol*. 2003;28:647–650.
43. Basparini G, Saponaro G, Gasparini D, et al. The use of ropivacaine in therapeutic treatment of oral aphthosis. *Biomed Res Int*. 2018;1868254.
44. Bankvall M, Sjoberg F, Gale G, et al. The oral microbiota of patients with recurrent aphthous stomatitis. *J Oral Microbiol*. 2014;6.
45. Kim Y, Choi YS, Baek KJ, et al. Mucosal and salivary microbiota associated with recurrent aphthous stomatitis. *BMC Microbiol*. 2016;16:57.
46. Pearce FL, Befus AD, Bienenstock J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J Allergy Clin Immunol*. 1984;73:819–823.
47. Kowolik MJ, Muir KF, MacPhee IT. Di-sodium cromoglycate in the treatment of recurrent aphthous ulceration. *Br Dent J*. 1978;144:384–389.
48. Das SK, Das V, Gulati AK, et al. Deglycyrrhizinated liquorice in aphthous ulcers. *J Assoc Physicians India*. 1989;37:647.
49. Babaee N, Mansourian A, Momen-Heravi F, et al. The efficacy of a paste containing Myrtus communis (myrtle) in the management of recurrent aphthous stomatitis: a randomized controlled trial. *Clin Oral Investig*. 2010;14(1):65–70.
50. Mousavi F, Mojaver YN, Asadzadeh M, et al. Homeopathic treatment of minor aphthous ulcer: a randomized, placebo-controlled clinical trial. *Homeopathy*. 2009;98(3):137–141.

148

Asthma

John Nowicki, ND, and *Michael T. Murray*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Recurrent attacks of dyspnea, cough, and expectoration of tenacious mucoid sputum
- Prolonged expiration phase with generalized wheezing and musical rales
- Eosinophilia, increased serum immunoglobulin E (Ig E), positive food/inhalant allergy tests

GENERAL CONSIDERATIONS

Bronchial asthma is a hypersensitivity disorder characterized by bronchospasm, mucosal edema, and excessive excretion of viscous mucus that can lead to ventilatory insufficiency. Asthma affects approximately 7% of the population of the United States and causes 4210 deaths per year. Although it occurs at all ages, it is most common in children below age 10. There is a 2:1 male:female ratio in children, which equalizes by the age of 30.[1]

Major factors involved in asthma include the following:

- Hypersensitivity of the airways
- Beta-adrenergic blockade
- Cyclic nucleotide imbalance in airway smooth muscle
- Release of inflammatory mediators from mast cells

The incidence of asthma is rising rapidly in the United States, especially in children. Reasons often given to explain the rise in asthma include the following:

- Increased stress on the immune system due to factors such as greater chemical pollution in the air, water, insect allergens from mites and cockroaches, and food
- Earlier weaning and earlier introduction of solid foods to infants
- Food additives
- Higher incidence of obesity[2]
- Genetic manipulation of plants, resulting in food components with greater allergenic tendencies

In addition, multiple genetic variables may make certain individuals more susceptible to asthma. For example, researchers have demonstrated that a deficiency in the glutathione S-transferase M1, a gene involved in response to oxidative stress, may also make those with this deletion more susceptible to asthmatic attacks, thus supporting the need for antioxidant therapy in these individuals.[3] The *ADAM33* gene on chromosome 20p13 has been linked to the pathogenesis involved in airway remodeling (see later mediators section) and is probably a factor in corticosteroid resistance in some patients with asthma.[4] Other research teams have identified genes on chromosomes 7 and 12 as probable players in the pathogenesis of asthma.[5,6]

Major Categories

Asthma has typically been divided into two categories: extrinsic and intrinsic. Extrinsic or atopic asthma is generally considered an immunologically mediated condition with a characteristic increase in serum IgE. Intrinsic asthma is associated with a bronchial reaction that is due not to antigen–antibody stimulation but rather such factors as chemicals, cold air, exercise, infection, agents that activate the alternative complement pathway, and emotional upset.

*Previous edition contributor

Asthma is often clinically classified according to the frequency of symptoms, forced expiratory volume in 1 second (FEV_1), and peak expiratory flow rate.

Diagnostic Considerations

The U.S. National Asthma Education and Prevention Program (NAEPP) guidelines for the diagnosis and management of asthma (Table 148.1) state that a diagnosis of asthma begins by assessing whether any of the indicators is present:

- Wheezing—high-pitched whistling sounds on expiration—especially in children (Lack of wheezing and a normal chest examination do not exclude asthma.)
- History of any of the following:
  - Cough, particularly worse at night
  - Recurrent wheeze
  - Recurrent difficulty in breathing
  - Recurrent chest tightness
- Symptoms occur or worsen in the presence of:
  - Exercise
  - Viral infection
  - Animals with fur or hair
  - House dust mites (in mattresses, pillows, upholstered furniture, carpets)
  - Mold
  - Smoke (tobacco, wood)
  - Pollen
  - Changes in weather
  - Strong emotional expression (laughing or crying hard)
  - Airborne chemicals or dusts
  - Menstrual cycles
- Symptoms that occur or worsen at night, awakening the patient

Spirometry plays a central role in the management of asthma and should be performed at the time of initial diagnosis, after treatment is initiated and symptoms are stabilized, whenever control of symptoms deteriorates, and every 1 or 2 years on a regular basis.

Causes

Asthma is caused by a complex interaction of environmental and genetic factors. The strongest risk factor for developing asthma is a history of atopic disease. The presence of atopic dermatitis increases the risk of asthma by three- to fourfold. Allergies and the response of the immune system are obviously involved in asthma.

Inflammation and Th1/Th2 Balance

Imbalances in T-helper cell immune responses appear to be a fundamental mechanism of immunologically mediated airway inflammation. $CD4^+$ T-helper cells are generally categorized into Th1 and Th2 cells. Via the release of interferons and interleukin 2, the Th1 pathway is increased in the immunological responses to cancer, multiple sclerosis, viruses, and type IV hypersensitivities. Th2 responses are related to increases in interleukins 4, 6, 9, and 13; IgE, eosinophilia; and activated B-cell humoral immunity. Clinical conditions that reflect increased Th2 responses include asthma and atopic syndromes as well as allergies. Research involving asthmatic subjects demonstrates a normal Th1 gene expression but a constant upregulation of Th2-specific genes, leading to Th2 predominance.[7] Although it is unclear what the cause of this exaggerated Th2 response is in asthma, it seems that genetics, fungi, metal toxicities, nutrition, viruses, and pollution are factors in this upregulation (Fig. 148.1).[8] The "hygiene hypothesis" is also gaining ground in the standard medical literature. It asserts that by minimizing exposure to infectious agents owing to lifestyle choices based on hygienic concerns, the dominance of Th2 immune responses to environmental allergens has been favored and thus also the probable encouragement of asthma and atopic diseases.[9,10]

Mediators

Both extrinsic and intrinsic factors involved primarily with Th2 imbalances trigger the cytokine-activated release of mast cell–derived chemical mediators. These mediators are responsible for bronchoconstriction, mucus production, and other signs and symptoms in the majority of cases. These mediators are either preformed within granules or generated from membrane-bound phospholipids. The preformed mediators include histamine, various chemotactic peptides such as eosinophilic chemolactic factor (ECF) and high-molecular-weight neutrophil

TABLE 148.1 National Asthma Education and Prevention Program (NEAPP) Classification of Asthma Severity Before Treatment in Adults and Youths 12 Years and Older[a]

Component of Severity		PERSISTENT		
	Intermittent	Mild	Moderate	Severe
Symptoms	≤2 days/week	>2 days per week but not daily	Daily	Throughout the day
Night-time awakenings	≤2x/month	3–4×/month	>1×/week but not nightly	Often 7×/week
Short-acting β-agonist use for symptoms	≤days/week	>2 days per week but not > 1×/day	Daily	Several times per day
Interference with normal activity	None	Minor limitation	Some limitation	Extremely limited
Pulmonary function	Normal FEV_1 between exacerbations; FEV_1 ≥80% predicted; FEV_1/FVC normal	FEV_1 <80% predicted; FEV_1/FVC normal	FEV_1 ≥60% but <80% predicted; FEV_1/FVC reduced ≥5%	FEV_1 <60%; FEV_1/FVC reduced >5%
Exacerbations (consider frequency and severity)	0–1 per year		≥2 per year	

FEV_1, Forced expiratory volume in 1 second; *FVC*, forced vital capacity.
[a]Severity level is determined in accordance with the worst impairment category.
Data from National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma. *Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma*. Bethesda, MD: National Heart, Lung, and Blood Institute; 2007.

chemotactic factor (NCF), proteases, glycosidases, and heparin proteoglycan. The membrane-derived agents include lipoxygenase products such as leukotrienes (LTs) and the so-called slow-reacting substance of anaphylaxis (SRS-A), prostaglandins (PGs), thromboxanes (TXs), and platelet-activating factor (PAF).

These mediators are diverse in chemical composition and modes of action and account for many of the signs and symptoms of asthma. Their actions include bronchial smooth muscle constriction (histamine, LTC_4, LTD_4 and LTE_4, PGF_{2a}, PGD_2, and PAF), mucosal edema (increased permeability, histamine, LTC_4, LTD_4, and PAF), vasodilation (PGD_2 and PGE_2), mucous plugging (histamine, hydroxyeicosatetraenoic [HETE] acids, and LTC_4), inflammatory cell infiltrate (NCF, ECF-A, HETEs, LTB_4, and PAF), and desquamation of epithelium (proteases and glycosidases, together with lysosomal enzymes and basic proteins derived from neutrophils and eosinophils). In chronic asthma, eventual airway remodeling is a consequence of these mediator effects. The cellular and mediator elements responsible for airway remodeling involve the anomalous interface between the airway epithelium and the underlying mesenchymal tissues. This process is highlighted by the induction of growth factors that encourage fibroblastic and smooth muscle proliferation and the deposition of matrix proteins, which cause the thickening of the airway wall linked to bronchial hyperresponsiveness and fixed airflow obstruction.[4]

Mild episodic asthma differs from moderate to severe sustained asthma in that the latter is largely dependent on a subacute/chronic inflammation of the bronchi with infiltration of eosinophils, neutrophils, and mononuclear cells, whereas episodic asthma is due primarily to bronchial smooth muscle contraction.

Lipoxygenase Products

The most potent chemical mediators in asthma are the lipoxygenase products (i.e., leukotrienes) (Fig. 148.2). The leukotrienes composing SRS-A (LTC_4, LTD_4, LTE_4) are 1000 times more potent as stimulators of bronchial constriction than histamine. Researchers have observed that patients with asthma have an imbalance in arachidonic acid metabolism, leading to a relative increase in lipoxygenase products.[11] Platelets from patients with asthma show a 40% decrease in cyclooxygenase-derived metabolites and a 70%

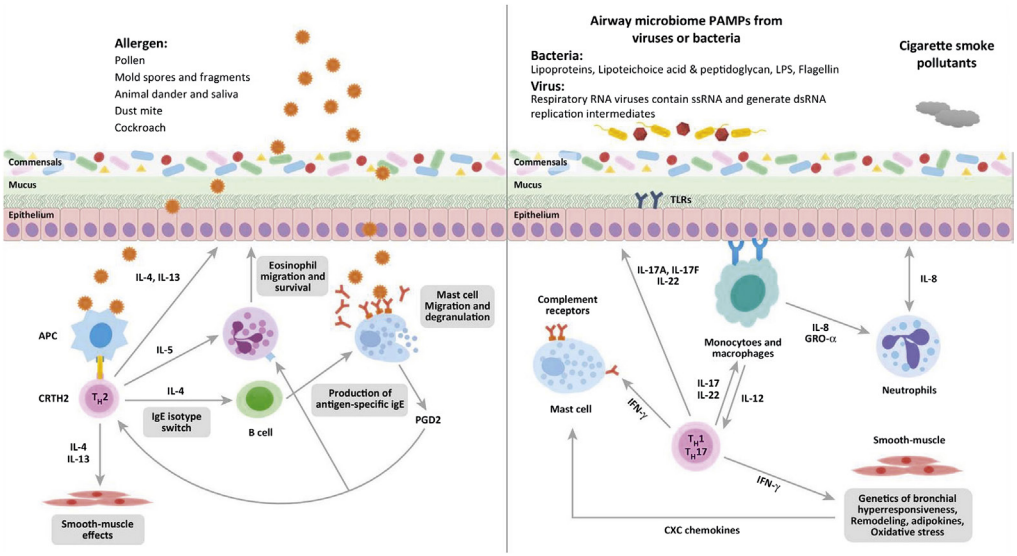


Fig. 148.1 Potential triggers and innate immune response of eosinophilic (Th2-dependent) and neutrophilic (non-Th2-dependent) asthma. (From Earl CS, An SQ, Ryan RP. The changing face of asthma and its relation with microbes. *Trends Microbiol.* 2015;23[7]:408–418. PubMed PMID: 25840766.)

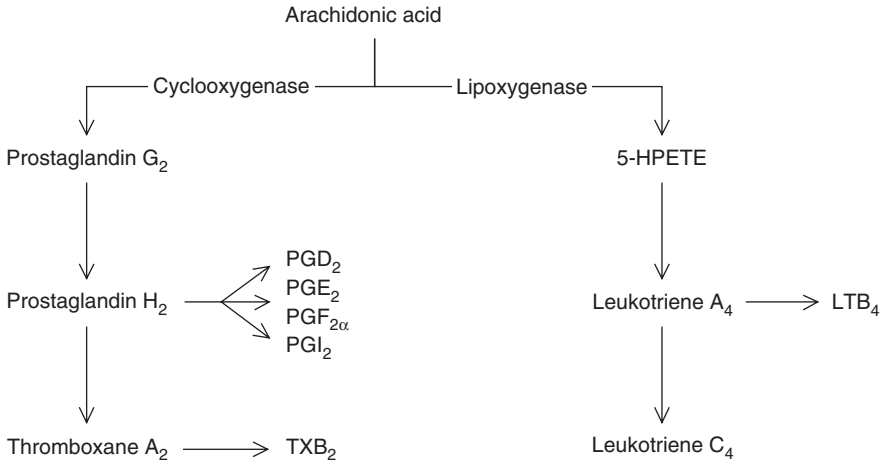


Fig. 148.2 Arachidonic Acid Metabolism.

increase in lipoxygenase products. This pathophysiologic alteration is further aggravated in "aspirin-induced asthma." Aspirin and other nonsteroidal anti-inflammatory drugs (e.g., indomethacin, phenylbutazone) inhibit cyclooxygenase while promoting lipoxygenase.[12,13] The net result is a shunting of arachidonic acid toward the lipoxygenase pathway and the production of excessive levels of leukotrienes.

Autonomic Nervous System

The relationship between the autonomic nervous system and bronchial asthma represents an interaction between parasympathetic and sympathetic innervation and beta$_2$ adrenergic receptors (which are localized in lung tissue and react to circulating catecholamines).[14] Stimulation of the parasympathetic vagus nerve results in airway constriction. This vagal mechanism involves the release of acetylcholine at the synapse, subsequent binding to its receptor on smooth muscle tissue, and subsequent formation of cyclic guanosine monophosphate (cGMP). Accumulation of cGMP or a relative deficiency in cyclic adenosine monophosphate (cAMP), or both, will result in constriction of the airway smooth muscle and degranulation of mast cells and basophils. Decreased sympathetic activity or diminished beta$_2$ receptor numbers or sensitivity also promote the cyclic nucleotide imbalance. Some of the mediators discussed earlier block beta$_2$ receptors and elevate cGMP levels both directly and indirectly.

Adrenal Gland

The activity of the adrenal gland is important in asthma because of the hormones cortisol and epinephrine. Cortisol is an effective prime activator of beta receptors, and epinephrine is considered to be the prime stimulator of beta receptors. It has been suggested that during asthmatic attacks, there is a relative deficiency of cortisol and epinephrine (which stimulates beta$_2$ receptors to catalyze the formation of cAMP from AMP). This leads to a decrease in the cAMP:cGMP ratio, resulting in bronchial constriction.

Vaccinations

Pertussis Vaccine

An evaluation of health criteria among 448 children and adolescents in Britain who had received only breast milk for the first 6 months of life and, in particular, on the first day after birth produced some interesting findings.[15] All of the children were weaned after 1 year of age and were older than 4 years at the time the parents responded. The mean age was 7.87 years. In response to the question "Has your child ever been diagnosed as asthmatic?" there were 30 positive answers (6.72%). The surprise came when the researchers classified the respondents according to whether they had received the pertussis vaccine.

Among the 243 immunized children, 26 were diagnosed as having asthma (10.69%). In contrast, of the 203 children who had not been immunized, only 4 had asthma (1.97%). The relative risk of developing asthma from the pertussis vaccine was 5.43 in this study. Even though all the children who received the pertussis vaccine received other vaccinations, the researchers suspected that the statistical evidence focused on pertussis. Among the children who did not receive the pertussis vaccine, most had received some other vaccination. Of the 91 subjects of the study who received no vaccines, only 1 had asthma, compared with 3 with asthma in the 112 who had other vaccinations. Therefore the relative risk of developing asthma is about 1% in children receiving no immunizations, 3% in those receiving vaccinations other than pertussis, and 11% in those receiving the pertussis vaccine. Another notable finding in the group not immunized to pertussis is that 16 developed whooping cough compared with only 1 in the immunized group.

Influenza Vaccine

One evaluation of more than 9600 children was employed to determine the safety of the cold-adapted trivalent intranasal influenza virus vaccine in children. Although this relatively new vaccination was deemed safe for children and adolescents, a significantly increased relative risk of 4.06 was observed in children 18 to 35 months of age for asthma and associated reactive airways disease.[16]

Environmental Toxins

The atmosphere is awash with a mixture of toxic substances, and inhalation of these toxicants can contribute to significant injury to the pulmonary system. Industrial materials, particulates from mining and combustion, agricultural chemicals, cigarette smoke, ozone, nitrogen oxides, and toxic metals have all been implicated in the pathophysiology of lung disease. Although these agents belong to different classes of chemicals, they may activate similar biochemical pathways, including inducing the recruitment and activation of macrophages, activation of mitogen-activated protein kinases, inhibition of protein synthesis, and production of interleukin-1 beta.[17]

Particulate Matter

Rapid industrialization and urbanization in many parts of the world have exposed more people to air pollutants now than at any point in human history. Exposure to $PM_{0.1}$ activates signaling pathways (e.g., nuclear factor kappa B [NF-κB], nicotinamide adenine dinucleotide phosphate [NADPH] oxidase) that induce inflammation, generate reactive oxygen species, and lead to cell death, which may explain the association between long-term particle accumulation and chronic respiratory diseases.[18] Several studies have explored the relationship between childhood asthma and respiratory problems with exposure to vehicle exhaust. Children in the Netherlands living within 100 m of a freeway had significantly more cough, wheeze, rhinitis, and asthma than those living farther away.[19] Among children living within 150 m of a main road, the risk of wheeze increased with increasing proximity by an odds ratio of 1.17 (95% confidence interval [CI] 1.01–1.36) per 30-m increment.[20]

Fine and ultrafine diesel exhaust particles can reach small airways, including the alveolar/gas-exchange regions of the lung, exacerbating asthma symptoms. Direct exposure to diesel exhaust has been linked to the development of asthma, which persists even after exposure ceases.[21]

Ozone

Asthma-related visits to the emergency department (ED) are associated with ozone levels. A study in New Jersey reported that ED visits for asthma occurred 28% more frequently when the mean ozone levels were greater than 0.06 ppm than when they were less than 0.06 ppm.[22] Children exposed to higher levels of ground-level ozone have greater rates of asthma and rhinitis than children in areas with less ozone.[23]

Acrolein

Acrolein (a contact herbicide used, for example, to control weeds and algae in irrigation canals) is a potent respiratory irritant and may have a prominent role as an environmental trigger of asthma attacks through air-pollutant emissions and tobacco smoke. Chronic residential exposure to outdoor acrolein was found to increase the prevalence-odds of having at least one asthma attack in the previous 12 months by approximately 8%.[24]

Arsenic

Chronic upper and lower respiratory problems, including dyspnea, asthma, and cough, were noted in a study from India with persons

consuming groundwater with arsenic levels between 11 and 50 ppm.[25] A dose-related decrease in lung function exists with increasing levels of baseline water and urinary arsenic.[26] For every 1-standard-deviation increase in baseline water arsenic exposure, there was a decrease in FEV_1 (–46.5 mL; $p = 0.0005$) and forced vital capacity (FVC; –53.1 mL; $p < 0.01$). This inverse association between arsenic exposure and FVC was consistent across sexes, remained significant in never-smokers, and was strongest in male smokers.

Cobalt

Respiratory effects from cobalt inhalation are well documented. Asthma, pneumonia, wheezing, respiratory irritation, and fibrosis have all been reported as occupational health hazards, especially in workers exposed to cobalt metal powder, cobalt salts, and cobalt-containing dusts.[27] Exposure to cobalt alone produces an allergic-like asthmatic condition.

Occupational Agricultural Exposure

Occupational exposures in the agricultural industry are associated with numerous lung diseases, including asthma. Production agriculture produces a variety of exposures, including organic dusts from livestock barns and confinements and chemical toxicants from fermentation and bacterial degradation of grain and animal wastes, pesticides, microorganisms, and bacterial endotoxins. Asthma-like syndrome is associated with swine confinement workers and grain elevator operators, with acute symptoms occurring in as many as 50%.[28]

Pesticides may contribute to asthma among farmers and has been associated with wheeze among U.S. farmers. For farmers, the organophosphates chlorpyrifos, malathion, and parathion have all been positively associated with wheeze.[29] Farmers are more likely to be diagnosed with nonatopic asthma than atopic asthma compared with other occupational groups, although high-pesticide-exposure events have been associated with a doubling of both allergic and nonallergic asthma in farmers.[30] Coumaphos, heptachlor, parathion, and ethylene dibromide all have odds ratios of greater than 2.0 for allergic asthma.

Mold

Exposure to mold and dampness in buildings is a major factor in the asthma epidemic. Studies have shown that dampness or mold in houses causes 21% of asthma in the United States and a 30% to 50% increase in asthma and asthma-related health problems.[31] The incidence may be higher; one study found that 67% of adult-onset asthma developed after working in a water-damaged office building.[32] The primary mechanisms for damp-building toxicity include immunological (e.g., stimulation, suppression, autoimmunity), toxic (e.g., neurotoxicity, genotoxicity, reproductive damage), and inflammatory. The Institute of Medicine report commissioned by the Centers for Disease Control and Prevention (CDC) and released in 2004 concluded that respiratory conditions, including asthma, have sufficient evidence of causation by mold or damp buildings.[33]

Drugs

Aspirin/NSAIDs

Aspirin-exacerbated respiratory disease (AERD) is an asthma phenotype and has a prevalence of 2% to 25% in the asthma population.[34] Precipitation of asthma attacks by the ingestion of aspirin and other NSAIDs is considered a hallmark of this syndrome. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase and promote lipoxygenase. The net result is a shunting of arachidonic acid toward the lipoxygenase pathway and the production of excessive levels of leukotrienes. AERD is chronic, and the respiratory mucosal inflammatory disease persists even with avoidance of cyclooxygenase 1 (COX-1) inhibitors.

Beta Blockers

In almost all patients with asthma, nonselective beta blockers cause bronchoconstriction and increase bronchial hyperresponsiveness to methacholine and histamine.[35] The regular use of noncardioselective beta blockers was demonstrated to cause a 13.5% decrease in FEV_1. Topical applications should be avoided as well because severe bronchoconstriction and fatal attacks have been reported with topical ophthalmic application of beta blockers.[36]

Other

Several other medications have been shown to cause bronchospasm or mimic asthma, including angiotensin-converting enzyme (ACE) inhibitors, cholinergic agonists, cholinomimetic alkaloids, chemotherapeutic agents, diuretics, corticosteroids, antibiotics, and radiocontrast dyes.[37]

THERAPEUTIC CONSIDERATIONS

As with many complex diseases, the initial cause or causes of asthma (e.g., hypochlorhydria) may initiate a sequence of events that becomes self-propagating (e.g., hypochlorhydria-induced food allergies leading to increased intestinal permeability and then to increased susceptibility to food allergy). Effective treatment requires control of both the initiating cause or causes and the induced physiological abnormalities.

General

Hypochlorhydria

Fractional gastric analyses in 200 children with asthma showed that 80% of them had gastric acid secretions below normal levels.[38] Hypochlorhydria may initiate a sequence of events that becomes self-propagating. The high occurrence of decreased gastric acid output may predispose these children to food allergies and has a major effect on the success of rotation or elimination diets and, if not corrected, on the development of additional food allergies (i.e., hypochlorhydria-induced food allergies leading to increased intestinal permeability, which then increases the susceptibility to food allergy).

Increased Intestinal Permeability

The presence of food allergies is thought to be responsible for patients with asthma having been shown to have "leaky guts."[39] Altered intestinal barrier function permits increased dietary antigen transport across the intestinal barrier and subsequent exposure of these antigens to the mucosal immune system, leading to the development of antigen-specific responses. This overwhelms the immune system, increasing the likelihood of developing additional allergies as well as increasing the circulation of bronchoconstrictive compounds. It is essential to identify offending foods as soon as possible so as to avoid the development of further allergies.

Candida albicans

An overgrowth of the common yeast *Candida albicans* in the gastrointestinal tract has been implicated as a causative factor in allergic conditions, including asthma. Apparently, the acid protease produced by *C. albicans* is the responsible allergen.[40] Appropriate anticandidal therapy may result in significant clinical improvement of asthma in many cases.

Food Additives

Vitally important in the control of asthma is the elimination of food additives.[41] Artificial dyes and preservatives are widely used in foods, beverages, and drugs. The most common coloring agents are azo dyes—tartrazine (yellow dye no. 5), sunset yellow, amaranth, and coccine (red)—and the nonazo dye pate blue. The most commonly used

preservatives in food are sodium benzoate, 4-hydroxybenzoate esters, and sulfur dioxide.

Tartrazine, benzoates, sulfur dioxide, and sulfites have been reported to cause asthma attacks in susceptible individuals.[41,42] Tartrazine is commonly found in processed foods, vitamin preparations, and prescription drugs and acts as a cyclooxygenase inhibitor, thus inducing asthma, especially in children. Approximately 4% to 14% of individuals with asthma are sensitive to acetylsalicylic acid and may react to this dye.[43]

Various sulfites are commonly used in prepared foods. It is estimated that 2 to 3 mg of sulfites are consumed each day by the average U.S. citizen, whereas an additional 5 to 10 mg are ingested by wine and beer drinkers.[42] The largest sources are the salads, vegetables (particularly potatoes), and avocado dip served in restaurants. A customer can ingest 25 to 100 mg of metabisulfite in as little as one restaurant meal. It has been postulated that a molybdenum deficiency may be responsible for sulfite sensitivity.[44] Sulfite oxidase, the enzyme responsible for neutralizing sulfites, is molybdenum dependent.

Salt

Strong evidence indicates that an increased intake of salt increases bronchial reactivity and mortality from asthma.[45,46] Dietary patterns of high fat, sugar, and salt are significantly associated with asthma prevalence (odds ratio [OR] = 1.13, 95% confidence interval [CI] = 1.03, 1.24) and current severe asthma (OR = 1.23, 95% CI = 1.03, 1.48).[47] The degree of bronchial reactivity to histamine is positively correlated with the 24-hour urinary sodium excretion and rises with increased dietary sodium. Because the severity of asthma correlates with the degree of bronchial reactivity, the severity of asthma can clearly be influenced by alterations in dietary sodium consumption.

Estrogen and Progesterone

For female patients who experience severe asthma symptoms, it may be useful to consider therapeutic regimens that encourage a decrease in hormonal fluctuations. Some studies find estrogen withdrawal to increase lung contractility.[48] However, other studies find little relationship between estrogen use and asthmatic symptoms.[49–51] Estrogen[52] and progesterone are both known to act as smooth muscle relaxants. It may be that the airways of premenopausal and postmenopausal women may respond differently to exogenous hormone replacement and therefore need to be assessed independently with this intervention.

One interesting review of more than 90 papers published from 1966 to 2001 found that during the premenstrual and menstrual phases, when hormone levels are low, women with asthma have been found to experience increased asthma exacerbations, increased hospitalizations for asthma, and decreased pulmonary function. Additionally, women who experienced decreased fluctuations in hormone levels either by becoming pregnant or by starting oral contraceptive therapy tended to have improvements in pulmonary function and fewer requirements for medication.[48] Natural hormone replacement may offer a safer approach than synthetic hormones, although no studies to verify the safety or efficacy of these natural forms of female sex hormones have been conducted. In the longer term, natural therapeutics designed to balance estrogen and progesterone, along with specific recommendations from this chapter, may offer the most risk-free relief for those premenopausal and postmenopausal women who suffer from asthmatic exacerbations (see Chapters 196 and 212).

Dehydroepiandrosterone

Decreased levels of dehydroepiandrosterone (DHEA) have been shown to be common in postmenopausal women with asthma compared with matched controls.[53] One study of 55 asthmatic and 20 healthy postmenopausal women aged 48 to 60 before hormone replacement therapy and after 6 months of transdermal 17b-estradiol (E2) and medroxyprogesterone acetate treatment demonstrated a significant increase in serum DHEA levels in the asthmatic group, with no change in the control group.[54] Whether DHEA itself produces any therapeutic benefit in asthma remains to be demonstrated; however, given its important role in proper immune function, an ability to produce positive effects is certainly possible.

Melatonin

There is some concern regarding elevated melatonin levels contributing to increased airway inflammation in patients with nocturnal asthmatic exacerbations.[55,56] In a study of people with nocturnal asthma, the peak measured serum melatonin level was significantly and inversely correlated with overnight change in FEV_1 in subjects with nocturnal asthma but not in those with nonnocturnal asthma or healthy controls. An interesting note is that this study observed a delayed release of melatonin in the patients with nocturnal asthma. Therefore it is theoretically possible that supplementation combined with an earlier sleep regimen may help regulate this late peaking of melatonin and possibly mitigate symptoms. Nevertheless, the practitioner may want to avoid giving this supplement to patients with asthma, especially the nocturnal type, until more studies are conducted regarding melatonin's immunotherapeutic effect.

Diet

Beneficial Foods

A number of publications corroborate the notion that people who have a diet rich in fruits and vegetables have a lower risk of poor respiratory health.[57–59] This effect is most likely due to the increased levels of antioxidants in such foods. One epidemiological review[60] found that among children, consumption of fresh fruit, particularly fruit high in vitamin C, has been related to a lower prevalence of asthma symptoms and better lung function. This effect was observed even at low levels of fruit consumption (one or two servings per week vs. fewer than one serving per week), which suggests that a small increase in fruit intake could be beneficial. This same review discussed the consumption of fish as also being related to lower airway hyperreactivity among children and better lung function in adults.

A study of Scottish adults also found a dose–response relationship between fruit consumption and pulmonary function, whereby increased fruit consumption led to decreases in phlegm and better pulmonary function.[61] In addition, a "reasonable" intake of fruit (>180 g/day), whole grains (>45 g/day), and moderate alcohol consumption (one to three glasses a day) was associated with a 139-mL-higher FEV_1 and a 50% lower prevalence of chronic obstructive pulmonary disease versus diets that did not meet these intake requirements.[57] Finally, one study of 607 asthma patients and 864 controls highlighted apples and moderate red wine consumption as sources of antioxidants that decreased asthma severity.[62]

Dietary intake of soy foods may be helpful because the soy isoflavone genistein is associated with reduced severity of asthma. Although this effect may be due to some antioxidant action, studies have also shown that genistein is able to block eosinophil leukotriene C(4) synthesis and inhibit the pathway of NF-κB and tumor necrosis factor-α (TNF-α) in patients with asthma.[63–65]

Food Allergy

Many studies have indicated that food allergies play an important role in asthma (see Chapter 14).[66–70] Adverse reactions to food may be immediate or delayed. Double-blind food challenges in children have

shown that immediate-onset sensitivities are usually due (in decreasing order of frequency) to egg, fish, shellfish, nuts, and peanuts, whereas the foods most commonly associated with delayed onset include (in decreasing order of frequency) milk, chocolate, wheat, citrus, and food colorings.[68] Elimination diets have been successful in identifying allergens and treating asthma and are a particularly valuable diagnostic and therapeutic tool in infants.[71] Elimination of common allergens during infancy (first 2 years) has been shown to reduce allergic tendencies in high-risk children (e.g., strong familial history).[72]

Breastfeeding

Many studies have demonstrated the protective effect of breastfeeding in the prevention of asthma. One study examined the association of breastfeeding and the presence of chronic respiratory symptoms among 5182 Brazilian schoolchildren 7 to 14 years of age. Ninety percent of the mothers in this population had breastfed their infants. After adjusting for potential confounding factors, these researchers revealed that children who had not been breastfed were more likely to have a medical diagnosis of asthma.[73] One Iraqi study additionally showed that the vitamin C content of breast milk was significantly correlated with the maternal intake of vitamin C; low vitamin C intake by the mother translates to low intake by the child, thus leaving him or her more susceptible to oxidative stress.[74]

The Canadian Asthma Primary Prevention study collected 2 years of data in which researchers chose 545 infants who were considered at high risk for asthma on the basis of a history of familial atopy. These children were broken down into control and intervention groups. The interventions included (1) measures to control house dust; (2) recommendations for avoidance of pets, environmental tobacco smoke, and day care during the first year; and (3) only breastfeeding or the use of partially hydrolyzed whey formula until at least the age of 4 months. At 1 year of age, the risk of asthma was significantly reduced by 34%. At 2 years of age, significantly fewer children had asthma in the intervention group than in the control group (16.3% vs. 23%), and 60% fewer of those in the intervention group had persistent asthma. A 90% reduction in recurrent wheeze was seen in the intervention group compared with the control group.[75] Studies like these are quite useful to elucidate the multitude of changes necessary to affect multifactorial diseases like asthma in an effective manner.

Antibiotics, Probiotics, and Mucosal IgA

In a combined analysis of seven studies involving more than 12,000 youngsters, researchers at the University of British Columbia found that those prescribed antibiotics before their first birthday were more than twice as likely as untreated kids to develop asthma.[76] Additionally, if they had multiple courses of antibiotics, it bumped up the risk even higher—16% for every course of the drugs taken before age 1. There are a couple of explanations for this association between antibiotic use and asthma. One is that antibiotics contribute to a state of "excess hygiene," leading to a reduced exposure to microbes. This, in turn, creates an oversensitive immune system, mounting an over-the-top allergic reaction to pollen and dust mites and ultimately resulting in asthma. The second explanation is that antibiotics have a negative effect on the normal flora of the gastrointestinal tract and respiratory passages. Some studies have shown that giving probiotics (active cultures of *Lactobacillus* and *Bifidobacterium* species) lowers the risk of atopic allergic diseases like asthma and eczema. Some of this protective effect may be mediated by mucosal IgA, which participates in antigen elimination. In a cohort of 237 allergy-prone infants given a combination of four probiotic strains or placebo, it was shown that supplementation with probiotics increased fecal IgA and calprotectin while reducing inflammatory markers (e.g., α_1-antitrypsin and TNF-α).[77] In infants with a high fecal IgA concentration at the age of 6 months, the risk of having any allergic disease or any IgE-associated (atopic) disease before the age of 2 years was cut by nearly 50%. High intestinal IgA in early life is associated with minimal intestinal inflammation and indicates a reduced risk for IgE-associated allergic diseases.

Vegan Diet

A long-term trial of a vegan diet (elimination of all animal products) provided significant improvement in 92% of the 25 treated patients who completed the study (9 dropped out).[78] Improvement was determined by a number of clinical variables, including vital capacity, FEV_1, and physical working capacity, as well as by biochemical indices such as haptoglobin, IgM, IgE, cholesterol, and triglyceride levels in the blood. The researchers also found a reduction in the vulnerability to infection. Importantly, although 71% of the patients responded within 4 months, 1 year of therapy was required before the 92% level was reached.

The diet excluded all meat, fish, eggs, and dairy products. Drinking water was limited to spring water (chlorinated tap water was specifically prohibited). Coffee, ordinary tea, chocolate, sugar, and salt were also excluded. Herbal spices were allowed, and water and herbal teas were allowed up to 1.5 L/day. Vegetables eaten freely were lettuce, carrots, beets, onions, celery, cabbage, cauliflower, broccoli, nettles, cucumber, radishes, Jerusalem artichokes, and all beans except soybeans and green peas. Potatoes were allowed in restricted amounts. A number of fruits were also eaten freely: blueberries, cloudberries, raspberries, strawberries, black currants, gooseberries, plums, and pears. Apples and citrus fruits were not allowed, and grains were either restricted or eliminated.

The beneficial effects of this dietary regimen are probably related to three areas:

- Elimination of food allergens
- Altered prostaglandin metabolism
- Increased intake of antioxidant nutrients and magnesium

In regard to altered prostaglandin metabolism, the avoidance of dietary sources of arachidonic acid (derived from animal products) appears to be significant. The prostaglandins and leukotrienes derived from arachidonic acid contribute significantly to the allergic reaction in asthma. The decreased availability of arachidonic acid as a substrate of these inflammatory compounds appears to explain some aspects of the efficacy of the vegan diet. The benefits of altering prostaglandin metabolism are further discussed later, as is the role of increased dietary antioxidants in preventing asthma.

Aside from the patients' improvement in health from the vegan diet, there was a significant reduction in healthcare costs (the patients had been receiving corticosteroids and other drugs and therapies for an average of 12 years), and according to the authors, patients changed their attitudes in terms of taking greater responsibility for their own health.

Nutrition

Omega-3 Fatty Acids

Epidemiological studies have shown that children who eat fish more than once a week have one-third the risk of asthma of children who do not eat fish regularly.[79] Several clinical studies have shown that increasing the intake of omega-3 fatty acids through supplementation with fish oils containing eicosapentaenoic acid and docosahexaenoic acid offers significant benefits in asthma, as noted by improvements in airway responsiveness to allergens as well as improvements in respiratory function.[80,81] These benefits are related to an increased ratio of omega-3 to omega-6 fatty acids in cell membranes, thereby reducing

the availability of arachidonic acid. In particular, the ingestion of omega-3 fatty acids leads to a significant shift in leukotriene synthesis from the extremely inflammatory 4-series to the less inflammatory 5-series leukotrienes. This shift is directly related to improvements in asthma symptoms.[82] The benefits may take as long as 1 year to become apparent because it seems to take time to turn over cellular membranes in favor of the omega-3 fatty acids.

Tryptophan Metabolism and Pyridoxine Supplementation

Children with asthma have been shown to have a metabolic defect in tryptophan metabolism and reduced platelet transport for serotonin.[83,84] Tryptophan is converted to serotonin, a known bronchoconstricting agent in asthmatics. High serotonin levels in the blood and sputum are common findings in patients with asthma and are reflected by an elevated urinary level of 5-hydroxyindoleacetic acid (5-HIAA), the breakdown product of serotonin. The levels of 5-HIAA in the urine correlate well with the severity of asthmatic symptoms. Double-blind clinical studies have shown that patients benefit from either a tryptophan-restricted diet[83] or pyridoxine supplementation[84,85] to correct the blocked tryptophan metabolism.

Pyridoxine may also be of direct benefit to patients with asthma. In one study, plasma and erythrocyte pyridoxal phosphate levels in 15 adult patients with asthma were significantly lower than in 16 controls.[84] Oral supplementation with 50 mg of pyridoxine twice daily to seven of the patients failed to produce a substantial elevation of these low levels. However, all patients reported a dramatic decrease in the frequency and severity of wheezing as well as asthma attacks while taking the supplements. In a study of 76 children with asthma, pyridoxine at a dosage of 200 mg daily produced significant reductions in symptoms and in dosages of bronchodilators and corticosteroids. However, a double-blind study failed to demonstrate any significant improvement with vitamin B_6 supplementation in patients who depended on steroids to control symptoms.[86]

Although vitamin B_6 supplementation may not help patients on steroids, it is definitely indicated in patients with asthma being treated with the drug theophylline. Theophylline significantly depresses pyridoxal-5-phosphate levels.[87] In addition, another study has shown that vitamin B_6 supplementation can significantly reduce the typical side effects of theophylline (e.g., headaches, nausea, irritability, sleep disorders).[88]

Antioxidants

The substantial increase in the prevalence of asthma over the past 20 years can be partially explained by the reduced dietary intake of antioxidant nutrients like beta-carotene and vitamins A, C, and E, as well as the mineral cofactors essential for antioxidant defense mechanisms, such as zinc, selenium, and copper.[89] Patients undergoing acute asthmatic distress are known to have lowered serum total antioxidants.[90] Genetic influences may also play a role in the need for antioxidants (see "General Considerations" earlier in the chapter).[3]

One study of 158 children with moderate to severe asthma revealed that supplementation of 50 mg/day of vitamin E and 250 mg/day of vitamin C conferred significant protection against ozone-induced reductions in pulmonary function.[91] Antioxidants are thought to provide important defense mechanisms for maintaining the redox state of the lungs. This protection is significant because oxidizing agents can both stimulate bronchoconstriction and increase hyperactivity to other agents. Asthma appears to be another disease process that is influenced greatly by antioxidant mechanisms. Analgesics like acetaminophen, known to deplete antioxidant levels such as glutathione in animals, should be used with caution in asthmatic patients.[92]

Vitamin C

Vitamin C is the major antioxidant substance present in extracellular fluid lining the airway surfaces.[93] Vitamin C intake in the general population appears to correlate with asthma, indicating that low vitamin C (in the diet and the blood) is an independent risk factor for asthma. In a survey of 771 persons with current asthma, 352 with former asthma, and 15,418 without asthma, lower vitamin C concentrations were observed among those with current or former asthma than those who had never had asthma.[94] Children of smokers have a higher rate of asthma (cigarette smoke is known to deplete respiratory vitamins C and E levels), and symptoms of ongoing asthma in adults appear to be increased by exposure to environmental prooxidants and decreased by vitamin C supplementation.[74]

Nitrogen oxides are examples of oxidants that can arise from both endogenous and exogenous sources. Vitamin C has been shown to offer significant protection against nitrogen oxide damage in the lungs of animal models.[95]

Both treated and untreated patients with asthma have been shown to have significantly lower levels of ascorbic acid in serum and leukocytes.[95] From a clinical perspective, it appears that patients with asthma have a higher need for vitamin C, with the majority of studies demonstrating significant improvements in respiratory measures and asthma symptoms as a result of supplementing the diet with 1 to 2 g of vitamin C daily.[96] This dosage is recommended based on the increasing exposure to inhaled oxidants, along with the growing appreciation of the antioxidant function of vitamin C in the respiratory system.

High-dose vitamin C therapy may also help asthma by lowering histamine levels.[97] The importance of vitamin C as a natural antihistamine has emerged for several reasons, including concern over the safety of antihistamine medications and the recently recognized immune-suppressing effects of histamine. In the initial stages of an immune response, histamine amplifies the immune response by increasing capillary permeability and smooth muscle contraction, thus enhancing the flow of immune factors to the site of infection. Subsequently, histamine exerts a suppressive effect on the accumulated white blood cells (WBCs) in an attempt to contain the inflammatory response.

Vitamin C prevents the secretion of histamine by WBCs and increases the detoxification of histamine. One study examined the antihistaminic effect of acute and chronic vitamin C administration and its effect on WBC (neutrophil) function in healthy men and women. In the chronic administration arm, 10 subjects ingested a placebo during weeks 1, 2, 5, and 6 and 2 g/day of vitamin C during weeks 3 and 4. Fasting blood samples were collected after the initial 2-week period (baseline) and at the end of weeks 4 and 6. Blood vitamin C levels rose significantly after vitamin C administration, whereas blood histamine levels fell by 38% during the weeks vitamin C was given. The ability of WNCs to move in response to an infection (chemotaxis) increased by 19% during vitamin C administration and fell 30% after vitamin C withdrawal. However, these changes were linked to histamine concentrations. Chemotaxis was greatest when histamine levels were the lowest. In the part of the study looking at the acute effects of vitamin C, blood histamine concentrations and chemotaxis did not change 4 hours after a single dose of vitamin C. This result suggests that vitamin C will lower blood histamine only if it is taken over time. Individuals prone to allergy or inflammation are encouraged to increase their consumption of vitamin C through supplementation.[97]

In a small study of patients with asthma and documented exercise-induced bronchoconstriction, the subjects participated in a randomized, placebo-controlled, double-blind crossover trial.[98] They entered the study on their usual diets and were placed on either 2 weeks of ascorbic acid supplementation (1500 mg/day) or placebo, followed

by a 1-week washout period, before crossing over to the alternative treatment. The ascorbic acid diet significantly reduced ($P < 0.05$) the maximum fall in postexercise FEV_1 (–6.4 %) compared with the usual diet (–14.3%) and a placebo diet (–12.9%). Asthma symptom scores significantly improved on the ascorbic acid diet compared with the placebo and usual diets. Postexercise inflammatory mediators were also significantly lower with ascorbic acid supplementation.

Flavonoids

Flavonoids appear to be key antioxidants in the treatment of asthma. Various flavonoids, chief among them being quercetin, have been shown to inhibit the following[99–102]:

- Histamine release from mast cells and basophils when stimulated by antigens and other ligands
- Phospholipase A2 in neutrophils
- Lipoxygenase
- Anaphylactic contraction of smooth muscle
- Phosphodiesterase in the lung (resulting in increased cAMP levels)
- Biosynthesis of SRS-A
- Calcium influx

In addition, quercetin has both a vitamin C–sparing effect and a direct stabilizing effect on membranes, including mast cells.

Flavonoid-rich extracts such as those from grape seed, pine bark, green tea, or *Ginkgo biloba* may prove even more helpful than quercetin in the treatment of asthma because of their enhanced bioavailability. In particular, the proanthocyanidins from grape seed or pine bark extracts appear to have an affinity for the lungs (for more information, see Chapter 106). In a randomized, placebo-controlled, double-blind study involving 60 subjects 6 to 18 years of age, a proprietary pine bark extract (Pycnogenol) significantly improved pulmonary function and asthma symptoms compared with placebo. Specifically, the Pycnogenol group was able to reduce or discontinue the use of rescue inhalers more often than the placebo group. There was also a significant reduction of urinary leukotrienes in the Pycnogenol group.[103]

In another study, a flavonoid preparation derived from purple passion fruit peel (PFP) was studied in a 4-week randomized, placebo-controlled, double-blind trial in asthma patients. The dosage of the PFP extract was 150 mg daily. The prevalence of wheeze, cough, and shortness of breath was reduced significantly in the group treated with PFP extract, whereas the placebo caused no significant improvement. PFP extract supplementation also resulted in a marked increase in FEV_1, whereas placebo showed no effect.[104]

Carotenes

Carotenes are powerful antioxidants that may increase the integrity of the epithelial lining of the respiratory tract and act as substrates for lipoxygenase, possibly competing with arachidonic acid and thereby decreasing leukotriene formation.[105] Some studies have shown that patients with asthma have reduced plasma antioxidant potential due to low whole-blood carotenoids (beta-carotene, lycopene, alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin),[106] in particular low lycopene levels,[107] thus making them more susceptible to the damaging effects of oxidative stress. Lycopene may emerge as the most useful supplemental carotenoid. In animal models of asthma, lycopene supplementation suppressed Th2 responses and reduced eosinophilic infiltrates in the bronchoalveolar lavage fluid, lung tissue, and blood as well as the number of mucus-secreting cells in the airways.[108]

In a proof-of-concept human study, asthmatic adults ($n = 32$) consumed a low-antioxidant diet for 10 days and then commenced a randomized crossover trial involving three 7-day treatment arms (placebo, tomato extract [45 mg lycopene per day], and tomato juice [45 mg lycopene per day]).[109] With the consumption of a low-antioxidant diet, plasma carotenoid concentrations decreased, Asthma Control Score worsened, lung function (FEV_1 and FVC) decreased, and sputum neutrophils increased. Treatment with both tomato juice and extract reduced airway neutrophil influx. Treatment with tomato extract also reduced sputum neutrophil elastase activity. This short-term study indicates that antioxidant status, particularly in terms of carotenoids, modifies some parameters in asthma.

There have been two double-blind studies of lycopene supplementation (30 mg/day) in exercise-induced asthma. One failed to show any benefit,[110] whereas another showed that in some patients, it prevented airway constriction and reduced FEV_1.[111]

Vitamin E

Vitamin E's activity as an antioxidant, lipoxygenase inhibitor, and phospholipase inhibitor makes it a useful supportive agent in asthma treatment.[112]

Selenium

Reduced selenium levels have been demonstrated in asthma patients.[113–115] Glutathione peroxidase, a selenium-dependent metalloenzyme, is important for reducing hydroperoxyeicosatetraenoic acid (HPETE) to HETE acid, thereby reducing leukotriene formation. Reduced levels of glutathione peroxidase have also been reported for patients with asthma.[113] Supplemental selenium appears warranted to address any deficiency of glutathione peroxidase. In addition, supplemental selenium may reduce the production of leukotrienes by ensuring the optimal activity of glutathione peroxidase.

Vitamin B_{12}

Jonathan Wright, MD, believes that "B_{12} therapy is the mainstay in childhood asthma."[116] In one clinical trial, weekly intramuscular injections of 1000 mg produced definite improvements in patients with asthma.[117] Of 20 patients, 18 showed less shortness of breath on exertion as well as improved appetite, sleep, and general condition. Vitamin B_{12} appears to be especially effective in sulfite-sensitive individuals. It offers the best protection when given orally (1–4 mcg) before challenge compared with other pharmacological agents (e.g., cromolyn sodium, atropine, doxepin).[118] The mode of action is the formation of a sulfite–cobalamin complex that blocks sulfite's effect.

Magnesium

In 1912 Trendelenburg demonstrated that magnesium relaxed bovine bronchial smooth muscle in vitro.[119] Later, uncontrolled clinical studies revealed magnesium's beneficial effect in the treatment of patients with acute attacks of bronchial asthma.[120] Unfortunately, this promising line of research was dropped as antihistamines and bronchodilators became available. However, the advent of calcium channel blockers for the treatment of asthma generated renewed interest in the therapeutic use of magnesium for asthma. In fact, intravenous magnesium (2 g of magnesium sulfate infused every hour up to a total of 24.6 g) is now a well-proven and clinically accepted measure to halt an acute asthma attack as well as acute exacerbations of chronic obstructive pulmonary disease.[121–125]

Although these initial studies used injectable magnesium, it has been demonstrated that this is not necessary to restore magnesium status except in the case of an emergency situation such as an acute heart attack or an acute asthma attack.[126] Oral magnesium therapy is an effective measure to raise body magnesium stores, but it will usually take 6 weeks to achieve significant elevations in tissue magnesium concentrations. Oral supplementation appears to be warranted because low levels of plasma magnesium have been found in asthmatic patients,[92] and dietary magnesium intake is independently related to lung function and the severity of asthma.[127] Several double-blind studies in adults and children with asthma have demonstrated improvements in respiratory function, antioxidant status (i.e., increased glutathione

concentrations), reduced reactivity to chemical challenge to methacholine, and measures of asthma control and quality of life.[128–130] The dosages used ranged from 300 mg a day in children to 340 mg a day in adults, usually in divided dosages.

Isotonic nebulized magnesium has also proved useful as an adjunct treatment to standard bronchodilation therapies in patients with severe asthma, with a greater response in those with life-threatening asthma.[131] A randomized controlled trial investigated the effect of inhaled magnesium sulfate on the treatment response in emergency department (ED) patients with moderate to severe asthma.[132] Subjects allocated to the study group were treated with a standard protocol, plus 3 mL of 260 mmol/L solution of magnesium sulfate every 20 to 60 minutes. The control group was treated with nebulized saline as a placebo in addition to the standard protocol. The results demonstrate that adding nebulized magnesium sulfate to standard therapy in patients with moderate to severe asthma attacks led to greater and faster improvement in PEFR, respiratory rate, oxygen saturation, and respiratory rate and reduces hospitalization rates.

Vitamin D

Vitamin D deficiency is linked to increased airway reactivity, poorer lung function, and worse asthma control.[133] One study in more than 1000 children with asthma showed that 35% were vitamin D–insufficient, as defined by a level of 30 ng/mL or less of 25-hydroxyvitamin D.[134] After adjusting for age, sex, body mass index, income, and treatment group, insufficient vitamin D status was associated with higher odds of hospitalization or emergency department visits (OR 1.5). In addition to correcting a vitamin D insufficiency, vitamin D supplementation may improve asthma control by blocking the cascade of inflammation-causing proteins in the lung as well as increasing the production of interleukin-10, which has anti-inflammatory effects. Preliminary clinical evidence is encouraging, especially in the prevention of childhood asthma, at a dosage of 1200 IU per day of vitamin D_3.[135] Although there is conflicting evidence from clinical trials, results from in vivo and in vitro studies in animals and humans have suggested that supplementation with vitamin D may ameliorate several hallmark features of asthma, and vitamin D deficiency may influence the inflammatory response in the airways.[136]

Botanical Medicines

Asthma patients commonly employ self-treatment with botanicals. A cross-sectional analysis of 601 adults with asthma found that 14% used either herbal products, coffee, or black tea to treat their condition. Unfortunately, this study illustrated that those who used these methods had a higher incidence of hospitalizations.[137] Because of the possibility of improper use of botanicals and the inability of the users to recognize the need for acute conventional interventions, it is recommended that before using natural therapies, patients with asthma consult a naturopathic physician or other qualified practitioner who understands the proper use of botanicals and can assess the asthmatic patient's severity of risk.

The most popular historical herbal treatment of asthma involved the use of *Ephedra sinensis* (Ma huang) in combination with herbal expectorants. This approach appeared to have considerable merit because *Ephedra* and its alkaloids have proven to be effective as bronchodilators in treating mild to moderate asthma and hay fever.[138,139] *Ephedra* preparations containing ephedrine alkaloids are no longer sold in the United States because of safety concerns. However, ephedra extracts without ephedrine are still legally sold.

Hedera helix (Ivy)

In Europe, herbal preparations containing extracts from the leaves of ivy are popular for the relief of cough as well as asthma. In 2007 more than 80% of herbal expectorants prescribed in Germany comprised ivy extract and amounted to nearly 2 million prescriptions nationwide. Ivy leaf contains saponins that show expectorant, mucolytic, spasmolytic, bronchodilatory, and antibacterial effects. The mucolytic and expectorant actions of ivy are based on indirect $beta_2$ adrenergic effects, and these actions are due to the saponins α-hederin and hederacoside C, the latter of which is metabolized to α-hederin when ingested.[140] The indirect effect involves α-hederin, which inhibits the intracellular uptake of $beta_2$ receptors and leads to an increased $beta_2$-adrenergic response of the cell.

A 2003 meta-analysis of three double-blind studies in children showed that the ivy preparations used were significantly superior to placebo.[141] Among the three trials, one study compared ivy leaf extract cough drops to placebo, one compared suppositories to drops, and one tested syrup against drops. The reviewers concluded that ivy leaf extract preparations are effective with respect to the improvement of respiratory function in children with chronic bronchial asthma but noted that the study had a meager database to assess. In the only placebo-controlled double-blind study reviewed, 24 children with asthma between the ages of 4 and 12 were given a dry ivy leaf extract (35 mg) in cough drops or placebo for 3 days with a washout of 3 to 5 days before crossing over to the other treatment. The superiority of ivy leaf extract over placebo was noted by small improvements in airway resistance, residual volume, vital capacity, FVC, and FEV_1 when the baseline measurements were compared with day 3 at 3 hours postdosing (morning dose).

Glycyrrhiza glabra

Licorice root has a long history of use as an anti-inflammatory and anti-allergic agent, as documented in the scientific literature (see Chapter 85). The primary active component of licorice root in this application is glycyrrhetinic acid, a compound that has shown cortisol-like activity. In particular, glycyrrhetinic acid has been shown to inhibit phospholipase A_2, the enzyme responsible for cleaving arachidonic acid from the phospholipid membrane pool and initiating eicosanoid synthesis.[142] Licorice is also an expectorant, which is useful in the treatment of asthma.

Lobelia inflata

Indian tobacco contains the alkaloid lobeline, an efficient expectorant. Although it has a long history of use in asthma, it does promote bronchoconstriction and is a respiratory stimulant in vitro.[143] This appears to suggest a cholinergic effect in the respiratory system; it also binds to nicotine acetylcholine receptors in the ganglions, thus promoting the release of epinephrine and norepinephrine. It is this action on adrenal hormone secretion that is responsible for lobelia's therapeutic effects.[144] Lobelia has traditionally been used in combination with other botanical agents, such as *Capsicum frutescens* and *Symphlocarpus factida.*

Capsicum frutescens

Experimental evidence has shown that capsaicin, cayenne pepper's major active component, induces long-lasting desensitization of airway mucosa to various mechanical and chemical irritants.[145] This effect is probably due to capsaicin-induced depletion of substance P (which normally increases vascular permeability and flow) in the respiratory tract nerves. Substance P is an undecapeptide associated with "neurogenic inflammation" via a direct effect and a synergistic action with histamine on the peripheral nervous system.[146] The respiratory

and gastrointestinal tracts have large numbers of substance P–containing neurons. Because of its location and physiological action, it is believed to play an important role in atopic conditions such as asthma and atopic dermatitis. Therefore depletion of substance P may be beneficial in these conditions.

Zizyphi fructus

The jujube plum has been used extensively in Chinese medicine for the treatment of asthma and allergic rhinitis.[147] It contains 100 to 500 nmol/g of cyclic-AMP (cAMP) per dry weight, a concentration 10 times greater than that of any other plant or animal tissue thus far reported in the literature.[148] It also contains a beta-adrenergic receptor stimulator that raises cAMP levels. These experimental findings, in conjunction with *Zizyphi*'s long historical use, strongly support its clinical use.

Thea sinensis

Green tea is useful as an adjunctive in asthma treatment due to its methylxanthine and antioxidant components (see Chapter 60).

Allium Family

Onions and garlic inhibit lipoxygenase and cyclooxygenases, which generate TxA_2, PGD_2, and PGE_2.[149] Oral pretreatment of guinea pigs (that were sensitized to ovalbumin) with 1 mL of an alcohol/onion extract markedly reduced the asthmatic response to allergen inhalation challenges. Onion contains quercetin, which may account for some of its pharmacological effect,[150] but the major protective actions appear to be related to its content of benzyl and other isothiocyanates (mustard oils).[151] Although the mechanism of action is unknown, it has been suggested that it is due to the inhibition of the biosynthesis of arachidonic acid metabolites.

Tylophora asthmatica

The leaves of *Tylophora asthmatica* have been used extensively in Ayurvedic medicine in asthma and other respiratory tract disorders. The mode of action of *Tylophora* is unknown but is thought to be due to the alkaloids, especially tylophorine, which have been reported to possess antihistamine and antispasmodic activity, as well as inhibition of mast-cell degranulation.[152,153] However, a more central mechanism may be responsible for the clinical effects in asthma.

Several double-blind clinical studies have shown *Tylophora* to be beneficial for asthma.[154–157] In one study of 135 patients, those given 200 mg of *Tylophora* leaves twice daily for 6 days demonstrated improvements in symptoms and respiratory function during the treatment period and for up to 2 weeks after treatment.[154]

In another double-blind study of 103 patients, those receiving 40 mg of the dry alcoholic extract of *Tylophora indica* daily for only 6 days demonstrated significant improvement in symptoms of asthma compared with a placebo group.[156] At the end of the first week, 56% had complete to moderate improvement, compared with 31.6% of the 92 patients receiving the placebo. At the end of 4 weeks, the respective figures were 32% and 23.8%; at 8 weeks, 23.8% and 8.4%; and at 12 weeks, 14.8% and 7.2%. The incidence of side effects such as nausea, partial diminution of taste for salt, and slight mouth soreness was 16.3% in the *Tylophora* group and 6.6% in the placebo group. These results, as well as the results from an additional study, indicate that the benefits of *Tylophora* are short-lived.[157,158]

Ginkgo biloba

G. biloba contains several unique terpene molecules known collectively as ginkgolides that antagonize platelet-activating factor (PAF), a key chemical mediator in asthma, inflammation, and allergies. Ginkgolides compete with PAF for binding sites and inhibit the various events induced by PAF. The antiasthmatic effects of orally administered or inhaled ginkgolides have been shown to produce improvements in respiratory function and to reduce bronchial reactivity in several double-blind studies.[158,159] Treatment consisted of 120 mg of the pure ginkgolides daily—a dosage that is currently expensive to achieve using the 24% ginkgo flavonglycoside and 6% total terpenoid *G. biloba* extract.

Aloe vera

In one study, the oral administration of an extract of *Aloe vera* for 6 months was shown to produce good results in the treatment of asthma in some individuals of various ages.[160] The extract was produced from the supernatant of fresh leaves stored in the dark at 4°C for 7 days. Subjecting the leaves to dark and cold results in an increase in the polysaccharide fraction. One gram of the crude extract obtained from leaves stored in a cold, dark environment produced 400 mg of neutral polysaccharide, compared with only 30 mg produced from leaves not subjected to cold or dark. The dosage was 5 mL of a 20% solution of the *A. vera* extract in saline twice daily for 24 weeks, and 11 of 27 patients (40%) without corticosteroid dependence felt better at the study's conclusion. The mechanism of action is thought to be via restoration of protective mechanisms, followed by augmentation of the immune system.

Coleus forskohlii

Coleus forskohlii extracts may be particularly useful in asthma because increasing intracellular levels of cAMP result in relaxation of bronchial muscles and relief of respiratory symptoms. Forskolin has been shown to have remarkable effects in relaxing constricted bronchial muscles in patients with asthma (see Chapter 69).[161,162] These studies used inhaled doses of pure forskolin. Whether orally administered forskolin in the form of *C. forskohlii* extract would produce similar bronchodilatory effects has yet to be determined. However, on the basis of the plant's historic use and additional mechanisms of action, it appears likely.

Boswellia

The Indian Ayurvedic botanical *Boswellia* is known for its ability to inhibit leukotriene biosynthesis.[163] In one double-blind, placebo-controlled study, bronchial asthma was reduced in 70% of 40 patients treated with gum resin at 300 mg three times daily for 6 weeks, whereas only 27% of the control group improved. The disappearance of physical symptoms and signs such as dyspnea and rhonchi; fewer attacks; increased FEV_1, vital capacity, and peak expiratory flow rate (PEFRs); and decreased eosinophilia counts and sedimentation rates were recorded as measures of improvement.[163]

Acupuncture and Acupressure

In traditional Chinese medicine, chronic asthmatic symptomatology is usually characterized as a lung or spleen deficiency. This model considers that acute symptoms may be caused by environmental invasion from cold wind (environmental factors) or an internal condition stemming from a lung heat condition (increased inflammation and eosinophilia). Chronic asthma is considered more of a weakness in the lung itself or a weakness of the spleen, which is responsible for nourishing the lung *qi* (or vitality). In traditional Chinese medicine, the emotion of grief is also said to weaken lung *qi.*

In one prospective randomized study of patients with chronic asthma, 41 patients with chronic obstructive asthma were randomly assigned to receive acupuncture in addition to standard care, acupressure and standard care, or standard care alone. For each subject, 20 acupuncture treatments were given, and self-administered acupressure was performed daily for 8 weeks. On the St. George's Respiratory

Questionnaire, the acupuncture subjects showed an average 18.5-fold improvement, whereas the improvement for the acupressure-only subjects was 6.57-fold. Additionally, for patients who received acupressure, the irritability domain score exhibited an 11.8-fold improvement.[164] Another study involved 44 patients receiving bona fide or sham acupressure. They received five treatments per week lasting 16 minutes each for 4 weeks. Using pulmonary function and dyspnea scores, 6-minute walking distance measurements, and State Anxiety Scale scores, the acupressure group had significant improvements in breathing and less anxiety compared with those of the sham group.[165]

THERAPEUTIC APPROACH

The effective treatment of asthma requires the consideration and control of many aspects. In particular, the specific underlying defects and initiating factors must be determined because many different defects and metabolic abnormalities result in the same clinical picture of asthma. The development of an appropriate treatment plan includes the following five steps:

1. Determining and rectifying the underlying defect that allows the development of sensitization
2. Determining and balancing the underlying metabolic defect that causes an excessive inflammatory response
3. Finding potential allergens and developing a lifestyle, diet, and environment that allow the allergens to be avoided
4. Modulating the inflammatory process to limit the severity of the response
5. Preparing an effective treatment for the bronchoconstriction of the acute attack

Environment

The most important preventative measures are to decrease exposure through decreased pollution in the work and home environments, the use of airway protection during the most-exposed work tasks, and cessation of smoking. Airborne allergens such as pollen, dander, and dust mites[166] are often difficult to avoid entirely, but measures can be taken to reduce exposure. Eliminating dogs, cats, carpets, rugs, upholstered furniture, and other surfaces where allergens can collect is a great first step. Also, methods to discourage the presence of dust mite and cockroach antigen should be used, although pesticide exposure should be minimized. If environmental exposures cannot be controlled entirely, make sure that the bedroom is as allergy-proof as possible. Have the patient encase the mattress in an allergen-proof plastic; wash sheets, blankets, pillowcases, and mattress pads every week; consider using bedding material made from Ventflex, a special hypoallergenic synthetic material; and install an air purifier. The best mechanical air purifiers are high-efficiency particulate-arresting filters that can be attached to central heating and air-conditioning systems. These units are available from suppliers of heating and air-conditioning units.

Environmental chemicals around the home should be removed. These include paints, solvents, new furniture, chemical cleaners, and scented candles/air fresheners, to name a few. Building materials containing formaldehyde (e.g., carpeting, cabinetry) should also be avoided. Perfume, cologne, hair spray, lotions, antiperspirant, and scented soaps and shampoos often contain several chemicals that should be avoided.

Water-damaged buildings must be repaired, and all mold, bacterial, and other organism growth must be addressed by professionals experienced in mold remediation.

Diet

Organic, mostly plant-based foods should be consumed when possible. Any foods known to cause adverse reactions should also be avoided. Elimination of the offending foods and incorporation of an oligoantigenic diet are primary in reducing inflammation. Elimination diets have been successful in identifying allergens and treating asthma, especially in infants. The patient who has many food allergies may have to use a 4-day rotation diet. In the early stages of treatment, mild dietary tryptophan reduction should be useful but is not critical unless there is a metabolic defect in tryptophan metabolism. Garlic and onions should be used liberally unless the patient reacts to them. If the patient is willing or his or her asthma is unresponsive to this recommended therapy, a vegan diet should be tried (for a minimum of 4 months), with the possible exception of coldwater fish. Moderate fruit consumption, especially of apples, should also be encouraged.

Supplements

- Vitamin B_6: 25 to 50 mg two times a day
- Vitamin B_{12}: 1000 mcg/day (oral) or weekly injection; evaluate for efficacy after 6 weeks
- Vitamin C: 10 to 30 mg/kg in divided dosages
- Vitamin D: 1000 to 8000 IU daily. Monitor blood levels to determine needed dosage because there is wide variability. Those whose blood levels do not rise in response to supplementation should have their $1,25(OH)2D_3$ checked. Also, Vitamin K_2 should always be prescribed with Vitamin D.
- Vitamin E: 100 to 200 IU daily
- Magnesium: 200 to 400 mg three times a day
- Quercetin: 400 mg 20 minutes before meals or enzymatically modified isoquercitrin (EMIQ) 100 mg daily.
- Grape seed extract (95% PCO content) or pine bark extract (e.g., Pycnogenol): 150 to 300 mg daily
- Lycopene: 30 to 45 mg/day
- Selenium: 200 mcg/day

Botanical Medicines

Choose one or more of the following botanical medicines.

Hedera helix

Ivy leaf is available primarily in tincture and fluid extract, and the dry-powdered extract comes in the form of capsules and tablets. Based on clinical studies, the daily dosages should deliver the following equivalent to dried herbal substance: 1 to 5 years: 150 mg; 6 to 12 years: 210 mg; over 12 years: 420 mg. The typical dosage of a 4:1 powdered extract for adults and children over 12 years of age is 100 mg daily.

Lobelia inflata

- Dried herb: 0.2 to 0.6 g three times a day
- Tincture: 15 to 30 drops three times a day
- Fluid extract: 8 to 10 drops three times a day

Glycyrrhiza glabra

- Powdered root: 1 to 2 g
- Fluid extract (1:1): 2 to 4 mL (½–1 Tsp)
- Solid (dry-powdered) extract (4:1): 250 to 500 mg

Camellia sinensis

Liberal use (green tea only)

Tylophora asthmatica

200 mg of *Tylophora* leaves or 40 mg of the dry alcoholic extract two times daily

Coleus forskolii

Extract standardized to contain 18% forskolin: 50 mg (9 mg of forskolin) two to three times daily

Counseling

For patients who respond to emotional crises with asthmatic attacks, counseling is important. Counseling is also important for children with moderate to severe asthma, who may develop behavioral problems.

Acupuncture and Acupressure

Regular acupuncture and home acupressure treatments should be used.

IN ACUTE ATTACK

An acute asthma attack can be a medical emergency. If necessary, the patient should be referred to an emergency department immediately.

There are two more natural approaches that can be considered, but research support is only anecdotal. Intravenous (IV) magnesium can quickly relieve bronchial spasm. This should only be done by those with proper training and facilities. Dr. Bastyr recommended an herbal protocol using 1 tsp every 15 minutes of a tincture containing equal parts capsicum, lobelia, and skunk cabbage, which the senior editor found consistently effective. Before prescribing, practitioners are advised to try this intense intervention themselves first. If the patient does not immediately respond to either of these two interventions, then emergency intervention is required.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Fanta CH. Asthma. *N Engl J Med*. 2009;360(10):1002–1014.
2. Murphy DM, O'Byrne PM. Recent advances in the pathophysiology of asthma. *Chest*. 2010;137(6):1417–1426.
3. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, et al. Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico. *Thorax*. 2004;59:8–10.
4. Cakebread JA, Haitchi HM, Holloway JW, et al. The role of ADAM33 in the pathogenesis of asthma. *Springer Semin Immunopathol*. 2004;25:361–375. Epub 2003 Nov 15.
5. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science*. 2004;304:300–304.
6. Raby BA, Silverman EK, Lazarus R, et al. Chromosome 12q harbors multiple genetic loci related to asthma and asthma-related phenotypes. *Hum Mol Genet*. 2003;12:1973–1979.
7. Joos L, Carlen Brutsche IE, Laule-Kilian K, et al. Systemic Th1- and Th2-gene signals in atopy and asthma. *Swiss Med Wkly*. 2004;134:159–164.
8. Miller A. The etiologies, pathophysiology, and alternative/complementary treatment of asthma. *Altern Med Review*. 2001;6:20–47.
9. McGeady SJ. Immunocompetence and allergy. *Pediatrics*. 2004;113(suppl 4):1107–1113.
10. von Mutius E. Infection: friend or foe in the development of atopy and asthma? The epidemiological evidence. *Eur Respir J*. 2001;18:872–881.
11. Yen SS, Morris HG. An imbalance of arachidonic acid metabolism in asthma. *Biochem Biophys Res Commun*. 1981;103:774–779.
12. Tan Y, Collins-Williams C. Aspirin-induced asthma in children. *Ann Allergy*. 1982;48:1–5.
13. Vanderhoek JY, Ekborg SL, Bailey JM. Nonsteroidal anti-inflammatory drugs stimulate 15-lipoxygenase/leukotriene pathway in human polymorphonuclear leukocytes. *J Allergy Clin Immunol*. 1984;74:412–417.
14. Scanlon RT. Asthma. A panoramic view and a hypothesis. *Ann Allergy*. 1984;53:203–212.
15. Odent MR, Culpin EE, Kimmel T. Pertussis vaccination and asthma. Is there a link? *JAMA*. 1994;72:592–593.
16. Bergen R, Black S, Shinefield H, et al. Safety of cold-adapted live attenuated influenza vaccine in a large cohort of children and adolescents. *Pediatr Infect Dis J*. 2004;23:138–144.
17. Wong J, Magun BE, Wood LJ. Lung inflammation caused by inhaled toxicants: a review. *Int J Chron Obstruct Pulmon Dis*. 2016;11:1391–1401. PubMed PMID: 27382275.
18. Traboulsi H, Guerrina N, Iu M, et al. Inhaled pollutants: the molecular scene behind respiratory and systemic diseases associated with ultrafine particulate matter. *Int J Mol Sci*. 2017;18(2):Pii: E243. PubMed PMID: 28125025.
19. Van Vliet P, Knape M, de Hartog J, Janssen N, Harssema H, Brunekreef B. Motor vehicle exhaust and chronic respiratory symptoms in children living near freeways. *Environ Res*. 1977;74:122–132. PubMed PMID: 9339225.
20. Venn A, Yemaneberhan H, et al. Proximity of the home to roads and the risk of wheeze in an Etiopian population. *Occup Environ Med*. 2005;62(6):376–380. PubMed PMID: 15901884.
21. Wade JF, Newman LS. Diesel asthma, reactive airway disease following overexposure to locomotive exhaust. *J Occup Med*. 1993;35(2):149–154. PubMed PMID: 8433186.
22. Weisel CP, Cody RP, Lioy PJ. Relationship between summertime ambient ozone levels and emergency department visits for asthma in central new jersey. *Environ Health Perspect*. 1995;103(suppl 2):97–102. PubMed PMID: 7614954.
23. Kim BJ, Kwon JW, Seo JH, et al. Association of ozone exposure with asthma, allergic rhinitis, and allergic sensitization. *Ann Allergy Asthma Immunol*. 2011;107(3):214–219.e1. PubMed PMID: 21875539.
24. deCastro BR. Acrolein and asthma attack prevalence in a representative sample of the United States adult population 2000-2009. *PLoS One*. 2014;9(5):e96926. PubMed PMID: 24816802.
25. Das D, Bindhani B, Mukherjee B, et al. Chronic low-level arsenic exposure reduces lung function in male population without skin lesions. *Int J Public Health*. 2014;59(4):655–663. PubMed PMID: 24879317.
26. Parvez F, Chen Y, et al. Arsenic exposure and impaired lung function. Findings from a large population-based prospective cohort study. *Am J Respir Crit Care Med*. 2013;188(7):813–819. PubMed PMID: 23848239.
27. Davison AG, Haslam PL, Corrin B, Coutts II , et al. Interstitial lung disease and asthma in hard-metal workers: bronchoalveolar lavage, ultrastructural, and analytical findings and results of bronchial provocation tests. *Thorax*. 1983;38(2):119–128. PubMed PMID: 6857569.
28. Respiratory health hazards in agriculture. *Am J Respir Crit Care Med*. 1998;158(5 Pt 2):S1–S76. PubMed PMID: 9817727.
29. Hoppin JA, Umbach DM, London SJ, et al. Pesticides and adult respiratory outcomes in the agricultural health study. *Ann NY Acad Sci*. 2006;1076:343–354. PubMed PMID: 17119214.
30. Hoppin JA, Umbach DM, London SJ, et al. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. *Eur Respir J*. 2009;34(6):1296–1303. PubMed PMID: 19541724.
31. Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air*. 2007;17(4):284–296. PubMed PMID: 17661925.
32. Cox-Ganser JM, White SK, Jones R, et al. Respiratory morbidity in office workers in a water-damaged building. *Environ Health Perspect*. 2005;113(4):485–490. PubMed PMID: 15811840.
33. *Institute of Medicine. Damp Indoor Spaces and Health*. Washington, DC: National Academies Press; 2004. https://www.nap.edu/read/11011/chapter/1. Accessed November 10, 2018.
34. Ledford DK, Wenzel SE, Lockey RF. Aspirin or other nonsteroidal inflammatory agent exacerbated asthma. *J Allergy Clin Immunol Pract*. 2014;2(6):653–657. PubMed PMID: 25439353.
35. Okayama M, Yafuso N, Nogami H, et al. A new method of inhalation challenge with propranolol: comparison with methacholine-induced bronchoconstriction and role of vagal nerve activity. *J Allergy Clin Immunol*. 1987;80(3 Pt 1):291–299. PubMed PMID: 3305663.
36. Dunn TL, Gerber MJ, Shen AS, et al. The effect of topical ophthalmic instillation of timolol and betaxolol on lung function in asthmatic patients. *Am Rev Respir Dis*. 1986;133(2):264–268. PubMed PMID: 3946922.
37. Covar RA, Macomber BA, Szefler SJ. Medications as asthma triggers. *Immunol Allergy Clin North Am*. 2005;25(1):169–190. PubMed PMID: 15579370.
38. Bray GW. The hypochlorhydria of asthma in childhood. *Q J Med*. 1931;24:181–197.
39. Benard A, Desreumeaux P, Huglo D, et al. Increased intestinal permeability in bronchial asthma. *J Allergy Clin Immunol*. 1996;97:1173–1178.
40. Akiyama K, Shida T, Yasueda H, et al. Atopic asthma caused by Candida albicans acid protease. *Case reports. Allergy*. 1994;49:778–781.
41. Freedman BJ. A diet free from additives in the management of allergic disease. *Clin Allergy*. 1977;7:417–421.
42. Stevenson DD, Simon RA. Sensitivity to ingested metabisulfites in asthmatic subjects. *J Allergy Clin Immunol*. 1981;68:26–32.
43. MacCara ME. Tartrazine: a potentially hazardous dye in Canadian drugs. *Can Med Assoc J*. 1982;126(8):910–914. PubMed PMID: 7074487.
44. Papaioannou R, Pfeiffer CC. Sulfite sensitivity—unrecognized threat. Is molybdenum deficiency the cause? *J Orthomol Psych*. 1984;13:105–110.
45. Carrey OJ, Locke C, Cookson JB. Effect of alterations of dietary sodium on the severity of asthma in men. *Thorax*. 1993;48:714–718.
46. Burney PG. A diet rich in sodium may potentiate asthma. Epidemiologic evidence for a new hypothesis. *Chest*. 1987;91:143–148.
47. Barros R, Moreira A, Padrao P, et al. Dietary patterns and asthma prevalence, incidence and control. *Clin Exp Allergy*. 2015;45(11):1673–1680. PubMed PMID: 25818037.
48. Haggerty CL, Ness RB, Kelsey S, et al. The impact of estrogen and progesterone on asthma. *Ann Allergy Asthma Immunol*. 2003;90:284–291.
49. Lange P, Parner J, Prescott E, et al. Exogenous female sex steroid hormones and risk of asthma and asthma-like symptoms: a cross sectional study of the general population. *Thorax*. 2001;56:613–616.
50. Hepburn MJ, Dooley DP, Morris MJ. The effects of estrogen replacement therapy on airway function in postmenopausal, asthmatic women. *Arch Intern Med*. 2001;161:2717–2720.
51. Ensom MH, Chong G, Zhou D, et al. Estradiol in premenstrual asthma: a double-blind, randomized, placebo-controlled, crossover study. *Pharmacotherapy*. 2003;23:561–571.

52. Mueller JE, Frye C, Brasche S, et al. Association of hormone replacement therapy with bronchial hyper-responsiveness. *Respir Med*. 2003;97:990–992.
53. Weinstein RE, Lobocki CA, Gravett S, et al. Decreased adrenal sex steroid levels in the absence of glucocorticoid suppression in postmenopausal asthmatic women. *J Allergy Clin Immunol*. 1996;97:1–8.
54. Kos-Kudla B, Ostrowska Z, Marek B, et al. Circadian serum levels of dehydroepiandrosterone sulphate in postmenopausal asthmatic women before and after long-term hormone replacement. *Endocr Regul*. 2001;35:217–222.
55. Sutherland ER, Ellison MC, Kraft M, et al. Elevated serum melatonin is associated with the nocturnal worsening of asthma. *J Allergy Clin Immunol*. 2003;112:513–517.
56. Sutherland ER, Martin RJ, Ellison MC, et al. Immunomodulatory effects of melatonin in asthma. *Am J Respir Crit Care Med*. 2002;166:1055–1061.
57. Denny SI, Thompson RL, Margetts BM. Dietary factors in the pathogenesis of asthma and chronic obstructive pulmonary disease. *Curr Allergy Asthma Rep*. 2003;3:130–136.
58. McKeever TM, Scrivener S, Broadfield E, et al. Prospective study of diet and decline in lung function in a general population. *Am J Respir Crit Care Med*. 2002;165:1299–1303.
59. Tabak C, Smit HA, Heederik D, et al. Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the MORGEN study). *Clin Exp Allergy*. 2001;31:747–755.
60. Romieu I, Trenga C. Diet and obstructive lung diseases. *Epidemiol Rev*. 2001;23:268–287.
61. Kelly Y, Sacker A, Marmot M. Nutrition and respiratory health in adults: findings from the health survey for Scotland. *Eur Respir J*. 2003;21:664–671.
62. Shaheen SO, Sterne JA, Thompson RL, et al. Dietary antioxidants and asthma in adults: population-based case-control study. *Am J Respir Crit Care Med*. 2001;164:1823–1828.
63. Smith LJ, Holbrook JT, Wise R, et al. Dietary intake of soy genistein is associated with lung function in patients with asthma. *J Asthma*. 2004;41(8):833–843.
64. Kalhan R, Smith LJ, Nlend MC, et al. A mechanism of benefit of soy genistein in asthma: inhibition of eosinophil p38-dependent leukotriene synthesis. *Clin Exp Allergy*. 2008;38(1):103–112.
65. Liu XJ, Zhao J, Gu XY. The effects of genistein and puerarin on the activation of nuclear factor-kappaB and the production of tumor necrosis factor-alpha in asthma patients. *Pharmazie*. 2010;65(2):127–131.
66. Bock SA. Food-related asthma and basic nutrition. *J Asthma*. 1983;20:377–381.
67. Oehling A. Importance of food allergy in childhood asthma. *Allergol Immunopathol (Madr)*. 1981;(suppl 9):71–73.
68. Ogle KA, Bullocks JD. Children with allergic rhinitis and/or bronchial asthma treated with elimination diet. A five-year follow-up. *Ann Allergy*. 1980;44:273.
69. Businco L, Falconieri P, Giampietro P, et al. Food allergy and asthma. *Pediatr Pulmonol Suppl*. 1995;11:59–60.
70. Bircher AJ, Van Melle G, Haller E, et al. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy*. 1994;24:367–374.
71. Hodge L, Yan KY, Loblay RL. Assessment of food chemical intolerance in adult asthmatic subjects. *Thorax*. 1996;51:805–809.
72. Hide DW, Matthews S, Matthews L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. *J Allergy Clin Immunol*. 1994;93:842–846.
73. Romieu I, Werneck G, Ruiz Velasco S, et al. Breastfeeding and asthma among Brazilian children. *J Asthma*. 2000;37:575–583.
74. Omenaas E, Fluge O, Buist AS, et al. Dietary vitamin C intake is inversely related to cough and wheeze in young smokers. *Respir Med*. 2003;97:134–142.
75. Becker A, Watson W, Ferguson A, et al. The Canadian asthma primary prevention study: outcomes at 2 years of age. *J Allergy Clin Immunol*. 2004;113:650–656.
76. Marra F, Lynb L, Coombes M, et al. Does antibiotic exposure during infancy lead to development of asthma? A systematic review and meta-analysis. *Chest*. 2006;129:610–618.
77. Kukkonen K, Kuitunen M, Haahtela T, et al. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. *Pediatr Allergy Immunol*. 2010;21(1 Pt 1):67–73.
78. Lindahl O, Lindwall L, Spangberg A, et al. Vegan diet regimen with reduced medication in the treatment of bronchial asthma. *J Asthma*. 1985;22:45–55.
79. Hodge L, Salome CM, Peat JK, et al. Consumption of oily fish and childhood asthma risk. *Med J Aust*. 1996;164:137–140.
80. Arm JP, Horton CE, Spur BW, et al. The effects of dietary supplementation with fish oil lipids on the airway response to inhaled allergen in bronchial asthma. *Am Rev Respiratory Dis*. 1989;139:1395–1400.
81. Dry J, Vincent D. Effect of a fish oil diet on asthma. Results of a 1-year double-blind study. *Int Arch Allergy Apply Immunol*. 1991;95:156–157.
82. Broughton KS, Johnson CS, Pace BK, et al. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am J Clin Nutr*. 1997;65:1011–1017.
83. Unge G, Grubbstrom J, Olsson P, et al. Effects of dietary tryptophan restrictions on clinical symptoms in patients with endogenous asthma. *Allergy*. 1983;38:211–212.
84. Reynolds RD, Natta CL. Depressed plasma pyridoxal phosphate concentrations in adult asthmatics. *Am J Clin Nutr*. 1985;41:684–688.
85. Collipp PJ, Goldzier III S, Weiss N, et al. Pyridoxine treatment of childhood asthma. *Ann Allergy*. 1975;35:93–97.
86. Sur S, Camara M, Buchmeier A, et al. Double-blind trial of pyridoxine (vitamin B_6) in the treatment of steroid-dependent asthma. *Ann Allergy*. 1993;70:147–152.
87. Shimizu T, Maeda S, Mochizuki H, et al. Theophylline attenuates circulating vitamin B_6 levels in children with asthma. *Pharmacology*. 1994;49:392–397.
88. Bartel PR, Ubbink JB, Delport R, et al. Vitamin B_6 supplementation and theophylline-related effects in humans. *Am J Clin Nutr*. 1994;60:93–99.
89. Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population. *Thorax*. 1994;49:171–174.
90. Katsoulis K, Kontakiotis T, Leonardopoulos I, et al. Serum total antioxidant status in severe exacerbation of asthma: correlation with the severity of the disease. *J Asthma*. 2003;40:847–854.
91. Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, et al. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *Am J Respir Crit Care Med*. 2002;166:703–709.
92. Oladipo OO, Chukwu CC, Ajala MO, et al. Plasma magnesium in adult asthmatics at the lagos university teaching hospital, nigeria. *East Afr Med J*. 2003;80:488–491.
93. Hatch GE. Asthma, inhaled oxidants, and dietary antioxidants. *Am J Clin Nutr*. 1995;61:625S–630S.
94. Ford ES, Mannino DM, Redd SC. Serum antioxidant concentrations among U.S. adults with self-reported asthma. *J Asthma*. 2004;41:179–187.
95. Fewtrell CM, Gomperts BD. Effect of flavone inhibitors of transport ATPase on histamine secretion from rat mast cells. *Nature*. 1977;265:635–656.
96. Bielory L, Gandhi R. Asthma and vitamin C. *Ann Allergy*. 1994;73:89–96.
97. Johnston CS, Martin LJ, Cai X. Antihistamine effect of supplemental ascorbic acid and neutrophil chemotaxis. *J Am Coll Nutr*. 1992;11:172–176.
98. Tecklenburg SL, Mickleborough TD, Fly AD, et al. Ascorbic acid supplementation attenuates exercise-induced bronchoconstriction in patients with asthma. *Respir Med*. 2007;101(8):1770–1778.
99. Hope WC, et al. In vitro inhibition of the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. *Biochem Pharmacol*. 1983;32:367–371.
100. Middleton Jr E, Drzewiecki G, Quercetin Krishnarao D. An inhibitor of antigen-induced human basophil histamine release. *J Immunol*. 1981;127:546–550.
101. Foreman JC. Mast cells and the actions of flavonoids. *J Allergy Clin Immunol*. 1984;73:769–774.

102. Hope WC, Welton AF, Fiedler-Nagy C, et al. in vitro inhibition of the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. *Biochem Pharmacol*. 1983;32:367–371.
103. Lau BH, Riesen SK, Truong KP, et al. Pycnogenol as an adjunct in the management of childhood asthma. *J Asthma*. 2004;41(8):825–832.
104. Watson RR, Zibadi S, Rafatpanah H, et al. Oral administration of the purple passion fruit peel extract reduces wheeze and cough and improves shortness of breath in adults with asthma. *Nutr Res*. 2008;28(3):166–171.
105. Grosch W, Laskawy G. Co-oxidation of carotenes requires one soybean lipoxygenase isoenzyme. *Biochem Biophys Acta*. 1979;575:439–445.
106. Wood LG, Gibson PG. Reduced circulating antioxidant defences are associated with airway hyper-responsiveness, poor control and severe disease pattern in asthma. *Br J Nutr*. 2010;103(5):735–741.
107. Riccioni G, Bucciarelli T, Mancini B, et al. Plasma lycopene and antioxidant vitamins in asthma: the PLAVA study. *J Asthma*. 2007;44(6):429–432.
108. Hazlewood LC, Wood LG, Hansbro PM, et al. Dietary lycopene supplementation suppresses Th2 responses and lung eosinophilia in a mouse model of allergic asthma. *J Nutr Biochem*. 2011;22(1):95–100.
109. Wood LG, Garg ML, Powell H, et al. Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept. *Free Radic Res*. 2008;42(1):94–102.
110. Falk B, Gorev R, Zigel L, et al. Effect of lycopene supplementation on lung function after exercise in young athletes who complain of exercise-induced bronchoconstriction symptoms. *Ann Allergy Asthma Immunol*. 2005;94(4):480–485.
111. Neuman I, Nahum H, Ben-Amotz A. Reduction of exercise-induced asthma oxidative stress by lycopene, a natural antioxidant. *Allergy*. 2000;55(12):1184–1189.
112. Panganamala RV, Cornwell DG. The effects of vitamin E on arachidonic acid metabolism. *Ann N Y Acad Sci*. 1982;393:376–391.
113. Misso NL, Powers KA, Gillon RL, et al. Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. *Clinical Exp Allergy*. 1996;26:838–847.
114. Stone J, Hinks LJ, Beasley R, et al. Reduced selenium status of patients with asthma. *Clin Sci (Colch)*. 1989;77:495–500.
115. Kadrabova J, Mad'aric A, Kovacikova Z, et al. Selenium status is decreased in patients with intrinsic asthma. *Biol Trace Elem Res*. 1996;52:241–248.
116. Wright JV. Treatment of childhood asthma with parenteral vitamin B_{12}, gastric re-acidification, and attention to food allergy, magnesium, and pyridoxine. *J Nutr Med*. 1990;1:277–282.
117. Simon SW. Vitamin B_{12} therapy in allergy and chronic dermatoses. *J Allergy*. 1951;2:183–185.
118. Garrison R, Somer E. *The Nutrition Desk Reference, Ch 5. Vitamin Research: Selected Topics*. New Canaan, CN: Keats; 1985.
119. Trendelenburg P. Physiologische und pharmakologische untersuchungen an der isolierten bronchial muskulatur. *Arch Exp Pharmacol Ther*. 1912;69:79.
120. Haury VG. Blood serum magnesium in bronchial asthma and its treatment by the administration of magnesium sulfate. *J Lab Clin Med*. 1940;26:340–344.
121. Skobeloff EM, Spivey WH, McNamara RM, et al. Intravenous magnesium sulfate for the treatment of acute asthma in the emergency department. *JAMA*. 1989;262:1210–1213.
122. Okayama H, Aikawa T, Okayama M, et al. Bronchodilating effect of intravenous magnesium sulfate in bronchial asthma. *JAMA*. 1987;257:1076–1078.
123. Noppeen M, Vanmaele L, Impens N, et al. Bronchodilating effect of intravenous magnesium sulfate in acute severe bronchial asthma. *Chest*. 1990;97:373–376.
124. Skorodin MS, Tenholder MF, Yetter B, et al. Magnesium sulfate in exacerbations of chronic obstructive pulmonary disease. *Arch Intern Med*. 1995;155:496–500.
125. McLean RM. Magnesium and its therapeutic uses: a review. *Am J Med*. 1994;96:63–76.
126. Gullestad L, Oystein Dolva L, et al. Oral versus intravenous magnesium supplementation in patients with magnesium deficiency. *Magnes Trace Elem*. 1991-92;10:11–16.
127. Britton J, Pavord I, Richards K, et al. Dietary magnesium, lung function, wheezing, and airway hyper-reactivity in a random adult population sample. *Lancet*. 1994;344:357–362.
128. Gontijo-Amaral C, Ribeiro MA, Gontijo LS, et al. Oral magnesium supplementation in asthmatic children: a double-blind randomized placebo-controlled trial. *Eur J Clin Nutr*. 2007;61(1):54–60.
129. Bede O, Nagy D, Surányi A, et al. Effects of magnesium supplementation on the glutathione redox system in atopic asthmatic children. *Inflamm Res*. 2008;57(6):279–286.
130. Kazaks AG, Uriu-Adams JY, Albertson TE, et al. Effect of oral magnesium supplementation on measures of airway resistance and subjective assessment of asthma control and quality of life in men and women with mild to moderate asthma: a randomized placebo controlled trial. *J Asthma*. 2010;47(1):83–92.
131. Hughes R, Goldkorn A, Masoli M, et al. Use of isotonic nebulised magnesium sulphate as an adjuvant to salbutamol in treatment of severe asthma in adults: randomised placebo-controlled trial. *Lancet*. 2003;361:2114–2117.
132. Hossein S, Pegah A, Davood F, et al. The effect of nebulized magnesium sulfate in the treatment of moderate to severe asthma attacks: a randomized clinical trial. *Am J Emerg Med*. 2016;34(5):883–886. PubMed PMID: 26964827.
133. Mak G, Hanania NA. Vitamin D and asthma. *Curr Opin Pulm Med*. 2011;17(1):1–5.
134. Brehm JM, Schuemann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*. 2010;126(1):52–58.
135. Urashima M, Segawa T, Okazaki M, et al. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr*. 2010;91(5):1255–1260.
136. Hall SC, Agrawal DK. Vitamin D and bronchial asthma: an overview of data from the past 5 years. *Clinical Therapeutics*. 2017;39(5):917–929. 28449868.
137. Blanc PD, Kuschner WG, Katz PP, et al. Use of herbal products, coffee or black tea, and over-the-counter medications as self-treatments among adults with asthma. *J Allergy Clin Immunol*. 1997;100:789–791.
138. Gilman AG, Goodman LS, Gilman A. *Goodman and Gilman's the Pharmacologic Basis of Therapeutics*. New York: MacMillan; 1980.
139. American Pharmaceutical Association. *Handbook of Nonprescription Drugs*. 8th ed. Washington, DC: American Pharmaceutical Association; 1986.
140. Sieben A, Prenner L, Sorkalla T, et al. Alpha-hederin, but not hederacoside C and hederagenin from *Hedera helix*, affects the binding behavior, dynamics, and regulation of beta 2-adrenergic receptors. *Biochemistry*. 2009;48(15):3477–3482.
141. Hofmann D, Hecker M, Völp A. Efficacy of dry extract of ivy leaves in children with bronchial asthma–a review of randomized controlled trials. *Phytomedicine*. 2003;10(2-3):213–220.
142. Okimasu E, Moromizato Y, Watanabe S, et al. Inhibition of phospholipase A2 and platelet aggregation by glycyrrhizin, an anti-inflammatory drug. *Acta Med Okayama*. 1983;37:385–391.
143. Cambar PJ, Shore SR, Aviado DM. Bronchopulmonary and gastrointestinal effects of lobeline. *Arch Int Pharmacodyn Ther*. 1969;177:1–27.
144. Halmagyi DF, Kovacs A, Neumann P. Adrenalcortical pathway of lobeline protection in some forms of experimental lung edema of the rat. *Dis Chest*. 1958;33:285–296.
145. Lundberg JM, Saria A. Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature*. 1983;302:251–253.
146. Payan DG, Levine JD, Goetzl EJ. Modulation of immunity and hypersensitivity by sensory neuropeptides. *J Immunol*. 1984;132:1601–1604.
147. Cyong J, Otsuka Y. A pharmacological study of the anti-inflammatory activity of Chinese herbs. *Acupunc Electrother Res*. 1982;7:173–202.
148. Hanabusa K, Cyong J, Takahashi M. High-level of cyclic AMP in jujube plum. *Planta Med*. 1981;42:380–384.

149. Vanderhoek J, Makheja A, Bailey J. Inhibition of fatty acid oxygenases by onion and garlic oils. Evidence for the mechanism by which these oils inhibit platelet aggregation. *Bioch Pharmacol*. 1980;29:3169–3173.
150. Dorsch W, Weber J. Prevention of allergen-induced bronchial obstruction in sensitized guinea pigs by crude alcoholic onion extract. *Agents Actions*. 1984;14:626–629.
151. Dorsch W, Adam O, Weber J, et al. Antiasthmatic effects of onion extracts—detection of benzyl- and other isothiocyanates (mustard oils) as antiasthmatic compounds of plant origin. *Euro J Pharmacol*. 1984;107:17–24.
152. Udupa AL, Udupa SL, Guruswamy MN. The possible site of anti-asthmatic action of *Tylophora asthmatica* on pituitary-adrenal axis in albino rats. *Planta Med*. 1991;57:409–413.
153. Gopalkrishnan C, Shankaranarayanan D, Nazimudeen SK, et al. Effect of tylophorine, a major alkaloid of *Tylophora indica,* on immunopathological and inflammatory reactions. *Indian J Med Res*. 1980;71:940–948.
154. Gupta S, George P, Gupta V, et al. *Tylophora indica* in bronchial asthma—a double blind study. *Ind J Med Res*. 1979;69:981–989.
155. Thiruvengadam KV, Haranath K, Sudarsan S, et al. *Tylophora indica* in bronchial asthma (a controlled comparison with a standard anti-asthmatic drug). *J Indian Med Assoc*. 1978;71:172–176.
156. Shivpuri DN, Singhal SC, Prakash D. Treatment of asthma with an alcoholic extract of *Tylophora indica.* A cross-over, double-blind study. *Ann Allergy*. 1972;30:407–412.
157. Shivpuri DN, Menon MP, Prakash D. A crossover double-blind study on *Tylophora indica* in the treatment of asthma and allergic rhinitis. *J Allergy*. 1969;43:145–150.
158. Wilkens JH, Wilkens H, Uffmann J, et al. Effects of a PAF-antagonist (BN 52063) on bronchoconstriction and platelet activation during exercise induced asthma. *Br J Clin Pharmacol*. 1990;29:85–91.
159. Guinot P, Brambilla C, Duchier J, et al. Effect of BN 52063, a specific PAF-acether antagonist, on bronchial provocation test to allergens in asthmatic patients. A preliminary study. *Prostaglandins*. 1987;34:723–731.
160. Shida T, Tagi A, Nishimura H, et al. Effect of Aloe extract on peripheral phagocytosis in adult bronchial asthma. *Planta Med*. 1985;51:273–275.
161. Lichey J, Friedrich T, Priesnitz M, et al. Effect of forskolin on methacholine-induced bronchoconstriction in extrinsic asthmatics. *Lancet*. 1984;2:167.
162. Bauer K, Dietersdorfer F, Sertl K, et al. Pharmacodynamic effects of inhaled dry powder formulations of fenoterol and colforsin in asthma. *Clin Pharmacol Ther*. 1993;53:76–83.
163. Wildfeuer A, Neu IS, Safayhi H, et al. Effects of boswellic acids extracted from a herbal medicine on the biosynthesis of leukotrienes and the course of experimental autoimmune encephalomyelitis. *Arzneimittelforschung*. 1998;48:668–674.
164. Maa SH, Sun MF, Hsu KH, et al. Effect of acupuncture or acupressure on quality of life of patients with chronic obstructive asthma: a pilot study. *J Altern Complement Med*. 2003;9:659–670.
165. Wu HS, Wu SC, Lin JG, et al. Effectiveness of acupressure in improving dyspnoea in chronic obstructive pulmonary disease. *J Adv Nurs*. 2004;45:252–259.
166. Van Woerden H. Dust mites living in human lungs—the cause of asthma? *Med Hypotheses*. 2004;63:193–197.

Atherosclerosis

John Nowicki, ND, and *Michael T. Murray*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Characteristically associated with high blood pressure, weak pulse, and wide pulse pressure
- Symptoms and signs depend on the arteries involved and degree of obstruction: angina, leg cramps (intermittent claudication), gradual mental deterioration, weakness, or dizziness
- May also occur without symptoms
- Diagonal earlobe crease

GENERAL CONSIDERATIONS

Atherosclerosis refers to the process that is the underlying pathology in a group of clinical entities collectively referred to as cardiovascular disease (CVD) including: heart disease (atherosclerosis of the coronary arteries); coronary artery disease (CAD); and myocardial, pulmonary, and cerebral infarction. Despite a steady decline in the age-adjusted death toll since 1980, CVD remains the number-one cause of death in the United States, where it is responsible for 1 of every 2.8 deaths. In 2015 nearly 634,000 deaths were attributed to heart disease and nearly 140,000 deaths to stroke in the United States.

Understanding Atherosclerosis

To fully understand the important ways in which various natural measures described in this chapter affect the health of the arteries and treatment of CVD, it is necessary to examine closely the structure of an artery and the process of atherosclerosis.

Structure of an Artery

An artery is divided into three major layers:

- The intima, representing the endothelium or internal lining of the artery, consists of a layer of endothelial cells lined by glycosaminoglycans (GAGs) to protect them from damage as well as to promote repair. Beneath the surface cells is the internal elastic membrane, composed of a layer of GAGs and other ground substance compounds, which supports the endothelial cells and separates the intima from the smooth muscle layer.
- The media consists primarily of smooth muscle cells. Interposed among the cells are GAGs and other ground substance structures that provide support and elasticity to the artery.
- The adventitia, or external elastic membrane, consists primarily of connective tissue, including GAGs; it provides structural support and elasticity to the artery.

The Process of Atherosclerosis

The lesions of atherosclerosis are initiated in response to injury to or a disruption of the normal functioning of the arterial endothelium.[1] The progression of atherosclerosis is detailed as follows:

1. The initial step is damage or dysfunction of the vascular endothelium. Damage results from weakening of the GAG layer, which protects the endothelial cells, due to the same factors that damage the endothelial cells (e.g., insulin resistance, reactive oxygen and

* Previous edition contributor

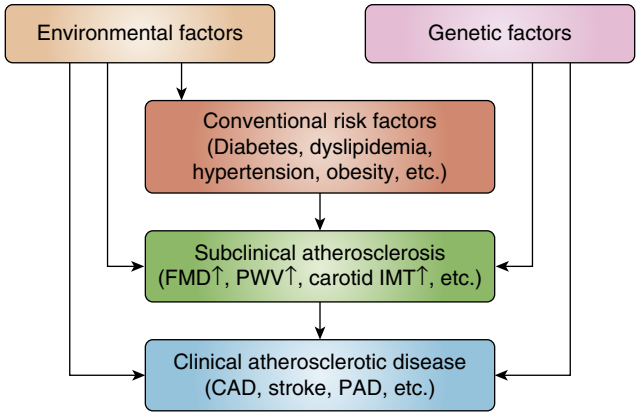


Fig. 149.1 Atherosclerosis is a multifactorial disease, and the development of atherosclerotic disease involves the interaction of many genetic and environmental factors, as well as conventional risk factors, such as diabetes, dyslipidemia, hypertension and obesity. *CAD*, coronary artery disease; *FMD*, flow-mediated vasodilation; *IMT*, intima-media thickness; *PAD*, peripheral artery disease; *PWV*, pulse wave velocity. (From Katakami N, Kaneto H, Shimomura I. Carotid ultrasonography: a potent tool for better clinical practice in diagnosis of atherosclerosis in diabetic patients. *J Diabetes Investig*. 2014;5[1]:3-13.)

nitrogen species, impaired repair processes, metal toxicity, hyperhomocysteinemia, and inhibition of either nitric oxide production or availability).

2. Once the endothelial cells have been sufficiently damaged, sites of injury become more permeable to plasma constituents, especially lipoproteins. The binding of lipoproteins to GAGs leads to a breakdown in the integrity of the ground substance matrix and causes an increased affinity for cholesterol. Simultaneously, monocytes, T lymphocytes, and platelets adhere to the damaged area, where they release growth factors that stimulate smooth muscle cells to migrate from the media into the intima and replicate.
3. Local concentration of lipoproteins, monocytes, and platelets leads to the migration of smooth muscle cells from the media into the intima, where they undergo proliferation. Smooth muscle cells dump cellular debris into the intima, leading to the further development of plaque.
4. Formation of a fibrous cap (consisting of collagen, elastin, and GAGs) over the intimal surface occurs. Fat and cholesterol deposits accumulate.
5. Plaque continues to grow until eventually it either blocks the artery directly or ruptures to form a clot that travels the general circulation until it occludes a blood vessel. Plaque instability is associated with a significantly greater risk for myocardial infarction (MI) or stroke.[1] Thus targeting plaque stabilization appears to be more clinically important than simply enlarging the lumen.

Causative Factors

Prevention of CVD involves reducing and, when possible, eliminating various risk factors (Fig. 149.1). Risk factors are divided into two primary categories: major risk factors and other risk factors. Box 149.1 lists the major risk factors. Risk for a heart attack increases with the number of major risk factors (Table 149.1).

In addition to these well-accepted major risk factors, numerous others have occasionally been shown to be more significant (Box 149.2). It is also important to develop a strategic approach to plaque stabilization by addressing endothelial dysfunction, increased local and systemic inflammation, increased reactive oxygen species, the activation of mast cells, and the infiltration and activation of macrophages.

BOX 149.1 Major Risk Factors for Atherosclerosis

- Smoking
- Elevated blood cholesterol levels
- High blood pressure
- Diabetes
- Physical inactivity
- Other risk factors

TABLE 149.1 Association of Risk Factors With the Incidence of Atherosclerosis

Major Risk Factors	Increase In Incidence
Presence of one of the major risk factors	30%
High cholesterol and high blood pressure	300%
High cholesterol and a smoker	350%
High blood pressure and a smoker	350%
Smoker, high blood cholesterol, and high blood pressure	720%

BOX 149.2 Other Risk Factors for Atherosclerosis

- Insulin resistance
- Low thyroid function (see Chapter 182)
- Low antioxidant status
- Elevations of C-reactive protein
- Low levels of essential fatty acids
- Increased platelet aggregation
- Increased fibrinogen formation
- Low levels of magnesium and potassium
- Elevated levels of homocysteine
- "Type A" personality

Determining a Patient's Risk

To help determine a patient's overall risk for having a heart attack or stroke, the following risk-determinant scale may prove useful. Although this risk assessment does not take into consideration several other important factors—such as the level of C-reactive protein, lipoprotein(a)[Lp(a)], fibrinogen, and coping style—the score provides a good indication of a patient's risk for a heart attack or stroke (Table 149.2).

Clinical Evaluation

Clinical cardiovascular assessment may include various laboratory and imaging such as the tests listed in Box 149.3.

Risk Factors

Smoking

Statistical evidence reveals smokers have a 70% greater risk of death from CVD than nonsmokers.[2] The more cigarettes smoked and the longer the period of years a person has smoked, the greater the risk of dying from a heart attack or stroke. Overall, the average smoker dies 7 to 8 years sooner than the nonsmoker.

Tobacco smoke contains more than 4000 chemicals, of which more than 50 have been identified as carcinogens. These chemicals are extremely damaging to the cardiovascular system. Many of these

TABLE 149.2 Risk-Determination Scale for Heart Disease and Stroke

	SCALE OF RISK				
	1	2	3	4	5
Blood pressure (systolic)	<125	125–134	135–149	150–164	≥165
Blood pressure (diastolic)	<90	90–94	95–104	105–114	≥115
Smoking (cigarettes per day)	None	1–9	10–19	20–29	≥30
Heredity I[a]	None	>65	50–64	35–49	<35
Heredity II[b]	0	1	2	4	≥4
Diabetes duration (years)	0	1–5	6–10	11–15	>15
Total cholesterol (mg/dL)	<200	200–224	225–249	250–274	≥275
HDL-C (mg/dL)	≥75	65–74	55–64	35–54	<35
Total cholesterol/ HDL-C ratio[c]	<3	3–3.9	4–4.9	5–6.4	≥6.5
Exercise (hours per week)	>4	3–4	2–3	1–2	0.1
Supplemental EPA/ DHA (mg) intake	>600	400–599	200–399	100–199	<100
Supplemental vitamin C (mg) and vitamin E (IU) intake	>400	251–499	250	125–249	0–124
Average daily servings of fruits and vegetables	>5	4–5	3	1–2	0
Age	<35	36–45	46–55	56–65	>65
Subtotals					

HDL-C, high-density-lipoprotein cholesterol.
[a]Age of patient when he or she had a heart attack or stroke.
[b]Number of immediate family members having had a heart attack before the age of 50.
[c]The value of the total cholesterol is to be divided by the value of the HDL-C. Risk = sum of all five columns; 14–20 = very low risk; 21–30 = low risk; 31–40 = average risk; 41–50 = high risk; ≥51 = very high risk.

BOX 149.3 Assessment of the Cardiovascular System

- Laboratory tests
- Total cholesterol
- Low-density-lipoprotein cholesterol
- High-density-lipoprotein cholesterol
- C-reactive protein
- Lipoprotein(a)
- Fibrinogen
- Homocysteine
- Ferritin (an iron-binding protein)
- Lipid peroxides
- Exercise stress test
- Electrocardiography
- Echocardiography

chemicals are carried in the bloodstream on LDL cholesterol where they either directly damage the lining of the arteries, or they oxidize the LDL molecule, which then damages the arteries. Exposure of human plasma to the gas phase of cigarette smoke produces oxLDL which strongly correlates with cardiovascular disease. An elevated cholesterol level makes the effect of smoking on the cardiovascular system even worse because more cigarette toxins will be carried through the vascular system. Smoking also contributes to elevated cholesterol presumably by damaging feedback mechanisms in the liver that control how much cholesterol is being created.[3] Smoking promotes platelet aggregation and elevated fibrinogen levels, two other significant independent risk factors for heart disease and strokes. In experimental models, PAHs found in the tar fraction of cigarette smoke have been shown to accelerate atherosclerosis.[4] In addition, cigarette smoking is a contributing factor to high blood pressure.[5]

Convincing evidence links environmental (secondhand or passive) smoke to heart disease mortality and morbidity. In the United States it is estimated that more than 37,000 coronary heart disease (CHD) deaths each year are attributable to environmental smoke. Meta-analysis of ten epidemiological studies indicates a consistent dose–response effect related to exposure.[6] Individuals exposed to secondhand smoke have higher homocysteine and fibrinogen (biomarkers of cardiovascular disease risk) levels than nonsmokers.[7] Evidence suggests that nonsmokers appear to be more sensitive to smoke, including its deleterious effects on the cardiovascular system. Environmental tobacco smoke actually has a higher concentration of some toxic constituents. Pathophysiological and biochemical data after short- and long-term environmental tobacco smoke exposure show changes in the lining of the arteries and in platelet function as well as exercise capacity, similar to those in active smoking. Passive smoking is a relevant risk factor for CVD.

The magnitude of risk reduction achieved by smoking cessation in patients with CVD is quite significant. Results from a detailed meta-analysis showed a 36% reduction in relative risk of mortality for patients with CAD who quit compared with those who continued smoking.[8]

Various measures, including nicotine-containing skin patches or chewing gum, acupuncture, and hypnosis, have been shown to provide some benefit in helping patients quit smoking. In a systematic review of the efficacy of interventions intended to help people to stop smoking, data were analyzed from 188 randomized controlled trials.[9] Encouragement to stop smoking by physicians during a routine office call resulted in a 2% cessation rate after 1 year. Supplementary measures such as follow-up letters or visits had an additional effect. Behavioral modification techniques such as relaxation, rewards and punishments, and avoiding trigger situations in group or individual sessions led by a psychologist had no greater effect than the 2% rate achieved by simple advice from a physician. Eight studies with acupuncture have produced an overall effectiveness rate of roughly 3%. Hypnosis has been judged to be ineffective, even though trials have shown a success rate of 23%. Hypnosis was judged to be ineffective because no biochemical marker was used to accurately determine effectiveness. Nicotine replacement therapy (gum or patch) is effective in about 13% of smokers who seek help in quitting. All together, these results are not encouraging. It appears that the best results occur when people quit "cold turkey." Box 149.4 lists 10 tips to help patients stop smoking.

Elevated Blood Cholesterol Levels

The evidence overwhelmingly demonstrates that elevated cholesterol levels greatly increase the risk of death due to CVD. It is recommended that the total blood cholesterol level should be less than 200 mg/dL. In

BOX 149.4 Tips to Help Patients Stop Smoking

- List all the reasons why you want to quit smoking and review them daily.
- Set a specific day to quit, tell at least ten friends that you are going to quit smoking, and then . . . DO IT!
- Throw away all cigarettes, butts, matches, and ashtrays.
- Use substitutes. Instead of smoking, chew on raw vegetables, fruits, or gum. If your fingers seem empty, play with a pencil.
- Take one day at a time.
- Realize that 40 million Americans have quit. If they can do it, so can you!
- Visualize yourself as a nonsmoker with a fatter pocketbook, pleasant breath, unstained teeth, and the satisfaction that comes from being in control of your life.
- Join a support group. Call the local branch of the American Cancer Society and ask for referrals. You are not alone.
- When you need to relax, perform deep breathing exercises rather than reaching for a cigarette.
- Avoid situations that you associate with smoking.
- Each day, reward yourself in a positive way. Buy yourself something with the money you have saved or plan a special reward as a celebration for quitting.

addition, it is recommended that the LDL-C should be less than 100 mg/dL, the high-density-lipoprotein cholesterol (HDL-C) should be greater than 40 mg/dL in men and 50 mg/dL in women, and the triglyceride level should be less than 150 mg/dL. Keep in mind, however, that except at very high levels, cholesterol is not the culprit but rather an indirect measure of oxLDL which is the culprit.

Cholesterol is transported in the blood by lipoproteins. The major categories of lipoproteins are very-low-density lipoprotein (VLDL), LDL, and HDL. Because VLDL and LDL are responsible for transporting fats (primarily triglycerides and cholesterol) from the liver to body cells, whereas HDL is responsible for returning fats to the liver, elevations of either VLDL or LDL are associated with an increased risk for developing atherosclerosis, the primary cause of a heart attack or stroke. In contrast, elevations of HDL-C are associated with a lower risk of heart attacks.

The ratios of total cholesterol to HDL-C and LDL-C to HDL-C are referred to as the *cardiac risk factor ratios* because they reflect whether cholesterol is being deposited into tissues or broken down and excreted. The ratio of total cholesterol to HDL-C should be no higher than 4.2, and the ratio of LDL-C to HDL-C should be no higher than 2.5. The risk of heart disease can be reduced dramatically by lowering LDL-C while simultaneously raising HDL-C levels. For every 1% drop in the LDL-C level, the risk of a heart attack drops by 2%. Conversely, for every 1% increase in HDL-C level, the risk for a heart attack drops by 3% to 4%.[10]

Further refinement of determining risk. Although LDL-C is referred to as "bad cholesterol," some forms are worse than others. For example, oxidized LDL-C is a persistent proinflammatory trigger for the progression of atherosclerosis and plaque rupture and highly predictive of cardiovascular disease. LDL-C molecules of higher density are associated with greater risk than larger, less dense molecules. Small, dense LDL-C has a greater content of apolipoprotein CIII (apo CIII); in addition, apoB particles are more atherogenic than larger, less dense LDL-Cs and are markers for CVD risk.[11] In a small trial of nondiabetic subjects, researchers determined that these smaller particles were more heavily and preferentially glycated over the larger, more buoyant LDL-C particles, strongly suggesting an explanation for why these particles are more likely to participate in atherogenesis and highlighting the importance of avoiding hyperglycemia and excessive glycation.[12]

Another marker that deserves mention is Lp(a), a plasma lipoprotein whose structure and composition closely resemble those of LDL-C but with an additional molecule of an adhesive protein called apolipoprotein(a). Elevated plasma levels of Lp(a) are an independent risk factor for CHD, particularly in those patients with elevated LDL-C levels. In one analysis, a high level of Lp(a) was shown to carry with it a ten times greater risk for heart disease than an elevated LDL-C level.[13] That is because LDL-C alone lacks the adhesive apolipoprotein(a). As a result, LDL-C does not easily stick to the walls of the artery. An elevated LDL-C level carries less risk than a normal or even low LDL-C combined with an elevated Lp(a). Levels of Lp(a) below 20 mg/dL are associated with a low risk of heart disease; levels between 20 and 40 mg/dL are associated with a moderate risk; and levels above 40 mg/dL are associated with an extremely high risk of heart disease.

Elevations of triglycerides. A large body of accumulating evidence indicates that hypertriglyceridemia (HTG) is an independent risk factor for CVD. Multivariate analysis of 8-year follow-up data from the large-scale Prospective Cardiovascular Munster Study found HTG to be an independent risk factor for major coronary events after controlling for LDL-C and HDL-C.[14] HTG combined with elevated LDL-C and a high LDL-C:HDL-C (>5) increased the risk for a CHD event by approximately sixfold. A large meta-analysis of 17 prospective trials reported that an 88 mg/dL (1.0 mmol/L) increase in plasma triglyceride levels significantly increased the relative risk of CVD by approximately 30% in men and 75% in women. The corresponding rates were somewhat lower (14% and 37%, respectively) but still statistically significant after adjustment for HDL-C level.[15]

This increased risk is mediated through the metabolic interrelationships between serum triglyceride (TG) levels and other risk factors, such as the atherogenic lipid profile (low HDL-C levels and elevated small dense LDL levels), insulin resistance, a prothrombotic propensity, and low-grade systemic inflammation. TG-lowering strategy in patients with HTG is an important clinical goal in reducing the risk of not only atherosclerosis but also the metabolic syndrome and diabetes.

Inherited elevations of cholesterol and triglycerides. Elevations of blood cholesterol, triglycerides, or both can be due to genetic factors. These conditions are referred to as *familial hypercholesterolemia (FH)*, *familial combined hyperlipidemia (FCH)*, and *familial hypertriglyceridemia (FHTG)*. Relatively speaking, these disorders are among the most common inherited diseases, affecting about 1 in every 500 people.

The problem in FH is a defect in the receptor protein for LDL-C in the liver. Normally, the LDL-C receptor is responsible for removing cholesterol from the blood. When the liver cell takes up the LDL-C after it has bound to the receptor, it signals the liver cell to stop making cholesterol. In FH, the defect in the LDL-C receptor results in the liver not receiving the message to stop making cholesterol.

Damage to the LDL-C receptor occurs with normal aging and in several disease states. Diabetes may be the most significant disease owing to increased glycosylation of the receptor proteins. As a result of damage to the LDL-C receptor, cholesterol levels tend to rise with age. In addition, a diet high in saturated fat and cholesterol decreases the number of LDL-C receptors, thereby reducing the feedback mechanism that tells the liver cell to decrease cholesterol synthesis.

Lifestyle and dietary changes can increase the function or number of LDL-C receptors or both. The most dramatic effects are in people without inherited causes of elevated cholesterol or triglycerides or both, but even people with FH can benefit.

FCH and FHTG result in defects that are similar to those of FH. In FCH, the basic defect appears to be an accelerated production of VLDL

in the liver. These individuals may have only a high blood triglyceride level, only a high cholesterol level, or both. In FHTG, there is only an elevation in blood triglyceride levels, and HDL-C levels tend to be low. The defect in FHTG is that VLDL particles made by the liver are larger than normal and carry more triglycerides. FHTG is aggravated by diabetes, gout, and obesity.

Diabetes

Atherosclerosis is one of the key underlying factors in the development of many chronic complications of diabetes. Individuals with diabetes have a twofold to threefold higher risk of dying prematurely of heart disease or stroke than persons who do not have diabetes, and 55% of deaths in patients with diabetes are caused by CVD. However, even mild insulin resistance and poor glucose control have both been shown to have a dramatic effect on the incidence and progression of CVD. For more information, see Chapter 165.

Elevated Blood Pressure

Elevated blood pressure is often a sign of considerable atherosclerosis and a major risk factor for heart attack or stroke. In fact, the presence of hypertension is generally regarded as the most significant risk factor for stroke. For more information, see Chapter 179.

Physical Inactivity

Physical activity refers to "bodily movement produced by skeletal muscles that requires energy expenditure" and produces healthy benefits. Exercise, a type of physical activity, is defined as "a planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness." Physical inactivity denotes a level of activity less than that needed to maintain good health. Physical inactivity characterizes most Americans, because roughly 54% of adults report little or no regular leisure physical activity, and there is also a sharp decline in regular exercise among children and adolescents.[1]

Physical activity protects against the development of CVD and favorably modifies other CVD risk factors, including high blood pressure, blood lipid levels, insulin resistance, and obesity. Physical activity is also important in the treatment and management of patients with CVD or increased risk including those who have hypertension, stable angina, a prior MI, peripheral vascular disease, heart failure, or who are recovering from a cardiovascular event.

Environmental Toxins

Particulate matter. Cumulative epidemiological and experimental data have shown that exposure to air pollutants leads to increased cardiovascular ischemic events and enhanced atherosclerosis. In approximately 43 million adults from the APHEA2 (Air Pollution and Health: A European Approach) study, it was found that daily cardiovascular mortality increased 1.5% for every 20 μg/m^3 increase in PM_{10}.[16] Ultrafine particles (<0.18 μm) enhance early atherosclerosis, partly due to their high content in redox cycling chemicals and their ability to synergize with known proatherogenic mediators in the promotion of tissue oxidative stress as well as through alterations of the anti-inflammatory function of plasma HDL (Fig. 149.2).[17]

$PM_{2.5}$ levels are also positively associated with increases in carotid intima-media thickness (CIMT). A study of 798 residents of the Los Angeles area found that for each 10 μg/m^3 of $PM_{2.5}$, CIMT increased by 5.9%.[18] Those patients on lipid-lowering agents had almost triple the increase in CIMT (15.8%) for each 10 μg/m^3 rise in $PM_{2.5}$, and in women over the age of 60, CIMT increased an average of 19.2%.

Persistent and nonpersistent organic pollutants

PCBs. Swedish seniors with higher levels of polychlorinated biphenyls (PCBs) 206, 170, 156, 153, 138, and 118 had more carotid artery atherosclerosis than those with lower PCB levels.[19] PCBs 170 and 156 both doubled the risk, and PCBs 206 and 153 increased the risk by 65%, 138 by 46%, and 118 by 16%.

Bisphenol A (BPA) and Phthalates. The Metabonomics and Genomics in Coronary Artery Disease study in the United Kingdom found that participants without coronary artery disease had a mean urinary BPA of 1.28 ng/mL, whereas participants with severe CAD had a mean of 1.53 ng/mL.[20] The PIVUS study found a positive association between BPA and carotid atherosclerosis with both thickness of the carotid intima and echogenicity of plaque in Swedish seniors.[21] Data from this study also revealed a positive association between carotid atherosclerosis and monomethyl phthalates (MMP), monoisobutyl phthalate (MiBP), and mono-2-ethylhexyl phthalate (MEHP).

Toxic metals

Mercury. Mercury is a powerful cardiac toxicant, and its presence in fish may completely negate the positive benefit of the omega-3 oils. Elevated hair mercury is associated with a 60% increase in risk of having an acute MI, a 68% greater likelihood of having CVD, and a 56% greater risk of having coronary heart disease.[22] As hair mercury increases, CIMT also increases.

Cadmium. The 1999 to 2006 NHANES trial found a 50% increase in blood cadmium was associated with a 35% increase in the risk of stroke and a 48% increased risk for heart failure.[23] Swedish adults (46–67 years of age) with blood cadmium levels in the fourth quartile were 80% more likely to have an acute coronary event and 90% more likely to have a stroke.[24]

Other Risk Factors

In addition to the major risk factors for CVD (e.g., smoking, elevations in cholesterol, elevated blood pressure, diabetes, and physical inactivity/obesity), a number of other factors have, on occasion, been shown to be more significant. See Box 149.2 for a list of several of the more than 300 different risk factors that have been identified.

Although there is considerable evidence that all these risk factors can play significant roles in the pathogenesis of atherosclerosis, much of the research has focused on the central roles of inflammatory processes and insulin resistance.[25] Inflammatory mediators influence many stages of atheroma development, from initial leukocyte recruitment to eventual rupture of the unstable atherosclerotic plaque. In particular, C-reactive protein (CRP), an acute-phase reactant that reflects different degrees of inflammation, has been identified as an independent risk factor for CAD. Although the CRP level has been shown to be a stronger predictor of cardiovascular events than the LDL-C level, screening for both biological markers provides better prognostic information than screening for either alone.[26]

Elevations in CRP are closely linked to insulin resistance and the metabolic syndrome.[27] The diagnostic criteria for the metabolic syndrome comprise the presence of at least three of the following metabolic risk factors in one person:

- Central obesity (a waist-to-hip ratio >1 for men and >0.8 for women)
- Atherogenic dyslipidemia (mainly triglycerides >150 mg/dL and low HDL-C [<40 mg/dL in men and <50 mg/dL in women])
- Hypertension (≥130/85 mm Hg)
- Insulin resistance or glucose intolerance (fasting blood sugar levels >101 mg/dL or HbA_{1c} 5.7%–6.4%)
- Prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor in the blood)
- Proinflammatory state (e.g., elevated high-sensitivity CRP in the blood)

The metabolic syndrome has become increasingly common in the United States, affecting more than 60 million U.S. adults. Insulin

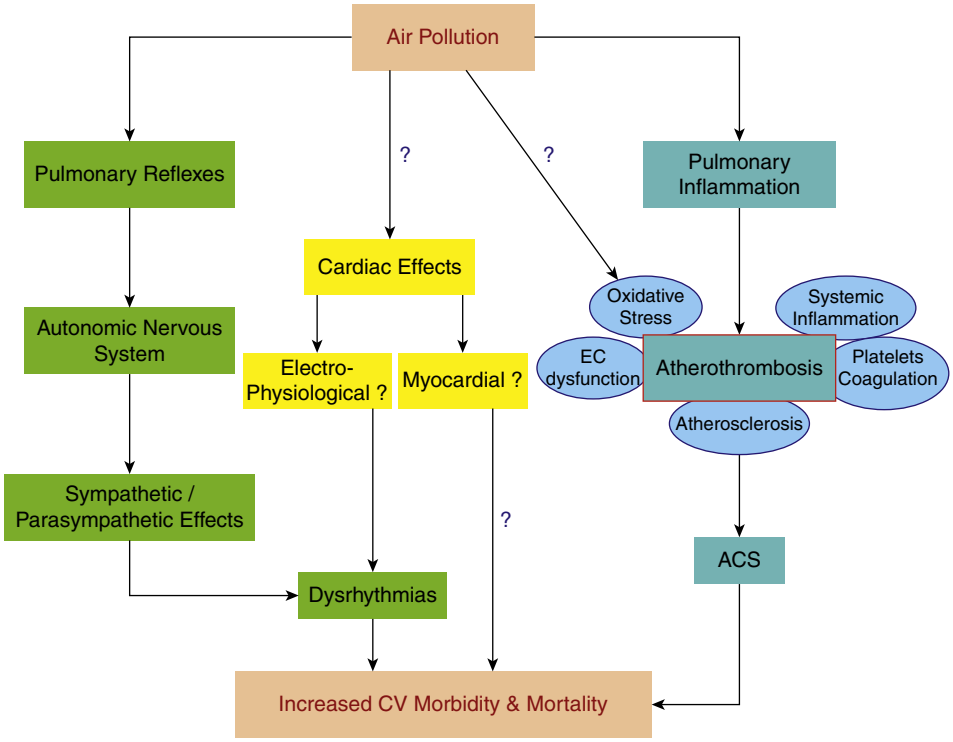


Fig. 149.2 Possible mechanisms that link air pollution with increased cardiovascular morbidity and mortality. (From Araujo JA, Nel AE. Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. *Part Fibre Toxicol.* 2009;6:24.)

resistance and metabolic syndrome are closely tied to obesity (particularly abdominal obesity), elevations in CRP, and a significant risk for CAD.

THERAPEUTIC CONSIDERATIONS—GENERAL GUIDELINES

Prevention of a heart attack or stroke involves reducing risk factors. The major risk factor of smoking was discussed earlier, and obesity, physical inactivity, diabetes, and hypertension are detailed in separate chapters. Significant evidence shows that simply adopting a healthy diet and lifestyle dramatically reduces CVD-related mortality. In a prospective trial enrolling more than 20,000 men and women, it was found that the combination of four healthy behaviors (nonsmoking, not physically inactive, moderate alcohol intake, and a plasma vitamin C indicative of at least 5 servings of fruit and vegetables per day) reduced total mortality fourfold compared with those who had none of these behaviors.[28]

Diet—General Guidelines

The dietary program detailed in Chapter 44 features a comprehensive dietary approach for both the prevention and treatment of CVD as well as the improvement of blood lipid profiles. It is important to reduce the intake of saturated fat and trans fatty acids and to increase the consumption of vegetables, fruit, dietary fiber, monounsaturated fats, and omega-3 fatty acids. One important dietary goal is improving the structure and composition of cell membranes by incorporating essential structural components, such as monounsaturated and omega-3 fatty acids, and by preventing oxidative and free radical damage to these structures by consuming a high level of antioxidants and phytochemicals.

Mediterranean Diet

One of the most widely studied dietary interventions in CAD is the traditional "Mediterranean diet,"[29] which reflects food patterns typical in some Mediterranean regions in the early 1960s. Unfortunately, the modern Mediterranean diet has deviated significantly from its healthful origin.

The original Mediterranean diet had the following characteristics:

- Olive oil is the principal source of fat.
- The diet centers on an abundance of plant food (fruits; potatoes, beans, and other vegetables; breads; pasta; nuts; and seeds).
- Foods are minimally processed, and people focus on seasonally fresh and locally grown foods.
- Fresh fruit is the typical daily dessert, with sweets containing concentrated sugars or honey consumed only a few times a week at the most.
- Dairy products (principally cheese and yogurt) are consumed daily in low to moderate amounts.
- Fish is consumed regularly.
- Poultry and eggs are consumed in moderate amounts (up to four times a week) or not at all.
- Red meat is consumed in low amounts.
- Wine is consumed in low to moderate amounts, normally with meals.

In one study, the effect of the Mediterranean diet on endothelial function and vascular inflammatory markers was studied in patients with metabolic syndrome.[30] Patients in the intervention group were instructed to follow the Mediterranean diet and received detailed advice on how to increase their daily consumption of whole grains, fruits, vegetables, nuts, and olive oil; patients in the control group followed the American Heart Association (AHA) diet. After 2 years, patients following the Mediterranean diet consumed more foods rich in monounsaturated fat, polyunsaturated fat, and fiber and had a lower

ratio of omega-6 to omega-3 fatty acids. In comparison with patients consuming the control diet, patients consuming the intervention diet had significantly reduced serum concentrations of CRP and other inflammatory mediators, improved endothelial function, and greater weight loss.

A study in elderly individuals analyzed the relationship between polyphenol intake from a Mediterranean diet and circulating inflammatory biomarkers and cardiovascular risk factors.[31] A total of 1139 high-risk participants were randomly assigned to a low-fat control diet or to two Mediterranean diets, supplemented with either extra virgin olive oil or nuts. Results showed increases in polyphenol intake measured as urinary total urinary polyphenol excretion (TPE) were associated with decreased inflammatory biomarkers, suggesting a dose-dependent anti-inflammatory effect of polyphenols. In addition, systolic and diastolic blood pressure (BP) decreased and plasma high-density lipoprotein cholesterol increased in parallel with increasing urinary TPE.

Although several components of the Mediterranean diet deserve special mention, it is important to stress that the total benefits reflect an interplay among many beneficial compounds rather than any single factor.[32]

Although the consumption of seafood has been shown to have beneficial effects on cardiovascular risk, these benefits can be challenged by the toxic metal content of the seafood. A cross-sectional study explored the association of seafood consumption and its estimated toxic metal contents (arsenic [As], cadmium [Cd], mercury [Hg], and lead [Pb]) with the lipid profile and lipid oxidation biomarkers in adults from a Spanish Mediterranean area without risk factors for CVD.[33] Researchers observed a mean seafood consumption of 74.9g per day (95% CI: 59.9–89.9), including 22.7g of shellfish per day (95% CI: 13.5–31.9) with moderate adherence to the Mediterranean diet. The estimated toxic metal contents were: 21.12 μg/kg per week As, 0.57 μg/kg per week InAs, 0.15 μg/kg per week Cd, 1.11 μg/kg per week Hg and 0.28 μg/kg per week Pb. An increase in shellfish consumption was associated with increases in the levels of LDLc, non-HDLc, APOB/A, and plasma oxLDL. The study authors concluded that in adults without risk factors for CVD, increasing shellfish consumption, even by a moderate amount, could favor a proatherogenic lipid profile and a higher level of oxidized LDL.

In addition, it is critical to follow a low-glycemic diet. A prospective trial with more than 48,000 participants highlighted the importance of good glycemic control for preventing CVD. Following a study population for an average of 8 years, researchers found that the consumption of foods with a high glycemic load increased the risk of CHD in women by 68%; women in the highest glycemic-load quartile had a relative risk of 2.2 for CHD compared with those in the lowest quartile.[34]

Olive Oil and Omega-3 Fatty Acids

One of the most important aspects of the Mediterranean diet may be the combination of olive oil (a source of monounsaturated fats and antioxidants) and the intake of omega-3 fatty acids. Olive oil consists not only of monounsaturated fatty acid (oleic acid), but also of several antioxidant agents that may also account for some of its health benefits. In addition to a mild effect in lowering LDL-C and triglycerides, olive oil increases the HDL-C level and helps prevent LDL-C from being damaged by free radicals.[35] Bioactive compounds in olive oil have also been shown to exhibit a potent capability to attenuate oxidative stress and improve endothelial function through their anti-inflammatory, antioxidant, and antithrombotic properties, therefore reducing the risk and progression of atherosclerosis.[36] Ironically, olive oil has often been inappropriately used as the placebo in trials of fish oils.

In addition to olive oil, the benefits of the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for cardiovascular health has been demonstrated in more than 300 clinical trials. These fatty acids exert considerable benefits in reducing the risk for CVD. Supplementation with EPA and DHA has little effect on cholesterol levels but does lower triglyceride levels significantly, as well as producing myriad additional beneficial effects, including reduced platelet aggregation; improved endothelial function and arterial flexibility; improved blood and oxygen supply to the heart; and a mild effect in lowering blood pressure by vasodilation and promotion of sodium excretion.[37]

The levels of EPA and DHA within red blood cells have been shown to be highly significant predictors of heart disease and is known as the omega-3 index. An omega-3 index of 8% is associated with the greatest protection, whereas an index of 4% is associated with the least protection. In one analysis, the omega-3 index was shown to be the most significant predictor of CAD compared with CRP; total cholesterol, LDL-C, or HDL-C; and homocysteine. Researchers subsequently determined that a total of a combined 1000 mg of EPA and DHA daily is required to achieve or surpass the 8% target of the omega-3 index.[38,39]

The findings with the omega-3 index are not surprising, because a wealth of information has documented a clear relationship between dietary intake of omega-3 fatty acids and the likelihood of developing CHD: the higher the omega-3 fatty acid intake, the lower the likelihood of CHD. It has been estimated that raising the levels of long-chain omega-3 fatty acids through diet or supplementation may reduce overall cardiovascular mortality by as much as 45%.[40,41]

In general, for preventive effects against CVD, the dosage recommendation is 1000 mg of EPA plus DHA a day; for lowering triglycerides, the dosage is 3000 mg. In a double-blind study, after 8 weeks of supplementation, a daily dosage of 3.4 g EPA plus DHA lowered triglycerides by 27%, but a lower dosage of 0.85 g had no effect. These results clearly indicate that the effective dosage for lowering triglycerides with fish oils requires dosages of 3 g EPA plus DHA a day.[42] In comparison with other triglyceride-lowering therapies, EPA has been found to inhibit cholesterol crystal formation, inflammation, and oxidative modification of atherogenic lipoprotein particles, thus interfering with the mechanisms of atherosclerosis, resulting in reduced numbers of cardiovascular events.[43]

Although the longer-chain omega-3 fatty acids exert more pronounced effects than alpha-linolenic acid (ALA), the shorter omega-3 fatty acid from vegetable sources, it is important to point out that the two populations with the lowest rates of heart attack have a relatively high intake of ALA: the Japanese population who inhabits Kohama Island and the inhabitants of Crete.[44,45] Typically, Cretans have a threefold higher serum concentration of ALA than members of other European countries, owing to their frequent consumption of walnuts and the vegetable purslane.[44] Of course, another important dietary factor in both the Kohamans and Cretans is their use of oleic acid–containing oils. However, although the oleic acid content of their diets offers some degree of protection, the rates of heart attack among the Kohamans and Cretans are much lower than rates in populations that consume only oleic acid sources and little ALA. The intake of ALA is viewed as a more significant protective factor than oleic acid.

Nuts and Seeds

Higher consumption of nuts and seeds has been shown to significantly reduce the risk of CVD in large epidemiological studies including the Nurses' Health Study, the Iowa Health Study, and the Physicians' Health Study.[46] Researchers estimate that substituting nuts for an equivalent amount of carbohydrates in an average diet resulted in a

30% reduction in heart disease risk. Researchers calculated an even more impressive risk reduction, 45%, when fat from nuts was substituted for saturated fats (found primarily in meat and dairy products). Nuts have a cholesterol-lowering effect, which partly explains this benefit, but they are also a rich source of arginine. By increasing nitric oxide levels, arginine may help to improve blood flow, reduce blood clot formation, and improve blood fluidity (i.e., the blood becomes less viscous and therefore flows through blood vessels more easily).

Walnuts appear to be especially beneficial because they are also a rich source of both antioxidants and ALA. In one study, hypercholesterolemic men and women were randomized to a cholesterol-lowering Mediterranean diet and a diet of similar energy and fat content in which walnuts replaced approximately 32% of the energy from monounsaturated fat (olive oil). Participants followed each diet for 4 weeks. In comparison with the Mediterranean diet, the walnut diet improved endothelial cell function (i.e., it increased endothelium-dependent vasodilation and reduced levels of vascular cell adhesion molecule-1). The walnut diet also significantly reduced total cholesterol (–4.4%) and LDL-C (–6.4%).[47]

Vegetables, Fruits, and Red Wine

An important contributor to the benefits noted with the Mediterranean diet is the focus on carotene- and flavonoid-rich fruits, vegetables, and beverages (e.g., red wine). Numerous population studies have repeatedly demonstrated that a higher intake of dietary antioxidants significantly reduces the risk of heart disease and stroke. Higher blood levels of antioxidant nutrients are also associated with lower levels of CRP.[48]

Two valuable sources of antioxidants in the Mediterranean diet are tomato products and red wine. Tomatoes are a rich source of the carotene lycopene. A systematic review and meta-analysis evaluated the effect of supplementing tomato and lycopene on CV risk factors and determined that the available evidence supports the view that increasing the intake of these has positive effects on blood lipids, blood pressure, and endothelial function.[49] In large clinical studies evaluating the relationship between carotene status and heart attack (acute MI), lycopene but not beta-carotene was shown to be protective. Lycopene exerts greater antioxidant activity compared with beta-carotene in general but specifically against LDL-C oxidation.[50]

The cardiovascular protection offered by red wine is popularly referred to as the "French paradox." Because the French population consumes more saturated fat than people in the United States and the United Kingdom do yet have a lower incidence of heart disease, red wine consumption has been suggested to be the reason. Presumably this protection is the result of flavonoids and other polyphenols in red wine that protect against oxidative damage to LDL-C as well as help reduce the levels of inflammatory mediators.[30,51] Moderate alcohol consumption alone has also been shown to be protective in some studies by exerting positive effects on ratios of HDL-C to LDL-C and CRP as well as levels of fibrinogen, although red wine typically exerts the most significant effects.[52]

Importantly, the effects of alcohol on CVD risk, morbidity, and total mortality are counterbalanced by alcohol's addictive and psychological effects.

The major benefits of red wine consumption in protecting against CVD may ultimately be the effects that the polyphenols have on improving endothelial cell function. In one study, 30 male patients with CHD were randomly assigned to take a red grape polyphenol extract (600 mg) or placebo. Flow-mediated dilation was measured after fasting and 30, 60, and 120 minutes after intake of the grape extract or placebo. The intake of the red grape polyphenol extract caused an increase in flow-mediated dilation, peaking at 60 minutes, which was significantly higher than the baseline values and the corresponding values at 60 minutes after the intake of placebo (4.52% vs. 2.64%).[53]

The consumption of green tea, like that of red wine, has also been shown in population studies to be associated with a reduced risk for CVD. As with red wine, much of the benefit of green tea consumption may be the result of several different mechanisms, including improving endothelial cell function. Green tea polyphenols (catechins) have been shown to decrease the oxidation of LDL-C, lower LDL-C levels, and improve the LDL-C:HDL-C ratio. In vitro studies have shown a dose-dependent response with green tea polyphenols in reducing several biomarkers associated with atherosclerosis and ischemia, including the inhibition of the endothelial cell–derived vascular cell adhesion molecule-1 as well as angiotensin II, platelet derived growth factor-BB, apolipoprotein E, and inducible nitric oxide synthase.[54]

Another mechanism of action for red wine and green tea polyphenols involves inhibiting the formation of new blood vessels within the vascular lesion. During the angiogenic process, new blood vessels develop from the existing microvascular bed. The initial event involves dilation of an existing blood vessel followed by an increase in vascular permeability and the degradation of extracellular matrices. This process allows endothelial cells to migrate into the lesion and proliferate, and these events are followed by the maturation of new blood vessels. The angiogenic process is controlled by two major proangiogenic factors: matrix metalloproteinases (MMPs), which degrade extracellular matrices, and vascular endothelial growth factor (VEGF), which strongly stimulates endothelial cell migration and proliferation and the formation of new blood vessels. Both red wine and green tea polyphenols have been shown to inhibit this process in vitro at concentrations conceivably achievable with oral intake.[54]

Foods and beverages rich in antioxidant content have shown benefit in fighting atherosclerosis. Pomegranate *(Punica granatum)* juice appears to be particularly useful. It is remarkably rich in antioxidants, such as soluble polyphenols, tannins, and anthocyanins. Animal research has indicated that components of pomegranate juice can retard atherosclerosis, reduce plaque formation, and improve arterial health. Human clinical studies have supported the role of pomegranate juice in benefiting heart health.[55–57]

In a randomized, placebo-controlled, double-blind study, the daily consumption of pomegranate juice for 3 months was evaluated for its effect on myocardial perfusion in 45 patients who had CHD and myocardial ischemia. Patients were randomly assigned into one of two groups: a pomegranate juice group (240 mL per day) or a placebo group, who drank a beverage of similar caloric content, amount, flavor, and color. Participants underwent electrocardiographic-gated myocardial perfusion single-photon emission computed tomographic scintigraphy at rest and during stress at baseline and 3 months. After 3 months, the extent of stress-induced ischemia decreased in the pomegranate group but increased in the control group. This benefit was observed without changes in cardiac medications, blood sugar, hemoglobin A_{1c}, weight, or blood pressure in either group.[55]

In another study, 10 patients with carotid artery stenosis (CAS) were evaluated to gauge the effect of pomegranate juice consumption on the progression of carotid lesions and changes in oxidative stress and blood pressure. The patients were supplemented with pomegranate juice (50 mL) for 1 year, and 5 patients continued for up to 3 years. In the control group (which did not consume pomegranate juice), common carotid intimal-medial thickness (IMT) increased by 9% during 1 year, whereas pomegranate juice consumption resulted in a significant IMT reduction, by up to 30%, after 1 year. In comparison with values obtained before pomegranate juice consumption, the patients' serum paraoxonase 1 (PON 1) activity was increased by 83%,

whereas serum LDL-C basal oxidative state and LDL-C susceptibility to copper ion–induced oxidation were both significantly reduced, by 90% and 59%, respectively, after 12 months. Furthermore, serum levels of antibodies against oxidized LDL-C were decreased by 19%; in parallel, serum total antioxidant status was increased by 130% after 1 year of pomegranate juice consumption. Systolic blood pressure was reduced after 1 year of pomegranate juice consumption by 21%. For all studied parameters, the maximal effects were observed after 1 year of pomegranate juice consumption.[56]

THERAPEUTIC CONSIDERATIONS—LOWERING CHOLESTEROL

Lowering total cholesterol (TC), as well as LDL-C and triglycerides (TG), is clearly associated with reducing CVD risk. Most of the benefits noted with lowering LDL-C are based on a large number of randomized clinical trials involving the use of the HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors known collectively as statin drugs. Statins owe their origin to red yeast *(Monascus purpureus)* fermented on rice. This traditional Chinese medicine has been used for its health-promoting effects in China for more than 2000 years. Red yeast rice is the source of a group of compounds known as monacolins (e.g., lovastatin, also known as monacolin K, one of the key monacolins in red yeast rice extract). The marketing of an extract of red yeast fermented on rice standardized for monacolin content as a dietary supplement in the United States caused controversy in 1997, because this agent contained a natural source of a prescription drug. The U.S. Food and Drug Administration (FDA) eventually ruled that red yeast rice products could be sold only if they were free of monacolin.

Although the data are clear that statin drugs can produce decreases in total mortality, cardiovascular events, hospitalizations, and the need for revascularization procedures in high-risk patients, the debate remains whether statin therapy represents the optimal treatment approach to the primary prevention of CAD in patients with only the risk factor of elevated LDL-C, especially in light of the growing acceptance of risk factors like CRP and the emerging role of nutrition.[58,59] For example, one study compared the "portfolio diet," comprising plant-based cholesterol-lowering foods, with lovastatin.[60] The participants were randomly assigned to undergo one of three interventions on an outpatient basis for 1 month: a diet low in saturated fat, based on milled whole-wheat cereals and low-fat dairy foods; the same diet plus lovastatin, 20 mg per day; or a diet high in plant sterols (1 g/1000 kcal), soy protein (21.4 g/1000 kcal), viscous fibers (9.8 g/1000 kcal), and almonds (14 g/1000 kcal). The control, statin, and dietary portfolio groups had mean (SE) decreases in LDL-C of 8%, 30.9%, and 28.6%, respectively. Respective reductions in CRP were 10%, 33.3%, and 28.2%. This study and subsequent studies showed that by diversifying the cholesterol-lowering components in the same dietary portfolio, the effectiveness of diet as a treatment of hypercholesterolemia was increased. Moreover, results comparable with those of a statin drug (with lipid-lowering effects similar to statins in terms of both LDL-C and size of LDL-C) were produced.[61,62]

Although individual dietary changes may produce benefit in improving blood lipids, the best clinical approach is to incorporate a broad-spectrum dietary approach that incorporates a wide array of dietary components shown to have a positive effect on lipid levels. For example, although a meta-analysis of 27 randomized controlled trials in which isolated soy protein supplementation was the only intervention demonstrated that soy protein supplementation was associated with a significant dose-dependent reduction in mean serum total cholesterol, LDL-C, and triglycerides, the effect shown (–5.26 mg/dL, –4.25 mg/dL, and –6.26 mg/dL, respectively) was less than when the soy protein was used in conjunction with other dietary interventions.[63] In addition, the effects of isolated soy protein appear to be considerably less than those achieved by increasing soy food consumption in general. Much of the cholesterol-lowering effect of soy foods may relate more to the isoflavone and soluble fiber content than to the protein. In an earlier meta-analysis based more on soy protein intake from other sources in addition to soy protein isolate, reductions were much more significant for total cholesterol (–23.2 mg/dL), LDL-C (–21.7 mg/dL), and triglycerides (–13.3 mg/dL) but still relatively modest from a clinical perspective.[64] Although these results do support the recommendation to increase soy protein intake in the dietary approach to hypercholesterolemia, given the relatively small effect of soy protein on lipids, it is imperative that other dietary recommendations also should be promoted, such as reducing the dietary intake of saturated fat, trans-fatty acids, and cholesterol, as well as increasing the dietary intake of monounsaturated fats, soluble fiber, and nuts.

Despite research documenting the benefits of nonpharmacological approaches, it is unlikely that lowering LDL-C with statin drugs will be supplanted as the primary therapy in lipid management and the prevention of CAD at any time in the near future. In 2011 it was estimated that more than one of every six adults—nearly 40 million people—was taking a statin drug to lower LDL-C. Therefore the focus with many patients will be on the support of statin therapy. For example, it appears that individuals taking statin drugs must supplement with coenzyme Q_{10} (CoQ_{10}). For the synthesis of cholesterol, not only is HMG-CoA reductase required, but CoQ10 is as well. Thus administration of these drugs might compromise the status of CoQ_{10} by decreasing its synthesis. Even modest dosages of various statins have been shown to lower serum levels of CoQ_{10}. Researchers have concluded that inhibition of CoQ_{10} synthesis by statin drugs could explain the most commonly reported adverse effects, especially fatigue and muscle pain, as well as more serious side effects such as rhabdomyolysis.[65,66] CoQ_{10} supplementation in subjects on statin drugs has also been shown to reduce markers of oxidative damage.

Statins are also gaining popularity as a prescription method to lower CRP. In one study, a group of 186 individuals with type 2 diabetes was selected to receive 10 mg of atorvastatin (Lipitor), 80 mg of atorvastatin, or a placebo for 30 weeks. In individuals given placebo, CRP levels increased by 6.6%, decreased by 15% in the 10-mg group, and by 47% in the 80-mg group. In a study with pravastatin, 40 mg daily lowered CRP levels by 13%.[67]

The Importance of Soluble Dietary Fiber in Lowering Cholesterol

Chapter 132 provides a complete discussion of the benefits of dietary fiber. It is well established that the soluble dietary fiber found in legumes, fruit, and vegetables is effective in lowering cholesterol levels.[68] The greater the degree of viscosity or gel-forming nature, the greater the effect a particular dietary fiber has on lowering cholesterol levels. Highly viscous, soluble fiber blends are showing greater effect than previously used fiber sources, leading to more reasonable dosage recommendations (the cholesterol-lowering effect of soluble fiber is clearly dose-dependent).[69,70] Table 149.3 shows the average dosages and reductions noted in clinical trials with soluble fiber supplements.

Many of the studies featured oat preparations containing either oat bran or oatmeal.[71] The overwhelming majority of these studies demonstrated that individuals with high cholesterol levels see significant reductions with frequent oatmeal or oat bran consumption. In contrast, individuals with normal or low cholesterol levels see little change. In individuals with high cholesterol levels (above 200 mg/dL), the consumption of the equivalent of 3 g of soluble oat fiber typically lowers total cholesterol by 8% to 23%. This finding is highly

TABLE 149.4 Comparison of Niacin With Lovastatin

Lipoprotein	Group	Week 10	Week 18	Week 26
LDL-C reduction	Lovastatin	26%	28%	32%
	Niacin	5%	16%	23%
HDL-C increase	Lovastatin	6%	8%	7%
	Niacin	20%	29%	33%
Lp(a) lipoprotein reduction	Lovastatin	0%	0%	0%
	Niacin	14%	30%	35%

HDL-C, High-density-lipoprotein cholesterol; *LDL-C,* low-density-lipoprotein cholesterol.

TABLE 149.3 Effect of Various Sources of Fiber on Serum Cholesterol Levels

Fiber	Dosage (g)	Typical Reduction in Total Cholesterol
Oat bran (dry)	50–100	20%
Guar gum	9–15	10%
Pectin	6–10	5%
Psyllium	10–20	10–20%
Vegetable fiber	27	10%

significant, because with each 1% drop in serum cholesterol level, there is a 2% decrease in the risk of developing heart disease. One bowl of ready-to-eat oat bran cereal or oatmeal contains approximately 3 g of fiber. Although oatmeal's fiber content (7%) is less than that of oat bran (15%–26%), it has been determined that the polyunsaturated fatty acids contribute as much to the cholesterol-lowering effects of oats as the fiber content. Although oat bran has a higher fiber content, oatmeal is higher in polyunsaturated fatty acids.

In an effort to lower cholesterol with dietary fiber, patients should be encouraged to eat 35 g of fiber daily from fiber-rich foods, a full listing of which can be found in Chapter 132. Achieving higher fiber intake is associated not only with lower cholesterol levels but also with lower inflammatory mediators like CRP.[72]

Natural Products to Lower Cholesterol Levels

In many cases, dietary therapy, although important, is not sufficient alone to reduce lipid levels to the desired ranges. Fortunately, several natural compounds can lower cholesterol levels and other significant risk factors for CAD. In fact, when all factors are considered (e.g., cost, safety, effectiveness), the natural alternatives presented here may offer significant advantages to the standard drug therapy.

Niacin

Since the 1950s niacin (vitamin B_3) has been known to be effective in lowering blood cholesterol levels. In the 1970s the famed Coronary Drug Project demonstrated that niacin was the only cholesterol-lowering agent to actually reduce overall mortality. Niacin typically lowers LDL-C levels by 16% to 23% and raises HDL-C levels by 20% to 33%. These effects, especially the effect on HDL-C, compare quite favorably with those of conventional cholesterol-lowering drugs.[73,74]

It is now known that niacin does much more than lower total cholesterol. Specifically, niacin has been shown to lower LDL-C, the more harmful Lp(a) lipoprotein, as well as triglyceride, CRP, and fibrinogen levels and simultaneously raise levels of beneficial HDL-C. Although niacin has demonstrated better overall results in reducing risk factors for CHD compared with other cholesterol-lowering agents, physicians are often reluctant to prescribe niacin. The reason is a widespread perception that niacin is difficult to work with because of the bothersome flushing of the skin associated with its use. In addition, because niacin is a widely available "generic" agent, no pharmaceutical company stands to generate the huge profits that the other lipid-lowering agents have brought them. As a result, niacin does not benefit from the intensive advertising that focuses on the statin drugs. Despite the advantages of niacin over other lipid-lowering agents, it accounts for less than 10% of all cholesterol-lowering prescriptions.

Several studies have compared niacin with standard lipid-lowering drugs including the statin drugs. These studies have shown significant advantages for niacin. In the first published clinical study comprising 136 patients, niacin was compared with lovastatin directly in the following groups of subjects: (1) those with LDL-C levels greater than 160 mg/dL and CHD, (2) those with more than two CHD risk factors, (3) those with both elevated LDL-C levels and more than two CHD risk factors, (4) those with LDL-C levels greater than 190 mg/dL and without CHD, and (5) those with fewer than two CHD risk factors.[75] This controlled, randomized, open-label, 26-week study was performed at five lipid clinics. Patients were first placed on a 4-week diet run-in period, after which eligible patients were randomly assigned to receive treatment with either lovastatin (20 mg per day) or niacin (1.5 g per day). On the basis of the LDL-C response and patient tolerance, the doses were sequentially increased to 40 and 80 mg per day of lovastatin or 3 and 4.5 g per day of niacin after 10 and 18 weeks of treatment, respectively. In the two patient groups, 66% of patients treated with lovastatin and 54% of patients treated with niacin underwent full dosage titration. Table 149.4 shows the results.

These results indicate that, although lovastatin produced a greater reduction in LDL-C, niacin provided better overall results despite the fact that fewer patients were able to tolerate a full dosage of niacin because of skin flushing. The percentage increase in HDL-C, a more significant indicator for CHD, was dramatically in favor of niacin (33% vs. 7%). Equally impressive was the percentage decrease in Lp(a) for niacin. Although niacin produced a 35% reduction in Lp(a) levels, lovastatin did not produce any effect. Niacin's effect on Lp(a) in this study confirmed a previous study showing that niacin (4 g per day) reduced Lp(a) levels by 38%. Moreover, a subsequent study showed similar reductions in Lp(a) in patients with diabetes.[76,77]

Another comparative study evaluated the lipoprotein responses to niacin, gemfibrozil, and lovastatin in patients with normal total cholesterol levels but low levels of HDL-C.[78] The first phase of the study compared lipoprotein responses with lovastatin and gemfibrozil in 61 middle-aged men with low levels of HDL-C. In the second phase, 37 patients agreed to take niacin; 27 patients finished this phase at a dose of 4.5 g per day. In the first phase, gemfibrozil therapy increased HDL-C levels by 10% and lovastatin did so by 6%. In the second phase, niacin therapy was shown to raise HDL-C by 30%.

Another comparative study involved niacin versus atorvastatin (Lipitor).[79] The average dosage was 3000 mg for niacin and 80 mg for atorvastatin. The patients selected had abnormal particle size of LDL-C in that the molecules were small and dense—these LDL-C molecules are considerably more atherogenic than larger, less dense LDL-Cs. The patients selected also had low levels (<40%) of a specific fraction of HDL-C associated with a greater protective effect than HDL-C alone. Although atorvastatin reduced total LDL-C levels substantially more than niacin, niacin was more effective in increasing LDL-C particle size and raising HDL-C and HDL2-C than atorvastatin (Table 149.5).

Because taking niacin at higher dosages (e.g., ≥3000 mg) can impair glucose tolerance, many physicians have avoided niacin therapy in

TABLE 149.5 The Effects of Atorvastatin (Lipitor) and Niacin on Lipid Profiles

	ATORVASTATIN		NIACIN		ATORVASTATIN + NIACIN	
Parameter	**Before**	**After**	**Before**	**After**	**Before**	**After**
Total LDL-C (mg/dL)	110	56	111	89	123	55
LDL-C peak diameter	251	256	253	263	250	263
Lipoprotein(a) (mg/dL)	45	44	37	23	54	35
HDL-C (mg/dL)	42	43	38	54	38	54
HDL2-C (%)	30	42	29	43	32	37
Triglycerides (mg/dL)	186	100	194	108	235	73

HDL-C, high-density-lipoprotein cholesterol; *LDL-C,* low-density-lipoprotein cholesterol.

diabetic patients, but newer studies with slightly lower dosages (1000–2000 mg) of niacin have not shown it to adversely affect blood sugar regulation.[80] For example, during a 16-week, double-blind, placebo-controlled trial, 148 patients with type 2 diabetes were randomized to placebo or 1000 or 1500 mg per day of niacin; in the niacin-treated groups, there was no significant loss of glycemic control, and the favorable effects on blood lipids were still apparent.[81] Other studies have actually shown hemoglobin A_{1C} to drop, indicating improvement in blood sugar control.[82]

The most common blood lipid abnormalities in patients with type 2 diabetes are elevated triglyceride levels, decreased HDL-C levels, and a preponderance of smaller, denser LDL-C particles. Niacin has been shown to address all of these areas more significantly than the statins or other lipid-lowering drugs.

In addition to lowering cholesterol and triglycerides, niacin exerts additional benefits in battling atherosclerosis. Specifically, niacin produces beneficial lipid-altering effects on particle distribution in patients with CAD that are not well reflected in typical lipoprotein analysis. In addition, systemic markers of inflammation decrease in patients receiving niacin. In one study, when a modest dosage of niacin (1000 mg daily) was added to existing therapy for 3 months in 54 subjects with stable CAD, participants experienced a 32% increase in large-particle HDL-C, an 8% decrease in small-particle HDL-C, an 82% increase in large-particle LDL-C, and a 12% decrease in small-particle LDL-C.[83] Niacin therapy also decreased lipoprotein-associated phospholipase A2 and CRP levels (20% and 15%, respectively). No significant changes from baseline were seen in any tested parameter in subjects who received placebo. These results indicate that the addition of niacin to existing medical regimens for patients with CAD and already well-controlled lipid levels improves the distribution of lipoprotein particle sizes and inflammatory markers in a manner expected to improve protection against a cardiovascular event.

Although niacin exerts significant benefit on its own, it does not appear to enhance the benefits of statins in well-controlled patients. The AIM-HIGH study funded by the National Heart, Lung, and Blood Institute recruited 3400 patients who were at risk for heart trouble despite the fact that their LDL-C was under control (<70 mg/dL) with the use of a statin drug [simvastatin (Zocor)]. The study ended 18 months early because no additional cardiovascular benefit was found in those taking niacin despite increases in HDL-C and reductions in triglycerides.[84]

In the Carotid Plaque Composition Study, 126 subjects with a history of CVD were randomized to atorvastatin or a combination of atorvastatin and niacin to evaluate the relationship between these therapies and the concentration of high-density lipid particles (HDL-p) and cholesterol efflux capacity (i.e., the ability of serum HDL [serum depleted of apolipoprotein B-containing lipoproteins] to remove cholesterol from macrophages).[85] Atorvastatin significantly reduced LDL-C levels by 39% (−65 mg/dL, $P<0.0001$) and apoB by 35% (−47 mg/dL, $P<0.0001$). Adding niacin to atorvastatin reduced both LDL-C and apoB levels by an additional 11% (−83 mg/dL and −63 mg/dL, respectively). After 1 year of treatment with atorvastatin, HDL-C levels increased by 11%, and combination therapy raised HDL-C by an additional 18%. Combination therapy also increased macrophage cholesterol efflux capacity but failed to improve ATP-binding cassette transporter A1 (ABCA1)-specific cholesterol efflux capacity, which may explain why clinical trials have shown that although niacin increases HDL-C levels, it does not reduce cardiac risk.

The side effects of niacin. The side effects of niacin are well known. The most common and bothersome side effect is the skin flushing that typically occurs 20 to 30 minutes after the niacin is taken. Other occasional side effects of niacin include gastric irritation, nausea, and liver damage. In an attempt to combat the acute reaction of skin flushing, several manufacturers began marketing "sustained-release," "timed-release," or "slow-release" niacin products. These formulations allow the niacin to be absorbed gradually, thereby reducing the flushing reaction. However, although these forms of niacin reduce skin flushing, earlier timed-release preparations proved to be more toxic to the liver than regular niacin. In one analysis, 52% of the patients taking the earlier sustained-release niacin preparations developed liver toxicity, whereas none of the patients taking immediate-release niacin did so.[86] "Intermediate-release" preparations appear to have solved this problem, as relatively large clinical trials have shown them to be extremely well tolerated even when combined with statin drugs.[87–89]

The safety and tolerability of intermediate-release niacin preparation have been evaluated in a multicenter study of 566 patients.[90] The target dose was achieved by 65% of patients. Flushing was the most common side effect (42%), as expected, and 9.7% withdrew because of this. Other drug-related adverse reactions occurred at low frequency (18.6%), and 8.7% withdrew for an adverse reaction other than flushing. Most adverse reactions were mild or moderate in severity. There was no hepatotoxicity or serious adverse event involving muscle. The researchers concluded that intermediate-release nicotinic acid was well tolerated, and these results support its use in the management of patients with an increased cardiovascular risk due to low HDL-C.

Inositol hexaniacinate has long been used in Europe to lower cholesterol levels and to improve blood flow in intermittent claudication. It yields slightly better clinical results than standard niacin but is much better tolerated in terms of both flushing and, more importantly, long-term side effects.[91,92]

Regardless of the form of niacin being used, periodic checking (at least every 3 months) of cholesterol and liver function is indicated. Niacin should not be used in patients with preexisting liver disease or elevations in liver enzymes. For these patient groups, policosanol, garlic, or pantethine are recommended.

For best results, niacin should be taken at night, because most cholesterol synthesis occurs during sleep. If pure crystalline niacin is being used, it should begin with a dosage of 100 mg a day and be carefully increased over 4 to 6 weeks to the full therapeutic dosage of 1.5 to 3 g per day. If a timed-released preparation or inositol hexaniacinate is being used, a 500-mg dose should be given at night and increased to 1500 mg after 2 weeks. If after 1 month of therapy the dosage of 1500 mg per day fails to effectively lower LDL-C, the dosage should be increased to 3000 mg.

Plant Sterols and Stanols

Phytosterols and phytostanols are structurally similar to cholesterol and can act in the intestine to lower cholesterol absorption by displacing cholesterol from intestinal micelles. Because phytosterols and phytostanols are poorly absorbed themselves, blood cholesterol levels will likely drop owing to increased excretion. These compounds are showing up in functional foods (e.g., margarine and other spreads, orange juice), as well as in dietary supplements.[93]

Phytosterols and phytostanols are effective in lowering LDL-C in some people. A meta-analysis of 41 trials showed that a daily intake of 2 g of stanols or sterols reduces LDL-C by 10%.[93] Administration of higher dosages produced little additional benefit. Effects of phytosterols and phytostanols are additive with diet or drug interventions: Eating foods low in saturated fat and cholesterol and high in stanols or sterols can reduce LDL-C by 20%; adding sterols or stanols to statin medication is more effective than doubling the statin dose alone. Individuals most likely to respond have been identified as having high cholesterol absorption and low cholesterol biosynthesis. Phytosterols and phytostanols have been shown to have antiplatelet and antioxidant effects as well.[94–97]

Phytosterol or phytostanol intake at higher dosages may reduce carotenoid absorption. Human subjects consuming 6.6 g per day phytosterols showed cholesterol-adjusted plasma reduction of alpha- and beta-carotene levels (–19% to –23%), lutein (–14%), and lycopene (–11%). However, this effect was partially reversed by increased fruit and vegetable intake.[98]

Pantethine

Pantethine is the stable form of pantetheine, the active form of vitamin B_5 or pantothenic acid. Pantothenic acid is the most important component of coenzyme A (CoA). This enzyme is involved in the transport of fats to and from cells, as well as to the energy-producing compartments in cells. Without coenzyme A, the cells' fats could not be metabolized to energy.

Pantethine has significant lipid-lowering activity, whereas pantothenic acid has little if any effect in lowering cholesterol and triglyceride levels owing to pantethine's ability to be converted to cysteamine. Pantethine administration (standard dosage 900 mg per day) has been shown to significantly reduce serum triglyceride (−32%), total cholesterol (−19%), and LDL-C (−21%) levels and to increase HDL-C (+23%) levels.[99,100] It appears to be especially useful in lowering blood lipids in diabetic patients.[101–103]

The lipid-lowering effects of pantethine are most impressive when its toxicity (virtually none) is compared with that of conventional lipid-lowering drugs. Its mechanism of action is due to the inhibition cholesterol synthesis and acceleration of the use of fat as an energy source.

Garlic (*Allium sativum*) and Onion (*Allium cepa*)

Garlic offers significant protection against heart disease and stroke by lowering blood cholesterol levels even in apparently healthy individuals. According to the results of numerous double-blind placebo-controlled studies in patients with initial cholesterol levels above 200 mg/dL, supplementation with commercial preparations providing a daily dose of at least 10 mg of alliin or a total allicin potential of 4000 mg can lower total serum cholesterol levels by about 10% to 12% and LDL-C by about 15%. HDL-C usually increases by about 10%, and triglyceride levels typically drop by 15%. However, most trials using lower dosages of allicin fail to produce a lipid-lowering effect.[104–108]

Although the effects of supplemental garlic preparations on cholesterol levels are modest, the combination of lowering LDL-C and raising HDL-C can greatly improve the HDL-C:LDL-C ratio, a significant goal in the prevention of heart disease and stroke. Garlic preparations have also demonstrated blood pressure–lowering effects, inhibition of platelet aggregation, reduction of plasma viscosity, promotion of fibrinolysis, prevention of LDL-C oxidation, and an ability to exert positive effects on endothelial function, vascular reactivity, and peripheral blood flow (see Chapter 51 for more information).

TABLE 149.6 Comparative Effects on Blood Lipids of Several Natural Compounds

	Niacin	Garlic	Policosanol	Pantethine
Total cholesterol (% decrease)	18	10	24	19
LDL-C (% decrease)	23	15	25	21
HDL-C (% increase)	32	31	15	23
Triglycerides (% decrease)	26	13	5	32

HDL-C, high-density-lipoprotein cholesterol; *LDL-C,* low-density-lipoprotein cholesterol.

Comparing Natural Cholesterol-Lowering Agents

Numerous natural compound products can effectively improve cholesterol and triglyceride levels. Of the several described earlier, niacin produces the best overall effect. However, the others do have a place in the clinical management of hyperlipidemia and the prevention of atherosclerosis (Table 149.6).

Typically, along with dietary and lifestyle recommendations, niacin (1500–3000 mg at night) reduces total cholesterol by 50 to 75 mg/dL within the first 2 months in patients with initial total cholesterol levels above 250 mg/dL. In patients with initial cholesterol levels above 300 mg/dL, it may take 4 to 6 months before cholesterol levels begin to reach recommended levels. Once cholesterol levels are reduced below 200 mg/dL, the dosage of niacin is reduced by 500 mg for 2 months. If the cholesterol levels creep up above 200 mg/dL, the dosage of niacin is raised back up to previous levels. If the cholesterol level remains below 200 mg/dL, then the niacin is dropped by another 500 mg and the cholesterol levels are rechecked in 2 months, with niacin therapy at previous levels if levels have exceeded 200 mg/dL. Continue with this reduction in dosage until niacin can be stopped entirely and cholesterol levels remain below 200 mg/dL.

Pantethine is recommended primarily to patients who have HTG, especially patients with diabetes. As stated earlier, pantethine has demonstrated excellent effects in diabetics. It not only improves cholesterol and triglyceride levels but also normalizes platelet lipid composition and function as well as blood viscosity.

With regard to elevations in Lp(a), both niacin and vitamin C have shown an ability to reduce Lp(a) levels dramatically (35% and 27% reductions, respectively). In addition, it is important to rule out low thyroid function (hypothyroidism) in all cases of elevated blood lipids, especially elevated Lp(a).

It is well established that patients with overt hypothyroidism are prone to CAD because of increased LDL-C and decreased HDL-C. What is not as well established is the significance of "subclinical" hypothyroidism in terms of risk for cardiovascular mortality. One study demonstrated that patients with subclinical hypothyroidism (defined in the study as normal T3 and free thyroxine index with a raised TSH) were shown to have significantly elevated levels of LDL-C as well as elevated Lp(a).[109] For more information, see Chapter 180.

THERAPEUTIC CONSIDERATIONS—ANTIOXIDANT STATUS

Dietary antioxidant nutrients like lycopene, lutein, selenium, vitamin E, and vitamin C have been shown in epidemiological studies to offer significant protection against the development of CVD. Fats and cholesterol are particularly susceptible to free radical damage. When damaged, fats and cholesterol form lipid peroxides and oxidized cholesterol, which can then damage the artery walls and accelerate the progression of atherosclerosis. Antioxidants block the formation of these damaging compounds.

Although diets rich in antioxidant nutrients have consistently shown tremendous protection against CVD, clinical trials using antioxidant vitamins and minerals have produced inconsistent results.[110,111] The human antioxidant system represents a complex scenario of interacting components, which may explain the inconsistent findings. It is unlikely that any single antioxidant would prove to be effective, especially in the absence of a supporting cast. Most antioxidants require some sort of "partner" antioxidant, allowing them to work more efficiently. The most salient example of this is the partnership between the two primary antioxidants in the human body—vitamins C and E. Vitamin C is an "aqueous phase" antioxidant, and vitamin E is a "lipid phase" antioxidant. Although some studies have shown that supplementation with these nutrients reduces atherosclerotic lesions, more protection is likely required to ensure optimal effect.[112]

In addition to vitamin C, vitamin E also requires selenium and CoQ_{10} to work efficiently. Further adding to the shortcomings of many of the studies on antioxidant nutrients is the lack of consideration of the importance of phytochemicals and plant-derived antioxidants that, in addition to exerting benefit on their own, are well known to potentiate the activities of vitamin and mineral antioxidants. Phytochemicals like carotenes (especially lycopene and lutein) and flavonoids are especially important in fighting against free-radical damage. Most scientific reviews on antioxidant supplements devote significant attention to the studies using beta-carotene, because they have involved more than 70,000 subjects, but such studies fail to differentiate the facts that synthetic beta-carotene was used, and that beta-carotene is of little importance in protecting against LDL-C oxidation. (Unlike lycopene and lutein, beta-carotene does not become incorporated into LDL-C effectively, although it may help protect the endothelium.)

Lutein may be one of the most significant carotenes in the battle against atherosclerosis. Based on analysis of the different subtypes of LDL-C, lycopene, beta-carotene, and cryptoxanthin were mainly located in the larger, less dense LDL-C particles, whereas lutein and zeaxanthin were found preferentially in the smaller, denser LDL-C particles.[113] Because the smaller, denser LDL-C subtype is most easily oxidized, lutein and zeaxanthin are particularly important in protecting against damage to LDL-C.

The support of nonantioxidant vitamins and minerals may also be important in supporting the effectiveness of antioxidants. Taking a multivitamin/multimineral supplement seems appropriate. In one double-blind study, CRP levels were significantly lower in the multivitamin group than in the placebo group, with the reduction in CRP levels most evident in patients who had elevated levels (≥1 mg/L) at baseline.[114] Researchers found that serum vitamin B_6 and vitamin C levels were inversely associated with CRP level.

TABLE 149.7 Effect of Increasing Doses of Vitamin E on Oxidation Parameters

Dosage (mg/day)	Lag Time[a]	Propagation Rate[b]
0	94	7.8
25	99	8
50	100	7.9
100	106	7.7
200	111	7.5
400	116	6.8
800	120	6.5

[a]The time before oxidation occurs after the addition of an oxidizing agent. The higher the number, the greater the beneficial effect.
[b]The rate at which lipid peroxidation progresses. The lower the number, the greater the beneficial effect.

Vitamin E

Oxidation of LDL to oxLDL is a crucial step in the development of atherosclerosis. Although clinical studies have shown inconsistent effects, vitamin E plays a role in the protection against oxidation of LDL-C because it can be easily incorporated into the LDL-C molecule (Table 149.7). In a placebo-controlled, randomized study, 12 weeks of vitamin E supplementation (800 International Units (IU) per day) increased LDL resistance to oxidation without changing the serum lipid profile or lipoprotein lipid composition.[115] There is a clear-cut dosage effect (i.e., the higher the dosage of vitamin E, the greater the degree of protection against oxidative damage to LDL-C). Doses greater than 400 IU are often required to produce clinically significant effects.[116–118]

Improvements have also been noted in insulin sensitivity and plasma lipids in non–insulin-dependent diabetic patients.

Several large population studies have demonstrated that vitamin E levels may be more predictive of incipient heart attack or stroke than total cholesterol levels.[117–120] Although the consumption of red wine has been suggested as the reason behind the French paradox, described earlier, higher vitamin E levels also provide a viable explanation.[117–119]

Vitamin E may offer additional benefit in protecting against heart disease and strokes by its ability to:

- Reduce LDL-C peroxidation and increase plasma LDL-C breakdown
- Inhibit excessive platelet aggregation
- Increase HDL-C levels
- Increase fibrinolytic activity
- Reduce CRP levels
- Improve endothelial cell function
- Improve insulin sensitivity

Two early large-scale studies with relatively low dosages of vitamin E supplementation demonstrated a significant reduction in the risk of heart attack or stroke. The Nurses' Health Study of 87,245 nurses concluded that those who took 100 IU of vitamin E daily for more than 2 years had a 41% lower risk of heart disease compared with nonusers of vitamin E supplements.[119] The Physicians' Health Study of 39,910 male health care professionals found similar results: a 37% lower risk of heart disease with the intake of more than 30 IU of supplemental vitamin E daily.[120] Subsequent studies have been equivocal.[110]

Large-scale studies examining the effect of vitamin E supplementation in patients with existing CAD have also shown somewhat conflicting results.[111,112] Some of the disappointing results may have been due to the choice of synthetic vitamin E (DL-alpha tocopherol) in one of

TABLE 149.8 Intervention Trials With Vitamin E for Secondary Prevention

Study	Number of Subjects	Dosage	Duration	Outcome
HOPE (2000)	9541	400 IU (d-alpha)	4.5 years	No effect
SPACE (2000)	196	800 IU (d-alpha)	1.4 years	-70%
GISSI (1999)	11,324	300 IU (synthetic)	3.5 years	-35% (vs. placebo)
CHAOS (1996)	9541	400–800 IU (d-alpha)	1.4 years	-47%

CHAOS, Cambridge Heart Antioxidant Study; *HOPE,* Heart Outcomes Protection Evaluation Trial; *GISSI,* Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico; *SPACE,* Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease.

the large studies versus the more active natural form (D-alpha tocopherol). In addition, interference by statin drugs in vitamin E and CoQ_{10} metabolism increases the needs for both compounds (Table 149.8).

Vitamin E and CoQ_{10} work synergistically, and each is required for the regeneration of the other. For example, CoQ_{10} is present in the blood in both oxidized (inactive) and reduced (active) forms. During times of increased oxidative stress or low vitamin E levels, more CoQ_{10} will be converted to its oxidized (inactive form). Thus by providing higher levels of vitamin E, the biological activity and function of CoQ_{10} are enhanced, and vice versa. Several studies in humans and animals have shown that the combination of vitamin E and CoQ_{10} works better than either one alone. For example, in a study in baboons, where supplementation with vitamin E alone reduced CRP levels, cosupplementation with CoQ_{10} significantly enhanced this effect of vitamin E. Similar results have been seen in other animal studies on other factors associated with atherosclerosis, including LDL-C oxidation and lipid peroxide content within the aorta.[120–123]

In addition to CoQ_{10}, vitamin E also requires adequate selenium status for optimal antioxidant effects. Selenium functions primarily as a component of the antioxidant enzyme glutathione peroxidase. This enzyme works closely with vitamin E to prevent free-radical damage to cell membranes. Studies looking only at vitamin E's ability to reduce cancer and heart disease are often faulty because they fail to factor in the critical partnership between selenium and vitamin E, not to mention the interrelationship between vitamin E and CoQ_{10}. Several studies have clearly demonstrated that low selenium status is significantly associated with CAD.[124,125] Failure to cosupplement with selenium as well as vitamin C and CoQ_{10} may be a major reason for the inconsistent results in intervention trials with vitamin E supplementation alone.

Finally, the importance of prescribing the full complement of the eight vitamin E isomers cannot be overstated. Most of the cardiovascular research with vitamin E has been on the synthetic DL alpha-tocopherol. The problem with this approach is more fully discussed in Chapter 127, Vitamin Toxicities and Therapeutic Monitoring.

Vitamin C

Vitamin C works as an antioxidant in aqueous (water) environments in the body—both outside and inside human cells. It is the first line of antioxidant protection in the body. Its primary antioxidant partner is vitamin E, as this antioxidant is fat-soluble. Along with CoQ_{10}, vitamin C is also responsible for regenerating oxidized vitamin E in the body, thus potentiating the antioxidant benefits of vitamin E.[126] Vitamin C also works along with antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. Vitamin C has been shown to be extremely effective in preventing LDL-C from being oxidized, even in smokers.[127] Vitamins C and E supplementation (500 mg and 272 IU daily, respectively) for 6 years has been shown to reduce the progression of carotid atherosclerosis by 53% in men and 14% in women.[128]

A high dietary intake of vitamin C has been shown to significantly reduce the risk of death from heart attacks and strokes, as well as all other causes including cancer in numerous population studies.[129] One of the most detailed studies analyzed the vitamin C intake of 11,348 adults over 5 years and divided them into three groups: (1) less than 50 mg dietary intake daily; (2) greater than 50 mg daily dietary intake with no vitamin C supplementation; and (3) greater than 50 mg dietary intake a day plus vitamin C supplementation (estimated to be ≥300 mg).[129] Analysis showed that the standardized mortality ratio, a comparison with the average death rate, was up to 48% lower in the high vitamin C intake group than in the low-intake group for CVD and overall mortality. These differences correspond to an increase in longevity of 5 to 7 years for men and 1 to 3 years for women.

Dozens of observational and clinical studies have shown that vitamin C levels correspond to total cholesterol and HDL-C.[130–132] In one of the best-designed studies, it was shown that the higher the vitamin C content of the blood, the lower the total cholesterol and triglycerides and the higher the HDL-C.[132] The benefits for HDL-C were particularly impressive. For each 0.5 mg/dL increase in vitamin C content of the blood, there was an increase in HDL-C of 14.9 mg/dL in women and 2.1 mg/dL in men. This study demonstrates that the association of vitamin C and HDL-C levels persists even in well-nourished individuals with normal serum levels of vitamin C who supplement their diets with additional vitamin C. Vitamin C therapy also has positive effects on the fibrinolytic system, and has been shown to reduce CRP and Lp(a)

In summary, vitamin C lowers the risk of CVD by[126–134]:

- Acting as an antioxidant
- Strengthening the collagen structures of the arteries
- Lowering total cholesterol, Lp(a), and blood pressure
- Raising HDL-C levels
- Inhibiting platelet aggregation
- Promoting fibrinolysis
- Reducing markers of inflammation

Grape Seed and Pine Bark Extracts

One of the most beneficial groups of plant flavonoids are the proanthocyanidins (also referred to as *procyanidins* or *procyanidolic oligomers* [PCOs]). Although PCOs exist in many plants, as well as in red wine, commercially available sources of PCO include extracts from grape seeds and the bark of the maritime pine (Landes).[135] These extracts offer protection via several different mechanisms, including their antioxidant activity and effects on endothelial cells. For more information, see Chapter 106.

THERAPEUTIC CONSIDERATIONS—MISCELLANEOUS RISK FACTORS

Platelet Aggregation

Excessive platelet aggregation is another independent risk factor for heart disease and stroke. Once platelets aggregate, they release potent

compounds that dramatically promote the formation of the atherosclerotic plaque, or they can form a clot that can lodge in small arteries and produce a heart attack or stroke. The adhesiveness of platelets is determined largely by the types of fats in the diet and the level of antioxidants. Although saturated fats and cholesterol increase platelet aggregation, omega-3 oils (both short-chain and long-chain) and monounsaturated fats have the opposite effect.[136,137]

In addition to the monounsaturated and omega-3 fatty acids, antioxidant nutrients, and flavonoids, vitamin B_6 also inhibits platelet aggregation and lowers blood pressure and homocysteine levels.[138,139] In one study, the effect of vitamin B_6 (pyridoxine HCl) supplementation on platelet aggregation, plasma lipids, and serum zinc levels was determined in 24 healthy male volunteers (19–24 years old) given either pyridoxine at a dosage of 5 mg/kg body weight or a placebo for 4 weeks.[138] Results demonstrated that pyridoxine inhibited platelet aggregation by 41% to 48%, but there was no change in the control group. Pyridoxine prolonged both bleeding and coagulation time but not over the physiological limits. It had no effect on platelet count. Pyridoxine was also shown to lower total plasma lipids and cholesterol levels considerably from pretreatment levels. Total plasma lipids were reduced from 593 to 519 mg/dL, and total cholesterol was reduced from 156 to 116 mg/dL. HDL-C increased from 37.9 to 48.6 mg/dL. Serum zinc levels increased from 96 to 138 mg/dL.

In another study, a significant inverse graded relation was observed between the serum level of pyridoxal-5-phosphate (P5P) and both CRP and fibrinogen.[140] The odds ratio for CAD risk related to low P5P concentrations after adjustments for the major classic CAD risk factors, including CRP and fibrinogen, was 1.89. In addition, the CAD risk as a result of low P5P was additive when considered in combination with elevated CRP concentrations or with an increased LDL-C:HDL-C ratio.

These results provide clear evidence of the possible role of vitamin B_6 supplementation in reducing the risk of atherosclerotic mortality.

Garlic preparations standardized for alliin content as well as garlic oil have also demonstrated inhibition of platelet aggregation. In one study, 120 patients with increased platelet aggregation were given either 900 mg per day of a dried garlic preparation containing 1.3% alliin or a placebo for 4 weeks.[141] In the garlic group, spontaneous platelet aggregation disappeared, the microcirculation of the skin increased by 47.6%, plasma viscosity decreased by 3.2%, diastolic blood pressure dropped from an average of 74 to 67 mm Hg, and fasting blood glucose concentration dropped from an average of 89.4 to 79 mg/dL.

Fibrinogen

Early clinical studies encouraged detailed epidemiological investigations into the possible link between fibrinogen and CVD.[142] The first such study was the Northwick Park Heart Study in the United Kingdom. This large study involved 1510 men 40 to 64 years of age who were randomly recruited and tested for a range of clotting factors including fibrinogen. At the 4-year follow-up, there was a stronger association between cardiovascular deaths and fibrinogen levels than that for cholesterol. This association has now been confirmed in at least five other prospective epidemiological studies.

Natural therapies designed to promote fibrinolysis include exercise, omega-3 oils, niacin, garlic, and nattokinase. In addition, the Mediterranean diet alone significantly reduces fibrinogen and other markers of inflammation.[143] Adherence to the Mediterranean diet was shown to be associated with a 20% lower CRP level, 17% lower interleukin-6 level, 15% lower homocysteine level, and 6% lower fibrinogen level.

Natto is a traditional Japanese food prepared from fermented soybeans by *Bacillus subtilis.* Nattokinase is a serine proteinase isolated from natto that has potent fibrinolytic and thrombolytic activity. An open-label, self-controlled clinical trial was conducted on subjects of the following groups: healthy volunteers (healthy group), patients with cardiovascular risk factors (cardiovascular group), and patients undergoing dialysis (dialysis group). All subjects ingested two capsules of nattokinase (2000 fibrinolysis units per capsule) daily orally for 2 months. The laboratory measurements were performed on the screening visit and then regularly after the initiation of the study. After 2 months of administration, fibrinogen, factor VII, and factor VIII decreased by 9%, 14%, and 17%, respectively, for the healthy group; 7%, 13%, and 19%, respectively, for the cardiovascular group; and 10%, 7%, and 19%, respectively, for the dialysis group. These results highlight the potential of nattokinase as a natural fibrinolytic.[144]

Homocysteine

Homocysteine is an intermediate in the conversion of the amino acid methionine to cysteine. If a person is functionally deficient in folic acid, vitamin B_6, or vitamin B_{12}, there will be an increase in homocysteine. Elevated homocysteine levels are an independent risk factor for developing a heart attack, stroke, or peripheral vascular disease. Elevations in homocysteine are found in approximately 20% to 40% of patients with heart disease and are significantly associated with CAD.[145–147] A meta-analysis found that for each increase of 5 μmol/L in homocysteine, the risk of CHD events increased by approximately 20%, independently of traditional CHD risk factors.[148]

A number of interrelated atherogenic mechanisms are thought to be involved with hyperhomocysteinemia. These mechanisms include advanced thickening and smooth muscle cell proliferation of endothelial vessel wall intima, enhanced lipid deposition in the vessel wall, forced detachment of endothelial cells, activation of leukocytes and thrombocytes, increased LDL-C oxidation, initiation of platelet thromboxane synthesis, enhanced oxidative stress induced by peroxide formation during homocysteine oxidation, and prothrombotic coagulation interference. Homocysteine is thought to promote atherosclerosis by directly damaging the artery, reducing the integrity of the vessel wall, and interfering with the formation of proper collagen.

Although supplementation with the active form of folate, 5-methylfolate (400 mcg daily), alone can reduce homocysteine levels in many subjects, given the importance of vitamins B_{12} and B_6 for proper homocysteine metabolism, all three should be used together. In one study, the suboptimal levels of these nutrients in men with elevated homocysteine levels were 56.8%, 59.1%, and 25% for folic acid, vitamin B_{12}, and vitamin B_6, respectively, indicating that folate supplementation alone would not lower homocysteine levels in many cases.[149] In other words, folate supplementation will lower homocysteine levels only if there are adequate levels of vitamins B_{12} and B_6.

In 1998 the FDA mandated the fortification of food products with folic acid. Although homocysteine levels have decreased modestly since then, the effect on mortality has been minor at best.[150] This reflects the importance of more aggressive supplementary measures to reduce homocysteine-associated cardiovascular risk, as well as the importance of supplementing with the most active forms to enhance bioavailability.

"Type A" Personality

"Type A" behavior is characterized by an extreme sense of time urgency, competitiveness, impatience, and aggressiveness. This behavior carries with it a twofold increase in CHD compared with non–type A behavior.[151-152] Particularly damaging to the cardiovascular system is the regular expression of anger. In one study, the relationship between habitual anger coping styles, especially anger expression, and serum lipid concentrations were examined in 86 healthy subjects.[153] Habitual

anger expression was measured on four scales: aggression, controlled affect, guilt, and social inhibition. A positive correlation between serum cholesterol level and aggression was found. The higher the aggression score, the higher the cholesterol level. A negative correlation was found between the ratio of LDL-C to HDL-C and controlled affect score—the greater the ability to control anger, the lower this ratio. In other words, people who learn to control anger experience a significant reduction in the risk for heart disease, whereas an unfavorable lipid profile is linked with a predominantly aggressive (hostile) anger coping style.

Anger expression also plays a role in CRP levels. In one study, greater anger and severity of depressive symptoms, separately and in combination with hostility, were significantly associated with elevations in CRP in apparently healthy men and women.[154] Other mechanisms explaining the link between the emotions, personality, and CVD include increased cortisol secretion, endothelial dysfunction, hypertension, and increased platelet aggregation and fibrinogen levels.[138] These associations are independent of potential confounding factors.

Ten tips that can help patients improve their coping strategies include:

1. Do not starve your emotional life. Foster meaningful relationships. Provide time to give and receive love in your life.
2. Learn to be a good listener. Allow the people in your life to really share their feelings and thoughts uninterruptedly. Empathize with them; put yourself in their shoes.
3. Do not try to talk over somebody. If you find yourself being interrupted, relax; do not try to outtalk the other person. If you are courteous and allow someone else to speak, eventually (unless he or she is extremely rude) he or she will respond likewise. If not, explain that he or she is interrupting the communication process. You can do this only if you have been a good listener.
4. Avoid aggressive or passive behavior. Be assertive but express your thoughts and feelings in a kind way to help improve relationships at work and at home.
5. Avoid excessive stress in your life as best you can by avoiding excessive work hours, poor nutrition, and inadequate rest. Get as much sleep as you can.
6. Avoid stimulants like caffeine and nicotine. Stimulants promote the fight-or-flight response and tend to make people more irritable in the process.
7. Take time to build long-term health and success by performing stress-reduction techniques and deep breathing exercises.
8. Accept gracefully those things over which you have no control. Save your energy for those things that you can do something about.
9. Accept yourself. Remember that you are human and will make mistakes from which you can learn along the way.
10. Be more patient and tolerant of other people. Follow the golden rule.

Other Nutritional Factors

Magnesium and Potassium

Magnesium and potassium are absolutely essential to the proper functioning of the entire cardiovascular system. Their critical roles in preventing heart disease and strokes are now widely accepted. In addition, there is a substantial body of knowledge demonstrating that magnesium or potassium supplementation or both are effective in treating a wide range of CVDs, including angina, arrhythmias, congestive heart failure, and high blood pressure. In many of these applications, magnesium or potassium supplementation or both have been used for more than 50 years.

Because the role of potassium in the cardiovascular system is described in detail in Chapter 179, the focus here is on magnesium. Most Americans do not consume enough of this important mineral. The average intake of magnesium by healthy adults in the United States ranges from 143 to 266 mg/day. This level is well below even the recommended daily allowance (RDA) of 350 mg for men and 300 mg for women. Food choices are the main reason. Because magnesium occurs abundantly in whole foods, most nutritionists and dietitians assume that most Americans get enough magnesium in their diets. However, most Americans are not eating whole, natural foods. They are consuming large quantities of processed foods. Because food processing refines a large portion of magnesium, most Americans are not getting the RDA for magnesium.

The best dietary sources of magnesium are tofu, legumes, seeds, nuts, whole grains, and green leafy vegetables. Fish, meat, milk, and most commonly eaten fruits are quite low in magnesium. Most Americans consume a low-magnesium diet because their diet is high in low-magnesium foods such as processed foods, meat, and dairy products.

People dying of heart attacks have been shown to have lower heart magnesium levels than people of the same age dying of other causes.[155] Low serum magnesium (≤0.80 mmol/L) has been associated with an increased risk of coronary heart disease mortality (hazard ratio: 1.36, 95% CI: 1.09–1.69) and sudden cardiac death (hazard ratio: 1.54, 95% CI: 1.12–2.11) as well as subclinical atherosclerosis (expressed as increased carotid intima-media thickness: +0.013 mm, 95% CI: 0.005–0.020).[156] Low magnesium levels contribute to atherosclerosis and CVD via many mechanisms including the promotion of endothelial dysfunction by generating a proinflammatory, prothrombotic, proatherogenic environment.[157]

Intravenous magnesium therapy has emerged as a valued treatment measure in acute MI.[158–160] The major obstacle to it becoming the preferred method for saving a person's life may be a financial interest. Magnesium is inexpensive compared with high-tech, high-priced, genetically engineered drugs currently being promoted by drug companies. The treatment of acute MI is big business in the United States. Each year more than 1.5 million U.S. citizens experience acute MI. Although many other parts of the world are now using magnesium therapy for acute MI because of its effectiveness, low cost, safety, and ease of administration, it plays second fiddle to the high-tech drugs in the United States.

Several well-designed studies involving more than 4000 patients have demonstrated that intravenous magnesium supplementation during the first hour of admission to a hospital for acute MI produces a favorable effect in reducing immediate and long-term complications as well as death rates. The beneficial effects of magnesium in acute MI relate to its ability to:

- Improve energy production within the heart
- Dilate the coronary arteries, resulting in improved delivery of oxygen to the heart
- Reduce peripheral vascular resistance, resulting in reduced demand on the heart
- Inhibit platelets from aggregating and forming blood clots
- Reduce the size of the infarct (blockage)
- Improve heart rate and arrhythmias

Vitamin D Deficiency

Data from more than 8000 individuals enrolled in the NHANES 2001 to 2004 study indicate that individuals with vitamin D (25-OH) levels below 30 ng/mL were more likely to be at high risk for CVD (OR 1.32), for CHD (OR 1.48), and for both CHD and heart failure (OR 3.52).[161] A prospective cohort study of more than 3000 men and women found that those in the lowest quartile of 25-hydroxy vitamin D had a hazard ratio of 2.22 for cardiovascular mortality and 2.08 for all-cause mortality.[162]

THERAPEUTIC CONSIDERATIONS—PREVENTING A RECURRENT HEART ATTACK

People who have experienced a heart attack or stroke and live through it are extremely likely to experience another. The primary prevention of subsequent cardiovascular events is to control the major cardiac risk factors (e.g., hyperlipidemia, hypertension, cigarette smoking, diabetes, physical inactivity). The most popular "secondary" recommendation to reduce the risk of a subsequent heart attack being given by most physicians is low-dose aspirin (e.g., 325 mg per day or every other day). However, there may be effective alternatives, especially for those who cannot tolerate aspirin therapy. It is becoming standard to recommend dosages of aspirin lower than 325 mg (e.g., 50–150 mg per day or every other day). To date these lower dosages have not been shown to be effective in reducing CVD mortality.

Aspirin

Aspirin has been shown to decrease the risk of CVD events in both primary and secondary trials. The first primary prevention study was the Physicians' Health Study, a randomized double-blind placebo-controlled trial of 22,071 apparently healthy men. The study was terminated early owing principally to a statistically extreme 44% reduction in the risk of a first MI with the use of 325 mg of aspirin every other day. Since this study, three additional randomized trials including both men and women have shown aspirin to be effective in the primary prevention of MI: the Thrombosis Prevention Trial, the Hypertension Optimal Treatment Study, and the Primary Prevention Project. Among the 55,580 subjects, aspirin use was associated with a statistically significant 32% reduction in the risk of a first MI and a significant 15% reduction in the risk of all other important vascular events, but it had no significant effects on nonfatal stroke or vascular death. Evaluation of the data from the Physicians' Health Study indicated that those with the highest CRP had the greatest decrease in risk, 55.7%, versus 13.9% in those with the lowest CRP. It seems reasonable to reserve the use of aspirin as a primary prevention strategy for individuals with high CRP values.[163]

Regarding the secondary prevention of MI, there have been seven prospective randomized placebo-controlled trials involving almost 15,000 survivors of heart attack that have examined the use of aspirin to reduce the incidence of recurrent heart attack and death. These trials used several doses of aspirin, ranging from 325 to 1500 mg per day and enrolled patients at various intervals after infarction ranging from 4 weeks to 5 years. None of the studies demonstrated a statistically significant reduction in mortality with aspirin use. However, when all the results from these studies were pooled, aspirin was shown to reduce the mortality rate from all causes as well as cardiovascular deaths. The mortality rate for all causes in the aspirin group was 5.8%, compared with 8.3% in the placebo group, indicating a reduction in mortality by 30% with aspirin.[164,165]

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with a significant risk of peptic ulcer. However, most studies documenting the relative frequency of peptic ulcers as a consequence of NSAIDs have focused on their use in the treatment of arthritis and headaches. The risk of gastrointestinal bleeding due to peptic ulcers has been evaluated for aspirin at daily dosages of 300, 150, and 75 mg, respectively. Essentially there is an increased risk of gastrointestinal bleeding due to peptic ulcers at all dosage levels. However, the dosage of 75 mg per day was associated with a 40% reduction in ulcers compared with 300 mg per day and 30% compared with 150 mg per day.[166]

Because it is unknown whether 75 mg per day of aspirin is helpful in preventing a second heart attack, most physicians recommend at least 100mg. To prevent stroke, the dosage necessary appears to be 160mg. These dosage recommendations carry with them a significant risk for developing a peptic ulcer but may be appropriate for high-risk patients unwilling to adopt the natural approach.

Dietary Alternatives to Aspirin

Several studies have shown that dietary modifications are not only more effective in preventing recurrent heart attack than aspirin but can also reverse the blockage of clogged arteries. In addition to the studies with the Mediterranean diet, three famous studies deserve special mention. The first was the Lifestyle Heart Trial conducted by Dean Ornish.[167] In this study, subjects with heart disease were divided into a control group and an experimental group. The control group received regular medical care, and the experimental group was asked to eat a low-fat vegetarian diet for at least 1 year. The diet included fruits, vegetables, grains, legumes, and soybean products. Subjects were allowed to consume as many calories as they wished. No animal products were allowed except egg whites and 1 cup of nonfat milk or yogurt daily. The diet contained approximately 10% fat; 15% to 20% protein; and 70% to 75% carbohydrates—that is, predominantly complex carbohydrates from whole grains, legumes, and vegetables.

The experimental group was also asked to perform stress reduction techniques such as breathing exercises, stretching exercises, meditation, imagery, and other relaxation techniques for an hour each day and to exercise for at least 3 hours each week. At the end of the year, the subjects in the experimental group showed significant overall regression of atherosclerosis of the coronary blood vessels. In contrast, subjects in the control group who were being treated with regular medical care and following the standard AHA diet actually showed progression of their disease. Ornish stated, "This finding suggests that conventional recommendations for patients with CHD (such as a 30% fat diet) are not sufficient to bring about regression in many patients."

Numerous population studies have demonstrated that people who consume a diet rich in omega-3 oils from either fish or vegetable sources have a significantly reduced risk of developing heart disease. Two famous intervention trials upheld this protective effect. In the Dietary and Reinfarction Trial (DART), it was only when the intake of omega-3 fatty acids (from fish) was increased that future heart attacks were reduced.[168] In another study, the Lyon Diet Heart Study, increasing the intake of omega-3 fatty acids from plant sources (alpha-linolenic acid) was found to offer the same degree of protection as increased fish intake.[169]

Preventing a Subsequent Stroke

To prevent a subsequent stroke and promote recovery from a stroke, *Ginkgo biloba* extract should be added to the program as described earlier for preventing a subsequent heart attack. The extract of *G. biloba* leaves standardized to contain 24% ginkgo flavonglycosides and 6% terpenoids has been the subject of more than 300 published scientific papers and more than 40 double-blind studies in the treatment of decreased blood supply to the brain (cerebrovascular insufficiency). *G. biloba* extract has also been shown to enhance stroke recovery (see Chapter 93 for more information).

Other Considerations

Angiography, Coronary Artery Bypass Surgery, or Angioplasty

A significant challenge for the clinician is determining when a patient should be referred for angiography, coronary artery bypass surgery, or angioplasty. As is fully discussed in Chapter 82, these procedures are used far more frequently than is justified by objective evaluation of their appropriateness and efficacy. Chapter 145 also provides advice for patient care when angiography, coronary artery bypass surgery, or angioplasty is unavoidable.

TABLE 149.9 Food Choices for Lowering Cholesterol

Decrease Consumption of the Following:	Substitute With the Following:
Red meat	Fish and white-meat poultry
Hamburgers and hot dogs	Soy-based alternatives
Eggs	Egg Beaters and similar products, tofu
High-fat dairy products	Low-fat or nonfat dairy products
Butter, lard, and other saturated fats	Vegetable oils
Ice cream, pies, cake, cookies, etc.	Fruits
Refined cereals, white	Whole grains, whole wheat bread, etc.
Fried foods, fatty snack foods	Vegetables, fresh salads
Salt and salty foods	Low-sodium foods, light salt
Coffee and soft drinks	Herbal teas, fresh fruit, and vegetable juices

Intravenous Chelation Therapy

Intravenous ethylenediamine tetraacetic acid chelation therapy is a useful yet controversial procedure and is fully discussed in Chapter 146.

Earlobe Crease

The presence of a diagonal earlobe crease has been recognized as a sign of CVD since 1973. More than 30 studies have been reported in the medical literature. The earlobe is richly vascularized, and a decrease in blood flow over an extended period of time is believed to result in collapse of the vascular bed. This collapse leads to a diagonal crease.[170,171]

In one study, angiographs performed on 205 consecutive patients showed an 82% accuracy in predicting heart disease, with a false-positive rate of 12% and a false-negative rate of 18%. In another study of 112 consecutive patients, the earlobe crease was highly correlated with demonstrable heart disease and less strongly with previous MI.[171]

The crease is seen more commonly with advancing age, until the age of 80, when the incidence drops dramatically. However, the association with heart disease is independent of age. Although the presence of an earlobe crease does not prove heart disease, it strongly suggests it, and examination of the earlobe is an easy screening procedure. The correlation does not hold with Asians, Native Americans, or children with Beckwith's syndrome.[171]

THERAPEUTIC APPROACH

There is little doubt that in most cases atherosclerosis is directly related to diet and lifestyle. Treatment and prevention include reducing all known risk factors. For many patients, this goal requires a major change in diet and lifestyle. Because so many factors are known to be involved in atherosclerosis, any treatment plan must be individualized to assure optimal results. What follows is a general approach that must be tailored according to the clinical picture and the patient's willingness to adopt it.

BOX 149.5 Foods Typically Containing Partially or Totally Hydrogenated Vegetable Oils and Trans Isomers

- Virtually all refined and processed foods
- Margarine
- Cakes
- Cookies
- Candies
- Doughnuts
- Bread
- Canned soups
- Crackers
- Processed cheese
- Canned foods
- Cerealsd
- Snack foods
- Salad oils, except olive oil, which is recommended

Dietary Recommendations

Follow the dietary guidelines given in Chapter 44 (a modified version of the Mediterranean diet). Table 149.9 provides additional guidance in dietary selections, and Box 149.5 lists foods to avoid because of their trans-fatty acid content. Specifically, it is important to:

- Eat less saturated fat and cholesterol by reducing or eliminating the amounts of animal products in the diet.
- Increase the consumption of fiber-rich plant foods (fruits, vegetables, grains, legumes, and raw nuts and seeds).
- Increase the consumption of monounsaturated fats and omega-3 fatty acids.
- Follow a low-glycemic diet.

Lifestyle Recommendations

- Achieve ideal body weight.
- Exercise aerobically on a regular basis.
- Do not smoke.

Supplements

- High-potency multivitamin and mineral formula
- Vitamin C: 250 to 500 mg 3 times a day
- Vitamin E (mixed tocopherols): 200 to 400 IU per day
- Grape seed or pine bark extract: 100 mg per day
- Fish oil supplement: minimum 1000 mg of EPA + DHA
- Vitamin D: 1000 to 4000 IU/day (should usually be complemented with vitamin K_2)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Viles-Gonzalez JF, Anand SX, Valdiviezo C, et al. Update in atherothrombotic disease. *Mt Sinai J Med*. 2004;71:197–208.
2. Qiao Q, Tervahauta M, Nissinen A, et al. Mortality from all causes and from coronary heart disease related to smoking and changes in smoking during a 35-year follow-up of middle-aged Finnish men. *Eur Heart J*. 2000;21:1621–1626.
3. Imamura H, Tanaka K, Hirae C, et al. Relationship of cigarette smoking to blood pressure and serum lipids and lipoproteins in men. *Clin Exp Pharmacol Physiol*. 1996;23:397–402.
4. Penn A, Snyder C. Arteriosclerotic plaque development is 'promoted' by polynuclear aromatic hydrocarbons. *Carcinogenesis*. 1988;9(12):2185–2189. PMID: 3142695.
5. Levenson J, Simon AC, Cambien FA. Cigarette smoking and hypertension. Factors independently associated with blood hyperviscosity and arterial rigidity. *Arteriosclerosis*. 1987;7:572–577.
6. Kritz H, Schmid P, Sinzinger H. Passive smoking and cardiovascular risk. *Arch Intern Med*. 1995;155:1942–1948.
7. Venn A, Britton J. Exposure to secondhand smoke and biomarkers of cardiovascular disease risk in never-smoking adults. *Circulation*. 2007;115(8):990–995. PMID: 17296856.
8. Critchley JA, Capewell S. Mortality risk reduction associated with smoking cessation in patients with coronary heart disease: a systematic review. *JAMA*. 2003;290:86–97.
9. Law M, Tang JL. An analysis of the effectiveness of interventions intended to help people stop smoking. *Arch Intern Med*. 1995;155:1933–1941.
10. Wilson PW. High-density lipoprotein, low-density lipoprotein and coronary artery disease. *Am J Cardiol*. 1990;66:A7–A10.
11. Ip S, Lichtenstein AH, Chung M. Systematic review: association of low-density lipoprotein subfractions with cardiovascular outcomes. *Ann Intern Med*. 2009;150(7):474–484.
12. Davidson MH. Apolipoprotein measurements: is more widespread use clinically indicated? *Clin Cardiol*. 2009;32(9):482–486.
13. Schaefer EJ, Lamon-Fava S, Jenner JL, et al. Lipoprotein(a) levels and risk of coronary heart disease in men. The lipid research Clinics coronary primary prevention trial. *JAMA*. 1994;271:999–1003.
14. Kannel WB, Vasan RS. Triglycerides as vascular risk factors: new epidemiologic insights. *Curr Opin Cardiol*. 2009;24(4):345–350.
15. Stalenhoef AF, de Graaf J. Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. *Curr Opin Lipidol*. 2008;19(4):355–361.
16. Analitis A, Katsouyanni K, Dimakopoulou K, et al. Short-term effects of ambient particles on cardiovascular and respiratory mortality. *Epidemiology*. 2006;17(2):230–233. PMID: 16477266.
17. Araujo JA, Nel AE. Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. *Particle Fibre Toxicol*. 2009;6:24. PMID: 19761620.
18. Kunli N, Jerrett M, Mack WJ, et al. Ambient air pollution and atherosclerosis in Los Angeles. *Environ Health Perspect*. 2005;113(2):201–206. PMID: 15687058.
19. Lind PM, Van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. *Environ Health Perspect*. 2012;120(1):38–43. PMID: 22222676.
20. Melzer D, Gates P, Osborne NJ, et al. Urinary bisphenol A concentration and angiography-defined coronary artery stenosis. *PLoS ONE*. 2012;7(8):e43378. PMID: 22916252.
21. Lind PM, Lind L. Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly. *Atherosclerosis*. 2011;218(1):207–213. PMID: 21621210.
22. Virtanen JK, Voutilainen S, Rissanen TH, Mursu J, et al. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in Eastern Finland. *Arterioscler Thromb Vasc Biol*. 2005;25(1):228–233. PMID: 15539625.
23. Peters JL, Perlstein TS, Perry MJ, Mcneely E, Weuve J. Cadmium exposure in association with history of stroke and heart failure. *Environmental Research*. 2010;110(2):199–206. PMID: 20060521.
24. Barregard L, Sallsten G, Fagerberg B, Borné Y, et al. Blood cadmium levels and incident cardiovascular events during follow-up in a population-based cohort of Swedish adults: the Malmö Diet and Cancer Study. *Environ Health Perspect*. 2016;124(5):594–600. PMID: 26517380.
25. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analysis. *BMJ*. 2000;321:199–204.
26. Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347:1557–1565.
27. Lee WY, Park JS, Noh SY, et al. C-reactive protein concentrations are related to insulin resistance and metabolic syndrome as defined by the ATP III report. *Int J Cardiol*. 2004;97:101–106.
28. Khaw KT, Wareham N, Bingham S, et al. Combined impact of health behaviours and mortality in men and women: the EPIC-Norfolk prospective population study. *PLoS Med*. 2008;5(1):e12.
29. De Lorgeril M, Salen P, Martin J-L, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation*. 1999;99:779–785.
30. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA*. 2004;292:1440–1446.
31. Medina-Ramon A, Casas R, Tressserra-Rimbau A, et al. Polyphenol intake from a Mediterranean diet decreases inflammatory biomarkers related to atherosclerosis: a substudy of the PREDIMED trial. *Br J Clin Pharmacol*. 2017;83(1):114–128. PMID: 27100393.
32. Martinez-Gonzalez MA, Sanchez-Villegas A. The emerging role of Mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? *Eur J Epidemiol*. 2004;19:9–13.
33. Aranda N, Valls RM, Romeu M, et al. Consumption of seafood and its estimated heavy metals are associated with lipid profile and oxidative lipid damage on healthy adults from a Spanish Mediterranean area: a cross-sectional study. *Environ Res*. 2017;156:644–651. PMID: 28463823.
34. Sieri S, Krogh V, Berrino F, et al. Dietary glycemic load and index and risk of coronary heart disease in a large Italian cohort: the EPICOR study. *Arch Intern Med*. 2010;170(7):640–647.
35. Alarcon de la Lastra C, Barranco MD, Motilva V, et al. Mediterranean diet and health: biological importance of olive oil. *Curr Pharm Des*. 2001;7:933–950.
36. Summerhill V, Karagodin V, Grechko A, Myasoedova V, et al. Vasculoprotective role of olive oil compounds via modulation of oxidative stress in atherosclerosis. *Front Cardiovasc Med*. 2018;5:188. PMID: 30622950.
37. Bucher HC, Hengstler P, Schindler C, et al. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med*. 2002;112:298–304.
38. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med*. 2004;39:212–220.
39. Harris WS. The omega-3 index as a risk factor for coronary heart disease. *Am J Clin Nutr*. 2008;87(6):S1997–S2002.
40. Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA*. 2002;287:1815–1821.
41. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med*. 2002;346:1113–1118.
42. Skulas-Ray AC, Kris-Etherton PM, Harris WS, et al. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr*. 2011;93(2):243–252.
43. Preston Mason R. New insights into mechanisms of action for omega-3 fatty acids in atherothrombotic cardiovascular disease. *Curr Atheroscler Rep*. 2019;21(1):2. PMID: 30637567.
44. Sandker GW, Kromhout D, Aravanis C. Serum cholesterol ester fatty acids and their relation with serum lipids in elderly men in Crete and The Netherlands. *Eur J Clin Nutr*. 1993;47:201–208.
45. Kagawa Y, Nishizawa M, Suzuki M, et al. Eicosapolyenoic acids of serum lipids of Japanese Islanders with low incidence of cardiovascular diseases. *J Nutr Sci Vitaminol (Tokyo)*. 1982;28:441–453.

46. Hu FB, Stampfer MJ. Nut consumption and risk of coronary heart disease: a review of epidemiologic evidence. *Curr Atheroscler Rep*. 1999;1:204–209.
47. Ros E, Nunez I, Perez-Heras A, et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation*. 2004;109:1609–1614.
48. Ford ES, Liu S, Mannino DM, et al. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. *Eur J Clin Nutr*. 2003;57:1157–1163.
49. Cheng HM, Koutsidis G, Lodge JK, Ashor A, Siervo M, Lara J. Tomato and lycopene supplementation and cardiovascular risk factors: a systematic review and meta-analysis. *Atherosclerosis*. 2017;257:100–108. PMID: 28129549.
50. Weisburger JH. Lycopene and tomato products in health promotion. *Exp Biol Med*. 2002;227:924–927.
51. Williams MJ, Sutherland WH, Whelan AP, et al. Acute effect of drinking red and white wines on circulating levels of inflammation-sensitive molecules in men with coronary artery disease. *Metabolism*. 2004;53:318–323.
52. Rimm EB, Williams P, Fosher K, et al. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ*. 1999;319:1523–1528.
53. Skaltsounis AL, Kremastinos DT. Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. *Eur J Cardiovasc Prev Rehabil*. 2005;12(6):596–600.
54. Oak MH, El Bedoui J, Schini-Kerth VB. Antiangiogenic properties of natural polyphenols from red wine and green tea. *J Nutr Biochem*. 2005;16(1):1–8.
55. Sumner MD, Elliott-Eller M, Weidner G, et al. Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *Am J Cardiol*. 2005;96(6):810–814.
56. Aviram M, Rosenblat M, Gaitini D, et al. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin Nutr*. 2004;23(3):423–433.
57. Esmaillzadeh A, Tahbaz F, Gaieni I, et al. Concentrated pomegranate juice improves lipid profiles in diabetic patients with hyperlipidemia. *J Med Food*. 2004;7(3):305–308.
58. Pedersen TR. Pro and con: low-density lipoprotein cholesterol lowering is and will be the key to the future of lipid management. *Am J Cardiol*. 2001;87:B8–B12.
59. Ong HT. The statin studies: from targeting hypercholesterolaemia to targeting the high-risk patient. *QJM*. 2005;98(8):599–614.
60. Jenkins DJ, Kendall CW, Marchie A, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. *JAMA*. 2003;290:502–510.
61. Jenkins DJ, Kendall CW, Faulkner DA, et al. Long-term effects of a plant-based dietary portfolio of cholesterol-lowering foods on blood pressure. *Eur J Clin Nutr*. 2008;62(6):781–788.
62. Gigleux I, Jenkins DJ, Kendall CW, et al. Comparison of a dietary portfolio diet of cholesterol-lowering foods and a statin on LDL particle size phenotype in hypercholesterolaemic participants. *Br J Nutr*. 2007;98(6):1229–1236.
63. Reynolds K, Chin A, Lees KA, et al. A meta-analysis of the effect of soy protein supplementation on serum lipids. *Am J Cardiol*. 2006;98(5):633–640.
64. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med*. 1995;333(5):276–282.
65. Langsjoen PH, Langsjoen AM. The clinical use of HMG CoA-reductase inhibitors and the associated depletion of coenzyme Q10: a review of animal and human publications. *Biofactors*. 2003;18:101–111.
66. Rundek T, Naini A, Sacco R, et al. Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. *Arch Neurol*. 2004;61:889–892.
67. Albert MA, Danielson E, Rifai N, et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA*. 2001;286:64–70.
68. Glore SR, Van Treeck D, Knehans AW, et al. Soluble fiber and serum lipids: a literature review. *J Am Diet Assoc*. 1994;94:425–436.
69. Vuksan V, Jenkins DJ, Spadafora P, et al. Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes: a randomized controlled metabolic trial. *Diabetes Care*. 1999;22:913–919.
70. Vuksan V, Sievenpiper JL, Owen R, et al. Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. *Diabetes Care*. 2000;23:9–14.
71. Ripsin CM, Keenan JM, Jacobs DR, et al. Oat products and lipid lowering: a meta-analysis. *JAMA*. 1992;267:3317–3325.
72. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from National Health and Nutrition Examination Survey data. *J Nutr*. 2004;134:1181–1185.
73. Canner PL, Berge KG, Wenger NK. Fifteen year mortality in coronary drug project patients: long-term benefit with niacin. *J Am Coll Cardiol*. 1986;8:1245–1255.
74. DiPalma JR, Thayer WS. Use of niacin as a drug. *Annu Rev Nutr*. 1991;11:169–187.
75. Illingworth DR, Stein EA, Mitchel YB, et al. Comparative effects of lovastatin and niacin in primary hypercholesterolemia. *Arch Intern Med*. 1994;14:1586–1595.
76. Carlson LA, Hamsten A, Asplund A. Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidaemic subjects treated with nicotinic acid. *J Intern Med*. 1989;226:271–276.
77. Pan J, Lin M, Kesala RL, et al. Niacin treatment of the atherogenic lipid profile and Lp(a) in diabetes. *Diabetes Obes Metab*. 2002;4:255–261.
78. Vega GL, Grundy SM. Lipoprotein responses to treatment with lovastatin, gemfibrozil, and nicotinic acid in normolipidemic patients with hypoalphalipoproteinemia. *Arch Intern Med*. 1994;154:73–82.
79. Van JT, Pan J, Wasty T, et al. Comparison of extended-release niacin and atorvastatin monotherapies and combination treatment of the atherogenic lipid profile in diabetes mellitus. *Am J Cardiol*. 2002;89:1306–1308.
80. Rindone JP, Achacoso S. Effect of low-dose niacin on glucose control in patients with non–insulin-dependent diabetes mellitus and hyperlipidemia. *Am J Ther*. 1996;3:637–639.
81. Grundy SM, Vega GL, McGovern ME, et al. Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of Niaspan trial. *Arch Intern Med*. 2002;162:1568–1576.
82. Kane MP, Hamilton RA, Addesse E, et al. Cholesterol and glycemic effects of Niaspan in patients with type 2 diabetes. *Pharmacotherapy*. 2001;21:1473–1478.
83. Kuvin JT, Dave DM, Sliney KA, et al. Effects of extended-release niacin on lipoprotein particle size, distribution, and inflammatory markers in patients with coronary artery disease. *Am J Cardiol*. 2006;98(6):743–745.
84. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med*. 2011;15;365(24):2255–2267.
85. Ronsein GE, Hutchins PM, Isquith D, Vaisar T, Zhao XQ, Heinecke JW. Niacin therapy increases high-density lipoprotein particles and total cholesterol efflux capacity but not ABCA1-specific cholesterol efflux in statin-treated subjects. *Arterioscler Thromb Vasc Biol*. 2016;36(2):404–411. PMID: 26681752.
86. McKenney JM, Proctor JD, Harris S, et al. A comparison of the efficacy and toxic effects of sustained- vs immediate-release niacin in hypercholesterolemic patients. *JAMA*. 1994;271:672–677.
87. Goldberg AC. A meta-analysis of randomized controlled studies on the effects of extended-release niacin in women. *Am J Cardiol*. 2004;94:121–124.
88. Guyton JR. Extended-release niacin for modifying the lipoprotein profile. *Expert Opin Pharmacother*. 2004;5:1385–1398.
89. Rubenfire M. Impact of medical subspecialty on patient compliance to treatment study group. Safety and compliance with once-daily niacin extended-release/lovastatin as initial therapy in the Impact of Medical Subspecialty on Patient Compliance to Treatment (IMPACT) study. *Am J Cardiol*. 2004;94:306–311.

90. Vogt A, Kassner U, Hostalek U, et al. Evaluation of the safety and tolerability of prolonged-release nicotinic acid in a usual care setting: the NAUTILUS study. *Curr Med Res Opin*. 2006;22(2):417–425.
91. Welsh AL, Ede M. Inositol hexanicotinate for improved nicotinic acid therapy. *Int Rec Med*. 1961;174:9–15.
92. El-Enein AMA, Hafez YS, Salem H, et al. The role of nicotinic acid and inositol hexaniacinate as anticholesterolemic and antilipemic agents. *Nutr Rep Intl*. 1983;28:899–911.
93. Ostlund Jr RE. Phytosterols and cholesterol metabolism. *Curr Opin Lipidol*. 2004;15:37–41.
94. Clifton PM, Noakes M, Ross D, et al. High dietary intake of phytosterol esters decreases carotenoids and increases plasma plant sterol levels with no additional cholesterol lowering. *J Lipid Res*. 2004;45:1493–1499.
95. Miettinen TA, Gylling H. Plant stanol and sterol esters in prevention of cardiovascular diseases. *Ann Med*. 2004;36:126–134.
96. Kozlowska-Wojciechowska M, Jastrzebska M, Naruszewicz M, et al. Impact of margarine enriched with plant sterols on blood lipids, platelet function, and fibrinogen level in young men. *Metabolism*. 2003;52:1373–1378.
97. Yoshida Y, Niki E. Antioxidant effects of phytosterol and its components. *J Nutr Sci Vitaminol (Tokyo)*. 2003;49:277–280.
98. de Jong A, Plat J, Mensink RP. Metabolic effects of plant sterols and stanols. *J Nutr Biochem*. 2003;14:362–369.
99. Arsenio L, Bodria P, Magnati G, et al. Effectiveness of long-term treatment with pantethine in patients with dyslipidemias. *Clin Ther*. 1986;8:537–545.
100. Gaddi A, Descovich GC, Noseda P, et al. Controlled evaluation of pantethine, a natural hypolipidemic compound, in patients with different forms of hyperlipoproteinemia. *Atherosclerosis*. 1984;50:73–83.
101. Coronel F, Tomero F, Torrente J, et al. Treatment of hyperlipemia in diabetic patients on dialysis with a physiological substance. *Am J Nephrol*. 1991;11:32–36.
102. Donati C, Bertieri RS, Barbi G. Pantethine, diabetes mellitus and atherosclerosis: clinical study of 1045 patients. *Clin Ter*. 1989;128:411–422.
103. Hiramatsu K, Nozaki H, Arimori S. Influence of pantethine on platelet volume, microviscosity, lipid composition and functions in diabetes mellitus with hyperlipidemia. *Tokai J Exp Clin Med*. 1981;6:49–57.
104. Lawson LD, Wang ZJ, Papdimitrou D. Allicin release under simulated gastrointestinal conditions from garlic powder tablets employed in clinical trials on serum cholesterol. *Planta Med*. 2001;67:13–18.
105. Lawson LD, Wang ZJ. Tablet quality: a major problem in clinical trials with garlic supplements. *Forsch Komplmentarmed*. 2000;7:45.
106. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nutr J*. 2002;1:4.
107. Alder R, Lookinland S, Berry JA, et al. A systematic review of the effectiveness of garlic as an anti-hyperlipidemic agent. *J Am Acad Nurse Pract*. 2003;15:120–129.
108. Stevinson C, Pittler MH, Erst E. Garlic for treating hypercholesterolemia: a meta-analysis of randomized clinical trials. *Ann Intern Med*. 2000;133:420–429.
109. Kung AW, Pang RW, Janus ED. Elevated serum lipoprotein(a) in subclinical hypothyroidism. *Clin Endocrinol (Oxf)*. 1995;43:445–449.
110. Clarke R, Armitage J. Antioxidant vitamins and risk of cardiovascular disease: review of large-scale randomised trials. *Cardiovasc Drugs Ther*. 2002;16:411–415.
111. Vivekananthan DP, Penn MS, Sapp SK, et al. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet*. 2003;361:2017–2023.
112. Salonen RM, Nyyssonen K, Kaikkonen J, et al. Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression: the antioxidant supplementation in atherosclerosis prevention (ASAP) study. *Circulation*. 2003;107:947–953.
113. Lowe GM, Bilton RF, Davies IG. Carotenoid composition and antioxidant potential in subfractions of human low-density lipoprotein. *Ann Clin Biochem*. 1999;36:323–332.
114. Church TS, Earnest CP, Wood KA, et al. Reduction of C-reactive protein levels through use of a multivitamin. *Am J Med*. 2003;115:702–707.
115. Baldi S, Innocenti M, Frascerra S, et al. Effects of hemodialysis and vitamin E supplements on low-density lipoprotein oxidizability in end-stage renal failure. *J Nephrol*. 2013;26(3):549–555. PMID: 22941872.
116. Princen HM, Van Duyvenvoorde W, Buytenhek R, et al. Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arterioscler Thromb Vasc Biol*. 1995;15:325–333.
117. Gey KF, Puska P, Jordan P. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am J Clin Nutr*. 1991;53:S326–S334.
118. Bellizzi MC, Franklin MF, Duthie GG. Vitamin E and coronary heart disease: the European paradox. *Eur J Clin Nutr*. 1994;48:822–831.
119. Stampfer MJ, Hennekens CH, Manson JE. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med*. 1993;328:1444–1449.
120. Rimm EB, Stampfer MJ, Ascherio A. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*. 1993;328:1450–1456.
121. Kaikkonen J, Nyyssonen K, Tomasi A, et al. Antioxidative efficacy of parallel and combined supplementation with coenzyme Q10 and d-alpha-tocopherol in mildly hypercholesterolemic subjects: a randomized placebo-controlled clinical study. *Free Radic Res*. 2000;33:329–340.
122. Wang XL, Rainwater DL, Mahaney MC, Stocker R. Cosupplementation with vitamin E and coenzyme Q10 reduces circulating markers of inflammation in baboons. *Am J Clin Nutr*. 2004;80:649–655.
123. Thomas SR, Leichtweis SB, Pettersson K, et al. Dietary cosupplementation with vitamin E and coenzyme Q(10) inhibits atherosclerosis in apolipoprotein E gene knockout mice. *Arterioscler Thromb Vasc Biol*. 2001;21:585–593.
124. Yegin A, Yegin H, Aliciguzel Y, et al. Erythrocyte selenium-glutathione peroxidase activity is lower in patients with coronary atherosclerosis. *Jpn Heart J*. 1997;38:793–798.
125. Bor MV, Cevik C, Uslu I, et al. Selenium levels and glutathione peroxidase activities in patients with acute myocardial infarction. *Acta Cardiol*. 1999;54:271–276.
126. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci*. 1989;86:6377–6381.
127. Harats D, Ben-Naim M, Dabach Y. Effect of vitamin C and E supplementation on susceptibility of plasma lipoproteins to peroxidation induced by acute smoking. *Atherosclerosis*. 1990;85:47–54.
128. Salonen RM, Nyyssonen K, Kaikkonen J, et al. Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression. The Antioxidant Supplementation Atherosclerosis Prevention (ASAP) study. *Circulation*. 2003;107:947–953.
129. Simon JA. Vitamin C and cardiovascular disease: a review. *J Am Coll Nutr*. 1992;11:107–125.
130. Howard PA, Meyers DG. Effect of vitamin C on plasma lipids. *Ann Pharmacother*. 1995;29:1129–1136.
131. Jacques PF, Sulsky SI, Perrone GA. Ascorbic acid and plasma lipids. *Epidemiology*. 1994;5:19–26.
132. Hallfrisch J, Singh VN, Muller DC. High plasma vitamin C associated with high plasma HDL- and HDL2 cholesterol. *Am J Clin Nutr*. 1994;60:100–105.
133. Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr*. 2003;22:18–35.
134. Tousoulis D, Antoniades C, Tountas C, et al. Vitamin C affects thrombosis/fibrinolysis system and reactive hyperemia in patients with type 2 diabetes and coronary artery disease. *Diabetes Care*. 2003;26:2749–2753.
135. Shi J, Yu J, Pohorly JE, et al. Polyphenolics in grape seeds: biochemistry and functionality. *J Med Food*. 2003;6:291–299.
136. Freese R, Mutanen M. Alpha-linolenic acid and marine long-chain n-3 fatty acids differ only slightly in their effects on hemostatic factors in healthy subjects. *Am J Clin Nutr*. 1997;66:591–598.
137. Smith RD, Kelly CN, Fielding BA, et al. Long-term monounsaturated fatty acid diets reduce platelet aggregation in healthy young subjects. *Br J Nutr*. 2003;90:597–606.
138. Lam SC, Harfenist EJ, Packham MA, et al. Investigation of possible mechanisms of pyridoxal 5′-phosphate inhibition of platelet reactions. *Thromb Res*. 1980;20:633–645.

139. Sermet A, Aybak M, Ulak G. Effect of oral pyridoxine hydrochloride supplementation on in vitro platelet sensitivity to different agonists. *Arzneimittelforschung*. 1995;45:19–21.
140. Friso S, Girelli D, Martinelli N, et al. Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk. *Am J Clin Nutr*. 2004;79:992–998.
141. Kiesewetter H, Jung F, Pindur G. Effect of garlic on thrombocyte aggregation, microcirculation, and other risk factors. *Int J Clin Pharmacol Ther Toxicol*. 1991;29:151–155.
142. Ernst E. Fibrinogen: an important risk factor for atherothrombotic diseases. *Ann Med*. 1994;26:15–22.
143. Chrysohoou C, Panagiotakos DB, Pitsavos C, et al. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: the ATTICA Study. *J Am Coll Cardiol*. 2004;44:152–158.
144. Hsia CH, Shen MC, Lin JS, et al. Nattokinase decreases plasma levels of fibrinogen, factor VII, and factor VIII in human subjects. *Nutr Res*. 2009;29(3):190–196.
145. Boushey C, Beresford S, Omenn G, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA*. 1995;274:1049–1057.
146. Gauthier GM, Keevil JG, McBride PE. The association of homocysteine and coronary artery disease. *Clin Cardiol*. 2003;26:563–568.
147. Bozkurt E, Keles S, Acikel M, et al. Plasma homocysteine level and the angiographic extent of coronary artery disease. *Angiology*. 2004;55:265–270.
148. Humphrey LL, Fu R, Rogers K, et al. Homocysteine level and CHD incidence: a systematic review and meta-analysis. *Mayo Clin Proc*. 2008;83(11):1203–1212.
149. Ubbink JB, Vermaak WJ, wan der Merwe A, et al. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr*. 1993;57:47–53.
150. Anderson JL, Jensen KR, Carlquist JF, et al. Effect of folic acid fortification of food on homocysteine-related mortality. *Am J Med*. 2004;116:158–164.
151. Matthews KA, Haynes SG. Type A behavior pattern and coronary disease risk. *Am J Epidemiol*. 1986;123:923–960.
152. Strike PC, Steptoe A. Psychosocial factors in the development of coronary artery disease. *Prog Cardiovasc Dis*. 2004;46:337–347.
153. Muller MM, Rau H, Brody S. The relationship between habitual anger coping style and serum lipid and lipoprotein concentrations. *Biol Psychol*. 1995;41:69–81.
154. Suarez EC. C-reactive protein is associated with psychological risk factors of cardiovascular disease in apparently healthy adults. *Psychosom Med*. 2004;66:684–691.
155. Maier JA. Low magnesium and atherosclerosis: an evidence-based link. *Mol Aspects Med*. 2003;24:137–146.
156. Kieboom BC, Niemeijer MN, Leening MJ, et al. Serum magnesium and the risk of death from coronary heart disease and sudden cardiac death. *J Am Heart Assoc*. 2016;5(1):pii: e002707. PMID: 26802105.
157. Maier JA, Malpuech-Brugere C, Zimowska W, et al. Low magnesium promotes endothelial cell dysfunction: implications for atherosclerosis, inflammation and thrombosis. *Biochim Biophys Acta*. 2004;1689:13–21.
158. Hampton EM, Whang DD, Whang R. Intravenous magnesium therapy in acute myocardial infarction. *Ann Pharmacother*. 1994;28:212–219.
159. Teo KK, Yusuf S. Role of magnesium in reducing mortality in acute myocardial infarction: a review of the evidence. *Drugs*. 1993;46:347–359.
160. Schecter M, Kaplinsky E, Rabinowitz B. The rationale of magnesium supplementation in acute myocardial infarction: a review of the literature. *Arch Intern Med*. 1992;152:2189–2196.
161. Kim DH, Sabour S, Sagar UN, et al. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National health and nutrition examination Survey 2001 to 2004). *Am J Cardiol*. 2008;102(11):1540–1544.
162. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008;168(12):1340–1349.
163. Eidelman RS, Hebert PR, Weisman SM, et al. An update on aspirin in the primary prevention of cardiovascular disease. *Arch Intern Med*. 2003;163:2006–2010.
164. Weisman SM, Graham DY. Evaluation of the benefits and risks of low-dose aspirin in the secondary prevention of cardiovascular and cerebrovascular events. *Arch Intern Med*. 2002;162:2197–2202.
165. Willard JE, Lange RA, Hillis LD. The use of aspirin in ischemic heart disease. *N Engl J Med*. 1992;327:175–181.
166. Weil J, Colin-Jones D, Langman M. Prophylactic aspirin and risk of peptic ulcer bleeding. *BMJ*. 1995;310:827–830.
167. Ornish D. Can lifestyle changes reverse coronary heart disease? *Lancet*. 1990;336:129–133.
168. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fiber intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*. 1989;2:757–761.
169. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343:1454–1459.
170. Elliot WJ. Ear lobe crease and coronary artery disease. *Am J Med*. 1983;75:1024–1032.
171. Elliott WJ, Powell LH. Diagonal earlobe creases and prognosis in patients with suspected coronary artery disease. *Am J Med*. 1996;100:205–211.

Atopic Dermatitis (Eczema)

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Chronic, pruritic, inflammatory skin
- Dry, hyperkeratotic skin
- Lesions include excoriations, papules, eczema (patches of erythema, exudation, and scaling with small vesicles formed within the epidermis), and lichenification (hyperpigmented plaques of thickened skin with accentuated furrows)
- Scratching and rubbing lead to lichenification, most commonly in the antecubital and popliteal flexures
- Personal or family history of atopy

GENERAL CONSIDERATIONS

Atopic dermatitis (eczema) is a common condition with a prevalence in the United States of up to 10.2% of the adult population and 13% of children.[1] This prevalence is four times higher than when this textbook was first published in 1985.

The current etiopathogenesis of atopic dermatitis (AD) involves the interplay of the following:

- Cutaneous barrier dysfunction and mutations in genes coding for skin proteins (filaggrin)
- Dysregulation of the immune system and the role of thymic stromal lymphopoietin in allergic inflammation
- Environmental factors

Filaggrin and Epidermal Barrier Dysfunction

A genetic basis for AD has long been recognized. Family history of atopic disease is a major risk factor. Before characterization of the human genome, heritability studies combined with family-based linkage studies supported the understanding of AD as a complex trait, in that interactions between genes and environmental factors and the interplay between multiple genes contribute to disease manifestation. More than 100 published reports on genetic association studies through mid-2009 implicated 81 genes, in 46 of which at least one positive association with AD had been demonstrated. Of these, the gene encoding filaggrin (FLG) has been most consistently replicated. Most candidate gene studies to date have focused on adaptive and innate immune response genes, but there is increasing interest in genes governing skin barrier dysfunction.[2]

Filaggrin is a protein that facilitates the aggregation of keratin filaments; it is otherwise known as filament aggregating protein. It binds cytokeratin into tonofilaments and, when proteolyzed, forms small hygroscopic molecules including amino acids, which comprise natural moisturizing factor (NMF). NMF is responsible for hydration of the stratum corneum. Robust associations of filaggrin mutations have been replicated in several populations, especially in those with northern European ancestry, and it is now accepted that 15% of cases of AD are attributable to such mutations.[3] If an individual is born with one filaggrin mutation, the risk of AD is 40%.

But additional acquired stressors to the skin barrier are required to initiate inflammation. Sustained hapten access through a defective barrier stimulates a Th1-to-Th2 shift in immunophenotype, which in turn further impairs the barrier. Secondary *Staphylococcus aureus* colonization not only amplifies inflammation but also further stresses the barrier in AD. These observations suggest a new "outside-to-inside, back to outside" paradigm for the pathogenesis of AD. This new concept is providing impetus for the development of new categories of "barrier repair" therapy.[4]

Immune Dysregulation, Thymic Stromal Lymphopoietin, and Allergic Inflammation

Immunoglobulin E (IgE) is elevated in up to 80% of patients with AD. It is clear that activation of type 2 T-helper (Th2) cells in response to

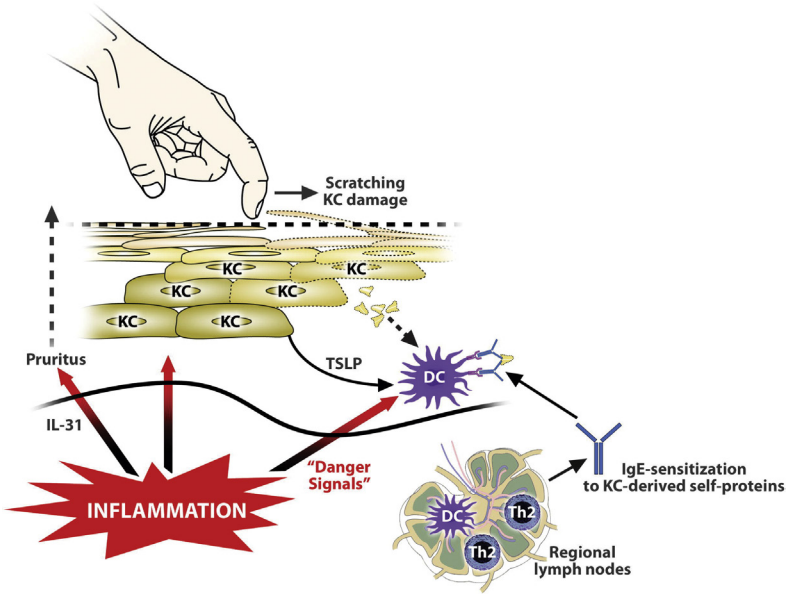


Fig. 150.1 Mechanisms of immunoglobulin E (IgE) sensitization to epidermal self-proteins in patients with atopic dermatitis. Inflammation induces pruritus through interleukin (IL)-31 production. Scratching results in cellular damage and release of membranous and intracellular compounds from keratinocytes (KCs) and possibly other skin cells. The local dendritic cells (DCs) subjected to thymic stromal lymphopoietin (TSLP) will induce a Th2 response and the generation of specific IgE directed against these self-proteins. IgE will bind to Fc ε RI on dendritic cells in the skin and thereby amplify the immune response and inflammatory reaction in the skin. (From Tang TS, Bieber T, Williams HC. Does "autoreactivity" play a role in atopic dermatitis? *J Allergy Clin Immunol*. 2012;129:1209–1215.)

antigens plays a major role in AD. Thymic stromal lymphopoietin (TSLP) is an IL-7–like cytokine secreted by barrier-defective skin and is found at high levels in skin biopsies from patients with lesional AD.[5] TSLP is a critical factor linking responses at interfaces between the body and environment (skin, airway, gut, ocular tissues, etc.) to Th2 responses (Fig. 150.1). Environmental factors such as Toll-like receptor ligands, a Nod2 ligand, viruses, microbes, allergen sources, helminths, diesel exhaust, cigarette smoke, and chemicals trigger TSLP production. Proinflammatory cytokines, Th2-related cytokines, and IgE also induce or enhance TSLP production, indicating cycles of amplification. Skin barrier injury, increased epidermal endogenous protease activity, and less epidermal Notch signaling, all of which have been reported in AD, and keratinocyte-specific loss of retinoid X receptors and treatment of skin with agonists for vitamin D receptor in mice induce TSLP production, Th2 response, or AD-like inflammation.[6]

TSLP profoundly triggers Th2 commitment and is increased when stimulated by phthalates.[7] Phthalates are used in a large variety of products, from enteric coatings of pharmaceutical pills and nutritional supplements to viscosity control agents, gelling agents, film formers, stabilizers, dispersants, lubricants, binders, emulsifying agents, and suspending agents. End applications include adhesives and glues, agricultural uses, building materials, personal care products, medical devices, detergents and surfactants, packaging, children's toys, modeling clay, waxes, paints, printing inks and coatings, pharmaceuticals, food products, and textiles. Phthalates are also frequently used in soft plastic fishing lures, caulk, paint pigments, and sex toys made of "jelly rubber." Phthalates are used in a variety of household applications, such as shower curtains, vinyl upholstery, adhesives, floor tiles, food containers and wrappers, and cleaning materials. Pervasive exposure to phthalates may therefore play a role in the pathogenesis of AD. Cosmetic colorants have also been shown to upregulate TSLP and aggravate dermatitis.[8]

TSLP sensitizes the lungs to allergens and may help explain the frequently observed evolution of AD to asthma, known as the *atopic march*.

Development of atopic disease is also strongly influenced by the colonization of skin and intestinal microflora in early infancy. The microflora in the skin is dependent on the degree of hydration and the integrity of the skin barrier. Dry skin predisposes to *Staphylococcus aureus* colonization. Low gut microbiota diversity is associated with a Th2-dominant pattern as well as with food sensitization.[9]

Dupilumab is a fully humanized, monoclonal antibody targeting the alpha subunit of the interleukin (IL)-4 receptor to block signaling of IL-4 and IL-13. An in-depth study of lesional and nonlesional skin during dupilumab therapy found that modulating IL-4/IL-13 signaling through IL-4Rα antagonism in patients with AD led to statistically significant improvement in AD after 4 weeks of treatment, compared with placebo.[10]

The study also demonstrated that dupilumab suppressed mRNA expression in lesional skin of genes related to activation of T cells, dendritic cells, eosinophils, inflammatory pathways, type 2 cytokines, and downregulated genes responsible for epidermal hyperplasia (S100A and K16 genes). Blocking IL-4/IL-13 may not only improve inflammation in AD but may also restore skin barrier function as a result of significant increases in claudin, loricrin, filaggrin, and lipid product levels. These results show promising new insights into the role of type 2 cytokines in AD and suggest that inhibition of IL-4/IL-13 has the potential to reverse multiple molecular defects in patients with AD.

Gastric *Helicobacter pylori* directly stimulates epidermal cells to secrete TSLP, and *H. pylori* antibody is positive in up to 70% of patients with AD.[11] One study has shown that the treatment of infection, demonstrated by a reduction in C-urea breath test and anti–*H. pylori* antibody titers, resulted in partial improvement in patients with AD.[12] Another study found an inverse relationship between *H. pylori* positivity and AD in children.[13]

Galectins are a family of β-galactoside-binding lectins with one or two conserved carbohydrate-recognition domains (CRDs) that play various roles in skin physiology and pathology.[14] Galectin-3 contains one CRD following a nonlectin region of some 120 amino acids made of tandem repeats of short proline-/glycine-rich sequences. Galectin-3 is a proinflammatory mediator in AD by its promotion of polarization toward a Th2 immune response by regulating the functions of dendritic cells (DCs) and T cells.[15] Galectin-3 can enhance IgE synthesis by B cells and thus may play a role in the hyperactive immune response in IgE-associated atopic eczema/dermatitis syndrome.[16] Therapies that reduce galectin-3 may therefore be beneficial in AD (see section on treatment).

Environmental Factors: Atopic Dermatitis and Allergy

Sensitization to foods triggers isolated skin symptoms in about 30% of children. These symptoms include immediate reactions within minutes after ingesting food without exacerbation of AD and early and late exacerbations of AD. It is important to identify clinically relevant sensitizations to foods using skin-prick tests; atopy patch testing; specific IgE and IgG4 blood tests; and double-blind, placebo-controlled food challenges to initiate appropriate dietary interventions and avoid unnecessary dietary restrictions. A defective skin barrier and increased intestinal permeability appear to facilitate allergen sensitization. Appropriate skin care to maintain skin barrier function and dietary avoidance of identified food allergens during infancy may help prevent further allergen sensitization, thereby reducing the severity of AD and food allergies.[17] However, a study published in 2017 identified clinically active food allergies in 26.13% of 88 children with AD. No association with disease severity was found. This may be explained by this study only looking at IgE, which misses other food-related immunological factors.[18]

In February 2018 the first pilot study was published that identified microbial signatures specific for food allergy in children with AD using 16S rRNA sequencing techniques followed by statistical machine-learning approaches.[19] Six bacterial species discriminated between the presence and absence of food allergy in these children with AD. The fecal microbiome of children with AD and food allergy harbored significantly more *Escherichia coli* and *Bifidobacter pseudocatenulatum,* and less *Bifidobacter breve, Bifidobacter adolescentis, Faecalibacterium prausnitzii,* and *Akkermansia muciniphila* than children with AD without food allergy ($p = 0.001$). *F. prausnitzii* and *A. muciniphila* have gained interest because of their immunemodulatory and mucosal tolerance properties. *F. prausnitzii* is one of the most abundant species in the human intestinal microbiome. Its decreased abundance has been associated with several diseases, including allergic disease and AD *F. prausnitzii* is the chief source of butyrate in the colon, used as fuel by colonocytes, with important anti-inflammatory effects. *F. prausnitzii* also secretes anti-inflammatory modulators, stimulates IL-10–producing Treg cells and regulates the balance between effector and regulatory T cells. *A. muciniphila* plays a role in the immunological homeostasis of the gut mucosa and gut barrier.

Bifidobacteria and *E. coli* have been associated with food allergy and AD in other studies. *Bifidobacteria* is reduced in the feces of children with a confirmed cow's milk allergy.

Predictors of Severity

One longitudinal study of children examined a number of risk factors that may predict the severity of AD in children.[20] This study surveyed 137 children and followed them through four visits over a period of 1½ years. Parameters such as age at onset, social class, ethnic group, child's atopy, family history of atopy, and other potential risk factors were recorded. This study revealed the following: (1) Children with AD whose eczema started during the first year of life were more likely to have severe disease than were those whose eczema started later, (2) a history of atopy (asthma, hay fever, or both) was associated with severe AD, and (3) children with eczema who lived in an urban area were at increased risk of severe disease compared with their counterparts who lived in a rural environment. This urban environmental risk was independent of ethnicity.

A study published in 2018 concluded that short duration of breastfeeding, absence of older siblings, parental passive smoking, food allergens along with aeroallergens, and excessive cleanliness should be considered as negative prognostic factors, leading to a higher Scoring Atopic Dermatitis (SCORAD) score in children with AD.[21] Another study found that more than three fast-food meals per week increased the overall risk of AD by 70%. At least one course of a broad-spectrum antibiotic in early life increased the risk of AD by 41%, with an additional 7% increase in risk from each additional course of antibiotics. Reduced diversity of intestinal flora is a recognized risk factor, as is lack of exercise and traffic-related air pollution.

Protective factors include ultraviolet light exposure, which enhances filaggrin breakdown into an immunosuppressive isoform; maternal contact with farm animals during pregnancy, high fish intake during pregnancy (lowers AD risk by 25%–43%); consumption of unprocessed milk; helminth infection during pregnancy; dog and high-level endotoxin exposure in early life (through farm animals); and thumbsucking.[22]

Comorbidities

Three large data sets, including more than 1 million people, found that AD was associated with only a very small increased risk for cardiovascular disease.[23] Obesity in childhood is a positive association, although there is conflicting evidence about obesity in adults and AD.[24]

THERAPEUTIC CONSIDERATIONS

The myriad of prescription and over-the-counter medications commonly used to treat AD fail to address the root cause of the condition. Topical medications used to treat AD include emollients, corticosteroids, antibiotics, calcineurin inhibitors, and crisaborole, a boron-based PDE-4 inhibitor. Oral antihistamines are used for pruritus. Severe eczema has also been treated with systemic immunomodulatory agents (e.g., cyclosporine) and systemic corticosteroids. None of these medications has shown effective long-term ability to cure AD. Side effects such as growth retardation, cutaneous complications, sedation, and allergic reactions are a major concern.[25] Furthermore, a number of these medications can result in a rebound flare-up after discontinuation that is usually worse than the primary lesions.

Naturopathic medicine generally attempts to understand the underlying causes of AD and thus attends to additional factors, including those affecting the digestive system, such as food allergies and microbial overgrowth. Although no one treatment option may be universally effective, patient-specific combination therapeutics acting in synergy are encouraged for optimal efficacy.

Food Allergy, Gut Permeability, and Microbial Overgrowth

Numerous studies have documented the role of food allergy in AD (also see Chapter 14). Studies have also shown that breastfeeding offers significant prophylaxis against AD as well as allergies in general.[26]

One study of 100 infants concluded that breastfeeding should be promoted for the primary prevention of allergy.[27] Interestingly, further studies by the same group suggest that breastfed infants with allergies should be treated by allergen avoidance; in some cases breastfeeding cessation is recommended to avoid traces of food antigens in breast milk.[28] The development of AD in breastfed infants was once believed to be the result of the transfer of allergic antigens in the breast milk, in which mothers were instructed to avoid the common food allergens (especially milk, eggs, and peanuts and, to a lesser extent, fish, soy, wheat, citrus, and chocolate).[29] Maternal avoidance of these common allergens is associated with complete resolution in some cases. However, more recent studies have found that earlier introduction of common food allergens, rather than later, may be more efficacious in preventing the development of food allergies, at least for peanuts. In a prospective controlled study, regular consumption of peanut protein in infants from 4 to 11 months of age with atopic dermatitis or egg allergy was associated with a lower prevalence of peanut allergy (1.9%) at 60 months of age compared with peanut avoidance (13.7%). Other studies demonstrated that earlier introduction of cow's milk protein and egg powder were also associated with decreased risk for milk and egg allergy, respectively.[30]

In older or formula-fed infants, milk, eggs, and peanuts appear to be the most common food allergens inducing AD. In one study, these three foods were implicated in 81% of all cases of childhood AD,[31] whereas in another study, 60% of children with severe AD had a positive food challenge to one or two of the following: egg, cow's milk, peanut, fish, wheat, or soybean. One randomized controlled trial found that in those individuals with a positive radioallergosorbent test to eggs, an egg-free diet was associated with an improvement in the severity of AD, with the greatest effect seen in those most severely affected.[32] Although eggs are a major suspect, virtually any food can be the offending agent.[33]

Diagnosis of food allergy is usually best achieved via the elimination diet and double-blind placebo challenge method. This approach is especially useful in childhood eczema. Elimination of milk products, eggs, peanuts, tomatoes, and artificial colors and preservatives results in significant improvement in at least 75% of cases.[31,33,34] If laboratory methods are used to identify food allergies in eczema, the most useful method (although not perfect) appears to be the enzyme-linked immunosorbent assay (ELISA) IgE and IgG_4 (see Chapter 14).[35]

The presence of food allergies is thought to be partially responsible for persons with AD having a "leaky gut."[36] As a result of this increased gut permeability, there is an increased antigen load on the immune system, which subsequently overwhelms the immune system and increases the likelihood of developing additional allergies. Permeability studies using urinary mannitol and lactulose confirm that hypoallergenic diets do decrease gut permeability and are associated with improvements in atopic eczema. It is essential to identify offending foods as soon as possible to avoid increasing gut permeability. Trying to deal with multiple food allergies is often a difficult task for the patient because the diet is often unrealistically restrictive. The elimination of allergenic foods and restoration of normal intestinal permeability appear to stop the development of new allergies.[37]

Patients should be encouraged to find out whether, if they can avoid offending foods for a period of 6 to 12 months, their antibody levels to allergens will decrease to the point that they can tolerate the reintroduction of some foods at least twice a week. The loss rate of food allergy in patients with AD after 1 year was 26% for the five major allergens (egg, milk, wheat, soy, and peanut) and 66% for other food allergens.[38]

Gluten Sensitivity

Many patients with celiac disease also have atopic disorders. Of patients with gastrointestinal (GI) symptoms and mucosal lesions but negative results from serological or genetic tests for celiac disease, 30% had reduced GI and atopic symptoms when placed on a gluten-free diet.[39]

Candida albicans. An overgrowth of the common yeast *Candida albicans* in the gastrointestinal tract has been implicated as a causative factor in allergic conditions, including AD. Elevated levels of anticandidal antibodies are common in atopic individuals. Furthermore, the severity of lesions tends to correlate with the level of IgE antibodies in relation to candidal antigens. Appropriate anticandidal therapy (see Chapter 159) may result in significant clinical improvement of AD.[40,41]

Nutritional Supplements

Probiotics

Because the intestinal flora plays a major role in the health of the host, especially regarding AD, probiotic therapy is especially indicated. Studies evaluating the administration of the probiotic *Lactobacillus rhamnosus* strain GG alone or in conjunction with *Lactobacillus reuteri* to infants with AD and cow's milk allergy demonstrated a significant reduction of the severity of eczema.[42–45]

However, the one randomized controlled trial[44] that prescribed both probiotic strains used a skim milk powder preparation for the placebo group. Patients were also allowed to continue corticosteroid treatment. Both of these factors may have confounded any beneficial results because it was noted that corticosteroid use increased in the placebo group during the active treatment period, possibly indicating an allergic reaction to the milk proteins in the placebo. Another interesting note is the finding in this study that the positive therapeutic effect of probiotics was more pronounced in patients with an allergic constitution, as evidenced by positive responses to the skin-prick test and increased IgE levels. This suggests that these tests may be useful in forecasting who may be most responsive to probiotic therapy. A meta-analysis published in 2016 showed evidence that supports the use of synbiotics (combination of probiotics and prebiotics) for the treatment of AD in children age 1 year or older.[46] Finally, a spore-based probiotic has shown preliminary evidence of improving markers of leaky gut.[47] Although more study is warranted, it seems reasonable to include probiotics in a therapeutic regimen given their safety and overall health benefits.

Essential Fatty Acids

Supplementing the diet of AD patients with evening primrose, borage, or black currant oil (commercial sources of gamma-linolenic acid) may prove helpful. Several double-blind studies with evening primrose oil (typically using dosages of at least 3000 mg daily, providing 270 mg of gamma-linolenic acid) have shown benefit.[48–50] The benefits of omega-6 oils are primarily in skin barrier function. However, overall, the therapeutic results appear to be more favorable with omega-3 oil supplementation than with omega-6 oils. Several studies with evening primrose oil failed to demonstrate any therapeutic benefit over a placebo. In the largest of these studies and one of the highest in methodological quality, no benefit of evening primrose oil could be demonstrated.[51] Similarly, one study of 140 people, including 69 children, showed few beneficial effects of borage oil for AD.[52]

Dietary enrichment with "fish oil" supplements providing eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) or simply eating

more fatty fish (e.g., mackerel, herring, salmon) results in significant incorporation of omega-3 fatty acids into the membrane phospholipid pools. Flaxseed oil, which contains alpha-linolenic acid, the precursor omega-3 fatty acid, may benefit patients with AD. However, because the degree of clinical improvement correlates with the increase in the concentration of DHA in serum phospholipids, and fish oils are much more effective in raising DHA levels than flaxseed oil, supplementation with EPA/DHA or increasing the consumption of cold-water fish is likely to produce better overall results in AD than flaxseed oil.[53]

Epidemiological studies have found protective associations between fish intake in pregnancy, lactation, infancy, and childhood and atopic outcomes. Similar studies of fish oil supplements have also found protective and therapeutic effects.[54]

Vitamin D

The serum concentration of 25(OH)D3 in 95 patients with AD was not statistically different from 58 control subjects. However, the frequency of bacterial skin infection was higher in patients with AD with lower 25(OH)D_3 levels, and after supplementation, both mean objective SCORAD and SCORAD index were significantly lower ($P < .05$).[55]

Vitamin E

One single-blind analysis evaluated 96 patients given either 400 international units of vitamin E or a placebo daily for 8 months. Significant improvement was reported in approximately 60% of the treatment group (compared with 2% among the placebo group), with significant reductions in serum IgE levels.[56]

The combination of vitamin D_3 1600 IU and vitamin E 600 IU daily significantly reduced SCORAD ratings.[57]

Botanical Medicines

The use of botanical medicines in AD can be generally divided into two categories: internal and external. Licorice *(Glycyrrhiza glabra)* appears to be useful in either application. Internally, licorice preparations can exert significant anti-inflammatory and antiallergic effects (see Chapter 85). These benefits are perhaps best exemplified in several double-blind studies featuring a licorice-containing Chinese herbal formula.[58] In addition to licorice, the formula contained the following:

- *Ledebouriealla seseloides*
- *Potentilla chinensis*
- *Clematis chinensis*
- *Clematis armandi*
- *Rehmania glutinosa*
- *Paeonia lactiflora*
- *Lophatherum gracile*
- *Dictamnus dasycarpus*
- *Tribulus terrestris*
- *Schizonepeta tenuifolia*

Interest in this formula by a group of researchers began after a patient with AD experienced tremendous improvement after taking a decoction prescribed by a Chinese doctor.

Several double-blind studies have confirmed this benefit. In one study, 40 adult patients with long-standing, refractory, widespread AD were randomized to receive 2 months' treatment of either the active formula or a placebo decoction, followed by a crossover to the other treatment after a 4-week washout period.[59] The treatment group demonstrated significant superiority over the placebo in clinical evaluation. In addition, of the 31 patients completing the study, 20 preferred the active formula, whereas only 4 preferred the placebo. There was also a subjective improvement in itching and sleep during the active treatment phase. No side effects were reported, although many complained about the poor palatability of the decoction.

Similar results were demonstrated in a double-blind study in children.[60] These positive preliminary studies will hopefully be followed by more extensive investigations to determine proper dosages and perhaps different forms of administration (e.g., pills, tablets, or capsules vs. decoctions).

Licorice undoubtedly plays a major role in the effectiveness of the Chinese herbal formula. At this time, until the benefits of the other components can be confirmed, it makes the most sense to base the dosage level on the level of delivered licorice.

With regard to using licorice topically, good results are likely to be obtained by using commercial preparations featuring pure glycyrrhetinic acid. Several studies have shown glycyrrhetinic acid to exert an effect similar to that of topical hydrocortisone in the treatment of eczema, contact and allergic dermatitis, and psoriasis. In one study, 9 of 12 patients with intractable eczema noted marked improvement, and 2 noted mild improvement, when an ointment containing glycyrrhetinic acid was applied topically. In another study, 93% of the patients with eczema applying glycyrrhetinic acid demonstrated improvement compared with 83% using cortisone.[61]

A proprietary topical cream of *Rheum palmatum, Scutellaria baicalensis, Cnidium monnieri,* dipotassium glycyrrhizinate, *Sanguisorba officinalis,* and *Ailanthus altissima* in a base of ceramides, hyaluronic acid, shea butter, and vitamin E has demonstrated anti-inflammatory, antiallergic, antimicrobial, antipruritic, and barrier-repair effects: 46% reduction in SCORAD in 3 weeks, equal efficacy in severe and nonsevere AD, 60% reduction in itching after 3 weeks, and 32% improvement in skin moisture level after 3 weeks (Dermatest, Germany). A clinical trial is currently in process comparing this cream with another proprietary oat-based over-the-counter (OTC) product.

Other Therapeutic Considerations

Endocrine Factors

Patients with hypothyroidism and eczema respond well to thyroid hormone supplementation.[62]

Scratching Cessation

Scratching is extremely detrimental in AD because it breaks the skin, which aids bacterial ingress and promotes barrier dysfunction and lichenification. Factors that limit itching therefore promote healing and prevent recurrence. Some behavior modification techniques have proved valuable in reducing the exacerbation of AD symptoms caused by scratching.[25]

Wet wraps are an effective barrier to scratching while also increasing hydration as well as the penetration of medication and emollients and promoting more restful sleep, and using them is simple. After applying medication and emollient, the affected areas are wrapped in warm, wet gauze or, as an alternative, 100% cotton socks with the toe portion cut off. Apply a dry layer over the wet layer, and leave it on overnight for 5 to 14 days.[63]

Bleach baths

Bleach baths (1/4 cup household bleach added to bathwater) have been shown to reduce *S. aureus* and diminish AD severity.[64]

For those patients who do not have a bathtub or prefer showering, a bleach body wash is another option. An open-label trial of 0.006% sodium hypochlorite body wash gel, left on the skin for 1 to 2 minutes before showering off, decreased *S. aureus* colonization and markedly improved symptoms and quality of life in children by 2/3 after 2 weeks of daily use.[65]

Psychological Approach

Although the mechanism of the exact relationship between stress and AD has not been fully elucidated, it has been established through both

clinical and physiological pathways that psychological stress is a significant contributor to a patient's AD disease course through its direct and indirect effects on immune response, cutaneous neuropeptide expression, and skin barrier function.[66] Emotional tension can provoke and aggravate itching in patients with AD, and according to a number of studies, patients with AD show higher levels of anxiety, hostility, and neurosis than matched controls.[66] Studies employing psychotherapy have shown promising results, with one study showing reductions in the use of corticosteroids for up to 2 years.[25]

Humor

Starting in 2001 a fascinating series of studies was done by Dr. Hajime Kimata, an allergist at Unitika Central Hospital in Uji-City, Japan. The first mention Kimata made of this therapeutic intervention was in a letter to the *Journal of the American Medical Association* in 2001.[67]

Kimata had a series of studies published over a 9-year period. In his letter, he gives credit to Norman Cousins for giving him the idea for his research. Cousins had authored a book, *Anatomy of an Illness,* published in 1979, wherein he credited his relief from the pain of ankylosing spondylitis to watching comedies on TV. In Kimata's first trial, 26 patients with AD who were all allergic to dust mites and most of whom were also allergic to cedar pollen and cat dander were studied. After going 72 hours with no medication, they underwent skin-prick tests before and after viewing *Modern Times*, a comedy starring Charlie Chaplin. The size of the resulting wheal was measured. A similar procedure was repeated before and after an 87-minute video featuring weather information. The wheal responses to dust mites, cedar pollen, and cat dander were significantly reduced after watching Chaplin, and the effect lasted for hours. Watching the weather had no effect on the wheal size.[67]

His succeeding studies built on this finding, consistently showing that humor and positive emotions ameliorated symptoms of AD.[68] In 2015 Kimata was awarded the Ig Nobel Prize for a study showing that 30 minutes of kissing with a lover or spouse behind closed doors while listening to soft music can reduce allergic reactions among atopic and allergic patients.[69]

Homeopathy

A study of 17 patients with intractable AD treated with **individualized homeopathy** showed over 50% improvement in overall impression and in their skin condition by all patients.[70]

An observational longitudinal study was conducted on 325 children (37.9%) with atopic diseases out of 857 children consecutively examined from 1998 to 2014 at the Homeopathic Clinic of Lucca in Italy: 127 children were affected by asthma, 72 by allergic rhinitis, and 126 (36%) by AD. Follow-up on AD was achieved with 104 (48.8%), and 65 (62.5%) of them reported a major improvement or resolution. Children with AD who started homeopathic treatment at less than 4.9 years of age were assessed 5 to 10 years later, and complete remission of AD was obtained in 84.2%. Children with two or three atopic diseases at the first visit were completely cured in 40% of cases.[71]

A study was conducted in a homeopathic general practice in Buenos Aires, Argentina, of 42 patients with AD spontaneously seeking homeopathic treatment. Of these patients, 21 had other atopic comorbidities, and 28 (66.7%) were moderate or severe cases. Significant differences were observed when comparing the first and last consultations in the mean percentage of affected skin area, 21.1% versus 5.5% respectively ($P = 0.002$), and in the change in the four visual analog scale (VAS) scores: area of AD, 31.1 ($P < 0.0001$); itch, 35.0 ($P < 0.0001$); general well-being, 11.1 ($P < 0.0188$); and sleep, 17.4 ($P < 0.0073$). In this study, 12 individualized homeopathic medicines were prescribed; sulfur accounted for 60% of cases with good treatment response.[72]

A third study of homeopathic treatment compared with conventional treatment of AD conducted in Berlin, Germany, was a prospective, multicenter, comparative, observational, nonrandomized rater-blinded study of 135 children (48 treated with homeopathy, 87 with conventional medicine) with mild to moderate AD. SCORAD showed no significant differences between groups at 36 months. Total costs were higher in the homeopathic versus conventional group: EUR 200.5 versus EUR 78.86 ($p = 0.005$).[73]

ENVIRONMENTAL CONSIDERATIONS

Dust Mite Exposure

In patients with dust mite sensitization and eczema, sublingual immunotherapy has shown benefit.[74] Paradoxically, lower levels of eczema have been found in children with higher levels of dust mite exposure, so reducing house dust in early infancy could increase the risk of AD.[75]

Microwave Exposure

One fascinating study of cell phone use continuously for an hour showed significant increases in the allergic response to dust and pollen in adults with eczema.[76] Microwave radiation exposure also increased plasma levels of substance P and vasoactive intestinal peptide in patients with AD. This study raises the concern that microwave radiation emitted from cell phones may actually increase sensitivity to specific allergens in some patients.

THERAPEUTIC APPROACH

Effective management requires relief from and prevention of itching while the underlying abnormalities are being treated. Barrier dysfunction must be addressed. Food allergens must be detected and controlled, phthalates should be avoided and detoxification carried out, and the presence of *H. pylori* should be determined and treated.

Diet

The patient should begin a 4-day rotation diet and eliminate all major allergens (milk, eggs, and peanuts account for the offending food in approximately 81% of cases). As the patient improves, allergens can slowly be reintroduced, and a stringent rotation diet can be modified. Cold-water fish such as wild salmon, mackerel, herring, sardine, and halibut should be included, and other animal products should be limited.

Supplements

- High-potency multiple vitamin and mineral formula
- Vitamin D dosed to achieve and maintain an optimal level of 65 ng/dL
- Vitamin E: 400 international units daily (mixed tocopherols)
- Fish oil: 1000 to 3000 mg EPA+DHA daily
- Borage oil: 1 Tbsp daily
- Probiotics: 5 to 10 billion viable *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cells daily *or* a spore-based probiotic
- Modified citrus pectin to lower galectin-3 levels: 5 to 6 grams daily on empty stomach

Topical Treatment

Kamedis CALM Eczema Therapy and Eczema Wash

Patients should also try to take the following measures:

- Avoid sweating and rough-textured clothing.
- Wash clothing with mild soaps only and rinse thoroughly.
- Avoid exposure to chemical irritants and any other agent that might cause skin irritation.

Psychological Approach

It should be determined whether the patient is experiencing significant levels of anxiety, depression, or hostility. If so, he or she may be helped to resolve these problems or referred to a psychotherapist for professional assistance. Laughter from comedies and humorous films can be recommended for their salutary effect on AD.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Silverberg JI, Hanifin JM. Adult eczema prevalence and associations with asthma and other health and demographic factors: a US population-based study. *J Allergy Clin Immunol*. 2013;132(5):1132–1138.
2. Barnes KC. An update on the genetics of atopic dermatitis: scratching the surface in 2009. *J Allergy Clin Immunol*. 2010;125(1):16–29.
3. Parras A, Anta H, Santos-Galindo M, et al. Autism-like phenotype and risk gene mRNA deadenylation by CPEB4 mis-splicing. *Nature Genetics*. 2006;38:441–446.
4. Elias PM, Schmuth M. Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. *Curr Opin Allergy Clin Immunol*. 2009;9(5):437–446.
5. Liu Yong-Jun. Thymic stromal lymphopoietin: master switch for allergic inflammation. *JEM*. 2006;203:2269–2273.
6. Takai T. TSLP expression: cellular sources, triggers, and regulatory mechanisms. *Allergol Int*. 2012;61(1):3–17.
7. Shigeno T, Katakuse M, Fujita T, et al. Phthalate ester-induced thymic stromal lymphopoietin mediates allergic dermatitis in mice. *Immunology*. 2009;128(suppl 1):e849–e857.
8. Yang G, Lee HE, Lim KM, et al. Potentiation of skin TSLP production by a cosmetic colorant leads to aggravation of dermatitis symptoms. *Chem Biol Interact*. 2018;284:41–47.
9. Taniuchi S, Soejima K, Hatano Y, et al. Dual factors may be necessary for development of atopic march in early infancy. *J Nippon Med Sch*. 2018;85(1):2–10. https://doi.org/10.1272/jnms.2018_85-1.
10. Hamilton JD, Suárez-Fariñas M, Dhingra N, Cardinale I, Li X, Kostic A, et al. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol*. 2014;134:1293–1300.
11. Kido M, Tanaka J, Aoki N, et al. *Helicobacter pylori* promotes the production of thymic stromal lymphopoietin by gastric epithelial cells and induces dendritic cell-mediated inflammatory Th2 responses. *Infect Immun*. 2010;78(1):108–114.
12. Galadari IH, Sheriff MO. The role of *Helicobacter pylori* in urticaria and atopic dermatitis. *Skinmed*. 2006;5(4):172–176.
13. Pedulla M, Fierro V, Del Tufo E, et al. *Helicobacter pylori* immunization and atopic dermatitis in South Italian children. *United European Gastroenterol J*. 2014;2(4):263–267.
14. Wu NL, Liu FT. The expression and function of galectins in skin physiology and pathology. *Exp Dermatol*. 2018;27(3):217–226.
15. Larsen L, Chen HY, Liu FT. Galectin-3 and the skin. *J Dermatol Sci*. 2011;64(2):85–91.
16. Kimata H. Enhancement of IgE production in B cells by neutrophils via galectin-3 in IgE-associated atopic eczema/dermatitis syndrome. *Int Arch Allergy Immunol*. 2002;128:168–170.
17. Hauk PJ. The role of food allergy in atopic dermatitis. *Curr Allergy Asthma Rep*. 2008;8(3):188–194.
18. Mavroudi A, Karagiannidou A, Xinias I, et al. Assessment of IgE-mediated food allergies in children with atopic dermatitis. *Allergol Immunopathol*. 2017;45(1):77–81.
19. Fieten KB, Totte JEE, Levin E, et al. Fecal microbiome and food allergy in pediatric atopic dermatitis: a cross-sectional pilot study. *Int Arch Allergy Immunol*. 2018;175:77–84.
20. Ben-Gashir MA, Seed PT, Hay RJ. Predictors of atopic dermatitis severity over time. *J Am Acad Dermatol*. 2004;50:349–356.
21. Fotopoulou M, Iordanidou M, Vasilejou E, et al. A short period of breastfeeding in infancy, excessive house cleaning, absence of older sibling, and passive smoking are related to more severe atopic dermatitis in children. *Eur J Dermatol*. 2018;28(1):56–63.
22. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy*. 2014;69:3–16.
23. Standl M, Tesch F, Baurecht H. Association of atopic dermatitis with cardiovascular risk factors and diseases. *J Invest Dermatol*. 2016.
24. Ali Z, Suppli Ulrik C, Agner T, et al. Is atopic dermatitis associated with obesity? a systematic review of observational studies. *J Eur Acad Dermatol Venereol*. 2018.
25. Hanifin JM, Cooper KD, Ho VC, et al. Guidelines of care for atopic dermatitis, developed in accordance with the American Academy of Dermatology (AAD)/American Academy of Dermatology Association Administrative Regulations for Evidence-Based Clinical Practice Guidelines. *J Am Acad Dermatol*. 2004;50:391–404.
26. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet*. 1995;346:1065–1069.
27. Isolauri E, Tahvanainen A, Peltola T, et al. Breast-feeding of allergic infants. *J Pediatr*. 1999;134:27–32.
28. Arvola T, Moilanen E, Vuento R, et al. Weaning to hypoallergenic formula improves gut barrier function in breast-fed infants with atopic eczema. *J Pediatr Gastroenterol Nutr*. 2004;38:92–96.
29. Cant AJ, Bailes JA, Marsden, et al. Effect of maternal dietary exclusion on breast fed infants with eczema: two controlled studies. *Br Med J*. 1986;293:231–233.
30. Elizur A, Katz Y. Timing of allergen exposure and development of food allergy: treating before the horse is out of the barn. *Curr Opin Allergy Clin Immunol*. 2016;16(2):157–164.
31. Burks AW, Williams LW, Mallory SB, et al. Peanut protein as a major cause of adverse food reaction in patients with atopic dermatitis. *Allergy Proc*. 1989;10:265–269.
32. Lever R, MacDonald C, Waugh P, et al. Randomised controlled trial of advice on an egg exclusion diet in young children with atopic eczema and sensitivity to eggs. *Pediatr Allergy Immunol*. 1998;9:13–19.
33. de Maat-Bleeker F, Bruijnzeel-Koomen C. Food allergy in adults with atopic dermatitis. *Monogr Allergy*. 1996;32:157–163.
34. Van Bever HP, Docx M, Stevens WJ. Food and food additives in severe atopic dermatitis. *Allergy*. 1989;44:588–594.
35. Gondo A, Saeki N, Tokuda Y. IgG4 antibodies in patients with atopic dermatitis. *Br J Dermatol*. 1987;117:301–310.
36. Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol*. 1996;97:985–990.
37. Agata H, Kondo N, Fukutomi O, et al. Effect of elimination on food-specific IgE antibodies and lymphocyte proliferative responses to food antigens in atopic dermatitis patients exhibiting sensitivity to food allergens. *J Allergy Clin Immunol*. 1993;91:668–679.
38. Sampson HA, Scanlon SM. Natural history of food hypersensitivity in children with atopic dermatitis. *J Pediatr*. 1989;115:23–27.
39. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac gluten sensitivity. *Gastroenterology (Review)*. 2015;148(6):1195–1204.
40. Savolainen J, Lammintausta K, Kalimo K, et al. *Candida albicans* and atopic dermatitis. *Clin Exp Allergy*. 1993;23:332–339.
41. Adachi A, Horikawa T, Ichihashi M, et al. Role of *Candida* allergen in atopic dermatitis and efficacy of oral therapy with various antifungal agents. *Arerugi*. 1999;48(7):719–725.
42. Isolauri E, Arvola T, Sutas Y, et al. Probiotics in the management of atopic eczema. *Clin Exp Allergy*. 2000;30:1604–1610.
43. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol*. 1997;99:179–185.
44. Rosenfeldt V, Benfeldt E, Nielsen SD, et al. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol*. 2003;111:389–395.
45. Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev*. 2007;4:CD006475.
46. Chang YS, et al. Synbiotics for prevention and treatment of atopic dermatitis: meta-analysis of randomized clinical trials. *JAMA Pediatr*. 2016;170(3):236–242.
47. McFarlin BK, Henning AL, Bowman EM, et al. Oral spore-based probiotic supplementation was associated with reduced incidence of post-prandial dietary endotoxin, triglycerides, and disease risk biomarkers. *World J Gastrointest Pathophysiol*. 2017;8(3):117–126.
48. Stewart JCM, Morse PF, Moss M, et al. Treatment of severe and moderately severe atopic dermatitis with evening primrose oil (Epogram): a multi-center study. *J Nutr Med*. 1991;2:9–15.

49. Hederos CA, Berg A. Epogam evening primrose oil treatment in atopic dermatitis and asthma. *Arch Dis Child*. 1996;75:494–497.
50. Fiocchi A, Sala M, Signoroni P, et al. The efficacy and safety of gamma-linolenic acid in the treatment of infantile atopic dermatitis. *J Int Med Res*. 1994;22:24–32.
51. Berth-Jones J, Graham-Brown RA. Placebo-controlled trial of essential fatty acid supplementation in atopic dermatitis. *Lancet*. 1993;341:1557–1560.
52. Takwale A, Tan E, Agarwal S, et al. Efficacy and tolerability of borage oil in adults and children with atopic eczema: randomised, double blind, placebo controlled, parallel group trial. *BMJ*. 2003;327:1385.
53. Soyland E, Funk J, Rajka G, et al. Dietary supplementation with very long-chain n-3 fatty acids in patients with atopic dermatitis: a double-blind, multicentre study. *Br J Dermatol*. 1994;130:757–764.
54. Kremmyda LS, Vlachava M, Noakes PS, et al. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. *Clin Rev Allergy Immunol*. 2011;41(1):36–66.
55. Samochocki Z, Bogaczewicz J, Jeziorkowska R, et al. Vitamin D effects in atopic dermatitis. *JAAD*. 2013;69(2):238–244.
56. Tsoureli-Nikita E, Hercogova J, Lotti T, et al. Evaluation of dietary intake of vitamin E in the treatment of atopic dermatitis: a study of the clinical course and evaluation of the immunoglobulin E serum levels. *Int J Dermatol*. 2002;41:146–150.
57. Javanbakht MH, et al. Randomized controlled trial using vitamins E and D supplementation in atopic dermatitis. *J Dermatolog Treat*. 2011;22(3):144–150.
58. Atherton DJ, Sheehan MP, Rustin MH, et al. Treatment of atopic eczema with traditional Chinese medicinal plants. *Pediatr Dermatol*. 1992;9:373–375.
59. Sheehan MP, Rustin MH, Atherton DJ, et al. Efficacy of traditional Chinese herbal therapy in adult atopic dermatitis. *Lancet*. 1992;3:40 13–17.
60. Sheehan MP, Atherton DJ. A controlled trial of traditional Chinese medicinal plants in widespread non-exudative atopic eczema. *Br J Dermatol*. 1992;126:179–184.
61. Evans FQ. The rational use of glycyrrhetinic acid in dermatology. *Br J Clin Pract*. 1958;12:269–274.
62. Barnes B. Thyroid therapy in dermatology. *Cutis*. 1971;8:581–583.
63. Nicol NH, et al. Wet wrap therapy in children with moderate to severe atopic dermatitis in a multidisciplinary treatment program. *J Allergy Clin Immunol Pract*. 2014;2(4):400–406.
64. Huang JT, Abrams M, Tlougan B, Rademaker A, Paller AS. Treatment of *Staphylococcus aureus* colonization in atopic dermatitis decreases disease severity. *Pediatrics*. 2009;123(5):e808–e814. https://doi.org/10.1542/peds.2008-2217.
65. Jancin B. *Body Wash Offers Alternative to Bleach Baths for Atopic Dermatitis*; 2014. https://www.mdedge.com/pediatrics/article/85964/pediatrics/body-wash-offers-alternative-bleach-baths-atopic-dermatitis. Accessed 8 Sept 2019.
66. Jordan J, Whitlock F. Emotions and the skin: the conditioning of scratch responses in cases of atopic dermatitis. *Br J Dermatol*. 1972;86:574–585.
67. Kimata H. Effect of humor on allergen-induced wheal reactions. *JAMA*. 2001;14(6):738. 285.
68. Kimata H. Modulation of fecal polyamines by viewing humorous films in patients with atopic dermatitis. *Eur J Gastroenterol Hepatol*. 2010;22(6):724–728.
69. Hongo J. Japanese doctor wins Ig nobel for research on kissing. *Wall Street Journal*. 2015.
70. Itamura R, Hosoya R. *Homeopathy*. 2003;92(2):108–114.
71. Rossi E, et al. Homeopathy in paediatric atopic diseases: short- and long-term. *Homeopathy*. 2016;105(3):217–224.
72. Eizayaga JE1, Eizayaga JI. Prospective observational study of 42 patients with atopic dermatitis treated with homeopathic medicines. *Homeopathy*. 2012;101(1):21–27.
73. Roll S, et al. Comparative effectiveness of homoeopathic vs. conventional therapy in usual care of atopic eczema in children: long-term medical and economic outcomes. *PLoS One*. 2013;8(1):e54973.
74. Cadario G, et al. Sublingual immunotherapy efficacy in patients with atopic dermatitis and house dust mites sensitivity: a prospective pilot study. *Curr Med Res Opin*. 2007;23(10):2503–2506.
75. Harris JM, Williams HC, White C, et al. Early allergen exposure and atopic eczema. *Br J Dermatol*. 2007;156(4):698–704.
76. Kimata H. Enhancement of allergic skin wheal responses by microwave radiation from mobile phones in patients with atopic eczema/dermatitis syndrome. *Int Arch Allergy Immunol*. 2002;129:348–350.

151

Attention Deficit Hyperactivity Disorder

Joseph Katzinger, ND, Michael T. Murray, ND,* and *Michael R. Lyon*, MD*

OUTLINE

DIAGNOSTIC SUMMARY

- A neurodevelopmental disorder that begins in early childhood with persistence into adolescence and adulthood
- Typified by one or more symptoms of disabling inattentiveness, hyperactivity, and impulsivity
- Commonly accompanied by comorbid conditions, such as mood disorders or learning disabilities, with increased risk for multiple mental health and social difficulties

GENERAL CONSIDERATIONS

As updated in the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5), attention deficit hyperactivity disorder (ADHD) is defined as a condition characterized by developmentally appropriate inattention and impulsivity with or without hyperactivity. ADHD has three presentations:

Predominantly hyperactive-impulsive presentation:

- Most symptoms (six or more for children, five or more for adults) are in the hyperactivity-impulsivity categories.
- Fewer than six symptoms of inattention are present, although inattention may still be present to some degree.

Predominantly inattentive presentation:

- The majority of symptoms (six or more for children, five or more for adults) are in the inattention category and fewer than six symptoms of hyperactivity-impulsivity are present, although hyperactivity-impulsivity may still be present to some degree.
- Children with this presentation are less likely to act out or have difficulties getting along with other children. They may sit quietly, but they are not paying attention to what they are doing. Therefore the child may be overlooked, and parents and teachers may not notice that he or she has ADHD.

Combined presentation:

- Six or more symptoms (five or more for adults) of inattention and six or more symptoms of hyperactivity-impulsivity are present.

To be diagnosed with ADHD, an individual must have symptoms for 6 or more months and to a degree that is inconsistent with developmental level and that has a direct negative effect on social and academic/occupational activities. The same symptoms apply to adults as well as children, although the examples of how they manifest may be different.

Symptoms of inattention per the DSM-5 include the following:

- Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or during other activities (e.g., overlooks or misses details, work is inaccurate)
- Often has difficulty sustaining attention in tasks or play activities (e.g., has difficulty remaining focused during lectures, conversations, or lengthy reading)
- Often does not seem to listen when spoken to directly (e.g., mind seems elsewhere, even in the absence of any obvious distraction)
- Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., starts tasks but quickly loses focus and is easily sidetracked)

*Previous edition contributor

- Often has difficulty organizing tasks and activities (e.g., difficulty managing sequential tasks; difficulty keeping materials and belongings in order; messy, disorganized work; has poor time management; fails to meet deadlines)
- Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (e.g., schoolwork or homework; for older adolescents and adults, preparing reports, completing forms, reviewing lengthy papers)
- Often loses things necessary for tasks or activities (e.g., school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones)
- Is often easily distracted by extraneous stimuli (for older adolescents and adults, may include unrelated thoughts)
- Is often forgetful in daily activities (e.g., doing chores, running errands; for older adolescents and adults, returning calls, paying bills, keeping appointments)

Symptoms of hyperactivity per the DSM-5 may include the following:

- Often fidgets with or taps hands or feet or squirms in seat
- Often leaves seat in situations when remaining seated is expected (e.g., leaves his or her place in the classroom, in the office or other workplace, or in other situations that require remaining in place)
- Often runs about or climbs in situations where it is inappropriate (Note: In adolescents or adults, may be limited to feeling restless.)
- Often unable to play or engage in leisure activities quietly
- Is often "on the go," acting as if "driven by a motor" (e.g., is unable to be or uncomfortable being still for an extended time, as in restaurants, meetings; may be experienced by others as being restless or difficult to keep up with)
- Often talks excessively
- Often blurts out an answer before a question has been completed (e.g., completes people's sentences; cannot wait for turn in conversation)
- Often has difficulty waiting his or her turn (e.g., while waiting in line)
- Often interrupts or intrudes on others (e.g., butts into conversations, games, or activities; may start using other people's things without asking or receiving permission; for adolescents and adults, may intrude into or take over what others are doing)

ADHD characteristics are frequently associated with difficulties in school, in both learning and behavior, as well as at work among adults. If not intensively managed, a child with ADHD will likely experience academic impairment, increased risk of injuries, and problems with self-esteem and socialization. Later in adolescence and adulthood, those with ADHD have a high risk of experiencing depression or anxiety, substance abuse and addictions, traffic accidents, financial problems, vocational underachievement, and social problems. Indeed, a 2015 large cohort of Danish individuals with ADHD found a significant increase in risk for mortality among individuals with ADHD, which remained after adjustment for related conditions, such as substance abuse disorder.[1] Nevertheless, ADHD is a condition that can be transcended, and many with ADHD have achieved a high level of personal success.

Although influenced by the region, investigator, and diagnostic criteria, ADHD has been estimated to affect approximately 6% to 7% of children and 5% of adults (for whom we have prevalence data) in the United States.[2] The worldwide prevalence is slightly less, with a 2015 meta-analysis of 41 studies and 27 countries finding an overall prevalence of 3.4%.[3] The economic cost associated with ADHD has been estimated to range from $143 billion to $266 billion per year in the United States alone.[4] Clinical observation and epidemiological surveys typically report a greater incidence in boys than girls (approximately 3–4:1), although this gap may to some degree be due to less teacher-based referrals, for whom boys' behavior is seen as more disruptive. Approximately 3.5% of children in the United States take at least one prescription drug for ADHD every day, a rate consistently higher than other Western countries.[5] Onset is usually by 3 years of age, although the diagnosis is often not made until later when the child is in school.[6]

Etiology

The behavioral and cognitive manifestations of ADHD arise from evidence associating this disorder with diminished function of polysynaptic dopaminergic circuits belonging to executive centers within the brain's prefrontal cortex.[7,8] These executive centers are largely inhibitory in nature and are responsible for impulse control and the ability to maintain sustained attention.[9]

Evidence from studies using magnetic resonance imaging (MRI), positron emission tomographic (PET) scanning, single-photon emission computed tomographic scan imaging, and electroencephalograms (EEGs) suggests that the brains of those with ADHD exhibit differences both morphologically and metabolically from normal controls, particularly with regard to the prefrontal executive centers.[10,11] Various disturbances in dopaminergic activity within these brain centers have remained the primary molecular defects implicated in ADHD, although other neurotransmitters (particularly norepinephrine) have also been incriminated. Decreased sensitivity of the dopaminergic (D4) receptor and heightened dopamine reuptake by the presynaptic dopamine transporter were both suggested to result in diminished dopaminergic activity within executive centers. More recently, task-activated functional MRI (fMRI) suggests that functional deficiencies may be more widespread throughout the brain, in multiple neuronal networks, and not just limited to executive function. For example, a meta-analysis of 55 fMRI studies found that ADHD may be better described "as a disorder underpinned by dysfunctions in multiple large-scale brain networks." This includes both hypoactivation, such as observed in the frontoparietal executive control network, putamen, and ventral attention network, as well as potentially compensatory hyperactivation in the default network and visual circuits.[12] Other areas where deficits have been found include temporal information processing and timing, speech and language, memory span, processing speed and response time variability, arousal and activation, and motor control.[13] However, all of these defects are not necessarily permanent, because as children with ADHD grow up, the brain may develop a normal level of functional activity, and the anatomical variations lessen. In addition, ADHD symptoms also tend to improve.[14]

Genetic factors play a significant role in the etiology of ADHD.[15] Twin studies suggest that ADHD has a heritability of 70% to 80% in both children and adults, and parents and siblings of those with ADHD have a five- to tenfold elevated risk themselves.[13] Differences have been identified in genes encoding for both the D2 and the D4 dopamine receptors in ADHD. Other studies have linked ADHD with genetic polymorphisms associated with increased activity of the presynaptic dopamine transporter (which would result in increased uptake of dopamine). The gene most frequently implicated in GWAS studies is CDH13, which is involved in cell adhesion and neurodevelopmental processes. However, recent analyses suggest that although up to 70% of the genetic component of ADHD may be explained by common variants, the effect of any single single-nucleotide polymorphism (SNP) or copy-number variation is very small and should be viewed as a polygenic combination, comprising both common and rare variants.[16] Other genetic factors contributing to ADHD may include inherited tendencies toward allergic states, decreased immune competence, and various genetic polymorphisms, resulting in a diminished capacity to detoxify drugs, heavy metals, and xenobiotics. In other

words, although there is little doubt that genetics are a predisposing factor, like most health conditions, environmental and dietary factors may play a significant role in how and whether these genetic factors manifest to produce ADHD in an individual.

THERAPEUTIC CONSIDERATIONS

Conventional Drugs

The role of nutritional and environmental factors as the underlying cause of ADHD has been increasingly recognized. Despite significant advances in the use of nutritional therapies for ADHD, the prevailing conventional approach to the treatment of ADHD relies almost entirely on amphetamine drugs for purely symptomatic relief. These drugs, like Ritalin (methylphenidate), Adderall (amphetamine and dextroamphetamine), Concerta (methylphenidate), and Vyvanse (lisdexamfetamine dimesylate), improve ADHD symptoms primarily by potentiating the neurotransmitter dopamine within all brain regions, including those affected in ADHD. These medications reportedly improved behavior and cognitive functioning in approximately 75% of children in formal placebo-controlled trials. However, the success of treatment when studied in actual clinical practice might be significantly lower. Furthermore, follow-up studies failed to demonstrate long-term benefits with these stimulant medications. Additionally, these drugs are associated with a high prevalence of adverse effects, such as decreased appetite, sleep problems, anxiety, and irritability. Some of the long-term effects of these drugs could be extremely detrimental to both brain function and behavior.[17–19] For example, one of the largest and longest-term studies, the Multimodal Treatment Study of Children with ADHD (MTA), found that despite initial reports of treatment success, the long-term effectiveness was more questionable, and the risk of side effects, including growth suppression in children, may have been underappreciated.[20]

Nonstimulant drugs like atomoxetine (Strattera) have been promoted as a safe alternative to parents. However, the drug has its own set of problems, including studies that showed that children and teenagers who took atomoxetine were more likely to have suicidal thoughts than children and teenagers with ADHD who did not take it.[21]

The bottom line is that every effort should be made to treat ADHD without the long-term reliance on medications.

One of the primary goals in many cases of ADHD treatment is to enhance cognitive function. Perhaps up to 50% of people with ADHD have definable learning disabilities, and most have measurable cognitive disturbances. Particularly disabling is the diminishment in nonverbal working memory exhibited by most patients with ADHD.[22,23] This feature of ADHD results in a diminished sense of time, as well as a decreased ability to hold events or tasks in the mind. Tardiness, missed appointments, procrastination, poor task planning, and failure to meet deadlines are all examples of how diminished working memory can result in serious consequences, particularly in adulthood.

ENVIRONMENTAL NEUROTOXINS

Environmental factors that contribute to the development of ADHD may begin at or even before conception. Maternal-to-fetal transport of various neurotoxins can occur readily during pregnancy. A woman who has an ongoing exposure to or a significant body burden of neurotoxic substances (e.g., heavy metals like lead and mercury, solvents, pesticides, polychlorinated biphenyls [PCBs], alcohol, or other drugs of abuse) may herself exhibit features consistent with ADHD and give birth to a child who presents with symptoms of ADHD. In such cases, it might be assumed that ADHD is inherited when it is actually acquired. Children remain susceptible to neurotoxins after birth, and some of these agents have been shown to be common among children in North America.[24,25]

Polybrominated Diphenyl Ethers

For example, exposure to polybrominated diphenyl ethers (PBDEs) is fairly common among children, and both prenatal and postnatal exposure have been associated with increases in hyperactive and aggressive behavior, as well as decreased attention and executive function, and it may be that PBDEs potentially target the prefrontal cortex.[26]

Perfluorooctane sulfonate and β-Hexachlorocyclohexane

In a large Norwegian birth cohort, early life exposure (e.g., breast milk concentrations) to perfluorooctane sulfonate (PFOS) and β-hexachlorocyclohexane (β-HCH) were each associated with nearly a twofold increase in the risk for ADHD diagnosis by a median age of 13 years.[27]

Bisphenol A

Early exposure to bisphenol A (BPA) was also found to be associated with ADHD, as found in a 2018 systematic review and meta-analysis of existing data.[28]

Maternal Drug Use

Maternal tobacco and drug use have also been associated with a higher risk of ADHD.[29–31] One study suggested that up to 25% of all behavioral disorders in children can be attributed to exposure to cigarette smoking during pregnancy.[29] In addition, long-term, low-level lead intoxication in North American children was reported to exist at an alarmingly high incidence.[25,32]

Lead

The Centers for Disease Control and Prevention estimated that about 2% of American children younger than age 6 currently meet the criteria for lead toxicity at a level that has been associated with cognitive deficits and behavioral disturbances (more than 10 ug/dL whole blood lead).[32] Low-level lead intoxication has also been associated with addictive behaviors and impulsivity, suggesting neurological changes consistent with the reward deficiency syndrome, as described earlier.[33] And recent data suggest that a cutoff of 10 ug/dL is not protective; a 2017 systematic review and meta-analysis found that even levels as low as <3 ug/dL were associated with significant increases in ADHD symptoms.[34] A recent analysis of 75 participants with ADHD also found an interaction between lead exposure and the dopamine receptor D2 receptor (DRD2) gene on frontal lobe cortical thickness, suggesting a toxin–gene interaction that affects brain morphology.[35] In keeping with these data, pilot studies demonstrated improvement in ADHD behaviors in some children with moderate elevations in blood lead levels who were treated with intravenous ethylenediaminetetraacetic acid chelation.[36]

Other Metals

In addition to lead, other toxic metals, such as mercury, cadmium, and aluminum, as well as pesticides and PCBs, are nearly ubiquitous contaminants arising from dental amalgams, food, air, and drinking water, and these agents may act synergistically to impair neurological function and development in susceptible children. The Consumer's Union of the United States conducted a large study looking at the level of human exposure to a wide range of pesticides in the U.S. food supply. In this startling report, it was demonstrated that human exposure to pesticides is far greater than ever previously estimated and that children are at particularly high risk for neurotoxic effects from regular inadvertent pesticide exposure from common foods.[37]

Organophosphate Pesticides

Quite concerning is that an analysis of National Health and Nutrition Examination Survey (NHANES) data suggests that children with levels only higher than the median for a common urinary metabolite of organophosphate pesticides (dimethyl alkylphosphate [DMAP]) have roughly twice the odds of developing ADHD as those with undetectable levels.[38] Interactions between organophosphate pesticides and genetic polymorphisms have also been observed; a study conducted in Taiwan found that not only did children with ADHD have higher levels of an organophosphate pesticide metabolite (dimethylphosphate [DMP]), but also those who had higher exposure, a polymorphism in the dopamine receptor D4 gene (DRD4), and higher levels of oxidative stress had a nearly twelvefold risk for developing ADHD.[39]

DIETARY AND LIFESTYLE FACTORS

Food Additives, Sugar, and the Feingold Hypothesis

The hypothesis that food additives can cause hyperactivity in children stemmed from the research of Benjamin Feingold, MD, and is commonly referred to as the "Feingold hypothesis." According to Feingold, many hyperactive children, perhaps 40% to 50%, are sensitive to artificial food colors, flavors, and preservatives.[40]

Feingold's claims were based on his experience with more than 1200 cases in which food additives were linked to learning and behavior disorders. Since Feingold's presentation to the American Medical Association in 1973, the role of food additives as a contributing cause of hyperactivity has been hotly debated in the scientific literature. In actuality, however, researchers focused on only 10 food dyes versus the 3000 food additives with which Feingold was concerned.

At first glance, it appears that the majority of the double-blind studies designed to test the hypothesis showed essentially negative results. However, upon closer examination of these studies and further investigation into the literature, it becomes evident that food additives do play a major role in hyperactivity.[41–43] Overwhelming evidence was produced in several of these studies.

In a more recent study, 153 3-year-old and 144 8- to 9-year-old children from the general population (in other words, the study was not conducted on children with the specific diagnosis of ADHD) were challenged with either a drink that contained sodium benzoate and an artificial food coloring mix or a placebo mix. The main outcome measure was a global hyperactivity aggregate, based on observed behaviors and ratings by teachers and parents, plus, for the 8- to 9-year-old children, a computerized test of attention. The results showed that the children given the artificial food coloring agents definitely had a statistically significant adverse reaction on hyperactivity and behavior. The results were so clear that the authors concluded that "Artificial colors or a sodium benzoate preservative (or both) in the diet result in increased hyperactivity in 3-year-old and 8/9-year-old children in the general population."[44]

It is interesting to note that although the U.S. studies were largely negative, the reports from the United Kingdom, Australia, and Canada were more supportive. Feingold contended that there is a conflict of interest on the part of the Nutrition Foundation, an organization supported by the major food manufacturers—Coca-Cola, Nabisco, General Foods, and so forth. It appears significant that the Nutrition Foundation financed most of the negative studies. The conflict of interest arises because these companies would suffer economically if food additives were found to be harmful. Other countries have significantly restricted the use of artificial food additives because of the possible harmful effects.

In looking at all of the data on the role of food additives in ADHD, the following conclusions can be made: virtually Every study (both negative and positive) demonstrated that some hyperactive children consistently react with behavioral problems when challenged by specific food additives or specific foods. Critics of the hypothesis ignore the significance of the clear (reproducible under double-blind conditions) individual results. The bottom line is that some children definitely react quite strongly to foods or food additives, warranting the exclusion of these compounds for at least 10 days to judge their significance in all cases of ADHD.

In addition to eliminating food additives, there appears to be an interrelationship between sugar consumption and artificial food dyes. One study demonstrated that destructive-aggressive and restless behavior significantly correlated with the amount of sugar consumed.[45] The higher the sugar intake, the worse the behavior. In another study, researchers performed 5-hour oral glucose tolerance tests on 261 hyperactive children, with the result that 74% displayed abnormal glucose tolerance or hypoglycemia.[46] There is also a positive side to this relationship; in 2017 *Pediatrics* published results showing not only were sugar, candy, cola, and similar dietary factors associated with a ADHD diagnosis, but lower adherence to the Mediterranean diet was associated with a risk for ADHD of over sevenfold.[47] This strongly suggests that changing dietary patterns may have a significant effect.

Individual Nutrients and Attention Deficit Hyperactivity Disorder

Essential Fatty Acids

Numerous studies have shown that children with ADHD have a measurable reduction in tissue levels of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared with age-matched controls. The ratio of omega-3 to omega-6 is also likely to be lower, which may be as or more significant than omega-3 levels alone. A 2016 meta-analysis of studies that measured both omega-3 and omega-6 levels found elevated levels of omega-6/omega-3, particularly the ratio of arachidonic acid to eicosapentaenoic acid (AA/EPA).[48] Omega-3 (EPA+DHA) supplementation in ADHD has been studied extensively and is considered a sensible intervention even by many mainstream physicians. Of particular importance is the recognition that omega-3 fatty acid supplementation improves many symptoms of ADHD, including impulsive-oppositional behavior, a symptom not typically helped by the pharmaceutical treatment of ADHD.[49] A 2017 randomized trial also found that omega fatty acid supplementation had similar benefits to methylphenidate and that adding omega fatty acids to methylphenidate reduced the adverse effects of the drug.[50]

Because these omega-3 fatty acids are critical in the structure and function of brain cells, it is thought that low levels play a key role in ADHD. The nerve endings in the areas of the brain affected in ADHD should be rich in DHA because they are highly fluid and should be composed of approximately 80% DHA. DHA deficiency has also been shown to result in increased permeability of the blood–brain barrier in animal studies and is thought to play a critical role in protecting the brain from an influx of neurotoxic compounds.[51,52]

Human breast milk is also rich in DHA, and several studies suggested that formula-fed children are at twice the risk of developing ADHD as those who are breastfed.[53] The role of DHA in brain development, intelligence, and possible protection against ADHD finally led to its inclusion in many infant formulas and other foods.

Besides fatty acids, inadequate provision of other nutrients during fetal development and early childhood may also play a significant role in the development of ADHD as well as its treatment.[54,55] In addition, children with ADHD often show multiple nutrient deficiencies, highlighting the importance of broad-spectrum nutritional support. In particular, the following minerals are critical supplements in the management of ADHD.

Magnesium

Magnesium levels in serum, red blood cells, and hair were all shown to be low in the majority of children with ADHD.[56] These children also demonstrated improved behavior when administered magnesium supplements.[57]

Zinc

Lower hair and serum zinc levels were shown to frequently accompany ADHD.[58] Those children with low serum zinc were also more frequently found to have lower levels of free fatty acids, suggesting that abnormalities in fatty acid metabolism may, at least in part, result from zinc deficiency.[59] It was also shown that lower hair zinc levels correlated with a poorer response to treatment with amphetamines. Several clinical trials demonstrated the positive effects of zinc supplementation in hyperactive children.[60,61] An elevated copper/zinc ratio has also been observed.[62]

Iron

Anemia from iron deficiency is estimated to affect approximately 20% of infants, and many more are thought to have milder iron deficiencies without anemia, leaving them at risk for impairment of brain development.[63,64] Iron deficiency was found to be even much more common in children with ADHD. One study demonstrated that iron supplementation in nonanemic children with ADHD resulted in diminished ADHD symptoms within 30 days.[65] A more recent study demonstrated improvement in ADHD symptoms in children with ADHD and low iron stores.[66] A 2018 systematic review and meta-analysis found both lower ferritin levels among children with ADHD and more severe symptoms among those with iron deficiency.[67] It has also been suggested that iron deficiency may exacerbate lead exposure toxicity.

Vitamin D

Lower serum levels of vitamin D have been observed among children with ADHD compared with healthy controls.[68] An inverse association between cord vitamin D levels and ADHD in toddlers has also been observed.[69] Although clinical trials of vitamin D supplementation alone have not yet been conducted, when given as an adjunct to methylphenidate, vitamin D has been shown to improve some symptoms of ADHD.[70]

Food Allergy

There is a very strong relationship between allergies and ADHD, including food allergies.[71–75] In one study, demonstrable brainwave EEG changes occurred immediately after ingestion of a previously identified food allergy.[75] Food allergies and other allergic disorders were also associated with a higher incidence of recurrent ear infections (otitis media).[76] In turn, recurrent otitis media were associated with an increased risk of ADHD.[77] Both food allergy and ADHD were associated with sleep disturbances, which may, in turn, contribute to a worsening of ADHD symptoms. Heavy snoring and sleep apnea are particularly prevalent in allergic children and may significantly contribute to ADHD symptoms.[78,79] Studies demonstrated improved sleep in children with ADHD during a low-allergy potential (oligoantigenic) diet. Food allergy elimination or desensitization can be as effective as drug therapy in reducing ADHD symptoms.[80–84] In 2011 the *Lancet* published the results of the Impact of Nutrition on Children With ADHD (INCA) study, a randomized controlled trial conducted in the Netherlands and Belgium, and found that a strict elimination diet had a significant benefit on symptoms in 64% of children. Although immunoglobulin G (IgG) antibodies were not predictive of which foods induced symptoms, reintroduction of bothersome foods did induce symptoms, leading the authors to conclude that "dietary intervention should be considered in all children with ADHD, provided parents are willing to follow a diagnostic restricted elimination diet for a 5-week period, and provided expert supervision is available."[85]

Obstructive Sleep Apnea

It has been repeatedly demonstrated that attentional deficits are exceedingly common (95%) in both children and adults with obstructive sleep apnea (OSA).[86] Likewise, OSA is found in up to 30% of children diagnosed with ADHD, and this may be central to the etiology of their attentional problems. In children, chronic nasal or tonsillar/pharyngeal congestion is the most common etiological factor leading to OSA, although childhood obesity is also becoming a leading cause. OSA should be carefully considered in all children with ADHD, and health care providers should be familiar with the signs and symptoms suggestive of OSA as well as the local resources available for diagnosis and treatment. Children and parents will usually be unaware of the presence of OSA. However, clues such as nocturnal mouth breathing, snoring, enuresis, daytime urinary incontinence, and daytime drowsiness should strongly suggest a need to rule out OSA, usually by referring the patient for nocturnal oximetry testing or polysomnography. Because questionnaires and physical examination are relatively insensitive to pick up OSA in children, a high index of suspicion is essential. OSA results in severe sleep fragmentation and frequent hypoxic episodes, which, in turn, reduces a child's intellectual capacity, reduces attentional performance, and diminishes the child's quality of life. Children with sleep-disordered breathing should be treated. Efforts should be made to reduce nasal congestion and decrease recurrent upper respiratory infections. However, in those children with OSA who do not clearly respond to conservative measures, tonsilloadenoidectomy has been proven to have a substantial and sustainable benefit in ADHD symptoms and comorbidities. Parents need to be made aware of the substantial risks of untreated OSA versus the risks and/or benefits of this surgery.[87]

Dysbiosis, Gastrointestinal Permeability, and Intestinal Parasites

Probiotic supplementation with *Bifidobacteria* and *Lactobacilli* may be helpful in the treatment of ADHD. These organisms function as part of the first line of defense in gut immunity and have been shown to restore the altered gut permeability due to food allergies.[88,89] In addition, a stool analysis to rule out parasitical infection or altered bacterial flora appears advisable due to disturbances in gut mucosal immunity (discussed in the following). Interestingly, in a clinical trial in which 75 infants were given *Lactobacillus rhamnosus GG* or placebo during the first 6 months of life, by age 13, 17.1% of those given placebo developed either ADHD or Asperger's syndrome, whereas none in the probiotic group did. Healthy children also had higher levels of fecal *Bifidobacterium* during the first 6 months of life.[90]

IMMUNE SYSTEM IMPAIRMENT

It has been shown that subtle immune dysfunction may be a prominent problem in ADHD. Both cellular and humoral immunity were shown to be abnormal in children with ADHD compared with age-matched controls.[91] Plasma complement levels were found to be lower in children with ADHD.[92] Immune dysfunction in ADHD may be either directly inherited or may be a result of nutritional, toxicological, or atopic factors. ADHD may also be, in part, an autoimmune disorder. Antineural antibodies were found in the blood and cerebrospinal fluid in ADHD.[93,94] ADHD has been associated with a number of autoimmune conditions, including multiple sclerosis, type 1 diabetes, and inflammatory bowel disease.[95,96] Gut mucosal immunity may also be significantly impaired in ADHD, leading to increased susceptibility to gut pathogens and food allergies.

COGNITIVE-BEHAVIORAL THERAPIES

All children exhibiting symptoms suggestive of ADHD should undergo thorough neurobehavioral and cognitive assessment. Many of the features often associated with gifted children can be mistaken for symptoms of ADHD. Likewise, children with mood disorders or learning disabilities, those from abusive environments, and those who have suffered serious trauma may benefit from specific psychological or cognitive-behavioral interventions and may not respond appropriately to ADHD-specific pharmacotherapy. In addition, cognitive-behavioral therapies that can be implemented in schools and in the home environment have been shown to be efficacious in the treatment of ADHD.[97] Cognitive-behavioral therapies also appear to be as effective in older adults as they are in children.[98]

NEUROFEEDBACK

In neurofeedback (EEG biofeedback) treatment, individuals are provided with real-time feedback about their brainwave activity through electronic instrumentation. This feedback allows the subject to learn self-regulation of brainwave intensity and frequency. Measurements of brain activity in many individuals with ADHD demonstrate cortical slowing and diminished brainwave intensity in the prefrontal region and frontal lobes. Neurofeedback treatment is designed to train individuals to increase the production of brainwave patterns that reduce or eliminate this cortical slowing and thus reduce or eliminate many associated ADHD symptoms. Evidence supporting neurofeedback as an effective treatment in ADHD is accumulating, including studies showing cessation of methylphenidate (Ritalin) in children without loss of treatment effect.[99–103]

Rapid transcranial magnetic stimulation, a treatment used in many centers as a safer alternative to electroconvulsive therapy, has begun to show promise in the treatment of a variety of neuropsychiatric conditions. This treatment may hold promise in the treatment of ADHD, particularly in those children with more severe forms of the disorder, with serious comorbidities, or in those who are resistant to treatment.[104]

BOTANICAL MEDICINES

Procyanidolic Oligomers

Extracts from grape seed skin and the bark of the maritime pine such as Pycnogenol are rich sources of procyanidolic oligomers—one of the most beneficial groups of plant flavonoids. These extracts may prove useful in the treatment of ADHD due to their broad-spectrum antioxidant effects alone, because increased oxidative damage is believed to be a central factor in ADHD.

To date, four studies have been conducted in ADHD with Pycnogenol. In two of the studies, children with ADHD supplemented with Pycnogenol (1 mg/kg body weight per day) showed improved antioxidant status.[105,106] A third study not only confirmed this antioxidant effect but also showed improvements in hyperactivity.[107] In the most detailed study, 61 children with ADHD supplemented with 1 mg/kg/day Pycnogenol or placebo over 4 weeks showed that 1-month Pycnogenol administration caused a significant reduction of hyperactivity and improved attention and visual-motor coordination and concentration in children with ADHD.[108] No positive effects were found in the placebo group. These results point to an option to use grape seed or pine bark extract as a nutritional adjunct in ADHD.

Ginkgo biloba Extract

Two early pilot studies, one in children (in combination with American ginseng) and the other in adults, showed some beneficial effects attributed to supplementation with *Gingko biloba* extract.[109,110] Specifically, these studies showed improvements in inattentiveness, hyperactivity, and socialization. A more recent randomized trial found that both teacher and parent ratings improved when *Gingko* was given with methylphenidate versus methylphenidate and placebo.[111]

L-Theanine

L-theanine, an amino acid found in green tea, shows promise in improving sleep quality in children with ADHD. This amino acid is known to reduce anxiety, increase concentration, improve sleep quality, and stabilize mood without sedation.[112,113] It also improves cerebral dopaminergic activity. In a recently completed double-blind, placebo-controlled trial on L-theanine in ADHD, 90 boys with ADHD were found to have significant improvements in sleep quality as measured by nocturnal actigraphy with 400 mg L-theanine a day.[114]

THERAPEUTIC APPROACH

Many alternative and complementary therapies have been offered for the treatment of ADHD in children and adults. Some have good evidence for safety and efficacy, whereas the evidence for others is still lacking. A reasonable approach with a high probability of success will be multimodal and will address as many contributing factors as are practical. A basic workup ruling out common disorders that may manifest as ADHD is prudent because these may require specific treatments, and delay of treatment may be harmful. Celiac disease, depression, lead poisoning, sleep apnea, and visual or auditory problems are some of the problems that should be included in the differential diagnosis. Other contributory factors, such as increased intestinal permeability; food allergies or intolerances; and iron, magnesium, zinc, vitamin D, and omega-3 fatty acid deficiencies, may be assessed through laboratory testing or simply treated empirically if financial constraints prevent ordering noninsured tests.

In general, a stepwise, rational approach is often successful.

Step 1

Assisting parents in eliminating junk foods, fast foods, and food additives from the diet (especially sugary foods and beverages, white flour products, and deep-fried foods) while increasing the quantity of whole foods is an important first step. If a child consumes more whole grains, fruits, vegetables, clean protein sources, pure water, and fiber, health benefits are inevitable. In some cases, converting to a whole-foods, Mediterranean–based diet can result in marked improvements in behavior and cognitive performance. The initial adoption of a very restrictive elimination diet for at least 5 weeks, under nutritional supervision, is also likely to improve symptoms and identify problematic foods following food reintroduction. For those ready to navigate the difficulties of this diet, it may be an excellent starting point.

All sources of environmental toxin exposure need to be identified and eliminated.

Step 2

Nutritional supplementation is often helpful and usually includes a pharmaceutical-grade fish oil supplement or DHA/EPA concentrate; high-potency multivitamin/trace mineral supplement; additional calcium, magnesium, vitamin D, and zinc (additional iron is also beneficial in children and adolescents if anemia or low iron stores are documented); and potent antioxidant support (e.g., pine bark [Pycnogenol] or grape seed extract). Although there is evidence to support each of these interventions, when combined, as well as in the context of a whole-foods diet, the results are usually significant.

Step 3

Gastrointestinal rehabilitation is a concept familiar to practitioners of natural medicine. This involves therapeutic steps to reduce intestinal permeability, improve nutrient absorption, and increase the immune response to gut pathogens while diminishing hypersensitivities. A useful approach follows the mnemonic "ANT PIE" (abstain, nourish, toxins/detoxification, probiotics, identify, eliminate).

Abstain from junk foods, deep-fried foods, sugary drinks, and other foods that harm or irritate the gut, as well as unnecessary drugs or excessive alcohol.

Nourish the digestive tract with nutrients that support gut healing. Functional foods that combine low-allergy-potential protein and a number of nutrients helpful for gastrointestinal healing are readily available.

Toxins/detoxification refers to the avoidance of pesticides by eating organic foods and consuming nutrients that improve the efficiency of detoxification processes, such as L-glutamine, *N*-acetylcysteine, dietary fiber, and cruciferous vegetables. Regular exercise and stress reduction are also important parts of this step.

Probiotics are therapeutic bacteria (and some yeasts) that may improve the gastrointestinal microecology, improve immune response toward gut pathogens, reduce immune hypersensitivities, and stimulate gut repair.

Identify allergenic or intolerant foods and gut pathogens. Food allergies (type I or immediate hypersensitivity food reactions) can be identified by skin-prick testing, radioallergosorbent testing, or enzyme-linked immunosorbent assay testing for immunoglobulin-E antifood antibodies. Patients with evidence of type I hypersensitivity food allergies (e.g., postprandial swelling of the lips, urticaria, wheezing) should be referred to an allergist for definitive testing, because serious anaphylaxis could occur without proper avoidance. Delayed hypersensitivity reactions (usually type III mediated) are best identified with a properly conducted elimination test diet, followed by carefully observed reintroductions of individual food items eliminated in the test diet. IgG antifood antibodies were not found to be predictive in the INCA study.

Intestinal parasites, yeast, and pathogenic bacteria may also be identified by stool testing, *Candida* and *Helicobacter pylori* serology, breath testing, and urinary organic acids.

Eliminate foods found to be allergic or intolerant with elimination test diet. Gut pathogens, parasites, and *Candida* overgrowth may be treated.

This approach to gut rehabilitation follows a rational order that provides for optimal physiological responses to treatment. The results of following this program to its completion in children with ADHD can be quite dramatic.

Step 4

Nutraceuticals can often provide additional improvements in behavior and cognition and may be a necessary intervention earlier on if parents are pressured to submit their child to drug intervention. For example, *G. biloba* extract and/or L-theanine may provide some benefit. Cognitive-behavioral therapy should also be initiated, because it is likely to have a more sustained effect than pharmaceutical interventions.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Dalsgaard S, Østergaard SD, Leckman JF, et al. Mortality in children, adolescents, and adults with attention deficit hyperactivity disorder: a nationwide cohort study. *Lancet*. 2015;385(9983):2190–2196. PMID:25726514.
2. Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. *Neurotherapeutics*. 2012;9(3):490–499. PMID:22976615.
3. Polanczyk GV, Salum GA, Sugaya LS, et al. Annual research review: a meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *J Child Psychol Psychiatry*. 2015;56(3):345–365. PMID:25649325.
4. Doshi JA, Hodgkins P, Kahle J, et al. Economic impact of childhood and adult attention-deficit/hyperactivity disorder in the United States. *J Am Acad Child Adol Psychiat*. 2012;51:990–1000. PMID:23021476.
5. Bachmann CJ, Wijlaars LP, Kalverdijk LJ, et al. Trends in ADHD medication use in children and adolescents in five western countries, 2005-2012. *Eur Neuropsychopharmacol*. 2017;27(5):484–493. PMID:28336088.
6. Centers for Disease Control and Prevention (CDC). Increasing prevalence of parent-reported attention-deficit/hyperactivity disorder among children—United States, 2003 and 2007. *MMWR Morb Mortal Wkly Rep*. 2010;59:1439–1443.
7. Giedd J, Castellanos F, Casey B, et al. Quantitative morphology of the corpus callosum in attention deficit hyperactivity disorder. *Am J Psychiatry*. 1994;151:665–669.
8. Levy F. The dopamine theory of attention deficit hyperactivity disorder (ADHD). *Aust N Z J Psychiatry*. 1991;25:277–283.
9. Barkley R. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull*. 1997;121:65–94.
10. Amen D, Carmichael B. High-resolution brain SPECT imaging in ADHD. *Ann Clin Psychiatry*. 1997;9:81–86.
11. Lazzaro I, Gordon E, Whitmont S, et al. Quantified EEG activity in adolescent attention deficit hyperactivity disorder. *Clin Electroencephalogr*. 1998;29:37–42.
12. Cortese S, Kelly C, Chabernaud C, et al. Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. *Am J Psychiatry*. 2012;169(10):1038–1055. PMID:22983386.
13. Faraone SV, Asherson P, Banaschewski T, et al. Attention-deficit/hyperactivity disorder. *Nat Rev: Disease Primers*. 2015;1:15020. PMID:27189265.
14. Shaw P, Gornick M, Lerch J, et al. Polymorphisms of the dopamine D4 receptor, clinical outcome and cortical structure in attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry*. 2007;64:921–931.
15. Khan SA, Faraone SV. The genetics of attention-deficit/hyperactivity disorder: a literature review of 2005. *Curr Psychiatry Rep*. 2006;8:393–397.
16. Akutagava-Martins GC, Rohde LA, Hutz MH. Genetics of attention-deficit/hyperactivity disorder: an update. *Expert Rev Neurother*. 2016;16(2):145–156. PMID:26651394.
17. Wigal SB. Efficacy and safety limitations of attention-deficit hyperactivity disorder pharmacotherapy in children and adults. *CNS Drugs*. 2009;23(suppl 1):21–31.
18. Berman S, Kuczenski R, McCracken J, London E. Potential adverse effects of amphetamine treatment on brain and behavior: a review. *Mol Psychiatry*. 2009;14:123–142.
19. Advokat C. Update on amphetamine neurotoxicity and its relevance to the treatment of ADHD. *J Attention Disorders*. 2007;11:8–16.
20. Hinshaw SP, Arnold LE the MTA Cooperative Group. Attention-deficit hyperactivity disorder, multimodal treatment, and longitudinal outcome: evidence, paradox, and challenge. *WIREs Cognitive Science*. 2015;6:39–52. PMID:26262927.
21. Bangs ME, Tauscher-Wisniewski S, Polzer J, et al. Meta-analysis of suicide-related behavior events in patients treated with atomoxetine. *J Am Acad Child Adolesc Psychiatry*. 2008;47:209–218.
22. Denckla M. Biological correlates of learning and attention: what is relevant to learning disability and attention-deficit hyperactivity disorder? *J Dev Behav Pediatr*. 1996;17:114–119.
23. Barkley R. Attention-deficit/hyperactivity disorder, self-regulation, and time: toward a more comprehensive theory. *J Dev Behav Pediatr*. 1997;18:271–279.
24. Xu X, Nembhard W, Kan H, et al. Urinary trichlorophenol levels and increased risk of attention deficit hyperactivity disorder among U.S. school-aged children. *Occup Environ Med*. 2011;68:557–561.
25. Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect*. 2010;118:1654–1667.
26. Vuong AM, Yolton K, Xie C, et al. Childhood polybrominated diphenyl ether (PBDE) exposure and neurobehavior in children at 8 years. *Environ Res*. 2017;158:677–684. PMID:28734254.
27. Lenters V, Iszatt N, Forns J, et al. Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: a multi-pollutant analysis of a Norwegian birth cohort. *Environ Int*. 2019;125:33–42. PMID:30703609.
28. Rochester JR, Bolden AL, Kwiatkowski CF. Prenatal exposure to bisphenol A and hyperactivity in children: a systematic review and meta-analysis. *Environ Int*. 2018;114:343–356. PMID:29525285.
29. Milberger S, Biederman J, Faraone S, et al. Further evidence of an association between attention-deficit/hyperactivity disorder and cigarette smoking: findings from a high-risk sample of siblings. *Am J Addict*. 1997;6:205–217.
30. Williams G, O'Callaghan M, Najman J, et al. Maternal cigarette smoking and child psychiatric morbidity: a longitudinal study. *Pediatrics*. 1998;102:e11.
31. Froehlich T, Lanphear B, Kahn R, et al. Association of tobacco and lead exposures with attention-deficit/hyperactivity disorder. *Pediatrics*. 2009;124:e1054–e1063.
32. Centers for Disease Control and Prevention. Preventing Lead Poisoning in Young Children. www.cdc.gov/nceh/lead/publications/prevleadpoisoning.pdf. Accessed February 14, 2012.
33. Brockel B, Cory SD. Lead, attention, and impulsive behavior: changes in a fixed-ratio waiting-for-reward paradigm. *Pharmacol Biochem Behav*. 1998;60:545–552.
34. He J, Ning H, Huang R. Low blood lead levels and attention-deficit hyperactivity disorder in children: a systematic review and meta-analysis. *Environ Sci Pollut Res Int*. 2017. PMID:28780688.
35. Kim JI, Kim JW, Lee JM, et al. Interaction between DRD2 and lead exposure on the cortical thickness of the frontal lobe in youth with attention-deficit/hyperactivity disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;82:169–176. PMID:29169998.
36. David O, Hoffman S, Sverd J, et al. Lead and hyperactivity: behavioral response to chelation: a pilot study. *Am J Psychiatry*. 1976;133:1155–1158.
37. Kenney J, Groth E, Benbrook C. *Worst first: high risk insecticide uses, children's foods and safer alternatives*. Consumer's Union of the United States: Washington, DC.
38. Bouchard MF, Bellinger DC, Wright RO, et al. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. *Pediatrics*. 2010;125(6):e1270–e1277. PMID:20478945.
39. Chang CH, Yu CJ, Du JC, et al. The interactions among organophosphate pesticide exposure, oxidative stress, and genetic polymorphisms of dopamine receptor D4 increase the risk of attention deficit/hyperactivity disorder in children. *Environ Res*. 2018;160:339–346. PMID:29054088.
40. Feingold N. *Why your child is hyperactive*. New York, NY: Random House; 1975.
41. Schauss A. Nutrition and behavior. Complex interdisciplinary research. *Nutr Health*. 1984;3:9–37.
42. Rippere V. Food additives and hyperactive children: a critique of Conners. *Br J Clin Psych*. 1983;22:19–32.
43. Rimland B. The Feingold diet: an assessment of the reviews by Mattes, by Kavale and Forness and others. *J Learn Disabil*. 1983;16:331–333.
44. McCann D, Barrett A, Cooper A, et al. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet*. 2007;370:1560–1567.

45. Prinz R, Roberts W, Hantman E. Dietary correlates of hyperactive behavior in children. *J Consult Clin Psych*. 1980;48:760–769.
46. Langseth L, Dowd J. Glucose tolerance and hyperkinesis. *Food Cosmet Toxicol*. 1978;16:129–133.
47. Ríos-Hernández A, Alda JA, Farran-Codina A, et al. The Mediterranean diet and ADHD in children and adolescents. *Pediatrics*. 2017;139(2). pii: e20162027. PMID:28138007.
48. LaChance L, McKenzie K, Taylor VH, et al. Omega-6 to Omega-3 fatty acid ratio in patients with ADHD: a meta-analysis. *J Can Acad Child Adolesc Psychiatry*. 2016;25(2):87–96. PMID:27274744.
49. Bloch M, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry*. 2011;50:991–1000.
50. Barragán E, Breuer D, Döpfner M. Efficacy and safety of omega-3/6 fatty acids, methylphenidate, and a combined treatment in children with ADHD. *J Atten Disord*. 2017;21(5):433–441. PMID:24464327.
51. Transler C, Eilander A, Mitchell S, et al. The impact of polyunsaturated fatty acids in reducing child attention deficit and hyperactivity disorders. *J Atten Disord*. 2010;14:232–246.
52. Hussain S, Roots B. Effect of essential fatty acid deficiency and immunopathological stresses on blood brain barrier (B-BB) in Lewis rats: a biochemical study. *Biochem Soc Trans*. 1994;3:338.
53. Uauy R, De AI. Human milk and breast feeding for optimal mental development. *J Nutr*. 1995;125:S2278–S2280.
54. Sinn N. Nutritional and dietary influences on attention deficit hyperactivity disorder. *Nutr Rev*. 2008;66:558–568.
55. Schnoll R, Burshteyn D, Cea-Aravena J. Nutrition in the treatment of attention-deficit hyperactivity disorder: a neglected but important aspect. *Appl Psychophysiol Biofeedback*. 2003;28:63–75.
56. Kozielec T, Starobrat HB. Assessment of magnesium levels in children with attention deficit hyperactivity disorder (ADHD). *Magnes Res*. 1997;10:143–148.
57. Starobrat HB, Kozielec T. The effects of magnesium physiological supplementation on hyperactivity in children with attention deficit hyperactivity disorder (ADHD). Positive response to magnesium oral loading test. *Magnes Res*. 1997;10:149–156.
58. Arnold L, Votolato N, Kleykamp D, et al. Does hair zinc predict amphetamine improvement of ADD/hyperactivity? *Int J Neurosci*. 1990;50:103–107.
59. Bekaroglu M, Aslan Y, Gedik Y, et al. Relationships between serum free fatty acids and zinc, and attention deficit hyperactivity disorder: a research note. *J Child Psychol Psychiatry*. 1996;37:225–227.
60. Bilici M, Yildirim F, Kandil S, et al. Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28:181–190.
61. Arnold LE, DiSilvestro RA. Zinc in attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol*. 2005;15:619–627.
62. Viktorinova A, Ursinyova M, Trebaticka J, et al. Changed plasma levels of zinc and copper to zinc ratio and their possible associations with parent- and teacher-rated symptoms in children with attention-deficit hyperactivity disorder. *Biol Trace Elem Res*. 2016;169(1):1–7. PMID:26063047.
63. Konofal E, Lecendreux M, Arnulf I, et al. Iron deficiency in children with attention-deficit/hyperactivity disorder. *Arch Pediatr Adolesc Med*. 2004;158:1113–1115.
64. Lahat E, Heyman E, Livne A, et al. Iron deficiency in children with attention deficit hyperactivity disorder. *Israel Med Assoc J*. 2011;13:530–533.
65. Sever Y, Ashkenazi A, Tyano S, et al. Iron treatment in children with attention deficit hyperactivity disorder: a preliminary report. *Neuropsychobiology*. 1997;35:178–180.
66. Konofal E, Lecendreux M, Arnulf I, et al. Effects of iron supplementation on attention deficit hyperactivity disorder in children. *Pediatr Neurol*. 2008;38:20–26.
67. Tseng PT, Cheng YS, Yen CF, et al. Peripheral iron levels in children with attention-deficit hyperactivity disorder: a systematic review and meta-analysis. *Sci Rep*. 2018;8(1):788. PMID:29335588.
68. Goksugur SB, Tufan AE, Semiz M, et al. Vitamin D status in children with attention-deficit-hyperactivity disorder. *Pediatr Int*. 2014;56(4):515–519. PMID:24417979.
69. Mossin MH, Aaby JB, Dalgård C, et al. Inverse associations between cord vitamin D and attention deficit hyperactivity disorder symptoms: a child cohort study. *Aust N Z J Psychiatry*. 2017;51(7):703–710. PMID:27694636.
70. Mohammadpour N, Jazayeri S, Tehrani-Doost M, et al. Effect of vitamin D supplementation as adjunctive therapy to methylphenidate on ADHD symptoms: a randomized, double blind, placebo-controlled trial. *Nutr Neurosci*. 2018;21(3):202–209. PMID:27924679.
71. Pelsser L, Buitelaar J, Savelkoul H. ADHD as a (non) allergic hypersensitivity disorder: a hypothesis. *Pediatr Allergy Immunol*. 2009;20:107–112.
72. Tryphonas H, Trites R. Food allergy in children with hyperactivity, learning disabilities and/or minimal brain dysfunction. *Ann Allergy*. 1979;42:22–27.
73. Carter C, Urbanowicz M, Hemsley R, et al. Effects of a few food diet in attention deficit disorder. *Arch Dis Child*. 1993;69:564–568.
74. Boris M. Foods and food additives are common causes of the attention deficit hyperactivity disorder in children. *Ann Allergy*. 1994;72:462–468.
75. Uhlig T, Merkenschlager A, Brandmaier R, et al. Topographic mapping of brain electrical activity in children with food-induced attention deficit hyperkinetic disorder. *Eur J Pediatr*. 1997;156:557–561.
76. Nsouli T, Nsouli S, Linde R, et al. Role of food allergy in serous otitis media. *Ann Allergy*. 1994;73:215–219.
77. Hagerman RJ, Falkenstein MA. An association between recurrent otitis media in infancy and later hyperactivity. *Clin Pediatr*. 1987;26:253–257.
78. Marcotte A, Thacher P, Butters M, et al. Parental report of sleep problems in children with attentional and learning disorders. *J Dev Behav Pediatr*. 1998;19:178–186.
79. McColley S, Carroll J, Curtis S, et al. High prevalence of allergic sensitization in children with habitual snoring and obstructive sleep apnea. *Chest*. 1997;111:170–173.
80. Kaplan B, McNicol J, Conte R, et al. Sleep disturbance in preschool-aged hyperactive and nonhyperactive children. *Pediatrics*. 1987;80:839–844.
81. Kaplan BJ. Dietary replacement in preschool-aged hyperactive boys. *Pediatrics*. 1989;83:7–17.
82. Egger J, Carter C, Graham P, et al. Controlled trial of oligoantigenic treatment in the hyperkinetic syndrome. *Lancet*. 1985;1:540–545.
83. Schulte-Körne G, Deimel W, Gutenbrunner C, et al. Effect of an oligo-antigen diet on the behavior of hyperkinetic children. *Z Kinder Jugendpsychiatr*. 1996;3:176–183.
84. Egger J, Stolla A, McEwen L. Controlled trial of hyposensitisation in children with food-induced hyperkinetic syndrome. *Lancet*. 1992;339:1150–1153.
85. Pelsser LM, Frankena K, Toorman J, et al. Effects of a restricted elimination diet on the behaviour of children with attention-deficit hyperactivity disorder (INCA study): a randomised controlled trial. *Lancet*. 2011;377(9764):494–503. PMID:21296237.
86. Youssef N, Ege M, Angly S, et al. Is obstructive sleep apnea associated with ADHD? *Ann Clin Psychiatry*. 2011;23:213–224.
87. Wei J, Bond J, Mayo M, et al. Improved behavior and sleep after adenotonsillectomy in children with sleep-disordered breathing: long-term follow-up. *Arch Otolaryngol Head Neck Surg*. 2009;135:642–646.
88. Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. *Antonie Van Leeuwenhoek*. 1996;70:347–358.
89. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol*. 1997;99:179–185.
90. Pärtty A, Kalliomäki M, Wacklin P, et al. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatr Res*. 2015;77(6):823–828. PMID:25760553.
91. Mittleman B, Castellanos F, Jacobsen L, et al. Cerebrospinal fluid cytokines in pediatric neuropsychiatric disease. *J Immunol*. 1997;159:2994–2999.
92. Warren R, Odell J, Warren W, et al. Is decreased blood plasma concentration of the complement C4B protein associated with attention-deficit hyperactivity disorder? *J Am Acad Child Adolesc Psychiatry*. 1995;34:1009–1014.
93. Swedo S, Leonard H, Kiessling L. Speculations on antineuronal antibody-mediated neuropsychiatric disorders of childhood. *Pediatrics*. 1994;93:323–326.

94. Kiessling L, Marcotte A, Culpepper L. Antineuronal antibodies: tics and obsessive-compulsive symptoms. *J Dev Behav Pediatr*. 1994;15:421–425.
95. Instanes JT, Halmøy A, Engeland A, et al. Attention-deficit/hyperactivity disorder in offspring of mothers with inflammatory and immune system diseases. *Biol Psychiatry*. 2017;81(5):452–459. PMID:26809250.
96. Hegvik TA, Instanes JT, Haavik J, et al. Associations between attention-deficit/hyperactivity disorder and autoimmune diseases are modified by sex: a population-based cross-sectional study. *Eur Child Adolesc Psychiatry*. 2018;27(5):663–675. PMID:28983730.
97. Miranda A, Presentacion M. Efficacy of cognitive-behavioral therapy in the treatment of children with ADHD, with and without aggressiveness. *Psychol Schools*. 2000;37:169–182.
98. Solanto MV, Surman CB, Alvir JMJ. The efficacy of cognitive-behavioral therapy for older adults with ADHD: a randomized controlled trial. *Atten Defic Hyperact Disord*. 2018;10(3):223–235. PMID:29492784.
99. Gevensleben H, Holl B, Heinrich H, et al. Neurofeedback training in children with ADHD: 6-month follow-up of a randomised controlled trial. *Eur Child Adolesc Psychiatry*. 2010;19:715–724.
100. Bakhshayesh A, Hansch S, Wyschkon A, et al. Neurofeedback in ADHD: a single-blind randomized controlled trial. *Eur Child Adolesc Psychiatry*. 2011;20:481–491.
101. Isolauri E, Sutas Y, Makinen-Kiljunen S, et al. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. *J Pediatr*. 1995;127:550–557.
102. Monastra VJ, Monastra DM, George S. The effects of stimulant therapy, EEG biofeedback, and parenting style on the primary symptoms of attention-deficit/hyperactivity disorder. *Appl Psychophysiol Biofeedback*. 2002;27:231–249.
103. Fuchs T, Birbaumer N, Lutzenberger W, et al. Neurofeedback treatment for attention-deficit/hyperactivity disorder in children: a comparison with methylphenidate. *Appl Psychophysiol Biofeedback*. 2003;28:1–12.
104. Quintana H. Transcranial magnetic stimulation in persons younger than the age of 18. *J of ECT*. 2005;21:88–95.
105. Dvoráková M, Sivonová M, Trebatická J, et al. The effect of polyphenolic extract from pine bark, Pycnogenol on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD). *Redox Rep*. 2006;11:163–172.
106. Chovanová Z, Muchová J, Sivonová M, et al. Effect of polyphenolic extract, Pycnogenol, on the level of 8-oxoguanine in children suffering from attention deficit/hyperactivity disorder. *Free Radic Res*. 2006;40.
107. Dvoráková M, Jezová D, Blazícek P, et al. Urinary catecholamines in children with attention deficit hyperactivity disorder (ADHD): modulation by a polyphenolic extract from pine bark (pycnogenol). *Nutr Neurosci*. 2007;10:151–157.
108. Trebatická J, Kopasová S, Hradecná Z, et al. Treatment of ADHD with French maritime pine bark extract, Pycnogenol. *Eur Child Adolesc Psychiatry*. 2006;15:329–335.
109. Lyon MR, Cline JC, Totosy de Zepetnek J, et al. Effect of the herbal extract combination *Panax quinquefolium* and *Ginkgo biloba* on attention-deficit hyperactivity disorder: a pilot study. *J Psychiatry Neurosci*. 2001;26:221–228.
110. Niederhofer H. *Ginkgo biloba* treating patients with attention-deficit disorder. *Phytother Res*. 2010;24:26–27.
111. Shakibaei F, Radmanesh M, Salari E, et al. *Ginkgo biloba* in the treatment of attention-deficit/hyperactivity disorder in children and adolescents. A randomized, placebo-controlled, trial. *Complement Ther Clin Pract*. 2015;21(2):61–67. PMID:25925875.
112. Juneja LR, Chu DC, Okobu T, et al. L-theanine: a unique amino acid of green tea and its relaxation effect in humans. *Trends Food Science Technol*. 1999;10:199–204.
113. Kobayashi K. Effects of L-theanine in the release of alpha-brain waves in human volunteers. *Nippon Nogeikagako Kaishi*. 1998;72:153–157.
114. Lyon M. The effects of L-theanine in boys with attention deficit hyperactivity disorder (ADHD): a randomized placebo controlled trial. *Alt Med Rev*. 2011;16(4):348–354.

152

Autism

Sidney MacDonald Baker, MD

OUTLINE

MORGAN, A NONVERBAL 5-YEAR-OLD

My new patient, Morgan, a nonverbal 5-year-old who had been diagnosed with autism, emerged from his car seat with a full bladder and worried face. After a trip to the toilet, we met outdoors for a swing test. As Morgan watched the swing support my 160-pound frame, his face shifted from worried to puzzled. I dismounted and lifted Morgan onto the generous web of small rope that formed a sturdy wood-framed seat. His mom—who had traveled with her son from Texas to Sag Harbor, New York, to consult me—was puzzled, too, that this was how our appointment began. With all the tests that Morgan had endured in the 2 years since his autism diagnosis, none had had anything to do with swings.

And this swing was a big one: suspended by a rope through a pulley 30 feet overhead then attached with an adjustable tie to an adjacent tree trunk. After drawing Morgan to the secure back of the seat, and with the swing a few steps back in its range, I let it go. Morgan's response, reflected in his mom's eyes, was grateful surprise, and soon he was embraced by the full range of the swing's trajectory. I touched the swing seat to give it a half turn and saw the expression on Morgan's face blossoming to one of pure joy. As with all such children who had taken this test over the past 25 years, it was my joy to say, "Morgan, you are a terrific swinger. You are fearless. You gave your smile to those tree branches way up there. I am proud of you." Morgan smiled and did hand stims as we walked indoors to my consultation room after he had a given me a good workout in propelling him high into the leafy world over our heads.

Morgan now knew that this doctor's visit was going to be different. We toured the adjacent rooms to help him locate himself, then settled down in my consultation room with its couch and two comfortable chairs. He ignored a big basket of toys, choosing instead to drift between where his mom was seated and the space near my chair, where he reached out to touch me after the first hour. My conversation with his mom focused first

on Morgan's strengths. I had given her a 26-page questionnaire to complete before our appointment. I had received, extracted, and contemplated her answers before the visit, sorting all of Morgan's symptom data by frequency/severity and coding all the symptoms that invited a therapeutic intervention. Page 2 focused on strengths, which is always the place to begin.

Identifying Strengths

In previous doctors' offices, Morgan had heard conversations about all the things that were wrong with him. Here, he heard his mom's elaboration of the details connecting his strengths to his medical history.

Strength	Mild	Moderate	Severe	Occasional	Frequent	Always
Wants to be liked		x				x
Sensitive to people's feelings		x				x
OK if parent leaves		x				x
Good with computer						x
Strong desire to do things						x
Especially attractive			x			
Cuddly		x			x	
Happy		x			x	
Sensitive/affectionate		x			x	
Likes to be held		x			x	
Accepts new clothes					x	
Pleasant/easy to care for					x	
Answers parent					x	
Follows instructions					x	
Pronounces words well					x	
Unusual memory					x	
Good with math					x	
Good climbing					x	
Responsible				x		
Good throwing and catching				x		
Swimming				x		
Bold, free of fear				x		
Likes to be swaddled				x		
Physically coordinated	x					

Beginning with a child's strengths lets the child, as well as the parents, know that the practitioner is paying attention to them, celebrating them, and listening to them. The child feels seen by me and might also better see him- or herself through my eyes. That was to be the theme of my relationship with Morgan and his mom: beginning with his strengths and going forward with a focus on him as an individual for every aspect of his treatment for autism.

Morgan's mom had never had a doctor take an interest in the details of her son's symptoms, only those that supported the autism label. But, as I explained to her (pausing to retell Morgan what a good job he had done on the swing), with a patient with autism, as well as with a patient with any chronic disease, it is clinically important to review *all aspects* of the patient's health. It is with these specific details that the functional medical doctor and the family can make joint decisions about options for treatment. The entire symptom checklist I use for all in-depth consultations can be found in Chapter 137, Let the Data Speak, of this book.

Let the Data Speak

Morgan's treatment plan was built on the previously described data enriched by his mother's narrative as recorded in her completion of the "Chronological History Form." The following six excerpts exemplify the value of such a format:

1–2 months. Breastfeeding well established. Healthy growth. Colicky baby. Poor sleeping—Perhaps 3 hours per night total. Great eye contact. Coos. Responsive. Smiles.

Lifting head. Tends to cry when placed on tummy.

7–8 months. Razzing. First hand clap. Getting into crawling stance. Sits "mostly unsupported," using pillows.

Immunizations (DPT and Hib1)—"Got a fever and felt lousy for a week."

13–14 months.

11/20/09 Immunizations. MMR, HepA, pneumococcal, conjugate vaccine. "Sick for days." Seems like it was about a week of sickness. Fever, crying, fatigue, no appetite.

December—Starts to shake head "no" and say "na-na"

Mouths word "up" to be picked up. Doesn't say it again. Very high activity level. Poor attention. Not gaining enough new skills; development seems to be at a standstill.

19–20 months.

Morgan says, "Dive me dat!" Heard once and not again.

Extreme hyperactivity. Very intense sensory seeking. Running back and forth, throwing body against the walls. Either understimulated or overstimulated—never at stasis.

Toe walking. Flapping.

25–26 months.

11/3/2010—Autism diagnosis.

December: Establishing therapies. Home program. Starts making progress.

Dec. 2010—The whole family is ill. Morgan is "sick for 2 weeks." Loses weight. Refusing all foods except bacon, waffles, and noodles. Morgan is "not vocalizing, quiet and unhappy."

We start ABA. Rapid improvement.

45–46 months.

July—Eliminate casein. Easy adjustment.

Headaches stop completely. Constipation improves within 1 to 2 days. 2 weeks later, eliminated gluten. No noticeable changes.

Visits to developmental pediatrician and allergist/immunologist. Concerns: pale, bloated belly, constipation, inflamed ring around anus, allergic shiners, seasonal allergies that seem to last all year, repeated pink eye and upper respiratory infections. Recommendations are not helpful or have no impact.

I start digestive enzymes. It relieves the constipation, but his stools become poorly formed; mushy.

Now he has intermittent diarrhea/mushy stools and constipation.

Treatment Plan

Morgan's treatment plan at the time of our first consultation was initialed at a clinic specializing in autism and included the following:

Diets	Doing now?	Very Good	Good	No Response	Bad	Very Bad	Don't Know	Negative, Then Good	Comments:
Gluten-free	x						x		
Casein-free	x	x							
Yeast-free	x	x							
Specific carbohydrate diet	x	x							

Therapies	Taking now?	Very Good	Good	No Response	Bad	Very Bad	Don't Know	Negative, Then Good	Comments:
Lovaas	x	x							
Occupational therapy			x						
Speech therapy	x			x					

Medication or Supplement (please mark the response with lowercase x's	Taking now?	Very Good	Good	No Response	Bad	Very Bad	Don't Know	Negative, Then Good	Comments:
Digestive enzymes	x		x						
Probiotics	x						x		
B6 and magnesium	x						x		
Folic acid	x						x		
Melatonin		x							Sleeps well presently
Multivitamin, high potency	x						x		
Vitamin B_3 (niacin)	x						x		
Vitamin B_6	x						x		
Magnesium	x			x					
DHA-rich oils	x		x						
EPA-rich oils	x		x						
Cod liver oil	x		x						
Flax oil	x						x		

Morgan was a boy with many strengths, of which one was a very well-organized and bright mom. His symptoms, laboratory work, and response to previous treatment justified optimism in his prognosis. As rich as has been my experience in predicting treatment outcome, picking the winners remains an uncertain task in which positive response to previous treatments counts higher than other variables. The fact of his long list of symptoms of neuromuscular irritability, despite supplementation with magnesium, was disconcerting, although perhaps explained by bowel issue that would have limited his magnesium tolerance.[1]

Restore the Gut

Actionable findings from laboratory tests previously ordered at an autism clinic near his home were as follows:

Stool: *Dientamoeba fragilis* trophozoites, the growth of a non-*Candida* yeast, and *Rhodotorula* species.

Blood: Nutritional profile showed unmet needs for B vitamins, vitamins A and E, and alpha-tocopherol.

Plan:

1. Treat the amoebic infection with trimethoprim-Sulfamethoxazole and paromomycin as a red herring in his autism spectrum.
2. Cover the antimicrobials with an antifungal—he was already on *Saccharomyces boulardii.*

After a hiatus for treatment by his local physician for a question of strep versus fifth disease, Morgan responded dramatically to immune restoration treatment via probiotics (discussed later in the chapter), as his mother reported:

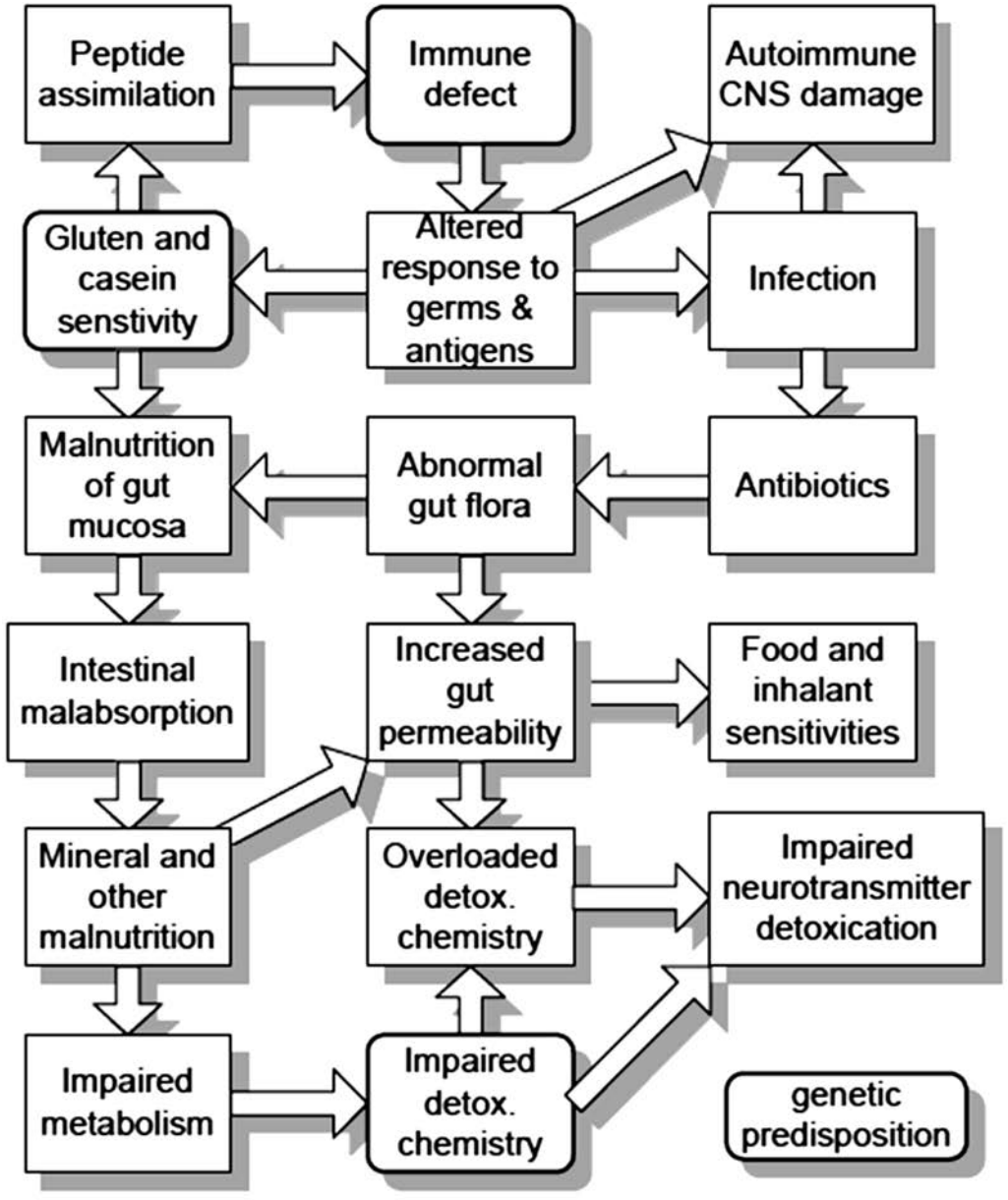
> *Huge improvement in overall immune functioning! Morgan has not been ill at all this summer. He attended 4 separate summer camps (each camp had attendance of 15–20 4-year-olds) and did not become ill! Previously attendance in these activities would result in*

bacterial and viral infections that would essentially negate his ability to attend. He has NEVER been able to tolerate that level of exposure! He has been at school for 3 weeks and has not become ill. Additionally, his seasonal and mold allergies have been very mild.

The immune restoration treatment has not produced significant improvements in symptoms such as weakness in his hands, poor fine motor, balance, and attention. Stimming remains.

Restore Immune Balance

Various supplements were on Morgan's plan, but the turning point came with the addition of helminthic therapy in the form of *Hymenolepis diminuta* cysticercoids, after which all of his autism symptoms cleared. He has since emerged as a brilliant and articulate. His mom recently updated me on his progress: "The school recently had a guest speaker, children's author and Newbery Medal winner Jack Gantos. Morgan was the darling of the event when he answered all of the prized author's questions with innocent humor and unique perspective. His teacher said he was totally engaged by the author and 'on.' The school is slowly recognizing that the strange, socioeconomically disadvantaged, disheveled-looking autistic child that managed somehow to transfer into their exclusive school is actually a benefit to their campus, as he may someday pull up their standardized test scores."



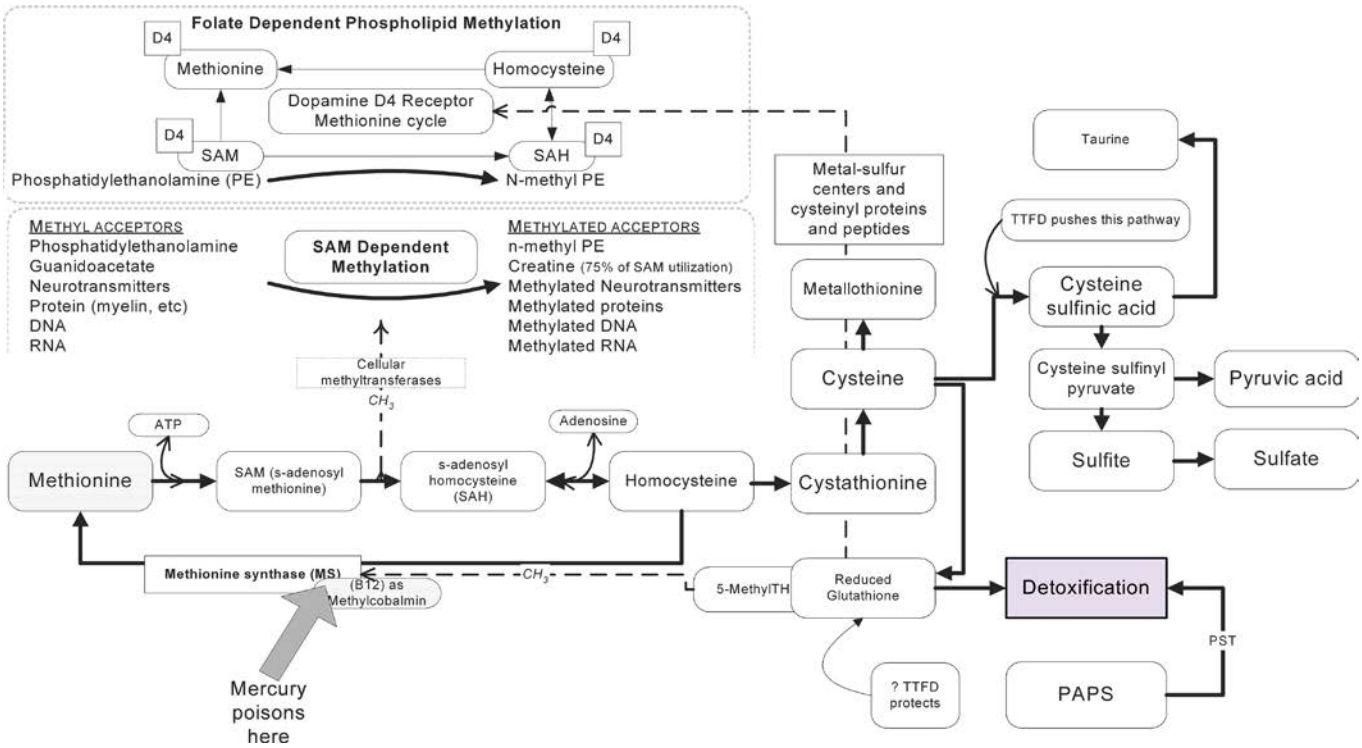
A model for relationships among "alternative approaches to autism spectrum problems

A Historical Perspective for a Comprehensive Model

Morgan's story is typical of the many children for whom the word *restoration* is the key to understanding a therapeutic strategy designed to evoke Nature's strong impulse toward healing by meeting special unmet needs, and/or eliminating toxins, allergens, and microorganisms. A consensus among 30 scientists, practitioners, and parents was formed in 1995 when Bernard Rimland, PhD; Jon Pangborn, PhD; and I organized a meeting in Dallas, Texas, in which the spirit among attendees from diverse backgrounds was to find common ground. The accompanying diagram summarized the landscape of biochemistry, toxicology, and immunology that could, we agreed, help organize thinking for each of us as we pursued our various efforts to seek actionable options for individual children affected by autism. Now, more than two decades later, our vision has been vindicated by research findings and the kinds of clinical outcomes illustrated by Morgan's response to treatment.

Our meeting took place three decades after the publication of Dr. Rimland's book challenging the medical view that had been current since the 1940s that autism was due to cold mothering.[2] That challenge unfolded when genetic research was fed by the reality that everything is genetic, as indicated by the "genetic predisposition" in the lower righthand corner of the diagram that evolved during 3 days of brainstorming. What was missed in the enthusiasm of advocates of the genetic etiology of autism was the impossibility of a genetic epidemic. As the prevalence of autism rose dramatically in the past two decades, those of us who were at that meeting and came up with the boxes in our diagram felt increasingly justified by our portrayal of the arrows. They give a clear impression that we are dealing with a *system,* which is a different reality an *entity*. Read the appendix by Crookshank in Ogden and Richards's linguistic classic, *The Meaning of Meaning* (Harcourt, Brace Jovanovich, 1959). It presents a serious concern that the medical profession's failure to make distinctions among names, ideas, and things will lead to increasing problems for the medical profession as the burden shifts from acute illnesses to chronic disease.

Conventional medical practitioners seemed incapable of avoiding the language and thinking about autism as if it were an entity that causes symptom until the word *spectrum* entered our citadel like the Trojan horse into Troy. Using the word *spectrum* combined with autism brought a simplicity to thinking about children with the defining symptoms in behavior, speech, and movement that opened our minds to the full picture of symptoms that drew attention to immune and digestive disruptions. For parents, this shift gave rise to a dispiriting feeling as the diagnostic label lost the implication that "we know what it is" and became one of being lost in a poorly defined space.

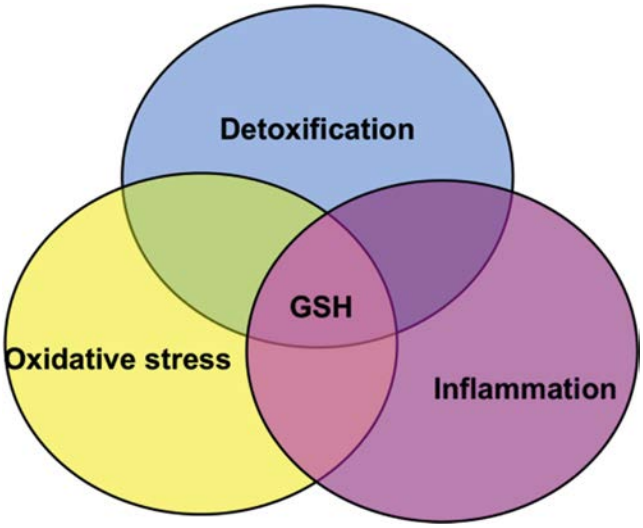


Definition of that space came as our original meeting in Dallas in 1995 gave rise to an organization to which Dr. Rimland gave the name Defeat Autism Now! (DAN!). Keen to meet again, we were able to afford future meetings by inviting an audience of parents, professionals, and scientist to come, listen, and participate. Richard Deth, PhD, of Tufts University pointed us to Jill James, PhD, of the University of Arkansas to add deep insight into problems of oxidative stress and the particular burdens of certain toxins (lead, mercury) on the chemistry in which glutathione was the threatened moiety in a vicious biochemical cycle breakable only by large doses of such nutrients as methylcobalamin and methyl folate. Here is the chart that Drs. Deth and James helped me draw. More biochemists and immunologists have since come on board and stayed as the Autism Research Institute's role as a meeting organizer shifted back to support of research and a new entity, the Medical Academy for Pediatric Special Needs, inherited the role of training physicians in the biochemistry, toxicology, and immunology that provide the basis for medical management of individual children on the autism spectrum.

Dan Rossignol, MD, has maintained his leadership role in the teaching program and in collaboration with Richard Frye, MD/PhD, published a stream of papers offering validation of the common sense of the approaches outlined in this chapter as well as peer-reviewed research supporting their scientific basis.

Readers who have a strong affiliation with the Institute for Functional Medicine (IFM) will recognize in this chapter a strong influence shared by IFM, DAN!, and MAPS. IFM did not sponsor a meeting focused on children's health until 2017, and as of this writing, the future of such a focus is uncertain.

The DAN! and MAPS path to discovery of effective treatment options has yielded dramatic clinical response in the children under the care of physicians who have been trained in the commonsense application of dietary and medical interventions aimed at finding in each individual child those elements in diet, supplementation, and natural and pharmaceutical medicine that evoke Nature's buoyant impulse toward healing. Going back to 1967, a registry kept by the Autism Research Institute (ARI) under the leadership of Dr. Rimland and Steven Edelson, PhD, recorded ratings of pharmaceuticals, nutritional supplements, and dietary measures and has yielded a treasure trove that should be examined by readers of this chapter.[3] It offers the clinician a detailed picture of the failure of conventional pill-for-an-ill practice and strong support for natural remedies plus antifungal medicines.



Dr. Rossignol's literature review cited herein is an invaluable resource, but ARI's presentation of good, neutral, and bad responses to various treatments given to children in the autism spectrum offers, on a single page, a clear message that will shorten the learning curve of any practitioner who may feel intimidated when crossing the bridge to the seemingly unfamiliar landscape of the autism spectrum. Once there, it will become clear to the explorer that the territory is not some exotic colony beyond the margins of mainstream medicine. On the contrary, autism is simply the core of pathology found in the most vulnerable members of our culture who are exposed to the stressors relating to micro- and commensal organisms, detoxification, and oxidative stress. After our DAN! group had welcomed new participants, the accompanying diagram began to coalesce around a theme that was to match that of the evolving understanding about all chronic illness and support a view of treatment options that is more of a guide than a shopping list. The diagram could be replaced in our current way of thinking by a single sentence: "While some would say that 'everything is autoimmune until proven otherwise' reading the chapters in this book written by world leaders in autoimmunity brings one to the conclusion that everything after all is infectious until proven otherwise (including autoimmune diseases)."[4] I believe that to be the most important sentence written in the field of chronic illness in my five decades as a doctor. Its author, Dr. Yehuda Shoenfeld, embraces the decisions we clinicians make every day and the way we speak with our patients and colleagues about options for the prevention and treatment of chronic illness. The language spoken by those who care and are cared for in contemporary culture is full of particularities. These items are embodied in words such as "disease entities" that preserve an ancient mythology that is kept alive in the classification of disease. Our system for naming illnesses wrongly guides our thinking about concepts by modeling them on the Linnaean categorization of live or once-living entities. How can we escape the muddle embodied in the confusion of concepts with entities that is so deeply engrained in the way we name and blame diseases as if they are things that cause symptoms?

CAUSES OF COMPLEX DISEASE

Loss of Immune Tolerance

Dr. Yehuda Shoenfeld's statement guides us to grasp that, whatever name we give it, the cause of chronic illness is the loss of immune tolerance. If everything we see in the realm of chronic illness is a manifestation of the loss of immune tolerance, then restoration of immune tolerance is the goal we strive to achieve. Nowhere more so than in the autism spectrum do we find so rich a set of symptoms presenting the ways sensitivity can be expressed in the human. Nowhere more so than in the autism spectrum do we discover resources for the recovery of immune tolerance that comes with the answer to a conceptually simple question: Does this individual have a special unmet need to get, avoid, or be rid of something, which, if taken care of, would favor Nature's buoyant impulse toward healing?

The word *spectrum* came into contemporary medical parlance as did the Greeks' horse into Troy, giving medical professionals a secure metaphor while parents were deprived of the (false) security of knowing the precise name of the "thing" that is causing their affected child's problems. Autism's history is full of cruelty, starting with a consensus formed in the mid-20th century that children who could not learn speech and were devastated by odd postures and behaviors and troubled by hypersensitivity to noise, touch, smell, taste, and bright lights were this way because of care given by "refrigerator mothers."

As second-year medical students at Yale in 1962, we were rarely shown movies, perhaps considering the risk of sleep. We witnessed two films at the Child Study Center. In one, the dignified doctor explained to the parents of a child with autism that it was his mother's fault. In another, a developmentally impaired child's parents were cautioned to "not look for answers." We students were told that it would be unlikely that we'd ever see an autistic child, rare as was that disease. I had seen but not grasped institutionalized individuals during my internship's rotation in the Southbury Training School. My first contact with an autistic child was when the fist of a speechless 14-year-old resident of another institution shattered the bridge of my glasses with a stroke to my nose. The precision and force of that hit produced in me the afterthought that he was saying to me, "You are looking into me with your ophthalmoscope, but you are not seeing **me**." I was struck and resolved to learn from other children I would meet.

One was referred to me by a former patient in a prepaid healthcare plan where I practiced for 7 years leading up to my directorship of the Gesell Institute of Child Development. This child of my patient's neighbor had developed a roaring case of eczema after a course of antibiotics. Could I see the child for the minute needed to cover a

prescription for nystatin? I agreed, saw the child's skin in a quick visit, and called in the script, and 6 weeks later, my former patient reported that the eczema was cured, and so was the child's autism. It was the dawn of my understanding that, within Dr. Yehuda Shoenfeld's model, the proliferation of yeasts gave a stimulus in which skin inflammation resulted in a case of mistaken identity in which immune defenses against yeasts targeted the child's skin as they had also been doing all along to her senses.

Is autism a matter of autoimmunity where yeasts are the exclusive trigger to an inflammatory response that affects the brain? No, but such a consideration deserves priority among all diagnostic and treatment considerations. Is autism the exclusive manifestation of the loss of immune tolerance evidenced by the increase of chronic illness in our population? No, but the increase in attention deficit disorder, arthritis, asthma, autism spectrum disorder, autoimmune disorders, developmental/learning disorders, cancer, cardiovascular problems, cerebral palsy, cystic fibrosis, diabetes, epilepsy, food allergies, obesity, respiratory allergies, sickle cell anemia, and spina bifida is more likely to have one cause than different causes for different diseases.

Unity of Disease Causes

The perception of unity in diseases with diverse presentations is easy if we notice that all living things share the same fundamental challenges to their health: bugs, toxins, and fire. In more technical language, these threats have to do with the following:

- Our interaction with internal and external predatory and commensal organisms
- Detoxification and ridding of our own metabolic end-products and environmental toxins
- Oxidative stress as expressed in the protection from being burned by our own metabolic fire

The appealing odor of the essential oils of plants supports the belief that the ancient substances offer themselves to us for our use as they have for plants in the noted three domains since the dawn of life. This textbook does not have an independent chapter discussing essential oils, which are known to many readers who value their role in healing. I will only add here that the marketplace, as I have found it, suffers from the same enthusiasm as contaminates thinking in both conventional and natural medicine: an enchantment with complexity. Purveyors of essential oils tend to multiply the options beyond the capacity of beginners and fail to promote the fact that a dozen or so such oils will do to cover the bases for which these oils were evolved in plants long before so-called higher organisms evolved. These oils addressed the same problems that have existed for living organisms since our planet became oxygenated and referred to in the previous list as bugs, toxins, and fire. A selection of a dozen or so oils may be used therapeutically by patients willing to read and seek advice on how to select the ones that together cover the three bases. The fact that essential oils, being low-molecular-weight lipids, pass through the skin and may travel from the bottom of a person's feet to the brain in 20 minutes is especially important in the context of knowing that any contaminants of such oils may join them it that passage. Thus shopping for oils must be guided by a need to ensure documentation of purity.

The simplicity of the challenges to health of bugs, toxins, and fire argues for a corresponding simplicity in considering the options for all individuals with chronic illness.

The word *special* inserts itself in the thinking—as stated previously—needed to solve the mystery of a person's illness. The term *special needs* has been around for a long time in the delivery of social and educational services for children and adults. Natural medicine's focus is treating not the disease but the individual, where "special needs" applies to therapeutic options. We begin in our consideration of each child's options with the one question—about unmet special needs to get, avoid, or be rid of items—that will favor healing. The answers to such a question come from the patient's narrative, the implication of certain symptoms, laboratory testing, and thumbs testing, defined as the introduction of a therapeutic trial in which a positive result generates a thumbs-up, a negative response generates a thumbs-down, a mixed response generates one thumb up and one down, and no response generates thumbs-sideways. Of all the tests in my toolbox, the thumbs test is the most easily explained and interpreted by patients and parents.

Shifting Thinking

The attachment of the word *spectrum* to autism reveals a new way of thinking in medicine and that is compatible with that of naturopathic medicine. The word *spectrum* joins affected children more loosely than a designation of a disease name. It was introduced in medical writing in 1985[5] but did not enter common lay and medical parlance until late in the 1990s. The word may be applied to all diseases, but its use belongs exclusively to autism in the sense that referring to someone as being on or "in the spectrum" is universally understood as "autistic."[6] A spectrum is a continuum: both wide and deep. It is a metaphor embracing various features of different individuals. The traditional medical way of speaking and thinking about a disease used the word *entity* and carried the thought of something "well defined" by a limited number of symptoms or laboratory tests.

Shifting from "entity" to "spectrum" directs thinking away from the selection of a treatment for *this disease* to tailoring treatment for *this individual*. Failure to understand the implication of this shift creates misunderstandings among doctors and patients. The confusion starts with the implication that a disease is a thing. Diseases are not things but ideas we form about people grouped by similar symptoms and laboratory tests. The danger in this confusion is neglect of the fundamental scientific principle in biology—and medicine—that each person is unique and that the individual, not the disease, is the proper target of treatment.

Understanding that autism spectrum disorder happens to an individual with manifestations on a spectrum of severity allows us to better understand chronic disease and other challenges to human health in the modern age. As different as conditions such as Alzheimer's and autism may look to specialized practitioners, they may be much more similar than we think: the manifestation of environmental, social, and epigenetic conditions I will discuss herein that are compromising human immunity and brain function.

AUTISM SPECTRUM DISORDERS

The Troubling Rise in Prevalence

Autism spectrum disorder (ASD) is an umbrella term given to a wide variety of developmental brain disorders that affect behavior, sociability, and communication. The typical presentation of ASD includes an inability to make eye contact, limited to no social interaction with others, inflexibility, and repetitive behaviors, including hand flapping, various movements called stimming, and repetitive play. The severity of symptoms in those identified as being on the autism spectrum is highly variable. Some children diagnosed with autism, like Morgan before treatment, are highly relational and enjoy social interaction. Others disappear completely into their own world and find social interaction impossible. The common denominator is that those diagnosed with ASD are presenting with behavioral and immunological dysfunction.

In 2014 the Centers for Disease Control and Prevention (CDC) estimated that ASD affected 1 in 45 children. In 2015 the estimate

was 1 in 43 children. The most current number of children diagnosed with ASD, released by the CDC in November 2017, is that 1 in every 36 children is on the spectrum.[7] Three times more boys than girls are affected. That ratio was four to one for many years and, as I reported in 2014,[8] it dropped suddenly in 2013 in the wake of a year when the girls' symptom data were more dispersed, suggesting that an increase in the variance of the data points to instability in the biological system—the autism spectrum—that must surely be environmental.

Doctors trained in the 1980s or earlier, as I was, were told in medical school that it would be unlikely they would ever encounter more than one or two people with autism over the course of their entire career. Now autism has become so prevalent that primary care doctors, particularly pediatricians and family physicians, often have several patients with ASD. ASD is so common, in fact, that the American Academy of Pediatrics recommends that every child be routinely screened for autism. The standard screening is the Modified Checklist for Autism in Toddlers (M-CHAT), usually administered between 16 and 30 months. Early screening is primarily recommended because of the benefits of early intervention in lessening the severity of symptoms. When delays are found on M-CHAT or clinically, steps must be taken to support optimal biochemistry, as discussed later in this chapter, and stop toxic exposures that may be harming a child's brain functions.

Although functional medical practitioners are unequivocal that autism rates have skyrocketed for reasons that cannot be contributed solely to genetics or epigenetics, there continues to be debate in the mainstream medical community about whether the rise in autism is due to a real increase in numbers or simply to better diagnosis. Work done by Stanford-educated atmospheric research scientist Cynthia Nevison, PhD, puts that debate to bed. Nevison's systematic comparison of temporal trends in autism prevalence in the United States revealed that between 75% and 80% of the tracked increase in autism is due to actual increase, whereas 20% to 25% may be attributed to changing diagnostic criteria.[9]

Etiology of Autism

We practitioners should not be misled from considering the environmental causes of ASD in an individual patient when we hear the constant refrain from conventional medicine that the "cause of autism is unknown." We should, moreover, take the lessons we learn from the many children who recover completely from having been autistic to become brilliant and talented individuals.

That said, it is true that we do not have a clear picture of autism's chain of causation. We know it is not caused by refrigerator mothers. We know that the autism we are seeing in the current epidemic does not have a genetic origin in the usual sense. We know that there is a viral shadow over the landscape of many children's stories and laboratory tests, with a particular focus on the measles vaccine virus derived from the measles, mumps, and rubella (MMR) immunization. Whereas the conventional medical doctor is adamant that we do not know what causes autism (therefore making it impossible to avoid as well as to treat), the functional medical practitioner understands that the cause of brain and immune dysfunction that falls under the umbrella term ASD is multifactorial and may be found in a given individual. The likely environmental triggers of autism include are discussed next.

Toxin Exposure and Autism Spectrum Disorders

Although the cause of ASD is still unclear, the role of environmental toxicants in increasing the risk for this disorder has been repeatedly shown. For example, insurance claim data covering one third of the U.S. population was reviewed to see if there was an association with known toxicant-induced disorders and ASD. Congenital malformations of the reproductive system, known to be environmentally induced, were used as a surrogate for environmental toxicant exposures. It was found that males with congenital reproductive malformations were 283% more likely to also have the diagnosis of ASD. A study of 192 twins was done to determine the roles of genetics and environment on the development of ASD.[10] These researchers found that environmental factors carried 55% of the etiological weight, whereas genetics only accounted for 37%.

Maternal exposure to environmental chemicals and body burden has also been associated. Mothers with chemical sensitivity were three times more likely to have a child with autism and 2.3 times more likely to have a child with attention deficit hyperactivity disorder (ADHD). The risk of being a parent of a child diagnosed with ASD is increased by a factor of 7.3 for occupational exposure to lacquers, 4.7 for varnish, 2.7 for xylene, 3.1 for solvents, and 6.9 for asphalt.[11] A study in San Francisco revealed that women occupationally exposed to toxicants have a significantly higher risk of having a child with ASD.[12] Women exposed to disinfectants at work were 4 times more likely to have a child on the spectrum, and those exposed to exhaust and combustion by-products were 12 times more likely.

The presence of low-molecular-weight phthalate metabolites in a mother's urine during her third trimester of pregnancy has been significantly correlated to the child having greater problems with social cognition, social awareness, and social communications by the age of 7.[13] In a Swedish study that followed families over a 5-year period and focused on the home environment and health, the researchers found a connection between vinyl flooring and the diagnosis of autism, Asperger's, and Tourette's syndromes.[14] In addition to increased rates of these diagnoses in children whose parents had vinyl flooring in the bedroom, risk was also associated with parental smoking, condensation on the windows, and reduced ventilation in the home.

Between 2010 and 2015, several articles were published on the relationship between air pollution and ASD. The first was a case-control study with 383 children with ASD and 2829 control children who had speech and language impairment from North Carolina and West Virginia.[15] Exposures to ambient levels of metals, particulates, and volatile organic compounds were assessed via pollutant data on the area where the child's parents lived during pregnancy. Significant associations were found between ASD risk and exposure to the polycyclic aromatic hydrocarbon quinolone (odds ratio [OR] 1.4), styrene (OR 1.8), and methylene chloride (OR 1.4). A group of 284 children with ASD in the San Francisco Bay area was compared with over 600 neurologically normal children to see if there was any difference in air pollutant exposures between the two groups.[16] The researchers found that children living in the San Francisco Bay area with higher levels of atmospheric mercury were 92% more likely to have been diagnosed with ASD. Areas with the highest vinyl chloride levels increase ASD risk by 75%, high cadmium levels by 54%, trichloroethylene by 47%, nickel by 46%, and diesel exhaust by 44%. Another study in California found that children with ASD were far more likely to be living in areas with high vehicular exhaust.[17] During the first year of life, exposure to either nitrogen dioxide (NO_2), particulate matter (PM)2.5, or PM10 all more than doubled the child's risk of developing ASD. In a large cohort of over 49,000 Taiwanese children, those with the highest exposure to NO_2 had a 340% increased risk of developing ASD.[18] In addition to the increased risk from NO_2, each increase of 10 ppb in ambient ozone increased ASD risk by 54%, and each 10-ppb increase in carbon monoxide (CO) levels increased the risk of autism by 37%.

Toxic Exposures During Gestation

Toxic exposures during gestation, especially exposure to prenatal ultrasound,[19] acetaminophen, and endocrine disruptors, have been

implicated in autism. The fact that autism affects at least three times as many males as females raises the possibility that the timing of prenatal exposure through disruption of the embryonic development, which follows a different hormonal course in boys versus girls, could play a role.

Pre- and Postnatal Exposure to Acetaminophen in Combination With Oxidative Stress

An exhaustive literature review by a team of researchers from Harvard and Duke Universities found that one contributing factor in the increased prevalence of autism is increased exposure to acetaminophen, which, in the presence of inflammation and oxidative stress, becomes neurotoxic to infants.[20] It blocks glutathione synthesis, further jeopardizing an individual with a detoxification pathway that is overloaded or compromised by other toxins.

Overexposure to Aluminum

Intracellular aluminum in autism brain tissue has been identified as a causative factor in ASD.[21] Mechanisms by which the human brain is exposed to potentially toxic amounts of aluminum include the synergistic effect of multiple aluminum-containing vaccinations given simultaneously and aluminum in medications such as parenteral nutrition[22] and vitamin K given to neonates, as well as via personal hygiene products and ingestion.[23] In addition, many water supplies and foods are contaminated with aluminum.

A Depleted Microbiome

Overuse of antibiotics during pregnancy and infancy, along with dietary deficiencies, excessive hygiene, and inadequate exposure to soil and other microbes, has resulted in an unhealthy microbiome for many. The antibiotic effect translates directly to consideration of the overgrowth of yeast and other fungi.

Toxic Metal Poisoning in Infancy

Lead and mercury interfere with our body's principal detoxification pathway, creating a vicious cycle.[24]

Loss and Restoration of Immune Tolerance

ASD, like all chronic illness, involves a loss of immune tolerance. If everything we see in the realm of chronic illness is a manifestation of the loss of immune tolerance, then the restoration of immune tolerance is the goal we strive to achieve.

A THREE-STEP PROCESS FOR CARING FOR CHILDREN WITH ASD

Step 1: Listen. There are many ways of listening. Listening begins with hearing the patient or the parent voice the child's story, in which the strengths, symptoms, life events, and previous responses to treatment and dietary intervention are the key elements. No other medical condition is more demanding or richer than the autism spectrum in terms of the details of these elements for presenting options for treatment. A system for coding the actionable implication of symptoms is described in Chapter 137, Let the Data Speak, of this book.

One way of gathering the patient's story of strengths, symptoms, changes, treatment responses, exposures, and life events is a checklist, and the other is a free-form aid for simply listing everything that describes how your patient feels, looks, and acts. Each has its merits. Neither is easy, but no one is better than the patient or the parent at getting it done. Just as one has to endure the pain of a needle to gather information from the blood, one has to exercise patience to get the full picture of a person's health.

Having that picture will reward you in two ways. First, the picture is full of messages about treatment options. Second, when the data are organized properly, they provide a means for helping the parent and the patient with autism see through the physician's eyes. How many times have I asked a patient how things are going and have been told that the stimming and toe walking have not changed? When I raise the question of what has happened with the hand scratching, constipation, and sleep problems, I may get a surprised reaction, "Oh, I forgot about those symptoms!" When the absence of those symptoms is noted and celebrated, families feel a sense of progress that helps support decisions about next steps.

Step 2: Test. Next come various signals from laboratory tests—looking at the body, as well as the blood, urine, stool, saliva, and spinal fluid, with various devices for picturing the shape, movements, and function of tissues and organs that are hidden from view. Finally, as we begin our tailoring, we observe what happens after each step in "trying on" treatments.

Step 3: Try and Evaluate. Most interventions should be considered tests until a positive response turns them into a treatment based on feedback from the patient and/or the laboratory. Healing can be felt and observed and sometimes confirmed by changes in laboratory tests, and in some laboratory tests, such as measures of heavy metals provoked by chelators such as ethylene diamine tetra-acetic acid (EDTA) and dimercaptosuccinic acid (DMSA), a clinical response to the test material may convey as strong of a message as the laboratory result. Beyond that, every treatment is what I call a "thumbs test": a way to ask the body how it feels in response to a change. Every illness, including ASD, is a signal to change. Once a change has been made, there is no more persuasive evidence than what the body tells us as to the value of that change. We can try on various treatments and evaluate their effectiveness by reading the patient before us as opposed to rigorously following a given protocol.

Tailoring Treatment to Fit Individuals

The conventional medical approach posits that autism cannot be successfully cured, only that the symptoms can be managed. The functional/natural/integrative medical approach posits that actionable treatment options exist to help with *tailoring* treatments to fit *individuals* on the autism spectrum, and that with careful tailoring, many children diagnosed with ASD can improve so much as to lose their diagnoses. The fit is best determined by scrupulous attention.

Imagine tailoring a suit of clothing. You try it on and then make adjustments to suit the individual until you have a good fit. A good fit does not mean attempting to suppress symptoms only. A good fit means taking action to favor Nature's strong impulse toward healing.

To achieve such a fit requires discovering and dealing with unmet needs, avoiding triggers, and detoxifying the body if necessary. Unmet needs include nutrients (vitamins, minerals, fatty acids, amino acids, and accessory nutritional factors), light, and rhythmic integration. Triggers include germs, allergens, and toxic exposures. If triggers are present in the body, detoxification will be a seminal step in healing. Toxins may be elementary (e.g., lead and mercury), synthetic (e.g., pesticides), or poisons made by living things such as plants and germs. Once we start thinking this way, the treatment process switches from being the old-fashioned "doctor knows best" and "do what the doctor says" to a different approach: "listen to the patient" and "work together."

TREATMENT

Step 1

In my practice, for years I have avoided doing a large set of laboratory tests before seeing the patient. It saves money, and the interventions I

may begin may produce benefits that will affect laboratory tests, thus making them invalid by the time a commonsense treatment option has been engaged. Toward the end of an initial visit, I demonstrate the injection of a dose 64.5 mcg/kg that will be repeated by the parent once every 3 days from methylcobalamin solution 25 mg/mL into a child's upper outer buttock, violating the prohibition of that location because the slow absorption in buttock fat reduces the benign but, to parents, curious appearance of pink urine resulting from renal overflow. A full explication by Jim Neubrander, MD, of the use of methyl B_{12} can be found on his website.[25]

Step 2

My next step is to find the dose of magnesium that achieves bowel tolerance. It is a precondition to all other interventions. Magnesium supplementation is free of any risk except in patients with poor renal function. Magnesium citrate is my top choice for a diagnostic trial of supplementation. The idea is to take it in escalating doses, starting in a range of 100 to 200 mg in an adult and 50 to 100 mg in a child, to fine "bowel tolerance"—that is, the point of bowel frequency and looseness that is a bit too much, and then back down a bit.

Magnesium taurate is another option for patients with very light-colored stools. Many forms of magnesium citrate capsules are available, and of course, magnesium citrate solution is available as a cathartic—used for "bowel prep" before lower bowel endoscopy or surgery. The dose of approximately 4000 mg found in such preparations in the 6- to 10-oz bottles sold at pharmacies gives a good indication of the general safety of oral magnesium supplementation. Very few individuals with unmet needs for magnesium cannot tolerate an oral dose that would be needed for a therapeutic trial. If so, they can apply magnesium cream (CALM is a brand found in most health food stores) liberally to smooth skin at bedtime. CALM comes as a powder for making a solution for children who do not swallow pills.

The idea with all of these options is to start with a low dose, usually first in the evening, and then increase by adding a dose in the morning, then more in the evening, then more in the morning, and so on until you reach "bowel tolerance" as signaled by loose bowel movements (BMs). At that point, you may back off to a dose that is associated with symptom relief and comfortable bowels.

Children with autism have an increased incidence of constipation and other gastrointestinal disorders.[26] Establishing a dose of magnesium at bowel tolerance provides a way of dealing with constipation. Most importantly, it lets us see improvement in neuromuscular irritability symptoms and allows us to expect the alleviation of other, less noticeable problems that are solved by meeting magnesium needs. These symptoms of neuromuscular irritability are described in Chapter 137, Let the Data Speak. Keep in mind, therefore, that as important as magnesium is in correcting "uptight" symptoms, those symptoms are not the only target of magnesium supplementation. Magnesium, with its partner pyridoxal 5 phosphate, is a cofactor in dozens of steps in every aspect of body chemistry. Many of these processes are relatively silent compared with those related to tension in the nerves and muscles. With that principle in mind, you may ask why I am so fixated on treating neuromuscular irritability instead of "treating autism." Removing a pebble from your shoe is not just for your foot but also for the journey.

Because various kinds of magnesium (magnesium sulfate) and high doses (4000 mg) of magnesium citrate are used as cathartics, patients and practitioners get confused into thinking that magnesium is just a temporary treatment for the constipation and fail to understand that people who have been under—and remain under—stress may have an ongoing need for magnesium supplementation that is magnified by the restricted diet common in many autistic kids.

Step 3

Once bowel tolerance has been achieved for magnesium with at least one reliable BM per day, the next step is a thumbs test with *Saccharomyces boulardii* to be given in escalating doses with activated charcoal as needed to quench the Herxheimer reaction (a die-off reaction, also called Herx, explained herein) symptoms that confirm the suspicion of a yeast problem that is nearly always indicated by answers on the questionnaire and a history of antibiotics.

Children with autism have a problem moving their tongues. We see in the many children who recover that they knew all the words, and some children teach us, in the midst of a sudden recovery, that they can go from very limited to fluid speech in a few days. What's going on when that happens? The research and review of contemporary science by Woody McGinnis, MD, brought the interactions among the midbrain and its far reaches in the gut and the higher brain to the attention of the autism community. We now understand that substances in the gut pass through the bloodstream and affect or touch the midbrain. This touch is based on the midbrain's ancient job of "tasting" the blood for signals that are needed to regulate automatic body functions shared by all animals.

In recent history, humans have experienced changes in diet, enormous increases in exposure to novel toxins and antibiotics, and the disappearance of beneficial gut organisms. These changes result in new signals coming from the gut to the midbrain. These signals cause confusion and result in damage to the automatic movements of organs of digestion, including the tongue and the gut. In humans, the tongue does double duty as an organ of digestion and speech. Both of these can be impaired when the autonomic nervous system is affected. Nonverbal children diagnosed with ASD have impaired function in the midbrain. The conventional medical approach is to treat the symptoms by intensive therapy with a speech pathologist. The consensus among professionals is that speech therapy works for children. It provides benefits that would not be obtained without that intervention.

Although early behavioral intervention can be helpful, the functional physician understands that detoxification takes precedence over speech therapy to get to the root causes of this autonomic dysfunction. I have found that successfully treating yeast overgrowth in nonverbal children with ASD is the key to resolving this autonomic problem. Morgan's story is a case in point. As the result of being on high doses of antifungal medication, Morgan went from having no speech to being a highly articulate, precocious talker over the course of just a few months.

Just recently, I treated a 4-year-old boy who was deep in the autism spectrum with very little speech. After seeing him respond to *S. boulardii* with a big Herx reaction relieved by activated charcoal, I uncovered that he had a significant yeast overgrowth meriting a change to trials of pharmaceutical antifungals. I decided to change to a pharmaceutical to go after the yeasts that persisted in showing up in his MOAT test from Great Plains Lab, where indications of *Aspergillus* turned up with elevation of 5-hydroxymethyl-2-fuoic. Watching to be sure his aspartate aminotransferase (AST) and alanine aminotransferase (ALT) stayed level, I increased his dose of Sporanox to 600 mg per day: three times the adult dose. This increase was done in tortuous steps of Herx reactions and wonderful breakthroughs in which we saw a new boy emerging. After getting to 600 mg, my patient came to his mom and said, "Mommy, I am all better now." In his new school (where they didn't know he had been diagnosed with ASD), his language ability was tested to be at the 6-year-old level. As an experienced solo practitioner, I have the leeway to be guided by a decision process in which the stakes are very high, the risk is tolerable, and the odds seemed pretty good. As the work of Dr. McGinnis and this case illustrate, a leaky midbrain can let in toxins that tie up a patient's tongue and adjacent parts of the nervous system.

Saccharomyces boulardii, Herx, and Activated Charcoal

Why do I move forward in this description without having first addressed changes in diet, which we agree are a foundation to nearly all natural medical interventions? Because asking parents of a child in the autism spectrum to make serious dietary changes—if they have not already started a gluten-free diet—is like asking them to convert to a different religion. Whether it has to do with introducing solid foods to babies in the villages of Chad, Africa, well before they are weaned at, say, age 2 or requesting a cattle rancher to go vegan, asking people to change the dietary habits of a child with serious sensory issues is an obstacle to be avoided even at the cost of perfection. Of course, we will soon move toward dietary changes that might ideally be good to have done at the beginning. To ask for it up-front as a sort of precondition to engaging in "treatments" in the form of thumbs testing is a challenge that is, I think, unnecessarily overburdening to families.

Saccharomyces (sugar fungus) is the genus name of common yeasts such as *Saccharomyces cerevisiae* (baker's and brewer's yeast). *Saccharomyces boulardii* is a closely related yeast whose exact classification and distinction from *Saccharomyces cerevisiae* are debated but that clinically acts as if it kills off pathogenic yeast and supports the development of normal gut flora. Taking *S. boulardii* may cause three main initial results (intense initial aggravation of symptoms typically due to Herx, nothing happens all, or marked benefit) that can be expected with any antifungal, except that the intensity of the Herx may be more than with any other antifungal. Charcoal (see box) is the answer to such a Herx and usually quenches it to make it stop or become tolerable. Apart from Herx reactions, this microbial supplement has no toxicity. It resides in the gut, where it joins the other flora until shortly after discontinuation and then disappears. A stool culture may grow out a yeast identified by the laboratory as *S. cerevisiae* because the laboratory cannot distinguish between these two strains.

Activated Charcoal—A Panacea

Parents who have witnessed the dramatic effects of charcoal in quenching a die-off reaction taught me years ago that it is helpful in many situations that, however mysterious, have no apparent relationship to a die-off reaction. We all have seen kids plunge into the depths of a really bad day of volatile moods, dominant among which are irritability and depression but could include all dysfunctional manifestations. In these situations, charcoal is a kind of panacea. The harm in using it every day is that it interferes with the absorption of nutrients from food, supplements, or medicine. If it is taken on a stomach that has been empty for an hour or will stay empty for an hour (20 minutes in a pinch), one need not worry about negative effects from up to 15 doses per week.

The *S. boulardii* dosage can be increased safely to multiples of the original dose to explore the possibility of overcoming partial resistance of the targeted fungus. The option of switching to other "systemic" antifungals, such as terbinafine (Lamisil), ketoconazole (Nizoral), itraconazole (Sporanox), and fluconazole (Diflucan), is remarkably safe but cannot compete with the complete safety of *S. boulardii*.

As I have emphasized, this trial should not be undertaken without first achieving control of constipation, usually with magnesium citrate capsules (150 mg) or CALM powder or liquid, such as is found in 10-oz bottles, provided as a cathartic but can be portioned out to be taken "to bowel tolerance" just short of risking an embarrassing urgency of BMs. As that dose is approached, the idea is to back off until reliable daily BMs are achieved with a frequency of at least once, preferably twice or three times.

When yeasts are killed by *S. boulardii*, they burst open, releasing their toxins in larger measure than when they are alive. The resulting mischief (the Herx reaction) may consist of the very symptoms that are the target of the diagnostic trial. Herx reactions may result in new symptoms of any kind and are occasionally quite disconcerting, but not harmful, and can usually be managed by taking activated charcoal. It comes as a 500-mg fine black powder, preferably refined from coconut. Its capacity to absorb poisons of all kinds is astonishing, as is its effectiveness.

Activated charcoal absorbs not only the targeted toxins but just about anything else it encounters, including medications, supplements, and nutrients. It is intended only for temporary use—as, for example, to get through a few days of Herx. An interval of at least 30 minutes should separate it (before or after) from medications, supplements, and food so that it neither absorbs medication and supplements nor is absorbed by food. It will impart a greenish-black hue to the stool and may be constipating, necessitating a sometimes-substantial hike in the dose of magnesium to keep toxins from lagging in their trip to the toilet. Observation of the time taken from the first dose until its appearance in the toilet gives a rough idea of one's "transit time," which should be about 12 hours from eating a food until its waste products appear in the toilet.

Activated charcoal should be recommended only if Herx symptoms emerge. Even if such symptoms are mild—or perhaps especially so—charcoal's effect in reversing symptoms helps to document that what your patient is experiencing is, in fact, probably a Herx reaction. I say "probably" because charcoal works for just about everything. Individuals—especially the parents of children who have been through Herx with the benefit of charcoal's dramatic neutralization of negative symptoms—find that charcoal can alleviate a wide variety of symptoms, such as malaise, hyperactivity, fatigue, headache, allergic reactions, and so forth, when taken occasionally as needed. It is not, as stated previously, for routine use.

It was 1831. In front of his distinguished colleagues at the French Academy of Medicine, Professor Touery drank a lethal dose of strychnine and lived to tell the tale. He had combined the deadly poison with activated charcoal. http://www.emedicinehealth.com/activated_charcoal/article_em.htm

S. boulardii. Klaire Labs (Reno, Nevada) now provides *S. boulardii* in powder form to facilitate the high doses that I recommend as needed to be able to walk away from a diagnostic trial with a strong conviction that it was given a fair trial. It is gratifying to hear from a patient or parent who has been skeptical of giving a high dose and struggling through the Herx reactions and charcoal and reports a sudden change to a dramatic success. The doses can be given the morning and evening because we are simply feeding a colony of temporarily commensal organisms that really know how to kill their cousins—a bit like the model of steps taken in a crime family. Uncomfortable Herx symptoms can be managed with 500 to 2000 mg of charcoal up to 5 times in 24 hours. A small amount of frozen grape juice concentrate thawed to a liquid provides a dark-colored, intensely sweet vehicle that may get charcoal past the vigilant senses of a child.

For some patients, the trial of *S. boulardii* is sufficient to control a yeast problem without any risk beyond the Herx reactions, which stop when the critical dose is achieved. At the very least, the trial of *S. boulardii* is sufficient to prove the existence of a yeast problem that may then be confronted with more careful dietary methods or a trial of systemic antifungal drugs or natural medicines.

The space given to magnesium and antifungal medicines and helminthic therapy (discussed in the following section) represents the

priority claimed in the implication of Dr. Yehuda Shoenfeld's point to the effect that all chronic illness is autoimmune. Of all the treatments that make up the toolkit for helping children in the spectrum recover, the ones that worked for Morgan represent a very good place to start.

Once all these diagnostic steps are in place, I have the following tests done, along with others that may be called for based on responses to the steps:
Zinc (*International Classification of Diseases* [ICD-10] code E60)
Ferritin (ICD-10 code V78.0)
Carnitine, (ICD-10 code E71.40)
Vitamin D_3 (ICD-10 code E55.9)
Vitamin A (ICD-10 code E56.9)
Beta-carotene (ICD-10 code E50.9)
Serum AST and ALT (ICD-10 code R94.6)
Thyroid-stimulating hormone (TSH) 244.9 (ICD-10 E03.9)
Complete blood count (CBC) with differential (ICD-10 D64.9)
Cortisol morning and evening (ICD-10 code E27.40)
Testosterone (ICD-10 code E27.40)
Dehydroepiandrosterone (DHEA) (ICD-10 code E27.40)
Pregnenolone (ICD-10 code E27.40)
Amino acid profile (ICD-10 code E72.9)
Lipid profile (ICD-10 code Z13.220)

The next step will usually be a trial of helminthic therapy, for which 6 biweekly doses are sufficient to judge efficacy for the *Hymenolepis diminuta* cysticercoids (HDC). Human and pig whipworm, human hookworm, and *Trichuis suis* ova (TSO) are other options available in a currently existing informal market. HDCs are supplied internationally by Biomerestoraton.com with a preservative. I provide HDCs shipped overnight with freezer packs by FedEx to recipients in the United States in a consultative context with referring practitioners. Other than allergy desensitization methods, antifungals and helminths are two effective options for the restoration of immune tolerance. This is not to say that the restoration of immune tolerance is not a function of healing provoked by many applications of the healing arts. In my experience with patients with severe chronic illness in forms that are as explicit as alopecia or autoimmune thyroiditis, these two interventions stand above all other treatment options.

Helminthic Therapy

HDCs are an organism native to the gut—part of the families of microorganisms that are essential to human health. The use of what is generally called "helminthic therapies" is not widely known. The popular view of "worms" is guided by experience with pinworms as well as by false claims that many individuals with chronic illness suffer from resident helminths. The functional medical practitioner needs to understand, however, that an absence of helminths is a major driver in autoimmune disorders, and reintroducing helminths into the human body is an essential component of the successful treatment of ASD and other chronic diseases.

Background: 10,000 years ago, our 200th great-grandmother was inventing agriculture while primitive men were out hunting and gathering. A beetle and a rat were watching Grandma planting seeds that were to become grain. The beetle said, "These humans are so cool! They are doing this for us." Ever since, the grain supplies of our planet have been host to grain beetles and rats whose parts and poops we consume safely and unaware. The transient, lively presence of HDCs in our digestive tracts restores immune tolerance that is present in populations living the old-fashioned ways whose microbiome naturally includes helminths.

Grain Beetle

Other organisms have also coevolved with us to live peacefully in our digestive tracts, and we humans have paid a high price for losing them. As a Peace Corps Volunteer in Chad in the 1960s, I took care of large numbers of Africans who were mostly fit and healthy, although sometimes afflicted with trauma or fumigating infections. The Chadian patterns of health and disease looked very different from Europeans'. We never saw an African living the old-fashioned way who had *any* allergies, autoimmune diseases, gallstones, appendicitis, or any of the kinds of skin or cardiovascular diseases so common among Americans.

Forty years later, researchers have established that these contrasting patterns of health are related to the absence in the digestive tract of Americans and Europeans of various organisms that were common among people living in less hygienic conditions. It is now uncontested that the rise in the prevalence of allergic and autoimmune diseases in Europe and America is due to the absence in our digestive system of organisms that have been regarded as parasitical. Without beneficial worms (symbionts), an underutilized immune system will attack itself. The phrase "loss of immune tolerance" applies to those of us who, during recent generations, have collectively suffered the absence of various sorts of worms. As a result, we suffer a lack of tolerance (allergy) to mold, pollens, dust, foods, and our own tissues (autoimmunity).

Tolerance is the treasure of all complex systems: social, political, mechanical, and biological. Its partner is diversity. As the organisms inhabiting the human digestive tract have become less diverse, the tolerance of the immune system has decreased, which is another contributing factor in the rise of ASD, as well as other autoimmune and allergic problems in those of us who have enjoyed the benefits of toilets and clean water. By introducing any of several options (human hookworms, pig whipworm eggs [TSO], or HDCs), the immune system regains tolerance so that inflammation in different tissues in different people is healed.

Helminth therapy for ASD: My experience with TSO and HDCs over the past decade is rich in cases with patients with all sorts of autoimmune and allergic problems. Given the high stakes involved in chronic illness and reasonable odds of success with Primobiotic use and the absence of significant risk, a trial of six doses at 2-week intervals is an option worthy of consideration to treat ASD. HDCs are, at first, a test in which we seek a result indicated by thumbs up, down, or sideways before deciding about continuing.

Primobiotics are not the treatment for any specific symptom of ASD. They are, like other probiotics, an option for all individuals with chronic illness to weigh by considering the BROCS, which are described later in this chapter. In that consideration, we often tend to overlook the stakes when focusing on the odds. That omission may occur even when the cost is tolerable, the potential benefit is great, and the risk is close to zero, as it is with the use of HDCs. When the stakes are high, as they are with any chronic illness, the odds of success with any proposed treatment may be less decisive than the stakes.

Complications: About one in a hundred patients acquires a rat tapeworm, which presents no significant risk and is expelled after a

dose of Biltricide (praziquantel). We have found that patients who have an initial puzzling mixture of positive and negative responses often have yeast problems that have not been treated with sufficiently aggressive therapy, including restriction of sugar intake.

Patients respond well to visiting the question of an inadequately addressed yeast problem. Response to HDCs is more robust and less confusing when such measures are taken into consideration.

Contraindications: Immunosuppressive drugs, systemic steroids, anesthesia, antiparasitic medicines, and antimetabolites. Helminth therapy depends on the participation of an active immune system.

Probiotics: Patients must be told to look at the label of any probiotics and discontinue those that specify any organisms with the name *Lactobacillus* or that begin with the abbreviation "L." or "l.," as in *L. acidophilus*. Primobiotics will work in the presence of such probiotics, but they work better without them. *Lactobacilli* that are the agents of fermentation in yogurt and kefir and other fermented foods are not a problem when these foods are consumed. Other probiotics are okay. Options for *Lactobacillus*-free probiotics from Klaire Laboratory include Ther-Biotic Factor 4 and BioSpora.

It usually takes only a matter of weeks, during which a small number (between 5 and 30) of HDCs can restore tolerance in a person's immune system to produce a large benefit, with a risk that is close to zero. A placebo-controlled double-blind study, conducted by Joel Weinstock, MD, and his colleagues, showed that pig whipworm resulted in dramatic improvements in 44.8% of those receiving the therapy, compared with 17.4% for those who took the placebo. No patient suffered adverse effects.[27]

Chelation

When chelation first appeared as a treatment for autism among pioneering doctors in the autism community in 2000, my original reaction was profound skepticism. I soon learned that I had been wrong, not only from my own patients' results with it but also from the data collected by the ARI.[28] Of all the treatments that were monitored by the ARI's parent reporting project, EDTA and DMSA chelation was not only the one with the highest score for positive/negative results, but it also climbed the ratings within a couple of years from its inclusion in the reporting module in 2001.

At an annual meeting of the DAN! think tank, I asked the physician attendees to raise their hands if they had seen positive responses to EDTA and DMSA that occurred so soon after the beginning of their administration, creating a strong impression of efficacy based on a mechanism other than heavy metal removal. Note that the latter mechanism was not to take place as monitored by serial 6-hour urine testing over months of treatment.

James Adams reported that children receiving just one dose of DMSA had positive responses comparable to those who were treated with multiple doses.[29] There appears, in other words, to be two separate features of the heavy metal/chelation picture. First, there is good evidence[30] pointing to a goal of minimizing lead and mercury levels to as low a level as can be achieved. The Lanphear paper responded to findings in a study of lead toxicity in the Boston study showing that children's cognitive testing had no significant improvement when blood levels were reduced from above 45 to below 25 ug/dL. Lanphear's review showed that cognitive health improved only when lead levels were brought below 10 ug/dL and improved more so between 5 and 0 than between 10 and 5. These data put us in a position in which maximizing the removal of heavy metals is justified to the point of continuing chelation until no further lowering can be achieved with additional bouts of chelation on a biweekly schedule. Here we find a disconcerting gap between the data used to drive public policy and what we use for children under our care, in whom the Lanphear's data oblige us to treat for maximum effect as measured by urinary output from an EDTA/DMSA chelation.

EDTA and DMSA are chelating medicines that bind to heavy metals and other toxins and send them out in the urine. They can be swallowed, injected, or given by suppository. I prefer to use suppositories, which provide very good absorption and results.

Both medications can be used for testing and for treatment. EDTA and DMSA are each "strong" in ways that make for a good partnership. I find in my patients that a 500-mg suppository of EDTA will pull a substantial amount of mercury and lead into the urine, and then, if followed 6 to 7 hours later with a DMSA suppository, another—sometimes larger—amount of mercury and lead comes out in the next 6 to 7 hours. The partnership of the two aligns with the observation that EDTA, while cleaning, tends to throw up some "dust" consisting of mercury and lead that would best be captured before it can settle back down in the body.

Whether you go by the ARI data showing parental evaluations bringing chelation to the top of treatment ratings or by the exquisite study by Lanphear et al., measuring children's body burden of heavy metals with an EDTA and DMSA loading test and following through with biweekly—allowing the body burden to equilibrate—chelation is the gold standard. Various protocols are in use for heavy metal detoxification. However, when I ask families who have done such protocols, I find that they have no objective test results that document the start, course, and end of the treatment.

Now we come to the second feature of the heavy metal/chelation picture. There was a consensus, referred to previously, that EDTA and DMSA often produce benefits for patients with ASD with an immediate timing that is consistent with a mechanism other than the necessarily slow process of heavy metal removal. And James Adams's report of sustained clinical improvement after just one chelation further suggests that there is something odd going on in this clinical landscape. Be that as it may, we are obliged to take very seriously the fact that we are living in an environment that exposes children to a burden of toxins and that the children on the autism spectrum have measurable weaknesses in detoxification.

Elevated Body Burden of Lead, Mercury, and Other Neurotoxins

Chelating agents are used to test for the body burden of lead and mercury and other neurotoxic chemicals. That burden is inside cells. Neither a blood level nor urine level will provide an accurate view of the starting point for considering further chelation unless the burden is high. Careful studies of lead poisoning have demonstrated that the improvement in key measures of health and behavior does not begin to occur until the blood levels of lead in the blood are below what has until recently been considered safe: 10 ug/dL. When lead levels drop from 5 to 0, the improvement in cognitive function is markedly better than in the range from 10 to 5. In the context of this international pooled analysis, "better" meant something quite different from what the scientists and intuition would predict. Most of us would expect that as the burden of lead gets closer to zero, smaller increments of improvement would occur—sort of like the law of diminishing returns, which says, for example, that continuing to add fertilizer to your lawn reaches a point where each handful produces much less of an effect than previous handfuls. In the case of removing lead, one might expect the rate of improvement to diminish as zero is approached.

Lanphear's study has shown that as one gets closer to zero, the positive effect is the highest.[31] For the child with ASD with a high body burden of lead, it is worth the effort to pursue a target of as close to zero as can be achieved, as long as it is done safely. To do so means that

one has to assess the size of the problem and then track progress over time to see the rate of improvement and find the point at which further chelation produces no further improvement in not blood but urine concentrations after a challenge with DMSA.

Testing for heavy metal burden may include blood levels to start with but not without giving a test dose of DMSA to see how much lead, mercury, and so forth will appear in the urine over the next 6 hours.

DMSA Suppository Loading Test

The Doctor's Data Lab is used for toxic metal testing. The protocol recommended here is different from the standard challenge testing protocol.

On three consecutive nights, the patient is administered 500-mg DMSA suppositories. On the first night at bedtime, administer one suppository. Nothing more is required until the second night at bedtime. On the second night, right after the bladder is emptied, administer the second DMSA suppository and begin a 6-hour urine collection—that means all urine passed during and including any produced at the 6-hour mark. If sleep continues a little beyond 6 hours, that's okay, but the collection must not exceed 7 hours. Repeat the same procedure on the third night.

The reason for doing three nights' collection is that the peak excretion of heavy metals tends to occur after the second or third night's dose of DMSA. The reason for doing the collection at night is that nighttime is the phase of the circadian cycle when detoxification is most active. When the levels of excretion are initially high, the discrepancy between nights 2 and 3 may be quite wide. With ongoing treatment after the initial tests, the gap should diminish and obviate the need for the double collection. In the early phases, a single measurement—instead of having the average of two—may bring a less reliable picture of the trend. A trend is very helpful in predicting the overall duration.

For a child, the trauma of suppositories may be alleviated/prevented by instructing the parent to do the following:

a. Place a hand mirror on the floor and invite the child to squat over it so as to get a good view of the anus.
b. "This is the hole where your poops come out. We call it your "____"
c. Explain that sometimes the doctor finds it better to put medicine in there, especially when the medicine is smelly.
d. Offer a whiff of the suppository. "See how smelly this is? It would not be fun to swallow it, would it?"
e. "Putting the suppository into your anus feels funny—like pooping backward—but it does not hurt."

For the treatment phase, the same routine is followed for the three nights every 2 weeks.

RESTORATION

Morgan's story illustrates the two steps that I have witnessed since 1977 and 2007, respectively, to beckon the word *restoration* into our efforts to promote Nature's buoyant impulse toward healing. First was a diagnostic trial of *S. boulardii* and the sequence of antifungal pharmaceuticals in search of the best fit, and finally was helminthic therapy, which was the dramatic final step in supporting Nature's job. A third approach to promoting tolerance is embodied in various methods to achieve desensitization. It was in the medical family of clinical ecology—later to become environmental medicine—that I learned the side of my medical paradigm that has to do with avoiding allergens and toxins or ridding them from our environment, and that continues to be part of my toolkit, minus the methods for "neutralization" that I practiced for many years and admire in the hands of colleagues who can restore tolerance by those methods. The more I learned about finding out what my patients were sensitive to, the more I became curious about *why* they were sensitive. The what and why lists are short, and yeast and mutualistic organisms turn out to be the major factors in the loss of immune tolerance. The list cautions against the danger of being blinded by the obvious to the point of ignoring other causes of sensitivity.

What Sensitive To?	Why Sensitive?
Food	Unmet needs[a]
Inhalants (dust, dander, mold)	Bad digestion
Microorganisms	Bad flora, esp. yeast
Chemicals	Toxins (Al, Hg, Pb, and novel chemicals)
	Adrenal insufficiency
	Invasive life events

[a]Essential nutrients, accessory nutrients, light, love, rhythmic integration, mutualistic organisms.
From Baker, SM. *Detoxification and Healing.* New York: McGraw-Hill; 2003.

Diet

For ASD, a diet free of sugars and starches is absolutely critical. Whether it comes in the form of Dr. Natasha Campbell-McBride's gut and psychology syndrome diet (GAPS); the specific carbohydrate diet (SCD), which is based on the work of Sidney Hass, MD; the diet more recently presented in Dr. David Perlmutter's book *Grain Brain;* the paleo diet, popularized by Loren Cordain, PhD; or the super immunity diet presented by Joel Fuhrman MD, the common feature of diets that can help resolve many of the symptoms of autism, including self-injurious behavior (which I discuss in more detail in the following discussion), is the restriction of carbohydrates. Disagreements about what is the right diet for everyone have been debated publicly and have produced confusion in all of us for the past half century, but a consensus has finally emerged. Dr. Mark Hyman's 2018 book *FOOD: What the Heck Should I Eat?* provides a commonsense standard.

A healthy diet to treat ASD:

1. Must consist of real, whole, fresh organic foods
2. Must be free of refined sugars
3. Must be gluten-free
4. Must be low in casein and soy (casein and soy are better off avoided.)
5. Must consist of a diversity of foods

One of the greatest strengths in children with autism is an iron will, which makes it difficult for families affected by autism to implement new diets. Children on the autism spectrum also have problems with midbrain regulation of the muscles of chewing, swallowing, digesting, and pooping, making those functions more problematic for them than for neurotypical children.

Mainstream medicine posits that there are no "proven" treatments for autism. Granted, there are a few drugs that are "approved" for the symptoms of children with autism. For instance, risperidone and aripiprazole are both antipsychotics approved by the U.S. Food and Drug Administration (FDA) to treat irritability caused by ASD. The natural medical doctor's job is not to find the cure for "autism" but to find out what works for the individual patient. If we want to find out if a given dietary intervention, supplement, or other treatment works for one particular child, we can put it to the thumbs test.

Implement dietary changes for at least 6 weeks. Parents, teachers, grandparents, and practitioners who are unable to change a child's diet are passing up the most potent therapy we have, a therapy that is certainly better and often more effective than all the drugs. Understanding that should mobilize families to prove the point for themselves.

Eliminating sugar, restricting carbohydrates, and eating a diet of real foods are the first steps to resolving self-injurious behavior and other symptoms of autism that are often exacerbated by food additives,[32] pesticide and herbicide residues, and other contaminants. A diet free of gluten and casein should be implemented at the beginning of any effort to make a therapeutic plan for a child on the autism spectrum. No other step is more demanding of parental understanding of the following aspects of dietary changes:

A. Background of reasoning and science and the experience of countless physicians and parents
B. Potential for producing an immediate and very dramatic breakthrough
C. Need from support from an experienced diet counselor and/or parent

The Yeast Problem

In the children I have treated who have recovered from autism to become not just normal but usually gifted with various cognitive talents, a yeast problem has been an important factor in all such children, so naturally I feel that this consideration is high on the list of options for testing and treatment. Symptoms, such as those discussed Chapter 137, Let the Data Speak, are a good guide to patient selection, but no child should be deprived of a diagnostic trial of a dietary thumbs test. I describe it here as a matter of protocol, with the understanding that many new patients on the autism spectrum come to us on very restricted diets with little latitude for experimentation until measures beyond the basics (gluten-, casein-, and sugar-free) can be implemented. The following may fit situations in which the patient's diet is so yeasty as to perhaps be an obstacle to further thinking. Otherwise, the following information may be a resource to share with parents as a glimpse down the path that will begin with less resistance and more focus. Keep in mind that the population of patients on the spectrum share a profound sensitivity expressed in the domains of the nervous and immune systems. The word *senses,* as in touch, taste, vision, hearing, and olfaction, has the same function as does the immune system in terms of receiving, remembering, and responding to environmental stimuli. In other words, the immune system and this aspect of the central nervous system are a functional unit. As such, it is not surprising to find that children on the spectrum have allergic, autoimmune, and sensory issues that gather under the term *loss of tolerance.* Food is obviously one environmental stimulus that engages the senses and immune system in challenging ways. By that token, the avoidance of a bothersome food can, in some instances—such as gluten—bring about marvelous changes in a matter of days. But let's focus here on yeasts.

Yeasts and other molds are the only organisms to be found in the air we breathe, in food (e.g., on the surfaces of fruits and as used for leavening and fermenting), as well as being members of our microflora, where they are subject to overgrowth from the consumption of antibiotics and sugars. Even milk can have fairly large amounts of yeast in it. Our relationship with them crosses the boundaries among commensal, infectious, and allergic interactions. When the latter case is put under scrutiny, various tests can indicate sensitivity, and the most precise test in terms of establishing pathological sensitivity is strict avoidance followed by a challenge. Extreme sensitivity to molds and yeasts found in food requires strict yeast/mold avoidance for at least 5 days, followed by a challenge to implicate symptoms that have cleared and then been provoked.

Usually, a simplified yeast-/mold-free diet is sufficient to make this point. Many foods consumed nowadays are heavily yeasty or moldy, and avoidance of these is often enough to establish the relationship between sensitivity to yeast/mold and various symptoms, which may include craving bread, vinegar, and sugar. A simplified yeast- and mold-free diet focuses on avoiding the yeasty substances normally found in a healthy diet, including the following, which may be put in a handout for your patients.

Leavened foods: Breads, bagels, pastries, pretzels, crackers, pizza dough, and rolls are usually made with yeast as leavening. Biscuits, muffins, and soda bread, as well as waffles, pancakes, and some cookies made with baking soda or baking powder, are allowed. "Essene" bread is made with sprouted grain, and no yeasts or baking powder/soda is used. Baked goods made with baking soda or baking powder are permitted on a yeast-free diet.

For the yeast-sensitive person, sourdough bread offers no advantage, and even when sourdough bread is labeled "yeast-free," it should not be considered so from the standpoint of people avoiding yeasts and molds.

Dairy products: Cheese is a product fermented from a variety of molds and bacteria. Some of these are quite distantly related to the family of molds referred to as yeasts, and some yeast-sensitive people can eat even quite strong cheeses without difficulty. During the yeast avoidance that is designed to detect yeast sensitivity, all cheeses should be avoided. Milk products known as "processed" are usually prepared with a substance called rennet. Artificial types of rennet are derived from mold products and, therefore, are not safe for the person trying to avoid yeast. Other milk products such as cottage cheese and sour cream may be made by adding vinegars or lactic acid to milk and are, therefore, "yeasty." Yogurt is fermented milk, and the germ used to make yogurt is a *Lactobacillus* or *Acidophilus* species. These bacteria are healthy, normal inhabitants of the human intestinal tract and vagina and are not related to yeasts or molds that are pathogenic. Yogurt without fruit can be consumed by people who are yeast-sensitive even though the milk itself, as mentioned, may have some yeast in it.

Juices: The juices of all fruits and berries contain yeasts that came from their skin. Ciders, apple juices, commercial frozen or reconstituted orange juices, and the juices of berries have an impressive content of yeasts and can be extremely troublesome for yeast-sensitive people. During the trial avoidance of yeasts, then, juices must be avoided. For the treatment of ASD, juices should be avoided anyway because of their glycemic index.

Dried fruits, condiments, and sauces: Most sauces and condiments are made with some vinegar or other products of fermentation as well as sugars. This includes salad dressings, barbecue sauce, tomato sauce, soy sauce, miso, tamari sauce, mincemeat, horseradish, sauerkraut, pickles, and olives. Dried fruits themselves are, of course, very "yeasty" because the drying process generally reduces the whole fruit to a small size while the surface yeast has had a chance to proliferate during drying. Salad dressing can be made with oil, lemon juice (in place of vinegar), and some spices.

Mushrooms: Mushrooms, which are fungi, are the product of molds that live in a lacy network underground and periodically push up a mushroom as part of their reproductive processes. The mushroom itself is relatively unrelated to yeasts, and some yeast-sensitive people can eat mushrooms. During a trial avoidance of molds and yeasts, mushrooms should be avoided. The same applies to truffles.

Sugar and Carbohydrates: Some yeast-sensitive people have a lot of trouble with carbohydrates of any kind, and for most, the consumption of refined sugar is bothersome. Anyone going to the trouble of removing yeast is well advised to eliminate refined carbohydrates, such as sugars, white rice, and white flour.

The following yeast-free diet outline addresses only the yeast question, not gluten, casein, or other issues.

Dairy Products

Permitted: butter, margarine, mozzarella cheese, feta, milk (cow or goat), yogurt (plain)

Not permitted: yogurt with fruit, aged cheeses, processed cheeses (which are often made with an artificial rennet derived from fungi)

Cereals/Grains

Permitted: soda bread, cakes, waffles, pancakes, popovers, cookies, biscuits, barley, corn, oatmeal, grits, rice, pasta (check label), amaranth, buckwheat, quinoa

Not permitted: bread, sourdough bread (except that made with *Lactobacillus* culture), rolls, breakfast cereals, crackers (check label), nongrains

Meat, Poultry

All permitted (preferably organic to avoid antibiotics)

Vegetables

Permitted: all fresh vegetables, tofu (fresh), legumes (beans)

Not permitted: mushrooms, olives, capers, tempeh

Fruits

Permitted: pineapple, avocado, banana, any fruit you can peel, but no fruit juice

Not permitted: berries, melons (they are not yeasty but tend to cross-react for people with mold allergy), prunes, dates, figs, raisins, cherries, commercial juices (concentrate, premium, in cans, in bottles)

Nuts

Permitted: almonds, almond butter, cashew, cashew butter, walnut, brazil, macadamia, filbert, pistachio, pecan, chestnut, water chestnut

Not permitted: peanut, peanut butter

Oils

All oils are **permitted.**

Beverages

Permitted: herb teas, mineral water, soda water, artificial sweeteners (but they are not good for you)

Not permitted: fruit juice

Miscellaneous

Permitted: English mustard (made without vinegar), salad dressing made with oil and lemon juice

Not permitted: catsup, mayonnaise, French mustard (made with vinegar), salad dressing made with vinegar, **sweets,** Chinese food (admittedly a broad and tempting category, but in general, Chinese food is poorly tolerated by yeast-sensitive individuals)

Artificial Sweeteners Implicated in Brain Dysfunction

Richard Wurtman, MD, and John Fernstrom, PhD, neurologists at the Massachusetts Institute of Technology, have pointed to aspartame's potential interference with neurotransmitter function.[33] The vigilant practitioner occasionally finds patients whose symptoms are relieved by abstinence from aspartame. The first sip of a diet soda tells the brain, "here comes sugar," raising metabolic expectations and mobilizing vital processes. The brain tells the body to get the insulin because sugar is on its way. Insulin levels begin to rise, only to find that what has been imbibed is empty of the calories the insulin is there to manage. It is as if you made a date for dinner, told your friend to order for you, and then failed to show up. The insulin grabs whatever real sugar is available and pushes it into the cells. This causes blood sugar to drop, providing a truthful signal to the brain that the body needs sugar. This cycle is the real roller coaster behind the artificial sweetener story and the mechanism behind the diet soda habit that I see in so many patients. Artificial sweeteners are all "bad" in the sense that they compromise the metabolism, fooling the body's chemistry with a false promise that calories are about to arrive. For thousands of years, our species has used sweet taste as a perfect test for what is good to eat. Refined sugar is, strictly speaking, an artificial sweetener because in its pure form it was unknown in the human. But it is not, at least, duplicitous at the cellular level.

Dietary Tests

Start with all the additives. If a food has a label with anything but the name of the food on it, then do not give that food during the time when the diet test is taking place. Sensitivity is the issue. The test of avoidance to see if a person may experience a positive change that may take as little as 5 days, after which the avoidance may be followed with a confirmatory challenge. If the results are equivocal, repeat avoidance testing is required to make sure that a suspect food deserves avoidance, considering that we wish to avoid diets that verge on being too limiting. Rarely, the avoidance of a food may require as long as 3 weeks to be confirmatory. Such is the case with gluten. The research of Alissio Fasano, MD, shows that gluten opens the tight junctions in almost everyone. An elimination diet serving as a test should put gluten near the top of the list and be given more than the 5-day minimum for such tests.

Oxalates. Susan Owens was an early member of the DAN! movement. She has developed a website (http://www.lowoxalate.info) and a Facebook group (Trying_Low_Oxalates) with more than 15,000 members. Her work has benefited vast numbers of people who have been able to untangle the complexities of oxalates for their benefit in confronting a variety of vexing clinical problems. I encourage you and your patients to become informed and make use of her efforts in ways that, I must say, have not been fully grasped and appreciated by the DAN! movement.

Foods rich in oxalate, such as the spinach, kale, Swiss chard, and arugula found in the green smoothies that have been popularized as a way of "cleaning up" the diet, can be harmful in large quantities. The complexities of oxalate are increased by the role of oxalate-producing bacteria and yeasts that are part of our microbiome. Quirks in our own biochemistry add to the total production. This biochemistry is fueled by the consumption of foods with amino acids that convert to oxalate. In addition, dietary calcium and oxalate have interactions that result in a paradox. A high-oxalate food, spinach, reduces calcium absorption even though spinach is a good source of calcium. The oxalate binds calcium in the gut and prevents its absorption so that the calcium oxalate ends up in the toilet. Thus calcium (and magnesium) supplements lower oxalate absorption. In body fluids—destined to pass through the kidneys to become urine—calcium and oxalate combine to form kidney stones (if citrate levels in the urine are insufficient to solubilize the supersaturated calcium oxalate solution). One must consume the calcium with the meal to bind the oxalates in the gut and allow them to be excreted in the feces. Consuming the calcium between meals, however, can increase oxalate stone formation because binding will take place after absorption.

Oxalate crystals can accumulate in many tissues, including the brain. Oxalate accumulation figures in chronic fatigue syndrome, and oxalate crystals in the urine can be irritants that cause vulvar pain that can be misdiagnosed as infectious or hormonal. A family history of kidney stones, vulvar pain, fibromyalgia, and even stroke or cardiovascular disease may alert you to the need to look for oxalate issues in a child with ASD. A rich source of information on the subject of oxalates is available at Susan's website and Facebook address.

A change in diet is not the only way to evaluate. Arguably the most informative test to have done in a child on the autism spectrum—or for that matter, with any mysterious chronic illness—is a measure of

urinary organic acids. This test was first provided by Andy Braille, PhD, and Richard Lord, PhD, at Metametrix and is now available from Great Plains Laboratory and Genova Diagnostics. Testing for the key organic acids—produced not by the patient but by the fungal flora of the patient's microbiome—is a very useful clinical tool.

Urinary Organic Acids

William Shaw, PhD, at Great Plains Laboratory has continued to develop testing that reveals the presence of some of the key germs that produce compounds that are closely associated with toxic and immunological damage in children on the autism spectrum. The test not only allows documentation of yeast and other fungi, *Clostridia,* and other harmful microorganisms but also provides a way of tracking progress when it comes to treatment. The urinary organic acid test reveals other features of the metabolic "fire" and many aspects of our biochemistry, including energy production. The urine organic acid test is the most valuable among all that take a biochemical inventory leading to actionable options for safe intervention.

BROCS TEST FOR ANY PROPOSED MEDICAL TREATMENT FOR ASD

B stands for benefit, in which the doctor and patient share.
R stands for risk, in which the doctor's part is called liability.
O stands for odds, or the probability of the desired benefit.
C stands for cost, as in the expenditure of time and resources.
S stands for stakes, shared variably by the doctor and patient.

These considerations apply to choices confronting doctors and patients when trying to help a patient severely affected by ASD. When weighing the options for treatment, we may keep in mind that nearly all chronic illnesses have to do with the loss of immune tolerance. Once we accept that proposition, then the question of tailoring the treatment to a particular individual is simplified when we consider that the individual, not the disease, is the target of treatment. How do we know which people will be cured or benefited or will be nonresponders? We do not know. That's where the idea of a safe therapeutic trial comes in. Indeed, it's the only way to find out.

If you have explored the terrain of thumbs testing, methyl B_{12} injection, magnesium to bowel tolerance, yeasty dysbiosis, helminthic therapy, and heavy metal testing and chelation and seen the benefits of a sugar-, casein-, gluten-free organic diet, you will have reached an altitude where the view will offer various options as your thumbs embrace a view of responsiveness. If antifungal approaches have yielded Herx reactions and some improvement, I suggest an effort to engage with a high dose of Sporanox with monitoring of AST and ALT in case it returns a sudden miracle, such as I have seen. This is the time to weigh the BROCS.

The menu of choices will differ depending on whether you have a responder or not. The order of the following tests and treatments will depend on the view past your thumbs, which includes the following options:

1. **Induction of glutathione and its partners in dealing with oxidative stress.** At the 2016 Institute for Functional Medicine Meeting, Albena Dinkova-Kostova, PhD, from Dr. Paul Talay's team at Johns Hopkins, and Eleanor Rogan, PhD, revealed the path to raising glutathione levels by inducing them as well as estrogen quinone hydroxylase and other agents for the protection of DNA, RNA, and other vital players that need to hang on to their electrons. Broccoli sprout extract has since been through various steps to produce its ideal in the form of Avmacol.
2. **Induction of NRF2 and NRF1 with Protandim.** Because it is sold in a multilevel marketing mode, I am not sure how to handle this recommendation. You can email Erin at katepande@yahoo.com. I have done my homework on it, and so has David Perlmutter. Get microbial organic acids testing (MOAT) and MYCOTOX testing from Great Plains Laboratory. Whether you know already or not, these data will help you answer the key question in the antifungal sphere, "Have we done everything we can for this patient?"
3. **Antiviral therapy.** The immune system of autistic children tends to be both weakened and defensive. So-called thymus helper 1 (Th1) immunity is down, and thymus helper 2 (Th2) is up. The most helpful image to illustrate this situation is an unbalanced see-saw. The low end corresponds to mechanisms that deal with viruses, fungi, and cancer cells: all foes with which we humans have nothing but unfriendly encounters. The high end represents an enhanced activity of the immune system's branch offices that deal with more complex defensive decisions regarding food, bacteria, and other foreign agents (antigens), among which are friends, foes, and cases of mistaken identity giving rise to allergy and autoimmunity. The see-saw is an unbalanced Th1–Th2 relationship.

Th1 cells are the important cellular participants in defensive activities of the immune system. Th2 refers to immunity derived from antibodies, which are sticky proteins made specifically against everything internal and external that you encounter in life; these serve to label and handle foods, germs, toxins, and so forth.

Viral or fungal infections lower Th1 immunity. Low Th1 immunity leads to difficulty in resisting viral and fungal infections. Here is another vicious cycle in which it is hard to tell which came first: the infection or the lowered cellular immunity. We know that measles vaccine virus has been isolated from the biopsied gut lymphoid tissue of a number of the autistic children in whom it was tested. Did this virus cause the lowered Th1, or did the virus take hold because Th1 immunity was already lowered—perhaps from the effects of antibiotics or overvaccination or both?

When we find evidence of viral infection—including the isolation of measles vaccine virus from gut lymph nodes as well as spinal fluid—then the cause-versus-effect dilemma becomes secondary to therapeutic considerations, which are especially confusing in kids who may have severe symptoms of autism but seem to get sick less frequently than their neurotypical siblings and neighbors. Those kids may not be getting sick because their immune systems are activated by already being sick.

Jaquelyn McCandless, MD, has shown that low-dose naltrexone raises Th1 and is an antiviral, not because it kills viruses but because it rebalances immune function by other means. Dr. McCandless presented evidence that low-dose naltrexone (LDN) can help children with autism, but not without some caveats. I refer practitioners to the latest edition of Dr. McCandless's *Children With Starving Brains: A Medical Treatment Guide for Autism Spectrum Disorder,* published just before her untimely death in 2014.

Ridding children of the persisting presence of harmful viruses can be a helpful treatment, but we do not know if the apparent effectiveness of "antiviral" treatments stems from their antiviral properties or results from a different mechanism. The benefits of LDN have been demonstrated in AIDS, so the hope regarding the immunological benefits of LDN is well founded. The benefits of other antiviral treatments, such as acyclovir (Zovirax) and related medicines (e.g., Valtrex), may stem from the fact they combat viruses belonging to the large family of herpes viruses. These drugs also lower levels of adenosine in the body (see the diagram to find adenosine at the tip of the curved arrow between SAH and homocysteine).

The diagram shows the effect of the antiviral Acyclovir on adenosine levels. It is what Dr. McCandless cited in her book tying the antiviral drug effects to the same with LDN. Its levels are, in turn, also affected by the interplay between dietary gluten and the enzyme DPP-IV that digests gluten and also functions as a regulatory protein (CD26) in the immune system.

OTHER CONSIDERATIONS IN TREATING ASD

Taurine

Notice that taurine is at the end of the line in the previous diagram. With that in mind, here is Edward's story. This 8-year-old patient with ASD had light-colored stools. Edward came to see me with his mother and grandmother. I explained to the family how bile cuts dietary fat in the body much the same way that soap cuts grease when we wash dishes. Formed in the liver during the night, bile descends to the gallbladder to await the need for digesting (breaking up, cutting, dissolving) dietary fat in the morning. Instead of lard, which is used with a mineral in soap, the liver uses cholesterol to make bile. Instead of a caustic mineral such as potash (potassium carbonate), taurine steps forward, pretending successfully to be a mineral, and joins with cholesterol to make the bile that helps digests fat. If taurine is in short supply, cholesterol has no partner and is left in the gallbladder to form gallstones, which are not rocks but pebbles of hard cholesterol.

After I explained all this, we decided to start Edward on a trial of taurine at a dose of 1000 mg in the evening to see if it would darken his stools and help his itchy skin and digestive issues. Mind you, taurine has other very important roles in biochemistry, not the least of which is the quenching of the ring of hypochlorite (bleach). Macrophages use taurine to protect themselves from the bleach they use to kill ingested microorganisms. If Edward's stools would darken (not a long shot), it would reveal that his previous shortage of taurine had been giving him trouble with bile.

Two months later, Edward's family returned. Edward's grandmother announced that she took taurine and her gallbladder pain stopped immediately. An angry skin condition she had suffered from for decades cleared up. For years she had visited dermatologists who had confidently diagnosed eczema without being able to make the link to a special sensitivity to chlorine that resolved with her use of taurine. Meanwhile, Edward's stools had darkened as the taurine supplement satisfied his needs in bile formation, cleared his itchy skin, and presumably in the several other, more silent ways that taurine plays its role of pretending to be a mineral in his body's chemistry.

I have seen taurine supplementation result in lasting health benefits like these in my practice more times than I can count. Do you ask each patient the color of his or her stool?

Gene Variants DQB1 and DRB1

I was asked to testify in a divorce case in a state with a law that mandates immunization to the hilt. The judge was, moreover, noted for her anti-anti-vax position. The affluent husband insisted on catch-up immunization for his two boys, one of whom was on the spectrum and seemed to have gotten there after immunization during infancy. The boys tested positive for DQB1 and DRB1 alleles. Individuals with DQB1 and DRB1 mutations are at significant risk of having a serious negative reaction to vaccines with aluminum as an adjuvant. The test can be ordered from the laboratory with a request for "Please do: HLA-DR/DQ Low Resolution Typing, CPT Code 81375, Includes DRB1, DQB1." A small percentage of individuals with different variants (alleles) of the DQB1 and DRB1 families of genes react to the aluminum that is used in the vaccines. The reaction may take the form of many different autoimmune conditions and can produce lifelong disability and continued risk with further exposure to aluminum, such as is found in baking powders and buffered aspirin. The risk may be as low as 1 in 100. If you were invited to take a flight on an airplane with a 1 in 100 risk of crashing, would you do so? Would you consider taking such a flight once, 10 times, 50 times? You get the point. No one knowing the odds and with one of the DQB1 or DRB1 markers would allow vaccination with an aluminum-containing vaccine. At least 75 scientific peer-reviewed articles support this recent research. The public health and commercial entities with an interest in this research have not made a public response, which is, indeed, one that will be difficult to manage in terms of the overall risk/benefit with regard to mass immunization. The literature on this is covered in Dr. Shoenfeld's book.[34]

The judge returned from a brief recess, and the case was decided in the mother's (and children's) favor. If the question of vaccines comes up, I'd say the BROCS strongly indicate having these variants tested. Although the public has become increasingly aware of methylenetetrahydrofolate reductase (MTHFR) variants, when it comes to genetic testing, DQB1 and DRB1 testing may be more important.

Electroconvulsive Therapy

Let's go to a darker part of this landscape, where we find nonresponders. I had a patient who seemed to respond to just about everything for a few days and then went back to breaking everything in his house, including his parents' hearts. Brett grew to well over 6 feet tall and weighed 500 pounds. He had lost 300 pounds in the past couple of years, but that's about the only good thing that has happened for him in the 26 years I have known him. He can, at times, be cheerful, but the injuries he has self-inflicted and delivered to his caretakers have been repetitive tragedies. I met Dr. Lee E. Wachtel, medical director of the Behavioral Unit at the Johns Hopkins School of Medicine in Baltimore, MD, and learned about electroconvulsive therapy (ECT) for a similar patient. With the right technique, Dr. Wachtel explained, ECT could be transformative.

Dr. Wachtel published the first report of the use of ECT in an 8-year-old child. "Self-injury included slapping and punching his head as well as banging his head on his knees and shoulders, with daily rates averaging 109.3 attempts hourly based on 24-h data collection."[39]

During the 5 years since the onset of his self-injury, 17 psychotropic drugs had failed to provide relief, and 2 of them (sertraline and fluoxetine) made him worse. Only clomipramine and fluphenazine led to small reductions in self-injury. The following interventions failed: "behavioral assessments and interventions including, but not limited to multiple functional analyses, antecedent analyses, preference assessments, reinforcement-based interventions (i.e., functional communication training, differential reinforcement procedures, noncontingent reinforcement), response reduction procedures (i.e., brief physical holds, contingent application of helmet), and bilateral arm restraints and protective equipment (i.e., padding)." The ECT resulted in a profound reduction in the rates of self-injurious behavior.[35]

Brett lived a thousand miles away, but miraculously, a kindly psychiatrist who was simply making rounds when Brett was hospitalized for a leg he broke on a trampoline peeked into his room as a courtesy. He knew about Dr. Wachtel's protocol and managed to carry it out, and Brett had a year of respite. Then the kindly doctor moved far away, and the nuances of how to tailor the ECT to Brett went with him. The only other treatment that worked for Brett to calm his violent episodes and uncontrollable rages has been cannabis. Now that cannabis has become legal in his state, it is doing a pretty good job. Cannabis may prove of medicinal benefit for many patients with ASD in the coming years.

If your patient's life is controlled by self-injurious behavior, ECT may be a reasonable intervention. A consultation with a neurologist is helpful.

Fasting

Dr. Wachtel's reports of the use of ECT compared with the results of psychotropic drugs, as mentioned previously, certainly make the drugs seem of limited effect in extreme cases. Given that ECT itself is an extreme measure, I wonder whether extreme dietary interventions might first deserve more serious consideration. The model of extremity in dietary matters is fasting. Many children have been reported to calm down and or be relieved of various severe symptoms during abstinence from food. Preparation for radiological examinations and surgery and loss of appetite during acute illness are widely recognized as having transient beneficial effects. Although many of the children I have treated have followed restricted diets (e.g., sugar, gluten, casein, yeast), none has done so in ways that would seem therapeutic in the broader sense. Many with gastrointestinal problems have been found to tolerate only a few foods (e.g., chicken, rice, squash) while continuing to suffer from symptoms that are barely controlled by diet. Fasting, on the other hand, requires complete abstinence from food. It is a practice with a long history in human experience and a recent upsurge of interest in its medical benefits. I believe that the resistance to the notion of fasting in children may be overcome by the comparative danger of some of the symptoms many children endure without relief when using other interventions. Just as Dr. Wachtel was the first to try the extreme measure of ECT for an 8-year-old, another researcher or practitioner will try fasting a child with uncontrolled, continuous self-injurious behavior.

Supervised fasting has been a foundational protocol in the natural hygiene and naturopathic medicine traditions. Trevor Salloum, ND, first wrote the first modern, scientific chapter on fasting almost three decades ago for this book. This chapter has since been extensively updated by Alan Goldhammer, DC (see Chapter 37, Fasting, and supporting Appendix).

Several sources are of value to any practitioner who wishes to learn about fasting. The classic reference was written by early naturopath Herbert Shelton, ND: *Fasting Can Save Your Life.* Trevor Salloum, ND, wrote *Fasting: Signs and Symptoms: A Clinical Guide* for consumers. *Fasting: The Ultimate Diet* by Alan Cott, MD, is a fascinating little book by one of the pioneers of orthomolecular psychiatry. In no other concept or practice did I feel as misinformed as I was for decades on the subject of fasting. I made a sudden turnaround when biochemist T. Colin Campbell, PhD, told me the personal story of his cure by fasting from a condition for which he had consulted a top neurologist. The neurologist told him he would be dead in 6 months from his progressive disease. He consulted another top neurologist, who offered the opinion that he would commit suicide in 6 months. It goes to show the value that can sometimes come from a second opinion! As it turned out, Dr. Campbell undertook a medically supervised fast, and his health was completely restored.

Unmet Needs for Calcium Connected to Self-Injurious Behaviors

Mary Coleman, MD, a lone pioneer in autism research at the academic level in the 1970s, visited my office in the 1990s and told me about an association between eye-poking and unmet needs for calcium. She had a patient who poked incessantly at his eyes. His mom had consulted Dr. Coleman about the eye-poking, and Dr. Coleman asked her to collect a 24-hour urine specimen that was needed to document what Dr. Coleman thought would be a problem of unmet needs for calcium—leading to eye-poking in some autistic children. Twenty-four-hour urine collections in any child are a challenge, and especially so in children who are not toilet trained and have many uncontrolled, hyperactive behaviors.

The boy's mother was unable to collect the urine, and the boy poked out his own eye. Returning to consult Dr. Coleman after this tragic event, the mom found a way to collect the urine, which revealed the suspected low calcium level. The boy was started on a calcium supplement and stopped poking his other eye, saving his vision.

I was haunted by this story and spoke of it again in 2014 with Dr. Coleman. She pointed out the value of the urine test for calibrating calcium intake to the patient's need. I asked whether the level at which eye-poking stopped could be found by simply trying an escalating dosage regimen without urine collection. She said yes. I asked her what she believed was the root cause of eye-poking. One boy in Dr. Coleman's practice who was able to explain, once he became verbal, said that his eye-poking had been a response to an intense itching behind the eye.

Many children seem to prefer pain to itching, and I have seen patients with ASD injure their skin by scratching it raw. Sometimes a self-inflicted pain, such as wrist biting, may change the intensity of another pain in the body. The father of one of my wrist-biting patients told me of his own need to press hard on his eyes to blunt the pain of severe migraine. We must keep in mind that various self-injurious behaviors may be pain substitutions.

These examples illustrate that self-injurious behavior is likely *done for a reason*. Once the practitioner understands that self-injurious behaviors may be motivated by sensory challenges such as incessant itching or extreme pain, our own motivation to get to the bottom of the behavior is magnified. When confronted with any mysterious repetitive behavior, consider that remedies such as calcium supplementation may, in some individuals, also have benefits beyond the specific indications that I have described. To find out if such a treatment works, try it on the individual patient and let the results speak for themselves. Take into consideration the risk, probabilities, potential benefits, and the stakes. When the stakes are high, as in self-injurious behavior, a decision to give a safe treatment—such as calcium supplementation in the form of calcium citrate—can be reasonable even if the probability of success seems low. The dose would range from 1 to 6 g daily over a few weeks, depending on body weight.

Lithium

Head pain (e.g., cluster headaches) has been shown to be responsive to a form of lithium.[36,40,41] Lithium has many medicinal uses in low doses (5–100 mg of lithium orotate), as well as in high doses (900–1200 mg of lithium carbonate). Low-dose lithium is found in some well water because the wells draw from natural water tables, which can contain a generous concentration of lithium salts. Several comparative studies reveal a lower prevalence of anxiety and hyperactivity in consumers of such water compared with people living in low-lithium areas.[37] Low-dose lithium may also help with anxiety in the child with ASD. In a nonverbal individual practicing self-injurious behavior that may be indicative of underlying severe pain, a trial of lithium is simply common sense.

Testosterone

A mild form of congenital adrenal hyperplasia may be spotted by aggressive or hyperactive behavior accompanied by elevated testosterone levels. This is best handled not by suppressing testosterone levels but by recognizing the underlying adrenal problem of underproduction of cortisol so that the pituitary's stimulation evokes production of what the adrenals are able to produce: androgens. Subreplacement doses of hydrocortisone are the logical answer to a problem that must be considered when seeing aggressive behavior in patients with ASD.

Zinc

White spots on the fingernails and stretch marks in various parts of the skin are indications not seen in younger children with unmet needs for zinc. A serum zinc level is, therefore, an important part of blood screening. The consensus in a meeting of trace element researchers that I attended years ago was that the cutoff in displaying laboratory zinc test results should be ignored in favor of accepting a level of at least above 80 ug/dL.

Cholesterol

See the Great Plains Laboratory's website for an excellent body of information on the question of low cholesterol levels and their treatment with Sonic Cholesterol. While you are there, order William Shaw's excellent autism book's most recent edition. Only because of the availability of that guide do I have the leeway to focus here on subjects with which I am particularly familiar without having to pretend omniscience.

Thiamine Tetrahydrofurfuryl Disulfide

Thiamine tetrahydrofurfuryl disulfide (TTFD) was first identified by Derrick Lonsdale, MD, a professor and head of a department at the Cleveland Clinic. He retired in 1982 to open a private office to focus on the care of individual children, as contrasted with the study of diseases. Dr. Lonsdale brought his deep understanding of biochemistry to the ARI's meetings and introduced us to the potential of TTFD, a "fatty" form of vitamin B_1 (thiamine), to be of benefit to some children on the autism spectrum. My experience with it has shown that its potential in different children has a wide range. The effects of its use are dazzling in some children, although no prior testing will pick the winners. Here is the protocol I have used with remarkable success in many children. A 21-day trial is a reasonable thumbs test.

A. **Apply TTFD cream, 0.5 mL (½ mL), on a patch of skin at bedtime.** Instruct your patient to choose a location (e.g., the lower legs and feet) that is easily washed the next morning, to remove the skunky/garlicky odor that may form there. **Reapply it in the morning** if there will be no social problems arising from having a kid smell like a skunk. Otherwise, wait until later in the day when out of the public nose and then **apply the second daily dose**, repeating it at bedtime. Afternoon and evening doses can be separated by only a couple of hours.

B. **After 3 days, apply ½ mL topical reduced glutathione twice daily to another part of the skin.** Reduced glutathione (often abbreviated GSH) is the body's main detoxifying "usher" that combines with various toxic molecules that need to be taken from the body. Note whether the odor increases consequent to the reduced glutathione. In some instances, the odor will be barely perceptible until the addition of the reduced glutathione. The enhancement of the odor on the skin after the application of GSH provides persuasive evidence that the GSH gets on board through the skin and forms a detoxifying partnership with TTFD.

C. **After 3 days of that combination, add 0.5 mL twice daily of topical alpha-lipoic acid,** which is another sulfur-containing molecule and is a partner of vitamin B_1 in key steps in chemistry in addition to having a role in detoxification that is exemplified by its use in treating, for example, mushroom poisoning. The enhancement of odor with alpha-lipoic acid provides evidence for its partnership in the process we are trying to achieve.

D. **Keep up all three creams/gels for at least 1 month and observe any improvement in symptoms.** If a rash develops at the site of the application, add the following two supplements: molybdenum 300 mcg daily, biotin 1 mg daily. If the rash does not resolve, discontinue. It may be preferable to start the molybdenum and biotin when starting the TTFD. This idea is based on the experience of a child who went off TTFD and GSH for a bad rash, waited for the rash to go away, started the molybdenum and biotin, and got a report from school of marked cognitive and behavioral improvement before she had restarted the TTFD and GSH—which, this time, were not associated with any rash. If hyperactivity occurs, try activated charcoal, approximately 500 mg four times daily, to quench it. If the hyperactivity persists, stop the treatment.

Postscript about TTFD and GSH creams: We do not know yet whether the role of TTFD in helping kids with autism resolve their symptoms has to do with heavy metal detoxification directly, for which there is some evidence (mercury in the urine), or with energy metabolism, where it plays a very crucial role. Considering that detoxification is the most energy-demanding part of chemistry (other than growth in children), it may not make that much difference, or it may be some of each. We do not know the exact nature (beyond membership in the family of sulfur-bearing molecules called mercaptans) of the compounds that appear on the skin, and it is speculative to consider that mercury is brought out by that route. The smell on the fingers of a person applying TTFD and GSH represents the same phenomenon.

Both TTFD and GSH have important roles in the body's synthetic chemistry (construction), so the benefits associated with their intended use in detoxification (sanitation) may be attributable to the multiple jobs TTFD, lipoic acid, and GSH have in energy metabolism and the making of new molecules.

OTHER LABORATORY TESTING

Complete Blood Count and Comprehensive Metabolic Panel

The CBC and comprehensive metabolic (CMP) tests are the bedrock of traditional medicine and are necessary to rule out the failure of basic body functions and organs—bone marrow, liver, kidneys—but they are generally of little value until pathology has substantially progressed. Platelet count stands out in many autistic children as a key indicator of inflammation and, as such, is useful for tracking progress. The red blood cell indices, measuring their average size and volume as MCH, MCHC, and MCV, are of interest because large red blood cells (high MCV) occur with a deficiency of vitamin B_{12} and/or folic acid. Children who are very responsive overall to high doses of B_{12} and folic acid may not reflect that special need with the elevated levels of MCV (i.e., large red blood cells) that go with deficiency of these two vitamins. Thus the CBC's indices are of often of little value for tracking the benefits of B_{12} and folate supplementation. A low MCV—typical of iron deficiency (and certain inherited abnormalities of red blood cell size)—is, however, a solid indicator that will increase with correction by iron supplementation.

Thyroid

Hypothyroidism can mimic some symptoms of autism and impair development. TSH can check for this problem. The problem with thyroid testing is the so-called "reference range." It is a "normal" but not necessarily healthy range and may certainly not reflect the optimal level

for a given individual. Endocrinologist disagree, but experts will say that a TSH of close to 1 is normal. That means that a level of above 1, which will include most tests done on children, leaves some doubt about the question at hand, namely whether the patient would benefit from a supplement of either iodine or thyroid hormone. In an adult, such a question is quite easily resolved by a thumbs test—a trial of escalating thyroid supplementation over a period of 3 to 6 weeks. An adult can reliably report changes in body warmth and energy. It is a tricky problem in children, and given that thyroid problems are reaching epidemic levels and have a serious but often subtle implication for child development, it must be confronted thoughtfully by practitioners.

Iron

Low iron can cause attention deficient problems and has also linked to lowered IQ. Many physicians believe that a low red blood cell count, hemoglobin level, or hematocrit (anemia) is a reliable indicator of iron deficiency, and they do not measure serum ferritin, which is the only good indicator of iron status. Serum iron levels are not reliable because iron is withdrawn from circulation under conditions of stress (infection), so a low serum iron becomes a false indicator of deficiency. Iron supplements may be constipating and may be resisted by parents coping with a child who already has such problems. Iron supplementation must be recommended with magnesium, if at all.

Ammonia and Lactic Acid

The accuracy of blood ammonia level testing depends on the blood being drawn in the laboratory where the test is performed to avoid any delay.

This testing may determine whether mitochondrial dysfunction, associated with low energy production and hypotonia, exists. Mitochondrial dysfunction may be treatable with coenzyme Q_{10} and L-carnitine supplementation.

Cholesterol

There is a subset of children with ASD who have low cholesterol levels and benefit from supplementation. A cholesterol count of less than 145 mg/dL has been shown to be detrimental, increasing defiance and irritability in neurotypical children. Cholesterol is needed to synthesize steroid hormones (estrogen, testosterone, cortisol) and to make bile. Conventional medical doctors continue to perpetuate the idea that cholesterol is detrimental, but in patients with ASD, low cholesterol can be problematic. Supplementation with cholesterol may be in order.

Urinary and Stool Measures

Urinary porphyrin concentrations can reflect increased heavy metal or pesticide levels in the kidney and are markers of the metal burden in the body.

Urinary neopterin is a marker of inflammation and tends to reflect autoimmunity in some children with autism. Elevated neopterin often predicts positive responses to anti-inflammatory treatments.

Urinary oxidized DNA and RNA are markers of oxidative stress inside the cell,[26] and children with elevated levels often have improvements with antioxidants.

Urinary isoprostane is a marker of oxidative stress outside the cell.[26] Again, antioxidants can be helpful when this is elevated.

Urinary pyrroles. A condition called pyroluria may also underlie some cases of autism. Pyroluria, or pyrrole disorder, was described by Carl Pfeiffer, MD, in his 1976 book *Mental and Elemental Nutrients.* William Walsh, PhD, a biochemist who has continued Pfeiffer's work, explains that this quirk in biochemistry, also known as the mauve factor, is a common feature of behavioral and emotional disorders. It is detectable as a purple metabolite in the urine on testing paper. Children with pyroluria are severely deficient in zinc, vitamin B_6, and arachidonic acid (an omega-6 fatty acid). Walsh explains further: "certain pyrroles called kryptopyrroles (literally, 'hidden pyrroles') bind with B_6, then zinc to deplete the body's supply. Common symptoms include explosive temper, mood swings, poor short-term memory, and frequent infections. These patients are easily identified by their inability to tan, poor dream recall, abnormal fat distribution, and sensitivity to light and sound. The decisive laboratory test is analysis for kryptopyrroles (the 'mauve factor') in urine."[38]

The treatment for pyroluria includes zinc and B_6 supplements (P5P if unresponsive), along with omega-6 essential fatty acids.

Stool testing can check for the presence of inflammation, dysbiosis (increased levels of yeast and abnormal bacteria), digestion, and absorption.

Omega-6 and Omega-3 Fatty Acids

The omega-6 and omega-3 families of molecules are distinctive in human chemistry. The human body can make dozens of them, with two noteworthy exceptions. One exception is a member of the so-called omega-6 family, and the other is the source of all the members of the omega-3 family. These fatty acids are needed for making not only the flexible membrane that encloses each of the 10 billion cells in the body but also the messenger molecules that these cells use to communicate with one another. Linolenic acid (LA) and alpha-linolenic acid (ALA) must come from dietary sources. They are essential in two ways. We cannot make them, and we cannot live without them. Getting LA from the diet is not usually a problem; it is well distributed in food. ALA is more difficult. It is found only in flax oil (edible linseed oil 40% ALA), walnut oil (10%), and soy that is grown in northern climates (10%).

When I was a child, there was no such thing as vegetable oil. We had Crisco, olive oil, and butter. Not until the 1940s were large steel presses invented for extracting vegetable oils from various seeds, such as corn. This provided consumers with cheap, easily distributed cooking fats. The traces of ALA found in such oils were removed from the vegetable oils that became widely available in grocery stores because ALA quickly becomes rancid, which limits its shelf life. The result is that our food supply is short on the very one element that we must get from our diet.

Signs of unmet needs for essential fatty acids (EFAs) include dull and dry skin, dandruff, unmanageable hair, and brittle or fraying nails. The abundance of television ads to rectify these problems testify to an epidemic of EFA deficits in those living in modern industrial cultures. Blood testing readily documents such deficits. The appearance of the skin, hair, and nails is sufficient to drive a decision to supplement with omega-3 oils such as flax, fish, or krill oil.

When a child with ASD shows the previously noted symptoms, I recommend daily omega-3 supplementation given in the morning, which is the best time for the body to absorb and metabolize it. The dosage is 1 teaspoon per year of life per day. So a 2-year-old would be given 2 teaspoons in the morning.

Delta-6 desaturase deficiency: The nutritional/biochemical problem that causes unmet needs for omega-6 fatty acids is common. The essential dietary source of the oils in the omega-6 family are "regular" vegetable oils. The first step in the synthesis of downstream members of the family is the insertion of a double bond into the sixth C-C bond from the delta end of the 16-carbon structure of the essential fatty acid molecule. Delta-6-desaturase deficiency worsens with age; alcohol consumption; sugar consumption; saturated fat consumption; and deficiencies of Mg, Zn, B_3. It is common in patients with eczema, multiple sclerosis, hypertension, premenstrual syndrome, hyperactivity, and autoimmune problems. It can be treated by giving primrose, borage, or black currant seed oils, which are sources of fatty acids that help the body better synthesize omega-6s. These should not be routinely prescribed but may be tried in cases where a deficiency in delta-6 desaturase is suspected.

METHYLATION

It is important for the functional practitioner to understand how methylation, methyl-B_{12}, and most of the other nutrients work in the autism spectrum. The same nutrients, interestingly, are relevant to all chronic illnesses as well as to aging. Why? Because medical treatments aimed at individuals—rather than disease—are based on fundamentals of biochemistry that are shared by all of us.

SAM: Recall that taurine, which is like an amino acid but plays roles on stage for minerals, is at the last stop in the previous diagram. Look at the upper right-hand corner of the diagram. There he is—glad that you recognize him as he gets off the bus. See how he has been transformed on a trip that started all the way on the left-hand side of the diagram as methionine?

Now follow the arrows that start with methionine and notice that adenosine triphosphate (ATP) gets into the game with methionine and becomes s-adenosyl methionine (SAM). SAM, under the trademark "SAMe," is used as a treatment for osteoarthritis, depression, ADHD, and a buildup of bile in the gall bladder, as is taurine. SAMe may be effective in doses ranging from 400 to 1200 mg daily in divided doses. It has been used for patients with Alzheimer's disease, pain, migraine, and bursitis, so it provides a good example of a remedy that may be useful for certain individuals and does not have the same effect in certain diseases.

Follow the arrow that goes upward as SAM goes to work as the distributor of CH_3 in the long list of methylations that hovers over the first part of our bus route. Creatine, the stuff you can find in big jars in the health food store where muscle-builders go for "energy," is indeed a molecule that is a crucial player in energy transfer in the body and certainly works to give some people more energy (see Chapter 139, Sports Nutrition, for a more complete discussion). The restlessness that may be a side effect from taking SAMe may be minimized by starting with a low dose and increasing slowly. SAM's role in methylation (adding CH_3) in the chemistry of neurotransmitters, proteins, RNA, and DNA strongly makes it a reasonable supplement to use in treating autism, especially considering that it is safe and has a much lower chance of producing negative effects than pharmaceutical medications. Unfortunately, my experience with it is that, although worth a try, it does not often fulfill the hopes we attach to it based on its place in biochemistry.

SAH: S-adenosyl homocysteine (SAH) is what is left after the CH_3 leaves. Then adenosine gets off the bus. Adenosine is, as mentioned previously, mischievous when it accumulates. High levels promote inflammation, and that effect may be seen as what happens when the high adenosine level creates a stoppage on SAM. Recall that adenosine levels are decreased by the antiviral medication acyclovir.

Homocysteine: As we arrive at homocysteine, we have already found relevance in the diagram to treatment with SAMe, creatine, antiviral medications, and a gluten-free diet. Now we arrive at a crisis point in the bus trip. Methionine has lost a CH_3, and adenosine has come and gone, leaving a very hot, sticky molecule called homocysteine. I say sticky because its sulfur atom is very exposed. Sulfur is both stinky and sticky. The odor of sulfur that appears when you strike a match is distinctive in that it sticks to the fecal odors that you may wish to cancel before leaving the toilet. Homocysteine is hot in the sense that it is like an ingot in the blacksmith's tongs. It is ready to be fashioned into something useful, but it cannot be simply put down and kept around without the risk of damage. The crisis involves the choice of what to do with homocysteine: quench it, returning it to its original state as methionine, or send it further along the bus route.

Quenching is not a simple as the blacksmith cooling an ingot with water. Homocysteine must get a methyl group put back on. That process is depicted in the diagram where the dashed line with a CH_3 in it catches up with the U-turn that homocysteine has made. The CH_3 gets on board right at the point of the arrow showing mercury poisoning the process.

Mercury is only one of many toxins that can impair the crucial step of remethylation of homocysteine (quenching the valuable but dangerous ingot). This step and its investigation by Richard Deth, PhD, are of interest in understanding the pathology of ASD and other conditions. Does the vulnerability of this step to various environmental toxins explain its importance in the autism spectrum? Or is the special complexity of the remethylation process—involving a very delicate dance engaging vitamin B_{12}, 5-methyl-tetrahydrofolate, homocysteine, and SAM—the source of its vulnerability? Both factors are probably at work, but the bottom line is that this step deserves our attention in all efforts to treat autism. Those efforts include the following:

- **Injections of high doses of methyl-B_{12}.** This was described previously and is mentioned here to emphasize the point that the injections are not to correct a deficiency but to bring a strong force to bear on a step that needs a good (safe) kick to get it working. That's why oral doses are not able to do the job.
- **Oral doses of folinic acid.** Again, not to correct a deficiency but to ensure sufficient amounts for that kind of folic acid that is needed for a task that is not shown on the diagram.
- **Methylenetetrahydrofolate reductase (MTHFR).** An enzyme is a large molecule that embraces small ones so that the small ones join or come apart. Enzymes are named in ways that reveal their function and end in *-ase.* Then scientists abbreviate them to letters such as MTHFR that give them an important sound.

MTHFR is indeed important. This enzyme converts a molecule called 5,10-methylenetetrahydrofolate to a molecule called 5-methyltetrahydrofolate. To oxidize a molecule means to steal its electron. So reduction means to give it an electron. That's what it takes to get homocysteine to take the U-turn back to methionine. That's the gift that requires several contributors, among which MTHFR is a major player.

The code MTHFR refers to the gene that provides the instructions for making this big molecule, in whose embrace a major part of homocysteine's return trip takes place. Mutations (malformation of the gene) of the MTHFR gene occur with varied prevalence in different ethnic/racial groups and range from 1 in 10 to 1 in 5 individuals in most groups. The list of medical conditions and diseases for which 1 of 40 different mutations is a risk factor includes everything from heart attacks to cancer, depression, and schizophrenia. The good news is that supplementation of various activated folics is the preventive measure and/or remedy for all of them. Testing for MTHFR mutations has become a common medical practice. Many patients who consult me have found out that their child with ASD has an MTHFR mutation. What it means, really, is that they need supplementation with methyl folate, and if they do so, their risk factors are normalized. It need not be a subject for grief or drama in the realm of genetics. Methyl folate is at the heart of the biochemistry that was previously referred to in terms of the contribution of Dr. James and Dr. Deth. Look at the chart of methylation and see where mercury does its dirty work.

GLUTATHIONE

Oral administration: The problem with oral administration is that GSH is disassembled in the digestive tract and needs to be reassembled in the liver and then disassembled for export to other parts of the body. GSH is very sticky, so sending it around intact is problematic. Oral GSH can be very effective. I used oral GSH to cure long-standing inflammation of the eyes in a patient who had seen many doctors and tried many other remedies. The induction of GSH with glucoraphanin (Avmacol) is more efficient.

Transdermal and intravenous administration: Transdermal preparations and intravenous administration are usually more effective than oral glutathione administration. There is a way of "packaging" GSH inside membranes, like the ones that enclose every cell in the body. These membranes are made of fat (lipid) molecules. These travel through the lymph ducts from the intestine up through the chest and are delivered directly into a vein under the left collarbone. They stay in the circulation, giving the serum a milky appearance that lasts for a few hours before it is absorbed by body tissues. GSH packaged in microscopical fat balls, called liposomes, takes advantage of this distribution of ingested fat to distribute GSH.

Induction of GSH is the top choice in terms of safety, cost, and long-term efficacy.

CONCLUSION

We are in the midst of an epidemic of inflammatory disease with environmental causes. Autism is probably the most malignant, and research on its origins has been delayed by mistaken searches for genetics while it became clear that such could simply not be the case. We continue to hear that "the cause" has not been found so that a cure must await further research. We who are on the front lines of functional medicine must do our best to learn from each other as we treat each individual patient, case by case, and find ways of supporting Nature's strong impulse toward healing. We must tailor our treatments to the needs of each individual patient while heeding the experience of our peers on the familiar landscape of detoxification, microbes, and oxidative stress. Morgan's mom's report on the way his school now values his gifts is a testimony to what a determined parent and clinician can do together to lessen symptoms and even cure autism spectrum disorders.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Magnesium references. Durlach J. Clinical aspects of chronic magnesium deficit. In: Cantin M, Selig M, eds. *Magnesium in Health and Disease*. New York: Spectrum Press; 1980:883–909.
2. Durlach J. *Le Magnésium en Pratique Clinique*. Paris: Médicales Internationales; 1985.
3. Holl M, Baker S. Magnesium status as determined by retention of an intramuscular magnesium load: repletion and monitoring of symptoms. *J of the Am Dietetic Assn*. 1990;(suppl 90):37.
4. Seelig M. *Magnesium Deficiency in the Pathogenesis of Disease*. 367ff. New York: Plenum; 1980.
5. Baker SM. Magnesium deficiency in primary care and preventive medicine: symptom profiles in relation to magnesium loading studies. *Magnesium and Trace Elements*, 1991–1992;10:251–262
6. Rimland B. *Infantile Autism. The Syndrome and its Implication for a Neural Theory of Behavior*. East Norwalk, CT: Appleton-Century-Crofts; 1964.
7. Autism Research Institute, Parent Ratings Of Behavioral Effects Of Biomedical Interventions ARI Publ. 34 March 2009. https://www.autism.com/pdf/providers/ParentRatings2009.pdf. Accessed 25 Jan 2020.
8. Rose NE, Shoenfeld Y, eds. *Infection and Autoimmunity*. San Diego, CA: Elsevier; 2005.
9. Gillberg C. Asperger's syndrome and recurrent psychosis—a case study. *J Autism Dev Disord*. 1985;15(4):389–397.
10. Baker S. Autism spectrum disorder as a model for thinking differently about patients with complex disease. *N A J Med Sci*. 2012;5(3):193–197.
11. Zablotsky B, Black LI, Blumberg SJ. *Estimated Prevalence of Children With Diagnosed Developmental Disabilities in the United States, 2014–2016. NCHS Data Brief, no 291*. Hyattsville, MD: National Center for Health Statistics; 2017. https://www.cdc.gov/nchs/products/databriefs/db291.htm. Accessed 25 Jan 2020.
12. Baker SM, Milivojevich A, Kraycar T, Holt B, Gade S. Secular trend of sex ratio and symptom patterns among, children with autism spectrum disorders. *GAHM*. 2014;3(3):92.
13. Nevison CD. A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. *Environ Health*. 2014;13:73.
14. Hallmayer J, Cleveland S, Torres A, et al. Genetic heritability and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry*. 2011;68(11):1095–1102. PubMed PMID: 21727249.
15. McCanlies EC, Fekedulegn D, Mnatsakanova A, et al. Parental occupational exposures and autism spectrum disorder. *J Autism Dev Disord*. 2012;42(11):2323–2334. PubMed PMID: 22399411.
16. Windham GC, Sumner A, Li SX, et al. Use of birth certificates to examine maternal occupational exposures and autism spectrum disorders in offspring. *Autism Res*. 2013;6(1):57–63. PubMed PMID: 23361991.
17. Miodovnik A, Engel SM, Zhu C, et al. Endocrine disruptors and childhood social impairment. *Neurotoxicol*. 2011;32(2):261–267. PubMed PMID: 21182865.
18. Larsson M, Weiss B, Janson S, Sundell J, Bornehag CG. Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6-8 years of age. *Neurotoxicol*. 2009;30(5):822–831. PubMed PMID: 19822263.
19. Kalkbrenner AE, Daniels JL, Chen JC, Poole C, Emch M, Morrissey J. Perinatal exposure to hazardous air pollutants and autism spectrum disorders at age 8. *Epidemiol*. 2010;21(5):631–641. PubMed PMID: 20562626.
20. Windham GC, Zhang L, Gunier R, Croen LA, Grether JK. Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay area. *Environ Health Perspect*. 2006;114(9):1438–1444. PubMed PMID: 16966102.
21. Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, McConnell R. Residential proximity to freeways and autism in the CHARGE study. *Environ Health Perspect*. 2011;119(6):873–877. PubMed PMID: 21156395.
22. Jung CR, Lin YT, Hwang BF. Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan. *PLoS One*. 2013;8(9):e75510. PubMed PMID: 24086549.
23. Casanova MF. Autism as a sequence: from heterochronic germinal cell divisions to abnormalities of cell migration and cortical dysplasias. *Med Hypotheses*. 2014;83(1):32–38.
24. Parker W, Hornik CD, Bilbo S, et al. The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *J Int Med Res*. 2017;45(2):407–438.
25. Mold, M., Umar, D. and King, A. Aluminium in brain tissue in autism. Journal of Trace Elements in Medicine and Biology, 46, pp.76-82. Accessed March 2018.
26. Code of Federal Regulations Title 21, Sec. 201.323. Aluminum in large and small volume parenterals used in total parenteral nutrition. Revised as of April 1, 2019. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=201.323. Accessed 25 Jan 2020.
27. Exley C. Human exposure to aluminium. *Environ Sci Processes Impacts*. 2013;15:1807–1816.
28. Deth RC. Autism: a redox/methylation disorder. *Glob Adv Health Med*. 2013;2(6):68–73. Available from: https://doi.org/10.7453/gahmj.2013.087. [Online]. Accessed 25 Jan 2020.
29. James SJ, Cutler P, Melnyk S. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr*. 2004;80:1611–1617.
30. Samar HI, Voigt RG, Slavica KK, et al. Incidence of gastrointestinal symptoms in children: a population-based study. *Pediatrics*. 2009;124(2):680–686.
31. *Trichuris suis* Therapy for Active Ulcerative Colitis: A randomized controlled trial. *Gastroenterology*. 2005;128:825–832. http://tinyurl.com/Weinstock-2005-paper. Accessed 25 Jan 2020.
32. Autism Research Institute. Parent Ratings of Behavioral Effects of Biomedical Interventions. ARI Publ. 34/March 2009. http://www.autism.com/pdf/providers/ParentRatings2009.pdf. Accessed 25 Jan 2020.
33. Adams JB, Baral M, Geis E, Mitchell J, et al. Safety and efficacy of oral DMSA therapy for children with autism spectrum disorders: part B—behavioral results. *BMC Clinic Pharmacology*. 2009;9:17. Available from: https://doi.org/10.1186/1472-6904-9-17. [Online]. Accessed 25 Jan 2020.
34. Bruce P. Lanphear,1,2 Richard Hornung,1,2,3 et al. Low-Level Environmental Lead Exposure and Children's Intellectual Function: an international pooled analysis. *Environ Health Perspect*. 2005;113(7):894–899.
35. Lanphear RH, Khoury J, et al. "Low-Level Environmental Lead Exposure and Children's Intellectual Function: An International Pooled Analysis" by Bruce P. *Environ Health Perspect*. 2005;113(7):894–899.
36. Center for science in the public interest. Food dyes: a rainbow of risks, 2010. http://www.autism.com/pdf/providers/ParentRatings2009.pdf. Accessed 25 Jan 2020.
37. Maher TJ, Wurtman RJ. Possible neurologic effects of aspartame, a widely used food additive. *Environ Health Perspect*. 1987;75:53–57.
38. Shoenfeld Y, Agmon-Levin N. *Vaccines and Autoimmunity*. New York: Wiley; 2015.
39. Wachtel LE, Contrucci-Kuhn SA, Griffin MG, et al. ECT for self-injury in an autistic boy. *Eur Child Adolesc Psychiatry*. 2009;18(7):458–463.
40. Abdel-Maksoud MB, Nasr A, Abdul-Aziz. A lithium treatment in cluster headache. Review of literature. *Eur J Psychiat*. 2009;23(1). Zaragoza ene.-mar. https://doi.org/10.4321/S0213-61632009000100006.
41. Schrauzer GN, Shrestha KP. Lithium in drinking water and the incidences of crimes, suicides, and arrests related to drug addictions. *Biol Trace Elem Res*. 1990;25(2):105–113.
42. Walsh Research Institute. Walsh Research Institute, 2020. www.walshinstitute.org. Accessed 25 Jan 2020.

Bacterial Sinusitis

Michael T. Murray, ND,* and *Elaine Roe, MD*

OUTLINE

DIAGNOSTIC SUMMARY

- History of acute viral respiratory infection, dental infection, or nasal allergy
- Purulent nasal discharge
- Nasal obstruction; facial pain, pressure, and fullness; or both
- Worsening of symptoms after initial improvement if less than 10 days' duration, or no reduction in symptoms if more than 10 days' duration
- Transillumination showing opaque sinus
- Chronic infection, often producing no symptoms other than mild postnasal discharge, a musty odor, or a nonproductive cough

GENERAL CONSIDERATIONS

The most common predisposing factor in acute bacterial sinusitis, defined as a symptomatic inflammation of the paranasal sinuses and nasal cavity, is a viral upper respiratory infection. Allergic rhinitis and other factors that interfere with normal protective mechanisms, including asthma and exposure to cigarette smoke, may precede the viral infection and therefore are the more likely predisposing factors. Any factor that induces edema of the mucous membranes may result in obstruction of meatal drainage. The transudate that is produced serves as a suitable medium for bacterial overgrowth, with the most common pathogens in adults being *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* and *Staphylococcus aureus.*[1,2]

Although up to 90% of adults with a viral upper respiratory tract infection have concurrent viral sinusitis, only 0.5% to 2.0% will progress to acute bacterial sinusitis. The diagnosis of acute bacterial sinusitis cannot be made with any one sign or symptom, such as fever or pain, and radiographic evidence cannot adequately distinguish between viral and bacterial sinusitis. An algorithm has been suggested for the diagnosis in which the following three conditions must be present: (1) purulent nasal discharge; (2) nasal obstruction or facial pain, pressure, or fullness, or both; (3) if the duration is less than 10 days, there is a worsening of symptoms after an initial improvement, *or* if more than 10 days, the symptoms are not decreasing.[3] The diagnosis is made similarly in children but also allows for a severe onset, characterized by a temperature of greater than or equal to 39°C/102.2°F and purulent nasal discharge for at least 3 consecutive days.[4]

In chronic bacterial sinusitis, an allergic background is commonly present, and in 10% to 12% of cases of chronic maxillary sinusitis, there is an underlying dental infection.[5]

Although vasoconstrictors and antihistamines cause transient relief, their chronic use is contraindicated because there is usually a reflex reaction after continual administration.

Cigarette smokers should be advised to quit smoking. Secondhand exposure is also a contributing factor.

THERAPEUTIC CONSIDERATIONS

Antibiotics

Although antibiotic therapy is the dominant treatment for acute and chronic bacterial sinusitis, it is of limited value.[6] A detailed analysis to determine the evidence for the effectiveness of antibiotic treatment in acute maxillary sinusitis in adults by assessing the methodological quality of placebo-controlled, double-blind, randomized trials concluded: "The effectiveness of antibiotic treatment in acute maxillary sinusitis in a general practice population is not based sufficiently on evidence."[7] In 2018 a Cochrane review of more than 15 trials and 3000 healthy adult participants found that only 5 to 11 more people per 100 with acute rhinosinusitis would be cured faster if they received antibiotics instead of either placebo or no treatment. Additionally, although imaging results or specific clinical symptoms may somewhat improve the cure rates with antibiotics, the very low incidence of serious complications and the high rate of adverse events led the authors to conclude "there is no place for antibiotics for people with uncomplicated acute rhinosinusitis."[8] Nonetheless, in severe or unresponsive cases, antibiotics may be appropriate, and most reviews have focused on acute sinusitis that is not strictly defined as having a bacterial etiology. In a Cochrane review, it was shown that although 80% of participants treated without antibiotics improved within 2 weeks, antibiotics had a small treatment effect in patients with uncomplicated acute sinusitis in a primary care setting with symptoms for more than 7 days (this longer duration of symptoms

* Previous edition contributor

supports a diagnosis of bacterial sinusitis). Newer, more potent antibiotics (e.g., lactam antibiotics) appear to be more effective than penicillin, amoxicillin, and other less potent antibiotics.[9] Amoxicillin and amoxicillin-clavulanate are considered first-line antibiotics. Topical antibiotics via nasal irrigation may also be used.

In children, it is also questionable that antimicrobial therapy is of significant benefit for uncomplicated sinusitis.[10] Overuse of antibiotics in children with sinusitis or otitis media is a growing concern because it is leading to antibiotic-resistant strains of bacterial pathogens. Antibiotics are currently used in 82% of children with acute sinusitis, although the majority of these infections would likely resolve on their own.[11] However, when controlled for symptoms that suggest a diagnosis of acute *bacterial* sinusitis, antibiotics are associated with a higher cure rate (50% vs. 14%) and lower treatment failure (14% vs. 68%) than placebo.[12]

In chronic sinusitis, antibiotics are also of little or no benefit.[13] Clearly, the most rational approach seems to be to address the underlying cause of chronic sinusitis (e.g., respiratory or food allergens) along with providing supportive therapy (e.g., saline nasal sprays, immune-enhancing herbs, natural decongestants).

Allergy

Studies indicate that most patients with chronic sinusitis, perhaps as many as 84%, have allergies.[14,15] Patients with chronic sinusitis should be aggressively screened for environmental and food allergies. Environmental control requires the elimination of dust mites (warm-water washing at a temperature of least 58°C), use of air-filtering vacuum cleaners, installation of an air cleaner with a high-efficiency particulate air filter, and whatever methods are necessary to maintain the humidity under 50%. Some particularly sensitive patients may need to have all pets removed, along with carpeting and featherbedding.[16]

Mucolytics

Airway mucociliary clearance depends on the properties and volume of secreted mucus, ciliary function, and mucociliary interactions. In chronic sinusitis, mucus viscoelasticity is higher than the optimal values for mucociliary clearance. Mucolytic agents such as *N*-acetylcysteine (NAC) and proteolytic enzymes can reduce viscoelasticity and promote mucociliary clearance.[17]

NAC is the most commonly used mucolytic agent. The free sulfhydryl group of NAC interacts with the disulfide bonds of mucus glycoproteins, thereby breaking the protein network into less viscous strands. Although NAC is often used as a 10% solution by dilution with saline, sodium bicarbonate, and sterile water, it can also be used orally for sinusitis, and it has been shown to be effective for chronic bronchitis.[18]

Proteolytic enzymes may break down complex proteins at the site of inflammation, exert some antimicrobial effects, or act directly on the naked peptide region of mucus glycoproteins. Trypsin, chymotrypsin, *Serratia* peptidase, bromelain, and streptokinase are the proteolytic enzymes that can break down mucus glycoproteins and other proteins when they are administered topically. When *Serratia* peptidase was given at a dose of 30 mg/day for 4 weeks to patients with chronic sinusitis, it significantly reduced the viscosity but not the elasticity of nasal mucus.[19]

It has been reported that the ratio of the viscosity to the elasticity is an important determinant of mucociliary transport. When *Serratia* peptidase was administered at the same dose to patients with chronic bronchitis, it significantly increased mucociliary clearance.[20] A multicenter, double-blind, placebo-controlled study of 193 subjects suffering from various acute or chronic ear, nose, or throat disorders, including sinusitis, demonstrated a greater efficacy and rapid action of the peptidase against all the symptoms examined.[21] Orally administered bromelain has also shown benefit in the treatment of chronic sinusitis.[22] A small study among children with acute sinusitis found that treatment with bromelain alone resulted in faster recovery than when combined with standard therapies or with standard therapy alone.[23]

Nasal Saline

Intranasal saline irrigation improves sinusitis symptoms by washing away secretions, allergens, and irritants. A 2015 Cochrane review found that although existing trials have been too small and had a high risk of bias, nasal saline therapy may have possible benefit for upper respiratory tract infections.[24] Among individuals with frequent sinusitis, it has also been shown to improve symptoms and sinus-related quality of life while reducing the need for medication.[25]

Nasal Steroids

Inhaled glucocorticoids may be of use in acute sinusitis and are commonly used in chronic sinusitis.[26] A 2013 review of four studies and nearly 2000 adults and children with acute sinusitis found that intranasal steroids were associated with a greater likelihood of resolution or improvement versus placebo (relative risk [RR] 1.11).[27]

Pelargonium sidoides (South African Geranium)

Extracts from the rhizomes and tubers of *Pelargonium sidoides* have been shown to exert a number of beneficial effects in upper respiratory tract infections, particularly acute bronchitis, an indication for which it is an approved drug in Germany (see Chapter 155, Bronchitis and Pneumonia). *P. sidoides* has demonstrated immune-enhancing effects as well as antibacterial and antiviral effects and the ability to prevent adhesion of bacteria to epithelial cells.[28] In one double-blind, placebo-controlled trial, 103 patients with acute rhinosinusitis of presumably bacterial origin were given an ethanolic extract of *P. sidoides* (EPs 7630) or matching placebo at a dose of 3 mL three times daily for a maximum 22 days.[29] The mean decrease in the Sinusitis Severity Score was 5.5 points in the EPs 7630 group compared with 2.5 points in the placebo group, a difference of 3.0 points. This result was confirmed by all secondary parameters, indicating a more favorable course and a faster recovery in the EPs 7630 group.

THERAPEUTIC APPROACH

In acute sinusitis, the immediate therapeutic goals are to reestablish drainage and clear the acute infection. Various measures can be used: local application of heat, local use of volatile oils and botanicals with antibacterial properties, and immune system support (see Chapter 136).

Because chronic bacterial sinusitis is often secondary to allergy, long-term control depends on isolation and elimination of the food or airborne allergens and correction of the underlying problem that allowed the allergy to develop. During the acute phase, the elimination of common food allergens (milk, wheat, eggs, citrus, corn, and peanuts) is indicated until a more definitive diagnosis can be made.

Local applications of heat have been shown to be effective in alleviating both the short- and long-term symptoms of allergic rhinitis.[30]

Supplements

- Vitamin C: 500 mg three times a day
- Bioflavonoids: 1000 mg/day
- Vitamin A: 5000 IU/day or beta-carotene (but only if single-nucleotide polymorphisms [SNPs] are checked to rule out those who do not convert beta-carotene to actual vitamin A): 25,000 IU/day
- NAC: 200 mg three times a day

Serratia Peptidase or Bromelain

- *Serratia* peptidase (enteric coated): 50 mg three times a day between meals
- Bromelain (1200–1800 MCU): 250 mg three times a day

Botanicals

Echinacea sp.

- Dried root (or as tea): 0.5 to 1 g
- Freeze-dried plant: 325 to 650 mg
- Juice of aerial portion of *Echinacea purpurea* stabilized in 22% ethanol: 2 to 3 mL
- Tincture (1:5): 2 to 4 mL (½–1 tsp)
- Fluid extract (1:1): 2 to 4 mL (½–1 tsp)
- Solid (dry powdered) extract (6.5:1 or 3.5% echinacoside): 150 to 300 mg

Hydrastis canadensis

The dosage should be based on berberine content. Because there is a wide range of quality in goldenseal preparations, standardized extracts are recommended. Three-times-a-day dosages follow:

- Dried root or as infusion (tea): 2 to 4 g
- Tincture (1:5): 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content): 250 to 500 mg

Pelargonium sidoides

Dosage recommendations for EPs 7630 or equivalent preparation:

- Adults: 3 mL three times a day or two 20-mg tablets three times a day for up to 14 days
- Children: ages 7 to 12 years, 30 drops (1.5 mL) three times a day; age 6 years or less, 10 drops (0.5 mL) three times a day

Local Treatment

- Intranasal douche with saline water or *Hydrastis* tea
- Swabbing of passages with oil of bitter orange
- Menthol or eucalyptus packs over sinuses (care should be taken to avoid irritation)

Physical Therapy

- Local applications of hot packs
- Diathermy: 30 minutes (discontinue if pain increases without drainage)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES[31]

1. Gwaltney Jr JM, Scheld WM, Sande MA, et al. The microbial etiology and antimicrobial therapy of adults with acute community-acquired sinusitis: a fifteen-year experience at the University of Virginia and review of other selected studies. *J Allergy Clin Immunol*. 1992;90:457–461. PMID: 1527337.
2. Sokol W. Epidemiology of sinusitis in the primary care setting: results from the 1999-2000 Respiratory Surveillance Program. *Am J Med*. 2001;111(suppl 9A):19S24S. PMID: 11755439.
3. Rosenfeld RM. Clinical practice. Acute sinusitis in adults. *N Engl J Med*. 2016;375(10):962–970. PMID: 27602668.
4. Wald ER1, Applegate KE, Bordley C, et al. Clinical practice guideline for the diagnosis and management of acute bacterial sinusitis in children aged 1 to 18 years. *Pediatrics*. 2013;132(1):e262–e280. PMID: 23796742.
5. Mehra P. Jeong D Maxillary sinusitis of odontogenic origin. *Curr Infect Dis Rep*. 2008;10(3):205. PMID: 18510882.
6. Stalman WA, van Essen GA, van der Graaf Y. Determinants for the course of acute sinusitis in adult general practice patients. *Postgrad Med J*. 2001;77:778–782.
7. Stalman W, van Essen GA, van der Graf Y. Maxillary sinusitis in adults: an evaluation of placebo-controlled double-blind trials. *Fam Pract*. 1997;14:124–129.
8. Lemiengre MB, van Driel ML, Merenstein D, et al. Antibiotics for acute rhinosinusitis in adults. *Cochrane Database Syst Rev*. 2018;9:CD006089. PMID: 30198548.
9. Ahovuo-Saloranta A, Borisenko OV, et al. Antibiotics for acute maxillary sinusitis. *Otolaryngol Head Neck Surg*. 2008;139(4):486–489.
10. Cohen R. The antibiotic treatment of acute otitis media and sinusitis in children. *Diagn Microbiol Infect Dis*. 1997;27:35–39.
11. Shapiro DJ, Gonzales R, Cabana MD, Hersh AL. National trends in visit rates and antibiotic prescribing for children with acute sinusitis. *Pediatrics*. 2011;127(1):28–34. PMID: 21187311.
12. Wald ER1, Nash D, Eickhoff J. Effectiveness of amoxicillin/clavulanate potassium in the treatment of acute bacterial sinusitis in children. *Pediatrics*. 2009;124(1):9–15. PMID: 19564277.
13. Dohlman AW, Hamstreet MPB, Odrezin GT, et al. Subacute sinusitis: are antimicrobials necessary? *J Allergy Clin Immunol*. 1993;91:1015–1023.
14. Gutman M, Torres A, Keen KJ, et al. Prevalence of allergy in patients with chronic rhinosinusitis. *Otolaryngol Head Neck Surg*. 2004;130:545–552.
15. Emanuel IA, Shah SB. Chronic rhinosinusitis: allergy and sinus computed tomography relationships. *Otolaryngol Head Neck Surg*. 2000;123:687–691.
16. Evans III R. Environmental control and immunotherapy for allergic disease. *J Allergy Clin Immunol*. 1992;90:462–468.
17. Majima Y. Mucoactive medications and airway disease. *Paediatr Respir Rev*. 2002;3:104–109.
18. Grandjean EM, Berthet P, Ruffmann R, et al. Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of published double-blind, placebo-controlled clinical trials. *Clin Ther*. 2000;22:209–221.
19. Majima Y, Inagaki M, Hirata K, et al. The effect of an orally administered proteolytic enzyme on the elasticity and viscosity of nasal mucus. *Arch Otorhinolaryngol*. 1988;244:355–359.
20. Nakamura S, Hashimoto Y, Mikami M, et al. Effect of the proteolytic enzyme serrapeptase in patients with chronic airway disease. *Respirology*. 2003;8:316–320.
21. Mazzone A, Catalani M, Costanzo M, et al. Evaluation of *Serratia* peptidase in acute or chronic inflammation of otorhinolaryngology pathology: a multicentre, double-blind, randomized trial versus placebo. *J Int Med Res*. 1990;18:379–388.
22. Ryan RE. A double-blind clinical evaluation of bromelains in the treatment of acute sinusitis. *Headache*. 1967;7:13–17.
23. Braun JM, Schneider B, Beuth HJ. Therapeutic use, efficiency and safety of the proteolytic pineapple enzyme Bromelain-POS in children with acute sinusitis in Germany. *In Vivo*. 2005;19(2):417–421. PMID: 15796206.
24. King D, Mitchell B, Williams CP, et al. Saline nasal irrigation for acute upper respiratory tract infections. *Cochrane Database Syst Rev*. 2015;4:CD006821. PMID: 25892369.
25. Rabago D1, Zgierska A, Mundt M, et al. Efficacy of daily hypertonic saline nasal irrigation among patients with sinusitis: a randomized controlled trial. *J Fam Pract*. 2002;51(12):1049–1055. PMID: 12540331.
26. Fokkens W, Lund V, Bachert C, et al. EAACI position paper on rhinosinusitis and nasal polyps executive summary. *EAACI Allergy*. 2005;60(5):583. PMID: 15813802.
27. Zalmanovici Trestioreanu A, Yaphe J. Intranasal steroids for acute sinusitis. *Cochrane Database Syst Rev*. 2013;12:CD005149. PMID: 24293353.
28. Brendler T, van Wyk BE. A historical, scientific and commercial perspective on the medicinal use of *Pelargonium sidoides (Geraniaceae)*. *J Ethnopharmacol*. 2008;119(3):420–433.
29. Bachert C, Schapowal A, Funk P, et al. Treatment of acute rhinosinusitis with the preparation from *Pelargonium sidoides* EPs 7630: a randomized, double-blind, placebo-controlled trial. *Rhinology*. 2009;47(1):51–58.
30. Yerushalmi A, Karman S, Lwoff A. Treatment of perennial allergic rhinitis by local hyperthermia. *Proc Natl Acad Sci U S A*. 1982;79:4766–4769.
31. For references on botanical recommendations, see Chapters 64 and 75.

Benign Prostatic Hyperplasia

Geovanni Espinosa, ND, LAc, IFMCP, CNS, and *Ralph Esposito, ND, LAc*

OUTLINE

DIAGNOSTIC SUMMARY

- Symptoms of bladder outlet obstruction (progressive urinary frequency, urgency and nocturia, hesitancy, and intermittency with reduced force and caliber of urine)
- Enlarged, nontender prostate
- Uremia with prolonged obstruction

GENERAL CONSIDERATIONS

Benign prostatic hyperplasia (BPH) is the fourth most common diagnosis in older men.[1] More than 50% of men over age 50 years are affected, and 90% of men will have an enlarged prostate by the time they are 80 years old. Progression to urinary retention may occur, with an accompanying risk of recurrent urinary tract infections, bladder calculi, and occasionally renal insufficiency. Management options for BPH include exercise, low-fat diet, supplementary medications, minimally invasive therapies, and prostate surgery.

The most common symptoms of BPH are lower urinary tract symptoms (LUTS), such as frequent urination, urgency to urinate, nocturia, weak urinary stream, incomplete bladder emptying, straining to void, and an intermittent stream. A patient may have multiple symptoms but be bothered primarily by one. Prostate cancer, bladder cancer, overactive bladder, urinary tract infections, prostatitis, urethral stricture, and bladder stones can also cause LUTS and must be ruled out before a diagnosis of BPH is made.[2]

Risk Factors

Abdominal obesity and genetic factors appear to be the main risk factors for the development of BPH, which appears to run in families. If one or more first-degree relatives are affected, an individual is at greater risk of developing this disorder.[1,3] Waist size, body mass index, and increases in body weight appear to increase the volume of the prostate gland.[4]

Hormones and Benign Prostatic Hyperplasia

The role of androgens in the development of prostate enlargement is evident, owing to the fact that BPH does not develop in men who have been castrated before puberty and therefore have greatly depleted levels of circulating androgens. Furthermore, in men with BPH, medical or surgical castration has been shown to lead to a reduction in prostate volume.[5]

For testosterone to have any effect on the prostate, it must be converted to dihydrotestosterone (DHT) by the enzyme 5-alpha-reductase.[6] DHT has twice as great an effect on the prostate as testosterone.[7]

Although testosterone levels decline with age, the concentration of DHT remains constant in both younger and older men, even with a low serum level.[5] Circulating DHT, by virtue of its low serum plasma concentration and tight binding to plasma proteins, is of diminished importance as a circulating androgen affecting prostate growth.[8]

However, much of the latest research has questioned the medical paradigm of testosterone as a cause for BPH. In fact, two recent review papers have found no correlation between testosterone replacement therapy and worsening LUTS or BPH.[9,10] It has been proposed that testosterone may be protective against BPH. Several studies have shown improvement or no change in the International Prostate Symptom Score (IPSS), transrectal ultrasound (TRUS) prostate volume, LUTS, and maximum flow rate in men on testosterone replacement.[9] It is suspected that the mechanism is multifactorial. Low testosterone levels are a risk factor for metabolic syndrome, which can lead to autonomic hypertonicity, chronic pelvic inflammation, and lower nitric oxide (NO) levels. Low NO levels worsen bladder detrusor activity and cause urethral contractions, thus worsening BPH. Interestingly, testosterone improves NO levels, thus displaying the protective impact of testosterone on LUTS.

Most of the research on BPH and its pharmaceutical treatment has focused on modulating DHT by inhibiting the enzyme 5-alpha-reductase. Other intriguing and accumulating research has illustrated the effects of estrogen or, more importantly, the estrogen:testosterone ratio in aging men and its effects on BPH.

In animal models, estrogen has shown molecular changes in the prostate of the aging dog.[11] The evidence is less clear in humans. Estrogens in the male are predominantly the products of peripheral aromatization of testicular and adrenal androgens.[12] Although the testicular and adrenal production of androgens declines with aging, levels of total plasma estradiol do not decline. In fact, estradiol remains relatively constant or even increases with age in men. This increase has been attributed to increased aromatase activity in the aging male, who often also has an increased accumulation of body fat. Fat cells contain aromatase, an enzyme that converts certain androgens to estrogen. The resulting disproportion in the estrogen:testosterone ratio may increase up to 40% in some men.[13]

By limiting aromatase or inhibiting the binding of estrogen to prostate cells, it may be possible to reduce BPH or slow its progression. Yet as we are now discovering, the interplay of androgens, testosterone and DHT metabolites, estrogens, and their respective receptors is intricately involved in the growth of prostatic tissue. DHT has gained a reputation for being problematic and the cause for BPH; however, 3-beta-diol and 3-alpha-diol, two DHT metabolites, may affect prostate growth via their binding activity of estrogen receptor (ER)-alpha and ER-beta.[14] ER-alpha induces prostatic stromal proliferation, whereas ER-beta induces apoptosis in luminal, basal, and stromal BPH cells.[15] Interestingly 3-beta-diol binds to ER-beta, reducing prostatic growth, and has been inversely correlated with IPSS.[14] ER-alpha is upregulated in BPH, along with aromatase, resulting in increased estrogens and ER-alpha, subsequently worsening BPH.[16] These findings suggest there is a unique interplay between androgens, estrogens, and their respective metabolites and receptors. It may not be fair to indict one single hormone as a causal factor for BPH, instead requiring consideration of the complete hormonal milieu.

Metabolic Syndrome as a Risk Factor

Metabolic syndrome (MetS) is a cluster of conditions including abdominal obesity, impaired glycemia, hypertension, hypertriglyceridemia, low high-density lipoprotein (HDL), and hyperlipidemia.[17] It is very well known that together, these conditions increase the risk of type 2 diabetes and cardiovascular disease.[18] However, what is less apparent but is certainly a health concern for men is the risk associated with MetS and BPH/LUTS.[19]

There is a significant body of evidence suggesting there is a strong clinical association between BPH and MetS.[20–22] Not only has the correlation between the two been very well established in the literature, but MetS has also been correlated with the severity of LUTS[21] and increased prostatic growth[17] and is a predictor of the clinical progression of BPH.[23] BPH/LUTS is also positively associated with the number of MetS components, with the research showing an increased risk of experiencing an IPSS of greater than 7, a total prostate volume of greater than or equal to 30cc, and a postvoid residual (PVR) of greater than or equal to 50 mL with an increasing number of MetS abnormalities.[20] This suggests that the more components of MetS a man experiences, the more likely he is to experience BPH/LUTS.

With lack of exercise also being a significant risk factor for LUTS[24] together with MetS, it would be prudent for practitioners to address MetS as an underlying cause of BPH and LUTS in older men.

Toxins as Risk Factors

Endocrine-disrupting chemicals (EDCs) are defined as exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process. EDCs include the biologically persistent organochlorine compounds (chlorinated pesticides and polychlorinated biphenyls [PCBs] and dioxins) and brominated flame retardants (PBDE), as well as nonpersistent plastics (bisphenol A), plasticizers (phthalates), organophosphate pesticides, fungicides, and herbicides.

It is well established that steroidal hormones (testosterone and estrogen) increase the risk of BPH, and cytochrome P450 (CYP) enzymes, especially CYP1A1, CYP1B1, and CYP17, metabolize these hormones. Thus functional polymorphisms in these genes and exposure to EDCs may further increase BPH risk. A study of 100 subjects with newly diagnosed BPH and 100 age-matched healthy male controls evaluated this potential risk.[25] Levels of p,p′-DDE and Endosulfan-α were found to be significantly higher among subjects with BPH compared with controls, and CYP17 polymorphism was observed to be significantly associated with subjects with BPH compared with controls, indicating that these factors may be important risk factors for BPH.

Although cadmium is a known antagonist of zinc and increases the activity of 5-alpha-reductase, its concentration in the prostate and its effects are unclear. Several studies have produced conflicting results.[26,27] An epidemiological study from India examined the association of cadmium (Cd) and lead (Pb) in the pathophysiology and progression BPH in 116 patients with the condition.[28] Prostatic acid phosphatase activity, prostate-specific antigen, maximum urinary flow rate, and redox status of BPH patients were correlated with Cd and Pb contents in the prostate. Increased levels of lipid peroxidase, a marker of oxidative damage, and increased glutathione peroxidase, thought to be via a compensatory action, are the possible mechanism as to which Cd contributes to prostatic hyperplasia.

Bisphenol A (BPA) has been shown to exert endocrine-disrupting effects on reproduction, development, metabolism, and cancer in humans, and findings in animal studies have linked BPA with BPH. In BPA-treated rats, the weight of the ventral prostate significantly increased, with a corresponding depletion of the antioxidant defense system and an increase in oxidative stress in epididymal sperm.[29] An additional rodent study induced BPH with testosterone and then treated the rats with BPA (10, 30, or 90 μg/kg daily), 17β-estradiol (50.0 μg/kg daily), or vehicle for 4 weeks. Rats treated with low-dose BPA (10 μg/kg) had a significant increase in relative prostate weight, and for prostate lobes, BPA 10μg/kg/day significantly increased the relative weight of the ventral prostate as well as the weight and relative weight of the dorsolateral prostate.[30]

DIAGNOSTIC CONSIDERATIONS

International Prostate Symptom Score

A useful subjective assessment tool for BPH patients is the IPSS. The IPSS is a modification of the American Urological Association (AUA) Symptom Index, adding a single question assessing the quality-of-life or bother score based on the patient's perception of the problem. The IPSS is a questionnaire that assesses the degree of LUTS and the quality of life of patients with BPH.

Patients can fill out the IPSS form before their examinations, but as little as possible interference from the health care provider and personnel is recommended (Fig. 154.1). A score of 7 or less is considered mildly symptomatic, 8 to 19 points is considered moderate, and a score of 20 to 35 points is considered severely symptomatic. Both the AUA index and IPSS questionnaire—although not specific for BPH, prostate volume, urinary flow rate, PRV, or bladder outlet obstruction—have been validated and are sensitive enough to be used in evaluating symptoms and selecting treatment.[31]

A good physical examination should include a digital rectal examination (DRE) to determine the approximate consistency and shape of the patient's prostate and abnormalities suggestive of prostate cancer,

International Prostate Symptom Score (I-PSS)

Patient Name: _____ **Date of birth:** _____ **Date completed** _____

In the past month:	Not at all	Less than 1 in 5 times	Less than half the time	About half the time	More than half the time	Almost always	Your score
1. Incomplete emptying How often have you had the sensation of not emptying your bladder?	0	1	2	3	4	5	
2. Frequency How often have you had to urinate less than every two hours?	0	1	2	3	4	5	
3. Intermittency How often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Urgency How often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Weak stream How often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Straining How often have you had to strain to start urination?	0	1	2	3	4	5	
	None	**1 Time**	**2 Times**	**3 Times**	**4 Times**	**5 Times**	
7. Nocturia How many times did you typically get up at night to urinate?	0	1	2	3	4	5	
Total I-PSS score							

Score 1–7: *Mild* 8–19: *Moderate* 20–35: *Severe*

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?	**0**	**1**	**2**	**3**	**4**	**5**	**6**

Fig. 154.1 International Prostate Symptom Score (IPSS) questionnaire. (From Bope E, Kellerman R, Rkel, R. *Conn's current therapy 2011*. Philadelphia: Saunders; 2011. International Prostate Symptom Score [IPSS] questionnaire, available from the Urological Sciences Research Foundation at http://www.usrf.org/questionnaires/UA_SymptomScore.html. Accessed September 26, 2011. Used with permission.)

BPH, or prostatitis. The approximate size of the prostate can be determined with a DRE; however, inadvertent misestimations can be made even by physicians who are highly experienced. Prostate size can be determined most accurately by a TRUS or magnetic resonance imaging (MRI).

Urine and Blood Tests

Patients with suspected BPH should undergo dipstick urinalysis testing or microscopical urinalysis to screen for infection and hematuria, proteinuria, pyuria, glycosuria, and ketonuria. If a urine dipstick is positive, a urine culture may be necessary to determine whether the patient's LUTS are independent of BPH.

Prostate-Specific Antigen

Because the symptoms of BPH and prostate cancer can be similar, measurement of prostate-specific antigen (PSA) is often used to differentiate BPH from prostate cancer. Recent studies show there are high levels of false-positive and false-negative PSA test results; therefore the benefits and risks of the test should be discussed with the patient. PSA testing is one of the tools used to assess for prostate cancer and its severity, along with imaging, PSA density, staging, and Gleason score. This remains a controversial test, however, owing to its lack of specificity, which can lead to the overdiagnosis and overtreatment of indolent prostate cancers.[32,33] One European study showed that it takes 1 of 48 men treated for prostate cancer to be saved from prostate cancer death

over a 10-year period, yet the conversation comparing the benefits versus harms of PSA screening in overall survival is still largely debated in the medical society.[32,34]

PSA and BPH

PSA is a glycoprotein secreted by the glands of the prostate; it is released in the serum with disruption of normal prostatic tissue due to prostate cancer, inflammation, BPH, or trauma.

Finasteride (5-mg dose) and dutasteride (0.5-mg dose), both of which are 5-alpha-reductase inhibitors used for the treatment of BPH and male-pattern baldness (1-mg dose of finasteride), will lower PSA levels by approximately 50% regardless of the dose.[35] Ejaculation and DRE have been reported to increase PSA levels, but studies have shown the effects to be variable or insignificant.[36,37] Prostate biopsy, however, will usually cause a substantial elevation of PSA levels. Therefore, after biopsy, PSA testing should be postponed for at least 3 to 6 weeks.[38,39] Although an elevated level indicates prostate cancer in about 90% of cases, it must be kept in mind that midrange elevations in PSA can be caused by BPH and that in some instances, there may be prostate cancer without an elevation of PSA levels. One approach to distinguish the two conditions when PSA is elevated is to measure the ratio of free to total PSA: more free PSA (fPSA) than complexed PSA suggests BPH rather than prostate cancer. A ratio of around 20% or greater for fPSA is considered more likely to represent BPH than cancer.[40] The 4K score, an algorithm utilizing total PSA, fPSA, intact PSA, and hK2, is being integrated into medical practices because it has the highest level of evidence of its ability to identify aggressive prostate cancer before biopsy.[41]

PSA density (PSAD) can be used to differentiate between prostate cancer and BPH in men with PSAs between 4 and 10 ng/mL and normal DRE results. PSAD should be higher in men with prostate cancer than in those with benign disease because cancer causes a greater elevation in PSA per prostate volume in comparison with BPH. To determine PSAD, the patient's PSA can be divided by prostate volume (as determined by a TRUS examination). Studies show that a PSAD greater than 0.15 may mean a higher risk of prostate cancer. A lower PSAD would likely indicate that the patient has BPH. This method should not conclusively determine whether a patient has BPH or prostate cancer, but it can be one piece of the puzzle.[42]

THERAPEUTIC CONSIDERATIONS

Left untreated, BPH eventually obstructs the bladder outlet, leading to the retention of urine and eventual kidney damage. Because this situation is potentially life-threatening, proper surgical treatment is crucial. Surgery may also be indicated in patients who have failed medical therapy or have recurrent infections, hematuria, or renal insufficiency. In the past, medical treatment involved a procedure called transurethral resection of the prostate (TURP). Because this surgery is associated with the risk of considerable morbidity (sexual dysfunction, incontinence, and bleeding)[43] and often makes matters worse, it should be avoided unless absolutely necessary. Surgical procedures that use thermal microwaves or a laser to reduce hyperplastic tissue are also available. Generally, these newer procedures are less expensive than TURP and have fewer complications, although subsequent therapies are often required.[44] Green-light therapy or photoselective vaporization of the prostate is a newer option that has similar outcomes to TURP, with a reduced risk of bleeding.[45]

Lifestyle/Exercise

There is an inverse association between physical activity[22] and BPH and a positive association between abdominal obesity and BPH.[44] In fact, sedentary men are at higher risk of LUTS/BPH, with even nonsedentary activities such as field work and casual walking proving to be protective against BPH.[46]

The ingestion of more calories may encourage abdominal obesity and sympathetic nervous system activity, both of which can increase the risk of BPH. Increased sympathetic activity, which is the "fight-or-flight" arm of the autonomic nervous system, may cause the prostate's smooth muscle to contract, resulting in a worsening of LUTS. Furthermore, hyperinsulinemia and insulin resistance caused by increased caloric ingestion, especially of high sugar content, may further complicate BPH/LUTS via a hypersympathetic response, thus resulting in constriction of the internal urethral sphincter.[46–48] However, a higher caloric intake does not seem to increase BPH risk when accompanied by increased physical activity.[49]

It is thus possible that physical activity may serve a threefold purpose:

It may increase blood flow to the area, allowing the body to remove wastes efficiently.

It can decrease the sympathetic stress responses, thus relaxing prostatic tissue.

It can reduce excess abdominal weight, which increases overall lower body pressure, thus relaxing the prostate/rectal region and improving blood flow into and out of the area.

Dietary and Nutritional Factors

Diet appears to play a critical role in the health of the prostate. Diets high in overall fat (not specific to the type of fat) are associated with an increased risk of BPH, as are diets high in red meat (whether conventional meats were used in comparison with the grass-fed kind is unknown). Conversely, according to a recent large randomized trial, diets high in protein and vegetables are associated with a decreased risk.[44] A special emphasis should be considered for onions, garlic, and other *Allium* plants because higher intakes of these foods have been found to be associated with a decreased BPH risk.[50]

It is particularly important to avoid pesticides, increase fruit consumption,[51] increase the intake of zinc and essential fatty acids, decrease coffee consumption,[52] decrease butter consumption, avoid margarine,[51] and keep cholesterol levels below 200 mg/dL. A moderate intake of alcohol can possibly benefit BPH but make LUTS worse.[53]

High-Protein Diet

The research evaluating protein intake and BPH is mixed. Some evidence shows that a high-protein diet (total calories: 44% protein, 35% carbohydrate, 21% fat) can inhibit 5-alpha-reductase, whereas a low-protein diet (10% protein, 70% carbohydrate, and 20% fat) may stimulate the enzyme.[54] On the other hand, an 8-year study of 3523 men with BPH found that total protein intake is positively associated with BPH, with the association being slightly stronger for animal protein intake than vegetable protein.[44] A study of Chinese farmers also revealed a correlation between a higher intake of animal protein and the incidence of BPH (91.1% in those eating diets high in animal protein vs. 11.8% in those not eating animal protein).[55]

A possible theoretical reason why high protein intake may not be helpful relates to a greater osmolar load, which may influence urinary output and thus impose an undue extra burden on an already taxed system.[7] Therefore we recommend against ingesting excess animal protein as a means to increase total protein intake. Instead, high-quality, plant-derived protein and protein from cold-water fish in moderate amounts is probably a reasonable recommendation until we know more about the relationship between protein intake and BPH.

Zinc

Studies conducted in the 1970s showed that zinc supplementation reduced the size of the prostate and the symptomatology of BPH in the majority of patients.[56] A more recent randomized trial comprising 4770 participants indicates a possible protective role of zinc against BPH. In that investigation, BPH was assessed over 7 years and defined in terms of medical or surgical treatment or repeated elevation (above 14) on the IPSS questionnaire. Diet, alcohol, and the use of supplements were assessed via a food frequency questionnaire. Higher zinc intake was associated with a significantly reduced risk for BPH.[49] The mechanism probably involves zinc's ability to inhibit 5-alpha-reductase[57] and/or its ability to inhibit prolactin.[58] Prolactin has been shown to increase the uptake of testosterone by the prostate, thereby leading to increased levels of DHT by providing additional substrate.[59]

Alcohol

Although only beer raises prolactin levels, higher alcohol intake may be associated with BPH. In a 17-year study of 6581 men in Hawaii, it was noted that an alcohol intake of at least 25 ounces per month was directly correlated with the diagnosis of BPH.[60]

The association was most significant for beer, wine, and sake and less so for distilled spirits. Most other recent studies confirm a protective effect of alcohol with regard to BPH when consumption is kept under three alcoholic beverages daily because anything above this amount may increase obstructive and irritative LUTS in men.[43,61,62]

Amino Acids

The combination of glycine, alanine, and glutamic acid (in the form of two 6-grain capsules administered three times daily for 2 weeks and one capsule three times daily thereafter) has been shown in several studies to relieve many BPH symptoms. In a controlled study of 45 men, nocturia was relieved or reduced in 95%, urgency was reduced in 81%, frequency was reduced in 73%, and delayed micturition was alleviated in 70%.[63] These results have also been reported in other controlled studies.[64] The mechanism of action is unknown but is likely related to the amino acids acting as inhibitory neurotransmitters and reducing the feeling of a full bladder. In other words, amino acid therapy is only palliative.

Cholesterol

The association among cholesterol, BPH, and LUTS is mixed. High levels of serum lipids have been associated with cardiovascular disease and include elevated serum low-density lipoprotein (LDL) cholesterol, decreased serum HDL cholesterol, and increased serum triglycerides. In the case of BPH, however, as described by Parsons, no positive association has been shown for cholesterol.[65] In fact, hyperinsulinemia with hyperlipidemia and low HDL are risk factors for prostatic inflammation and BPH.[47]

Beta-Sitosterol

Beta-sitosterol is one of several plant sterols (cholesterol is the main animal sterol) found in almost all plants. High levels are found in rice bran, wheat germ, corn oil, and soybeans; peanuts and their products—such as peanut oil, peanut butter, and peanut flour; and *Serenoa repens,* avocados, pumpkin seed, *Pygeum africanum,* and cashew fruit. The ability of beta-sitosterol and other phytosterols to lower cholesterol has been well documented.[66] Beta-sitosterol has also been shown to improve BPH. One double-blind study comprised 200 men receiving beta-sitosterol (20 mg) or placebo three times daily.[67] The beta-sitosterol group experienced an increase in maximum urinary flow rate from a baseline of 9.9 to 15.2 mL/s and a decrease in mean residual urinary volume of 30.4 from 65.8 mL.

In yet another study, 177 patients with benign prostate enlargement were randomized.[68] Patients received 130 mg of beta-sitosterol each day and were monitored for over 6 months. Measurements of the IPSS, urinary flow, and residual urine in the bladder after voiding were recorded. On average, urinary flow values increased by 4.5 mL/s, whereas residual urine volumes decreased by a substantial 33.5 mL. The IPSS showed a statistically significant improvement.[68]

Furthermore, a large systematic review analyzing 519 men among four randomized, placebo-controlled trials found that beta-sitosterol, although it did not reduce prostate volume, did improve urinary flow measures via IPSS, urodynamic measure, mean urine flow, residual urine volume, and nocturia.[69]

Vitamin D

Krystal and colleagues showed that vitamin D supplementation was associated with a reduced risk of BPH, but the dosage was imprecise. The association in this 4770-subject trial was observed only among men who used both multivitamins and single vitamin D supplements. There were no associations of supplement use with BPH risk, with the exception of a trend toward decreased BPH risk with increased intake of supplemental vitamin D.[49] Although this study lacked data on the frequency, dose, and duration of vitamin D use, the results are intriguing enough to support further research addressing whether high-dose supplementation will have any benefit for BPH. Numerous studies have shown that vitamin D_3 may have protective effects against prostate cancer.[70]

The mechanism of vitamin D's favorable effect on BPH is by attaching this molecule to vitamin D receptors on the prostate and bladder and inhibiting prostate growth, lowering excessive contractility, and reducing inflammation.[71]

Lycopene

Commonly found in watermelon and tomatoes, lycopene is a carotenoid known for its strong antioxidative effects. Levels in the prostate appear inversely correlated with disorders. However, controlled intervention trials, although promising, have been small and typically in conjunction with other interventions, such as the botanical medicines discussed in the next section.

Botanical Medicines

Botanical medicines in the treatment of BPH have been a popular recommendation in Europe for decades.[72] The chance of clinical success with any of the botanical treatments of BPH appears to be determined by the degree of obstruction, as indicated by the volume of residual urine. For levels of less than 50 mL, the results are usually excellent. For levels between 50 and 100 mL, the results are usually quite good. With residual urine levels between 100 and 150 mL, it will be tougher to produce a significant improvement within the customary 4- to 6-week period. If the volume of residual urine is greater than 150 mL, saw palmetto extract and other botanical medicines alone are unlikely to produce any significant improvement.

Serenoa repens (Saw Palmetto)

The liposterolic extract of the fruit of the saw palmetto palm tree (also known as *Sabal serrulata*), native to Florida, has been shown to significantly improve the signs and symptoms of BPH in numerous clinical studies (see Chapter 112). The mechanism of action is related to inhibition of DHT binding to both the cytosolic and nuclear androgen receptors, inhibition of 5-alpha-reductase, and interference with intraprostatic estrogen receptors. Owing to these effects, the results of most randomized trials have been excellent. Systematic literature reviews and meta-analyses of clinical trials report excellent results using

Serenoa, comparing quite favorably with those of finasteride (Proscar) and tamsulosin (Flomax) in terms of efficacy but with a better side-effect profile. It appears that the effect of *Serenoa* extract is most obvious in the early stages of BPH (i.e., in cases of mild to moderate hypertrophy) because the clinical research has clearly shown that roughly 90% of men with mild to moderate BPH experience improvement in symptoms during the first 4 to 6 weeks after starting *Serenoa* extract (320 mg per day of the liposterolic extract). However, it is likely that *Serenoa* extract may show little if any clinical benefit in more advanced cases of BPH. Chapter 112 offers a complete discussion of the pharmacology of *Serenoa.*

Furthermore, although *Serenoa* is often very effective on its own, better results may be achieved by combining *Urtica dioca* root extract (discussed herein) with *Serenoa* extract. One double-blind study involving 431 patients found this combination to produce clinical benefit equal to that of finasteride.[73] In a more recent long-term study, the efficacy and tolerability of this combination was investigated in elderly male patients suffering from LUTS caused by BPH.[74] In total, 257 patients were randomized to treatment with the combination (320 mg of *Serenoa* and 240 mg of *Urtica* extracts per day) or placebo. A 24-week double-blind treatment was followed by an open control period of 24 weeks, during which all patients were given the *Serenoa* and *Urtica* combination. Patients treated with the botanical combination exhibited a substantially greater reduction in total IPSS after 24 weeks of double-blind treatment than did patients in the placebo group. This applied to obstructive as well as to irritative symptoms and to patients with moderate symptoms as well as those with severe symptoms at baseline. Patients randomized to placebo showed a marked improvement in LUTS (as measured by the IPSS) after being switched to the botanical combination. Like the extract of *Serenoa, Urtica* extract appears to interact with the binding of DHT to cytosolic and nuclear receptors.[75] In vitro studies also show that lignans found in *Urtica* may modulate hormonal effects because of their affinity for sex hormone–binding globulin.[74] Although earlier studies have shown promising results, a large recent Cochrane review suggests that the benefits of *Serenoa,* even at high doses, may be best utilized as a combination therapy. Studies have shown benefits when combined with *Urtica,* as described previously, but also with selenium and lycopene. The combination of selenium, lycopene, and *S. repens* shows improved IPSS and urinary function tests.[76]

Furthermore, this same Cochrane review claims *Serenoa* alone was no better than placebo but also that it was equally as effective as tamsulosin in reducing IPSS scores.[77] This thus raises the question, if *Serenoa* is no better than placebo but equal to tamsulosin, then is either no better than placebo?

Cernilton

Cernilton, an extract of rye-grass flower pollen, has been used to treat prostatitis and BPH in Europe for more than 35 years.[78] It has been shown to be quite effective in several double-blind clinical studies in the treatment of BPH,[79,80] where the overall success rate is about 70%.[81] Patients who respond typically experience less nocturia and diurnal frequency (a reduction of about 70%) as well as significant reductions in residual urine volume.[73] The extract has been shown to exert some anti-inflammatory action and to produce a contractile effect on the bladder while simultaneously relaxing the urethra. In addition, Cernilton contains a substance that inhibits the growth of prostate cells.[75]

In one study, the clinical efficacy of Cernilton in the treatment of symptomatic BPH was examined over a 1-year period.[79] Seventy-nine men averaging 68 years of age (range 62–89) with a mean baseline prostatic volume of 33.2 cm^2 were given 63 mg Cernilton pollen extract twice daily for 12 weeks. As a result, their average urinary maximum flow rate increased from 5.1 to 6 mL/s. The average flow rate increased from 9.3 to 11 mL/s. Residual urine volume decreased from 54.2 mL to less than 30 mL. Clinical efficacy, based on symptoms, was as follows:
Urgency or discomfort: improved by 76.9%
Dysuria: improved by 71.4%
Nocturia: improved by 56.8%
Incomplete emptying: improved by 66.2%
Prolonged voiding: improved by 64.1%
Delayed voiding: improved by 62.2%
Intermittency: improved by 60.6%
Postvoid dribbling: improved by 42.7%

Overall, 85% of the test subjects experienced benefit: 11% reporting "excellent," 39% "good," 35% "satisfactory," and 15% "poor" results.

A summary review of two placebo-controlled studies, two comparative trials (both lasting 12–24 weeks), and three double-blind studies of 444 men showed that although Cernilton did not improve urinary flow rates, residual volume, or prostate size compared with placebo or the comparative study agents, it did improve self-rated urinary symptom scores and reduced nocturia compared with placebo and an amino acid mixture.[75] Clearly, more long-term studies of Cernilton need to be conducted to elucidate the terms of its usefulness as an alternative or adjunct to *Serenoa.*

Last, a study by Preuss and associates illustrates the potential value of a combination of nutrients used for BPH. In this randomized trial, 144 subjects were randomized to Cernilton, saw palmetto, beta-sitosterol, vitamin E, or placebo. After 3 months, the combination group had significantly less nocturia, frequency, and overall BPH symptoms, without any adverse side effects.[82]

Ganoderma lucidum

Of the many medicinal mushrooms utilized in naturopathic medicine, *Ganoderma lucidium* has shown the most promise in improving BPH symptoms and LUTS in older men. This medical mushroom has been shown to inhibit 5-alpha-reductase in the ventral prostate of rats.[83] Two follow-up randomized, placebo-controlled trials tested the use of *G. lucidum* in adult men with LUTS. The primary outcomes assessed for changes in the IPSS. The two studies exhibited a significant reduction in IPSS using 6 mg daily of *G. lucidum.*[84,85] There was no added benefit at doses of 60 mg, suggesting a higher dose is not necessary to reduce LUTS.[84]

Interestingly, no changes were noted in quality-of-life scores, peak urinary flow, mean urinary flow, residual urine, prostate volume, serum PSA, or testosterone levels.[84,85] This would suggest that the benefits observed from *Ganoderma* are not dependent on the ability to inhibit 5-alpha-reductase, as was seen in earlier animal trials. This is significant because reductions in PSA and the inhibition of androgen and DHT receptors in the prostate may affect screening for BPH and prostate cancer. Therefore supplementing with *Ganoderma* may improve symptoms and still allow for proper PSA screening and monitoring.

Vaccinium macrocarpon

Vaccinium macrocarpon, better known as cranberry fruit, has been popularized for its use in urinary tract infections. However, the use of this fruit has also shown promise in the management of LUTS and BPH in men. Two studies have assessed the efficacy of *Vaccinium* extract in improving the IPSS and quality-of-life scores, along with clinical parameters such as PSA, PVR, average urinary flow rate (Q ave), and maximum urinary flow rate (Q max). Findings from both studies suggest that *Vaccinium* improves the IPSS, along with significantly improving urinary parameters such as Q max, Q ave, and

PVR.[86,87] One of the studies assessed the benefits of PSA reduction, showing this fruit was able to reduce PSA in men with histologically confirmed nonbacterial chronic prostatitis.[86] Possible mechanisms, although unclear, suggest *V. macrocarpon* improves detrusor contraction and activation, improves the micturition reflex, reduces inflammation, and possibly improves the dynamic and static prostatic components of voiding.[86,87]

Pygeum africanum

The bark of *Pygeum africanum,* an evergreen tree native to Africa, has historically been used in the treatment of urinary tract disorders. The major active components of the bark are fat-soluble sterols and fatty acids. Virtually all of the research on *Pygeum* has featured a *Pygeum* extract standardized to contain 14% triterpenes, including beta-sitosterol and 0.5% n-docosanol. This extract has been extensively studied in both experimental animals and humans in clinical trials (see Chapter 107). A study on rat prostatic cells suggests that the therapeutic effect of *Pygeum* may be due in part to the inhibition of growth factors epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor I (IGF-I), which are responsible for prostatic overgrowth.[88] Additional possible benefits of *Pygeum* have been suspected to be due to its ability to inactivate androgen receptors, inhibit cellular proliferation, and reduce inflammation.[89–91]

Numerous clinical trials with more than 600 patients have shown *Pygeum* extract to be effective in reducing the symptoms and clinical signs of BPH, especially in early cases.[92] However, in a double-blind study that compared *Pygeum* extract with extract of saw palmetto, the latter produced a greater reduction of symptoms and was better tolerated.[93] In addition, the effects on objective parameters, especially urine flow rate and residual urine content, were better in the clinical studies with saw palmetto. However, there may be circumstances in which *Pygeum* is more effective than saw palmetto. For example, saw palmetto has not been shown to produce some of *Pygeum*'s effects on prostate secretion. Of course, the two extracts have somewhat overlapping mechanisms of action and can be used in combination.

Urtica dioica (Stinging Nettle)

Extracts of the root of *Urtica dioica* have also been shown to be effective in the treatment of BPH. Three double-blind studies have shown it to be more effective than a placebo.[94,95] The most recent study, conducted in Iran, was a 6-month double-blind, placebo-controlled, randomized, partial crossover, comparative trial of *Urtica* with placebo in 620 patients.[96] Both groups continued the medication up to 18 months, and only those who continued the therapy had favorable results. No side effects were identified in either group.

THERAPEUTIC APPROACH

Therapeutic goals for BPH are aimed at the following goals:
Normalize prostate nutrient levels.
Restore steroid hormones to normal levels.
Inhibit excessive conversion of testosterone to DHT.
Inhibit DHT-receptor binding.
Reduce inflammation.
Eliminate excess estradiol production.
Limit promoters of the hyperplastic process (e.g., prolactin).

Severe BPH resulting in significant acute urinary retention may require catheterization for relief; a sufficiently advanced case may not respond rapidly enough to therapy and may require the short-term use of an alpha$_1$ antagonist by itself or combined with a 5-alpha-reductase medication or surgical intervention.

Lifestyle

Patients should exercise, between four to six times a week, and properly stretch the prostate/urogenital area to increase blood flow.

Diet

Initially, the diet should be higher in vegetable protein, low in carbohydrates, low in animal fats, and high in unsaturated oils. After the patient responds, a less strict whole-foods, balanced approach can be used. The patient should limit alcohol and coffee intake; avoid drug-, pesticide- and hormone-contaminated foods; and limit cholesterol-rich foods. Soy foods should be used regularly.[97]

Supplements

Zinc: 30 to 45 mg/day (picolinate preferred, maximum of 6 months; reduce dosage to 15–30 mg/day after 6 months; consider monitoring copper status in long-term therapy)
Glycine: 200 mg/day
Glutamic acid: 200 mg/day
Alanine: 200 mg/day
Vitamin D3: 2000 to 5000 IU/day

Botanicals

Liposterolic extract of saw palmetto *(S. repens)* (standardized at 85%–95% fatty acids and sterols): 160 mg two times a day
Flower pollen extract (e.g., Cernilton): 63 mg two to three times a day
P. africanum extract (14% triterpene content): 50 to 100 mg/day
U. dioica extract: 120 to 150 mg two times a day
Beta-sitosterol: 60 to 130 mg/day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bushman W. Etiology, epidemiology, and natural history of benign prostatic hyperplasia. *Urol Clin North Am*. 2009;36(4):403–415. https://doi.org/10.1016/j.ucl.2009.07.003.
2. Sanda MG, Beaty TH, Stutzman RE, Childs B, Walsh PC. Genetic susceptibility of benign prostatic hyperplasia. *J Urol*. 1994;152(1):115–119.
3. Wang J-Y, Fu Y-Y, Kang D-Y. The association between metabolic syndrome and characteristics of benign prostatic hyperplasia: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2016;95(19):e3243. https://doi.org/10.1097/MD.0000000000003243.
4. Dahle SE, Chokkalingam AP, Gao Y-T, Deng J, Stanczyk FZ, Hsing AW. Body size and serum levels of insulin and leptin in relation to the risk of benign prostatic hyperplasia. *J Urol*. 2002;168(2):599–604. https://doi.org/10.1016/S0022-5347(05)64687-3.
5. Bartsch G, Rittmaster R, Klocker H. Dihydrotestosterone and the concept of 5alpha-reductase inhibition in human benign prostatic hyperplasia. *World J Urol*. 2002;19(6):413–425. https://doi.org/10.1007/s00345-002-0248-5.
6. Imperato-McGinley J, Zhu YS. Androgens and male physiology the syndrome of 5α-reductase-2 deficiency. In: *Molecular and Cellular Endocrinology*. vol. 198. ; 2002:51–59. https://doi.org/10.1016/S0303-7207(02)00368-4.
7. Wright AS, Thomas LN, Douglas RC, Lazier CB, Rittmaster RS. Relative potency of testosterone and dihydrotestosterone in preventing atrophy and apoptosis in the prostate of the castrated rat. *J Clin Invest*. 1996;98(11):2558–2563. https://doi.org/10.1172/JCI119074.
8. Partin A, Rodriguez R. The molecular biology, endocrinology, and physiology of the prostate and seminal vesicles. In: Walsh PC, ed. *Campbell's Urology*. Philadelphia: Saunders; 2002:1237–1296.
9. DeLay KJ, Kohler TS. Testosterone and the prostate. *Urol Clin North Am*. 2016;43(3):405–412. https://doi.org/10.1016/j.ucl.2016.04.011.
10. Baas W, Köhler TS. Testosterone replacement therapy and BPH/LUTS. what is the evidence? *Curr Urol Rep*. 2016;17(6):46. https://doi.org/10.1007/s11934-016-0600-8.
11. Barrack ER, Berry SJ. DNA synthesis in the canine prostate: effects of androgen and estrogen treatment. *Prostate*. 1987;10(1):45–56.
12. Gooren LJG, Toorians AWFT. Significance of oestrogens in male (patho) physiology. *Ann Endocrinol (Paris)*. 2003;64(2):126–135. https://doi.org/MDOI-AE-04-2003-64-2-0003-4266-101019-ART15 [pii].
13. Prezioso D, Denis LJ, Klocker H, et al. Estrogens and aspects of prostate disease. *Int J Urol*. 2007;14(1):1–16. https://doi.org/10.1111/j.1442-2042.2006.01476.x.
14. Asiedu B, Anang Y, Nyarko A, et al. The role of sex steroid hormones in benign prostatic hyperplasia. *Aging Male*. 2017;20(1):17–22. https://doi.org/10.1080/13685538.2016.1272101.
15. Wynder JL, Nicholson TM, DeFranco DB, Ricke WA. Estrogens and male lower urinary tract dysfunction. *Curr Urol Rep*. 2015;16(9):61. https://doi.org/10.1007/s11934-015-0534-6.
16. Hullender Rubin LE, Opsahl MS, Wiemer KE, Mist SD, Caughey AB. Impact of whole systems traditional Chinese medicine on in-vitro fertilization outcomes. *Reprod Biomed Online*. 2015;30(6):602–612. https://doi.org/10.1016/j.rbmo.2015.02.005.
17. Pan JG, Jiang C, Luo R, Zhou X. Association of metabolic syndrome and benign prostatic hyperplasia in Chinese patients of different age decades. *Urol Int*. 2014;93(1):10–16. https://doi.org/10.1159/000354026.
18. Eckel RH, Alberti KGMM, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet (London, England)*. 2010;375(9710):181–183. https://doi.org/10.1016/S0140-6736(09)61794-3.
19. Calogero AE, Burgio G, Condorelli RA, Cannarella R, La Vignera S. Epidemiology and risk factors of lower urinary tract symptoms/benign prostatic hyperplasia and erectile dysfunction. *Aging Male*. 2018:1–8. https://doi.org/10.1080/13685538.2018.1434772.
20. Park YW, Kim SB, Kwon H, et al. The relationship between lower urinary tract symptoms/benign prostatic hyperplasia and the number of components of metabolic syndrome. *Urology*. 2013;82(3):674–679. https://doi.org/10.1016/j.urology.2013.03.047.
21. Pashootan P, Ploussard G, Cocaul A, de Gouvello A, Desgrandchamps F. Association between metabolic syndrome and severity of lower urinary tract symptoms (LUTS): an observational study in a 4666 European men cohort. *BJU Int*. 2015;116(1):124–130. https://doi.org/10.1111/bju.12931.
22. Yin Z, Yang J-R, Rao J-M, Song W, Zhou K-Q. Association between benign prostatic hyperplasia, body mass index and metabolic syndrome in Chinese men. *Asian J Androl*. 2014;0(0):0. https://doi.org/10.4103/1008-682X.148081.
23. Kwon H, Kang HC, Lee JH. Relationship between predictors of the risk of clinical progression of benign prostatic hyperplasia and metabolic syndrome in men with moderate to severe lower urinary tract symptoms. *Urology*. 2013;81(6):1325–1329. https://doi.org/10.1016/j.urology.2013.01.042.
24. Marshall LM, Holton KF, Parsons JK, et al. Lifestyle and health factors associated with progressing and remitting trajectories of untreated lower urinary tract symptoms among elderly men. *Prostate Cancer Prostatic Dis*. 2014;17(3):265–272. https://doi.org/10.1038/pcan.2014.22.
25. Kumar V, Banerjee BD, Datta SK, et al. Association of CYP1A1, CYP1B1 and CYP17 gene polymorphisms and organochlorine pesticides with benign prostatic hyperplasia. *Chemosphere*. 2014;108:40–45. PubMed PMID: 24875910.
26. Lahtonen R. Zinc and cadmium concentrations in whole tissue and in separated epithelium and stroma from human benign prostatic hypertrophic glands. *Prostate*. 1985;6(2):177–183.
27. Sinquin G, Morfin RF, Charles JF, Floch HH. Testosterone metabolism by homogenates of human prostates with benign hyperplasia: effects of zinc, cadmium and other bivalent cations. *J Steroid Biochem*. 1984;20(3):773–780.
28. Pandya C, Gupta S, Pillai P, et al. Association of cadmium and lead with antioxidant status and incidence of benign prostatic hyperplasia in patients of Western India. *Biological Trace Element Research*. 2013;152(3):316–326. PubMed PMID: 23479318.
29. Chitra KC, Latchoumycandane C, Mathur PP. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*. 2003;185(1–2):119–127. PubMed PMID: 12505450.
30. Wu JH, Jiang XR, Liu GM, Liu XY, He GL, Sun ZY. Oral exposure to low-dose bisphenol A aggravates testosterone-induced benign hyperplasia prostate in rats. *Toxicology and Industrial Health*. 2011;27(9):810–819. PubMed PMID: 21415097.
31. Barry MJ. Evaluation of symptoms and quality of life in men with benign prostatic hyperplasia. *Urology*. 2001;58:25–32. https://doi.org/10.1016/S0090-4295(01)01300-0.
32. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med*. 2009;360(13):1320–1328. https://doi.org/10.1056/NEJMoa0810084.
33. Woolf S. Screening for prostate cancer with prostate-specific antigen: an examination of the evidence. *N Engl J Med*. 1995;333(21):1401–1405. https://doi.org/10.1056/NEJM199511233332107.
34. Hayes JH, Barry MJ. Screening for prostate cancer with the prostate-specific antigen test. *JAMA*. 2014;311(11):1143. https://doi.org/10.1001/jama.2014.2085.
35. Andriole GL, Guess HA, Epstein JI, et al. Treatment with finasteride preserves usefulness of prostate-specific antigen in the detection of prostate cancer: results of a randomized, double-blind, placebo-controlled clinical trial. *Urology*. 1998;52(2):195–202. https://doi.org/10.1016/S0090-4295(98)00184-8.
36. Zisman A, Soffer Y, Siegel YI, Paz A, Lindner A. Postejaculation serum prostate-specific antigen level. *Eur Urol*. 1997;32(1):54–57.
37. Effect of digital rectal examination on serum prostate-specific antigen in a primary care setting. The Internal Medicine Clinic Research Consortium. *Arch Intern Med*. 1995;155(4):389–392.
38. Oesterling JE, Rice DC, Glenski WJ, Bergstralh EJ. Effect of cystoscopy, prostate biopsy, and transurethral resection of prostate on serum prostate-specific antigen concentration. *Urology*. 1993;42(3):276–282. https://doi.org/10.1016/0090-4295(93)90616-I.
39. Hara N, Koike H, Nobushita T, et al. Kinetics of serum total and free prostate-specific antigen (PSA) after extended multisite prostate biopsy: comparison among biopsy, transurethral resection of the prostate (TURP), and biopsy plus TURP. *Urol Oncol Semin Orig Investig*. 2010;28(4):355–359. https://doi.org/10.1016/j.urolonc.2008.10.014.

40. Prestigiacomo AF, Stamey TA. Clinical usefulness of free and complexed PSA. *Scand J Clin Lab Invest*. 1995;55(S221):32–34. https://doi.org/10.3109/00365519509090561.
41. Lamy P-J, Allory Y, Gauchez A-S, et al. Prognostic biomarkers used for localised prostate cancer management: a systematic review. *Eur Urol Focus*. 2017. https://doi.org/10.1016/j.euf.2017.02.017.
42. Allan RW, Sanderson H, Epstein JI. Correlation of minute (0.5 MM or less) focus of prostate adenocarcinoma on needle biopsy with radical prostatectomy specimen: role of prostate specific antigen density. *J Urol*. 2003;170(2 Pt 1):370–372. https://doi.org/10.1097/01.ju.0000074747.72993.cb.
43. Dull P, Reagan RW, Bahnson RR. Managing benign prostatic hyperplasia. *Am Fam Physician*. 2002;66(1).
44. Suzuki S, Platz EA, Kawachi I, Willett WC, Giovannucci E. Intakes of energy and macronutrients and the risk of benign prostatic hyperplasia. *Am J Clin Nutr*. 2002;75:689–697.
45. Zang Y-C, Deng X-X, Yang D-R, et al. Photoselective vaporization of the prostate with GreenLight 120-W laser versus transurethral resection of the prostate for benign prostatic hyperplasia: a systematic review with meta-analysis of randomized controlled trials. *Lasers Med Sci*. 2016;31(2):235–240. https://doi.org/10.1007/s10103-015-1843-1.
46. Lee HW, Kim SA, Nam JW, Kim MK, Choi BY, Moon HS. The study about physical activity for subjects with prevention of benign prostate hyperplasia. *Int Neurourol J*. 2014;18(3):155. https://doi.org/10.5213/inj.2014.18.3.155.
47. Vignozzi L, Gacci M, Cellai I, et al. Fat boosts, while androgen receptor activation counteracts, BPH-associated prostate inflammation. *Prostate*. 2013;73(8):789–800. https://doi.org/10.1002/pros.22623.
48. Wolin KY, Grubb RL, Pakpahan R, et al. Physical activity and benign prostatic hyperplasia-related outcomes and nocturia. *Med Sci Sport Exerc*. 2015;47(3):581–592. https://doi.org/10.1249/MSS.0000000000000444.
49. Kristal AR, Arnold KB, Schenk JM, et al. Dietary patterns, supplement use, and the risk of symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *Am J Epidemiol*. 2008;167(8):925–934. https://doi.org/10.1093/aje/kwm389.
50. Galeone C, Pelucchi C, Talamini R, et al. Onion and garlic intake and the odds of benign prostatic hyperplasia. *Urology*. 2007;70(4):672–676. https://doi.org/10.1016/j.urology.2007.06.1099.
51. Lagiou P, Wuu J, Trichopoulou A, Hsieh CC, Adami HO, Trichopoulos D. Diet and benign prostatic hyperplasia: a study in Greece. *Urology*. 1999;54:284–290. https://doi.org/10.1016/S0090-4295(99)00096-5.
52. Gass R. Benign prostatic hyperplasia: the opposite effects of alcohol and coffee intake. *BJU Int*. 2002;90(7):649–654.
53. Parsons JK, Kashefi C. Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. *Eur Urol*. 2008;53(6):1228–1235. https://doi.org/10.1016/j.eururo.2008.02.019.
54. Kappas A, Anderson KE, Conney AH, Pantuck EJ, Fishman J, Bradlow HL. Nutrition-endocrine interactions: induction of reciprocal changes in the delta 4-5 alpha-reduction of testosterone and the cytochrome P-450-dependent oxidation of estradiol by dietary macronutrients in man. *Proc Natl Acad Sci U S A*. 1983;80(24):7646–7649. https://doi.org/10.1073/pnas.80.24.7646.
55. Zhang S-X, Yu B, Guo S-L, Wang Y-W, Yin C-K. Comparison of incidence of BPH and related factors between urban and rural inhabitants in district of Wannan. *Zhonghua Nan Ke Xue*. 2003;9:45–47.
56. Fahim M, Fahim Z, Der R, Harman J. Zinc treatment for reduction of hyperplasia of prostate. *Fed Proc*. 1976;35:361.
57. Om AS, Chung KW. Dietary zinc deficiency alters 5 alpha-reduction and aromatization of testosterone and androgen and estrogen receptors in rat liver. *J Nutr*. 1996;126:842–848.
58. Login IS, Thorner MO, MacLeod RM. Zinc may have a physiological role in regulating pituitary prolactin secretion. *Neuroendocrinology*. 1983;37:317–320. https://doi.org/10.1159/000123568.
59. Farnsworth WE, Slaunwhite WR, Sharma M, et al. Interaction of prolactin and testosterone in the human prostate. *Urol Res*. 1981;9(2):79–88.
60. Chyou P-H, Nomura AMY, Stemmermann GN, Hankin JH. A prospective study of alcohol, diet, and other lifestyle factors in relation to obstructive uropathy. *Prostate*. 1993;22(3):253–264. https://doi.org/10.1002/pros.2990220308.
61. Gass R. Benign prostatic hyperplasia: the opposite effects of alcohol and coffee intake. *BJU Int*. 2002;90(7):649–654. https://doi.org/10.1046/j.1464-410X.2002.03001.x.
62. Bradley CS, Erickson BA, Messersmith EE, et al. Evidence of the impact of diet, fluid intake, caffeine, alcohol and tobacco on lower urinary tract symptoms: a systematic review. *J Urol*. 2017;198(5):1010–1020. https://doi.org/10.1016/j.juro.2017.04.097.
63. Damrau F. Benign prostatic hypertrophy: amino acid therapy for symptomatic relief. *J Am Geriatr Soc*. 1962;10:426–430.
64. Feinblatt HM, Gant JC. Palliative treatment of benign prostatic hypertrophy; value of glycine-alanine-glutamic acid combination. *J Maine Med Assoc*. 1958;49(3):99–101 (passim).
65. Parsons J. Lifestyle factors, benign prostatic hyperplasia, and lower urinary tract symptoms. *Curr Opin Urol*. 2011;21(1):1–4. https://doi.org/10.1097/MOU.0b013e32834100c9.
66. Tilvis RS, Miettinen TA. Serum plant sterols and their relation to cholesterol absorption. *Am J Clin Nutr*. 1986;43(1):92–97.
67. Berges RR, Windeler J, Trampisch HJ, Senge T. Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia. Beta-sitosterol Study Group. *Lancet (London, England)*. 1995;345(8964):1529–1532.
68. Klippel KF, Hiltl DM, Schipp B. A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. German BPH-Phyto Study group. *Br J Urol*. 1997;80(3):427–432.
69. Wilt TJ, Ishani A, MacDonald R, Stark G, Mulrow CD, Lau J. Beta-sitosterols for benign prostatic hyperplasia. In: Wilt TJ, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd; 1999:CD001043. https://doi.org/10.1002/14651858.CD001043.
70. Li H, Stampfer MJ, Hollis JBW, et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. Franco EL, ed. *PLoS Med*. 2007;4(3):e103. https://doi.org/10.1371/journal.pmed.0040103.
71. Adorini L, Penna G, Fibbi B, Maggi M. Vitamin D receptor agonists target static, dynamic, and inflammatory components of benign prostatic hyperplasia. *Ann N Y Acad Sci*. 2010;1193:146–152. https://doi.org/10.1111/j.1749-6632.2009.05299.x.
72. Buck AC. Phytotherapy for the prostate. *Br J Urol*. 1996;78(3):325–336.
73. Habib FK, Ross M, Lewenstein A, Zhang X, Jaton JC. Identification of a prostate inhibitory substance in a pollen extract. *Prostate*. 1995;26(3):133–139.
74. Lopatkin N, Sivkov A, Schläfke S, Funk P, Medvedev A, Engelmann U. Efficacy and safety of a combination of Sabal and Urtica extract in lower urinary tract symptoms—long-term follow-up of a placebo-controlled, double-blind, multicenter trial. *Int Urol Nephrol*. 2007;39(4):1137–1146. https://doi.org/10.1007/s11255-006-9173-7.
75. MacDonald R, Ishani A, Rutks I, Wilt TJ. A systematic review of Cernilton for the treatment of benign prostatic hyperplasia. *BJU Int*. 2000;85(7):836–841.
76. Minutoli L, Bitto A, Squadrito F, et al. *Serenoa repens*, lycopene and selenium: a triple therapeutic approach to manage benign prostatic hyperplasia. *Curr Med Chem*. 2013;20(10):1306–1312.
77. Tacklind J, MacDonald R, Rutks I, Stanke JU, Wilt TJ. *Serenoa repens* for benign prostatic hyperplasia. In: Tacklind J, ed. *Cochrane Database of Systematic Reviews*. vol. 12. Chichester, UK: John Wiley & Sons, Ltd; 2012:CD001423. https://doi.org/10.1002/14651858.CD001423.pub3.
78. Wilt T, Ishani A, Mac Donald R. *Serenoa repens* for benign prostatic hyperplasia. Tacklind J, ed. *Cochrane Database Syst Rev*. 2002;3:CD001423. https://doi.org/10.1002/14651858.CD001423.
79. Yasumoto R, Kawanishi H, Tsujino T, et al. Clinical evaluation of long-term treatment using cernitin pollen extract in patients with benign prostatic hyperplasia. *Clin Ther*. 1995;17(1):82–87.
80. Buck AC, Cox R, Rees RW, Ebeling L, John A. Treatment of outflow tract obstruction due to benign prostatic hyperplasia with the pollen

extract, cernilton. A double-blind, placebo-controlled study. *Br J Urol*. 1990;66(4):398–404.
81. Dutkiewicz S. Usefulness of Cernilton in the treatment of benign prostatic hyperplasia. *Int Urol Nephrol*. 1996;28(1):49–53.
82. Preuss HG, Marcusen C, Regan J, Klimberg IW, Welebir TA, Jones WA. Randomized trial of a combination of natural products (cernitin, saw palmetto, B-sitosterol, vitamin E) on symptoms of benign prostatic hyperplasia (BPH). *Int Urol Nephrol*. 2001;33(2):217–225.
83. Liu J, Fujita R, Sato M, et al. The effect of strain, growth stage, and cultivating condition of *Ganoderma lucidum* on 5α-reductase inhibition. *J Wood Sci*. 2005;51(2):189–192. https://doi.org/10.1007/s10086-004-0645-z.
84. Noguchi M, Kakuma T, Tomiyasu K. Effect of an extract of *Ganoderma lucidum* in men with lower urinary tract symptoms: a double-blind, placebo-controlled randomized and dose-ranging study. *Asian J Androl*. 2008;10:651–658.
85. Noguchi M, Kakuma T, Tomiyasu K, et al. Randomized clinical trial of an ethanol extract of *Ganoderma lucidum* in men with lower urinary tract symptoms. *Asian J Androl*. 2008;10(5):777–785. https://doi.org/10.1111/j.1745-7262.2008.00361.x.
86. Vidlar A, Vostalova J, Ulrichova J, et al. The effectiveness of dried cranberries *(Vaccinium macrocarpon)* in men with lower urinary tract symptoms. *Br J Nutr*. 2010;104:1181–1189. https://doi.org/10.1017/S0007114510002059.
87. Vidlar A, Student V, Vostalova J, et al. Cranberry fruit powder (Flowens™) improves lower urinary tract symptoms in men: a double-blind, randomized, placebo-controlled study. *World J Urol*. 2016;34(3):419–424. https://doi.org/10.1007/s00345-015-1611-7.
88. Yablonsky F, Nicolas V, Riffaud JP, Bellamy F. Antiproliferative effect of *Pygeum africanum* extract on rat prostatic fibroblasts. *J Urol*. 1997;157(6):2381–2387.
89. Papaioannou M, Schleich S, Prade I, et al. The natural compound atraric acid is an antagonist of the human androgen receptor inhibiting cellular invasiveness and prostate cancer cell growth. *J Cell Mol Med*. 2009;13(8b):2210–2223. https://doi.org/10.1111/j.1582-4934.2008.00426.x.
90. Lawson RK. Role of growth factors in benign prostatic hyperplasia. *Eur Urol*. 1997;32(suppl 1):22–27.
91. Paubert-Braquet M, Cave A, Hocquemiller R, et al. Effect of *Pygeum africanum* extract on A23187-stimulated production of lipoxygenase metabolites from human polymorphonuclear cells. *J Lipid Mediat Cell Signal*. 1994;9(3):285–290.
92. Duvia R, Radice G, Galdini R. Advances in the phytotherapy of prostatic hypertrophy. *Med Prax*. 1983;4:143–148.
93. Belaiche P, Lievoux O. Clinical studies on the palliative treatment of prostatic adenoma with extract of *Urtica* root. *Phyther Res*. 1991;5(6):267–269. https://doi.org/10.1002/ptr.2650050608.
94. Romics I. Observations with Bazoton in the management of prostatic hyperplasia. *Int Urol Nephrol*. 1987;19(3):293–297.
95. Sökeland J. Combined *Sabal* and *Urtica* extract compared with finasteride in men with benign prostatic hyperplasia: analysis of prostate volume and therapeutic outcome. *BJU Int*. 2000;86(4):439–442.
96. Safarinejad MR. *Urtica dioica* for treatment of benign prostatic hyperplasia: a prospective, randomized, double-blind, placebo-controlled, crossover study. *J Herb Pharmacother*. 2005;5(4):1–11.
97. Wong SYS, Lau WWY, Leung PC, Leung JCS, Woo J. The association between isoflavone and lower urinary tract symptoms in elderly men. *Br J Nutr*. 2007;98(06):1237–1242. https://doi.org/10.1017/S0007114507787433.

FURTHER READING

Bent S, Kane C, Shinohara K, et al. Saw palmetto for benign prostatic hyperplasia. *N Engl J Med*. 2006;354(6):557–566.
Gerber GS. Phytotherapy for benign prostatic hyperplasia. *Curr Urol Rep*. 2002;3(4):285–291.
Hartmann RW, Mark M, Soldati F. Inhibition of 5-alpha-reductase and aromatase by PHL-0081, a combination of PY102 *(Pygeum africanum)* and UR102 *(Urtica dioica)* extracts. *Phytomedicine*. 1996;3(2):121–128.
Lopatkin N, Sivkov A, Walther C, et al. Long-term efficacy and safety of a combination of *Sabal* and *Urtica* extract for lower urinary tract symptoms: a placebo-controlled, double-blind, multicenter trial. *World J Urol*. 2005;23(2):139–146.
Maar K. Regression of the symptoms of prostatic adenomas: results of 6 months' conservative treatment using ERU capsules. *Fortschr Med*. 1987;105(1):18–20.
Wilt TJ, Ishani A, Stark G, et al. Saw palmetto extracts for treatment of benign prostatic hyperplasia: a systematic review. *JAMA*. 1998;280:1604–1609.
Yalla SV, Sullivan MP, Lecamwasam HS, et al. Correlation of American Urological Association symptom index with obstructive and nonobstructive prostatism. *J Urol*. 1995;153:674–679.

155

Bronchitis and Pneumonia

John Nowicki, ND, and *Michael T. Murray*, ND*

OUTLINE

GENERAL CONSIDERATIONS

The respiratory system is the primary interface for inhaled compounds and the center for gas exchange in the body. Alveoli are tiny, balloon-shaped structures that allow the rapid exchange of oxygen and carbon dioxide between the lungs and pulmonary capillaries (Fig. 155.1). For gas exchange to occur, the molecules must be capable of traversing three structures: the alveolar epithelium, the interstitial space, and the capillary endothelium. If ventilation is reduced because of lung disease, or perfusion is decreased through small-vessel vasculitis, lung function is impaired. In addition, inhalation of foreign particles or infection can cause inflammation and mucus production, which may act as potential barriers to gas exchange if these secretions are found on the respiratory surfaces.

The airway distal to the larynx is normally sterile owing to several protective mechanisms, both mechanical and humoral. The mucus-covered ciliated epithelium that lines the lower respiratory tract propels sputum to the larger bronchi and trachea, evoking the cough reflex. The respiratory secretions contain substances that exert nonspecific antimicrobial actions: alpha$_1$ antitrypsin, lysozyme, and lactoferrin. At the level of the alveoli, potent defense mechanisms are present, including alveolar macrophages, a rich vasculature capable of rapidly delivering lymphocytes and granulocytes, and an efficient lymphatic drainage network.

Bronchitis is inflammation of the mucous membranes that line the bronchi, the airways that carry air to and from the lungs. Pneumonia is inflammation of lung tissue caused by a bacterial, viral, or fungal infection in one or both lungs accompanied by infiltration and inflammation of the alveoli. Both acute bronchitis and pneumonia are characterized by the development of a cough with or without the production of sputum.

Acute bronchitis often occurs during the course of an acute viral illness such as the common cold or influenza. Viruses cause about 90% of cases of acute bronchitis. Chronic bronchitis is one type of chronic obstructive pulmonary disease (COPD) and is characterized by recurrent episodes of bronchitis for at least 3 months over 2 or more consecutive years. In chronic bronchitis, innate immune cells, including macrophages and neutrophils, increase the levels of airway inflammation through the excessive secretion of cytokines and chemokines that recruit and activate other immune cells and release tissue-destructive proteases.[1] Several pulmonary toxicants have been found to be associated with chronic bronchitis, including particulate matter (i.e., air pollution), organic dusts (e.g., grain, hay, animal by-products, microorganisms), silicates, gases (e.g., nitrous oxides, methane, ozone), mycotoxins, pesticides, and metals (e.g., arsenic, cadmium,

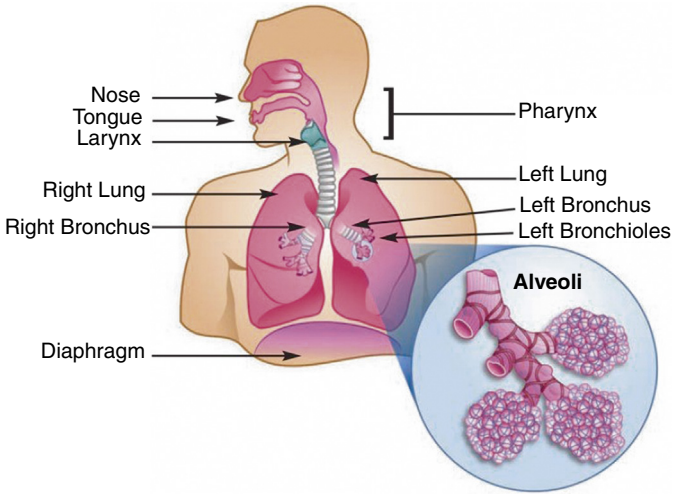


Fig. 155.1 The human respiratory system. (From Quintero D, Guidot DM. Focus on the lung. *Alcohol Res Clin Rev.* 2010;33[3]:219–228. PubMed PMID: 23584063.)

* Previous edition contributor

lead). Cigarette smoking is the major risk factor for COPD and continues to be one of the leading causes of cigarette smoke–related death worldwide.[2]

Although pneumonia may appear in healthy individuals, it is usually seen in those who are immune compromised, particularly drug and alcohol abusers. The growing population of those with chronic lung diseases and other debilitating illnesses and a history of the use of respiratory therapy, immunosuppressive drugs, and other such technologies has contributed to the further increase of nosocomial and opportunistic pneumonias, which have high mortality rates. Acute pneumonia is the seventh-leading cause of death in the United States.[3] It is particularly dangerous in the elderly.

In healthy individuals, pneumonia most often follows an insult to the host defense mechanisms: viral infection (especially influenza), cigarette smoke and other noxious fumes, impairment of consciousness (which depresses the gag reflex, allowing aspiration), neoplasms, and hospitalization (Table 155.1). In immunocompetent, nonelderly adults, cigarette smoking is the strongest independent risk factor for invasive pneumococcal disease.[4]

DIAGNOSTIC SUMMARY

Bronchitis

The diagnosis of acute bronchitis is usually made by ruling out other causes of an acute cough—such as pneumonia, the common cold, acute asthma, or an exacerbation of chronic obstructive pulmonary disease (Fig. 155.2).

TABLE 155.1 Etiologies of Common Pneumonias

Type	Percentage
Viral (influenza)	20 (3)
Mycoplasmal	10–20
Bacterial	12
Bacterial superimposed on viral	6
Chlamydia	10
Unknown cause (Legionnaires' disease, toxic)	38

Data from Branch WT Jr. *Office practice of medicine*. Philadelphia: Saunders; 1982: 57–76.

In patients with the presumed diagnosis of acute bronchitis, viral cultures, serological assays, and sputum analyses should not be routinely performed because the responsible organism is rarely identified in clinical practice.

In patients with acute cough and sputum production suggestive of acute bronchitis, the absence of the following findings reduces the likelihood of pneumonia sufficiently to eliminate the need for a chest radiograph: (1) heart rate greater than 100 beats per minute; (2) respiratory rate greater than 24 breaths per minute; (3) oral body temperature above 38°C; and (4) chest examination findings of focal consolidation, egophony, or fremitus.

Pneumonia

The diagnosis of pneumonia is usually made by physical examination and confirmed by a chest x-ray. Common physical examination findings include:

- Rales (a bubbling or crackling sound)—Rales on one side of the chest and rales heard while the patient is lying down are strongly suggestive of pneumonia.
- Rhonchi (abnormal rumblings indicating the presence of thick fluid)
- Percussion—A dull thud instead of a healthy hollow, drum-like sound indicates certain conditions that suggest pneumonia, including:
  - Consolidation (a condition in which the lung becomes firm and inelastic)
  - Pleural effusion (fluid buildup in the space between the lungs and the surrounding lining)

Examination of the sputum suggestive of infection includes the presence of blood; a positive Gram stain; and thick, opaque, yellow-, green-, or brown-colored sputum. Sputum culture and sensitivity are not always helpful in identifying the cause of pneumonia due to contamination of the sample with throat or mouth bacteria.

A urine test (Binax NOW, Binax Inc., Scarborough, Maine) can detect *Streptococcus pneumonia* or *Legionella pneumophila* antigens within 15 minutes. It may identify up to 77% of pneumonia cases and may rule out the infection in 98% of patients who do not have *S. pneumoniae*. However, the test is not very useful in diagnosing *S. pneumoniae* as a cause of pneumonia in children because the organism is common in this population with or without pneumonia. *L. pneumophila* is the bacterium that causes Legionnaires' disease, a severe form of pneumonia.

A chest x-ray is nearly always taken to confirm a diagnosis of pneumonia, but a positive result is not necessary to make the clinical diagnosis. A positive chest x-ray for pneumonia may reveal lung infiltrates or complications of pneumonia such as pleural effusions.

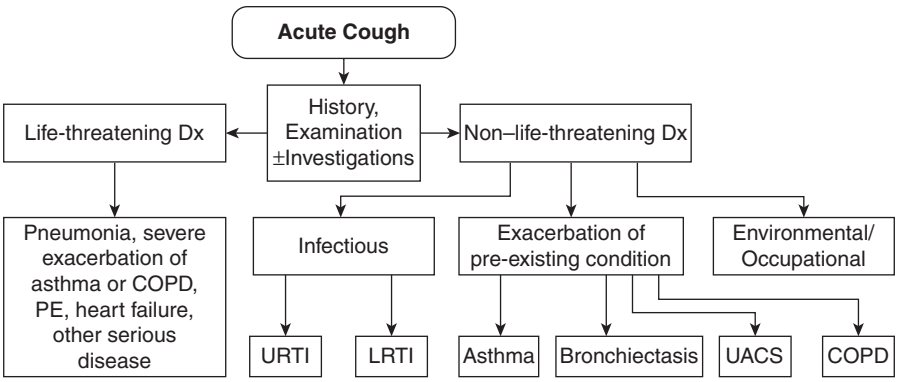


Fig. 155.2 Algorithm for assessment of acute cough in patients 15 or more years of age. *COPD,* Chronic obstructive pulmonary disease; *LRTI,* lower respiratory tract infection; *UACS,* upper airway cough syndrome; *URTI,* upper respiratory tract infection. (From Dicpinigaitis PV, Colice GL, Goolsby MJ, Rogg GI, Spector SL, Winther B. Acute cough: a diagnostic and therapeutic challenge. Cough. 2009;5:11. PubMed PMID: 20015366.)

Special Considerations With Pneumonia

There are more than 100 types of bacteria, viruses, and fungi known to cause bronchitis or pneumonia. The three most common forms of pneumonia are viral, mycoplasmal, and pneumococcal.

Viral pneumonia. Viral pneumonia is most often caused by one of several viruses: adenovirus, influenza, parainfluenza, and respiratory syncytial virus. Viral pneumonia is responsible for about 30% of pneumonia cases and may develop as a complication of viral upper respiratory infections. Immunocompromised individuals are at risk for more serious viral pneumonia. Antibiotics are of no value in treating viral pneumonia unless a secondary bacterial infection develops.

Clinical summary for viral pneumonia.

- People who are at risk for more serious viral pneumonia are often immunocompromised.
- Antibiotics are of no value in viral pneumonia.
- Symptoms of viral pneumonia often begin slowly and may not be severe at first.
- The most common symptoms of viral pneumonia are as follows:
  - Cough (some patients with pneumonia may cough up mucus or even bloody mucus)
  - Fever, which may be mild or high
  - Shaking chills
  - Shortness of breath (may only occur when on merely climbing stairs)

Mycoplasmal pneumonia. *Mycoplasma* is a genus of bacteria that lack cell walls. Mycoplasmal pneumonia is caused by the bacterium *Mycoplasma pneumoniae.* Various studies suggest that *M. pneumoniae* is responsible for 15% to 50% of all cases of pneumonia in adults and even more than those in school-age children. It is often referred to as "walking pneumonia" because symptoms tend to be milder than pneumonia caused by other organisms. *M. pneumoniae* infections are one of the most common etiologies of community-acquired pneumonia (CAP). Antibiotics are usually not necessary but may speed recovery. Effective classes of antibiotics that may be effective against *M. pneumoniae* include macrolides, quinolones, and tetracyclines.

Clinical summary for mycoplasmal pneumonia.

- Most commonly occurs in children or young adults
- Insidious onset over several days
- Nonproductive cough, minimal physical findings, temperature generally less than 102°F
- Headache and malaise are common early symptoms
- White blood cell count is normal or slightly elevated
- X-ray pattern is patchy or inhomogeneous

Pneumococcal pneumonia. Pneumococcal pneumonia (due to *S. pneumoniae*) is the most common bacterial pneumonia and the most common cause of pneumonia requiring hospitalization. Careful clinical judgment is necessary in determining the severity of the disease and the status of the patient's immune system because it is often necessary to administer antibiotics or to refer for hospitalization, especially for elderly or immunocompromised patients.

Unfortunately, most reports show an increase in resistance rates to antibiotic therapy and an increase in the proportion of highly resistant strains.[5–7] In two multinational studies, the worldwide prevalence of penicillin- and macrolide-resistant *S. pneumoniae* ranged from 18% to 22% and from 24% to 31%, respectively.[8,9] Given this information, it is important to consider natural treatments in cases resistant to antibiotics or as an adjunctive treatment to strengthen the immune response and increase the therapeutic effect.

Clinical summary for pneumococcal pneumonia.

- Pneumonia is usually preceded by upper respiratory tract infection.
- There is a sudden onset of shaking, chills, fever, and chest pain.
- Sputum is pinkish or blood-specked at first, then becomes rusty at the height of the infection, and finally becomes yellow and mucopurulent during resolution.
- Gram-positive diplococci are present in the sputum smear.
- A rapid urine test (Binax NOW) for *S. pneumoniae* antigens is positive.
- Initially, chest excursion is diminished on the involved side, breath sounds are suppressed, and fine inspiratory rales are heard.
- Later, classic signs of consolidation appear (bronchial breathing, crepitant rales, dullness).
- Leukocytosis is present.
- Radiograph shows lobar or segmental consolidation.

THERAPEUTIC CONSIDERATIONS

The basic approach in the treatment of bronchitis and pneumonia is to use an expectorant, mucolytic, and immune-supportive nutrients. Although antibiotics are of limited value in acute bronchitis, they certainly play a role in treating pneumonia.

Expectorants

Botanical expectorants have a long history of use in bronchitis and pneumonia. Because impaired cough reflexes have been thought to play a role in recurrent bronchitis and pneumonia,[10] it seems reasonable that these botanicals would be useful in helping relieve this condition and preventing recurrences. Botanical expectorants act to increase the quantity, decrease the viscosity, and promote expulsion of the secretions of the respiratory mucous membranes. Many also have antibacterial and antiviral activity. Some expectorants are also antitussives; however, *Lobelia inflata,* a commonly used expectorant, helps promote the cough reflex.[11] Therefore *Lobelia* may be more effective at clearing the lungs than other expectorants when the cough is productive. Other commonly used expectorants include *Glycyrrhiza glabra* (licorice), *Pelargonium sidoides* (South African geranium), *Hedera helix* (ivy), and wild cherry bark.

Pelargonium sidoides (South African Geranium)

P. sidoides is a medicinal plant in the geranium family that is native to South Africa. *Umckaloaba,* its common name, is a close approximation of the word in the Zulu language that means "severe cough" and is a testimony to its effect in bronchitis. The plant has an intricate grouping of thick dark-red rhizomes and tubers underground that allows it to withstand the frequent grass fires in its habitat. Extracts from the rhizomes and tubers have been shown to exert a number of effects beneficial in upper respiratory tract infections, particularly bronchitis. Most of the research has been conducted using an ethanolic extract known as EPs 7630 (also marketed as Umcka), and it is an approved drug for the treatment of acute bronchitis in Germany. It is produced using 11% ethanol to yield a drug/extract ratio of 1:8 to 10. The primary active ingredients include highly oxygenated coumarins (e.g., umckalin) and polyphenolic compounds.[12]

Research with EPs 7630 shows that it exerts a three-pronged approach in acute bronchitis: (1) it enhances immune function; (2) it has some antimicrobial effects, including antimycobacterial[13] and antiviral activity,[14] and appears to inhibit the attachment of bacteria, viruses, and perhaps other organisms to mucous membranes of the respiratory tract[12]; and (3) it acts as an expectorant.[12] These effects are mediated by the activation of macrophages (with the involvement of cytokine interferon-gamma) and the consequent increase in the production of nitric oxide (NO) (Fig. 155.3). Regarding its antiviral effects, EPs 7630, at concentrations up to 100 mcg/mL, interfered with

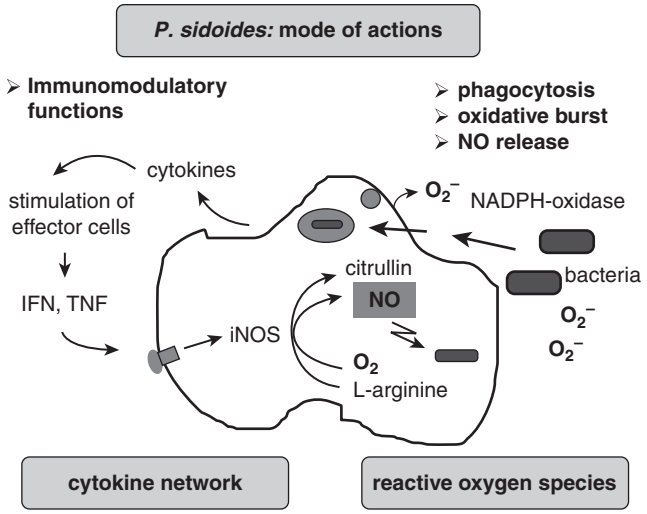


Fig. 155.3 Illustration of cytotoxic defense mechanisms of activated macrophages induced by the root extract of *Pelargonium sidoides*. (From Kolodziej H. Antimicrobial, antiviral, and immunomodulatory activity studies of *Pelargonium sidoides* 9Eps 7630) in the context of health promotion. Pharmaceuticals (Basel). 2011;4[10]:1295–1314. PubMed PMID: 27721327.)

the replication of seasonal influenza A virus strains (H1N1, H3N2), respiratory syncytial virus, human coronavirus, parainfluenza virus, and coxsackievirus but did not affect replication of highly pathogenic avian influenza A virus (H5N1), adenovirus, or rhinovirus.[14]

A 2008 meta-analysis of four randomized clinical trials (RCTs) of EPs 7630 comprising 1647 patients with acute bronchitis supports its safety and efficacy in acute bronchitis.[15] Inclusion criteria required patients to have been diagnosed with acute bronchitis within 48 hours and that they should not have received antibiotic therapy and had no obvious contraindications to therapy. The primary outcome or review of efficacy for most of the trials involved changes in the Bronchitis Severity Score (BSS), which includes the symptoms of coughing, expectoration, chest pain, dyspnea, and wheezing from baseline versus after 7 days of treatment. Included in this review was a double-blind placebo-controlled study in 468 adults with recent onset of acute bronchitis who were given either placebo or EPs 7630 for 1 week. The results showed a significantly greater improvement in symptoms in the treatment group compared with the placebo group. On average, participants who received the real treatment were able to return to work 2 days earlier than those given placebo. In another included study, 742 children with acute bronchitis showed a drop of at least 80% in the severity of component symptoms of the BSS within 2 weeks of therapy, and over 88% of the treating physicians rated the performance as "successful."

In 2018 a review of eight RCTs found that the early administration of EPs 7630 not only reduced the severity of symptoms resulting from infection of the airways but also preponed the start of symptom improvement, with a reduction of illness duration and an earlier resumption of usual activities in the affected patients.[16]

Additional studies provide evidence of the safety and efficacy of EPs 7630 in acute bronchitis as well as further insight on dosage. In one study, 406 patients with acute bronchitis were randomly assigned to one of four parallel treatment groups—10-mg EPs 7630 tablets three times a day (30-mg group), 20-mg EPs 7630 tablets three times a day (60-mg group), 30-mg EPs 7630 tablets three times a day (90-mg group), or placebo three times a day (control group)—for a treatment period of 7 days.[17] The primary endpoint was the change in the total BSS score from baseline to day 7. Between day 0 and day 7, the mean BSS score decreased by 2.7 (control group), 4.3 (30-mg group), 6.1 (60-mg group), and 6.3 (90-mg group), respectively. These results indicated that the 20-mg tablets of EPs 7630 taken three times daily constitute the optimal dose. Similar results were seen in a study of 400 children with acute bronchitis using the same dosage assessment.[18]

In another study, 200 children with acute bronchitis were randomized to receive either EPs 7630 in liquid form or placebo for 7 consecutive days.[19] Dosage was based on age: from ages 1 to 6 years, 10 drops three times daily; from ages 6 to 12 years, 20 drops three times daily; and from ages 12 to 18 years, 30 drops three times daily. From baseline to day 7, the mean BSS score improved significantly more for EPs 7630 compared with placebo (3.4 vs. 1.2 points). On day 7, secondary measures of treatment outcome were significantly better, satisfaction with treatment more pronounced, onset of effect faster, and time of bed rest shorter compared with placebo.

In clinical studies with umcka in more than 2500 adults and children, adverse events occurred on par with a placebo and mainly consisted of mild gastrointestinal complaints and skin rashes. There were no known drug interactions.[12]

Hedera helix (Ivy)

In Europe, herbal preparations containing extracts from the leaves of ivy enjoy great popularity for the relief of cough as well as asthma. In 2007 more than 80% of herbal expectorants prescribed in Germany comprised ivy extract and amounted to nearly 2 million prescriptions nationwide. Ivy leaf contains saponins that show expectorant, mucolytic, spasmolytic, bronchodilatory, and antibacterial effects. The mucolytic and expectorant action of ivy is based on indirect β_2-adrenergic effects, and this action is due to the saponins α-hederin and hederacoside C, the latter of which is metabolized to α-hederin when ingested. The indirect effect is that α-hederin inhibits the intracellular uptake of β_2 receptors and leads to an increased β_2-adrenergic response of the cell.[20]

Ivy is often used as a monopreparation, with very good safety, compliance, and efficacy ratings from postmarketing surveillance studies in both acute and chronic bronchitis.[21,22]

A randomized, placebo-controlled, double-blind trial was conducted to assess the efficacy and safety of ivy leaves cough liquid in the treatment of 181 adult patients with acute cough. [23] Participants were treated with either ivy leaves cough liquid containing EA 575 or with placebo three times a day for 1 week. The evaluation of the visual analogue scale (VAS), BSS, and Verbal Category Descriptive (VCD) score revealed that subjects treated with ivy leaves cough liquid showed statistically significant and clinically relevant reductions in cough severity, severity of symptoms associated with cough, and bronchitis compared with the placebo group. In addition, an early onset of efficacy was observed: significant reductions of cough severity were detected within 48 hours after the first intake. Treatment advantage was observed at all following visits and even 7 days after the end of treatment compared with placebo.

One double-blind study in acute bronchitis used a combination of ivy and thyme (*Thymus vulgaris*).[24] The 361 patients with acute bronchitis and 10 or more coughing fits during the day, onset of bronchial mucus production with impaired ability to cough up at a maximum of 2 days before recruitment, and a BSS score of greater than or equal to 5 points were randomly assigned to an 11-day treatment (5.4 mL three times daily) with either thyme–ivy combination syrup or placebo syrup. The mean reduction in coughing fits on days 7 to 9 relative to baseline was 68.7% under the thyme–ivy combination compared with 47.6% under the placebo. In the thyme–ivy combination group, a 50% reduction in coughing fits from baseline was

reached 2 days earlier compared with the placebo group. The symptoms of acute bronchitis (BSS) improved rapidly in both groups, but the regression of symptoms was faster, and the responder rates compared with placebo were higher at visit 2 (83.0% vs. 53.9%) and visit 3 (96.2% vs. 74.7%) under the treatment of the thyme–ivy combination. Treatment was well tolerated, with no difference in the frequency or severity of side effects between the thyme–ivy combination and placebo groups.

Mucolytics

A mucolytic agent should be used to improve the quality of the mucus secretions so as to promote expectoration. Guaifenesin (also known as glycerol guiacolate) is a derivative of a compound originally isolated from beech wood. Guaifenesin is an approved over-the-counter expectorant and mucolytic. Alternatives include N-acetylcysteine and bromelain.

N-Acetylcysteine

N-acetylcysteine (NAC) has an extensive history of use as a mucolytic in the treatment of acute and chronic lung conditions. It directly splits the sulfur linkages of mucoproteins, thereby reducing the viscosity of bronchial and lung secretions. As a result, it improves bronchial and lung function, reduces cough, and improves oxygen saturation in the blood.

NAC is helpful in all lung and respiratory tract disorders, especially chronic bronchitis and COPD. A detailed analysis of 39 trials concluded that oral NAC reduces the risk of exacerbations (severe worsening) and improves symptoms in patients with chronic bronchitis compared with placebo.

In addition to its effects as a mucolytic, NAC increases the synthesis of glutathione, a major antioxidant for the entire respiratory tract and lungs. The typical dosage for NAC is 200 mg three times daily.

Bromelain

Bromelain is a useful adjunctive therapy for bronchitis and pneumonia owing to its fibrinolytic, anti-inflammatory, and mucolytic actions as well as enhancement of antibiotic absorption.[25] Bromelain's mucolytic activity is responsible for its effectiveness in respiratory tract diseases, including pneumonia, bronchitis, and sinusitis.[26]

IMMUNE AND BARRIER FUNCTION SUPPORT

Vitamin C

In the early part of the 20th century, before the advent of effective antibiotics, many controlled and uncontrolled studies demonstrated the efficacy of large doses of vitamin C in bronchitis and pneumonia but only when they were started on the first or second day of infection.[27] If administered later, vitamin C tended only to lessen the severity of the disease. Researchers also demonstrated that in pneumonia, white blood cells take up large amounts of vitamin C.

The value of vitamin C supplementation in elderly patients with pneumonia was demonstrated in a double-blind study of 57 elderly patients hospitalized for severe acute bronchitis and pneumonia.[28] The patients were given either 200 mg/day of vitamin C or a placebo. Patients were assessed by clinical and laboratory methods (vitamin C levels in the plasma, white blood cells, and platelets; sedimentation rates; and white blood cell counts and differential). Patients receiving vitamin C demonstrated substantially increased vitamin C levels in all tissues, even in the presence of an acute respiratory infection. Using a clinical scoring system based on major symptoms of respiratory infections, results indicated that the patients receiving the vitamin C fared significantly better than those on placebo. The benefit of vitamin C was most obvious in patients with the most severe illness, many of whom had low plasma and white blood cell levels of vitamin C on admission.

Vitamin A

Vitamin A supplementation appears to be of value, especially in children with measles. This may be because of the increased rate of excretion of vitamin A found during severe infections such as pneumonia. One study evaluated 29 patients with pneumonia and sepsis and found that their mean excretion rate of vitamin A was 0.78 mmol/day. Subjects with fever excreted significantly more retinol than did those without fever. A remarkable 34% of the patients excreted more than 1.75 mmol/day of retinol, which is equivalent to 50% of the U.S. recommended daily allowance.[29]

This may be particularly important for children. A randomized, double-blind trial of 189 children with measles (average age 10 months) in South Africa evaluated the efficacy of vitamin A in reducing complications. Providing 400,000 IU (120 mg of retinyl palmitate), one-half on admission and one-half a day later, reduced the death rate by more than 50% and the duration of pneumonia, diarrhea, and hospital stay by 33%.[30]

However, another study did not show any benefit from vitamin A supplementation. The difference may be due to the lower dose (100,000 IU) used in the second study or that vitamin A was not limited to children with pneumonia as a complication of measles, a condition known to also decrease vitamin A levels.[31]

Evidence also indicates there are positive results using vitamin A concomitantly with zinc supplementation. One study of 2482 children aged 6 months to 3 years revealed that those children given initial high doses of vitamin A followed by 4 months of elemental zinc (10 mg/day for infants and 20 mg/day for children older than 1 year) brought about a reduced incidence of pneumonia, which was not seen in the group given only vitamin A.[32]

Vitamin E

Patients with influenza complicated by pneumonia experience a sharp rise in lipid peroxidation (LPO) products, especially those who are seriously ill. Administration of α-tocopherol promotes a significant decrease in the levels of lipid peroxidation products and a more benign clinical course.[33]

Garlic

Allium sativum (garlic) has exhibited a broad spectrum of antibiotic activity against both gram-positive and gram-negative bacteria.[34] In vitro studies have demonstrated garlic to be an effective antibacterial agent against *S. pneumoniae*.[35] Therefore its use should be considered in cases of antibiotic resistance or as an adjunct to antibiotic therapy. Alternatively, berberine-containing plants like *Hydrastis canadensis* (goldenseal) may be helpful.

Bottle Blowing and Salt Pipes

A Swedish study was carried out with 145 adults hospitalized for community-acquired pneumonia.[36] These patients were divided into three groups. Group A was given early mobilization with no breathing-associated exercises, group B was instructed to sit up and take 20 deep breaths 10 times daily, and group C was instructed to sit up and blow bubbles in a bottle containing 10 mL water through a plastic tube 20 times on 10 occasions daily. In this study, length of hospitalization was significantly modified in groups B and C: group A patients were hospitalized for a mean of 5.3 days, group B for 4.6 days, and group C for only 3.9 days. The number of days with fever was lowest in the bottle-blowing group. It should be noted that early mobilization itself is known to significantly decrease hospital stays in pneumonia patients.[37] Despite the positive clinical results, C-reactive protein levels, peak expiratory flow, and vital capacity were not significantly affected.

Although it is not completely understood why the patients who performed bottle blowing had shorter hospital stays, it seems that the changes in respiratory pressure associated with this exercise may be involved in providing an environment for more efficient bacterial clearance. Another study also found decreased impairment of pulmonary function and an increase in total lung capacity in patients who had undergone coronary artery bypass.[38] This modality or another similar activity, like playing a wind instrument, may well prove useful as a means of decreasing the frequency and duration of respiratory events in patients who are vulnerable to respiratory infections like pneumonia.

An alternative to bubble blowing is the use of a salt pipe. These pipes are inhaler-type devices containing tiny salt particles said to ease breathing. The practice originated in central Europe, where individuals with respiratory complaints would spend time in salt caves or mines to help relieve their breathing problems.

THERAPEUTIC APPROACH

As previously mentioned, the basic approach is to use an expectorant, mucolytic, and immune-supportive nutrients to help resolve the condition. Some general physical modalities and measures that may be helpful include the following:

- Diathermy to chest and back: 30 min/day
- Mustard poultice: once/day
- Lymphatic massage: three times a day
- Postural drainage: three times a day
- Bottle-blowing therapy: blowing bubbles in a bottle containing 10 mL water through a plastic tube 20 times on 10 occasions a day
- Getting plenty of rest
- Drinking enough liquids
- Using a humidifier

Expectorants

Choose one or more of the following:

Lobelia inflata

- Dried herb: 50 to 200 mg three times a day
- Tincture: 10 to 20 drops three times a day
- Fluid extract: 8 to 10 drops three times a day

Glycyrrhiza glabra

- Powdered root: 1 to 2 g three times a day
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp) three times a day
- Solid (dry-powdered) extract (4:1): 250 to 500 mg three times a day

Pelargonium sidoides

Dosage recommendations for EPs 7630 or equivalent preparation are as follows:

Adults: 1.5 mL three times a day or 20-mg tablets three times a day for up to 14 days

Children: age 7 to 12 years, 20 drops (1 mL) three times a day; age 6 years or less, 10 drops (0.5 mL) three times a day

Hedera helix

Ivy leaf is available primarily in tincture and fluid extract forms and the dry powdered extract in capsules and tablets. Based on clinical studies, the daily dosages are to deliver the following equivalent to dried herbal substance: 1 to 5 years: 150 mg; 6 to 12 years: 210 mg; above 12 years: 420 mg. Therefore the typical dosage for adults and children over 12 years of age for a 4:1 dried powdered extract is 100 mg daily.

Mucolytics

Choose one or more of the following.

Guaifenesin

Adults and children 12 years of age and older: 200 to 400 mg every 4 hours. It is inadvisable to take more than 2400 mg in a 24-hour period.

The dosage for children age 6 to 11 years is 100 to 200 mg every 4 hours and no more than 1200 mg in a 24-hour period. For children age 2 to 5 years, 50 to 100 mg every 4 hours and no more than 600 mg in 24 hours. Guaifenesin is not recommended for children under 2 years of age.

N-Acetylcysteine

- 200 mg three times a day

Bromelain (1200–1800 Milk Clotting Units [MCU])

- 500 to 750 mg three times a day between meals

Supplements

- Vitamin A: 50,000 IU/day for 1 week or beta-carotene 200,000 IU/day (Note: Vitamin A should not be used in menstruating women owing to possible teratogenic effects.)
- Vitamin C: 500 mg every 2 hours
- Vitamin E: 200 IU/day
- Choose one of the following:
  Bioflavonoids (mixed citrus): 1000 mg/day
  Grape seed *(Vitis vinifera)* extract (95% procyanidolic oligomers) 150 to 300 mg/day
  Pine bark extract *(Pinus pinaster)* 150 to 300 mg/day
- Zinc: 30 mg/day

Additional Recommendations for Pneumococcal Pneumonia

Choose one or both of the following.

Garlic

A commercial garlic product should provide a daily dose equal to at least 4000 mg of fresh garlic, which translates to at least 10 mg alliin or a total allicin potential of 4000 mcg (see Chapter 50, *Allium cepa*).

Hydrastis canadensis

Given berberine's broad-spectrum antimicrobial activity and immune-enhancing effects, berberine-containing plants are an important consideration. The dosage should be based on berberine content. Because of the wide range of quality in goldenseal preparations, standardized extracts are recommended. The following dosages are intended to be given three times daily:

- Dried root or as an infusion (tea): 2 to 4 g
- Tincture (1:5): 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content): 250 to 500 mg

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Tetley TD. Inflammatory cells and chronic obstructive pulmonary disease. *Curr Drug Targets Inflamm Allergy*. 2005;4(6):607–618. PubMed PMID: 17305517.
2. Centers for Disease Control and Prevention (CDC). Smoking-attributable mortality, years of potential life lost, and productivity losses – United States, 2000-2004. *MMWR. Morbidity Mortality Weekly Report*. 2008;57(45):1226–1228. PubMed PMID: 19008791.
3. Centers for Disease Control and Prevention. *National Vital Statistics Reports*. 2003;52(9):9.
4. Nuorti JC, Butler JC, Farley MM, et al. Cigarette smoking and invasive pneumococcal disease. *N Engl J Med*. 2000;342:681–689.
5. Bauer T, Ewig S, Marcos MA, et al. *Streptococcus pneumoniae* in community-acquired pneumonia: how important is drug resistance? *Med Clin North Am*. 2001;85:1367–1379.
6. Cunha BA. Clinical relevance of penicillin-resistant *Streptococcus pneumoniae*. *Semin Respir Infect*. 2002;17:204–214.
7. Garau J. Treatment of drug-resistant pneumococcal pneumonia. *Lancet Infect Dis*. 2002;2:404–415.
8. Felmingham D. Evolving resistance patterns in community-acquired respiratory tract pathogens: first results from the PROTEKT global surveillance study. Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin. *J Infect*. 2002;44(suppl A):3–10.
9. Jacobs MR, Felmingham D, Appelbaum PC, et al. The Alexander Project 1998–2000: susceptibility of pathogens isolated from community-acquired lower respiratory tract infection to commonly used antimicrobial agents. *J Antimicrob Chemother*. 2003;52:229–246.
10. Niimi A, Matsumoto H, Ueda T, et al. Impaired cough reflex in patients with recurrent pneumonia. *Thorax*. 2003;58:152–153.
11. Cambar PJ, Shore SR, Aviado DM. Bronchopulmonary and gastrointestinal effects of lobeline. *Arch Int Pharmacodyn*. 1969;177:1–27.
12. Brendler T, van Wyk BE. A historical, scientific and commercial perspective on the medicinal use of Pelargonium sidoides (Geraniaceae). *J Ethnopharmacol*. 2008;119(3):420–433.
13. Kim CE, Griffiths WJ, Taylor PW. Components derived from *Pelargonium* stimulate macrophage killing of Mycobacterium species. *J Appl Microbiol*. 2009;106(4):1184–1193.
14. Michaelis M, Doerr HW, Cinatl Jr J. Investigation of the influence of EPs([R]) 7630, a herbal drug preparation from *Pelargonium sidoides,* on replication of a broad panel of respiratory viruses. *Phytomedicine*. 2011;18(5):384–386.
15. Agbabiaka TB, Guo R, Ernst E. *Pelargonium sidoides* for acute bronchitis: a systematic review and meta-analysis. *Phytomedicine*. 2008;15(5):378–385.
16. Careddu D, Pettenazzo A. *Pelargonium sidoides* extract EPs 7630: a review of its clinical efficacy and safety for treating acute respiratory tract infections in children. *Int J General Med*. 2018;11:91–98. PubMed PMID: 29563828.
17. Matthys H, Lizogub VG, Malek FA, et al. Efficacy and tolerability of EPs 7630 tablets in patients with acute bronchitis: a randomised, double-blind, placebo-controlled dose-finding study with a herbal drug preparation from *Pelargonium sidoides*. *Curr Med Res Opin*. 2010;26(6):1413–1422.
18. Kamin W, Maydannik V, Malek FA, et al. Efficacy and tolerability of EPs 7630 in children and adolescents with acute bronchitis—a randomized, double-blind, placebo-controlled multicenter trial with a herbal drug preparation from *Pelargonium sidoides* roots. *Int J Clin Pharmacol Ther*. 2010;48(3):184–191.
19. Kamin W, Maydannik VG, Malek FA, et al. Efficacy and tolerability of EPs 7630 in patients (aged 6-18 years old) with acute bronchitis. *Acta Paediatr*. 2010;99(4):537–543.
20. Sieben A, Prenner L, Sorkalla T, et al. Alpha-hederin, but not hederacoside C and hederagenin from *Hedera helix,* affects the binding behavior, dynamics, and regulation of beta 2-adrenergic receptors. *Biochemistry*. 2009;48(15):3477–3482.
21. Stauss-Grabo M, Atiye S, Warnke A, et al. Observational study on the tolerability and safety of film-coated tablets containing ivy extract (Prospan Cough Tablets) in the treatment of colds accompanied by coughing. *Phytomedicine*. 2011;18(6):433–436.
22. Hecker M, Runkel F, Voelp A. Treatment of chronic bronchitis with ivy leaf special extract–multicenter post-marketing surveillance study in 1,350 patients. *Forsch Komplementarmed Klass Naturheilkd*. 2002;9(2):77–84.
23. Schaefer A, Kehr MS, Giannetti BM, Bulitta M, Staiger C. A randomized, controlled, double-blind, multi-center trial to evaluate the efficacy and safety of a liquid containing ivy leaves dry extract (EA 575) vs. placebo in the treatment of adults with acute cough. *Pharmazie*. 2016;71(9):504–509. PubMed PMID: 29441845.
24. Kemmerich B, Eberhardt R, Stammer H. Efficacy and tolerability of a fluid extract combination of thyme herb and ivy leaves and matched placebo in adults suffering from acute bronchitis with productive cough: a prospective, double-blind, placebo-controlled clinical trial. *Arzneimittelforschung*. 2006;56(9):652–660.
25. Kelly GS. Bromelain: a literature review and discussion of its therapeutic applications. *Altern Med Rev*. 1996;1:243–257.
26. Rimoldi R, Ginesu F, Giura R. The use of bromelain in pneumological therapy. *Drugs Exp Clin Res*. 1978;4:55–66.
27. Klenner FR. Virus pneumonia and its treatment with vitamin C. *South Med Surg*. 1948;110(2):36–38.
28. Hunt C, Chakravorty NK, Annan G, et al. The clinical effects of vitamin C supplementation in elderly hospitalized patients with acute respiratory infections. *Int J Vitam Nutr Res*. 1994;64:212–219.
29. Stephensen CB, Alvarez JO, Kohatsec J. Vitamin A is excreted in the urine during acute infection. *Am J Clin Nutr*. 1994;60:88–92.
30. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. *N Engl J Med*. 1990;323:160–164.
31. Kjolhede CL, Chew FJ, Gadomski AM, et al. Clinical trial of vitamin A as adjuvant treatment for lower respiratory tract infections. *J Pediatr*. 1995;126:807–812.
32. Bhandari N, Bahl R, Taneja S, et al. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomised controlled trial in an urban slum. *BMJ*. 2002;324:1358.
33. Nagibina MV, Neifakh EA, Krylov VF, et al. The treatment of pneumonias in influenza using antioxidants. *Ter Arkh*. 1996;68:33–35.
34. Sivam GP. Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr*. 2001;131:1106S–1108S.
35. Dikasso D, Lemma H, Urga K, et al. Investigation on the antibacterial properties of garlic *(Allium sativum)* on pneumonia causing bacteria. *Ethiop Med J*. 2002;40:241–249.
36. Bjorkqvist M, Wiberg B, Bodin L, et al. Bottle-blowing in hospital-treated patients with community-acquired pneumonia. *Scand J Infect Dis*. 1997;29:77–82.
37. Mundy LM, Leet TL, Darst K, et al. Early mobilization of patients hospitalized with community-acquired pneumonia. *Chest*. 2003;124:883–889.
38. Westerdahl E, Lindmark B, Almgren SO, et al. Chest physiotherapy after coronary artery bypass graft surgery—a comparison of three different deep breathing techniques. *J Rehabil Med*. 2001;33:79–84.

156

Carpal Tunnel Syndrome

Terry M. Elder, DC, and *Robert M. Martinez*, DC, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Insidious onset of numbness and tingling, primarily in the first three fingers, often bilaterally
- Pain over the palmar surface of the wrist, which may extend proximally up to the forearm or shoulder
- Pain often worse at night that may wake the patient
- Positive Tinel's and Phalen's signs
- Neurodiagnostic testing showing altered nerve conduction of the median nerve at the carpal tunnel

GENERAL CONSIDERATIONS

Carpal tunnel syndrome (CTS) involves compression of the median nerve in the carpal tunnel. Sensory impairment occurs in the first three digits and the lateral one half of the fourth digit of the hand. Pain may be felt in the palm, anterior wrist, forearm, and proximally to the shoulder. Loss of fine motor skills and strength in abduction and opposition of the thumb may develop. Atrophy of the opponens pollicis muscle may occur.[1]

Anatomy of the Carpal Tunnel

The floor and sides of the carpal tunnel are formed by the carpal bones; the roof is formed by the flexor retinaculum. The median nerve lies between the flexor retinaculum and the finger flexors and pollicus longus tendons and their tenosynovium (Fig. 156.1). The flexor retinaculum acts to prevent bowstringing of the flexor tendons. When the flexors contract, especially with the wrist in flexion, the tendons in the carpal tunnel compress the median nerve between the tenosynovium and the flexor retinaculum.

Etiology

Most cases of CTS are considered idiopathic. It should be noted that the term *idiopathic* in the medical literature does not include any mention of mechanical dysfunction or its subsequent neurological consequences. Nonspecific flexor tenosynovitis has been reported as the most common cause of CTS. The most common histological findings in CTS are noninflammatory synovial fibrosis[2] and vascular proliferation. Conceptually, it helps to think of factors that cause the canal to get smaller or factors that cause the contents to swell.

Causes of CTS include the following[3]:

- Increased volume of canal contents/edema (nonspecific flexor tenosynovitis, obesity, pregnancy, oral contraceptives)
- Trauma (fracture, repetitive wrist flexion)
- Aberrant anatomy (cysts, lipomas, arthritic spurs)
- Infections (septic arthritis, Lyme disease)
- Metabolic conditions (diabetes, hypothyroidism)
- Inflammatory conditions (rheumatoid arthritis, gout, connective tissue disease)

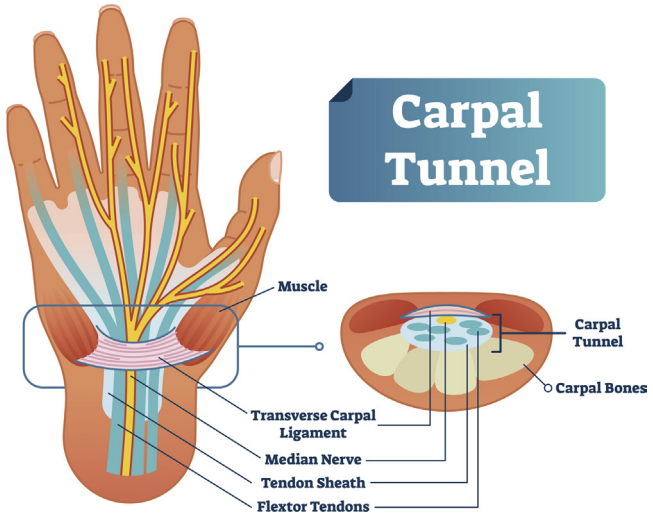


Fig. 156.1 The carpal tunnel is a passageway through which nine flexor tendons and the median nerve pass to supply function, feeling, and movement to the thumb, index, middle, and half of the ring finger. (VectorMine/iStock.com.)

* Previous edition contributor

Risk Factors and Frequency of Occurrence

CTS most often occurs after age 30, and women are affected three times as often as men.[4] Repetitive strain injury (RSI) from light-duty work as a cause of CTS is not well supported by the current literature.[5] However, a study of 501 participants, 156 of whom were diagnosed with CTS, showed the following risk factors: repetitive activities with a flexed or extended wrist, hysterectomy without oophorectomy, and menopausal women who had their last menstrual period 6 to 12 months earlier.[6] Others report an increased incidence of CTS due to pregnancy, hypothyroidism, diabetes, and recent menopause.[7]

Although uncommon, acute carpal tunnel syndrome has been reported after anticoagulation therapy.[8] Complications appear to be secondary to intraneural hemorrhage of the median nerve. In addition, features of CTS, predominantly sensory peripheral polyneuropathy, were found in 18% of individuals with lifetime low-level exposures to drifts containing organophosphate pesticides.[9]

DIAGNOSTIC CONSIDERATIONS

There is no single reference standard for diagnosis of the syndrome; a combination of symptoms, signs, and tests should be used to characterize the disorder.[1]

History

CTS typically has an insidious onset of intermittent tingling or numbness, often bilaterally, of the first three fingers and the lateral one half of the fourth digit. Symptoms are frequently worse at night, often waking the patient. As the condition progresses, loss of fine motor skills and loss of grip strength, with "things slipping from my fingers," are commonly reported. Relief is gained from shaking the affected hand or hands. Katz and Stirrat[10] developed a self-administered hand diagram test that has an 80% sensitivity and a 90% specificity for CTS. Loss of finger dexterity and atrophy are late complaints.

Physical Examination

Examination may show decreased sensation over the distribution of the median nerve to sharp, light touch and vibration (128-Hz tuning fork). Surprisingly, many patients are not able to localize the symptoms to the median distribution and feel that the whole hand is involved, which may be due to autonomic nerve involvement. Tingling in the distribution of the median nerve produced by tapping on the wrist crease (positive Tinel's sign), tingling in the distribution of the median nerve produced by holding the wrist in forced flexion for 60 to 90 seconds (Phalen's test), or tingling produced by forcibly extending the wrist and applying pressure over the median nerve (reverse Phalen's test) are signs of CTS. Early on, the perceived loss of strength may be due to loss of sensory feedback, not loss of strength. Manual muscle tests may elicit weakness of the LOAF muscles—the muscles innervated by the median nerve in the hand: **l**umbricals first and second, **o**pponens pollicus, **a**bductor pollicus, and **f**lexor pollicis brevis. As CTS advances, atrophy of the thenar eminence and weakness of the thumb abductor muscle may develop.

Other Diagnostic Tests

The carpal compression test, in which the examiner exerts direct compression with the thumbs over the patient's carpal tunnel for 30 seconds and reproduces the symptoms, was reported as having 89% sensitivity and 96% specificity when performed with the Durkan gauge.[11] Other studies have not reproduced these findings but have reported that "Tinel's, Phalen's, Reverse Phalen's, and carpal tunnel compression tests are more sensitive, as well as being specific tests for the diagnosis of tenosynovitis of the flexor muscles of the hand, rather than being specific tests for CTS."[12,13] Weakness of the thumb abductor muscle is a strong indication to order neurophysiological testing (nerve conduction studies [NCSs] and/or electromyography). In CTS, NCSs may show motor and sensory latencies across the wrist.

Magnetic resonance imaging (MRI) and high-definition ultrasound imaging can measure the dimensions of the carpal tunnel and size of the median nerve, but these tests are expensive and not needed in uncomplicated CTS.

DIFFERENTIAL DIAGNOSIS

When the clinical picture is not clear, other sources of paresthesia should be considered, such as compression of the brachial plexus in the thoracic outlet, impingement of the median nerve under the pronator teres muscle, and entrapment of either the radial or ulnar nerves. The initial presentation of complex regional pain syndrome (reflex sympathetic dystrophy) may appear to be CTS.

THERAPEUTIC CONSIDERATIONS

Many patients report spontaneous recovery from CTS. CTS from pregnancy most often is self-resolving. The waxing and waning symptoms of CTS can give a false sense of success from conservative treatment. The cost of conservative care in persistent CTS may be greater than a carpal tunnel release when performed as an office procedure.[14] In cases of progressive neurological deficit and pain, an open release procedure may be the best choice of treatment.[15] In the Netherlands, only open procedures are compensated by the state plan because of their high rate of success and low rate of complications. Unless the surgeon has good skills, the more expensive endoscopic procedures have poorer outcomes.[16]

Physical Medicine

Splinting

Initially, the most recommended treatment is 4 weeks in a neutral wrist splint worn full time, which provides better results than night-only splinting, but night-only splinting has higher compliance. Splinting is most effective when it is started within 3 months of the onset of CTS. Specialized splints have not been proven more effective than a good-quality, well-fitted, off-the-shelf splint.[17,18] Splinting should not be done after carpal tunnel release.[19]

Therapeutic Ultrasound

Ultrasound (US) therapy for 2 weeks is generally not beneficial, but one trial showed significant symptom improvement from US (1 MHz, 1.0 W/cm^2, pulse 1:4, 15 minutes per session) after 20 sessions over 7 weeks that was maintained at 6 months.[20] The cost of 15 to 20 sessions of US treatment may be prohibitive.

Yoga and Stretching

One small preliminary study described 10 yoga poses held for 30 seconds, each followed by 10 to 15 minutes of relaxation, that resulted in significantly reduced pain and increased grip strength persisting for more than 8 weeks.[21]

Tests recording carpal tunnel pressure showed a significant rise in pressure with wrist flexion, wrist extension, making a fist, holding objects, and isolated isometric flexion of a finger against resistance. But after 1 minute of specific stretching–loading exercises, intratunnel pressures dropped to normal levels and remained normal for more than 15 minutes.[22]

Manual Manipulation

Studies of manipulation show benefits from soft tissue and joint mobilization of the forearm, wrist, and hand but not from spinal manipulation alone.

A case report of manipulative treatment to the cervical spine, right elbow, and right wrist performed three times per week for 4 weeks reported improved grip strength; both Tinel's and Phalen's tests became negative, and NCSs improved.[23]

In 1993 Sucher[24] described the treatment of four patients with myofascial release and self-stretching techniques performed three to five times daily. Posttreatment comparison MRI demonstrated increased dimensions in the carpal tunnel, and NCSs showed a reduction in distal latencies or an increase in motor response amplitudes. Nerve and tendon gliding exercise produced no changes in Phalen's test, Tinel's test, or NCSs but reduced the number of reported surgeries.[25]

Low-Level Laser Therapy

One small (N = 11) randomized, double-blind, placebo-controlled crossover trial studied low-level laser therapy (LLLT) combined with microcurrent transcutaneous electrical nerve stimulation (TENS).[26] Eleven patients with mild to moderate symptoms of CTS who had failed to improve with standard medical or surgical treatment were treated with real or sham local applications of LLLT and microcurrent for 3 to 4 weeks. After treatment, there was a significant improvement in scores on the McGill Pain Questionnaire, sensory and motor latency scores with electromyography (EMG), and Tinel's and Phalen's tests; these improvements were not found after sham treatment.

Hydrotherapy

An anecdotal naturopathic treatment to relieve CTS pain is immersing the wrist and hand in hot water for 3 minutes, then immersing in cold water for 30 seconds, repeated three to five times once or twice a day. It is important to ensure that the hot-water immersion is deeper than the cold.

Acupuncture

A randomized controlled study comparing prednisolone (20 mg for 2 weeks, then 10 mg for 2 weeks) to eight sessions of acupuncture over 4 weeks showed acupuncture to be effective in symptom control, but acupuncture decreased distal motor latency, and prednisolone did not.[27]

One acupuncture study with the puncture of PC-7 and PC-6 on the affected side demonstrated a positive response in 35 of 36 patients (14 of whom were previously treated unsuccessfully with surgery).[28]

Ergonomics

There is insufficient epidemiological evidence that computer work causes CTS.[4] Other studies have not demonstrated that work practices (other than high-force work, like that done by meatpackers) have a direct influence on CTS.[29]

Nutrition

Vitamin B Supplementation

The increased incidence of CTS since its initial description by Phalen in 1950 parallels the increased presence of pyridoxine antimetabolites (hydrazine dyes, isoniazid, hydralazine, dopamine, penicillamine, and oral contraceptives) in the environment as well as the intake of excessive protein.

Ellis and coworkers[30–32] demonstrated the efficacy of vitamin B_6 supplementation for CTS with 50 mg initially and increased to 200 to 300 mg. An even greater effect was seen when vitamins B_6 and B_2 were given together, possibly as a result of riboflavin-dependent enzymes, which convert pyridoxine to pyridoxal 5′-phosphate. Two randomized controlled trials suggest that vitamin B_6 is no better than placebo; however, given its safety profile, vitamin B_6 can be considered in the treatment of CTS.[33,34] Failure of vitamin B_6 supplementation to relieve CTS could be due to a lack of riboflavin or a genetic defect that does not allow sufficient levels of vitamin B_6 to be converted to the active P5P form.

Medications

Steroid injection proximal to the carpal tunnel provides relief for up to 8 weeks, but 50% of those receiving the steroid injection require surgery within a year. Oral prednisolone (20 mg for 2 weeks, then 10 mg for 2 weeks) provides short-term relief over 8 weeks. Nonsteroidal anti-inflammatory drugs and diuretics were found to provide no reduction in symptoms and should *not* be used because of their potential side effects.[35]

No studies were found evaluating the use of the "natural" anti-inflammatory medications (bromelain, curcumin, *Boswellia,* various oils). Remedies that address noninflammatory synovial fibrosis[2] and vascular proliferation would theoretically be beneficial.

Surgery

Surgery for CTS should not be considered before 6 months of more conservative treatment, but "In general, the management is surgery for persistent (not resolving after 1 year) or deteriorating (worsening clinically plus or minus deterioration on nerve conduction studies) CTS." Surgery should not be delayed beyond 3 years. Open carpal tunnel release surgery is one of the most commonly performed outpatient surgeries and is less expensive than endoscopic procedures. The abstract in the review of the Cochrane Library[36] reports no difference in long-term results between the procedures, but pain is reduced during the first 2 weeks after endoscopic surgery compared with open procedures. Surgery with early mobilization,[19] surgery with oral homeopathic *Arnica* and topical *Arnica* ointment,[37] and surgery with controlled cold therapy[38] all showed benefits over surgery alone.

THERAPEUTIC APPROACH FOR MILD/MODERATE IDIOPATHIC CARPAL TUNNEL SYNDROME

Approximately 50% of patients will improve spontaneously. Identify and reduce causes of strain and vibration, and prevent repeated trauma.

Physical Medicine

Regular wrist stretching, yoga, and exercises. The key to these exercises is performing them several times daily and "breaking up" activities that are strenuous and repetitive. Nerve/tendon gliding may be helpful.

Splint. For moderate persistent CTS, full-time splinting in neutral is recommended. Splinting is less effective if it is not started within 3 months of onset or if the splints are only worn at night.

Acupuncture. PC-7 and PC-6 on the affected side should be needled. Laser acupuncture can be considered.

Manipulation/deep-tissue mobilization. Manipulation for CTS can be very effective if a comprehensive manipulation protocol is utilized. In the short term, manipulation of the distal radial ulnar expansion/gapping and the "opponens roll maneuver" (Kaltenborn) to increase the length of the transverse carpal ligament are very effective. Some musculoskeletal etiological factors must be considered to understand the effectiveness of manipulation procedures.

The distal radioulnar joint is functionally a homologue of the ankle mortice joint. During the late midstance phase of gait with full dorsiflexion, the tibia and the fibula have to separate due to the width of the talus. A joint restriction that prevents this from happening causes an adaptation that leads to looser (hypermobile) tarsal joints and a pronated foot. The same process occurs in the wrist. During dorsiflexion of the wrist, the radius and ulna must also separate to accommodate the shape of the carpals.

A joint restriction that does not allow for this separation or gapping also leads to hypermobile carpal joints that are the support structure for the carpal tunnel. The mechanical support for the carpal tunnel fails, leading to the collapse of the roof of the carpal tunnel, which is the transverse carpal ligament. The transverse carpal ligament mechanically rests on the median nerve, which leads to the symptoms of CTS. It should also be noted that if atrophy is prevalent during CTS examination, there is a high probability that the median nerve axon distal to the tunnel has undergone Wallerian degeneration (the axon has died). Any treatment modality at this point requires that the axon must regrow to reinnervate the distal structures. This regrowth process could take a minimum of 2 to 3 months, and the first sensation that would return would be pain. This, I believe, is why many research studies fail to show significant effectiveness in the treatment of CTS.

In addition to the previously mentioned treatment protocols, a comprehensive treatment must also include the entire upper extremity, the upper thoracic spine, and the lower cervical spine. In her editorial, a second commentary on "Multifocal Neuropathy: Expanding the Scope of Double Crush Syndrome," Catherine Curtin, MD, uses a new term called *multifocal neuropathy*. The double-crush theory as theorized by Upton and McComas in *Lancet* in 1973 stated that a peripheral nerve may only develop symptomology if there is more than one compression site along the length of the entire nerve. Curtin stated that treatment should include metabolic, postural, and secondary entrapments for effective treatment to occur. One omission that has led to much controversy and misunderstanding concerning the double-crush theory or the new term *multifocal neuropathy* is mechanical traction of the nerve. All studies and articles have focused on entrapment or compression and fail to recognize the effect of mechanical traction on nervous tissue, which could cause continuous firing of pain fibers. As noted in Curtin's editorial, postural changes should be a part of any CTS treatment.

Manipulation and postural exercise would be the best treatment to effect this change. A review of the *National University of Health Sciences (NUHS) Postgraduate Neuroanatomy Study Guide* (National University of Health Sciences, Lombard, IL), reveals that the C5, C6, and C7 nerve roots; first rib; scalenes; pectoralis minor; three different sites at the elbow; and the carpal tunnel (10 sites) can all be affected by compression, entrapment, or mechanical nerve traction. Therefore effective carpal treatment should include all the tissues and joints along the path of the median nerve.

Contrast hydrotherapy. Immerse for 3 minutes in hot water followed by a 30-second immersion in cold water three to five times daily. Heat alone is contraindicated.

Diet

All sources of hydrazines should be avoided.

All foods containing yellow dyes should be avoided.

Daily protein intake should be limited to a maximum of 0.75 g/kg body weight.

Supplements

- Pyridoxine: 50 to 200 mg/day: administer in divided dosages of 25 to 50 mg two to four times a day
- If no response to pyridoxine, pyridoxal 5′-phosphate: 10 mg two to four times a day
- Riboflavin: 5 to 10 mg/day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Goodyear-Smith F, Arroll B. What can family physicians offer patients with carpal tunnel syndrome other than surgery? A systematic review of nonsurgical management. *Ann Fam Med*. 2004;2:267–273.
2. Mackinnon SE, Novak CB. Repetitive strain in the workplace. *J Hand Surg [Am]*. 1997;22:2–18.
3. Viera AJ. Management of carpal tunnel syndrome. *Am Fam Physician*. 2003;68:265–272. 279–280.
4. Stevens JC, Sun S, Beard CM, et al. Carpal tunnel syndrome in Rochester, Minnesota, 1961 to 1980. *Neurology*. 1988;38:134–138.
5. Thomsen JF, Gerr F, Atroshi I. Carpal tunnel syndrome and the use of computer mouse and keyboard: a systematic review. *BMC Musculoskelet Disord*. 2008;9:134. https://doi.org/10.1186/1471-2474-9-134. Published online 2008 October 6. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2569035/. Accessed 20 Sept 2019.
6. de Krom MC, Kester AD, Knipschild PG, et al. Risk factors for carpal tunnel syndrome. *Am J Epidemiol*. 1990;132:1102–1110.
7. Ashworth N. Carpal tunnel syndrome. *Am Fam Physician*. 2007;75(3):381–383.
8. Dussa CU, Gul A. An unusual cause of carpal tunnel syndrome. *Acta Orthopaedica Belgica*. 2005;71(2):236–238. PubMed PMID: 16152862.
9. Ophir A, Karakis I, Richter ED, et al. An uncommon pattern of polyneuropathy induced by lifetime exposures to drift containing organophosphate pesticides. *Neurotoxicology*. 2014;45:338–346. PubMed PMID: 25128617.
10. Katz JN, Stirrat CR. A self-administered hand diagram for the diagnosis of carpal tunnel syndrome. *J Hand Surg [Am]*. 1990;15:360–363.
11. Durkan JA. The carpal compression test: an instrument device for diagnosing carpal tunnel syndrome. *Orthp Rev*. 1994;23(6):522–525.
12. El Miedany Y, Ashour S, Youssef S, et al. Clinical diagnosis of carpal tunnel syndrome: old tests—new concepts. *Joint Bone Spine*. 2008;75(4):451–457. Epub 2008 May 2.
13. Wainner RS, Boninger ML, et al. Durkan gauge and carpal compression test: accuracy and diagnostic test properties. *J Orthop Sports Phys Ther*. 2000;30(11):676–682.
14. Korthals-de Bos IB, Gerritsen AA, van Tulder MW, et al. Surgery is more cost-effective than splinting for carpal tunnel syndrome in the Netherlands: results of an economic evaluation alongside a randomized controlled trial. *BMC Musculoskelet Disord*. 2006;7:86. https://doi.org/10.1186/1471-2474-7-86. Published online 2006 November 16. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1660539/?tool=pubmed. Accessed 20 Sept 2019.
15. Carlson H, Colbert A, Frydl J, et al. Current options for nonsurgical management of carpal tunnel syndrome. *Int J Clin Rheumtol*. 2010;5(1):129–142.
16. Derkash RS, Weaver JK, Berkeley ME, et al. Office carpal tunnel release with wrist block and wrist tourniquet. *Orthopedics*. 1996;19(7):589–590.
17. Gravlee JR, Van Durme DJ. Braces and splints for musculoskeletal conditions. *Am Fam Physician*. 2007;75(3):342–348.
18. Walker WC, Metzler M, Cifu DX, et al. Neutral wrist splinting in carpal tunnel syndrome: a comparison of night-only versus full-time wear instructions. *Arch Phys Med Rehabil*. 2000;81:424–429.
19. Cook AC, Szabo RM, Birkholz SW, et al. Early mobilization following carpal tunnel release: a prospective randomized study. *J Hand Surg [Br]*. 1995;20(2):228–230.
20. Ebenbichler GR, Resch KL, et al. Ultrasound treatment for treating the carpal tunnel syndrome: randomised "sham" controlled trial. *BMJ*. 1998;316:731–735.
21. Garfinkel MS, Singhal A, Katz WA, et al. Yoga-based intervention for carpal tunnel syndrome: a randomized trial. *JAMA*. 1998;280:1601–1603.
22. Seradge H, Jia YC, Owens W. In vivo measurement of carpal tunnel pressure in the functioning hand. *J Hand Surg Am*. 1995;20(5):855–859.
23. Valente R, Gibson H. Chiropractic manipulation in carpal tunnel syndrome. *J Manipulative Physiol Ther*. 1994;17:246–249.
24. Sucher BM. Myofascial manipulative release of carpal tunnel syndrome: documentation with magnetic resonance imaging. *J Am Osteopath Assoc*. 1993;93:1273–1278.
25. Rozmaryn LM, Dovelle S, Rothman ER, et al. Nerve and tendon gliding exercises and the conservative management of carpal tunnel syndrome. *J Hand Ther*. 1998;11. 171–117.
26. Naeser MA, Hahn KA, Lieberman BE, et al. Carpal tunnel syndrome treated with low-level laser and microamperes transcutaneous electric nerve stimulation: a controlled study. *Arch Phys Med Rehabil*. 2002;83:978–988.
27. Yang CP, Hsieh CL, Wang NH, et al. Acupuncture in patients with carpal tunnel syndrome: a randomized controlled trial. *Clin J Pain*. 2009;25(4):327–333.
28. Chen GS. The effect of acupuncture treatment on carpal tunnel syndrome. *Am J Acupunct*. 1990;18:5–9.
29. Chell J, Stevens A, Davis TR. Work practices and histopathological changes in the tenosynovium and flexor retinaculum in carpal tunnel syndrome in women. *J Bone Joint Surg Br*. 1999;81(5):868–870.
30. Ellis JM, Folkers K. Clinical aspects of treatment of carpal tunnel syndrome with vitamin B6. *Ann N Y Acad Sci*. 1990;585:302–320.
31. Folkers K, Ellis J. Successful therapy with vitamin B6 and vitamin B2 of the carpal tunnel syndrome and need for determination of the RDA's for vitamin B6 and B2 disease states. *Ann N Y Acad Sci*. 1990;585:295–301.
32. Folkers K, Wolaniuk A, Vadhanavikit S. Enzymology of the response of carpal tunnel syndrome to riboflavin and to combined riboflavin and pyridoxine. *Proc Nat Acad Sci U S A*. 1984;81:7076–7078.
33. Spooner GR, Desai HB, Angel JF, et al. Using pyridoxine to treat carpal tunnel syndrome: randomized control trial. *Can Fam Phys*. 1993;39:2122–2127.
34. Stransky M, Rubin A, Lava NS, et al. Treatment of carpal tunnel syndrome with vitamin B6: a double-blind study. *South Med J*. 1989;82:841–842.
35. Chang MH, Chiang HT, Lee SS, et al. Oral drug of choice in carpal tunnel syndrome. *Neurology*. 1998;51(2):390–393.
36. Scholten RJPM, Mink van der Molen A, Uitdehaag BMJ, et al. Surgical treatment options for carpal tunnel syndrome. *Cochrane Database Sys Rev*. 2007;4: Art. No.: CD003905. https://doi.org/10.1002/14651858.CD003905.pub3.
37. Jeffrey SL, Belcher HJ. Use of Arnica to relieve pain after carpal-tunnel release surgery. *Altern Ther Health Med*. 2002;8(2):66–68.
38. Hochberg J. A randomized prospective study to assess the efficacy of two cold-therapy treatments following carpal tunnel release. *J Hand Ther*. 2001;14(3):208–215.

157

Celiac Disease

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- A chronic intestinal malabsorption disorder caused by an intolerance to gluten
- Bulky, pale, frothy, foul-smelling, greasy stools with increased fecal fat
- Weight loss and signs of multiple vitamin and mineral deficiencies
- Increased levels of serum antiendomysium and/or transglutaminase antibodies
- Diagnosis confirmed by jejunal biopsy

GENERAL CONSIDERATIONS

Celiac disease, also known as nontropical sprue, gluten-sensitive enteropathy, or celiac sprue, is characterized by malabsorption and an abnormal small intestinal structure that reverts to normal on the removal of dietary gluten. The protein gluten and its polypeptide derivative gliadin are found primarily in wheat, barley, and rye grains. Symptoms most commonly appear during the first 3 years of life, after cereals are introduced into the diet. A second peak incidence occurs during the third decade, and although celiac disease is often thought of as a disease diagnosed early in life, more diagnoses are made in adulthood than in childhood.[1] It is not unusual for individuals to experience symptoms for 4.5 to 9 years before a confirmed diagnosis. A screening of more than 13,000 individuals found that those with digestive complaints such as constipation, diarrhea, and/or abdominal pain had a 1:56 chance of having celiac disease, a risk increase of more than twofold that of the general population.[2]

The prevalence of celiac disease has increased dramatically, and this is not solely because of increased detection. Until a few decades ago, celiac disease was believed to be relatively rare (approximately 1 case in 5000 within the United States). Now, however, celiac disease is thought to affect approximately 1% of most populations and approximately 3 million people in the United States. Gluten sensitivity has become quite common, and the incidence continues to increase. Interestingly, the amount of wheat consumed today is far less than the amount consumed in the 19th century. The average American consumed 225 pounds of wheat flour per person in 1880 versus 132.5 pounds of wheat flour per person in 2011.[3] However, celiac disease remains largely undiagnosed.[4,5] Undetected celiac disease carries with it increased mortality, indicating that widespread screening may be economically justified.

Chemistry of Grain Proteins

Gluten, a major component of the wheat endosperm, is composed of gliadins and glutenins. Only the gliadin portion has been demonstrated to activate celiac disease. In rye, barley, and oats, the proteins that appear to activate the disease are termed secalins, hordeins, and avenins, respectively, and prolamines collectively. Cereal grains belong to the family *Gramineae.* The closer a grain's taxonomic relationship to wheat, the greater is its ability to activate celiac disease. Rice and corn, two grains that do not appear to activate celiac disease, are further removed taxonomically from wheat. The taxonomic relationship of the grains is shown in Fig. 157.1.

Gliadins are single polypeptide chains that range in molecular weight from 30,000 to 75,000, with a high glutamine and proline content. There are at least 50 toxic epitopes (i.e., antigenic determinants) in gluten peptides exerting cytotoxic, immunomodulatory, and gut-permeating activities. Gliadins have been divided into four major electrophoretic fractions: alpha-, beta-, gamma-, and omega-gliadin, and are distinguished based on their amino acid sequences. Alpha-gliadin is believed to be the fraction most capable of activating celiac disease, although beta- and gamma-gliadin are also capable of doing so. Omega-gliadin does not appear to activate the disease, although it has the highest content of glutamine and proline. As seen in Fig. 157.2, each of the various peptide sequences in α-gliadin has different toxic activities: cytotoxic, immunomodulatory, zonulin release, and interleukin (IL)-8 release. Gliadin that has been subjected to complete hydrolysis does not activate celiac disease in susceptible individuals.[6]

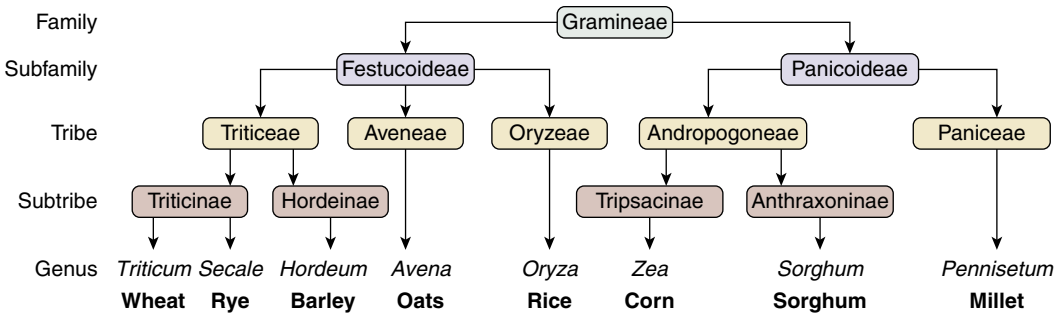


Fig. 157.1 Taxonomic relationship of the major cereal grains. (From Kasarda DD, Okita TW, Bernardin JE, Baecker PA, Nimmo CC, Lew, EJ, Dietler MD, Greene FC. *Proc Nat Acad Sci US*. 1984;81[15]:4712–4716.)

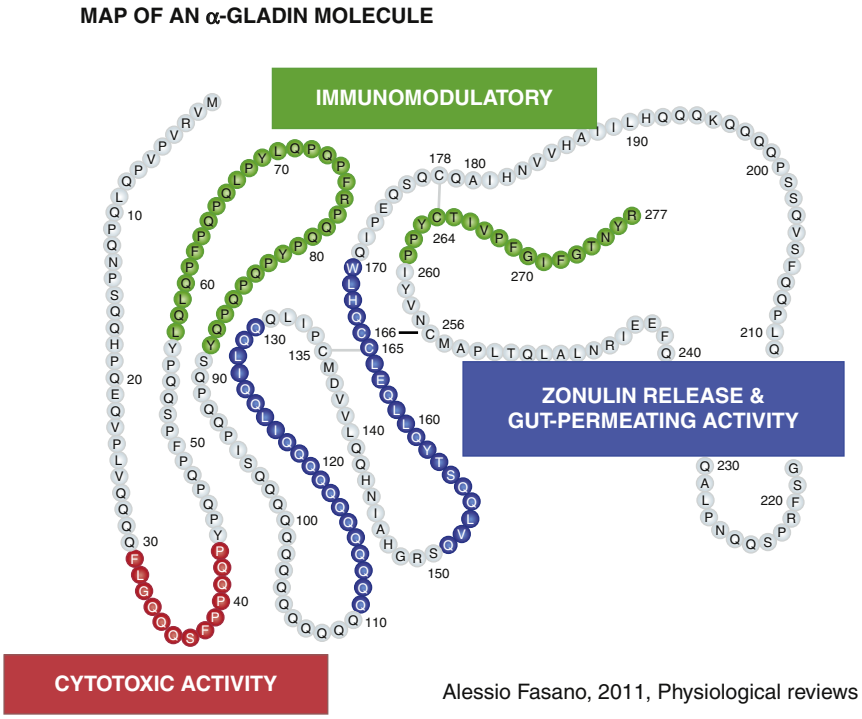


Fig. 157.2 Map of a molecule.

Zonulin

Zonulin is a protein modulator of intercellular tight junctions. It has been shown to induce a significant and reversible increase in gastroduodenal and small intestinal permeability and is involved in tolerance/immune response balance. The hybridization of wheat to dramatically increase gluten (gliadin) content and its overconsumption, multiple times a day, every day, in the typical diet, chronically disrupts this tolerance/immune response balance. Zonulin release is stimulated by contact with gliadin (alpha-gliadin is the most effective). The enterocytes that release zonulin occur in the jejunum and distal ileum but not in the colon. When exposed to gliadin, zonulin receptor-positive IEC6 and Caco2 cells release zonulin, leading to the rearrangement of the cell cytoskeleton, loss of occluding-ZO1 protein–protein interaction, and increased intestinal permeability to macromolecules.[7] Chemokine receptor CXCR3 is the receptor for specific gliadin peptides that cause zonulin release and a subsequent increase in intestinal permeability (Fig. 157.3). In vitro research with chemokine receptor CXCR3 transfectants found that several peptides, especially 33-mer, from gliadin bind to the receptor. Zonulin and CXCR3 are overexpressed in the intestinal mucosa of patients with celiac disease and autoimmune disease.

Human zonulin was discovered to be prehaptoglobin-2 (pre-HP2) and was known in earlier research as an "inactive" precursor for HP2. Haptoglobin, identified as a marker of inflammation several decades ago, is an α2–globulin found in human plasma at a concentration of 82 to 236 mg/dL. It is an acute-phase plasma protein whose primary role appears to be to bind the free hemoglobin released when erythrocytes are damaged, thus decreasing the oxidative damage of the freed iron. It is composed of four polypeptide chains: two α chains and two β chains connected by disulfide bridges. Human haptoglobin occurs primarily as three major phenotypes: HP1-1, HP2-1, and HP2-2. The phenotypes are differentiated by the presence of an α1 chain (HP1-1) or an α2 chain (HP2-2) with a combination of α1 and α2 chains distinguishing HP2-1.

Gliadin causes the release of zonulin in the 80% of the population with HP2. Although HP1-1 has a much lower chance of an immunological reaction, the probability is still not zero. There is growing recognition of the nonimmunological reaction to wheat, which is identified as nonceliac gluten sensitivity (NCGS). Table 157.1 provides an estimate of the percentage of the North American population in each haptoglobin category, along with clinical responses and clinical

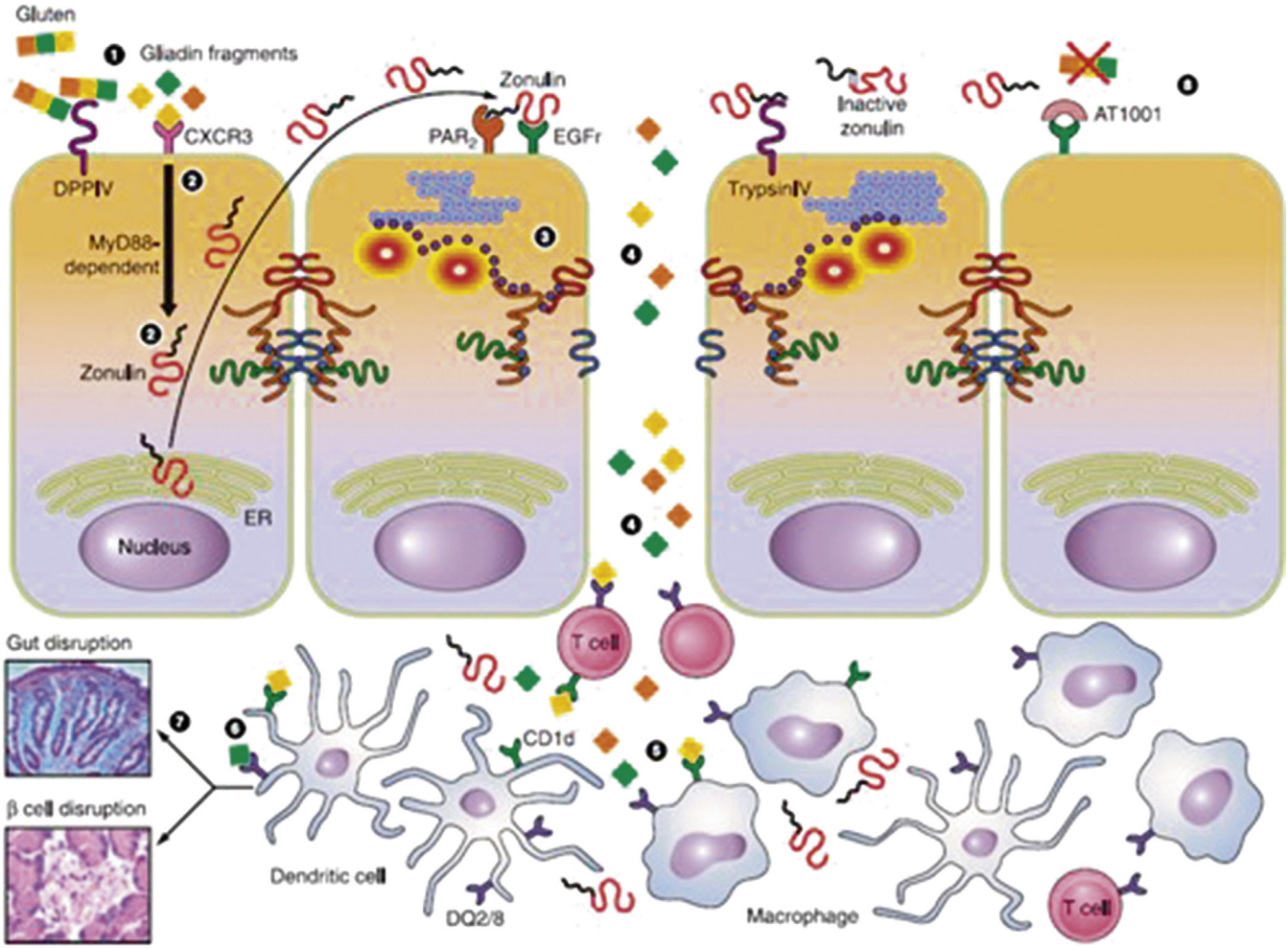


Fig. 157.3 Gliadin release of zonulin to open tight junctions and activate immune system. (From Lammers KM, Lu R, Brownley J, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. *Gastroent*. 2008 Jul;135[1]:194–204.)

TABLE 157.1 Summary of Clinical Responses to Wheat and Clinical Recommendations

Response to Wheat	% Pop.	Characteristics	Intervention
Safe	34%	HP1-1; no grain antibodies	None needed
Inconsistent, dose-dependent response (some research is labeling severe form nonceliac gluten sensitivity [NCGS])	43%	HP2-1; low to elevated blood zonulin	Limit wheat, rye, barley; optimize protein digestion; reseed with *Bifidobacteria* and other healthy gut bacteria.
Immune reactions to grains	15%	Antibodies to some grain proteins	Avoid all grains.
Celiac disease	3%	HP2-1 or HP2-2; elevated antibodies to gliadin; HLA-DQ2 or DQ8	Strictly avoid wheat, rye, barley; may need to avoid other grains as well.
Autoimmune disease	5%	HP2-1 or HP2-2; elevated antiself antibodies	Strictly avoid all allergens.

HLA, Human leukocyte antigen.

recommendations. Although this may be inconsistent with "official" numbers, considering the significant underdiagnosis of these conditions, the research suggests this is an accurate reflection of actual occurrence.

Pathogenesis

Celiac disease appears to have a genetic etiology because it is associated with specific human leukocyte antigen (HLA) molecules—HLA-DQ2 in 95% of patients and DQ8 in the remainder. These gene loci are believed to be linked to the immunological recognition of antigens and specific T-cell–regulated immune responses.

Various hypotheses have been proposed to explain the pathogenesis of celiac disease. Currently, the most likely relates to abnormalities in the immune response rather than some "toxic" property of gliadin. Sensitization to gliadin occurs both in humoral and cell-mediated immunity, and it appears that T-cell dysfunction is the main factor responsible for the enteropathy.[6] A number of circulating antibodies that are specific for celiac disease, particularly antiendomysial antibody

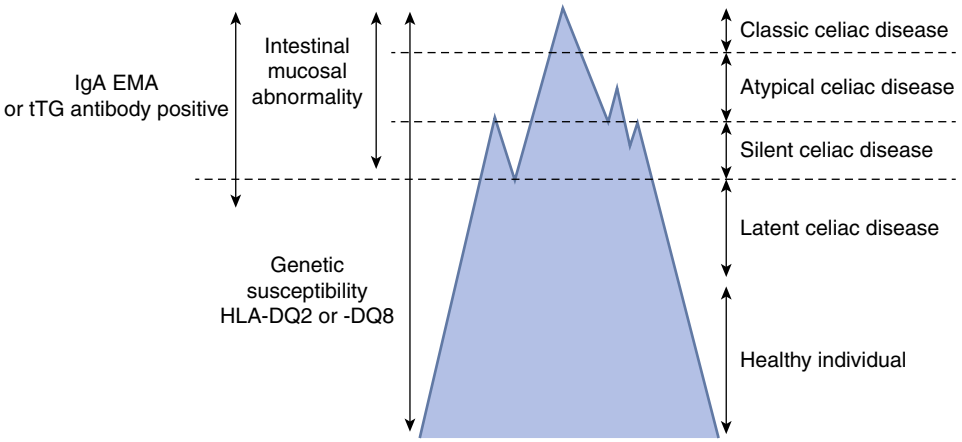


Fig. 157.4 The spectrum of celiac disease. (Feldman M, Friedman LS, Brandt LJ. Sleisenger and Fordtran's gastrointestinal and liver disease e-book: pathophysiology, diagnosis, management. 2010. https://clinicalgate.com/celiac-disease-and-refractory-celiac-disease/#f0010.)

(AEA), have been identified. These serological markers have been used successfully to screen patients and to estimate the true prevalence of celiac disease in the general population. The discovery that tissue transglutaminase (tTG) is the autoantigen for AEA led to the development of an improved enzyme-linked assay using recombinant tTG, which has been shown to be highly sensitive and specific for celiac disease. Tissue transglutaminase is a ubiquitous, predominantly cytoplasmic enzyme that can be released extracellularly, particularly in response to tissue wounding and stress. The observation that anti-tTG antibody titers fall and can become undetectable during a gluten-free diet suggests that tTG–gliadin complexes stimulate gluten-specific T cells to induce anti-tTG antibody production. T and B cells likely recognize different parts of this antigen complex, with T cells reacting to the smaller gliadin peptides and B cells responding to the larger tTG enzyme.[6]

Interestingly, breastfeeding appears to have a prophylactic effect, and breastfed babies have a decreased risk of developing celiac disease.[8,9] The reduced risk of celiac disease was even more pronounced in infants who continued to be breastfed after dietary gluten was introduced. Not surprisingly, the risk of celiac disease was greater when gluten was introduced in the diet in large amounts than when introduced in small or medium amounts. The early introduction of cow's milk is also believed to be a major etiological factor.[10] Research in the past few years has clearly indicated that breastfeeding and delayed administration of cow's milk and cereal grains are primary preventive steps that can greatly reduce the risk of the development of celiac disease.

CLINICAL ASPECTS

The histological lesions of celiac disease are often indistinguishable from changes caused by tropical sprue, food allergy, diffuse intestinal lymphoma, and viral gastroenteritis. Furthermore, celiac disease often leads to the development of multiple food allergies; disaccharidase deficiency, causing lactose intolerance; and increased intestinal permeability.[11] Improving nutritional status, even something as simple as taking a multiple B vitamin, appears to produce significant improvement in quality of life in patients with celiac disease. In one double-blind study, 65 patients with celiac disease who were on a strict gluten-free diet for several years were randomized to a daily dose of 0.8 mg folic acid, 0.5 mg cyanocobalamin, and 3 mg pyridoxine or placebo for 6 months.[12] After vitamin supplementation, homocysteine levels dropped a median of 34%, accompanied by a significant improvement in well-being, notably anxiety and depressed mood.

Celiac disease exhibits a range of clinical presentations (Fig. 157.4). Atypical celiac disease involves gluten-sensitive enteropathy that manifests only as extraintestinal signs and symptoms such as short stature, anemia, and osteoporosis. Silent celiac disease also involves fully expressed gluten enteropathy, but patients are asymptomatic, and the diagnosis is typically discovered after serological testing. A combination of serological, genetic, and histological data has led to the identification of latent celiac disease (normal-villus architecture on a gluten-containing diet but have had or will have gluten-sensitive villus atrophy) as well as potential celiac disease (elevated gluten antibodies but negative intestinal biopsy).

Associated Conditions

Perhaps the most significant condition associated with celiac disease is an early death. A landmark study looked at almost 30,000 patients between 1969 and 2008 and examined deaths in four groups: those with full-blown celiac disease, those with inflammation of the intestine but not full-blown celiac disease, those with latent celiac disease or gluten sensitivity, and a control group with no evidence of celiac disease or gluten sensitivity.[13] The findings were significant: compared with the control group, there was a 39% increased risk of death in those with celiac disease, 72% increased risk in those with gut inflammation related to gluten, and 35% increased risk in those with gluten sensitivity but no celiac disease.

A review article lists 55 health conditions linked to celiac disease and gluten sensitivity, including irritable bowel disease, inflammatory bowel disease, anemia, migraines, epilepsy, fatigue, canker sores, osteoporosis, rheumatoid arthritis, lupus, multiple sclerosis, and almost all other autoimmune diseases.[14] Thyroid abnormalities, insulin-dependent diabetes mellitus, psychiatric disturbances (including schizophrenia), dermatitis herpetiformis, and urticaria have also been linked to gluten intolerance.[1] A more ominous association is the increased risk for malignant neoplasms seen in celiac patients, especially for non-Hodgkin's lymphoma.[15] The hypothesis that gluten is a contributing factor in some cases of schizophrenia is substantiated by epidemiological, clinical, and experimental studies.[16–19] Pepsin hydrolysates of wheat gluten have demonstrated opioid activity. This activity is believed to be the factor responsible for the association between wheat consumption and schizophrenia. Gluten sensitivity has also been weakly linked to mood scores, cognitive impairment, and autism.[20–22]

The effects of gut inflammation associated with altered permeability are not restricted to the digestive system. For example, animal models of type 1 diabetes have shown that intestinal permeability, with

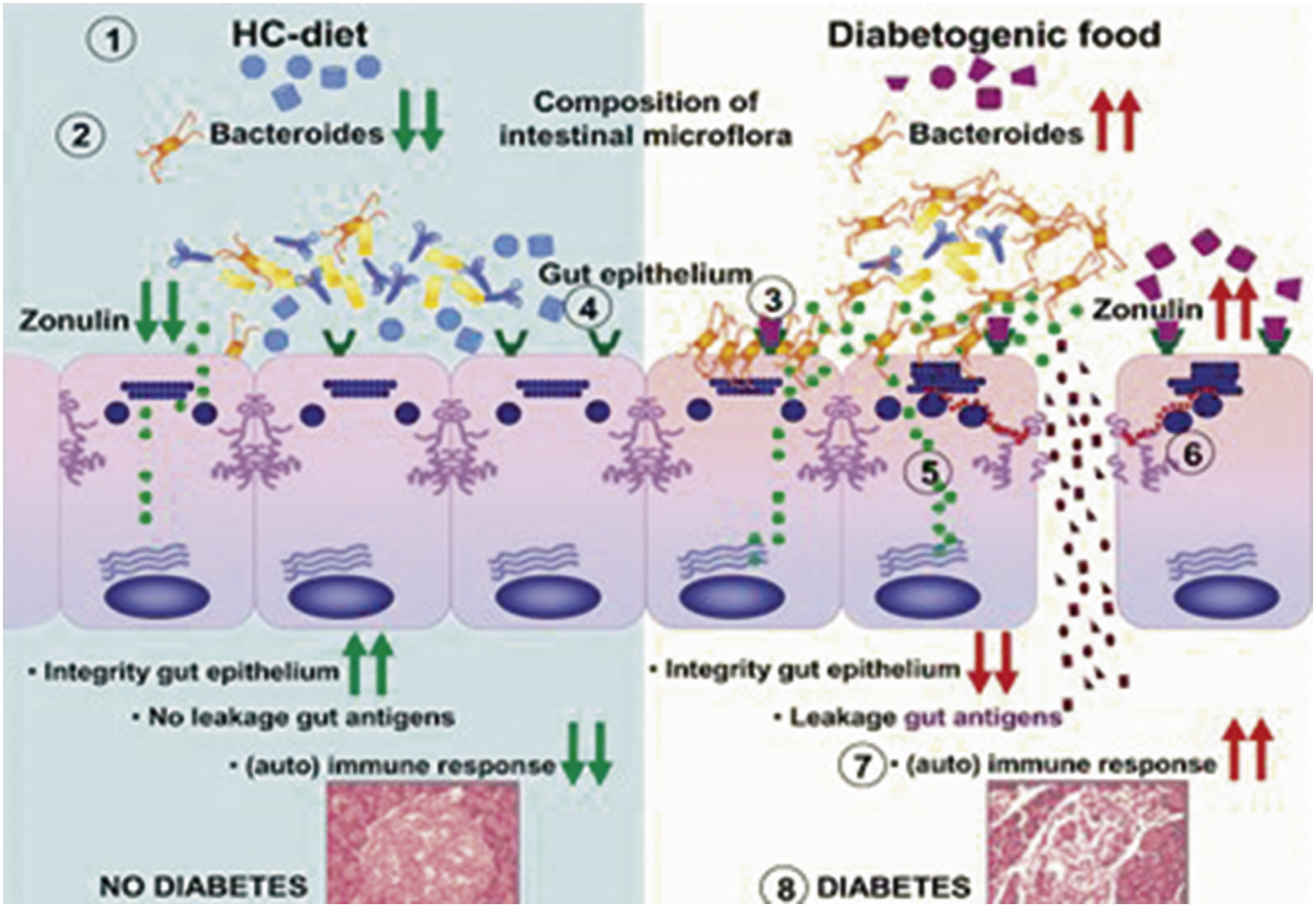


Fig. 157.5 Diet affects the composition of the intestinal microflora, and gluten interaction influences diabetes development. (From Visser J, Rozing J, Sapone A, et al. Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. *Ann NY Acad Sci.* 2009 May;1165:195–205.)

resultant inflammation, plays a role in the autoimmune destruction of pancreatic islet cells. There appears to be a link between antibodies to Glo-3a (a wheat-related protein), zonulin up-regulation, and islet cell autoimmunity in children at increased risk for type 1 diabetes.[23] In individuals who went on to develop type 1 diabetes, elevated serum zonulin was detected in 70% of subjects during the pre–type 1 diabetes phase and preceded the onset of disease by 3.5 ± 0.9 years.[24] Increased lactulose permeability (with normal mannitol) appears to precede the detectable clinical onset of disease, suggesting that the small intestine participates in the pathogenesis of the disease. The composition of the gut microbiota combined with gluten intake affects intestinal integrity, which affects the development of diabetes (Fig. 157.5).

Zonulin is also associated with the development and/or progression of chronic inflammatory lung and neurological disease. The blockade of zonulin reduces the severity of acute lung injury by reducing capillary leak, diminishing the accumulation of polymorphonuclear leukocytes (PMNs) in the interstitial and alveolar components, and attenuating levels of proinflammatory mediators.[25] There is some evidence that the expression of zonulin correlates with the aggressiveness of gliomas and may indicate the degree of disturbance of the blood-brain barrier and the walls of the blood vessels.[26]

DIAGNOSIS

As an initial screening for celiac disease, serological tests are an excellent starting point, and they may be followed by a biopsy of the small intestine for a definitive diagnosis. Before the introduction of highly sensitive and specific serological markers, endoscopy with jejunal biopsy was the definitive diagnostic procedure. To fully understand a patient's response to gluten, the key laboratory tests are haptoglobin type, serum zonulin, HLA type, antibodies to wheat proteins, and antiself antibodies. Of these serological markers, the test for human antitissue transglutaminase antibodies (IgA anti-tTG) is the most widely used, primarily owing to a lower cost compared with antiendomysium (IgA EMA) and a greater sensitivity compared with antigliadin antibodies (AGAs). IgA EMA can detect celiac disease with a sensitivity and specificity of 90% and 99%, respectively. Anti-tTG also has a high sensitivity (99%) and specificity (>90%) for identifying celiac disease. An equivocal result on tTG testing should be followed by IgA EMA. With IgA EMA testing, it is recommended that a total serum IgA level be checked in parallel because celiac patients with IgA deficiency may be unable to produce the antibodies on which these tests depend, leading to a false-negative result.

Genetic tests for HLA-DQ2 and DQ8 have poor positive predictive value but are considered up to 100% sensitive and are therefore able to assist in ruling out celiac disease. The progression of an immunological reaction to gliadin to autoimmune disease appears to be dependent on several factors, especially HLA class.

Endoscopy/gastroscopy and biopsy should still be performed if clinically warranted.

THERAPEUTIC CONSIDERATIONS

Diet

Once the diagnosis has been established, a gluten-free diet is indicated. This diet should not contain any wheat, rye, barley, triticale, or oats. Species falling in the genus *Triticum* are almost certain to be harmful to gliadin-sensitive patients. Although the hybridization of wheat to higher concentrations of gluten has clearly aggravated the problem, this does not mean that so-called "ancient" wheats are safe for those who react to gliadin. *Triticum spelta* (spelt or spelta), *Triticum polonicum* (Polish wheat or kamut), and *Triticum monococcum* (einkorn or small spelt) still contain gliadin and must also be avoided. They may be acceptable in modest dosages for those who are wheat intolerant but do not react immunologically. Buckwheat and millet are often excluded as well. Although buckwheat is not in the grass family, and millet appears to be more closely related to rice and corn, buckwheat and millet contain prolamines with similar antigenic activity to the alpha-gliadin of wheat.

In addition, other foods should be rotated, and milk and milk products should be eliminated until the patient's intestinal structure and function return to normal.

Patient Response

Usually, clinical improvement will be apparent within a few days or weeks (30% respond within 3 days, another 50% within 1 month, and 10% within another month). However, 10% of patients respond only after 24 to 36 months of gluten avoidance.[1]

If the patient does not appear to respond, the following should be considered:

- The diagnosis is incorrect.
- The patient is not adhering to the diet or is being exposed to hidden sources of gliadin.
- There is an associated disease or complication, such as zinc deficiency.

The U.S. Food and Drug Administration (FDA) has strict criteria and labeling requirements for food manufacturers to label a food "gluten-free." However, even with this assurance, it seems that many people on a gluten-free diet are ingesting enough gluten in their diets to cause issues. Eating as little as 10 mg of gluten is enough to cause issues in some especially sensitive individuals, whereas most patients with celiac disease can tolerate up to 100 mg per day. A detailed analysis to determine the amount of gluten consumed by measuring the level of gluten in the stool and urine in people with celiac disease and those without celiac disease who were following a gluten-free diet.[27] The results showed that the average inadvertent exposure to gluten by people with celiac disease on a gluten-free diet was estimated to be between 150 and 400 mg/d using the stool test and between 300 and 400 mg/d using the urine test. These results indicate that patients with celiac disease, as well as those with gluten sensitivity or intolerance, should be on a gluten-digesting enzyme (discussed later in the chapter) as well as a gluten-free diet. Clinical trials with gluten-digesting enzymes combined with a gluten-free diet show a statistically significant, dose-dependent reduction in the severity and frequency of symptoms as well as improved intestinal biopsy results in patients with mild to moderate celiac disease.

Multivitamin/multimineral supplementation in patients with celiac disease appears indicated. In addition to treating any underlying deficiency, supplementation provides the necessary cofactors for growth and repair. OCTN2, the specific carnitine receptor, has been found in intestinal cells and is decreased in patients with celiac disease compared with normal subjects. A randomized, double-blind-versus-placebo parallel study evaluated the effect of L-carnitine treatment on fatigue in adult patients with celiac disease.[28] The results showed that fatigue was significantly reduced in the L-carnitine group, and because L-carnitine is involved in muscle energy production, its decreased absorption due to OCTN2 reduction might explain muscular symptoms in patients with celiac disease. Other potential nutritional deficiencies include iron, zinc, calcium, copper, fat-soluble vitamins, and dietary fiber. Celiac disease is refractive to dietary therapy if an underlying zinc deficiency is present.[29]

SUPPLEMENTS

Pancreatic Enzymes

Low fecal elastase-1, a marker for pancreatic insufficiency, is common among individuals with celiac disease. Even when following a gluten-free diet, celiac patients with chronic diarrhea are likely to have low fecal elastase.

The effect of pancreatic enzyme substitution therapy in the 2 months after the initial diagnosis of celiac disease (gluten enteropathy) was investigated in one double-blind study. The study sought to clarify the benefit of pancreatic enzyme therapy because previous studies had shown pancreatic insufficiency in 8% to 30% of patients with celiac disease. In this study, patients followed a gluten-free diet, the standard treatment for celiac disease, and received either two capsules of pancreatic enzymes with each meal (6–10 capsules a day with each capsule containing lipase 5000 IU, amylase 2900 IU, and protease 330 IU) or two placebo capsules with meals. Complete nutritional and anthropomorphic evaluations were conducted at days 0, 30, and 60. Results indicated that pancreatic enzyme supplementation enhances the clinical benefit of a gluten-free diet during the first 30 days but does not provide any greater benefit than the placebo after 60 days. These results support the use of pancreatic enzyme preparations in the first 30 days after a diagnosis of celiac disease[30] (see Chapter 100 for a complete discussion).

Alternatively, a superior choice to pancreatic enzymes is enzyme preparations containing glutenases. A mixture of two gluten-specific recombinant proteases (Latiglutenase) that degrades gluten proteins into small, physiologically irrelevant fragments has been shown to mitigate gluten-induced intestinal mucosal injury as well as effect symptomatic improvements in clinical trials, and it is particularly effective in patients with celiac disease who remain seropositive despite a gluten-free diet; however, this preparation is not currently available commercially.[27] Two options with confirmed glutenase activity are available: prolyl endopeptidase from genetically modified *Aspergillus niger* (AN-PEP) and dipeptidyl peptidase IV (DPP-IV) from *Aspergillus oryzae.* Of the two, AN-PEP is the most effective in degrading gluten more completely, including the immunogenic epitopes.[31] (See Chapter 95 for a comprehensive discussion.) DPP-IV targets both gliadin and casein (milk protein) and is resistant to breakdown by other digestive enzymes. DPP-IV is thought to be one of the key enzymes responsible for the digestion of these proteins and is known to be found in lower amounts in the intestinal mucosa of individuals with celiac disease and also to have an inverse correlation with the level of mucosal damage among those with as well as without celiac disease. The lower the DPP-IV, the more significant the damage to the intestinal lining. Preparations containing DPP-IV are often recommended to safeguard against any hidden sources of gluten. They are not a substitution for a gluten-free diet.[32]

THERAPEUTIC APPROACH

The therapeutic approach is straightforward: eliminate all sources of gliadin (see Appendix 6), eliminate dairy products initially, correct

underlying nutritional deficiencies, treat any associated conditions, and determine and eliminate all food allergens. If the patient does not begin to respond within 1 month, reconsider the diagnosis and search for hidden sources of gliadin.

The discovery of the zonulin pathway enables an opportunity for both diagnostic and therapeutic applications to be developed. The strategy to facilitate repair of the damaged intestinal mucosa involves three steps: (1) stop the damage by avoiding known gastrointestinal toxins, (2) reestablish a healthy microbial flora, and (3) stimulate regeneration. In addition, reestablishing the zonulin-dependent intestinal barrier function may provide a mechanism by which processes such as autoimmune disease, inflammatory conditions, and neoplastic disorders may be halted in their development and possibly reversed.

There is interesting evidence supporting the use of the zonulin inhibitor AT1001 in the prevention of autoimmune diseases such as type 1 diabetes and celiac disease. Pretreatment with AT1001 has been shown to prevent the loss of intestinal barrier function, the appearance of auto-antibodies, and the onset of disease and protected against the insult of pancreatic islets and, therefore, of the insulitis responsible for the onset of T1D.[33] The protective effects of AT1001 were also demonstrated in a small study of 20 patients on a gluten-free diet randomized to a 3-day AT1001 treatment (12 mg once daily) or placebo with a 2.5-g oral gluten challenge on the second day.[34] In the placebo group, a significant 70% increase in intestinal permeability was observed after gluten challenge compared with no changes in permeability in the AT1001-treated group. In addition, AT1001 has been shown to reduce proinflammatory cytokine production and gastrointestinal symptoms in patients with celiac disease exposed to gluten.[35] The maintenance of a strict gluten-free diet is difficult in the United States because of the ubiquitous distribution of gliadin and other activators of celiac disease in processed foods. Patients must be encouraged to read labels carefully to avoid hidden sources of gliadin, as it is found in some brands of soy sauce, modified food starch, ice cream, soup, beer, wine, vodka, whiskey, and malt. Patients should also be encouraged to consult resources for patient education and information on gluten-free recipes. Supplemental glutenases may be helpful to offset a low level of inadvertent gluten ingestion but are not a substitute for a gluten-free diet.

RESOURCES FOR PATIENTS

Gluten Intolerance Group
31214 124th Ave. SE
Auburn, WA 98092
Website: http://www.gluten.net
Celiac Sprue Association
P.O. Box 31700
Omaha, NE 68131-0700
Website: www.csaceliacs.org
Celiac Disease Foundation
13251Ventura Blvd., Suite 1
Studio City, CA 91604
Website: http://celiac.org

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rubio-Tapia A, Murray JA. Celiac disease. *Curr Opin Gastroenterol*. 2010;26(2):116–122.
2. Fasano A, Berti I, Gerarduzzi T, Not T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med*. 2003;163(3):286–292. PubMed PMID: 12578508.
3. United States Department of Agriculture (USDA) Economic Resource Service. *Wheat's Role in the U.S. Diet*; 2016. www.ers.usda.gov/topics/crops/wheat/wheats-role-in-the-us-diet.aspx. Accessed June 15, 2018.
4. Rewers M. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? *Gastroenterology*. 2005;128(4 suppl. 1):S47–S51.
5. Tack GJ, Verbeek WH, Schreurs MW, et al. The spectrum of celiac disease: epidemiology, clinical aspects and treatment. *Nat Rev Gastroenterol Hepatol*. 2010;7(4):204–213.
6. Dewar D, Pereira SP, Ciclitira PJ. The pathogenesis of coeliac disease. *Int J Biochem Cell Biol*. 2004;36:17–24.
7. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, et al. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol*. 2006;41(4):408–419. PubMed PMID: 16635908.
8. Persson LA, Ivarsson A, Hernell O. Breast-feeding protects against celiac disease in childhood: epidemiological evidence. *Adv Exp Med Biol*. 2002;503:115–123.
9. Ivarsson A, Hernell O, Stenlund H, et al. Breast-feeding protects against celiac disease. *Am J Clin Nutr*. 2002;75:914–921.
10. Faellstroem SP, Winberg J, Andersen HJ. Cow's milk induced malabsorption as a precursor of gluten intolerance. *Acta Paediatr Scand*. 1965;54:101–115.
11. Saalman R, Dahlgren UI, Fallstrom SP, et al. Avidity progression of dietary antibodies in healthy and coeliac children. *Clin Exp Immunol*. 2003;134:328–334.
12. Hallert C, Svensson M, Tholstrup J, et al. Clinical trial: B Vitamins improve health in coeliac patients living on a gluten-free diet. *Aliment Pharmacol Ther*. 2009;29(8):811–816. PubMed PMID: 19154566.
13. Ludvigsson JF, Montgomery SM, Ekbom A, et al. Small-intestinal histopathology and mortality risk in celiac disease. *JAMA*. 2009;302(11):1171–1178.
14. Rubio-Tapia A, Kyle RA, Kaplan EL, et al. Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology*. 2009;137(1):88–93.
15. Green PH, Fleischauer AT, Bhagat G, et al. Risk of malignancy in patients with celiac disease. *Am J Med*. 2003;115:191–195.
16. Dohan FC, Harper EH, Clark MH, et al. Is schizophrenia rare if grain is rare? *Biol Psychiatry*. 1984;19:385–399.
17. Dohan FC, Gasberger JC. Relapsed schizophrenics: earlier discharge from the hospital after cereal-free, milk-free diet. *Am J Psychiatry*. 1973;130:685–688.
18. Paroli E. Opioid peptides from food (the exorphins). *World Rev Nutr Diet*. 1988;55:58–97.
19. Ludvigsson JF, Osby U, Ekbom A, et al. Coeliac disease and risk of schizophrenia and other psychosis: a general population cohort study. *Scand J Gastroenterol*. 2007;42(2):179–185.
20. Ludvigsson JF, Reutfors J, Osby U, et al. Coeliac disease and risk of mood disorders: a general population-based cohort study. *J Affect Disord*. 2007;99(1–3):117–126. Epub 2006 Oct 6.
21. Hu WT, Murray JA, Greenaway MC, et al. Cognitive impairment and celiac disease. *Arch Neurol*. 2006;63(10):1440–1446.
22. Millward C, Ferriter M, Calver S, et al. Gluten- and casein-free diets for autistic spectrum disorder. *Cochrane Database Syst Rev*. 2004;2:CD003498.
23. Simpson M, Mojibian M, Barriga K, et al. An exploration of Glo-3a antibody levels in children at increased risk for type 1 diabetes mellitus. *Pediatr Diabetes*. 2009;10(8):563–572. PubMed PMID: 19622083.
24. Sapone A, de Magistris L, Pietzak M, Clemente MG, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes*. 2006;55(5):1443–1449. PubMed PMID: 16644703.
25. Rittirsch D, Flierl MA, Nadeau BA, Day DE, et al. Zonulin as prehaptoglobin2 regulates lung permeability and activates the complement system. *Am J Physiol Lung Cell Mol Physiol*. 2013;304(12):L863–L872. PubMed PMID: 23564505.
26. Skardelly M, Armbruster FP, Meixensberger J, Hilbig H. Expression of zonulin, c-kit, and glial fibrillary acidic protein in human gliomas. *Transl Oncol*. 2009;2(3):117–120 .PubMed PMID: 19701495.
27. Syage JA, Kelly CP, Dickason MA, et al. Determination of gluten consumption in celiac disease patients on a gluten-free diet. *Am J Clin Nutr*. 2018;107(2):201–207.
28. Ciacci C, Peluso G, Iannoni E, et al. L-carnitine in the treatment of fatigue in adult celiac disease patients: a pilot study. *Dig Liver Dis*. 2007;39(10):922–928. PubMed PMID: 17693145.
29. Love AHG, Elmes M, Golden M, et al. Zinc deficiency and celiac disease. In: McNicholl B, McCarthy CF, Fottrell PF, eds. *Perspectives in Coeliac Disease. Proceedings of the 3rd International Symposium on Coeliac Disease*. Baltimore: University Press; 1978:335–342.
30. Carroccio A, Iacono G, Montalto G, et al. Pancreatic enzyme therapy in childhood celiac disease: a double-blind prospective randomized study. *Dig Dis Sci*. 1995;40:2555–2560.
31. Janssen G, Christis C, Kooy-Winkelaar Y, et al. Ineffective degradation of immunogenic gluten epitopes by currently available digestive enzyme supplements. *PLoS One*. 2015;10(6):e0128065. https://doi.org/10.1371/journal.pone.0128065.
32. Krishnareddy S, Stier K, Recanati M, Lebwohl B, Green PH. Commercially available glutenases: a potential hazard in coeliac disease. *Therap Adv Gastroenterol*. 2017;10(6):473–481.
33. Watts T, Berti I, Sapone A, et al. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type 1 diabetes in BB diabetic-prone rats. *Proc Natl Acad Sci U S A*. 2005;102(8):2916–2921. PubMed PMID: 15710870.
34. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac subjects: a proof of concept study. *Aliment Pharmacol Ther*. 2007;26(5):757–766. PubMed PMID: 17697209.
35. Kelly CP, Green PH, Murray JA, et al. Safety, tolerability and effects on intestinal permeability of larazotide acetate in celiac disease: results of a phase IIB 6-week gluten-challenge clinical trial. *Gastro*. 2009;136(5). A–474.

Cervical Dysplasia

Angela Sadlon, ND, and *Michael T. Murray*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Abnormal Papanicolaou (Pap) smears (atypical squamous cells of undetermined significance [ASC-US]; low-grade squamous intraepithelial lesions [LGSILs]; high-grade squamous intraepithelial lesions [HGSILs])
- Human papillomavirus (HPV) testing for the presence of high-risk strains
- Colposcopy, endocervical curettage, cervical biopsies

Since the Bethesda System of reporting was first established in 1988, it has been revised several times. The current U.S. cervical disease management guidelines were last updated in 2012 and include the recommended frequency of Pap smear testing and algorithms for managing high-risk HPV strains, ASC-US, atypical cells for which high-grade lesions cannot be ruled out (ASC-H), LGSILs, and HGSILs.[1–3] Recommendations for HPV testing and cytology testing and the combination of the two, HPV vaccinations, colposcopy and biopsies, follow-up cytology testing, and colposcopy based on abnormal results are complex and often change. Guidelines differ depending on the patient's age, history of previous abnormal cytology testing, pregnancy status, previous exposure to diethylstilbestrol, immunosuppression status (e.g., kidney transplants), human immunodeficiency virus (HIV) status, and hysterectomy for benign indications and prior history of cervical intraepithelial neoplasia (CIN). It is recommended that the clinician consult the latest available guidelines for screening and evaluation with colposcopy, biopsy, and follow-up testing from the American College of Obstetricians and Gynecologists, the American Cancer Society, and/or the U.S. Preventive Services Task Force.

A possible future risk factor not included in the current guidelines is the patient's ethnicity. Although more studies are needed, there seem to be racial differences in the time it takes for progression (e.g., ASC-US to LSIL) or regression (e.g., LSIL to ASC-US) to occur. A 10-year study[4] of 5472 women (self-reporting as either white, black, Asian, or Hispanic), all with the same access to health care and follow-ups, showed that blacks had the slowest progression rate from ASC-US to HSIL (expected time = 28.1 months) and the slowest regression rate from HSIL to ASC-US (expected time = 49 months) without intervention. Conversely, Hispanics had the fastest progression rate (expected time = 17.6 months) as well as the fastest regression rate (expected time = 27.6 months). The rates for whites were approximately 21 months for progression and 36 months for regression, and those for Asians were approximately 24.5 months for progression and 41 months for regression. Although the causes of these observations are still considered unknown, the authors proposed a biological explanation that HPV-16 sequence variations (e.g., E6 hybridization, African 1 or 2 variants, Asian variants, Hispanic variants) may be an influencing factor.[4] If there proves to be reproducible evidence of racial differences, the treatment options presented to the patient may vary based on that race's speed of progression. It may also help guide the length of natural therapy, showing a possibility that Hispanics may expect to have a shorter therapeutic protocol for regression, whereas blacks may expect to have a longer therapeutic protocol.

Currently, HPV is identified with tissue collection during a Pap smear, either as a default if the Pap smear is abnormal or as ordered by the physician. For greater than a decade, research has been testing other, less invasive ways to detect HPV.[5–10] Menstrual pads with a filter, allowing the blood to be collected and tested, seem to show the most promise. The accuracy of menstrual blood in comparison with cervical sampling for HPV ranged from 67% to 100% for sensitivity and 86% to 99% for specificity.[5–7,9,10] However, in one study, the specificity was as low as 45.5%.[10]

GENERAL CONSIDERATIONS

Cervical dysplasia is generally regarded as a precancerous lesion with risk factors similar to those of cervical cancer.[11] Therefore this discussion focuses on the following lifestyle and nutritional factors that appear to be cofactors in the development and progression of cervical dysplasias and ultimately cervical cancer[11,12]:

- Early age of first intercourse
- Multiple sex partners (in heterosexual women without barrier contraception)
- Other infectious agents (*Chlamydia,* herpesvirus)
- History of genital warts

*Previous edition contributor

- Immunosuppression (HIV, transplant patient, chronic corticosteroid therapy, chemotherapy)
- Smoking
- Oral contraceptive use
- Pregnancy
- Many nutritional factors

RISK FACTORS (TABLE 158.1)

Human Papillomavirus

Papillomaviruses are small DNA viruses around 50 to 55 nm. They are nonenveloped viruses. Papillomaviruses are found in not just humans but also cats, rabbits, and nonhuman primates. Papillomaviruses have an affinity for skin and mucous membranes (genitals, mouth, larynx, and esophagus). The DNA specifics of papillomavirus are summarized in Leto's 2011 review article.[13]

The human papillomavirus can be classified into cutaneous, mucosal, cutaneous and/or mucosal, and cutaneous associated with epidermodysplasia verruciformis.[13] For the purpose of this section, only the mucosal will be discussed. There are 40 HPV mucosal types ranging in number from 6 to 114. High-risk types 16 and 18 are found approximately 70% of the time in cervical cancer and 50% of the time in CIN3.[13] Other high-risk HPV types include 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82.[13–15] There are four types that are possibly high risk: 26, 53, 67, and 70.[15] And there are 12 low-risk types for carcinogenesis: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108.[14,15] Women can be infected with more than one type of HPV, and the highest incidence of this is with younger women at the peak of their sexual activity or women with an impaired immune response.[14,15]

TABLE 158.1 Risk Factors in Cervical Dysplasia/Cancer[a]

Risk Factor	Relative Risk
Smoking (10+ cigarettes/day)	3.06
Multiple sex partners (2–5)	3.46
First intercourse before age 18	2.76
Deficient dietary β-carotene (<5000 IU/day)	2.813
Deficient dietary vitamin C (<30 mg/day)	6.716

[a]The actual values for the absolute risk of the various risk factors are as yet somewhat controversial. The numbers listed here represent the author's summarization of the literature. These risks are not linearly additive because they are usually closely related; more extensive multivariant analysis will be necessary to determine the actual relative risk of each.

According to a 1999 study, 99.7% of cervical cancer is associated with long-term persistent HPV infection (usually HPV type 16 or 18), which is easily transmitted by genital-to-genital contact.[16] The time from exposure to the appearance of a lesion or an abnormal Pap smear can range from a few weeks to decades (Fig. 158.1).

The Centers for Disease Control and Prevention (CDC) reports that nearly 80 million Americans are currently infected with HPV. About 14 million people in the United States become infected each year.[17] For most people (9 out of 10), the HPV infections will resolve without intervention within 2 years.[3,17] This suggests that the host immunity is able to defend against the development of clinical disease and that HPV infections are often transient and result in minor manifestations such as ASC-US. For other individuals, especially women, HPV results in a clinical expression to which the host immune response has not been able to respond effectively. The result is clinical disease that can include flat or raised genital warts; cervical, vaginal, vulvar, or perianal dysplasias; or progression to invasive cancers of the same site.[17] The first step in the development of dysplasias or invasive cancers is viral entry. A complex interaction of host immunity, viral load, viral type, and host susceptibility determines the natural course of the disease.

Histological Considerations

The principal reservoir of HPV is the moist mucosa and the cutaneous epithelial tissue in adjacent areas. Ninety-five percent of cervical dysplasias and cancers originate in the squamocolumnar junction of the cervical os.[11] In adolescence, glandular epithelium covers much of the exocervix, but as adolescence progresses, the columnar epithelium is gradually replaced by squamous cells. This actively growing area seems to be more susceptible to multiple insults and HPV, probably because of the metaplastic nature of the conversion process and the inflammatory process of metaplasia.

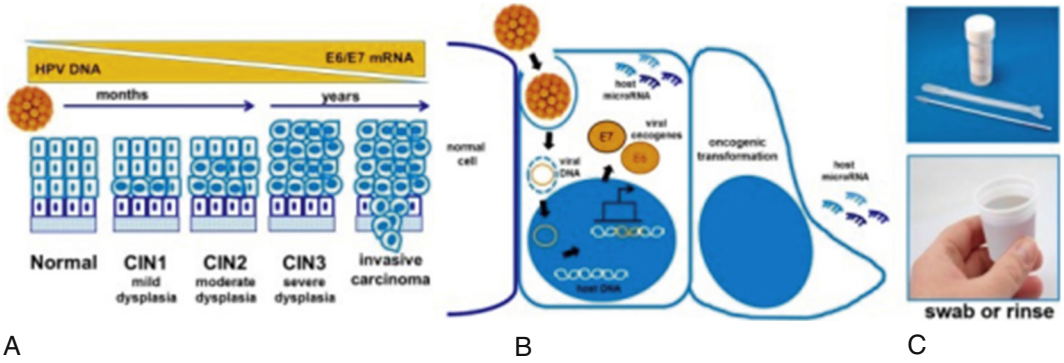


Fig. 158.1 Timeline of cervical cancer progression. (a) Human papillomavirus (HPV) infects the basal cells of the cervical epithelium *(dark blue)* exposed by wounding, leading to cytological abnormalities. Expression of viral oncogenes E6/E7 accompanies malignant transformation. (b) HPV infection pathway. After viral entry, viral replication is synchronous with host cellular DNA replication. Integrated and episomal viral DNA produce E6 and E7 oncoproteins. E6 targets p53 for degradation and prevents apoptosis, whereas E7 inactivates retinoblastoma (Rb) tumor suppressors, promoting cell-cycle progression. The end result of these processes is cellular transformation. HPV infection also alters the transcription of host microRNAs. (c) Samples for detection of HPV E6/E7 mRNA or host microRNA. *CIN,* Cervical intraepithelial neoplasia. (Shah SS, Senapti S, Klacsmann F, Miller DL, Johnson JJ, Chang HC, Stack MS. Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. *Cancers* (Basel). 2016;8[9]:pii: E85. PubMed PMID: 27618102.)

Sexual Transmission

Early age at first intercourse, multiple sexual contacts without the use of condoms, or both are associated with an increased risk of cervical dysplasia/carcinoma.[11,12] From this and other evidence, it has been suggested that cervical cancer is a sexually transmitted disease in the sense that the implicated infectious agent, HPV, is easily transmitted by genital-to-genital contact. Because the time from exposure to the appearance of a lesion can range from weeks to decades, it is almost impossible to identify individuals who transmit the virus.[17]

Genital-to-oral transmission is suggested to be possible owing to the detection of HPV types 6, 11, and 16 in some oropharyngeal cancers, but oral HPV lesions are in fact rare. Nonsexual exposure to the virus may occur from examination tables, doorknobs, tanning beds, and other inanimate objects, but it is difficult to document and prove.

Other infectious agents such as herpes simplex, *Chlamydia,* and HIV may serve as cofactors for HPV. These agents may alter cervical immunity, contribute to inflammation, facilitate entry of HPV into the basal cells, accelerate the replication of HPV in the host cell nucleus, and coexist with HPV infection.

Smoking

A significant risk factor for cervical cancer and cervical dysplasia is cigarette smoking: smokers have an approximately threefold increased incidence compared with nonsmokers (one study[18] showed the increase to be as high as seventeenfold in women ages 20–29).[18–21] Several hypotheses have been proposed to explain this association:

- Smoking may depress immune function, allowing a sexually transmitted agent to promote abnormal cellular development, leading to the onset of cervical dysplasia.
- Smoking induces vitamin C deficiency, and vitamin C levels are significantly depressed in smokers.[22]
- Cervical cells may concentrate nicotine.
- There may be unrecognized associations between smoking and sexual behavior.[18–21]

Oral Contraceptives

Earlier studies suggested that the use of oral contraceptives (OCs) increased the risk of cervical neoplasia, both the invasive and precancerous types. More recent studies controlled for sexual history have not confirmed this association. Three large, well-controlled studies looked at invasive cervical cancer and OC use and did not find statistically significant associations compared with women who never used OCs.[23–25] There was no overall change in the risk of invasive cervical cancer; however, one of the three studies[23] found a modestly increased risk in long-term users of OCs. The other two studies failed to find a significantly increased risk of invasive cervical cancer even with long-term OC use. Two other studies assessed OC use and the risk of cervical dysplasia, and neither found any statistically significant associations.[26,27]

A more disturbing aspect is that OC use has been associated with an increased incidence of adenocarcinoma, a rare cancer of the cervix. This is a less common variant of squamous cervical cancer. It appears that the incidence of this disease has increased over the past several decades, whereas the incidence of invasive squamous cervical cancer has decreased since the OC pill was introduced. Two studies found a modest but statistically significant increased risk of invasive cervical adenocarcinoma in women who had used OCs for more than 12 years.[24,28]

OCs are known to potentiate the adverse effects of cigarette smoking and to decrease the levels of numerous nutrients, including vitamins C, B_6, and B_{12} as well as folic acid, riboflavin, and zinc.[29]

Toxicant Exposure

A study evaluating the effect of depleted uranium weapons used in the Balkan war on the incidence of cervical intraepithelial neoplasia (CIN) found that the incidence of precancerous lesions of the cervix in areas near the borders of former Yugoslavia may be influenced by environmental factors such as exposure to depleted uranium due to the bombings.[30] Compared with histologically normal tissues, endometrial cancer, hyperplasia, and CIN revealed significantly increased levels of toxic metals (Cd and Pb), an altered status of Cu and Mn, and an elevated Cu:Zn ratio.[31]

Xenoestrogens are environmental estrogens that have endocrine effects, acting as both estrogen agonists and antagonists. In human cervical cancer cells (HeLa cells), dichlorodiphenyltrichloroethane (DDT) variably but significantly suppressed bcl-2 protein expression.[32] DDT has also been shown to mimic estradiol stimulation of breast cancer cells. However, these results should be interpreted with caution, because in vitro effects cannot be reliably extrapolated to chronic, low-dose exposure in vivo effects.

THERAPEUTIC CONSIDERATIONS

A follow-up Pap test is used to determine what course of action is needed. Many cases of mild cervical dysplasia (ASC-US, LSILs) will go away spontaneously. The median time required for progression from cervical dysplasia to carcinoma in situ ranges from 86 months for LSIL to 12 months for HSIL. In LSIL, the natural approaches provided in this chapter can be followed with a follow-up Pap smear and colposcopy at 3 months. If colposcopy finds abnormal tissue, endocervical curettage may be appropriate. Prescribing a patient diagnosed with HSIL a colposcopy with endocervical curettage is recommended.

Dietary Factors

Numerous nutritional factors have been implicated as cofactors in the development of cervical dysplasia. Although many single nutrients may play a significant role (particularly beta-carotene and retinoids, folate, pyridoxine, and vitamin C), it is important to recognize that a large proportion (67%) of patients with cervical cancer have multiple nutrient deficiencies or abnormal anthropometric measurements. Significant abnormalities have been found in height-to-weight ratios, triceps skin-fold thickness, midarm muscle circumference, serum albumin levels, total iron-binding capacity, hemoglobin levels, creatinine height index, prothrombin time, and lymphocyte count. Many other patients have marginal but "normal" nutritional status as determined by these cursory evaluations,[33] suggesting that multiple nutrient deficiencies are probably the rule rather than the exception.

Vitamin assessment by biochemical evaluation (plasma and red cell folate; serum beta-carotene; vitamins A, B_{12}, and C; erythrocyte transketolase for thiamine determination; erythrocyte glutathione reductase for riboflavin determination; and erythrocyte aspartate transaminase for pyridoxine determination) in patients with untreated cervical cancer shows that at least one abnormal vitamin level was present in 67% of patients, whereas 38% displayed multiple abnormal parameters.[34]

There is a correlation between elevated total plasma homocysteine levels (>9.12 mumol/L) and cervical dysplasia.[35] Because homocysteine levels are influenced by folate status, this may be an indirect way to determine whether there may be a folate deficiency.

General dietary factors are also important. A high fat intake has been associated with an increased risk for cervical cancer, whereas a diet rich in fruits and vegetables is believed to offer significant protection against carcinogenesis, probably owing to the higher intake of fiber, beta-carotene, and vitamin C.[20] Total serum carotene and tocopherol levels and their association with dietary intakes and the risk of newly diagnosed CIN and invasive cervical cancer were evaluated in a case-control study in Brazil.[36] Increasing concentrations of serum

lycopene were negatively associated with CIN 1, CIN 3, and cervical cancer. Increasing concentrations of serum alpha and gamma tocopherols and higher dietary intakes of dark green and deep yellow vegetables/fruits were associated with nearly 50% decreased risk of CIN 3. In another case-control study, 239 women with squamous cell carcinoma of the cervix from the tumor registry in Buffalo, New York, completed a questionnaire, whereby researchers investigated the relationships between intakes of selected dietary nutrients and food groups and risk of cervical cancer.[37] Significant reductions in risk of cervical cancer of approximately 40% to 60% were observed for women in the highest versus the lowest tertiles of dietary fiber, vitamin C, vitamin E, vitamin A, α-carotene, beta-carotene, lutein, and folate.

A 2003 study also suggests a lower antioxidant status (in this case coenzyme Q_{10} [CoQ_{10}] and tocopherols) was seen in patients with various grades of CIN and cervical cancer compared with controls.[38]

Nutritional Supplements

Several key individual supplements are discussed later, but a combination of products may work best. One study showed that multiple vitamin and mineral formulas, vitamins A and E, and calcium were significantly associated with a lower risk of cervical cancer and a lower HPV viral load.[39] The study enrolled 1096 women between the ages of 18 and 65 and included 328 HPV-positive women, 166 controls, 90 women with CIN 1, and 72 women with CIN 2 or 3. Multiple vitamins and minerals, vitamins A and E, and calcium were significantly associated with a lower risk of CIN 2 or 3. The patients who took the multiple vitamins and minerals had a lower HPV viral load and a significantly decreased frequency of CIN 1.

Vitamin A and Beta-Carotene

A minor association appears to exist between dietary retinoids and the risk of cervical cancer or dysplasia as well as a strong inverse correlation between beta-carotene intake and the risk of cervical cancer or dysplasia.[40–43] Although only 6% of patients with untreated cervical cancer have below-normal serum vitamin A levels, 38% have stage-related abnormal levels of beta-carotene.[34] Low serum beta-carotene levels are associated with a threefold greater risk for severe dysplasia,[42] and serum vitamin A and beta-carotene levels were found to be significantly lower in patients with cervical dysplasia than in a control group (54 vs. 104 mg/dL for vitamin A and 21.3 vs. 13.9 mcg/dL for beta-carotene).[44,45] However, the serum levels of retinoic acid (vitamin A) may not be reflective of the cellular levels of retinoic acid,[46,47] meaning even if the serum retinoic acid level is normal, it may still be low in the cervical tissue.

Response rates to intervention with carotenoids have been inconsistent. In a double-blind, randomized, placebo-controlled trial composed of more than 100 women who used either 30 mg/day of beta-carotene or placebo,[48] cervical biopsies were performed before treatment and after 6 and 24 months. Persistence of CIN 3 resulted in the patient's removal from the study. Of the 124 women included, 21 were not randomized because they moved, became pregnant, or voluntarily withdrew or the pathological review of their initial cervical biopsies did not confirm CIN 2 or 3. Of the remaining 103 women, 33 experienced lesion regression, 45 had persistent or progressive disease, and 25 women did not complete the study and were considered nonresponders.

The overall regression rate (32%) was similar between the beta-carotene and placebo arms and when stratified for CIN grade. HPV typing of 99 women showed that 77% were HPV positive and 23% HPV negative at enrollment. HPV-positive lesions were subdivided into indeterminate-, low-, and high-risk categories. The response rate was highest for women with no HPV detected (61%), lower for those ranked at indeterminate or low risk (30%), and lowest for those classified at high risk (18%). In conclusion, beta-carotene did not enhance the regression of high-grade CIN, especially in HPV-positive subjects.[48]

Other intervention studies also fared poorly for beta-carotene:

- No difference in regression of CIN 1 lesions after 12 months with 30 mg of beta-carotene daily versus placebo[49]
- No regression of CIN lesions with 10 mg/day of beta-carotene versus placebo[50]
- After 9 months, lower regression rates of CIN 1 to 3 with 30 mg/day beta-carotene versus placebo[51]
- Slightly increased progression of ASCUS and CIN 1 with 30 mg/day of beta-carotene versus no treatment[52]

However, naturopathically, carotenoids and retinols can offer several types of protection. They improve the integrity and function of the epithelial tissues, regulate cellular differentiation, provide antioxidant properties, and enhance immune system function (see Chapter 136).[47]

In an HPV-immortalized human ectocervical epithelial cell line, retinoids downregulated the cell proliferative activity of epidermal growth factor receptor (EGFR) expression.[53]

Topical vitamin A was used in a study of 301 women who received either four consecutive 24-hour applications (using a collagen sponge in a cervical cap) of retinoid or placebo followed by two more applications at 3 and 6 months. Retinoic acid increased the complete regression rate of moderate dysplasia from 27% in the placebo group to 43% in the treatment group. The women with severe cervical dysplasia did not improve.[54] In a University of Arizona study, vitamin A was delivered to 20 women via a cervical cap. In 10 of 20 women, cervical dysplasia completely disappeared. Of the 10 patients with a complete response, 5 had mild dysplasia, and 5 had moderate dysplasia.[55] There were too few patients with severe dysplasia to be evaluated.

Vitamin A suppositories have also been used as part of a multifactorial treatment plan using oral folic acid, vitamin C, and carotenes with topical vitamin A suppositories and herbal vitamin suppositories. Other patients with more severe disease were treated with a topical "escharotic treatment." This study of atypia, mild, moderate, and severe dysplasia and carcinoma in situ comprised 43 women.[56,57] Of these, 38 returned to a normal disease-free state, 3 had partial improvements, 2 stayed the same, and none progressed to a more severe state of dysplasia during the course of the natural treatment protocol.

Newer research has illuminated a possible biochemical explanation for the improvements seen with retinoic acid and abnormal cervical cells.[46,47] An enzyme that metabolizes retinoic acid (CYP26A1) was expressed in LSIL, HSIL, and cervical cancer. It was not present in normal cervical epithelium. The authors theorized that the overexpression of CYP26A1 in the cervix may contribute to the development and progression of cervical cell abnormalities.[46,47]

It seems that vitamin A, instead of beta-carotenes, may be a better recommendation for cervical dysplasia. With vitamin A (retinyl palmitate), it is important to watch for symptoms of excess, with the first usually being a low-grade headache. If this occurs, the vitamin A dosage should be lowered or discontinued. As a reminder, vitamin A dosages in excess of 10,000 IU/d are not considered safe during pregnancy.

Vitamin C

A significant decrease in vitamin C intake and plasma levels occurs in patients with cervical dysplasia, and it has been documented that inadequate vitamin C intake is an independent risk factor for the development of premalignant cervical disease and carcinoma in situ.[43,58,59] Vitamin C is known to do the following[29]:

- Act as an antioxidant
- Strengthen and maintain normal epithelial integrity
- Improve wound healing

- Enhance immune function
- Inhibit carcinogen formation

Folate

Low folate levels are implicated in many cases of cervical dysplasia, although this link is less now with the widespread folic acid fortification of the food supply. When cervical cells lack folate, they become "macrocytic" in the same way as red blood cells (RBCs). Cervical cytological abnormalities related to folate deficiency precede hematologic abnormalities by many weeks.[60,61] Before fortification of the food supply with folic acid, it was the most common vitamin deficiency in the world and was especially common in women who were pregnant or taking oral contraceptives (OCs).[61,62] It is possible that many abnormal cytological smears in the past reflected folate deficiency rather than "true" dysplasia.[52,54,61]

Even with food fortification, folate is still a factor in many cases of cervical dysplasia. This observation is particularly applicable to patients taking OCs. It has been hypothesized that OCs induce a localized interference with folate metabolism, and although serum levels may be increased, tissue levels at end-organ targets, such as the cervix, may be deficient.[63,64] This is consistent with the observation that tissue status as measured by erythrocyte folate is typically decreased (especially in those with cervical dysplasia), whereas serum levels may be normal or even increased.[65] OCs are believed to induce the synthesis of a macromolecule that inhibits folate uptake by cells. In controlled clinical studies in women with cervical dysplasia taking OCs, folic acid supplementation (10 mg/day) has resulted in the improvement or normalization of Pap smears.[63,66,67] Regression rates for patients with untreated cervical dysplasia are typically 1.3% for mild and 0% for moderate dysplasia. When patients were treated with folic acid, the regression-to-normal rate, as determined by colposcopy/biopsy examination, was observed to be 20% in one study,[67] 63.7% in another,[66] and 100% in another.[63] Furthermore, the progression rate of cervical dysplasia in untreated patients is typically 16% at 4 months, a figure matched in the placebo group in one study, whereas the folate-supplemented group had a 0% progression rate.[64] These figures were achieved despite the fact that the women remained on OCs.

Lower folate status has been shown to enhance the effect of the other risk factors for cervical dysplasia. For example, low RBC folate appears to be a major risk factor for HPV infection of the cervix.[66–68] In particular, higher circulating concentrations of folate are independently associated with a lower likelihood of becoming positive for high-risk human papillomaviruses (HR-HPVs) and of having a persistent HR-HPV infection and a greater risk for HSIL.

Vitamin B_{12} supplementation should always accompany folate supplementation to rule out the possibility that the latter may be masking an underlying vitamin B_{12} deficiency. In addition, women with higher concentrations of plasma folate who also had sufficient plasma vitamin B_{12} had 70% lower odds of being diagnosed with cervical dysplasia.[69]

Pyridoxine

Vitamin B_6 status, as determined by erythrocyte transaminase, is decreased in one third of patients with cervical cancer.[70] Decreased pyridoxine status would have a significant effect on the metabolism of estrogens and tryptophan while also impairing the immune response.

Selenium

Low selenium levels in the diet and blood have been reported to be significantly lower in patients with cervical dysplasia. In one study, significantly lower selenium and zinc levels were found in both HSIL and cervical cancer patients compared with the control group. The activity of the selenium-containing antioxidant enzyme glutathione peroxidase was significantly lower in the patients with HSIL or cancer than with the control group, and total antioxidant ability decreased from the control group to those with CIN to those with cancer. Increased glutathione peroxidase activity resulting from increased selenium intake is believed to be the factor responsible for selenium's anticarcinogenic effect, although other factors may be of equal significance.[71]

A 2002 study did not find a correlation with serum selenium (67.5–185.0 ng/mL) levels and a relationship to invasive cervical cancer in 227 invasive cases, 127 in-situ cases, and 526 controls.[72]

Copper:Zinc Ratio, Zinc, and Retinol

An increase in the serum copper:zinc ratio is a nonspecific reaction to inflammation or malignancy. In a study involving gynecological cancer, it was suggested that the serum copper:zinc ratio "is clinically of utmost importance, since it constitutes a tool with which to establish the extent of the cancer."[73] A ratio above 1.95 indicated malignancy in 90% of the patients in that study. Elevated ratios are also seen in various conditions, including the following:

- OC use
- Pregnancy
- Acute and chronic infections
- Chronic liver disease
- Inflammatory conditions

Therefore the serum copper:zinc ratio should not be used to predict malignancy in patients with these conditions. A decrease in available zinc may explain why retinol-binding protein was either absent or undetectable in 80% of dysplastic tissue samples compared with 23.5% in normal tissue. A large study of 206 women found an inverse relationship between serum levels of both retinol and zinc and the incidence of cervical dysplasia.[74]

Indole-3-Carbinol/Diindolylmethane

Indole-3-carbinol (I3C) is a phytochemical found in vegetables of the cabbage family. It is converted in the stomach to several compounds, including diindolylmethane (DIM). I3C and DIM are antioxidants and potent stimulators of natural detoxifying enzymes in the body. Studies have shown that increasing the intake of vegetables of the cabbage family or taking I3C or DIM as a dietary supplement significantly increases the conversion of estrogen from cancer-producing forms to nontoxic breakdown products.[75,76] The body breaks down estrogen in several ways. It can be converted into a substance called 16-α-hydroxyestrone, a compound that promotes estrogen-dependent cancer. Another method of breakdown produces 2-hydroxyestrone, which does not stimulate cancer cells. Women with HSIL have altered estrogen metabolism, with a higher level of 16-α hydroxyestrone and fewer 2-hydroxyestrogen metabolites than normal.[77]

Given the ability of I3C or DIM to improve estrogen metabolism and possibly exert anti-HPV activity, these agents are very good candidates in the treatment of cervical dysplasia.[76] Preliminary studies are very encouraging. In one double-blind, placebo-controlled study of 30 women with HSIL (biopsy-proved CIN 2 or 3), the women were given either 200 or 400 mg of I3C or a placebo for 12 weeks.[77] In 4 of 8 patients in the group who took 200 mg per day of I3C and 4 of 9 in the 400-mg group, there was complete regression of their severe dysplasia, compared with none of the placebo group. HPV was detected in 7 of 10 placebo patients, in 7 of 8 in the 200 mg/day group, and in 8 of 9 in the 400 mg/day group.

DIM was used in another study of 64 patients with HSIL (biopsy-proved CIN 2 or 3) who were scheduled for loop electrosurgical excision procedure (LEEP). The patients were randomized 2:1 to receive DIM at approximately 2 mg/kg/day for 12 weeks or placebo.

Although there was no statistically significant difference in any outcome between the DIM and placebo groups, the overall results with DIM showed an improved Pap smear in 49% (22 out of 45), with either a less severe abnormality or normal result. Colposcopy also improved in 25 subjects in the DIM group (56%).[78]

Botanical Medicines

Green Tea

Constituents of green tea, namely polyphenol E and epigallocatechin-3-gallate (EGCG), have been effective against HPV-infected cervical cells and lesions in both laboratory and clinical studies. Green tea appears to induce apoptosis of HPV-infected cervical cells and also to arrest cell cycles, modify gene expression, and inhibit tumor formation.[79,80]

A clinical study confirmed these findings in patients through the use of either topical application via a green tea polyphenol ointment and/or oral ingestion of a green tea polyphenol capsule or an EGCG capsule. All treatment groups improved more than the placebo group (50%–75% vs. 10%), but those given the topical treatment improved most significantly.[80]

Coriolus Versicolor

The *Coriolus versicolor* mushroom has a mucopolysaccharide and polysaccharide protein complex that composes its cell wall. These complexes are high in beta-glucans. Studies done with mucopolysaccharides indicate many beneficial health benefits, such as anticarcinogenic, immune-modulating, antimicrobial, and anti-inflammatory effects, to name a few.[81] There have been at least three studies on *C. versicolor* and HPV.[82–84] Unfortunately, no further details can be provided because the studies are all in Bulgarian. However, the Bogdanova study[82] used *Coriolus* to study the ability to reverse the early stages of cervical cancer and reduce the risk factors of recurring HPV virus.

Miscellaneous Considerations

Vaginal Depletion Pack

The vaginal depletion pack (or "vag pack") has a long history of effective use by naturopathic and eclectic physicians in the treatment of cervical dysplasia. Although its mechanism of action has not yet been elucidated, it is thought to work by promoting the sloughing of the superficial cervical cells, particularly those that are abnormal. It is effective as part of a multifactorial nutritional and topical treatment approach.[56,57] (See Appendix 14 for a complete description of this technique.)

Escharotic Treatment

The escharotic treatment is a topical herbal cryotherapy treatment of the cervix used to remove abnormal cells. It involves the use of zinc chloride mixed with *Sanguinaria canadensis,* a botanical. A full description of this protocol is given in Appendix 2. The escharotic treatment is especially indicated for CIN 2 and CIN 3, both HGSILs, but only when a satisfactory colposcopy has been performed by a clinician. In addition, the use of the escharotic treatment, rather than a LEEP or conization, must fall within the guidelines outlined under "Therapeutic Approach." The escharotic treatment is best implemented twice a week, with 2 full days between treatments. The zinc chloride solution must be made by a compounding pharmacy as a prescription item.

A 2009 case report involved a 20-year-old female with CIN 2, 3 who refused conventional recommendation and instead received escharotic treatment twice weekly for 5 weeks and oral vitamins and botanicals. She had a complete reversal of the diagnosis at her 4-month and 10-month check-ups. She was followed for 5 years and remained negative for abnormal Pap smears.[85]A case report involving a 28-year-old female with CIN 3 who followed the same escharotic treatment protocol (as the 2009 case report) had similar results.[86]

THERAPEUTIC APPROACH

Treatment of cervical dysplasia requires proper monitoring and coordination of care if one practitioner is doing the workup with colposcopy/biopsies and another is proceeding with natural or integrated treatment approaches. The basic approach is to eliminate all factors known to be associated with cervical dysplasia and to optimize the patient's nutritional status. In particular, smoking and OC use are eliminated, and the patient follows the supplementation program listed here.

The guidelines provide further insight into the appropriate medical care of cervical dysplasia.

A. Criteria for Naturopathic Protocol
   1. ASC-US
   2. ASC-US with documented HPV
   3. ASC-H: endocervical curettage is negative or positive with a satisfactory colposcopy.
   4. Low-grade squamous intraepithelial neoplasia: endocervical curettage is negative, with a satisfactory colposcopy.
   5. High-grade squamous intraepithelial neoplasia: endocervical curettage is negative, with a satisfactory colposcopy.
   6. ASC-H: endocervical curettage is positive, with a satisfactory colposcopy, but either the patient is at low risk for more serious disease, has a low-risk type of HPV, or even if the patient does not have low-risk HPV, the natural treatment protocol may be recommended at the discretion of the practitioner.
   7. Low-grade squamous intraepithelial neoplasia: endocervical curettage is positive, with a satisfactory colposcopy, but either the patient is at low risk for more serious disease or has a low-risk type of HPV or the practitioner may recommend the natural treatment protocol at his or her discretion.
   8. High-grade squamous intraepithelial neoplasia: endocervical curettage is positive, with a satisfactory colposcopy, but the patient is at low risk, or even if not, the natural treatment protocol may be recommended at the discretion of the practitioner and considered carefully after colposcopy, biopsies, and careful evaluation.
   9. It is possible to treat carcinoma in situ in select cases, but this is definitely a judgment call and should be considered very carefully after colposcopy, biopsies, and careful follow-up.

B. Referrals for Colposcopy With Biopsies
   1. ASC-US if HPV DNA testing is positive for high-risk HPV; if no HPV testing is done, then the Pap test should be repeated twice at 4- to 6-month intervals. If HPV typing is negative for high-risk types, then the Pap test should be repeated in 12 months.
   2. ASC-H
   3. Low-grade squamous intraepithelial lesions
   4. High-grade squamous intraepithelial lesions
   5. Atypical glandular cells of undetermined significance (AGUS); endometrial biopsy is needed as well.
   6. Adenocarcinoma in situ (AIS): endometrial biopsy is needed as well.
   7. Pap smear diagnosis of microinvasion or frank invasion
   8. Endometrial cells present in a postmenopausal woman; also calls for an endometrial biopsy even if the cells are benign.

9. A patient who may not follow through with the recommended follow-up Pap smear after an abnormal Pap result
10. Visible unknown cervical lesion regardless of the Pap smear test result
11. Initial examination of a DES daughter
12. Unexplained or persistent cervical bleeding
13. Vulvar condyloma with abnormal Pap test result
14. To be used for follow-up after treatment plan is completed, especially in high-grade squamous intraepithelial lesions

C. Referrals for Conization or LEEP
1. Pap test results show more than one grade of dysplasia different from that seen on colposcopy or reported on in the biopsy.
2. Biopsy shows squamous intraepithelial lesions with three to four quadrants involved.
3. Unsatisfactory colposcopy with any degree of squamous intraepithelial lesions on biopsy
4. The patient may not be a good candidate for ongoing treatment and the closer follow-up required by alternative treatments.
5. No improvement in pathology with the initial naturopathic plan or repeated alternate plan
6. If AGUS is found on the Pap test and no disease is detected on colposcopy, biopsies and endocervical curettage are indicated.
7. If AIS is found on the Pap test and no disease is detected on colposcopy, biopsies and endocervical curettage are indicated.

D. At the Discretion of the Practitioner and Patient
1. Positive endocervical curettage with any degree of squamous intraepithelial lesions. A more assertive approach is recommended.
2. High-risk patients: the last Pap test more than 1 year previous, a history of genital warts, a history of cervical dysplasia, smokers, multiple sex partners with lack of safe-sex practices. In these cases, a more proactive and assertive approach is recommended.

E. Referral for Probable Hysterectomy
1. Microinvasive cervical cancer
2. Frank invasive cervical cancer
3. Adenocarcinoma

Diet

Increase fruits and vegetables, especially those yellow and orange in color. A general immune-supportive diet is considered prudent—high in fiber, vegetables, fruits, nuts, and seeds; low in fat and saturated fat.

Nutritional Supplements

- High-potency multiple vitamin and mineral formula
- Folate or methyltetrahydrofolate: 10 mg/day for 3 months, then 2.5 mg/day for 1 year
- Vitamin B_{12} (methylcobalamin): 1 mg/day
- Retinyl palmitate: 50,000 IU/day for 1 month, then decrease to 15 to 25,000 IU/day until repeat testing is normal. Refer to the previous section on vitamin A for cautions.
- Vitamin C: 1 to 3 g/day for 3 to 12 months
- Vitamin E: 200 IU/day for 3 to 12 months
- Selenium: 200 to 400 mcg/day for 3 to 12 months
- Zinc: 20 to 30 mg/day for 3 to 12 months
- Choose one of the following:
  - I3C: 200 to 400 mg/day for 3 months
  - DIM: 2.2 mg per kg body weight/day

Botanical Medicines

- Green tea extract (>90% total polyphenol content): 150 to 300 mg/day for 3 to 12 months

Sample Treatment Protocols

Atypical Cells of Undetermined Significance

- Topical
  Week 1: Insert vitamin A suppository every night for 6 nights.
  Week 2: Insert herbal vaginal suppository every night for 6 nights.
  Week 3: Repeat vitamin A.
  Week 4: Repeat herbal.
  Weeks 5 to 12: Insert green tea suppository 2 nights a week.
- Supplementation as previously noted for a minimum of 3 months

Low-Grade Squamous Intraepithelial Lesions

- Topical
  Week 1: Insert vitamin A suppository every night for 6 nights.
  Insert vag pack suppository on night 7.
  Week 2: Insert herbal suppository every night for 6 nights.
  Insert vag pack suppository on night 7.
  Week 3: Repeat vitamin A.
  Insert vag pack suppository on night 7.
  Week 4: Repeat herbal suppository.
  Insert vag pack suppository on night 7.
  Weeks 5 to 12: insert green tea suppository twice a week.
- Supplementation as previously noted for a minimum of 3 months

High-Grade Squamous Intraepithelial Lesions

- Topical
  Escharotic treatment twice a week for 5 weeks
  The last treatment is followed by 1 month of suppository routine as in LGSIL, then a green tea suppository twice a week for weeks 5 through 12.
- Supplementation as previously noted for a minimum of 3 months

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Smith RA, Andrews KS, Brooks D, et al. Cancer screening in the United States, 2017: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin*. 2017;67(2):100–121.
2. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for colposcopy and cervical pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin*. 2012;62(3):147–172.
3. Kauffman RP, Griffin SJ, Lund JD, Tullar PE. Current recommendations for cervical cancer screening: do they render the annual pelvic examination obsolete? *Med Princ Pract*. 2013;22(4):313–322.
4. Martingano D, Renson A, Martingano AJ, Martingano FX. Variations in progression and regression of precancerous lesions of the uterine cervix on cytology testing among women of different races. *J Am Osteopath Assoc*. 2018;118(1):8–18.
5. Arbyn M, Verdoodt F, Snijders PJ, et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. *Lancet Oncol*. 2014;15(2):172–183.
6. Tong TR, Chan OW, Chow TC, Yu V, Leung KM, To SH. Detection of human papillomavirus in sanitary napkins: a new paradigm in cervical cancer screening. *Diagn Cytopathol*. 2003;28(3):140–141.
7. Kim SR, Song SY, Kim DS, et al. Pad—a new self-collection device for human papillomavirus. *Int J STD AIDS*. 2007;18(3):163–166.
8. Harper DM, Raymond M, Noll WW, Belloni DR, Duncan LT, Cole BF. Tampon samplings with longer cervicovaginal cell exposures are equivalent to two consecutive swabs for the detection of high-risk human papillomavirus. *Sex Transm Dis*. 2002;29(11):628–636.
9. Budukh A, Palayekar V, Maheshwari A, et al. Menstrual pad, a cervical cancer screening tool, a population-based study in rural India. *Eur J Cancer Prev*. 2017.
10. Lee B, Cho HY, Jeon KJ, et al. Detection of high-risk human papillomavirus using menstrual blood in women with high-grade squamous intraepithelial lesions or high-risk human papillomavirus infections: a pilot study. *J Obstet Gynaecol Res*. 2016;42(3):319–324.
11. Kumar V, Robbins SL. *Robbins Basic Pathology*. 8th ed. Philadelphia, PA: Saunders/Elsevier; 2007.
12. de Vet HC, Sturmans F. Risk factors for cervical dysplasia: implications for prevention. *Public Health*. 1994;108(4):241–249.
13. Leto M, Santos Júnior GF, Porro AM, Tomimori J. Human papillomavirus infection: etiopathogenesis, molecular biology and clinical manifestations. *An Bras Dermatol*. 2011;86(2):306–317.
14. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348(6):518–527.
15. Schmitt M, Depuydt C, Benoy I, et al. Multiple human papillomavirus infections with high viral loads are associated with cervical lesions but do not differentiate grades of cervical abnormalities. *J Clin Microbiol*. 2013;51(5):1458–1464.
16. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999;189(1):12–19.
17. Human papillomavirus (HPF). Centers for Disease Control and Prevention. www.cdc.gov/hpv/parents/questions-answers.html. Accessed January 13, 2018.
18. Clarke EA, Morgan RW, Newman AM. Smoking as a risk factor in cancer of the cervix: additional evidence from a case-control study. *Am J Epidemiol*. 1982;115(1):59–66.
19. Lyon JL, Gardner JW, West DW, Stanish WM, Hebertson RM. Smoking and carcinoma in situ of the uterine cervix. *Am J Public Health*. 1983;73(5):558–562.
20. Marshall JR, Graham S, Byers T, Swanson M, Brasure J. Diet and smoking in the epidemiology of cancer of the cervix. *J Natl Cancer Inst*. 1983;70(5):847–851.
21. Clarke EA, Hatcher J, McKeown-Eyssen GE, Lickrish GM. Cervical dysplasia: association with sexual behavior, smoking, and oral contraceptive use? *Am J Obstet Gynecol*. 1985;151(5):612–616.
22. Pelleter O. Vitamin C and tobacco. *Int J Vitam Nutr Res*. 1977;16:147–169.
23. Parazzini F, la Vecchia C, Negri E, Maggi R. Oral contraceptive use and invasive cervical cancer. *Int J Epidemiol*. 1990;19(2):259–263.
24. Brinton LA. Oral contraceptives and cervical neoplasia. *Contraception*. 1991;43(6):581–595.
25. Kjaer SK, Engholm G, Dahl C, Bock JE, Lynge E, Jensen OM. Case-control study of risk factors for cervical squamous-cell neoplasia in Denmark. III. Role of oral contraceptive use. *Cancer Causes Control*. 1993;4(6):513–519.
26. Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *J Natl Cancer Inst*. 1993;85(12):958–964.
27. Coker AL, McCann MF, Hulka BS, Walton LA. Oral contraceptive use and cervical intraepithelial neoplasia. *J Clin Epidemiol*. 1992;45(10):1111–1118.
28. Ursin G, Peters RK, Henderson BE, d'Ablaing G, Monroe KR, Pike MC. Oral contraceptive use and adenocarcinoma of cervix. *Lancet*. 1994;344(8934):1390–1394.
29. Webb JL. Nutritional effects of oral contraceptive use: a review. *J Reprod Med*. 1980;25(4):150–156.
30. Papathanasiou K, Gianoulis C, Tolikas A, et al. Effect of depleted uranium weapons used in the Balkan War on the incidence of cervical intraepithelial neoplasia (CIN) and invasive cancer of the cervix in Greece. *Clin Exp Obstet Gynecol*. 2005;32(1):58–60. PubMed PMID: 15864941.
31. Rzymski P, Niedzielski P, Rzymski P, Tomczyk K, Kozak L, Poniedzialek B. Metal accumulation in the human uterus varies by pathology and smoking status. *Fertil Steril*. 2016;105(6):1511–1518. PubMed PMID: 26921623.
32. Ndebele K, Graham B, Tchounwou PB. Estrogenic activity of coumestrol, DDT, and TCDD in human cervical cancer cells. *Int J Environ Res Public Health*. 2010;7(5):2045–2056. PubMed PMID: 20623010.
33. Orr JW, Wilson K, Bodiford C, et al. Nutritional status of patients with untreated cervical cancer. I. Biochemical and immunologic assessment. *Am J Obstet Gynecol*. 1985;151(5):625–631.
34. Orr JW, Wilson K, Bodiford C, et al. Nutritional status of patients with untreated cervical cancer. II. Vitamin assessment. *Am J Obstet Gynecol*. 1985;151(5):632–635.
35. Thomson SW, Heimburger DC, Cornwell PE, et al. Effect of total plasma homocysteine on cervical dysplasia risk. *Nutr Cancer*. 2000;37(2):128–133.
36. Tomita LY, Longatto Filho A, Costa MC, et al. Diet and serum micronutrients in relation to cervical neoplasia and cancer among low-income Brazilian women. *Int J Cancer*. 2010;126(3):703–714.
37. Ghosh C, Baker JA, Moysich KB, Rivera R, Brasure JR, McCann SE. Dietary intakes of selected nutrients and food groups and risk of cervical cancer. *Nutr Cancer*. 2008;60(3):331–341.
38. Palan PR, Mikhail MS, Shaban DW, Romney SL. Plasma concentrations of coenzyme Q10 and tocopherols in cervical intraepithelial neoplasia and cervical cancer. *Eur J Cancer Prev*. 2003;12(4):321–326.
39. Hwang JH, Kim MK, Lee JK. Dietary supplements reduce the risk of cervical intraepithelial neoplasia. *Int J Gynecol Cancer*. 2010;20(3):398–403.
40. La Vecchia C, Franceschi S, Decarli A, et al. Dietary vitamin A and the risk of invasive cervical cancer. *Int J Cancer*. 1984;34(3):319–322.
41. Romney SL, Palan PR, Duttagupta C, et al. Retinoids and the prevention of cervical dysplasias. *Am J Obstet Gynecol*. 1981;141(8):890–894.
42. Wylie-Rosett JA, Romney SL, Slagle NS, et al. Influence of vitamin A on cervical dysplasia and carcinoma in situ. *Nutr Cancer*. 1984;6(1):49–57.
43. Ramaswamy G, Krishnamoorthy L. Serum carotene, vitamin A, and vitamin C levels in breast cancer and cancer of the uterine cervix. *Nutr Cancer*. 1996;25(2):173–177.
44. Dawson E, Nosovitch J, Hannigan E. Serum vitamin and selenium changes in cervical dysplasia. *Fed Proc*. 1984;43:612.
45. Romney SL, Palan PR, Basu J, Mikhail M. Nutrient antioxidants in the pathogenesis and prevention of cervical dysplasias and cancer. *J Cell Biochem Suppl*. 1995;23:96–103.
46. Osanai M. Cellular retinoic acid bioavailability in various pathologies and its therapeutic implication. *Pathol Int*. 2017;67(6):281–291.
47. Osanai M, Lee GH. Increased expression of the retinoic acid-metabolizing enzyme CYP26A1 during the progression of cervical squamous neoplasia and head and neck cancer. *BMC Res Notes*. 2014;7:697.

48. Keefe KA, Schell MJ, Brewer C, et al. A randomized, double blind, Phase III trial using oral beta-carotene supplementation for women with high-grade cervical intraepithelial neoplasia. *Cancer Epidemiol Biomarkers Prev*. 2001;10(10):1029–1035.
49. Fairley C, Tabrizi S, Chen S, et al. A randomized clinical trial of beta-carotene vs. placebo for the treatment of cervical HPV infections. *Int J Gynecol Cancer*. 1996;6(3):225–230.
50. de Vet HC, Knipschild PG, Willebrand D, Schouten HJ, Sturmans F. The effect of beta-carotene on the regression and progression of cervical dysplasia: a clinical experiment. *J Clin Epidemiol*. 1991;44(3):273–283.
51. Romney SL, Ho GY, Palan PR, et al. Effects of beta-carotene and other factors on outcome of cervical dysplasia and human papillomavirus infection. *Gynecol Oncol*. 1997;65(3):483–492.
52. Mackerras D, Irwig L, Simpson JM, et al. Randomized double-blind trial of beta-carotene and vitamin C in women with minor cervical abnormalities. *Br J Cancer*. 1999;79(9–10):1448–1453.
53. Sah JF, Eckert RL, Chandraratna RA, Rorke EA. Retinoids suppress epidermal growth factor-associated cell proliferation by inhibiting epidermal growth factor receptor-dependent ERK1/2 activation. *J Biol Chem*. 2002;277(12):9728–9735.
54. Meyskens FL, Surwit E, Moon TE, et al. Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-trans-retinoic acid: a randomized trial. *J Natl Cancer Inst*. 1994;86(7):539–543.
55. Graham V, Surwit ES, Weiner S, Meyskens FL. Phase II trial of beta-all-trans-retinoic acid for cervical intraepithelial neoplasia delivered via a collagen sponge and cervical cap. *West J Med*. 1986;145(2):192–195.
56. Hudson T. Consecutive case study research of carcinoma in situ of cervix implying local escharotic treatment combined with nutritional therapy. *J Naturopath Med*. 1991;2:6–10.
57. Hudson T. Escharotic treatment for cervical dysplasia and carcinoma in situ. *J Naturopath Med*. 1993;4:23.
58. Wassertheil-Smoller S, Romney SL, Wylie-Rosett J, et al. Dietary vitamin C and uterine cervical dysplasia. *Am J Epidemiol*. 1981;114(5):714–724.
59. Romney SL, Duttagupta C, Basu J, et al. Plasma vitamin C and uterine cervical dysplasia. *Am J Obstet Gynecol*. 1985;151(7):976–980.
60. Van Niekerk W. Cervical cytological abnormalities caused by folic acid deficiency. *Acta Cytol*. 1966;10:67–73.
61. Kitay DZ, Wentz WB. Cervical cytology in folic acid deficiency of pregnancy. *Am J Obstet Gynecol*. 1969;104(7):931–938.
62. Streiff RR. Folate deficiency and oral contraceptives. *JAMA*. 1970;214(1):105–108.
63. Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. *JAMA*. 1973;226(12):1421–1424.
64. Butterworth CE, Hatch KD, Macaluso M, et al. Folate deficiency and cervical dysplasia. *JAMA*. 1992;267(4):528–533.
65. Harper JM, Levine AJ, Rosenthal DL, et al. Erythrocyte folate levels, oral contraceptive use and abnormal cervical cytology. *Acta Cytol*. 1994;38(3):324–330.
66. Butterworth CE, Hatch KD, Soong SJ, et al. Oral folic acid supplementation for cervical dysplasia: a clinical intervention trial. *Am J Obstet Gynecol*. 1992;166(3):803–809.
67. Butterworth CE, Hatch KD, Gore H, Mueller H, Krumdieck CL. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am J Clin Nutr*. 1982;35(1):73–82.
68. Flatley JE, McNeir K, Balasubramani L, et al. Folate status and aberrant DNA methylation are associated with HPV infection and cervical pathogenesis. *Cancer Epidemiol Biomarkers Prev*. 2009;18(10):2782–2789.
69. Piyathilake CJ, Macaluso M, Alvarez RD, Bell WC, Heimburger DC, Partridge EE. Lower risk of cervical intraepithelial neoplasia in women with high plasma folate and sufficient vitamin B12 in the post-folic acid fortification era. *Cancer Prev Res (Phila)*. 2009;2(7):658–664.
70. Ramaswamy PG, Natarajan R. Vitamin B6 status in patients with cancer of the uterine cervix. *Nutr Cancer*. 1984;6(3):176–180.
71. Patterson BH, Levander OA. Naturally occurring selenium compounds in cancer chemoprevention trials: a workshop summary. *Cancer Epidemiol Biomarkers Prev*. 1997;6(1):63–69.
72. Thompson FE, Patterson BH, Weinstein SJ, et al. Serum selenium and the risk of cervical cancer among women in the United States. *Cancer Causes Control*. 2002;13(6):517–526.
73. Brandes JM, Lightman A, Drugan A, Zinder O, Cohen A, Itskovtiz J. The diagnostic value of serum copper/zinc ratio in gynecological tumors. *Acta Obstet Gynecol Scand*. 1983;62(3):225–229.
74. Liu T, Soong SJ, Alvarez RD, Butterworth CE. A longitudinal analysis of human papillomavirus 16 infection, nutritional status, and cervical dysplasia progression. *Cancer Epidemiol Biomarkers Prev*. 1995;4(4):373–380.
75. Zeligs M. Diet and estrogen status: the cruciferous connection. *J Med Food*. 1998;1:67–81.
76. Newfield L, Goldsmith A, Bradlow HL, Auborn K. Estrogen metabolism and human papillomavirus-induced tumors of the larynx: chemo-prophylaxis with indole-3-carbinol. *Anticancer Res*. 1993;13(2):337–341.
77. Bell MC, Crowley-Nowick P, Bradlow HL, et al. Placebo-controlled trial of indole-3-carbinol in the treatment of CIN. *Gynecol Oncol*. 2000;78(2):123–129.
78. Del Priore G, Gudipudi DK, Montemarano N, Restivo AM, Malanowska-Stega J, Arslan AA. Oral diindolylmethane (DIM): pilot evaluation of a nonsurgical treatment for cervical dysplasia. *Gynecol Oncol*. 2010;116(3):464–467.
79. Sah JF, Balasubramanian S, Eckert RL, Rorke EA. Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. *J Biol Chem*. 2004;279(13):12755–12762.
80. Ahn WS, Yoo J, Huh SW, et al. Protective effects of green tea extracts (polyphenon E and EGCG) on human cervical lesions. *Eur J Cancer Prev*. 2003;12(5):383–390.
81. Hoffmann D. *Medical Herbalism: The Science and Practice of Herbal Medicine*. Rochester, Vt: Healing Arts Press; 2003.
82. Bogdanova J. Coriolus versicolor—innovation in prevention of oncogynecological diseases, especially HPV. *Akush Ginekol (Sofiia)*. 2008;47(suppl 3):51–53.
83. Borisov S. Coriolus-MRL-a new addition to the arsenal for complex treatment of oncogynecological diseases. *Akush Ginekol (Sofiia)*. 2009;48(suppl 2):31.
84. Trifonov G. Coriolus-MRL-a new addition to the arsenal for complex treatment of oncogynecological diseases. *Akush Ginekol (Sofiia)*. 2009;48(suppl 1):18.
85. Swanick S, Windstar-Hamlin K, Zwickey H. An alternative treatment for cervical intraepithelial neoplasia II, III. *Integr Cancer Ther*. 2009;8(2):164–167.
86. Windstar K, Dunlap C, Zwickey H. escharotic treatment for ECC-positive CIN3 in Childbearing Years: a case report. *Integr Med (Encinitas)*. 2014;13(2):43–49.

159

Chronic Candidiasis

Michael T. Murray, ND

OUTLINE

GENERAL CONSIDERATIONS

In the past 30 years, overgrowth in the gastrointestinal (GI) tract of the usually benign yeast *Candida albicans* has become increasingly recognized as a complex medical syndrome known as chronic candidiasis, or the yeast syndrome (Fig. 159.1).[1,2] Specifically, the overgrowth of *C. albicans* is believed to cause a wide variety of symptoms in virtually every system of the body, with the GI, genitourinary, endocrine, nervous, and immune systems being most susceptible.[3]

Although chronic candidiasis has been clinically defined for a long time, it was not until Orion Truss published *The Missing Diagnosis* and William Crook published *The Yeast Connection* that the public and many physicians became aware of the magnitude of the problem.[1,2]

Normally, *C. albicans* lives harmoniously in the inner creases and crevices of the digestive tract (and vaginal tract in women). However, when this yeast overgrows, immune system mechanisms are depleted, or when the normal lining of the intestinal tract is damaged, the body can absorb yeast cells, particles of yeast cells, and various toxins.[3] As a result, there may be significant disruption of body processes, resulting in the development of the "yeast syndrome."

Women are eight times more likely to experience candidiasis compared with men, likely the result of estrogen, oral contraceptive use, and the higher number of prescriptions for antibiotics (Box 159.1).[4] Fatigue, allergies, immune system malfunction, depression, chemical sensitivities, and digestive disturbances are some of the symptoms patients with the yeast syndrome may experience.[3]

Causal Factors

Chronic candidiasis is a classic example of a multifactorial condition (Box 159.2). Therefore the most effective treatment involves addressing and correcting the factors that predispose to *C. albicans* overgrowth and includes much more than killing the yeast with antifungal agents, whether synthetic or natural.

Antibiotics

Prolonged antibiotic use is believed to be the most important factor in the development of chronic candidiasis. Antibiotics, through the elimination of the normal intestinal bacteria that prevent yeast overgrowth and suppression of the immune system, strongly promote the overgrowth of *Candida.*

Syndromes Related to the Yeast Syndrome

Small intestinal bacterial overgrowth (SIBO) and the leaky gut syndrome are often associated with *C. albicans* overgrowth and may produce identical symptoms to the yeast syndrome. For further discussion of small intestinal bacterial overgrowth and leaky gut syndrome, see Chapters 9 and 19.

DIAGNOSIS

Questionnaire

One of the most useful screening methods for determining the likelihood of yeast-related illness is a comprehensive questionnaire (see Appendix 1).

Diagnosis of the yeast syndrome is primarily based on clinical judgment from a detailed medical history and patient questionnaire. In addition, laboratory techniques such as stool cultures for *C. albicans* and measurement of antibody levels to *C. albicans* or *C. albicans* antigens in the blood may aid in the diagnosis and can be used to confirm the diagnosis.

Comprehensive Digestive Stool Analysis

Rather than simply culture a stool sample for the presence of *C. albicans,* the comprehensive digestive stool analysis (CDSA) is more clinically useful (discussed in detail in Chapter 28). This battery of integrated diagnostic laboratory tests evaluates digestion, intestinal function, intestinal environment, and absorption by carefully examining the stool. It is a useful tool in determining

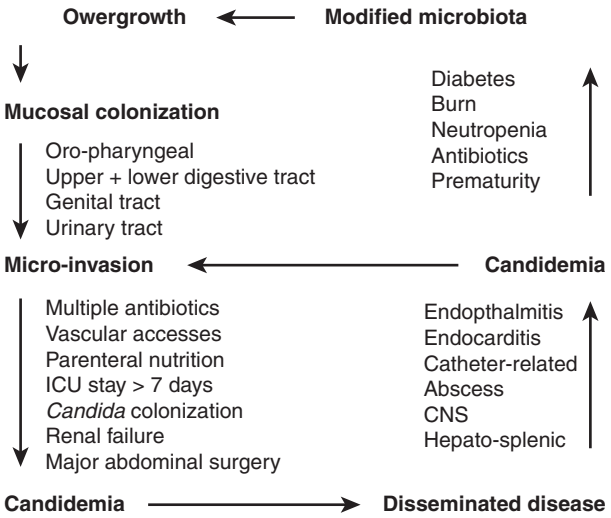


Fig. 159.1 Pathophysiology of chronic candidiasis. (From Eggimann P, Bille J, Marchetti O. Diagnosis of invasive candidiasis in the ICU. *Ann Intensive Care.* 2011;1:37. PubMed PMID: 21906271.)

the digestive disturbance that is likely to be the underlying factor responsible for *C. albicans* overgrowth. In addition, the CDSA may determine that the symptoms are not related to *C. albicans* overgrowth but, rather, to conditions such as SIBO and/or the leaky gut syndrome.

Antibody and Antigen Levels

Another laboratory method to confirm the presence of *C. albicans* overgrowth is measuring the level of antibodies to *Candida* or the level of antigens in the blood.[3,5] Although these tests are rarely necessary, some patients and physicians may desire confirmation that *C. albicans* is a responsible factor in the patient's health equation. In this situation, blood studies can be helpful and may also be used as a way of monitoring therapy.

THERAPEUTIC CONSIDERATIONS

A comprehensive approach is more effective in treating chronic candidiasis than simply trying to kill *C. albicans* with a drug or natural anti–*C. albicans* agent. Drugs like nystatin, ketoconazole, and Diflucan, as well as various natural anti–*C. albicans* agents, rarely produce significant long-term results because they fail to address the underlying factors that promote *C. albicans* overgrowth. In many cases, it is useful to try to eradicate *C. albicans* from the system, preferably with the help of natural anti–*C. albicans* therapies such as timed-release caprylic acid preparations, enteric-coated volatile oil preparations, or fresh garlic preparations. A follow-up stool culture and *C. albicans* antigen determination will confirm if the *C. albicans* has been eliminated. If *C. albicans* has been eradicated but symptoms persist, the symptoms are unrelated to an overgrowth of *C. albicans* and may be caused by SIBO. In this scenario, pancreatic enzymes and berberine-containing plants like goldenseal can be helpful.

Diet

A number of dietary factors appear to promote the overgrowth of *C. albicans.* The most important factors are a high intake of sugar, milk and other dairy products, foods containing a high content of yeast or mold, and food allergies/sensitivities.

BOX 159.1 Typical Chronic Candidiasis Patient Profile

Sex: Female

Age: 15 to 50 years

General Symptoms
- Chronic fatigue
- Loss of energy
- General malaise
- Decreased libido

Gastrointestinal Symptoms
- Thrush
- Bloating, gas
- Intestinal cramps
- Rectal itching
- Altered bowel function

Genitourinary System Complaints
- Vaginal yeast infection
- Frequent bladder infections
- Endocrine system complaints
- Primarily menstrual complaints

Nervous System Complaints
- Depression
- Irritability
- Inability to concentrate

Immune System Complaints
- Allergies
- Chemical sensitivities
- Low immune function

History
- Chronic vaginal yeast infections
- Chronic antibiotic use for infections or acne
- Oral birth control usage
- Oral steroid hormone usage

Associated Conditions
- Premenstrual syndrome
- Sensitivity to foods, chemicals, and other allergens
- Endocrine disturbances
- Eczema
- Psoriasis
- Irritable bowel syndrome

Other
- Craving for foods rich in carbohydrates or yeast

BOX 159.2 Predisposing Factors to *Candida albicans* Overgrowth

- Decreased digestive secretions
- Dietary factors
- Impaired immunity
- Nutrient deficiency
- Drugs (particularly antibiotics)
- Impaired liver function
- Underlying disease states
- Altered bowel flora
- Prolonged antibiotic use

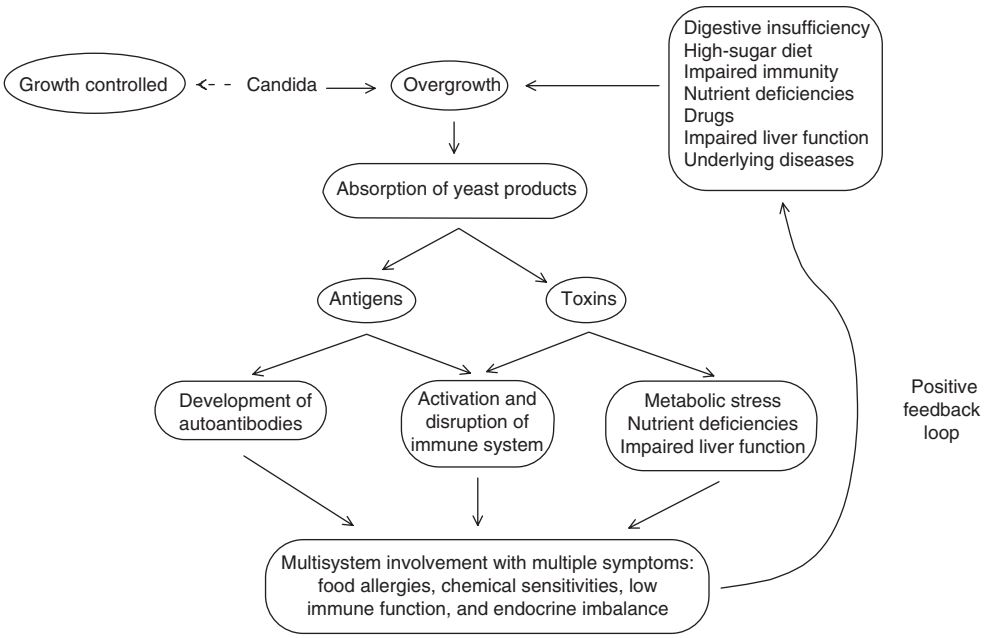


Fig. 159.2 The vicious cycle of chronic candidiasis.

Sugar

Sugar is the chief nutrient of *C. albicans*. It is well accepted that restriction of sugar intake is a necessity in the treatment of chronic candidiasis. Most patients do well by avoiding refined sugar and large amounts of honey, maple syrup, and fruit juice.[1–4]

Milk and Other Dairy Products

Several reasons to restrict or eliminate the intake of milk in patients with chronic candidiasis include the following:

- High lactose content promotes the overgrowth of *Candida*.
- Cow's milk and dairy products are some of the most frequent food allergens.
- Milk may contain trace levels of antibiotics, which can further disrupt the GI bacterial flora and promote *Candida* overgrowth.[1–4]

Mold- and Yeast-Containing Foods

Many experts recommend that individuals with chronic candidiasis avoid foods with a high content of yeast or mold, including alcoholic beverages, cheeses, dried fruits, and peanuts. Although many patients with chronic candidiasis may be able to tolerate these foods, it is best to eliminate these foods from the diet until the situation is resolved.[1–4]

Food Allergies

Food allergies are another common finding in patients with the yeast syndrome.[3] Enzyme-linked immunosorbent assay tests, which determine both immunoglobulin E– and immunoglobulin G–mediated food allergies, are often helpful in identifying food allergies.

Hypochlorhydria

Gastric hydrochloric acid, pancreatic enzymes, and bile all inhibit the overgrowth of *C. albicans* and prevent its penetration into the absorptive surfaces of the small intestine. Decreased secretion of any of these important digestive components can lead to overgrowth of *C. albicans* in the GI tract. Therefore the restoration of normal digestive secretions through the use of supplemental hydrochloric acid, pancreatic enzymes, and substances that promote bile flow is critical in the treatment of chronic candidiasis. The CDSA can provide valuable information in identifying which factor is most important.

People on antiulcer drugs, such as Tagamet (cimetidine) and Zantac (ranitidine), often develop *C. albicans* overgrowth in the stomach.[6] This occurrence highlights the importance of hydrochloric acid in the prevention of *C. albicans* overgrowth. Restoring proper levels of gastric acid with supplemental hydrochloric acid is often useful in chronic candidiasis.

Pancreatic enzymes can also be useful in the treatment of chronic candidiasis. As well as being necessary for protein digestion, proteases are responsible for keeping the small intestine free from parasites (including bacteria, yeast, protozoa, and intestinal worms).[7,8] A lack of proteases or other digestive secretions greatly increases an individual's risk of having an intestinal infection, including chronic *C. albicans* infections of the GI tract.

Enhancing Immunity

Recurrent or chronic infections, including chronic candidiasis, are characterized by a depressed immune system. The cycle of chronic candidiasis makes it difficult for individuals to overcome the condition (Fig. 159.2); a compromised immune system leads to infection, and infection leads to damage of the immune system, further weakening resistance.

The importance of a healthy immune system in protecting against *C. albicans* overgrowth is well known by physicians who work with patients having acquired immunodeficiency syndrome (AIDS) or those who may be taking immunosuppressive medications. The occurrence of *C. albicans* overgrowth in these conditions provides considerable evidence that improving immune function is essential in resolving chronic candidiasis.

In addition, patients with chronic candidiasis often have other chronic infections, likely secondary to a weakened immune system. Typically, this depression of immune function is related to decreased thymus function, primarily presenting as depressed cell-mediated immunity. Although expensive laboratory tests can document this depression, a history of repeated viral infections (including the common cold), outbreaks of cold sores or genital herpes, and prostatic (men) or vaginal (women) infections provides enough evidence to support reduced immune function.

BOX 159.3 Triggers to Impaired Immunity in Candidiasis

- Antibiotic use
- Corticosteroid use
- Other drugs that suppress the immune system
- Nutrient deficiency
- Food allergies
- High-sugar diet
- Stress

Causes of Depressed Immune Function in Candidiasis

Regarding the immune system, a triggering event such as antibiotic use or nutrient deficiency (Box 159.3) can lead to immune suppression, allowing *C. albicans* to overgrow and become more firmly entrenched in the lining of the GI tract. Once the organism attaches itself to the intestinal cells, it competes with the cell and ultimately the entire body for nutrition—potentially robbing the body of vital nutrients. In addition, *C. albicans* secretes a large number of mycotoxins and antigens and is referred to as a "polyantigenic" organism because more than 79 distinct antigens have been identified.[9,10]

Restoring Proper Immune Function

Restoring proper immune function requires a comprehensive approach involving lifestyle, stress management, exercise, diet, nutritional supplementation, glandular therapy, and the use of plant-based medicines.

Perhaps the most effective intervention in reestablishing a healthy immune system is employing measures designed to improve thymus function. Oral candidiasis occurs in roughly 90% of HIV-positive individuals, and the clinical remission of oral candidiasis has been shown to be critically dependent on T-cell function.[11,12] Presumably, improving thymus-mediated immunity is also critical in the remission of chronic candidiasis. Promoting optimal thymus gland activity involves the following:

- Prevention of thymic involution or shrinkage by ensuring adequate dietary intake of antioxidant nutrients, such as carotenes, vitamin C, vitamin E, zinc, and selenium
- The use of nutrients that are required in the manufacture or action of thymic hormones

Promoting Detoxification

Patients with chronic candidiasis may exhibit multiple chemical sensitivities and allergies, indicating that detoxification reactions are stressed. Therefore their liver function must be supported. When the liver is even slightly damaged by chemical toxins, immune function is severely compromised. Improving the health of the liver and promoting detoxification may be one of the most critical factors in the successful treatment of candidiasis.

The immune system–suppressing effect of nonviral liver damage has been repeatedly demonstrated in experimental animal studies and human studies. For example, when the liver of a rat is damaged by a chemical toxin, immune function is severely hindered.[13] Liver injury is also linked to *C. albicans* overgrowth, as evident in studies of mice demonstrating that when the liver is even slightly damaged, *C. albicans* runs rampant through the body.[14]

A rational approach to aiding the body's detoxification involves the following:

- A diet focused on fresh fruits and vegetables, whole grains, legumes, nuts, and seeds
- A healthy lifestyle, including avoiding alcohol and exercising regularly

BOX 159.4 Indications of the Need for Detoxification

- More than 20 lb overweight
- Diabetes
- Presence of gallstones
- History of heavy alcohol use
- Psoriasis
- Natural and synthetic steroid hormone use
  - Anabolic steroids
  - Estrogens
  - Oral contraceptives
- High exposure to certain chemicals or drugs
  - Cleaning solvents
  - Pesticides
  - Antibiotics
  - Diuretics
  - Nonsteroidal anti-inflammatory drugs
  - Thyroid hormone
  - History of viral hepatitis

- A high-potency multiple vitamin and mineral supplement
- Lipotropic formulas and silymarin to protect the liver and enhance liver function
- A 3-day fast at the change of each season

If any of the factors in Box 159.4 apply to the patient, enhancing detoxification is a major therapeutic goal.

Lipotropic Factors

The nutrients choline, betaine, and methionine are often beneficial in enhancing liver function and detoxification reactions. These compounds, referred to as lipotropic agents, promote the flow of fat and bile to and from the liver. In essence, they produce a "decongesting" effect on the liver and promote improved liver function and fat metabolism. Formulas containing lipotropic agents are useful in enhancing detoxification reactions and other liver functions. Lipotropic formulas have been used for a wide variety of conditions, including a number of liver disorders such as hepatitis, cirrhosis, and chemical-induced liver disease. The daily dosage should be 1000 mg of choline and 1000 mg of methionine or cysteine, or both.

Lipotropic formulas appear to increase the levels of two important liver substances: S-adenosyl-methionine, the major lipotropic compound in the liver, and glutathione, one of the major detoxifying compounds in the liver.[15,16]

Promoting Elimination

A diet that focuses on high-fiber plant foods should be sufficient to promote proper elimination by supplying an ample amount of dietary fiber. If additional support is needed, fiber formulas can be prescribed. These formulas are composed of natural plant fibers derived from psyllium seed, kelp, agar, pectin, and plant gums like karaya and guar. Alternatively, fibers can be purified semisynthetic polysaccharides like methylcellulose and carboxymethylcellulose sodium. Psyllium-containing laxatives are the most popular and usually the most effective. Fiber formulas most closely approximate the natural mechanism that promotes a bowel movement. In the treatment of candidiasis, 3 to 5 g of soluble fiber at bedtime should be recommended, especially if antiyeast therapies are employed, to ensure that dead yeast cells are excreted and not absorbed.

Probiotics

Intestinal flora plays a major role in the health of the host, especially in the battle against GI tract infection.[17,18] Intestinal flora is intimately involved in the host's nutritional status and affects immune system function, cholesterol metabolism, carcinogenesis, and aging. Although probiotic supplements such as *Lactobacillus acidophilus* and *Bacterium bifidum* can be used to promote overall good health, the four primary uses related to chronic candidiasis are the promotion of a proper intestinal environment, postantibiotic therapy, vaginal yeast infections, and urinary tract infections.

The dosage of a commercial probiotic supplement is based on the number of live organisms. The ingestion of 5 to 10 billion viable *L. acidophilus* or *B. bifidum* cells daily is a sufficient dosage for most people. Amounts exceeding these may induce mild GI disturbances, whereas smaller amounts may not be able to colonize the GI tract.

See Chapters 104 and 105 for a more comprehensive discussion of these useful agents.

Natural Antiyeast Agents

A number of natural agents have proven activity against *C. albicans.* Rather than relying on these agents as a primary therapy, it is important to address the factors that predispose one to chronic candidiasis, especially a lack of either hydrochloric acid or pancreatic enzymes. The five natural agents recommended against *C. albicans* are as follows:

- Caprylic acid
- Berberine-containing plants
- Garlic
- Enteric-coated volatile oil preparations
- *Propolis*

Most patients can achieve benefits from the natural agents described rather than the use of pharmaceuticals. Antiyeast therapy can produce a die-off (Herxheimer) reaction due to the rapid killing of the organism and subsequent absorption of large quantities of yeast toxins, cell particles, and antigens. The Herxheimer reaction refers to a worsening of symptoms as a result of this die-off. The Herxheimer reaction can be minimized by the following means:

- Following the dietary recommendations for a minimum of 2 weeks before taking an antiyeast agent
- Supporting the liver
- Starting any antiyeast medications in low doses and gradually increasing dosages over 1 month to achieve full therapeutic dosage

Caprylic Acid

Caprylic acid is a naturally occurring fatty acid that has been reported to be an effective antifungal compound in the treatment of candidiasis.[19,20] Because caprylic acid is readily absorbed in the intestines, it is necessary to take timed-release or enteric-coated caprylic acid formulas to allow for gradual release throughout the entire intestinal tract.[21] The standard dose for these delayed-release preparations is 1000 to 2000 mg with meals.

Chitosan

Chitosan is a natural polysaccharide derived from the outer skeleton of shellfish composed of β-linked D-glucosamine and N-acetyl-D-glucosamine. It is used in the treatment of obesity, high cholesterol, and Crohn's disease. In addition, a randomized, single-blind, clinical trial investigated the antifungal effects of low-molecular-weight chitosan solution on *C. albicans* in denture stomatitis in comparison with nystatin suspension.[22] After 2 weeks of treatment, the chitosan solution significantly decreased the erythematous surface area, burning sensation, and time required for clinical improvement, making it a promising candidate for use as an antifungal mouthwash.

Berberine-Containing Plants

Berberine-containing plants include goldenseal *(Hydrastis canadensis),* barberry *(Berberis vulgaris),* Oregon grape *(Berberis aquifolium),* and goldthread *(Coptis chinensis).* Berberine, an alkaloid, has been extensively studied in both experimental and clinical settings for its antibiotic activity. Berberine exhibits a broad spectrum of antibiotic activity, having shown antibiotic activity against bacteria, protozoa, and fungi, including *C. albicans.*[23–29] Berberine's action in inhibiting *C. albicans,* as well as pathogenic bacteria, prevents the overgrowth of yeast, which is a common side effect of antibiotic use.

Diarrhea is a common symptom in patients with chronic candidiasis. Berberine has shown remarkable antidiarrheal activity in even the most severe cases. Positive clinical results have been shown with berberine in relieving diarrhea in cases of cholera, amebiasis, giardiasis, and other causes of acute GI infection (e.g., *Escherichia coli, Shigella, Salmonella,* and *Klebsiella*) and may also relieve the diarrhea seen in patients with chronic candidiasis.[30–36]

The dosage of any berberine-containing plant should be based on berberine content. Because there is a wide range of quality in goldenseal preparations, standardized extracts are preferred. Three-times-a-day dosages are as follows:

- Dried root or infusion (tea), 2 to 4 g
- Tincture (1:5), 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1), 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content), 250 to 500 mg

Note that the dosage recommendations for berberine would be 25 to 50 mg three times daily or a daily dose of up to 150 mg. This dosage is consistent with the dosage range in the positive clinical studies in patients with GI infections. For children, a dosage based on body weight is appropriate. The daily dose would be the equivalent of 5 to 10 mg of berberine/kg (2.2 lb) body weight.

Berberine and berberine-containing plants are generally nontoxic at the recommended dosages. However, berberine-containing plants are not recommended for use during pregnancy, and higher dosages may interfere with vitamin B metabolism.[37]

Allium sativum. Garlic has demonstrated significant antifungal activity. Its inhibition of *C. albicans* in both animal and test tube (in vitro) studies has shown it to be more potent than nystatin, gentian violet, and six other reputed antifungal agents.[38–40] The active component is allicin—the pungent and odorous principle of garlic.

The clinical use of garlic features commercial preparations designed to offer the benefits of garlic without the odor. These preparations are designed to delay the formation of allicin until the enteric-coated tablet is delivered to the small and large intestines.

The treatment of chronic candidiasis requires a daily dose of at least 10 mg allicin or a total allicin potential of 4000 mcg. This amount is equal to approximately one clove (4 g) of fresh garlic. Higher dosages of these preparations usually result in the odor of garlic being detectable.

Enteric-Coated Volatile Oils

Volatile oils from oregano, thyme, peppermint, and rosemary are all effective antifungal agents. In one study, the sensitivity of 30 different strains of *C. albicans* to 12 essential oils was compared with the three mainly used drugs (clotrimazole, fluconazole, and itraconazole).[41] Mint, basil, lavender, tea tree oil, winter savory, and oregano essential oils inhibited both the growth and the activity of *C. albicans* more efficiently compared with the mainly used drugs (especially clotrimazole). The results showed that the essential oils primarily affected the cell wall and the membranes of the yeast. An additional study compared the anti–*C. albicans* effect of oregano oil with that of caprylic acid.[42] Although the minimum inhibitory concentration of oregano oil was

less than 0.1 mg/mL, and the 0.1% survival of *C. albicans* occurred at a concentration of 45 mg/mL, the minimum inhibitory concentration of caprylic acid was less than 500 mg/mL, and 0.1% survival occurred at a concentration of 5000 mg/mL. These results indicate that the anti–*C. albicans* activity of oregano oil is more than 100 times more potent than caprylic acid. Because volatile oils are quickly absorbed and associated with inducing heartburn, an enteric coating is recommended to ensure delivery to the small and large intestines. An effective dosage for an enteric-coated volatile oil preparation is 0.2 to 0.4 mL twice daily between meals.

Melaleuca alternifolia. In one open study, 27 patients with AIDS and oral candidiasis clinically refractory to fluconazole were randomized to receive either an alcohol-based or alcohol-free *Melaleuca alternifolia* oral solution 4 times daily for 2 to 4 weeks. Overall, 60% of patients demonstrated a clinical response to this oral solution (seven patients were cured, and eight patients clinically improved) at the 4-week evaluation.[43]

Propolis

Propolis has shown considerable in vitro antimicrobial activity against *C. albicans,* as well as an ability to enhance the effectiveness of conventional antifungal drugs.[44–47] Its cytotoxic activity against *C. albicans,* along with its immune-enhancing effects, make propolis a strong candidate for the treatment of chronic candidiasis.

THERAPEUTIC APPROACH

The following is a comprehensive, step-by-step approach to the successful elimination of chronic candidiasis.

Step 1. Identify and address predisposing factors:
- Eliminate the use of antibiotics, steroids, immune-suppressing drugs, and birth control pills (unless medically necessary).
- Perform a CDSA.
- Follow the specific recommendations if the identifiable predisposing factor is dietary factors, impaired immunity, impaired liver function, or an underlying disease state.

Step 2. Recommend the *C. albicans* control diet:
- Eliminate refined and simple sugars.
- Eliminate milk and other dairy products.
- Eliminate foods with a high content of yeast or mold, including alcoholic beverages, cheeses, dried fruits, melons, and peanuts.
- Eliminate all known or suspected food allergies.

Step 3. Provide nutritional support:
- A high-potency multiple vitamin and mineral formula
- Additional antioxidants
- 1 Tbs of flaxseed oil daily

Step 4. Support immune function:
- Promote a positive mental attitude.
- Help patients deal with stress by teaching positive stress-coping techniques.
- Recommend avoiding alcohol, sugar, smoking, and elevated cholesterol levels, which can impair immune function.
- Recommend plenty of rest and good sleep.
- Follow the supplement recommendations given in Chapter 136 on immune support.

Step 5. Promote detoxification and elimination:
- Recommend 3 to 5 g of a water-soluble fiber source, such as guar gum, psyllium seed, or pectin at night.
- If necessary, recommend lipotropic factors and silymarin to enhance liver function.

Step 6. Recommend probiotics:
- Dose: 5 to 10 billion viable *L. acidophilus* and *B. bifidum* cells daily

Step 7. Use appropriate antiyeast therapy:
- Ideally, use the recommended nutritional or herbal supplements, or both, to help control against yeast overgrowth and promote a healthy bacterial flora.
- If necessary, use prescription antiyeast drugs appropriately.

These steps should resolve chronic candidiasis in most cases. If a patient follows these guidelines and fails to achieve significant improvement or complete resolution, further evaluation is necessary to determine whether chronic candidiasis is the underlying factor. Repeat stool cultures and antigen levels are often helpful. If the organism has not been eradicated, stronger prescription antibiotics can be used, along with the other general recommendations.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Truss O. *The Missing Diagnosis.* Birmingham, AL: C. O. Truss; 2983.
2. Crook WG. *The Yeast Connection*. Jackson, TN: Professional Books; 1984.
3. Kroker GF. Chronic candidiasis and allergy. In: Brostoff J, Challacombe SJ, eds. *Food Allergy and Intolerance*. Philadelphia: WB Saunders; 1987:850–872.
4. Crook WG. *The Yeast Connection and the Woman*. Jackson, TN: Professional Books; 1995.
5. Bauman DS, Hagglund HE. Correlation between certain polysystem chronic complaints and an enzyme immunoassay with antigens of Candida albicans. *J Advancement Med*. 1991;4:5–19.
6. Boero M, Pera A, Andriulli A, et al. Candida overgrowth in gastric juice of peptic ulcer subjects on short- and long-term treatment with H2-receptor antagonists. *Digestion*. 1983;28:158–163.
7. Rubinstein E, Mark Z, Haspel J, et al. Antibacterial activity of the pancreatic fluid. *Gastroenterol*. 1985;88:927–932.
8. Sarker SA, Gyr K. Non-immunological defence mechanisms of the gut. *Gut*. 1992;33:987–993.
9. Iwata K. Toxins produced by Candida albicans. *Contrib Microbiol Immunol*. 1977;4:77–85.
10. Axelsen NH. Analysis of human Candida precipitins by quantitative immunoelectrophoresis: a model for analysis of complex microbial antigen-antibody systems. *Scand J Immunol*. 1976;5:177–190.
11. Farah CS, Elahi S, Drysdale K, et al. Primary role for CD4(+) T lymphocytes in recovery from oropharyngeal candidiasis. *Infect Immun*. 2002;70:724–731.
12. Fidel Jr PL. Immunity to Candida. *Oral Dis*. 2002;8(suppl 2):69–75.
13. Klein A, Pappas SC, Gordon P, et al. The effect of nonviral liver damage on the T-lymphocyte helper/suppressor ratio. *Clin Immunol Immunopathol*. 1988;46:214–220.
14. Abe F, Nagata S, Hotchi M. Experimental candidiasis in liver injury. *Mycopathologia*. 1987;100:37–42.
15. Barak AJ, Beckenhauer HC, Junnila M, et al. Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. *Alcohol Clin Exp Res*. 1993;17:552–555.
16. Zeisel SH, Da Costa KA, Franklin PD, et al. Choline, an essential nutrient for humans. *FASEB J*. 1991;5:2093–2098.
17. Isolauri E. Probiotics in human disease. *Am J Clin Nutr*. 2001;73. 1142S–116S.
18. Sullivan A, Nord CE. The place of probiotics in human intestinal infections. *Int J Antimicrob Agents*. 2002;20:313–319.
19. Keeney EL. Sodium caprylate. A new and effective treatment of moniliasis of the skin and mucous membrane. *Bull Johns Hopkins Hosp*. 1946;78:333–339.
20. Neuhauser I, Gustus EL. Successful treatment of intestinal moniliasis with fatty acid resin complex. *Arch Intern Med*. 1954;93:53–60.
21. Schwabe AD, Bennett LR, Bowman LP. Octanoic acid absorption and oxidation in humans. *J Appl Physiol*. 1964;19:335–337.
22. Atai Z, Atai M, Amini J, Salehi N. In vivo study of antifungal effects of low-molecular-weight chitosan against Candida albicans. *J Oral Sci*. 2017;59(3):425–430. PubMed PMID: 28904319.
23. Hahn FE, Ciak J. Berberine. *Antibiotics*. 1976;3:577–588.
24. Amin AH, Subbaiah TV, Abbasi KM. Berberine sulfate. Antimicrobial activity, bioassay, and mode of action. *Can J Microbiol*. 1969;15:1067–1076.
25. Johnson CC, Johnson G, Poe CF. Toxicity of alkaloids to certain bacteria. II. Berberine, physostigmine, and sanguinarine. *Acta Pharmacol Toxicol*. 1952;8:71–78.
26. Kaneda Y, Torii M, Tanaka T, et al. In vitro effects of berberine sulphate on the growth of Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis. *Ann Trop Med Parasitol*. 1991;85:417–425.
27. Subbaiah TV, Amin AH. Effect of berberine sulphate on Entamoeba histolytica. *Nature*. 1967;215:527–528.
28. Ghosh AK, Rakshit MM, Ghosh DK. Effect of berberine chloride on *Leishmania donovani*. *Indian J Med Res*. 1983;78:407–416.
29. Mahajan VM, Sharma A, Rattan A. Antimycotic activity of berberine sulphate. An alkaloid from an Indian medicinal herb. *Sabouraudia*. 1982;20:79–81.
30. Gupte S. Use of berberine in treatment of giardiasis. *Am J Dis Child*. 1975;129:866.
31. Bhakat MP, Nandi N, Pal HK, et al. Therapeutic trial of berberine sulphate in non-specific gastroenteritis. *Indian Med J*. 1974;68:19–23.
32. Kamat SA. Clinical trial with berberine hydrochloride for the control of diarrhoea in acute gastroenteritis. *J Assoc Physicians India*. 1967;15:525–529.
33. Desai AB, Shah KM, Shah DM. Berberine in the treatment of diarrhoea. *Indian Pediatr*. 1971;8:462–465.
34. Sharma R, Joshi CK, Goyal RK. Berberine tannate in acute diarrhoea. *Indian Pediatr*. 1970;7:496–501.
35. Choudhry VP, Sabir M, Bhide VN. Berberine in giardiasis. *Indian Pediatr*. 1972;9:143–146.
36. Rabbani GH, Butler T, Knight J, et al. Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic *Escherichia coli* and *Vibrio cholerae*. *J Infect Dis*. 1987;155:979–984.
37. Kowalewski Z, Mrozikiewicz A, Bobkiewicz T, et al. Toxicity of berberine sulfate. *Acta Pol Pharm*. 1975;32:113–120 [in Polish].
38. Moore GS, Atkins RD. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. *Mycologia*. 1977;69:341–348.
39. Sandhu DK, Warraich MK, Singh S. Sensitivity of yeasts isolated from cases of vaginitis to aqueous extracts of garlic. *Mykosen*. 1980;23:691–698.
40. Prasad G, Sharma VD. Efficacy of garlic (*Allium sativum*) treatment against experimental candidiasis in chicks. *Br Vet J*. 1980;136:448–451.
41. Bona E, Cantamessa S, Pavan M, et al. Sensitivity of *Candida albicans* to essential oils: are they an alternative to antifungal agents? *J App Microbiol*. 2016;121(6):1530–1545. PubMed PMID: 27568869.
42. Stiles JC, Sparks W, Ronzio RA. The inhibition of *Candida albicans* by oregano. *J Appl Nutr*. 1995;47:96–102.
43. Vazquez JA, Zawawi AA. Efficacy of alcohol-based and alcohol-free melaleuca oral solution for the treatment of fluconazole-refractory oropharyngeal candidiasis in patients with AIDS. *HIV Clin Trials*. 2002;3:379–385.
44. Stepanovic S, Antic N, Dakic I, et al. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microbiol Res*. 2003;158:353–357.
45. Ota C, Unterkircher C, Fantinato V, et al. Antifungal activity of propolis on different species of *Candida*. *Mycoses*. 2001;44:375–378.
46. D'Auria FD, Tecca M, Scazzocchio F, et al. Effect of propolis on virulence factors of *Candida albicans*. *J Chemother*. 2003;15:454–460.
47. Martins RS, Pereira Jr ES, Lima SM, et al. Effect of commercial ethanol propolis extract on the in vitro growth of *Candida albicans* collected from HIV-seropositive and HIV-seronegative Brazilian patients with oral candidiasis. *J Oral Sci*. 2002;44:41–48.

Chronic Fatigue Syndrome

John Nowicki, ND, Michael T. Murray, ND,* and *Mark Dreher*, PhD*

OUTLINE

DIAGNOSTIC SUMMARY

- Mild fever
- Recurrent sore throat
- Painful lymph nodes
- Muscle weakness
- Muscle pain
- Prolonged fatigue after exercise
- Recurrent headache
- Migratory joint pain
- Depression
- Sleep disturbance (hypersomnia or insomnia)

INTRODUCTION

Chronic fatigue syndrome (CFS), now also called myalgic encephalomyelitis, describes varying combinations of symptoms, including recurrent fatigue, sore throat, low-grade fever, swollen lymph nodes, headache, muscle and joint pain, intestinal discomfort, emotional distress, depression, and loss of concentration. Although newly defined and currently popular, CFS is not a new disease. References to a similar condition in the medical literature go back as far as the 1860s. In the past, CFS has been known by various names, including the following:

- Chronic mononucleosis-like syndrome or chronic Epstein–Barr virus (EBV) syndrome
- Yuppie flu
- Postviral fatigue syndrome
- Postinfectious neuromyasthenia
- Chronic fatigue and immune dysfunction syndrome (CFIDS)
- Iceland disease
- Royal Free Hospital disease

In addition, the symptoms of CFS mirror the symptoms of neurasthenia, a condition first described in 1869.

Definition

In response to the growing interest, CFS was formally defined in 1988 by a consensus panel convened by the Centers for Disease Control and Prevention (CDC) in an attempt to establish a guide for evaluating patients with chronic fatigue of unknown cause by clinical physicians and researchers.[1]

The CDC established a formal (and controversial) set of diagnostic criteria (Box 160.1). These are controversial for many reasons, including that psychological symptoms are both a minor criterion and potential grounds for exclusion. One of the major complaints from physicians about the CDC definition is that it appears better suited for research than for clinical purposes. A significant problem with the CDC criteria is that they ignore many of the common symptoms reported by patients with CFS (Table 160.1).

The British and Australian criteria for the diagnosis of CFS are less strict than those of the CDC.[2] In particular, the minor diagnostic criteria are not required, and the major diagnostic criteria are not as strict. For example, in the Australian definition, the major criterion is simply fatigue at a level that causes disruption of daily activities in the absence of other medical conditions associated with fatigue.

Using the CDC criteria, the prevalence of CFS in individuals suffering from chronic fatigue in the United States is thought to be about 11.5%. Using the British criteria, it is about 15%, and using the Australian criteria, it is about 38%.[2]

* Previous edition contributor

BOX 160.1 Centers for Disease Control and Prevention Diagnostic Criteria for Chronic Fatigue Syndrome

Major Criteria

- New onset of fatigue causing 50% reduction in activity for at least 6 months
- Exclusion of other illnesses that can cause fatigue

Minor Criteria

- Presence of 8 of the 11 symptoms listed, or 6 of the 11 symptoms and 2 of the 3 signs

Symptoms

1. Mild fever
2. Recurrent sore throat
3. Painful lymph nodes
4. Muscle weakness
5. Muscle pain
6. Prolonged fatigue after exercise
7. Recurrent headache
8. Migratory joint pain
9. Neurological or psychological complaints
   - Sensitivity to bright light
   - Forgetfulness
   - Confusion
   - Inability to concentrate
   - Excessive irritability
   - Depression
10. Sleep disturbance (hypersomnia or insomnia)
11. Sudden onset of symptom complex

Signs

1. Low-grade fever
2. Nonexudative pharyngitis
3. Palpable or tender lymph nodes

TABLE 160.1 Frequency of Symptoms in Chronic Fatigue Syndrome

Symptom/Sign	Frequency (%)
Fatigue	100
Low-grade fever	60–95
Muscle pain	20–95
Sleep disorder	15–90
Impaired mental function	50–85
Depression	70–85
Headache	35–85
Allergies	55–80
Sore throat	50–75
Anxiety	50–70
Muscle weakness	40–70
Postexercise fatigue	50–60
Premenstrual syndrome (women)	50–60
Stiffness	50–60
Visual blurring	50–60
Nausea	50–60
Dizziness	30–50
Joint pain	40–50
Dry eyes and mouth	30–40
Diarrhea	30–40
Cough	30–40
Decreased appetite	30–40
Night sweats	30–40
Painful lymph nodes	30–40

Etiology

Infectious Agents

Owing in part to the similarity of CFS to acute or chronic infection, it was initially thought to be caused by a virus (e.g., EBV mononucleosis).[3] It now seems clear that CFS is not caused by any single recognized infectious disease agent. The CDC's four-city surveillance study found no association between CFS and infection by a wide variety of human pathogens, including EBV, human retroviruses, herpesvirus, rubella, *Candida albicans,* and others. No one pathogen appears to be causing CFS. However, the possibility remains that CFS may have multiple causes leading to a common endpoint, in which case, some viruses or other infectious agents may play a contributing role (Fig. 160.1).

Immune System Abnormalities

There is little argument that a disturbed immune system plays a central role in CFS. A variety of such abnormalities have been reported in CFS patients (Box 160.2). Although no specific immunological dysfunction pattern has been recognized, the most consistent abnormality is a decreased number or activity of natural killer (NK) cells.[4–7] NK cells received their name because of their ability to destroy cells that have become cancerous or infected by viruses. In fact, for a time, CFS was also referred to as *low natural killer cell syndrome* (LNKS).

Other consistent findings include a reduced ability of lymphocytes, key in the battle against viruses, to respond to stimuli.[7] One reason for this lack of response may be reduced activity or decreased production of interferon. Although both low and high levels of interferon have been reported in CFS, levels are depressed in most cases. When interferon levels are low, reactivation of latent viral infection is likely. Conversely, when interferon (as well as other chemical mediators like interleukin-1) levels are high, many of the symptoms may be related to the physiological effects of interferon. When interferon is used as a therapy in cancer and viral hepatitis, the side effects produced are similar to the symptoms of CFS.

Other Causes of Chronic Fatigue

Chronic fatigue can be caused by various physical and psychological factors. Box 160.3 lists the major causes of chronic fatigue in an order of importance that represents how common the cause is among sufferers of chronic fatigue in the general population. The list is based on the findings of several large studies as well as the author's clinical experience (CFS is listed under a broader category of impaired immune function).

Chronic Fatigue Syndrome, Fibromyalgia, and Multiple Chemical Sensitivities

Fibromyalgia (FM) and multiple chemical sensitivities (MCS), like CFS, are recognized disorders with a substantial overlap of symptomatology.[4,5,8,9] The only difference in the diagnostic criteria for FM and CFS is the requirement of musculoskeletal pain in fibromyalgia and fatigue in CFS. The likelihood of being diagnosed as having fibromyalgia or CFS depends on the type of physician consulted. Specifically, if a rheumatologist or orthopedic specialist is consulted, the patient is much more likely to be diagnosed with fibromyalgia. (Box 160.4 presents the diagnostic criteria for fibromyalgia.)

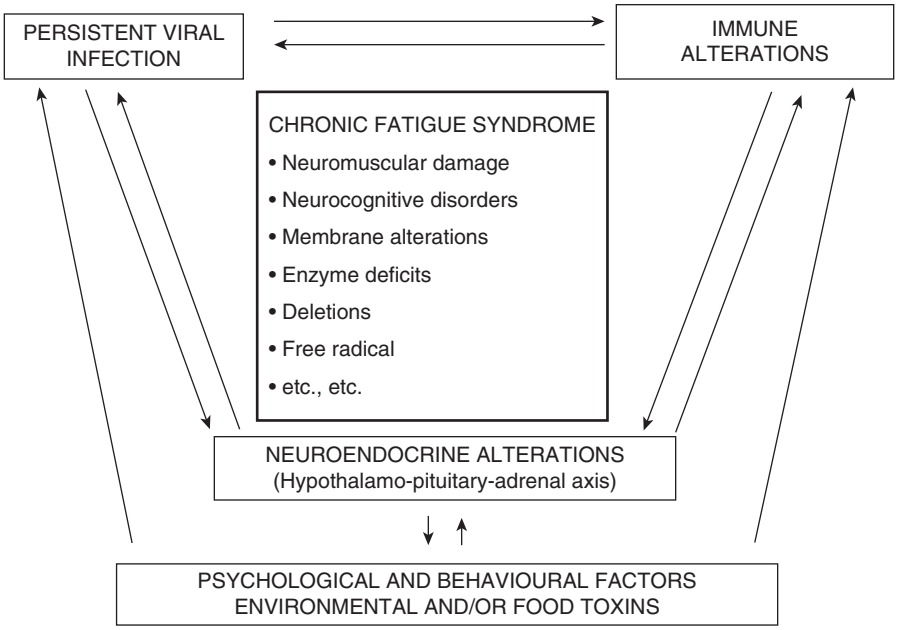


Fig. 160.1 Pathogenic Hypotheses of Chronic Fatigue Syndrome. (From Eligio P. Delia R, Valeria G. EBV chronic infections. *Mediterr J Hematol Infect Dis.* 2010;2[1]:e2010022. PubMed PMID: 21415952.)

BOX 160.2 Immunological Abnormalities Reported for Chronic Fatigue Syndrome

- Elevated levels of antibodies to viral proteins
- Decreased activity of natural killer cells
- Low or elevated antibody levels
- Increased or decreased levels of circulating immune complexes
- Increased cytokine (e.g., interleukin-2) levels
- Increased or decreased interferon levels
- Altered ratio of T-helper cells to T-suppressor cells

One group of researchers carefully compared the symptomatology of 90 patients who had been diagnosed as having CFS, MCS, or FM (30 in each category).[9] Using the same questionnaire for all 90 patients, 70% of the patients diagnosed with FM and 30% of those with MCS met the CDC criteria for CFS. Particularly significant was the observation that 80% of both the FM and MCS patients met the CFS criteria of fatigue lasting more than 6 months, with a 50% reduction in activity. More than 50% of the CFS and FM patients reported adverse reactions to various chemicals.

DIAGNOSIS

A great number of factors must be considered when evaluating a patient with chronic fatigue. Women tend to be at higher risk than men.[10] A detailed medical history and review of body systems goes a long way toward identifying important factors. The goal is to identify as many factors as possible that may be contributing to the patient's feeling of fatigue. For example, if a patient has heart disease, diabetes, or some other health condition and the condition or the drug he or she is taking is clearly responsible for the fatigue, the treatment of the fatigue becomes secondary to the treatment of his or her underlying health condition.

In many cases of chronic fatigue, further evaluation is necessary. The next steps can include a complete physical examination and laboratory studies. In the physical examination, it is important to look for clues that may point to the cause of chronic fatigue. For example, swollen lymph nodes may indicate a chronic infection, and the presence of a diagonal crease on both earlobes usually indicates impaired blood flow to the brain, a significant cause of fatigue in the elderly.

BOX 160.3 Causes of Chronic Fatigue

- Preexisting physical condition
  - Diabetes
  - Heart disease
  - Lung disease
  - Rheumatoid arthritis
  - Chronic inflammation
  - Chronic pain
  - Cancer
  - Liver disease
  - Multiple sclerosis
- Prescription drugs
  - Antihypertensives
  - Anti-inflammatory agents
  - Birth control pills
  - Antihistamines
  - Corticosteroids
  - Tranquilizers and sedatives
- Depression
- Stress and/or low adrenal function
- Impaired liver function, environmental illness, or both
- Impaired immune function
  - Chronic *Candida* infection
  - Other chronic infections
- Food allergies
- Hypothyroidism
- Hypoglycemia
- Anemia and nutritional deficiencies
- Sleep disturbances
- Cause unknown

BOX 160.4 Diagnostic Criteria for Fibromyalgia[a]

Major Criteria

- Generalized aches or stiffness of at least three anatomical sites for at least 3 months
- Six or more typical reproducible tender points
- Exclusion of other disorders that can cause similar symptoms

Minor Criteria

- Generalized fatigue
- Chronic headache
- Sleep disturbance
- Neurological and psychological complaints
- Joint swelling
- Numbness or tingling sensations
- Irritable bowel syndrome
- Variation of symptoms in relation to activity, stress, and weather changes

[a]Fulfillment of all three major criteria and four or more minor criteria required.
Data from Murphree R. Treating and beating fibromyalgia. http://drrodgermurphree.com/fibromyalgia/.

Plasma neuropeptide Y (PNY) has been reported as a potential biomarker for symptom severity in CFS.[11] Clinical research has demonstrated a significant correlation of PNY with stress, negative mood, general health, depression, and impaired cognitive function. However, expensive laboratory tests are best not ordered unless they are absolutely necessary. For example, if it is quite obvious that the patient has impaired immunity, it does not make much sense to perform elaborate and expensive blood tests on immune function because the results of these tests are not likely to influence the method of treatment.

Of particular value are assessments of liver detoxification function, bowel dysbiosis, and gastrointestinal permeability (see Chapters 9, 19, and 28).

THERAPEUTIC CONSIDERATIONS

Because chronic fatigue and CFS are generally multifactorial conditions, the therapeutic approach typically involves multiple therapies that address different facets of the clinical picture.

A person's energy level, as well as his or her emotional state, is determined by an interplay between two primary factors—internal focus and physiology. Many people with chronic fatigue focus on how tired they are. They repeatedly reaffirm their fatigue to themselves and to anyone who will listen. Their physiology includes not only the chemicals and hormones circulating in the body but also the way they hold their bodies (usually slouched) and the way they breathe (shallow). In most patients with chronic fatigue, both the mind and the body must be addressed. The most effective treatment is a comprehensive program designed to help people use their minds, attitudes, and physiologies to fuel higher energy levels.

Underlying Physiological Dysfunctions

Depression

The first factor to address is any underlying depression. Depression is one of the major causes of chronic fatigue, and it is a common feature of CFS. In the absence of a preexisting physical condition, depression is generally regarded as the most common cause of chronic fatigue. However, it is often difficult to determine whether the depression preceded the fatigue or vice versa. Depression is fully discussed in Chapter 142.

Stress

Stress is another factor to consider in the patient with chronic fatigue or CFS (Fig. 160.2). Stress can be the underlying factor in the patient with depression, low immune function, or another cause of chronic fatigue (see Chapter 140 for guidelines on assessing the role of stress).

One of the tools recommended to rate stress levels is the Social Readjustment Rating Scale developed by Holmes and Rahe.[12] The scale was originally designed to predict the likelihood of a person getting a serious disease due to stress. Various life-changing events are numerically rated according to their potential for causing disease. Even events commonly viewed as positive, such as an outstanding personal achievement, carry stress with them.

Impaired Liver Function, Environmental Illness, or Both

Exposure to food additives, solvents (e.g., cleaning materials, formaldehyde, toluene, benzene), pesticides, herbicides, toxic metals (e.g., lead, mercury, cadmium, arsenic, nickel, aluminum), and other toxins can greatly stress the liver and detoxification processes.[13,14] This exposure can lead to a condition labeled by many naturopathic and nutrition-oriented physicians as the "congested liver," "sluggish liver," or the more recently coined "impaired hepatic detoxification." These terms signify a reduced ability of the liver to detoxify. The congested or sluggish liver is characterized by a diminished bile flow, a condition known in medical terms as cholestasis or steatohepatitis, whereas the term *impaired hepatic detoxification* refers to decreased Phase I or II enzyme activity, or both. Phase I detoxification rates in excess of Phase II activity also cause toxicity problems owing to excessive accumulation of activated intermediates. In addition to exposure to toxic chemicals, various other agents and conditions, as listed in Box 160.5, can cause impairment of bile flow within the liver.

Although many of the conditions listed in Box 160.5 are typically associated with alterations in laboratory tests of liver function (e.g., serum bilirubin, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, γ-glutamyl transpeptidase), these tests alone may not be adequate to evaluate liver function because they are elevated only when the liver has been significantly damaged, and many of these conditions in the initial or "subclinical" stages of chronic fatigue may yield normal laboratory values.

Although there are more sensitive tests to determine the functional activity of the liver, such as the serum bile acid assay and various clearance tests, clinical judgment based on medical history remains the major diagnostic tool for the "sluggish liver" or impaired hepatic detoxification enzymes, the presence of chronic fatigue being the hallmark symptom.

People with a sluggish liver may also complain of the following:

- Depression
- General malaise
- Headaches
- Digestive disturbances
- Allergies and chemical sensitivities
- Premenstrual syndrome
- Constipation

Not surprisingly, people exposed to toxic chemicals often complain of the same symptoms. Many toxic chemicals (especially solvents) and toxic metals have an affinity for nervous tissue, giving rise to various psychological and neurological symptoms[14,15]:

- Depression
- Headaches

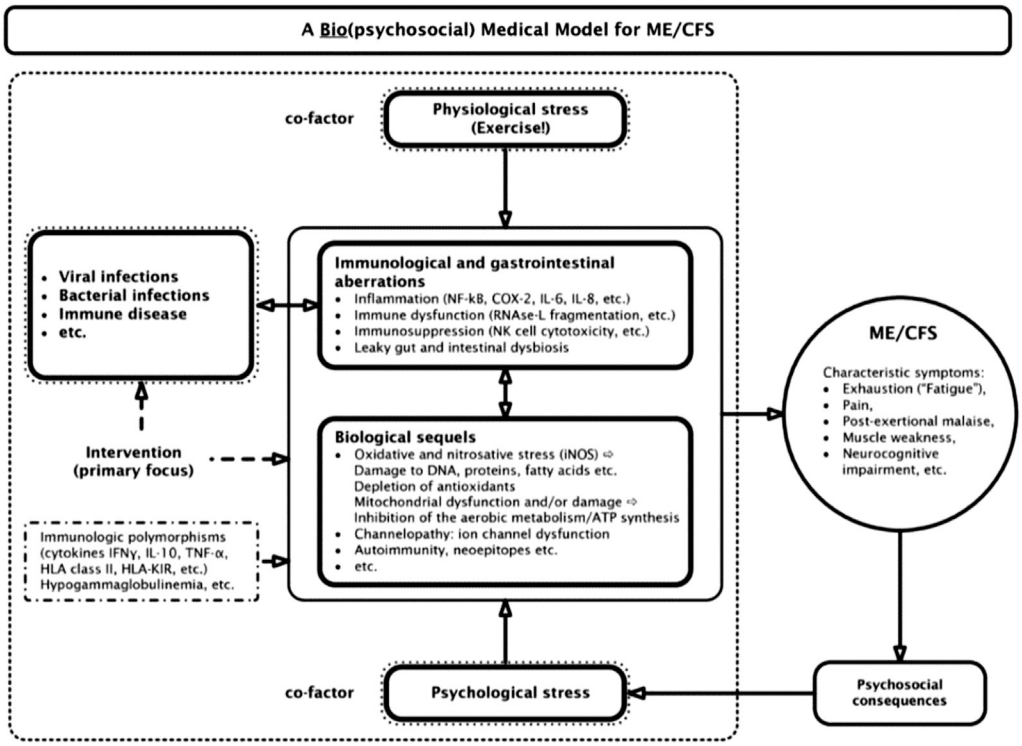


Fig. 160.2 Biological model that may explain "fatigue" and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) by organic abnormalities and cause and effect relationships. *COX-2*, cyclo-oxygenase 2; *iNOS*, inducible nitric oxide synthase; *PUFA*, polyunsaturated fatty acid; *NF-κB*, nuclear factor κB. (From Maes M, Twisk FN. Chronic fatigue syndrome: Harvey and Wessley's [bio]psychosocial model versus a bio[psychosocial] model based on inflammatory and oxidative and nitrosative stress pathways. *BMC Med.* 2010;8:35. PubMed PMID: 20550693.)

BOX 160.5 Causes of Cholestasis

- Dietary factors
  - Saturated fat
  - Refined sugar
  - Low fiber intake
  - Obesity
- Diabetes
- Presence of gallstones
- Alcohol
- Endotoxins and other gut-derived bacterial toxins
- Hereditary disorders such as Gilbert's syndrome
- Pregnancy
- Natural and synthetic steroid hormones
- Anabolic steroids
- Estrogens
- Oral contraceptives
- Certain chemicals or drugs
- Cleaning solvents
- Pesticides
- Antibiotics
- Diuretics
- Nonsteroidal anti-inflammatory drugs
- Thyroid hormone
- Viral hepatitis

- Mental confusion
- Mental illness
- Tingling in the extremities
- Abnormal nerve reflexes
- Other signs of impaired nervous system function

A hair mineral analysis is a possible screening test for metal toxicity. Refer to Chapter 22 (Metal Toxicity: Assessment of Exposure and Retention) for further discussion.

A multiclinic research study of chronically ill patients, many of whom were diagnosed as suffering from CFIDS, evaluated the efficacy of a comprehensive detoxification program. Patients were placed on a hypoallergenic diet and given a dietary food supplement rich in nutrients that assist in liver detoxification. These patients reported a 52% reduction in symptoms after 10 weeks, and symptom improvement was mirrored by normalization of hepatic Phase I and Phase II detoxification.[15]

Gastrointestinal Permeability

Excessive gastrointestinal permeability, as measured by the lactulose-mannitol absorption test (see Chapter 19), is a common finding in CFS.[16]

Hepatic Detoxification Functions

A treatment program using hypoallergenic rice protein food replacement formula to support hepatic Phases I and II detoxification was provided to patients who fulfilled the classic criteria of the CDC

BOX 160.6 Questionnaire for the Recognition of Impaired Immune Function

- Do you get more than two colds per year?
- When you catch a cold, does it take more than 5 to 7 days to get rid of the symptoms?
- Have you ever had infectious mononucleosis?
- Do you have herpes?
- Do you suffer from chronic infections of any kind?

and National Institutes of Health for CFS. The treatment resulted in symptom reduction, with clinical improvement being paralleled by improved hepatic detoxification function.[17]

Impaired Immune Function, Chronic Infection, or Both

When the immune system is impaired, infections can linger, and fatigue can persist. There is a good reason for this: fatigue is the body's response mechanism to infection because the immune system works best when the body is at rest.

To determine the role that the immune system is playing in patients with chronic fatigue, the series of questions listed in Box 160.6 can be used during the patient interview to indicate an impaired immune system. Chapter 18 describes in substantial detail the laboratory methodologies for assessing immune function.

Chronic Candidal Infection

One of the most common findings in individuals with impaired immune function is gastrointestinal overgrowth of *Candida albicans*. Candidal overgrowth is now becoming recognized as a complex medical syndrome also known as the "yeast syndrome" and "chronic candidiasis." This overgrowth is believed to cause a wide variety of symptoms in virtually every system of the body, with the gastrointestinal, genitourinary, endocrine, nervous, and immune systems being the most susceptible. Box 160.7 lists the profile of a typical patient with chronic candidiasis (see Chapter 159 for a comprehensive discussion).

The diagnosis of chronic candidiasis is often difficult because there is no specific diagnostic test. Stool cultures and elevated antibody levels to *Candida* are useful diagnostic aids, but they should not be relied on for diagnosis. The best method for diagnosing chronic candidiasis in most cases is a detailed medical history and patient questionnaire (see Appendix 1). Box 160.8 lists the factors that typically predispose a patient to candidal overgrowth.

Food Allergies

As far back as 1930, chronic fatigue was recognized as a key feature of food allergies.[18] Originally, Rowe and Rowe[19] used the term *allergic toxemia* to describe a syndrome that included the symptoms of fatigue, muscle and joint aches, drowsiness, difficulty in concentration, nervousness, and depression. Around the 1950s, this syndrome began to be referred to as the "allergic tension-fatigue syndrome."[19] With the popularity of CFS, many physicians and others are forgetting that food allergies can lead to chronic fatigue. Furthermore, between 55% and 85% of individuals with CFS have allergies. For more information on food allergies, see Chapter 14.

Hypothyroidism

Hypothyroidism is a common cause of chronic fatigue. However, the condition is often overlooked. The reason for this may be the reliance on standard blood measurements of thyroid hormone levels as the method of diagnosis.[20–22] Undiagnosed hypothyroidism is a serious concern because failure to treat such a critical and underlying problem

BOX 160.7 Typical Profile of Patient With Chronic Candidiasis

- Gender: female
- Age: 15 to 50 years

General Symptoms
- Chronic fatigue
- Loss of energy
- General malaise
- Decreased libido

Gastrointestinal Symptoms
- Thrush
- Bloating, gas
- Intestinal cramps
- Rectal itching
- Altered bowel function

Genitourinary System Complaints
- Vaginal yeast infection
- Frequent bladder infections
- Primarily menstrual complaints

Nervous System Complaints
- Depression
- Irritability
- Inability to concentrate

Immune System Complaints
- Allergies
- Chemical sensitivities
- Low immune function

Past History
- Chronic vaginal yeast infections
- Chronic antibiotic use for infections or acne
- Oral birth control use
- Oral steroid hormone use

Associated Conditions
- Premenstrual syndrome
- Sensitivity to foods, chemicals, and other allergens
- Endocrine disturbances
- Psoriasis
- Irritable bowel syndrome

BOX 160.8 Factors Predisposing to Candidal Overgrowth

- Impaired immune function
- Antiulcer drugs
- Broad-spectrum antibiotics
- Cellular immunodeficiency
- Corticosteroids
- Diabetes mellitus
- Excessive sugar in the diet
- Intravascular catheters
- Intravenous drug use
- Lack of digestive secretions
- Oral contraceptive agents

reduces the effectiveness of every other measure designed to increase energy levels. For more information, see Chapter 182.

Hypoglycemia

The association between hypoglycemia and fatigue is well known. Numerous studies have shown that depressed individuals suffer from hypoglycemia.[23–26] Because depression is the most common cause of chronic fatigue, hypoglycemia must always be ruled out (see Chapter 181).

Hypoadrenalism

Adrenal exhaustion was first proposed as a cause of chronic fatigue more than 50 years ago by Tintera.[27] A small but growing body of evidence now supports the role of a disruption of the hypothalamic–pituitary–adrenal (HPA) axis in CFS.[28] Salivary tests of patients with chronic fatigue show lower cortisol levels on wakening.[29] Other research suggests that there is no specific change to the HPA axis in CFS and that the observed changes are of multifactorial etiology, with some factors occurring as a consequence of the illness. This second school of thought suggests that the HPA axis might play a role in exacerbating or perpetuating symptoms late in the course of the illness but not as a primary etiology.[30]

Either way, one of the major symptoms of glucocorticoid deficiency is debilitating fatigue. Glucocorticoid insufficiency is also characterized by a stressing event followed by feverishness, arthralgias, myalgias, adenopathy, postexertional fatigue, exacerbation of allergic responses, and disturbances of mood and sleep (i.e., the typical presentation of CFS). These symptoms are seen in partial or subclinical adrenal insufficiency, which may be detected only by the adrenocorticotropic hormone (ACTH) stimulation test or other endocrine testing. Glucocorticoids have a profound endogenous immunosuppressive effect. In subclinical adrenal insufficiency, this may allow for the symptoms of chronic fatigue, including exacerbation of allergic responses, enhanced antibody titers to various viral antigens, and elevations in cytokine levels. A group of CFS researchers believes that these patients form a heterogeneous group with various infectious and noninfectious antecedents.[28] They feel that CFS does not represent a discrete disease with a singular cause but rather a clinical condition. CFS is analogous to a number of complex medical conditions, such as hypertension, in which various direct and indirect factors lead to the development of the clinical syndrome.

The researchers hypothesize that in CFS, specific pathophysiological antecedents such as acute infection, stress, and preexisting or concurrent psychiatric illness may ultimately converge in a final common biological pathway, resulting in the clinical syndrome of CFS. They believe that their data and those of others suggest that a reduction in adrenocortical secretion is an important pathophysiological component in the development of many biological and behavioral features of the syndrome.

For example, in one of their studies, 30 patients with classically defined CFS were compared with 72 normal volunteers and patients.[31] The CFS patients were found to have significantly reduced evening cortisol levels and low 24-hour urinary-free cortisol excretion. The CFS patients also had elevated basal ACTH concentrations and increased adrenocortical sensitivity to ACTH but a reduced maximal response, and they showed an attenuated net integrated ACTH response to corticotrophin-releasing hormone. These results are most compatible with a mild central adrenal insufficiency secondary to either a deficiency of corticotrophin-releasing hormone (CRH) or some other central stimulus to the pituitary–adrenal axis. The authors feel that the hyperresponsiveness of the adrenal cortex to ACTH in patients with CFS may reflect a secondary adrenal insufficiency in which adrenal ACTH receptors have become hypersensitive owing to inadequate exposure to ACTH. The reduction in response to large doses of ACTH might suggest overall adrenal atrophy. The evidence suggests that the mild hypocortisolism in these patients reflects a defect at or above the level of the hypothalamus, resulting in a deficiency in the release of CRH or other secretagogues, or both, that serve to activate the pituitary–adrenal axis.

Endotoxins

Endotoxins are lipopolysaccharides (LPSs) from the cell walls of gram-negative bacteria. One bacterial cell contains approximately 3.5 million LPS molecules. Upon bacterial cell death, cell-wall lysis occurs, releasing LPS to interact with intestinal cell membranes and surface proteins, triggering local and systemic inflammatory reactions. Patients with diagnosed CFS have been shown to have significantly higher circulating LPS levels than healthy controls (119.43 pg/mL versus 74.74 pg/mL).[32] They also have higher levels of CD 14 (49 µg/mL versus 39 µg/mL in controls) and high sensitivity C-reactive protein (46 mg/L versus 34 mg/L in controls). In addition, CFS patients had lower intestinal microbiome diversity with significantly diminished *Firmicutes* population.

Mind and Attitude

The mind and attitude play a critical role in determining the status of the immune system and energy levels. Many patients with chronic fatigue (including CFS) are either depressed or just seem to have lost a real enthusiasm for life. It is not easy to have much enthusiasm when you do not have much energy, but the two usually go hand in hand. Related to these psychological difficulties, it has been shown that there is a lack of social support for CFS patients.[33] It is a question of whether the lack of energy affects the CFS patient's ability to maintain a relationship, or vice versa, or possibly both.

Cognitive-behavioral therapy has shown some effective results in clinical use.[34,35] The first step is to convey to CFS patients that they can get better. Many patients with CFS are told that this is "something they will have to live with" and that "there is no cure." Achieving or maintaining a positive mental attitude is critical to good health and high energy levels, especially in patients with CFS. To achieve a positive mind-set, a person must exercise or condition the attitude, much as one would condition the body. To help patients, physicians can prescribe mental exercises such as visualizations, goal setting, affirmations, and empowering questions, as detailed in Chapter 142.

Diet

Energy level appears to be directly related to the quality of foods routinely ingested. Patients should be encouraged to adhere to the dietary guidelines given in Chapter 44. It is especially important to eliminate or restrict caffeine and refined sugar.

Although acute caffeine consumption provides stimulation, regular caffeine intake may actually lead to chronic fatigue. Although mice fed one dose of caffeine demonstrated significant increases in their swimming capacity, when the dose of caffeine was given repeatedly for 6 weeks, a significant decrease in the mice's swimming capacity was observed.[36]

Several studies have found caffeine intake to be extremely high in individuals with psychiatric disorders. Another interesting finding is that the degree of fatigue experienced is often related to the quantity of caffeine ingested. In one survey of hospitalized psychiatric patients, 61% of those ingesting at least 750 mg/day (at least five cups of coffee) complained of fatigue, compared with 54% of those ingesting 250 to 749 mg/day and only 24% of those ingesting less than 250 mg/day.[37]

In patients who routinely drink coffee, abrupt cessation of coffee drinking will probably result in symptoms of caffeine withdrawal, including fatigue, headache, and an intense desire for coffee.[38,39] Fortunately, this withdrawal period does not last more than a few days.

Nutritional Supplements

Nutritional supplementation is essential in the treatment of chronic fatigue. A deficiency of virtually any nutrient can produce the symptoms of fatigue as well as render the body more susceptible to infection. Individuals with chronic fatigue require, at the bare minimum, a high-potency multivitamin-multimineral formula along with extra vitamin C (3000 mg/day in divided doses) and magnesium (500–1200 mg/day in divided doses).

Magnesium

An underlying magnesium deficiency, even if subclinical, can result in chronic fatigue and symptoms similar to those of CFS. In addition, low red blood cell magnesium levels, a more accurate measure of magnesium status than routine blood analysis, have been found in many patients with chronic fatigue and CFS. The literature demonstrates that magnesium deficiency is not necessarily due to low dietary intake,[40] and several studies have shown good results with supplementation with improvements in magnesium stores.

For example, in one double-blind, placebo-controlled trial, 32 patients with CFS received an intramuscular injection of either magnesium sulfate (1 g in 2 mL of injectable water) or a placebo (2 mL of injectable water) for 6 weeks. At the end of the study, 12 of the 15 patients receiving magnesium reported, on the basis of strict criteria, significantly improved energy levels, improved emotional state, and less pain. In contrast, only 3 of the 17 placebo patients reported that they felt better, and only 1 reported improved energy levels.[41]

This study seems to confirm some impressive results obtained in clinical trials during the 1960s on patients suffering from chronic fatigue.[42–45] These studies used oral magnesium and potassium aspartate (1 g each) rather than injectable magnesium. Between 75% and 91% of the nearly 3000 patients studied experienced relief of fatigue during treatment with the magnesium and potassium aspartate. In contrast, the number of patients responding to a placebo was between 9% and 26%. The beneficial effect was usually noted after only 4 to 5 days, but sometimes it took as long as 10 days to achieve results. Patients usually continued treatment for 4 to 6 weeks; afterward, fatigue frequently did not return.

Injectable magnesium is not necessary to restore magnesium status.[46] Absorption studies indicate that magnesium is easily absorbed orally when it is bound to aspartate or citrate. In addition, both of these compounds may also help fight fatigue. Aspartate feeds into the Krebs cycle, the final common pathway for the conversion of glucose, fatty acids, and amino acids to chemical energy (adenosine triphosphate [ATP]), whereas citrate is itself a component of the Krebs cycle. Krebs cycle components, including aspartate, citrate, fumarate, malate, and succinate, usually provide a better mineral chelate; evidence suggests that minerals chelated to the Krebs cycle intermediates are better absorbed, used, and tolerated compared with inorganic or relatively insoluble mineral salts, including magnesium chloride, oxide, or carbonate.[46,47]

Essential Fatty Acids

In one study, essential fatty acid supplementation was shown to improve the clinical picture and red blood cell phospholipid membrane profile of patients with postviral fatigue. The preparation given contained linoleic, gamma-linolenic, eicosapentaenoic, and docosahexaenoic acids and was given as eight 500-mg capsules per day over a 3-month period.[48] In another study, an essential fatty acid supplement rich in eicosapentaenoic acid (EPA) was given daily to a female patient with a 6-year history of unremitting symptoms of CFS.

Within 6 to 8 weeks, the EPA-rich essential fatty acid supplementation led to a marked clinical improvement in the symptoms of CFS.[49] Previous research suggests that CFS is associated with pathophysiological brain changes, such as an enlargement in cerebral ventricular volume.[50] More clinical research is needed.

Nicotinamide Adenine Dinucleotide

Nicotinamide adenine dinucleotide (NADH), the active coenzyme form of vitamin B_3, has been shown to be effective in countering the negative effects of jet lag on cognition and wakefulness.[51] It is also known to encourage energy production through increased ATP generation. One small trial of NADH found a beneficial effect and improvement in quality of life in patients with CFS. This study supplemented the stabilized oral absorbable form in a 26-subject randomized, double-blind, placebo-controlled crossover study. Subjects were randomly assigned to receive either 10 mg of NADH or placebo for 1 month. After a 1-month washout period without supplement, subjects were then crossed to the alternate regimen for a final 1-month period. Within this cohort of 26 patients, 8 of 26 (31%) responded favorably to NADH, in contrast to 2 of 26 (8%) to placebo. No severe adverse effects were observed related to NADH,[52] nor have studies in animals shown any toxicity, even at megadoses.[53] More clinical research is necessary to fully elucidate the possible beneficial effects of NADH in patients with CFS.

L-Carnitine

Carnitine is an essential nutrient for the transport of long-chain fatty acids into the mitochondrial matrix. One randomized controlled crossover study compared the efficacy of L-carnitine with amantadine, a drug known to relieve fatigue in patients with multiple sclerosis. Each intervention was given for 2 months, with a 2-week washout period between medicines. In 30 patients with CFS, L-carnitine or amantadine was given as the first medicine. Amantadine was poorly tolerated by the CFS patients, of whom only 15 were able to complete the 8 weeks of treatment. In those individuals who completed 8 weeks of treatment, there was no statistically significant difference in any of the clinical parameters. With L-carnitine, there was a statistically significant clinical improvement in 12 of the 18 studied parameters after 8 weeks of treatment, with none of the clinical parameters showing any deterioration. The greatest improvement took place between weeks 4 and 8 of L-carnitine treatment. Only one patient, owing to diarrhea, was unable to complete the eight-treatment course.[54] L-Carnitine is extremely safe, with no significant side effects having been reported in any of the human clinical studies.

Coenzyme Q10 (CoQ10)

CoQ10 plays a role as a mitochondrial nutrient that acts as an essential cofactor to produce ATP in the mitochondria.[55] Plasma CoQ10 has been found to be significantly lower ($P = 0.00001$) in CFS patients than in controls. Symptoms such as fatigue and neurocognitive impairment may be related to CoQ10 depletion. In one randomized controlled trial, 43 patients with CFS were randomly assigned to receive either ubiquinol-10 (150 mg/day) or placebo every day for 12 weeks.[56] Although no direct improvement in fatigue was observed, measured with Chandler's Fatigue Scale, ubiquinol-10 supplementation improved several CFS symptoms (e.g., nighttime awakenings), which may have longer-term effects on reducing fatigue in CFS patients.

Melatonin

Dim-light melatonin onset (DLMO) is a naturally occurring event that may account for delayed-sleep-phase syndrome in 10% of patients with chronic insomnia. Research on DLMO concluded that a certain subset of CFS patients may respond to the therapeutic use of melatonin.

In one study, 29 patients with CFS were asked to take 5 mg of melatonin at bedtime for 3 months.[57] Several variables were measured using the Checklist Individual Strength (CIS) questionnaire. The authors determined that the total score ($P = 0.006$), as well as the subscores of fatigue ($P = 0.017$), concentration ($P = 0.031$), motivation ($P = 0.010$), and activity ($P = 0.008$), improved significantly after 90 days of melatonin use. Additionally, in those patients with late DLMO (>21.30 hours), overall CIS scores and its subscores were better than in those CFS patients with early DLMO.

Botanical Medicines

Several botanical medicines support adrenal function and may offer significant benefits in CFS. Most notable are adaptogens, such as Chinese ginseng *(Panax ginseng)*, Siberian ginseng *(Eleutherococcus senticosus)*, rhodiola (*Rhodiola rosacea*), and ashwaganda (*Withania somnifera*). The effects of these herbs as "adaptogens" are discussed in Chapter 140, Stress Management. Of these herbal adaptogens, both Siberian ginseng and rhodiola have shown effects specific to CFS.

Siberian Ginseng *(E. senticosus)*

In addition to supporting adrenal function and acting as a nonspecific adaptogen, Siberian ginseng has been shown to exert a number of beneficial effects on immune function that may be useful in the treatment of CFS. In one double-blind study, 36 healthy subjects received either 10 mL of a fluid extract of ginseng or placebo daily for 4 weeks.[58] The group receiving the ginseng demonstrated significant improvements in various immune system parameters. Most notable were a significant increase in T-helper cells and an increase in NK cell activity—both of which are of value in the treatment of CFS.

Rhodiola rosea

Rhodiola rosea (artic root) is a popular plant in traditional medical systems in Eastern Europe and Asia, where it has traditionally been recommended to help combat fatigue and restore energy. In one randomized, placebo-controlled trial of 60 patients with stress-related fatigue, Rhodiola was found to have an antifatigue effect that increased mental performance, particularly the ability to concentrate, and decreased the cortisol response to awakening stress.[59]

Licorice Root *(Glycyrrhiza glabra)*

Considering the possible roles of viral infection and hypoadrenalism in CFS, licorice root, with its antiviral and glucocorticoid-potentiating properties (see Chapter 85 for documentation of these properties), would seem to be an ideal botanical for this condition.[60] Unfortunately, this has not been rigorously evaluated, although an excellent response in a single patient has been reported.[61] The whole root must be used to ensure glucocorticoid-potentiating glycyrrhizic and glycyrrhetinic acid activity.

Other Therapies

Breathing, Posture, and Bodywork

Proper care of the body is critical to the achievement of high levels of energy. Breathing with the diaphragm, good posture, and bodywork (e.g., massage, spinal manipulation) are all important in helping relieve the stress that is a common contributor to fatigue.

Exercise

Exercise alone has been demonstrated to have a tremendous effect on mood and the ability to handle stressful life situations.[62] Regular exercise has also been shown to lead to improved immune status. For CFS patients, regular exercise has been shown to lead to a significant increase (up to 100%) in NK cell activity.[63,64] Although more strenuous exercise is required to benefit the cardiovascular system, light to moderate exercise may be best for the immune system. One study found that immune function was significantly increased by the practice of tai chi exercises.[65] Tai chi is a martial art technique that features movement from one posture to the next in a flowing motion that resembles dance.

Research suggests that light to moderate exercise stimulates the immune system, whereas intense exercise (e.g., training for the Olympics) can have the opposite effect.[36,66] Graded exercise therapies, in which the patient begins with gradual walking and weight exercises and increases duration and intensity as is comfortable over time, may be the best approach.[67] Although there is a concern for overtraining, one study has shown this method to be superior to relaxation and flexibility exercises.[68]

THERAPEUTIC APPROACH

Successful treatment of CFS requires a comprehensive diagnostic and therapeutic approach. Especially important is identifying underlying factors that may be affecting the patient's energy levels or immune system. The strong correlation between CFS, FM, and MCS suggests that all may respond to hepatic detoxification, food allergy control, and a gut-restoration diet. Special attention should be paid to the advice on immune support in Chapter 136.

Diet

Food allergies should be identified and controlled. Water consumption should increase, whereas the consumption of caffeine-containing drinks and alcohol should stop. A diet of whole organically grown foods should be encouraged. Hypoglycemia should be controlled through the elimination of sugar and other refined foods and the regular consumption of small meals and snacks.

Lifestyle

The patient should be taught diaphragmatic breathing and proper posture. A regular exercise program should be prescribed; low-intensity activities may produce the greatest benefits.

Supplementation

- High-potency multivitamin and mineral formula according to guidelines given in Chapter 44
- Vitamin C: 500 to 1000 mg three times a day
- Vitamin E (mixed tocopherols): 200 to 400 IU/day
- Magnesium bound to citrate or Krebs cycle intermediates: 200 to 300 mg three times a day
- Pantothenic acid: 250 mg/day
- NADH: 10 mg/day on an empty stomach
- L-Carnitine: 1500 to 2000 mg/day in divided doses (if unresponsive, try acetyl-L-carnitine)
- Fish oil: 5 g/day for at least 3 to 4 months
- Coenzyme Q10: 200 to 300 mg/day

Botanical Medicines

- Siberian ginseng *(E. senticosus)*
  - Dried root: 2 to 4 g
  - Tincture (1:5): 10 to 20 mL
  - Fluid extract (1:1): 2 to 4 mL
  - Solid (dry powdered) extract (20:1 or standardized to contain >1% eleutheroside E): 100 to 200 mg
- *R. rosea*
  - The therapeutic dose varies according to the rosavin content. The typical dosage is 200 to 300 mg/day of the extract standardized to contain 3% rosavins and 0.8% to 1% of salidroside.

- Licorice root *(G. glabra)*
  - Powdered root: 1 to 2 g
  - Fluid extract (1:1): 2 to 4 mL
  - Solid (dry-powdered) extract (4:1): 250 to 500 mg

Counseling

The patient should either be counseled directly or referred to a professional counselor for cognitive-behavioral therapy[69] and to establish a regular pattern of mental, emotional, and spiritual affirmations.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Holmes GP, Kaplan J, Gantz N, et al. Chronic fatigue syndrome: a working case definition. *Ann Intern Med*. 1988;108:387–389.
2. Bates DW, Schmitt W, Buchwald D, et al. Prevalence of fatigue and chronic fatigue syndrome in a primary care practice. *Arch Intern Med*. 1993:2759–2765.
3. Centers for Disease Control and Prevention. *Chronic Fatigue Syndrome. Causes*. http://www.cdc.gov/cfs/general/causes/index.html. Accessed October 15, 2010.
4. Shafran SD. The chronic fatigue syndrome. *Am J Med*. 1991;90:731–739.
5. Kyle DV, deShazo RD. Chronic fatigue syndrome: a conundrum. *Am J Med Sci*. 1992;303:28–34.
6. Caligiuri M, Murray C, Buchwald D, et al. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. *J Immunol*. 1987;139:3306–3313.
7. Gupta S, Vayuvegula B. A comprehensive immunological analysis in chronic fatigue syndrome. *Scand J Immunol*. 1991;33:319–327.
8. Komaroff AI, Goldenberg D. The chronic fatigue syndrome: definition, current studies and lessons for fibromyalgia research. *J Rheumatol*. 1989;16:23–27.
9. Buchwald D, Garrity DL. Comparison of patients with chronic fatigue syndrome, fibromyalgia and multiple chemical sensitivities. *Arch Intern Med*. 1994;154:2049–2053.
10. Reid S, Chalder T, Cleare A, et al. Chronic fatigue syndrome. *Clinical Evidence*. 2008;08:1101–1115.
11. Fletcher MA, Rosenthal M, Antoni M, et al. Plasma neuropeptide Y: a biomarker for symptom severity in chronic fatigue syndrome. *Behav Brain Funct*. 2010;6:76–82.
12. Holmes TH, Rahe RH. The social readjustment rating scale. *J Psychosom Res*. 1967;11:213–218.
13. Seaton A, Jeelinek EH, Kennedy P. Major neurological disease and occupational exposure to organic solvents. *Q J Med*. 1992;305:707–712.
14. Rutter M, Russell-Jones R, eds. *Lead Versus Health: Sources and Effects of Low Level Lead Exposure*. New York: John Wiley; 1983.
15. Bland JS, Barrager E, Reedy RG, et al. A medical food-supplemented detoxification program in the management of chronic health problems. *Altern Ther Health Med*. 1995;1:62–71.
16. Rigden S. Entero-hepatic resuscitation program for CFIDS. CFIDS Chron. *Spring*. 1995:46–49.
17. Rigden S, Barrager E, Bland JS. Evaluation of the effect of a modified entero-hepatic resuscitation program in chronic fatigue syndrome patients. *J Adv Med*. 1998;11(4):247–262.
18. Rowe AH, Rowe Jr A. *Food Allergy: Its Manifestations and Control and the Elimination Diets. A Compendium*. Springfield, IL: CC Thomas; 1972.
19. Breneman JC. *Basics of Food Allergy*. Springfield, IL: CC Thomas; 1977.
20. Barnes BO, Galton L. *Hypothyroidism: The Unsuspected Illness*. New York: Thomas Crowell; 1976.
21. Langer SE, Scheer JF. *Solved: The Riddle of Illness*. New Canaan, CT: Keats; 1984.
22. Gold M, Pottash A, Extein I. Hypothyroidism and depression, evidence from complete thyroid function evaluation. *JAMA*. 1981;245:1919–1922.
23. Winokur A, Maislin G, Philips J, et al. Insulin resistance after glucose tolerance testing in patients with major depression. *Am J Psychiatry*. 1988;145:325–330.
24. Wright JH, Jacisin J, Radin N, et al. Glucose metabolism in unipolar depression. *Br J Psychiatry*. 1978;132:386–393.
25. Schauss AG. Nutrition and behavior: complex interdisciplinary research. *Nutr Health*. 1984;3:9–37.
26. Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr*. 1981;24:362–366.
27. Tintera JW. The hypoadrenocortical state and its management. *NY State J Med*. 1955;55:1869–1876.
28. Demitrack MA. Chronic fatigue syndrome: a disease of the hypothalamic-pituitary-adrenal axis? *Ann Med*. 1994;26:1–3.
29. Roberts AD, Wessely S, Chalder T, et al. Salivary cortisol response to awakening in chronic fatigue syndrome. *Br J Psychiatry*. 2004;184:136–141.
30. Cleare AJ. The HPA axis and the genesis of chronic fatigue syndrome. *Trends Endocrinol Metab*. 2004;15:55–59.
31. Demitrack MA, Dale JK, Straus SE, et al. Evidence for impaired activation of hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J Clin Endocrinol Metab*. 1991;73:1224–1234.
32. Giloteaux L, Goodrich JK, Walters WA, et al. Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome*. 2016;4(1):30. PubMed PMID: 27338587.
33. Prins JB, Bos E, Huibers MJ, et al. Social support and the persistence of complaints in chronic fatigue syndrome. *Psychother Psychosom*. 2004;73:174–182.
34. Sharpe M, Hawton K, Simkin S, et al. Cognitive behavior therapy for the chronic fatigue syndrome: a randomized controlled trial. *BMJ*. 1996;312:22–26.
35. LaManca JJ, Sisto SA, DeLuca J, et al. Influence of exhaustive treadmill exercise on cognitive functioning in chronic fatigue syndrome. *Am J Med*. 1998;105:S59–S65.
36. Estler CJ, Ammon HP, Herzog C. Swimming capacity of mice after prolonged treatment with psychostimulants. I. Effects of caffeine on swimming performance and cold stress. *Psychopharmacology*. 1978;58:161–166.
37. Greden JF, Fontaine P, Lubetsky M, et al. Anxiety and depression associated with caffeinism among psychiatric inpatients. *Am J Psychiatry*. 1978;135:963–966.
38. Chou T. Wake up and smell the coffee: caffeine, coffee, and the medical consequences. *West J Med*. 1992;157:544–553.
39. Hughes JR, Higgins ST, Bickel WK, et al. caffeine self-administration, withdrawal, and adverse effects among coffee drinkers. *Arch Gen Psychiatry*. 1991;48:611–617.
40. Manuel Y, Keenoy B, Moorkens G, et al. Magnesium status and parameters of the oxidant-antioxidant balance in patients with chronic fatigue: effects of supplementation with magnesium. *J Am Coll Nutr*. 2000;19:374–382.
41. Cox IM, Campbell MJ, Dowson D. Red blood cell magnesium and chronic fatigue syndrome. *Lancet*. 1991;337:757–760.
42. Ahlborg H, Ekelund LG, Nilsson CG. Effect of potassium-magnesium aspartate on the capacity for prolonged exercise in man. *Acta Physiol Scand*. 1968;74:238–245.
43. Hicks JT. Treatment of fatigue in general practice: a double blind study. *Clin Med*. 1964:85–90.
44. Friedlander HS. Fatigue as a presenting symptom: management in general practice. *Curr Ther Res*. 1962;4:441–449.
45. Shaw Jr DL, Chesney MA, Tullis IF, et al. Management of fatigue: a physiologic approach. *Am J Med Sci*. 1962;243:758–769.
46. Gullestad L, Oystein Dolva L, Birkeland K, et al. Oral versus intravenous magnesium supplementation in patients with magnesium deficiency. *Magnes Trace Elem*. 1991;10:11–16.
47. Lindberg JS, Zobitz MM, Poindexter JR, et al. Magnesium bioavailability from magnesium citrate and magnesium oxide. *J Am Coll Nutr*. 1990;9. 48-45.
48. Behan PO, Behan WM, Horrobin D. Effect of high doses of essential fatty acids on the postviral fatigue syndrome. *Acta Neurol Scand*. 1990;82:209–216.
49. Puri BK, Holmes J, Hamilton G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. *Int J Clin Pract*. 2004;58:297–299.
50. Lange G, Holodny AI, DeLuca J, et al. Quantitative assessment of cerebral ventricular volumes in chronic fatigue syndrome. *Appl Neuropsychol*. 2001;8:23–30.
51. Birkmayer GD, Kay GG, Vurre E. Stabilized NADH (ENADA) improves jet lag-induced cognitive performance deficit. *Wien Med Wochenschr*. 2002;152:450–454.
52. Forsyth LM, Preuss HG, MacDowell AL, et al. Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome. *Ann Allergy Asthma Immunol*. 1999;82:185–191.
53. Birkmayer JG, Nadlinger K. Safety of stabilized, orally absorbable, reduced nicotinamide adenine dinucleotide (NADH): a 26-week oral tablet admin-

istration of ENADA/NADH for chronic toxicity study in rats. *Drugs Exp Clin Res*. 2002;28:185–192.
54. Plioplys AV, Plioplys S. Amantadine and L-carnitine treatment of chronic fatigue syndrome. *Neuropsychobiology*. 1997;35:16–23.
55. Maes M, Mihaylova I, Kubera M, et al. Coenzyme Q10 deficiency in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another factor explaining the early mortality in ME/CFS due to cardiovascular disorder. *Neuro Endocrinol Lett*. 2009;30(4):470–476.
56. Fukuda S, Nojima J, Kajimoto O, Yamaguti K, Nakatomi Y, Kuratsune H, Watanabe Y. Ubiquinol-10 supplementation improves autonomic nervous function and cognitive function in chronic fatigue syndrome. *Biofactors*. 2016;42(4):431–440. PubMed PMID: 27125909.
57. Van Heukelom RO, Prins JB, Smits MG, et al. Influence of melatonin on fatigue severity in patients with chronic fatigue syndrome and late melatonin secretion. *Eur J Neurol*. 2006;13:55–60.
58. Bohn B, Nebe CT, Birr C. Flow-cytometric studies with *Eleutherococcus senticosus* extract as an immunomodulatory agent. *Arzniemitteforschung*. 1987;37:1193–1196.
59. Olsson EM, von Schéele B, Panossian AG. A randomised, double-blind, placebo-controlled, parallel-group study of the standardised extract shr-5 of the roots of *Rhodiola rosea* in the treatment of subjects with stress-related fatigue. *Planta Med*. 2009;75(2):105–112.
60. Brown D. Licorice root: potential early intervention for chronic fatigue syndrome. *Q Rev Nat Med*. 1996:95–96.
61. Baschetti R. Chronic fatigue syndrome and liquorice (letter). *N Z Med J*. 1995;108:156–157.
62. Farmer ME, Locke BZ, Moscicki EK, et al. Physical activity and depressive symptoms: the NHANES 1 Epidemiologic Follow-Up Study. *Am J Epidemiol*. 1988;1328:1340–1351.
63. Fiatarone MA, Morley JE, Bloom ET, et al. The effect of exercise on natural killer cell activity in young and old subjects. *J Gerontol*. 1989;44:M37–M45.
64. Makinnon LT. Exercise and natural killer cells: what is their relationship? *Sports Med*. 1989;7:141–149.
65. Sun XS, Xu Y, Xia YJ. Determination of E-rosette-forming lymphocytes in aged subjects with Taichiquan exercise. *Int J Sport Med*. 1989;10:217–219.
66. Fitzgerald L. Exercise and the immune system. *Immunol Today*. 1988;9:337–339.
67. Friedberg F. Does graded activity increase activity? A case study of chronic fatigue syndrome. *J Behav Ther Exp Psychiatry*. 2002;33:203–215.
68. Wallman KE, Morton AR, Goodman C, et al. Randomised controlled trial of graded exercise in chronic fatigue syndrome. *Med J Aust*. 2004;180:444–448.
69. Deale A, Chalder T, Marks L, et al. Cognitive behavior therapy for chronic fatigue syndrome: a randomized controlled trial. *Am J Psychiatry*. 1997;154:408–414.

161

Congestive Heart Failure

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Left ventricular failure—exertional dyspnea, cough, fatigue, orthopnea, cardiac enlargement, rales, gallop rhythm, and pulmonary venous congestion
- Right ventricular failure—elevated venous pressure, hepatomegaly, dependent edema
- Both left and right ventricular failure
- Diagnosis confirmed by echocardiography

GENERAL CONSIDERATIONS

Congestive heart failure (CHF) refers to the inability of the heart to pump enough blood. The contractile function of the heart is governed by five primary factors:

- The contractile state of the myocardium
- The preload of the ventricle
- End-diastolic volume
- Impedance to left ventricular ejection
- Heart rate

Chronic CHF is most often due to the long-term effects of high blood pressure, previous myocardial infarction, disorder of a heart valve, cardiomyopathy, or chronic lung disease. These conditions produce CHF by affecting one or more of the primary determinants of myocardial contractile function. (Precipitating or exacerbating factors in CHF are presented in Box 161.1.)

One of the most serious consequences of reduced cardiac output is a reduction in renal blood flow and the glomerular filtration rate, which in turn leads to fluid retention and dilutional hyponatremia (Fig. 161.1). In addition, activation of the renin–angiotensin–aldosterone system leads to an increase in peripheral vascular resistance, increased ventricular afterload, and increased levels of circulating vasopressin, which also serves as a vasoconstrictor and antidiuretic.

To compensate for the reduced cardiac output, several mechanisms occur: tachycardia, increased activation of the sympathetic nervous system, ventricular dilation, and hypertrophy. Although the increase in sympathetic activity increases cardiac output by increasing heart rate and force of contraction, it also leads to increased vascular resistance.

The signs and symptoms of CHF depend on which ventricle has failed. The clinical symptoms of left ventricular failure are dominated by symptoms of pulmonary congestion and edema, whereas right ventricular failure is characterized by signs of systemic venous congestion and peripheral edema. Weakness, fatigue, and shortness of breath are common to both right and left ventricular failure as well as biventricular failure.

DIAGNOSTIC CONSIDERATIONS

CHF is most effectively treated via natural measures in the early stages. Hence, early diagnosis and prevention by addressing causative factors are imperative. The first symptom of CHF is usually shortness of breath. A chronic nonproductive cough may also be the first manifesting symptom. Patients suspected of having CHF should have an extensive cardiovascular evaluation, including a complete physical examination to look for the characteristic signs of CHF (e.g., peripheral signs of heart failure, enlarged and sustained left ventricular impulse, diminished first heart sound, gallop rhythm), electrocardiography, and echocardiography.

THERAPEUTIC CONSIDERATIONS

In the initial stages of CHF, natural measures designed to address the underlying cause (e.g., hypertension) or improve the metabolic functions of the myocardium (described later, as well as measures described in Chapter 145) are often effective. In later stages, however, medical treatment involving the use of diuretics and angiotensin-converting enzyme (ACE) inhibitors, digitalis glycosides, or both is indicated in most cases. The measures described here can be used as adjunctive therapy in these more severe cases. The New York Heart Association (NYHA) staging system for CHF can be used in an effort to better identify likely respondents to natural therapy alone (Table 161.1). In general, excellent clinical results can be expected in stages I and II with the use of the natural measures described in this chapter.

Nutritional Supplements

The natural approach focuses on improving myocardial energy production because CHF is always characterized by a state of energy

BOX 161.1 Precipitating or Exacerbating Factors in Congestive Heart Failure

- Low levels of essential fatty acids
- Increased platelet aggregation
- Increased demand
  - Anemia
  - Fever
  - Infection
  - Fluid overload
  - Increased sodium intake
  - High environmental temperature
  - Renal failure
  - Hepatic failure
  - Thyrotoxicosis
  - Arteriovenous shunt
  - Respiratory insufficiency
  - Emotional stress
  - Pregnancy
  - Obesity
- Arrhythmias
- Pulmonary embolism
- Ethanol ingestion
- Nutrient deficiency
- Uncontrolled hypertension
  - Drugs
  - Beta-adrenergic blockers
  - Antiarrhythmic drugs
  - Sodium-retaining drugs
    - Corticosteroids
    - Nonsteroidal anti-inflammatory drugs

depletion. This impaired energy production is often the result of a nutrient or coenzyme deficiency (e.g., magnesium, thiamine, coenzyme Q_{10} [CoQ_{10}], carnitine). The dietary recommendations given in Chapter 174 are appropriate for most patients with CHF, especially if the CHF is due to long-term hypertension. Of particular importance is a diet low in sodium and high in potassium. A high intake of sodium greatly exacerbates the hemodynamic aspects of CHF. Sodium intake should be restricted to below 1.8 g daily. Furthermore, the dietary intake of several nutrients is usually well below recommended levels in patients with CHF.[1] Most notably low are magnesium, calcium, zinc, copper, manganese, thiamine, riboflavin, and folic acid. A high-potency multivitamin/multimineral formula is critical in these patients, especially if they are taking a diuretic.

Magnesium

Low magnesium levels (particularly white blood cell [WBC] magnesium) are commonly found in patients with CHF. This association is extremely significant because magnesium levels have been shown to correlate directly with survival rates. In one study, CHF patients with normal levels of magnesium had 1- and 2-year survival rates of 71% and 61%, respectively, compared with rates of 45% and 42%, respectively, in patients with lower magnesium levels.[2] These results are not surprising considering that magnesium deficiency is associated with cardiac arrhythmias, reduced cardiovascular prognosis, worsened ischemia, and increased mortality in acute myocardial infarction.

The magnesium deficiency probably stems from a combination of inadequate intake and increased wasting due to overactivation of the renin–angiotensin–aldosterone system, as is commonly seen in patients with heart failure. It can also be the effect of diuretics like furosemide.

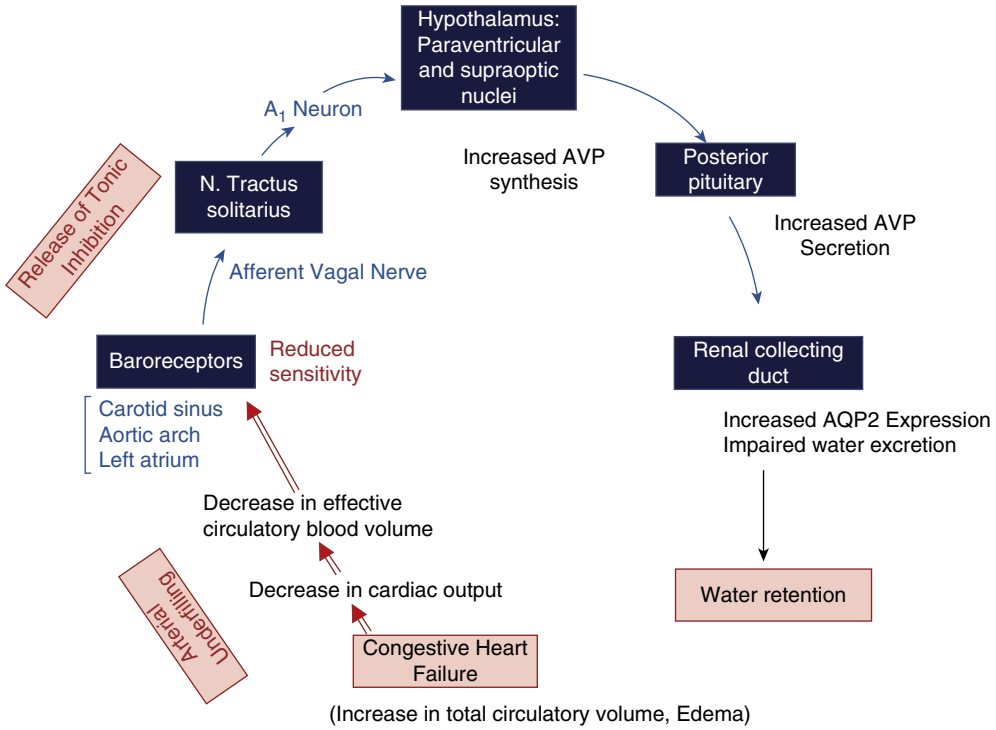


Fig. 161.1 Pathological role of AVP in impaired water excretion in congestive heart failure. *AQP2,* aquaporin-2; *AVP,* arginine vasopressin. (Ishikawa, SE. Hyponatremia associated with heart failure: pathological role of vasopressin-dependent impaired water excretion. *J Clin Med.* 2015;4[5]:933–947. PubMed PMID: 26239456.)

TABLE 161.1 Stages of Congestive Heart Failure as Defined by the New York Heart Association

Stage	Symptoms
Stage I	Patient is symptom-free at rest and with treatment.
Stage II	Patient experiences impaired heart function with moderate physical effort. Shortness of breath with exertion is common. There are no symptoms at rest.
Stage III	Even minor physical exertion results in shortness of breath and fatigue. There are no symptoms at rest.
Stage IV	Symptoms such as shortness of breath and signs such as lower extremity edema are present when the patient is at rest.

In addition to providing benefits of its own in CHF, magnesium supplementation prevents the magnesium depletion caused by the conventional drug therapy for CHF (e.g., digitalis, diuretics, and vasodilators such as beta blockers and calcium channel blockers). Magnesium supplementation has even been shown to produce positive effects in patients with CHF receiving conventional drug therapy even if serum magnesium levels are normal.[3] However, magnesium supplementation is not indicated in patients with renal failure, because this condition predisposes them to hypermagnesemia—a significant risk factor for mortality.[4] ACE inhibitors appear to produce a magnesium-sparing effect in patients on furosemide.[5] Typical dosages are 200 to 300 mg one to three times per day of magnesium in the citrate form. Oral magnesium can be effective in raising WBC magnesium and potassium levels.[6] It is highly important to monitor serum magnesium levels so as to prevent hypermagnesemia in patients with renal impairment as well as those on digoxin. Magnesium significantly reduces the frequency and complexity of ventricular arrhythmias in digoxin-treated patients with CHF even without the presence of digoxin toxicity, but too much magnesium may interfere with digoxin.[7]

Thiamine

Interest has increased regarding the potential role of thiamine deficiency in CHF. Thiamine was the first B vitamin discovered, hence its designation as vitamin B_1. It is well established that thiamine deficiency can result in the cardiovascular manifestations of "wet beriberi," sodium retention, peripheral vasodilation, and heart failure. It is also well established that furosemide (Lasix), the most widely prescribed diuretic, has been shown to cause thiamine deficiency in animals and patients with CHF. Diuretic use, changes in dietary habits, and altered thiamine absorption and metabolism have all been identified as possible mechanisms of thiamine deficiency in heart failure.[8]

Although severe thiamine deficiency is relatively uncommon except in alcoholics, many Americans do not consume the recommended daily allowance of 1.5 mg, especially elderly patients in hospitals or nursing homes. To gauge the prevalence of thiamine deficiency in the geriatric population, 30 consecutive patients visiting a university outpatient clinic in Tampa, Florida, were tested for thiamine levels. Depending on the thiamine measurement, plasma versus red blood cell thiamine, low levels (defined as a level below the lowest reference range for younger age groups) were found in 57% and 33%, respectively.[9]

These results highlight the growing body of evidence that a significant percentage of the geriatric population is deficient in one or more of the B vitamins. Given the essential role of thiamine and other B vitamins in normal human physiology, especially cardiovascular and brain function, routine supplementation of B vitamins in this age group appears to be worthwhile.

The association between thiamine deficiency and long-term furosemide use was discovered in 1980, when it was shown that after only 4 weeks of furosemide use, thiamine concentrations and the activity of the thiamine-dependent enzyme transketolase were significantly reduced. The first study looking at thiamine as a potential adjunct in the treatment of CHF showed only modest benefits. However, several subsequent studies have shown that daily doses of 80 to 240 mg of thiamine daily improved the clinical picture, as indicated by a 13% to 22% increase of left ventricular ejection fraction.[10,11] This is quite significant, because an increase in ejection fraction is associated with a greater survival rate in patients with CHF. Thiamine has also been shown to improve cardiac function, urine output, and weight loss in patients with CHF.[12] In one study, biochemical evidence of severe thiamine deficiency was found in 98% of patients receiving at least 80 mg/day of furosemide and in 57% of patients taking 40 mg furosemide daily.[13]

Given the possible benefit, lack of risk, and low cost of thiamine supplementation, administration of 200 to 250 mg of thiamine daily appears to be warranted in patients with CHF, especially if they are on furosemide.

Carnitine

Normal heart function is critically dependent on adequate concentrations of carnitine and CoQ_{10}. These compounds are essential in the transport of fatty acids into the myocardium and mitochondria for energy production. Although the normal heart stores more carnitine and CoQ_{10} than it needs, if the heart does not have a good supply of oxygen, carnitine and CoQ_{10} levels quickly decrease. Both of these agents have shown benefit in the treatment of CHF.

Several double-blind clinical studies have shown that carnitine supplementation improves cardiac function in patients with CHF.[14–16] In one double-blind study, only 1 month of treatment (500 mg three times daily) was necessary to cause a significant improvement in heart function.[15] The longer carnitine was used, the more dramatic was the improvement. After 6 months of use, the carnitine group demonstrated an increase in maximum exercise time of 25.9% and a 13.6% increase in ventricular ejection fraction. Another double-blind study showed that after 6 months of treatment, maximum exercise time on the treadmill increased by 16.4%, and the ejection fraction increased by 12.1%.[16]

Even more obvious benefits were seen in a 3-year study of 80 patients with moderate to severe heart failure (NYHA classifications III to IV) caused by dilated cardiomyopathy. After a period of stable cardiac function up to 3 months, patients were randomly assigned to receive either carnitine (2 g/day orally) or placebo. After a mean of 33.7 months of follow-up (range, 10–54 months), 70 patients were in the study: 33 in the placebo group and 37 in the carnitine group. At the time of analysis, 63 patients were alive. Six deaths occurred in the placebo group and one death in the carnitine group. Survival analysis showed that patients' survival was statistically significant in favor of the carnitine group.[17]

A meta-analysis of 17 randomized controlled trials with 1625 CHF patients demonstrated that L-carnitine treatment was associated with considerable improvement in overall efficacy, left ventricular ejection fraction, stroke volume, and cardiac output.[18] In addition, treatment with L-carnitine resulted in significant decreases in serum levels of brain natriuretic peptide, N-terminal probrain natriuretic peptide, left ventricular end-systolic dimension, left ventricular end-diastolic dimension, and left ventricular end-systolic volume.

Coenzyme Q_{10}

Numerous studies have also shown CoQ_{10} supplementation to be extremely effective in the treatment of CHF. Most of these studies used

CoQ_{10} as an adjunct to conventional drug therapy. In one of the early studies, 17 patients with mild CHF received 30 mg/day of CoQ_{10}.[19] All patients improved, and 9 (53%) became asymptomatic after 4 weeks. In another early study,[20] patients with congestive heart failure due to either atherosclerosis or high blood pressure were treated with CoQ_{10} at a dosage of 30 mg/day for 1 to 2 months.[20] Of these patients, 55% reported subjective improvement, 50% showed a decrease in NYHA classification, and 30% showed a "remarkable" decrease in chest congestion as seen on chest radiography. Patients with mild disease tended to improve more often than those with more severe disease. Subjective improvements in these patients—including increased cardiac output, stroke volume, cardiac index, and ejection fraction—were confirmed by various objective tests. These results were consistent with CoQ_{10} producing an increased force of heart muscle contraction (a positive inotropic effect) similar to but less potent than the effect of digitalis.[21,22]

Three more studies have also shown CoQ_{10} to be effective in significantly improving heart function in patients with CHF. In a double-blind Scandinavian study of 80 patients, participants were given either CoQ_{10} (100 mg/day) or placebo for 3 months and then crossed. The improvements noted with CoQ_{10} were actually found to be more positive than those obtained from conventional drug therapy alone.[23] In another double-blind study, 641 patients with CHF received either CoQ_{10} (2 mg/kg) or a placebo for 1 year.[24] The number of patients requiring hospitalization or experiencing serious consequences due to CHF was significantly reduced in the CoQ_{10} group compared with the placebo group.

In the largest study to date, a total of 2664 patients in NYHA classes II and III were enrolled in an open study in Italy.[25] The daily dosage of CoQ_{10} was 50 to 150 mg orally for 90 days, with the majority of patients (78%) receiving 100 mg/day. After 3 months of CoQ_{10} treatment, the proportions of patients with improvement in clinical signs and symptoms were as follows:

- Cyanosis: 78.1%
- Edema (fluid retention): 78.6%
- Pulmonary edema: 77.8%
- Enlargement of liver area: 49.3%
- Venous congestion: 71.8%
- Shortness of breath: 52.7%
- Heart palpitations: 75.4%
- Sweating: 79.8%
- Subjective arrhythmia: 63.4%
- Insomnia: 66.2%
- Vertigo: 73.1%
- Nocturnal urination: 53.6%

Improvement of at least three symptoms occurred in 54% of patients, indicating a significantly improved quality of life (QoL) with CoQ_{10} supplementation. The results also showed a low incidence of side effects; only 36 patients (1.5%) reported mild side effects attributed to CoQ_{10}.

These positive results with CoQ_{10}, however, were not seen in one clinical trial. In this double-blind study, 55 patients with CHF NYHA classes III and IV, ejection fraction of less than 40%, and peak oxygen consumption less than 50% during standard therapy were randomly assigned to receive CoQ_{10} (200 mg) or placebo. Analysis indicated that there were no changes in ejection fraction, peak oxygen consumption, or exercise duration in either group. Possible explanations for failure to achieve a therapeutic benefit in this study may include the insufficient strength of CoQ_{10} to produce significant effects in more severe stages of CHF or the fact that blood levels of CoQ_{10} did not reach sufficient levels. Although the mean serum concentration of CoQ_{10} increased from 0.95 mcg/mL to 2.2 μg/mL in 19 of 22 patients on CoQ_{10}, blood levels were below the suggested threshold of 2.5 mcg/mL.[26]

Arginine

Another amino acid of value in CHF is arginine, although via totally different mechanisms from those of other nutrients. Patients with CHF are less able to achieve peripheral vasodilation during exercise due to endothelial dysfunction. Because the endothelial cells make the natural vasodilator nitric acid from arginine, several researchers have evaluated the efficacy of arginine in improving CHF. The first study of orally administered arginine showed promising results. In a randomized double-blind placebo-controlled study of 5.6 to 12.6 g/day of oral L-arginine, peripheral blood flow was found to increase by 29%; in addition, 6-minute walking distance increased by 8% and arterial compliance by 19%.[27] Subsequent studies have shown arginine supplementation to improve endothelial cell and renal function (as shown by improvements in glomerular filtration rate, natriuresis, and plasma endothelin level) in patients with CHF.[28,29] The use of arginine is cautioned by a report in one study in survivors of myocardial infarction, where supplementation with arginine (9 g/day for 6 months) was associated with an increase in mortality compared with the placebo group (8.6% vs. 0%).[30]

Botanical Medicines

Crataegus oxyacantha (Hawthorn)

Preparations of *Crataegus* sp. appear to be quite useful in CHF, especially in the early stages as a sole agent and in the later stages in combination with digitalis cardioglycosides. The effectiveness of *Crataegus* in CHF has been repeatedly demonstrated in double-blind studies.[31–33] For example, 30 patients with CHF (NYHA stage II) were assessed in a randomized double-blind study.[32] Treatment consisted of a *Crataegus* extract standardized to contain 15-mg procyanidin oligomers per 80-mg capsule. Treatment duration was 8 weeks, and the substance was administered at a dosage of one capsule taken twice daily. The group receiving the *Crataegus* extract showed a statistically significant advantage over placebo in terms of changes in heart function as determined by standard testing procedures. Systolic and diastolic blood pressures were also mildly reduced. As in all other studies with *Crataegus* extracts, no adverse reactions occurred.

In another study, 78 patients with CHF (NYHA stage II) were given either 600 mg of standardized *Crataegus* extract or placebo daily.[33] The parameter used to measure effectiveness was the patient's working capacity on a bicycle ergometer. After 56 days of treatment, the *Crataegus* group had a mean increase of 25 W compared with the placebo group's increase of only 5 W. In addition, the *Crataegus* group experienced a mild but significant reduction in systolic blood pressure (171–164 mm Hg) and heart rate (115–110 beats per minute). There was no change in blood pressure or heart rate in the placebo group.

In patients with NYHA stage III CHF, *Crataegus* may not be sufficient to produce clinical effects. In a double-blind, placebo-controlled trial of 120 ambulatory patients with NYHA class II to III CHF, patients received conventional medical therapy as tolerated and were randomized to either hawthorn 450 mg twice daily or placebo for 6 months. The primary outcome at 6 months was a change in the 6-minute walk distance. Secondary outcomes included QoL measures, peak oxygen consumption, and anaerobic threshold during maximal treadmill exercise testing, NYHA classification, left ventricular ejection fraction (LVEF), neurohormones, and measures of oxidative stress and inflammation. There were no significant differences between groups in the 6-minute walk distance or on measures of QoL, functional capacity, neurohormones, oxidative stress, or inflammation. However, a modest difference in LVEF favored hawthorn.[34]

Terminalia arjuna

A traditional Ayurvedic botanical for cardiac failure has been shown to be effective in controlled clinical studies. Twelve patients with severe refractory congestive heart failure (class IV NYHA) received an extract (500 mg every 8 hours) from the bark of *Terminalia arjuna* or placebo for 2 weeks. Those receiving the medicinal plant experienced, according to echocardiographic evaluation, statistically significant improvements in several parameters of cardiac function, such as end-systolic volume and left ventricular ejection fraction (LVEF). A second, uncontrolled phase of the study using a combination of *T. arjuna* with conventional medication found that after 2 years, nine patients showed a remarkable improvement to NYHA class II, with the other three improving to class III.[35] In addition, a 12-week, double-blind, parallel, randomized, placebo-controlled, add-on clinical trial of arjuna extract in patients with CHF demonstrated improvement in functional capacity, antioxidant reserves, and symptom-related QoL domains.[36]

THERAPEUTIC APPROACH

Treatment with diet and the natural agents as described earlier is effective in the initial stages of CHF (i.e., NYHA stages I and II; Fig. 161.2). In later stages, adjunctive drug therapy is usually necessary. Treatment is designed to address the underlying pathophysiology and to improve myocardial function through improved energy production.

Diet

Patients should be encouraged to achieve their ideal body weight, restrict sodium intake (below 1.8 g daily), increase the consumption of plant foods in the diet, reduce the intake of saturated fat, and follow the other dietary guidelines given for lowering blood pressure.

Exercise

Exercise training can produce significant benefits in patients with heart failure. A review of meta-analyses and randomized, controlled trials of exercise training in patients with heart failure demonstrated that exercise training improves functional capacity, QoL, hospitalization, and systolic and diastolic function in patients with heart failure (Fig. 161.3).[37] Exercise is beneficial for patients with heart failure with or without systolic dysfunction, although subtle differences in prescriptions may be required.

Sauna

Far-infrared sauna (FIR) has shown benefit for individuals with diagnosed CHF (NYHA functional class II or III). Compared with a control group that was only treated with bed rest, the FIR sauna group experienced improvement in endothelial-dependent dilation of the brachial artery after only 10 sauna sessions.[38] Whereas the control group had no change in improved blood flow or CHF symptoms, 17/20 in the sauna group reported an improvement in clinical symptoms. FIR daily for 4 weeks produced statistically significant reductions in systolic blood pressure and significant improvements in left ventricular ejection fraction, exercise tolerance, increased peak respiratory flow, and anaerobic threshold in patients with CHF.[39]

Nutritional Supplements

Magnesium: 200 to 400 mg three times a day
Thiamine: 200 to 250 mg a day
L-Carnitine: 500 to 1000 mg three times a day
CoQ_{10}: 100 to 300 mg a day
Arginine: 1000 to 2000 mg three times a day

Botanical Medicines

- *C. oxyacantha* (Hawthorn) extract (1.8% vitexin–4% rhamnoside or 10% procyanidin content): 200 to 300 mg three times a day
- *T. arjuna* extract: 500 mg three times a day

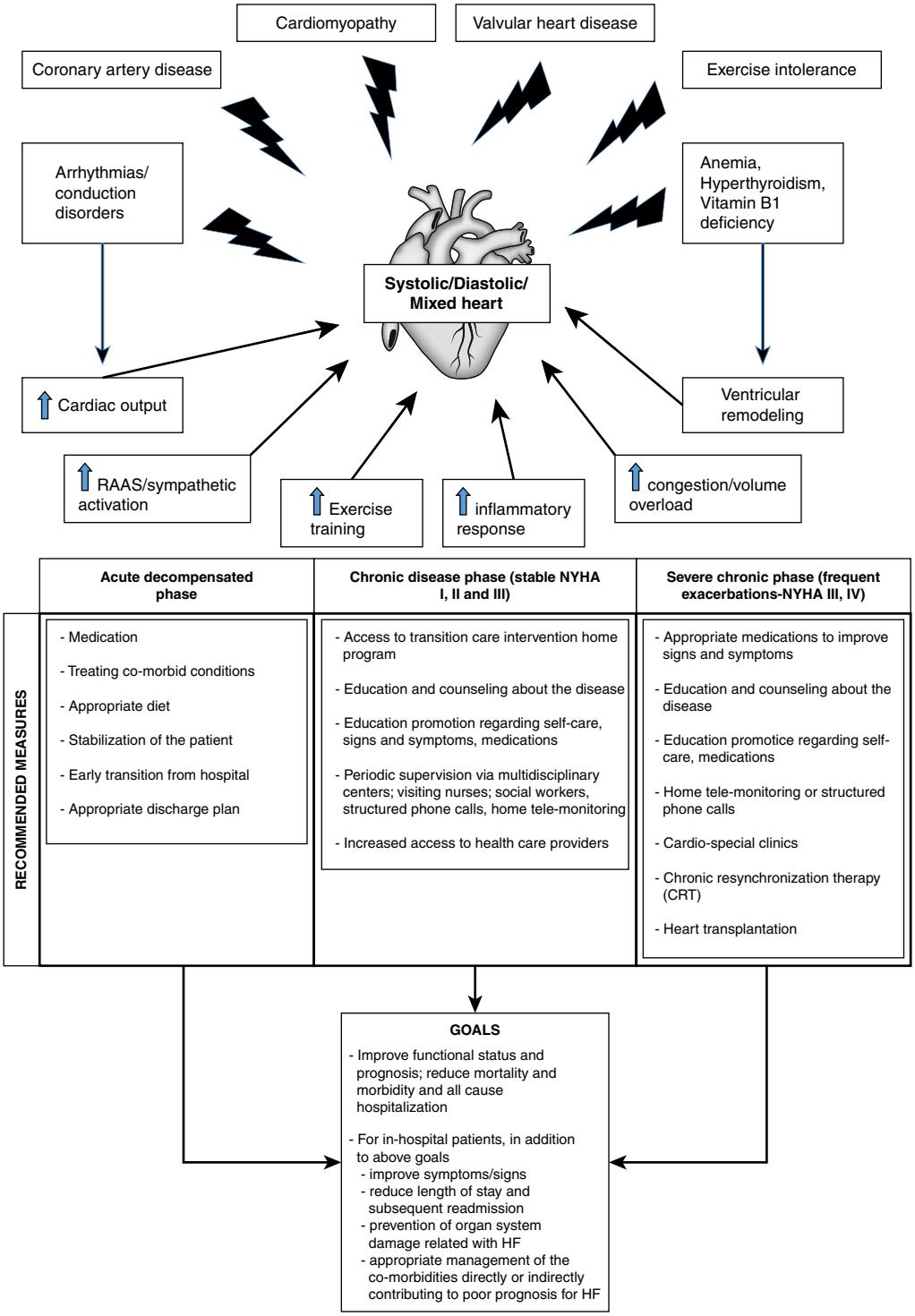


Fig. 161.2 A schematic diagram showing the pathogenic mechanism for heart failure, as well as the important recommended measures so as to meet the goals of heart failure treatment. (From Inamdar AA, Inamdar, AC. Heart failure: diagnosis, management and utilization. *J Clin Med.* 2016;5[7]:pii, E62. PubMed PMID: 27367736.)

Fig. 161.3 Clinical or prognostic markers of change in heart failure patients undertaking exercise training. (Smart, N. Exercise training for heart failure patients with and without systolic dysfunction: an evidence-based analysis of how patients benefit. *Cardiol Res Pract*. 2011;pii:837238. PubMed PMID: 20953365.)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gorelik O, Almoznino-Sarafian D, Feder I, et al. Dietary intake of various nutrients in older patients with congestive heart failure. *Cardiology*. 2003;99:177–181.
2. Gottlieb SS, Baruch L, Kukin ML. Prognostic importance of serum magnesium concentration in patients with congestive heart failure. *J Am Coll Cardiol*. 1990;16:827–831.
3. Gottlieb SS. Importance of magnesium in congestive heart failure. *Am J Cardiol*. 1989;63:39G–42G.
4. Cohen N, Almoznino-Sarafian D, Zaidenstein R, et al. Serum magnesium aberrations in furosemide (frusemide) treated patients with congestive heart failure: pathophysiological correlates and prognostic evaluation. *Heart*. 2003;89:411–416.
5. Oladapo OO, Falase AO. Serum and urinary magnesium during treatment of patients with chronic congestive heart failure. *Afr J Med Med Sci*. 2000;29:301–303.
6. Cohen N, Alon I, Almoznino-Sarafian D, et al. Metabolic and clinical effects of oral magnesium supplementation in furosemide-treated patients with severe congestive heart failure. *Clin Cardiol*. 2000;23: 433–436.
7. Crippa G, Sverzellati E, Giorgi-Pierfranceschi M, et al. Magnesium and cardiovascular drugs: interactions and therapeutic role. *Ann Ital Med Int*. 1999;14:40–45.
8. Jain A, Mehta R, Al-Ani M, Hill JA, Winchester DE. Determining the role of thiamine deficiency in systolic heart failure: a meta-analysis and systemic review. *J Cardiac Fail*. 2015;21(12):1000–1007. PubMed PMID: 26497757.
9. Chen MF, Chen LT, Gold M. Plasma and erythrocyte thiamine concentration in geriatric outpatients. *J Am Coll Nutr*. 1996;15:231–236.
10. Leslie D, Gheorghiade M. Is there a role for thiamine supplementation in the management of heart failure? *Am Heart J*. 1996;131:1248–1250.
11. Mendoza CE, Rodriguez F, Rosenberg DG. Reversal of refractory congestive heart failure after thiamine supplementation: report of a case and review of literature. *J Cardiovasc Pharmacol Ther*. 2003;8:313–316.
12. DiNicolantonio JJ, Niazi AK, Lavie CJ, O'Keefe JH, Ventura HO. Thiamine supplementation for the treatment of heart failure: a review of the literature. *Congestive Heart Fail*. 2013;19(4):214–222. PubMed PMID: 23910704.
13. Zenuk C, Healey J, Donnelly J, et al. Thiamine deficiency in congestive heart failure patients receiving long term furosemide therapy. *Can J Clin Pharmacol*. 2003;10:184–188.
14. Goa KL, Brogden RN. L-carnitine. A preliminary review of its pharmacokinetics, and its therapeutic use in ischemic cardiac disease and primary and secondary carnitine deficiencies in relationship to its role in fatty acid metabolism. *Drugs*. 1987;34:1–24.
15. Mancini M, Rengo F, Lingetti M, et al. Controlled study on the therapeutic efficacy of propionyl-l-carnitine in patients with congestive heart failure. *Arzneimittelforschung*. 1992;42:1101–1104.
16. Pucciarelli G, Matsursi M, Latte S, et al. The clinical and hemodynamic effects of propionyl-L-carnitine in the treatment of congestive heart failure. *Clin Ter*. 1992;141:379–384.
17. Rizos I. Three-year survival of patients with heart failure caused by dilated cardiomyopathy and L-carnitine administration. *Am Heart J*. 2000;139(2 Pt 3):S120–S123.
18. Song X, Qu H, Yang Z, Rong J, Cai W, Zhou H. Efficacy and safety of L-carnitine treatment for chronic heart failure: a meta-analysis of randomized controlled trials. *Biomed Res Int*. 2017;6274854. PubMed PMID: 28497060.
19. Ishiyama T, Morital Y, Toyama S, et al. A clinical study of the effect of coenzyme Q on congestive heart failure. *Jpn Heart J*. 1976;17:32–42.
20. Tsuyusaki T, Noro C, Kikawada R. Mechanocardiography of ischemic or hypertensive heart failure. In: Yamamura Y, Folkers K, Ito Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q*. vol. 2. Amsterdam: Elsevier/ North-Holland Biomedical Press; 1980:273–288.
21. Judy WV, Stogsdill WW, Folkers K. Myocardial effects of co-enzyme Q10 in primary heart failure. In: Folkers K, Yamamura Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q*. vol. 4. Amsterdam: Elsevier Science; 1984:353–367.
22. Vanfraechem JHP, Picalausa C, Folkers K. Coenzyme Q10 and physical performance in myocardial failure. In: Folkers K, Yamamura Y, eds. *Biomedical and Clinical Aspects of Coenzyme Q*. vol. 4. Amsterdam: Elsevier Science; 1984:281–290.
23. Hofman-Bang C, Rehnquist N, Swedberg K, et al. Coenzyme Q10 as an adjunctive treatment of congestive heart failure. *J Card Fail*. 1995;1:101–107.
24. Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q10 therapy in patients with congestive heart failure: a long-term multicenter randomized study. *Clin Investig*. 1993;71:S134–S136.
25. Baggio E, Gandini R, Plancher AC. Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure: CoQ10 drug surveillance investigators. *Mol Aspects Med*. 1994;15:s287–s294.
26. Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q10 in patients with congestive heart failure. *Ann Intern Med*. 2000;132:636–640.
27. Rector TS, Bank A, Mullen KA, et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. *Circulation*. 1996;93:2135–2141.
28. Hambrecht R, Hilbrich L, Erbs S, et al. Correction of endothelial dysfunction in chronic heart failure: additional effects of exercise training and oral l-arginine supplementation. *J Am Coll Cardiol*. 2000;35:706–713.
29. Watanabe G, Tomiyama H, Doba N. Effects of oral administration of l-arginine on renal function in patients with heart failure. *J Hypertens*. 2000;18:229–234.
30. Schulman SP, Becker LC, Kass DA, et al. l-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA*. 2006 Jan 4;295(1):58–64.
31. O'Conolly M, Jansen W, Bernhoft G. Treatment of decreasing performance: therapy using standardized crataegus extract in advanced age. *Fortschr Med*. 1986;104:805–808.
32. Leuchtgens H. Crataegus Special Extract WS 1442 in NYHA II heart failure. A placebo controlled randomized double-blind study. *Fortschr Med*. 1993;111:352–354.
33. Tauchert M, Ploch M, Hubner WD. Effectiveness of the hawthorn extract LI 132 compared to ACE inhibitor captopril: multicentre double-blind study with 132 NYHA stage II. *MMW Munch Med Wochenschr*. 1994;136(suppl 1):S27–S33.
34. Zick SM, Vautaw BM, Gillespie B, et al. Hawthorn Extract Randomized Blinded Chronic Heart Failure (HERB CHF) trial. *Eur J Heart Fail*. 2009;11(10):990–999.
35. Bharani A, Ganguly A, Bhargava KD. Salutary effect of *Terminalia arjuna* in patients with severe refractory heart failure. *Int J Cardiol*. 1995;49: 191–199.
36. Maulik SK, Wilson V, Seth S, et al. Clinical efficacy of water extract of stem bark of *Terminalia arjuna* (Roxb. Ex DC.) Wight % Arn. in patients of chronic heart failure: a double-blind, randomized controlled trial. *Phytomedicine*. 2016;23(11):1211–1219. PubMed PMID: 26988798.
37. Smart N. Exercise training for heart failure patients with and without systolic dysfunction: an evidence-based analysis of how patients benefit. *Cardiol Res Pract*. 2010;2011. pii: 837238 PubMed PMID: 20953365.
38. Kihara T, Biro S, Imamura M, et al. Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. *J Am Coll Cardiol*. 2002;39(5):754–759. PubMed PMID: 11869837.
39. Miyamoto H, Kai H, Nakaura H, et al. Safety and efficacy of repeated sauna bathing in patients with chronic systolic heart failure: a preliminary report. *J Card Fail*. 2005;11(6):432–436. PubMed PMID: 16105634.

Constipation

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Infrequent bowel movements (typically three times or fewer per week)
- Difficulty during defecation (straining during more than 25% of bowel movements or a subjective sensation of hard stools)
- Sensation of anorectal obstruction/blockage for at least 25% of defecations
- Large, hard stools or small, pellet-like stools
- Loose or insufficient stools rarely present without the use of laxatives
- In children, withholding behaviors (infants and toddlers may withhold bowel motion to avoid pain of passing stool)

GENERAL CONSIDERATIONS

An old-time naturopathic belief is that "disease begins in the colon." There appears to be great wisdom in that statement: improper elimination of waste products has significant health repercussions. Constipation is the most common digestive complaint in the United States. More than 4 million Americans have frequent constipation, accounting for 2.5 million physician visits per year. A review conducted in the United States reported a prevalence range from 2% to 27%, with most estimates falling between 12% and 19%.[1] However, differences in the definition of constipation have led to a wide range of reported prevalence (i.e., between 1% and 80%). More than $725 million is spent on laxative products each year in America.

Constipation is characterized by infrequent bowel movements, decreased urgency, and/or difficult stool passage. Despite a wide variation between individuals, normal whole-gut transit time is between 30 and 40 hours.[2] Patients with constipation have an increased gut transit time compared with healthy controls, with the upper limit of normal considered to be 70 h. Although frequency varies among individuals, one bowel movement per day should be considered normal. Some people consuming a high-fiber diet may have up to three bowel movements per day. Three or more days without a bowel movement may certainly be concerning because the stool becomes harder and more difficult to pass. Constipation can most simply be defined by one or a combination of frequency, size, consistency, and ease of elimination.[3]

A variety of risk factors are associated with constipation. Increased age, lower socioeconomic status, and lower level of education have been strongly linked with an increased likelihood of constipation. In addition, lower parental education, physical inactivity, medications, depression, physical and sexual abuse, and everyday life events also contribute to constipation.

Constipation among older people is far more common than among younger people. Common causes of constipation in the elderly are linked to several factors, including a lack of proper diet, a lack of adequate fluid intake, a lack of adequate physical activity, illness, and the use of drugs. In addition, female gender has been identified as a risk factor. Hormonal factors, damage to the innervation of the pelvic floor musculature related to childbirth or gynecological surgery, and genital prolapse may explain the high prevalence of constipation in women.[4] Severe constipation is markedly seen in elderly women compared with male individuals.[5]

Chronic constipation can have a significant negative effect on health-related quality of life and has been associated with psychological distress in severely affected patients. Constipation has the potential to cause patients to curtail work, school, and social activities. It is important for clinicians to have a clear understanding of the different pathophysiological mechanisms associated with constipation, understand the different testing modalities and treatments that are available, including their appropriateness and limitations, and tailor that knowledge to the management of individual patients.

Etiology

Constipation can be divided into two categories: primary (functional) and secondary (organic). Primary causes are intrinsic problems of

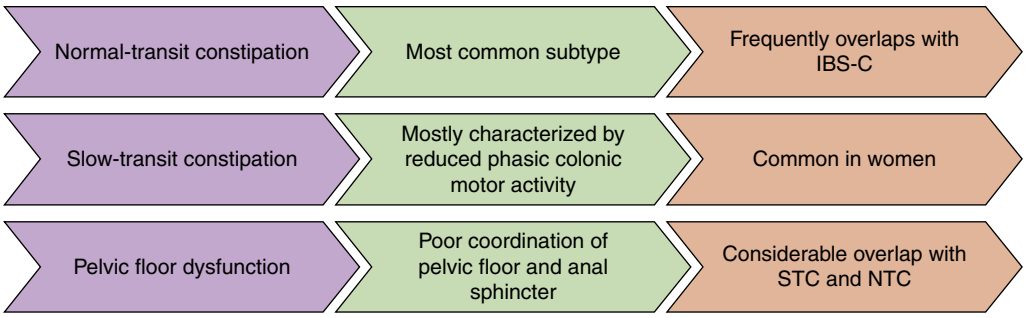


Fig. 162.1 Types of constipation. Primary (idiopathic) constipation can be conceptually categorized into three main types: normal transit, slow transit, and pelvic floor dysfunction. *IBS-C,* Constipation predominant irritable bowel syndrome; *STC,* slow-transit constipation. (From Tack J, Muller-Lissner S, Stanghellini V, Boeckxstaens G, Kamm MA, Simren M, Galmiche JP, Fried M. Diagnosis and treatment of chronic constipation—a European perspective. *Neurogastroenterol Motil.* 2011;23[8]:697–710. PubMed PMID: 21605282.)

colonic or anorectal function, whereas secondary causes are related to organic disease, systemic disease, or medications. Primary causes can be further subdivided into normal-transit constipation, slow-transit constipation, and anorectal dysfunction according to their pathophysiology (Fig. 162.1). Organic causes of constipation are the least common and include Hirschsprung's disease, anorectal malformation, spina bifida, and hypothyroidism. Functional constipation is usually caused by one or a combination of the following: dehydration, dysbiotic bowel flora, food sensitivity, and inadequate fiber intake.

Pathogenesis is multifactorial, with focuses on the type of diet, genetic predisposition, colonic motility, and absorption, as well as behavioral, biological, and pharmaceutical factors. Furthermore, low-fiber dietary intake, inadequate water intake, sedentary lifestyle, irritable bowel syndrome (IBS), failure to respond to urge to defecate, and slow transit have been revealed to be associated with a predisposition. Table 162.1 provides a more complete list of causes of constipation.

Several factors are vital to normal gut motility, including immune and nervous system function, bile acid metabolism and mucus secretion, and the gastrointestinal microbiota. An imbalance or dysfunction in any of these components may contribute to aberrant gut motility and, consequently, symptoms of constipation (Fig. 162.2). Normal-transit constipation is the most common form of constipation seen by clinicians. Although patients report symptoms they believe are consistent with constipation, such as the presence of hard stools or a perceived difficulty with evacuation, stool transit is not delayed, and stool frequency is typically within the normal range.[6] Slow-transit constipation causes infrequent bowel movements (typically less than once per week) and is most common in young women. Colonic transit time is prolonged in these patients, and patients may complain of associated bloating and abdominal discomfort because they do not feel the urge to defecate.[7] Defecation disorders (DDs) are a group of functional and anatomical abnormalities of the anorectum that lead to symptoms of constipation. Patients with DDs present with significant straining, often spending large amounts of time on the toilet daily, with frequent position changes and enema use. Dyssynergia, probably the most common functional DD, is an acquired behavioral DD resulting from poor toilet habits, painful defecation, obstetrical or back injury, or brain–gut dysfunction.[8]

Constipation in Children

Normal bowel movement frequency ranges from four stools per day in the first week of life to 1.2 per day at 4 years of age.[9] Constipation in children usually occurs at three distinct points in time: after starting formula or processed foods (while an infant), during toilet training in toddlerhood, and soon after starting school (nursery school or kindergarten). Factors that may contribute to functional constipation include pain, fever, dehydration, dietary and fluid intake, psychological issues, toilet training, medicines, and family history of constipation. As with adults, increasing fiber content usually produces the desired result. For children with a history of constipation, the initial step may be the elimination of milk and other dairy products from the diet. It is well accepted that cow's milk intolerance (either allergy or lactose intolerance) can produce diarrhea. Dairy intolerance is also a major cause of childhood constipation.[10] About 70% of cases of childhood constipation are improved with the elimination of cow's milk from the diet and substituting with nondairy milk alternatives (e.g., rice, almond, hemp, oat). Kids with constipation who respond to milk elimination also experience a decreased frequency of allergy symptoms (e.g., runny nose, eczema, asthma).

Slippery elm is effective in treating constipation in children from 4 months of age and above. The increased bulk stimulates peristalsis, and its mucilaginous properties create soft, slippery stools. From 12 months of age, freshly ground flax seeds can be added to pureed fruit/vegetables or mixed into water to increase fiber intake. Psyllium husks are slightly more abrasive than slippery elm and flax and thus may not be suitable to all children. Mineral oil and stimulant laxatives should be avoided unless necessary.

Microbiota

Research between intestinal diseases and gut microbiota has gradually revealed a connection between constipation and intestinal flora disturbance, providing a theoretical basis for microbial treatment in chronic constipation. Dysbiosis may be an effect as well as a cause of constipation.[11] Significant differences in bowel flora populations have been found in constipated individuals compared with nonconstipated individuals. Fecal microbiota composition is correlated with colonic transit time, and the colonic mucosal microbiota composition is associated with constipation status. More specifically, the abundance of *Bacteroides, Lactococcus,* and *Roseburia* was found to correlate with faster gut transit time, whereas *Faecalibacterium* was directly correlated to slower transit time.[12]

Gut microbiota modulation can be affected by probiotics, prebiotics, symbiotics, postbiotics, antibiotics, and fecal microbiota transplantation (FMT). Probiotics that have demonstrated a beneficial effect on bowel motion frequency and orofecal transit time include the following:

- *Bifidobacterium longum* 46 and 2C[13]
- *Bifidobacterium lactis* Bb-12, LKM512, and DN-173 010[14,15]
- *Lactobacillus casei* Shirota[16]

TABLE 162.1 Causes of Constipation

- Dietary
  - Highly refined, low-fiber foods
  - Inadequate fluid intake
  - Black tea
- Physical inactivity
  - Inadequate exercise
  - Prolonged bed rest
- Pregnancy
- Advanced age
- Psychogenic disorders
  - Anxiety
  - Depression
- Food sensitivities
- Dysbiosis
- Enemas (chronic use)
- Medications
  - Anesthetics
  - Antacids (aluminum and calcium salts)
  - Anticholinergics (bethanechol, carbachol, pilocarpine, physostigmine, ambenonium)
  - Anticonvulsants
  - Antidepressants (tricyclics, monoamine oxidase inhibitors)
  - Antihistamines
  - Antihypertensives (beta-adrenergic blocking agents, calcium channel blockers)
  - Anti-parkinsonism drugs
  - Antipsychotics (phenothiazines)
  - Bismuth salts
  - Diuretics
  - Iron salts
  - Laxatives and cathartics (chronic use)
  - Muscle relaxants
  - Nonsteroidal anti-inflammatory drugs
  - Opioids
  - Oral contraceptives
  - Progesterone
- Toxic metals
  - Arsenic
  - Lead
  - Mercury
- Metabolic abnormalities
  - Low potassium stores
  - Hyper- and hypocalcemia
  - Kidney disease
- Endocrine abnormalities
  - Hypothyroidism
  - Diabetes
  - Pituitary disorders
  - Hyperparathyroidism
- Structural abnormalities
  - Anatomical abnormalities in bowel structure
- Bowel diseases
  - Diverticulosis
  - Irritable bowel syndrome (alternating diarrhea and constipation)
  - Tumor
- Neurogenic abnormalities
  - Nerve disorders of the bowel
  - Aganglionosis
  - Autonomic neuropathy
  - Hirschsprung's disease
  - Spinal cord disorders
  - Trauma
  - Multiple sclerosis
  - Tabes dorsalis
  - Disorders of the splanchnic nerve
    - Tumors
    - Trauma
  - Cerebral disorders
  - Stroke
  - Parkinsonism
  - Neoplasm

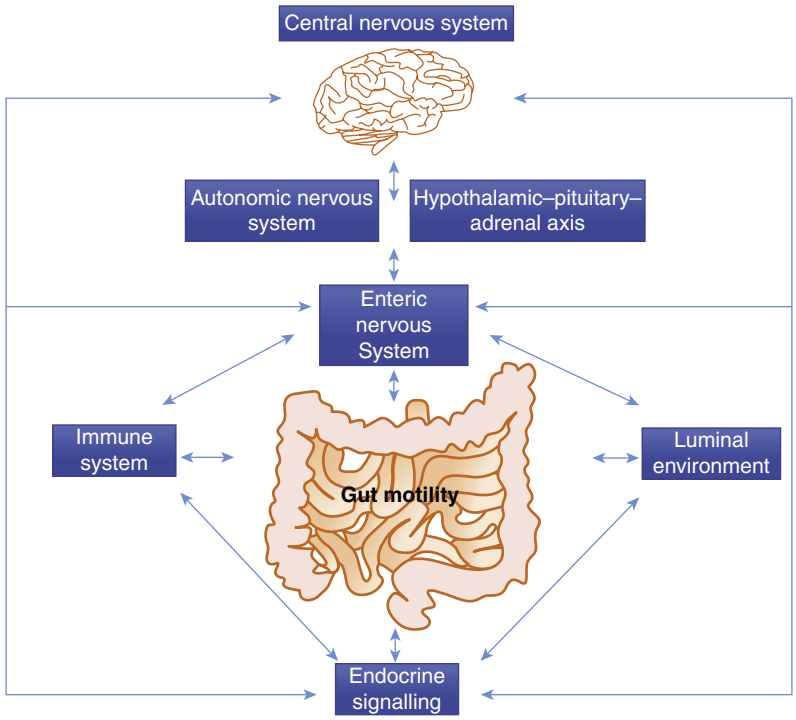


Fig. 162.2 Factors that control gut motility. (From Dimidi E, Christodoulides S, Scott SM, Whelan K. Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. *Adv. Nutr.* 2017;8[3]:484–494. PubMed PMID: 28507013.)

- *Bifidobacterium animalis*[10]
- *Lactobacillus rhamnosis*[17]
- *Propionibacterium freudenreichii*[12]
- *Lactobacillus plantarum* SN13T[18]
- *Lactobacillus reuteri* DSM 17938[19]

Toxins

Although uncommon, lead poisoning should be considered as a differential diagnosis in cases of unexplained acute abdominal pain in both adults and children. Although most cases of lead intoxication come from occupational exposures, traditional remedies, specifically Ayurvedic medicines, have been reported to contain toxic amounts of lead. A retrospective, observational case series from a tertiary care center in India evaluated the charts of patients who underwent blood lead level (BLL) testing as a part of a workup for unexplained abdominal pain between 2005 and 2013, and patients with lead intoxication (BLLs >25 µg/dL) were identified.[20] BLLs were tested in 786 patients, and high levels were identified in 75 (9.5%) of patients, of which a majority (73 patients, 9.3%) had a history of Ayurvedic medication intake, and only 2 had occupational exposure. Five randomly chosen Ayurvedic medications were analyzed, and lead levels were exceptionally elevated (14–34,950 ppm) in all of them. Besides abdominal pain, other presenting complaints were constipation, hypertension, neurological symptoms, and acute kidney injury. Discontinuing the Ayurvedic medicines and chelation with d-penicillamine led to an improvement in symptoms and reduction in BLLs in all patients within 3 to 4 months.

DIAGNOSTIC CONSIDERATIONS

Diagnosing constipation is primarily based on clinical history, physical examination, and initial workup to rule out secondary causes. A standardized definition of constipation is given in the Rome IV criteria for functional gastrointestinal disorders (FGIDs). According to the Rome IV criteria, functional constipation is defined when two or more of the following symptoms are present for the past 3 months with an onset at least 6 months before diagnosis: (1) straining during at least 25% of defecations, (2) lumpy or hard stools in at least 25% of defecations, (3) sensation of incomplete evacuation for at least 25% of defecations, (4) sensation of anorectal obstruction/blockage for at least 25% of defecations, (5) manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor), and (6) fewer than 3 defecations per week.[21] In addition, loose stools are rarely present without the use of laxatives, and the patient does not meet Rome IV criteria for irritable bowel syndrome.

A history and physical examination are often all that is needed to confirm the diagnosis of constipation. The physical examination should include an assessment of "alarm" signs and symptoms (i.e., fever, abdominal distention, anorexia, nausea, vomiting, weight loss, or poor weight gain). On abdominal examination, distention or a palpable "mass" may be appreciated in the lower abdomen. If treatment with fiber supplements and/or laxatives is unsuccessful, further workup is required. Blood tests such as complete blood count (CBC); thyroid function tests; blood glucose; and serum levels of electrolytes, calcium, and magnesium help rule out organic causes of constipation. Imaging studies, including abdominal x-ray, ultrasound, colonic transit tests, defecography, magnetic resonance (MR) defecography, and colonoscopy, may also provide information on the etiology of stool retention (Fig. 162.3). Currently, there is no uniform standardization of tests, and robust normative data for all measures of function remain inadequate, particularly regarding age and gender stratification.

THERAPEUTIC CONSIDERATIONS

Constipation will often respond to self-management strategies that include a high-fiber diet, plentiful fluid consumption, and exercise. These recommendations are well accepted. In fact, there is agreement across the medical community that these recommendations should constitute the first step in the treatment of chronic constipation. The treatment time for constipation can range from a few days in simple cases to more than 12 months in severe cases.

Dietary Factors

Fiber. It is well accepted that increasing dietary fiber is an effective treatment of chronic constipation. High levels of dietary fiber increase both the frequency and quantity of bowel movements, decrease the transit time of stools, decrease the absorption of toxins from the stool, and appear to be a preventive factor in several diseases. The recommended daily intake is 25 to 35 grams of fiber from dietary sources. However, higher amounts may be more optimal for health, as the human evolutionary diet contained approximately 100 grams of daily fiber. Most Western diets provide only 10 to 15 grams of fiber per day. The health-promoting effects of insoluble fiber from the bran of grains is well documented and extends beyond just its laxative effects.[22] The benefits of reduced stool transit time and increased fecal frequency may have more profound long-term benefits beyond the comfort that accompanies bowel regularity.[23] In addition, a systematic review showed that soluble fiber improved constipation symptoms in IBS, with varying effects on abdominal pain.[24]

A whole foods–based diet focusing on a large intake of vegetables and fruits as well as unrefined whole grains and legumes should be foundational. Foods particularly effective in relieving constipation are bran and prunes. The typical recommendation for bran is ½ cup of bran cereal, increasing to 1.5 cups over several weeks. A minimum of six to eight glasses of water should be consumed when using bran. Whole prunes, as well as prune juice, also possess good laxative effects. In one study, subjects suffering from chronic constipation received either dried prunes (50 g per day providing 6 g of fiber [about 10 prunes]) or psyllium (11 g per day providing 6 g of fiber) for 3 weeks each, in a crossover trial with a 1-week washout period.[25] Subjects maintained a daily symptom and stool diary. The number of complete spontaneous bowel movements per week (primary outcome measure) and stool consistency scores improved significantly with dried prunes compared with psyllium. However, straining and global constipation symptoms did not differ significantly between treatments. Four to eight ounces of prune juice or 5 to 10 prunes is usually an effective dose.

Exercise

Moderate to vigorous exercise, such as jogging, brisk walking, and water aerobics, is associated with a decreased prevalence of constipation. In multivariate analysis, women who reported daily physical activity had a 44% reduction in risk of constipation (prevalence ratio [PR] = 0.56, 95% confidence interval [CI]; 0.44–0.70).[26] One study reported that in the preparation for colonoscopy, patients who did walking exercise had better colon cleansing than those who rested.[27] Most walking patients (97.3%) considered walking exercise more comfortable than taking the polyethylene glycol solution. In addition, two studies on children have reported a negative association between physical activity and constipation. In the first study, children with moderate (615–1230 counts/15 seconds) and total activity (≥1231 counts/15 seconds) at the age of 2 years had significantly less functional constipation in the fourth year of life.[28] Additionally, children with physical activity of more than the World Health Organization (WHO) recommendation of 60 min/day

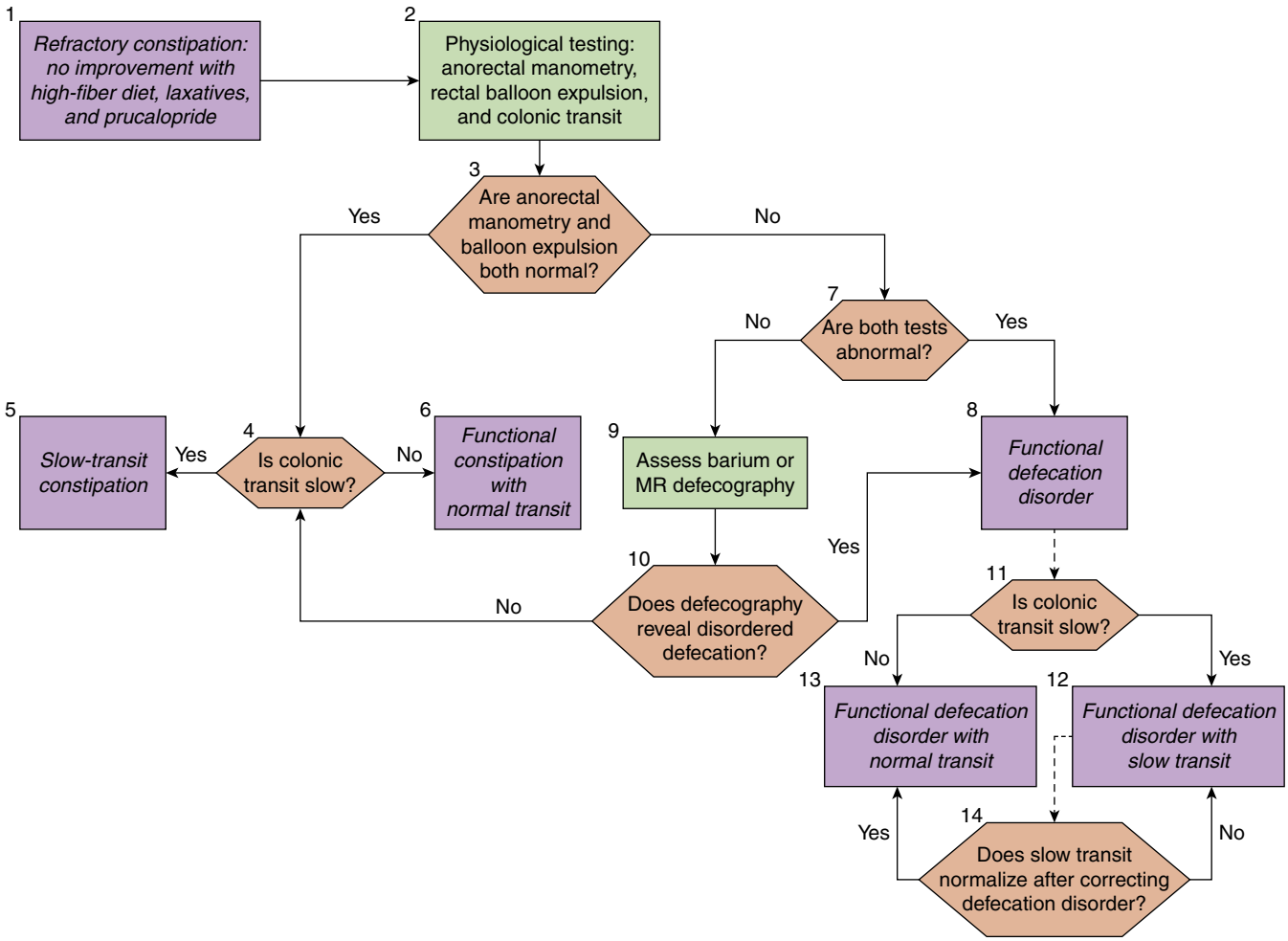


Fig. 162.3 Refractory constipation and difficult defecation. (1) Patients who fulfill the criteria for functional constipation and those who have not improved with an increase in dietary fiber and the use of simple laxatives, and with no alarm features, often warrant further physiological assessment. (2) The three key physiological investigations are anorectal manometry, the balloon expulsion test, and a colonic transit study. (3, 4) If both anorectal manometry and balloon expulsion are normal, the results of colonic transit testing enable characterization of the disorder as functional constipation with slow (5) or normal transit (6). (7, 8) If both manometry and the rectal balloon expulsion test are abnormal, this is sufficient to diagnose a functional defecation disorder. (9) If only one of the anorectal manometry and balloon expulsion is abnormal, further testing using barium or magnetic resonance defecography may be used to confirm or exclude the diagnosis. (10) If defecography reveals features of disordered defecation, a diagnosis of a functional defecation disorder can be made. (8) If defecography is not abnormal, then the patient does not fulfill criteria for the diagnosis of a functional defecation disorder; further diagnosis then depends on the presence or absence of colonic transit delay (see previous 4–6). (11–13) Treatment of choice for disordered defecation is biofeedback. If there is no adequate response to therapy, further investigation may be considered at this point. The presence of a functional defecation disorder does not exclude the diagnosis of slow colonic transit. Thus depending on the results of the colonic transit study, the patient can be characterized as suffering from a functional defecation disorder with slow (12) or normal colonic transit. (13, 14) Slow colonic transit may result from a defecation disorder. If it is felt appropriate to distinguish between the two possibilities, the colonic transit evaluation may be repeated after correction of the defecation disorder. If transit normalizes, the presumption is that the delay was secondary to the defecation disorder; if not, the delayed colonic transit is presumed to be a comorbid condition, which may require therapy if there is no clinical improvement with the treatment of functional defecation disorder. (From Tack J, Muller-Lissner S, Stanghellini V, Boeckxstaens G, Kamm MA, Simren M, Galmiche JP, Fried M. Diagnosis and treatment of chronic constipation—a European perspective. *Neurogastroenterol Motil.* 2011;23[8]:697–710. PubMed PMID: 21605282.)

had significantly less functional constipation in the fourth year of life. The second study was a cross-sectional evaluation that looked at factors associated with constipation in 1900 schoolchildren from ages 7 to 12 in Turkey.[29] Siblings with health problems, history of constipation in family members, abnormal oral habits, and little regular sporting activity were more common in constipated children than in those who were nonconstipated. Considering that insufficient physical activity and excessive sedentary behaviors are associated with constipation, incorporating exercise is advised as a preventative and, in some cases, therapeutic option.

Nutritional Factors

Magnesium

Magnesium-containing cathartics are used worldwide to treat chronic constipation.[30] Absorbable magnesium powders, such as magnesium citrate, alginate, oxalate, or amino acid chelate, can be used to help relax and promote healthy peristalsis of the smooth muscle of the intestinal tract. In addition, magnesium salts are known for their osmotic effects, accelerating intestinal transit time and leading to better stool consistency. A study in healthy human subjects demonstrated that high doses of orally administered magnesium sulfate accelerate small intestinal transit time and modulate antroduodenal motility in the fasting but not in the postprandial state.[31] A placebo-controlled, double-blind, randomized trial investigated whether a natural mineral water rich in magnesium sulfate and sodium sulfate (Donat Mg, 13g/L of dissolved mineral substances) may help improve bowel function.[32] A total of 106 otherwise-healthy subjects with functional constipation were randomly assigned to consume 300 or 500 mL of a natural mineral water, compared with placebo water, over a course of 6 weeks. Subjects documented complete spontaneous bowel movements, spontaneous and overall bowel movements/week, stool consistency, GI symptoms, and general well-being in a diary. For the 75 subjects in the 500-mL arms, stool consistency of spontaneous bowel movements ($p < 0.001$) and the subjectively perceived symptoms concerning constipation ($p = 0.005$) improved significantly with the natural mineral water compared with placebo.

Vitamin C

Vitamin C to bowel tolerance can be used as a bowel flush and may have added benefits in immune-challenged individuals.

Microbial Therapy

Microbial treatment mainly includes bacterial preparations such as probiotics, prebiotics, symbiotics, and FMT. Due to their safety, convenience, and curative effect, probiotic preparations have been widely accepted. Microbial treatment improves clinical symptoms, promotes the recovery of intestinal flora, and has no complications during the treatment process.

Prebiotics

Prebiotics are defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health."[33] *Lactobacilli* and *Bifidobacteria* feed on dietary plant fibers, allowing them to have strong populations and produce a tremendous amount of organic acids with far-reaching health benefits. Short-chain fatty acids help feed and fuel the cells of the colon and prevent dysbiosis and inflammation in the gut. Lactulose is a disaccharide manufactured from lactose that is poorly digested in the intestines. Lactulose is highly bifidogenic and is used in conventional medicine mainly for its laxative effects. Ingestion of lactulose was found to cause statistically significant increases in the frequency, weight, volume, and water content of stools and to produce stools of softer consistency compared with both baseline and placebo.[34] An intake of 15 g/day of either inulin or short-chain fructose oligosaccharides (FOSs) results in significant increases in bifidobacterial levels and increases the absorption of calcium and magnesium in the large intestine.[35,36] Several studies were conducted to evaluate the effects of galactooligosaccharide (GOS) administration on bowel function. Consuming 9 g/day of GOS was found to increase defecation frequency (from 5.9–7.1 movements per week) in elderly subjects over a 2-week treatment period.[37] There was also a trend for easier defecation during the GOS phase of the trial. Randomized controlled trials evaluating the laxative effects of GOS in healthy adult subjects with a tendency to constipation found that a daily dose of 5 or 10 g of GOS produced significant improvements in defecation frequency.[38] Foods high in prebiotics include artichokes, garlic, onion, leeks, asparagus, chives, legumes, peas, fruit, and okra.

Probiotics

Probiotics have long been used for the treatment of constipation. Studies have shown that specific probiotics may help decrease gut transit time in people with or without constipation. Meta-analyses of randomized controlled trials have demonstrated efficacy for several probiotic strains. A systematic review of randomized controlled trials combining data from a variety of probiotic strains found a significant reduction in whole-gut transit time (12.4-hr reduction; 95% CI: −22.3 to −2.5 hr) and increased stool frequency by an extra 1.3 bowel movements/week (95% CI: 0.7–1.9 bowel movements/week).[39] Stool consistency was also significantly improved. Another systematic review evaluating the capacity of probiotics to speed intestinal transit time found that probiotics could significantly reduce intestinal transit time, with large effect sizes seen in two probiotic strains: *Bifidobacterium lactis* HN019 and *Bifidobacterium lactis* DN-173 010.[40]

The mechanism of action of probiotics may involve the enteric nervous system. In a mouse study, administration of *Lactobacillus reuteri* has been shown to modulate reflexes that communicate with the brain.[41] In addition, *L. reuteri* has been shown to selectively increase the excitability of myenteric neurons in rats and interact with the gut–brain axis through the modulation of enteric sensory nerves that affect gut motility and pain perception.[42]

Botanicals

Although there is a paucity of research surrounding the use of herbal medicine in constipation, several herbs have properties that may provide benefit. Carminative herbs work by relaxing sphincters in the gastrointestinal tract and may aid in the treatment of constipation. Examples of carminative herbs include *Mentha piperita, Foeniculum vulgare, Zingiber officinalis, Melissa officinalis, Chamomilla recutita, Rosmarinus officinale, Curcuma longa,* and *Cinnamomum zeylanicum.* Demulcents, such as *Ulmus fulva, Althea officinalis,* and *Glycyrrhiza glabra,* are also sometimes used to ease constipation. From the whole plant, *Aloe vera* latex is a laxative that is regulated by the U.S. Food and Drug Administration.[43]

Laxatives

Bulk-forming fiber supplements are preferred to other forms of laxatives. Fiber formulas act as bulking agents and can be composed of natural soluble fibers derived from psyllium seed, kelp, agar, pectin, and plant gums like karaya and guar. Fiber formulas may also be composed of purified semisynthetic polysaccharides like methyl-cellulose and carboxymethyl cellulose sodium. Psyllium-containing laxatives are the most popular and usually the most effective. Psyllium is derived from the seed of the plant *Plantago ovago* native to Iran and India. The laxative properties of psyllium are due to the swelling of the husk when it contacts water. This forms a gelatinous mass and keeps the feces hydrated and soft. The resulting bulk stimulates a reflex contraction of the walls of the bowel, followed by emptying. Bulk-forming fiber formulas most closely approximate the natural mechanism that promotes a bowel movement and are both safe and effective in the treatment of chronic constipation.[44]

In difficult cases, the use of other laxatives is required to improve symptoms (Table 162.2). Conventional guidelines recommend daily polyethylene glycol (PEG) at a dose of 1 to 1.5 gm/kg per day for 3 to 6 days for initial fecal disimpaction, followed by a daily maintenance dose of 0.4 gr/kg per day for at least 2 months to prevent reaccumulation. A stimulant laxative should be added if PEG alone does not cause disimpaction after 2 weeks of treatment. Neuromuscular agents, such

TABLE 162.2 Types of Laxatives

Type of Laxative	How It Works	Side Effects
Bulk-forming fibers (psyllium, guar, methylcellulose)	Absorb water to form soft, bulky stool, prompting normal contraction of intestinal muscles	Bloating, gas, cramping, choking, or increased constipation if not taken with enough water
Oral osmotics (magnesium hydroxide)	Draw water into colon from surrounding body tissues to allow easier passage of stool	Bloating, cramping, diarrhea, nausea, gas, increased thirst
Oral stool softeners (docusate)	Add moisture to stool to allow strain-free bowel movements	Throat irritation, cramping
Oral stimulants (senna and cascara)	Trigger rhythmic contractions of intestinal muscles to eliminate stool	Belching, cramping, diarrhea, nausea, urine discoloration
Rectal stimulants (glycerine suppositories)	Trigger rhythmic contractions of intestinal muscles to eliminate stool	Rectal irritation, stomach discomfort, cramping

Cautions and Warnings

Do not use senna for more than 7 days unless directed by your doctor.

Stimulant laxatives should not be used in patients with abdominal pain, nausea or vomiting, intestinal obstruction, inflammatory bowel disease, or appendicitis or during pregnancy or lactation.

Do not take more than the recommended amount. Excessive laxative use or inadequate fluid intake may lead to significant fluid and electrolyte imbalance.

as tegaserod, a serotonin type 4 receptor antagonist, may also be used to stimulate peristalsis and fluid secretion.

Senna is a natural stimulant laxative that works by increasing the strength of contraction of the intestinal muscles. The laxative components are compounds known as sennosides. Senna should be used occasionally because tolerance can develop, and long-term use can lead to dependence. Senna is recommended to be taken on an empty stomach. Six to eight glasses of liquid should be consumed daily while taking senna or any other laxative.

Stimulant laxatives may cause abdominal cramping, nausea, and increased mucus secretion. Less common side effects are associated with chronic use and are usually related to loss of potassium and other electrolytes (e.g., muscle spasms, weakness, fatigue). A benign blackish-brown pigmentation of the lining of the colon (pseudomelanosis coli) may occur with prolonged use (at least 4 months) of senna due to the anthraquinones. This condition is generally reversible within 4 to 15 months after discontinuation. More significant side effects, including a sudden change in bowel habits that persists over a period of 2 weeks, rectal bleeding, or failure to have a bowel movement after use, require immediate attention.

Stimulant laxatives may decrease the absorption of drugs that pass through the GI tract. Individuals taking oral medications should talk to their pharmacist or physician before self-medicating with senna.

Senna may potentiate the action of digoxin and other heart medications due to potassium depletion. The use of senna with thiazide diuretics and corticosteroids may further decrease potassium levels.

If stimulant laxatives are used for long periods of time, the bowels will need to be "retrained." Table 162.3 provides recommended rules for reestablishing bowel regularity. The results of this procedure may take 4 to 6 weeks.

TABLE 162.3 Rules for Bowel Retraining

- Find and eliminate known causes of constipation.
- Never repress an urge to defecate.
- Eat a high-fiber diet, focusing on fruits and vegetables.
- Drink six to eight glasses of fluid per day.
- Sit on the toilet at the same time every day (even when the urge to defecate is not present), preferable immediately after breakfast or exercise.
- Exercise for at least 20 minutes, three times per week.
- Discontinue laxatives (except to reestablish bowel activity as described herein) and enemas.
- Week 1
  - Every night before bed, take a stimulant laxative containing either cascara or senna. Take the lowest amount necessary to reliably ensure a bowel movement every morning.
- Weekly
  - Each week, decrease dosage by ½. If constipation recurs, go back to the previous week's dosage. Decrease dosage if diarrhea occurs.

THERAPEUTIC APPROACH

General Recommendations

Constipation is a symptom, not a disease. The most common causes are inadequate consumption of dietary fiber, inadequate fluid intake, and/or a sedentary lifestyle. Determining the cause is the first step in treatment. In most cases, constipation is not serious and responds quickly to dietary and supplement strategies.

Diet

Follow the guidelines given in Chapter 5, Philosophy of Naturopathic Medicine.

- Obtain 25 to 35 g of fiber daily from dietary sources.
- Drink at least six to eight glasses of water per day.
- Four to eight ounces of prune juice or 5 to 10 prunes is effective.
- Incorporate a variety of prebiotic foods.

Nutritional Supplements

- Psyllium: 5 g one to two times per day. Mix each 5 g dose in at least 8 ounces of water immediately before consumption. After taking a dose of soluble fiber, drink a second glass of water.
- Probiotics
- Vitamin C: 1000 mg/hour to bowel tolerance
- Magnesium: 300 mg to 2000 mg depending on individual sensitivity and response. Dosage can be increased and repeated until the desired outcome is achieved.

Botanical Medicines

- Consider demulcent and/or carminative herbs.
- Senna: Follow manufacturer's instructions on package label. The usual dosage recommendation is based on sennoside content: 15 to 30 mg sennosides at bedtime.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Higgins PD, Johanson JF. Epidemiology of constipation in North America: a systematic review. *Am J Gastroenterol*. 2004;99:750–759.
2. Kim ER, Rhee PL. How to interpret a functional or motility test—colon transit study. *J Neurogastroenterol Motil*. 2012;18:94–99. PubMed PMID: 22323993.
3. Tobias N, Mason D, Lutkenhoff M, Stoops M, Ferguson D. Management principles of organic causes of childhood constipation. *J Pediatr Health Care*. 2008;22(1):12–23.
4. Chiarelli P, Brown W, McElduff P. Constipation in Australian women: prevalence and associated factors. *Int Urogynecol J Pelvic Floor Dysfunct*. 2000;11:71–78.
5. Vazquez Roque M, Bouras EP. Epidemiology and management of chronic constipation in elderly patients. *Clin Interv Aging*. 2015;10:919–930. PubMed PMID: 26082622.
6. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterol*. 2006;130(5):1480–1491. PubMed PMID: 16678561.
7. Dinning PG, Smith TK, Scott SM. Pathophysiology of colonic causes of chronic constipation. *Neurogastroenterol Motil*. 2009;21(suppl 2):20–30. PubMed PMID: 19824935.
8. Rao SS. Dyssynergic defecation and biofeedback therapy. *Gastroenterol Clin North Am*. 2008;37(3):569–586. PubMed PMID: 18793997.
9. Weaver L, Steiner H. The bowel habit of young children. *Arch Dis Child*. 1984;59(7):649–652.
10. Iacono G, Cavataio F, Montalto G, et al. Intolerance of cow's milk and chronic constipation in children. *N Engl J Med*. 1998;339(16):1100–1104.
11. Khalif IL, Quigley EM, Konovitch EA, et al. Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. *Dig Liver Dis*. 2005;37(11):838–849.
12. Parthasarathy G, Chen J, Chen X, et al. Relationship between microbiota of the colonic mucosa vs feces and symptoms, colonic transit, and methane production in female patients with chronic constipation. *Gastroenterol*. 2016;150:367–379.
13. Pitkala K, Strandberg TE, Finne-Soveri UH, et al. Fermented cereal with specific bifidobacteria normalizes bowel movements in elderly nursing home residents. A randomized, controlled trial. *J Nutr Health Aging*. 2007;11(4):305–311.
14. Matsumoto M, Ohishi H, Benno Y. Impact of LKM512 yoghurt on improvement of intestinal environment of the elderly. *FEMS Immunol Med Microbiol*. 2001;31(3):181–186.
15. Meance S, Cayuela C, Turchet P, et al. A fermented milk with a Bifidobacterium probiotic strain DN-173 010 shortened oro-fecal gut transit time in elderly. *Microb Ecol Health Dis*. 2001;13:217–222.
16. Koebnick C, Wagner I, Leitzman P, et al. Probiotic beverage containing Lactobacillus casei Shirota improves gastrointestinal symptoms in patients with chronic constipation. *Can J Gastroenterol*. 2003;17:655–659.
17. Ouwehand AC, Lagstrom H, Soumalainen T, et al. Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin in the elderly. *Ann Nutri Metabol*. 2002;46:159–162.
18. Higashikawa F, Noda M, Awaya T, Nomura K, Oku H, Sugiyama M. Improvement of constipation and liver function by plant-derived lactic acid bacteria—a double-blind, randomized trial. *Nutrition*. 2010;26(4):367–375.
19. Ojetti V, Petruzziello C, Migneco A, Gnarra M, Gasbarrini A, Franceschi F. Effect of Lactobacillus reuteri (DSM 17938) on methane production in patients affected by functional constipation: a retrospective study. *Eur Rev Med Pharmacol Sci*. 2017;21(7):1702–1708.
20. Mehta V, Midha V, Mahajan R, et al. Lead intoxication due to ayurvedic medications as a cause of abdominal pain in adults. *Clin Toxicol (Philadelphia)*. 2017;55(2):97–101. PubMed PMID: 27957879.
21. Schmulson MJ, Drossman DA. What is new in Rome IV? *J Neurogastroenterol Motil*. 2017;23(2):151–163. PubMed PMID: 28274109.
22. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr Res Rev*. 2010;23:65–134.
23. Huang YG, Ji JD, Hou QN. A study on carcinogenesis of endogenous nitrite and nitrosamine, and prevention of cancer. *Mutat Res*. 1996;358:7–14.
24. Bijkerk CJ, Muris JWM, Knottnerus JA, Hoes AW, de Wit NJ. Systematic review: the role of different types of fibre in the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther*. 2004;19:245–251. PubMed PMID: 14984370.
25. Attaluri A, Donahoe R, Valestin J, Brown K, Rao SS. Randomized clinical trial: dried plums (prunes) vs. psyllium for constipation. *Aliment Pharmacol Ther*. 2011;33(7):822–828.
26. Dukas L, Willett WC, Giovannucci EL. Association between physical activity, fiber intake, and other lifestyle variables and constipation in a study of women. *Am J Gastroenterol*. 2003;98(8):1790–1796. PubMed PMID: 12907334.
27. Kim HS, Park DH, Kim JW, et al. Effectiveness of walking exercise as a bowel preparation for colonoscopy: a randomized controlled trial. *Am J Gastroenterol*. 2005;100(9):1964–1969. PubMed PMID: 16128940.
28. Driessen LM, Kiefte-de Jong JC, Wijtzes A, et al. Preschool physical activity and functional constipation: the Generation R study. *J Pediatr Gastroenterol Nutr*. 2013;57(6):768–774. PubMed PMID: 23857342.
29. Inan M, Aydiner CY, Tokuc B, et al. Factors associated with childhood constipation. *J Paediatr Child Health*. 2007;43(10):700–706. PubMed PMID: 17640287.
30. Constipation Guideline Committee of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Evaluation and treatment of constipation in infants and children: recommendations of the North American Society for pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2006;43(3):e1–e13. PubMed PMID: 16954945.
31. Vu MK, Nouwens MA, Biemond I, Lamers CB, Masclee AA. The osmotic laxative magnesium sulphate activates the ileal brake. *Aliment Pharmacol Ther*. 2000;14(5):587–595. PubMed PMID: 10792122.
32. Bothe G, Coh A, Auinger A. Efficacy and safety of a natural mineral water rich in magnesium and sulphate for bowel function: a double-blind, randomized, placebo-controlled study. *Eur J Nutr*. 2017;56(2):491–499. PubMed PMID: 26582579.
33. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr*. 1995;125(6):1401–1412. PubMed PMID: 7782892.
34. Bass P, Dennis S. The laxative effects of lactulose in normal and constipated subjects. *J Clin Gastroenterol*. 1981;3(suppl 1):23–28.
35. Tahiri M, Tressol JC, Arnaud J, Bornet FR, Bouteloup-Demange C, Feillet-Coudray C, et al. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am J Clin Nutr*. 2003;77(2):449–457. PubMed PMID: 12540407.
36. Tahiri M, Tressol JC, Arnaud J, Bornet FR, Bouteloup-Demange C, Feillet-Coudray C, et al. Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J Bone Miner Res*. 2001;16(11):2152–2160. PubMed PMID: 11697813.
37. Teuri U, Korpela R. Galacto-oligosaccharides relieve constipation in elderly people. *Ann Nutr Metab*. 1998;42:319–327.
38. Niittyen L, Kajander K, Korpela R. Galacto-oligosaccharides and bowel function. *Scand J Food Nutr*. 2007;51:62–66.
39. Dimidi E, Christodoulides S, Fragkos KC, Scott SM, Whelan K. The effect of probiotics on functional constipation in adults: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2014;100(4):1075–1084.
40. Miller LE, Ouwehand AC. Probiotic supplementation decreases intestinal transit time: meta-analysis of randomized controlled trials. *World J Gastroenterol*. 2013;19(29):718–4725.
41. Wang B, Mao YK, Diorio C, et al. Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *FASEB J*. 2010;24(10):4078–4088. PubMed PMID: 20519636.
42. Kunze WA, Mao YK, Wang B, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J Cell Mol Med*. 2009;13(8B):2261–2270. PubMed PMID: 19210574.
43. Radha MH, Laxmipriya NP. Evaluation of biological properties and clinical effectiveness of Aloe vera: a systematic review. *J Tradit Complement Med*. 2014;5(1):21–26. PubMed PMID: 26151005.
44. Suares NC, Ford AC. Systematic review: the effects of fiber in the management of chronic idiopathic constipation. *Aliment Pharmacol Ther*. 2011;33(8):895–901.

163

Cystitis and Interstitial Cystitis/ Painful Bladder Syndrome

Geovanni Espinosa, ND, LAc, IFMCP, CNS, and *Ralph Esposito, ND, LAc*

OUTLINE

DIAGNOSTIC SUMMARY

- Burning pain on urination
- Increased urinary frequency, urgency, nocturia
- Turbid, foul-smelling, or dark urine
- Lower abdominal pain
- Urinalysis showing significant pyuria and bacteriuria with bacterial cystitis
- Pain in the pelvis (suprapubic) or between the vagina and anus in women or between the scrotum and anus in men (perineal)
- Chronic pelvic pain
- A persistent urgent need to urinate
- Pain during sexual intercourse

GENERAL CONSIDERATIONS

Bladder infections in women are surprisingly common: 10% to 20% of all women have urinary tract discomfort at least once a year, 37.5% of women with no history of urinary tract infection (UTI) will have such an infection within 10 years, and 2% to 4% of apparently healthy women have elevated levels of bacteria in their urine, indicative of an unrecognized UTI.[1] Women with a history of recurrent UTI will typically have an episode at least once every year.[2] Recurrent bladder infections can be a significant problem because 55% will eventually involve the upper urinary tract (i.e., the kidneys). Recurrent kidney infections can cause abscess formation, disseminated intravascular coagulation, acute respiratory distress syndrome, sepsis, and chronic progressive renal damage, resulting in scarring and, for some, kidney failure.

Except in infants, UTIs are much less common in males than females and in general indicate an anatomical abnormality, a prostate infection, or rectal intercourse. Table 163.1 lists incidence by age and gender.

Interstitial cystitis (IC)/painful bladder syndrome (PBS) is a chronic bladder disorder characterized by chronic pelvic pain (CPP) and irritative voiding symptoms. IC/PBS is not due to infection; it is characterized by symptoms similar to cystitis, and patients may also report otherwise undiagnosed CPP. The symptoms of IC/PBS can overlap with such conditions as endometriosis, recurrent UTI, CPP, overactive bladder, and vulvodynia. Studies have indicated that IC affects 52 to 67 per 100,000 people in the United States.[3] Some investigators believe these numbers are vastly underestimated owing to lack of proper diagnosis.

Cystitis and Irritants

Endogenous and exogenous irritants, including bacterial lipopolysaccharide (LPS), acid, turpentine, mustard oil, croton oil, and acrolein, have been used to induce cystitis in experimental studies. Cyclophosphamide is an antineoplastic agent, and hemorrhagic cystitis is a common complication observed in patients treated with this drug. Cyclophosphamide is metabolized by the liver to acrolein, and the presence of acrolein is thought to be the cause of cystitis in patients who receive cyclophosphamide.[4] Chemical irritants directly damage the urothelium and other cells of the bladder, resulting in various degrees of erosion of the mucosa, edema, hemorrhage, and leukocyte infiltration of the bladder wall.[5] Although the cause of chronic bladder pain is yet unknown, it is plausible that irritants may contribute to the etiology.

TABLE 163.1 Incidence of Urinary Tract Infection According to Age and Gender

Age Group	Incidence	Male:Female Ratio
Neonatal	1	1.5:1
Preschool age	1.5–3	1:10
School age	1.2	1:30
Reproductive age	3–5	1:50
Geriatric	10–30	1:1.5

Modified from Rubin RH. Infections of the urinary tract. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American; 1997.

TABLE 163.2 Results of Women Presenting With Typical Urinary Tract Infection Symptoms and No Fever, Vaginal Symptoms, or Toxicity

Diagnosis	Percentage of Subjects
Upper urinary tract infection	20
Bladder infection	40
Low levels of bacteria in urethra	16
Urethral *Chlamydia*	8
Unsuspected vaginitis	4
Other (e.g., herpes, gonorrhea, pelvic inflammatory disease)	12

Modified from Reilly BM. *Practical Strategies in Outpatient Medicine*. Philadelphia: Saunders; 1984: 277.

TABLE 163.3 Bacteriological Findings (Percentage Positive) in Outpatients and Inpatients With Urinary Tract Infections

Bacterial Species	Outpatients	Inpatients
Escherichia coli	89.2	52.7
Proteus mirabilis	3.2	12.7
Klebsiella pneumoniae	2.4	9.3
Enterococcus	2	7.3
Enterobacter aerogenes	0.8	4
Pseudomonas aeruginosa	0.4	6
Proteus spp.	0.4	3.3
Serratia marcescens	0	3.3
Staphylococcus epidermidis	1.6[a]	0.7
Staphylococcus aureus	0	0.7

[a]Recent evidence suggests that this may be more prevalent than is currently thought—in sexually active college-age women, it may account for as much as 20% of asymptomatic urinary tract infections.
Modified from Rubin RH. Infections of the urinary tract. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American; 1997.

DIAGNOSIS

The diagnosis of bladder infection is imprecise because clinical symptoms and the presence of significant amounts of bacteria in the urine do not correlate well. As indicated in Table 163.2, only 60% of women with the typical symptoms of UTI actually have significant levels of bacteria in their urine. Equally important, however, is the fact that 20% have the more potentially serious involvement of the upper tract.

In general, the diagnosis is made according to signs and symptoms and urinary findings. A pelvic examination is indicated if there is a history consistent with vaginitis or cervicitis or if there is confusion regarding diagnosis. Microscopical examination of the infected urine shows high levels of white blood cells (WBCs) and bacteria. Culturing the urine determines the quantity and type of bacteria involved. As shown in Table 163.3, *Escherichia coli* (from the colon) is by far the most common. The presence of fever, chills, and low back pain can indicate involvement of the kidneys. Those with recurrent infections should be examined by intravenous urogram to determine whether a structural abnormality is present.[6]

IC/PBS can also be difficult to diagnose because the symptoms overlap with a variety of other disorders, including endometriosis, UTI, CPP, overactive bladder (OAB), and vulvodynia.[7,8] Because there is no definitive diagnostic test, IC/PBS remains a diagnosis of exclusion. The presence of additional symptoms caused by other pain generators can confuse the diagnosis even further. Patients may not receive an accurate diagnosis for years. The median time between the development of IC/PBS symptoms and the diagnosis is approximately 5 years.[9]

CPP is pain lasting 6 months or longer that is severe enough to affect daily functioning or requires medical care.[7] The specific etiology of CPP is often unknown and may be multifactorial.[10] Gynecological conditions that can cause CPP include endometriosis, adhesions, pelvic inflammatory disease (PID), cysts, and polyps.[10] However, a retrospective cohort analysis of a large primary care database in the United Kingdom found that only 20.2% of all cases of CPP had a gynecological etiology,[11] and up to 55% of women had no known pathological cause for their pelvic pain.[12] Gastrointestinal diagnoses represented 37.7% of cases, with irritable bowel syndrome (IBS) accounting for 29.1%. Cystitis accounted for 30.8% of diagnoses in this population of women with noncyclic pain lasting for 6 months or longer. Up to one half of the women in primary care practices who have CPP may have more than one diagnosis for their pain. As described previously, it is common for a patient with CPP to have both endometriosis and IC/PBS.[10]

Physical examination is a critical component of diagnosing IC/PBS. In patients with IC/PBS, this can be emotionally distressing because of pain, so it is important that the physician proceed slowly and carefully.[13] Because the bladder is a pain generator in IC/PBS, tenderness with single-digit examination of the trigonal area (bladder base or urethra) can help establish a diagnosis of IC/PBS, as can pain in the pelvic floor or levator ani.[7] Patients with IC/PBS tend to have urethral or bladder tenderness, whereas those with vestibulodynia have vestibular tenderness.[14] Tenderness on single-digit examination of the vaginal fornices can help distinguish endometriosis from IC/PBS.[13]

Urinalysis can rule out hematuria, and urine culture is required to identify bladder infection.[13] Cytology and computed tomography (CT) with double contrast when indicated (hematuria, history of smoking, 40 years of age and older) can help rule out urinary tract malignancies.[15] Patients with results suggesting a malignancy should be referred to a urologist. Several optional diagnostic tests can help diagnose IC/PBS. The presence of glomerulations seen on cystoscopy with hydrodistension may aid in the diagnosis of IC/PBS. A negative cystoscopic evaluation should never be used to rule out IC/PBS, because many patients with early IC/PBS will not have glomerulations.[16] The potassium sensitivity test (PST) may indicate a defective bladder lining. The PST involves intravesical instillation of a potassium solution, which triggers symptoms of pain and urgency in patients with abnormal permeability of the bladder surface.[17] Intravesical instillation of an anesthetic cocktail can be used as a diagnostic tool as well as a treatment. Known as the "anesthetic bladder challenge," this test can help localize pain to the bladder.[18,19]

Collection of Urine Specimen for Culture

The optimal clinical method for obtaining a urine sample is the voided midstream specimen. It involves cleaning of the urethral meatus or vaginal vestibule before the sample is collected. If vaginal epithelial cells are present, a new specimen should be collected. To avoid repeating the collection, a satisfactory technique for the female, called the "clean catch," consists of spreading the labia and cleaning the area with two gauze sponges moistened with an antimicrobial solution and a dry gauze sponge. The washing is accomplished by making a single front-to-back motion with each of the two moist sponges and then the dry sponge. While the labia are still held apart, a small amount of urine is allowed to pass into the toilet (or bedpan); then a midstream specimen is collected in a sterile container and immediately closed.

Occasionally, the urine must be collected via catheterization. This is a more invasive procedure and carries with it a 1% to 2% chance of initiating a UTI via the introduction of microorganisms into the bladder. Suprapubic aspiration is the most accurate method of urine collection, but obviously, it is also the most invasive one.

Examination of the Collected Urine

Several methods are routinely employed in the detection of bacteria in the urine. They range from the use of dipsticks to microscopical examination and culture. For most accurate determinations, the urine should be examined within 1 hour. If examination must be delayed, refrigeration at 58°C preserves the urine for most routine examinations. However, culturing requires that the urine not be refrigerated for more than 8 hours.

Dipstick Methods

The modern examination of urine specimens typically involves the use of reagent strips, which are dipped into the urine and removed. Parts of each dipstick are impregnated with chemicals that react with specific substances in the urine to produce various colors. Color standards, with which the color can be compared, are provided. Careful attention must be paid to match the dipstick to the color standard at the appropriate time. Instructions accompany commercially prepared dipsticks.

Dipsticks are invaluable for qualitative and rough quantitative analysis. Typically, they provide information on pH, protein, glucose, ketones, bilirubin, hemoglobin, leukocyte esterase, nitrite, and urobilinogen. Some dipsticks also allow for the detection of WBCs and bacteria (including semiquantitative cultures).

Urinary infections typically increase the number of WBCs present. The leukocyte esterase test is used to detect WBCs in the urine. Because many common organisms contain enzymes that reduce nitrate in the urine to nitrite (Box 163.1), the measurement of urinary nitrite provides an inexpensive and rapid way to detect significant bacteriuria, but the findings should be confirmed by culture. Although both tests have a high specificity (approximately 95%), the leukocyte esterase test is approximately 80% to 90% sensitive, whereas the nitrite test is only approximately 50% sensitive compared with a quantitative culture as the gold standard. However, the combination of both tests improves the sensitivity up to 96%.[20,21]

Microscopic Examination

Microscopic examination should be performed within the first hour after collection. A drop of fresh urine or a drop of resuspended sediment from centrifuged fresh urine is placed on a microscope slide, covered with a coverslip, and examined with the high-dry objective under reduced illumination. The presence of more than 10 bacteria per field in an unstained specimen suggests a bacterial count of more than 100,000/mL of urine. Smears may also be made using a Gram stain and examined under the oil immersion objective. The presence of WBCs further indicates an infectious process. The presence of large quantities of protein, WBC casts, or both in the micrographic examination may be indicative of renal involvement, most commonly pyelonephritis.

BOX 163.1 Nitrates and Biological Organisms

Organisms That Reduce Nitrates to Nitrites

Citrobacter spp.
Escherichia coli
Klebsiella pneumoniae
Proteus spp.
Pseudomonas spp.
Serratia marcescens
Shigella
Staphylococcus spp. (most)

Organisms That Do Not Reduce Nitrates to Nitrites

Neisseria gonorrhoeae
Streptococcus faecalis
Mycobacterium tuberculosis

Urine Culture

Typically, only quantitative cultures are used. After the introduction of diluted urine to the suitable medium and incubation, the colonies are counted and multiplied by the dilution factor to yield the bacterial count per milliliter. Bacteriuria is considered significant if it is more than 100,000/mL, but even 1000/mL is considered clinically significant in the presence of symptoms characteristic of UTI. Most physicians are now employing semiquantitative tests[22] using dipsticks or glass slides coated with culture media. Colonies are counted, and the appearance is compared within 12 to 24 hours of incubation. For recurrent or chronic infection, sensitivity studies are often performed.

In more than 95% of UTIs, a single bacterial species is the problem. When mixed bacterial species are grown, the probability of contamination is high. Recurrence of UTI after the initial bacterial infection is resolved is common.[23]

Staphylococcus epidermidis, diphtheroids, and lactobacilli are commonly found in the distal urethra but rarely cause UTI. Symptoms of recurrent UTI include urgency, frequency, nocturia, and pelvic pain. Diagnosis is established by a positive urine culture.[24]

COEXISTING CONDITIONS AND DIFFERENTIAL DIAGNOSIS

Although bacterial cystitis usually appears acutely and by itself, IC/PBS typically manifests with comorbidities that include endometriosis, IBS, vulvodynia, and OAB.

A detailed medical history and physical examination are the basis for diagnosing the cause of CPP. The use of questionnaires, such as the Pelvic Pain, Urgency Frequency Questionnaire (PUF), or O'Leary Sant Questionnaire (OLS) indices, may elicit information about urinary symptoms. Tenderness of the bladder base on pelvic examination is a characteristic feature of IC/PBS that may help distinguish it from other causes of CPP.[13] Optional tests, including laparoscopy and diagnostic imaging, may also be helpful.[9] Additional useful information may be gained from the PST or intravesical anesthetic challenge.[17]

Endometriosis is characterized by the presence of endometrial-like glands and stroma outside the uterine cavity. Symptoms include pain in the lower abdomen, dysmenorrhea, and dyspareunia

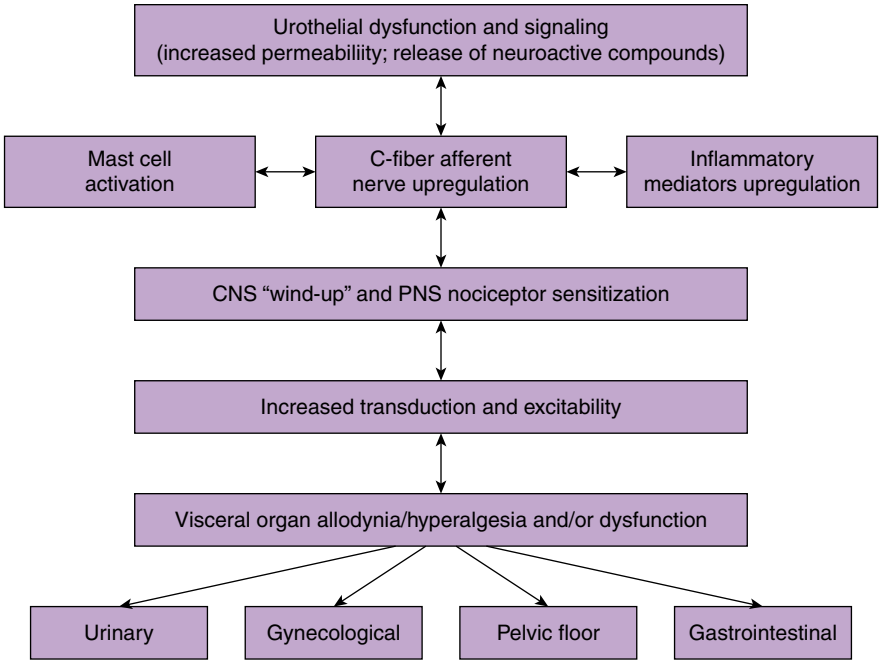


Fig. 163.1 Potential etiological cascade and pathogenesis underlying painful bladder syndrome (PBS)/interstitial cystitis (IC). It is likely that PBS/IC has a multifactorial etiology that may act predominantly through one or more pathways resulting in the typical symptom complex. There is a lack of consensus regarding the etiology or pathogenesis of PBS/IC, but a number of proposals include a "leaky epithelium," release of neuroactive compounds at the level of the urinary bladder with mast cell activation, "awakening" of C-fiber bladder afferents, and upregulation of inflammatory mediators including cytokines and chemotactic cytokines (chemokines). Inflammatory mediators can affect central nervous system (CNS) and peripheral nervous system (PNS) neural circuitry, including central "wind-up" and nociceptor sensitization, resulting in chronic bladder pain and voiding dysfunction. BPS/IC is associated with diseases affecting other viscera and pelvic floors. (From Gonzalez EJ, Arms L, Vizzard MA. The role(s) of cytokines/chemokines in urinary bladder inflammation and dysfunction. BioMed Res Int. 2014;120525. PubMed PMID: 24738044.)

and can also include such voiding symptoms as dysuria and frequency.[25] Endometriosis and IC/PBS frequently coexist in the same patient. In one study of women with CPP and bladder tenderness on examination, more than 70% had both endometriosis and IC/PBS.[26]

OAB is characterized by urgency with or without urge incontinence and usually includes frequency and nocturia. The key symptom is urgency.[27] OAB and IC/PBS can coexist in the same patient. Urgency can be caused by detrusor overactivity, which usually can be demonstrated through urodynamic testing. Urgency is a common symptom of both OAB and IC/PBS, although the cause differs. In patients with OAB, urgency results in a strong desire to avoid leakage, whereas in patients with IC/PBS, urgency results in a strong need to void to relieve pain caused by bladder fullness.[28]

Vulvodynia is characterized by vulvar discomfort, often reported as burning pain. Pain can occur during intercourse, during vigorous activity, after intercourse, or even at rest. The etiology of vulvodynia is unknown, and the diagnosis is one of exclusion. Symptoms of vulvodynia that overlap those of IC/PBS include pain and dyspareunia but not frequency or nocturia; this can help distinguish vulvodynia from IC/PBS. It is also possible for a patient to have both IC/PBS and vulvodynia. The history and physical examination are important in diagnosing vulvodynia. A history of chronic pain at the vestibule lasting more than 3 months can suggest vulvodynia. Infectious causes may include viral, bacterial, or fungal organisms; therefore these must be ruled out as causes of vulvar pain. Vulvar dermatoses may also account for vulvar symptoms.[29] Tenderness to pressure with a cotton swab at the vestibule is a hallmark of vulvodynia,[29] whereas tenderness at the bladder base is typical of IC/PBS.

THERAPEUTIC CONSIDERATIONS

The primary goal in the natural approach to treating infectious cystitis is enhancing normal host protective measures against UTI. Specifically, this means enhancing the flow of urine by achieving and maintaining proper hydration, promoting a pH that inhibits the growth of the organisms, preventing bacterial adherence to the endothelial cells of the bladder, and enhancing the immune system. In addition, several botanical medicines with antimicrobial activity can be employed.

Increasing Urine Flow

Increased urine flow can easily be achieved by increasing the quantity of liquids consumed. Ideally, these liquids should be in the forms of pure water and herbal teas. Fresh fruit and vegetable juices should be diluted with at least twice the amount of water. The patient should be encouraged to drink at least 2 L of liquid, with at least half being simply pure water. The patient should also be advised to avoid such liquids as soft drinks, concentrated fruit drinks, coffee, and alcoholic beverages.

Chronic Interstitial Cystitis

In addition to the general measures given later for cystitis, the focus for interstitial cystitis is on enhancing the integrity of the interstitium along with the lining of the bladder wall. The "leaky bladder urothelium" theory postulates that there is a problem with the glycosaminoglycan layer of the bladder epithelium, which results in making the bladder wall more permeable to potassium, causing inflammation and pain (Fig. 163.1).

The elimination of food allergies to reduce inflammation appears to be a valid goal, even though the association between food allergies and cystitis is not well established. Food allergies have been shown to produce cystitis in some patients. Repeated ingestion of a food allergen could easily explain the chronic nature of interstitial cystitis. For a complete discussion of food allergies, see Chapter 14.

Education of patients with IC/PBS helps empower them to control their symptoms and to be active participants in their own therapy. Patients should be counseled to avoid triggers that lead to increased IC/PBS symptoms. They should also be encouraged to reduce stress and use support groups to deal with the effect IC/PBS has on their daily lives. Dietary changes can provide relief of symptoms.[30]

Herati et al.[31] established the prevalence and characteristics of food sensitivities in patients with IC/PBS and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Validated questionnaires containing a list of 175 comestibles as well as pain questions were mailed to 325 patients with IC/PBS and 286 with CP/CPPS. The researchers found that although patients with IC/PBS were more likely to be food sensitive than patients with CP/CPPS, these questionnaires showed that the symptoms of patients with both IC/PBS and CP/CPPS were aggravated by similar comestibles, such as grapefruit juice, spicy foods, alcohol, and caffeinated coffee. The findings also revealed that the symptoms of both groups of patients were improved by certain comestibles, namely, water and an antacid containing calcium glycerophosphate (Prelief).[31] In 2007, a study done at Long Island University reported that over 90% of patients with interstitial cystitis experience an increase in symptoms when they consume certain foods and beverages, especially coffee, tea, soda, alcoholic beverages, citrus fruits and juices, artificial sweeteners, and hot pepper.[30,32]

Food restrictions have not been conclusively proved to help slow the progression of the disease or to improve its course. Usually food restriction should be weighed against the decrease in the patient's quality of life. After 2 weeks, patients are usually asked to start adding one food item back into their diets every 3 days to identify any specific alimentary sensitivity.

Medical treatments include tricyclic antidepressants (amitriptyline being the most popular),[33] anticholinergics to reduce symptoms of urinary urgency, and antihistamines for their efficacy in decreasing mast cell activation and reducing inflammation.[34,35]

Mast cells, once thought to be responsible for allergic reactions, have in some studies been shown to be critically important in interstitial cystitis. Current evidence from clinical and laboratory studies confirms that they play a central role in IC/PBS.[36] Histamine causes pain, swelling, and scarring and prevents the lining of the bladder from healing.[37,38]

Pentosan polysulfate sodium (Elmiron) is a U.S. Food and Drug Administration (FDA)-approved oral treatment heparinoid compound that is thought to replenish the defective bladder lining. According to one placebo-controlled, randomized clinical trial, when it is taken orally, only 1% to 3% of the active drug reaches the bladder. Based on levels of improvement and side effects, treatment can be continued for 3 months and then extended as needed,[39] which may be necessary for proper epithelial healing.

Cimetidine, a histamine-2 antagonist traditionally used for acid reflux, has been used for IC/PBS with potential benefits. It has been shown to improve pain, nocturia, and overall symptom scores by about 50% in patients with IC/PBS, yet it showed no improvements in dysuria, frequency, relief after voiding, or incomplete emptying.[40] Given the complex nature of IC/PBS, this is not surprising—it is suspected that it is not simply a histamine-driven condition, which is further confirmed with histological studies showing that cimetidine did not change mast-cell or B-cell concentrations but still improved patient symptoms.[41]

Second-line therapies include immunosuppressive agents like prednisone, which was used to treat severe IC/PBS in one small clinical trial.[42] Thirty patients with the Hunner's ulcer subtype of IC/PBS showed considerable improvement after endoscopic submucosal injection of triamcinolone.[43] Cyclosporine has been shown in clinical trials to relieve the symptoms of severe IC/PBS.[44] Symptoms generally recur after treatment is discontinued.

Intravesical therapy can be used for flare management. Dimethylsulfoxide (DMSO) is the only FDA-approved intravesical IC/PBS treatment.[45] Intravesical instillation of anesthetic can bring immediate relief. A study of patients with IC/PBS receiving intravesical instillation of heparin and alkalinized lidocaine showed that this treatment provided immediate and sustained relief of pain and urgency.[19]

Various intravesical cocktails use combinations of heparin, lidocaine, sodium bicarbonate, gentamicin, and glucocorticoids for the treatment of patients with IC/PBS.[46]

Muscle relaxants, used to treat increased muscle spasticity of the pelvic floor associated with CPP, appear to have a beneficial effect. Cyclobenzaprine is an agent closely related to the tricyclic antidepressants. It is used at starting doses of 10 mg daily, which can be prescribed up to three times daily. Tizanidine is a centrally acting alpha$_2$ agonist shown to be superior to placebo in treating spasticity in several conditions. In addition, clonazepam has been useful in treating neuropathic pain.[47]

The American Urological Association recognizes the multiple facets of IC/PBS, suggesting the first line of therapy must be personalized, focusing on patient education, self-care practices, behavioral modification, and stress management.[48]

Alternative Treatments

Biofeedback is another aspect of physical therapy that can provide beneficial results for pelvic pain. Through biofeedback, the patient can learn to control the musculature of the pelvic floor by visualizing the activity of the muscle on a computer and using the visual feedback to achieve conscious control over muscular contractions, thus breaking the cycle of spasm. Ger et al. studied 60 patients with intractable rectal pain, 70% of whom were diagnosed with levator spasm. Biofeedback was performed on 14 of the 60 patients for 30- to 60-minute sessions a week for 6 weeks. In 6 of the 14 patients (43%), pain relief was rated as good or excellent at the 15-month follow-up.[49] A prospective trial using biofeedback and a rectal manometric balloon included 16 patients with levator ani syndrome. The median pain score before biofeedback therapy was 8 (on a 0–10 linear analog scale) and significantly improved to a median score of 2 after therapy. All patients reported nontender musculature after treatment.[50]

Herbal Medicine

Katherine Whitmore, a urologist from Drexel University, found that the Chinese herbs *Cornus,* garden rhubarb, *Psoralea,* and *Rehmannia* decreased pain after 4 weeks in 61% of the subjects studied ($n = 25$). In 3 months, an additional 22% of the subjects had a significant response.[51] The most consistent research evidence was found for *Centella asiatica* (gotu kola) and horsetail *(Equisetum arvense).*[51] *Centella* appears to address some other pathological features of chronic interstitial cystitis. Specifically, *Centella* extracts have been shown to improve the integrity of the connective tissue composing the interstitium as well as to heal ulcerations of the bladder.[52] Horsetail *(E. arvense)* has astringent properties.[53]

Aloe vera, also known as the "burn plant," has been used extensively to heal and relieve the pain of burns. In a small open-label, double-blind crossover study using a freeze-dried whole-leaf *A, vera* concentrate, three 600-mg capsules were given twice daily for 3 months, followed

or preceded by placebo. Of 12 patients with interstitial cystitis, 8 completed the study, and 7 had significant symptom relief.[54]

L-Arginine

L-arginine is an essential amino acid that increases the production of nitric oxide (NO), which is dependent on the enzyme nitric oxide synthetase (NOS). NO and NOS have antibacterial, smooth muscle–relaxing, hormone-releasing, and immune-modulating (by increasing T-cell counts) properties. In a randomized, double-blind trial of oral L-arginine in the treatment of interstitial cystitis, NOS production was found to be decreased in patients with interstitial cystitis and increased in patients with UTI compared with controls. L-arginine (1.5 g/day) was given to 10 patients with interstitial cystitis for 6 months. The urinary NOS was then measured and found to have increased, and clinical symptoms of frequency and nocturia improved in 8 of the 10 patients.[55] The same authors performed a randomized, double-blind trial using 1.5 g of L-arginine per day for 3 months. At the end of this period, 6 (29%) of the 21 patients in the L-arginine group and 2 (8%) of the 25 patients in the placebo group were clinically improved. When an intent-to-treat analysis was done, no significant difference was found between the L-arginine and placebo groups, although Likert scales showed a significant difference in pain intensity and global scores, favoring those taking L-arginine.[56] Another randomized controlled trial with L-arginine found no improvement in symptoms scores of interstitial cystitis compared with controls.[57] The efficacy of L-arginine, NO, and NOS in the treatment of interstitial cystitis remains controversial.

Quercetin

Quercetin is a naturally occurring flavonoid that inhibits histamine release from mast cells and has anti-inflammatory and antioxidant properties. It is rich in onions, seeds, citrus fruits, olive oil, tea, and red wine. A quercetin-containing compound was studied in 22 patients with interstitial cystitis given 500 mg twice a day for 4 weeks. Two patients dropped out of the study. Of the remaining 20 patients, 57% had a significant decrease in the O'Leary Sant Symptom and Problem indices and a decreased global assessment of pain (Likert scale; range 0–10).[56] A randomized, placebo-controlled trial is needed to determine the efficacy of quercetin.

Cranberry Juice *(Vaccinium macrocarpon)*

Cranberries and cranberry juice have been used for the treatment and prevention of UTI for several hundred years. Several clinical studies of benefit in patients with UTI have shown mixed results. In one study, 0.5 L/day of cranberry juice produced beneficial effects in 73% of the subjects (44 females and 16 males) with active UTI.[58] Furthermore, withdrawal of cranberry juice in the people who benefited resulted in recurrent bladder infection in 61%.

In vitro experiments have demonstrated that cranberry reduces adherence of *Escherichia coli* to uroepithelial cells. *E. coli* accounts for up to 80% of UTI acquired in the community. The effect of cranberry on *E. coli* adherence is dose dependent; exposure to cranberry also can displace preattached *E. coli.*[59] For bacteria to infect, they must first adhere to the mucosa. By interfering with adherence, cranberry juice greatly reduces the likelihood of infection and helps the body fight it off. This is the most likely explanation of cranberry juice's positive effects in bladder infections.

A large double-blind, placebo-controlled study evaluated the efficacy of 300 mL of cranberry juice in 153 women (average age 78.5) with confirmed bacteriuria.[60] Even this small an amount dramatically decreased the level of bacteria in the women's urine and the frequency of recurrence of infection. Interestingly, the researchers used saccharin-sweetened juice. Because saccharin is a suspected carcinogen, we strongly recommend avoiding this artificial sweetener.

In one study of seven juices (cranberry, blueberry, grapefruit, guava, mango, orange, and pineapple), only cranberry and blueberry demonstrated inhibitory effects.[61] Blueberry juice *(Vaccinium augustifolium)* is a strong cousin of cranberry juice and a suitable alternative to cranberry juice in bladder infections.

It must be pointed out that most cranberry juices on the market contain one-third cranberry juice mixed with water and sugar. Because sugar has such a detrimental effect on the immune system,[62–64] the use of sweetened cranberry juice cannot be recommended. Fresh cranberry (unsweetened) or blueberry juice is preferred to avoid the deleterious effects of sugar consumption.

Cranberry extracts are also available commercially in pill form. To compare the efficacy and cost of tablets with fresh juice for the prevention of UTI, 150 sexually active women between 21 and 72 years of age were studied in a randomized controlled trial for 1 year.[65] Tablets were taken twice daily, as well as juice at 250 mL three times daily. Both cranberry juice and cranberry tablets significantly decreased the number of patients experiencing at least one symptomatic infection per year (20% and 18%, respectively) compared with placebo (32%). The mean annual cost of cranberry tablets was $624, whereas the juice cost $1400. Furthermore, total consumption of antibiotics was less in both treatment groups compared with placebo. Although the cranberry tablets may be more cost-effective, it is important to remember that sufficient fluid intake is still important, so ample water should be ingested if tablets are used.

Of interest, in a large trial of cranberry juice compared with placebo juice conducted among 376 British inpatients 60 years of age and older, the incidence of symptomatic UTI was also lower than anticipated in the placebo group, but there was no significant difference between groups.[66] Both the active and placebo juices contained ascorbic acid.

Another randomized controlled trial of 305 individuals with neuropathic bladder after spinal cord injury found that the ingestion of cranberry tablets did not reduce rates of UTI relative to the standard regimen.[67]

A large study looked at 319 college women presenting with acute UTI. In this double-blind, randomized trial, participants were followed up until a second UTI or for 6 months, whichever came first. The study concluded that an 8-oz dose of low-calorie cranberry juice twice daily gave no protection against the risk of recurrent UTI among college-aged women.[68] The research juice was formulated to be similar to the commercially available Ocean Spray and was sweetened with Splenda (sucralose), exactly as is the retail juice. Batches of the tested juice were standardized to 112 mg per day of proanthocyanidin content. The placebo juice was formulated by Ocean Spray to imitate the flavor (sugar and acidity) and color of the cranberry beverage but without any cranberry content. In addition to other food and pharmaceutical-grade substances, both juices contained ascorbic acid in their formulations.

The most recent meta-analysis identified cranberry as a useful preventive therapeutic in UTIs, with analysis of more than 1400 women in seven clinical trials. Cranberry was shown to reduce recurrence in women by 26%. The authors, however, still question cranberry as a treatment option, and the most effective form and dose are unclear.[69]

Last, there is a theoretical concern regarding the use of cranberry juice and the formation of kidney stones. Because cranberries are considered an oxalate-containing food, it is thought that this may encourage the formation of oxalate-rich kidney stones. However, urinary oxalate excretion does not increase after drinking cranberry juice, and none of the studies using cranberries has reported an increased incidence of kidney stones.[70]

Acidify or Alkalinize?

Although many practitioners believe that acidification of the urine is the best approach in addressing cystitis, several arguments can be made for alkalinizing the urine. First, it is often difficult to acidify the urine. Many popular methods of attempting to acidify the urine, such as ascorbic acid supplementation and the drinking of cranberry juice, have little effect on pH at commonly prescribed doses.

The best argument for alkalinizing the urine is that it appears to be more effective, especially in women without pathogenic bacteria in their urine. The best method for alkalinizing the urine appears to be the use of citrate salts (e.g., potassium citrate, sodium citrate). These salts are rapidly absorbed and metabolized without affecting gastric pH or producing a laxative effect. They are excreted partly as carbonate, thus raising the pH of the urine.

Potassium citrate, sodium citrate, or both have long been employed in the treatment of lower UTI. They are often used as a "holding exercise" until the results of a urine culture become available. Some clinical studies support this practice. For example, in one study, women presenting with symptoms of UTI were given 4 g of sodium citrate every 8 hours for 48 hours.[71] Of the 64 women evaluated, 80% had relief of symptoms, 12% had deterioration of symptoms, and 91.8% rated the treatment as acceptable. Of the 64 women, 19 were shown to have positive bacterial cultures. There was more variation in response to treatment in the group with proved bacterial infection, with those having symptoms of urethral pain (7 of 10) and dysuria (13 of 18) improving more than those with symptoms of frequency (9 of 17) and urgency (6 of 13). These results were similar to those of a previous study demonstrating significant symptomatic relief and abacteriuria in 80% of the 159 women studied.[72]

Because of these positive results, it has been suggested that urine culture could be restricted to those women who fail to respond to alkalinization therapy. Restricting urine cultures to nonresponders would also lower health care costs.

Acupuncture

Acupuncture is attracting the interest of patients with all kinds of urological conditions. The use of acupuncture also gives urologists an opportunity to collaborate with other health care professionals, such as acupuncturists.[73]

Patients with IC/PBS can potentially gain benefit from 10 to 20 sessions of acupuncture.[51] Alraek has been able to show in a study with 61 women that a traditional Chinese medicine diagnosis can be useful in cystitis.[74]

In a study of 14 patients, Rapkin and Kames found that 6 to 8 weeks of acupuncture reduced the pain of interstitial cystitis.[75]

One reported a case study involves a 31-year-old woman whose symptoms of interstitial cystitis were reduced with acupuncture to the kidney and bladder meridians.[76]

In a Norwegian study, 67 adult women with a history of recurrent lower UTI were randomized into three groups: one receiving acupuncture treatment, one receiving sham acupuncture, and one given no treatment. A statistically significant 85% of patients in the acupuncture group were free of cystitis during the 6-month observational period, compared with 58% in the sham group and only 36% in the control group.[77] Although more clinical studies are necessary to thoroughly elucidate the efficacy of acupuncture, this safe treatment method seems to be a reliable choice as a primary or adjunctive treatment option. Some practitioners also combine acupuncture with the Chinese herbal tradition, which can also be useful in the treatment of long-term chronic conditions.

Nutrient Supplementation

D-Mannose

D-mannose is a simple sugar that acts similarly to cranberry juice in that it helps prevent the pili of *E. coli* and other bacteria from adhering to the bladder wall. In fact, D-mannose is contained in cranberry juice but in lower doses than are available through specific supplementation. Although there are no clinical trials available to support the use of D-mannose powder, a handful of research articles explaining its function strongly support its use.

One in vitro study evaluated the effect of D-mannose on 73 *E. coli* strains of epithelial vaginal and buccal cells from women who suffered recurrent UTI. Of the strains from urinary, vaginal, or anal isolates, 66 (90%) demonstrated adherence to vaginal epithelial cells. When applied, D-mannose completely inhibited the adherence of 25 strains (42%) to epithelial cells and inhibited an additional 11 strains (18%) by at least 50%. Similar results were obtained with buccal cells. The inhibitory effect of D-mannose was similar regardless of the origin of the strains.[78] Another study of Sprague Dawley rats given intravesicular injections of D-mannose found significant improvement in bacteruria in a dose-dependent manner in the animals given D-mannose.[79]

More recent studies further support the use of D-mannose and a prophylactic and possible therapeutic for chronic UTIs. Domenici et al. showed that 1.5 g twice daily of D-mannose for 3 days and then 1.5 g daily for 7 subsequent days resulted in negative urine culture, improvements in symptoms, and improved Urinary Tract Infection Symptoms Assessment (UTISA) scores. Further, it proved to be effective as a prophylaxis for 6 months.[80] Further studies confirm the benefit of D-mannose in acute cystitis in combination with other therapeutics such as cranberry.[81]

As a combination therapy, D-mannose has shown benefit when used with berberine, arbutin, birch, and forskolin in uncomplicated recurrent UTIs.[82] This combination not only targeted uropathogens but also improved vaginal microflora overall, thus providing direct UTI effects but also lateral benefit.

Jonathan Wright recommends an adult dose of 0.5 to 1 tsp three times daily mixed in a little water. It is sweet-tasting and has been used successfully in appropriately reduced doses for pediatric UTI as well. Admittedly, more research is necessary to fully evaluate the usefulness and efficacy of this therapy.

Botanical Medicines

Many herbs have been used through the centuries in the treatment of UTIs (Table 163.4). The following sections discuss several whose use is supported by both folk medicine and scientific evidence.

Arctostaphylos uva ursi

Most research has focused on the urinary antiseptic component, arbutin, of *Arctostaphylos uva ursis* (bearberry or upland cranberry), which typically comprises 6.3% to 9.6% of the leaves.[83] Arbutin is hydrolyzed to hydroquinone and glucose in the body. Hydroquinone is most effective in an alkaline urine. However, crude plant extracts are more effective medicinally than isolated arbutin.[83] *A. uva ursi,* reported to be especially active against *E. coli,* also has diuretic properties.[84] However, recent research has challenged this theory. It is believed that the main antimicrobial constituent, arbutin, requires an alkaline environment to be activated to hydroquinone. In fact, uropathogens may deconjugate arbutin independent of urinary pH and still prove efficacious. This also explains the clinical benefits seen when combining cranberry and *A. uva ursi.*[85]

The prophylactic effect of a standardized *A. uva ursi* extract on recurrent cystitis was evaluated in a double-blind study in 57 women.[86] At the end of 1 year, 5 of 27 women in the placebo group

TABLE 163.4 Bacteriological Susceptibility to Nutrients and Botanical Medicines

Bacterial Species	Agent
Escherichia coli	*Allium sativum, Hydrastis canadensis, Arctostaphylos uva ursi*
Klebsiella pneumoniae	*A. sativum, H. canadensis*
Proteus mirabilis	*A. sativum, H. canadensis*
Pseudomonas aeruginosa	*H. canadensis*
Staphylococcus saprophyticus	*A. sativum, H. canadensis*

had a recurrence, whereas none of the 30 women receiving *A. uva ursi* extract had a recurrence. No side effects were reported in either group. These impressive results indicate that the regular use of *A. uva ursi,* like cranberry, may prevent bladder infections.

A. uva ursi has also been shown to be helpful in increasing the susceptibility of antibiotic-resistant bacteria to antibiotics like beta-lactams. A Japanese group studied the effect of corilagin, a polyphenolic compound from *A. uva ursi,* used against methicillin-resistant *Staphylococcus aureus.* Corilagin reduced the minimal inhibitory concentration of oxacillin and other beta-lactam antibiotics by 100- to 2000-fold. The effect of corilagin and oxacillin was synergistic and bactericidal. Some other antibiotics studied were not enhanced by corilagin. The authors also mentioned that catechins, compound P, and epicatechin gallate from green tea, as well as baicalin from *Scutellaria amoena,* may also produce similar effects.[87]

Although there has been worry about the use of *A. uva ursi,* due to the content of arbutin and hydroquinone, there has been no direct human data indicating arbutin or hydroquinone conjugates to be toxic. It appears that humans are able to eliminate the hydroquinone as conjugates at a rate that is negligible to humans. However, it would be prudent to be aware of toxic signs, including the following[88]:

- Ringing in the ears
- Nausea
- Vomiting
- Sense of suffocation
- Shortness of breath
- Convulsions
- Delirium
- Collapse

Allium sativum

Garlic has been shown to have antimicrobial activity against many disease-causing organisms, including those associated with urinary tract infections, such as the following[89,90]:

- *E. coli*
- *Proteus* spp.
- *Klebsiella pneumoniae*
- *Staphylococcus* spp.
- *Streptococcus* spp.

Hydrastis canadensis

Goldenseal is among the most effective of the herbal antimicrobial agents. Its long history of use by herbalists and naturopathic physicians for the treatment of infection is well documented in the scientific literature. Of particular importance here is its efficacy against *E. coli, Proteus* species, *Klebsiella* species, *Staphylococcus* species, *Enterobacter aerogenes* (large dose required), and *Pseudomonas* species.[91,92]

THERAPEUTIC APPROACH

Although most cases of cystitis are relatively benign, it is extremely important that the patient be properly diagnosed, treated, and monitored. Proper monitoring includes having the patient notify the physician of any change in his or her condition. If the original culture was positive, it is appropriate to follow up with another culture 7 to 14 days after treatment was started. Citrates can be used to ameliorate symptoms. Because of the possibility of a kidney infection, patients should notify their physician if there is fever, low back pain, nausea, or vomiting. Pyelonephritis requires immediate antibiotic therapy and sometimes hospitalization.

Although the occasional acute bladder infection is easily treated, treating women with chronic cystitis can be a significant challenge. Long-term success requires determining the underlying cause: structural abnormalities, excessive sugar consumption, food allergies, nutritional deficiencies, chronic vaginitis, local foci of infection (e.g., prostate, kidneys), and current or childhood sexual abuse. All potential causes must be evaluated and resolved.

Although the causative agents of cystitis have not changed over time, bacterial resistance to antibiotics is fast becoming an issue; even one-time cases of simple acute cystitis have become recalcitrant.[87,93] Resistance to common antibiotics—including trimethoprim-sulfamethoxazole, beta-lactam drugs, and fluoroquinolone—is emerging at an alarming rate. In this milieu, it makes sense to use natural therapeutics that involve fluid intake to protect the urinary tract, immune support, plus nutrient and botanical medicines as first-line therapy in nonpyelonephritic cases and as adjunctive therapy to increase the efficacy of antibiotic treatment if necessary.

General Measures

Patients should be advised to drink large amounts of fluids (at least 2 L/day), including at least 0.5 L of unsweetened cranberry or 0.25 L of blueberry juice.

Patients should be taught to urinate after intercourse. Women who develop bladder infections after intercourse should wash the labia and urethra with a strong tea of *H. canadensis* (2 tsp/cup) both before and after. If this is inadequate, a dilute solution of povidone-iodine usually proves effective.

Diet

UTIs are mostly ascending infections caused by bacteria derived from stools. Because alterations in the bacterial composition of stools depend on the diet, it is reasonable that the risk for infection will change with dietary modifications. Patients should avoid all simple sugars, refined carbohydrates, full-strength fruit juice (diluted fruit juice is acceptable), and food allergens. Yogurt products with live probiotics are also recommended. Patients should restrict calories and eat liberal amounts of garlic and onions.

Patients with IC/PBS should exclude coffee, tea, soda, alcoholic beverages, citrus fruits and juices, artificial sweeteners, and hot pepper from their diets. Other foods, such as gluten and dairy products, may also be suspect. Although no scientific data exist to support or reject this hypothesis, the authors believe, on the basis of clinical experience, that patients benefit from the exclusion of these foods.[94] An elimination/challenge diet may be worthwhile for this group of patients.

Nutritional Supplements for Bacterial Cystitis

- D-mannose powder: 0.75 tsp three times a day
- Vitamin C: 500 mg every 2 hours
- Bioflavonoids: 1000 mg/day
- Vitamin A: 25,000 IU/day

- Beta-carotene: 200,000 IU/day
- Zinc: 30 mg/day
- Choline: 1000 mg/day

Nutritional Supplements for Nonbacterial Interstitial Cystitis/Painful Bladder Syndrome

- L-arginine: 1500 mg in divided doses
- Quercetin: 500 mg twice a day

Botanical Medicines for Bacterial Cystitis

Dosages are three-times-a-day quantities:

A. uva ursi

- Dried leaves or as a tea: 1.5 to 4 g (1–2 tsp)
- Freeze-dried leaves: 500 to 1000 mg
- Tincture (1:5): 4 to 6 mL (1–1.5 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Powdered solid extract (10% arbutin): 250 to 500 mg

H. canadensis

- Dried root (or as tea): 1 to 2 g
- Freeze-dried root: 500 to 1000 mg
- Tincture (1:5): 4 to 6 mL (1–1.5 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Powdered solid extract (8% alkaloid): 250 to 500 mg
- Sandalwood oil: 1 to 2 drops

Botanical Medicines for Nonbacterial Interstitial Cystitis/Painful Bladder

Centella asiatica

- Dried herb: a tea can be made of the dried leaf, to be taken three times a day.
- Powdered herb (available in capsules): 1000 to 4000 mg, three times a day
- Tincture (1:2 w/v, 30% alcohol): 30 to 60 drops (equivalent to 1.5–3 mL; there are 5 mL in a teaspoon), three times a day
- Standardized extract: 50 to 250 mg two to three times a day. Standardized extracts should contain 40% asiaticoside, 29% to 30% asiatic acid, 29% to 30% madecassic acid, and 1% to 2% madecassoside.

Equisetum arvense

- Standardized dose: 300 mg three times a day, standardized to contain 10% to 15% silica
- Herbal infusion (tea): 2 to 3 tsp three times a day. Hot water is poured onto the herb, steeped for 5 to 10 minutes, and taken as directed.
- Tincture (1:5): 1 to 4 mL three times a day
- External (compresses): 10 g of herb per 1 L water a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Najar MS, Saldanha CL, Banday KA. Approach to urinary tract infections. *Indian J Nephrol*. 2009;19(4):129–139. https://doi.org/10.4103/0971-4065.59333.
2. Branch W. *Office Practice of Medicine*. 4th ed. Philadelphia: Saunders; 2002.
3. Nickel JC. Interstitial cystitis. *Med Clin North Am*. 2004;88(2):467–481. https://doi.org/10.1016/S0025-7125(03)00151-2.
4. Bogart LM, Berry SH, Clemens JQ. Symptoms of interstitial cystitis, painful bladder syndrome and similar diseases in women: a systematic review. *J Urol*. 2007;177(2):450–456. https://doi.org/10.1016/j.juro.2006.09.032.
5. Howard FM. Chronic pelvic pain. *Obstet Gynecol*. 2003;101(3).
6. Hanno PM. Interstitial cystitis and related disorders. In: Walsh PC, Retik AB, Vaughan EDJ, eds. *Campbell's Urology*. Philadelphia: Saunders; 2015:631–670.
7. Driscoll A, Teichman JM. How do patients with interstitial cystitis present? *J Urol*. 2001;166(6):2118–2120.
8. ACOG Committee on Practice Bulletins—Gynecology. ACOG Practice Bulletin No. 51. Chronic pelvic pain. *Obstet Gynecol*. 2004;103(3):589–605.
9. Daniels J, Gray R, Hills RK, et al. Laparoscopic uterosacral nerve ablation for alleviating chronic pelvic pain: a randomized controlled trial. *JAMA*. 2009;302(9):955. https://doi.org/10.1001/jama.2009.1268.
10. Zondervan KT, Yudkin PL, Vessey MP, Dawes MG, Barlow DH, Kennedy SH. Patterns of diagnosis and referral in women consulting for chronic pelvic pain in UK primary care. *Br J Obstet Gynaecol*. 1999;106(11):1156–1161.
11. Howard F. Physical examination. In: Howard FM, Perry CP, Carte r JE, El-Minawi AM, eds. *Pelvic Pain: Diagnosis and Management*. Philadelphia: Lippincott Williams & Wilkins; 2000:26–42.
12. McCormack WM. Two urogenital sinus syndromes. Interstitial cystitis and focal vulvitis. *J Reprod Med*. 1990;35(9):873–876.
13. Ottem DP, Teichman JMH. What is the value of cystoscopy with hydrodistension for interstitial cystitis? *Urology*. 2005;66(3):494–499. https://doi.org/10.1016/j.urology.2005.04.011.
14. Erickson DR, Tomaszewski JE, Kunselman AR, et al. Do the National Institute of Diabetes and Digestive and Kidney Diseases cystoscopic criteria associate with other clinical and objective features of interstitial cystitis? *J Urol*. 2005;173(1):93–97. https://doi.org/10.1097/01.ju.0000146466.71311.ab.
15. Teichman JM. Potassium sensitivity testing in the diagnosis and treatment of interstitial cystitis. *Infect Urol*. 2003;16:87–94.
16. Moldwin R, Brettschneider N. The use of intravesical anesthetics to aid in the diagnosis of interstitial cystitis. In: *Poster Presented at Research Insights Into Interstitial Cystitis: A Basic and Clinical Science Symposium*. Alexandria, VA; 2005:108–115.
17. Nickel JC, Moldwin R, Lee S, Davis EL, Henry RA, Wyllie MG. Intravesical alkalinized lidocaine (PSD597) offers sustained relief from symptoms of interstitial cystitis and painful bladder syndrome. *BJU Int*. 2009;103(7):910–918. https://doi.org/10.1111/j.1464-410X.2008.08162.x.
18. Bass PF, Jarvis JA, Mitchell CK. Urinary tract infections. *Prim Care*. 2003;30(1):41–61, v-vi.
19. John AS, Boyd JC, Lowes AJ, Price CP. The use of urinary dipstick tests to exclude urinary tract infection. *Am J Clin Pathol*. 2006;126(3):428–436. https://doi.org/10.1309/C69RW1BT7E4QAFPV.
20. Rubin R. Infections of the urinary tract. In: Dale D, Federman D, eds. *Scientific American Medicine*. New York: Scientific American; 1997.
21. Dason S, Dason JT, Kapoor A. Guidelines for the diagnosis and management of recurrent urinary tract infection in women. *Can Urol Assoc J*. 2011;5(5):316–322. https://doi.org/10.5489/cuaj.11214.
22. Hooton TM. Recurrent urinary tract infection in women. *Int J Antimicrob Agents*. 2001;17(4):259–268.
23. ACOG Committee on Practice Bulletins—Gynecology. ACOG practice bulletin. Medical management of endometriosis. Number 11, December 1999 (replaces Technical Bulletin Number 184, September 1993). Clinical management guidelines for obstetrician-gynecologists. *Int J Gynaecol Obstet*. 2000;71(2):183–196.
24. Chung MK, Chung RP, Gordon D. Interstitial cystitis and endometriosis in patients with chronic pelvic pain: The "Evil Twins" syndrome. *JSLS J Soc Laparoendosc Surg. 9(1):25-29*.
25. Abrams P. Describing bladder storage function: overactive bladder syndrome and detrusor overactivity. *Urology*. 2003;62(5 suppl 2):28–37.
26. Evans RJ. Overlapping Symptoms. *Female Patient (Parsippany)*. 2006;31(suppl):5–10.
27. Reed BD. Vulvodynia: diagnosis and management. *Am Fam Physician*. 2006;73(7):1231–1238.
28. Shorter B, Lesser M, Moldwin RM, Kushner L. Effect of comestibles on symptoms of interstitial cystitis. *J Urol*. 2007;178(1):145–152. https://doi.org/10.1016/j.juro.2007.03.020.
29. Herati AS, Shorter B, Tai J, Lesser M, Moldwin RM. Differences in food sensitivities between female interstitial cystitis/painful bladder syndrome (IC/PBS) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) patients. *J Urol*. 2009;181(4):22. https://doi.org/10.1016/S0022-5347(09)60074-4.
30. Friedlander JI, Shorter B, Moldwin RM. Diet and its role in interstitial cystitis/bladder pain syndrome (IC/BPS) and comorbid conditions. *BJU Int*. 2012;109(11):1584–1591. https://doi.org/10.1111/j.1464-410X.2011.10860.x.
31. van Ophoven A, Hertle L. Long-term results of amitriptyline treatment for interstitial cystitis. *J Urol*. 2005;174(5):1837–1840.
32. Dell JR. Building a successful interstitial cystitis practice in gynecology. *Pr Build Today*. 2003;49:4–11.
33. Theoharides TC, Sant GR. Hydroxyzine therapy for interstitial cystitis. *Urology*. 1997;49(suppl 5A):108–110.
34. Shupp Byrne DE, Sedor JF, Soroush M, McCue PA, Mulholland SG. Interaction of bladder glycoprotein GP51 with uropathogenic bacteria. *J Urol*. 2001;165(4):1342–1346.
35. Saini R, Gonzalez RR, Te AE. Chronic pelvic pain syndrome and the overactive bladder: the inflammatory link. *Curr Urol Rep*. 2008;9(4):314–319.
36. Patel BN, Evans RJ. Overactive bladder and pain: management strategies. *Curr Urol Rep*. 2010;11(6):379–384. https://doi.org/10.1007/s11934-010-0141-5.
37. Kahn BS, Stanford EJ, Mishell DR, Rosenberg MT, Wysocki S. Management of patients with interstitial cystitis or chronic pelvic pain of bladder origin: a consensus report. *Curr Med Res Opin*. 2005;21(4):509–516. https://doi.org/10.1185/030079905X38178.
38. Dancel R, Mounsey A, Handler L. Medications for treatment of interstitial cystitis. *Am Fam Physician*. 2015;91(2):116–117.
39. Thilagarajah R, Witherow RO, Walker MM. Oral cimetidine gives effective symptom relief in painful bladder disease: a prospective, randomized, double-blind placebo-controlled trial. *BJU Int*. 2001;87(3):207–212.
40. Soucy F, Grégoire M. Efficacy of prednisone for severe refractory ulcerative interstitial cystitis. *J Urol*. 2005;173(3):841–843; discussion 843. https://doi.org/10.1097/01.ju.0000153612.14639.19.
41. Cox M, Klutke JJ, Klutke CG. Assessment of patient outcomes following submucosal injection of triamcinolone for treatment of Hunner's ulcer subtype interstitial cystitis. *Can J Urol*. 2009;16(2):4536–4540.
42. Sairanen J, Tammela TLJ, Leppilahti M, et al. Cyclosporine A and pentosan polysulfate sodium for the treatment of interstitial cystitis: a randomized comparative study. *J Urol*. 2005;174(6):2235–2238. https://doi.org/10.1097/01.ju.0000181808.45786.84.
43. Parkin J, Shea C, Sant GR. Intravesical dimethyl sulfoxide (DMSO) for interstitial cystitis—a practical approach. *Urology*. 1997;49(suppl 5A):105–107.
44. Theoharides TC, Whitmore K, Stanford E, Moldwin R, O'Leary MP. Interstitial cystitis: bladder pain and beyond. *Expert Opin Pharmacother*. 2008;9(17):2979–2994. https://doi.org/10.1517/14656560802519845.
45. Curtis Nickel J, Baranowski AP, Pontari M, Berger RE, Tripp DA. Management of men diagnosed with chronic prostatitis/chronic pelvic pain syndrome who have failed traditional management. *Rev Urol*. 2007;9(2):63–72.
46. Hanno PM, Burks DA, Clemens JQ, et al. AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. *J Urol*. 2011;185(6):2162–2170. https://doi.org/10.1016/j.juro.2011.03.064.
47. Ger GC, Wexner SD, Jorge JM, et al. Evaluation and treatment of chronic intractable rectal pain—a frustrating endeavor. *Dis Colon Rectum*. 1993;36(2):139–145.
48. Heah SM, Ho YH, Tan M, Leong AF. Biofeedback is effective treatment for levator ani syndrome. *Dis Colon Rectum*. 1997;40(2):187–189.
49. Whitmore KE. Complementary and alternative therapies as treatment approaches for interstitial cystitis. *Rev Urol*. 2002;4(suppl 1):S28–S35.

50. Fam A. Use of titrated extract of *Centella asiatica* (TECA) in bilharzial baldder lesions. *Int Surg*. 1973;58:451–452.
51. Etrebi A, Ibrahim A, Zaki K. Treatment of ulcer bladder with asiaticoside. *J Egypt Med Assoc*. 1975;58(5-6):324–327.
52. Findings reported by Desert Harvest. www.desertharvest.com. Accessed November 1, 2001.
53. Smith SD, Wheeler MA, Foster Jr HE, Weiss RM. Improvement in interstitial cystitis symptom scores during treatment with oral L-Arginine. *J Urol*. 1997;158(3):703–708. https://doi.org/10.1016/S0022-5347(01)64297-6.
54. Korting GE, Smith SD, Wheeler MA, Weiss RM, Foster HE. A randomized double-blind trial of oral L-arginine for treatment of interstitial cystitis. *J Urol*. 1999;161(2):558–565.
55. Cartledge JJ, Davies A–M., Eardley I. A randomized double–blind placebo–controlled crossover trial of the efficacy of l–arginine in the treatment of interstitial cystitis. *BJU Int*. 2000;85(4):421–426.
56. Papas PN, Brusch CA, Ceresia GC. Cranberry juice in the treatment of urinary tract infections. *Southwest Med*. 1966;47(1):17–20.
57. Guay DRP. Cranberry and urinary tract infections. *Drugs*. 2009;69(7):775–807. https://doi.org/10.2165/00003495-200969070-00002.
58. Avorn J, Monane M, JH G, RJ G, Choodnovskiy I, LA L. Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *JAMA*. 1994;271(10):751–754.
59. Ofek I, Goldhar J, Zafriri D, Lis H, Adar R, Sharon N. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. *N Engl J Med*. 1991;324(22):1599–1599. https://doi.org/10.1056/NEJM199105303242214.
60. Sanchez A, Reeser JL, Lau HS, et al. Role of sugars in human neutrophilic phagocytosis. *Am J Clin Nutr*. 1973;26(11):1180–1184.
61. Ringsdorf WM, Cheraskin E, Ramsay RR. Sucrose, neutrophilic phagocytosis and resistance to disease. *Dent Surv*. 1976;52(12):46–48.
62. Bernstein J, Alpert S, Nauss K. Depression of lymphocyte transformation following oral glucose ingestion. *Am J Clin Nutr*. 1977;30:613.
63. Stothers L. A randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women. *Can J Urol*. 2002;9(3):1558–1562.
64. McMurdo MET, Bissett LY, Price RJG, Phillips G, Crombie IK. Does ingestion of cranberry juice reduce symptomatic urinary tract infections in older people in hospital? A double-blind, placebo-controlled trial. *Age Ageing*. 2005;34(3):256–261.
65. Lee BB, Haran MJ, Hunt LM, et al. Spinal-injured neuropathic bladder antisepsis (SINBA) trial. *Spinal Cord*. 2007;45(8):542–550.
66. Barbosa-Cesnik C, Brown MB, Buxton M, Zhang L, DeBusscher J, Foxman B. Cranberry juice fails to prevent recurrent urinary tract infection: results from a randomized placebo-controlled trial. *Clin Infect Dis*. 2011;52(1):23–30.
67. Fu Z, Liska D, Talan D, Chung M. Cranberry reduces the risk of urinary tract infection recurrence in otherwise healthy women: a systematic review and meta-analysis. *J Nutr*. 2017;147(12):2282–2288. https://doi.org/10.3945/jn.117.254961.
68. Brinkley L, McGuire J, Gregory J, Pak CY. Bioavailability of oxalate in foods. *Urology*. 1981;17(6):534–538.
69. Munday PE, Savage S. Cymalon in the management of urinary tract symptoms. *Genitourin Med*. 1990;66(6):461.
70. Spooner JB. Alkalinisation in the Management of Cystitis. *J Int Med Res*. 1984;12(1):30–34. https://doi.org/10.1177/030006058401200105.
71. Ripoll E, Bunn T. The role of acupuncture in the treatment of urologic conditions. *World J Urol*. 2002;20(5):315–318. https://doi.org/10.1007/s00345-002-0297-9.
72. Alraek T, Aune A, Baerheim A. Traditional Chinese medicine syndromes in women with frequently recurring cystitis: frequencies of syndromes and symptoms. *Complement Ther Med*. 2000;8(4):260–265. https://doi.org/10.1054/ctim.2000.0399.
73. Rapkin AJ, Kames LD. The pain management approach to chronic pelvic pain. *J Reprod Med*. 1987;32(5):323–327.
74. Lyons P. Acupuncture treatment for interstitial cystitis: a case report. *Am Acad Med Acupunct*. 2001. http://www.medicalacupuncture.org.ezproxy.med.nyu.edu. Accessed November 1, 2007.
75. Aune A, Alraek T, Lihua H, Baerheim A. Acupuncture in the prophylaxis of recurrent lower urinary tract infection in adult women. *Scand J Prim Health Care*. 1998;16(1):37–39.
76. Schaeffer AJ, Chmiel JS, Duncan JL, Falkowski WS. Mannose-sensitive adherence of Escherichia coli to epithelial cells from women with recurrent urinary tract infections. *J Urol*. 1984;131(5):906—910.
77. Michaels EK, Chmiel JS, Plotkin BJ, Schaeffer AJ. Effect of D-mannose and D-glucose on Escherichia coli bacteriuria in rats. *Urol Res*. 1983;11(2):97–102. https://doi.org/10.1007/BF00256954.
78. Domenici L, Monti M, Bracchi C, et al. D-mannose: a promising support for acute urinary tract infections in women. A pilot study. *Eur Rev Med Pharmacol Sci*. 2016;20(13):2920–2925.
79. Vicariotto F. Effectiveness of an association of a cranberry dry extract, D-mannose, and the two microorganisms *Lactobacillus plantarum* LP01 and *Lactobacillus paracasei* LPC09 in women affected by cystitis. *J Clin Gastroenterol*. 2014;48:S96–S101. https://doi.org/10.1097/MCG.0000000000000224.
80. Genovese C, Davinelli S, Mangano K, et al. Effects of a new combination of plant extracts plus d -mannose for the management of uncomplicated recurrent urinary tract infections. *J Chemother*. 2017:1–8. https://doi.org/10.1080/1120009X.2017.1393587.
81. Parejo I, Viladomat F, Bastida J, Codina C. A single extraction step in the quantitative analysis of arbutin in bearberry *(Arctostaphylos uva-ursi)* leaves by high-performance liquid chromatography. *Phytochem Anal*. 2001;12(5):336–339. https://doi.org/10.1002/pca.602.
82. Frohne D. The urinary disinfectant effect of extract from leaves uva ursi. *Planta Med*. 1970;18(1):1–25. https://doi.org/10.1055/s-0028-1099743.
83. Siegers C, Bodinet C, Ali SS, Siegers C-P. Bacterial deconjugation of arbutin by *Escherichia coli*. *Phytomedicine*. 2003;10(suppl 4):58–60.
84. Larsson B, Jonasson A, Fianu S. Prophylactic effect of UVA-E in women with recurrent cystitis: a preliminary report. *Curr Ther Res*. 2014;53(4):441–443. https://doi.org/10.1016/S0011-393X(05)80204-8.
85. Manges AR, Johnson JR, Foxman B, O'Bryan TT, Fullerton KE, Riley LW. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *N Engl J Med*. 2001;345(14):1007–1013. https://doi.org/10.1056/NEJMoa011265.
86. *Merck Index*. 10th ed. Rahway, NJ: Merck.
87. Sharma VD, Sethi MS, Kumar A, Rarotra JR. Antibacterial property of *Allium sativum* Linn.: in vivo & in vitro studies. *Indian J Exp Biol*. 1977;15(6):466–468.
88. Elnima EI, Ahmed SA, Mekkawi AG, Mossa JS. The antimicrobial activity of garlic and onion extracts. *Pharmazie*. 1983;38(11):747–748.
89. Amin AH, Subbaiah TV, Abbasi KM. Berberine sulfate: antimicrobial activity, bioassay, and mode of action. *Can J Microbiol*. 1969;15(9):1067–1076.
90. Johnson CC, Johnson G, Poe CF. Toxicity of alkaloids to certain bacteria. II. Berberine, physostigmine, and sanguinarine. *Acta Pharmacol Toxicol (Copenh)*. 1952;8(1):71–78.
91. Gupta K. Emerging antibiotic resistance in urinary tract pathogens. *Infect Dis Clin North Am*. 2003;17(2):243–259.
92. O'Hare PG, Hoffmann AR, Allen P, Gordon B, Salin L, Whitmore K. Interstitial cystitis patients' use and rating of complementary and alternative medicine therapies. *Int Urogynecol J*. 2013;24(6):977–982. https://doi.org/10.1007/s00192-012-1966-x.

164

Dermatitis Herpetiformis

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Pruritic papulovesicular eruption, usually on the extensor surfaces
- Most common in middle-aged white males but may be seen in individuals of any age
- Pathognomic granular IgA deposits at the dermal–epidermal junction; confirmed by immunofluorescence biopsy of unaffected skin in close proximity to an active lesion
- Asymptomatic celiac disease (gluten-sensitive enteropathy) in 75% to 90% of patients

GENERAL CONSIDERATIONS

Dermatitis herpetiformis (DH) is the most common extraintestinal manifestation of gluten-sensitive enteropathy (celiac disease; Fig. 164.1). The incidence of DH is decreasing, whereas the opposite is true for celiac disease (CD). Presently, the prevalence ratio of the disorders is 1:8. Subclinical CD is a prerequisite for developing DH. It remains a mystery why only some patients with undiagnosed CD develop DH. The increased age at diagnosis and less severe small intestinal damage in both DH and CD suggest changes in environmental factors, such as a lowered lifetime gluten load.[1]

Patients with DH demonstrate serum IgA antibodies against epidermal transglutaminase and tissue transglutaminase.[2] The putative autoantigen of DH is epidermal transglutaminase. DH has been referred to as "celiac disease of the skin."[3] Just as in celiac disease (CD), a gluten-free diet (GFD) is most often all that is required to resolve the lesions. The response is slow, sometimes taking months, in comparison to the gastrointestinal symptoms (GI) of CD that typically respond in days or weeks.[1] Absorption studies (see Chapter 19) can be used to assess the degree of enteropathy.

Almost all individuals with CD have celiac-associated antibodies and specific pairs of allelic variants in two HLA genes, *HLA-DQ2* (>90%) and *HLA-DQ8* (5%–10%).[4] Only 3% of individuals with one or both of these alleles develop CD, yet up to 40% of the general population has one of them. Therefore their presence is not diagnostic of CD, but their absence excludes a diagnosis of CD. Genetic testing is available for the assessment of CD. Findings of inflammatory changes in jejunal biopsies, ranging from lymphocytic enteritis to various degrees of villous atrophy, are the gold standard for CD diagnosis, even in the absence of negative serology for CD.

In the past, CD was considered to be an uncommon disease, affecting mostly children and limited to people of European ancestry. Now it is known that CD may be discovered at any age and is one of the most common chronic diseases worldwide, with a prevalence of 1% to 2%.[5] The mean age of adult CD diagnosis is 45 years, although up to 20% of patients are diagnosed at age 60 or older. CD is underdiagnosed in adults partly because they may lack the classic symptoms of diarrhea or signs of malabsorption. Clinical suspicion may arise from manifestations such as anemia, skin disorders (in addition to DH), neurological disease, osteoporosis, and abnormal liver function tests.[6]

Patients with CD have an increased incidence of other immune-mediated disorders in comparison to the general population, including thyroid disorders, type 1 diabetes mellitus, and other skin diseases such as psoriasis, atopic dermatitis, vitiligo, rosacea, systemic lupus erythematosus, alopecia areata, chronic urticaria, recurrent aphthous stomatitis, and oral lichen planus.[7] (For more comprehensive discussion, see Chapter 157.)

DH has a predilection for the elbows, knees, and buttocks. Skin biopsy shows granular or fibrillar IgA deposits.[8] The characteristic skin

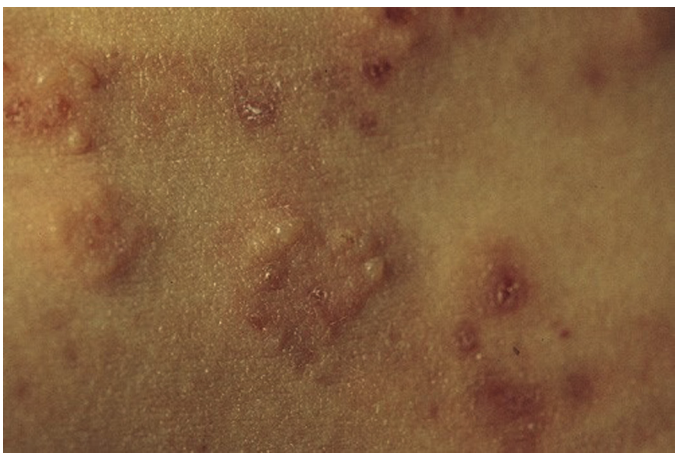


Fig. 164.1 Dermatitis Herpetiformis.

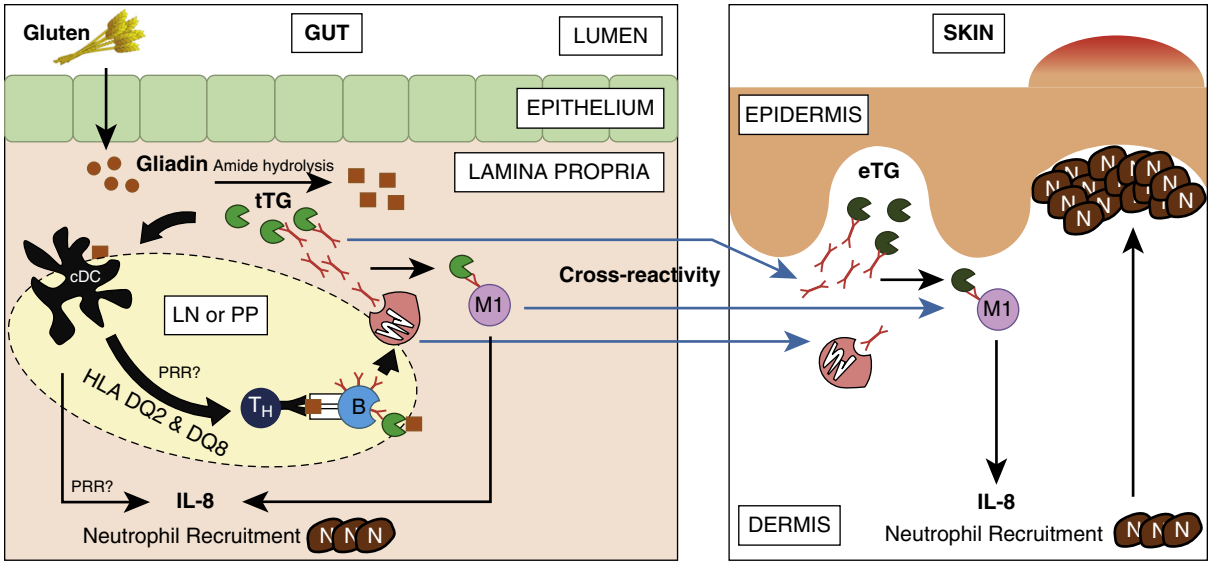


Fig. 164.2 Cross-reactivity Hypothesis of Dermatitis Herpetiformis in Celiac Disease.

lesions found in patients with DH are extremely itchy grouped vesicles most frequently located on extensor surfaces. Intense pruritus is the predominant symptom; however, DH is a clinical chameleon and can present with excoriations, eczematous lesions, or minimal patterns of discrete erythema or digital purpura.[9]

PATHOGENESIS OF DH: FROM GUT TO SKIN

At the present time, the immunopathogenesis of DH is believed to originate from occult CD in the gut with a tissue transglutaminase (TG2) autoantigen response, and possibly also an IgA epidermal transglutaminase (TG3) autoantibody response (Fig. 164.2). This results in an immune complex deposition of high-avidity IgA TG3 antibodies to the TG3 enzyme in the papillary dermis. This mechanism is supported by GFD results. TG3 and TG2 antibodies in serum disappear with the diet, the small intestine heals, and eventually the rash clears. However, the IgA–TG3 complexes in the dermis disappear quite slowly with the GFD, presumably because the active TG3 enzyme in the complexes results in cross-linkage of the complex to the dermal structures.[10]

THERAPEUTIC CONSIDERATIONS

Gluten

The most important factor in the treatment of patients with DH is the elimination of all sources of gluten. Frazer's criteria for the diagnosis of gluten-sensitive enteropathy (improvement on a GFD and relapse after reintroduction) have been used in many studies and have shown conclusively that the rash and villous atrophy of DH are largely gluten dependent.[11–16] Gluten elimination results in an improvement in virtually all patients, including the elimination of the reticulin and gluten antibodies found in patients with DH.[3,17]

Further study of the wheat connection has shown that the gliadin polypeptide of gluten is most likely the key antigen. Indirect immunofluorescence shows antibodies to gliadin in the sera of 45% of patients with DH. The titer and correlation increase with increasing severity of the disease; 81% of patients with severe jejunal abnormalities show antibodies to gliadin.[18]

Despite the published benefits of a GFD in the treatment of DH for more than 30 years, this treatment is still often omitted from conventional dermatology and medical textbooks. The advantage of a GFD over drugs like dapsone (the most widely prescribed drug for DH) is obvious because this drug is often associated with severe side effects. In contrast, with a GFD:

- Most patients (more than 65%) experience complete resolution, and the rest improve substantially.
- There is complete resolution of the enteropathy associated with DH.
- Harsh medications can be eliminated or substantially reduced.
- Most patients experience an improved sense of well-being.

Also of importance is that individuals who used a GFD rather than drugs are protected against developing non-Hodgkin's lymphoma (NHL)[19] because the risk of NHL is significantly increased in DH and CD.

Food Allergy

Although gluten control is critical in the treatment of patients with DH, some (about 35%) are not adequately helped. In particular, only 50% of patients totally eliminate the cutaneous IgA deposits and develop normal jejunal tissue. This is probably because of the presence of other food allergies that, although not initiating, developed as a result of the increased leakage of macromolecules across the damaged intestinal mucosa. Milk has been found to be significantly problematic in some patients.[20,21] Using a sensitive enzyme-linked immunoassay (ELISA), 75% of DH patients were shown to have serum antibodies reactive against gliadin, bovine milk, or ovalbumin.[22] These results suggest that other food sensitivities are implicated in DH. An elemental diet followed by careful food reintroduction usually produces better results than a simple gluten-free diet.[23]

Para-Aminobenzoic Acid

Para-aminobenzoic acid (PABA) has been used successfully in the control of DH, even in those patients who are not controlling the gluten content of their diet.[24] However, because its use is recommended only for symptom control and it probably does not result in repair of the villous atrophy, it is not recommended as the treatment of choice but rather as an adjunct in unresponsive cases or to assist in particularly severe cases.

Treatment of Nutritional Deficiencies

Evaluations for deficiencies of iron, zinc, calcium, fat-soluble vitamins, and folic acid should be conducted; also, the possibility of homocysteinemia, osteopenia, and osteoporosis should be assessed and treated if found.

THERAPEUTIC APPROACH

After all sources of gluten and gliadin have been eliminated (see Appendix 6 for the gluten content of foods), a careful search should be made for other food allergies (for more detail, see Chapter 14). Once allergens have been identified and avoided, a therapeutic regimen similar to that for atopic dermatitis (see Chapter 150) should be used. Patience is necessary because a response may take several weeks to 6 months to be seen.

Diet

Use a healthy, whole-foods diet free of processed foods, gluten-containing grains, and foods found to be allergenic.

Supplement

PABA: 5 g/day until remission (maximum of 3 months)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Reunala T, Salmi TT, Hervonen K, et al. Dermatitis herpetiformis: a common extraintestinal manifestation of coeliac disease. *Nutrients*. 2018;10(5):602.
2. Rose C, Armbruster FP, Ruppert J, et al. Autoantibodies against epidermal transglutaminase are a sensitive diagnostic marker in patients with dermatitis herpetiformis on a normal or gluten-free diet. *J Am Acad Dermatol*. 2009;61(1):39–43.
3. Fry L, Leonard JN, Swain F, et al. Long term follow-up of dermatitis herpetiformis with and without dietary wheat gluten withdrawal. *Br J Derm*. 1982;107:631–640.
4. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. *Lancet*. 2018;391:70–81.
5. Collin P, Vilppula A, Luostarinen L, et al. Review article: Coeliac disease in later life must not be missed. *Aliment Pharmacol Ther*. 2018;47:563–572.
6. Rodrigo L. Celiac disease. *World J Gastroenterol*. 2006;12:6585–6593.
7. Rodrigo L, Beteta-Gorriti V, Alvarez N, et al. Cutaneous and mucosal manifestations associated with celiac disease. *Nutrients*. 2018;10(7):800. https://doi.org/10.3390/nu10070800.
8. Ko CJ, Colegio OR, Moss JE, et al. Fibrillar IgA deposition in dermatitis-herpetiformis: an underreported pattern with potential clinical significance. *J Cutan Pathol*. 2010;37(4):475–477.
9. Pfeiffer C. Dermatitis herpetiformis: a clinical chameleon. *Hautarzt*. 2006;57(11):1021–1028.
10. Hietikko M, Hervonen K, Salmi T, et al. Disappearance of epidermal transglutaminase and IgA deposits from the papillary dermis of dermatitis herpetiformis patients after a long-term gluten-free diet. *Br J Dermatol*. 2018;178:e198–e201. https://doi.org/10.1111/bjd.15995.
11. Leonard J, Haffenden G, Tucker W, et al. Gluten challenge in dermatitis herpetiformis. *N Engl J Med*. 1983;308:816–819.
12. Savilahti E, Reunala T. Is dermatitis herpetiformis a gluten-sensitive enteropathy? *Int J Dermatol*. 1990;10:706–708.
13. Reunala T. Dermatitis herpetiformis: coeliac disease of the skin. *Ann Med*. 1998;30:416–418.
14. Kumar V, Zane H, Kaul N. Serologic markers of gluten-sensitive enteropathy in bullous diseases. *Arch Derm*. 1992;128:1474–1478.
15. Frodin T, Gotthard R, Hed J, et al. Gluten-free diet for dermatitis herpetiformis: the long term effect on cutaneous, immunological and jejunal manifestations. *Acta Derm Venereol (Stockholm)*. 1981;61:405–411.
16. Garioch JJ, Lewis HM, Sargent SA, et al. 25 years' experience of a gluten-free diet in the treatment of dermatitis herpetiformis. *Br J Dermatol*. 1994;131:541–545.
17. Fry L. Dermatitis herpetiformis: problems, progress and prospects. *Eur J Dermatol*. 2002;12:523–531.
18. Volta U, Cassani F, DeFranchis R, et al. Antibodies to gliadin in adult coeliac disease and dermatitis herpetiformis. *Digestion*. 1984;30:263–270.
19. Lewis HM, Renaula TL, Garioch JJ, et al. Protective effect of gluten-free diet against development of lymphoma in dermatitis herpetiformis. *Br J Derm*. 1996;135:363–367.
20. Engquist A, Pock-Steen OC. Dermatitis herpetiformis and milk-free diet. *Lancet*. 1971;2:438–439.
21. Pock-Steen OC, Niordson AM. Milk sensitivity in dermatitis herpetiformis. *Br J Derm*. 1970;83:614–619.
22. Barnes RM, Lewis-Jones MS. Isotype distribution and serial levels of antibodies reactive with dietary protein antigens in dermatitis herpetiformis. *J Clin Lab Immunol*. 1989;30:87–91.
23. Kadunce DP, McMurry MP, Avots-Avotins A, et al. The effect of an elemental diet with and without gluten on disease activity in dermatitis herpetiformis. *J Invest Dermatol*. 1991;97:175–182.
24. Zarafonetis CJ, Johnwick EB, Kirkman LW, et al. Paraaminobenzoic acid in dermatitis herpetiformis. *Arch Dermatol Syph*. 1951;63:115–132.

165

Diabetes Mellitus Types I and II

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

Elevated blood glucose is determined by the following:

- Fasting (overnight): venous plasma glucose concentration greater than or equal to 126 mg/dL on at least two separate occasions.
- Following ingestion of 75 g of glucose: venous plasma glucose concentration greater than or equal to 200 mg/dL at 2 hours postingestion and at least one other sample during the 2-hour test.
- A random blood glucose level of 200 mg/dL or more plus the presence of suggestive symptoms.
- Classic symptoms of polyuria, polydipsia, and polyphagia.
- Fatigue, blurred vision, poor wound healing, periodontal disease, and frequent infections are often manifesting symptoms as well in type 2 diabetes.

GENERAL CONSIDERATIONS

Diabetes is a chronic disorder of carbohydrate, fat, and protein metabolism characterized by fasting elevations of blood glucose levels and a greatly increased risk of cardiovascular disease, renal disease, and neuropathy. Diabetes is divided into two major categories: types 1 and 2. Type 1 diabetes (T1DM) occurs most often in children and adolescents. For this reason, it is often referred to as juvenile-onset diabetes. About 5% to 10% of all diabetic patients have T1DM. (Box 165.1 lists the major complications of diabetes.)

T1DM is an autoimmune disease caused by the destruction of the beta cells of the pancreas, which manufacture insulin. Positive antibodies against beta cells or insulin occur in 75% of patients with T1DM. Why the immune system is activated to attack the pancreas is not fully clear, but viral infection, food sensitivities, and chemical or free radical damage are likely possibilities, combined with genes that may predispose to T1DM. These individuals will require lifelong insulin for the control of blood glucose levels. The individual with T1DM must learn how to manage blood glucose levels on a day-by-day basis, modifying insulin types and dosages as necessary according to meals eaten, liver production of glucose, and the results of regular blood glucose testing.

Type 2 diabetes (T2DM) historically has had an onset after age 40 in overweight individuals, but today it is seen even in pediatric patients because of the obesity epidemic, which affects all age groups in America. It is generally thought that up to 90% of all those with diabetes have T2DM. Initially, insulin levels are typically elevated in T2DM, indicating a loss of sensitivity to insulin by the body's cells. Obesity is a major contributing factor to this loss of insulin sensitivity. Approximately 90% of individuals categorized as having T2DM are obese. The achievement of an ideal body weight in these patients is often associated with the restoration of normal blood glucose levels. Even if T2DM has progressed to the point where insulin deficiency is present, weight loss nearly always results in significant improvements in blood glucose control and dramatic reductions in other health risks, such as cardiovascular disease (Table 165.1).

BOX 165.1 Major Complications of Diabetes

- Cardiovascular disease: Adults with diabetes have death rates from cardiovascular disease about two to four times higher than that for adults without diabetes.
- Hypertension: About 75% of adults with diabetes have high blood pressure.
- Retinopathy: Diabetes is the leading cause of blindness among adults.
- Renal disease: Diabetes is the leading reason for dialysis treatment, accounting for 43% of new cases.
- Neuropathy: About 60% to 70% of people with diabetes have mild to severe forms of nervous system damage. Severe forms of diabetic nerve disease are a major contributing cause of lower-extremity amputations.
- Amputations: More than 60% of lower-limb amputations in the United States occur among people with diabetes.
- Periodontal disease: Almost one third of people with diabetes have severe periodontal (gum) disease.
- Pain: Many people with diabetes fall victim to chronic pain due to conditions such as arthritis, neuropathy, circulatory insufficiency, or muscle pain (fibromyalgia).
- Depression: This is a common accompaniment of diabetes. Clinical depression can often begin to occur even years before diabetes is fully evident. It is difficult to treat in those with poorly controlled diabetes.
- Autoimmune disorders: Thyroid disease, inflammatory arthritis, and other diseases of the immune system commonly add to the suffering of people with diabetes.

TABLE 165.1 Differences Between Type 1 and Type 2 Diabetes

Features	Type I	Type 2
Age at onset	Usually younger than 40 years	Usually older than 40 years
Proportion of all diabetics	<10%	>90%
Family history	Uncommon	Common
Appearance of symptoms	Rapid	Slow
Obesity at onset	Uncommon	Common
Insulin levels	Decreased	Normal to high initially, decreased after several years
Insulin resistance	Occasional	Often
Treatment with insulin	Always	Usually not required

T2DM is a disease characterized by progressive worsening of glycemic control, which starts with mild alterations in postprandial glucose homeostasis followed by an increase in fasting plasma glucose and often ultimately a lack of production of insulin and the need for insulin therapy.

There are other types of diabetes, such as latent autoimmune diabetes of the adult, sometimes termed *type 1.5*. This is a slower-onset autoimmune type of diabetes that occurs later in life, often after people reach 35 years of age. Diabetes may also occur as a result of chronic pancreatitis and other insults to the pancreas. Gestational diabetes, another type, affects about 4% of all pregnant women, adding up to about 135,000 cases in the United States each year. This occurs in women who were not diabetic before they became pregnant but developed diabetes during pregnancy. Gestational diabetes occurs more frequently among African Americans, Hispanic/Latino Americans, and American Indians. It is also more common among obese women and women with a family history of diabetes. After pregnancy, 5% to 10% of women with gestational diabetes are found to have T2DM. Women who have had gestational diabetes have a 20% to 50% chance of developing diabetes in the following 5 to 10 years. The least common types of diabetes are genetic disorders, such as neonatal diabetes and mature-onset diabetes of youth, which are generally due to faulty genes causing impaired insulin function.

Prediabetes and Metabolic Syndrome

Prediabetes (also called "impaired glucose tolerance") is characterized by a glycosylated hemoglobin (A_{1c}) from 5.7% to 6.4%, a fasting glucose between 100 and 125 mg/dL, and/or a postprandial glucose of 140 to 199 mg/dL. It is the first step in insulin resistance and estimated to affect more than 60 million Americans. Many people with prediabetes will go on to develop full-blown T2DM despite the fact that prediabetes is usually reversible and, in most cases, diabetes can be completely avoided through dietary and lifestyle changes. Factors implicated in contributing to prediabetes, insulin resistance, and the progression to T2DM include a diet high in refined carbohydrates, particularly high-fructose corn syrup; an elevated intake of saturated fats; overeating due to increased portion sizes of food; increases in inflammatory markers; lack of exercise; industrial pollution; abdominal weight gain; hormonal imbalances; inadequate sleep; and nutritional deficiencies.

Research increasingly indicates that prediabetes is accompanied by serious health risks, especially an increased risk for cardiovascular disease. Individuals with prediabetes often meet the criteria for the metabolic syndrome (MetS). This is a cluster of factors that together carry a significantly greater risk for cardiovascular disease and the development of T2DM. They include the following:

- Greater waist-to-hip ratio
- Two of the following:
  - Triglycerides greater than 150 mg/dL
  - High-density lipoprotein cholesterol (HDL-C) less than 40 mg/dL for men, less than 50 mg/dL for women
  - Blood pressure equal to or greater than 130/85 mm Hg
  - Fasting plasma glucose equal to or greater than 100 mg/dL

By this definition and using data from the National Health and Nutrition Examination Survey (2012), the prevalence of MetS in the United States is 34.2% among men and women aged 18 and above.[1] Among adolescents and using a similar definition, approximately 5.8% meet the established criteria.[2] In addition to an elevated risk for cardiovascular disease and diabetes, individuals with MetS report poorer health-related quality of life, both physically and mentally.[3]

DIAGNOSTIC CONSIDERATIONS

The classic symptoms of T1DM are frequent urination, weight loss, impaired wound healing, infections, and excessive thirst and appetite. Such individuals may suffer from diabetic ketoacidosis upon diagnosis, and they are usually lean in presentation. In T2DM, the symptoms are generally milder and may go unnoticed. For that reason and others, many people with T2DM do not even know that they have the disease. Abdominal obesity, fatigue, blurred vision, poor wound healing, periodontal disease, and frequent infections are often manifesting symptoms of T2DM.

The standard method for diagnosing diabetes involves the measurement of blood glucose levels. The initial measurement is generally a fasting blood glucose taken after the patient has avoided food for at least 10 hours but not more than 16. The normal reading is between 70 and 99 mg/dL. If a person has a fasting blood glucose measurement greater than 126 mg/dL (7 mmol/L) on two separate occasions, the diagnosis is diabetes. As mentioned previously, a fasting glucose greater than 100 but less than 126 mg/dL is classified as prediabetes.

A postprandial and a random glucose determination are also helpful in diagnosing diabetes. A postprandial measurement is usually made 1 to 2 hours after a meal, whereas a random measurement is

TABLE 165.2 Criteria for Response to the Glucose Tolerance Test

Type	Criteria
Normal	No elevation > 160 mg/dL (9 mmol/L); <150 mg/dL (8.3 mmol/L) at end of first hour, below 120 mg/dL (6.6 mmol/L) at end of second hour
Flat	No variation of more than ± 20 mg/dL (1.1 mmol/L) from fasting value
Prediabetic	Blood glucose levels of 140 mg/dL (7.8 mmol/L) to 180 mg/dL (10 mmol/L) at end of second hour
Diabetic	>180 mg/dL (10 mmol/L) during first hour, 200 mg/dL (11.1 mmol/L) or higher at end of first hour, 150 mg/dL (8.3 mmol/L) or higher at end of second hour

one that is made at any time during the day without regard to the time of the last meal. Any reading greater than 200 mg/dL (11 mmol/L) is considered indicative of diabetes (Table 165.2).

Glycosylated Hemoglobin

The measurement glycosylated $HgbA_{1c}$ is a valuable laboratory test for evaluating long-term blood glucose levels. Proteins that have glucose molecules attached to them (glycosylated peptides) are elevated several-fold in diabetic patients. Normally, about 4.6% to 5.7% of hemoglobin is combined with glucose. An $HgbA_{1c}$ from 5.7% to 6.4% indicates prediabetes. An $HgbA_{1c}$ of 6.5% or higher, particularly when done as a screening test, can diagnose diabetes and is particularly helpful in patients with nondiagnostic fasting blood sugar levels. Nonetheless, it is best coupled with a fasting blood glucose measurement and a 2-hour postprandial glucose level for a more accurate diagnosis. Because the average life of a red blood cell (RBC) is 120 days, the $HgbA_{1c}$ assay represents time-averaged values for blood glucose over the preceding 2 to 4 months. An $HgbA_{1c}$ at 5% indicates that the glucose median for the previous 3 months was around 100 mg/dL; for each digit of elevation in the percentage, a rough addition of 35 mg/dL is followed. Thus an $HgbA_{1c}$ of 7% means that on average over the preceding 3 months, the patient's blood glucose was 170 mg/dL. The $HgbA_{1c}$ index is extremely valuable in providing a simple, useful method for assessing the effectiveness of treatment as well as patient compliance; it should be checked every 3 to 6 months.[4]

Criteria for the Screening and Diagnosis of Diabetes[4]

	Prediabetes	Diabetes
A_{1c}	5.7–6.4%[a]	≥6.5%[b]
FPG	100–125 mg/dL (5.6–6.9 mmol/L)[a]	≥126 mg/dL (7.0 mmol/L)[b]
OGTT	140–199 mg/dL (7.8–11.0 mmol/L)[a]	≥200 mg/dL (11.1 mmol/L)[b]
RPG	—	≥200 mg/dL (11.1 mmol/L)[c]

[a]For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at the higher end of the range.
[b]In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.
[c]Only diagnostic in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.
FPG, Fasting plasma glucose; *OGTT,* oral glucose tolerance test; *RPG,* random plasma glucose.

RISK FACTORS FOR TYPE 1 DIABETES

In T1DM the insulin-producing cells of the pancreas are ultimately destroyed, in most cases by the body's own immune system, but what triggers this destruction can vary from one case to another. Genetic factors may predispose a person to damage to the insulin-producing cells through either impaired defense mechanisms, immune system sensitivity, or a defect in tissue-regeneration capacity. The entire set of genetic factors linked to T1DM have been termed "susceptibility genes" because they modify the risk of diabetes but are neither necessary nor sufficient for disease to develop.[5] Rather than acting as the primary cause, the genetic predisposition simply sets the stage for the environmental or dietary factor to initiate the destructive process.[6] The very term *predisposition* clearly indicates that something else must occur: less than 10% of those with increased genetic susceptibility for T1DM actually develop the disease.[7]

In detailed studies, the concordance rate for developing T1DM in identical twins was only 23% in one study[8] and 38% in another.[9] If one twin develops T1DM after age 24, the concordance rates drops all the way down to 6%, meaning that the other twin is at very low risk for developing the disease. These results and others indicate that in most cases, even where there is a true genetic predisposition, environmental and dietary factors may be more important in determining whether diabetes will develop.[10]

Additional evidence supporting the need to focus on dietary and environmental triggers follows:

- There has been a threefold to tenfold increase in the number of people with T1DM throughout the world over the past 40 years. Such a rise simply cannot be explained by an increased number of people genetically predisposed to T1DM. Changes to the human genetic code across large populations take more than one generation.[11]
- The rate of T1DM can increase dramatically when children in areas where T1DM is relatively rare move to developed countries.[12] For example, the rate of T1DM increased by nearly fourfold in one 10-year period in children of Asian origin moving to Great Britain, and the rate increased more than sevenfold in Polynesians migrating to New Zealand.[13,14] Genetic factors cannot explain such a rapid change.

Environmental and Dietary Risk Factors

Accumulating data indicate that abnormalities of the gut immune system and microbiome may play a fundamental role in the development of the immune attack on beta cells and the subsequent development of T1DM.[15] The intestinal immune system serves a vital role in processing the many food and microbial antigens to protect the body from infection and allergy. What appears to happen in the development of some cases of T1DM is the development of antibodies by the gut immune system that ultimately attack the beta cells. Possibly an underlying factor that may contribute to T1DM is poor protein digestion.

Poorly digested dietary proteins can cross-react with antigens on or within the beta cells of the pancreas. In humans, two proteins that have had the highest degree of incrimination are those found in milk (which contains bovine serum albumin and bovine insulin) and wheat (which contains gluten). For example, dietary bovine insulin differs from human insulin by only three amino acids. If a person develops antibodies to bovine insulin, there is a good chance that these antibodies will also attack their own insulin. In addition to causing antibody-mediated destruction of the beta cells, bovine insulin is able to activate T cells in those predisposed to diabetes in a manner that can also lead to beta-cell destruction by direct attack from T-killer cells.

Strong evidence implicates dietary factors like cow's milk and gluten as important triggers of the autoimmune process that leads to T1DM. In contrast, breastfeeding has been identified as an important factor in

establishing proper intestinal immune function and reducing the risk of T1DM. It is well known that breastfeeding confers a reduction in the risk of food allergies as well as better protection against both bacterial and viral intestinal infections. In case-controlled studies, patients with T1DM were more likely to have been breastfed for less than 3 months and to have been exposed to cow's milk or solid foods before 4 months of age. A critical review and analysis of all relevant citations in the medical literature indicate that early cow's milk exposure may increase the risk by about 1.5 times.[14,16] In addition, although the risk of diabetes associated with exposure to cow's milk was first thought to relate only to intake during infancy, additional studies showed that ingestion at any age may increase the risk of T1DM.

There is also considerable evidence that sensitivity to gluten—the major protein component of wheat, rye, and barley—may also play a role. Gluten sensitivity produces celiac disease, another autoimmune disorder. Celiac disease, like T1DM, is associated with abnormalities in intestinal immune function. And as in the case of diabetes, breastfeeding appears to have a preventive effect, whereas the early introduction of cow's milk is believed to be a major causative factor. The risk of developing T1DM is higher in children with celiac disease. Not surprisingly, the highest level of antibodies to cow's milk proteins is found in people with celiac disease.[17]

Enteroviruses and Type 1 Diabetes

Population-based studies, as well as prospective studies, have strengthened the hypothesis that T1DM can be the result of viral infection both during pregnancy and after birth.[18,19] A working theory in this regard is that the immune system becomes slightly confused as to which proteins to attack—the food-based ones such as those from dairy or gluten or the similar proteins on the pancreatic beta cells or insulin. When the person then has a viral infection, the increased stimulation of the immune system is the key that prompts it to become more active, and those confused immune cells begin to damage the pancreas. Gastrointestinal infections due to enteroviruses (e.g., polioviruses, coxsackieviruses, echoviruses) and rotavirus are common, especially in children. All of these viruses replicate in the gut and stimulate the intestinal immune system, which may then activate the insulin-specific immune cells to seek out and destroy beta cells. These viruses and others are also capable of infecting pancreatic beta cells, causing the leukocytes to attack and destroy the beta cells in an attempt to kill the virus. Gastrointestinal viral infections may also increase intestinal permeability and enhance the antibody response to dietary bovine insulin as a result of increased absorption of the intact protein. The severe "leaky gut"—or increased permeability of the small intestine that occurs during and for some time after rotavirus infections (one of the most common causes of acute diarrheal illness in children)—exposes the gut-associated immune cells to large quantities of intact protein.

Vitamin D Deficiency

Cod liver oil may offer significant protection against the development of diabetes because of its high content of vitamin D. The use of cod liver oil became popular during the 1890s to treat rickets, a vitamin D–deficiency disease characterized by an inability to calcify the bone matrix, resulting in softening of the skull bones, bowing of the legs, spinal curvature, and enlarged joints. Beginning in the 1930s, vitamin D was added to milk at a level of 100 IU per 8 oz. As a result, rickets is now uncommon in most developed countries.

Emerging evidence indicates that vitamin D supplementation from cod liver oil and other sources during early childhood can prevent not only rickets but also T1DM.[20] In fact, vitamin D fortification may offset some of the "diabetogenic" effect of cow's milk, but the dosage level in milk may not be sufficient to do so; the level that was shown to be protective was about 2000 IU—much higher than the amount typically ingested from the consumption of vitamin D–fortified milk.

The most extensive study looking at vitamin D and T1DM enrolled all pregnant women in northern Finland who were due to give birth in 1966 (more than 12,000 women), and their children were then monitored until December 1997.[21] Final analysis of 10,366 enrollees demonstrated that children who regularly took vitamin D, primarily from cod liver oil, had an 80% reduced risk of developing T1DM, whereas those who had a vitamin D deficiency actually had a 300% increased risk of developing the disease. One study found that the use of vitamin D from cod liver oil during pregnancy significantly reduced the frequency of T1DM in the offspring.[22] Furthermore, studies looking at vitamin D status in the blood of newly diagnosed individuals with T1DM have found much lower levels of the vitamin in these patients than in healthy controls. Because vitamin D can be produced in the body by the action of sunlight on the skin, lack of sun exposure during childhood may also play a role and partially explain the higher T1DM rates in northern countries. Vitamin D in recent research has been shown to prevent autoimmune conditions, including those that attack beta cells, from developing in the body, and observational studies have shown a dose-dependent degree of protection.[23]

This research indicates that ensuring adequate vitamin D supplementation during pregnancy and early childhood may reduce the risk of T1DM. Vitamin D is important for the normal development of the immune system. In addition, it has been shown that vitamin D inhibits some of the autoimmune reactions that target the beta cells.

Omega-3 Fatty Acid Deficiency

In addition to the strong case that can be made for vitamin D as a protective factor, an equally strong case can be made for the benefits of the omega-3 fatty acids in cod liver oil and other fish oils. Human studies have shown that when essential fatty acids (EFAs) are given, the onset of T1DM was significantly reduced. Also, higher levels of n-3 polyunsaturated fatty acids in RBCs have also been associated with reduced risk.[24] For one thing, cod liver oil also provides both EPA and DHA, which are vital EFAs in humans. Other studies support the benefit of supplementing EFAs in pregnant women and children. The mechanisms responsible for this effect may be related to improved cell membrane function, leading to enhanced antioxidant status and the reduced formation of inflammatory compounds called cytokines.[25]

Nitrates

Clear links between increased levels of nitrates (from both dietary sources and water) and an increased rate of T1DM have been established. Nitrates are produced by agricultural runoff from fertilizers and are found in cured or smoked meats such as ham, hot dogs, bacon, and jerky to keep the food from spoiling. Nitrates react within the body to form compounds known as nitrosamines. (Note: The U.S. Department of Agriculture [USDA] requires all manufacturers of processed meats to add vitamin C to their products to prevent the formation of nitrosamines.) Nitrates and nitrosamines are known to cause diabetes in animals. Infants and young children are believed to be particularly vulnerable to the harmful effects of nitrate exposure.

One of the most alarming features of T1DM is that it is becoming much more prevalent, with a current growth rate of 3% per year worldwide. Some areas have been hit particularly hard, such as Finland, Great Britain, Canada, and the United States. Increased nitrate exposure may be a key factor; the nitrate levels in ground and surface waters of agricultural regions have increased over the past 40 years owing to the use of nitrogen fertilizers. Nitrate contamination occurs in geographical patterns related to the amount of nitrogen contributed by fertilizers, manure, and airborne sources such as automobile and

industrial emissions. Nitrate exposure may explain why some geographical pockets have substantially higher rates of T1DM.[26,27]

Circumstantial evidence from population-based studies also suggests that a higher dietary intake of nitrates from smoked/cured meats is associated with a significantly higher risk for T1DM. These foods severely stress body defense mechanisms and are to be avoided. The habit of feeding children hot dogs, cold cuts, and ham would be a good one for parents to break. Health food stores now carry nitrate-free alternatives to these toxic food choices. Also, investing in a high-quality water purifier is good insurance against ingesting nitrate-contaminated drinking water.

EARLY TREATMENT AND POSSIBLE REVERSAL OF TYPE 1 DIABETES

Early intervention in T1DM designed to affect the autoimmune or oxidative process theoretically may be capable of lengthening the "honeymoon" phase or even completely reversing the process. This goal appears to have two candidates: niacinamide and epicatechin. Removing gluten and dairy from the diet and supporting gut health as well as immune system balance are also important considerations.

Niacinamide

Niacinamide, also called nicotinamide, has been shown to prevent some of the immune-mediated destruction of the pancreatic beta cells and may actually help reverse the process in some patients.[28,29] Observations that niacinamide can prevent the development of T1DM in experimental animals led to several pilot clinical trials that initially confirmed these observations and suggested that if given soon enough at the onset of diabetes, niacinamide could help restore beta cells or at least slow down their destruction. In one of the first pilot studies of people newly diagnosed with T1DM, seven patients were given 3 g of niacinamide daily, and nine were given a placebo. After 6 months, five patients in the niacinamide group and two in the placebo group were still not taking insulin and had normal blood glucose and $HgbA_{1c}$. At 12 months, three patients in the niacinamide group but none in the placebo group were in clinical remission.[30]

The results of this pilot study and others suggest that niacinamide can prevent T1DM from progressing in some patients if given soon enough at the onset of diabetes by helping restore beta cells. As of 2004, there had been 12 studies of niacinamide treatment in recent-onset T1DM or T1DM of less than 5 years' duration and residual beta-cell mass. Ten of these were double-blind placebo-controlled studies, of which half showed a positive effect compared with placebo in terms of prolonged non–insulin-requiring remission, lower insulin requirements, improved metabolic control, and increased beta-cell function as determined by secretion of a substance known as C-peptide. The main differences between the positive and negative studies in recent-onset T1DM were older age and higher baseline fasting C-peptide in positive studies.[31–34]

Although some of the studies have shown positive results, it is important to point out that two large studies designed to evaluate the effectiveness of niacinamide in preventing the development of T1DM in high-risk individuals—such as siblings of children who developed T1DM or in individuals who already show elevations in antibodies directed against the beta cells—did not show niacinamide to be effective. The first of these, the German Nicotinamide Intervention Study, did not show much of an effect with 1.2 g of niacinamide daily, and results from the larger study, the European Nicotinamide Diabetes Intervention Trial, did not show benefit with dosages as high as 3 g a day.[35,36] A possible shortcoming of these studies was the choice of a timed-released niacinamide. It is possible that such a formulation did provide the peak levels of niacinamide required to block autoimmune mechanisms such as cytokine production.[37]

In the best-case scenario, niacinamide will likely work for only a few individuals with T1DM of recent onset. Nonetheless, the fact that some patients have had a complete reversal of their disease makes a trial of niacinamide worth the effort, especially because there is currently no reasonable alternative.

The dosage recommendation is based on body weight: 25 to 50 mg of niacinamide for every 2.2 lb of body weight or a maximum dosage of 3 g/day in divided doses. Niacinamide is generally well tolerated and without side effects. In fact, no side effects have been reported in clinical trials in T1DM. It does not cause the flushing of the skin characterizing high dosages of niacin. However, because it could possibly harm the liver, a blood test for liver enzymes should be performed every 3 months to rule out liver damage.

Epicatechin

The second natural compound that may offer benefit is epicatechin. The line of research on its potential role in T1DM of recent onset began with examining the bark of the Malabar kino tree *(Pterocarpus marsupium)*. This botanical medicine has a long history of use in India as a treatment for diabetes. Initially, epicatechin extracted from the bark was shown to prevent beta-cell damage in rats. Further research indicated that both epicatechin and a crude alcohol extract of *P. marsupium* were actually able to promote the regeneration of functional pancreatic beta cells in diabetic animals.[38]

Green tea *(Camellia sinensis)* extract appears to be a better choice than extracts of *P. marsupium* because the epicatechin content in a high-quality green tea extract is actually higher than that found in extracts of *P. marsupium.* Second, green tea extract exerts a broader range of beneficial effects. Green tea polyphenols also exhibit significant antiviral activity against rotavirus and enterovirus—two viruses suspected of causing T1DM.[39] Last, green tea extract is considerably easier to find commercially than *P. marsupium.* Recommended dosages for children below age 6 is 50 to 150 mg; for those 6 to 12 years old, 100 to 200 mg; and for children older than 12 years old and adults, 150 to 300 mg. The green tea extract should have a polyphenol content of 80% and be decaffeinated.

Although the focus in the research has been on the reversal of T1DM, epicatechin may have more meaningful effects in prevention; epicatechin effectively prevented T1DM in nonobese diabetic (NOD) mice. At 32 weeks of age, 66.7% of control mice had overt diabetes, whereas only 16.6% of epicatechin-treated mice became diabetic. Consistently, epicatechin-treated mice had significantly higher plasma insulin levels but lower glycosylated hemoglobin concentrations compared with control mice. Treatment with epicatechin elevates circulating anti-inflammatory cytokine interleukin-10 levels, ameliorates pancreatic insulitis, and improves pancreatic islet mass—all important factors that may help prevent T1DM by modulating immune function and thereby preserving islet mass.

RISK FACTORS IN TYPE 2 DIABETES

Several factors are involved in the development and progression of diabetes. The most well-accepted major risk factor for T2DM is obesity or, more precisely, excess body fat. Approximately 80% to 90% of individuals with T2DM are obese (a body mass index above 30). When adipocytes, particularly those around the abdomen, become full of fat, they secrete several biological products (e.g., resistin, leptin, tumor necrosis factor, free fatty acids, cortisol) that dampen the effect of insulin, impair glucose utilization in skeletal muscle, promote glucose production

by the liver, and impair insulin release by pancreatic beta cells. Also important is that as the number and size of adipocytes increase, there is a reduction in the secretion of compounds that promote insulin action, including a novel protein produced by fat cells known as adiponectin. Adiponectin is associated not only with improved insulin sensitivity but also with anti-inflammatory activity; moreover, it lowers triglycerides and blocks the development of atherosclerosis, or hardening of the arteries. The net effect of these negative actions of fat cells is that they severely stress blood glucose control mechanisms while also leading to the development of the major complication of diabetes: atherosclerosis. Because of all these newly discovered hormones secreted by adipocytes, many experts now consider the adipose tissue a member of the endocrine system (e.g., the pituitary, adrenals, and thyroid).[40]

Measurements of blood levels of adiponectin and other hormones secreted by fat cells may turn out to be the most meaningful predictors of the likelihood of developing T2DM as well as gestational diabetes.[41,42]

In the early stages of the increased metabolic stress produced by the various secretions of adipocytes and the lack of adiponectin, blood glucose levels remain normal despite insulin resistance because pancreatic beta cells compensate by increasing insulin output. As metabolic stress increases and insulin resistance becomes more significant, the conventional explanation is that eventually the pancreas cannot compensate and elevations in blood glucose levels develop. As the disease progresses from insulin resistance to full-blown diabetes, the pancreas starts to "burn out" and produces less insulin. Avoiding this occurrence is a key therapeutic goal and is achievable with good diabetes care and if a patient's $HgbA_{1c}$ remains at 5.7 or less. (See Box 165.2 for risk factors.)

Genetics of Type 2 Diabetes and Obesity

In studies of identical twins, the concordance rate was between 70% and 90% for T2DM. This high concordance points to a strong genetic relationship. Data from family studies also provide additional support: children with one parent with T2DM have an increased risk of developing diabetes at some point in their lives. If both parents have the disease, the risk in offspring is nearly 40%. However, even with the strongest predisposition, diabetes can be avoided in most cases.[43]

The Case of the Pima Indians

The Pima Indians of Arizona have the highest rate of T2DM and obesity anywhere in the world. Research has demonstrated a strong genetic predisposition, but even with this strong tendency, the high rate of T2DM in this group is clearly related to diet and lifestyle. The Pima Indians living traditionally in Mexico still cultivate corn, beans, and potatoes as their main staples, plus a limited amount of seasonal vegetables and fruits such as zucchini squash, tomatoes, garlic, green pepper, peaches, and apples. The Pimas of Mexico also make heavy use of wild and medicinal plants in their diet. They work hard, have no electricity or running water in their homes, and walk long distances to bring in drinking water or wash their clothes. They use no modern household devices; consequently, food preparation and household chores require extra effort from the women. In contrast, the Pima Indians of Arizona are largely sedentary and follow the dietary practices of typical Americans. The results are astounding. Although roughly 16% of U.S. Native Americans have T2DM, 50% of Arizona Pimas have T2DM, and 95% of them are overweight or obese. T2DM is a rarity among the Mexican Pimas, and only about 10% can be classified as obese. The average difference in body weight between the Arizona and Mexican Pima men and women was more than 60 lb.[44]

Further evidence that diet and lifestyle appear to be able to overcome even the strongest genetic predisposition is shown by some of the intervention studies among Pima Indians. When these people were placed on a more traditional diet along with physical exercise, their blood glucose levels improved dramatically, and they lost weight. The focus right now, by various medical organizations such as the National Institute of Health, in dealing with the epidemic of diabetes and obesity among the Pima Indians is to educate children on the importance of exercise and dietary choices to reduce diabetes risk.

Other Genetic and Racial Factors

Other racial and ethnic groups beside Pima Indians that have a higher tendency to develop T2DM include other Native Americans, African Americans, Hispanic Americans, Asian Americans, Australian Aborigines, and Pacific Islanders. It is important for all these higher-risk groups to learn that when they follow the traditional dietary and lifestyle practices of their original cultures, their rates of diabetes will be extremely low. It appears that these groups are simply highly sensitive to the "Western diet" and lifestyle.

Diet, Exercise, Lifestyle, and Diabetes Risk

Findings from the U.S. government's Third National Health and Nutrition Examination Surveys (NHANES) make it clear that diabetes is a disease of diet and lifestyle. Of individuals with T2DM, 69% did not exercise at all or did not engage in regular exercise, 62% ate fewer than five servings of fruits and vegetables per day, 65% consumed more than 30% of their daily calories from fat and more than 10% of total calories from saturated fat, and 82% were either overweight or obese.[45]

Insights into the independent role of the modern lifestyle versus diet and obesity in the development of T2DM can be gleaned from the Old Order Amish. These 30,000 or so individuals, whose ancestors arrived on U.S. shores in the 18th century, maintain religious and cultural beliefs that preclude regular use of modern conveniences such as electrical appliances, telephones, and cars, and they have a physically active lifestyle. By comparison, the 200 million typical Americans living alongside them have, over the past 250 years, willingly adopted the advances of modern technology, making life less physically demanding.

Although the typical Amish diet and rate of obesity do not differ from those of the typical American, the rate of diabetes among the Amish is considerably less—about 50% lower. Although the percentage of Amish with impaired glucose tolerance (prediabetes) is about the same as in other whites in America, apparently not as many Amish go on to develop diabetes. This suggests that physical activity has a

BOX 165.2 Risk Factors for Type 2 Diabetes Mellitus

- Family history of diabetes (i.e., parent or sibling with type 2 diabetes)
- Obesity
- Increased waist-to-hip ratio
- Age: increasing age is associated with increased risk, beginning at age 45
- Race/ethnicity (e.g., African American, Hispanic American, Native American/Canadian, Native Australian or New Zealander, Asian American, Pacific Islander)
- Previously identified impaired fasting glucose or impaired glucose tolerance
- History of gestational diabetes (diabetes during pregnancy) or delivery of a baby weighing more than 9 lb
- Hypertension (blood pressure >140/90 mm Hg)
- Triglyceride level >250 mg/dL
- Low levels of adiponectin, elevated levels of fasting insulin
- Polycystic ovary syndrome (to be considered in any adult woman who is overweight with both acne and infertility)

protective effect against T2DM independent of obesity or percentage of body fat.[46,47]

Results from other studies corroborate this hypothesis. Lifestyle changes alone are associated with a 58% reduced risk of developing diabetes among those at high risk because they show evidence of impaired glucose tolerance (as based on results from the Diabetes Prevention Program—a large intervention trial of more than 1000 subjects). The two major goals of the program were a minimum of 7% weight loss/weight maintenance and a minimum of 150 min/week of physical activity similar in intensity to brisk walking.[48]

A Diet High in Refined Carbohydrates

Dietary carbohydrates play a central role in the causes, prevention, and treatment of T2DM. In an effort to qualify carbohydrate sources as acceptable or not, two indices have been developed: the glycemic index (GI) and glycemic load (GL). The GI is a numerical value that expresses the rise in blood glucose after a particular food is eaten. The standard value of 100 is based on the rise seen with the ingestion of glucose. The GI ranges from about 20 for fructose and whole barley to about 98 for a baked potato. The insulin response to carbohydrate-containing foods is similar to the rise in blood sugar. The GI is often used as a guideline in dietary recommendations for people with either diabetes or hypoglycemia. In addition, eating foods with a lower GI is associated with a reduced risk for obesity and diabetes.[49–51]

One of the shortcomings of the GI is that it tells us only about the quality of the carbohydrates, not the quantity. Obviously, quantity matters too, but measurement of the GI of a food is not related to portion size. That is where the GL comes into play. The GL takes the GI into account but provides much more accurate information than the GI alone. The GL is calculated by multiplying the amount of carbohydrate in a serving of food by that food's GI (compared with glucose) and then dividing it by 100. The higher the GL, the greater the stress on insulin. In Appendix 7, we provide the GI and GL for many common foods.

Research studies are just starting to use the GL as a more sensitive marker for the role of diet in chronic conditions like diabetes and heart disease. The preliminary results are showing an even stronger link in predicting diabetes than the one shown for the GI.[49,51] Researchers are also showing that a high-GL diet is also associated with an increased risk for heart disease. For example, when researchers from the Nurse's Health Study used GL measures to assess the effect of carbohydrate consumption on women, they found that high-GL diets (and, by extension, high-GI foods and greater total carbohydrate intake) correlated with even more significantly greater risk for heart disease than the GI because of lower levels of protective HDL-C and higher triglyceride levels.[52] Increased risk for diabetes and heart disease started, on average, at a daily GL of 161. Therefore we recommend using the information in Appendix 7 to help determine how to prevent the total daily GL from exceeding 150. Keep in mind that the GL is based on the stated serving size; the larger the serving size, the greater the GL.

The Importance of Dietary Fiber in Reducing the Risk of Developing Diabetes

Population studies, as well as clinical and experimental data, show diabetes to be one of the diseases most clearly related to an inadequate intake of dietary fiber. Different types of fiber possess different actions. The type of fiber that exerts the most beneficial effects on blood sugar control is the water-soluble form. Included in this class are hemicelluloses, mucilages, gums, and pectins. These types of fiber are capable of slowing down the digestion and absorption of carbohydrates, thereby preventing rapid rises in blood sugar. They are also associated with increasing the sensitivity of tissues to insulin and improving the uptake of glucose by the muscles, liver, and other tissues, thereby preventing a sustained elevation of blood sugar.[53,54]

Particularly good sources of water-soluble fiber are legumes, oat bran, nuts, seeds, psyllium seed husks, pears, apples, and most vegetables. Large amounts of plant foods must be consumed to obtain adequate levels of dietary fiber, although beans, peas, and legumes are overall the best sources for high fiber intake in relatively easy amounts to ingest. Even the simple change from white flour products to whole-grain versions is associated with a reduced risk for T2DM[55,56]; our recommendation is to consume at least 35 g of fiber a day from various food sources, especially vegetables. Fiber supplements can also be taken to achieve greater effects in lowering the GI.

The Wrong Types of Fats

Dietary fat also plays a central role in the likelihood of developing T2DM. Large controlled trials have shown that a reduction of fat intake as part of a healthy lifestyle, combined with weight reduction and exercise, reduces the risk for T2DM. However, more important than the amount of fat in the diet is the *type* of fat consumed.[57] The types of dietary fats linked to T2DM include saturated fats and trans fatty acids (partially hydrogenated vegetable oils) taken in large amounts along with a relative insufficiency of monounsaturated and omega-3 fatty acids.

One of the key reasons why dietary fats appear to be related to the risk for T2DM is that they determine cell membrane composition. That is, a "bad fat" pattern leads to reduced membrane fluidity, which in turn causes reduced insulin binding to receptors on cellular membranes, reduced insulin action, or both. Particularly harmful to cell membrane function are margarine, vegetable oil shortening, and other foods containing trans fatty acids and partially hydrogenated oils. These fatty acids interfere with the body's ability to use important essential fatty acids (EFAs). One study estimated that by substituting polyunsaturated vegetable oils for margarine containing partially hydrogenated vegetable oil, the likelihood of developing T2DM could be reduced by 40%.[58]

In contrast to the dampening of insulin sensitivity caused by margarine and saturated fats, clinical studies have shown that monounsaturated fats and omega-3 oils improve insulin action.[59] Adding further support is the fact that population studies have also indicated that the frequent consumption of monounsaturated fats such as olive oil, raw or lightly roasted nuts and seeds, nut oils, and omega-3 fatty acids from fish protect against the development of T2DM. Healthy omega-3 fish include wild salmon, trout, sardines, halibut, and herring. All of this evidence indicates that altered cell membrane composition and fluidity play a critical role in the development of T2DM.

One of the most useful food groups to reduce the risk of T2DM is nuts. Studies have shown that consumption of nuts is inversely associated with the risk of T2DM, independent of known risk factors for T2DM, including age, obesity, family history of diabetes, physical activity, smoking, and other dietary factors.[60] In addition to providing beneficial monounsaturated and polyunsaturated fats that improve insulin sensitivity, nuts are also rich in fiber and magnesium and have a low GI. Higher intakes of fiber and magnesium and foods with a low GI has been associated with a reduced risk of T2DM in several population-based studies. Eating mostly raw or lightly roasted fresh nuts and seeds rather than commercially roasted and salted nuts and seeds should be advocated.

Low Intake of Antioxidant Nutrients

Cumulative free-radical damage leads to cellular aging and is a major factor contributing to T2DM as well as many other chronic degenerative diseases. Several large population-based studies have shown that

the higher the intake of fruit and vegetables, the better blood glucose levels are controlled and the lower the risk for T2DM.[61] Many factors could explain this inverse correlation. Fruits and vegetables are good sources of fiber and also provide many nutrients and antioxidants. Even something as simple as the regular consumption of salads is associated with a reduced risk for T2DM.[62]

Studies looking at levels of individualized antioxidants have also shown similar inverse correlations—the higher the level of vitamin C, vitamin E, or carotenes, for example, the lower the risk for T2DM.[63–65] Likewise, the lower the levels of antioxidants and higher the levels of fats damaged by free radicals (lipid peroxides), the greater the risk for T2DM.[66] In one study, 944 men 42 to 60 years of age were followed closely for 4 years. None of them had diabetes at the beginning of the study. At the end of this time, 45 men had developed diabetes. The researchers found that a low vitamin E concentration was associated with 3.9-fold (390%) increased risk for T2DM in the study subjects.[67]

Free Radicals and Diabetes

One of the hallmark features of T2DM is the presence of higher levels of free radicals and prooxidants,[68] particularly an increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[69] These compounds are also activated by high blood glucose and elevated levels of saturated fat and, as already mentioned, are produced in the abdominal fat cells of individuals who are overweight or obese.

These compounds greatly stress antioxidant mechanisms; they directly oxidize and damage cellular components such as DNA, proteins, and cell membrane fatty acids. In addition to their ability to directly inflict damage on these structures, ROS and RNS indirectly induce damage to tissues by activating several inflammatory compounds, such as nuclear factor-kappa B, which ultimately leads to both insulin resistance and impaired insulin secretion.

Environmental Toxins

Persistent Organic Pollutants

Persistent organic pollutants (POPs) include such chemical compounds as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), hexachlorobenzene (HCB), organophosphates, dichlorodiphenyldichloroethylene (DDE), and bisphenol A. These compounds have been linked to the development of T2DM. In addition, research indicates that the body load of POPs is not only a significant predictor of T2DM but may also be a more significant risk factor than obesity.[70]

Individuals in the top quintile of exposure to six common POPs have a 37.7-fold increased risk of diabetes—much stronger than any other known risk factor. Because many POPs block insulin receptor sites, decrease glucose transporter type 4 (GLUT-4) activity in muscles, and decrease insulin production, a causal relationship appears highly probable. Beginning in the 1960s, the production of synthetic organic chemicals began to escalate along with the incidence of diabetes (Fig. 165.1). The total load of toxicants may be the strongest contributing factor in the development of diabetes, with data so compelling that some researchers now label these toxicants as "diabetogens."

More convincing is the correlation between body load of POPs and the risk of metabolic syndrome, as shown in Fig. 165.2. The association is synergistic. When the relationship between POP levels and diabetes risk is examined, the case becomes even more compelling. Those in the top 10% of transnonachlor level, a common termiticide used in North America for decades, have a remarkable twelvefold increased risk of developing diabetes. Those with levels of organochlorine pesticides in the top quartile have an odds ratio of 5.3 for metabolic syndrome.[71]

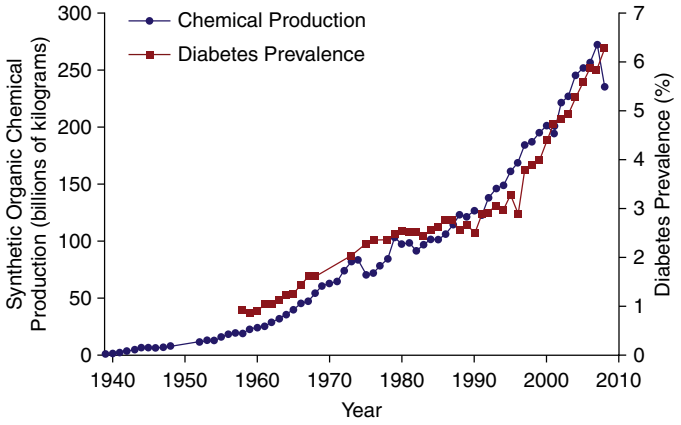


Fig. 165.1 The diabetes epidemic correlates with the release of persistent organic pollutants (POPs) into the environment. (From Ionnou GN, Bryson CL, Boyko EJ. Prevalence and trends of insulin resistance, impaired fasting glucose, and diabetes. *J Diabetes Complications.* 2007;21[6]:363–370.)

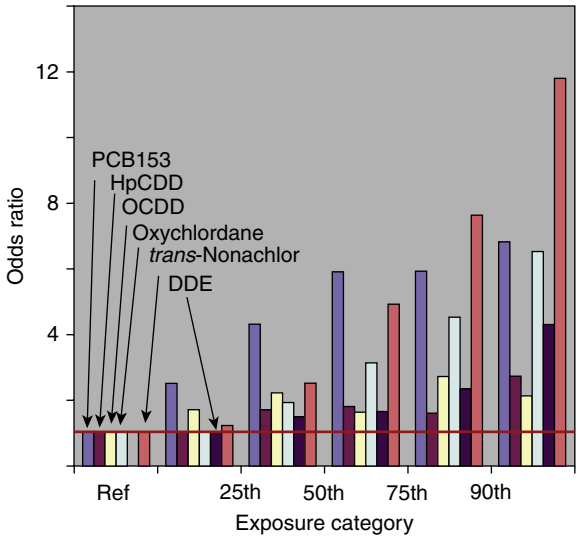


Fig. 165.2 Diabetes risk (odds ratio [OR]) for individual persistent organic pollutants (POPs) according to percentiles. *Blue rectangle,* PCB153; *red rectangle,* HpCDD; *yellow rectangle,* OCDD; *green rectangle,* oxychlordane; *pink rectangle,* trans-nonachlor; *purple rectangle,* DDE. Joseph Pizzorno, ND, Is the Diabetes Epidemic Primarily Due to Toxins?, *Integr Med (Encinitas).* 2016;15(4):8–17.

Unfortunately, direct measurement of POP levels is difficult and very expensive. However, a good indirect measure is gamma-glutamyltransferase (GGTP). An elevated level of GGTP is a strong predictor of diabetes risk. Those with levels above 40 IU/L have a twentyfold increased risk.[72]

Arsenic

Arsenic exposure occurs primarily through diet and water. A surprising 13 million people in the United States use public water that exceeds the Environmental Protection Agency (EPA) limit of 10 ug/L. There is a direct correlation between the amount of arsenic in a person's body and the risk of diabetes. In this case the primary mechanism appears to be the result of damaged pancreatic beta cells with resultant decrease insulin production.[73]

Arsenic has been shown to increase diabetes risk in a dose-dependent fashion. Comparing participants at the 80th versus the 20th percentiles, the odds ratios (ORs) for T2DM were 3.58 for the total level of arsenic, 1.57 for dimethylarsenate, and 0.69 for arsenobetaine.[74]

Bisphenol A and Phthalates

Higher urinary bisphenol A (BPA) concentrations are associated with type 2 diabetes, with an OR of 1.39 per 1-standard-deviation increase in BPA concentration.[75] BPA blocks insulin receptor sites resulting in insulin resistance. This increases the incidence of diabetes as well as obesity, especially the accumulation of visceral fat. The threshold for doubling the risk for diabetes is 5.0 ug/L urine.

Phthalates, such as di-2-ethyl-hexl phthalate, diethyl phthalate, dibutyl phthalate, dimethyl phthalate, dibenzyl phthalate, and diisononyl phthalate, are associated with the development of T2DM and obesity by interfering with various cell-signaling pathways involved in weight and glucose homeostasis.[76] Mitochondrial inhibition by phthalates likely contributes significantly to their role in obesity and diabetes.

Ambient Air Pollutants

A 3+ year study of overweight and obese Latino children from Los Angeles, California, showed significant effects of elevated NO_2 and particulate matter (PM) with an aerodynamic diameter of less than 2.5 ($PM_{2.5}$) on insulin homeostasis and beta-cell function that were independent of body fat percentage.[77] Epidemiological studies have also shown that greater exposure to NO_2 and $PM_{2.5}$ is associated with a greater risk for T2DM in adults.[78]

Lifestyle Management Versus Drugs to Prevent Type 2 Diabetes

Several well-designed large trials have shown that lifestyle and dietary modifications can be used to effectively prevent T2DM. That fact has not dissuaded drug companies from sponsoring studies attempting to prevent diabetes with drugs. However, the degree of prevention with drugs pales in comparison with that of diet and lifestyle. For example, in one of the most celebrated studies, 3234 subjects with impaired glucose tolerance (prediabetes) were randomly assigned to be in a group receiving a placebo, the blood glucose–lowering drug metformin (850 mg twice daily), or a lifestyle modification program with the goals of at least a 7% weight loss and at least 150 minutes of physical activity per week. The average follow-up was 2.8 years. The incidence of diabetes was 11, 7.8, and 4.8 cases per 100 person-years in the placebo, metformin, and lifestyle groups, respectively. The lifestyle intervention reduced the incidence of diabetes by 58% and metformin by 31% compared with placebo. Clearly, the lifestyle intervention was significantly more effective than metformin—a drug with sometimes serious side effects.[79]

Environmental Toxicity

Given the fact that environmental pollutants can increase the risk of developing T2DM, steps can be taken to reduce patients' exposure to them. This can be done, for example, by eating organic foods, using natural cleaning agents in the home, and avoiding chemical pesticides. These are all valid steps in preventing environmental toxins undermining the regulation of insulin.

CLINICAL MONITORING OF DIABETES

Knowledge and awareness are the greatest allies of people with diabetes. An individual with diabetes who makes a strong commitment to learning about his or her condition and accepts the lead role in a carefully supervised monitoring program that breaks away from the standard recommended by the American Diabetes Association (ADA) greatly improves the likelihood that he or she will lead a long and healthy life. On the other hand, individuals who remain blissfully ignorant about their disease and refuse to undergo regular testing or self-monitoring are far more likely to face years of unnecessary suffering and, more often than not, catastrophic health problems.

Unless it is properly managed and supervised, diabetes can be viewed as a state of biochemical and hormonal anarchy that will lead to organ injury and accelerated aging. Many of the complex control systems that faithfully govern and protect the body are damaged in the diabetic individual. For such a person to regain control, he or she must learn how to maintain an intimate awareness of blood sugar, risk factors for atherosclerosis (hardening of the arteries), blood pressure, body mass index, level of fitness, and other factors that determine the risk of developing diabetic complications and experiencing an erosion of his or her quality of life.

Fortunately, diabetic patients who do develop a keen awareness of these risk factors through regular testing and a properly supervised self-monitoring program are also those who are much more likely to benefit from changes in lifestyle: diet, supplements, and when necessary, medications.

Urinary Glucose Monitoring

The measurement of glucose in the urine is now entirely passé. Until the mid-1970s, the only option that diabetic patients had to monitor blood glucose was indirectly through urine glucose testing. Normally, the kidneys are able to conserve all of the glucose in the blood that they constantly filter. However, if blood glucose gets too high, the kidneys become unable to conserve all of the glucose, which then begins to appear in the urine. Because the average diabetic patient's kidneys can completely conserve glucose until the blood glucose reaches about 200 to 250 mg/dL (10 mmol/L), a negative urine glucose reading indicates that the blood glucose since the time of the previous voiding has been less than 200 to 250 mg/dL (10 mmol/L). Therefore the measurement of glucose in the urine is only a crude measurement of blood glucose control and is completely worthless in detecting severe hypo- or hyperglycemia.[80] Thus urinary glucose monitoring is of little value in determining the success of blood glucose control, and it does not provide adequate feedback when lifestyle, diet, or other treatments are adjusted. These days, all diabetic patients should own a glucometer and know how to test their own blood glucose levels.

Urinary Ketone Testing

In any circumstance when the body must derive its primary source of energy from fat, ketones are produced as a by-product. If the level of ketone production is high enough, ketones appear in the urine. In the patient with T1DM or T2DM who cannot produce any innate insulin, ketones appear in the urine when there is a severe deficiency in or activity of insulin. In general this is associated only with T1DM because the vast majority of patients with T2DM do not develop ketoacidosis. This can occur if a patient with insulin-dependent diabetes accidentally or purposefully forgets to take insulin. It can also occur when such a patient becomes ill or injured or is given high doses of cortisone-related drugs. All of these phenomena may result in a severe loss of insulin effectiveness, resulting in the cells' inability to take up and use glucose. In such circumstances, blood glucose rises to high levels, high amounts of fat are used by cells that cannot take in glucose, and the blood becomes polluted with toxic levels of acidic ketones. Severe dehydration occurs rapidly because the kidneys are unable to conserve water in the presence of such extraordinary levels of blood glucose. This dangerous state is referred to as diabetic ketoacidosis, and it must be treated as a medical emergency, usually necessitating intravenous

BOX 165.3 Optimal Range for Self-Monitored Blood Glucose

- Fasting or before meals: 80 to 110 mg/dL (4.4–6.7 mmol/L)
- 2 hours after eating (postprandial): <140 mg/dL (7.8 mmol/L)
- At bedtime: 100 to 140 mg/dL (5.6–7.8 mmol/L)
- Note that these are whole-blood values that typically run 10 mg/dL (0.6 mmol/L) higher than serum values. To avoid confusing numbers, some home glucose-monitoring kits, even those using whole-blood samples, are now calibrating to serum levels. Check the glucose monitor's documentation to find out if it is set up to determine whole-blood or serum glucose levels.
- Slightly higher values may be acceptable in the elderly or in young children because of their higher risk of developing dangerous hypoglycemia.

BOX 165.4 Optimal Schedule for Self-Monitored Blood Glucose

1. Test on awakening and just before each meal. Ideal blood sugar before meals is <120 mg/dL (6.7 mmol/L).
2. Test 2 hours after each meal. Ideal blood sugar 2 hours after meals is <140 mg/dL (7.7 mmol/L).
3. Test at bedtime. Ideal blood sugar at bedtime is <140 mg/dL (7.7 mmol/L).

insulin, large amounts of intravenous fluids, and careful monitoring, usually in an intensive care unit. Ignoring ketoacidosis can rapidly lead to death.

Because of the events just outlined, the testing of urine or, even better, blood for ketones remains an important part of monitoring only in patients with T1DM who have no remaining pancreatic function. The presence of urine or blood ketones accompanied by high blood sugar readings can be interpreted to determine how far along the ketoacidosis has developed and what type of medical attention is required. For this reason, all patients with T1DM should test their urine for ketones frequently during acute illness or severe stress, especially when blood glucose levels are consistently elevated (>300 mg/dL [16.7 mmol/L]); regularly during pregnancy; or when symptoms suggestive of ketoacidosis, such as nausea, vomiting, or abdominal pain, are present.

Self-Monitoring of Blood Glucose

Since its introduction, self-monitoring of blood glucose (SMBG) has revolutionized the management of diabetes.[81] The publication of the landmark Diabetes Control and Complications Trial,[82] which examined intensive glucose control in people with T1DM, and the United Kingdom Prospective Diabetes Study,[83] which examined intensive glucose control in those with T2DM, scientifically proved that the most important factor in determining the long-term risk of serious diabetic complications in both types of diabetes is blood glucose control. Patients who do not maintain vigilant awareness of their blood glucose and who do not make every effort to keep their blood sugar under tight control can expect a significant increase in their risk of serious health problems such as eye, kidney, and heart disease as well as a whole host of other problems, including depression, fatigue, impotence, and chronic infections. SMBG is important for a number of reasons[84]:

1. Modifications of treatment to achieve appropriate blood glucose control
2. Detection and diagnosis of hypoglycemia
3. Adjusting care in response to daily life circumstances (e.g., food intake, exercise, stress, illness)
4. Detection and treatment of severe hyperglycemia
5. Increasing compliance with therapy (helps combat apathy and denial)
6. Improvement in motivation because of immediate positive and negative feedback (Boxes 165.3 and 165.4)

Type 1 Diabetes and Self-Monitoring of Blood Glucose

Without question, all individuals with T1DM must monitor their blood glucose frequently if they want to achieve and maintain good health. In the absence of diabetes, the pancreas monitors blood glucose continuously and adjusts its insulin output depending on moment-by-moment changes. To achieve blood glucose levels that are consistently as close to normal as possible, those with T1DM must replicate this natural situation as closely as possible. This means that they must monitor their blood glucose frequently, and they must learn to use this information to make ongoing adjustments to their insulin injections, diet, and exercise.

Intensive insulin therapy allows a diabetic patient to achieve near-normal levels of blood glucose, along with enjoying improved lifestyle flexibility. With conventional, infrequent insulin injections, the diabetic patient must structure meals and other aspects of lifestyle around his or her injections or face serious abnormalities of blood glucose. On the other hand, with intensive insulin therapy that relies on rapid-acting, short-duration insulin or the use of an insulin pump (an electronic device that provides a continuous injection of short-acting insulins with extra boluses before meals), the timing and size of doses can be adjusted to suit the events of the day.[85] Even though it may involve multiple injections (usually before each meal and often at bedtime) and blood glucose measurements up to six times or more each day, intensive insulin therapy results in greater dietary and lifestyle freedom, a higher quality of life and well-being, and near nondiabetic blood glucose control, which is vital for long-term health.

Type 2 Diabetes and Self-Monitoring of Blood Glucose

Self-monitoring of blood glucose has an important place in the management of T2DM as well. Each such patient lies somewhere on a spectrum that ranges from mild glucose intolerance (accompanied by insulin resistance and higher-than-normal levels of insulin) to more advanced forms (with more severe insulin resistance, the potential for high levels of blood glucose, ketoacidosis, and partial or nearly complete pancreatic failure with an accompanying lack of insulin). Depending on the severity of the individual's diabetes, SMBG plays a varying role. Each such patient should own a blood glucose monitor and have become intimately familiar with its use. Even those whose blood glucose is well controlled through diet, lifestyle, and supplements should measure their blood glucose regularly.

Numerous dietary factors, supplements, exercise, stress, and illness can all have a significant effect on blood glucose control. Becoming intimately aware of how all these factors influence diabetes will help motivate these patients to make positive changes and provide immediate feedback as to the success of any changes that have been made.

Those whose disease is more advanced and who have diminished pancreatic insulin production may also benefit from efforts to establish consistently near-normal blood glucose control using intensive insulin therapy similar to that of patients with T1DM.[86] A C-peptide blood test can provide an estimate of how much insulin a patient is producing and is one way to help determine the appropriateness of using insulin. If patients with T2DM are placed on an intensive insulin therapy program, they must perform SMBG as frequently as those with T1DM who are receiving intensive insulin therapy (usually before and 2 hours after each meal).

TABLE 165.3 Interpreting Levels of C-Peptide

C-Peptide Results	Interpretation
Normal	Insulin production at normal level
Below normal	Newly diagnosed type 1 diabetic or chronic, long-term type 2 diabetic
Above normal	Newly diagnosed type 2 diabetic or a benign pancreatic tumor (insulinoma; rare)
Undetectable	Chronic type 1 diabetic or postsurgical removal of pancreas (rare)

Many patients with advanced T2DM have diminished insulin production (evidenced by lower-than-normal C-peptide levels). A common way to achieve optimal blood glucose in these individuals is to give one injection of the new, long-acting insulin glargine (Lantus), which provides a smooth, continual release of insulin for 24 hours, along with diet and other medication. Patients on this type of program must measure their blood glucose frequently (usually before and 2 hours after each meal).

C-Peptide Determination

Often it is important to know whether a diabetic patient's pancreas is making insulin, and if so, how much. This assessment can greatly influence treatment, especially in a patient hoping to avoid or cease using injected insulin. The level of pancreatic insulin production can also partially determine the type of medication or natural health products that are more likely to be effective. Once it is known how well the pancreas is producing insulin, the focus may be shifted toward replacing deficiencies in insulin production, stimulating insulin production, preserving pancreatic function, reducing insulin resistance, or a combination of these therapeutic efforts.

One way to determine the level of insulin production is by measuring C-peptide. The pancreas manufactures a large protein called *proinsulin* first. A piece of this protein (C-peptide) is then snipped off by enzymes, and both C-peptide and the remaining insulin are released into the bloodstream. Injected insulin has no C-peptide, nor does the body ever produce antibodies against it, as it can against insulin. Patients with T1DM and those who have injected insulin even once are at high risk of having developed insulin antibodies, which can destroy the molecule. The benefits of measuring C-peptide are helpful for both patients with T1DM and those with T2DM, but generally more so for those with T2DM. C-peptide can uncover how much insulin the pancreas is making, which may help determine how much of a T1DM pancreas is still active. It may even sometimes make it possible, with alternative care, to stabilize the patient's condition. In T2DM, high C-peptide levels confirm that the patient is highly insulin resistant. If the C-peptide is low, it indicates that the pancreas is so damaged that some type of insulin therapy will be required (Table 165.3).

Physician Monitoring

Although patients with diabetes must take charge of their illness and be in control of their diet, lifestyle, and glucose monitoring, they are rarely successful without professional guidance. Numerous studies have determined that physician monitoring through laboratory measurements of blood glucose levels can have a major effect on a diabetic patient's long-term health.

One of the key determinants of blood glucose control is the $HgbA_{1c}$ test (see earlier discussion). Unlike direct measurements of blood glucose, which detect the level at the moment of testing, the $HgbA_{1c}$ test reflects the average level of blood glucose over the preceding 3 months. Studies have shown that the level of $HgbA_{1c}$ closely correlates with the level of risk for diabetic complications. However, an $HgbA_{1c}$ may not be entirely accurate. A patient may have steady, regulated blood sugars that return an $HgbA_{1c}$ of 6%, or he or she may have very high numbers in combination with hypoglycemic events, which can also—because the $HgbA_{1c}$ is an average, median index—show the same $HgbA_{1c}$ of 6%. Having an $HgbA_{1c}$ level of less than 5.5% or less is ideal and indicates that blood glucose levels have averaged in a range that is essentially nondiabetic (meaning that the patient is suffering no damage because of his or her glucose level). Owing to the great importance of adequate glucose control, all patients with diabetes should have their $HgbA_{1c}$ levels measured every 3 to 4 months, depending on the stability of their condition. If the $HgbA_{1c}$ number is not clearly known to be the result of good control or fluctuating highs and lows, then a second laboratory test, called GlycoMark, is a good one to consider.[87] The GlycoMark assay measures blood levels of 1,5-anhydroglucitol. 1,5-AG is found in nearly all foods and is ingested in a regular diet. Once ingested, 1,5-AG is nearly 100% nonmetabolized and remains in a relatively constant amount in the blood and tissues. When blood glucose exceeds 180 mg/dL for any period of time, the kidney attempts to reabsorb as much glucose back into the blood as it can. During times of glucosuria, the additional amount of glucose in the kidney blocks 1,5-AG from being reabsorbed into the blood, and 1,5-AG is excreted in the urine at a higher rate than normal. Due to the lack of 1,5-AG being reabsorbed, blood levels of 1,5-AG decrease immediately and continue to decrease until glucose values go below 180 mg/dL. It is this competitive inhibition of 1,5-AG from glucose that allows GlycoMark to accurately reflect any hyperglycemic episodes over 180 mg/dL. The GlycoMark test has been shown to be more accurate than an $HgbA_{1c}$ and offers the physician a clearer picture of how well a patient's glucose levels are being controlled and also shows postprandial spikes more clearly.

Although it is clear that optimal blood glucose control is critical to the health of patients with diabetes, several other risk factors must be carefully monitored in all diabetic patients. Early detection of problems through a program of regular screening and monitoring will enable preventive efforts and treatments to be put in place before serious complications or catastrophic problems occur. Table 165.4 provides a checklist for the proper evaluation and monitoring of patients with diabetes.

THE COMPLICATIONS OF DIABETES

In diabetes, problematic regulation of glucose levels can lead to acute complications, and long-term elevations of blood glucose cause inflammatory and oxidative damage that will lead to chronic disease progression and the development of complications (Box 165.5).

Acute Complications

As described previously, the acute complications of diabetes may represent a medical emergency and possibly a life-or-death situation. Any diabetic patient experiencing any symptom even remotely suggestive of an acute complication should consult medical care immediately. The major acute complications of diabetes are hypoglycemia, ketoacidosis, and nonketogenic hyperosmolar syndrome.

Hypoglycemia

Hypoglycemia is usually seen in T1DM. It is the result of excessive insulin injection, decreased or delayed food ingestion, use of alcohol or drugs that interfere with the liver's production of glucose, or an unaccustomed increase in exercise. Severe hypoglycemia can also occur unpredictably in

TABLE 165.4 Clinical Management of the Patient With Diabetes

	Quarterly	Annually
Review Management Plan		
Blood glucose self-monitoring results	X	X
Medication/insulin regimen	X	X
Nutritional plan	X	X
Exercise program	X	X
Psychosocial support	X	X
Physical Examination		
Weight	X	X
Height (for child/adolescent)	X	X
Sexual maturation (for child/adolescent)	X	X
Skin, including insulin injection sites	X	X
Feet: pulses, capillary refill, color, sensation, nails, skin, ulcers	X	X
Neurological: reflexes, proprioception, vibratory sensation, touch (distal temperature sensation, distal pinprick or pressure sensation, standardized monofilament)		X
Regular retinal examination	X	X
Dilated retinal examination		X
Electrocardiogram		X
Laboratory Tests		
Fasting or random plasma glucose (normal/target range: 80–120 mg/dL before meals)	X	X
Glycosylated hemoglobin (A1c) (target range: <7% in adults, 7.5–8.5, age dependent)	X	X
Urinalysis (glucose, ketones, microalbumin, protein, sediment)	X	X
Complete Cardiovascular Profile		
Test/target		X
Cholesterol <200 mg/dL		X
Triglycerides <200 mg/dL		X
Low-density lipoprotein <130 mg/dL		X
High-density lipoprotein <35 mg/dL		X
Lipoprotein (a) <40 mg/dL		X
C-reactive protein <1.69 mg/L		X
Fibrinogen <400 mg/L		X
Homocysteine <16 μmole/L		X
Ferritin 60–200 mcg/L (if elevated, transferrin saturation; if elevated, genetic testing for hemochromatosis only once)		X
Lipid peroxides < normal		X
Serum creatinine (in adults; in children only if protein is present in urine)	X	X

patients with "brittle" T1DM or in any patient on insulin or sulfonylurea drugs who neglects the need for the proper monitoring of blood glucose. Daytime hypoglycemic episodes are usually recognized by symptoms such as sweating, nervousness, tremor, and hunger. Nighttime hypoglycemia may be without symptoms or manifest as night sweats, unpleasant dreams, or early-morning headache.

The earliest autonomic symptom of hypoglycemia is hunger and occurs when the glucose is at or below 65 to 70 mg/dL. Patients may also experience irritability, anxiety, heart palpitations, pallor, and sweating. Neuroglycopenic symptoms, which occur when the brain becomes starved of glucose, start with blurry vision and can progress to headache, tiredness, abnormal behavior, slurred speech, unconsciousness, and seizures. Treatment of hypoglycemia follows the 15-15 rule, whereby patients are told to ingest 15 g of carbohydrate and then recheck their glucose in 15 minutes. If the glucose is still below 80 mg/dL, another 15 g of carbohydrate should be taken and the glucose rechecked in an hour. When glucose sinks below 55 mg/dL, the patient will probably need help from another person, and when it is below 20 mg/dL, a seizure is highly likely to follow, and glucagon should be injected. Any hypoglycemic event should be recorded and reported to a physician.

BOX 165.5 Acute and Chronic Complications of Diabetes

Acute Complications

- Hypoglycemia
- Diabetic ketoacidosis
- Nonketotic hyperosmolar hyperglycemia

Chronic Complications

- Atherosclerosis and other vascular lesions
- Retinopathy and cataracts
- Neuropathy
- Nephropathy
- Poor wound healing
- Foot ulcers

Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is most commonly seen in patients with newly diagnosed T1DM when they have infections; when they have deliberately or accidentally omitted their insulin; and under other circumstances such as trauma, myocardial infarction or stroke, during surgery, and with dental abscess and other types of physiological stress. The lack of insulin leads to extremely high levels of blood glucose and a buildup of acidic ketone molecules as fat stores are burned to provide energy. If progressive, ketoacidosis can lead to numerous metabolic problems and even coma or death. Because ketoacidosis is a medical emergency, its prompt recognition is imperative. Patients should be taught to check for ketones in their urine or blood when their glucose is above 250 mg/dL for more than a few hours, if they are feverish or have an infection, if they do not "feel well," and regularly during pregnancy because DKA is usually fatal to the fetus. The symptoms of DKA include fruity breath, disorientation, abdominal tenderness, polyuria and polydipsia, hyperventilation, and signs of dehydration. Treatment of DKA depends on the severity of the situation and where the glucose level is—it can require injecting insulin, eating as well as insulin injection, or referral to an emergency department.

Hyperosmolar Hyperglycemic State

The hyperosmolar hyperglycemic state (HHS) occurs mostly in older patients with T2DM, usually in the seventh decade of life. It develops gradually, taking days to weeks to manifest itself. There is no ketoacidosis with this condition, but it has a higher mortality because it tends to occur in patients who have other serious problems, such as acute illness, recent surgery, congestive heart failure, renal dysfunction, or cardiovascular disease, or who are taking certain drugs, are victims of elder abuse or neglect, or are simply noncompliant with their diabetic therapy protocols. The diagnostic criteria include a glucose level above 600 mg/dL, profound dehydration, other changes in pH, and some alteration in consciousness. Patients may present with drowsiness, coma, visual changes, sensory deficits, and even paralysis or seizures.

HHS is a medical emergency, and the patient must be taken to an emergency room. Injecting insulin can cause severe complications, so hospital care is best.

Chronic Complications

Much more common than the acute complications of diabetes are certain long-term complications, described as follows. The main four areas of the body affected most by diabetic complications are the eyes, kidneys, nerves, and endothelial lining. These four areas do not require insulin to absorb glucose into their cells, as do the liver, muscle, and fat cells, so when glucose levels are elevated in uncontrolled diabetes, unregulated glucose floods those cells and causes significant damage. Thus they are the key sites for diabetic complications.

Atherosclerosis

Atherosclerosis and other vascular lesions are the underlying factors in the development of many chronic complications of diabetes. Individuals with diabetes have a four- to sixfold higher risk of dying prematurely of heart disease or stroke than do nondiabetic individuals, and 55% of deaths among patients with diabetes are caused by cardiovascular disease. The majority of those with T2DM have hypertension, and many of the diet and lifestyle habits of patients with diabetes—such as eating poorly and not exercising—combined with nutrient deficiencies can accelerate the risk of developing cardiovascular disease.

Retinopathy

Diabetic retinopathy is the leading cause of blindness in the United States for people between the ages of 20 and 64. In this condition, the retina is damaged by microhemorrhages, scarring, and the attachment of glucose molecules (glycosylation) to structural proteins in the retina. Studies have shown that 20 years after a diagnosis of diabetes, 80% of those with T1DM and 20% of those with T2DM have significant retinopathy. Diabetic patients are also prone to developing cataracts—opacities that occur in the lens of the eye as a result of oxidative damage to the delicate protein structures of the lens.

Neuropathy

Neuropathy usually involves the loss of peripheral nerve function and is characterized by tingling sensations, numbness, and a characteristic burning pain (neuropathic pain). It commonly occurs noticeably in the feet but can also spread elsewhere in the body, such as to the autonomic nerves of the gastrointestinal tract, causing diarrhea, constipation, and/or gastroparesis. If it progresses, it can affect deeper nerves of the autonomic nervous system, resulting in disturbances in stomach emptying and, later, impaired cardiac function, alternating bouts of diarrhea and constipation, and an inability to empty the bladder. Impotence is a common occurrence and is caused by damage to the small blood vessels of the penis as well as neuropathy of the autonomic nerves controlling blood flow into the penis. Approximately 60% of all people with diabetes eventually develop neuropathy. The main problem of peripheral neuropathy is that lack of feeling in the feet can lead patients to develop sores and lesions that they do not notice. These may then ulcerate, leading to gangrene and amputation.

Nephropathy

Nephropathy due to diabetes accounts for 40% of the cases of severe kidney disease and, in the United States, is the most common reason for end-stage renal disease, hemodialysis, and kidney transplantation. In addition to monitoring blood glucose levels, it is important to monitor kidney function with various laboratory measurements (random microalbuminuria, 24-hour urine protein, blood urea nitrogen, uric acid, creatinine and creatinine clearance, and glomerular filtration rate). Angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) are part of standard care because they have been shown to protect the kidneys from diabetic damage.

Poor Wound Healing and Foot Ulcers

Poor wound healing is common in patients with diabetes for several reasons, such as the microvascular changes leading to poor circulation and the functional deficiency of nutrients. Foot ulcers are common in individuals with diabetes because of microvasculature changes leading to a poor blood supply, peripheral neuropathy, poor wound healing, poor nutrition, and immune system dysfunction, all of which can promote chronic infections in the feet. Apart from trauma, diabetic wounds are the leading cause of limb amputations in the United States. More than 50% of lower-limb amputations in the United States (70,000 each year) are due to diabetic foot ulcers.

Immune System Dysfunction

Immune system dysfunction often begins long before a diagnosis of diabetes is made. In fact, in many cases, a recurrent yeast infection of the vagina or skin is the first clue leading to the detection of diabetes. Immune system problems are made worse by poor glucose control, which puts the diabetic patient at risk for serious infections or complications of simple infections. Susceptibility to chronic, hidden infections in the oral cavity, blood, or respiratory tract may be a primary underlying cause of the increased risk for cardiovascular disease among diabetic patients.

Depression and Cognitive Difficulties

Depression and cognitive difficulties are common among those with diabetes. In fact, depression may begin to occur when the individual first starts to develop insulin resistance, decades before the onset of T2DM. The brain is more sensitive to its need for glucose than any other organ, and it appears that brain cells may suffer from some degree of glucose deprivation when insulin resistance occurs.[88] Depression is also much more common among overweight and obese individuals, probably owing to the combined effect of insulin resistance and diminished self-esteem. Cognitive changes begin to occur after the first severe hypoglycemic episode in people with diabetes. Hypoglycemia is profoundly stressful to the brain and, if severe hypoglycemia occurs many times, significant cognitive impairment can ensue. Uncontrolled diabetes is also associated with an increased risk of developing Alzheimer's disease.

Contributors to the Long-Term Complications of Diabetes

The major contributors to the long-term complications of diabetes are listed here, followed by a brief description of each factor along with coping measures:

- Poor glucose control
- Glycosylation of proteins
- Intracellular accumulation of sorbitol
- Increased oxidative damage
- Nutrient deficiency
- Elevated levels of homocysteine
- Hypertension
- Endothelial cell dysfunction

Poor Glucose Control

A large body of evidence indicates that good blood glucose control significantly reduces the development of complications. The largest and most extensive study to date in T1DM is the Diabetes Control and Complications Trial, whereas the largest and longest study on patients with T2DM is the United Kingdom Prospective Diabetes Study. Both

studies conclusively demonstrated that improved blood glucose control reduces the risk of developing long-term complications of diabetes, especially retinopathy, nephropathy, and neuropathy. Maintaining $HgbA_{1c}$ levels near normal (equal to or less than 7%) can dramatically help reduce the risk of eye problems (up to 76%), nerve damage (up to 60%), and kidney disease (up to 56%).

Glycosylation of Proteins

As described previously, the term *glycosylation* refers to the binding of glucose to proteins. The poorer the glucose control, the greater the binding of glucose molecules to proteins. This binding leads to changes in the structure and function of the protein. Among the adverse effects of excessive glycosylation are inactivation of enzymes, inhibition of regulatory molecule binding, and the formation of abnormal protein structures. For example, when glucose molecules bind to cholesterol-carrying low-density lipoprotein (LDL) molecules, they block LDL from binding to receptors on the liver that tell the liver to cease manufacturing cholesterol. As a result, the liver "thinks" that there is a shortage of cholesterol in the body and continues to produce more and to release it into the blood. This is one reason why diabetes is almost always associated with high cholesterol levels.

In addition to keeping blood glucose levels as close to ideal as possible, high intakes of antioxidants—especially vitamins C and E, flavonoids, and alpha-lipoic acid (discussed later)—help reduce glycosylation.

Intracellular Accumulation of Sorbitol

Sorbitol is a sugar molecule formed from glucose within cells. In people without diabetes, once sorbitol is formed, it is quickly broken down into fructose. This conversion to fructose is critical because it allows any excess sorbitol to be excreted from the cell, and sorbitol cannot exit the cell once it is formed. If sorbitol levels continue to increase within cells, they create an osmotic effect.

When there is an increase in the concentration of soluble compounds (e.g., sorbitol) that the cell cannot get rid of, the cell leaks small molecules like amino acids, inositol, glutathione, niacin, vitamin C, magnesium, and potassium to maintain osmotic balance. Because these compounds function to protect cells from damage, their loss results in increased susceptibility to damage.

Intracellular accumulation of sorbitol is a major factor in the development of most complications of diabetes, as evidenced by the fact that elevated sorbitol levels are found in high concentrations in the tissues commonly involved in the major diabetic complications: the lens of the eye, nerve cells, kidney cells, and the cells that line blood vessels.

In addition to controlling blood glucose levels, vitamin C and flavonoids like quercetin, grape seed extract, and bilberry extract can help lower intracellular sorbitol levels. Sorbitol accumulation, by the way, has nothing to do with eating foods that contain sorbitol.

Increased Oxidative Damage

Increased oxidative stress is a major factor in the development of the chronic complications of diabetes. As previously stated, individuals with diabetes typically have elevated levels of free radicals and oxidative compounds.[89] These highly reactive compounds bind to and destroy cellular compounds, cause damage all over the body, and increase insulin resistance. They also greatly increase the inflammatory process by adding fuel to their destructive fire via the increased formation of inflammatory mediators like C-reactive protein, interleukin-6, and tumor necrosis factor-alpha. One of the critical goals in diabetes prevention and treatment is to flood the body with a high level of antioxidant compounds to counteract the negative effects of free radicals and prooxidants. Implementation of this goal is achieved by following the dietary and supplementary strategies given, which promote weight loss, establish better glucose control, and replete antioxidants in the body. In addition to the basic supplementation program, supplementing the diet with super antioxidants like alpha-lipoic acid and flavonoid-rich extracts is often useful in further boosting antioxidant protection.

Nutrient Deficiency

A deficiency of any one of several nutrients has been shown to contribute to several chronic complications of diabetes. Studies have found that nutrient supplementation helps patients with diabetes control their glucose levels; it can also lower their blood pressure and protect their bodies from diabetic complications. In general, the risk of long-term complications of diabetes is inversely proportional to micronutrient status. Sometimes the symptoms of nutrient deficiency can closely mimic those of a chronic complication of diabetes. For example, vitamin B_{12} deficiency is characterized by numbness, "pins and needles" sensations, or a burning feeling in the hands or feet—symptoms virtually identical to diabetic neuropathy. Although vitamin B_{12} supplementation has been used with some success in treating diabetic neuropathy, it is not clear whether this success is due to correction of a vitamin B_{12} deficiency state or the normalization of the deranged vitamin B_{12} metabolism seen in diabetic patients.

High-potency multivitamin/multimineral supplementation is critical to the management of diabetes. Supplying the diabetic patient with additional key nutrients improves blood glucose control and reduces the development of the major long-term complications of diabetes.

Elevated Levels of Homocysteine

Elevated homocysteine levels constitute an independent risk factor for heart attack, stroke, and peripheral vascular disease. In addition, recent research has implicated elevations of homocysteine in the development of long-term complications of diabetes, especially diabetic retinopathy.[90]

Hypertension

Blood pressure control is essential in preventing the complications of diabetes, especially renal disease, retinopathy, and stroke. Maintaining blood pressure in the normal range (120/80–140/80 mm Hg) can reduce the risk of heart disease and stroke by approximately 33% to 50% and can reduce microvascular disease (eye, kidney, and nerve disease) by approximately 33%.

Endothelial Cell Dysfunction

A single layer of endothelial cells lines all the body's blood vessels, serving as a metabolically active interface between the components of blood and the blood vessel. These cells regulate many important aspects of blood flow: coagulation, clot formation, and the formation of key regulatory compounds, including those that control blood pressure. Endothelial cells are susceptible to damage by oxidized LDL cholesterol (LDL-C) and other free radicals—hence the importance of a high dietary intake of antioxidants, flavonoids, key supplemental antioxidants like vitamins C and E, and alpha-lipoic acid. All of these factors have been shown to improve endothelial cell function and are critical in the battle against vascular disease in diabetes.[91–94]

THERAPEUTIC CONSIDERATIONS

Diet Therapy in Managing Diabetes

The optimal diet for the treatment of diabetes is virtually the same as the program presented in Chapter 44. The difference is that there must often be an even stricter avoidance of foods with a high carbohydrate

concentration (see Appendix 7). The strictness of the diet with regard to the intake of carbohydrates must be based on the patient's ability to maintain satisfactory blood glucose measurements and $HgbA_{1c}$ levels and to achieve/maintain ideal body weight. Obviously, the poorer the control, the more the carbohydrate intake must be restricted. Initially, some people with diabetes—especially those who have poorly controlled blood glucose levels—may have to avoid meals with a total GL of more than 20 and space these meals at least 3 hours apart. Higher-GL meals can be consumed if one of the special natural products designed to slow gastric emptying and blunt after-meal blood glucose levels is used (these compounds are discussed later in the chapter).

Clinical Studies With Diet Therapy in Type 1 Diabetes

Numerous clinical studies have shown impressive results in improving blood glucose control when diets high in fiber and low in GL are followed. This holds true in both adults and children as well as in both T1DM and T2DM. In a study involving children, 8- to 13-year-olds in Melbourne, Australia were divided into two groups: one group followed the ADA's exchange-program diet, and the other was instructed to eat low-GI foods.[95] Although there was no change in A_{1c} in the exchange diet (8.6%), the group eating the low-GI diet dropped from 8.6% to 8%—an acceptable value in children. Rates of excessive blood glucose levels were 66% for the exchange diet versus 35% for those eating low-GI foods. Although these results are good, what the study really highlighted was the effect of eating a low-GI diet on the quality of life. There were significantly fewer family conflicts, fewer limitations placed on family activities, and fewer difficulties in meal selections. Furthermore, parents and children alike showed a clear preference for the low-GI diet.

Similar results have been seen in adults, including pregnant women, with T1DM following a high-fiber, low-GI diet.[96–99] What these studies and others indicate is that low-GI and low-GL diets are emerging as the most scientifically proved dietary support for T1DM. We have taken the proved diet to a much higher level by also considering the role of fats on insulin action.

Although ketogenic diets have become popular for T2DM, they should be used with caution in T1DM. In an observational study, 11 adults with type 1 diabetes following a ketogenic diet (<55 g carbohydrate per day) for a mean of 2.6 years showed excellent effects on HbA_{1c} levels (5.3 ± 0.4%), and participants spent 74% and 3% of their time in the euglycemic and hyperglycemic ranges, respectively, with little daily glycemic variability. They did spend about 3.6% of the time with blood sugar levels of greater than 3.0 mmol/L or 54 mg/dL, and participants experienced a median (range) of 0.9 (0.0–2.0) daily episodes of hypoglycemia. In addition, total cholesterol, LDL-C, total cholesterol/HDL-C ratio, and triglycerides were above the recommended range in 82%, 82%, 64%, and 27% of participants, respectively. These results indicate that ketogenic diets in adults with T1DM result in excellent HbA_{1c} levels and little glycemic variability but may also be associated with dyslipidemia and a high number of hypoglycemic episodes.[100]

Clinical Studies with Diet Therapy in Type 2 Diabetes

Diet alone can often be effective as the sole factor in treating and reversing T2DM. Other lifestyle factors and supplements are important, but the treatment of T2DM begins with diet. The critical dietary focus based on results from clinical trials is that the diet should be low in GI and GL. The actual composition of the macronutrients is less as important as the quality of the carbohydrates consumed. A low-GI and low-GL diet is the most scientifically proven approach, especially considering not only the diet's effect on blood glucose levels but also its effects in reducing the sequelae of diabetes, such as high cholesterol levels, cardiovascular disease, hypertension, and complications of diabetes.[101]

In addition to low GL, another key goal is to get the total fiber intake from foods to at least 40 g/day. In one study, the effects of two diets on blood glucose levels were compared,[102] because higher fiber intake is known to lower average daily glucose levels. One diet contained 24 g of dietary fiber as 8 g of soluble fiber and 16 g of insoluble fiber on the basis of ADA recommendations, whereas the other provided a total of 50 g as 25 g of soluble fiber and 25 g of insoluble fiber. Both diets had the same calorie level and percentages of fat, carbohydrate, and protein. After 6 weeks, the average daily blood glucose levels were 13 mg/dL lower in the group on the higher-fiber diet. Furthermore, a high-fiber diet has also been found to lower the total area under the curve for 24-hour blood glucose levels; in addition, it lowered insulin concentrations and reduced total cholesterol concentrations by 6.7%, triglyceride concentrations by 10.2%, and very low-density lipoprotein cholesterol (VLDL-C) concentrations by 12.5%. This study showed that a high intake of dietary fiber, particularly of the soluble type, above the level recommended by the ADA, improves glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations in patients with T2DM. Similar studies looking at a low- versus a high-GI diet have clearly shown the advantages of the former.[101,103]

As far as specific eating plans, the Mediterranean, Dietary Approaches to Stop Hypertension (DASH) and plant-based diets are examples of healthful eating patterns that have shown positive results in clinical research. The diabetes plate method can also be helpful. This method provides a visual guide showing how to control calories (by featuring a smaller plate) and carbohydrates (by limiting them to what fits in one-quarter of the plate) and puts an emphasis on low-carbohydrate (or nonstarchy) vegetables. The bottom line is that there is great flexibility for patients to construct a personalized, health-promoting diet that works.[104]

Because weight loss is often a major goal in T2DM, please consult Chapter 201, Obesity, for additional recommendations. Diet and exercise combinations have shown significant effects in promoting weight loss and improved blood sugar control in patients with T2DM.[104]

Psychological Support in Diabetes

Helping people with diabetes deal with their diagnosis, develop a sense of empowerment, and make important lifestyle changes is an extremely important aspect of proper medical care. It is imperative to analyze the emotional aspects of diabetes and manage any negativity or sense of being overwhelmed, which is very common in patients with diabetes and can impair their capacity to follow their protocols and maintain good blood sugar control. A book called *Diabetes Burnout: What to Do When You Can't Take It Anymore,* by William Polonsky, published by the ADA, is highly useful reading material for patients with diabetes when they are feeling stressed by their condition. Cognitive-behavioral therapy has proven to be especially effective in helping adolescents with T1DM deal with their disease, leading to improvements in both mood and blood glucose control.[105]

Dealing With Stress

Stress adversely affects blood glucose control because higher stress levels are associated with higher blood glucose levels in both T1DM and T2DM. There is a simple explanation for this. Exposure to stress—whether it be physical, mental, or emotional—leads to activation of the body's "stress response" and causes increased secretion of the adrenal gland hormones adrenaline and cortisol. Among other things, these hormones cause blood glucose levels to rise and also blunt the response to insulin. In addition, they have a negative effect on the immune system. Because stress seems to be an inevitable part of modern living, it is critical to develop effective methods to deal with it. Some studies

have shown that positive methods for dealing with stress, such as yoga and relaxation training, can improve blood glucose control, especially in individuals who are anxious or are experiencing significant stress in their lives.[104,106,107]

Exercise and Diabetes

Exercise is absolutely essential in the prevention and management of diabetes. Exercise directly improves insulin sensitivity and blood glucose control from a combination of increased lean muscle mass and an improvement in muscle cell metabolism.[108] Exercise also has profound benefits for the cardiovascular system, directly as well as indirectly through improvements in blood lipids (especially an improvement in HDL-C, the "good cholesterol"). Exercise also decreases symptoms of anxiety and depression, improves sexual functioning, and improves confidence and self-esteem. Importantly, exercise has been shown to help attain and sustain weight loss.[109] Three types of exercise are important for people with diabetes: aerobic, strength training, and stretching.

Aerobic Exercises

Aerobic exercises, such as walking, jogging, aerobic dance classes, cycling, and swimming, produce rises in heart and respiratory rates. These sorts of activities are foundational in both the prevention and treatment of diabetes. In its simplest form, an aerobic exercise program would consist of a schedule of regular walking combined with cycling, swimming, or the use of low-impact indoor exercise equipment such as an elliptical trainer. Patients should engage in at least 30 minutes of aerobic exercise five times a week.

Strength Training

Strength training is important for many reasons, including that it leads to increased production of secretory molecules, called myokines, from contracting muscle fibers. Myokines are beneficial modulators of obesity, metabolic syndrome, and type 2 diabetes.[40]

Strength training should occur for 15 to 30 minutes five times a week. Working initially with a trainer to learn proper technique and a rotating regimen of weights is the healthiest way to go. Anaerobic weight training can burn 19 times the amount of glucose as aerobic exercise and protects against the loss of lean body mass while also increasing its size. The loss of lean muscle mass means that there is less tissue to actively absorb glucose. The more muscle one has, the easier it becomes to control blood glucose. Also, patients with diabetes are particularly prone to developing chronic musculoskeletal complaints. Most of these problems are related to increased susceptibility to injuries of joints, ligaments, and muscles due to the loss of muscle mass.

Stretching Exercises

Stretching exercises should be done daily. Stretching is extremely important because most people with diabetes suffer from premature stiffening of the spine and joints. Stretching on a daily basis helps maintain flexibility and avoid the chronic pain problems that occur frequently in patients with diabetes due to stiff muscles and joints.

Nutritional Supplements

The proper treatment of diabetes with natural medicine involves trying to achieve ideal blood glucose control and to reach metabolic targets as well as to reduce the risk of complications by focusing on the following four areas:

1. Providing optimal nutrient status
2. Reducing after-meal elevations in blood glucose levels
3. Improving insulin function and sensitivity
4. Preventing nutritional and oxidative stress

Even though natural products can have significant effects on their own, the proper and effective treatment of diabetes requires the careful integration of diet and lifestyle changes along with any required medication and then natural medicines. Furthermore, all those with T1DM and many with T2DM also require conventional medical treatment (with oral drugs or insulin), depending on the adequacy of pancreatic insulin production (this can be determined by the C-peptide level) and the individual's response to dietary and lifestyle measures. The most important determining factor as to whether or not a patient will require the use of drugs or insulin is the adequacy of blood glucose control.

Providing Optimal Nutritional Status

In addition to eating a nutrient-dense diet, a high-potency multivitamin/multimineral (MV) supplement is an absolute must for people with diabetes. Follow the guidelines given in Appendix 9. The person with diabetes has such an increased need for many nutrients that supplementation is critical. Supplying him or her with additional key nutrients has been shown to improve blood glucose control as well as to help prevent or reduce the development of the major complications of diabetes. Taking an MV supplement has also been shown to boost immune function and reduce infections in patients with diabetes.[110] The best MV is not a "one a day" but instead a high-potency product recommended by a physician who specializes in alternative medicine. Specific examples of nutrients for which the patient with diabetes has increased requirements include chromium, vitamin C, vitamin E, certain B vitamins, manganese, magnesium, potassium, and zinc.

Whenever a patient with diabetes adds significant nutrient, fiber, or botanical medicines to his or her protocol, glucose monitoring is recommended because oral or injectable medicines may have to be reduced. A physician should be involved in all decisions regarding natural supplementation.

Chromium

Chromium is vital to proper blood glucose control because it functions in the body as a key constituent of what is referred to as the "glucose tolerance factor." Chromium works closely with insulin in facilitating the uptake of glucose into cells. Without chromium, insulin's action is blocked, and glucose levels are elevated. Evidence indicates that marginal chromium status is common in the United States. A chromium deficiency may be an underlying contributing factor to the tremendous number of Americans who have diabetes and hypoglycemia and are obese.

More than 20 clinical studies have focused on chromium supplementation in diabetes. In some of these studies in T2DM, supplementing the diet with chromium has been shown to decrease fasting glucose levels, improve glucose tolerance, lower insulin levels, and decrease total cholesterol and triglyceride levels while increasing HDL-C levels. Although there are also studies that have not shown chromium to exert much effect in improving glucose tolerance in diabetes, there is no argument that chromium is an important mineral in blood glucose metabolism. However, it appears that chromium supplementation is likely to produce meaningful improvements in glycemic control only in people who are deficient in this essential trace element.[111]

It appears to see benefits in people with diabetes, they must supplement between 400 and 600 mg per day. Chromium polynicotinate and chromium picolinate may offer the best results because chromium-rich yeast failed to produce any significant benefit in the most recent trials.[112] In contrast, several recent studies with chromium picolinate in combination with biotin at dosages of 600 mcg and 2 mg, respectively, showed considerable benefit in helping patients with T2DM improve blood sugar control: fasting glucose levels dropped 10

mg/dL, and $HgbA_{1c}$ levels dropped 0.54%.[113] Improvements in blood lipids and atherogenic indices were also noted in other studies.[114]

Vitamin C

Because the transport of vitamin C into cells is enhanced by insulin,[115] many people with diabetes suffer from a relative deficiency of vitamin C within their cells even if they consume an adequate amount of vitamin C in their diets, which many do not. For many reasons, people with diabetes must take extra vitamin C as well as increase the consumption of vitamin C–rich, low-GI foods.

In addition to its role as an antioxidant, vitamin C is required in immune functions and the manufacture of collagen, the main protein substance of the human body. Because collagen is such an important protein for the structures that hold the body together (e.g., connective tissue, cartilage, tendons), vitamin C is vital for wound repair, healthy gums, and the prevention of easy bruising. A chronic, latent vitamin C deficiency leads to a number of problems for the patient with diabetes, including increased capillary permeability, poor wound healing, elevated cholesterol levels, and a depressed immune system. Vitamin C supplementation has been shown to exert a mild effect in improving glucose control, as evident by a slightly lower $HgbA_{1c}$ in the vitamin C group (8.5%) compared with placebo (9.3%) in one double-blind study.[116] Probably more important than any significant effect on improving blood glucose control is the fact that vitamin C supplementation has been shown to reduce the formation of compounds linked to the development of diabetic complications.

In one study of vitamin C supplementation in T2DM, 30 patients who were 45 to 70 years old and had not only T2DM but also hypertension were randomly assigned in a double-blind manner to take either 500 mg of ascorbic acid by mouth or a placebo daily for 4 weeks. Vitamin C supplementation decreased systolic blood pressure from 142.1 to 132.3 mm Hg and diastolic blood pressure from 83.9 to 79.5 mm Hg. Additional analytic methods designed to measure vascular resistance also demonstrated significant improvements in arterial stiffness in the group taking vitamin C. These results indicate that vitamin C supplementation is effective in improving the elasticity and function of blood vessels in patients with T2DM.[117]

The attempt to prevent sorbitol accumulation with drugs has failed because of severe side effects. In contrast, vitamin C can accomplish what these drugs could not—provide safe and effective inhibition of sorbitol accumulation. In one study comprising young adults with T1DM, the baseline measurement of sorbitol in RBCs was nearly double in these patients compared with those without diabetes despite "adequate" dietary intakes of vitamin C. Vitamin C supplementation at a dose of either 100 or 600 mg normalized RBC sorbitol within 30 days. This correction was independent of changes in diabetic control as monitored by fasting glucose or $HgbA_{1c}$. In fact, overall diabetic control during the study was moderate to poor, indicating that vitamin C's effect was not dependent on glucose concentration.

Vitamin C inhibits the enzyme aldose reductase, which converts glucose to sorbitol.[118] At concentrations higher than 100 µmol, vitamin C reduced sorbitol production by about 30%. Levels between 600 and 900 µmol reduced sorbitol production by about 50%. These concentrations are attainable with vitamin C supplementation. In fact, the normal level of vitamin C in plasma and RBCs is 40 to 120 µmol. Supplementing with 500 to 1500 mg of vitamin C a day boosts blood levels into the higher range required to inhibit sorbitol production.

Although vitamin C supplementation is necessary, patients should not rely exclusively on it to meet all their vitamin C requirements. Vitamin C–rich foods are also rich in compounds such as flavonoids and carotenes, which work to enhance the effects of vitamin C while also exerting favorable effects of their own.

Vitamin E

Vitamin E functions primarily as an antioxidant in protecting against damage to cell membranes. Nerve cells are particularly vulnerable when vitamin E levels are low. Compounding the matter is that diabetic patients appear to have an increased requirement for vitamin E. At higher dosages in the range of 400 to 800 IU, vitamin E improves the action of insulin and has a number of beneficial effects that may aid in preventing the long-term complications of diabetes. Actions of vitamin E:

- Prevents free radical damage to LDL-C and the vascular lining[119–121]
- Improves the functioning of blood vessels and the cells that line them[122,123]
- Increases the concentration of magnesium within cells[124,125]
- Decreases the level of C-reactive protein and other inflammatory compounds[126,127]
- Increases the level of glutathione—an important intracellular antioxidant—within cells[128]
- Improves the rate of conduction of electrical impulses through the nervous system[129]
- Improves blood flow to the eye and mitigates diabetic retinopathy[130]
- In diabetic nephropathy, it improves kidney function and leads to a significant decrease in urine protein and protein-to-creatinine ratio, serum tumor necrosis factor-α, lipid peroxides, advanced glycation end products, and insulin concentrations[131]

Vitamin E supplementation may be particularly helpful for patients with the haptoglobin (Hp) 2-2 genotype, a subgroup that comprises 2% to 3% of the general population. Hp, a major antioxidant protein, is a determinant of cardiovascular events in patients with T2DM. The Hp gene is polymorphic, with two common alleles, 1 and 2. The Hp 2 allelic protein product provides inferior antioxidant protection compared with the Hp 1 product. In a large study of more than 1400 patients with diabetes 55 years of age or older with the Hp 2-2 genotype randomized to vitamin E (400 IU a day) or placebo, the rates of myocardial infarction, stroke, and cardiovascular death 18 months after initiating the study were significantly reduced in those receiving vitamin E (2.2%) compared with placebo (4.7%), a greater than 50% reduction.[132]

It should be noted that in one study in patients with T2DM, treatment with either 500 mg of α-tocopherol or mixed tocopherols significantly increased systolic blood pressure (approximately 6–7 mm Hg) versus placebo, indicating that some patients may have a hypertensive reaction.[133] Patients should be monitored to rule out this negative effect.

Niacin and Niacinamide

Enzymes that contain niacin (vitamin B_3) play an important role in energy production; fat, cholesterol, and carbohydrate metabolism; and the manufacture of many body compounds, including sex and adrenal hormones. Niacin, like chromium, is an essential component of the glucose tolerance factor, making it a key nutrient for hypoglycemia and diabetes.

In addition to offering possible benefits in T1DM, niacinamide may also help in T2DM. Eighteen patients with T2DM of normal body weight who failed to respond to oral antihyperglycemic drugs were randomly assigned to one of three treatments for 6 months: (1) insulin plus nicotinamide (500 mg three times daily), (2) insulin plus placebo, or (3) an oral antihyperglycemic drug plus niacinamide (500 mg three times daily). The parameters assessed included C-peptide, $HgbA_{1c}$, and both fasting and mean daily blood glucose levels. With detailed analysis, niacinamide administration was the only significant factor accounting for the improvement of C-peptide release. The data indicated that niacinamide improves C-peptide release and blood glucose control in patients with T2DM who had previously failed to respond to oral antihyperglycemic drugs alone.[134]

Vitamin B_6

Vitamin B_6 supplementation appears to offer significant protection against the development of diabetic neuropathy.[135] Patients with diabetes with neuropathy have been shown to be deficient in vitamin B_6 and to benefit from supplementation.[136] The neuropathy of a vitamin B_6 deficiency is indistinguishable from diabetic neuropathy. Individuals with long-standing diabetes or who are developing signs of peripheral nerve abnormalities should definitely supplement their diets with vitamin B_6, which is also important in preventing other diabetic complications.

Vitamin B_6 supplementation can be a safe and effective treatment for gestational diabetes. When 14 women with gestational diabetes were given 100 mg of vitamin B_6 daily for 2 weeks in one study, the diagnosis was eliminated in 12 of the 14 women.[137]

Magnesium

Like manganese and chromium, magnesium is also involved in glucose metabolism. Considerable evidence indicates that patients with diabetes should eat foods rich in magnesium as well as take supplemental magnesium—the reasons being that more than one half of all people with diabetes show evidence of magnesium deficiency, and magnesium may prevent some of the complications of diabetes, such as retinopathy and heart disease. Magnesium levels are usually low in patients with diabetes and lowest in those with diabetic complications like retinopathy and neuropathy. Clinical studies have shown that magnesium supplementation (usually 400–500 mg/day) improves insulin response and action, glucose tolerance, and the fluidity of the RBC membrane in patients with diabetes.[138,139] Higher magnesium intake is also associated with a reduced risk of progression from prediabetes to diabetes.[140]

Magnesium supplementation is especially helpful for gestational diabetes as well as preterm labor, preeclampsia, and small for gestational age or intrauterine growth restriction. In a double-blind study of 70 women aged 24 to 34 with gestational diabetes, the subjects were given either 250 mg of magnesium (oxide) or placebo daily for 6 weeks. Results demonstrated significant improvements in several blood parameters beyond blood sugar, including homeostatic model assessment of insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hs-CRP), and malonaldehyde (MDA). In addition, only 3 of the 35 newborns from mothers taking magnesium were afflicted with hyperbilirubinemia (8.8%) compared with 10 in the placebo group (29.4%).[141]

Effects of Magnesium Supplementation in Gestational Diabetes[141]

	Magnesium Group	Placebo Group	*p*-Value
Fasting blood sugar (mg/dL)	9.8% decrease (95.1–85.8)	2.5% increase (91.4–93.7)	<0.001
HOMA-IR	13% decrease (3.1–2.7)	46.8% increase (3.2–4.7)	<0.001
Triglycerides (mg/dL)	1.2% decrease (173–171)	21% increase (166.5–201.5)	0.005
Very low-density cholesterol (mg/dL)	0.6% decrease (34.1–33.9)	28.3% increase (33.1–39.9)	0.005
hs-CRP (nanograms/dL)	7.5% decrease (5731–5305)	12.8% increase (6101.1–6881.1)	0.03
MDA (micrograms/Liter)	12.5% decrease (4.0–3.5)	8.8% increase (3.4–3.7)	0.01

Because the study consisted of women with hypomagnesemia (i.e., serum levels below 1.82 mg/dL), results with magnesium supplementation may be more apparent in those with lower magnesium levels. However, in a follow-up analysis in these same patients, quantitative results of reverse-transcription polymerase chain reaction (RT-PCR) demonstrated that compared with the placebo, magnesium supplementation upregulated gene expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and glucose transporter 1 (GLUT-1) and downregulated gene expression of oxidized low-density lipoprotein receptor (LDLR). These results would indicate possible beneficial effects are not limited to either hypomagnesia or in only those with gestational diabetes.[142]

The recommended dietary intake (RDI) for magnesium is 400 to 420 mg/day for adult males and 300 to 320 mg/day for adult females. Patients with diabetes may need as much as twice this amount because they tend to lose excessive magnesium through their kidneys.[139] Most of the magnesium should be derived from the diet. The average intake of magnesium by healthy U.S. adults ranges from 143 to 266 mg/day. This is obviously far below the RDI. Food choices are the main reason. Although magnesium occurs abundantly in whole foods, food processing refines out a large portion of it. The best dietary sources of magnesium are tofu, seeds, nuts, and green leafy vegetables. Fish, meat, milk, and the most commonly eaten fruits are low in magnesium. Most Americans consume a low-magnesium diet because they eat a lot of refined foods, meat, and dairy products.

In addition to favoring a diet rich in magnesium, patients with diabetes should supplement it with 300 to 500 mg of magnesium. For best results, highly absorbable sources of magnesium like magnesium aspartate or citrate should be taken. Diabetic patients should also be sure to get at least 25 mg of vitamin B_6 per day because the level of vitamin B_6 within body cells appears to be intricately linked to the cells' magnesium content. In other words, without vitamin B_6 (as well as vitamin E), magnesium will not get inside the cells and will therefore be useless.

Zinc

Zinc deficiency, like chromium deficiency, has also been suggested to play a role in the development of diabetes.[143] Although severe zinc deficiency is rare in developed countries, many individuals in the United States have marginal zinc deficiency. This is particularly common in the elderly population as well as in people with diabetes. Zinc is involved in virtually all aspects of insulin metabolism: synthesis, secretion, and utilization. Zinc also has a protective effect against beta-cell destruction and has well-known antiviral effects. Patients with diabetes typically excrete too much zinc in the urine and therefore require supplementation (e.g., 30 mg of zinc daily). Zinc is also found in good amounts in nuts and seeds.

Manganese

Manganese functions in many enzyme systems, including those involved in blood glucose control, energy metabolism, and thyroid hormone function. Manganese also functions in the antioxidant enzyme superoxide dismutase (SOD). Patients with diabetes have been shown to have only one-half the manganese of normal individuals. In a large epidemiological study, dietary manganese intake was inversely proportional to type 2 diabetes incidence.[144] A good daily dose of manganese for a patient with diabetes is 1.5 to 3 mg.

Biotin

Biotin is a member of the B-vitamin family; it functions in the manufacture and utilization of carbohydrates, fats, and amino acids. Without biotin, sugar metabolism is severely impaired. Biotin supplementation

has been shown to enhance insulin sensitivity and increase the activity of the enzyme glucokinase—which is responsible for the first step in the utilization of glucose by the liver. Glucokinase concentrations in patients with diabetes are low. Evidently, supplementing the diet with high doses of biotin improves glucokinase activity and glucose metabolism. In one study, 16 mg of biotin daily resulted in a significant lowering of fasting blood glucose levels and improvements in blood glucose control in patients with T1DM. In another study in patients with T2DM, similar effects were noted with 9 mg of biotin daily. Biotin therapy has also been shown to be helpful in the treatment of diabetic neuropathy.[145,146]

Omega-3 Fatty Acids From Fish Oil Supplements

Omega-3 fatty acids are vital supplements for patients with diabetes. They offer significant protection against heart disease in patients with diabetes, helping lower lipids and blood pressure. They are anti-inflammatory and promote insulin sensitivity. Omega-3 oils are usually almost completely lacking in the basic diet of patients with diabetes. Foods that contain these oils include oily fish such as wild salmon, sardines, herring, trout, and mackerel; walnuts; grass-fed beef; wild game; omega-3 eggs; and ground flax, hemp, and chia seeds.

Initially, there were concerns that omega-3 fatty acid supplementation might adversely affect blood glucose control based on earlier studies, but many of these studies were conducted with lower-quality fish oil products that contained significant amounts of cholesterol and lipid peroxides. Two intensive investigations, one conducted at Oxford University and the other at the Mayo Clinic, analyzed data from 18 double-blind clinical trials involving 823 participants followed for an average of 12 weeks.[147,148] Dosages of fish oil (18% eicosapentaenoic acid [EPA] and 12% docosahexanoic acid [DHA]) ranged from 3 to 18 g/day. Both evaluations came to the same conclusions. Fish oil supplementation has no adverse effect on glycemic control, but it does appear to offer the same protection against cardiovascular disease in people with diabetes that it does in people without diabetes. A more recent analysis of 45 randomized controlled trials involving 2674 people with T2DM, omega-3 fatty acid supplementation was associated with significant reductions in LDL-C, VLDL-C, triglycerides, and HbA_{1c}.[149] The researchers did note the heterogeneity in the studies, however, that is likely explained by the poorer quality of products used in many of the early studies. It is important for patients with diabetes to ingest a high-quality fish oil product that is guaranteed not to be rancid or to contain mercury, solvents, polychlorinated biphenyls (PCBs), or other toxins. The combined total EPA + DHA level should be approximately 1000 mg daily.

Reducing After-Meal Elevations in Blood Glucose Levels

Elevations of blood glucose levels after a meal can wreak biochemical havoc in any patient with diabetes. In fact, an elevation in postprandial blood glucose levels is the major contributor to the development of diabetic complications, especially cardiovascular disease and diseases of the microvasculature (retinopathy, neuropathy, and nephropathy). For example, patients who have a normal fasting blood glucose measurement but an average 2-hour postprandial glucose level greater than 200 mg/dL (11 mmol/L) have a threefold increase in the incidence of diabetic retinopathy.[150] Therefore blunting of the after-meal increase in blood glucose levels is an important goal.

In addition to following low-carbohydrate dietary guidelines to reduce postprandial blood glucose levels (eating low-GI/low-GL meals), several natural products can be used. The best supplements in this regard are fiber supplements and natural glucosidase inhibitors.

Fiber Supplements

Fiber supplements have been shown to enhance blood glucose control, decrease insulin levels, and reduce the number of calories absorbed by the body. The best fiber sources for reducing postprandial blood glucose levels, lowering cholesterol levels, and promoting weight loss are those that are rich in water-soluble fibers, such as glucomannan (from konjac root), psyllium, guar gum, defatted fenugreek seed powder or fiber, seaweed fibers (alginate and carrageenan), and pectin.

Clinical studies have repeatedly shown that after-meal blood glucose levels decrease as soluble fiber viscosity increases. This relationship has also been shown to hold true for the other physiological benefits produced by soluble fibers, including increased insulin sensitivity, diminished appetite, significant weight control, improved bowel movements, and decreased serum cholesterol.[151,152]

When taken with water before meals, these types of fiber bind to the water in the stomach and small intestine to form a gelatinous, viscous mass that not only slows down the absorption of glucose but also induces a sense of satiety (fullness) and reduces the absorption of calories.

One of the most viscous naturally occurring dietary fibers is glucomannan, a soluble fiber obtained from grinding the root of konjac, a plant that has been used as a food and remedy for thousands of years in Asia. Highly refined and uniquely processed glucomannan possesses the greatest viscosity of any single dietary fiber. It is three times more viscous than guar and approximately seven times more viscous than psyllium. Konjac fiber is now easily available in the form of noodles made with konjac root.

PGX is a novel natural polysaccharide matrix composed of three natural compounds (glucomannan, alginate, and xanthan gum) combined in a proprietary process that leads them to coalesce and form an entirely new matrix that is one of the most viscous fibers ever discovered.[153,154] By binding glucomannan with alginate and xanthan gum, the viscosity of glucomannan can be amplified to a viscosity three to five times higher than that of glucomannan alone. PGX reduces the glycemic index of any food or beverage by 15% to 70% and also reduces postprandial glucose levels when added to or taken with foods.[155,156] In a double-blind study with an earlier version of PGX, 3 weeks of supplementation with meals was shown to lower postprandial blood glucose by approximately 20% and insulin secretion by approximately 40%, producing a whole-body insulin-sensitivity index improvement of nearly 50%.[157] It was also shown to reduce total cholesterol (12.4%), LDL-C (22.3%), the LDL-C:HDL-C ratio (15%), and serum fructosamine (5%). The typical dosage for PGX is 2.5 to 5 grams before meals. PGX is discussed further in Chapter 201, Obesity.

Natural Glucosidase Inhibitors

Starches, complex carbohydrates, and even simple sugars (disaccharides) like sucrose are broken down in the digestive tract into glucose by the action of certain enzymes. Among the most important of these are the α-glucosidases that line the intestines. Because these enzymes are essential for the breakdown of starches, complex carbohydrates, maltose, and sucrose into absorbable glucose molecules, their inhibition can diminish the after-meal rise in both glucose and insulin.

Acarbose (Precose) and miglitol (Glyset) are approved drugs for treating diabetes by inhibiting α-glucosidase. Although clinical studies have shown them to be effective, they are also characterized by a high frequency of mild to moderate gastrointestinal side effects, such as flatulence, diarrhea, and abdominal discomfort. Although these side effects generally diminish in frequency and intensity with time, few patients are willing to deal with them. Natural alternatives include berberine and mulberry extract.

Berberine

Berberine is an alkaloid found in many plants (see Chapter 86, *Hydrastis canadensis* (Goldenseal) and Other Berberine-Containing Botanicals). In the early 1900s, purified berberine also emerged as a popular natural medicine in China. Berberine exerts a number of

TABLE 165.5 Influence of Mulberry and Glyburide Treatments on Blood Glucose, Glycosylated Hemoglobin, and Serum Lipids of Patients With Type 2 Diabetes

	GLYBURIDE			MULBERRY		
Parameter	**Before**	**After**	**Change (%)**	**Before**	**After**	**Change (%)**
Fasting blood glucose (mg/dL)	154.4	141.8	–8	152.7	110.5	–27
$HgbA_{1c}$ (%)	12.5	12.4	0	12.5	11.2	–10
Cholesterol (mg/dL)	190	182	–4	193.7	170.3	–12
LDL cholesterol (mg/dL)	102.5	95.5	–7	102.1	78.7	–23
HDL cholesterol (mg/dL)	49.8	51.3	+3	50.1	59.2	+18
Triglycerides (mg/dL)	199.5	180	–10	200.4	168	–16
Free fatty acids (pmol/dL)	589.8	580	–2	590.1	520	–12

HDL, high-density lipoprotein; *LDL*, low-density lipoprotein.

interesting effects beneficial in T2DM, including inhibition of glucosidase. A meta-analysis of 27 published clinical trials using berberine as a therapeutic agent in T2DM, elevated blood lipids, and high blood pressure showed quite convincingly that in the treatment of T2DM, berberine along with lifestyle intervention lowered the level of fasting blood sugar levels, after-meal blood sugar levels, and glycosylated hemoglobin (HbA_{1c}) more than lifestyle intervention alone or placebo. When berberine was compared with oral hypoglycemic drugs used in T2DM, there was no statistical significance between treatment with berberine and these drugs. In other words, the clinical results seen with berberine were on par with those of the drugs.[158]

Much of berberine's health benefits in T2DM may be the result of modulating the gut microbiota. One of the easy links to this line of thought is that berberine has a very low oral bioavailability. The gut absorption rate of berberine is about 9%, and systemic oral bioavailability is only about 1% of an administered dosage. With so many double-blind studies showing clinical effects, it is thought to work via the microbiome specifically by increasing level of *Akkermansia muciniphila* because its colonization in the gut is inversely associated with diabetes, obesity metabolic syndrome, and low-grade chronic inflammation.[159]

Morus indica

The mulberry plant *(Morus indica)* is probably best known as food for silkworms, but it has also been highly regarded in traditional Chinese and Japanese medicine for human use. It has been shown to possess significant hypoglycemic effects in animal studies, and it contains an effective α-glucosidase inhibitor along with other compounds that appear to improve blood glucose control.[160] Mulberry extract has been studied in T2DM, and the results are excellent. In one study, researchers decided to investigate its effect on blood and RBC lipids as well as to compare its blood glucose–lowering actions with those of the oral antihyperglycemic drug glyburide.[161] Patients were given either dried mulberry leaves at a dosage of 3 g/day or one tablet of glyburide (5 mg/day) for 4 weeks. Mulberry therapy significantly improved diabetic control in patients with T2DM (Table 165.5). The results clearly show that fasting blood glucose concentrations were significantly lowered with mulberry therapy, suggesting that it is effective in controlling diabetes. Compared with glyburide treatment, mulberry therapy significantly reduced the fasting blood glucose concentrations of patients with diabetes (27%; $P < 0.01$). However, no significant differences were observed in blood glucose concentrations between pretreatments and posttreatments with glyburide (P 0.05). Mulberry extract was also superior to the approved drug in its ability to decrease $HgbA_{1c}$, total cholesterol, LDL-C, and triglycerides. It also resulted in an increase in HDL-C (the "good cholesterol"). Although these changes were not statistically significant ($P > 0.05$), there are strong suggestions that this natural product is clearly superior to an established pharmaceutical agent (see Table 165.5).

In addition to the benefits for blood glucose levels and blood lipids, mulberry therapy was also shown to reduce the amount of lipid peroxidation to the cell membranes of RBCs, indicating a significant antioxidant effect. Additionally, mulberry therapy significantly decreased membrane cholesterol in patients with T2DM.

Improving Insulin Function and Sensitivity

The first step in improving insulin function and sensitivity is achieving ideal body weight and following the dietary and lifestyle recommendations given earlier, including taking a high-potency multivitamin/multimineral supplement to make sure that the body has all essential vitamins and minerals required for proper insulin sensitivity. If additional support is necessary to bring blood glucose levels under control, we would recommend using in isolation or in scientifically formulated combinations one or more of the following: *Gymnema sylvestre* extract, bitter melon, *P. quinquefolius* (American ginseng) or *Panax ginseng*, and fenugreek seed extract. We also recommend increasing the intake of onions and garlic.

Gymnema sylvestre

Gymnema is a plant from India that has long been used as a treatment for diabetes. Recent scientific investigation has upheld its effectiveness in both T1DM and T2DM. Gymnema extracts have been shown to enhance glucose control in diabetic dogs and rabbits. Interestingly, in animals that have their pancreas removed, gymnema has no apparent effects, suggesting that it enhances the production or activity of insulin. There is evidence in animal studies that gymnema promotes the regeneration of insulin-producing beta cells in the pancreas. Studies in humans also seem to support the possibility of pancreatic regeneration.[162]

An extract of the leaves of *G. sylvestre* given to 27 patients with T1DM on insulin therapy was shown to reduce insulin requirements and fasting blood glucose levels as well as to improve blood glucose control.[163] These results indicate that gymnema enhances the action of insulin because these patients with diabetes were not recently diagnosed. Clinical experience also shows that gymnema has a significant benefit in decreasing cravings for carbohydrates and enabling patients to follow a lower-carbohydrate diet.

With regard to T2DM, gymnema extract appears to work by enhancing the action of insulin. In one study, 22 patients with T2DM were given gymnema extract along with their oral antihyperglycemic drugs.[164] All demonstrated improved blood glucose control, 21 of the 22 were able to reduce their drug dosage considerably, and 5 were able to discontinue their medication and maintain blood glucose control with the gymnema extract alone.

The dosage for gymnema extract (standardized to contain 24% gymnemic acid) can range from 200 mg twice a day to 2400 mg/day. No side effects have been reported from gymnema extract.

Momordica charantia

In addition to being eaten as a vegetable in Asia, unripe bitter melon has been used extensively in folk medicine as a remedy for diabetes. The blood glucose–lowering action of the fresh juice or extract of the unripe fruit has been clearly established in modern scientific studies in both T1DM and T2DM.

Bitter melon is composed of several compounds with confirmed blood glucose–lowering properties. Charantin, extracted by alcohol, is a hypoglycemic agent composed of mixed steroids that is more potent than the oral hypoglycemic drug tolbutamide. Bitter melon also contains an insulin-like polypeptide, polypeptide-P, which lowers blood glucose levels when injected like insulin into patients with T1DM. Because it appears to have fewer side effects than insulin, bitter melon has been suggested as a replacement therapy for some patients, although there is little likelihood of this application ever being developed. Fortunately, drinking as little as 2 oz of the juice has shown good results in clinical trials.[165,166]

Unripe bitter melon is available primarily at Asian grocery stores. Health food stores may have bitter melon extracts, but the fresh juice is probably the best to use because this was what was used in the studies. Bitter melon juice is difficult to make palatable. As its name implies, it is quite bitter, so it is recommended that patients plug their noses and take a 2-oz shot of the juice. The dosage of other forms should approximate this dose.

Panax quinquefolium and *Panax ginseng*

Research conducted at the University of Toronto's Risk Factor Modification Center has uncovered important properties of some ancient natural medicines. In their first studies and published reports, the group used whole powdered American ginseng root *(Panax quinquefolium)* and found that a dose of about 3 g before each meal reduced postprandial blood glucose significantly in patients with T2DM.[167–170]

P. ginseng can also be helpful. In a double-blind controlled study, 36 patients with non–insulin-dependent diabetes were treated for 8 weeks with ginseng extract at 100 or 200 mg or with a placebo. Ginseng elevated mood, improved psychophysiological performance, and reduced fasting blood glucose and body weight. The 200-mg dose improved glycated hemoglobin, serum aminoterminalpropetide concentrations, and physical activity.[171]

Fenugreek

Fenugreek seeds have demonstrated significant antidiabetic effects in experimental and clinical studies. The active principles are the special soluble fiber of fenugreek along with the alkaloid trigonelline and 4-hydroxyisoleucine. Fenugreek appears to be helpful in both types of diabetes. Defatted fenugreek seed powder given twice daily at a 50-g dose to patients with T1DM led to a significant reduction in fasting blood glucose and improved the results of glucose tolerance tests.[172] A 54% reduction in 24-hour urinary glucose excretion and significant reductions in LDL-C and VLDL-C and triglyceride values also occurred. In patients with T2DM, the addition of 15 g of powdered fenugreek seeds soaked in water significantly reduced postprandial glucose levels during the meal tolerance test.[173] However, that is a very large dose and impractical for daily supplementation. In another study, 25 patients with T2DM randomly received 1 g/day of fenugreek seed extract or placebo capsules for 2 months.[174] Complex analysis of the data found that the group taking the fenugreek seed extract had improved blood glucose measurements (e.g., fasting blood glucose levels dropped from 148.3–119.9 mg/dL), but there was also a significant decrease in insulin output. This finding indicates that there was a significant improvement in insulin sensitivity, an effect that is most likely due to the 4-hydroxyleucine.

Allium cepa and *Allium sativum*

Onions and garlic appear to have significant blood glucose–lowering action. The active principles are believed to be the sulfur-containing compounds allyl propyl disulfide and diallyl disulfide oxide (allicin), respectively, although other constituents such as flavonoids may play a role as well.

Although garlic generally has more potent effects, onions can be given at higher doses, and the active compounds appear to be more stable than allicin. Graded doses of onion extracts (1 mL of extract = 1 g of whole onion) at levels sometimes found in the diet (i.e., 1–7 oz of onion) reduced blood glucose levels during an oral glucose tolerance test in a dose-dependent manner. The effects are similar in both raw and boiled onion extracts, indicating that the active components are probably stable.[175]

Garlic has a wide range of additional well-documented effects that are useful for patients with diabetes, including helping improve blood glucose control, lower cholesterol and blood pressure, and inhibit some of the factors associated with increased risk for vascular complications of diabetes, such as increased fibrinogen levels.

Preventing Nutritional and Oxidative Stress

Diabetes is characterized by increased nutritional and oxidative stress. Because individuals with diabetes typically have elevated levels of free radicals and oxidative compounds, they are much more likely to experience damage. These highly reactive compounds bind to and destroy cellular compounds. They also greatly increase the inflammatory process by adding fuel to their destructive fire via increased formation of inflammatory mediators like C-reactive protein.

One of the critical goals in nutritionally supporting individuals with diabetes is to flood the body with a high level of antioxidant compounds to counteract the negative effects of free radicals and prooxidants. This goal is implemented by using the recommendations given earlier, along with taking a flavonoid-rich extract and alpha-lipoic acid.

Flavonoids

Flavonoids are extremely important considerations in both T1DM and T2DM, both in terms of possibly improving glycemic control and definitely in the prevention of diabetes complications. A variety of individual flavonoids, like quercetin, as well as flavonoid-rich extracts such as extracts of proanthocyanidolic oligomers (see Chapter 106), *Silybum marianum* (see Chapter 113), *Vaccinium myrtillus* (see Chapter 122), and *Craetagus oxyacantha* (see Chapter 71), have shown favorable mechanisms in blood sugar control, insulin secretion, prevention of oxidative damage, and inhibition of glycosylation and sorbitol accumulation.

In individuals with diabetes who are already showing signs of long-term complications, it is extremely important to take a flavonoid-rich extract. Because certain flavonoids concentrate in specific tissues, it is possible to take flavonoids that target those tissues. For example, because the flavonoids of bilberry *(V. myrtillus)* have an affinity for the eye, including the retina, bilberry is probably the best choice in a patient with diabetes who is already exhibiting signs of diabetic retinopathy. The point is to identify which flavonoid or flavonoid-rich extract is most appropriate for the patient and then to direct the patient to take it according to the recommended dosage. There is tremendous overlap among the mechanisms of action and benefits of flavonoid-rich extracts; it is important to take the one that is most specific to the individual's needs (Table 165.6).

TABLE 165.6 Flavonoids Used in the Treatment of Diabetes and Diabetic Complications

Flavonoid-Rich Extract	Daily Dose	Indication
Bilberry extract (25% anthocyanidins)	160–320 mg	Best choice in diabetic retinopathy or cataracts.
Ginkgo biloba extract (24% Ginkgo flavonglycosides)	120–240 mg	Best choice for most people > age 50. Protects the brain and vascular lining. Very important in improving blood flow to the extremities in treating neuropathy and foot ulcers.
Grape seed or pine-bark extract (95% procyanidolic oligomers)	150–300 mg	Systemic antioxidant; best choice for most people < age 50, especially if retinopathy, hypertension, easy bruising, and poor wound healing exist. Also specific for the lungs, varicose veins, and protection against cardiovascular disease.
Green tea extract (60%–70% total polyphenols)	150–300 mg	Best choice in the early stage of type 1 diabetes or if there is a family history of cancer.
Hawthorn extract (10% procyanidins)	150–300 mg	Best choice in cardiovascular disease or hypertension.
Milk thistle extract (70% silymarin)	100–300 mg	Best choice if there are signs of impaired liver function.
Mixed citrus flavonoids	1000–2000 mg	Least expensive choice but may not provide same level of benefit; use if no complication is present.
Quercetin	150–300 mg	Good choice if allergies, symptoms of prostate enlargement or bladder irritation, or eczema are also present.

Alpha-Lipoic Acid

Alpha-lipoic acid is a vitamin-like substance that is often described as "nature's perfect antioxidant." Alpha-lipoic acid is a small molecule that is efficiently absorbed and easily crosses cell membranes. Unlike vitamin E, which is primarily fat soluble, and vitamin C, which is water soluble, α-lipoic acid can quench either water- or fat-soluble free radicals both inside the cell and in the intracellular spaces. Furthermore, α-lipoic acid extends the biochemical lives of vitamins C and E as well as other antioxidants.

Although α-lipoic acid's primary benefit is as an antioxidant, it has also been shown to lead to an improvement in blood glucose metabolism as well as blood flow to peripheral nerves and even to stimulate the regeneration of nerve fibers.[176] Alpha-lipoic acid is an approved drug in Germany for the treatment of diabetic neuropathy. In fact, it has been successfully used in Germany for more than 40 years. The beneficial effects of α-lipoic acid in diabetic neuropathy at a dosage of 400 to 600 mg daily have been confirmed in several double-blind studies.[177,178]

Recommendations for Specific Chronic Complications

Following are additional recommendations for dealing with the specific complications of diabetes. The most important method for reducing the risk of these complications is achieving optimal blood glucose control.

Elevated Cholesterol

Key natural products used to lower cholesterol levels in diabetes are soluble fiber, garlic, berberine, and niacin. These agents are also discussed in Chapter 149. Berberine may yield the best results because it also addresses blood sugar control and hypertension. Regarding niacin, because taking niacin at higher doses (e.g., 3000 mg or more) can impair glucose tolerance, many physicians have avoided niacin therapy in patients with diabetes. However, studies with slightly lower doses (1000–2000 mg) have not shown that niacin affects the regulation of blood glucose adversely.[179] For example, during a 16-week double-blind placebo-controlled trial, 148 patients with T2DM were randomized to placebo or 1000 or 1500 mg/day of niacin; in the niacin-treated groups, there was no significant loss of glycemic control, and the favorable effects on blood lipids were still apparent.[180] Other studies have actually shown $HgbA_{1c}$ to drop, indicating improved glycemic control.[181]

The most common blood lipid abnormality in patients with T2DM is elevated triglyceride levels; decreased HDL cholesterol levels; and a preponderance of smaller, denser LDL particles. Niacin has been shown to address all of these areas much more significantly than statins or other lipid-lowering drugs. However, one reason that niacin may not be as popular as it should be is the side effect of skin flushing—rather like a prickly heat rash—which typically occurs 20 to 30 minutes after the niacin is taken and disappears within about the same time frame. Other occasional side effects include gastric irritation, nausea, and liver damage. The risk of liver damage is especially pertinent. Patients with diabetes who are overweight often develop fatty livers, which are now considered to be as damaging as livers injured by alcoholism and hepatitis C. Moreover, a fatty liver may facilitate the development of fibrosis and cirrhosis. Giving niacin to a patient with a fatty liver may put extra stress on that vital organ. Therefore in an overweight patient with diabetes, high-dose niacin is to be used only under a physician's supervision. To reduce the side effect of skin flushing, an intermediate-release niacin may be prescribed, one that is identical in its dissolution pattern to the prescription product Niaspan. This product is best taken just before going to bed because most people sleep right through any flushing reaction that might occur.

If regular niacin is being used, a dosage of 500 mg a night may be tried for 1 week. The dose can then be increased to 1000 mg the next week and 1500 mg the following week. The 1500-mg dose should be given for 2 months before checking the response, and the dose can then be adjusted up or down as needed. Time-release niacin products like Niaspan can be used at the full dosage of 1000 to 2000 mg at night from the beginning. Regardless of the form of niacin being used, periodic checking (at least every 3 months) of cholesterol, $HgbA_{1c}$, and liver function is strongly indicated.

Retinopathy and Cataracts

Diabetic retinopathy has two forms: (1) "simple" retinopathy consisting of burst blood vessels, hemorrhages, and swelling and (2) proliferative retinopathy with newly formed vessels, scarring, more serious hemorrhage, and retinal detachment. Laser photocoagulation therapy is an important treatment for the more severe type of proliferative retinopathy but is still not indicated in milder forms because of the risk of vision loss. That is, there is a risk that the side effects of this treatment may outweigh the benefits.

As with other complications of diabetes, prevention is the best treatment, with the key factor being the maintenance of optimal blood glucose control. $HgbA_{1c}$ should be monitored frequently.

Extremely important in the battle against retinopathy are flavonoid-rich extracts, especially bilberry, pine bark, and grape seed extracts.

The flavonoids in these extracts exert many benefits in diabetes, including an ability to increase intracellular vitamin C levels, decrease the leakiness and breakage of capillaries, prevent easy bruising, and serve as potent antioxidants. These effects are of particular value in dealing with the microvascular abnormalities of diabetes. Because the flavonoids in bilberry, pine bark, and grape seed extract have an affinity for the blood vessels of the eye and improve circulation to the retina, they are particularly helpful in slowing the progression of diabetic retinopathy, as evidenced by positive results in more than a dozen clinical trials (see Chapters 106 and 122).

Neuropathy

In addition to α-lipoic acid and the basic supplementation program, three additional natural medicines, along with acupuncture, deserve mention here.

Gamma-linolenic acid (GLA) has been shown to improve and prevent diabetic neuropathy. Diabetes is associated with a substantial disturbance in EFA metabolism. One of the key disturbances is the impairment in the process of converting linoleic acid to GLA. As a result, providing GLA in the form of borage, evening primrose, or black currant oil can bypass some of this disturbance. In the GLA Multicenter Trial, 111 patients with mild diabetic neuropathy were given either GLA at a dose of 480 mg/day or a placebo for 1 year. Sixteen parameters were assessed, including conduction velocities, hot and cold thresholds, sensation, tendon reflexes, and muscle strength. After 1 year, all 16 parameters improved, 13 of them to a statistically significant degree. Treatment was more effective in patients with diabetes who were relatively well controlled than in those who were poorly controlled. The latter finding highlights the need for a comprehensive approach in controlling blood glucose levels rather than expecting a single physiological aid (i.e., GLA) to compensate for poor control.[182]

Benfotiamine is a fat-soluble form of thiamine, or vitamin B_1, that is more effective in raising blood thiamine levels (up to 120%–240% vs. regular thiamine). In studies in patients with diabetes, benfotiamine decreased advanced glycosylated end-product formation, decreased the aldose reductase pathway, and reduced oxidative cellular damage.[183] However, results in the treatment of diabetic neuropathy and nephropathy in small clinical trials with benfotiamine alone have shown modest to no benefit.[184,185] It is possible that benfotiamine can be usefully combined with α-lipoic acid. In a small study in patients with T1DM, treatment with 600 mg of benfotiamine plus 300 mg of α-lipoic acid produced better results in reducing the effects of hyperglycemia than benfotiamine alone.[186]

Capsaicin is the active component of cayenne pepper *(Capsicum frutescens),* which stimulates and then blocks the small nerve fibers that transmit the pain impulse by depleting these fibers of a transmitting substance known as substance P. In numerous double-blind studies, topically applied capsaicin has been shown to be of considerable benefit in relieving the pain of diabetic neuropathy. Roughly 80% of people with diabetic neuropathy experience great relief in this way. Commercial ointments containing 0.025% or 0.075% capsaicin are available over the counter. The patient should apply the 0.075% cream twice daily to the affected area (the first covering his or her hand with plastic wrap to prevent the capsaicin from coming in contact with the eyes or mucous membranes). It may take a few days for the cream to start working, and it will continue to work only with regular application.

Acupuncture can also be helpful in improving neuropathy. The scientific investigation of acupuncture in diabetes includes both experimental and clinical studies. For example, animal experiments have shown that acupuncture can act on the pancreas to enhance insulin synthesis, increase the number of receptors on target cells, and accelerate the utilization of glucose, resulting in the lowering of blood glucose.[187] However, the best documentation for the clinical application of acupuncture is in treating chronic painful diabetic neuropathy. In one clinical study, 77% of patients treated with acupuncture noted a significant improvement in their symptoms, with 21% noting that their symptoms were completely eliminated.[188] That success rate is excellent considering the long-standing nature of the condition in most of the cases and the fact that no side effects were observed.

Nephropathy

Many of the previous recommendations also apply to preventing diabetic nephropathy. In fact, a strong reason for adhering to the dietary principles outlined previously is to protect the diabetic kidneys. Especially important, again, is dietary fiber. Dietary fibers (especially the water-soluble type) are fermented in the colon to produce short-chain fatty acids. These by-products are the primary fuel for colonic cells and, if present in high amounts, greatly increase the colon's waste-removal capabilities. It has been shown that in the presence of a diet high in fermentable fiber, the colon turns into the "second kidney" as it collects nitrogenous wastes out of the blood and disposes of them via the feces. This has been shown to greatly reduce the workload and stress on the kidneys themselves.[189]

Highlighting just how important some basic supplement recommendations are in halting the progression of diabetic nephropathy are the results of a study of 30 patients with T2DM with elevated albumin in their urine. These patients received vitamin C (1250 mg) and vitamin E (680 IU) daily or a matching placebo for 4 weeks, followed by a 3-week washout period before being switched to the other arm.[190] The results were that the vitamins were successful in reducing urinary albumin levels by an average of nearly 20%, indicating that antioxidant therapy may halt or slow down the progression of kidney disease in patients with diabetes.

If a patient has developed serious kidney failure, then following the low-protein, low-potassium standard diet becomes necessary; unfortunately, that does not promote good glucose control, which can then worsen kidney function. The main goal is to prevent end-stage renal disease from developing in the first place.

If drugs are necessary, the ACE inhibitors and ACE receptor blockers offer the greatest benefits in dealing with diabetic nephropathy.[191] They are now often prescribed in low doses to help prevent nephropathy even in the absence of high blood pressure. Alternatively, a special preparation of bonito peptides has been shown to exert anti-ACE activity (see Chapter 179 for more information) and may prevent the need for ACE-inhibitor drugs.

Poor Wound Healing

A deficiency of virtually any essential nutrient can lead to impaired wound healing. Key nutrients include vitamin C and zinc, both of which are often deficient in patients with diabetes. Taking a high-potency multivitamin/multimineral formula should improve nutritional status and promote proper wound healing. For topical application, pure (100%) *Aloe vera* gel can be used. The wound-healing effects of *Aloe vera* are well known. It contains a number of compounds that promote wound healing, including vitamin C, vitamin E, and zinc. It has been shown to stimulate many factors important to wound repair and should be applied to affected areas (not open wounds) two to three times daily. Another option is a topical ointment called Amerigel, featuring an oak extract *(Quercus rubra)*, which contains tannins, including quercitannic acid, catechin, ellagitannin, and proanthocyanidin, that are readily absorbed into damaged skin.

Foot Ulcers

Lack of blood supply, poor wound healing, and peripheral neuropathy are key factors in the development of diabetic foot ulcers. Key strategies

in prevention and treatment are proper foot care (including professional care of nails and calluses), preferably by a podiatrist; regular physician's examination of the feet; avoidance of injury; avoidance of tobacco in any form; and the use of methods to improve the local circulation. Proper foot care includes keeping the feet clean, dry, and warm and wearing well-fitting shoes. Tobacco use in any form constricts the peripheral blood vessels and can lead to more serious peripheral vascular disease, including severe arterial blockages. Diabetes and smoking are like nitro plus glycerin—a sure recipe for catastrophe. Circulation can be improved by exercising regularly, avoiding sitting cross-legged or in other positions that compromise circulation, and massaging the feet lightly upward. *Ginkgo biloba* or grape seed extract can also be used to support optimal circulation.

THERAPEUTIC APPROACH

Effective treatment of patients with diabetes requires the careful integration of wide-ranging therapies and patients who are willing to substantially improve their lifestyles. T2DM is usually the result of many years of chronic metabolic insult and, although treatable with the natural metabolic approach presented here, requires persistence for its ultimate resolution. Although much of the information presented in this chapter focuses on T2DM, it is equally appropriate for T1DM, with the exception that, according to current information, the patient with T1DM will always require insulin.

The first step in the therapy of either T1DM or T2DM is a thorough diagnostic workup. Of particular importance is identifying any of the complications of diabetes. The patient's diet, environment, and lifestyle should be carefully studied to rule out any exposure to agents that may be inducing his or her glucose intolerance.

A diet, exercise, and supplement program that meets the patient's individual needs and his or her current level of glucose tolerance should be developed. For maximum efficacy, the patient's ideal body weight must be achieved (see Chapter 201 for additional recommendations).

In treating patients with severe or uncontrolled diabetes, a high level of awareness of the acute diabetic complications must be maintained. If the patient is on insulin, the physician should be adept at using, changing, and adapting insulin types and doses according to the individual's particular needs.

Finally, the patient must be monitored carefully, particularly if he or she is on insulin or has relatively uncontrolled diabetes. Patient symptomatology, home glucose monitoring, and the $HgbA_{1c}$ test are at present the best ways to monitor progress. It is important to recognize that as these therapies take effect, the patient's drug dosages must be altered and that a good working relationship with the physician will greatly aid the healing process. The ultimate goal is to reestablish normal glycemia and prevent the development of (or ameliorate) the complications of diabetes.

Diet

The optimal healthy diet detailed in Chapter 44 is clearly the diet of choice. All simple, processed, and concentrated carbohydrates must be avoided. A low-GI diet rich in high-fiber foods should be stressed, and sources of healthy fats should be ingested. Low-GI vegetables, particularly onions and garlic, are useful, and their liberal ingestion should be encouraged.

Supplementation for Type 1 Diabetes

The recommended supplementation program depends on the degree of blood glucose control, as evidenced by self-monitored blood glucose and $HgbA_{1c}$ levels.

Recently diagnosed T1DM

- Foundation supplements
  - High-potency multivitamin/multimineral supplement
  - Fish oils: 1000 mg of EPA and DHA (combined total) a day
  - Vitamin C: 500 to 1500 mg a day
  - Vitamin E (mixed tocopherols): 400 to 800 IU a day
- Vitamin D: 4000 to 8000 IU a day (vitamin K_2 should always be used with vitamin D)
- Niacinamide: 25 to 50 mg/kg a day
- Green tea extract: The recommended dosage for children 6 years of age or younger is 50 to 150 mg; for children 6 to 12 years old, 100 to 200 mg; for children older than 12 years of age and adults, 150 to 300 mg. The green tea extract should have a polyphenol content of 80% and be decaffeinated.

Level 1: Achievement of targeted blood glucose levels, $HgbA_{1c}$ levels less than 7%, no lipid abnormalities, no signs of complications

- High-potency multivitamin/multimineral supplement
- Fish oils: 600 mg each of EPA and DHA a day
- Vitamin C: 500 to 1500 mg a day
- Vitamin E (mixed tocopherols): 400 to 800 IU a day
- Alpha-lipoic acid: 400 to 600 mg a day
- Grape seed, pine bark, or green tea extract (or other appropriate flavonoid-rich extract): 100 to 300 mg a day

Level 2: Failure to achieve targeted blood glucose levels, $HgbA_{1c}$ above 7%

- Level 1 supplements plus:
  - *G. sylvestre* extract (24% gymnemic acid): 200 mg twice a day
  - Biotin: 8 mg twice a day
- Optional:
  - Bitter melon juice: 2 to 4 oz a day

Supplementation for Type 2 Diabetes

The recommended supplementation program depends on the degree of blood glucose control, as evidenced by self-monitored blood glucose and $HgbA_{1c}$ levels.

Level 1: Achievement of targeted blood glucose and $HgbA_{1c}$ levels less than 7%, no lipid abnormalities, no signs of complications

- High-potency multivitamin/multimineral supplement
- Greens drink: one serving a day
- Fish oils: 600 mg each of EPA and DHA a day
- Vitamin C: 500 to 1500 mg a day
- Vitamin E (mixed tocopherols): 400 to 800 IU a day
- Grape seed, pine bark, or green tea extract (or other appropriate flavonoid-rich extract): 100 to 150 mg a day
- Alpha-lipoic acid: 400 to 600 mg a day
- PGX (glucomannan, or other soluble source at equivalent dosage to PGX): 2500 to 5000 mg before meals

Level 2: Failure to achieve targeted blood glucose levels, $HgbA_{1c}$ above 7%

- Level 1 support plusOne of the following:
  - Berberine: 500 mg two or three times daily just before meals
  - Mulberry extract: equivalent to 1000 mg dried leaf three times a day
  - *G. sylvestre* extract (24% gymnemic acid): 200 mg twice a day
  - Fenugreek extract: 1 g a day
  - Garlic: minimum of 4000 mcg of allicin per day

If self-monitored blood glucose levels do not improve after 4 weeks of following the recommendations for the current level, move to the next highest level. For example, if the patient starts out having an $HgbA_{1c}$ level of 8.2% and a fasting blood glucose level of 130 mg/dL, he or she should start on level 2 support. After 4 weeks, if the average reading has not dropped to less than 110 mg/dL, a prescription medication (either an oral hypoglycemic drug or insulin) will be required.

Additional Supplements for the Prevention and Treatment of Diabetic Complications

For high cholesterol levels and other cardiovascular risk factors
Total cholesterol greater than 200 mg/dL or LDL-C greater than 135 mg (100 mg if history of heart attack); HDL-C below 45 mg/dL; lipoprotein(a) above 40 mg/dL; or triglycerides above 150 mg/dL

- Berberine: 500 mg two or three times daily just before meals
- Niacin (intermediate release): 1000 to 2000 mg at bedtime
- Garlic: minimum of 4000 mcg of allicin a day

For hypertension

- Choose one of the following:
  - Berberine: 500 mg two or three times daily just before meals
  - Garlic: minimum of 4000 mcg of allicin a day
  - Coenzyme Q10: 100 to 200 mg a day
  - Anti-ACE peptides from bonito: 1500 mg/day
- Choose one of the following:
  - Hawthorn extract (10% procyanidins or 1.8% vitexin with 4% rhamnoside): 100 to 250 mg three times a day
  - Olive leaf extract (17%–23% oleuropein content): 500 mg twice a day
  - Hibiscus: three 240-mL servings a day or an extract providing 10 to 20 mg anthocyanidins a day

For diabetic retinopathy

- Bilberry extract: 160 to 320 mg daily or grape seed extract 150 to 300 mg a day

For diabetic neuropathy

- Gamma-linolenic acid from borage, evening primrose, or blackcurrant oil: 480 mg a day
- Benfotiamine: 600 mg a day
- Capsaicin (0.075%) cream: applied to affected area twice a day

For diabetic nephropathy

- Follow previous recommendations for high blood pressure unless kidney function falls below 40% of normal. Be cautious in recommending magnesium and potassium supplements.
- Benfotiamine: 600 mg a day

For poor wound healing

- *A. vera* gel: Applied to affected areas twice a day

For diabetic foot ulcers

- *G. biloba* extract: 120 to 240 mg a day or grape seed extract 150 to 300 mg a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Moore J, Chaudhary N, Akinyemiju T. Metabolic syndrome prevalence by race/ethnicity and sex in the United States, national health and nutrition examination Survey, 1988-2012. *Prev Chronic Dis*. 2017;14:E24. https://doi.org/10.5888/pcd14.160287.
2. Cook S, Auinger P, Li C, et al. Metabolic syndrome rates in United States adolescents, from the national health and nutrition examination Survey, 1999-2002. *J Pediatr*. 2008;152(2):165–170.
3. Ford ES, Li C. Metabolic syndrome and health-related quality of life among U.S. adults. *Ann Epidemiol*. 2008;18(3):165–171.
4. American Diabetes Association. Standards of medical care in diabetes—2018. *Diabetes Care*. 2018;41(suppl 1).
5. Kelly MA, Mijovic CH, Barnett AH. Genetics of type 1 diabetes. *Best Pract Res Clin Endocrinol Metab*. 2001;15:279–291.
6. Akerblom HK, Vaarala O, Hyoty H, et al. Environmental factors in the etiology of type 1 diabetes. *Am J Med Genet*. 2002;115:18–29.
7. Knip M, Akerblom HK. Environmental factors in the pathogenesis of type 1 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 1999;107(suppl 3):S93–S100.
8. Kaprio J, Tuomilehto J, Koskenvuo M, et al. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia*. 1992;35:1060–1067.
9. Redondo MJ, Yu L, Hawa M, et al. Heterogeneity of type 1 diabetes: analysis of mono-zygotic twins in Great Britain and the United States. *Diabetologia*. 2001;44:354–362. [Erratum in Diabetologia 2001;44:927].
10. Metcalfe KA, Hitman GA, Rowe RE, et al. Concordance for type 1 diabetes in identical twins is affected by insulin genotype. *Diabetes Care*. 2001;24:838–842.
11. Onkamo P, Vaananen S, Karvonen M, et al. Worldwide increase in incidence of type 1 diabetes: the analysis of the data on published incidence trends. *Diabetologia*. 1999;42:1395–1403. [Erratum in Diabetologia 2000;43:685].
12. Feltbower RG, Bodansky HJ, McKinney PA, et al. Trends in the incidence of childhood diabetes in south Asians and other children in Bradford, UK. *Diabet Med*. 2002;19:162–166.
13. Bodansky HJ, Staines A, Stephenson C, et al. Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population. *BMJ*. 1992;304:1020–1022.
14. Elliott RB. Epidemiology of diabetes in Polynesia and New Zealand. *Pediatr Adolesc Endocrinol*. 1992;21:66–71.
15. Zheng P, Li Z, Zhou Z. Gut microbiome in type 1 diabetes: a comprehensive review. *Diabetes Metab Res Rev*. 2018;34(7):e3043. https://doi.org/10.1002/dmrr.3043. Epub 2018 Jul 17.
16. Hypponen E, Kenward MG, Virtanen SM, et al. Infant feeding, early weight gain, and risk of Type 1 diabetes. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care*. 1999;22:1961–1965.
17. Monetini L, Cavallo MG, Manfrini S, et al. Antibodies to bovine beta-casein in diabetes and other autoimmune diseases. *Horm Metab Res*. 2002;34:455–459.
18. Richardson SJ, Morgan NG. Enteroviral infections in the pathogenesis of type 1 diabetes: new insights for therapeutic intervention. *Curr Opin Pharmacol*. 2018;43:11–19. https://doi.org/10.1016/j.coph.2018.07.006.
19. Allen DW, Kim KW, Rawlinson WD, Craig ME. Maternal virus infections in pregnancy and type 1 diabetes in their offspring: systematic review and meta-analysis of observational studies. *Rev Med Virol*. 2018;28(3):e1974. https://doi.org/10.1002/rmv.1974.
20. EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*. 1999;42:51–54.
21. Hypponen E, Laara E, Reunanen A, et al. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet*. 2001;358:1500–1503.
22. Stene LC, Ulriksen J, Magnus P, et al. Use of cod liver oil during pregnancy associated with lower risk of Type 1 diabetes in the offspring. *Diabetologia*. 2000;43:1093–1098.
23. Brekke HK, Ludvigsson J. Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. *Pediatr Diabetes*. 2007;8(1):11–14.
24. Norris JM, Yin X, Lamb MM, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. *JAMA*. 2007;298(12):1420–1428.
25. Krishna Mohan I, Das UN. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. *Nutrition*. 2001;17:126–151.
26. Zhao HX, Mold MD, Stenhouse EA, et al. Drinking water composition and childhood-onset type 1 diabetes mellitus in Devon and Cornwall, England. *Diabet Med*. 2001;18:709–717.
27. Parslow RC, McKinney PA, Law GR, et al. Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. *Diabetologia*. 1997;40:550–556.
28. Kretowski A, Mysliwiec J, Szelachowska M, et al. Nicotinamide inhibits enhanced in vitro production of interleukin-12 and tumour necrosis factor-alpha in peripheral whole blood of people at high risk of developing type 1 diabetes and people with newly diagnosed type 1 diabetes. *Diabetes Res Clin Pract*. 2000;47:81–86.
29. Kolb H, Burkart V. Nicotinamide in type 1 diabetes: mechanism of action revisited. *Diabetes Care*. 1999;22(suppl 2):B16–B20.
30. Cleary JP. Vitamin B3 in the treatment of diabetes mellitus: case reports and review of the literature. *J Nutr Med*. 1990;1:217–225.
31. Pocoit F, Reimers JI, Andersen HU. Nicotinamide: biological actions and therapeutic potential in diabetes prevention. *Diabetologia*. 1993;36:574–576.
32. Pozzilli P, Andreani D. The potential role of nicotinamide in the secondary prevention of IDDM. *Diabetes Metabol Rev*. 1993;9:219–230.
33. Visalli N, Cavallo MG, Signore A, et al. A multi-centre randomized trial of two different doses of nicotinamide in patients with recent-onset type 1 diabetes (the IMDIAB VI). *Diabetes Metab Res Rev*. 1999;15:181–185.
34. Crino A, Schiaffini R, Manfrini S, et al. A randomized trial of nicotinamide and vitamin E in children with recent onset type 1 diabetes (IMDIAB IX). *Eur J Endocrinol*. 2004;150:719–724.
35. Schatz DA, Bingley PJ. Update on major trials for the prevention of type 1 diabetes mellitus: the American diabetes prevention trial (DPT-1) and the European nicotinamide diabetes intervention trial (ENDIT). *J Pediatr Endocrinol Metab*. 2001;14(suppl 1):619–622.
36. Gale EA, Bingley PJ, Emmett CL, et al. European nicotinamide diabetes intervention trial (ENDIT) group. European nicotinamide diabetes intervention trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet*. 2004;363:925–931.
37. Kretowski A, Mysliwiec J, Szelachowska M, et al. Nicotinamide inhibits enhanced in vitro production of interleukin-12 and tumour necrosis factor-alpha in peripheral whole blood of people at high risk of developing type 1 diabetes and people with newly diagnosed type 1 diabetes. *Diabetes Res Clin Pract*. 2000;47:81–86.
38. Chakravarthy BK, Gupta S, Gode KD. Functional beta cell regeneration in the islets of pancreas in alloxan induced diabetic rats by (-)-epicatechin. *Life Sci*. 1982;31:2693–2697.
39. Mukoyama A, Ushijima H, Nishimura S, et al. Inhibition of rotavirus and enterovirus infections by tea extracts. *Jpn J Med Sci Biol*. 1991;44:181–186.
40. Chung HS, Choi KM. Adipokines and myokines: a pivotal role in metabolic and cardiovascular disorders. *Curr Med Chem*. 2018;25(20):2401–2415.
41. Sattar N. Biomarkers for diabetes prediction, pathogenesis or pharmacotherapy guidance? Past, present and future possibilities. *Diabet Med*. 2012;29(1):5–13.
42. Iliodromiti S, Sassarini J, Kelsey TW, et al. Accuracy of circulating adiponectin for predicting gestational diabetes: a systematic review and meta-analysis. *Diabetologia*. 2016;59(4):692–699.
43. Ingelsson E, McCarthy MI. Human genetics of obesity and type 2 diabetes mellitus: past, present, and future. *Circ Genom Precis Med*. 2018;11(6):e002090.
44. Bennett PH. Type 2 diabetes among the Pima Indians of Arizona: an epidemic attributable to environmental change? *Nutr Rev*. 1999;57:S51–S54.
45. Nelson KM, Reiber G, Boyko EJ. Diet and exercise among adults with type 2 diabetes: findings from the third National health and nutrition examination Survey (NHANES III). *Diabetes Care*. 2002;25:1722–1728.
46. Snitker S, Mitchell BD, Shuldiner AR. Physical activity and prevention of type 2 diabetes. *Lancet*. 2003;361:87–88.

47. Hsueh WC, Mitchell BD, Aburomia R, et al. Diabetes in the old Order amish: characterization and heritability analysis of the amish family diabetes study. *Diabetes Care*. 2000;23:595–601.
48. The Diabetes Prevention Program (DPP). Description of lifestyle intervention. *Diabetes Care*. 2002;25:2165–2171.
49. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. *Am J Clin Nutr*. 2002;76:S274–S280.
50. Jenkins DJ, Kendall CW, Augustin LS, et al. Glycemic index: overview of implications in health and disease. *Am J Clin Nutr*. 2002;76:S266–S273.
51. Riccardi G, Rivellese AA, Giacco R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. *Am J Clin Nutr*. 2008;87(1):S269–S274.
52. Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in U.S. women. *Am J Clin Nutr*. 2000;71:1455–1461.
53. Wursch P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes: a review with special emphasis on cereals rich in beta-glucan. *Diabetes Care*. 1997;20:1774–1780.
54. Slama G. Dietary therapy in type 2 diabetes oriented towards postprandial blood glucose improvement. *Diabetes Metab Rev*. 1998;14(suppl 1):S19–S24.
55. Montonen J, Knekt P, Jarvinen R, et al. Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am J Clin Nutr*. 2003;77:622–629.
56. Fung TT, Hu FB, Pereira MA, et al. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *Am J Clin Nutr*. 2002;76:535–540.
57. Hung T, Sievenpiper JL, Marchie A, et al. Fat versus carbohydrate in insulin resistance, obesity, diabetes and cardiovascular disease. *Curr Opin Clin Nutr Metab Care*. 2003;6:165–176.
58. Salmeron J, Hu FB, Manson JE, et al. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr*. 2001;73:1019–1026.
59. Rivellese AA, De Natale C, Lilli S. Type of dietary fat and insulin resistance. *Ann N Y Acad Sci*. 2002;967:329–335.
60. Jiang R, Manson JE, Stampfer MJ, et al. Nut and peanut butter consumption and risk of type 2 diabetes in women. *JAMA*. 2002;288:2554–2560.
61. Sargeant LA, Khaw KT, Bingham S, et al. Fruit and vegetable intake and population glycosylated haemoglobin levels: the EPIC-Norfolk Study. *Eur J Clin Nutr*. 2001;55:342–348.
62. Williams DE, Wareham NJ, Cox BD, et al. Frequent salad vegetable consumption is associated with a reduction in the risk of diabetes mellitus. *J Clin Epidemiol*. 1999;52:329–335.
63. Reunanen A, Knekt P, Aaran RK, et al. Serum antioxidants and risk of non-insulin dependent diabetes mellitus. *Eur J Clin Nutr*. 1998;52:89–93.
64. Feskens EJ, Virtanen SM, Rasanen L, et al. Dietary factors determining diabetes and impaired glucose tolerance: a 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care*. 1995;18:1104–1112.
65. Ruhe RC, McDonald RB. Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. *J Am Coll Nutr*. 2001;5:S363–S369.
66. Facchini FS, Humphreys MH, DoNascimento CA, et al. Relation between insulin resistance and plasma concentrations of lipid hydroperoxides, carotenoids, and tocopherols. *Am J Clin Nutr*. 2000;72:776–779.
67. Salonen JT, Nyyssonen K, Tuomainen TP. Increased risk of non-insulin diabetes mellitus at low plasma vitamin E concentrations: a four year follow-up study in men. *BMJ*. 1995;311:1124–1127.
68. Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*. 2003;17:24–38.
69. Evans JL, Goldfine ID, Maddux BA, et al. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes*. 2003;52:1–8.
70. Lee DH, Lee IK, Song K, et al. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. *Diabetes Care*. 2006;29(7):1638–1644.
71. Lee DH, Lee IK, Song K, et al. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Diabetologia*. 2007;50(9):1841–1851.
72. Lee DH, Ha MH, Kim JH, et al. Gamma-glutamyltransferase and diabetes: a 4 year follow-up study. *Diabetologia*. 2003;46:359–364.
73. Liu S, Guo X, Wu B, et al. Arsenic induces diabetic effects through beta-cell dysfunction and increased gluconeogenesis in mice. *Scientific Reports*. 2014;4:6894.
74. Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, et al. Arsenic exposure and prevalence of type 2 diabetes in US adults. *JAMA*. 2008;300(7):814–822.
75. Lang IA, Galloway TS, Scarlett A, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. *JAMA*. 2008;300(11):1303–1310.
76. Stojanoska MM, Milosevic N, Milic N, Abenavoli L. The influence of phthalates and bisphenol A on the obesity development and glucose metabolism disorders. *Endocrine*. 2016. [Epub ahead of print]. PubMed PMID: 27822670.
77. Alderete TL, Habre R, Toledo-Corral CM, et al. Longitudinal associations between ambient air pollution with insulin sensitivity, beta-cell function, and adiposity in Los Angeles Latino children. *Diabetes*. 2017: Pii:db161416. [Epub ahead of print]. PubMed PMID: 28137791.
78. Weinmayr G, Hennig F, Fuks K, Nonnemacher M, et al. Long term exposure to fine particulate matter and incidence of type 2 diabetes mellitus in a cohort study: effects of total and traffic-specific air pollution. *Environ Health*. 2015;14:53. PubMed PMID: 26087770.
79. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346:393–403.
80. Goldstein DE, Little RR. Monitoring glycemia in diabetes: short-term assessment. *Endocrinol Metab Clin North Am*. 1997;26:475–486.
81. American Diabetes Association. Tests of glycemia in diabetes (position statement). *Diabetes Care*. 1997;20(suppl 1):518–520.
82. DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;32:9977–9986.
83. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;352:837–853.
84. Bertrand S, Aris-Jilwan N, Reddy S, et al. Recommendations for the use of self-monitoring of blood glucose (SMBG) in diabetes mellitus. *Diabetes Care*. 1992;20:14–16.
85. DAFNE Study Group. Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: dose adjustment for normal eating (DAFNE) randomized controlled trial. *Diabet Med*. 2003;20(suppl 3):4–5.
86. Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*. 1995;28:103–117.
87. Dungan KM. 1,5-anhydroglucitol (GlycoMarktm) as a marker of short-term glycemic control and glycemic excursions. *Expert Rev Mol Diagn*. 2008;8(1):9–19.
88. Lingenfelser T, Overkamp D, Renn W, et al. Insulin-associated modulation of neuroendocrine counterregulation, hypoglycemia perception, and cerebral function in insulin-dependent diabetes mellitus: evidence for an intrinsic effect of insulin on the central nervous system. *J Clin Endocrinol Metab*. 1996;81:1197–1205.
89. Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. *Biochim Biophys Acta*. 2014;1840(9):2709–2729.
90. Hoogeveen EK, Kostense PJ, Eysink PE, et al. Hyperhomocysteinemia is associated with the presence of retinopathy in type 2 diabetes mellitus. *Arch Intern Med*. 2000;160:2984–2990.
91. De Mattia G, Laurenti O, Fava D. Diabetic endothelial dysfunction: effect of free radical scavenging in type 2 diabetic patients. *J Diabetes Complications*. 2003;17(suppl 2):30–35.
92. Price KD, Price CS, Reynolds RD. Hyperglycemia-induced ascorbic acid deficiency promotes endothelial dysfunction and the development of atherosclerosis. *Atherosclerosis*. 2001;158:1–12.
93. Heitzer T, Finckh B, Albers S, et al. Beneficial effects of alpha-lipoic acid and ascorbic acid on endothelium-dependent, nitric oxide-mediated

vasodilation in diabetic patients: relation to parameters of oxidative stress. *Free Radic Biol Med*. 2001;31:53–61.
94. Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radic Biol Med*. 2000;28:1806–1814.
95. Gilbertson HR, Brand-Miller JC, Thorburn AW, et al. The effect of flexible low glycemic index dietary advice versus measured carbohydrate exchange diets on glycemic control in children with type 1 diabetes. *Diabetes Care*. 2001;24:1137–1143.
96. Giacco R, Parillo M, Rivellese AA, et al. Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. *Diabetes Care*. 2000;23:1461–1466.
97. Buyken AE, Toeller M, Heitkamp G, et al. Glycemic index in the diet of European outpatients with type 1 diabetes: relations to glycated hemoglobin and serum lipids. *Am J Clin Nutr*. 2001;73:574–581.
98. Toeller M, Buyken AE, Heitkamp G, et al. Prevalence of chronic complications, metabolic control and nutritional intake in type 1 diabetes: comparison between different European regions. EURODIAB Complications Study group. *Horm Metab Res*. 1999;31:680–685.
99. Kalkwarf HJ, Bell RC, Khoury JC, et al. Dietary fiber intakes and insulin requirements in pregnant women with type 1 diabetes. *J Am Diet Assoc*. 2001;101:305–310.
100. Leow ZZX, Guelfi KJ, Davis EA, Jones TW, Fournier PA. The glycaemic benefits of a very-low-carbohydrate ketogenic diet in adults with Type 1 diabetes mellitus may be opposed by increased hypoglycaemia risk and dyslipidaemia. *Diabet Med*. 2018. https://doi.org/10.1111/dme.13663.
101. Ojo O, Ojo OO, Adebowale F, Wang XH. The effect of dietary glycaemic index on glycaemia in patients with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Nutrients*. 2018;10(3):E373. https://doi.org/10.3390/nu10030373.
102. Chandalia M, Garg A, Lutjohann D, et al. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med*. 2000;342:1392–1398.
103. Brynes AE, Lee JL, Brighton RE, et al. A low glycemic diet significantly improves the 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor. *Diabetes Care*. 2003;26:548–549.
104. American Diabetes Association. 4. Lifestyle management: standards of medical care in diabetes—2018. *Diabetes Care*. 2018;41(suppl 1):S38–S50. https://doi.org/10.2337/dc18-S004.
105. Grey M, Boland EA, Davidson M, et al. Coping skills training for youths with diabetes on intensive therapy. *Appl Nurs Res*. 1999;12:3–12.
106. Kumar V, Jagannathan A, Philip M, et al. Role of yoga for patients with type II diabetes mellitus: a systematic review and meta-analysis. *Complement Ther Med*. 2016;25:104–112.
107. Lane JD, McCaskill CC, Ross SL, et al. Relaxation training for NIDDM: predicting who may benefit. *Diabetes Care*. 1993;16:1087–1094.
108. Boule NG, Haddad E, Kenny GP, et al. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA*. 2001;286:1218–1227.
109. Pronk NO, Wing RR. Physical activity and long term maintenance of weight loss. *Obesity Res*. 1994;2:587–589.
110. Barringer T, Kirk J, Santaniello A, et al. Effects of a multivitamin and mineral supplement on infection and quality of life. *Ann Intern Med*. 2003;138:365–371.
111. Suksomboon N, Poolsup N, Yuwanakorn A. Systematic review and meta-analysis of the efficacy and safety of chromium supplementation in diabetes. *J Clin Pharm Ther*. 2014;39(3):292–306.
112. Kleefstra N, Houweling ST, Bakker SJ, et al. Chromium treatment has no effect in patients with type 2 diabetes in a Western population: a randomized, double-blind, placebo-controlled trial. *Diabetes Care*. 2007;30(5):1092–1096.
113. Albarracin CA, Fuqua BC, Evans JL, et al. Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled overweight to obese patients with type 2 diabetes. *Diabetes Metab Res Rev*. 2008;24(1):41–51.
114. Geohas J, Daly A, Juturu V, et al. Chromium picolinate and biotin combination reduces atherogenic index of plasma in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Am J Med Sci*. 2007;333(3):145–153.
115. Cunningham JJ. The glucose/insulin system and vitamin C: implications in insulin-dependent diabetes mellitus. *J Am Coll Nutr*. 1998;17:105–108.
116. Eriksson J, Kohvakka A. Magnesium and ascorbic acid supplementation in diabetes mellitus. *Ann Nutr Metab*. 1995;39:217–223.
117. Mullan BA, Young IS, Fee H, et al. Ascorbic acid reduces blood pressure and arterial stiffness in Type 2 diabetes. *Hypertension*. 2002;40:804–809.
118. Vincent TE, Mendiratta S, May JM. Inhibition of aldose reductase in human erythrocytes by vitamin C. *Diabetes Res Clin Pract*. 1999;43:1–8.
119. Anderson JW, Gowri MS, Turner J, et al. Antioxidant supplementation effects on low-density lipoprotein oxidation for individuals with type 2 diabetes mellitus. *J Am Coll Nutr*. 1999;18:451–461.
120. Astley S, Langrish-Smith A, Southon S, et al. Vitamin E supplementation and oxidative damage to DNA and plasma LDL in type 1 diabetes. *Diabetes Care*. 1999;22:1626–1631.
121. Pinkney JH, Downs L, Hopton M, et al. Endothelial dysfunction in type 1 diabetes mellitus: relationship with LDL oxidation and the effects of vitamin E. *Diabet Med*. 1999;16:993–999.
122. Skyrme-Jones RA, O'Brien RC, Berry KL, et al. Vitamin E supplementation improves endothelial function in type 1 diabetes mellitus: a randomized, placebo-controlled study. *J Am Coll Cardiol*. 2000;36:94–102.
123. Gazis A, White DJ, Page SR, et al. Effect of oral vitamin E (alpha-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. *Diabet Med*. 1999;16:304–311.
124. Paolisso G, Tagliamonte MR, Barbieri M, et al. Chronic vitamin E administration improves brachial reactivity and increases intracellular magnesium concentration in type 2 diabetic patients. *J Clin Endocrinol Metab*. 2000;85:109–115.
125. Barbagallo M, Dominguez LJ, Tagliamonte MR, et al. Effects of vitamin E and glutathione on glucose metabolism: role of magnesium. *Hypertension*. 1999;34:1002–1006.
126. Upritchard JE, Sutherland WH, Mann JI. Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care*. 2000;23:733–738.
127. Devaraj S, Jialal I. Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. *Free Radic Biol Med*. 2000;29:790–792.
128. Gokkusu C, Palanduz S, Ademoglu E, et al. Oxidant and antioxidant systems in NIDDM patients: influence of vitamin E supplementation. *Endocr Res*. 2001;27:377–386.
129. Tutuncu NB, Bayraktar M, Varli K. Reversal of defective nerve conduction with vitamin E supplementation in type 2 diabetes: a preliminary study. *Diabetes Care*. 1998;21:1915–1918.
130. Bursell SE, Clermont AC, Aiello LP, et al. High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. *Diabetes Care*. 1999;22:1245–1251.
131. Khatami PG, Soleimani A, Sharifi N, Aghadavod E, Asemi Z. The effects of high-dose vitamin E supplementation on biomarkers of kidney injury, inflammation, and oxidative stress in patients with diabetic nephropathy: a randomized, double-blind, placebo-controlled trial. *J Clin Lipidol*. 2016;10(4):922–929.
132. Milman U, Blum S, Shapira C, et al. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2-2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol*. 2008;28(2):341–347.
133. Ward NC, Wu JH, Clarke MW, et al. The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *J Hypertens*. 2007;25(1):227–234.
134. Polo V, Saibene A, Pontiroli AE. Nicotinamide improves insulin secretion and metabolic control in lean type 2 diabetic patients with secondary failure to sulphonylureas. *Acta Diabetol*. 1998;35:61–64.
135. Jones CL, Gonzalez V. Pyridoxine deficiency: a new factor in diabetic neuropathy. *J Am Pod Assoc*. 1978;68:646–653.

136. Solomon LR, Cohen K. Erythrocyte O_2 transport and metabolism and effects of vitamin B6 therapy in type 2 diabetes mellitus. *Diabetes*. 1989;38:881–886.
137. Coelingh-Bennick HJT, Schreurs WHP. Improvement of oral glucose tolerance in gestational diabetes. *Br Med J*. 1975;3:13–15.
138. Barbagallo M, Dominguez LJ, Galioto A, et al. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med*. 2003;24:39–52.
139. Lima Mde L, Cruz T, Pousada JC, et al. The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. *Diabetes Care*. 1998;21:682–686.
140. Hruby A, Meigs JB, O'Donnell CJ, Jacques PF, McKeown NM. Higher magnesium intake reduces risk of impaired glucose and insulin metabolism and progression from prediabetes to diabetes in middle-aged Americans. *Diabetes Care*. 2014;37(2):419–427.
141. Asemi Z, Karamali M, Jamilian M, et al. Magnesium supplementation affects metabolic status and pregnancy outcomes in gestational diabetes: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr*. 2015;102:222–229.
142. Jamilian M, Samimi M, Faraneh AE, et al. Magnesium supplementation affects gene expression related to insulin and lipid in patients with gestational diabetes. *Magnes Res*. 2017;30(3):71–79.
143. Salgueiro MJ, Krebs N, Zubillaga MB, et al. Zinc and diabetes mellitus: is there a need of zinc supplementation in diabetes mellitus patients? *Biol Trace Elem Res*. 2001;81:215–228.
144. Wang X, Zhang M, Lui G, et al. Associations of serum manganese levels with prediabetes and diabetes among ≥60-year-old Chinese adults: a population-based cross-sectional analysis. *Nutrients*. 2016;8(8): pii:E497. https://doi.org/10.3390/nu8080497.
145. Maebashi M, Makino Y, Furukawa Y, et al. Therapeutic evaluation of the effect of biotin on hyperglycemia in patients with non-insulin dependent diabetes mellitus. *J Clin Biochem Nutr*. 1993;14:211–218.
146. Koutsikos D, Agroyannis B, Tzanatos-Exarchou H. Biotin for diabetic peripheral neuropathy. *Biomed Pharmacother*. 1990;44:511–514.
147. Farmer A, Montori V, Dinneen S, et al. Fish oil in people with type 2 diabetes mellitus. *Cochrane Database Syst Rev*. 2001;3:CD003205.
148. Montori VM, Farmer A, Wollan PC, et al. Fish oil supplementation in type 2 diabetes: a quantitative systematic review. *Diabetes Care*. 2000;23:1407–1415.
149. O'Mahoney LL, Matu J, Price OJ, et al. Omega-3 polyunsaturated fatty acids favourably modulate cardiometabolic biomarkers in type 2 diabetes: a meta-analysis and meta-regression of randomized controlled trials. *Cardiovasc Diabetol*. 2018;17(1):98. https://doi.org/10.1186/s12933-018-0740-x.
150. Bell DS. Importance of postprandial glucose control. *South Med J*. 2001;94:804–809.
151. Vuksan V, Jenkins DJ, Spadafora P, et al. Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes: a randomized controlled metabolic trial. *Diabetes Care*. 1999;22:913–919.
152. Jenkins DJ, Kendall CW, Axelsen M, et al. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Curr Opin Lipidol*. 2000;11:49–56.
153. Abdelhameed AS, Ang S, Morris GA, et al. An analytical ultracentrifuge study on ternary mixtures of konjac glucomannan supplemented with sodium alginate and xanthan gum. *Carbohydrate Polymers*. 2010;81:141–148.
154. Harding SE, Smith IH, Lawson CJ, et al. Studies on macromolecular interactions in ternary mixtures of konjac glucomannan, xanthan gum and sodium alginate. *J Carbpol*. 2010;10:1016–1020.
155. Brand-Miller JC, Atkinson FS, Gahler RJ, et al. Effects of PGX, a novel functional fibre, on acute and delayed postprandial glycaemia. *Eur J Clin Nutr*. 2010;64(12):1488–1493.
156. Jenkins AL, Kacinik V, Lyon MR, et al. Reduction of postprandial glycemia by the novel viscous polysaccharide PGX in a dose-dependent manner, independent of food form. *J Am Coll Nutr*. 2010;29(2):92–98.
157. Vuksan V, Sievenpiper JL, Owen R, et al. Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. *Diabetes Care*. 2000;23:9–14.
158. Lan J, Zhao Y, Dong F, et al. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. *J Ethnopharmacol*. 2015;161:69–81.
159. Di Pierro F. Impact of berberine on human gut bacteria. *Nutrafoods*. 2018;17:5–8.
160. Chen F, Nakashima N, Kimura I, et al. Hypoglycemic activity and mechanisms of extracts from mulberry leaves (folium mori) and cortex mori radicis in streptozotocin-induced diabetic mice. *Yakugaku Zasshi*. 1995;115:476–482.
161. Andallu B, Suryakantham V, Lakshmi Srikanthi B, et al. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. *Clin Chim Acta*. 2001;314:47–53.
162. Porchezhian E, Dobriyal RM. An overview on the advances of *Gymnema sylvestre:* chemistry, pharmacology and patents. *Pharmazie*. 2003;58:5–12.
163. Shanmugasundaram ER, Rajeswari G, Baskaran K, et al. Use of Gymnema sylvestre leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. *J Ethnopharmacol*. 1990;30:281–294.
164. Baskaran K, Ahamath BK, Shanmugasundaram KR, et al. Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin dependent diabetes mellitus patients. *J Ethnopharmacol*. 1990;30:295–305.
165. Srivastava Y, Venkatakrishna-Bhatt H, Verma Y, et al. Antidiabetic and adaptogenic properties of Momordica charantia extract: an experimental and clinical evaluation. *Phytother Res*. 1993;7:285–289.
166. Welihinda J, Karunanaya EH, Sheriff MHR, et al. Effect of *Momordica charantia* on the glucose tolerance in maturity onset diabetes. *J Ethnopharmacol*. 1986;17:277–282.
167. Vuksan V, Stavro MP, Sievenpiper J, et al. American ginseng *(Panax quinquefolius)* reduces postproandial glycemia in nondiabetic subjects with type 2 diabetes mellitus. *Arch Intern Med*. 2000;160:1009–1013.
168. Vuksan V, Sievenpiper J, Wong J, et al. American ginseng *(Panax quinquefolius)* attenuates postprandial glycemia in a time dependent but not dose dependent manner in healthy individuals. *Am J Clin Nutr*. 2001;73:753–758.
169. Vuksan V, Stavro MP, Sievenpiper J, et al. American ginseng improves glycemia in individuals with normal glucose tolerance: effect of dose and time escalation. *J Am Coll Nutr*. 2000;19:738–744.
170. Sievenpiper J, Arnason JT, Leiter LA, et al. Variable effects of American ginseng: a batch of American ginseng *(Panax quinquefolius)* with a depressed ginsenoside profile does not affect postprandial glycemia. *Eur J Clin Nutr*. 2003;57:243–248.
171. Sotaniemi EA, Haapakoski E, Rautio A. Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care*. 1995;18:1373–1375.
172. Sharma RD, Raghuram TC, Rao NS. Effect of fenugreek seeds on blood glucose and serum lipids in type 1 diabetes. *Eur J Clin Nutr*. 1990;44:301–306.
173. Mada Z, Abel R, Samish S, et al. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *Eur J Clin Nutr*. 1988;42:51–54.
174. Gupta A, Gupta R, Lal B. Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Assoc Physicians India*. 2001;49:1057–1061.
175. Sharma KK, Gupta RK, Gupta S, et al. Antihyperglycemic effect of onion: effect on fasting blood sugar and induced hyperglycemia in man. *Ind J Med Res*. 1977;65:422–429.
176. Rochette L, Ghibu S, Muresan A, Vergely C. Alpha-lipoic acid: molecular mechanisms and therapeutic potential in diabetes. *Can J Physiol Pharmacol*. 2015;93(12):1021–1027.
177. Reljanovic M, Reichel G, Rett K, et al. Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Alpha Lipoic Acid in Diabetic Neuropathy. *Free Radic Res*. 1999;31:171–179.
178. Jacob S, Ruus P, Hermann R, et al. Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebo-controlled pilot trial. *Free Radic Biol Med*. 1999;27:309–314.

179. Rindone JP, Achacoso S. Effect of low-dose niacin on glucose control in patients with non-insulin-dependent diabetes mellitus and hyperlipidemia. *Am J Ther*. 1996;3:637–639.
180. Grundy SM, Vega GL, McGovern ME, et al. Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of niaspan trial. *Arch Intern Med*. 2002;162:1568–1576.
181. Kane MP, Hamilton RA, Addesse E, et al. Cholesterol and glycemic effects of Niaspan in patients with type 2 diabetes. *Pharmacotherapy*. 2001;21:1473–1478.
182. Keen H, Payan J, Allawi J, et al. Treatment of diabetic neuropathy with gamma-linolenic acid. The gamma-Linolenic Acid Multicenter Trial Group. *Diabetes Care*. 1993;16(1):8–15.
183. Stirban A, Negrean M, Stratmann B, et al. Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. *Diabetes Care*. 2006;29(9):2064–2071.
184. Stracke H, Gaus W, Achenbach U, et al. Benfotiamine in diabetic polyneuropathy (BENDIP): results of a randomised, double blind, placebo-controlled clinical study. *Exp Clin Endocrinol Diabetes*. 2008;116(10):600–605.
185. Alkhalaf A, Klooster A, van Oeveren W, et al. A double-blind, randomized, placebo-controlled clinical trial on benfotiamine treatment in patients with diabetic nephropathy. *Diabetes Care*. 2010;33(7):1598–1601.
186. Du X, Edelstein D, Brownlee M. Oral benfotiamine plus alpha-lipoic acid normalises complication-causing pathways in type 1 diabetes. *Diabetologia*. 2008;51(10):1930–1932.
187. Forst T, Pohlmann T, Kunt T, et al. The influence of local capsaicin treatment on small nerve fibre function and neurovascular control in symptomatic diabetic neuropathy. *Acta Diabetol*. 2002;39:1–6.
188. Rains C, Bryson HM. Topical capsaicin. A review of its pharmacological properties and therapeutic potential in post-herpetic neuralgia, diabetic neuropathy and osteoarthritis. *Drugs Aging*. 1995;7:317–328.
189. Hu H. A review of treatment of diabetes by acupuncture during the past forty years. *J Tradit Chin Med*. 1995;15:145–154.
190. Abuaisha BB, Costanzi JB, Boulton AJ. Acupuncture for the treatment of chronic painful peripheral diabetic neuropathy: a long-term study. *Diabetes Res Clin Pract*. 1998;39:115–121.
191. Younes H, Alphonse JC, Behr S, et al. Role of fermentable carbohydrate supplements with a low-protein diet in the course of chronic renal failure: experimental bases. *Am J Kidney Dis*. 1999;33:633–646.

Endometriosis

Bethany Montgomery Hays, MD, and *Tori Hudson*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Endometriosis, the presence of endometroid tissue outside the uterus, is a heterogeneous condition characterized by a triad of symptoms: dysmenorrhea (pain with menses), dyspareunia (pain with intercourse), and infertility. It is now separated into superficial peritoneal endometriosis, ovarian endometriomas or "chocolate cysts" (hemorrhagic cysts with endometrial lining containing old blood and debris), and deeply infiltrating endometriosis. Some consider these to be different but closely related diseases.[1]
- Physical examination reveals one or more of the following: tenderness of the pelvic organs, cul-de-sac, or both; enlarged or tender ovaries (although endometriomas are often soft and therefore not palpable); a uterus that is tipped or flexed backward and may lack mobility; and other fixed pelvic structures.
- Pelvic ultrasound, although useful in the detection and differentiation of endometriomas from other types of cysts, is often unhelpful or negative in cases absent significant ovarian disease.
- Definitive diagnosis requires laparoscopy or laparotomy, visualizing endometrial implants within the pelvic cavity, and/or confirmatory biopsy of implants.

GENERAL CONSIDERATIONS

Endometriosis affects 10% to 15% of menstruating women of childbearing age. Although published estimates of the incidence vary depending on the methodology of diagnosis and the geographical location of the study, the exact incidence is unknown because most women undergoing the laparoscopic evaluation needed for a reliable diagnosis have pain and/or infertility, and as many as half of these (high-risk) women are found to have endometriosis.[2]

*Previous edition contributor

Genetics

A common risk factor for endometriosis is heredity. A woman with a mother or a sister who has endometriosis is at increased risk,[3] and "risk genes" such as aromatase, which converts androgens to estrogens), and 17-beta HSD type 2, which converts estrone to the more powerful estradiol (but not type 1, which does the reverse), have been identified. Common low-penetrance genes such as GSTM1, GSTT1, and CYP1B1 strongly involved in detoxification and protection of DNA are associated with increased susceptibility.[4] Additionally, the genetics of COMT may affect both estrogen metabolism[5] and pain perception in patients with endometriosis. Patients with low-activity COMT have higher adrenaline/noradrenaline levels, have more anxiety, and are more susceptible to nociceptive pain.[6] It is likely that heredity interacting with the environment creates a greater likelihood for developing endometriosis and its symptoms.

Menstrual History and Lifestyle Factors

Women with shorter menstrual cycles and a longer duration of flow, more painful periods, and the use of intrauterine devices have been found to be at higher risk for having endometriosis. Lack of exercise from an early age, a high-fat diet, and increased or unbalanced estrogen levels have been suggested as factors in the development of endometriosis. These factors have in common higher levels of estrogen and xenoestrogens, as well as poorer ability to detoxify them.

Personal History and Stress

A greater number of women with adhesions, endometriosis, or both have reported abuse histories.[7] This may be mediated through stress

effects on the immune system functions, which we will explore next, or epigenetic changes in relevant genes.

Immune System

A growing body of information implicates the immune system in the pathophysiology of endometriosis. Abnormalities of cytokines and adhesion molecules, increased numbers and activation of peritoneal macrophages, changes in T cells and natural killer (NK) cells (which normally remove cellular debris from retrograde menstruation), and the presence of increased autoimmunity are notable findings in endometriosis. It is not known whether the immune system changes are primary (causative) in the transformation of totipotent cells of the pelvic peritoneum or the failure to remove viable cells from retrograde menstrual effluent, or if its aberrations are secondary, that is, in response to the development and invasion of the endometrial implant.

Estrogen Levels (Production and Metabolism) and Xenoestrogens

Endometriosis has long been known to be an estrogen-related disease. Men don't get it. Women don't get it premenarche. It occurs during the reproductive period, and the incidence increases in perimenopause, during the period of increased estrogen and decreased progesterone. It regresses at menopause or with oophorectomy. It responds to estrogen-lowering agents such as gonadotropin-releasing hormone (GNRH) agonists, danazol, and aromatase inhibitors.[8] However, none of these drugs, although they may improve symptoms, improves fertility. Endometriosis may return in menopausal women put on hormone replacement therapy, although many tolerate low-dose therapy. Because endometrial implants make their own estrogen, as many as one third of implants are out of sync with the eutopic endometrium,[9–11] suggesting that the hormonal signals from the ovary are being overridden by the production of local estrogens. More recently, aromatase has been found to be present in endometrial implants but not in eutopic endometrium.[12] However, aromatase inhibitors have not been found as helpful in managing disease as it was once thought they would be.[13] The benefits of oral contraceptives, long thought to prevent endometriosis and ameliorate its symptoms,[14] are currently in debate. A meta-analysis in 2011 found that the apparent protective effect may have been due to the postponement of surgical evaluation and suggested that this treatment was "not sufficiently substantiated."[15]

Numerous lines of research have confirmed the association of endometriosis with higher levels of endogenous, exogenous, and xenoestrogens (polychlorinated biphenyls [PCBs], weed killers, plastics, detergents, household cleaners, tin can liners) and altered estrogen metabolism. Prenatal factors such as exposure to xenoestrogens have also been seen in these women.[16]

ETIOLOGICAL THEORIES

The origin of endometriosis remains a mystery. Many theories have been proposed, but none fits all the findings in patients with endometriosis. Establishing a correct hypothesis leads to targeted research, which benefits patients. Following an incorrect hypothesis leads to confusion, expensive and misleading research, and incorrect results, which do not benefit or only partially benefit patients.[17] It is possible that part of the confusion regarding the origin of endometriosis is that, as noted before, different locations of endometriosis and depth of disease may represent entirely different diseases.[18] It is also likely that the origin of endometriosis is multifactorial.

Retrograde Flow

Retrograde flow, the predominant theory, first proposed in 1927 by Sampson, proposes that during menses, menstrual effluent flows backward through the fallopian tubes, and living endometrial cells implant in the pelvic cavity. The problem with this theory is that more than 90% of menstruating women without endometriosis have retrograde flow as well. Women get endometriosis after tubal ligation and, rarely, after menopause. Finally, eutopic endometrial cells are genetically different from endometriosis cells. Thus this theory has been challenged, and questions have been raised regarding the biochemical and immunological factors causing the implantation of endometrial tissue within the pelvic environment.[19] The persistence of this theory, however, suggests that there may be factors in the menstrual effluent, such as inflammatory cytokines or autoantibodies, that stimulate or support endometriosis in nearby pelvic tissues. Finally, the eutopic endometrium of these women appears to be different from the endometrium of normal women.[12] Alterations in apoptosis, proliferation, adhesive and invasive behavior, angiogenesis, neurogenesis, lymphangiogenesis, hormone production and responsiveness, and immunological regulation differ between women with and without endometriosis.[20] Implants found in odd locations, such as the brain, nose, and lungs, suggest the transportation of endometrial tissue through lymphatic channels and/or blood vessels.[20]

Cellular Transformation

Another theory suggests that that "totipotent" (stem) cells are somehow triggered to transform. Some researchers believe the implants to be of embryological origin. Indeed, genetics specific to stem cells have been identified in endometriosis.[21,22] A closely related theory suggests that pieces of the embryonic female reproductive tract (Mullerian cells) were left behind during developmental migration and that these cells, when activated, transform in some way to become endometriosis. The growing understanding of the genetics of these aberrant cells is likely to shed light on the true origin of endometriosis in the near future.

Environmental Toxins

A recurring question is whether environmental toxins, many of which are estrogen mimics, are involved or even causative in endometriosis. Research on endometriosis in baboons suggests activation by environmental toxins that mimic estrogens.[23] Exposure to radiation and dioxin has been associated with a higher frequency of endometriosis.[24,25] The highest incidence of endometriosis appears to be in Belgium, which is also the home to the highest level of dioxin pollution in the world.[26] Two studies, however, one in Belgium, found no significantly increased risk associated with dioxins or polychlorinated biphenyls (PCBs).[27] The other study, in Italy, found no significantly increased risk of endometriosis in women who had high levels of dioxin in their blood.[28] These studies also point out the difficulty of studying a fat-soluble chemical that may not show up in the blood. Although there is currently no widely accepted epidemiological evidence linking any one class of chemicals to the risk of endometriosis, there does appear to be some link with estrogen-like compounds in the environment,[29] called xenoestrogens, which can interact with the estrogen receptor and disrupt estrogen metabolism.

In addition to estrogen mimetics, there are a number of environmental toxins that have strong associations but for which there is no clear mechanism. For example, women with endometriosis have significantly higher levels (median 0.57 micro g/mL vs. median 0.18 micro g/mL) of high-molecular-weight Di-(2-ethylhexyl)-phthalate (DEHP) than controls.[30] However, the levels do not correlate with severity. Several studies also show a correlation with perfluorocarbon levels.[31]

Immune Origins

Immunological alterations may exist in women with endometriosis. Lack of proper immune surveillance in the pelvis following menstrual retrograde flow may allow endometrial cells to implant rather than be phagocytized and removed.[32] Alteration of the inflammatory response to the implanted tissue is then involved in progression and

severity of disease. These alterations may be generational and begin in utero.[33] Women with endometriosis have been found to have suppressed NK-cell activity in their peritoneal fluid,[34,35] high levels of immunoglobulins (Igs) IgG and IgM,[36] and high levels of autoantibodies against ovary and endometrial cells.[37,38] Macrophages, cytokines, T lymphocytes, and tumor necrosis factor-alpha (TNF-α) are all found in greater numbers in the peritoneal fluid in early stages of endometriosis.[39] Both types of immunity, cell-mediated and humoral, have been implicated in endometriosis, with immunological defects present with even minimal disease.[40] Growth factors, angiogenic factors, and lipid peroxidation in the peritoneal fluid may stimulate endometrial cell growth.[41–43] Targeting of these proinflammatory compounds and blocking their action with anti-inflammatory antioxidants and natural compounds are being investigated as new treatment strategies.

Oxidative Stress

The presence of high levels of iron from retrograde flow creates oxidative stress, another factor linked to causation in endometriosis. The contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress.[44]

Genetic Origins

Endometriosis is now considered to be of multifactorial origin by most researchers.[45] Grouping of endometriosis cases within families has long been found in clinical studies,[46,47] population-based studies,[48] and studies of twins,[49-51] all of which suggest the importance of genetics in the origins of endometriosis. Several abnormalities in detoxification enzymes, tumor-suppressor genes, and antioxidant genes, interacting in multistep fashion, may be involved in both the development and the progression of the disease,[52] but no single genes that are primarily responsible have been identified. Epigenetic influences from the gut and the environment and prenatal influences also underline the importance of genetics, regardless of the theory of origins we ultimately accept.

Gut Dysfunction and Pathology

The search for a unifying theory of the origins of endometriosis that explains all the research quickly leads to the gastrointestinal tract.[53] Its close proximity to the most common sites of endometriosis; the frequent involvement of large and small bowel; dietary approaches that ameliorate symptoms; the inflammatory nature of the disease; and the effect of higher levels of endogenous estrogens, xenoestrogens, and other toxins ingested orally make looking to the gut for origins and to find preventive and curative measures a necessity. Let's look at the evidence implicating the gut:

1. Because family genetics is an important association, we must remember that family members share their microbiome as well as their genes.
2. The amount of pain is not proportional to the amount of disease, suggesting that both the disease and the pain come from a third cause: the gut.
3. Although associated with endometriosis, the cause or causes of infertility are not known. One theory linked tubal transport of the fertilized egg to dysbiosis.
4. Dietary interventions improve endometriosis symptoms.[54]
5. One of the most frequent sites of endometrial implants is the cul-de-sac between the uterus and rectum. One of the most common extraperitoneal sites is the rectovaginal septum just below the cul-de-sac. These sites, in close proximity to the rectum, would share the highest levels of toxins and toxic bacteria.
6. The gut microbiota is not only essential for physiological gastrointestinal function but also acts as a central regulator of a variety of inflammatory and proliferative conditions. "[W]e hypothesize that the gut microbiota may be involved crucially in the onset and progression of endometriosis."[55]
7. Alterations in gut bacteria and the gut-associated immune system in inflammatory bowel diseases produce an exudate that closely approximates the peritoneal fluid in early endometriosis (i.e., high levels of cytokines and macrophages).
8. Women with endometriosis are more likely to be diagnosed with pelvic inflammatory disease (altered bacteria likely sourced from the gut) and irritable bowel syndrome (IBS), both of which are associated with dysbiosis.[51]
9. Inflammation produces adhesions, a common finding in patients with endometriosis.
10. Eighty percent of the immune system is found in the gut. The immune response to altered contents in the gut (food, toxins, and the microbiome) produce many if not all of the altered immune system findings in endometriosis. Endometriosis and IBS share a picture of chronic inflammation.[51]
11. TNF-α has been shown to be in higher quantities in the peritoneal fluid of women with endometriosis. TNF-α has been shown to induce accelerated differentiation of stem cells. TNF-α is thought to be a critical factor in the induction and maintenance of endometrial implants.[56,57]
12. Autoimmunity is now both seen in patients with endometriosis and closely linked to gut pathology.
13. The pelvic peritoneum can be altered by benign and malignant forms of any of the three cell types of the female genital tract: serous (tubal) cells, endometrial cells, and mucinous (cervical) cells. These diseases are called endosalpingosis, endometriosis, and pseudomyxoma peritonei when benign and are called epithelial ovarian cancer (serous, endometrioid, and mucinous) when malignant. Although tubal cells might flow retrograde, cervical cells are not likely to go "upstream," making the transformation of peritoneal cells more likely than retrograde flow as an etiology. Thus we must look for reasons for the transformation of the peritoneal "totipotential cells."
14. Epithelial ovarian cancer of all three types (serous/tubal, endometrioid/endometrial, and mucinous/cervical) occurs more often in patients with endometriosis. This disease (epithelial ovarian cancer) is deadly because it is rarely found "early." In fact, the ovarian disease may start in multiple locations on the peritoneal surface and simply predominate in the epithelium of the ovary because the peritoneal surface reflected over the ovary is more richly supplied with estrogen, allowing what may start as peritoneal disease to progress more rapidly in that location.
15. Patients with endometriosis are more likely to develop endometrioid ovarian cancer and perhaps ovarian cancer of other types, suggesting cellular transformation and issues of altered environment.
16. The cells that become endometriosis are stem cells.[22]
17. Peritoneal stem cells are responding to something that is going on in the pelvis. Gastrointestinal immune activation is a likely candidate.

DIAGNOSIS

The clinical diagnosis of endometriosis is presumptive. A definitive diagnosis requires surgical evaluation and biopsy. Endometriosis is rarely seen before menarche. Although in some cases symptoms begin with the onset of menstruation, in most patients, symptoms

begin later and worsen progressively over time, suggesting the importance of stimulation of the disease by estrogens and/or the accumulation of xenoestrogens with time. It should be noted that pre-menarche girls with abdominal pain are usually diagnosed with gut problems, not gynecological problems, unless they have signs of infection. Although the triad of symptoms of endometriosis is dysmenorrhea, dyspareunia, and infertility, two of these—dyspareunia and infertility—may not be issues for young women having menstrual cramps, so the diagnosis may be missed. Acute pain may occur for only a few hours at the onset of menses or be present throughout the month. In some women, vomiting, diarrhea, and fainting can occur concurrently, suggesting a vagal component, with the intense pelvic/abdominal cramping and pains that are likened to contractions in childbirth. These are largely the symptoms of a heavy menstrual flow trying to pass through a small cervical outlet and autonomic stimulation from abdominal organs. In many women, these symptoms disappear after first childbirth (especially when the cervix is dilated by labor.) Because these symptoms also occur in conditions likely to produce retrograde flow, it is hard to state which comes first: heavy, painful periods or endometriosis. Other sensations include rectal pain producing a chronic bearing-down sensation, pressure in the lower back, and pain sometimes radiating down the legs, likely related to irritation of the psoas muscle, which attaches to the lower spine and courses retroperitoneally through the pelvis and down the front side of the leg, placing it immediately under the pelvic peritoneum, where implants may cause the muscle to tighten and spasm. The correlation between pain and the extent of disease is weak. Women with fixed ovaries and large endometriomas may report only mild discomfort, whereas those with visibly smaller lesions may report severe chronic pain. Pain therefore may be caused not by the endometriosis but by whatever causes endometriosis.[58] Some research suggests that the severity of symptoms is correlated with the depth of the lesions rather than the number or size of lesions.[59,60] Symptoms may also include pain with urination or bowel movements; bleeding from ectopic sites of endometrial implants, such as the nose or bowels; and fatigue.

Endometriomas, endometroid involvement of the epithelium reflected over the ovaries, are found in two of three patients with endometriosis. It is likely that endometriosis simply grows fastest on the peritoneal surface reflecting over the estrogen-producing ovary. This may also be true of epithelial ovarian cancer, leading the diagnosing surgeon to conclude that epithelial cancers *begin* in the ovary and spread to the peritoneum when, in fact, they may *begin* throughout the peritoneal cavity. (No wonder we don't "find them soon enough.") The association of endometriosis and ovarian cancer was first reported by Sampson in 1925. The origin of ovarian cancer is unknown, but both endometriosis and certain ovarian cancers share genetic changes (loss of p53 and PTEN and ARID1A).[61]

Endometriosis is associated with infertility and miscarriage and is often found in women at laparoscopic evaluation for infertility. The amount of endometriosis does not always correlate with infertility. Some women with small peritoneal implants are infertile, whereas some women with severe disease may become pregnant. Data suggest that excessive amounts of free radicals accompany endometriosis. Tubal scarring, adhesions, and unruptured follicles are common findings in women with endometriosis and concurrent infertility. Alterations of eutopic endometrium are also found in patients with endometriosis. Autoantibodies, including those to sperm, have been found. All of these as well as the inflammatory environment of the pelvis may contribute to poor ovulation, tubal transport, and implantation of the fertilized egg.

Physical Findings

Physical examination reveals one or more of the following: tenderness of the pelvic area, cul-de-sac, or both; enlarged or tender ovaries; a uterus that tips backward (and may be retroflexed as well as retroverted) and lacks mobility due to adhesions; and other fixed pelvic structures. Endometrial tissue can be visible in surgical scars, in the vagina, and on the cervix. Physical examination during the first or second day of menses may accentuate tender areas or nodules in the cul-de-sac or in the septum between the rectum and vagina.

An ultrasound examination of the pelvis can be useful in ruling out other pathology and tumors but is not a definitive tool in diagnosing endometriosis. It can detect a fluid-filled mass on the ovary and determine the size, characteristics, and consistency of the cyst, suggesting endometrioma, but it often fails to identify peritoneal disease.

Laboratory Findings

A blood test, CA-125, can have positive results in endometriosis but cannot differentiate among endometriosis, uterine fibroids, malignancies, and even normal tissue.

A definitive diagnosis of endometriosis can only be accomplished with laparoscopy or laparotomy. Endometrial implants, endometriomas, or both are then visualized, and a presumptive diagnosis is made. Only biopsy gives the definitive answer.

THERAPEUTIC CONSIDERATIONS

Endometriosis is a multifactorial disease. Therefore therapeutic approaches must be individualized for maximum benefit to each patient. Numerous drugs, surgical procedures, and integrative approaches have been tried. Most medical approaches to date have been only mildly to moderately successful in ameliorating symptoms and consist of hormone intervention aimed at lowering estrogen effects or nonsteroidal anti-inflammatory drugs (NSAIDs) aimed at decreasing inflammation. Only surgery has been successful in eradicating disease and stopping the feed-forward mechanisms that drive estrogen production, inflammatory cascades, and visceral hypersensitivity (pain).[62] And only surgery has proven effective in improving fertility. Recurrence rates are high, so surgical intervention should be timed to closely precede attempts at conception. These factors suggest that we are not getting at causes—that is, we are focusing our attention too far "downstream."

Therapeutic approaches should be aimed at the following:

a. Minimizing symptoms because there are significant quality-of-life issues for these women
b. Slowing or arresting the progression of disease, especially before desired childbearing, to preserve fertility and avoid or delay surgery
c. Improving fertility rates

A multifactorial approach to causes suggests the following areas to address therapeutically:

1. Lowering estrogen levels and improving estrogen metabolism
2. Decreasing xenoestrogens and toxic exposures
3. Improving gut health
4. Approaches that decrease inflammation

Approaches that have multiple effects will be starred (*).

Diet

Diet is the common denominator in a multifactorial approach to endometriosis.[63] Sadly, research on diet and endometriosis is sparse.[64]

Several dietary principles are key in a natural medicine approach to endometriosis, as discussed in the following subsections.

Reduce Inflammatory Foods and Increase Anti-inflammatory Foods

Perhaps the most inflammatory food is gluten*. Producing zonulin, which causes leaky gut, gluten increases the gut-associated immune system reactivity to intraluminal irritants and antigens, inducing inflammatory cytokines interleukin (IL)-6 and TNF-α. Additionally, removing gluten will often decrease carbohydrate consumption and help prevent insulin resistance (discussed later in the chapter). Consuming higher amounts of anti-inflammatory prostaglandin precursors (omega-3* and certain omega-6 fatty acids, discussed later in the chapter) and blocking leukotrienes (with omega-3 eicosapentaenoic acid [EPA]) may be more effective and safer than NSAIDs, the current first approach for pelvic pain. Decreasing "flesh foods"* (dairy, red meat, eggs), which contain high amounts of arachidonic acid (the inflammatory prostaglandin series 2 precursor), is also recommended. This approach may also limit toxins excreted in cow's milk and antigens in meat and dairy that drive the inflammatory process. Avoiding insulin resistance*, a diabetes precursor and major cause of inflammation and driver of obesity, with its accompanying increase in estrogen, is critical as well. Anti-inflammatory herbs and vegetables*, discussed later in the chapter, complete the four major parts of an anti-inflammatory diet: good fat, balanced carbohydrates, avoidance of gluten and inciting food antigens, and the addition of anti-inflammatory plants. Avoiding abdominal obesity (a stress-related disease) because of the inflammogenic properties of visceral adiposity may also be helpful.[65]

Enhance Detoxification Mechanisms, Especially Estrogen Metabolism

By increasing the intake of vegetables*, specifically those that enhance Phase I and Phase II detoxification in the liver, improving metabolism of estrogen and providing methylation factors, cell damage from toxins and estrogen metabolites can be limited. Methylation-supporting (lipotropic factors)[66] and detoxification-supporting vegetables to be increased include carrots, beets (carotene-containing vegetables; see later discussion), and cruciferous vegetables and green leafy vegetables. Indole-3-carbinol—found in broccoli, Brussels sprouts, cabbage, kale, and cauliflower—favors the production of less active 2OH metabolite of estrogen, thereby decreasing the more potent 16OH metabolite and the more dangerous DNA-damaging 4OH metabolite.[67] Other liver-supporting foods include artichokes, lemons, dandelion greens, watercress, and burdock root. Onions, garlic, and leeks contain organosulfur compounds, which enhance the immune system and induce enzymes that detoxify in the liver. They also provide the sulfate groups that modulate estrogen effects and are a major Phase II pathway for estrogens. In addition, they contain the bioflavonoid quercetin, which is known to stimulate the immune response, protect against oxidation, block the inflammatory response, and inhibit tumor growth by lowering aromatase.[68] Adding a tablespoon of milk thistle seeds that have been soaked and ground can also help liver function because silymarin is known to increase Phase I detoxification. Organically grown vegetables are expected to contain lower levels of pesticides and herbicides that may also mimic estrogen. In some studies, fruit was associated with increased risk (more than two servings/day). It is likely that the extra sugar is not helpful, whereas most studies show a significant benefit from vegetables. This may be mediated through beneficial effects on the microbiome.[69]

Increase Soy/Isoflavones and Lignans

The isoflavones in soy products and the lignans in flax seeds* may also be important in a dietary approach to endometriosis. Although some claim that these phytoestrogen compounds should be avoided, thinking that they would stimulate the growth of endometriotic implants, much as estrogen does, the evidence supports the conclusion that soy is beneficial in the diets of women with endometriosis.[70] A recent study in an endometriosis model in mice showed a dramatic lowering of estrogen receptors; inflammatory markers; and HIF-1a, a tumor marker, in mice given genistein in various doses.[71]

Improving Gut Function and the Microbiome

Foods high in fiber are associated with optimal transit time in the intestines and an optimal balance of friendly microorganisms within the large intestines (see Chapter 132). These microorganisms, better known as the microbiome, must remain healthy to crowd out flora that produces beta-glucuronidase, which deconjugates estrogens and allows them to recycle back through the body (called enterohepatic circulation). Freshly ground flax seeds*, high vegetable roughage (cellulose), and omega-3 fatty acids* all improve transit time in the large bowel, preventing absorption of some of the toxic load as well as feeding healthy bacteria. These "dual-use" foods are easily incorporated into the diet in smoothies, muffins, and dishes that combine fish and vegetables (fish tacos, for example). Magnesium*-rich foods, or magnesium taken as a supplement (see following discussion), are also useful for this purpose. Increased fiber content (24g/day) was shown in Women's Heath Initiative (WHI) to lower IL-6 and TNF-α.[72] Seasonings such as turmeric (curcumin) protect against environmental carcinogens, decrease inflammation, and increase bile secretion. Fresh flax seeds increase anti-inflammatory omega-6 fatty acids. Seasoning with *Fucus* (a seaweed) helps stimulate T-cell production and absorb toxins.[73] Studies suggest that a predominantly vegetarian diet emphasizing less protein and more fiber can lead to a decrease of biologically active unconjugated estrogens in the blood plasma.[74] Vegetarian diets are also of greater value owing to their lower fat content. Diets containing large amounts of animal protein, especially in the form of red meat, contain large amounts of arachidonic acid, which promotes inflammatory prostaglandins and thus inflammation and pain. Being higher on the food chain, the animals that produce red meat carry a greater burden of toxins from the environment. Adding vegetable protein, soy, nut butters (e.g., almond), and salmon to our diets tips the inflammatory pathway toward the anti-inflammatory prostaglandins that inhibit tumor growth—both benign and malignant.

Increase Omega-3 Fatty Acids and Reduce Trans Fats

Omega-3 fats, in addition to their anti-inflammatory function, improve membrane function, making membranes more fluid and accommodating to receptors. Omega-3 fats are precursors for series 3 anti-inflammatory prostaglandins and block the cyclooxygenase-2 (COX2) enzyme as well as the lipoxygenase enzyme. The arachidonic acid in flesh foods increases prostaglandin E 2 (PGE2) and the inflammatory series 2 prostaglandin, which increases aromatase, adding to the feed-forward production of estrogens in endometriosis.[75] Lowering fat and increasing essential fatty acids (EFAs) and short-chain polyunsaturated fatty acids (FAs)[76]; adding unfried, low-mercury, low-contamination fish to the diet[77]; and decreasing saturated fats[78] have all been shown to be beneficial.

The consumption of trans fats appears to increase the risk of endometriosis,[79] whereas long-chain omega-3 fats appear to be protective. Twelve years of prospective data from the Nurses' Health Study II, which began in 1989, were analyzed for dietary fat and its association with many health problems, including endometriosis.[80] Although there was no association with total fat consumption and endometriosis risk, those women with the highest consumption of long-chain omega-3 fatty acids were 22% less likely to be diagnosed with endometriosis compared with women who had the lowest intake of these fats. Those women who had the highest intake of trans unsaturated fats were 48% more likely to be diagnosed with endometriosis.

Foods to Avoid

Foods to decrease include sugar, caffeine, dairy, red meat, and alcohol. Sugar is known to increase estrogen levels.[81] A study of 50 women with endometriosis examined the effect of the reduction of higher glycemic carbohydrates, the addition of omega-3 and omega-9 fatty acids, and the elimination of foods containing caffeine and tyramine; after 8 weeks, there was a significant reduction in symptoms.[82] Endometriosis is found to be specifically correlated with caffeine consumption. Women consuming 5 to 7 g of caffeine monthly had a 1.2 times greater incidence of endometriosis, whereas those consuming more than 7 g had a 1.6 times greater increase.[83] The Environmental Protection Agency estimates that 90% of human dioxin exposure is through food, primarily meat and dairy products.[84] Cheese, particularly cottage cheese, and milk cause the lipid pathway to be tipped toward prostaglandins and leukotrienes, which cause inflammation, smooth muscle contraction, and vascular constriction. Alcohol consumption depletes stores of B vitamins in the liver and affects the metabolism of estrogen.

Nutritional Supplements

A number of nutritional supplements have been looked at related to the amelioration of symptoms, the development of endometriosis, and the preservation of fertility. The side effects of these supplements are minimal.

Estrogen Metabolism Facilitators

A number of supplements have been studied and found to be beneficial in lowering estrogen levels and improving estrogen metabolism. Because there is a clear benefit to lowering estrogen to slow the progression of disease, this same approach is helpful in patients with endometriosis. Important areas of estrogen production and metabolism to address are as follows: (1) aromatase, the main estrogen-producing enzyme; (2) 17OH dehydrogenase, the balance between the more potent estradiol and the less potent estrone; (3) Phase I detoxification, which involves directing the metabolites away from 16OH estrone (CPY3A4) and 4OH estrone (CYP1B1) to the least toxic 2OH estrogen (CYP1A1); and (4) Phase II detoxification, which involves methylation, sulfation, and glucuronidation to prevent DNA damage by oxidative intermediates and make the estrogens water soluble so that they can be excreted in urine or feces. There are numerous foods and natural substances that are effective in improving the overall estrogen picture in patients with endometriosis.

Aromatase Inhibitors

Because estrogen is being produced locally in the endometriosis implant, aromatase inhibitors (AIs) would seem to be an effective treatment. Pharmaceutical AIs have not been as successful as expected and are reserved for patients who do not respond to first-line therapies. A number of plant substances have been shown to be aromatase inhibitors. Because they have few if any side effects, and some do double duty (COX-2 inhibitors), they should be included in a comprehensive approach to endometriosis:

COX-2 inhibitors (see discussion of Essential Fatty Acids later in the chapter)

Essential fatty acids* (discussed later in the chapter)

Indole-3-Carbinol

Indole-3-carbinol (I3C) or DIM (a metabolic product of I3C in the gut) have been shown to improve Phase I detoxification of estrogens favoring the less potent 2OH estrone pathway. Although the 2OH estrone can also make DNA-damaging quinones, they are less adhesive to the DNA and therefore are probably only important when levels are very high (as can be seen with certain single-nucleotide polymorphisms [SNPs] of CYP1A1). These metabolites can be measured in the blood or urine to ensure that levels are low before starting I3C or DIM.

Calcium-D-Glucarate

Calcium-d-glucarate is a beta-glucuronidase inhibitor. By preventing the action of bacterially produced beta-glucuronidase in the gut, this supplement interrupts enterohepatic circulation of estrogen, allowing it to be removed in the stool and lowering the levels of estrogen in the system.

Sources of methyl groups, sulfate groups, and glucuronides are important to provide the Phase II substrates that make estrogens water soluble so that they can be excreted. Most patients can obtain these substrates from foods, as noted previously. Rarely, s-adenosyl methionine or NaSO4 will be needed as a supplement.

Methylation Factors

Methyl groups (functional single-carbon atoms) are involved in numerous biochemical reactions in the human body. They are critical to the reading of DNA and should be considered pertinent to the activation of pelvic peritoneal and immune cells in endometriosis.

As noted previously, B vitamins help the liver inactivate estrogen via the Phase II methylation pathway,[85] helping detoxify the DNA-damaging 4OH estrogen metabolite. Because it appears that changes in reading of the DNA in totipotent/stem cells may be critical to the development of endometriosis, attention to methylation in young women would seem to be important. Additionally, adequate methylation is critical for fertility and the developing embryo and could decrease the infertility and pregnancy losses seen in women with endometriosis. Because several enzymes are required to convert folic acid (an inexpensive, man-made folate) to 5-methyl folate (MTHF), the key folate for donating methyl groups to the methionine/homocysteine cycle, B-vitamin supplements containing 5-methyl folate are preferred. Pyridoxine as P5P, thiamine, riboflavin, and B_{12} as methyl or hydroxy cobalamine are also important in this process.

Lipotropic Factors

Supplements that contain choline, betaine, and methionine, the "lipotropic factors," promote the flow of fat and bile (containing estrogen metabolites) from the liver and facilitate excretion in the gut.[86] Because of their prominent role in methylation, making useable single-carbon CH3 molecules available, lipotropic factors may also play a role in the epigenetics of this disease.[87]

Vitamin C

A number of studies suggest that oxidative stress is a prominent feature of endometriosis.[88] Antioxidants are known to work in sequence, suggesting that they should be taken together in to fully reduce oxidants.

Studies with the use of vitamin C show increases in cellular immunity, decreases in autoimmune progression, and decreases in fatigue.[89] It is a potent antioxidant and is a cofactor for many biosynthetic and gene regulatory enzymes. It accumulates in phagocytic cells and enhances phagocytosis such as may be needed to "clean up" the peritoneal cavity after retrograde menstruation.[90] In addition, vitamin C enhances immunity and decreases capillary fragility and tumor growth, all of which are evident at varying levels in women with endometriosis. Studies on autoimmune progression point to the effectiveness of high levels of vitamin C.[90,91]

Beta-Carotene

Beta-carotene helps enhance immunity. Studies show that the use of beta-carotene increased T-cell levels after 7 days.[92] In addition, beta-carotene was shown to be protective against early stages of tumor growth.[91] Retinoids can moderate the effects of IL-6, which has been implicated in endometriosis.[94] Impairment of phagocytosis is seen in vitamin A–deficient states.[95] Although vitamin A was used in the latter

study, one third of beta-carotene is converted to retinol, the active form of vitamin A. Additional studies suggest that immune function is affected more by carotenoids than by vitamin A.[96] A significant portion of the population has genetics limiting the conversion of beta-carotene to vitamin A and therefore requires preformed vitamin A as a supplement (see Chapter 57) to ensure proper immune system function.

Vitamin E

Because parallels have been found between abnormal tumor growth in cancer and abnormal growth of lesions in endometriosis, vitamin E supplementation may be advantageous. Vitamin E helps correct abnormal progesterone:estradiol ratios in patients with mammary dysplasia (increased growth of cells).[97] Vitamin E has been shown to be of benefit in women with dysmenorrhea, a common complaint in patients with endometriosis.[98] When taken as a supplement, care should be taken to avoid synthetic d,l tocopherols, which could have a negative effect. Free radicals may contribute to the inflammation, damage to DNA, and excessive growth of endometrial tissue; vitamin E and N-acetyl cysteine, another antioxidant, can act to inhibit this proliferation.[99,100]

Selenium

Selenium aids in the synthesis of antioxidant enzymes responsible for detoxification reactions within the liver. In addition, selenium stimulates white blood cells and thymic function.[101,102]

In a mouse model, decreased selenium levels are associated with suboptimal cell-mediated immunity, decreased T cells, and associated inflammation.[103] Individuals with low selenium are also vulnerable to yeast infections,[104] a common clinical finding in patients with endometriosis.

N-Acetylcysteine

The rate-limiting precursor for glutathione, the number one antioxidant and detoxification molecule in cells, is N-acetylcysteine (NAC). It has been shown to limit the damage of estrogen quinones and serves as an important addition to an antioxidant regimen. Additionally, many women have glutathione transferase (GSTM1) null status and are not able to use glutathione as well for detoxification. These women need higher levels to ensure utilization of alternative transferases.

Resveratrol

Recently studies have shown that resveratrol, a property of red grapes in juice or wine, may have beneficial effects in endometriosis.[105] Most studies to date have been in animal models, however.[106] Resveratrol interacts with estrogen receptor (ER)-beta to slow growth and upregulates MnSOD, both of which may be helpful in endometriosis.[107]

Pine Bark (Pycnogenol)

Pycnogenol is a special standardized extract from the bark of the French maritime pine. It is composed of polyphenols, several phenolic acids, catechins, taxifolin, and procyanidins. In laboratory research, Pycnogenol was found to selectively inhibit matrix metalloproteinases and inflammatory cells and inhibit cyclooxygenases 1 and 2. Its role in endometriosis was evaluated in a study of 58 women who were surgically diagnosed with endometriosis. After confirming regular menstruation and ovulation for 3 months, they were randomized to receive either Pycnogenol 30 mg twice daily for 48 weeks or a GNRH agonist, leuprorelin acetate depot, 3.75 mg intramuscularly (IM) every 4 weeks for 24 weeks. After 4 weeks on Pycnogenol, patients slowly but steadily improved, experiencing a 33% reduction in symptoms (from severe to moderate). The leuprorelin group had a greater response within the treatment period but relapsed after 24 weeks posttreatment. The Pycnogenol group had the added benefit of maintaining regular menses and normal estrogen levels during treatment, whereas the leuprorelin group had suppressed menstruation and drastically lowered estrogen levels during treatment. Five of the women taking Pycnogenol became pregnant.[108]

Essential Fatty Acids

A number of studies have looked at the fat content in the diet and found that saturated and trans fats are detrimental in endometriosis, whereas long-chain unsaturated fats are beneficial. Long-chain unsaturated FAs provide the precursors for prostaglandins, a group of highly potent pro- and anti-inflammatory molecules. Two main groups of FAs are particularly important: the omega-6 group and the omega-3 group (named for the first carbon atom containing an unsaturated or double bond at the omega end of the molecule). Omega-6 fatty acids (from flaxseed, canola, pumpkin, soy, and walnuts) are precursors for gamma-linolenic acid (GLA; found in borage, black currant, and evening primrose oils), leading to either the series 1 prostaglandins, which are anti-inflammatory, or to arachidonic acid (AA), which, via the COX-2 enzyme, becomes the highly inflammatory series 2 prostaglandins. Another source of AA is animal fats via the enzyme phospholipase A2. Numerous supplements, including *Glycyrrhiza glabra* and quercetin, affect phospholipase A2, blocking the release of AA from membranes. AA, via lipoxygenase, can also be a precursor for leukotrienes, which are known to increase pelvic pain during menses. The omega-3 alpha-linolenic acid (from flaxseed, canola, pumpkin, soy, and walnuts) provides the precursor for EPA and the series 3 prostaglandins, which are anti-inflammatory. EPA is both a COX-2 inhibitor and a lipoxygenase inhibitor, which might be especially effective in endometriosis. Many botanical supplements act as COX-2 inhibitors, including *Zingiber officinale, Curcuma longa,* quercetin , *Boswellia serrata,* and *Withania somnifera.* Lipoxygenase inhibitors include quercetin, *Allium cepa, Allium staivum, Curcuma longa, Silybum marianum,* and *Boswellia serrata.*

In a 2001 study looking at the role of EFA ratios on the viability of endometrial cells and their production of inflammatory cytokines, endometrial cells from women with and without endometriosis attending an infertility clinic were studied in vitro. The culture media was supplemented with various ratios of omega-3 and omega-6 polyunsaturated fatty acids. The investigators found that the higher the ratio of inflammatory omega-6 to anti-inflammatory omega-3 fatty acids, the longer was the survival time and secretion of IL-8 in cells from women with and without endometriosis.[109]

Prostaglandins can be prospasmodic or antispasmodic, depending on the prostaglandins that are being produced; can inhibit or promote tumor growth; and as noted earlier, can stimulate estrogen production via upregulation of aromatase.[110] (See Chapter 57 for a more complete discussion of this complex topic.)

Long-chain FAs, saturated fats, and trans fats make up a large percentage of the lipid bilayer of cells. Polyunsaturated FAs, because of their "bent" configuration in space, allow more fluidity in the membrane. This facilitates the insertion of proteins such as receptors in the membrane, making it more functional. Whereas long-chain unsaturated FAs are "bent," trans fats and saturated fats are "straight." This allows them to stack tightly in the lipid bilayer, excluding proteins and receptors.

Castor oil packs to the abdomen have been used successfully by many women. They are usually applied by soaking a flannel sheet in organic castor oil and applying it to the lower abdomen with a plastic covering topped with a heating pad for 15 to 30 minutes. It is postulated that the castor oil may provide alterations of prostaglandin metabolism in the pelvis. In any event, the 15 to 30 minutes of "quiet time" and relaxation are beneficial to many women.

Botanical Medicines

The herbs appropriate for acute pain relief in endometriosis are the same herbs commonly used for menstrual cramps, such as valerian, crampbark, and black cohosh, in addition to other traditional herbs that might be helpful, such as wild yam, chamomile, blue cohosh, ginger, passion flower, and false unicorn root.

Vitex Agnus Castus

Vitex agnus castus, also known as chaste tree, has traditionally been used as a treatment for hormonal imbalances in women. Through its action on the pituitary gland, chaste tree increases progesterone production via an increase in luteinizing hormone. This herb is useful for fibroids, premenstrual syndrome, and perimenopausal times of increased estrogen. With its use, less estrogen is available to stimulate endometrial tissue.[111]

Taraxacum Officinale

Taraxacum officinale, also known as dandelion root, is one of the most detoxifying herbs. It works principally on the liver and gallbladder to help remove waste products. By supporting the liver, excessive estrogens and toxins can be deactivated. Researchers in Japan have found a link between dandelion and antitumor activity.[112] Additionally, dandelion leaf contains vitamins A, C, and K; calcium; and choline.

Xanthoxylum Americanum

Xanthoxylum americanum, or prickly ash, is known for its specific effects on capillary engorgement and sluggish circulation. Prickly ash stimulates blood flow throughout the body and enhances the transport of oxygen and nutrients, in addition to removing cellular waste products. For women with pelvic congestion, this herb enhances circulation throughout the pelvis.

Leonorus Cardiaca

Leonorus, or motherwort, is antispasmodic and gently soothes the nerves. Because women with endometriosis generally experience uterine cramps and pain, motherwort is useful in promoting relaxation during times of extreme "bearing-down" pain in the uterus and other regions.[62] As a mild sedative, motherwort helps women get needed rest during their menstrual periods.

Turska's Formula

Turska's formula is a well-used old-time naturopathic treatment for decreasing aberrant cancer cell growth. A tincture of this formula is useful in endometriosis owing to the similarities in cell growth found in the pelvis. This formula contains *Aconite napellus* (monkshood), *Gelsemium sempervirens* (yellow jasmine), *Bryonia alba* (bryony), and *Phytolacca americana* (poke root). Monkshood and yellow jasmine contain various alkaloids that have been known to disrupt the assembly of microtubules, which eventually aid in the formation of undifferentiated mesenchymal cells. Quite possibly, these herbal alkaloids interfere with the induction of abnormal ectopic lesions within the pelvis (consistent with the theory of cells left behind in embryonic development). Bryonia is also known to provide antitumor effects. Poke root contains glycoproteins, which are known to stimulate lymphocyte transformation for immune enhancement. In addition, poke root also has anti-inflammatory properties. Owing to its toxic properties, however, this tincture can be provided only by a licensed health professional.

Bioidentical Progesterone

Progesterone has the beneficial effect of downregulating estrogen receptors, thereby decreasing estrogen effects in the normal breast and endometrium. Progesterone, however, is difficult to measure in blood or urine because of its many pathways of use, metabolism, and excretion. Additionally, many, if not most, articles on progesterone are actually written about man-made progestins, which have a very different biological profile. Progesterone induces a secretory endometrium, preparing it for normal menstruation (or pregnancy). Progesterone in the form of artificial progestins has long been used to control the overgrowth of eutopic endometrium when administering estrogens, so it is not surprising that progestins have been proposed as a treatment for endometriosis. The use of bioidentical progesterone in the treatment of endometriosis is not well accepted because endometriosis appears to be progesterone resistant. In eutopic endometrium, however, progesterone may produce benefits for women with endometriosis. In the perimenopausal period, when estrogens are high and progesterone from the corpus luteum is low or absent, endometriosis is often seen to flourish. Balancing estrogen and progesterone would be expected to have the benefits of (1) lowering estrogen effect and decreasing menstrual flow ergo retrograde menstruation; (2) decreasing uterine cramping and pain ergo retrograde flow; and (3) improving psychological distress and lowering catecholamines, thereby reducing pain. Bioidentical progesterone is usually not used alone as a treatment for endometriosis but as part of a comprehensive natural treatment plan. Bioidentical progesterone creams can be applied in various regimens. In women attempting pregnancy, application should begin *after* ovulation to avoid inhibiting ovulation or interfering with the establishment of the corpus luteum. Administration can be transdermal (15–30 mg/day in divided doses), preferably on nonfatty areas of the skin, such as the forearm, to avoid deposition in fat. Micronized progesterone in cream or oil can be used vaginally. An oral micronized progesterone approved by the U.S. Food and Drug Administration (FDA) is available if sedating effects are desired. (Note: It is delivered in peanut oil, so allergic patients will have to get an alternative from a formulating pharmacy.) An FDA-approved vaginal preparation is also available and recommended in luteal-phase defects in women attempting pregnancy. If a luteal-phase deficiency is suspected, progesterone application should continue until the placental production of progesterone is well established (around 12 weeks) and then weaned slowly to prevent a sudden change in progesterone level, which could lead to uterine irritability. For the treatment of nonpregnant patients, antispasmodic, anticramping effectiveness may require higher doses, and the application may need to be extended into the menstrual phase of the cycle. Bioidentical progesterone is usually not used alone as a treatment for endometriosis but as part of a comprehensive natural treatment plan.

A COMPREHENSIVE APPROACH TO THE PATIENT WITH ENDOMETRIOSIS

Because very young women can have symptoms that cannot be differentiated from early endometriosis, such as heavy periods and cramping, addressing these symptoms early may prevent or delay the onset and severity of disease as well as preserve fertility.

Assessment of gastrointestinal function, the microbiome, and the diet is a critical first step that will have far-reaching effects outside of endometriosis. These are areas young women do not often wish to address, but heavy, painful periods may provide an impetus for early correction and the establishment of good habits, which can have long-lasting effects. Simplifying the recommendations may be helpful. The primary dietary recommendation is to increase the consumption of high-fiber vegetables, especially crucifers, *Allium family,* beta-carotene, and fruits high in antioxidants. Decrease sources of saturated and trans fats, and increase the consumption of foods rich in omega-3 fatty acids, especially fatty fish (salmon, herring, sardines, mackerel). Sources of sugar, caffeine, and alcohol should be limited.

Pharmaceutical approaches with oral contraceptives and NSAIDs and progestin-eluting intrauterine devices (IUDs), GNRH agonists, and aromatase inhibitors have been the mainstay of conventional treatment. However, all are fraught with side effects. Oral contraceptives would be the best approach if pregnancy prevention is desired because they have the overall effect of lowering estrogen levels and decreasing menstrual flow with the most acceptable side-effect profile.

Supplements

- An antioxidant combination containing vitamin C 500 mg, beta-carotene, vitamin E (as d-mixed tocopherols) 200 IU, and selenium 100 to 200 mcg. Adding these together improves the body's ability to detoxify and remove free radicals while keeping the doses relatively low.
- N-acetylcysteine 800 to 1000 mg/day
- Pycnogenol: 60 to 150 mg a day and/or resveratrol
- Fish oil: 1000 mg EPA + docosahexaenoic acid (DHA) a day for inflammation and to improve bowel function and the health of gut flora
- B complex: containing folate as 5-methyl folate, B6 as P5P, cobalamin as methyl or hydroxocobalamin, thiamine, choline or betaine, and methionine, to improve the epigenetic health and estrogen metabolism. Dosing will be patient specific.

Botanical Medicines

- Chaste tree, dandelion root, prickly ash, and motherwort tincture (equal parts): 1½ tsp to 1 tsp three times a day
- Turska's formula (*Aconite* 1½ drams, *Bryonia* 1½ drams, *Gelsemium* 1½ drams, Phytolacca 3 drams, and ½ dram water): five drops four times a day

Topical

- Progesterone cream (Table 166.1; Option 1 should only be used in anovulatory women not attempting pregnancy)
- Castor oil packs to the abdomen

Stress Reduction

- Stress reduction to relieve pain and lower cortisol steal. Many options are available: yoga, meditation, HeartMath, transcutaneous electrical nerve stimulation (TENS), progressive relaxation.

TABLE 166.1 Progesterone Cream Schedule

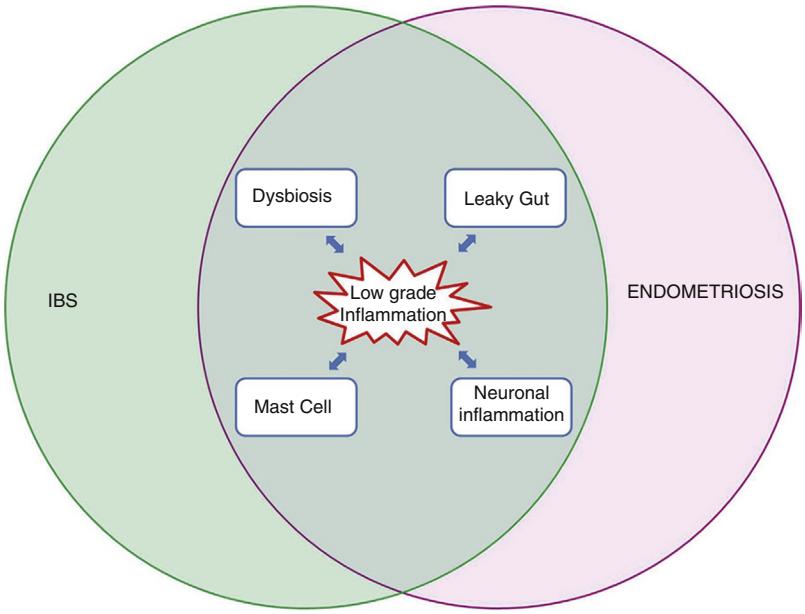
Option 1	Days 1–7	No cream
	Days 8–28	¼ to ½ tsp twice a day
Option 2	Days 1–14	No cream
	Days 15–28	¼ to ½ tsp twice a day
Option 3	Days 1–21	No cream
	Days 22–28	¼ to ½ tsp twice a day

Surgery

Surgical treatment of endometriosis is both the first and last line of treatment in conventional medical practice. Laparoscopic surgery has advanced dramatically in the past 40 years. Endometriosis surgery, however, can be quite difficult because of adhesions and distortion of the anatomy. The surgical approach usually includes confirmation of the presumed diagnosis with biopsy, identification of the extent and location of disease, debulking disease and lysing adhesions, removal of endometriomas, ablation of the uterosacral nerves by electrocautery, laser or endocoagulation, and presacral neurectomy or hysterectomy with bilateral salpingo-oophorectomy as a last resort. Damage to the bowel, bladder, ureters, and blood vessels is not unusual and is commonly related to the depth of disease and the experience of the operator. The management of ovarian disease at surgery varies; some surgeons prefer to keep as much ovarian tissue as possible, and some will sacrifice severely damaged adnexa to allow for regular ovulation each month on the remaining, less damaged side. Surgery has been shown to increase pregnancy rates in the immediate postoperative period.[113]

REFERENCES

See www.expertconsult.com for a complete list of references.



(Vigano D, Zara F, et al. Irritable bowel syndrome and endometriosis: New insights for old diseases. *Dig Liver Dis*. 2018 Mar;50[3]:213–219. https://doi.org/10.1016/j.did2018.12017. Epub 2017 Dec 27.)

REFERENCES

1. McKinnon B, Mueller M, Montgomery G. Progesterone resistance in endometriosis: an acquired property? *Trends Endocrinol Metab*. 2018;29(8):535–548. https://doi.org/10.1016/j.tem.2018.05.006. Epub 2018 Jun 19.
2. Parasar P, Ozcan P, et al. Endometriosis: epidemiology, Diagnosis and Clinical Management. *Curr Obstet Gynecol Rep*. 2017;6(1):34–41. published online 2017 Jan 27. https://doi.org/10.1007/s13669-017-0187-1.
3. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril*. 1999;71:701–710.
4. Arvanitis DA, Goumenou AG, et al. Low-penetrance genes are associated with increased susceptibility to endometriosis. *Fertility Steril*. 2001;76(6):1202–1206.
5. Hevir N, Ribic-Pucelj M, et al. Disturbed balance between phase I and II metabolizing enzymes in ovarian endometriosis: a source of excessive hydroxy-estrogens and ROS? *Mol Cell Endocrinol*. 2013;267(1–2):74–84.
6. Segall SK, Maixner W, et al. Janus molecule 1: dichotomous effects of COMT in neuropathic vs nociceptive pain modalities CNA. *Neurol Disord Drug Targets*. 2012;11(3):222–235.
7. Liebermann C, Kohl Schwartz AS, et al. Maltreatment during childhood: a risk factor for the development of endometriosis? *Hum Reprod*. 2018. https://doi.org/10.1093/humrep/dey111. [Epub ahead of print].
8. Kitawaki J, Kado N, et al. Endometriosis: the pathophysiology as an estrogen-dependent disease. *J Ster Biochem Mol Biol*. 2003;83:149–155.
9. Schweppe KW, Wynn RM. Ultrastructural changes in endometriotic implants during the menstrual cycle. *Obstet Gynecol.* 1981;58:465–473.
10. Metzger DA, Olive DL, Haney AF. Limited hormonal responsiveness of ectopic endometrium: histologic correlation with intrauterine endometrium. *Hum Pathol*. 1988;19:1417–1424.
11. Huhtinen K, Saloniemi-Heinonen T, et al. Intra-tissue steroid profiling indicates differential progesterone and testosterone metabolism in the endometrium and endometriosis lesions. *J Clin Endocrinol Metab*. 2014;99(11):e2188–e2197.
12. Noble L, Simpson E, Johns A, et al. Aromatase expression in endometriosis. *J Clin Endo Metab*. 1996;81:174–179.
13. Colette S, Donnez J. Are aromatase inhibitors effective in endometriosis treatment? *Expert Opin Investig Drugs*. 2011. [Epub ahead of print].
14. Ferrero S, Evangelisti G, Barra F. Current and emerging treatment options for endometriosis. *Expert Opin Pharmacother*. 2018:1–17. https://doi.org/10.1080/14656566.2018.1494154. [Epub ahead of print].
15. Vercellini P, Eskenazi B, Consonni D, Somigliana E, Parazzini F, Abbiati A, et al. Oral contraceptives and risk of endometriosis: a systematic review and meta-analysis. *Hum Reprod Update*. 2011;17(2):159–170.
16. Buck Louis GM, Chen Z, et al. Persistent lipophilic environmental chemicals and endometriosis: the ENCO study. *Environ Health Perspect*. 2012;120:811–816. https://doi.org/10.1289/ehp.1104432. Accessed March 14, 2012.
17. Koninckx PR, Ussia A, et al. Evidenced-based medicine: pandora's box of medical and surgical treatment of endometriosis. *J Minim Invasive Gynecol*. 2018;25(3):360–365. https://doi.org/10.1016/j.jmig.2017.11.012. Epub 2017 Nov 24.
18. Gordts S, Koninckx PR, Brosens I. Pathogenesis of deep endometriosis. *Fertil Steril*. 2017;108:872–885.
19. Dmowski W, Steele R, Baker G, et al. Deficient cellular immunity in endometriosis. *Am J Obstet Gynecol*. 1981;141:377–383.
20. Jerman LF, Hey-Cunningham AJ. The role of the lymphatic system in endometriosis: a comprehensive review of the literature. *Biol Reprod*. 2015;92(3):64. https://doi.org/10.1095/biolreprod.114.124313 Epub2015 Jan 14.
21. Pacchiarotti A, Caserta D, Sbracia M, Moscarini M. Expression of oct-4 and c-kit antigens in endometriosis. *Fertil Steril*. 2010. [Epub ahead of print].
22. Fettback PB, Pereira RM, Roaha AM. Expression of stem cell-related genes in the endometrium and endometriotic lesions: a pilot study. *Gynecol Endocrinol*. 2016;32(1):82–86. https://doi.org/10.3109/09513590.2015.1092135. Epub 2015 Oct 1.
23. Rier S, Martin D, Bowman R, et al. Immunoresponsiveness in endometriosis: implications of estrogenic toxicants. *Envir Health Perspect*. 1995;103(suppl 7):151–156.
24. Ranton J, Golden J. Radiation-induced endometriosis in *Maccaca mulatta*. *Radiat Res*. 1991;126:141–146.
25. Rier S, Martin D, Bowman R, et al. Endometriosis in rhesus monkeys *(Maccaca mulatta)* following chronic exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol*. 1993;21:431–441.
26. Koninckx P, Braet P, Kennedy S, et al. Dioxin pollution and endometriosis in Belgium. *Hum Reprod*. 1994:91001–91002.
27. Pauwels A, Schepens P, D'Hooghe T, et al. The risks of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-controlled study of infertile women. *Hum Reprod*. 2001;16:2050–2055.
28. Eskenazi B, Mocarelli P, Warner M, et al. Serum dioxin concentrations and endometriosis: a cohort sudy in Seveso, Italy. *Environ Health Perspect.* 2002;110:629–634.
29. Myers J, Guillette Jr L, Palanza P, et al. The emerging science of endocrine disruption. *Science and Culture Series*. Erice, Italy: International Seminar on Nuclear War and Planetary Emergencies, 28th Session; 2003.
30. Cobellis L, Latini G, De Felice C, et al. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod*. 2003;18(7):1512–1515.
31. Campbell S, Raza M, Pollack AZ. Perfluoroalkyl substances and endometriosis in US women in NHANES 2003-2006. *Reprod Toxicol*. 2016;65:230–235. PubMed PMID: 27544573.
32. Leibovic D, Mueller M, Taylor R. Immunobiology of endometriosis. *Fertil Steril*. 2001;75:1–10.
33. Wei M, Chen X, Zhao Y. Effects of prenatal environmental exposures on the development of endometriosis in female offspring. Sage. *J Reprod Sci*. 23(9):1129–1138. Article first published online: February 22, 2016; Issue published: September 1, 2016.
34. Wilson T, Hertzog P, Angus D, et al. Decreased natural killer cell activity in endometriosis patients: relationship to disease pathogenesis. *Fertil Steril*. 1994;62(5):1086–1088.
35. Oosterlynick D, Meuleman C, Waer M, et al. Immunosuppressive activity of peritoneal fluid in women with endometriosis. *Obstet Gynecol*. 1993;82(2):206–211.
36. Gleicher N, el-Roeiy A, Confino E, et al. Is endometriosis an autoimmune disease? *Obstet Gynecol*. 1987;70:115–122.
37. Gleicher N, Pratt D. Abnormal (auto)immunity and endometriosis. *Int J Gynecol Obstet*. 1993;40(suppl):S21–S27.
38. Mathur S, Peress M, Williamson H, et al. Autoimmunity to endometrium and ovary in endometriosis. *Clin Exp Immunol*. 1982;50:259–266.
39. Halme J, Becker S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. *Am J Obstet Gynecol*. 1983;145:333–337.
40. Nomiyama M, Hachisuga T, Sou H, et al. Local immune response in infertile patients with minimal endometriosis. *Gynecol and Obstet Invest*. 1997;44:32–37.
41. Oosterlynck D, Cornillie F, Waer M, et al. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. *Fertil Steril*. 1991;56:45–51.
42. Chegini N. Peritoneal molecular environment, adhesion formation, and clinical implication. *Front Biosci*. 2002;1:e91–e115.
43. Bazvani R, Templeton A. Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis. *Reproduction*. 2002;123:217–226.
44. Yamaguchi K, Mandai M, Toyokuni S, et al. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. *Clin Cancer Res*. 2008;14(1):32–40. https://doi.org/10.1158/1078-0432.CCR-07-1614.
45. Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis of endometriosis. *Int J Reprod Med*. 2014;2014:9. https://doi.org/10.1155/2014/179515.
46. Simpson J, Elias S, Malinak L, et al. Heritable aspects of endometriosis. *Am J Obstet Gynecol*. 1980;137:327–331.

47. Kennedy S, Mardon H, Barlow D. Familial endometriosis. *J Assist Reprod Genet*. 1995;12:32–34.
48. Stefansson H, Geirsson R, Steinthorsdottir V, et al. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod*. 2002;17:555–559.
49. Moen M. Endometriosis in monozygotic twins. *Acta Obstet Gynecol Scand*. 1994;73:59–62.
50. Hadfield R, Mardon H, Barlow D, et al. Endometriosis in monozygotic twins. *Fertil Steril*. 1997;68:941–942.
51. Treloar S, O'Connor D, O'Connor V, et al. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril*. 1997;71:701–710.
52. Wang Y, Guo S. Statistical methods for detecting genomic alterations through array-based comparative genomic hybridization (CGH). *Front Biosci*. 2004;9:540–549.
53. Vigano D, Zara F, et al. Irritable bowel syndrome and endometriosis: new insights for old diseases. *Dig Liver Dis*. 2018;50(3):213–219. https://doi.org/10.1016/j.did2018.12017. Epub 2017 Dec 27.
54. Giamberardino MA, Costantini R, Affaitati G, Fabrizio A, Lapenna D, Tafuri E, et al. Viscero-visceral hyperalgesia: characterization in different clinical models. *Pain*. 2010;151:307–322. https://doi.org/10.1016/j.pain.2010.06.023.
55. Laschke MW, Menger MD. The gut microbiota: a puppet master in the pathogenesis of endometriosis? *Am J Obstet Gynecol*. 2016;215(1):68.e1–e4. https://doi.org/10.1016/j.ajog.2016.02.036 Epub 2016 Feb 18.
56. Kohchi C1, Tanabe Y, Noguchi K, Mizuno D, Soma G. Induction of differentiation in embryonic stem cells by 26-kD membrane-bound tumor necrosis factor (TNF) and 17-kD free TNF. *Vivo*. 1996;10(1):19–27.
57. Iwabe T, Harada T, et al. Tumor necrosis factor-a promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J Clin Endcrinol Metab*. 2000;85:824–829.
58. The practice Committee of the American Society for Reproductive Medicine. The treatment of pelvic pain associated with endometriosis: a committee opinion. *Fertil Steril*. 2014;101(4):927–935.
59. Koninckx P, Meuleman C, Mourman P, et al. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertil Steril*. 1991;55(4):759–765.
60. Koninckx P, Martin D. Treatment of deeply infiltrating endometriosis. *Curr Opin Obstet & Gynec*. 1994;6(3):231–241.
61. Birrer MJ. The Origin of Ovarian Caner—Is It Getting Clearer? 2010. *N Engl J Med*. 2010;363:1574–1575. https://doi.org/10.1056/NEJMe1009527.
62. Issa B, Onon TS, Agrawal A, Shekhar C, Morris J, Hamdy S, Whorwell PJ. Visceral hypersensitivity in endometriosis: a new target for treatment? *Gut*. 2012;61(3):367–372.
63. Jurkiewicz-Przondziono J, Kwiakowska-Pamula A. Influence of diet on the risk of developing endometriosis. *GinekolPol*. 2017;88(2):96–102. https://doi.org/10.56033/GPa2017.0017.
64. Hansen SO, Knudsen UB. Endometriosis dysmenorrhea and diet. *Eur J Obstet Gynecol Repsond Biol*. 2013;169(2):162–171. https://doi.org/10.1016/j.ejogrb.2013.03.028 Epub 2013 May 2.
65. Galland L. Diet and Inflammation. *Nutr Clin Pract*. 2010;25(6):634–640. https://doi.org/10.1177/0884533610385703.
66. Guo SW. Epigenetics of endometriosis. *Mol Hum Reprod*. 2009;15:587–607.
67. Michnovicz J, Bradlow H. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr Cancer*. 1991;16:59–66.
68. Leibovitz BE, Mueller JA. Bioflavonoids and polyphenols: medical applications. *J Opt Nutr*. 1993;2:17–35.
69. Parazzini F, Vigano P. Diet and endometriosis risk: a literature review. *Reprod BioMed Online*. 2013;26:323–336.
70. Nagata C, Takatsuka N, Kawakami N, et al. Soy product intake and premenopausal hysterectomy in a follow-up study of Japanese women. *Eur J Clin Nutr*. 2001;55(9):773–777.
71. Sutrisno Sutrisno, Aprina Hardianti, et al. Genistein modulates the estrogen receptor and suppresses angiogenesis and inflammation in the murine model of peritoneal endometriosis. *J Tradit Complement Med*. 2018;8(2):278–281. Published online 2017 Apr 25. https://doi.org/10.1016/j.jtcme.2017.03.002.
72. Ma Y, Hébert JR, Li W. *Association between dietary fiber and markers of systemic inflammation in the Women's Health Initiative Observational Study Nutrition*; 2008.
73. Leung A. Encyclopedia of common natural ingredients used in food. *Drugs and Cosmetics*. New York: Wiley & Sons; 1980.
74. Goldin B, Adlercreutz H, Dwyer JT, et al. Effect of diet on excretion of estrogens in pre- and postmenopausal women. *Cancer Res*. 1981;41:3771–3773.
75. Missmer SA, Chavarro JE, Malspeis S, et al. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;25(6):1528–1535.
76. Mozaffarian D, Aro A, Willett WC. Health effects of trans-fatty acids: experimental and observational evidence. *Eur J Clin Nutr*. 2009.
77. He K, Liu K, Daviglus ML. Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish with biomarkers of inflammation and endothelial activation (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *Am J Cardiol*. 2009.
78. Missmer SA, Chavarro JE, Malspeis S, et al. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;25(6):1528–1535.
79. Missmer SA, Chavarro JE. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;(6):1528–1535. https://doi.org/10.1093/humrep/deq044. Epub 2010 Mar 23.
80. Missmer S, Chavarro JE, Malspeis S, et al. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;25(6):1528–1535.
81. Yudkin J, Elisa O. Dietary sucrose and oestradiol concentration in young men. *Ann Nutr Metab*. 1988;32:53–55.
82. Mathias JR, Franklin R, Quast DC, et al. Relation of endometriosis and neuromuscular disease of the gastrointestinal tract: new insights. *Fertil Steril*. 1998;70(1):81–88.
83. Grodstein F, Goldman M, Ryan L, et al. Relation of female infertility to consumption of caffeinated beverages. *Am J Epidemiol*. 1993;137:1353–1360.
84. Tremblay L. Reproductive toxins conference—pollution prevention network. *Endomet Assoc Newsletter*. 1996;17(5–6):13–15.
85. Biskind M, Biskind G. Effect of vitamin B complex deficiency on inactivation of estrone in the liver. *Endocrinol*. 1942;31:109–114.
86. Murray M, Pizzorno J. Immune support. *Encyclopedia of Natural Medicine*. Rocklin, CA: Prima Publishing; 1998.
87. Szczpearnska M, Mostowska A, et al. Polymorphic variants of folate and choline metabolism genes and the risk of endometriosis-associated infertility. *Eur J Obstet Gynecol Reprod Biol*. 2011;157(1):67–72. https://doi.org/10.1016/j.ejogrb.2011.02.003. Epub 2011 Mar 22.
88. Harlev A, Gupta S, et al. Targeting oxidative stress to treat endometriosis. *Expert Opin Ther Targets*. 2015;19(11):1447–1464. https://doi.org/10.1517/14728222.2015.1077226. Epub 2015 Aug 10.
89. Anderson R. The immunostimulatory, anti-inflammatory and anti-allergic properties of ascorbate. *Adv Nut Res*. 1984;6:19–45.
90. Carr AC, Maggini S. Vitamin C and Immune Function. *Nutrients*. 2017;9(11):1211. https://doi.org/10.3390/nu9111211. Published online 2017 Nov 3.
91. Leibovitz B, Siegel B. Ascorbic acid and the immune response. *Adv Exp Med Biol*. 1981;135:1–25.
92. Alexander M, Newmark H, Miller RG. Oral beta-carotene can increase the number of okT4+ cells in human blood. *Immunol Lett*. 1985;9:221–224.
94. Sawatsri S, Desai N, Rock JA, et al. Retinoic acid suppresses interleukin-6 production in human endometrial cells. *Fertil Steril*. 2000;73(5):1012–1019.
95. Ongsakul M, Sirisinha S, Lamb A. Impaired blood clearance of bacteria and phagocytic activity in vitamin A deficient rats. *Proc Soc Exp Biol Med*. 1985;178:204–208.
96. Watson R, Prabhala R, Plezia P, et al. Effect of beta-carotene on lymphocyte subpopulations in elderly humans: evidence for a dose-response relationship. *Am J Clin Nutr*. 1991;53:90–94.
97. London R, Sundaram G, Schultz M, et al. Endocrine parameters and alpha-tocopherol therapy of patients with mammary dysplasia. *Cancer Res*. 1981;41:3811–3813.
98. Butler EB, McKnight E. Vitamin E in the treatment of primary dysmenorrhea. *Lancet*. 1955;1:844–847.

99. Foyouzi N, Berkkanoglu M, Arici A, et al. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. *Fertil Steril*. 2004;82(suppl 3):1019–1022.
100. Murphy AA, Santanam N, Parthasarathy S. Endometriosis: a disease of oxidative stress? *Semin Reprod Endocrinol*. 1998;16(4):263–273. Review.
101. Kiremidjian-Schumacher L, Stotzky G. Selenium and immune responses. *Environ Res*. 1987;42:277–303.
102. Arthur JR, McKenzie RC, et al. Selenium in the Immune System. *J Nutr*. 2003;133(5):147S–149S. https://academic.oup.com/jn/article/133/5/1457S/4558526. Accessed July 15, 2018.
103. Spallholz J, Martin J, Gerlach M, et al. Immunologic responses of mice fed diets supplemented with selenite selenium. *Proc Soc Exp Biol Med*. 1973;143:685–689.
104. Hoffmann PR, Berry MJ. The influence of selenium on immune response. *Mol Nutr Food Res*. 2008;52(11):1273–1280.
105. Ricci AG, Olivares CN, et al. Natural therapies assessment for the treatment of endometriosis. *Hum Reprod*. 2013;28(1):176–188. https://doi.org/10.1093/humrep/des369. Epub 2012 Oct 18.
106. Kolahdouz Mohammadi R, Arabiou T, et al. Resveratrol and endometriosis: In vitro and animal studies and underlying mechanisms (Review). *Biomed Pharmacother*. 2017;91:220–228. https://doi.org/10.1016/j.biopha.2017.04.078. Epub 2017 Apr 28.
107. Robb EL, Stuart JA. Resveratrol interacts with estrogen receptor-β to inhibit cell replicative growth and enhance stress resistance by upregulating mitochondrial superoxide dismutase. *Free Radic Biol Med*. 2011. [Epub ahead of print].
108. Kohama T, Herai K, Inoue M. Effect of French maritime pine bark extract on endometriosis as compared with leuprorelin acetate. *J Reprod Med*. 2007;52(8):703–708.
109. Gazvani MR, Smith L, Haggarty P, et al. High omega-3:omega-6 fatty acid ratios in culture medium reduce endometrial-cell survival in combined endometrial gland and stromal cell cultures from women with and without endometriosis. *Fertil Steril*. 2001;76:717–722.
110. Moncada S, Salmon J. Leukocytes and tissue injury: the use of eicosapentaenoic acid in the control of white cell activation. *Wien Klin Wochenschr*. 1986;98:104–106.
111. Decapite L. Histology, anatomy and antibiotic properties of Vitex agnus castus. *Ann Facul Agr Univ Studi Perugia*. 1967;22:109–126.
112. Baba K, Abe S, Mizuno D. Antitumor activity of hot water extract of dandelion, Taraxacum officinale—correlation between antitumor activity and timing of administration. *Yakugaku Zasshi*. 1981;101:538–543.
113. Jacobson TZ, Duffy JM, Barlow D, Farquhar C, Koninckx PR, Olive D. Laparoscopic surgery for subfertility associated with endometriosis. *Cochrane Database Syst Rev*. 2010;(1):CD001398.

167

Epilepsy

Gaetano Morello, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Recurrent seizures
- Characteristic electroencephalographic changes accompanying seizures
- Mental status abnormalities or focal neurological symptoms potentially persisting for hours postictally

GENERAL CONSIDERATIONS

The word *epilepsy* derives from the Greek word *epilepsia,* which means "to take hold of" or "to seize." Epilepsy is not a disease in itself but rather a symptom of disease. The epilepsies are a group of disorders characterized by sudden, recurrent, episodic changes in neurological function caused by abnormalities in the electrical activity of the brain.[1–4] Each episode of neurological dysfunction is called a seizure. Seizures are termed *convulsive* when accompanied by motor manifestations or *nonconvulsive* when accompanied by sensory, cognitive, or emotional events. Epilepsy can occur because of a number of abnormalities, such as neurological injuries, structural brain lesions, or some systemic diseases. Epilepsy is termed *idiopathic* when there is neither a history of neurological insult nor other apparent neurological dysfunction.[3] Whatever the etiology, the common denominator in all these conditions is the epileptic attack or seizure.

Epidemiology

The reported incidence and prevalence rates of epilepsy have varied widely because of uncontrolled methodological factors.[5] According to the best available data, the prevalence of chronic recurrent epilepsy is about 10 per 1000, and it has been estimated that 10% of the population will have one or more seizures at some point in life. The cumulative lifetime incidence of epilepsy is approximately 3%.[6] An additional 2% to 5% of children experience febrile convulsions during the first several years of life. About 10% of these children, especially those in whom the febrile seizure is prolonged, develop epilepsy later in life.[1] Prospective studies show that more than 60% of newly diagnosed patients enter remission with conventional treatment.[7]

Etiology

Epilepsy may be idiopathic, cryptogenic, or symptomatic.[8] Idiopathic epilepsies are generally genetic in origin and account for 70% to 80% of all cases of epilepsy. The role of genetic factors in the pathogenesis is complex because of diverse conditions leading to the common symptom of seizures. In general, the prevalence of seizures in close relatives is three times that of the overall population.[1] William Lennox meticulously studied the relationship of epilepsy to genetics from 1934 through 1958, using twin studies. He concluded that epilepsy was a condition in which genetic and exogenous factors interact in all patients, with variable weighting in each particular case.[9]

This understanding sparked the concept that many factors play a role in the epileptic condition. Cryptogenic epilepsies are those in which an underlying cause is suspected but the etiology remains undetected. Finally, epilepsies for which there is an underlying structural cause or major metabolic derangement are considered symptomatic, for a probable cause has been identified.[4] As shown in Table 167.1, probable causes can be determined by age at onset of seizures. Box 167.1 lists the most common etiological factors.

Classification

The current classification of epileptic seizures is based on clinical and electroencephalographic criteria proposed by the International League Against Epilepsy. Box 167.2 lists the clinical features.

TABLE 167.1 Presumptive Causes of Epilepsy According to Age at Onset of First Seizure

Age at Onset	Presumptive Causes
Birth to 2 years	Birth injury, degenerative brain disease
2–19 years	Congenital birth injury, febrile thrombosis, head trauma, infection (meningitis or encephalitis)
20–34 years	Head trauma, brain neoplasm
35–54 years	Brain neoplasm, head trauma, stroke
55 years and older	Stroke, brain neoplasm

Data from Hauser WA, Hesdorfer DC. *Epilepsy: Frequency, Causes, and Consequences*. New York: Demos; 1990; Freeman JM, Vining EPG, Pillas DJ. *Seizures and Epilepsy in Childhood: A Guide for Parents*. Baltimore: Johns Hopkins University Press; 1990.

BOX 167.1 The Most Common Etiological Factors in Epilepsy

- Brain damage before or at birth (congenital malformations, anemia, fetal infection)
- Head trauma significant enough to injure the brain and cause gliosis
- Central nervous system infections such as meningitis, encephalitis, brain abscess, neurosyphilis, rabies, tetanus, falciparum malaria, toxoplasmosis, and cysticercosis of the brain
- Metabolic disorders: hypocalcemia, hypoglycemia, hypoparathyroidism, phenylketonuria, and withdrawal from alcohol and drugs
- Brain tumors and other space-occupying lesions
- Stroke and other vascular disorders
- Degenerative brain disease
- Genetic disease
- Toxic conditions
- Idiopathic causes

BOX 167.2 Classification of Epileptic Seizures in Adults

Partial (Focal, Local) Seizures

- Simple partial seizures (consciousness not impaired)
- Motor signs
- Somatosensory or special sensory symptoms
- Autonomic symptoms or signs
- Psychic symptoms
- Complex partial (consciousness impaired)
- Simple partial onset followed by impaired consciousness
- Consciousness impaired at onset
- Partial seizures evolving to generalized seizures (tonic, clonic, or tonic-clonic)
- Simple partial seizures evolving to generalized seizures
- Complex partial seizures evolving to generalized seizures
- Simple partial seizures evolving to partial seizures evolving to generalized seizures

Generalized Seizures (Convulsive or Nonconvulsive)

- Absence seizures
- Typical (brief stare, eye flickering, no emotion)
- Atypical (associated with movement)
- Myoclonic seizures
- Clonic seizures
- Tonic seizures
- Tonic-clonic seizures
- Atonic seizures
- Unclassified seizures

Modified from Willmore LJ, Ferrendelli JA. Epilepsy. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American; 1997: 11, XII-1-14.

DIAGNOSIS

Differentiating between partial or focal seizures and generalized seizures is of great clinical significance. Partial seizures begin focally with a specific sensory, motor, or psychic aberration that reflects the affected part of the cerebral hemisphere.[5] These types of seizures may remain localized, whereas generalized seizures appear to spread from their onset and usually affect both consciousness and motor function.[2–5] Differentiation is important because partial seizures are usually indicative of focal brain disorders such as tumors or gliosis, whereas generalized seizures rarely have a definable etiology (although some studies now implicate metabolic disorders).

An eyewitness account of a typical attack can be of great value in classifying the seizure. Past trauma, infections, and the use of drugs and alcohol should be fully explored, as should the family history. A complete neurological examination should be performed as a preliminary screen for neoplasms. Laboratory work should include the following[4]:

- Serum glucose and calcium
- Complete blood cell count
- Liver and kidney function tests
- Serological test for syphilis
- Skull radiographs
- An electroencephalogram (EEG)
- A computed tomography (CT) scan
- Magnetic resonance imaging (MRI)

Examination of the cerebrospinal fluid is indicated if infection or meningeal neoplasm is suspected.[1] MRI is considered the gold standard in evaluating epilepsy because the resolution is superior to that of the CT scan.[6] Newer studies also employ EEG recording during functional MRI scanning to map normal and pathological brain function.[10] Epilepsy should not be diagnosed on the basis of a solitary seizure. The recurrence rate after a single seizure is approximately 27% over 3 years.[11]

PATHOPHYSIOLOGY

The hallmark of the altered physiological state of epilepsy is a rhythmic, repetitive, synchronous discharge of many neurons in a localized area of the brain.[3] This discharge pattern is easily recorded on the EEG during an attack. However, the cause of the abnormal discharges is still not known. Research shows that the synchronous depolarization of masses of neurons is the result of a combination of increased excitatory mechanisms and decreased inhibitory mechanisms.[12]

Seizures can be induced in experimental models by blocking inhibitory mechanisms. For example, agents that block the action of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) are potent convulsants in humans. Conversely, the antiepileptic drugs phenobarbital and benzodiazepines enhance GABA, thus supporting the inhibitory mechanism. In some forms of chronic focal epilepsy, inhibitory terminals on neurons in areas around cortical gliotic lesions are in fact diminished.

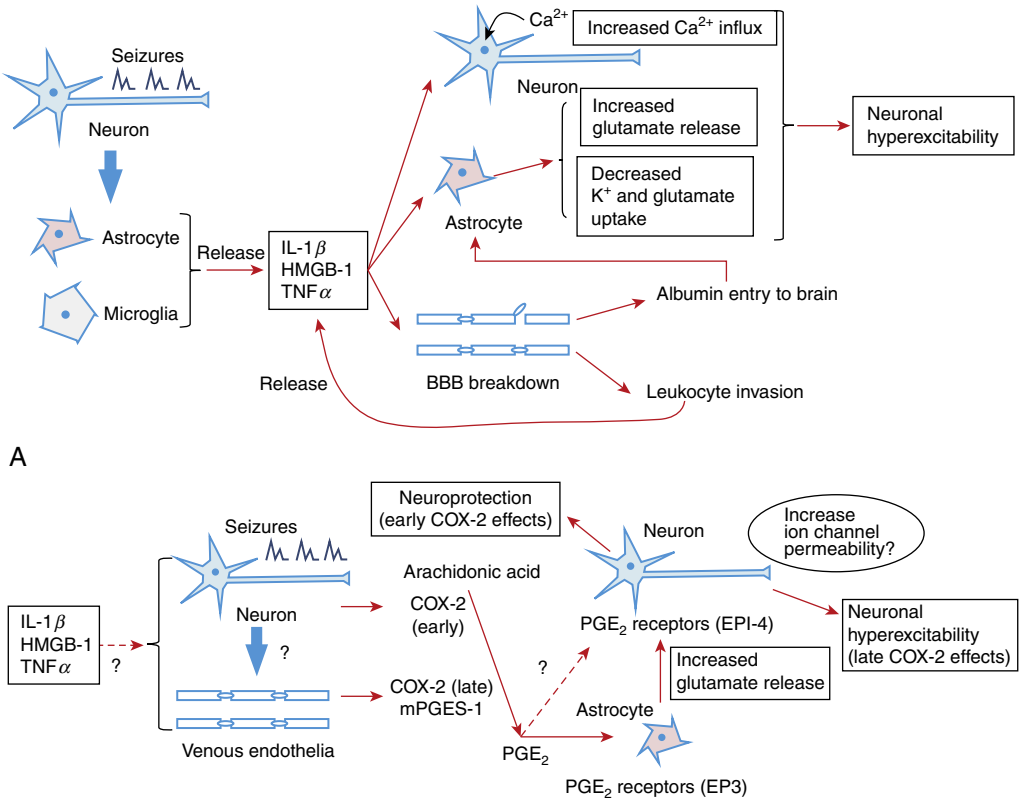


Fig. 167.1 Proposed inflammatory mechanisms in epileptogenesis. (a) Epileptic seizures induce the release of cytokines from glial cells, thereby (1) increasing the influx of neuronal calcium, (2) enhancing extraneuronal glutamate concentration, (3) decreasing K^+ and glutamate uptake by glia, and (4) impairing the blood–brain barrier (BBB). BBB breakdown leads to albumin entry and leukocyte invasion into the brain, resulting in a further release of inflammatory cytokines. Such inflammatory responses cause an induction of neuronal hyperexcitability, reoccurrence of seizures, and finally, the development of refractory epilepsy. (b) Seizures induce cyclooxygenase-2 (COX-2) in neurons (early phase) and vascular endothelial cells (late phase) and mPGES-1 in endothelial cells. These inducible prostaglandin (PG) synthases cooperate to produce PGE2, most likely in endothelial cells. Endothelial PGE2 might cause neuronal hyperexcitability by enhancing glutamate release from astrocytes via the glial EP3 receptor, whereas neuronal PGs may protect neurons against seizures. (From Shimada T, Takemiya T, Sugiura H, Yamagata K. Role of inflammatory mediators in the pathogenesis of epilepsy. *Med Inflamm.* 2014;901–902. PubMed PMID: 25197169.)

Other research demonstrates the instability of the nerve cell membranes in patients with epilepsy. During seizure activity, intracellular Ca^{2+} stored in the cell organelles is released and moves toward the inner cell membranes, where it binds to specific Ca^{2+}-receptive proteins, causing conformational changes in the protein's structure. These changes cause the transmembranal Ca^{2+}, K^+, and Na^+ channels to pathologically remain open, potentiating the excitatory state and hence the convulsive activity.[1,2]

Accumulating preclinical and clinical evidence suggests that there is a positive feedback cycle between epileptogenesis and brain inflammation. Epileptic seizures increase key inflammatory mediators, which in turn cause secondary damage to the brain and increase the likelihood of recurrent seizures (Fig. 167.1).

The earliest symptomatology, or aura, that is generated by a focal discharge provides the best clue to the localization and occasionally to the characterization of the responsible lesion. For example, a generalized seizure after an aura of a peculiar abdominal sensation or ill-defined odor indicates a lesion in the temporal lobe. A Jacksonian seizure beginning in the fingers and spreading up the arm before becoming generalized and producing a coma indicates a focal lesion in the convexity of the opposite motor cortex.[1]

THERAPEUTIC CONSIDERATIONS

Environmental Toxins

Metals

Metals such as lead, mercury, cadmium, and aluminum can induce seizures by disrupting neural function.[13] Metal toxicity should be ruled out as a possible cause in all seizures. A hair mineral analysis provides the most cost-effective screening method for the detection of heavy metals. Urine testing may be beneficial in examining the long-term, low-dose, chronic exposure to toxic compounds and qualitatively reflect excretion of an unknown fraction of the total body pools of assimilated metals. Metal mobilization testing using a chelating agent such as calcium disodium ethylenediaminetetraacetic acid or dimercaptosuccinic acid provides the best estimation of body burden for mercury and lead (see Chapter 35).

Neurotoxic Chemicals

A great number of commonly present environmental toxicants adversely affect the central and peripheral nervous systems, especially

in the fetus and children. These adverse effects are a result of both direct and indirect action and result in cognitive, mood, and movement disorders and have been linked to the major chronic neurological diseases. Chlorinated pesticides, for example, impair the sodium channels in the neurons, preventing proper transmission of the nerve impulse. Solvents are fat soluble and therefore readily cross the blood–brain barrier. Once in the brain, they diminish the axonal transmission of electrical impulses along the neurons. Minimizing exposure and improving the body's ability to eliminate neurotoxic agents become important aspects of the therapeutic approach. Chapter 35 discuss the effect of these neurotoxins.

Dietary Considerations

Hypoglycemia

Focal neurological deficits associated with hypoglycemia have been well described in adults with diabetes,[14] and some researchers believe that hypoglycemia is the most important metabolic cause of seizures.[13] Researchers have found the following[13,15–18]:

- Serum glucose levels are unusually low before a seizure.
- Of patients with epilepsy, 50% to 90% have constant or periodic low blood sugar.
- Of patients with epilepsy, 70% or more have abnormal glucose tolerance tests.

Although the correlation of blood sugar abnormalities and epilepsy seems well documented, the mechanism of action is unknown. It has been suggested that low blood sugar could impair adenosine triphosphate (ATP) production in nerve cells, reducing the efficacy of the sodium ATPase pump. A defective sodium pump allows for increased intracellular sodium concentrations, which depolarize the cell membrane and thus lowers the firing threshold.

Observational studies have pointed to leptin as a factor that may help in regulating the occurrence of seizures. Animal studies have confirmed that injecting leptin reduces the occurrence and severity of seizures. It is well established that low blood sugar results in low circulating leptins levels, suggesting another possible mechanism of action.[19]

Single measurements of serum glucose levels are inadequate to determine glycemic status; only an extended glucose tolerance test will give a complete picture.

Ketogenic Diet

Originally introduced by Wilder in 1921,[20] the ketogenic diet (KD) has a long history of use for the reduction of seizure activity.[21–23]

The diet is composed of more than 90% fat by weight; low in carbohydrates; and adequate in proteins, vitamins, and minerals. The low carbohydrate intake inhibits fat metabolism, resulting in the production of excessive levels of ketone bodies (acetone, acetoacetic acid, and beta-hydroxybutyric acid), the intermediary oxidation products. Presumably, the beneficial effects of such a diet are due to its induction of metabolic acidosis, which corrects an underlying tendency of patients with epilepsy toward the spontaneous development of alkalosis.[20–22] This acidification is thought to normalize nerve conductivity, irritability, and membrane permeability.

Another possible benefit of the KD may be in augmenting ATP production by utilizing ketone bodies in place of glucose. It has been documented that many patients with epilepsy are hypoglycemic, thus compromising mitochondrial ATP production. The KD may provide an alternative route for balancing this deficit.[24]

The two main types of KDs are (1) the classic diet and (2) the medium-chain triglyceride diet. The classic KD produces ketosis by limiting the intake of carbohydrates and protein to less than 10% of energy combined. The medium-chain triglyceride ketogenic diet uses medium-chain triglyceride fat to produce ketosis. This allows for a larger intake of carbohydrates and protein.[20]

Research continues to document the efficacy of these types of dietary therapy. For example, one study of 27 children from 1 to 16 years of age using the classic diet found that 40% experienced a reduction of seizures of more than 50%, with 25% becoming seizure-free.[25] However, 35% discontinued the diet owing to difficulty in following the rigorous guidelines. A review from 1996 concluded that the KD had efficacy in one third to one half of childhood epilepsy cases and was partially effective in another one third of cases.[26] A review article from 1997 states that the success rate of the KD "greatly exceeds that of the medications" and that its side effects are fewer and the therapy cheaper.[27]

One prospective nonrandomized study measured the nutrient intakes, growth, and biochemical indexes of 30 children from 1 to 16 years of age who had intractable epilepsy before and after a 4-month protocol using a KD. Fourteen children on the classic diet and 11 on the medium-chain triglyceride diet completed the study. The results indicated that linear growth was maintained in patients from baseline to 4 months on both therapies. However, body weight decreased for children on both diets, which could be a result of inadequate energy intake. Protein intake met recommendations for both diets. In the medium-chain triglyceride group, there was a 0.7 decrease in the ratio of total cholesterol to high-density lipoprotein ratios at 4 months. All biochemical indices, including albumin levels, remained within normal limits.[20] Longer-term evaluations may show eventual unwanted changes in these parameters. The authors concluded that the medium-chain triglyceride diet may be more nutritionally adequate and thus confer an advantage over the classic KD.

The long-term risks of a high-fat diet are well known, and a KD may prove unhealthy for a growing child. One retrospective investigation found that the linear growth of some children might be slowed.[28] When treating children on a KD, clinicians should recommend adequate intake of energy and protein, a higher proportion of unsaturated to saturated dietary fats, and also consider vitamin and mineral supplements.[20] Small, frequent meals may be appropriate and may decrease hypoglycemic episodes.

In a study appearing in *Seizure,* researchers evaluated the long-term efficacy and tolerability of the KD in pediatric patients with drug-resistant epilepsy. The results pointed to good long-term effects of the KD on seizure frequency, EEG readings, and neurological development.[29]

The Atkins diet, which gained great popularity in past years as a weight-loss tool, theoretically may be useful to treat epilepsy.[30] Like the KD, it, too, produces a ketotic state, but it creates this effect with less restriction on protein intake. In one pilot study, 6 patients ranging from 7 to 52 years of age were prescribed the Atkins diet for intractable focal and multifocal epilepsy. Five of the patients maintained moderate to large ketosis for periods of 6 weeks to 24 months, and 3 experienced seizure reduction. As a result, they were able to reduce their antiepileptic medications.[31] Larger trials are necessary to explore whether the Atkins diet may be useful to treat patients with epilepsy.

Leptin

Leptin is a protein hormone that plays a key role in regulating appetite and metabolism. Acting on the receptors of the hypothalamus, it inhibits hunger by counteracting the effects of neuropeptide Y and anandamide, two powerful feeding stimulants. Research demonstrates the ability of leptins to act on receptors that activate signaling proteins, which, in turn, trigger changes that reduce brain excitability and thus seizures frequency and intensity.[19,32]

The anticonvulsant action of leptin was tested in animal seizure models by either injecting leptin directly into the cortex or

administering it intranasally. Focal seizures in these animals were induced by neocortical injections of 4-aminopyridine, an inhibitor of voltage-gated K^+ channels. Results showed that seizures were briefer and less frequent upon coinjection of 4-aminopyridine and leptin. In mice, intranasal administration of leptin produced elevated brain and serum leptin levels and delayed the onset of chemical convulsant pentylenetetrazole-induced generalized convulsive seizures.[33]

The theorized mechanism of action may involve leptin's effect in activating two signaling proteins known as JAK2 (Janus kinase) and PI3K (phosphatidylinositol 3-kinase). These proteins blocked nerve impulses triggered by the neurotransmitter glutamate, thus reducing the severity and frequency of seizures. PI3K is also involved in regulating GLUT4, a cytoplasmic protein essential for sugar regulation. This may be another important mechanism of action of elevated leptins.[33,34]

Although these preliminary studies show that leptin can reduce seizures in acute settings, leptin's usefulness in chronic cases is yet to be determined. One challenge would be the fact that leptin has a relatively short half-life (15–30 minutes), leading to dosing problems. However, creating a physiological environment that improves blood leptin levels through dietary and supplemental intervention could prove therapeutic.

There appears to be a link to the KD because this diet increases the amount of circulating leptins, perhaps another plausible mechanism of action of KD. Future research may involve testing foods that have a propensity to increase circulating leptins and examining their effect on seizure frequency and intensity.[34]

Another link to the anticonvulsant action of leptin is hypoglycemia. It has been established that low blood sugar is associated with an increase in seizure formation, and hypoglycemic individuals also have low leptin levels. This may explain in part why hypoglycemia is associated with the occurrence of seizures.

Although the use of leptin as an anticonvulsant is in its early stages of research, the strategy of increasing blood levels of leptin to reduce seizure formation warrants consideration.

Food Allergy

There has been little research into the correlation between food allergy and epilepsy, with only anecdotal case histories[35,36]; single-case, double-blind, placebo-controlled studies[37,38]; and uncontrolled studies being reported. It is postulated that patients with epilepsy may have allergic reactions in the brain that are similar to the swelling, anoxia, and inflammatory chemical reactions seen at other sites of local allergic reactions.[35]

One uncontrolled study evaluated oligoantigenic diets in 63 children with epilepsy. Of 45 children with recurrent headaches, hyperkinetic behavior, or abdominal symptoms, 25 ceased to have seizures during diet therapy, and 11 had fewer seizures. Headaches, abdominal pain, and hyperkinetic behavior resolved in all patients whose seizures ceased and in some of those whose seizures did not cease. Reintroduction of foods reproduced symptoms. Of 24 children with generalized epilepsy, 18 recovered or improved, as did 18 of 21 children with partial epilepsy. In double-blind provocation, 15 of 16 children experienced recurrence of symptoms, including seizures in 8, whereas none improved in the placebo group. Another group of 18 children who had epilepsy alone had no improvement with dietary change.[39] This study suggests that food allergy should be suspected in patients with epilepsy who suffer from multiple other symptoms of food allergy (see Chapter 15).

Another study evaluated two females, 5 and 23 years of age, who had focal occipital epilepsy with cerebral calcifications and were not responding well to antiepileptic therapy. Both had celiac disease as well as documented folic acid deficiency (a common side effect of most antiepileptic drugs). A gluten-free diet combined with supplementation with folic acid (dosage not reported) led to complete normalization of the EEG in the 5-year-old and cessation of seizures. The 23-year-old experienced significant improvement in her EEG and enhanced seizure control. Folic acid returned to the normal range within several months.[40]

A larger study looked more closely at the association between celiac disease and epilepsy. A total of 43 patients (15 male) between 4 and 31 years of age were evaluated for the association between celiac disease, epilepsy, and cerebral calcifications. Intestinal biopsy on the 31 patients with cerebral calcifications of unexplained origin and epilepsy found a flat intestinal mucosa in 24, suggesting celiac disease. CT scans showed cerebral calcifications in 5 of the 12 patients with celiac disease and epilepsy. Antibodies to gluten and folic acid serum concentrations were measured, and histocompatibility leukocyte antigen typing was done in most patients. Only two patients with cerebral calcifications and epilepsy had gastrointestinal symptoms at the time of biopsy. A gluten-free diet in epilepsy was found to be inversely related to the duration of epilepsy before the diet and to the patient's age at the beginning of the diet.

Celiac disease should be considered in all cases of epilepsy and cerebral calcifications of unexplained origin, especially when the epilepsy is characterized by occipital seizures and the calcification is located bilaterally in the posterior regions.[41] One work involving 72 children with epilepsy and 202 controls revealed significantly higher rates of eczema in the mothers and rhinitis in the siblings of the patients with epilepsy, as well as a generally higher incidence of allergic pathologies in both of these groups compared with the controls. Additionally, a significantly higher incidence of allergy to cow's milk as well as asthma was documented in the children with epilepsy with respect to the control group. Prick tests gave a significantly higher rate of positive results for cow's milk proteins in the patients with epilepsy versus controls.[42]

Mitochondrial ATP

Direct research into the possible benefits, with regard to seizure formation, of optimizing mitochondrial ATP production is minimal. However, there is peripheral evidence supporting this idea. For example, it is well accepted that individuals with mitochondrial diseases compromising ATP production have an increase in seizure frequency. This may be related to ATP production and the importance of this respiratory chain substrate in controlling the stability of cell membranes.[43]

In one observational study, 37 family members with a genetic mitochondrial disease (a mild defect in the NADH-ubiquinone oxidoreductase step) showed a significant increase in seizures (22% of the group developed epilepsy).[44]

Studies of subjects with mitochondrial encephalopathies have consistently shown epileptic seizures as a main recognized symptom. Partial seizures, chiefly with elementary motor symptoms, and focal or multifocal EEG epileptiform activity characterized the epileptic presentation in 71% of these patients.[45]

These results support the possibility that compromised ATP production may be playing a role in provoking seizures.

Supplementation with ubiquinone has shown positive effects in improving symptomatology produced by mitochondrial encephalopathies. A study of patients with mitochondrial encephalopathies (which can lead to seizures) showed a significant improvement in fatigability and muscle endurance after the administration of supplemental ubiquinone. Ubiquinone has been shown to have significant benefits in several neurological disorders (e.g., Parkinson's disease, Huntington's chorea). Thus using this agent as part of the anticonvulsant protocol may have some merit.[46–48]

Even the positive effects of the KD may have some relationship to mitochondrial functionality. One study showed that animals placed on a KD had a 46% increase in the density of mitochondria in neuronal tissues. KD has been hypothesized to work on several levels, and it is plausible that increasing mitochondrial density, and thus ATP production, could be one possible mechanism.[49,50]

Microbiome

The gut–brain axis is a two-way communication system between the central nervous system (CNS) and the gastrointestinal (GI) tract. It is becoming increasingly recognized that the presence of a healthy and diverse gut microbiome is important to cognitive and emotional well-being. Recent research is showing that a relationship may exist between GI disorders, particularly those with an immunological pathogenesis, and neurological diseases, pointing to a probable new pathogenic pathway involving the microbiome and the gut–brain axis.[51,52]

Some hypotheses propose blood–brain barrier (BBB) disruption leading to inflammation through autoimmune mechanisms. This may involve etiologies from food allergens and bacteria disrupting the "gut barrier" and imparting systemic consequences through the translocation of pathogen-associated molecular patterns (PAMPs) and other molecular entities.

Interestingly, in a recent publication in the *Journal of Pediatric Neurosciences,* the authors review the case of a 10-month-old infant admitted for drug-resistant epilepsy associated with GI discomfort, secondary to a milk allergy. They noted seizure frequency up 15 to 20 episodes daily with no effective anticonvulsant drug therapy. The only effective intervention turned out to be a diet free of cow's milk, initiated due to gastroenteritis and surprisingly stopping the seizures. This case demonstrated the exposure of the BBB to neurological episodes related to systemic inflammation, again highlighting the gut–brain axis.[53]

The human intestinal microbiome consists of trillions of bacteria affecting human health on various levels and, interestingly, having numerous dynamic interactions with our immune systems. It has been well established that gut microbes control pathogenic microbial growth, assist in digestive function, and support the functionality of the important intestinal epithelium (gut–blood barrier). The relationship is definitely cooperative and one that has far-reaching effects.[54,55]

Evidence continues to mount supporting the premise that the gut microbiome and immune system are closely connected and can influence each other. An important observation is that immune modulation by the microbiome is not exclusive to the GI environment but, rather, affects other systems in a variety of ways.[56,57]

Therefore it is not surprising that gut-residing bacteria are involved in the pathogenicity of autoimmune disorders. For example, patients with Crohn's disease or ulcerative colitis presented abnormal intestinal microbiome, characterized by depletion of two bacterial genera—the phyla *Firmicutes* and the *Bacteroidetes*.[58] In other words, the alteration of the microbiome may turn out to be an important treatment consideration for Crohn's.

This research has led scientists to continue evaluating the relationships between the gut microbiome and epileptic seizures. In that vein, a recent study looked at the relationship between the microbiome and patients with drug-resistant epilepsy. The question was whether or not an altered microbiome (dysbiosis) was involved in the mechanism of drug-resistant epilepsy. Patients were grouped into drug-resistant (n = 42) and drug-sensitive (n = 49) groups, and another 65 were used as healthy controls. Fecal samples were collected from all the study participants, and the microbiome composition was analyzed.

The researchers found that the gut microbiome of the drug-resistant epilepsy group was significantly altered, with an abnormal increased level of rare bacteria. Conversely, the gut microbiome makeup of the drug-sensitive epilepsy group was similar to that of the healthy controls. Also, patients with four seizures per year or fewer showed an increase in *Bifidobacteria* and *Lactobacillus* compared with those with more than four seizures per year.[59] The researchers concluded that dysbiosis or an altered gut microbiome may be involved in the mechanism of drug-resistant epilepsy, and thus restoring the gut microbiome may be a novel therapeutic approach for drug-resistant epilepsy.

In another study appearing in *Beneficial Microbes,* researchers examined the effectiveness of probiotics for controlling seizures in 45 patients with drug-resistant epilepsy. The study patients were given a probiotic mixture for 4 months. Assessment of the participants was carried out before and after the administration of probiotics; including number of seizures; patient's quality of life; and levels of CD-14, interleukin 6, and γ-aminobutyric acid. Of the patients, 28.9% showed a reduction of greater than 50% in the number of epileptic seizures and a significant improvement in quality of life. The researchers concluded that probiotics may contribute to improving seizure control and quality of life in patients with drug-resistant epilepsy.[60]

An area that is gaining significant recognition in the treatment of the challenging *Clostridium difficille* infections of the GI tract is fecal microbiome transplantation (FMT). FMT is another exciting and promising field in gut microbiome research that involves the reconstruction of the gut microbial environment by the transplantation of healthy bacteria from a human donor.

In a recently published report, FMT cured epilepsy in the case of a patient with Crohn's disease. This was the first case to not only achieve remission of Crohn's disease but also resolve neurological symptoms in a girl with a 17-year history of epilepsy. More impressive was the fact that after a 20-month follow-up, FMT therapy had proven its efficacy in preventing relapse of seizures, even after withdrawing the antiepileptic drugs. This again highlights the role of the microbiome–gut–brain axis and hints at a potential new treatment for epilepsy through reconstruction of the gut microbiome.

Nutritional Considerations

Pyridoxine

Two types of vitamin B_6–related seizures are known in newborns and infants younger than 18 months of age: vitamin B_6-deficient and vitamin B_6-dependent. They cause similar neurologic symptoms and EEG abnormalities; if not treated, they carry a prognosis of significant developmental disabilities.[62]

Pyridoxine-dependent seizure is an autosomal, recessively inherited inborn error of metabolism. In affected patients, long-lasting seizures usually begin in infancy, but they may also appear up to 3 years of age and recur. They are resistant to conventional anticonvulsants. The condition ends fatally if diagnosis and administration of pyridoxine in pharmacological doses are delayed too long.[63] The diagnosis of pyridoxine dependency should be suspected in every infant with convulsions in the first 18 months of life. Certain clinical features may be especially suggestive, including the following:

- Seizures of unknown origin in a previously normal infant without an abnormal gestational or perinatal history
- A history of severe convulsive disorders
- The occurrence of long-lasting focal or unilateral seizures, often with partial preservation of consciousness
- Irritability, restlessness, crying, and vomiting preceding the actual seizure

MRI with spectroscopy may be a useful tool in the neuroimaging evaluation of parenchymal changes, despite a normal-appearing brain MRI in patients with pyridoxine-dependent seizures.[64] Because atypical presentations of pyridoxine-responsive seizures have been

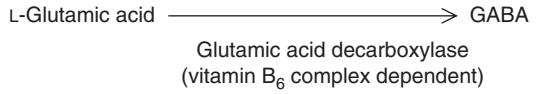


Fig. 167.2 The conversion of glutamic acid to gamma-aminobutyric acid.

reported, it has been recommended that an empiric trial of parenteral pyridoxine be tried in any neonate or infant with long-lasting convulsions, especially when no clear-cut etiology is present.[62,65] A 100- to 200-mg intravenous dose or 20-mg dose every 5 minutes to a total of 200 mg is recommended. If the seizures stop, it is likely that the child has pyridoxine-responsive seizures.[62,65] The chance to identify a pyridoxine-responsive seizure is lost if pyridoxine is given together with or after most anticonvulsant drugs.[65]

Although vitamin B_6-deficient seizures are promptly corrected by the administration of dietary amounts of pyridoxine, vitamin B_6-dependent seizures require continuous high-dose supplementation in the range of 25 to 50 mg/day.[62,65-67]

Although the mechanism by which pyridoxine decreases seizure activity is not fully understood, it is undoubtedly related to its role as a necessary cofactor in the metabolism of various neurotransmitters, the production of which depends on amino acid decarboxylation. Once absorbed, pyridoxine is phosphorylated to pyridoxal-5-phosphate, a coenzyme in the reaction converting glutamic acid to GABA (Fig. 167.2). GABA, as mentioned earlier, is an important inhibitory neurotransmitter.

One proposed mechanism for pyridoxine dependency is that pyridoxal phosphate does not bind with its usual affinity to glutamic acid decarboxylase, resulting in reduced GABA production. In these patients, higher-than-normal levels of pyridoxine are required to allow for the activity of this enzyme.[62,65-67]

One study of infants and children with uncontrollable infantile spasms or their sequelae found improvement in 2 to 14 days using oral pyridoxine phosphate (20–50 mg/kg). Of the 17 total subjects, 3 had complete relief, 6 showed transient relief, and 8 showed a marked reduction in seizures and improvement in their EEGs.[67] Some adverse reactions were seen, including elevated transaminases, nausea, and vomiting.

Administration of vitamin B_6 to patients with epilepsy must be strictly monitored. Although some improvements have been noted, daily doses in the range of 80 to 400 mg have been shown to interfere with commonly used anticonvulsants.[68]

Folic Acid

Serum and red blood cell folate levels are reduced in up to 90% of patients receiving phenytoin, carbamazepine, or barbiturates. These drugs interfere with the intestinal uptake of folic acid at the mucosal level. Lamotrigine and zonisamide appear to not be associated with low levels of folic acid. Most reports state that valproate does not reduce folate levels, although some of the literature is inconsistent. A convincing argument now suggests that routine folic acid supplementation is important for adults receiving antiepileptic medication.[69]

The U.S. Food and Drug Administration (FDA) has directed that oral tablets of folic acid not exceed 1 mg because of concerns brought forward more than 30 years ago that larger amounts might counteract the antiseizure effects of antiepileptic drugs and increase the frequency of seizures in some children.[70] Although that concern is no longer considered valid by epilepsy specialists, the dose restriction has not been lifted. Supplementation with folic acid appears safe even up to doses as high as 15 mg/day[69] and can protect both against birth defects for women of childbearing potential and also lower homocysteine levels, a risk factor for cardiac disease for adults using antiepileptic medications.

Thiamine

In an interesting study, 16 of 50 consecutive neurological patients with a diagnosis of thiamine deficiency showed epileptic manifestations. It is possible that thiamine deficiency may promote epileptic episodes in those who have a subclinical predisposition to seizures. For example, the stimulation of nerve fibers in vitro causes them to lose significant amounts of thiamine pyrophosphate in the surrounding fluid, suggesting a significant role for thiamine in nerve-to-nerve conduction. In addition, thiamine deficiency may be accompanied by low concentrations of GABA, which, as noted earlier, is significant in seizure disorders. The authors suggested that, in patients with late-onset epilepsy, thiamine deficiency may be considered one of the possible causes.[71]

Taurine

Taurine is one of the most abundant amino acids in the mammalian brain.[72,73] It is involved in hyperpolarizing neurons by changing ion permeability and may mimic the effects of GABA and glycine.

Since the early 1970s, considerable clinical and experimental evidence has accumulated supporting the anticonvulsive activity of taurine.[72-85] The primary mode of action responsible for taurine's activity in epilepsy is its membrane-stabilizing effects (i.e., it seems to normalize the flow of Na^+, K^+, and Ca^{2+} into and out of the cell).[72-74]

In addition to acting as a GABA-like neurotransmitter,[72-75] taurine may also help decrease seizure activity by increasing GABA levels through the enhancement of glutamic acid decarboxylase.

Patients with epilepsy have been shown to have significantly lower levels of taurine in platelets than control patients.[76] Although several studies have shown lower taurine levels in the plasma, serum, and urine of patients with epilepsy, other research has yielded disparate results.[77-80] The cellular concentration of taurine as determined by platelet level is thought to be of much greater significance.

The first clinical trials of taurine were undertaken by Barbeau and Donaldson[81] in 1973 and Bergamini and colleagues[82] in 1974. The results were encouraging, and several additional reports have confirmed taurine's anticonvulsant effects.[72,74,81-87] However, the rate of effectiveness of taurine is far below the level where it could be recommended as a standard treatment for epilepsy. At this time, there is no consensus on the seizure types for which taurine is most suitable, nor is there agreement on an appropriate dose. Even more, it is questionable whether taurine may even have the ability to effectively traverse the blood–brain barrier to gain an effect.[88]

At a daily oral dose of 0.05 to 0.3 g/kg in one study and 750 mg in another, taurine has demonstrated efficacy in some cases of intractable epilepsy, decreasing seizures by more than 30% in 11 of 34 subjects.[86,87] This is highly significant because these patients were unresponsive to any other anticonvulsants. Patients with partial epilepsy demonstrated the best results, with those achieving the highest taurine concentrations showing the best response.

The positive clinical response of some patients with epilepsy to supplemental taurine and the apparent cellular deficiency of taurine suggest that taurine supplementation be tried in most patients. Therapeutic monitoring of platelet or plasma taurine levels may prove useful.

Magnesium

Patients with epilepsy have been shown to have significantly lower serum magnesium levels, with seizure severity correlating with the level of hypomagnesemia.[89-92] Although the mechanism of action is not fully understood, in uncontrolled trials, magnesium has been shown to be of benefit in the control of seizures.[89,93] It has also been postulated that like those of folic acid, magnesium levels may be lowered by antiepileptic medications.[92]

Pfeiffer[93] found that a magnesium deficiency induces muscle tremors and convulsive seizures and reported success in controlling the seizure activity of 30 patients with epilepsy with 450 mg/day of magnesium.

Manganese

The link between epilepsy and manganese was first suggested in 1963, when Hurley and associates[94] observed that manganese-deficient rats were more susceptible to seizures than manganese-replete animals and that manganese-deficient animals exhibit an epileptic-like EEG. This prompted researchers to measure manganese concentrations in patients with epilepsy. Low manganese levels in whole blood and hair have been found in patients with epilepsy, and those with the lowest levels typically have the highest seizure activity.[95-99]

Manganese plays a significant role in cerebral function, as it is a critical cofactor for glucose utilization within the neuron, adenylate cyclase activity, and neurotransmitter control. Optimal central nervous system function requires proper manganese levels. Manganese supplementation may be helpful in controlling seizure activity in some patients.[100]

Zinc

Children with epilepsy have been found to have significantly lower levels of serum zinc, especially those with West's or Lennox syndrome.[91,101] More importantly, it appears that patients with epilepsy may have an elevated copper:zinc ratio.[101] Seizures may be triggered when zinc levels fall, as in the absence of adequate taurine. Although the exact role of zinc or the copper:zinc ratio is not clearly understood, it appears to involve either the storage or binding of GABA.[101]

Zinc supplementation appears to be warranted, especially in light of the observation that anticonvulsants may cause zinc deficiency.[102]

Choline, Betaine, Dimethylglycine, and Sarcosine

Choline, betaine (N,N,N,-trimethylglycine), dimethylglycine (DMG), and sarcosine have exhibited some anticonvulsant activity in human and animal studies. Choline is converted to betaine when it acts as a methyl donor, whereas betaine is converted to DMG when it donates a methyl group. This is most evident in the transformation of homocysteine to methionine. Supplemental betaine has also been shown to be effective in preventing various induced seizures in rats and in alleviating seizures in humans with homocystinuria.[103-107]

DMG has also been shown to block induced seizures in rats and mice.[105,106] Roach and Carlin[108] reported a striking decrease in seizure frequency in a patient with long-standing mental retardation when 90 mg of DMG was administered twice daily. Despite treatment with phenobarbital and carbamazepine, the patient had an average of 16 to 18 generalized seizures each week. Within 1 week of starting DMG, seizure frequency dropped to three per week. Two attempts to withdraw the DMG caused dramatic increases in seizure frequency.

It has been suggested that glycine and betaine may act indirectly on glycine metabolism and glycine-mediated neuronal inhibition, enhance GABA activity, or may have a nonspecific effect on biological membranes.

Vitamin D

The association of anticonvulsant drugs with disorders of mineral metabolism—including hypocalcemia, rickets, and osteomalacia—is well documented.[109] Conflicting results have been published concerning the serum levels of vitamin D in patients with epilepsy. Studies have reported increased,[110] normal,[111] and decreased levels.[112]

Supplemental vitamin D intake is recommended when climatic conditions or the patient's lifestyle do not allow adequate exposure to sunlight.[113] Supplementing the diets of 23 patients with epilepsy with 4000 to 16,000 IUs of vitamin D resulted in a significant decrease in the number of seizures, indicating a possible therapeutic effect.[114] Doses of this magnitude can, however, be toxic, requiring careful monitoring.

Vitamin E, Selenium, and Antioxidants

Vitamin E and selenium function synergistically in many physiological functions. Vitamin E deficiency is known to produce seizures,[115] and antiepileptic drugs have been shown to decrease the levels of vitamin E[116,117] as well as those of other antioxidants, like beta-carotene. Studies have shown both vitamin E and selenium to be low in patients with epilepsy.[115,118] Phosphate diesters of vitamins E and C have been shown to prevent the formation of epileptogenic foci and to attenuate seizure activity in animal models of epilepsy.[119] Supplementation with vitamin E and selenium may result in improved control of seizures.[115]

A double-blind placebo-controlled study of 24 children with epilepsy unresponsive to antiepileptic drugs found that supplementation with 400 IU/day of vitamin E provided a significant reduction in the number of seizures in 10 of 12 children. The 2 unresponsive children were noncompliant. All the children continued their normal doses of antiepileptic drugs. No benefit was seen in the 12 placebo children, and, as expected, no negative side effects resulted from the vitamin E therapy.[120]

One report described four children with reduced glutathione peroxidase activity, intractable seizures, multiple infections, and resistance to anticonvulsant treatment. All had seizures within the first 6 months of life. There was no evidence of known inborn errors of metabolism; however, all four patients had low intracellular levels of glutathione peroxidase. Two had normal whole-blood selenium levels, whereas the other two had low levels. Once anticonvulsant therapy was stopped and selenium administered, all four patients experienced clinical improvement.[121]

For epileptic discharges induced in animal models, other antioxidants, such as alpha-lipoic acid and those found in green tea, have been shown to scavenge radical oxygen species and may be prophylactic.[119,122]

Essential Fatty Acids

Animal studies have demonstrated that long-chain polyunsaturated fatty acids may alleviate or prevent cerebrovascular pathophysiology and can improve the competence of the blood–brain barrier.[123] One human 5-patient study using 5 g/day of a 65% omega-3 fatty acid spread demonstrated a marked reduction in both the frequency and strength of epileptic seizures.[124] However, three hospitalized patients with schizophrenia treated with gamma-linolenic acid and linoleic acid in the form of evening primrose oil developed temporal lobe epilepsy following ingestion of the oil. This epilepsy ceased once carbamazepine treatment was initiated.[125] It may be important to monitor the use of oils and fatty acids in closely treating epilepsy; also, more research is needed to determine which agents are most beneficial. At this point, omega-3 fatty acids seem to be the best choice, and evening primrose oil should be used with caution.

Melatonin

It is well documented that melatonin (*N*-acetyl-5-methoxytryptamine) is a powerful antioxidant. Melatonin is often used to treat abnormalities in sleep–wake cycles, jet lag, cancer, and Parkinson's disease. Children with epilepsy have a higher incidence of sleep problems, and epilepsy is exacerbated by sleep deprivation.[126] Animal studies have demonstrated melatonin to have antiepileptic activity. Although not entirely clear, it is possible that melatonin exerts an antiexcitotoxic neuroprotective effect by lowering lipid peroxidation and raising coenzyme Q_{10} levels within the central nervous system.[127]

One randomized, double-blind, placebo-controlled trial included 31 children 3 to 12 years of age who had been taking sodium valproate (10 mg/kg per day) for 6 months and were seizure-free. Of these children, 16 received a fast-release 3-mg melatonin tablet and 15 received a placebo, all 1 hour before bedtime. Using standard Quality of Life in Childhood Epilepsy scales, it was revealed that attention, memory, language, anxiety, behavior, and other cognitive processes all improved in the group receiving melatonin. The general health score also improved marginally. Although not objectively assessed, perceptible increases in appetite and the quality of sleep were reported by most in the melatonin group.[128] Given that high doses have been well tolerated in adults (up to 300 mg/day),[129] it may be possible to slowly increase the 3-mg dose of melatonin for even better results.

Botanical Medicines

Chinese Herbal Medicines

Botanical medicines have played a significant role in the treatment of epilepsy in both the Eastern and Western healing arts. Western scientific methodologies have been used to evaluate the efficacy of the Chinese herbal medicine combination known as saiko-keishi-to (SK).[130] SK demonstrated dramatic therapeutic effects on some difficult cases of epilepsy that had long been unsuccessfully treated with standard allopathic anticonvulsive drugs. SK is a combination of nine botanical drugs:

- *Bupleuri radix* (5 g)
- *Scutellaria radix* (3 g)
- *Pinelliae tuber* (5 g)
- *Paeoniae radix* (6 g)
- *Cinnamon cortex* (2 g)
- *Zizyphi fructus* (4 g)
- *Ginseng radix* (30 g)
- *Glycyrrhizae radix* (1.5 g)
- *Zingiber rhizoma* (2 g)

In one experiment, seizure-like activity was induced in snail neurons by the drug pentylenetetrazol.[131,132] SK was found to do the following:

- Inhibit the intracellular shift of Ca^{2+} toward the cell membrane
- Inhibit the binding of Ca^{2+} to Ca^{2+}-receptive membrane proteins and the Ca^{2+}-calmodulin complex
- Inhibit the conformational changes of the Ca^{2+}-receptive membrane proteins
- Inhibit the pathological transmembrane current of Na^+, K^+, and Ca^{2+}

The researchers presumed that SK's experimental effects were similar to its mode of action in humans. When an attempt was made to isolate purified chemicals from the component herbs, the crude drug's efficacy was lost. This suggests a synergistic effect between the component botanical agents.

Clinical research has shown some benefit in patients with epilepsy. One study reported that 8 of 28 patients with epilepsy experienced a 25% decrease in the number of seizures after 8 weeks of treatment.[132]

More recent research has shown that SK also has a protective effect on neuron damage produced by various factors, including stress. This may point to possible antioxidant-specific compounds within the formula, affecting the central nervous system. Supporting this hypothesis is the fact that the same researchers found SK to have preventive effects on abnormal expression of one of the seizure-related genes.[133] Research showing that the cyclic nucleotides involved in the pathophysiology of seizures has elucidated a possible mechanism for the action of several botanical medicines used in the treatment of seizure activity.[134] Cyclic adenosine monophosphate (cAMP) has been demonstrated to depress electrical activity in cat hippocampal slices, whereas cyclic guanosine monophosphate can produce seizure-like discharge in the same tissues.[135] In addition, cAMP inhibits Ca^{2+} from binding to intracellular proteins like calmodulin, resulting in a decrease in the extrusion of neurotransmitters into the synapse.

Coleus forskolii. The botanical *Coleus forskolii,* an important plant medicine in the Ayurvedic treatment of epilepsy, activates adenylate cyclase, thus increasing the levels of cAMP.[136,137] This action is thought to be due to forskolin, a diterpene, which has been found to increase adenylate cyclase activity by 530%.[137] This is the greatest activity found in 100 plants studied for the ability to increase cAMP. The same study found that seven of the nine botanicals in SK—*B. radix, C. cortex, G. radix, P. radix, G. radix, S. radix,* and *Z. rhizoma*—were found to display significant cAMP enhancement, either by enhancing adenylate cyclase or inhibiting cAMP phosphodiesterase.[137] *B. radix,* a key component of the SK formula, was found to increase cAMP by 170%.

Ginkgo biloba. *Ginkgo biloba* is well known for its usefulness as a memory aid, for cerebral insufficiency, and for its powerful antioxidant properties. However, some of the literature has cited the possibility that it may be responsible for precipitating seizures in two individuals with prior well-controlled seizures. In both patients (78 and 84 years of age), seizures commenced after taking gingko, and they were both seizure-free after the ginkgo was discontinued. The flavonoid components in gingko have been shown to exert GABAergic activity as partial agonists at benzodiazepine receptor sites, and this activity could play a role.[138] As a prudent measure, patients with a seizure history should probably stay away from this botanical until more research has been conducted.

Cannabinoids

There has been interest in the use of cannabis for the treatment of epileptic seizures for hundreds of years. In fact, the first studies on the medical use of cannabis actually date back thousands of years; Sumerian and Akkadian records indicate the use of a medicinal plant (probably cannabis) for nocturnal convulsions.[139-141] Records in the Arabic and Islamic literature also identify cannabis as a treatment for seizures and epilepsy.[142]

The first detailed descriptions of the efficacy of cannabis as an antiseizure medication were reported by W.B. O'Shaughnessy, a physician at the Medical College of Calcutta. Dr. O'Shaughnessy tested the behavioral effects of various preparations of cannabis on healthy animals and military assistants. It was during this period that he reported the impressive antiseizure effect on an infant girl with recurrent convulsive seizures.[143]

During the 20th century, the interest in the use of cannabis declined because of the illegality of the plant and thus the inability to carry out proper clinical trials. However, in recent years, interest in cannabis-based products has shifted dramatically, and we are now beginning to see the evidence beyond just anecdotal reports.

With more than 100 different cannabinoids and numerous terpenes, the plant has a plethora of active components adding to its vast therapeutic potential and complexity. It has been established that the cannabinoids interact with numerous cellular pathways implicated in a range of diseases. In a nutshell, cannabinoids act as ligands, modulating numerous receptors and, consequently, their downstream biological pathways. Interestingly, although cannabinoids may have similar structures, their actions vary significantly.

In general, research surrounding epileptic seizures has focused on nonpsychotropic cannabinoids; these are cannabinoid extracts void of tetrahydrocannabinol (THC) because this psychotropic cannabinoid is not suitable for seizure treatment. Instead, researchers have focused on cannabidiol (CBD), a nonpsychotropic cannabinoid with an impressive anticonvulsant profile and devoid of adverse psychoactive effects.

This accumulation of safety data has spawned an increasing use of CBD extracts for seizure disorders, specifically in children.

Endocannabinoid System

To better understand cannabinoids and their effect on human physiology, it is important to have an overview of the endocannabinoid system (ECS). The ECS has been extensively studied in regards to its biochemistry and physiology. It is a complex system made up of receptors, ligands, and enzymes, with far-reaching effects. Regarded as one of the largest receptor systems in the human body, the ECS is able to send and deliver important information to numerous cell types and impart important downstream physiological actions.

Research over the years has revealed that modulating the activity of the ECS through cannabinoids plant compounds found in cannabis, like CBD, holds therapeutic promise for a number conditions, ranging from anxiety disorders to Parkinson's, Huntington's disease, multiple sclerosis, neuropathic pain, atherosclerosis, myocardial infarction, stroke, hypertension, glaucoma, metabolic syndrome, osteoporosis, and probably one of the most exciting and best-studied areas, epileptic seizures.[144,145]

Endocannabinoid Receptors, Ligands, and Enzymes

The ECS includes a group of endogenous cannabinoid receptors, ligands, and enzymes that are found in both the CNS and the peripheral nervous system (PNS) and throughout most organ systems in the body.

Cannabis contains compounds similar to these ligands known as cannabinoids, with an affiliation not only with these receptors but also affecting G-protein–coupled receptor 55 (GPR55), adenosine receptor, tumor necrosis factor-alpha (TNF-α), transient receptor potential of vanilloid type 1 (TRPV1), and others. Some of the cannabinoids found in cannabis are well known, whereas others are just starting to gain attention. Tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabidivarin (CBDV), and cannabichromene (CBC) are just some of the interesting active components. New research is giving us valuable information about the possibilities of these cannabinoids being used as medicines.

The two main endocannabinoid receptors are cannabinoid receptor type 1 (CB1) receptors, found in the CNS as well as peripheral organs and tissues, and cannabinoid receptor type 2 (CB2) receptors, predominantly present in the immune system and associated structures. Both of these receptors are the main molecular targets of the endocannabinoid ligands, anandamide and 2-arachidonoglycerol (2-AG).

Although endocannabinoid receptors and ligands are often what we associate with the ECS, there is actually more. Enzymes also play an important role in the process. For example, the enzyme fatty acid amide hydrolase (FAAH) breaks down anandamide, pulling it out of circulation. Anandamide is an important endocannabinoid that helps reduce the feelings of anxiety and stress. Elevated levels of FAAH will reduce the levels of anandamide, leading to amplified feelings of anxiety. Interestingly, it may be a deficiency in endocannabinoid activity that is a contributing factor in seizure disorders and other conditions like multiple sclerosis, chronic fatigue, fibromyalgia, migraines, irritable bowel syndrome (IBS), and others.[146] This deficiency could be due to lifestyle, drug abuse, genetic predisposition, poor diet, omega-3 imbalance, and possible environmental toxins. In 2003 Ethan Russo, MD, coined the term *clinical endocannabinoid deficiency syndrome* (CEDS), reflecting the observations seen in clinical research.[147]

There are numerous chronic conditions where symptomatic success has been difficult to achieve and often involves using a plethora of prescription medications. The concept of toning the ECS opens up a whole new arena of treatment possibilities involving not only seizure disorders but other conditions as well.

CBD for Seizures

For centuries, there have been numerous cases treated with cannabis for epileptic seizures. Unfortunately, the lack of properly controlled clinical trials has clouded the outcomes and understanding of mechanisms of action. However, recent research has given rise to high-quality placebo-controlled trials of purified CBD extracts showing significantly better outcomes than placebo in treating specific types of seizure disorders. For example, in a large, prospective, single-center, open-label study recently published in *Epilepsy Behavior,* 72 children and 60 adults with treatment-resistant epilepsy (TRE) were given CBD at 5 mg/kg/day and titrated up to a maximum dosage of 50 mg/kg/day (most patients landed at treatment dosages between 20 and 30 mg/kg/day) The objective of this study was to characterize the changes in adverse events, seizure severity, and frequency in response to a pharmaceutical formulation of highly purified cannabidiol (CBD).

Data evaluation of the pediatric and adult subgroups revealed similar outcomes. Both groups showed significant improvements in both seizure frequency and severity by week 12 that sustained over the 48-week treatment period.[148]

What can be called a breakthrough study in CBD use for seizures was published in the *New England Journal of Medicine* in May 2017. This was the first randomized, placebo-controlled, double-blind trial of CBD in Dravet syndrome (a rare genetic epileptic encephalopathy). One hundred and twenty patients with Dravet syndrome participated in the study that involved 23 centers in the USA and Europe. These patients varied in ages from 2.3 to 18.4 years (mean age 9.8 years) and were randomized to receive either 20 mg/kg/day of CBD in two divided doses or placebo.

The baseline was set to include patients who had at least four convulsive seizures during a preceding 4-week period. Duration of treatment was 14 weeks, including a 2-week titration phase. Compared with the start of the treatment, the median monthly frequency of convulsive seizures (defined as the sum of tonic-clonic, tonic, clonic, and atonic seizures) decreased from 12.4 to 5.9 in the CBD group and from 14.9 to 14.1 in the placebo group.[149]

This trial was considered a "game-changer" because, for the first time, we had robust evidence that CBD added on to preexisting antiepileptic drug treatment reduced the frequency of convulsive seizures in children and young adults with Dravet syndrome.

Another study appearing in the *Lancet*—titled "Cannabidiol in Patients with Seizures Associated with Lennox-Gastaut Syndrome [LGS; LGS is characterized by two or more types of seizures, mental retardation, and a particular EEG pattern with a slow spike-and-waves pattern], a Double-Blind, Placebo-Controlled Phase 3"—also showed positive outcomes. This study demonstrated that CBD, given as an adjunct treatment to patients with LGS taking anticonvulsant drugs, significantly decreased the frequency of monthly drop seizures compared with placebo.

The multicenter study enlisted 171 patients with LGS (mean age 15 years) whose seizures failed to respond to treatment with antiepileptic drugs. Patients were randomized to receive either 20 mg/kg of oral CBD (86 patients) or placebo (85 patients) for 14 weeks.

A key study objective was to measure the baseline change in the number of atonic, tonic, and tonic-clonic seizures (drop seizures) recorded during the treatment period in those randomized to receive CBD compared with those receiving placebo.

Before the treatment, patients had a baseline median frequency of 74 drop seizures per month. Compared with placebo-treated patients, those on CBD had a significantly higher median decrease in drop seizures—22% versus 44%, respectively. Importantly, improvements in drop seizure rates in patients on CBD were detected in the first month of treatment and maintained.

Additional (secondary) endpoints also supported CBD's efficacy: 44% of the patients had a 50% or higher reduction in drop seizures compared with 24% of placebo controls, and the total seizure frequency was significantly reduced in 41% of CBD-treated patients versus 14% in placebo. Also important to note is that CBD was generally well tolerated and safe.[150]

SUMMARY

After centuries of anecdotal reports, there is now conclusive evidence that cannabinoids have a place in treating seizure disorders. Double-blind, randomized clinical trials have shown CBD to be effective in treating two difficult epileptic disorders: Dravet and LGS. In fact, the FDA has approved CBD (Epidolex) oral solution for the treatment of these two epileptic disorders for patients 2 years of age and older. This is the first FDA-approved drug that contains a purified natural drug substance derived from cannabis.

These are very exciting times for cannabinoid research because this approval will open the doors to expand research into more epileptic seizure disorders. In many ways, we are just beginning to scratch the surface in uncovering the incredible treasure chest that lies within the family of cannabinoid molecules. The advantages of using CBD to treat certain forms of epileptic seizures are that they have fewer side effects than common antiepileptic drugs and are generally considered safe even at high doses. This has been proven by the clinical trials and supports the premise that many effective natural plant products have fewer side effects. It appears we have entered into a new era in seizure treatments where cannabinoids may become the treatment of choice.

Acupuncture

From work on animal models, it has been suggested that the mechanism of acupuncture in suppressing epileptic seizures involves the release of neurotransmitters such as endogenous opioid peptides, serotonin, and GABA.[151] Other studies have shown possible seizure inhibition through decreasing neuronal nitric oxide synthase transcription in the hippocampus.[152,153]

In humans, effective use of acupuncture, scalp needling, electroacupuncture, and embedding of acupuncture points to treat epilepsy and status epilepticus has been reported. However, one sham-controlled study of 34 patients found no benefit.[154] This research group reported similar results with a study of 29 patients. In a second study, many of the patients reported changes in their sense of well-being.[155] In both clinical trials, drug-resistant patients who had been considered intractable were given nearly identical treatments save one or two points, also without benefit. Given the individualized nature of traditional Chinese medicine, this may not be a reasonable representation of the acupuncture modality. Additionally, it may be possible that more benign cases of epilepsy may be more responsive, even if intractable cases are not.

Auricular, or ear, acupuncture was developed by Nogier, a French neurologist, in the 1950s. Unlike traditional Chinese acupuncture, which uses the meridian system, ear acupuncture uses points on the ear that represent different areas on the body, similar to the way reflexology works with points on the foot. In one study, epileptiform behaviors in rats were induced by intrahippocampal injection of penicillin. Radioimmunoassay techniques demonstrated that levels of somatostatin, aspartic acid, glutamine, and GABA were increased. One hour later, the lower-half auricular lobules of seizure rats, containing the ear-points Pizhixia and Shenmen, were electrically stimulated. After auricular treatment, the convulsion behaviors of the rats improved. Concomitantly, the content of the somatostatin, aspartic acid, and glutamine in the seizure rats' hippocampi was significantly decreased, whereas the content of glycine, taurine, and GABA had increased. These results suggest that ear-point electrical stimulation has antiepilepsy effects. Physiologically, given that amino acid concentrations may be involved in the pathogenesis of an epileptic seizure, auricular treatment might be involved in decreasing the content of the somatostatin, aspartic acid, and glutamine and increasing that of glycine, taurine, and GABA in the hippocampi of seizure rats.[156,157]

Ear acupuncture may then be more effective than traditional body acupuncture. More studies are necessary to determine the effectiveness of both auricular and body acupuncture in the treatment of epilepsy. The negative side effect profile of acupuncture is low, although one case report describes an episode of convulsive syncope, which occurred immediately after the insertion of acupuncture needles into the bilateral ST-36 acupuncture point.[158] Given the extraordinarily common use of these points and the extremely rare appearance of side effects due to acupuncture, this case report should be considered an anomaly.

Yoga and Meditation

Yoga teaches a variety of physical postures, breathing exercises, and meditation techniques with the goal of improving both physical and mental health. There are a number of types of yoga emphasizing harmony between the physical and energetic aspects of the body. It has been documented that yoga has a physiological effect that may offer benefit to the patient with epilepsy.[159]

Research has indicated that the meditative types of yoga (e.g., Sahajaea yoga) have the most benefits in reducing the intensity and occurrence of seizures. A Cochrane Review of yoga among patients with epilepsy showed significant improvement in one particular study involving 32 patients with epilepsy divided into an active group and two control groups. The results showed that the active group had a 50% reduction in the number of seizures at 1-year follow-up. Although these results are statistically significant, the authors concluded that further research with larger numbers was needed.[159-161]

Trigeminal Nerve Stimulation

Intractable epilepsy, which affects more than 1 million Americans and is often resistant to drug treatment, may be ameliorated by a procedure known as trigeminal nerve stimulation (TNS). The trigeminal nerve, which extends into the brain from the face and forehead, is known to play a role in the inhibition of seizures, and it has been shown that stimulating this nerve can reduce their occurrence.[162] TNS involves using a stimulator the size of a cell phone that fits into a pocket; its wires are connected to the forehead with adhesives. Electrical impulses are transmitted to the nerve, resulting in reduced seizure frequency.[162]

In a completed clinical trial, patients experienced significant reductions in seizure occurrence. The study demonstrated that those participating in the study experienced a 66% reduction of seizures in the first 3 months, a 56% reduction after 6 months, and a 59% reduction after 12 months, and one of the subjects experienced a nearly 90% reduction in seizures after 1 year. The therapy shows promise as an alternative mode of neurostimulation, especially because it is not invasive (compared with vagus nerve stimulation, which requires surgery).[163,164]

Exercise

There has been understandable caution in recommending exercise to patients with epilepsy, owing to fear of injury and the possibility of seizure induction. However, a review of the literature shows that there is no increase in the frequency of seizures with exercise. Some studies have shown that exercise reduces seizure frequency, specifically in patients with drug-resistant epilepsy. In one study, 15 women resistant to drug therapy engaged in 1-hour physical exercise sessions twice a week for 15 weeks. Seven patients had 27 seizures during the 30 exercise sessions; the self-reported seizure rate was significantly reduced

by the end of the 15-week period (2.9 seizures per week had dropped to 1.7 seizures per week). There were also other benefits to the exercise program, including reduced muscle spasms, improved sleep, and increased energy.[165]

In a review study, the authors discuss the positive effects of physical exercise programs in experimental models of epilepsy. Findings from animal studies show that exercise seems to modulate neuronal vulnerability to epileptic insults. The review also concludes that metabolic, electrophysiologic, and immunohistochemical evaluations have confirmed the positive influence of exercise on the patient with epilepsy.[166]

These findings seem to suggest that physical exercise should be considered and integrated into the therapeutic approach for reducing the intensity and frequency of seizures.

Patients with epilepsy should be advised to avoid situations that could provoke further seizures or could pose dangers in the event that a seizure should occur. Also, they should be made aware of state laws regarding epilepsy and driving. Patients with epilepsy should avoid known initiators of seizure activity (e.g., bright flashing lights, sudden loud sounds). Therapy should continue at full dose until the patient has been seizure-free for at least 2 years. Dosage reduction should then be gradual over a period of several months. Patients with epilepsy who are not controlled with natural therapies require drug therapy.

Patients should eliminate pyridoxine antagonists (isoniazid, cycloserine, hydralazine, dopamine, penicillamine), oral contraceptives, alcohol, and hydrazine dyes (Food, Drug, and Cosmetic Act yellow no. 5).

Note: Status epilepticus, a prolonged seizure lasting longer than 30 minutes that is not likely to stop spontaneously, is a medical emergency that inevitably results in serious neurological damage or death if untreated. Immediate hospitalization is required, preferably with ambulance or aid car transport, with the necessary equipment to maintain the patient's airway, assist in ventilation, and administer intravenous glucose and antiepileptic drugs.

Because endogenous depression is more prevalent in persons with epilepsy than in the general population,[167] monitoring for depressive illness is important in patients with epilepsy.

Diet

All sugar and other refined carbohydrates should be eliminated, and protein levels should be moderated. Food allergens should be identified and eliminated. Small frequent meals are best. If a ketotic effect is desired, all carbohydrates should be strictly limited and fat intake increased. Furthermore, adequate intake of energy and protein, plus a higher proportion of omega-3 unsaturated to saturated dietary fats, should be assured. Finally, vitamin and mineral supplements should be used with ketotic diets (ketotic diets should be used only under professional supervision).

Lifestyle

Regular sleep patterns should be encouraged, along with some form of physical exercise.

Nutritional Supplements

(The following are adult doses; reduce proportionately for children.)

- Vitamin B_6: 50 mg three times a day (if ineffective, try P5'P 5 mg three times a day)
- Folic acid: 0.4 to 4 mg/day (activated folates probably a better choice)
- Vitamin E: 400 IU/day (mixed tocopherols suggested)
- Taurine: 500 mg three times a day
- Magnesium: 300 mg three times a day
- Manganese: 10 mg three times a day
- Selenium: 100 mcg/day
- Zinc: 25 mg/day
- Ubiquinone: 100 mg/day
- Melatonin: 3 mg a day in children, increase up to 20 mg or more in adults

Botanical Medicines

- Saiko-keishi-to: 300 mL before bedtime
- *G. biloba*: contraindicated at this time
- CBD: 5 to 30 mg/Kg

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Willmore LJ, Ferrendelli JA. Epilepsy. In: Dale DC, Federman DD, eds. *Scientific American medicine*. New York: Scientific American; 1997:11.
2. Pedley TA. The epilepsies. In: Bennett JC, Plum F, eds. *Cecil textbook of medicine*. Philadelphia: WB Saunders; 1996:2113–2125.
3. Hauser WA, Hesdorfer DC. *Epilepsy: frequency, causes, and consequences*. New York: Demos; 1990.
4. Freeman JM, Vining EPG, Pillas DJ. *Seizures and epilepsy in childhood: a guide for parents*. Baltimore: Johns Hopkins University Press; 1990.
5. Sander JW, Shorvon SD. Incidence and prevalence studies in epilepsy and their methodological problems: a review. *J Neurol Neurosurg Psychiatry*. 1987;50:829–839.
6. Shneker BF, Fountain NB. Epilepsy. *Dis Mon*. 2003;49:426–478.
7. Kwan P, Sander JW. The natural history of epilepsy: an epidemiological view. *J Neurol Neurosurg Psychiatry*. 2004;75:1376–1381.
8. Edwards JC. Seizure types, epilepsy syndromes, etiology, and diagnosis. *CNS Spectr*. 2001;6:750–755.
9. Vadlamudi L, Andermann E, Lombroso CT, et al. Epilepsy in twins: insights from unique historical data of William Lennox. *Neurology*. 2004;62:1127–1133.
10. Hamandi K, Salek-Haddadi A, Fish DR, et al. EEG/functional MRI in epilepsy: the Queen Square experience. *J Clin Neurophysiol*. 2004;21: 241–248.
11. Schroeder SA, Krupp MA, Tierney LM, et al. *Current medical diagnosis and treatment*. Norwalk, CT: Appleton & Lange; 1989.
12. Dichter MA, Ayala GF. Cellular mechanisms of epilepsy: a status report. *Science*. 1987;237:157–164.
13. Allen RB. Nutritional aspects of epilepsy. *Int Clin Nutr Rev*. 1983;3:3–9.
14. Lahat E, Barr J, Bistritzer T. Focal epileptic episodes associated with hypoglycemia in children with diabetes. *Clin Neurol Neurosurg*. 1995;97:314–316.
15. Mackay G, Borbash H. The glucose tolerance curve in epilepsy. *Ment Sci*. 1931;72:83–85.
16. Harrad RA, Kennedy PG. Hypoglycemia-induced epilepsy during lactation. *Br Med J (Clin Res Ed)*. 1982;285:607–608.
17. Tyson F, Otis L, Joyce FT. A study of blood sugar in epileptics. *Am J Med Sci*. 1935;190:164–169.
18. Nielsen JM. Convulsions of undetermined etiology. *Arch Neurol Psych*. 1934;31:1055–1061.
19. Brennan AM, Mantzoros CS. Drug Insight: the role of leptin in human physiology and pathophysiology—emerging clinical applications. *Nat Clin Pract Endocrinol Metab*. 2006;2(6):318–327.
20. Liu YM, Williams S, Basualdo-Hammond C, et al. A prospective study: growth and nutritional status of children treated with the ketogenic diet. *J Am Diet Assoc*. 2003;103:707–712.
21. Signore JM. Ketogenic diet containing medium-chain triglycerides. *J Am Diet Assoc*. 1973;62:285–290.
22. Barborha CJ. Ketogenic diet treatment of epilepsy in adults. *JAMA*. 1928;91:73–78.
23. Barborha CJ. Epilepsy in adults, results of treatment by ketogenic diet in one hundred cases. *Arch Neurol Psych*. 1930;23:904–914.
24. Bough K, et al. Energy metabolism as part of the anticonvulsant mechanism of the ketogenic diet. *Epilepsia*. 2008;49(suppl 8):91–93.
25. Batchelor L, Nance J, Short B. An interdisciplinary team approach to implementing the ketogenic diet for the treatment of seizures. *Pediatr Nurs*. 1997;23:465–471.
26. Prasad AN, Stafstrom CF, Holmes GL. Alternative epilepsy therapies: the ketogenic diet, immunoglobulins, and steroids. *Epilepsia*. 1996;37(suppl 1):S81–S95.
27. Swink TD, Vining EP, Freeman JP. The ketogenic diet. *Adv Pediatr*. 1997;44:297–329.
28. Williams S, Basualdo-Hammond C, Curtis R, et al. Growth retardation in children with epilepsy on the ketogenic diet: a retrospective chart review. *J Am Diet Assoc*. 2002;102:405–407.
29. Dressler A, Stöcklin B, Reithofer E, et al. Long-term outcome and tolerability of the ketogenic diet in drug-resistant childhood epilepsy: the Austrian experience. *Seizure*. 2010;19(7):404–408.
30. Kossoff EH. More fat and fewer seizures: dietary therapies for epilepsy. *Lancet Neurol*. 2004;3:415–420.
31. Kossoff EH, Krauss GL, McGrogan JR, et al. Efficacy of the Atkins diet as therapy for intractable epilepsy. *Neurology*. 2003;61:1789–1791.
32. Vezzani A, Sperk G, Colmers WF, Neuropeptide Y. emerging evidence for a functional role in seizure modulation. *Trends Neurosci*. 1999;22:25–30.
33. Xu L, Rensing N, Yang XF. Leptin inhibits 4-aminopyridine- and pentylenetetrazole-induced seizures and AMPAR-mediated synaptic transmission in rodents. *J Clin Investigat*. 2008;118(1):272–280.
34. Fruhbeck G. Intracellular signalling pathways activated by leptin. *Biochem J*. 2006;393:7–20.
35. MacKarness R. *Chemical victims*. London: Pan Books; 1980.
36. Mandell M, Scanlon LW. *Dr. Mandell's 5-day allergy relief system*. New York: Pan Books.
37. Crayton JW, Stone T, Stein G, et al. Epilepsy precipitated by food sensitivity: report of a case with double-blind placebo-controlled assessment. *Clin Electroencephalogr*. 1981;12:192–198.
38. Campbell MB. Allergy and epilepsy. In: Speer F, ed. *Allergy of the nervous system*. Springfield, IL: Thomas; 1970:59–78.
39. Egger J, Carter CM, Soothill JF, et al. Oligoantigenic diet treatment of children with epilepsy and migraine. *J Pediatr*. 1989;114:51–58.
40. Ventura A, Bouquet F, Sartorelli C, et al. Celiac disease, folic acid deficiency and epilepsy with cerebral calcifications. *ACTA Paediatr Scand*. 1991;80:559–562.
41. Gobbi G, Bouquet F, Greco L, et al. Celiac disease, epilepsy and cerebral calcifications. the italian working group on coeliac disease and epilepsy. *Lancet*. 1992;340:439–443.
42. Frediani T, Lucarelli S, Pelliccia A, et al. Allergy and childhood epilepsy: a close relationship? *Acta Neurol Scand*. 2001;104:349–352.
43. Rosing HS, Hopkins LC, Wallace DC. Maternally inherited mitochondrial myopathy and myoclonic epilepsy. *Ann Neurol*. 1985;17:228–237.
44. Torberg T, Ellisiv M, Jan A. Epilepsy in a mitochondrial disorder. *J Neurol Neurosurg Psychiatr*. 1991;54:1073–1076.
45. Chen RS, Huang CC, Chu NS. Coenzyme Q10 treatment in mitochondrial encephalomyopathies. *Eur Neurol*. 1997;37:212–218.
46. Flint BM, Matthews RT. Coenzyme Q10 in the central nervous system and its potential usefulness in the treatment of neurodegenerative diseases. *Mol Aspects Med*. 1997;18(s):s169–s179.
47. Shults CW, Flint BM, Fontaine D. Absorption, tolerability and effects on mitochondrial activity of oral Coenzyme Q10 in parkinsonian patients. *Neurology*. 1998;50:793–795.
48. Koroshetz WJ, Jenkins BG, Rosen BR, Flint Beal M. Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. *Ann Neurol*. 1997;41:160–165.
49. Bough KJ, Wetherington J, Hassel B, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *AnnNeurol*. 2006;60:223–235.
50. Bough KJ. Energy metabolism as part of the anticonvulsant mechanism of the ketogenic diet. *Epilepsia*. 2008;49(suppl 8):91–93.
51. Falsaperla R, Pavone P, Miceli Sopo S, Mahmood F, Scalia F, Corsello G, et al. Epileptic seizures as a manifestation of cow's milk allergy: A studied relationship and description of our pediatric experience. *Expert Rev Clin Immunol*. 2014;10:1597–1609.
52. Maqsood R, Stone TW. The gut-brain axis, BDNF, NMDA and CNS disorders. *Neurochem Res*. 2016;41:2819–2835.
53. Falsaperla R, Romano C, Pavnone P, et al. The gut-brain axis: a new pathogenic view of neurologic symptoms - description of a pediatric case. *J Pediatr Neurosci*. 2017;12(1):105–108.
54. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet*. 2003;361(9356):512–519.
55. Sears CL. A dynamic partnership: celebrating our gut flora. *Anaerobe*. 2005;11(5):247–251.
56. Cerf-Bensussan N, Gaboriau-Routhiau V. The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol*. 2010;10(10):735–744.

57. Vital M, Harkema JR, Rizzo M, Tiedje J, Brandenberger C. Alterations of the murine gut microbiome with age and allergic airway disease. *J Immunol Res*. 2015. Article ID 892568.
58. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. In: *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(34):13780–13785.
59. Peng A, Qiu X, Lai W, Zhang L, et al. Altered composition of the gut microbiome in patients with drug-resistant epilepsy. *Epilepsy Res*. 2018;147:102–107.
60. Gomez-Equilaz M, Ramon-Trapero JL, Perez-Martinez, et al. The beneficial effect of probiotics as a supplementary treatment in drug-resistant epilepsy: a pilot study. *Beneficial Microbes*. 2018:1–8.
61. He Z, Cui BT, Zhang T, et al. Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease: the first report. *World J Gastroenterol*. 2017;23(19):3565–3568.
62. Crowell GF, Roach ES. Pyridoxine-dependent seizures. *Am Fam Physician*. 1983;27:183–187.
63. Akre B, Rasmussen M, Lundby R. Pyridoxine-dependent seizures. *Tidsskr Nor Laegeforen*. 2004;124:162–164.
64. Alkan A, Kutlu R, Aslan M, et al. Pyridoxine-dependent seizures: magnetic resonance spectroscopy findings. *J Child Neurol*. 2004;19:75–78.
65. Goutieres F, Aicardi J. Atypical presentations of pyridoxine-dependent seizures: a treatable cause of intractable epilepsy in infants. *Ann Neurol*. 1985;17:117–120.
66. Bankier A, Turner M, Hopkins IJ. Pyridoxine dependent seizures: a wider clinical spectrum. *Arch Dis Child*. 1983;58:415–418.
67. Nakazawa M, Tadataka M, Takeda H, et al. High-dose vitamin B_6 therapy for infantile spasms: the effect and adverse reactions. *Brain Dev*. 1983;5:193–197.
68. Hansson O, Sillanpaa M. Letter: pyridoxine and serum concentration of phenytoin and phenobarbitone. *Lancet*. 1976;1:256.
69. Morrell MJ. Folic acid and epilepsy. *Epilepsy Curr*. 2002;2:31–34.
70. Reynolds EH. Mental effects of anticonvulsants and folic acid metabolism. *Brain*. 1968;91:197–214.
71. Keyser A, De Bruijn SF. Epileptic manifestations and vitamin B_1 deficiency. *Eur Neurol*. 1991;31:121–125.
72. Braverman E, Pfeiffer CC. *The healing nutrients within*. New Canaan, CT: Keats; 1987.
73. van Gelder NM. A central mechanism of action for taurine, osmoregulation, bivalent cations, and excitation threshold. *Neurochem Res*. 1983;8:687–699.
74. Huxtable R, Barbeau A, eds. *Taurine*. New York: Raven Press; 1976.
75. Okamoto K, Kimura H, Sakai Y. Effects of taurine and GABA on Ca spikes and Na spikes in cerebellar Purkinje cells in vitro: intrasomatic study. *Brain Res*. 1983;260:249–259.
76. Goodman HO, Shihabi Z, Oles KS. Antiepileptic drugs and plasma and platelet taurine in epilepsy. *Epilepsia*. 1989;30:201–207.
77. Collins BW, Goodman HO, Swanton CH, et al. Plasma and urinary taurine in epilepsy. *Clin Chem*. 1988;34:671–675.
78. Huxtable RJ, Laird H, Lippincott SE, et al. Epilepsy and the concentration of plasma amino acids in humans. *Neurochem Int*. 1983;5:125–135.
79. Janjua N, Metrakos J, van Gelder N. Plasma amino acids in epilepsy. In: Anderson VE, Hauser WA, Penry JK, et al., eds. *Genetic basis of the epilepsies*. New York: Raven Press; 1982:181–197.
80. Monaco F, Mutani R, Durelli L, et al. Free amino acids in serum of patients with epilepsy: significant increase in taurine. *Epilepsia*. 1975;16:245–249.
81. Barbeau A, Donaldson J. Taurine in epilepsy. *Lancet*. 1973;2:387.
82. Bergamini L, Mutani R, Delsedime M, et al. First clinical experience on the antiepileptic action of taurine. *Eur Neurol*. 1974;11:261–269.
83. Takahashi R, Nakane Y. Clinical trial of taurine in epilepsy. In: Barbeau A, Huxtable RJ, eds. *Taurine and neurological disorders*. New York: Raven Press; 1978:375–385.
84. Nakane Y, Takahashi R. Clinical experience of taurine for the treatment of epilepsy. *Sulfur-Containing Amino Acids*. 1978:173–180.
85. Striano S, Buscaino GA. 1st results on the effects of taurine in human epilepsy. *Acta Neurol*. 1974;29:537–542.
86. Fukuyama Y, Ochiai Y. Therapeutic trial by taurine for intractable childhood epilepsies. *Brain Dev*. 1982;4:63–69.
87. Pasantes-Morales H, Chapparro H, Otero E. Clinical study on the effect of taurine on intractable epileptics (author's transl). *Rev Invest Clin*. 1981;33:373–378.
88. Birdsall TC. Therapeutic applications of taurine. *Alt Med Rev*. 1998;3:128–136.
89. Jooste PL, Wolfswinkel JM, Schoeman JJ, et al. Epileptic-type convulsions and magnesium deficiency. *Aviat Space Environ Med*. 1979;50:734–735.
90. Benga I, Baltescu V, Tilinca R, et al. Plasma and cerebrospinal fluid concentrations of magnesium in epileptic children. *J Neurol Sci*. 1985;67:29–34.
91. Shoji Y, Sasazaki Y, Suzuki M, et al. Serum magnesium and zinc in epileptic children. *Brain Dev*. 1983;5:200–212.
92. Oladipo OO, Ajala MO, Okubadejo N, et al. Plasma magnesium in adult Nigerian patients with epilepsy. *Niger Postgrad Med J*. 2003;10:234–237.
93. Pfeiffer CC. *Mental and elemental nutrients: a physician's guide to nutrition and health care*. New Canaan, CT: Keats Pub; 1975.
94. Hurley LS, Wooley DE, Rosenthal F, et al. Influence of manganese on susceptibility of rats to convulsions. *Am J Physiol*. 1963;204:493–496.
95. Carl GF, Keen CL, Gallagher BB, et al. Association of low blood manganese concentrations with epilepsy. *Neurology*. 1986;36:1584–1587.
96. Dupont CL, Tanaka Y. Blood manganese levels in children with convulsive disorder. *Biochem Med*. 1985;33:246–255.
97. Sampson P. Low manganese level may trigger epilepsy. *JAMA*. 1977;238:1805.
98. Papavasilious PS, Kutt H, Miller ST, et al. Seizure disorders and trace metals: manganese in epileptics. *Neurology*. 1979;29:1466–1473.
99. Sohler A, Pfeiffer CC, Casey R. A direct method for the determination of manganese in whole blood: patients with seizure activity have low blood levels. *Orthomol Psych*. 1979;8:275–280.
100. Pfeiffer CC, LaMola S. Zinc and manganese in the schizophrenias. *Orthomol Psych*. 1983;12:215–234.
101. Barbeau A, Donaldson J. Zinc, taurine and epilepsy. *Arch Neurol*. 1974;30:52–58.
102. Lewis-Jones MS, Evans S, Culshaw MA. Cutaneous manifestations of zinc deficiency during treatment with anticonvulsants. *Br Med J (Clin Res Ed)*. 1985;290:603–604.
103. Ghoz EH, Freed WJ. Effects of betaine on seizures in the rat. *Pharmacol Biochem Behav*. 1985;22:635–640.
104. Freed WJ, Gillin JC, Wyatt RJ. Anticonvulsant properties of betaine. *Epilepsia*. 1979;20:209–213.
105. Freed WJ. Prevention of strychnine-induced seizures and death by the N-methylated glycine derivatives betaine, dimethylglycine and sarcosine. *Pharmacol Biochem Behav*. 1985;22:641–643.
106. Freed WJN. N-dimethylglycine, betaine, and seizures. *Arch Neurol*. 1984;41:1129–1130.
107. Smolin LA, Benevenga NJ, Barlow S. The use of betaine for the treatment of homocystinuria. *J Pediatr*. 1981;99:467–472.
108. Roach ES, Carlin LN. N-dimethylglycine for epilepsy. *N Engl J Med*. 1982;307:1081–1082.
109. Hahn TJ. Drug-induced disorders of vitamin D and mineral metabolism. *Clin Endocrinol Metab*. 1980;301:107–127.
110. Williams C, Netzloff M, Folkerts L, et al. Vitamin D metabolism and anticonvulsant therapy. *South Med J*. 1984;77:834–836, 842.
111. Winstein RS, Bryce GF, Sappington LJ, et al. Decreased serum ionized calcium and normal vitamin D metabolite levels with anticonvulsant drug treatment. *J Clin Endocrinol Metab*. 1984;58:1003–1009.
112. Gough H, Goggin T, Bissessar A, et al. A comparative study of the relative influence of different anticonvulsant drugs, UV exposure, and diet on vitamin D and calcium metabolism in out-patients with epilepsy. *QJM*. 1986;59:569–577.
113. Riancho JA, del Arco C, Arteaga R, et al. Influence of solar radiation on vitamin D levels in children on anticonvulsant drugs. *Acta Neurol Scand*. 1989;79:296–299.
114. Christiansen C, Rodbro P, Sjo O. "Anticonvulsant action" of vitamin D in epileptic patients? A controlled pilot study. *Br Med J*. 1974;22:58–259.

115. Ogunmekan AO. Vitamin E deficiency and seizures in animals and man. *Can J Neurol Sci*. 1979;6:43–45.
116. Hojo Y. Subject groups high and low in selenium. *Bull Environ Contam Toxicol*. 1981;26:466–471.
117. Verrotti A, Greco R, Latini G, et al. Obesity and plasma concentrations of alpha-tocopherol and beta-carotene in epileptic girls treated with valproate. *Neuroendocrinology*. 2004;79:157–162.
118. Kataoka K, Kanamori N, Oishi M, et al. Vitamin E status in pediatric patients receiving antiepileptic drugs. *Dev Pharmacol Ther*. 1989;14:96–101.
119. Mori A, Yokoi I, Noda Y, et al. Natural antioxidants may prevent posttraumatic epilepsy: a proposal based on experimental animal studies. *Acta Med Okayama*. 2004;58:111–118.
120. Ogunmekan AO, Hwang PA. A randomized, double-blind-placebo-controlled clinical trial of d-alpha-tocopheryl acetate as add-on therapy for epilepsy in children. *Epilepsia*. 1989;30:84–89.
121. Weber GF, Maertens P, Meng, et al. Glutathione peroxidase deficiency and childhood seizures. *Lancet*. 1991;337:1443–1444.
122. Meyerhoff JL, Lee JK, Rittase BW, et al. Lipoic acid pretreatment attenuates ferric chloride-induced seizures in the rat. *Brain Res*. 2004;1016:139–144.
123. Farkas E, de Wilde MC, Kiliaan AJ, et al. Systemic effects of dietary n-3 PUFA supplementation accompany changes of CNS parameters in cerebral hypoperfusion. *Ann N Y Acad Sci*. 2002;977:77–86.
124. Schlanger S, Shinitzky M, Yam D. Diet enriched with omega-3 fatty acids alleviates convulsion symptoms in epilepsy patients. *Epilepsia*. 2002;43:103–104.
125. Vaddadi KS. The use of gamma-linolenic acid and linoleic acid to differentiate between temporal lobe epilepsy and schizophrenia. *Prostaglandins Med*. 1981;6:375–379.
126. Zaiwalla Z, Stores G. Sleep and arousal disorders in childhood epilepsy. *Electroencephalogr Clin Neurophysiol*. 1989;72:107.
127. Yalcin A, Kilinc E, Kocturk S, et al. Effect of melatonin cotreatment against kainic acid on coenzyme Q10, lipid peroxidation and Trx mRNA in rat hippocampus. *Int J Neurosci*. 2004;114:1085–1097.
128. Gupta M, Aneja S, Kohli K. Add-on melatonin improves quality of life in epileptic children on valproate monotherapy: a randomized, double-blind, placebo-controlled trial. *Epilepsy Behav*. 2004;5:316–321.
129. James SP, Sack DA, Rosenthal NE. Melatonin administration in insomnia. *Neuropsychopharmacology*. 1990;3:19–23.
130. Sugoya A, Tsuda T, Yasuda K, et al. Effects of Chinese herbal medicine "saiko-keishi-to" on intracellular calcium and protein behavior during pentylenetetrazol-induced bursting activity in snail neuron. *Planta Med*. 1985:2–6.
131. Sugoya A, Tsuda T, Yasuda K, et al. Effects of Chinese herbal medicine "saiko-keishi-to" on transmembrane ionic current of snail neurons. *Planta Med*. 1985:60–61.
132. Nagakubo S, Niwa S, Kumagai N, et al. Effects of TJ-960 on Sternberg's paradigm results in epileptic patients. *Jpn J Psychiatry Neurol*. 1993;47:609–620.
133. Sugaya A. Cellular mechanism of seizure discharge and its normalization by a herbal mixture prescription "saikokeishito-ka-shakuyaku" (SK). *Yakugaku Zasshi*. 2001;121(5):295–317. Review. Japanese.
134. Narita Y, Satowa H, Kokubu T, et al. Treatment of epileptic patients with the Chinese herbal medicine "Saiko-Keishi-To" (SK). *IRCS J Med Sci*. 1982;10:88–89.
135. Ferrendelli JA. Roles of biogenic amines and cyclic nucleotides in seizure mechanisms. *Adv Neurol*. 1986;44:393–400.
136. Seamon KB, Daly JW. Forskolin: a unique diterpene activator of cyclic AMP-generating systems. *J Cyclic Nucleotide Res*. 1981;7:201–224.
137. Kanatoni H, Tanimoto J, Hidaka K, et al. Search for adenylate cyclase activator in medicinal plants. *Planta Med*. 1985:182–185.
138. Granger AS. Ginkgo biloba precipitating epileptic seizures. *Age Ageing*. 2001;30:523–525.
139. Elsohly M. *Marijuana and the Cannabinoids*. Totowa: Humana Press; 2007.
140. Friedman D, Sirven JI. Historical perspective on the medical use of cannabis for epilepsy: ancient times to the 1980s. *Epilepsy Behav*. 2017;70(Pt B):298–301.
141. Russo EB. Cannabis and epilepsy: an ancient treatment returns to the fore. *Epilepsy Behav*. 2017;70(Pt B):292–297.
142. Russo EB, Jiang HE, Li X, et al. Phytochemical and genetic analyses of ancient cannabis from Central Asia. *J Exp Bot*. 2008;59:4171–4182.
143. O'Shaughnessy WB. On the preparations of the Indian hemp, or Gunjah: Cannabis indica their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases.
144. McPartland JM, Guy GW, Di Marzo V. Care and feeding of the endocannabinoid system: a systematic review of potential clinical interventions that upregulate the endocannabinoid system. *PLoS One*. 2014.
145. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease—successes and failures. *FEBS J*. 2013;280:1918–1943.
146. Donvito Giulia, Nass Sara R, Wilkerson Jenny L, Curry Zachary A, Schurman Lesley D, Kinsey Steven G, Lichtman Aron H. The Endogenous Cannabinoid System; A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. *Neuropsychopharmacology*. 2017.
147. Russo EB. Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? *Neuroendocrinol Lett*. 2004;25:31–39.
148. Szaflarski P, Bebin Elizabeth Martina, Cutter Gary, et al. Cannabidiol improves frequency and severity of seizures and reduces adverse events in an open-label add-on prospective study. *Epilepsy Behavior*. 2018:S1525–5050. pii.
149. Devinsky O, Cross JH, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med*. 2017;376:2011–2020.
150. Thiel EA, Marsh ED, French JA, Mazurkiewicz-Beldzinska M, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome: a randomized, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2018;391(10125):1085–1096.
151. Wu D. Mechanism of acupuncture in suppressing epileptic seizures. *J Tradit Chin Med*. 1992;12:187–192.
152. Huang ZN, Yang R, Chen G, et al. Effect of electroacupuncture and 7-NI on penicillin-induced epilepsy and their relation with intrahippocampal NO changes. *Sheng Li Xue Bao*. 1999;51:508–514.
153. Yang R, Huang ZN, Cheng JS. Anticonvulsion effect of acupuncture might be related to the decrease of neuronal and inducible nitric oxide synthases. *Acupunct Electrother Res*. 2000;25:137–143.
154. Stavem K, Kloster R, Rossberg E, et al. Acupuncture in intractable epilepsy: lack of effect on health-related quality of life. *Seizure*. 2000;9:422–426.
155. Kloster R, Larsson PG, Lossius R, et al. The effect of acupuncture in chronic intractable epilepsy. *Seizure*. 1999;8:170–174.
156. Shu J, Liu RY, Huang XF. The effects of ear-point stimulation on the contents of somatostatin and amino acid neurotransmitters in brain of rat with experimental seizure. *Acupunct Electrother Res*. 2004;29:43–51.
157. Allen IC, Grieve A, Griffiths R. Differential changes in the content of amino acid neurotransmitters in discrete regions of the rat brain prior to the onset and during the course of homocysteine-induced seizures. *J Neurochem*. 1986;46:1582–1592.
158. Cole M, Shen J, Hommer D. Convulsive syncope associated with acupuncture. *Am J Med Sci*. 2002;324:288–289.
159. Panjwani U, Selvamurthy W, Singh SH, Gupta HI, Thakur L. Effect of sahaja yoga practice on seizure control and changes in patients with epilepsy. *Ind J Med Res*. 1996;103:165–172.
160. Yardi N. Yoga for control of epilepsy. *Seizure*. 2001;10(1):7–12.
161. Ramaratnam S, Sridharan K. *Yoga for epilepsy*. Oxford, UK: Cochrane Rev Cochrane Lib; 2001:2.
162. DeGiorgio CM, Shewmon DA, Whitehurst T. Trigeminal nerve stimulation for epilepsy. *Neurology*. 2003;61(3):421–422.
163. DeGiorgio CM, Murray D, Markovic D. Trigeminal nerve stimulation for epilepsy: long-term feasibility and efficacy. *Neurology*. 2009;72(10):936–938.
164. DeGiorgio CM, Shewmon A, Murray D. Pilot study of trigeminal nerve stimulation (TNS) for epilepsy: a proof-of-concept trial. *Epilepsia*. 2006 Jul;47(7):1213–1215.
165. Eriksen HR, Ellertsen B, Grønningsaeter H. Physical exercise in women with intractable epilepsy. *Epilepsia*. 1994;35(6):1256–1264.
166. Arida RM, Scorza FA, Cavalheiro EA. Favorable effects of physical activity for recovery in temporal lobe epilepsy. *Epilepsia*. 2010;51(suppl 3):76–79.
167. Penovich PE, Eck KE, Economou VV. Recommendations for the care of women with epilepsy. *Cleve Clin J Med*. 2004;71(suppl 2):S49–S57.

168

Erythema Multiforme

Michael Traub, ND, DHANP, FABNO, John Nowicki, ND, and *Michael T. Murray, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Sudden onset of symmetrical erythematous, edematous, macular, papular, urticarial, bullous, or purpuric skin lesions
- Evolves into "target lesions" (lesions with clear centers and concentric erythematous rings)
- Characteristic first site: dorsum of the hand
- Characteristic distribution: extensor surfaces of extremities, with relative sparing of head and trunk
- Although rare, oral manifestations ranging from tender superficial erythematous and hyperkeratotic plaques to painful, deep, hemorrhagic bullae and erosions
- Tendency to recur in spring and fall

GENERAL CONSIDERATIONS

The term *erythema multiforme* (EM) includes a wide range of clinical expressions, from exclusive oral erosions (oral EM) to mucocutaneous lesions ranging from mild (EM minor) to severe involvement of multiple mucosal membranes (EM major, Stevens–Johnson syndrome [SJS]) or with involvement of a large area of the total body surface (toxic epidermal necrolysis [TEN]). EM, SJS, and TEN are all cutaneous hypersensitivity reactions to specific antigens. SJS and TEN were once classified as distinct entities but are now widely accepted as having similar etiopathogeneses, differing only in severity as measured by the percentage of body surface involvement. Identifying EM is critical because reexposure to the offending antigens is thought to allow for progression to more severe reactions, such as SJS and TEN.[1] Although significant differences exist among EM minor, EM major, SJS, and TEN regarding severity and clinical expression, all variants share two common features: typical or less typical cutaneous target lesions and satellite-cell or more widespread necrosis of the epithelium. Clinically, EM major is characterized by typical or raised atypical targets located on the extremities and/or face (Fig. 168.1). SJS is diagnosed when lesions are flat, atypical targets or purpuric maculae that are widespread or distributed on the trunk.[2]

Among the great number of suspected etiological factors, the differentiating feature between EM and SJS/TEN is that herpes simplex virus or mycoplasmal infections are involved in roughly 90% of cases of EM minor, whereas 80% of cases of SJS and TEN are caused by systemic drugs, mainly anticonvulsants, sulfonamides, nonsteroidal anti-inflammatory drugs, allopurinol, and antibiotics. It is possible that some cases of EM associated with a variety of respiratory, gastrointestinal, or urinary tract infections may represent a single specific delayed-sensitivity reaction to the bacterial endotoxin lipopolysaccharide.[3] Various vaccines (e.g., *Vaccinia;* tuberculosis; poliomyelitis; human papillomavirus; bacille Calmette-Guerin [BCG]; diphtheria, tetanus, and pertussis [DTaP]; measles, mumps, and rubella [MMR]; hepatitis B; meningococcal; pneumococcal; rabies), food additives (e.g., benzoates, nitrobenzene), food allergies, chemicals (e.g., perfume, terpenes), and other infectious organisms have all induced EM. Compounds such as formaldehyde, acrylonitrile, and chlorinated hydrocarbons have also been associated with case reports of either EM, SJS, or TEN.[4]

All variants of EM appear to have a common mechanism. Evidence points to a T-cell–mediated immune reaction to the precipitating agent, which leads to a cytotoxic immunological attack on keratinocytes that express nonself antigens. Subsequent subepithelial and intraepithelial vesiculation leads to widespread blistering and erosions.[5]

Human leukocyte antigen (HLA)-DQ3 correlates closely with recurrent EM and may be a marker to distinguish herpes-associated EM from other skin diseases.[6] Biopsy can help predict disease progression from EM lesions to SJS or TEN.[7]

EM typically occurs in young adults 20 to 40 years of age and is more common in women compared with men (1.5:1.0). The reported prevalence rate of EM is less than 1%, and prevalence rates of oral EM lesions vary from 35% to 65% among patients with cutaneous lesions.[8] The number of EM cases is increasing in cancer patients who have been prescribed immunotherapy or endocrine therapy drugs or are receiving radiotherapy.

THERAPEUTIC CONSIDERATIONS

Potassium Iodide

Potassium iodide has historically been used to treat various erythematous disorders, including the following:

- EM
- Erythema nodosum
- Nodular vasculitis
- Acute febrile neutrophilic dermatosis
- Subacute nodular migratory panniculitis

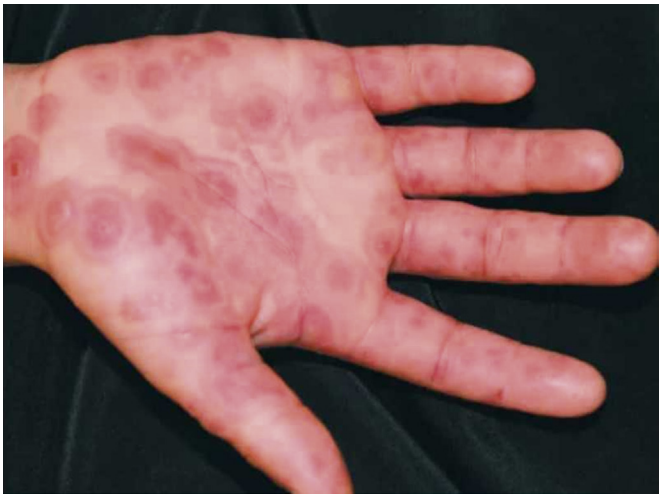


Fig. 168.1 Erythema multiforme.

Clinical studies have documented dramatic success with potassium iodide.[9–11] The mechanism is apparently related to the suppression of the generation of oxygen intermediates such as hydrogen peroxide and hydroxyl radicals by stimulated neutrophils.[12] Importantly, iodine therapy has occasionally been associated with various adverse skin reactions and gastrointestinal discomfort; it should never be used in pregnant women in the last trimester owing to its effect in suppressing the fetal thyroid.[13,14]

Zinc

Zinc sulfate (0.025%–0.05%) has been used locally at the site of herpetic infection and to prevent the relapse of postherpetic EM.[15]

Topical Vitamin A

Ocular involvement of EM can be treated with topical vitamin A ointment.[16]

THERAPEUTIC APPROACH

A careful search should be made to determine the initiating factor. Underlying infections should be treated, all unnecessary medications stopped, and an anti-inflammatory program initiated. In herpes-associated EM, antiviral treatment initiated after EM has erupted has shown no benefit. Symptomatic treatment—including local skin care, analgesics, and soothing mouthwashes—is important. Liquid antiseptics help prevent superinfection. In the severe form of EM, there may be extensive lesions and an inability to ingest foods. A liquid diet may be necessary. Intravenous fluid replacement with electrolytes and nutrients should be started as early as possible. The use of systemic corticosteroids is controversial, with some believing that it may increase the risk of complications.

Supplements

- Potassium iodide: 100 mg three times daily for 4 to 6 weeks, to be discontinued if there are adverse reactions
- Zinc sulfate: 0.025% to 0.05% solution applied locally to postherpetic lesions
- Topical vitamin A ointment for ocular lesions

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Studdiford J, Oppenheim L, McCann E, Altshuler M. Erythema multiforme after meningitis vaccine: patient safety concerns with repeat immunization. *Pharmacother J Hum Pharmacol Drug Ther*. 2006;26:1658–1661.
2. Assier H, Bastuji-Garin S, Revuz J, et al. Erythema multiforme with mucous membrane involvement and Stevens-Johnson syndrome are clinically different disorders with distinct causes. *Arch Dermatol*. 1995;131(5):539–543.
3. Shelley WB. Bacterial endotoxin (lipopolysaccharide) as a cause of erythema multiforme. *JAMA*. 1980;243(1):58–60. PubMed PMID: 6985676.
4. House RA, Jakubovic H, Wong L, Holness DL. Work-related toxic epidermal necrolysis? *Journal of Occupational Med*. 1992;34(2):135–139. PubMed PMID: 1597767.
5. Scully C, Bagan J. Oral mucosal diseases: Erythema multiforme. *Br J Oral Maxillofac Surg*. 2008;46. 90–53.
6. Kämpgen E, Burg G, Wank R. Association of herpes simplex virus-induced erythema multiforme with the human leukocyte antigen DQw3. *Arch Dermatol*. 1988;124(9):1372–1375.
7. Hosaka H, Ohtoshi S, Nakada T, et al. Erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis: frozen-section diagnosis. *J Dermatol*. 2010;37(5):407–412.
8. Samim F, Auluck A, Zed C, Williams PM. Erythema multiform: a review of epidemiology, pathogenesis, clinical features, and treatment. *Dental Clinics of North Am*. 2013;57(4):583–596. PubMed PMID: 24034067.
9. Horio T, Danno K, Okamoto H, et al. Potassium iodide in erythema nodosum and other erythematous dermatoses. *J Am Acad Dermatol*. 1983;9:77–81.
10. Schultz EJ, Whiting DA. Treatment of erythema nodosum and nodular vasculitis with potassium iodide. *Br J Dermatol*. 1976;94:75–78.
11. Kelly F. Iodine in medicine and pharmacy since its discovery: 1811-1961. *Proc R Soc Med*. 1961;54:831–836.
12. Miyachi Y, Niwa Y. Effects of potassium iodide, colchicine, and dapsone on the generation of PMN derived oxygen intermediates. *Br J Dermatol*. 1982;107:209–214.
13. Zone JJ. Potassium iodide and vasculitis. *Arch Dermatol*. 1981;117:758–759.
14. Sterling JB, Heymann WR. Potassium iodide in dermatology: a 19th century drug for the 21st century-uses, pharmacology, adverse effects, and contraindications. *J Am Acad Dermatol*. 2000;43(4):691–697.
15. Brody I. Topical treatment of recurrent herpes simplex and post-herpetic erythema multiforme with low concentrations of zinc sulphate solution. *Br J Dermatol*. 1981;104:191–194.
16. Hafsi W, Badri T. Erythema Multiforme. *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2018-2017 Dec 18.

169

Fibrocystic Breast Disease

Bethany Montgomery Hays, MD, and *Tori Hudson*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

Abnormalities of the breast, both benign and malignant, are increasing.[1] In one study, 16% of women who visited a doctor over a ten-year period had breast complaints. Only 4% of these women turned out to have breast cancer. Yet failure to diagnose breast cancer (often slow to develop and related to lifetime exposure to estrogen)[2] is the most common reason for a physician to be sued for malpractice.[3] Therefore attention to breast complaints and the relationship between benign and malignant lesions is paramount to women's health.

Characteristics of fibrocystic changes of the breast are as follows:

- They are common in reproductive-age women, usually worsening in the luteal phase and in perimenopause and improving after menopause, suggesting a relationship to elevated levels of estrogen (or estrogenic compounds) and/or imbalanced or decreased progesterone.
- They are sometimes asymptomatic or occurring throughout the month but often present as painful, lumpy breasts premenstrually and as part of the premenstrual syndrome (PMS)—also suggesting their relationship to elevated estrogen or estrogen unbalanced by progesterone.
- They are histologically characterized by fibrosis, ductal scarring, and cysts (of various sizes) which may be lined with epithelial cells, apocrine metaplasia, or unlined. The relationship of fibrocystic changes to breast cancer appears to depend on whether the remaining ductal and lobular tissue is found to be proliferative and whether there is atypia.[4]
- Paradoxically, as compared to women with breast cancer, they occur more frequently in women who have nursed multiple infants,[5] suggesting increased exposure to bacteria or antigenic substances in the milk.
- Mammographically, they are are dense, both increasing the risk of cancer[6] and making it difficult to see and diagnose embedded cancers.

*Previous edition contributor

GENERAL CONSIDERATIONS

Fibrocystic breasts or fibrocystic changes of the breast (no longer called "fibrocystic disease"[2,7]) is, in fact, a condition of clinical significance more than just annoyance. The condition is now referred to as fibrocystic changes (FCC), and its relationship to cancer risk has been debated in the past.[8] Currently, breast cancer risk is thought to be related to proliferation and atypia of the epithelial (ductal) component.[9] In fact, FCC may exist on a spectrum of changes that lead to breast cancer.[10] Perhaps the question should be: Do the same things that cause FCC over time cause breast cancer? And more importantly, can we use fibrocystic breast symptoms to begin treatments that will prevent later cancers? Studies of fibrocystic disease are surprisingly few since the 1980s. There is, however, a new understanding of fibrosis, its causes, and its relationship to breast cancer.[11] This new understanding points us to the extracellular matrix as well as the epithelial component of the breast and their response to the following:

1. Inflammation
2. Elevated estrogen/xenoestrogens
3. The breast microbiome

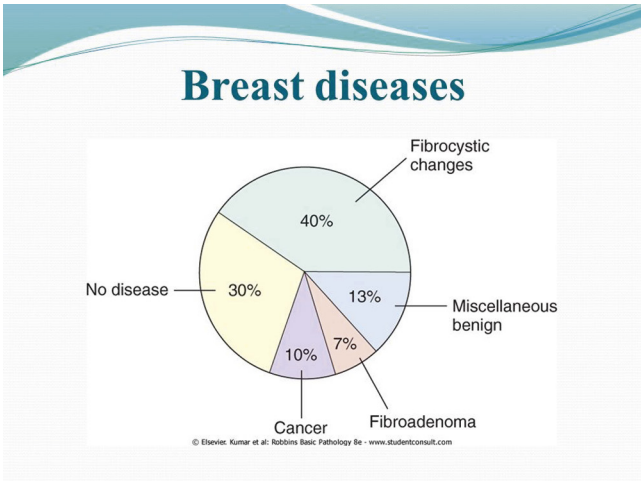
It is important to reconsider whether the condition of FCC, like many diseases, is another response of an organ or tissue to a hostile environment.

In this age of increasing rates of breast cancer, tender or lumpy breasts are among the most common reasons women consult their health practitioners for assessment and treatment. Many women

experience premenstrual discomfort and fullness of the breasts. When bilateral, cyclic, and repetitive and occurring in the luteal phase of the menstrual cycle, these symptoms are clearly hormonally related and suggest an effect of both estrogen *and* progesterone or, more specifically, the balance of the two. Symmetrical nodularity, particularly in the upper outer quadrant, is extremely common on physical examination. These areas may be so tender that palpation is difficult. An asymmetrical (unilateral) mass that can be clearly described (size, shape, distance from the nipple and quadrant of the breast) is a "dominant mass" and must be diagnosed or followed until a firm diagnosis is made by examination under a microscope, resolution (by either spontaneous resolution after one or more menstrual periods or by aspiration of a cyst without recurrence), or occasionally by a history of its presence without change for many years. It is the role of the practitioner to keep any dominant mass on his or her radar screen until convinced of its continuing benign nature. A fixed or changing mass or one accompanied by skin changes, tissue edema, or axillary nodes is clearly problematic and should be examined by mammography and diagnosed by biopsy.

Fibrocystic changes in the breast are so common (estimates vary, sometimes as high as 64%[12] in symptomatic women, although found in up to 54% of *asymptomatic* women in one autopsy study[13]) that they should not be labeled a "disease." Because these changes are frequent even in older women at a subclinical level, they should be considered a "functional change."[12]

It is likely that fibrocystic changes are a common response to the interactions of this unique organ with its environment.



Because many women have symptoms that wax and wane and resolve with strategies that lower estrogen or improve at menopause, and because there is a significant placebo effect in studies, FCC has not received much study or interest. But ask any woman about the discomfort of a mammogram or the annoyance of having to wear a "supportive" bra to bed or the recommendation that she consider psychotherapy, and she will tell you she would like answers and treatment.

ANATOMY AND PHYSIOLOGY OF THE BREAST

Breasts exist for two reasons: to feed babies and to feel good *to their owner* (releasing oxytocin) during sex. (The fact that they are extraordinarily interesting to the opposite sex should have a secondary place in how we care for them.) Both functions require a sensitive nipple, which will cause the release of oxytocin from the pituitary when stimulated.

The nipple has 8 to 10 lactiferous ducts connecting to lactiferous sinuses or ampullae underneath the pigmented areola. These dilated areas of the duct collect small amounts of milk to be expressed by the action of the nursing infant's tongue and jaws. The ducts are connected to 15 to 20 lobes composed of lobules of milk-producing alveoli or acini, with each lobe functioning semiautonomously to reproduce the amount of milk removed at each nursing before the next feeding. Additionally, the breast creates a formula ideal for the gestational age of the infant at birth and later to provide the nutritional needs for the growing infant.[14] (Contemplate how the same breast can completely feed a 2-lb premature newborn and a 1-year old toddler 10 times that size without appreciably changing the size or number of lobules.). The "growing edge" of the ductal system is the ducts within the lobule, which connect to the milk-producing acini (terminal duct lobular unit [TDLU]). These are the most cellularly active parts of the breast. It is in this location that cancers most often begin. The immature ductal system is stimulated to grow into lobules under the influence of increasing levels of estrogen and progesterone at menarche and to mature into milk-producing or mature lobules during pregnancy. Breast duct cells that have never undergone this maturation divide 20 times more often than cells that have acquired the mature phenotype.[15] Once the lobule has matured, it will remain relatively well differentiated under the influence of monthly progesterone until menopause when it regresses, leaving the more "at-risk" undifferentiated ductal system. The differentiated or mature lobule is less frequently the site of cancer, but when it occurs here, it can be more aggressive (lobular breast cancer).

The breast is also composed of connective tissue or stroma surrounding the acini (shown in the accompanying figure in light pink) and more dense stroma surrounding the lobules (shown in dark pink). This tissue is laid down by fibroblasts, which are sensitive to inflammation from various causes.

Each duct and its acinus are surrounded by myoepithelial cells that contract under the influence of oxytocin, pushing the milk to the lactiferous sinuses and allowing the infant to empty virtually the entire breast while suckling and compressing only the areola. The myoepithelial cells also rest upon a basement membrane around the ducts, which ductal cancers and lobular cancers must either break through or be allowed through, and are considered "natural tumor suppressors."[16]

Extracellular Matrix and Fibrosis

The TDLU is embedded in a myxomatous stroma containing fibroblasts, adipocytes, lymphocytes, macrophages, and plasma cells, as well as extracellular matrix (ECM) containing collagen; glycosaminoglycans, such as hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratin sulfate, heparin sulfate, and other polar molecules[17]; and soluble factors (minerals, hormones, cytokines, growth factors and enzymes) at a specific pH and oxygen content. This partly liquid/partly solid matrix both supports the milk-producing lobules and ducts and allows them to expand and contract during milk production and storage. The development of the normal breast and the development of breast cancer depend on the "cross-talk" between these stromal and epithelial elements.[18] The stromal elements surround, nourish, and communicate with the ductal and milk-producing cells.[19] They are subject to epigenetic changes that have been shown to determine both the development of cancer and its ability to invade and metastasize.[20] Fibrocytes in the ECM respond to chronic inflammatory signals.[6] Fibrosis is defined by the overgrowth, hardening, and/or scarring of various tissues and is attributed to excess deposition of ECM components, including collagen.[21] In other words, fibrosis is a response of the ECM to chronic inflammation. "Fibrosis is the end result of chronic inflammatory reactions induced by a variety of stimuli including

persistent infections, autoimmune reactions, allergic responses, chemical insults, radiation, and tissue injury."[22]

This fibrous "scar" tissue, laid down in response to inflammatory signals, both compresses the ducts and obstructs them, producing cysts of various sizes, and through cross-talk and epigenetic changes, it produces alterations in the ductal and lobular epithelium. When these alterations are proliferative, the character of the cysts is different than when the epithelium is flattened, and the risk of breast cancer increases. (See later discussion of cysts.) Only fibrosis with involution of the lobules and nonproliferative changes in the epithelium qualifies as the type of fibrocystic change that does not increase the risk of breast cancer. It is likely that benign epithelium in a background of abnormal ECM, proliferative epithelium, and proliferative epithelium with atypia represents a continuum leading to cancer.

The stromal tissue, especially fibroblasts and immune cells, responds to the environment, which arrives via the blood supply and exits via the lymphatics. It also responds to the contents of the ductal lumen, consisting of the milk being produced, excreted immune cells, and the breast microbiome. The ductal cells also respond to both the ECM and the luminal environment, which, when hostile, can induce metaplastic changes (apocrine metaplasia[23–25]), hyperplasia, atypia, and ultimately, cancer. "Most chronic fibrotic disorders have in common a persistent irritant that sustains the production of growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines, which stimulate the deposition of connective tissue elements that progressively remodel and destroy normal tissue architecture."[19] In fibrocystic breasts, these chronic irritant signals are likely arriving at the ECM via both the blood and the luminal contents.

Breast Cysts

The breast makes and reabsorbs small amounts of fluid in the ducts throughout life. If the duct is obstructed or scarred by infection or injury, or if fibrosis or fibrotic extracellular matrix is laid down, cysts can form behind the scarred or compressed portion of the duct.[26] Cysts can also form due to excessive secretions created by hyper-cellularity of the ducts and by poor drainage.[27] The former, called *type 2*[28] (compressed ducts), occurs in ~45% of cases and usually do not recur after needle aspiration. They have fluid that is a transudate of serum with a low K^+/Na^+ ratio. The latter, called type 1 (excessive secretions), occurs in ~55% of cases. They are more likely multiple, associated with apocrine metaplasia, often recur after aspiration, and have a high K^+/Na^+ ratio.[20,29,30] This is the classic picture of fibrocystic changes. Because both types of cysts can coexist in the same patient and one has an increased risk of breast cancer but the other does not, studies of fibrocystic breasts have shown conflicting results with regard to the risk of breast cancer. The current consensus is that breast cancer risk is related to the degree of cellular proliferation and atypia of the ductal cells.

Breast Microbiome

Breast tissue has its own microbiome.[31] Studies have recently been done looking at whether a bacterial population exists in the normal uninfected breast and whether that tissue microbiome changes or is different in women with infection or cancer.[30,32,33] Not surprisingly, both are true. Researchers found higher numbers of bacteria that are able to cause DNA damage and fewer *Lactobacillus* in the patients with breast cancer.[31] *Helicobacter pylori,* a known initiator of carcinogen production, has been found in the 4/66 breast milk samples.[34] There is evidence that some bacteria "prepare suitable microenvironments for tumor cell growth."[35]

The breast is one of the tissues "open" to the environment in the female (as are the nasopharynx, cervix/uterus, and bladder), so it should not be surprising to find bacteria and viruses (and occasionally parasites) in the ducts. Its microbiome, however, is different from the gut, mouth, or overlying skin. Mastitis is now thought to be due to dysbiosis or imbalance of the breast microbiome rather than the simple invasion of bacteria.[36] Acute mastitis is caused by pathological organisms (usually staph or strep). It occurs most often at the initiation of breastfeeding when previously unused ducts must dilate and acini and lobules must contract and clear the colostrum and milk being produced. (This early risk may be in part the reason that colostrum is composed primarily of immune cells *and* immunoglobulins.) Acute mastitis often goes untreated and clears spontaneously. When severe, it is usually treated with oral antibiotics. Recently, it has been shown that acute mastitis can be treated with an oral *pro*biotic containing *Lactobacillus salivarius* CECT 5713 or *Lactobacillus gasseri* CECT 5714. With no other treatment than continuing to nurse, the probiotic group had complete resolution by 14 days.[37] A second study compared the *Lactobacillus fermentum* CECT 5716 and *L. salivarius* CECT 5713 with antibiotics. The probiotic groups showed both significant lowering of pathogenic bacteria in the milk and better improvement in pain compared with antibiotics.[38] Another study prevented mastitis by pretreatment with *L. salivarius* in late pregnancy.[39] How do oral probiotics reach the breast? It may be the same way the breast microbiome is established: via immune cells from the gut that carry live bacteria through the bloodstream to the ECM and deliver them to the lumen of the acini and ducts.[35] Completing the loop of connection between the breast microbiome and fibrocystic changes, treatment of *H. pylori* in the gut has been found to be successful in the resolution of FCC symptoms in the breast.[32]

A much less common entity, "plasma cell mastitis"[40] is thought to be caused by chronic irritation of the ducts from secretory products that can produce an autoimmune reaction with infiltration of plasma cells.[41] Because it is most often seen in multiparous women, it may be an immune reaction to antigens seen in previous episodes of lactation. Free fatty acids are known to produce a marked inflammatory response in breast trauma, mimicking cancer as a hard, spiculated mass with microcalcifications on mammogram. Fat-laden macrophages differentiate its microscopical appearance from that of cancer (also thought to be an aberration of the healing process by some[42]).

These "open" channels to the outside world (oropharynx, cervix/uterus, rectum/anus, and bladder/prostate) are protected by locally increased lymphatics; by metaplasia of the interfacing epithelium; by iodine[43]; and in the breast, by lactoperoxidase, lysozyme, and a number of proteins secreted in the milk.[44] Dysbiosis of the breast, like that of the gut,[45] is a likely source of inflammatory signals[32] that can induce changes in the fibroblasts of the ECM,[18,46] causing both fibrocystic changes and potentially cancers. Because fibrosis (a tissue response to inflammation) is found almost universally in the breast (if autopsy studies are to be believed), it would appear that fibrocystic changes are a normal or at least common response to the degree of inflammation in the environment.

A number of additional protective mechanisms exist to prevent infection from the outside from entering the body via the ductal system. The ends of the ducts at the nipple are often sealed by desquamated epithelial cells producing a plug. When removed by the suckling infant or during sex, oral bacteria are more likely to enter, and the microbiome changes. Properties of the milk (lactoferrin, white blood cells [WBCs], various iodinated proteins, and immunoglobulins) act as antibacterial agents in the duct and in the milk being produced. If these fail, infection in the duct can produce oxidative stress, tissue damage, ductal scarring, and ultimately, cysts and fibrosis.

TABLE 169.1 Possible Causes of an Estrogen Effect

1. Overproduction of estrogen
2. Poor metabolism or excretion of estrogen
3. Exogenous estrogen or estrogen therapy
4. Xenoestrogens and metalloestrogens
5. Unbalanced estrogen and progesterone, with estrogen dominance or luteal-phase progesterone deficit
6. Pregnancy
7. Overabundance of estrogen receptors

Hormonal Milieu

The relationship of fibrocystic changes to cyclic mastalgia, increased lumpiness before menses, PMS, increased symptoms in perimenopause, and the resolution of most symptoms after menopause strongly suggest that fibrocystic changes are related to elevated estrogen and, in some cases, the decreased or unbalanced progesterone found at these times.[47,48] FCC has also been related to polycystic ovary syndrome (PCOS), a syndrome related to increased estrogen and insulin resistance.[49,50] The relationship between FCC and estrogen is so strong that many early studies implied that this hormone milieu was causative of FCC. It is more likely that it is important but ancillary. Both the immune system and the ductal system are under the influence of estrogen, progesterone, and estrogen and chemicals from the environment. Some of these environmental toxins are preferentially secreted in the milk.[51] Prolactin has been shown to be normal or mildly elevated in serum but also chronobiological (i.e., related to time of day, day of cycle, and also sleep related).[52] Differences in response to prolactin between normal patients and patients with FCC have been documented.[53] When breast symptoms are cyclic and present throughout the breast, more often than not, they are caused by or exacerbated by too much estrogen *effect.* Possible causes are listed in Table 169.1.

High levels of estrogen and progesterone in pregnancy or during the first few weeks of oral contraceptive use produce growth and differentiation of the milk-producing alveoli, which can also be uncomfortable for some women. These symptoms usually subside as the pregnancy progresses or the endogenous estrogen levels fall with oral contraceptive pill (OCP) suppression. Because hormones are closely linked to FCC and breast symptoms, they offer considerable opportunity for prevention and treatment. Although the traditional approach to hormonal treatment for FCC has been estrogen or prolactin-lowering drugs, which have substantial side effects, each of the noted causes of excess estrogen effect can be addressed with diet, supplements, and herbal therapies.

Environmental Toxins

A growing number of new-to-nature-chemicals in the environment that negatively affect humans are xenoestrogens. The breasts are particularly susceptible. Xenoestrogens can exert both genomic and nongenomic (cell-surface–receptor) effects.[54] Bisphenol A,[55] Glyphosate, plastics,[56] pesticides, (herbicides, insecticides, rodenticides),[57] chemicals used in hydraulic fracturing,[58] a panoply of chemicals in personal care products,[59] and more importantly, combinations of chemicals in doses considered unimportant when taken individually[60] are shown to create cellular changes in susceptible tissues such as the breast. These chemicals are now showing up in groundwater. We are all exposed.

In addition to man-made chemicals, environmental contamination with metalloestrogens, which are small ionic metals that activate the estrogen receptor (ER), are becoming a problem. Breast cysts can be induced by aluminum, a common component of underarm deodorant.[61] Aluminum can cause genomic instability and proliferation in breast epithelial cells. At lower levels, methyl mercury promotes the proliferation of breast cancer cells.[62] Other metalloestrogens, such as cadmium, are used in many everyday products, such as cosmetics, household items, and processed foods.[63] Prenatal exposure to arsenic has been shown to increase the number of mammosphere-forming cells and increase branching, epithelial cells, and density of the breast at puberty.[64,65]

Approaches that help a woman limit her exposure to chemicals in cosmetics, food additives, and household cleaning fluids, such as water filtration and the like, would be expected to have a positive effect if started early enough.

DIFFERENTIAL DIAGNOSIS

Benign breast lesions include benign neoplastic and nonneoplastic groups. The critical factor in the differential diagnosis is the identification of premalignant and malignant lesions.

Mass

The management of a breast mass is critical to the early and accurate diagnosis of benign versus malignant breast disease. Proper diagnosis and management of a breast mass should include the following:

1. A careful examination looking for dominant masses. Examination of a suspicious mass should preferably be done in the first week after a period when hormone levels are low.
2. A dominant mass is cancer until proven otherwise by its disappearance, its removal and microscopical diagnosis, by aspiration and resolution, or occasionally by long-term follow-up without change. The practitioner's job is not done until he or she is satisfied that there is no cancer. This does not mean every lump has to be surgically removed.
3. Breast symptoms while on hormone therapy of any sort should not be ignored. Practitioners commonly get the blame if there is a cancer or one develops.
4. Unilateral or asymmetrical masses or symptoms unrelated to menses require careful evaluation even though they are most often benign.
5. Never tell the patient "everything is fine" when she presents with a complaint even if you feel no mass. Tell her that you don't see anything to worry about, but if things change or she continues to have concern, she should be sure to return until you are both satisfied there is nothing to worry about. Encourage a return visit. Women know things about their bodies they can't always verbalize.

Lumps in the breast due to FCC are often difficult to describe because they meld with the dense areas of fibrosis. The fibrocystic breast is, by definition, lumpy. Because the fibrous component makes them tender and dense and the cystic component makes them lumpy, and these symptoms often get worse after ovulation, it is best to examine the breasts on day 4 to 9 of the menstrual cycle. If the examination is not reassuring and the examination is in the luteal phase, a return visit or an ultrasound is reasonable. Other benign masses include fibroadenoma; phyllodes tumor, which can be benign, borderline, or malignant; hamartoma; fibromatosis; and cysts of various sizes. Cysts that are proliferative have a 1.5 to 2.0 times higher risk of becoming cancers, and cysts with atypia have a relative risk of developing cancer as high as 3.9 to 13.[66]

Inflammation

As noted before, the breast ductal system is open to the outside world and is therefore a portal of entry for bacteria and viruses. An altered microbiome, cytokine signals (such as in patients with diabetes),

radiation, and trauma can all produce inflammation, which may incite fibroblast activity. Inflammation in the breast can present as pain, redness, or a mass, which should be addressed and treated.

Pain

Mastalgia refers to any breast pain severe enough to cause concern. Breast pain is categorized as cyclic (related to hormones) or noncyclic (related to drugs, pathology, or extramammary causes). Cyclic breast pain, because it is related to higher estrogen/progesterone effect, has been shown to be a risk factor for later breast cancer.[67] A careful history and physical can help rule out extramammary or nonhormonal causes of pain, such as costochondritis, cervical radiculopathy, myocardial ischemia, pneumonia, esophageal spasm, pleuritis, rib fracture, or shingles.[68]

HISTORY AND PHYSICAL EXAMINATION

Five major findings in benign breast disease that should be carefully evaluated are as follows:

1. Mass (size, shape, consistency, dominant, how long there, tenderness, history of trauma); cystic or solid
2. Pain (localized or general, constant or cyclic, drug or supplement related), which suggests inflammation
3. Discharge from nipple (spontaneous or expressed, history of breastfeeding, color, smell, volume)
4. Enlargement/swelling of the breast (related to menses, localized or unilateral, painful), suggesting tumor, edema, or lymphedema
5. Skin changes (dimpling, nipple retraction, peau d'orange, redness), suggesting underlying disease

These features should be carefully documented in the chart.

A common trap is the belief that the practitioner can differentiate benign from malignant disease on physical examination. Although the history and physical examination, mammogram, and ultrasound can increase or decrease the level of suspicion of malignancy, only histology on a specimen obtained by fine-needle biopsy, core biopsy, or open excision of suspicious lesions can determine whether a lesion is malignant, and even these methods are not perfect.[69]

Mammography is an appropriate way to obtain further information in women with a question of fibrocystic changes or a dominant mass. As stated before, dense fibrous areas make mammographic diagnosis difficult. In this situation, ultrasound of a mass can be helpful in differentiating a fibrocystic area from a cystic or solid lesion. Findings on routine mammogram, the risk of mammography, and whether and when to perform screening mammograms are important topics for discussion elsewhere.

Thermography can be useful in women with dense fibrocystic breasts. It is important to remember that increased temperature is a sign of inflammation and therefore an upstream indicator of potential risk. Thermograms have not been shown to be as accurate as mammography for identifying early cancers (which may be a benefit from the standpoint of overdiagnosis in the perimenopause) and therefore provide a different type of information (about inflammation) that may be helpful in decision making.

THERAPEUTIC CONSIDERATIONS

From the foregoing discussion, it should be clear that there are at least three areas that should be addressed in the treatment of fibrocystic symptoms: estrogen effect, inflammation, and the microbiome.

Because excessive estrogen or altered sensitivity to estrogen is the dominant theory of etiology,[70,71] interventions that modulate endogenous estrogens or limit exposure to exogenous estrogen or estrogen-like substances warrant consideration as potential treatments for symptomatic fibrocystic breast conditions and will be discussed first.

Premenstrual syndrome, which shares a common hormonal picture, is discussed elsewhere (see Chapter 212). In fact, breast pain is often a part of the PMS complex of symptoms. The factors discussed here are not covered in depth in Chapter 212 and are particularly relevant to fibrocystic breast disease.

Approaches that address excessive production or poor metabolism of estrogen include the following:

1. Inhibition of aromatase with diet and treatment of inflammation with curcumin, omega-3 fatty acids, and an anti-inflammatory diet (gluten-free and low in arachidonate)
2. Improved metabolism of estrogen by CYP-450 enzymes aimed at discouraging the 16OH and 4OH pathways, improving Phase II detoxification and preventing DNA adduct formation with adequate intracellular glutathione. Discouraging alcohol; preventing insulin resistance; addressing obesity; and encouraging crucifers, allium-containing vegetables, and B vitamins are all effective strategies.
3. Protection of glucuronidated estrogens from beta-glucuronidase in the gut (calcium-d-glucarate) preventing reabsorption of partially metabolized estrogens
4. Attention to regular excretion using fiber, especially freshly ground organic flax seed and omega-3 fatty acids, magnesium, and so forth
5. Blocking estrogen receptors with weaker estrogens, such as estriol[21] or phytoestrogens

Once elevated estrogens have been addressed, balancing estrogen with adequate progesterone can be helpful. Progesterone appears to improve iodine absorption,[42] in addition to its antiestrogen and pro-differentiation effects. Addition of progesterone before addressing estrogen, however, can put the breast in a pseudopregnancy or continuous luteal phase in which pain and cysts get worse.

Inflammation

Because the ECM responds to inflammatory signals from the bloodstream as well as from the bacteria that make up the breast microbiome, and because inflammation is known to increase estrogen production by increasing the activity of aromatase, a number of nutritional, supplemental, and herbal preparations would be expected to be useful in the treatment of FCC. Needless to say, the earlier they are instituted, the more likely they are to be successful; once significant fibrosis occurs, treatment is more difficult.

Treating the Breast Microbiome

Because the breast microbiome is a relatively new concept, therapies have not been established that might affect it. From the studies of treatment of mastitis with probiotics, however, it is apparent that oral probiotics have an effect on the breast microbiome and should be considered. Bacterial species that have been cultured from normal breasts may be beneficial and include *L. salivarius* CECT 5713, *L. gasseri* CECT 5714, *L. fermentum* CECT 5716, and possibly other friendly bacteria cultured from human milk.

Dietary Considerations

Although the literature is confusing with regard to whether dietary changes have a significant role in prevention and treatment,[22] this is likely due to the complexity of causes of FCC; the diversity of genetics involved in estrogen effect and inflammation in a given woman; and the likelihood that there is a long latent period between fibrosis, cyst formation, proliferative changes, and cancers.[72] There are, however, general principles in lowering the estrogen effect and reducing

inflammation that should be effective in most women (see previous suggestions). Additionally, the Mediterranean-type diet has been shown to positively affect the breast microbiome.[73]

Obesity[74] and type 2 diabetes are both associated with breast pathology and later breast cancer, so dietary changes in these patients should be included in therapy for FCC.

Methylxanthines

Epidemiologically,[21,75,76] experimentally,[77–80] and clinically,[81] there is strong evidence supporting an association between methylxanthine consumption and fibrocystic symptoms, although little research appears to have been done since 1996.[82] The methylxanthines—caffeine, theophylline, and theobromine—are all known to be competitive inhibitors of cyclic mononucleotide phosphodiesterase, which catalyzes the hydrolysis (removal) of cyclic adenosine monophosphate (cAMP), thereby elevating its level in breast tissue. In turn, increased levels of cAMP (a so-called second messenger) stimulate protein-kinase activities and causes the overproduction of epithelial elements and fibrous tissue.[21] Minton et al. found that levels of cAMP in fibrocystic breasts were higher than normal and even higher in women with breast cancer.[83]

In one study, limiting methylxanthines (coffee, tea, cola, chocolate, and caffeinated medications) in the diet resulted in improvement in 97.5% of the 45 women who completely abstained and in 75% of the 28 who limited their consumption. Those who continued with little change in their methylxanthine consumption showed little improvement.[84] According to this study, women may have varying thresholds of response to methylxanthines. Other studies have shown no association between methylxanthines and fibrocystic breast disease.[85–87] This disparity may have to do with the difference between causation, which is unlikely, and symptom exacerbation, which is likely, as well as genetic susceptibility in a given woman.

Fiber

Fiber is both anti-inflammatory by virtue of its support of a healthy gut microbiome and antiestrogen by virtue of its effect on estrogen excretion. A comparison between the diets of 354 women with "benign proliferative epithelial disorders of the breast" and those of 354 matched controls and 189 unmatched controls found an inverse association between dietary fiber and the risk of benign proliferative epithelial disorders of the breast.[88] An increased intake of dietary fiber may be associated with a reduced risk of both benign breast disease and breast cancer.[89]

Soy

It is likely that soy, when metabolized to equol, presents a weak estrogen to the receptor in high numbers, which displace the stronger estradiol and estrone, effectively decreasing the estrogen effect.[90]

An uncontrolled study of 64 premenopausal women with FCC who consumed soy protein for 1 year used breast-enhanced scintigraphy testing (BEST) to evaluate change in inflammation, using angiogenesis and mitochondrial activity, which vary with tissue metabolic activity. The women and their physicians reported subjective and objective reduction in both breast tenderness and FCC. Evaluation by BEST also showed that a nonstatistical reduction occurred in both the average and maximal breast tissue activity. There was a statistically significant reduction ($P < 0.01$) in the variability of tissue activity, however.[91] A study of the concentrations of the plasma isoflavones genistein and daidzein and the risk of benign breast disease in China showed an inverse relationship for both benign and malignant breast diseases.[92]

Fats and Essential Fatty Acids

Increased calories, fat intake, and saturated fat are associated with benign breast disease.[93,94] Because these are older studies, it is not known whether the increased risk is associated with fat intake per se or the intake of foods containing more xenoestrogens. Because of their anti-inflammatory properties as well as their effects on bowel regularity, omega-3 fatty acids in the diet and as supplements have been shown to be helpful in fibrocystic breasts.[95,96]

Nutritional Supplements

Evening Primrose Oil

Studies of evening primrose oil (EPO) are contradictory.[97] This may point to the difference between measuring essential fatty acids versus measuring ratios of fatty acids, as well as the function of the delta 5, 6, and 9 desaturases. Studies of the omega-3 fats appear consistent in showing a lower incidence of symptoms. The omega-6 fatty acids, such as EPO, may be confusing based upon whether they are converted to anti-inflammatory prostaglandins or converted to inflammatory arachidonate. Because EPA inhibits the pathway of arachidonate to prostaglandin E2 as a COX-2 inhibitor, it should always be given concurrently with EPO.

Several studies have examined EPO for both mastalgia and breast cysts. In general, these studies are old and included small numbers of subjects.[98–102] A more recent meta-analysis showed no difference between EPO-treated patients and controls.[103]

A single study done looking at breast cyst response to EPO was also disappointing. Two hundred women with breast cysts were randomly assigned either six capsules per day of EPO or placebo for 1 year. Although there were fewer cysts in the EPO group (92 vs. 113), this difference was not statistically significant.[104]

These studies demonstrate the importance of adequately powered studies using the correct essential fats at the correct dosage for a long enough time to accomplish change in both symptoms and disease. It is logical to assume that dietary fats and EPO would be more likely to prevent fibrosis and cyst formation when started at a young age and possibly to inhibit progression than to be effective in relieving symptoms or reversing well-established fibrosis and cyst formation.

Vitamin E

Studies of vitamin E were generally small and of poor quality due to the failure to differentiate between the available forms of tocopherols. The mode of action remains unclear but is likely related to the effect of tocopherols on the inflammatory effects of oxidative stress in the ECM or ductal system.[105]

A very small (23 patients) study of alpha-tocopherol in the 1980s was shown to relieve many PMS symptoms, particularly FCC.[106,107] The same authors demonstrated that vitamin E is clinically useful in relieving pain and tenderness, whether cyclic or noncyclic.[108] When larger numbers of women were studied by the same authors, vitamin E did not fare so well, showing no significant effects either subjectively or objectively.[109,110]

In 2004 Bespalov et al. studied the effects of supplementation with a combination of beta-carotene 5 mg/d, vitamin E (alpha-tocopherol 5 mg), vitamin C (30 mg), and garlic powder (150 mg) in 66 women. There was a reduction in the severity of mastalgia, PMS, infrequent menses, and menstrual cramping as well as a reduction in "palpable symptoms of fibromatosis" in 75% of the women compared with 45% of women on placebo.[111]

In 2009 another small double-blind, placebo-controlled trial of vitamin E supplementation (200 mg) in 150 women in Iran, chewable tablets containing either vitamin E (200 mg) or placebo were given twice daily for 4 months. The results at 2 months were dramatically

better for 70% of the group receiving vitamin E than for those on placebo, but there was no further improvement at 4 months.[112]

More recently (2015), a brief study (2 months) of 80 patients with cyclic mastalgia was done by Shobeiri et al. This study compared vitamin E and vitamin B_6. A trend toward improvement in symptoms was found in both groups.[113]

Although there is a suggestion that vitamin E is effective for the symptoms of fibrocystic breasts, much more work needs to be done to confirm this. It is unlikely this will happen. However, the safety profile of this treatment is excellent.

Vitamin A

A review in 2004 points out the difficulty in studying the relationship of vitamin intake, vitamin levels, levels in breast tissue, and outcome. Their review of the research is inconclusive with regard to vitamin A and breast cancer, let alone benign breast disease, which is often used as the "control."[114]

Vitamin A is involved in cellular proliferation and differentiation.[115] The carotenoids additionally have antioxidant properties.[116] Vitamin A has been only minimally studied for fibrocystic breasts. However, there are retinoid receptors in breast tissue, which can modulate breast changes, including their effect on iodine processing.[42] In a study of 12 women with fibrocystic breast disease, women were given 150,000 IU of vitamin A daily for 3 months (by current standards, a massive dose). Five of the nine patients who completed the study had full or partial remission of their fibrocystic breast disease. However, some patients developed mild side effects, leading 2 of the original 12 to withdraw because of headaches, and 1 patient required a reduction in dosage.[117] Beta-carotene would appear to be a safer approach (see Chapter 127). However, genetic inability to convert beta-carotene to retinol must be considered because a significant portion of the population has genomic variations that impair the conversion of beta-carotene to vitamin A (see Chapters 57 and 125).

Thyroid and Iodine

The breast is a site that concentrates iodine (a known antimicrobial) and contains both the NIS (sodium iodide symporter) and lactoperoxidase (oxidizing iodide to iodine). Both the milk produced and the tissue producing it show increased concentrations of iodine,[118] presumably to protect the nursing infant as well as the breast tissue itself from pathogenic bacteria and the ravages of inflammation.

Experimental iodine deficiency in rats resulted in a mammary dysplasia histologically similar to human FCC when either estrogen or testosterone was administered.[119] In animals treated with perchlorate to block the NIS, treatment with thyroxin did not improve the breast abnormalities, suggesting that the cause was iodine deficiency and not hypothyroidism.[120] In humans, benign breast disease, including cysts and fibrocystic changes, were associated with nodular thyroid disease and Hashimoto's thyroiditis in 54.9% and 47.4%, respectively, versus 29.2% in a control group[121] and has been shown to improve with thyroid treatment,[122] possibly due to the breast's ability to deiodinate T3 and T4 and use the iodine.[42] Thyroid hormone replacement therapy in women with mastodynia resulted in improvement based upon a thyroid-releasing hormone (TRH)–induced prolactin response.[123] More recent studies show that the antineoplastic effects of iodine require either elemental iodine (I^2) or a tissue that can both absorb iodide (NIS) and convert it to iodine via peroxidases such as lactoperoxidase.[124,125] These results suggest that subclinical hypothyroidism, iodine deficiency, or both may be etiological factors in both FCC and breast cancer.[126] Iodine absorption increases in pregnancy and lactation. This may explain the decrease in density of fibrocystic appearance after pregnancy and lactation.[127]

In studies in rats and humans, it was shown that there is a hierarchical response to the type of iodine compound used in correcting the abnormalities in mammary tissue due to iodine deprivation. Although iodide corrects the cystic spaces and partially corrects the ductal cell proliferation, it produces more side effects and more disruption of thyroid function. Iodine in its elemental form corrects the entire disease process, including the fibrosis. Additionally, iodide or iodine, excreted in the milk or into the ductal lumen, may change the microbiome of the milk. Both the beneficial effects on the microbiome and the direct effects on the ECM influence the cause of FCC.

Since 1975, four clinical trials of iodide or iodine have been performed in women with FCC[6]:

- An uncontrolled study with sodium iodide (Lugol's, which contains only ~5% iodine) and a protein-bound iodide (iodine caseinate or I-)
- A prospective controlled crossover study of patients failing iodide treatment who switched to molecular iodine (aqueous molecular iodine-I^2, 0.08 mg/kg)
- A prospective, controlled, double-blind study with molecular iodine (aqueous iodine-I^2, 0.07–0.09 mg/kg)
- A controlled study of IoGen (an iodide/iodate combination that produces approximately 50% iodine in the stomach)

The results from these studies indicate that although treatment with iodides was effective in about 70% of subjects, it was associated with a high rate of side effects (e.g., altered thyroid function in 4%, iodinism in 3%, and acne in 15%). Results with elemental iodine were equally if not more effective but were not associated with significant side effects. The most significant side effect with molecular iodine was a short-term increase in breast pain, which seemed to correspond to a softening of the breast and disappearance of fibrous tissue plaques on physical examination. The more recent study using IoGen (a mix of sodium iodide and sodium iodate that generates approximately 50% molecular iodine in the stomach resulted in 7 of 28 patients in the 3.0 mg group and 5 of 27 in the 6.0-mg group exhibiting a reduction in physician-assessed symptoms and findings compared with none of the 1.5-mg or placebo groups. Over 50% of the women on the drug reported a reduction in pain compared with only 8.3% of the placebo group.[128] The dose of molecular iodine was 0.07 to 0.09 mg/kg/d in the first three studies and a somewhat lower dose of 3 to 6 mg (1.5–3 mg of iodine) of IoGen in the fourth. These doses appear to be safe and effective in the treatment for FCC.

Although iodine (I^2) may be an effective treatment for FCC,[6,129] it has been shown that iodine stimulates the transcriptional activity of estrogen receptor alpha in breast cancer cells,[130] and because of the induction of lactoperoxidase or perhaps as an oxidant itself,[131] iodine may increase 4OH estrone production. Other researchers believe that lactoperoxidase is not present in cancer cells, and therefore the presence of higher amounts of iodide in tumors is unimportant.[42] More recent studies show that elemental iodine induces a caspase-independent and mitochondrial-mediated apoptosis pathway.[132] These conflicting studies make the use of iodine in the population most at risk (perimenopausal women) problematic.

The iodine supplements used in these studies are no longer clinically available in the United States. Although there appear to be some elemental iodine products on the market, none has been studied. If iodine is to be used clinically, care must be taken to select the correct form of iodine and not overdose with iodides, which affect the thyroid or induce iodism. Women at increased risk of breast cancer may not be candidates for iodine (I^2) therapy until further studies are available to identify who benefits and who does not. Increased iodine intake via seaweed uncontaminated by I^{131} should, however, be safe and is supported by both the lower risk of breast cancer both pre- and

postmenopause in Asian women consuming seaweed as a source of iodine[42] as well as animal studies adding seaweed to the diet without negative effect on the thyroid.[133–135]

Herbal Supplements

Vitex Agnus-Castus (Chasteberry)

Chaste tree berry has been a very important plant for the treatment of PMS, including breast complaints and premenstrual dysphoric disorder. The evidence for the use of chaste tree berry in the treatment of mastalgia has been reported in both randomized and nonrandomized studies. A large open study (no control group) of 1634 women who experienced cyclic mastalgia as part of their PMS reported that 81% of participants rated it as a very good treatment for their mastalgia after 3 months of therapy.[136] In a prospective, multicenter trial using chaste tree in 50 patients with premenstrual cyclic mastalgia, 43 women were treated daily with chaste tree for three consecutive menstrual cycles. By the end of the study period, cyclic mastalgia decreased significantly, along with a smaller degree of improvement even 3 months after stopping the plant. Thirty-eight women rated the effectiveness as moderate to excellent, with five reporting no effect.[137] In a randomized controlled trial, 97 women with cyclic mastalgia showed a significant decrease in the intensity and duration of pain after one or two treatment cycles compared with placebo.[138] In a randomized trial of premenstrual dysphoric disorder, comparing chaste tree with a selective serotonin reuptake inhibitor (SSRI), 58% of patients being treated with chaste tree had an improvement in their cyclic mastalgia, and 68% of patients had improvements in their psychological symptoms.[139] In a randomized, placebo-controlled trial, 170 women were given chaste tree or placebo for three consecutive cycles. The improvement in breast pain was greater in the chaste tree group (52%) compared with the placebo group (24%).[140]

Vitex agnus castus is well tolerated. The most frequent adverse events are nausea, headache, gastrointestinal disturbances, menstrual disorders, acne, pruritus, and erythematous rash; however, all are mild and reversible.[141]

Chamomile *(Matricaria chamomilla)*

A small, randomized placebo-controlled study of chamomile tincture (5 gtts tid) in 60 patients showed a significant reduction of symptoms in women with cyclic mastalgia.[142] Chamomile has been reported to be both a galactogog[143] and to inhibit lactation.[98] The mode of action is unknown; however, *Matricaria chamomilla* has been shown to have antibacterial and antioxidant potential.[144] Chamomile is generally safe, although gastrointestinal side effects have been seen,[145] and allergic reactions and can occur, showing cross-reactivity with echinacea, feverfew, and milk thistle.[146]

Inositol, Betaine, Boswellia

A combination of B vitamins with myo-inositol 200 gm, *Boswellia serrata* 50 mg, and betaine 175 mg (Eumastos) was studied in 76 women in a 2016 randomized controlled study and showed statistically significant improvement in pain relief (56% vs. 17%), breast density reduction (60% vs. 9%), and diminution of size of mass (40% vs. 16%). There were no side effects.[147]

Curcumin

Curcumin, the yellow pigment in turmeric, has the advantage of having both anti-inflammatory and antioxidant properties.[148] It has been shown to have effects on radiation-induced fibrosis as well.[149,150]

Other Considerations

Liver Function

Because FCC may be related to an increased level of and sensitivity to estrogen and the liver is the primary site of estrogen clearance, any factor (e.g., cholestasis, alcohol intake, environmental pollution, or other hepatic compromise) that interferes with proper liver function may lead to an increased estrogen effect and worse symptoms. The previously discussed approaches to lower estrogens should be systematically addressed in these patients.

Colon Function

Breast disease has been linked to the Western diet and bowel function. There is an association between epithelial dysplasia in the nipple aspirates of breast fluid and the frequency of bowel movements. Women having fewer than three bowel movements per week have a risk of FCC that is 4.5 times greater than those having at least one a day.[151]

This association is probably due to reabsorption of deconjugated estrogens; the bacterial flora in the large intestine transforming endogenous and exogenous sterols and fatty acids into various toxic metabolites, and the effect on the gut microbiome.[152] Fecal microorganisms are capable of synthesizing estrone, estradiol, and 17-methoxyestradiol as well as metabolizing estrogen sulfate and glucuronate conjugates, resulting in the absorption of bacteria-derived estrogens and reabsorption of previously conjugated human estrogens. Diet plays a major role in determining colonic microflora, transit time, and the concentration of absorbable metabolites.[153]

Recent studies of the breast microbiome suggest that taking probiotics orally can alter the breast microbiome in beneficial ways and should be considered. (See earlier discussion of the microbiome.)

Women on vegetarian diets excrete two to three times more conjugated estrogens via the gut than those who are omnivorous, who also reabsorb more estrogens.[104] Omnivorous women have 50% higher mean levels of unconjugated estrogens. Supplementation with *L. acidophilus* has been shown to lower fecal β-glucuronidase.[154] Calcium-d-glucarate blocks β-glucuronidase, improving the excretion of estrogens conjugated with glucuronide. Both should be beneficial to women with FCC.

Pharmaceuticals

Pharmaceutical approaches to benign breast symptoms, including oral contraceptives, Tamoxifen, Danazol,[155] Centchroman (ormeloxifene),[156] and Goserelin (luteinizing hormone–releasing hormone [LHRH] analog), are all aimed at reducing estrogen effect. All have significant side effects. Bromocriptine and Lisuride maleate are dopamine agonists aimed at blocking the release of prolactin. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used by mouth and topically for breast pain.[157] Their anti-inflammatory effects both locally as a cyclooxygenase-2 (COX-2) inhibitor and as an aromatase inhibitor have been reported. Although the reports on some drugs (e.g., Tamoxifen) indicating effectiveness in preventing breast cancer are encouraging, little is reported on reducing cysts or fibrosis.

NONPHARMACOLOGIC THERAPEUTIC APPROACH

Because the origins of FCC are likely related to the causes of fibrosis (inflammation, autoimmune issues, and genetics) in relationship to the environment (breast microbiome, radiation, and trauma) and stimulated by estrogen excess (estrogen/progesterone imbalance, genetics, xenoestrogens), treatment should be aimed at early avoidance of inflammation and improved gut function and ongoing treatments that lower and balance estrogen levels. The use of these approaches is likely to decrease both symptoms problematic to the patient and diagnostic dilemmas problematic to the practitioner.

Some women have noncyclic FCC, and others have cyclic premenstrual breast tenderness and lumpiness, so the therapeutic approach outlined in Chapter 212 may be more appropriate for individual needs.

The therapy recommended here also includes some of the key factors discussed in Chapter 212.

Diet

Dietary changes should be aimed at avoiding the development of obesity[158] and insulin resistance (a known cause of inflammation), improving the microbiome of the gut and the breast, and improving disturbed steroid metabolism. Avoidance of sugar, caffeine, and alcohol at least during the luteal phase of the cycle is helpful. Avoiding gluten in the diet as an inflammagen can be beneficial for some. Avoiding dairy, which contains both high levels of estrogens from the pregnant cows included among the milk suppliers and high level of toxins from the environment, can be useful. The diet should be primarily vegetarian (replete with aromatase inhibitors and phytoestrogens from vegetables, with large amounts of dietary fiber to encourage bowel regularity. All methylxanthines should be eliminated until symptoms are relieved. At that point, methylxanthines can be reintroduced in small amounts because they are tolerated by many patients.

Reducing the saturated fat content of the diet while increasing the consumption of complex carbohydrates has been shown to reduce the severity of premenstrual breast tenderness and swelling as well as the nodularity in some women in one very old study.[159] Reducing the dietary fat intake to 20% of total calories results in significant decreases in circulating estrogens in women with benign breast disease in one study,[160] but other studies have not shown an effect of a diet of increased vegetables and fruit, low fat, and higher grains on benign proliferative breast disease[161] or when breast cancer was the end point.[162] Recommendations on fat intake should emphasize the quality and origins of the fat rather than the total amount, especially in prediabetic women who will likely substitute fat with higher sugar and carbs, to their detriment. Emphasis should be placed on increased vegetable rather than fruit intake.

The diet should emphasize organic, whole, unprocessed foods (whole grains, legumes, vegetables, fruits, nuts, and seeds) to limit food contaminated with herbicides, such as glyphosate, and insecticides, which are xenoestrogens. Patients should be encouraged to drink at least 48 oz of water daily to facilitate the excretion of estrogens and xenoestrogens.

Exogenous estrogens (hormone replacement therapy and environmental toxins)[163,164] should be avoided if possible, as they may exacerbate the condition (hormone replacement therapy[163] and environmental toxins[164]). Birth control pills have been used to lower the ovarian output of estrogen and are helpful in some once the increased estrogen period (first 1–3 months) is past.[165] However, the effect on the breast of the progestins in the pill is likely negative.

Nutritional Supplements

- B vitamins containing 5-methylfolate and B_{12} (preferably in a form not containing cyanide). Doses should be individualized.
- Lipotropic factors: choline 1000 mg and methionine 1000 mg
- Betaine: 175 mg bid
- NAC: 1000 mg/day as an antioxidant
- Indole-3-carbinol (I3C) or Di-indoylmethane (DIM), provided 2OH estrogen metabolites are not already high
- Calcium d-glucarate: 200 to 500 mg bid
- Vitamin E: 400 to 800 IU/day of d-alpha-tocopherol (take care to avoid synthetic dL forms)
- Beta-carotene: 50,000 to 300,000 IU/day
- Iodine (aqueous molecular iodine): 0.07 to 0.09 mg/kg (a prescription item) or Lugol's iodine (95% iodide and 5% iodine 5–10 gtts/d; iodine caseinate [iodized casein I-] 10 mg/d; see earlier precautions)
- Evening primrose oil: 1500 mg twice a day in combination with omega-3 fatty acids
- Omega-3 fatty acids 1000 mg/day of eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)
- Probiotics containing a mixture of lactobacillius species with guaranteed live organisms at the date of expiration
- Magnesium: to bowel tolerance for constipation and PMS symptoms other than breast
- Fiber as organic ground flax seeds: 1 to 2 tsp/day

Botanical Medicines

- Vitex (chaste berry): The usual dosage of chaste berry extract (often standardized to contain 0.5% agnuside) in tablet or capsule form is 175 to 225 mg per day. If using the liquid extract, the typical dosage is 2 to 4 mL (½–1 tsp) a day.
- *Boswellia serrata* (resin) standardized to 60%; boswellic acid: 200 mg/day
- Chamomile as tea or extracted
- Curcumin as a spice added to food or a bioavailable supplement (200–600 mg/day)

Mechanical

Breast self-massage can be amazingly helpful in women with painful breasts. A lymphatic massage technique of *gentle* manipulation of the breast from the areola outward and sweeping up into the armpit followed by a circular motion with hands on either side of the breast, usually in the prone position, relieves the congestion of the breast due to hormonal imbalance.[166] Practitioners of thermography have found that this also decreases some of the signs of inflammation and congestion in the tail of the breast and the underarm area (personal communication). Herbalist Susan Weed recommends using essential oils and combining massage with breast self-examination. (http://www.susunweed.com/herbal_ezine/April09/breasthealth.htm). Avoid using progesterone or other hormone creams directly on the breast.

SURGICAL INTERVENTION

Treatment of asymptomatic, fluid-filled cysts with aspiration is no longer recommended.[167] Surgical treatment should be reserved for suspicious dominant masses and used liberally in this situation to avoid failure to diagnose breast cancers. Surgical treatment of fibrocystic breasts with subcutaneous mastectomy should be a last resort and used only when pain is severe and the breast tissue has been almost completely replaced with fibrosis.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Ghent WR, Skin BA, et al. Iodine replacement in fibrocystic disease of the breast. *Can J Surg*. 1993;36(6):453–460.
2. Zhang F, Swanson SM, van Breemen RB, et al. Equine estrogen metabolite 4-hydroxyequilenin induces DNA damage in the rat mammary tissues: formation of single-strand breaks, apurinic sites, stable adducts, and oxidized bases. *Chem Res Toxicol*. 2001;14(12):1654–1659.
3. Physician Insurers Association of America. *Breast Cancer Study*. 3rd ed. Rockville, MD: Physician Insurers Association of America, Spring; 2002 [Google Scholar].
4. Navarro Silvera SA, Rohan TE. Benign proliferative epithelial disorders of the breast: a review of the epidemiologic evidence. *Breast Cancer Res Treat*. 2008;110:397–409.
5. Minami Y, Ohuchi N, et al. Risk factors for benign breast disease according to Histopathological type: comparisons with risk factors for breast cancer. *Jpn J Cancer Res*. 1998;89:116–123.
6. Tice JA, O'Meara ES, et al. Benign breast disease, mammographic breast density and breast cancer. *J Natl Cancer Inst*. 2013;104(4):1043–1049.
7. Breast masses (breast lumps). Merck Manual: Professional Version. https://www.merckmanuals.com/professional/gynecology-and-obstetrics/breast-disorders/breast-masses-breast-lumps. Accessed 7/29/18.
8. Fibrocystic breasts. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/fibrocystic-breasts/symptoms-causes/syc-20350438. Accessed 7/29/18.
9. Zendehdel M, Niakan B, Keshtkar A, Rafiei E, Salamat F. Subtypes of benign breast disease as a risk factor for breast cancer: a systematic review and meta-analysis protocol. *Iran J Med Sci*. 2018;43(1):1–8.
10. Dyrstad SW, Yan Y, Fowler AM, Colditz GA. Breast cancer risk associated with benign breast disease: systematic review and meta-analysis. *Breast Cancer Res Treat*. 2015;149(3):569–575.
11. Reilkoff RA, Bucala R, et al. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol*. 2011;11(6):427–435.
12. Kumar S, Rai R, Das V, Kumar S, Dwivedi V, Agrawal GG. Visual analogue scale for assessing breast nodularity in non-discrete lumpy breasts: the Lucknow-Cardiff breast nodularity scale. *Breast*. 2010;19(3):238–242.
13. Sarnelli R. Squartini F Fibrocystic condition and 'at risk' lesions in asymptomatic breasts: a morphologic study of postmenopausal women. *Clin Exp Obstetr Gynecol*. 1991;18(4):271–279.
14. Moles L, Manzano S, et al. Bacteriological, biochemical, and immunological properties of colostrum and mature milk from mothers of extremely preterm infants. *J Pediatr Gastroenterol Nutr*. 2015;60(1):120–126.
15. Pihan GA, Doxsey SJ. The mitotic machinery as a source of genetic instability in cancer. *Semin Cancer Biol*. 1999;9:289–302.
16. Hu M, Polyak K. Microenvironmental regulation of cancer development. *Curr Opin Genet Dev*. 2008;18:27–34.
17. Pischinger A. *The Extracellular Matrix and Ground Regulation-Basis for a Holistic Biological Medicine*. Berkley, CA: North Atlantic Books; 2007.
18. Seo BR, Bhardwaj P, et al. Obesity-dependent changes in interstitial ECM mechanics promote breast tumorigenesis. *Sci Tranl Med*. 2015;7(301):301ra130.
19. Bissel MJ. Hall HG how does the extracellular matrix direct gene expression? *J Theor Biol*. 1982;99:31–68.
20. Soysal SD1, Tzankov A, Muenst SE. Role of the tumor microenvironment in breast cancer. *Pathobiology*. 2015;82(3–4):142–152. https://doi.org/10.1159/000430499. Epub 2015 Aug 31.
21. Egblad M, Rasch MG et al. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr Opin Cell Biol*. Author manuscript; available in PMC 2011 Oct 1. Published in final edited form as: *Curr Opin Cell Biol*. 2010;22(5):697–706. Published online 2010 Sep 6.
22. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol*. 2008;214(2):199–210.
23. Apocrine. Wikipedia. https://en.wikipedia.org/wiki/Apocrine. Accessed 9/20/18.
24. Wellings SR, Alpers CE. Apocrine cystic metaplasia: subgroups pathology and prevalence in cancer-associated versus random autopsy breasts. *Hum Path*. 1987;18:381–386.
25. Celis JE, Gromov P. Apocrine Cysts of the Breast-Biomarkers, origin, enlargement and relation with cancer. *Molecul Cellular Proteomics*. 2006;5:462–483.
26. Angeli A, Dogliotti L, et al. Mammary cysts: pathophysiology and biochemistry international journal of radiation applications and instrumentation. Part B. *Nucl Med Biol*. 1987;14(4):397–406.
27. Angeli A, Naldoni C, et al. Biochemistry and Categorization of breast cyst fluid: relation to breast cancer risk. *J Steroid Biochem Molec Biol*. 1994;49(4–6):333–339.
28. Celis JE, Gromov P, et al. Apocrine cysts of the breasts: biomarkers, origin, enlargement and with cancer phenotype. *Molecul Cellular Proteomics*. 2006;5:462–483.
29. Dixon JM, Scott WN, et al. Natural history of cystic disease. *Br J Surg*. 1985;72:190–192.
30. Dixon JM, Miller WR, et al. The morphological basis of human breast cyst populations. *Br J Surg*. 1983;70:604–606.
31. Urbaniak C, Gloor GB, et al. The microbiota of breast tissue and its association with breast cancer. *Appl Environ Microbiol*. 2016;82(16):5039–5048. https://doi.org/10.1128/AEM.01235-16. Print 2016 Aug 15.
32. Hieken TJ, Chen J, Hoskin TL, et al. The microbiome of aseptically collected human breast tissue in benign and malignant disease. *Sci Rep*. 2016;6:30751. https://doi.org/10.1038/srep30751.
33. Meng S, Chen B, Yang J, et al. Study of microbiomes in aseptically collected samples of human breast tissue using needle biopsy and the potential role of in situ tissue microbiomes for promoting malignancy. *Front Oncol*. 2018;8:318. https://doi.org/10.3389/fonc.2018.00318. eCollection 2018.
34. Kast RE. Some fibrocystic breast change may be caused by sexually transmitted *H. pylori* during oral nipple contact: supporting literature and case report of resolution after gut H. pylori eradication treatment. *Medical Hypotheses*. 2007;68:1041–1046.
35. Yazdi HR, Movafagh A et al. Evaluation of Methylobacterium radiotolerance and *Sphyngomonas yanoikoaie* in Sentinel Lymph Nodes of Breast Cancer Cases. *Asian Pac J Cancer Prev*. 17(Cancer Control in Western Asia Special Issue):279–285.
36. Fernandez L, Langa S, et al. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res. Mar*. 2013;69(1):1–10.
37. Jiménez E, Fernández L, Maldonado A, et al. Oral administration of lactobacilli strains isolated from breast milk as an alternative for the treatment of infectious mastitis during lactation. *Appl Env Microbiol*. 2008;74:4650–4655.
38. Arroyo R, Martín V, Maldonado A, Jiménez E, Fernández L, Rodríguez JM. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk. *Clin Infect Dis*. 2010;50:1551–1558.
39. Cárdenas N, Arroyo R, Manzano S, Jiménez E, Martín V, Rodríguez JM. Prevention of infectious mastitis by oral administration of *Lactobacillus salivarius* PS2 during late pregnancy. *Clin Infect Dis*. 2016;62:568–573.
40. Ming J, Meng G, et al. Clinical characteristics and surgical modality of plasma cell mastitis: analysis of 91 cases. *Am Surg*. 2013;79(1):54–60.
41. Dixon JM, Ravisekar O, Chetty U, et al. Periductal mastitis and duct ectasia: different conditions with different aetiologies. *Br J Surg*. 1996;83:820–822.
42. Dvorak HF. Tumors: wounds that do not heal—Redux. *Cancer Immunol Res*. 2015;3(1):1–11.
43. Aceves C, Anguiano B, et al. Is iodine a Gatekeeper of the Integrity of the mammary gland? *J Mammary Gland Biol Neoplasia*. 2005;10(2):189–196.
44. Martinez-Costa C, Silvestre MD, et al. Effects of refrigeration on the bactericidal activity of human milk. *J Pediatr Gastroenterol Nutr*. 2007;45(2):275–277.
45. Xuan C, Shamonki J, et al. Microbial dysbiosis is associated with human breast cancer. *PLoS One*. 2014;9(1):e8374.
46. Lisanti MP, Tsirgos A, et al. JNK1 stress signaling is hyper-activated in high breast density and the tumor stroma-connecting fibrosis, inflammation, and stemness for cancer prevention. *Cell Cycle*. 2014;13(4):580–599.
47. Thalabard JC, Sitruk-Ware R, Kuttenn F, Mauvais-Jarvis P. Endocrine markers in benign breast diseases. *Zentralbl Gynakol*. 1986;108(6):354–358.

48. Mauvais-Jarvis P, Sitruk-Ware R, Kutten F, Sterkers N. Luteal phase insufficiency: a common pathophysiological factor in development of benign and malignant breast disease. In: Bulbrook RD, Taylor DJ, eds. *Commentaries on Research in Breast Disease*. New York: Alan R. Liss; 1979:25–29.
49. Gumus II , Koktener A, Dogan D, Turhan NO. Polycystic ovary syndrome and fibrocystic breast disease: is there any association? *Arch Gynecol Obstet*. 2009;280:249–253.
50. D'Amelio R, Farris M, Grande S, Feraudo E, Iuliano A, Zichella L. Association between polycystic ovary and Fibrobrocystic breast disease. *Gynecol Obstet Invest*. 2001;51:134–137. https://doi.org/10.1159/000052909.
51. Van Herwaarden AE, Wagenaar E, et al. Breast cancer resistance protein (Bcrp1/Abcg2) reduces systemic exposure of the dietary carcinogens aflatoxin B1, IQ and Trp-P-1 but also mediates their secretion into breast milk. *Carcinogenesis*. 2006;27(1):123–130. Epub 2005 Jul 6.
52. Dogliotti L, Orlandi F, et al. The endocrine basis of benign breast disorders. *World J Surg*. 1989;13:674–679.
53. Dogliotti L, Faggiuolo R, Ferusso A, et al. Prolactin and thyrotropin response to thyrotropin releasing hormone in premenopausal women with fibrocystic disease of the breast. *Hormone Res*. 1985;21:137.
54. Watson CS, Ku G, et al. Rapid actions of xenoestrogens disrupt normal estrogenic signaling. *Steroids*. 2014;81:36–42.
55. Vinas R, Watson CS. Bisphenol s disrupts estradiol-induced nongenomic signaling in a rat pituitary cell line: effects on cell functions. *Environ-Health Perspect*. 2013;121:352–358.
56. Thompson RC, Moore CJ, vom Saal FS, Swan SH. Plastics, the environment and human health: current consensus and future trends. *Philos Trans R Soc Lond B Biol Sci*. 2009;364:2153–2166.
57. Mesnage R, Séralini GE. Editorial: Toxicity of pesticides on health and environment. *Front Public Health*. 2018;6:268. https://doi.org/10.3389/fpubh.2018.00268. eCollection 2018.
58. Sapouckey SA, Kassotis DC, et al. Prenatal exposure to unconventional oil and gas operation chemical mixtures altered mammary gland development in adult female mice. *Endocrinology*. 2018;159(3):1277–1289. https://doi.org/10.1210/en.2017-00866.
59. Taylor KW, Troester MA, Herring AH, et al. Associations between personal care product use patterns and breast cancer risk among white and black women in the sister study. *Environ Health Perspect*. 2018;126(2):027011. https://doi.org/10.1289/EHP1480.
60. Kortenkamp A. Low dose mixture effects of endocrine disruptors: implications for risk assessment and epidemiology. *Int J Androl*. 31, 233–240.
61. Darbre PD. Aluminium and the human breast. *Morphologie*. 2016;100(329):65–74.
62. Gaudet HM, Christensen E, et al. Methylmercury promotes breast cancer cell proliferation. *Toxicol Rep*. 2018;5:579–584.
63. Wallace DR. Nanotoxicology and metalloestrogens: possible involvement in breast cancer. *Toxics*. 2015;3(4):390–413.
64. Parodi DA, Greenfield M, et al. Alteration of the mammary gland development and gene expression y in utero exposure to arsenic. *Reprod Toxicol*. 2015;54:66–75.
65. Byrne C, Divekar SD, et al. Metals and breast cancer. *J Mammary Gland Biol Neoplasia*. 2013;18(1):64–73.
66. Guray M, Sahin AA. Benign breast diseases: classification, diagnosis, and management. *Oncologist*. 2006;11:435–449.
67. Plu-Bureau G1, Lê MG, SitrukWareR TJC. Cyclical mastalgia and breast cancer risk: results of a French cohort study. *Cancer Epidemiol Biomarkers Prev*. 2006;15(6):1229–1231.
68. Onstad M, Stuckey A. Benign breast disorders. *Obstet Gynecol Clin N Am*. 2013;40:459–473. https://doi.org/10.1016/j.ogc.2013.05.004.
69. Knuttel FM, Menezes GL, van Diest PJ, Witkamp AJ, van den Bosch MA, Verkooijen HM. Meta-analysis of the concordance of histological grade of breast cancer between core needle biopsy and surgical excision specimen. *Br J Surg*. 2016;103(6):644–655. Epub 2016 Mar 15.
70. Hindi-Alexander MC, Zielezny MA, Montes N, et al. Theophylline and fibrocystic breast disease. *J Allergy Clin Immunol*. 1985;75(6):709–715.
71. Horner NK, Lampe JW. Potential mechanisms of diet therapy for fibrocystic breast conditions show inadequate evidence of effectiveness. *J Am Diet Assoc*. 2000;100(11):1368–1380.
72. Li W, Ray RM, et al. Dietary and other risk factors in women having fibrocystic breast conditions with and without concurrent breast cancer: a nested case-control study in Shanghai, China. *Int J Cancer*. 2005;115(6):981–993.
73. Shively CA, Register TC et al. *Consumption of Mediterranean versus Western Diet Leads to Distinct Mammary Gland Microbiome Populations*. https://doi.org:10.1016/j.celrep.2018.08.078.
74. Seo BR, Bhardwaj P, et al. Obesity-dependent changes in interstitial ECM mechanics promote breast tumorigenesis. *Sci Transl Med*. 2015;7(301):301ra130. https://doi.org/10.1126/scitranslmed.3010467.
75. Boyle CA, Berkowitz GS, LiVolsi VA, et al. Caffeine consumption and fibrocystic breast disease: a case-control epidemiologic study. *J Natl Cancer Inst*. 1984;72:1015–1019.
76. La Vecchia C, Franceschi S, Parazzini F, et al. Benign breast disease and consumption of beverages containing methylxanthines. *J Natl Cancer Inst*. 1985;74(5):995–1000.
77. B1 B, Hindi-Alexander M, Fetouh S. Methylxanthines and fibrocystic breast disease: a study of correlations. *Nurse Pract*. 1990;15(3):36–38. 43-44.
78. Minton JP, Abou-Issa H, Reiches N, et al. Clinical and biochemical studies on methylxanthine-related fibrocystic breast disease. *Surgery*. 1981;90:299–304.
79. Minton JP, Foecking MK, Webster DJT, et al. Caffeine, cyclic nucleotides, and breast disease. *Surgery*. 1979;86:105–109.
80. Ernster VL, Mason L, Goodson III WH, et al. Effects of caffeine-free diet on benign breast disease: a random trial. *Surgery*. 1982;91:263–267.
81. Russell LC1. Caffeine restriction as initial treatment for breast pain. *Nurse Pract*. 1989;14(2):36–37, 40.
82. Literature search by author, Snedeker SM1, Diaugustine RP. Hormonal and environmental factors affecting cell proliferation and neoplasia in the mammary gland. *Prog Clin Biol Res*. 1996;394:211–253.
83. Minton JP, Foecking MK, Webster DJT, Mathews RI-I. Response of fibrocystic breast disease to caffeine withdrawal and correlation of cyclic nucleotides with breast disease. *Am J Obstet Gynecol*. 1979;135:157.
84. Minton JP, Abou-Issa H, Reiches N, et al. Clinical and biochemical studies on methylxanthine-related fibrocystic breast disease. *Surgery*. 1981;90:299–304.
85. Lubin F, Ron E, Wax Y, et al. A case-control study of caffeine and methylxanthine in benign breast disease. *JAMA*. 1985;253:2388–2392.
86. Shairer C, Brinton LA, Hoover RN. Methylxanthine and benign breast disease. *Am J Epidemiol*. 1986;124:603–611.
87. Marshall J, Graham S, Swanson M. Caffeine consumption and benign breast disease: a case-control comparison. *Am J Public Health*. 1982;72:610–612.
88. Baghurst PA, Rohan TE. Dietary fiber and risk of benign proliferative epithelial disorders of the breast. *Int J Cancer*. 1995;63:481–485.
89. Farvid MS, Eliassen AH, et al. Dietary fiber intake in young adults and breast cancer risk. *Pediatrics*. 2016;137(3). http://pediatrics.aappublications.org/content/pediatrics/early/2016/01/28/peds.2015-1226.full.pdf. Accessed 10/30/19.
90. Setchell KDR, Clerici C, et al. S-Equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr*. 2005;81(5):1072–1079.
91. Fleming RM. What effect, if any, does soy protein have on breast tissue? *Integr Cancer Ther*. 2003;2:225–228.
92. Lampe JW, Nahino Y et al. *Plasma isoflavones and fibrocystic breast conditions and breast cancer among women in Shanghai, China.*
93. Lubin F, Wax Y, et al. Nutritional factors associated with benign breast disease etiology: a case-control study. *Am J Clin Nutr*. 1989;50(3):551–556.
94. Gateley CA, Maddox PR, et al. Plasma fatty acid profiles in benign breast disorders. *Br J Surg*. 1992;79(5):407–409.

95. Mirhashemi SM1, Sahmani M1, Salehi B2, et al. Metabolic response to omega-3 fatty acids and vitamin E Co-supplementation in patients with fibrocystic breast disease: a randomized, double-blind, placebo-controlled trial. *Arch Iran Med*. 2017;20(8):466–473.
96. Shannon J, King IB, et al. Erythrocyte fatty acids and risk of proliferative and nonproliferative fibrocystic disease in women in Shanghai, China. *Am J Clin Nutr*. 2009;89(1):265–276.
97. Murshid KR. A review of mastalgia in patients with fibrocystic breast changes and the non-surgical treatment options. *J Taibah Univ Med Sci*. 2011;6(1):1–18.
98. Blommers J, de Lange-De Klerk E, Kuik D, et al. Evening primrose oil controlled trial. *Am J Obstet Gynecol*. 2002;187(5):1389–1394.
99. Pye J, Mansel RE, Hughes LE. Clinical experience of drug treatment for mastalgia. *Lancet*. 1985;2:373–377.
100. Pashby N, Mansel RE, Hughes LE, et al. A clinical trial of evening primrose oil in mastalgia. *Br J Surg*. 1981;68:801–824.
101. Gately C, Miers M, Mansel R, et al. Drug treatments for mastalgia: 17 years experience in the cardiff mastalgia clinic. *J R Soc Med*. 1992;85(1):12–15.
102. Pruthi S, Wahner-Roedler DL, et al. Vitamin E and evening primrose oil for management of cyclical mastalgia: a randomized pilot study. *Altern Med Rev*. 2010;15(1):59–67.
103. Srivastava A, Mansel RE, Arvinda N. Evidence-based management of mastalgia: a meta-analysis of randomized trials. *Breast*. 2007;16(5):503–512. https://doi.org/10.1016/j.breast.2007.03.003.
104. Mansel R, Harrison B, Melhuish J, et al. A randomized trial of dietary intervention with essential fatty acids in patients with categorized cysts. *Ann NY Acad Sci*. 1990;586:288–294.
105. Kelley DS, Bendich A. Essential nutrients and immunologic functions. *Am J Clin Nutr*. 1996;63:994S–996S.
106. London RS, Sundaram GS, Schultz M, et al. Endocrine parameters and alpha-tocopherol therapy of patients with mammary dysplasia. *Cancer Res*. 1981;41:3811–3813.
107. London RS, Sundaram G, Manimekalai S, et al. The effect of alpha-tocopherol on premenstrual symptomatology: a double-blind study. II. Endocrine correlates. *J Am Coll Nutr*. 1984;3:351–356.
108. London R, Sundaram G, Manimekalai S, et al. Mammary dysplasia: endocrine parameters and tocopherol therapy. *Nutr Res*. 1982;7:243.
109. Myer EC, Sommers DK, Reitz CJ, et al. Vitamin E and benign breast disease. *Surgery*. 1990;107:549–551.
110. London RS, Sundaram GS, Murphy L, et al. The effect of vitamin E on mammary dysplasia: a double-blind study. *Obstet Gynecol*. 1985;65:104–106.
111. Bespalov V, Barash N, Ivanova O, et al. Study of an antioxidant dietary supplement Karinat in patients with benign breast disease. *Vopr Onkol*. 2004;50(4):467–472.
112. Parsay S, Olfati F, Nahidi S. Therapeutic effects of vitamin E on cyclic mastalgia. *Breast J*. 2009;15(5):510–514.
113. Shobeiri F, Oshvandi K, Nazari M. Clinical effectiveness of vitamin E and vitamin B6 for improving pain severity in cyclic mastalgia. *Iran J Nurs Midwifery Res*. 2015;20(6):723–727.
114. Zhang SM. Role of vitamins in the risk, prevention and treatment of breast cancer. *Curr Opin Obstetr Gynecol*. 2004;16(1):19–25.
115. Blomhoff R. Introduction: overview of vitamin A metabolism and function. In: Blumhoff R, ed. *Vitamin A in Health and Disease*. New York: Marcel Dekker, Inc; 1994:1–35.
116. Frei B. Reactive oxygen species and antioxidant vitamins : mechanisms of action. *Am J Med*. 1994;97(suppl 3A):5S–13S.
117. Band PR, Deschamps M, Falardeau M, et al. Treatment of benign breast disease with vitamin A. *Prev Med*. 1984;13:549–554.
118. Strum JM. Site of iodination in rat mammary gland. *Anal Rec*. 1978;192(2):235–244.
119. Eskin BA, Bartushka DG, Dunn MR, et al. Mammary gland dysplasia in iodine deficiency. *JAMA*. 1967;200:691–695.
120. Eskin BA, Shuman R, Krouse T, Merion JA. Rat mammary gland atypia produced by iodine blockade with perchlorate. *Cancer Res*. 1975;35:2332–2339.
121. Anil C, Guney T, et al. The prevalence of benign breast diseases in patients with nodular goiter and Hashimoto's thyroiditis. *J Endocrinol Invest*. 2015;38(9):971–975. https://doi.org/10.1007/s40618-015-0269-8. Epub 2015 Apr 1.
122. Daro AF, Gollin HA, Samos FH. The effect of thyroid on cystic mastitis. *J Int Coll Surg*. 1964;41:58–59.
123. Peters F, Pickardt CR, et al. Thyroid hormones in benign breast disease. Normalization of exaggerated responsiveness to thyrotropin-releasing hormone. *Cancer*. 1985;56(5):1082–1085.
124. Pruitt KM, Reiter B. Biochemistry of peroxidase systems: antimicrobial effects.. In: Tenovuo JO, Pruitt KM, eds. *The Lactoperoxidase System: Chemistry and Biological Significance*. New York: Dekker; 1985:272. ISBN 0-8247-7298-9.
125. Soriano O, Delgado G, et al. Antineoplastic effect of iodine and iodide in methylbenz[a]anthracene-induced mammary tumors: association between lactoperoxidase and estrogen-adduct production. *Endocr Relat Cancer*. 2011;18(4):529–539. https://doi.org/10.1530/ERC-11-0065. Print 2011 Aug.
126. Garcia-Solis P, Alfaro Y, et al. Inhibition of N-methyl-N-nitrosourea-induced mammary carcinogenesis by molecular iodine (I2) but not by iodide (I−) treatment: evidence that I2 prevents cancer promotion. *Molecul Cellular Endocrinology*. 2005;231:49–57.
127. Haagensen CD. *Disease of the Breast*. Philadelphia, PA: WB Saunders; 1971:62.
128. Kessler JH. The effect of supraphysiologic levels of iodine on patients with cyclic mastalgia. *Breast J*. 2004;10(4):328–336.
129. Rosner H, Moller W, et al. Antiproliferative/cytotoxic effects of molecular iodine, povidone-iodine and Lugol's solution in different human carcinoma cell lines. *Oncol Lett*. 2016;12(3):2159–2162. Epub 2015 Jul 5.
130. He S, Wang B, et al. Iodine stimulates estrogen receptor signaling and its systemic level is increased in surgical patients duet o topical absorption. *Oncotarget*. 2017;9(1):375–384.
131. Hanson AA, Rogan EG, Cavalieri EL. Synthesis of adducts formed by iodine oxidation of aromatic hydrocarbons in the presence of deoxyribonucleosides and nucleobases. *Chem Res Toxicol*. 1998;11(10):120–128.
132. Shrivastava A, Tiwari M, et al. Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving the mitochondria-mediated pathway. *J Biol Chem*. 2006;281(28):19762–19771. Epub 2006 May 5.
133. Teas J, Harbison ML, Gelman RS. Dietary seaweed *(Laminaria)* and mammary carcinogenesis in rats. *Cancer Res*. 1984;44:2758–2761.
134. Yamamoto I, Maruyama H, Moriguchi M. The effect of dietary seaweeds on 7,12-dimethyl-benz[a]anthracene-induced mammary tumorigenesis in rats. *Cancer Lett*. 1987;35:109–118.
135. Funahashi H, Imai T, Tanaka Y, et al. Wakame seaweed suppresses the proliferation of 7,12-dimethylbenz(a)-anthracene-induced mammary tumors in rats. *Jpn J Cancer Res*. 1999;90:922–927.
136. Loch E, Selle H, Boblitz N. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *Vitex agnus castus*. *J Womens Health Gend Based Med*. 2000;9:315–320.
137. Berger D, Schaffner W, Schrader E, et al. Efficacy of *Vitex agnus castus* L. extract Ze 440 in patients with pre-menstrual syndrome. *Arch Gynecol Obstet*. 2000;264:150–153.
138. Halaska M, Beles P, Gorkow C, et al. Treatment of cyclical mastalgia with a solution containing a *Vitex agnus castus* extract: results of a placebo-controlled double-blind study. *Breast*. 1999;8(4):175–181.
139. Atmaca M, Kumru S, Tezcan E. Fluoxetine versus *Vitex agnus castus* extract in the treatment of premenstrual dysphoric disorder. *Hum Psychopharmacol*. 2003;18:191–195.
140. Schellenberg R. Treatment for the premenstrual syndrome with agnus castus fruit extract: prospective, randomised, placebo controlled study. *Br Med J*. 2001;322:134–137.
141. Chasteberry. *Drugs and Lactation Database (LactMed) [Internet]*. Bethesda (MD): National Library of Medicine (US); 2006. PMID: 30000866.
142. Saghafi N, Rhkhshandeh H, et al. Effectiveness of Matricaria chamomilla (chamomile) extract on pain control of cyclic mastalgia: a double-blind randomised controlled trial. *J Obstet Gynaecol*. 2018;38(1):81–84. https://doi.org/10.1080/01443615.2017.1322045. Epub 2017 Oct 26.
143. Silva FV, Dias F, Costa G, Campos MDG. Chamomile reveals to be a potent galactogogue: the unexpected effect. *J Matern Fetal Neonatal Med*.

2018;31(1):116–118. https://doi.org/10.1080/14767058.2016.1274300. Epub 2017 Apr 10. PMID: 28000519.
144. Munir N, Iqbal AS, et al. Evaluation of antioxidant and antimicrobial potential of two endangered plant species Atropa belladonna and *Matricaria chamomilla*. *Afr J Tradit Complement Altern Med*. 2014;11(5):111–117. eCollection 2014.
145. Maleki-Saghooni N, Karimi FZ, et al. The effectiveness and safety of Iranian herbal medicines for treatment of premenstrual syndrome: a systematic review. *Avicenna J Phtomed*. 2018;8(2):96–113.
146. Chamomile. *Drugs and Lactation Database (LactMed) [Internet]*. Bethesda (MD): National Library of Medicine (US); 2006. PMID: 30000867.
147. Pasta V, Gullo G, Giuliani A, et al. An association of boswellia, betaine and myo-inositol (Eumastós) in the treatment of mammographic breast density: a randomized, double-blind study. *Eur Rev Med Pharmacol Sci*. 2015;19(22):4419–4426 (ISSN: 2284-0729).
148. Ammon HP, Wahl MA. Pharmacology of curcuma longa. *Planta Med*. 1991;57(1):1–7.
149. Lee JC, Kinniry PA, Arguiri E, et al. Dietary curcumin increases antioxidant defenses in lung, ameliorates radiation-induced pulmonary fibrosis, and improves survival in mice. *Radiat Res*. 2010;173(5):590–601.
150. Cho YJ, Yi CO, et al. Curcumin attenuates radiation-induced inflammation and fibrosis in rat lungs. *Korean J Physiol Pharmacol*. 2013;17(4):267–274.
151. Petrakis NL, King EB. Cytological abnormalities in nipple aspirates of breast fluid from women with severe constipation. *Lancet*. 1981;2:1203–1204.
152. Hentges DJ. Does diet influence human fecal microflora composition? *Nutr Rev*. 1980;38:329–336.
153. Goldin B, Aldercreutz H, Dwyer JT, et al. Effect of diet on excretion of estrogens in pre- and post-menopausal women. *Cancer Res*. 1981;41:3771–3773.
154. Goldin B, Gorback S. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. *Am J Clin Nutr*. 1984;39:756–761.
155. von Fallois J, Etterli-Billenkamp U, Schindler EM, Schindler AE. Danazol for treatment of fibrocystic mastopathy. *Zentralbl Gynakol*. 1995;117:457–465.
156. Rajswaroob U, Kannan R, et al. Effectiveness of centchroman on regression of fibroadenosis and mastalgia. *J Clin Diagn Res*. 2016;10(10):PC10–PC14.
157. Colak T, Ipek T, et al. Efficacy of topical nonsteroidal antiinflammatory drugs in mastalgia treatment. *J Am Coll Surg*. 2003;196(4):525–530.
158. Chen YY, Fang WH, Wang CC, et al. Examining the associations among fibrocystic breast change, total lean mass, and percent body fat. *Sci Rep*. 2018;8(1):9180. https://doi.org/10.1038/s41598-018-27546-3.
159. Boyd NF, McGuire V, Shannon P, et al. Effect of a low fat high-carbohydrate diet on symptoms of cyclical mastopathy. *Lancet*. 1988;2:128–132.
160. Rose DP, Boyar AP, Cohen C, et al. Effect of a low-fat diet on hormone levels in women with cystic breast disease. I. Serum steroids and gonadotropins. *J Natl Cancer Inst*. 1987;78:623–626.
161. Rohan TE, Negassa A, et al. Low-fat dietary pattern and risk of benign proliferative breast disease: a randomized, controlled dietary modification trial. *Cancer Prev Res (Phila)*. 2008;1(4):275–284.
162. Prentice RL, caan B, et al. Low-fat dietary pattern and risk of invasive breast cancer: the Women's health initiative randomized controlled dietary modification trial. *JAMA*. 2006;295(6):6299–6242.
163. Goehring C, Morabia A. Epidemiology of benign breast disease, with special attention to histologic types. *Epidemiol Rev*. 1997;19:310–327.
164. Gray JM. Rasanayagam S State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health*. 2017;16:94.
165. Rohan TE, Miller AB. A cohort study of oral contraceptive use and risk of benign breast disease. *Int J Cancer*. 1999;82(2):191–196.
166. Pelton R, Pelton T, Vint VC. *How to Prevent Breast Cancer*. New York, NY: Simon and Schuster; 1995.
167. Rao R, Ludwig K et al. Select choices in benign breast disease: an initiative of the American Society of Breast Surgeons for the American Board of Internal Medicine Choosing Wisely Campaign. *Ann Surg Oncol*. 2018;25:2795–2800. http://doi.org/10.1245/s10434-018-6584-5.

Fibromyalgia Syndrome

John Nowicki, ND, and *John C. Lowe*, MA, DC*

OUTLINE

INTRODUCTION

Fibromyalgia diagnosis is a challenging and long process, especially among primary care physicians. There are a variety of factors involved, including the patient's attitude, the patient's personal history, comorbidities, and sociodemographic characteristics (Fig. 170.2). The 13 most common symptoms associated with the pain and tenderness of fibromyalgia syndrome (FMS)[2–4] are listed in Box 170.1.

Diagnostic Summary

- Common: 2% to 13% of population; approximately 80% to 90% women
- Chronic widespread pain involving axial pain and pain on the left, right, upper, and lower parts of body[1]
- Abnormal tenderness at 11 or more of 18 specific anatomical tender point (TnP) sites[1] (Fig. 170.1)

Differential Diagnosis

The purpose of the 1990 American College of Rheumatology (ACR) criteria for FMS was to distinguish patients with a putative primary disorder designated FMS from those with similar symptoms due to other distinguishable medical disorders. The two major criteria for FMS are chronic (longer than 3 months) widespread pain and tenderness.[1] The criteria were established mainly for use in research that would eventually identify the underlying pathological mechanism of the symptoms, but they have come to serve as diagnostic criteria in clinical practice.

Some theorists have argued that widespread pain and tenderness at predictable anatomical sites are features of many medical disorders.[5] Their argument is true only of hypothyroidism and cellular resistance to thyroid hormone, especially when the associated symptoms of FMS are also taken into account. When most patients with hypothyroidism or cellular resistance to thyroid hormone meet the ACR criteria for FMS and are effectively treated with thyroid hormone therapy, they no longer meet the FMS criteria.[6–15] This finding indicates that these patients' FMS symptoms and signs are a distinct clinical phenotype of inadequate thyroid hormone tissue regulation.[16] It also justifies a trial of thyroid hormone therapy to distinguish whether a patient's FMS symptoms and signs are features of hypothyroidism or cellular resistance to thyroid hormone.

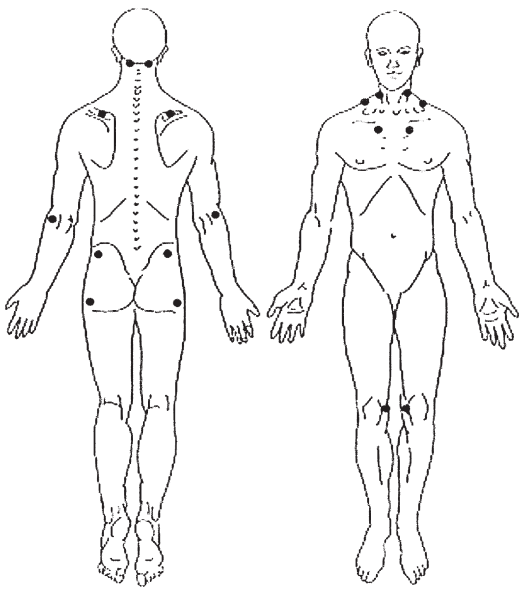


Fig. 170.1 Locations of 18 Anatomical Tender Point Sites. (Copyright John C. Lowe.)

* Previous edition contributor

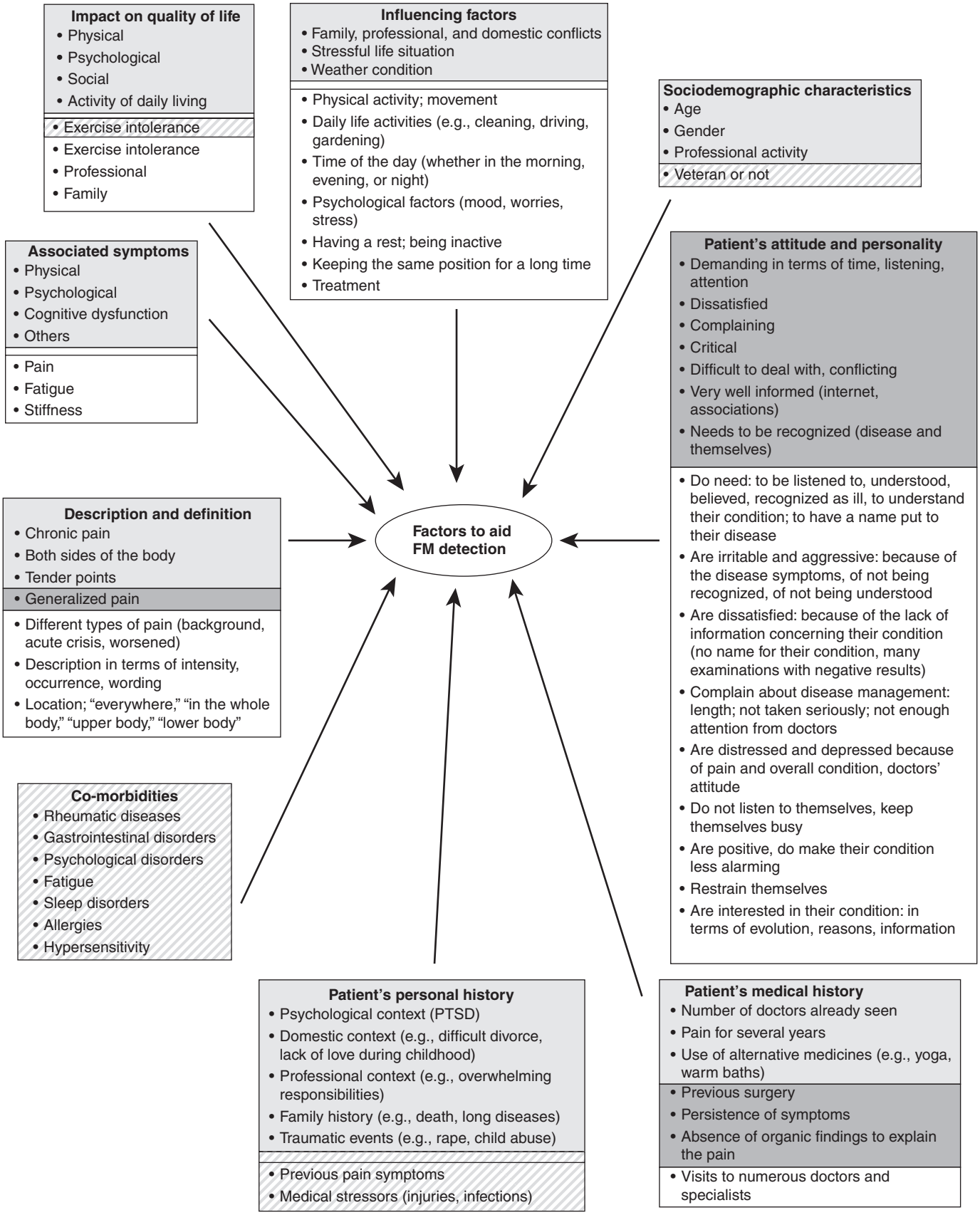


Fig. 170.2 Conceptual Model of Factors That Could Help Primary Care Physicians in the Detection of Fibromyalgia in Practice. (Baron R, Perrot S, Guillemin I, et al. Improving the primary care physicians' decision making for fibromyalgia in clinical practice: development and validation of the Fibromyalgia Detection [FibroDetect] screening tool. *Health Qual Life Outcomes.* 2014;12:128. PubMed PMID: 25341959.)

BOX 170.1 Most Common Associated Symptoms in Fibromyalgia Syndrome

- Fatigue
- Stiffness
- Headache
- Sleep disturbance
- Irritable bowel
- Depression
- Cognitive dysfunction
- Anxiety
- Coldness
- Paresthesias
- Sicca symptoms
- Exercise intolerance
- Dysmenorrhea

From Lowe JC. *The Metabolic Treatment of Fibromyalgia.* Boulder, CO: McDowell Publishing; 2000; Awad EA. Pathological changes in fibromyalgia. *First International Symposium on Myofascial Pain and Fibromyalgia.* Minneapolis, 1989; Rodolico C, Toscano A, Benvenga S, et al. Myopathy as the persistently isolated symptomatology of primary autoimmune hypothyroidism. *Thyroid.* 1998;8:1033–1038; Lowe JC, Honeyman-Lowe G. Fibromyalgia and thyroid disease. Paper presented and discussed in Grenoble, France May 6 (Conference of the French Fibromyalgia Association of Région Rhône-Alpes) and in Toulon, France, May 11 (Centre Hospitalier Intercommunal), 2000; Wirz-Justice A. Platelet research in psychiatry. *Experientia.* 1988;44:145–152.

The theorists' argument does not apply to other medical disorders, however, and is refuted by two lines of evidence.[17] First, studies have not established that chronic widespread pain and multiple TnPs are features of other medical disorders. Second, many patients with rheumatoid arthritis, osteoarthritis, Lyme disease, systemic lupus erythematosus, Chiari malformation, and spinal stenosis also meet the ACR criteria for FMS, but when these other disorders are effectively treated, the patients still meet the criteria for FMS.[18–20]

The argument is false, then, that chronic widespread pain and tenderness are features of many medical disorders. Nonetheless, the argument highlights the need to differentiate FMS from other disorders with similar symptoms that may lead the clinician to misdiagnose FMS. The main disorders the clinician should distinguish from FMS are arthritis, myopathy, polymyalgia rheumatica, diabetic polyneuropathy, ankylosing spondylitis, discopathy, cardiac or pleural pain,[21] multiple muscle myofascial pain syndromes,[22] and lupus erythematosus. A given patient may, of course, concurrently meet the criteria for FMS and one or more other disorders with overlapping symptoms. FMS can usually be distinguished from medical disorders other than hypothyroidism and cellular resistance to thyroid hormone by careful pathognomy. However, diagnostic scrutiny will show that the symptoms and signs of most FMS patients are indistinguishable from those of the subclass of hypothyroid and thyroid hormone–resistant patients for whom pain is a predominant symptom.

GENERAL CONSIDERATIONS

Focus of the Conventional Fibromyalgia Research Community

The serotonin deficiency hypothesis is the oldest proposed mechanism of FMS.[23–25] As a research-inspiring concept, it constituted the core theoretical underpinning of the rheumatology paradigm of FMS. This hypothesis proposed that a central nervous system (CNS) serotonin deficiency reduced the efficiency of the brainstem–spinal cord descending antinociceptive system, lowering the threshold for pain perception.[26] By 2000, the serotonin deficiency hypothesis had been effectively refuted[16] by three lines of evidence: First, the only site of low serotonin among FMS patients was their platelets.[26–28] Second, serotonin-increasing drugs were no more effective than placebos[29,30–34] and exacerbated some patients' FMS status.[35] And third, the low brain blood flow of FMS patients[36,37] contradicts a serotonin deficiency; serotonin is a potent vasoconstrictor,[38] and a low CNS level would produce cerebral vasodilation and increased blood flow.

This was further documented in the 2005 publication of the state of that paradigm.[39] The central theoretical viewpoint of the former paradigm had shifted from a serotonin deficiency to a generalized problem of augmented pain processing resulting in a hyperalgesic state,[39,40] which is plausibly explained by the extraordinarily high levels of substance P in patients with FMS.[41] But researchers espousing the former paradigm have failed to address the most plausible mechanism of the patients' high levels of substance P (see the following section). Instead, their main efforts have been in developing pharmaceuticals to manage FMS pain. This has led to the approval of several drugs by the U.S. Food and Drug Administration for FMS and the recommendation that patients undergo education about FMS as well as psychotherapy and aerobic, strength, and flexibility training.[40]

Environmental Toxicants

More than 50% of FMS patients reported adverse reactions to various chemicals. It has been suggested that an elevated total burden of toxic metals, such as cadmium and mercury, may induce worsening of diverse muscle pains with various etiologies. One study evaluated the frequency and clinical relevance of metal allergy in fibromyalgia (FM) patients.[42] Fifteen female FM patients and 10 healthy age-matched women (controls) were included in the study. Metal allergy was measured by a lymphocyte transformation test, MELISA. All FM patients tested positive for at least one of the metals tested. The most frequent reactions were to nickel, followed by inorganic mercury, cadmium, and lead. Some healthy controls responded to inorganic mercury in vitro, but most of the tests were negative. Dental metal restorations and avoidance of known sources of toxic metals were recommended, implemented, and achieved to reduce metal exposure. An objective health assessment was performed 5 years after treatment, which showed that half of the patients no longer fulfilled the FM diagnosis, 20% had improved, and the remaining 30% still had FM. All patients reported subjective health improvement. Additionally, mercury and other toxic elements may interfere with the bioavailability of essential nutrients, which may aggravate symptoms.

Hypometabolism Hypothesis of Fibromyalgia Syndrome

The hypometabolism hypothesis posits that FMS is chronic hyperalgesia and other symptoms and signs of hypometabolism due to hypothyroidism, partial cellular resistance to thyroid hormone, or other metabolism-impeding factors. Among the other factors said to be responsible are pernicious diet, nutritional deficiencies, low physical fitness, and metabolism-impeding drugs. The term *hypometabolism* refers to the global impact on the patient of the underlying factors, most of which are catabolic and inhibitory, although some are anabolic or excitatory.[16]

Considerable evidence indicates that inadequate thyroid hormone regulation (ITHR) due to hypothyroidism or cellular resistance to thyroid hormone is the underlying mechanism of the two main features of FMS: chronic widespread pain and abnormal tenderness. ITHR of metabolic processes in cells of the brainstem–spinal cord descending antinociceptive system can result in chronically enhanced pain perception. Impairment of the antinociceptive system in FMS patients results in the following:

- Spontaneous or ongoing pain
- Tenderness (lowering of the pain threshold to mechanical stimuli)
- Hyperalgesia (increased responsiveness to noxious stimuli)[43,44]

ITHR can impair the antinociceptive system by two mechanisms. First, ITHR can severely increase the production of substance P, which is extremely high in the cerebrospinal fluid (CSF) of tested FMS patients.[41] Substance P is released from the terminals of nociceptive neurons[45] and assists the summation of slow nociceptive signals.[46] This facilitation amplifies the transmission of nociceptive signals in the spinal cord.[47] Thyroid hormone normally inhibits the synthesis and secretion of substance P in many CNS cells. It does so by repressing the transcription of the preprotachykinin-A gene. Preprotachykinin-A is the precursor of substance P and its cognate substance P receptor.[48,49] Lowering thyroid hormone levels by thyroidectomy increased the substance P level in astrocytes,[49] the anterior pituitary,[50–52] many brain nuclei,[53] and, most relevant to pain, the dorsal horns of the lumbar spinal cord. The increase in dorsal horn substance P was highly elevated (100%),[54,55] as in FMS patients.[41] Thyroid hormone treatment lowered the substance P level in the anterior pituitary,[51] brain nuclei,[56] and dorsal horns.[55] Excess thyroid hormone reduced substance P to subnormal levels.[52]

The second mechanism by which ITHR can reduce the effectiveness of the antinociceptive system is by reducing the synthesis and secretion of norepinephrine (NE) in cells of the brainstem locus ceruleus. Adequate NE is essential to the normal function of the descending antinociceptive system.[57,58]

The antinociceptive pathways that descend from the brainstem to the dorsal horns contain two types of neurons: those that secrete serotonin and others that secrete NE.[20,57,59,60] Serotonin secretion by the neurons is tonically augmented by NE secretion. Normal serotonin secretion is therefore dependent on NE secretion.[55] The serotonin stimulates interneurons to secrete opiates.[57,59,61] These then inhibit transmission partly by blocking the release of neurotransmitter substances such as glutamate and substance P from the afferent neurons.[62] They also block calcium influx and potassium efflux from the afferent terminals,[63] mainly those of types C and A delta fibers.[64] The decreased potassium efflux hyperpolarizes terminals, inhibiting the transmission of nociceptive signals to spinothalamic neurons that otherwise would transmit the signals to the brain.[65] Low NE secretion by descending neurons, however, may reduce the secretion of serotonin selectively at dorsal horn interneurons and secondarily reduce opiate secretion. As a result, the transmission of nociceptive signals in the CNS will increase, thus heightening pain perception.

That decreased NE production is involved in the heightened pain perception of FMS patients is indicated by low metabolites of both dopamine and NE in patients' CSF.[27] That inadequate T_3 regulation of locus ceruleus neurons accounts for the low NE production is suggested by the crucial role T_3 plays in the synthesis of both dopamine and NE. The locus ceruleus is the brain site with the heaviest concentration of T_3.[66–68] Thyroid hormone regulates the activity levels of two rate-limiting enzymes in dopamine and NE synthesis.[69] One enzyme, tyrosine hydroxylase, catalyzes the conversion of tyrosine to levodopa, which in turn is converted to dopamine.[70] Tyrosine hydroxylase activity in the noradrenergic neurons of the locus ceruleus is low in hypothyroidism.[71] Low activity of the enzyme and reduced conversion of levodopa to dopamine may be responsible for low dopamine levels in the striatum, hypothalamus, and superior cervical ganglia in hypothyroidism.[69] Thyroid hormone therapy increases the activity of tyrosine hydroxylase.[71] The second enzyme, dopamine-β-hydroxylase, catalyzes the conversion of dopamine to NE. Low activity of the enzyme in hypothyroidism can reduce NE levels.[69] Unfortunately, NE levels in the antinociceptive system and other tissues in thyroid disorders have not been studied extensively[72] enough to support this putative mechanism.

By raising substance P levels and possibly lowering NE levels in the spinal cord, ITHR can thus plausibly heighten FMS patients' pain perception. Patients' pain, however, is probably compounded by other factors. First, most FMS patients are physically inactive because of their pain,[73,74] although low motor drive from low dopamine levels[27] probably contribute to their inactivity. Their low physical activity level may further contribute to the inefficiency of the antinociceptive system.[75–77]

ITHR can also plausibly account for the other symptoms and objectively verified abnormalities of FMS: muscle and joint pain, paresthesias, cognitive dysfunction, depression, cold intolerance, exercise intolerance, weakness and fatigue, dry skin and mucous membranes, constipation, dysmenorrhea and menorrhagia,[78,79] increased platelet α_2-adrenergic receptor density,[80,81] reduced brain blood flow,[82] reduced peripheral blood flow,[83] sleep disturbance,[84] deficient slow-wave sleep,[85] hypotension,[86] blunted sympathetic response to stress,[87] stiffness and swelling,[88] irritable bowel syndrome,[89] excessive urination,[84] high serum hyaluronic acid,[90] low procollagen III,[91] high ground substance proteoglycans,[92] low pyridinoline[93] and hydroxyproline,[94] glycolysis abnormalities,[95] low concentrations of high-energy phosphates in erythrocytes and muscle cells,[96,97] and low growth hormone and somatomedin C levels.[98] The cellular and genomic actions of thyroid hormone can explain the hormone's relationship to all of these factors.[16] Especially important is thyroid hormone's effect on the adrenergic system. If the hypometabolism hypothesis is verified at some point in the future, the definition of fibromyalgia will include an explanation of FMS as a condition of α-adrenergic dominance.

Experimental Support for the Hypometabolism Hypothesis of Fibromyalgia Syndrome

The hypothesis that ITHR is an underlying mechanism of FMS has considerable experimental support.[99] Many researchers have noted the virtually identical features of FMS, hypothyroidism, and the peripheral form of cellular resistance to thyroid hormone.[16,100–115]

Several research groups have used thyroid function testing to determine the incidence of thyroid disease among FMS patients. Each group has reported an incidence higher than in the general population.[100–110] A thorough analysis of all the available evidence indicates that approximately 90% of FMS patients have some form of thyroid disease, either primary or central hypothyroidism or cellular resistance to thyroid hormone.[116,117]

In two studies, 15 female FMS patients had low resting metabolic rates (RMRs) and basal axillary temperatures compared with 15 matched healthy controls. In both studies, the controls' mean RMR was within the reference range predicted by equations. However, in the first study, the mean RMR of FMS patients was 29.2% below normal based on their gender, age, height, and weight.[118] In the second study, FMS patients' mean RMR was 32.5% below normal.[119] In both studies, FMS patients' basal temperatures were significantly lower than those of controls. In the first study,[118] FMS patients' mean basal temperature was 96.95°F (36.08°C). In the second study,[119] the mean temperature of FMS patients was 96.38°F (35.77°C).

Thyroid Supplementation

The only clinical trials in which patients have been fully relieved of FMS have involved orally administered thyroid hormone. It should be noted, however, that in each study patients also used other metabolism-regulating therapies (see later discussion on metabolism-regulating therapies other than thyroid hormone). Euthyroid and hypothyroid FMS patients fully recovered in five open but highly systematic trials[6–10]; three double-blind, placebo-controlled crossover trials[11–13]; and a randomized, double-blind, placebo-controlled trial.[14] In another randomized, double-blind trial, patients with FMS had limited improvement with the use of transdermal

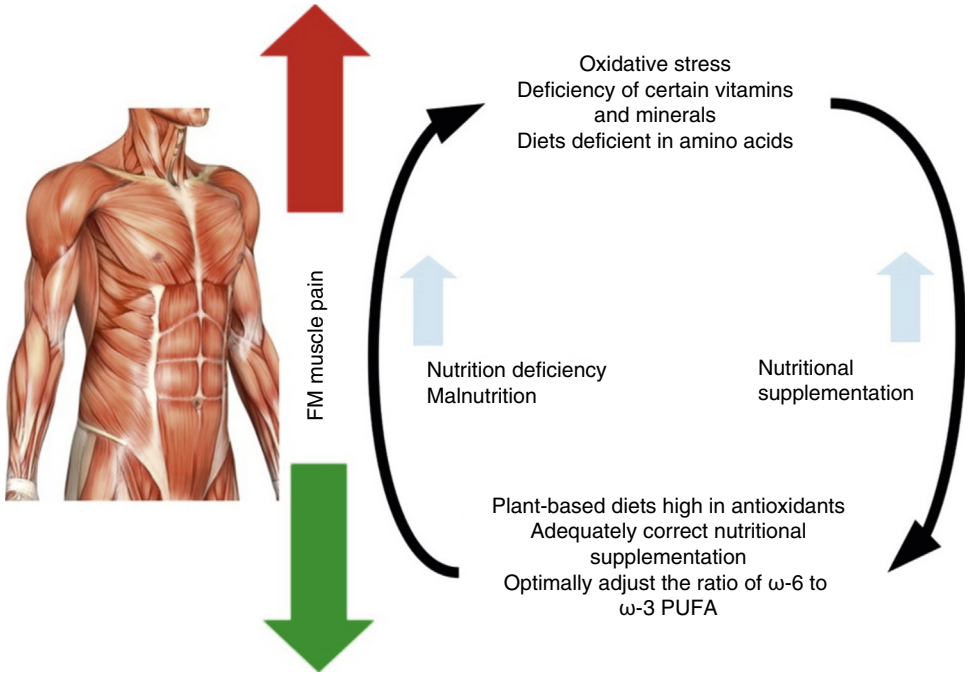


Fig. 170.3 The Relationship Between Nutrition and Fibromyalgia-Related Musculoskeletal Pain. (Bjorklund G, Dadar M, Chirumbolo S, Aaseth J. Fibromyalgia and nutrition: therapeutic possibilities? *Biomed Pharmacother.* 2018;103:531–538. PubMed PMID: 29677539.)

T_3.[120] A 1- to 5-year follow-up study compared the status of control patients with FMS with patients with FMS who had either hypothyroidism or cellular resistance to thyroid hormone and underwent metabolic therapy, including the use of thyroid hormone. Although control patients' FMS status deteriorated, treated patients recovered and maintained their recovery throughout the follow-up period. The results of this study were the first to demonstrate the long-term effectiveness of an FMS treatment.[15]

Metabolism-Regulating Therapies Other Than Thyroid Hormone

Thyroid hormone therapy is necessary in most cases of FMS to ensure recovery, but it is not sufficient. For example, in a study of 77 euthyroid patients with FMS,[6] those who declined to adopt a wholesome diet, take nutritional supplements, and exercise to tolerance were among the 25% who failed to benefit from T_3 therapy. This result is consistent with years of clinical experience supporting the necessity for patients to use other metabolism-regulating therapies in addition to thyroid hormone. A common experience, for example, is a recovered patient stopping the use of B complex vitamins only to suffer a partial recurrence of FMS symptoms. The symptoms are again relieved when the vitamin use resumes.

The most common other metabolism-regulating therapies that patients must use are a wholesome diet, nutritional supplementation, and exercise to tolerance. These therapies are necessary for most patients to recover fully. However, in most cases, they are not sufficient and must be used along with thyroid hormone therapy. This is shown by studies indicating that these therapies alone provide some improvement but that patients do not recover fully (i.e., they continue to meet the criteria for FMS). In addition to the metabolic therapies, most FMS patients also require some physical treatment for full relief of their FMS pain.

Wholesome Diet

Various diets and dietary supplements have been tested as FMS treatments. Mild-to-moderate improvements in FMS status have been reported for vegetarian diets[121,122]; elimination of the excitotoxins monosodium glutamate and aspartame[123]; *Chlorella pyrenoidosa* (a unicellular freshwater green alga rich in proteins, vitamins, and minerals)[124,125]; an uncooked vegan diet consisting of berries, fruits, vegetables, roots, nuts, and germinated seeds and sprouts[126]; and a strict, low-salt, uncooked vegan diet rich in lactobacteria.[127]

Nutritional Therapies

Long clinical experience shows that a wide array of nutritional supplements is necessary if FMS patients are to improve significantly or recover (Fig. 170.3). As the sole treatment, however, supplements provide only mild improvement except for the rare patient.

Studies indicate that vitamins B_1, B_6, B_{12},[56,128–136] C,[137–141] and E and beta-carotene[142] have antinociceptive properties. Other reports indicate that various nutritional supplements improve FMS status: the Myers' cocktail (intravenous formula of B vitamins, vitamin C, calcium, and magnesium)[143]; 5-hydroxytryptophan[144,145]; S-adenosylmethionine[146–149]; magnesium and malic acid[150,151]; combined aloe vera extracts, plant saccharides, freeze-dried fruits and vegetables, *Dioscorea,* and a vitamin/mineral complex[152]; collagen hydrolysat[153]; and a blend of ascorbigen and broccoli powder.[154]

Exercise to Tolerance

The results of studies of exercise in the treatment of FMS have been mixed: some have shown no improvement in FMS status[35,69,155–158] or only minimal improvement.[159–164] Cardiovascular exercise has provided the most improvement,[161–164] especially low-intensity endurance training.[164,165] Endurance exercise is important in reducing the physical limitations associated with FMS.[166] The importance of physical fitness and the high metabolic efficiency it provides[167,168] was shown by deficient slow-wave sleep, inducing FMS-like symptoms in sedentary and not aerobically fit students.[23] This finding suggests that low metabolic efficiency due to ITHR renders some individuals susceptible to FMS.[16]

Vigorous exercise tends to exacerbate patients' FMS symptoms.[72,73] As a result, some patients avoid exercise altogether and consequently are not physically fit.[166,169–171] The most plausible explanation for the exacerbations of vigorous exercise is the high density of α_2-adrenergic receptors on FMS patients' platelets.[172] The platelet density of α_2-adrenergic receptors is a reliable indicator of the receptor density in the CNS.[173] Binding of catecholamines to a high density of the receptors on cells of most tissues inhibits energy metabolism. The inhibition of energy metabolism during vigorous exercise, mediated by the high density of the receptors, appears to worsen symptoms severely. During the early phase of treatment, then, exercise should be mild enough to avoid or minimize catecholamine secretion.[16] With effective thyroid hormone therapy, the density of α_2-adrenergic receptors decreases and the density of β-adrenergic receptors increases, enabling cells to respond appropriately to high levels of catecholamines. The shift away from α_2-adrenergic receptor dominance probably explains, in part, the FMS patient's ability to engage in vigorous physical activity after receiving thyroid hormone therapy.[6,7,8,11–13,15]

Physical Treatment

Extensive clinical experience and clinical trials[7,8,11–13] show that despite the use of integrated metabolic therapies, many patients require physical treatment to fully relieve their FMS pain.[174–177] Several studies have shown that spinal manipulation, soft tissue manipulation, and trigger point therapy provide palliative improvement in some FMS symptoms, especially pain.[178–181]

The most common lesions that exacerbate FMS symptoms are myofascial trigger points and spinal joint fixations.[16,175,182] However, any nociception-generating neuromusculoskeletal lesion may exacerbate FMS patients' pain, probably owing to their high levels of substance P.[40] Neuromusculoskeletal lesions can also disturb sleep.[183–185] This can, in turn, increase FMS symptoms.[23]

It appears, then, that for most FMS patients to improve or recover, integrated metabolic therapies that include the use of thyroid hormone are necessary and sufficient, whereas no single metabolic therapy is sufficient in itself. In addition, physical treatment may be necessary to eliminate most FMS patients' pain completely.

THERAPEUTIC CONSIDERATIONS

As fully discussed previously, because metabolic hypofunction is primarily driven by thyroid dysfunction, fully understanding the patient's cellular thyroid function underlies the therapeutic approach.

Laboratory Testing for Thyroid Status

Thyroid Function Tests

Before beginning the treatment for a patient with FMS, his or her thyroid status should be determined with thyroid function tests. Primary hypothyroidism (thyroid hormone deficiency due to subnormal function of the thyroid gland) in most patients can be determined with the standard thyroid panel:

- Free T_3, free T_4, and thyroid-stimulating hormone (TSH)

In untreated primary hypothyroidism, the TSH is elevated. The recently revised upper limit of the serum TSH reference range is 2.5 μIU/mL.[186]

Thyroid Antibodies

The most common cause of primary hypothyroidism is autoimmune thyroiditis. The presence of this disorder can be determined by the patient's titer of thyroglobulin and thyroid peroxidase (microsomal) antibodies. It is especially important to order tests of antibody levels in patients with FMS. Many patients who have elevated thyroid antibodies have also had thyroid function test results within the reference ranges for many years.[187,188] However, compared with people without chronic widespread musculoskeletal complaints, those with such complaints were found to have a significantly higher incidence of thyroid microsomal antibodies (16% vs. 7.3%, $P < 0.01$). The prevalence of antibodies was also significantly higher in women than men (20.4% vs. 11.6%, $P < 0.02$), but thyroid function test results did not differ significantly between those with and without musculoskeletal complaints.[120] This study indicates that in patients with autoimmune thyroiditis, thyroid hormone levels too low to properly regulate the CNS antinociceptive system and properly inhibit the production of substance P can escape detection by thyroid function tests, including the TSH.

Thyroid Hormone Therapy Based on Initial Thyroid Status

Patients with FMS whose test results indicate hypothyroidism should begin therapy with a thyroid hormone preparation containing both T_4 and T_3. Many hypothyroid FMS patients do not benefit from T_4 alone no matter how high the dosage.[189] Most do benefit, however, from the use of T_4/T_3 preparations in a 4:1 ratio. The dosage range at which most patients improve or recover with T_4/T_3 preparations is that which was used throughout the 20th century, without harmful effects,[190] before TSH assays came into widespread use in the early 1970s: 76 mcgT_4 and 18 mcg T_3 to 152 mcg T_4 and 36 mcg T_3.[16,191]

The thyroid hormone therapy used in studies in which hypothyroid and euthyroid FMS patients recovered[6–14] was not conventional T_4-replacement therapy. Although T_4 was used in some studies,[9,10,14,15] it was T_4/T_3 preparations and T_3 alone used in a way that violated the mandates of T_4 replacement. Specifically, patients were permitted to use thyroid hormone despite laboratory test results indicating that they were euthyroid, and doses were not titrated according to TSH levels but by patients' clinical responses to particular doses.

Effective doses usually suppressed TSH levels. Despite this, patients did not have symptoms of thyrotoxicosis, nor did they have evidence of thyrotoxicosis according to electrocardiography, bone densitometry, or serum and urine biochemical test results.

Patients with FMS whose laboratory test results indicate euthyroidism should begin with T_3. In studies of the thyroid status of FMS patients at intake, approximately 33% were euthyroid according to thyroid function testing.[192,193] Of these patients, approximately 75% have partial peripheral cellular resistance to thyroid hormone according to four criteria[115,116]:

1. The patients are euthyroid (according to laboratory thyroid function test results) before beginning the use of T_3.
2. The patients recover from their hypothyroid-like FMS symptoms and signs with supraphysiological doses of T_3.
3. The patients have high serum-free T_3 levels after beginning T_3 therapy.
4. The patients have no evidence of tissue thyrotoxicosis, according to the results of serial electrocardiograms (ECGs), serum and urine biochemical tests, and bone densitometry.

These patients usually benefit only from immediate-release T_3 (*not* sustained-release T_3) in a single daily dose.[16] They are likely to respond only to supraphysiological doses of T_3. Effective T_3 dosages for most patients are between 25 and 125 mcg. For some patients, however, safe and effective doses are far higher.[16]

Some FMS patients have both hypothyroidism and cellular resistance to thyroid hormone.[8] Most of these patients improve or recover only with T_3 therapy, typically supraphysiological doses.

The Basis of Dosage Adjustments

After a patient's thyroid status has been determined and thyroid hormone therapy has begun, dosage changes should be titrated according to symptom status and physiological measures, not according to thyroid function test results. Thyroid function tests are of no value in finding the safest and most effective dosage for any particular patient.[194] There is no scientific justification for inferring the metabolic status of cells (other than the thyrotrophs of the anterior pituitary) from thyroid function test results. The belief that tissue metabolic status can be accurately inferred from TSH or thyroid hormone levels is a postulate, not a research-derived conclusion. This postulate was found to be false, at least for rats, by studies showing that the levels of T_3 and T_4 in the cells of different tissues cannot be accurately predicted from plasma levels of TSH, T_3, or T_4.[195,196] Moreover, some studies show that TSH levels correlate poorly with measures of the tissue effects of thyroid hormone, such as the speed of the relaxation phase of the Achilles reflex.[197] Conversely, other studies show that the patient's symptoms and signs are far more accurate and reliable indicators of tissue metabolic status than are the results of thyroid function tests.[194,198]

The resting metabolic rate (RMR) measured with indirect calorimetry is the most reliable measurement of oxidative metabolism, which is strongly regulated by thyroid hormone. In a study designed to find a correlation between patients' RMRs and their TSH levels, a significant correlation was obtained only by log-transforming the TSH values.[199] The lack of a significant correlation without log transformation means that the TSH is not a useful tool for determining whether an individual patient's thyroid hormone dosage is providing him or her with sufficient oxidative metabolism. Preferably, clinicians will include indirect calorimetry in their clinical practices. However, this instrument is not necessary for finding patients' safe and effective dosages.

Rehabilitation Model: Data-Driven Clinical Decisions

The clinician should distinguish as clearly as possible all the various factors impairing the individual patient's metabolism. Next, the clinician should provide the patient with an individualized treatment regimen designed to correct, eliminate, or compensate for the causative factors. The treatment regimen that is most effective for guiding the FMS patient to recovery conforms to a rehabilitation model, which requires baseline and repeated monitoring of the patient's clinical status through objective assessment methods. Thus clinical decisions, such as thyroid hormone dosage adjustments, are data driven.

Objective Assessment of Patient Status

In patients with FMS, recovery of normal metabolism, a symptom-free state, and full function occurs gradually over a range of 2 to 6 months. Because of the time involved, it is best for the clinician to quantify the patient's clinical status through objective measures at weekly or biweekly intervals. Scores from these measures should be posted as data points to line graphs after each evaluation. The patient's baseline score on each measure (taken before therapy is begun) is the initial data point on each graph.

Next, a line should be drawn through the data points to create a trend line that makes obvious, on visual inspection, changes in the patient's clinical status. The trend lines of the data points on the graphs thus help the clinician assess the effectiveness of the patient's regimen at any point during treatment. In this way, the lines enable the clinician to make informed, data-driven decisions about any treatment changes needed to optimize clinical results.

The five line graphs in Fig. 170.4 show the changes in FMS measures during metabolic rehabilitation of a euthyroid FMS patient reported by Honeyman-Lowe.[9] Typically, all measures change together in the direction of improvement, no improvement, or worse. The patient's subjective status usually corresponds closely to what the trend lines indicate.

During the course of the rehabilitation process, patients and clinicians often lose sight of the severity of the patient's clinical status before treatment. When patients occasionally experience a brief or mild exacerbation of symptoms, they are especially prone to forget the progress they have made. For some, this state of mind can lead to the false conclusion that they have made no progress at all. This false conclusion is quickly corrected, however, by a review of the patients' graphs, which show visually and objectively their real progress.

The five objective assessment methods that should be used at each patient evaluation are described as outlined in the following discussion. The clinician should also perform a physical examination at each evaluation to further assess the patient's changing tissue metabolic status.

Pain Distribution Body Form

Four studies have shown that the most sensitive indicator for changes in FMS status is the pain distribution as a percentage of the body in pain.[11–13,163,200] This is convenient in that chronic widespread pain is one of the two major criteria for the assessment of FMS status.[1]

Small changes in pain distribution are assessed by using a form containing drawings of the body. The patient precisely shades in where he or she has had aching, pain, soreness, or tenderness since the last evaluation. Next, a template is placed over the patient's shaded form, which shows the body form divided into 36 areas. Each of the 36 divisions has a percentage value.[16,11–13] To obtain the patient's pain distribution, the values of the shaded divisions are totaled. This percentage number is placed on a graph as a data point. The data points for each evaluation are connected by a line showing the trend of the pain distribution over time.

Mean Pressure/Pain Threshold of Tender Point Sites

The second major criterion for the assessment of FMS is the presence of tender point sites (TnPs).[1] A modified version of the TnP examination measures the pressure/pain threshold of TnPs with algometry (numeric values in kilograms per square centimeter). This provides more precise quantification than the 1990 ACR method and enables the clinician to make more objective, evidence-based decisions.[201] The mean of the threshold values is calculated and posted to a line graph.

FibroQuest Symptoms Survey

The FibroQuest Symptoms Survey assessment form consists of 100-mm visual analog scales (VASs), 1 for each of the 13 most common associated FMS symptoms.[2] These symptoms are also characteristic symptoms of hypothyroidism and the peripheral form of cellular resistance to thyroid hormone (see Box 170.1).[16]

The patient estimates the intensity of each symptom by marking the appropriate point on the scale (1–10). The values for each marked symptom are added, and this total symptom intensity is then divided by the number of symptoms the patient marked at intake (baseline). The mean score is then posted to a line graph.

Fibromyalgia Impact Questionnaire

The Fibromyalgia Impact Questionnaire (FIQ)[202] is a measure of the patient's functional status. The total score from the FIQ is posted as a data point to a line graph. The assessment of functional ability is highly pertinent to the care of FMS patients. For example, in a study of the functional abilities of 1735 female FMS patients ages 31 to 78, researchers concluded that the average patient had less ability in activities of daily living than the average community-dwelling woman in her 80s.[203]

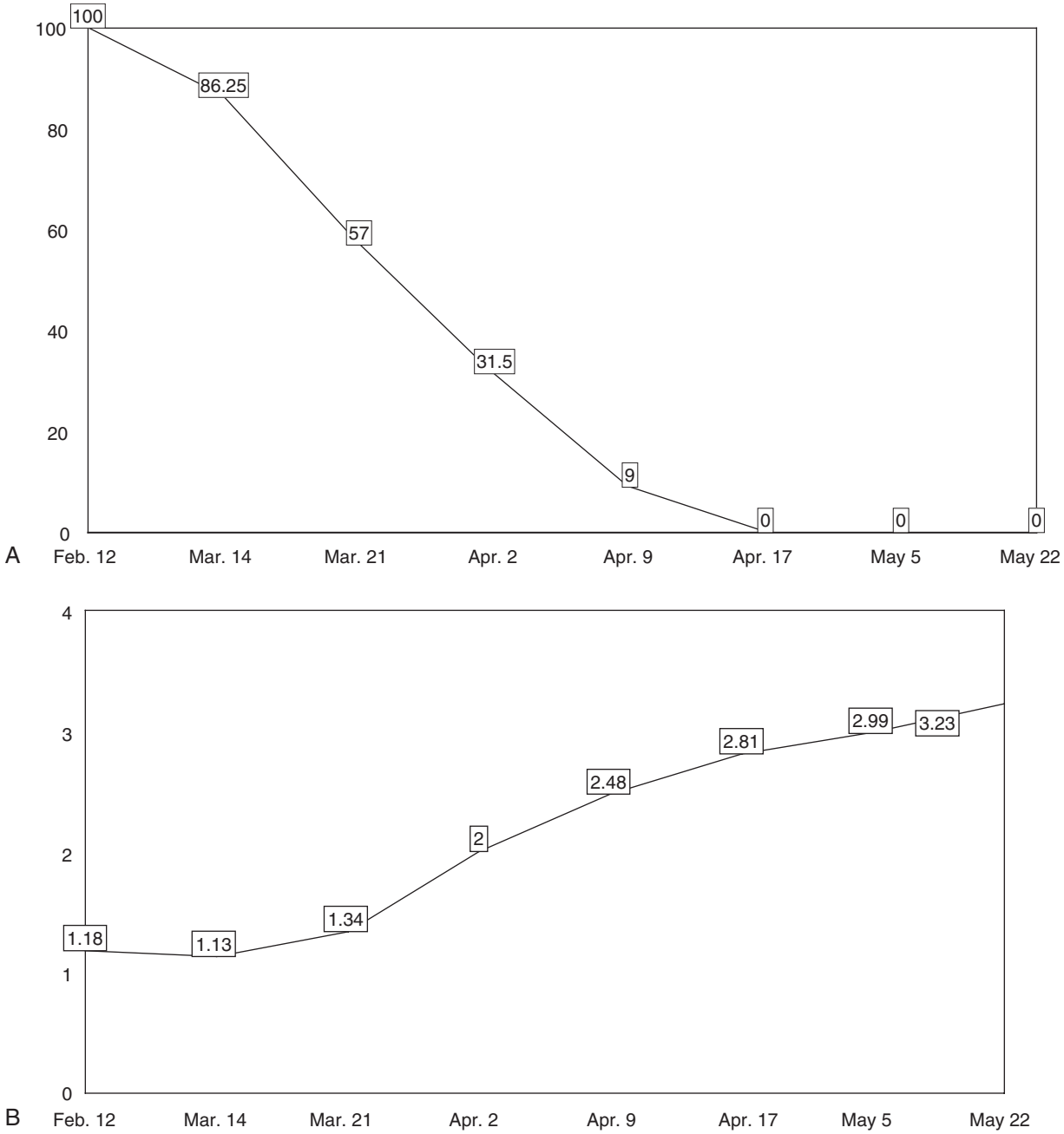


Fig. 170.4 (A) Pain distribution. Scores show the percentage of 36 body divisions containing pain at each evaluation (determined by the patient's pain drawing). At each evaluation, the percentage was posted to the graph. (B) Increases in mean tender point pressure/pain threshold during metabolic rehabilitation.

Zung's Self-Rating Depression Scale

Depression is so common among FMS patients that an assessment specific for the presence and severity of depression is used in addition to the VAS scale for depression. Zung's is one such assessment tool.[204] The score from the depression scale is posted to a line graph.

Physical Examination

When the treatment for the patient with FMS includes thyroid hormone, the five FMS measures should be used in conjunction with physiological tests that assess tissue responses to the hormone. The most convenient physiological tests are the relaxation phase of the Achilles' reflex, basal pulse rate, and basal body temperature. A combination of physiological measurements is a more reliable indicator of tissue metabolic status than are the results of laboratory thyroid function tests.

In the early 20th century, indirect calorimeters came into use to measure patients' RMRs.[205] Based on the patient's measured oxygen consumption, the instrument calculates the patient's estimated 24-hour resting energy expenditure. Thyroid hormone potently and exquisitely regulates oxidative metabolism. Because of this, during the mid-20th century, indirect calorimeters were used as the most relevant instrument for the diagnosis of hypometabolism (subnormal oxidative metabolism) due to hypothyroidism and thyroid hormone resistance. In recent years, handheld and other types of convenient-to-use, affordable, and accurate indirect calorimeters have become available for clinical use. Two studies have shown that patients with FMS have abnormally low RMRs compared with matched controls.[118,119]

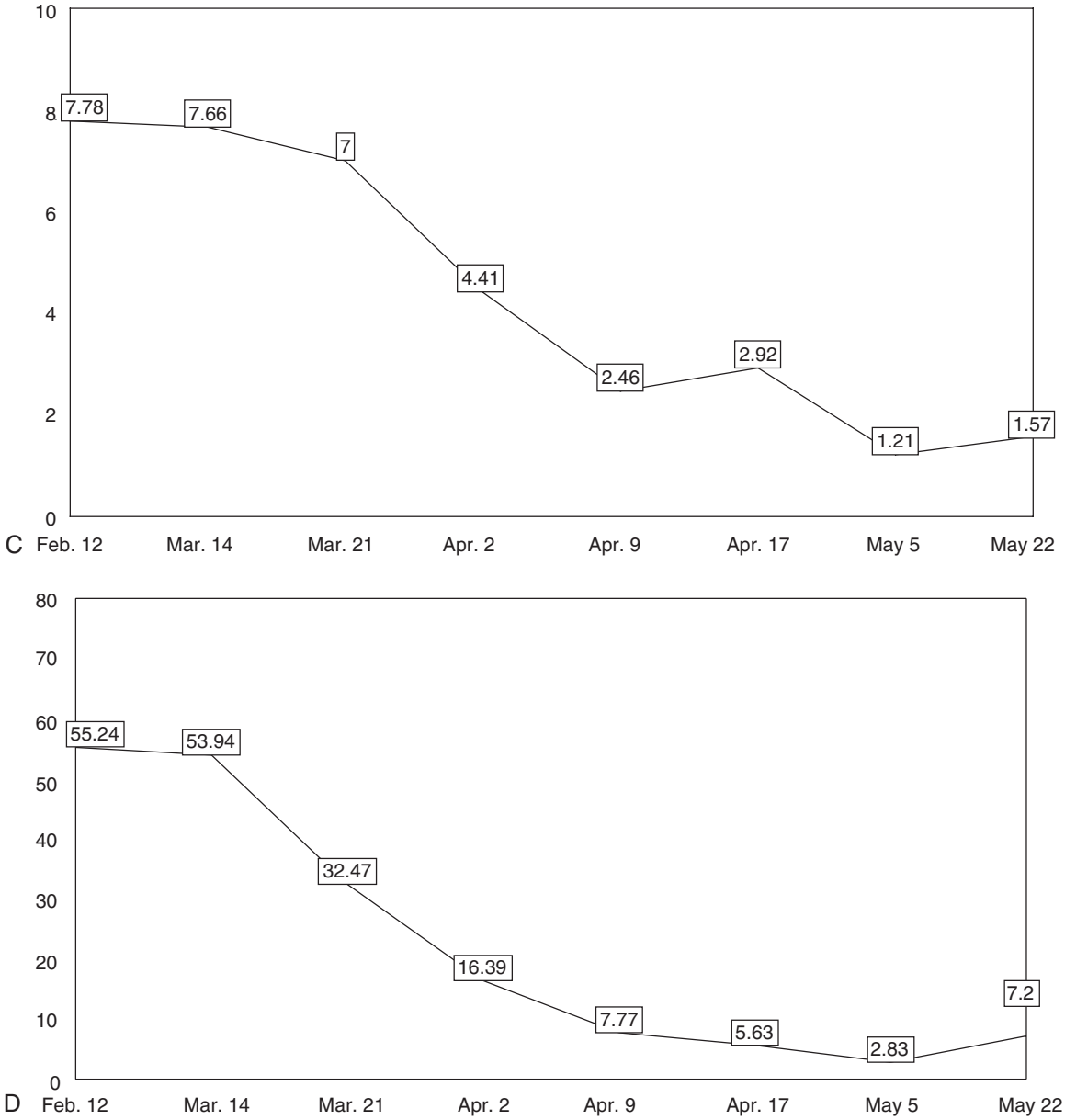


Fig. 170.4, cont'd (C) Decreases in mean intensity of fibromyalgia syndrome symptoms as calculated from the FibroQuest form. (D) Decreasing scores on the Fibromyalgia Impact Questionnaire.

Integrated Metabolic Therapies Essential for All Patients With Fibromyalgia Syndrome

The majority of patients with FMS must control or eliminate at least four metabolism-impeding factors. First, for the approximately 90% of FMS patients who have some form of thyroid disease,[116,117] the proper preparation and dosage of thyroid hormone are necessary for the patient's recovery.

Second, most patients must also modify their diets so that they minimize the intake of arachidonic acid. A high intake of arachidonic acid can increase body levels of both the proinflammatory eicosanoids prostaglandin E2 and leukotriene B4,[206] which can contribute to chronic pain.[207] Patients must also reduce their intake of refined carbohydrate. ITHR of glycolysis, the citric acid cycle, and the electron transport chain results in low production of adenosine triphosphate (ATP) and creatine phosphate. Dysglycemia and insulin resistance due to the intake of refined carbohydrates can worsen the low production of high-energy phosphates due to ITHR.[16]

Third, most patients must take a wide array of nutritional supplements. Various nutrients are synergistic to thyroid hormone in providing optimal intracellular metabolism, and patients must provide themselves with optimal amounts. For example, a deficiency of vitamin B_1 can render patients intolerant of even low dosages of thyroid hormone—dosages too low to be therapeutic.[208] In addition, supplemental thyroid hormone can increase the expenditure of vitamin B_1 in cells. If a patient has a marginal deficiency of the vitamin, supplemental thyroid hormone could induce a frank deficiency. This can result in a beriberi-type of cardiomyopathy.[208]

Fourth, most patients must also exercise to tolerance. Biochemical treatments such as thyroid hormone, optimal diet, and nutritional supplements provide the patient with increased metabolic capacity. Sufficiently vigorous physical activity, however, is necessary for optimizing metabolism in most tissues and for priming the descending nociceptive inhibitory system of the CNS.

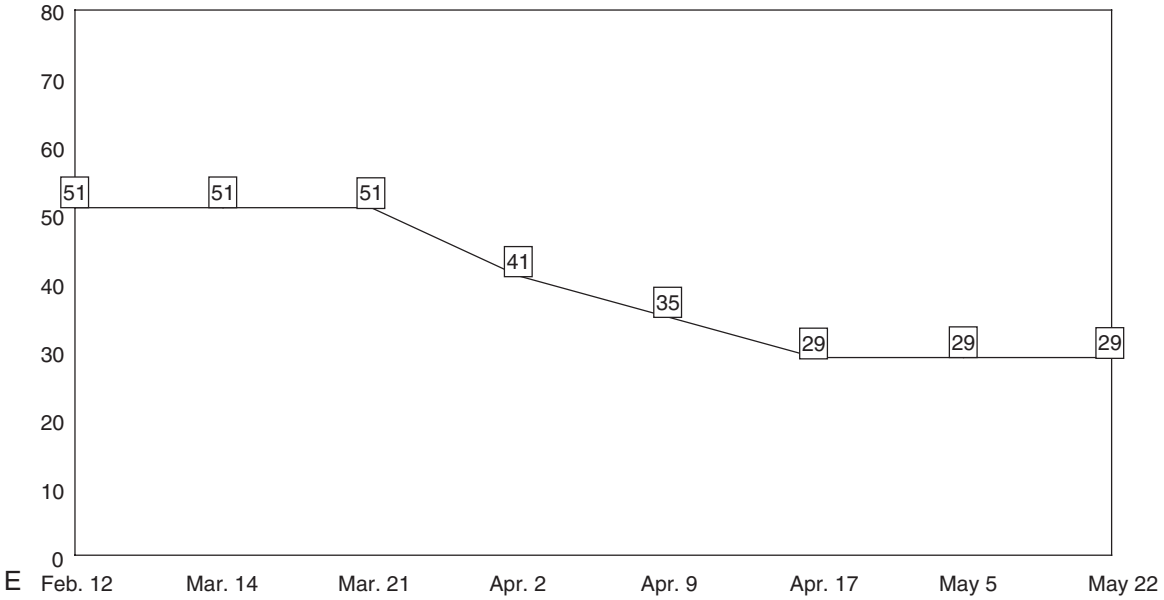


Fig. 170.4, cont'd (E) Decreasing scores on Zung's Self-Rating Depression Scale.

Additional Metabolic Therapies Necessary for Some Patients

Adrenocortical Hypofunction

Some patients must be treated for adrenocortical hypofunction. Low cortisol levels can produce some symptoms that are the same as those associated with FMS. These include weakness, fatigue, exaggerated responses to stress, exercise intolerance, hypotension, and low nociception threshold. Low cortisol levels can also render the patient intolerant of a dosage of thyroid hormone high enough to be fully therapeutic. The reason is that a minimally effective dosage of thyroid hormone is likely to accelerate the hepatic clearance of cortisol.[209] This typically exacerbates the symptoms of cortisol deficiency symptoms.

Metabolism-Impairing Medications

Several classes of medications may be significant in the development or perpetuation of FMS symptoms. Because of this, some patients must cease taking some metabolism-impairing medications, and they must convert from the maintenance to as-needed use of others.

Beta blockers impair metabolism directly by reducing the density of β-adrenergic receptors on cell membranes and rendering α_2-adrenergic receptors predominant in the regulation of cell function, exactly as hypothyroidism does. In some patients, this shift in adrenoceptor isoform balance creates a general mental and physical inhibitory state that may be indistinguishable from hypothyroidism or the associated symptoms of FMS.[210] The shift in adrenergic receptor isoform may also impair the function of the CNS's descending antinociceptive system, resulting in the pain and tenderness characteristic of FMS.

Narcotics, tranquilizers, and muscle relaxants are commonly prescribed as FMS treatments. Patients should be weaned from these as soon as possible because they can render patients disinclined to engage in vigorous physical activity. As the patient's physical fitness level declines, anergia and pain perception increase.

Some antidepressants (also commonly prescribed for FMS) and decongestants can cause tachycardia when they are used simultaneously with exogenous thyroid hormone. Because of this, patients using these drugs must reduce the dosages or stop their use to be able to take a dosage of thyroid hormone high enough to be therapeutic.

THERAPEUTIC APPROACH

Assessment of Fibromyalgia Syndrome Status

The patient's FMS status should be evaluated before treatment is begun and at intervals throughout the course of treatment. Reevaluations are best performed weekly to biweekly and before each increase in thyroid hormone dosage. If patients have taken measurements at home, such as the basal temperature, they should provide the clinicians with a record of these measurements. A physical examination and five FMS measures should be used at each evaluation:

- Pain distribution as the percentage of 36 body divisions containing pain, according to a body drawing
- Mean TnP score in kg/cm^2 measured by algometry
- Symptom intensity estimate according to visual analog scales (FibroQuest Symptoms Survey)
- Functional status according to the Fibromyalgia Impact Questionnaire
- Depression according to the Zung's Self-Rating Depression Scale

Scores for each assessment instrument should be posted to a line graph so that the trend of the scores is obvious on visual inspection. Changes in the patient's individualized treatment regimen should be based on the trend of scores on the line graphs combined with the physical examination and the clinician's and patient's subjective assessments. This process is continued (usually for 2–6 months) until the scores on the line graphs are representative of normal, the patient no longer meets the ACR criteria for FMS, and the patient is symptom-free and fully functional.

Patient Safety

All patients must have an electrocardiogram before beginning metabolic rehabilitation and, if appropriate, at intervals throughout the treatment process. Although dysrhythmic conditions are usually not obstacles to metabolic treatment, it is essential to be aware of them before treatment with thyroid hormone.

Patients should be educated extensively on the possible signs and symptoms of thyrotoxicosis and its management should overstimulation occur. When a patient's history indicates, he or she should

be tested for possible adrenocortical hypofunction. If testing reveals decreased cortisol production, the patient should be treated with physiological doses of cortisol at least temporarily before the use of thyroid hormone is initiated.

Thyroid Hormone Therapy

If the FMS patient is hypothyroid, he or she should begin treatment with 60 mg of desiccated thyroid or a synthetic T4/T3 preparation with a 4:1 ratio. The dosage should be increased by 60 mg of desiccated thyroid at approximately 3- to 4-week intervals until an optimal dosage has been reached.

If the patient is euthyroid, he or she should begin treatment with immediate-release T3, usually with a starting dosage of 50 mcg. The dosage is increased at weekly to biweekly intervals by 12.5 mcg. Dosage increases should continue until the patient is symptom-free, no longer meets the ACR criteria for FMS, is fully functional, and has reference-range physiological measures such as the basal body temperature and basal pulse rate.

Wholesome Diet

If they have not done so already, patients should adopt a diet that both stabilizes blood sugar regulation and insulin levels and reduces pain perception. Such a diet involves lean meats—especially poultry—fish, fruits, vegetables, and minimal intake of whole grains. It also minimizes the intake of foods high in arachidonic acid (which increases the production of the proinflammatory eicosanoids prostaglandin E2 and leukotriene B4[194]) and additive excitotoxins such as monosodium glutamate and aspartame. The author recommends that patients use organic foods and purified drinking water to decrease the quantity of chemical contaminants ingested.

Nutritional Supplements

Patients should take, at minimum, the following nutrients in the approximate dosages given:

High-potency multiple vitamin and mineral
Vitamin B_{12}: 3000 to 5000 mcg a day of methylcobalamin
Vitamin C: 500 mg to 1 g three times a day
Vitamin E (mixed tocopherols): 800 to 1600 IU a day
Carotenoids (mixed): 15 mg a day
Calcium: 2000 mcg a day in divided dosages
Magnesium (preferably as aspartate, citrate, malate, or glycinate): 1000 mg a day in divided dosages
Fish oils: 1000 to 2000 mg EPA+DHA a day

Exercise to Tolerance

Exercise is essential to help normalize metabolism. It is recommended that patients engage in forms of exercise that they enjoy and gradually increase the intensity as their symptoms diminish. Weight-bearing exercise is encouraged because it supports bone health. Aerobic exercise is essential to strengthen the cardiovascular and pulmonary systems. Toning activities build lean muscle mass, which in turn improves metabolism. A combination of these forms of exercise is best. Many patients who have become severely deconditioned owing to their FMS symptoms find warm-water exercise an ideal way to begin.

Physical Treatment

Myofascial therapy to eliminate trigger points in muscles and spinal manipulation to regain flexibility of the spine and other joints are essential for completely eliminating some patients' FMS pain. Both trigger points and spinal lesions can be perpetuated by self-sustaining skeletal muscle contractures. The contractures may result from deficient intramuscular high-energy phosphate production due to ITHR.[20,22,196] Both forms of treatment may raise the nociceptive threshold and increase mobility.

If an examination indicates the need, orthotics should be prescribed to relieve pain-inducing chronic postural muscle tension. The therapeutic aim is to reduce nociceptive input of somatic origin to the CNS.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. *Arthritis Rheum*. 1990;33:160–172.
2. Wolfe F. Diagnosis of fibromyalgia. *J Musculoskel Med*. 1990;7:53–69.
3. Yunus MB, Masi AT. Fibromyalgia, restless legs syndrome, periodic limb movement disorder, and psychogenic pain. In: McCarty DJ, Koopman WJ, eds. *Arthritis and Allied Conditions: A Textbook of Rheumatology*. Philadelphia: Lea & Febiger; 1993:1383–1405.
4. Nielson WR, Grace GM, Hopkins M, et al. Concentration and memory deficits in patients with fibromyalgia syndrome. *J Musculoskel Pain*. 1995;3:123.
5. Brady DM, Schneider MJ. Commentary: fibromyalgia syndrome: a new paradigm for differential diagnosis and treatment. *J Manipulative Physiol Ther*. 2001;24:529–541.
6. Lowe JC. Results of an open trial of T_3 therapy with 77 euthyroid female fibromyalgia patients. *Clin Bull Myofascial Ther*. 1997;2:35–37.
7. Lowe JC, Eichelberger J, Manso G, et al. Improvement in euthyroid fibromyalgia patients treated with T_3. *J Myofascial Ther*. 1994;1:16–29.
8. Lowe JC. T3-induced recovery from fibromyalgia by a hypothyroid patient resistant to T_4 and desiccated thyroid. *J Myofascial Ther*. 1995;1: 26–31.
9. Honeyman GS. Metabolic therapy for hypothyroid and euthyroid fibromyalgia: two case reports. *Clin Bull Myofascial Ther*. 1997;2:19–49.
10. Teitelbaum J, Bird B. Effective treatment of severe chronic fatigue: a report of a series of 64 patients. *J Musculoskel Pain*. 1995;3:91–110.
11. Lowe JC, Garrison R, Reichman A, et al. Effectiveness and safety of T_3 therapy for euthyroid fibromyalgia: a double-blind, placebo-controlled response-driven crossover study. *Clin Bull Myofascial Ther*. 1997;2:31–57.
12. Lowe JC, Garrison R, Reichman A, et al. Triiodothyronine (T_3) treatment of euthyroid fibromyalgia: a small-n replication of a double-blind placebo-controlled crossover study. *Clin Bull Myofascial Ther*. 1997;2:71–88.
13. Lowe JC, Reichman A, Yellin J. The process of change with T_3 therapy for euthyroid fibromyalgia: a double-blind placebo-controlled crossover study. *Clin Bull Myofascial Ther*. 1997;2:91–124.
14. Teitelbaum J, Bird B, Greenfield RM, et al. Effective treatment of CFS and FMS: a randomized, double-blind placebo controlled study. *J Chronic Fatigue Syndr*. 2001;8:3–28.
15. Lowe JC, Reichman AJ, Yellin J. A case-control study of metabolic therapy for fibromyalgia: long-term follow-up comparison of treated and untreated patients. *Clin Bull Myofascial Ther*. 1998;3:65–79.
16. Lowe JC. *The Metabolic Treatment of Fibromyalgia*. Boulder, CO: McDowell Publishing; 2000.
17. Lowe J. The Brady/Schneider proposal: a misdirected reclassification of fibromyalgia. *Dyn Chir*. 2002;20(12, 14):25–27.
18. Rosner MJ. *Decompression of craniovertebral stenosis leads to improvement in FMS and CFIDS symptoms*. http://www.nfra.net/NewRosner1.htm. Accessed August 5, 2004.
19. Alarcón GS, Bradley LA, Hadley MN, et al. *Does Chiari malformation contribute to fibromyalgia symptoms?* http://www.nfra.net/Chiari5.htm. Accessed August 5, 2004.
20. Mense S, Simons DG, Russell IJ. *Muscle Pain: Understanding Its Nature, Diagnosis, and Treatment*. Philadelphia: Lippincott Williams & Wilkins; 2001.
21. Yunus MB. Fibromyalgia syndrome and myofascial pain syndrome: clinical features, laboratory tests, diagnosis, and pathophysiologic mechanisms. In: Rachlin ES, ed. *Myofascial Pain and Fibromyalgia: Trigger Point Management*. St. Louis: Mosby-Year Book; 1994:3–29.
22. Travell JG, Simons DG. *Myofascial Pain and Dysfunction: The Trigger Point Manual*. vol. 2. Baltimore: Williams & Wilkins; 1992.
23. Moldofsky H, Scarisbrick P. Induction of neurasthenic musculoskeletal pain syndrome by selective sleep stage deprivation. *Psychosom Med*. 1976;38:35–44.
24. Moldofsky H, Warsh JJ. Plasma tryptophan and musculoskeletal pain in non-articular rheumatism ("fibrositis syndrome"). *Pain*. 1978;5:65–71.
25. Moldofsky H. Rheumatic pain modulation syndrome: the interrelationship between sleep, central nervous system serotonin, and pain. *Adv Neurol*. 1982;33:51–57.
26. Russell IJ. Neurochemical pathogenesis of fibromyalgia syndrome. *J Musculoskel Pain*. 1999;7:183–191.
27. Russell IJ, Vaeroy H, Javors M, et al. Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. *Arthritis Rheum*. 1992;35:550–556.
28. Russell IJ, Vipraio GA. Serotonin (5HT) in serum and platelets (PLT) from fibromyalgia patients (FS) and normal controls (NC). *Arthritis Rheum*. 1994;37(suppl):S214.
29. O'Malley PG, Balden E, Tomkins G, et al. Treatment of fibromyalgia with antidepressants: a meta-analysis. *J Gen Intern Med*. 2000;15: 659–666.
30. Carette S, Bell M, Reynolds WJ, et al. A controlled trial of amitriptyline, cyclobenzaprine, and placebo in fibromyalgia. *Arthritis Rheum*. 1992;35(suppl 9):112.
31. Carette S, Bell M, Reynolds WJ, et al. Comparison of amitriptyline, cyclobenzaprine, and placebo in the treatment of fibromyalgia: a randomized, double-blind clinical trial. *Arthritis Rheum*. 1994;37:32–40.
32. Carette S. What have clinical trials taught us about the treatment of fibromyalgia? *J Musculoskel Pain*. 1995;3:133–140.
33. Bennett RM. Fibromyalgia review. *J Musculoskel Pain*. 1997;5:85–96.
34. Bennett RM. Antidepressants do not have better results than placebo in the treatment of fibromyalgia in Brazil. *Curr Rheumatol Rep*. 2002;4: 284–285.
35. Isomeri R, Mikkelsson M, Latikka P, et al. Effects of amitriptyline and cardiovascular fitness training on pain in patients with primary fibromyalgia. *J Musculoskel Pain*. 1993;1:253–260.
36. Kwiatek R, Barnden L, Tedman R, et al. Regional cerebral blood flow in fibromyalgia: single-photon-emission computed tomography evidence of reduction in the pontine tegmentum and thalami. *Arthritis Rheum*. 2000;43:2823–2833.
37. Mountz JM, Bradley LA, Modell JG, et al. Fibromyalgia in women. Abnormalities of regional cerebral blood flow in the thalamus and the caudate nucleus are associated with low pain threshold levels. *Arthritis Rheum*. 1995;38:926–938.
38. Vanhoutte PM. 5-hydroxytryptamine and vascular disease. *Fed Proc*. 1983;42(2):233–237.
39. Wallace DJ, Clauw DJ, eds. *Fibromyalgia and Other Central Pain Syndromes*. Philadelphia: Lippincott Williams & Wilkins; 2005.
40. Arnold LM, Clauw DJ. Fibromyalgia syndrome: practical strategies for improving diagnosis and patient outcomes. *Am J Med*. 2010;123(6):S2.
41. Vaeroy H, Helle R, Forre O, et al. Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. *Pain*. 1988;32:21–26.
42. Stejskal V, Ockert K, Bjorklund G. Metal-induced inflammation triggers fibromyalgia in metal-allergic patients. *Neur Lett*. 2013;34(6):559–565.
43. Mense S. Descending antinociception and fibromyalgia. *Z Rheumatol*. 1998;57(suppl 2):23–26.
44. Henriksson KG, Mense S. Pain and nociception in fibromyalgia: clinical and neurobiological considerations on aetiology and pathogenesis. *Pain Rev*. 1994;1:245–260.
45. Messlinger K. What is a nociceptor? *Anaesthesist*. 1997;46:142–153.
46. Baranauskas G, Nistri A. Sensitization of pain pathways in the spinal cord: cellular mechanisms. *Prog Neurobiol*. 1998;54:349–365.
47. Satoh M. Transmission and modulation of nociceptive information in the spinal dorsal horn. *Nippon Yakurigaku Zasshi*. 1993;101:289–298.
48. Mendelson SC, Quinn JP. Characterization of potential regulatory elements within the rat preprotachykinin-A promoter. *Neurosci Lett*. 1995;184:125–128.
49. Too HP, Marriott DR, Wilkin GP. Preprotachykinin-A and substance P receptor (NK1) gene expression in rat astrocytes in vitro. *Neurosci Lett*. 1994;182:185–187.
50. Jonassen JA, Mullikin-Kirkpatrick D, McAdam A, et al. Thyroid hormone status regulates preprotachykinin-A gene expression in male rat anterior pituitary. *Endocrinology*. 1987;121:1555–1561.

51. Lam KS, Lechan RM, Minamitani N, et al. Vasoactive intestinal peptide in the anterior pituitary is increased in hypothyroidism. *Endocrinology*. 1989;124:1077–1084.
52. Jones PM, Ghatei MA, Wallis SC, et al. Differential response to neuropeptide Y, substance P, and vasoactive intestinal polypeptide in the rat anterior pituitary gland to alterations in thyroid hormone status. *J Endocrinol*. 1994;143:393–397.
53. Savard P, Blanchard LM, Merand Y, et al. Serotonin, 5-hydroxyindoleacetic acid and substance P content of discrete brain nuclei in rats made hypo- or hyperthyroid in the neonatal period: effect of growth hormone treatment. *Brain Res*. 1984;317:239–245.
54. Savard P, Merand Y, Bedard P, et al. Comparative effects of neonatal hypothyroidism and euthyroidism on TRH and substance P content of lumbar spinal cord in saline and PCPA-treated rats. *Brain Res*. 1983;277:263–268.
55. Savard P, Blanchard LM, Merand Y, et al. Influences of both thyroid and bovine growth hormones on substance P, thyrotropin-releasing hormone, serotonin and 5-hydroxyindoleacetic acid contents in the lumbar spinal cord of developing rats. *Brain Res*. 1984;315:105–110.
56. Dupont A, Dussault JH, Rouleau D, et al. Effect of neonatal thyroid deficiency on the catecholamine, substance P, and thyrotropin-releasing hormone contents of discrete rat brain nuclei. *Endocrinology*. 1981;108:2039–2045.
57. Hammond DL. Pharmacology of central pain-modulating networks (biogenic amines and nonopioid analgesics). In: Fields HL, Dubner R, Cervero F, eds. *Advances in Pain Research and Therapy. Proceedings of the Fourth World Congress on Pain: Seattle*. vol. 9. New York: Raven; 1985:499–511.
58. Hammond DL. Control systems for nociceptive afferent processing: the descending inhibitory pathways. In: Yaksh TL, ed. *Spinal Afferent Processing*. New York: Plenum Press; 1986:363–390.
59. Dubner R, Bennett GJ. Spinal and trigeminal mechanisms of nociception. *Ann Rev Neurosci*. 1983;6:381–418.
60. Cooper JR, Bloom FE, Roth RH. *The Biochemical Basis of Neuropharmacology*. 6th ed. New York: Oxford University Press; 1991.
61. Archer T, Arwestrem E, Jonsson G, et al. Noradrenergic-serotonergic interactions and nociception in the rat. *Eur J Pharmac*. 1986;120:295–307.
62. Riedel W, Neeck G. Nociception, pain, and antinociception: current concepts. *Z Rheumatol*. 2001;60:404–415.
63. Carr DB, Lipkowski AW. Neuropeptides and pain. *Agressologie*. 1990;31:173–177.
64. Chaouch A, Besson JM. Peripheral and spinal mechanisms of nociception. *Rev Neurol (Paris)*. 1986;142:173–200.
65. Jessell TM, Kelly DD. Pain and analgesia. In: Kandel ER, Schwartz JH, Jessell TM, eds. *Principles of Neural Science*. Norwalk, CT: Appleton & Lange; 1991:385–389.
66. Rozanov CB, Dratman MB. Immunohistochemical mapping of brain triiodothyronine reveals prominent localization in central noradrenergic systems. *Neuroscience*. 1996;74:897–915.
67. Gordon JT, Kaminski DM, Rozanov CB, et al. Evidence that 3,3¢,5-triiodothyronine is concentrated in and delivered from the locus coeruleus to its noradrenergic targets via anterograde axonal transport. *Neuroscience*. 1999;93:943–954.
68. Dratman MB, Gordon JT. Thyroid hormones as neurotransmitters. *Thyroid*. 1996;6:639–647.
69. de Lonlay A, Blouquit MF, Valens M, et al. Tyrosine hydroxylase and dopamine b-hydroxylase inductions evoked by reserpine in the superior cervical ganglion of developing eu- and hypothyroid rats. *J Auton Nerv Syst*. 1991;36:33–38.
70. Schwartz JH, Kandel ER. Synaptic transmission mediated by second messengers. In: Kandel ER, Schwartz JH, Jessell TM, eds. *Principles of Neural Science*. Norwalk, CT: Appleton & Lange; 1991:173–193.
71. Claustre J, Balende C, Pujol JF. Influence of the thyroid hormone status on tyrosine hydroxylase in central and peripheral catecholaminergic structures. *Neurochem Int*. 1996;28:277–281.
72. Mano T, Sakamoto H, Fujita K, et al. Effects of thyroid hormone on catecholamine and its metabolite concentrations in rat cardiac muscle and cerebral cortex. *Thyroid*. 1998;8:353–358.
73. Nørregaard J, Lykkegaard JJ, Mehlsen J, et al. Exercise training in treatment of fibromyalgia. *J Musculoskel Pain*. 1996;5:71–79.
74. Verstappen FTJ, van Santen-Hoeufft HMS, van Sloun S, et al. Fitness characteristics of female patients with fibromyalgia. *J Musculoskel Pain*. 1995;3:45–58.
75. Clark WC, Yang JC, Janal MN. Altered pain and visual sensitivity in humans: the effects of acute and chronic stress. *Ann N Y Acad Sci*. 1986;467:116–129.
76. Guieu R, Blin O, Pouget J, et al. Nociceptive threshold and physical activity. *Can J Neurol Sci*. 1992;19:69–71.
77. Suaudeau C, Costentin J. Long lasting increase in nociceptive threshold induced in mice by forced swimming: involvement of an endorphinergic mechanism. *Stress*. 2000;3:221–227.
78. DeGroot LJ, Larsen PR, Refetoff S, et al. *The Thyroid and Its Diseases*. 5th ed. New York: Wiley; 1984.
79. DeGowin EL, DeGowin RL. *Bedside Diagnostic Examination*. 3rd ed. New York: Macmillan; 1976.
80. Kunos G, Ishac EJN. Mechanism of inverse regulation of alpha 1- and beta-adrenergic receptors. *Biochem Pharmacol*. 1987;36:1185–1191.
81. Kunos G, Vermes-Kunos I, Nickerson M. Effects of thyroid state on adrenoceptor properties. *Nature*. 1974;250:779–781.
82. Constant EL, de Volder AG, Ivanoiu A, et al. Cerebral blood flow and glucose metabolism in hypothyroidism: a positron emission tomography study. *J Clin Endocrinol Metab*. 2001;86:3864–3870.
83. Stewart JH, Evans WF. Peripheral blood flow in myxedema. *Arch Intern Med*. 1942;69:808.
84. Watanakunakorn C, Hodges RE, Evans TC. Myxedema: a study of 400 cases. *Arch Intern Med*. 1965;116:183–190.
85. Kales A, Heuser G, Jacobson A, et al. All night sleep studies in hypothyroid patients, before and after treatment. *J Clin Endocrinol Metab*. 1967;27:1593–1599.
86. Gay RG, Raya TE, Lancaster LD, et al. Effects of thyroid state on venous compliance and left ventricular performance in rats. *Amer J Physiol*. 1988;254:H81–H88.
87. Guttler RB, Shaw JW, Otis CL, et al. Epinephrine-induced alterations in urinary cyclic AMP in hyper- and hypothyroidism. *J Clin Endocrinol Metab*. 1975;41:707–711.
88. Kohn LD. Connective tissue. In: Werner SC, Ingbar SH, eds. *The Thyroid*. New York: Harper & Row Publishers; 1978.
89. Thompson WG, Heaton KW. Functional bowel disorders in apparently healthy people. *Gastroenterology*. 1980;79:283–288.
90. Asboe-Hansen G. The variability in the hyaluronic acid content of the dermal connective tissue under the influence of thyroid hormone. *Acta Derm Venereol*. 1950;30:221–229.
91. Faber J, Horslev-Petersen K, Perrild H, et al. Different effects of thyroid disease on serum levels of procollagen III N-peptide and hyaluronic acid. *J Clin Endocrinol Metab*. 1990;71:1016–1021.
92. Smith TJ, Murata Y, Horwitz AL, et al. Regulation of glycosaminoglycan accumulation by thyroid hormone in vitro. *J Clin Invest*. 1982;70:1066–1073.
93. Nakamura H, Mori T, Genma R, et al. Urinary excretion of pyridinoline and deoxypyridinoline measured by immunoassay in hypothyroidism. *Clin Endocrinol*. 1996;44:447–451.
94. Foscolo G, Roiter I, De Menis E, et al. Bone metabolism in primary hypothyroidism in the adult. *Minerva Endocrinol*. 1991;7–10:16.
95. Eisinger J, Dupond JL, Cozzone PJ. Anomalies de la glycolyse au cours des fibromyalgies: étude biologique et thérapeutique. *Lyon Méd*. 1996;27:2180–2181.
96. Taylor DJ, Rajagopalan B, Radda GK. Cellular energetics in hypothyroid muscle. *Eur J Clin Invest*. 1992;22:358–365.
97. Sharma VK, Banerjee SP. Beta-adrenergic receptors in rat skeletal muscle: effects of thyroidectomy. *Biochim Biophys Acta*. 1978;539:538–542.
98. Chernausek SD, Underwood LE, Utiger RD, et al. Growth hormone secretion and plasma somatomedin-C in primary hypothyroidism. *Clin Endocrinol (Oxf)*. 1983;19:337–344.
99. Lowe JC, Yellin J. Inadequate thyroid hormone regulation as the main mechanism of fibromyalgia: a review of the evidence. *Thyroid Sci*. 2008;3(6):R1–R14.

100. Wilson J, Walton JN. Some muscular manifestations of hypothyroidism. *J Neurol Neurosurg Psychiatry*. 1959;22:320–324.
101. Fessel WJ. Myopathy of hypothyroidism. *Ann Rheum Dis*. 1968;27:590–596.
102. Bland JH, Frymoyer JW. Rheumatic syndromes of myxedema. *N Engl J Med*. 1970;282:1171–1174.
103. Golding DN. Hypothyroidism presenting with musculoskeletal symptoms. *Ann Rheum Dis*. 1970;29:10–14.
104. Hochberg MC, Koppes GM, Edwards CQ, et al. Hypothyroidism presenting as a polymyositis-like syndrome: report of two cases. *Arthritis Rheum*. 1976;19:1363–1366.
105. Beetham Jr WP. Diagnosis and management of fibrositis syndrome and psychogenic rheumatism. *Med Clin North Am*. 1979;63:433–439.
106. Wilke SW, Sheeler LR, Makarowski WS. Hypothyroidism with presenting symptoms of fibrositis. *J Rheumatol*. 1981;8:626–631.
107. Delamere JP, Scott DL, Felix-Davies DD. Thyroid dysfunction and rheumatic diseases. *J R Soc Med*. 1982;75:102–106.
108. Sonkin LS. Endocrine disorders and muscle dysfunction. In: Gelb H, ed. *Clinical Management of Head, Neck, and TMJ Pain and Dysfunction: A Multidisciplinary Approach to Diagnosis and Treatment*. Philadelphia: Saunders; 1985:137–170.
109. Awad EA. Histopathological changes in fibrositis. In: Fricton JR, Awad E, eds. *Advances in Pain Research and Therapy: Myofascial Pain and Fibromyalgia*. vol. 17. New York: Raven Press; 1990:249–258.
110. Awad EA. *Pathological Changes in Fibromyalgia*. Minneapolis: First International Symposium on Myofascial Pain and Fibromyalgia; 1989.
111. Lowe JC, Cullum M, Graf Jr L, et al. Mutations in the c-erbA beta 1 gene: do they underlie euthyroid fibromyalgia? *Med Hypotheses*. 1997;48:125–135.
112. Alajouanine T, Nick J. De l'existence d'une myopathie d'origine hypothyroidienne. *Paris Med*. 1945;35:346.
113. Bergouignan M, Vital C, Bataille JM. Hypothyroid myopathies. Clinical and histopathological aspects. *Presse Med*. 1967;75:1551–1556.
114. Aarflot T, Bruusgaard D. Association between chronic widespread musculoskeletal complaints and thyroid autoimmunity. Results from a community survey. *Scand J Prim Health Care*. 1996;14:111–115.
115. Rodolico C, Toscano A, Benvenga S, et al. Myopathy as the persistently isolated symptomatology of primary autoimmune hypothyroidism. *Thyroid*. 1998;8:1033–1038.
116. Lowe JC, Honeyman-Lowe G. Fibromyalgia and thyroid disease. Paper presented and discussed in Grenoble, France May 6 (Conference of the French Fibromyalgia Association of Région Rhône-Alpes) and in Toulon, France, May 11 (Centre Hospitalier Intercommunal), 2000.
117. Lowe JC, Honeyman-Lowe G. Thyroid disease and fibromyalgia syndrome. *Lyon Méditerranée Médical: Médecine du Sud-Est*. 2000;36:15–17.
118. Lowe JC, Yellin J, et al. Female fibromyalgia patients: lower resting metabolic rates than matched healthy controls. *Med Sci Monitor*. 2006;12(8):CR1–CR8.
119. Lowe, JC, Honeyman, G, Yellin, J. Lower resting metabolic rate and basal body temperature of fibromyalgia patients compared to matched healthy controls. *Thyroid Sci*. 1006;1(9):CLS1–CLS18.
120. Starlanyl DJ, Jeffrey JL, Roentsch G, et al. The effect of transdermal T_3 (triiodothyronine) on geloid masses found in patients with both fibromyalgia and myofascial pain: double-blinded, crossover N of 1 clinical study. *Myalgies Internat*. 2001;2:8–18.
121. Donaldson MS, Speight N, Loomis S. Fibromyalgia syndrome improved using a mostly raw vegetarian diet: an observational study. *BMC Complement Altern Med*. 2001;1:7.
122. Azad KA, Alam MN, Haq SA, et al. Vegetarian diet in the treatment of fibromyalgia. *Bangladesh Med Res Counc Bull*. 2000;26:41–47.
123. Smith JD, Terpening CM, Schmidt SO, et al. Relief of fibromyalgia symptoms following discontinuation of dietary excitotoxins. *Ann Pharmacother*. 2001;35:702–706.
124. Merchant RE, Andre CA. A review of recent clinical trials of the nutritional supplement Chlorella pyrenoidosa in the treatment of fibromyalgia, hypertension, and ulcerative colitis. *Altern Ther Health Med*. 2001;7:79–91.
125. Merchant RE, Carmack CA, Wise CM. Nutritional supplementation with Chlorella pyrenoidosa for patients with fibromyalgia syndrome: a pilot study. *Phytother Res*. 2000;14:167–173.
126. Hanninen O, Kaartinen K, Rauma A, et al. Antioxidants in vegan diet and rheumatic disorders. *Toxicology*. 2000;155:45–53.
127. Kaartinen K, Lammi K, Hypen M, et al. Vegan diet alleviates fibromyalgia symptoms. *Scand J Rheumatol*. 2000;29:308–313.
128. Mauro GL, Martorana U, Cataldo P, et al. Vitamin B_{12} in low back pain: a randomised, double-blind, placebo-controlled study. *Eur Rev Med Pharmacol Sci*. 2000;4:53–58.
129. Franca DS, Souza AL, Almeida KR, et al. B vitamins induce an antinociceptive effect in the acetic acid and formaldehyde models of nociception in mice. *Eur J Pharmacol*. 2001;421:157–164.
130. Waikakul W, Waikakul S. Methylcobalamin as an adjuvant medication in conservative treatment of lumbar spinal stenosis. *J Med Assoc Thai*. 2000;83:825–831.
131. Kuwabara S, Nakazawa R, Azuma N, et al. Intravenous methylcobalamin treatment for uremic and diabetic neuropathy in chronic hemodialysis patients. *Intern Med*. 1999;38:472–475.
132. Flynn MA, Irvin W, Krause G. The effect of folate and cobalamin on osteoarthritic hands. *J Am Coll Nutr*. 1994;13:351–356.
133. Eckert M, Schejbal P. Therapy of neuropathies with a vitamin B combination: symptomatic treatment of painful diseases of the peripheral nervous system with a combination preparation of thiamine, pyridoxine and cyanocobalamin. *Fortschr Med*. 1992;20(110):544–548.
134. Leuschner J. Antinociceptive properties of thiamine, pyridoxine and cyanocobalamin following repeated oral administration to mice. *Arzneimittelforschung*. 1992;42:114–115.
135. Seror P. Sciatica cured by vitamin B_{12}. *Rev Rhum Mal Osteoartic*. 1989;56:344.
136. Dordain G, Aumaitre O, Eschalier A, et al. Vitamin B_{12}, an analgesic vitamin? Critical examination of the literature. *Acta Neurol Belg*. 1984;84:5–11.
137. Goldfarb AH. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. *Can J Appl Physiol*. 1999;24:249–266.
138. Narchi H, Thomas M. A painful limp. *J Paediatr Child Health*. 2000;36:277–278.
139. Lytle RL. Chronic dental pain: possible benefits of food restriction and sodium ascorbate. *J Appl Nutr*. 1988;40:95–98.
140. Hodges RE, Hood J, Canham JE, et al. Clinical manifestations of ascorbic acid deficiency in man. *Am J Clin Nutr*. 1971;24:432–443.
141. Kinsman RA, Hood J. Some behavioral effects of ascorbic acid deficiency. *Am J Clin Nutr*. 1971;24:455–464.
142. Hirschmann JV, Raugi GJ. Adult scurvy. *J Am Acad Dermatol*. 1999;41:895–906.
143. Gaby AR. Intravenous nutrient therapy: the "Myers' cocktail. *Altern Med Rev*. 2002;7:389–403.
144. Caruso I, Sarzi Puttini P, Cazzola M, et al. Double-blind study of 5-hydroxytryptophan versus placebo in the treatment of primary fibromyalgia syndrome. *J Intern Med Res*. 1990;18:201–209.
145. Puttini PS, Caruso I. Primary fibromyalgia syndrome and 5-hydroxy-tryptophan: a 90-day open study. *J Intern Med Res*. 1992;20:182–189.
146. Jacobsen S, Danneskiold-Samsoe B, Andersen RB. Oral S-adenosylmethionine in primary fibromyalgia: a double-blind clinical evaluation. *Scand J Rheumatol*. 1991;20:294–302.
147. Tavoni A, Vitali C, Bombardieri S, et al. Evaluation of S-adenosylmethionine in primary fibromyalgia: a double-blind crossover study. *Am J Med*. 1987;83(5A):107–110.
148. Volkmann H, Norregaard J, Jacobsen S, et al. Double-blind, placebo-controlled cross-over study of intravenous S-adenosyl-L-methionine in patients with fibromyalgia. *Scand J Rheumatol*. 1997;26:206–211.
149. Lieber CS, Packer L, S-Adenosylmethionine. molecular, biological, and clinical aspects: an introduction. *Am J Clin Nutr*. 2002;76:S1148–S1150.

150. Abraham GE, Flechas JD. Management of fibromyalgia: rationale for the use of magnesium and malic acid. *J Nutr Med*. 1992;3:49–59.
151. Russell IJ, Michalek JE, Flechas JD, et al. Treatment of fibromyalgia syndrome with Super Malic: a randomized, double blind, placebo controlled, crossover pilot study. *J Rheumatol*. 1995;22:953–958.
152. Dykman KD, Tone C, Ford C, et al. The effects of nutritional supplements on the symptoms of fibromyalgia and chronic fatigue syndrome. *Integr Physiol Behav Sci*. 1998;33:61–71.
153. Olson GB, Savage S, Olson J. The effects of collagen hydrolysat on symptoms of chronic fibromyalgia and temporomandibular joint pain. *Cranio*. 2000;18:135–141.
154. Bramwell B, Ferguson S, Scarlett N, et al. The use of ascorbigen in the treatment of fibromyalgia patients: a preliminary trial. *Altern Med Rev*. 2000;5:455–462.
155. Mengshoel AM. Effect of physical exercise in fibromyalgia. *Tidsskr Nor Laegeforen*. 1996;116:746–748.
156. McCain GA. Role of physical fitness training in the fibrositis/fibromyalgia syndrome. *Am J Med*. 1986;81(3A):73–77.
157. Mengshoel AM, Komnæ HB, Forre O. The effects of 20 weeks of physical fitness training in female patients with fibromyalgia. *Clin Exp Rheumatol*. 1992;10:345–349.
158. Nichols DS, Glen TM. Effects of aerobic exercise on pain perception, affect, and level of disability in individuals with fibromyalgia. *Phys Ther*. 1994;74:327–332.
159. Bailey A, Starr L, Alderson M, et al. A comparative evaluation of a fibromyalgia rehabilitation program. *Arthritis Care Res*. 1999;12:336–340.
160. Wolfe F. The clinical syndrome of fibrositis. *Am J Med*. 1986;81:7–14.
161. McCain GA. Nonmedicinal treatments in primary fibromyalgia. *Rheumat Dis Clin North Am*. 1989;15:73–90.
162. McCain GA, Bell DA, Mai FM, et al. A controlled study of the effects of a supervised cardiovascular fitness training program on the manifestations of primary fibromyalgia. *Arthritis Rheum*. 1988;31:1135–1141.
163. Wigers SH, Stiles TC, Vogel PA. Effects of aerobic exercise versus stress management treatment in fibromyalgia. *Scand J Rheumatol*. 1996;25:77–86.
164. Burckhardt CS, Mannerkorpi K, Hedenberg L, et al. A randomized, control clinical trial of education and physical training for women with fibromyalgia. *J Rheumatol*. 1994;21:714–720.
165. Mengshoel AM, Førre O. Physical fitness training in patients with fibromyalgia. *J Musculoskel Pain*. 1993;1:267–272.
166. Clark SR, Burckhardt C, Campbell S, et al. Fitness characteristics and perceived exertion in women with fibromyalgia. *J Musculoskel Pain*. 1993;1:191–197.
167. Poelhman ET, Berke EM, Joseph JR, et al. Influence of aerobic capacity, body composition, and thyroid hormones on the age-related decline in resting metabolic rate. *Metabolism*. 1992;41:915–921.
168. Broeder CE, Burrhus KA, Svanevik LS, et al. The effects of either high-intensity resistance or endurance training on resting metabolic rate. *Am J Clin Nutri*. 1992;55:802–810.
169. Mengshoel AM, Haugen M. Health status in fibromyalgia: a follow-up study. *J Rheumatol*. 2001;28:2085–2089.
170. Bennett RM, Clark SR, Goldberg L, et al. Aerobic fitness in patients with fibrositis: a controlled study of respiratory gas exchange and ^{133}xenon clearance from exercising muscle. *Arthritis Rheum*. 1989;32:454–460.
171. Klug GA, McAuley E, Clark S. Factors influencing the development and maintenance of aerobic fitness: lessons applicable to the fibrositis syndrome. *J Rheumatol Suppl*. 1989;19:30–39.
172. Bennett RM, Clark SR, Campbell SM, et al. Symptoms of Raynaud's syndrome in patients with fibromyalgia: a study utilizing the Nielsen test, digital photoplethysmography and measurements of platelet alpha 2-adrenergic receptors. *Arthritis Rheum*. 1991;34:264–269.
173. Wirz-Justice A. Platelet research in psychiatry. *Experientia*. 1988;44:145–152.
174. Lowe JC, Honeyman-Lowe G. The metabolic rehabilitation of fibromyalgia patients. In: Chaitow L, ed. *Fibromyalgia Syndrome: A Practitioner's Guide to Treatment*. Edinburgh: Churchill Livingstone; 2000:131–143.
175. Lowe JC, Honeyman-Lowe G. Facilitating the decrease in fibromyalgic pain during metabolic rehabilitation: an essential role for soft tissue therapies. *J Bodywork Mov Ther*. 1998;2:208–217.
176. Honeyman-Lowe G. Ultrasound treatment of the fibromyalgia patient. Paper presented at the French Fibromyalgia Association of Région Rhône-Alpes: Grenoble, France, 2000.
177. Honeyman-Lowe G. Ultrasound treatment of the fibromyalgia patient. *Lyon Méditerranée Médical: Médecine du Sud-Est*. 2000;36:30–31.
178. Pioro-Boisset M, Esdaile JM, Fitzcharles MA. Alternative medicine use in fibromyalgia syndrome. *Arthritis Care Res*. 1996;9:13–17.
179. Gamber RG, Shores JH, Russo DP, et al. Osteopathic manipulative treatment in conjunction with medication relieves pain associated with fibromyalgia syndrome: results of a randomized clinical pilot project. *J Am Osteopath Assoc*. 2002;102:321–325.
180. Hains G, Hains F. A combined ischemic compression and spinal manipulation in the treatment of fibromyalgia: a preliminary estimate of dose and efficacy. *J Manipulative Physiol Ther*. 2000;23:225–230.
181. Blunt KL, Rajwani MH, Guerriero RC. The effectiveness of chiropractic management of fibromyalgia patients: a pilot study. *J Manipulative Physiol Ther*. 1997;20:389–399.
182. Offenbacher M, Stucki G. Physical therapy in the treatment of fibromyalgia. *Scand J Rheumatol Suppl*. 2000;113:78–85.
183. Lowe JC. The myofascial genesis of unpleasant thoughts and emotions: its neural basis. *Dig Chiro Econ*. 1989;32(78):80–81.
184. Harding SM. Sleep in fibromyalgia patients: subjective and objective findings. *Am J Med Sci*. 1998;315:367–376.
185. Moldofsky H. Sleep and fibrositis syndrome. *Rheum Dis Clin North Am*. 1989;15:91–103.
186. National Academy of Clinical Chemists. Available online at: http://www.nacb.org/lmpg/thyroid_LMPG_PDF.stm. Accessed July 5, 2004.
187. Volpé R. Autoimmune thyroiditis. In: Braverman LE, Utiger RD, eds. *Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text*. Philadelphia: Lippincott; 1991:921–933.
188. Wikland B. Redefining hypothyroidism: a paradigm shift. *Thyroid Sci*. 2008;3(1):1.
189. Lowe JC. Thyroid hormone replacement therapies: ineffective and harmful for many hypothyroid patients. *Thyroid Sci*. 2006;1(1):C1–C21.
190. Pearce CJ, Himsworth RL. Total and free thyroid hormone concentrations in patients receiving maintenance replacement treatment with thyroxine. *Br Med J*. 1984;288:693.
191. Hamburger JI. Strategies for cost-effective thyroid function testing with modern methods. In: Hamburger JI, ed. *Diagnostic Methods in Clinical Thyroidology*. New York: Springer-Verlag; 1989:63–109.
192. Lowe JC. Thyroid status of 38 fibromyalgia patients: implications for the etiology of fibromyalgia. *Clin Bull Myofascial Ther*. 1997;2:47–64.
193. Lowe JC, Reichman A, Honeyman GS, et al. Thyroid status of fibromyalgia patients (abstract). *Clin Bull Myofascial Ther*. 1998;3:69–70.
194. Fraser WD, Biggart EM, O'Reilly DS, et al. Are biochemical tests of thyroid function of any value in monitoring patients receiving thyroxine replacement? *Br Med J (Clin Res Ed)*. 1986;293:808–810.
195. Escobar-Morreale HF, del Rey FE, Obregón MJ, et al. Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. *Endocrinology*. 1996;137:2490–2502.
196. Escobar-Morreale HF, Obregón MJ, Escobar del Rey F, et al. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest*. 1995;96:2828–2838.
197. Costin G, Kaplan SA, Ling SM. The Achilles reflex time in thyroid disorders. *J Pediatr*. 1970;76:277–282.
198. Johansen K, Hansen JM, Skovsted L. Myxedema and thyrotoxicosis: relations between clinical state and concentrations of thyroxine and triiodothyronine in blood. *Acta Med Scand*. 1978;204:361–364.
199. Al-Adsani H, Hoffer LJ, Silva JE. Resting energy expenditure is sensitive to small dose changes in patients on chronic thyroid hormone replacement. *J Clin Endocrinol Metab*. 1997;82:1118–1125.
200. Wigers SH, Skrondal A, Finset A, et al. Measuring change in fibromyalgic pain: the relevance of pain distribution. *J Musculoskel Pain*. 1997;5:29–41.
201. Chaves TC, Nagamine HM, de Sousa LM, et al. Comparison between the reliability levels of manual palpation and pressure pain threshold

in children who reported orofacial pain. *Man Ther*. 2010;15(5):508–512.
202. Burckhardt CS, Clark SR, Bennett RM. The fibromyalgia impact questionnaire: development and validation. *J Rheumatol*. 1991;18:728–733.
203. Jones J, Rutledge DN, Jones KD, et al. Self-assessed physical function levels of women with fibromyalgia: a national survey. *Women's Health Issues*. 2008;18(5):406–412.
204. Zung WW. A self-rating depression scale. *Arch Gen Psychiatry*. 1965;12:63–70.
205. Harris J, Benedict F. A biometric study of human basal metabolism. *Proc Sci USA*. 1918;4(12):370–373.
206. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr*. 2000;71(suppl 1):S343–S348.
207. Seaman DR. The diet-induced proinflammatory state: a cause of chronic pain and other degenerative diseases? *J Manipulative Physiol Ther*. 2002;25:168–179.
208. Travell JG, Simons DG. *Myofascial Pain and Dysfunction: The Trigger Point Manual*. vol. 1. Baltimore: Williams & Wilkins; 1983.
209. Christy NP. The adrenal cortex in hypothyroidism. In: Braverman LE, Utiger RD, eds. *Werner's the Thyroid: A Fundamental and Clinical Text*. Philadelphia: JB Lippincott Co; 1991:1045–1049.
210. Hoffman BB, Lefkowitz RJ. Adrenergic receptor antagonists. In: Gilman AG, Rall TW, Nies AS, Tayleor P, eds. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*. New York: Pergamon Press; 1990:221–243.

171

Gallstones

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- May be asymptomatic or cause biliary colic with irregular pain-free intervals of days or months
- Real-time ultrasonography provides a definitive diagnosis.

GENERAL CONSIDERATIONS

Gallstones are another example of a "Western diet"–induced disease. In the United States, autopsy studies have shown that gallstones exist in approximately 20% of women and 8% of men older than age 40. Conservative estimates suggest that about 20 million Americans (10% of the U.S. population) and 5% to 22% of the population in the Western world have gallstones.[1] Each year, 1 million more develop gallstones. More than 300,000 gallbladders are removed each year in the United States owing to the presence of gallstones.[2–5] Gallstones are also considered a significant risk factor for gallbladder cancer.[6,7]

The bile components include bile salts, bilirubin, cholesterol, phospholipids, fatty acids, water, electrolytes, and other organic and inorganic substances.[8] Table 171.1 shows the characteristics of the major bile components. Gallstones arise when a normally solubilized component of bile becomes supersaturated and precipitates (Fig. 171.1).

Gallstones can be divided into four major categories:

- Pure cholesterol
- Pure pigment (calcium bilirubinate)
- Mixed—containing cholesterol and its derivatives along with varying amounts of bile salts, bile pigments, and inorganic salts of calcium
- Stones composed entirely of minerals

Pure stones, either cholesterol or calcium bilirubinate, are extremely rare in the United States, where recent studies indicate that approximately 80% of the stones are of the mixed variety. The remaining 20% of the stones are composed entirely of minerals, principally calcium salts, although some stones contain oxides of silicon and aluminum.[2–4]

PATHOGENESIS

The formation of gallstones has been divided into three steps:

- Bile supersaturation
- Nucleation and initiation of stone formation
- Enlargement of the gallstone by accretion

Cholesterol and Mixed Stones

The requisite step in the formation of cholesterol and mixed stones is cholesterol supersaturation of bile within the gallbladder. Bile solubility, as well as bile supersaturation, is based on the relative molar concentrations of cholesterol, bile acids, phosphatidylcholine (lecithin), and water. Because free cholesterol is water insoluble, it must be incorporated into a lecithin–bile salt micelle (Fig. 171.2). Using triangular coordinates, it is possible to demonstrate the solubility of cholesterol in bile (Fig. 171.3).

As illustrated in the figures, either an increase in cholesterol secretion or a decrease in bile acid or lecithin secretion will lead the bile to become supersaturated. Once the bile is supersaturated, stone formation is initiated by such factors as biliary stasis, infection, and increased mucin secretion by the gallbladder epithelium. Once the stone begins to form, its radius increases at an average rate of 2.6 mm/year, eventually reaching a size of a few millimeters to more than a centimeter. Symptoms typically occur an average of 8 years after formation begins. Cholelithiasis is present in 95% of patients with cholecystitis.[8]

Risk Factors for Cholesterol and Mixed Stones

The major risk factors for the development of cholesterol and mixed gallstones include the following[2–4]:

- Diet
- Gender
- Race
- Obesity
- High caloric intake
- Estrogens

TABLE 171.1 Characteristics of the Major Bile Components

Component	Percentage of Bile	Water Solubility	Physiochemical Properties
Cholesterol	5	Very poor	Will precipitate from aqueous solutions
Bile salts	69–90	Soluble; have polar and nonpolar regions	Capable of solubilizing cholesterol and phospholipid in aqueous phase
Phospholipid	2–25	Poor	Fits between bile salt molecules, thus increasing their capacity to solubilize cholesterol

Modified from Rubenstein E, Federman DD. *Scientific American Medicine*. New York: Scientific American; 1986;4: VI-1–VI-10.

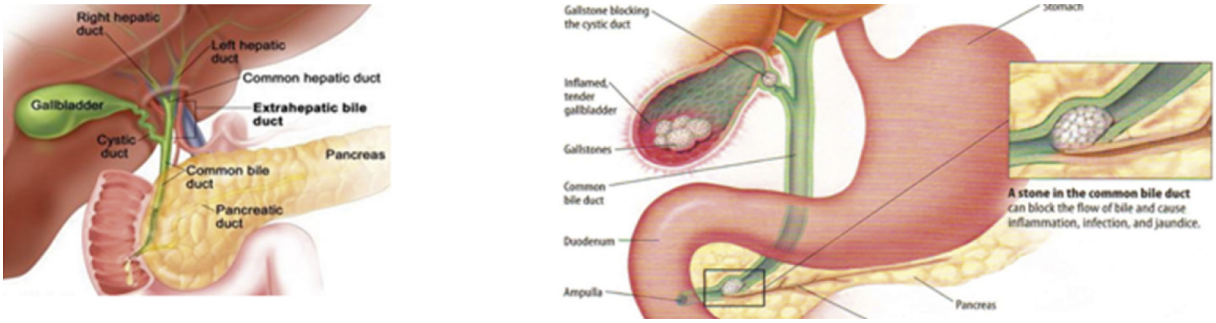


Fig. 171.1 Gallstones.

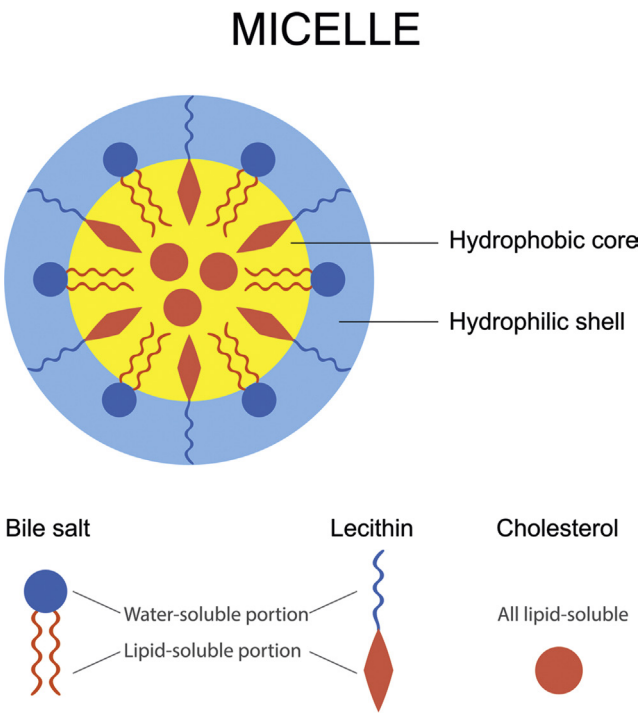


Fig. 171.2 Mixed micelle. (From FancyTapis/iStock.com.)

- Gastrointestinal diseases (especially Crohn's disease and cystic fibrosis)
- Drugs
- Age

The role of a low-fiber, high-fat diet in the development of gallstones, as well as other dietary factors, is discussed later. The other factors are briefly discussed here.

Gender

The frequency of gallstones is two to four times greater in women than in men. Women are thought to be predisposed to gallstones because of either increased cholesterol synthesis or suppression of bile acids by estrogens. Pregnancy, the use of oral contraceptives or other causes of elevated estrogen levels, and tamoxifen use greatly increase the incidence of gallstones.

Genetic and Ethnic

The prevalence of gallstones appears to have some genetic aspects. Gallstones are most common in Native American women over age 30. Nearly 70% of this group have gallstones. In contrast, only 10% of black women over 30 have gallstones. The difference in the prevalence rate between ethnic and genetic groups reflects the extent of cholesterol saturation of the bile. The degree to which dietary factors affect this value probably outweighs genetic factors.[9]

Obesity

Obese subjects are at risk of developing gallstones by being overweight and when initially losing weight.[10] Obesity causes increased activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, with increased secretion of cholesterol in the bile as a result of increased cholesterol synthesis. Therefore obesity is associated with a significantly increased incidence of gallstones due to biliary cholesterol saturation.

It is important to note that during active weight reduction, changes in body fat and diet can promote gallstone problems. During the first stages of weight loss, biliary cholesterol saturation initially increases.[4] The secretion of all biliary lipids is reduced during weight loss, but the secretion of bile acids decreases more than that of cholesterol. Once the weight is stabilized, bile acid output returns to normal levels, whereas the cholesterol output remains low. The net effect is a significant reduction in cholesterol saturation. In prescribing diet therapies for obese patients with a high risk of gallstones, it should be recognized that prolonged dietary fat reduction can also promote biliary stasis,[1] thus contributing to cholesterol saturation. Studies show that at least 10 g of fat per day is necessary to assure proper gallbladder emptying.[11]

Gastrointestinal Tract Diseases

Malabsorption of bile acids from the terminal ileum disturbs the enterohepatic circulation, thereby reducing the bile acid pool and the rate of secretion of bile. Diseases associated with this phenomenon include Crohn's disease and cystic fibrosis.

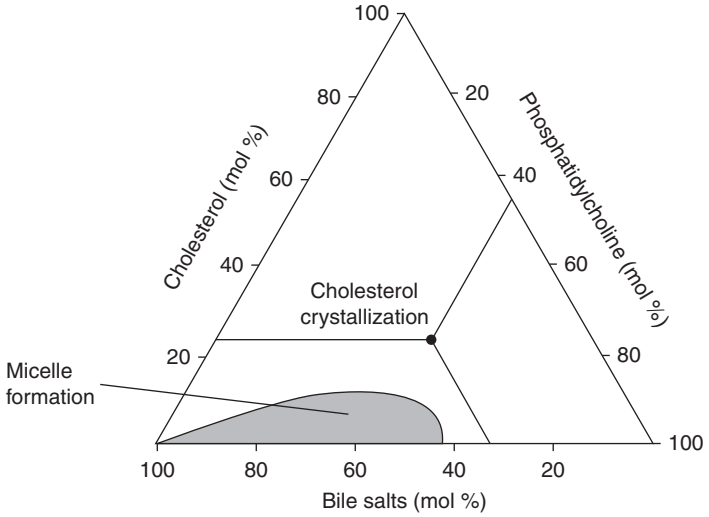


Fig. 171.3 Cholesterol solubility in bile.

Drugs

Tamoxifen treatment in postmenopausal patients with breast cancer greatly increases gallstones. One retrospective cohort study of 703 women demonstrated that after 5 years, the incidence of stone formation in the tamoxifen-treated patients was 37.4%, whereas it was 2% in patients who did not receive tamoxifen.[12] Most gallstones became apparent after 3 years.

In addition to oral contraceptives and other estrogens, other drugs that increase the risk of gallstones include the cephalosporin ceftriaxone, octreotide, HMG-CoA reductase inhibitors,[1] and possibly other lipid-lowering drugs.

Age

Gallstones have been reported from fetal age to extreme old age, but the average patient is 40 to 50 years old. A decline in the activity of cholesterol 7-α-hydroxylase with age[1] leads to an increase in biliary cholesterol hypersecretion[13] and thus cholesterol saturation, with an accelerated formation of gallstones.

Hypothyroidism

Patients with hypothyroidism are more prone to have high serum cholesterol levels. The mechanisms of thyroid hormones on cholesterol are multifactorial, including influencing the synthesis, absorption, and usage of cholesterol. Disturbances in lipid metabolism that occur during hypothyroidism, particularly in the cholesterol pathway, change the rate of bile excretion and lead to the formation of gallstones. In regression multivariate analysis, it has been concluded that the level of serum thyroid-stimulating hormone (TSH) was an independent factor that could be considered a risk factor for the formation of common bile duct stones (odds ratio [OR] = 3.07; 95% confidence interval [CI], 1.51–6.3).[14]

Environmental Pollutants

Metal contamination of both natural and anthropogenic origin has an adverse effect on human health. A study explored the effects of metal contamination on gallstone formation by comparing two environmentally contrasting populations in the Huelva Province of Southwest Spain.[15] The study population resided in an area with high metal abundance derived from the naturally metal-enriched bedrock and historical mining activities in the region of the Iberian Pyrite Belt, whereas the control population resided in the National Park of Sierra de Aracena and Picos de Aroche. The patients from the study group had a higher risk of metal exposure through contaminated soil, particle matter in the air, and consumption of local water and food products. Results demonstrated that metal exposure was related to a higher tendency of forming black-pigment gallstones in the study group compared with the control group, and the gallstones from the study group contained more abundant metal components, such as copper, iron, nickel, and zinc. In addition, a study in India identified a positive correlation between nickel, cadmium, and chromium in water and a high prevalence of gallbladder disease in adjacent villages in Vaishali District, Bihar.[16]

Organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) historically used in the agricultural control of pests. A case-control study from China showed that high levels of p,p′-DDE and p,p′-DDT residues were risk factors for gallstone formation.[17] Because of their lipophilic property, OCPs can accumulate in adipose tissue and other organs. β-HCH and p′,p′-DDE levels in adipose tissues were found to be higher in patients with gallstones and strongly related to gallstone disease.[18]

Risk Factors for Pigmented Gallstones

Risk factors for pigmented gallstones are not related to diet as much as they are to geography, sun exposure, and severe diseases. Pigmented gallstones are more common in Asia because of the higher incidence of parasitical infections of the liver and gallbladder by various organisms, including the liver fluke *Clonorchis sinensis.* Bacteria and protozoa can cause stasis or act as nucleating agents. In the United States, pigmented stones are usually caused by chronic hemolysis or alcoholic cirrhosis of the liver.

THERAPEUTIC CONSIDERATIONS

Gallstones are easier to prevent than to reverse. Primary treatment, therefore, involves reducing the controllable risk factors discussed earlier. Once gallstones have formed, therapeutic intervention involves avoiding aggravating foods and employing measures that increase the solubility of cholesterol in bile. If symptoms persist or worsen, cholecystectomy should be considered.

A number of dietary factors are important in the prevention and treatment of gallstones. Foremost is the elimination of foods that can produce symptoms. Also important are increasing dietary fiber, eliminating food allergies, and reducing the intake of refined carbohydrates and animal protein. There is a protective effect of vegetables and fruits

against gallbladder cancer, and red meat (beef and mutton) was found to be associated with an increased risk of gallbladder carcinogenesis.[19] Because gallstones are a risk factor for gallbladder cancer,[6,7,19] appropriate nonlithogenic dietary practices will protect against both cancer and the development of gallstones.

Other treatment measures involve the use of nutritional lipotropic compounds, herbal choleretics, and other natural compounds in an attempt to increase the solubility of bile.

Biliary cholesterol concentration and serum cholesterol levels do not seem to correlate.[20] Although some authors have reported an inverse correlation between high-density-lipoprotein cholesterol and bile saturation and a direct correlation between low-density-lipoprotein cholesterol and bile saturation,[21,22] more detailed research does not appear to support these correlations.[20] There does, however, seem to be an association between increased levels of serum triglycerides and bile saturation.[23]

Asymptomatic Gallstones

The natural history of silent or asymptomatic gallstones supports the contention that elective cholecystectomy is not warranted. Although there is a cumulative chance of developing symptoms—10% at 5 years, 15% at 10 years, and 18% at 15 years—a patient should never experience discomfort if controllable risk factors are eliminated or reduced.[3]

Diet

Dietary Fiber

The hypothesis that the main cause of gallstones is the consumption of fiber-depleted refined foods has considerable research support.[4,5] As mentioned earlier, in population studies, gallstones are associated with the Western diet. Such a diet, high in refined carbohydrates and fat and low in fiber, leads to a reduction in the synthesis of bile acids by the liver and a lower bile acid concentration in the gallbladder.

Another way in which fiber may prevent gallstone formation is by reducing the absorption of deoxycholic acid.[4,5] This compound is produced from bile acids by bacteria in the intestine. Deoxycholic acid greatly lessens the solubility of cholesterol in bile.

Dietary fiber has been shown both to decrease the formation of deoxycholic acid and to bind deoxycholic acid and promote its excretion in the feces. This greatly increases the solubility of cholesterol in the bile. A diet high in fiber, especially those fibers capable of binding to deoxycholic acid (e.g., predominantly the water-soluble fibers found in vegetables and fruits, pectin, oat bran, and guar gum), is extremely important in both the prevention and reversal of most gallstones.[5]

Interestingly, diets rich in legumes with high concentrations of water-soluble fibers are associated with an increased risk for gallstones.[9] Chileans, Pima Indians, and other North American Indians have the highest prevalence rates for cholesterol gallstones, and all consume a diet rich in legumes. Evidently, legume intake increases biliary cholesterol saturation because of legumes' saponin content.[9] A study conducted in the Netherlands showed just the opposite because legume intake was shown to offer significant protection against gallstones.[24] Until this issue is clarified, it might be best to restrict legume intake in individuals with existing gallstones.

Vegetarian Diet

A vegetarian diet has been shown to protect against gallstone formation.[5,25] A recent study in England compared a large group of healthy nonvegetarian women with a group of vegetarian women. Ultrasound diagnosis showed that gallstones occurred significantly less frequently in the vegetarian group.

Although this may simply be a result of the increased fiber content of the vegetarian diet, other factors may be equally important. Animal proteins, such as casein from dairy products, have been shown to increase the formation of gallstones in animals, whereas vegetable proteins, such as soy, were shown to be preventive against gallstone formation.[4,5,26]

Food Allergies

Since 1948, J. C. Breneman, author of *Basics of Food Allergy,* has used a successful therapeutic regimen to prevent gallbladder attacks: allergy elimination diets. The idea that food allergies cause gallbladder pain has some support in the scientific literature.[27–30] A 1968 study revealed that 100% of a group of patients were free from symptoms while they were on a basic elimination diet (beef, rye, soybean, rice, cherry, peach, apricot, beet, and spinach).[27] Foods inducing symptoms, in decreasing order of their occurrence, were as follows:

- Egg
- Pork
- Onion
- Fowl
- Milk
- Coffee
- Citrus
- Corn
- Beans
- Nuts

The addition of eggs to the diet caused gallbladder attacks in 93% of the patients.

Several mechanisms have been proposed to explain the association of food allergy and gallbladder attacks. Breneman believes that the ingestion of allergy-causing substances causes swelling of the bile ducts, resulting in impairment of bile flow from the gallbladder.

Buckwheat

Buckwheat is a well-known alternative for those avoiding wheat for hypoallergenic purposes. When three groups of eight hamsters were given a buckwheat-, soy-, or casein-based diet, a Japanese research group demonstrated that buckwheat can significantly decrease gallstone formation and reduce the concentration of cholesterol in the gallbladder, plasma, and liver of hamsters compared with the casein diet.[31] Even though soy is a known potent gallstone attenuator itself,[32] these researchers found that the positive effects of buckwheat were far stronger than those of soy. Gallstones were clearly visible in all eight hamsters fed the casein diet, whereas two of seven hamsters fed the soy diet (29%) and none of the buckwheat-fed animals had gallstones. Studies in rats have also corroborated these findings.[33] Rats consuming high-protein buckwheat flour (PBF) had a 33% decrease in serum cholesterol and a significant decrease in liver cholesterol compared with rats consuming casein.[34] In addition, consumption of PBF for 10 days significantly suppressed adipose tissue weight and the hepatic activity of fatty acid synthase in rats fed cholesterol-free diets compared with the consumption of casein, and PBF significantly decreased the incidence of cholesterol gallstones and the lithogenic index in mice fed cholesterol-enriched diets for 37 days. The authors suggest that buckwheat can enhance bile acid synthesis and fecal excretion of steroidal compounds, and PBF has strong activities against hypercholesterolemia, obesity, and gallstone formation. Buckwheat may be useful to treat patients with both high cholesterol and gallstones and may reduce the proliferation of colon cancer cells.[35] The authors also postulated that higher levels of arginine and glycine may play a role in buckwheat's protective function.

Sugar

A 4-year study evaluated 111 cases of biliary tract cancer and compared them with 480 controls for various dietary parameters, including

sugar intake. The major finding was an increased risk associated with the intake of sugars, either monosaccharides or disaccharides, independent of other energy sources. Sugar may be a risk factor for biliary tract cancer based on the relationship among sugars, blood lipids, and gallstone formation. The author also noted an association between the intake of sugar and gallstones. Diets high in refined carbohydrates have been found to be associated with increased cholesterol saturation of the bile.[36,37] A 25-year follow-up study of 860 men, 54 of whom developed symptomatic gallstones, also found a positive association between sugar consumption and gallstones.[38]

Caloric Restriction

In a case-controlled study in which 200 patients with gallstones were compared with 98 controls, total caloric and carbohydrate intake and serum triglyceride levels were higher in the patients with gallstones. Dietary intake of refined carbohydrates was higher in female patients with gallstones, whereas male patients with gallstones had an increased fat intake.[39] In another study, 76 patients whose gallstones were detected by ultrasound were matched to control subjects without cholelithiasis. It was found that high caloric intake of greater than 2500 kcal/day and diets rich in carbohydrates and saturated fats significantly increased gallstone risk.[40] One intriguing note to this study was the finding that alcohol consumption of 20 to 40 g/day was protective.

However, caloric restriction must be instituted carefully, because rapid weight loss[41] and fasting[42] increase the risk of gallstones (see earlier section on obesity). For example, in 179 obese patients, 9% of whom had preexisting gallstones, a low-calorie (605 kcal) diet resulted in 11% of the patients developing gallstones either during or within 6 months of completing the diet.[41] Another study of a 925-kcal diet found that 12.8% of the 47 female patients displayed gallstones at week 17 as determined by sonography. Those who developed gallstones had significantly higher baseline triglyceride and total cholesterol levels than those who did not. They also had a significantly greater rate of weight loss.[43] A 1-year matched cohort study of consecutively enrolled adults in a commercial weight-loss program using very low-calorie diets (VLCDs) or a low-calorie diet (LCD) assessed the risk of symptomatic gallstones requiring hospital care and/or cholecystectomy.[44] During the 6361 person-years, 48 and 14 gallstones requiring hospital care occurred in the VLCD and LCD groups, respectively, and of the 62 gallstone events, 38 (61%) resulted in cholecystectomy. Albeit low, the risk of symptomatic gallstones requiring hospitalization or cholecystectomy was threefold greater with VLCD than LCD during the 1-year weight-loss program.

Coffee

Although coffee can promote symptoms of gallstones, it may also inhibit their formation. In one study, 400 mL of regular coffee and 165 mL of regular and decaffeinated coffee were assessed for their effect on cholecystokinin secretion. Regular coffee at both dosages and decaffeinated coffee caused significant gallbladder contractions compared with the control sodium chloride in six healthy regular coffee drinkers.[45] Another study of 80,898 female nurses between the ages of 34 and 59 years found that drinking four cups of caffeinated coffee per day was associated with a 28% lowered risk of developing symptoms of gallstones. Even one to three cups seemed to have some protective effect.[46] Five prospective cohort studies involving 227,749 participants and 11,477 gallstone disease cases found that coffee consumption was significantly associated with a reduced risk of gallstone disease in a dose–response relationship.[47]

It may be that women who began to drink coffee regularly before or during early gallstone formation were able to either inhibit the development of stones or clear any small stones due to the enhanced gallbladder contractions from multiple daily coffee doses. In women who already have large stones present, increased contractions from coffee may exacerbate their condition and cause untoward symptoms.

Nutritional Supplements

Lecithin (Phosphatidylcholine)

As the main cholesterol solubilizer in bile, lecithin in low concentrations may be a causative factor for many individuals with gallstones. For example, a pure bile salt micelle requires 50 molecules to enclose a single molecule of cholesterol, whereas a mixed bile salt/phospholipid micelle requires only seven molecules.[8] Studies have shown that the ingestion of lecithin can have the direct effect of solubilizing cholesterol.[48] As little as 100 mg of lecithin taken three times a day will increase the concentration of lecithin in the bile, whereas larger doses (up to 10 g) produce even greater increases.[49,50] This is significant because an increased lecithin content of bile usually increases the solubility of cholesterol. However, no significant effects on gallstone dissolution have been obtained using lecithin supplementation alone.

Nutrient Deficiencies

Deficiencies of either vitamin E or C have been shown to cause gallstones in experimental studies in animals.[51,52]

Olive Oil

A popular lay remedy for gallstones is the olive oil "liver flush." There are several variations. A typical one involves drinking 1 cup of unrefined olive oil with the juice of two lemons in the morning for several days.

Many people tell tales of passing huge stones while on the liver flush. However, what they think are gallstones are simply soft, saponified complexes of minerals, olive oil, and lemon juice produced within the gastrointestinal tract.[53]

The olive oil liver flush is undesirable for patients with gallstones for several reasons. First, consuming a large quantity of any oil results in contraction of the gallbladder, which may increase the likelihood that a stone will block the bile duct. This may result in cholecystitis, requiring immediate surgery to prevent death. Second, oleic acid, the main component of olive oil, has been shown to increase the development of gallstones in rabbits and rats by increasing the content of cholesterol in the gallbladder.[54,55] Although this effect has not yet been observed in humans, the animal research suggests that it is unwise to use an olive oil liver flush as a treatment for gallbladder disease.[56] This recommendation is not meant to obviate the use of the liver flush as a viable method of liver detoxification. Rather, it is a caution against its use in patients with gallstones.

Fish Oils

Although human studies appear not to be available at this time, some provocative animal studies exist. For example, one study compared a diet high in lard with a diet high in fish oils in 21 male African green monkeys. One group was fed 0.8 mg cholesterol per kilocalorie and 42% of the calories as fat, with one half of the fat calories derived from lard, whereas the other group was fed a similar diet with an isocaloric substitution of menhaden oil instead of the lard. After 2 to 3 years, necropsies were performed, and it was found that 67% of the animals fed the lard diet had cholesterol gallstones compared with 22% of the animals fed the fish oil. The cholesterol saturation index of the gallbladder bile was also higher in the lard-fed group, although there was no difference in bile acids. There was a significantly greater rate of biliary phospholipid secretion in the fish oil–fed animals compared with the lard-fed animals.[57]

A study found similar effects in a prairie dog model using a diet more typical of human habits: 12 animals consumed a standard control diet, whereas 16 consumed a lithogenic 1.2% cholesterol diet. Half the animals in each group were supplemented with 200 mg/kg/day of omega-3 fatty acids from eicosapentaenoic and docosahexaenoic acids. After 14 days, those fed the diet with added fish oil showed an inhibition of gallstone formation accompanied by a significant decrease in biliary calcium and total protein concentration. This antilithogenic effect may be due to an enhancement in the stability of biliary phospholipid-cholesterol vesicles.[58] In mice, n-3 polyunsaturated fatty acids attenuated gallstone formation through increasing the levels of bile phospholipids and suppressing bile mucin formation.[59]

Lipotropic Factors and Botanical Choleretics

The naturopathic approach to the treatment of gallstones has typically involved the use of lipotropic and choleretic formulas. Lipotropic factors are, by definition, substances that hasten the removal or decrease the deposition of fat in the liver through their interaction with fat metabolism. Compounds commonly employed as lipotropic agents include choline, methionine, betaine, folic acid, and vitamin B_{12}.

These nutritional factors are often used with herbal cholagogues and choleretics. Cholagogues stimulate gallbladder contraction to promote bile flow, whereas choleretics increase bile secretion by the liver.

Many herbal choleretics have a favorable effect on the solubility of bile. Choleretics that are appropriate to use in the treatment of gallstones include *Taraxacum officinale,* silymarin from *Silybum marianum, Cynara scolymus,* and *Curcuma longa.* In addition, *Peumus boldo* contains alkaloids, such as boldine, that are useful in the treatment of gallstones.

One study in rats given a diet that promoted gallstones demonstrated that the animals supplemented with 0.5% dietary curcumin for 10 weeks had only a 26% incidence of gallstone formation compared with a 100% incidence in the group fed the lithogenic diet alone.[60] This effect was found to be dose dependent.

Chemical Dissolution of Gallstones

Several successful nonsurgical alternatives for the treatment of gallstones now exist. These entail using a complex of plant terpenes alone or, preferably, in combination with oral bile acids. Because the formation of the stone depends on either increased accumulation of cholesterol or reduced levels of bile acids or lecithin, decreasing gallbladder cholesterol levels or increasing bile acid or lecithin levels should result in the dissolution of the stone. Chemical dissolution is especially indicated in the treatment of gallstones in the elderly, who cannot withstand the stress of surgery, and in other cases where surgery is contraindicated.

Bile Acids

The use of bile acids, such as ursodeoxycholic acid and tauroursodeoxycholic acid, is effective in dissolving small, uncalcified cholesterol gallstones. About 15% of all patients with cholesterol gallstones meet these criteria. Treatment with bile acids will lead to complete dissolution in about 90% of cases after 6 months of therapy. Once dissolved, it is important to follow the recommendations for gallstone prevention to reduce the risk for recurrence. Bile acid preparations are available by prescription only and represent a safe and effective nonsurgical alternative for many people with gallstones.[60,61] The typical dosage is 12 mg per kg body weight daily.

Terpenes

Gallstone dissolution by a natural terpene combination of menthol, menthone, pinene, borneol, cineol, and camphene has been demonstrated in several studies.[62] This nonsurgical approach to gallstone removal offers an effective alternative to surgery and has been demonstrated to be safe even when it has been implemented for prolonged periods of time (up to 4 years).

Combined Therapy

Although terpenes are effective alone, the best results appear to be achieved when plant terpene complexes are used in combination with bile acid therapy.[63–65] This combined approach offers better results than either bile acids or plant terpenes used alone.[64,65] Furthermore, because a lower dose of bile acid can be used, there is a significant reduction in the risk of complications or side effects and in the cost of bile acid therapy. Because menthol is the major component of this formula, peppermint oil, especially enteric-coated capsules, may offer similar results.

Lifestyle

Sunbathing

Because most cholesterol gallstones contain a central pigmented nucleus with radial or lamellar pigmented bands alternating with layers of crystalline cholesterol, one researcher speculated that activation of the pigmentary system by ultraviolet light might lead to increased concentrations of indole metabolites in the bile, triggering their polymerization. In a case-controlled study of 206 white-skinned individuals, a positive attitude toward sunbathing was associated with twice the risk of cholelithiasis as a negative attitude. When the effect of sunbathing attitude was considered for individual skin pigmentation types, the association was found to be almost entirely restricted to those who always burn after long sunbathing. In this group, the relative risk was a remarkable 25.6% for a positive attitude toward sunbathing versus a negative one.[66]

Social Stress

Using a fish model, one research experiment examined the effect of stress on the gallbladder function. In this work, fish were subjected to prolonged cohabitation with dominant individuals. It was found that this chronic social stress could increase bile retention, increase gallbladder hypertrophy, and inhibit gallbladder emptying.[67] This study suggests that chronic stress may be a predictor of gallbladder dysfunction and eventual gallstone formation.

THERAPEUTIC APPROACH

As is typical of most diseases, gallstones are much easier to prevent than to reverse. The risk factors and causes of gallstones are well known, and in most cases, a healthy diet rich in fiber, with a limitation of excess calories and saturated fats, is adequate prevention.

Once gallstones have developed, measures to avoid gallbladder attacks and increase the solubility of the bile are necessary. To limit the incidence of symptoms, the foods to which an individual is intolerant or allergic must be determined (see Chapter 14) and, along with fatty foods, avoided. The solubility of the bile can be increased by following the dietary guidelines and using the nutritional and herbal supplements recommended here.

Diet

Patients should increase their intake of vegetables, fruits, dietary fiber (especially the gel-forming or mucilaginous fibers such as flaxseed, oat bran, guar gum, and pectin), and buckwheat. They should reduce their consumption of saturated fats, refined carbohydrates, cholesterol, sugar, and animal proteins. All fried foods should be avoided.

An allergy elimination diet can be used to reduce gallbladder attacks.

Water

Consumption of six to eight glasses of water is necessary each day to maintain the water content of bile.

Nutritional Supplements

- Vitamin C: 1 to 3 g/day
- Vitamin E: 200 to 400 IU/day
- Phosphatidylcholine: 500 mg/day
- Choline: 1 g/day
- l-Methionine: 1 g/day
- Fiber supplement (guar gum, pectin, psyllium, or oat bran): minimum of 5 g/day

Botanical Medicines

Choose one or more of the following:

- Dandelion (*Taraxacum officinale)* (three times/day)
  - Dried root: 4 g
  - Fluid extract (1:1): 4 to 8 mL
  - Solid extract (4:1): 250 to 500 mg
- *Pneumus boldo* (three times/day)
  - Dried leaves (or by infusion): 250 to 500 mg
  - Tincture (1:10): 2 to 4 mL
  - Fluid extract (1:1): 0.5 to 1 mL
- Milk thistle *(Silybum marianum)*
  - Sufficient dosage according to form to yield 70 to 210 mg of silymarin three times/day
- Artichoke *(Cynara scolymus)*
  - Extract (15% cynarin): 500 mg three times/day
- Curcumin: 300 mg three times/day

Gallstone-Dissolving Formula

- Menthol: 30 mg
- Menthone: 5 mg
- Pinene: 15 mg
- Borneol: 5 mg
- Camphene: 5 mg
- Cineol: 2 mg
- Citral: 5 mg
- Phosphatidylcholine: 50 mg
- Medium-chain triglycerides: 125 mg
- Chenodeoxycholic acid: 750 mg

The dosage for this formula is to take it three times daily if used in combination with meals.

Note: Peppermint oil in an enteric-coated capsule can be used instead at a dosage of 1 to 2 capsules (0.2 mL per capsule) three times daily between meals.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Kalloo AN, Kantsevoy SV. Gallstones and biliary disease. *Primary Care*. 2001;28:591–606, vii.
2. Robbins SL, Cotran RS, Kumar V. *Pathologic Basis of Disease*. Philadelphia: Saunders; 1984.
3. Tierney Jr LM, McPhee SJ, Papadakis MA. *Current Medical Diagnosis and Treatment*. Stamford, CT: Appleton & Lange; 1997:630–632.
4. Weisberg HF. Pathogenesis of gallstones. *Ann Clin Lab Sci*. 1984;14:243–251.
5. Trowell H, Burkitt D, Heaton K. *Dietary Fibre, Fibre-Depleted Foods and Disease*. London: Academic Press; 1985:289–304.
6. Rizvi TJ, Zuberi SJ. Risk factors for gall bladder cancer in Karachi. *J Ayub Med Coll Abbottabad*. 2003;15:16–18.
7. Vitetta L, Sali A, Little P, et al. Gallstones and gall bladder carcinoma. *Aust N Z J Surg*. 2000;70:667–673.
8. Rubenstein E, Federman DD. *Scientific American Medicine*. New York: Scientific American; 1986.
9. Nervi F, Covarrubias C, Bravo P, et al. Influence of legume intake on biliary lipids and cholesterol saturation in young Chilean men. *Gastroenterology*. 1989;96:825–830.
10. Mathus-Vliegen EM, Van Ierland-Van Leeuwen ML, Terpstra A. Determinants of gallbladder kinetics in obesity. *Dig Dis Sci*. 2004;49:9–16.
11. Festi D, Colecchia A, Larocca A, et al. Review: low caloric intake and gallbladder motor function. *Aliment Pharmacol Ther*. 2000;14(suppl 2):51–53.
12. Akin ML, Uluutku H, Erenoglu C, et al. Tamoxifen and gallstone formation in postmenopausal breast cancer patients: retrospective cohort study. *World J Surg*. 2003;27:395–399.
13. Wang DQ. Aging per se is an independent risk factor for cholesterol gallstone formation in gallstone susceptible mice. *J Lipid Res*. 2002;43:1950–1959.
14. Ajdarkosh H, Khansari MR, Sohrabi MR, Shamspour N, Abodolahi N, Zamani F. Thyroid dysfunction and choleduocholithiasis. *Middel East J Dig Dis*. 2013;5(3):141–145. PubMed PMID: 24829684.
15. Parviainen A, Suarez-Grau JM, Perez-Lopez R, Nieto JM, Garrido CJ, Cobo-Cardenas G. Combined microstructural and mineralogical phase characterization of gallstones in a patient-based study in SW Spain – implications for environmental contamination in their formation. *Sci Total Environ*. 2016;573:433–443. PubMed PMID: 27572536.
16. Unisa S, Jagannath P, Dhir V, Khandelwal C, Sarangi L, Roy TK. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. *HPB (Oxford)*. 2011;13:117–125.
17. Su Y, Dai Y, Lin Y, Gao X, Han Y, Zhao B. Serum organochlorine pesticide residues and risk of gallstone disease: a case-control study in Xiamen. *Ann Epidemiol*. 2012;22(8):592–597. PubMed PMID: 22695391.
18. Ji G, Xu C, Sun H, et al. Organochlorine pesticides induced hepatic ABCG5/G8 expression and lipogenesis in Chinese patients with gallstone disease. *Oncotarget*. 2016;7(23):33689–337602. PubMed PMID: 27203212.
19. Pandey M, Shukla VK. Diet and gallbladder cancer: a case-control study. *Eur J Cancer Prev*. 2002;11:365–368.
20. Marks JW, Cleary PA, Albers JJ. Lack of correlation between serum lipoproteins and biliary cholesterol saturation in patients with gallstones. *Dig Dis Sci*. 1984;29:1118–1122.
21. Petitti DB, Friedman GD, Klatsky AL. Association of a history of gallbladder disease with a reduced concentration of high-density-lipoprotein cholesterol. *N Engl J Med*. 1981;304:1396–1398.
22. Thornton JR, Heaton KW, Macfarlane DG. A relation between high-density-lipoprotein cholesterol and bile cholesterol saturation. *Br Med J*. 1981;283:1352–1354.
23. Van der Linder W, Bergman F. An analysis of data on human hepatic bile: relationship between main bile components, serum cholesterol and serum triglycerides. *Scand J Clin Lab Invest*. 1977;37:741–747.
24. Thijs C, Knipschild P. Legume intake and gallstone risk: results from a case-control study. *Int J Epidemiol*. 1990;19(3):660–663.
25. Pixley F, Wilson D, McPherson K, et al. Effect of vegetarianism on development of gallstones in women. *Br Med J*. 1985;291:11–12.
26. Kritchevsky D, Klurfeld DM. Gallstone formation in hamsters: effect of varying animal and vegetable protein levels. *Am J Clin Nutr*. 1983;37:802–804.
27. Breneman JC. Allergy elimination diet as the most effective gallbladder diet. *Ann Allergy*. 1968;26:83–87.
28. Necheles H, Rappaport BZ, Green R, et al. Allergy of the gallbladder. *Am J Dig Dis*. 1949;7:238–241.
29. Walzer M, Gray I, Harten M, et al. The allergic reaction in the gallbladder: experimental studies in rhesus monkeys. *Gastroenterology*. 1943;1:565–572.
30. De Muro P, Ficari A. Experimental studies on allergic cholecystitis. *Gastroenterology*. 1946;6:302–314.
31. Tomotake H, Shimaoka I, Kayashita J, et al. A buckwheat protein product suppresses gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamsters. *J Nutr*. 2000;130:1670–1674.
32. Kritchevsky D, Klurfeld DM. Influence of vegetable protein on gallstone formation in hamsters. *Am J Clin Nutr*. 1979;32:2174–2176.
33. Tomotake H, Shimaoka I, Kayashita J, et al. Stronger suppression of plasma cholesterol and enhancement of the fecal excretion of steroids by a buckwheat protein product than by a soy protein isolate in rats fed on a cholesterol-free diet. *Biosci Biotechnol Biochem*. 2001;65:1412–1414.
34. Tomotake H, Yamamoto N, Yanaka N, et al. High protein buckwheat flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility. *Nutrition*. 2006;22(2):166–173. PubMed PMID: 16459229.
35. Liu Z, Ishikawa W, Huang X, et al. A buckwheat protein product suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by reducing cell proliferation. *J Nutr*. 2001;131:1850–1853.
36. Moerman CJ, Bueno de Mesquita HB, Runia S. Dietary sugar intake in the etiology of biliary tract cancer. *Int J Epidemiol*. 1993;22:207–214.
37. Thornton JR, Emmett PM, Heaton KW. Diet and gall stones: effects of refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism. *Gut*. 1983;24:2–6.
38. Moerman CJ, Smeets FW, Kromhout D. Dietary risk factors for clinically diagnosed gallstones in middle-aged men: a 25-year follow-up study (the Zutphen Study). *Ann Epidemiol*. 1994;4:248–254.
39. Tandon RK, Saraya A, Paul S, et al. Dietary habits of gallstone patients in northern India: a case control study. *J Clin Gastroenterol*. 1996;22:23–27.
40. Caroli-Bosc FX, Deveau C, Peten EP, et al. Cholelithiasis and dietary risk factors: an epidemiologic investigation in Vidauban, Southeast France. General Practitioner's Group of Vidauban. *Dig Dis Sci*. 1998;43:2131–2137.
41. Kamrath RO, Plummer LF, Sadur CN. Cholelithiasis in patients treated with a very-low-calorie diet. *Am J Clin Nutr*. 1992;56:255S–257S.
42. van Erpecum KJ, van Berge Henegouwen GP. Intestinal aspects of cholesterol gallstone formation. *Dig Liver Dis*. 2003;35(suppl 3):S8–S11.
43. Spirt BA, Graves LW, Weinstock R. Gallstone formation in obese women treated by a low-calorie diet. *Int J Obes Relat Metab Disord*. 1995;19:593–595.
44. Johansson K, Sundstrom J, Marcus C, Hemmingsson E, Neovius M. Risk of symptomatic gallstones and cholecystectomy after a very-low-calorie diet or low-calorie diet in a commercial weight loss program: 1-year matched cohort study. *Int J Obes (London)*. 2014;38(2):279–284. PubMed PMID: 23736359.
45. Douglas BR, Jansen JB, Tham RT. Coffee stimulation of cholecystokinin release and gallbladder contraction in humans. *Am J Clin Nutr*. 1990;52:553–556.
46. Leitzmann MF, Stampfer MJ, Willett WC, et al. Coffee intake is associated with lower risk of symptomatic gallstone disease in women. *Gastroenterology*. 2002;123:1823–1830.
47. Zhang YP, Li WQ, Sun YL, Zhu RT, Wang WJ. Systematic review with meta-analysis: coffee consumption and the risk of gallstone disease. *Aliment Pharmacol Ther*. 2015;42(6):637–648. PubMed PMID: 26198295.
48. Kasbo J, Tuchweber B, Perwaiz S, et al. Phosphatidylcholine-enriched diet prevents gallstone formation in mice susceptible to cholelithiasis. *J Lipid Res*. 2003;44:2297–2303. Epub July 1, 2003.
49. Tuzhilin SA, Drieling DA, Narodetskaja RV, et al. The treatment of patients with gallstones by lecithin. *Am J Gastroenterol*. 1976;65:231–235.
50. Hanin I, Ansell GB. *Lecithin. Technological, Biological, and Therapeutic Aspects*. New York: Plenum Press; 1987.

51. Jenkins SA. Vitamin C and gallstone formation: a preliminary report. *Experientia*. 1977;33:1616–1617.
52. Dam H, Christensen F. Alimentary production of gallstones in hamsters. *Acta Pathol Microbiol Scand*. 1952;30:236–242.
53. Sies CW, Brooker J. Could these be gallstones? *Lancet*. 2005;365(9468):1388.
54. Lee SP, Tassman-Jones C, Carlisle V. Oleic acid-induced cholelithiasis in rabbits. *Am J Pathol*. 1986;124:18–24.
55. Beynen AC. Dietary monounsaturated fatty acids and liver cholesterol. *Artery*. 1988;15:170–175.
56. Baggio G, Pagnan A, Muraca M, et al. Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. *Am J Clin Nutr*. 1988;47:960–964.
57. Scobey MW, Johnson FL, Parks JS. Dietary fish oil effects on biliary lipid secretion and cholesterol gallstone formation in the African green monkey. *Hepatology*. 1991;14(4 Pt 1):679–684.
58. Magnuson TH, Lillemoe KD, High RC, et al. Dietary fish oil inhibits cholesterol monohydrate crystal nucleation and gallstone formation in the prairie dog. *Surgery*. 1995;118:517–523.
59. Kim JK, Cho SM, Kang SH, et al. N-3 polyunsaturated fatty acid attenuates cholesterol gallstones by suppressing mucin production with a high cholesterol diet in mice. *J Gastroenterol Hepatol*. 2012;27(11):1745–1751. PubMed PMID: 22849613.
60. Hussain MS, Chandrasekhara N. Effect on curcumin on cholesterol gallstone induction in mice. *Indian J Med Res*. 1992;96:288–291.
61. Di Ciaula A, Wang DQ, Wang HH, et al. Targets for current pharmacologic therapy in cholesterol gallstone disease. *Gastroenterol Clin North Am*. 2010;39(2):245–264.
62. Gaby AR. Nutritional approaches to prevention and treatment of gallstones. *Altern Med Rev*. 2009;14(3):258–267. PubMed PMID: 19803550.
63. Somerville KW, Ellis WR, Whitten BH, et al. Stones in the common bile duct: experience with medical dissolution therapy. *Postgrad Med J*. 1985;61:313–316.
64. Ellis WR, Bell GD, Middleton B, et al. Adjunct to bile-acid treatment for gallstone dissolution: low-dose chenodeoxycholic acid combined with a terpene preparation. *Br Med J*. 1981;282:611–612.
65. Ellis WR, Somerville KW, Whitten BH, et al. Pilot study of combination treatment for gallstones with medium dose chenodeoxycholic acid and a terpene preparation. *Br Med J*. 1984;289:153–156.
66. Pavel S. Sunbathing and gallstones. *Lancet*. 1992;339:241.
67. Earley RL, Blumer LS, Grober MS. The gall of subordination: changes in gall-bladder function associated with social stress. *Proc R Soc Lond B Biol Sci*. 2004;271:7–13.

172

Gastroesophageal Reflux Disease (GERD)

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Burning sensation in the esophagus, regurgitation, teeth erosion
- Symptoms chronic and periodic
- Epigastric tenderness and guarding
- Gastric analysis showing acid in all cases, with hypersecretion in about one half of the patients with duodenal ulcers
- Ulcer crater or deformity, usually occurring at the duodenal bulb (duodenal ulcer) or pylorus (gastric ulcer) on radiography or fiberoptic examination
- Positive test for occult blood in stool

GENERAL CONSIDERATIONS

Gastroesophageal reflux disease (GERD), informally known as heartburn, is a chronic relapsing condition in which repeated exposure of the esophagus to gastric contents provokes symptoms, complications, or histological changes and impairs quality of life. GERD is a common condition, with up to 25% of the general population experiencing symptoms at least one time per month.[1] The incidence of reflux is increasing because of the growing numbers of people with obesity, increased longevity, and the increased use of medications that affect esophageal function.[2] In fact, the prevalence of GERD is 50% higher in the United States in studies carried out after 1995 compared with those carried out before 1995.[3]

The degree of esophageal mucosal injury and the frequency and severity of symptoms are determined by the degree and duration of esophageal acid exposure. However, this is not always the case. A multiple-site, double-blind, randomized clinical trial failed to demonstrate a correlation between the severity of self-reported heartburn symptoms and the presence of endoscopically graded esophagitis.[4] Although there was no correlation between severity and underlying esophagitis, there was a strong correlation between the frequency of heartburn episodes and increasing severity of esophagitis.

Common symptoms include the sensation of burning in the esophagus, chest pain, chronic cough, hoarseness, regurgitation, throat clearing, and teeth erosion. Atypical symptoms such as dysphagia, chronic postnasal drip, laryngitis, dental erosions, and asthma may also be related to GERD and may represent the only clinical presentation of the disease. Other proposed associations include sinusitis, idiopathic pulmonary fibrosis, and recurrent otitis media.[5] In cases of atypical GERD, the cause may be difficult to establish because pH testing does not accurately classify these patients, and placebo-controlled studies with high-dose proton-pump inhibitors (PPIs) show very inconsistent results in this group of patients.[6]

Etiology and Pathogenesis

The pathogenesis of GERD is multifactorial. Mechanisms include (1) motor abnormalities, such as transient lower esophageal sphincter (LES) relaxation, impaired LES resting tone, impaired esophageal acid clearance, and delayed gastric emptying; (2) anatomical factors, such as hiatal hernia; (3) visceral hypersensitivity; and (4) impaired mucosal resistance.[4] Contact between esophageal mucosa with refluxate composed of acid, bile, pepsin, and pancreatic enzymes leads to esophageal injury.

The LES is a segment of smooth muscle at the distal end of the esophagus that acts as a physical barrier preventing gastric contents from refluxing into the esophagus. This sphincter typically opens in response to peristalsis, allowing food and liquids to pass into the stomach. A minority of patients with GERD have a constantly weak, low-pressure LES, which permits reflux every time the pressure in the stomach exceeds the LES pressure (i.e., when LES pressure is <6 mm Hg).[7] In patients with GERD, transient LES relaxations account for 48% to 73% of reflux episodes and thus account for most GERD episodes.[8]

The most common cause of reflux esophagitis is overeating. Other common causes include obesity, cigarette smoking, chocolate, fried foods, carbonated beverages, alcohol, and caffeine. Factors that decrease LES tone include endogenous hormones (e.g., cholecystokinin, progesterone in

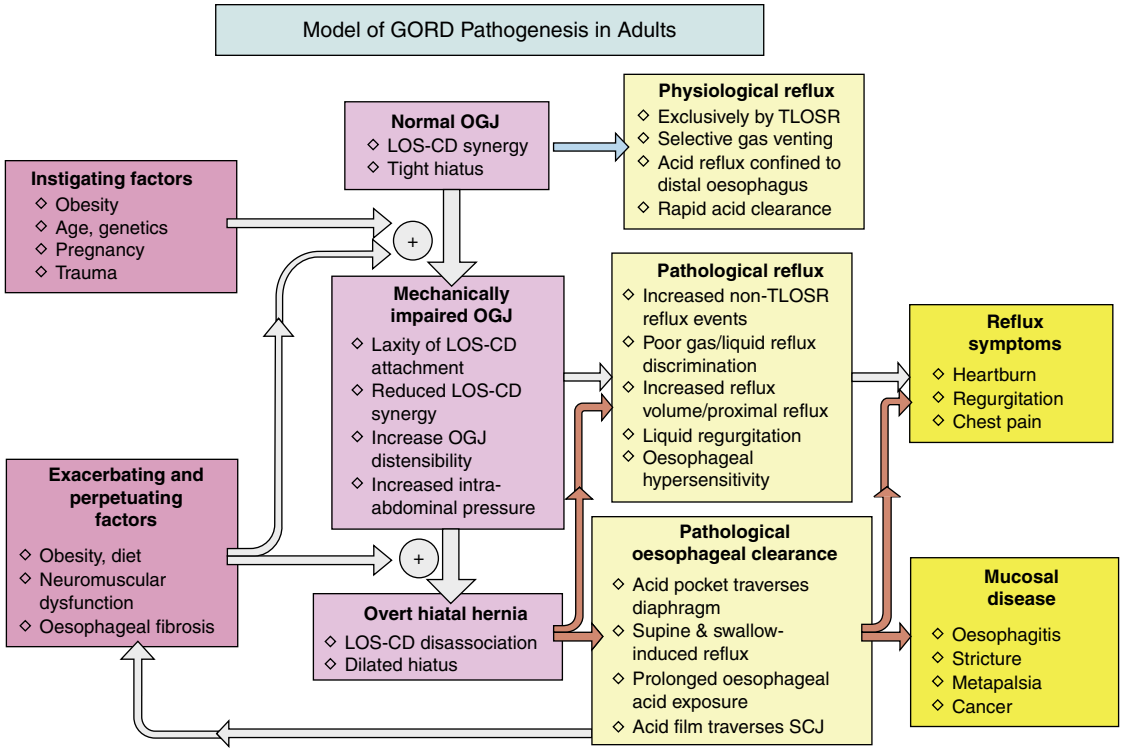


Fig. 172.1 Model of Gastroesophageal Reflux Disease (GERD) Pathogenesis. With the accumulation of these "hits," physiological reflux is transformed into pathological reflux, manifest either symptomatically and/or with mucosal disease. Abdominal obesity plays a dominant role, with its effect mediated through progressive degradation of the OGJ, culminating in the development of overt hiatus hernia. *CD,* crural diaphragm; *LOS,* lower (o)esophageal sphincter; *OGJ,* (o)esophagogastric junction; *TLOSR,* transient lower (o) esophageal sphincter relaxation. (From Boeckxstaens G, El-Serag HB, Smout AJ, Kahrilas PJ. Symptomatic reflux disease: the present, the past and the future. *Gut.* 2014;63[7]:1185–1193. PubMed PMID: 24607936.)

pregnancy); medications (e.g., nitrates, calcium channel blockers); foods (e.g., high-fat meals); and voluntary habits like smoking, caffeine, and alcohol (Fig. 172.1). Symptoms may be particularly bad when the individual is lying down. Studies in healthy volunteers have identified reflux episodes during sleep and the postprandial period resulting from an increased number of inappropriate LES relaxations.[9] During the night, LES relaxation and esophageal reflux occurred only during transient arousals from sleep or when the subjects were fully awake but not during stable sleep. Reduced salivation during or immediately before sleep accounts for markedly prolonged acid clearance times and increased esophageal acid exposure and thus may be a significant causative factor in GERD.[10]

The process of esophageal acid clearance involves peristalsis as well as the swallowing of bicarbonate and is an important protective mechanism against the development of GERD. Impaired esophageal clearance can be caused by an increase in the volume of the refluxate and occasionally by an underlying condition such as scleroderma. In experimentally induced or spontaneous reflux, patients with GERD have been found to present acid clearance times that are two to three times longer than those of subjects without GERD.[11]

Hiatal hernia has been shown to be present in ≥90% of patients with reflux esophagitis.[12] A study assessing the role of hiatal hernia in patients with Barrett's esophagus found a 2-cm or longer hernia in 96% of the patients and 72% of the patients with short segment Barrett's esophagus.[13] It is unclear if hiatal hernias are an initiating factor in GERD. However, hernias clearly play a role in sustaining GERD, accounting for the chronicity of the disease. A hiatal hernia may act as a reservoir for acid-containing material that is subsequently refluxed into the esophagus when the patient swallows, leading to the delayed acid clearance observed in patients with GERD with hiatal hernia.[14]

A traditional naturopathic understanding of reflux suggests that hypochlorhydria (i.e., low stomach acid) is a significant etiological factor in the development of GERD. It is hypothesized that food is inadequately broken down and stays in the stomach for longer periods of time, leading to an increase in gastric pressure and reflux of gastric contents into the esophagus. In many cases, clinical experience has shown that hydrochloric acid supplementation may benefit rather than aggravate GERD.

The ability of the esophageal mucosa to withstand injury is a determining factor in the development of GERD, and the major defense of the epithelium relies solely on the epithelium itself. The epithelial defense consists of three main components: (1) the cell membranes and the intercellular junction complex, which limit the rate of hydrogen ion penetration into the intercellular space or cell cytosol; (2) the presence of cellular and intercellular buffers (e.g., bicarbonate, proteins, phosphate) that neutralize back-diffusing luminal acid; and (3) the presence of cell membrane ion transporters that serve to extrude acid from the cell cytosol when intracellular pH falls to acidic levels.[15] When aggravating factors, such as alcohol, heat, osmolarity, or smoke-derived chemicals, overwhelm the esophageal defense, mucosal injury occurs.

The severity of GERD varies from nonerosive disease to significant esophageal damage. Barrett's esophagus, erosive esophagitis, and esophageal carcinoma are severe complications associated with GERD. Endoscopy reveals that most symptomatic patients do not have mucosal breaks or other esophageal injuries. Therefore the terms "nonerosive reflux disease" and "functional heartburn" have been introduced to redefine and characterize GERD into separate entities rather than a continuous spectrum.[16]

Microbiota

Esophageal inflammation is associated with an altered microbiota in GERD, Barret's esophagus, eosinophilic esophagitis, and cancer. Despite numerous observations by scientists from the late 19th century describing bacteria in the acidic environment of the stomach, there was a widely held view that the gastric secretions of hydrochloric acid and the proteolytic enzyme pepsin, as well as the reflux of bile acids in the stomach, the thickness of the mucus layer, and the effectiveness of gastric peristalsis, ensured a sterile stomach.[17] *Helicobacter pylori* was once believed to be the only species able to colonize the human stomach. *H. pylori* is the most common chronic infection in the world and disrupts the normal gastric physiology and morphology. Research surrounding *H. pylori* has increased the understanding of how it can modify its own microclimate, and further investigations with modern techniques have shown that the microbiota of the stomach involves hundreds of phylotypes with a microbial density between 10^1 and 10^3 colony-forming units (CFU)/g.[18] Similar to the rest of the intestinal microbiome. This is a dynamic situation, with significant fluctuations in microbial density resulting from many factors, including ingestion of foods and gastric pH.

A study of the bacterial flora of the oral cavity and lower esophagus, obtained by esophageal brushings and biopsy samplings, revealed that *Streptococcus viridans* is the most common bacterium, with an occurrence rate of 95% to 98%.[19] The pathogenesis of GERD might be driven by increasing gram-negative bacteria of the esophageal microbiome because lipopolysaccharide upregulates gene expression and increases proinflammatory cytokine production.[20] In patients with severe GERD, histological changes have been reported characterized by T-lymphocyte–predominant inflammation with hyperplasia of papillary and basal cells, suggesting the inflammation may be cytokine mediated rather than the result of the usually attributed acid chemical injury.[21] Bacterial diversity decreases with the transition from nonatrophic gastritis to intestinal metaplasia and then to gastric cancer, with a decrease in the number of *Porphyromonas, Neisseria,* and *Streptococcus sinensis* but with a relative increase in *Lactobacillus coleohominis* and *Lachnospiraceae*.[22] Further study into the microbiota of the stomach and esophagus, including exploration of host immunity and dysbiosis of the microbiota, would be especially productive.

Medications

The use of certain medications can lead to the emergence of GERD and can also exacerbate existing reflux symptoms. For example, the combination of calcium channel blockers and warfarin is an independent risk factor for GERD.[23] Mechanisms by which drugs cause or aggravate reflux include a reduction in LES pressure, delayed gastric emptying, and inducing/facilitating esophageal inflammation and damage (Table 172.1).[24]

TABLE 172.1 Drugs and GERD

Reducing LES Pressure	Inducing/Facilitating Esophageal Inflammation	Delayed Gastric Emptying
• Beta-adrenergic agonists • Alpha-adrenergic antagonists • Anticholinergics • CCBs/nitrates • Benzodiazepines • Estrogen • Progesterone • Theophylline • SSRIs • Tricyclic antidepressants	• Bisphosphonates • Aspirin and NSAIDs • Iron salts • Ascorbic acid • Potassium chloride • Quinidine • Tetracycline • Doxycycline • Clindamycin • Chemotherapeutic agents	• Calcium channel blockers

CCB, Calcium channel blocker; *GERD,* gastroesophageal reflux disease; *NSAID,* nonsteroidal anti-inflammatory drug; *SSRI,* selective serotonin reuptake inhibitor.
Adapted from Mungan Z, Pinarbasi Simsek B. Which drugs are risk factors for the development of gastroesophageal reflux disease? Turk J Gastroenterol. 2017;28(Suppl 1):S38–S43. PubMed PMID: 29199166.

DIAGNOSTIC CONSIDERATIONS

The diagnosis of gastroesophageal reflux disease based on subjective evaluation symptoms alone can be difficult. Heartburn is a reasonably sensitive symptom for the diagnosis of GERD, although it does not reliably predict esophagitis. In the absence of alarm symptoms, empiric treatment with acid suppression is warranted. Researchers have developed multiple questionnaires to improve the accuracy of GERD diagnosis, but many have limited sensitivity and specificity.[25] The double-contrast barium swallow has a low sensitivity to diagnose GERD but may be beneficial to identify complications of GERD.[26] The role of esophageal manometry is limited to accurate placement of a pH-measuring device and should not be used to make or confirm a diagnosis of GERD. pH testing has reasonable sensitivity and specificity for the diagnosis of GERD. The sensitivity of upper endoscopy to diagnose GERD is lower than that of pH tests.[27]

THERAPEUTIC CONSIDERATIONS

Most individuals who suffer heartburn and acid reflux self-treat their symptoms, and it is only when the condition becomes intractable or chronic that professional help is sought.[28] A variety of over-the-counter products is currently available in the United States and worldwide for the symptomatic treatment of heartburn, acid indigestion, and acid reflux disorders. These include numerous liquid and tablet antacid formulations, antacid/alginates, histamine H_2-receptor antagonists (H_2RAs), proton-pump inhibitors, and bismuth-containing formulations. Proton-pump inhibitors (PPIs) are the mainstay of both acute and chronic reflux treatment, and they have a clear advantage over H_2RAs because they can increase gastric pH for as long as 15 to 21 hours compared with 8 hours for H_2RAs.[29] Although these products may be necessary when symptoms persist, most individuals improve with diet and lifestyle changes. Natural medicines are worthy of use before implementing additional pharmaceutical interventions.

Diet and Lifestyle Factors

Although it is generally recommended to advise lifestyle changes, the evidence for the efficacy of these recommendations in the management of GERD is mostly anecdotal. Yet many people note symptom aggravation after consuming certain foods, such as chocolate, coffee, alcohol, fatty foods, citrus fruits, spicy foods, and other acidic agents. Considering individual uniqueness, it is prudent to limit recommendations to a generally healthy diet and avoidance of (or limited use of) specific food items or activities that, in the experience of the patient, evoke symptoms.

Weight reduction is an important goal in the treatment of GERD. A structured weight-loss program can lead to complete resolution of symptoms in most overweight individuals with GERD. A decrease in the body mass index (BMI) of as little as 3.5 kg/m^2 has been shown to lead to a nearly 40% reduction in the risk for GERD.[30] A prospective cohort

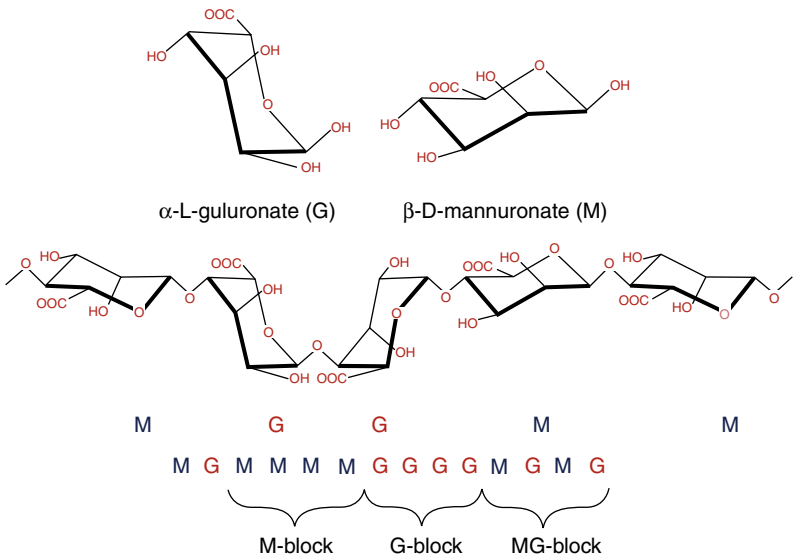


Fig. 172.2 Chemical Structure of Alginate. (From Immunological and technical considerations in application of alginate-based microencapsulation systems. https://www.researchgate.net/Chemical-structure-of-alginate-Linear-block-polymers-of-b-d-mannuronate-M-and_fig3_264826259. Accessed October 2, 2018.)

study measured the effect of weight loss on GERD symptoms in 322 overweight/obese (BMI: 25–39.9 kg/m^2) adult subjects.[31] At 6 months, most of the subjects (97%) lost weight, and compared with baseline, there was a significant decrease in the overall prevalence of GERD and the mean GERD symptom score. Overall, 81% of the subjects had a reduction in GERD symptom scores, 65% had a complete resolution of reflux symptoms, and 15% had a partial resolution of reflux symptoms.

Nutritional Factors

Alginates

Alginates are natural polysaccharide polymers isolated from brown seaweed *(Phacophycae)* and can be classified as dietary fiber. Alginates are block copolymers of L-guluronic and D-mannuronic acid residues connected by 1:4 glycosidic linkages. The relative proportions of D-mannuronic and L-guluronic acids are species dependent and can be influenced by growth conditions (Fig. 172.2).

For decades, formulations containing alginate have been marketed worldwide for the symptomatic treatment of heartburn and esophagitis. Alginate should be combined with calcium carbonate because both the calcium and carbonate serve essential functions in the beneficial effect. When the alginate formulation reaches the acidic environment of the stomach, the alginate forms a pliable gel. At the same time, the calcium carbonate mixes with gastric acid to produce carbon dioxide bubbles that get trapped in the gel, causing it to float to the top of the stomach contents. The free calcium ions released bind with alginic acid, providing strength to raft formation. The resultant alginate complex literally is like a foam raft sitting on top of the stomach contents. Several studies have demonstrated that the alginate raft can preferentially move into the esophagus in place, or ahead, of acidic gastric contents during episodes of GERD.[32] The raft literally acts as a physical barrier to block reflux episodes. The raft-forming process takes less than a minute, and the raft can survive in the stomach for as long as 4 hours.[33] As the alginate complex makes its way through the intestinal tract, the alginate is partially digested and behaves as other dietary fibers until it is finally passed out of the body.

Fourteen human clinical trials and detailed meta-analyses have demonstrated alginate formulations to be a well-proven treatment for GERD. The most recent meta-analysis showed that alginate-based products were clearly more effective than placebo or antacids.[34] Pooling the data from these studies produced a high degree of statistical significance ($P = .001$). Subjects taking alginate were 4.42 times more likely to have complete resolution of their symptoms compared with those taking placebo or antacids. The comparison to stronger acid-blocking medications, such as PPIs and H_2-receptor antagonists, was less clear and did not reach statistical significance largely because most studies were using alginate in conjunction with a PPI. Table 172.2 summarizes the studies used in the meta-analysis.

In one of the more recent double-blind studies, 110 patients with symptoms of GERD were given either alginate chewable tablets or a placebo for 7 consecutive days.[35] The primary endpoint compared the change in overall Reflux Disease Questionnaire (RDQ) symptom score (combined heartburn/regurgitation/dyspepsia). After 7 days, there was a highly statistically significant greater decrease in overall RDQ symptom score in the alginate group compared with the placebo group ($P = 0.0033$), as well as a statistically significant greater decrease for each of the symptoms independently. These results were achieved without any side effects.

When comparing alginate with a PPI, it is important to distinguish between the two basic forms of GERD. The first is characterized by erosion of the esophagus (ERD), and the second involves no esophageal erosion (NERD). The differentiation is important because patients with NERD do not respond well to PPIs. Around the world, the NERD form is the most common. Patients suffering from NERD are ideal candidates for alginate therapy. However, even in the erosive form, alginate is effective. In a detailed open-label study that included patients with either ERD or NERD, alginate, given as the only treatment, was found to be "well-tolerated and effective in reducing heartburn by modifying esophageal acid exposure time, number of acid refluxes and their proximal migration."[36]

A double-blind study evaluated the efficacy and safety of alginate compared with omeprazole (Prilosec) in 195 adult subjects with NERD.[37] Subjects were given either alginate three times a day or omeprazole once daily. The primary efficacy endpoint was the percentage of patients achieving adequate heartburn or regurgitation relief at day 28. Results showed no difference in effectiveness between the two treatments, and although the average symptom score was slightly lower in the alginate group, it was not statistically significant.

Considering the confirmed efficacy, remarkable safety profile, and lack of side effects, alginate therapy should be considered as a first-line approach to symptomatic relief. It is also safe for use during pregnancy.

TABLE 172.2 Characteristics of Studies Included in This Systematic Review

Study	Study Design	GERD Diagnosis and/or Severity	Comparators (*N*)	Duration (Formulation)	Outcome	Results
Placebo or Antacid as Comparators						
Beeley and Warner	Randomized, double blind, three arm, crossover, single center	Typical symptoms and presence of hiatal hernia on barium	Alginate (28) vs. alginate + antacid (28) vs. placebo (28)	2 weeks (tablet)	Improvement in regurgitation	Alginate (19/28) vs. alginate + antacid (25/28) vs. placebo (12/28)
Stanciu and Bennett	Randomized, single blind, three arm, parallel group, multicenter	Typical symptoms	Alginate + antacid (20) vs. antacid (20) vs. placebo (20)	2 weeks (tablet)	Global improvement of symptoms	Alginate + antacid (11/20) vs. antacid (5/20) vs. placebo (7/20)
Barnardo et al.	Randomized, double blind, crossover, single center	Typical symptoms and reflux on barium	Alginate + antacid (26) vs. antacid (26)	6 weeks (tablet)	Global acceptability of treatment	Alginate + antacid (21/26) vs. antacid (5/26)
Chevrel	Randomized, open label, crossover, single center	Typical symptoms and reflux on barium	Alginate (44) vs. antacid (44)	2 weeks (liquid)	Global improvement of symptoms	Alginate (37/44) vs. antacid (10/44)
Lang and Dougall	Randomized, parallel group, multicenter	Reflux dyspepsia of pregnancy	Alginate + antacid (50) vs. antacid (47)	2 weeks (liquid)	Improvement in nighttime reflux symptoms	Alginate (41/50) vs. antacid (36/47)
Chatfield	Randomized, double blind, parallel group, multicenter	Typical symptoms ≥2 days/week	Alginate + antacid (46) vs. placebo (48)	4 weeks (liquid)	Global improvement of symptoms	Alginate + antacid (39/46) vs. placebo (17/48)
Giannini et al.	Randomized, open label, parallel group, multicenter	Typical symptoms ≥3 days/week	Alginate + antacid (87) vs. antacid (92)	2 weeks (liquid)	Complete absence of symptoms	Alginate + antacid (71/87) vs. antacid (68/92)
Lai et al.	Randomized, open label, parallel group, single center	Typical symptoms and EGD without erosions	Alginate (69) vs. antacid (65)	6 weeks (tablet)	Global improvement of symptoms assessed by physician	Alginate (42/65) vs. antacid (18/56)
Thomas	Randomized, double blind, parallel group, single center	Typical symptoms ≥5 days/week	Alginate + antacid (56) vs. placebo (54)	1 week (tablet)	Overall treatment response	Alginate + antacid (47/56) vs. placebo (34/54)
Proton Pump Inhibitor or Histamine-2 Receptor Antagonist as Comparators						
Bennett et al.	Randomized, parallel group, single center	Typical symptoms and positive pH test	Alginate + antacid (19) vs. alginate + antacid + H_2RA (17)	6 weeks (tablet)	Global improvement of symptoms	Alginate + antacid (12/19) vs. alginate + antacid + H_2RA (15/17)
Goves et al.	Randomized, single blind, parallel group, multicenter	Typical symptoms ≥2 days/week	Alginate (337) vs. PPI (333)	2 weeks (liquid)	Complete resolution of symptoms	Alginate (27/337) vs. PPI (90/333)
Poynard et al.	Randomized, open label, parallel group, multicenter	Typical symptoms ≥2 days/week	Alginate (180) vs. 5HTR agonist (173)	4 weeks (liquid)	Global improvement of symptoms	Alginate (158/180) vs. 5HTR agonist (120/173)
Manabe et al.	Randomized, open label, parallel group, multicenter	Typical symptoms ≥2 days/week and EGD without erosions	Alginate + PPI (26) vs. PPI (31)	4 weeks (liquid)	Complete resolution of regurgitation	Alginate + PPI (18/26) vs. PPI (20/31)
Pouchain et al.	Randomized, double blind, parallel group, multicenter	Typical symptoms ≥2 days/week	Alginate (120) vs. PPI (121)	1 week (liquid)	Self-assessed heartburn/pain relief	Alginate (62/120) vs. PPI (74/121)
Chiu et al.	Randomized, double blind, parallel group, multicenter	Typical symptoms ≥2 days/week and EGD without erosions	Alginate (92) vs. PPI (91)	4 weeks (liquid)	Relief of heartburn or regurgitation	Alginate (49/92) vs. PPI (46/91)

EGD, Upper endoscopy; *GERD,* gastroesophageal reflux disease; *H_2RA,* histamine-2 receptor antagonist; *PPI,* proton pump inhibitor; 20 mg omeprazole daily; *5HTR,* serotonin receptor, 20 mg cisapride daily; 400 mg cimetidine four times daily.

Melatonin

Melatonin may block the production of nitric oxide synthase, which may help lower the degree of LES relaxation. Melatonin also stimulates the production of bicarbonate, which protects against the action of gastric acid production.

Several studies suggest melatonin is of therapeutic value in patients with GERD. In one study, 36 individuals with GERD were selected and placed into four groups: control; 3 mg melatonin at bedtime; 20 mg of omeprazole twice daily; and 3 mg of melatonin and 20 mg of omeprazole twice daily for 2 months.[38] After 8 weeks, those using

the melatonin alone had a significant reduction in GERD symptoms, increase in LES pressure (i.e., better LES tone), increase in serum gastrin, a reduction in gastric basal acid output, and an increase in serum melatonin levels. Although omeprazole was also effective in reducing GERD symptoms, it did not significantly alter LES tone.

A randomized clinical trial evaluated the effects of 6 mg melatonin plus vitamin B_6 (25 mg), vitamin B_{12} (50 mcg), folic acid (10 mg), tryptophan (100 mg), methionine (100 mg), and betaine (100 mg) or 1 capsule (20 mg) omeprazole per day in the evening for 40 days in individuals (141 men, 210 women; mean age 44 years) with at least one episode of moderate to severe heartburn.[39] After 40 days, individuals using the natural product combination had a 100% reduction in symptoms as measured by a five-point severity scale, compared with a 65.7% reduction in symptoms in the omeprazole group. As an observation, the 34.3% of the subjects who experienced persistence of their GERD symptoms with the use of omeprazole had complete resolution of their symptoms after 40 days with the natural combination of vitamins, melatonin, and amino acids.

Limonene

D-limonene is a major constituent of citrus oils and is used as a fragrance and flavoring agent in a variety of products. Because of its gastric acid–neutralizing effect and its support of normal peristalsis, it has been used for relief of heartburn and GERD.[40] In a pilot study, 19 patients with GERD or chronic heartburn were given 1000 mg D-limonene daily or every other day. After 14 days, 89% of patients reported a complete remission of symptoms. As a follow-up to this pilot study, 13 participants with GERD or chronic heartburn were randomized to 1000 mg D-limonene once daily, 1000 mg D-limonene every other day, or placebo; 29% of participants in the treatment group experienced significant relief by day 4, and 86% experienced complete relief of all symptoms by day 14.[41]

Botanical Medicines

Demulcent herbs soothe irritated tissue. In the gastrointestinal tract, these substances may help relieve irritation in the esophagus and stomach associated with GERD. Botanical medicines that may help include *Ulmus fulva, Althea officinalis, Plantago major, Pistacia lentiscus, Aloe vera,* and *Glycyrrhiza glabra.*

Glycyrrhiza glabra (Licorice)

Licorice has a mucoprotective effect because it stimulates or accelerates the differentiation of glandular cells and increases mucus formation and secretion from the gastric mucosa.[42] Because glycyrrhizinic acid (GA) has aldosterone-like side effects, a procedure was developed to remove GA from licorice, resulting in deglycyrrhizinated licorice (DGL). Licorice is also anti-inflammatory and prevents ulcer formation. Clinical studies have demonstrated no significant differences in recurrence rates of symptoms between cimetidine and DGL drug regimens.[43] GutsyGum is a chewing gum developed to alleviate the symptoms of GERD that contains calcium carbonate and a proprietary blend of licorice extract, papain, and apple cider vinegar. A double-blind, placebo-controlled crossover study examined the efficacy of GutsyGum in alleviating the symptoms of GERD after a refluxogenic meal compared with placebo gum.[44] Although many of the secondary symptoms, such as pain, nausea, and belching, were lower after GutsyGum, the difference was not statistically significant. However, the primary symptoms of heartburn and acid reflux were significantly lower in GutsyGum than in placebo treatment. The standard dosage for DGL is two to four 380-mg chewable tablets between meals or 20 minutes before meals. DGL therapy should be continued for at least 8 to 16 weeks after a full therapeutic response.

Althea officinalis (Marshmallow)

Although no clinical trials have investigated the effects of marshmallow on GERD, marshmallow contains 5% to 10% polysaccharides that provide a soothing and protective barrier on the mucous membranes of the esophagus and stomach, which may provide benefit.

Ulmus fulva (Slippery elm)

Slippery elm acts as a demulcent that produces a soothing film that extends over mucous membranes as well as acting as an anti-inflammatory agent. The bark of slippery elm is thought to be responsible for the healing effects due to the high percentage of mucilaginous polysaccharides. It is used to alleviate pain from irritated and inflamed mucosa lining the esophagus and gastrointestinal tract.[45]

Pistacia lentiscus (Mastic)

Mastic is a resin obtained from the mastic tree *(Pistacia lentiscus)*. Originally liquid, it is sun-dried into drops of hard, brittle, translucent resin. When chewed, the resin softens and becomes a bright white, opaque gum. The flavor is bitter at first, but after chewing, the gum releases a refreshing, slightly piney or cedar flavor. People in the Mediterranean region have used mastic gum as a medicine for gastrointestinal ailments for several thousand years. Although no human studies are currently available, animal studies have demonstrated that mastic significantly reduced the intensity of gastric mucosal damage induced by pyloric ligation, aspirin, and phenylbutazone.[46]

THERAPEUTIC APPROACH

Physicians should be judicious in prescribing acid-suppressing medications because these medications cause immune dysfunction that persists after discontinuation of the drug.[47] The use of PPIs in infants should be closely monitored in the light of changes in the gut microbiota and the oropharyngeal and respiratory tract colonization, potentially with pathogenic flora.

GENERAL RECOMMENDATIONS

General therapeutic objectives include symptom relief, reducing the risk of Barrett's esophagus and cancer, and improving quality of life. Many individuals experience heartburn nocturnally, when recumbent, and elevating the head of the bed to physically reduce gastroesophageal reflux is a standard medical recommendation in these cases. Lying on the left side while sleeping is also recommended because this position appears to alleviate reflux.

Smoking and alcohol should be eliminated. An exercise program may be implemented, as physical activity has some protective effect.[48]

Diet

Foods that precipitate attacks or aggravate symptoms should be identified and eliminated. The evening meal should be consumed 2-3 hours before retiring, as going to bed with a full stomach increases the likelihood of reflux. Overeating causes the stomach to distend, which may also increase symptoms. If necessary, a weight-reduction program may be implemented.

Supplements

- Alginate preparations are available as liquids and chewable tablets. The general recommendation is that the formula should provide a dosage of 500 to 1000 mg of alginate. The preparation is to be taken after meals and at nighttime as needed up to four times daily. For nighttime use, it is recommended to take 30 minutes before bedtime and avoid lying down for 30 minutes.

- Melatonin: 3 to 6 mg 30 to 45 minutes before bed

Botanicals

- Deglycyrrhizinated licorice: 380 to 760 mg three times a day 20 minutes before meals
- Marshmallow: powder, 1 teaspoon three times per day in cold water
- Slippery elm: powder, 1 teaspoon with food up to 4 times per day in cold water

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Moayyedi P, Talley NJ. Gastro-esophageal reflux disease. *Lancet*. 2006;367: 2086–2100.
2. Pandolfino JE, Kwiatek MA, Kahrilas PJ. The pathophysiologic basis for epidemiologic trends in gastroesophageal reflux disease. *Gastroenterol Clin Nor Am*. 2008;37:827–843.
3. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut*. 2014;63(6):871–880. PubMed PMID: 23853213.
4. Decktor D, Korn S, Stauffer L, Tipping R, Irvin J, Ciccone P. Intermittent heartburn and gastroesophageal reflux disease (GERD): effects of symptom frequency and severity on erosive mucosal changes. *Am J Gastroenterol*. 1998;93: 1614–1614.
5. Richter JE. Gastroesophageal reflux disease. *Best Prac Res Clin Gastroenterol*. 2007;21:609–631.
6. De Giorgi F, Palmiero M, Esposito I, Mosca F, Cuomo R. Pathophysiology of gastro-oesophageal reflux disease. *Acta Otorhinolaryngologica Italica*. 2006;26(5):241–246. PubMed PMID: 17345925.
7. Richter J. Do we know the cause of reflux disease? *Eur J Gastroenterol Hepatol*. 1999;1(supp 1):S3–S9.
8. Mittal RK, McCallum RW. Characteristics and frequency of transient relaxations of the lower esophageal sphincter in patients with reflux esophagitis. *Gastroenterol*. 1988;95:593–599.
9. Dent J, Dodds WJ, Friedman RH, et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. *J Clin Invest*. 1980;65:256–267. PubMed PMID: 7356677.
10. Orr WC, Robinson MG, Johnson LF. Acid clearance during sleep in the pathogenesis of reflux esophagitis. *Dig Dis Sci*. 1981;26:423–427. PubMed PMID: 7249882.
11. Kahrilas PJ. Gastroesophageal reflux disease and its complications. In: Feldman M, ed. *Sleisinger and Fordtran's Gastrointestinal and Liver Disease*. 6th ed. Philadelphia: WB Saunders Company; 1998:498–516.
12. Ott DJ, Gelfand DW, Chen YM, Wu WC, Munitz HA. Predictive relationship of hiatal hernia to reflux esophagitis. *Gastrointest Radiol*. 1985;10(4):317–320. PubMed PMID: 4054494.
13. Cameron AJ. Barrett's esophagus: prevalence and size of hiatal hernia. *Am J Gastroenterol*. 1999;94(8):2054–2059. PubMed PMID: 10445527.
14. Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. *Gastroeneterol*. 1987;92(1):130–135. PubMed PMID: 3781181.
15. Orlando RC. Pathophysiology of gastroesophageal reflux disease: offensive factors and tissue resistance. In: Orlando RC, ed. *Gastroesophageal Reflux Disease*. New York: Marcel Dekker; 2000:165–192.
16. Fass R. Distinct phenotypic presentations of gastroesophageal reflux disease: a new view of the natural history. *Dig Dis*. 2004;22(2):100–107. PubMed PMID: 15383749.
17. Yang I, Neil S, Suerbaum S. The microbiota of the stomach. *FEMS Microbiol Rev*. 2013;37:736–761.
18. Hunt RH, Camilleri M, Crowe SE, et al. The stomach in health and disease. *Gut*. 2015;64:1650–1668.
19. Norder Grusell E, Dahlen G, Ruth M, et al. Bacterial flora of the human oral cavity, and the upper and lower esophagus. *Dis Esophagus*. 2013;26(1): 84–90.
20. Yang L, Francois F, Pei Z. Molecular pathways: pathogenesis and clinical implications of micobiome alteration in esophagitis and Barrett esophagus. *Clin Cancer Res*. 2012;18(8):2138–2144.
21. Dunbar KB, Agoston AT, Odze RD, et al. Association of acute gastroesophageal reflux disease with esophageal histologic changes. *J Am Med Assoc*. 2016;315(19):2104–2112.
22. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R, et al. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. *Scientific Reports*. 2014;4:4202.
23. Nakaji G, Fujihara M, Fukata M, et al. Influence of common cardiac drugs on gastroesophageal reflux disease: multicenter questionnaire survey. *Int J Clin Pharmacol Ther*. 2011;49(9):555–562. PubMed PMID: 21888868.
24. Mungan Z, Pinarbasi Simsek B. Which drugs are risk factors for the development of gastroesophageal reflux disease? *Turk J Gastroenterol*. 2017;28(suppl 1):S38–S43. PubMed PMID: 29199166.
25. Numans ME, de Wit NJ. Reflux symptoms in general practice: diagnostic evaluation of the Carlsson-Dent gastro-oesophageal reflux disease questionnaire. *Aliment Pharmacol Ther*. 2003;17(8):1049–1055. PubMed PMID: 12694087.
26. Dibble C, Levine MS, Rubesin SE, Laufer I, Katzka DA. Detection of reflux esophagitis on double-contrast esophagrams and endoscopy using the histological findings as the gold standard. *Abdom Imaging*. 2004;29(4):421–425. PubMed PMID: 14716454.
27. Lacy BE, Weiser K, Chertoff J, et al. The diagnosis of gastroesophageal reflux disease. *Am J Med*. 2010;123(7):583–592. PubMed PMID: 20493461.
28. Jones R, Lydeard S. Prevalence of symptoms of dyspepsia in the community. *Lancet*. 1989;298(6665):30–32. PubMed PMID: 2492849.
29. Richter JE. Gastroesophageal reflux disease. *Best Pract Res Clin Gastroenterol*. 2007;21:609–631.
30. Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camargo Jr CA. Body-mass index and symptoms of gastroesophageal reflux in women. *N Eng J Med*. 2006;354(22):2340–2348. PubMed PMID: 16738270.
31. Singh M, Lee J, Gupta N, et al. Weight loss can lead to resolution of gastroesophageal reflux disease symptoms: a prospective intervention trial. *Obesity (Silver Spring)*. 2013;21(2):284–290. PubMed PMID: 23532991.
32. Mandel KG, Daggy BP, Brodie DA, Jacoby HI. Review article: alginate-raft formulations in the treatment of heartburn and acid reflux. *Aliment Pharmacol Ther*. 2000;14(6):669–690. PubMed PMID: 10848650.
33. Dettmar PW, Gil-Gonzalez D, Fisher J, et al. A comparative study on the raft chemical properties of various alginate antacid raft-forming products. *Drug Dev Ind Pharm*. 2018;44(1):30–39. PubMed PMID: 28836872.
34. Leiman DA, Riff BP, Morgan S. Alginate therapy is effective treatment for GERD symptoms: a systematic review and meta-analysis. *Dis Esophagus*. 2017;30(5):1–9.
35. Thomas E, Wade A, Crawford G, Jenner B, Levinson N, Wilkinson J. Randomised clinical trial: relief of upper gastrointestinal symptoms by an acid pocket-targeting alginate-antacid (Gaviscon Double Action)—a double-blind, placebo-controlled, pilot study in gastro-oesophageal reflux disease. *Aliment Pharmacol Ther*. 2014;39:595–602.
36. Savarino E, de Bortoli N, Zentilin P, et al. Alginate controls heartburn in patients with erosive and nonerosive reflux disease. *World J Gastroenterol*. 2012;18(32):4371–4378.
37. Chiu CT, Hsu CM, Wang CC, et al. Randomised clinical trial: sodium alginate oral suspension is non-inferior to omeprazole in the treatment of patients with non-erosive gastroesophageal disease. *Aliment Pharmacol Ther*. 2013;38:1054–1064.
38. Kandil TS, Mousa AA, El-Gendy AA, et al. The potential therapeutic effect of melatonin in gastro-esophageal reflux disease. *BMC Gastroenterol*. 2010;10:7–16.
39. De Souza Pereira R. Regression of gastroesophageal reflux disease symptoms using dietary supplementation with melatonin, vitamins and amino acids: comparison with omeprazole. *J Pineal Res*. 2006;41:195–200.
40. Sun J. D-limonene: safety and clinical applications. *Altern Med Rev*. 2007;12(3):259–264. PubMed PMID: 18072821.
41. Patrick L. Gastroesophageal reflux disease (GERD): a review of conventional and alternative treatments. *Altern Med Rev*. 2011;16(2):116–133. PubMed PMID: 21649454.
42. van Marle J, Aarsen PN, Lind A, et al. Deglycyrrhizinised liquorice (DGL) and the renewal of rat stomach epithelium. *Eur J Pharmacol*. 1981;72:219–225.
43. Morgan AG, McAdam WA, Pacsoo C, et al. Comparison between cimetidine and Caved-S in the treatment of gastric ulceration, and subsequent maintenance therapy. *Gut*. 1982;23:545–551.
44. Brown R, Sam CH, Green T, Wood S. Effect of GutsyGum™, a novel gum, on subjective ratings of gastro esophageal reflux following a refluxogenic meal. *J Diet Supplements*. 2015;12(2):138–145. PubMed PMID: 25144853.
45. Martin BR, Seaman DR. Dietary and lifestyle changes in the treatment of a 23-year-old female patient with migraine. *J Chiropr Med*. 2015;14(3):205–211. PubMed PMID: 26778934.

46. Al-Said MS, Ageel AM, Parmar NS, Tariq M. Evaluation of mastic, a crude drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity. *J Ethnopharmacol*. 1986;15(3):271–278. PubMed PMID: 3724207.
47. Birlutiu V, Luca CM, Birlutiu RM. Streptococcus agalactiae meningoencephalitis associated with gastroesophageal reflux disease and chronic proton pump inhibitors use, in a 9month-old infant: a case report. *BMC Pediatrics*. 2018;18(1):21. PubMed PMID: 29390957.
48. Nocon M, Labenz J, Willich SN. Lifestyle factors and symptoms of gastro-oesophageal reflux—a population-based study. *Aliment Pharmacol Ther*. 2006;23(1):L 169–174. PubMed PMID: 16393294.

173

Glaucoma: Acute (Angle Closure) and Chronic (Open Angle)

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

Acute Glaucoma

- Increased intraocular pressure, usually unilateral
- Severe throbbing pain in eye, with markedly blurred vision
- Pupil moderately dilated and fixed
- Absence of pupillary light response
- Nausea and vomiting common

Chronic Glaucoma

- Persistent elevation of intraocular pressure associated with pathological cupping of optic discs
- Asymptomatic in the early stages
- Gradual loss of peripheral vision, resulting in tunnel vision
- Insidious onset in older individuals

Normotensive Glaucoma

- Normal intraocular pressure with no pathological cupping of optic discs
- Asymptomatic in the early stages
- Gradual loss of peripheral vision, resulting in tunnel vision
- Insidious onset in older individuals, more common in women than men
- Low blood pressure a common underlying feature

GENERAL CONSIDERATIONS

Glaucoma refers to increased intraocular pressure (IOP) resulting from an imbalance between the production and outflow of aqueous humor. Obstruction to outflow is the main factor responsible for this imbalance in closed-angle glaucoma. Acute glaucoma can occur only with the closure of a preexisting narrow anterior chamber angle, whereas in chronic open-angle glaucoma the anterior chamber appears normal.

In the United States, approximately 3 million people have glaucoma, which is undetected in 25% of them.[1] The chronic open-angle type, for which there appears to be no consistent anatomical basis, accounts for 70% to 75% of these cases. Histologically, however, there is a strong correlation between the content and composition of collagen, glycosaminoglycans, extracellular matrix, and the glaucomatous eye.[2]

Collagen is the most abundant protein in the body, including the eye. In the eye, it provides tensile strength and integrity to the tissues (e.g., cornea, sclera, lamina cribrosa, trabecular meshwork, vitreous). Inborn errors of collagen metabolism (e.g., osteogenesis imperfecta, Ehlers–Danlos syndrome, Marfan syndrome) are often associated with ocular complications: glaucoma, myopia, retinal detachment, ectopia lentis, and blue sclera. Morphological changes in the lamina cribrosa (the scleral area that is pierced by the optic nerve fibers and blood vessels), trabecular meshwork (the connective tissue network through which aqueous humor must pass to reach the canal of Schlemm), and papillary blood vessels in the eye have all been observed in glaucomatous eyes. These changes may result in elevated increased ocular pressure (IOP) readings or, perhaps more significantly, lead to the progression of peripheral vision loss. Changes in collagen structure explain the following:

- Similar peripheral vision loss in patients with normal and elevated IOP
- Cupping of the optic disc even at low IOP levels
- No apparent anatomical reason for decreased aqueous outflow

In some cases, glaucoma develops in people with normal IOP. Referred to as low-tension glaucoma or normotensive glaucoma, this form accounts for approximately 25% to 30% of all cases of glaucoma

in the United States. Because elevated IOP is not a factor in normal-tension glaucoma, other factors must be responsible for the optic nerve damage. Suggested causes include:

- Reduced blood flow
- Early nerve cell death
- Nerve irritation
- Excess glutamate production
- Autoimmune disease

Normotensive glaucoma is more common in women than in men and affects adults averaging 60 years of age. A common risk factor for normotensive glaucoma is low blood pressure.

Toxic Metals

Toxic metals, such as lead, are known to increase the risk of hypertension by multiple mechanisms, including renal and oxidative damage, reducing available nitric oxide, and increasing circulating vasoconstrictive prostaglandins. It has been hypothesized that toxic metals in the blood may accumulate in the eyes, which may result in the elevation of intraocular pressure.[3] A study evaluating the relationship between toxic metals and intraocular pressure found that blood lead and blood mercury levels were associated with a 14% to 21% increase in IOP.[4] A cross-sectional study of 5198 participants older than 19 years of age examined the association between toxic metal levels and open-angle glaucoma (OAG) with low- and high-teen baseline intraocular pressure.[5] After adjusting for potential confounders, the low-teen OAG was positively associated with log-transformed blood cadmium levels (odds ratio [OR] = 1.41; 95% confidence interval [CI], 1.03–1.93). However, in this study, blood lead and blood mercury were not associated with disease prevalence. Although further studies are needed to better assess the significance of toxic metals in glaucoma, the toxic metal body load should be explored as a potential contributing factor in patients with IOP.

DIAGNOSIS

Patients with OAG initially have no symptoms, which is why this disease can be so insidious. Physical examination may reveal slight cupping of the optic disc and narrowing of the visual fields (Fig. 173.1). Tonometry is key to confirmation of the diagnosis.

The primary challenge with acute glaucoma is early recognition because a delay in referral for surgical intervention increases the risk of blindness. Table 173.1 provides an overview of the differential diagnosis of the inflamed eye.

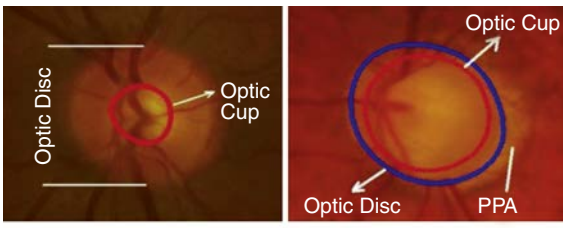


Fig. 173.1 Comparison of the Optic Disc Area of the (A) Normal and (B) Glaucomatous Eye. The cup boundary is shown with the red outline in both images, and the disc boundary is shown with blue outline in (B) only. There is a significantly larger cup in relation to the size of the optic disc in the glaucoma image compared with the normal image. Inferior-sector peripapillary atrophy (PPA) in the glaucoma image (B) is also evident, possibly due to concomitant erosion of the inferior neuroretinal rim tissue. (Haleem MS, Han L, Hemert JV, et al. Regional image features model for automatic classification between normal and glaucoma in fundus and scanning laser ophthalmoscopy [SLO] images. *J Medical Syst.* 2016;40[6]:132. PubMed PMID: 27086033.)

THERAPEUTIC CONSIDERATIONS

Treatment and prevention of both acute and chronic glaucoma depend on the reduction of IOP and improvement of collagen metabolism, particularly in the optic disc and trabecular meshwork.

The optic disc is composed of the lamina cribrosa, optic nerve fibers, and blood vessels. The lamina cribrosa is a meshlike network rich in collagen through which the optic nerves and blood vessels must pass (Fig. 173.2). The morphological changes in the collagen of the eye (i.e., lamina cribrosa, papillary vessels, and trabecular meshwork) precede pressure changes. Therefore primary prevention of the breakdown of the ground substance and collagen framework is important here, as it is in other conditions involving collagen abnormalities (i.e., atherosclerosis, rheumatoid arthritis, and periodontal disease).

Corticosteroids

The importance of collagen destruction in the etiology of glaucoma is apparent in corticosteroid-induced glaucoma.[2] Corticosteroid use should be discouraged in the patient with glaucoma because it is known to inhibit the biosynthesis of collagen and glycosaminoglycans, thereby worsening the patient's glaucoma.[2]

Diet, Supplements, Botanicals, and Lifestyle

Vitamin C

Of foremost importance in achieving collagen integrity are optimal tissue concentrations of ascorbic acid (AA). Furthermore, AA has been demonstrated to lower IOP levels in many clinical studies.[6–9] A daily dose of 0.5 g/kg, whether in single or divided doses, reduces the IOP by an average of 16 mm Hg.[9] Near-normal tension levels were achieved in some patients unresponsive to acetazolamide (a carbonic anhydrase inhibitor) and 2% pilocarpine (a miotic agent).[10] Note, however, that oral supplementation at this dosage will likely cause osmotic diarrhea.

The hypotonic action of AA on the eye is long-lasting if supplementation is continued, and intravenous administration results in an even greater initial reduction in IOP. The patient must be monitored to determine the appropriate individual dose because some patients respond to as little as 2 g/day, whereas others will respond only to extremely high doses (e.g., 35 g/day). Abdominal discomfort as a side effect of high doses is common but usually resolves after 3 to 4 days.[9]

The proposed mechanisms by which AA lowers IOP include the following:

- Increased blood osmolarity
- Diminished production of aqueous fluid by the ciliary epithelium
- Improved outflow of aqueous fluid

However, the role of AA in collagen formation may be the key to its antiglaucomatous action.

Flavonoids

To further aid normal collagen metabolism, flavonoids should be supplemented, particularly anthocyanosides (the blue-red pigments found in berries) and proanthocyanosides. These compounds elicit an AA-sparing effect, improve capillary integrity, and stabilize the collagen matrix by preventing free radical damage, inhibiting enzymatic cleavage of the collagen matrix, and cross-linking with collagen fibers directly to form a more stable collagen matrix.[11] *Vaccinium myrtillus* (European bilberry) extract is particularly rich in these flavonoid and anthocyanidin compounds and has been used in Europe, with good results in reducing myopia, improving nocturnal vision, and reversing diabetic retinopathy.

TABLE 173.1 Differential Diagnosis of the Inflamed Eye

	Acute Conjunctivitis	Acute Iritis	Acute Glaucoma	Corneal Trauma or Infection
Incidence	Very common	Common	Uncommon	Common
Discharge	Moderate to copious	None	None	Watery or purulent
Vision	No effect	Slightly blurred	Markedly blurred	Usually blurred
Pain	None	Moderate	Severe	Moderate to severe
Conjunctival injection	Diffuse, mostly fornices	Circumcorneal	Diffuse	Diffuse
Cornea	Clear	Usually clear	Steamy	Clarity may change
Pupillary size	Normal	Small	Dilated and fixed	Normal
Pupillary light response	Normal	Poor	None	Normal
Intraocular pressure	Normal	Normal	Elevated	Normal
Anterior chamber		Normal depth	Very shallow	Normal depth
Iris	Normal	Dull, swollen	Congested and bulging	Normal unless infected
Smear	Causative organisms	No organisms	No organisms	Causative organisms if there is infection

Modified from Distelhorst JS, Hughes GM. Open-angle glaucoma. *Am Fam Physician*. 2003;67:1937–1944.

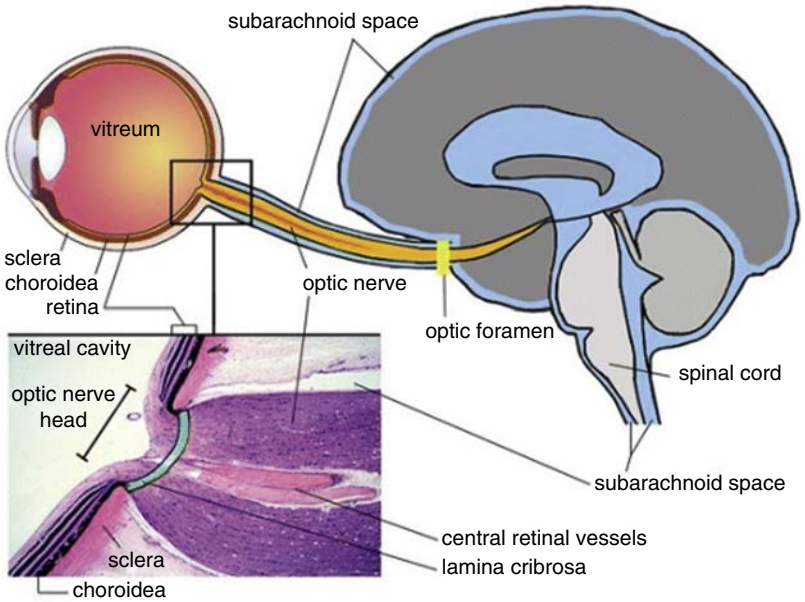


Fig. 173.2 Section of Eye, Optic Nerve Head, and Brain, Illustrating the Subarachnoid Space. The lamina cribrosa *(green)* is a sieve-like structure in the posterior part of the sclera and allows the passage of the retinal ganglion cell axons and the central retinal vessels. The optic nerve is surrounded by cerebrospinal fluid *(blue)* in the subarachnoid space. (Woslyn P, Van Dam D, Audenaert K, Killer HE, De Deyn PP, De Groot V. A new glaucoma hypothesis: a role of glymphatic system dysfunction. *Fluids Barriers CNS*. 2015;12:16. PubMed PMID: 26118970.)

Mirtogenol, a combination of bilberry anthocyanoside extract (160 mg Mirtoselect) and pine bark extract (80 mg Pycnogenol), was studied in 38 asymptomatic subjects with intraocular hypertension. Patients were given either Mirtogenol (20 subjects) or were not treated (18 subjects). After 2 months of supplementation with Mirtogenol, the mean IOP decreased from a baseline of 25.2 to 22.2 mm Hg. After 3 months of treatment with Mirtogenol, the IOP was significantly lowered compared with that of untreated controls ($P < 0.05$) to 22 mm Hg. No further improvement was found after 6 months. Of the 20 patients taking Mirtogenol, 19 had a decreased IOP after 3 months. Only marginal effects on the IOP were found in the 18 control subjects. No side effects were observed. Ocular blood flow (central retinal, ophthalmic, and posterior ciliary arteries) improved both in the systolic and diastolic components as measured by ultrasound. After 3 months of treatment, the improvement in ocular blood flow was significant compared with both baseline and the control group.[10] Rutin has also been demonstrated to lower IOP when used as an adjunct in patients unresponsive to miotics alone.[12]

In patients with normotensive glaucoma (NTG), *Ginkgo biloba* extract (GBE) may be helpful, based on the results of two double-blind studies. In the first study, which comprised healthy human volunteers, GBE (120 mg daily) significantly increased end-diastolic velocity in the ophthalmic artery (23% change), with no change in the placebo group. GBE did not alter arterial blood pressure, heart rate, or IOP.[13] In the second study, patients with NTG received either 40 mg GBE or placebo three times daily for 4 weeks, followed by a washout period of 8 weeks, then 4 weeks of the opposite treatment. After GBE treatment, a

significant improvement in visual field indices was recorded, showing that GBE improves preexisting visual field damage in some patients with NTG.[14]

Allergen Avoidance

The successful treatment of chronic glaucoma by antiallergy measures has been reported in the literature.[15] In one study, many of the 113 patients demonstrated an immediate rise in IOP of up to 20 mm Hg (in addition to other typical allergic symptoms) when challenged with the appropriate allergen, whether foodborne or environmental. The author speculated that the known allergic responses of altered vascular permeability and vasospasm could result in the congestion and edema characteristic of glaucoma.

Magnesium

Because channel-blocking drugs benefit some glaucoma patients, a group of researchers at the University Eye Clinic in Basel, Switzerland, decided to evaluate the effect of supplemental magnesium, which has been referred to as "nature's physiological calcium-channel blocker." The trial included 10 glaucoma patients (6 with primary open-angle glaucoma and 4 with normal-tension glaucoma). All patients had digital cold-induced vasospasm. Magnesium was given at a dose of 121.5 mg twice daily for 1 month. After 4 weeks of treatment, the visual fields improved, as noted by a decrease in the mean defect and square root of loss variance scores. All three nail fold–capillaroscopic parameters improved, as well as digital temperature. These results demonstrate that magnesium supplementation improves the peripheral circulation and seems to have a beneficial effect on the visual field in patients with glaucoma.[16]

To evaluate the effect of oral magnesium therapy on ocular blood flow and visual field perimetry indices in patients with normotensive glaucoma (NTG), 15 patients with NTG (the study group) received 300 mg of oral magnesium (citrate) for 1 month, whereas 15 patients with NTG (the control group) received no treatment. In the study group, significant improvements were noted in visual field measurements (e.g., mean deviation improved from –3.7 at baseline to –2.5 and pattern standard deviation improved from 3.6 baseline to 2.8). There was no change in ocular blood flow, so the exact mechanism of magnesium's effect is not known.[17]

Chromium

A case-controlled study of 400 consecutive eye patients, 52 of whom had ocular hypertension glaucoma, or both, found that primary open-angle glaucoma was strongly associated with a deficiency of erythrocyte chromium and average daily intake of ascorbic acid, and elevations of erythrocyte vanadium, which is chromium's principal antagonist.

Ascorbic acid and chromium potentiate insulin receptors, which help sustain strong focusing activity in the ciliary muscle. Deficiencies of either ascorbic acid or chromium were associated with elevated intraocular pressure, which tends to stretch the normal eye, thereby reducing focusing power.[18]

Fish Oil

In an interesting exploratory study, rabbits were fed food soaked with cod liver oil, resulting in a drop in IOP from 25 to 11 mm Hg. Intramuscular injections of cod liver oil produced a dose-dependent reduction in IOP. When the animals were taken off cod liver oil, their IOP returned to baseline. Control animals given liquid lard or safflower oil experienced no change in IOP.[19] Preliminary studies in humans with docosahexaenoic acid have had encouraging results.[20]

Caffeine

Many physicians instruct patients with glaucoma to avoid coffee, although clinical data supporting this practice are insufficient. To estimate the effect of coffee drinking on IOP, the effects of the consumption of regular coffee (180 mg caffeine in 200 mL of coffee) and decaffeinated coffee (3.6 mg caffeine in 200 mL of coffee) were compared in patients with normotensive glaucoma or ocular hypertension in a double-blind crossover study. IOP was monitored in both groups at 30, 60, and 90 minutes after coffee ingestion. In patients with normotensive glaucoma who drank regular caffeine-containing coffee, the mean changes in IOP at 30, 60, and 90 minutes were 0.9, 3.6, and 2.3 mm Hg, respectively; in those who drank decaffeinated coffee, they were 0.75, 0.70, and 0.4 mm Hg, respectively. The corresponding values in patients with ocular hypertension were as follows: after regular coffee, 1.1, 3.4, and 3 mm Hg; and after decaffeinated coffee, 0.6, 0.9, and 0.5 mm Hg, respectively. This study showed clearly that subjects who drank regular coffee experienced a greater elevation in IOP, which may be clinically significant.[21]

Exercise

Exercise can lead to an immediate and prolonged reduction in IOP. Within 5 minutes of starting exercise, IOP initially increases and then gradually decreases to its lowest level 60 minutes after exercise. The drop in IOP is approximately 23% in normal individuals, whereas people with glaucoma usually experience a greater drop and longer duration of postexercise recovery than do those with normal eyes.[22] Specifically, the drop in IOP for glaucoma patients after walking/jogging and running was 7.2% and 12.7%, respectively, more than the decrease in IOP experienced by persons with normal eyes. Similarly, the mean duration of the IOP-lowering effect after running was approximately 84 minutes in glaucomatous eyes and 63 minutes in normal eyes. The mechanism of acute lowering of IOP is independent of systemic blood pressure and level of sympathetic stimulation but may be partly due to increased serum osmolarity.

Exercise appears to be effective in lowering IOP in sedentary subjects engaging in moderate to heavy exercise but is somewhat less effective in physically fit subjects.[23] However, individuals who are more physically fit tend to have lower IOP. If one stops exercising, the effect wears off in 3 weeks. Although exercise may not be effective in lowering IOP in everyone, it can lead to significant improvements in many. One study found that IOP was lowered by at least 2 mm Hg by exercise in 34% of subjects; however, 57% had no change in IOP, whereas 9% had an IOP elevation.[24]

Coleus forskohlii

In clinical studies, forskolin from *Coleus forskohlii* was shown to greatly reduce IOP when it was applied directly to the eyes (see Chapter 69, *Coleus forskohlii*). Oral administration might prove effective as well. In an open-label pilot study, 16 patients with OAG and stable IOP underwent treatment with different topical drugs and were given a supplement containing 15 mg of forskolin along with rutin (200 mg), thiamin (0.7 mg), and riboflavin (0.8 mg) for 40 days, which produced a further decrease in IOP by roughly 20% of the initial value.[25,26]

Other Factors

Helicobacter pylori

Several studies have found an association between *Helicobacter pylori* infection and OAG.[26] A particularly interesting 2-year study found that successful eradication of *H. pylori* resulted in decreased IOP.[27] See Chapter 198 for a discussion of the treatment of *H. pylori.*

THERAPEUTIC APPROACH

Acute closed-angle glaucoma is an ocular emergency; such patients should be referred immediately to an ophthalmologist or emergency room. Unless adequately treated within 12 to 48 hours, the patient will become permanently blind within 2 to 5 days. An asymptomatic eye with a narrow anterior chamber angle may convert spontaneously to angle-closure glaucoma. The process can be precipitated by anything that dilates the pupil, such as atropine and epinephrine-like drugs. Typical signs and symptoms include extreme pain; blurring of vision; conjunctivitis; and a fixed, dilated pupil.

Agents that dilate the pupils must be strictly avoided in any patient suspected of having glaucoma.

Lifestyle

All patients who are sedentary should be advised to exercise. However, their IOP must be regularly monitored to determine whether they are in the small percentage that will experience an elevation in IOP.

Supplements

- Vitamin C: 0.1 to 0.5 g/kg per day in divided doses
- Bioflavonoids (mixed): 1000 mg/day
- Magnesium: 300 mg/day
- Chromium: 100 mcg/day

Botanical Medicines

For the prevention and treatment of chronic open-angle glaucoma, either bilberry extract or a proanthocyanoside extract, alone or in combination, is indicated as follows:

- *V. myrtillus* extract (25% anthocyanidin content): 160 to 240 mg a day
- Pine bark extract (Pycnogenol) or grape seed extract (95% proanthocyanidin content): 150 to 300 mg a day

  For normotensive glaucoma:
- *Ginkgo biloba* extract: 120 to 320 mg a day

  For open-angle glaucoma:
- *C. forskohlii:*
  - Forskolin: 5 to 10 mg two to three times a day
  - Standardized extract (10% forskolin): 250 mg one to two times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311(18):1901–1911.
2. Wallace DM, Murphy-Ullrich JE, Downs JC, O'Brien CJ. The role of matricellular proteins in glaucoma. *Matrix Biol*. 2014;37:174–182.
3. DuVal G, Grubb BR, Bentley PJ. Mercury accumulation in the eye following administration of methylmercury. *Exp Eye Res*. 1987;44(1):161–164. PubMed PMID: 3556450.
4. Park S, Choi NK. Associations of blood heavy metal levels with intraocular pressure. *Ann Epidemiol*. 2016;26(8):546–550. PubMed PMID: 27497680.
5. Lee SH, Kang EM, Kim GA, et al. Three toxic heavy metals in open-angle glaucoma with low-teen and high-teen intraocular pressure: a cross-sectional study from South Korea. *PLoS One*. 2016;11(10):e0164983. PubMed PMID: 27768724.
6. Fishbein S, Goodstein S. The pressure lowering effect of ascorbic acid. *Ann Ophthalmol*. 1972;4:487–491.
7. Linner E. The pressure lowering effect of ascorbic acid in ocular hypertension. *Acta Ophthalmol (Copenh)*. 1969;47:685–689.
8. Shen TM, Yu MC. Clinical evaluation of glycerin-sodium ascorbate solution in lowering intraocular pressure. *Chinese Med J (Engl)*. 1975;1:64–68.
9. Virno M, Bucci M, Pecori-Giraldi J, et al. Oral treatment of glaucoma with vitamin C. *Eye Ear Nose Throat Monthly*. 1967;46:1502–1508.
10. Steigerwalt RD, Gianni B, Paolo M, et al. Effects of Mirtogenol on ocular blood flow and intraocular hypertension in asymptomatic subjects. *Mol Vis*. 2008;14:1288–1292.
11. Patel S, Mathan JJ, Vaghefi E, Braakhuis AJ. The effect of flavonoids on visual function in patients with glaucoma or ocular hypertension: a systematic review and meta-analysis. *Graefes Arch Clin Exp Ophthalmol*. 2015;253(11):1841–1850.
12. Stocker F. New ways of influencing the intraocular pressure. *N Y St J Med*. 1949;49:58–63.
13. Chung HS, Harris A, Kristinsson JK, et al. Ginkgo biloba extract increases ocular blood flow velocity. *J Ocul Pharmacol Ther*. 1999;15(3):233–240.
14. Quaranta L, Bettelli S, Uva MG, et al. Effect of Ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. *Ophthalmology*. 2003;110(2):359–362.
15. Raymond LF. Allergy and chronic simple glaucoma. *Ann Allergy*. 1964;22:146–150.
16. Gaspar AZ, Gasser P, Flammer J. The influence of magnesium on visual field and peripheral vasospasm in glaucoma. *Ophthalmologica*. 1995;209:11–13.
17. Aydin B, Onol M, Hondur A, et al. The effect of oral magnesium therapy on visual field and ocular blood flow in normotensive glaucoma. *Eur J Ophthalmol*. 2010;20(1):131–135.
18. Lane BC. Diet and glaucomas. *J Am Coll Nutr*. 1991;10:536.
19. McGuire R. Fish oil cuts lower ocular pressure. *Med Tribune*. 1991;19:25.
20. Cellini M, Caramazza N, Mangiafico P, et al. Fatty acid use in glaucomatous optic neuropathy treatment. *Acta Ophthalmol Scand Suppl*. 1998;227:41–42.
21. Avisar R, Avisar E, Weinberger D. Effect of coffee consumption on intraocular pressure. *Ann Pharmacother*. 2002;36:992–995.
22. Qureshi IA. The effects of mild, moderate, and severe exercise on intraocular pressure in glaucoma patients. *Jpn J Physiol*. 1995;45:561–569.
23. Qureshi IA. Effects of exercise on intraocular pressure in physically fit subjects. *Clin Exp Pharmacol Physiol*. 1996;23:648–652.
24. Era P, Parssinen O, Kallinen M, et al. Effect of bicycle ergometer test on intraocular pressure in elderly athletes and controls. *Acta Ophthalmol (Copenh)*. 1993;71:301–307.
25. Pescosolido N, Librando A. Oral administration of an association of forskolin, rutin and vitamins B1 and B2 potentiates the hypotonising effects of pharmacological treatments in POAG patients. *Clin Ter*. 2010;161:e81–e85.
26. Kountouras J, Zavos C, Chatzopoulos D. Induction of apoptosis as a proposed pathophysiological link between glaucoma and *Helicobacter pylori* infection. *Med Hypotheses*. 2004;62:378–381.
27. Kountouras J, Mylopoulos N, Chatzopoulos D, et al. Eradication of *Helicobacter pylori* may be beneficial in the management of chronic open-angle glaucoma. *Arch Intern Med*. 2002;162:1237–1244.

174

Gout

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Acute onset, frequently nocturnal, with typically monoarticular joint pain, involving the metatarsophalangeal joint of the big toe in about 50% of cases
- Elevated serum uric acid level
- Asymptomatic periods between acute attacks
- Identification of urate crystals in joint fluid
- Aggregated deposits of monosodium urate monohydrate (tophi) chiefly in and around the joints of the extremities but also in subcutaneous tissue, bone, cartilage, and other tissues
- Uric acid kidney stones
- Familial disease; 95% males

GENERAL CONSIDERATIONS

Gout is a common type of arthritis caused by an increased concentration of uric acid (the final breakdown product of purine metabolism) in biological fluids. In gout, uric acid crystals (monosodium urate) are deposited in joints, tendons, kidneys, and other tissues, where they cause considerable inflammation and damage.[1] Gout is a condition characterized biochemically by increased serum uric acid levels, leukotriene levels, and neutrophil accumulation. Gout may lead to debilitation owing to the tophaceous deposits around the joints and tendons; renal involvement may result in kidney failure due to either parenchymal disease or urinary tract obstruction.

Gout is associated with affluence and is often called the "rich man's disease." Throughout history, the sufferer of gout has been depicted as a portly, middle-aged man sitting in a comfortable chair with one foot resting painfully on a soft cushion as he consumes great quantities of meat and wine. In fact, the traditional picture does have some basis because meats, particularly organ meats, are high-purine foods, whereas alcohol inhibits uric acid secretion by the kidneys. Gout is primarily a disease of adult males, with more than 95% of sufferers being men older than age 30. The incidence of diagnosed gout cases has been estimated at 2.13% of the 2009 U.S. population, although 10% to 20% of the adult population has hyperuricemia. Gout is a strong predictor of the metabolic syndrome and an increased risk for type 2 diabetes.[1–4]

Causes of Gout

Gout is classified into two major categories: primary and secondary. Primary gout accounts for about 90% of all cases, whereas secondary gout accounts for only 10%. Primary gout is usually idiopathic (i.e., the underlying metabolic defect is unknown). However, there are several genetic defects in which the exact cause of the elevated uric acid is known.[1] The synthesis and degradation of purines are summarized in Fig. 174.1.

The term *secondary gout* refers to those cases in which the elevated uric acid level is secondary to some other disorder, such as excessive breakdown of cells or some form of renal disease. Diuretic therapy for hypertension and low-dose aspirin therapy are also important causes of secondary gout because they cause decreased uric acid excretion.

The increased serum uric acid level observed in primary idiopathic gout can be divided into three categories:

- Increased synthesis of uric acid, found in a majority of individuals
- Reduced ability to excrete uric acid, typical of a smaller group (about 30%)
- Overproduction of uric acid, as well as underexcretion of uric acid—a small minority

Although the exact metabolic defect in gout is unknown in the majority of cases, gout is one of the most controllable metabolic diseases. Box 174.1 summarizes the causes of gout.

About 200 to 600 mg of uric acid is excreted daily in the urine of an adult male. This is two thirds of the amount produced, the rest being excreted in the bile and other gastrointestinal tract secretions. The dietary component of uric acid is usually 10% to 20%, but in an individual with significant hyperuricemia, 1 mg/100 mL may be added to the serum concentration of uric acid through the diet, enough to increase precipitation into the tissues if the individual is near the saturation threshold.

Almost all of the plasma urate is filtered at the glomerulus; only the small amount bound to protein is not filtered. Renal excretion is peculiar in that about 80% of the filtered uric acid is reabsorbed in the proximal tubule of the nephron. Actually, the distal tubule secretes most of the uric acid found in the urine. Distal to this site, some postsecondary reabsorption occurs. These events are summarized in Fig. 174.2.

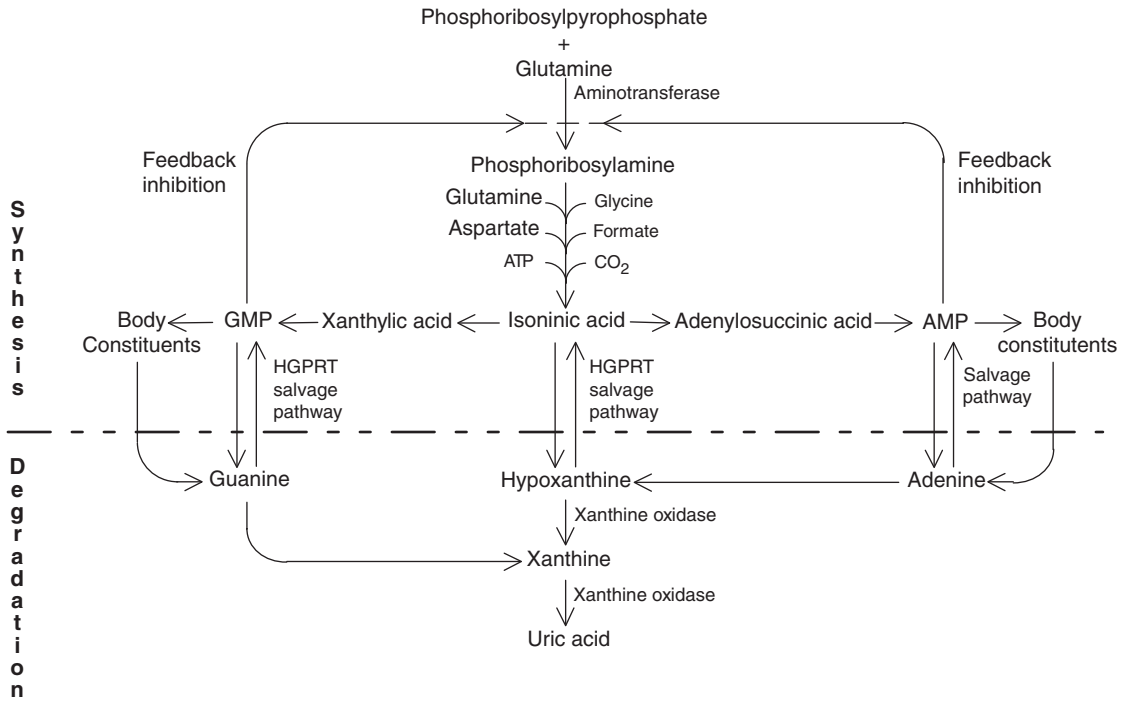


Fig. 174.1 Purine synthesis and degradation. (From Nutrition Foundation. *Nutrition reviews' present knowledge in nutrition*. 5th ed. Washington, DC: Nutrition Foundation; 1984: 740–756.)

BOX 174.1 Causes of Hyperuricemia

Metabolic

- Increased production of purine (primary)
- Idiopathic
- Specific enzyme defects (e.g., Lesch–Nyhan syndrome, glycogen storage disease)
- Decreased enzyme activity (e.g., hypoxanthine-guanine phosphoribosyltransferase is decreased in 1%–2% of adults with gout)
- Increased enzyme activity (e.g., phosphoribosylpyrophosphate synthetase)
- Increased production of purine (secondary)
- Increased turnover of purines
- Myeloproliferative disorders
- Lymphoproliferative disorders
- Carcinoma and sarcoma (disseminated)
- Chronic hemolytic anemia
- Cytotoxic drugs
- Psoriasis
- Increased de novo synthesis (e.g., glucose-6-phosphatase deficiency)
- Increased catabolism of purines
- Fructose ingestion or infusion
- Exercise

Renal

- Decreased renal clearance of uric acid (primary)
- Intrinsic kidney disease
- Decreased renal clearance of uric acid (secondary)
- Functional impairment of tubular secretion
- Drug induced (e.g., thiazides, probenecid, salicylates, ethambutol, pyrazinamide)
- Hyperlacticemia (e.g., lactic acidosis, alcoholism, toxemia of pregnancy, chronic beryllium disease)
- Hyperketoacidemia (e.g., diabetic ketoacidosis, fasting, starvation)
- Diabetes insipidus
- Bartter syndrome
- Chronic lead intoxication
- Glucose-6-phosphatase deficiency

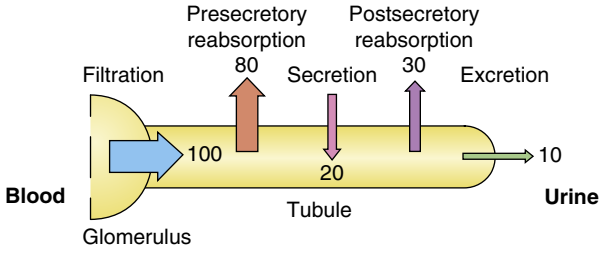


Fig. 174.2 Normal uric acid excretion.

Uric acid is a highly insoluble molecule; at pH 7.4 and body temperature, the serum is saturated at 6.4 to 7 mg/100 mL. Although higher concentrations do not necessarily result in urate deposition (some unknown factor in serum appears to inhibit urate precipitation), the chance of an acute attack is greater than 90% when the level is above 9 mg/100 mL (Table 174.1). Lower temperatures decrease the saturation point of uric acid, which may explain why urate deposits tend to form in areas such as the pinna of the ear, where the temperature is lower than the mean body temperature (Table 174.2). Uric acid is insoluble below pH 6 and can precipitate as the urine is concentrated in the collecting ducts and passed to the renal pelvis.

Signs and Symptoms

The first attack of gout is characterized by intense pain, usually involving only one joint. The first joint of the big toe is affected in nearly one half of the first attacks and is at some time involved in more than 90% of individuals with gout. If the attack progresses, fever and chills will appear. The first attacks usually occur at night and are generally preceded by a specific event, such as dietary excess, alcohol ingestion, trauma, certain drugs, or surgery.

The classic description of gout was offered in 1683 by Sydenham, an English physician who suffered from gout. Little has changed in the clinical picture of gout in more than 300 years. Sydenham's classic description is as follows:

> *The victim goes to bed and sleeps in good health. About two o'clock in the morning he is awakened by a severe pain in the great toe;*

TABLE 174.1 Prevalence of Gouty Arthritis by Maximum Urate Level

Serum Urate (mg/100 mL)	Men (%)	Women (%)
<6	0.6	0.08
6–6.9	1.9	3.3
7–7.9	16.7	17.4
8–8.9	25	0
9+	90	0

Data from Faller J, Fox IH. Ethanol-induced hyperuricemia: evidence for increased urate production by activation of adenine nucleotide turnover. *N Engl J Med.* 1982;307:1598–1602.

TABLE 174.2 Solubility of Urate Ion as a Function of Temperature in 140 mM Na^+

Temperature (°C)	Maximum Solubility (mg/100 mL)
37	6.8
35	6
30	4.5
25	3.3
20	2.5
15	1.8
10	1.2

Data from Faller J, Fox IH. Ethanol-induced hyperuricemia: evidence for increased urate production by activation of adenine nucleotide turnover. *N Engl J Med.* 1982;307:1598–1602.

> *more rarely in the heel, ankle, or instep. The pain is like that of a dislocation, and yet parts feel as if cold water were poured over them. Then follows chills and shivers, and a little fever. The pain which at first was moderate, becomes more intense. With its intensity the chills and fever increase. After a time this comes to a height, accommodating itself to the bones and ligaments of the tarsus and metatarsus. Now it is a violent stretching and tearing of the ligaments—now it is a gnawing pain and now a pressure and tightening. So exquisite and lively meanwhile is the feeling of the part affected, that it cannot bear the weight of bedclothes nor the jar of a person walking in the room. The night is passed in torture, sleeplessness, turning the part affected, and perpetual change of posture; the tossing about of the body being as incessant as the pain of the tortured joint, and being worse as the fit comes on. Hence the vain effort by change of posture, both in the body and the limb affected, to obtain an abatement of pain.*

Subsequent attacks are common, with the majority of such individuals having another attack within 1 year. However, nearly 7% never have a second attack. Chronic gout rarely is an issue, owing to the advent of dietary therapy and drugs that lower uric acid levels. When it does occur, chronic gout is due to poor compliance or inadequate response to treatment, or it may arise in patients with high flare frequency, tophi, and the inability to maintain serum urate levels below 6 mg/dL.[2] Some degree of kidney dysfunction occurs in nearly 90% of subjects with gout, and there is a higher risk of kidney stones.

THERAPEUTIC CONSIDERATIONS

The current standard medical treatment of acute gout is the administration of colchicine, the anti-inflammatory drug originally isolated from the plant *Colchicum autumnale* (autumn crocus, meadow saffron). Colchicine has no effect on uric acid levels; rather, it stops the inflammatory process by inhibiting neutrophil migration into areas of inflammation.

More than 75% of patients with gout show a major improvement in symptoms within the first 12 hours after receiving colchicine. However, up to 80% of patients are unable to tolerate an optimal dose because of gastrointestinal side effects, which may precede or coincide with clinical improvement.

Colchicine may also cause bone marrow depression, hair loss, liver damage, depression, seizures, respiratory depression, and even death. Other anti-inflammatory agents are also used in acute gout, including various nonsteroidal anti-inflammatory drugs (NSAIDs), indomethacin, phenylbutazone, naproxen, and fenoprofen.

Once the acute episode has resolved, a number of measures are taken to reduce the likelihood of recurrence:

- Drugs like allopurinol or febuxostat to keep uric acid levels within a normal range
- Controlled weight loss in obese individuals
- Avoidance of known precipitating factors, such as heavy alcohol consumption or a diet rich in purines or refined carbohydrates
- Low doses of colchicine to prevent further acute attacks

Several dietary factors are known to cause gout: consumption of alcohol, especially beer and hard liquor; foods high in purine content (e.g., organ meats, meat, yeast, poultry); fats; refined carbohydrates, especially high-fructose corn syrup; and overconsumption of calories.[5] Individuals with gout are typically obese; they are prone to hypertension, the metabolic syndrome, and diabetes and are at a greater risk for cardiovascular disease. Obesity is probably the most important dietary factor. Thiazide and loop diuretics are also associated with a higher risk of incident gout and a higher rate of gout flares.[5]

In concept, the naturopathic approach for chronic gout does not differ substantially from the standard medical approach. However, naturopaths focus on dietary and herbal measures to keep uric acid levels within the normal range rather than on the use of drugs. In the conventional medical treatment of gout, drugs that inhibit xanthine oxidase are too often relied upon. Allopurinol, a structural isomer of hypoxanthine (a naturally occurring purine in the body), has been the mainstay treatment for decades. However, in February 2009, the U.S. Food and Drug Administration approved febuxostat (Uloric), another xanthine oxidase inhibitor; it is a more effective treatment for lowering and maintaining serum urate levels.[6] Febuxostat is beginning to supplant allopurinol in the conventional management of gout. Uricosuric agents (probenecid, sulfinpyrazone, and benzbromarone) are used as second-line therapy for patients with underexcretion of uric acid.

Xenobiotics

The risk of hyperuricemia is increased in subjects with higher serum concentrations of organochlorine (OC) pesticides, polychlorinated dibenzodioxins (PCDDs), and dioxin-like polychlorinated biphenyls (PCBs).[7] Chronic low-level arsenic exposure is associated with hyperuricemia and gout. In men, the adjusted odds ratio (OR) for hyperuricemia comparing highest to lowest quartiles of total arsenic was 1.84 (95% confidence interval [CI], 1.26–2.68), and in women, the OR was 1.26 (95% CI, 0.77–2.07).[8] Serum uric acid increases in proportion to body load of perfluorinated hydrocarbons (PFOA and PFOS).[9] Interestingly, low levels of PFOA or PFOS show little effect initially and then rapid elevation of uric acid levels.

An additional item of concern relates to lead toxicity. A secondary type of gout, sometimes called saturnine gout, can result from lead toxicity. Historically, saturnine gout was due to the consumption of alcoholic beverages stored in containers with lead in them. An unexpected and fairly common source of lead appears to be leaded crystal because port wine elutes lead when stored in a crystal decanter.[10] Lead concentration increases with storage time, reaching toxic levels

after several months. Even a few minutes in a crystal glass results in a measurable increase in the level of lead in the wine. Lead promotes hyperuricemia as a result of decreasing renal urate excretion. Among patients in the highest blood lead level (BLL) quartile (mean, 3.95 µg/dL), the prevalence of gout was 6.05% (95% CI, 4.49%–7.62%) compared with 1.76% (CI, 1.10%–2.42%) among those in the lowest quartile (mean, 0.89 µg/dL).[11] Each doubling of BLL was associated with an unadjusted OR of 1.74 (CI, 1.47–2.05) for gout and 1.25 (CI, 1.12–1.40) for hyperuricemia.

Dietary Considerations

The dietary treatment of gout involves the following guidelines:

- Elimination of alcohol intake
- Low purine intake
- Achievement of ideal body weight
- Liberal consumption of complex carbohydrates
- Low fat intake
- Low protein intake
- Liberal fluid intake

Alcohol

Alcohol increases uric acid production by accelerating purine nucleotide degradation; it also reduces uric acid excretion by increasing lactate production (a result of ethanol oxidation), which impairs kidney function. The net effect is a significant increase in serum uric acid levels. This explains why alcohol consumption is often a precipitating factor in acute attacks of gout. Using a 24-hour diet recall, beer consumption was significantly associated with hyperuricemia, with an adjusted OR of 1.28 for men who consumed five or more cans of beer daily compared with those who did not drink.[12] In many individuals, the elimination of alcohol is all that is necessary to reduce uric acid levels and prevent gouty arthritis.

Low-Purine Alkaline-Ash Diet

A low-purine diet has been the mainstay of the dietary therapy of gout for many years. However, with the advent of potent drugs that lower uric acid levels, many physicians lower serum urate levels without subjecting the patient to the inconvenience and deprivation associated with a purine-free diet. However, dietary restriction of purines is recommended to reduce metabolic stress. Foods with high purine levels should be entirely omitted. These include organ meats, meats, shellfish, yeast (brewer's and baker's), herring, sardines, mackerel, and anchovies. Foods with moderate levels of protein should be curtailed as well. These include dried legumes, spinach, asparagus, fish, poultry, and mushrooms. In an analysis of 1247 recurrent gout attacks occurring over a 1-year period in 633 participants, the multivariate OR for recurrent gout attacks for each increasing quintile of purine consumption were 1.17 (95% CI 0.88, 1.55). 1.38 (95% CI 1.02, 1.87), 2.21 (95% CI 1.62, 3.01), and 4.76 (95% CI 3.37, 6.74), respectively.[13]

An alkaline-ash diet is recommended in the dietary treatment of gout because a more alkaline pH increases uric acid solubility. An alkaline-ash diet was shown to increase uric acid excretion from 302 mg/day at pH 5.9 to 413 mg/day at pH 6.5.[14]

Weight Reduction

Excess weight is associated with an increased rate of gout. Weight reduction in obese individuals significantly reduces levels of serum uric acid levels.[15] Weight reduction should involve the use of a low-glycemic diet designed to improve insulin sensitivity. Such a diet also helps manage the elevated cholesterol and triglycerides that are common in obesity.

Carbohydrates, Fats, and Protein

Refined carbohydrates and saturated fats should be kept to a minimum because the former increases uric acid production, whereas the latter increases uric acid retention. In addition, one of the key dietary goals in the treatment of gout appears to be the enhancement of insulin sensitivity.[15] Acute and hypercaloric ingestion of fructose raises serum urate levels via the unregulated phosphorylation of fructose by fructokinase, leading to local ATP depletion and increased AMP production. Exposure to two or more sugar-sweetened beverages (SSBs) per day is associated with a 1.78-fold increased risk of gout in men and a 3.05-fold increased risk in women.[16,17] Fructose consumption is associated with an increased risk of gout (relative risk [RR] = 1.62, 95% CI, 1.28–2.03) when comparing the highest and lowest quantiles of consumption.[18]

Protein intake should not be excessive (i.e., >0.8 g/kg per day) because it has been shown that uric acid synthesis may be accelerated in both normal and gouty patients by a high protein intake. After adjusting for confounders, men in the highest quintiles of total meat and seafood intake have an increased risk of incident gout of 41% and 51%, respectfully.[19] Adequate protein is necessary (0.8 g/kg daily). However, amino acids decrease the resorption of uric acid in the renal tubules, thus increasing uric acid excretion and reducing serum uric acid concentrations.

Fluid Intake

Liberal fluid intake keeps the urine dilute and promotes the excretion of uric acid. Furthermore, dilution of the urine reduces the risk of kidney stones.

Nutritional Supplements

Eicosapentaenoic Acid

Eicosapentaenoic acid (EPA) supplementation may prove useful in the treatment of gout. EPA limits the production of the proinflammatory leukotrienes, the mediators of much of the inflammation and tissue damage observed in gout.

Folic Acid

Folic acid has been shown to inhibit xanthine oxidase, the enzyme responsible for producing uric acid.[20] Research has demonstrated that a derivative of folic acid is an even greater inhibitor of xanthine oxidase than allopurinol, suggesting that folic acid at pharmacological doses may be an effective treatment for gout.[21] Positive results in the treatment of gout have been reported, but the data are incomplete and uncontrolled.[22]

Quercetin

The bioflavonoid quercetin has demonstrated several effects in experimental studies, indicating its possible benefit to individuals with gout.[23,24] Quercetin may offer significant protection by inhibiting the following:

- Xanthine oxidase in a similar fashion to the drug allopurinol[25]
- Leukotriene synthesis and release
- Neutrophil accumulation and enzyme release

One clinical study evaluated the effect of 4 weeks of oral supplementation of quercetin on plasma uric acid levels.[24] Twenty-two healthy males (19–60 years) with baseline plasma uric acid concentration in the higher, but still considered healthy, range (339 (SD 51) µmol/l) were enrolled in the randomized, double-blinded, placebo-controlled, crossover trial. The intervention included one tablet containing 500 mg quercetin daily for 4 weeks, compared with placebo, with a 4-week washout period between treatments. After quercetin treatment, plasma

uric acid concentrations were significantly lowered by –26•5 µmol/l (95% CI, –7•6, –45•5; $P = 0.008$). These results are quite promising and indicate a possible preventive effect against recurrent gout attacks.

For more information on the pharmacology of quercetin, see Chapter 81.

Vitamin C

Megadoses of vitamin C are probably contraindicated in individuals with gout because vitamin C may increase uric acid levels in a small number of individuals. A single large dose (4 gm) of vitamin C resulted in a significant increase in urate excretion, but no change in the serum urate concentration, in 10 patients (5 with gout).[25] Although there are reports that moderate dosages of vitamin C may lower serum uric acid levels by increasing uric acid excretion, neither effect was seen in patients with gout taking vitamin C at a dosage of 500 mg/day. No clinically significant effect was seen on either measurement in either patients taking allopurinol or not.[26]

Niacin

High doses of niacin (i.e., above 50 mg/day) are probably contraindicated in the treatment of gout because niacin competes with uric acid for excretion.[27]

Botanical Medicines

Cherries

Consuming one-half pound of fresh or canned cherries per day has been long recommended in lowering uric acid levels and preventing attacks of gout based upon historical use and early clinical investigation. In a more recent analysis to assess this use, a study was conducted by researchers from the Boston University School of Medicine in 633 people with gout who were recruited and followed online for a year. Participants answered questions about gout onset, symptoms, risk factors, medications, and whether they ate cherries or took cherry extract, and for how long. When the researchers analyzed the participant responses, they found that cherry intake (defined as one-half cup or 10–12 cherries or the equivalent in extract form) over a 2-day period was associated with a 35% lower risk for gout attacks and that cherry extract intake was associated with a 45% lower risk. The risk for gout attacks was reduced by 75% when cherry intake was combined with allopurinol. These benefits persisted even after taking into account factors that can affect gout risk, such as gender, obesity, purine intake (in foods that can increase gout risk), plus the use of alcohol, diuretics, and antigout medications.[28]

These results are consistent with a series of pilot studies conducted by one group of researchers using a cherry juice concentrate.[29] In the first randomized controlled trial (RCT), presented in 2007, patients with gout experienced a significant decrease in the number of acute gout attacks within 4 months of initiating ingestion of the cherry juice concentrate ($P < 0.05$), an effect that was not seen in the control group ingesting pomegranate juice concentrate. Of patients ingesting cherry juice concentrate, 55% were attack-free and stopped their regular intake of nonsteroidal anti-inflammatory drugs within 60 days of initiating consumption of cherry juice concentrate. None of the patients in the group ingesting pomegranate juice discontinued any of their medications. Similar results were shown in the three other trials, leading the researchers to conclude "Our studies suggest that cherry juice concentrate reduces acute attacks of gout when it is consumed over a period of 4 months."[30]

Although some studies have not shown an effect on serum urate levels, one study showed a clear effect on urate metabolism. Researchers measured plasma urate, antioxidant, and inflammatory markers in 10 healthy women who consumed two servings (280 g) of cherries after an overnight fast.[31] Blood and urine samples were taken before the cherry dose and at 1.5, 3, and 5 hours afterward. Plasma urate decreased 5 hours after the cherry consumption by an average of 30 mmol/L. This reduction correlated with an increase in urine urate excretion. Plasma C-reactive protein and nitric oxide concentrations decreased slightly after the 3-hour mark.

Cherries, hawthorn berries, blueberries, and other dark red and blue berries are rich sources of anthocyanidins and proanthocyanidins. These compounds are flavonoid molecules, which give these fruits their deep red-blue color, and are remarkable in their ability to prevent collagen destruction. Anthocyanidins and other flavonoids affect collagen metabolism in many ways:

- They have the unique ability to actually cross-link collagen fibers, resulting in reinforcement of the natural cross-linking of collagen that forms the collagen matrix of connective tissue (e.g., ground substance, cartilage, tendon).
- They prevent free radical damage through their potent antioxidant and free-radical–scavenging action.
- They inhibit enzymatic cleavage of collagen from enzymes secreted by leukocytes during inflammation.
- They prevent the release and synthesis of compounds that promote inflammation, such as histamine, serine proteases, prostaglandins, and leukotrienes.

Zingiber officinalis (Ginger)

Ginger has important anti-inflammatory and analgesic properties (see Chapter 130, *Zingiber officinale* (Ginger)). In a study involving monosodium urate crystal-induced joint inflammation in mice, administration of 6-shogaol, an isolated active principle from ginger, reduced lysosomal enzymes, reduced lipid peroxidation, and decreased the activity of tumor necrosis factor-alpha (TNF-α).[32]

THERAPEUTIC APPROACH

As stated earlier, the naturopathic approach to the prevention and treatment of gout does not differ substantially from the standard medical approach. The basic approach involves the following:

- Dietary and herbal measures that maintain uric acid levels within the normal range
- Controlled weight loss in obese individuals
- Avoidance of known precipitating factors (e.g., heavy alcohol consumption and a high-purine diet)
- The use of nutritional substances to prevent further acute attacks
- The use of herbal and nutritional substances to inhibit the inflammatory process

Diet

Patients should be advised to eliminate alcohol intake, maintain a low-purine diet, increase consumption of complex carbohydrates, decrease consumption of simple carbohydrates, maintain a low-fat intake, optimize protein intake (0.8 g/kg daily), and consume liberal quantities of fluid. Urinary 24-hour uric acid levels can be used to monitor compliance with a purine-free diet (maintained below 0.8 g/day).

In addition, liberal amounts (250–500 g/day) of cherries, blueberries, and other anthocyanoside-rich (i.e., red-blue) berries (or extracts), should be consumed.

Nutritional Supplements

- Fish oils: 3000 mg eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) per day
- Folic acid: 1 to 4 mg/day

Botanical Medicines

Choose one of the following:

- Cherry fruit extract (10:1): 500 to 1000 mg three times a day
- Grape seed extract (>95% procyanidolic oligomers): 100 to 300 mg/day
- Pine bark extract (>90% procyanidolic oligomers): 100 to 300 mg/day
- Anthocyanoside extracts (e.g., *Vaccinium myrtillus*): equivalent to 80 mg anthocyanoside content a day
- Quercetin: 200 to 400 mg three times a day between meals (Note: Consider EMIQ at a dosage of 100–200 mg/day; see Chapter 81 for more information.)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Richette P, Bardin T. Gout. *Lancet*. 2010;375(9711):318–328.
2. Brook RA, Forsythe A, Smeeding JE, et al. Chronic gout: epidemiology, disease progression, treatment and disease burden. *Curr Med Res Opin*. 2010;26(12):2813–2821.
3. Hernández-Cuevas CB, Roque LH, et al. First acute gout attacks commonly precede features of the metabolic syndrome. *J Clin Rheumatol*. 2009;15(2):65–67.
4. Choi HK, De Vera MA, Krishnan E. Gout and the risk of type 2 diabetes among men with a high cardiovascular risk profile. *Rheumatology (Oxford)*. 2008;47(10):1567–1570.
5. Singh JA, Reddy SG, Kundukulam J. Risk factors for gout and prevention: a systematic review of the literature. *Curr Opin Rheumatol*. 2011;23(2):192–202.
6. Schumacher Jr HR, Becker MA, Wortmann RL, et al. Effects of febuxostat versus allopurinol and placebo in reducing serum urate in subjects with hyperuricemia and gout: a 28-week, phase III, randomized, double-blind, parallel-group trial. *Arthritis Rheum*. 2008;59(11):1540–1548.
7. Lee YM, Bae SG, Lee SH, et al. Persistent organic pollutants and hyperuricemia in the US general population. *Atherosclerosis*. 2013;230(1):1–5. PubMed PMID: 23958244.
8. Kuo CC, Weaver V, et al. Arsenic exposure, hyperuricemia, and gout in US adults. *Environ Int*. 2015;76:32–40. PubMed PMID: 25499256.
9. Geiger SD, Xiao J, Shankar A. Positive association between perfluoroalkyl chemicals and hyperuricemia in children. *Am J Epidemiol*. 2013;177(11):1255–1262. PubMed PMID: 23552989.
10. Graziano JH, Blum C. Lead exposure from lead crystal. *Lancet*. 1991l;337:141–142.
11. Krishnan E, Lingala B, Bhalla V. Low-level lead exposure and the prevalence of gout: an observational study. *Ann Intern Med*. 2012;157(4):233–241. PubMed PMID: 22910934.
12. Yu KH, See LC, et al. Dietary factors associated with hyperuricemia in adults. *Semin Arthritis Rheum*. 2008;37(4):243–250. PubMed PMID: 17570471.
13. Zhang Y, Chen C, Choi H, et al. Purine-rich foods intake and recurrent gout attacks. *An Rheum Dis*. 2012;71(9):1448–1453. PubMed PMID: 22648933.
14. Kanbara A, Hakoda M, Seyama I. Urine alkalization facilitates uric acid excretion. *Nutr J*. 2010;9:45. Published online 2010 October 19. https://doi.org/10.1186/1475-2891-9-45.
15. Dessein PH, Shipton EA, Stanwix AE, et al. Beneficial effects of weight loss associated with moderate calorie/carbohydrate restriction, and increased proportional intake of protein and unsaturated fat on serum urate and lipoprotein levels in gout: a pilot study. *Ann Rheum Dis*. 2000;59:539–543.
16. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ*. 2008;336(7639):309–312. 18244959.
17. Choi HK, Willett W, Curhan G. Fructose-rich beverages and risk of gout in women. *JAMA*. 2010;304(20):2270–2278. PubMed PMID: 21068145.
18. Jamnik J, Rehman S, Blanco Mejia S, et al. Fructose intake and risk of gout and hyperuricemia: a systematic review and meta-analysis of prospective cohort studies. *Br Med J*. 2016;6(10):e013191. PubMed PMID: 27697882.
19. Choi HK, Atkinson K, et al. Purine-rich foods, dairy and protein intake, and the risk of gout in men. *N Engl J Med*. 2004;350(11):1093–1103. 15014182.
20. Lewis AS, Murphy L, McCalla C, et al. Inhibition of mammalian xanthine oxidase by folate compounds and amethopterin. *J Biol Chem*. 1984;259:12–15.
21. Oster KA. Folic acid and xanthine oxidase. *Ann Intern Med*. 1977;86:367.
22. Flouvier B, Devulder B. Folic acid, xanthine oxidase, and uric acid. *Ann Intern Med*. 1978;88(2):269.
23. Zhang C, Wang R, Zhang G, Gong D. Mechanistic insights into the inhibition of quercetin on xanthine oxidase. *Int J Biol Macromol*. 2018;112:405–412. PubMed PMID: 29410028.
24. Shi Y, Williamson G. Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomised, double-blinded, placebo-controlled, cross-over trial. *Br J Nutr*. 2016;115(5):800–806. https://doi.org/10.1017/S0007114515005310. Epub 2016 Jan 20.
25. Stein HB, Hasan A, Fox IH. Ascorbic acid-induced uricosuria: a consequence of megavitamin therapy. *Ann Intern Med*. 1976;84:385–388.
26. Stamp LK, O'Donnell JL, Frampton C, et al. Clinically insignificant effect of supplemental vitamin C on serum urate in patients with gout: a pilot randomized controlled trial. *Arthritis Rheum*. 2013;65(6):1636–1642. https://doi.org/10.1002/art.37925.
27. Gershon SL, Fox IH. Pharmacologic effects of nicotinic acid on human purine metabolism. *J Lab Clin Med*. 1974;84:179–186.
28. Zhang Y, Neogi T, Chen C, et al. Cherry consumption and the risk of recurrent gout attacks. *Arthritis Rheum*. 2012;64(12):4004–4011.
29. Schlesinger N, Rabinowitz R, Schlesinger M. Pilot studies of cherry juice concentrate for gout flare prophylaxis. *J Arthritis*. 2012;1:1–5.
30. Schlesinger N, Schlesinger M. Previously reported prior studies of cherry juice concentrate for gout flare prophylaxis. *Arthritis Rheum*. 2013;65(4):1135–1136. https://doi.org/10.1002/art.37864.
31. Jacob R, Spinozzi G, Simon V, et al. Consumption of cherries lowers plasma urate in healthy women. *J Nutr*. 2003;133:1826–1829.
32. Sabina EP, Rasool M, Matthew L, Ezilrani P, Indu H. 6-Shogaol inhibits monosodium urate crystal induced inflammation – and in vivo and in vitro study. *Food Chem Toxicol*. 2010;48(1):229–235. PubMed PMID: 19819286.

Hair Loss in Women

Michael T. Murray, ND

OUTLINE

INTRODUCTION

In clinical practice, one of the most common complaints from female patients is excessive hair loss. In most cases it is not severe alopecia; rather, it is the perception that hair loss is occurring at an increasing rate. Often these complaints are dismissed because minor hair loss is difficult to quantify, and it is certainly not a life-threatening disorder. However, the patient wants an answer as to why it is happening, so it is important to address it. Physicians should also be careful not to underestimate the emotional impact of hair loss for some patients.

BASIC PHYSIOLOGY OF THE HAIR CYCLE

Between 100,000 and 350,000 hair follicles occupy the human scalp, but not all these hairs undergo cyclic phases of growth and rest. During the growth (anagen) phase, there is active genetic expression of protein synthesis. As the hair matures, it enters a resting stage (telogen). Then the hair bulb migrates outward and eventually is sloughed. It is during this migratory phase that the stage is set for new hair to fill the remaining papilla after the original hair is lost. Age, pathology, and a wide variety of nutritional and hormonal factors influence the duration of the hair cycle. Hair loss is a normal part of aging. By the age of 40 or so, the rate of hair growth slows down. New hairs do not grow in as quickly as old ones fall out.

The hair-pull test can help determine the relative formation of new hair. It involves taking a few strands between the thumb and forefinger and pulling on them gently. Anagen hairs should remain rooted in place, whereas hairs in telogen should come out easily. By knowing approximately how many hairs were pulled and the number that came out, the percentage of hair follicles in a telogen state can be determined. Thus, if 20 hairs were pulled and 2 came out, the frequency of telogen hair follicles is 10%. As a very rough guide, a 10% telogen frequency is excellent, up to 25% is typical, and over 35% is problematic. If after the pull test there are still hairs in the balding areas, it is regarded as a positive test.

DIFFERENTIAL DIAGNOSIS

Hair loss can be broadly divided into two types: focal and diffuse. Diffuse hair loss is most often due to *telogen effluvium*—an umbrella term denoting nonscarring alopecia characterized by diffuse hair shedding, often with an acute onset, caused by a metabolic or hormonal stress or by medications. Generally, recovery occurs when the precipitating factors are dealt with. Women can also experience male- or female-pattern hair loss (previously called androgenic alopecia) (Fig. 175.1). Focal hair loss is most often secondary to an underlying disorder that may cause nonscarring or scarring alopecia. Nonscarring focal alopecia is usually caused by tinea capitis or alopecia areata, although patchy hair loss may also be caused by traction alopecia or trichotillomania. Scarring alopecia is rare and has several causes, most often discoid lupus erythematosus.[1]

CAUSES OF HAIR LOSS IN WOMEN AND THERAPEUTIC CONSIDERATIONS

Six common causes of hair loss in women are discussed here: (1) high levels of androgens, (2) drugs, (3) nutritional deficiencies, (4) hypothyroidism, (5) the presence of antigliadin antibodies; and (6) exposure to environmental toxicants.

Androgenic Female-Pattern Hair Loss

Women can suffer from androgen-related hair loss, just like men.[2–5] The female pattern, however, is more diffuse than the characteristic male pattern, so it was originally referred to as "diffuse androgen-dependent alopecia"; now, however, it is referred to as either female- or male-pattern hair loss.[2] It is a relatively common condition. In fact, one report states that it affects approximately 30% of women before the age of 50.[2] Although genetic factors are clearly significant, androgen excess, insulin resistance, polycystic ovary syndrome, and low antioxidant (i.e., reduced glutathione) status are also associated with female-pattern hair loss.[3–11] Three possible recommendations to help slow down this genetically predisposed process are as follows: (1) improve blood glucose regulation through dietary, lifestyle, and supplementary measures (see Chapter 181); (2) increase antioxidant intake; (3) consider saw palmetto extract; and (4) consider hormone replacement therapy.

Reactive oxygen species have been shown to play a central role (along with testosterone) in male-pattern baldness. Higher levels of these damaging compounds are found in the hair follicles of

Fig. 175.1 Ludwig pattern of hair loss in women. (Vujovic A, Del Marmol V. The female pattern hair loss: review of etiopathogenesis and diagnosis. Biomed Res Int. 2014;2014:767628. PubMed PMID: 24812631.)

men (and presumably women) with male-pattern baldness.[7] This appears to be due to lower levels of glutathione. The use of glutathione-sparing antioxidants like vitamin C and flavonoids seems appropriate.

Saw palmetto extract is a popular recommendation for benign prostatic hyperplasia (BPH). Its effects in BPH occur via several mechanisms, including an ability to inhibit the formation and transport of dihydrotestosterone (DHT). This potent androgen is formed from testosterone by the action of the enzyme 5-α-reductase. The activity of this enzyme is increased in BPH, as well as in both male- and female-pattern baldness.[8] Saw palmetto extract may offer some benefit in androgen-related alopecia by reducing the formation of DHT. This is the same mechanism as the drug finasteride (Propecia), which is often used in the treatment of female-pattern hair loss.[11] Saw palmetto extract also inhibits the transport of DHT to nuclear receptor sites and therefore may prove even more useful. The dosage for the extract standardized to contain 85% to 95% fatty acids and sterols is 320 mg/day.

Drug-Induced Hair Loss

A long list of drugs can cause hair loss, but it should not be assumed that simply because a woman is complaining of hair loss and taking one of these drugs that the drug is the single cause. Some drugs, most notably chemotherapy agents like fluorouracil, are certainly causative because they are such powerful inhibitors of hair growth. When medically appropriate, natural alternatives to the suspected drug or drugs should be employed (Table 175.1).

Nutritional Deficiency

A deficiency of any number of nutrients can lead to significant hair loss. Deficiency of vitamin C manifests as scurvy and can present with corkscrew hairs.[12] Telogen effluvium and alopecia areata have been associated with deficiencies of iron and zinc,[13,14] and generalized hair loss occurs in conjunction with deficiencies in selenium, iron, biotin, and essential fatty acids.[15] In addition, serum vitamin D and folate levels tend to be lower in patients with alopecia areata compared with controls.[16] The patient's nails should be examined for the characteristic white lines indicating poor wound healing of the nail bed even with the most minor of trauma, which may be a sign of a low zinc level. The backs of the arms should be examined for hyperkeratosis, a common sign of vitamin A deficiency. The skin of the elbows and general health of the skin should also be evaluated, looking for the dry skin associated with a deficiency of essential fatty acids. To determine iron status, a serum ferritin evaluation is recommended.

In evaluating serum ferritin levels, it is important to know that many reference laboratories report low ferritin levels (e.g., 10–30 mg/L) to be within the normal range. If the serum ferritin is less than 30 mg/L, iron replacement is indicated. When serum ferritin levels fall below this, hair growth and regeneration are impaired because the body seeks to conserve the remaining iron.[17] There is a very strong association between low body iron stores and diffuse hair loss in women when the serum ferritin level is at or below 30 mg/L.[18,19]

Women with noticeable generalized hair loss typically suffer from deficiencies of all these nutrients. The treatment of hair loss secondary to nutritional deficiency is straightforward—the dietary intake of these nutrients must be increased through appropriate supplementation. One caveat is that many of these women turn out to be hypochlorhydric. In these cases, hydrochloric acid supplementation at meals may be all that is necessary. A general recommendation for women with hair loss related to nutritional status is to take a high-potency multivitamin/multimineral formula that contains iron along with 1 tablespoon of flaxseed oil per day. If their serum ferritin levels are below 30 mg/L, such women can consume iron-rich foods and supplement with additional iron, either 30 mg of iron bound to succinate, fumarate, or other chelate twice daily between meals. If this recommendation results in abdominal discomfort, 30 mg taken with meals three times daily can be suggested. After 2 months, serum ferritin levels should be reassessed. Improvements in serum ferritin often correlate with improved health of the hair and the halting of excessive hair loss.

TABLE 175.1 Classes of Drugs That Can Cause Hair Loss

Class	Examples
Antibiotics	Gentamicin, chloramphenicol
Anticoagulants	Warfarin, heparin
Antidepressants	Fluoxetine, desipramine, lithium
Antiepileptic drugs	Valproic acid, phenytoin
Cardiovascular drugs	Angiotensin converting enzyme inhibitors, beta blockers
Chemotherapy drugs	Doxorubicin, vincristine, etoposide
Endocrine drugs	Bromocriptine, clomiphene, danazol
Gout medications	Colchicine, allopurinol
Lipid-lowering drugs	Gemfibrozil, fenofibrate
Nonsteroidal anti-inflammatory drugs	Ibuprofen, indomethacin, naproxen
Ulcer medications	Cimetidine, ranitidine

Van Neste DJJ, Rushton H. Hair problems in women. *Clin Dermatol.* 1997;15:113–125.

Hypothyroidism

It is well known that hair loss is one of the cardinal signs of hypothyroidism. Using blood levels of thyroid hormones as the criterion, an estimated 1% to 4% of the adult population has moderate to severe hypothyroidism, and another 10% to 12% has mild hypothyroidism.[20] The prevalence of hypothyroidism in American women is estimated to be as high as 20%, even among practitioners of conventional medicine.

Antigliadin Antibodies

The protein gluten and its polypeptide derivative, gliadin, are found primarily in wheat, barley, and rye. It appears that antibodies to gliadin can elicit cross-reacting antibodies that attack the hair follicles, leading to alopecia areata—an autoimmune disease characterized by areas of virtually complete hair loss.[21]

Celiac disease, also known as nontropical sprue, gluten-sensitive enteropathy, or celiac sprue, is characterized by malabsorption and an abnormal small intestinal structure that reverts to normal on the removal of dietary gluten. Evidence is growing that many people with gluten intolerance do not have overt gastrointestinal symptoms. Instead, they may demonstrate gluten intolerance insidiously as hair loss. Rather than testing for antigliadin antibodies in patients with general hair loss or alopecia areata, the test for human antitissue transglutaminase antibodies (IgA anti-tTG) is recommended because it is more sensitive than the test for antigliadin antibodies (see Chapter 157). This recommendation is especially important in the presence of any gastrointestinal symptoms pointing to celiac disease (Box 175.1).

BOX 175.1 Key Diagnostic Features of Celiac Disease

- Bulky, pale, frothy, foul-smelling, greasy stools with increased fecal fat
- Weight loss and signs of multivitamin and mineral deficiencies
- Increased levels of serum gliadin antibodies
- Diagnosis confirmed by jejunal biopsy

Toxicant Exposure

Toxin metal poisoning can also lead to alopecia or thinning of the hair. Excess intake of the trace element selenium can cause increased hair fragility and global hair loss.[22] Hair loss has also resulted from exposure to mercury,[23] arsenic,[24] copper, and lead.[25] Alopecia is often one of the distinctive clinical manifestations of thallium toxicity.[26] A case report of a man who developed severe alopecia during 12 of his 20 years as a glassworker determined the cause to be chronic poisoning by cadmium and bismuth compounds.[27]

CONCLUSION

Hair loss in women should not be dismissed. Although some hair loss is a natural part of the aging process, complaints of excessive or accelerated hair loss should be investigated and treated appropriately. Clinical studies have investigated the psychological impact of increasing hair loss in women and found that it is a significant source of anxiety, fear, and depression.[2,5] Conversely, increasing the health of the hair results in an improvement in the general health, especially the mental health, of these patients.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Santiago-Casas Y, Vilá LM, McGwin Jr G, et al. Association of discoid lupus with clinical manifestations and damage accrual in profile: a multiethnic lupus cohort. *Arthritis Care Res (Hoboken)*. 2011. [Epub ahead of print]. https://doi.org/10.1002/acr.21581.
2. Mounsey AL, Reed SW. Diagnosing and treating hair loss. *Am Fam Physician*. 2009;80(4):356–362.
3. Van Neste DJJ, Rushton H. Hair problems in women. *Clin Dermatol*. 1997;15:113–125.
4. Azziz R, Sanchez LA, Knochenhauer ES, et al. Androgen excess in women: experience with over 1000 consecutive patients. *J Clin Endocrinol Metab*. 2004;89:453–462.
5. Price VH. Androgenetic alopecia in women. *J Investig Dermatol Symp Proc*. 2003;8:24–27.
6. Birch MP, Lalla SC, Messenger AG. Female pattern hair loss. *Clin Exp Dermatol*. 2002;27:383–388.
7. Giralt M, Cervello I, Nogues MR, et al. Glutathione, glutathione S-transferase and reactive oxygen species of human scalp sebaceous glands in male pattern baldness. *J Invest Dermatol*. 1996;107:154–158.
8. Legro RS, Carmina E, Stanczyk FZ, et al. Alterations in androgen conjugate levels in women and men with alopecia. *Fertil Steril*. 1994;62:744–750.
9. Cela E, Robertson C, Rush K, et al. Prevalence of polycystic ovaries in women with androgenic alopecia. *Eur J Endocrinol*. 2003;149:439–442.
10. Matilainen V, Laakso M, Hirsso P, et al. Hair loss, insulin resistance, and heredity in middle-aged women: a population-based study. *J Cardiovasc Risk*. 2003;10:227–231.
11. Shum KW, Cullen DR, Messenger AG. Hair loss in women with hyperandrogenism: four cases responding to finasteride. *J Am Acad Dermatol*. 2002;47:733–739.
12. Magiorkinis E, Beloukas A, Diamantis A. Scurvy: past, present and future. *Eur J Int Med*. 2011;22(2):147–152. PubMed PMID: 21402244.
13. Deloche C, Bastien P, Chadoutaud S, et al. Low iron stores: a risk factor for excessive hair loss in non-menopausal women. *Eur J Dermatol*. 2007;17(6):507–512. PubMed PMID: 17951130.
14. Saper RB, Rash R. Zinc: an essential micronutrient. *Am Fam Phys*. 2009;79(9):768–772. PubMed PMID: 20141096.
15. Daniells S, Hardy G. Hair loss in long-term or home parenteral nutrition: are micronutrient deficiencies to blame? *Curr Opin Clin Nutri Metabol Care*. 2010;13(6):690–697. PubMed PMID: 20823774.
16. Thompson JM, Mirza MA, Park MK, Qureshi AA, Cho E. The role of micronutrients in alopecia areat: a review. *Am J Clin Dermatol*. 2017;18(5):663–679. PubMed PMID: 28508256.
17. Kantor J, Kessler LJ, Brooks DG, et al. Decreased serum ferritin is associated with alopecia in women. *J Invest Dermatol*. 2003;12:985–988.
18. Moeinvaziri M, Mansoori P, Holakooee K, et al. Iron status in diffuse telogen hair loss among women. *Acta Dermatovenerol Croat*. 2009;17(4):279–284.
19. Deloche C, Bastien P, Chadoutaud S, et al. Low iron stores: a risk factor for excessive hair loss in non-menopausal women. *Eur J Dermatol*. 2007;17(6):507–512.
20. Arem R, Escalante D. Subclinical hypothyroidism: epidemiology, diagnosis, and significance. *Adv Intern Med*. 1996;41:213–250.
21. Corazza GR, Andreani ML, Venturo N, et al. Celiac disease and alopecia areata: report of a new association. *Gastroenterology*. 1995;109:1333–1337.
22. Agarwal P, Sharma S, Agarwal US. Selenium toxicity: a rare diagnosis. *Indian J Dermatol*. 2016;82(6):690–693. PubMed PMID: 27716724.
23. Haas NS, Shih R, Gochfeld M. A patient with postoperative mercury contamination of the peritoneum. *J Toxicol Clin Toxicol*. 2003;41(2):175–180. PubMed PMID: 12733856.
24. Ghosh A. Evaluation of chronic arsenic poisoning due to consumption of contaminated ground water in West Bangal, India. *Int J Prevent Med*. 2013;4(8):976–979. PubMed PMID: 24049627.
25. Fraitag S, Bodemer C. Neonatal erythroderma. *Curr Opin Pediatr*. 2010;22:438–444.
26. Mulkey JP, Oehme FW. A review of thallium toxicity. *Veterinary Hum Toxicol*. 1993;35(5):445–453. PubMed PMID: 8249271.
27. Bachanek T, Staroslawska E, Wolanska E, Jarmolinska K. Heavy metal poisoning in glass worker characterized by severe. *Annal Agricultur Environ Med*. 2000;7(1):51–53. PubMed PMID: 10865245.

176

Hepatitis

Cory Szybala, ND, Mollie Parker Szybala, ND, MPH, and *Peter B. Bongiorno*, ND, MSAc, LAc*

OUTLINE

GENERAL CONSIDERATIONS

Hepatitis refers to inflammation *(-itis)* of the liver *(hepar-)*. Inflammation lasting less than 6 months is known as acute hepatitis, whereas that lasting longer than 6 months is called chronic hepatitis. In people with acute hepatitis who do not progress to chronic disease, all the symptoms, signs, and blood test abnormalities return to normal without permanent or long-term sequelae. People who progress from acute to chronic hepatitis are at risk of progressing to cirrhosis and its complications, such as portal hypertension and hepatocellular carcinoma (HCC). *Hepatitis* is a term that encompasses many inflammatory causes, such as autoimmune liver disease, obesity, alcoholic liver disease, and some medications and herbs. Only hepatitis caused by a virus (viral hepatitis) is potentially infectious to others (Fig. 176.1). This chapter focuses on viral hepatitis A, B, and C.

Hepatitis A is caused by a virus of the *Picornaviridae* family and is transmitted via the fecal–oral (enteric) route by person-to-person contact. Before its identification in 1973, hepatitis A was known as infectious hepatitis. It is the most common cause of acute viral hepatitis in the United States, making up approximately one half of all reported cases of viral hepatitis. The incidence has declined significantly since the implementation of vaccination for those at increased risk of infection. Although infection with the hepatitis A virus (HAV) does not lead to chronic disease, it can relapse up to 6 months after the acute illness has resolved, and it accounted for an estimated 100 deaths each year in the United States in the prevaccine era.[1,2]

Hepatitis B is caused by a virus of the *Hepadnaviridae* family and is transmitted via the parenteral route through blood or blood products, through sexual contact, or from mother–child transmission during pregnancy and childbirth. Before its identification in 1963, it was known as serum hepatitis. Approximately 250 million people worldwide, including an estimated 850,000 to 2.2 million (estimates dependent on the inclusion of immigrants from endemic countries) people in the United States, are chronically infected with the hepatitis B virus (HBV). Chronic carriers are defined through blood work as a positive hepatitis B surface antigen (HBsAg) and a positive hepatitis B core antibody (HBcAb).[3–5]

Hepatitis C is caused by a virus of the *Flaviviridae* family and is transmitted via the percutaneous route through blood-to-blood contact. Current or former intravenous drug users; anyone who received a blood or a blood-product transfusion or donated organ before 1992; anyone who received clotting factor concentrates before 1987; individuals on long-term hemodialysis; individuals born to a mother infected with the hepatitis C virus (HCV); and those who may be at increased risk for exposure to infected blood, such as health care or public safety workers, are at increased risk of infection.[5] Unlike the case with HBV, sexual contact is an inefficient means of HCV transmission. Before its identification in 1989, it was known as non-A, non-B hepatitis. An estimated 185 million people worldwide, 2.4 million of whom are in the United States, have been exposed to and are living with chronic HCV, and approximately 400,000 people per year die of HCV-related liver disease.[6,7]

Symptoms and Signs

Acute viral hepatitis may be asymptomatic or may be an extremely debilitating disease manifesting with jaundice, flu-like symptoms, decreased appetite, abdominal pain, nausea, diarrhea, vomiting, joint pain, and fatigue. Hepatitis A never leads to chronic disease. For hepatitis B, the probability of developing chronic disease in unimmunized individuals is approximately 6% to 10% in older children and adults, 25% to 50% in children aged 1 to 5 years, and greater than 90% in infants. Hepatitis C progresses to chronicity in 75% to 85% of newly infected individuals, with females and children being less likely to progress to chronic diseases than male adults.[5,8,9] The symptoms of chronic hepatitis vary from virtually nonexistent to liver failure or liver cancer, relentless fatigue, and signs and symptoms of decompensated cirrhosis, such as ascites, variceal bleeding, and hepatic encephalopathy.[5]

*Previous edition contributor

Disease	Pathogen	Symptoms	Incubation period	Method of transmission	Diagnostic test
Hepatitis A	HAV, Picornaviridae	Fever, headache, malaise, jaundice	2-6 weeks	Ingestion	IgM antibodies
Hepatitis B	HBV, Hepadnaviridae	Severe liver damage, chronic disease occurs	3-26 weeks	Parenteral, sexual contact	IgM antibodies
Hepatitis C	HCV, Flaviviridae	Same as HBV, more chronic	2-33 weeks	Parenteral	PCR of viral RNA
Hepatitis D	HDV, Deltaviridae	Severe liver damage, high mortality rate	6-26 weeks	Parenteral, when co-infected with HBV	IgM antibodies
Hepatitis E	HEV, Caliciviridae	Pregnant women may be at high risk and show high mortality, not chronic disease	2-6 weeks	Ingestion	IgM antibodies, PCR of viral RNA

Fig. 176.1 Features of different types of viral hepatitis. (From Saleem A, Akhtar MF, Mushtag MF, Saleem M, Muhammad ST, Akhtar B, Sharif A, Peerzada S. Current trends in the treatment of hepatitis C: interventions to avoid adverse effects and increase effectiveness of anti-HCV drugs. *EXCLI J.* 2016;15:578–588. PubMed PMID: 28096788.)

DIAGNOSTIC CONSIDERATIONS

A diagnosis of hepatitis is made via a combination of a thorough history; physical examination; laboratory tests, including serial liver function tests (aspartate aminotransferase [AST] and alanine aminotransferase [ALT], which are known together as transaminases); hepatitis-specific serologies; and in some instances, imaging studies. Because these evaluations typically do not accurately correlate with the extent of damage done to the liver, a liver biopsy is often necessary in chronic cases to determine the grade (degree of inflammation) and stage (degree of fibrosis) of the disease, as well as the need for therapy.[10,11]

Prevention

All people with chronic liver disease or chronic hepatitis of any etiology should receive immunizations for hepatitis A and B if they have not already been exposed to these viruses. The hepatitis A vaccination was first approved by the U.S. Food and Drug Administration (FDA) in 1995, and since its implementation, the incidence has significantly declined. The development of the hepatitis B vaccine represents one of the most important advances in medicine. This is the first and only vaccine in history that can simultaneously prevent liver cancer, cirrhosis, and a sexually transmitted disease, hepatitis B. The FDA approved the hepatitis B vaccine in 1981, and the improved version has been available since 1986. It is considered safe to administer the hepatitis B vaccine to pregnant women if needed. Infants born to HBsAg-positive mothers should receive both the hepatitis B vaccination and the hepatitis B immune globulin (HBIG) within 12 hours of birth. See Box 176.1 for a list of those at increased risk for hepatitis B and who therefore should receive the hepatitis B vaccination.[5,12]

There is currently no vaccine available to prevent HCV. Many barriers exist, slowing the vaccine development, including the not-well-defined immune responses that prevent persistence, the lack of immune correlates, and one of the major barriers, the complex HCV population of mutant strains, known as quasispecies, that can exist in a person infected with hepatitis C.[13]

The hepatitis A virus can be killed by heating foods to 185ºF (85ºC) for 1 minute and by disinfecting surfaces with a 1:100 household bleach solution. It is best to avoid eating raw or partially cooked mollusks (clams, oysters, mussels, and scallops) because these fish often live in HAV-contaminated rivers and seas. When traveling to areas of the world known to have a high incidence of hepatitis A, it is especially important to eat well-cooked foods and to drink only bottled water. Sanitizing diaper-changing tables is also important because hepatitis A–infected infants are typically a silent source for the spread of hepatitis A infection. Meticulous hand washing is of great importance after using the bathroom, before eating a meal, and before preparing food for others.[1]

The avoidance of unprotected sex and injection drug use will greatly reduce the likelihood of infection with HBV or HCV. Employing a barrier such as a condom, dental dam, female condom, or finger cot will decrease the risk of transmission and acquisition of both viruses. Although the risk of sexual transmission of

BOX 176.1 Groups Recommended to Receive the Hepatitis B Vaccination[5]

- All newborns at birth
- Older children who never received a vaccination
- Individuals with multiple sex partners and susceptible sex partners of infected persons
- Individuals who are being evaluated or treated for a sexually transmitted infection (STI)
- Men who have sex with men
- Injection drug users and susceptible sex partners of infected persons
- Susceptible contacts of infected persons
- Health care and public safety workers who may be exposed to blood on the job
- Individuals with chronic liver disease, HIV infection, and end-stage renal disease
- The residents and staff of facilities for developmentally disabled individuals
- Anyone traveling to regions with intermediate or high rates (HBsAg prevalence of ≥2%) of hepatitis B
- Unvaccinated adults between the ages of 19 and 59 with diabetes mellitus
- Any individual seeking long-term protection

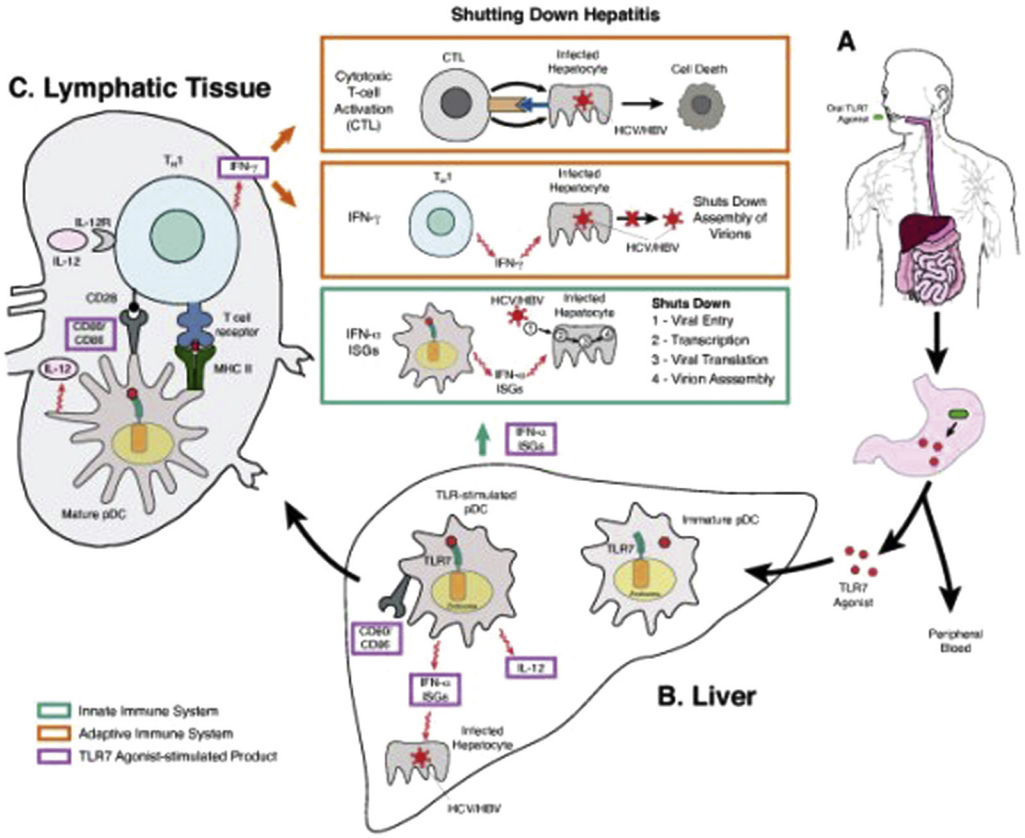


Fig. 176.2 TLR7 stimulation mediates an endogenous type I interferon response. (From Funk F, Kottilil S, Gilliam B, Talwani R. Ticking the TLR7 to cure viral hepatitis. *J Transl Med.* 2014;12:129. PubMed PMID: 24884741.)

HCV is rare, protected sex is recommended if a person engages in anal sex, has multiple sex partners, has frequent prostate infections, has open cuts or sores on the genitalia, or is menstruating. People with hepatitis B or C should avoid sharing anything that may contain even the tiniest amount of their blood, including toothbrushes, needles, razors, and nail clippers. A drop of blood so minuscule that it cannot be seen by the human eye may contain hundreds or even thousands of HBV and/or HCV particles. Even meticulous cleaning may not totally eradicate the virus from a needle. If a person needs unused needles but cannot obtain them, he or she should seek out a needle-exchange program. Alternatively, needle use can be limited to autodestruct syringes, which are designed to self-destruct after one use. Of course, the best advice for a person who continues to use illicit drugs is to discontinue this activity immediately and seek help at a drug rehabilitation center. Also, anyone who intends to get a tattoo or have a body part pierced should deal only with establishments that are clean and that adhere to meticulous sterilization practices.

THERAPEUTIC CONSIDERATIONS

Conventional Therapies

The goals for treatment of chronic hepatitis include sustained viral suppression or total elimination of the virus to slow or prevent progression to cirrhosis, hepatic decompensation, HCC, and to prevent transmission to others. Although not curable, conventional treatment options for hepatitis B include either nucleoside/nucleotide analogs (NAs) or pegylated interferon. Interferons (IFNs) are a family of proteins made naturally by the body that have antiviral, antiproliferative, and immunomodulatory properties (Fig. 176.2). IFN and pegylated IFN have both been shown to be effective in reducing HBV replication and inducing disease remission, although pegylated IFN is preferred due to its increased effectiveness and similar side-effect profile. However, the efficacy of IFN is limited to a narrow population of HBV patients. In addition, its numerous side effects, difficult subcutaneous route of administration, cost, and associated high relapse rate after medication is discontinued have hampered its use for patients with chronic HBV. There are currently five FDA-approved NAs for the treatment of chronic HBV. NAs are quite effective for suppressing HBV replication, induction of disease remission, and the improvement of long-term outcomes. However, the length of time during which patients are treated is not predefined and is determined by specific parameters that may differ from one patient to another, and treatment may be indefinite for some. Furthermore, long-term use of NAs can be complicated by the selection of antiviral-resistant mutations.[4]

In contrast to hepatitis B, hepatitis C can be cured, resulting in improved survival, reduced morbidity, and higher quality of life. The goal of treatment is a sustained virologic response (SVR), or an absence of the virus in the blood 12 weeks after the completion of a treatment regimen. Treatment choice is based on multiple factors, including genotype, efficacy, duration, side-effect profile, drug interactions, history of prior treatment, and stage of fibrosis.[14] The length and success of HCV treatment are also dependent on a variety of factors (Box 176.2). With the approval of new interferon-free regimens in 2014, a major advance in the treatment of HCV occurred, leading to SVR in over 90% of HCV-infected individuals. Interferon-free regimens have helped reduce the number of adverse events (AEs), complex treatment regimens, and the potential for the development of drug-resistance,

BOX 176.2 Factors Affecting Hepatitis C Sustained Virologic Response (SVR) Rates[6]

- Age
- Fibrosis and cirrhosis
- Genotype
- Polymorphisms
- Insulin resistance
- Viral load
- Race
- Statin use

but one barrier still exists: the high price tag associated with each regimen. This change in treatment underscores the need for clinicians to be aware of the effects of natural and integrative therapies on the progression of HCV, potential interactions, and of the potential benefits of these therapies for chronic hepatitis C.[6]

Several nutrients and herbs, discussed later, have been shown to inhibit viral reproduction, improve immune system function, and stimulate regeneration of the damaged liver cells.

Lifestyle Recommendations

Sleep

Experimental studies have demonstrated that sleep deprivation results in poorer immune function, such as reduced natural killer cell activity, suppressed interleukin-2 production, and increased levels of circulating proinflammatory cytokines.[15,16] One study showed that survival was inversely associated with sleep disturbance among 156 patients with cirrhosis. The study's authors suggested that tailored behavioral interventions might help improve overall health-related quality of life and could potentially improve patient outcomes.[17] Sleep problems and chronic fatigue have been reported in patients with chronic hepatitis C, with about 50% to 70% of individuals reporting such complaints. Subjects administered IFN-α demonstrated increased waking after sleep onset, decreased sleep efficiency, and reduced stage 3 and/or 4 sleep.[18,19] Evidence also suggests that impairments in sleep quality exist independent of antiviral therapy with IFN-α and before the advanced stages of liver disease.[20] Sleep deprivation has also been found to attenuate antibody response to hepatitis A and B immunization.[21,22] In terms of sleep duration, individuals with less than 7 hours of regular sleep are approximately three times more likely to develop a cold when exposed to nasally introduced rhinovirus than those with 8 hours or more of sleep.[23] It may therefore be prudent to encourage proper sleep habits and a duration of 8 hours or more to optimize immune and antiviral benefits in the patient with hepatitis as well.

In a 2017 study, resveratrol, at a dose of 19.8 mg/day, was determined to reduce sleep disturbance in patients receiving pegylated-IFN-a-2b and Ribavirin therapy. This was a randomized, double-blind, placebo-controlled study of 60 participants with chronic hepatitis C; 30 receiving resveratrol and 30 receiving a placebo (N-acetylcysteine (NAC) 600 mg and lactoferrin 23.6 g). Addition of resveratrol significantly reduced anxiety; depression; sleep disturbance; serum inflammatory markers, including AST, ALT, C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6; and histological characteristics, including steatosis and fibrosis. At this time, the mechanism of action for resveratrol is speculative, but it is thought that the phenolic content of resveratrol can alter cellular metabolism to reduce cellular inflammation and improve function. Reduction of inflammatory cytokines likely improves sleep modulation. Consumption of polyphenols likely alters microbial metabolism and alters the composition of microflora, affecting anxiety, depression, and fatigue. It is unknown if resveratrol alone would have the same benefits in the absence of lactoferrin and NAC.[24]

Exercise

Light to moderate exercise has been shown to be well tolerated and beneficial for direct and indirect (extrahepatic) symptoms associated with acute and chronic liver disease.[25,26] Studies demonstrating the incorporation of exercise into the standard of care (SOC) treatment plan for HCV showed that patients can safely participate in an exercise program, create clear changes in the way they perceive their bodies and its capacities, and improve their self-confidence, all of which can lead to far-reaching changes in the way this disease and the constraints of treatment are perceived.[27] Exercise has also been shown to improve symptoms of fatigue, one of the most common and bothersome symptoms affecting people with chronic hepatitis; enhance cardiovascular function; and reduce total body fat, which is important to all people with liver disease. When total body fat is reduced, the fat content of the liver is also reduced, often resulting in a significant reduction in elevated liver enzymes.[27] Thus it is recommended that all patients with chronic hepatitis incorporate an exercise program combining weight training and aerobics into their daily routine, as tolerated, once they have been medically cleared by their physicians.

Diet

Nutritional treatment decisions for hepatitis should be based on modifiable factors associated with accelerated liver disease, including alcohol consumption, obesity, fluid retention, and insulin resistance.[28–30] The most recommended, and researched, dietary pattern for hepatitis is the Mediterranean diet because of its well-documented effects on cardio-metabolic health. Individual components of this diet include reduced carbohydrate intake; increased monounsaturated and omega-3 fatty acid intake; and an increase in whole foods, including whole grains, fruits, and vegetables. The consumption of a diet high in saturated fats, simple carbohydrates (e.g., sugar, white flour, fruit juice, honey), oxidized fatty acids (fried oils), sodium, and animal products has been shown to be detrimental.[31,32] Alcohol should be completely avoided.

The gut–liver–axis (GLA), a relatively novel concept, has been strongly implicated in the progression of hepatitis.[33] The GLA is characterized by the movement of nutrients and metabolites between the small intestine, portal circulation, and bile acid.[33] When there is microbial dysbiosis and disruption of the intestinal barrier, bacteria and their products (including endotoxins) travel to the liver, causing liver damage.[33,34] Elevated bacterial lipopolysaccharide (LPS) causes metabolic endotoxemia and liver inflammation. This both causes and worsens liver disease.[33,34]

HCV infection alters the gut microbial composition.[35,36] It is unknown exactly how this occurs, but it is likely (1) from the direct interaction of HCV and the microbiota and/or (2) mediated via the immune system.[35,36] One study in patients with chronic HBV or HCV has shown that increased microbial translocation and LPS-induced inflammation is predictive of the progression of liver disease[34] The most concerning sequelae of altered GLA function is an increased risk of hepatocellular carcinoma (HCC).[37]

To reduce the risk of HCC by influencing the GLA, it is important to eat a diet that supports the growth of diverse microflora, repairs the intestinal barrier, and decreases LPS levels. Although most research in this field has been performed on mice, eating a high-fat diet (the standard American diet) increases LPS levels and metabolic endotoxemia.[38] Likewise, a high-sugar diet drastically modifies the gut

microbial composition, increases gut permeability, elevates blood endotoxins, and increases liver inflammation.[38,39] Both diets reduce the diversity of the gut flora, which has been linked to a decrease in health.[39–41] To promote the growth of a diverse microflora, eating a diet rich in polyphenols, fiber, and resistant starches is important.[42–44] Probiotic foods including kefir, yogurt, kimchi, miso, and sauerkraut may improve dysbiosis, improve microflora composition, and reduce LPS levels.[45,46] Additionally, the avoidance of xenobiotics in foods should be emphasized.[40]

Patients presenting with liver cirrhosis are likely to be undernourished and sarcopenic.[47] These qualities lead to a decreased quality of life and poor prognosis. Nutritional therapy should support liver regeneration and prevention and correction of nutritional deficiencies. Common nutritional deficiencies with cirrhosis include vitamins A, B, D, E, K, zinc, branched-chain amino acids, and carnitine. Adequate nutrition and prevention of muscle wasting help improve a patient's ability to perform daily activities and reduce complications associated with cirrhosis and HCC.[47]

Flavonoids

Flavonoids are a class of secondary plant metabolites, including common fruits and vegetables, that have been shown to exhibit anti-inflammatory, antithrombogenic, antidiabetic, anticancer, neuroprotective, and antiviral activities. There are a number of ongoing studies looking at the effects flavonoids have on viral infections, including hepatitis B and C.

One such flavonoid, proanthocyandin, found in the blueberry fruit and leaves, has been found to inhibit the replication of HCV,[48] and rats fed diets high in blueberries were protected from developing acute hepatitis.[49] Naringenin, a flavonoid found in grapefruit and other citrus fruits, has been shown to have anti-inflammatory, antioxidant, antiviral, and lipid-lowering properties.[50] Its lipid-lowering properties are specific for very low-density lipoprotein (VLDL) cholesterol; it is suspected that the hepatitis C virus may "hitch a ride" on cholesterol. Studies have demonstrated that naringenin can inhibit or reduce steatosis (the deposition of fat in the liver)[51,52] in addition to inhibiting HCV viral replication.[53,54] It is important to remember that grapefruit contains furanocoumarins and flavonoids, which can inhibit components of the cytochrome P450 drug metabolism pathway, leading to toxic levels of many medications.[55,56]

Caffeine

The mechanisms by which caffeine exerts its hepatoprotective effects are still under investigation, but a growing number of studies are demonstrating a multifactorial etiology, including detoxification, antioxidation, and antifibrinogenesis properties.[57] Although the research is early, there does appear to be dose-dependent response between coffee intake (up to 4 cups daily) and reduced mortality from chronic liver disease. Two population-based studies demonstrated that those who consumed 2 cups of coffee a day were less likely to have elevated serum liver enzymes and were less likely to have chronic liver disease, compared with those who did not drink coffee.[58,59] The protective effect of coffee drinking on the development of cirrhosis and its complications, including HCC, has also been well documented.[60–65]

Higher coffee consumption was associated with less hepatic steatosis, a lower ratio of serum AST/ALT, and lower α-fetoprotein levels in patients with hepatitis C. Patients who drank more than 2 to 3 cups of coffee a day had a lower incidence of disease progression and less fibrosis on histological evaluation compared with patients with HCV who drank less coffee.[60,66] Similar results were not seen in patients who got caffeine from other sources or who consumed decaffeinated coffee. Caffeine may also reduce the fatigue associated with HCV and/or chronic liver disease. Although the exact coffee intake necessary to obtain benefits is unclear, it appears reasonable for patients with hepatitis C to drink 2 to 4 cups of regular coffee daily.

Whey Protein

A by-product of the cheese-making process, whey contains biologically active ingredients that have been shown to enhance immune function, protect against free-radical damage, improve cellular glutathione levels, and reduce overall viral load.[67] A preliminary trial found that 24 g of whey protein daily reduced serum ALT levels and increased plasma glutathione levels in people with hepatitis B but not those with hepatitis C.[68] In a pilot study where whey protein concentrate was supplemented daily in patients with chronic HCV, a significant decrease was noted in viral load, inflammatory markers, and serum ALT and AST. Unfortunately, this study did not disclose an optimal dose, duration, or potential for interaction with ongoing therapy.[67]

Nutritional Supplements

Vitamins C and E

Patients with hepatitis C treated with pegylated interferon and ribavirin were found to have higher SVRs and to be less likely to experience ribavirin-associated anemia when they supplemented this antiviral treatment with vitamins C and E.[69,70] It should be kept in mind that vitamin C increases iron absorption, and many patients with hepatitis C already have elevated iron stores.

Vitamin E levels have been shown to be lower in the serum and liver tissue of people with active hepatitis and those who later develop liver cancer from chronic hepatitis.[71,72] In one preliminary trial of adults with hepatitis C, administering 1200 IU/day of vitamin E for 8 weeks appeared to reduce liver damage.[73] In another study, 544 IU of vitamin E/day for 24 weeks improved the response to interferon/antioxidant therapy, although the results did not reach statistical significance.[74]

Zinc

Zinc is essential to the normal functioning of the immune system and may protect the liver from oxidative damage. Some researchers believe that zinc may protect the body from viruses, including the common cold. A deficiency of this trace element is common in patients with active HCV, HCC, and/or cirrhosis, and serum levels of zinc are inversely correlated with the progression of HCV. Therefore, due to zinc's complex antioxidant effects and the known deficiency state in patients with HCV, zinc supplementation is seen as a viable adjunctive therapy.[75] Oral zinc supplementation (150 mg/day) may slow HCV progression and reduce the incidence of HCC in these patients.[76,77] Zinc supplementation has also been shown to improve functional hepatic reserve, hepatic encephalopathy, and general nutritional status.[47] In addition, zinc supplementation may improve the response to interferon treatment in patients with chronic hepatitis C.[78,79] One study using 17 mg of zinc twice daily (in the form of a zinc complex of L-carnosine) enhanced the response to interferon therapy in patients with chronic hepatitis C.[80] However, it is not clear whether this benefit was due primarily to the zinc or the carnosine. Although daily doses up to 100 mg of zinc may boost the immune system and improve the response to interferon, an excess of this amount may be immunosuppressive. There are also adverse effects associated with excessive zinc consumption, including nausea, vomiting, and diarrhea—all possible side effects of HCV antiviral SOC therapy.

Vitamin D

Patients with chronic hepatitis C often have lower serum levels of 25 hydroxy (OH) vitamin D than individuals without hepatitis C, and approximately one third of these patients are severely vitamin D–deficient.[81] Decreased vitamin D levels have been independently correlated with both the severity of inflammation and hepatic fibrosis on histological evaluation and a reduced incidence of SVRs to therapy with pegylated interferon (PI) plus ribavirin (RBV).[82–85] Additional preliminary findings have suggested that supplementation with vitamin D (1000–4000 IU/day), with the goal of achieving a serum level greater than 32 ng/mL, may improve SVRs in some patients with HCV treated with antiviral therapy. These results suggest that the immunomodulatory properties of vitamin D may act in synergy with this antiviral therapy. Further evaluation revealed that tissue expression of cytochrome P450 25-hydroxylating liver enzymes CYP27A1 paralleled with vitamin D levels and inversely correlated with hepatic necroinflammatory activity. Although further research is expected, because population-based studies have concluded that up to 75% of Americans are deficient in vitamin D,[182] it seems reasonable for all patients with hepatitis C to add vitamin D to their antiviral regimens.

Branched-Chain Amino Acids

In advanced liver disease, such as cirrhosis, patients are often unable to maintain adequate levels of branched-chain amino acids (BCAAs) due to increased demand from ammonia detoxification and energy production.[47] Inadequate BCAA availability causes a reduction of protein synthesis in the liver, resulting in hypoalbuminemia, sarcopenia, edema, and ascites.[86] Supplementing with BCAA has been shown to improve hypoalbuminemia, insulin resistance, immune function, and fatty-acid metabolism; reduce complications of cirrhosis (e.g., esophageal varices, ascites); ameliorate oxidative stress; and inhibit angiogenesis and hepatocarcinogenesis.[47,87] A multicenter randomized controlled trial (RCT) of 622 patients with HCV-related liver cirrhosis showed that oral BCAA supplementation, at a dose of 12 g/day, inhibited hepatocarcinogenesis in obese (body mass index [BMI] >25) patients.[86] The BCAA granules studied contained L-valine, L-leucine, and L-isoleucine at a 1.2:2:1 ratio.[47] General recommendations for the administration of BCAA varies between Japanese, American, and European treatment guidelines.[86]

Acetyl-L-Carnitine

L-carnitine is required for the transportation of fatty acids into the mitochondria of cells. HCV has been found to alter fatty-acid metabolism in hepatocytes by inducing adipogenesis, enhancing lipid droplet formation, and then using these lipids for viral assembly.[88] In vitro studies have shown the inhibition of lipid droplet formation using L-carnitine, suggesting it may have antiviral properties and inhibit the HCV replication process. Additionally, L-carnitine is a well-known antioxidant that has been proven to help decrease hepatocyte damage from HCV.[88] A 2008, placebo-controlled, randomized, double-blind study of 125 cirrhotic patients with chronic hepatitis B or C showed that L-carnitine is able to reduce serum ammonia levels and improve neuropsychological functions compared with placebo.[89] Studies by the same group have demonstrated additional benefits of acetyl-L-carnitine supplementation in patients with HCV, including reduction of fatigue and depression, improved cognition, and an increase in quality of life.[90–92]

Selenium

Selenium is an essential trace element that plays a pivotal role in immune function, including activation of the enzyme glutathione peroxidase, a well-known mediator of oxidative stress. In a deficient state, general immune dysfunction, cancer, and liver cirrhosis have all been documented, the latter showing a proportional decline with the severity of liver injury.[93,94] These findings help confirm that selenium supplementation may be an important addition to current viral hepatitis therapies.[95]

Whole-blood and plasma selenium levels in 59 patients with chronic liver pathologies, including alcoholic and viral liver cirrhosis, were found to be significantly lower than in healthy controls.[96] Another study of hepatocellular carcinoma cell lines reveals that liver cancer cells are able to acquire a selective survival advantage that is prominent under conditions of selenium deficiency and oxidative stress.[97] Oxidative stress is a well-known feature in late-stage cirrhotic liver disease, and subsequent studies have found it to occur much earlier than previously thought.[98] Yu and colleagues found that selenium deficiency correlated with the development of HCC in patients with chronic hepatitis B, and those who supplemented with 200 mcg of selenium daily were less likely to develop HCC compared with nonsupplemented individuals.[99,100] Given this information and the safety of selenium, it is reasonable to employ a supplementary dose of 50 to 100 mg of selenium/day in a hepatitis protocol.[101]

S-Adenosyl Methionine

S-adenosyl methionine (SAMe) functions mainly as a methyl group donor but is less known for its regulation of hepatocyte growth, death, and differentiation. It has been hypothesized that lower hepatic methionine adenosyltransferase (MAT) activity may play a role in the pathogenesis and progression of cirrhosis as well as predisposition to HCC.[102] In a mouse model, SAMe has been shown to enhance interferon's antiviral properties.[103] In a small pilot study performed on G1 HCV nonresponders to pegylated interferon plus ribavirin, it was demonstrated that those patients who were retreated with the same regimen but supplemented with 400 mg SAMe tablets per day displayed both improved early viral kinetics and interferon signaling. This led to enhanced interferon responsiveness and resulted in a higher percentage of patients achieving SVR.[104]

SAMe, at an oral dose of 1600 mg/day, has also been shown to subjectively reduce pruritis and fatigue related to intrahepatic cholestasis due to viral hepatitis.[105]

Probiotics

Probiotics and the gut microbiota seem to be playing a larger role in liver health than previously thought. The GLA, a relatively new concept, helps us better understand the pathophysiology of several hepatopathies, including viral hepatitis. Studies have demonstrated a direct relationship between HBV and HCV and an overproduction of proinflammatory cytokines and hepatic inflammation. One study demonstrated the direct effects of lactitol, a prebiotic known to increase the number of beneficial bacteria species (e.g., *Bifidobacterium* and *Lactobacillus*), on the reduction of endotoxins in patients with hepatitis B or C.[106] Another study looking at the GLA found that those infected with HCV had an increased risk for intestinal permeability, the inciting factor of gut dysbiosis and the proinflammatory effects of liver cirrhosis and HCC.[107] More research is needed to completely understand the effects of probiotics on liver disease, but the link between gut dysbiosis and endotoxemia has been well documented, and any therapies used to treat dysbiosis should be considered when treating those patients with hepatitis.[108]

Glandulars

Liver Extracts

The oral administration of liver extracts has been used in the treatment of many chronic liver diseases since 1896. Numerous investigations

into the therapeutic efficacy of liver extracts have demonstrated that these extracts promote hepatic regeneration and are quite effective in the treatment of chronic liver disease, including chronic active hepatitis.[109–111] For example, in one double-blind study, 556 patients with chronic hepatitis were given either 70 mg of a liver extract or a placebo three times daily.[111] At the end of 3 months, the group receiving the liver extract had far lower liver enzyme (aminotransaminase) levels, suggesting that liver extract may have an anti-inflammatory effect on the liver. This study must be interpreted with caution because the cause of chronic hepatitis was not clearly stated.

Thymus Extracts

The effectiveness of orally administered bovine thymus in viral hepatitis is reflective of broad-spectrum immune system enhancement, presumably mediated by improved thymus gland activity. Several double-blind studies in both acute and chronic hepatitis B have shown thymus extracts to be moderately effective at best. In these studies, a therapeutic effect was noted by accelerated decreases of liver enzymes (transaminases), elimination of the virus, and a higher rate of seroconversion to anti-HBe[112,113] (see Chapter 136 for additional information on the thymus gland).

Botanical Medicines

The use of botanical medicine, in conjunction with other forms of complementary and alternative medicine (CAM), has been increasing among patients with liver disease even though long-term clinical trials have yet to confirm the safety and efficacy of many of these treatments. It is due to this increased public interest, as well as known historical uses and lack of quality research, that the scientific community has taken an interest in botanical medicine. Unfortunately, among the several hundred plants that have been examined, there are only a handful of quality studies looking at safety, therapeutic effectiveness, and mechanisms of action. Of all those herbs studied, the most promising for the treatment of viral hepatitis include licorice *(Glycyrrhiza glabra),* silymarin (the flavonoid complex from milk thistle, *Silybum marianum*), and an ayurvedic herbal combination Liv-52 (containing *Capparis spinosa, Cichorium intybus, Soalnum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium, Tamarix gallica,* and *Phyllanthus amarus*).[114–116,183]

Glycyrrhiza glabra (Licorice Root)

Many hepatitis trials have looked at the triterpenoid saponin, glycyrrhizin, found in the roots of the licorice plant. Licorice, and its main constituent glycyrrhizin, has been suggested to exert many pharmacological actions beneficial to the treatment of acute and chronic hepatitis, including antioxidant, antihepatotoxic, choleretic, antiviral, and immune-modulating actions. These actions have been proven to reduce serum alanine transaminase and aspartate transaminase values, inhibit immune-mediated cytotoxicity against hepatocytes, suppress hepatitis B surface antigen (HBsAg) secretion, and antagonize nuclear factor-kappa B (NF-κB), a transcription factor that activates genes encoding inflammatory cytokines.[80,115,116]

Licorice has been shown to stimulate the production of the body's natural supply of interferon. This may account for its popularity in Japan, where it has been used in the treatment of chronic viral hepatitis for more than 20 years.[117–122] When used intravenously, licorice has been demonstrated to lower liver enzymes, suggesting a potential benefit in the treatment of hepatitis C, and decrease the risk of HCV-associated HCC.[123–125] A product by the name Stronger Neominophagen C (SNMC) consists of 200 mg of glycyrrhizin, 100 mg of cysteine, and 2000 mg of glycine in 100 mL of physiological saline. When administered intravenously, liver enzymes were decreased in approximately 40% of patients, but with little to no change in HCV load.[117–121] Furthermore, the beneficial effect of SNMC on the reduction of liver enzymes was short-lived: after discontinuation of this supplement, ALT elevations returned. A similar outcome was found by others.[123]

In a study of 453 Japanese patients diagnosed with chronic hepatitis C, 84 were treated with SNMC at a dosage of 100 mL/day for 8 weeks, followed by treatments two to seven times weekly for periods up to 16 years. The rates of cumulative HCC and cirrhosis in year 10 were 7% and 12%, respectively; in year 15, they were 12% and 21%, respectively. And in a cohort of patients with HCV who were unresponsive to interferon therapy, intravenous glycyrrhizin decreased the risk of progression to HCC.[126] Unfortunately, the beneficial results of glycyrrhizin in hepatitis are not consistent. When lower doses of oral glycyrrhizin were used, significant reductions in ALT levels were not observed. Many have concluded that although licorice may reduce ALT elevations, long-term beneficial effects on HCV-associated fibrosis have not been shown.[127]

The main adverse effect of licorice administration is the production of its aldosterone-like effects, including fluid retention, hypokalemia, and hypertension; thus, licorice should be avoided in patients with a history of ascites, hypertension, or renal failure and those using digitalis preparations.[115,116]

Silybum marianum (Milk Thistle)

The origins of the effects of milk thistle on the liver can be traced back to ancient Roman times when Pliny the Elder (AD 23–79) referred to the milky juice of this plant as being excellent for "carrying off bile." John Gerard, a 16th-century British herbalist, recommended milk thistle for "expelling melancholy," a symptom attributed to liver disease during that era. In Germany, during the 19th century, doctors commonly treated jaundice and other liver diseases with an extract from milk thistle seeds. In 1949 German researchers found that milk thistle appeared to protect the livers of animals exposed to high doses of carbon tetrachloride, a potent hepatotoxin.[128]

In 1968 it was found that the active ingredients in milk thistle, a mixture of flavolignans and a flavonoid, are in the seed and consists of silybin, silydianin, and silychristin, collectively referred to as silymarin. Due to its cytoprotectant, antioxidant, anti-inflammatory, and antifibrotic properties, silymarin is the most used natural compound for hepatic disorders worldwide.[129] In the United States, milk thistle is most commonly used for treating viral hepatitis and cirrhosis of the liver. Unfortunately, the trials that have been completed have produced conflicting results, even with the standardization (between 70% and 80%) of silymarin.[130] It is hypothesized that silymarin's hepatoprotective properties come from the following:

- Acting as an antioxidant and reducing reactive oxygen species
- Increasing the intracellular content of glutathione, superoxide dismutase, and catalase
- Inhibiting the formation of leukotrienes and cellular damage
- Stimulating hepatocyte regeneration

Silymarin has been used to treat both acute and chronic hepatitis of varying etiologies. In a study with 29 patients with acute viral hepatitis, those treated with silymarin showed greater improvements in serum levels of bilirubin and liver enzymes compared with the placebo group.[131]

Other investigators specifically evaluating the antiviral effect of silymarin on patients with hepatitis C concluded that improvements in some symptoms occurred when silymarin was administered orally but that it failed to exert an antiviral effect or result in significant reductions in ALT or improvements in ultrasound abnormalities related to hepatitis C.[132–134]

In humans, flavonoids have a low bioavailability, owing to extensive first-pass metabolism; thus, the plasma levels needed to exert an antiviral effect may not be possible. Discrepant results reported by various investigators may be due to a lack of homogeneity among doses and routes of administration.

A newer form of silymarin that binds it to phosphatidylcholine (referred to as silymarin phytosome) may provide greater benefit. A growing body of research indicates that phosphatidylcholine-bound silymarin is better absorbed and produces better clinical results than the unbound variety.[135–140] These benefits were demonstrated in a study involving 232 patients with chronic hepatitis (viral, alcoholic, or chemically induced) treated with silymarin phytosome at either 120 mg twice a day or 120 mg three times a day for up to 120 days.[140] Liver function returned to normal faster in patients given silymarin phytosome compared with both the commercially available silymarin and a placebo.

Intravenous silibinin is capable of the greatest suppression of hepatitis C viral replication. The first study that provided clear evidence of the antiviral effects of silymarin in humans with hepatitis C was published in 2008 by Ferenci and colleagues.[141] These investigators found that such patients who were nonresponders to SOC treatment were able to achieve undetectable HCV RNA levels after intravenous administration of silibinin for 15 days. Although these results proved to be temporary, the antiviral effect of intravenous silibinin was permanent for one patient with hepatitis C who received this treatment for 2 weeks after liver transplantation. This suggests that silibinin may be capable of preventing graft reinfection with HCV.[142] Further studies are needed to confirm these promising results.

Phyllanthus amarus

Phyllanthus amarus is an important herb in the Ayurvedic system of medicine commonly used for liver disorders. An array of phytochemical studies looking at this herb demonstrated a wide spectrum of pharmacological activities, including antiviral, antibacterial, anti-inflammatory, antimicrobial, anticancer, antioxidant, and hepatoprotective.[143] A preliminary report from 1988 demonstrated that 59% of patients with hepatitis B had lost the hepatitis B surface antigen when tested 15 to 20 days after treatment with a preparation of *P. amarus* (200 mg of the dried, powdered, sterilized plant in capsules three times a day).[144] In a 2011 in vitro study looking at the correlation between *P. amarus* (root and leaf) extracts and HCV replication, there were two HCV enzymes that were shown to be inhibited by the extracts, therefore also inhibiting HCV replication.[143] At this time, these results have not been confirmed by other researchers, indicating the need for more research in this field.[145–148]

Combination Approach with Antioxidants and Botanical Therapy

It has been well documented that the pathogenesis of chronic HCV is associated with a suboptimal immune response and intrahepatic oxidative stress. Therefore the addition of antioxidant therapies in the treatment of HCV may have beneficial effects on the prognosis and outcome of this disease.[149,150]

A report by Berkson describes three patients with advanced cirrhosis, portal hypertension, and esophageal varices secondary to chronic hepatitis C infection who were effectively treated with a low-cost combination program. Although these patients were originally given extremely poor prognoses, all "recovered quickly," showed improved liver enzymes, and were able to resume their normal daily activities. One patient revealed a substantial improvement in her condition within only 2 weeks. The other patients progressed to health within 4 to 7 months.[151]

Berkson's regimen was composed of oral supplementation of α-lipoic acid (600 mg/day in two divided doses), selenium (as selenomethionine at 400 mcg/day in two divided doses), and silymarin (900 mg/day in three divided doses). The subjects were also asked to take a B-complex vitamin, eat a diet high in fruits and vegetables, consume 4 oz or less of meat per meal, and drink eight glasses of water per day. They also took between 1000 and 6000 mg of vitamin C daily and 400 IU/day of vitamin E. They were encouraged to walk 1 mile three times a week. Only the α-lipoic acid/selenium/silymarin portion of the protocol was verified, and it is not known how strictly the other nutritional and lifestyle suggestions were followed.[151]

Although this protocol is documented in only three cases, it makes reasonable sense from a naturopathic standpoint, given the excellent safety record, reasonable cost, and known efficacy of the individual treatment options and is certainly worth trying, especially in patients failing to respond to conventional therapies. It is hoped that stringent clinical trials of this type of multiantioxidant/lifestyle combination protocol will be conducted soon.

One such study, by Melhem et al., in which 50 patients with chronic HCV were treated with oral (glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, l-glutathione, and alpha-tocopherol) and intravenous preparations (glycyrrhizin, ascorbic acid, l-glutathione, b-complex) demonstrated an enhanced response rate when looking at HCV RNA levels, liver enzymes, liver histology, and quality of life.[152]

Acupuncture and Chinese Medicine Herbs

The Chinese medicine diagnosis for hepatitis often includes consideration of damp heat in the liver and spleen channels. Acupuncture points and herbal formulas are often directed at clearing heat and damp to cool and drain these channels. Often efforts are made to also nourish liver blood and tone the liver and spleen.

In a study of 60 patients with hepatitis B, 30 were prescribed 30 minutes of acupuncture once a day for 4 to 6 weeks, whereas the other 30, the control group, continued their conventional treatment. Those in the treatment group had significantly shorter recovery times, greater symptom improvement, and lower interleukin-8 levels.[153] Studies have also investigated the use of acupuncture to improve secondary and comorbid symptoms, such as depression and myalgias. One 6-week trial categorized 28 patients: group 1 comprised 13 patients with high depression and myalgia scores, group 2 was made up of 11 patients with low depression but high myalgia scores, and group 3 consisted of 4 patients with high depression but low myalgia scores. Compared with baseline levels, significant improvement was shown in end-treatment depression and myalgia scores.[142] Although no positive outcomes regarding improved viral load using acupuncture have been shown, acupuncture may help other symptoms, improve immune balance, and lower comorbidities in patients with hepatitis. In a meta-analysis done by Zhao et al. in 2011, a positive correlation was found between interferon along with the use of Chinese herbal formulas and a higher sustained virologic response.[154] More studies are needed, but given the possible benefit and low risk, acupuncture and Chinese herbs may be reasonable therapies for patients with viral hepatitis.

Because the standard operating procedure for acupuncture mandates the use of clean-needle technique, the risk of hepatitis from acupuncture is considered extremely low.[155] Acupuncture may help improve symptoms and hepatic function. A patient should look for a practitioner who uses only disposable needles as opposed to resterilized needles.

Sho-saiko-to or "Xiao Chai Hu Tang"

Sho-saiko-to (named Xiao Chai Hu Tang in Chinese) is a botanical formulation containing seven herbs traditionally used to treat liver

and gastrointestinal disorders.[156] This formula has been shown in animal models to prevent liver injury and promote liver regeneration by inhibiting oxidative stress on lipid peroxidation in hepatocytes and hepatic stellate cells[157,158] and, in human trials, to prevent the progression of cirrhosis to HCC.[159] One prospective study of 260 patients also found that it increased the survival of those with chronic viral hepatitis by reducing progression to HCC.[160–162]

A Phase II trial at Sloan-Kettering Cancer Center showed that shosaiko-to may improve liver pathology in patients with HCV who are unable or choose not to receive interferon therapy.[163] In this open-label trial, 24 subjects were given 2.5 g granulated powder, three times daily, for 12 months. After 12 months of treatment, 38% of subjects had improved liver histology on fine-needle biopsy, 67% had reduced AST levels, and 75% had reduced ALT levels. Of note, this treatment is contraindicated with interferon drug therapy because there is an increased risk of pneumonitis,[164] and Sho-saiko-to has been reported to cause acute hepatitis.[165]

Hepatotoxic Supplements

The use of nutritional and herbal supplements has dramatically increased in popularity since the 1990s, especially in certain subgroups, including affluent societies, elderly individuals, nonsmoking females, and those with higher education levels. With such an increase in supplementation and limited oversight from either the FDA or practitioners, there is also more concern for toxicity due to unknown additives, dosage, and incorrect or off-label uses.[166]

Niacin, a supplement used mostly for hyperlipidemia, known to cause flushing, headache, and stomachache, may cause hepatotoxicity, depending on form and dosage. Immediate-release, or crystalline, niacin at doses above 500 mg and/or sustained-release forms at doses of 2 g/day or higher have been shown to contribute to hepatotoxicity and should be avoided in patients with hepatitis.[167–169] Other forms of niacin, including extended release (ER) and niacinamide, appear less likely to induce hepatic abnormalities and may be considered safer options.[170,171]

Vitamin A belongs to a group of compounds known as retinoids. About 80% to 90% of total-body stores of retinoids are found in hepatic stellate cells. Excessive consumption of vitamin A (acute doses of over 100,000 IU or chronic doses of 25,000–100,000 IU daily for over a year) may cause hypervitaminosis A; individual tolerability may vary. Hypervitaminosis A has been well documented to cause hepatotoxicity, including elevation of serum liver enzymes, cholestatic hepatitis, noncirrhotic portal hypertension, fibrosis, and cirrhosis.[166,172] Vitamin A's potential to cause liver toxicity has been shown to be enhanced with increased alcohol consumption, excessive intake of other fat-soluble vitamins, and vitamin C deficiency.

Iron has been demonstrated to generate reactive oxygen species, leading to point mutations, chromosomal damage, and inactivation of tumor-suppressor genes such a p53; therefore it is a direct hepatotoxin. Unless a patient is found to be iron deficient, iron supplementation should be avoided because iron has been shown to speed the progression and worsen the course of many liver diseases. This is especially true for alcoholic liver disease because alcohol and excess iron have been shown to have an additive harmful effect on the liver.[173]

Hepatotoxic Botanicals

Given the pharmacological nature of plant medicines, it is important to remember that some commonly used botanicals may be hepatotoxic and should be avoided in patients with hepatitis. Perhaps the most researched is *Symphytum officinale* (comfrey), which contains hepatotoxic pyrrolizidine alkaloids (PAs), such as lasiocarpine and symphytine, and their related N-oxides.[174] Although *Symphytum* is most commonly used as a topical vulnerary, the PAs may still be absorbed through the skin, and their use should be avoided in patients with liver disease. *Piper methysticum* (kava kava) has also been implicated in hepatic liver damage.[175] A number of these reported cases of kava toxicity seemed to involve poor documentation, preexisting conditions, kava overdose, or concomitant polypharmacy. Although considered safe at normal doses for people without liver disease, those with hepatic conditions should avoid kava kava as a precaution.[176]

There is a long list of other potentially hepatotoxic botanicals that should be discussed due to past and emerging research (Box 176.3). Many of these herbs are found in formulas that are known to be hepatotoxic, and others have been included based on constituent actions. Until more quality research is done to confirm the role of these botanicals in the pathophysiology of liver damage/disease, they should be used with caution in those with hepatitis.[177,178]

BOX 176.3 Botanicals Associated With Liver Damage[177,178]

- *Borago officinalis*
- *Camellia sinensis*
- *Larrea tridentata*
- *Hypericum perforatum*
- *Lycopodium serratum*
- *Valeriana officinalis*
- *Atractylis gummifera*
- *Chelidonium majus*
- *Cimicifuga racemosa*

THERAPEUTIC APPROACH

During the contagious phase (2–3 weeks before symptoms appear to 3 weeks after), good hygiene and avoiding close contact with others is important. Once a diagnosis has been made, work in a daycare center, restaurant, or other similar employment is not recommended. Chronic hepatitis requires a multifactorial, integrative approach that will include lifestyle and dietary measures, as well as possibly a combination of nutrients, botanicals, and conventional pharmaceuticals, as needed on an individual basis.

Lifestyle

In the acute phase, bed rest is important, with a slow resumption of activities as health improves. At least 8 hours of sleep is recommended during the acute and recovery phases. Exercise is recommended at the level of intensity safest for each patient, a level that may vary from person to person. Strenuous exercise should be avoided. Weight loss is recommended for those who are overweight or obese. Smoking tobacco and drinking alcohol should be completely avoided.[179,180]

Diet

A low-caloric, whole-foods, high-fiber diet low in saturated fats, oxidized fatty acids (fried oils), and simple carbohydrates (e.g., sugar, white flour, and fruit juice) is recommended.[179] Consumption of vegetable broths, diluted vegetable juices (diluted with 50% water), and herbal teas is helpful to avoid dehydration, especially in the presence of diarrhea. Foods such as flavonoid-rich blueberry, coffee, and grapefruit (if there are no interactions with medication) may be beneficial to the patient with chronic hepatitis. Prebiotic and probiotic foods should be emphasized to improve the GLA and reduce liver inflammation.[181]

Nutritional Supplements

Vitamin C: to bowel tolerance (10–50 g/day) in acute cases; 1000 mg three times/day in chronic cases. Caution in cases of iron overload when taken with iron sources.
Vitamin D: 1000 to 4000 IU a day
Vitamin E (mixed tocopherols): 1200 IU a day
Selenium: 50 to 200 mcg a day
Whey protein: for hepatitis B at 24 g a day
Liver extracts: 70 mg three times a day
Thymus extracts: equivalent to 120 mg pure polypeptides with molecular weights of less than 10,000 or roughly 750 mg of the crude polypeptide fraction a day

Botanical Medicines

Glycyrrhiza glabra (Licorice)

Powdered root: 1 to 2 g three times daily
Fluid extract (1:1): 2 to 4 mL (1–2 g) three times daily
Solid (dry powdered) extract (5% glycyrrhetinic acid content): 250 to 500 mg three times daily.
Intravenous administration may be the most effective form.
Note: chronic licorice administration may call for an increased intake of potassium-rich foods.

Silybum marianum (Milk Thistle)

Standardized extract: 70 to 210 mg three times daily, based on its silymarin content.
The best results are achieved at higher dosages (i.e., 150–300 mg three times daily). For this reason, standardized extracts are preferred.
Silybin bound to phosphatidylcholine: 120 mg two to three times daily between meals. Intravenous silibinin may be the most effective form.

Berkson Combination Antioxidant Approach

See the earlier section on antioxidant/botanical therapy for this specific protocol.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Matheny SC, Kingery JE. "*Hepatitis A.*" *AAFP Home*; 2012. www.aafp.org/afp/2012/1201/p1027.html.
2. Lai M, Chopra S. Hepatitis A virus infection in adults: epidemiology, clinical manifestations, and diagnosis. In: Hirsch MS, Baron EL, ed. *UpToDate.* Waltham, MA: UpToDate Inc. http://www.uptodate.com (Accessed on November 14, 2018.)
3. Lok ASF. Hepatitis B virus: screening and diagnosis. In: Mitty J, Esteban R, ed. *UpToDate.* Waltham, MA: UpToDate Inc. http://www.uptodate.com (Accessed on November 14, 2018.)
4. Wilkins T, Zimmerman D, Schade RR. Hepatitis B: diagnosis and treatment. *Am Fam Physician*. 2010;81(8):965–972. Retrieved from https://www.aafp.org/afp/2010/0415/p965.html#afp20100415p965-b3.
5. Centers for Disease Control and Prevention. *The ABCs of Hepatitis*; 2016. Retrieved from https://www.cdc.gov/hepatitis/Resources/Professionals/PDFs/ABCTable.pdf.
6. Wilkins T, Akhtar M, Gititu E. Diagnosis and management of hepatitis C. *Am Fam Physician*. 2015;91(12):835–842. Retrieved from https://www.aafp.org/afp/2015/0615/p835.html#afp20150615p835-b1.
7. Centers for Disease Control and Prevention. *Viral Hepatitis*. Centers for Disease Control and Prevention; 2018. Retrieved from www.cdc.gov/hepatitis/hcv/hcvfaq.htm#a3.
8. Strader DB, Seeff LB. The natural history of chronic hepatitis C infection. *Eur J Gastroenterol Hepatol*. 1996;8:324–328.
9. Seeff LB, Hoofnagle JH. National institutes of health consensus development conference: management of hepatitis C: 2002. *Hepatology*. 2002;36(suppl):S1–S2.
10. Rutherford AE. "*Overview of Acute Viral Hepatitis - Hepatic and Biliary Disorders*". Merck Manuals Professional Edition; 2017. www.merckmanuals.com/professional/hepatic-and-biliary-disorders/hepatitis/overview-of-acute-viral-hepatitis.
11. Rutherford AE. "*Overview of Chronic Hepatitis - Hepatic and Biliary Disorders*". Merck Manuals Professional Edition; 2017. www.merckmanuals.com/professional/hepatic-and-biliary-disorders/hepatitis/overview-of-chronic-hepatitis.
12. Ogholikhan S, Schwarz KB. "*Hepatitis Vaccines*" *Vaccines*. 2016;4(1):6. https://doi.org/10.3390/vaccines4010006.
13. Walker C. Designing an HCV vaccine: a unique convergence of prevention and therapy? *Curr Opin Virol*. 2017;23:113–119.
14. Chopra S, Pockros PJ. Overview of the management of chronic hepatitis C virus infection. In: Di Bisceglie AM, Bloom A, ed. *UpToDate.* Waltham, MA: UpToDate Inc. http://www.uptodate.com (Accessed on November 15, 2018).
15. Vgontzas AN, Zoumakis E, Bixler EO, et al. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab*. 2004;89(5):2119–2126.
16. Opp MR, Born J, Irwin MR. Sleep and the immune system. In: Ader R, ed. *Psychoneuroimmunology*. New York, NY: Academic Press; 2007.
17. Kanwal F, Gralnick IM, Rays RD, et al. Health related quality of life predicts mortality in patients with advanced chronic liver disease. *Clin Gastrolenterol Hepatol.* 2009;7:793–799.
18. Raison CL, Rye DB, Woolwine BJ, et al. Chronic interferon-alpha administration disrupts sleep continuity and depth in patients with hepatitis C: association with fatigue, motor slowing, and increased evening cortisol. *Biol Psychiatry*. 2010;15(68):942–949 (10).
19. Scott J, et al. Fatigue during treatment for hepatitis C virus: results of self-reported fatigue severity in two Phase IIb studies of simeprevir treatment in patients with hepatitis C genotype 1 infection. *BMC Infectious Dis*. 2014;14:465.
20. Carlson MD, Hilsabeck RC, Barakat F, et al. Role of sleep disturbance in chronic hepatitis C infection. *Curr Hepat Rep*. 2010;9(1):25–29.
21. Lange T, Perras B, Fehm HL, et al. Sleep enhances the human antibody response to hepatitis A vaccination. *Psychosom Med*. 2003;65(5):831–835.
22. Prather AA, Hall M, et al. Sleep and antibody response to hepatitis B vaccination. *Sleep*. 2012;35(8):1063–1069.
23. Cohen S, Doyle WJ, Alper CM, et al. Sleep habits and susceptibility to the common cold. *Arch Intern Med*. 2009;169(1):62–67.
24. Pennisi M, Bertino G, Gagliano C, et al. Resveratrol in hepatitis C patients treated with pegylated-interferon-α-2b and ribavirin reduces sleep disturbance. *Nutrients*. 2017;9(8).
25. Bi L, et al. Exercise and gastrointestinal function and disease: an evidence-based review of risks and benefits. 1(5):345–355.
26. McKenna O, Cunningham C, et al. Management of the extrahepatic symptoms of chronic hepatitis C: feasibility of a randomized controlled trial of exercise. 92(6):504–512.
27. Payan JL, Pillard F, Mascarell V, et al. Is physical activity possible and beneficial for patients with hepatitis C receiving pegylated interferon and ribavirin therapy? *Gastroenterol Clin Biol*. 2009;33(1 Pt 1):8–14.
28. Chopra S, Pockros PJ. Overview of the management of chronic hepatitis C virus infection. In: Di Bisceglie AM, Bloom A, ed. *UpToDate.* Waltham, MA: UpToDate Inc. http://www.uptodate.com (Accessed on November 20, 2018.)
29. Turati F, Trichopoulos D, Polessel J, et al. Mediterranean diet and hepatocellular carcinoma. *J Hepatol*. 2014;60(3):606–611.
30. Hickman IJ, Clouston AD, Macdonald GA, et al. Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. *Gut*. 2002;51:89–94.
31. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol*. 2017;67(4):829–846.
32. Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: evidence and plausible mechanisms. *Liver Int*. 2017;37(7):936–949.
33. Poeta M, Pierri L, Vajro P. Gut-liver axis derangement in non-alcoholic fatty liver disease. *Children (Basel)*. 2017;4(8).
34. Sandler NG, Koh C, Roque A, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology*. 2011;141(4):1220–1230. 1230.e1-3.
35. Heidrich B, Vital M, Plumeier I, et al. Intestinal microbiota in patients with chronic hepatitis C with and without cirrhosis compared with healthy controls. *Liver Int*. 2018;38(1):50–58.
36. Preveden T, Scarpellini E, Milić N, Luzza F, Abenavoli L. Gut microbiota changes and chronic hepatitis C virus infection. *Expert Rev Gastroenterol. Hepatol*. 2017;11(9):813–819. https://doi.org/10.1080/17474124.2017.1343663. Epub 2017. Jun 23. Review.
37. Tao X, Wang N, Qin W. Gut Microbiota and hepatocellular carcinoma. *Gastrointest Tumors*. 2015;2(1):33–40.
38. Asgharpour A, Cazanave SC, Pacana T, et al. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J Hepatol*. 2016;65(3):579–588.
39. Do MH, Lee E, Oh MJ, Kim Y, Park HY. High-glucose or -fructose diet cause changes of the gut microbiota and metabolic disorders in mice without body weight change. *Nutrients*. 2018;10(6):761. https://doi.org/10.3390/nu10060761. Published 2018 Jun 13.
40. Sharpton SR, Ajmera V, Loomba R. Emerging role of the gut microbiome in nonalcoholic fatty liver disease: from composition to function. *Clin Gastroenterol Hepatol*. 2019;17(2):296–306. https://doi.org/10.1016/j.cgh.2018.08.065.
41. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol*. 2015;31(1):69–75.
42. Bindels LB, Walter J, Ramer-Tait AE. Resistant starches for the management of metabolic diseases. *Curr Opin Clin Nutr Metab Care*. 2015;18(6):559–565.
43. Carrera-Quintanar L, López Roa RI, Quintero-Fabián S, Sánchez-Sánchez MA, Vizmanos B, Ortuño-Sahagún D. Phytochemicals that influence gut microbiota as prophylactics and for the treatment of obesity and inflammatory diseases. *Mediators Inflamm*. 2018;2018:9734845.
44. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes*. 2017;8(2):172–184.
45. Veiga P, Pons N, Agrawal A, et al. Changes of the human gut microbiome induced by a fermented milk product. *Sci Rep*. 2014;4:6328.
46. Wen L, Duffy A. Factors influencing the gut microbiota, inflammation, and type2 diabetes. *J Nutr*. 2017;147(7):1468S–1475S.

47. Nishikawa H, Osaki Y. Liver cirrhosis: evaluation, nutritional status, and prognosis. *Mediators Inflamm*. 2015;2015:872152. https://doi.org/10.1155/2015/872152. Epub2015 Sep 30.
48. Takeshita M, Ishida Y, Akamatsu E, et al. Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. *J Biol Chem*. 2009;284(32):21165–21176.
49. Wang YP, Cheng ML, Zhang BF, et al. Effects of blueberry on hepatic fibrosis and transcription factor Nrf2 in rats. *World J Gastroenterol*. 2010;16(21):2657–2663.
50. Hernández-Aquino E, Muriel P. Beneficial effects of naringenin in liver diseases: molecular mechanisms. *World J Gastroenterol*. 2018;24(16):1679–1707.
51. Mulvihill EE, Allister EM, Sutherland BG, et al. Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. *Diabetes*. 2009;58(10):2198–2210.
52. Felmlee DJ, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins–impact for the viral life cycle and pathogenesis of liver disease. *Viruses*. 2013;5(5):1292–1324. https://doi.org/10.3390/v5051292. Published 2013 May 22.
53. Nahmias Y, Goldwasser J, Casali M, et al. Apolipoprotein B-dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin. *Hepatology*. 2008;47(5):1437–1445.
54. Goldwasser J, et al. Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through a PPAR-mediated mechanism. *J Hepatol*. 2011;55:963–971.
55. Bailey DG, Malcolm J, Arnold O, et al. Grapefruit juice-drug interactions. *Br J Clin Pharmacol*. 1998;46:101–110.
56. Dahan A, Altman H. Food-drug interaction: grapefruit juice augments drug bioavailability: mechanism, extent and relevance. *Eur J Clin Nutr*. 2004;58:1–9.
57. Heath RD, Brahmbhatt M, Tahan AC, Ibdah JA, Tahan V. Coffee: the magical bean for liver diseases. *World J Hepatol*. 2017;9(15):689–696.
58. Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology*. 2005;129(6):1928–1936.
59. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology*. 2005;128(1):24–32.
60. Modi AA, Feld JJ, Park Y. Increased caffeine consumption is associated with reduced hepatic fibrosis. *Hepatology*. 2010;51(1):201–209.
61. Tverdal A, Skurtveit S. Coffee intake and mortality from liver cirrhosis. *Ann Epidemiol*. 2003;13(6):419–423.
62. Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterology*. 2007;132(5):1740–1745. Epub 2007 Mar 24.
63. Bravi F, Bosetti C, Tavani A, et al. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology*. 2007;46(2):430–435.
64. Wijarnpreecha K, Thongprayoon C, Ungprasert P. Impact of caffeine in hepatitis C virus infection: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol*. 2017;29(1):17–22. Review.
65. Wadhawan M, Anand AC. Coffee and liver disease. *J Clin Experimental Hepatol*. 2016;6(1):40–46.
66. Freedman ND, Everhart JE, Lindsay KL, et al. Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. *Hepatology*. 2009;50(5):1360–1369.
67. Elattar G, Saleh Z, El-Shebini S, et al. The use of whey protein concentrate in management of chronic hepatitis C virus - a pilot study. *Arch Med Sci*. 2010;6(5):748–755.
68. Watanabe A, Okada K, Shimizu Y, et al. Nutritional therapy of chronic hepatitis by whey protein (non-heated). *J Med*. 2000;31:283–302.
69. Brass CA, Piken E. Do Antioxidants ameliorate ribavirin related anemia in HCV patients. *Gastroenterology*. 1999;116(4):A1192–A1193.
70. Kawaguchi Y, Mizuta T, Takahashi K, et al. High-dose vitamins E and C supplementation prevents ribavirin-induced hemolytic anemia in patients with chronic hepatitis C. *Hepatol Res*. 2007;37(5):317–324.
71. Jain SK, Pemberton PW, Smith A, et al. Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. *J Hepatol*. 2002;36:805–811.
72. Evans JL, Goldfine ID. Alpha-lipoic acid: a multifunctional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. *Diabetes Technol Ther*. 2000;2:401–413.
73. Pande M, Flora SJ. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. *Toxicology*. 2002;177:187–196.
74. Suh JH, Shigeno ET, Morrow JD, et al. Oxidative stress in the aging rat heart is reversed by dietary supplementation with (R)-(alpha)-lipoic acid. *FASEB J*. 2001;15:700–706.
75. Stehbens WE. Oxidative stress, toxic hepatitis, and antioxidants with particular emphasis on zinc. *Exp Mol Pathol*. 2003;75(3):265–276.
76. Matsuoka S, Matsumura H, Nakamura H, et al. Zinc supplementation improves the outcome of chronic hepatitis C and liver cirrhosis. *J Clin Biochem Nutr*. 2009;45(3):292–303.
77. Palmer M, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology*. 1990;99(5):1408–1413.
78. Takagi H, Nagamine T, Abe T, et al. 2001 Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. *J Viral Hepat*. 2001;8(5):367–371.
79. Suzuki A, Lindor K, St Saver J, et al. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol*. 2005;43(6):1060–1066.
80. Bean P. The use of alternative medicine in the treatment of hepatitis C. *Am Clin Lab*. 2002;21:19–21.
81. Abu Mouch S, Fireman Z, Jarchovsky J, et al. Vitamin D supplement improve SVR in chronic hepatitis C (genotype 1) naive patients treated with peg interferon and ribavirin. *45th Annual Meeting of the European Association for the Study of the Liver (EASL* 2010): Vienna, Austria.
82. Gutierrez JA, Parikh N, Branch AD. Classical and emerging roles of vitamin D in hepatitis C virus infection. *Semin Liver Dis*. 2011;31(4):387–398.
83. Villar LM, Del Campo JA, Ranchal I, Lampe E, Romero-Gomez M. Association between vitamin D and hepatitis C virus infection: a meta-analysis. *World J Gastroenterol*. 2013;19(35):5917–5924.
84. Petta S, Cammà C, Scazzone C, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology*. 2010;51(4):1158–1167.
85. Bitetto D, Fabris C, Fornasiere E, et al. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. *Transplant Int*. 2011;24:43–50.
86. Takami T, Yamasaki T, Saeki I, Matsumoto T, Suehiro Y, Sakaida I. Supportive therapies for prevention of hepatocellular carcinoma recurrence and preservation of liver function. *World J Gastroenterol*. 2016;22(32):7252–7263.
87. Kawaguchi T, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology*. 2011;54(3):1063–1070.
88. Tsukuda Y, Suda G, Tsunematsu S, et al. Anti-adipogenic and antiviral effects of l-carnitine on hepatitis C virus infection. *J Med Virol*. 2017;89(5):857–866.
89. Malaguarnera M, Gargante MP, Cristaldi E, et al. Acetyl-L-carnitine treatment in minimal hepatic encephalopathy. *Dig Dis Sci*. 2008;53(11):3018–3025.
90. Malaguarnera M, Bella R, Vacante M, et al. Acetyl-l-carnitine reduces depression and improves quality of life in patients with minimal hepatic encephalopathy. *Scand J Gastroenterol*. 2011;46(6):750–759.
91. Malaguarnera M, Vacante M, Motta M, et al. *Metab Brain Dis*. 2011;26:281. https://doi.org/10.1007/s11011-011-9260-z.
92. Malaguarnera M, Vacante M, et al. The supplementation of acetyl-l-carnitine decreases fatigue and increases quality of life in patients with hepatitis C treated with pegylated interferon-α 2b plus ribavirin. *J Interferon Cytokine Res*. 2011;31(9).
93. Khan MS, Dilawar S, Ali I, Rauf N. The possible role of selenium concentration in hepatitis B and C patients. *Saudi J Gastroenterol*. 2012;18(2):106–110.
94. Ko WS, Guo CH, Yeh MS, et al. Blood micronutrient, oxidative stress, and viral load in patients with chronic hepatitis C. *World J Gastroenterol*. 2005;11(30):4697–4702.

95. Rayman MP. The importance of selenium to human health. *Lancet*. 2000;356(9225):233–241.
96. Czuczejko J, Zachara BA, Staubach-Topczewska E, et al. Selenium, glutathione and glutathione peroxidases in blood of patients with chronic liver diseases. *Acta Biochim Pol*. 2003;50:1147–1154.
97. Irmak MB, Ince G, Ozturk M, et al. Acquired tolerance of hepatocellular carcinoma cells to selenium deficiency: a selective survival mechanism? *Cancer Res*. 2003;63:6707–6715.
98. Jain SK, Pemberton PW, Smith A, et al. Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. *J Hepatol*. 2002;36:805–811.
99. Yu MW, Horng IS, Hsu KH, et al. Plasma selenium levels and risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. *Am J Epidemiol*. 1999;150:367–374.
100. Yu SY, Zhu YJ, Li WG. Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. *Biol Trace Elem Res*. 1997;56(1):117–124.
101. Navarro-Alarcón M, López-G de la Serrana H, Pérez-Valero V, López-Martínez MC. Selenium concentrations in serum of individuals with liver diseases (cirrhosis or hepatitis): relationship with some nutritional and biochemical markers. *Sci Total Environ*. 2002;291(1–3):135–141.
102. Mato JM, Lu SC. Role of S-adenosyl-L-methionine in liver health and injury. *Hepatology*. 2007;45:1306–1312.
103. Duong FH, Christen V, Filipowicz M, et al. S-Adenosylmethionine and betaine correct hepatitis C virus induced inhibition of interferon signaling in vitro. *Hepatology*. 2006;43(4):796–806.
104. Feld JJ, Modi AA, El-Diwany R, et al. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. *Gastroenterology*. 2011;140(3):830–839.
105. Anstee QM, Day CP. S-adenosylmethionine (SAMe) therapy in liver disease: a review of current evidence and clinical utility. *J Hepatol*. 2012;57(5):1097–1109.
106. Cesaro C, Tiso A, Del Prete A, et al. Gut microbiota and probiotics in chronic liver diseases. *Digestive Liver Dis*. 2011;43:431–438.
107. Preveden T, Scarpellini E, Milic N, Luzza F, Abenavoli L. Gut microbiota changes and chronic hepatitis C virus infection. *Expert Rev Gastroenterol Hepatol*. 2017;11(9):813–819.
108. Wilson Gratz S, Mykkanen H, El-Nezami HS. Probiotics and gut health: a special focus on liver diseases. World J Gastroenterol. *201;*16(4):403–410.
109. Ohbayashi A, Akioka T, Tasaki H. A study of effects of liver hydrolysate on hepatic circulation. *J Therapy*. 1972;54:1582–1585.
110. Sanbe K, Murata T, Fujisawa K, et al. Treatment of liver disease—with particular reference to liver hydrolysates. *Jap J Clin Exp Med*. 1973;50:2665–2676.
111. Fujisawa K, Suzuki H, Yamamoto S, et al. Therapeutic effects of liver hydrolysate preparation on chronic hepatitis: a double blind, controlled study. *Asian Med J*. 1984;26:497–526.
112. Galli M, Crocchiolo P, Negri C, et al. Attempt to treat acute type B hepatitis with an orally administered thymic extract (Thymomodulin): preliminary results. *Drugs Exptl Clin Res*. 1985;11:665–669.
113. Bortolotti F, Cadrobbi P, Criverllaro C, et al. Effect of an orally administered thymic derivative, Thymodulin, in chronic type B hepatitis in children. *Curr Ther Res*. 1988;43:67–72.
114. Malnick S, Zimhony O. Herbal medicines have no proven efficacy for Hepatitis C. *Evidence-based Healthcare*. 2002;6:132–133.
115. Rajaratnam M, Prystupa A, Lachowska-Kotowska P, Zaluska W, Filip R. Herbal medicine for treatment and prevention of liver disease. *J Pre-clin Clin Res*. 2014;8(2):55–60.
116. Verma S, Thuluvath PJ. Complementary and alternative medicine in hepatology: review of the evidence of efficacy. *Clin Gastroenterol Hepatol*. 2007;5(4):408–416.
117. Suzuki H, Ohta Y, Takino T, et al. Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis: double blind trial. *Asian Med J*. 1984;26:423–438.
118. Mori K, Sakai H, Suzuki S, et al. Effects of glycyrrhizin (SNMC. Stronger Neo-Minophagen C) in hemophilia patients with HIV-1 infection. *Tohoku J Exp Med*. 1990;162:183–193.
119. Eisenburg J. Treatment of chronic hepatitis B. Part 2: effect of glycyrrhizinic acid on the course of illness. *Fortschr Med*. 1992;110:395–398.
120. Acharya SK, Dasarathy S, Tandon A, et al. A preliminary open trial on interferon stimulator (SNMC) derived from Glycyrrhiza glabra in the treatment of subacute hepatic failure. *Indian J Med Res*. 1993;98:69–74.
121. Arase Y, Ikeda K, Murashima N, et al. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer*. 1997;79:1494–1500.
122. Fiore C, Eisenhut M, Krausse R, et al. Antiviral effects of Glycyrrhiza species. *Phytother Res*. 2008;22(2):141–148.
123. van Rossum TG, Vulto AG, Hop WC, et al. Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis C. *Am J Gastroenterol*. 2001;96(8):2432–2437.
124. Arase Y, Ikeda K, Murashima N, et al. The superiority of laparoscopic examination in predicting hepatocellular carcinoma after interferon therapy for chronic type C hepatitis. *Dig Endosc J Pharmacol*. 1997;10:613–620.
125. Veldt BJ, Hansen BE, Ikeda K, et al. Long-term clinical outcome and effect of glycyrrhizin in 1093 chronic hepatitis C patients with non-response or relapse to interferon. *Scand J Gastroenterol*. 2006;41(9):1087–1094.
126. Ikeda K, Arase Y, Kobayashi M, et al. A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1249 patients. *Dig Dis Sci*. 2006;51(3):603–609.
127. Orlent H, Hansen BE, Willems M, et al. Biochemical and histological effects of 26 weeks of glycyrrhizin treatment in chronic hepatitis C: a randomized phase II trial. *J Hepatol*. 2006;45(4):539–546. Epub 2006 Jun 30.
128. Post-White J, Ladas EJ, Kelly KM. Advances in the use of milk thistle (Silybum marianum). *Integr Cancer Ther*. 2007;6(2):104–109.
129. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, et al. Hepatoprotective effect of silymarin. *World J Hepatol*. 2014;6(3):144–149.
130. Rainone F. Milk thistle. *Am Fam Physician*. 2005;72(7):1285–1292.
131. Deak G, Muzes G, Lang I, et al. Immunomodulator effect of silymarin therapy in chronic alcoholic liver diseases. *Orv Hetil*. 1990;131:1291–1292. 1295-1296.
132. Tanamly MD, Tadros F, Labeeb S, et al. Randomised double-blinded trial evaluating silymarin for chronic hepatitis C in an Egyptian village: study description and 12-month results. *Dig Liver Dis*. 2004;36:752–759.
133. Gabbay E, Zigmond E, Pappo O, et al. Antioxidant therapy for chronic hepatitis C after failure of interferon: results of phase II randomized, double-blind placebo controlled clinical trial. *World J Gastroenterol*. 2007;13:5317–5323.
134. Torres M, Rodriguez-Serrano F, Rosario DJ, Rodriguez-Perez F, Toro DH. Does silybum marianum play a role in the treatment of chronic hepatitis C? *PRHSJ*. 2004;23(2):69–74.
135. Schandalik R, Gatti G, Perucca E. Pharmacokinetics of silybin in bile following administration of silipide and silymarin in cholecystectomy patients. *Arzneimittelforschung*. 1992;42:964–968.
136. Barzaghi N, Crema F, Gatti G, et al. Pharmacokinetic studies on IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. *Eur J Drug Metab Pharmacokinet*. 1990;15:333–338.
137. Vailati A, Aristia L, Sozze E, et al. Randomized open study of the dose-effect relationship of a short course of IdB 1016 in patients with viral or alcoholic hepatitis. *Fitoterapia*. 1993;44:219–228.
138. Moscarella S, Giusti A, Marra F, et al. Therapeutic and antilipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease: preliminary results. *Curr Ther Res*. 1993;53:98–102.
139. Buzzelli G, Moscarella S, Giusti A, et al. A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB 1016) in chronic active hepatitis. *Int J Clin Pharmacol Ther Toxicol*. 1993;31:456–460.
140. Marena C, Lampertico M. Preliminary clinical development of silipide: a new complex of silybin in toxic liver disorders. *Planta Med*. 1991;57(suppl 2):A124–A125.
141. Ferenci P, Scherzer TM, Kerschner H, et al. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated

interferon/ribavirin therapy. *Gastroenterology*. 2008;135(5):1561–1567. Epub 2008 Aug 3.
142. Neumann UP, Biermer M, Eurich D, et al. Successful prevention of hepatitis C virus (HCV) liver graft reinfection by silibinin mono-therapy. *J Hepatol*. 2010;52(6):951–952. Epub 2010 Mar 15.
143. Ravikumar YS, Ray U, Nandhitha M, et al. Inhibition of hepatitis C virus replication by herbal extract: *Phyllanthus amarus* as potent natural source. *Virus Res*. 2011;158(1-2):89–97.
144. Thyagarajan SP, Subramanian S, Thirunalasundari T, et al. Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. *Lancet*. 1988;2:764–766.
145. Leelarasamee A, Trakulsomboon S, Maunwongyathi P, et al. Failure of *Phyllanthus amarus* to eradicate hepatitis B surface antigen from symptomless carriers. *Lancet*. 1990;335:1600–1601.
146. Blumberg BS, Millman I, Venkateswaran PS, et al. Hepatitis B virus and primary hepatocellular carcinoma: treatment of HBV carriers with *Phyllanthus amarus*. *Vaccine*. 1990;8:S86–S92.
147. Berk L, de Man RA, Schalm SW, et al. Beneficial effects of *Phyllanthus amarus* for chronic hepatitis B, not confirmed. *J Hepatol*. 1991;12: 405–406.
148. Milne A, Hopkirk N, Lucas CR, et al. Failure of New Zealand hepatitis B carriers to respond to *Phyllanthus amarus*. *N Z Med J*. 1994;107:243.
149. Yamamoto Y, Yamashita S, Fujisawa A, Kokura S, Yoshikawa T. Oxidative stress in patients with hepatitis, cirrhosis, and hepatoma evaluated by plasma antioxidants. *Biochem Biophys Res Commun*. 1998;247(1):166–170.
150. Calland N, et al. Polyphenols inhibit hepatitis C virus entry by a new mechanism of action. *J Virol*. 2015;89(19):10053–10063.
151. Berkson BM. A conservative triple antioxidant approach to the treatment of hepatitis C: combination of alpha lipoic acid (thioctic acid), silymarin, and selenium: three case histories. *Med Klin (Munich)*. 1999;94(suppl 3):84–89.
152. Melhem A, et al. Treatment of chronic hepatitis C virus infection via antioxidants: results of a Phase I clinical trial. *J Clin Gastroenterol*. 2005;39(8):737–742.
153. Neumann UP, Biermer M, Eurich D, et al. Successful prevention of hepatitis C virus (HCV) liver graft reinfection by silibinin mono-therapy. *J Hepatol*. 2010;52(6):951–952. Epub 2010 Mar 15.
154. Zhao S, et al. Interferon plus Chinese herbs are associated with higher sustained virological response than interferon alone in chronic Hepatitis C: a meta-analysis of randomised trials. *Antiviral Res*. 2011;89(2):156–164.
155. White A. A cumulative review of the range and incidence of significant adverse events associated with acupuncture. *Acupunct Med*. 2004;22(3):122–133.
156. Bensky D, Gamble A. *Chinese Herbal Medicine: Materia Medica*. Revised Ed. Seattle: Eastland Press.
157. Shimizu I. Sho-saiko-to: japanese herbal medicine for protection against hepatic fibrosis and carcinoma. *J Gastroenterol Hepatol*. 2000;15(suppl):D84–D90.
158. Sakaida I, et al. Herbal medicine Sho-saiko-to (TJ-9) prevents liver fibrosis and enzyme-altered lesions in rat liver cirrhosis induced by a choline-deficient L-amino acid-defined diet. *J Hepatol*. 1998;28:298–306.
159. Yamashiki M, et al. Herbal medicine 'Sho-saiko-to' induces tumour necrosis factor-alpha and granulocyte colony-stimulating factor in vitro in peripheral blood mononuclear cells of patients with hepatocellular carcinoma. *J Gastroenterol Hepatol*. 1996;11:137–142.
160. Oka H, Yamamoto S, Kuroki T, et al. Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). *Cancer*. 1995;76(5):743–749.
161. Lee JK, Kim JH, Shin HK. Therapeutic effects of the oriental herbal medicine Sho-saiko-to on liver cirrhosis and carcinoma. *Hepatol Res*. 2011;41(9):825–837.
162. Qi F, Wang Z, et al. Traditional Chinese Medicine and related active compounds: a review of their role on hepatitis B virus infection. *Drug Discov Ther*. 2013;7(6):212–224.
163. Deng G, Kurtz RC, Vickers A, et al. A single arm phase II study of a Far-Eastern traditional herbal formulation (sho-sai-ko-to or xiao-chai-hu-tang) in chronic hepatitis C patients. *J Ethnopharmacol*. 2011;136(1):83–87. https://doi.org/10.1016/j.jep.2011.04.008.
164. Mizushima Y, Oosaki R, Kobayashi M. Clinical features of pneumonitis induced by herbal drugs. *Phytother Res*. 1997;11:295–298.
165. Hsu LM, Huang YS, Tsay SH, et al. Acute hepatitis induced by Chinese hepatoprotective herb, xiao-chai-hu-tang. *J Chin Med Assoc*. 2006;69(2):86–88.
166. Stickel F, Kessebohm K, Weimann R, Seitz HK. Review of liver injury associated with dietary supplements. *Liver Int*. 2011;31(5).
167. Coppola A, Brady PG, Nord HJ. Niacin-induced hepatotoxicity: unusual presentations. *South Med J*. 1994;87(1):30–32.
168. Figge HL, Figge J, Souney PF, et al. Comparison of excretion of nicotinuric acid after ingestion of two controlled release nicotinic acid preparations in man. *J Clin Pharmacol*. 1988;28:1136–1140.
169. Dalton TA, et al. Hepatotoxicity associated with sustained-release niacin. *Am J Med*. 1992;93(1):102–104.
170. National Institutes of Health. Niacin. (n.d.). Retrieved from https://livertox.nih.gov/Niacin.htm on December 5, 2018.
171. Cervantes-Laurean D, McElvaney NG, Moss J. Niacin. In: Shils ME, Olson JA, Shike M, Ross AC, eds. *Modern Nutrition in Health and Disease*. Baltimore: Williams and Wilkins: 401–411.
172. Inkeles SB, Connor WE, Illingworth DR. Hepatic and dermatologic manifestations of chronic hypervitaminosis A in adults. report of two cases. *Am J Med*. 1986;80(3):491–496.
173. Pietrangelo A. Mechanisms of iron hepatotoxicity. *J Hepatol*. 2016;65(1):226–227.
174. Stickel F, Seitz HK. The efficacy and safety of comfrey. *Public Health Nutr*. 2000;3(4A):501–508.
175. Moulds RF, Malani J. Kava: herbal panacea or liver poison? *Med J Aust*. 2003;178:451–453.
176. Teschke R. Kava, kavapyrones and toxic liver injury. *Z Gastroenterol*. 2003;41:395–404.
177. Licata A, Macaluso FS, Craxi A. Herbal hepatotoxicity: a hidden epidemic. *Intern Emerg Med*. 2013;8(1):13–22. https://doi.org/10.1007/s11739-012-0777-x.
178. Stournaras E, Tziomalos K. Herbal medicine-related hepatotoxicity. *World J Hepatol*. 2015;7(19):2189–2193.
179. Rusu E, et al. Effects of lifestyle changes including specific dietary intervention and physical activity in the management of patients with chronic hepatitis C - a randomized trial. *Nutri J*. 2013;12(119):1–12.
180. Nobili V, Carter-Kent C, Feldstein AE. The role of lifestyle changes in the management of chronic liver disease. *BMC Med*. 2011;9(70):1–7.
181. Vajro P, Paolella G, Fasano A. Microbiota and gut-liver axis: their influences on obesity and obesity-related liver disease. *J Pediatr Gastroenterol Nutr*. 2013;56(5):461–468.
182. Ginde AA, Liu MC, Camargo Jr CA. Demographic differences and trends of vitamin D insufficiency in the U.S. population, 1988-2004. *Arch Intern Med*. 2009;169(6):626–632.
183. Yarnell E AK. Herbal medicine for viral hepatitis. *Altern Complement Ther*. 2010;16(3):151–157.

177

Herpes Simplex

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Acute or recurrent viral infection of the skin or mucous membranes characterized by the appearance of grouped vesicles on an erythematous base, frequently occurring about the mouth (herpes gingivostomatitis), lips (herpes labialis), genitals (herpes genitalis), and conjunctiva and cornea (herpes keratoconjunctivitis)
- Incubation period of 2 to 12 days, average of 6 to 7
- Positive culture or polymerase chain reaction (PCR), with the latter being more sensitive
- Regional lymph nodes sometimes tender and swollen
- Outbreak may follow minor infections, trauma, hormonal fluctuations, stress (emotional, dietary, and environmental), and sun exposure
- Viral shedding, leading to possible transmission, during primary infection, recurrences, and (asymptomatically) between recurrences (Fig. 177.1)

GENERAL CONSIDERATIONS

More than 70 viruses compose the *Herpesviradae family*. Of these, four are important in human disease: herpes simplex virus (HSV), varicella zoster virus, Epstein–Barr virus, and cytomegalovirus. Serological methods have distinguished two types of HSV, which have been designated HSV-1 and HSV-2. HSV-1 is frequently acquired in early childhood. More than one third of the world's population has recurrent HSV. Although 80% of seropositive individuals do not have clinically apparent recurrences, the virus is still shed asymptomatically. The prevalence of HSV-1 infection has declined steadily from 60% when first measured between 1979 and 1980. The most pronounced decline was among 14- to 19-year-olds, in whom the prevalence fell from 39% to 30% ($P < 0.01$) from 1999 to 2004 and from 2005 to 2010, respectively. HSV-2 prevalence also declined from 21% between 1988 and 1994 to 16% between 2007 and 2010. However, racial disparities in HSV-2 prevalence have widened, as this decline did not occur among black individuals. Thus rates of HSV-2 infection were highest among black women (50%), followed by black men (32%), white women (15%), and white men (7%). These differences were found to exist at every level of sexual activity and thus do not reflect individual behaviors. In all, 87% of HSV-2 seropositive persons did not know they had genital herpes.[1]

Previously, HSV-1 was primarily isolated from extragenital sites, whereas genital infections were caused primarily by HSV-2. By the year 2000, however, HSV-1 had replaced HSV-2 as the primary cause of genital lesions, likely due to orogenital contact.[2] A retrospective review of genital HSV isolates collected in a university student health service showed that HSV-1 accounted for 78% of all genital isolates in this population by 2001, compared with 31% of isolates in 1993.[3] Individuals who are exposed to HSV and have asymptomatic primary infections may experience an initial clinical episode of genital herpes months to years after becoming infected.

Herpes outbreaks on the hands and fingers (herpes whitlow) is increasingly recognized, probably as result of digital–genital contact.

Recurrence Rate

After the resolution of the primary infection, HSV probably becomes a dormant inhabitant within sensory or autonomic trigeminal or lumbosacral ganglia or both (Fig. 177.2). Recurrences develop at or near the sites of primary infection and may be precipitated by many different stimuli:

- Sunburn
- Sexual activity
- Menses
- Stress
- Food allergy
- Drugs
- Certain foods

Patients with primary genital HSV need to be counseled that recurrence is expected. The frequency of recurrence depends on the severity and duration of the initial episode, the infecting serotype, and the host. In one series of 457 patients with HSV-2 primary infection, 89% had one recurrence during a follow-up of 391 days.[4] Furthermore, 38% of patients had as many as 6 recurrences, and 20% had more than 10. Recurrent infection is more common with HSV-2 than HSV-1. The magnitude of this effect was evaluated in a prospective study of 137 patients with a first symptomatic episode of genital herpes. The likelihood of recurrence was much higher with HSV-2 infections (60% vs. 14% with HSV-1).[5]

Herpes viruses: life cycle

- Virus can bind several host receptors
  - Envelope fuses with plasma membrane
- Capsid moves to nucleus
  - Uses host polymerse to replicate
- Lytic infection
  - mRNA moves to cytoplasm
  - Proteins built, assembled
  - Virus exocytosed from plasma membrane

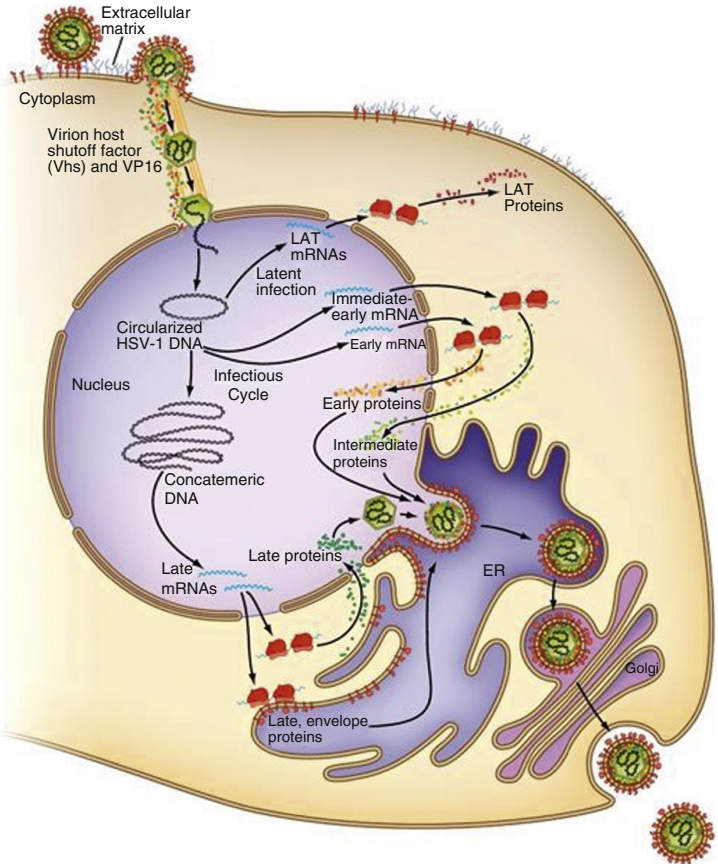


Fig. 177.1 Life Cycle of Herpes Simplex Virus.

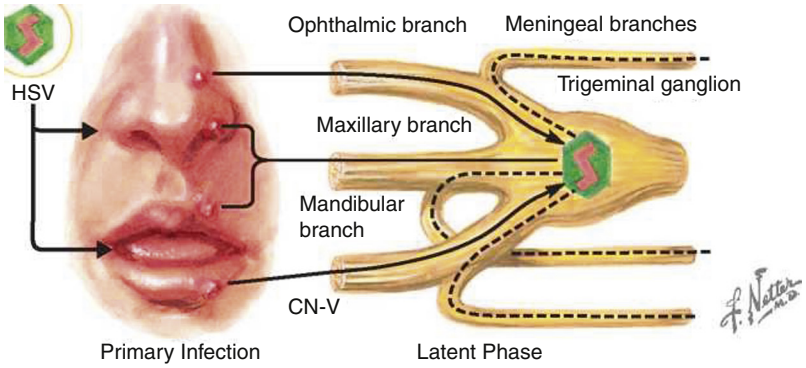


Fig. 177.2 Herpes Simplex Virus in Trigeminal Ganglion.

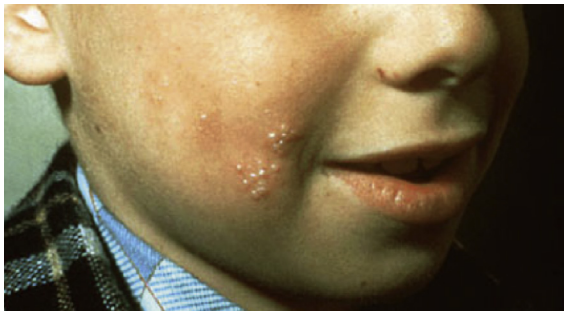
Immunological Aspects

Because not everyone exposed to HSV develops clinical infection, it appears that host defense mechanisms are paramount in protecting against infection. Chronic, persistent labial and genital infections are seen in immunosuppressed individuals. The cell-mediated immune system is undoubtedly the major factor in determining the outcome of herpes exposure—that is, whether it leads to resistance, latent infection, or clinical disease.

DIAGNOSIS

Isolation of HSV from tissue scrapings is the gold standard for diagnosis. The availability of type-specific serology using surface glycoproteins (gG2 and gG1 for HSV-2 and HSV-1, respectively) to distinguish HSV-1 and HSV-2 enables the clinician to determine whether the patient is at risk of acquisition or has evidence of prior infection with either subtype.[5] A positive serology indicates present or past infection. Positive serological testing for HSV-2 indicates anogenital infection,

but positive serological testing for HSV-1 can be consistent with either anogenital or orolabial infection.[6]



THERAPEUTIC CONSIDERATIONS

Prevention of recurrent herpes is recommended to decrease the number and severity of outbreaks, reduce asymptomatic viral shedding, and prevent transmission to a partner and/or fetus. Three or more people may benefit when one person takes preventive measures. Education regarding transmission and asymptomatic viral shedding is imperative.

Enhancement of the host's immunological status is a key goal in the control of herpes infection. In addition to general immune support (for a complete discussion, see Chapter 136), one of the key natural measures to strengthen cell-mediated immunity is the use of polypeptide-rich bovine thymus extracts. These have been shown to be effective in preventing both the number and severity of recurrent infections in immune-suppressed individuals.[7] Thymus extract appears to increase the lymphoproliferative response to HSV, natural killer cell activity, and interferon production, thus preventing viral activation by potentiating these cell-mediated immune responses. To date, no clinical trials have been published on thymus extract and the prevention or treatment of HSV infections.

Nutritional Supplements

Zinc

Oral supplementation with zinc (50 mg/day) has been shown to be effective in laboratory studies.[8] Although zinc is an effective inhibitor of HSV replication in vitro, its effect in vivo is probably related to its role in enhancing cell-mediated immunity. The topical application of 0.01% to 0.025% zinc sulfate solutions has also been shown to be effective in both ameliorating symptoms and inhibiting recurrences of HSV infection.[9]

Vitamin C

Both the oral consumption and topical application of vitamin C increase the rate of healing of herpetic ulcers. In a randomized, double-blind study, an ascorbic acid–containing pharmaceutical formulation (Ascoxal) applied with a soaked cotton-wool pad three times daily for 2 minutes resulted in patients reporting fewer days with scabs and fewer cases of worsening of symptoms. Cultures yielded herpes complex viruses significantly less frequently in the treatment group.[10] In another study, 20 patients with herpes labialis were treated with a complex of 600 mg of water-soluble bioflavonoids and 600 mg of vitamin C given orally in equal increments three times daily. Twenty episodes of herpes labialis were treated with a complex of 1000 mg of water-soluble bioflavonoids and 1000 mg of vitamin C in equal increments five times daily. Ten episodes were treated with a lactose preparation. This approach was maintained for 3 days after the recognition of symptoms. The water-soluble bioflavonoid–vitamin C complex was shown to reduce vesiculation and to prevent disruption of the vesicular membrane. The therapy was most beneficial when it was initiated at the beginning of the disease. Those treated with the 1000-mg regimen saw their blisters heal in 4.4 days, compared with 10 days for the placebo group. Optimal remission of symptoms was observed in 4.2 ± 1.7 days with the 600-mg dosage of the water-soluble bioflavonoid/ascorbic acid complex.[11] Vitamin C has also been employed intravenously with benefit in the treatment of patients with HSV infection, including patients with AIDS.[12]

Lysine and Arginine

A lysine-rich/arginine-poor diet has become a popular treatment for HSV infections. This approach came from research showing that lysine has antiviral activity in vitro because of its antagonism of arginine metabolism.[13] HSV replication requires the synthesis of arginine-rich proteins, and arginine itself is suggested to be an operon coordinate inducer.[14] A preponderance of lysine over arginine is believed to act as either an allosteric enzyme inhibitor or an operon coordinate repressor.

Double-blind studies on the effectiveness of lysine supplementation with uncontrolled avoidance of arginine-rich foods have shown inconsistent results.[14–17] These outcomes may be due to the relatively low levels of lysine used (1200 mg/day) and the severity of the cases in some of the studies (placebo and treated groups had lesions for 40% of the time in one negative study).[14,15] In one study, lysine was given at a larger dosage (1 g three times daily) along with the dietary restriction of nuts, chocolate, and gelatin.[16] At 6 months, lysine was rated as effective or very effective by 74% of those receiving lysine compared with only 28% of those receiving the placebo. The mean number of outbreaks was 3.1 in the lysine group compared with 4.2 in the placebo group.

Theoretically, this approach should be effective because in vitro studies have shown that HSV replication depends on adequate levels of arginine and low levels of lysine.[17] As dibasic amino acids, they compete with each other for intestinal transport, and rats fed a lysine-rich diet displayed a 60% decrease in brain arginine levels, although there was no change in serum levels.[12] Because HSV is believed to reside in the ganglia during latency, lysine supplementation seems appropriate. In some patients, withdrawal from lysine is followed by relapse within 1 to 4 weeks.[17] However, there are no published data to support the avoidance of arginine-rich foods to prevent or treat HSV. In fact, in one study, the opposite was shown to be true: arginine inactivated HSV-2 and inhibited genital HSV infection in mice.[18]

Topical Preparations

Melissa officinalis

One of the most widely used topical preparations in the treatment and prevention of herpes outbreaks is a concentrated extract (70:1) of *Melissa officinalis* (lemon balm). Rather than a single antiviral chemical, *Melissa* contains several components that work together to prevent the virus from infecting human cells. When *Melissa* cream was used in patients with an initial herpes infection, results from comprehensive trials in three German hospitals and a dermatology clinic demonstrated that there was not a single recurrence.[19] In other words, by using the cream, not a single patient with a first herpes outbreak developed another cold sore.

Furthermore, it was noted in these studies that the *Melissa* cream produced an interruption of the infection and promoted healing of the herpes blisters much faster than normal. The control group receiving other topical creams had a healing period of 10 days, whereas the group receiving the *Melissa* cream healed completely within 5 days.

The *Melissa* cream was also studied in patients suffering from recurrent cold sores. Researchers found that if subjects used this cream regularly, they would either stop having recurrences or experience a tremendous reduction in the frequency of recurrences (an average cold sore–free period of >3.5 months).[20]

The *Melissa* cream should be applied to the lips two to four times daily during an active recurrence.[20] It can be applied fairly thickly (1–2 mm). Detailed toxicology studies have demonstrated that it is extremely safe and suitable for long-term use. Extracts of other species of the *Lamiaceae* family have also shown in vitro efficacy against the adsorption, but not replication, of HSV-1 and HSV-2, including *Mentha piperita, Prunella vulgaris, Rosmarinus officialis, Salvia officinalis,* and *Thymus vulgaris.*[21]

Salvia officinalis

A combination of topical *Salvia officinalis* extract and rhubarb root extract proved to be as effective as topical acyclovir cream in a German double-blind, comparative, randomized trial of 149 patients with herpes labialis, and the herbal combination was more effective in relieving HSV-associated pain.[22]

Glycyrrhiza glabra

Another popular ingredient for the topical treatment and prevention of herpes outbreaks is glycyrrhetinic acid. This triterpenoid component of *Glycyrrhiza glabra* (licorice root) inhibits both the growth and cytopathic effects of herpes simplex as well as vaccinia, Newcastle disease, and vesicular stomatitis viruses.[23] Topical glycyrrhetinic acid has been shown in clinical studies to be helpful in reducing the healing time and pain associated with cold sores and genital herpes.[24–26]

Resveratrol

Resveratrol (3,5,4′-trihydroxy-transstilbene) is a natural component of certain foods such as grape skins; it has been shown to have anti-HSV activity in vitro. Intriguing research in mice has shown that the topical application of a cream rich in resveratrol (12.5%–25%) effectively blocked replication of the virus and stopped lesion eruption if applied early and frequently.[5,27] No human studies have been reported; however, subsequent in vitro studies have yielded data indicating that resveratrol suppresses HSV-induced activation of nuclear factor (NF)-kappaB within the nucleus and impairs the expression of essential immediate-early, early, and late HSV genes and the synthesis of viral DNA.[28]

Vitamin E and Other Antioxidants

In two uncontrolled trials, topical vitamin E decreased pain and healing time in oral herpes. Lesions responded best when the content of a vitamin E capsule was applied every 4 hours.[29] In a clinical trial, a combination of oral acyclovir with alpha-tocopherol, selenium, L-methionine, and coenzyme Q_{10} induced significantly faster healing with significantly reduced incidence of relapse compared to the control group that received acyclovir alone, confirmed by decreased viral load and increased antiviral cytokine and peroxynitrite plasma levels. Plasma antioxidant capacity was also significantly higher in the combination group.[30]

THERAPEUTIC APPROACH

The therapeutic aim is to shorten the current attack and prevent recurrences. Support of the immune system is of primary importance, necessitating control of food allergens and the optimization of nutrients necessary for cell-mediated immunity. Inhibition of HSV replication through lysine supplementation may be useful. Strengthening the immune system can be effective in reducing the frequency, duration, and severity of recurrences.

Diet

A diet that avoids major food allergens and is rich in antioxidants is recommended.

Supplements

- Vitamin C: 2000 mg/day
- Bioflavonoids: 1000 mg/day
- Zinc: 25 mg/day
- Lysine: 1000 mg three times daily
- Vitamin E 400 iu, selenium 200 mcg, coenzyme Q_{10} (ubiquinol) 100 mg

Topical Treatments

- Sunblock
- Ice: 10 minutes on, 5 minutes off during prodrome
- Zinc sulfate solution: 0.025% solution applied three times a day
- *Melissa* cream: applied twice a day
- Glycyrrhetinic acid: applied twice a day
- Vitamin E: applied every 4 hours

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lafferty WE. The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. *Herpes*. 2002;9:51–55.
2. Roberts CM, Pfister JR, Spear SJ. Increasing proportion of herpes simplex virus type 1 as a cause of genital herpes infection in college students. *Sex Transm Dis*. 2003;30:797–800.
3. Benedetti J, Corey L, Ashley R. Recurrence rates in genital herpes after symptomatic first-episode infection. *Ann Intern Med*. 1994;121(11):847.
4. Reeves WC, Corey L, Adams HG, et al. Risk of recurrence after first episodes of genital herpes. Relation to HSV type and antibody response. *N Engl J Med*. 1981;305(6):315.
5. Docherty JJ, Smith JS, Fu MM, et al. Effect of topically applied resveratrol on cutaneous herpes simplex virus infections in hairless mice. *Antiviral Res*. 2004;61:19–26.
6. Whittington WL, Celum CL, Cent A, Ashley RL. Use of a glycoprotein G-based type-specific assay to detect antibodies to herpes simplex virus type among persons attending sexually transmitted disease clinics. *Sex Transm Dis*. 2001;28(2):99.
7. Aiuti F, Sirianni M, Stella A, et al. A placebo-controlled trial of thymic hormone treatment of recurrent herpes simplex labialis infection in immunodeficient hosts. *Int J Clin Pharm Ther Toxicol*. 1983;21:81–86.
8. Fitzherbert J. Genital herpes and zinc. *Med J Aust*. 1979;1:399.
9. Brody I. Topical treatment of recurrent herpes simplex and post-herpetic erythema multiforme with low concentrations of zinc sulphate solution. *Br J Dermatol*. 1981;104:191–213.
10. Hovi T, Hirvimies A, Stenvik M, et al. Topical treatment of recurrent mucocutaneous herpes with ascorbic acid-containing solution. *Antiviral Res*. 1995;27:263–270.
11. Terezhalmy Gt, Bottomley WK, Pelleu GB. The use of water-soluble bioflavinoid-ascorbic acid complex in the treatment of recurrent herpes labialis. *Oral Surg Oral Med Oral Pathol*. 1978;45(1):56–62.
12. Cathcart RF. Vitamin C in the treatment of acquired immune deficiency syndrome (AIDS). *Med Hypotheses*. 1984;14:423–433.
13. Griffith R, DeLong DC, Nelson JD. Relation of arginine-lysine antagonism to herpes simplex growth in tissue culture. *Chemotherapy*. 1981;27:209–213.
14. DiGiovanna JJ, Blank H. Failure of lysine in frequently recurrent herpes simplex infection. *Arch Dermatol*. 1984;120:48–51.
15. Griffith R, Norins A, Kagan C. A multicentered study of lysine therapy in herpes simplex infection. *Dermatologica*. 1978;156:257–267.
16. McCune MA, Perry HO, Muller SA. Treatment of recurrent herpes simplex infections with L-lysine monohydrochlorite. *Cutis*. 1984;34:366–373.
17. Griffith RS, Walsh DE, Myrmel KH, et al. Success of L-lysine therapy in frequently recurrent herpes simplex infection. *Dermatologica*. 1987;175:183–190.
18. Ikeda K, Yamasaki H, Minami S, et al. Arginine inactivates human herpesvirus 2 and inhibits genital herpesvirus infection. *Int J Mol Med*. 2012;30(6):1307–1312.
19. Wolbling RH, Leonhardt K. Local therapy of herpes simplex with dried extract from Melissa officinalis. *Phytomedicine*. 1994;1:25–31.
20. Koytchev R, Alken RG, Dundarov S. Balm mint extract (Lo-701) for topical treatment of recurring herpes labialis. *Phytomedicine*. 1999;6:225–230.
21. Nolkemper S, Reichling J, Stintzing FC, et al. Antiviral effect of aqueous extracts from species of the Lamiaceae family against herpes simplex virus type 1 and type 2 in vitro. *Planta Med*. 2006;72(15):1378–1382.
22. Saller R, Büechi S, Meyrat R, Schmidhauser C. Combined herbal preparation for topical treatment of herpes labialis. *Forsch Komplementarmed Klass Naturheilkd*. 2001;8(6):373–382.
23. Pompei R, Pani A, Flore O, et al. Antiviral activity of glycyrrhizic acid. *Experientia*. 1980;36:304.
24. Partridge M, Poswillo D. Topical carbenoxolone sodium in the management of herpes simplex infection. *Br J Oral Maxillofac Surg*. 1984;22:138–145.
25. Csonka G, Tyrrell D. Treatment of herpes genitalis with carbenoxolone and cicloxolone creams: a double blind placebo controlled trial. *Br J Vener Dis*. 1984;60:178–181.
26. Ikeda T, Yokomizo K, Okawa M, et al. Anti-herpes virus type 1 activity of oleanane-type tripterpenoids. *Biol Pharm Bull*. 2005;28(9):1779–1781.
27. Faith SA, Sweet TJ, Bailey E, et al. Resveratrol suppresses nuclear factor-kappa B in herpes simplex virus infected cells. *Antiviral Res*. 2006;72(3):242–251.
28. Fink M, Fink J. Treatment of herpes simplex by alpha-tocopherol (vitamin E). *Br Dent J*. 1980;148:246.
29. Faith SA, Sweet TJ, Bailey E, et al. Resveratrol suppresses nuclear factor-kappa B in herpes simplex virus infected cells. *Antiviral Res*. 72:242–251.
30. De Luca C, Kharaeva Z, Raskovic D, et al. Coenzyme Q(10), vitamin E, selenium, and methionine in the treatment of chronic recurrent viral mucocutaneous infections. *Nutrition*. 2012;28(5):509–514.

178

HIV/AIDS: Naturopathic Medical Principles and Practice

Steven C. Milkis, ND, and *Kevin L. Conroy, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- A diagnosis of human immunodeficiency virus–positive (HIV+) infection is most commonly made after a positive HIV 1/0/2 antigen/antibody (fourth-generation) test to identify HIV antibodies by enzyme-linked immunosorbent assay (ELISA) and/or HIV-1 p24 antigen. A positive result is reflexed to distinguish between HIV-1 and HIV-2 infection.
- Primary HIV infection (acute retroviral syndrome) resembles common influenza. Most persons experience this syndrome 2 to 6 weeks after initial inoculation with the virus; it often goes unnoticed and undiagnosed as HIV owing to its similarity to the flu. Signs and symptoms can include fever, lymphadenopathy, skin rash, pharyngitis, myalgia, arthralgia, headache, diarrhea, and oral ulcerations. Laboratory findings might include leukopenia, thrombocytopenia, and elevated transaminases. Primary infection is also characterized by a high level of virus production, high concentrations of viral particles and RNA in plasma, and a rapid and steep decline in CD4+ T-helper cells. Peak viral titers can reach 10^7 virions per milliliter during this phase. Viral production can range up to 10 billion copies per day. After the initial viremia, high levels of viral p24 antigen appear.
- An insidious onset of HIV infection may manifest as an acquired immunodeficiency syndrome (AIDS); associated opportunistic infection (OI); or unexplained progressive fatigue, weight loss, fever, diarrhea, or generalized lymphadenopathy. It is often something that is discovered after other possible causes for illness are ruled out, resulting in delayed diagnosis.
- AIDS, for an individual aged 6 or older, is diagnosed after positive serology and either a CD4+ T-cell count below 200/mm^3 or the presence of a designated AIDS-indicator condition (from the Centers for Disease Control and Prevention [CDC] guidelines of 2014).
- The lower the CD4+ count and the higher the viral load, the higher the risk of contracting OIs, neoplasms, or neurological abnormalities, and the higher the mortality rate.
- Groups at high risk for contracting HIV include injection drug users who share needles, homosexual and bisexual men, hemophiliacs and others receiving transfused blood or other blood products (highest risk to recipients before May 1985, when regular screening of the blood supply began), regular sex partners of people in the aforementioned groups, heterosexual individuals with greater than one sex partner in the past 12 months, and those who have had unprotected sex during the previous 6 months.

INTRODUCTION

HIV has been described as a retrovirus containing two identical single strands of RNA. It enters the body via the transfer of body fluids and subsequently binds to and infects CD4-expressing cells of the human immune system (T-helper lymphocytes, blood monocytes, tissue macrophages, Langerhans cells in the skin, and microglial and multinucleated cells in the central nervous system). AIDS is a secondary syndrome typically resulting from long-term HIV infection. AIDS is defined by the following: levels of CD4+ cells or the presence of certain specific indicator conditions or secondary malignancies known to be associated with HIV. Many theories exist as to what causes AIDS, ranging from HIV itself to multifactorial (environmental and infectious) assaults on the immune system. In this science's infancy, there is no definitive answer, but current medical thinking is that AIDS is caused by HIV.

Focus and Goals of Chapter

This chapter is written for the naturopathic physician, although conventionally trained physicians and other holistic practitioners may find

the perspective, treatment principles, and some of the treatment suggestions helpful. The field of HIV/AIDS medicine is rapidly changing. HIV/AIDS was only first reported to the CDC in 1981, and it has since exploded into an international pandemic. Highly active antiretroviral drug therapy (HAART), introduced in 1996, is the use of multiple drugs in combination, with the goal of decreasing viral load and increasing CD4+ T-cell counts, thereby prolonging life and preventing the onset of complicating conditions related to the immune system decline. Although this innovation resulted in a dramatic slowing in the death rate from AIDS, with it came complicated drug side effects, drug interactions, and unique symptoms that require frequent follow-up between the patient and physician and affect the quality of life of individuals on those regimens. These complications from HIV treatment have become less and less common over the past several years with the advent of new, safer, and more effective medications. In addition, changing treatment guidelines that include starting treatment on diagnosis (vs. the older guideline of waiting until the CD4+ count drops to a low level) have resulted in patients being in a much healthier state and making actual AIDS diagnoses less and less common.

Studies indicate that more than 79% of HIV+ persons are using some form of complementary/alternative medicine (CAM) in their treatment, resulting in improvements in their quality of life and better outcomes.[1] The goal of this chapter is to help the physician better understand the condition and to appropriately guide this affected population with unique and complicated health issues.

BIOLOGY OF HIV

Two types of HIV have been identified. HIV-1 is found throughout the world. The majority of infections in the United States are caused by HIV-1. HIV-2 was first identified in West Africa in 1986 and has been found to have a genetic sequence approximately 50% similar to HIV-1. HIV-2 is considered less virulent than HIV-1 and shows lower rates of sexual and perinatal transmission and a lower viral load with a slower rate of CD4 cell decline. This results in a slower rate of disease progression in infected people.[2] Research shows that the virus is mutating, and multiple subtypes or clades are spreading throughout the world.[1] The existence of multiple clades raises the theoretical possibility that a single individual could become infected with multiple subtypes of the virus. Research has not conclusively demonstrated the existence of a "superinfection," but the possibility of this phenomenon would significantly enhance virulence as well as increase resistance to present treatments.

Existing research has failed to isolate an intact HIV entity, although fragments believed to belong to the HIV virus have been isolated and identified. Despite this shortcoming, the virus is believed to consist of a bilayer lipid envelope covered with envelope glycoproteins (gp120 receptor and gp41 transmembrane), a viral core containing two single strands of RNA and the reverse transcriptase enzyme (needed to translate the viral RNA into DNA that can affect human cells), and structural core proteins (p24 capsid protein and p18 matrix protein). It is believed that HIV binds to CD4-expressing cells of the immune system and, through a series of steps, is brought into the cell, replicates itself, and incorporates its genome into the host cell's genome.

The various classes of antiretroviral medications have been developed to interrupt viral replication during various steps in this process. The key to understanding the mechanisms of action of the various medications is to understand those steps.

Fusion is the process by which the HIV particle contacts and binds to the target cell. This occurs through the interaction of the viral gp120 and gp41 transmembrane proteins with CD4 and chemokine receptors on the cell's membrane. Entry inhibitors are designed to inhibit this process by altering the cellular receptor sites or the process that takes the virion into the cell.

Transcription occurs when the viral reverse transcriptase enzyme translates the viral RNA into a double strand of DNA. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) are designed to disrupt this process.

Integration occurs when the double strand of proviral DNA migrates to the cell's nucleus and is inserted into the host's chromosome via the integrase enzyme. The provirus is then permanently integrated into the host DNA. It can remain essentially dormant (equilibrium of viral production and destruction) or become activated and initiate the production of large chains of new viral RNA subunits independent of the host cell's replication process. Integrase strand transfer inhibitors (ISTIs) prevent the integrase enzyme from inserting the viral DNA into the host DNA; thus it is unable to produce new virus material.

After a period of normal cell function, the infected cell becomes activated, initiating *transcription* of the proviral DNA into mRNA, which is then *translated* in the cytoplasm to produce long chains of viral polypeptides. *Cleavage* occurs when the protease enzyme cuts up these viral polypeptides. The pieces are then joined together to form new retroviruses. Protease inhibitors block the action of the protease enzyme and thereby prevent the reassembly of new viral polypeptides.

Packaging is a poorly understood step in this process, where the new RNA viruses are wound up tightly and encapsulated into new viral particles. *Budding* occurs when the HIV nucleocapsid containing the new viral RNA moves to the host cell's membrane and binds to the inside of the membrane. It is then enveloped by the membrane as it leaves the cell. As of yet, there are not any approved medications that target these steps of viral replication.

TRANSMISSION

HIV has been found in body fluids and tissues that contain lymphocytes. This includes blood and blood products, semen, vaginal fluids, breast milk, cerebrospinal fluid, synovial fluid, pleural fluid, amniotic fluid, and infected tissues. It has also been found in small amounts in saliva, tears, feces, and urine. According to the CDC, the only documented cases of transmission are via blood, semen, vaginal secretions, breast milk, and transplanted organs from an infected person. Variables affecting transmission rates include the infectivity of the source partner (higher viral load), the presence of sexually transmitted diseases (STDs) (especially with ulcerations), and the type of contact (increased risk with anal intercourse or vaginal intercourse during menses because blood is present).

The most significant routes of transmission are via sexual contact (vaginal, anal, and less frequently, oral intercourse), intravenous drug use (sharing contaminated needles and syringes), transfusion of blood or blood products (highest risk before May 1985 when screening of the blood supply in the United States for HIV began), and from an HIV-infected mother to her baby (before or during birth or through breastfeeding). Transmission can also potentially occur via percutaneous needlestick, mucous membrane exposure, or exposure of infected fluid into an open wound. Studies show the risk of seroconversion after a needlestick from an HIV+ source to be 0.25% per exposure. They also show that there have been no known seroconverters after splash exposure. The CDC has identified one documented case of transmission from acupuncture.

There is no evidence that HIV can be transmitted via air, water, insects, or dried body fluids. Universal infection control precautions as developed by the CDC should always be employed by health care

workers to minimize the risk of HIV transmission in the health care setting. Always assume that all blood and body fluids are potentially hazardous and that all patients are potentially infected.[3]

THE IMMUNE SYSTEM'S RESPONSE

Both the humoral and cellular immune systems have a role in the body's response to HIV infection. The humoral response involves the initial production of transient immunoglobulin M (IgM) antibodies to viral core and envelope proteins. Permanent immunoglobulin G (IgG) antibodies are subsequently established (this may take several weeks) to core p24 and envelop gp160, gp120, and gp41, corresponding to the resolution of clinical symptoms. The development of these IgG antibodies results in a rapid decrease in circulating virions and p24 antigen titers. *Seroconversion* occurs when the body starts making antibodies to HIV antigens and those antibodies become detectable by conventionally used laboratory tests (enzyme-linked immunoassays).

There is a window of time between initial infection (when patients remain antibody-negative or seronegative as measured by EIA tests) and when seroconversion (antibody-positive or seropositive by EIA) occurs. The length of this window varies depending on the individual and can range from 10 to 14 days to 6 months. The cellular immune response is characterized by the elevation of various cytokines (including interferons and tumor necrosis factor), CD4+ lymphopenia, and CD8+ lymphocytosis. Cytotoxic T cells reduce the plasma viremia, whereas natural killer (NK) cells may be involved with killing infected T cells.

CLINICAL PROGRESSION TO AIDS

The progression of HIV in an untreated state is broken down into three stages: stage 1 is acute HIV infection, stage 2 is clinical latency (or HIV inactivity/dormancy), and stage 3 is AIDS.

The overall natural progression of HIV infection can be broken up into three types: rapid (AIDS develops within 3 years of infection and seroconversion), intermediate (AIDS develops more slowly, between 3–10 years after infection), and long-term non-progression (AIDS does not develop for >8–10 years after infection).

Assuming no antiretroviral treatment, a typical scenario of HIV infection might unfold as follows: Starting with stage 1, within 2 to 3 weeks of initial infection with the virus, an acute retroviral syndrome occurs. This resembles a common flu and is often missed in individuals without obvious risk factors or awareness about HIV. Recovery and seroconversion usually follow in 2 to 3 weeks. After recovery from the initial acute syndrome, HIV plasma concentrations decline to a viral "set point," and equilibrium is established between the production and destruction of CD4+ cells, which denotes the beginning of stage 2 or clinical latency. The level of viral replication after acute infection and seroconversion coupled with the CD4+ count is predictive of long-term prognosis. A lower level of replication with a higher CD4+ count indicates a longer asymptomatic course. Conversely, a higher level of replication with lower CD4+ counts generally indicates a shorter asymptomatic course. Over time, a gradual decline in T-cell numbers begins with a concurrent gradual increase in the amount of virus in the body. This period can last 5 to 15 years. Eventually there are not enough T cells for the body's immune system to function properly, and stage 3 or AIDS is the result. This is when the risk of OIs and other so-called AIDS indicator conditions increases, with the ultimate result being death.[4]

There are two unique groups of patients that do not follow this typical scenario. The first group is termed *long-term non-progressors*. These individuals are thought to make up less than 5% of the total HIV+ population. They are antiretroviral naïve, their viral load remains <5000 copies/mm^3, and CD4 count remains in a normal range, and they never manifest any AIDS-defining illness or OI. The second group is a subset of the long-term non-progressors called *elite controllers*. These individuals maintain even lower viral loads and higher CD4 counts and are thought to number approximately 1 in 200 HIV+ individuals. The reasons for why these individuals are able to maintain this status are unclear (and may even be different between the two groups), but they likely involve some sort of interplay between genetic factors in the individual host and the genetics of the specific virus. Both of these groups are getting considerable attention from researchers who are working on finding a cure for HIV.

Box 178.1 lists the CDC's 2014 revised surveillance case definition of AIDS for adults and children (6 years of age or older). Note that the criteria are different both for children less than 1 year old and children who are 1 to 5 years old. This definition creates stages of HIV infection based on CD4+ cell counts/percentages and/or clinical symptoms. The primary criterion used is positive serology (i.e., positive for antibodies to HIV or the presence of p24 antigen via fourth-generation HIV 1/0/2 antigen/ antibody test and/or positive HIV antigen detection via polymerase chain reaction [PCR] or another specific HIV antigen test). Given a positive serology, a diagnosis of stage 3 HIV or AIDS is given when CD4+ counts fall below 200/mm^3 (<14%), any of the noted AIDS-defining conditions occurs, or both.[5]

Clinically, patients tend to be susceptible to certain complications (both infectious and noninfectious) on the basis of their CD4+ cell counts.[6] Although there is always individual variation, these data are helpful in predicting probable clinical presentations. Generally, lower CD4+ cell count and/or a higher viral load represents a greater risk for complications and should always be factored into any such consideration. It is notable that these issues are becoming less and less common, given the current standard of care and the use of antiretroviral medications by most patients.

- When the CD4+ cell count is greater than 500/mm^3, a patient could manifest acute retroviral syndrome, candidal vaginitis, persistent generalized lymphadenopathy (PGL), Guillain–Barré syndrome, myopathy, or aseptic meningitis.
- When the CD4+ count is between 200 and 500/mm^3, possible complications include pneumococcal or other bacterial pneumonia, pulmonary tuberculosis, herpes zoster, oropharyngeal candidiasis/thrush, cryptosporidiosis (self-limited), Kaposi sarcoma, oral hairy leukoplakia, cervical and anal dysplasia/cancer, B-cell lymphoma, anemia, mononeuritis multiplex, idiopathic thrombocytopenic purpura, Hodgkin's lymphoma, or lymphocytic interstitial pneumonitis.
- When the CD4+ count is below 200/mm^3, complications might include *Pneumocystis jirovecii* pneumonia (previously known as PCP pneumonia), disseminated histoplasmosis and coccidioidomycosis, miliary/extrapulmonary tuberculosis, progressive multifocal leukoencephalopathy (PML), wasting, peripheral neuropathy, HIV-associated dementia, cardiomyopathy, vacuolar myelopathy, progressive polyradiculopathy, or non-Hodgkin's lymphoma.
- When the CD4+ count is below 100/mm^3, complications might include disseminated herpes simplex, toxoplasmosis, cryptococcosis, chronic cryptosporidiosis, microsporidiosis, or candidal esophagitis.
- When the CD4+ count is below 50/mm^3, complications might include disseminated cytomegalovirus (CMV), disseminated *Mycobacterium avium* complex (MAC), or central nervous system lymphoma.

BOX 178.1 Centers for Disease Control and Prevention (CDC) Revised Surveillance Case Definition for HIV Infection—United States, 2014

For adults and children aged 6 years and older:

Stage 0: When a previous negative or indeterminate HIV test result has been documented within 180 days of the first confirmed positive HIV test of any type

Stage 1: CD4+ T-lymphocyte count of ≥500/microliter (μl) or ≥26%; no AIDS-defining condition present

Stage 2: CD4+ T-lymphocyte count of 200 to 499/μL or 14% to 25%; no AIDS-defining condition present

Stage 3 (AIDS): CD4+ T-lymphocyte count <200 /μL or <14% or documentation of an AIDS-defining condition

Stage unknown: no information of CD4+ T-lymphocyte count or percentage; no information on presence of AIDS-defining conditions

NOTE: There are separate CD4+ T-lymphocyte–based criteria for children <1 year old and 1 to 5 years old.

The specific AIDS-indicator conditions include the following:

Bacterial infections, multiple or recurrent (only among children <6 years old)

Candidiasis of esophagus, bronchi, trachea, or lungs

Cervical cancer, invasive

Coccidioidomycosis, disseminated or extrapulmonary

Cryptococcosis, extrapulmonary

Cryptosporidiosis, chronic intestinal (>1 month's duration)

Cytomegalovirus disease (other than liver, spleen, or lymph nodes), onset at age >1 month

Cytomegalovirus retinitis (with loss of vision)

Encephalopathy, attributed to HIV

Herpes simplex with chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)

Histoplasmosis, disseminated or extrapulmonary

Isosporiasis, chronic intestinal (>1 month's duration)

Kaposi sarcoma

Lymphoma—Burkitt's, immunoblastic, primary (of brain)

Mycobacterium avium complex or *Mycobacterium kansasii,* disseminated or extrapulmonary

Mycobacterium tuberculosis of any site, pulmonary, disseminated, or extrapulmonary

Mycobacterium, other species, or unidentified species, disseminated, or extrapulmonary

Pneumocystis jirovecii (previously known as *"Pneumocystis carinii"*) pneumonia

Pneumonia, recurrent

Progressive multifocal leukoencephalopathy

Salmonella septicemia, recurrent

Toxoplasmosis of brain, onset at age >1 month

Wasting syndrome attributed to HIV—involuntary weight loss >10% of baseline plus chronic diarrhea (>2 loose stools/day for >30 days) or chronic weakness and documented enigmatic fever >30 days

[a]In all stages laboratory confirmation is required.

From Selik RM, Mokotoff, ED, Branson B, et al. Revised surveillance case definition for HIV infection—United States, 2014. *MMWR Morb Mortal Wkly Rep.* 2014, April 11;63(RR03):1–10.

BOX 178.2 Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health Care Settings as of 2006

Screening for HIV Infection

- Routine testing for all patients 13 to 64 years old (at least once in their life; more frequently if risk factors are present)
- Pregnant women
- All patients initiating treatment for tuberculosis
- All patients seeking treatment or evaluation at clinics treating sexually transmitted diseases
- All persons with signs or symptoms consistent with HIV or with an opportunistic infection consistent with HIV/AIDS

Repeat Screening

- Annual screening (minimally) for persons in high-risk groups (includes injection-drug users and their sex partners, persons who exchange sex for money or drugs, sex partners of HIV-infected persons, and men who have sex with men [MSM] or heterosexual persons who themselves or whose sex partners have had more than one sex partner since their most recent HIV test)
- Potential screening every 3 to 6 months for persons with ongoing high risks
- Patients and their prospective partners before initiating a new sexual relationship
- Any person whose blood or body fluid is the source of an occupational exposure to a health care provider should be informed of the incident and tested at the time of the exposure

Data from Branson BM, Handsfield HH, Lampe MA, et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Morb Mortal Wkly Rep.* 2006, September 22;55(RR14):1—17; DiNenno EA, Prejean J, Irwin K, et al. Recommendations for HIV Screening of gay, bisexual, and other men who have sex with men—United States, 2017. *MMWR Morb Mortal Wkly Rep.* 2017, August 11;66(31):830–832.

DIAGNOSIS AND WORKUP

Diagnostic Testing

Box 178.2 lists the most recent CDC guidelines for HIV testing (from 2006 and 2017).[7,8] These guidelines advise routine HIV screening of adults, adolescents, and pregnant women in health care settings in the United States. The recommendations also call for reducing barriers to HIV testing. In April 2013 the U.S. Preventive Services Task Force issued similar recommendations.

Standard serological testing for the presence of HIV begins with the fourth-generation HIV 1/0/2 antigen/antibody test that uses synthetic peptide or recombinant protein antigens to detect IgM and IgG antibodies (via enzyme-linked immunoassay technique) and monoclonal antibodies to detect the presence of p24 antigen. If this is positive, then a reflex test is done to distinguish HIV-1 from HIV-2 infection. The inclusion of p24 antigen detection in HIV screening has allowed for a positive result to be found 3 to 5 days before seroconversion (when HIV-specific antibodies begin to appear), thus allowing for earlier detection of an infection than was previously possible with the earlier test methods. This test has a sensitivity of between 99.76% and 100% for HIV-1 and 100% for HIV-2.[9]

A number of rapid HIV tests have been developed and are currently approved for use by the U.S. Food and Drug Administration (FDA). They all use similar technology to detect antibodies and vary in category assigned by the Clinical Laboratory Improvement Amendments (CLIA) from "waived" to "moderately complex." Options include Chembio DPP HIV-1/2, Chembio Sure Check HIV1/2 Assag, Clearview HIV 1/2 Stat-Pak, Determine HIV-1/2 Ag/Ab Combo Test, INSTI HIV-1/HIV-2 Rapid Antibody Test, MedMira Reveal G3 Rapid HIV-1 Antibody Test, OraQuick Advance Rapid HIV-1/2 Antibody Test, and Uni-Gold Recombigen HIV.[10,11] Additionally, there are self-administered tests that have been FDA approved. Home Access

Health Corp. (www.HomeAccess.com/shop) produces a kit to detect HIV-1 where the specimen is self-obtained at home and mailed in for analysis. Results are available by phone as soon as the next business day. The OraQuick In-Home HIV Test provides results at home in 20 minutes. These tests use either whole blood, serum, plasma, or oral fluids and have sensitivity and specificity rates of 99% to 100%.[12–14]

If an HIV screening test is negative and risk factors suggest likely infection, the test should be repeated at 6-week, 3-month, and 6-month intervals. There is no reason to repeat if one of those tests comes back positive.

Although routine testing is now recommended, HIV testing remains voluntary and should be performed only with the patient's knowledge and understanding. Patients should be informed either orally or in writing of testing recommendations and that testing will be performed unless they decline (termed "opt-out" screening). A patient's decision to decline an HIV test should be documented in the medical record. It is recommended that HIV screening consent be incorporated into the patient's general informed consent for medical care form in the same way other screening or diagnostic tests are. A separate consent form for HIV testing is not recommended. Risk screening should be incorporated into routine primary care and risk reduction counseling offered as applicable. All states require that newly diagnosed cases of HIV/AIDS be reported to local health departments and that providers have case reporting forms available.

Medical History

Initiation of care of an HIV+ patient should begin with a standard medical history, including a detailed diet history, exercise patterns, and a complete review of systems. A number of additional historical details should be obtained as well (Box 178.3).

BOX 178.3 Historical Data Recommended for HIV-Positive Patients

- Dates of infection and subsequent diagnosis, as well as probable source of infection (IV drug use, sexual contact, transfusion). If date of infection is unknown, the naturopathic physician should determine whether there was any history of acute retroviral syndrome. Additionally, past and current risk factors for HIV exposure should be determined. This information helps estimate overall health and vital force and long-term prognosis.
- Vaccination history and adverse reactions to past vaccines.
- History of other sexually transmitted diseases. This information should include date and duration of infection as well as therapies (efficacy, adverse reactions, and duration of treatment). Particular infections to screen for include syphilis, gonorrhea, *Chlamydia,* herpes simplex (all types), hepatitis (A, B, and C or E), and HPV (skin, genital, or anal).
- Chronological history of HIV-related problems, including history of OIs or cofactor viruses/infections (mononucleosis, EBV, molluscum contagiosum, CMV, or yeast infections—vaginal, gastrointestinal, skin), skin rashes or other lesions, oral lesions or tongue coating, lymphadenopathy, fevers, night sweats, weight loss, diarrhea, anorexia, fatigue, malaise, shortness of breath, or cough.
- Specific for females, history of abnormal Pap smears and frequency of gynecological examinations.
- HIV viral load and trend of CD4 count (both currently and historically if known); good indicators of the patient's susceptibility to OIs, indication for and effectiveness of HAART, as well as long-term prognosis.
- History of all past and present HAART, history of any prophylactic antibiotic prescription medications, and all other current medications and supplements, with duration of treatment, response and side effects, intolerance, and allergies.
- Family history of chronic disease, with particular emphasis on cardiovascular disease (including lipid problems), diabetes, and cancer.
- History of psychoemotional trauma and issues (abuse history, anxiety, depression).
- Patients' spiritual lives and support systems, life goals, and meaning of HIV in their lives.
- Clear identification of complete medical care team as well as reasons for seeking naturopathic medical care.
- The initial physical examination must be both comprehensive and appropriately focused as directed by the history. In addition, all patients should have particularly thorough examinations of the mouth and throat, skin, and genitalia. If a full pelvic examination and Pap smear cannot be done on a new female patient at the initial evaluation, these should be scheduled for shortly thereafter (the exception being if one of their other providers does this at appropriate intervals).

CMV, Cytomegalovirus; *EBV,* Epstein–Barr virus; *HAART,* highly active antiretroviral drug therapy; *HIV,* human immunodeficiency virus; *HPV,* human papillomavirus; *IV,* intravenous; *OI,* opportunistic infection.

Laboratory Assessment and Monitoring

Basic initial screening tests for all HIV+ patients should include a complete blood count (CBC) and a fasting serum chemistry panel (to monitor liver, kidney, and pancreatic function; electrolytes; blood proteins; glucose; and lipids). The baseline serum albumin level can serve as an independent predictor of prognosis in HIV+ women.[15] Additional testing should include a urinalysis, screening for STDs (syphilis, herpes simplex, *Chlamydia,* and gonorrhea—including oral/urinary/rectal areas), hepatitis (A, B, and C), toxoplasmosis, tuberculosis, and varicella (if unknown history of chickenpox or shingles). An optional test is glucose-6-phosphate dehydrogenase (based on risk factors).[13] Female patients should have regular Pap smears with initial testing for human papillomavirus (HPV) because there is a significant increase in cervical cancer rates in HIV+ women.[16] A recent development is the use of the anal Pap smear in male patients engaging in anal sex. Anal squamous cell cancer is similar to cervical cancer in that it is caused by HPV infection, and rates are significantly increased in HIV+ men compared with the general population. Currently, there continues to be controversy about the use of this test, with no clear consensus on whether to make it part of routine screening.[13] Studies are ongoing, which should help clarify this. One such study is the multicenter Anchor Study, begun in 2017 and involving 5058 participants over an 8-year study period. The goal of the study is to determine whether routine anal screening, detection, and removal of high-grade squamous intraepithelial lesions (HSILs) will reduce the occurrence of more severe anal cancers in HIV+ men.[17]

HIV-specific testing includes measurement of viral load and T-cell counts as part of the initial evaluation and then at 3- to 6-month intervals. The viral load test detects the presence of viral RNA in the plasma or DNA in white blood cells (WBCs) and uses PCR branched-chain DNA (bDNA) or nucleic acid sequence–based amplification technology. A number of viral load tests exist, with varying levels of sensitivity. The most sensitive test currently available detects virus to 20 copies per cubic millimeter of plasma.[18] Below any specific test's threshold, the viral load is deemed "undetectable." This means that the virus may still exist but in concentrations in the blood below the ability of the given test to detect. Additionally, note that these assays are not reflective of viral presence or concentration in body compartments beyond the blood (e.g., tissues, cerebrospinal fluid, breast milk, seminal fluid, vaginal fluid).

Other key tests involve T-cell counts. CD4+ helper cells and CD8+ suppressor cells are quantified (absolute numbers and relative

percentages), along with the CD4:CD8 ratio. These tests are known as CD4:CD8 ratio panels. This information is used to stage HIV, predict susceptibility to various OIs, and monitor response to antiretroviral therapy. Many factors can influence CD4+ cell counts, including total WBCs, acute infection, certain medications (e.g., corticosteroids, interferon), and other medical conditions. There are also seasonal and diurnal variations in these counts.

In 2014, the guidelines on HIV-specific testing were altered as a reflection of the success of HAART. Once a person's CD4+ count is above 500 cells/mm^3, his or her immune system is thought to be fully reconstituted and functional. As long as the CD4+ count has remained above 500 cells/mm^3 and he or she has had an undetectable viral load for 2 years, then continuing to check the CD4+ count is now considered optional. From this time on, all that is now checked is HIV viral load once yearly, and as long as this remains undetectable, then the CD4+ is expected to stay stable and in a safe range. If the viral load becomes consistently detectable, then it would become necessary to check CD4+ cell counts again.[19]

Resistance to antiretroviral medications is a growing problem in treatment-naive patients and remains a potential problem in patients already undergoing this therapy. Therefore resistance testing is now recommended at the time of diagnosis as part of baseline screening as well as before changing therapy because of the failure of a current regimen. Two types of tests are available: genotypic and phenotypic. There are advantages and disadvantages to each, but in general, this testing can help identify antiviral medications that the patient might be either resistant or susceptible to and thereby allow the physician to formulate the most effective medication regimen.[13]

THERAPEUTIC CONSIDERATIONS

Medical Management of HIV and AIDS

The medical understanding and treatment of HIV/AIDS continue to evolve. AIDS is a complex, multifactorial disease that has both immunodeficiency and autoimmune inflammatory aspects involving virtually every system of the body. HIV enters the body and infects CD4+-expressing cells of the immune system. It inserts itself into cellular DNA and can lie dormant indefinitely. Those cells can be activated for the reproduction and multiplication of HIV virions by antigens, oxidative stress, proinflammatory cytokines, and overstressed liver detoxification and immune systems.

Conventional medical management of HIV/AIDS revolves around the following treatment principles:

- Frequent monitoring, including laboratory and physical examination
- Vaccinations administered as appropriate (may include hepatitis A, hepatitis B, influenza, mumps-measles-rubella [MMR], pneumonia tetanus-diphtheria, varicella, HPV, and travel-related vaccines)[13]
- HAART for inactivation or slowing HIV replication and increasing CD4+ cell counts
- Antibiotic prophylaxis for patients with abnormally low CD4 lymphocyte counts
- Antimicrobial treatment of OIs
- Symptomatic care of HIV- or HAART-induced adverse drug reactions
- Radiation, chemotherapy, or both for HIV-related neoplasms
- Psychosocial support via counseling, clinical social work, and medication

Up-to-date resources should always be consulted before making any management decisions. The AIDS information website, administered by the U.S. Department of Health and Human Services (https://aidsinfo.nih.gov), provides an excellent resource for understanding all aspects of HIV/AIDS, including determining the most up-to-date conventional treatment guidelines as well as information on prevention, FDA-approved and investigational drugs, clinical trials, and vaccinations. Another fine resource is the Johns Hopkins HIV Guide (https://www.hopkinsguides.com/hopkins/index/Johns_Hopkins_HIV_Guide). This HIV guide provides detailed information on the diagnosis and management of HIV and its associated OIs, medications, and resistance. Note that some areas of the site require a subscription for access. Johns Hopkins University School of Medicine/Knowledge Source Solutions LLC also publishes a helpful text called the *Medical Management of HIV Infection* (2012, 16th ed, by J. G. Bartlett, J. E. Gallant, and P. A. Pham). It can be ordered online. Excellent resources also exist for both clinician and patient education. These include the websites www.positivelyaware.com, www.poz.com, and www.thebody.com. These sites each have their strengths, but all have valuable information on various aspects of HIV, including signs, symptoms, conventional treatments, detailed drug information, some patient-centered complementary treatments, as well as future possibilities for treatment.

HIV Prevention Strategy and the Continuum of Care

Over the past several years, with the success and availability of HAART medications throughout the world, there has been a shift in focus in HIV care and management from how to cope with so many very sick people to thinking about how we can prevent of the further spread of HIV. This has resulted in a strategy known as "treatment as prevention." It is based on recent studies showing that if a person's HIV viral load is undetectable, then there is an extremely low risk of that person having enough free virus in his or her system to infect someone else.[20] Worldwide, the strategy for prevention of HIV spread is known as "90/90/90," and the goal is to have it implemented by the year 2020. This means that 90% of people who are HIV+ will know it. And of those, 90% will be on antiretroviral therapy. And of those, 90% will have an undetectable viral load. Therefore those HIV+ individuals who are being treated and undetectable will not be vectors for the further spread of the disease. This will ultimately result in far fewer new infections.

In the United States this prevention strategy is effectively being implemented by targeting at-risk populations with education about transmission, getting at-risk people tested regularly (to identify individuals who are positive), and then getting into care when there is a positive test result. The National HIV Care Continuum has identified and monitors the steps required from the time a person is diagnosed with HIV to achieve viral suppression. It involves testing to achieve the diagnosis; linking them to care; and making sure they are engaged in and retained in care, that they are prescribed and take antiretroviral therapy, and they get to the point where they are virally suppressed and remain that way.[21]

The other key piece of the worldwide prevention strategy is the use of pre-exposure prophylaxis (PrEP). In the United States the CDC first issued guidelines about PrEP use in 2012, and they have since gone through some minor revisions, but the principle and use are unchanged. The concept is that a person who is HIV negative but at risk for contracting HIV takes a daily antiretroviral medication (initially approved with Truvada and now Descovy is an option as well; both are discussed in the section on antiretroviral therapy that follows) that prevents them from becoming infected should they be exposed to the virus. This implementation of this strategy was initially based on four key studies, the iPrEx, TDF2, Partners PrEP, and Bangkok Tenofovir studies.[22–25] These studies variously followed both heterosexual and homosexual discordant couples and men and women using intravenous drugs in multiple locations. All the studies showed a significant

reduction in risk of acquiring a new HIV infection—up to 92%, with the best results coming with rigorous adherence to the regime of daily use. Based on this, PrEP has become a key HIV prevention recommendation in high-risk individuals. The CDC maintains excellent resources with regard to PrEP for both consumers and providers. This includes detailed instructions on how to administer Truvada and how to monitor patients.[24] Note that regular HIV testing (every 3 months) is part of the process because it is critical that any individual who has contracted the virus despite the PrEP be identified so that they can be placed on effective HAART treatment (Truvada alone is insufficient and is likely to lead to resistance issues). Note also that the use of barrier methods such as condoms is still recommended to protect against other sexually transmitted infections as well as further lower the risk of HIV transmission.

On October 3, 2019 the FDA approved a second medication, Descovy, for PrEP. Descovy is similar to Truvada in that it contains two similar medications but with one of them being a safer variant. The approval was based on preliminary data from the ongoing DISCOVER trial. In this multi-year phase 3 trail, 5300 HIV at-risk cisgender men having sex with men and transgender women having sex with men were provided with PrEP with either Truvada or Descovy. After 96 weeks, Descovy was found to be non-inferior to Truvada in preventing HIV infection. Efficacy was strongly correlated with daily dosing. Note that Descovy also has advantages over Truvada in areas of renal safety and bone density. While Truvada will end up being more economical than Descovy due to its age, Descovy could be an excellent option for any individual with renal or bone density issues.[26]

For the naturopathic physician, practical actions toward prevention can include the following:

- Talk to patients about sex to determine risk factors, educate about how HIV and other sexually transmitted infections are transmitted, and educate about safer sex practices.
- Recommend HIV testing at appropriate intervals based on individual risk factors and CDC guidelines.
- Recommend PrEP as appropriate. This includes helping patients to access the medication should they desire to use it.

Naturopathic Management of HIV and AIDS

The naturopathic medical management of HIV and AIDS revolves around a number of treatment principles that should guide the physician in helping patients to optimize their health, slow disease progression, improve their quality of life, and improve immune function.

Specific goals include the following:

- Enhance overall integration with primary care physicians and build a caregiving team.
- Complement and enhance the positive effects of conventional medical treatment and minimize the negative effects.
- Establish a foundation of health and wellness on the basis of naturopathic principles.
- Establish a core nutrient protocol:
  - Replace nutrient deficiencies known to exist with HIV.
  - Provide other nutrients to support optimal immune function.
- Provide therapies to address constitutional symptoms, medication side effects, and symptoms of immunosuppression.
- Provide specific antiviral therapy and immunomodulation using other, nondrug treatments.

Enhance Overall Integration With Primary Care Physicians and Build a Caregiving Team

In the area of HIV, naturopathic physicians should lead the effort to optimize integrated care. A holistic provider who identifies advantages of all available treatments and encourages positive relationships with all available providers creates a unique and trusting relationship with patients who have often experienced lifetimes of fear, discrimination, victimization, and abuse. Naturopathic medicine offers an important opportunity to empower HIV+ patients.

One of the first priorities is to ensure that each HIV+ patient has a complete care-providing team beginning with a conventional Western medicine HIV specialist. HIV specialists ensure the accessibility of HAART and have the greatest familiarity with the multitude of signs, symptoms, adverse reactions, and other issues unique to the HIV+ population. A growing body of literature reports that HIV+ patients have better objective indices of health (low viral load and high CD4 counts), better compliance with medications, and better long-term survival when they work with HIV specialists.[27,28]

The specialist's obligations constitute a full-time job, precluding many other aspects of care that have demonstrated efficacy for the overall health of HIV+ patients. Additional providers can support HIV+ patients with general health and lifestyle promotion, specific nutrient and other nondrug recommendations, social services, psychoneuroimmunological support, counseling, personal empowerment, and end-of-life care. In fact, although naturopathic physicians might be legally able to prescribe prophylactic antimicrobial therapy and HAART in a growing number of states, the greater need is to provide nondrug holistic care in the areas just mentioned.

Complement and Enhance the Positive Effects of Conventional Medical Treatment and Minimize the Negative Effects

One of the paradigms of natural/preventative/holistic medicine is to minimize the need for higher-force (drug, surgery, radiation) interventions. This goal remains valid in the care of patients with HIV, but there are no therapies known to be as effective as HAART in suppressing viral load or increasing CD4 T-lymphocyte cell numbers. CAM providers may be the best practitioners to teach HIV+ patients that there are no realistic s"alternatives" to HAART.

Some HIV+ patients may never need HAART, and others may be resistant to HAART, but the option of HAART should always be considered, and its use is the current standard of care; therefore it should not be discouraged. With or without HAART, there is significant evidence that HIV+ patients receive many benefits from nondrug therapies.[29] Naturopathic physicians must actively understand, integrate, and use the entire therapeutic order (most subtle, lower-force through most high-force, often-invasive therapies) to ensure that each patient has the best options to maximize his or her quality of life as well as length of life. Finally, physicians working with HIV must stay current with the global effort to find solutions for this pandemic to provide the best possible treatment options to match patients' short-term needs while also including strategic health goals and future possibilities.

Factors to be considered in the holistic care of HIV+ patients include complications (from immune suppression) of health challenges that existed before HIV infection. In addition, the social, psychological, and spiritual challenges that exist with the diagnosis of HIV create a need for health care that extends far beyond the best drug or nutrient necessary for optimal health. Holistic physicians have a vital role in the care of each patient with HIV.

The majority of HIV+ patients in North America are using HAART. Many of those same patients are also using CAM therapies. Therefore one of the first priorities of the naturopathic provider should be to ensure that the patient is safe from adverse drug–nutrient interactions. To

ensure safety, it is necessary to understand the mechanisms of HAART as well as those of common nutrients being used by HIV+ patients.

Conventional Treatment: Highly Active Antiretroviral Therapy

The use of HAART has been the standard of care in HIV treatment since 1996 when multiple classes of medications came into existence and it was found that using multiple drugs of multiple classes was more effective at suppressing HIV virus as well as preventing resistance issues than single drug/class regimes.

In terms of when to start HAART, the risks of OIs and other serious complications increase exponentially when CD4 cell counts drop below 200/μL. Until 2015, the standard of care was to initiate HAART when the CD4 count dropped below 350/μL in asymptomatic patients, those with an AIDS-defining illness (regardless of CD4 count), pregnant women, patients with HIV-associated nephropathy, or hepatitis B coinfection (when treatment of the hepatitis is indicated). HAART initiation could be considered when CD4 cell counts are greater than 350/μL in discordant relationships (one partner is HIV+ and the other HIV−), rapid CD4 decline, advanced age, or high viral load (more than 100,000 copies per milliliter).[30]

In September 2015, the World Health Organization (WHO) published new guidelines on the treatment and prevention of HIV infection (http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/). The main outcome of these new recommendations is that HAART should be initiated in most patients upon diagnosis. This was based on two studies, the START Study (Strategic Timing of Anti-Retroviral Treatment Trial) and the HPTN 052 Trial (HIV Prevention Trials Network 052 study).[20,31,32] These were large studies at multiple sites around the world. The START study concluded that immediate initiation of antiretroviral therapy significantly reduced both AIDS-related and non–AIDS-related adverse events in that population. The HPTN 052 study concluded that antiretroviral therapy is highly effective at preventing heterosexual transmission of HIV if viral suppression is achieved and maintained in the positive partner. From this, the WHO issued the new guidelines recommending HAART be initiated in everyone upon diagnosis (with some case-specific caveats). The U.S. guidelines (published July 14, 2016) made similar recommendations for HAART initiation.[33]

The choice of specific antiretrovirals for either initial regimens or the modification of existing therapy is complex, and there are many factors to consider. Additionally, the guidelines change frequently. The best resource for the most current recommendations is the "Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV," available on the AIDS information website https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/0. The most recent guidelines were updated as of May 30, 2018.

Six main classes of HAART are currently being used in the care of HIV+ patients in various combinations designed to disrupt the life cycle of the virus at multiple junctures.[30] These include the following:

- Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs, or "nukes")
- Nonnucleoside reverse transcriptase inhibitors (NNRTIs, or "non-nukes")
- Protease inhibitors (PIs)
- Entry inhibitors (including fusion inhibitors, CCR5 inhibitors, and monoclonal antibodies)
- Integrase strand transfer inhibitors
- Pharmacokinetic enhancers

Over the past several years, a number of single-tablet, fixed-dose, multiclass formulations have been developed. This has helped to simplify dosing and decrease pill burden for patients using HAART. These single-tablet regimens (STRs) are rapidly becoming the most commonly used forms of HAART as of 2019.

As a result of the rapidly evolving resistance of HIV to specific drugs, there has historically been great pressure to quickly develop and approve new medications. As a result, many HAART medications have been generally fast-tracked through the FDA approval process. Therefore new drugs have been typically prescribed as soon as they are approved and usually before the full extent of their adverse reactions are known. Because the adverse drug reactions and interactions with HAART are myriad and potentially quite harmful, the clinician is advised to consult up-to-date resources to determine specific issues with each individual medication.

Nucleoside/nucleotide reverse transcriptase inhibitors. NRTIs were the first class of antiretroviral drugs approved for use in the United States by the FDA. These drugs work during the transcription phase of the HIV life cycle by competitive inhibition of HIV reverse transcriptase (RT), leading to DNA chain termination. Nucleoside (or nucleotide) analogues act as substrates and bind to the active site of the RT enzyme. Then they are added to the new chain of DNA. Once they have been inserted, the normal links between pieces of the chain will not form, and the viral chain will be terminated without being inserted into the cell's DNA.

The following is a list of NRTIs; all are nucleoside analogues except tenofovir, which is the only nucleotide analogue that is FDA-approved:

- 3TC: lamivudine (Epivir)
- ABC: abacavir (Ziagen; patient must be HLA-B*5701 negative if used)
- AZT: azidothymidine, zidovudine (Retrovir; the first FDA-approved HIV medication in 1986)
- DDC: zalcitabine (Hivid; no longer manufactured)
- DDI: didanosine (Videx, Videx EC)
- D4T: stavudine (Zerit)
- FTC: emtricitabine (Emtriva)
- TDF: tenofovir disoproxil fumarate (Viread, a nucleotide)
- TAF: tenofovir alafenamide (a "prodrug" form of TDF)

As of 2019, it is extremely rare to see AZT, DDC, DDI, or D4T used because there are many safer and easier-to-use alternatives.

There are several combination formulations of NRTIs in existence:

- Trizivir: 3TC, AZT, and ABC (no longer in use)
- Combivir: 3TC and AZT (no longer in use)
- Epzicom: 3TC and ABC
- Truvada: FTC and TDF
- Descovy: FTC and TAF
- Cimduo: 3TC and TDF

Truvada and, as of October 2019, Descovy have both been approved for use as PrEP for individuals in high-risk categories of exposure to HIV. Either one is given as a single tablet once daily. Daily use is important for maintaining efficacy.

In April of 2016 the FDA approved tenofovir alafenamide (TAF) for use. It is a "prodrug" form of TDF that is used in much lower doses and becomes activated once it's inside individual cells. It has a reduced profile of adverse effects versus its predecessor, TDF, which can affect kidney function as well as create problems with bone mineral density. The TAF form alleviates these issues. TAF is not available as a stand-alone but is found along with FTC as Descovy (as well as some specific single-tablet formulations). It is approved for the same uses as Truvada in HIV infection but is not approved for PrEP or post-exposure prophylaxis (PEP).

NRTIs have effectively become the "backbone" of all HAART regimes. Unless there are resistance issues, every regime will contain Truvada, Descovy, or Epzicom, either directly or as part of an STR.

The NRTIs are fairly well tolerated after introduction (initial mild to severe nausea usually resolves), with minimal long-term adverse

effects. Exceptions include AZT and the remaining "D" drugs (DDI and D4T). A common side effect of AZT is bone marrow suppression, leading to macrocytic anemia.[34] The D drugs are less commonly used today except in the case of multidrug resistance. They have been associated with mild to severe peripheral neuropathy and, of greater concern, pancreatitis.[35] These side effects manifest secondary to neural inhibition of mitochondrial DNA polymerase and reduced mitochondrial DNA content.[36] Therefore patients who have been on D drug therapy should be provided with ample mitochondrial support. Acetyl-L-carnitine, coenzyme Q_{10} (CoQ_{10}), essential fatty acids (EFAs), α-lipoic acid, and B vitamins have been shown to decrease signs or progression of these side effects when taken in large doses.[37–41] Additionally, the thymidine analogues (d4T, ddI, AZT) have been associated with lipoatrophy, also as a result of mitochondrial toxicity. Because the NRTIs have been on the market longer than the other drugs, it is no surprise that they are backed with the greatest amount of research regarding nutrient interactions. Although there are no studies indicating that vitamins or other nutrients deplete AZT, there is evidence that AZT may deplete cellular levels of carnitine, copper, zinc, and vitamin B_{12}.[42,43] With the exception of carnitine, these nutrients can be found in a good hypoallergenic multivitamin/multimineral supplement. In addition to deficiency caused by NRTI drugs, carnitine deficiency has been found in HIV+ patients not on HAART. Further, there are many other indications for carnitine in patients with HIV, making this nutrient a high priority for supplementation.

Some nutrients have been found to enhance the efficacy of NRTIs when taken in combination with these drugs, including vitamin E, zinc, and folate.[44–46] These nutrients are easily supplemented through a nutritious diet full of whole grains, colorful vegetables, and fruits and a good multivitamin/multimineral supplement.

Overall, NRTIs are fairly well tolerated, and some have the additional benefit of being active against hepatitis B virus (3TC, FTC, TDF).[47–49]

Nonnucleoside reverse transcriptase inhibitors. The second class of HAART comprises the NNRTIs. NNRTIs also affect the transcription process and work by allosterically binding to HIV-1 reverse transcriptase and inhibiting both RNA- and DNA-directed DNA polymerase functions of the RT enzyme.

The following is a list of the NNRTIs:

- EFV: efavirenz (Sustiva)
- ETV: etravirine (Intelence)
- DLV: delavirdine (Rescriptor; rarely used)
- NVP: nevirapine (Viramune XR)
- DOR: doravirine (Pifeltro)
- RPV: rilpivirine (Edurant)

NNRTI drugs are also fairly well tolerated after introduction, with low long-term toxicity. Other than an initial transient mild to severe skin rash, typical side effects (depending on the specific drug) include insomnia, abnormal dreams, elevated liver enzymes, and gynecomastia (all manageable with naturopathic support).[50–52] Resistance to this class of HAART can occur easily if the viral load is unsuppressed, so this type of medication may not be an optimal choice for someone who is inconsistent with taking medications. No nutrients are known to be depleted by NNRTIs or to enhance their efficacy.

Large randomized, double-blind, placebo-controlled studies have shown that when used in combination with NRTIs, NNRTIs produce sustained reductions in plasma viral loads and improvements in immunological responses.[53–56] The advantages of this type of protocol include the fewest number of side effects, excellent long-term viral suppression, and ease of dosing (fewer pills).

Protease inhibitors. The third class of HAART is the PIs. PIs intervene during the cleavage part of the viral life cycle by binding competitively to the substrate site of the viral protease (the enzyme responsible for the posttranslational processing), resulting in inhibition of the enzyme and the production of immature virus particles.

The following is a list of the PIs:

- ATV: atazanavir (Reyataz)
- DRV: darunavir (Prezista)
- FPV: fosamprenavir (Lexiva)
- IDV: indinavir (Crixivan)
- LPV-r: lopinavir + ritonavir (Kaletra; fixed-dose combination)
- NFV: nelfinavir (Viracept)
- SQV: saquinavir (Invirase)
- TPV: tipranavir (Aptivus)

Although PIs are effective at viral suppression, they are associated with the greatest number and most significant adverse drug reactions, and as of 2018, with the availability of equally effective classes with much fewer adverse reactions, they are being used less frequently. Adverse reactions include chronic and persistent gastrointestinal (GI) abnormalities; lipodystrophy and lipoatrophy; elevations of cholesterol and triglycerides; insulin resistance leading to diabetes; and liver, kidney, and musculoskeletal complaints.

Note that PIs are typically used in a "boosted" form. That is, they are given along with another class of HAART called a pharmacokinetic enhancer that inhibits the enzyme responsible for their metabolism and thereby raises their blood levels and their effectiveness. They have traditionally been given as two separate medications; however, there are three single-tablet combinations available. Kaletra (lopinavir + ritonavir) is the oldest example of this. Newer options include Evotaz (atazanavir + cobicistat) and Prezcobix (darunavir + cobicistat).

In understanding these "boosted" forms of HAART, it is essential to realize that NNRTIs and PIs work by inhibiting cytochrome P450 3A4 enzyme metabolism. Inhibition of this enzyme slows down the metabolic breakdown of the drug and effectively prolongs higher blood levels, which results in better viral suppression. Many foods, nutrients, and botanicals have been known to upregulate this enzyme function, and two have gained noteworthy attention. Some small studies (of fewer than 25 subjects) in healthy HIV-negative (HIV−) individuals as well as in vitro studies have demonstrated that garlic and St. John's wort decrease NNRTI and PI drug levels. In the human trials, subjects initiated NNRTI or PI therapy to establish the drug levels theoretically necessary to suppress viral load. Garlic or St. John's wort was then introduced at levels typically used for therapeutic effect. Blood levels were then measured and found to be lower than the levels believed to be necessary to maintain optimal viral suppression. Garlic or St. John's wort was then discontinued and NNRTI or PI blood levels subsequently measured and found to be back in the therapeutic range.[57–60] Although these studies certainly do not conclusively demonstrate that garlic and St. John's wort interfere with HAART viral suppression (small number of participants, single antiretroviral vs. typical multidrug HAART protocol, HIV− vs. HIV+, drug-naive vs. long-term HAART), these therapies should be avoided in patients with HIV on HAART. The lesson learned from these studies is to be intimately aware of the mechanisms of action when adding new non-drug therapies intended for patients on multidrug treatments. The potential for drug–nutrient interactions also should encourage and reinforce the use of the multitude of low-force interventions (e.g., diet, lifestyle, homeopathy, physical medicine, counseling) for conditions commonly treated by garlic or St. John's wort to avoid potentially decreasing NNRTI or PI blood levels and risking increases in drug resistance.

Milk thistle may be considered for the treatment of significant gastrointestinal (GI) and liver complications with PIs. To date, there have been several studies investigating the drug interactions of this herb. In one with 10 HIV−, drug-naive patients taking milk thistle at 175 mg three times daily, investigators concluded that milk thistle did not significantly alter blood levels of indinavir despite a decrease of as much

as 25% in trough levels.[61] Another study showed no effect on indinavir levels in healthy subjects.[62] Yet another tested cellular uptake of ritonavir in the presence of various herbal substances including milk thistle (silybinin) and found no change in the efflux of ritonavir or any effect on CYP 3A4.[63] An in vivo study with coadministration of nifedipine and silymarin in 16 healthy male volunteers concluded that silymarin is not a potent CYP 3A4 inhibitor in vivo.[64] Silymarin was also found to be safe with other PI combinations.[65]

The best study to date involved 15 HIV+ individuals taking darunavir and ritonavir as it is actually used in practice (600/100 mg twice daily) and giving them silymarin (150 mg three times daily) for 14 days. High-performance gas-liquid chromatography was used to measure serum concentrations of the darunavir before and at regular intervals after its ingestion through to 12 hours after taking it. Values were compared between day 0 and day 14. This study's findings showed no effect on the serum concentrations of darunavir from the silymarin. Therefore it was concluded that coadministration of silymarin with darunavir/ritonavir was safe and no dose modifications were necessary.[65]

Currently, there is no evidence suggesting that milk thistle should be avoided by HIV+ patients on HAART. No nutrients are known to be depleted by PIs or to enhance their efficacy. However, if GI side effects are present, it could lead to micro- and macronutrient deficiencies.

Entry inhibitors. There are now three classes of entry inhibitors: fusion inhibitors, chemokine coreceptor antagonists (CCR5 inhibitors), and monoclonal antibodies. They are designed to prevent the HIV virion from initially entering the CD4+ cell through its membrane.

The following is a list of the entry inhibitors:

- ENF: T-20 or enfuvirtide (Fuzeon)
- MVC: maraviroc (Selzentry)
- IBA: ibalizumab (Trogarzo)

ENF, T-20, or enfuvirtide (Fuzeon), is a fusion inhibitor that was approved by the FDA in 2003. It blocks the fusion of the virus with the target-cell membrane by binding with cell receptor gp41 proteins and thereby preventing the virus from entering the cell. This drug is unique because it is the only HAART medication administered via injection (two subcutaneous injections per day into the upper arm, thigh, or abdomen). Because of its cost and difficulty of use, Fuzeon was used only as "last resort" or salvage therapy during the mid-2000s in heavily treatment-experienced individuals in the presence of extensive viral resistance and is not designed to be used alone. With the approval of numerous new medications, Fuzeon is no longer used. It was fairly well tolerated, with the exception of injection-site irritation, allergic reactions, and an increased risk of bacterial pneumonia. There are no known drug–nutrient interactions of any clinical significance.

MVC, or maraviroc (Selzentry), is a chemokine coreceptor antagonist (CCR5 inhibitor) approved by the FDA in 2007. After HIV binds to CD4 receptors on the T-cell membrane, it then binds to one of two coreceptors, CCR5 or CXCR4. MVC is an antagonist that specifically blocks CCR5 coreceptors and prevents HIV from binding to them so that it cannot enter the cell. The limitation with this drug is that different viruses may express various coreceptors: CCR5, CXCR4, or both. A relatively expensive test called a tropism assay must be run in any individual in whom the use of MVC is being considered. This test determines whether the specific virus present binds to CCR5, CXCR4, or both types of receptors. MVC will be effective only in a patient with a CCR5-tropic virus. Significant possible side effects include an allergic reaction (rash) and liver toxicity. Other side effects can include cough, fever, cold, muscle/joint pain, stomach pain, and dizziness. If MVC is used, doses of other medications may have to be adjusted (including but not limited to NNRTIs, PIs, oral contraceptives, certain other antimicrobials). Another potential issue is that other cells of the immune system exhibit CCR5 receptors, and MVC may affect them and potentially increase the risk of other infections and cancers. As of the date of writing, this has not been seen in clinical trials.

IBA or ibalizumab (Trogarzo) is a monoclonal antibody approved for use in HIV by the FDA in March 2018. It is the first of this type of biologic medication approved for use as a treatment for HIV. It was specifically approved for use in individuals with multiple resistances to other HIV medications. It is not approved for use other than in this multidrug-resistance scenario. It works by binding to CD4 receptors on the T-lymphocytes and interferes with HIV from properly binding to those same receptors, thus blocking HIV's entry into the cell. Notably, it does not interfere with the CD4 lymphocyte's normal functioning. It is administered by intravenous infusion, with a single initial loading dose of 2000 mg, followed by 800 mg infusions every 2 weeks. It must be combined with other oral antiretrovirals, and resistance testing is imperative to identify those medications with at least some anti-HIV activity to ensure success. The main common side effects include diarrhea, dizziness, nausea, and rash. It can also trigger immune reconstitution inflammatory syndrome (IRIS). The most common laboratory abnormalities include creatinine and bilirubin elevations. There are no known drug interactions between Trogarzo and other medications (including the HIV antiretrovirals).

Integrase strand transfer inhibitors. ISTIs are one of the newer classes of antiretroviral medications, first approved for use by the FDA in 2007. These drugs disrupt the integrase enzyme, which facilitates the incorporation of HIV DNA into human DNA in the T cell and thereby prevents viral replication at that site.

Four ISTIs currently exist:

- RAL: raltegravir (Isentress)
- DTG: dolutegravir (Tivicay)
- EVG: elvitegravir (Vitekta; no longer available as a stand-alone; found only in the STRs Genvoya and Stribild)
- BIC: bictegravir (approved by the FDA in February 2018 as part of a new STR medication; it will likely not be approved as a stand-alone)

Both RAL and DTG are used quite extensively in many multidrug regimes.

ISTIs are relatively well tolerated, are easy to take, and have demonstrated a remarkable ability to increase CD4 counts and drop viral load to undetectable levels in highly treatment-experienced patients with significant resistance issues. They are now part of many first-line initial regimes based on this effectiveness and tolerability. The most common side effects include diarrhea, nausea, headache, and fever. Some other possible issues may include hypersensitivity reaction, anemia, and liver toxicity. They are metabolized by glucuronidation, and there is no interaction with the CYP450 system. Care should be taken with any drug that affects glucuronidation. Divalent cations (calcium, magnesium, zinc, iron) can bind ISTIs and can potentially interfere with absorption and activity. This includes vitamins, minerals, and antacids containing any of these minerals, and it is recommended that ISTIs not be taken with 2 hours of any of these formulations.

Pharmacokinetic enhancers. The PKs are medications that have no inherent anti-HIV activity but are used in low doses in combination with other classes of medications to boost their blood levels and make them more effective. This mechanism was previously mentioned with regard to "boosted" PIs. The PKs work by inhibiting CYP-3A4, which is a key enzyme in the detoxification pathway of certain antiretroviral medications. Because they affect CYP-3A4, there are significant interactions between any medication, herb, or nutritional supplement that involve this enzyme. Caution is therefore advised.

- RTV: ritonavir (Norvir) has been in use for the longest and has traditionally been used with protease inhibitors. It has previously been

categorized as a protease inhibitor but is included here because this most closely describes its function. It is not particularly well tolerated, with significant GI side effects, and is rarely used anymore.

- COBI: cobicistat (Tyboost) is the newest of this class and is typically better tolerated. It can raise serum creatinine and decrease creatinine clearance, an effect that is reversible when stopped. It is used in some STRs (Stribild and Genvoya) and two boosted PIs (Evotaz and Prezcobix).

Single-tablet regimens. Over the past several years, there has been a trend in the medical treatment of HIV toward the use of multiclass combination STRs. The STRs are single tablets that are taken once daily and consist of a full complement of fixed doses of the medications needed to effectively suppress HIV replication. These formulations have allowed for the simplification of HIV treatment, resulted in a decrease in issues with adverse effects, and significantly reduced the daily pill burden for those individuals taking them. The STRs are now commonly used as initial treatment as well as an alternative for patients who have been on other, more complicated regimes. Side effects and interactions are all related to the individual drugs that make up each single tablet, and it is recommended that each be researched. As previously mentioned, all STRs (with the sole exception of Juluca) have as their backbone NRTIs, to which some other class of medication is added. In some instances, a PK is included; others do not include a PK.

NRTI- and NNRTI-based STRs include the following:

- Atripla: FTC, TDF, EFV
- Complera: FTC, TDF, RPV
- Delstrigo: 3TC, TDF, DOR (approved by the FDA August 2019; note that it contains TDF rather than the safer TAF)
- Odefsey: FTC, TAF, RPV
- Symfi and Symfi Lo: 3TC, TDF, EFV (both approved by the FDA in 2018)

NRTI- and ISTI-based STRs include the following:

- Biktarvy: FTC, TAF, BIC (approved by the FDA in November 2017 as maintenance therapy in already virally suppressed individuals; now used as an initial regime)
- Dovato: DTG, 3TC (approved by the FDA in April 2019 as an initial regime in individuals where ABC, TAD, TAF cannot be used)
- Genvoya: FTC, TAF, EVG, COBI
- Stribild: FTC, TDF, EVG, COBI

NNRTI: and ISTI based:

- Juluca: DTG, RPV (approved by the FDA in November 2017 for maintenance therapy in an already virally suppressed individual)

NRTI- and PI-based STRs include the following:

- Symtuza: FTC, TAF, DRV, COBI (the first of the PI-based fixed-dose single-tablet treatments; FDA approved on July 17, 2018)

New Approaches. One promising new approach to antiretroviral therapy involves the use of long-acting injectable medications as an alternative to daily oral regimens. These are given at predetermined intervals and alleviate the need for patients to take daily medications. The Latte 2 trial is a Phase IIb trial that used an investigational ISTI (cabotegravir) with an existing NNRTI (rilpivirine). Once the study subjects were virally suppressed on an oral medication regime, they were switched to the injectable medication combination at either 4- or 8-week intervals, with another group maintaining the oral regime as controls. After 32 weeks, 94% of the 4-week group, 95% of the 8-week group, and 91% of the oral group maintained viral suppression. At the 96-week mark, 87% of the 4-week group, 94% of the 8-week group, and 84% of the oral group maintained viral suppression. The main side effects were site reactions and pain, both of which were more pronounced in the 4-week versus 8-week group. The patients involved in the study reacted quite favorably to it.[66,67] The results at 96 weeks for the 8-week study group are quite impressive, and the use of injectable antiretrovirals may prove to be a viable strategy for treating individuals who are in situations where they cannot effectively maintain a daily oral regimen.

Establish a Foundation of Health and Wellness on the Basis of Naturopathic Principles

The naturopathic standard of incorporating healthy diet and lifestyle practices cannot be overemphasized for HIV+ individuals. These patients are likely to be taking a large number of medications, nutritional supplements, or both, resulting in additional biochemical stress on the body. Therefore each patient should be clearly encouraged and continually reminded to maintain dietary habits designed to ensure optimal GI health and nutritional intake.

The most important recommendation is to encourage a high intake of filtered or safe water and fiber so as to decrease oxidative stress and reduce the toxic load of the HIV and medications.[68] These patients should also be encouraged to maintain an optimal intake of protein.[69,70] Protein requirements can be as high as 100 to 150 g per day, particularly when patients are experiencing malabsorption and/or diarrhea; it may be necessary to provide supplemental protein to prevent weight loss. Studies using large doses of whey protein and amino acids (L-glutamine, L-arginine) have proved effective in reversing or preventing HIV-induced wasting.[71–73] Essential fatty acids (EFAs) have also been found to be deficient in HIV+ patients. Studies have demonstrated that HIV+ patients consuming generous amounts of EFAs have an increased body cell mass and a decreased risk of progression to AIDS[74–76] as well as improved symptoms of depression.[77]

HIV+ patients and healthy subjects ingesting fruit juices or a fruit-vegetable concentrate on a long-term basis were found to have higher micronutrient and antioxidant levels.[78,79] From this, it can be concluded that HIV+ patients should be continually encouraged to include colorful vegetables and fruits in their diets to help maintain levels of essential micronutrients and fiber.[80,81]

HIV+ patients commonly require appetite stimulation, digestive enzyme support, or both to combat the adverse effects of HIV, HAART, and prophylactic antibiotics on the GI system.[82,83] Further, with high oxidation already present in the digestive system, all patients should be strongly encouraged to avoid additional GI stressors such as high-sodium foods, high-fructose corn syrup in processed foods, alcohol, caffeine, fried foods, and cigarette smoke. Raw eggs, unpasteurized milk, undercooked meat or fish, and potentially contaminated foods must also be avoided to decrease the risk of gastrointestinal OIs and parasites.

Naturopathic physicians should exercise extreme caution in considering allergy-elimination diets in HIV+ patients owing to the likelihood of nutrient deficiencies, maldigestion, and malabsorption caused by HIV itself and HAART medications. A safer alternative is to recommend replacement of common food allergens one food at a time to make sure that patients can maintain replacement diets without decreasing caloric and nutrient intake over time. Removing food vices and irritating foods may be one of the most challenging aspects of moving HIV+ patients toward optimal wellness because there are so many other higher-priority challenges and a vital need to maintain body mass.

Lifestyle factors should be part of the naturopathic foundation with each HIV+ patient. Aerobic exercise has been demonstrated to provide benefit to individuals with immunodeficiency diseases, particularly through stress alleviation and mood enhancement. HIV+ individuals had increases in CD4, CD8, and NK cells immediately after aerobic exercise, and long-term exercise has demonstrated increases in immune parameters.[84,85] HIV+ individuals practicing Tai qi demonstrated a greater overall perception of health and significant improvements in several measures of physical function compared with

controls.[86] Other patients practicing yoga demonstrated an increased quality of life (QOL)[87,88] and reported increased self-confidence, reduced blood pressure,[89] and a quicker return to athletic activities after medical interventions.[90]

All patients should be encouraged to create an optimal sleeping environment and to sleep 8 to 10 hours each night. Sleep is essential for the repair and rebuilding of tissues and has been demonstrated to increase circulating NK cells and lymphocytes.[91,92]

Screen for obstructive sleep apnea. Uncontrolled sleep apnea can result in a host of adverse health issues that can be alleviated if the issue is identified and treated. Simple in-office screening can be done using the STOP-BANG questionnaire (http://www.stopbang.ca/osa/screening.php). Based on a patient's answers and situation, a score is obtained that gives a low, intermediate, or high likelihood of the presence of obstructive sleep apnea. If there is intermediate or high risk, clinicians should consider referring the patient for further evaluation with a qualified sleep medicine specialist.

HIV+ patients involved with group activities have better prognoses as well as decreased stress and anxiety. Anecdotal evidence links long-term AIDS survival with one or more of the following: having a positive attitude toward the illness, participating in health-promoting behaviors, engaging in spiritual activities, and taking part in activities that support the HIV+ community. Structured, brief group intervention for bereavement due to the loss of loved ones has been shown to decrease plasma cortisol and improve several immune markers in patients with HIV.[93,94] Other forms of stress relief and management have proved effective for HIV+ patient health care. Cognitive-behavioral stress management is a guided form of relaxation training. It has been shown to improve the quality of life in HIV+ women, decrease herpes simplex 2 antibody titers in HIV+ men, improve HIV laboratory values, and have lasting effects on quality of life and psychological well-being of patients.[95–100] Mindfulness-based stress reduction was able to demonstrate increased mean CD4 counts in patient HIV+ ART-naïve patients.[101]

Engaging in prayer, including distance healing, has demonstrated fewer new AIDS-defining illnesses, decreased illness severity, significantly fewer doctor visits, fewer hospitalizations, and significant improvements in mood.[102,103] For individuals not on HAART, participation in spiritual activities (prayer, meditation, affirmations, psychic healing, visualizations) resulted in a reduced risk of death over a 1-year period.[104] Finally, laughter has been associated with improvement in WBC values and decreased stress.[105]

Establish a Core Nutrient Protocol

Replace known nutrient deficiencies caused by HIV. Vitamin and mineral replenishment has been demonstrated to be efficacious in HIV+ patients.[106–109] A double-blind placebo-controlled study involving 1078 pregnant Tanzanian women infected by HIV reported that taking vitamin A (preformed vitamin A and beta-carotene) and a low-cost multivitamin (vitamins B, C, and E) resulted in delayed progression to AIDS as well as a delay in the requirement to initiate antiretroviral therapy. Adding the low-cost multivitamins was statistically significant versus placebo or vitamin A alone.[110] Numerous vitamin and mineral deficiencies have been identified with HIV disease progression.[111] These deficiencies are likely due to loss, poor absorption, or rapid use and consumption.

Box 178.4 summarizes the nutrients that have been found to be deficient in patients with HIV. Typical daily doses required to replete deficiencies taken in divided doses throughout the day are noted in parentheses followed by the rationale or benefits.

Nutrient deficiencies (beta-carotene, vitamin D, acetyl-L-carnitine, dehydroepiandrosterone [DHEA], testosterone, reduced glutathione, and catalase) not commonly included in a multivitamin/multimineral supplement must be replaced through specific dietary regimens or through additional supplementation. Natural forms of beta-carotene should be used because evidence suggests that synthetic forms can increase the risk of lung cancer in individuals who smoke.[112] Consider acetyl-L-carnitine as a high-priority nutrient, despite its high cost, on the basis of the number of benefits.

Provide other nutrients to support optimal immune function. Box 178.5 summarizes nutrients that have been found to replace or reduce other nutrient deficiencies or have beneficial action in patients with HIV, their daily doses divided throughout the day, and effects of supplementation.

All these nutrients have benefits, but they significantly increase the overall cost and pill burden. Further, despite the positive evidence and outcomes demonstrated integrating CAM with HIV, there have been a few recent studies, including a systemic review, that have reported CAM therapies potentially interfering with HAART.[113–116] In these negative studies, the antiretroviral therapies referenced are no longer commonly being prescribed outside of less developed regions of world, the forms (chelates) of CAM that were used were often inferior forms, the populations being studied were often nutritionally unique (African), and many of the findings were debatable. These negative studies clearly emphasize the need to understand the mechanisms and interactions for the best integration of CAM and HAART.

With pill burden and efficacy in mind, it is suggested that priority be given to the most essential (EFAs and DHEA) and lower-cost (vitamin C) nutrients or to address specific symptoms, as described in the next section.

Provide Therapies to Address Constitutional Symptoms, Medication Side Effects, and Symptoms of Immunosuppression

The following are some approaches for specific health conditions and OIs. The naturopathic physician should endeavor to identify and remove the cause, tonify systems, and treat each patient individually.

Candida albicans/Oral Thrush. Overgrowth of this fungal organism may occur as a result of HIV itself (a low CD4+ count, reducing the body's immune capacity) or secondary to prophylactic antibiotic treatment. Orally, it can cause pain and make eating difficult, which can lead to reduced caloric intake and subsequent problems. In the intestines, it can cause inflammation and compromise nutrient absorption. In the esophagus, it can cause pain and should be dealt with swiftly via antifungal pharmaceuticals.

For treatment, patients should avoid foods that promote yeast growth, such as simple carbohydrates, sugar, baked goods, and starchy vegetables. These options should be considered:

- Probiotics: Provide and support healthy flora via the consumption of fermented foods as well as using supplements with at least 8 billion colony-forming units (CFUs), best taken with food. Beneficial species include *Lactobacillus acidophilus/bifidus; Lactobacillus* GG, a well-researched strain from Germany; and *Saccharomyces boulardii,* which is best for antibiotic-induced colitis and can help displace pathogenic flora.[117–121]
- Garlic (4 g/day, approx. = 1 clove, best taken before meals): Garlic is a potent antifungal found to spare nonpathogenic flora; note that patients on HAART should not take supplemental garlic.[122] Consider incorporating it into the diet.
- Oregano oil (300 mg best taken away from food): This is also a potent antifungal.[123]
- Nystatin, clotrimazole, and fluconazole: These are pharmaceutical antifungals.

BOX 178.4 Nutrient Deficiencies in HIV Patients

- Vitamin A (15,000–30,000 IU taken with food) slows progression to AIDS and decreases mortality, improves growth in infants, decreases stunting associated with chronic diarrhea, and prevents GI deterioration in mothers and infants.[253–256] May increase the risk of HIV transmission by breastfeeding but has no effect on mortality by 24 months.[257] Deficiencies can be due to decreased dietary intake, poor GI absorption, high urinary loss, impaired hepatic protein synthesis, and increased needs due to chronic infection.
- Beta-carotene (60–120 mg/150,000 IU taken with food) replenishment increased CD4+ count, CD4/CD8 ratios, and total lymphocyte count and decreased mortality.[258–260] Deficiency found in all HIV+ patients is likely due to poor digestion, decreased free-radical elimination, and high lipid peroxidation.[261]
- Folate (400 mcg) normalized cell differentiation. Deficiency is most likely due to malabsorption; AZT-induced deficiency may increase the risk of bone marrow toxicity.[262,263]
- Vitamin B_1 (50 mg) replenishment is associated with increased survival in HIV+ patients and decreased progression to AIDS.[264–266]
- Vitamin B_6 (50 mg) is essential in nucleic acid and protein metabolism and cellular and humoral immune responses.[267,268] Vitamin B_6 repletion both alone and in conjunction with CoQ_{10} increased circulating IgG, CD4+ cells, and CD4/CD8 ratios.[269]
- Vitamin B_{12} (1000 mcg hydroxy-, methyl- or cyanocobalamin best taken via intramuscular injections 3 times a week—alternate form is daily 1000 mcg sublingual) is important in several parameters of immune function, in proper cell differentiation and nerve function, and in decreasing homocysteine levels. Vitamin B_{12} repletion can improve lymphocyte counts, CD4/CD8 ratios, and NK-cell activity.[270] Supplementation has also been found to reverse AIDS dementia complex when associated with low levels.[271] Deficiency has been associated with an increased risk of progression to AIDS and with HIV disease in general.[272–274]
- Vitamin D (5000 IU) deficiency commonly found in urban HIV-infected men with suppressed viral load and CD4 >200; tobacco use was correlated with severe deficiency.[275] Undetectable levels of vitamin D in HIV+ patients correlated with more advanced HIV infection, lower CD4 count, and higher levels of TNF-α.[276] Repletion of vitamin D decreased bone turnover markers[277] and improved some clinically important HIV immune markers in children and young adults.[278] Lower bone-mineral density was found in HIV patients using tenofovir/emtricitabine combination (TDF, FTC).[279] Recently, it was determined that switching from combination therapy to Darunavir/ritonavir monotherapy resulted in significant improvements in vitamin D.[280] Vitamin D with calcium was also demonstrated to minimize bone loss with the initiation of therapy.[281]
- Vitamin E (800 IU daily—mixed tocopherols taken with food) is indicated to decrease lipid peroxidation, protect against AZT-induced oxidative damage to cardiac mitochondria, normalize immune function, and slow progression to AIDS.[282–287] Deficiency is found in most HIV+ patients with wasting and in progression to AIDS.[288]
- Copper (2 mg) can inhibit HIV protease and viral replication.[289] Deficiencies of copper are associated with AZT therapy and AIDS.[290,291]
- Magnesium (300 mg) deficiency is found in AIDS patients.[292,293]
- Selenium (400 mcg) to suppress the progression of HIV-1, decrease viral burden, and provide indirect improvement of CD4 count as well as reducing the rate of decline.[294,295] Selenium has been found to decrease HIV-associated mortality, decrease anxiety in HIV+ recreational drug users, and decrease hospitalizations and costs of caring for HIV+ patients.[296–299] The severity of deficiency in patients progressing to AIDS may be due to decreased calorie and protein intake, malabsorption, and various infections.[300]
- Zinc (15 mg—optimal intake levels have not been determined) has been found to decrease the frequency of OIs.[301,302] Deficiency is noted in all HIV+ patients progressing to AIDS.[303] Higher pre-ART Zn levels were associated with significant increases in CD4% at 48 weeks.[304] Zinc was also found to decrease diarrhea and upper respiratory tract infections in HIV+ infants.[305]
- Acetyl-L-carnitine (2–6 g, best taken away from other proteins to optimize absorption) is essential for proper energy supply as well as critical metabolic functions. Deficiency is common in HIV+ patients and increases the risk for alterations in fatty-acid oxidation.[306–308] Repletion has also been linked with a reduction in serum triglycerides, decreased risk of wasting, increase in CD4 cells and reduced apoptosis, increased levels of serum insulin-like growth factor, reduction in mitochondrial neurotoxicity, and treatment of peripheral neuropathy.[309–314] It has also been demonstrated as an effective treatment for NRTI-induced lactic acidosis.[315]
- DHEA (15–50 mg taken with food) repletion helps increase CD4 count, stimulate immune function, and improve quality of life.[316,317]
- Testosterone (intramuscular injection weekly) is indicated if serum levels are low to decrease the loss of lean body and muscle mass.[318]
- Glutathione/GSH (increased through selenium and N-acetyl-cysteine or whey protein powder) has been found to decrease disease progression and mortality.[319–322] Cysteine with glycine increased insulin sensitivity, body composition, and muscle strength in older HIV-infected patients.[323] Deficiencies are found in both symptom-free HIV+ and AIDS patients.[324,325]
- Many of these nutrients can be replenished through the use of a highly nutritious diet and a good multivitamin. A few multivitamins have been specifically developed to replace the deficiencies of HIV+ patients and include high doses of carotenoids, B vitamins, antioxidants, and sometimes digestive enzymes. Taking one multivitamin designed specifically for HIV reduces pill burden and enhances compliance. Conversely, it is generally more cost-effective to use a basic multivitamin and supplement individually with additional nutrients.

AIDS, Acquired immunodeficiency syndrome; *ART,* antiretroviral therapy; *AZT,* azidothymidine/zidovudine; *CoQ_{10},* coenzyme Q_{10}; *DHEA,* dehydroepiandrosterone; *GI,* gastrointestinal; *GSH,* growth-stimulating hormone; *HIV,* human immunodeficiency virus; *NK,* natural killer; *NRTI,* nucleoside/tide reverse transcriptase inhibitors; *OI,* opportunistic infection; TNF-α, tumor necrosis factor-alpha.

Cardiovascular disease. The issue of cardiovascular disease is becoming increasingly problematic and prevalent, particularly in the aging HIV+ population. The adverse effects on serum lipids and glucose that accompany the use of HAART medications (specifically PIs and D-type NRTIs) significantly promote cardiovascular disease, particularly in patients already at high risk (where there are predisposing factors such as a positive family history).

All patients on HAART should be encouraged to institute a preventive diet, adopt a healthy lifestyle, and implement exercise recommendations to promote cardiovascular health. Exercise in particular has been demonstrated to increase functional aerobic capacity, eliminate functional aerobic impairment, and improve measures of exercise performance (improved peak oxygen consumption, oxygen pulse, tidal volume, ventilation, and leg power).[124,125] Garlic should be avoided owing to its adverse interaction with HAART medications. The following specific nutrients should be considered:

- Acetyl-L-carnitine (2–6 g best taken away from other proteins to optimize absorption) repletion has also been linked with a reduction in serum triglycerides.
- CoQ10 (100–300 mg) may enhance cardiovascular function.[126]
- Arginine (7.4 g taken away from food) decreases atherosclerosis.[127]

BOX 178.5 Nutrients That Have Been Found to Replace or Reduce Other Nutrient Deficiencies or Have Beneficial Actions in HIV Patients

- *Silybum marianum*/milk thistle (300 mg standardized extract away from HAART) is indicated for all patients on HAART to improve liver function, decrease liver damage, and increase the antioxidant activity of blood cells.[326]
- NAC (2–8 g best taken away from other nutrients but may need to be taken with food to decrease GI distress) is indicated to prevent loss of sulfur-containing amino acids, increase GSH, decrease TNF-α activity, and increase CD4 cell count.[327–329]
- CoQ_{10} (100–300 mg) optimized mitochondrial function and replaced deficiency.[330,331] Repletion with CoQ_{10} increased circulating IgG, CD4+ cells, and CD4/CD8 ratios.[332,333]
- Vitamin C (2–6 g) when combined with vitamin E significantly lowered oxidative stress and has demonstrated a trend toward lowered viral load.[334]
- Alpha-lipoic acid (600 mg) protected the liver, inhibited viral replication, increased intracellular GSH, increased CD4/CD8 ratios, and potentially decreases peripheral neuropathic pain by its antioxidant effect on nervous tissue.[335–340]
- L-arginine (7.4 g taken away from food) improved lean body mass and increased NK-cell activity. Must be used with caution because large amounts of arginine can aggravate herpesvirus outbreaks. Prophylactic L-lysine may prevent or reduce this effect.
- EFAs (5 g taken with food; best from fish oil) improved lean body mass,[341] improved outcomes from ART,[342] increased NK-cell activity, and were beneficial as adjuvant therapy in patients with tuberculosis.
- L-Methionine (1000 mg taken away from other proteins) slowed the decline of CD4 cells.[343]
- Phosphatidylcholine-containing food or supplements can offer protection against HAART-induced hepatoxicity.[344]

ART, Antiretroviral therapy; *CoQ_{10},* coenzyme Q_{10}; *EFAs,* essential fatty acids; *GSH,* growth-stimulating hormone; *HAART,* highly active antiviral therapy; *IgG,* immunoglobulin G; *NAC,* N-acetylcysteine; *NK,* natural killer; *TNF-α,* tumor necrosis factor-alpha.

- EFAs (up to 6 g daily taken with food) have been clearly demonstrated to lower triglycerides and markers of inflammation including, IL-6 and TNF-α,[128–130] as well as lower lipids.[131]

Diarrhea. Diarrhea is a common complaint and an issue that can greatly affect quality of life. An HIV+ person might develop diarrhea for numerous reasons; the specific etiology guides the choice of treatment. Causes might include HIV-associated enteropathy, HAART side effects (see specific medications—this is particularly an issue with PIs), antibiotic side effects, GI infections, and food allergies or intolerances. An osmotic-type diarrhea can also result from the consumption of too many indigestible gel caps (from medications and supplements) building up in the intestinal lumen.

The first treatment priority is to remove the cause if at all possible. There is an increased incidence of gluten intolerance in HIV+ individuals.[132] Lactose intolerance and irritable bowel syndrome are also possibilities. Food allergies/sensitivities should be cautiously ruled out, with consideration given to the patient's general nutritional status to ensure the safety of trial food eliminations. Stool cultures and testing for ova and parasites should be considered to screen for suspected infectious causes, and patients should be treated as indicated for each microbial etiology. All nutrient supplementation may be stopped on a temporary basis to screen for an osmotic cause and then reintroduced one at a time to determine potential causes.

Steps for prevention include avoiding unfiltered tap water, unpasteurized milk or dairy products, ice made from unfiltered tap water, raw fruits and vegetables unless they can be peeled personally or washed with appropriate antimicrobial agents, raw or rare meat and fish, meat or shellfish that is not hot when served, and food from street vendors. Dehydration can be prevented by replacing electrolytes; broths, soups, fruit and vegetable juices, and high-nutrient drinks should be included.

Prevent malnutrition; if diarrhea is present, malabsorption of nutrients is a likely consequence. Ensure adequate micro- and macronutrient intake with a multivitamin/multimineral supplement and maintain adequate protein intake (requirements may increase to 100–150 g/day). Initial dietary intervention may include simplifying the diet (BRAT diet: *b*ananas, *r*ice, *a*pplesauce, *t*oast) or using 1 tbsp tomato juice combined with 1 tbsp sauerkraut juice (a protocol taught by Dr. John Bastyr to two generations of naturopathic students and used with considerable clinical success by the senior editor to facilitate electrolyte replacement and promote enterocyte healing). Carob powder is an astringent that can help symptomatically. Begin with 1 tsp in applesauce and increase up to 6 tsp/day as needed.

Consider these additional supplements:

- Provide and support healthy flora as described earlier in the care of candidal infection.[117–121]
- L-Glutamine (9–40 g best taken away from other proteins) is an amino acid that provides fuel for small intestinal enterocytes. Using a powder is most efficient and cost-effective. Start with 3 g three times daily. This can be increased up to 40 g daily if necessary. This high dose can lead to psychoses, so the patient should be monitored closely.[133]
- Diphenoxylate and atropine (Lomotil), loperamide (Imodium), and psyllium husk (Metamucil) are common pharmaceutical interventions.

Herpes simplex virus/herpes zoster/shingles. All conditions with a herpes-type virus etiology are considered together because the treatment approach is similar for each. The sequelae of herpesviruses tend to manifest on the skin in a dermatomal pattern of distribution. These symptoms can occur secondary to various types of stress, hormonal fluctuations, or a poor immune system response. First and foremost, try to identify and remove causes of stress (including dietary, emotional, and sunlight exposure) and support immune function. Secondarily, address the symptoms (most often, pain is at the forefront).

Naturopathic physicians should consider the following:

- Dietary suggestions include eliminating foods high in L-arginine (promotes herpes replication), such as chocolate, nuts, and peanuts, and increasing foods high in L-lysine (antagonizes L-arginine), such as whole grains, dairy, fish, lima beans, and soy.
- L-lysine: 1000 mg three times daily away from food during an outbreak; 500 mg three times daily away from food prophylactically.[134]
- Monolaurin: this antiviral, taken at 300 to 600 mg three times daily, is effective against encapsulated DNA viruses.[135]
- *Melissa officinalis:* an antiviral botanical used topically.[136]
- *Glycyrrhiza glabra:* an antiviral oral and topical botanical.[137]
- Acyclovir/valacyclovir: pharmaceutical antiviral therapy.

Kaposi sarcoma. Kaposi sarcoma (KS) is an HIV-associated neoplasm of vascular origin that tends to occur when CD4+ counts fall below 500/mm^3. KS is rarely found outside of the HIV+ population and is an issue that is becoming far less common in this era of early treatment through HAART. The etiology of AIDS-associated KS is poorly understood, but several factors are thought to contribute, including the presence of human herpesvirus (HHV) type 8, a compromised immune response, and hormones (it rarely occurs in women, and androgen therapy causes increased proliferation of the tumors). It is most commonly found in iron-rich geographical areas.

Interestingly, 90% of AIDS-related cases in the United States occur in homosexual men, making it rare in heterosexual men, women, and intravenous drug users.

KS begins as macules or elevated papules and can progress to large plaques or nodules. The color ranges from pink to purple or brownish-black. The lesions may be round or oval and do not blanch when pressure is applied to them. The lesions often appear first on the skin of the upper body or mucosal surfaces. In their aggressive form they can become widely disseminated and can manifest anywhere on the skin, mucous membranes, lymph nodes, or viscera (including the GI tract from the mouth to anus, lungs, liver, spleen, or pancreas).

KS tumors secrete increased amounts of angiogenic growth factors, TNF-α, IL-6, basic fibroblast growth factor, platelet-derived growth factor, and oncostatin-M.[138–143]

The level of immunosuppression of an affected person determines the clinical course of KS in AIDS. Currently, there are no known cures; the various local and systemic approaches are aimed at palliation.

Treatment may involve the use of chemotherapeutic agents such as doxorubicin as well as standard HAART. Surgical excision can successfully remove a lesion, but there is a high rate of recurrence at the affected site. Other researched options that have produced varying results include radiation therapy, cryotherapy with liquid nitrogen, and electrical stimulation with direct current.

The following treatments should also be considered:

- Topical and systemic retinoids[144]
- Topical vitamin D (1, 25-dihydroxyvitamin D3)[145]
- Iron has been shown to stimulate the growth of KS cells in vitro, whereas iron chelators reduced their growth.[146,147] Given this information, it may be sensible to limit iron intake and ensure that serum iron and ferritin levels are at the low end of normal.
- The use of various cytokine inhibitors and antiangiogenesis compounds might have a benefit for reducing the proliferation of these vascular tumors.
- Because there are no known curative therapies and the palliative treatment of KS is difficult at best, naturopathic physicians have an opportunity to be creative and contribute in this area. Given the association between KS and HHV type 8, it may be beneficial to use antiherpetic therapies if a patient is known to be herpes simplex virus–positive.[148] Topical application of a medicinal peat preparation has resulted in remission of topical KS lesions in several patients. Glutathione (NAC as a precursor) could be helpful in the treatment of KS lesions as well.[149–151]

Lipodystrophy. Lipodystrophy is a redistribution of adipose tissue from the extremities to the trunk. It often manifests with a Cushingoid "buffalo hump" over the upper thoracic region of the back or with truncal obesity. Self-image issues are often most significant for patients experiencing this condition. Lipodystrophy can occur as a result of alterations in lipid metabolism secondary to HAART or simply chronic HIV infection (greater than 15 years).[152] Given the appearance of the buffalo hump, it is possible that adrenal abnormalities contribute to the problem. Increases in serum cortisol lead to increases in insulin release from the pancreas, which drives blood glucose into storage in adipose cells. This leads to increased truncal obesity,[153–156] a physiological mechanism that bears consideration in the therapeutic approach as well as in future research. Lipodystrophy has also been found to increase with lack of exercise.[157] In addition to correcting dietary nutrient insufficiencies, supplementing nutrients as per the general protocol, increasing exercise, and ensuring that blood glucose is appropriately regulated, the use of the following can be considered:

- L-Glutamine (10 g in divided doses away from food)[158,159]
- Acetyl-L-carnitine (2–6 g away from food) to optimize mitochondrial function[160–162]
- Uridine (36 g three times daily for 10 consecutive days per month) to normalize subcutaneous fat[163]
- Testosterone (10 g of testosterone gel daily) in HIV+ men with abdominal obesity and low testosterone; decreased whole-body, total, and abdominal fat mass; and increased lean body mass[164]
- Nandrolone decanoate (150 mg intramuscularly [IM] every 2 weeks) may increase lean body mass more effectively than testosterone.[165]
- Tesamorelin (Egrifta), a growth hormone–releasing factor analogue (2 mg per subcutaneous injection given daily), decreases visceral adipose tissue and triglycerides without aggravating glucose.[166]
- Dimethyl sulfoxide (DMSO)/bromelain (5% bromelain compounded by the Key pharmacy in DMSO gel applied two to three times daily). Topical applications have resulted in the reduction of Cushingoid adipose deposits.
- Conventional treatment may include surgical removal of the truncal adipose deposits. Although this may not be the most desirable approach owing to its invasiveness, patients who undergo this procedure tend to be happy afterward because of their more positive self-image.
- Buccal injections of polylactic acid (PLA/Nufill) have been found to be safe and can reduce the cosmetic signs and depressive symptoms of lipodystrophy and wasting.[167]

Macrocytic anemia. Macrocytic anemia can be a secondary effect from the malabsorption of nutrients or a side effect of HAART (AZT causes bone marrow toxicity; PIs may cause malabsorption).[168,169] The following treatments can be considered:

- Vitamin B_{12} (hydroxocobalamin/methylcobalamin/cyanocobalamin 1000 mcg/mL given IM three times a week) is an important nutrient for proper cell division and differentiation.[170] If an IM injection is unavailable, sublingual tablets at 1000 mcg/day can be used to bypass intestinal absorption issues.
- Folic acid (activated form recommended) and vitamin B complex (both found in a good multivitamin supplement) are indicated for proper cell division, differentiation, and improved vascular reactivity.[171–173]

Neuropathy. This is usually manifested in the periphery, particularly in the feet. It can work its way proximally as it increases in severity. It is most often seen either as a result of long-term HIV infection or as a side effect of the D-type NRTIs and their toxic effect on the mitochondria in neurons.[174,175] Diabetes mellitus type 2 can also be a factor, as can overdoses of vitamin B_6 (greater than 200 mg/day).[176]

The following treatments can be considered:

- Acetyl-L-carnitine (2–6 g best taken away from other proteins to optimize absorption) decreases the risk for alterations in fatty-acid oxidation and energy supply, improves critical metabolic functions, and decreases pain.[177]
- EFAs (5 g/day with food) are anti-inflammatory agents and components of healthy cell membranes.[178]
- Vitamin B_{12} (1000 mcg/mL IM three times weekly) is an important nutrient for proper cell division and differentiation.
- Vitamin B complex (1 mL IM three times weekly) is indicated for proper cell division and differentiation.[179,180]
- CoQ_{10} (100–300 mg) is indicated to optimize mitochondrial function.[181,182]
- Alpha-lipoic acid (600 mg/day) may decrease pain.[183]

Psychological conditions. Issues such as depression, anxiety, posttraumatic stress disorder, sexual dysfunction, and substance abuse are extremely common in the HIV+ population. These problems potentially have physiological as well as psychological components but may not be adequately addressed by other providers.

Suicidal ideations and suicide attempts can also be an issue for this population. As quality of life deteriorates, the burden of ongoing

BOX 178.6 AIDS Wasting Syndrome Criteria

AIDS wasting syndrome must meet one of the following criteria:
Unintentional weight loss of >10% over 12 months
Unintentional weight loss of >7.5% over 6 months
BCM loss of <5% over 6 months
Men: BCM <35% of TBW and BMI <27 kg/m^2
Women: BCM <23% of TBW and BMI <27 kg/m^2
BMI <20 kg/m^2

BCM, body cell mass; *BMI,* body mass index; *TBW,* total body weight.

medical treatment increases, and close friends die, the desire to live can wane. It is critical that practitioners always be vigilant for signs of suicidal thinking or loss of hope in patients. These signs should be taken seriously, and there should be no hesitation in bringing in experienced professional help to assist these patients.

In contemplating the treatment of any psychological issue in this patient population, extreme caution must be exercised owing to issues of polypharmacy and drug–nutrient interactions. Many of these patients are taking a substantial number of pharmaceutical medications. Interactions among many nutrients and these medications and even among the medications themselves are likely. Before instituting any treatment, all potential interactions must be researched, identified, and considered.

An example of this problem is the potential use of St. John's wort, a commonly used herbal antidepressant. As previously discussed, St. John's wort is an inducer of the cytochrome P-450 detoxification enzyme 3A4 in the liver and has been found to reduce serum levels of various HAART medications. Therefore it can theoretically reduce the effectiveness of those medications and potentially lead to increased issues with resistance. The use of St. John's wort should be avoided in any patient who is undergoing HAART.

Modalities such as counseling, lifestyle modification, and homeopathy can be quite useful in treating these psychological conditions. Meditation and mindfulness have been found to be effective for psychological health. For example, a mindfulness-based stress reduction meditation program showed stabilization of CD4+ T-lymphocyte counts independent of antiretroviral medication use.[184] In another study, mantra repetition—a word or phrase with spiritual associations repeated silently throughout the day—was found to reduce anger as well as to increase spiritual faith and spiritual connectedness; it was inversely associated with non-HIV-related intrusive thoughts and positively associated with quality of life, total existential spiritual well-being, and meaning/peace.[185,186] Additional therapies to consider are as follows:

- Multivitamin supplementation (B complex, C, and E) resulted in a reduction in the risk of elevated depressive symptoms and improvement in quality of life.[187]
- Androgens (DHEA 50 mg or testosterone—in supraphysiological doses of 300 mg IM weekly) can improve mood, quality of life, and overall survivability and also decrease fatigue.[188,189]
- Amino acids (if levels are found to be low by specialty laboratory analysis) should be prescribed in combinations tailored to replenish deficiencies.[190,191]
- Zinc (25 mg/day) can serve to normalize levels and improve the efficacy of antidepressants.[192]
- EFAs (5 g/day with food) have been found to be useful as adjunctive treatment in depressive disorders.[193,194]
- SAMe (1600 mg/day) has been shown to be as effective as imipramine (150 mg) in treating major depression and is better tolerated.[195]

Wasting. In 1987, the CDC defined HIV-associated wasting syndrome as an involuntary weight loss of greater than 10% of baseline body weight accompanied by chronic fever, weakness, or diarrhea. A more complete definition is available in *The AIDS Reader*[196] (Box 178.6).

Body cell mass (BCM) can be measured via bioimpedance assay. Research shows that BCM is a better predictor of survival than CD4 counts. Key issues to be addressed with patients include perceived weight loss, changes in appetite, diarrhea, energy level, and difficulties in performing activities of daily living.

The etiology of wasting is most likely multifactorial in nature; some possible etiologies include the following[197]:

- Decreased food intake—anorexia (decreased desire to eat or loss of appetite with corresponding decrease in food intake) or nausea secondary to medications, systemic illness, or GI pathology; finances; dependence on others; poor food choices.
- Malabsorption/chronic diarrhea—results in decreased nutrient intake; influenced by many pathologies and dysfunctions (affecting any part of the GI tract: oral, esophageal, stomach, pancreatic, biliary, hepatic, and small and large intestine); problems might include infections, medication side effects, enzyme deficiencies, and malignancies; all might result in the inability to absorb macro- and micronutrients.
- Alterations of metabolism—studies show that resting energy expenditure (REE) is higher in HIV/AIDS patients than in HIV– controls; studies also show the REE does not downregulate if anorexia or malabsorption is present (therefore the combination of decreased caloric intake with a metabolic rate that does not adjust to that state would result in a negative energy balance and weight loss).[198]
- Cytokine abnormalities—not well understood; TNF and IL-1 may induce anorexia and affect lipid, protein, and carbohydrate utilization; an example is cachexia in cancer.
- Endocrine abnormalities—thyroid abnormalities, adrenal insufficiency (cortisol, DHEA), hypogonadism (testosterone), growth hormone deficiency.
- Alcohol abuse—studies show that progression to AIDS is much quicker in HIV+ patients who consume large amounts of ethyl alcohol (ETOH) versus normal expectations; ETOH can stimulate HIV production, suppress immune defenses (lower CD4 cell counts), and deplete tissue micronutrients (specifically, antioxidants).

As per general recommendations, the naturopathic physician should endeavor to remove the cause if it is identifiable and removal is feasible. Then he or she should identify and remove possible food allergies/intolerances (gluten and dairy in particular) and ensure adequate and appropriate nutritional intake via dietary counseling; patients should also be helped to access social service assistance if necessary. Patients are likely to need superdoses of nutrients. Protein intake should be increased to 100 to 150 g/day. Patients should be active and engage in daily exercise.

The following therapies can be considered:

- EFAs (5 g/day with food), which are essential in a healthy diet[199]
- Acetyl-L-carnitine (2–6 g/day) to optimize fatty acidic metabolism[200]
- L-Glutamine (10–40 g), an essential amino acid for enterocytes[201,202]
- DHEA (50 mg) to promote lean body mass[203]
- Melatonin (20 mg at bedtime) to decrease cachexia and TNF[204]
- Cannabis (four times daily) or oral dronabinol (5–10 mg four times daily) to increase daily caloric intake and body weight[205]
- Megastrol acetate (a synthetic hormone similar to progesterone; 400 mg) to decrease cachexia[206]

BOX 178.7 Botanicals That Have Demonstrated Efficacy in Treating HIV

- *Glycyrrhiza glabra*/licorice (1500 mg) inhibited HIV fusion and viral transcription.[345–347] It should not be used in patients with a history of hypertension or with renal or cardiac problems.
- *Curcuma longa*/tumeric (1200 mg) inhibited HIV integrase and proteases and viral transcription and decreased nuclear factor-kappa B.[348–350]
- *Olea* sp./olive leaf extract (2–6 g) increased NK-cell function and was effective against HIV and herpesviruses.[351,352]
- *Phyllanthus amarus* (1200 mg) demonstrated in vitro and ex vivo HIV-1 inhibition of reverse transcriptase, receptors, and proteases and is also effective against HBV.[353]
- *Lentinus edodes*/shitake mushrooms (1–5 mg IV twice weekly) inhibited reverse transcriptase, increased CD4 counts, and decreased p24 (surface marker).[354,355]
- *Andrographis paniculata* (1500 mg) inhibited fusion, viral replication, and HIV-1 cell-to-cell transmission. It also stimulated objective measures of immune function, increased the effectiveness of AZT, protected against liver damage, and decreased diarrhea.[356] Toxicity questions remain; further study is necessary.
- *Silybum marianum*/milk thistle (300 mg extract) improved liver function and antioxidant activity of blood cells.
- *Hyssopus officinalis*/hyssop (1- to 4-mL tincture) exhibited anti-HIV activity and inhibited the integration of proviral genome into host genome.[357,358]
- *Prunella vulgaris*/self-heal (10 mg/mL IV) inhibited HIV replication and binding and prevented cell-to-cell infection.[359,360]
- *Rosmarinus officinalis*/rosemary (use liberally in food) decreased HIV replication and protease activity.[361]
- *Momordica charantia*/bitter melon (6 oz fresh juice) normalized CD4+/CD8+ ratios and inactivated viral DNA.[362]
- *Spirulina platensis*/blue-green algae (use liberally in food) reduced fusion and viral production.[363]
- *Scutellaria baicalensis*/skullcap (6–15 g whole root) inhibited HIV-1 reverse transcriptase.[364]
- *Podophyllum* resin (25% solution as a single topical application) resulted in resolution of hairy leukoplakia.[365]
- *Melaleuca leucadendron*/tea tree oral solution was effective for fluconazole-refractory oropharyngeal candidiasis in patients with AIDS.[366]
- *Hypericum perforatum*/St. John's wort (900 mg) inhibited protein kinase C and viral uncoating, fusion, and assembly.[367] Do not use in patients on NNRTI or PI.
- *Allium sativa*/garlic (4 g fresh garlic) selectively killed HIV-1 infected cells in vitro.[368] It should not be used in patients on NNRTI or PI.

AIDS, Acquired immunodeficiency syndrome; *AZT,* azidothymidine/zidovudine; *HBV,* hepatitis B virus; *HIV,* human immunodeficiency virus; *IV,* intravenous; *NK,* natural killer; *NNRTI,* nonnucleoside reverse transcriptase inhibitor; *PI,* protease inhibitor.

- Testosterone in hypogonadal men to increase lean body mass and muscle strength[207]
- Anabolic steroids (nandrolone decanoate, oxandrolone, oxymetholone) to increase lean body mass[208,209]
- Thalidomide, a cytokine modulator, to increase BCM and extracellular fluid[210]

Provide Specific Antiviral Therapy and Immunomodulation Using Other Nondrug Treatments

This section summarizes the botanical, homeopathic, and physical medicine studies that have been found to have potential for patients with HIV. They are not substitutes for HAART. This list is intended to assist health providers in finding additional nondrug solutions that resonate with the unique needs of individual HIV+ patients. Further, the majority of the following studies are based on initial or empirical findings encouraging additional areas for research and exploration.

Botanical Medicines

Box 178.7 includes botanicals that have demonstrated efficacy in treating HIV. Care must be taken to understand the constituents and metabolism of these substances to ensure that there are no interactions with the patient's medications or other treatments.

Homeopathy

Homeopathy may offer a most interesting possibility in the care of HIV+ patients. The *British Journal of Homeopathy* published a report on the use of constitutional homeopathic remedies (30C-1M doses) given to 129 asymptomatic HIV+ patients in India. As a result, 12 patients became ELISA negative after 3 to 16 months.[211] In another study, 100 HIV+ patients between 17 and 50 years of age (71% men) were randomized to receive either a single homeopathic remedy or a placebo. The experimental group receiving remedies showed statistically significant increases in CD4+ T cells after 6 months.[212]

Other studies in homeopathy have demonstrated success with symptom care in HIV.[213] For example, Bissuel and colleagues[214] reported on the use of homeopathic sulfamethoxazole and trimethoprim (Bactrim) in preventing the hypersensitivity reaction commonly associated with this drug. In a randomized, double-blind, placebo-controlled trial of 24 HIV+ adults with baseline CD4+ counts between 125 and 500/mL, as reported by Brewitt and colleagues,[215] oral doses of homeopathic growth hormone resulted in statistically significant improvements in CD4 cells, CD8 cells, erythrocyte sedimentation rate (ESR), weight gain, and viral load; reduced occurrences of OI; and increased platelet counts.

The use of constitutional homeopathy should be considered an essential aspect of the care of all HIV+ patients considering the amazing potential, minimal side effects, and overall affordability.

Physical medicine and acupuncture. Physical medicine is another low-cost therapy that HIV+ patients can seek through a naturopathic provider. A growing body of evidence suggests that these therapies also play a pivotal role in the care of HIV+ patients:

- Whole-body hyperthermia (twice weekly at 108°F for 20 minutes) may elevate CD4 count, but extreme caution must be used in heat-intolerant individuals.[216]
- Ozone (rectal and aural insufflation) may inactivate HIV-1.[217–219]
- Acupuncture and moxibustion (three treatments weekly) have been demonstrated to decrease diarrhea, decrease nausea, and decrease cravings for illicit drugs (cocaine and heroin).[220–223] Acupuncture has also been found to be clearly effective in reducing attrition and decreasing pain in peripheral neuropathy.[224]
- Electroacupuncture (by continually stimulating acupuncture/conductance skin points associated with nervous and immune systems) has been demonstrated to ameliorate the complications of HIV-related peripheral neuropathy, raise CD4+ counts, and raise other lymphocyte counts.[225,226]
- Massage therapy has been demonstrated to increase NK cells, CD3 and CD4 cell counts, and CD4:CD8 ratios; improve quality of life; decrease health care costs; and decrease anxiety and symptoms of depression.[227–230]
- Reiki therapy with music has been found to reduce stress, anxiety, and depression.[231] Other studies have demonstrated increased

growth and development and overall behavior in HIV-exposed newborns as well as improved immune preservation in HIV+ children in the absence of antiretroviral therapy.[232,233]
- Therapeutic touch can reduce anxiety in children.[234]
- Cranioelectrical stimulation (microcurrent 0.1 mA at 100 Hz to alligator clips attached to the earlobes 20 minutes twice daily) can decrease anxiety, insomnia, and depression.[235]
- Movement therapies may also be useful:
  - Aerobic exercise has been demonstrated to provide benefit to individuals with immunodeficiency diseases, particularly through stress alleviation and mood enhancement. One exercise study demonstrated that HIV+ individuals had significant improvements in both CD8 and NK cells.[236–239]
  - Tai chi has demonstrated a greater overall perception of health and significant improvements in all functional measures in 13 HIV+ individuals compared with controls.[240]
  - Yoga has shown improvements in self-confidence and a return to athletic activities after the intervention.[241,242]

Provide Education and Guidance to Patients Seeking Alternatives to Conventional Treatment

As mentioned earlier, there are currently no known therapies as effective as HAART in suppressing viral load or increasing CD4 lymphocyte cell numbers, thereby supporting normal immune function. CAM providers must avoid the temptation to allow their patients to believe otherwise. Additionally, as HAART has proven to be so efficacious in reducing the incidence of AIDS and other HIV-associated adverse events, it is now the standard of care that it should be started upon diagnosis.

If a patient is aware of the risk of a high HIV viral load, low CD4 count, or both, yet still does not desire to initiate HAART or antibiotic prophylaxis, ensure that this patient has access to a conventional Western HIV specialist. This specialist can provide clear education on conventional Western medical approaches to HIV and help diagnose and treat OIs should they arise. Concurrently, initiate a foundation of care that includes a healthy diet and lifestyle, nutritional support, and additional therapies as described earlier. Furthermore, ensure that the patient is scheduled for frequent follow-ups with health care providers to regularly screen for and monitor potential or existing OIs.

If a sole practitioner is treating a patient who refuses to undergo conventional Western medical and pharmaceutical intervention, specific use of the following therapies should be considered to decrease viral load and increase CD4 cell count:
- *Curcuma longa*/turmeric (1200 mg) to inhibit HIV integrase, proteases, and viral transcription
- *Olea* sp./olive leaf extract (2–6 g) to increase NK-cell function and oppose HIV and herpesviruses
- *Phyllanthus amarus* (1200 mg), which has been shown to inhibit HIV-1, reverse transcriptase, receptors, and proteases in vitro and ex vivo
- *Lentinus edodes*/shiitake mushrooms (1–5 mg IV twice a week), which can increase CD4 and decrease p24 (a surface marker)
- Ozone (rectal and aural insufflations), which may inactivate HIV-1

For patients seeking advice on the discontinuation or interruption of HAART (a structured treatment interruption [STI] or "drug holiday"), naturopathic physicians should ensure that patients clearly communicate this desire to their primary care physicians and HIV specialists. Treatment interruptions may be indicated in the following situations[30]:
- Severe or life-threatening toxicity, unexpected inability to take oral medications, or lack of availability of antiretroviral medication (a short-term interruption)
- Where the patient has to be relieved of the inconvenience, toxicity, and cost of antiretroviral therapy
- Where the response to salvage therapy (treatment regimens used in patients who have failed other HAART regimens) can be improved by allowing the re-emergence of wild-type virus (the predominant virus type in a given individual as unaffected by HAART therapy)

Potential problems with treatment interruptions include viral rebound, immune decompensation, and clinical progression of HIV. Multiple studies have been done over time to examine strategies for discontinuing and reintroducing HAART as well as to evaluate the safety of such discontinuations; however, there has been no clear resulting consensus.[243–249] The most recent studies include the SMART, TRIVACAN, and DART trials, which have not had positive outcomes.[250–252] The SMART study was the largest, with more than 5000 subjects, and interrupted HAART when the CD4 cell count was greater than 350/mm^3 and reinitiated treatment when it fell below 250/mm^3. This trial was discontinued when there was a clearly increased risk of disease progression and death compared with the arm on continuous HAART. The current recommendation of the Department of Health and Human Services is that HAART interruptions should not be recommended except in the case of clinical trials or an acute problem.[13]

THERAPEUTIC APPROACH

HIV is a chronic disease with no known cure; naturopathic physicians are encouraged to apply the principles of naturopathic medicine in the search for treatments and refinements of care. In addition, naturopathic physicians must be familiar with all aspects of this disease because HIV+ patients are actively seeking treatment options unique to naturopathic medical training. Naturopathic medicine already offers important possibilities in the care of HIV, and all fields in health care must collaborate to find solutions for this worldwide health problem. Each patient should have a complete and appropriate caregiving team, including an infectious disease expert, primary care physician, and specialist providing psychosocial support.

Diet

A natural, whole-foods diet and a high caloric intake are recommended. This diet should be low in simple carbohydrates (e.g., sugar, white flour, fruit juice, honey, processed foods), low in natural and synthetically saturated fats, and low in oxidized or trans fatty acids. It should also be high in protein, fiber, and filtered water. Patients should abstain from alcohol, nicotine, and caffeine. Digestive stimulation is recommended if appropriate. Broad-spectrum allergy-elimination diets should generally be avoided (dependent on an individual's nutritional status).

Lifestyle

Aerobic or mind–body exercise (e.g., tai qui, yoga) and 8 to 10 hours of sleep each night are optimal. Rule out obstructive sleep apnea if suspected. Prayer, spiritual activities, and activities that support the HIV+ community, guided forms of stress relief, and relaxation training are beneficial.

Nutritional Supplements

Potential interactions with HAART must always be investigated in considering the use of any nutritional or botanical supplement.

- A high-potency hypoallergenic multivitamin/multimineral supplement, preferably designed specifically to replace known nutrient deficiencies of HIV
- Beta-carotene (150,000 IU/day best taken as food)
- Vitamin D (5000 IU/day best taken with food)
- DHEA (15–50 mg/day best taken with food)
- Acetyl-L-carnitine (2–6 g/day in divided doses best taken away from other proteins)
- EFAs (5 g/day taken with food)
- Probiotics (8–12 billion CFU a day taken with meals)
- Botanical medicines:
  Silybum marianum/milk thistle extract (300 mg/day) for all patients on HAART
- Homeopathic medicines:
  Constitutional intake and remedy for each patient
  Acute remedies as indicated
- Physical medicine and acupuncture:
  Whole-body hyperthermia—use extreme caution in patients with heat intolerance or peripheral neuropathies.
  Movement therapies—as indicated
  Acupuncture and electroacupuncture—as indicated

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Takebe Y, Kusagawa S, Motomura K. Molecular epidemiology of HIV: tracking AIDS pandemic. *Pediatr Int*. 2004;46:236–244.
2. Bartlett JG, Gallant JE, Pham PE. *2009-10 Medical Management of HIV Infection*. Johns Hopkins University School of Medicine/Knowledge Source Solutions, LLC; 2009.
3. HIV Transmission. *Centers for Disease Control and Prevention, HIV/AIDS, HIV Basics*; 2018. Available at: https://www.cdc.gov/hiv/basics/transmission.html. Accessed August 5, 2018.
4. Fauci AS, Pantaleo G, Stanley S, et al. Immunopathogenic mechanisms of HIV infection. *Ann Intern Med*. 1996;124:654–663.
5. Selik RM, Mokotoff ED, Branson B, et al. Revised surveillance case definition for HIV infection – United States, 2014. *MMWR Morb Mortal Wkly Rep, Recomm Rep*. 2014;63(RR03):1–10.
6. Hanson DL, Chu SY, Farizo KM, et al. Distribution of CD4+ T lymphocytes at diagnosis of acquired immunodeficiency syndrome–defining and other human immunodeficiency virus–related illnesses. *Arch Intern Med*. 1995;155:1537–1542.
7. Branson BM, Handsfield HH, Lampe MA, et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Morb Mortal Wkly Rep*. 2006;55(RR14):1–17.
8. DiNenno EA, Prejean J, Irwin K, et al. Recommendations for HIV screening of gay, bisexual, and other men who have sex with men – United States, 2017. *Morb Mortal Wkly Rep*. 2017;66(31):830–832.
9. Branson BM, Owen SM, Wesolowski LG, et al. *Laboratory testing for the diagnosis of HIV infection: updated recommendations.* Centers for Disease Control and Prevention; Published June 27, 2014:1–66. Available at https://doi.org/10.15620/cdc.23447. Accessed August 5, 2018.
10. Centers for Disease Control and Prevention. Division of HIV/AIDS Prevention. Department of Health and Human Services. *Rapid HIV tests suitable for use in clinical settings (CLIA-moderate complexity)*. https://www.cdc.gov/hiv/pdf/testing/rapid-hiv-tests-clinical-moderate-complexity.pdf. Updated 8/10/16. Accessed August 5, 2018.
11. Centers for Disease Control and Prevention. Division of HIV/AIDS Prevention. Department of Health and Human Services. *Rapid HIV tests suitable for use in non-clinical settings (CLIA-waived)*. Updated 6/11/18. Accessed Aug 5, 2018.
12. Centers for Disease Control and Prevention. *Department of Health and Human Services, FDA-Approved Rapid HIV Antibody Screening Tests*; 2008. Available at: http://www.cdc.gov/hiv/topics/testing/re-comparison.htm. Accessed June 27, 2010.
13. Bartlett JG, Gallant JE, Pham PA. *Medical Management of HIV Infection 2009-2010*. Durham, NC: Johns Hopkins University School of Medicine; Knowledge Source Solutions LLC; 2009.
14. Home HIV Testing. Centers for Disease Control and Prevention. *Division of HIV/AIDS Prevention.* Department of Health and Human Services. https://www.cdc.gov/hiv/testing/hometests.html. Updated Oct 16, 2015. Accessed August 5, 2015.
15. Feldman JG, Burns DN, Gange SJ, et al. Serum albumin as a predictor of survival in HIV-infected women in the Women's Interagency HIV Study. *AIDS*. 2000;14:863–870.
16. Hawes SE, Critchlow CW, Faye Niang MA, et al. Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. *J Infect Dis*. 2003;188:555–563.
17. Palefsky JM, Schmelz M, Berry-Lawhorn JM, et al. The Anchor Study. https://anchorstudy.org. Accessed August 5, 2018.
18. Verhofstede C, Van Wanzeele F, Reynaerts J, et al. Viral load assay sensitivity and low level viremia in HAART treated HIV patients. *J Clin Virol*. 2010;47(4):335–339.
19. *Laboratory Testing: Plasma HIV-1 RNA (Viral Load) and CD4 Count Monitoring.* U.S. Dept of Health and Human Services. AIDS Info. https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv/458/plasma-hiv-1-rna—viral-load—and-cd4-count-monitoring. Last Updated: May 1, 2014. Accessed August 6, 2018.
20. Cohen M, Chen Y, McCauley M, et al. Final results of the HPTN 052 randomized controlled trial: antiretroviral therapy prevents HIV transmission. *J Int AIDS Soc*. 2015;18(suppl 4):20479.
21. Continuum of Care. *Policy, Planning, and Strategic Communication.* Centers for Disease Control and Prevention. Division of HIV/AIDS Prevention. Department of Health and Human Services. https://www.cdc.gov/hiv/policies/continuum.html. Last updated July 23, 2018. Accessed August 6, 2018.
22. Grant RM, Lama JR, Anderson PL, et al. Preexposure Chemoprophylaxis for HIV prevention in men who have sex with men. *New Eng J Med*. 2010;363:2587–2599.
23. Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral Preexposure prophylaxis for heterosexual HIV transmission in Botswana. *New Eng J Med*. 2012;367:423–434.
24. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *New Eng J Med*. 2012;367:399–410.
25. Choopanya K, Martin M, Suntharasamai P, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomized, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2013;381(9883):2083–2090.
26. Highlights of Prescribing Information. http://www.gilead.com/-/media/files/pdfs/medicines/hiv/descovy/descovy_pi.pdf. Accessed January 10, 2020.
27. Golin CE, Smith SR, Reif S. Adherence counseling practices of generalist and specialist physicians caring for people living with HIV/AIDS in North Carolina. *J Gen Intern Med*. 2004;19:16–27.
28. Page J, Weber R, Somaini B, et al. Quality of generalist vs. specialty care for people with HIV on antiretroviral treatment: a prospective cohort study. *HIV Med*. 2003;4:276–286.
29. Furler MD, Einarson TR, Walmsley S, et al. Use of complementary and alternative medicine by HIV-infected outpatients in Ontario, Canada. *AIDS Patient Care STDS*. 2003;17:155–168.
30. Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.* Department of Health and Human Services. http://www.aidsinfo.hih.gov/ContentFiles/Adultand AdolescentGL.pdf (December 1, 2009). Accessed June 30, 2010. 1–161.
31. World Health Organization. *Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV*. http://apps.who.int/iris/bitstream/handle/10665/186275/9789241509565_eng.pdf;jsessionid=47C27347995253AAE13E119BFE7CD6A3?sequence=1. ISBN: 978 92 4 150956 5. Published September 2015. Accessed November 17, 2019.
32. Lundgren JD, Babiker AG, et al. The INSIGHT START Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. *N Engl J Med*. 2015;373(9):795–807.
33. US DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents – A Working Group of the Office of AIDS Research Advisory Council (OARAC); *Guidelines for the Use of Antiretroviral Agents in HIV—Infected Adults and Adolescents*; https://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL003464.pdf. Published July 14, 2016. Accessed November 17, 2019.
34. Pepe R, Molavi A. Zidovudine for the treatment of HIV infection. *Am Fam Physician*. 1990;42:437–443.
35. Fleisher R, Boxwell D, Sherman KE. Nucleoside analogues and mitochondrial toxicity. *Clin Infect Dis*. 2004;38:e79–e80.
36. Reiss P, Casula M, de Ronde A, et al. Greater and more rapid depletion of mitochondrial DNA in blood of patients treated with dual (zidovudine+didanosine or zidovudine+zalcitabine) vs. single (zidovudine) nucleoside reverse transcriptase inhibitors. *HIV Med*. 2004;5:11–14.
37. Famularo G, Moretti S, Marcellini S, et al. Acetyl-carnitine deficiency in AIDS patients with neurotoxicity on treatment with antiretroviral nucleoside analogues. *AIDS*. 1997;11:185–190.
38. Folkers K, Langsjoen P, Nara Y, et al. Biochemical deficiencies of coenzyme Q_{10} in HIV-infection and exploratory treatment. *Biochem Biophys Res Commun*. 1988;153:888–896.
39. Osmundsen H, Clouet P. Metabolic effects of omega-3 fatty acids. *Biofactors*. 2000;13:5–8.

40. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med*. 1995;19:227–250.
41. Falguera M, Perez-Mur J, Piug T, et al. Study of the role of vitamin B12 and folinic acid supplementation in preventing hemologic toxicity of zidovudine. *Eur J Haematol*. 1995;55:97–102.
42. Moretti S, Famularo G, Marcellini S, et al. L-carnitine reduces lymphocyte apoptosis and oxidant stress in HIV-1-infected subjects treated with zidovudine and didanosine. *Antioxid Redox Signal*. 2002;4: 391–403.
43. Baum MK, Javier JJ, Mantero-Atienza E, et al. Zidovudine-associated adverse reactions in a longitudinal study of asymptomatic HIV-1-infected homosexual males. *J Acquir Immune Defic Syndr*. 1991;4:1218–1226.
44. De la Asuncion JG, del Olmo ML, Sastre J, et al. AZT treatment induces molecular and ultrastructural oxidative damage to muscle mitochondria: prevention by antioxidant vitamins. *J Clin Invest*. 1998;102:4–9.
45. Mocchegiani E, Veccia S, Ancarani F, et al. Benefit of oral zinc supplementation as an adjunct to zidovudine (AZT) therapy against opportunistic infections in AIDS. *Int J Immunopharmacol*. 1995;17:719–727.
46. Herzlich BC, Ranginwala M, Nawabi I, et al. Synergy of inhibition of DNA synthesis in human bone marrow by azidothymidine plus deficiency of folate and/or vitamin B12? *Am J Hematol*. 1990;33:177–183.
47. Bani-Sadr F, Palmer P, Scieux C, et al. Ninety-six-week efficacy of combination therapy with lamivudine and tenofovir in patients coinfected with HIV-1 and wild-type hepatitis B virus. *Clin Infect Dis*. 2004;39:1062–1064.
48. Nelson M, Schiavone M. Emtricitabine (FTC) for the treatment of HIV infection. *Int J Clin Pract*. 2004;58:504–510.
49. Dore GJ, Cooper DA, Pozniack AL, et al. Efficacy of tenofovir disoproxil fumarate in antiretroviral therapy-naive and -experienced patients coinfected with HIV-1 and hepatitis B virus. *J Infect Dis*. 2004;189:1185–1192.
50. Lochet P, Peyriere H, Lotthe A, et al. Long-term assessment of neuropsychiatric adverse reactions associated with efavirenz. *HIV Med*. 2003;4: 62–66.
51. Kontorinis N, Dieterich DT. Toxicity of non-nucleoside analogue reverse transcriptase inhibitors. *Semin Liver Dis*. 2003;23:173–182.
52. Mira JA, Lozano F, Santos J, et al. Gynaecomastia in HIV-infected men on highly active antiretroviral therapy: association with efavirenz and didanosine treatment. *Antivir Ther*. 2004;9:511–517.
53. Robbins GK, De Gruttola V, Shafer RW, et al. Comparison of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med*. 2003;349:2293–2303.
54. Shafer RW, Smeaton LM, Robbins GK, et al. Comparison of four-drug regimens and pairs of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med*. 2003;349:2304–2315.
55. Gulick RM, Ribaudo HJ, Shikuma CM, et al. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. *N Engl J Med*. 2004;350:1850–1861.
56. van Leth F, Phanuphak P, Ruxrungtham K, et al. Comparison of first-line antiretroviral therapy with regimens including nevir-apine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomized open-label trial, the 2NN Study. *Lancet*. 2004;363:1253–1263.
57. Piscitelli SC, Burstein AH, Chaitt D, et al. Indinavir concentrations and St. John's wort. *Lancet*. 2000;355:547–548.
58. Piscitelli SC, Burstein AH, Welden N, et al. The effect of garlic supplements on the pharmacokinetics of saquinavir. *Clon Infect Dis*. 2002;34:234–238.
59. Gallicano K, Foster B, Choudhri S. Effect of short-term administration of garlic supplements on single-dose ritonavir pharmacokinetics in healthy volunteers. *Br J Clin Pharmacol*. 2003;55:199–202.
60. Zhou S, Chan E, Pan SQ, et al. Pharmacokinetic interactions of drugs with St John's wort. *J Psychopharmacol*. 2004;18:262–276.
61. Piscitelli SC, Formentini E, Burstein AH, et al. Effect of milk thistle on the pharmacokinetics of indinavir in healthy volunteers. *Pharmacotherapy*. 2002;22:551–556.
62. DiCenzo R, Shelton M, Jordan K, et al. Coadministration of milk thistle and indinavir in healthy subjects. *Pharmacotherapy*. 2003;23:866–870.
63. Patel J, et al. In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. *Am J Ther*. 2004;11(4):262–277.
64. Fuhr U, Beckmann-Knopp S, Jetter A, et al. The effect of silymarin on oral nifedipine pharmacokinetics. *Planta Med*. 2007;73(14):1429–1435.
65. Molto J, Valle M, Miranda C, et al. Effect of milk thistle on the pharmacokinetics of darunavir-ritonavir in HIV-infected patients. *Antimicrob Agents Chemother*. 2012;56(6):2837–2841.
66. Margolis DA, Gonzalez-Garcia J, Stellbrink HJ, et al. Long acting intramuscular cabotegravir and rilpivirine in adults with HIV-1infection (Latte-2): 96-week results of a randomized, open-label, phase 2b, non-inferiority trial. *Lancet*. 2017;390(1010):1499–1510.
67. Kerrigan D, Mantsios A, Gorgolas M, et al. Experiences with long acting injectable ART: a qualitative study among PLHIV participating in a Phase II study of cabotegravir + rilpivirine (Latte-2) in the United States and Spain. *PLOS ONE*. 13(1):e0190487.
68. Lule JR, Mermin J, Ekwaru JP, et al. Effect of home-based water chlorination and safe storage on diarrhea among persons with human immunodeficiency virus in Uganda. *Am J Trop Med Hyg*. 2005;73(5): 926–933.
69. Crenn P, Rakotoanbinina B, Raynaud JJ, et al. Hyperphagia contributes to the normal body composition and protein-energy balance in HIV-infected asymptomatic men. *J Nutr*. 2004;134:2301–2306.
70. Williams SB, Bartsch G, Muurahainen N, et al. Protein intake is positively associated with body cell mass in weight-stable HIV-infected men. *J Nutr*. 2003;133:1143–1146.
71. Agin D, Gallagher D, Wang J, et al. Effects of whey protein and resistance exercise on body cell mass, muscle strength, and quality of life in women with HIV. *AIDS*. 2001;15:2431–2440.
72. Micke P, Beeh KM, Buhl R. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. *Eur J Nutr*. 2002;41:12–18.
73. Rathmacher JA, Nissen S, Panton L, et al. Supplementation with a combination of beta-hydroxy-beta-methylbutyrate (HMB), arginine, and glutamine is safe and could improve hemological parameters. *J Parenter Enteral Nutr*. 2004;28:65–75.
74. de Luis Roman DA, Bachiller P, Izaola O, et al. Nutritional treatment for acquired immunodeficiency virus infection using an enterotropic peptide-based formula enriched with n-3 fatty acids: a randomized prospective trial. *Eur J Clin Nutr*. 2001;55:1048–1052.
75. Pichard C, Sudre P, Karsegard V, et al. A randomized double-blind controlled study of 6 months of oral nutritional supplementation with arginine and omega-3 fatty acids in HIV-infected patients. Swiss HIV Cohort Study. *AIDS*. 1988;12:53–63.
76. Bell SJ, Chavali S, Bistrian BR, et al. Dietary fish oil and cytokine and eicosanoid production during human immunodeficiency virus infection. *J Parenter Enteral Nutr*. 1996;20:43–49.
77. Ravi S, Khalili H, Abbasian L. Effect of omega-3 fatty acids on depressive symptoms in HIV-positive individuals: a randomized, placebo-controlled clinical trial. *Ann Pharmacother*. 2016;50(10):797–807.
78. Winkler P, Ellinger S, Boetzer AM, et al. Lymphocyte proliferation and apoptosis in HIV-seropositive and healthy subjects during long-term ingestion of fruit juices or a fruit-vegetable-concentrate rich in polyphenols and antioxidant vitamins. *Eur J Clin Nutr*. 2004;58: 317–325.
79. Arendt BM, Boetzer AM, Lemoch H, et al. Plasma antioxidant capacity of HIV-seropositive and healthy subjects during long-term ingestion of fruit juices or a fruit-vegetable-concentrate containing antioxidant polyphenols. *Eur J Clin Nutr*. 2001;55:786–792.
80. Ichimura T, Otake T, Mori H, et al. HIV-1 protease inhibition and anti-HIV effect of natural and synthetic water-soluble lignin-like substances. *Biosci Biotechnol Biochem*. 1999;63:2202–2204.
81. Hendricks KM, Dong KR, Tang AM, et al. High-fiber diet in HIV-positive men is associated with lower risk of developing fat deposition. *Am J Clin Nutr*. 2003;78:790–795.
82. Carroccio A, Di Prima L, Di Grigoli C, et al. Exocrine pancreatic function and fat malabsorption in human immunodeficiency virus-infected patients. *Scand J Gastroenterol*. 1999;34:729–734.
83. Koch J, Garcia-Shelton YL, Neal EA, et al. Steatorrhea: a common manifestation in patients with HIV/AIDS. *Nutrition*. 1996;12:507–510.

84. Ullum H, Palmo J, Halkjaer-Kristensen J, et al. The effect of acute exercise on lymphocyte subsets, natural killer cells, proliferative responses, and cytokines in HIV-seropositive persons. *J Acquir Immune Defic Syndr*. 1994;7:1122–1133.
85. LaPerriere A, Antoni MH, Ironson G, et al. Effects of aerobic exercise training on lymphocyte subpopulations. *Int J Sports Med*. 1994;15: S127–S130.
86. Galantino ML, Findley T, Krafft L, et al. Blending traditional and alternative strategies for rehabilitation: measuring functional outcomes and quality of life issues in an AIDS population, Eighth World Congress of International Rehabilitation Medicine Association. *Monduzzi Editore*. 1997;1:713–716.
87. Mawar N, Katendra T. Sudarshan Kriya yoga improves quality of life in healthy people living with HIV. *Indian J Med Res*. 2015;141(1):90–99.
88. Agarwal RP, Kumar A, Lewis JE. A pilot feasibility and acceptability study of yoga/meditation on the quality of life and markers of stress in persons living with HIV who also use crack cocaine. *J Altern Complement Med*. 2015;21(3):152–158.
89. Cade WT, Reeds DN, Mondy KE, et al. Yoga lifestyle intervention reduces blood pressure in HIV-infected adults with cardiovascular disease risk factors. *HIV Med*. 2010;11(6):379–388.
90. Rehse A. Body movement workshop for people with HIV/AIDS. *Int Conf AIDS*. 1992;8:126.
91. Born J, Lange T, Hansen K, et al. Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol*. 1997;158:4454–4464.
92. Moldofsky H. Sleep and the immune system. *Int J Immunopharmacol*. 1995;17:649–654.
93. Goodkin K, Feaster DJ, Asthana D, et al. A bereavement support group intervention is longitudinally associated with salutary effects on the CD4 cell count and number of physician visits. *Clin Diagn Lab Immunol*. 1998;5:382–391.
94. Goodkin K, Feaster DJ, Tuttle R, et al. Bereavement is associated with time-dependent decrements in cellular immune function in asymptomatic human immunodeficiency virus type 1-seropositive homosexual men. *Clin Diagn Lab Immunol*. 1996;3:109–118.
95. Lechner SC, Antoni MH, Lydston D, et al. Cognitive-behavioral interventions improve quality of life in women with AIDS. *J Psychosom Res*. 2003;54:253–261.
96. Cruess S, Antoni M, Cruess D, et al. Reductions in herpes simplex virus type 2 antibody titers after cognitive behavioral stress management and relationships with neuroendocrine function, relaxation skills, and social support in HIV-positive men. *Psychosom Med*. 2000;62:828–837.
97. McCain NL, Munjas BA, Munro CL, et al. Effects of stress management on PNI-based outcomes in persons with HIV disease. *Res Nurs Health*. 2003;26:102–117.
98. Antoni MH, Cruess S, Cruess DG, et al. Cognitive-behavioral stress management reduces distress and 24-hour urinary free cortisol output among symptomatic HIV-infected gay men. *Ann Behav Med*. 2000;22:29–37.
99. Cruess DG, Leserman J, Petitto JM, et al. Psychosocial-immune relationships in HIV disease. *Semin Clin Neuropsychiatry*. 2001;6:241–251.
100. Berger S, Schad T, von Wyl V, et al. Effects of cognitive behavioral stress management on HIV-1 RNA, CD4 cell counts and psychosocial parameters of HIV-infected persons. *AIDS*. 2008;22(6):767–775.
101. SeyedAlinaghi S, Jam S, et al. Randomized controlled trial of mindfulness-based stress reduction delivered to HIV+ patients in Iran: effects on CD4+, T lymphocyte count and medical and psychological symptoms. *Psychosom Med*. 2012;74(6):620–627.
102. Sicher F, Targ E, Moore II D, et al. A randomized double-blind study of the effect of distant healing in a population with advanced AIDS: report of a small scale study. *West J Med*. 1988;169:356–363.
103. Eller LS, Kirksey KM, et al. A randomized controlled trial of an HIV/AIDS Symptom Management Manual for depressive symptoms. *AIDS Care*. 2013;25(4):391–399.
104. Fitzpatrick AL, Standish LJ, Berger J, et al. Survival in HIV-1-positive adults practicing psychological or spiritual activities for one year. *Altern Ther Health Med*. 2007;13(5):18–20. 22-24.
105. Bennett MP, Zeller JM, Rosenberg L, et al. The effect of mirthful laughter on stress and natural killer cell activity. *Altern Ther Health Med*. 2003;9:38–45.
106. Jiamton S, Pepin J, Suttent R, et al. A randomized trial of the impact of multiple micronutrient supplementation on mortality among HIV-infected individuals living in Bankok. *AIDS*. 2003;17:2461–2469.
107. Fawzi W, Msamanga G. Micronutrients and adverse pregnancy outcomes in the context of HIV infection. *Nutr Rev*. 2004;62:269–275.
108. Rabeneck L, Palmer A, Knowles JB, et al. A randomized controlled trial evaluating nutrition counseling with or without oral supplementation in malnourished HIV-infected patients. *J Am Diet Assoc*. 1998;98: 434–438.
109. Shivakoti R, Christian P, Yang WP, et al. Prevalence and risk factors of micronutrient pre- and post-antiretroviral therapy among adverse multi-country cohort of HIV-infected adults. *Clin Nutr*. 2016;35(1):183–189.
110. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med*. 2004;351:23–32.
111. Baum MK, Shor-Posner G, Lu Y, et al. Micronutrients and HIV-1 disease progression. *AIDS*. 1995:1051–1056.
112. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst*. 1996;88:1550–1559.
113. Jalloh MA, Gregory PJ, Hein D. Dietary supplement interactions with antiretrovirals: a systemic review. *Int J STD AIDS*. 2017;28(1):4–15.
114. Shivakoti R, Gupte N, Yang WT, et al. Pre-antiretroviral therapy serum selenium concentrations predict WHO stages 3,4 or death but not virologic failure post-therapy. *Nutrients*. 2014;6(11):5061–5078.
115. Sudfeld CR, Aboud S, Kupka R, et al. Effect of selenium supplementation on HIV-1 RNA detection in breast milk of Tanzanian women. *Nutrition*. 2014;30(9):1081–1084.
116. Flax VL, Bentley ME, Combs GF. Plasma and breast-milk selenium in HIV-infected Malawian mothers are positively associated with infant selenium status but are not associated with maternal supplementation; results of the Breastfeeding, Antiretrovirals, and Nutrition study. *Am J Clin Nutri*. 2014;99(4):950–956.
117. Williams AB, Yu C, Tashima K, et al. Evaluation of two self-care treatments for prevention of vaginal candidiasis in women with HIV. *J Assoc Nurses AIDS Care*. 2001;12:51–57.
118. Wagner RD, Pierson C, Warner T, et al. Probiotic effects of feeding heat-killed *Lactobacillus acidophilus* and *Lactobacillus casei* to *Candida albicans*-colonized immunodeficient mice. *J Food Prot*. 2000;63:638–644.
119. Collins EB, Hardt P. Inhibition of *Candida albicans* by *Lactobacillus acidophilus*. *J Dairy Sci*. 1980;63:830–832.
120. Wagner RD, Pierson C, Warner T. Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. *Infect Immun*. 1997;65:4165–4172.
121. Berg R, Bernasconi P, Fowler D, et al. Inhibition of *Candida albicans* translocation from the gastrointestinal tract of mice by oral administration of *Saccharomyces boulardii*. *J Infect Dis*. 1993;168:1314–1318.
122. Lemar KM, Muller CT, Plummer S, et al. Cell death mechanisms in the human opportunistic pathogen *Candida albicans*. *J Eukaryot Microbiol*. 2003;50:685–686.
123. Stiles JC, Sparks W, Ronzio RA. The inhibition of *Candida albic*ans by oregano. *J Appl Nutr*. 1995;47(4):96–102.
124. Hand GA, Phillips KD, Dudgeon WD, et al. Moderate intensity exercise training reverses functional aerobic impairment in HIV-infected individuals. *AIDS Care*. 2008;20(9):1066–1074.
125. Perna FM, LaPerriere A, Klimas N, et al. Cardiopulmonary and CD4 cell changes in response to exercise training in early symptomatic HIV infection. *Med Sci Sports Exerc*. 1999;31(7):973–979.
126. Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q_{10} therapy in patients with congestive heart failure: a long-term multicenter randomized study. *Clin Invest*. 1993;71(suppl):S134–S136.
127. Gornik HL, Creager MA. Arginine and endothelial and vascular health. *J Nutr*. 2004;134(suppl):S2880–S2887.
128. Paranadi A, Astalos BF, Mangili A. Short communication: effects of omega -3 fatty acids on triglycerides and high-density lipoprotein subprofiles

in HIV-infected persona with hypertriglyceridemia. *AIDS Res Hum Retrviruses*. 2014;30(8):800–805.
129. Metkus TS, Timpone J, Leaf D, et al. Omega-3 fatty acid therapy reduces triglycerides and IL-6 in hypertriglyceridemic HIV patients. *HIV Med*. 2013;14(9):530–539.
130. Peters BS, Wierzbicki AS, Moyle G, Brockmeyer N. The effect of a 12 week course of omega-e polyunsaturated fatty acids on lipid parameters in hypertriglyceridemic adult HIV-infected patients undergoing HAART: a randomized, placebo-controlled pilot trial. *Clin Ther*. 2012;34(1):67–76.
131. Noone EJ, Roche HM, Nugent AP, et al. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in health human subjects. *Br J Nutr*. 2002;88:243–251.
132. Quinones-Galvan A, Lifshitz-Guinzberg A, Ruiz-Arguelles GJ. Gluten-free diet for AIDS-associated enteropathy. *Ann Intern Med*. 1990;113:806–807.
133. Bushen OY, Davenport JA, Lima AB, et al. Diarrhea and reduced levels of antiretroviral drugs: improvement with glutamine or alanyl-glutamine in a randomized controlled trial in northeast Brazil. *Clin Infect Dis*. 2004;38:1764–1770.
134. McCune MA, Perry HO, Muller SA, et al. Treatment of recurrent herpes simplex infections with L-lysine monohydrochloride. *Cutis*. 1984;34: 366–373.
135. Hornung B, Amtmann E, Sauer G. Lauric acid inhibits the maturation of vesicular stomatitis virus. *J Gen Virol*. 1994;75:353–361.
136. Dimitrova Z, Dimov B, Manolova N, et al. Antiherpes effect of *Melissa officinalis* L. extracts. *Acta Microbiol Bulg*. 1993;29:65–72.
137. Pompei R, Flore O, Marccialis MA, et al. Glycyrrhizic acid inhibits virus growth and inactivates virus particles. *Nature*. 1979;281:689–690.
138. Barillari G, Sgadari C, Palladino C, et al. Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of basic fibroblast growth factor and the alpha v beta 3 integrin. *J Immunol*. 1999;163:1929–1935.
139. Murakami-Mori K, Mori S, Bonavida B, et al. Implication of TNF receptor-I-mediated extracellular signal-related kinases 1 and 2 (ERK1/2) activation in growth of AIDS-associated Kaposi's sarcoma cells: a possible role of a novel death domain protein MADD in TNF-alpha-induced ERK1/2 activation in Kaposi's sarcoma cells. *J Immunol*. 1999;162: 3672–3679.
140. Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood*. 2000;96:2562–2567.
141. Ghyka G, Alecu M, Halalau F, et al. Human leukocyte interferon treatment associated with IL-2 in the non-AIDS related Kaposi's sarcoma. *Rom J Intern Med*. 1991;29:75–83.
142. Koster R, Blatt LM, Streubert M, et al. Consensus-interferon and platelet-derived growth factor adversely regulate proliferation and migration of Kaposi's sarcoma cells by control of c-myc expression. *Am J Pathol*. 1996;149:1871–1885.
143. Aboulafia DM. The epidemiology, pathogenesis and treatment of Kaposi's sarcoma. *STEP Perspect*. 1995;7:9–12.
144. Aboulafia DM, Norris D, Henry D, et al. 9-cis-retinoic acid capsules in the treatment of AIDS-related Kaposi sarcoma: results of a phase 2 multicenter clinical trial. *Arch Dermatol*. 2003;139:178–186.
145. Masood R, Nagpal S, Zheng T, et al. Kaposi sarcoma is a therapeutic target for vitamin D(3) receptor agonist. *Blood*. 2000;96:3188–3194.
146. Simonart T. Iron: a target for the management of Kaposi's sarcoma? *BMC Cancer*. 2004;4:1.
147. Simonart T, Degraef C, Andrei G, et al. Iron chelators inhibit the growth and induce the apoptosis of Kaposi's sarcoma cells and of their putative endothelial precursors. *J Invest Dermatol*. 2000;115:893–900.
148. Schwartz RA. Kaposi's sarcoma: an update. *J Surg Oncol*. 2004;87: 146–151.
149. Albini A, Morini M, D'Agostini F, et al. Inhibition of angiogenesis-driven Kaposi's sarcoma tumor growth in nude mice by oral N-acetylcysteine. *Cancer Res*. 2001;61:8171–8178.
150. Mallery SR, Clark YM, Ness GM, et al. Thiol redox modulation of doxorubicin mediated cytotoxicity in cultured AIDS-related Kaposi's sarcoma cells. *J Cell Biochem*. 1999;73:259–277.
151. Mallery SR, Bailer RT, Hohl CM, et al. Cultured AIDS-related Kaposi's sarcoma (AIDS-KS) cells demonstrate impaired bioenergetic adaptation to oxidant challenge: implication for oxidant stress in AIDS-KS pathogenesis. *J Cell Biochem*. 1995;59:317–328.
152. Calza L, Manfredi R, Chiodo F. Lipodystrophy and lipid metabolism alterations in HIV-infected patients receiving highly active antiretroviral therapy (HAART). *Recenti Prog Med*. 2004;95:265–275.
153. Gougeon ML, Penicaud L, Fromenty B, et al. Adipocytes targets and actors in the pathogenesis of HIV-associated lipodystrophy and metabolic alterations. *Antivir Ther*. 2004;9:161–177.
154. Kino T, Chrousos GP. Human immunodeficiency virus type-1 accessory protein Vpr: a causative agent of the AIDS-related insulin resistance/lipodystrophy syndrome? *Ann N Y Acad Sci*. 2004;1024: 153–167.
155. Manco M, Calvani M, Mingrone G. Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabetes Obes Metab*. 2004;6:402–413.
156. Walker UA. Lactic acidosis in HIV-patients: diagnosis and treatment. *MMW Fortschr Med*. 2004;146(Spec No 1):65–67.
157. Gavrila A, Tsiodras S, Doweiko J, et al. Exercise and vitamin E intake are independently associated with metabolic abnormalities in human immunodeficiency virus-positive subjects: a cross-sectional study. *Clin Infect Dis*. 2003;36:1593–1601.
158. Hankard RG, Haymond MW, Darmaun D. Role of glucose in the regulation of glutamine metabolism in health and in type 1 insulin-dependent diabetes. *Am J Physiol Endocrinol Metab*. 2000;279:E608–E613.
159. M'bemba J, Cynober L, de Bandt P, et al. Effects of dipeptide administration on hypoglycaemic counterregulation in type 1 diabetes. *Diabetes Metab*. 2003;29:412–417.
160. Coker M, Coker C, Darcan S, et al. Carnitine metabolism in diabetes mellitus. *J Pediatr Endocrinol Metab*. 2002;15:841–849.
161. Derosa G, Cicero AF, Gaddi A, et al. The effect of L-carnitine on plasma lipoprotein(a) levels in hypercholesterolemic patients with type 2 diabetes mellitus. *Clin Ther*. 2003;25:1429–1439.
162. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. *Mol Aspects Med*. 2004;25:455–473.
163. Sutinen J, Walker UA. Uridine supplementation for the treatment of antiretroviral therapy-associated lipoatrophy: a randomized, double-blind, placebo-controlled trial. *Antivir Ther*. 2007;12(1):97–105.
164. Bhasin S, Parker RA, Sattler F, et al. Effects of testosterone supplementation on whole body and regional fat mass and distribution in human immunodeficiency virus-infected men with abdominal obesity. *J Clin Endocrinol Metab*. 2007;92(3):1049–1057. Epub 2006 Dec 12.
165. Gold J, Batterham MJ, Rekers H, et al. Effects of nandrolone decanoate compared with placebo or testosterone on HIV-associated wasting. *HIV Med*. 2006;7(3):146–155.
166. Falutz J, Allas S, Mamputu JC, et al. Long-term safety and effects of tesamorelin, a growth hormone-releasing factor analogue, in HIV patients with abdominal fat accumulation. *AIDS*. 2008;22(14):1719–1728.
167. Onesti MG, Renzi LF, Paoletti F, et al. Use of polylactic acid in face lipodystrophy in HIV positive patients undergoing treatment with antiretroviral drugs (HAART). *Acta Chir Plast*. 2004;46:12–15.
168. Romanelli F, Empey K, Pomeroy C. Macrocytosis as an indicator of medication (zidovudine) adherence in patients with HIV infection. *AIDS Patient Care STDS*. 2002;16:405–411.
169. Wolf K, Tichelli A, Battegay M. Anemia, neutropenia and thrombocytopenia: hematological findings and HIV. *Schweiz Rundsch Med Prax*. 2001;90:1775–1779.
170. Markle HV. Cobalamin. *Crit Rev Clin Lab Sci*. 1996;33:247–356.
171. Robinson AR, Mladenovic J. Lack of clinical utility of folate levels in the evaluation of macrocytosis or anemia. *Am J Med*. 2001;110:88–90.
172. Davis RE, Icke GC. Clinical chemistry of thiamin. *Adv Clin Chem*. 1983;23:93–140.
173. Grigoletti SS, Guindani G, Moraes RS, et al. Short term folinic acid supplementation improves vascular reactivity in HIV-infected individuals; a randomized trial. *Nutrition*. 2013;29(6):886–891.

174. Williams D, Geraci A, Simpson AD. AIDS and AIDS-treatment neuropathies. *Curr Pain Headache Rep*. 2002;6:125–130.
175. Dalakas MC, Semino-Mora C, Leon-Monzon M. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2,3-dideoxyxytidine (ddC). *Lab Invest*. 2001;81:1537–1544.
176. Coker M, Coker C, Darcan S, et al. Carnitine metabolism in diabetes mellitus. *J Pediatr Endocrinol Metab*. 2002;15:841–849.
177. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. *Mol Aspects Med*. 2004;25:455–473.
178. Nowak G, Grant DF, Moran JH. Linoleic acid epoxide promotes the maintenance of mitochondrial function and active Na+ transport following hypoxia. *Toxicol Lett*. 2004;147:161–175.
179. Chong ZZ, Lin SH, Maiese K. The NAD+ precursor nicotinamide governs neuronal survival during oxidative stress through protein kinase B coupled to FOXO3a and mitochondrial membrane potential. *J Cereb Blood Flow Metab*. 2004;24:728–743.
180. Kannan K, Jain SK. Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H_2O_2-treated U937 monocytes. *Free Radic Biol Med*. 2004;36:423–428.
181. Crestanello JA, Doliba NM, Babsky AM, et al. Effect of coenzyme Q_{10} supplementation on mitochondrial function after myocardial ischemia reperfusion. *J Surg Res*. 2002;102:221–228.
182. Alleva R, Tomasetti M, Andera L, et al. Coenzyme Q blocks biochemical but not receptor-mediated apoptosis by increasing mitochondrial antioxidant protection. *FEBS Lett*. 2001;503:46–50.
183. Antonawich FJ, Fiore SM, Welicky LM. Regulation of ischemic cell death by the lipoic acid-palladium complex, Poly MVA, in gerbils. *Exp Neurol*. 2004;189:10–15.
184. Creswell JD, Myers HF, Cole SW, et al. Mindfulness meditation training effects on CD4+ T lymphocytes in HIV-1 infected adults: a small randomized controlled trial. *Brain Behav Immun*. 2009;23(2):Y184–Y188.
185. Bormann JE, Gifford AL, Shively M, et al. Effects of spiritual mantram repetition on HIV outcomes: a randomized controlled trial. *J Behav Med*. 2006;29(4):359–376.
186. Lyon ME, Garvie PA, Kao E. Spirituality in HIV-infected adolescents and their families: FAmily CEntered (FACE) Advance Care Planning and medication adherence. *J Adolesc Health*. 2011;48(6):633–636.
187. Smith Fawzi MC, Kaaya SF, et al. Multivitamin supplementation in HIV-positive pregnant women: impact on depression and quality of life in a resource-poor setting. *HIV Med*. 2007;8(4):203–212.
188. Piketty C, Jayle D, Leplege A, et al. Double-blind placebo-controlled trial of oral dehydroepiandrosterone in patients with advanced HIV disease. *Clin Endocrinol (Oxf)*. 2001;55(3):325–330.
189. Knapp PE, Storer TW, Herbst KL, et al. Effects of a supraphysiological dose of testosterone on physical function, muscle performance, mood, and fatigue in men with HIV-associated weight loss. *Am J Physiol Endocrinol Metab*. 2008;294(6):E1135–E1143.
190. Moller SE. Plasma amino acid profiles in relation to clinical response to moclobemide in patients with major depression. *J Affect Disord*. 1993;27:225–231.
191. Neumeister A, Nugent AC, Waldeck T, et al. Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. *Arch Gen Psychiatry*. 2004;61:765–773.
192. Nowak G, Siwek M, Dudek D, et al. Effect of zinc supplementation on antidepressant therapy in unipolar depression: a preliminary placebo-controlled study. *Pol J Pharmacol*. 2003;55:1143–1147.
193. Su KP, Huang SY, Chiu CC, et al. Omega-3 fatty acids in major depressive disorder: a preliminary double-blind placebo-controlled trial. *Eur Neuropsychopharmacol*. 2003;13:267–271.
194. Peet M, Horrobin DF. A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch Gen Psychiatry*. 2002;59:913–919.
195. Delle Chiaie R, Pancheri P, Scapicchio P. Efficacy and tolerability of oral and intramuscular S-adenosyl-L-methionine 1,4-butanedisulfonate (SAMe) in the treatment of major depression: comparison with imipramine in 2 multicenter studies. *Am J Clin Nutr*. 2002;76:S1172–S1176.
196. Steinhart CR. HIV-associated wasting in the era of HAART: a practice-based approach to diagnosis and treatment. *AIDS Read*. 2001;11:557–560, 566–569.
197. Standish LJ, Calabrese C, Galantino ML. *AIDS and Complementary and Alternative Medicine Current Science and Practice.* New York: Churchill Livingstone.
198. Kosmiski L. Energy expenditure in HIV infection. *Am J Clin Nutr. December*. 2011;94(6):1677S–1682S.
199. Fewtrell MS, Abbott RA, Kennedy K, et al. Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. *J Pediatr*. 2004;144:471–479.
200. Wutzke KD, Lorenz H. The effect of l-carnitine on fat oxidation, protein turnover, and body composition in slightly overweight subjects. *Metabolism*. 2004;53:1002–1006.
201. Clark RH, Feleke G, Din M, et al. Nutritional treatment for acquired immunodeficiency virus-associated wasting using beta-hydroxy beta-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study. *J Parenter Enteral Nutr*. 2000;24:133–139.
202. Bushen OY, Davenport JA, Lima AB, et al. Diarrhea and reduced levels of antiretroviral drugs: improvement with glutamine or alanyl-glutamine in a randomized controlled trial in northeast Brazil. *Clin Infect Dis*. 2004;38:1764–1770.
203. Christeff N, Melchior JC, Mammes O, et al. Correlation between increased cortisol: DHEA ratio and malnutrition in HIV-positive men. *Nutrition*. 1999;15:534–539.
204. Lissoni P, Paolorossi F, Tancini G, et al. Is there a role for melatonin in the treatment of neoplastic cachexia? *Eur J Cancer*. 1996;32A:1340–1343.
205. Haney M, Gunderson EW, Rabkin J, et al. Dronabinol and marijuana in HIV-positive marijuana smokers: caloric intake, mood, and sleep. *J Acquir Immune Defic Syndr*. 2007;45(5):545–554.
206. Batterham MJ, Garsia R. A comparison of megestrol acetate, nandrolone decanoate and dietary counseling for HIV associated weight loss. *Int J Androl*. 2001;24:232–240.
207. Corcoran C, Grinspoon S. The use of testosterone in the AIDS wasting syndrome. *AIDS Clin Care*. 1999;11:25–26, 33–34.
208. Batterham MJ, Garsia R. A comparison of megestrol acetate, nandrolone decanoate and dietary counseling for HIV associated weight loss. *Int J Androl*. 2001;24:232–240.
209. Hengge UR, Stocks K, Faulkner S, et al. Oxymetholone for the treatment of HIV-wasting: a double-blind, randomized, placebo-controlled phase III trial in eugonadal men and women. *HIV Clin Trials*. 2003;4:150–163.
210. Kaplan G, Thomas S, Fierer DS, et al. Thalidomide for the treatment of AIDS-associated wasting. *AIDS Res Hum Retroviruses*. 2000;16:1345–1355.
211. Rastogi DP, Singh VP, Dey SK. Evaluation of homeopathic therapy in 129 asymptomatic HIV carriers. *Br Homeop J*. 1993;1:4–8.
212. Rastogi DP, Singh VP, Singh V, et al. Homeopathy in HIV infection: a trial report of double-blind placebo controlled study. *Br Homeop J*. 1999;88:49–57.
213. Ullman D. Controlled clinical trials evaluating the homeopathic treatment of people with human immunodeficiency virus or acquired immune deficiency syndrome. *J Altern Complement Med*. 2003;9:133–141.
214. Bissuel F, Cotte L, Crapanne JB, et al. Trimethoprim-sulphamethoxazole rechallenge in 20 previously allergic HIV-infected patients after homeopathic. *AIDS*. 1995;9:407–408.
215. Brewitt B, Standish LJ. High dilution growth factors/cytokines: positive immunological, hematological, and clinical effects in HIV/AIDS patients. *Eleventh Int Conf AIDS*. 1996; Abstract TH 4108.
216. Owens SD, Gasper PW. Hyperthermic therapy for HIV infection. *Med Hypotheses*. 1995;44:235–242.
217. Shallenberger F. Selective compartmental dominance: an explanation for a noninfectious, multifactorial etiology for acquired immune deficiency syndrome (AIDS), and a rationale for ozone therapy and other immune modulating therapies. *Med Hypotheses*. 1998;50:67–80.

218. Carpendale MT, Freeberg JK. Ozone inactivates HIV at noncytotoxic concentrations. *Antiviral Res*. 1991;16:281–292.
219. Garber GE, Cameron DW, Hawley-Foss N, et al. The use of ozone-treated blood in the therapy of HIV infection and immune disease: a pilot study of safety and efficacy. *AIDS*. 1991;5:981–984.
220. Anastasi JK, McMahon DJ. Testing strategies to reduce diarrhea in persons with HIV using traditional Chinese medicine: acupuncture and moxibustion. *J Assoc Nurses AIDS Care*. 2003;14:28–40.
221. Avants SK, Margolin A, Chang P, et al. Acupuncture for the treatment of cocaine addiction: investigation of a needle puncture control. *J Subst Abuse Treat*. 1995;12:195–205.
222. Washburn AM, Fullilove RE, Fullilove MT, et al. Acupuncture heroin detoxification: a single-blind clinical trial. *J Subst Abuse Treat*. 1993;10:345–351.
223. Chang BH, Sommers E. Acupuncture and the relaxation response for treating gastrointestinal symptoms in HIV patients on HAART. *Acupunct Med*. 2011;29(3):180–187.
224. Shiflett SC, Schwartz GE. Effects of acupuncture in reducing attrition and mortality in HIV-infected men with peripheral neuropathy. *Explore*. 2011;7(3):148–154.
225. Galantino ML, Eke-Okoro ST, Findley TW, et al. Use of noninvasive electroacupuncture for the treatment of HIV-related peripheral neuropathy: a pilot study. *J Altern Complement Med*. 1999;5:135–142.
226. Ye F, Chen S, Liu W. Effects of electro-acupuncture on immune function after chemotherapy in 28 cases. *J Tradit Chin Med*. 2002;22:21–23.
227. Diego MA, Field T, Hernandez-Reif M, et al. HIV adolescents show improved immune function following massage therapy. *Int J Neurosci*. 2001;106:35–45.
228. Birk TJ, McGrady A, MacArthur RD, et al. The effects of massage therapy alone and in combination with other complementary therapies on immune system measures and quality of life in human immunodeficiency virus. *J Altern Complement Med*. 2000;6:405–414.
229. Gregory R, Gilles C, Aude A. Effects of massage therapy on anxiety, depression, hyperventilation and quality of life in HIV infected patients; a randomized controlled trial. *Complement Ther Med*. 2017;32:109–114.
230. Poland RE, Gertsik L, Favreau JT, et al. Open-label, randomized, parallel-group controlled clinical trial of massage for treatment of depression in HIV-infected subjects. *J Altern Complement Med*. 2013;19(4):334–340.
231. Bremner MN, Blake BJ, Wagner VD, Pearcey SM. Effects of Reiki with music compared to music only among people living with HIV. *J Assoc Nurses AIDS Care*. 2016;27(5):635–647.
232. Scafidi F, Field T. Massage therapy improves behavior in neonates born to HIV-positive mothers. *J Pediatr Psychol*. 1996;21:889–897.
233. Shor-Posner G, Hernandez-Reif M, et al. Impact of a massage therapy clinical trial on immune status in young Dominican children infected with HIV-1. *J Altern Complement Med*. 2006;12(6):511–516.
234. Ireland M. Therapeutic touch with HIV-infected children: a pilot study. *J Assoc Nurses AIDS Care*. 1998;9:68–77.
235. Taylor DN, Wallace JG, Masdeu JC. Perception of different frequencies of cranial transcutaneous electrical nerve stimulation in normal and HIV-positive individuals. *Percept Mot Skills*. 1992;74:259–264.
236. Ullum H, Palmo J, Halkjaer-Kristensen J, et al. The effect of acute exercise on lymphocyte subsets, natural killer cells, proliferative responses, and cytokines in HIV-seropositive persons. *J Acquir Immune Defic Syndr*. 1994;7:1122–1133.
237. LaPerriere A, Antoni MH, Ironson G, et al. Effects of aerobic exercise training on lymphocyte subpopulations. *Int J Sports Med*. 1994;15(suppl 3):S127–S130.
238. Ciccolo JT, Jowers EM, Bartholomew JB. The benefits of exercise training for quality of life in HIV/AIDS in the post-HAART era. *Sports Med*. 2004;34:487–499.
239. Vazquez E. Don't just sit there. *Posit Aware*. 1996;7:23–25.
240. Galantino ML, Findley T, Krafft L, et al. Blending traditional and alternative strategies for rehabilitation: measuring functional outcomes and quality of life issues in an AIDS population. Eighth world congress of international rehabilitation medicine association. *Monduzzi Editore*. 1997;1:713–716.
241. Telles S, Naveen KV. Yoga for rehabilitation: an overview. *Indian J Med Sci*. 1997;51:123–127.
242. Rehse A. Body movement workshop for people with HIV/AIDS. *Int Conf AIDS*. 1992;8:126.
243. Yerly S, Fagard C, Gunthard HF, et al. Drug resistance mutations during structured treatment interruptions. *Antivir Ther*. 2003;8:411–415.
244. Dybul M, Chun TW, Yoder C, et al. Short-cycle structured intermittent treatment of chronic HIV infection with highly active antiretroviral therapy: effects on virologic, immunologic, and toxicity parameters. *Proc Natl Acad Sci U S A*. 2001;98:15161–15166.
245. Martinez-Picado J, Morales-Lopetegi K, Wrin T, et al. Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions. *AIDS*. 2002;16:895–899.
246. Prado JG, Shintani A, Bofill M, et al. Lack of longitudinal intrapatient correlation between p24 antigenemia and levels of human immunodeficiency virus (HIV) type 1 RNA in patients with chronic HIV infection during structured treatment interruptions. *J Clin Microbiol*. 2004;42:1620–1625.
247. Lawrence J, Mayers DL, Hullsiek KH, et al. Structured treatment interruption in patients with multidrug-resistant human immunodeficiency virus. *N Engl J Med*. 2003;349:837–846.
248. Ortiz GM, Wellons M, Brancato J, et al. Structured antiretroviral treatment interruptions in chronically HIV-1-infected subjects. *Proc Natl Acad Sci U S A*. 2001;98:13288–13293.
249. Ruiz L, Ribera E, Bonjoch A, et al. Role of structured treatment interruption before a 5-drug salvage antiretroviral regimen: the Retrogene Study. *J Infect Dis*. 2003;188:977–985.
250. El-Sadr WM, Lundgren JD, Neaton JD, et al. Strategies for management of antiretroviral therapy (SMART) study group. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*. 2006;355(22):2283–2296.
251. Danel C, Moh R, Minga A, et al. CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivican ANRS 1269 trial): a randomized trial. *Lancet*. 2006;9527(367):1981–1989.
252. DART trial team. Fixed duration interruptions are inferior to continuous treatment in African adults starting therapy with CD4 cell counts <200/μL. *AIDS*. 2008;22(2):237–247.
253. Semba RD, Miotti PG, Chiphangwi JD, et al. Infant mortality and maternal vitamin A deficiency during human immunodeficiency virus infection. *Clin Infect Dis*. 1995;21:966–972.
254. Melikian G, Mmiro F, Ndugwa C, et al. Relation of vitamin A and carotenoid status to growth failure and mortality among Ugandan infants with human immunodeficiency virus. *Nutrition*. 2001;17:567–572.
255. Villamor E, Mbise R, Spiegelman D, et al. Vitamin A supplements ameliorate the adverse effect of HIV-1, malaria, and diarrheal infections on child growth. *Pediatrics*. 2002;109:1–10. E6.
256. Filteau SM, Rollins NC, Coutsoudis A, et al. The effect of antenatal Vitamin A and beta-carotene supplementation on gut integrity of infants of HIV-infected South African women. *J Pediatr Gastroenterol Nutr*. 2001;32:464–470.
257. Fawzi WW, Msamanga GI, Hunter D, et al. Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. *AIDS*. 2002;16:1935–1944.
258. Coodley GO, Nelson HD, Loveless MO, et al. Beta-carotene and HIV infection. *J Acquir Immune Defic Syndr*. 1993;6:272–276.
259. Alexander M, Newmark H, Miller RG. Oral beta-carotene can increase the number of OKT4+ cells in human blood. *Immunol Lett*. 1985;9:221–224.
260. Melikian G, Mmiro F, Ndugwa C, et al. Relation of vitamin A and carotenoid status to growth failure and mortality among Ugandan infants with human immunodeficiency virus. *Nutrition*. 2001;17:567–572.
261. Ullrich R, Schneider T, Heise W, et al. Serum carotene deficiency in HIV-infected patients. Berlin Diarrhoea/wasting syndrome study group. *AIDS*. 1994;8:661–665.
262. Falguera M, Perez-Mur J, Piug T, et al. Study of the role of vitamin B12 and folinic acid supplementation in preventing hemologic toxicity of zidovudine. *Eur J Haematol*. 1995;55:97–102.

263. Herzlich BC, Ranginwala M, Nawabi I, et al. Synergy of inhibition of DNA synthesis in human bone marrow by azidothymidine plus deficiency of folate and/or vitamin B12? *Am J Hematol*. 1990;33:177–183.
264. Arici C, Tebaldi A, Quinzan GP, et al. Severe lactic acidosis and thiamine administration in an HIV-infected patient on HAART. *Int J STD AIDS*. 2001;12:407–409.
265. Shoji S, Furuishi K, Misumi S, et al. Thiamine disulfide as a potent inhibitor of human immunodeficiency virus (type-1) production. *Biochem Biophys Res Comm*. 1994;205:967–975.
266. Muri RM, Von Overbeck J, Furrer J, et al. Thiamin deficiency in HIV-positive patients: evaluation by erythrocyte trans-ketolase activity and thiamin pyrophosphate effect. *Clin Nutr*. 1999;18:375–378.
267. Baum MK, Mantero-Atienza E, Shor-Posner G, et al. Association of vitamin B6 status with parameters of immune function in early HIV-1 infection. *J Acquir Immune Defic Syndr*. 1991;4:1122–1132.
268. Trakatellis A, Dimitriadou A, Trakatelli M. Pyridoxine deficiency: new approaches in immunosuppression and chemotherapy. *Postgrad Med J*. 1997;73:617–622.
269. Folkers K, Morita M, McRee Jr J. The activities of coenzyme Q10 and vitamin B6 for immune responses. *Biochem Biophys Res Commun*. 1993;193:88–92.
270. Tamura J, Kubota K, Murakami H, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol*. 1999;116:28–32.
271. Herzlich BC, Schiano TD. Reversal of apparent AIDS dementia complex following treatment with vitamin B12. *J Intern Med*. 1993;233:495–497.
272. Tang AM, Graham NM, Chandra RK, et al. Low serum vitamin B-12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. *J Nutr*. 1997;127:345–351.
273. Rule SA, Hooker M, Costello C, et al. Serum vitamin B12 and transcobalamin levels in early HIV disease. *Am J Hematol*. 1994;47:167–171.
274. Burkes RL, Cohen H, Krailo M, et al. Low serum cobalamin levels occur frequently in the acquired immune deficiency syndrome and related disorders. *Eur J Haematol*. 1987;38:141–147.
275. Wasserman P, Rubin DS. Highly prevalent vitamin D deficiency and insufficiency in an urban cohort of HIV-infected men under care. *AIDS Patient Care STDS*. 2010;24(4):223–227.
276. Haug CJ, Aukrust P, Haug E, et al. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. *J Clin Endocrinol Metab*. 1998;83(11):3832–3838.
277. Eckard AR, O'Riordan MA, Rosebush JC, et al. Effects of vitamin D supplementation on bone mineral density and bone markers in HIV-infected Youth. *J Acquir Immune Defic Syndro*. 2017;76(5):539–546.
278. Stallings VA, Schall JI, Hediger ML. High-dose vitamin D3 supplementation in children and young adults with HIV. A randomized, placebo-controlled trial. *Pediatr Infect Dis J*. 2015;34(2):e32–e40.
279. Anonymous. Tenofovir/emtricitabine combination results in lower bone-mineral density. *AIDS Patient Care STDS*. 2010;24(4):265–266.
280. Hamzah L, Tiraboschi JM, Iveson H, et al. Effects of vitamin D, bone and the kidney of switching from fixed-dose tenofovir disoproxil fumarate/emtricitabine/efavirenz to darunavir/ritonavir monotherapy; a randomized, controlled trial (MIDAS). *Antivir Ther*. 2016;21(4):287–296.
281. Overton ET, Chan ES, Brown TT. Vitamin D and calcium attenuate bone loss with antiretroviral therapy initiation: a randomized trial. *Ann Intern Med*. 2015;162(12):815–824.
282. Allard JP, Aghdassi E, Chau J, et al. Effects of vitamin E supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS*. 1998;12:1653–1659.
283. Favier A, Sappey C, Leclerc P, et al. Antioxidant status and lipid peroxidation in patients infected with HIV. *Chem Biol Interact*. 1994;91:165–180.
284. Edeas MA, Claise C, Vergnes L, et al. Protective effects of the lipophilic redox conjugate tocopheryl succinyl-ethyl ferulate on HIV replication. *FEBS Lett*. 1997;418:15–18.
285. de la Asuncion JG, Del Olmo ML, Gomez-Cambronero LG, et al. AZT induces oxidative damage to cardiac mitochondria: protective effect of vitamins C and E. *Life Sci*. 2004;76:47–56.
286. Wang Y, Huang DS, Liang B, et al. Nutritional status and immune responses in mice with murine AIDS are normalized by vitamin E supplementation. *J Nutr*. 1994;124:2024–2032.
287. Tang AM, Graham NM, Semba RD, et al. Association between serum vitamin A and E levels and HIV-1 disease progression. *AIDS*. 1997;11:613–620.
288. Pacht ER, Diaz P, Clanton T, et al. Serum vitamin E decreases in HIV-seropositive subjects over time. *J Lab Clin Med*. 1997;130:293–296.
289. Davis DA, Branca AA, Pallenberg AJ, et al. Inhibition of the human immunodeficiency virus-1 protease and human immuno-deficiency virus-1 replication by bathocuproine disulfonic acid Cu1+. *Arch Biochem Biophys*. 1995;322:127–134.
290. Baum MK, Javier JJ, Mantero-Atienza E, et al. Zidovudine-associated adverse reactions in a longitudinal study of asymptomatic HIV-1-infected homosexual males. *J Acquir Immune Defic Syndr*. 1991;4:1218–1226.
291. Beach RS, Mantero-Atienza E, Shor-Posner G, et al. Specific nutrient abnormalities in asymptomatic HIV-1 infection. *AIDS*. 1992;6:701–708.
292. Moreno Diaz MT, Ruiz Lopez MD, Navarro Alarcon M, et al. Magnesium deficiency in patients with HIV-AIDS. *Nutr Hosp*. 1997;12:304–308.
293. Seguro AC, de Araujo M, Seguro FS, et al. Effects of hypokalemia and hypomagnesemia on zidovudine (AZT) and didanosine (ddI) nephrotoxicity in rats. *Clin Nephrol*. 2003;59:267–272.
294. Hurwitz BE, Klaus JR, Llabre MM, et al. Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation: a randomized controlled trial. *Arch Intern Med*. 2007;167(2):148–154.
295. Kamwesiga J, Mutabazi V, et al. Effect of selenium supplementation on CD4+ T-cell recovery, viral suppression and morbidity of HIV-infected patients in Rwanda: a randomized controlled trial. *AIDS*. 2015;29(9):1045–1052.
296. Baum MK, Miguez-Burbano MJ, Campa A, et al. Selenium and interleukins in persons infected with human immunodeficiency virus type 1. *J Infect Dis*. 2000;182(suppl 1):S69–S73.
297. Baum MK, Shor-Posner G, Lai S, et al. High risk of HIV-related mortality is associated with selenium deficiency. *J Acquir Immun Defic Synd Hum Retrovirol*. 1997;15:370–374.
298. Shor-Posner G, Lecusay R, Miguez MJ, et al. Psychological burden in the era of HAART: impact of selenium therapy. *Int J Psychiatry Med*. 2003;33:55–69.
299. Burbano X, Miguez-Burbano MJ, McCollister K, et al. Impact of a selenium chemoprevention clinical trial on hospital admissions of HIV-infected participants. *HIV Clin Trials*. 2002;3:483–491.
300. Rayman MP. The argument for increasing selenium intake. *Proc Nutr Soc*. 2002;61:203–215.
301. Mocchegiani E, Muzzioli M. Therapeutic application of zinc in human immunodeficiency virus against opportunistic infections. *J Nutr*. 2000;130(suppl S5):S1424–S1431.
302. Koch J, Neal EA, Schlott MJ, et al. Zinc levels and infections in hospitalized patients with AIDS. *Nutrition*. 1996;12:515–518.
303. Mocchegiani E, Veccia S, Ancarani F, et al. Benefit of oral zinc supplementation as an adjunct to zidovudine (AZT) therapy against opportunistic infections in AIDS. *Int J Immunopharmacol*. 1995;17:719–727.
304. McDonald CM, Manji KP, Kisenge R, et al. Daily zinc but not multivitamin supplementation reduces diarrhea and upper respiratory tract infections in Tanzanian infants; a randomized, double-blind, placebo-controlled clinical trial. *J Nutri*. 2015;145(9):2153–2160.
305. Bunupuradah T, Ubolyam S, Hansudewechakul R. Correlation of selenium and zinc levels to antiretroviral treatment outcomes in Thai HIV infected children without severe HIV symptoms. *Eur J Clin Nutr*. 2012;66(8):900–905.
306. Mintz M. Carnitine in human immunodeficiency virus type 1 infection/acquired immune deficiency syndrome. *J Child Neurol*. 1995;10(suppl 2):S40–S44.
307. De Simone C, Famularo G, Tzantzoglou S, et al. Carnitine depletion in peripheral blood mononuclear cells from patients with AIDS: effect of oral L-carnitine. *AIDS*. 1994;8:655–660.
308. Saheki T. Carnitine as a vitamin-like biofactor. *Nippon Rinsho*. 1999;57:2270–2275.

309. De Simone C, Tzantzoglou S, Famularo G, et al. High dose L-carnitine improves immunologic and metabolic parameters in AIDS patients. *Immunopharmacol Immunotoxicol*. 1993;15:1–12.
310. Harmon WG, Dadlani GH, Fisher SD, et al. Myocardial and pericardial disease in HIV. *Curr Treat Options Cardiovasc Med*. 2002;4:497–509.
311. Chariot P, Gherardi R. Myopathy and HIV infection. *Curr Opin Rheumatol*. 1995;7:497–502.
312. Di Marzio L, Moretti S, D'Alo S, et al. Acetyl-L-carnitine administration increases insulin-like growth factor 1 levels in asymptomatic HIV-1-infected subjects: correlation with its suppressive effect on lymphocyte apoptosis and ceramide generation. *Clin Immunol*. 1999;92:103–110.
313. Virmani MA, Biselli R, Spadoni A, et al. Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. *Pharmacol Res*. 1995;32:383–389.
314. Scarpini E, Sacilotto G, Baron P, et al. Effect of acetyl-L-carnitine in the treatment of painful peripheral neuropathies in HIV+ patients. *J Periph Nerv Syst*. 1997;2:250–252.
315. Claessens YE, Cariou A, Monchi M, et al. Detecting life-threatening lactic acidosis related to nucleoside-analog treatment of human immunodeficiency virus-infected patients, and treatment with L-carnitine. *Crit Care Med*. 2003;31:1042–1047.
316. Wisniewski TL, Hilton CW, Morse EV, et al. The relationship of serum DHEA-S and cortisol levels to measures of immune function in human immunodeficiency virus-related illness. *Am J Med Sci*. 1993;305:79–83.
317. Abrams DI, Shade SB, Couey P, et al. Dehydroepiandrosterone (DHEA) effects on HIV replication and host immunity: a randomized placebo-controlled study. *AIDS Res Hum Retroviruses*. 2007;23(1):77–85.
318. Dobs A. Role of testosterone in maintaining lean body mass and bone density in HIV-infected patients. *Int J Impot Res*. 2003;15(suppl 4):S21–S25.
319. De Rosa SC, Zaretsky MD, Dubs JG, et al. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest*. 2000;30:915–929.
320. Micke P, Beeh KM, Buhl R. Effects of long-term supplementation with whey proteins on plasma glutathione levels of HIV-infected patients. *Eur J Nutr*. 2002;41:12–18.
321. Droge W, Hack V, Breitkreutz R, et al. Role of cysteine and glutathione in signal transduction, immunopathology and cachexia. *Biofactors*. 1998;8:97–102.
322. Herzenberg LA, De Rosa SC, Dubs JG, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci U S A*. 1997;94:1967–1972.
323. Nguyen D, Hsu JW, Jahoor F, Sekhar RV. Effect of increasing glutathione with cysteine and glycine supplementation on mitochondrial fuel oxidation, insulin sensitivity, and body composition in older HIV-infected patients. *J Clin Endocrinol Metab*. 2014;99(1):169–177.
324. Jahoor F, Jackson A, Gazzard B, et al. Erythrocyte glutathione deficiency in symptom-free HIV infection is associated with decreased synthesis rate. *Am J Physiol*. 1999;276:E205–E211.
325. Sbrana E, Paladini A, Bramanti E, et al. Quantitation of reduced glutathione and cysteine in human immunodeficiency virus-infected patients. *Electrophoresis*. 2004;25:1522–1529.
326. Jacobs BP, Dennehy C, Ramirez G, et al. Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. *Am J Med*. 2002;113:506–515.
327. Breitkreutz R, Pittack N, Nebe CT, et al. Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *J Mol Med*. 2000;78:55–62.
328. Akerlund B, Jarstrand C, Lindeke B, et al. Effect of N-acetylcysteine (NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. *Eur J Clin Pharmacol*. 1996;50:457–461.
329. Spada C, Treitinger A, Reis M, et al. The effect of N-acetylcysteine supplementation upon viral load, CD4, CD8, total lymphocyte count and hematocrit in individuals undergoing antiretroviral treatment. *Clin Chem Lab Med*. 2002;40:452–455.
330. Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U S A*. 1990;87:4879–4883.
331. Folkers K, Langsjoen P, Nara Y, et al. Biochemical deficiencies of coenzyme Q10 in HIV-infection and exploratory treatment. *Biochem Biophys Res Commun*. 1988;153:888–896.
332. Folkers K, Morita M, McRee Jr J. The activities of coenzyme Q10 and vitamin B6 for immune responses. *Biochem Biophys Res Commun*. 1993;193:88–92.
333. Folkers K, Hanioka T, Xia LJ, et al. Coenzyme Q10 increases T4/T8 ratios of lymphocytes in ordinary subjects and relevance to patients having the AIDS related complex. *Biochem Biophys Res Commun*. 1991;176:786–791.
334. Allard JP, Aghdassi E, Chau J, et al. Effects of vitamin E supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS*. 1998;12:1653–1659.
335. Berkson BM. A conservative triple antioxidant approach to the treatment of hepatitis C: combination of alpha lipoic acid (thioctic acid), silymarin, and selenium: three case histories. *Med Klin (Munich)*. 1999;94(suppl 3):84–89.
336. Grieb G. Alpha-lipoic acid inhibits HIV replication. *Med Monatsschr Pharm*. 1992;15:243–244.
337. Suzuki YJ, Aggarwal BB, Packer L. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochem Biophys Res Commun*. 1992;189:1709–1715.
338. Han D, Tritschler HJ, Packer L. Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line. *Biochem Biophys Res Commun*. 1995;207:258–264.
339. Fuchs J, Schofer H, Milbradt R, et al. Studies on lipoate effects on blood redox state in human immunodeficiency virus infected patients. *Arzneimittelforschung*. 1993;43:1359–1362.
340. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med*. 1995;19:227–250.
341. De Luis DA, Bachiller P, Palacios T, et al. Nutritional treatment for ambulatory patients with acquired immunodeficiency virus infection and previous weight loss using a formula enriched with omega 3fatty acids; a randomized prospective trial. 2010; 14(5):449–454.
342. Kabagambe EK, Ezeamama AE, Guwatudde D, et al. Plasma n-6 fatty acid levels are associated with CD4 cell counts, hospitalizations, and mortality in HIV-Infected patients. *J Acquir Immune Defic Syndr*. 2016;73(5):598–605.
343. Van Brummelen R, du Toit D. L-methionine as immune supportive supplement: a clinical evaluation. *Amino Acids*. 2007;33(1):157–163.
344. Otegbayo JA, Kuti MA, Ogunbode O, et al. Livolin ameliorates elevations in alanine transaminase in HIV infected patient commencing highly active antiretroviral therapy. *Afr J Med Sci*. 2012;41(4):417–422.
345. Hattori T, Ikematsu S, Koito A, et al. Preliminary evidence for inhibitory effect of glycyrrhizin on HIV replication in patients with AIDS. *Antiviral Res*. 1989;11:255–261.
346. Hirabayashi K, Iwata S, Matsumoto H, et al. Antiviral activities of glycyrrhizin and its modified compounds against human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 1 (HSV-1) in vitro. *Chem Pharm Bull (Tokyo)*. 1991;39:112–115.
347. De Clercq E. Current lead natural products for chemotherapy of human immunodeficiency virus (HIV) infection. *Med Res Rev*. 2000;20:323–349.
348. Barthelemy S, Vergnes L, Moynier M, et al. Curcumin and curcumin derivatives inhibit Tat-mediated transactivation of type 1 human immunodeficiency virus long terminal repeat. *Res Virol*. 1988;149:43–52.
349. Taher MM, Lammering G, Hershey C, et al. Curcumin inhibits ultraviolet light induced human immunodeficiency virus gene expression. *Mol Cell Biochem*. 2003;254:289–297.
350. De Clercq E. Current lead natural products for chemotherapy of human immunodeficiency virus (HIV) infection. *Med Res Rev*. 2000;20:323–349.
351. Konlee M. A new triple combination therapy. *Posit Health News*. 1998;17:12–14.
352. Lee-Huang S, Zhang L, Huang PL, et al. Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochem Biophys Res Commun*. 2003;307:1029–1037.

353. Notka F, Meier G, Wagner R. Concerted inhibitory activities of Phyllanthus amarus on HIV replication in vitro and ex vivo. *Antiviral Res*. 2004;64:93–102.
354. Ngai PH, Ng TB. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. *Life Sci*. 2003;73:3363–3374.
355. Gordon M, Bihari B, Goosby E, et al. A placebo-controlled trial of the immune modulator, lentinan, in HIV-positive patients: a phase I/II trial. *J Med*. 1998;29:305–330.
356. Calabrese C, Berman SH, Babish JG, et al. A phase I trial of Andrographalide in HIV positive patients and normal volunteers. *Phytother Res*. 2000;14:333–338.
357. Gollapudi S, Sharma HA, Aggarwal S, et al. Isolation of a previously unidentified polysaccharide (MAR-10) from *Hyssop officianalis* that exhibits strong activity against human immunodeficiency virus type 1. *Biochem Biophys Res Commun*. 1995;210:145–151.
358. Kreis W, Kaplan WH, Freeman J, et al. Inhibition of HIV replication by *Hyssop officianalis* extracts. *Antiviral Res*. 1990;14:323–337.
359. Yao XJ, Wainberg MA, Parniak MA. Mechanism of inhibition of HIV-1 infection in vitro by purified extract of *Prunella vulgaris*. *Virology*. 1992;187:56–62.
360. Tabba HD, Chang RS, Smith KM. Isolation, purification, and partial characterization of prunellin, an anti-HIV component from aqueous extracts of *Prunella vulgaris*. *Antiviral Res*. 1989;11:263–273.
361. Paris A, Strukelj B, Renko M, et al. Inhibitory effect of carnosic acid on HIV-1 protease in cell-free assays. *J Nat Prod*. 1993;56:1426–1430.
362. Rebultan SP. Bitter melon therapy: an experimental treatment of HIV infection. *AIDS Asia*. 1995;2:6–7.
363. Hayashi K, Hayashi T, Kojima I. A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. *AIDS Res Hum Retroviruses*. 1996;12:1463–1471.
364. Li BQ, Fu T, Yan YD, et al. Inhibition of HIV infection by baicalin: a flavonoid compound purified from Chinese herbal medicine. *Cell Mol Biol Res*. 1993;39:119–124.
365. Gowdey G, Lee RK, Carpenter WM. Treatment of HIV-related hairy leukoplakia with podophyllum resin 25% solution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1995;79:64–67.
366. Vazquez JA, Zawawi AA. Efficacy of alcohol-based and alcohol-free melaleuca oral solution for the treatment of fluconazole-refractory oropharyngeal candidiasis in patients with AIDS. *HIV Clin Trials*. 2002;3:379–385.
367. Takahashi I, Nakanishi S, Kobayashi E, et al. Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their antiretroviral activity. *Biochem Biophys Res Commun*. 1989;165:1207–1212.
368. Tatarintsev AV, Vrzhets PV, Ershov DE, et al. The ajoene blockade of integrin-dependent processes in an HIV-infected cell system. *Vestn Ross Akad Med Nauk*. 1992;(11–12):6–10.

Hypertension

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Elevated blood pressure: 120 to 129/<80 mm Hg
- Stage 1 hypertension: 130 to 139/80 to 89 mm Hg
- Stage 2 hypertension: 140 and higher/90 and higher mm Hg

GENERAL CONSIDERATIONS

Elevated blood pressure (BP) is a major risk factor for a heart attack or stroke. In fact, it is generally regarded as the most significant risk factor for stroke. More than 70 million Americans have high BP, including more than half (54.3%) of all Americans 65 to 74 years of age and almost three-quarters (71.8%) of all African Americans in the same age group (Box 179.1). Additionally, 37% of U.S. adults have a blood pressure of 120 to 139/80 to 89 mm Hg, which is associated with a greater likelihood of developing not only hypertension and cardiovascular disease but also diabetes and cognitive impairment.[1]

Individuals with a normal diastolic pressure (<80 mm Hg) but elevated systolic pressure (>140 mm Hg), meaning an increased pulse pressure (>60 mm Hg), usually suffer from decreased compliance of the aorta (arteriosclerosis) and have a twofold increased risk of cardiovascular death compared with those whose systolic pressure is normal (<120 mm Hg).[2] Increased stroke volume due to aortic regurgitation, thyrotoxicosis, fever, and so on can also be a cause of increased pulse pressure.

Hypertension is also classified as to the cause. However, more than 90% of patients with hypertension are classified as suffering from essential hypertension, in which no discernible cause is given. Patients with essential hypertension are further divided into groups based on the level of renin, an enzyme secreted by the juxtaglomerular cells of the kidneys and linked with aldosterone in a negative feedback loop. Renin plays a central role in vascular reactivity through its effects in generating the vasoconstricting peptide angiotensin II. Renin secretion is influenced primarily by the individual's fluid-volume status and salt intake.

Patients with low-renin essential hypertension have low renin activity. In these patients, aldosterone production is not being suppressed, leading to a mild degree of hyperaldosteronism, with its resulting increased sodium retention, fluid volume, and BP. The same phenomenon occurs in patients with normal-renin hypertension, indicating that such patients may be at the end of a continuum of those with essential hypertension. Patients with low renin levels can also display an increased sensitivity to angiotensin II.

Patients with normal-renin essential hypertension typically are insulin resistant and display abdominal obesity. However, several studies have found that hyperinsulinemia and insulin resistance are present even in lean hypertensive patients without non–insulin-dependent diabetes mellitus, suggesting that there is a strong relationship between insulin sensitivity and BP. Several theories have been suggested to explain this connection. The most plausible states that because insulin

BOX 179.1 Classification of Blood Pressure

Optimal
- Systolic <120 mm Hg
- Diastolic <80 mm Hg

Prehypertension
- Systolic 120 to 139 mm Hg
- Diastolic 80 to 89 mm Hg

Stage 1 Hypertension
- Systolic 140 to 159 mm Hg
- Diastolic 90 to 99 mm Hg

Stage 2 Hypertension
- Systolic 160 and higher mm Hg
- Diastolic 100 and higher mm Hg

BOX 179.2 Classifications of Hypertension

I. Essential hypertension (>90% of all cases of hypertension)
  A. Low renin
  B. Normal renin
  C. High renin
II. Renal etiology
  A. Chronic pyelonephritis
  B. Acute and chronic glomerulonephritis
  C. Polycystic renal disease
  D. Renovascular stenosis or renal infarction
  E. Most other severe renal diseases (e.g., arteriolar nephrosclerosis, diabetic nephropathy)
  F. Renin-producing tumors
III. Endocrine etiology
  A. Adrenocortical hyperfunction
  B. Cushing's disease and syndrome
  C. Primary hyperaldosteronism
  D. Congenital or hereditary adrenogenital syndromes (17-hydroxylase and 11-hydroxylase defects)
  E. Pheochromocytoma
  F. Myxedema
  G. Acromegaly
IV. Neurogenic etiology
  A. Psychogenic
  B. Diencephalic syndrome
  C. Familial dysautonomia (Riley–Day)
  D. Polyneuritis (acute porphyria, lead poisoning)
  E. Increased intracranial pressure (acute)
  F. Spinal cord section (acute)
V. Miscellaneous
  A. Toxemia of pregnancy
  B. Acute intermittent porphyria
  C. Coarctation of aorta
  D. Increased intravascular volume (excessive transfusion, polycythemia vera)
  E. Polyarteritis nodosa
  F. Hypercalcemia
  G. Medications (e.g., glucocorticoids, cyclosporine, oral contraceptives)

modifies ion transport across the cell membrane, insulin insensitivity can lead to decreased cytosolic magnesium levels and increased cytosolic calcium levels within the vascular smooth muscle, resulting in increased vascular reactivity. Patients with normal-renin essential hypertension typically do not respond to sodium restriction.

Patients with high-renin essential hypertension make up approximately 15% of patients with essential hypertension. The elevation in renin (and associated high BP) is thought to be secondary to an increase in sympathetic nervous system activation.

The categorization of BP by renin level does not remain constant in each patient. For example, a patient might be labeled as having low-renin essential hypertension due to insulin resistance secondary to obesity. If the patient lost weight, regained insulin sensitivity, and yet the BP did not normalize, he or she would then be categorized as having normal- or high-renin essential hypertension. The categories based on renin level are primarily useful in identifying possible therapeutic interventions, as shown in Box 179.2.

White-Coat Hypertension

White-coat hypertension has been defined as the persistent elevation of BP at the clinic or office only. Its prevalence may be as high as 20% to 45% of people diagnosed as hypertensive.[1] It appears to be more frequent in women, older patients, and persons with stage 1 hypertension. White-coat hypertension should not be confused with the *white-coat effect,* which signifies the difference in BP between the office and daytime ambulatory BP and occurs in patients with white-coat hypertension as well as in those with other causes of hypertension. The current conventional wisdom among naturopathic physicians is to treat white-coat hypertension as if it were essential hypertension. The reason for this stance is that current data suggest that the pressor response elicited mirrors real-life reactions to stress; that is, studies have suggested that white-coat hypertension is not an innocent phenomenon.[3,4] In regard to the latter, in a 21-year study comprising 536 men, those with a white-coat effect had a significantly higher rate of mortality than normotensive men and were nearly twice as likely to develop sustained hypertension.[4]

Ambulatory BP monitoring is a clinically useful tool for assessing suspected white-coat hypertension and cardiovascular risk.[5] In patients with confirmed white-coat hypertension, drug treatment is usually not indicated; instead, treatment should consist of lifestyle and dietary modifications, weight reduction, regular exercise, smoking cessation, and correction of glucose and lipid abnormalities.[1] In addition, semiannual or annual follow-up with ambulatory BP monitoring is advised.

Etiology

Essential hypertension is most likely the result of any number of factors that disrupt the regulation of arterial pressure and fluid volume. Vascular, hormonal, renal, and neurological factors function in a complex interrelationship to maintain normal BP; disruption of any single facet disrupts the entire system and creates a cascading effect on regulatory mechanisms. Although genetic factors play a role, dietary, lifestyle, psychological, and environmental factors are the underlying causes in most cases of essential hypertension. Dietary factors include obesity; high sodium-to-potassium ratio; a low-fiber, high-sugar diet; high saturated fat and low omega-3 fatty acid intake; and a diet low in calcium, magnesium, and vitamin C. Important lifestyle factors that may cause high BP include stress, lack of exercise, and smoking. The dietary factor that has received the greatest attention is salt intake. Between 40% and 60% of patients with hypertension are salt sensitive. This factor is discussed later in the chapter under "Therapeutic Considerations."

Exposure to toxic metals, such as lead, mercury, cadmium, and arsenic, may also be a significant factor in some patients. The kidneys are end-organ targets of several toxins. Although studies of blood lead levels have not consistently shown an association, it is important to point out that blood lead levels reflect primarily acute exposure.[6–8] Studies looking at bone lead, for example, have upheld that exposure to heavy metals is associated with an increased risk of hypertension.[9] Specifically, the data suggest that lead has an acute effect on BP via either a recent exposure or cumulative dose. These associations were demonstrated in a cross-sectional analysis of 2001 to 2002 data from a community-based cohort in Baltimore, Maryland, of 964 men and women aged 50 to 70 years (40% African American, 55% white, 5% other race/ethnicity). Both blood lead and tibial lead were determined, along with systolic and diastolic BP. Blood lead was a strong and consistent predictor of both systolic and diastolic BP in models adjusted and not adjusted for race/ethnicity and socioeconomic status. Tibial lead was also associated with hypertension status before and after adjustment for race/ethnicity and socioeconomic status.[10]

THERAPEUTIC CONSIDERATIONS

Because more than 90% of patients with higher-than-optimal blood pressure have either prehypertension or stage 1 hypertension, most can be brought under control through changes in diet and lifestyle.[11] In fact, in head-to-head comparisons, many nondrug therapies, such as diet, exercise, and relaxation therapies, have proved to be superior to drugs in cases of prehypertension and stage 1 hypertension. For stage 2 hypertension, pharmacological therapy may be necessary. Ideally, drug treatment should be used only until the dietary, lifestyle, and supplement strategies take hold. However, long-term drug therapy is sometimes required.

Pharmacotherapy of Hypertension

For many years, the first drug of choice for high BP was a thiazide diuretic alone or in combination with a beta blocker. Because of the questionable effectiveness of this combination in reducing the cardiovascular death rate and the side effects noted in numerous studies, this approach has somewhat fallen out of favor but is still quite popular. The most common treatment is a diuretic used alone or in combination with newer medications designed to relax the arteries, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, and calcium channel blockers.

The use of a diuretic or any of the previously mentioned other drugs alone is referred to as a "step 1" therapy. Thiazide diuretics are still the most popular step 1 drugs but may soon be displaced by calcium channel blockers or ACE inhibitors. Beta blockers are not suitable step 1 drugs because of their known side effects.[12] Step 2 therapy comprises two medications, step 3 uses three, and the step 4 approach involves four. Physicians are instructed to use single therapies before going on to the combinations, unless compelling indications are present, such as diabetes or heart failure.[13]

Other types of BP-lowering medications that can be used in step 3 or 4 approaches include those that act on the central nervous system, such as clonidine, methyldopa, and reserpine, as well as some that are potent dilators of the blood vessels, like nitroprusside sodium, hydralazine, prazosin, minoxidil, and hydralazine. However, these drugs have fallen out of favor with the development of the newer calcium channel blockers and ACE inhibitors. Nonetheless, they may be appropriate in certain situations.

Overview of Dietary and Lifestyle Factors

Hypertension is closely related to lifestyle and dietary factors. Some of the important lifestyle factors linked to hypertension include smoking, stress, and lack of exercise. The most important dietary factors include obesity; high sodium-to-potassium ratio; low-fiber, high-sugar diet; high saturated fat and low essential fatty acids (EFAs) intake; a diet low in calcium, magnesium, or vitamin C; and excessive alcohol or caffeine intake (Fig. 179.1).

Several of these dietary and lifestyle factors are also discussed in Chapter 148 because the health of the arteries is critical to maintaining normal BP.

Stress

Stress can be the causative factor of hypertension in many instances, although, as in other health conditions, it has more to do with the response to and processing of stress than with stress itself. Relaxation techniques such as deep-breathing exercises, biofeedback, autogenics, transcendental meditation, yoga, progressive muscle relaxation, and hypnosis have all been shown to have some value in lowering BP.[14] Although the effect is only modest, a stress reduction technique is a necessary component of a natural BP-lowering program.

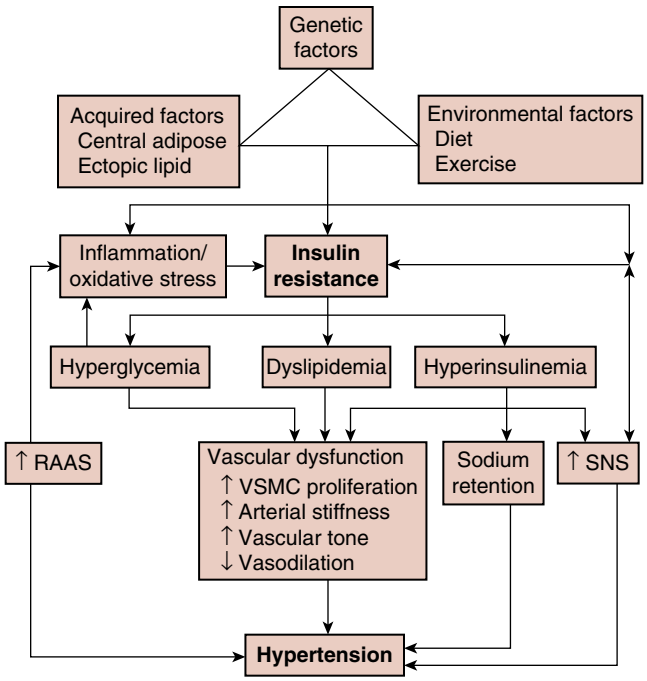


Fig. 179.1 Summary of putative pathophysiological mechanisms in the development of hypertension. (From Mugo MN, Stump CS, Rao PG, et al. Hypertension and diabetes mellitus. In: Black HR, Elliott WJ, editors. Hypertension: A Companion to Braunwald's Heart Disease. Elsevier; 2007. p. 409.)

One of the most powerful methods of producing less stress and more energy in the body is diaphragmatic breathing. Regular short exercise sessions of slow, regular diaphragmatic breathing have also been shown to lower BP in hypertensive individuals in several studies.[15,16] One study in particular has shed light on the effect of breathing in hypertension.[17] Volunteers with normal BP were taught shallow breathing. Measurement of the amount of sodium and potassium then excreted in their urine indicated that shallow breathing led to the retention of sodium in the body. It was suggested that this breathing pattern may play a causative role in some cases of hypertension as a result of the retention of sodium. However, slow breathing (six breaths per minute) has also been shown to improve oxygen saturation, exercise tolerance, and baroreflex sensitivity.[18]

RESPeRATE is a medical device that interactively guides the user toward slow, regular breathing by synchronizing voluntary respiration to musical tones. When used for 15 minutes daily, this device can lead to a significant reduction in BP. In one 8-week study, systolic BP was reduced by 10.0 mm Hg and diastolic BP by 3.6 mm Hg, but not in the controls, and greater BP reduction was observed in those who demonstrated increased compliance with the device.[19]

Exercise

Epidemiological studies have consistently demonstrated an inverse association between physical activity (or fitness) and hypertension. In addition to this evidence, clinical trials in hypertensive patients have clearly established that regular exercise is an effective treatment for high BP.[20,21] Although it is generally thought that the greater the intensity of aerobic exercise, the greater the hypotensive effect, it has been shown that even mild to moderate aerobic exercise in as few as three exercise sessions per week with durations as short as 20 minutes produces a hypotensive effect.[21] The degree of BP reduction from a regular exercise program is typically in the range of 5 to 10 mm Hg for both the systolic and diastolic readings. Patients with prehypertension and stage

1 hypertension can typically bring their BP readings into the normal range with regular exercise.

Dietary Recommendations

Obesity is the major dietary cause of hypertension, and achieving their ideal body weight is the most important therapeutic goal for most patients with any form of hypertension. From prehypertension to chronic renal failure, weight loss can lead to complete elimination of the health issue, significant improvement, or at least a reduction in the number of prescriptions needed.[22,23]

Vegetarians generally have lower BP levels and a lower incidence of hypertension and other cardiovascular diseases than nonvegetarians.[25]Although dietary levels of sodium do not differ significantly between these two groups, a vegetarian diet typically contains more potassium, complex carbohydrates, EFAs, fiber, calcium, magnesium, and vitamin C and less saturated fat and refined carbohydrates, all of which have a favorable influence on BP.

An increased intake of fruits and vegetables has been shown to lower BP.[24] This effect may be due to increased antioxidant concentrations. Hypertensive patients have been shown to have increased oxidative stress, and antioxidants have been shown to block angiotensin II–induced increases in BP as well as to promote proper nitric oxide synthesis.[25,26]

The most useful foods for people with hypertension include the following:

- Celery
- Garlic and onions
- Nuts and seeds or their oils for their EFA content
- Cold-water fish (e.g., salmon, mackerel)
- Green leafy vegetables for their calcium and magnesium
- Whole grains and legumes for their fiber
- Foods rich in vitamin C, like broccoli and citrus fruits
- Foods rich in active flavonoids, including berries, cherries, grapes, and small red kidney beans

Celery contains 3-n-butyl phthalide, a compound that has been found to lower BP. In animals, a small amount of this compound lowered BP by 12% to 14% and cholesterol by about 7%.[27] The equivalent dose in humans can be supplied in about four to six ribs of celery. The research was prompted by the father of one of the researchers, who, after eating a quarter pound of celery daily for 1 week, observed that his BP had dropped from 158/96 to 118/82.

Garlic and onions are also important foods for lowering BP. Although most research has focused on the cholesterol-lowering properties of these vegetables, both have also been shown to lower BP in hypertension.[28] In addition, commercial garlic supplements may be of benefit. The usual response to the use of garlic is fairly modest, a reduction of roughly 8 to 11 mm Hg for the systolic and 5 to 8 mm Hg for the diastolic BP (see Chapters 62 and 63 for a full discussion and dosages).

Dietary Approaches to Stop Hypertension (DASH)

Clinical studies of Dietary Approaches to Stop Hypertension (DASH) were funded by the National Heart, Lung, and Blood Institute (NHLBI) to evaluate the efficacy of a system of dietary recommendations in the treatment of hypertension. The DASH diet is rich in fruits, vegetables, and low-fat dairy foods and low in saturated and total fat. It is also low in cholesterol; high in dietary fiber, potassium, calcium, and magnesium; and moderately high in protein.

The first study showed that a diet rich in fruits, vegetables, and low-fat dairy products can reduce BP in the general population and people with hypertension.[29] The original DASH diet did not require either sodium restriction or weight loss—the two traditional dietary tools to control BP—to be effective.[30] The second study from the DASH research group found that coupling the original DASH diet with sodium restriction is more effective than dietary manipulation alone.[31] In the first trial, the DASH diet produced a net BP reduction of 11.4 and 5.5 mm Hg systolic and diastolic, respectively, in patients with hypertension. In the second trial, sodium intake was also quantified at a "higher" intake of 3300 mg/day, an "intermediate" intake of 2400 mg/day, and a "lower" intake of 1500 mg/day. Compared with the control diet, the DASH diet was associated with a significantly lower systolic BP at each sodium level. The DASH diet with the lower sodium level led to a mean systolic BP that was 7.1 mm Hg lower in participants without hypertension and 11.5 mm Hg lower in participants with hypertension. These results are clinically significant and indicate that sodium intake below the recommended level of 2400 mg daily can significantly and quickly lower BP.

Table 179.1 lists a brief description of the components of the DASH eating plan on the basis of a 2000-calorie daily diet.

Potassium and Sodium

Considerable evidence indicates that a diet low in potassium and high in sodium is associated with hypertension and plays a major role in the development of cancer and cardiovascular disease (e.g., heart disease, hypertension, stroke).[32,33] In regard to hypertension, there is overwhelming evidence that dietary sodium chloride (salt) is a major cause of raised BP and that a modest reduction in salt intake can lower BP, which is predicted to reduce cardiovascular disease because there is a direct relation between salt intake and cardiovascular risk.[34] In advising a patient to decrease salt consumption, one must ensure that he or she is getting enough iodine from alternative sources. Conversely, a diet high in potassium and low in sodium is protective against cardiovascular diseases. In the case of hypertension, as evident from the second DASH study and others, this type of diet can be therapeutic.

It is a well-established fact that excessive consumption of dietary sodium chloride coupled with diminished dietary potassium is a common cause of high BP, especially in "salt-sensitive" individuals. However, numerous studies have shown that in many cases, sodium restriction alone does not significantly improve BP control—it must be accompanied by high potassium intake. In a typical Western diet, only 5% of sodium intake comes from the natural constituents in food. Prepared foods contribute 45% of the sodium intake, 45% is added during cooking, and another 5% is added as a condiment.

Most Americans have a potassium-to-sodium (K:Na) ratio of less than 1:2. Epidemiological and experimental research suggests that a dietary K:Na ratio of greater than 5:1 (as was apparently typical of human evolutionary diets) is necessary to maintain health. However, even this level may not be optimal. A natural diet rich in fruits and vegetables can produce a K:Na ratio greater than 100:1 because most fruits and vegetables have a K:Na ratio of at least 50:1.

Nutritional Supplements

Potassium

Many studies have shown that increased dietary potassium intake can lower BP.[35] In addition, several studies have shown that potassium supplementation alone can produce significant reductions in BP in hypertensive subjects. Results from 33 randomized controlled trials (2609 participants) in which potassium supplementation was the only difference between the intervention and control conditions were used in one meta-analysis. Using a random-effects model, potassium supplementation was associated with a significant reduction in mean systolic and diastolic BP of 4.44 and 2.45 mm Hg, respectively. The effects of potassium supplementation appeared to be enhanced in subjects who had a high intake of sodium, indicating that this is an important recommendation for the prevention and treatment of hypertension in those who are unable to reduce their intake of sodium. The dosage of potassium typically used in the studies ranged from 2.5 to 5 g/day.

TABLE 179.1 Basic Components of the DASH Diet (2000 Calories a Day)

Food Group	Daily Servings	Serving Sizes	Examples	Significance of Each Food Group to the Dash Diet Pattern
Grains and grain products	7–8	1 slice bread ½ cup dry cereal ½ cup cooked rice, pasta, or cereal	Whole-wheat bread, English muffin, pita bread, bagels, cereals, grits, oatmeal	Major sources of energy and fiber
Vegetables	4–5	1 cup raw leafy vegetables ½ cup cooked vegetables 6 oz vegetable juice	Tomatoes, potatoes, squash, broccoli, turnips, greens, collards, kale, spinach, artichokes, sweet potatoes, beans	Rich sources of potassium, magnesium, and fiber
Fruits	4–5	6 oz fruit juice 1 medium fruit ¼ cup dried fruit ½ cup fresh, frozen, or canned fruit	Apricots, bananas, dates, oranges, orange juice, mangoes, melons, peaches, pineapples, prunes, raisins, strawberries, tangerines	Important sources of potassium, magnesium, and fiber
Low-fat or nonfat dairy foods	2–3	8 oz milk 1 cup yogurt 5 oz cheese	Skim or 1% milk, skim or low-fat yogurt, part-skim mozzarella cheese, nonfat cheese	Major sources of calcium and protein
Meats, including poultry and fish	≤2	3 oz cooked meats, poultry, or fish	Select only lean; trim away visible fats; broil, roast, or boil instead of frying; remove skin from poultry	Rich sources of protein and magnesium
Nuts, seeds, and legumes	4–5 weekly	5 oz or 1⁄3 cup nuts ½ oz or 2 tbsp seeds ½ cup cooked legumes	Almonds, filberts, mixed nuts, peanuts, walnuts, sunflower seeds, kidney beans, lentils	Rich sources of energy, magnesium, potassium, protein, and fiber

DASH, Dietary Approaches to Stop Hypertension.

In one study, 37 adults with hypertension received either 2.5 g/day of potassium, 2.5 g/day of potassium plus 480 mg/day of magnesium, or a placebo for 8 weeks; then they were crossed over to receive one of the other treatments for another 8 weeks; and then they were crossed over again in another 8 weeks.[36] The potassium supplementation lowered systolic BP an average of 12 mm Hg and diastolic BP an average of 16 mm Hg. Interestingly, the additional magnesium offered no further reduction in BP; nonetheless, magnesium supplementation has been shown to be helpful in other studies (discussed later).

Potassium supplementation may be especially useful in the treatment of hypertension in persons above age 65, who often do not fully respond to antihypertensive drugs. In one double-blind study, 18 untreated elderly patients (average age 75 years) with a systolic BP of greater than 160 mm Hg, a diastolic BP of greater than 95 mm Hg, or both were given either potassium chloride (2.5 g of potassium) or a placebo each day for 4 weeks.[37] After this relatively short treatment period, the group receiving the potassium experienced a drop of 12 mm Hg in their systolic BP and 7 mm Hg in their diastolic BP. These results compare quite favorably with the reduction of BP produced by drug therapy—and without the negative effects and side effects.[38]

Potassium supplements are available by prescription and over the counter (OTC). However, the U.S. Food and Drug Administration (FDA) restricts the amount of potassium available in OTC potassium supplements to 99 mg per dose because of problems associated with high-dosage prescription potassium salts. Salt substitutes such as the popular brands NoSalt and Nu-Salt are, in fact, potassium chloride and provide 530 mg of potassium per 1⁄6 tsp. The prescription and OTC supplements are either potassium salts (chloride and bicarbonate), potassium bound to various mineral chelates (e.g., aspartate, citrate), or food-based potassium sources. Potassium chloride preparations are the most popular by prescription and are available in a vast array of formulations (e.g., timed-release tablets, liquids, powders, and effervescent tablets) and flavors. Potassium salts are commonly prescribed in the dosage range of 1.5 to 3 g/day. However, potassium salts can cause nausea, vomiting, diarrhea, and ulcers when given in pill form at high dosages. These effects are not seen when potassium levels are increased through the diet only. This difference highlights the advantages of using foods or food-based potassium supplements rather than pills to meet the human body's high potassium requirements.

Potassium supplementation is relatively safe except for patients with kidney disease. Their inability to maintain appropriate potassium homeostasis may result in heart arrhythmias and other consequences of potassium toxicity. Potassium supplementation is also contraindicated if used in combination with a number of prescription medications, including digitalis, potassium-sparing diuretics, and the ACE inhibitor class of antihypertensive drugs.

Magnesium

Potassium interacts with magnesium in many body systems, and low intracellular potassium levels may be the result of low magnesium intake. It is therefore appropriate to supplement magnesium (400–1200 mg/day in divided doses) along with potassium. This may also lower BP.

A meta-analysis of 14 clinical trials that tested the effects of magnesium supplementation on hypertension demonstrated clear dose-dependent BP reductions—a drop of 4.3 mm Hg systolic and 2.3 mm Hg diastolic for each 10 mmol/day increase in magnesium dose.[39]

In one double-blind clinical study, 21 male patients with hypertension were given 600 mg/day of magnesium (as magnesium oxide) or placebo.[40] Mean BP (the average between the systolic and diastolic) decreased from 111 to 102 mm Hg. The patients who responded best were those with reduced red blood cell (RBC) potassium. After therapy with magnesium, the levels of intracellular sodium, potassium, and magnesium normalized, suggesting that one of the ways in which magnesium lowers BP is through activation of the cellular membrane pump moving sodium out of the cell and potassium into the cell.

Considerable evidence indicates that a high intake of magnesium is associated with lower BP in population studies. The principal source of magnesium in early studies was water. Water that is high in minerals like magnesium is often referred to as "hard water." Numerous studies have demonstrated an inverse correlation between water hardness and high BP.[41]

In the Honolulu Heart Study, systolic BP was 6.4 mm Hg lower and diastolic BP 3.1 mm Hg lower in the group with the highest magnesium intake compared with that having the lowest magnesium intake.[42]

Studies of magnesium supplementation in the treatment of hypertension have yielded mixed results. Although the overall results in a meta-analysis of the data are quite favorable, it appears that the hypertensive patients who respond are those taking a diuretic who have a high level of renin, with low RBC magnesium, or with elevated intracellular sodium or a decreased intracellular potassium.

The recommended daily intake for magnesium in hypertensive patients appears to be approximately 6 to 10 mg/kg body weight.

Magnesium is available in several forms. Although most are equally well absorbed, magnesium bound to aspartate or the Krebs cycle intermediates (malate, succinate, fumarate, and citrate) is usually preferable to magnesium oxide, gluconate, sulfate, or chloride. Absorption studies indicate that magnesium is easily absorbed orally, especially when it is bound to citrate (and presumably aspartate and other members of the Krebs cycle).[43,44] In addition, magnesium bound to aspartate or Krebs cycle intermediates may also help with fatigue. Aspartate feeds into the Krebs cycle, the final common pathway for the conversion of glucose, fatty acids, and amino acids to chemical energy (adenosine triphosphate, or ATP), whereas citrate, fumarate, malate, and succinate are actual components of the Krebs cycle. Minerals chelated to the Krebs cycle intermediates are better absorbed, used, and tolerated compared with inorganic or relatively insoluble mineral salts, including magnesium chloride, oxide, or carbonate. In addition, although inorganic magnesium salts often cause diarrhea at higher dosages, organic forms of magnesium generally do not.

In general, magnesium is well tolerated. Magnesium supplementation can sometimes cause a looser stool, particularly magnesium sulfate (Epsom salts), hydroxide, or chloride. Magnesium supplementation must be used with great care in patients with kidney disease or severe heart disease (such as high-grade atrioventricular block).

Calcium

Population-based studies have suggested a link between hypertension and a low intake of calcium.[36] However, the association is not as strong as the one for magnesium and potassium. In addition to the epidemiological data, several clinical studies have demonstrated that calcium supplementation can lower BP in hypertension, but the results have been inconsistent.[45]

To clarify the effectiveness of calcium supplementation in patients with hypertension, a double-blind, placebo-controlled crossover study was performed on 46 patients with either salt-sensitive or salt-resistant hypertension.[46] During the calcium supplementation phase, patients received 1.5 g/day of calcium (as calcium carbonate) for 8 weeks. The calcium supplementation was found to effectively reduce BP in black patients and in salt-sensitive patients but not in those having salt-resistant hypertension. Better results have been found for calcium citrate versus calcium carbonate.[47]

Another group that appears to respond to calcium supplementation is elderly patients with hypertension. One study used 24-hour monitoring of BP to evaluate the effect of calcium supplementation on essential hypertension in elderly hospitalized patients. The mean systolic and diastolic BPs over a 24-hour period declined by 13.6 mm Hg and 5 mm Hg, respectively, in patients whose diets were supplemented with 1 g of elemental calcium.[48]

Vitamin C

Population-based and clinical studies have shown that the higher the intake of vitamin C, the lower the BP. The results from two double-blind trials have confirmed a modest BP-lowering effect with vitamin C supplementation in people with mild elevations of BP.[49,50] One of the key findings of these studies was that a daily dose of 500 mg produced the same benefit as higher doses (i.e., 1000 and 2000 mg daily). Vitamin C supplementation can produce decreases of up to 4.5 mm Hg in the systolic and 2.5 mm Hg in the diastolic BP.

One of the mechanisms by which vitamin C exerts this antihypertensive effect is by promoting the excretion of lead. Chronic exposure to lead from environmental sources, including drinking water, is associated with hypertension and increased cardiovascular mortality. Areas with a soft water supply have an increased lead concentration in drinking water due to the increased acidity of the water, and people living in these areas may be predisposed to hypertension. It should be noted that soft water is also low in calcium and magnesium, two minerals that have also been shown to protect against hypertension.

Vitamin C is likely to be more effective when it is used with other antioxidant nutrients. The combination of 500 mg of vitamin C, 600 mg of α-tocopherol, 200 mg of zinc sulfate, and 30 mg of beta-carotene daily produced mild reductions in the systolic BP compared with the placebo phase both in subjects receiving antihypertensive therapy and those who were normotensive.[51]

Folic Acid and Vitamin B_6

Folic acid and vitamin B_6 reduce plasma homocysteine levels—a known contributor to atherosclerosis. A 2-year trial of folic acid and vitamin B_6 therapy to lower homocysteine was associated with a reduction of 3.7 mm Hg in systolic BP and a reduction of 1.9 mm Hg in diastolic BP.[52] Vitamin B_6 supplementation alone has also been shown to lower BP. In one study, vitamin B_6 supplementation at a single daily oral dose of 5 mg/kg for 4 weeks in 20 people with hypertension demonstrated significant reductions in systolic and diastolic BPs as well as serum norepinephrine levels.[53] The effects on BP were significant; systolic pressure dropped from 167 to 153 mm Hg, and the diastolic pressure dropped from 108 to 98 mm Hg.

Omega-3 Oils

More than 60 double-blind studies have demonstrated that fish oil supplements are effective in lowering BP.[54,55] Typically, fish oils produced a reduction of 2.1 mm Hg in the systolic BP and 1.6 mm Hg in the diastolic BP at a dose of 3000 mg of omega-3 (EPA/DHA) daily. In one double-blind, placebo-controlled, randomized clinical trial, healthy men and women consumed a control oil or fish oil providing 0.7g or 1.8g EPA + DHA per day (intakes achievable through diet), in random order, each for 8 weeks to examine the effect of fish oil on systolic and diastolic BP.[56] Findings indicated that in adults with isolated systolic hypertension, daily doses of EPA + DHA as low as 0.7 g showed clinically meaningful BP reductions. Flaxseed oil may also lower BP. In one study, along with reducing the intake of saturated fat, 1 tbsp/day of flaxseed oil reduced both the systolic and diastolic readings by up to 9 mm Hg.[57] Another study found that for every absolute 1% increase in body alpha-linolenic acid content, there was a decrease of 5 mm Hg in the systolic, diastolic, and mean BPs.[58]

Arginine

Arginine, an amino acid, is important in the formation of nitric oxide. This compound plays a central role in determining the tone of blood vessels and renal function. Specifically, it exerts a relaxing effect on

blood vessels, thereby improving blood flow as well as renal plasma flow and the glomerular filtration rate. Normally the body makes enough arginine even when the diet is lacking. However, in some instances, the body may not be able to keep up with increased requirements, in which case supplementation may prove useful. In high BP, even in mild cases, there appears to be a derangement of endothelial nitric oxide production, especially in the kidneys.

Low levels of NO are associated with impaired endothelial function. Asymmetrical dimethylarginine (ADMA), an analog of L-arginine, is a naturally occurring product of metabolism found in human circulation. Elevated levels of ADMA inhibit NO synthesis and therefore impair endothelial function and thus promote atherosclerosis and hypertension. Experimental data from cell culture and animal experiments and cross-sectional studies in humans suggest an association between elevated ADMA concentrations and cardiovascular diseases.

In a randomized, double-blind, placebo-controlled study investigating endothelial function as measured by brachial artery flow-mediated dilatation (FMD), serum ADMA and serum L-arginine levels in 49 hypercholesterolemic individuals were compared with those of individuals with normal cholesterol levels.[59] People with hypercholesterolemia had impaired endothelial function, increased ADMA levels, and decreased L-arginine/ADMA ratios. ADMA levels were inversely correlated to the endothelial-dependent vasodilatation, and intravenous infusion of L-arginine normalized the L-arginine/ADMA ratio as well as the endothelial function.

A 2005 study investigated the relationship between ADMA plasma levels and endothelium-dependent vasodilation in 36 patients with never-treated essential hypertension and 8 normotensive healthy subjects.[60] Subjects with hypertension had impaired brachial artery FMD, increased ADMA levels, and increased L-arginine plasma concentrations compared with normotensive controls. These measures were inversely correlated with ADMA levels, independently accounting for 34% of the interindividual variability in peak flow-mediated dilatation. Infusion of L-arginine improved the endothelial function.

Arginine supplementation has been shown to be beneficial in several cardiovascular diseases, including hypertension. By increasing nitric oxide levels, arginine supplementation improves blood flow, reduces the formation of blood clots, and improves blood fluidity. In hypertension, the degree of improvement offered by arginine supplementation can be quite significant in some cases.[61,62] In general, however, a dosage of 4 g three times daily will produce only modest decreases (e.g., 5 mm Hg) in systolic BP with little meaningful change in diastolic BP.[63] Arginine supplementation may prove to be most beneficial in younger subjects with essential hypertension because older adult patients with hypertension appear to have a derangement in NO-dependent renal mechanisms. In a study comparing the renal response with an intravenous infusion of arginine in young and aged patients with essential hypertension, arginine induced a significant increase in renal plasma flow, arginine glomerular filtration rate, and natriuresis and kaliuresis in the younger subjects, without changes in filtration fraction.[64] These effects were not observed in older subjects.

One trial investigated the influence of a combination of L-arginine with the vitamins B_6, folic acid, and B_{12} (TELCOR Arginin plus) on endothelial dysfunction in subjects aged 40 to 65 years with mild to moderate BP elevation not treated with antihypertensive drugs.[65] Individuals were randomly assigned to either the dietetic product ($n = 40$) or a matching placebo ($n = 41$) for 3 months with open follow-up for a further 3 months. The primary efficacy analysis demonstrated a statistically significant superiority of the combination of L-arginine with B vitamins over placebo in improving and restoring impaired endothelial function and lowering BP in patients with mild to moderate blood pressure elevation.

Anti-ACE Peptides

Various naturally occurring peptides have been shown to inhibit ACEs, including peptides from milk, chicken, and fish. The best-studied are composed of a purified mixture of nine small peptides (proteins) derived from muscle of the fish bonito (a member of the tuna family).[66–68] Anti-ACE bonito peptides do not appear to produce the side effects typical of ACE inhibitors (according to human safety studies) and do not lower BP in people with normal BP, even when administered at levels 20 times greater than the dose level that lowers BP in people with high BP. A possible reason is that its mechanism of action in inhibiting ACEs is different from that of the drugs. Research bears out this theory. The drugs indiscriminately block ACEs by interfering with their action, whereas the bonito anti-ACE peptides interact much differently. ACEs convert angiotensin I to angiotensin II by cleaving off a small peptide. Drugs work by directly blocking this action. Naturally occurring anti-ACE peptides work differently; ACEs actually react with the peptides instead of angiotensin. In addition to competing with angiotensin via this effect, anti-ACE peptides are transformed into even more potent inhibitors of ACEs. Technically speaking, the bonito anti-ACE peptides are considered a "prodrug" because the transformed peptides exert an 800% greater activity level.

Four clinical studies have shown that fish-derived anti-ACE peptides (three with the bonito peptides and one with a dipeptide from sardine) exert significant BP-lowering effects in people with high BP.[67–69] The degree of BP reduction in these studies was quite significant, typically reducing the systolic BP by at least 10 mm Hg and the diastolic BP by 7 mm Hg in people with hypertension. Greater reductions are seen in people with higher initial BP readings.

Coenzyme Q_{10}

Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone, is an essential component of the mitochondria. Although CoQ_{10} can be synthesized within the body, deficiency states have been reported. In hypertension, CoQ_{10} deficiency has been shown to be present in 39% of patients. This finding alone suggests a need for CoQ_{10} supplementation. However, CoQ_{10} appears to provide benefits beyond the correction of a deficiency.

The majority of studies exploring CoQ10 in the treatment of high BP have been uncontrolled or have used CoQ10 in combination with conventional antihypertensive medical treatments, making these studies difficult to interpret. A Cochrane review on CoQ_{10} in the treatment of hypertension (12 clinical trials, 362 patients) concluded that in hypertensive patients, CoQ_{10} has the potential to lower systolic and diastolic BP without significant side effects.[70] Among all included studies, decreases in systolic BP ranged from 11 to 17 mm Hg and in diastolic BP from 8 to 10 mm Hg. In 3 of the 12 studies, CoQ_{10} was given in addition to existing antihypertensive medication, and in one of these, more than 50% of the patients were able to cease taking at least one antihypertensive medication during the trial. These results are consistent with some of the uncontrolled studies. For example, in one uncontrolled study, the dose of CoQ_{10} was adjusted in 109 patients with essential hypertension according to clinical response and blood CoQ_{10} levels (the aim was to attain blood levels >2 mg/mL). The average CoQ_{10} dose was 225 mg/day in addition to these patients' usual antihypertensive regimen. The need for antihypertensive medication declined gradually; after a mean treatment period of 4.4 months, about half of the patients were able to discontinue between one and three of their drugs.[71]

It is important to remember that the antihypertensive effect of CoQ_{10} is usually not seen until after 4 to 12 weeks of therapy. Thus CoQ_{10} is not a typical BP-lowering drug; rather, it seems to correct some metabolic abnormality, which in turn has a favorable influence on BP. For more information, see Chapter 79.

L-Citrulline

Supplementation with citrulline, an NO-boosting agent, has shown promise as a BP-lowering intervention in adults with hypertension, with animal evidence for atherogenic-endothelial protection. The cardiovascular health benefits of L-citrulline are largely predicated on the capacity for citrulline to increase L-arginine availability for NO biosynthesis. In spontaneously hypertensive rats, L-citrulline treatment prevents hypertension by increasing the L-arginine/ADMA ratio.[72] In obese men with prehypertension or stage 1 hypertension, those treated with watermelon extract containing 6 g/day of L-citrulline/L-arginine, compared with placebo, for 6 weeks exhibited reduced ankle and brachial systolic blood pressure (–12 ± 4 and –15 ± 3 mm Hg, respectively), ankle and brachial diastolic blood pressure (–8 ± 2 and –8 ± 2 mm Hg, respectively), and carotid wave reflection, which may suggest improved arterial function.[73] A pilot study of middle-aged, prehypertensive men and women evaluated the effects of watermelon supplementation (L-citrulline/L-arginine, 2.7g/ 1.3g per day) on aortic BP and arterial function in individuals with prehypertension.[74] Compared with placebo, 6 weeks of watermelon extract supplementation improved peripheral vascular tone (decreased augmentation index and pulse wave velocity) and led to a significant reduction in aortic systolic blood pressure (–9 ± 3 vs. –2 ± 3 mm Hg) and nonsignificant reductions in brachial artery systolic blood pressure (–9 ± 7 vs. –3 ± 7 mm Hg).

Forty volunteers consumed 6 g of watermelon extract daily ($n = 20$; age 48.7 ± 1.9 years, 10 men) or a placebo ($n = 20$; age 47.4 ± 1.2 years, 11 men) in a 6-week randomized, double-blind, experimental, and placebo-controlled study to evaluate the effect of supplementation with watermelon extract on the BP and sympathovagal balance of individuals with prehypertension or hypertension.[75] BP and cardiac autonomic modulation were measured. Although there was no significant change in sympathovagal balance from the beginning (1.7 ± 0.1) to the end of the study (1.7 ± 0.4), watermelon extract promoted a significant reduction in systolic (137.8 ± 3.9–126.0 ± 4.0 mm Hg, $p < 0.0001$) and diastolic (79.2 ± 2.2–72.3 ± 2.0 mm Hg, $p < 0.001$) BP.

Accumulating human clinical trials provide evidence that L-citrulline and watermelon extract reduce peripheral and central (aortic) BP and improve resting BP and arterial stiffness in adults with prehypertension and those with hypertension.[76] In addition, L-citrulline appears to promote adaptations to physiological and environmental stressors to reduce vessel wall stiffness and attenuates any sympathetic-mediated hypertensive response.[77]

Caffeine

Caffeine consumption from coffee or tea can produce an immediate, short-lived increase in BP, and regular coffee drinking has been associated with slight increases in BP. However, it is generally thought that a tolerance to the hypertensive effects of caffeine develops in habitual coffee or tea drinkers.[78–79] Yet some studies have shown that with repeated administration of caffeine, a persistent pressor effect is produced. For example, in 11 short-term trials looking at the pressor effect of caffeine consumption (ranging from 14 to 79 days), the average dose of five cups of coffee per day was associated with an increase of 2.4 mm Hg in systolic BP and an increase of 1.2 mm Hg in diastolic BP.[80] Although the overall benefit of long-term avoidance of caffeine (from coffee, tea, chocolate, cola drinks, and some medications) on BP is unclear, it appears that some patients seem to respond quite favorably to caffeine avoidance. It should therefore be attempted in patients with hypertension.

Botanical Medicines

Crataegus Species

Extracts of hawthorn berries as well as of the flowering tops are widely used by physicians in Europe because of their cardiovascular effects. Several studies, including double-blind trials, have demonstrated that hawthorn extracts are effective in lowering BP and improving heart function.[81,82] However, the BP-lowering effect of hawthorn is mild. It typically takes 2 to 4 weeks before this agent begins to exert any effect. For more information, see Chapter 82.

Olive *(Olea europaea)*

The leaves of the olive tree (*Olea europaea* L.) have been used since ancient times to combat high BP, and this agent has significant support in animal and human studies both as an antihypertensive and in lowering cholesterol. The active substances are oleuropein (a polyphenolic iridoid glycoside),[83] oleacein, and oleanolic acid, which act as natural calcium channel blockers. Hydroxytyrosol is a metabolite of oleuropein that exerts antioxidant effects. Olive extracts are often standardized for hydroxytyrosol, but this compound is devoid of any significant antihypertensive effect. Oleuropein is also found in the olive's fruit and oil but in significantly smaller quantities than the leaf.

In an initial small double-blind study of patients with essential hypertension, 12 consulting for the first time and 18 currently on antihypertensive drugs, olive leaf extract at a dose of 400 mg four times daily for 3 months produced a modest yet statistically significant decrease in BP, with no side effects.[84]

Additional studies have used Benolea (EFLA943), standardized to oleuropein (16%–24%), and polyphenols. In a preliminary clinical study, 20 monozygotic adult twin pairs with mild hypertension received either a dose of 500 or 1000 mg daily for 8 weeks or a placebo. After 8 weeks, BP remained unchanged from baseline in the placebo group and the 500-mg/day group but was significantly decreased for the group receiving 1000 mg/day (137 vs. 126).[83]

In another study with Benolea, 232 patients with hypertension were given either the olive leaf extract (500 mg twice a day) or captopril (12.5 mg twice daily). The mean BP at baseline was 149.3/93.9 mm Hg in the group receiving olive leaf extract and 148.4/93.8 mm Hg in the group on captopril. Mean reductions in systolic BP from baseline to the end of the study were 11.5 and 13.7 mm Hg in the olive leaf extract and captopril groups, respectively, with parallel reductions in diastolic BP of 4.8 and 6.4 mm Hg, respectively.[85]

Allium Sativum and *Allium Cepa*

A meta-analysis of published and unpublished randomized controlled trials of garlic preparations was conducted to determine the effect of garlic on BP relative to placebo.[28] Eight trials (seven double blind, one single blind) were identified as meeting analytic criteria. In total, 415 subjects were included in the analysis. All trials used a dried garlic powder standardized to contain 1.3% allicin at a dose of 600 to 900 mg daily (corresponding to 7.8 and 11.7 mg, respectively, of allicin or the equivalent of approximately 1.8–2.7 g of fresh garlic daily). The meta-analysis concluded that garlic preparations designed to yield allicin can lower systolic and diastolic BP over a 1- to 3-month period. The typical drop from pooled data was 11 mm Hg in the systolic BP and 5 mm Hg in the diastolic BP.

Hibiscus Sabdariffa

Hibiscus tea and extracts prepared from the dried flowers (calyces) of *Hibiscus sabdariffa* have demonstrated antihypertensive properties in clinical trials. The active components are anthocyanidin glycosides. A systematic review and meta-analysis of randomized controlled trials

indicated a significant effect of *H. sabdariffa* supplementation in lowering both systolic blood pressure (weighted mean difference –7.58 mm Hg) and diastolic blood pressure (weighted mean difference –3.53 mm Hg).[86]

One double-blind, placebo-controlled clinical trial was conducted in 65 prehypertensive and mildly hypertensive adults 30 to 70 years of age who were not taking antihypertensive medications. They were given either three 240-mL servings per day of brewed hibiscus tea or a placebo beverage for 6 weeks. At 6 weeks, hibiscus tea was found to have lowered systolic BP compared with placebo (by 7.2 vs. 1.3 mm Hg). Diastolic BP was also lower, although this change did not differ from placebo. Participants with higher systolic BP at baseline showed a greater response to the hibiscus treatment.[87]

In another double-blind study, the effect of hibiscus tea was compared with that of black tea in 60 patients with diabetes and hypertension who were not taking antihypertensive or lipid-lowering drugs. The mean systolic BP in the hibiscus group decreased from 134.4 mm Hg at the beginning of the study to 112.7 mm Hg after 1 month, whereas it increased from 118.6 to 127.3 mm Hg in the group drinking black tea. The intervention had no statistically significant effect on the mean diastolic BP in either group.[88]

Another study with hibiscus tea did show an effect on diastolic BP (reducing it by 10.7%), as well as systolic BP (reducing it by 11.2%), after 12 days of treatment.[89]

Two clinical studies featured the use of a standardized extract of hibiscus in patients with high BP. In one double-blind study, 193 patients with hypertension were given either hibiscus extract (250 mg of total anthocyanins daily) or 10 mg of lisinopril (control group). The results showed that the hibiscus extract decreased BP from 146.48/97.77 to 129.89/85.96 mm Hg, reaching an absolute reduction of 17.14/11.97 mm Hg. The hibiscus treatment showed a therapeutic effectiveness of 65.12% as well as tolerability and safety of 100%. BP reductions and therapeutic effectiveness were lower than those obtained with lisinopril. Hibiscus treatment lowered plasma ACE activity from 44 to 30 U/L.[90]

Similar effects on BP were shown in another double-blind study with a standardized hibiscus extract (a daily dose of 9.6 mg of total anthocyanins) compared with captopril (50 mg/day). Hibiscus extract decreased the systolic BP from 139.05 to 123.73 mm Hg and the diastolic BP from 90.81 to 79.5 mm Hg.[91]

Viscum Album

Viscum album, or mistletoe, exhibits hypotensive action in animal studies. The crude extract of *V. album* possesses smooth-muscle–relaxant effects mediated through the voltage-dependent Ca(++) channel blockade, which may explain its spasmolytic and vasorelaxant activity.[92] *V. album* has been shown to inhibit the excitability of the vasomotor center in the medulla oblongata and to possess cholinomimetic activity.[87]

Its hypotensive activity may depend on the form in which the mistletoe is administered and the host tree from which it was collected. Studies indicate that aqueous extracts are more effective; the highest hypotensive activity was demonstrated by a macerate of leaves of mistletoe parasitizing on willow and gathered in January (see Chapter 134 for further discussion).

THERAPEUTIC APPROACH

Elevated Blood Pressure (120–129/<80 mm Hg) or White-Coat Hypertension

- Reduce excessive weight (see Chapter 192 for more information).
- Substantially reduce or eliminate salt (sodium chloride) intake.
- Follow a healthy lifestyle. Avoid alcohol, caffeine, and smoking. Exercise and use stress-reduction techniques.
- Follow a potassium-rich diet that is high in fiber and consistent with either the Mediterranean or DASH diet.
- Increase dietary consumption of celery, garlic, and onions.
- Reduce or eliminate the intake of animal fats while increasing the intake of monounsaturated vegetable oils.
- Supplement the diet with the following:
  - A high-potency multivitamin and mineral formula
  - Vitamin C: 500 to 1000 mg three times a day
  - Magnesium: 600 to 1200 mg/day
  - Fresh garlic: the equivalent of 4000 mg/day
  - Omega-3 fatty acids such as flaxseed oil, 1 tbsp/day, or fish oils, 3 g total EPA/DHA content per day

If BP has not returned to normal after following these recommendations for a period of 3 months, the recommendations for stage 1 hypertension, given next, may be followed.

Stage 1 Hypertension (130–139/80–89 mm Hg)

- Employ all the measures mentioned earlier, plus the following:
  - CoQ_{10}: 100 mg two to three times a day
  - Anti-ACE peptides from bonito: 1500 mg/day
  - L-citrulline: 3 g/day to 10 g/day
  - Take one of the following:
    - Hawthorn extract (10% procyanidins or 1.8% vitexin–4%-rhamnoside): 100 to 250 mg three times a day
    - Olive leaf extract (17%–23% oleuropein content): 500 mg twice a day
    - Hibiscus: three 240-mL servings a day or an extract providing 10 to 20 mg anthocyanidins a day

These guidelines should be followed for 1 to 3 months. If the BP has not dropped below 135/85, the patient may have to be placed on antihypertensive medications.

Stage 2 Hypertension (140+/90+ mm Hg)

Drug intervention is required. All the measures mentioned previously should be employed. When satisfactory control over the high BP has been achieved, the patient can taper off the medications gradually.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Egan BM, Lackland DT, Jones DW. Prehypertension: an opportunity for a new public health paradigm. *Cardiol Clin*. 2010;28(4):561–569.
2. Thomas F, Blacher J, Benetos A, et al. Cardiovascular risk as defined in the 2003 European blood pressure classification: the assessment of an additional predictive value of pulse pressure on mortality. *J Hypertens*. 2008;26(6):1072–1077.
3. Munakata M, Saito Y, Nunokawa T, et al. Clinical significance of blood pressure response triggered by a doctor's visit in patients with essential hypertension. *Hypertens Res*. 2002;25:343–349.
4. Strandberg TE, Salomaa V. White coat effect, blood pressure and mortality in men: prospective cohort study. *Eur Heart J*. 2000;21:1714–1718.
5. Addison C, Varney S, Coats A. The use of ambulatory blood pressure monitoring in managing hypertension according to different treatment guidelines. *J Hum Hypertens*. 2001;15:535–538.
6. Nawrot TS, Thijs L, Den Hond EM, et al. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis. *J Hum Hypertens*. 2002;16:123–131.
7. Gerr F, Letz R, Stokes L, et al. Association between bone lead concentration and blood pressure among young adults. *Am J Ind Med*. 2002;42:98–106.
8. Pizent A, Jurasovie J, Telisman S. Blood pressure in relation to dietary calcium intake, alcohol consumption, blood lead, and blood cadmium in female nonsmokers. *J Trace Elem Med Biol*. 2001;15:123–130.
9. Telisman S, Jurasovic J, Pizent A, et al. Blood pressure in relation to biomarkers of lead, cadmium, copper, zinc, and selenium in men without occupational exposure to metals. *Environ Res*. 2001;87:57–68.
10. Martin D, Glass TA, Bandeen-Roche K, et al. Association of blood lead and tibia lead with blood pressure and hypertension in a community sample of older adults. *Am J Epidemiol*. 2006;163(5):467–478.
11. Egan BM, Zhao Y, Axon RN, et al. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. *JAMA*. 2010;303(20):2043–2050.
12. Wiysonge CS, Bradley H, Mayosi BM. Beta-blockers for hypertension. *Cochrane Database Syst Rev*. 2007;24(1):CD002003.
13. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA*. 2003;289(19):2560–2572.
14. Blumenthal JA, Sherwood A, Gullette EC, et al. Biobehavioral approaches to the treatment of essential hypertension. *J Consult Clin Psychol*. 2002;70:569–589.
15. Grossman E, Grossman A, Schein MH, et al. Breathing-control lowers blood pressure. *J Hum Hypertens*. 2001;15:263–269.
16. Schein MH, Gavish B, Herz M, et al. Treating hypertension with a device that slows and regularises breathing: a randomised, double-blind controlled study. *J Hum Hypertens*. 2001;15:271–278.
17. Anderson DE, Bagrov AY, Austin JL. Inhibited breathing decreases renal sodium excretion. *Psychosom Med*. 1995;57:373–380.
18. Bernardi L, Porta C, Spicuzza L, et al. Slow breathing increases arterial baroreflex sensitivity in patients with chronic heart failure. *Circulation*. 2002;105:143–145.
19. Schein MH, Gavish B, Baevsky T, et al. Treating hypertension in type II diabetic patients with device-guided breathing: a randomized controlled trial. *J Hum Hypertens*. 2009;23(5):325–331.
20. Arakawa K. Exercise: a measure to lower blood pressure and reduce other risks. *Clin Exp Hypertens*. 1999;21:797–803.
21. Moreira WD, Fuchs FD, Ribeiro JP, et al. The effects of two aerobic training intensities on ambulatory blood pressure in hypertensive patients: results of a randomized trial. *J Clin Epidemiol*. 1999;52:637–642.
22. Fogari R, Zoppi A, Corradi L, et al. Effect of body weight loss and normalization on blood pressure in overweight non-obese patients with stage 1 hypertension. *Hypertens Res*. 2010;3(3):236–242.
23. Navaneethan SD, Yehnert H, Moustarah F, et al. Weight loss interventions in chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol*. 2009;4(10):1565–1574.
24. Yasunari K, Maeda K, Nakamura M, et al. Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reactive protein. *Hypertension*. 2002;39:777–780.
25. John JH, Ziebland S, Yudkin P, et al. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *Lancet*. 2002;359:1969–1974.
26. Ortiz MC, Manriquez MC, Romero JC, et al. Antioxidants block angiotensin II-induced increases in blood pressure and endothelin. *Hypertension*. 2001;38:655–659.
27. Tsi D, Tan BKH. Cardiovascular pharmacology of 3-n-butylphthalide in spontaneously hypertensive rats. *Phytother Res*. 1997;11:576–582.
28. Silagy CA, Neil HA. A meta-analysis of the effect of garlic on blood pressure. *J Hypertens*. 1994;12:463–468.
29. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med*. 1997;336:1117–1124.
30. Moore TJ, Conlin PR, Ard J, et al. DASH (Dietary Approaches to Stop Hypertension) diet is effective treatment for stage 1 isolated systolic hypertension. *Hypertension*. 2001;38:155–158.
31. Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med*. 2001;344:3–10.
32. Jansson B. Dietary, total body, and intracellular potassium-to-sodium ratios and their influence on cancer. *Cancer Detect Prev*. 1990;14:563–565.
33. Langford HG. Dietary potassium and hypertension: epidemiological data. *Ann Intern Med*. 1983;98:770–772.
34. He FJ, MacGregor GA. Salt, blood pressure and cardiovascular disease. *Curr Opin Cardiol*. 2007;22(4):298–305.
35. Whelton PK, He J. Potassium in preventing and treating high blood pressure. *Semin Nephrol*. 1999;19:494–499.
36. Patki PS, Singh J, Gokhale SV, et al. Efficacy of potassium and magnesium in essential hypertension: a double-blind, placebo-controlled, crossover study. *BMJ*. 1990;301:521–523.
37. Fotherby MD, Potter JF. Potassium supplementation reduces clinic and ambulatory blood pressure in elderly hypertensive patients. *J Hypertens*. 1992;10:1403–1408.
38. Thijs L, Amery A, Birkenhager W, et al. Age-related effects of placebo and active treatment in patients beyond the age of 60 years: the need for a proper control group. *J Hypertens*. 1990;8:997–1002.
39. Jee SH, Miller III ER, Guallar E, et al. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. *Am J Hypertens*. 2002;15:691–696.
40. Motoyama T, Sano H, Fukuzaki H. Oral magnesium supplementation in patients with essential hypertension. *Hypertension*. 1989;13:227–232.
41. Whelton PK, Klag MJ. Magnesium and blood pressure: review of the epidemiologic and clinical trial experience. *Am J Cardiol*. 1989;63:G26–G30.
42. Joffres MR, Reed DM, Yano K. Relationship of magnesium intake and other dietary factors to blood pressure. The honolulu heart study. *Am J Clin Nutr*. 1987;45:469–475.
43. Lindberg JS, Zobitz MM, Poindexter JR, et al. Magnesium bioavailability from magnesium citrate and magnesium oxide. *J Am Coll Nutr*. 1990;9:48–55.
44. Bohmer T, Roseth A, Holm H, et al. Bioavailability of oral magnesium supplementation in female students evaluated from elimination of magnesium in 24-hour urine. *Magnes Trace Elem*. 1990;9:272–278.
45. Cappuccio FP, Elliott P, Allender PS, et al. Epidemiologic association between dietary calcium intake and blood pressure: a meta-analysis of published data. *Am J Epidemiol*. 1995;142:935–945.
46. Meese RB, Gonzales DG, Casparian JM, et al. The inconsistent effects of calcium supplements upon blood pressure in primary hypertension. *Am J Med Sci*. 1987;294:219–224.
47. Sowers JR, Zemel MB, Standley PR, et al. Calcium and hypertension. *J Lab Clin Med*. 1989;114:338–348.
48. Takagi Y, Fukase M, Takata S, et al. Calcium treatment of essential hypertension in elderly patients evaluated by 24 H monitoring. *Am J Hypertens*. 1991;4:836–839.
49. Hajjar IM, George V, Sasse EA, et al. A randomized, double-blind, controlled trial of vitamin C in the management of hypertension and lipids. *Am J Ther*. 2002;9:289–293.

50. Fotherby MD, Williams JC, Forster LA, et al. Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons. *J Hypertens*. 2000;18:411–415.
51. Galley HF, Thornton J, Howdle PD, et al. Combination oral antioxidant supplementation reduces blood pressure. *Clin Sci (Lond)*. 1997;92:361–365.
52. van Dijk RA, Rauwerda JA, Steyn M, et al. Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol*. 2001;21:2072–2079.
53. Aybak M, Sermet A, Ayyildiz MO, et al. Effect of oral pyridoxine hydrochloride supplementation on arterial blood pressure in patients with essential hypertension. *Arzneimittelforschung*. 1995;45:1271–1273.
54. Geleijnse JM, Giltay EJ, Grobbee DE, et al. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *J Hypertens*. 2002;20:1493–1499.
55. Cicero AF, Ertek S, Borghi C. Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. *Curr Vasc Pharmacol*. 2009;7(3):330–337.
56. Minihane AM, Armah CK, Miles EA, et al. Consumption of fish oil providing amounts of eicosapentanoic acid and docosahexanoic acid that can be obtained from the diet reduces blood pressure in adults with systolic hypertension: a retrospective analysis. *J Nutri*. 2016;146(3):516–523. PubMed PMID: 26817716.
57. Singer P. Alpha-linolenic acid vs. long-chain n-3 fatty acids in hypertension and hyperlipidemia. *Nutrition*. 1992;8:133–135.
58. Berry EM, Hirsch J. Does dietary linolenic acid influence blood pressure? *Am J Clin Nutr*. 1986;44:336–340.
59. Boger RH, Bode-Boger SM, Szuba A, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation*. 1998;98(18):1842–1847. PubMed PMID: 9799202.
60. Perticone F, Sciacqua A, Maio R, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *J Am Coll Cardiol*. 2005;46(3):518–523. PubMed PMID: 16053968.
61. Kelly BS, Alexander JW, Dreyer D, et al. Oral arginine improves blood pressure in renal transplant and hemodialysis patients. *JPEN J Parenter Enteral Nutr*. 2001;25:194–202.
62. Kelly JJ, Williamson P, Martin A, et al. Effects of oral L-arginine on plasma nitrate and blood pressure in cortisol-treated humans. *J Hypertens*. 2001;19:263–268.
63. Ast J, Jablecka A, Bogdanski P, et al. Evaluation of the antihypertensive effect of L-arginine supplementation in patients with mild hypertension assessed with ambulatory blood pressure monitoring. *Med Sci Monit*. 2010;16(5):CR266–CR271.
64. Campo C, Lahera V, Garcia-Robles R, et al. Aging abolishes the renal response to L-arginine infusion in essential hypertension. *Kidney Int Suppl*. 1996;55:S126–S128.
65. Menzel D, Haller H, Wilhelm M, Robenek H. L-arginine and B vitamins improve endothelial function in subjects with mild to moderate blood pressure elevation. *Eur J Nutri*. 2018;57(2):557–568. PubMed PMID: 27817128.
66. Fujita H, Yoshikawa M. LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology*. 1999;44:123–127.
67. Fujita H, Yamagami T, Ohshima K. Effects of an ace-inhibitory agent, katsuobushi oligopeptide, in the spontaneously hypertensive rat and in borderline and mildly hypertensive subjects. *Nutr Res*. 2001;21:1149–1158.
68. Fujita H, Yasumoto R, Hasegawa M, et al. Antihypertensive activity of "Katsuobushi oligopeptide" in hypertensive and borderline hypertensive subjects. *Jpn Pharmacol Ther*. 1997;25:147–151.
69. Kawasaki T, Seki E, Osajima K, et al. Antihypertensive effect of valyl-tyrosine, a short chain peptide derived from sardine muscle hydrolysate, on mild hypertensive subjects. *J Hum Hypertens*. 2000;14:519–523.
70. Ho MJ, Bellusci A, Wright JM. Blood pressure lowering efficacy of coenzyme Q10 for primary hypertension. *Cochrane Database Syst Rev*. 2009:CD007435.
71. Langsjoen P, Langsjoen P, Willis R, et al. Treatment of essential hypertension with coenzyme Q10. *Mol Aspects Med*. 1994;15(suppl):S265–S272.
72. Chien SJ, Lin KM, Kuo HC, et al. Two different approaches to restore renal nitric oxide and prevent hypertension in young spontaneously hypertensive rats: l-citrulline and nitrate. *Trans Res*. 2014;163(1):43–52. PubMed PMID: 24113064.
73. Figueroa A, Sanchez-Gonzalez MA, Wong A, Arjmandi BH. Watermelon extract supplementation reduces ankle blood pressure and carotid augmentation index in obese adults with prehypertension or hypertension. *Am J Hypertens*. 2012;25(6):640–643. PubMed PMID: 22402472.
74. Figueroa A, Sanchez-Gonzalez MA, Perkins-Veazie PM, Arjmandi BH. Effects of watermelon supplementation on aortic blood pressure and wave reflection in individuals with prehypertension: a pilot study. *Am J Hypertens*. 2011;24(1):40–44. PubMed PMID: 20616787.
75. Massa NM, Silva AS, Toscano LT, Silva JD, Persuhn DC, Goncalves Mda C. Watermelon extract reduces blood pressure but does not change sympathovagal balance in prehypertensive and hypertensive subjects. *Blood Pressure*. 2016;25(4):244–248. PubMed PMID: 26947668.
76. Allerton TD, Proctor DN, Stephens JM, Dugas TR, Spielmann G, Irving BA. L-citrulline supplementation: impact on cardiometabolic health. *Nutrients*. 2018;10(7). pii: E921. PubMed PMID: 30029482.
77. Sanchez-Gonzalez MA, Koutnik AP, Ramirez K, Wong A, Figueroa A. The effects of short-term L-citrulline supplementation on wave reflection responses to cold exposure with concurrent isometric exercise. *Am J Hypertens*. 2013;26(4):518–526. PubMed PMID: 23467207.
78. Nurminen ML, Niittynen L, Korpela R, et al. Coffee, caffeine and blood pressure: a critical review. *Eur J Clin Nutr*. 1999;53:831–839.
79. Jee SH, He J, Whelton PK, et al. The effect of chronic coffee drinking on blood pressure: a meta-analysis of controlled clinical trials. *Hypertension*. 1999;33:647–652.
80. Hartley TR, Lovallo WR, Whitsett TL, et al. Caffeine and stress: implications for risk, assessment, and management of hypertension. *J Clin Hypertens (Greenwich)*. 2001;3:354–361.
81. Walker AF, Marakis G, Morris AP, et al. Promising hypotensive effect of hawthorn extract: a randomized double-blind pilot study of mild, essential hypertension. *Phytother Res*. 2002;16:48–54.
82. Walker AF, Marakis G, Simpson E, et al. Hypotensive effects of hawthorn for patients with diabetes taking prescription drugs: a randomised controlled trial. *Br J Gen Pract*. 2006;56(527):437–443.
83. Perrinjaquet-Moccetti T, Busjahn A, Schmidlin C, et al. Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces blood pressure in borderline hypertensive monozygotic twins. *Phytother Res*. 2008;22(9):1239–1242.
84. Cherif S, Rahal N, Haouala M, et al. A clinical trial of a titrated Olea extract in the treatment of essential arterial hypertension. *J Pharm Belg*. 1996;51:69–71.
85. Susalit E, Agus N, Effendi I, et al. Olive *(Olea europaea)* leaf extract effective in patients with stage-1 hypertension: comparison with Captopril. *Phytomedicine*. 2011;18(4):251–258.
86. Serban C, Sahebkar A, Ursoniu S, Andrica F, Banach M. Effect of sour tea (*Hibiscus sabdriffa* L.) on arterial hypertension: a systematic review and meta-analysis of randomized controlled trials. *J Hypertens*. 2015;33(6):1119–1127. PubMed PMID: 25875025.
87. McKay DL, Chen CY, Saltzman E, et al. *Hibiscus sabdariffa* L. tea (tisane) lowers blood pressure in prehypertensive and mildly hypertensive adults. *J Nutr*. 2010;140(2):298–303.
88. Mozaffari-Khosravi H, Jalali-Khanabadi BA, Afkhami-Ardekani M, et al. The effects of sour tea *(Hibiscus sabdariffa)* on hypertension in patients with type II diabetes. *J Hum Hypertens*. 2009;23(1):48–54.
89. Haji Faraji M, Haji Tarkhani A. The effect of sour tea *(Hibiscus sabdariffa)* on essential hypertension. *J Ethnopharmacol*. 1999;65(3):231–236.
90. Herrera-Arellano A, Miranda-Sánchez J, Avila-Castro P, et al. Clinical effects produced by a standardized herbal medicinal product of *Hibiscus sabdariffa* on patients with hypertension: a randomized, double-blind, lisinopril-controlled clinical trial. *Planta Med*. 2007;73(1):6–12.
91. Herrera-Arellano A, Flores-Romero S, Chávez-Soto MA, et al. Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine*. 2004;11(5):375–382.
92. Khan T, Ali S, Qayyum R, Hussain I, Wahid F, Shah AJ. Intestinal and vascular smooth muscle relaxant effect of *Viscum album* explains its medicinal use in hyperactive gut disorders and hypertension. *BMC Complementary Alternat Med*. 2016;16:251. PubMed PMID: 27465545.

Hyperthyroidism

Alan G. Christianson, NMD, and *Michael T. Murray, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Weakness; sweating; weight loss; nervousness; loose stools; heat intolerance; irritability; fatigue; tachycardia; warm, thin, moist skin; stare; tremor
- Diffuse nonpainful goiter
- Increased T4, free T4, and free T4 index
- Failure of thyroid suppression with T3 administration
- In Graves' disease: goiter (often with bruit), ophthalmopathy

GENERAL CONSIDERATIONS

Hyperthyroidism, also known as thyrotoxicosis, denotes a group of clinical disorders characterized by increased levels of tetraiodothyronine (T4) and/or triiodothyronine (T3). The autoimmune disorder Graves' disease accounts for up to 85% of all cases of hyperthyroidism. It is more common in women than men (ratio 8:1) and typically begins between the ages of 20 and 40. Diffuse, nonpainful goiter with hyperthyroidism is the most common presentation of Graves' disease. Less common signs and symptoms include exophthalmos, pretibial myxedema and other skin changes, nail changes (acropachy), and, in some groups, paralysis. The exophthalmos and skin changes can progress independently from thyroid dysfunction (euthyroid Graves' disease), making it difficult to predict the course of the disease. Ophthalmopathy and pretibial myxedema are caused by thyroid antibodies that cross-react with antigens in fibroblasts and adipocytes behind the eye (Fig. 180.1). Although no single immunological abnormality explains all the clinical features of the disease, the common denominator is the presence of antibodies against thyroid-stimulating hormone (TSH) receptors.

Disease Risk

Gender

The majority of original cases described by Parry, von Basedow, and Graves occurred in women. The female-to-male ratio in published series is 7:1 to 10:1, but the ratio in those with ophthalmic complications is about 1:1.

Stress

Recent stress has been recognized as a precipitating factor since Graves' disease was first recognized. In fact, the most common precipitating event is an "actual or threatened separation from an individual upon whom the patient is emotionally dependent."[1] Studies support the long-held observation that the onset of Graves' disease often follows emotional shock, specifically some sort of loss, such as divorce, death, or difficult separation.[2]

Genetics

Graves' disease is statistically more prevalent in some human leukocyte antigen (HLA) haplotypes, such as HLA-B8 and HLA-DR3 in Caucasians, HLA-Bw35 in Japanese populations, and HLA-Bw46 in Chinese populations.[1] HLA identical twins have a 50% chance of presenting with Graves' disease if one twin is affected, and there is a 9% chance for fraternal twins.[3] Some haplotypes appear to offer protection from manifesting Graves' disease. Genetic haplotype does not seem to affect the clinical course of Graves' disease or the response to treatment.

Smoking

Smoking is known to raise the risk and severity of ophthalmopathy among patients with Graves' disease.[4,5] Germline polymorphisms of detoxification genes and genes belonging to the major DNA

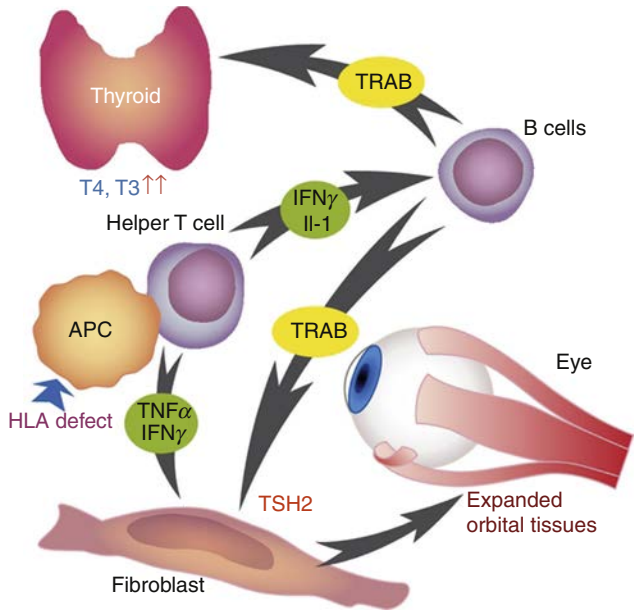


Fig. 180.1 Schematic representation of the cascade of events in the pathogenesis of Graves' ophthalmopathy. (From Duntas LH. The evolving role of selenium in the treatment of Graves' disease and ophthalmopathy. *J Thyroid Res.* 2012:736161. PubMed PMID: 22315699.)

repair-apoptosis pathways might have an important role in disease susceptibility, and because some of these genes are regulated by thyroid hormones, they may affect the patient outcomes. One prospective case-controlled study aimed to assess the influence of the GST, CYP, and TP53 gene polymorphisms in the risk of Graves' disease.[6] The authors concluded that GSTP1, CYP1A1, and TP53 germline polymorphisms may be associated with smoking-related Graves' disease susceptibility and configure a risk profile for the disease. However, these polymorphisms did not influence the patients' response to treatment.

Iodine Supplementation

An interesting study evaluated the effects of mandatory consumption of iodized salt in a whole population of 267,330 inhabitants of Galicia.[7] The incidence of thyrotoxicosis, diagnosed as elevated T4 and suppressed TSH levels, increased throughout the whole study period, with 4.89 new cases per 100,000 population. The rate of increase among females (8.03) was much greater than that among males (1.34). The increased incidence of thyrotoxicosis comprised both nodular and diffuse goiters. The authors concluded that dietary iodine supplementation in iodine-sufficient areas can increase the incidence of thyrotoxicosis in susceptible individuals.

Globally, similar scenarios have occurred. When a population's intake of iodine suddenly increases, even to appropriate levels, many individuals lapse into hyperthyroid states. This is more common among the elderly, those with positive thyroid antibodies, and those with marginal selenium status. Iodine from other sources in doses above 600 mcg can trigger Graves' disease and toxic multinodular goiter. Common sources include potassium iodide, an iodine/potassium iodide (Iodoral) supplement, medications such as amiodarone, and imaging contrast agents.[7]

Infection

The TSH receptor antibodies in Graves' disease share a similar structure with antibodies against several known pathogens. It has been shown that cases of Graves' have started during or shortly after acute infection with *Yersinia enterocolitica* and *Borrelia burgdorferi*.[8]

Drugs

In older patients with hyperthyroidism, a toxic reaction to prescription drugs must be considered. Exposure to iodine-containing contrast agents during medical procedures is a common scenario. Some sources recommend TSH screening before administration of iodine-containing contrast agents.[9] Another drug-induced cause of hyperthyroidism in the elderly is the use of amiodarone, an antiarrhythmic drug.[10]

Toxins

Workers exposed to 2,3,7,8-TCDD, a dioxin contaminant of chlorophenoxy herbicides, have significantly higher free T4 levels.[11] Children with high perinatal exposure to PCDD/Fs show no change in thyroid hormone levels at age 1 or 2 but have significantly elevated T3 levels at age 5.[12] A similar elevation of FT4 was noted at birth in babies exposed to dioxins in utero and through breast milk.[13] The elevation of FT4 in the exposed infants reached statistical significance at week 1 and week 11. By week 11, TSH concentrations were also statistically higher in the children receiving dioxin with their breast milk.

Perchlorate is known to competitively inhibit iodide uptake at the sodium iodide symporter and has been used in the past in the diagnosis and treatment of thyrotoxicosis.[14] Workers in an ammonium perchlorate production plant were found to have a 38% reduction in 14-hour thyroid radioactive iodine uptake.[15]

Other

Other factors that may initiate Graves' disease include corticosteroid use and local trauma to the thyroid. In addition, symptoms of hyperthyroidism vary somewhat in older adults, with apathy, tachycardia, and weight loss being more common. This response is more probable in those with subclinical hyperthyroidism (lack of symptoms, normal T4, but low TSH).

DIAGNOSIS

Clinical Presentation

The typical clinical presentation of Graves' disease is a young adult female complaining of the following:

- Nervousness
- Irritability
- Sweating
- Palpitations
- Insomnia
- Tremor
- Frequent stools
- Unexplained weight loss
- Metrorrhagia

Physical examination often reveals a smooth, diffuse, nontender goiter; tachycardia, especially after exercise; loud heart sounds (often a systolic murmur); mild proptosis, lid retraction, lid lag, and tremor; and exaggerated deep tendon reflexes.

Other signs and symptoms may include muscle weakness and fatigue, anxiety, heat intolerance, and pretibial myxedema. However, in atypical cases, especially in elderly patients, any or all of the previously mentioned symptoms may be absent. This presentation is known as apathetic thyrotoxicosis and has been known to present in the elderly, with the only symptom being depression. Special care must be taken to avoid missing the diagnosis with this presentation. A screening laboratory test for suppressed TSH is helpful in these patients. Also, it is useful to keep in mind that an increase in thyroid hormone can present as a worsening of already present cardiac symptoms such as angina pectoris, congestive heart failure, and atrial fibrillation.

TABLE 180.1 Relative Frequency of Primary Causes of Hyperthyroidism

Etiology	Relative Frequency[a]
Graves' disease	70%–85%
Toxic multinodular goiter	5%–15%
Toxic uninodular goiter	3%–30%
Thyroiditis (including so-called hashitoxicosis)	4%–23%
Drug induced	Commonly in the elderly; rare in the young except in cases where iodine is administered to a population
Postradiation	

[a]Overall frequency and relative frequency may be altered in certain populations (e.g., the elderly, individuals living in regions of endemic goiter).

The skin can show characteristic changes, becoming moist, warm, and finely textured. Perspiration increases as a response to the increased body temperature. Pigment changes such as vitiligo can be associated with Graves' disease, as well as increased pigmentation of areas such as skin creases and the knuckles. Hair may thin or fall out in patches or altogether (alopecia). Nails may separate prematurely from the nail bed (onycholysis). Localized, nonpitting edema typically occurs along the shins (myxedema) but may occur elsewhere, generally on the extensor surfaces, and is often pruritic and red.

Additional symptoms associated with Graves' disease, include glucose intolerance, dyspnea, polyuria and polydipsia, myopathy, paralysis, parkinsonian, and others.[16,17]

Although in severely hyperthyroid states symptoms are predictable, some symptoms can be paroxysmal, manifesting in both mild hyperthyroid and hypothyroid states. These include anxiety, palpitations, weight gain, fatigue, and insomnia. This is relevant in managing patients for either hypothyroidism or hyperthyroidism because it is not safe to assume that patients have increased or decreased levels of thyroid hormone based solely on symptoms.

Differential Diagnosis

Graves' disease is the most common diagnosis of hyperthyroidism, but several other conditions present with a similar symptom picture. The second most common cause is toxic nodular goiter, which includes toxic adenoma and multinodular goiter. Other causes of hyperthyroidism include early Hashimoto's thyroiditis (also known as hashitoxicosis), painless thyroiditis, and radiation thyroiditis. Hyperthyroidism can also result from exogenous causes, including iatrogenic hyperthyroidism, factitious hyperthyroidism (often seen in dieters who are taking thyroxine for weight loss), and iodine-induced hyperthyroidism (Jod–Basedow disease).

Rare causes of hyperthyroidism include thyroid carcinoma, ectopic hyperthyroidism, trophoblastic tumors (e.g., hydatidiform mole, choriocarcinoma, embryonic carcinoma of the testis), excessive TSH (e.g., pituitary adenoma, nonneoplastic pituitary secretion of TSH), and struma ovarii.

Table 180.1 indicates the relative frequency of the various causes of hyperthyroidism.

Laboratory Diagnosis

The laboratory is where the definitive diagnosis is made, and several tests are available to assess thyroid function and functional anatomy (Table 180.2).

In most cases, serum TSH will be suppressed below 0.2, and free T3 and free T4 may be elevated. Hyperthyroid morbidity and mortality risks are present even with TSH suppression alone. The autoimmune activity associated with Graves' disease involves both B and T lymphocytes and is directed at four specific thyroid antigens: the sodium/iodide symporter (NIS), thyroglobulin (Tg), thyroid peroxidase (TPO), and the thyrotropin receptor (TSH-R). TSH receptor antibodies (TSH-R Ab) or thyroid-stimulating immunoglobulins (TSIs) are present in 80% of cases of Graves' disease.

TABLE 180.2 Thyroid Function Tests in Hyperthyroidism

	Serum TSH	Serum T4	Serum T3	Free T4	24-h Radioiodine Uptake (Thyroid)
Hyperthyroidism, untreated	Low	High	High	High	High
Hyperthyroidism, toxicosis	Low	Normal	High	High	Normal

If a nodule or diffuse swelling is present on palpation or ultrasound, a thyroid uptake scan should be performed to guide treatment and rule out malignancy. The determination of serum antibodies can also be useful in ruling out cancer in patients with firm lobular goiters. High antibody levels suggest chronic thyroiditis in these cases. The presence of a nodule in the absence of abnormal thyroid function tests always warrants ultrasound and possible biopsy. Iodine scans show diffuse heightened iodine uptake in Graves' disease and localized heightened iodine uptake, also known as "hot" nodules, in toxic nodular goiter. "Cold" nodules on scans are regions of poor iodine uptake and are assumed to be cancerous until proven otherwise.

THERAPEUTIC CONSIDERATIONS

The objective of the natural treatment of Graves' disease and hyperthyroidism is to reduce symptoms while trying to reestablish normal thyroid status. Few treatments besides antithyroid drugs (ATDs), radiation, or surgery will have a significant effect on the immediate severity of hyperthyroid symptoms. Nonetheless, adjunctive treatments and Naturopathic lifestyle recommendations can lower the dose or duration of ATDs or increase the likelihood of disease remission when combined with conventional treatment.

Practical steps include reduction of risk factors (e.g., stress, smoking, excess iodine intake) and increased rest. Stress control is the single most important action the patient can take to assist normalization of the thyroid. The patient should avoid anything that will excite him or her and increase agitation. Counseling may be incorporated to prevent a return to a stress-generating lifestyle. Rest should also be increased.

Comorbidities

Patients with autoimmune thyroid disease are more likely than the general population to suffer from the following[18]:

- Celiac disease
- Osteoporosis
- Mitral valve prolapse
- Type 1 diabetes

CONVENTIONAL TREATMENT OF HYPERTHYROIDISM

Conventional first-line treatment of hyperthyroidism in the United States focuses on radioactive iodine ablation. Advantages include a high rate of response and lack of ongoing suppression. Disadvantages include progression to hypothyroidism and an elevated risk of nonlocalized cancers and parathyroid disease.

Surgery to remove all or part of the gland is less commonly used for hyperthyroidism. It is a consideration when the patient is very young or pregnant and iodine or ATDs are less preferred. It can also be considered when the gland is enlarged enough to create anatomical impingement on adjacent structures or cosmetic concerns. Disadvantages include laryngeal nerve damage, parathyroid abnormalities through localized trauma or inadvertent removal, and general surgical complications.

Globally, ATDs are more commonly relied on as mainstays of treatment for hyperthyroidism. Most cases can be given a trial of an ATD for 18 to 24 months; these individuals may not need ongoing treatment afterward. The disadvantage is that they can have hepatic and hematologic toxicities and monitoring is required.

ATDs work by entering the thyroid via active transport mechanisms, accumulating inside the thyroid, and inhibiting binding of iodine to tyrosine, thus disrupting hormone synthesis. In Europe and Japan, ATD treatment is considered a first-line approach for Graves' and Plummer's diseases. Most cases are known to resolve within 18 months, and most remain euthyroid or hypothyroid for life. In the United States, radioactive iodine (RAI) is considered first-line therapy, with ATDs only being used for initial management.[19] However, according to a recent study examining the frequency of antithyroid drug prescription in the United States, methimazole has become the most frequently prescribed antithyroid drug, indicating a shift toward pharmacological treatment as the primary treatment option in Graves' disease.[20]

Fluoride in the form of sodium fluoride is a natural compound with antithyroid properties significant enough to allow for its use instead of conventional ATD or in conjunction with ATDs allowing for lower doses of medications. Historically, sodium fluoride and fluorotyrosine were used as oral antithyroid agents before propylthiouracil (PTU) and methimazole. Common doses of PTU used to manage adult hyperthyroidism range from 200 to 450 mg/day in divided doses and 5 to 20 mg/d in divided doses for methimazole. Fluoride is listed as a pregnancy category C agent and is known to cause gastrointestinal side effects at doses starting at 3 mg/kg and toxicity at doses starting at 5 mg/kg.

In the early management of hyperthyroidism, 4 to 6 weeks of ATD are needed to see clinical benefit because of the high amount of protein-bound iodine already stored within the thyroid gland. Excess levels of both T4 and T3 are known to be reabsorbed through enterohepatic recirculation. It has been shown that cholestyramine can speed the elimination of thyroid hormone by blocking enterohepatic recirculation.[18] It is plausible that dietary fibers such as rice bran or ground flax may have similar properties.

Diet

Balanced, Whole-Foods Diet

Small, frequent, high-calorie meals should be consumed to compensate for the increase in metabolism. Protein should be supplemented if the patient is nutritionally depleted. Caffeine-containing foods and other stimulants should be avoided. In mild cases, consumption of large amounts of raw *Brassica* family foods and raw soy products may be adequately combined with restricted iodine consumption to control symptoms.

Dietary Goitrogens

Some foods contain goitrogenic substances that prevent the utilization of iodine. These compounds are primarily isothiocyanates that are similar in action and structure to propylthiouracil. They are found in such foods as turnips, cabbage, rutabagas, mustard, cassava root, soybeans, peanuts, pine nuts, and millet. However, these foods are unreliable in the treatment of hyperthyroidism for the following reasons:

- The goitrogen content is quite low and has minimal beneficial effect on hyperthyroidism.
- Cooking inactivates goitrogens.
- No substantial documentation exists that these naturally occurring goitrogens interfere with thyroid function to any significant degree when dietary iodine levels are adequate.

Because iodine is found in kelp and other seaweeds, vegetables grown near the ocean, seafood, iodized salt, and some nutritional supplements should be avoided.[21] Great care must be exercised when using this approach because results are inconsistent, and a delay in effective treatment can harm the patient. If the patient does not respond within a few weeks of treatment, conventional intervention is required.

NUTRITIONAL SUPPLEMENTS

Iodine

Worldwide, iodine deficiency continues to be a problem, but in developed countries, iodine excess is more common. Sources of iodine include additives to food products (e.g., salt and iodine used to sterilize pipes in dairies) and in medical products (e.g., povidone/iodine washes, iodine-containing drugs such as amiodarone, radiographic dyes).

The effects of iodine in patients with hyperthyroidism include the following:

- Large doses of iodine temporarily reduce symptoms by stopping hormone synthesis (Wolff–Chaikoff effect).
- The thyroid can remain suppressed or can eventually resume hormone synthesis at a reduced, former, or even increased rate (escape from Wolff–Chaikoff).[22]
- Excess iodine can trigger hyperthyroidism (Jod–Basedow disease) in a euthyroid person or can trigger an overactive thyroid to return to normal.

From the brief descriptions of these three possibilities, aside from the initial suppression of thyroid function, the action of iodine is unpredictable.

- Iodine intake should be kept on the low end of the normal therapeutic range; 150 to 250 mcg/day for adults.

L-Carnitine

L-Carnitine is a ubiquitous nutrient in tissues, where it plays an important role in energy metabolism and may be beneficial in cases of hyperthyroidism.[23] Carnitine is already known for its important implications for cardiovascular disease, Alzheimer's disease, acquired immunodeficiency syndrome, and several other conditions (see Chapter 63).

Calcium

Calcium metabolism is altered in hyperthyroidism, and patients with Graves' disease are more susceptible to osteoporosis.

Selenium

Emerging research is showing that selenium deficiency influences the generation of free radicals, the conversion of T4 to T3, and the autoimmune thyroid process. Selenium administration may be effective and safe in patients with Graves' disease and with mild forms of Graves' orbitopathy.

A prospective, case-control study of 198 patients from Australia aimed to determine whether serum selenium levels are reduced in patients with Graves' disease with orbitopathy compared with those without orbitopathy.[24] Results showed that mean serum selenium levels were significantly lower in individuals with Graves' orbitopathy than in those without orbitopathy. In addition, mean selenium levels appeared to decrease in parallel with increasing severity of orbitopathy.

One randomized, double-blind, placebo-controlled trial compared the effect of selenium (an antioxidant agent) or pentoxifylline (an anti-inflammatory agent) in 159 patients with mild Graves' orbitopathy.[25] The patients were given selenium (100 μg twice daily—a very modest dosage), pentoxifylline (600 mg twice daily), or placebo (twice daily) orally for 6 months. Selenium, but not pentoxifylline, was shown to improve quality of life, reduce ocular involvement, and significantly slow the progression of the disease. Moreover, a 6-month follow-up confirmed the benefits of the 6-month selenium treatment.

Antioxidants

Oxygen free radicals and cytokines play a pathogenic role in Graves' disease as well as in the development of Graves' orbitopathy. Patients with Graves' disease who received supplementation with a mixture of antioxidants (selenium, beta-carotene, vitamin C, and vitamin E) in addition to therapy with methimazole achieved euthyroidism faster than patients taking methimazole alone.[26] A prospective, nonrandomized, comparative study of patients with mild or moderately severe, active, newly diagnosed Graves' ophthalmopathy found that 82% of patients treated with oral antioxidants showed improvement in symptoms compared with only 27% of the control group.[27] Although the sample size was small, the encouraging results warrant further prospective studies.

Vitamin D

Low serum vitamin D status is associated with the development of autoimmune diseases, including hyperthyroidism and new-onset Graves' disease. A quantitative meta-analysis of t20 case-control studies found that patients with autoimmune thyroid disease had lower levels of 25(OH)D and were more likely to be deficient in 25(OH)D compared with controls.[28] Subgroup analyses results showed that patients with Graves' disease and Hashimoto's thyroiditis also had lower 25(OH)D levels and were more likely to have a 25(OH)D deficiency. In addition, considering the increase in metabolic rate and systemic functioning, vitamin D supplementation is recommended to maintain bone mineral density and to maintain circulating vitamin D concentrations.

Vitamin A

Vitamin A is vital for proper thyroid function, and deficiency is common in thyroid disorders. In a study of 190 patients with goiter, serum levels were significantly decreased in those with hyperthyroidism or hypothyroidism.[29]

Botanical Medicines

Table 180.3 lists medicinal plants that have traditionally been used in the treatment of hyperthyroidism. Unfortunately, these plants have not been adequately evaluated in clinical studies.

Lycopus Species, *Lithospermum officinale,* and *Melissa officinalis*

Aqueous freeze-dried extracts of *Lycopus* species, *Lithospermum officinale,* and *Melissa officinalis* have been studied both in vivo and in vitro, with preliminary results supporting their use in the treatment of Graves' disease. However, additional research is necessary before these plants can be relied on clinically.

Effects noted for these three plants include an ability to inhibit the effects of exogenous TSH on the thyroid glands of rats, block the effects of TSH on the TSH receptor sites located on thyroid membranes, inhibit the peripheral deiodination of T4 to T3, and block the effects of antithyroid immunoglobulins on TSH receptors.[30–32]

Eight isolated compounds, mainly oxidation products of derivatives of 3,4-dihydroxycinnamic acid, were found to be responsible for most of these effects.[33] The blocking effects of the isolated compounds of the TSH receptors were reversible, and none of the compounds combined irreversibly, damaged, or altered the TSH receptors in situ.

TABLE 180.3 Medicinal Plants Traditionally Used in the Treatment of Hyperthyroidism

Medicinal Plant	Traditional Indications
Valerian officinalis	Nervine effect
Scutellaria lateriflora	Nervine effect
Cactus grandiflorus	Heart tonic; used with elevated pulse
Iris versicolor	Traditionally used for hyperthyroidism
Fucus spp.	To be used with caution because a high iodine content can improve symptoms at first and then later cause aggravation
Lycopus spp.	Blocks the TSH receptors; blocks peripheral conversion of T4 to T3
Lithospermum officinale	Blocks the TSH receptors
Melissa officinalis	Blocks the TSH receptors

TSH, Thyroid-stimulating hormone.
Data from Felter HW. *The Eclectic Material Medica, Pharmacology and Therapeutics.* Portland, OR: Eclectic Medical Publications; 1985 (first published Cincinnati, 1922); Hoffman D. *The New Holistic Herbal: A Herbal Celebrating the Wholeness of Life.* Rockport, MA: Element;1990.

An alcohol extract of *Lycopus europaeus* administered orally to rats caused a long-lasting decrease in T3 levels, presumably due to reduced peripheral T4 deiodination. A pronounced reduction in TSH was also observed 24 hours later, as were declines in luteinizing hormone and testosterone.[34] Water extracts did not have as strong an effect, explaining the lack of results in some earlier studies. This finding is consistent with the observation that the active constituents are the unoxidized phenolic compounds, which are not present in the aqueous extract.

Although these findings support the traditional use of these plants for patients with hyperactive thyroids, there is no research on the clinical use of these plants or documented guidelines for dosage recommendations. Anecdotally, most who have attempted to use botanical medication to treat Graves' disease have found it to be ineffective.

THERAPEUTIC APPROACH

Acute Graves' disease is not easily treated by naturopathic methods. In most cases, there is no guarantee that natural treatments will alleviate the symptoms. Thyroid storm is a potentially fatal complication and should be treated aggressively with antithyroid drugs, radio ablation, or thyroidectomy. In mild cases, natural therapeutics can manage the symptoms well but must be monitored carefully to avoid sudden exacerbation of the illness. Patients will often do well for a time and then relapse. Mild cases should be treated symptomatically and allowed to return to a euthyroid status if possible. Rarely are natural antithyroid treatments adequate as monotherapies. If iodine is used, then ablative treatment should also be scheduled, as the risk of escape, with a potential for a return to even more intense symptoms, increases with time.

The allopathic treatment of Graves' disease is unusual in that there are three relatively equal treatments from which to choose. As physicians, it is best to educate patients about the advantages and disadvantages of each treatment option and work together to decide which treatment best fits their needs. Importantly, decision making is difficult while patients are in a thyrotoxic state. For these individuals, symptomatic relief must be rendered before a decision is made.

Antithyroid medications can manage symptoms indefinitely while waiting for a possible return to euthyroid status. The main consideration is they can have immunosuppresant and hepatotoxic effects.

Monitoring is required and changing therapies may be needed. If these drugs are not well tolerated, then ablative therapies must be considered. Surgery has a higher rate of euthyroid results than radioactive iodine but carries a greater risk of serious complications. Regardless of the treatment selected, supportive treatment should be rendered.

Diet

A whole-foods diet with increased levels of calories, micronutrients, and protein is recommended to meet the increased metabolic needs of the patient with hyperthyroidism. In mild cases, consumption of large amounts of raw *Brassica* family foods and raw soy products may be adequately combined with restricted iodine consumption to control symptoms.

Supplements

- Fluoride: 3 to 10 mg/day
- Indole 3 carbinol: 200 to 600 mg/day
- Selenium: 60 to 200 µcg/day

Botanical Medicines

- *Lycopus* species, *L. officinale,* or *M. officinalis*
- Dried herb: 1 to 3 g or by infusion three times a day
- Tincture (1:5): 2 to 6 mL three times a day
- Fluid extract (1:1): 1 to 3 mL three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Larson PR, Ingbar SH. The thyroid gland. In: Wilson JD, Foster DW, eds. *Williams' Textbook of Endocrinology*. Philadelphia: WB Saunders; 1992:367–487.
2. Sonino N, Girelli ME, Boscaro M, et al. Life events in the pathogenesis of Graves' disease: a controlled study. *Acta Endocrinol (Copenh)*. 1993;128:293–296.
3. Doniach D. Humoral and genetic aspects of thyroid autoimmunity. *Clin Endocrinol Metab*. 1975;4:267–268.
4. Winsa B, Karlsson A. Graves' disease, endocrine ophthalmopathy and smoking. *Acta Endocrinol (Copenh)*. 1993;128:156–160.
5. Bartalena L, Bogazzi F, Tanda ML, et al. Cigarette smoking and the thyroid. *Eur J Endocrinol*. 1995;133:507–512.
6. Bufalo NE, Santos RB, Cury AN, et al. Genetic polymorphisms associated with cigarette smoking and the risk of Graves' disease. *Clinical Endocrinology (Oxf)*. 2008;68(6):982–987. PubMed PMID: 17980001.
7. Galofre JC, Fernandez-Calvet L, Rios M, et al. Increased incidence of thyrotoxicosis after iodine supplementation in an iodine sufficient area. *J Endocrinol Invest*. 1994;17:23–27.
8. Benvenga S, Guarneri F, Vaccaro M, et al. Homologies between proteins of Borrelia burgdorferi and thyroid autoantigens. *Thyroid*. 2004;14(11):964–966.
9. Cerqueira C, Knudsen N, Ovesen L. Association of iodine fortification with incident use of antithyroid medication: a Danish nationwide study. *J Clin Endo Met*. 2009;94(7):2400–2405.
10. Saam T, Hess T, Kasperk C, et al. Prevalence of latent and manifest hyperthyroidism in an iodine-deficient area: non-selected patient population admitted for CT studies with iodine-containing contrast agents. *Rofo*. 2005;177(9):1250–1254.
11. Calvert GM, Haring Sweeney M, Deddens J, Wall DK. Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Occup Environ Med*. 1997;56:270–276. PubMed PMID: 10450245.
12. Su PH, Chen JY, Chen JW, Wang SL. Growth and thyroid function in children with in utero exposure to dioxin: a 5-year follow-up study. *Pediatr Res*. 2010;67(2):205–210. PubMed PMID: 20091939.
13. Pluim HJ, de Vijlder JJ, Olie K, et al. Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. *Environ Health Perspect*. 1993;101(6):504–508. PubMed PMID: 8137779.
14. Tonacchera M, Pinchera A, Dimida A, et al. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid*. 2004;14(12):1012–1019. PubMed PMID: 15650353.
15. Braverman LE, He X, Pino S, Cross M, et al. The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. *J Clin Endocrinol Metab*. 2005;90(2):700–706 PubMed PMID: 15572417.
16. Trivalle C, Doucet J, Chassagne P, et al. Differences in the signs and symptoms of hyperthyroidism in older and younger patients. *J Am Geriatr Soc*. 1996;44:50–53.
17. Paul DT, Mollah FH, Alam MK, et al. Glycemic status in hyperthyroid subjects. *Mymensingh Med J*. 2004;13:71–75.
18. Tsai WC, Pei D, Wang TF, et al. The effect of combination therapy with propylthiouracil and cholestyramine in the treatment of Graves' hyperthyroidism. *Clin Endocrinol (Oxf)*. 2005;62(5):521–524.
19. Hashizume K, Ichikawa K, Nishii Y, et al. Effect of administration of thyroxine on the risk of postpartum recurrence of hyperthyroid Graves' disease. *J Clin Endocrinol Metab*. 1992;75:6–10.
20. Emiliano AB, Governale L, Parks M, Cooper DS. Shifts in propylthiouracil and methimazole prescribing practices: antithyroid drug use in the United States from 1991 to 2008. *J Clin Endocrinol Metab*. 2010;95(5):2227–2233. PubMed PMID: 20335447.
21. Newberne PM. Naturally occurring food-borne toxicants. In: Shils ME, Young VR eds. *Modern Nutrition in Health and Disease.* Philadelphia: Lea & Febiger; 689–690.
22. Philippou G, Koutras DA, Piperingos G, et al. The effect of iodide on serum thyroid hormone levels in normal persons, in hyperthyroid patients, and in hypothyroid patients on thyroxine replacement. *Clin Endocrinol (Oxf)*. 1992;36:573–578.
23. Benvenga S, Ruggeri RM, Russo A, et al. Usefulness of L-carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab*. 2001;86:3579–3594.
24. Khong JJ, Goldstein RF, Sanders KM, et al. Serum selenium status in Graves' disease with and without orbitopathy: a case-control study. *Clinical Endocrinology (Oxf)*. 2014;80(6):905–910. PubMed PMID: 24372054.
25. Marcocci C, Kahaly GJ, Krassas GE, et al. Selenium and the course of mild Graves' orbitopathy. *N Engl J Med*. 2011;364(20):1920–1931. PubMed PMID: 21591944.
26. Vrca VB, Skreb F, Cepelak I, Romic Z, Mayer L. Supplementation with antioxidants in the treatment of Graves' disease; the effect on glutathione peroxidase activity and concentration of selenium. *Clinica Chimica Acta*. 2004;341(1–2):55–63. PubMed PMID: 14967159.
27. Bouzas EA, Karadimas P, Mastorakos G, Koutras DA. Antioxidant agents in the treatment of Graves' ophthalmopathy. *Am J Ophthalmol*. 2000;129(5):618–622. 10844053.
28. Wang J, Lv S, Chen G, et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients*. 2015;7(4):2485–2498. PubMed PMID: 25854833.
29. Smolle J, Wawschinek O, Hayn H, Eber O. Vitamin A and carotene in thyroid disease. *Acta Medicine Austriaca*. 1983;10(2–3):71–73. PubMed PMID: 6880574.
30. Winterhoff H, Sourgens H, Kemper FH. Antihormonal effects of plant extracts. Pharmacodynamic effects of *Lithospermum officinale* on the thyroid gland of rats: comparison with the effects of iodide. *Horm Metabol Res*. 1983;15:503–507.
31. Aufmkolk M, Ingbar JC, Amir SM, et al. Inhibition by certain plant extracts of the binding and adenylate cyclase stimulatory effects of bovine thyrotropin in human thyroid membranes. *Endocrinology*. 1984;115:527–534.
32. Aufmkolk M, Kohrle J, Gumbinger H, et al. Antihormonal effects of plant extracts. Iodothyronine deiodinase of rat liver is inhibited by extracts and secondary metabolites of plants. *Horm Metab Res*. 1984;16:188–192.
33. Aufmkolk M, Amir SM, Kubota K, et al. The active principles of plant extracts with antithyroid activity: oxidation products of derivatives of 3,4-dihydrooxycinnamic acid. *Endocrinology*. 1985;116:1677–1686.
34. Winterhoff H, Gumbinger HG, Vahlensieck U, et al. Endocrine effects of *Lycopus europaeus* L. following oral application. *Arzneimittelforschung*. 1994;44:41–45.

181

Hypoglycemia

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Blood glucose level at or below 40 to 50 mg/dL
- A normal response curve during the first 2 to 3 hours of a glucose tolerance test (GTT), followed by a decrease of 20 mg or more below the fasting glucose level during the final hours of the test, with symptoms developing during the decrease

INTRODUCTION

Both low and high blood glucose levels can cause significant physiological dysfunction. Normally, the body maintains blood sugar levels within a narrow range through the coordinated effort of several glands and their hormones (Fig. 181.1). If these control mechanisms are disrupted, hypoglycemia (low blood sugar) or hyperglycemia (high blood sugar) may result.

The beta cells of the pancreas respond to the rise in blood glucose levels after meals by secreting the hormone insulin, which lowers blood glucose by increasing the rate at which glucose is taken up by cells throughout the body. Reductions in blood glucose, as occur during food deprivation or exercise, cause the release of the hormone glucagon by the alpha cells of the pancreas. Glucagon stimulates the release of glucose stored in body tissues as glycogen, especially the liver. Rapidly falling blood sugar levels, anger, fright, and stress may stimulate the release of epinephrine and corticosteroids by the adrenal glands. These hormones provide a quicker breakdown of stored glucose for extra energy during a crisis or increased need.

Americans overstress these control mechanisms through improper diet and lifestyle. As a result, diabetes and hypoglycemia are common diseases.

Hypoglycemia can be divided into two main categories: reactive hypoglycemia and fasting hypoglycemia. Reactive hypoglycemia, the most common, is characterized by the development of symptoms of hypoglycemia 3 to 5 hours after a meal and may herald the early onset of type 2 diabetes.

Despite this rather straightforward designation into reactive and fasting hypoglycemia, there are underlying factors that can lead to more precise designations. Hypoglycemia can further result from insulinoma, noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS), postbariatric surgery, dumping syndrome, insulin autoimmunity, or postprandial syndrome.[1] Reactive hypoglycemia may also result from the use of oral hypoglycemic drugs (Box 181.1).

Some researchers suggest that reactive hypoglycemia should instead be termed *idiopathic postprandial syndrome*. Although symptoms exist and are related to rapid drops in blood glucose, the absolute glucose levels are not reliable indicators of the syndrome. Many asymptomatic controls have glucose levels below 50, and many symptomatic patients have normal postprandial glucose levels.[2–4] Fasting hypoglycemia is rare because it usually appears only in severe disease states, such as pancreatic tumors, extensive liver damage, prolonged starvation, autoantibodies against insulin or its receptor, various cancers, or from excessive exogenous insulin in patients with diabetes. Pregnant women with diabetes who use insulin or oral glycemic medications also have a high incidence of asymptomatic hypoglycemic events.[5]

Hypoglycemia can promote untoward physiological changes in the body. Insulin-induced hypoglycemia is known to increase the levels of C-reactive protein (CRP), a known cardiac risk factor.[6] Glucose is the primary fuel for the brain, and thus low levels initially affect the brain. Symptoms of hypoglycemia can range from mild to severe and may include the following:

- Headache
- Depression, anxiety, irritability, and other psychological disturbances
- Blurred vision
- Excessive sweating
- Mental confusion
- Incoherent speech
- Nocturnal hypoglycemic episodes
- Bizarre behavior
- Convulsions

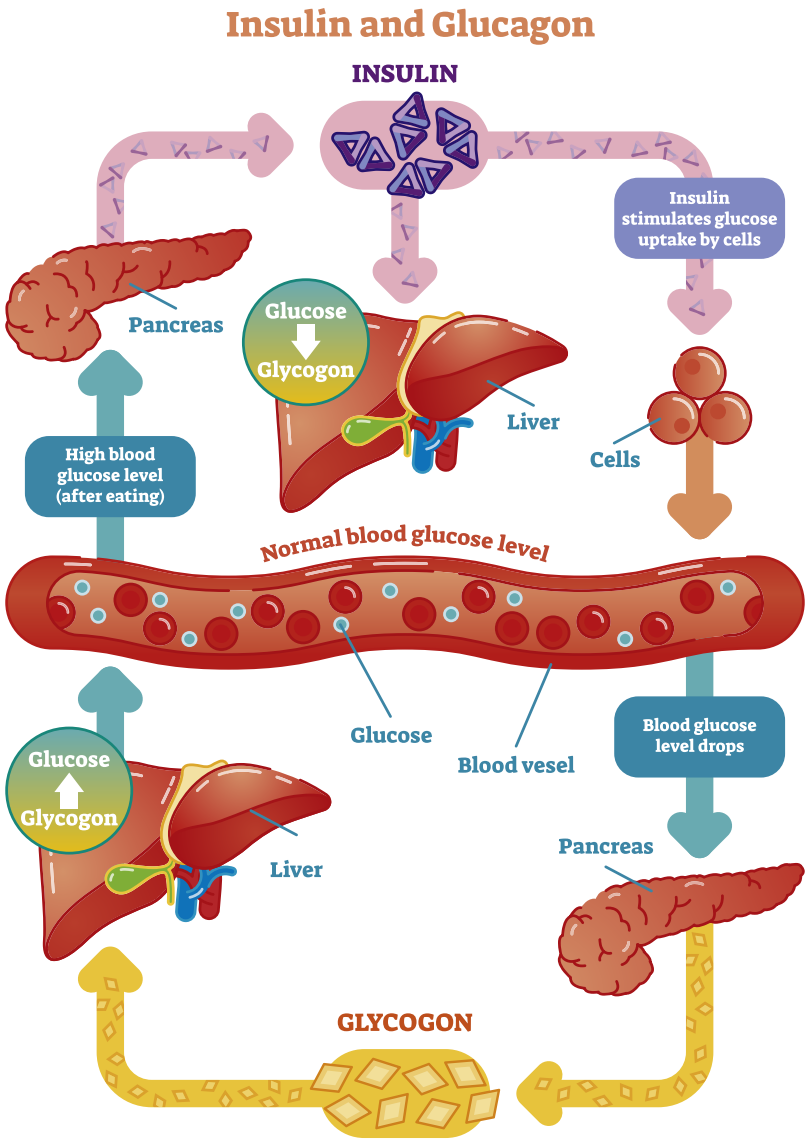


Fig. 181.1 The cycle of glucose control.

BOX 181.1 Oral Hypoglycemic Drugs

Alpha-glucosidase inhibitors
- Acarbose (Precose)
- Miglitol (Glyset)

Biguanide
- Metformin (Glucophage)

Meglitinides
- Repaglinide (Prandin)
- Nateglinide (Starlix)

Sulfonylureas
- Acetohexamide
- Chlorpropamide (Diabinese)
- Tolbutamide (Orinase)

Sulfonylureas (second generation)
- Glipizide (Glucotrol)
- Glibenclamide, Glyburide (DiaBeta, Micronase, Glynase)
- Glimepiride (Amaryl)

Thiazolidinediones
- Rosiglitazone (Avandia)
- Pioglitazone (Actos)

The heart is also dependent on glucose. In a study in Japan, oral glucose tolerance (OGTT) was assessed in 116 nondiabetic patients with coronary artery disease.[7] Reactive hypoglycemia was observed in 24% (28 individuals) of the patients. These patients exhibited significantly elevated insulin levels at 1 hour. Furthermore, a significant increase in the white blood cell count during OGTT was observed in the reactive hypoglycemia group compared with the nonreactive hypoglycemia group.

DIAGNOSTIC CONSIDERATIONS

Clinical hypoglycemia is identified by the modified Whipple's criteria, consisting of (1) central nervous system symptoms, including confusion, aberrant behavior, or coma; (2) a simultaneous blood glucose level equal to or less than 40 mg/dL; and (3) relief of these symptoms by the administration of glucose.[1] The normal fasting blood glucose level is between 65 and 100 mg/dL. A fasting plasma blood glucose measurement greater than 126 mg/dL on two separate occasions is diagnostic of diabetes.[8] Although the most specific criterion for the presence of hypoglycemia is a blood glucose level of 40 mg/dL or less, a blood glucose level below 50 mg/dL should arouse clinical suspicion.[1]

TABLE 181.1 Criteria of Response to the Glucose Tolerance Test

Diagnosis	Response
Normal	No elevation >200 mg <200 mg at the end of the first hour <140 mg at the end of the second hour Never <20 mg below fasting
Flat	No variation more than ± 20 mg from fasting value
Prediabetic	>140 mg at the end of the second hour
Diabetic	≥200 mg at the end of the second hour
Reactive hypoglycemia	A normal 2- or 3-hour response curve, followed by a decrease of ≥20 mg from the fasting level during the final hours
Probable reactive hypoglycemia	A normal 2- or 3-hour response curve, followed by a decrease of 10–20 mg from the fasting level during the final hours
Flat hypoglycemia	An elevation of >20 mg, followed by a decrease of ≥20 mg below the fasting level
Prediabetic hypoglycemia	A 2-hour response identical to the hypoglycemic prediabetic response but showing a hypoglycemic response during the final 3 hours
Hyperinsulinism	A marked hypoglycemic response with a value of <50 mg during the third, fourth, or fifth hour

A more functional test of blood sugar control is the OGTT. It is used in the diagnosis of both reactive hypoglycemia and diabetes, although it is rarely required for the latter. After fasting for at least 12 hours, a baseline blood glucose measurement is collected. The subject then consumes a glucose-containing drink. The amount consumed is based on body weight: 1.75 g/kg. Blood sugar levels are measured at 30 minutes, 1 hour, and then hourly for up to 6 hours. Blood sugar levels greater than 200 mg/dL indicate diabetes. Levels below 50 mg/dL indicate reactive hypoglycemia. Table 181.1 further explains in detail how to interpret the results of a GTT.

The Glucose–Insulin Tolerance Test

Relying on blood sugar levels alone is often not enough to diagnose hypoglycemia. It is now widely recognized that the signs and symptoms of hypoglycemia can occur in individuals having blood glucose levels well above 50 mg/dL, and there is a wide overlap between symptomatic patients and asymptomatic controls.[3] Many of the symptoms linked to hypoglycemia appear to be the result of increases in insulin or epinephrine. It has been recommended that insulin or epinephrine (adrenaline) be measured during a GTT because symptoms often correlate better with elevations in these hormones than with glucose levels.[9,10] Several studies have shown that the glucose–insulin tolerance test (G-ITT) leads to a greater sensitivity in the diagnosis of both hypoglycemia and diabetes than the standard GTT.[10,11]

The G-ITT uses a standard 6-hour GTT coupled with measurements of insulin levels. The G-ITT appears to be one of the best diagnostic indicators for faulty sugar metabolism.[11] As many as two thirds of subjects with suspected diabetes or hypoglycemia who have normal GTTs will demonstrate abnormal insulin tolerance tests. Table 181.2 lists the various patterns seen with the G-ITT.

24-Hour Continuous Glucose Monitoring

Continuous glucose monitors (CGMs) are electronic diagnostic systems that require the insertion of a sensing catheter under the skin of the patient's abdomen. The catheter contains a miniaturized electronic device that measures blood sugar and then sends this information every few seconds to a pager-sized computer module worn on the patient's belt for up to a week. The portable computer module translates and records blood sugar data, which can then be downloaded to a larger computer. A graph showing the average blood sugar reading every 5 minutes (288 blood sugar readings per day) can then be generated and studied in relation to food intake, appetite, food cravings, hypoglycemic symptoms, medication, and exercise. CGMs have been shown to be very useful tools in the diagnosis and monitoring of blood sugar control.[12,13]

TABLE 181.2 Criteria for the Glucose–Insulin Tolerance Test

Pattern	Response
Pattern 1	Normal fasting insulin 0–30 units. Peak insulin at 0.5–1 hour. The combined insulin value for the second and third hours is <60 units. This pattern is considered normal.
Pattern 2	Normal fasting insulin. Peak at 0.5–1 hour with a "delayed return to normal." Second- and third-hour levels between 60 and 100 units are usually associated with hypoglycemia and are considered borderline for diabetes; values >100 units considered definite diabetes.
Pattern 3	Normal fasting insulin. Definite diabetes.
Pattern 4	High fasting insulin. Definite diabetes.
Pattern 5	Low insulin response. All tested values for insulin <30. If this response is associated with elevated blood sugar levels, it probably indicates insulin-dependent diabetes ("juvenile pattern").

Using a CGM, Michael R. Lyon, MD, has discovered that most people with weight problems and insulin resistance go through their days with remarkably fluctuating blood sugar, or increased glycemic volatility. Rather than the absolute glucose level being the prime determinant of hypoglycemic symptoms, Lyon has found that feelings of hypoglycemia occur when the blood sugar drops rapidly, even when it is above the normal range (i.e., between 70 and 100 mg/dL) (Fig. 181.2). In such cases, symptoms of hypoglycemia can range from mild to severe and include such things as food cravings, headache, depression, anxiety, irritability, blurred vision, excessive sweating, and mental confusion.

Rather than referring to these patients as suffering from hypoglycemia, Lyon and Michael T. Murray, ND, propose that they suffer from a new clinical entity referred to as "elevated glycemic volatility."[14] These authors believe that such volatility is at the heart of most weight problems. Their data indicate that rapidly fluctuating blood sugar levels are generally related to some degree of insulin resistance and made worse by more-than-moderate consumption of foods with a high glycemic effect.

The Hypoglycemic Index

In borderline cases of hypoglycemia, the hypoglycemic index may aid in the diagnosis. This value is determined by calculating the fall in blood glucose levels during the 90-minute period before reaching the lowest point divided by the value of the lowest point. A hypoglycemic index greater than 0.8 usually indicates reactive hypoglycemia.

The Hypoglycemia Questionnaire

In many cases, when all is considered, the most useful measure in the diagnosis of hypoglycemia remains an assessment of symptoms. In general, when symptoms appear 3 to 4 hours after eating and disappear

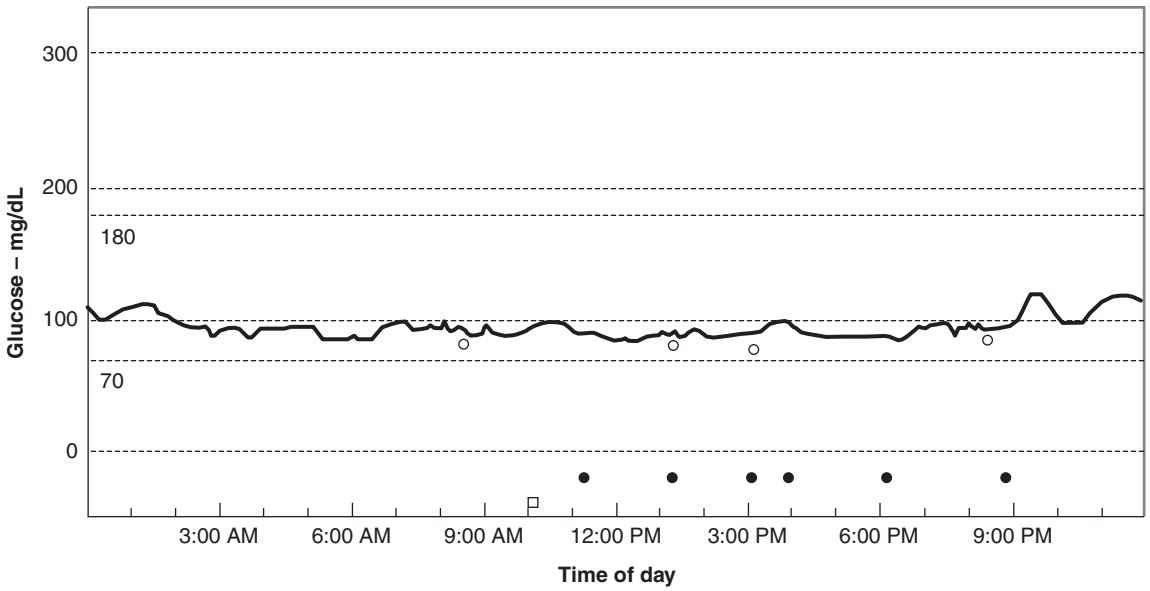


Fig. 181.2 Blood sugar stabilization from fiber supplementation (same patient as in Fig. 181.1). (Courtesy Dr. Michael Lyon.)

with the ingestion of food, hypoglycemia should be considered. The questionnaire shown in Table 181.3 is an excellent screening method for hypoglycemia.

GENERAL CONSIDERATIONS

In the 1970s, hypoglycemia was a popular "self-diagnosis" for a long list of symptoms; every symptom on the questionnaire in Table 181.3 was linked to hypoglycemia. Although all these symptoms may be due to hypoglycemia, many cases have other etiologies. The tremendous public interest in hypoglycemia and sugar intake was fueled by popular books like *Sugar Blues,* by William Duffy; *Hope for Hypoglycemia,* by Broda Barnes; and *Sweet and Dangerous,* by John Yudkin. The popularity of these books and the diagnosis of hypoglycemia were met with much skepticism from the medical community. Editorials in the *Journal of the American Medical Association* and the *New England Journal of Medicine* during the 1970s denounced this public interest in hypoglycemia and tried to invalidate the concept.[15,16]

Research has provided an ever-increasing amount of information concerning the roles that refined carbohydrates and the faulty control of blood sugar play in many disease processes. *Metabolic syndrome* or *syndrome X* describes a cluster of abnormalities that owe their existence largely to a high intake of refined carbohydrates, leading to the development of hypoglycemia, excessive insulin secretion, and glucose intolerance followed by diminished insulin sensitivity and leading to high blood pressure, elevated cholesterol levels, obesity, and ultimately, type 2 diabetes. Metabolic syndrome is discussed in greater detail later.

Hypoglycemia, or idiopathic postprandial syndrome, is without question a valid clinical entity. A substantial amount of information indicates that hypoglycemia is caused by an excessive intake of refined carbohydrates.[17,18] Although most medical and health organizations, as well as the U.S. government, have recommended that no more than 10% of the total caloric intake be derived from refined sugars added to foods, added sugar accounts for roughly 30% of the total calories consumed by most Americans.[19] The average American consumes more than 100 lb of sucrose and 40 lb of high-fructose corn syrup each year. This sugar addiction plays a major role in the high prevalence of ill health and chronic disease in the United States.

TABLE 181.3 Hypoglycemia Questionnaire

	None	Mild	Moderate	Severe
Crave sweets	0	1	2	3
Irritable if a meal is missed	0	1	2	3
Feel tired or weak if a meal is missed	0	1	2	3
Dizzy when standing suddenly	0	1	2	3
Frequent headaches	0	1	2	3
Poor memory (forgetful) or concentration	0	1	2	3
Feel tired an hour or so after eating	0	1	2	3
Heart palpitations	0	1	2	3
Feel shaky at times	0	1	2	3
Afternoon fatigue	0	1	2	3
Vision blurs on occasion	0	1	2	3
Depression or mood swings	0	1	2	3
Overweight	0	1	2	3
Frequently anxious or nervous	0	1	2	3

Total Scoring: <5, hypoglycemia not likely a factor; 6 to 15, hypoglycemia a likely factor; 15, hypoglycemia extremely likely.

Health Effects of Hypoglycemia

Hypoglycemia and the Brain

The brain depends on glucose as an energy source. During hypoglycemic events, brain dysfunction develops, resulting in dizziness, headache, clouding of vision, blunted mental acuity, emotional instability, confusion, and abnormal behavior.

The association between hypoglycemia and impaired mental function is well known. However, the role of hypoglycemia in various psychological disorders is often overlooked. For example, despite numerous studies of depressed individuals showing a high percentage of abnormal glucose or insulin tolerance tests, rarely is hypoglycemia considered, and rarely are depressed individuals offered dietary therapy.[20,21] There is no explanation for this oversight among so many

physicians, especially because dietary therapy (usually simply eliminating refined carbohydrates from the diet) is occasionally all that is necessary for effective therapy in patients who suffer from depression due to reactive hypoglycemia.

Aggressive and Criminal Behavior

A strong yet controversial link exists between hypoglycemia and aggressive or criminal behavior. Several controlled studies among psychiatric patients and habitually violent and impulsive criminals have shown that reactive hypoglycemia (as determined by an OGTT) is a common finding.[22,23] Furthermore, during the GTT, abnormal and sometimes emotionally explosive behavior is often observed. In one study, reactive hypoglycemia was shown to induce fire-setting behavior in arsonists.[24]

Several large studies involving more than 6000 inmates in 10 correctional institutions in three states have evaluated the effect of dietary intervention (i.e., the elimination of refined sugar) on antisocial or aggressive behavior.[25,26]

In the first study, 174 incarcerated juvenile delinquents were placed on a sugar-restricted diet, and another 102 offenders were placed on a control diet.[25] During the 2-year study, the incidence of antisocial behavior was reduced by 45% in the treatment group. The most significant changes were in the reduction of assaults (83%), theft (77%), "horseplay" (65%), and refusal to obey orders (55%). Antisocial behavior changed most in those charged with assault, robbery, rape, aggravated assault, auto theft, vandalism, child molestation, arson, and possession of a deadly weapon.

In the largest study, 3999 incarcerated juveniles were studied over a period of 2 years.[26] This study limited the dietary revisions to replacing sugary soft drinks with fruit juices and high-sugar snacks with nonrefined carbohydrate snacks (e.g., replacing a candy bar with popcorn). When the 1121 young men on the sugar-restricted diet were compared with the 884 on the control diet, there were significant differences: suicide attempts were reduced by 100%, the need for restraints to prevent self-injury was reduced by 75%, disruptive behavior was reduced by 42%, and assaults and fights were reduced by 25%. Interestingly, the dietary changes did not seem to affect the behavior of female subjects. This lack of effect seems to indicate that men and women may react to hypoglycemia differently. From an anthropological and evolutionary view, this makes sense. Low blood sugar levels were undoubtedly an internal signal for men to hunt for food.

The link between hypoglycemia and aggressive behavior also extends to men without a history of criminal activity. In one study, a GTT was given to a group of men who did not have a history of aggressive behavior or hypoglycemia.[18] In these subjects, a significant correlation was found between the tendency to become mildly hypoglycemic and scores on questionnaires used to measure aggression. The results indicate that aggressiveness often coincides with hypoglycemia.

Premenstrual Syndrome

The premenstrual syndrome (PMS) is a recurrent condition of women characterized by troublesome yet often ill-defined symptoms that usually appear 7 to 14 days before menstruation. The syndrome affects about one third of women between 30 and 40 years of age, about 10% of whom may have a significantly debilitating form.

An authority on PMS, Guy Abraham, MD, attempted to clarify the different forms by subdividing PMS into four distinct subgroups (A, C, D, and H).[27] Each subgroup is linked to specific symptoms, hormonal patterns, and metabolic abnormalities (see Chapter 212 for further information).

PMS-C is associated with increased appetite, craving for sweets, headache, fatigue, fainting spells, and heart palpitations. GTTs on patients with PMS-C during the 5 to 10 days before their menses typically displayed a flattening of the early part of the curve and reactive hypoglycemia, whereas during other parts of the menstrual cycle, the GTT was normal. A flat early portion of the GTT curve usually implies excessive secretion of insulin in response to sugar consumption. This appears to be hormonally regulated, but other factors may also be involved.[28] Sodium chloride ingestion enhances the insulin response to sugar ingestion, and decreased magnesium levels in the pancreas can result in the increased secretion of insulin in response to glucose. Regardless of the cause, women with PMS-C appear to be extremely sensitive to hypoglycemia.

Migraine Headaches

Migraine headaches are probably caused by excessive dilation (expansion) of a blood vessel in the head (see Chapter 198). Migraines are a surprisingly common disorder, affecting 15% to 20% of men and 25% to 30% of women at some time in their lives. More than one half of the patients have a family history of the illness. Since 1933, hypoglycemia has been shown to be a common precipitating factor in migraine headaches.[29]

Several studies have found that by eliminating refined sugar from the diets of migraine sufferers with confirmed hypoglycemia, significant improvement may be seen. In one study of 48 migraine sufferers with reactive hypoglycemia, 27 (56%) showed an improvement in symptoms of greater than 75%, 17 (35%) showed an improvement of greater than 50%, and 4 (8%) showed an improvement of greater than 25%.[30]

Atherosclerosis, Intermittent Claudication, and Angina

Substantial evidence indicates that reactive hypoglycemia or impaired glucose tolerance is a significant factor in the development of atherosclerosis. Although a high sugar intake leads to elevations in triglyceride and cholesterol levels, the real culprit may be the elevations of insulin.[31] Abnormal GTTs and elevations in insulin secretion are common findings in patients with heart disease.[32,33] High sugar consumption and reactive hypoglycemia can also be a cause of angina and intermittent claudication.[34,35]

Metabolic Syndrome

The term *metabolic syndrome* is used to describe a set of cardiovascular risk factors that includes glucose or insulin disturbances, high blood cholesterol and triglyceride levels, elevated blood pressure, and android obesity. Other terms to describe this syndrome include *syndrome X, Reaven's syndrome, insulin resistance syndrome,* and *atherothrombogenic syndrome.*[36,37]

The underlying common denominator in the metabolic syndrome is an elevated insulin level, along with insulin resistance. There is little doubt that an elevated intake of refined carbohydrates contributes to these elevations. An increased intake of simple sugar may first lead to hypoglycemia and potentially to diabetes. There exists considerable scientific evidence that the development of type 2 diabetes is preceded by elevations of serum insulin values and insulin insensitivity due to the prolonged consumption of refined sugars.[38] In most cases these defects presented themselves decades before the development of diabetes. Hypoglycemia, increased insulin secretion, metabolic syndrome, and type 2 diabetes can be viewed as a progression of the same illness—maladaptation to the Western diet.

THERAPEUTIC CONSIDERATIONS

Dietary Factors

Dietary carbohydrates play a central role in the cause, prevention, and treatment of hypoglycemia. Simple carbohydrates, or sugars, are

quickly absorbed by the body, resulting in a rapid elevation in blood sugar and stimulating a corresponding excessive elevation in serum insulin levels. It is thought by some that the assortment of natural, simple sugars in fruits and vegetables have an advantage over sucrose and other refined sugars in that they are balanced by a wide range of nutrients that aid in the utilization of the sugars. This is true. Perhaps more importantly, sugars found in whole, unprocessed foods are absorbed slower because they are contained within cells and are associated with fiber and other food elements.

Problems with carbohydrates begin during the refining process, which increases their rate of absorption and removes essential nutrients. Virtually all the vitamin and trace mineral content is removed from white sugar, white breads, pastries, and many breakfast cereals. When high-sugar foods are eaten alone, blood sugar levels rise quickly, producing a strain on blood sugar control. Eating foods high in simple sugars from any source (e.g., sucrose, honey, maple syrup) is harmful to blood sugar control. Large amounts of fruit juice, and even vegetable juice, may be problematic for individuals with hypoglycemia because the cell disruption characteristic of juicing increases the percentage of the sugars in the juices.

More than one half of the carbohydrates consumed in the United States are in the form of sugars added to processed foods as sweetening agents.[19] Patients should be instructed to read food labels carefully for clues to sugar content, noting that various words are used to describe refined simple carbohydrates. Any of the following might appear on the label as sugar: *sucrose, glucose, maltose, lactose, fructose, corn syrup, evaporated cane juice,* and *white grape juice concentrate.*

A Closer Look at Simple Carbohydrates

Glucose is not particularly sweet-tasting compared with fructose and sucrose. It is found in abundant amounts in fruits, honey, sweet corn, and most root vegetables. Glucose is also the primary repeating sugar unit of most complex carbohydrates.

Fructose, or fruit sugar, is the primary carbohydrate in many fruits, maple syrup, and honey. Fructose is very sweet, roughly 1.5 times sweeter than sucrose. Although fructose has the same chemical formula as glucose, its structure is quite different. To be used by the body, fructose must be converted to glucose within the liver.

Many physicians have recommended that individuals with diabetes or hypoglycemia avoid fruits and fructose. However, research challenges this. Fructose does not cause a rapid rise in blood sugar levels. Because fructose must be changed to glucose in the liver to be used by the body, blood glucose levels do not rise as rapidly with fructose consumption as they do with that of other simple sugars. For example, the ingestion of sucrose results in an immediate elevation in the blood sugar level. Although most individuals with diabetes or hypoglycemia cannot tolerate sucrose, most can tolerate moderate amounts of fruits and fructose without loss of blood sugar control. In fact, fructose and fruits are not only better tolerated than white bread and other refined carbohydrates, but they also produce more gradual elevations in blood sugar levels, compared with most sources of complex carbohydrates (starch).[39] In addition, fructose at concentrations found in most single servings of fruit (~10 g) has been shown to enhance sensitivity to insulin.[40]

Regular fruit consumption may help control sugar cravings and promote weight loss in overweight individuals. Although studies have shown aspartame (NutraSweet), glucose, and sucrose to increase the appetite, fructose has been shown in several double-blind studies to decrease the calories and fat consumed. Typically, subjects are given food or drink containing an equivalent caloric amount of fructose or other sweetener 30 minutes to 2.5 hours before consuming as much food as they want at a dinner buffet. Consistently, subjects receiving the fructose-sweetened food or drink consume substantially fewer calories and fat compared with the groups receiving aspartame, sucrose, or glucose.[41–43]

TABLE 181.4 Glycemic Index of Isocaloric Amounts of Some Common Foods

	Food	Glycemic Index
Sugars	Fructose	20
	Glucose	100
	Honey	75
	Maltose	105
	Sucrose	60
Fruits	Apples	39
	Bananas	62
	Orange juice	46
	Oranges	40
	Raisins	64
Vegetables	Beets	64
	Carrot, cooked	36
	Carrot, raw	31
	Potato, baked	98
	Potato (new), boiled	70
Grains	Bran cereal	51
	Bread, white	69
	Bread, whole grain	72
	Corn	59
	Corn flakes	80
	Oatmeal	49
	Pasta	45
	Rice	70
	Rice, puffed	95
	Wheat cereal	67
Legumes	Beans	31
	Lentils	29
	Peas	39
Other foods	Ice cream	36
	Milk	34
	Nuts	13

Modified from Truswell AS. Glycaemic index of foods. *Eur J Clin Nutr.* 1992;46(suppl 2):S91–S101.

The Glycemic Index and Glycemic Load

The glycemic index (GI) and the glycemic load (GL) are two helpful methods of categorizing foods based on the ability of the food to alter blood sugar.

The GI was developed in 1981 to express the rise of blood glucose after a particular food is eaten.[44] The standard value of 100 is based on the rise seen with the ingestion of glucose. The GI ranges from about 20 for fructose and whole barley to about 98 for a baked potato. The insulin response to carbohydrate-containing foods is similar to the rise in blood sugar.

The GI is used as a guideline for dietary recommendations for people with either diabetes or hypoglycemia (see Table 181.4). People with blood sugar problems are advised to avoid foods with high values and choose carbohydrate-containing foods with lower

values. However, the GI should not be the only dietary guideline. For example, high-fat foods like ice cream and sausage may have a low GI, but because a diet high in fat has been shown to impair glucose tolerance, these foods are not good choices for people with hypoglycemia or diabetes.

The GL uses the information the GI provides in a more comprehensive way to assess the effect of carbohydrate consumption.[45] Although the GI reveals how quickly a food's carbohydrate content can raise blood glucose levels, it still does not explain how much of the blood sugar–increasing carbohydrate is in a certain food.

The dietary GL is defined as the value of a food's GI divided by 100 and then multiplied by its available carbohydrate content. A GL of 20 or more is considered high, 11 to 19 is considered intermediate, and 10 or less is considered low. The GI of watermelon, for example, is 72, and a typical serving of 120 g has 6 g of available carbohydrate. Therefore the GL for watermelon is

$$(72/100) \times 6 = 4.32$$

Even though the GI for watermelon is fairly high, the GL is low, indicating that within reasonable serving amounts, it does not adversely stress blood sugar control. Appendix 6 provides a complete listing of foods and their GI, fiber content, and GL.

The GL reinforces the idea that foods containing natural soluble and insoluble fibers, as well as whole foods that are minimally processed, remain better choices in terms of glycemic influence and insulin response.[46]

Further research indicates that diets with a high GL are directly linked to conditions such as diabetes[38]; coronary heart disease[47]; and colon,[48] ovarian,[45] and pancreatic[49] cancers.

The Importance of Fiber

Population studies and clinical and experimental data show blood sugar disorders to be clearly related to inadequate dietary fiber intake.[19,50] These results indicate that although the intake of refined sugars should be curtailed, the intake of complex carbohydrate sources that are rich in fiber should be increased.

The term *dietary fiber* refers to the components of the plant cell wall as well as the indigestible residues from plant foods. Different types of fibers possess different actions. The water-soluble forms exert the most beneficial effects on blood sugar control and include hemicelluloses, mucilages, gums, and pectins. These types of fiber are capable of the following actions:

- Slowing down the digestion and absorption of carbohydrates, thereby preventing rapid rises in blood sugar
- Increasing cell sensitivity to insulin, thereby preventing the excessive secretion of insulin
- Improving uptake of glucose by the liver and other tissues, thereby preventing a sustained elevation of blood sugar (Fig. 181.3)

For a full discussion of the importance of fiber, see Chapter 132.

The majority of fiber in most plant cell walls is water soluble. Particularly good sources of water-soluble fiber are legumes, oat bran, nuts, seeds, psyllium seed husks, pears, apples, and most vegetables. Patients should be advised to consume a large amount of plant food to obtain adequate levels of dietary fiber. A daily intake of 50 g is a healthful goal.

PolyGlycoPlex

Based on their work with CGM, Drs. Lyon and Murray have uncovered many important findings on how to effectively reduce blood sugar volatility. For example, they confirmed earlier work that although a low-glycemic diet is very important in reducing blood sugar levels, it has little effect in eliminating blood sugar volatility.[51] The most effective way to reduce glycemic volatility is a low-glycemic diet along with the use of a novel matrix of soluble fibers known as PolyGlycoPlex (PGX). Several double-blind studies have shown a reduction of postprandial hypoglycemia by PGX in a dose-dependent manner, independent of food form.[52–54] The PGX matrix produces a higher level of viscosity, gel-forming properties, and expansion with water compared with any other known fiber. This translates to a significant reduction in the glycemic effect of any food or meal. The typical dosage is 1.5 to 5 g before meals.

Chromium

Chromium is vital to proper blood sugar control because it functions in the body as a key constituent of the "glucose tolerance factor." Without chromium, the action of insulin is blocked, and glucose levels are elevated. A chromium deficiency may be an underlying factor to the tremendous number of Americans who have hypoglycemia, have diabetes, or are obese.[55] Evidence exists that marginal chromium deficiency is quite common in the United States and may be responsible for many cases of reactive hypoglycemia.[56]

In one double-blind crossover study of eight female patients, 200 mcg of chromium (as chromium chloride) given twice daily for 3 months alleviated hypoglycemic symptoms and the glucose nadir at 2 to 4 hours after a glucose load.[56] In addition, insulin binding improved, and the number of insulin receptors increased.[57,58]

Lifestyle Factors

Alcohol

Alcohol consumption severely stresses blood sugar control and is often a contributing factor to hypoglycemia. Alcohol induces reactive hypoglycemia by interfering with normal glucose utilization as well as increasing the secretion of insulin. The resultant drop in blood sugar produces a craving for food, particularly foods that quickly elevate blood sugar, as well as a craving for more alcohol. In the presence of more alcohol, the increased sugar consumption aggravates the reactive hypoglycemia, again due to alcohol-induced impairment of normal glucose utilization and increased secretion of insulin.

Hypoglycemia is an important complication of acute and chronic alcohol abuse. Hypoglycemia aggravates the mental and emotional problems of the alcoholic and the withdrawing alcoholic with symptoms such as the following[59]:

- Sweating
- Tremor
- Rapid heartbeat
- Anxiety
- Hunger
- Dizziness
- Headache
- Visual disturbance
- Decreased mental function
- Confusion
- Depression

Although acute alcohol ingestion induces hypoglycemia, chronic alcohol use leads to hyperglycemia and diabetes. Eventually the body becomes insensitive to the augmented insulin release caused by the alcohol. In addition, alcohol itself can cause insulin resistance even in healthy individuals.[60] There is also evidence from large population studies that alcohol intake is strongly correlated with diabetes.[19,61] The greater the alcohol intake, the more likely it is that an individual will have or develop diabetes.

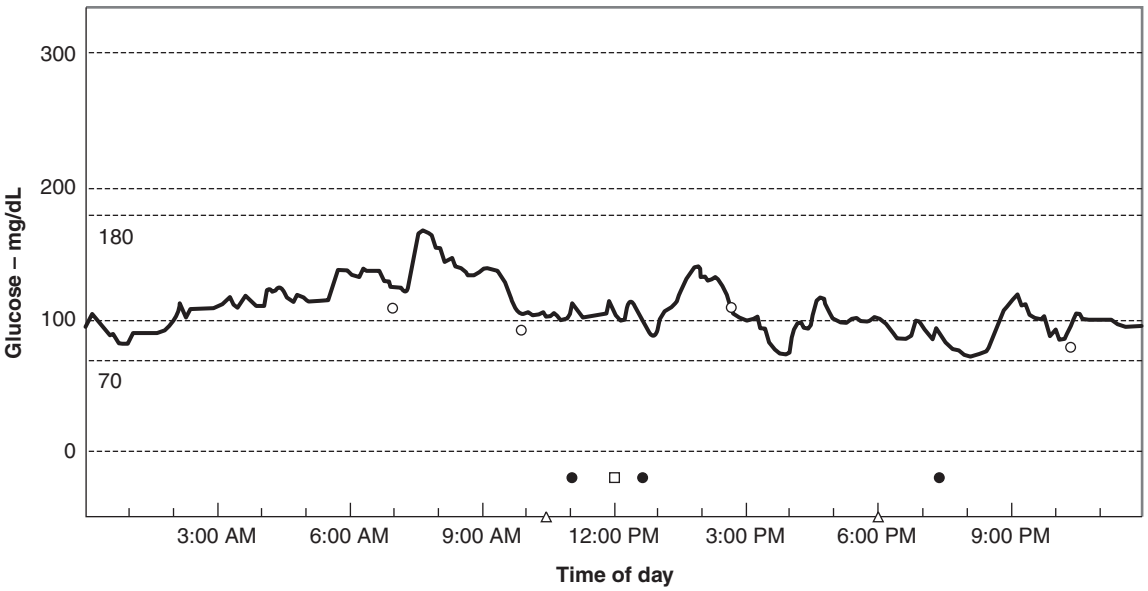


Fig. 181.3 Blood sugar fluctuation revealed by 24-hour monitoring. (Courtesy Dr. Michael Lyon.)

Exercise

Regular exercise has been well documented to prevent type 2 diabetes, and it improves many aspects of glucose metabolism, including enhancing insulin sensitivity and improving glucose tolerance in those who are already diabetic. Some effects of exercise on blood sugar control may stem from the fact that exercise increases tissue chromium concentrations.[62]

Toxin Exposure

A significant amount of research demonstrates the effects of toxins and toxicants on glycemic control. Although most results demonstrate hyperglycemic effects, others have the opposite effect. For example, vanadium potentiates the action of insulin and lowers blood glucose levels by inhibiting some tyrosine-protein phosphatases increasing phosphorylation levels of various insulin pathway intermediaries.[63] There have been reports of severe hypoglycemia that may threaten life in vanadium acute intoxication.[64] Hypoglycin-A (methylene cyclopropyl-alanine, MCPA), a compound found in ackee fruit, causes hypoglycemic encephalopathy in a dose-dependent manner and has been implicated in ackee-fruit poisoning in Africa and West Indies.[65]

THERAPEUTIC APPROACH

The primary treatment of hypoglycemia is the use of dietary therapy to stabilize blood sugar levels. Reactive hypoglycemia is not a disease; it is a complex set of symptoms caused by faulty carbohydrate metabolism induced by an inappropriate diet.

Diet

All simple, processed, and concentrated carbohydrates, as well as food choices with a high GL, must be avoided. Foods rich in soluble fiber, such as legumes and low-glycemic vegetables, should be consumed regularly. Frequent small meals may be more effective than larger meals further apart in stabilizing blood sugar levels. Alcohol must be avoided because it can cause hypoglycemia. Further dietary recommendations mirror those in Chapter 165.

Supplements

Many essential nutrients are critical for proper carbohydrate metabolism. Taking a multivitamin–multimineral formula providing at least the recommended dietary intake levels appears warranted.

Chromium: 200 to 400 mcg/day
PGX: 1500 to 5000 mg before meals

Exercise

Because the beneficial effects of exercise in improving insulin sensitivity decrease within 3 days after exercise and are no longer evident after 1 week, a sustained program is required.[66] A graded long-term exercise program should be developed that is appropriate to the individual's fitness level and interest; it should elevate the heart rate to at least 60% of its maximum for half an hour three times a week.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Galati SJ, Rayfield EJ. Approach to the patient with postprandial hypoglycemia. *Endocr Pract*. 2014;20(4):331–340.
2. Chalew SA, Koetter H, Hoffman S, et al. Diagnosis of reactive hypoglycemia: pitfalls in the use of the oral glucose tolerance test. *South Med J*. 1986;79:285–287.
3. Palardy J, Havrankova J, Lepage R, et al. Blood glucose measurements during symptomatic episodes in patients with suspected postprandial hypoglycemia. *N Engl J Med*. 1989;321:1421–1425.
4. Kwentus JA, Achilles JT, Goyer PF. Hypoglycemia etiologic and psychosomatic aspects of diagnosis. *Postgrad Med*. 1982;71:99–104.
5. Yogev Y, Ben-Haroush A, Chen R, et al. Undiagnosed asymptomatic hypoglycemia: diet, insulin, and glyburide for gestational diabetic pregnancy. *Obstet Gynecol*. 2004;104:88–93.
6. Galloway PJ, Thomson GA, Fisher BM, et al. Insulin-induced hypoglycemia induces a rise in C-reactive protein. *Diabetes Care*. 2000;23:861–862.
7. Murata M, Adachi H, Oshima S, Kurabayashi M. Asymptomatic reactive hypoglycemia and Inflammatory reaction in patients with coronary artery disease. *Int Heart J*. 2018;59(4):705–712.
8. World Health Organization. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications*. Geneva: Department of Non-communicable Disease Surveillance; 1999.
9. Chalew SA, McLaughlin JV, Mersey JH, et al. The use of the plasma epinephrine response in the diagnosis of idiopathic postprandial syndrome. *JAMA*. 1984;251:612–615.
10. Hadji-Georgopoulus A, Schmidt MI, Margolis S, et al. Elevated hypoglycemic index and late hyperinsulinism in symptomatic postprandial hypoglycemia. *J Clin Endocrinol Metab*. 1980;50:371–376.
11. Fabrykant M. The problem of functional hyperinsulinism on functional hypoglycemia attributed to nervous causes. 1. Laboratory and clinical correlations. *Metabolism*. 1955;4:469–479.
12. Gross TM, Mastrototaro JJ. Efficacy and reliability of the continuous glucose monitoring system. *Diabetes Technol Ther*. 2000;2(suppl 1):S19–S26.
13. Sabbah H, McCulloch K, Frederickson L, et al. Detailed data from a continuous glucose monitoring system (CGMS) facilitates comprehensive diabetes management recommendations. *Diabetes*. 2000;49(suppl 1):718.
14. Murray MT, Lyon MR. *Hunger Free Forever*. New York: Atria; 2008.
15. No authors. Statement on hypoglycemia. *JAMA*. 1973;223:682.
16. Cahill Jr GF, Soeldner JS. A non-editorial on non-hypoglycemia. *N Engl J Med*. 1974;291:905–906.
17. Hofeldt FD. Patients with bona fide meal-related hypoglycemia should be treated primarily with dietary restriction of refined carbohydrate. *Endocrinol Metab Clin North Am*. 1989;18:185–201.
18. Sanders LR, Hofeldt FD, Kirk MC, et al. Refined carbohydrate as a contributing factor in reactive hypoglycemia. *South Med J*. 1982;75:1072–1075.
19. National Research Council. *Diet and Health: Implications for Reducing Chronic Disease Risk*. Washington, DC: National Academy Press; 1989.
20. Winokur A, Maislin G, Phiillips JL, et al. Insulin resistance after glucose tolerance testing in patients with major depression. *Am J Psychiatry*. 1988;145:325–330.
21. Wright JH, Jacisin JJ, Radin NS, et al. Glucose metabolism in unipolar depression. *Br J Psychiatry*. 1978;132:386–393.
22. Schauss AG. Nutrition and behavior: complex interdisciplinary research. *Nutr Health*. 1984;3:9–37.
23. Benton D. Hypoglycemia and aggression: a review. *Int J Neurosci*. 1988;41:163–168.
24. Virkkunen M. Reactive hypoglycemic tendency among arsonists. *Acta Psychiatr Scand*. 1984;69:445–452.
25. Schoenthaler SJ. Diet and crime: an empirical examination of the value of nutrition in the control and treatment of incarcerated juvenile offenders. *Int J Biosocial Res*. 1983;4:25–39.
26. Schoenthaler SJ. The northern California diet-behavior program: an empirical evaluation of 3,000 incarcerated juveniles in Stanislaus County Juvenile Hall. *Int J Biosocial Res*. 1983;5:99–106.
27. Abraham GE. Nutritional factors in the etiology of the premenstrual tension syndromes. *J Reprod Med*. 1983;28:446–464.
28. Walsh CH, O'Sullivan DJ. Studies of glucose tolerance, insulin and growth hormone secretion during the menstrual cycle in healthy women. *Ir J Med Sci*. 1975;144:18–24.
29. Critchley M. Migraine. *Lancet*. 1933;1:123–126.
30. Dexter JD, Roberts J, Byer JA. The five hour glucose tolerance test and effect of low sucrose diet in migraine. *Headache*. 1978;18:91–94.
31. Mykkanen L, Laakso M, Pyorala K. High plasma insulin levels associated with coronary heart disease in the elderly. *Am J Epidemiol*. 1993;137:1190–1202.
32. Yudkin J. Metabolic changes induced by sugar in relation to coronary heart disease and diabetes. *Nutr Health*. 1987;5:5–8.
33. Pyorala K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care*. 1979;2:131–141.
34. Bansal S, Toh SH, LaBresh KA. Chest pain as a presentation of reactive hypoglycemia. *Chest*. 1983;84:641–642.
35. Hanson M, Bergentz SE, Ericsson BF, et al. The oral glucose tolerance test in men under 55 years of age with intermittent claudication. *Angiology*. 1987;38:469–473.
36. Hjermann I. The metabolic cardiovascular syndrome: syndrome X, Reaven's syndrome, insulin resistance syndrome, atherothrombogenic syndrome. *J Cardiovasc Pharmacol*. 1992;20:S5–S10.
37. Maseri A. Syndrome X. Still an appropriate name. *J Am Coll Cardiol*. 1991;17:1471–1472.
38. Willett W, Manson J, Liu S. Glycemic index, glycemic load, and risk of type 2 diabetes. *Am J Clin Nutr*. 2002;76:274S–2780S.
39. Gregersen S, Rasmussen O, Larsen S, et al. Glycaemic and insulinaemic responses to orange and apple compared with white bread in non-insulin dependent diabetic subjects. *Eur J Clin Nutr*. 1992;46:301–303.
40. Koivisto VA, Yki-Jarvinen H. Fructose and insulin sensitivity in patients with type 2 diabetes. *J Intern Med*. 1993;233:145–153.
41. Rodin J. Effects of pure sugar vs. mixed starch fructose loads on food intake. *Appetite*. 1991;17:213–219.
42. Rodin J. Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *Am J Clin Nutr*. 1990;51:428–435.
43. Spitzer L, Rodin J. Effects of fructose and glucose preloads on subsequent food intake. *Appetite*. 1987;8:135–145.
44. Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr*. 1981;34:362–366.
45. Lukaczer D. Sweet, hot and deadly: the link between glycemic index, inflammation, and cardiovascular disease: evolving research and practical applications. Portland, OR In: *Proceedings of the American Association of Naturopathic Physicians 18th Annual Convention and Exposition*; 2003.
46. Granfeldt Y, Eliasson AC, Bjorck I. An examination of the possibility of lowering the glycemic index of oat and barley flakes by minimal processing. *J Nutr*. 2000;130:2207–2214.
47. Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in U.S. women. *Am J Clin Nutr*. 2000;71:1455–1461.
48. Franceschi S, Dal Maso L, Augustin L, et al. Dietary glycemic load and colorectal cancer risk. *Ann Oncol*. 2001;12:173–178.
49. Michaud DS, Liu S, Giovannucci E, et al. Dietary sugar, glycemic load, and pancreatic cancer risk in a prospective study. *J Natl Cancer Inst*. 2002;94:1293–1300.
50. Trowell H, Burkitt DP. *Western Diseases: Their Emergence and Prevention*. Cambridge, MA: Harvard University Press; 1981.
51. Brynes AE, Lee JL, Brighton RE, et al. A low glycemic diet significantly improves 24-h blood glucose profile in people with type 2 diabetes, as assessed using the continuous glucose MiniMed monitor [Letter to the Editor]. *Diabetes Care*. 2003;26:548–549.
52. Jenkins AL, Kacinik V, Lyon M, et al. Effect of adding the novel fiber, PGX®, to commonly consumed foods on glycemic response, glycemic index and GRIP: a simple and effective strategy for reducing post

prandial blood glucose levels: a randomized, controlled trial. *Nutr J*. 2010;9:58.
53. Brand-Miller JC, Atkinson FS, Gahler RJ, et al. Effects of PGX, a novel functional fibre, on acute and delayed postprandial glycaemia. *Eur J Clin Nutr*. 2010;64(12):1488–1493.
54. Jenkins AL, Kacinik V, Lyon MR, et al. Reduction of postprandial glycemia by the novel viscous polysaccharide PGX, in a dose-dependent manner, independent of food form. *J Am Coll Nutr*. 2010;29(2):92–98.
55. Anderson RA. Chromium, glucose tolerance, and diabetes. *Biol Trace Elem Res*. 1992;32:19–24.
56. Anderson RA, Polansky MM, Bryden NA, et al. Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism*. 1987;36:351–355.
57. McCarty MF. Chromium and other insulin sensitizers may enhance glucagon secretion: implications for hypoglycemia and weight control. *Med Hypoth*. 1996;46:77–80.
58. Anderson RA. Nutritional factors influencing glucose/insulin system: chromium. *J Am Coll Nutr*. 1997;16:404–410.
59. Wyngaarden JB, Smith LH, Bennett JC, eds. *Cecil Textbook of Medicine*. Philadelphia: WB Saunders; 1992.
60. Hirata Y. Diabetes and alcohol. *Asian Med J*. 1988;31:564–569.
61. Selby JV, Newman B, King MC, et al. Environmental and behavioral determinants of fasting plasma glucose in women: a matched co-twin analysis. *Am J Epidemiol*. 1987;125:979–988.
62. Vallerand AL, Cuerrier JP, Shapcott D, et al. Influence of exercise training on tissue chromium concentrations in the rat. *Am J Clin Nutr*. 1984;39:402–409.
63. Niu X, Xiao R, Wang N, et al. The molecular mechanisms and rational design of anti-diabetic vanadium compounds. *Curr Top Med Chem*. 2016;16(8):811–822.
64. Boulassel B, Sadeg N, Roussel O, Perrin M, Belhadj-Tahar H. Fatal poisoning by vanadium. *Foren Sci Int*. 2011;206(1–3):e79–e81.
65. Vashishtha VM. Outbreaks of hypoglycemic encephalopathy in Muzaffarpur, India: are these caused by toxins in litchi fruit? the counterpoint. *Indian Pediatr*. 2016;53(5):399–402. PubMed PMID: 27254049.
66. Sato Y, Nagasaki M, Nakai N, et al. Physical exercise improves glucose metabolism in lifestyle-related diseases. *Exp Biol Med (Maywood)*. 2003;228:1208–1212.

182

Hypothyroidism

Alan G. Christianson, NMD

OUTLINE

DIAGNOSTIC SUMMARY

- Depression
- Dry skin
- Headaches
- Lethargy or fatigue
- Memory problems
- Menstrual problems
- Hyperlipidemia
- Recurrent infections
- Sensitivity to cold
- Thinning of head hair
- Muscle pain
- Voice changes
- Weight gain

GENERAL CONSIDERATIONS

Thyroid gland hormones are required for mitochondrial metabolism in every cell of the body. As a result, a deficiency of thyroid hormones can affect virtually all bodily functions. The severity of symptoms in adults ranges from mild deficiency states that may not be apparent with standard blood tests (e.g., early-stage Hashimoto's) to severe deficiency states that can be life-threatening (e.g., myxedema).

The pathophysiology of hypothyroidism may result from the lack of stimulation by the pituitary gland, defective hormone synthesis, impaired cellular conversion of T4 to T3, or a decrease in the cellular uptake of active hormones. The pituitary gland regulates thyroid activity through the secretion of thyroid-stimulating hormone (TSH). The combination of low thyroid hormone and elevated TSH blood levels usually indicates defective thyroid hormone synthesis, which is defined as *primary hypothyroidism.* When TSH and thyroid hormone levels are both low, the pituitary gland is responsible, a situation termed *secondary hypothyroidism. Tertiary hypothyroidism* develops as a result of a deficiency of thyroid hormone-releasing hormone (THRH) from the hypothalamus. Normal thyroid hormone and TSH blood levels combined with low functional thyroid activity (as defined by a low basal metabolic rate) suggest cellular hypothyroidism.

Most estimates on the rate of hypothyroidism are based on an elevated TSH and a suppressed level of T4. Using these criteria, it is estimated that 5% to 10% of the adult population suffers from hypothyroidism.[1–4] The Colorado Thyroid Disease Prevalence Study suggests that no fewer than 10% of the adult population is affected, with the rate exceeding 20% among senior citizens.[5]

Thyroid disease of all types is more common among females. Reports suggest a 4:1 female-to-male ratio. Hypothyroidism is more prevalent among whites and Mexican Americans (5.7%) than among African Americans (1.7%).[6] The rate of hypothyroidism increases steadily with advancing age.

If symptoms and basal body temperature were used for diagnosis, it is likely that the true rate of hypothyroidism may be approximately 25% of the adult population and significantly higher among the elderly.

Symptoms of Hypothyroidism

Psychological

The human brain is sensitive to the slightest fluctuations of thyroid hormone. Depression, weakness, and fatigue are usually the first symptoms of hypothyroidism.[7] Later, the hypothyroid individual has difficulty concentrating and may experience memory lapses.

Metabolic

A deficiency of thyroid hormones can lead to substantial suppression of the basal metabolic rate. Moderate weight gain and sensitivity to cold (demonstrated by cold hands or feet) are common.

Cholesterol and triglyceride levels are increased even in mild forms of hypothyroidism. Before the current generation of thyroid tests, cholesterol was used as a marker of thyroid function. This may explain

the increased rate of heart disease due to atherosclerosis in individuals with hypothyroidism.[8]

In addition, hypothyroidism can cause increases in capillary permeability and slow lymphatic drainage, resulting in the swelling of tissue (edema) as well as fluid retention, commonly noticed in the lower extremities.

Endocrine

Various hormonal symptoms can exist in hypothyroidism. The most common are decreased libido in both genders and menstrual abnormalities in women. Women with mild hypothyroidism may have prolonged, heavy menstrual bleeding with a shorter menstrual cycle. Female infertility may also result. If a woman with untreated hypothyroidism becomes pregnant, there is an increased risk for miscarriage, premature delivery, and stillbirth. Hypotheses for the occurrence of these conditions include hyperprolactinemia from increased production of TRH leading to ovulatory dysfunction,[9] and hypothyroidism interferes with normal physiological pulsatile gonadotrophin-releasing hormone secretion, which is a prerequisite for normal follicular development and ovulation.[10]

Adrenal dysfunction is a common comorbidity with primary and secondary hypothyroidism. Patients with thyroid antibodies are more likely to possess adrenal cortex antibodies associated with Addison's disease. Furthermore, cortisol secretion may increase to compensate for low T3 levels because cortisol raises the cell membrane's permeability to T3.

Skin, Hair, and Nails

Most individuals with hypothyroidism have dry, rough skin covered with fine superficial scales along with coarse, dry, and brittle hair. Hair loss can be quite severe and is generally diffuse and uniform as opposed to patchy. The nails become thin and brittle and typically show transverse grooves.

Muscular and Skeletal

Muscle weakness and joint stiffness are predominant features of hypothyroidism. Some individuals with hypothyroidism may also experience muscle and joint pain as well as general tissue tenderness. Slow reflexes are common, and a slow Achilles reflex time can be diagnostic.

Cardiovascular

Overt and subclinical hypothyroidism predispose individuals to atherosclerosis as a result of increases in cholesterol and triglycerides, as well as elevated levels of homocysteine and C-reactive protein (CRP).[11] Hypothyroidism can also lead to hypertension, reduced cardiac function, and bradycardia.

Other Manifestations

Shortness of breath, constipation, and impaired kidney function are other common features of hypothyroidism.

Causes of Hypothyroidism

Iodine Deficiency

About 95% of all cases of clinical hypothyroidism are primary. The thyroid gland adds iodine to the amino acid tyrosine to create thyroid hormones. Iodine deficiency leads to hypothyroidism, the development of an enlarged thyroid gland (i.e., goiter), or both.

Goiters are estimated to affect more than 200 million people worldwide, and in more than 95% of cases, the cause is an iodine deficiency. Iodine deficiency is now quite rare in the United States and other industrialized countries due to the addition of iodine to table salt.

Few people in the United States are considered iodine-deficient, yet some still develop goiters. Prolonged thyrotropin (TSH) elevation can lead to thyrocyte hypertrophy, which can manifest as a goiter. Goiters generally regress as thyrotropin is suppressed but in rare cases may require surgical resection.

Nodules can also form within the gland during or independent of output dysfunction. Approximately 20% of adult patients have nodules on palpation, and random screening by ultrasound reveals nodules in up to 50% of the population. About 5% of nodules may be cancerous. Goiters or nodules in patients with adequate but marginal iodine intake may result from excessive ingestion of dietary or environmental goitrogens. See Nutritional Considerations for details on dietary goitrogens.

Environmental goitrogens include perchlorate, iodine more than 290 mcg/day, fluoride, and mercury. Medications that induce goiters and suppress thyroid function include amiodarone, carbamazepine, lithium, potassium iodide, phenobarbitone, phenytoin, and rifampin.

Hashimoto's Thyroiditis

Hashimoto's thyroiditis is an autoimmune inflammatory disorder and is the most frequent cause of clinical hypothyroidism in the United States. The antibodies that bind to the thyroid (specifically against the thyroid peroxidase enzyme, thyroglobulin, and TSH receptors) are formed and prevent the manufacturing of sufficient levels of thyroid hormone. In addition to binding to thyroid tissue, these antibodies may also bind to the adrenal glands, pancreas, and acid-producing cells of the stomach (parietal cells). Autoimmune thyroid disease can exist in the absence of serum hypothyroidism, although those with autoimmune thyroid disease progress to hypothyroidism in almost all cases. The presence of Hashimoto's disease raises the risk of developing other autoimmune diseases. Hashimoto's thyroiditis is formally a histological diagnosis. However, the presence of serum antibodies against thyroid proteins such as thyroglobulin (TG) or thyroid peroxidase (TPO) is often sufficient for diagnosis; 10% to 15% of individuals may be antibody-negative.

If a histological assessment is done via biopsy, fine-needle aspiration, or postthyroidectomy, the typical finding is cellular infiltration of immune cells, especially lymphocytes.

Variables that predict Hashimoto's thyroiditis include a family history of thyroid disease of any type and exposure to environmental triggers. Environmental toxins generate free radicals, which activate immune responses and may sensitize immune cells to surrounding thyroid proteins.

Chemicals documented to correlate with Hashimoto's disease include the following:

- Iodine (in excess or when intake increases significantly)
- Perchlorate
- Fluoride
- Lithium
- Mercury
- Bisphenol A
- Teflon

Toxins

Many groups of chemicals have thyroid-disrupting potential. These chemicals have various mechanisms of action and, when combined, may have synergistic effects.[12] Many aspects of the many components of thyroid production and effects can be adversely affected, such as decreased TSH production, decreased T4 production, poor or inconsistent conversion of T4 to T3, reduced cellular response to T3, decreased absorption of iodine, and impaired mitochondrial response to T3 limiting adenosine triphosphate (ATP) production. In addition,

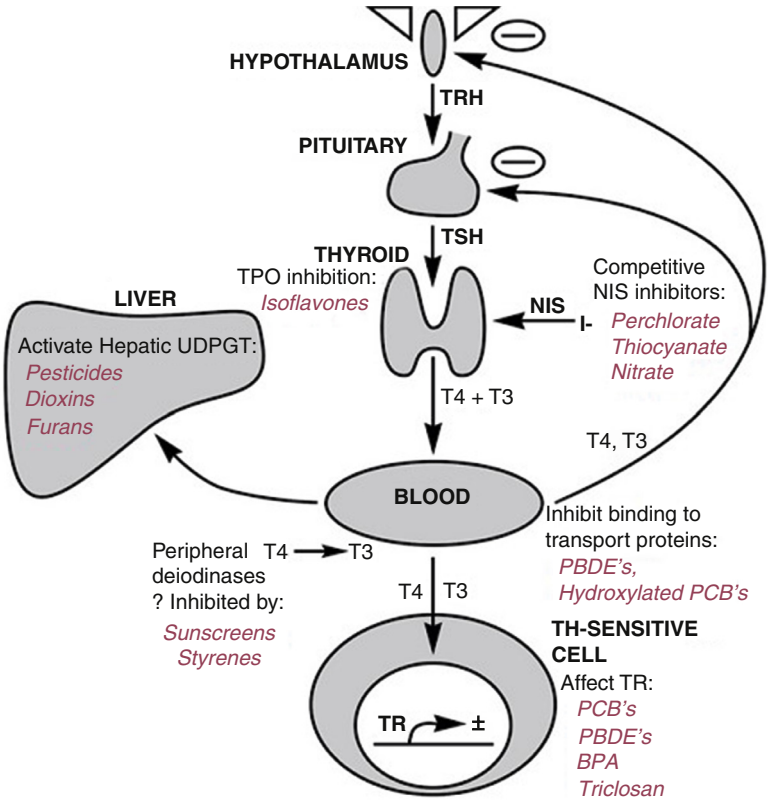


Fig. 182.1 Mechanisms of various thyroid disruptors. (From Pearce EN, Braverman LE. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab.* 2009 Dec;23[6]:801–813. Review. PubMed PMID: 19942155.)

the function of the sodium iodide symporter (NIS) or TPO can be inhibited or stimulated by chemicals, and transthyretin (TTR), a transport protein, may be a source for competitive binding of environmental chemicals. Fig. 182.1 demonstrates the mechanisms of action of several toxicants.[13]

PCBs, Dioxins, and Furans

Polychlorinated biphenyls (PCBs) and thyroxine both consist of two connected benzene rings with halogens attached. PCBs have chlorine attached, and thyroxine has iodine. PCBs inhibit the production, activation, and utilization of thyroid hormones. There is a dose-dependent decrease in total T4 among U.S. adults with exposure to dioxin-like toxic equivalents (TEQs) at levels similar to those found in the general U.S. population.[14] Several animal studies confirm that exposure to PCBs or dioxin results in reduction of serum thyroid hormone levels, especially T4.[15,16] Dioxins and furans adversely affect the thyroid hormone–metabolizing enzymes uridine-diphosphate-glucuronyl transferase, iodothyronine deiodinase, and sulfotransferases found in the liver and brain. Hydroxylated PCBs also inhibit iodothyronine sulfotransferase activity, adversely affecting peripheral metabolism of thyroid hormones. German children showed a significant positive correlation between PCB serum levels and elevation of TSH, as well as a significant inverse correlation between serum PCB levels and FT4.[17] U.S. Air Force personnel participating in operation Ranch Hand (the spraying of broadleaf defoliants) during the Vietnam War were found to have high serum tetrachlorodibenzo-p-dioxin (TCDD) levels that were significantly associated with elevated levels of TSH.[18] Thus PCBs reduce peripheral thyroid hormone levels, inducing a compensatory increase in TSH.

PCBs, dioxins, and furans have demonstrated in vitro activity in binding transthyretin, blocking the transport of thyroxine.[19] Hydroxylated PCBs bind to TTR with a stronger binding affinity than their parent compounds.

Elevated antithyroid antibodies, both antiperoxidase and antithyroglobulin, were found in workers in a PCB manufacturing plant.[20] However, no difference was found in the levels of T4 and TSH in these TCDD workers than in the control group. Swedish women who consumed at least two meals of fish from the Baltic Sea each month showed a significant inverse correlation between serum PCB levels and TT3 and an insignificant inverse correlation with TT4.[21]

DDT was found to be higher in a group of hypothyroid Indian women than in euthyroid female controls, whereas Dieldrin was significantly associated with low T4 levels.[22] Canadian newborns had significantly lower T4 concentrations in their cord blood when pentachlorophenols (PCPs) were also present.[23]

Organophosphate Pesticides

Individuals with acute organophosphate pesticide (OP) poisoning have revealed a host of hormonal alterations, including elevations of ACTH, cortisol, and prolactin. Interestingly 31.8% of these individuals also experienced sick euthyroid syndrome with low FT3, FT4, or TSH. OP pesticide formulators had significantly low TT3 and nonsignificantly elevated TSH levels,[24] and persons using backpack sprayers with dithiocarbamates (also acetylcholinesterase inhibitors) had increased TSH levels.[25]

Mercury

Mercury accumulates in the thyroid and reduces the uptake of iodine by binding to the sodium/iodide transporting molecule. Mercury also inhibits deiodinase function in the peripheral tissues, preventing the production of triiodothyronine (T3) from thyroxine (T4).

Methylmercury, particularly when combined with a selenium deficiency, inhibits D1, the predominant deiodinase in the liver, kidney, and intestines.

Both adolescents and adults in the 2007 to 2008 National Health and Nutrition Examination Survey (NHANES) had a negative association between blood mercury levels and total T3 and T4.[26] Adolescents also showed a higher risk for both antithyroglobulin and anti-TPO antibodies. Women in the same NHANES cohort with blood mercury of greater than 1.8 ug/L (approximately the 80th percentile) were more likely to have antithyroglobulin antibodies, than those with blood mercury of less than 0.4 ug/L.[27]

Perchlorate

Newborn TSH levels in cities with 100% perchlorate-contaminated drinking water were significantly higher than those in newborns in cities with noncontaminated water.[28] Perchlorate is known to competitively inhibit iodide uptake at the sodium iodide symporter and has been used in the past in the diagnosis and treatment of thyrotoxicosis.

Cadmium

Increasing serum cadmium levels is associated with elevating TSH and lowering FT4 levels. Cadmium has been shown to reduce TSH levels,[29] although this effect is completely blunted in animals given zinc and selenium.[30] Cadmium accumulates primarily in the kidneys and at lower levels in the lungs, and liver and thyroid hyperplasia and hypertrophy are often seen with chronic cadmium toxicity.

Polybrominated Flame Retardants

Brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), pentabromophenol (PBP), and tetrabromobisphenol A (TBBPA), have a structural resemblance to thyroxine (T4) and are potent competitors for T4 binding to TTR. PBDEs suppress TH-regulated gene expression in humans and interfere with TH signaling.[31] Several animal studies indicate that PBDEs decrease the levels of circulating thyroid hormones,[32–34] and perinatal maternal exposure reduces thyroid hormones pre- and postnatally.[35,36]

Phthalates

Phthalates have various mechanisms of action on thyroid homeostasis, including interfering with the activity of the NIS,[37] inhibiting T3 uptake in cells,[38] and competitively binding to TTR.[39] Significant mild negative correlations were found between TT4 and FT4 and urinary phthalate monoesters in pregnant women exposed to di-n-butyl-phthalate (DBP).[40] In adult men, an inverse association exists between urinary concentration of mono(2-ethylhexyl) phthalate (MEHP) and serum levels of FT4 and TT3.[41]

Other Causes

Other causes of clinical hypothyroidism include thyroid surgery, ablation, and postpartum hypothyroidism, which is a transient form of hypothyroidism, affecting 5% to 10% of women in the United States. Annual follow-up should be offered to any woman who develops postpartum hypothyroidism because 25% of these women will develop overt hypothyroidism within the next 5 years.[42]

SUBCLINICAL HYPOTHYROIDISM AND HYPOTHYROIDISM SYNDROME

Subclinical hypothyroidism is defined as elevated TSH levels with normal serum thyroid levels. Using this criterion, subclinical hypothyroidism is a relatively common finding in primary care, affecting 2% to 7% of adults. Subclinical hypothyroidism has been shown to be linked to an increased risk for cardiovascular disease.[43]

Thyroid disease in the absence of overt laboratory findings is more accurately known as hypothyroid syndrome and can be defined by the following clinical findings:

- The presence of hypothyroid symptoms
- The absence of other explanatory diseases
- Possible functional thyroid abnormalities, as found on basal body temperature (BBT) and Achilles reflex testing

In addition, one or more of the following objective findings will help confirm the presence of thyroid disease:

- Suboptimal blood levels
- Abnormal antibody studies
- Abnormal findings on ultrasound
- Abnormal findings on fine-needle aspiration of the thyroid

DIAGNOSTIC CONSIDERATIONS

Before the use of blood measurements, it was common to diagnose hypothyroidism using basal body temperature (BBT; i.e., the temperature of the body at rest) and Achilles reflex time (reflexes are slowed in hypothyroidism). With the advent of sophisticated laboratory measurement of thyroid hormones in the blood, these "functional" tests were no longer used Normal BBT is 97.6°F to 98.2°F. Many consider BBT to be an indicator of thyroid status, yet it is affected by many other variables, including adrenal function, body composition, activity level, menstrual status, and immune function. Therefore it has little specificity for thyroid function. Furthermore, reliance on BBT as a factor superseding blood levels in determining optimal thyroid dosing can expose patients to dangerous supraphysiologic doses of thyroid hormones. BBT can, however, be used as a general indicator within the context of a complete clinical evaluation. Instructions for taking BBT are outlined in Appendix 10.

Clinical Assessment

A clinical score based on the Colorado Thyroid Disease Prevalence Study has been created to simplify the process of symptom evaluation in patients with suspected hypothyroidism. In this study of more than 25,000 people, it was shown that many patients with significant symptoms did not have clinical hypothyroidism. The symptoms listed in Table 182.1 serve as the best predictors of hypothyroidism.

Laboratory Evaluation

When clinically indicated, thyroid screening ideally includes a marker of thyroid regulation (e.g., TSH), markers of thyroid output (e.g., free T4, free T3), and markers of thyroid inflammation (e.g., thyroid microsomal antibody, thyroid peroxidase antibody, antithyroglobulin antibody). The American Thyroid Association recommends TSH screening every 5 years beginning at age 35.

The diagnosis of hypothyroidism by laboratory methods is primarily based on the results of total T4, free T4, free T3, and TSH levels (Table 182.2). TSH is a more sensitive test for both hyper- and hypothyroidism than free T4. The diagnosis is straightforward in overt cases (Table 182.3). In subclinical cases, the diagnosis is clear, but the benefit of treatment in the absence of symptoms is debated. An elevation in TSH with a normal T4 level is considered subclinical. The accepted normal TSH range is extremely broad, 0.35 to 5.50 mIU/mL. Some physicians do not recommend treatment unless the TSH is greater than 10 mIU/mL. Given the importance of adequate thyroid hormone to human health, the recommendation presented here is more aggressive.

Patients with a combination of subclinical hypothyroidism and objective signs of autoimmune thyroiditis can benefit from TSH reduction. This can be achieved through diet and lifestyle changes and possible thyroid replacement therapy.

TABLE 182.1 Accuracy of 12 Symptoms and Signs in the Diagnosis of Primary Hypothyroidism

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Score IF Present
Symptoms					
Impairment of hearing	22	98	90	53	1
Diminished sweating	54	86	80	65	1
Constipation	48	85	76	62	1
Paraesthesia	52	83	75	63	1
Hoarseness	34	88	73	57	1
Weight increase	54	78	71	63	1
Dry skin	76	64	68	73	1
Physical Signs					
Slow movements	36	99	97	61	1
Periorbital puffiness	60	96	94	71	1
Delayed ankle reflex	77	94	92	80	1
Coarse skin	60	81	76	67	1
Cold skin	50	80	71	62	1
Sum of all symptoms and signs					12

Data from Zulewski H, Müller B, Exer P, et al. Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. *J Clin Endcrinol Metab.* 1997;82:771–776.

TABLE 182.2 Normal Value of Serum Thyroid Hormone

T4	4.8–13.2 mg/dL
Free T4	0.9–2 ng/dL
T3	80–220 ng/dL
Thyroid-stimulating hormone	0.35–5.50 mIU/mL

THERAPEUTIC CONSIDERATIONS

Fig. 182.2 provides an algorithm for managing patients with hypothyroidism; it combines clinical evaluation, BBT, and laboratory evaluation. Although the conventional diagnostic model may be restrictive, relying solely on symptoms or BBT to determine the need for thyroid treatment often leads to several attempts at proper dosing, side effects, and limited to no benefits. Furthermore, if such measures are used as the sole factors for determining dosing strategies, many will end up on dangerous supraphysiologic doses. Adult patients who have reached full physiological doses of thyroid and have TSH scores lower than 1.0 should be considered to have maximal improvement from thyroid treatment. Because supraphysiologic doses speed the rate of T4 degradation, further increases in dosage will not yield lasting benefit. Higher doses should also be discouraged because a chronically suppressed TSH raises the risk of dementia, atrial fibrillation, and osteoporosis.

Patients with "hypothyroid syndrome" may have a positive clinical response to thyroid treatment when they possess the following characteristics:

- Objective signs of early Hashimoto's disease. This would include positive serum antibodies or abnormalities on thyroid anatomy evidenced by examination or ultrasound.
- Pertinent hypothyroid symptoms. These include the highly sensitive symptoms gleaned from the Colorado study delineated earlier.
- Suboptimal thyroid blood levels. TSH scores greater than 2.5 increase the likelihood of Hashimoto's disease and hypothyroid symptoms.

Patients with less sensitive hypothyroid symptoms, such as overweight, depression, or poor libido, yet lacking the previously listed three criteria, may respond poorly to thyroid treatment even if they possess low BBTs or delayed Achilles tendon reflexes.

Diet/Iodine Status

Although the key role of iodine deficiency in hypothyroidism has been known for over a century, there are clearly problems with even modestly excessive levels. There is a correlation between iodine intake and autoimmune thyroid disease. In one Furans study, a group of 45 adults with hypothyroidism secondary to Hashimoto's thyroiditis were divided into two groups. One group was placed on a low-iodine diet of under 100 micrograms per day of iodine, whereas the other group did not modify their diets. Of those on the low-iodine diet, 78.3% returned to euthyroid status with no other treatment, as opposed to 45% in the control group.[44] The average TSH score changed from 14.28 IU/mL to 3.28 IU/mL in those who recovered.

Physiological/Hormonal Considerations

Thyroid-binding globulin (TBG) is a protein generated by the liver that transports thyroid hormones, including 75% of circulating T4. Certain factors may lead to increased TBG levels, including pregnancy, oral estrogen supplementation from birth control pills, and hormone replacement therapy. Tamoxifen therapy for breast cancer and liver diseases also increase TBG levels. High TBG levels can hinder the availability of free thyroid hormone for use in the peripheral tissues, thus contributing to hypothyroid symptoms. Depending on the clinical picture, it may be useful to moderate hormonal excesses by decreasing the intake of oral estrogens, changing to nonoral regimens that do not involve first-pass metabolism through the liver, or helping the liver efficiently metabolize estrogens.

Iodine and Tyrosine

Thyroid hormones are made from iodine and the amino acid tyrosine, which is found in a wide variety of animal- and plant-based protein foods.

TABLE 182.3 Laboratory Findings in Thyroid Disease

Disease	Primary Hypothyroidism	Secondary Hypothyroidism	Tertiary Hypothyroidism	Peripheral Hypothyroidism
THRH	High/normal	High	Low/normal	Normal/high
TSH	High	Normal/low	Low/normal	Normal/high
Free T4	Low	Low	Low	Normal
Free T3	Low/normal	Low/normal	Low/normal	Low
Reverse T3	Normal	Normal	Low/normal	Normal/high

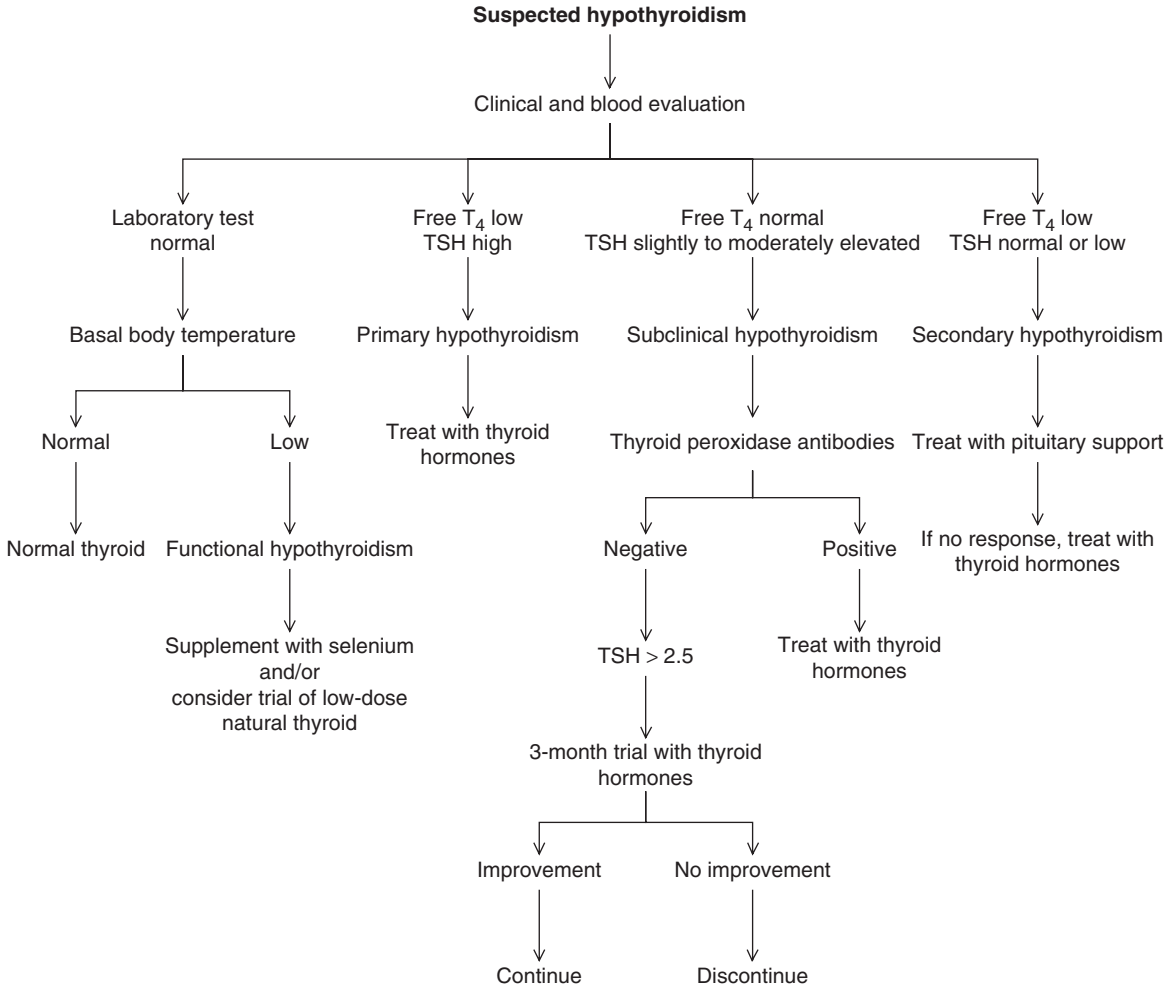


Fig. 182.2 Hypothyroidism diagnostic flowchart.

The recommended dietary allowance (RDA) for iodine in adults is 150 mcg. The average intake of iodine in the United States is estimated to be more than 600 mcg/day. However, intake has decreased substantially in the past several decades due to decreased use of iodized table salt (more people now eat prepared foods, which typically use salt that has no added iodine), dairies using antibiotics instead of iodine for cleaning udders, and bakers switching from iodine-based to bromine-based leavening agents. Too much iodine can inhibit thyroid gland synthesis. Because the only role of iodine in the body is to provide for thyroid hormone synthesis, it is recommended that dietary levels or supplementary iodine not exceed 600 mcg/day for any length of time. Individuals with an iodine intake under 300 mcg daily have the lowest rates of development of thyroid disease or thyroid disease progression.[45]

Vitamins and Minerals

Zinc, vitamin E, and vitamin A function together in the manufacturing of thyroid hormone. A deficiency of any of these nutrients results in lower levels of active thyroid hormone production. Low zinc levels, as well as hypothyroidism, are common in the elderly, indicating a possible correlation.[46] Riboflavin (B_2), niacin (B_3), pyridoxine (B_6), and vitamin C are also necessary for thyroid hormone manufacture.

The trace minerals zinc, copper, and selenium are required cofactors for iodothyronine deiodinase, the enzyme that converts T4 to the more active T3. This enzyme has several different forms, each requiring a different trace mineral. Supplementation with zinc has been shown to reestablish normal thyroid function in patients with hypothyroidism who were zinc-deficient even though they had normal serum T4 levels.

Individuals living in areas where selenium is deficient have a greater incidence of thyroid disease. Although a selenium deficiency does not decrease the conversion of T4 to T3 in the thyroid or the pituitary, it does result in a decrease in this conversion in other cells of the body.[47] People with a selenium deficiency have elevated levels of T4 and TSH, and supplementation with selenium results in a decrease in T4 and TSH and a normalization of thyroid activity.[48] Moreover, selenium is known to decrease thyroid antibody levels in autoimmune thyroid conditions.[49] Antioxidant supplementation has been shown to increase thyroid function in rats with methimazole-induced hypothyroidism. Statistically significant results were reported in rats given vitamin C, vitamin E, and *Curcuma longa* (turmeric) extract with decreased abnormal thyroid weight changes, less suppression of T4 and T3, and attenuated increases in cholesterol levels.[50] Furthermore, in one animal study, thyroid hormone abnormalities also correlated with decreased glutathione antioxidant status in testicular tissues.[51] Although there are no human studies to corroborate these findings, given the safety and beneficial profile of these supplements, it seems a reasonable approach to consider them in an anti-inflammatory and antioxidant treatment regimen.

Exercise

Exercise stimulates thyroid gland secretion and increases tissue sensitivity to thyroid hormone. Many of the health benefits of exercise may be a result of improved thyroid function. A consistent effect of dieting is a decrease in the metabolic rate as the body strives to conserve fuel. Exercise has been shown to prevent the decline in metabolic rate in response to dieting.

Thyroid Hormone Replacement

If nutritional and lifestyle interventions are inadequate to reestablish normal thyroid activity, the prescription of thyroid hormones is necessary. Several thyroid hormone preparations are available, each with advantages and disadvantages. Conventional treatment involves the administration of oral tetraiodothyronine/levothyroxine sodium (T4). Levothyroxine preparations are bioidentical to human tetraiodothyronine and readily absorbed orally. The half-life of tetraiodothyronine is roughly 7 to 10 days. Therefore once-daily dosing is adequate for steady blood levels. For maximum and consistent absorption, it is best to take thyroid hormones at least 1 hour before food and beverages other than water. Morning administration is preferred to mimic the circadian thyroid cycle.

Dosages are titrated to reduce TSH and to elevate T4 to normal ranges. The most common starting dose is 50 mcg (0.05 mg), and the most common ending doses range from 88 to 137 mcg for women and 100 to 150 mcg for men.

Some patients who remain clinically hypothyroid may poorly deiodinate levothyroxine (T4) into triiodothyronine (T3). In these cases, oral T3 is given. Like T4, T3 (Cytomel or liothyronine) is well absorbed orally and is easily bound by many nutrients. The half-life of T3 is approximately 2½ days, so once-daily dosing is recommended. Common doses are 5 to 10 mcg daily.

Many naturopathic physicians prefer the use of natural desiccated thyroid (NDT). NDT contains all thyroid hormones, not just thyroxine. Studies in rats whose thyroids were removed demonstrated that normal tissue levels of T4 and T3 were achieved only with an infusion of T4 and T3, and not by T4 alone.[52] The advantage of NDT is that it provides T4, T3, T2, and relevant amino acids and micronutrients (including 0.2% iodine per table). The main drawback often cited by those who prefer thyroxine is that NDT lacks consistency. However, according to the U.S. Pharmacopoeia (USP), these preparations must contain not less than 85% and not more than 115% of the labeled amount of T4 and not less than 90% and not more than 110% of T3. The labeled amount is 38 mcg of T4 and 9 mg T3 per grain (65 mcg). The equivalencies of the thyroid agents based on clinical response are as follows:

- 100 mcg of T4 (e.g., Synthroid)
- 20 to 25 mcg of T3 (e.g., Cytomel)
- 1 grain of desiccated thyroid
- 12.5 mcg of T3 + 50 mcg of T4 (e.g., Thyrolar)

THERAPEUTIC APPROACH

Natural treatment strategies for normalizing thyroid function vary depending on the diagnosis of an autoimmune hypothyroid condition, Wilson's syndrome, or subclinical or clinical hypothyroidism.

Treatments for Autoimmune Hypothyroidism (Hashimoto's Disease)

Thyroid Replacement

The use of thyroid replacement achieves two objectives in the patient with autoimmune hypothyroidism: the normalization of decreased thyroid hormone levels and the suppression of thyroid activity, thus decreasing autoimmune processes. Either desiccated or synthetic thyroid replacement may be used in sufficient doses to decrease TSH to 0.1 or 1.5, the levels found in healthy euthyroid populations. It should be noted that some reports say that TSH values equal to or less than 0.1 mIU/mL may carry a risk of numerous hyperthyroid side effects as well as atrial fibrillation. Desiccated thyroid may be beneficial in stimulating blocking antibodies to antithyroid antibodies and by acting as decoys for thyroid antibodies. Some patients recover from Hashimoto's thyroiditis after an extended treatment time with thyroid hormone and no longer need maintenance replacement. Some patients may not benefit from desiccated thyroid, leading to an adverse reaction to antigenic thyroid substances. These patients should promptly be placed on a combination of synthetic T4 and T3 thyroid instead. In either case, frequent TSH and antibody tests are essential to verify the suppression of thyroid activity and monitor autoimmune activity. Often in early Hashimoto's disease, a hyperthyroid picture is more prevalent. In this case thyroid replacement may be contraindicated.

Diet/Lifestyle

Food elimination, detoxification, and gut-healing options may be useful treatments aimed at ameliorating a possible root factor of antigenic autoimmune activity (see Chapter 14).

Other Recommendations

Dehydroepiandrosterone (DHEA) is a beneficial supplement in the treatment of several autoimmune conditions.[53] Although many clinical studies have employed high dosages (100–200 mg/day), much lower physiological doses ranging from 5 to 10 mg/day in women and 10 to 20 mg/day in men may be beneficial and safe. Laboratory work should be done to confirm physiological ranges with DHEA supplementation. DHEA should be used with caution, particularly in patients at risk for developing hormone-dependent cancers.[54]

Two hundred mg/day of selenium is recommended for Hashimoto's autoimmune thyroiditis, especially in patients who have high titers of antithyroid peroxidase (TPO) antibodies.

Treatments for Nonautoimmune Overt or Subclinical Hypothyroid Disorder

Diet

The diet should be sufficient in vitamins and trace minerals needed for thyroid hormone production and activation, including selenium, zinc, B vitamins, and vitamin D. Sources of zinc include seafood (especially oysters), beef, oatmeal, chicken, liver, spinach, nuts, and seeds. Copper

is found in liver and other organ meats, eggs, yeast, beans, nuts, and seeds. Sources of B vitamins include yeast, whole grains, and liver. A great source of selenium is unshelled Brazil nuts. Organically grown foods are recommended because of their higher levels of trace minerals and reduced levels of toxins.

Supplements

- Zinc: 20 to 30 mg/day
- Copper: 1 to 2.5 mg/day
- Selenium: 200 to 400 mcg/day
- Vitamin C: 1 to 3 g/day in divided doses
- Vitamin E (mixed tocopherols): 200 to 400 IU/day

Thyroid Hormones

Thyroid hormone replacement options include the following:

- Desiccated natural thyroid: ¼ grain initially; can be adjusted incrementally up or down as needed
- T4 (Levothyroxine): 25 to 200 mcg
- T4 and T3 Use 10:1 T4/T3 Ratio for isolated T4 and T3 medications such as Synthroid and Cytomel.

Compounded thyroid medication is not recommended due to variabilities in production.

Brand consistency is recommended for thyroid medication. Do not prescribe generic medications. Both of these practices can lead to fluctuating thyroid levels due to variables in inactive ingredients from one product to the next.

Reevaluation of TSH T4, T3, free T3, and free T4 levels 4 to 6 weeks after initiating therapy is recommended. The goal of treatment is to normalize serum hormone levels and improve patient symptoms. After stabilization of dosage, periodic evaluations are based on the individual patient's needs. Follow-up is recommended at least once a year.

Thyroid hormone replacement therapy is to be taken on an empty stomach to increase absorption. The patient should avoid taking thyroid hormones at the same time as other medications and supplements (especially those containing iron), which may affect absorption. Once-a-day dosage generally produces stable increases in thyroid hormone levels.

Thyroid hormone dosage during pregnancy will likely have to be adjusted because estrogens increase serum thyroid hormone–binding globulin, thereby reducing the level of free thyroxine. Careful monitoring is required during pregnancy. Dose requirements may increase by 30% to 50% during pregnancy and return to prepregnancy levels shortly after delivery.

Detoxification

All patients with hypothyroidism without apparent cause should have their toxic load measured and treated.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wang C, Crapo LM. The epidemiology of thyroid disease and implications for screening. *Endocrinol Metab Clin North Am*. 1997;26:189–218.
2. Weetman AP. Hypothyroidism: screening and subclinical disease. *BMJ*. 1997;314:1175–1178.
3. Banovac K, Zakarija M, McKenzie JM. Experience with routine thyroid function testing: abnormal results in 'normal' populations. *J Fla Med Assoc*. 1985;72:835–839.
4. Arem R, Escalante D. Subclinical hypothyroidism: epidemiology, diagnosis, and significance. *Adv Intern Med*. 1996;41:213–250.
5. Canaris GJ, Manowitz NR, Mayor G, et al. The Colorado thyroid disease prevalence study. *Arch Intern Med*. 2000;160:526–534.
6. Aoki Y, Belin RM, Clickner R, et al. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999-2002). *Thyroid*. 2007;17(12):1211–1223.
7. Esposito S, Prange Jr AJ, Golden RN. The thyroid axis and mood disorders: overview and future prospects. *Psychopharmacol Bull*. 1997;33:205–217.
8. Dean JW, Fowler PB. Exaggerated responsiveness to thyrotrophin releasing hormone: a risk factor in women with artery disease. *Br Med J (Clin Res Ed)*. 1985;290:1555–1561.
9. Staub JJ, Althaus BU, Engler H, et al. Spectrum of subclinical and overt hypothyroidism: effect on thyrotropin, prolactin, and thyroid reserve, and metabolic impact on peripheral target tissues. *Am J Med*. 1992;92(6):631–642. PubMed PMID: 1605145.
10. Wakim AN, Polizotto SL, Buffo MJ, Marrero MA, Burholt DR. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cells. *Fertil Steril*. 1993;59(6):1187–1190. PubMed PMID: 8495763.
11. Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. *J Clin Endocrinol Metab*. 2003;88:2438–2444.
12. Mastorakos G, Karoutsou EI, Mizamtsidi M, et al. The menace of endocrine disruptors on thyroid hormone physiology and their impact on intrauterine development. *Endocrine*. 2007;31(3):219–237.
13. Pearce EN, Braverman LE. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab*. 2009;23(6):801–813. Review. PubMed PMID: 19942155.
14. Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and DDE in adults. *Environ Health Perspect*. 2007;115(8):1197–1203.
15. Hallgren S, Darnerud PO. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats-testing interactions and mechanisms for thyroid hormone effects. *Toxicol*. 2002;177(2–3):227–243. PubMed PMID: 12135626.
16. Martin L, Klaassen CD. Differential effects of polychlorinated biphenyl cogeners on serum thyroid hormone levels in rats. *Toxicol Sci*. 2010;117(1):36–44. PubMed PMID: 20573785.
17. Osius N, Karmaus W, Kruse H, Witten J. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ Health Perspect*. 1999;107:843–849. PubMed PMID: 10504153.
18. Pavuk M, Schecter AJ, Akhtar FZ, Michalek JE. Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) levels and thyroid function in Air Force veterans of the Vietnam war. *Ann Epidemiol*. 2003;13(5):335–343. PubMed PMID: 12821272.
19. Lans MC, Spiertz C, Brouwer A, Koeman JH. Different competition of thyroxine binding to transthyretin and thyroxine binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *Europ J Pharm Envir Toxicol Pharmacol Sect*. 1994;270:129–136. PubMed PMID: 8039542.
20. Langer P, Tajtakova M, Fodor G. Increased thyroid volume and prevalence of thyroid disorders in a area heavily polluted by polychlorinated biphenyls. *Europ J Endocrin*. 1998;139:402–409. PubMed PMID: 9820616.
21. Hagmar L, Rylander L, Dyremark E, Klasson-Wehler E, Erfurth EM. Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. *Int Arch Occup Environ Health*. 2001;74:184–188. PubMed PMID: 11355292.
22. Rathore M, Bhatnagar P, Mathur D, Saxena FN. Burden of organochlorine pesticides in blood and its effect on thyroid hormones in women. *Sci Total Environ*. 2002;295(1–3):207–215. PubMed PMID: 12186288.
23. Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Pentachlorophenol and hyroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. *Environ Health Perspect*. 2002;110:411–417. PubMed PMID: 11940460.
24. Zaidi SS, Bhatnagar VK, Gandhi SJ, Shah MP, Kulkarni PK, Saiyed HN. Assessment of thyroid function in pesticide formulators. *Hum Exp Toxicol*. 2000;19(9):497–501. PubMed PMID: 11204551.
25. Steenland K, Cedillo L, Tucker J, et al. Thyroid hormones and cytogenetic outcomes in backpack sprayers using ethylenebis(dithiocarbamate) (EBDC) fungicides in Mexico. *Environ Health Perspect*. 1997;105(10):1126–1130. PubMed PMID: 9349837.
26. Chen A, Kim SS, Chung E, Dietrich KN. Thyroid hormones in relation to lead, mercury, and cadmium exposure in the National Health and Nutrition Examination Survey, 2007-2008. *Environ Health Perspect*. 2013;121(2):181–186. PubMed PMID: 23164649.
27. Gallagher CM, Meliker JR. Mercury and thyroid autoantibodies in U.S. women, NHANES 2007-2008. *Environ Int*. 2012;40:39–43. PubMed PMID: 22280926.
28. Brechner RJ, Parkhurst GD, Humble WO, Brown MB, Herman WH. Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona. *J Occup Environ Med*. 2000;42:777–782. PubMed PMID: 10953814.
29. Iijima K, Otake T, Yoshinaga J, et al. Cadmium, lead, and selenium in cord blood and thyroid hormone status of newborns. *Biol Trace Elem Res*. 2007;119(1):10–18.
30. Hammouda F, Messaoudi I, El Hani J, et al. Reversal of cadmium-induced thyroid dysfunction by selenium, zinc, or their combination in rat. *Biol Trace Elem Res*. 2008. [Epub ahead of print].
31. Zheng J, He CT, Chen SJ, Yan X, et al. Disruption of thyroid hormone (TH) levels and TH-regulated gene expression be polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and hydroxylated PCBs in e-waste recycling workers. *Envrion Int*. 2017;102:138–144. PubMed PMID: 28245931.
32. Fowles JR, Fairbrother A, Beacher-Steppan L, Kerkvliet NI. Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology*. 1994;86(1–2):49–61. PubMed PMID: 8134923.
33. Zhou T, Ross DG, DeVito MJ, Crofton KM. Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol Sci*. 2001;61(1):76–82. PubMed PMID: 11294977.
34. Stoker TE, Laws SC, Crofton KM, et al. Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicol Sci*. 2004;78(1):144–155. PubMed PMID: 14999130.
35. Kim TH, Lee YJ, Lee E, et al. Effects of gestational exposure to decarbromodiphenyl ether on reproductive parameters, thyroid hormone levels, and neuronal development in Sprague-Dawley rats offspring. *J Toxicol Environ Health A*. 2009;72(21–22):1296–1303. PubMed PMID: 20077200.
36. Kodavanti PR, Coburn CG, Moser VC, et al. Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. *Toxicol Sci*. 2010;116(1):297–312. PubMed PMID: 20375078.
37. Breous E, Wenzel A, Loos U. The promotor of the human sodium/iodide symporter responds to certain phthalate plasticisers. *Mol Cell Endocrinol*. 2005;244(1–2):75–78. PubMed PMID: 16257484.
38. Shimada N, Yamauchi K. Characteristics of 3,5,3'-triiodothyronine (T3)-uptake system of tadpole red blood cells: effect of endocrine-disrupting chemicals on cellular T3 response. *J Endocrinol*. 2004;183(3):627–637. PubMed PMID: 15590988.
39. Ishihara A, Nishiyama N, Sugiyama S, Yamauchi K. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail thransthyretin and thyroid hormone receptor. *Gen Comp Endocrinol*. 2003;134(1):36–43. PubMed PMID: 13129501.
40. Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Hum Reprod*. 2007;22(10):2715–2722. PubMed PMID: 17704099.

41. Meeker JD, Calafat AM, Hauser R. Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environ Health Perspect*. 2007;115(7):1029–1034. PubMed PMID: 17637918.
42. Othman S, Phillips DI, Parkes AB, et al. A long-term follow-up of postpartum thyroiditis. *Clinical Endocrinology (Oxf)*. 1990;32(5):559–564. PubMed PMID: 2364561.
43. Razvi S, Weaver JU, Vanderpump MP, et al. The incidence of ischemic heart disease and mortality in people with subclinical hypothyroidism: reanalysis of the Whickham Survey cohort. *J Clin Endocrinol Metab*. 2010;95(4):1734–1740.
44. Yoon SJ, Choi SR, Kim DM, et al. The effect of iodine restriction on thyroid function in patients with hypothyroidism due to Hashimoto's thyroiditis. *Yonsei Med J*. 2003;44(2):227–235.
45. Preedy, et al. *Comprehensive Handbook of Iodine: Nutritional, Biochemical, Pathological and Therapeutic Aspects*. 1st ed. Elsevier; 2009.
46. Prasad A. Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects: an update. *Nutr Rev*. 1983;41:197–208.
47. Meinhold H, Campos-Barros A, Behne D, et al. Effects of selenium and iodine deficiency on iodothyronine deiodinases in brain, thyroid and peripheral tissue. *Acta Med Austriaca*. 1992;19:8–12.
48. Berry MJ, Larsen PR. The role of selenium in thyroid hormone action. *Endocr Rev*. 1992;13:207–219.
49. Gärtner R, Gasnier BCH, Dietrich JW, et al. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrinol Metab*. 2002;87:1687–1691.
50. Deshpande UR, Joseph LJ, Patwardhan UN, et al. Effect of antioxidants (vitamin C, E and turmeric extract) on methimazole induced hypothyroidism in rats. *Indian J Exp Biol*. 2002;40:735–738.
51. Choudhury S, Chainy GB, Mishro MM. Experimentally induced hypo- and hyper-thyroidism influence on the antioxidant defense system in adult rat testis. *Andrologia*. 2003;35:131–140.
52. Escobar-Morreale HF, Obregón MJ, Escobar del Rey FE, et al. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest*. 1995;96:2828–2838.
53. van Vollenhoven RF. Dehydroepiandrosterone for the treatment of systemic lupus erythematosus. *Expert Opin Pharmacother*. 2002;3:23–31.
54. Monograph DHEA. *Altern Med Rev*. 2001;6:314–318.

Infectious Diarrhea

Robert Kachko, ND, LAc, and *Peter B. Bongiorno, ND, MSAc, LAc*

OUTLINE

DIAGNOSTIC SUMMARY

- Increased bowel movement frequency (usually more than three bowel movements per day)
- Increased stool liquidity
- Abdominal pain
- A sense of fecal urgency
- Fecal incontinence
- Perianal discomfort
- Blood, mucus, or both possibly present in the stool

GENERAL CONSIDERATIONS

Diarrhea is not only an important clinical entity but has also played a central role in the course of human history. Since the ancient times of Herodotus, the "father of history," diarrhea has had a particularly significant effect on the course of military strategies and consequently on world events. Infectious diarrhea has modified the wartime outcomes of the Roman Empire and the Crusades as well as the relatively modern-day military campaigns of Henry V, the Civil War, World War I, and World War II.[1] Walt Whitman, who visited the sick and wounded Union soldiers in Washington, D.C., hospitals, wrote that war consisted of "about 999 parts diarrhea to 1 part glory."[1]

Although there is considerable variation in normal bowel habits, diarrhea is defined as the passage of loose or watery stools, typically at least three times in a 24-hour period.[2,94] Acute diarrhea occurs for 14 days or fewer, persistent diarrhea for more than 14 but fewer than 30 days, and chronic diarrhea is longer than 30 days' duration. Diarrheal disease can be classified into three major clinical syndromes: acute watery diarrhea, bloody diarrhea, and persistent diarrhea. For most patients, any increase in stool mass, stool frequency, or stool fluidity is perceived as diarrhea. For most adults, this consists of a daily stool production in excess of 250 g containing 70% to 95% water. More than 14 L of fluid may be lost daily in severe cases.[3] Low-volume, painful, bloody diarrhea is known as dysentery (or invasive diarrhea).

Even today diarrheal illness is a common cause of morbidity and mortality. Infectious agents cause more than 200 million cases of diarrhea in the United States yearly.[4] Although it is a major cause of mortality in developing nations, infectious diarrhea also has significant costs in developed nations in terms of hospitalization and lost time.[5] Intestinal infection is the most common cause of diarrhea worldwide and is responsible for the deaths of 3 to 4 million individuals annually, the majority of whom are preschool-age children.[6] According to the World Health Organization, nearly 2 million children die each year because of diarrheal illness.[1] Despite these alarming statistics, approximately 90% of cases of acute diarrhea are mild and self-limited and respond within 5 days to simple rehydration therapy or antidiarrheal agents.[7] The prognosis in research-rich settings is significantly better than in resource-poor settings.

INFECTIOUS AGENTS AND SYMPTOMS

An increasingly wide variety of viral and bacterial agents are being recognized as causes of serious diarrheal illness.[4] Overall, the causes of infectious diarrhea can be categorized as viral, bacterial, or parasitic. In general, these pathogens create a diarrheal effect by inhibiting intestinal absorption and increasing secretion; in causing inflammation, they promote secretory and exudative diarrheal symptoms. Most cases of acute diarrhea are likely viral; stool cultures are positive in only 1.5%

to 5.6% of samples in most studies. In severe cases, however, bacterial etiology is more common: in a study of 173 community-dwelling healthy adults with severe diarrhea, a bacterial etiology was discovered in 87% of cases.[8]

Viral Agents

Viruses are important causes of diarrhea. In healthy adults, the clinical manifestation of viral diarrhea is generally an acute, self-limited gastroenteritis. Although at least 25 different bacteria and protozoa can cause an identical clinical syndrome, greater than 75% of diarrhea-associated cases of gastroenteritis are caused by viruses. Viruses are suspected when vomiting is prominent, the incubation period is longer than 14 hours, and the entire illness is over in less than 4 days. Viral pathogens are likely when there are no warning signs of bacterial infection (e.g., high fever, bloody diarrhea, severe abdominal pain, more than 6 stools in 24 hours) and there are no epidemiological clues from the history (i.e., travel, sexual contact, antibiotic use) suggesting an alternative diagnosis.[9]

Rotavirus

Rotavirus is one of the most ubiquitous agents of diarrhea. It can contribute to an acute, dehydrating diarrhea in children and is estimated to cause more than 800,000 annual deaths of young children in developing countries.[10] Rotavirus-associated diarrheal disease is seasonal; in the United States it begins in the autumn in the Southwest and ends in the spring in the Northeast. Rotaviruses are spread via a fecal–oral route that includes person-to-person transmission. Although the precise mechanisms of disease have yet to be fully elucidated, rotaviruses are known to infect the villous enterocytes of the small intestine and cause a watery diarrhea. In addition to diarrhea, patients often have fever and vomiting.[11]

Parvovirus (Norwalk Virus)

Often categorized within the family of the Norwalk virus are the caliciviruses, also referred to as Norwalk-like viruses or small, round-structured viruses. The Centers for Disease Control and Prevention (CDC) estimates that 66% of 13.8 million cases of food-related illnesses are caused by these viruses each year in the United States. This family of viruses is often suspected when acute gastroenteritis sweeps through a semiclosed community (e.g., family, school, residential home, hospital, ship, dormitory).[9] These viruses are transmitted through contaminated food and water or from person to person. Sources of outbreaks have included well water, raspberries, lunch meat, and, in several instances, oysters.[11] Individuals infected by caliciviruses experience nausea/vomiting, diarrhea, abdominal cramping, and headache; low-grade fever, malaise, and myalgias also occur.[11]

Cytomegalovirus

Cytomegalovirus (CMV) is present in a latent state in most people, having been acquired either at birth or through sexual or parenteral exposure. CMV comes out of latency when there is strong allergenic or antigenic stimulation of the immune system. Often those with severe immunodeficiency also have activation and replication of CMV. When CMV becomes active in the bowel wall, gastrointestinal disease may occur. The disease is seen primarily in patients with severe immune deficiency such as acquired immunodeficiency syndrome (AIDS), transplantation, and cancer chemotherapy[9] and is a major cause of diarrhea in these conditions.

Epstein–Barr Virus

By the age of 20 years, almost all adults are infected with Epstein–Barr virus (EBV). This virus infects B cells and then persists for life because these cells proliferate indefinitely. Proliferation is usually controlled by virus-specific cytotoxic T lymphocytes. EBV causes diarrhea mainly in immunocompromised patients.

Bacterial Agents

Escherichia coli

At least four distinct varieties of diarrheogenic *Escherichia coli* exist: enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enteroadherent. In North America, the most prominent diarrheogenic *E. coli* is the EHEC serotype, *E. coli* O157:H7.[11] Contaminated ground beef is the most common vehicle of transmission because contaminating bacteria are distributed throughout the meat during grinding and are not killed if hamburgers are undercooked. After an incubation period from 1 to 8 days, typical symptoms include abdominal cramping and diarrhea that can present as mild, moderate, or severe. Early in the disease course, bowel movements may be loose and watery; in time, however, they may contain gross blood. A subset of patients, often children, may develop the severe, life-threatening hemolytic-uremic syndrome, characterized by the clinical triad of hemolytic anemia, renal failure, and thrombocytopenia.[11]

Campylobacter jejuni

Campylobacter is part of the intestinal flora in many mammals. Contaminated and improperly cooked meat contributes significantly to the spread of disease. Unpasteurized dairy products, contaminated water, and other foods also may encourage transmission. Symptoms begin 1 to 7 days after ingestion of the bacteria and consist of fever, headache, and malaise. These may herald the onset of diarrhea and abdominal cramps by 1 to 2 days. The nature of the diarrhea varies from watery to bloody. *Campylobacter* enterocolitis is usually acute and self-limited over 7 to 10 days. Rarely, relapses, complications, severe disease, and death may occur.[11]

Clostridium difficile

C. difficile infection has become the most common cause of infectious diarrhea in hospitalized patients[12] and is most likely to affect the elderly. *Clostridium* can be catalyzed by the use of antibiotics.

Salmonella

The spread of resistant *Salmonella* strains continues to increase worldwide,[13] and there are more than 2200 currently known serotypes. Spread chiefly through contaminated food, *Salmonella enteritidis* accounts for 85% of all salmonella infections in the United States. Other prominent salmonella infections include *Salmonella typhi* and *Salmonella paratyphi,* which are responsible for typhoid fever. Nontyphoidal salmonellosis may present as fever, gastroenteritis-related diarrhea, and localized infections of the gastrointestinal tract, endothelial surfaces, pericardium, meninges, lungs, joints, bones, urinary tract, or soft tissues. Typhoidal symptoms include a gradual onset of fever, headache, arthralgias, pharyngitis, constipation, anorexia, and abdominal discomfort.

Shigella

Among the bacterial enteric pathogens, *Shigella* is unique in that ingestion of fewer than 200 organisms and possibly as few as 10 organisms may cause clinically apparent disease. *Shigella,* like other enteric pathogens, may be transmitted through contaminated food and water. However, person-to-person spread and transmission by flies may also occur because so few organisms are necessary to cause disease.[11] The incubation period is usually 1 to 4 days. Adults present with nonbloody diarrhea with or without fever and possible gripping abdominal pain, urgency, and relief with defecation. As the infection progresses,

episodes may increase, with the presence of mucus and blood in the stool. Infections often resolve spontaneously in 4 to 8 days for mild cases or 3 to 6 weeks in severe cases.

Yersinia enterocolitica

Yersinia enterocolitica, like *Salmonella* and *Shigella,* is a member of the *Enterobacteriaceae* family. *Yersinia* species are significantly less frequent causes of bacterial enterocolitis in North America than elsewhere. Pathogenic strains of *Y. enterocolitica* are usually transmitted through fecally contaminated food or water but infrequently are transmitted by contaminated blood products. A 4- to 7-day incubation period occurs, followed by watery to bloody diarrhea. This organism is invasive and demonstrates a propensity for lymphoid tissues, causing mesenteric lymphadenitis that may be clinically mistaken for acute appendicitis.[11] Disease usually resolves within 1 to 4 weeks, but occasionally complicating septicemia may develop, usually in patients with underlying disease.

Laribacter hong-kongensis[14]

Described and characterized in 2001, *Laribacter hong-kongensis* is a novel genus and species originally isolated from the blood and empyema pus cultures of a cirrhotic patient with bacteremic empyema thoracis. Since then, it has been described in six patients with diarrhea in Hong Kong and Switzerland. Although more extensive epidemiological studies are necessary, there is reason to consider its role in future studies of diarrheal outbreaks worldwide.

Parasitic Agents

Diarrheal diseases caused by parasites still constitute the greatest single worldwide cause of illness and death. The problem is more severe in underdeveloped countries with poor sanitation, but even in the United States, diarrheal diseases are a major cause of sickness and death. Furthermore, the ease and frequency of worldwide travel and increased migration to the United States are resulting in growing numbers of parasitic infections. In addition to normal inhabitants of the gastrointestinal system acting as parasites, there are also significant diarrheal diseases associated with protozoa and helminths.

Giardia lamblia is the most frequent cause of parasitic enteritis in the United States and should be suspected in cases where history suggests recent hiking and drinking out of streams. Waterborne infection is also important in the developed world, particularly as a result of contamination of domestic water supplies with the cysts of *Giardia intestinalis* and *Cryptosporidium parvum.*[6] Other diarrhea-associated parasites include *Entamoeba histolytica, Microsporidium, Isospora belli,* and *Strongyloides* species.

Although the most commonly reported symptoms of parasitic infection are abdominal pain and cramping with concomitant explosive diarrhea, these symptoms do not occur in all cases. In fact, a growing number of individuals are experiencing milder-than-usual gastrointestinal symptoms due to parasitic infections or symptoms not traditionally considered to be linked to parasitic infections. For example, in some cases of irritable bowel syndrome, indigestion, and poor digestion, parasites may be causing the symptoms. In addition, parasitic infections are often an unsuspected cause of chronic illness and fatigue.

DIAGNOSTIC CONSIDERATIONS

Patients presenting with bloody diarrhea, persistent fever, severe and unremitting abdominal pain, and/or symptoms of severe dehydration warrant in-office evaluation. Immune-compromised/immune-suppressed patients, those with the potential for cardiovascular compromise, or those with a history of inflammatory bowel disease may warrant hospitalization. The initial assessment should include careful history (including travel history and other sources of recent exposure from food or occupational hazard) and physical examination to assess for volume depletion. Diarrhea of small bowel origin is more typically of large volume, watery, and associated with abdominal bloating, cramping, and gas.[15] Fever and bloody stool are rare. In contrast, diarrhea of large bowel origin is typically lower volume and more frequent but is more likely to present with painful bowel movements, fever, and blood/mucus. Visibly bloody stool is most often caused by *E. coli* O157:H7, *Shigella, Salmonella,* or *Campylobacter.*[16] The timing of recent food intake may provide some nonspecific clues to etiology; early symptoms (<6 hours postingestion) suggest the presence of preformed toxin (likely from *Staphylococcus aureus* or *Bacillus cereus*). An onset of greater than 16 hours suggests viral or bacterial infection. An onset between 8 and 16 hours may indicate *Clostridium perfringens* infection. Recent antibiotic use or hospitalization greater than 72 hours should be considered (particularly if within the prior 3 months), especially to rule out a high likelihood of *C. difficile.*

The physical examination to assess for signs of dehydration or more serious illness should include an assessment of skin turgor, dryness of mucous membranes, hypotension, and altered mental state. An abdominal examination may show signs of generalized tenderness and pain with percussion, as well as general distension.

Laboratory Diagnosis

Numerous laboratory procedures exist for the assessment of patients with diarrhea. Stool diagnostic studies may be used if available in cases of dysentery, moderate to severe disease, and symptoms lasting more than 7 days. Other potentially concerning components of the history that may warrant stool testing include more than 6 loose stools within 24 hours, severe abdominal pain, or signs of frank blood in the stool. When visible blood was present in the stool, more than one third of cases were caused by Shiga toxin–producing *E. coli* (STEC) O157:H7 (making this the most common STEC in the United States).[4] Consideration in bloody diarrhea should also be made for the presence of amebiasis, especially in recent travelers.

Bacterial cultures using selective media, detection of pathogen-specific genes using polymerase chain reaction (PCR), electron microscopic examination and antigen detection for viruses, and direct examination with or without the use of special stains for protozoa are some of the plethora of diagnostic tests available. Despite this wide array, a microbiological cause is identified in only 50% of patients at best.[14] One hospital survey of more than 30,000 stool specimens sent for testing revealed a specific pathogen in only 5.6% of cases. In descending order, the most commonly identified pathogens were *Campylobacter jejuni, Salmonella, Shigella,* and *E. coli* O157:H7.[4]

Confounding this issue, it has been shown that substantial variation exists in the use of stool testing, which typically does not correlate with the clinical characteristics of affected patients.[4,17] One study has identified several important independent variables that may be predictive of positive stool culture in adult patients with a clinical picture of infectious diarrhea. These variables are month of presentation, fever, duration of abdominal pain at presentation, and requirement of intravenous fluid therapy. Surprisingly, this study demonstrated that neither a history of bloody diarrhea nor persistent diarrhea was associated with positive stool culture.[17]

Testing for ova and parasites is of low yield in most cases and should be run only when diarrheal illness persists for more than 7 days.[4] In the case of persistent diarrhea, parasites most likely to be discovered include *Giardia, Cryptosporidium,* and *E. histolytica,* and assessment should include ova and parasite inspection of three specimens separated by at least 24 hours.

THERAPEUTIC CONSIDERATIONS

Conventional Medicines

Although appropriate in certain cases, the antibiotics and opiates used to treat diarrhea are also well known to have their limitations because many of these drugs adversely affect gastrointestinal motility; furthermore, increasing numbers of pathogens are proving resistant to antimicrobial agents. Increasing numbers of isolates resistant to antimicrobial agents and the risk of worsened illness (e.g., hemolytic uremic syndrome with STEC O157:H7) further complicate antimicrobial and antimotility drug use.[4] Additionally, available antiperistaltic or antisecretory drugs to reduce the severity of diarrhea can cause serious side effects in children.[10]

Generally, selective antibiotic treatment is recommended for traveler's diarrhea, *Shigella,* and *Campylobacteria* infection. Bismuth salts (e.g., Pepto-Bismol) can be used for travel abroad to coat the intestinal lining and help prevent infection. The role of antibiotic therapy in salmonellosis and *E. coli* O157:H7 infection remains unclear, although in general, enterohemorrhagic *E. coli* should not be treated with empiric antibiotics. Avoidance of antimotility agents in bloody diarrhea is emphasized, especially when illness is caused by *E. coli* O157:H7, which could increase the risk of subsequent hemolytic-uremic syndrome. In select at-risk populations, wider use of vaccines, including the oral typhoid vaccine and the oral cholera vaccine (available only outside the United States), is recommended.[4]

Some newer therapeutic approaches include 5-HT_2 and 5-HT_3 receptor antagonists, calcium-calmodulin antagonists, and alpha-receptor agonists. These may be useful to avoid adverse effects on gastric motilility.[6]

Underlying and Predisposing Factors

Although a pathogenic agent is often responsible for infectious diarrhea, a number of host factors can predispose individuals to experience illness. Poor digestive function, which is often characterized by low stomach acid output or achlorhydria and inadequate pancreatic enzyme output, should be explored in patients who are susceptible to infectious diarrhea. In these cases, hydrochloric acid and pancreatic enzyme supplementation may be advisable. Immunoglobulin A (IgA) antibodies help discourage epithelial adherence of pathogenic organisms. Depressed levels of secretory IgA can leave the immune system of the gastrointestinal tract feeble and unable to deal with pathogenic infection. Decreased intestinal motility, which can be caused by chronic stress and high sugar intake, can also allow microbes to flourish in an intestinal environment. Recurrent infectious diarrhea may be encouraged by food allergy and sensitivities as well.

The clinician should also be aware that many drugs may actually increase susceptibility to infectious diarrheal illness. These include proton-pump inhibitors, antifolate drugs, and antibiotics. Hospitalized patients who receive proton-pump inhibitors are at increased risk of *C. difficile*–induced diarrhea.[18–20] As in the case of achlorhydria, it is likely that inhibition of upper gastrointestinal digestive acids by proton-pump inhibitors may allow pathogens and undigested food to reach the intestines without being properly broken down. Also, antibiotic-associated diarrhea is known to occur with broad-spectrum antibiotics.[21] Antibiotics can disrupt local bowel flora, allowing pathogenic organisms to flourish, and are well-known to leave patients susceptible to diarrhea (see "Probiotics" later in the chapter).

Hydration/Electrolyte Balance

It is of paramount importance to keep the patient with diarrhea well hydrated and to ensure electrolyte balance. This is most crucial in children. Of course, rehydration does not treat the diarrhea itself, which will persist until the infection resolves. Signs of dehydration may include decreased or absent urination, decreased skin turgor, and dry tongue. Rehydration using solutions of glucose, sodium, and potassium is appropriate. In cases of severe dehydration with weight loss of more than 10% or unconsciousness, intravenous rehydration is indicated.[22] Otherwise, oral rehydration solutions can be quite effective, given that glucose absorption via sodium–glucose cotransport remains intact during episodes of diarrhea.

Diet

Prevention of infectious diarrhea may be accomplished by avoiding undercooked meat or seafood, unpasteurized milk, or soft cheese.[4] Once the patient has symptoms, the use of the traditional "BRAT diet" can usually help decrease gastric motility. The components of this diet are foods that can slow peristalsis and tend to be more binding. These are bananas, white rice, apples, plain white toast or bread, and tea.

High-fat meals and dairy-based foods (due to secondary lactose malabsorption, which may last for several weeks to months after an infectious diarrheal event) should be avoided.

Evidence indicates that larger, less frequent meals are more taxing to the digestive and absorptive capacities of the gastrointestinal system. In one porcine study, 3-week-old piglets were assigned to one of four dietary regimens and were subsequently all infected with rotavirus, followed 24 hours later with enteropathogenic *E. coli.* The dietary regimen designed to tax the digestive and absorptive capacities of the piglets (a high nutrient intake with a three-times-a-day feeding) significantly produced the most prolonged diarrhea as well as the most advanced colonization of the gut by hemolytic enteropathogenic *E. coli* and persistent shedding of rotavirus. The same nutrient intake divided into 24 equal increments and administered hourly produced a less severe response. The least severe diarrhea was seen in piglets fed one-third the nutrient intake either hourly or three times a day.[23]

Traditional dietary preparations for diarrhea such as carrot soup and products based on rice can be useful because of their high absorbance capacity. They have been known to reduce stool output and the duration of diarrhea, although they may not necessarily diminish the intestinal loss of water and electrolytes.[22,24]

One study evaluated the effects of green banana and pectin on intestinal permeability in 57 boys with persistent diarrhea. Green banana and pectin contain nondigestible dietary sources of colonic short-chain fatty acids. The patients were given a week's treatment with a rice-based diet containing either cooked green banana, pectin, or a rice diet alone. Intestinal permeability was assessed before and after treatment by giving a lactulose-mannitol (LM) drink and measuring urinary recovery after 5 hours. Treatment with banana significantly reduced lactulose recovery, increased mannitol recovery, and decreased the LM ratio, indicating an improvement of permeability. Pectin produced similar results. Permeability changes were associated with a 50% reduction in stool weights, which correlated strongly with the LM ratio.[25] In a study of 154 male infants aged between 1 and 30 months, commonly allergenic soy- and casein-based diets were not helpful in alleviating persistent diarrhea, whereas yogurt and broken-down amino acid formulas were helpful in encouraging a significant reduction in stool output and in the duration of diarrhea.[26] Other studies also suggest, as an adjunctive measure for patients who experience vomiting, the administration of a small amount of glucose.[22]

Supplements

Vitamin A

The administration of vitamin A to children can reduce the incidence of severe diarrhea. One double-blind controlled trial examined 900 children from ages 1 to 5 with acute diarrhea. These young subjects

were assigned to receive either 60 mg of vitamin A or a placebo. They were followed up at home on alternate days until they recovered from the diarrheal episode. In all study children, those treated with vitamin A had a significantly lower risk of persistent diarrhea. However, there was no change in the duration or average stool frequency. Interestingly, the group who was not breastfed had a shorter average duration of diarrhea. In these children, the mean number of stools passed after the intervention, the proportion of episodes lasting greater than 14 days, and the percentage of children who passed watery stools on any study day were also significantly lower in those treated with vitamin A.[27]

Folic Acid and Vitamin B_{12}

Studies are beginning to emerge correlating low levels of folic acid and vitamin B_{12} with increased susceptibility to diarrhea. Folic acid deficiency is known to promote malabsorption due to the altered structure of the intestinal mucosal cells.[28] Thirteen patients diagnosed with megaloblastic anemia suffering from chronic diarrhea showed significant histological changes of the ileal mucosa, including inflammation, atrophy, erosion and flattening of the villi, lymphatic ectasia, and focal fibrosis. When given folate and cyanocobalamin supplementation, these patients quickly demonstrated decreased diarrheal episodes as well as intestinal restoration.[29]

Antifolate chemotherapeutic drugs such as methotrexate can contribute to folic acid and vitamin B_{12} deficiencies and can cause or contribute to diarrheal symptomatology. Cancer patients treated with this class of drugs who had lowered folic acid and vitamin B_{12} were found to be more susceptible to subsequent development of serious drug-related toxicities such as myelosuppression, diarrhea, mucosal toxicity, and infection. This suggests that toxicity is related to relative folate deficiency in some cancer patients.[30] In this study, nutritional supplementation led to a marked reduction in toxicity and the abolition of treatment-related deaths without affecting the efficacy of the antineoplastic drugs.

Tangential to the conversation of infectious diarrhea and folic acid, there is a concern for neural tube defects (NTDs) with folic acid and vitamin B_{12} deficiency. It has been suggested that gastrointestinal disturbances such as those caused by diarrhea might negatively affect the availability of these vitamins to the pregnant mother and fetus, thereby increasing the risk of birth defects. In one study evaluating the risk of neural tube defects with periconceptional diarrhea, it was observed that one or more episodes of periconceptional diarrhea were associated with an increased risk of NTD-affected pregnancies compared with no episodes of diarrhea. This association was independent of fever, obesity, maternal age, maternal birthplace, income, prior unproductive pregnancy, and dietary plus multivitamin folate intake.[31] Whether the cause of diarrhea is infectious or of any other origin, it is prudent to ensure proper folic acid and vitamin B_{12} nutrition in women who are trying to conceive as well as in newly pregnant women.

Folic acid supplementation appears to be without side effects, even at high doses (e.g., 15 mg/day). Patients with certain genetic polymorphisms, including COMT and/or MAO, may experience aggravations from high doses of methyl-donors secondary to impaired ability to metabolize increased neurotransmitter availability (a concept known as methyl-trapping). It has been reported that 8 of 14 healthy human subjects who consumed 15 mg/day of folic acid for 1 month developed abdominal distention, flatulence, nausea, anorexia, sleep disturbances with vivid dreams, malaise, and irritability.[32] This, however, has not been confirmed in a double-blind clinical study[33] and other investigations.[34,35] Vitamin B_{12} is not carcinogenic, teratogenic, or mutagenic. It is considered safe even at 1000 times the recommended daily allowance. However, during such treatment, care should be given to not take large amounts of folic acid because this may mask the nerve damage associated with vitamin B_{12} deficiency.

Zinc

In recent years, the nutritional importance of zinc has been established, and zinc deficiency and its symptoms have been well recognized. Diarrhea has been found to be one of the clinical manifestations of zinc deficiency. Zinc has demonstrated an antimicrobial effect on enteric pathogens such as *S. typhi, Salmonella, E. coli, Enterobacter, Shigella, Staphylococcus albus, Streptococcus pyogenes,* and *Vibrio cholerae*[36,37] and may contribute to the treatment of diarrhea. Zinc absorption occurs throughout the small intestine, not only in the duodenum, jejunum, and ileum. Many illnesses distinguished by chronic diarrhea entail insufficient absorption of zinc. In fact, in some cases of chronic enteropathies in infants, like celiac disease and cystic fibrosis, a deficiency of zinc has been isolated.[38]

Good dietary sources of zinc are meat, fish, and to a lesser extent, human milk. *Acrodermatitis enteropathica* is a rare but severe disease in which skin lesions, chronic diarrhea, and recurring infections are the main symptoms. The disease is related to the malfunctioning of intestinal absorption of zinc and can be treated by administering pharmacological doses of zinc orally. The amount of zinc absorbed in the small intestine is influenced by other nutrients: some compounds such as dietary fiber and phytates (which are found in foods including soy, wheat bran, peas, carob, and brown rice) may inhibit this process, whereas picolinic and citric acid facilitate it. Citric acid is thought to be the ligand that accounts for the high level of bioavailability of zinc in human milk.[38]

As a cautionary note, it is theoretically possible that some of the dietary treatments used by practitioners of natural medicine for persistent postenteritis diarrhea—such as elimination of cow's milk and increased fiber and carob powder as well as use of soy formula in infants—can contribute to a scarce supply of zinc and therefore promote the persistence of diarrhea itself.[38] Although these therapeutic approaches are being used, zinc supplementation may be warranted, especially if the patient tends to have diarrheal symptoms.

Glutamine

Glutamine is the most abundant amino acid in the blood. As one of the principal fuels used by the intestinal lining cells, it accounts for 35% of their energy production. Although glutamine is readily available in the diet and synthesized in the body, supplementation improves the energy metabolism of the gastrointestinal mucosa, thus stimulating regeneration.[39] Glutamine prevents intestinal mucosal damage and has been shown to decrease bacterial leakage across the intestines after they are damaged, presumably by stimulating repair.[40] Clearly, glutamine holds promise for enhancing the repair of mucosal injury by a wide range of infections or toxic agents and thus has great potential as a new oral rehydration and nutritional therapeutic for patients with enteric infection, malnutrition, or chemotherapy-induced or radiation-induced enteritis.[40]

Animal models have definitely shown the usefulness of this amino acid in diarrhea. Glutamine has been shown to enhance sodium and water absorption in a rabbit model of cholera and *Cryptosporidium*-infected piglet intestine and has proven effective in a bovine model of *Cryptosporidium* as well. One rat model of cholera toxin–induced diarrhea also showed that alanyl-glutamine (a more stable derivative of glutamine) enhanced water and electrolyte intestinal absorption even better than the traditional glucose solutions.[41]

In one double-blind clinical study of 128 healthy children ages 6 to 24 months old with acute diarrhea, 63 received 0.3 g/kg/day of glutamine, and 65 controls received a placebo for 7 days. The

average duration of diarrhea in the glutamine-treated group was significantly shorter than that in the placebo group (3.4 days vs. 4.57 days, respectively).[42]

Glutamine, even at high doses, is without side effects and is well tolerated.[41] A typical dosage of glutamine is 100 mg three times a day (see Chapter 84 for a more comprehensive discussion of glutamine).

Probiotics

Lactobacillus and *Saccharomyces*

Probiotics, translated as "for life," are bacteria residing in the intestine that are considered beneficial to health. The most important healthful bacteria are *Lactobacillus acidophilus* and *Bifidobacterium bifidum.* Other bacteria (including such beneficial strains as *Lactobacillus casea, Lactobacillus fermentum, Lactobacillus salivores,* and *Lactobacillus brevis*) also become established in the gut (see Chapter 105 for an extensive discussion of these useful clinical agents).

A substantially growing body of scientific evidence has now demonstrated that lactobacilli, *Saccharomyces,* and fermented foods play a significant role in human health. Probiotics are thought to have a protective effect against acute diarrheal disease and have been shown to be successful in the treatment or prevention of various types of infectious diarrhea, including that due to rotavirus, *C. difficile,* and traveler's diarrhea.[5,43,44] A review of the literature suggests that *Lactobacillus rhamnosus* GG (LGG) and *Saccharomyces boulardii* had the most compelling evidence of efficacy; they reduced the duration of disease by 24 hours in acute gastroenteritis and were both likely to improve outcomes in antibiotic-associated diarrhea.[95] Corroborating earlier reviews,[45,46] a Cochrane meta-analysis supports the use of probiotics to shorten the duration (reduced risk of diarrhea lasting 4 or more days by 59%) and reduce stool frequency in acute infectious diarrhea.[47]

Studies show that probiotic supplementation may also prevent future nosocomial rotaviral gastroenteritis, thus demonstrating an immune-modulating effect of probiotics,[5,48] possibly by significantly increasing the number of cells secreting IgA. *Lactobacillus* sp. have also shown remarkable ability to inhibit *E. coli* O157:H7, but not *Salmonella,* in refrigerated storage.[49] *Lactobacillus* may be considered as a future addition to stored foods used for low immune status and high susceptibility to *E. coli* O157:H7.

Children are especially susceptible to infectious diarrhea and its sequelae. In a recent Cochrane review of children with antibiotic-associated diarrhea, *L. rhamnosus* or *S. boulardii* at 5 to 40 billion colony-forming units/day was shown to be effective at improving symptoms, with a reasonable number needed to treat (NNT).[50] In one double-blind, placebo-controlled study, a standard infant formula was supplemented with the probiotics *Bifidobacteria bifidum* and *Streptococcus thermophilus.* Subjects in the probiotic group developed diarrhea at a statistically lower rate than those in the control group (7% vs. 31%). Additionally, rotavirus shedding was decreased statistically in the probiotic group (10% vs. 39%).[44] A second randomized placebo-controlled trial evaluated probiotics in a different but also high-risk population. In this case, 204 undernourished Peruvian children received LGG or placebo. Although a difference in rates of diarrhea was observed, where the treated group had statistically fewer episodes of diarrhea, the reduction was minor (from 6 episodes per child per year to 5.2 episodes per child per year). Interestingly, children who were breastfed did not seem to benefit at all, raising the possibility that probiotics provide a similar action as breast milk in the prevention of infection.[51] In a third double-blind study, 81 children aged 1 to 36 months were enrolled in a trial and randomly assigned at admission to receive 6×10^9 CFUs of LGG or placebo twice daily orally for the duration of their hospital stay. The *Lactobacillus*-treated children enjoyed a dramatically reduced risk of nosocomial diarrhea of 6.7%, versus 33.3% in those who were not given the probiotic. Once again, the use of LGG significantly reduced the risk of rotavirus gastroenteritis (2.2% vs. 16.7%).[52]

S. boulardii, also known as *Saccromyces cerevisiae,* is a nonpathogenic probiotic yeast that may be most helpful in diarrhea caused by *C. difficile,* a pathogen most likely to affect the elderly. Although in the conventional medical world vancomycin is the preferred treatment of severe cases, *S. boulardii* alone or in combination with vancomycin has been reported to be an effective therapeutic alternative for recurrent infection.[12] Although it is generally safe, a few case reports have demonstrated that fungemia and sepsis are rare complications of the administration of *S. boulardii* in immunocompromised patients,[53] in whom it may be contraindicated. The daily adult dose is usually 1 g/day in divided doses (500 mg twice a day) for at least 4 weeks.

L. acidophilus has been shown to correct the increase of gram-negative bacteria observed after the administration of broad-spectrum antibiotics, as occurs with any acute or chronic diarrhea.[54–59] Similarly, a mixture of *B. bifidum* and *L. acidophilus* inhibited the lowering of fecal flora induced by ampicillin and maintained the equilibrium of the intestinal ecosystem.[57]

Although it is commonly believed that acidophilus supplements are not effective if taken during antibiotic therapy, the research actually supports the use of *L. acidophilus* during antibiotic administration.[57,58] Reductions of friendly bacteria or superinfection with antibiotic-resistant flora, or both, may be prevented by administering *L. acidophilus* products during antibiotic therapy. A dose of at least 15 to 20 billion organisms is required. Probiotic supplements should, however, be taken as temporally far away from the antibiotic as possible.

Evidence indicates that the conventional medical world is beginning to take notice of the benefits of giving *Lactobacillus* with antibiotic therapy. In one double-blind, controlled study, the antibiotic containing ampicillin (250 mg) and cloxacillin (250 mg) with or without protected lactobacilli was evaluated in 740 patients undergoing cataract surgery. The incidence of diarrhea in patients receiving antibiotic alone was 13.3%, compared with 0% in patients receiving antibiotic with protected lactobacilli.[21] Although antibiotics should be used judiciously and only in cases where the benefits outweigh the risks, adjunctive therapy may decrease the untoward effects of antibiotic therapy on the gastrointestinal system.

Although many excellent companies provide high-quality probiotic products, it is difficult to sort through all of the manufacturers' claims of superiority, and some products have been shown to contain no active *L. acidophilus.*[60] Only high-quality, laboratory-tested probiotics should be used.

Botanical Medicines

Berberine-Containing Plants

Berberine-containing plants include goldenseal *(Hydrastis canadensis),* barberry *(Berberis vulgaris),* Oregon grape *(Berberis aquifolium),* and goldthread *(Coptis chinensis).* Berberine, an alkaloid, has been extensively studied in both experimental and clinical settings for its antibiotic activity. Berberine exhibits a broad spectrum of antibiotic activity, having shown antimicrobial activity against bacteria, protozoa, and fungi, including *Candida albicans.*[61–67]

Berberine's antibiotic action against some of these pathogens is actually stronger than that of the antibiotics commonly used for the disease these pathogens cause. Berberine-containing plants should be considered in infectious processes involving the previously mentioned organisms. Berberine's action in inhibiting *Candida,* as well as pathogenic bacteria, prevents the overgrowth of yeast, which is a common side effect of antibiotic use.

Diarrhea is a common symptom in patients with chronic candidiasis. Berberine has shown remarkable antidiarrheal activity in even

the most severe cases. Positive clinical results have been shown with berberine in relieving diarrhea in cases of cholera, amebiasis, giardiasis, and other causes of acute gastrointestinal infection (e.g., due to *E. coli, Shigella, Salmonella,* and *Klebsiella*) and may also relieve the diarrhea seen in patients with chronic candidiasis.[68–74]

The dosage of any berberine-containing plant should be based on berberine content. Because there is a wide range of quality in goldenseal preparations, standardized extracts are preferred. Three-times-a-day dosages are as follows:

- Dried root or as infusion (tea), 2 to 4 g
- Tincture (1:5), 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1), 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content), 250 to 500 mg

Note that the dosage recommendations for berberine would be 25 to 50 mg three times a day or a daily dosage of up to 150 mg. This dosage is consistent with the dosage range in the positive clinical studies in patients with gastrointestinal infections. For children, a dosage based on body weight is appropriate. The daily dosage would be the equivalent of 5 to 10 mg of berberine/kg (2.2 lb) body weight.

Berberine and berberine-containing plants are generally nontoxic at the recommended dosages; however, berberine-containing plants are not recommended for use during pregnancy, and higher dosages may interfere with B vitamin metabolism.

Potentilla tormentilla (Tormentil Root)

Tormentil root contains more than 15% tannic acid, giving this plant high astringent capacity[75]; it may be useful in treating infectious diarrhea, shortening the duration of rotavirus diarrhea, and decreasing the requirement for rehydration solutions.[10] A randomized double-blind placebo-controlled trial was conducted at a children's hospital in St. Petersburg, Russia. In this study, 40 children ranging in age from 3 months to 7 years with rotavirus diarrhea were divided into two groups: a treatment group consisting of 20 children given 3 drops of tormentil root extract per year of life three times a day until discontinuation of diarrhea or a maximum of 5 days and a control group of 20 children who received a placebo.

The duration of diarrhea in the group treated with tormentil root extract was 60% less than that in the placebo group (3 days, compared with 5 days in the control group). In the treatment group, 8 of 20 (40%) children were diarrhea-free 48 hours after admission to the hospital, compared with 1 of 20 (5%) in the control group. Children in the treatment group also received smaller volumes of parenteral fluids than subjects in the control group.[10] For adults, a reasonable dose is 60 drops of tincture twice daily. Some clinicians find the powdered herb to be more effective in adults at a dosage of 1/4 tsp twice daily.[75]

Allium sativum (Garlic)

Garlic appears to affect DNA and RNA synthesis and is being investigated as an effective antibiotic. In vitro antimicrobial sensitivity tests on *E. coli, Shigella* sp., *Salmonella* sp., and *Proteus mirabilis* found significant effects of garlic along with ciprofloxacin and ampicillin. The gram-negative diarrheagenic pathogens from the stool samples were highly sensitive to garlic, whereas ciprofloxacin was most effective against *E. coli.* The differences were inferred to result from genetic differences among the organisms and differences in the modes of action of the antibiotics. Most interestingly, no pathogens were resistant to garlic, which led the authors to conclude it was a promising antimicrobial agent.[76]

Other Antidiarrheal Botanicals

A recent review of the available evidence indicates the use of herbal compounds in children or adolescents suffering from acute diarrhea. An herbal preparation containing *Matricaria chamomilla* (German chamomile) was shown to significantly reduce the duration of diarrhea and frequency of bowel movements compared with placebo.

Carob bean juice was shown to be superior to placebo in reducing the required intake of a standard rehydration solution, total stool output, and the overall duration of diarrhea. The more commonly available carob powder, at a dosage of 15 g daily (in divided doses), has been used successfully in children.[77]

Other Antiparasitic Botanicals

A wide variety of botanicals have been used alone or in combination with anti-inflammatory and demulcent herbs to specifically stop parasitic infections and heal the digestive tract. Curcumin has been shown to protect against castor oil– and carrageenan-induced diarrhea in rat models.[78] The Brazilian *Ocimum selloi* essential oil produced a significant reduction in the severity and frequency of diarrhea produced by castor oil while also reducing transit time in mice.[79] A Thai herbal formula called Pikutbenjakul—which contains *Piper longum, Piper sarmentosum, Piper interruptum, Plumbago indica,* and *Zingiber officinale*—has shown promise in in vitro work against some *Vibrio* species, *Salmonella, Shigella, E. coli,* and *S. aureus.*[80] Unfortunately, human clinical trials are lacking to corroborate the safety and efficacy of their use. Future studies should evaluate the potential benefits and toxicities of the following antiparasitic herbs: *Artemisia absinthium* (wormwood), *Chenopodium abrosioides* (wormseed), *Curcuma longa* (turmeric), *Phytolacca decandra* (pokeweed, pokeroot), *Juglans* species (black and white walnut), and *Tanacetum vulgare* (tansy).

Homeopathy

A meta-analysis of three double-blind clinical trials of diarrhea found that individualized homeopathic treatment decreases the duration of acute childhood diarrhea. In this review, 242 children ages 6 months to 5 years were analyzed as one group. Children were randomized to receive either an individualized homeopathic medicine or placebo to be taken as a single dose after each unformed stool for 5 days. Parents recorded daily stools on diary cards, and health workers made home visits daily to monitor the children. The duration of diarrhea was defined as the time until there were fewer than three unformed stools per day for 2 consecutive days. A meta-analysis of the effect–size difference of the three studies was also conducted. Combined analysis showed a statistically significant duration of diarrhea of 3.3 days in the homeopathy group versus 4.1 in the placebo group.

Homeopathics commonly included as possible remedies for diarrhea include podophyllum (which is well known for its almost universal application for traveler's diarrhea[81]), aloe, china, mercurius, phosphorus, nux vomica, sulfur, aconite, veratrum album, and arsenicum. Although these results must still be evaluated further with larger-scale studies, given the safety and historical effectiveness of homeopathy, it should be thoroughly considered as part of the natural medicine practitioner's therapeutic choices.

Acupuncture

From a traditional Chinese medicine perspective, diarrhea is generally due to an imbalance of large intestinal regulation as well as deficiency of the spleen and stomach. Although there are variations depending on a particular patient's presentation, the four basic diagnoses are diarrhea due to Cold-Damp, Damp-Heat, Spleen-Yang Depletion, and Kidney Yang deficiency. Typically, diarrhea caused by infectious pathogens falls into the category of Damp-Heat and may present with pain, mucus, and blood in the stool.

A few studies have shown the effect that acupuncture[82–84] and Chinese herbal preparations have on intestinal movement.[2,85] One

study demonstrated that intestinal peristalsis was accelerated significantly by acupuncture at the abdomen but suppressed by moxibustion, which is the warming of certain acupuncture points with nearby burning of *Artemisia argyi*.[83] Depending on the desired result, the acupuncture practitioner may want to focus on moxa therapy for some cases where slower peristalsis is desired. Two studies measured the influence of electrical stimulation of acupuncture points including ST-36 (Zusanli) and SP-6 (Sanyinjiao) on the myoelectrical activity of the small intestine; as a result, a clear ability of acupuncture to alter intestinal motility was recorded. One of these trials employed 3 Hz of electroacupuncture on the ST-36 (Zusanli) point and demonstrated increased regularity of the gastric myoelectrical system.[84] However, another evaluation did not find acupuncture plus electrostimulation to be effective enough to normalize gastric dysrhythmia after pretreatment with atropine.[86]

Animal research has supported the historical use of acupuncture for infectious diarrhea. The effect of acupuncture was evaluated in the treatment of 32 young pigs with induced enteropathogenic *E. coli* diarrhea. The animals were all inoculated with *E. coli* and then divided into three groups. One group, the controls, remained untreated; a second group was treated with the antibiotic enrofloxacin; and the third group was treated with traditional acupuncture using GV-1 (Jiaochao). Using histopathological evaluation by hematoxylin-and-eosin stain, severe infiltration of inflammatory cells in the lamina propria was observed in the ascending and descending colon and in the fundic stomach of the untreated control group. Destruction of the fundic gland's architecture and necrotic lesions were also observed. However, in these same anatomic locations, the animals treated with acupuncture or antibiotics maintained mucosa of the colon and stomach that were relatively similar to those of the normal group.[87] Another corroborating similar study of 34 animals employed Du-20 (Bai-hui), BL-20 (Pishu), CV-12 (ZongWan), and ST-25 (TianShu). This study found no extra benefit from using electrical stimulation.[88]

Many Chinese practitioners find benefit in using acupuncture and Chinese herbs together to decrease the side effects associated with Western medicine.[89] One study of Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang) on diarrhea induced by pilocarpine, barium chloride, or castor oil found a significant reduction when the herbal combination was dosed at 1000 mg/kg. This herb may mechanistically act to inhibit excessively accelerated small intestinal movement. Another trial comprised 162 children suffering from chronic protracted diarrhea; it involved randomly dividing them into two groups and observing them clinically. In the Chinese herbal medicine group, Xiang Cheng San was externally applied to the umbilicus of each patient; in a second group, Western drugs were routinely given. The results showed that the therapeutic effects were markedly better in the herbal medicine group.[90] Although no control was used in this experiment, the results are encouraging. A wider variety of patent, granular, and loose herb combinations are used to treat various Chinese diagnoses for infectious diarrhea. Future clinical research will help elucidate their use.

Finally, complex chronic immune deficiency conditions leave patients quite susceptible to pathogenic infection and are difficult to treat. Diarrhea affects more than 60% of persons living with human immunodeficiency virus (HIV)/AIDS. AIDS patients already on a polypharmaceutical regimen have shown good responses to acupuncture and moxibustion used to alleviate symptoms of diarrhea.[91,92] In one pilot study, 15 HIV-positive men with chronic diarrhea received the same acupuncture/moxibustion treatment for six sessions over a 3-week period. In all subjects, stool frequency was reduced by approximately one episode per day. Stool consistency also improved from baseline to week 3 and week 4 by more than 1 point on Hansen's stool consistency scale. Once again, more studies are necessary, but preliminary results are promising for antidiarrheal therapy that does not require additional pharmaceuticals.

Overall, Chinese medicine modalities have proved to be quite safe. In one 6-year Japanese study, the results of 84 therapists and 65,482 treatments were evaluated. Participants were required to report all adverse events. In total, only 94 (0.14%) adverse events were recorded, and none was serious (serious events included conditions like pneumothorax, infection, and spinal cord injury). The authors concluded that serious or severe adverse events are rare in standard practice. Although more research is clearly necessary to elucidate the benefits of acupuncture, given the little risk and long history of positive anecdotal evidence, it is certainly worth using.[93] More research on Chinese herbs is also necessary to elucidate the toxicity in those preparations that appear therapeutically beneficial.

THERAPEUTIC APPROACH

Overall, natural treatments can adequately manage most cases of non–life-threatening diarrhea. Importantly, diarrhea itself is an eliminative function used by the body to clear toxins and should not be completely suppressed. Of course, chronic diarrhea and diarrhea that causes extreme or rapid electrolyte and water loss may require conventional interventions. It is imperative to maintain hydration and electrolyte balance. Nowhere is this need more immediate than in children younger than 5 years of age.

Underlying Factors

Addressing underlying factors such as low stomach and pancreatic output and depressed levels of IgA helps decrease the chances of pathogenic infection. When possible, eliminating iatrogenic causes such as proton-pump inhibitors and antibiotics also helps prevent recurrences. Diets low in refined sugar and cow's milk dairy are encouraged. Food allergies and intolerances should be assessed in those with recurrent infections. Methods to relieve stress also help encourage proper digestive function.

Proper treatment with either an antibiotic or a natural alternative requires close monitoring by standard laboratory methods (e.g., repeating multiple stool samples 2 weeks after initiation of therapy).

Diet

The BRAT diet may be useful to discourage large volume loss in infectious diarrhea. Green banana and pectin fiber may be a useful part of the BRAT diet. Small, frequent meals (hourly, if possible) may be best, with glucose administration in the case of vomiting. Avoidance of soy, casein, and other known allergenic foods may be beneficial.

Nutritional Supplements

Dosages are for adults unless otherwise indicated.

- Vitamin A: 60 mg/day in children and up to 50,000 IU/day for 1 to 2 days for adults with infections. Caution against high doses (above 10,000 IU/day in pregnant women).
- Folate: 1 mg/day
- Vitamin B_{12} (methylcobalamin): 600 to 1000 mcg/day
- Zinc picolinate: 30 mg/day
- Glutamine: 100 mg twice a day
- *Lactobacillus* sp. and *Bifidobacteria sp.* 6 to 10 billion CFU of LGG or other high-quality probiotic twice a day orally. For prevention of antibiotic-induced diarrhea, a dosage of at least 15 to 20 billion organisms is required. Probiotic supplements should be taken as far away from the antibiotic as possible (2–4 hours is ideal). In children younger than age 6 experiencing antibiotic-induced diarrhea,

the probiotic should be taken every day of the antibiotic dose and continued for 1 week after discontinuing the antibiotic.
- *S. boulardii:* Specifically to treat *C. difficile,* a daily adult dose of 1 g/day in divided doses (500 mg twice a day) for at least 4 weeks. Can be used adjunctively with vancomycin.

Botanicals

- Berberine: Dosage recommendations for berberine would be 25 to 50 mg three times a day or a daily dosage of up to 150 mg. For children, a dosage based on body weight is appropriate. The daily dosage would be the equivalent of 5 to 10 mg of berberine/kg (2.2 lb) body weight. See earlier discussion for more details.
- Tormentil: 60 drops of tincture twice a day or powdered herb at 1/4 tsp twice a day. In children, 3 drops of tormentil root extract per year of life three times a day until discontinuation of diarrhea or a maximum of 5 days.
- Carob powder: 15g daily (in divided dose), mixed into applesauce or similar mixture

Homeopathy

- Typical remedies for diarrhea include podophyllum, aloe, china, mercurius, phosphorus, nux vomica, sulfur, aconite, veratrum album, and arsenicum. The specific choice of remedy should be based on the individual patient and his or her presentation.

Acupuncture

- Acupuncture points may include ST-36 (Zusanli) and SP-6 (Sanyinjiao), DU-20 (Bai-hui), BL-20 (Pishu), CV-12 (ZongWan), and ST-25 (TianShu).
- Acupuncture is appropriate for stress relief as well as in the direct treatment of intestinal issues and is excellent for treating HIV/AIDS-related diarrhea.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lim ML, Wallace MR. Infectious diarrhea in history. *Infect Dis Clin North Am*. 2004;18:261–274.
2. Li YL. Clinical and experimental study on the treatment of children diarrhea by granule of children-diarrhea fast-stopping. *Zhong Xi Yi Jie He Za Zhi*. 1991;11:79–82.
3. Cotran RS, Kumar V, Collins T. *Robbin's Pathologic Basis of Disease*. 6th ed. Philadelphia: Saunders; 1984.
4. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis*. 2001;32:331–351.
5. Majamaa H, Isolauri E, Saxelin M, et al. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr*. 1995;20:333–338.
6. Farthing MJ. Diarrhoea: a significant worldwide problem. *Int J Antimicrob Agents*. 2000;14:65–69.
7. McQuaid KR. Alimentary tract. In: Tierney LM, McPhee SJ, Papadakis MA, eds. *Current Medical Diagnosis and Treatment*. New York: Lange Medical Books/McGraw-Hill; 2000:562.
8. Dryden MS, Gabb RJ, Wright SK. Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. *Clin Infect Dis*. 1996;22:1019.
9. Goodgame RW. Viral causes of diarrhea. *Gastroenterol Clin North Am*. 2001;30:779–795.
10. Subbotina MD, Timchenko VN, Vorobyov MM, et al. Effect of oral administration of tormentil root extract *(Potentilla tormentilla)* on rotavirus diarrhea in children: a randomized, double blind, controlled trial. *Pediatr Infect Dis J*. 2003;22:706–711.
11. Procop GW. Gastrointestinal infections. *Infect Dis Clin North Am*. 2001;15:1073–1108.
12. Stroehlein JR. Treatment of *Clostridium difficile* infection. *Curr Treat Options Gastroenterol*. 2004;7:235–239.
13. Chiappini E, Galli L, Pecile P, et al. Results of a 5-year prospective surveillance study of antibiotic resistance among *Salmonella enterica* isolates and ceftriaxone therapy among children hospitalized for acute diarrhea. *Clin Ther*. 2002;24:1585–1594.
14. Woo PC, Kuhnert P, Burnens AP, et al. *Laribacter hongkongensis:* a potential cause of infectious diarrhea. *Diagn Microbiol Infect Dis*. 2003;47:551–556.
15. Wanke CA. Small intestinal infections. *Curr Opin Gastroenterol*. 1994;10:59.
16. Riddle MS, DuPont HL, Connor BA. ACG clinical guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. *Am J Gastroenterol*. 2016;111:602–622.
17. Chan SS, Ng KC, Lam PK, et al. Predictors of positive stool culture in adult patients with acute infectious diarrhea. *J Emerg Med*. 2002;23:125–130.
18. Dial S, Alrasadi K, Manoukian C, et al. Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *CMAJ*. 2004;171:33–38.
19. Khatami SS, Mukunda B, Ravakhah K. Coinfection with *Giardia lamblia* and *Clostridium difficile* after use of ranitidine. *Am J Med Sci*. 2004;327: 91–93.
20. Cunningham R, Dale B, Undy B, et al. Proton pump inhibitors as a risk factor for *Clostridium difficile* diarrhoea. *J Hosp Infect*. 2003;54:243–245.
21. Ahuja MC, Khamar B. Antibiotic associated diarrhoea: a controlled study comparing plain antibiotic with those containing protected lactobacilli. *J Indian Med Assoc*. 2002;100:334–335.
22. Girardet JP, Fontaine JL. Current treatment of acute diarrhea in infants. *Ann Pediatr (Paris)*. 1988;35:609–612.
23. Lecce JG, Clare DA, Balsbaugh RK, et al. Effect of dietary regimen on rotavirus—*Escherichia coli* weanling diarrhea of piglets. *J Clin Microbiol*. 1983;17:689–695.
24. Pietschnig B, Javaid N, Haschke F, et al. Acute diarrheal diseases: treatment with carrot-rice viscous solution is more effective than ORS solution. *Monatsschr Kinderheilkd*. 1992;140:426–430.
25. Rabbani GH, Teka T, Saha SK, et al. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. *Dig Dis Sci*. 2004 Mar;49(3):475–484.
26. de Mattos AP, Ribeiro TC, Mendes PS, et al. Comparison of yogurt, soybean, casein, and amino acid-based diets in children with persistent diarrhea. *Nutr Res*. 2009;29(7):462–469.
27. Bhandari N, Bahl R, Sazawal S, et al. Breast-feeding status alters the effect of vitamin A treatment during acute diarrhea in children. *J Nutr*. 1997;127:59–63.
28. Carruthers LB. Chronic diarrhea treated with folic acid. *Lancet*. 1946;1:849–850.
29. Ayala E, Frisancho O, Chacon Yupanqui P. Histological changes of the distal ileum in chronic diarrhea associated with megaloblastic anaemia. *Rev Gastroenterol Peru*. 2004;24:117–121.
30. Calvert H. Folate status and the safety profile of antifolates. *Semin Oncol*. 2002;29(2 suppl 5):3–7.
31. Felkner M, Hendricks K, Suarez L, et al. Diarrhea: a new risk factor for neural tube defects? *Birth Defects Res Part A Clin Mol Teratol*. 2003;67:504–508.
32. Hunter R, Barnes J, Oakeley HF, et al. Toxicity of folic acid given in pharmacological doses to healthy volunteers. *Lancet*. 1970;1:61–63.
33. Hellstrom L. Lack of toxicity of folic acid given in pharmacological doses to healthy volunteers. *Lancet*. 1971;1:59–61.
34. Sheehy T. Folic acid: lack of toxicity. *Lancet*. 1973;1:37.
35. Boss G, Ragsdale RA, Zettner A, et al. Failure of folic acid (pteroglutamic acid) to affect hyperuricemia. *J Lab Clin Med*. 1980;96:783–789.
36. Surjawidjaja JE, Hidayat A, Lesmana M. Growth inhibition of enteric pathogens by zinc sulfate: an in vitro study. *Med Princ Pract*. 2004;13:286–289.
37. Basit N, Mahmood I, Siddiqui F, et al. Studies on the inhibitory effects of zinc heptanoate on microorganisms. *G Batteriol Virol Immunol*. 1979;72:10–20.
38. Giorgi PL, Catassi C, Guerrieri A. Zinc and chronic enteropathies. *Pediatr Med Chir*. 1984;6:625–636.
39. Souba WW. Glutamine. A key substrate for the splanchnic bed. *Annu Rev Nutr*. 1991;11:285–308.
40. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. *Arch Surg*. 1990;125:1040–1045.
41. Carneiro-Filho BA, Bushen OY, Brito GA, et al. Glutamine analogues as adjunctive therapy for infectious diarrhea. *Curr Infect Dis Rep*. 2003;5:114–119.
42. Yalcin S, Yurdakok K, Tezcan I, et al. Effect of glutamine supplementation on diarrhea, interleukin-8 and secretory immunoglobulin A in children with acute diarrhea. *J Pediatr Gastroenterol Nutr*. 2004;38:494–501.
43. Markowitz JE, Bengmark S. Probiotics in health and disease in the pediatric patient. *Pediatr Clin North Am*. 2002;49:127–141.
44. Saavedra JM, Bauman NA, Oung I, et al. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet*. 1994;344:1046–1049.
45. Goossens D, Jonkers D, Stobberingh E, et al. Probiotics in gastroenterology: indications and future perspectives. *Scand J Gastroenterol Suppl*. 2003;239:15–23.
46. Rosenfeldt V, Michaelsen KF, Jakobsen M, et al. Effect of probiotic *Lactobacillus* strains in young children hospitalized with acute diarrhea. *Pediatr Infect Dis J*. 2002;21:411–416.
47. Allen SJ, Martinez EG, Gregorio GV, et al. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Syt Rev*. 2010;11:CD003048.
48. Isolauri E, Juntunen M, Rautanen T, et al. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. *Pediatrics*. 1991;88:90–97.
49. Brashears MM, Durre WA. Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherichia coli* O157:H7 during growth and refrigerated storage. *J Food Prot*. 1999;62:1336–1340.
50. Goldenberg J. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev*. 2015;12:CD004827.
51. Oberhelman RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of *Lactobacillus GG* to prevent diarrhea in undernourished Peruvian children. *J Pediatr*. 1999;134:15–20.
52. Szajewska H, Kotowska M, Mrukowicz JZ, et al. Efficacy of *Lactobacillus GG* in prevention of nosocomial diarrhea in infants. *J Pediatr*. 2001;138:361–365.

53. Lestin F, Pertschy A, Rimek D. Fungemia after oral treatment with *Saccharomyces boulardii* in a patient with multiple comorbidities. *Dtsch Med Wochenschr*. 2003;128:2531–2533.
54. Hentges DJ, ed. *Human Intestinal Microflora in Health and Disease*. New York: Academic Press; 1983.
55. Shahani KM, Ayebo AD. Role of dietary lactobacilli in gastrointestinal microecology. *Am J Clin Nutr*. 1980;33:2448–2457.
56. Friend BA, Shahani KM. Nutritional and therapeutic aspects of lactobacilli. *J Appl Nutr*. 1984;36:125–152.
57. Zoppi G, Deganello A, Benoni G, et al. Oral bacteriotherapy in clinical practice. I. The use of different preparations in infants treated with antibiotics. *Eur J Ped*. 1982;139:18–21.
58. Gotz VP, Romankiewics JA, Moss J. Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. *Am J Hosp Pharm*. 1979;36:754–757.
59. Zoppi G, Balsamo V, Deganello A, et al. Oral bacteriotherapy in clinical practice. II. The use of different preparations in the treatment of acute diarrhoea. *Eur J Ped*. 1982;139:22–24.
60. Hughes VL, Hillier SL. Microbiologic characteristics of *Lactobacillus* products used for colonization of the vagina. *Obstet Gynecol*. 1990;75:244–248.
61. Hahn FE, Ciak J. *Berberine. Antibiotics*. 1976;3:577–588.
62. Amin AH, Subbaiah TV, Abbasi KM. Berberine sulfate: antimicrobial activity, bioassay, and mode of action. *Can J Microbiol*. 1969;15:1067–1076.
63. Johnson CC, Johnson G, Poe CF. Toxicity of alkaloids to certain bacteria. II. Berberine, physostigmine, and sanguinarine. *Acta Pharmacol Toxicol (Copenh)*. 1952;8:71–78.
64. Kaneda Y, Torii M, Tanaka T, et al. In vitro effects of berberine sulfate on the growth and structure of *Entamoeba histolytica, Giardia lamblia* and *Trichomonas vaginalis*. *Ann Trop Med Parasitol*. 1991;85:417–425.
65. Subbaiah TV, Amin AH. Effect of berberine sulfate on *Entamoeba histolytica*. *Nature*. 1967;215:527–528.
66. Ghosh AK, Rakshit MM, Ghosh DK. Effect of berberine chloride on *Leishmania donovani*. *Indian J Med Res*. 1983;78:407–416.
67. Majahan VM, Sharma A, Rattan A. Antimycotic activity of berberine sulphate. An alkaloid from an Indian medicinal herb. *Sabouraudia*. 1982;20:79–81.
68. Gupte S. Use of berberine in the treatment of giardiasis. *Am J Dis Child*. 1975;129:866.
69. Bhakat MP. Therapeutic trial of berberine sulphate in non-specific gastroenteritis. *Indian Med J*. 1974;68:19–23.
70. Kamat SA. Clinical trial with berberine hydrochloride for the control of diarrhoea in acute gastroenteritis. *J Assoc Physicians India*. 1967;15:525–529.
71. Desai AB, Shah KM, Shah DM. Berberine in the treatment of diarrhoea. *Indian Pediatr*. 1971;8:462–465.
72. Sharma R, Joshi CK, Goyal RK. Berberine tannate in acute diarrhea. *Indian Pediatr*. 1970;7:496–501.
73. Choudry VP, Sabir M, Bhide VN. Berberine in giardiasis. *Indian Pediatr*. 1972;9:143–146.
74. Rabbani GH, Butler T, Knight J, et al. Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic *Escherichia coli* and *Vibrio cholerae*. *J Infect Dis*. 1987;155:979–984.
75. Mitchell WA. *Plant Medicine in Practice: Using the Teachings of John Bastyr*. St. Louis: Churchill Livingstone; 2003.
76. Eja ME, Asikong BE, Abriba C, et al. A comparative assessment of the antimicrobial effects of garlic *(Allium sativum)* and antibiotics on diarrheagenic organisms. *Southeast Asian J Trop Med Public Health*. 2007;38(2):343–348.
77. Loeb H, Vandenplas Y, Wursch P, Guesry P. Tannin-rich pod for treatment of acute-onset diarrhea. *J Pediatr Gastroenterol Nutr*. 1989;8:480–485.
78. Gnanasekar N, Perianayagam JB. Influence of sodium curcuminate on castor oil-induced diarrhea in rats. 36(3):177–178.
79. Franca CS, Menezes FS, Costa LC, et al. Analgesic and antidiarrheal properties of *Ocimum selloi* essential oil in mice. *Fitoterapia*. 2008;79(7–8):569–573. Epub 2008 Jul 11.
80. Kondo S, Sattaponpan C, Phongpaichit S, et al. Antibacterial activity of Thai medicinal plants Pikutbenjakul. *J Med Assoc Thai*. 2010;93(suppl 7):S131–S135.
81. Reichenberg J, Ullman R. Podophyllum: a nearly routine medicine for traveler's diarrhea. *Townsend Lett Doctors Patients*. 2004;252:32–33.
82. Iwa M, Sakita M. Effects of acupuncture and moxibustion on intestinal motility in mice. *Am J Chin Med*. 1994;22:119–125.
83. Tabosa A, Yamamura Y, Forno ER, et al. Effect of the acupoints ST-36 (Zusanli) and SP-6 (Sanyinjiao) on intestinal myoelectric activity of Wistar rats. *Braz J Med Biol Res*. 2002;35:731–739.
84. Chang CS, Chou JW, Ko CW, et al. Cutaneous electrical stimulation of acupuncture points may enhance gastric myoelectrical regularity. *Digestion*. 2002;66:106–111.
85. Saitoh K, Kase Y, Ishige A, et al. Effects of Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang) on diarrhea and small intestinal movement. *Biol Pharm Bull*. 1999;22:87–89.
86. Chang CS, Chou JW, Wu CY, et al. Atropine-induced gastric dysrhythmia is not normalized by electroacupuncture. *Dig Dis Sci*. 2002;47:2466–2472.
87. Park ES, Jo S, Seong JK, et al. Effect of acupuncture in the treatment of young pigs with induced *Escherichia coli* diarrhea. *J Vet Sci*. 2003;4:125–128.
88. Hwang YC, Jenkins EM. Effect of acupuncture on young pigs with induced enteropathogenic *Escherichia coli* diarrhea. *Am J Vet Res*. 1988;49:1641–1643.
89. Chavez C. Prickly business: the finer points of acupuncture. *Posit Aware*. 1995:14–15.
90. Yang S. Application of xiang cheng san in treatment of chronic protracted diarrhea in children. *J Tradit Chin Med*. 1995;15:214–219.
91. Huson C. Acupuncture and traditional Oriental medicine in the treatment of HIV and AIDS. *STEP Perspect*. 1996;8:2–3.
92. Anastasi JK, McMahon DJ. Testing strategies to reduce diarrhea in persons with HIV using traditional Chinese medicine: acupuncture and moxibustion. *J Assoc Nurses AIDS Care*. 2003;14:28–40.
93. Yamashita H, Tsukayama H, Tanno Y, et al. Adverse events in acupuncture and moxibustion treatment: a six-year survey at a national clinic in Japan. *J Altern Complement Med*. 1999;5:229–236.
94. Shane AL, Mody RK, Crump JA, et al. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. *Clin Infect Dis*. 2017;65:e45.
95. Guarino A. Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol*. 2015;49(suppl 1):S37–S45.

Infertility, Female

Leah Hechtman, MSci (RHHG), BHSc (Nat), ND

OUTLINE

DIAGNOSTIC SUMMARY

- Inability to conceive a child after 12 months of regular unprotected intercourse (at least twice weekly) with the same male partner and in the absence of male causes

GENERAL CONSIDERATIONS

Females are believed to contribute to 50% of infertility cases as the primary (30%) and combined (20%) factor. Recent Centers for Disease Control and Prevention (CDC) infertility statistics include the following[1]:

- Percentage of women aged 15 to 44 with impaired fecundity: 12.1%
- Percentage of married women aged 15 to 44 who are infertile: 6.7%
- Number of women aged 15 to 44 who have ever used infertility services: 7.3 million
- Percentage of women aged 15 to 44 who have ever used infertility services: 12.0%

Infertility statistics suggest that one in seven couples in the United States experiences this condition. It is defined by a lack of conception after 12 months of regular unprotected intercourse (at least twice weekly) with the same male partner and in the absence of male causes. After 12 months, intervention and treatment are recommended. It is advisable to initiate earlier intervention and treatment for women over 35 years of age. After this time, the recommendations are that women should receive a comprehensive review and discussion surrounding natural or assisted conception owing to age-related concerns.

A person's fertility is a reflection of his or her general health and well-being. Because we are genetically constructed to pass on the best information for the next generation, the optimal ages for reproduction are believed to be between 18 and 35 years for a female and 16 and 40 years for a male.

Delaying the decision to have children contributes to the proportion of couples who are involuntarily childless. Demographic[2] and clinical studies[3] have shown that women experience optimal fertility before the age of 30 to 31 years. After the age of 31, the probability of conception declines rapidly, but this can be partly compensated for by continued insemination for additional cycles. In addition, the probability of an adverse pregnancy outcome starts to increase at about the same age.[3]

Thereafter, fertility gradually decreases, with acceleration toward the age of 40 years. In women, fertility remains relatively stable until 30 years of age, generally producing more than 400 pregnancies per 1000 exposed women per year; it then begins to decrease substantially.[4] By 45 years of age, the fertility rate is only 100 pregnancies per 1000 exposed women.[5] Already at the age of 40 to 41 years, half of women will have completely lost their capacity for reproduction. It is generally accepted that reproductive aging is in fact ovarian aging and is related to the decreasing quantity and quality of the pool of follicles preserved in the ovaries.[6]

To achieve a normal conception, the following are needed[4]:

- Production of viable sperm with respect to motility, morphology, count, and DNA integrity
- Transport of sperm through the male genital tract and deposition in the female genital tract, usually near the cervix of the uterus during sexual intercourse
- Normal oocyte production (ovulation) by the ovaries
- Transport of sperm and the oocyte within the female genital tract to the site of fertilization in one fallopian tube
- Penetration of sperm into the oocyte, fertilization, development of a preembryo, and its transport to and implantation in the uterus

The reader is directed to Chapter 180, Infertility, Male, for specific information for the male partner. Additionally, Chapter 185 covers the definitions of infertility—primary, secondary, and unexplained.

Fecundity is defined as the couple's chance of conception in a single menstrual cycle. The normal monthly success rate for couples trying to conceive naturally at age 25 is 25%. This figure decreases with increasing age, particularly after 35 years of age for women. It was once believed that a woman's age was the only major contributing factor; however, it is now known that male fertility declines with age concurrent with abnormalities evident in semen parameters.

TABLE 184.1 Causes of Female Infertility

Ovulation disorders (40%)	Aging
	Diminished ovarian reserve
	Endocrine disorder (e.g., hypothalamic amenorrhea, hyperprolactinemia, thyroid disease, adrenal disease)
	Polycystic ovary syndrome
	Premature ovarian failure
	Tobacco use
Tubal factors (30%)	Obstruction (e.g., history of pelvic inflammatory disease, tubal surgery)
Endometriosis (15%)	
Other (approximately 10%)	
Uterine/cervical factors (>3%)	Congenital uterine anomaly
	Fibroids
	Endometrial polyps
	Poor-quality/quantity cervical mucus (caused by smoking, infection); mucous hostility (sperm antibodies)
	Uterine synechiae or adhesions (Asherman's syndrome)

Data from Jose-Miller, A., Boyden, JW, Frey, KA. Infertility. *Am Fam Physician.* 2007;75(6):849–856.

The probability of a couple's fecundity is calculated to determine the monthly chance a couple has of conceiving a child. It factors in a number of variables and considerations for both the male and female partners. In diagnosing the cause of infertility, it is important to consider all variables as pieces of a couple's reproductive and fertility health. The clinical presentation may suggest either blatant fertility impediments (e.g., genetic abnormality) or each partner may have minor health concerns that, when combined, constitute major impediments to a successful fertility outcome (Table 184.1).

Assisted Reproductive Technologies and Natural Medicine

The statistics suggest that 4.6% of U.S. infants born in 2005 (95% uncertainty range: 2.8%–7.1%) resulted from ovulation treatments not involving assisted reproductive technologies (ARTs). National ART Surveillance System data indicate that ART treatments currently account for 1.2% of total U.S. live births, 16% of U.S. twins, and 38% of U.S. triplets or higher-order (quadruplets or more) live-born infants.[7] Therefore the prudent clinician must be aware of potential interactions and consider each treatment protocol accordingly. It is advisable for clinicians to recommend the following approach to their patients for safe, integrated treatment:

- Before an in vitro fertilization (IVF) cycle, a 3- to 4-month preconception program for both partners should be encouraged to ensure appropriate nutritional status and detoxification to optimize successful outcome.
- Any causative or contributing factors that may hinder the success of an IVF cycle should be investigated and eliminated thoroughly.
- Any preexisting health conditions during the preconception window of 3 to 4 months must be addressed and treated.
- During an IVF cycle, it is crucial to enable optimal communication between the naturopath and fertility specialist to prevent any interactions. Dietary and lifestyle recommendations should be encouraged; however, nutritional and/or herbal medicine prescriptions must consider potential negative interactions with IVF medications. As such, all prescriptions must be individualized.
- Once IVF has concluded and a successful outcome has been attained, treatment can be modified to support the pregnancy, focusing on miscarriage prevention in the first trimester and then adjusting as the pregnancy continues.

Optimizing Natural Fertility

Natural conception is clearly the ideal scenario for any couple. It is therefore necessary to assess a few key variables, including ovulation detection and the timing and frequency of intercourse. Before infertility is diagnosed, the current recommendations are to encourage couples to attempt conception for a minimum of 12 months of unprotected, appropriately timed intercourse. The time period is reduced in couples where the female is older than 35 years. In these couples, a diagnosis of infertility is achieved after 6 months.

A couple's fertility is generally highest in the first few months of unprotected sex and declines gradually thereafter. If no conception occurs within the first 3 months, monthly fecundity decreases substantially among those who continue their efforts to conceive.[8] Therefore couples who are likely to conceive naturally are likely to do so in a few short cycles. In cases where conception takes longer, other impediments may be present. These may be physical or genetic factors, which may require assistance from ART. However, the cause may simply be a compounding health variable such as a blatant nutritional deficiency. Appropriate investigations, as discussed later on in this chapter, will elucidate the approach required.

Couples should be encouraged to attempt conception during the fertile window. A recently ovulated egg will survive for only a few hours (maximum of 24 hours); however, sperm can survive for up to 5 to 6 days in the presence of fertile-quality cervical fluid. The fertile window is best defined as the 6-day interval ending on the day of ovulation.[9] As estrogen increases in the female in the lead-up to ovulation, she produces fertile-quality cervical fluid, which protects the sperm from the acidic pH of the vagina, provides a medium in which it can travel, and provides nutritional sustenance for it to survive on its journey.

A widely held misconception is that frequent ejaculations decrease male fertility. A retrospective study analyzed 9489 men with normal semen quality, sperm concentrations, and motility and found that profiles remained normal even with daily ejaculation.[10] Of more importance is the finding that males with abnormalities such as oligozoospermia, lowered sperm count, and poor motility concerns may be improved with more frequent (daily) ejaculation.[10]

In supporting patients with natural conception, ovulation detection is a key component. Box 184.1 includes the main methods available and highlights their most appropriate application.

DIAGNOSTIC CONSIDERATIONS

Female Reproductive Assessment

A thorough assessment of the female patient is crucial to accurately determine her general and fertility health. Some of these assessments may require referral to a fertility specialist, gynecologist, or endocrinologist; however, thorough questioning should be conducted by the naturopath to elucidate a full history and assess causative or contributing factors (Table 184.2). All fertility patients should be screened with the queries in Table 184.3, and stage 1 investigations should be conducted with all fertility patients (Table 184.4).

Miscarriage

The comprehensive coverage of the topic of miscarriage is beyond the scope of this chapter. Because the incidence is as high as one in four pregnancies,[11] the prudent clinician should be informed about the background, useful assessments, and treatment approaches to miscarriage as part of the holistic approach to female fertility.

BOX 184.1 Ovulation Detection

Fertility charting incorporates consideration of basal body temperature (waking temperature) and changes in both cervical fluid and cervical positions.

Waking temperature is a hindsight measurement that confirms ovulation occurrence. It is only able to assist in *predicting* ovulation once a woman is aware of her cyclic changes. It is used to confirm the luteal shift to progesterone and is beneficial for assessing the lengths of the follicular and luteal phases. It is also used to assess progesterone stability in the luteal phase to support implantation and pregnancy; it can also confirm anovulatory cycles. Progesterone causes the endometrium to support the implantation of a fertilized ovum. It also causes temperature to rise perceptibly, typically 0.4°F/0.2°C. Temperature is best taken immediately on waking after a minimum of 6 hours of unbroken sleep via oral assessment.

Fertile-quality cervical fluid is produced 3 to 5 days before ovulation in response to increasing estrogen levels before the luteinizing hormone (LH) surge. Sperm can theoretically survive for 5 to 6 days in the presence of fertile-quality cervical fluid. The role of cervical fluid is multifaceted; it buffers the pH of the vagina to provide a hospitable environment for sperm survival; provides a medium for sperm to swim in on their journey through the female's reproductive system; provides nutritional supplementation for sperm survival; and acts as a lubricant to increase sexual pleasure and increase frequency of intercourse, thus providing more opportunities for conception. Assessment should be conducted from the vagina (inserting a finger into the vaginal opening and extracting fluid for assessment) rather than relying on toilet paper or underwear changes.

Cervical position assessment is the most controversial of self-assessments, with reproductive specialists often discrediting its value. This is because the position of the cervix is affected by numerous variables, including the timing of bowel movements. The cervical tissue is responsive to fluctuations in estrogen and produces physical and tangible changes when ovulation is approaching. Signs of fertility include a softening, opening of the cervical os, increased wetness from cervical fluid, and a lengthening of the vaginal canal as the cervix shortens away from the vaginal opening. Signs of infertility include a closed os, hardening, shortening, and dryness.

Urine-based ovulation testing detects the LH surge that occurs before ovulation, typically occurring 24 to 36 hours before ovulation. Testing can produce false positives and is inadvisable in women with polycystic ovary syndrome (PCOS) or other, similar conditions due to LH increases in these populations. Additionally, because sperm require an optimal 2 days' travel time, detection of the LH surge can often be too late to optimize conception. It is therefore advisable to make sure that all variables are synchronistic rather than isolated. Additionally, there are some women whose LH surge is shorter (12 hours or less). This can occur in those with hyperprolactinemia and other conditions that interfere with follicle-stimulating hormone (FSH)/LH secretion from the anterior pituitary. In these individuals it is advisable to encourage LH testing twice a day (i.e., morning and night), as they can often "miss" the surge.

Saliva assessment testing and tools base their justification on the premise that with increasing estrogen fluctuations, changes in cervical fluid are synchronistic with other fluid media, including saliva. Fertility potential is detected by the presence of a "ferning" pattern in the saliva (viewed by microscopy) suggestive of a synchronicity in cervical fluid changes that enable sperm travel. This method is inappropriate for older patients owing to natural estrogen reductions and is also not optimal for hypothyroid patients because of the relationship between estrogen and thyroid function. Additionally, caution with patients experiencing estrogen displacement, such as those with fibroids and/or endometriosis, is advisable.

TABLE 184.2 Fertility Enquiry

Assessment	Elaboration and Explanation
Age	What are the ages of the couple?
Fertility history	How long have they been trying to conceive, and have they ever conceived previously (together/separately)? Do they have any idea why they have not been able to conceive?
Sexual history	Potential sexually transmitted disease exposure, symptoms of genital inflammation (e.g., vaginal discharge, dysuria, abdominal pain, fever)
Medical history	Genetic disorders, endocrine disorders, history of pelvic inflammatory disease
Medication history	Hormone therapy, contraceptive pills, psychotropics, nonsteroidal anti-inflammatory drugs (NSAIDs)
Surgical history	Such as previous reproductive or genitourinary surgery
Contraception	When it was ceased and the likely speed of its reversibility
Fertile times	Whether the couple engage in regular intercourse during fertile times
Lifestyle factors	Diet, exercise, alcohol, smoking cessation, recreational drug use, caffeine, environmental toxin screen
Prior paternity	Previous fertility
Psychosexual issues	Interference with conception
Pubertal development	Poor progression can suggest underlying reproductive issue; initial query regarding age of menarche and secondary sexual characteristics to eliminate Turner's syndrome and the like
Physical examination	Breast formation Galactorrhea Genitalia (e.g., patency, development, masses, tenderness, discharge) Signs of hyperandrogenism (e.g., hirsutism, acne, clitoromegaly) Body mass index and waist:hip ratio to assess weight effect

Modified from Hechtman, L, 2018, Clinical naturopathic medicine. Sydney, Australia: Elsevier; 2011.

TABLE 184.3 Stage 1 Investigations

Assessment	Timing in Cycle	Justification
Follicle-stimulating hormone (FSH)	Day 2–3	Stimulates follicle development. High levels can indicate menopause or declining fertility; query primary ovarian failure. Useful to assess ratio comparative to luteinizing hormone to ensure that hormone status is optimal. Eliminates primary ovarian failure.
Luteinizing hormone (LH)	Day 2–3 or preovulation (day 13)	Triggers ovulation when surges; excessive levels may indicate polycystic ovary syndrome (PCOS). Useful to assess ratio comparative with FSH to ensure hormone status is optimal.
Progesterone (P4)	Day 21 (7 days postovulation)	Evaluates adequacy of progesterone; confirms ovulation. Eliminates luteal-phase defect.
17-OH-progesterone (17 hydroxy progesterone)	Follicular phase of cycle—preferably day 2–3	Ensures adrenal involvement is eliminated from affecting ovulation and progesterone insufficiency.
Prolactin (PRL)	Any day	Inhibits ovarian production of estrogen, inhibitory role with progesterone if high, stimulates production of breast milk. Ensure women sit relaxed for 20 min before blood draw for accuracy of result.
Estradiol (E2)	Day 2–3	Stimulates egg maturation and endometrial maturation for implantation; responsible for fertile-quality cervical fluid
Testosterone (TT), free androgen index (FAI), androstenedione	Any day	Eliminate PCOS or testosterone dominance
Specific hormone-binding globulin (SHBG)	Any day	Evaluates if concentration of SHBG is affecting the amount of testosterone available to body tissues
Transvaginal ultrasound	Day 7–11	Evaluates follicle maturation, ovulation, endometrial thickness, and character. General assessment of pelvic organs for diagnosing abnormalities of the uterus and ovaries. Assesses thickness and appearance of endometrium to be followed. Enables antral follicle count.
β-hCG	Any day	Eliminate pregnancy or tumor.
Anti-Müllerian hormone (AMH)	Day 2–3	Assesses ovarian reserve (best when combined with antral follicle count through ultrasound)
General Health Screen		
CBC, UEC, LFT, iron studies, TSH and urinary iodine (24-hour or morning spot); plasma zinc and serum copper (including ratio); ceruloplasmin, red blood cell folate, active B_{12}, homocysteine, 25[OH] D, Fasting glucose, cholesterol profile, celiac screen, celiac HLA, Pap smear	NA	General health assessments to eliminate other abnormalities
General Prepregnancy Screen		
Blood group and agglutinins, infectious disease screening, rubella immunity	NA	General prepregnancy assessments
High Cervical Swab and Urinalysis		
Bacterial culture, screen for sexually transmitted infections	NA	Assess for infective pathogen compromising fertility. General urinalysis to eliminate underlying infection or abnormality. Urogenital infections have been found to play a part in the genesis of miscarriage[297] and infertility[298]; however, patients may be unaware of their presence owing to the asymptomatic nature of many infections. Screening for a range of genitourinary infections is necessary in preconception care to detect possible infection and thus barriers to conception. The most common and essential infections that require screening include the following: Primary genitourinary tract infections: *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium/hominis, Neisseria gonorrhea* Secondary genitourinary tract infections: *Gardnerella vaginalis,* Group B *Streptococcus,* beta-hemolytic *Streptococcus, Staphylococcus aureus, Staphylococcus millerii*

Continued

TABLE 184.3 Stage 1 Investigations—cont'd

Assessment	Timing in Cycle	Justification
Other Fertility Assessments		
Waking temperature, cervical fluid assessment cervical position assessment, ovulation testing (urine), ovulation testing (saliva)	Various	Assessment and interpretation of self-directed assessments (if used). See Box 179.1 for further discussion.
Other Considerations		
Salivary hormone screen, including reproductive and adrenal	NA	Salivary screen provides an advantage over blood levels in yielding significantly greater accuracy for interpretation of findings. The adrenal hormone profile determines adrenal function and may help determine the presence or extent of acute and/or chronic mental and/or physical stress. Prolonged stress has been shown to have suppressive effects on the fertility of both the male and female. Of importance, it is likely to have a profound effect on gonadotropin release (FSH, LH).
Nutrient and toxic element screening	NA	Assessment of toxic elements, including aluminium, arsenic, cadmium, lead, and mercury is crucial so that these can be eliminated as causative or contributing factors. It is widely accepted that excessive exposure to heavy metals has detrimental effects on fertility[299] and must therefore be assessed and remedied during the preconception period. Pre- and postchelation challenge testing should be considered for some patients.
Environmental screen	NA	Other environmental assessments including those that check for porphyrins, PCBs, chlorinated pesticides, volatile solvents, phthalates, parabens, and other toxins. These should additionally be considered owing to their deranging effects on reproductive function, endocrinology, gamete development, and thus embryological potential.

CBC, Complete blood count; *HLA,* human leukocyte antigen; *LFT,* liver function test; *PCB,* polychlorinated biphenyl; *TSH,* thyroid-stimulating hormone; *UEC,* urea, electrolytes, creatinine.
Modified from Hechtman, L. *Clinical Naturopathic Medicine*. Sydney, Australia: Elsevier; 2011.

TABLE 184.4 Stage 2 Investigations

Assessment	Timing in Cycle	Justification
Thyroid antibodies and reverse T3	NA	Indicated if thyroid function appears compromised, ovulation potential is reduced, or patient appears to have implantation issues
Sperm antibodies (serum)	NA	Determines whether antibodies are present against the partner's sperm. If blood results are positive, cervical mucus sperm antibodies may be required.
Laparoscopy, hysteroscopy, and salpingoscopy	Before ovulation	Inspection of uterotubal junctions. Diagnosis and treatment of pelvic diseases (endometriosis) or adhesions. Hysteroscopy complements hysterosalpingography (HSG) in revealing pathology that disturbs the shape of the endometrial cavity, especially intrauterine adhesions, submucosal fibroids, and endometrial polyps. These can be diagnosed with HSG, but hysteroscopy is required to locate the pathology accurately and treat it accordingly.
HSG ± selective salpingogram (if indicated)	Day 7	Vaginal examination and injection of radiographic fluid into the uterus and fallopian tubes on x-ray. Determines whether fallopian tubes are clear and uterine cavity is normal.
Clomid challenge test	Day 2–3, FSH and estradiol Day 10, FSH	Determines ovarian reserve and if pregnancy can occur before assisted reproductive techniques are implemented.
Curettage or endometrial biopsy	Day 26 (just before menses are expected)	Reveals the tissue structure of the endometrium. Estimates normal ovulation owing to timing. Not advised regularly because of risk of scarring.
Methylation genetics and profile	NA	Methylation testing to assess SAM:SAH ratio and levels as well as folate cycle intermediates to determine methylation function. Addition of methylation genetics to assess competence of pathways due to contribution of effect to impede optimal fertility.
Other	NA	If all results show no abnormality or if the patient is older than 38 years, the clinician is advised to consider a number of miscarriage assessments to improve outcomes because a number of these assessments are associated with implantation failure as well as miscarriage (see Table 184.5)

FSH, Follicle-stimulating hormone; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.
Modified from Hechtman, L. *Clinical Naturopathic Medicine*. Sydney, Australia: Elsevier; 2011.

TABLE 184.5 Miscarriage Screen

Key

General medical or naturopathic referral

Reproductive endocrinology and infertility subspecialist

Research interest in recurrent miscarriage

Assessment	Justification	Interpretation
On presentation: at any time in the ovarian (menstrual) cycle		
General health screen, including CBC, LFT, UEC, fasting glucose, blood group, and agglutinins	General medical checkup	Various; assess for compounding factors.
Red blood cell folate, active B_{12}	Unsuspected folate deficiency; if low, test fasting serum homocysteine.	Folate supplementation in vitamin B_{12} deficiency masks pernicious anemia and the risk of subacute spinal cord degeneration. RBC and serum are not definitive assessment tools for these nutrients. Additional assessments may be warranted.
Fasting homocysteine	Elevations can correlate with vitamins B_9 and B_{12} findings, presence (or absence) of MTHFR polymorphism and coagulation or vascular abnormalities	Treat as indicated with high doses of vitamins B_6, B_9, and B_{12}. Correlate findings with vitamins B_9 and B_{12} and MTHFR status.
Thyroid function tests (TFTs)	Although mild hypothyroidism is strongly associated with anovulation, even mild hyperthyroidism is strongly associated with miscarriage	It is important to keep all aspects of a TFT well within normal limits: avoid even slightly excessive thyroid replacement in cases of hypothyroidism.
Antithyroglobulin Abs (TG Abs), thyroid peroxidase Abs (TPO Abs), TSH receptor Abs	The presence of thyroid antibodies can occur with a normal TFT (especially TPO Abs) and is strongly correlated with a first-trimester miscarriage	Miscarriage is increased in the presence of any of the thyroid Abs directly correlating with immune modulation requirements of gestation (i.e., implantation and throughout the first trimester at individual increments).
Fasting plasma glucose	Assess for preclinical diabetes, If raised, assess insulin and HbA_{1c} (± GTT) and assess for PCOS factors	Miscarriage is increased in overt diabetes and PCOS.
Serum testosterone, free androgen index, SHBG, ± androstenedione	Screen for PCOS. If results are positive, confirm with day 7 ultrasound.	Miscarriage is more likely to occur due to disordered follicular development and oocyte function.
Serum gliadin antibodies (IgG, IgA), serum IgA levels, and antibodies to tissue transglutaminase or endomysial antibodies. If the patient is already on a low-gluten or gluten-free diet, referral for celiac gene screen is advisable. Note that it is only 95% accurate, and a negative reading is not conclusive.	Asymptomatic celiac disease an occasional (though not unequivocal) cause of unexplained infertility or repeated miscarriage. Underdiagnosed in young adult women (population prevalence 1:250 and satisfies WHO criteria for population screening[300]).	Confirmation is via small bowel biopsy needed for diagnosis; however, this will require consumption of gluten for 3 months. Objective clinical opinion is required to warrant this necessity in the fertility context. Note that missing celiac disease in a woman with otherwise unexplained reproductive dysfunction might prove embarrassing when, later, it manifests more classically. Classic presentation may include marked anemia and/or general nutritional deficiencies
Pregnancy infection screen (rubella, Hep B, Hep C+, HIV, toxoplasmosis, CMV, EBV)	Each infection can correlate with miscarriage eventualities (dependent on each infection and timing in pregnancy)	As indicated with each assessment
Serum copper, plasma zinc, and ceruloplasmin	Wilson's disease, a rare copper storage disorder, can present in young women with repeated miscarriage before other symptoms and signs develop (liver disease, Kayser–Fleischer rings, cerebral dysfunction).[301] Screen for high copper and ceruloplasmin levels; confirm with elevated 24-hour urinary copper excretion (and then liver biopsy). In assessing zinc status, a 1:1 zinc:copper ratio should be observed. Zinc supplementation can then be calculated on an as-required basis.	Population prevalence is ~1:30,000 (in any ethnic group); ATP7B mutant gene carrier frequency is ~1:90; more than 100 mutations are known and affected people are usually compound heterozygotes. Possible mechanism: raised copper levels in uterine secretions compromising mitochondrial function in the conceptus. Additionally, raised copper levels are antagonistic to zinc absorption and will warrant supplementation. Depending on copper reading, treatment with copper-binding agents may be required and has been shown to reverse pregnancy losses.
Prolactin and βhCG	Assess effect of these hormonal levels on pregnancy sustenance and achievement.	Address as indicated; typically, hormonal modulation is required to ensure that hormone levels are optimal for each stage of pregnancy (and preconceptually).

TABLE 184.5 Miscarriage Screen—cont'd

Assessment	Justification	Interpretation
Karyotype, peripheral blood (both partners)	Assess for balanced chromosomal translocation in either partner. If an imbalance is detected, couples are prone not only to conceive embryos with unbalanced translocations; their unstable meiotic spindles also make otherwise unremarkable aneuploidies more common.	Referral for genetic counseling to estimate unbalanced chromosomal segregation patterns and initiate PGD if required.
Hysterosalpingogram	To assess tubal patency and fallopian tube structure	Address as relevant
Antinuclear antibody, anticardiolipin antibody (IgM, IgG), lupus inhibitor/anticoagulant, IgA	Screening for the antiphospholipid syndrome, either secondary to SLE or, more commonly but often less aggressively, primary	Laboratory testing for anticardiolipin antibody is not well standardized, and interpretations of isolated elevations are difficult. SLE requires referral to immunologist or rheumatologist. Treatment of definite cases is treated through allopathic means with low-molecular-weight heparin, vaginal progesterone (immunosuppressive locally), corticosteroids, and occasionally plasmapheresis. Naturopathic means include immune modulation, blood coagulation normalization, and dietary modification to support appropriate immune and hematologic systems.
CA-125	Presence does not automatically confirm cancer; however, it can correlate with cancerous conditions, endometriosis, or proliferative disorders	In instances of endometriosis can indicate increased development, poor management, or contributing factors to miscarriage
Anti-TjA antibody (anti-PP1Pk hemolysin)	Rare but well described[302]	Plasmapheresis and immunoglobulin replacement during pregnancy can enable term delivery[303]
Thrombophilia screen	Protein S, protein C, activated protein C (APC) resistance, antithrombin III	Protein S deficiency: second-trimester miscarriage increase × 7.4. Protein C deficiency: less convincing APC resistance: first trimester × 2.1, second trimester × 3.317
Thrombophilia PCR testing (DNA assessments)	Test for factor V Leiden, prothrombin G20210A, MTHFR C677T, and A1298C	Meta-analysis[304] reports the following increased risks of miscarriage with homozygosity: Factor V Leiden: first trimester × 2.1, second trimester × 3.3. Prothrombin G20210: first trimester × 2.3, second trimester × 2.3. MTHFR alleles (increase miscarriage risk with elevated homocysteine): × 4.3 (if controlled with adequate folate may actually protect against miscarriage and intrauterine growth retardation). The naturopathic model also acknowledges the effect of MTHFR on folate metabolism and its subsequent effects on ensuring that DNA replication within each cell (including the cells of the fetus) is consistent. Additionally, folate is responsible for a number of other roles in the body. Therefore an MTHFR abnormality can correlate with a number of other health concerns regardless of homocysteine status.
Day 2–3 of the menstrual cycle		
Serum FSH, LH, estradiol (E2) Note: ovulation tracking over 2 cycles is advisable (E2, LH, and P4)	Reduced oocyte numbers: serum FSH > 11 U/L PCOS: suspected when LH:FSH (U/L) > 2:1. In either case, E2 > 200 pmol/L = more severe. Correlate any findings with a day 7 ultrasound.	Low oocyte numbers: risk of early menopause; IVF may be indicated if also infertile. PCOS: requires additional treatment changes, incidence correlates highly with increased miscarriage risk.
Anti-Müllerian hormone (AMH)	Reduced levels may indicate failing ovarian reserve. Increased levels can indicate PCOS.	Interpretation is controversial; however, in instances of reduced AMH, refer to fertility specialist and possibly concurrent ART.
Day 7 of the menstrual cycle (or estimated 1 week before ovulation) Transvaginal ultrasound		
Uterine assessment	Normal endometrium is >5 mm thick and echolucent (thick echogenic endometrium means hyperplasia or polyp; if it is thin, consider endometritis). Assess for fibroid or diagnose other uterine abnormalities.	Treat specific to finding (i.e., thin endometrium will require progesterone support; thick endometrium will respond to uterine tonics, such as herbal medicines) and antioxidant regulation. For fibroid-specific treatment, see Chapter 223.

Continued

TABLE 184.5 Miscarriage Screen—cont'd

Assessment	Justification	Interpretation
Ovarian assessment	Follicles present in the ovary indicate the typical number of eggs achievable: many peripheral follicles indicate PCOS; few follicles can indicate premenopause (or oopause).	If PCOS, low follicle numbers or endometriotic cyst, specialist management is indicated, possibly including IVF.
Vaginal and cervical swabs and culture (cervical swabs are often indicated for the most accurate interpretation)	Vaginosis, either *Gardneralla vaginalis* or other organismal overgrowth, with loss of *Lactobacillus*. Primary genitourinary infections: *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium or hominis, Neisseria gonorrhoeae* Secondary genitourinary infections: *Gardnerella vaginalis,* group B *Streptococcus,* beta-hemolytic *Streptococcus, Staphylococcus aureus, Staphylococcus millerii*	Urogenital infections have been found to play a part in the genesis of miscarriage[14,305] and infertility[298]; however, patients may be unaware of their presence due to the asymptomatic nature of many infections. Midtrimester (and later) pregnancy losses can occur after internal cervical os becomes covered by growing sac, at 13 weeks. Vaginal flora modulation is required. Herbal douches, probiotic supplementation, and in some instances, antibiotic treatment.
7 days postovulation (typically days 21–28)		
Serum progesterone (P4)	Screen for ovulation confirmation	If <35 nmol/L, consider further investigation confirming ovulation. Support progesterone cascades as required.
12 days postovulation (premenstrual phase)		
Premenstrual endometrial biopsy for dating	Check integrated action of almost 2 weeks of progesterone on decidualizing the endometrium; confirm progesterone falling to low level and negative βhCG day before test. Extremely time-critical investigation: usefulness lost if performed in midluteal phase.	Often deficient stromal decidualization in disorders of ovulation (adjust treatment as indicated if present). A deficiency can also indicate endometrial atrophy (which can be untreatable) or, rarely, a molecular defect: in these two cases, gestational surrogacy may be the only treatment option. Best screen for rare molecular bases for decidualization failure causing implantation failure.
Premenstrual endometrial biopsy for lymphocyte (T-cell) subsets on immunocytochemistry [with adjunct T-cell subsets in blood]	Specialist labs can look for relative abundance of peripheral NK cells (CD57+) versus normal uterine (CD56+) NK cells in relation to T-cell numbers[306]	If unfavorable, consider vaginal progesterone during early pregnancy. Relevance of findings is conflictual. Naturopathic treatment measures adapt to module the immune response in relevant instances.

ART, Assisted reproductive technology; *βhCG,* beta-human chorionic gonadotropin; *CBC,* complete blood count; *CMV,* cytomegalovirus; *EBV,* Epstein–Barr virus; *FSH,* follicle-stimulating hormone; *GTT,* glucose tolerance test; *IgA,* immunoglobulin A; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *IVF,* in vitro fertilization; *LFT,* liver function test; *LH,* luteinizing hormone; *MTHFR,* methylene-tetra-hydro folate reductase; *NK,* natural killer; *PCOS,* polycystic ovary syndrome; *PCR,* polymerase chain reaction; *PGD,* pregenetic diagnosis; *RBC,* red blood cell; *RM,* recurrent miscarriage; *SHBG,* sex hormone–binding globulin; *SLE,* systemic lupus erythematosus; *TPO,* thyroid peroxidase antibodies; *TSH,* thyroid-stimulating hormone; *UEC,* urea, electrolytes, creatinine; *WHO,* World Health Organization.
Data from Jansen R., Gee A. Testing for miscarriage. *O&G Magazine*. 2008:10(2):48–52.

Implantation of the developing embryo into the receptive endometrium is a critical and key event in the establishment of early pregnancy. The unique process of cell adhesion of the trophoblast to the endometrium at the time of implantation and its subsequent invasion into the maternal tissue is dynamically balanced through the expression of specific cell adhesion molecules and endocrine, paracrine, and autocrine signals. The process can be viewed as a series of distinct events, many of which are similar to those of an inflammatory reaction and are greatly influenced by the factors present in the uterine microenvironment, including hormones, growth factors, and inflammatory and proinflammatory cytokines.[12] The secretion of a number of factors achieves modulation of the immunological response of the trophoblast. Cytokines produced at the fetal-maternal interface play a key role in regulating maternal tolerance to the fetus and successful pregnancy.

Although approximately 25% of all recognized pregnancies result in miscarriage, less than 5% of women will experience two consecutive miscarriages, and only 1% experience three or more.[11] Recurrent pregnancy loss is a disease distinct from infertility, defined by two or more failed pregnancies.[13] When the cause is unknown, each pregnancy loss merits careful review to determine whether specific evaluation may be appropriate. Although the general consensus is to evaluate after three or more losses, it is more in keeping with the naturopathic paradigm to review all patients even after the first and more comprehensively after the second or subsequent miscarriage.

The risk of miscarriage is highest immediately after implantation. It is thought that around 50% of all fertilized eggs do not survive, coming away with a normal (or slightly late) period. This is often referred to as an "unnoticed miscarriage" because it is usually not formally acknowledged by the woman, who is never aware of her pregnancy.

In summarizing the possible clinical scenarios, it is useful to group them into three distinct categories:

1. For a healthy, viable pregnancy to initiate and continue successfully, a number of biochemical, anatomical, and molecular events must occur in a systematic and timely manner. Any deviation can disrupt the process and abort the pregnancy.
2. The overlapping clinical continuum of presentation can conflict with or complicate the fertility presentation. Each clinical presentation

has the potential to present as infertility (occult implantation failure), subclinical or clinical miscarriage in the first trimester, a fetal loss in the midtrimester before viability, a premature birth, or as a birth defect presenting at or after term. Each presentation is unique and must be considered holistically and individually.

3. The number of similarities between previous miscarriages (if any) can enable the clinician to ascertain the most definitive scenario and develop and adjust treatment accordingly.
4. As seen in Table 184.5, various conditions can be associated causally or coincidentally with miscarriage.

Classic Causes of Miscarriage

- Genetic/chromosomal: 60% or more of early miscarriages are due to chromosomal abnormalities.
- Age: after age 40, one third of pregnancies end in miscarriage.
- Hormonal abnormalities: luteal-phase deficiencies (low progesterone) are the most common cause. This can relate to hyperprolactinemia.
- Metabolic abnormalities: poorly controlled blood sugar levels or diabetes mellitus and polycystic ovary syndrome increase miscarriage risk.
- Uterine abnormalities: distortion of the uterine cavity can account for 15% to 20% of miscarriages.
- Antiphospholipid syndrome: this can account for 3% to 15% of pregnancy losses.
- Thrombophilias: this creates an increased risk of thrombosis and consequent increased risk of pregnancy loss, especially in the second half of pregnancy.
- Male factors: abnormal sperm DNA can result in pregnancy loss (see Chapter 185 for a full discussion).
- Unexplained: no explanation is found in 50% to 75% of couples.

Unexplained Causes of Miscarriage

When the causes of miscarriage are unexplained, the naturopath can provide the most beneficial support and care. A miscarriage, especially a first-trimester loss, can occur for any number of natural biological reasons. A marked deficiency in any nutrient (macro or micro) can theoretically cause a miscarriage. A full discussion of macro- and micronutrients and their relation to pregnancy loss is offered later in this chapter. Additionally, the naturopath's consideration of the patient's general health and comprehensive review of all other factors is invaluable.

THERAPEUTIC CONSIDERATIONS

Supporting Female Fertility

On being asked to consider a patient with infertility, it is crucial for the practitioner to assess several factors initially to determine the best approach to treatment. As in the case of male fertility, naturopathic treatment cannot address all variables, such as genetic factors or overt physical impediments; however, it can attenuate various presentations. In the clinician's initial assessment, the prime objective is to assess the patient fairly and holistically. Therefore consideration should be given to the patient's previous pregnancy, duration of infertility, age of partner, severity of present pathology, and other factors. For example, in the case of a 41-year-old female who has had no prior pregnancies, has been infertile for 2 years, and suffers from endometriosis, it is crucial to initiate integrative care with a reproductive endocrinology and infertility (REI)/fertility specialist because ART is likely to be required. If this patient had sought consultation 4 years earlier, time constraints would not be as severe, and the naturopathic paradigm would possibly be of help. Unfortunately, age is one of the variables that cannot be modified. Similarly, if the couple incorporates donor gametes, the clinician must acknowledge the potential limitations to treatment.

A useful summary to delineate the possible approaches is as follows:

- Treatable conditions: Some patients present with blatant deficiencies or disease processes that require interventions to improve their natural fertility. Most people respond positively to a preconception program focusing on detoxification, which enables conception more readily at the conclusion.
- Untreatable subfertility: After naturopathic intervention, some infertility factors may not be possible to address, or alternatively, referral for ART/IVF may be required.
- Untreatable female sterility: This can occur in instances of mature patients, genetic disorders, or previous conditions that compromise fertility. Adoption or donor oocytes are the only possibilities for couples in this group who wish to have a family. Donor oocytes may not be an option for all patients.

Naturopathic Preconception Treatment

As in the case of male infertility, during naturopathic preconception treatment, the most optimal approach for females is to encourage a program that correlates with the final stages of gamete development (oocytes). Although women are born with all of their oocytes at birth, there are limited treatment options to remedy the primordial stage of development (unless we can modify potential epigenetic factors). Because the primary follicle development from the primordial follicle takes up to 120 days, preconception care is best prescribed within this time period. Following this treatment approach, we can successfully influence additional stages of oocyte development (secondary → antral → Graafian). By influencing the oocyte's environment during development and the correlating general health of the patient, practitioners are able to positively influence the development of oocytes and address and attenuate related health concerns.

The principles of the preconception approach are to attenuate preexisting conditions; optimize nutritional repletion; detoxify the body from dietary, lifestyle, and environmental perspectives; and encourage a healthy approach to parenting on all levels. It is imperative to acknowledge that nutritional repletion is required throughout the preconception period and that prescriptions may require higher quantities than typical to optimize the patient's status. Fertility occurs when a person's individual health is optimal. The notion of a healthy body being a fertile one cannot be ignored. As discussed in Chapter 185, the body is primed to pass genetic material only when the environment and conditions are at their best. If survival from an evolutionary perspective is compromised, fertility will be hindered. Everything a person eats, drinks, experiences, or is exposed to can and will influence his or her fertility. True naturopathic fertility supports, acknowledges, and considers absolutely all variables for holistic health. Couples should be encouraged to participate in treatment for 3 to 4 months to properly address all genetic and epigenetic variables.

The concept of preconception care was pioneered by Foresight, the Association for the Promotion of Preconceptual Care. It was established in the United Kingdom in 1978 and is a preconception program that incorporates dietary and lifestyle advice to enhance fertility, thereby increasing the chances of a healthy conception. Readers may wish to review this research in more detail for additional information. However, published studies lack sufficient evidence owing to poor study design and delivery (one was provided as a letter to an editor and is referred to as a study); nevertheless, the evidence of successful outcomes cannot be refuted. It is hoped that further investigation into this highly effective approach will be conducted in the future.

If the practitioner is incorporating ART, it is beneficial to inform patients that cotreatment is likely to produce the best outcome. In

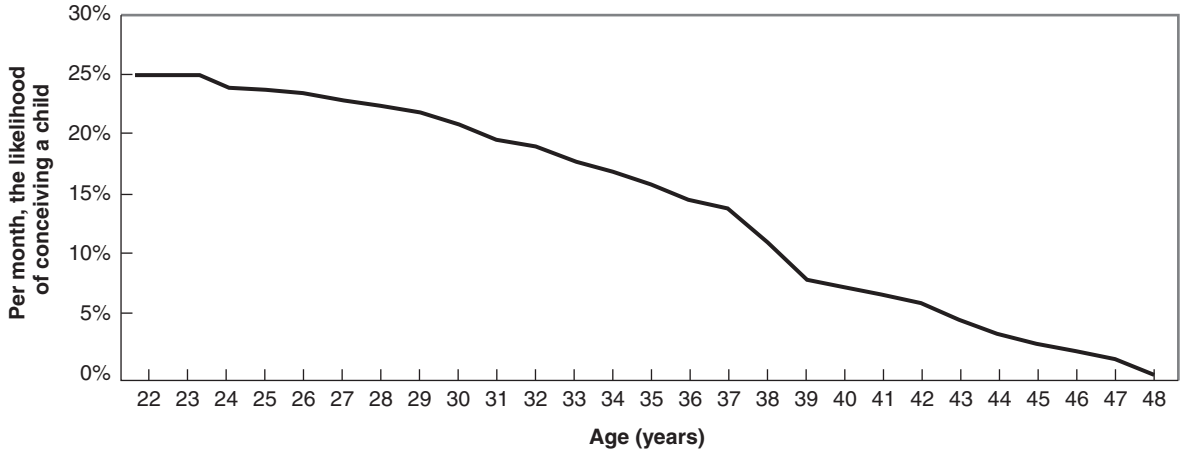


Fig. 184.1 Effect of Age on Fertility.

one study, 65% of couples who had previously undergone multiple IVF cycles were able to conceive within 2 years of a preconception program.[14]

It is important to acknowledge that most fertility patients are incorporating several treatment options concurrently.[15] It is therefore prudent to point out the scope of all treatments and to discuss individual treatment protocols openly so as to foster a collaborative approach and reduce the risk of negative interactions.

Fertility-Damaging Factors

Age. The biggest preventable cause of infertility is undoubtedly a woman's age. Approximately 20% of women wait until after age 35 to begin their families. Several factors have contributed to this trend, including contraception choices, career pathways, marrying at older ages, high divorce rates, financial security, or—most concerning—the fact that many women simply do not realize that their fertility begins to decline in their late 20s, with a rapid decrease from age 35 onward. After 35 years of age, a women's fertility declines rapidly in conjunction with the deteriorating quality of her oocytes (Fig. 184.1). She may enter oopause in her 30s, depending on her genetic makeup, thus further reducing the chances of a successful fertility outcome. Regardless of a woman's health, age-related changes cannot be prevented or modified. The age of a woman's oocytes is fixed, and as she ages further, fertility potential undergoes a statistical decline. Although over 95% of healthy couples will conceive within 3 years of trying when the woman is 35 years of age, that figure drops to 77% when she reaches age 38. By the age of 41, less than half of the healthy couples will conceive after 3 years of trying.

A number of considerations regarding the effects of age on fertility should be considered. These include the following[4]:

- Ovarian function: As women age, fertility declines owing to normal age-related changes in the ovaries. Women are born with all the oocytes they will ever have, with approximately 300,000 follicles remaining at menarche. They will then lose between 20 to 30 follicles each cycle. Of the oocytes remaining at puberty, it is believed that approximately 360 to 400 of these follicles will mature over 30 years and be released by the body, with the remainder being lost in each cycle. The rest will undergo atresia, a degenerative process that occurs regardless of whether a woman is pregnant, has normal menstrual cycles, uses birth control, or is undergoing infertility treatment. Smoking appears to accelerate atresia and is linked to earlier menopause.
- Ovarian reserve: Ovarian reserve declines with each cycle. Diminished ovarian reserve is usually age related and occurs because of the natural loss of eggs and decrease in the average quality of the eggs that remain. The remaining eggs become "poor responders" to follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thus shortening the menstrual cycles. Young women may also have reduced ovarian reserve because of smoking, a family history of premature menopause, prior ovarian surgery, or even if they have no known risk factors.
- Genetic abnormalities: Aging affects oocyte quality and increases the risk of neonatal genetic abnormalities. As a female ages, her risk for having a child with Down syndrome and total chromosomal abnormalities increases dramatically. At 20 years of age, her risk for producing a child with Down syndrome is 1 in 1667 (1 in 526 total risk for any chromosomal abnormalities); at 35 years of age, that risk increases to 1 in 378 (1 in 192). At 45 years of age, the risk increases again to 1 in 30 (1 in 21).[16]
- Miscarriage risk: Older oocytes increase the risk of miscarriage. The risk of miscarriage remains at 10% between 15 and 29 years of age; it then increases to 12% at 30 to 34 years of age and to 18% at 35 to 39 years of age. Thereafter, it escalates significantly to 34% at 40 to 44 years of age and to 53% after the age of 45.[16]

Weight balance. For optimal conception, women must ensure that their body fat percentage is between 20% and 25% to support fertility and the subsequent health of their child. A body fat percentage below 17% can result in anovulation, with some research suggesting that it can take as long as 2 years before regular conception occurs even after body fat percentage has been corrected.[17] Women with a body mass index (BMI) below 19 who have irregular or absent menses should be advised to gain weight to improve their chance of conception. Malnutrition can be a relatively simple solution for conception difficulties (provided mental health issues are addressed).

Although anorexia or underweight is a concern, obesity poses a similar and significant risk for infertility. The effect of obesity is much more comprehensive than that of underweight, and the necessary treatment is easier to prescribe.

Obesity affects female reproduction by disturbing the general body metabolism, hormone metabolism, and the follicular environment. Obesity is associated with the following:

- Suboptimal oocyte quality and development, oocyte fertilization, and embryo development and implantation[18,19]
- Adverse effect on ovarian reserve[20,21]
- LH surge and corpus luteum dysfunction[22–24]

The risk of infertility in obese women is three times that of normal-weight women.[25] A systematic review of the literature concluded that weight-loss interventions were likely to result in ovulation improvements and pregnancies. Miscarriage rates were not affected by weight-loss interventions.[26] In a retrospective cohort study, meaningful weight

loss (10% of their maximum weight) was found to increase conception rates (88% vs. 54%) and live birth rates (71% vs. 37%) in patients with infertility compared with women who did not lose weight.[27]

The effect of obesity on reproductive function is as follows[28]:

- Ovulation potential: Ovulation returns with a relatively modest degree of weight loss from diet and exercise. Approximately 90% of obese women will resume ovulation if they lose more than 5% of their pretreatment weight, and 30% will conceive. In very obese women, these statistics are higher for pregnancy rates than can be expected from a single IVF cycle. This recommendation is crucial and encouraging for obese women wishing to have children.
- IVF outcome: The success rate of ART is significantly reduced in obese women. IVF success rates may be reduced by as much as 25% in obese patients and 50% in very obese patients. A retrospective cohort study of 4609 women with obesity found a 68% reduction in live births from an ART cycle compared with women of normal weight.[29] It has been proposed that altered endometrial gene expression in obese patients may contribute to the lower implantation rates and increased miscarriage rates seen in obese infertile patients.[30] Obesity also promotes inflammatory markers that are negatively correlated with pregnancy rates in women undergoing IVF treatment.[31] In women undergoing ART, obesity has a greater effect on fertility in women under 35 years of age. After age 35, age becomes a more important factor in regard to the chance of conception than obesity.[18,32] IVF centers are very reluctant to perform fertility treatment on women with a BMI greater than 35 and generally recommend a target BMI of 30 before starting treatment.[33]
- Miscarriage: The risk of miscarriage in the first trimester increases from 12% to 15% in normal-weight women under 37 years of age; for women with a BMI above age 35, it increases to 31%. The rate of recurrent miscarriage increases fourfold in obese women.
- Birth defects: Maternal obesity has a detrimental effect on fetal development (e.g., three times the risk of neural tube defects and structural heart defects). Folic acid supplementation is less effective in preventing neural tube defects, so high-dose supplementation is required for obese women (it is always beneficial to calculate nutrient requirements based on the patient's presenting weight, using Clark's rule).
- Pregnancy complications: Pregnancy complications are more common in obese women (gestational diabetes is six times more common), and pregnancy-induced hypertensive diseases are more common (specifically, gestational hypertension and preeclampsia). Complications increase the risk of premature delivery and/or the need for cesarean section. Babies born to obese women have heavier birth weights (fetal macrosomia), which increases the risk of traumatic vaginal delivery. Additionally, there is a higher complication rate for cesarean and vaginal deliveries in obese women (e.g., excessive blood loss, thromboembolic disease, and postoperative infection).
- Pregnancy outcomes: Increasing BMI increases the risk of stillbirth (twice the risk vs. normal-weight women).
- The newborn: Maternal obesity carries long-term risks for the newborn infant (e.g., increased risk of being overweight as an adult and the likelihood that these children will have weight-related diseases in adulthood).

A recent prospective cohort study of 501 couples found that the body composition both partners needs to be taken into account. In couples where both partners are obese, a longer time to pregnancy was observed than in leaner couples.[34] A recent Cochrane review was unable to suggest any evidence-based recommendations on the best interventions for improving pregnancy outcomes for women who are overweight or obese[35]; however, an individualized weight-loss program involving dietary, lifestyle, and exercise changes should be implemented.

Immunological considerations. Although this topic is beyond the scope of this chapter, the immunological considerations associated with successful conception and a healthy birth are worthy of review. Because optimal production of gametes requires a healthy immune response, any deviation from "normal" can significantly affect the outcome. In females, any immunological aspect that can affect general health, conception, implantation, and the maintenance of pregnancy can cause problems. A number of miscarriage factors (including implantation factors) relate to autoimmune diseases. Therefore stabilizing and modulating the immune response is a key objective of treatment. The immune system must "switch itself off" at conception, implantation, and a number of key stages in the first trimester. Therefore consideration of these variables is crucial.

Environmental factors. Increased industrialization and the use of agricultural chemicals has contributed to an increased exposure to thousands of chemicals now associated with a negative effect on male and female fertility. Exposure to environmental toxins such as radiation (including mobile phone use),[36] heavy metals, and chemicals can cause oxidative stress and damage to DNA, leading to a negative effect on female fertility.[37]

A comprehensive discussion of the environmental effects on fertility is provided in Chapter 185. The reader is specifically directed to the discussion regarding estrogen exposure and general environmental pollutants. Specific considerations for women should acknowledge the effect of substances more commonly associated with females, including makeup, fragrances, hair dyes, nail polishes, and other beauty products. All of these can have a negative effect on reproductive function and hormone cascades. It is therefore prudent to reduce such exposures as much as possible, specifically within the preconception window and during the first trimester, when embryogenesis and placentogenesis are paramount.

Of greater concern is the fact that gestational exposure to carcinogens, endocrine disruptors, and other toxins has been shown to affect more than one generation in some cases. Most studies unfortunately have not investigated effects beyond the third (F2) generation. Further studies are required in this intriguing area of genetics, especially because the F2 generation may have experienced the exposure directly. Chemicals known to induce phenotypic effects in unexposed generations include alloxan,[38] cyclophosphamide,[39] orthoaminoasotoluol,[40] benzpyrene,[41] diethylstilbestrol,[42] and vinclozolin.[43] The underlying mechanisms are not known, and in most cases, inherited genetic lesions cannot be ruled out, especially in cases of mutagens (e.g., cyclophosphamide). As time progresses, it is likely that these chemicals will induce greater damage to subsequent generations due to their cumulative effects and earlier generational effect.

A full discussion on potential environmental effects is provided in Chapter 35.

Sleep irregularities. Shift work is associated with negative effects on the regularity of the menstrual cycle and reproductive function and also increases the risk of an adverse pregnancy outcome. In one study, the effect of shift work on nurses (all less than 40 years of age) was reviewed; the investigators evaluated sleep, menstrual function, pregnancy outcome, and reproductive function. It was found that 53% of the women studied noted menstrual changes owing to shift work, changes that clearly could affect fertility.[44] It is therefore essential that women wishing to achieve pregnancy normalize their cycles and find a way to optimize and regulate their sleep patterns.

Lifestyle

Smoking. Both active and passive cigarette smoking are known to have a significant effect on both pregnancy rates and long-term ovarian function. Additionally, smokers are more likely to have a premature menopause, thus making smoking one of the most readily

avoidable causes of infertility. Smoking appears to reduce fertility by having a direct effect on the uterus, oocytes, and embryos by increasing the thickness of the zona pellucida.[45] It becomes difficult for the sperm to penetrate the increased thickness of the zona pellucida. Overall, research indicates that smoking can age oocytes prematurely by as much as 10 years. Considering that the average age for conception is 30 years and the average age at which fertility begins to decline steeply is 38 years, it appears prudent to recommend immediate smoking cessation coupled with systematic antioxidant therapy.

On review of the research, smoking has been shown to decrease ovarian vascularization and reduce oocyte maturation.[46] It is linked with premature ovarian aging (premature menopause) and an increased risk of miscarriage.[47] It is important to note that smoking, either current or historical, is strongly correlated with negative effects on the health of the oocytes. A trial comprising 14,779 women between the ages of 18 and 23 years showed that current smokers and ex-smokers had an increased risk of menstrual symptoms and miscarriage compared with women who had never smoked.[48] Of more importance, it was found that women who smoked 20 or more cigarettes a day and for the longest periods were most at risk. Additionally, those who started smoking at a younger age had the worst outcome.

A 2010 Cochrane review found that active and passive smoking reduced fertility and decreased the chance of a healthy live birth in both fertile and infertile populations.[49] Additionally, a number of meta-analyses have proved that smokers perform poorly in IVF/ART procedures compared with their nonsmoking counterparts.[50,51] Smoking has been associated with delayed conception and a significant decrease in the odds for pregnancy and live delivery per IVF/ART cycle. There is also a marked increase in the odds for spontaneous miscarriage and ectopic pregnancy.[52] Cigarette smoke is composed of many toxic chemicals and pro-oxidants that increase reactive oxygen species (ROS). Each stage of reproductive function—folliculogenesis, steroidogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow, and uterine myometrium—is a target for cigarette smoke components.[52,53]

Caffeine. Patients should be encouraged to avoid caffeine during the preconception period and during pregnancy. Caffeine has been shown to increase time to conception[54–56] and has also been linked with endometriosis and an increased risk of spontaneous abortion.[57] Caffeine has been shown to increase dopamine production, which in turn stimulates progesterone secretion, thus inhibiting the production of prolactin (owing to the antagonistic and regulatory interrelationship between progesterone and prolactin). Any prolactin abnormality, either excess or deficiency, can affect a woman's fertility negatively. According to several studies, as little as one caffeinated beverage a day can lead to a temporary reduction in conception.[58,59] Owing to its prolactin-modulating effects, caffeine appears to increase early follicular estrogen levels.[60] Caffeine is also likely to interfere with adrenal function and associated cortisol secretion. In addition, caffeine's diuretic effects increase the loss of nutrients that are beneficial to fertility. It is important to note that the quantity of coffee has a direct correlation with pregnancy rates. Women who drink less than one cup of coffee a day are twice as likely to conceive compared with moderate coffee drinkers.[55]

Caffeine has been shown to reach the follicular fluid. Increased serum caffeine levels are associated with a decrease in the number of harvested eggs in IVF treatment.[61] Some studies have found that coffee intake is unrelated to fertility. In women undergoing ART, coffee intake was unrelated to treatment outcomes, but consumption of caffeinated herbal tea had lower odds of achieving pregnancy during treatment.[62] In a prospective cohort study of fertile women, it was found that caffeine in general does not have negative effect on time to pregnancy.[63] Similarly, a recent prospective cohort study of 2135 women found that there was no significant association between coffee intake and fecundity. Once again, caffeinated tea was associated with slight reductions in female fecundability.[64]

Alcohol. A review of the literature regarding alcohol intake and female fertility indicates that there are many factors that can affect each woman uniquely. There are strong associations with alcohol intake and miscarriage[65]; excessive alcohol consumption is associated with hyperprolactinemia,[66] adverse effects on oocyte retrieval, and a lower chance of subsequent pregnancy in IVF[67]; and a minimum of 50% reduction in conception can be seen with intake as low as one drink per week during a menstrual cycle.[68] Additionally, research suggests that alcohol produces negative effects on blastocyst development and implantation and increases estrogen levels, causing a subsequent reduction in FSH secretion and suppressing folliculogenesis, thus leading to anovulation and infertility.[69] Acute alcohol consumption can cause increases in estradiol, testosterone, and LH levels.[70] Regular alcohol use leads to overproduction of ROS, which is associated with delayed conception. A Danish study demonstrated that alcohol might exacerbate age-related infertility. Women over the age of 30 who consumed more than seven alcohol servings per week had an increased risk of infertility.[71] Clinical studies to date have been inconclusive in their findings; however, a recent systematic review and meta-analysis of the literature concluded that female alcohol consumption is associated with reduced fertility and that the association is dose dependent.[72] It is advisable to recommend the cessation of alcohol intake during the preconception period. Conclusive evidence exists regarding fetal abnormalities and associated alcohol intake during pregnancy. This evidence can often be sufficient to motivate patients. Because detoxification is encouraged during the preconception period, the avoidance of alcohol is recommended.

Marijuana. As discussed in Chapter 61, marijuana contains cannabinoids, which have been shown to impair signaling pathways, alter hormonal regulation, and interfere with the timing of implantation.[73] Additionally, it is believed to cause ovulatory abnormalities and to disrupt ovarian function,[74] and regular marijuana use results in an elevated risk of primary infertility compared with nonusers.[52] Furthermore, marijuana has been shown to have harmful effects on the developing fetus.[75] Therefore avoidance is strongly recommended.

NUTRITIONAL CONSIDERATIONS

The Fertility Diet

There are countless papers documenting dietary modifications to support and improve a woman's fertility. Specifically, research suggests that dietary modulation improves ovulation, conception, and the birth of a healthy child.

The strongest evidence in favor of dietary modification to support fertility can be seen in the prospective study developed from the information ascertained from the Nurses' Health Study II (NHS-II).[76] Of importance, the information acquired was from 2 years before the report of infertility in this study, thus enabling a preconception interpretation. Information included dietary and lifestyle characteristics (body weight, physical activity) and medical health factors. In this intriguing study, the participants were followed for 8 years.

The "high-fertility diet" thus characterized includes the following:

- Lower intakes of trans fatty acids and greater intake of monounsaturated fats[77]
- Lower intake of animal protein and greater intake of vegetable protein[78]
- Higher intake of high-fiber, low-glycemic carbohydrates

- Higher dietary sources of dairy foods (Note: This is controversial because there are conflicting studies on this.[79,80])
- Higher dietary sources of nonheme iron
- Higher frequency of multivitamin use
- More likely to consume coffee, tea, and alcohol
- More likely to be physically active (vigorous activity for 30 minutes or more daily)
- Less likely to smoke
- Less likely to have long menstrual cycles
- More likely to be a recent user of the oral contraceptive pill
- Weight balance: BMI between 20 and 25

Researchers concluded that these components of a fertility diet are conducive to the most successful outcome. When these approaches are combined with the control of body weight and increased physical activity, the majority of infertility problems involving ovulation may be prevented.

In another trial, researchers noted that the Mediterranean diet increased the chance of a successful pregnancy in those undergoing IVF/intracytoplasmic sperm injection (ICSI). It was found that the high content of B vitamins (especially vitamin B_6) combined with the high intake of vegetable oils may be the mechanism responsible for this positive outcome.[81] These findings are supported by a case-control study of 485 Spanish women that showed improved fertility in women who adhered to a Mediterranean diet compared with those who followed a Western dietary pattern.[82] Adequate folate intake is associated with female fertility, and a study found that a higher adherence to a Mediterranean diet was significantly related to a greater folate intake.[83]

In another cohort study, adherence to the Dutch dietary recommendations (characterized by high intake of whole grains, monounsaturated or polyunsaturated oils, vegetables, fruit, meat or meat replacers, and fish) before IVF treatment was associated with an increased probability of pregnancy.[84]

Another aspect of diet that needs to be considered is fat intake. High-fat diets have been associated with changes in reproductive function, including altered menstrual cycle length, as well as changes in reproductive hormone levels and embryo quality in ART settings. These effects on reproductive function are present in normal-weight women, and the effect is worsened in obesity.[85] Trans fatty acid intake is associated with reduced fecundability.[86] A recent prospective cohort study of 60 women undergoing IVF treatment found a negative correlation between the trans fatty acid index and IVF outcomes.[87]

Advanced glycation end products (AGEs) have been shown to have a negative effect on ART outcomes and appear to be related to diminishing ovarian reserve or abnormally functioning folliculogenesis.[88] Dietary AGEs are formed during the cooking process (grilled, burned, or barbequed foods), especially in animal-derived foods that are high in fat and protein.[89]

The evidence is mounting that a healthy diet can reduce the risk of infertility. With regard to pregnancy loss, results from the NHS-II cohort found no relationship between preconception adherence to any one specific dietary pattern and risk of pregnancy loss.[90]

L-Arginine

Arginine is a precursor of nitric oxide synthesis and is required for angiogenesis, embryogenesis, fertility generally, and hormone secretion.[91] Additionally, it is required for the replication of cells, making it essential for oocyte development and embryo formation.

One study[92] produced notable results. The specific population was "poor responders"—women who had failed to achieve an adequate number of mature follicles and/or adequate serum estradiol levels after gonadotrophin stimulation. This trial indicated that arginine supplementation (oral at 16 g/day) improved ovarian response, endometrial receptivity, and pregnancy rates. The women were divided into two groups: (1) flareup gonadotrophin-releasing hormone analog (GnRHa) plus elevated pure follicle-stimulating hormone (pFSH) (n = 17) or (2) flareup GnRHa plus elevated pFSH plus oral L-arginine (n = 17). Results indicated that group 2 (the arginine group) demonstrated a lower cancellation rate, increased number of oocytes on collection, and increased embryo quality and transfer compared with the control group. Additionally, 3 of 17 women in the arginine group conceived, whereas none in the control group did so.

The literature regarding arginine and fertility outcome is impressive; however, although the trial showed very promising results, it assessed only a small sample of women, too small to allow definitive conclusions. Further research into the potential of this promising adjunct during ART procedures is recommended.

L-Carnitine

Similar to its role in male reproductive function, L-carnitine plays a vital role in fatty acid metabolism. It is derived from the amino acids lysine and/or methionine and works synergistically with coenzyme Q_{10}. It is essential in the transport of fatty acids into the mitochondria; a deficiency results in a decrease in fatty acid concentrations in the mitochondria and reduced energy production. Prescriptions must use the L form and not the DL form to ensure effectiveness and safety. The mitochondria are crucial components of oocyte development, acceptance of the spermatozoa, and subsequent embryogenesis. It is the mRNA within the oocyte that requires the power of the mitochondria to allow conception to occur. Therefore the prescription of carnitine is specifically indicated in women who are experiencing implantation issues and/or presenting with aging oocytes. In addition to mitochondrial energy production, L-carnitine improves female fertility by reducing cellular stress and maintaining hormonal balance.[93]

In mouse studies, carnitine has been found to exert a protective effect on mouse oocytes, reducing damage to the oocyte's cytoskeleton and also mitigating embryo apoptosis induced by incubation in the peritoneal fluid of patients with endometriosis.[94] Although peritoneal fluid has a strong effect on endometriosis, it helps regulate endometrial lining production and implantation factors. A small in vitro study of follicular fluid from 22 infertile women with mild endometriosis undergoing IVF treatment found that L-carnitine with N-acetylcysteine prevented oocyte damage.[95] As such, although human studies are lacking, the prescription of carnitine for patients experiencing endometriosis and/or implantation failure due to endometrial factors is recommended.

A trial in 170 women with clomiphene-resistant polycystic ovary syndrome (PCOS) found that administering L-carnitine (3 g/day) in conjunction with clomiphene citrate (250 mg/day) from day 3 until day 7 of the cycle significantly improved both the ovulation and pregnancy rates. The lipid profile and BMI of the women were also improved.[96]

A small prospective study (n = 24) of acetyl-L-carnitine supplementation (1 g/day) over 16 weeks in women with stress-induced hypothalamic amenorrhea found a significant increase in LH levels in hypo-LH women, via modulation of the hypothalamic–pituitary–gonad (HPG) axis. LH response to naloxone was restored in the treatment group.[97] These results were supported in a follow-up study of 27 women with functional hypothalamic amenorrhea who were administered L-carnitine (500 mg/day) with acetyl-L-carnitine (250 mg/day) over 12 weeks. LH plasma levels increased (in hypo-LH women), whereas cortisol and amylase levels decreased significantly.[98]

Antioxidants

Compared with male fertility, there is little evidence supporting the use of specific antioxidants. However, the consensus remains that the

use of antioxidants combined with a good diet improves fertility in women.[15] One Cochrane review assessed the use of vitamin supplementation in the prevention of miscarriage. It concluded that patients given multivitamins plus iron and folic acid, compared with controls, had reduced risk for stillbirth, but there was no effect on miscarriage risk.[99] The same review concluded that there is a lack of evidence to support antioxidant supplementation for reducing the risk of miscarriage. Therefore it is highly likely that any vitamin prescription, provided that it is prescribed holistically and effectively, will have a positive effect on female fertility.

Transgenerational epigenetic effects include both the physiological and behavioral (intellectual) transfer of information across generations. Although in most cases the underlying molecular mechanisms are not understood, modification of the chromosomes that pass to the next generation through gametes is sometimes involved; this is called transgenerational epigenetic inheritance.

The oocyte, a highly sensitive structure, is highly vulnerable to its external environment and external influences. Every female is born with all of her oocytes. As such, they are thus exposed to everything she experiences throughout her entire life. Additionally, from an epigenetic perspective, a woman's oocytes are also exposed to everything to which her mother and even her maternal grandmother were exposed.

Oxidative stress has a detrimental effect on female fertility by increasing time to conception, decreasing fertilization rates, decreasing oocyte penetration function and viability, decreasing implantation, and increasing loss of implantation.[100]

The oocyte requires a regular antioxidant supply to continue to reduce oxidative damage both from current circumstances and historical (or transgenerational) exposure. Glutathione is the major nonenzymatic antioxidant found in oocytes and embryos.[52] During conception, the mRNA is responsible for using antioxidant reserves to address any DNA fragmentation or oxidation from both the sperm and the oocyte. The very act of conception and the subsequent travel of the embryo through the fallopian tubes before implantation generate free-radical damage. Because oxidative stress has been shown to hinder optimal fertility,[101,102] it is imperative to provide sufficient support for the oocyte so that conception and implantation can occur.

It is important to note that physiological levels of ROS are required for healthy oocyte development and better IVF outcomes. It is a prerequisite for the first meiotic phase (meiosis I) and is also required for folliculogenesis; however, if levels are abnormally high, they are negatively associated with oocyte and embryonic development and pregnancy outcomes. Antioxidant intake is important to suppress ROS buildup and maintain physiological levels of free radicals for homeostasis and proper cell functioning.[103] Excessive ROS production (through lifestyle factors such as advanced age, obesity, smoking, alcohol intake, recreational drug use, and exposure to environmental pollutants) can overpower the body's natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions, leading to unexplained infertility.[52,103–105]

Women with idiopathic infertility have lower total antioxidant status in the peritoneal fluid, and it has been suggested that peritoneal fluids with increased ROS and decreased antioxidant status diffuse into the fallopian tubes and can damage the sperm, which is highly sensitive to oxidative stress.[105] Oocyte quality is also affected by oxidative stress, limiting successful ovulation and fertilization. Follicular fluid contains high levels of antioxidants that protect oocytes from ROS damage, and a small pilot study has shown that higher levels of melatonin (a powerful antioxidant) in the follicular fluid are associated with both the quantity and quality of oocytes and can predict IVF outcomes.[106] Selenium and glutathione peroxidase levels have been found to be lowest in follicular fluid in patients with unexplained infertility, and antioxidant capacity levels were found to be higher in follicles of patients whose oocytes fertilized successfully.[107]

Oxidative stress causes alterations in certain protein pathways, the abnormal expression of which could possibly lead to the pathophysiology of infertility.[103]

In women undergoing IVF treatment, it was found that increased intakes of β-carotene, vitamin C, and vitamin E were associated with time to pregnancy, but the effect of individual antioxidants varies by age and BMI.[104] Although is it clear that antioxidant status plays a role in reproduction and infertility, the success of antioxidant supplementation in female subfertility is not so clear. A 2013 Cochrane review found that the quality of the evidence in the studies was low and that antioxidant supplementation was not associated with an increased live birth rate or clinical pregnancy rate.[108] This conclusion was supported by a later trial of women with unexplained subfertility undergoing IVF treatment, which showed that daily oral antioxidant supplementation (multivitamins and minerals) did not improve oocyte quality and pregnancy rates.[109]

R-Alpha Lipoic Acid

In both its lipid- and water-soluble forms, alpha-lipoic acid is a powerful antioxidant found in virtually all cells of the human body, scavenging the free radicals implicated in oxidative stress. It assists in the chelation of heavy metals, thus minimizing the risk of cellular damage and regenerating other antioxidants, including vitamins C and E, coenzyme Q_{10}, and glutathione.[110]

Vitamin A

Vitamin A is a powerful antioxidant that assists in cellular growth and differentiation, both crucial for embryogenesis and placentogenesis.[111] It is required for gene expression and cellular differentiation in organogenesis and embryonic development[112] as well as for immunity, regulatory functions, the integrity of epithelial tissue, and the health of the cilia in the fallopian tubes. It has a direct relationship with zinc, and the deficiency of zinc has been linked with infertility, miscarriage, and the development of cleft palate. It is a cofactor of 3-β-dehydrogenase in steroidogenesis (specifically for estrogen production), and deficiencies may result in impaired enzyme activity.[113] Additionally, low concentrations of vitamin A are associated with anovulation,[114] and plasma levels have been observed to be decreased in women suffering from habitual miscarriage.[115] A Cochrane review has found no evidence, however, to support the use of vitamin A supplementation to reduce the risk of miscarriage.[99]

Supplementation during pregnancy is inadvisable due to the potential for malformation and congenital abnormalities. However, supplementation in the preconception period is strongly advised. Owing to its lipid solubility and the liver's high storage capacity, it is advisable to keep prescriptions below 10,000 IU/day. It is also important to note that cross-placental transfer occurs primarily with lipid-soluble nutrients. Therefore cessation of vitamin A supplementation is advisable as a cautionary measure immediately before conception attempts.

An alternative is to prescribe carotenoids (β-carotene), owing to their lower toxic potential. An additional benefit is that they have a role in ovarian oocyte and follicular maturation and function.[116] However, 15% to 25% of whites are unable to convert β-carotene to vitamin A, so it is not a substitute for vitamin A (see also Chapters 57 and 125).

Coenzyme Q_{10}

Coenzyme Q_{10}, also known as ubiquinone (ubidecarenone) or ubiquinol, is an endogenous enzyme involved in intracellular adenosine triphosphate (ATP) production; it serves as a cofactor in oxidative stress respiration for the citric acid cycle and the electron transport chain.[117] It

is a fat-soluble antioxidant and free-radical scavenger found in every cell in the body and plays a vital role in all energy-dependent processes. It is required in the maintenance of cell membrane integrity and cell functioning and is therefore specifically important for all new cells in the body and the development of cells in their maturation process. The presence of coenzyme Q_{10} in human follicular fluid indicates its potential role in reproductive outcomes. Higher coenzyme Q_{10} levels were seen in the follicular fluid of mature oocytes and good-grade embryos compared with immature eggs and poor-grade embryos, respectively.[118,119] It is endogenously produced from tyrosine in a 17-step process that requires riboflavin (B_2), niacinamide (B_3), pantothenic acid (B_5), pyridoxine (B_6), cobalamin (B_{12}), folic acid (B_9), vitamin C, tetrahydrobiopterin, and trace elements as cofactors. Therefore an ample supply of these key nutrients is essential for the production of this enzyme. Additionally, it can be expected that patients with thyroid irregularities (both hyper- and hypothyroid) would require additional coenzyme Q_{10} owing to its reliance on tyrosine as an initial building block.

In women with clomiphene-resistant PCOS, it was found that supplementing with coenzyme Q_{10} (180 mg/day) in conjunction with clomiphene citrate resulted in significantly improved ovarian response, luteinization (midluteal progesterone), endometrial thickness, ovulation, and most importantly, pregnancy rate compared with treatment with clomiphene citrate alone.[120] The use of coenzyme Q_{10} supplementation (600 mg/day) in women undergoing IVF procedures resulted in reduced aneuploidy and increased pregnancy rates compared with placebo.[121] Clinical trials are very limited; however, there are countless papers supporting its inclusion in culture media used in an IVF setting, and it is considered a crucial component to mimic various reproductive fluids incorporated in natural conception. In the clinical setting, its role can be extrapolated fairly conclusively; therefore, its use should certainly be considered because its clinical and therapeutic effectiveness are irrefutable. Because dietary sources are poor choices for optimal effect, supplementation is essential. In choosing supplements, it is essential to select the ubiquinol form because it is the active antioxidant.

Specifically in the context of female fertility, coenzyme Q_{10} can be seen to support mitochondrial function and to have positive applications for maturing oocytes, which are at greater risk of oxidative damage. Oxidative damage is typically seen in the zona pellucida of the oocyte, thus preventing the acrosome reaction with sperm (fertilization). Receptivity of the zona pellucida is strongly correlated with the antioxidant status of the oocyte, and coenzyme Q_{10} is believed to be the most specific antioxidant for this application. Understandably, requirements for coenzyme Q_{10} will be greater for more mature oocytes (women above 35 years of age) or women undergoing ART procedures owing to the increased oxidative effect on the oocyte of such procedures.

Vitamin C

Vitamin C is the body's primary water-soluble antioxidant. It is protective against free radicals, protects folic acid and vitamin E from oxidation, and has been shown to improve fertility outcomes in women with luteal-phase defects.[122] The ovary is considered the primary site of ascorbic acid accumulation because the midcycle change of retention and excretion of ascorbic acid is one of the main markers of ovulation. In the literature, vitamin C is seen to be crucial for collagen biosynthesis and its positive effect on the growth and repair of the ovarian follicle and the development of the corpus luteum[123]; it may also facilitate luteal steroidogenesis. It is necessary for the maturation of the preovulatory follicle and reduces the risk of preeclampsia when prescribed concurrently with vitamin E. Deficiency increases the risk of miscarriage, spontaneous rupture of membranes, and brain tumors in the offspring.

Vitamin C is best coprescribed with bioflavonoids, which provide the additional benefits of strengthening capillaries, preventing miscarriage, and preventing breakthrough bleeding during the pregnancy. Evidence levels are still low, and according to a Cochrane review, there is insufficient evidence to support the use of vitamin C supplementation alone, or in combination with other supplements, to help prevent stillbirth, poor fetal growth, preterm birth, or preeclampsia.[124]

Vitamin E

Vitamin E is a powerful lipid-soluble antioxidant that is essential for fertility and reproduction. It is a plant-derived fat-soluble vitamin that exists in eight different isomers. Alpha-tocopherol is the most biologically active form for conception and is the focus in nutrition research. It has been shown to maintain the health of the ovaries[125]; help premenstrual syndrome (PMS) sufferers, including relief of affective and physical symptoms[126]; relieve benign breast disease[127]; and ameliorate menstrual migraine,[128] indicating effects in the regulation of hormonal activity. The literature suggests that it may be of benefit in infertile women by reducing scar formation (especially keloid scarring when applied topically).[129] This may make it particularly useful to those suffering from endometriosis and/or complicated reproductive conditions such as Asherman's syndrome. Additionally, one study found that women suffering from infertility and endometriosis had lower levels of vitamin E and increased lipid peroxidation.[130] This was confirmed by another study indicating that levels of vitamin E were low in infertile patients compared with controls. This research suggests that the antioxidant capacity was compromised in infertile women and may cause subsequent risk of oxidative damage.[131]

A recent study found that higher levels (10–15ml/dL) of serum vitamin E were associated with higher-quality embryos in women undergoing IVF treatment.[132]

As far as intervention trials go, one trial assessing outcomes in women with unexplained infertility undergoing ART showed that vitamin E (400 IU/day) combined with clomiphene citrate resulted in increased endometrial thickness. However, no statistical difference was found in implantation or ongoing pregnancy rates compared with clomiphene citrate treatment without vitamin E.[133] A double-blind, randomized controlled trial (RCT) of 105 infertile women with PCOS undergoing IVF treatment showed that clinical pregnancy and implantation rates were significantly higher in the treatment group compared with placebo. Treatment consisted of supplementation with vitamin E (400 mg/day) and vitamin D_3 (50,000 IU/once every 2 weeks) over an 8-week period. Oxidative stress was not reduced, and antioxidant status was not increased, in the treatment group in this study, and it was concluded that the positive results were due to the increase in vitamin D_3 levels of the participants.[134] Further high-quality trials are needed in this area. A 2015 Cochrane review found no evidence to support vitamin E supplementation in pregnancy to prevent stillbirth, baby death, preterm birth, preeclampsia, or low-birthweight babies.[135]

Selenium

Selenium is a powerful antioxidant with marked immune-modulating properties. Deficiency has been linked to female infertility, miscarriage, preeclampsia, gestational diabetes, fetal growth restriction, preterm labor, and obstetrical cholestasis.[136] Supplementation appears to reduce the risk of miscarriage, especially in association with thyroid autoantibodies.[137]

Supplementation has been suggested as a powerful supportive nutrient to regulate and control thyroid autoimmunity, specifically thyroid peroxidase (TPO) antibodies.[137] Selenium is essential in thyroid hormone synthesis because several of the enzymes involved are selenoproteins.[138] Selenium also plays a role in the immune system and the coagulation system.[139,140]

In a double-blind, randomized clinical trial, selenium substitution decreased TPO antibody levels in euthyroid subjects and increased quality of life.[140] In another trial, it also decreased TPO antibody levels in hypothyroid patients treated with T4 substitution.[141] Specifically in this specialized population, lowered selenium levels have been observed in the hair of women with recurrent miscarriage compared with controls who have had successful pregnancies[139]; decreased serum selenium has also been observed in women who suffered from miscarriage in the first trimester as well as recurrent miscarriage.[142]

Serum and follicular fluid levels of selenium appear to be reduced in women undergoing IVF, and this has been associated with nonfertilization of the harvested oocytes.[143] Multivitamin/mineral supplementation has been shown to normalize selenium in serum and follicular fluid in women undergoing IVF.[144] It has been shown that selenium levels in hair correlate positively with follicle number and oocyte yield after ovarian stimulation, suggesting that selenium has a positive effect on ovarian response to gonadotrophin therapy for IVF.[145]

In pregnancy, selenium supplementation (100 mcg/day) reduces prelabor membrane rupture and preeclampsia.[146,147] In a preliminary study of healthy women, dietary selenium was significantly associated with lower odds of luteal phase deficiency. A 10-mcg increase in average daily selenium consumption decreased the odds of luteal-phase deficiency by 20%. Luteal-phase deficiency refers to inadequate progesterone secretion by the corpus luteum, which may render the endometrium less receptive to implantation and result in infertility or early pregnancy loss.[148]

Zinc

More than 200 enzymes require zinc for their activity; these include the enzymes required for the production of the nucleic acids in DNA and RNA.[149] Zinc has specific antioxidant properties required for the reproductive system and protects the oocytes from free radicals and reactive oxygen species.[150] It enables reproduction, ovulation, fertilization, and the development of the oocyte.[151,152] Deficiency may result in altered synthesis/secretion of follicle-stimulating hormone or luteinizing hormone[153] because it has been reported to have a role in the synthesis, transport, and peripheral action of hormones. Low zinc status is associated with low circulating concentrations of several hormones.[154]

Deficiency may produce symptoms such as abnormal ovarian development, increased risk of miscarriage, or increased risk of teratogenicity.[155] It is considered to be the single most important nutrient for preconception and pregnancy and is frequently disrupted with the use of an oral contraceptive pill or a copper intrauterine device. In animal trials, low zinc levels were associated with impaired ovulation and a larger number of deteriorated oocytes[151]; in such circumstances, supplementation may be beneficial. Caution is advised because an excessive intake of zinc can be detrimental[156]; doses should be calculated on the basis of assessments and clinical findings.

In the preconception period, zinc supplementation used to enhance fertility will further support a healthy pregnancy and subsequent healthy birth. Poor maternal zinc status is associated with a number of adverse pregnancy outcomes, including low birth weight, premature delivery, complications of labor and delivery, and congenital anomalies in the offspring.[157] Intrafollicular zinc levels in women with endometriosis have been observed to be higher in those women who successfully achieved pregnancy after IVF than in those who did not become pregnant.[158] Additionally, requirements increase during pregnancy because marginal zinc deficiency has been shown to be prevalent during this time.[159] Monitoring is crucial because suboptimal levels are associated with growth retardation in the fetus.[159] In one study, supplementation of 25 mg from at least 19 weeks' gestation resulted in significantly greater birth weight.[160] Additionally, a meta-analysis of supplementation trials indicates that zinc supplementation produced a 14% reduction in premature deliveries.[161] However, a Cochrane review found that although zinc supplementation in pregnancy leads to a slight reduction in preterm births, it does not prevent other problems such as low birth weight or preeclampsia.[162]

B-Complex Vitamins

It is imperative to take the B-group vitamins in a stable, combined formulation owing to their interrelation and interdependence in a number of key reactions in the body. Their synergy of effect can be seen in countless scenarios; however, some patients may require additional key B vitamins such as folate for methylenetetrahydrofolate reductase (MTHFR), polymorphisms, or vitamin B_6 in instances of luteal-phase defects or progesterone insufficiency.

Deficiencies of this group of vitamins have been associated with a number of fertility problems, including fetal abnormalities such as neural tube defects,[163] neonatal or perinatal death, low birth weight, and miscarriage.[164,165] It is important to consider that alcohol, carbohydrate-rich diets, and the oral contraceptive pill increase the body's requirements for B vitamins. Therefore any couple wanting to optimize their fertility may require additional B vitamins to prepare themselves for conception. Acknowledging that stress also increases requirements for B vitamins and that stress has been identified as playing a major role in couples who are having difficulty conceiving, the importance of adequate B-vitamin intake is further emphasized.

Vitamin B_1 (Thiamine)

In animal models, vitamin B_1 has been shown to stabilize the membranes of newly generated neuronal cells during embryogenesis concurrent with slowing cell death. Additionally, it has been shown to be involved in the plasma membrane transformation of uterine epithelial cells during pregnancy.[166] It plays a key role in reproductive function, with deficiency associated with altered cellular differentiation and proliferation and interference with hormonal processes, thus increasing the risk of miscarriage.[167] Additionally, when taken preconceptually in combination with vitamins B_3 and B_6, it has been shown to contribute to the prevention of oral-facial clefts.[168]

Vitamin B_2 (Riboflavin)

Vitamin B_2 is crucial for energy production, antioxidant defense, and numerous enzyme systems that rely on the presence of B vitamins. Insufficient levels can lead to altered estrogen and progesterone levels, often causing irregular menstruation. Supplementation often addresses irregularities such as general menstrual difficulties, irregular menses, PMS, and infertility.[169]

Vitamin B_2 is essential for the development of the fetus; according to animal studies, deficiency appears to affect embryonic growth and cardiac development. Therefore supplementation has been encouraged to prevent these complications.[170] Low dietary intake has also been associated with low birth weight[171] and has been shown in one study to increase the risk of congenital heart defects in the offspring of mothers who did not take vitamin supplements in the periconceptual period or consume sufficient dietary saturated fatty acids.[172]

Vitamin B_6 (Pyridoxine)

Vitamin B_6 is required for the metabolism of amino acids, lipids, pathways of gluconeogenesis, and synthesis of neurotransmitters during the periconception period.[111] It is required for the synthesis of prostaglandins; it also plays a key role in conjunction with vitamins B_9 and B_{12} in their regulation of homocysteine, which, when elevated, has been linked to infertility and recurrent spontaneous miscarriage.[173,174] Additionally, elevated homocysteine concentrations in follicular fluid

are also associated with poor oocyte and embryo qualities in patients with PCOS who are undergoing assisted reproduction,[175] thus further supporting the B-vitamin combination.

In addition, vitamin B_6 helps reduce elevated prolactin levels; support luteal-phase defects by increasing progesterone secretion; increase the influx of magnesium into the myometrium; improve the absorption of zinc; and prevent preeclampsia, toxemia, and infarction of the placenta. Serum levels are often low in hyperemesis gravidarum, and a deficiency may be associated with a higher incidence of gestational diabetes.

A study examining a Mediterranean diet in couples undergoing IVF demonstrated an increase in plasma vitamin B_6 levels and, in those with high adherence to the diet, a 40% increase in the probability of pregnancy.[81]

Vitamin B_9 (Folate, Folinic Acid or L-5MTHF)

Folic acid is required for healthy DNA and RNA synthesis, optimal protein synthesis, and the regulation of gene expression.[176] Impaired folate metabolism disturbs endometrial maturation and results in poor oocyte quality. The most important variation in folate metabolism in terms of effect is the MTHFR polymorphism, which could be one reason for unexplained infertility.[177]

Deficiency can be corrected with supplementation, which is recommended for at least 3 months for any woman planning pregnancy.[178] The primary justification for its preconceptual prescription is to prevent neural tube defects and spina bifida, as confirmed by a 2015 Cochrane review[179]; however, it must be supplemented for the first 28 days of gestation to achieve this goal.[180] Therefore a preconceptual prescription for 3 months before conception is the safest option because most women are unaware of their pregnancy status until it is confirmed after a minimum of 14 days.

Supplementation of folic acid is associated with a decreased incidence of ovulatory infertility[76,181] and has been identified as being important for oocyte quality and maturation.[152] A 2016 Cochrane review found no evidence to support the use of folic acid supplementation for reducing the risk of early or late miscarriage or stillbirth.[99]

Folate and vitamin B_{12} levels have been found to be largely inadequate among women undergoing IVF treatment.[182] Inadequate folate results in elevated homocysteine and alters DNA methylation negatively, influencing oocyte and early embryo quality.[183] Inadequate B-vitamin status and raised concentrations of homocysteine are also associated with the early loss of pregnancy, as evidenced by a systematic review, which found that folate-deficiency–induced hyperhomocysteinemia posed a risk for placenta-mediated diseases such as preeclampsia, spontaneous abortion, and placental abruption.[184]

In a prospective cohort study of women undergoing IVF (n = 232), live birth rates were 20% higher among women with the highest amount of supplemental folate intake (>800 mcg/day) compared with women taking the lowest amount (<400 mcg/day). This study also suggested that folate supplementation was superior to dietary folate.[185] Similarly, in a study of 100 women, higher serum concentrations of folate and vitamin B_{12} before ART treatment were associated with higher live birth rates.[186] Contrary to these positive findings, a 2014 study found that neither folic acid supplementation nor folate status increased the chance of achieving a pregnancy after ART in women with unexplained infertility.[187]

Folate intake has been related to a lower frequency of sporadic anovulation in young healthy women,[188] and folic acid supplement use was also associated with a shorter time to pregnancy among a large cohort of 3895 women.[189] In women undergoing IVF treatment, higher urinary bisphenol A (BPA) concentrations were associated with a 66% lower probability of implantation. This effect was ameliorated by higher levels of dietary folate intake (more than 400 mcg/day).[190]

Preconception folic acid intake may increase fertility and increase a woman's chance of becoming pregnant. Doses higher than recommended for the prevention of neural tube defects may offer the greatest benefit, as might the additional intake of vitamin B_{12}.

Vitamin B_{12} (Hydroxo-, Adenosyl- or Methyl-Cobalamin)

It is common for women to take folate in isolation and not acknowledge its copartner in all metabolic reactions, vitamin B_{12}. Vitamins B_9 and B_{12} work together to ensure that the replication of DNA and RNA in all cells is uniform and regulated. In one longitudinal study, it was noted that although folate levels decreased slightly during pregnancy and remained decreased for up to 6 weeks postdelivery, vitamin B_{12} progressively declined during pregnancy and reached marginal or deficient levels in some individuals.[191] Clinicians are reminded that supplementation of either vitamin B_9 or B_{12} in isolation can cause a rebound anemia of the other nutrient and that pernicious anemia can be masked with supplementation of one vitamin in isolation, as evident on the basis of red blood cell indices. Furthermore, low maternal levels of vitamin B_{12} have been associated with a threefold risk of neural tube defects,[192] again confirming its combined role with folate.

Vitamin B_{12} deficiency has been linked to infertility and recurrent spontaneous miscarriage.[193] Moreover, pregnancy has been shown to occur after correction of this deficiency.[193] In addition, it is prudent to acknowledge the collaborative role of vitamin B_{12} in cases of elevated homocysteine levels.

Vitamin D

The Third National Health and Nutrition Examination Survey (NHANES III) reported a prevalence of vitamin D deficiency of between 25% and 57% among adults.[194] It is important to note that the accepted levels of this vitamin in this analysis are below what naturopaths would consider a safe, therapeutic level for optimal health. Therefore it can be hypothesized that the deficiency percentage is actually much higher. Each week a new paper emerges highlighting an additional application for this crucial vitamin. Its main functions include calcium homeostasis; modeling and remodeling of bone (supporting the skeletal health of both mother and child); immune activation; cell differentiation, proliferation, and growth; and many others.

In the context of female fertility, the active form of vitamin D, 1-25-dihydroxyvitamin D3, has been shown to regulate the transcription and function of genes associated with placental invasion, normal implantation, and angiogenesis.[195] Vitamin D is a powerful immune modulator and therefore an effective prescription for those who experience autoimmune conditions that affect fertilization, implantation, and an increased risk of miscarriage. This is because of the vitamin D receptors found in cells involved in the immune response. Specifically, in fertilization, it is believed to have receptors on the zona pellucida of the oocyte as well as in the head of the sperm. Researchers have theorized that the acrosome reaction with the zona pellucida is enabled by an adequate vitamin D status. Vitamin D receptors are also found in the ovaries, uterus, placenta, and endometrium.[196] Additionally, this vitamin appears to be involved in the acceptance of the sperm by the oocyte, whereby the vitamin D receptors act as gatekeepers to enable immune regulation and thus prevent rejection. Although the effect of vitamin D deficiency is unclear, no association with ovarian reserve or risk of spontaneous abortion has been observed[197,198]; however, a positive association of vitamin D levels with endometrial thickness has been observed.[199] Maternal vitamin D deficiency has also been suggested to be an independent risk factor for preeclampsia, and a number of animal studies highlight the fact that vitamin D deficiency is associated with infertility.[200]

There has been an increased level of interest in the effect of vitamin D on fertility, as evidenced by a search on the literature, and although some studies have yielded positive findings, similar numbers of studies have found no association.

Studies of women undergoing IVF treatment found that higher serum and follicular fluid levels of 25(OH)D had increased pregnancy rates. Each ng/mL increase in follicular fluid 25(OH)D increased the chance of becoming pregnant by 6%.[201–203] Similar results were found in women undergoing single blastocyst transfer—vitamin D deficiency was found to impair pregnancy.[204] These findings were not supported in other studies.[205–208] One study even found that that higher follicular fluid levels of 25(OH)D negatively affected embryo quality and led to poorer IVF outcome.[209]

Other studies have failed to find a significant association between serum and follicular fluid 25(OH)D levels and IVF outcome.[210,211] In women undergoing euploid embryo transfer, vitamin D status was unrelated to pregnancy outcomes.[212]

A case-control study compared early pregnancy levels of vitamin D between women who took 12 to 24 months to get pregnant with age-matched women conceiving in less than 1 year and found no association.[213] A recent study of 70 infertile women found that 64% of the women were vitamin D deficient, but this did not correlate with anti-Müllerian hormone levels.[214]

A retrospective cohort study found that the relationship between vitamin D status and pregnancy rates differed by race. In non-Hispanic white women, pregnancy rates declined with lower levels of vitamin D, whereas in Asian women, the reverse association was seen.[215] A study in donor–recipient IVF cycles showed that insufficient vitamin D levels of the recipients (rather than the donors) was associated with lower pregnancy rates, suggesting that the effects of vitamin D may be mediated through the endometrium rather than through the follicle or oocyte.[216] Once again, however, these results were not supported by another study, which found no difference in pregnancy rates in recipients who had differing vitamin D levels (normal, insufficient, or deficient).[217]

Given the heterogeneity of findings, little can be conclusively stated from the results on vitamin D and fertility. Vitamin D deficiency might be detrimental to fertility, but it is not clear whether higher levels of vitamin D confer additional benefit once sufficiency has been achieved.

A Cochrane review concluded that supplementing with vitamin D during pregnancy may reduce the risk of preeclampsia, low birth weight, and preterm birth. However, vitamin D and calcium supplementation combined increases the risk of preterm birth.[218] At this stage, the clinician is advised to use serum levels to ascertain the best clinical approach and be mindful of not overprescribing.

Calcium

Calcium is an important nutrient for conception, embryogenesis, and gestation. It is required for oocyte maturation, fertilization,[219] and bone formation of both mother and child. It has also been shown to exhibit a protective effect against preeclampsia. A Cochrane review emphasized that calcium supplementation appeared to almost halve the risk of preeclampsia,[220] which supports the prescription of calcium for all preconceptual women whose dietary intake is inadequate.[221]

Iodine

Iodine deficiency has increased in the past two decades because of changes in the dairy industry. The primary supplementation for most people consisted of their dairy intake owing to the iodine solutions used to clean the dairy equipment.[222] This practice has been modified, and today, chloride and bromide solutions are used instead. More than 30% of nonpregnant women of childbearing age have suboptimal urinary iodine concentrations.[223,224]

Adequate dietary intake of iodine preconceptually is imperative because it can minimize the risk of thyroid-related disorders and/or mental retardation in the infant, defects that are commonly seen in the offspring of deficient mothers. Additionally, low iodine has been shown to negatively affect the development of the central nervous system in the fetus and can hinder conception owing to its role as a nutrient donator for the T3 and T4 hormones that regulate thyroid function and thus ovulation.

Maturing oocytes are highly dependent on healthy thyroid hormone levels for optimal reproductive function[225]; deficiency may result in a spectrum of disorders, including miscarriage, stillbirth, mental retardation, and cretinism (deaf-mutism and spasticity).[226,227] Additionally, thyroid autoimmunity is significantly higher among infertile women than among fertile women and increases the rate of miscarriage.[228] Therefore iodine can help supply building blocks for thyroid hormones that are compromised by the autoimmune process.

Subclinical hypothyroidism may be associated with ovulatory dysfunction and adverse pregnancy outcomes; it is therefore crucial to assess thyroid (and iodine) status for both infertile males and females because correction of this condition can reverse or prevent infertility.[229] In one study, 4980 women (462 of whom were pregnant) were tested for iodine status. Repeated miscarriage and stillbirth were associated with higher levels of deficiency, which increased the risk of reproductive failure.[230] A recent population-based prospective cohort study of 501 women found that moderate to severe iodine deficiency is associated with a 46% decrease in fecundability.[231] A 2017 Cochrane review found no conclusive evidence for the benefits or harms of iodine supplementation in women before, during, or after pregnancy. Iodine supplementation appears to decrease the likelihood of postpartum hyperthyroidism and increases the likelihood of digestive intolerance in pregnancy.[232]

As early as 1939, Kemp[233] acknowledged a link between a deficiency of iodine and stillbirth of unknown origin. In the late 1970s, Potter et al.[234] discussed the decline in stillbirth rates after iodine supplementation, highlighting the positive relationship that ensued between iodine (when taken at the correct dose) and a reduced incidence of stillbirth. Current research[235] indicates that 2000 babies continue to be stillborn every year. In spite of advances in technology, the cause of one third of these deaths is unknown.

It is recognized that a diagnosis of hypothyroidism based on thyroid-stimulating hormone (TSH) values in isolation may not suffice to diagnose mild to moderate iodine deficiency in the pregnant woman.[236] It is therefore prudent to assess the urinary iodine status of each patient either as a morning spot urine test, 24-hour urinary excretion test, or iodine:creatinine ratio, as recommended by the World Health Organization.[237,238]

Iron

Iron is required for the formation of red blood cells, subsequent transport of oxygen to the tissues via hemoglobin, and nucleic acid metabolism, as well as being involved in numerous enzyme systems within the body.[111] Because there may be a risk of hemochromatosis, it is imperative that all patients be assessed thoroughly before prescription to ensure that their health is prioritized. If supplementation is needed, patients should address this deficiency in the preconceptual period to help prevent iron deficiency during the pregnancy.[221] Results from the NHS-II suggest that women who consume iron supplements have a significantly lower risk of ovulatory infertility than women who do not.[107,239]

In one article, iron supplements up to 40 mg or more were shown to reduce the risk of ovulatory infertility by up to 60% and to improve the chances of conception compared with women who did not take iron supplements.[239] The link between iron deficiency and infertility has been confirmed by a number of other articles.[240] Therefore practitioners should study the patient's iron profile and ensure that all components are within the normal range, aiming to achieve serum ferritin stores of 80 ng/mL before conception and saturation of greater than 30%. It is imperative to assess a full iron study profile because ferritin can be elevated due to inflammatory processes in the body and give a false sense of iron repletion. This is especially important because some women are unable to take iron supplements during the first trimester owing to their tendency to aggravate nausea.

Magnesium

Magnesium is involved in numerous reactions in the body relevant to fertility, including cell signaling and energy production. Women experiencing spontaneous abortion have been observed to have lower plasma magnesium levels than healthy controls,[241] and those experiencing associated stress regarding their fertility will benefit from both magnesium and B vitamin supplementation. Stress is associated with impaired fertility, including miscarriage and failed pregnancy outcomes.[242]

Essential Fatty Acids

Women undergoing preconception care are encouraged to consume a diet rich in essential fatty acids (EFAs)[221] because omega-3 fatty acids are required to maintain the lipid bilayer in all cell membranes and to serve as precursors for prostaglandin synthesis. Specifically, in the prevention of miscarriage, omega-3 fatty acids (4 g/day providing 795 mg of docosahexaenoic acid [DHA] and 1190 mg of eicosapentaenoic acid [EPA]) have been found to improve the velocity of blood flow in the uterine arteries in women with recurrent miscarriage due to impaired uterine perfusion.[243]

Supplementation with omega-3 fatty acids has been shown to improve endometrial function. In vitro studies of decidualized cells have identified a decrease in prostaglandin production by up to 80%, indicating that prescription is beneficial for all women but most importantly, those suffering from endometriosis or endometrial insufficiency.[244] Additionally, supplementation is believed to assist in reducing spontaneous preterm births associated with intrauterine inflammation.[245] In a North America cohort, women with the lowest intake of omega-3 fatty acids had lower fecundability than women with higher intakes.[86] DHA has been associated with a reduced risk of anovulation in a cohort of healthy, regularly menstruating women.[246]

The role of EFAs in IVF treatment has received a little more attention. A study of women undergoing IVF treatment found that omega-3 fatty acid intake has a beneficial effect on fertility. Women with a higher alpha-linolenic acid (ALA) intake had higher baseline estradiol levels, and ALA and DHA intake was associated with improved embryo morphology.[247] A study investigating the associations between serum levels of polyunsaturated fatty acids (PUFAs) and embryo implantation in women undergoing IVF found that an increased ratio of omega-6 to omega-3 was more important than individual levels of PUFAs in increasing implantation and pregnancy rates.[248] In overweight and obese women undergoing IVF in Australia, intake of PUFAs, specifically omega-6 PUFAs and linoleic acid and possibly omega-3, was associated with improved pregnancy rates; however, there was no association with live birth rates.[249] A cohort study among 105 women undergoing IVF treatment in Iran found that serum levels of EPA were significantly higher in women who achieved pregnancy compared with those who did not.[250]

Probiotics

Alterations in the microflora of the vagina and subsequent genital and intrauterine infections[251] have been linked to reproductive failure and adverse pregnancy outcomes such as preterm labor, miscarriage, and spontaneous preterm birth.[251–253] In one study, during the first half of pregnancy, women with altered vaginal flora of bacterial origin were four times more likely to have a spontaneous preterm birth compared with the overall preterm birth rate.[254]

Bacterial vaginosis is associated with ascending infections, which may lead to tubal factor infertility. Selected *Lactobacillus* strains are typically used for treating bacterial vaginosis because the human vaginal microbiota is a predominantly *Lactobacillus* community.[255]

Botanical Medicines

Actaea racemosa (Black Cohosh)

Actaea racemosa has historically been described by the eclectics as a uteroovarian tonic for "pains of a dull aching character; dragging pains in the womb, with sense of soreness; the dull tensive pains incident to reproductive disorders of the female, as well as the annoying pains accompanying pregnancy; false pains; after pains."[256] Its modern clinical application focuses heavily on mitigating menopausal complaints and regulating the estrogen cascade specifically in this life stage; however, its potential benefits are far broader, and it shows promise in assisting fertility. In one trial, women with unexplained fertility who were not responding to clomiphene citrate were divided randomly into two groups, both of which received clomiphene. However, one group was also given 120 mg of *A. racemosa* on days 1 to 12 of the cycle. Results showed that pregnancy rates were higher in the *A. racemosa* plus clomiphene group compared with the group that received clomiphene is isolation. The A. *racemosa* group had increased LH, progesterone, and estradiol, in addition to increased endometrial thickness.[257]

A subsequent trial of women with unexplained infertility who were undergoing clomiphene induction compared clomiphene citrate cycles with follicular-phase supplementation with *A. racemosa* (120 mg/day on days 1–12 of the cycle) or ethinyl estradiol. Results showed that *A. racemosa* supplementation improved endometrial thickness, follicular maturation, and estradiol levels. There was no statistically significant difference in clinical pregnancy rates between the two treatment groups.[258] Similar results were seen in a study of women with PCOS and infertility. Treatment with clomiphene citrate alone or clomiphene citrate combined with *A. racemosa* (120 mg/day) found that the *A. racemose* group had improved cycle outcomes and pregnancy rates.[259]

Alchemilla vulgaris (Lady's Mantle)

Unfortunately, there is little research on this valuable herb. *Alchemilla vulgaris* has a strong clinical and traditional use as a therapeutic astringent, which makes it an effective prescription for patients experiencing menorrhagia or frequent early miscarriage. Its tannin content supports the antihemorrhagic property, and *A. vulgaris* has traditionally been prescribed to halt bleeding and heal wounds (internal or external).[260] *A. vulgaris* is used to treat female reproductive problems, including menstrual disorders; to prevent miscarriage; and as an aid to conception.[261]

Angelica sinensis (Dang Gui)

Angelica sinensis is a traditional Chinese herbal medicine with a gentle warming action. It is believed to nourish the blood, restore the body's natural balance, and serve as a female tonic for a number of gynecological conditions, including amenorrhea. It appears to contain components that can increase uterine tone and relax the uterine musculature. The two active constituents most associated with this function include ligustilide[262] (an essential oil component) and ferulic acid.[263] Like many other herbal medicines, it is supported by a long history of use

and clinical efficacy. In the absence of human studies, animal experiments have demonstrated that compounds in *A. sinensis* also regulate prostaglandin synthesis and can regulate experimentally induced inflammatory responses due to prostaglandin release.[264] Additionally, thromboxane A2, which increases blood viscosity and promotes blood clotting, appears to be regulated by this herb.[265] These mechanisms may explain its strong reputation as a blood tonic. *A. sinensis* is specifically prescribed for women with compromised fertility who also have blood disorders, menorrhagia, and reproductive inflammatory responses such as those present in endometriosis. It is important to remember that the very process of fertilization and implantation is a marked inflammatory event, which may explain the efficacy of *A. sinensis* as an antimiscarriage herbal medicine serving to promote and sustain conception.

A. sinensis is known to have spasmolytic effects on the uterus, specifically in the treatment of dysmenorrhea. It appears to normalize uterine activity and ease pelvic blood flow, which relieves pelvic congestion and pain. Traditionally it has been combined with *Paeonia lactiflora*[266] and *Ligusticum wallichii*[263] for menstrual pain that is aggravated by the cold. Modern herbalists extrapolate that usage to suggest that *A. sinensis* is a relaxing blood mover that can improve circulation to the uterus and thus assist in normalizing the endometrial lining and supporting implantation.

Asparagus racemosus (Shatavari)

A. racemosus is an Ayurvedic botanical renowned for its effects on the female reproductive system; it has been used for this purpose since the 16th century. *A. racemosus* is believed to function as an aphrodisiac, promoting strength and enhancing sexual appetite while preventing miscarriage and supporting healthy fertility. The roots were traditionally boiled in milk and consumed with sugar owing to their ability to promote libido and to increase *ojas* (which can be likened to the naturopathic version of "vital force"). In Western herbal medicine, Shatavari is primarily prescribed as a female reproductive herb; in animal studies, it has been observed to exert estrogenic effects.[267] Unfortunately, few well-blinded human clinical studies are to be found in the literature; however, in Ayurveda, this herb has been used safely over long periods of time, even during pregnancy and lactation.[268]

Chamaelirium luteum (False Unicorn Root)

An important herbal medicine for reproductive disorders, *C. luteum* is a restricted herb owing to its threatened status from excessive wild crafting. Therefore practitioners are encouraged to prescribe other herbal medicines containing similar steroidal saponins, such as *Dioscorea villosa* (wild yam) and to prescribe *Chamaelirium luteum* if the clinical objective is not achieved. Additionally, the combination of *A. racemosus* (Shatavari) and *D. villosa* (wild yam) may be an even better choice owing to the synergistic action of these herbs.

C. luteum has a strong traditional application as an antimiscarriage herbal medicine. It is undoubtedly one of the most effective herbal medicines for this purpose. The rhizomes appear to function as a uterine tonic and, since the time of the eclectics, have been prescribed to remedy any imbalance or symptom in the female reproductive tract. King's dispensatory documents the use of *C. luteum* for treating repeated and successive miscarriages, and Ellingwood[269] highlights its use for uterine displacement, particularly where there is threatened abortion, saying that it should be combined with *Viburnum prunifolium* to optimize this effect. It has a strong traditional application in the regulation of ovarian function and is particularly indicated to assist in the regulation of menstrual issues experienced in the first half of the cycle.[270] Additionally, it is described as an ovarian amphoteric because of its ability to regulate and normalize ovarian function.[270] Unfortunately, its precise mechanism of action is unknown; however, researchers have determined that it contains steroidal saponins, which are converted through digestion into the sapogenin diosgenin. This constituent has been shown to produce effects on ovarian function via its action on the hypothalamic–pituitary axis.

Paeonia lactiflora (White Peony)

Paeonia lactiflora has long been used in traditional Chinese medicine for its effects on the female reproductive system. In Chinese medicine, *P. lactiflora* is used in a formulation known as Dang-Gui-Shao-Yao-San, a combination of medicinal herbs used for the treatment of ovulatory disorders and thus to promote a good fertility outcome. Another formulation, Unkei-to (a traditional Japanese herbal medicine consisting of Shakuyaku [*Paeonia radix, Paeonia lactiflora Pallas*] and Keihi [*Cinnamomi cortex, Cinnamomum cassia Blume*]) demonstrates stimulatory effects on the ovulatory process and human granulosa cells in vitro.[271] This formulation has been found to promote steroidogenesis, cytokine secretion, 17 beta-estradiol secretion, and progesterone secretion in highly luteinized granulosa cells obtained from IVF patients.

Tribulus terrestris (Tribulus)

Tribulus terrestris is generally thought of as a male tonic; however, it is used extensively in Western herbal medicine for the treatment of female reproductive disorders, including infertility.

A common clinical use of *Tribulus* involves its FSH-stimulating properties at the start of the menstrual cycle in initiating ovulation and improving conception rates. Most of the evidence for this claim, although clinically relevant, lacks research substantiation.

In a clinical context, one can expect to see a marked increase in cervical fluid within a few days of taking the prescription. This increase precedes ovulation and supports conception outcome markedly. It is especially beneficial for patients who have delayed or absent ovulation because it regulates the ratio of follicle-stimulating hormone to luteinizing hormone. Patients are generally more aware of their increased fertility, libido, and release of cervical fluid. This has a positive effect on their mood and confidence in their fertility.

Most articles and documentation refer back to a poorly designed study by Tabakova et al.,[272] who conducted a nonrandomized trial in 51 women with endocrine sterility to assess the effect of Tribestan (standardized *Tribulus* extract) on infertility. Although the results of this study were encouraging, larger studies of better design are needed to evaluate the effectiveness of Tribestan in treating female infertility. Subjects took Tribestan, 1 to 2 tablets (250 mg per tablet) by mouth three times daily, either on days 5 to 14 of the cycle (schedule I) or on days 1 to 12 of the cycle (schedule II) for the first 3 months. After a washout period, the patients were treated with Tribestan, 1 to 2 tablets by mouth three times daily days 1 to 12 of the cycle, plus either Stimovul, 1 to 2 tablets daily on days 5 to 14 of the cycle, or clomiphene citrate 1 to 2 tablets daily on days 5 to 9 of the cycle. The authors found that 67% of the patients treated with Tribestan had normalized ovulation with or without pregnancy. Compared with other drugs, Tribestan was inferior to Stimovul alone. Of 36 patients, 20 went on to receive combination treatment.

Vitex agnus castus (Chaste Tree)

Vitex agnus castus, one of the best-known adjuncts to fertility treatment, is beneficial for anovulatory cycles, hyperprolactinemia, hypothalamic dysfunction, hypothalamic–pituitary–ovarian (HPO) axis modulation, and promoting a regular and normal menstrual cycle. It is well known for its beneficial effects in PMS, and numerous trials have been undertaken in multiple countries to evaluate its effectiveness. It

has been proved to alleviate symptoms associated with latent hyperprolactinemia, whereby it is characterized by lower-than-normal progesterone secretion and normal to mildly elevated prolactin levels. These characteristics are believed to cause a number of menstrual disorders, including typical PMS symptoms, altered menstrual cycle lengths, and amenorrhea. Causative factors appear to relate to an alteration in the hypothalamic–pituitary axis. *V. agnus castus* has been shown to correct menstrual irregularities,[273] including amenorrhea,[274] specifically when caused by latent hyperprolactinemia, owing to its ability to inhibit prolactin secretion.[275] This is achieved by competitively binding to dopamine D2 and opioid receptors, suggesting a dopaminergic effect.[276]

An uncontrolled study using a blend of *V. agnus castus* combined with green tea extract and nutrients (L-arginine; vitamins E, B_6, and B_{12}; folate; as well as iron, magnesium, zinc, and selenium) administered to 30 women who had been unsuccessful in their attempt to conceive despite trying for 6 to 36 months also observed beneficial results.[277] After 5 months of treatment, 5 women out of 15 in the treatment group were pregnant (33% vs. 0%, $P < 0.01$). Four of the five women had healthy live births.

A randomized, double-blind, controlled trial of *V. agnus castus* compared with placebo was shown to improve hormone levels, facilitate the recurrence of menstruation in women with amenorrhea, and ameliorate pregnancy outcome in women with fertility problems.[278] Women were given a registered product called Mastodynon, and the results were impressive. In those with amenorrhea or luteal-phase insufficiency, pregnancy occurred in the active group more than twice as often as in the placebo group, with minimal side effects.

Another study used a preparation containing *V. agnus castus* as well as other botanicals.[279] Ninety-six women with secondary amenorrhea, luteal insufficiency, or idiopathic infertility received either the *Vitex* combination or placebo over three menstrual cycles. Of these women, 15 went on to conceive (7 with amenorrhea, 4 with idiopathic infertility, and 4 with luteal insufficiency).

THERAPEUTIC APPROACH

General Measures

- Avoid exposure to free radicals.
- Identify and eliminate exposure to environmental hazards, including pesticides, solvents, heavy metals, and other toxins
- Use effective stress-reduction techniques and employ psychological counseling if needed.[280,281]
- Avoid cigarette smoking, alcohol, and recreational drugs
- Avoid douches, vaginal sprays, scented tampons, or other feminine products that change the pH of the vagina and disturb vaginal microecology.
- Ensure that weight is within a healthy body mass index (20%–25%).
- Avoid medications that affect the quality of cervical fluid, including antihistamines, some cough mixtures, dicyclomine, progesterone (taken before or at ovulation), propantheline, tamoxifen, and others.
- Avoid all personal lubricants (unless sperm-friendly types are chosen) because several studies show that some common lubricants kill sperm as effectively as contraceptive jellies do.[282–294]

Diet

- Encourage the principles of the fertility diet.
- Avoid dietary sources of free radicals, saturated fats, hydrogenated oils, trans fatty acids, and cottonseed oil.
- Increase consumption of good dietary sources of antioxidant vitamins, carotenes, and flavonoids (dark vegetables and fruits) as well as essential fatty acids (nuts and seeds).
- Follow the recommendation of daily consumption of 8 to 12 servings of vegetables and 1 to 2 servings of fresh fruits.
- Optimize protein intake from both vegetarian and organic animal sources.
- Ensure that hydration requirements are met using a 30-mL/2.2-lb (1-kg) formula to calculate requirements individually and optimize hydration and renal function.
- Eliminate caffeine, alcohol, sugar, and artificial substances (preservatives, colorings, additives). Artificial sweeteners have been found to be associated with unfavorable embryo development.[295] Sugared beverages, independent of their caffeine content, are associated with lower oocyte and embryo quality in women undergoing IVF treatment.[296]
- Encourage sufficient essential fatty acid intake in the form of half a cup of raw nuts or seeds, cold-pressed oils, and sustainably farmed fish (from organic sources).

Nutritional Supplements

Supplementation is best achieved by incorporating the repletion model (as previously discussed). A comprehensive summary of all nutrients is listed in Table 184.6; however, an individualized prescription based on a thorough assessment is advisable to accurately determine the most essential supplements required.

Botanical Medicines

Actaea racemosa (Black Cohosh)

- Fluid extract (1:2): 2 to 4 mL (0.5–1 tsp) three times daily
- Extracts equivalent to dry herbal medicine: 42.25 mg/day

Alchemilla vulgaris (Lady's Mantle)

Supplementation is best achieved by incorporating the repletion model (as previously discussed). A comprehensive summary of all nutrients is listed in Table 184.6; however, an individualized prescription based on a thorough assessment is advisable to accurately determine the most essential supplements required.

- Fluid extract (1:1): 6 to 12 mL/day

Angelica sinensis (Dang Gui)

- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp) three times a day

Asparagus racemosus (Shatavari)

- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp) three times a day

Paeonia lactiflora (White Peony)

- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp) three times a day

Tribulus terrestris (Tribulus)

- Standardized extract, equivalent to dried herb (aerial parts of leaf), standardized to contain furostanol saponins as protodioscin 12.22 mg/1 g, 9 to 36 g per day.

Vitex agnus castus (Chaste Berry)

- The usual dosage of chaste berry extract (often standardized to contain 0.5% agnuside) in tablet or capsule form is 175 to 225 mg per day. If using the liquid extract, the typical dosage is 2 to 4 mL (1/2–1 tsp) per day.

TABLE 184.6 Summary of Nutrient Considerations to Promote Fertility

Vitamin A[a]	3000–10000 IU/day
Vitamin B_1 (thiamine)	50–100 mg/day
Vitamin B_2 (riboflavin)	50 mg/day
Vitamin B_3 (niacin)	50–200 mg/day
Vitamin B_5 (pantothenic acid)	50–200 mg/day
Vitamin B_6 (pyridoxine)	50–250 mg/day
Vitamin B_9 (folate/folinic acid/L-5MTHF)	800–5000 mcg (dependent on methylation status and homocysteine levels)
Vitamin B_{12} (hydroxo-, adenosyl- or methyl-cobalamin)	800–5000 mcg (reflecting vitamin B_9 with a 1:1 ratio where possible, choice of form of B_{12} dependent on methylation status)
Vitamin C	1000–4000 mg/day in divided doses
Vitamin D3[b]	1000–10,000 IU/day pending blood levels (decrease to 1000 IU/d in summer and 2000 IU/d in winter when repleted)
Vitamin E (mixed tocopherols and tocotrienols)	500–1000 IU/day
Vitamin K[c]	2–75 mg/day
Beta carotene	10–30 mg/day
Coenzyme Q_{10}	100–800 mg/day in divided doses
Calcium	1000–1500 mg/day
Chromium[d]	100–1000 mcg/day
Copper[e]	2–4 mg/day
Iodine[f]	200–400 mcg/day (decrease to 200 mcg/day when repleted)
Iron[g]	10–100 mg/day
Magnesium	400–800 mg/day
Selenium	150–300 mcg/day
Zinc	40–80 mg/day (decrease to 25 mg/day when repleted)
Total omega-3 essential fatty acids	1000–5000 mg/day
Docosahexaenoic acid (DHA)	400–700 mg/day
Eicosapentaenoic acid (EPA)	800–1000 mg/day
Total omega-6 essential fatty acids	1000–2000 mg/day
Evening primrose oil	1000–1500 mg/day
L-Arginine	3000–10,000 mg/day
L-Carnitine	1000–4000 mg/day
Probiotics (mixed strains)	$25–50 \times 10^9$/day

[a]Retinol equivalents (REs) are now used: 1 mcg RE = 1 mcg retinol = 6 mcg beta-carotene = 12 mcg other carotenoids. Avoid during pregnancy. Ensure that prescription is based on need due to fat solubility and potential for placental transfer in second trimester. Monitor dose carefully. Alternatively, beta-carotene may be a more appropriate prescription.
[b]Prescribe based on pathology results.
[c]Consider prescription in instances of coagulation disorders. Monitor closely to ensure no drug interaction is present.
[d]Dose dependent on blood sugar level control and weight requirements. Calculate based on patient's weight.
[e]Avoid in instances of Wilson's disease (assess before prescription) and ensure that prescription is recommended only when zinc:copper ratio is considered.
[f]Assessment before prescription is essential to determine dose. Should only be conducted when thyroid function values can be reviewed.
[g]Prescription must have pathology interpretation before recommendation to determine required dosage.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. *Key Statistics from the National Survey of Family Growth*. Centre for Disease Control and Prevention, US Department of Health and Human Services; 2017. https://www.cdc.gov/nchs/nsfg/key_statistics.htm. Accessed October 27, 2019.
2. Wood JW. Fecundity and natural fertility in humans. *Oxf Rev Reprod Biol.* 1989;11:61–109.
3. Noord-Zaadstra BM, Looman CW, Alsbach H, et al. Delaying childbearing: effect of age on fecundity and outcome of pregnancy. *Br Med J.* 1991;302:1361–1365.
4. Hechtman L. *Clinical Naturopathic Medicine*. Sydney, Australia: Elsevier; 2018.
5. Heffner LJ. Advanced maternal age: how old is too old? *NEJM*. 2004;(351):1927–1929.
6. te Velde ER, Pearson PL. The variability of female reproduction ageing. *Hum Reprod Update*. 2002;(8):141–154.
7. Wright VC, Chang J, Jeng G, et al. Assisted reproductive technology surveillance: United States, 2005. *MMWR Surveill Summ*. 2008;57(5):1–23.
8. Gnoth C, Godehardt E, Frank-Hermann P, et al. Time to pregnancy: results of the German prospective study and impact on the management of infertility. *Hum Reprod*. 2003;18:1959–1966.
9. Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation: effects on the probability of conception, survival of the pregnancy and sex of the baby. *NEJM*. 1995;333:1517–1521.
10. Levitas E, Lunenefeld E, Weiss N, et al. Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fert Steril*. 2005;83:1680–1686.
11. ASRM, Recurrent pregnancy loss, ASRM Patient's Fact Sheet, 08/2008.
12. Das C, Senthil Kumar V, Gupta S, et al. Network of cytokines, integrins and hormones in human trophoblast cells. *J Reproduct Immunol*. 2002;53:257–268.
13. ASRM (American Society of Reproductive Medicine). Definitions of infertility and recurrent pregnancy loss. *Fertil Steril*. 2008;90:3.
14. Ward N. Preconceptual care questionnaire research project. *J Nutr Environ Med*. 1995;5:205–208.
15. Stankiewicz M, Smith C, Alvino H, et al. The use of complementary medicine and therapies by patients attending a reproductive medicine unit in South Australia: a prospective survey. *Aust NZ J Obstet Gynaecol*. 2007;47(2):145–149.
16. ASRM. *Age and Fertility: A Guide for Patients*. American Society for Reproductive Medicine; 2003.
17. Bolúmar F, Olsen J, Rebagliato M, et al. Body mass index and delayed conception: a European Multicentre study on infertility and subfecundity. *Am J Epidemiol*. 2000;151(11):1072–1079.
18. Jungheim ES, Travieso JL, Hopeman MM. Weighing the impact of obesity on female reproductive function and fertility. *Nutr Rev*. 2013;71(suppl 1):S3–S8.
19. Pantasri T, Norman RJ. The effects of being overweight and obese on female reproduction: a review. *Gynecol Endocrinol*. 2014;30(2):90–94.
20. Bernardi LA, Carnethon MR, de Chavez PJ, et al. Relationship between obesity and anti–Müllerian hormone in reproductive–aged African American women. *Obesity*. 2017;25(1):229–235.
21. Kiranmayee D, Talla Praveena YH, Sriharibabu M, Kavya K, Mahalakshmi M. The effect of moderate physical activity on ovarian reserve markers in reproductive age women below and above 30 Years. *J Hum Reproduct Sci*. 2017;10(1):44.
22. Chosich J, Bradford AP, Allshouse AA, Reusch JE, Santoro N, Schauer IE. Acute recapitulation of the hyperinsulinemia and hyperlipidemia characteristic of metabolic syndrome suppresses gonadotropins. *Obesity*. 2017;25(3):553–560.
23. Kuokkanen S, Polotsky AJ, Chosich J, et al. Corpus luteum as a novel target of weight changes that contribute to impaired female reproductive physiology and function. *Sys Biol Reproduct Med*. 2016;62(4):227–242.
24. Roth LW, Allshouse AA, Bradshaw–Pierce EL, et al. Luteal phase dynamics of follicle–stimulating and luteinizing hormones in obese and normal weight women. *Clin Endocrinol*. 2014;81(3):418–425.
25. Skrzypek M, Wdowiak A, Marzec A. Application of dietetics in reproductive medicine. *Ann Agricult Environ Med: AAEM*. 2017;24(4):559.
26. Best D, Avenell A, Bhattacharya S. How effective are weight-loss interventions for improving fertility in women and men who are overweight or obese? A systematic review and meta-analysis of the evidence. *Hum Reproduct Update*. 2017;23(6):681–705.
27. Kort JD, Winget C, Kim SH, Lathi RB. A retrospective cohort study to evaluate the impact of meaningful weight loss on fertility outcomes in an overweight population with infertility. *Fertil Steril*. 2014;101(5):1400–1403.
28. ESHRE Capri Workshop Group. Nutrition and reproduction in women. *Hum Reproduct Update*. 2006;12(3):193–207.
29. Moragianni VA, Jones SM, Ryley DA. The effect of body mass index on the outcomes of first assisted reproductive technology cycles. *Fertil Steril*. 2012;98(1):102–108.
30. Comstock IA, Diaz-Gimeno P, Cabanillas S, et al. Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis. *Fertil Steril*. 2017;107(3):740–748.
31. Buyuk E, Asemota OA, Merhi Z, et al. Serum and follicular fluid monocyte chemotactic protein-1 levels are elevated in obese women and are associated with poorer clinical pregnancy rate after in vitro fertilization: a pilot study. *Fertil Steril*. 2017;107(3):632–640.
32. Luke B, Brown MB, Stern JE, Missmer SA, Fujimoto VY, Leach R. Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates. *Hum Reproduct*. 2011;26(1):245–252.
33. Lighten A. *A Weighty Issue: Managing Reproductive Problems in the Obese*. Vol. 9. Sydney IVF: Conceptions; 2009.
34. Sundaram R, Mumford SL, Buck Louis GM. Couples' body composition and time-to-pregnancy. *Hum Reproduct*. 2017;32(3):662–668.
35. Opray N, Grivell RM, Deussen AR, Dodd JM. Directed preconception health programs and interventions for improving pregnancy outcomes for women who are overweight or obese. *Cochrane Database Sys Rev*. 2015;7.
36. Nazıroğlu M, Yüksel M, Köse SA, Özkaya MO. Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signaling pathways in females and males. *J Membrane Biol*. 2013;246(12):869–875.
37. Chalupka S, Chalupka AN. The impact of environmental and occupational exposures on reproductive health. *J Obstet, Gynecol, Neonatal Nurs*. 2010;39(1):84–102.
38. Spergel G, Levy LJ, Goldner MG. Glucose intolerance in the progeny of rats treated with single subdiabetogenic dose of alloxan. *Metabolism*. 1971;20:401–413.
39. Hales BF, Crosman K, Robaire B. Increased postimplantation loss and malformations among the F2 progeny of male rats chronically treated with cyclophosphamide. *Teratol*. 1992;45:671–678.
40. Popova NV. Transgenerational effect of orthoaminoasotoluol in mice. *Cancer Lett*. 1989;46:203–206.
41. Csaba G, Inczefi-Gonda A. Transgenerational effect of a single neonatal benzpyrene treatment on the glucocorticoid receptor of the rat thymus. *Hum Exp Toxicol*. 1998;17:88–92.
42. Newbold RR, Padilla-Banks E, Jefferson WN. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinol*. 2006;147:S11–S17.
43. Anway MD, Cupp AS, Uzumcu M, et al. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*. 2005;308:1466–1469.
44. Labyak S, Lava S, Turek F, et al. Effects of shift work on sleep and menstrual function in nurses. *Health Care Women Internat*. 2002;23:703–714.
45. Shiloh H, Lahav Baratz S, Koifman M, et al. The impact of cigarette smoking on zona pellucida thickness of oocytes and embryos prior to transfer into the uterine cavity. *Hum Reprod*. 2004;19(1):157–159.
46. Motejlek K, Palluch F, Neulen J. Impairs angiogenesis during maturation of human oocytes. *Fertil Steril*. 2006;86(1):186–191.
47. Waylen AL, Jones GL, Ledger WL. Effect of cigarette smoking upon reproductive hormones in women of reproductive age: a retrospective analysis. *Reprod Biomed Online*. 2010;20(6):861–865.
48. Mishra GD, Dobson AJ, Schofield MJ. Cigarette smoking, menstrual symptoms and miscarriage among young women. *Aust N Z J Public Health*. 2000;24(4):413–420.

49. Anderson K, Norman RJ, Middleton P. Preconception lifestyle advice for people with subfertility. *Cochrane Database Syst Rev*. 2010;4:CD008189.
50. Feichtinger W, Papalambrou K, Poehl M, et al. Smoking and in vitro fertilization: a meta-analysis. *J Assist Reprod Genet*. 1997;14(10):596–599.
51. Waylen AL, Metwally M, Jones GL, et al. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis. *Hum Reprod Update*. 2009;15(1):31–44. Epub 2008 Oct 15.
52. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reproduct Biol Endocrinol*. 2012;10(1):49.
53. Dechanet C, Anahory T, Daude JM, et al. Effects of cigarette smoking on reproduction. *Hum Reprod Update*. 2011;17(1):76–95.
54. Bolumar F, Olsen J, Rebagliato M, et al. Caffeine intake and delayed conception: a European multicenter study on infertility and subfecundity. *Am Epidemiol*. 1997;145:324–334.
55. Wilcox AJ, Weinberg C, Baird DD. Caffeinated beverages and decreased fertility. *Lancet*. 1988;2(8626–7):1453–1456.
56. Stanton CK, Gray RH. Effects of caffeine consumption on delayed conception. *Am Epidemio*. 1995;142(12):1322–1329.
57. Fernandes O, Sabharwal M, Smiley T, et al. Moderate to heavy caffeine consumption during pregnancy and relationship to spontaneous abortion and abnormal fetal growth: a meta-analysis. *Reprod Toxicol*. 1998;12:435–444.
58. Casas M, Ferrer S, Calaf J, et al. Dopaminergic mechanisms for caffeine induced decrease in fertility? *Lett Lancet*. 1989;1:731.
59. Hofman GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Hum Reprod Update*. 2007;13(3):209–223.
60. Lucero J, Harlow BL, Barbieri RL, et al. Early follicular phase hormone levels in relation to patterns of alcohol, tobacco, and coffee use. *Fertil Steril*. 2001;76:723–729.
61. Al-Saleh I, El-Doush I, Grisellhi B, Coskun S. The effect of caffeine consumption on the success rate of pregnancy as well various performance parameters of in-vitro fertilization treatment. *Med Sci Monitor*. 2010;16(12):CR598–605.
62. Gormack AA, Peek JC, Derraik JG, Gluckman PD, Young NL, Cutfield WS. Many women undergoing fertility treatment make poor lifestyle choices that may affect treatment outcome. *Hum Reprod*. 2015;30(7):1617–1624.
63. Hatch EE, Wise LA, Mikkelsen EM, et al. Caffeinated beverage and soda consumption and time to pregnancy. *Epidemiology (Cambridge, Mass.)*. 2012;23(3):393.
64. Wesselink AK, Wise LA, Rothman KJ, et al. Caffeine and caffeinated beverage consumption and fecundability in a preconception cohort. *Reprod Toxicol*. 2016;62:39–45.
65. Windham GC, Fenster L, Swan SH. Moderate maternal and paternal alcohol consumption and the risk of spontaneous abortion. *Epidemiology*. 1992;3:364–370.
66. Mendelson JH. Alcohol effects on reproductive function in women. *Psychiatry Letter*. 1986;4(7):35–38.
67. Dondorp W, de Wert G, Pennings G, et al. Lifestyle-related factors and access to medically assisted reproduction. *Hum Reprod*. 2010;25(3):578–583. ESHRE Task Force on Ethics and Law, including Epub 2010 Jan 18.
68. Hakim RB, Gray RH, Zacur H. Alcohol and caffeine consumption and decreased fertility. *Fertil Steril*. 1998;70(4):632–637.
69. Gill J. The effects of moderate alcohol consumption on female hormone levels and reproductive function. *Alcohol*. 2000;35(5):417–423.
70. Schliep KC, Zarek SM, Schisterman EF, et al. Alcohol intake, reproductive hormones, and menstrual cycle function: a prospective cohort study, 2. *Am J Clin Nutr*. 2015;102(4):933–942.
71. Tolstrup JS, Kjær SK, Holst C, et al. Alcohol use as predictor for infertility in a representative population of Danish women. *Acta obstetricia et gynecol Scand*. 2003;82(8):744–749.
72. Fan D, Liu L, Xia Q, et al. Female alcohol consumption and fecundability: a systematic review and dose-response meta-analysis. *Scientific Rep*. 2017;7(1):13815.
73. Battista N, Pasquariello N, Di Tommaso M, et al. Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *J Neuroendocrinol*. 2008;20(suppl 1):82–89.
74. Mueller BA, Daling JR, Weiss NS, et al. Recreational drug use and the risk of primary infertility. *Epidemiology*. 1990;1(3):195–200.
75. Addis A, Moretti ME, Ahmed Syed F, et al. Fetal effects of cocaine: an updated meta-analysis. *Reprod Toxicol*. 2001;15:341–369.
76. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Diet and lifestyle in the prevention of ovulatory disorder infertility. *Obstet Gynecol*. 2007;110:1050–1058.
77. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Dietary fatty acid intakes and the risk of ovulatory infertility. *Am J Clin Nutr*. 2007;85(1):231–237.
78. Chavarro J, Rich-Edwards JW, Rosner BA, et al. Protein intake and ovulatory infertility. *Am J Obstet Gynec*. 2008;198(2):210.e1–210.e7.
79. Wise LA, Wesselink AK, Mikkelsen EM, et al. Dairy intake and fecundability in 2 preconception cohort studies. *Am J Clin Nutr*. 2016;105(1):100–110.
80. Kim K, Wactawski-Wende J, Michels KA, et al. Dairy food intake is associated with reproductive hormones and sporadic anovulation among healthy premenopausal women–3. *J Nutr*. 2016;147(2):218–226.
81. Vujkovic M, de Vries JH, Lindemans J, et al. The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. *Fertil Steril*. 2010;94(6):2096–2101.
82. Toledo E, Lopez-del Burgo C, Ruiz-Zambrana A, et al. Dietary patterns and difficulty conceiving: a nested case–control study. *Fertil Steril*. 2011;96(5):1149–1153.
83. Monteagudo C, Mariscal-Arcas M, Palacin A, Lopez M, Lorenzo ML, Olea-Serrano F. Estimation of dietary folic acid intake in three generations of females in Southern Spain. *Appetite*. 2013;67:114–118.
84. Twigt JM, Bolhuis ME, Steegers EA, et al. The preconception diet is associated with the chance of ongoing pregnancy in women undergoing IVF/ICSI treatment. *Hum Reprod*. 2012;15:des157.
85. Hohos NM, Skaznik-Wikiel ME. High-fat diet and female fertility. *Endocrinology*. 2017;158(8):2407–2419.
86. Wise LA, Wesselink AK, Tucker KL, et al. Dietary fat intake and fecundability in 2 preconception cohort studies. *Am J Epidemiol*. 2017;187(1):60–74.
87. Eskew AM, Wormer KC, Matthews ML, Norton HJ, Papadakis MA, Hurst BS. The association between fatty acid index and in vitro fertilization outcomes. *J Assisted Reprod Genet*. 2017;1–6.
88. Merhi Z. Advanced glycation end products and their relevance in female reproduction. *Hum Reprod*. 2013:det383.
89. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc*. 2010;110(6):911–916.
90. Gaskins AJ, Rich-Edwards JW, Hauser R, et al. Prepregnancy dietary patterns and risk of pregnancy loss. *Am J Clin Nutr*. 2014;100(4):1166–1172.
91. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids*. 2009;37(1):1–17. Epub 2009 Mar 20.
92. Battaglia C, Salvatori M, Maxia N, et al. Adjuvant L-arginine treatment for in-vitro fertilization in poor responder patients. *Hum Reprod*. 1999;14(7):1690–1697.
93. Agarwal A, Sengupta P, Durairajanayagam D. Role of L-carnitine in female infertility. *Reprod Biol Endocrinol*. 2018;16(1):5.
94. Mansour G, Abdelrazik H, Sharma RK, et al. L-carnitine supplementation reduces oocyte cytoskeleton damage and embryo apoptosis induced by incubation in peritoneal fluid from patients with endometriosis. *Fertil Steril*. 2009;91(suppl 5):2079–2086. Epub 2008 Apr 18.
95. Giorgi VS, Da Broi MG, Paz CC, Ferriani RA, Navarro PA. N-acetyl-cysteine and L-carnitine prevent meiotic oocyte damage induced by follicular fluid from infertile women with mild endometriosis. *Reprod Sci*. 2016;23(3):342–351.
96. Ismail AM, Hamed AH, Saso S, Thabet HH. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. *Eur J Obstet Gynecol Reprod Biol*. 2014;180:148–152.
97. Genazzani AD, Lanzoni C, Ricchieri F, et al. Acetyl-L-carnitine (ALC) administration positively affects reproductive axis in hypogonadotropic

women with functional hypothalamic amenorrhea. *J Endocrinol Invest*. 2011;34(4):287–291.
98. Genazzani AD, Despini G, Czyzyk A, et al. Modulatory effects of l-carnitine plus l-acetyl-carnitine on neuroendocrine control of hypothalamic functions in functional hypothalamic amenorrhea (FHA). *Gynecol Endocrinol*. 2017;33(12):963–967.
99. Balogun OO, da Silva Lopes K, Ota E, et al. Vitamin supplementation for preventing miscarriage. *Cochrane Database Syst Rev*. 2016;(5):CD004073.
100. Ruder EH, Hartman TJ, Blumberg J, et al. Oxidative stress and antioxidants: exposure and impact on female fertility. *Hum Reprod Update*. 2008;14(4):345–357. Epub 2008 Jun 4.
101. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2005;3:28.
102. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril*. 2003;79(4):829–843.
103. Gupta S, Ghulmiyyah J, Sharma R, Halabi J, Agarwal A. Power of proteomics in linking oxidative stress and female infertility. *BioMed Res Int*. 2014;2014.
104. Ruder EH, Hartman TJ, Reindollar RH, Goldman MB. Female dietary antioxidant intake and time to pregnancy among couples treated for unexplained infertility. *Fertil Steril*. 2014;101(3):759–766.
105. Pereira AC, Martel F. Oxidative stress in pregnancy and fertility pathologies. *Cell Biol Toxicol*. 2014;30(5):301–312.
106. Tong J, Sheng S, Sun Y, et al. Melatonin levels in follicular fluid as markers for IVF outcomes and predicting ovarian reserve. *Reproduction*. 2017;153(4):443–451.
107. Tvrdá E, Kňažická Z, Lukáčová J, Lukáč N. *Antioxidants, Nutrition And Fertility*. 2011:407–416.
108. Showell MG, Brown J, Clarke J, Hart RJ. *Antioxidants for female subfertility*. Cochrane Lib; 2013.
109. Youssef MA, Abdelmoty HI, Elashmwi HA, et al. Oral antioxidants supplementation for women with unexplained infertility undergoing ICSI/IVF: randomized controlled trial. *Hum Fertil*. 2015;18(1):38–42.
110. Bilska A, Włodek L. Lipoic acid: the drug of the future? *Pharmacol Rep*. 2005;57(5):570–577.
111. Cetin I, Berti C, Calabrese S. Role of micronutrients in the periconceptional period. *Hum Reprod Update*. 2010;16(1):80–95.
112. Morriss-Kay GM, Sokolova N. Embryonic development and pattern formation. *Faseb J*. 1996;10(9):961–968.
113. Lithgow DM, Politzer WM. Vitamin A in the treatment of menorrhagia. *S Afr Med J*. 1977;51(7):191–193.
114. Al-Azemi MK, Omu AE, Fatinikun T, et al. Factors contributing to gender differences in serum retinol and alpha-tocopherol in infertile couples. *Reprod Biomed Online*. 2009;19(4):583–590.
115. Simşek M, Naziroğlu M, Simşek H, et al. Blood plasma levels of lipoperoxides, glutathione peroxidase, beta carotene, vitamin A and E in women with habitual abortion. *Cell Biochem Funct*. 1998;16(4):227–231.
116. Palan PR, Cohen BL, Barad DH, et al. Effects of smoking on the levels of antioxidant beta carotene, alpha tocopherol and retinol in human ovarian follicular fluid. *Gynecol Obstet Invest*. 1995;39(1):43–46.
117. Bhagavan HN, Chopra RK. Coenzyme Q10 absorption, tissue uptake, metabolism, and pharmacokinetics. *Free Radic Res*. 2006;40(5):445–453.
118. Turi A, Giannubilo SR, Brugè F, et al. Coenzyme Q10 content in follicular fluid and its relationship with oocyte fertilization and embryo grading. *Arch Gyneco Obstetr*. 2012;285(4):1173–1176.
119. Akarsu S, Gode F, Isik AZ, Dikmen ZG, Tekindal MA. The association between coenzyme Q10 concentrations in follicular fluid with embryo morphokinetics and pregnancy rate in assisted reproductive techniques. *J Assis Reprod Gene*. 2017;34(5):599–605.
120. El Refaeey A, Selem A, Badawy A. Combined coenzyme Q10 and clomiphene citrate for ovulation induction in clomiphene-citrate-resistant polycystic ovary syndrome. *Reprod Biomed Online*. 2014;29(1):119–124.
121. Bentov Y, Hannam T, Jurisicova A, Esfandiari N, Casper RF. Coenzyme Q10 supplementation and oocyte aneuploidy in women undergoing IVF-ICSI treatment. *Clin Med Insights: Reproductive Health*. 2014;8. CMRH–S14681.
122. Henmi H, Endo T, Kitajima Y, et al. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect. *Fertil Steril*. 2003;80:459–461.
123. Luck MR, Jeyaseelan I, Scholes RA. Ascorbic acid and fertility. *Biol Reprod*. 1995;52(2):262–266.
124. Rumbold A, Ota E, Nagata C, Shahrook S, Crowther CA. Vitamin C supplementation in pregnancy. *Cochrane Database Syst Rev*. 2015;(2):CD004072.
125. Osiecki H. The Nutrient Bible. In: *Eagle Farm*. 6th ed. Queensland, Australia: Bioconcepts Publishing; 2004.
126. London RS, Murphy L, Litlowski KE, et al. Efficacy of alpha-tocopherol in the treatment of premenstrual syndrome. *J Repro Med*. 1983;28(8):503–508.
127. London RS, Sundaram GS, Murphy L, et al. Evaluation and treatment of breast symptoms in patients with the premenstrual syndrome. *J Repro Med*. 1987;32(6):400–404.
128. Ziaei S, Kazemnejad A, Sedighi A. The effect of vitamin E on the treatment of menstrual migraine. *Med Sci Monit*. 2009;15(1):CR16–CR19.
129. Palmieri B, Gozzi G, Palmieri G, et al. Vitamin E added silicone gel sheets for treatment of hypertropic scars and keloids. *Int J Dermatol*. 1995;34(7):506–509.
130. Campos Petean C, Ferriani RA, dos Reis RM, et al. Lipid peroxidation and vitamin E in serum and follicular fluid of infertile women with peritoneal endometriosis submitted to controlled ovarian hyperstimulation: a pilot study. *Fertil Steril*. 2008;90(6):2080–2085.
131. Mehendale SS, Kilari Bams AS, Deshmukh CS, et al. Oxidative stress-mediated essential polyunsaturated fatty acid alterations in female infertility. *Hum Fertil (Camb)*. 2009;12(1):28–33.
132. Bahadori MH, Sharami SH, Fakor F, Milani F, Pourmarzi D, Dalil-Heirati SF. Level of vitamin E in follicular fluid and serum and oocyte morphology and embryo quality in patients undergoing IVF treatment. *J Family Reprod Health*. 2017;11(2):74.
133. Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Genc Y. Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. *J Assist Reprod Gene*. 2012;29(4):325–328.
134. Fatemi F, Mohammadzadeh A, Sadeghi MR, et al. Role of vitamin E and D3 supplementation in Intra-Cytoplasmic Sperm Injection outcomes of women with polycystic ovarian syndrome: a double blinded randomized placebo-controlled trial. *Clin Nutr ESPEN*. 2017;18:23–30.
135. Rumbold A, Ota E, Hori H, Miyazaki C, Crowther CA. Vitamin E supplementation in pregnancy. *Cochrane Database Syst Rev*. 2015;(2):CD004069.
136. Mistry HD, Pipkin FB, Redman CW, Poston L. Selenium in reproductive health. *Am J Obstetr Gynecol*. 2012;206(1):21–30.
137. Prummel MF, Wiersinga WM. Thyroid autoimmunity and miscarriage. *Eur J Endocrinol*. 2004;150(6):751–755.
138. Rayman MP. The importance of selenium to human health. *Lancet*. 2000;356:233–241.
139. Al Kunani AS, Knight R, Haswell SJ, et al. The selenium status of women with a history of recurrent miscarriage. *Br J Obstet Gynaecol*. 2001;108:1094–1097.
140. Gartner R, Gasnier BC, Dietrich JW, et al. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrin Metab*. 2002;87:1687–1691.
141. Duntas LH, Mantzou E, Koutras DA. Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. *Euro J Endocrinol*. 2003;148:389–393.
142. Rayman MP, Rayman MP. The argument for increasing selenium intake. *Proc Nutr Soc*. 2002;61:203–215.
143. Mirone M, Giannetta E, Isidori AM. Selenium and reproductive function. A systematic review. *J Endocrinol Invest*. 2013;36(suppl 10):28–36.
144. Özkaya MO, Nazıroğlu M, Barak C, Berkkanoglu M. Effects of multivitamin/mineral supplementation on trace element levels in serum and follicular fluid of women undergoing in vitro fertilization (IVF). *Biol Trace Element Res*. 2011;139(1):1–9.
145. Dickerson EH, Sathyapalan T, Knight R, et al. Endocrine disruptor & nutritional effects of heavy metals in ovarian hyperstimulation. *J Assist Reprod Gene*. 2011;28(12):1223–1228.

146. Tara F, Rayman MP, Boskabadi H, et al. Selenium supplementation and premature (pre-labour rupture of membranes: a randomised double-blind placebo-controlled trial. *J Obstetr Gynaecol*. 2010;30(1):30–34.
147. Tara F, Maamouri G, Rayman MP, et al. Selenium supplementation and the incidence of preeclampsia in pregnant Iranian women: a randomized, double-blind, placebo-controlled pilot trial. *Taiwanese J Obstetr Gynecol*. 2010;49(2):181–187.
148. Andrews MA, Schliep KC, Wactawski-Wende J, et al. Dietary factors and luteal phase deficiency in healthy eumenorrheic women. *Hum Reprod*. 2015;30(8):1942–1951.
149. Prasad AS. Zinc: an overview. *Nutrition*. 1995;11(suppl 1):93–99.
150. Colagar AH, Marzony ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nutr Res*. 2009;29(2):82–88.
151. Favier A. The role of zinc in reproduction: hormonal mechanisms. *Biol Trace Elem Res*. 1992;32:363–382.
152. Ebisch IM, Thomas CM, Peters WH, et al. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Hum Reprod Update*. 2007;13(2):163–174. Epub 2006 Nov 11.
153. Bedwal RS, Bahuguna A. Zinc, copper and selenium in reproduction. [abstract only]. *Experientia*. 1994;50(7):626–640.
154. Plamer BF. Sexual dysfunction in uremia. *J Am Soc Nephrol*. 1999;10(6):1381–1388.
155. Breskin MW, Worthington-Roberts BS, Knopp RH, et al. First trimester serum zinc concentrations in human pregnancy. *Am J Clin Nutr*. 1983;38(6):943–953.
156. Favier A. Current aspects about the role of zinc in nutrition. *Rev Prat*. 1993;43(2):146–151.
157. Shad D, Sachdev HP. Zinc deficiency in pregnancy and fetal outcome. *Nutr Rev*. 2006;64(1):15–30.
158. Singh AK, Chattopadhyay R, Chakravarty B, Chaudhury K. Markers of oxidative stress in follicular fluid of women with endometriosis and tubal infertility undergoing IVF. *Reprod Toxicol*. 2013;42:116–124.
159. Cole CR, Lifshitz F. Zinc nutrition and growth retardation. *Pediatr Endocrinol Rev*. 2008;5(4):889–896.
160. Goldenberg RL, Tamura T, Neggers Y. The effect of zinc supplementation on pregnancy outcomes. *JAMA*. 1995;274(6):463–468.
161. Hess SY, King JC. Effects of maternal zinc supplementation on pregnancy and lactation outcomes. *Food Nutr Bull*. 2009;30(suppl 1):560–578.
162. Ota E, Mori R, Middleton P, et al. Zinc supplementation for improving pregnancy and infant outcome. *Cochrane Database Syst Rev*. 2015;(2).
163. Kondo A, Kamihira O, Ozawa H. Neural tube defects: prevalence, etiology and prevention. *Int J Urol*. 2009;16(1):49–57.
164. George L, Mills JL, Johansson AL, et al. Plasma folate levels and risk of spontaneous abortion. *JAMA*. 2002;288(15):1867–1873.
165. Hübner U, Alwan A, Jouma M, et al. Low serum vitamin B12 is associated with recurrent pregnancy loss in Syrian women. *Clin Chem Lab Med*. 2008;46(9):1265–1269.
166. Bâ A. Metabolic and structural role of thiamine in nervous tissues. *Cell Mol Neurobiol*. 2008;28(7):923–931. Epub 2008 Jul 19.
167. Bâ A. Alcohol and B1 vitamin deficiency-related stillbirths. *J Matern Fetal Neonatal Med*. 2009;22(5):452–457.
168. Krapels IP, van Rooij IA, Ocké MC, et al. Maternal dietary B vitamin intake, other than folate, and the association with orofacial cleft in the offspring. *Eur J Nutr*. 2004;43(1):7–14. Epub 2004 Jan 6.
169. Barron ML. Light exposure, melatonin secretion, and menstrual cycle parameters: an integrative review. *Biol Res Nurs*. 2007;9(1):49–69.
170. Chan J, Deng L, Mikael LG, et al. Low dietary choline and low dietary riboflavin during pregnancy influence reproductive outcomes and heart development in mice. *Am J Clin Nutr*. 2010;91(4):1035–1043. Epub 2010 Feb 17.
171. Haggarty P, Campbell DM, Duthie S, et al. Diet and deprivation in pregnancy. *Br J Nutr*. 2009;102(10):1487–1497. Epub 2009 Aug 17.
172. Smedts HP, Rakhshandehroo M, Verkleij-Hagoort AC, et al. Maternal intake of fat, riboflavin and nicotinamide and the risk of having offspring with congenital heart defects. *Eur J Nutr*. 2008;47(7):357365. Epub 2008 Sep 8.
173. Wouters MGAJ, Boers GHJ, Blom HJ, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. *Fertil Steril*. 1993;60:820–882.
174. Ronnenberg AG, Venners SA, Xu X, et al. Preconception B-vitamin and homocysteine status, conception, and early pregnancy loss. *Am J Epidemiol*. 2007;166(3):304–312. Epub 2007 May 2.
175. Berker B, Kaya C, Aytac R, et al. Homocysteine concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. *Hum Reprod*. 2009;24(9):2293–2302. Epub 2009 May 14.
176. Wong WY, Merkus HM, Thomas CM, et al. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertil Steril*. 2002;77:491–498.
177. Altmäe S, Stavreus-Evers A, Ruiz JR, et al. Variations in folate pathway genes are associated with unexplained female infertility. *Fertil Steril*. 2010;94(1):130–137.
178. Forges T, Monnier-Barbarino P, Alberto JM, et al. Impact of folate and homocysteine metabolism on human reproductive health. *Hum Reprod Update*. 2007;13(3):225–238.
179. De–Regil LM, Peña–Rosas JP, Fernández–Gaxiola AC, Rayco–Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database Syst Rev*. 2010;(10):CD007950. https://doi.org/10.1002/14651858.CD007950.pub2.
180. Johnston Jnr RB. Folic acid: prevention nutrition for preconception, the fetus and the newborn. *Neo Rev*. 2009;10(1):e10–e19.
181. Chavarro J, Rich-Edwards JW, Rosner BA, et al. Use of multivitamins, intake of B vitamins and risk of ovulatory infertility. *Fertil Steril*. 2008;89(3):668–676.
182. La Vecchia I, Paffoni A, Castiglioni M, et al. Folate, homocysteine and selected vitamins and minerals status in infertile women. *Eur J Contracep Reprod Health Care*. 2017;22(1):70–75.
183. Laanpere M, Altmäe S, Stavreus-Evers A, et al. Folate-mediated one-carbon metabolism and its effect on female fertility and pregnancy viability. *Nutr Rev*. 2010;68(2):99–113.
184. Ray JG, Laskin CA. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. *Placenta*. 1999;20:519–552.
185. Gaskins AJ, Afeiche M, Wright DL, et al. Dietary folate and reproductive success among women undergoing assisted reproduction. *Obstetr Gynecol*. 2014;124(4):801.
186. Gaskins AJ, Chiu YH, Williams PL, et al. Association between serum folate and vitamin B-12 and outcomes of assisted reproductive technologies. *Am J Clin Nutr*. 2015;102(4):943–950.
187. Murto T, Svanberg AS, Yngve A, et al. Folic acid supplementation and IVF pregnancy outcome in women with unexplained infertility. *Reprod Biomed Online*. 2014;28(6):766–772.
188. Gaskins AJ, Mumford SL, Chavarro JE, et al. The impact of dietary folate intake on reproductive function in premenopausal women: a prospective cohort study. *PloS One*. 2012;7(9):e46276.
189. Cueto HT, Riis AH, Hatch EE, et al. Folic acid supplementation and fecundability: a Danish prospective cohort study. *Eur J Clin Nutr*. 2016;70(1):66.
190. Mínguez-Alarcón L, Gaskins AJ, Chiu YH, et al. Dietary folate intake and modification of the association of urinary bisphenol A concentrations with in vitro fertilization outcomes among women from a fertility clinic. *Reprod Toxicol*. 2016;65:104–112.
191. Cikot R, Steegers-Theunissen R, Thomas C, et al. Longitudinal vitamin and homocysteine levels in normal pregnancy. *Br J Nutr*. 2007;85(01):49–58.
192. Ray JG, Wyatt PR, Thompson MD, et al. Vitamin B12 and the risk of neural tube defects in a folic-acid fortified population. *Epidemiol*. 2007;18(3):362–366.
193. Reznikoff-Etiévant MF, Zittoun J, Vaylet C, et al. Low vitamin B(12):level as a risk factor for very early recurrent abortion. *Eur J Obstet Gynecol Reprod Biol*. 2002;104(2):156–159.
194. IARC Working Group. *World health Organisation Citation, vitamin D and cancer, IARC working group Repots*. Lyon, France: International Agency for Research on Cancer; 2008.

195. Bodnar LM, Catov JM, Simhan HN, et al. Maternal vitamin D and the risk of preeclampsia. *J Clin Endocrin Metab*. 2007;22:3517–3522.
196. Shahrokhi SZ, Ghaffari F, Kazerouni F. Role of vitamin D in female reproduction. *Clin Chimica Acta*. 2016;455:33–38.
197. Drakopoulos P, van de Vijver A, Schutyser V, et al. The effect of serum vitamin D levels on ovarian reserve markers: a prospective cross-sectional study. *Hum Reprod*. 2016;32(1):208–214.
198. Amegah AK, Klevor MK, Wagner CL. Maternal vitamin D insufficiency and risk of adverse pregnancy and birth outcomes: a systematic review and meta-analysis of longitudinal studies. *PloS One*. 2017;12(3):e0173605.
199. Abdullah UH, Lalani S, Syed F, Arif S, Rehman R. Association of Vitamin D with outcome after intra cytoplasmic sperm injection. *J Maternal-Fetal Neonatal Med*. 2017;30(1):117–120.
200. Kinuta K, Tanaka H, Moriwake T, et al. Vitamin D is an important factor in estrogen biosynthesis of both male and female gonads. *Endocrinol*. 2000;141:1317–1324.
201. Ozkan S, Jindal S, Greenseid K, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertil Steril*. 2010;94(4):1314–1319.
202. Garbedian K, Boggild M, Moody J, Liu KE. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. *CMAJ Open*. 2013;1(2):E77–E82.
203. Paffoni A, Ferrari S, Viganò P, et al. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. *J Clin Endocrinol Metabol*. 2014;99(11):E2372–E2376.
204. Polyzos NP, Anckaert E, Guzman L, et al. Vitamin D deficiency and pregnancy rates in women undergoing single embryo, blastocyst stage, transfer (SET) for IVF/ICSI. *Hum Reprod*. 2014;29(9):2032–2040.
205. Abadia L, Gaskins AJ, Chiu YH, et al. Serum 25-hydroxyvitamin D concentrations and treatment outcomes of women undergoing assisted reproduction. *Am J Clin Nutr*. 2016;104(3):729.
206. Neville G, Martyn F, Kilbane M, et al. Vitamin D status and fertility outcomes during winter among couples undergoing in vitro fertilization/intracytoplasmic sperm injection. *Int J Gynecol Obstetr*. 2016;135(2):172–176.
207. Aflatoonian A, Arabjahvani F, Eftekhar M, Sayadi M. Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: a randomized clinical trial. *Iran J Reproduct Med*. 2014;12(9):595.
208. Asadi M, Matin N, Frootan M, Mohamadpour J, Qorbani M, Tanha FD. Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. *Arch Gynecol Obstetr*. 2014;289(4):865–870.
209. Anifandis GM, Dafopoulos K, Messini CI, et al. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reprod Biol Endocrinol*. 2010;8(1):91.
210. Aleyasin A, Hosseini MA, Mahdavi A, al e. Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. *Eur J Obstetr Gynecol Reproduct Biol*. 2011;159(1):132–137.
211. Firouzabadi RD, Rahmani E, Rahsepar M, Firouzabadi MM. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. *Arch Gynecol Obstetr*. 2014;289(1):201–206.
212. Franasiak JM, Molinaro TA, Dubell EK, et al. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. *Am J Obstetr Gynecol*. 2015;212(3). 315–e1.
213. Somigliana E, Paffoni A, Lattuada D, et al. Serum levels of 25-hydroxyvitamin D and time to natural pregnancy. *Gynecol Obstetr Invest*. 2016;81(5):468–471.
214. Lata I, Tiwari S, Gupta A, Yadav S, Yadav S. To study the vitamin D levels in infertile females and correlation of Vitamin D deficiency with AMH levels in comparison to fertile females. *J Hum Reproduct Sci*. 2017;10(2):86.
215. Rudick B, Ingles S, Chung K, Stanczyk F, Paulson R, Bendikson K. Characterizing the influence of vitamin D levels on IVF outcomes. *Hum Reprod*. 2012;27(11):3321–3327.
216. Rudick BJ, Ingles SA, Chung K, Stanczyk FZ, Paulson RJ, Bendikson KA. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. *Fertil Steril*. 2014;101(2):447–452.
217. Fabris A, Pacheco A, Cruz M, Puente JM, Fatemi H, Garcia-Velasco JA. Impact of circulating levels of total and bioavailable serum vitamin D on pregnancy rate in egg donation recipients. *Fertil Steril*. 2014;102(6):1608–1612.
218. De–Regil LM, Palacios C, Lombardo LK, Peña–Rosas JP. Vitamin D supplementation for women during pregnancy. *Cochrane Database Sys Rev*. 2016;1.
219. Martín-Romero FJ, Ortíz-de-Galisteo JR, Lara-Laranjeira J, et al. Store-operated calcium entry in human oocytes and sensitivity to oxidative stress. *Biol Reprod*. 2008;78(2):307–315. Epub 2007 Nov 14.
220. Hofmeyr GJ, Atallah AN, Duley L. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev*. 2006;3:CD001059.
221. Gardiner PM, Nelson L, Shellhaas CS, et al. The clinical content of preconception care: nutrition and dietary supplements. *Am J Obstet Gynecol*. 2008;199(6suppl 2):S345–S356.
222. Li M, Ma G, Boyages SC, Eastman CJ. Re-emergence of iodine deficiency in Australia. *Asia Pac J Clin Nutr*. 2001;10(3):200–203.
223. Pan Y, Caldwell KL, Li Y, et al. Smoothed urinary iodine percentiles for the US population and pregnant women: national health and nutrition examination survey, 2001-2010. *Eur Thyroid J*. 2013;2(2):127–134.
224. Stagnaro-Green A, Dogo-Isonaige E, Pearce EN, Spencer C, Gaba ND. Marginal iodine status and high rate of subclinical hypothyroidism in Washington DC women planning conception. *Thyroid*. 2015;25(10):1151–1154.
225. Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. *Nat Clin Pract Endocrinol Metab*. 2008;4(7):394–405. Epub 2008 May 27.
226. Government Australian. Dept of Health & Ageing Nutrient Reference Values for Australia and New Zealand. http://www.nhmrc.gov.au/_files_nhmrc/file/publications/synopses/n35.pdf. Accessed March 1, 2011.
227. Picciano MF. Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. *J Nutr*. 2003;133(6):S1997–S2002.
228. Redmond GP. Thyroid dysfunction and women's reproductive health. *Thyroid*. 2004;14(suppl 1):S5–S15.
229. Trokoudes KM, Skordis N. Infertility and thyroid disorders. *Curr Opin Obstet Gynecol*. 2006;18(4):446–451.
230. Dillon JC, Milliez J. Reproductive failure in women living in iodine deficient areas of West Africa. *BJOG*. 2000;107(5):631–636.
231. Mills JL, Buck Louis GM, Kannan K, et al. Delayed conception in women with low-urinary iodine concentrations: a population-based prospective cohort study. *Hum Reprod*. 2018;33(3):426–433. https://doi.org/10.1093/humrep/dex379.
232. Harding KB, Peña–Rosas JP, Webster AC, et al. *Iodine supplementation for women during the preconception, pregnancy and postpartum period*. Cochrane Lib; 2017.
233. Kemp MD. Iodine deficiency in relation to the stillbirth problem. *Can Med Assoc J*. 1939;41(4):356–361.
234. Potter JD, McMichael AJ, Hetzel BS. Iodization and thyroid status in relation to stillbirths and congenital anomalies. *Int J Epidemiol*. 1979;8(2):137–144.
235. Robinson J. Perinatal Society of Australia and New Zealand (PSANZ) Perinatal Mortality Group. http://www.stillbirthfoundation.org. Accessed April 25, 2010.
236. Melse-Boonstra A, Jaiswal N. Iodine deficiency in pregnancy, infancy and childhood and its consequences for brain development. *Best Pract Res, Clin Endocrin Metab*. 2010;24(1):29–38.
237. Dunn JT. Editorial: what's happening to our iodine? *Clin Endocrin Metab*. 1998;83:3398–3400.
238. WHO, UNICEF, ICCIDD. *Indicators for Assessing Iodine Deficiency Disorders and Their Control through Salt Iodisation*. WHO/NUT/94.6, WHO: Geneva; 1994.
239. Chavarro JE, Rich-Edwards JW, Rosner BA, et al. Iron intake and risk of ovulatory infertility. *Obstet Gynecol*. 2006;108:1145–1152.

240. Allen LH. Multiple micronutrients in pregnancy and lactation: an overview. *ACJN*. 2005;81(5):S1206–S1212.
241. Borella P, Szilagyi A, Than G, et al. Maternal plasma concentrations of magnesium, calcium, zinc and copper in normal and pathological pregnancies. *Sci Total Environ*. 1990;99(1–2):67–76.
242. Anderson K, Nisenblat V, Norman R. Lifestyle factors in people seeking infertility treatment: a review. *Aust N Z J Obstet Gynaecol*. 2010;50(1):8–20.
243. Lazzarin N, Vaquero E, Exacoustos C, et al. Low-dose aspirin and omega-3 fatty acids improve uterine artery blood flow velocity in women with recurrent miscarriage due to impaired uterine perfusion. *Fertil Steril*. 2009;92(1):296–300. Epub 2008 Aug 9.
244. Roman AS, Schreher J, Mackenzie AP, et al. Omega-3 fatty acids and decidual cell prostaglandin production in response to inflammatory cytokine IL-1beta. *Am J Obstet Gynecol*. 2006;195(6):1693–1699.
245. Arntzen KJ, Kjøllesdal AM, Halgunset J, et al. TNF, IL-1, IL-6, IL-8 and soluble TNF receptors I relation to chorioamnionitis and premature labor. *J Perinat Med*. 1998;26(1):17–26.
246. Mumford SL, Chavarro JE, Zhang C, et al. Dietary fat intake and reproductive hormone concentrations and ovulation in regularly menstruating women, 2. *Am J Clin Nutr*. 2016;103(3):868–877.
247. Hammiche F, Vujkovic M, Wijburg W, et al. Increased preconception omega-3 polyunsaturated fatty acid intake improves embryo morphology. *Fertil Steril*. 2011;95(5):1820–1823.
248. Jungheim ES, Frolova AI, Jiang H, Riley JK. Relationship between serum polyunsaturated fatty acids and pregnancy in women undergoing in vitro fertilization. *J Clin Endocrinol Metabol*. 2013;98(8):E1364–E1368.
249. Moran LJ, Tsagareli V, Noakes M, Norman R. Altered preconception fatty acid intake is associated with improved pregnancy rates in overweight and obese women undertaking in vitro fertilisation. *Nutrients*. 2016;8(1):10.
250. Mirabi P, Chaichi MJ, Esmaeilzadeh S, et al. The role of fatty acids on ICSI outcomes: a prospective cohort study. *Lipid Health Dis*. 2017;16(1):18.
251. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med*. 2000;342:1500–1507.
252. Simhan HN, Caritis SN, Krohn MA, et al. Elevated vaginal pH and neutrophils are associated strongly with early spontaneous preterm birth. *Am J Obstet Gynecol*. 2003;189:1150–1154.
253. Slattery MM, Morrison JJ. Preterm delivery. *Lancet*. 2002;360:1489–1497.
254. Verstraelen H, Verhelst R, Roelens K, et al. Temmerman Modified classification of Gram-stained vaginal smears to predict spontaneous preterm birth: a prospective cohort study. Am J Obstet Gynecol. 196(6):528e1–528e6
255. Mastromarino P, Hemalatha R, Barbonetti A, et al. Biological control of vaginosis to improve reproductive health. *Indian J Med Res*. 2014;140(suppl 1):S91.
256. Felter HW. *The Eclectic Materia Medica, Pharmacology and Therapeutics*. Portland, OR: Eclectic Materia Medica Publications; 1983. reprint.
257. Shahin A, Ismail A, Zahran K, et al. Adding phytoestrogens to clomiphene induction in unexplained infertility patients. *Biomed Online*. 2008;16:580–588.
258. Shahin AY, Ismail AM, Shaaban OM. Supplementation of clomiphene citrate cycles with *Cimicifuga racemosa* or ethinyl oestradiol—a randomized trial. *Reproduct Biomed Online*. 2009;19(4):501–507.
259. Shahin AY, Mohammed SA. Adding the phytoestrogen *Cimicifugae racemosae* to clomiphene induction cycles with timed intercourse in polycystic ovary syndrome improves cycle outcomes and pregnancy rates–a randomized trial. *Gynecol Endocrinol*. 2014;30(7):505–510.
260. Grieve M. A modern herbal. http://botanical.com/botanical/mgmh/l/ladman05.html. Accessed December 1, 2011.
261. *Alchemilla vulgaris* monograph. www.naturalstandard.com. Accessed April 19, 2010.
262. Mei QB, Tao JY, Cui B. Advanced in the pharmacological studies of radix *Angelica sinensis*. *Chin Med J Engl*. 1991;104(9):776–781.
263. Ozaki Y, Ma JP. Inhibitory effects of tetramethylpyrazine and ferulic acid on spontaneous movement of rat uterus. *Chem Pharm Bull*. 1990;38(6):1620–1623.
264. Ozaki Y. Antiinflammatory effect of tetramethylpyrazine and ferulic acid. *Chem Pharm Bull*. 1992;40(4):954–956.
265. Wang SR, Guo ZQ, Liao JZ. Experimental study on the effects of 18 kinds of Chinese herbal medicine for synthesis of thromboxane A2 and PGI2. *Chung Kuo Chung His I Chieh Ho Tsa Chih*. 1993;7(3):167–170.
266. Harada M, Suzuki M, Ozaki Y. Effect of Japanese angelica root and Peony root on uterine contraction in the rabbit in situ. *J Pharm Dyn*. 1984;7:304–311.
267. Pandey SK, Sahay A, Pandey RS, et al. Effect of *Asparagus racemosus* rhizome (Shatavari) on mammary gland and genital organs of pregnant rat. *Phytother Res*. 2005;19(8):721–724.
268. Goyal RK, Singh J, Lal H. *Asparagus racemosus*: an update. *Indian J Med Sci*. 2003;57:408.
269. Ellingwood F. *The American Materia Medica, Therapeutics and Pharmacognosy*. Portland, OR: Eclectic Materia Medica Publications; 1919.
270. Mill S. *The Dictionary of Modern Herbalism*. Melbourne: Lothian Publishing Company Pty Ltd.; 1985.
271. Sun WS, Imai A, Tagami K, et al. In vitro stimulation of granulosa cells by a combination of different active ingredients of unkei-to. *Am J Chin Med*. 2004;32(4):569–578.
272. Tabakova P, Dimitrov M, Ognyanov K, et al. *Clinical Study of Tribestan in Females with Endocrine Sterility. Documentation for Registration*; 1999 (unpublished).
273. Peters-Welte C, Albrecht M. Menstrual-cycle disorders and PMS: study on the use of Vitex-agnus castus. *TW Gynakologie*. 1994;7(1):49–52.
274. Amann W. Amenorrhoea. favourable effect of *Agnus castus* (Agnolyt) on amenorrhea. *Z Allg Med*. 1982;58(4):228–231.
275. Roeder D. Therapy of cyclical disorders with *Vitex agnus* castus. *Zieterschrift Fur Phytotherapie*. 1994;15(3):157–163.
276. Meier B, Berger D, Hoberg E, et al. Pharmacological activities of *Vitex agnus* castus in vitro. *Phytomedicine*. 2000;7(5):373–381.
277. Westphal LM, Polan ML, Trant AS, et al. A nutritional supplement for improving fertility in women: a pilot study. *J Reprod Med*. 2004;49(4):289–293.
278. Gerhard II , Patek A, Monga B, et al. Mastodynon® bei weiblicher Sterilität. *Forsch Komplementarmed*. 1998;5(6):272–278.
279. Dennehy C. The use of herbs and dietary supplements in gynecology: an evidence-based review. *J Midwifery Womens Health*. 2006;51(6):402–409.
280. Vahratian A, Smith YR, Dorman M, Flynn HA. Longitudinal depressive symptoms and state anxiety among women using assisted reproductive technology. *Fertil Steril*. 2011;95(3):1192–1194.
281. Louis GM, Lum KJ, Sundaram R, et al. Stress reduces conception probabilities across the fertile window: evidence in support of relaxation. *Fertil Steril*. 2011;95(7):2184–2189.
282. Anderson L, Lewis SE, McClure N. The effects of coital lubricants on sperm motility in vitro. *Hum Reprod*. 1998;13:3351–3356.
283. Frishman GN, Luciano AA, Maier DB. Evaluation of Astroglide, a new vaginal lubricant: effects of length of exposure and concentration on sperm motility. *Fertil Steril*. 1992;58:630–632.
284. Kutteh WH, Choe CH, Ritter JO, et al. Vaginal lubricants for the infertile couple: effect on sperm activity. *Int J Fertil Meno Stud*. 1996;41:400–404.
285. Miller B, Klein TA, Opsahl MS. The effect of surgical lubricant on in vivo sperm penetration of cervical mucus. *Fertil Steril*. 1994;6:1171–1173.
286. Tagatz GE, Okagake T, Sciarra JJ. The effect of vaginal lubricants on sperm motility and viability in vitro. *Am J Obstet Gynecol*. 1972:113 88–90.
287. Tulandi T, Plouffe LJ, McInnes RA. Effect of saliva on sperm motility and activity. *Fertil Steril*. 1982;38:721–723.
288. Tulandi T, McInnes RA. Vaginal lubricants: effect of glycerin and egg white on sperm motility and progression in vitro. *Fertil Steril*. 1984;41:151–153.
289. Ozgur K, Franken DR, Kaskar K, et al. The influence of a mineral oil overlay on the zona pellucida binding potential of human spermatozoa. *Andrologia*. 1995;27:155–159.
290. Wright RW, Ellington JE. *Effects of personal lubricants on in vitro fertilisation and embryo development*. Phoenix, AZ: Am Soc Androl; 2003.
291. Ellington JE, Short RA, Schimmels J. *Effect of New Intimate Moisturizer on Sperm Motility*. Phoenix, AZ: Am Soc Androl; 2003.

292. Ellington JE, Short RA. *Prevalence of vaginal dryness in trying to conceive couples*. Rancho Mirage, CA: Pacific Coast Reproductive Society Meeting; 2003.
293. Ellington JE, Schimmels J. The effects of vaginal lubricants on computer assisted sperm analysis parameters associated with cervical mucus penetration. *Fert & Ster.* 2004;82(suppl 2):S145–S146.
294. Agarwal TM, Said K, Seifarth DP, et al. Short changes in sperm motility and chromatin integrity following contact with vaginal lubricants. *Fert & Ster.* 2005;84(suppl 1):S73.
295. Setti AS, Braga DP, Halpern G, et al. Is there an association between artificial sweetener consumption and assisted reproduction outcomes? *Reprod Biomed Online*. 2018;36(2):145–153. https://doi.org/10.1016/j.rbmo.2017.11.004.
296. Machtinger R, Gaskins AJ, Mansur A, et al. Association between preconception maternal beverage intake and in vitro fertilization outcomes. *Fertil Steril*. 2017;108(6):1026–1033.
297. Penney GC. Preventing infective sequelae of abortion. *Hum Reprod*. 1997;12(suppl 11):107–112.
298. Gaudoin M, Rekha P, Morris A, et al. Bacterial vaginosis and past chlamydial infection are strongly and independently associated with tubal infertility but do not affect in vitro fertilization success rates. *Fertil Steril*. 1999;72(4):730–732.
299. Kasperczyk A, Kasperczyk S, Horak S, et al. Assessment of semen function and lipid peroxidation among lead exposed men. *Toxicol Appl Pharmacol*. 2008;228(3):378–384. Epub 2008 Jan 3.
300. Fasano A. Celiac disease: how to handle a clinical chameleon. *N Engl Med*. 2003;348:2568–2570.
301. Klee JG. Undiagnosed Wilson's disease as cause of unexplained miscarriage. *Lancet*. 2001;ii:423.
302. Vos GH, Fudenberg HH, Injo LUAN EMG, et al. A study of various antibodies and genetically determined serum groups among aborters producing anti-Tja-like hemolysis and non-aborters in Western Australia. *Acta Haematol*. 1967;38:231–239.
303. Fernandez-Jimenez MC, Jimenez-Marco MT, Hernandez D, et al. Treatment with plasmapheresis and intravenous immunoglobulin in pregnancies complicated with anti-PP1Pk or anti-Kimmunization: a report of two patients. *Vox Sang*. 2001;80:117–120.
304. Rey E, Kahn SR, David M, et al. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet*. 2003;361:901–908.
305. Hay PE, Lamont RF, Taylor-Robinson D, et al. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ*. 1994;308:295–298. 6924.
306. Ntrivalas EI, Bowser CR, Kwak-Kim J, et al. Expression of killer immunoglobulin-like receptors on peripheral blood NK cell subsets of women with recurrent spontaneous abortions or implantation failures. *Am Reprod Immunol*. 2005:215–221.
307. Rumhold A, Middleton P, Crowther CA. Vitamin supplementation for preventing miscarriage. *Cochrane Database Sys Rev*. 2005;(2): Art. No:cd004073. https://doi.org/10.1002/14651858.CD004073.pub2.

Infertility, Male

Leah Hechtman, MSci (RHHG), BHSc (Nat), ND

OUTLINE

DIAGNOSTIC SUMMARY

- Inability to conceive a child after 12 months of regular unprotected intercourse (at least twice weekly) with the same female partner and in the absence of female causes
- Sperm abnormalities confirmed by two properly performed semen analyses of sperm count, morphology, motility, or other aspects

GENERAL CONSIDERATIONS

Infertility affects about 6.7 million women and their partners in the United States, which equates to approximately 11% of the reproductive-age population.[1] One out of seven couples will experience difficulty conceiving. Infertility affects men and women equally, with approximately one third of cases being due to male factors, one third to female factors, and the remaining one third to joint issues. In the United States, it is estimated that about 6% of men between the ages of 15 and 50 years are infertile.[2]

Throughout the world, there has been a recent and dramatic decline in fertility.[3] It is estimated that 48.5 million couples are affected globally, with male-factor infertility counting for about 20% to 30% of these cases. It has been calculated that least 30 million men worldwide are infertile, with the highest rates in Africa and Eastern Europe.[4] A recent review identified an overall 57% decline in mean sperm concentration over the past 35 years, with a significant decline noted in North America, Europe, Asia, and Africa.[5]

Globally there has been a decline in childbirth that appears to be unrelated to the socioeconomic status of any given country; however, deferred childbearing and improved contraception are undoubtedly major factors. Population growth is below the replacement rate in several countries—such as Sri Lanka, Denmark, and Spain—where there have been no obvious increases in abortion rates or contraceptive use. This loss of fertility has affected Denmark to the point where approximately 7% of all newborn babies are now being conceived by assisted methods.[6] In the United States, some patients will require assisted reproductive technologies (ARTs) to conceive. Statistics indicate that in vitro fertilization (IVF) and similar treatments account for less than 3% of infertility services and approximately 0.07% of U.S. health care costs.

As a general rule, it is considered that 3 out of 5 couples conceive within 6 months of trying; 1 in 4 takes between 6 months and a year. For the rest, conception takes more than a year, indicating that there may be a problem. Therefore we can define primary infertility as existing when pregnancy has never occurred despite regular unprotected intercourse for a year or more. Secondary infertility is defined as existing if, despite having achieved a pregnancy in the past (which may or may not have resulted in the birth of a child), a couple is unable to conceive again after a year or more of regular unprotected intercourse.

Unexplained infertility occurs in approximately 5% to 10% of couples trying to conceive. Generally, assessments reveal limited information and find no apparent cause for infertility. The effort to determine whether unexplained infertility is a true diagnosis is often complicated by poor investigation or the lack of current assessment strategies. The naturopath can often elucidate answers in these situations by taking a holistic view in assessing the patients and thus producing positive outcomes.

Most causes of male infertility reflect abnormal sperm counts, morphology, or motility. Although it takes only one sperm to fertilize an egg, a male ejects nearly 200 million sperm in an average ejaculate. Because of the natural barriers in the female reproductive tract, only about 40 sperm ever reach the vicinity of an egg. There is a strong correlation between the number of sperm in an ejaculate and fertility.

Fertility is a reflection of general health and well-being and can also indicate latent or undiagnosed genetic abnormalities or other etiological considerations. These considerations are many and varied; thus a comprehensive, holistic review is essential. An overview of the causes of male infertility is given in Table 185.1.

TABLE 185.1 Semen Terminology[272]

Aspermia	An absence of semen despite male orgasm
Azoospermia	A complete absence of sperm (spermatozoa) in the semen
Oligozoospermia	Reduced number of normal motile sperm cells (spermatozoa) in the ejaculate (compared with *azoospermia,* which means no sperm in the ejaculate). It includes laborious terms such as *asthenozoospermia, teratozoospermia,* and *oligoasthenoteratozoospermia*
Teratozoospermia	Sperm with abnormal morphology
Necrospermia	Death of sperm
Oligoasthenoteratozoospermia	An unnecessarily long name that indicates low count, weak motility, and abnormal morphology

- A smooth, oval head that is 5 to 6 μm long and 2.5 to 3.5 μm around (smaller than the point of a needle)
- A well-defined cap (acrosome) that covers 40% to 70% of the sperm head
- No visible defect of neck, midpiece, or tail
- No fluid droplets in the sperm head that are bigger than half of the sperm head's size[7]

If a sperm deviates from normal, it is defined by the terminology given in Table 185.1.

SPERMATOGENESIS

In healthy young men, sperm are produced by repeated divisions of cells in small, coiled tubules within the testes at an average rate of approximately 100 million per day. Each spermatogenic cycle consists of six stages, and approximately five cycles are required to produce one mature sperm. From the beginning of the division of the stem cell to the appearance of mature sperm in the semen takes between 72 and 76 days. Therefore anything that the male experiences during spermatogenesis can affect mature sperm regardless of his health at the time of examination. Factors to consider would include illness, toxicity, trauma, nutritional status, and others.

The sperm spend 2 to 10 days passing through the epididymis, during which time they mature and become capable of swimming and penetrating oocytes. At the beginning of ejaculation, sperm are transported from the tail of the epididymis via the vas deferens to the urethra. The seminal vesicles, prostate gland, and Cowper's glands secrete most of the volume of semen; these secretions help deliver the sperm during ejaculation. The volume of liquid coming from the two epididymides is less than 5% of the total semen volume. Approximately 60% of the semen volume comes from the seminal vesicles and 30% from the prostate gland. The average semen volume for healthy men ejaculating every 2 days is 3 mL; the average sperm concentration is 85 million/mL (more specifics are given later). During ejaculation, the sperm and prostatic fluid are usually ejected first, and the seminal vesicle fluid follows. The seminal vesicle fluid coagulates, giving the semen a lumpy, gel-like appearance. Liquefaction occurs after 20 minutes or so, when the gel disappears.

DIAGNOSTIC CONSIDERATIONS

Semen Analysis

In a fertility context, the semen analysis forms the primary basis of assessment. It provides the clinician with a snapshot of the male's fertility and reflects his general health in the preceding 72 to 76 days.

An individual's semen quality can vary considerably between samples, even in males with normal semen parameters. In interpreting the assessment, it is imperative that the clinician acknowledge the important fact that a diagnosis is not achieved until an abnormality is confirmed by two separate investigations. As a result, at least two and occasionally three semen analyses are needed, each several weeks apart, to gain an accurate picture of an individual's *average* semen quality. It is well recognized that sperm count can be adversely affected by illness, especially fevers, which may temporarily suppress sperm count in normal males for several months. In this case the semen analysis should, of course, be delayed. Additionally, general recommendations such as in-clinic collection (vs. at-home collection), careful consideration of laboratory guidelines (abstinence timing, lubricant usage), and the standard of the andrology laboratory facility must be considered in reviewing the results. Table 185.2 outlines the World Health Organization's (WHO's) guidelines for assessing a semen analysis.

In 2010 the WHO released updated guidelines for semen analysis. The fourth edition (released in 1999, with a review in 2003) had presented a more positive picture of male fertility. The 2010 guidelines suggest that, overall, male fertility is declining. (Note that all andrology laboratories have adopted the new guidelines. The greatest difference can be noted by reviewing morphology, owing to differing criteria and percentage scales.)

A semen analysis can be conducted by a number of assessments, including the following:

- General semen analysis (SA): To assess motility, morphology, concentration, volume, appearance, and so forth
- Sperm chromatin integrity test: To determine the level of DNA damage to sperm
- Immunobead test: To assess for antibodies against sperm
- Semen culture: To assess for infection
- Retrograde ejaculatory testing: To assess for retrograde semen flow or obstruction if semen analysis yields an extremely low count
- Trial wash: To assess sperm factors and determine whether IVF or intracytoplasmic sperm injection (ICSI) is more appropriate, conducted before IVF and/or ICSI procedures
- MESA/TESE/PESE (microsurgical epididymal sperm aspiration/testicular sperm extraction/percutaneous epididymal sperm aspiration): To extract sperm from the testicles by a specialized procedure when sperm count is low/absent or if ejaculation is not possible

Male Reproductive Assessment

A thorough assessment of the male patient is crucial to accurately determine his general and fertility health. Some of these assessments may require referral to a fertility specialist, urologist, or endocrinologist; however, thorough questioning should be conducted by the naturopath to elucidate a full history and assess causative or contributing factors. Tables 185.3 through 185.6 highlight the most relevant assessments required in a fertility workup.

THERAPEUTIC CONSIDERATIONS

When the clinician is presented with a male patient complaining of infertility, the first step is to determine whether he has been given a thorough, comprehensive assessment. Assessment results can help determine whether the issue can be effectively treated because genetic factors or overt physical impediments will require additional interventions.

- *Treatable conditions:* One in eight infertile men has a treatable condition that can be overcome so that natural conception may become possible.

TABLE 185.2 Interpretation of Semen Analysis

Semen Parameter	Lowest Reference (Reference Range)	Interpretation and Treatment Objective
Standard Components of a Semen Analysis		
Abstinence	Parameters are defined based on abstinence of 3 days.	It is essential to ensure that males ejaculate and then count the required 3 days' abstinence.
Collection method	*Optimal collection is via masturbation; however, specialized condoms can be provided for males with religious restrictions. Additionally, standard lubricants can interfere with the accuracy of the reading and must be avoided. Andrology laboratories are able to supply alternatives.*	
Specimen	Semen sample must be complete.	Incomplete samples are frequent and will distort readings. Assessment can be determined only by reviewing a full sample owing to variations in prostatic secretion vs. epididymal involvement.
Analysis time	*Sample must be analyzed within 60 min and is best collected in the clinic environment to prevent complications.*	
Appearance	Nil debris, nil clumping, or viscosity changes, liquefaction complete.	Debris, clumping, viscosity, or liquefaction issues can suggest systemic congestion, poor hydration, poor elimination, or immune reactions (clumping especially). It can also indicate poor ejaculation frequency.
pH	>7.2 (7.2–7.8)	pH control is essential for sperm survival. An abnormally high or low semen pH can kill sperm or affect their ability to move or to penetrate an egg. The pH of the sample will be affected if there was a delay between sample collection and analysis. If the pH is <7.0 and the sample is azoospermic, there may be an obstruction of the ejaculatory ducts or congenital bilateral absence of the vas deferens (CBAVD). pH irregularities can relate to dietary intake and hydration level. Very acidic samples can indicate obstruction and require referral.
Volume	>1.5 mL (1.4–1.7 mL)	Volume can be affected by the period of abstinence (3 days are recommended), incomplete ejaculation, and retrograde ejaculation. It generally indicates dehydration; treatment should consist of hydration calculation based on weight and energy expenditure.
Concentration		
Sperm concentration Total sperm count	> 15 million/mL (12–16 million/mL) >39 million per ejaculate (33–46 million per ejaculate)	Concentration can be affected by a number of factors, including: • Incomplete collection of the sample • Health status of the individual • Obstruction • Genetic condition The finding of no sperm in the ejaculate suggests either an absence of sperm production or obstruction to sperm outflow. It is most important that an azoospermic semen sample be spun down to carefully examine whether the ejaculate contains even a few sperm. The naturopathic approach considers defects in internal processes and hormonal pathways as potential hindrances to an optimal count. Nutritional deficiencies are essential, and interferences with pathways for spermatogenesis require exploration.
Motility		
Total motility	40% (38%–42%) (>25% rapid, >40% progressive, >50% motile)	Sperm must be able to move forward (or "swim") through cervical mucus to reach an egg. A high percentage of sperm that cannot swim properly may impair a man's ability to father a child.
Progressive rating	>3	There are other important conditions that predominantly affect sperm motility, such as sperm autoimmunity, a condition that accounts for about 6% of male infertility. No movement (immotile sperm) may be due to structural problems in the sperm's tail or to death of sperm.
Progressive motility	>32% (31%–34%) with forward movement	
Vitality	>58% (55%–63%) live	The percentage of sperm that are alive (sperm vitality) is noted because this declines in association with genital tract infections and disorders of sperm transport through the genital tract. The proportion of live sperm is assessed if total motility is <50%. Low motility and high vitality could indicate a disturbance of the motility apparatus. If >75% of sperm are dead, immobilizing antisperm antibodies might be present, and testing is encouraged. Poor motility can often indicate autoimmune processes; infection; lack of mitochondrial energy to propel the sperm; or medication, alcohol or other toxins that affect semen quality.

Continued

TABLE 185.2 Interpretation of Semen Analysis—cont'd

Morphology		
Sperm morphology	4% (3%–4%) normal forms (Tygerberg criteria) Note: A trial wash can provide specificities of morphological abnormalities (i.e., head, neck, tail).	Sperm shape is an important predictive indicator of the sperm's fertilizing ability. Morphology is performed on Pap-stained sperm using the Strict Tygerburg criteria of assessment. These criteria have a strong correlation with the presence of abnormalities and clinical pregnancies and accept only sperm that are normal in every way. Morphology is often a direct reflection of generalized toxicity because semen is a by-product of the body and is a major eliminatory channel. Detoxification, avoidance of environmental toxins, and immaculate dietary practices are essential. Key nutrients in sperm structure must be considered, including protein, essential fatty acids (DHA), all antioxidants including coenzyme Q_{10}, zinc, vitamins C and E, and selenium.
Teratozoospermia index (TZI)	<1.64 TZI	
Specialized Additions to a Semen Analysis		
Immune Factors		
Peroxidase-positive leukocytes	<1.0 million/mL	The presence of white blood cells or bacteria indicates the presence of a genitourinary infection.
Semen culture	Negative	Ascertaining the type of infection is the primary objective, with subsequent targeted treatment to eradicate it. It is essential to assess the female partner to prevent cross-infection.
Mixed antiglobulin reaction (MAR) test (motile sperm with bound particles)	<50%	Antibodies attach to the surface of the sperm and reduce their life span, impairing their motility and ability to penetrate the partner's cervical mucus. Antibodies located on the sperm head may prevent the sperm from fertilizing the egg.
Immunobead test (motile spermatozoa with bound beads)	<50% sperm with adherent particles	Abstinence or a barrier method until the immune system is regulated is essential, along with concurrent autoimmune treatment with herbal medicines, dietary modifications, lifestyle modifications, and nutritional supplementation.
GAM or isotype	>20% positive >50% pathologically significant (except tail tip binding)	
Sperm DNA Damage		
SCIT (sperm chromatin integrity test)	DNA Fragmentation Index (DFI): Excellent DNA integrity (<15%) Good DNA integrity (15%–24%) Fair DNA integrity (25%–29%) Poor DNA integrity (>29%) High green stain (HG): Normal (<15%) Abnormal (15%–100%)	Various methods have been developed to measure strand breaks in sperm DNA in situ. Currently, there are four major tests of sperm DNA fragmentation, including the Comet, Tunel, SCIT, and the acridine orange test (AOT). DNA fragmentation can be attributed to various pathological conditions including cryptorchidism, cancer, varicocele, fever, age, infection, leukocytospermia, and others. Many environmental conditions can also affect DNA fragmentation, such as chemotherapy, radiation, prescribed medicines, air pollution, smoking, pesticides, chemicals, heat, and assisted reproductive technology (ART preparation protocols. Research indicates that sperm with high levels of DNA fragmentation have a lower probability of producing a successful pregnancy. Samples with a DNA fragmentation level greater than 29% are likely to have significantly reduced fertility potential, including a significant reduction in term pregnancies and an increased miscarriage rate. Sperm that appears to be normal by traditional semen analysis parameters may have extensive DNA fragmentation. It is normal for up to 1:5 sperm (20%) to have some DNA fragmentation. Mature sperm are protected from damage because 85% of the chromosomes are bound by protamines into a condensed, compact structure. If more than 20% of sperm have DNA damage, there is an increased risk of infertility, poor oocyte fertilization, defective/impaired embryo development, increased probability of implantation failure, miscarriage, and recurrent miscarriage (up to 3–4 times higher) and disease or childhood cancer in the next generation. Reasons for testing include unexplained infertility, low fertility rates, poor embryo quality, implantation failure after in vitro fertilization (IVF), recurrent miscarriage, exposure to environmental toxins, abnormal semen analysis, and in males above 45 years of age.

TABLE 185.2 Interpretation of Semen Analysis—cont'd

		Once male germ cells have completed meiosis, they lose their capacity for DNA repair, discard their cytoplasm (containing the defensive enzymes that protect most cell types from oxidative stress), and eventually become separated from the Sertoli cells that have nursed and protected them throughout their differentiation into spermatozoa. In this isolated state, spermatozoa must spend a week or so journeying through the male reproductive tract and, uniquely in our species, a further period (up to 3 or 4 days) in the female tract waiting for an egg. During this period of isolation, sperm DNA is vulnerable to damage by both xenobiotics and electromagnetic radiation. Such DNA damage is associated with male infertility; its aberrant repair in the fertilized egg may result in mutations in the embryo with the potential to either induce abortion or impair the health and fertility of the offspring. Treatment consists of environmental review and modification as well as exceptionally high doses of antioxidant prescription.
Other		
Seminal zinc	>2.4 mol per ejaculate	Low levels suggest that supplementation is required.
Seminal fructose	>13 mol per ejaculate	Normal levels are 300 mg/100 mL ejaculate. Absence may indicate that the man was born without seminal vesicles or may have a blockage of seminal vesicles. Referral is essential for further investigation.
Seminal neutral glucosidase	>20 mU per ejaculate	Alpha-glucosidase is a normal constituent of human semen produced mainly in the epididymis. It is significantly correlated with sperm count. Its activity is low in cases of epididymal obstruction.

Data from World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen,* 5th ed. Geneva, Switzerland: WHO; 2010, cited in Hechtman L. *Clinical Naturopathic Medicine*. Chatswood, Australia: Elsevier Australia, 2018.

- *Untreatable subfertility:* Three quarters of infertile men have sperm present in the semen, but in lower numbers than normal. In these cases, the problem cannot be solved without intervention. These men are often defined as subfertile because pregnancies may occur but at lower rates than usual. On average, more months of trying are needed to achieve conception, or conception may simply not occur. After naturopathic intervention, it may be possible to address infertility factors or, alternatively, offer a referral for ARTs/IVF.
- *Untreatable male sterility:* About one in nine infertile men has no sperm in his semen or testes and cannot be treated. Sperm-producing cells in the testes either have not developed or have been irreversibly destroyed. Adoption or donor insemination are the only possibilities for couples in this group who wish to have families.

In treating males for infertility, the optimal approach is to adopt a preconception program for both prospective parents. Preconception treatment adheres to the philosophy that the final stages of gamete production can be modified and influenced. The final stages of spermatogenesis last between 72 and 76 days. By influencing the environment to which sperm are exposed and optimizing the general well-being of the host, practitioners may be able to influence sperm development positively and to address and deal with related health concerns.

Of importance, acknowledging and addressing nutritional status throughout this developmental stage can significantly influence the prospective outcome. The concept of nutrient repletion is highly applicable within a fertility context. Optimal fertility is best achieved when prime health is realized. The repletion model indicates that prescriptions are required for a minimum of 3 months to properly address any deficiencies present and to ensure that all nutrients are used within each required pathway in the body. For example, zinc is involved in hundreds of pathways, including those that affect reproductive health and function. To rectify all possible deficiencies, sufficient zinc supplementation at a repletion dose (typically higher than in general prescriptions) for an extended time (at least 3 months) can enable optimal correction. At lower doses or doses of insufficient duration, zinc treatment may address only those health concerns that have the highest priority (i.e., immune function vs. sperm production).

Within the naturopathic framework, it is crucial to naturally support and attenuate all coexisting health conditions. Therefore treatment should be structured to acknowledge all health concerns; that is, to optimize the general health of the male patient as an underlying position.

Finally, it is always important to acknowledge that the most favorable fertility occurs when the individual's health is optimal. The body is primed to pass on genetic material only when the environment and other conditions are at their best. If survival from an evolutionary perspective is compromised, fertility will be hindered. Everything a person eats, drinks, experiences, or is exposed to can and will influence fertility. True naturopathic supports of fertility acknowledge and consider absolutely all variables of holistic health. Couples should be encouraged to participate in treatment for 3 to 4 months to properly address all genetic and epigenetic variables affecting the gamete.

Improving Sperm-Controlling and Sperm-Damaging Factors

The first step in improving sperm counts, morphology, and function is controlling factors that can damage or impair sperm formation. These include the following:

- Scrotal temperature
- Estrogen and xenoestrogen exposure
- Heavy metals
- Radiation
- Cigarettes, alcohol, and illicit drugs
- Obesity
- Infection
- Diabetes

TABLE 185.3 Fertility Inquiry

Assessment	Elaboration and Explanation
Age	What ages are the couple?
Fertility history	How long have they been trying to conceive, and have they ever conceived previously (together/separately)? Do they have any idea why they have not been able to conceive?
Sexual history	Sexually transmitted infection (STI) screen: Potential sexually transmitted disease exposure, symptoms of genital inflammation (e.g., urethral discharge, dysuria)
Medication history	Such as sulfasalazine (Azulfidine), methotrexate, colchicine, cimetidine (Tagamet), spironolactone (Aldactone), proton-pump inhibitors
Surgical history	Such as previous genitourinary surgery
Contraception	When it was ceased and the likely speed of its reversibility
Fertile times	Whether the couple engage in regular intercourse during fertile times
Lifestyle factors	Diet, exercise, alcohol, smoking cessation, recreational drug use, environmental toxin screen
Prior paternity	Previous fertility
Psychosexual issues (erectile, ejaculatory)	Interference with conception
Pubertal development	Poor progression suggests underlying reproductive issue
A history of undescended testes	Risk factor for infertility and testicular cancer
Previous genital infection (STI) or trauma	Risk of testis damage or obstructive azoospermia
Symptoms of androgen deficiency	Indicative of hypogonadism
Previous inguinal, genital, or pelvic surgery	Testicular vascular impairments, damage to vasa, ejaculatory ducts, ejaculation mechanisms
Medications, drug use	Transient or permanent damage to spermatogenesis
General health (diet, exercise, smoking)	General health screen

Modified from Hechtman L. *Clinical Naturopathic Medicine*. Chatswood, Australia: Elsevier Australia; 2018.

Scrotal Temperature

The scrotal sac normally keeps the testes at a temperature of between 94°F and 96°F. At temperatures above 96°F, sperm production is greatly inhibited or stopped completely. Heat stress modifies gene expression in the testes, causing impaired spermatogenesis. Sperm cells, being extremely vulnerable, respond by apoptosis and DNA damage.[8] Typically, the mean scrotal temperature of infertile men is significantly higher than that of fertile men. A reduction in the scrotal temperature in infertile men is often enough to make them fertile. This is best achieved by having them avoid tight-fitting underwear, tight jeans, and hot tubs.

In addition, the following exercise or exercise equipment can raise scrotal temperature, especially if a man is wearing synthetic fabrics, exceptionally tight shorts, or tight bikini underwear: rowing machines, simulated cross-country ski machines, treadmills, and jogging. After exercising, a man should allow his testicles to hang free to allow them to recover from heat buildup.

TABLE 185.4 Physical Examination

Assessment	Elaboration and Explanation
General examination	Acute/chronic illness, nutritional status
Genital examination	Assess for varicocele, testicular size, and other genital factors Testes: Small testes suggest spermatogenic failure Presence of vas deferens: may be congenitally absent Epididymides: thickening or cysts may suggest previous infection and resultant obstructive problems Varicoceles: detected when standing, coughing, or performing Valsalva maneuver Penis: assessed for abnormalities (e.g., Peyronie's disease) that may interfere with intercourse
Degree of virilization assessment	Assess for signs of virility Signs of androgen deficiency (e.g., increased body fat, decreased muscle mass, decreased facial and body hair, small testes, Tanner stage <5)
Prostate examination	Assess if history suggests prostatitis or a sexually transmitted infection

Modified from Hechtman L. *Clinical Naturopathic Medicine*. Chatswood, Australia: Elsevier Australia; 2018.

TABLE 185.5 Endocrinological Assessments

Assessment	Justification for Assessment
Follicle-stimulating hormone (FSH)	Assessment to ensure that hormonal status is optimal to eliminate hormonal abnormalities
Progesterone (P4)	Testosterone
Prolactin (PRL)	Testosterone is often normal (8–27 nmol/L) even in men with significant spermatogenic defects. Some men with severe testicular problems display a fall in testosterone levels and a rise in serum LH. These men should undergo evaluation for androgen deficiency. The finding of low serum testosterone and low LH suggests a hypothalamic–pituitary problem (e.g., prolactinoma; serum prolactin levels required).
Luteinizing hormone (LH)	FSH Elevated levels are seen when spermatogenesis is poor (primary in testicular failure); in normal men, the upper reference value is approximately 8 IU/L. In azoospermic men, 14 IU/L strongly suggests spermatogenic failure, 5 IU/L suggests obstructive azoospermia; a testis biopsy may be required to confirm that diagnosis.
Total testosterone, free testosterone	
Sex hormone–binding globulin (SHBG)	Evaluates if concentration of SHBG is affecting the amount of testosterone available to body tissues.
Dehydroepiandrosterone sulfate (DHEA-S), cortisol	Additional hormone levels should be reviewed on an individual basis, including a full adrenal profile if the effect of stress is considered relevant.

Modified from Hechtman L. *Clinical Naturopathic Medicine*. Chatswood, Australia: Elsevier Australia; 2018.

TABLE 185.6 Other Assessments

Assessment	Justification
General Health Assessments	
FBC, blood type	General health assessments to eliminate other abnormalities
Standard blood chemistries	
25[OH]D3	
Fasting glucose	
Cholesterol profile	
General sexually transmitted infection (STI) screen	
TSH and urinary iodine (24-hour or morning spot)	Query thyroid function and iodine status
Urinalysis/swab	
Infection screen	General urinalysis to eliminate underlying infection or abnormality. Urogenital infections have been found to play a part in the genesis of miscarriage and infertility. Most patients are unaware of their presence owing to the asymptomatic nature of these infections. The most common infections that require screening include *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, and Neisseria gonorrhoeae.*
Advanced Fertility Assessments	
Karyotyping and subsequent genetic testing	Advanced fertility assessments if previous results show no abnormality or if infertility remains unexplained. WHO guidelines suggest that peripheral blood karyotyping analysis can be diagnostically helpful. Abnormal genotype may be present in up to 12% of azoospermic men and 4% of oligospermic men. Cystic fibrosis screening is recommended for azoospermia if due to CBAVD. Optional screening for Y-chromosome microdeletion if sperm count is <5 million/mL.
Scrotal (testicular) ultrasonography	History of undescended testes or concern regarding testicular cancer.
Transrectal ultrasonography	If ejaculatory duct obstruction suspected.
MTHFR C677T/*MTHFR* 1298C	Indicated in instances of miscarriage, unexplained infertility, marked sperm abnormalities, or implantation issues.
Prothrombin G20210A	
Factor V Leiden	
Selenium assay	
Fasting homocysteine	
Other Considerations	
Nutrient and toxic element screening	Assessments of toxic elements, including aluminum, arsenic, cadmium, lead, and mercury, are crucial so as to eliminate them as causative or contributing factors. It is widely accepted that excessive exposure to heavy metals has a detrimental effect on fertility and must therefore be assessed and remedied during the preconception period. Pre- and post-chelation challenge testing is advisable for more definitive assessment.
Environmental effect	Other environmental assessments, including those that assess porphyrins, PCBs, chlorinated pesticides, volatile solvents, phthalates, parabens, and other toxins. These should additionally be considered due to their deranging effects on reproductive function, endocrinology, gamete development, and thus embryological potential.
Methylation profile and methylation genetics	Methylation defects are strongly associated with changes to sperm parameters.

CBAVD, Congenital bilateral absence of the vas deferens; *FBC,* full blood count; *PCB,* polychlorinated biphenyl; *TSH,* thyroid-stimulating hormone; *WHO,* World Health Organization.
Modified from Hechtman L. *Clinical Naturopathic Medicine.* Chatswood, Australia: Elsevier Australia; 2018.

Infertile men should wear boxer-type underwear and periodically apply a cold shower or ice to the scrotum. They can also choose to use a device called a testicular hypothermia device or "testicle cooler" to reduce scrotal temperatures. The testicle cooler looks like a jockstrap from which long, thin tubes have been extended. The tubes are attached to a small fluid reservoir filled with cold water that attaches to a belt around the waist. The fluid reservoir is also a pump that causes the water to circulate. When the water reaches the surface of the scrotum, it evaporates and keeps the scrotum cool. Because of evaporation, the reservoir must be filled every 6 hours or so. It is recommended that the testicle cooler be worn daily during waking hours. Most users claim that it is fairly comfortable and easy to conceal.[9]

Increased scrotal temperature can be due to the presence of a varicocele. A large varicocele can lead to scrotal temperatures high enough to inhibit sperm production and motility. Surgical repair may be necessary, but scrotal cooling should be tried first.

Estrogen and Xenoestrogen Exposure

According to experts on the effect of the environment and diet on fetal development, we now live in an environment that can be viewed as "a virtual sea of estrogens."[10,11] Increased exposure to environmental

BOX 185.1 Compounds That Exert Estrogenic Activity

- Alkylphenols (intermediate chemicals used in the manufacture of other chemicals)
- Atrazine (weed killer)
- 4-Methylbenzylidene camphor (4-MBC; sunscreen lotions)
- Butylated hydroxyanisole (BHA; food preservative)
- Bisphenol A (monomer for polycarbonate plastic and epoxy resin; antioxidant in plasticizers)
- Dichlorodiphenyldichloroethylene (one of the breakdown products of DDT)
- Dieldrin (banned insecticide)
- DDT (banned insecticide)
- Endosulfan (widely banned insecticide)
- Erythrosine, FD&C Red No. 3
- Ethinylestradiol (released into the environment as a xenoestrogen)
- Heptachlor (restricted insecticide)
- Lindane, hexachlorocyclohexane (restricted insecticide)
- Metalloestrogens (a class of inorganic xenoestrogens)
- Methoxychlor (banned insecticide)
- Nonylphenol and derivatives (industrial surfactants; emulsifiers for emulsion polymerization; laboratory detergents; pesticides)
- Pentachlorophenol (restricted general biocide and wood preservative)
- Polychlorinated biphenyls (PCBs; banned; formerly used in electrical oils, lubricants, adhesives, paints)
- Parabens (lotions)
- Phenosulfothiazine (a red dye)
- Phthalates (plasticizers)
- DEHP (plasticizer for polyvinyl chloride [PVC])
- Propyl gallate (used to protect oils and fats in products from oxidation)

estrogens and other environmental pollutants during fetal development as well as during the reproductive years is suggested to be a major cause of the tremendous rise in the incidence of disorders of development and function of the male sexual system[12] (Box 185.1).

One can best view the relationship between estrogens and male sexual development by examining the effects of the synthetic estrogen diethylstilbestrol (DES). Between 1945 and 1971, several million women were treated with DES. By 1970, the side effects of DES became better known. DES is now recognized to have led to substantial increases in the number of men suffering from developmental problems of the reproductive tract as well as decreased semen volume and sperm count.[10] Apart from having been used in humans, DES and other synthetic estrogens were used for 20 to 30 years in the livestock industry to fatten the animals and make them grow faster.

Although most synthetic estrogens like DES are now outlawed, many animals, both livestock and poultry, are still hormonally manipulated, especially dairy cows. Cow's milk contains substantial amounts of estrogen because of modern farming techniques. The rise in dairy consumption since the 1940s inversely parallels the drop in sperm counts. Avoidance of hormone-fed animal products, including milk products, is important for male sexual vitality, especially in men with low sperm counts or low testosterone levels.

There are reports that estrogens have been detected in drinking water.[11,13] Presumably they are recycled from excreted synthetic estrogens (birth control pills) at water treatment plants. These estrogens may be harmful to male sexual vitality because they are more potent—they do not bind to sex hormone–binding globulin (SHBG). Purified or spring water may be a suitable option to prevent exposure. It is also important to ensure that bottled water is avoided owing to the bisphenol A content of plastic bottles.

Other sources of estrogen in the environment (food, water, and air) can weaken male sexual vitality. For example, many of the chemicals with which we have contaminated our environment in the past 50 years are weakly estrogenic. Most of these chemicals, like polychlorinated biphenyls (PCBs), dioxin, and dichlorodiphenyltrichloroethane (DDT), are resistant to biodegradation and are recycled in our environment until they find a safe haven in our bodies. For example, even though DDT has been banned for nearly 30 years, it is still often found in the soil and in root vegetables such as carrots and potatoes. These toxic chemicals are known to interfere with spermatogenesis, but their effects during sexual development may be even more important.

All of the estrogenic factors previously discussed are thought to have their greatest effect during fetal development. On the basis of animal studies, these estrogens inhibit the multiplication of the Sertoli cells. The number of Sertoli cells is directly proportional to the number of sperm that can be produced because each Sertoli cell can support only a fixed number of germ cells that will develop into sperm. Sertoli cell multiplication occurs primarily during fetal life and before puberty and is controlled by follicle-stimulating hormone (FSH). In animal studies, estrogens administered early in life have been found to inhibit FSH secretion, resulting in a reduced number of Sertoli cells and, in adult life, diminished sperm counts.

One example of the impairment of male sexual development by environmental estrogens is the ability of vinclozolin, a fungicide used in the wine industry, to disrupt the fertility of male rats.[14] Alarmingly, just one exposure of a pregnant female rat to this fungicide was found to disrupt spermatogenesis in more than 90% of the male offspring for at least four generations via an effect exclusively transmitted through the male germline.

Environmental toxins are also linked to increasing testicular cancer rates, testicular dysgenesis syndrome (TDS), cryptorchidism, and hypospadias.[6,15] Whether the outcome is impaired spermatogenesis, TDS, testicular cancer, or any other disturbance may depend on the timing and nature of the xenobiotic attack and the genetic background on which these factors are acting. As such, a determination of the outcome will have to take into account the patient's polymorphism profile for proteins involved in detoxification, such as the cytochrome P450s and glutathione-*S*-transferases. The bottom line is that the environmental effect on spermatogenesis cannot be underestimated. Industrial growth since the end of World War II has introduced many complex chemicals into the environment that are novel to biological detoxification systems. Some of these molecules are reproductive toxicants, capable of impairing fertility and inducing developmental abnormalities in the embryo, including errors in normal sexual differentiation.

The power of reproductive toxicants that target the germline lies in their capacity to generate damage that can be passed down the generations via genetic or epigenetic means. A prime example is the effect of paternal smoking. Men who smoke heavily generate spermatozoa that may have high levels of DNA damage, largely as a result of oxidative stress. One of the consequences of this DNA damage is that the children of such men exhibit an increased incidence of childhood cancer.[16] Although we have traditionally focused on the ability of cigarette smoke to induce lung cancer, a far more sinister effect is its ability to induce DNA damage in the germline and thereby influence the health and well-being of future generations.

It is therefore advisable to discourage exposure to cigarette smoke in all male fertility patients and, in those with suspected heavy exposure, to initiate further investigations and specialized treatments to chelate and support detoxification.

Exposure Route

Environmental toxins may exert their genetic or epigenetic effects on the germline via several potential routes of exposure:

1. Women may be exposed to xenobiotics during pregnancy, thereby disrupting the normal differentiation of the germline in the fetus.
2. Women exposed to toxicants may transmit xenobiotics to their offspring via breast milk.
3. Because of acquired toxicity, men may contribute adverse effects on the integrity of DNA in the male germline. Toxicological studies in animal models reporting infertility, abortion, and birth defects as a result of male exposure to xenobiotics demonstrate that such associations are possible.[17] Epidemiological studies suggest that they are clinically significant.[6,15]

For a full discussion of potential environmental effects, see Chapter 35, Environmental Medicine.

Assessment

It is advisable to thoroughly assess all presenting patients for exposure to environmental toxins and, depending on the results, to offer appropriate treatment. Specific areas to consider include the following:

1. Full occupational exposure review
2. Nonoccupational exposure
   - The area in which the couple lives
   - Age of the couple's home
   - Ages of carpet, floor coverings, floorboards
   - Renovations—when, what, and how
   - Type of heating/air conditioning
   - Use of indoor or outdoor pesticides
   - Attached garage
   - Blinds, curtains
   - Cleaning products
   - Pool/spa/sauna
   - Pets—type, medicines, foods
   - Food sources
   - Water source
   - Prescription drugs
   - Others

It is best to recommend the avoidance of other exposures, such as those from renovation materials, cleaning products, personal toiletries, new furniture, new carpet or cabinets, plastics, gas, chemical gases, and many others. Organic, environmentally sound, sustainable practices are generally the safest.

For men who work in occupations that may affect fertility, it is important to wear protective clothing and follow all occupational health and safety guidelines.

Persistent organic pollutants, phthalates, and PCB serum levels in males have been associated with reductions of up to 30% in fecundity in couples trying to conceive.[18] An unexpected source of exposure to phthalates is via prescription medications, where phthalates and polymers are used as excipients to enable the timed release of active ingredients. A recent Danish case-control study included more than 18,500 males with poor semen quality, and more than 31,000 controls, from the Danish IVF Register over a 10-year period. Exposure to medicinal products containing ortho-phthalates and/or polymers within 90 days of testing was associated with a 30% and 71% increased risk of poor semen quality, respectively. The highest association was found in exposure to polymers from bisacodyl and sulfasalazine, alimentary tract and metabolism drugs.[19]

Heavy Metals

Sperm are also particularly susceptible to the damaging effects of heavy metals such as lead, cadmium, arsenic, and mercury.[12,20,21] The results of a recent meta-analysis of 20 case-control studies indicate that men with low fertility have higher semen levels of lead and cadmium and lower semen zinc levels. Lead and cadmium have a direct toxic effect on the testis and also inhibit the formation of sperm and induce sperm morphology changes, leading to a decline in the quality and quantity of semen.[22] A hair mineral analysis for heavy metals should be performed on all men with reduced sperm counts to rule out heavy metals as a cause.

Radiation

Cell phones operate at 400- and 2000-MHz frequency bands and emit radiofrequency electromagnetic waves (EMWs).[23] Cordless phones must be considered in this context as well; these use the 900-MHz, 1.9-GHz, 2.4-GHz, and 5.8-GHz bands. Reports of potential adverse effects of radiofrequency EMWs on the brain, heart, endocrine system, and DNA in humans and animals are commonly found in the literature. Specifically, from a fertility context, they have also been implicated in DNA strand breaks.[24] The relationship between cell phone use and male infertility remains unclear. Harmful EMWs emitted from cell phones may interfere with normal spermatogenesis and result in a significant decrease in sperm quality. Specific findings pertaining to sperm motility in humans have also been noted.[25,26]

In one observational study,[27] the use of cell phones decreased semen quality by decreasing the sperm count as well as the sperms' motility, viability, and normal morphology. This decrease in sperm parameters was dependent on the duration of daily exposure to cell phones and independent of initial semen quality. Of greatest importance was that sperm count, viability, and morphology declined as cell phone use increased. Specifically, the use of a cell phone for more than 4 hours a day caused a 25% drop in the number of sperm produced, and only 20% of these looked normal. These results were not supported in a longitudinal cohort study of 153 men attending a fertility clinic. Results were inconsistent between different patterns of cell-phone usage, and overall, no evidence was found for a relationship between cell phone use and semen quality.[28]

By comparison, a retrospective analysis of 468 men at a fertility clinic found that cell-phone storage in trouser pockets had an effect on sperm morphology and luteinizing hormone (LH) levels. Cell-phone storage in pockets was also significantly associated with increased varicocele, which in turn have an effect on sperm concentration and testosterone levels.[29] A 2014 meta-analysis reported a statistically significant decrease in sperm motility with mobile phone use, but no relationship with sperm concentration.[30] A second meta-analysis reported that mobile-phone use in epidemiological studies was not related to semen volume, motility, sperm concentration, or normal morphology. However, mobile-phone EMW was related to significant decreases in sperm motility in in vitro studies as well as decreases in motility and concentration in animal studies.[31] The authors of both of these meta-analyses, despite inconsistent results from a small of number studies, concluded that mobile-phone use may negatively affect semen quality. Studies have also shown a negative correlation between wireless Internet usage duration and the total sperm count. Wi-Fi was shown to have a more destructive effect than cell-phone use.[32] A recent in vitro study found that the effects of EMWs emitted from 3G+ Wi-Fi modems cause a significant decrease in sperm motility and velocity.[33]

It is therefore prudent to encourage limiting Wi-Fi use and exposure and discourage cell-phone use. It is recommended that male patients refrain as much as possible from storing cell phones in their pockets, owing to increased genital exposure to the frequency bands.

Cigarettes, Alcohol, Caffeine, and Illicit Drugs

Lifestyle exposures, including cigarette smoke, alcohol, and caffeine, have all been studied in relation to male reproductive health. Over the years, the focus has primarily been on semen quality and/or fertility.

More recently, the literature evaluating direct adverse effects of lifestyle exposures on sperm, chromosomes, and chromatin has grown because of concern that induced damage could be transmitted to offspring, causing transgenerational health effects.

Cigarette smoking. A common source of oxidants is cigarette smoking, which is associated with decreased sperm counts and sperm motility as well as a higher frequency of abnormal sperm.[34] Cigarette smoking, as well as the increase in environmental pollution, is thought to be a major contributor to the diminution in sperm counts seen in many industrialized nations over the past few decades.

Although passive and active smoking are known to lead to a number of health concerns, male patients are often complacent about its effect. However, heavy smokers have been shown to produce more than 20% less sperm. Tobacco smoke contains high levels of reactive oxygen species (ROS), which cause DNA damage. The cadmium, lead, and nicotine in cigarettes also cause DNA strand breaks.[35] Cigarette smoking accelerates DNA damage of the sperm, and smoking cessation is the quickest treatment strategy to reverse such damage and thus affect sperm morphology parameters positively. Stopping smoking for 3 months allows sperm quality to improve.[36]

When the sperm enters the oocyte during conception, the mRNA within the oocyte attempts to defragment the DNA and provide antioxidant support so as to improve sperm quality. It is important to note that the improvements to sperm occur before any oocyte maintenance. It is only once the sperm has been positively addressed that the mRNA and any antioxidant potential within the oocyte can address any oocyte deficiency, oxidative process, or DNA (or chromosomal) abnormality. When the male partner is a heavy smoker, it often means that there are simply insufficient reserves to repair the damage for both gametes; as a result, the oocyte fails, causing either fertilization deficits, a lack of implantation, or abnormalities in the embryo, thus initiating an early miscarriage or negative effects on the long-term health and fertility of the subsequent child.[37] The female's surveillance does not typically allow the compromised embryo to grow beyond early miscarriage because the chance of a healthy birth is then unlikely. Therefore this highly pertinent clinical discussion can motivate most male patients to take responsibility for their oxidative processes and commit to smoking cessation.

A systematic Cochrane review found that in *both* fertile and infertile populations, active and passive smoking are associated with reduced fertility and a decreased chance of producing a healthy, live infant. In males, cigarette smoking has been observed to impair sperm respiration, thus affecting the sperms' mitochondrial function[38] as well as causing a reduction in sperm motility and semen quality.[39] Reproductive hormone system disorders, dysfunction of spermatogenesis and the sperm maturation process, and impaired spermatozoa function have been observed in smokers. Tobacco smoking leads to reduced semen quality, including semen volume, sperm density, motility, viability, DNA fragmentation, seminal zinc levels, and normal morphology. The effects are directly correlated with cigarette quantity and duration of smoking.[40,41] A systematic review and meta-analysis (n = 5865 men) on the effects of cigarette smoking suggested an overall negative effect on semen parameters. An association was seen with reduced sperm count and motility, and the effect is greater in moderate and heavy smokers.[42]

It is therefore strongly recommended that all males cease both passive and active smoking for at least the preconception period and ideally beyond it.

Alcohol intake. Excessive alcohol consumption in men is strongly associated with diminished sperm function; however, there is a scarcity of comprehensive research in this area. The limited available data suggest that moderate or occasional alcohol intake does not affect semen quality as do regular high levels of consumption.[43–45]

A review of the literature in 2013 suggests that alcohol consumption alters sperm parameters (most commonly morphologically abnormal spermatozoa) and testicular pathology. It is noted that genetic factors, as well as nutritional deficiencies, may modulate the effect of alcohol on spermatogenesis.[43]

More recently, a cross-sectional study of 8344 healthy men in Europe and the United States found that moderate alcohol intake was not associated with a reduction in semen quality. However, this study only looked at alcohol consumed the week before the semen testing, which does not account for the long-term effects of alcohol consumption.[44] Another study of 347 men investigating the effect of the last 5 days' worth of alcohol intake found that alcohol intake was associated with impairment of most semen characteristics. There was a tendency toward lower semen parameters with higher intake of alcohol, but no statistically significant dose-response association was found.[45] Without further rigorous studies, the best available evidence suggests that alcohol intake and fertility are linked only with high levels of consumption (more than 8 drinks per week, or more than 40 g alcohol per day, depending on the study).[46]

A recent review of 15 cross-sectional studies has shown a detrimental effect of alcohol consumption on semen volume and morphology, mainly in daily, not occasional, consumers. This suggests that moderate consumption of alcohol should not adversely affect semen quality parameter.[47] As with smoking, abstaining from alcohol reverses semen parameter damage. A case report of a 6-year follow-up of a male patient showed that stopping alcohol consumption led to a rapid, dramatic improvement in semen characteristics. Normal semen parameters were observed after 3 months.[48]

From a naturopathic viewpoint, however, it is important to encourage avoidance for a number of reasons, including effects on the liver and detoxification as well as on endocrinology (especially estrogen pathways), calorie consumption, hyperglycemic effects, and others. It is prudent to recommend alcohol avoidance in the preconception period so as to ensure optimal functioning of the male body and subsequent positive effects on sperm parameters. It is of prime importance to recommend abstinence from binge drinking and regular heavy drinking.

Caffeine. High dosages of caffeine have been shown in vitro to increase protein oxidative damage in Sertoli cells, but moderate caffeine intake appears to be safe in male reproductive health. Although caffeine intake boosts semen volume, it also lowers the concentration.[49,50] Caffeine intake of less than 800 mg/day does not appear to affect motility, morphology, or DNA fragmentation.[51]

A recent systematic review of the relationship between caffeine and parameters of male fertility found that caffeine intake may negatively affect male reproductive function; however, the data to date are inconsistent and inconclusive.[52] An observational study on the effects of caffeine and alcohol on men at a fertility clinic found no association between caffeine or alcohol intake on semen parameters; however, pretreatment caffeine and alcohol intake did affect live birth outcome after infertility treatment with ART. Caffeine intake was associated with a lower probability of achieving live birth; however, alcohol intake was related to a higher probability of live birth. Alcohol intake was assessed on reported drinking habits over 1 year; therefore the effect of acute alcohol intake preceding ART was not assessed.[53]

Marijuana and other recreational drugs. The effects of marijuana and other recreational drugs are difficult to determine because their use is illegal; however, the illicit drugs that have been found to adversely affect male fertility are marijuana, opioid narcotics, methamphetamines, cocaine, and anabolic-androgenic steroids.[54,55] The use of such drugs generally should be discouraged, particularly because they have well-documented harmful effects on the developing fetus.[56]

A known fertility toxicant, marijuana contains constituents known as cannabinoids, which have been shown to impair signaling pathways, alter hormonal regulation, and complicate timing issues during embryo implantation.[57] In males, cannabinoids have been found to inhibit the mitochondrial respiration of sperm,[58] reduce testosterone production,[57] decrease sperm motility, compromise sperm morphology, and decrease sperm function, specifically capacitation and acrosome reactions.[59]

In vivo and in vitro studies have shown that cannabis may disrupt the hypothalamus–pituitary–gonadal axis, spermatogenesis, and sperm function (motility, capacitation, acrosome reaction).[60]

Consuming cannabis several times per week for 5 years results in a reduction in the volume and number of spermatozoa and changes sperm morphology and motility, reducing their fertilization capacity.[61]

Obesity, Weight Loss, and Exercise

Obese men are known to have lower sperm counts (up to 50%), reduced sperm motility, reduced spermatogenesis, increased DNA fragmentation in sperm, and increased levels of erectile dysfunction.[62] A meta-analysis of 21 studies demonstrated an increased risk of DNA fragmentation and azoospermia or oligozoospermia in overweight or obese males.[63]

Obesity can alter the physical and molecular structure of germ cells in the testes and ultimately affect the mature sperm.[64,65] Obesity induces a state of inflammation, and proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are increased in the serum, testicular tissue, and seminal plasma of obese males. In the testes, proinflammatory cytokines can directly impair the seminiferous epithelium. A proinflammatory state can also damage epididymal epithelium function, impeding sperm maturation and fertilization ability.[66]

Obesity also causes oxidative stress due to the excess of ROS, which causes damage to DNA and plasma membrane integrity in sperm.[67] Additionally, extra-abdominal weight can increase scrotal temperature. Hormonal changes are primarily responsible for such changes in obese men. The levels of total and free testosterone are reduced in obese men in proportion to the level of obesity. Estrogen is increased owing to the peripheral aromatization of androgens in adipose tissue. The estrogens produced have a negative-feedback effect on gonadotropin production, thereby reducing FSH. This reduction in FSH further reduces testosterone production and spermatogenesis. Additionally, increased body fat and a sedentary lifestyle are associated with raised testicular temperature, which further adversely affects spermatogenesis.[62] Obesity has been shown to affect semen parameters, including decreased sperm concentration, decreased sperm motility and vitality, and increased abnormal morphology.[67–70] Similarly, it was found that being underweight also had a negative effect on sperm quality. Maintaining an adequate or normal body weight is an important factor for male fertility,[71] and weight loss in obese men can increase sperm count and normal sperm morphology.[72]

Regular physical activity has beneficial effects on sperm parameters, as demonstrated in a cross-sectional study of 32 men that found a significant enhancement in semen volume, viability, motility, and morphology in the physically active men compared with sedentary men.[73]

A study of 419 sedentary men attending a fertility clinic found that moderate aerobic exercise training for 24 weeks favorably improved seminal markers of inflammation and oxidative stress and enhanced antioxidant defense system. This correlated with improvements in semen parameters, sperm DNA integrity, and pregnancy rate. Improvements were seen after 12 weeks of the trial.[74] These results are supported by additional studies showing that in healthy men, moderate-intensity aerobic exercise training can induce significant improvements in semen parameters and sperm DNA integrity, mainly through adaptations in the seminal antioxidant defense system and attenuating seminal markers of inflammation.[75–78] It should be noted that prolonged intensive exercise and training may lead to alterations in reproductive hormone levels; atrophy of the testicular germinal epithelium and adverse effects on spermatogenesis; and changes in semen parameters, including abnormal sperm morphology and reduced sperm motility.[77]

Sedentary Lifestyle

A study of 189 young men found that sperm concentration and total sperm count were directly related to physical activity. Measurement of physical and leisure time activities was not related to sperm motility and morphology in this study; however, TV watching has been inversely associated with sperm concentration and sperm count.[79] In another small study (n = 31) of healthy males, it was found that physically active men have a more anabolic hormonal environment and healthier semen production compared with their sedentary counterparts.[80] Physical activity also helps maintain a healthy weight: body mass index (BMI) and waist circumference are associated with a decrease in total sperm count and ejaculate volume.[81]

Infections and Infertility

Infections in the male genitourinary tract—including infections of the epididymis, seminal vesicles, prostate, bladder, and urethra—are thought to play a major role in many cases of infertility.[82] The exact extent of this role is largely unknown because of the lack of suitable diagnostic criteria coupled with the asymptomatic nature of many infections. The presence of antisperm antibodies or high levels of debris in the semen sample is considered to be a good indicator of chronic infection in the absence of other clinical findings.

A wide number of bacteria, viruses, and other organisms can infect the male genitourinary system. Table 185.6 offers a list of the more common potential causative agents.

DIETARY CONSIDERATIONS

A single sperm comprises the key nutritional ingredients (Fig. 185.1).

The dietary guidelines in Chapter 44 provide sound guidance for improving fertility. In particular, it is important to eat the right types of fats. Surrounding the entire sperm is a "shield" of essential fatty acids protecting its structure, enabling continuity of movement, and safeguarding the integrity of the precious genetic material within. The male's dietary intake must be assessed so as to ensure that all trans, rancid, and oxidized fats and excessive saturated fats are avoided. High intakes of saturated fats are negatively associated with sperm count and sperm concentration.[83–85] It is recommended to prescribe sufficient essential fatty acids from both supplemental and dietary sources. The presence of essential fatty acids ensures that sperm are kept fluid and flexible, which regulates the acrosome reaction, sperm-oocyte fusion, and sperm-oocyte fertilization.[86]

Because of the effects of fats and oils on agglutination and cell-membrane dynamics, certain fats are best avoided in infertile men, and consumption of others should be increased. Hydrogenated oils, trans fatty acids, and cotton oils should be avoided. Trans fats are unable to be endogenously synthesized, and excessive consumption contributes to fat accumulation in the testicular environment,[87] and trans-fat intake has been shown to be inversely related to sperm count and testicular volume.[88,89] Cottonseed is especially problematic because it may contain toxic residues as a result of the heavy spraying of cotton and its high levels of gossypol, a substance known to inhibit sperm function. In fact, gossypol is being investigated for possible use in a "male birth control pill." Its potential as an antifertility agent became known after

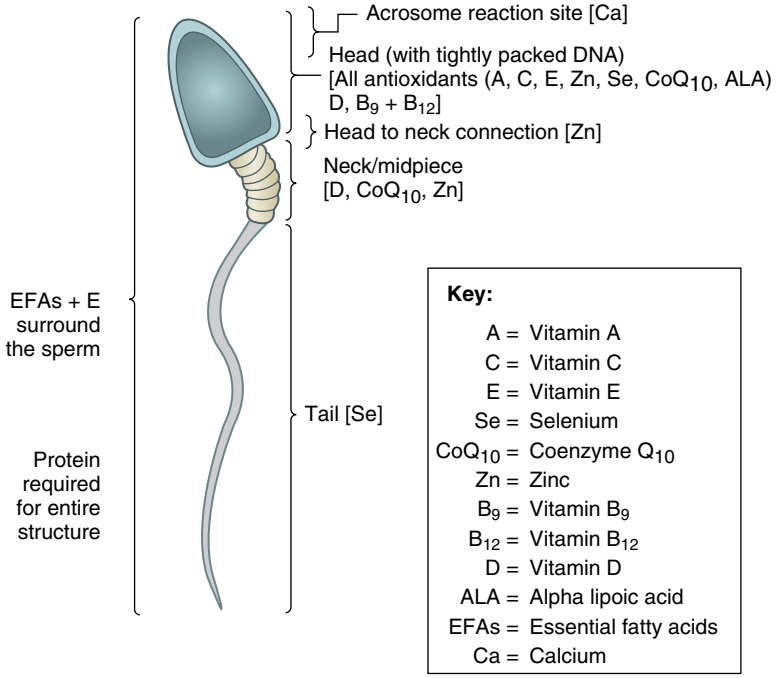


Fig. 185.1 Sperm morphology and composition.

studies demonstrated that men who had used crude cottonseed oil for cooking had low sperm counts followed by total testicular failure.[90] Excessive consumption of long-chain saturated fats from meat should also be avoided; these, combined with an inadequate intake of essential fatty acids, can change the fatty acid composition of the sperm membranes, thus reducing fluidity and interfering with sperm motility. The patient must be taught to read food labels carefully and avoid all sources of cottonseed oil and other damaging oils.

To promote proper sperm membrane function, it is important to supplement with the long-chain omega-3 fatty acids from fish oil because studies have shown that sperm motility and morphology strongly correlate with levels of omega-3 fatty acids, in particular, docosahexaenoic acid (DHA), in the sperm membrane.[83,91] One paper noted that excessive omega-6 compared with omega-3 fatty acids in seminal fluid decreased sperm concentration, sperm motility, and sperm morphology among patients with idiopathic oligoasthenoteratozoospermia.[92] It is therefore advisable to encourage omega-3 sources as a preference and to restrict the use of popular omega-6 cooking oils, such as soy, corn, and safflower. Supplemental goals should be to prescribe an optimal dose of 1000 to 2000 mg of eicosapentaenoic acid (EPA)+DHA daily, with additional dietary recommendations such as raw nuts and seeds; cold-pressed monounsaturated oils like olive, canola, and macadamia nut oils; avocados; and wild and sustainably farmed fish containing high essential fatty acids (EFAs).

In a double-blind, placebo-controlled, randomized controlled trial (RCT) of men with idiopathic oligoasthenoteratospermia (n = 238), it was shown that 1.84 g/day of omega-3 fatty acids (DHA and EPA) over 32 weeks resulted in a significant improvement of sperm cell total count and sperm cell concentration in the omega-3 groups compared with placebo.[93] An RCT of 57 men found that supplementation with DHA-enriched oil (990 mg of DHA and 135 mg of EPA per day) over a 10-week period resulted in a significant decrease in sperm DNA fragmentation compared with placebo. No significant effect was found on semen parameters.[94] Walnuts contain EFAs and are also a good source of antioxidants in the diet, and a study has shown that 75 g of walnuts per day for 3 months improved sperm vitality, motility, and morphology in a group of healthy men consuming a Western-style diet.[95]

Adequate dietary protein is also an important consideration. The entire sperm is dependent on the protein status of the male. Insufficient protein in the diet undermines the quality of the sperm, reflected primarily in their morphology but undoubtedly also in their inability to move adequately and in the right direction (motility). Similar to the rest of the body, if protein needs are not met, deterioration of bodily processes and structures can eventuate. It is crucial to thoroughly assess the protein levels of all male patients both in laboratory assessments (total protein status) and through dietary calculation. The standard calculation of 0.8 to 1.2 g protein per 2.2 lb (1 kg) body weight is a positive general rule; 0.8 g protein is best for more sedentary patients, and 1.2 g protein is more appropriate for more active types. For example, an active patient weighing 176 lb (80 kg) should be prescribed 96 g pure protein per day, ideally from a variety of sources. The quality of the protein is also important. A high intake of processed meats is associated with fewer morphologically normal sperm compared with a low intake. It's also associated with a higher risk of asthenozoospermia. Fish intake is related to higher sperm count and morphologically normal sperm.[96,97]

Hydration must also be optimized. Sperm are manufactured and spend time within semen. On reviewing the nutritional components of semen, it becomes clear that it is a by-product of the body, as is urine or feces. Therefore, if a male is dehydrated, it is understandable that the resultant coagulation of semen will increase, and exposure to toxin and by-products from eliminatory channels will occur. Thus optimal hydration can dilute semen, reduce sperm exposure, and improve motility considerably. Calculating a male's hydration requirements is best achieved using a 30-mL/2.2-lb (1-kg) rule; that is, for a 176-lb (80-kg) patient, minimal hydration requirements would be 2.4 L per day, with additional intake required for dehydration, exercise, and alcohol or caffeine intake.

A recent review of observational studies concluded that adherence to a healthy diet (e.g., the Mediterranean diet pattern and diets characterized by higher intakes of seafood, poultry, whole grains, fruits, and vegetables in non-Mediterranean countries) has been consistently associated with better semen parameters in a wide range of studies in North America, Europe, the Middle East, and East Asia.[98–100] It has been shown that following a diet characterized by higher intakes of

legumes, vegetables, cereals, fruits, and olive oil and low intakes of dairy, mayonnaise, margarines, sauces, snacks, and sweets was associated with semen quality—particularly sperm concentration and progressive motility—among men from couples planning pregnancy.[101] A study in men with asthenozoospermia showed an association between following a dietary nutrient intake pattern comprising mainly antioxidants, vitamin D, fiber, and polyunsaturated fatty acids with a significantly lower risk of asthenozoospermia.[102]

NUTRITIONAL SUPPLEMENTS

Antioxidants

A 2014 Cochrane review[103] assessed the effect of antioxidants on male subfertility by reviewing 48 trials and 4179 subfertile men. Of importance, the authors concluded that there is sufficient evidence to prove that antioxidant supplementation in subfertile males improves the outcomes of live births and pregnancy rates for subfertile couples undergoing ART cycles.

Other reviews found an improvement in sperm quality (predominantly motility, but including concentration and morphology), along with increased pregnancy rates, after antioxidant supplementation. Significant reductions in measures of sperm oxidative stress and DNA damage were found in 95% of the studies after oral antioxidant treatment.[104–106]

A study found that combined antioxidant therapy consisting of vitamin C (80 mg/day), vitamin E (40 mg/day) and coenzyme Q_{10} CoQ_{10} (120 mg/day) administered over a period 6 months in men with oligoasthenozoospermia (n = 169) resulted in significant improvements in sperm concentration and motility.[107] Similarly, combination therapy with L-carnitine (440 mg), CoQ_{10} (ubiquinol 30 mg), vitamin E (75IU), and vitamin C (12 mg), two or three times per day, for 3 to 6 months improved sperm concentration in infertile men.[108] A study of 120 men with idiopathic infertility showed that supplementation with NAC (600 mg/day) for 3 months resulted in improvements in semen volume, motility, and viscosity. Serum antioxidant capacity was increased, and the total peroxide and oxidative stress index were lower.[109] A clinical trial on the effects of curcumin in infertile men found that supplementation with curcumin (80 mg/day) over 10 weeks resulted in statistically significant improvements in semen parameters and total antioxidant capacity, compared with placebo.[110]

In greater detail, important points in these reports include the following:

- Antioxidant use was associated with a statistically significant increased pregnancy rate compared with control (pooled odds ratio [OR] 4.18, 95%; confidence interval [CI] 2.65–6.59; $P < 0.00001$).
- No studies reported evidence of harmful side effects of the antioxidant therapy used.
- One man in 20 will be affected by subfertility, and 3% to 80% of male-factor subfertility is believed to be due to oxidative stress.[111]
- Subfertile men are confirmed to have lower levels of antioxidants in their semen than fertile men.[111,112]
- Levels of ROS are significantly higher in infertile sperm samples compared with others from healthy controls; 25% to 80% of infertile men present with elevated ROS in the semen.[113]
- ROS cause fertility problems by
  a. Damaging the sperm membrane, which contains high levels of unsaturated fatty acids, which are susceptible to oxidation, thus affecting sperm motility and the ability of spermatozoa to break down the oocyte membrane (zona pellucida). Damage to the sperm membrane leads to loss of membrane integrity, increased permeability, and structural DNA damage.[112,113]
  b. Altering sperm DNA: the integrity of sperm DNA is one of the major determinants of normal fertilization and embryo growth in natural and assisted conception.

All antioxidants are concentrated in the head of the sperm to varying degrees. The general recommendation is to encourage the prescription of combination products to ensure that the greatest antioxidant potential is achieved. Antioxidants are responsible for protecting the DNA within the head of the sperm and reducing the workload of the mRNA in the eventual oocyte. By optimizing oxidant status, the survival and longevity of the sperm are also promoted, and the sperm is enabled to detect signals from the recently ovulated oocyte. A reduction in antioxidants is likely to present specifically as high DNA damage or poor morphology. However, individual antioxidants such as zinc are responsible for regulating hormone status and thus sperm count; therefore all must be considered in a holistic infertility treatment protocol.

Free-radical or oxidative damage to sperm is thought to be responsible for many cases of male infertility, high levels of free radicals having been found in the semen of approximately 40% of infertile men.[114–116] The following three factors combine to render sperm particularly susceptible to damage by free radicals:

- A high membrane concentration of polyunsaturated fatty acids (PFAs)
- Active generation of free radicals
- A lack of defensive enzymes

All of these factors combine to make the health of the sperm critically dependent on antioxidants. Although most free radicals are produced during normal metabolic processes, the environment contributes greatly to the free-radical load. Men exposed to higher levels of free radicals are much more likely to have abnormal sperm and sperm counts.[114–116] Antioxidants are required to protect sperm against oxidative damage (which may alter DNA) as well as to instigate cellular repair of damage caused by environmental factors or aging. In the healthy male, the seminal plasma is naturally rich in antioxidants because sperm are highly susceptible to the effects of ROS.

Sperm are extremely sensitive to free radicals because they depend so much on the integrity and fluidity of their cell membranes for proper function. Without proper membrane fluidity, enzymes are activated, possibly leading to impaired motility, abnormal structure, loss of viability, and ultimately death of the sperm.

The major determinant of membrane fluidity is the concentration of PFAs, particularly omega-3 fatty acids like DHA, which are highly susceptible to free-radical damage. The sperm have a relative lack of superoxide dismutase and catalase, which can prevent or repair oxidative damage. Adding to this more susceptible state is the fact that sperm generate large quantities of free radicals to help break down barriers to fertilization.

Vitamin C

Vitamin C improves all semen parameters. A marginal deficiency causes oxidative damage to sperm, resulting in reduced sperm motility and viability. Supplementation leads to improvement in both viability and motility, reduced numbers of abnormal sperm, and reduced sperm agglutination.[117,118]

The generation of ROS and associated links with infertility have been established and extensively studied. In particular, the effects of increased ROS in the serum, semen, and testicular tissues have been considered. Alterations in the testicular microenvironment and hemodynamics can increase the production of ROS and/or decrease local antioxidant capacity, resulting in the generation of excessive oxygen species.

Vitamin C (ascorbic acid), a major antioxidant present in extracellular fluid, is present at a high concentration in seminal fluid compared with blood plasma (364 vs. 40 mM) and is present in detectable amounts in sperm,[119] where it prevents sperm agglutination and oxidative damage. In infertile men, vitamin C has been found in reduced quantity in the seminal plasma.[120,121] Males with inadequate seminal vitamin C have also been observed to suffer from sperm DNA damage,[120] suggesting that a defect or inadequate intake of vitamin C may prompt ROS to cause breakage and oxidation of sperm DNA.

When dietary vitamin C was reduced from 250 to 5 mg/day in healthy human subjects, the ascorbic acid content in seminal fluid decreased by 50%, and the number of sperm with damage to their DNA rose by 91%.[122] These results indicate that dietary vitamin C plays a critical role in protecting against sperm damage and that low dietary vitamin C levels were likely to lead to infertility. A study examining vitamin C as an adjunct therapy for men after undergoing varicocelectomy (n = 115) showed that 3 months of daily vitamin C supplementation (500 mg/day) resulted in a significant improvement in sperm motility and morphology in the treatment group compared with placebo.[123] Another study has shown that supplementation with 1000 mg vitamin C per day for 6 months resulted in improvements in sperm concentration and motility in fertile but obese men. There was no change in semen volume or percentage of normal sperm morphology in the study.[124]

It is now well documented that cigarette smoking greatly reduces vitamin C levels throughout the body, and it is proved that smokers require at least twice as much vitamin C as nonsmokers. In one study, men who smoked one pack of cigarettes a day received either 0, 200, or 1000 mg of vitamin C. After 1 month, sperm quality improved in proportion to the level of vitamin C supplementation.[125] Nonsmokers appear to benefit from vitamin C as much as do smokers. In one study, 30 infertile but otherwise healthy men received either 200 or 1000 mg of vitamin C or placebo daily.[126] Sperm count, viability, motility, agglutination, abnormalities, and immaturity were measured weekly. After 1 week, the 1000-mg group demonstrated a 140% increase in sperm count, the 200-mg group a 112% increase, and the placebo group no change. After 3 weeks, both vitamin C groups continued to improve, with the 200-mg group catching up to the improvement of the 1000-mg group. One of the key improvements was observed in the number of agglutinated sperm. Sperm become agglutinated when antibodies produced by the immune system bind to them. Antibodies to sperm are often associated with chronic genitourinary tract or prostatic infection. When more than 25% of the sperm are agglutinated, fertility is very unlikely. At the beginning of the study, all three groups had more than 25% agglutinated sperm. After 3 weeks, the proportion of agglutinated sperm in the vitamin C groups dropped to 11%. Although this result is significant, the most impressive result of the study was that at the end of 60 days, several men in both of the vitamin C groups had impregnated their wives, compared with none in the placebo group. Therefore vitamin C supplementation can be very effective in treating male infertility, particularly if it is due to antibodies against sperm.

Vitamin E

Vitamin E supplementation appears to be especially warranted because this vitamin is the main antioxidant in various cell membranes, including those surrounding sperm. Free radicals, if left alone, lead to the peroxidation of phospholipids in the mitochondria of the sperm, making the sperm immotile. Vitamin E has been shown to play an essential role in inhibiting free-radical damage to the unsaturated fatty acids of the sperm membrane[127] and in enhancing the ability of sperm to fertilize an egg in an IVF setting. Additionally, it has been shown to protect DNA within the sperm from damage.[128] Infertile men have significantly lower serum and seminal vitamin E concentrations than fertile men.[129]

In one study, supplementation with vitamin E was found to decrease malondialdehyde concentration in sperm pellet suspensions and to improve sperm motility. Even more important, however, 11 of 52 (21%) treated infertile men impregnated their spouses, whereas none in the placebo group did so. After completion of the study, 26 of the placebo patients were switched to vitamin E; soon thereafter, 4 were able to successfully impregnate their spouses.[130] In another study, vitamin E (400 IU) and selenium (225 mcg) significantly improved sperm quality.[131] This was supported by a study in which a combined therapy of vitamin E (400 IU/day) and selenium (200 mcg/day) was administered for 6 months, resulting in a significant increase in sperm motility and a reduced percentage of defective spermatozoa.[132] A prospective randomized study compared the effectiveness of vitamin E alone, clomiphene citrate alone, or a combination of both treatments on semen parameters of 90 patients with idiopathic oligoasthenozoospermia (OAT). After 6 months, there was a significant improvement in sperm concentration and sperm motility in the combined-treatment group.[133]

Supplementation with vitamin E may also be useful for couples undergoing IVF. Vitamin E (200 mg/day for at least 3 months) was found to improve the in vitro fertilization rate of fertile normospermic males with low fertilization rates after 1 month of treatment, possibly by reducing the lipid peroxidation potential.[134] Beneficial results have also been observed in patients undergoing ICSI, where vitamin E helps prevent DNA fragmentation, thus improving ICSI outcomes.[135]

Vitamin A, Beta-Carotene, and Lycopene

Vitamin A is an antioxidant required for cellular growth and differentiation, gene expression and cellular differentiation, immunity, regulatory functions, and epithelial tissue integrity. It is necessary for the health of the testes and for sperm production. Low concentrations of vitamin A are associated with abnormal semen parameters in men,[136] and deprivation of vitamin A in animals has been shown to lead to a loss of spermatogenesis due to degeneration of the germ cells, which is restored once vitamin A is reintroduced.[137] Studies are lacking on the effects of vitamin A supplementation in men; however, studies of vitamin A in combination with other nutrients show improved effects on sperm motility and sperm count.[138] Beta-carotene levels are significantly reduced in immune-infertile men. Intake is associated positively with a higher sperm concentration as well as higher quantities of motile sperm.[139] In one study of 189 young, healthy men, higher β-carotene and lutein intake from food sources was associated with better sperm motility, whereas a moderate intake of β-carotene and vitamin C was associated with higher sperm concentration and sperm count.[140] The same study found that higher lycopene intake was associated with better sperm morphology, and lycopene may be even more useful than β-carotene. Lycopene is found in high concentrations in the testes and seminal plasma, and reduced levels have been demonstrated in men with infertility.[141] In one clinical trial, 30 men with idiopathic nonobstructive oligo-/astheno-/teratozoospermia were administered 2 mg of lycopene twice a day for 3 months. Twenty patients (66%) showed an improvement in sperm concentration, 16 (53%) had improved motility, and 14 (46%) showed improvement in sperm morphology. In patients showing an improvement, the median changes were 22 million/mL in concentration, 25% in motility, and 10% in morphology.[131] Lycopene levels in semen can be significantly increased with dietary intake of natural sources of lycopene.[142] A randomized controlled study found that regular consumption of tomato juice significantly increased seminal plasma lycopene levels and increased sperm motility. In this study, 44 infertile men consumed one can of tomato juice per day (30 mg lycopene) for 12 weeks. Improved results were seen after 6 weeks of the trial.[143]

Vitamin D

Vitamin D is a lipid-soluble vitamin that is required structurally and functionally. It enhances male and female fertility and facilitates the absorption of calcium. The vitamin D receptor and enzymes that metabolize vitamin D are found in the sperm head and midpiece of human sperm and in the testes and male reproductive tract of rats. It has been suggested to play an important role in the production and transport of sperm,[144] and the expression levels of vitamin D receptor in CYP24A1 in spermatozoa are positive markers for semen quality.[145] Studies show that men who are vitamin D deficient have a lower percentage of motile spermatozoa compared with those who have sufficient vitamin D[146,147]; however, both low and high vitamin D levels are associated with a decline in semen parameters.[148] This trend is supported by a further study investigating the association between vitamin D status and semen quality in 307 young, healthy men. High vitamin D levels were associated with lower total sperm count and percentage of normal morphology sperm.[149]

Vitamin D supplementation has been shown to increase testosterone levels in a small study ($n = 54$). All men in the study were vitamin D deficient at the start of the study and had testosterone levels at the lower end of the reference range.[150] This result was not confirmed in other studies. Other studies found that vitamin D supplementation did not affect testosterone levels.[147,151] A cross-sectional study of 1362 men did find that plasma 25(OH)D levels were positively associated with total and free testosterone levels.[152] Given the level of available evidence, supplementation with vitamin D might improve semen quality in at least some of the idiopathic cases of male infertility in a safe and noninvasive manner. Data from small intervention studies and association studies indicate that vitamin D supplementation may only be beneficial for men with vitamin D deficiency,[153] and this finding is supported by a recent prospective study of more than 1000 infertile men.[154] Vitamin D supplementation (5000 IU/day for 2 months) was found to improve sperm motility in idiopathic infertility patients who had low vitamin D levels.[155] Similarly, in a prospective pilot study of infertile men, the incidence of low vitamin D was 76.9% (mean serum vitamin D level 23.6 ng/mL). After treatment with vitamin D supplements, the rate of low sperm motility (<40%) improved.[156]

Selenium

Selenium is a critical antioxidant that is essential for male fertility because of its role in testosterone synthesis, normal sperm maturation, and motility[157]; moreover, clinical trials reveal that selenium has the ability to increase sperm motility and assist in the production of healthy spermatozoa.[158] Selenium is also required structurally because the sperm's capsular selenoprotein is involved in the stability and motility of the mature sperm and also forms part of the glutathione peroxidase antioxidant system, which is vital for spermatogenesis and protects the sperm against the effects of ROS.[159] Lack of selenium leads to atrophy of the seminiferous epithelium, testis volume reduction, and disorders of spermatogenesis and maturation of spermatozoa in the epididymis. Selenium in seminal plasma correlates with good spermatozoa concentrations, motility, and morphology.[160]

In animal studies, depletion of mitochondrial glutathione peroxidase has been found to cause impaired sperm quality and severe structural abnormalities in the midpiece of spermatozoa, leading to infertility.[161] The tail of the sperm relies on adequate selenium status to maintain its "whip-like" action. Without sufficient selenium, sperm are unable to swim in the right direction or may display marked immotility, thus preventing oocyte location and fusion. A supply of selenium for the selenoproteins in the testis is critical to spermatogenesis, and deficiency or an excess of dietary selenium may impair spermatogenesis, resulting in poor semen characteristics and quality and infertility.[162]

The effects of selenium on sperm motility are highlighted in a study involving a subgroup of individuals with poorly motile sperm and subsequent subfertility.[163] Over a 3-month period, the administration of selenium (either on it its own or as a combination of antioxidants, including vitamins A, C, and E) to males led to increased sperm motility compared with placebo. Five men (11%) achieved paternity in the treatment group, in contrast to none in the placebo group. This small study highlights the efficacy of selenium supplementation in subfertile men and suggests that improved selenium status can, in turn, improve sperm motility and the possibility of successful conception. Although this number may be seen as small, it was highly meaningful to those who were successful in conceiving—and all the more significant in terms of the cost and convenience of supplementation compared with IVF or ICSI.

Selenium (200 mcg/day) in combination with the antioxidant N-acetyl-cysteine (600 mg/day) was found to improve semen parameters in idiopathic oligo-asthenoterato-spermiaplasma males in a double-blind, placebo-controlled, randomized study undertaken over 6.5 months.[164] Improvements in sperm count, motility, and morphology were all observed; however, once supplementation stopped, the parameters reverted back to their readings at baseline in two spermatogenesis cycles. This study did not include pregnancy rate.

Zinc

Zinc is perhaps the most important trace mineral for male sexual function and is found in high concentrations within the prostate and testes; particularly high amounts are also found in the semen (approximately 2.5 mg of zinc is lost per ejaculate). It is involved in virtually every aspect of male reproduction, including hormone metabolism, spermatogenesis, and sperm motility.[165]

Zinc plays an important role in all human living cells; it is involved in the transcription of RNA, the replication of DNA, and the synthesis of protein, all of which are crucial for reproduction and fertility. Additionally, it protects against free-radical damage and ROS, which can impair sperm.[166] Deficiency of zinc in males can lead to gonadal dysfunction[165] and has been observed to be associated with idiopathic male infertility[166] and impotence.

Zinc levels are typically much lower in infertile men with low sperm counts, indicating that a low zinc status may be the contributing factor to infertility.[167,168] It has also been shown that zinc status directly correlates with an increase in sperm count as well as improvements in morphology and motility.[160,169] In considering sperm structure, zinc has been shown to influence motility and the head–neck connection of the sperm[170]; it is also important in the stabilization of cell membranes and sperm chromatin.[171] Zinc has been shown in vitro to improve seminal antioxidant states in infertile men, but it does not prevent sperm lipid peroxidation.[172] Finally, it has been shown to exert an antimicrobial effect on the seminal plasma, which is helpful if sperm antibodies or underlying genitourinary infection is present.[173]

Several studies have evaluated the effect of zinc supplementation on sperm counts and motility.[174–177] The results of all of the studies support the use of zinc supplementation in the treatment of oligospermia, especially in the presence of low testosterone levels. The effectiveness of zinc is best illustrated by a study in 37 men with infertility of greater than 5 years' duration whose sperm counts were less than 25 million/mL.[27] Blood testosterone levels were also measured. The men received a supplement of zinc sulfate (60 mg elemental zinc daily) for 45 to 50 days. In the 22 patients with initially low testosterone levels, the mean sperm count rose significantly from 8 to 20 million/mL. Testosterone levels also increased, and 9 of the 22 wives became pregnant during the study. This result is quite impressive, given the long-term nature of the infertility and the rapidity of the results. In contrast, in the 15 men

who had normal testosterone levels before the study, sperm counts increased slightly, but there was no change in testosterone levels, and no pregnancies occurred.

In a 3-month study of 45 asthenozoospermic men, it was found that supplementing with zinc sulfate (200 mg twice daily) alone, in combination with vitamin E, or in combination with both vitamin E and vitamin C was associated with improved sperm parameters, less oxidative stress, sperm apoptosis, and sperm DNA fragmentation compared with placebo. No difference in effect between the three treatment groups was found.[178] Two other studies have shown that supplementation with zinc sulfate (24 mg for 45–50 days, and 89 mg for 4 months) significantly increases testosterone, seminal zinc levels, and sperm count.[179] An additional study of zinc sulfate supplementation (220 mg/day for 3 months) in 110 men found that it resulted in increased semen volume, increased sperm motility, and normal sperm count. Antioxidant status was improved in spermatozoa and seminal plasma.[180] Oral zinc supplementation (440 mg zinc sulfate per day) successfully restored seminal catalase-like activity and improved sperm concentration and progressive motility in a group of asthenozoospermic men.[181] A recent systematic review and meta-analysis of the literature examined 22 studies including 2600 cases and 867 controls. The review concluded that seminal plasma zinc concentrations in infertile males were significantly lower than those of fertile males, and zinc supplementation could significantly increase the sperm quality of infertile males.[182]

Optimal zinc levels must be attained if optimal male sexual vitality is desired. Zinc deficiency is increasing throughout the world and is dependent on the country's soil content and on environmental legislation that prevents harmful farming practices. Additionally, owing to the negative effects of excess copper, it is advisable to check serum zinc and copper status. Where a deficiency state is diagnosed, it is important to ensure that a 1:1 ratio (or better) is achieved.

B Vitamins (Especially Folate and Vitamin B_{12})

Folate and vitamin B_{12} are concentrated within the head of the sperm and provide vital nutritional potential in sperm generation and survival. Folate and B_{12} are required for healthy DNA and RNA synthesis, normal protein synthesis, and the regulation of gene expression.[176] These nutrients are required to ensure that the DNA within the head of the sperm is structured appropriately and that each sperm (and the DNA within it) replicates identically.

Both folate and B_{12} facilitate spermatogenesis,[183] which is reliant on DNA synthesis[176] for germ cell growth and the rapid division of cells. Multiple studies have found that low levels of folic acid in seminal plasma are associated with increased sperm DNA damage,[184] whereas B_{12} deficiency is strongly associated with reduced sperm motility and count.[185] Because the human body has a high turnover of B_{12} and requires a continuous supply on a daily basis, supplementation is advisable for all men experiencing infertility regardless of proved deficiency state and especially for men who have sperm counts below 20 million/mL or a motility rate of less than 50%. In one study, 27% of men with sperm counts less than 20 million/mL who were given 1000 mcg/day of B_{12} were able to achieve a total sperm count in excess of 100 million/mL.[177] In another study, 57% of men with low sperm counts who took 6000 mcg/day demonstrated improvements.[186] A recent small study showed that infertile men with varicocele administered a multivitamin including vitamin B_{12} at 1 mcg/day, for 3 months, had lower sperm DNA fragmentation by about 22.1%.[187] Similarly, a study found that vitamin B_{12} (1 mcg/day for 3 months) as part of an oral antioxidant treatment improved sperm vitality, motility, and DNA integrity.[188]

Infertile men have lower concentrations of serum folic acid than fertile men; however, serum folate concentration was not found to correlate with any semen parameters.[189] In another study, seminal plasma folate concentration was found to be lower in infertile men than fertile men and was significantly correlated with low sperm concentration.[190] Folic acid supplementation (5 mg/day) in conjunction with zinc sulfate (220 mg/day) did not improve sperm quality in 83 subfertile men in a 16-week trial.[191] However, another study found that coadministration of folic acid (5 mg/day) and zinc sulfate (66 mg/day) over a 6-month period significantly improved sperm parameters after surgical repair of varicocele.[192]

It is important to remember that it is advisable to refrain from prescribing one nutrient without the other owing to the possibility of inducing a rebound anemia. Where this occurs, disorders of homocysteine metabolism such as methylenetetrahydrofolate reductase (MTHFR) polymorphisms may present with count or morphological issues. In men with the *MTHFR* gene polymorphisms, vitamin B_9 and vitamin B_{12} dietary intake was associated with a decrease in homocysteine and improvement of sperm concentration, motility, and morphology. The greatest benefits were seen in men with the T allele of *MTHFR* C677T polymorphism.[193]

A recent study investigating high-dose folic acid supplementation (5 mg/day) over 6 months found that methylation of promoter regions in several genes involved in cancer and neurobehavioral disorders was altered. Care must be taken in folic acid supplementation to avoid overdosage problems, particularly in patients who are homozygous for the *MTHFR* C677T polymorphism.[194]

Carnitine

Carnitine is derived from the amino acids lysine and/or methionine and plays a vital role in fatty acid metabolism. It works synergistically with CoQ_{10}, highlighting the importance of coprescription for optimal benefit. It is essential in the transport of fatty acids into the mitochondria, and deficiency results in a decrease in fatty acid concentrations in the mitochondria and reduced energy production. It is believed to have protective antioxidant effects and provides energy to the testicles and spermatozoa specifically. Several studies comparing fertile with infertile men found that fertile men had a statistically significant larger amount of carnitine in their seminal sample than the infertile men and that low levels of L-carnitine in the seminal plasma may be a potent marker for infertility.[195]

Carnitine concentrations are extremely high in the epididymis and sperm, suggesting a role in male reproductive function. The epididymis derives the majority of its energy requirements from fatty acids, as do the sperm, during transport through the epididymis. After ejaculation, the motility of sperm correlates directly with carnitine content; the higher the carnitine content, the more motile the sperm. Conversely, when carnitine levels are low, sperm development, function, and motility are drastically reduced.[114]

Several clinical studies have shown that carnitine supplementation can produce dramatic improvements in sperm counts and sperm motility. In the Italian Study Group on Carnitine and Male Infertility, 100 subjects were given 3000 mg of L-carnitine daily for 4 months.[196] Carnitine was able to increase sperm counts and sperm motility in both a qualitative and a quantitative manner, as follows:

- The number of ejaculated sperm per milliliter increased from 142 billion to 163 billion.
- The percentage of motile sperm increased from 26.9% to 37.7%.
- The percentage of sperm with rapid linear progression increased from 10.8% to 18%.
- The mean sperm velocity increased from 28.4% to 32.5%.

These results are even more impressive if results for only the patients with the poorest sperm motility are examined. This subgroup saw even more significant gains on all parameters. For example, the

percentage of motile sperm increased from 19.3% to 40.9%, and the percentage of sperm with rapid linear progression increased from 3.1% to 20.3%. These results have been confirmed in several double-blind studies.[197–201] Carnitine, administered in conjunction with other nutrients, has been shown to improve sperm motility in infertile men with idiopathic asthenozoospermia or asthenoteratozoospermia. Carnitine (1500 mg/day) was given in combination with vitamin C, CoQ_{10}, selenium, zinc, B_{12}, folic acid and vitamin E.[188,202] These results are supported by another randomized interventional study in which L-carnitine (2 g/day) was administered in conjunction with a multivitamin for 3 months. Improvements were seen in sperm concentration, sperm count, and sperm motility in men with idiopathic oligo- and/or asthenozoospermia. The results were more significant in L-carnitine combined with multivitamin than in either of those interventions alone, compared with control.[203] Similarly, in a 3-month study of 199 subfertile men, supplementation with a combination of L-carnitine (440 mg), L-arginine (250 mg), zinc (40 mg), vitamin E (120 mg), glutathione (80 mg), selenium (60 mcg), CoQ_{10} (15 mg), and folic acid (800 mcg) once per day resulted in a significantly better improvement in sperm density and motility compared with supplementation with L-carnitine (500 mg) twice per day.[204] In ART, where sperm is cryopreserved, L-carnitine had an enhancing effect on sperm motility and viability after cryopreservation.[205]

R-Alpha-Lipoic Acid

Alpha-lipoic acid is a powerful antioxidant indicated for its lipid and water solubility and because it assists in the chelation of heavy metals regardless of their storage site in the body. It is especially useful owing to its ability to regenerate other antioxidants, including vitamins C and E, CoQ_{10}, and glutathione.[206]

Alpha lipoic acid exhibits marked antioxidant activity to sperm in animal studies.[207–209] On review of the research, it appears to act as a shield for the sperm, forming a protective barrier around the midpiece (aqueous layer) and within the structure itself (lipid layer). This protection is crucial because it has been identified as one of the first places at which free radicals attack.[209] It is therefore useful to consider in patients with high DNA fragmentation levels. Additionally, animal studies reveal that alpha-lipoic acid improves sperm motility and viability, minimizes DNA damage,[209] and protects against bacterial lipopolysaccharides, which can induce acute inflammation[210]; it and may also assist with energy supply to the sperm.[209] In a randomized, triple-blind, placebo-controlled clinical trial of infertile men (n = 44), alpha-lipoic acid supplementation (600 mg/day) for 12 weeks improved sperm count, concentration, and motility and seminal levels of total antioxidant capacity.[211]

Coenzyme Q_{10}

CoQ_{10} is concentrated in the head and midpiece (neck) of the sperm. It is considered to be the most crucial and powerful antioxidant in sperm structure because of its role in mitochondrial energy release. It is believed to promote motility, foster sperm survival, and provide optimal energy to assist the sperm's travel on its journey to the oocyte.

As a fat-soluble antioxidant and free-radical scavenger, CoQ_{10} is required for the maintenance of cell membrane integrity and cell functioning. It is also specific for the health of all new cells, especially spermatozoa. CoQ_{10} in the seminal fluid and sperm[212] helps maintain optimal sperm motility.[213,214] Decreased levels have been found in the seminal plasma and spermatozoa of males with idiopathic and varicocele-associated asthenospermia.[215]

There have been a number of trials of CoQ_{10} supplementation in infertile men with idiopathic oligoasthenoteratospermia (OAT). A 26-week RCT of 212 infertile men with idiopathic OAT showed that daily supplementation with CoQ_{10} (300 mg) resulted in a statistically significant improvement in sperm count, sperm motility, and sperm morphology.[216] These results were confirmed in a 3-month study of supplementation with CoQ_{10} (200 mg/day), resulting in a significant improvement in sperm morphology in men with idiopathic OAT. The supplementation also resulted in higher catalase and superoxide dismutase (SOD) activity, indicating improved oxidative stress in seminal plasma.[217] A longer, 12-month study of 187 infertile men with idiopathic OAT found that treatment with CoQ_{10} (300 mg/day) resulted in significant improvements in sperm motility and sperm morphology. There was also a beneficial effect on pregnancy rate in the group.[218] In men with varicocele, supplementation with CoQ_{10} (100 mg/day) for 3 months resulted in a 40% increase in seminal plasma antioxidant status and a small improvement in semen parameters.[219] Administration of 200 mg/day of ubiquinol (reduced form of CoQ_{10}) over 26 weeks in a group of 228 men with unexplained infertility resulted in significant improvements in sperm density, motility, and morphology.[220] These results were supported in a 6-month trial of 60 men where 150 mg ubiquinol daily was found to increase sperm count and motility.[221] Although CoQ_{10} supplementation has been shown to be of benefit in improving various parameters of male infertility, CoQ_{10} intake from food was not found to be related to semen parameters (sperm concentration, total and progressive motility, and morphology) among subfertile men. A study of 211 participants showed that these men had a mean dietary CoQ_{10} intake of 19.2 mg/day (ranging from 2 to 247 mg/day). Intake estimates varied greatly and were based on a validated food frequency questionnaire.[222]

L-Arginine

The amino acid arginine is required for the replication of cells, making it essential in sperm formation. Nitric oxide synthase (NOS) uses L-arginine to synthesize nitric oxide, which can protect spermatozoa from lipid peroxidase damage. Via its role as a precursor to nitric oxide synthesis, arginine is required for angiogenesis, spermatogenesis, and hormone secretion.[223]

Some studies have shown that L-arginine can improve sperm count and motility.[185,224] Stress, in particular, has been found to decrease the levels of arginine in sperm-production pathways. Arginine supplementation is often but not always an effective treatment for male infertility. The critical determinant appears to be the level of oligospermia. If sperm counts are less than 20 million/mL, arginine supplementation is less likely to be of benefit. To be effective, the dose of L-arginine must be at least 4 g/day for 3 months. In perhaps the most favorable study, 74% of 178 men with low sperm counts had significant improvements in sperm counts and motility after arginine therapy.[225]

One double-blind, randomized, placebo-controlled crossover clinical trial examined the effects of Prelox, a combination of 80 mg/day of Pycnogenol and 3 g/day of L-arginine aspartate.[226] The results showed an improvement in semen parameters in 50 males with idiopathic infertility over a treatment period of 4 weeks. Also observed were significant increases in ejaculate volume, concentration, and number of spermatozoa as well as the percentage of vital spermatozoa compared with placebo. The percentage of spermatozoa with good progressing motility also increased significantly, whereas the percentage of immotile spermatozoa decreased. These results appear to be due to a combination of the antioxidant activity of Pycnogenol and/or the activity of L-arginine in stimulating the activity of endothelial NOS, leading to enhanced motility of spermatozoa. In a small pilot study, Pycnogenol (200 mg daily for 90 days) alone was shown to improve sperm morphology by 38% and the mannose receptor binding assay scores by 19%.[227]

Probiotics

Two small preliminary studies into the use of probiotics for improving male fertility have been carried out recently, opening new avenues for treatment of male infertility.

In one study, nine men with asthenozoospermic were administered two different antioxidant probiotic strains (*Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347) for 6 weeks. Each capsule contained an equal combination of both strains corresponding to 10^9 cfu/day. Results showed a significant improvement in sperm motility and a decrease in DNA fragmentation and intracellular ROS. Cell viability was not affected by the treatment. The authors concluded that the results were due to the antioxidant activity of the probiotic strains.[228]

The second randomized trial of 41 men with idiopathic oligoasthenoteratospermia found that treatment with a proprietary probiotic/prebiotic therapy (Flortec) over 6 months improved the quality and quantity of spermatozoa to a larger extent than placebo. Flortec is made up of *Lactobacillus paracasei* B21060 5×10^9 cells, arabinogalctan 1243 mg, oligo-fructosaccharides 700 mg, and L-glutamine 500 mg. The probiotic/prebiotic treatment improved the volume of the ejaculate, sperm concentration, number of ejaculated spermatozoa, motility, and the percentage of typical forms. The men's FSH, LH, and testosterone levels also improved. The improvements may be mediated by normalization of subtle alterations in hypothalamic–pituitary function, an antioxidant effect, and possibly even improvements in the prostatic microenvironment.[229] However, an alternative theory relating to the improvements seen in this study has been proposed.[230] Improvement in gut bacteria leads to improvement in intestinal mucosa barrier function, thereby resolving "leaky gut" and endotoxin-containing gut bacteria migrating into the systemic circulation. Endotoxin is a powerful immune stimulant, with recent data linking endotoxin activation of the immune system with impaired Leydig and Sertoli cell function via systemic inflammation and hypogonadism. Earlier reports have shown that *Lactobacillus* probiotics exert a beneficial effect on testicular function in mice via modulation of the immune system.[230]

Botanical Medicines

Panax ginseng (Korean Ginseng)

Current scientific investigation suggests that *Panax ginseng* (Chinese or Korean ginseng) may be supportive in the treatment of male infertility. Both botanicals have a long history of use as male "tonics." In studies with animals, *Panax* ginseng has been shown to improve the growth of the testes, raise sperm formation and testosterone levels, and increase sexual activity and mating behavior. It is classed as an energy tonic, indicated when there is lowered vitality and therefore impaired physical performance and sexual function. The active constituents (ginsenosides) have been shown to enhance nitric oxide production, which is useful in regulating the capacitating process of spermatozoa and the acrosome reaction, thus improving fertilization sperm motility.[231,232] Additionally, ginsenosides have been shown to affect different levels of the hypothalamic–pituitary–testicular axis,[233] which can assist in modulating stress-induced infertility or lowered testosterone from insufficient dehydroepiandrosterone (DHEA) synthesis. In clinical trials, *Panax* ginseng has been shown to increase testosterone levels as well as sperm counts and motility in patients with oligospermia, some of whom had varicocele,[233] and to improve erection and libido.[234,235]

An RCT of infertile male patients with varicocele ($n = 80$) found that supplementation with Korean red ginseng root powder (500 mg three times a day) resulted in significant improvements in sperm concentration, viability, motility, and morphology. The study participants were divided into four groups: nonvaricocelectomy with placebo or treatment; varicocelectomy with placebo or treatment. All groups except for the nonvaricocelectomy with placebo group showed similar improved sperm parameter results at the end of the trial.[236]

For additional information and references, see Chapter 99.

Pygeum africanum (Pygeum)

Pygeum africanum may be effective in improving fertility in cases where diminished prostatic secretion plays a significant role. *Pygeum* has been shown to increase prostatic secretions and improve the composition of the seminal fluid.[237–239] Specifically, *Pygeum* administration to men with decreased prostatic secretion has led to increased levels of total seminal fluid plus increases in alkaline phosphatase and protein content.

Pygeum appears to be most effective in men in whom alkaline phosphatase activity is reduced (i.e., <400 IU/cm^3) and there is no evidence of inflammation or infection (i.e., absence of white blood cells and immunoglobulin A[IgA]). The lack of IgA in the semen is a good indicator of clinical success. In one study, the patients with no IgA in their semen demonstrated an increase in alkaline phosphatase from 265 to 485 IU/cm^3.[239] In contrast, those with IgA showed only a modest increase, from 213 to 281 IU/cm^3.

Pygeum extract has also shown an ability, in a double-blind clinical trial, to improve the capacity of patients with benign prostatic hypertrophy (BPH) or prostatitis to achieve an erection, as determined by nocturnal penile tumescence.[240] BPH and prostatitis are often associated with erectile dysfunction and other sexual disturbances. Presumably by alleviating the underlying condition, *Pygeum* can improve sexual function.

Tribulus terrestris

Tribulus terrestris has been used traditionally in Ayurvedic medicine as a tonic and aphrodisiac and in European folk medicine to increase sexual potency. Protodioscin, one of the steroidal saponins, is considered the chief constituent responsible for the herb's effects on libido and sexual functioning. Of prime importance is correct sourcing of *Tribulus* to ensure its effectiveness. All of the data and clinical outcomes have been based on a leaf extract from Bulgaria, which has been shown to be highest in protodioscin. Therefore, if a *Tribulus* product is made from the root or fruit of the plant or is obtained from anywhere other than Eastern Europe, it will probably contain low levels of protodioscin and therefore not be effective.

In animal studies, *Tribulus* has been shown to increase the levels of certain sex hormones, including testosterone, and also to improve nitric oxide synthesis[241]; however, these same results have not been observed in some human studies.[242] One possible explanation is that there were differences in the extract and plant parts of the *Tribulus* used as well as the fact that many of the studies have comprised healthy males with normal testosterone levels rather than males with testosterone deficiency.

Tribulus appears to enhance male fertility through its ability to increase sperm count, viability, and libido; however, the published material is unclear, reporting older studies that were poorly designed and results that are not sufficiently definitive. A number of older papers supporting the extract product Tribestan highlight significant efficacy; however, owing to poor study design and lack of reliability, it is difficult to rely on these findings, which include increased ejaculate volume, sperm concentration, and motile sperm[243] and improved conception rates.[244] More recent studies have also produced conflicting results. In a recent prospective, randomized, double-blind, placebo-controlled trial, Tribestan (500 mg three times per day for 12 weeks) was found to successfully improve sexual function in men with mild to moderate erectile dysfunction ($n = 180$).[245] These results are in conflict with a previous study in which 400 mg of *Tribulus* treatment

(400 mg twice per day for 30 days) was found to be no better than placebo at improving erectile dysfunction, possibly due to suboptimal dosing and/or too short a study period.[246]

A recent in vitro study found that the addition of *Tribulus* to human sperm enhanced sperm motility, number of progressive motile spermatozoa, and curvilinear velocity. Sperm viability was also significantly enhanced.[247] *Tribulus* had an enhancing effect on sperm motility and viability after cryopreservation.[248] A human randomized trial of 30 male patients with idiopathic infertility found no significant improvement in levels of testosterone or semen parameters after 3 months of treatment with *Tribulus* (750 mg/day).[249] However, a trial of 65 men with abnormal semen evaluation found that administering Androsten (250 mg dried extract per capsule, including 37.5 mg protodioscin) three times per day over 12 weeks resulted in a significant enhancement of sperm count and motility but not morphology.[250] In conclusion, the exact role of *Tribulus* in male infertility is still inconclusive and controversial, and there may be a need to determine different mechanisms of action other than the current hypothesis that its desirable effects are due to androgen-enhancing properties.[251] Further large, robust studies are needed to reconcile the conflicting results to date.

Astragalus membranaceus

In experimental studies, *Astragalus membranaceus* has been observed to increase the motility of sperm in semen.[252] Studies show that *Astragalus* increases the motility of sperm in semen, but it also increases the motility of washed sperm, which is of special relevance to those seeking ART treatment.[253] Additionally, it has been shown to increase sperm motility and progression.[254]

Turnera diffusa (Damiana)

The traditional application of damiana was for "its positive aphrodisiac effects, acting energetically on the genito urinary organs of both genders where it was highly indicated for sexual weakness and debility,"[255] and "its ability to act as a stimulant tonic of the sexual apparatus especially if there is enfeeblement of the central nervous system."[256] Modern clinicians continue to prescribe damiana in this context and find it is especially beneficial when there is sexual debility, erectile difficulty, and depression. Human studies are lacking; however, a number of animal studies show promising supportive research. *Turnera diffusa* has been shown to facilitate the sexual behavior of male rats with sexual dysfunction, to reduce ejaculation latency,[257] to produce a restorative effect in sexually exhausted male rats, and to hasten their recovery.[258] It has also been observed to suppress aromatase activity, leading to the hypothesis that it may increase levels of testosterone.[259]

Mucuna pruriens (Velvet Bean)

Velvet bean has been used in Ayurvedic medicine for endurance against stress, general resistance against infection, retardation of the aging process, and eventual improvement of male sexual function; it has been known to alleviate disorders, including psychogenic impotence and unexplained infertility.[260] One paper showed that *M. pruriens* seed powder produced dramatic improvements in 70% of study participants and helped fight stress-mediated poor semen quality; it has also acted as a restorative and invigorating tonic/aphrodisiac in infertile subjects.[261] The same researchers reporting these positive effects determined that they were achieved through the regulation of steroidogenesis and resulting improvements in semen quality.[262] Specifically, this herbal treatment significantly improved testosterone, LH, dopamine, adrenaline, and noradrenaline levels in infertile men and reduced levels of FSH and prolactin. Sperm count and motility were also significantly improved in infertile men. In a follow-up study, it was found that treatment with *Mucuna pruriens* regulated steroidogenesis and improved semen quality and significantly improved testosterone, LH, dopamine, adrenaline, and noradrenaline levels in infertile men. Levels of FSH and prolactin were reduced. Sperm count and motility were also significantly recovered in infertile males after treatment.[263]

A 3-month study of *M. pruriens* seed powder administered to infertile men ($n = 180$) found a significant improvement in seminal plasma metabolic profile. Improvements were seen in alanine, citrate, glycerophosphocholine, histidine, and phenylalanine concentrations in seminal plasma.[264]

Withania somnifera (Withania)

Withania has shown considerable antistress and adaptogenic effects. To investigate the effects of *Withania* on male fertility, 75 normal healthy fertile men (control subjects) and 75 men undergoing infertility screening participated in a 3-month clinical trial. The men in the *Withania* group received 5 g of the powdered root daily. Before and after the treatment, semen analysis, antioxidant vitamins, and serum sex hormone levels were determined. Results showed that *Withania* inhibited lipid peroxidation and improved sperm count and motility. Treatment also significantly increased serum testosterone and LH and reduced the levels of FSH and prolactin (PRL), all beneficial effects in infertile men.[265]

A pilot study of 46 men with oligospermia found that supplementation with *Withania somnifera* (675 mg/d of high concentration, full-spectrum root extract) over 90 days resulted in a 167% increase in sperm count, 53% in semen volume, and 57% increase in sperm motility compared with placebo. Improvement and regulation of serum testosterone and LH levels were also observed in the treatment group.[266] The molecular mechanism of *W. somnifera* on semen quality was demonstrated to act via repairing disturbed concentrates of lactate, alanine, citrate, glycerophosphocholine, histidine, and phenylalanine in seminal plasma, thereby normalizing seminal plasma metabolites. *W. somnifera* actions include regulating reproductive hormones, fatty acid metabolism, and enzymatic activity of the TCA cycle.[267] A 3-month study into the effect of *W. somnifera* on stress-related male fertility found that treatment with *W. somnifera* powder (5 g/day dried root powder) resulted in a decrease in stress; improved antioxidant levels; and improvement in sperm concentration, motility, and liquefaction time. Treatment also resulted in increased serum testosterone and LH levels. Cortisol levels in the treatment groups were significantly decreased after treatment.[268] A 3-month treatment of normozoospermic, oligozoospermic, and asthenozoospermic men ($n = 75$) with *W. somnifera* (5 g/day root powder) showed that semen quality was improved by restoring the altered levels of intracellular ROS in spermatozoa and cell death and improving essential metal ion (Cu^{2+}, Zn^{2+}, Fe^{2+}, and Au^{2+}) concentrations.[269]

Nigella sativa (Black Cumin)

Nigella sativa is an herb displaying antioxidant properties. The main antioxidant components are thymoquinone and unsaturated fatty acids (linoleic and oleic acid). A study of infertile men with abnormal semen parameters ($n = 68$) found that treatment with *N. sativa* seed oil (2.5 mL twice daily) over 2 months improved sperm count, motility, morphology, and semen volume compared with placebo.[270] This study is supported by prior animal studies showing that *N. sativa* improves sperm parameters, semen Leydig cells, reproductive organs, and reproductive hormones.[271]

THERAPEUTIC APPROACH

Male infertility is a multifactorial condition, making a holistic treatment plan essential. Referral to a urologist or fertility specialist for a complete

evaluation is often necessary. It is advisable to encourage a detoxification program at the start of treatment so as to optimize spermatogenesis and the subsequent cohort of spermatozoa. Additionally, nutritional status should be optimized, environmental pollutants identified and eliminated, lifestyle practices modified, and fertility-enhancing botanicals consumed. It is also crucial to avoid all xenobiotics, pollutants, and toxicants owing to their disruptive endocrinological effects.

General Measures

Maintain scrotal temperature between 94°F and 96.8°F.

- Avoid exposure to free radicals.
- Identify and eliminate environmental pollutants.
- Stop or reduce all drugs, especially antihypertensives, antineoplastics such as cyclophosphamide, and anti-inflammatory drugs such as sulfasalazine.
- Use effective stress-reduction techniques and employ psychological counseling if needed.
- Avoid cigarette smoking and the use of recreational drugs.

Diet

- Avoid dietary sources of free radicals, saturated fats, hydrogenated oils, trans fatty acids, and cottonseed oil.
- Increase consumption of legumes, especially soy (high in phytoestrogens and phytosterols); good dietary sources of antioxidant vitamins, carotenes, and flavonoids (dark vegetables and fruits); and EFAs and zinc (nuts and seeds).
- Consume 8 to 12 servings of vegetables and 1 to 2 servings of fresh fruits daily.
- Optimize protein intake from both vegetarian and organic animal sources.
- Ensure that hydration requirements are met, using a 30-mL/2.2-lb (1-kg) rule.
- Eliminate caffeine, alcohol, sugar, and artificial substances (preservatives, colorings, additives).
- Ensure sufficient EFA intake several times a week in the form of a half a cup of raw nuts or seeds, cold-pressed oils (from organic sources), and sustainably farmed fish.

Nutritional Supplements

- High-potency multivitamin/mineral supplement
- Fish oils: 1000 to 2000 mg EPA+DHA
- Vitamin C: 500 to 1000 mg three times a day
- Vitamin E: 200 to 400 IU/day
- Beta-carotene: 15,000 to 30,000 IU/day (preferably as mixed carotenoids)
- Vitamin B_9 (folinic acid or L5MTHF): 1000 mcg/day
- Vitamin B12 (cyano- or methyl- cobalamin): 1000 mcg/day
- Zinc: 30 to 60 mg/day
- Selenium: 200 to 400 mcg/day
- Lycopene: 2 mg/day
- CoQ_{10}: 200 to 400 mg/day
- L-Carnitine: 2000 to 3000 mg/day
- L-Arginine: 2000 to 4000 mg/day

Botanical Medicines

Choose one or more of the following botanical medicines.

***Panax ginseng* (Korean Ginseng)**

- High-quality crude ginseng root: 1.5 to 2 g/day
- Standardized extract: Containing a minimum of 10.5 mg/mL ginsenosides with Rg1:Rb1 greater than or equal to 0.5 by high-performance liquid chromatography (HPLC), 1 to 6 mL/day

***Pygeum africanum* (Pygeum)**

- Standardized extract: 100 to 200 mg/day standardized to a 14% content of sterols in divided doses

***Tribulus terrestris* (Tribulus)**

- Standardized extract, equivalent to dried herb (aerial parts of leaf), standardized to contain furostanol saponins as protodioscin 12.22 mg/L g, 9 to 36 g/day
- Fluid extract (2:1): 7 to 21 mL/day

***Astragalus membranaceous* (Astragalus)**

- Dosages to be administered one to three times a day are as follows:
- Dried root (or as decoction or as a powder in capsules or tablets): 1 to 2 g
- Tincture (1:5): 2 to 4 mL
- Fluid extract (1:1): 1 to 2 mL solid (dry powdered) extract (standardized to contain 0.5% 4-hydroxy-3-methoxy isoflavone): 100 to 150 mg

***Turnera diffusa* (Damiana)**

- Dried leaves or as tea: 2 to 4 g/day
- Fluid extract (1:2): 3.0 to 6.0 mL/day
- Dry powdered extract (4:1): 500 to 1000 mg/day

***Mucuna pruriens* (Velvet bean)**

- Dosage equivalent to 5 g/day of the powdered dried seed

***Withania somnifera* (Withania)**

- Dosage equivalent to 5 g/day of the powdered root
- Dried powdered extract (root and leaves) 125 to 250 mg/day (standardized to contain 8% withanolide glycoside conjugates and 32% oligosaccharides)
- Fluid extract (2:1): containing a minimum of 4.0 mg/mL of withanosides, 2.5 to 5.0 mL/day

Nigella sativa

- Seed oil: 5 ml
- Fluid extract (1:2): 4 to 12 mL/day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Chandra A, Copen CE, Stephen EH. *Infertility and impaired fecundity in the United States, 1982-2010: data from the national survey of family growth*. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2013.
2. Brugh III VM, Lipshultz LI. Male factor infertility: evaluation and management. *Med Clin North Am*. 2004;88:367–385.
3. Virtanen HE, Jørgensen N, Toppari J. Semen quality in the 21st century. *Nat Rev Urol*. 2017;4.
4. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015 Apr 26;13(1):37.
5. Sengupta P, Dutta S, Krajewska-Kulak E. The disappearing sperms: analysis of reports published between 1980 and 2015. *Am J Mens Health*. 2016;19. 1557988316643383.
6. Skakkebaek NE, Jørgensen N, Main NE, et al. Is human fecundity declining? *Int J Androl*. 2006;29:2–12.
7. ASRM, Sperm Shape (Morphology): Does It Affect Fertility? ASRM Fact Sheet
8. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online*. 2015;30(1):14–27.
9. Zorgniotti AW, Cohen MS, Sealfon AI. Chronic scrotal hypothermia: results in 90 infertile couples. *J Urol*. 1986;135:944–947.
10. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproduction tract? *Lancet*. 1993;341:1392–1395.
11. Field B, Selub M, Hughes CL. Reproductive effects of environmental agents. *Semin Reprod Endocrinol*. 1990;8:44–54.
12. Joffe M. Infertility and environmental pollutants. *Br Med Bull*. 2003;68:47–70.
13. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproduction tract? *Lancet*. 1993;341:1392–1395.
14. Anway MD, Cupp AS, Uzumcu M, et al. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*. 2005;308:1466–1469.
15. Boisen K, Chellakooty M, Schmidt IM, et al. Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at 3 months of age. *J Clin Endocrinol Metab*. 2005;90:4041–4046.
16. Ji BT, Shu XO, Linet MS, et al. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. *J Natl Cancer Inst*. 1997;89:238–244.
17. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue Res*. 2005;322:33–41.
18. Buck Louis GM, Barr DB, Kannan K, Chen Z, Kim S, Sundaram R. Paternal exposures to environmental chemicals and time-to-pregnancy: overview of results from the LIFE study. *Andrology*. 2016;4(4):639–647.
19. Broe A, Pottegård A, Hallas J, Ahern TP, Fedder J, Damkier P. Association between use of phthalate-containing medication and semen quality among men in couples referred for assisted reproduction. *Hum Reprod*. 2018 Feb:1–9.
20. Zubair M, Ahmad M, Qureshi ZI. Review on arsenic-induced toxicity in male reproductive system and its amelioration. *Andrologia*. 2017;1.
21. de Angelis C, Galdiero M, Pivonello C, Salzano C, Gianfrilli D, Piscitelli P, Lenzi A, Colao A, Pivonello R. The environment and male reproduction: the effect of cadmium exposure on reproductive system and semen quality and its implication in fertility. *Reprod Toxicol* 2017;1.
22. Sun J, Yu G, Zhang Y, Liu X, Du C, Wang L, Li Z, Wang C. Heavy metal level in human semen with different fertility: a meta-analysis. *Biol Trace Elem Res*. 2017 Mar 1;176(1):27–36.
23. British Medical Association Board of Science and Education. Mobile phones and health: an interim report. *BMA Policy Report*. 2001;1–15.
24. Lai H, Singh NP. Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int J Radiat Biol*. 1996;69:513–521.
25. Fejes I, Zavaczki Z, Szollosi J, et al. Is there a relationship between cell phone use and semen quality? *Arch Androl*. 2005;51:385–393.
26. Davoudi M, Brossner C, Kuber W. The influence of electromagnetic waves on sperm motility. *Urol Urogynaecol*. 2002;19:18–22.
27. Agarwal A, Deepinder F, Sahrma RK, et al. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. *Fertil Ster*. 2008;1(89):124–128.
28. Lewis RC, Mínguez-Alarcón L, Meeker JD, Williams PL, Mezei G, Ford JB, Hauser R. EARTH Study Team. Self-reported mobile phone use and semen parameters among men from a fertility clinic. *Reprod Toxicol*. 2017;67:42–47.
29. Schauer I, Al-Ali BM. Combined effects of varicocele and cell phones on semen and hormonal parameters. *Wien Klin Wochenschr*. 2017; 13:1–6.
30. Adams JA, Galloway TS, Mondal D, Esteves SC, Mathews F. Effect of mobile telephones on sperm quality: a systematic review and meta-analysis. *Environ int*. 2014 Sep 30;70:106–112.
31. Liu K, Li Y, Zhang G, Liu J, Cao J, Ao L, Zhang S. Association between mobile phone use and semen quality: a systemic review and meta-analysis. *Androl*. 2014 Jul 1;2(4):491–501.
32. Yildirim ME, Kaynar M, Badem H, Cavis M, Karatas OF, Cimentepe E. What is harmful for male fertility: cell phone or the wireless internet? *Kaohsiung J Med Sci*. 2015 Sep 30;31(9):480–484.
33. Kamali K, Atarod M, Sarhadi S, et al. Effects of electromagnetic waves emitted from 3G+ wi-fi modems on human semen analysis. *Urologia*. 2017 Oct 1;84(4).
34. Saleh RA, Agarwal A, Sharma RK, et al. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril*. 2002 Sep;78:491–499.
35. Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online*. 2014 Jun 30;28(6):684–703.
36. Prentki Santos E, López-Costa S, Chenlo P, et al. Impact of spontaneous smoking cessation on sperm quality: case report. *Andrologia*. 2011 Dec 1;43(6):431–435.
37. Fuentes A, Muñoz A, Barnhart K, et al. Recent cigarette smoking and assisted reproductive technologies outcome. *Fertil Steril*. 2010 January;93(1):89–95.
38. Chohan KR, Badawy SZ. Cigarette smoking impairs sperm bioenergetics. *Int Braz J Urol*. 2010 Jan-Feb;36(1):60–65.
39. Gaur DS, Talekar MS, Pathak VP. Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. *Indian J Pathol Microbiol*. 2010 Jan-Mar;53(1):35–40.
40. Dai JB, Wang ZX, Qiao ZD. The hazardous effects of tobacco smoking on male fertility. *Asian J Androl*. 2015 Nov 1;17(6):954.
41. Taha EA, Ez-Aldin AM, Sayed SK, Ghandour NM, Mostafa T. Effect of smoking on sperm vitality, DNA integrity, seminal oxidative stress, zinc in fertile men. *Urol*. 2012 Oct 31;80(4):822–825.
42. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. *Eur Urol*. 2016 Oct 31;70(4):635–645.
43. La Vignera S, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? a review of literature. *Asian J Androl*. 2013 Mar 1;15(2):221.
44. Jensen TK, Swan S, Jørgensen N, et al. Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. *Hum Reprod*. 2014 Aug 1;29(8):1801–1809.
45. Hansen ML, Thulstrup AM, Bonde JP, Olsen J, Håkonsen LB, Ramlau-Hansen CH. Does last week's alcohol intake affect semen quality or reproductive hormones? a cross-sectional study among healthy young Danish men. *Reprod Toxicol*. 2012 Nov 30;34(3):457–462.

46. Barazani Y, Katz BF, Nagler HM, Stember DS. Lifestyle, environment, and male reproductive health. *Urol Clin North Am*. 2014 Feb 28;41(1):55–66.
47. Ricci E, Al Beitawi S, Cipriani S, Candiani M, Chiaffarino F, Viganò P, Noli S, Parazzini F. Semen quality and alcohol intake: a systematic review and meta-analysis. *Reprod Biomed online*. 2017 Jan 31;34(1):38–47.
48. Sermondade N, Elloumi H, Berthaut I, et al. Progressive alcohol-induced sperm alterations leading to spermatogenic arrest, which was reversed after alcohol withdrawal. *Reproduct Biomed online*. 2010 Mar 31;20(3):324–327.
49. Dias TR, Alves MG, Bernardino RL, et al. Dose-dependent effects of caffeine in human Sertoli cells metabolism and oxidative profile: relevance for male fertility. *Toxicol*. 2015 Feb 3;328:12–20.
50. Belloc S, Cohen-Bacrie M, Dalleac A, Amar E, Hazout A, de Mouzon J. Caffeine intake and sperm parameters. Analysis of a cohort of 4474 consecutive semen samples. *Fertil Steril*. 2013 Sep 1;100(3):S212.
51. Jensen TK, Swan SH, Skakkebæk NE, Rasmussen S, Jørgensen N. Caffeine intake and semen quality in a population of 2,554 young Danish men. *Am J Epidemiol*. 2010 Apr 15;171(8):883–891.
52. Ricci E, Viganò P, Cipriani S, et al. Coffee and caffeine intake and male infertility: a systematic review. *Nutri J*. 2017 December;16(1):37.
53. Karmon AE, Toth TL, Chiu YH, et al. Male caffeine and alcohol intake in relation to semen parameters and in vitro fertilization outcomes among fertility patients. *Androl*. 2017 Mar 1;5(2):354–361.
54. Fronczak CM, Kim ED, Barqawi AB. The insults of illicit drug use on male fertility. *J Androl*. 2012 Jul 8;33(4):515–528.
55. Christou MA, Christou PA, Markozannes G, Tsatsoulis A, Mastorakos G, Tigas S. Effects of anabolic androgenic steroids on the reproductive system of athletes and recreational users: a systematic review and meta-analysis. *Sports Med*. 2017 Sep 1;47(9):1869–1883.
56. Addis A, Moretti ME, Ahmed Syed F, et al. Fetal effects of cocaine: an updated meta-analysis. *Reprod Toxicol*. 2001;15:341–369.
57. Battista N, Pasquariello N, Di Tommaso M, et al. Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *J Neuroendocrinol*. 2008;20(suppl 1):82–89.
58. Badawy ZS, Chohan KR, Whyte DA, et al. Cannabinoids inhibit the respiration of human sperm. *Fertil Steril*. 2009;91(6):2471–2476. Epub 2008 Jun 18.
59. Rossato M. Endocannabinoids, sperm functions and energy metabolism. *Mol Cell Endocrinol*. 2008 Apr 16;286(1-2 suppl 1):S31–S35. Epub 2008 Feb 29.
60. du Plessis SS, Agarwal A, Syriac A. Marijuana, phytocannabinoids, the endocannabinoid system, and male fertility. *J Assist Reproduct Genetic*. 2015 Nov 1;32(11):1575–1588.
61. Alvarez S. Do some addictions interfere with fertility? *Fertil Steril*. 2015 Jan 31;103(1):22–26.
62. Lighten A. A weighty issue: managing reproductive problems in the obese. *Conceptions.* Sydney IVF; 2009;9.
63. Sermondade N, Faure C, Fezeu L, et al. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update*. 2012 Dec 12;19(3):221–231.
64. Stokes VJ, Anderson RA, George JT. How does obesity affect fertility in men–and what are the treatment options? *Clin Endocrinol*. 2015 May 1;82(5):633–638.
65. Palmer NO, Bakos HW, Fullston T, Lane M. Impact of obesity on male fertility, sperm function and molecular composition. *Spermatogenesis*. 2012 Oct 1;2(4):253–263.
66. Liu Y, Ding Z. Obesity, a serious etiologic factor for male subfertility in modern society. *Reproduct*. 2017 Oct 1;154(4):R123–R131.
67. Shukla KK, Chambial S, Dwivedi S, Misra S, Sharma P. Recent scenario of obesity and male fertility. *Androl*. 2014 Nov 1;2(6):809–818.
68. Guo D, Wu W, Tang Q, Qiao S, et al. The impact of BMI on sperm parameters and the metabolite changes of seminal plasma concomitantly. *Oncotarget*. 2017 Jul 25;8(30):48619.
69. Wang EY, Huang Y, Du QY, Yao GD, Sun YP. Body mass index effects sperm quality: a retrospective study in Northern China. *Asian J Androl*. 2017 March;19(2):234.
70. Oliveira JB, Petersen CG, Mauri AL, et al. Association between body mass index and sperm quality and sperm DNA integrity. A large population study. *Andrologia*. 2017 Aug 30.
71. Luque EM, Tissera A, Gaggino MP, et al. Body mass index and human sperm quality: neither one extreme nor the other. *Reproduc Fertil Develop*. 2017 Apr 10;29(4):731–739.
72. Håkonsen LB, Thulstrup AM, Aggerholm AS, et al. Does weight loss improve semen quality and reproductive hormones? results from a cohort of severely obese men. *Reproduct Health*. 2011 Aug 17;8(1):24.
73. Lalinde-Acevedo PC, Mayorga-Torres BJ, Agarwal A, du Plessis SS, Ahmad G, Cadavid ÁP, Maya WD. Physically active men show better semen parameters than their sedentary counterparts. *Inter J Fertil Steril*. 2017 October;11(3):156.
74. Maleki BH, Tartibian B. Moderate aerobic exercise training for improving reproductive function in infertile patients: a randomized controlled trial. *Cytokine*. 2017 Apr 30;92:55–67.
75. Maleki BH, Tartibian B, Chehrazi M. The effects of three different exercise modalities on markers of male reproduction in healthy subjects: a randomized controlled trial. *Reproduct*. 2017 Feb 1;153(2):157–174.
76. Maleki BH, Tartibian B. High-intensity exercise training for improving reproductive function in infertile patients: a randomized controlled trial. *J Obstet Gynaecol Can*. 2017 Jul 1;39(7):545–558.
77. Hajizadeh Maleki B, Tartibian B. Combined aerobic and resistance exercise training for improving reproductive function in infertile men: a randomized controlled trial. *Appl Physiol, Nutri, Metabol.* 2017 Aug 18;42(12):1293–1306.
78. Rosety MÁ, Díaz A, Rosety JM, Pery M, et al. Exercise improved semen quality and reproductive hormone levels in sedentary obese adults. *Nutri Hospital*. 2017;34(3).
79. Gaskins AJ, Mendiola J, Afeiche M, Jørgensen N, Swan SH, Chavarro JE. Physical activity and television watching in relation to semen quality in young men. *Br J Sports Med*. 2013 Feb 4. bjsports-2012.
80. Vaamonde D, Da Silva-Grigoletto ME, García-Manso JM, Barrera N, Vaamonde-Lemos R. Physically active men show better semen parameters and hormone values than sedentary men. *Eur J App Physiol*. 2012 Sep 1;112(9):3267–3273.
81. Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Louis GM. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Hum Reproduct*. 2014 Feb 1;29(2):193–200.
82. Purvis K, Christiansen E. Review: infection in the male reproductive tract: impact, diagnosis and treatment in relation to male infertility. *Int J Androl*. 1993;16:1–13.
83. Attaman JA, Toth TL, Furtado J, Campos H, Hauser R, Chavarro JE. Dietary fat and semen quality among men attending a fertility clinic. *Hum Reproduct*. 2012 Mar 13:des065.
84. Jensen TK, Heitmann BL, Jensen MB, et al. High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *Am J Clin Nutri*. 2013 Feb 1;97(2):411–418.
85. Dadkhah H, Kazemi A, Nasr-Isfahani MH, Ehsanpour S. The relationship between the amount of saturated fat intake and semen quality in men. *Iran J Nurs Midwifery Res*. 2017 Jan;22(1):46.
86. Lenzi A, Gandini L, Maresca V, et al. Fatty acid composition of spermatozoa and immature germ cells. *Mol Hum Reprod*. 2000;6(3):226–231.
87. Rato L, Alves MG, Cavaco JE, Oliveira PF. High-energy diets: a threat for male fertility? *Obesity Rev*. 2014 Dec 1;15(12):996–1007.
88. Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ, Torres-Cantero AM. Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Hum Reproduct*. 2014 Mar 1;29(3):429–440.
89. Mínguez-Alarcón L, Chavarro JE, Mendiola J, et al. Fatty acid intake in relation to reproductive hormones and testicular volume among young healthy men. *Asian J Androl*. 2017 Mar;19(2):184.
90. Weller DP, Zaneveld JD, Farnsworth NR. Gossypol. Pharmacology and current status as a male contraceptive. *Econ Med Plant Res*. 1985;1:87–112.

91. Gulaya NM, Margitich VM, Govseeva NM, et al. Phospholipid composition of human sperm and seminal plasma in relation to sperm fertility. *Arch Androl*. 2001;46(3):169–175.
92. Safarinejad MR, Hosseini SY, Dadkhah F, et al. Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. *Clin Nutr*. 2010 Feb;29(1):100–105.
93. Safarinejad MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic antioxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. *Andrologia*. 2011 Feb 1;43(1):38–47.
94. Martínez-Soto JC, Domingo JC, Cordobilla B, et al. supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation. *Sys BiolReprodut Med*. 2016 Nov 1;62(6):387–395.
95. Robbins WA, Xun L, FitzGerald LZ, Esguerra S, Henning SM, Carpenter CL. Walnuts improve semen quality in men consuming a Western-style diet: randomized control dietary intervention trial. *Biol Reprodut*. 2012 Oct 1;87(4):101.
96. Afeiche MC, Gaskins AJ, Williams PL, et al. Processed meat intake is unfavorably and fish intake favorably associated with semen quality indicators among men attending a fertility clinic. *J Nutri*. 2014 Jul 1;144(7):1091–1098.
97. Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi MR, Hekmatdoost A. Intake of food groups and idiopathic asthenozoospermia: a case–control study. *Hum Reproduct*. 2012 Aug 30;27(11):3328–3336.
98. Salas-Huetos A, Bulló M, Salas-Salvadó J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. *Hum Reproduct Update*. 2017 Mar 10:1–9.
99. Liu CY, Chou YC, Chao JC, Hsu CY, Cha TL, Tsao CW. The association between dietary patterns and semen quality in a general Asian population of 7282 males. *PloS one*. 2015 Jul 28;10(7):e0134224.
100. Karayiannis D, Kontogianni MD, Mendorou C, Douka L, Mastrominas M, Yiannakouris N. Association between adherence to the Mediterranean diet and semen quality parameters in male partners of couples attempting fertility. *Hum Reproduct*. 2016 Dec 16;32(1):215–222.
101. Oostingh EC, Steegers-Theunissen RP, de Vries JH, Laven JS, Koster MP. Strong adherence to a healthy dietary pattern is associated with better semen quality, especially in men with poor semen quality. *Fertil Steril*. 2017 Apr 30;107(4):916–923.
102. Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi MR, Hekmatdoost A. Nutrient patterns and asthenozoospermia: a case–control study. *Andrologia*. 2017 Apr 1;49(3).
103. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. *Antioxidants for male subfertility*. Cochrane Library; 2014 Jan 1.
104. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, El-Toukhy T. A systematic review of the effect of oral antioxidants on male infertility. *Reproduct Biomed Online*. 2010 Jun 30;20(6):711–723.
105. Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Hum Reproduct*. 2011 Jul 1;26(7):1628–1640.
106. Zini A, Al-Hathal N. Antioxidant therapy in male infertility: fact or fiction? *Asian J Androl*. 2011 May;13(3):374.
107. Kobori Y, Ota S, Sato R, Yagi H, Soh S, Arai G, Okada H. Antioxidant cosupplementation therapy with vitamin C, vitamin E, and coenzyme Q10 in patients with oligoasthenozoospermia. *Archiv Italiano Urol Androl*. 2014 Mar 28;86(1):1–4.
108. Gvozdjáková A, Kucharská J, Dubravicky J, Mojto V, Singh RB. Coenzyme Q10, α-tocopherol, and oxidative stress could be important metabolic biomarkers of male infertility. *Dis Markers*. 2015;2015.
109. Ciftci H, Verit A, Savas M, Yeni E, Erel O. Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. *Urol*. 2009 Jul 31;74(1):73–76.
110. Alizadeh F, Javadi M, Karami AA, Gholaminejad F, Kavianpour M, Haghighian HK. Curcumin nanomicelle improves semen parameters, oxidative stress, inflammatory biomarkers, and reproductive hormones in infertile men: a randomized clinical trial. *Phytother Res*. 2017 Nov 28.
111. Tremellen K, Miari G, Froilan D, et al. A randomized control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. *Aust New Zea J Ob Gyn*. 2007;47:216–221.
112. Atig F, Kerkeni A, Saad A, Ajina M. Effects of reduced seminal enzymatic antioxidants on sperm DNA fragmentation and semen quality of Tunisian infertile men. *J Assist Reproduct Genetic*. 2013 Jan 25:1–9.
113. Ko EY, Sabanegh ES, Agarwal A. Male infertility testing: reactive oxygen species and antioxidant capacity. *Fertil Steril*. 2014 Dec 31;102(6):1518–1527.
114. Agarwal A, Nallella KP, Allamaneni SS, et al. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online*. 2004;8:616–627.
115. Zini A, de Lamirande E, Gagnon C. Reactive oxygen species in semen of infertile patients: levels of superoxide dismutase- and catalase-like activities in seminal plasma and spermatozoa. *Int J Androl*. 1993;16:183–188.
116. Pasqualotto FF, Sharma RK, Nelson DR, et al. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril*. 2000;73:459–464.
117. Akmal M, Qadri JQ, Al-Waili NS, et al. Improvement in human semen quality after oral supplementation of vitamin C. *J Med Food*. 2006;9(3):440–442. Fall.
118. Colagar AH, Marzony ET. Ascorbic acid in human seminal plasma: determination and its relationship to sperm quality. *J Clin Biochem Nutr*. 2009 Sep;45(2):144–149. Epub 2009 Aug 28.
119. Patel SR, Sigman M. Antioxidant therapy in male infertility. *Urol Clin N Am*. 2008;35:319–330.
120. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. *Int J Androl*. 2006 Dec;29(6):569–575.
121. Kao SH, Chao HT, Chen HW, et al. Increase of oxidative stress in human sperm with lower motility. *Fertil Steril*. 2008 May;89(5):1183–1190. Epub 2007 Jul 31.
122. Fraga CG, Motchnik PA, Shigenaga MK, et al. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc Natl Acad Sci U S A*. 1991;88:11003–11006.
123. Cyrus A, Kabir A, Goodarzi D, Moghimi M. The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: a double blind placebo controlled clinical trial. *Int Braz J Urol*. 2015 Apr;41(2):230–238.
124. Rafiee B, Morowvat MH, Rahimi-Ghalati N. Comparing the effectiveness of dietary vitamin c and exercise interventions on fertility parameters in normal obese men. *Urol J*. 2016 Apr 16;13(2):2635–2639.
125. Dawson E, Harris W, Powell L. Effect of vitamin C supplementation on sperm quality of heavy smokers. *FASEB J*. 1991;5:A915.
126. Dawson EB, Harris WA, Rankin WE, et al. Effect of ascorbic acid on male fertility. *Ann NY Acad Sci*. 1987;498:312–323.
127. Aitken RJ, Clarkson JS, Hargreave TB, et al. Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia. *J Androl*. 1989;10:214–220.
128. Greco E, et al. *J Androl*. 2005;26(3):349–353.
129. Benedetti S, Tagliamonte MC, Catalani S, et al. Differences in blood and semen oxidative status in fertile and infertile men, and their relationship with sperm quality. *Reproduct Biomed Online*. 2012 Sep 30;25(3):300–306.
130. Suleiman SA, Ali ME, Zaki ZM, et al. Lipid peroxidation and human sperm mobility: protective role of vitamin E. *J Androl*. 1996;17:530–537.
131. Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility: a preliminary report. *Int Urol Nephrol*. 2002;34:369–372.
132. Moslemi MK, Tavanbakhsh S. Selenium–vitamin E supplementation in infertile men: effects on semen parameters and pregnancy rate. *Int J General Med*. 2011;4:99.
133. ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad A, Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenozoospermia: a prospective, randomized trial. *Androl*. 2015 Sep 1;3(5):864–867.

134. Geva E, Bartoov B, Zabludovsky N, et al. The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. *Fertil Steril*. 1996 Sep;66(3):430–434.
135. Greco E, Iacobelli M, Rienzi L, et al. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl*. 2005 May-Jun;26(3):349–353.
136. Al-Azemi MK, Omu AE, Fatinikun T, et al. Factors contributing to gender differences in serum retinol and alpha-tocopherol in infertile couples. *Reprod Biomed Online*. 2009 Oct;19(4):583–590.
137. Morales A, Cavicchia JC. Spermatogenesis and blood-testis barrier in rats after long-term vitamin A deprivation. *Tissue Cell*. 2002 Oct;34(5):349–355.
138. Ko EY, Sabanegh ES. The role of nutraceuticals in male fertility. *Urol Clin North Am*. 2014 Feb 28;41(1):181–193.
139. Eskenazi B, et al. Antioxidant intake is associated with semen quality in healthy men. *Hum Reproduct*. 2005;20(4):1006–1012.
140. Zareba P, Colaci DS, Afeiche M, et al. Semen quality in relation to antioxidant intake in a healthy male population. *Fertil Steril*. 2013 Dec 31;100(6):1572–1579.
141. Durairajanayagam D, Agarwal A, Ong C, Prashast P. Lycopene and male infertility. *Asian J Androl*. 2014 May;16(3):420.
142. Goyal A, Chopra M, Lwaleed BA, Birch B, Cooper AJ. The effects of dietary lycopene supplementation on human seminal plasma. *BJU Int*. 2007 Jun 1;99(6):1456–1460.
143. Yamamoto Y, Aizawa K, Mieno M, et al. The effects of tomato juice on male infertility. *Asia Pacific J Clini Nutri*. 2017 Mar 1;26(1):65–71.
144. Corbett ST, Hill O, Nangia AK. Vitamin D receptor found in human sperm. *Urol*. 2006;68(6):1345–1349.C.
145. Jensen MB. Vitamin D and male reproduction. *Nat Rev Endocrinol*. 2014 Mar 1;10(3):175–186.
146. Jensen MB, Bjerrum PJ, Jessen TE, et al. Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum Reproduct*. 2011 Jun 1;26(6):1307–1317.
147. Tirabassi G, Cutini M, Muscogiuri G, et al. Association between vitamin D and sperm parameters: clinical evidence. *Endocrine*. 2016 Dec 10:1–5.
148. Hammoud AO, Meikle AW, Peterson CM, Stanford J, Gibson M, Carrell DT. Association of 25-hydroxy-vitamin D levels with semen and hormonal parameters. *Asian J Androl*. 2012 Nov 1;14(6):855.
149. Ramlau-Hansen CH, Moeller UK, Bonde JP, Olsen J, Thulstrup AM. Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men. *Fertil Steril*. 2011 Mar 1;95(3):1000–1004.
150. Pilz S, Frisch S, Koertke H, et al. Effect of vitamin D supplementation on testosterone levels in men. *Horm Metab Res*. 2011 Mar;43(03):223–225.
151. Heijboer AC, Oosterwerff M, Schroten NF, et al. Vitamin D supplementation and testosterone concentrations in male human subjects. *Clin Endocrinol*. 2015 Jul 1;83(1):105–110.
152. Nimptsch K, Platz EA, Willett WC, Giovannucci E. Association between plasma 25-OH vitamin D and testosterone levels in men. *Clin Endocrinol*. 2012 Jul 1;77(1):106–112.
153. Boisen IM, Hansen LB, Mortensen LJ, Lanske B, Juul A, Jensen MB. Possible influence of vitamin D on male reproduction. *J Steroid Biochem Mol Biol*. 2016 Sep 28.
154. Blomberg Jensen M, Gerner Lawaetz J, Andersson AM, et al. Vitamin D deficiency and low ionized calcium are linked with semen quality and sex steroid levels in infertile men. *Hum Reproduct*. 2016 Aug 1;31(8):1875–1885.
155. Alzoubi A, Mahdi H, Al Bashir S, et al. Normalization of serum vitamin D improves semen motility parameters in patients with idiopathic male infertility. *Acta Endocrinol (1841-0987)*. 2017 Apr 1;13(2).
156. Waud K, Bocca S. Role of vitamin D deficiency in male fertility. *Fertil Steril*. 2015 Feb 1;103(2):e39.
157. Ursini F, Heim S, Kiess M, et al. Dual function of the selenoprotein PHGPx during sperm maturation. *Science*. 1999;285:1393. selenium on sperm motility in healthy men. *J Androl*. 2001;22: 764.
158. Vézina D, Mauffette F, Roberts KD, et al. Selenium-vitamin E supplementation in infertile men: effects on semen parameters and micronutrient levels and distribution. *Biol Trace Elem Res*. 1996 Summer;53(1-3):65–83.
159. Rayman MP, Rayman MP. The argument for increasing selenium intake. *Proc Nutr Soc*. 2002;61:203–215. 71.
160. Camejo MI, Abdala L, Vivas-Acevedo G, Lozano-Hernández R, Angeli-Greaves M, Greaves ED. Selenium, copper and zinc in seminal plasma of men with varicocele, relationship with seminal parameters. *Biol Trace Elem Res*. 2011 Dec 1;143(3):1247–1254.
161. Schneider M, Förster H, Boersma A, et al. Mitochondrial glutathione peroxidase 4 disruption causes male infertility. *FASEB J*. 2009 Sep;23(9):3233–3242. Epub 2009 May 5.
162. Ahsan U, Kamran Z, Raza I, Ahmad S, Babar W, Riaz MH, Iqbal Z. Role of selenium in male reproduction - a review. *Animal Reproduct Sci*. 2014 Apr 30;146(1):55–62.
163. Scott R, MacPherson A, Yates RW, et al. The effect of oral selenium supplementation on human sperm motility. *Br J Urol*. 1998 Jul;82(1):76–80.
164. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *J Urol*. 2009 Feb;181(2):741–751.
165. Wong WY, Thomas CM, Merkus JM, et al. Male factor subfertility: possible causes and the impact of nutritional factors. *Fertil Steril*. 2000;73:435–442.
166. Colagar AH, Marzony ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nutr Res*. 2009 Feb;29(2):82–88.
167. Prasad AS. Zinc in growth and development and spectrum of human zinc deficiency. *J Am Coll Nutr*. 1988;7:377–384.
168. El-Tawil AM. Zinc deficiency in men with Crohn's disease may contribute to poor sperm function and male infertility. *Andrologia*. 2003;35:337–341.
169. Chia SE, Ong C, Chua L, et al. Comparison of zinc concentration in blood and seminal plasma and various sperm parameters between fertile and infertile men. *J Androl*. 2000;21:53–57.
170. Bjorndahl L, Kvist U. Importance of zinc for human sperm head-tail connection. *Acta Physiol Scand*. 1982;126:51–55. as cited Seibel M. The role of nutrition and nutritional supplements in women's health *Fertil Steril*. October 1999;72(4):579–591.
171. Caldamone AA, Freytag MK, Cockett AT. Seminal zinc and male infertility. *Urol*. 1979;13. 280–228.
172. Ajina T, Sallem A, Haouas Z, Mehdi M. Total antioxidant status and lipid peroxidation with and without in vitro zinc supplementation in infertile men. *Andrologia*. 2017 Sep 1;49(7).
173. Carreras A, Mendosa C. Zinc levels in seminal plasma of infertile and fertile men. *Andrologia*. 1990;22:279–283.
174. Takihara H, Cosentino MJ, Cockett AT. Zinc sulfate therapy for infertile males with or without varicocelectomy. *Urol*. 1987;29:638–641.
175. Netter A, Hartoma R, Nakoul K. Effect of zinc administration on plasma testosterone, dihydrotestosterone and sperm count. *Arch Androl*. 1981;7:69–73.
176. Wong WY, Merkus HM, Thomas CM, et al. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertil Steril*. 2002;77:491–498.
177. Sandler B, Faragher B. Treatment of oligospermia with vitamin B12. *Infertil*. 1984;7:133–138.
178. Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. *Med Princ Pract*. 2008 Feb 19;17(2):108–116.
179. Ghareeb DA, Sarhan EM. Role of oxidative stress in male fertility and idiopathic infertility: causes and treatment. *J Diagn Tech Biomed Anal*. 2014;2(1):12. 2.
180. Alsalman AR, Almashhedy LA, Hadwan MH. Zinc supplementation attenuates lipid peroxidation and increases antiperoxidant activity in seminal plasma of iraqi asthenospermic men. *Life Sci J*. 2013;10(4):989–997.
181. Hadwan MH, Almashhedy LA, Alsalman AR. Oral zinc supplementation restores superoxide radical scavengers to normal levels in spermatozoa of Iraqi asthenospermic patients. *Int J Vitam Nutr Res*. 2015 Jan 1;85(3-4):165–173.
182. Zhao J, Dong X, Hu X, et al. Zinc levels in seminal plasma and their correlation with male infertility: a systematic review and meta-analysis. *ScientiReports*. 2016 Mar 2;6:22386.

183. Boxmeer JC, Smit M, Weber RF, et al. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. *J Androl*. 2007 Jul-Aug;28(4):521–527. Epub 2007 Feb 7.
184. Boxmeer JC, Smit M, Utomo E, et al. Low folate in seminal plasma is associated with increased sperm DNA damage. *Fertil Steril*. 2009 Aug;92(2):548–556.
185. Sinclair S. Male infertility: nutritional and environmental considerations. *Altern Med Rev*. 2000 Feb;5(1):28–38.
186. Kumamoto Y, Maruta H, Ishigami J, et al. Clinical efficacy of mecobalamin in treatment of oligozoospermia: results of a double-blind comparative clinical study. *Acta Urol Japan*. 1988;34:1109–1132.
187. Gual-Frau J, Abad C, Amengual MJ, et al. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. *Hum Fertil*. 2015 Jul 3;18(3):225–229.
188. Abad C, Amengual MJ, Gosálvez J, et al. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. *Andrologia*. 2013 Jun 1;45(3):211–216.
189. Murphy LE, Mills JL, Molloy AM, et al. Folate and vitamin B12 in idiopathic male infertility. *Asian J Androl*. 2011 Nov 1;13(6):856.
190. Yuan HF, Zhao K, Zang Y, et al. Effect of folate deficiency on promoter methylation and gene expression of Esr1, Cav1, and Elavl1, and its influence on spermatogenesis. *Oncotarget*. 2017 Apr 11;8(15):24130.
191. Raigani M, Yaghmaei B, Amirjannti N, et al. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. *Andrologia*. 2014 Nov 1;46(9):956–962.
192. Azizollahi G, Azizollahi S, Babaei H, Kianinejad M, Baneshi MR, Nematollahi-mahani SN. Effects of supplement therapy on sperm parameters, protamine content and acrosomal integrity of varicocelectomized subjects. *J Assisted Reproduct Genetic*. 2013 Apr 1;30(4):593–599.
193. Najafipour R, Moghbelinejad S, Aleyasin A, Jalilvand A. Effect of B9 and B12 vitamin intake on semen parameters and fertility of men with MTHFR polymorphisms. *Andrology*. 2017 Apr 25.
194. Aarabi M, San Gabriel MC, Chan D, et al. High-dose folic acid supplementation alters the human sperm methylome and is influenced by the MTHFR C677T polymorphism. *Hum Mol Genetic*. 2015 Aug 24;24(22):6301–6313.
195. Ng CM, Blackman MR, Wang C, et al. The role of carnitine in the male reproductive system. *Ann N Y Acad Sci*. 2004 Nov;1033:177–178.
196. Costa M, Canale D, Filicori M, et al. L-Carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility. *Andrologia*. 1994;26:155–159.
197. Vitali G, Parente R, Melotti C. Carnitine supplementation in human idiopathic asthenospermia: clinical results. *Drugs Exp Clin Res*. 1995;21:157–159.
198. Vicari E, La Vignera S, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertil Steril*. 2002;78:1203–1208.
199. Lenzi A, Sgrò P, Salacone P, et al. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. *Fertil Steril*. 2004;81:1578–1584.
200. Lenzi A, Lombardo F, Sgro P, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. *Fertil Steril*. 2003;79:292–300.
201. Balercia G, Regoli F, Armeni T, et al. Placebo-controlled, double-blind, randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic astheno zoospermia. *Fertil Steril*. 2005;84:662–671.
202. Busetto GM, Koverech A, Messano M, Antonini G, De Berardinis E, Gentile V. Prospective open-label study on the efficacy and tolerability of a combination of nutritional supplements in primary infertile patients with idiopathic astenoteratozoospermia. *Arch Ital Urol Androl*. 2012 Sep;84(3):137–140.
203. Aram JJ, Al-Shamma KJ, Al-Hassani AN. Effect of L-carnitine, multivitamins and their combination in the treatment of idiopathic male infertility. *Iraqi J Pharmaceutical Sci (ISSN: 1683–3597, ESSN: 2521–3512)*. 2017 Mar 28;21(1):14–20.
204. Lipovac M, Bodner F, Imhof M, Chedraui P. Comparison of the effect of a combination of eight micronutrients versus a standard mono preparation on sperm parameters. *Reproduct Biol Endocrinol*. 2016 Dec;14(1):84.
205. Banihani S, Agarwal A, Sharma R, Bayachou M. Cryoprotective effect of l-carnitine on motility, vitality and DNA oxidation of human spermatozoa. *Andrologia*. 2014 Aug 1;46(6):637–641.
206. Bilska A, Włodek L. Lipoic acid: the drug of the future? *Pharmacol Rep*. 2005 Sep-Oct;57(5):570–577.
207. Selvakumar E, Prahalathan C, Sudharsan PT, et al. Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm. *Toxicol*. 2006 Jan 5;217(1):71–78. Epub 2005 Oct 3.
208. Prahalathan C, Selvakumar E, Varalakshmi P. Modulatory role of lipoic acid on adriamycin-induced testicular injury. *Chem Biol Interact*. 2006 Mar 25;160(2):108–114. Epub 2006 Jan 24.
209. Ibrahim SF, Osman K, Das S, et al. A study of the antioxidant effect of alpha lipoic acids on sperm quality. *Clinics (Sao Paulo)*. 2008 Aug;63(4):545–550.
210. Aly HA, Lightfoot DA, El-Shemy HA. Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells in vitro. *Chem Biol Interact*. 2009 Dec 10;182(2-3):112–118. Epub 2009 Aug 21.
211. Haghighian HK, Haidari F, Mohammadi-asl J, Dadfar M. Randomized, triple-blind, placebo-controlled clinical trial examining the effects of alpha-lipoic acid supplement on the spermatogram and seminal oxidative stress in infertile men. *Fertil Steril*. 2015 Aug 1;104(2):318–324.
212. Mancini A, De Marinis L, Oradei A, et al. Coenzyme Q_{10} concentration in normal and pathological human seminal fluid. *J Androl*. 1994;15. 591–559.
213. Balercia G, Mosca F, Mantero F, et al. Coenzyme Q10 supplementation in infertile men with idiopathic asthenozoospermia: an open, uncontrolled pilot study. *Fertil Steril*. 2004;81:93–98.
214. Balercia G, Buldreghini E, Vignini A, et al. Coenzyme Q10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. *Fertil Steril*. 2009 May;91(5):1785–1792. Epub 2008 Apr 8.
215. Balercia G, Arnaldi G, Fazioli F, et al. Coenzyme Q_{10} levels in idiopathic and varicocele-associated asthenozoospermia. *Andrologia*. 2002;34:107–111.
216. Safarinejad MR. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. *J Urol*. 2009 Jul 31;182(1):237–248.
217. Nadjarzadeh A, Shidfar F, Amirjannati N, et al. Effect of Coenzyme Q10 supplementation on antioxidant enzymes activity and oxidative stress of seminal plasma: a double-blind randomised clinical trial. *Andrologia*. 2014 Mar 1;46(2):177–183.
218. Safarinejad MR. The effect of coenzyme Q10 supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study. *Int Urol Nephrol*. 2012 Jun 1;44(3):689–700.
219. Festa R, Giacchi E, Raimondo SE, et al. Coenzyme Q10 supplementation in infertile men with low-grade varicocele: an open, uncontrolled pilot study. *Andrologia*. 2014 Sep 1;46(7):805–807.
220. Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Effects of the reduced form of coenzyme Q 10 (ubiquinol) on semen parameters in men with idiopathic infertility: a double-blind, placebo controlled, randomized study. *J Urol*. 2012 Aug 31;188(2):526–531.
221. Thakur AS, Littarru GP, Funahashi I, Painkara US, Dange NS, Chauhan P. Effect of ubiquinol therapy on sperm parameters and serum testosterone levels in oligoasthenozoospermic infertile men. *J Clini Diagnostic Res: JCDR*. 2015 Sep;9(9):BC01.
222. Tiseo BC, Gaskins AJ, Hauser R, Chavarro JE, Tanrikut C, EARTH Study Team. Coenzyme Q10 Intake from food and semen parameters in a subfertile population. *Urol*. 2017 Apr 30;102:100–105.

223. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids*. 2009 May;37(1):1–17.
224. Scibona M, Meschini P, Capparelli S, et al. L-arginine and male infertility. *Minerva Urol Nefrol*. 1994 Dec;46(4):251–253.
225. Schacter A, Goldman JA, Zukerman Z. Treatment of oligospermia with the amino acid arginine. *J Urol*. 1973;110:311–313.
226. Stanislavov R, Nikolova V, Rohdewald P. Improvement of seminal parameters with Prelox: a randomized, double-blind, placebo-controlled, cross-over trial. *Phytother Res*. 2009 Mar;23(3):297–302.
227. Roseff SJ. Improvement in sperm quality and function with French maritime pine tree bark extract. *J Reprod Med*. 2002;47:821–824.
228. Valcarce DG, Genovés S, Riesco MF, Martorell P, Herráez MP, Ramón D, Robles V. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Beneficial Microbes*. 2017 Apr 26;8(2):193–206.
229. Maretti C, Cavallini G. The association of a probiotic with a prebiotic (Flortec, Bracco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: a pilot study. *Androl*. 2017 May 1;5(3):439–444.
230. Tremellen K, Pearce K. Probiotics to improve testicular function (Andrology 5: 439–444, 2017)–a comment on mechanism of action and therapeutic potential of probiotics beyond reproduction. *Androl*. 2017 Sep 1;5(5):1052–1053.
231. Zhang H, Zhou QM, Li XD, et al. Ginsenoside R(e) increases fertile and asthenozoospermic infertile human sperm motility by induction of nitric oxide synthase. *Arch Pharm Res*. 2006 Feb;29(2):145–151.
232. Zhang H, Zhou Q, Li X, et al. Ginsenoside Re promotes human sperm capacitation through nitric oxide-dependent pathway. *Mol Reprod Dev*. 2007 Apr;74(4):497–501.
233. Salvati G, Genovesi G, Marcellini L, et al. Effects of *Panax ginseng* C.A. Meyer saponins on male fertility. *Panminerva Med*. 1996 Dec;38(4):249–254.
234. Choi HK, Seong DH, Rha KH. Clinical efficacy of Korean red ginseng for erectile dysfunction. *Int J Impot Res*. 1995;7(3):181–187.
235. Kim TH, Jeon SH, Hahn EJ, Paek KY, Park JK, Youn NY, Lee HL. Effects of tissue-cultured mountain ginseng (*Panax ginseng* CA Meyer) extract on male patients with erectile dysfunction. *Asian J Androl*. 2009 Apr;11(3):356.
236. Park HJ, Choe S, Park NC. Effects of Korean red ginseng on semen parameters in male infertility patients: a randomized, placebo-controlled, double-blind clinical study. *Chinese J Integrative Med*. 2016 Jul 1;22(7):490–495.
237. Lucchetta G, Weill A, Becker N, et al. Reactivation of the secretion from the prostatic gland in cases of reduced fertility: biological study of seminal fluid modifications. *Urol Int*. 1984;39:222–224.
238. Menchini-Fabris GF, Giorgi P, Reini F, et al. New perspectives of treatment of prostato-vesicular pathologies with *Pygeum africanum*. *Arch Int Urol*. 1988;60:313–322.
239. Clavert A, Cranz C, Riffaud JP, et al. Effects of an extract of the bark of *Pygeum africanum* on prostatic secretions in the rat and man. *Ann Urol*. 1986;20:341–343.
240. Carani C, Salvioli C, Scuteri A, et al. Urological and sexual evaluation of treatment of benign prostatic disease using *Pygeum africanum* at high dose. *Arch Ital Urol Nefrol Androl*. 1991;63:341–345.
241. Gauthaman K, Ganesan AP. The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction: an evaluation using primates, rabbit and rat. *Phytomed*. 2008 Jan;15(1-2):44–54.
242. Neychev VK, Mitev VI. The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men. *J Ethnopharmacol*. 2005 Oct 3;101(1-3):319–323.
243. Protich M, Tsvetkov D, Nalbanski B, et al. Clinical trial of a tribestan preparation in infertile men. *Akush Ginekol. (Sofiia)*. 1983;22(4):326–329. as cited in National Standard monograph.
244. Adimoelja A. Phytochemicals and the breakthrough of traditional herbs in the management of sexual dysfunctions. *Int Androl*. 2002;23(S2):82–84. Published online.
245. Kamenov Z, Fileva S, Kalinov K, Jannini EA. Evaluation of the efficacy and safety of *Tribulus terrestris* in male sexual dysfunction—A prospective, randomized, double-blind, placebo-controlled. *Clin Trial Maturitas*. 2017 May 31;99:20–26.
246. Santos Jr CA, Reis LO, Destro-Saade R, Luiza-Reis A, Fregonesi A. *Tribulus terrestris* versus placebo in the treatment of erectile dysfunction: a prospective, randomized, double-blind study. *Actas Urol Español (English Ed)*. 2014 May 1;38(4):244–248.
247. Khaleghi S, Bakhtiari M, Asadmobini A, Esmaeili F. *Tribulus terrestris* extract improves human sperm parameters in vitro. *J Evidence-Based Comp Alter Med*. 2017 Jul;22(3):407–412.
248. Asadmobini A, Bakhtiari M, Khaleghi S, Esmaeili F, Mostafaei A. The effect of *Tribulus terrestris* extract on motility and viability of human sperms after cryopreservation. *Cryobiol*. 2017 Apr 30;75:154–159.
249. Roaiah MF, Elkhayat YI, Saleh SF, Abd El Salam MA. Prospective analysis on the effect of botanical medicine *(Tribulus terrestris)* on Serum testosterone level and semen parameters in males with unexplained infertility. *J Dietary Suppl*. 2017 Jan 2;14(1):25–31.
250. Salgado RM, Marques-Silva MH, Gonçalves E, Mathias AC, Aguiar JG, Wolff P. Effect of oral administration of *Tribulus terrestris* extract on semen quality and body fat index of infertile men. *Andrologia*. 2017 Jun 1;49(5).
251. Neychev V, Mitev V. Pro-sexual and androgen enhancing effects of *Tribulus terrestris* L.: fact or fiction. *J Ethnopharmacol*. 2016 Feb 17;179:345–355.
252. Hong CY, Ku J, Wu P. *Astragalus membranaceus* stimulates human sperm motility in vitro. *Am J Chin Med*. 1992;20(3-4):289–294.
253. Liu J, Liang Yin C. Effects of several Chinese herbal aqueous extracts on human sperm motility in vitro. *Andrologia*. 2004;36(2):78–83.
254. Dinchev D, Janda B, Evstatieva L, et al. Distribution of steroidal saponins in *Tribulus terrestris* from different geographical regions. *Phytochem*. 2008;69(1):176–186.
255. Felter HW, Lloyd JU. *Kings Am Dispensatory Eclectic Materia Medica Publi*; 1905.
256. Ellingwood F. *The american materia medica, therapeutics and pharmacognosy. Eclectic* Materia Medica Publications.
257. Arletti R, Benelli A, Cavazzuti E, et al. Stimulating property of *Turnera diffusa* and *Pffafia paniculata* extracts on the sexual behavior of male rats. *Psychopharmacol*. 1999;144:15–19.
258. Estrada-Reyes R, Ortiz-López P, Gutiérrez-Ortíz J, et al. *Turnera diffusa* Wild (Turneraceae) recovers sexual behavior in sexually exhausted males. *J Ethnopharmacol*. 2009 Jun 25;123(3):423–429. Epub 2009 Mar 31.
259. Zhao J, Dasmahapatra A, Khan S, et al. Anti-aromatase activity of the constituents from damiana *(Turnera diffusa)*. *J Ethnopharmacol*. 2008;120(3):387–393.
260. Tripathi YB, Upadhyay AK. Antioxidant property of *Mucuna pruriens*. *Curr Sci*. 2001;80:1377–1378.
261. Ahmad MK, Mahdi AA, Shukla KK, et al. Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men. *Fertil Steril*. 2008;90:627–635.
262. Ahmad MK, Mahdi AA, Shukla KK, et al. *Mucuna pruriens* improves male fertility by its action on the hypothalamus–pituitary–gonadal axis. *Fertil Steril*. 2009;92:1934–1940.
263. Shukla KK, Mahdi AA, Ahmad MK, et al. *Mucuna pruriens* improves male fertility by its action on the hypothalamus–pituitary–gonadal axis. *Fertil Steril*. 2009;92:1934–1940.
264. Gupta A, Mahdi AA, Ahmad MK, Shukla KK, Bansal N, Jaiswer SP, Shankhwar SN. A proton NMR study of the effect of *Mucuna pruriens* on seminal plasma metabolites of infertile males. *J Pharm Biomed Anal*. 2011 Jul 15;55(5):1060–1066.
265. Ahmad MK, Mahdi AA, Shukla KK, et al. *Withania somnifera* improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. *Fertil Steril*. 2010 Aug;94(3):989–996.
266. Ambiye VR, Langade D, Dongre S, Aptikar P, Kulkarni M, Dongre A. Clinical evaluation of the spermatogenic activity of the root extract of Ashwagandha *(Withania somnifera)* in oligospermic males: a pilot study. *Evid Based Complement Alternat Med*. 2013 Nov 28:2013.
267. Gupta A, Mahdi AA, Shukla KK, Ahmad MK, Bansal N, Sankhwar P, Sankhwar SN. Efficacy of *Withania somnifera* on seminal plasma metabolites of infertile males: a proton NMR study at 800MHz. *J Ethnopharmacol*. 2013 Aug 26;149(1):208–214.

268. Mahdi AA, Shukla KK, Ahmad MK, et al. *Withania somnifera* improves semen quality in stress-related male fertility. *Evid-Based Complement Alternat Med*. 2011 Jun 18:2011.
269. Shukla KK, Mahdi AA, Mishra V, Rajender S, Sankhwar SN, Patel D, Das M. *Withania somnifera* improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations. *Reproduct Biomed online*. 2011 May 31;22(5):421–427.
270. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of *Nigella sativa* L. seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. *Phytomed*. 2014 May 15;21(6):901–905.
271. Mahdavi R, Heshmati J, Namazi N. Effects of black seeds *(Nigella sativa)* on male infertility: a systematic review. *J Herbal Med*. 2015 Sep 30;5(3):133–139.
272. Hechtman L. *Clinical Naturopathic Medicine,* 5th ed. Australia: Elsevier; 2018.

186

Inflammatory Bowel Disease (Crohn's Disease and Ulcerative Colitis)

Gerard E. Mullin, MD

OUTLINE

DIAGNOSTIC SUMMARY

Crohn's disease:
- Abdominal pain the most common symptom—can be localized anywhere in the abdomen
- Complicated by intestinal obstruction, abscesses, fistulas, strictures, perianal disease
- Intermittent bouts of diarrhea or constipation, low-grade fever, and weight loss
- Frequently misdiagnosed as irritable bowel syndrome; time to diagnosis from onset is estimated to be approximately 8 years
- Ileocolonoscopy and capsule endoscopy are key diagnostic modalities
- Radiographic evidence of abnormality of the terminal ileum is characteristic
- Stools can be high in calprotectin—noninvasive inflammatory markers
- Genetic predisposition, environmental trigger (gut dysbiosis, medications, etc.), chronic full-thickness patchy intestinal inflammation
- Risk of digestive tract cancer higher than in the general population

Ulcerative colitis:
- Bloody diarrhea with cramps in the lower abdomen
- Mild abdominal tenderness, weight loss, and fever
- Involves only the colon; distinguishing point from Crohn's disease, which can involve any portion of the alimentary tract
- Inflammation is superficial and continuous, whereas Crohn's is full-thickness and tends to be patchy
- Diagnosis is confirmed by radiography and sigmoidoscopy
- Risk of colon cancer increased after 10 years of disease, universal distribution throughout colon and poorly controlled disease activity
- Genetic predisposition, triggers similar to those of Crohn's disease

GENERAL CONSIDERATIONS

Definition

Inflammatory bowel disease (IBD) is a general term for a group of chronic inflammatory disorders of the bowel. IBD is divided into two major categories: Crohn's disease and ulcerative colitis. Clinically, IBD is characterized by recurrent inflammatory involvement of specific intestinal segments, resulting in diverse clinical manifestations.

Crohn's Disease

Crohn's disease is characterized by a granulomatous inflammatory reaction comprising the entire thickness of the bowel wall. In approximately 50% of cases, however, the granulomas are either poorly developed or totally absent. The original description in 1932 by Crohn and colleagues localized the disease to segments of the ileum. However, the same granulomatous process may involve the buccal mucosa, esophagus, stomach, duodenum, jejunum, and colon. Rectal biopsies are routinely recommended during flexible sigmoidoscopy or colonoscopy because granulomas are most commonly found at this site. Crohn's disease of the small intestine is also known as regional enteritis. Involvement of the colon is known as Crohn's disease of the colon or granulomatous colitis; the latter designation is less accurate because granulomatous lesions develop in only some of these patients.

Ulcerative Colitis

In ulcerative colitis a nonspecific inflammatory response is limited largely to the colonic mucosa and submucosa. Well-developed granulomas are exceedingly rare. Crohn's disease and ulcerative colitis do have many features in common, however, and the diseases are discussed together when appropriate. Otherwise they are considered as separate entities. Bear in mind that 1% to 15% of IBD is characterized

as "indeterminate" when the disease has features of both Crohn's disease and ulcerative colitis. Over time, the disease may morph into one predominant disease pattern or remain indeterminate.

Common Features of Crohn's Disease and Ulcerative Colitis

- The colon is frequently involved in Crohn's disease and is invariably involved in ulcerative colitis.
- Although rarely, patients with ulcerative colitis who have total colon involvement may experience a so-called backwash ileitis. Thus both conditions may cause chronic inflammatory changes in the distal small intestine.
- Patients with Crohn's disease often have close relatives with ulcerative colitis, and vice versa.
- When there is no granulomatous reaction in Crohn's disease of the colon, the two lesions may resemble each other clinically as well as pathologically.
- There are many epidemiological similarities between the two diseases, including age, race, gender, and geographic distribution.
- The two conditions are associated with similar extraintestinal manifestations.
- There appear to be etiological parallels between the two conditions.
- Both conditions are associated with a higher frequency of colonic carcinoma and extraintestinal cancers.

Etiology

The epidemiological and etiological data on ulcerative colitis (UC) and Crohn's disease (CD) are quite similar.[1] The incidence and prevalence of the two diseases differ slightly, with most studies showing UC to be more common. Overall, the incidence of CD is rapidly rising in Western cultures.[2] The annual incidence of CD in North America is reported to be 3.1 to 20.2 per 100,000 with a prevalence of 201 per 100,000 population.[2] The current estimate of the incidence of UC in Western Europe and the United States is approximately 6 to 8 cases per 100,000; the estimated prevalence is approximately 286 cases per 100,000.[3] The incidence of CD is rising in Western cultures.[2] Overall, between 1 and 1.6 million Americans have IBD, with an overall prevalence of 504 per 100,000 in the population.[4] The incidence of IBD has been rising in newly industrialized countries in Africa, Asia, and South America, including Brazil and Taiwan.

IBD may occur at any age, but it most often occurs between the ages of 15 and 35 years. Females are affected slightly more frequently than males. White people have the disease two to five times more often than African or Asian Americans, and Jews have a threefold to sixfold higher incidence than non-Jews.[3]

Theories about the etiology of IBD can be divided into several groups, as follows (Fig. 186.1):

- Genetic predisposition
- Gut dysbiosis
- Immunological abnormality
- Dietary factors
- An assortment of miscellaneous concepts implicating psychosomatic, vascular, traumatic, and other mechanisms

Genetic Predisposition

Although the search for a specific genetic marker for IBD has been futile, several factors suggest a genetic predisposition. As already mentioned, IBD is two to five times more common among white than nonwhite people and three to six times more common among Jews than non-Jews. In addition, multiple members of the same family have CD or UC in 15% to 40% of cases.[5]

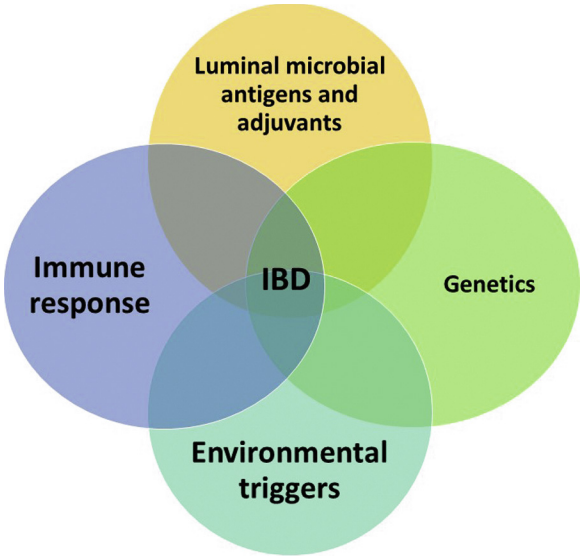


Fig. 186.1 Pathogenesis of Inflammatory Bowel Disease (IBD). The pathogenesis of IBD involves the interaction of four principal factors: genetics, gut microbiome, immune response, and environmental triggers.

Gut Dysbiosis

Many microorganisms have been hailed as putative causes of IBD; however, in spite of numerous attempts to confirm a bacterial, mycobacterial, fungal, or viral etiology, the idea that a transmissible agent is responsible for IBD is still a hotly debated subject. Viruses—rotavirus, Epstein–Barr virus, cytomegalovirus, measles virus, and an uncharacterized RNA intestinal cytopathic virus—and mycobacteria continue to be favored candidates. Gastrointestinal infections with *Aeromonas* bacteria (*Aeromonas sobria* and *Aeromonas hydrophila*) and the yeast *Candida albicans* can trigger a flare of disease, and all patients with IBD should be tested for these infections at the start and during the course of their illness.[6] Overall, a state of gut dysbiosis has been well characterized for both forms of IBD whereby bacteria and fungi have alterations in composition and biodiversity.[6]

Antibiotic Exposure

Before the 1950s, CD was found in select groups with a strong genetic component. Since that time there has been a rapid climb in developed countries, particularly the United States, and in countries that previously had virtually no reported cases. In fact, CD has spread like an epidemic since 1950. Are antibiotics to blame? Penicillin and tetracycline have been available in oral form since 1953. The annual increase in prescriptions of antibiotics parallels the rise in the annual incidence of CD. Comparative statistics have shown that wherever antibiotics have been used early and in large quantities, the incidence of CD is now quite high.

Over the years, researchers have sought to identify CD as an infectious process. The problem may be that the infectious agent is a component of the normal intestinal flora that suddenly produces immunostimulatory toxins or becomes invasive as a direct result of sublethal doses of antibiotics. Administration of sublethal amounts of antibiotics has been shown to induce a capacity for toxin production in intestinal organisms. When microbes are not given a full lethal dose, their usual response is to adapt and become even stronger in virulence and numbers. Other medications that have been implicated as etiopathogenic include nonsteroidal anti-inflammatory agents and, most recently, isotretinoin (Accutane). Antibiotic exposure is now being

TABLE 186.1 Dietary Components Can Favor the Risk of Inflammatory Bowel Disease

Dietary Component	Effect on IBD Risk	Reference
Animal protein	Increased	137
Heme iron, sulfur	Increased	138
Refined sugars	Increased	139
High fat (trans)	Increased	140
High *n*-6 PUFA/*n*-3	Increased	140
High trans fat	Increased	140
Fiber	Decreased	138
Fruit	Decreased	141
Vegetables	Decreased	141
High omega-3 fatty acids	Decreased	142

IBD, Inflammatory bowel disease; *PUFA,* polyunsaturated fatty acid.

linked more to CD than UC. A total of 15 observational studies (8748 IBD patients) were systematically evaluated and confirmed that antibiotic exposure was linked with CD but not with UC.[7] The antibiotics that were most strongly linked to CD onset included the penicillins, cephalosporins, metronidazole, and fluoroquinolones, whereas the effect of the tetracycline-family antibiotics was unclear.

Immune Mechanisms

An overwhelming amount of evidence points to immunological derangements in IBD, but whether they are causal or secondary phenomena remains unclear. Theories relating humoral mechanisms, immunological regulatory defects, and cell-mediated reactions to the etiology of IBD have all been proposed, but the current evidence seems to indicate that these derangements are probably secondary to the disease process.[8]

Dietary Factors

Despite the fact that a dietary etiology of CD is barely considered (if mentioned at all) in most standard medical and gastroenterology texts, several lines of evidence strongly support dietary factors as being the most important etiologically, as recently reviewed by Limketkai et al.[9]

The incidence of CD is increasing in cultures consuming the Western diet, but it is virtually nonexistent in cultures consuming a more primitive diet.[10–15] Food is the major factor in determining the intestinal environment, so the considerable change in dietary habits over the past century could explain the rising incidence of IBD. Several studies that have analyzed the preillness diets of patients with CD have found they habitually eat more refined sugar, chemically modified fats, and fast food and less raw fruits, vegetables, omega-3–rich foods, and dietary fiber than do healthy people (Table 186.1).[10–14] In one study, the preillness intake of refined sugar in patients with CD was nearly twice that in controls (122 g/day vs. 65 g/day).[14] One researcher found that before the onset of disease, patients with CD had eaten cornflakes more frequently than controls.[16] Although other researchers could not verify this specific finding, cornflakes are high in refined carbohydrates and are derived from a very common allergen (corn). Much of controversy over the role of preillness diet in the etiology of CD is largely due to the fact that the only way to assess this diet is from postdiagnostic interviews. Studies in which the interview has taken place within the first 6 months of diagnosis tend to be more supportive than studies in which the interview was conducted more than 7 months after diagnosis. Increased refined sugar intake and high overall carbohydrate intake precede the development of CD.[17] Along these lines, patients with UC show higher consumption of refined carbohydrates than do control subjects.[18] Increased consumption of chemically modified fats (e.g., those found in margarine) may be involved in the etiology of UC.[19] Interestingly, high consumption of a fast-food diet is an antecedent of both UC and CD.[20]

Another important dietary factor that is entirely overlooked in the standard texts is the role of food allergy. Support for this hypothesis is offered in clinical studies that have used an elemental diet, total parenteral nutrition, or an exclusion diet with great success in the treatment of IBD.[16,21–25] The role of food allergy is discussed in greater detail later in the chapter, as is the effect of dietary fiber in the etiology and treatment of IBD (see "Therapeutic Considerations").

A reduced intake of omega-3 oils and an increased intake of omega-6 oils are also being linked with the growing rise of CD in Japan. Because the genetic background of the Japanese is relatively homogeneous, this higher incidence is most likely due to the incorporation of Western foods in the diet. To examine the contribution of diet to the increased incidence of CD in Japan, the incidence and daily intake of a number of dietary components were compared annually between 1966 and 1985. The analysis showed that the greater incidence of CD was strongly correlated with increased dietary intake of total fat, animal fat, omega-6 fatty acids, animal protein, milk protein, and the ratio of omega-6 to omega-3 fatty acids. It was less correlated with intake of total protein, was not correlated with fish protein, and was inversely correlated with vegetable protein. Multivariate analysis showed that higher intake of animal protein was the strongest independent factor, followed by an increased ratio of omega-6 to omega-3 fatty acids.[26] Correction of this increased ratio by reduction of omega-6 oil intake and an increase of omega-3 oil intake may lead to significant clinical benefit through an effect on eicosanoid metabolism (discussed later).

Miscellaneous Factors

Psychosomatic factors such as mental and emotional stress can promote exacerbation of IBD, so stress management techniques may prove useful for some patients.[27]

THERAPEUTIC CONSIDERATIONS

IBD is the end result of a complex interplay of several factors. This section discusses the key nutritional, microbial, and toxic issues that must be addressed for the successful management of this difficult disease.

Control of Causative Factors

Natural History of Crohn's Disease

Little is known about the natural course of CD because virtually all patients with the disease undergo standard medical care (drugs and/or surgery) or alternative therapy. The only exceptions are patients in clinical trials who are assigned to the placebo group.[28–30] However, even these patients do not represent the natural course of the disease because they are seen frequently by physicians and other members of a healthcare team and are taking medication, even if it is only in the form of a placebo. If proper evaluation of therapies for IBD is to occur, there must be a greater understanding of its natural history. This is particularly important for alternative practitioners because it is commonly believed that standard medical care often interferes with the normal efforts of the body to restore health. Some aspects of the "natural" course of CD support this idea, especially when coupled with the limited efficacy of current medications and surgery and their known toxicity. However, heroic measures do have their place in many instances and should be used when appropriate.

Researchers in the National Cooperative Crohn's Disease Study (NCCDS) reviewed 77 patients who received placebo therapy in part 1 of

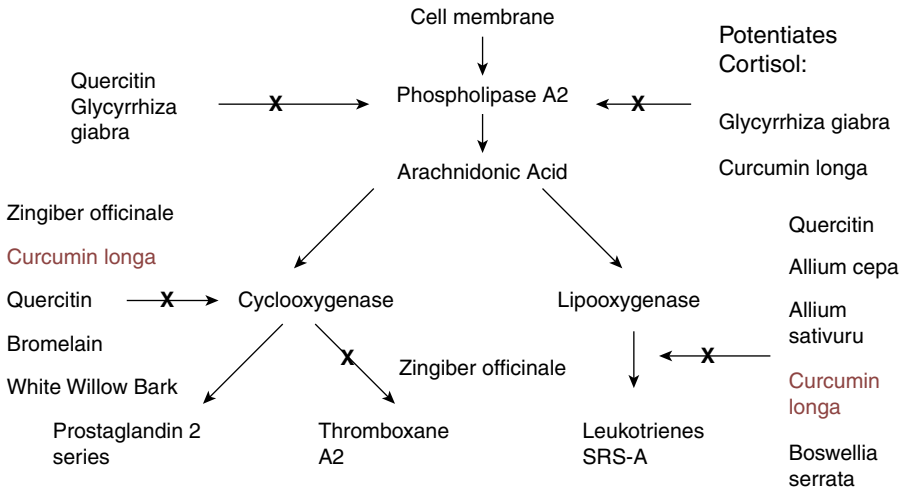


Fig. 186.2 Botanical Modulation of the Arachidonic Cascade. This figure illustrates the pathway by which arachidonic acid in plasma membranes is metabolized to proinflammatory eicosanoids such as PGE2, thromboxane A2, and so forth. Medications that are given for IBD work in large part by their ability to attenuate this pathway. However, botanicals found in nature and food at therapeutic doses can work on the exact same pathways and block the metabolism of arachidonic acid into proinflammatory mediators.

the 17-week study.[28,29] They all had active disease, as defined by a CD activity index (CDAI) higher than 150. Of the patients completing the study:

- None died.
- Only 7 (9%) suffered a major worsening of their disease (i.e., either a major fistula developed or the patient required abdominal surgery).
- Twenty-five (32%) suffered a lesser worsening (increase in the CDAI to >450 or presence of fever of 100°F for 2 weeks).
- Treatment was considered to have failed in 25 (32%) because their CDAIs remained higher than 150.
- Twenty (26%) achieved clinical remission.

On at least one occasion during the 17 weeks of therapy, 49% of the patients were found to have a CDAI lower than 150.

The patients who showed a favorable response to the placebo continued to be observed with placebo therapy for up to 2 years (part 1, Phase II). Interestingly, although none of these patients' intestinal radiographs showed worsening during Phase I or Phase II, 18% showed improvement. Of the patients whose disease responded to placebo (20; 26% of the 77), the majority (70%) remained in remission at 1 year, and a fair number (45%) remained in remission at 2 years. These results indicate that many patients undergo spontaneous remission, approximately 20% at 1 year and 12% at 2 years. However, when another factor is considered, the "success" of placebo therapy rises dramatically. Of patients in the placebo group who had no previous history of steroid therapy, 41% achieved remission after 17 weeks. In addition, 23% of this group continued in remission after 2 years, compared with only 4% of the group with a history of steroid use.

The European Cooperative Crohn's Disease Study (ECCDS), although different in some methodological details, is quite similar to the NCCDS.[28,30] In the ECCDS, 110 patients constituted the placebo group, 68 patients with prior treatment and 42 patients with no prior treatment. The results of the study showed that 55% of the total placebo group achieved remission by 100 days, 34% remained in remission at 300 days, and 21% remained in remission at 700 days. Like the NCCDS, the ECCDS demonstrated that patients with no prior therapy have a greater likelihood of remission.

Although one group of researchers did not advocate placebo therapy, they did carefully point out that once remission was achieved, 75% of the patients continued in remission at the end of 1 year and up to 63% at 2 years regardless of the maintenance therapy used. These results would suggest that the key is achieving remission, which, once attained, can be maintained by conservative nondrug therapy rather than the "medicines we are currently using with their limited efficacy and known toxicity."[28]

Eicosanoid Metabolism in Inflammatory Bowel Disease

Patients with IBD show greatly increased levels of inflammatory chemicals in the colonic mucosa, serum, and stool samples. Specifically, these patients show an increase in the synthesis of the lipoxygenase products, leukotrienes, and mono-hydroxyeicosatetraenoic acids (mono-HETEs).[31–35] These compounds are produced by neutrophils and are known to amplify the inflammatory process and cause smooth muscle contraction. Release of lipoxygenase products is promoted by activation of the alternative complement pathway. The therapeutic efficacy of sulfasalazine and corticosteroids is due to their effect on eicosanoid metabolism. Sulfasalazine is an inhibitor of both cyclooxygenase and neutrophil lipoxygenase, whereas corticosteroids inhibit phospholipase A_2 and thus block the release of arachidonic acid from the membrane phospholipid pool. Sulfasalazine also inhibits the degranulation of mast cells. Several naturally occurring compounds, such as the polyphenols (quercetin, curcumin, resveratrol, etc.), also interact favorably with these enzymes (Fig. 186.2).

The formation of these inflammatory compounds can be decreased by reducing or eliminating the consumption of omega-6–rich foods (corn, beef, liver, pork, lamb, and milk/dairy products) and increasing the consumption of omega-3 fatty acids through a higher intake of cold-water fish (salmon, mackerel, herring, and halibut). These fish are good sources of the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Meta-analyses of double-blind studies with fish oil supplements (2.7–5.1 g total omega-3 oils) have demonstrated an ability to prevent or delay relapses in both CD and UC.[36,37] The Cochrane Collaboration recently published a systematic review evaluating 214 publications and identified only 15 randomized controlled trials (RCT). Only four studies were of sufficient quality to be included in the analysis. Enteric-coated omega-3 essential fatty acid (EFA) supplementation reduced the 1-year relapse rate by half, with an absolute risk reduction of 31% and a number needed to treat (NNT) of only 3. A much larger RCT asked whether omega-3 fatty acids could sustain remission once it was achieved. Two randomized, double-blind, placebo-controlled studies (Epanova Program in Crohn's Study 1 [EPIC-1] and EPIC-2) were conducted between January 2003 and February 2007 at 98 centers in Canada, Europe, Israel, and the United

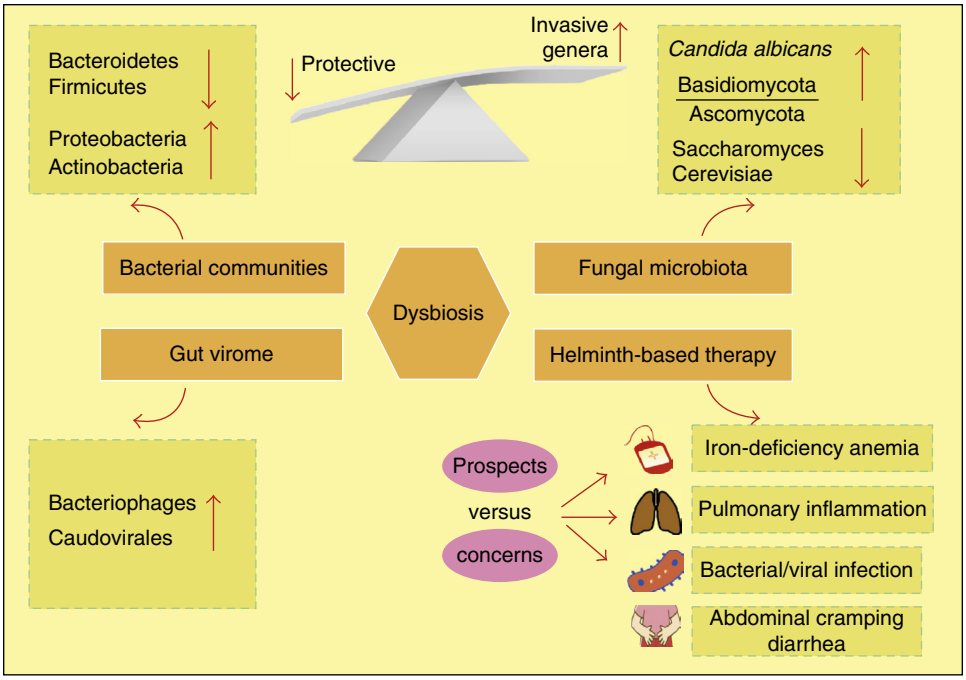


Fig. 186.3 Gut Microbiota Alteration and Immune Responses in Inflammatory Bowel Disease (IBD). The gut microbes, including bacteria, viruses and fungi, and dysfunctional immune responses, engaging Tregs, T-helper 1 (Th1), and Th17, are implicated in IBD pathogenesis. During homestasis, gut microbes induce an immune tolerance phenotype in the host, whilst in inflammatory conditions like IBD, antigens from dysbiotic microbes activate Th1 and Th17 cells, resulting in tissue injury, decreased mucus layer, and microbial penetration and persistence in the intestinal tissues. This mucosal injury results in further uptake of microbial antigens, TLR ligands, and viable organisms that perpetuate the immune responses. *DC, Dendritic cell; MMP,* matrix metalloproteinase; TGF, Transforming growth factor. (Published under. Creative Commons Attribution License [CC BY]. Zuo T, Ng SC. The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease. *Front Microbiol.* 2018;9:2247. Published online 2018 Sep 25. doi: 10.3389/fmicb.2018.02247)

States. Data from 363 and 375 patients with quiescent CD were evaluated in EPIC-1 and EPIC-2, respectively. Patients with a CDAI score of less than 150 were randomly assigned to receive either 4 g/day of omega-3 free fatty acids or placebo for up to 58 weeks. No other treatments for CD were permitted. Clinical relapse is defined by a CDAI score of 150 points or greater and an increase of more than 70 points from the baseline value, or initiation of treatment for active CD. In both EPIC-1 and EPIC-2, there were no significant differences in the CD relapse rate for placebo versus fish oils.[38] Flaxseed oil is also of value. Flaxseed oil contains alpha-linolenic acid, an essential omega-3 fatty acid that has anti-inflammatory effects and can be converted to EPA in limited amounts.[39]

Mucin Defects in Ulcerative Colitis

Mucins are an ill-defined class of high-molecular-weight, carbohydrate-rich (85% by weight of *N*-acetylgalactosamine, galactose, sialic acids, *N*-acetylglucosamine, and fructose) glycoproteins thought to be largely responsible for the viscous and elastic characteristics of secreted mucus. Alterations in mucin composition and content in the colonic mucosa have been reported in patients with UC.[39–42] The factors responsible for these changes appear to be a dramatic drop in the mucous content of the goblet cells (proportional to the severity of the disease) and a diminution of the major sulfomucin subfraction (designated "species IV," according to diethylaminoethanol-cellulose differentiation). In contrast, these abnormalities are not found in patients with CD. It is significant that although the mucin content of the goblet cells returns to normal during remission, the sulfomucin deficiency does not. The specific components of the sulfomucin and the cause of its lower concentration have not yet been determined. These mucin abnormalities are also thought to be a major factor in these patients' higher risk of colon cancer.

Many of the herbs used historically in the treatment of UC are demulcents; that is, agents that soothe irritated mucous membranes and promote the secretion of mucus. This effect appears to be very beneficial and supports the use of demulcents in UC.

Intestinal Microflora

The intestinal microflora is extraordinarily complex and contains more than 400 distinct microbial species.[43,44] Accurate simultaneous quantification of all possible species is not possible with current conventional culture techniques. In addition, measurement techniques such as stool cultures do not indicate bacterial metabolic activity, locations of growth within the gastrointestinal tract, or turnover rates. These last two factors may prove to be the more important determinants of the role of the intestinal bacterial flora in IBD than the actual numbers of specific bacterial species. In an effort to describe a nonspecific (qualitative or quantitative) alteration in the intestinal flora, the term *dysbiosis* is often used (see Chapters 9 and 28 for a full discussion of this important topic).[43]

The fecal flora of patients with IBD has been found to contain higher numbers of gram-positive anaerobic coccoid rods and *Bacteroides vulgatus*, a gram-negative rod.[43] Studies have indicated that these alterations in fecal flora are not secondary to the disease, and alterations in the metabolic activity of the various bacteria are thought to be more important than alterations in the number of bacteria per se. In addition, specific bacterial cell components (which vary even within the same species) are thought to be responsible for promoting lymphocyte cytotoxic activity against the colonic epithelial cells.[43,44] Fig. 186.3 illustrates the potential role of gut dysbiosis in IBD disease pathogenesis, and Fig. 186.4 demonstrates the interaction of dietary factors with the gut microbiome in disease risk.

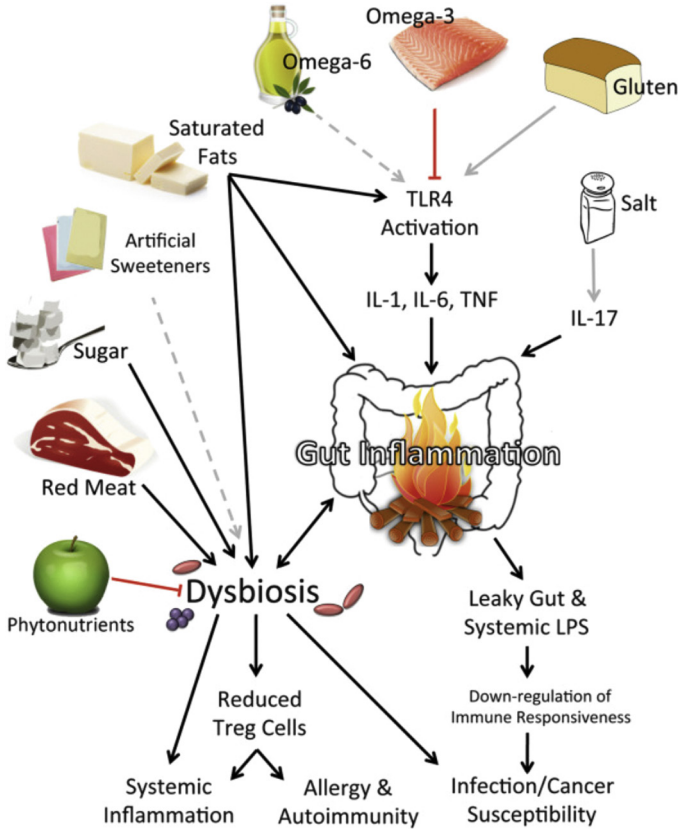


Fig. 186.4 Diagrammatic overview of the current mechanisms for macro-components of the modern diet altering susceptibility to infection, allergy, and autoimmunity. Solid black lines indicate that direct human evidence for enhancement is present; solid red lines indicate that direct human evidence of inhibition exists; gray lines indicate that only in vitro or animal model evidence exists currently; dotted lines indicate significant disagreement within the scientific literature. *IL-*, Interleukin; *TLR4,* Toll-like receptor 4; *TNF,* tumor necrosis factor; *Treg,* T-regulatory cell. (Published with permission under the terms of the Creative Commons Attribution License [http://creativecommons.org/licenses/by/4.0], *Nutr J.* 2014;13:61.[135] Published online 2014 Jun 17. https://doi.org/10.1186/1475/2891-13-61)

Carrageenan

Interestingly, researchers investigating the intestinal flora of UC often use the carrageenan (a sulfated polymer of galactose and D-anhydrogalactose extracted from red seaweeds, principally *Eucheuma spinosum* and *Chondrus crispus*) model to induce the disease in animals experimentally. In the initial experiments reported by Marcus and Watt[45] in 1969, 1% and 5% carrageenan solutions were provided as the exclusive source of oral fluids for guinea pigs. Over a period of several days, the animals lost weight, demonstrated anemia, and had bloody diarrhea. Gross anatomic studies after sacrifice at 20 and 45 days revealed a loss of haustral folds, mucosal granularity, pseudopolyps, and strictures; microscopic examination showed crypt abscesses, lymphocytic infiltration, capillary congestion of the lamina propria, and gross ulcerations. These results have since been confirmed by numerous investigators and in studies involving other animal species, including primates.[46–49]

In its native state, the carrageenan polymer has a molecular weight of 100,000 to 800,000 Da, but in the studies, it is degraded by mild acidic hydrolysis to yield products with weights in the vicinity of 30,000 Da. Carrageen compounds are used by the food industry as stabilizing and suspending agents, with polymers of different molecular weights being used for a variety of purposes. Typically, carrageenans used in the food industry have a molecular weight greater than 100,000 Da. Carrageenan is widely used in milk and chocolate milk products (e.g., ice cream, cottage cheese, milk chocolate) because of its ability to stabilize milk proteins.

As suggestive as the animal studies are in linking UC with carrageenan and despite the higher consumption of carrageenan in Western diets, at this time there appears to be no correlation between the human consumption of carrageenan and the development of UC. In one study, no lesions of IBD were observed in healthy human subjects fed enormous quantities of degraded carrageenan.[50] However, differences in intestinal bacterial flora are probably responsible for this discrepancy, because germ-free animals do not display carrageenan-induced damage.

On further examination, it was discovered that the bacterium linked to the carrageenan-induced damage in animals is a strain of *B. vulgatus.*[43] As mentioned earlier, this organism is found in much higher concentrations (six times as high) in the fecal cultures of patients with IBD. When all the data are evaluated, they appear to imply that although carrageenan can be metabolized into nondamaging components in most human subjects, those individuals with an overgrowth of *B. vulgatus* may be at risk. Strict avoidance of carrageenan appears warranted at this time for patients with IBD until further research clarifies its safety for them.

Aspirin and Intestinal Permeability

A very interesting study evaluated 30 patients with CD and 37 first-degree relatives of patients with CD for intestinal permeability by means of the lactulose/mannitol ratio. First-degree relatives had a 110% higher intestinal permeability after ingesting acetylsalicylic acid, compared with an increase of 57% in the control subjects. Thirty-five percent were "hyperresponders."[51] A familial permeability defect causing increased permeability would be a significant predisposing factor for CD, because a leaky gut is associated with a higher incidence of food allergy and greater absorption of intestinal toxins (see Chapter 19).

Endotoxemia and the Alternative Complement Pathway

Endotoxemia is associated with both CD and UC.[51,52] Endotoxemia-induced activation of the alternative complement pathway could explain some of the extraintestinal (i.e., outside the gastrointestinal tract) manifestations of IBD (discussed later). Whole-gut irrigation significantly reduces the endotoxin pool in the gut and has been shown to have a very beneficial antiendotoxemia effect.[53] Colonic irrigation may offer similar benefit. However, colonic irrigation during an acute inflammatory flare may be contraindicated.

Extraintestinal Manifestations

More than 100 disorders, known as extraintestinal lesions (EILs), constitute a diverse group of systemic complications of IBD.[54,55] The most common EIL in adults is arthritis, which is found in about 25% of patients. Two types are typically described, the more common being peripheral arthritis affecting the knees, ankles, and wrists. Arthritis is more common in patients with colon involvement. The severity of symptoms is typically proportional to disease activity.[54,55]

Less frequently, arthritis primarily affects the spine. Symptoms are low back pain and stiffness, with eventual limitation of motion. This EIL occurs predominantly in men with human leukocyte (HLA)-B_{27} and is fairly indistinguishable from typical ankylosing spondylitis. In fact, it may antedate the bowel symptoms by several years. There is probably a consistent underlying factor in both the progression of ankylosing spondylitis and IBD.[54,55]

Skin manifestations are also common, being seen in approximately 15% of patients. Typical lesions are erythema nodosum, pyoderma gangrenosum, and aphthous ulcerations. Recurrent aphthous stomatitis occurs in approximately 10% of patients.[54,55]

BOX 186.1 Causes of Malnutrition in Inflammatory Bowel Disease

Decreased oral intake
- Disease-induced (pain, diarrhea, nausea, anorexia)
- Iatrogenic (restrictive diets without supplementation)

Malabsorption (with corresponding diagnostic tests)
- Decreased absorptive surface due to disease or resection (D-xylose testing)
- Bile salt deficiency after resection (fecal bile acids, Mayo Clinic)
- Bacterial overgrowth (lactulose or glucose breath hydrogen and methane testing)
- Drugs (e.g., corticosteroids, sulfasalazine, cholestyramine)

Increased secretion and nutrient loss
- Protein-losing enteropathy (fecal alpha-1 antitrypsin 24 h clearance)
- Electrolyte, mineral, and trace mineral loss in diarrhea

Increased utilization and increased requirements
- Inflammation, fever, infection
- Increased intestinal cell turnover

Serious liver disease (i.e., sclerosing cholangitis, chronic active hepatitis, cirrhosis) is also a common EIL, affecting 3% to 7% of the patients with IBD. It probably relates to the increased endotoxin load associated with IBD. If patients are demonstrating liver enzyme abnormalities, hepatoprotection appears indicated, with botanical medicines such as *Silybum marianum* (i.e., silymarin) and curcumin.[56–58]

Other common EILs are as follows[54,55]:
- Thrombophlebitis
- Finger clubbing
- Ocular manifestations (episcleritis, iritis, and uveitis)
- Nephrolithiasis
- Cholelithiasis
- In children: failure to grow, thrive, and mature normally

Malnutrition

Many nutritional complications occur during the course of IBD.[59–61] Because these complications can have a significant influence on the morbidity and perhaps also the mortality of these patients, every effort should be made to ensure optimal nutritional status. The major mechanisms that contribute to nutritional depletion in patients with IBD are listed in Box 186.1.

A decreased food intake is the most important mechanism of nutritional deficiency in patients with IBD, and a deficient calorie intake is the most common nutritional deficit in patients requiring hospitalization. Often the patient feels significant pain, diarrhea, nausea, and/or other symptoms after a meal, resulting in a subtle diminution in dietary intake. Protein-calorie malnutrition and associated weight loss are prevalent in 65% to 75% of patients with IBD.[59]

Malabsorption can be anticipated in patients with extensive mucosal involvement of the small intestine and in those who have undergone resection of segments of the small intestine. Particularly common is fat malabsorption, which results in significant loss of calories as well as the loss of fat-soluble vitamins and minerals. Involvement of the ileum or surgical resection of that area typically leads to malabsorption of vitamins (in particular vitamin B_{12}) and bile acid. Because of the cathartic effect of bile acids on the colon, the malabsorption may result in chronic watery diarrhea. Electrolyte and trace mineral deficiencies should be suspected in patients with a history of chronic diarrhea, whereas calcium and magnesium deficiencies may be a result of chronic steatorrhea.

Increased secretion of protein into the intestinal lumen and nutrient loss due to the exudative and inflammatory nature of IBD are common. In particular, there is a significant loss of plasma proteins across the damaged and inflamed mucosa. The loss of protein may exceed the ability of the liver to replace plasma proteins despite a high protein intake. The chronic loss of blood often leads to iron depletion and anemia.

TABLE 186.2 Prevalence of Nutritional Deficiencies in Hospitalized Patients With Inflammatory Bowel Disease

Deficiency	Prevalence (%)
Hypoalbuminemia	25–80
Anemia	60–80
Iron deficiency	40
Low serum vitamin B_{12}	48
Low serum folate	54–64
Low serum magnesium	14–33
Low serum potassium	6–20
Low serum retinol	21
Low serum ascorbate	12
Low serum 25-OH-vitamin D	25–65
Low serum zinc	40–50

Data from Aoki K. A study of endotoxemia in ulcerative colitis and Crohn's disease. I. Clinical study. *Acta Med Okayama* 1978;32:147–158.

The drugs most commonly used in the allopathic treatment of IBD are corticosteroids and sulfasalazine, both of which increase nutritional needs. Corticosteroids are known to have the following effects:
- Stimulate protein catabolism
- Depress protein synthesis
- Decrease the absorption of calcium and phosphorus
- Increase the urinary excretion of ascorbic acid, calcium, potassium, and zinc
- Increase blood glucose, serum triglyceride, and serum cholesterol levels
- Increase the requirements for vitamin B_6, ascorbic acid, folate, and vitamin D
- Decrease bone formation
- Impair wound healing

Sulfasalazine has been shown to have the following effects[62]:
- Inhibits the absorption and transport of folate
- Decreases serum folate and iron
- Increases the urinary excretion of ascorbic acid

The last consideration in the causes of nutrient deficiency in patients with IBD involves the nutritional consequences of a chronic inflammatory and/or infectious disease. This topic has not been fully investigated; the only conclusion that currently can be drawn is that protein requirements may be raised in patients with acute exacerbations of IBD. An elevated erythrocyte sedimentation rate signifies a rise in both protein breakdown and protein synthesis. Typically, patients with IBD require perhaps as much as 25% more protein than the usual recommended allowance.[59–61]

Prevalence of Nutritional Deficiencies

The prevalence of nutritional deficiencies is quite high in hospitalized patients with IBD. Although it is generally agreed that the prevalence of nutritional deficiencies is greater in hospitalized patients (who typically are more severely ill) than in outpatients, a great number of ambulatory patients with CD display one or more nutrient deficiencies as well.[63] In addition to the deficiencies listed in Table 186.2, low levels of vitamin K, copper, niacin, and vitamin E have been reported.[59]

TABLE 186.3 Dietary Therapies for Inflammatory Bowel Disease

Diet	Rationale	Plan	Evidence
Elimination	Lower antigenic burden—up to 66% of patients with CD report food intolerances.	Eliminate known and suspected provocative foods for 2–4 weeks, then reintroduce one new food per day; process may take 2–3 months.	143,144
Enteral Nutrition	Multifactorial: lower antigenic stimulation	Enteral feeds via tube feed or ONS; induction/maintenance	145
Specific Carbohydrate Diet	Eliminate poorly digestible carbohydrates to limit fermentation in small bowel. Avoid complex carbohydrates.	Allowed: meat, fish, eggs, vegetables, nuts, low-sugar fruits, oils, honey Avoid: starches, grains, pasta, legumes, and breads	146,147
Low FODMAP	Decrease fermentable carbohydrates, poorly transported di-saccharides, gas production, osmotic diarrhea, etc.	Limit fructose, lactose, fructans, galactans, and sugar-alcohols.	148,149

CD, Crohn's disease; *FODMAP,* fermentable oligo-, di-, mono-saccharides and polyols; *ONS,* oral nutrition supplement.

Diet

The importance of correcting nutritional deficiencies in patients with IBD cannot be overstated. Deficiencies of both macronutrients and micronutrients cause alterations in gastrointestinal function and structure, which may lead to a vicious cycle—the secondary effects of malnutrition on gastrointestinal tract function and structure may further exacerbate malabsorption, further reducing nutrient status. Foremost in nutritional therapy is providing adequate caloric intake. It should be assumed that the majority of patients suffer from micronutrient deficiency, although the deficiency is often subclinical and can be detected only by appropriate laboratory investigation.

In general, patients with IBD should be started on therapeutic vitamin supplements of at least five times the recommended dietary allowances (RDAs). Several minerals may also have to be supplemented at equally high levels. Dietary treatment involves the use of either an elemental diet or an elimination diet (described later).

Elemental Diet

The elemental diet has been shown to be an effective nontoxic alternative to corticosteroids as the primary treatment of acute IBD (Table 186.3).[16,21–23] Such a diet is one that is purported to contain all essential nutrients, with protein being provided only as predigested or free-form amino acids. The improvements noted on an elemental diet, however, are probably not primarily related to nutritional improvement but could be a result of alterations in the intestinal flora (which have been noted to occur in patients consuming an elemental diet). A stronger case could be made for a secondary immune mechanism being bypassed during elemental feeding: the elemental diet is serving as an allergy-elimination diet.

The main drawbacks of the widespread use of elemental diets are their poor palatability and their hyperosmolality, which often result in diarrhea. In addition, hospitalization is often required for satisfactory administration, and relapse is quite common when patients resume normal eating. An elimination diet, rather than an elemental diet, may be a more acceptable alternative in the treatment of acute and particularly of chronic IBD.

Elimination (Oligoantigenic) Diet

Although food allergy has long been considered an important etiological factor in the pathogenesis of IBD, studies using an elimination diet in the treatment of IBD have been performed only recently (elimination diets are described in Chapter 14).[24–26] These studies demonstrate that an elimination diet is the primary therapy of choice in the treatment of chronic IBD. The most common offending foods were found to be wheat and dairy products.

An alternative approach is to determine the actual allergens with laboratory methods, preferably a method that measures reactions mediated by both immunoglobulins G (IgG) and E (IgE). The allergens are then avoided, or a rotary diversified diet is used as appropriate (see Chapter 46).

Minerals

Zinc

Zinc deficiency, a well-known complication of CD, occurs in approximately 45% of patients with the disorder.[64] Low serum zinc concentrations, low hair zinc levels, malabsorption of zinc, altered urinary excretion of zinc, and impaired taste acuity are commonly found in patients with CD.[60,64] The deficiency of zinc is due to low dietary intake, poor absorption, and excess fecal losses.[60–62,64] Zinc deficiency may be the direct cause of the following complications of CD[64–66]:

- Poor healing of fissures and fistulas
- Skin lesions (acrodermatitis)
- Hypogonadism
- Growth retardation
- Retinal dysfunction
- Depressed cell-mediated immunity
- Anorexia
- Chronic diarrhea

Many patients with CD show no response to oral or intravenous zinc supplementation because such patients appear to have a defect in the tissue transport of zinc. Intravenous supplementation results in a tremendous rise in urinary zinc excretion but insignificant clinical results. Several clinical trials using oral zinc sulfate have shown the same lack of positive effect.[67]

Administration of zinc in the oral picolinate form may be more advantageous than other forms of zinc, possibly improving both intestinal absorption and tissue transport, although one study displayed no significant difference in zinc absorption between zinc sulfate and zinc picolinate in patients with pancreatic insufficiency.[68] Zinc citrate may also be an appropriate alternative. In any event, every attempt should be made to ensure that adequate tissue stores of zinc are maintained because disease activity is correlated with zinc deficiency. Parenteral zinc administration may be needed in some cases. Although less than ideal, intravenous administration of zinc is often the only way to attain even marginal zinc levels in patients with CD.[67]

Magnesium

Magnesium deficiency is often seen in patients with IBD.[60,69,70] However, a poor correlation exists between serum magnesium levels—which may be low in only a few patients— and intracellular magnesium levels, which are commonly decreased in patients with IBD.[60]

Patients with low intracellular magnesium levels may present with the following problems[60]:

- Weakness
- Anorexia
- Hypotension
- Confusion
- Hyperirritability
- Tetany
- Convulsions
- Electrocardiographic or electroencephalographic abnormalities

These symptoms are generally responsive to parenteral magnesium supplementation.

A daily intravenous dose of 200 to 400 mg of elemental magnesium may be necessary for patients not responding to oral supplementation. Patients with IBD may require this route of supplementation owing to magnesium's somewhat cathartic action and poor absorption in those with a short bowel. Oral supplementation should be with magnesium chelates (i.e., citrate, aspartate) rather than inorganic magnesium salts (i.e., carbonate).

Iron

Iron deficiency anemia is very common in IBD, largely because of chronic blood loss through the gut.[60] Serum ferritin levels are the most useful indices of iron status. A serum ferritin concentration of greater than 55 ng/mL indicates adequate iron reserves in bone marrow, whereas a concentration of less than 18 ng/mL is highly predictive of iron deficiency (see Chapter 23 for further discussion). The clinician should attempt to increase the patient's iron stores by improving absorption, as with supplemental vitamin C, rather than through direct iron supplementation, which promotes intestinal infection.[71]

Calcium

Patients with IBD are also at risk for the development of calcium deficiency, probably owing to the following factors[60]:

- Loss of absorptive surfaces
- Steatorrhea
- Corticosteroid use
- Vitamin D deficiency

Potassium

Diarrheal diseases are often associated with potassium and other electrolyte deficiencies. Although symptoms of potassium deficiency are quite rare in patients with IBD, levels of this mineral are probably below optimum. In one study, nutritional support to correct potassium deficiency resulted in significantly reduced rates of surgical complications.[72]

Vitamins

Vitamin A

Low serum retinol levels are found in approximately 20% of patients with CD and are correlated with the activity of the disease.[60,66] Vitamin A can profoundly affect the metabolism and differentiation of the intestinal epithelial mucosa because it can increase the number of goblet cells, the production of mucins, and the secretion of mucus; moreover, it can restore normal barrier function. Preliminary case reports indicated that vitamin A may be of therapeutic use in CD.[73,74] However, long-term controlled trials have shown that vitamin A (50,000 IU twice daily) has no therapeutic effect in the majority of patient with CD.[75,76] It should be kept in mind, however, that certain individuals may respond to vitamin A therapy and that zinc supplementation will often normalize disturbances of vitamin A metabolism because zinc is a necessary component of retinol-binding protein.[66]

Vitamin D

Vitamin D deficiency is quite common in IBD, with laboratory evidence of this in 75% of patients with CD and 35% of patients with UC.[60,77] It is probably due to decreased absorption of 25-hydroxyvitamin D. Patients with IBD are at increased risk for the development of metabolic bone diseases such as osteoporosis and osteomalacia. This relates to previously mentioned factors in calcium deficiency. Vitamin D plays an important role in supporting proper immune regulation, much like probiotics; both influence T-regulatory cell function and dampen proinflammatory cytokines produced in animal models of IBD. Clinical studies using vitamin D at supraphysiological doses are under way.

Vitamin E

Vitamin E deficiency can occur in IBD, as observed in one case report.[78] The patient had a 25-year history of CD and had undergone multiple small bowel resections. Presenting symptoms relating to vitamin E deficiency were as follows:

- Bilateral visual field scotomata
- Generalized motor weakness
- A broad-based gait with marked ataxia
- Brisk reflexes
- A bilateral Babinski response

This patient's serum vitamin E concentration was 0.03 mg/dL (normal is 0.8–1.2 mg/dL), and in vitro peroxide hemolysis was 100% (normal is <10%). Supplementation with 270 IU/day of vitamin E eventually brought complete recovery over a period of 2 years.

Vitamin E supplementation is also indicated because of its ability to inhibit the formation of leukotrienes and reduce free-radical damage.[79]

Vitamin K

Vitamin K deficiency, resulting in the formation of abnormal prothrombin (deficient in gamma-carboxyglutamic acid), is quite common in patients with IBD.[80,81] It also contributes to the osteoporosis and osteopenia that are so often seen in patients with CD.[82] The best laboratory test to diagnose early vitamin K deficiency before lowered serum levels is to measure the incomplete gamma-carboxylation of specific serum proteins such as osteocalcin and prothrombin. Low vitamin K status has been linked to bone disease by incomplete carboxylation of osteocalcin and coronary artery disease by unknown mechanisms. Vitamin K_2 deficiency is tested my measuring PIVKA II (protein in the absence of vitamin K carboxylation) and/or uncarboxylated osteocalcin by laboratories such as Quest and LabCorp as well as many designer specialty laboratories.

Folic Acid

Low serum concentrations of folic acid are common in IBD, with reports of occurrence ranging between 25% and 64% of cases.[60,82–84] The drug sulfasalazine exacerbates the condition by interfering with folate-dependent enzymes and with the intestinal folate transport system.[85] A folate deficiency promotes further malabsorption through alteration in the structure of the intestinal mucosal cells.[86] These cells have a very rapid turnover (1–4 days) compared with red blood cells (RBCs; 3–4 months); therefore deficiency affects mucosal cells much earlier than RBCs. Testing CD patients for elevations in serum homocysteine or single-nucleotide polymorphisms (SNPs) for enzymes involved in the methylation of folate, such as methyl-tetrahydrofolate reductase, may provide valuable diagnostic tools for the early detection of aberrant folate metabolism in patients with CD. Patients with UC, on the other hand, require supplemental folate to reduce their higher risk of colon cancer.

TABLE 186.4 Randomized Controlled Clinical Trials of Curcumin in Ulcerative Colitis (UC)

Author	Design/#	Method	Control	Duration	Results
150	DBRCT Refractory Left-Sided UC	*Enema curcuma longa* (140 mg in 20 mL of water QD for 8 weeks) + 800 oral mesalamine BID *N* = 45	Oral Mesalamine plus placebo (enema)	8 wk	Induction of remission superior with curcumin 43% herb vs. 23% placebo
151	DBRCT Stable UC	Oral 1 gm BID curcumin *N* = 89	Mesalamine 2.4 gm daily or placebo	26 wk	Maintenance of remission superior with curcumin
152	DBRCT Mild to moderate UC refractory to maximal oral and topical doses of mesalamine	Oral 3 gm curcumin daily *N* = 50	Mesalamine (oral and topical same doses continue) or placebo oral and topical	4 wk	All superior in combined group 65% response $p < 0.001$; 35% endoscopic remission. Adverse events rare.

DBRCT, Double-blind, randomized controlled trial.

Vitamin B_{12}

There is a significant correlation between vitamin B_{12} absorption and the extent of terminal ileal disease and/or resection.[60,87] Overall, abnormal Schilling test results are found in 48% of patients with CD. When ileal resection exceeds 90 cm, the Schilling test result is abnormal in all patients and does not improve with time. If the length of the resection or the extent of the inflammatory lesion is less than 60 cm, adequate absorption may occur.

Ascorbic Acid

A low vitamin C intake is common in patients with IBD, particularly in those on a low-fiber diet.[60,88] Ascorbic acid levels in serum and leukocytes are significantly lower in patients with CD than in matched controls.[60,88] Vitamin C is thought to be particularly important in the prevention of fistula formation. It has been shown that patients with fistulas have lower ascorbic acid levels than patients without them.[89]

Antioxidant Defenses

Increased oxidative stress and decreased antioxidant defenses in the mucosa are hallmark features of IBD.[90] Patients with IBD have heightened oxidative stress and associated protein and DNA oxidative damage).[90] Likewise, IBD patients have serum and mucosal deficiencies in antioxidant defenses such as selenium-dependent glutathione peroxidase and copper-zinc superoxide dismutase, along with cofactors for these enzyme systems (i.e., zinc, copper) and others (i.e., ascorbate, vitamin E, vitamin A), as previously described in this chapter.[90]

Other Nutrients

Obviously, patients with IBD are at risk for the development of any nutrient deficiency, including nutrients not discussed here. However, there is probably no real need for laboratory investigation to determine micronutrient deficiencies other than for the nutrients discussed here.[60]

Recommendation for a High-Potency Multivitamin/Multimineral Formula

It is absolutely essential that patients with IBD take a high-potency multivitamin/multimineral supplement comprising all the known vitamins and minerals. Table 186.4 lists recommendations for an optimal intake range.

In addition, patients with IBD should consume additional antioxidants (rationale provided previously). The two primary antioxidants in the human body are vitamins C and E. Vitamin C is an "aqueous phase" antioxidant, whereas vitamin E is a "lipid phase" antioxidant. Recommended doses in patients with IBD are as follows:

- Vitamin E (mixed tocopherols with approximately 40% gamma-tocopherol): 400 to 800 IU
- Vitamin C (ascorbic acid): 1000 to 3000 mg

Botanical Medicines

Curcumin

Turmeric, the major spice in curry, is a natural spice made from the herb *Curcuma longa,* a member of the ginger family. In addition to being a culinary staple, it has been used in Ayurvedic medicine since ancient times. The major chemical constituents of turmeric are curcuminoids, the most prominent of which is curcumin. Recently, the investigational focus has shifted toward the role of curcumin as an intracellular signaling agent, and studies have demonstrated that, much like green tea polyphenols, curcumin is an inhibitor of nuclear factor (NF)-κB and leads to downstream regulation and inhibition of proinflammatory genes and cytokines (see Fig. 186.2).[91] Administration of curcumin has also been reported to modulate a host of other cytokines and signaling pathways, including iNOS, MMP-9, tumor necrosis factor-alpha (TNF-α), JNK, p38, AKT, JAK, ERK, and PKC.[92] Four studies involving curcumin administration in murine colitis models showed clinical and histopathological improvement and, where measured, decreased inflammatory cytokine production.[93–95] These findings were echoed in three further studies involving rodent models of colitis.[96–98]

In 2005 Holt and colleagues reported the preliminary results of a pilot study involving open-label administration of a curcumin preparation to five patients with UC and five patients with CD. Of these 10 patients, 9 reported improvement at the conclusion of the 2-month study. Of the five patients with UC, four were able to decrease or eliminate their medications.[99] In a larger randomized, double-blind, multicenter trial involving 89 patients with quiescent UC, administration of 1 g of curcumin twice daily resulted in both clinical improvement and a statistically significant decrease in the rate of relapse.[100] Given its excellent safety profile, its plausible mechanism for affecting inflammation, and the results just described, curcumin is poised to have a prominent role in the future management of IBD. Table 186.5 summarizes the results of the randomized controlled clinical trials for curcumin in ulcerative colitis.

Boswellia

The Ayurvedic herb *Boswellia serrata* (Indian frankincense) contains boswellic acids, which inhibit leukotriene biosynthesis in neutrophilic granulocytes by noncompetitive inhibition of 5-lipoxygenase.[101] During a small 6-week trial, 350 mg three times a day of *Boswellia* gum

TABLE 186.5 Optimal Range of Nutrients

Nutrient	Range for Adults
Vitamins	
Vitamin A (retinol)	5000 IU[a]
Vitamin A (from beta-carotene)	5000–25,000 IU
Vitamin D	100–400 IU
Vitamin E (D-alpha tocopherol)	100–200 IU
Vitamin K (phytonadione)	60–300 mcg
Vitamin C (ascorbic acid)	100–1000 mg
Vitamin B_1 (thiamin)	10–100 mg
Vitamin B_2 (riboflavin)	10–50 mg
Niacin	10–100 mg
Niacinamide	10–30 mg
Vitamin B_6 (pyridoxine)	25–100 mg
Biotin	100–300 mcg
Pantothenic acid	5–100 mg
Folic acid	400 mcg
Vitamin B_{12}	400 mcg
Choline	10–100 mg
Inositol	10–100 mg
Minerals	
Boron	1–6 mg
Calcium	250–500 mg
Chromium	200–400 mcg
Copper	1–2 mg
Iodine	50–150 mcg
Iron	15–30 mg[b]
Magnesium	250–500 mg
Manganese	10–15 mg
Molybdenum	10–25 mcg
Potassium	200–500 mg
Selenium	100–200 mcg
Silica	1–25 mg
Vanadium	50–100 mcg
Zinc	15–45 mg

[a]Women of childbearing age for whom becoming pregnant is a possibility should not take more than 2500 IU/day of retinol because of the possible risk of birth defects.
[b]Men and postmenopausal women rarely need supplemental iron.

resin was as effective as sulfasalazine (1000 mg three times a day) in reducing symptoms or laboratory abnormalities of patients with active UC.[102] The rate of remission was 82% with *Boswellia* and 75% with sulfasalazine.[103] In a randomized, double-blind study from Germany, a proprietary *Boswellia* extract, H15, was found as effective as mesalazine in improving symptoms of active CD.[104] Table 186.6 summarizes the results of the randomized controlled clinical trials for *Boswellia* in inflammatory bowel disease.

Quercetin

The plant flavonoid quercetin appears to be particularly indicated in the treatment of IBD. Flavonoids in general are considered natural biological response modifiers.[105,106] Quercetin, as perhaps the most pharmacologically active flavonoid, has remarkable effects on a variety

TABLE 186.6 Randomized Controlled Clinical Trials of *Boswellia* in Inflammatory Bowel Disease

Author	Design/#	Disease	Control	Duration
153	Open labeled Left-sided colitis *N* = 30	Colitis (UC, CD) *Boswellia* 900 mg QD *N* = 20	Sulfasalazine 1 gm/TID *N* = 10	6 wk
154	Open-labeled *Boswellia* 350 mg TID *N* = 34	UC colitis *Boswellia* 350 mg TID *N* = 34	Sulfasalazine 1 gm TID *N* = 8	6 wk
155	DBRCT *Boswellia* 2400 mg/D	CD in remission	Placebo	12 mo

DBRCT, Double-blind, randomized controlled trial; *CD,* Chron's disease; *UC,* ulcerative colitis.

of enzyme systems,[105,106] and many of the enzymes thus affected are important in the secretory, contractile, and motility processes associated with the inflammatory response: the release of histamine and other inflammatory mediators from mast cells, basophils, neutrophils, and macrophages; migration and infiltration of leukocytes; and smooth muscle contraction.

Quercetin's effect on these enzymes and processes is likely due to a common action—antagonizing calmodulin.[107] When bound to calmodulin, calcium activates a great variety of enzymes, including those involved in cyclic nucleotide metabolism, protein phosphorylation, secretory function, muscle contraction, microtubule assembly, glycogen metabolism, and calcium flux. Quercetin is believed to interact directly with calmodulin and calcium channels. This has been shown in many experimental studies and would explain quercetin's effect on such a wide number of enzymes.

Quercetin and many other flavonoids have been shown to be potent inhibitors of mast cell and basophil degranulation.[108–111] A generally accepted hypothesis for this action is that quercetin inhibits receptor-mediated calcium influx, thereby inhibiting the primary signal for degranulation. However, quercetin is also active under conditions in which the calcium channel mechanism is not operative, indicating that other mechanisms are responsible as well.

Quercetin has been shown to inhibit many of the inflammatory processes attributed to activated neutrophils.[112] This is probably due to its membrane-stabilizing action, potent antioxidant effect (which prevents the production of free radicals and inflammatory leukotrienes), and inhibition of the enzyme hyaluronidase (thus preventing the breakdown of the collagen matrix of connective tissue and ground substance). Quercetin's membrane-stabilizing effect could also account for its action in preventing mast cell and basophil degranulation. The effect also inhibits inflammation by decreasing neutrophil lysosomal enzyme secretion.[112] (Neutrophils and monocytes contain lysosomes that, on secretion of their contents, contribute greatly to the inflammatory process.)

Quercetin has been shown to inhibit many steps in eicosanoid metabolism. Probably of most significance in IBD is its inhibition of phospholipase A_2 and lipoxygenase enzymes.[105,106,113] The net result is a significant reduction in the formation of leukotrienes. Excessive leukotriene formation has been linked to asthma, psoriasis, atopic dermatitis, gout, and possibly cancer as well as IBD.[114,115] The leukotrienes C_4, D_4, and E_4 (composing the slow-reacting substances of

anaphylaxis [SRS-A]) are derived from arachidonic acid and are 1000 times as potent as histamine in promoting inflammation. Leukotrienes promote inflammation by causing vasoconstriction (thereby increasing vascular permeability) and other smooth muscle contraction and by promoting white blood cell (WBC) chemotaxis and aggregation. The reduction of leukotriene formation has significant anti-inflammatory effects, particularly in IBD. (For more information on quercetin, see Chapter 81). Despite the overwhelming evidence supporting the role of quercetin in preventing and improving inflammation in IBD, only three of six animal model–based studies showed a positive effect. Authors have speculated that the methylation of quercetin in the gut is variable in IBD, which could explain the inconsistent results observed thus far. Clinical trials in humans are thus far lacking, so no conclusions can be drawn.

Aloe Vera

Aloe vera gel has a dose-dependent inhibitory effect on the production of the reactive oxygen metabolites prostaglandin E2 and (at high doses) interleukin (IL)-8 by human colonic epithelial cells grown in tissue culture.[116] When administered via the oral route, aloe vera gel at 100 mL twice a day for 4 weeks produced a clinical response significantly more often than placebo (response ratio 5.6) in patients with UC.[117] Remission occurred in 30% of patients taking aloe vera gel and 7% of those receiving a placebo. In this clinical trial, aloe also reduced histological disease activity, whereas placebo did not. No significant side effects were described in the aforementioned trials on aloe vera for UC, although it should be noted that aloe vera gel is often used as a laxative. Acemannan, an extract of aloe vera, concentrated to a mucopolysaccharide (MPS) concentration of 30% of solid weight, has been demonstrated to reduce symptoms and indices of inflammation in controlled studies of patients with UC.[118]

Bastyr Formula (Modified Robert Formula)

Although no research has been done to document its efficacy, an old naturopathic remedy, the Robert formula or its modification (Bastyr formula), has a long history of use in IBD (Box 186.2). It is composed of the following botanical medicines:

- *Althea officinalis* (also known as marshmallow root)—a demulcent with soothing effects on the mucous membranes
- *Baptisia tinctora* (also known as wild indigo)—used for gastrointestinal infections
- *Echinacea angustifolia* (also known as purple coneflower)—antibacterial and used to promote normalization of the immune system (see Chapter 75)
- *Geranium maculatum*—a gastrointestinal hemostatic
- *Hydrastis canadensis* (also known as goldenseal)—inhibits the growth of many enteropathic bacteria (see Chapter 86)
- *Phytolacca americana* (also known as poke root)—used for healing ulcerations of the intestinal mucosa
- *Symphytum offinale* (also known as comfrey)—anti-inflammatory and a promoter of tissue growth and wound healing
- *Ulmus fulva* (also known as slippery elm)—a demulcent

The cabbage powder is used because of its documented ability to heal gastrointestinal ulcers (see Chapter 207), pancreatin is used to assist in the digestive process (see Chapter 100), niacinamide is used for its anti-inflammatory effects, and duodenal substance is used because it also heals gastrointestinal ulcers.

Butyrate Enemas in Ulcerative Colitis

The short-chain fatty acids (SCFAs)—mainly acetate, propionate, and butyrate—arise in the colon as end products of bacterial carbohydrate fermentation. These SCFAs function as primary energy sources for the luminal colon cells, especially in the distal segments. Decreased levels or decreased utilization of SCFAs leading to impaired cellular energetics have been hypothesized to play a major role in UC (Fig. 186.5). On the basis of this hypothesis, several clinical trials have been conducted with enemas providing SCFAs (usually composed of either butyrate as a sole agent in concentrations ranging from 80 to 100 mmol/L or SCFA combinations composed of acetate 60 mmol/L, propionate 25 mmol/L, and butyrate 40 mmol/L; Table 186.7). Excellent preliminary results in pilot studies have led to a well-designed double-blind study.[73,119]

In this study, 47 patients with distal UC randomly received either a combination of SCFAs, butyrate, or a placebo in an enema form retained for 30 minutes twice daily.[73] The primary end point of the study was the disease activity index composed of stool frequency, rectal bleeding, mucosal appearance, and physician rating of disease activity as well as endoscopic examination. After 8 weeks of therapy, there was a drop in activity in all three groups, with the butyrate group showing the lowest disease activity. Endoscopic examination demonstrated less disease in the butyrate and SCFA combination groups. Complete remission occurred in 47% receiving SCFAs, 38% receiving butyrate, and 25% receiving placebo. This study and others indicate that butyrate and SCFA enemas may prove to be useful adjuncts in the treatment of UC. There is a total of four studies demonstrating improved healing of patients with left-sided UC that is refractory to standard medical therapy.

Prebiotics

Prebiotics are nondigestible food ingredients that stimulate the growth or modify the metabolic activity of intestinal bacterial species that have the potential to improve the health of their human host. The criteria associated with the notion that a food ingredient should be classified as a prebiotic are that it remain undigested and unabsorbed as it passes through the upper part of the gastrointestinal tract and that it be a selective substrate for the growth of specific strains of beneficial bacteria (usually lactobacilli or bifidobacteria) rather than for all colonic bacteria. Prebiotic food ingredients include bran, psyllium husk, resistant (high-amylose) starch, inulin (a polymer of fructofuranose), lactulose, and various natural or synthetic oligosaccharides, which consist of short-chain complexes of sucrose, galactose, fructose, glucose, maltose, or xylose. The best-known effect of prebiotics is to increase fecal water content, relieving constipation. Bacterial fermentation of prebiotics yields SCFAs like butyrate. Several studies have suggested benefits of various prebiotics for the treatment of patients with UC. For instance, when oat bran at 60 g/day (supplying 20 g of dietary fiber) was given to patients with UC, fecal butyrate was increased by 36%, and abdominal pain improved.[120] A dietary supplement containing fish oil and two types of indigestible carbohydrate, FOS and xanthan gum,

BOX 186.2 Composition of the Bastyr Formula

- Eight parts *Althea officinalis*
- Four parts *Baptisia tinctora*
- Eight parts *Echinacea angustifolia*
- Eight parts *Geranium maculatum*
- Eight parts *Hydrastis canadensis*
- Eight parts *Phytolacca americana*
- Eight parts *Ulmus fulva*
- Eight parts cabbage powder
- Two parts pancreatin
- One part niacinamide
- Two parts duodenal substance

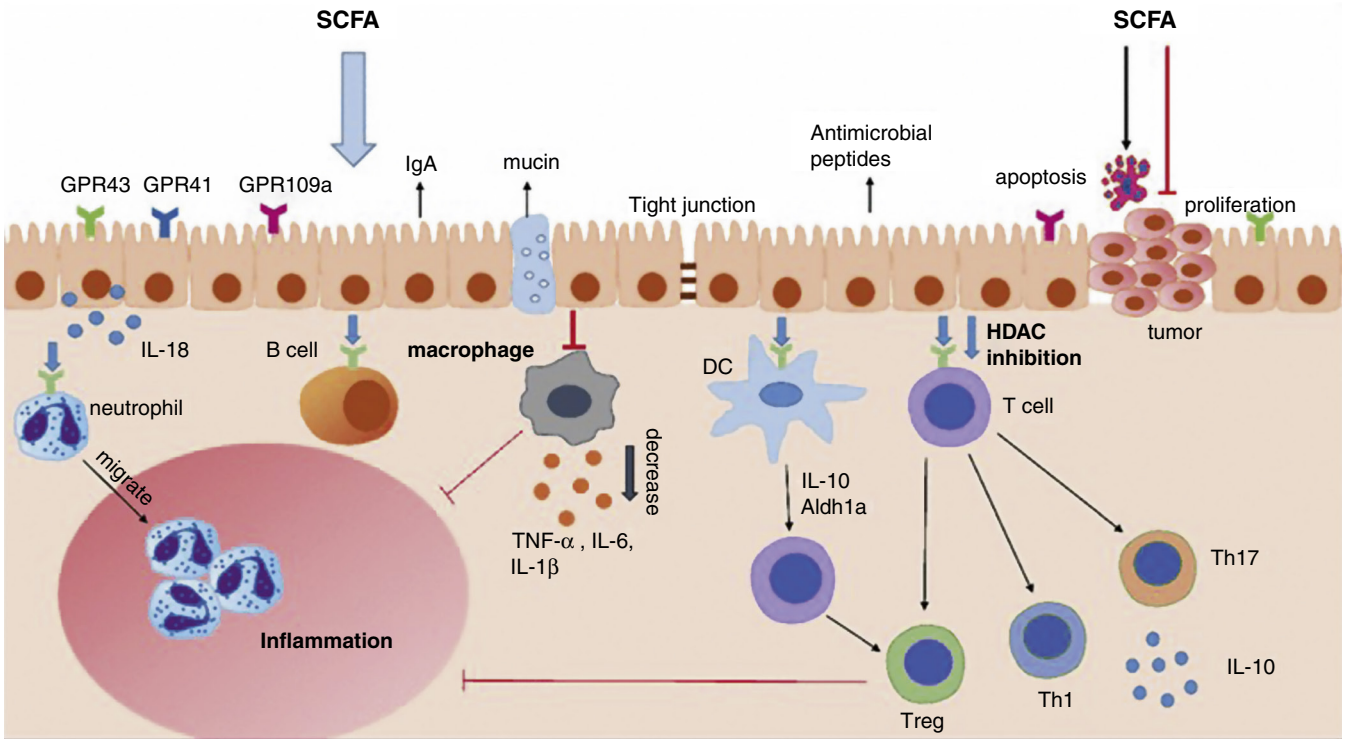


Fig. 186.5 Role of short-chain fatty acids (SCFAs) in the regulation of mucosal immune responses. SCFAs regulate intestinal mucosal immunity by exerting their effects on various immune cells. SCFAs regulate intestinal barrier integrity by inducing intestinal epithelial cell secretion of interleukin-18 (IL-18), antimicrobial peptides, and mucin and upregulating the expression of tight junctions. SCFAs induce neutrophil migration to inflammatory sites and enhance their phagocytosis ability. SCFAs regulate T-cell function not only through the GPCR pathway but also through the inhibition of histone deacetylases (HDAC). The differentiation of T cells is mediated both by SCFA regulation of dendritic cells (DCs) and the direct action of SCFAs on T cells. SCFAs regulate the generation of T-helper type-1 (Th1), T-helper type-17 (Th17), and regulatory T cells (Tregs) in different cytokine milieu. SCFAs also inhibit intestinal macrophage production of proinflamamtory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), through inhibition of HDAC, and possibly induce intestinal immunoglobulin A (IgA) production by B cells. Moreover, SCFAs inhibit carcinogenesis by promoting apoptosis and suppressing the proliferation of tumor cells. (Original figure published in Sun M, et al. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases.*J Gastroenterol*. 2017 Jan;52[1]:1–8. https://doi.org/10.1007/s00535-016-1242-9. Figure and text reproduced with permission by Springer Nature.)

TABLE 186.7 Clinical Trials of Butyrate Enemas for Left-Sided Ulcerative Colitis

Reference	Study Details	Condition	Outcome
156	RCT *N* = 35 100 mM Na-butyrate (*n* = 17) vs. saline (*n* = 18) × 20 days	UC in remission	IL-10/IL-12 increased. No clinical benefit for recurrence.
157	Open label *N* = 10 100 mM Na-butyrate × 6 weeks	Refractory proctosigmoiditis	60% improve, 40% remission
158	Open label *N* = 9 100 mM Na-butyrate +5-ASA × 2 weeks	Refractory proctosigmoiditis	7/9 histo/endo improvement
159	Single-blinded RCT *N* = 10 100 mM Na-butyrate or saline × 2 weeks, crossover	Refractory proctosigmoiditis	9/10 stool frequency, bleeding histology improved
160	DBRCT *N* = 40 40 mM Na-butyrate or saline × 6 weeks	Mild to moderate distal colitis	70% butyrate vs. 25% *P* improved clinically, urgency, bleeding
161	DBRCT *N* = 38 80 mM Na-butyrate or saline × 6 weeks	Moderate distal colitis	37% butyrate vs. 45% *P* improved clinically not significant
162	DBRCT *N* = 103 40 mM Na-butyrate enema BID or saline × 6 weeks	Distal UC	33% butyrate vs. 20% *P* improved NS, short-term colitis <6 mo improved $p < 0.03$, open-label extension 65% improved $p < 0.03$

DBRCT, Double-blind, randomized controlled trial; *IL,* interleukin; *NS,* not significant; *RCT,* randomized controlled trial; *UC,* ulcerative colitis.

TABLE 186.8 Summary of Clinical Trials of Probiotics for Inflammatory Bowel Disease

Disease	Design	*N*	Outcomes	Conclusions
CD	Post-Op Recurrence	6	1 benefit, 1 trend, 3 no benefit, 1 deterioration	Not recommended
CD	Induction	2	1 benefit, 1 no benefit	Not recommended
CD	Maintenance	9	0/6 Bacteria-based no benefit, 2 deterioration, 2/3 SCB possible benefit nonsmokers	Not recommended
UC	Induction	12	3/3 *Escherichia coli* Nissle 1917, 3/5 VSL#3 benefit, 2 others benefited; 8 overall	Recommended
UC	Maintenance	12	8 showed significant clinical benefit, 3/3 *E. coli* Nissle 1917	Recommended
Pouchitis	Induction	2	LGG no benefit, mixed *Lactobacillus/Bifidobacteria* benefit	Insufficient to recommend
Pouchitis	Secondary Prevention	2	1 of 2 benefit Gionchetti et al. 2003, $n = 40$	Insufficient to recommend
Pouchitis	Secondary Prevention	4	2 probiotics some benefit; 2 antibiotics-probiotics benefit	Recommended

CD, Chron's disease; *LGG,* Lactobacillus rhamnosus *GG; SCB,* Saccharomyces boulardii*; UC,* ulcerative colitis.

allowed a reduction of glucocorticoid dose as compared with a placebo in patients with steroid-dependent UC.[121] Other prebiotic trials for UC included a Japanese germinated barley foodstuff (GBF) containing hemicellulose-rich fiber; given at a dose of 20 to 30 g/day, it was found to increase stool butyrate concentration,[122] decrease the clinical activity index of patients with active disease,[123] and prolong remission in patients with inactive disease.[124] A mixture of *Bifidobacterium longum* and inulin-derived FOS administered for 1 month as monotherapy to patients with UC produced improvement in sigmoidoscopic appearance, histology, and several biochemical indices of tissue inflammation versus a placebo control.[125]

Probiotics in Inflammatory Bowel Disease

The term *probiotics* refers to beneficial bacteria and yeasts that can be administered orally to achieve a therapeutic benefit. The results of studies for the use of probiotics in IBD are summarized in Table 186.8. Overall, patients with UC do benefit from using probiotics during an active flare of their disease, and there is a benefit of probiotics for maintaining remissions. The studies to date have shown inconsistent results with all strains of probiotics for CD overall. However, supplementation with the beneficial yeast *Saccharomyces boulardii* is a safe and effective treatment for patients with CD, helping reduce diarrhea, intestinal inflammation, and the risk of relapse.[126,127] Common doses of *S. boulardii* are 250 mg three to four times a day. Likewise, supplementation with *Lactobacillus-GG* has proved to be of benefit in patients with CD; the customary dose is 10^{10} colony-forming units (CFUs) in enteric-coated tablets twice a day.[128,129]

THERAPEUTIC MONITORING AND EVALUATION

Fecal Calprotectin

Calprotectin is a protein secreted into the intestinal lumen in direct proportion to inflammation. Measurement of calprotectin in stool samples has been shown to be a sensitive and specific noninvasive assessment for inflammation in patients with IBD, and the test is helpful for distinguishing IBD from other, noninflammatory gastrointestinal conditions, such as irritable bowel syndrome.[130]

Crohn's Disease Activity Index

The CDAI was developed as a monitoring tool in the NCCDS.[131] It met the basic requirements necessary for the study—providing uniform clinical parameters that could be assessed and producing a consistent numerical index for recording the results of the study of several centers over several years.

In general, CDAI scores below 150 indicate a better prognosis than higher scores. The CDAI is a very useful way to monitor the progress of therapy.

BOX 186.3 Monitoring of the Pediatric Patient With Inflammatory Bowel Disease

History
- Appetite, extracurricular activities
- Type and duration of inflammatory bowel disease, frequency of relapses
- Severity and extent of ongoing symptoms
- Medication history

3-Day Diet Diary

Physical Examination
- Height, weight, arm circumference, triceps skinfold measurements
- Loss of subcutaneous fat, muscle wasting, edema, pallor, skin rash, hepatomegaly

Laboratory Tests
- Complete blood count and differential, reticulocyte and platelet counts, sedimentation rate, urinalysis
- Serum total proteins, albumin, globulin, and retinol-binding protein
- Serum electrolytes, calcium, phosphate, ferritin, folate, carotenes, tocopherol, and vitamin B_{12}
- Leukocyte ascorbate, magnesium, and zinc
- Creatinine height index, blood urea nitrogen/creatinine ratio

Monitoring of the Pediatric Patient

Pediatric patients with IBD present a particularly difficult problem in that it is often very difficult for them to achieve normal growth and development. Growth failure occurs in 75% of children with CD and in 25% of children with UC.[62] The pediatric patient with IBD should be evaluated at least twice yearly. Evaluation should include a pertinent history, clinical anthropometry, Tanner staging, and appropriate laboratory testing. Box 186.3 outlines the necessary components of a comprehensive, twice-yearly nutritional evaluation in pediatric patients with IBD. An aggressive nutritional program should be instituted, including supplements (it may be necessary to use enteral or parenteral methods in some patients), which is similar to the approach outlined for the adult patient, with the doses adjusted as appropriate.

The CDAI is not as accurate in monitoring the disease in children as it is in adults. To overcome this shortcoming, Lloyd-Still and Green[132] devised a clinical scoring system for IBD in children. It is divided into five major divisions (the maximum score is in parentheses): general activity (10), physical examination and clinical complications (30), nutrition (20), radiography (15), and laboratory (25). An elevated score (i.e., scores in the 80s) represents good status, whereas scores in the 30s and 40s represent severe disease.

THERAPEUTIC APPROACH

It is important to recognize that in some patients, CD and UC are life-threatening diseases that at times require emergency treatment. A small percentage of patients who have severe colitis may experience severe exacerbations requiring hospitalization. This is more common in patients with UC, who typically present with a fever of 101°F or higher; profuse, constant, loose, bloody stools; anorexia; and apathy and prostration; and although abdominal signs may be normal, physical examination will uncover a distended abdomen, tympany, absence of bowel sounds, and even rebound tenderness.

For the typical patient, IBD is a chronic disease requiring long-term therapy and follow-up. The first step is to identify and remove all factors that may be initiating or aggravating the inflammatory reaction, such as food allergens and carrageenan. The patient is started on a diet that maximizes macronutrients and micronutrients while minimizing aggravating foods and nonfoods.

A broad-based, individualized nutritional supplementation plan is necessary for all patients with IBD. Particularly important are the nutrients zinc, magnesium, folic acid, and vitamin A. Nutritional supplements are used as appropriate to correct deficiencies, normalize the inflammatory process, and promote healing of the damaged mucosa. Botanical medicines are used to promote healing and normalize the intestinal flora.

Diet

All allergens—as well as wheat, corn, dairy products, and carrageenan-containing foods—should be eliminated. The diet should be high in complex carbohydrates and low in sugar and refined carbohydrates. Fiber is poorly tolerated in Crohn's disease with luminal narrowing, whereas complex carbohydrates are prebiotic for fostering healthy enteric flora and have been shown to be helpful for ulcerative colitis.[133]

Supplements

- Multivitamin and mineral supplements with trace mineral cofactors for antioxidant systems
- Magnesium: 200 mg/day. Higher dosages may be required.
- Zinc picolinate or carnosine: 37.5 mg of zinc L carnosine or 50 mg of picolinate. Please be cautioned that long-term use of zinc supplements can deplete the body's copper storage and lead to neurologic problems.
- Vitamin E: 400 to 800 IU/day mixed tocopherols
- Fish oil: 4,000-5,000 mg of combined EPA/DHA daily.
- Choose one of the following:
  - Quercetin: 400 mg 20 minutes before meals
  - Grape seed extract (>95% procyanidolic oligomers): 150 to 300 mg/day
  - Pine bark extract (>90% procyanidolic oligomers): 150 to 300 mg/day
- Prebiotics (inulin, fructose oligosaccharides, etc.): 5 g/day
- Short-chain fatty acid enemas (60 mL of 80–100 mmol/L) nightly for left-sided colitis

Botanical Medicines

- Curcumin from turmeric (*C. longa*): 1000 two to three times a day before meals
- *Boswellia* extract: equivalent to 400 mg boswellic acids three times a day
- Aloe vera: Choose one of the following:
  - Aloe vera gel: oral preparations can be consumed at a dose of 100 mL a day . Diarrhea is a potential side effect. The preparation needs to be a "latex-free" distilled preparation that is 100% pure aloe gel. Otherwise there is a risk of toxicity.
  - Aloe vera juice: a variety of different preparations types and concentrations make accurate dose recommendations difficult. They can be consumed orally as a beverage or tonic.
  - Acemannan: 400 to 800 mg/day
- Bastyr formula: 2 to 3 "00" capsules with each meal

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gajendran M, Loganathan P, Catinella AP, Hashash JG. A comprehensive review and update on Crohn's disease. *Dis Mon*. 2018;64(2):20–57.
2. Kamm MA. Rapid changes in epidemiology of inflammatory bowel disease. *Lancet*. 2018;390(10114):2741–2742.
3. Vegh Z, Kurti Z, Lakatos PL. Epidemiology of inflammatory bowel diseases from west to east. *J Dig Dis*. 2017;18(2):92–98.
4. Kappelman MD, Moore KR, Allen JK, Cook SF. Recent trends in the prevalence of Crohn's disease and ulcerative colitis in a commercially insured US population. *Dig Dis Sci*. 2013;58(2):519–525.
5. Doecke JD, Simms LA, Zhao ZZ, et al. Genetic susceptibility in IBD: overlap between ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis*. 2013;19(2):240–245.
6. Sokol H, Leducq V, Aschard H, et al. Fungal microbiota dysbiosis in IBD. *Gut*. 2017;66(6):1039–1048.
7. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. *Scand J Gastroenterol*. 2018;53(1):1–7.
8. Lee SH, Kwon JE, Cho ML. Immunological pathogenesis of inflammatory bowel disease. *Intest Res*. 2018;16(1):26–42.
9. Limketkai BN, Wolf A, Parian AM. Nutritional Interventions in the patient with inflammatory bowel disease. *Gastroenterol Clin North Am*. 2018;47(1):155–177.
10. Levi AJ. Diet in the management of Crohn's disease. *Gut*. 1985;26:985–988.
11. Jarnerot J, Jarnmark I, Nilsson K. Consumption of refined sugar by patients with Crohn's disease, ulcerative colitis, or irritable bowel syndrome. *Scand J Gastroenterol*. 1983;18:999–1002.
12. Mayberry JF, Rhodes J, Newcombe RG. Increased sugar consumption in Crohn's disease. *Digestion*. 1980;20:323–326.
13. Grimes DS. Refined carbohydrate, smooth-muscle spasm and diseases of the colon. *Lancet*. 1976;1:395–397.
14. Thornton JR, Emmett PM, Heaton KW. Diet and Crohn's disease: characteristics of the pre-illness diet. *Br Med J*. 1979;279:762–764.
15. Heaton KW, Thornton JR, Emmett PM. Treatment of Crohn's disease with an unrefined-carbohydrate, fiber-rich diet. *Br Med J*. 1979;279:764–766.
16. Harries AD, Danis V, Heatley RV, et al. Controlled trial of supplemented oral nutrition in Crohn's disease. *Lancet*. 1983;1:887–890.
17. Reif S, Klein I, Lubin F, et al. Pre-illness dietary factors in inflammatory bowel disease. *Gut*. 1997;40:754–760.
18. James AH. Breakfast and Crohn's disease. *Br Med J*. 1978;2:1715–1716.
19. Dietary and other risk factors of ulcerative colitis: a case-control study in Japan. Epidemiology group of the research committee of inflammatory bowel disease in Japan. *J Clin Gastroenterol*. 1994;19:166–171.
20. Persson PG, Ahlbom A, Hellers G. Diet and inflammatory bowel disease: a case-control study. *Epidemiology*. 1992;3:47–52.
21. O'Morain C, Segal AW, Levi AJ. Elemental diet as primary treatment of acute Crohn's disease: a controlled trial. *Br Med J*. 1984;288:1859–1862.
22. Axelsson C, Jarnum S. Assessment of the therapeutic value of an elemental diet in chronic inflammatory bowel disease. *Scand J Gastroenterol*. 1977;12:89–95.
23. Voitk AJ, Echave V, Feller JH, et al. Experience with elemental diet in the treatment of inflammatory bowel disease: is this primary therapy? *Arch Surg*. 1973;107:329–333.
24. Borok G, Segal I. Inflammatory bowel disease: individualized dietary therapy. *S Afr Fam Pract*. 1995;16:393–399.
25. Jones VA, Workman E, Freeman AH, et al. Crohn's disease: maintenance of remission by diet. *Lancet*. 1985;2:177–180.
26. Shoda R, Matsueda K, Yamato S, et al. Epidemiologic analysis of Crohn's disease in Japan: increased dietary intake of w-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn's disease in Japan. *Am J Clin Nutr*. 1996;63:741–745.
27. Collins SM. Stress and the gastrointestinal tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G315–G318.
28. Thornton JR, Emmett PM, Heaton KW. Diet and ulcerative colitis. *Br Med J*. 1980;280:293–294.
29. Meyers S, Janowitz HD. "Natural history" of Crohn's disease: an analytical review of the placebo lesson. *Gastroenterology*. 1984;87:1189–1192.
30. Mekhjian HS, Switz DM, Melnyk CS, et al. Clinical features and natural history of Crohn's disease. *Gastroenterology*. 1979;77:898–906.
31. Malchow H, Ewe K, Brandes JW, et al. European cooperative Crohn's disease study (ECCDS): results of drug treatment. *Gastroenterol*. 1984;86:249–266.
32. Donowitz M. Arachidonic acid metabolites and their role in inflammatory bowel disease. *Gastroenterol*. 1985;88:580–587.
33. Ford-Hutchinson AW. Leukotrienes: their formation and role as inflammatory mediators. *Fed Proc*. 1985;44:25–29.
34. Sharon P, Stenson WF. Enhanced synthesis of leukotriene B4 by colonic mucosa in inflammatory bowel disease. *Gastroenterology*. 1984;86:453–460.
35. Musch MW, Miller RJ, Field M, et al. Stimulation of colonic secretion by lipoxygenase metabolites of arachidonic acid. *Science*. 1982;217:1255–1256.
36. Belluzzi A, Brignola C, Compieri M, et al. Effect of an enteric-coated fish oil preparation on relapses in Crohn's disease. *N Engl J Med*. 1996;334:1557–1560.
37. Loeschke K, Ueberschaer B, Pietsch A, et al. n-3 fatty acids only delay early relapse of ulcerative colitis in remission. *Dig Dis Sci*. 1996;41:2087–2094.
38. Feagan BG, Sandborn WJ, Mittmann U, et al. Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC randomized controlled trials. *JAMA*. 2008;299(14):1690–1697.
39. Mantzioris E, James MJ, Gibson RA, et al. Dietary substitution with alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am J Clin Nutr*. 1994;59:1304–1309.
40. Podolsky DK, Isselbacher KJ. Glycoprotein composition of colonic mucosa: specific alterations in ulcerative colitis. *Gastroenterol*. 1984;87:991–998.
41. Kim YS, Byrd JC. Ulcerative colitis: a specific mucin defect? *Gastroenterol*. 1984;87:1193–1195.
42. Boland CR, Lance P, Levin B, et al. Abnormal goblet cell glycoconjugates in rectal biopsies associated with an increased risk of neoplasia in patients with ulcerative colitis: early results of a prospective study. *Gut*. 1984;25:1364–1371.
43. Hentges DJ, ed. *Human Intestinal Microflora in Health and Disease.* New York: Academic Press; 1983:3–31.
44. Van de Merwe JP. The human faecal flora and Crohn's disease. *Ant Van Leeuwenhoek*. 1984;50:691–700.
45. Marcus R, Watt J. Seaweeds and ulcerative colitis in laboratory animals. *Lancet*. 1969;2:489–490.
46. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2018;390(10114):2769–2778.
47. Grasso P, Sharratt M, Carpanini FMB, et al. Studies on carrageenan and large bowel ulceration in mammals. *Food Cosmet Toxicol*. 1973;11:555–564.
48. Mottet NK. On animal models for inflammatory bowel disease [editorial]. *Gastroenterology*. 1972;62:1269–1271.
49. Bentiz KR, Goldberg L, Coulston F. Intestinal effect of carrageenans in the rhesus monkey (*Macaca mulatta*). *Food Cosmet Toxicol*. 1973;11:565–575.
50. Bonfils S. Carrageenan and the human gut. *Lancet*. 1970;2:414.
51. Hilsden RJ, Meddings JB, Sutherland LR. Intestinal permeability changes in response to acetylsalicylic acid in relatives of patients with Crohn's disease. *Gastroenterology*. 1996;110:1395–1403.
52. Aoki K. A study of endotoxemia in ulcerative colitis and Crohn's disease. I. Clinical study. *Acta Med Okayama*. 1978;32:147–158.
53. Aoki K. A study of endotoxemia in ulcerative colitis and Crohn's disease. II. Experimental study. *Acta Med Okayama*. 1978;32:297–316.
54. Wellman W, Fink PC, Schmidt FW. Whole-gut irrigation as antiendotoxinaemic therapy in inflammatory bowel disease. *Hepatogastroenterology*. 1984;31:91–93.
55. Allan RN. Extra-intestinal manifestations of inflammatory bowel disease. *Clin Gastroenterol*. 1983;12:617–632.
56. Pettei MJ, Davidson M. Extra gastrointestinal manifestations of inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 1985;4:689–691.
57. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. *Drugs*. 2001;61:2035–2063.

58. Hikino H, Kiso Y, Wagner H, et al. Antihepatotoxic actions of flavanolignins from Silybum marianum fruits. *Planta Medica*. 1984;50:248–250.
59. Kiso Y, Suzuki Y, Watanabe N, et al. Antihepatotoxic principles of Curcuma longa rhizomes. *Planta Medica*. 1983;49:185–187.
60. Rosenberg IH, Bengoa JM, Sitrin MD. Nutritional aspects of inflammatory bowel disease. *Annu Rev Nutr*. 1985;5:463–484.
61. Heatley HV. Nutritional implications of inflammatory bowel disease. *Scand J Gastroenterol*. 1984;19:995–998.
62. Motil KJ, Grand RJ. Nutritional management of inflammatory bowel disease. *Pediatr Clin North Am*. 1985;32:447–469.
63. Gee MI, Grace MG, Wensel RH, et al. Nutritional status of gastroenterology outpatients: comparison of inflammatory bowel disease with functional disorders. *J Am Diet Assoc*. 1985;85:1591–1599.
64. Fleming CR, Huizenga KA, McCall JT, et al. Zinc nutrition in Crohn's disease. *Dig Dis Sci*. 1981;26:865–870.
65. Kruis W, Rindfleisch GE, Weinzierl M. Zinc deficiency as a problem in patients with Crohn's disease and fistula formation. *Hepatogastroenterology*. 1985;32:133–134.
66. Schoelmerich J, Becher MS, Hoppe-Seyler P, et al. Zinc and vitamin A deficiency in patients with Crohn's disease is correlated with activity but not with localization or extent of the disease. *Hepatogastroenterology*. 1985;32:34–38.
67. Main AN, Hall MJ, Russell RI, et al. Clinical experience of zinc supplementation during intravenous nutrition in Crohn's disease: value of serum and urine zinc measurements. *Gut*. 1982;23:984–991.
68. Boosalis MG, Evans GW, McClain CJ. Impaired handling of oral zinc in pancreatic insufficiency. *Am J Clin Nutr*. 1983;37:268–271.
69. LaSala MA, Lifshitz F, Silverberg, et al. Magnesium metabolism studies in children with chronic inflammatory disease of the bowel. *J Ped Gastroenterol Nutr*. 1985;4:75–81.
70. Nyhlin H, Dyckner T, Ek B, Wester PO. Magnesium in Crohn's disease. *Acta Med Scand Suppl*. 1982;661:21–25.
71. Ward CG. Influence of iron on infection. *Am J Surg*. 1986;151:291–295.
72. Lehr L, Schober O, Hundeshagen H, et al. Total body potassium depletion and the need for preoperative nutritional support in Crohn's disease. *Ann Surg*. 1982;196:709–714.
73. Skogh M, Sunquist T, Taggeson C. Vitamin A in Crohn's disease. *Lancet*. 1980;1:766.
74. Dvorak AM. Vitamin A in Crohn's disease. *Lancet*. 1980;1:1303–1304.
75. Wright JP, Mee AS, Parfitt A, et al. Vitamin A therapy in patients with Crohn's disease. *Gastroenterology*. 1985;88:512–514.
76. Norrby S, Sjodahl R, Taggeson C. Ineffectiveness of vitamin A therapy in severe Crohn's disease. *Acta Chir Scand*. 1985;151:465–468.
77. Harries AD, Brown R, Heatley RV, et al. Vitamin D status in Crohn's disease: association with nutrition and disease activity. *Gut*. 1985;26:1197–1203.
78. Howard L, Ovesen L, Satya-Murti S, et al. Reversible neurological symptoms caused by vitamin E deficiency in a patient with short bowel syndrome. *Am J Clin Nutr*. 1982;36:1243–1249.
79. Scheppach W. Treatment of distal ulcerative colitis with short-chain fatty acid enemas: a placebo-controlled trial. *German-Austrian SCFA Study Group Dig Dis Sci*. 1996;41:2254–2259.
80. Krasinski SD, Russell RM, Furie BC, et al. The prevalence of vitamin K deficiency in chronic gastrointestinal disorders. *Am J Clin Nutr*. 1985;41:639–643.
81. Vitamin K deficiency in chronic gastrointestinal disorders. *Nutr Review*. 1986;44:10–12.
82. Schoon EJ, Muller MC, Vermeer C, et al. Low serum and bone vitamin K status in patients with longstanding Crohn's disease: another pathogenetic factor of osteoporosis in Crohn's disease? *Gut*. 2001;48:473–477.
83. Elsborg L, Larsen L. Folate deficiency in chronic inflammatory bowel diseases. *Scand J Gastroenterol*. 1979;14:1019–1024.
84. Hellberg R, Hulten L, Bjorn-Rasmussen E. The nutritional and haematological status before and after primary and subsequent resectional procedures for classical Crohn's disease and Crohn's colitis. *Acta Chir Scand*. 1982;148:453–460.
85. Franklin JL, Rosenberg IH. Impaired folic acid absorption in inflammatory bowel disease: effects of salicylazosulfapyridine (Azulfidine). *Gastroenterology*. 1973;64:517–525.
86. Carruthers LB. Chronic diarrhea treated with folic acid. *Lancet*. 1946;1:849–850.
87. Filipsson S, Hulten L, Lindstedt G. Malabsorption of fat and vitamin B12 before and after intestinal resection for Crohn's disease. *Scand J Gastroenterol*. 1978;13:529–536.
88. Hughes RG, Williams N. Leukocyte ascorbic acid in Crohn's disease. *Digestion*. 1978;17:272–274.
89. Gerson CD, Fabry EM. Ascorbic acid deficiency and fistula formation in regional enteritis. *Gastroenterology*. 1974;67:428–433.
90. Lih-Brody L, Powell SR, Collier KP, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci*. 1996;41:2078–2086.
91. Jobin C, Bradham CA, Russo MP, et al. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. *J Immunol*. 1999;163(6):3474–3483.
92. Duvoix A, Blasius R, Delhalle S, et al. Chemopreventive and therapeutic effects of curcumin. *Cancer Lett*. 2005;223(2):181–190.
93. Sugimoto K, Hanai H, Tozawa K, et al. Curcumin prevents and ameliorates trinitrobenzene sulfonic acid-induced colitis in mice. *Gastroenterology*. 2002;123(6):1912–1922.
94. Kim H, Kong H, Choi B, et al. Metabolic and pharmacological properties of rutin, a dietary quercetin glycoside, for treatment of inflammatory bowel disease. *Pharm Res*. 2005;22(9):1499–1509.
95. Deguchi Y, Andoh A, Inatomi O, et al. Curcumin prevents the development of dextran sulfate sodium (dss)-induced experimental colitis. *Dig Dis Sci*. 2007;52:2993–2998.
96. Jian YT, Mai GF, Wang JD, et al. Preventive and therapeutic effects of NF-kappaB inhibitor curcumin in rats colitis induced by trinitrobenzene sulfonic acid. *World J Gastroenterol*. 2005;11(12):1747–1752.
97. Zhang M, Deng C, Zheng J, et al. Curcumin inhibits trinitrobenzene sulphonic acid-induced colitis in rats by activation of peroxisome proliferator-activated receptor gamma. *Int Immunopharmacol*. 2006;6(8):1233–1242.
98. Sugimoto K, Hanai H, Tozawa K, et al. Curcumin prevents and ameliorates trinitrobenzene sulfonic acid-induced colitis in mice. *Gastroenterology*. 2002;123(6):1912–1922.
99. Holt PR, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. *Dig Dis Sci*. 2005;50(11):2191–2193.
100. Hanai H, Iida T, Takeuchi K, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol*. 2006;4(12):1502–1506.
101. Ammon HPH. Boswellic acids (components of frankincense) as the active principle in treatment of chronic inflammatory diseases. *Wien Med Wochenschr*. 2002;152(15–16):373–378.
102. Gupta I, Parihar A, Malhotra P, et al. Effects of gum resin of Boswellia serrata in patients with chronic colitis. *Planta Med*. 2001;67(5):391–395.
103. Gupta I, Parihar A, Malhotra P, et al. Effects of Boswellia serrata gum resin in patients with ulcerative colitis. *Eur J Med Res*. 1997;2(1):37–43.
104. Gerhardt H, Seifert F, Buvari P, et al. Therapy of active Crohn disease with Boswellia serrata extract H 15. *Z Gastroenterol*. 2001;39(1):11–17.
105. Middleton E. The flavonoids. *Trends Pharmaceut Sci*. 1984;5:335–338.
106. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol*. 1983;32:1141–1148.
107. Nishino H, Naitoh E, Iwashima A, et al. Quercetin interacts with calmodulin, a calcium regulatory protein. *Experientia*. 1984;40:184–185.
108. Middleton E, Drzewieki G. Flavonoid inhibition of human basophil histamine release stimulated by various agents. *Biochem Pharmacol*. 1984;33:3333–3338.
109. Middleton E, Drzewieki G. Naturally occurring flavonoids and human basophil histamine release. *Int Arch Allergy Appl Immunol*. 1985;77:155–157.
110. Amella M, Bronner C, Briancon F, et al. Inhibition of mast cell histamine release by flavonoids and bioflavonoids. *Planta Med*. 1985;1:16–20.
111. Pearce FL, Befus AD, Bienenstock J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J Allergy Clin Immunol*. 1984;73:819–823.
112. Busse WW, Kopp DE, Middleton E. Flavonoid modulation of human neutrophil function. *J Allergy Clin Immunol*. 1984;73:801–809.

113. Yoshimoto T, Furukawa M, Yamamoto S, et al. Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochem Biophys Res Common*. 1983;116:612–618.
114. Ford-Hutchinson AW. Leukotrienes: their formation and role as inflammatory mediators. *Fed Proc*. 1985;44:25–29.
115. Ford-Hutchinson AW. *J Allergy Clin Immunol*. 1984;74:437–440.
116. Langmead L, Makins RJ, Rampton DS. Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. *Aliment Pharmacol Ther*. 2004;19(5):521–527.
117. Langmead L, Feakins RM, Goldthorpe S, et al. Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. *Aliment Pharmacol Ther*. 2004;19(7):739–747.
118. Robinson M. Medical therapy of inflammatory bowel disease for the 21st century. *Eur J Surg Suppl*. 1998;582:90–98.
119. Steinhart AH, Brzezinski A, Baker JP. Treatment of refractory ulcerative proctosigmoiditis with butyrate enemas. *Am J Gastroenterol*. 1994;89:179–183.
120. Hallert C, Bjorck I, Nyman M, et al. Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. *Inflamm Bowel Dis*. 2003;9(2):116–121.
121. Seidner DLH, Lashner BAH, Brzezinski AH, et al. An oral supplement enriched with fish oil, soluble fiber, and antioxidants for corticosteroid sparing in ulcerative colitis: a randomized, controlled trial. *Clin Gastroenterol Hepatol*. 2005;3(4):358–369.
122. Bamba T, Kanauchi O, Andoh A, et al. A new prebiotic from germinated barley for nutraceutical treatment of ulcerative colitis. *J Gastroenterol Hepatol*. 2002;17(8):818–824.
123. Kanauchi O, Mitsuyama K, Homma T, et al. Treatment of ulcerative colitis patients by long-term administration of germinated barley foodstuff: multi-center open trial. *Int J Mol Med*. 2003;12(5):701–704.
124. Hanai H, Kanauchi O, Mitsuyama K, et al. Germinated barley foodstuff prolongs remission in patients with ulcerative colitis. *Int J Mol Med*. 2004;13(5):643–647.
125. Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*. 2005;54(2):242–249.
126. Plein K, Hotz J. Therapeutic effects of *Saccharomyces boulardii* on mild residual symptoms in a stable phase of Crohn's disease with special respect to chronic diarrhea: a pilot study. *Z Gastroenterol*. 1993;31:129–134.
127. Guslandi M, Mezzi G, Sorghi M, et al. *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. *Dig Dis Sci*. 2000;45:1462–1464.
128. Guandalini S. Use of *Lactobacillus*-GG in paediatric Crohn's disease. *Dig Liver Dis*. 2002;34(suppl 2):S63–S65.
129. Gupta P, Andrew H, Kirschner BS, et al. Is *Lactobacillus* GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. *J Pediatr Gastroenterol Nutr*. 2000;31:453–457.
130. Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. *World J Gastroenterol*. 2001;7:460–465.
131. Best WR, Becktel JM, Singleton JW, et al. Development of a Crohn's disease activity index. *Gastroenterology*. 1976;70:439–444.
132. Lloyd-Still J, Green OC. A clinical scoring system for chronic inflammatory bowel disease in children. *Dig Dis Sci*. 1979;24:620–624.
133. Kanauchi O, Mitsuyama K, Andoh A. The therapeutic impact of manipulating microbiota in inflammatory bowel disease. *Curr Pharm Des*. 2009;15(18):2074–2086.
134. Zhou M, et al. New frontiers in genetics, gut microbiota, and immunity: a Rosetta stone for the pathogenesis of inflammatory bowel disease. *Biomed Res Int*. 2017;2017:8201672.
135. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J*. 2014;13:61.
136. Sun M, et al. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *J Gastroenterol*. 2017;52(1):1–8.
137. Jantchou P, et al. Animal protein intake and risk of inflammatory bowel disease: the E3N prospective study. *Am J Gastroenterol*. 2010;105(10):2195–2201.
138. Ananthakrishnan AN, et al. High school diet and risk of Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis*. 2015;21(10):2311–2319.
139. Jarnerot G, Jarnmark I, Nilsson K. Consumption of refined sugar by patients with Crohn's disease, ulcerative colitis, or irritable bowel syndrome. *Scand J Gastroenterol*. 1983;18(8):999–1002.
140. Ananthakrishnan AN, et al. Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. *Gut*. 2014;63(5):776–784.
141. Hou JK, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol*. 2011;106(4):563–573.
142. Chan SS, et al. Association between high dietary intake of the n-3 polyunsaturated fatty acid docosahexaenoic acid and reduced risk of Crohn's disease. *Aliment Pharmacol Ther*. 2014;39(8):834–842.
143. Brown AC, Roy M. Does evidence exist to include dietary therapy in the treatment of Crohn's disease? *Expert Rev Gastroenterol Hepatol*. 2010;4(2):191–215.
144. Brown AC, Rampertab SD, Mullin GE. Existing dietary guidelines for Crohn's disease and ulcerative colitis. *Expert Rev Gastroenterol Hepatol*. 2011;5(3):411–425.
145. Yamamoto T, et al. Enteral nutrition for the maintenance of remission in Crohn's disease: a systematic review. *Eur J Gastroenterol Hepatol*. 2010;22(1):1–8.
146. Suskind DL, et al. Nutritional therapy in pediatric Crohn disease: the specific carbohydrate diet. *J Pediatr Gastroenterol Nutr*. 2014;58(1):87–91.
147. Cohen SA, et al. Clinical and mucosal improvement with specific carbohydrate diet in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr*. 2014;59(4):516–521.
148. Gearry RB, et al. Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease-a pilot study. *J Crohns Colitis*. 2009;3(1):8–14.
149. Prince AC, et al. Fermentable carbohydrate restriction (low FODMAP diet) in clinical Practice improves functional gastrointestinal symptoms in patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2016;22(5):1129–1136.
150. Singla V, et al. Induction with NCB-02 (curcumin) enema for mild-to-moderate distal ulcerative colitis - a randomized, placebo-controlled, pilot study. *J Crohns Colitis*. 2014;8(3):208–214.
151. Hanai H, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol*. 2006;4(12):1502–1506.
152. Lang A, et al. Curcumin in combination with Mesalamine induces remission in patients with mild-to-moderate ulcerative colitis in a randomized controlled trial. *Clin Gastroenterol Hepatol*. 2015;13(8):1444–1449 e1.
153. Gupta I, et al. Effects of gum resin of *Boswellia serrata* in patients with chronic colitis. *Planta Med*. 2001;67(5):391–395.
154. Gupta I, et al. Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis. *Eur J Med Res*. 1997;2(1):37–43.
155. Holtmeier W, et al. Randomized, placebo-controlled, double-blind trial of *Boswellia serrata* in maintaining remission of Crohn's disease: good safety profile but lack of efficacy. *Inflamm Bowel Dis*. 2011;17(2):573–582.
156. Hamer HM, et al. Effect of butyrate enemas on inflammation and antioxidant status in the colonic mucosa of patients with ulcerative colitis in remission. *Clin Nutr*. 2010;29(6):738–744.
157. Steinhart AH, Brzezinski A, Baker JP. Treatment of refractory ulcerative proctosigmoiditis with butyrate enemas. *Am J Gastroenterol*. 1994;89(2):179–183.
158. Hove H, Mortensen PB. Influence of intestinal inflammation (IBD) and small and large bowel length on fecal short-chain fatty acids and lactate. *Dig Dis Sci*. 1995;40(6):1372–1380.
159. Scheppach W, et al. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology*. 1992;103(1):51–56.
160. Vernia P, et al. Short-chain fatty acid topical treatment in distal ulcerative colitis. *Aliment Pharmacol Ther*. 1995;9(3):309–313.
161. Steinhart AH, et al. Treatment of left-sided ulcerative colitis with butyrate enemas: a controlled trial. *Aliment Pharmacol Ther*. 1996;10(5):729–736.
162. Jorgensen JR, Clausen MR, Mortensen PB. Oxidation of short and medium chain C2-C8 fatty acids in Sprague-Dawley rat colonocytes. *Gut*. 1997;40(3):400–405.

Insomnia and Other Sleep Disorders

John Nowicki, ND, and *Michael T. Murray, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Difficulty falling asleep (sleep-onset insomnia)
- Frequent or early awakening (sleep-maintenance insomnia)
- Early wakening
- Daytime symptoms such as fatigue, cognitive problems, mood disorders, and impaired psychomotor performance
- Affects one of every four people in the United States

GENERAL CONSIDERATIONS

Insomnia is one of the most common complaints seen by physicians. Within the course of a year, up to 30% of the population suffers from insomnia, and roughly 10% of the adult population has chronic insomnia (Fig. 187.1).[1] Many use over-the-counter (OTC) medications to combat the problem, and others seek stronger sedatives. Approximately 12.5% of the adult population uses a prescribed anxiolytic or sedative-hypnotic in the course of a year; about 2% of the population takes one on any given day. More than one half of these drugs, especially benzodiazepines, are prescribed by primary care physicians. Nearly 100 million prescriptions are written each year for these drugs.[2]

A thorough history and physical examination are indicated in the patient presenting with insomnia because it is a symptom that can have many causes (Table 187.1). Psychological factors account for 50% of all insomnias evaluated in sleep laboratories.[1] Insomnia is closely associated with affective disorders (see Chapter 142), and cognitive-behavioral therapy can produce effective improvements in sleep quality.[3] A detailed recreational, prescription, and nonprescription drug-use history, along with a dietary and beverage history, are also required to determine whether the patient is consuming any agents known to interfere with sleep. Examples are shown in Table 187.2. The listing of marijuana may seem confusing because users report improved sleep with use. However, this improved sleep appears primarily with occasional use. Chronic use has been consistently shown to increase sleep disorders. The mechanism is likely similar to that seen with alcohol—rebound and withdrawal.[4]

Sleep apnea is the most common example of sleep-disordered breathing. A high prevalence of insomnia has been observed in individuals with sleep apnea. First described in 1965, sleep apnea is a breathing disorder characterized by brief interruptions of breathing with subsequent hypoxia during sleep. These breathing pauses are almost always accompanied by snoring between apneic episodes, although not everyone who snores has this condition. Sleep apnea can also be characterized by choking sensations. Sleep apnea may happen several times an hour, ensuring the individual is never fully rested. The frequent interruptions of deep, restorative sleep often lead to excessive daytime sleepiness and may be associated with an early-morning headache. Approximately 18 million Americans are thought to suffer from sleep apnea.

Early recognition and treatment of sleep apnea are important because it is associated with marked daytime fatigue, irregular heartbeat, high blood pressure, heart attack, and stroke as well as memory loss and other intellectual capabilities. The patient usually does not know he or she has a problem and may not believe it when told. If a person snores heavily or if their sleep partner reports the individual has periods of interrupted breathing during sleep, it is important to consult a doctor. Sleep apnea should also be considered in anyone with significant daytime drowsiness or changes in intellectual function. Sleep apnea can be properly diagnosed through the services of a sleep disorder specialist, usually in a sleep laboratory. Home sleep studies are also available but require a sleep specialist for interpretation of the results.

Sleep apnea is most often caused by narrowing of the airway by pharyngeal structures, causing obstructive sleep apnea. With a narrowed airway, the person continues efforts to breathe, but air cannot easily flow into or out of the nose or mouth. This narrowing results in heavy snoring, periods of no breathing, and frequent arousals (causing abrupt changes from deep sleep to light sleep). Ingestion of alcohol and sleeping pills increases the frequency and duration of breathing pauses in people with sleep apnea. In some cases, sleep apnea occurs even if no airway obstruction or snoring is present. This form of sleep apnea, central sleep apnea, is caused by a loss of perfect control over breathing by the brain.

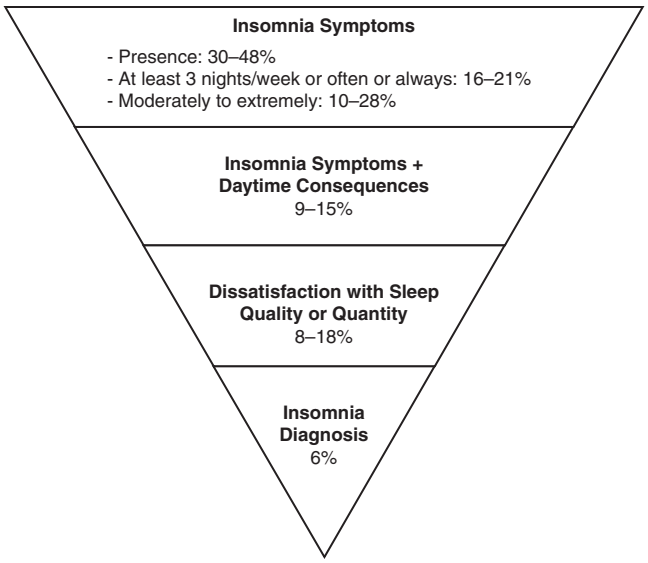


Fig. 187.1 Average prevalence of insomnia symptoms and diagnoses. (Maurice M.Ohayon, Epidemiology of insomnia: what we know and what we still need to learn, *Sleep Medicine Reviews*. 2002; 6(2): 97–111.)

TABLE 187.1 Causes of Insomnia[a]

Sleep-onset Insomnia	Sleep-maintenance Insomnia
Anxiety or tension	Depression
Environmental change	Environmental change
Emotional arousal	Sleep apnea
Fear of insomnia	Nocturnal myoclonus
Phobia of sleep	Hypoglycemia
Disruptive environment	Parasomnias
Pain or discomfort	Pain or discomfort
Caffeine	Drugs
Alcohol	Alcohol

[a]The boundary between the categories is not entirely distinct.

TABLE 187.2 Common Agents Known to Interfere With Sleep

- Alcohol
- Beta blockers
- Chocolate
- Coffee
- Marijuana
- Oral contraceptives
- Tea
- Thyroid preparations

In both obstructive and central sleep apnea, obesity is the major risk factor, and weight loss is the most important aspect of long-term management. People with sleep apnea experience periods of anoxia (oxygen deprivation of the brain) with each apneic episode, which ends in arousal and a reinitiation of breathing. Seldom does the sufferer awaken enough to be aware of the problem. However, the combination of frequent periods of oxygen deprivation (20 to several hundred times per night) and the greatly disturbed sleep can significantly diminish the sufferer's quality of life and lead to serious problems.[5]

The most common treatment of sleep apnea is the use of nasal continuous positive airway pressure (CPAP). In this procedure, the patient wears a mask over the nose during sleep, and pressure from an air blower forces air through the nasal passages. The air pressure is adjusted so that it is just enough to prevent the throat from collapsing during sleep. The pressure is constant and continuous. Nasal CPAP prevents airway closure while in use, but episodes of apnea return when CPAP is stopped or used improperly. Surgery to reduce soft tissue in the throat or soft palate should be used only as a last resort because it is often unsuccessful and may aggravate the condition. Laser-assisted uvulopalatoplasty is a highly promoted surgical option. In this procedure, lasers are used to surgically remove excessive soft tissue from the back of the throat and the palate. About 90% of sleep apnea sufferers receive benefit initially. However, within a year, many people regress or end up worse than before the procedure as a result of the formation of scar tissue.[5]

Normal Sleep Patterns

Human sleep is perhaps one of the least understood physiological processes. Its value to human health and proper functioning is without question. Sleep is absolutely essential to both the body and the mind. Impaired sleep, altered sleep patterns, and sleep deprivation impair mental and physical function.

Normal adult sleep–wake patterns repeat themselves on an approximately 24-hour cycle, of which sleep constitutes one third. Exactly how much sleep is required varies from one person to the next. Sleep tends to decrease with age, but whether this tendency is a normal or abnormal progression is unknown. A 1-year-old baby requires about 14 hours of sleep a day, a 5-year-old about 12 hours, and adults about 7 to 9 hours. Women tend to require more sleep than men. The elderly tend to sleep less at night but doze more during the day than do younger adults.

From observations of eye movement and electroencephalographic (EEG) recordings, sleep is divided into two distinct types: rapid eye movement (REM) sleep and non-REM sleep. During REM sleep, the eyes move rapidly and dreaming takes place. When people are awakened during non-REM sleep, they report that they were thinking about everyday matters but rarely report dreams.

Non-REM sleep is divided into stages 1 through 4 according to the level of EEG activity and ease of arousal. As sleep progresses, there is a deepening of sleep and slower brainwave activity until REM sleep ensues, when suddenly the brain becomes much more active. In adults, the first REM sleep cycle is usually triggered 90 minutes after going to sleep and lasts about 5 to 10 minutes. After the flurry of activity, brainwave patterns return to those of non-REM sleep for another 90-minute sleep cycle.

Each night, most adults experience five or more sleep cycles. REM sleep periods grow progressively longer as sleep continues; the last sleep cycle may produce a REM sleep period that can last about an hour. Non-REM sleep lasts approximately 50% of this 90-minute sleep cycle in infants and about 80% in adults. As people age, in addition to less REM sleep, they tend to awaken at the transition from non-REM to REM sleep.

The Importance of Adequate Sleep

Adequate sleep is absolutely necessary for long-term health and regeneration. Many physiological processes occur during sleep, but perhaps the most important are the increased secretion of growth hormone (GH) and the scavenging of free radicals in the brain.

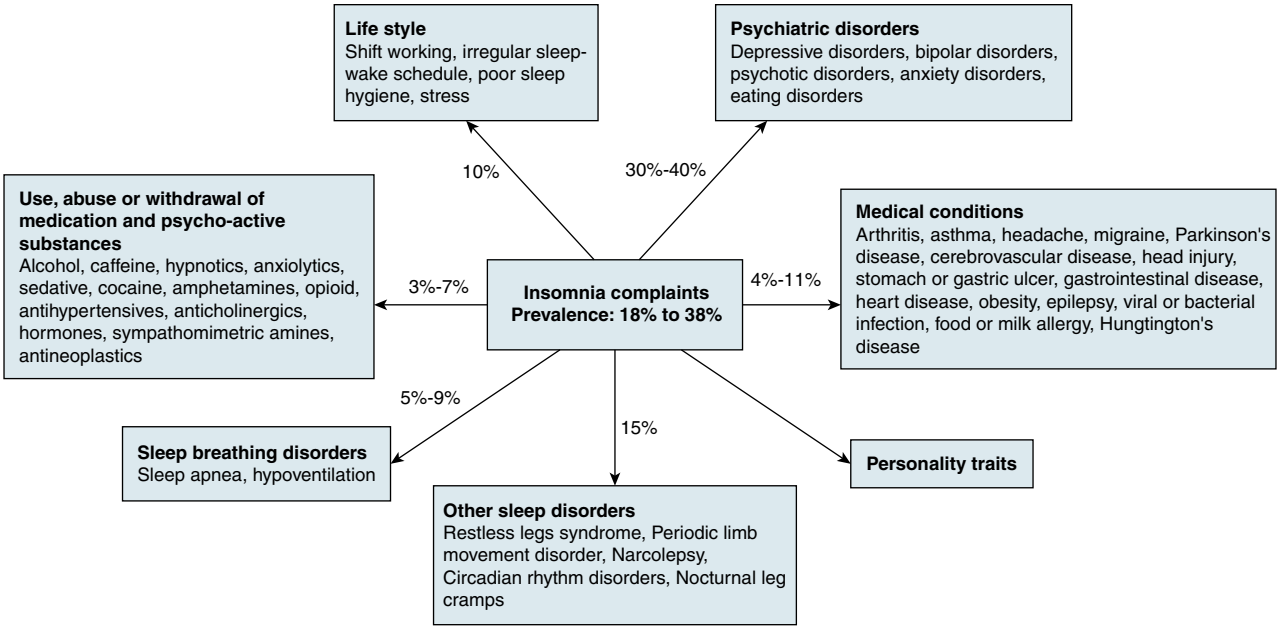


Fig. 187.2 Factors associated with insomnia. (From https://www.sleepeval.com/dyssomnia/impact.html.)

Many of the benefits of sleep are probably mediated through GH. As an anabolic hormone, GH has been called by some the "antiaging" hormone. GH stimulates tissue regeneration, liver regeneration, muscle building, breakdown of fat stores, normalization of blood sugar, and a host of other beneficial processes in the body. It also helps convert fat to muscle. Small amounts of GH are secreted at various times during the day, but essentially all secretion of GH occurs during sleep.

Sleep is required to ensure minimal neuronal damage from free radicals accumulated during waking. Most people can tolerate a few days without sleep and fully recover. However, chronic sleep deprivation accelerates aging of the brain, causes neuronal damage, and produces nighttime elevations in cortisol.[6]

THERAPEUTIC CONSIDERATIONS

Insomnia is initiated or maintained by several psychological and physiological factors, which should be addressed before inducing sleep pharmacologically (Fig. 187.2). Counseling and/or stress-reduction techniques (including biofeedback and hypnosis) may be indicated in many cases. The following topics are discussed in this chapter as they relate to the promotion of sleep:

- Exercise
- Progressive relaxation
- Nocturnal glucose levels
- Serotonin precursor and cofactor therapy
- 5-Hydroxytryptophan (5-HTP)
- Cofactors for serotonin synthesis
- Melatonin
- Restless legs syndrome and nocturnal myoclonus
- Botanicals with sedative properties

Lifestyle

Exercise

Regular physical exercise is known to improve general well-being and promote improvement in sleep quality.[3] Exercise should be performed in the morning or early evening, not before bedtime, and should be of moderate intensity. Usually 20 minutes of aerobic exercise at a heart rate between 60% and 75% of maximum (maximum heart rate is approximately 220 minus the patient's age in years) is sufficient.

Progressive Relaxation

Progressive relaxation is based on a simple procedure comparing tension with relaxation. Each muscle is first forcefully contracted for a period of 1 to 2 seconds and then relaxed. Because the procedure goes progressively through all the muscles of the body, a deep state of relaxation results. The procedure begins with contraction of the muscles of the face and neck, with the contraction held for at least 1 or 2 seconds. Next, the upper arms and chest are contracted and then relaxed, followed by the lower arms and hands. The process is repeated progressively down the body—abdomen, buttocks, thighs, calves, and feet. This whole practice is repeated two or three times or until sleep is produced.

Nocturnal Glucose Levels

Increased nocturnal blood glucose volatility may be an important cause of sleep-maintenance insomnia, especially when there are rapid drops in blood glucose levels. The brain is highly dependent on glucose as an energy substrate, and a quick drop in blood glucose level promotes awakening via the release of glucose regulatory hormones (e.g., epinephrine, glucagon, cortisol, and GH). Increased nocturnal blood glucose volatility and/or hypoglycemia must be ruled out in maintenance-type insomnia (see Chapter 181).

Serotonin Precursor and Cofactor Therapy

Serotonin is an important initiator of sleep. The synthesis of central nervous system (CNS) serotonin depends on the availability of tryptophan (discussed in more detail in Chapter 76, 5-HTP).

Tryptophan

L-Tryptophan has shown modest effects in the treatment of insomnia,[7–9] and although not every patient responded to L-tryptophan in clinical trials, those who have experienced dramatic relief. It is generally more effective in sleep-onset insomnia and less effective in sleep-maintenance insomnia. Advantages of L-tryptophan versus over-the-counter (OTC) and prescription pills include limited distortions

of normal sleep processes and no withdrawal symptoms. Doses smaller than 2000 mg are generally ineffective.

The sleep-promoting effect of L-tryptophan is believed to be due to enhanced serotonin synthesis, but there is evidence to suggest that other mechanisms may also be responsible or contributory, including L-tryptophan–enhanced melatonin synthesis. Administration of L-tryptophan causes a massive elevation of plasma melatonin concentration.[10] Although L-tryptophan reduces sleep latency, it exerts effects in normal subjects that are at odds with the serotonin system, such as reducing REM sleep and increasing non-REM sleep.[11,12] Drugs that prevent the conversion of tryptophan to serotonin enhance these effects. From this information, it is concluded that some of L-tryptophan's effects on sleep do not involve the serotonin or melatonin system. As discussed in Chapter 87, the effects of L-tryptophan can be negated by conversion via the kynurenine pathway. This conversion can be partially inhibited by niacin (30 mg is an appropriate dose), thereby enhancing the effects of L-tryptophan.

The insomnia-relieving and sleep-promoting actions of L-tryptophan appear to be cumulative, because it often takes a few nights for L-tryptophan to start working. In a double-blind study, the effects of 3 g of L-tryptophan on sleep performance, arousal threshold, and brain electrical activity during sleep were assessed in 20 males with chronic sleep-onset insomnia.[13] After a sleep laboratory screening night, all subjects received placebo for three consecutive nights; then 10 subjects received L-tryptophan and 10 received a placebo for six nights. All subjects received placebo on two withdrawal nights. L-Tryptophan had no effect on sleep latency during the first three nights of administration. However, on nights four through six of administration, sleep latency was significantly reduced. Consistent with other studies, this study found that unlike sleeping pills (benzodiazepines especially), L-tryptophan did not alter sleep stages, impair performance, or alter brain electrical activity during sleep. This study indicates that L-tryptophan should be used for a minimum of 1 week to gauge its effectiveness in chronic insomnia. Administration of high-dose L-tryptophan (4 g) during the day can promote sleep. This indicates that the consumption of foods high in tryptophan during the day may contribute to daytime sleepiness, and an evening meal high in tryptophan in relation to competing amino acids may promote sleep.

The important cofactors vitamin B_6, niacin, and magnesium should be administered along with the tryptophan to ensure its conversion to serotonin. Also, because other amino acids compete with tryptophan for transport into the CNS across the blood–brain barrier and insulin increases tryptophan uptake by the CNS, protein consumption should be avoided near administration, and a carbohydrate source such as fruit or fruit juice should accompany the tryptophan.

5-Hydroxytryptophan

5-HTP is one step closer to serotonin than L-tryptophan and does not depend on a transport system for entry into the brain. Several clinical studies have shown 5-HTP to produce dramatically better results than L-tryptophan in promoting and maintaining sleep.[14–17]

One of the key benefits of 5-HTP is its ability to increase REM sleep (typically by about 25%) while increasing deep sleep stages 3 and 4 without lengthening total sleep time.[11,12] The dosage recommendation of 5-HTP is 100 to 300 mg, 30 to 45 minutes before retiring. The patient should start with the lower dose for at least 3 days before increasing it. For more information, see Chapter 87.

Vitamin B Complex

B vitamins play a significant role in the manufacture of serotonin, and deficiencies can affect both sleep quality and sleep quantity. Niacin has been reported to have a sedative effect, probably owing to its peripheral dilating action and shunting of tryptophan metabolism toward serotonin synthesis. Cross-sectional data from the National Health and Nutrition Examination Surveys (NHANES) 2005–2006 found independent inverse associations between serum vitamin B_{12} and sleep duration as well as between folate and sleep disturbance.[18] A study of 87 adults aged 21 to 45 years found that insomniacs consumed significantly less energy, carbohydrates, folic acid, and vitamin B_{12} than normal sleepers.[19] Further, intakes of protein, fat, and thiamine were significantly different in insomniacs.

Magnesium

Magnesium is a cofactor in the synthesis of serotonin as well as an *N*-methyl-D-aspartic acid (NMDA) antagonist and gamma-aminobutyric acid (GABA) agonists and seems to play a key role in the regulation of sleep. One double-blind, randomized clinical trial was conducted to determine the efficacy of magnesium supplementation to improve insomnia in elderly individuals.[20] Forty-six elderly subjects randomly allocated into the magnesium or the placebo group received 500 mg magnesium or a placebo daily for 8 weeks. Compared with the placebo group, in the experimental group, dietary magnesium supplementation brought about statistically significant increases in sleep time, sleep efficiency, and concentration of serum melatonin and also resulted in a significant decrease in sleep-onset latency and serum cortisol concentration.

Melatonin

Melatonin is secreted by the pineal gland, and its production is based on light cues, increased at night and inhibited during the day. Supplementation with melatonin has been shown in several studies to be effective in helping induce and maintain sleep in children and adults, people with normal sleep patterns, and those with insomnia. However, the sleep-promoting effects of melatonin are most apparent only if melatonin levels are low.[21] In other words, melatonin has a sedative effect only when endogenous melatonin levels are low. Melatonin appears to be most effective in treating insomnia in the elderly, in whom low melatonin levels are common.[22]

In one study, 26 elderly insomniacs with lower-than-normal melatonin levels were given 1 to 2 mg of melatonin 2 hours before the desired bedtime for 1 week. Rapid- and slow-release melatonin preparations were used. Both sleep latency and sleep quality were evaluated. Although there was no discernible difference in sleep onset or sleep efficiency (time asleep as a percentage of total time in bed) between the two forms, the slow-release form yielded better effects on sleep maintenance.[23] A 2017 meta-analysis assessed the therapeutic effects of exogenous melatonin in treating primary sleep disorders.[24] The results showed that melatonin had significant effects on sleep-onset latency in primary insomnia, delayed-sleep-phase syndrome, and regulation of the non-24 sleep–wake syndrome compared with placebo.

Doses as low as 0.1 and 0.3 mg have been shown to produce a sedative effect when melatonin levels are low.[25] Common doses of melatonin range from 0.2 to 3 mg. Although melatonin appears to have no serious side effects at recommended doses, melatonin supplementation could conceivably disrupt the normal circadian rhythm. In one study, a dosage of 8 mg/day for only 4 days resulted in significant alterations in hormone secretions.[26] For more information, see Chapter 92.

Restless Legs Syndrome and Nocturnal Myoclonus

Restless legs syndrome and nocturnal myoclonus are significant causes of insomnia. Restless legs syndrome is characterized during waking by an irresistible urge to move the legs. Almost all patients with restless legs syndrome have nocturnal myoclonus,[1] a neuromuscular disorder characterized by repeated contractions of one or more muscle groups,

typically of the leg, during sleep. Each jerk usually lasts less than 10 seconds. The patient is normally unaware of the myoclonus and complains only of either frequent nocturnal awakenings or excessive daytime sleepiness, but questioning of the sleep partner often discovers the myoclonus.

If there is a family history of restless legs syndrome (present in about one third of all cases of the syndrome), high-dose folic acid, 35 to 60 mg daily, can be helpful.[27] Doses in this range require a prescription because the U.S. Food and Drug Administration limits the amount available per capsule to 800 mcg. Restless legs syndrome is also a common finding in patients with malabsorption syndromes.[27]

Serum ferritin levels should also be measured to determine iron stores. The association between low iron levels and restless legs syndrome was documented in clinical studies more than 30 years ago. One study reproduced these observations, finding serum ferritin levels to be lower in 18 patients with restless legs syndrome than in 18 control subjects.[28] Serum iron, vitamin B_{12}, folic acid, and hemoglobin levels did not differ in the two groups. A rating scale with a maximum score of 10 was used to assess the severity of symptoms of restless legs syndrome. Serum ferritin levels were inversely correlated with the severity of symptoms. Fifteen of the patients with the syndrome were treated with iron (ferrous sulfate) at a dosage of 200 mg three times daily for 2 months. The severity of restless legs syndrome improved by an average of 4 points in 16 patients with an initial ferritin level lower than 18 mg/L, by 3 points in 4 patients with ferritin levels between 18 and 45 mg/L, and by 1 point in 5 patients with ferritin levels between 45 and 100 mg/L. The conclusion of the study is an important contribution to the understanding of the development of restless legs syndrome in elderly patients, and iron supplements were found to produce a significant reduction in symptoms.

In addition to restless legs syndrome, low serum ferritin levels have been found in psychiatric patients experiencing a condition called akathisia, coming from a Greek word meaning "cannot sit down." Akathisia is a drug-induced state of agitation and can significantly impair restful sleep. The drugs that most commonly produce akathisia are antidepressants, such as fluoxetine (Paxil, Prozac) and sertraline (Zoloft). The level of iron depletion correlates with the severity of akathisia. Anyone suffering from drug-induced akathisia should ask his or her physician to perform a serum ferritin assessment. If serum ferritin levels are below 35 mg/L, the physician should recommend that the patient take 30 mg of iron bound to either succinate or fumarate twice daily between meals. If this recommendation causes abdominal discomfort, the patient should try 30 mg with meals three times daily.

Botanicals With Sedative Properties

Numerous plants have sedative action. Plants commonly prescribed as aids in promoting sleep include the following:

- *Valeriana officinalis*
- *Passiflora incarnata*
- *Humulus lupulus*
- *Scutellaria lateriflora*
- *Matricaria chamomilla*

Of the herbs listed, the one with the most clinical research is *Valeriana officinalis* (see Chapter 123). More than 20 double-blind clinical studies have substantiated valerian's ability to improve sleep quality and relieve insomnia.[29,30] Additional research is warranted, but these studies show that extracts of valerian root improve sleep quality and sleep latency. The studies demonstrated that valerian was as effective in reducing sleep latency as small doses of barbiturates or benzodiazepines. Although these latter compounds also increase morning sleepiness, valerian usually reduces morning sleepiness.

THERAPEUTIC APPROACH

Treatment should be conservative and include some means of addressing the psychological factors contributing to insomnia. Metabolically, the foremost component of treatment is the elimination of any factors known to disrupt normal sleep patterns, including the following:

- Stimulants (e.g., coffee, tea, chocolate, coffee-flavored ice cream)
- Alcohol
- Hypoglycemia
- Stimulant-containing herbs (e.g., ephedra, guarana)
- Marijuana and other recreational drugs
- Numerous OTC medications
- Prescription drugs

If this approach produces no response, more aggressive measures can be taken. Once a normal sleep pattern has been established, the recommended supplements and botanicals should be slowly decreased. If the patient suffers from restless legs syndrome, 5 to 10 mg/day of folic acid should be added to the therapy. Nocturnal myoclonus can be aided with 400 IU/day of natural vitamin E.

Lifestyle

The patient should institute a regular exercise program that elevates the heart rate to 60% to 75% of maximum for at least 20 minutes a day.

Supplements

The following supplements should be taken 45 minutes before bedtime:

- Niacin: 100 mg (decrease dose if uncomfortable flushing interferes with sleep induction)
- Vitamin B_6: 50 mg (if unresponsive, use P5'P)
- Magnesium: 250 to 500 mg
- Tryptophan: 3 to 5 g, or 5-HTP, 100 to 300 mg
- Melatonin: 3 mg

Botanical Medicines

The following botanical medicines should also be taken 45 minutes before bedtime:

Valeriana officinalis

- Dried root (or as tea): 2 to 3 g
- Tincture (1:5): 4 to 6 mL (1 to 1½ tsp)
- Fluid extract (1:1): 2 to 4 mL (½ to 1 tsp)
- Dry powdered extract (0.8% valerenic acid): 150 to 300 mg

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Roth T, Roehrs T. Insomnia: epidemiology, characteristics, and consequences. *Clin Cornerstone*. 2003;5:5–15.
2. Vermeeren A. Residual effects of hypnotics: epidemiology and clinical implications. *CNS Drugs*. 2004;18:297–328.
3. Montgomery P, Dennis J. A systematic review of non-pharmacological therapies for sleep problems in later life. *Sleep Med Rev*. 2004;8:47–62.
4. Angarita GA, Emadi N, Hodges S, Morgan PT. Sleep abnormalities associated with alcohol, cannabis, cocaine, and opiate use: a comprehensive review. *Addict Sci Clin Pract*. 2016;11(1):9. PMID: 27117064.
5. Victor LD. Treatment of obstructive sleep apnea in primary care. *Am Fam Physician*. 2004;69:561–568.
6. Leproult R, Copinschi G, Buxton O, et al. Sleep loss results in an elevation of cortisol levels the next evening. *Sleep*. 1997;20:865–870.
7. Hartmann E. l-Tryptophan a rational hypnotic with clinical potential. *Am J Psychiatry*. 1977;134:366–370.
8. George CF, Millar TW, Hanly PJ. The effect of l-tryptophan on daytime sleep latency in normals: correlation with blood levels. *Sleep*. 1989;12:345–353.
9. Thorleifsdottir B, Bjornsson JK, Kjeld M. Effects of l-tryptophan on daytime arousal. *Neuropsychobiology*. 1989;21:170–176.
10. Hajak G, Huether G, Blanke J. The influence of intravenous l-tryptophan on plasma melatonin and sleep in men. *Pharmacopsychiatry*. 1991;24:17–20.
11. Zarcone Jr VP, Hoddes E. Effects of 5-hydroxytryptophan on fragmentation of REM sleep in alcoholics. *Am J Psychiatry*. 1975;132:74–76.
12. Soulairac A, Lambinet H. Effect of 5-hydroxytryptophan, a serotonin precursor, on sleep disorders. *Ann Med Psychol (Paris)*. 1977;1:792–798.
13. Hartmann E, Elion R. The insomnia of 'sleeping in a strange place': effects of l-tryptophane. *Psychopharmacology*. 1977;53:131–133.
14. Wyatt RJ. The serotonin-catecholamine-dream bicycle: a clinical study. *Biol Psychiatry*. 1972;5:33–64.
15. Guilleminault C, Cathala HP, Castaigne P. Effects of 5-HTP on sleep of a patient with brain stem lesion. *Electroencephalogr Clin Neurophysiol*. 1973;34:177–184.
16. Wyatt RJ, Zarcone V, Engelman K. Effects of 5-hydroxytryptophan on the sleep of normal human subjects. *Electroencephalogr Clin Neurophysiol*. 1971;30:505–509.
17. Autret A, Minz M, Bussel B, et al. Human sleep and 5-HTP: effects of repeated high doses and of association with benserazide. *Electroencephalogr Clin Neurophysiol*. 1976;41:408–413.
18. Beydoun MA, Gamaldo AA, Canas JA, et al. Serum nutritional biomarkers and their associations with sleep among US adults in recent national surveys. *PLoS One*. 2014;9(8):e103490. PubMed PMID: 25137304.
19. Zadeh SS, Begura K. Comparison of nutrient intake by sleep status in selected adults in Mysore, India. *Nut Res Pract*. 2011;5(3):230–235. PubMed PMID: 21779527.
20. Abbasi B, Kimiagar M, Sadeghniiat K, Shirazi MM, Hedayati M, Rashidkhani B. The effect of magnesium supplementation on primary insomnia in elderly: a double-blind placebo-controlled clinical trial. *J Res Med Sci*. 2012;17(12):1161–1169. PubMed PMID: 23853635.
21. Nave R, Peled R, Lavie P. Melatonin improves evening napping. *Eur J Pharmacol*. 1995;275:213–216.
22. Olde Rikkert MG, Rigaud AS. Melatonin in elderly patients with insomnia: a systematic review. *Z Gerontol Geriatr*. 2001;34:491–497.
23. Haimov I, Lavie P, Laudon M, et al. Melatonin replacement therapy of elderly insomniacs. *Sleep*. 1995;18:598–603.
24. Auld F, Maschauer EL, Morrison I, Skene DJ, Riha RL. Evidence for the efficacy of melatonin in the treatment of primary adult sleep disorders. *Sleep Med Rev*. 2017;34:10–22. PubMed PMID: 28648359.
25. Dollins AB, Zhdanova IV, Wurtman RJ, et al. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc Natl Acad Sci U S A*. 1994;91:1824–1828.
26. Mallo C, Zaidan R, Faure A, et al. Effects of a four-day nocturnal melatonin treatment on the 24 h plasma melatonin, cortisol and prolactin profiles in humans. *Acta Endocrinol (Copenh)*. 1988;119:474–480.
27. Botez MI, Cadotte M, Beaulieu R, et al. Neurologic disorders responsive to folic acid therapy. *Can Med Assoc J*. 1976;115:217–223.
28. O'Keeffe ST, Gavin K, Lavan JN. Iron status and restless legs syndrome in the elderly. *Age Ageing*. 1994;23:200–203.
29. Hadley S, Petry JJ. *Valerian. Am Fam Physician.* 2003;67:1755–1758.
30. Stevinson C, Ernst E. Valerian for insomnia: a systematic review of randomized clinical trials. *Sleep Med*. 2000;1:91–99.

Intestinal Protozoan Infestation and Systemic Illness

Helen (Verhesen) Messier, and *Leo Galland*, AB, MD*

OUTLINE

INTRODUCTION

The gastrointestinal tract is the largest organ of immune surveillance in the body, home to two thirds of the total lymphocyte population.[1] Regulatory T cells play a crucial role among the lymphocytes responding to protozoan infestation, so parasitical infestation may alter the immune response environment, exerting a major effect on systemic responses, including allergy and autoimmunity.[2] Systemic immunological reactivity may occur in the absence of digestive complaints.[3–7]

PROTOZOA

Giardia spp.

Human giardiasis may provoke asthma,[8,9] urticaria,[10–14] arthritis,[5,14–17] and uveitis,[18] presumably by inducing immunological hypersensitivity. Urticaria induced by *Giardia* has been associated with specific anti-*Giardia* immunoglobulin-E (IgE), which is not found in patients with intestinal symptoms only. IgE production is associated with increased activity of vascular and intercellular adhesion molecules.[19] Using proteomics, *Giardia* has recently been found to release a number of specific proteins, known as excretory-secretory products (ESPs), which affect gene expression, secretion, signaling, metabolism, and immune responses in intestinal epithelial cells (Fig. 188.1).[20]

During *Giardia*–host cell interactions in vitro, specific proteins are released into the growth medium. The same types of proteins, mainly enzymes, are released from both the host and parasite (*black* in Fig. 188.1), but there are also parasite-specific proteins being released (*blue* in Fig. 188.1).[20]

Giardia may also provoke systemic illness through malabsorption or protein loss, which can occur without diarrhea.[21] Iron deficiency[22,23]; low levels of carotene and folate[24]; and abnormal absorption of vitamin A,[25–27] folic acid, and vitamin B_{12}[21] can result from chronic giardiasis and add to the burden of illness, even in patients who appear well nourished.[26] Giardiasis may also induce small intestinal bacterial overgrowth[21,28] and jejunal candidosis,[29] each of which may independently cause systemic symptoms. *G. lamblia* may also act as a vector for double-stranded RNA viruses.[30]

Giardiasis was identified in 61 of 218 consecutive patients presenting to the author's medical clinic with a chief complaint of chronic fatigue.[31] The symptoms of patients with and without giardiasis are shown in Table 188.1. Giardiasis was strongly associated with myalgia, muscle weakness, flu-like feelings, sweats, adenopathy, and a previous diagnosis of chronic fatigue immune dysfunction syndrome (CFIDS). Cure of giardiasis resulted in the clearing of fatigue and related "viral" symptoms (myalgia, sweats, flu-like feelings) in 70% of cases and in some palliation of fatigue in 18%. In 2012 a waterborne outbreak of giardiasis occurred in which more than 1200 individuals were exposed. Over a threefold increase in the risk for irritable bowel syndrome (IBS) and a fourfold risk for chronic fatigue was reported in the 3 years after the acute exposure.[32] Evidence of CD4 T-cell activation at least 5 years after exposure has also been documented among these individuals.[33] The association between intestinal protozoa and chronic fatigue in patients without prominent digestive complaints may not be limited to giardiasis. In an unpublished presentation, the author reported that 80% of patients with a diagnosis of CFIDS who were infected with the protozoan *Blastocystis hominis* showed significant improvement of fatigue associated with treatment that cleared the protozoa from stool specimens.[34] As in giardiasis, infestation with *B. hominis* has also been associated with several patterns of urticaria: acute, chronic, and pressure induced.[35–37]

Entamoeba histolytica

Chronic infestation with *Entamoeba histolytica* has been associated with autoimmune phenomena, including the appearance of antibodies to colonic epithelial cells[38] and the development of ulcerative colitis after the cure of amebic colitis.[39] Extraintestinal autoimmune reactions to intestinal amebiasis include a case of antiphospholipid antibody syndrome with deep vein thrombosis and pulmonary embolism[40] and the development of symmetrical polyarthritis mimicking rheumatoid arthritis.[5,41–44] Diarrhea, polyarthritis, and circulating antinuclear

*Previous edition contributor

antibodies developed in a U.S. serviceman heavily infested with *Endolimax nana*, an allegedly nonpathogenic ameba.[45] Metronidazole rapidly reversed all abnormalities. Amebic arthritis may be an example of parasitical rheumatism, an inflammatory polyarthropathy produced by circulating antigen–antibody complexes.[46,47] Reiter syndrome (arthritis, uveitis, and urethritis) has been reported as a complication of infection with two other intestinal protozoa, *Cryptosporidium*[48–50] and *Cyclospora*.[51] *Cyclospora cayetanensis* has also provoked Guillain-Barré syndrome, a severe autoimmune neuropathy.[52] Chagas heart disease, caused by the protozoan parasite *Trypanosoma cruzi*, may also have an autoimmune etiology because some individuals clearly produce autoantibodies and autoreactive T cells during infection.[53]

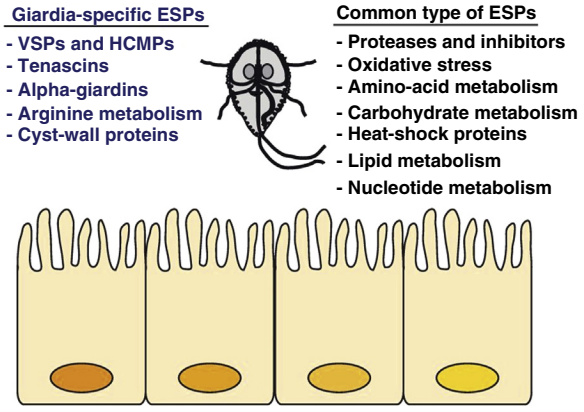


Fig. 188.1 Proteins secreted during *Giardia*–host cell interactions.

TABLE 188.1 Systemic Symptoms of Patients with Chronic Fatigue Immune Dysfunction Syndrome

Symptom	With Giardiasis (%) (*n* = 63)	Without Giardiasis (%) (*n* = 157)
Depression	61	41
Muscle weakness	46	19
Headache	41	36
Sore throat	41	11
Lymphadenopathy	36	8
Arthralgia	36	27
Myalgia	34	18
Flu-like symptoms	34	6
Poor exercise tolerance	30	10

Modified from Galland L, Lee M, Bueno H, et al. *Giardia lamblia* infection as a cause of chronic fatigue. *J Nutr Med.* 1990;2:27–32.

E. histolytica contains a soluble lectin that is mitogenic for T lymphocytes.[54,55] Activation of helper T cells by this lectin may induce replication of human immunodeficiency virus (HIV) in vivo. In one report, soluble *E. histolytica* protein, although not mitogenic itself, induced HIV replication in tissue culture of lymphocytes obtained from three of seven men with chronic HIV infection.[56] Infection with *E. histolytica* and other parasites may promote the development of acquired immunodeficiency syndrome in HIV-infected individuals.[57,58] Epidemiological evidence associated preexisting intestinal protozoan infection with the appearance of Kaposi's sarcoma among homosexual men in the United States.[59] Although the influence of treating intestinal protozoan infection on the course of HIV infection has not been systematically studied, the treatment of intestinal helminth infestation decreased the HIV viral load among African patients with AIDS.[60] Synergism between intestinal parasites and other lymphotrophic retroviruses was considered an explanation for the pathogenesis of Burkitt lymphoma[61] and adult T-cell leukemia and/or lymphoma.[62]

Helminths and Immune Modulation

In recent years, the potential for beneficial immune modulation with specific parasites has also emerged. Helminths, or multicellular organisms that are either parasitical or free-living, appear to downmodulate both the innate and adaptive immune systems and may block the same inflammatory pathways that are responsible for allergies and autoimmunity (Fig. 188.2).

Infection with parasitical worms causes the host immune system to polarize into a Th2 response (preventing Th1 or Th17 immune response) characterized by Th2 cytokines. Helminth ES products can cause the differentiation of macrophages toward the M2 phenotype, resulting in a Th2 immune response. ES products can also prevent dendritic cell synthesis of proinflammatory cytokines and promote the production of immunoregulatory molecules such as interleukin (IL)-10 and transforming growth factor-beta (TGF-β). A regulatory T cell (Treg) phenotype is also induced, promoting the protection/

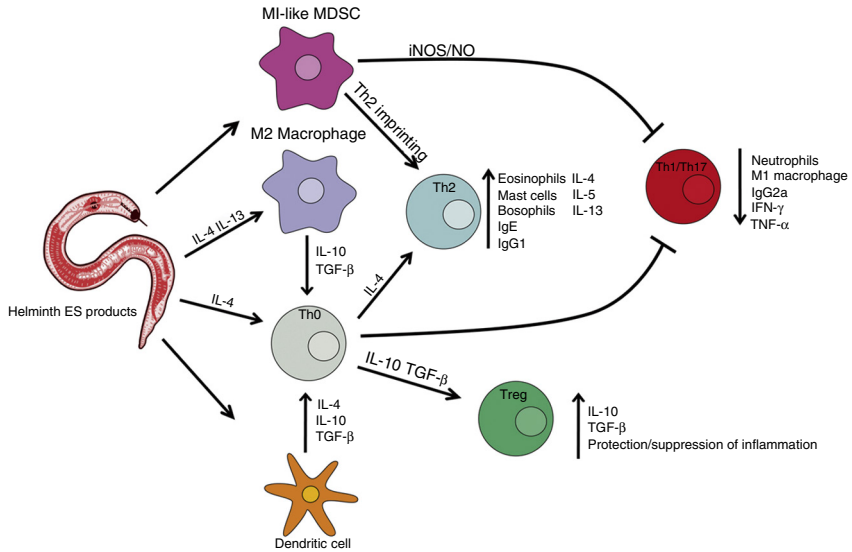


Fig. 188.2 Helminth excretory/secretory (ES) products effect on host immune cells.

suppression of inflammation produced by a Th1 autoimmune disease. Myeloid-derived suppressor cells (MDSCs) function as immunoregulators, producing reactive oxygen/nitrogen species that inhibit the function of T cells.[63]

Currently, one fourth of the world's population may be infected with helminths, and it has been proposed that for much of human history, this rate would have been near 100%, which has sparked the "old friends" hypothesis—that is, that humans have coevolved with various organisms, including helminths, that act as inducers of immunoregulatory circuits. Areas with higher infections rates also have lower rates of autoimmune disease, and human and animal trials are suggestive of a potential benefit in the treatment of inflammatory bowel disease (IBD), type 1 diabetes, multiple sclerosis, asthma, and celiac disease.[64,65] As the excretory products are better understood, it may be that specific molecular therapy can replace the use of live organisms, although clinical trials have generally proven safe.

DIAGNOSTIC CONSIDERATIONS

Protozoan infection may be diagnosed with stool examination; however, comparison of the results of stool microscopy and duodenal aspiration has consistently shown that stool may fail to contain identifiable parasites even at the height of acute giardiasis.[66,67] Some authorities have suggested empirical treatment for intestinal parasites in high-risk groups, such as immigrants to the United States from Asia, the Middle East, sub-Saharan Africa, Eastern Europe, Latin America, and the Caribbean, and have justified this approach on a cost-effective basis, given the safety of current medical therapies.[68] A similar case might be made for treating chronically ill patients at high risk for parasitical infection because of residence, travel, sexual practices, or the context in which illness occurred. Specific recommendations for laboratory testing are also available, including the recommendation for the use of a *Giardia/Cryptosporidium* enzyme immunoassay in areas where *Giardia* is more common and the use of a modified acid-fast stain when *Cryptosporidium, Cyclospora,* or *Cystoisospora* are suspected, for example.[69]

THERAPEUTIC CONSIDERATIONS

Artemisia annua

Numerous naturally occurring substances have antiprotozoan activity. The most extensively studied is *Artemisia annua* (sweet Annie or qinghao), a plant that yields the lactone artemisinin (qinghaosu), which is the basis for a new class of antimalarial compounds widely used in Asia and Africa.[70] Artemisinin is thought to owe its antiprotozoan effects to its content of endoperoxides and killing of parasites through oxidation. Its activity, at least in the treatment of Simian malaria, is enhanced by coadministration of cod liver oil and diminished by coadministration of vitamin E. Artemisinin has low toxicity. In addition to its antibiotic activity, it stimulates macrophages, an important component of the immune response to protozoan infestation.[71] It has demonstrated antiparasitical activity against protozoan parasites such as *Leishmania* spp., *Trypanosoma* spp., *Toxoplasma gondii, Neospora caninum, Eimeria tenella, Acanthamoeba castellanii, Naegleria fowleri, Cryptosporidium parvum, Giardia lamblia,* and *Babesia* spp., with both low cost and low toxicity.[72] Artemisinin may induce abortion if given during pregnancy.

Berberine

The alkaloid berberine can be extracted from the roots of several plant species, notably *Berberis aquifolium* (Oregon grape), *Hydrastis canadensis* (goldenseal) root, and *Coptis chinensis* (goldthread). Berberine has protostatic and protocidal activity against *E. histolytica, G. lamblia,* and *B. hominis.*[73–75] It has shown benefit in the treatment of giardiasis in children.[76]

Allium sativum and *Juglans nigra*

Allium sativum (garlic) and *Juglans nigra* (black walnut) have a long history of use as antimicrobials. Allicin inhibits the growth of *E. histolytica* in culture[77] and may be responsible for the antimicrobial activity of garlic.[78] Human studies on the efficacy of garlic and black walnut in treatment of protozoan infections are lacking.

Intestinal Bacterial Milieu

The intestinal bacterial milieu may be important in the treatment of protozoan infestation, especially for colonic organisms like *E. histolytica.* Pathogenic strains of *E. histolytica* are able to evade lysis by both classic and alternative pathways of complement. Intestinal bacteria, *Escherichia coli* in particular, are necessary for complement resistance and for amebic virulence.[79] Additionally, children with very high rates of infection with *E. histolytica* are more likely to be symptomatic (diarrheal disease) depending on the composition of their microflora, specifically an increase in *Prevotella copri* colonization.[80] Gitler and Mirelman[81] suggested that ingested bacteria lowered the redox potential within the parasite and allowed the amebae to escape destruction by oxidative enzymes.[81]

Animal studies have also documented that dysbiosis is associated with a decrease in the surface expression of CXCR2 on neutrophils, impairing neutrophil recruitment to the site of infection.[82] Mirelman et al.[83] reported that one can reversibly change the zymodeme patterns of *E. histolytica* isolates from nonpathogenic to invasive by culturing amebae with the gut flora of patients who have either invasive disease or no symptoms. Optimal treatment of protozoan infection requires not only the administration of antimicrobial substances but also strategies aimed at enhancing the function of intestinal resistance factors such as secretory immunoglobulin A (IgA), both neutrophil and phagocyte function, and the creation of a bacterial milieu that is not parasite friendly.

CONCLUSION

Intestinal protozoan infestation is a significantly underrecognized cause of systemic illness. Undiagnosed infestations with *Giardia* spp., *E. histolytica,* and other organisms have been associated with diverse diseases, such as arthritis; asthma; Reiter syndrome; urticaria and uveitis; CFIDS; increased rate of progression of HIV infection; and a wide range of systemic dysfunction, such as fatigue, malabsorption, muscle weakness, and myalgia. Effective eradication of protozoa can result in surprisingly quick and complete clinical response.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Targan SR, Kagnoff MF, Brogan MD, et al. Immunologic mechanisms in intestinal disease. *Ann Intern Med*. 1987;106:854–870.
2. Belkaid Y, Liesenfeld O, Maizels RM. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: induction and control of regulatory T cells in the gastrointestinal tract: consequences for local and peripheral immune responses. *Clin Exp Immunol*. 2010;160:35–41.
3. Wilhelm RE. Urticaria associated with giardiasis lamblia. *J Allergy*. 1958;28:351–353.
4. Kennou MF, Jenayah S, Rekhis M, et al. Skin manifestation of giardiasis: some clinical cases. *Arch Inst Pasteur Tunis*. 1980;57:257–260. [French].
5. Galland L. Intestinal protozoan infection is a common unsuspected cause of chronic illness. *J Advancement Med*. 1989;2:529–552.
6. Pasqui AL, Savini E, Saletti M, et al. Chronic urticaria and *Blastocystis hominis* infection: a case report. *Eur Rev Med Pharmacol Sci*. 2004;8: 117–120.
7. Giacometti A, Cirioni O, Antonicelli L, et al. Prevalence of intestinal parasites among individuals with allergic skin diseases. *J Parasitol*. 2003;89:490–492.
8. Di Prisco MC, Hagel I, Lynch NR, et al. Possible relationship between allergic disease and infection by *Giardia lamblia*. *Ann Allergy*. 1993;70:210–213.
9. Lopez-Brea M, Sainz T, Camarero C, et al. *Giardia lamblia* associated with bronchial asthma and serum antibodies, and chronic diarrhoea in a child with giardiasis. *Trans R Soc Trop Med Hyg*. 1979;73:600.
10. Harris RH, Mitchell JH. Chronic urticaria due to *Giardia lamblia*. *Arch Dermatol Syphilol*. 1949;59:587–589.
11. Webster BH. Human infection with *Giardia lamblia*. *Am J Dig Dis*. 1958;3:64–71.
12. Dellamonica P, Le Fichoux Y, Monnier B, et al. Dysenteric syndrome and urticaria in giardiasis. *Nouv Presse Med*. 1976;5:1913. [French].
13. Weisman BL. Urticaria and *Giardia lamblia* infection. *Ann Allergy*. 1979;42:91.
14. Farthing MJ, Chong SK, Walker-Smith JA. Acute allergic phenomena in giardiasis. *Lancet*. 1983;2:1428.
15. Goobar JP. Joint symptoms in giardiasis. *Lancet*. 1977;1:1010–1011.
16. Woo P, Panayi GS. Reactive arthritis due to infestation with *Giardia lamblia*. *J Rheumatol*. 1984;11:719.
17. Shaw RA, Stevens MB. The reactive arthritis of giardiasis: a case report. *JAMA*. 1987;258:2734–2735.
18. Carroll ME, Anast BP, Birch CL. Giardiasis and uveitis. *Arch Ophthalmol*. 1961;65:775–778.
19. Mahmoud MS, Salem AA, Rifaat MM. Human giardiasis as an etiology of skin allergy: the role of adhesion molecules and interleukin-6. *J Egypt Soc Parasitol*. 2004;34:723–737.
20. Ma'ayeh SY, Liu J, Peirasmaki D, et al. Characterization of the *Giardia intestinalis* secretome during interaction with human intestinal epithelial cells: the impact on host cells. *PLoS Negl Trop Dis*. 2017;11(12):e0006120. PMID: 29228011.
21. Sherman P, Liebman WM. Apparent protein-losing enteropathy associated with giardiasis. *Am J Dis Child*. 1980;134:893–894.
22. Gonen C, Yilmaz N, Yalcin M, et al. Diagnostic yield of routine duodenal biopsies in iron deficiency anaemia: a study from Western Anatolia. *Eur J Gastroenterol Hepatol*. 2007;19:37–41.
23. Kasirga E, Gülen H, Simşek A, et al. Coexistence of symptomatic iron-deficiency anemia and duodenal nodular lymphoid hyperplasia due to giardiasis: case report. *Pediatr Hematol Oncol*. 2009;26:57–61.
24. Brasitus TA. Parasites and malabsorption. *Clin Gastroenterol*. 1983;12:495–510.
25. Quihui-Cota L, Astiazarán-García H, Valencia ME, et al. Impact of *Giardia intestinalis* on vitamin A status in school children from northwest Mexico. *Int J Vitam Nutr Res*. 2008;78:51–56.
26. Astiazaran-Garcia H, Lopez-Teros V, Valencia ME, et al. *Giardia lamblia* infection and its implications for vitamin A liver stores in school children. *Ann Nutr Metab*. 2010;57:228–233.
27. Girard C, Dereure O, Blatière V, et al. Vitamin A deficiency phrynoderma associated with chronic giardiasis. *Pediatr Dermatol*. 2006;23:346–349.
28. Tompkins AM, Wright SG, Drasar BS, et al. Bacterial colonization of jejunal mucosa in giardiasis. *Trans R Soc Trop Med Hyg*. 1978;72:33–36.
29. Naik SR, Rau NR, Vinayak VK, et al. Presence of *Candida albicans* in normal and in *Giardia lamblia* infected human jejunum. *Ann Trop Med Parasitol*. 1978;72:493–494.
30. Den Hollander N, Riley D, Befus D. Immunology of *Giardia*. *Parasitol Today*. 1988;4:124–133.
31. Galland L, Lee M, Bueno H, et al. *Giardia lamblia* infection as a cause of chronic fatigue. *J Nutr Med*. 1990;2:27–32.
32. Wensaas KA1, Langeland N, Hanevik K, et al. Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. *Gut*. 2012;61(2):214–219. PMID: 21911849.
33. Hanevik K, Kristoffersen E, Svard S, et al. Human cellular immune response against *Giardia lamblia* 5 years after acute giardiasis. *J Infect Dis*. 2011;204(11):1779–1786. PMID: 21990423.
34. Galland L. Patient-centered diagnosis and treatment for chronic fatigue syndrome. *Presented at the Annual Scientific Session of the American Academy of Environmental Medicine.* Virginia Beach, VA; 1994.
35. Katsarou-Katsari A, Vassalos CM, Tzanetou K, et al. Acute urticaria associated with amoeboid forms of *Blastocystis* sp. subtype 3. *Acta Derm Venereol*. 2008;88:80–81.
36. Gupta R, Parsi K. Chronic urticaria due to *Blastocystis hominis*. *J Dermatol*. 2006;47:117–119.
37. Cassano N, Scoppio BM, Loviglio MC, et al. Remission of delayed pressure urticaria after eradication of *Blastocystis hominis*. *Acta Derm Venereol*. 2005;85:357–358.
38. Salem E, Zaki SA, Moneim WA, et al. Autoantibodies in amoebic colitis. *J Egypt Med Assoc*. 1973;56:113–118.
39. Sturgess I, Greenfield SM, Teare J, et al. Ulcerative colitis developing after amoebic dysentery in a haemophiliac patient with AIDS. *Gut*. 1992;33:408–410.
40. Korkmaz C, Harmanci E, Metintas I, et al. Antiphospholipid syndrome associated with intestinal amoebiasis. *Scand J Infect Dis*. 2001;33:938–940.
41. Zinneman HH. Ten cases of amebiasis with arthritic complaints. *Am J Dig Dis*. 1950;17:343–344.
42. Rappaport EM, Rossieu AX, Rosenblum LA. Arthritis due to intestinal amebiasis. *Ann Intern Med*. 1951;34:1224–1231.
43. Kasliwal RM. Colon and disease. *J Assoc Physicians India*. 1970;18:739–744.
44. Singh IP, Das SK, Sharma P, et al. Antibodies to *Entamoeba histolytica* in patients with rheumatoid arthritis. *Trop Gastroenterol*. 1985;6:141–144.
45. Burnstein SL, Liakos S. Parasitic rheumatism presenting as rheumatoid arthritis. *J Rheumatol*. 1983;10:514–515.
46. Bocanegra TS, Espinoza LR, Bridgeford PH, et al. Reactive arthritis induced by parasitic infestation. *Ann Intern Med*. 1981;94:207–209.
47. Solomons NW. Giardiasis: nutritional implications. *Rev Infect Dis*. 1982;4:859–869.
48. Cron RQ, Sherry DD. Reiter's syndrome associated with cryptosporidial gastroenteritis. *J Rheumatol*. 1995;22:1962–1963.
49. Collings S, Highton J. Cryptosporidium reactive arthritis. *N Z Med J*. 2004;117:1.
50. Sing A, Bechtold S, Heesemann J, et al. Reiter syndrome following protracted symptoms of *Cyclospora* infection. *Emerg Infect Dis*. 2001;7:453–454.
51. Connor BA, Johnson EJ, Soave R. Reiter syndrome following protracted symptoms of Cyclospora infection. *Emerg Infect Dis*. 2001;7:453–454.
52. Richardson Jr RF, Remler BF, Katirji B, et al. Guillain-Barré syndrome after *Cyclospora* infection. *Muscle Nerve*. 1998;21:669–671.
53. Bonney KM, Engman DM. Autoimmune pathogenesis of Chagas heart disease: looking back, looking ahead. *Am J Pathol*. 2015;185(6):1537–1547. PMID: 25857229.
54. Chen ZC, Herrmann F, Koleski F, et al. Mitogenic factor for T inducer/helper cells in *Entamoeba histolytica* extracts. *Acta Acad Med Wuhan*. 1985;5:213–216.
55. Petri Jr WA, Ravdin JI. Treatment of homosexual men infected with *Entamoeba histolytica*. *N Engl J Med*. 1986;315:393.
56. Croxson S, Mildvan D, Mathews H, et al. *Entamoeba histolytica* antigen-specific induction of human immunodeficiency virus replication. *J Clin Microbiol*. 1988;26:1304–1308.
57. Pearce RB, Abrams DI. *Entamoeba histolytica* in homosexual men. *N Engl J Med*. 1987;316:690–692.

58. Archer DL, Glinsman WH. Enteric infections and other co-factors in AIDS. *Immunol Today*. 1985;6:292–294.
59. Abrams DI. The relationship between Kaposi's sarcoma and intestinal parasites among homosexual males in the United States. *J Acquir Immune Defic Syndr*. 1990;3:S44–S46.
60. Wolday D, Mayaan S, Mariam ZG, et al. Treatment of intestinal worms is associated with decreased HIV plasma viral load. *J Acquir Immune Defic Syndr*. 2002;31:56–62.
61. Burkitt DP. The discovery of Burkitt's lymphoma. *Cancer*. 1983;51:1777–1786.
62. Tajima K, Tominaga S, Shimizu H, et al. A hypothesis on the etiology of adult T-cell leukemia/lymphoma. *Gann*. 1981;72:684–691.
63. From Smallwood TB, Giacomin PR, Loukas A, et al. Helminth immunomodulation in autoimmune disease. *Front Immunol*. 2017;8:453. PMID: 28484453.
64. Varyani F, Fleming JO, Maizels RM. Helminths in the gastrointestinal tract as modulators of immunity and pathology. *Am J Physiol Gastrointest Liver Physiol*. 2017;312(6):G537–G549. PMID: 28302598.
65. Smallwood TB, Giacomin PR, Loukas A, et al. Helminth immunomodulation in autoimmune disease. *Front Immunol*. 2017;8:453. PMID: 28484453.
66. Rosenthal P, Liebman WM. Comparative study of stool examinations, duodenal aspiration, and pediatric Entero-Test for giardiasis in children. *J Pediatr*. 1980;96:278–279.
67. Kamath KR, Murugasu R. A comparative study of four methods for detecting *Giardia lamblia* in children with diarrheal disease and malabsorption. *Gastroenterol*. 1974;66:16–21.
68. Muennig P, Pallin D, Sell RL, et al. The cost effectiveness of strategies for the treatment of intestinal parasites in immigrants. *N Engl J Med*. 1999;340:773–779.
69. Garcia LS, Arrowood M, Kokoskin E, et al. Laboratory diagnosis of parasites from the gastrointestinal tract. *Clin Microbiol Rev*. 2017;31(1). pii: e00025-17. PMID: 29142079.
70. Hien TT, White NJ. Qinghaosu. *Lancet*. 1993;341:603–608.
71. Tang W, Eisenbrand G. *Chinese Drugs of Plant Origin*. Berlin: Springer-Verlag; 1992.
72. Loo CS, Lam NS, Yu D, et al. Artemisinin and its derivatives in treating protozoan infections beyond malaria. *Pharmacol Res*. 2017;117:192–217. PMID: 27867026.
73. Kaneda Y, Torii N, Tanaka T, et al. In vitro effects of berberine sulfate on the growth and structure of *Entamoeba histolytica, Giardia lamblia* and *Trichomonas vaginalis*. *Ann Trop Med Parasitol*. 1991;85:417–425.
74. Subbaiah TV, Amin AH. Effect of berberine sulphate on *Entamoeba histolytica*. *Nature*. 1967;215:527–528.
75. Yang LQ, Singh M, Yap EH, et al. In vitro response of *Blastocystis hominis* against traditional Chinese medicine. *J Ethnopharmacol*. 1996;55:35–42.
76. Gupte S. Use of berberine in treatment of giardiasis. *Am J Dis Child*. 1975;129:866.
77. Mirelman D, Monheit D, Varon S. Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle in garlic extract *(Allium sativum)*. *J Infect Dis*. 1987;156:243–244.
78. Wright CW, Phillipson JD. Natural products and the development of selective antiprotozoal drugs. *Phytother Res*. 1990;4:127–139.
79. Wittner M, Rosenbaum RM. Role of bacteria in modifying virulence of *E. histolytica*: studies of amoebae from axenic cultures. *Am J Trop Med Hyg*. 1970;19:755–761.
80. Gilchrist CA, Petri SE, Schneider BN, et al. Role of the gut microbiota of children in diarrhea due to the Protozoan parasite *Entamoeba histolytica*. *J Infect Dis*. 2016;213(10):1579–1585. PMID: 26712950.
81. Gitler C, Mirelman D. Factors contributing to the pathogenic behavior of *Entamoeba histolytica*. *Annu Rev Microbiol*. 1986;40:237–261.
82. Watanabe K, Petri WA. Learning from the research on amebiasis and gut microbiome: is stimulation by gut flora essential for effective neutrophil mediated protection from external pathogens? *Gut Microbes*. 2019;10(1):100–104. PMID: 30252579.
83. Mirelman D, Bracha R, Chayen A, et al. *Entamoeba histolytica*: effect of growth conditions and bacterial associates on isoenzyme patterns and virulence. *Exp Parasitol*. 1986;62:142–148.

189

Irritable Bowel Syndrome

Gerard E. Mullin, MD

OUTLINE

DIAGNOSTIC SUMMARY

Irritable bowel syndrome (IBS) is a functional disorder of the large intestine with no evidence of accompanying structural defect; it is characterized by some combination of:

- Abdominal pain
- Altered bowel function, constipation, or diarrhea
- Hypersecretion of colonic mucus
- Dyspeptic symptoms (flatulence, nausea, anorexia)
- Varying degrees of anxiety or depression
- In some cases, also extraintestinal manifestations such as restless legs syndrome (RLS), migraine headaches, chronic fatigue, irritable bladder syndrome, and dyspareunia

IBS is presently diagnosed according to the Rome IV criteria (Box 189.1).

Less acceptable synonyms are *nervous indigestion, spastic colitis, mucous colitis,* and *intestinal neurosis.* Splenic flexure syndrome is a variant of IBS in which gas in the bowel leads to pain in the lower chest or the left shoulder. Many patients with IBS also have extraintestinal symptoms, including sexual dysfunction, fibromyalgia, dyspareunia, urinary frequency and urgency, poor sleep, menstrual difficulties, lower back pain, headaches, chronic fatigue, RLS, and insomnia. These conditions tend to increase in number with the severity of IBS. The pathophysiology of IBS is complex, but it is known to involve genetic factors, intolerance to food(s), gastrointestinal dysbiosis, altered gut motility, decreased intestinal barrier integrity, visceral hypersensitivity, and altered mucosal immune responses (Fig. 189.1). Extraintestinal symptoms (EISs) are important to recognize in patients with IBS because increased EISs portends a higher likelihood of greater IBS severity (Fig. 189.2).[1]

GENERAL CONSIDERATIONS

IBS is the most common gastrointestinal disorder seen in general practice, representing 30% to 50% of all referrals to gastroenterologists.[2,3] Determining incidence or prevalence figures is virtually impossible because many sufferers never seek medical attention. It has been estimated, however, that approximately 15% of the population has complaints of IBS, with women predominating in a ratio of 2:1 (it is likely that an equal number of men have IBS but that they do not report symptoms as often). The etiology of the greater colonic motility seen in IBS has been attributed to physiologic, psychological, and dietary factors.

Although IBS is often a diagnosis of exclusion, clinical judgment must be used to determine the extent of the diagnostic process required. A detailed history and physical examination has been shown to eliminate much of the vagueness involved in diagnosing IBS.[4] Distention, relief of pain with bowel movements, and the onset of loose or more frequent bowel movements with pain seem to correlate best with the diagnosis of IBS (any three, $P = 0.84$; just one, $P = 0.25$).[2]

In most cases, a comprehensive stool and digestive analysis (see Chapter 28, Stool Analysis), with special attention to dysbiosis, complete blood count, and measurements of erythrocyte sedimentation rate, free thyroid T3 hormone level, and celiac testing (antiendomysial antibody test; see Chapter 157, Celiac Disease), should be performed to establish the diagnosis and exclude other diseases. If diarrhea-predominant IBS symptoms are more pervasive, panendoscopy with duodenal, colonic, and terminal ileal biopsies should be obtained to rule out autoimmune conditions such as celiac disease, inflammatory bowel disease, lymphocytic colitis, and collagenous colitis. If food allergy is suspected as a contributor, stool testing for eosinophilic cationic protein should be considered (for a more complete discussion of laboratory tests to evaluate food allergy and/or adverse reactions, see Chapter 14, Food Hypersensitivities). If no discernible cause can be identified, screening for occult fecal blood and flexible sigmoidoscopy are indicated in patients younger than 50 years and colonoscopy in older patients.

THERAPEUTIC CONSIDERATIONS

Once other chronic conditions have been ruled out, there appear to be three major treatments to consider in the formulation of a therapeutic regimen for IBS:

- Increasing dietary fiber
- Eliminating allergic/intolerant foods

BOX 189.1 Rome IV Criteria for Irritable Bowel Syndrome

According to Rome IV, a diagnosis of IBS can be made if recurrent abdominal pain (at least once weekly) is associated with two or more of the following:

- Related to defecation;
- Associated with a change in frequency of stool; or
- Associated with a change in form (appearance) of stool.

The patient needs to have none of the following warning signs:

- Are greater than 50 years of age at onset, no previous colon cancer screening, and with the presence of symptoms.
- Recent change in bowel habit.
- Evidence of overt GI bleeding (i.e., melena or hematochezia).
- Nocturnal pain or passage of stools.
- Unintentional weight loss.
- Family history of colorectal cancer or inflammatory bowel disease.
- Palpable abdominal mass or lymphadenopathy.
- Evidence of iron-deficiency anemia on blood testing.
- Positive test for fecal occult blood.

GI, Gastrointestinal; *IBS,* irritable bowel syndrome.Data from Palsson OS, Whitehead WE, van Tilburg MA, et al. Rome IV diagnostic questionnaires and tables for investigators and clinicians. *Gastroenterology*. 2016;150:6:1481–1491.

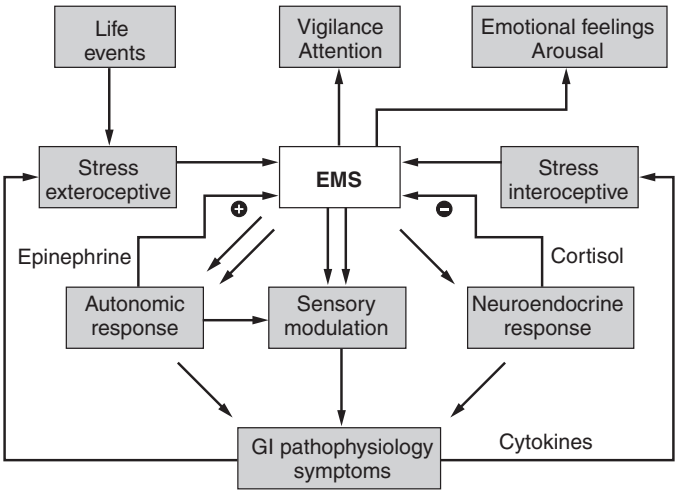


Fig. 189.1 Current understanding of irritable bowel syndrome (IBS) pathophysiology. Stress, abuse, and emotional feelings influence bowel symptoms via activation of the brain's central circuitry called the emotional motor system (EMS) to produce autonomic and neuroendocrine responses. Bowel symptoms then cause more distress, triggering the release of mediators (cytokines, cortisol, and adrenaline) that act on the EMS, producing a feed-forward cycle of bowel symptoms and emotional distress.

- Controlling psychological components
- Peppermint oil for spastic colon
- Acupuncture for pain modulation and motility regulation
- Melatonin as a stress adaptogen, as a mood stabilizer, and to promote restful sleep
- Small intestinal bacterial overgrowth, intestinal dysbiosis, and food allergies may be silent triggers of the disease.
- Dietary fermentable oligo-, di-, and monosaccharides and polyols (FODMAPS) appear to play a role in promoting disease activity and should be limited in IBS.

Diet

Dietary Fiber

The treatment of IBS through an increase in dietary fiber has a long, though irregular, history.[2] Patients with constipation are much more likely to show a response to dietary fiber than are those with diarrhea. One problem that has not been addressed in studies on the therapeutic use of dietary fiber is the role of food allergy. The type of fiber often used in both research and clinical practice is wheat bran.[5] Wheat and other grains are among the most commonly implicated foods in malabsorptive and allergic conditions, and food allergy is a significant etiological factor in IBS, so the use of wheat bran is usually contraindicated.

Increasing dietary fiber from fruit and vegetable sources rather than cereal sources may offer more benefit to some individuals, although in one uncontrolled clinical study, there was no significant difference in improvement when a diet composed of 30 g of fruit and vegetable fiber and 10 g of cereal fiber was compared with a diet consisting of the opposite ratio.[6] Although the two diets resulted in similar significant improvements in abdominal pain, bowel habits, and state of well-being, the presence of large quantities of potentially allergenic cereal fiber in both diets would probably have obscured any differences.

One type of fiber that may be useful and that is without the allergenic component of a wheat-based fiber is partially hydrolyzed guar gum (PHGG). The guar plant, *Cyamopsis tetragonolobus,* has been grown in India and Pakistan since ancient times. PHGG is a natural water-soluble dietary fiber derived from the guar plant.[7] One study of 134 patients found that consumption of 6 g/day of PHGG decreased the frequency of IBS symptoms such as abdominal spasms, flatulence, and abdominal tension.[8] The researchers concluded that PHGG works well in cases of altered intestinal motility and is easy to use because of its nongelling properties, unlike unhydrolyzed gum, which is much higher in viscosity and more difficult to incorporate into the diet. A recent study has shown that the guar gums when combined with the antibiotic rifaximin (Rifaxam) provide an additional benefit to eradicating dysbiosis in small intestinal bacterial overgrowth (SIBO) associated with IBS.[9] Put simply, for most cases of IBS, nonwheat sources of fiber, such as vegetables and fruits, may be the best choice to help reduce symptoms. For some cases of IBS, especially those with a strong diarrheal component, cooked vegetables in small quantities at first may be most helpful. Because each case is unique, clinical judgment and close patient monitoring are needed. In some cases, fiber may aggravate diarrhea and is therefore contraindicated. For a more detailed explanation of dietary fiber, see Chapter 132.

Food Allergy/Intolerance

The importance of food allergies in the etiology of IBS has been recognized since the early 1900s.[10,11] Later studies have further documented the association between food allergy and the irritable bowel.[12–15] The type of food allergy most significant in IBS is believed to be nonimmunologic, so food intolerance rather than food allergy may be a more appropriate diagnosis. According to double-blind challenge methodologies, the majority of patients with IBS (approximately two thirds) have at least one food intolerance, and some have multiple intolerances.[12] Foods rich in carbohydrates, as well as fatty food, coffee, alcohol, and hot spices, are most frequently reported to cause symptoms.[1] The most common allergens are dairy products (40%–44%) and grains (40%–60%).[14] Because in most cases the reaction appears to be related to prostaglandin synthesis or immunoglobulin G (IgG)–mediated rather than immunoglobulin E (IgE)–mediated reactions, skin tests and the IgE-radioallergosorbent test are poor indicators of food intolerance in these patients. The enzyme-linked immunosorbent assay (ELISA) allergen challenge test or ELISA IgE/IgG_4 may be a better indicator (see

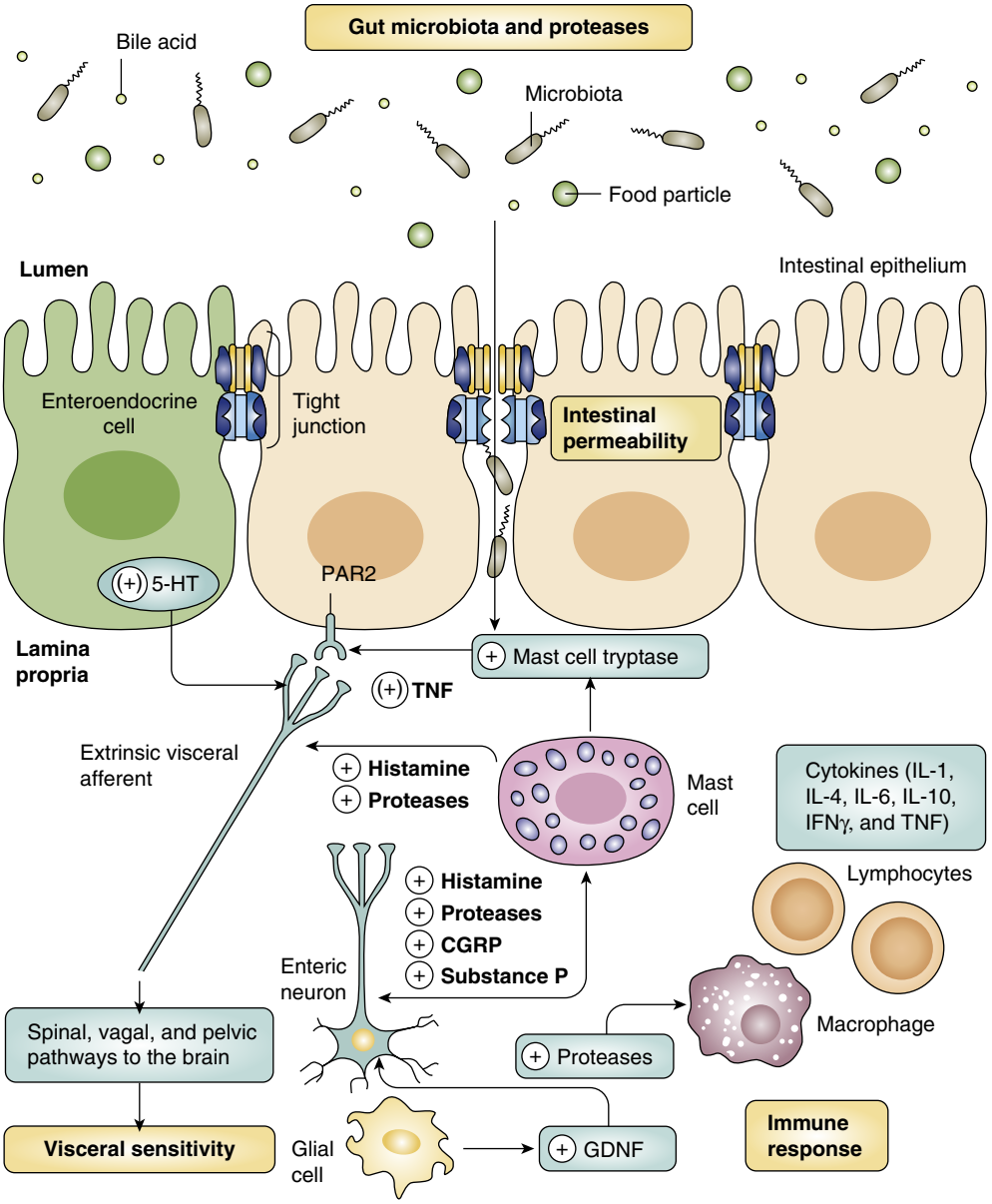


Fig. 189.2 Overview of the pathophysiology of irritable bowel syndrome (IBS). Although the etiology of IBS has not yet been completely elucidated, various factors have a role, including the composition of the gut microbiota, intestinal permeability, immune cell reactivity and sensitivity of the enteric nervous system, the brain–gut axis (spinal, vagal, or pelvic pathways), and the brain. The figure highlights those mediators that are probably involved in IBS pathology. The plus symbols indicate whether a mediator activates or inhibits its target cell; those in parentheses denote actions established in animal models, and those without parentheses are effects demonstrated in humans (human tissue). *5-HT,* 5-hydroxytryptamine (also known as serotonin); *CGRP,* calcitonin gene-related peptide; *GDNF,* glial cell-derived neurotrophic factor; *IL,* interleukin; *PAR2,* proteinase-activated receptor 2; *TNF,* tumor necrosis factor. (Figure and text obtained from Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. *Nat Rev Dis Primers.* 2016;2. https://doi.org/10.1038/nrdp.2016.14; with permission.)

Chapter 14, Food Hypersensitivities), although many sensitivities may still be undetectable by currently available laboratory procedures.[16] Many patients have noted marked clinical improvement with the use of elimination diets.[12-15,17]

Interestingly, many patients with IBS have associated symptoms suggestive of vasomotor instability (e.g., palpitations, hyperventilation, fatigue, excessive sweating, and headaches), which is consistent with food allergy/intolerance reactions.

Sugar

Meals high in refined sugar can contribute to IBS as well as small intestinal bacterial overgrowth by decreasing intestinal motility.[18] When blood glucose levels rise too rapidly, gastrointestinal tract peristalsis slows down. Because glucose is absorbed primarily in the duodenum and jejunum, the message affects this portion of the gastrointestinal tract most strongly. The result is that the duodenum and jejunum become atonic. A diet high in refined sugar may be the

most important reason that IBS is such a common condition in the United States.

Dietary FODMAPs

FODMAPs are short-chain carbohydrates that are osmotically active and fermentable (degradable by intestinal bacteria yielding large amount of gases, like hydrogen or carbon dioxide, thus causing abdominal bloating). FODMAPs include oligosaccharides such as fructans, which are chains of fructose with one glucose molecule on the end. Only minimal amounts of fructans may be absorbed in the human intestine. They may interfere with the absorption of fructose, thus aggravating symptoms in fructose malabsorption. Foods rich in fructans are wheat (white bread, pasta, pastries, cookies), onions, and artichokes; other not commonly problematic foods containing fructans are asparagus, leeks, garlic, chicory roots, and chicory-based coffee substitutes. Fructans with more than 10 molecules of fructose in a chain are known as inulins, and those with fewer than 10 fructoses are referred to as *fructo-oligosaccharides* or *oligofructoses*. Fructans cause problems mainly in fructose malabsorption. Galactans (e.g., stachyose and raffinose) are chains of fructose with one galactose molecule on the end. They act much like fructans. The main foods rich in galactans are legumes (soy, beans, chickpeas, lentils), cabbage, and brussels sprouts.[58-60]

Disaccharides. Lactose (milk sugar). Lactose is found in dairy products, but it may also be found in chocolate and other sweets, beer, preprepared soups and sauces, and so on. Lactose is poorly absorbed by individuals with lactose intolerance, SIBO, and small intestinal inflammation (Crohn's disease, celiac disease).

Monosaccharides. Fructose (fruit sugar). Fructose-rich foods include honey, dried fruits (prunes, figs, dates, or raisins), apples, pears, sweet cherries, peaches, agave syrup, watermelon, and papaya. Fructose is often added to commercial foods and drinks as high-fructose corn syrup. Fructose causes symptoms even in healthy people if ingested in excess and especially in those with fructose malabsorption or SIBO.

Polyols. These are also known as sugar alcohols (appearing as artificial sweeteners in commercial foods and drinks). Sorbitol may appear in "sugar-free chewing gum" and "low-calorie foods"; it appears naturally in stone fruits (peaches, apricots, and plums). Xylitol naturally appears in some berries. A pack of chewing gum containing sorbitol or xylitol may cause bloating or diarrhea in a healthy child and especially in persons with fructose malabsorption or SIBO. Other polyols—like mannitol, isomalt, erythritol, arabitol, erythritol, glycol, glycerol, lactitol, and ribitol—may be problematic in fructose malabsorption and SIBO.

Recent work indicates that FODMAP short-chain carbohydrates that are poorly absorbed in the small intestine are important triggers of functional gut symptoms. Open studies have suggested that three out of four patients with IBS will respond well symptomatically to the restriction of FODMAP intake,[19] as confirmed by a randomized, placebo-controlled rechallenge trial.[20] Breath hydrogen testing helps identify which specific sugars behave as FODMAPs in the individual. Due to a recent series of articles on the low-FODMAP diet in IBS, a number of systematic reviews and meta-analyses have been published, all showing efficacy for reducing IBS symptoms (Table 189.1).[18–20]

Nutritional Supplements

Probiotics

Probiotics are dietary supplements containing live microorganisms that, when ingested, exert a beneficial effect on the host. Among the most commonly used and studied species are *Lactobacillus, Bifidobacterium,* and *Saccharomyces boulardii.* The association between IBS and SIBO is well established.[21] Pimentel and colleagues have demonstrated that successful treatment of SIBO with antibiotics improved IBS symptoms.

The results of a meta-analysis of randomized controlled clinical trials using probiotics to treat IBS symptoms are shown in Figs. 189.3, 189.4, and 189.5.[21] Zhang and colleagues looked at a number of issues related to strain, duration, and dosing of probiotics. The authors report in their methodology section that 1392 randomized clinical trials were eligible using the Rome III criteria for the diagnosis of IBS. Of these, 21 met criteria for inclusion, and 17 were used for data synthesis. IBS symptoms and quality of life were improved for both single strains and multistrain formulations. IBS symptom improvement was not different whether the probiotic was administered for less than or greater than 8 weeks. Likewise, IBS symptom improvement was not different according to whether the probiotic daily dose was greater or less than 10 billion colony forming units.[61]

In summary, probiotics may prove to be key components in the comprehensive approach to treating IBS. Probiotic supplementation appears to be indicated in IBS (see Chapter 105, Probiotics).

Botanical Medicines

Enteric-Coated Volatile Oils

Peppermint oil, and presumably similar volatile oils, inhibits gastrointestinal smooth muscle action in both laboratory animal preparations and humans. Clinically, peppermint oil has been used to reduce colonic spasms during endoscopy,[27] and an enteric-coated peppermint oil (ECPO) capsule has been used in the treatment of IBS.[28] Enteric coating is believed to be necessary because menthol (the major constituent of peppermint oil) and other plant monoterpenes in peppermint oil are rapidly absorbed.[29] This rapid absorption tends to limit effects to the upper intestine, resulting in relaxation of the cardioesophageal sphincter and common side effects, such as esophageal reflux and heartburn, after administration. A transient hot, burning sensation in the rectum during defecation, due to unabsorbed menthol, has been noted by some patients taking the ECPO.

Most of the studies have used ECPO at a dosage of 0.2 mL twice daily between meals.[67] A meta-analysis of five studies supported the efficacy of peppermint oil in IBS.[30] A well-designed study of ECPO involved 110 patients with symptoms of IBS.[31] The patients took one capsule of either ECPO (0.2 mL) or a placebo three to four times daily 15 to 30 minutes before meals for 1 month. The effects of peppermint oil on IBS are summarized in Table 189.2. A summary of the findings of a meta-analysis conducted by the author and colleagues demonstrating the effects of ECPO on the major symptoms of IBS and associated adverse events is provided in Table 189.3. These results are impressive, especially given the safety of ECPO.

Herbal Formulas

For centuries, botanicals have been used for medicinal purposes; they are now a commonly used remedy for IBS. The appeal of botanicals likely stems from their accessibility and perceived safety as "natural" products. Various botanical preparations have been studied in IBS, with varied results. Of the 75 randomized trials for combination herbal therapy identified in one systematic review, only three double-blind, placebo-controlled trials were considered to be high quality. In a study of 116 patients, both standard and individualized combination Chinese herbal medicine significantly improved bowel symptoms. The benefit of individualized herbal treatment was maintained at 14 weeks after completion of treatment. Another study in 208 patients demonstrated efficacy with the herbal preparations STW 5 and STW 5-II.[32] The most recent of the three trials randomized 119 patients to a standard preparation of traditional Chinese medicine extracts containing 11 herbs or

TABLE 189.1 Studies of Randomized Trials of Low FODMAPs Dietary Intervention in Irritable Bowel Syndrome

First Author, Year, Reference No.	Country	Design	Sample Size	Intervention/Time	Definition of Response/Primary Outcome
Bohn, 2014[69]	Sweden	Parallel, multicenter	82	LFD vs. THD diet 4 weeks No follow-up	>50% reduction in IBS-SSS. LFD reduced the IBS-SSS from 337 (287–382) (median [25th–75th percentile]) to 231 (154–350) ($p = .001$). THD reduced the symptom score from 312 (250–346) to 240 (171–296) ($p < .001$). No difference in reduction.
Bohn, 2015[70]	Sweden	Parallel, single center	75	LFD vs. NICE diet guidelines 4 weeks No follow-up	>50% reduction in IBS-SSS. Change in IBS-SSS LFD vs. NICE was similar between groups ($P = 0.62$).
Eswaran, 2016[71]	USA	Parallel, single center	92	LFD vs. NICE diet guidelines 4 weeks No follow-up	Adequate relief of symptoms >50% of last 2 weeks of study. 52% of the LDF vs. 41% of the NICE group reported adequate relief of their IBS-D symptoms ($P = 0.31$).
Halmos, 2014[72]	Australia	Crossover with washout at least 21 days, single center	30	LFD vs. typical Australian diet 3 weeks of each diet with ≥3-week washout	10-mm difference on 100-mm VAS scale considered clinically significant. LFD had significant improvements in global symptoms compared with control diet for IBS (all $P < 0.001$).
McIntosh, 2017[73]	UK	Parallel, single center	40	LFD vs. HFD 3 weeks No follow-up	Symptoms were assessed using the IIBS-SSS. The IBS-SSS was reduced in the low-FODMAP diet group ($p < 0.001$) but not the high-FODMAP group.
Laatikainen, 2016[74]	UK	Crossover design, single center 4 weeks	87	Low-FODMAP (LF) rye bread vs. High-FODMAP (HF) rye bread.	IBS-SSS and VAS were used to assess individual symptoms. Abdominal pain, wind, and bloating improved on LF rye ($p < 0.05$) but IBS-SSS no different vs. HF rye.
Ong, 2010[62]	Australia	Crossover with washout, single center 7 days	15	LFD vs HFD 2 days No follow-up	Composite scale of GI symptoms based on 3-point Likert scale. HFD has higher scalae of symptoms (wind, abdominal pain, bloating; median 6; range 1–9) vs. LFD (2, 0–7), $p = 0.002$.
Pedersen, 2014[63]	Denmark	Parallel, single center	123 Web-based app	Dietary advice or probiotics: Intervention = LFD Control = probiotic (LGG)[a] or normal Danish diet 6 weeks No follow-up	>50% reduction in IBS-SSS. Reduction of IBS-SSS for LFD vs. control at 6 weeks (75; 95% CI 24–126; $P < 0.01$) but not for LGG vs. control (32; 95% CI 18–80; $P = 0.2$).
Peters, 2016[64]	UK	Parallel, single center	74	Gut-directed hypnotherapy, LFD vs. both. 6 weeks therapy 6 months follow-up	>20-mm improvement on 100-mm VAS for GI symptoms. Improvements in overall symptoms were observed from baseline to week 6 for hypnotherapy (mean difference [95% CI]: –33 [–41 to –25]), diet (–30 [–42 to –19]), and combination (–36 [–45 to –27]]). There were no differences across groups ($P = 0.67$).
Staudacher, 2012[65]	UK	Parallel, single center	41	LFD vs. Australian habitual diet 4 weeks No follow-up	Global symptoms rating scale not defined. Low-FODMAP diet had significant improvements in global symptoms ($P = 0.006$).
Staudacher, 2017[66]	UK	Crossover 2 × 2 factorial design, 2 centers	104	LFD or sham diet (placebo) vs. probiotic VSL#3 or placebo 4 weeks Follow-up period unclear	Mean IBS-SSS scores were lower for patients on the LFD (173±95) than sham (224 ± 89) ($P = .001$). LFD had a trend toward adequate symptom relief (57%) vs. the sham diet group (38%) ($P = .051$).

CI, Confidence interval; *FODMAP,* fermentable oligo-, di-, and monosaccharides and polyols; *GI,* gastrointestinal; *HFD,* high-fat diet; *IBS,* irritable bowel syndrome; ; *IBS-D,* IBS with diarrhea; *LFD,* low-fat diet; *LGG, Lactobacillus rhamnosus* GG; *SSS,* symptom severity scoring; *THD,* traditional habitual diet; *VAS,* visual analog scale.

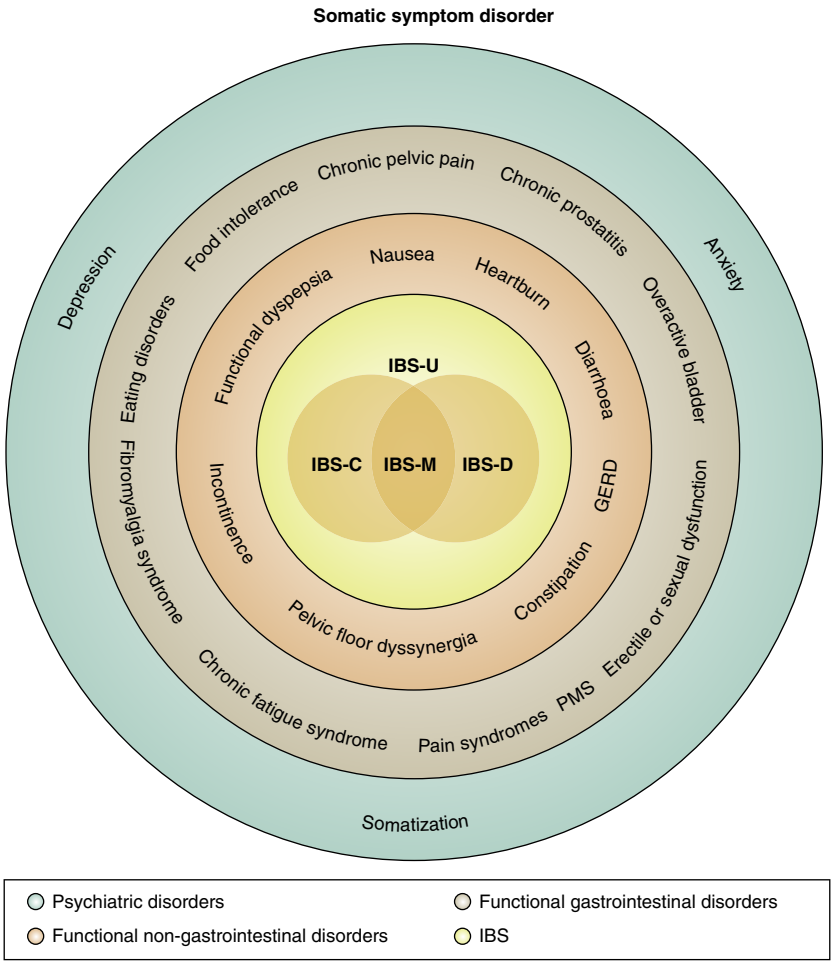


Fig. 189.3 Conditions associated with irritable bowel syndrome (IBS). A model of IBS and its associations with other clinical, intestinal, extraintestinal, and psychiatric conditions. *GERD,* gastroesophageal reflux disease; *IBS-C,* IBS with constipation; *IBS-D,* IBS with diarrhea; *IBS-M,* mixed-type IBS; *IBS-U,* unsubtyped IBS; *PMS,* premenstrual syndrome. (Figure and text obtained from Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. *Nat Rev Dis Primers*. 2016;2. https://doi.org/10.1038/nrdp.2016.14; with permission.)

placebo. However, the use of this herbal formulation for diarrhea-predominant IBS did not improve symptoms.

Although no research has been done to document its efficacy, an old naturopathic remedy called Robert's formula has a long history of use in this condition. The Tibetan herbal formula known as Padma Lax also showed efficacy for patients with constipation-predominant IBS in one 3-month double-blind, randomized pilot study. A small number of patients did have loose stools with this formula, but this effect was easily remedied by lowering the dosage.[33]

Miscellaneous Considerations

Psychological Factors

Mental/emotional problems—anxiety, fatigue, hostile feelings, depression, and sleep disturbances—are reported by almost all patients with IBS.[34] The severity and frequency of such symptoms tend to correlate with these psychological factors. Anxiety predicts a high degree of food-related symptoms in IBS.[1] Especially significant is sleep quality; poor sleep quality results in a rise in symptom severity.[35]

Several theories link psychological factors with the symptoms of IBS. The "learning model" holds that when exposed to stressful situations, some children learn to develop gastrointestinal symptoms to cope with the stress. Another theory holds that IBS is a manifestation of depression, chronic anxiety, or both. Personality assessments of IBS sufferers have shown them to have higher anxiety levels and a greater feeling of depression.[36] However, these studies were based on postmorbid personality assessments, and it has since been determined, with premorbid personality assessment, that IBS sufferers have normal personality profiles. Therefore many of the psychological symptoms may be either secondary to the bowel disturbances (particularly malabsorption) or due to a common etiological factor (e.g., stress, food allergy, or gut microbial dysbiosis; Figs. 189.6 and 189.7).[68]

Greater colonic motility during exposure to stressful situations has been shown to occur in both normal subjects and sufferers of IBS.[37] This finding apparently accounts for the greater abdominal pain and irregular bowel function seen in patients with IBS and normal subjects during periods of emotional stress. Distressed patients with IBS are also known to have decreased immune function, as evidenced by lower percentages of activated T cells and natural killer cells.[38] This finding suggests that patients with long-term IBS have lowered immune function, which may leave them susceptible to a host of other illnesses. Some researchers believe that IBS sufferers have difficulty adapting to life events, although this belief has not been well demonstrated in clinical studies. Psychotherapy, in the form of relaxation therapy,[39] biofeedback,[40] hypnosis,[40] counseling,[41] or stress-management training,[42] has been shown to reduce symptom frequency and severity and to enhance the results of standard medical treatment of IBS. Hypnosis, which the

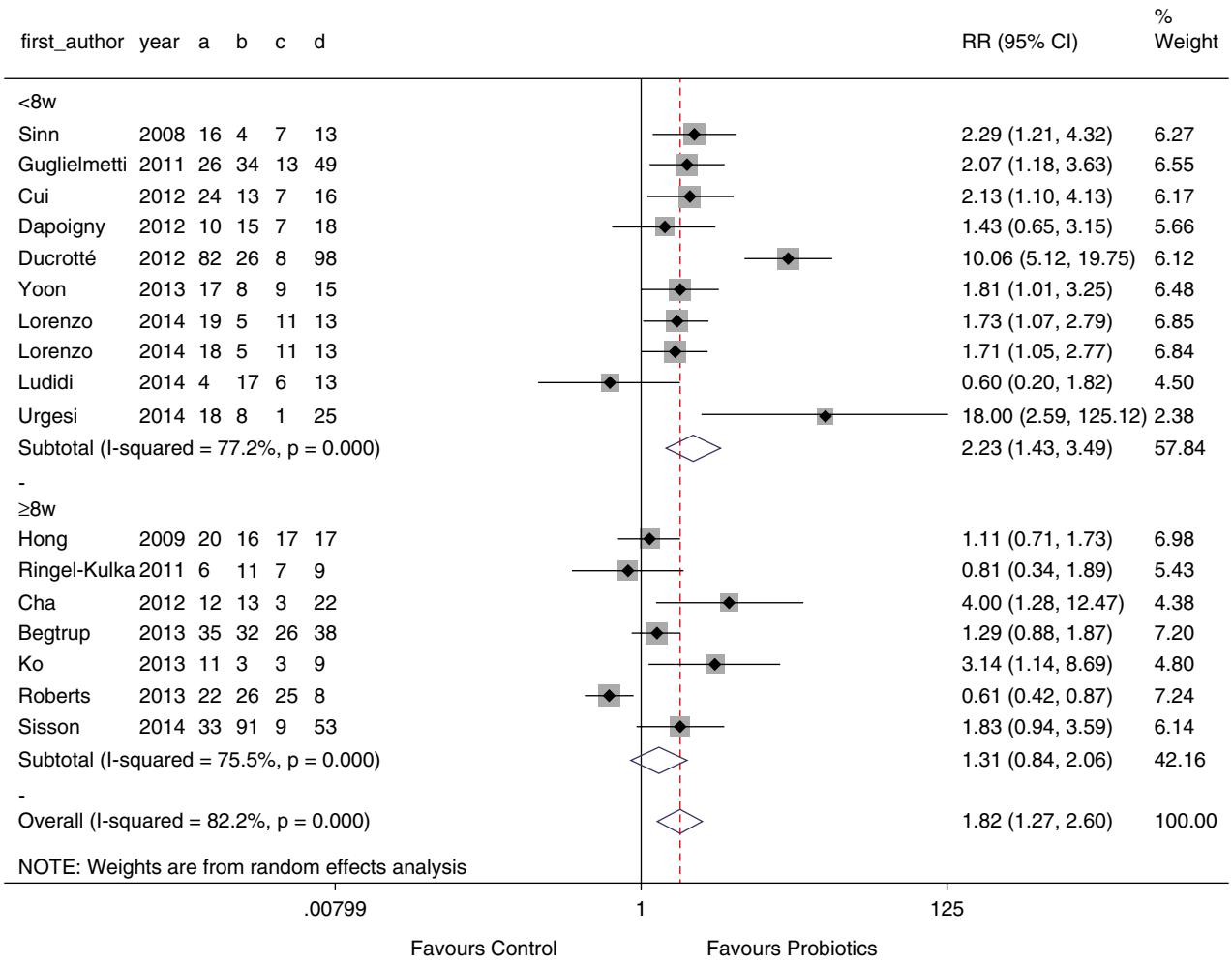


Fig. 189.4 Forrest plot of overall symptom response in patients with irritable bowel syndrome (IBS): subgroup probiotics duration. Overall, probiotics improved symptoms response in patients with IBS (n = 17 studies, relative risk [RR] 1.82, 95% confidence interval [CI] [1.27, 2.60]). Subgroup analysis shows that patients with IBS have improved symptom response irrespective of the duration of probiotics; intervention with benefit was demonstrated in patients receiving treatment for less than 8 weeks or greater than 8 weeks. (Figure obtained from Zhang Y, Li L, Guo C, Mu D, Feng B, Zuo X, Yanging L. Effects of probiotic type, dose, and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. *BMC Gastroenterology*. 2016;16:62. https://doi.org/10.1186/s12876-016-0470-z.)

patient may practice at home using simple compact disc recording equipment,[43] has been shown to reduce both fasting colonic motility[44] and rectal sensitivity.[45] In contrast to psychotherapeutic modalities, the use of anxiolytic drugs, a combination of tranquilizers and antispasmodics, or antidepressants has not yielded effective results.

Physical Medicine

An increase in physical exercise also appears helpful for patients with IBS. Many find that daily leisurely walks markedly reduce symptoms, probably owing to the known stress-reducing effects of exercise. A specific massage therapy known as foot reflexology was evaluated in a small study of 34 individuals; no benefit for patients with IBS was found.[46]

THERAPEUTIC APPROACH

Because IBS is a multifactorial disease, the approach to the patient requires consideration and integration of the following factors:

- Dietary fiber
- Determination and elimination of food allergies/intolerances
- Stress reduction
- Exercise

As necessary, peppermint oil and Robert's formula may be used to temporarily ameliorate symptoms. Also, because the diagnosis is typically made by exclusion, a careful diagnostic evaluation is always indicated.

Diet

Fiber-rich foods (see Chapter 132, Dietary Fiber) are to be increased, and foods that are allergenic, contain refined sugar, or are highly processed are to be eliminated, along with FODMAPs.

Supplements

Lactobacillus acidophilus: 1 to 2 billion live organisms per day

Botanical Medicine

Enteric-coated volatile oil preparations (e.g., peppermint oil): 0.2 mL caps once to twice daily between meals is the recommended protocol

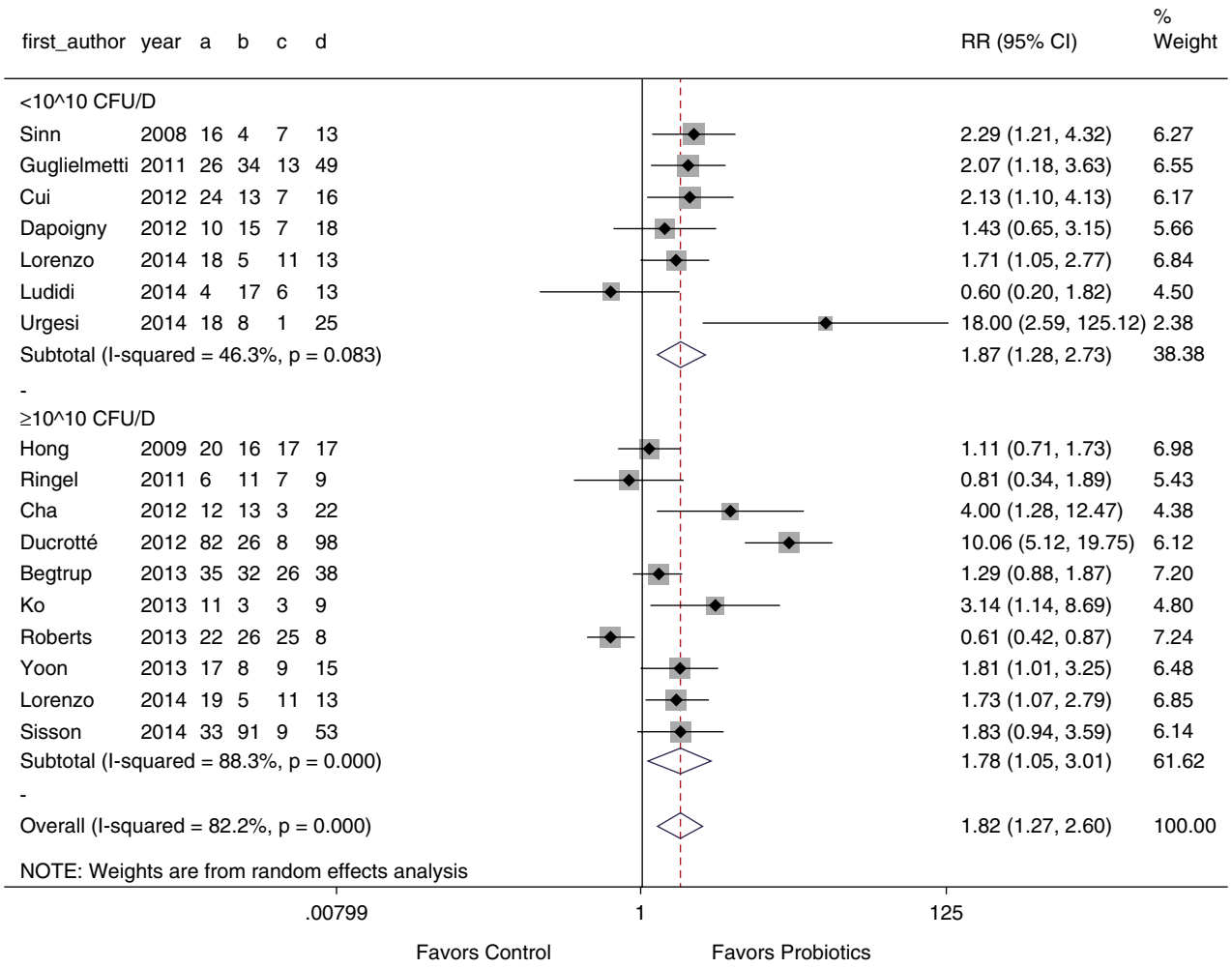


Fig. 189.5 Forrest plot of overall symptom response in patients with irritable bowel syndrome (IBS): subgroup probiotics dose. Overall, probiotics improved symptoms response in patients with IBS (n = 17 studies, relative risk [RR] 1.82, 95% confidence interval [CI)] [1.27, 2.60]). Subgroup analysis shows that patients with IBS have improved symptom response irrespective of the dose of probiotics; intervention with benefit was demonstrated in patients receiving treatment of less than 10^{41} colony-forming units (CFU) per day (n = 7 studies, RR 1.87, 95% CI [1.28, 2.73]) or greater than 10^{41} CFU per day (n = 10 studies, RR 1.78, 95% CI [1.05, 2.60]). (Figure obtained from Zhang Y, Li L, Guo C, Mu D, Feng B, Zuo X, Yanging L. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. *BMC Gastroenterology*. 2016;16:62. https://doi.org/10.1186/s12876-016-0470-z.)

TABLE 189.2 Characteristics of Included Studies of Enteric-Coated Peppermint Oil for Irritable Bowel Syndrome

Year	Author	Country	Design	Setting	*N* Enrolled	*N* Completed	Duration of therapy	Grade Quality of Evidence
2013	Alam[47]	Bangladesh	Double-blind RCT	University single-center	74	65	6 weeks	3
2016	Cash[48]	USA	Double-blind RCT	Multicenter	72	70	4 weeks	4
2005	Cappanni[49]	Italy	Double-blind RCT	University single-center	178	173	12 weeks	4
2007	Cappello[50]	Italy	Double-blind RCT	University single-center	57	50	4 weeks	4
1989	Carling[51]	Sweden	Double-blind, crossover, 3-arm RCT 1-week washout	2 university centers	40	38	2 weeks	3

TABLE 189.2 Characteristics of Included Studies of Enteric-Coated Peppermint Oil for Irritable Bowel Syndrome—cont'd

Year	Author	Country	Design	Setting	*N* Enrolled	*N* Completed	Duration of therapy	Grade Quality of Evidence
1984	Dew[52]	Wales	Double-blind cross over Washout period	Multicenter	29	29	2 weeks	2
1988	Lech[53]	Dutch	Double-blind RCT	University single-center	47	42	4 weeks	3
1997	Liu[54]	China	Double-blind RCT	University single-center	110	101	4 weeks	3
2009	Merat[55]	Iran	Double-blind RCT	University single- center	90	60	8 weeks	3
1979	Rees[29]	UK	Double-blind crossover Washout period Defined by recurrence of active symptoms	University single- center	18	16	3 weeks	2
1990	Schneider[56]	USA	Double-blind crossover RCT 2-week washout	University single- center	60	47	6 weeks	2
1988	Weiss[57]	Germany	Single-blind RCT	Hospital, single center	60	46	3 weeks	2

RCT, Randomized controlled trial.

TABLE 189.3 Summary of Findings for Enteric-Coated Peppermint Oil in Irritable Bowel Syndrome

Peppermint Oil Versus Placebo for Treatment of IBS
Patient or Population: Patients With Active IBS
Settings: Outpatients
Intervention: Enteric-Coated Peppermint Oil Capsules Versus Placebo

Illustrative Comparative Risk[a]

(95% CI)

Assumed

	RISK			CORRESPONDING RISK		
Outcomes NNT	Control	Peppermint Oil Versus Placebo	Risk Ratio (95% CI)	No. Participants (Studies)	Quality of the Evidence (GRADE)	
Global improvement in IBS symptoms	250 per 1000[b]	598 per 1000 (483–743)	RR 2.39(1.93–2.97)	507 (7 studies)	⊕⊕⊕⊕ High[c]	3 (2–4)
Improvement in abdominal	303 per	539 per 1000 (433–666)	RR 1.78 (1.43–2.20)	556 (6 studies)	⊕⊕⊕◯	4 (3–8)
pain	1000[b]				Moderate[d]	
Adverse events	21 per 1000[b]	29 per 1000 (18–47)	RR 1.40 (0.87–2.26)	671(8 studies)	⊕⊕◯◯ Low[e]	125 (29–∞)

GRADE Working Group grades of evidence: *high quality,* further research is very unlikely to change our confidence in the estimate of effect; *moderate quality,* further research is likely to have an important effect on our confidence in the estimate of effect and may change the estimate; *low quality,* further research is very likely to have an important effect on our confidence in the estimate of effect and is likely to change the estimate; *very low quality* we are very uncertain about the estimate.

[a]The basis for the assumed risk (e.g., the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

[b]Control group risk estimates come from control arm of meta-analysis, based on included trials.

[c]⊕⊕⊕⊕ High: downgraded on risk of bias, upgraded on large magnitude of effect.

[d]⊕⊕⊕◯ Moderate: downgraded on risk of bias.

[e]⊕⊕◯◯ Low: downgraded on risk of bias and imprecision.

CI, Confidence interval; *IBS,* irritable bowel syndrome; *NNT,* number needed to treat; *RR,* relative risk.

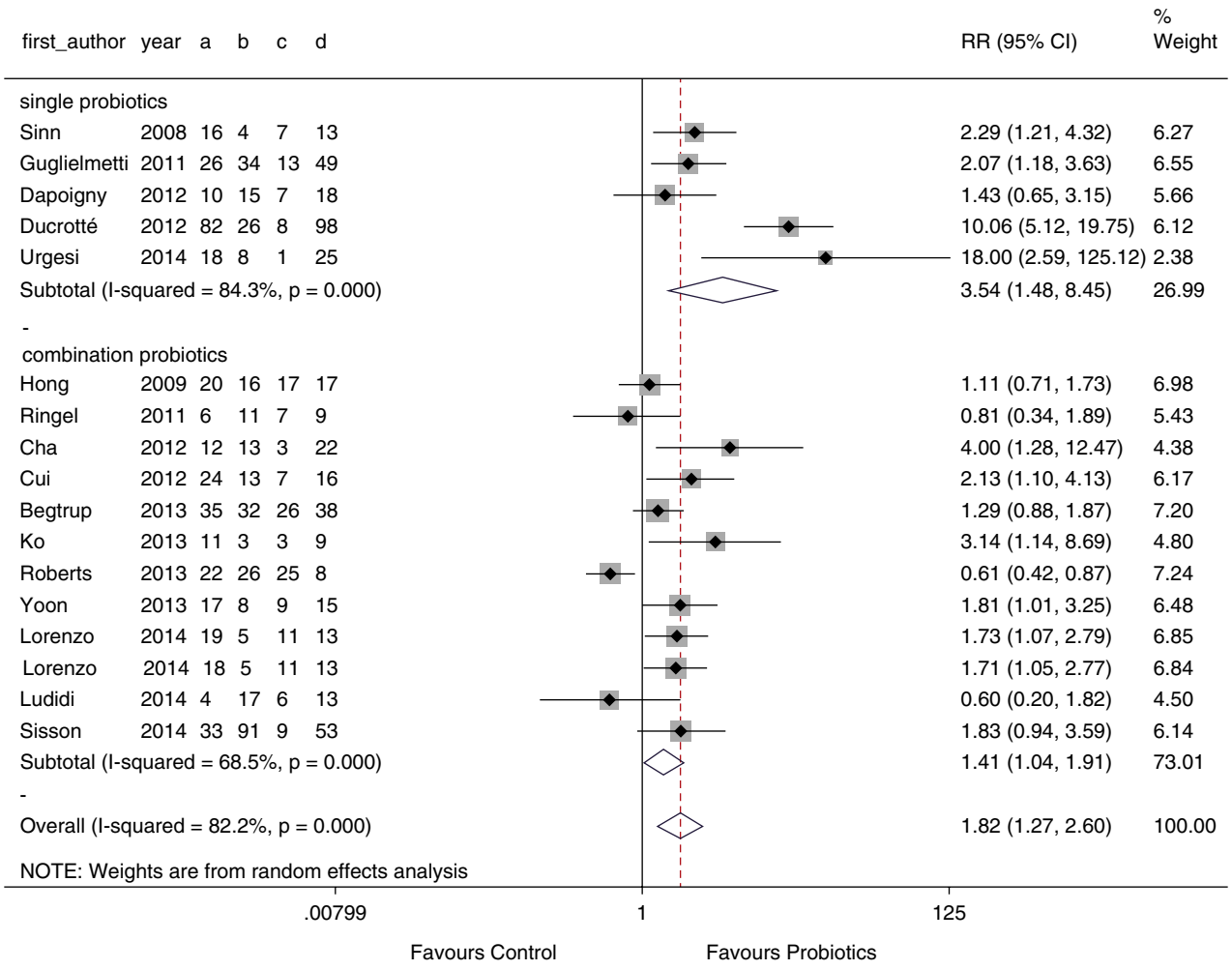


Fig. 189.6 Forrest plot of overall symptom response in patients with irritable bowel syndrome (IBS): subgroup probiotics duration. Overall, probiotics improved symptom response in patients with IBS (n = 17 studies, relative risk [RR] 1.82; 95% confidence interval [CI] [1.27, 2.60]). Subgroup analysis shows that patients with IBS have improved symptom response irrespective of the strain of probiotics used for the intervention. Benefit was demonstrated in patients receiving treatment with single strain (n = 5, RR 3.54, 95% CI [1.48, 8.45]) or multistrain probiotics (n = 12, RR 1.41, 95% CI [1.04, 1.91]). (Figure obtained from Y, Li L, Guo C, Mu D, Feng B, Zuo X, Yanging L. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. *BMC Gastroenterology*. 2016;16:62. https://doi.org/10.1186/s12876-016-0470-z.)

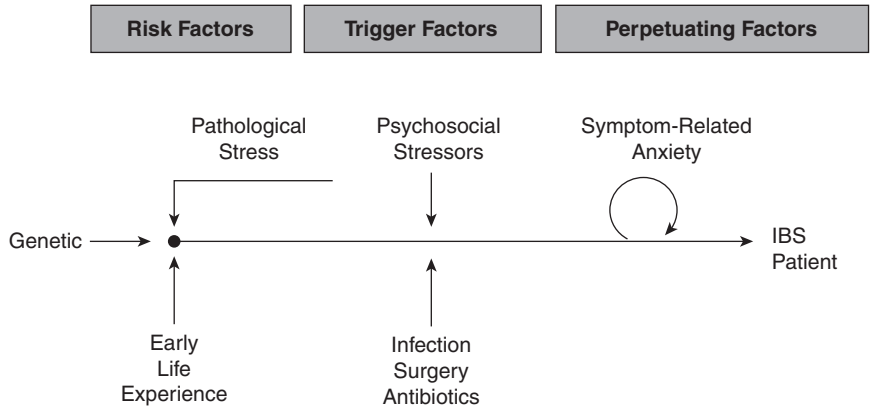


Fig. 189.7 Role of stress in the development and modulation of irritable bowel syndrome (IBS) symptoms. Different types of stressors may play a role in the permanent biasing of stress responsiveness, in transient activation of the stress response, and in the persistence of symptoms. (Figure and caption reprinted with permission from Mayer EA, Naliboff BD, Chang L, Coutinho SV. Stress and irritable bowel syndrome. *Am J Phy-Gastroint Liver Physiol*. 2001;280:G519–G524. https://doi.org/10.1152/ajpgi.2001.280.4.G519.)

Physical Therapy

The patient may be advised to take leisurely 20-minute walks daily.

Counseling

The clinician should help the patient develop an effective stress reduction program. Biofeedback may be particularly useful for patients with IBS.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Simren M, Mansson A, Langkilde AM, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion*. 2001;63:108–115.
2. Spiro H. *Clinical Gastroenterology*. 3rd ed. New York: Macmillan; 1983:713–735.
3. Longstreth GF. Irritable bowel syndrome: a multibillion-dollar problem. *Gastroenterol*. 1995;109:2029–2031.
4. Fielding JF. Detailed history and examination assist positive clinical diagnosis of the irritable bowel syndrome. *J Clin Gastroenterol*. 1983;5:495–497.
5. Cann PA, Read NW, Holdsworth CD. What is the benefit of coarse wheat bran in patients with irritable bowel syndrome? *Gut*. 1984;25:168–173.
6. Fielding JF, Kehoe M. Different dietary fibre formulations and the irritable bowel syndrome. *Ir J Med Sci*. 1984;153:178–180.
7. Slavin JL, Greenberg NA. Partially hydrolyzed guar gum: clinical nutrition uses. *Nutrition*. 2003;19:549–552.
8. Giaccari S, Grasso G, Tronci S, etal. Partially hydrolyzed guar gum: a fiber as coadjuvant in the irritable colon syndrome. *Clin Ter*. 2001;152:21–25.
9. Furnari M, Furnari A, Parodi, L, et al. The combination of rifaximin with partially hydrolysed guar gum is more effective than rifaximin alone in eradicating small intestinal bacterial overgrowth. *Aliment Pharm Ther*. epub August 22, 2010.
10. Hollander E. Mucous colitis due to food allergy. *Am J Med Sci*. 1927;174:495–500.
11. Gay L. Mucous colitis, complicated by colonic polyposis, relieved by allergic management. *Am J Dig Dis*. 1937;3:326–329.
12. Jones VA, McLaughlan P, Shorthouse M, et al. Food intolerance. A major factor in the pathogenesis of irritable bowel syndrome. *Lancet*. 1982;2:1115–1117.
13. Petitpierre M, Gumowski P, Girard JP. Irritable bowel syndrome and hypersensitivity to food. *Ann Allergy*. 1985;54:538–540.
14. Nanda R, James R, Smith H, et al. Food intolerance and the irritable bowel syndrome. *Gut*. 1989;30:1099–1104.
15. Gertner D, Powell-Tuck J. Irritable bowel syndrome and food intolerance. *Practitioner*. 1994;238:499–504.
16. Zwetchkenbaum J, Burakoff R. The irritable bowel syndrome and food hypersensitivity. *Ann Allergy*. 1988;61:47–49.
17. Drisko JA, Bischoff B, Hall MA, et al. Treating irritable bowel syndrome with a food elimination diet followed by food challenge and probiotics. *Am J Gastroenterol*. 2003;98:S276: From an abstract submitted for the 68th Annual Scientific Meeting of the American College of Gastroenterology.
18. Russo A, Fraser R, Horowitz M. The effect of acute hyperglycemia on small intestinal motility in normal subjects. *Diabetologia*. 1996;39:984–989.
19. Shepherd SJ, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. *J Am Diet Assoc*. 2006;106:1631–1639.
20. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomised, placebo-controlled evidence. *Clin Gastroenterol Hepatol*. 2008;6:765–771.
21. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol*. 2000;95(12):3503–3506.
22. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and Bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 2005;128(3):541–551.
23. Whorwell PJ, Altringer I, Morel J, et al. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol*. 2006;101(7):1581–1590.
24. Gawronska A, et , Dziechciarz P, Horvath A. A randomized double-blind placebo-controlled trial of Lactobacillus GG for abdominal pain disorders in children. Aliment Pharmacol Ther. 2007;25(2):177–184
25. Kajander K, Hatakka T, Poussa M, et al. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Aliment Pharmacol Ther*. 2005;22(5):387–394.
26. Kim HJ, Vazquez Roque MI, Camilleri M, et al. A randomized controlled trial of a probiotic combination VSL#3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil*. 2005;17(5):687–696.
27. Leicester RJ, Hunt RH. Peppermint oil to reduce colonic spasm during endoscopy. *Lancet*. 1982;2:989
28. Somerville KW, Richmond CR, Bell GD. Delayed release peppermint oil capsules (Colpermin) for the spastic colon syndrome. A pharmacokinetic study. *Br J Clin Pharmacol*. 1984;18:638–640.
29. Rees WD, Evans BK, Rhodes J. Treating irritable bowel syndrome with peppermint oil. *Br Med J*. 1979;2:835–836.
30. Pittler MH, Ernst E. Peppermint oil for irritable bowel syndrome: a critical review and metaanalysis. *Am J Gastroenterol*. 1998;93:1131–1135.
31. Liu JH, Chen GH, Yeh HZ, et al. Enteric-coated peppermint oil capsules in the treatment of irritable bowel syndrome: a prospective, randomized trial. *J Gastroenterol*. 1997;32:765–768.
32. Madisch A, Holtmann G, Plein K, et al. Treatment of irritable bowel syndrome with herbal preparations: results of a double-blind, randomized, placebo-controlled, multi-centre trial. *Aliment Pharmacol Ther*. 2004;19(3):271–279.
33. Sallon S, Ben-Arye E, Davidson R, et al. A novel treatment for constipation-predominant irritable bowel syndrome using Padma Lax, a Tibetan herbal formula. *Digestion*. 2002;65:161–171.
34. Svedlund J, Sjodin I, Dotevall G, et al. Upper gastrointestinal and mental symptoms in the irritable bowel syndrome. *Scand J Gastroenterol*. 1985;20:595–601.
35. Goldsmith G, Levin JS. Effect of sleep quality on symptoms of irritable bowel syndrome. *Dig Dis Sci*. 1993;38:1809–1814.
36. Ryan WA, Kelly MG, Fielding JF. The normal personality profile of irritable bowel syndrome patients. *Ir J Med Sci*. 1984;153:127–129.
37. Narducci F, Snape Jr WJ, Battle WM, et al. Increased colonic motility during exposure to a stressful situation. *Dig Dis Sci*. 1985;30:40–44.
38. Motzer SA, Jarrett M, Heitkemper MM, et al. Natural killer cell function and psychological distress in women with and without irritable bowel syndrome. *Biol Res Nurs*. 2002;4:31–42.
39. Blanchard EB, Greene B, Scharff L, et al. Relaxation training as a treatment for irritable bowel syndrome. *Biofeedback Self Regul*. 1993;18:125–132.
40. Goldsmith G, Patterson M. Irritable bowel syndrome. Treatment update. *Am Fam Physician*. 1985;31:191–195.
41. Schwarz SP, Taylor AE, Scharff L, et al. Behaviorally treated irritable bowel syndrome patients: a four-year follow-up. *Behav Res Ther*. 1990;28:331–335.
42. Shaw G, Srivastava ED, Sadlier M, et al. Stress management for irritable bowel syndrome. A controlled trial. *Digestion*. 1991;50:36–42.
43. Palsson OS, Turner MJ, Whitehead WE. Hypnosis home treatment for irritable bowel syndrome: a pilot study. *Int J Clin Exp Hypn*. 2006;54(1):85–99.
44. Whorwell PJ, Houghton LA, Taylor EE, et al. Physiological effects of emotion: assessment via hypnosis. *Lancet*. 1992;340:69–72.
45. Prior A, Colgan SM, Whorwell PJ. Changes in rectal sensitivity after hypnotherapy in patients with irritable bowel syndrome. *Gut*. 1990;31:896–898.
46. Tovey P. A single-blind trial of reflexology for irritable bowel syndrome. *Br J Gen Pract*. 2002;52:19–23.
47. Alam MS, Roy PK, Miah AR, et al. Efficacy of Peppermint oil in diarrhea predominant IBS—a double blind randomized placebo-controlled study. *Mymensingh Med J MMJ*. 2013;22(1):27–30.
48. Cash B, Epstein M, Shah S. A novel Delivery system of peppermint oil is an effective therapy for irritable bowel syndrome symptoms. *Digestive Dis Sci*. 2016;61(2):560–571. http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/878/CN-01166878/frame.html https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4729798/pdf/10620_2015_Article_3858.pdf. Accessed 14 Dec 2019.
49. Capanni M, Surrenti E, Biagini MR, Milani S, Surrenh C, Galli A. Efficacy of peppermint oil in the treatment of irritable bowel syndrome: a randomized, controlled trial. *Gazz Med Ital Arch Sci Med*. 2005;164(2):119–126. http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/601/CN-01262601/frame.html. Accessed 14 Dec 2019.
50. Cappello G, Spezzaferro M, Grossi L, Manzoli L, Marzio L. Peppermint oil (Mintoil((R))) in the treatment of irritable bowel syndrome: a pro-

spective double blind placebo-controlled randomized trial. *Dig Liver Dis*. 2007;39(6):530–536.
51. Carling L, Svedberg LE, Hulten S. Short term treatment of the irritable bowel syndrome: a placebo-controlled trial of peppermint oil against hyoscyamine. *Opuscula Med*. 1989;34(3):55–57.
52. Dew MJ, Evans BK, Rhodes J. Peppermint oil for the irritable bowel syndrome - a Multicenter trial. *Br J Clin Pract*. 1984;38(11–1):394.
53. Lech Y, Olesen KM, Hey H, Rask-Pedersen E, Vilien M, Ostergaard O. Treatment of irritable bowel syndrome with peppermint oil. A double-blind investigation with a placebo. *Ugeskrift Laeger*. 1988;150(40):2388–2389.
54. Liu JH, Chen GH, Yeh HZ, Huang CK, Poon SK. Enteric-coated peppermint-oil capsules in the treatment of irritable bowel syndrome: a prospective, randomized trial. *J Gastroenterol*. 1997;32(6):765–768.
55. Merat S, Khalili S, Mostajabi P, Ghorbani A, Ansari R, Malekzadeh R. The effect of enteric-coated, delayed-release peppermint oil on irritable bowel syndrome. *Digestiv Dis Sci*. 2010;55(5):1385–1390.
56. Schneider MMEOM. Efficacy of Colpermin in the treatment of patients with irritable bowel syndrome. *Gastroenterology*. 1990;98.
57. Weiss H, Rest J, Limbach HJ. Vitamin D3 level and age. Changes in the 1,25-dihydroxycholecalciferol level in various age groups in normal persons, in patients with osteoporosis, renal failure and liver cirrhosis. *Med Klin (Munich)*. 1988;83(20):660–666.
58. Altobelli E, et al. Low-FODMAP diet improves irritable bowel syndrome symptoms: a meta-analysis. *Nutrients*. 2017;9(9).
59. Schumann D, et al. Low fermentable, oligo-, di-, mono-saccharides and polyol diet in the treatment of irritable bowel syndrome: a systematic review and meta-analysis. *Nutrition*. 2018;45:24–31.
60. Singh R, et al. The role of diet in the treatment of irritable bowel syndrome: a systematic review. *Gastroenterol Clin North Am*. 2018;47(1):107–137.
61. Zhang Y, et al. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. *BMC Gastroenterol*. 2016;16(1):62.
62. Ong DK, Mitchell SB, Barrett JS, et al. Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. *J Gastroenterol Hepatol*. 2010;25(8):1366–1373.
63. Pedersen N, Vegh Z, Burisch J, et al. Ehealth monitoring in irritable bowel syndrome patients treated with low fermentable oligo-, di-, mono-saccharides and polyols diet. *World J Gastroenterol*. 2014;20(21):6680–6684.
64. Peters SL, Yao CK, Philpott H, Yelland GW, Muir JG, Gibson PR. Randomised clinical trial: the efficacy of gut-directed hypnotherapy is similar to that of the low FODMAP diet for the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther*. 2016;44(5):447–459.
65. Staudacher HM, Lomer MC, Anderson JL, et al. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J Nutri*. 2012;142(8):1510–1518.
66. Staudacher HM, Lomer MCE, Farquharson FM, et al. Diet low in FODMAPs reduces symptoms in patients with irritable bowel syndrome and probiotic Restores Bifidobacterium species: a randomized controlled trial. *Gastroenterology*. 2017.
67. Bensoussan A, Talley NJ, Hing M, et al. Treatment of irritable bowel syndrome with Chinese herbal medicine: a randomized controlled trial. *JAMA*. 1998;280(18):1585–1589.
68. Mayer EA, et al. V. Stress and irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol*. 2001;280(4):G519–G524.
69. Böhn L, Störsrud S, LiIjebo T, Collin L, Törnblom H, Simrén M. A multi-center, randomized, controlled, single-blind, comparative trial: low-FODMAP diet versus traditional dietary advice in IBS. *United Eur Gastroenterol J*. 2014;2(1):A3.
70. Böhn L, Störsrud S, Liljebo TM, Lindfors P, Törnblom H, Simren M. A randomized, controlled trial comparing a diet low in FODMAPs with traditional dietary advice in patients with IBS. *Gastroenterology*. 2015;148(4):S654.
71. Eswaran SL, Chey WD, Han-Markey T, Ball S, Jackson K. A randomized controlled trial comparing the low FODMAP diet vs. Modified NICE guidelines in US adults with IBS-D. *Am J Gastroenterol*. 2016;111(12):1824–1832.
72. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology*. 2014;146(1):67–75. e65.
73. McIntosh K, Reed DE, Schneider T, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut*. 2017;66(7):1241–1251.
74. Laatikainen R, Koskenpato J, Hongisto SM, et al. Randomised clinical trial: low-FODMAP rye bread vs. regular rye bread to relieve the symptoms of irritable bowel syndrome. *Aliment Pharmacol Ther*. 2016;44(5):460–470.

Kidney Disease

Thomas A. Kruzel, MT, ND

OUTLINE

DIAGNOSTIC SUMMARY

- General symptoms of fatigue, malaise, lethargy, decreased mental sharpness
- Skin may become ruddy, dry, itchy
- Edema, loss of appetite, nausea, vomiting, polyneuropathy, chest pain, dyspnea, muscle cramping, insomnia, electrolyte imbalance, unintentional weight loss, changes in urination
- Microalbuminuria with a normal glomerular filtration rate (GFR) in early disease
- Hypertension; abnormal heart and lung sounds
- Change in slope of serum creatinine levels over time correlates with disease process
- Worsening of GFR, casts, albumin, blood on urinalysis
- Progressive if not addressed, resulting in end-stage renal failure

GENERAL CONSIDERATIONS

Physiology of Kidney Function

Human kidneys are described as bean shaped and lie in the retroperitoneal space near the vertebrae. An adult kidney weighs approximately 125 to 170 g in males and between 115 and 155 g in females, and the kidneys are about 11 cm (4.3 in.) in length.

About 20% of the plasma entering the glomerulus is filtered at any given moment, with the kidneys filtering about 180 liters/day (125 mL/min). This means that approximately 3 liters of blood plasma is filtered 60 times/day, which allows the kidneys to maintain close control on the internal environment. The kidneys, in conjunction with the lungs, maintain tight control on blood pH to preserve the delicate balance between metabolic acidosis and alkalosis.

The renal corpuscles filtration barrier consists of three layers composed of the capillary endothelium, a basement membrane, and a single cell layer that comprises Bowman's capsule. The glomerular basement membrane (GBM) is a gel-like cellular meshwork of glycoproteins and proteoglycans surrounded by cells termed podocytes because of their intrusion, like filaments, into the spaces. The slits between the endothelial cells and the foot processes (podocytes) and Bowman's space constitute the path through which the filtrate passes. An electrical charge partially occludes the slits and provide selective barriers through which materials are filtered (Fig. 190.1).

Reabsorption and secretion are the primary functions of the renal corpuscle, and when the direction of flow is from the lumen to the peritubular capillaries, it is termed tubular reabsorption; movement in the opposite direction is termed *tubular secretion*. This must not be confused with secretion, which occurs when substances are pumped into the lumen against a gradient. As a rule, more substances undergo reabsorption than secretion.

The kidneys perform five major functions:

1. They regulate volume, osmolality, mineral composition, and acidity of the body while balancing ions such as sodium, potassium, chloride, calcium, magnesium, sulfate, phosphate, and hydrogen.
2. They excrete metabolic wastes, including urea, uric acid, creatinine, end products of hemoglobin metabolism, hormone metabolites, and other solutes.

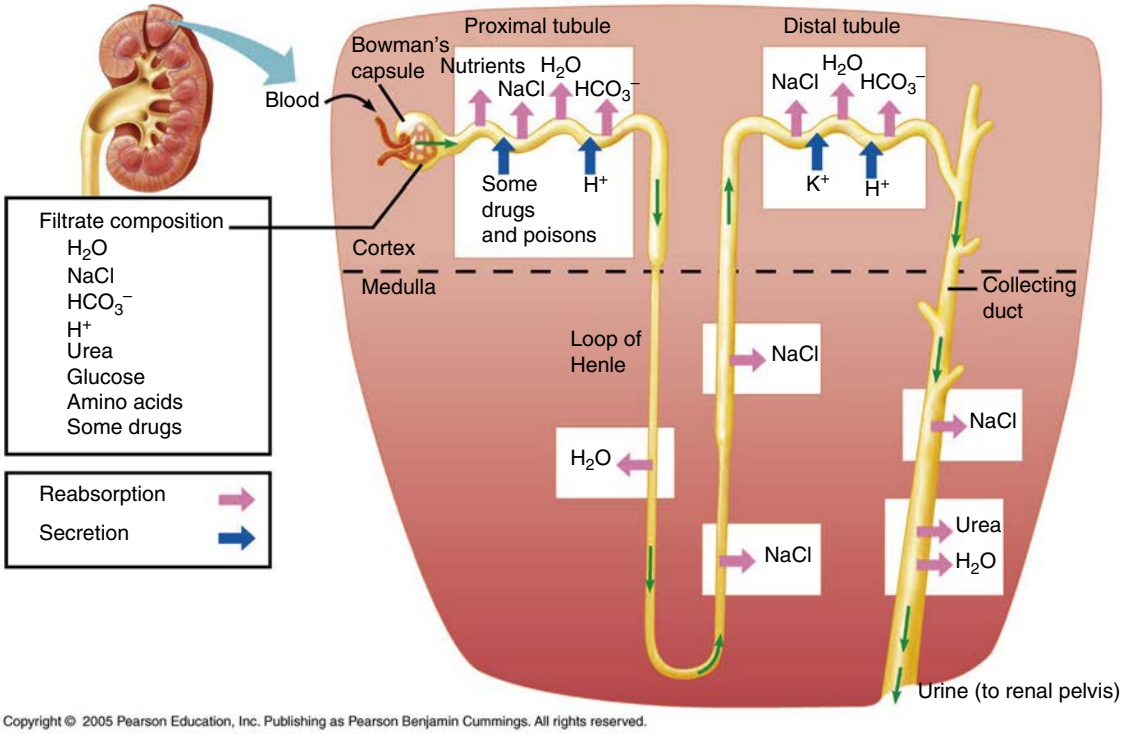


Fig. 190.1 Kidney excretion of toxins. (Copyright © 2005 Pearson Education, Inc. Publishing as Pearson Benjamin Cummings. All rights reserved.)

3. They regulate the excretion of foreign substances, such as chemicals, drugs, pesticides, food additives, and other metabolic waste products.
4. They are involved in gluconeogenesis, especially during prolonged fasting, and can supply up to 20% of the glucose from gluconeogenesis
5. They contribute to endocrine function conversion of 25(OU)D3 to 1,25-dihydroxyvitiman D3, erythropoietin, and renin.[1,2]

Prevalence

Chronic kidney disease is recognized as a public health problem worldwide. In the United States, the prevalence of chronic kidney disease (CKD) in the general population is approximately 14%. More than 30 million, or about 15% of, Americans have some form of CKD, and as shown in Fig. 190.2, the rate is increasing relentlessly. CKD, regardless of the type or cause, leads to complications of decreased kidney function, kidney failure, and cardiovascular disease (CVD). African Americans and Mexican Americans are more likely to develop CKD than Caucasians.[3–5]

End-stage renal disease (ESRD) is defined as the last stage (stage 5) of CKD. The number of persons progressing to ESRD continues to rise by about 21,000 cases per year, making it one of the most expensive diseases to manage medically.[6] African Americans are about 3.5 times more likely to develop ESRD than Caucasians, and Hispanics are 1.5 times more likely to develop ESRD than non-Hispanics.[7,8]

In children, the main causes of kidney disease are birth defects, hereditary disease, infections, systemic diseases, nephrotic syndrome, trauma, and blockage of the urinary tract or reflux. Kidney failure in children from birth to age 4 is usually the result of birth defects and inherited conditions, with hereditary diseases, nephrotic syndrome, and the effects of systemic disease seen between the ages of 5 to 14 years. Glomerular function diseases primarily affect older children, up to age 19.

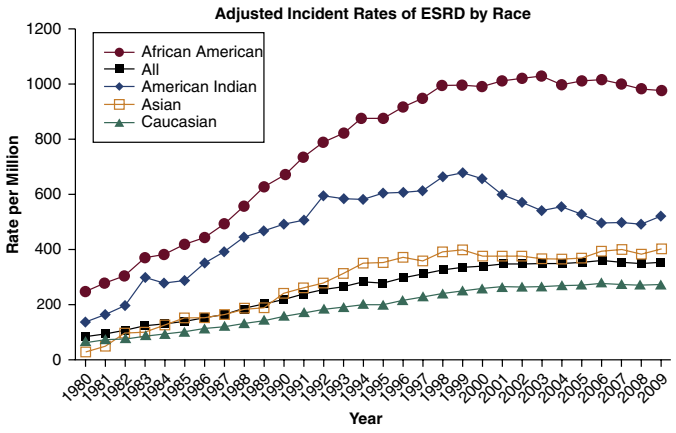


Fig. 190.2 Increasing prevalence of kidney disease. (https://www.google.com/url?sa=i&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwjt363iu97cAhVHJDQIHeJvCEcQjRx6BAgBEAU&url=https%3A%2F%2Fcolumbusblack.com%2Fblog-series-vol-1%2Fdr-chris-brown%2Fnot-the-end-but-a-new-beginning-end-stage-renal-disease-esrd.html&psig=AOvVaw0ytVQcZNM8zKwhsb02rcs0&ust=1533852312484646.)

Kidney disease affects children in various ways, ranging from treatable disorders without long-term consequences to life-threatening conditions to acute kidney disease that develops suddenly, lasts a short time, and then resolves. The effects of both acute and chronic kidney disease can be serious, with long-lasting consequences, or completely clear once the underlying cause has been treated. Children with CKD face additional challenges, such as negative self-image; behavioral, learning, and relationship problems; and delayed language and motor skills.[9]

In the allopathic model, the belief is that CKD generally does not go away with treatment and tends to become worse over time, eventually

leading to kidney failure. This is because symptoms are managed with medications in hopes of slowing down the progression of the disease. In contrast, naturopathic therapies act to remove the cause and restore normal homeostasis as per the laws of healing and the therapeutic order.[10]

One of the reasons CKD is increasing is that many patients do not have symptoms until later stages of the disease process. Additionally, many early cases are missed as a result of a lack of screening on routine physical examinations and/or adequate screening laboratory studies.

Risk Factors

Although CKD and ESRD are found universally, certain ethnic groups exhibit faster rates of disease progression than others. In a study comparing estimated creatinine clearance rates (eGFRcreat) and cystatin C levels, the authors found that kidney function decline varied significantly by race/ethnicity, and these differences were present even among those without albuminuria upon initial evaluation.[11] Overall, blacks and Hispanics had higher rates of decline compared with whites, whereas Chinese and whites had similar rates of kidney function decline. Additionally, in Hispanics, researchers found that rates of decline varied significantly by country of origin, with the highest among Dominicans and Puerto Ricans. Sociodemographics and comorbidities were not found to be a factor.[12]

In a review article by Stenvinkel, the rate of prevalence of CKD was significantly higher in men compared with women. Women with CKD are more likely to be found in stages 1 to 4 compared with men.[13] Obesity was found to convey a threefold risk of developing CKD compared with hypertension and diabetes. Several other studies looked at hyperlipidemia, hypertension, changes in estimated glomerular filtration (eGFR) rates, and inflammation and their relationship to development of CKD.[14–16]

Comorbid conditions, such as cardiovascular disease and diabetes, can accelerate the rate of decline in CKD, and CKD itself contributes to the acceleration of CVD and diabetes.

The GFR is estimated to decline about 1.0 mL min^{-1} per year in the elderly, and therefore deterioration of renal function can be considered a "normal" aging process. Rates of decline in the elderly are highly variable and depend on lifestyle, diet, and overall health status.

The decline in the eGFR depends on the CKD stage and baseline values, with a faster decline the higher the baseline eGFR and a slower rate of decline with lower baseline eGFRs. Baseline eGFR values are a better predictor of CKD progression than age, gender, and stage of renal disease.[17] It has also been suggested that social isolation, loneliness, and poverty may also contribute to deterioration in kidney function as well as other diseases.[18]

An association of blood type and the development of CKD has been examined, with the most prevalent blood group among renal failure patients being blood group A (45.7%), followed by blood group O (30.4%), blood group B (17.3%), and blood group AB (6.6%). This is due to the presence of lectins that stimulate the production of antibodies, with the circulating immune complexes affecting glomerular basement membrane function. The disruption causes changes that contribute to filtration and eventual loss of kidney function.[19,20]

ENVIRONMENTAL EXPOSURE AND KIDNEY DYSFUNCTION

Excessive Salt in Diet

Excessive salt consumption is another significant factor contributing to the degeneration of the kidneys.[21] Mean intake of sodium in a global population is estimated at 3.95 g/day (equivalent to 10.06 g/day of salt), and in the United States, the average intake of sodium is 3.4 g/day (equivalent to 8.7 g/day of salt).[22] Studies have shown a connection between NaCl intake and acid–base balance, with high-salt diets causing a decrease in pH and moderate metabolic acidosis.[23,24] In response to acidosis, the kidney implements adaptive processes to restore the acid–base balance. These include the removal of the nonmetabolizable anions, the conservation of citrate, the enhancement of kidney ammoniagenesis, and urinary excretion of ammonium ions, resulting in the lowering of urinary pH, hypocitraturia, hypercalciuria, and nitrogen and phosphate wasting.[25] Some researchers have hypothesized that excessive salt in the diet is a significant contributor to loss of kidney function with aging.

Excessive Phosphates in Diet

The primary sources of "hidden" phosphates are additives used as processing aids, such as acid balancing (especially in carbonated beverages), leavening of bread, color and moisture retention, anticaking, and flavorings. It appears that a substantial portion of the population consumes more than the tolerable upper limit of 4000 mg/d and significantly more than the Recommended Dietary Allowance (RDA) of 700 mg/d for adults. Eliminating phosphates is difficult for the kidneys. Excessive intake of phosphates damages the tubules, increases fibrosis, which blocks the blood vessels, and decreases the GFR. Increasing phosphate levels in the blood is one of the early signs of kidney failure.[26] Serum phosphorous levels in the "high-normal" range (≥4.0 mg/dL but < 4.5mg/dL) have been associated with a twofold-higher risk of developing new-onset CKD and ESRD in the general population.[27] An observational study showed that every 0.5-mg/dL increase in serum phosphorous demonstrated a 40% greater risk for incident ESRD.[28]

Toxic Metals

Cadmium, chromium, lead, mercury, platinum, and uranium are all nephrotoxic. Although each is significant, the most common, as well as the most researched, are cadmium and mercury.

Cadmium is a significant kidney toxicant. It has a worrisome half-life of over 10 years, making it very difficult to excrete. The kidney holds 50% of the total body burden of cadmium, which increases the nephrotoxicity. The current cadmium exposure experienced by individuals living in the United States has reached levels that adversely affect kidney health in a significant proportion of the population.[29] As the kidneys degenerate, they not only lose their ability to excrete toxins but are less able to perform other normal physiological functions.

The kidneys have a high affinity for mercury. Within a few hours of exposure, 50% of the mercury that gets into the blood ends up in the kidneys. Mercury damages both the glomeruli and the tubules. Proximal tubular necrosis, especially along the straight renal segments in the inner cortex and outer stripe of the outer medulla, is a prominent feature of inorganic mercury nephrotoxicity.[30] Much of the tissue damage appears to result from poisoning of the kidney mitochondria, so there is not enough adenosine triphosphate (ATP) for the cells to protect themselves from the toxins they are excreting.[31]

Persistent Organic Pollutants

Because persistent organic pollutants (POPs) are mostly fat soluble, they are particularly damaging to the kidneys. Many of these chemicals are classified as halogenated hydrocarbons and are so difficult to detoxify or excrete that they have half-lives measured in months to years.

Fluorinated hydrocarbons (e.g., polytetrafluoroethylene [PTFE] polymers such as Teflon, perfluorooctanoic acid [PFOA], and perflourooctanesulfonic acid [PFOS]) damage the kidneys by passive diffusion into the tubules, where they poison the mitochondria. The resulting damage creates inadequate energy production, impairing active excretion; increases oxidative stress as the damaged mitochondria leak highly oxidative electrons and oxygen; and causes cell death, producing progressive loss of kidney function.[32]

Glyphosate

Epidemiological research has found a strong correlation between glyphosate use and the kidney failure epidemic. Of course, association does not prove causation. Animal research shows that chronic exposure at very low dosages causes kidney damage. A 2-year study that involved 0.1 ppb of glyphosate in rats' drinking water resulted in cellular kidney abnormalities and significant chronic kidney deficiencies.[33] Research conducted in Sri Lanka found that those who drank well water contaminated with glyphosate had a higher incidence of kidney failure in proportion to concentrations starting at 0.7 ppb, and farmers spraying glyphosates in the fields had a 5.4-fold increased incidence of kidney disease.[34] The European standard for water contamination is 0.1 ppb, whereas the U.S. Environmental Protection Agency (EPA) standard is an inexplicable 700 ppb.[35]

Nonsteroidal Anti-Inflammatory Drugs

Most nonsteroidal anti-inflammatory drugs (NSAIDs) were initially available only by prescription. Later, when the patents expired, NSAIDs became available over the counter. Acetaminophen, aspirin, ibuprofen, naproxen, indomethacin, and COX-2 inhibitors have all been shown to cause kidney damage when used chronically.[36–38,74,75]

Chronic consumption (>3 years) of single and/or combinations of NSAIDs is known to cause irreversible analgesic nephropathy.[39] Fig. 190.3 summarizes the risks. As can be seen, these drugs greatly increase the risk of kidney failure. Note that this is kidney failure; loss of kidney function is much more prevalent. Discontinuing the use of NSAIDs results in some recovery of function even in patients with kidney failure. After 6 months of stopping NSAIDs, those with the most damaged kidneys still had a doubling of kidney function.

CLASSIFICATIONS OF CHRONIC KIDNEY DISEASE

There are several classifications of kidney disease, each with their own characteristics. Understanding the etiology of CKD is helpful in determining the therapy needed. This is specifically important in naturopathic medicine due to the large number of therapeutic options available to the clinician.

Minimal-Change Nephropathy

Minimal-change nephropathy, also termed minimal-change disease (MCD), is believed to be primarily a disease of children but is found in adults as well. It is most often seen between the ages of 24 to 36 months, with males showing a higher incidence. The male-to-female predominance changes, as well as the incidence of development, with advances in age.

The onset of the disease is insidious and usually discovered after the development of edema. Kidney biopsy shows minimal changes in the glomerular structure, tubules, and interstitial areas, and thus the name minimal-change nephropathy. Hypertension and a reduction in renal function are usually not seen, nor is hematuria or the presence of red cell casts found with glomerulonephritis. A low serum albumin and proteinuria, an elevated serum cholesterol, and edema are the hallmarks of this disease.

MCD is associated with primary idiopathic nephrotic syndrome but can be secondary to NSAID use; exposure to heavy metals, such as mercury and lead; and in children with AIDS. Additionally, Hodgkin's disease and lymphoproliferative disorders must also be kept in mind with a presentation of MCD.

Membrane-Proliferative Glomerulonephritis

Membrane-proliferative glomerulonephritis (MPGN) is generally considered to be an immune complex disorder characterized by mesangial cell proliferation and deposits in the basement membrane of the C3 component of complement and immunoglobulins. The condition can further be divided into types I and II depending on the morphological presentation, age of onset, serum concentrations of C3, and the presence of serum nephritic factors and lipodystrophy. As with many of the renal diseases, there are disagreements as to whether these are separate entities or part of a natural history of the disease.

Type I MPGN is the most common presentation and is a slowly progressive disease. About 30% to 40% of individuals affected will remain clinically stable even with persistent proteinuria. Type I disease is characterized by a persistent 1+ to 2+ proteinuria and urinary sediments, which may contain low levels of erythrocytes and red blood cell (RBC) casts. Occasionally, a marked hematuria along with higher levels of protein will be seen at the beginning of the disease, which will revert to chronic levels after the initial insult.

In type II MPGN disease, an acute nephritis with macroscopic hematuria is the most common presentation. This presentation is more likely to have accompanying edema, hypertension, and signs of renal failure compared with an acute type I episode. Because of this, type II MPGN disease progresses more rapidly to renal failure than type I. About 20% of patients will remain stable for considerable periods of time despite the larger protein losses and low levels of uremia.

MPGN may also be associated with other diseases, such as immune-complex diseases (systemic lupus erythematosus [SLE], mixed cryoimmunoglobulinemia), malignancy (leukemia, lymphomas, light-chain disease), chronic liver disease (chronic active hepatitis, cirrhosis), and infectious diseases (bacterial, endocarditis, AIDS), and with Down syndrome, drug addiction, sarcoidosis and sickle cell disease.

Membranous Nephropathy

Membranous nephropathy is the most common form of nephropathy found in adults (usually over 30) with an unexplained or idiopathic proteinuria. The diagnosis is made after renal biopsy, which shows a thickened glomerular basement membrane and subendothelial deposits. Medications or an associated systemic illness seem to play a role, and the disease may be present for years before it is detected.

The first presentation may be heavy proteinuria, followed soon after by the signs and symptoms of progressive renal failure. Microscopical hematuria may also be present in a few cases. Males are affected more often than females, and hypertension and azotemia may not be present until later in the disease process.

The subsequent nephrotic syndrome presents with malaise, edema, and anorexia. Occasionally anasarca is also present. Elevated cholesterol and triglyceride levels are found, and there may be a predisposition to the formation of thrombosis, especially of the renal veins.

Membranous nephropathy is believed to be an immune-mediated disease because immunoglobulin G (IgG) and complement C3 are found with special staining. These could be formed locally or be deposits of circulating immune complexes. Secondary antigens have been found associated with hepatitis B surface and e antigens as well as thyroid antigens associated with thyroiditis.

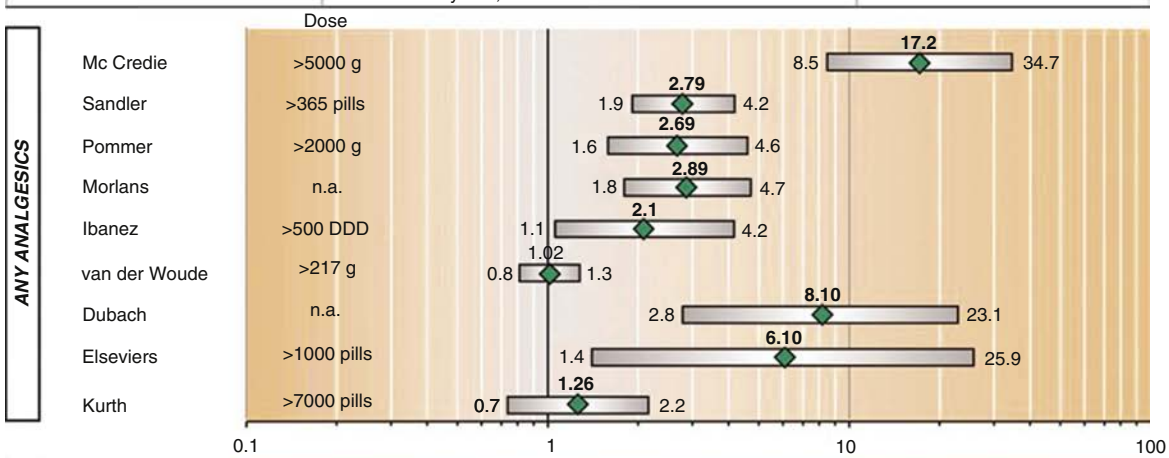
Immunoglobulin A Nephropathy

Immunoglobulin A (IgA) nephropathy, otherwise known as Berger's disease, represents about 10% of all patients who reach end-stage renal failure and is the most common form of primary glomerulonephritis found worldwide. The histopathological findings are similar to those of Henoch–Schönlein purpura, which may represent a more systemic form, and together with glomerulonephritis are termed primary IgA nephropathy. Secondary forms are the result of conditions in which circulating IgA becomes deposited in the glomerulus. This

A

Case-control studies	Cases	Controls	Lifetime dose
McCredie et al, Australia, 1982 [11]	80 women with RPN	80 healthy women	3 units/week for one year
Murray et al, USA, 1983 [12]	527 p. with ESRD	1047 hospitalized p.	almost daily for 30 days
Sandler et al, USA, 1989+1991 [13,14]	554 p. with newly diagnosed CRF	516 population based	daily for one year
Pommer et al, West Berlin, 1989 [15]	517 p. with ESRD	517 outpatient clinic p.	15 units/month for one year
Morlans et al, Barcelona, 1990 [16]	340 p. with ESRD	673 hospitalized p.	15 units/month for 30 days
Perneger et al, USA, 1994 [17]	716 p. with ESRD	361 population based	daily for one year
Fored et al, Sweden, 2001 [18]	926 p. with newly diagnosed CRF	998 population based	twice a week for 2 months
Ibanez et al, Barcelona, 2005 [19]	583 p. with ESRD	1190 hospitalized p.	15 units/month for 30 days
Van der Woude et al, Austria, Germany, 2007 [20]	907 p. with ESRD	3622 population based, no fenacetine intake	1 unit per month; no phenacetin intake
Prospective controlled cohort studies			
Dubach et al,Switzerland,1983 [21]	623 healthy women	621 healthy women	urine positive for paracetamol
	followed for 10 years, outcome decreased eGFR		
Elseviers and De Broe, Belgium, 1995 [22]	200 healthy subjects	200 healthy subjects	daily for 1 year with total > 1000 units
	followed for 7 years, outcome decreased eGFR		
Observational cohort studies			
Kurth et al, USA, 2004 [23]	4494 healthy male physicians, aged 40-84, followed for 15 years, outcome decreased eGFR		all kinds of analgesics, daily aspirin intake
Curham et al, USA, 2004 [24]	1697 healthy female nurses, aged 30-55, followed for 12 years, outcome decreased eGFR		all kinds of analgesics

B



C

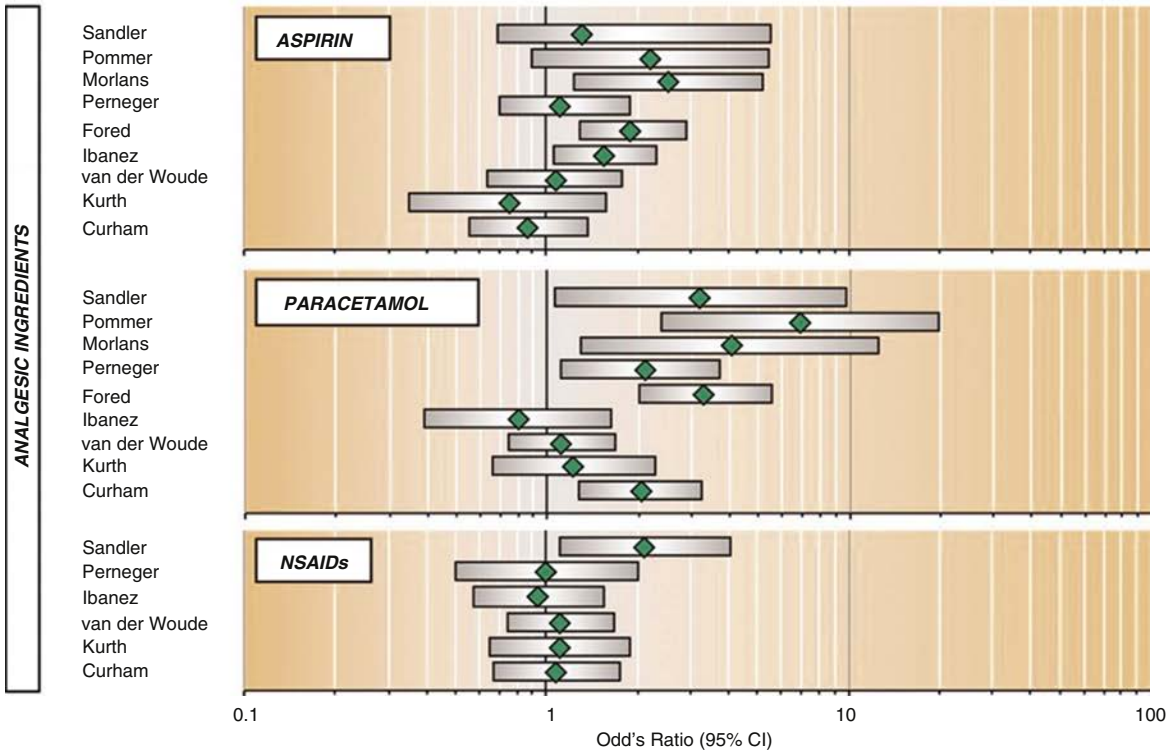


Fig. 190.3 Risk of kidney failure from long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs). (From Wei L, MacDonald TM, Jennings C, et al. Estimated GFR reporting is associated with decreased nonsteroidal anti-inflammatory drug prescribing and increased renal function. *Kidney Int.* 2013 Jul;84[1]:174–178.)

is commonly seen with celiac disease, chronic liver disease, dermatitis herpetiformis, psoriasis, ankylosing spondylitis, inflammatory bowel disease, IgA monoclonal gammopathies, HIV infection, certain cancers, and mycosis fungoides.

Although the disease is found in all age groups, children and young adults are more commonly affected, with a higher ratio of males to females. Most commonly, the person will present with macroscopic hematuria along with an upper respiratory tract infection. The person is usually asymptomatic but may present with fatigue, malaise, or muscle soreness or pain. Children and some adults may complain of loin pain. A distinction from post-streptococcal nephritis (PSN) can be made because the symptoms usually occur 10 to 14 days after infection, and the patient may have had a fever before the onset. Additionally, hypertension and peripheral edema, commonly seen with PSN, are usually not seen with IgA nephropathy.

Hematuria may last hours to days and may become intermittent with periods of no bleeding and then recur several months or years later after a febrile illness. Occasionally the hematuria is also accompanied by proteinuria of varying degrees, usually less than 1 g/day.

Henoch–Schönlein purpura, which is found more often in children, may also have associated arthralgia with a lack of joint swelling and inflammation; a skin rash primarily over the legs and lower trunk that rapidly becomes purpuric; and severe abdominal pain, ileus, and bloody diarrhea. Less commonly, the presentation will be a nephrotic syndrome with renal insufficiency and hypertension.

Acute Post-Streptococcal and Postinfectious Nephropathy

Acute post-streptococcal and post-infectious glomerulonephritis are a result of the deposition of circulating immune complexes in the glomerulus. This is often due to the presence of group A beta hemolytic streptococcus (BHS) after pharyngitis or a skin infection (cellulitis, impetigo). Other microorganisms, such as bacteria, viruses, and parasites, have been implicated as well.

Post-streptococcal glomerulonephritis (PSGN) is the most commonly encountered form of glomerulonephritis in children, with subclinical asymptomatic cases occurring 4 times more frequently than acute disease. There is some association with disease development in families, which may also be due to blood type and the presence of certain human leukocyte antigens (HLAs).[19,20] There appears to be a 2:1 male-to-female ratio even though susceptibility to BHS infection is not sex related.

The onset of the disease is usually abrupt and follows an infection with group A BHS. There is a latent period of 7 to 21 days after the infection before symptoms, such as edema and hematuria, occur. Periorbital edema is commonly observed. Hypertension may be present, resulting in headaches, visual disturbances, and altered sensorial states. Coma and convulsions may occur in severe cases. Circulatory congestion due to fluid retention results in dyspnea, cough, orthopnea, and cardiac and pulmonary changes suggesting pulmonary edema and congestive heart failure. PSGN should always be considered in patients presenting with cardiovascular or upper respiratory diseases of rapid onset.

Diagnosis is made by urinalysis positive for hematuria and RBC casts. However, white blood cell (WBC), tubular epithelial, and granular casts are also commonly found and reflect the degree of pathology present. Serum creatinine levels tend to remain stable, but blood urea nitrogen (BUN) levels may rise somewhat. Changes in electrolyte balance will parallel changes in metabolic acidosis or alkalosis and need to be monitored frequently. Anti-streptolysin O (ASO) titers begin to rise about 10 to 14 days post–streptococcal pharyngitis, peak at about 4 weeks, and return to normal levels between 1 and 6 months later. The magnitude of the titer does not seem to correlate to the degree of kidney involvement. Depression of hemolytic complement activity (C3) is present almost 100% of the time but also does not correlate to the degree of kidney involvement.

Rapidly Progressive Glomerulonephritis

Rapidly progressive glomerulonephritis (RPGN) is defined as an idiopathic primary glomerular disease characterized by a rapidly progressing deterioration in renal function over a period of days to weeks to a few months. The definition is somewhat complicated because a rapid deterioration of kidney function can occur with a variety of illnesses. The cause of illness must be ruled out before a diagnosis of RPGN can be obtained. If untreated, the condition will commonly progress to end-stage kidney failure or death.

Diagnosis is by kidney biopsy confirming renal failure to be related to circulating antiglomerular basement membrane antibodies. A relationship to primary alveolar disease, such as Goodpasture's disease, has been suggested but not proven. The disease can be divided into three types, suggesting a vasculitis type of disease, circulating immune complexes as a pathogenesis, and possibly a relationship to HLA antigens and specific disease activity.[40,41]

Nephrotic Syndrome

Nephrotic syndrome (NS) is a recognized condition that can be the result of several diseases and is not considered a disease itself but rather a collection of symptoms due to kidney failure. Nephrotic syndrome is characterized by very high levels of urinary protein, low levels of serum total protein, swelling of the extremities (especially around the eyes), and high serum cholesterol.

Nephrotic syndrome can be caused by diseases that affect the glomerular filtration of the kidneys, such as focal segmental glomerulosclerosis (FSGS) or membranous nephropathy and are termed primary NS. Systemic diseases such as diabetes or lupus (secondary NS) can also cause nephrotic syndrome. Most cases of nephrotic syndrome in adults have secondary causes, with diabetes being the most common.[42,43]

DIAGNOSIS OF CHRONIC KIDNEY DISEASE

As previously mentioned, many patients with CKD have no knowledge of its presence until it is at somewhat of an advanced stage. This is because of the body's substantial organ reserve and ability to adapt to chronic conditions, or the person believes that it is due to another disease or part of the aging process.

Signs and symptoms may be minimal at first, and the patient may complain of feeling run-down or fatigued. The skin may become ruddy, dry, itchy, and perhaps yellowish in color. Mental processes and movements may become slower, and as the disease advances, the person may become anorexic and have cramping, numbness, and tingling of the extremities and may complain of insomnia.

At a minimum, a fasting chemistry screen and lipid levels should be ordered and include BUN, creatinine, and uric acid levels to assess kidney filtration. A complete blood count (CBC) to determine whether there is anemia, along with a fasting glucose, hemoglobin A1c, and insulin level to rule out metabolic syndrome or diabetes, and inflammatory markers such as serum ferritin, high-sensitivity C-reactive protein (hsCRP), and erythrocyte sedimentation rate (ESR) should also be considered.

Additionally, a routine urinalysis with microscopical examination should be performed, and if albumin is present, then a total protein/creatinine ratio is required to assess protein loss. A 24-hour urine creatinine clearance can follow this to establish a baseline to evaluate patient response to therapy.[44]

Several parameters, if noted early on, suggest developing kidney disease. One of the earliest manifestations is the development of microalbuminuria with a normal (calculated) GFR.[45]

A change in the slope of serum creatinine levels over time is a predictor of decreasing kidney function, with a greater slope correlating with a greater decrease in function. The association of low serum albumin corresponding with a faster rate of GFR decline was more consistently noted in studies of diabetic patients than nondiabetic patients.

The effect that dyslipidemia has on kidney decline is based on high levels of total cholesterol, triglycerides, or low-density lipoprotein and low levels of high-density lipoprotein. Several studies looking at individual or the totality of these parameters showed varying results, suggesting that there may be some association.

Smoking seems to have an effect on changes in GFR, with smokers being found to have a greater rate of deterioration.[45]

The GFR can be estimated from serum creatinine levels by using prediction equations that also account for age, sex, race, and body size. Two such equations are the Cockcroft–Gault and the Abbreviated MDRD study equations. The MDRD equation has largely replaced the Cockcroft–Gault equation because it is believed to be more accurate and precise for persons with a GFR of less than approximately 90 mL/min per 1.73 m^2 and does not require height and weight. Thus it is easily calculated and can be included with routine laboratory panels.

$$C_{Cr} = \left\{ \frac{(140 - \text{age}) \times \text{weight}}{(72 \times S_{Cr})} \right\} \times 0.85 \text{ (if female)}:$$

(The Cockcroft and Gault formula (1973))

$$\text{eGFR} = 175 \times (S_{Cr})^{-10154^{\circ}} \times (\text{age})^{-0.203^{\circ}} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}: \text{(MDRD equation)}$$

$$C_{Cr}^{\circ}\text{(creatinine clearance)} = {}^{\text{mL}}/_{\text{minute}}$$

$$\text{Age} = \text{years}$$

$$\text{Weight} = \text{kg}$$

$$\text{SCr (serum creatinine)} = {}^{\text{mg}}/_{\text{dL}}$$

Although both equations are good for screening for potential kidney disease development, there is some question as to the validity of the MDRD equation in diabetic kidney disease, in patients with serious comorbid conditions, in "normal" patients, or in patients older than 70 years of age. In these cases, the Cockcroft–Gault equation may provide a better assessment.

The value of using both equations is in individuals with extremes of age and body size, severe malnutrition or obesity, diseases of skeletal muscle, paraplegia or quadriplegia, vegetarian diet, or rapidly changing kidney function. They can also be used to determine the dose of potentially toxic drugs that are excreted by the kidneys.

Other screening parameters of note are the serum calcium/phosphorus ratio and calcium X phosphorus, which is an assessment of the kidney's ability to balance calcium and phosphorus as well as to assess parathyroid function, response to vitamin D, and renal calcinosis. As serum calcium levels fall, there is a rise in phosphorus, resulting in symptoms of hypocalcemia. However, changes noted over time on routing chemistry screens may provide a clue for developing kidney disease. The normal calcium/phosphorus ratio = (2.5 ± 0.4), and the normal calcium X phosphorus = (N = < 40 and abnormal = 60 or >).

STAGES OF CHRONIC KIDNEY DISEASE

Kidney disease is recognized as a chronic, progressive disease and is classified according to the following stages:

Stage 1—Signs of mild kidney disease but with normal or better GFR (greater than 90% kidney function)

Stage 2—Signs of mild kidney disease with reduced GFR (indicating 60%–89% kidney function).

Stage 3—Signs of moderate chronic renal insufficiency (where the GFR indicates 40%–59% kidney function)

Stage 4—Signs of severe chronic renal insufficiency (where the GFR indicates 15%–29% kidney function).

Stage 5—Signs of end-stage renal failure (where the GFR indicates less than 15% kidney function).

THERAPEUTIC APPROACH

Case Management

Considering the many different causes of chronic kidney disease, comorbid disease management is as important as the management of the kidney disease itself. This has been one of the challenges facing physicians as medicine has become more compartmentalized, with specialists treating isolated organ systems. Polypharmacy is increasingly recognized as contributing to morbidity, negative health outcomes, and decreasing the quality of life.[46] Polypharmacy is particularly relevant to kidney function because many of these drugs, such as antihypertensive medications, directly affect kidney function.

In children affected with any type of kidney disease, it is important to consider that the kidney is still maturing. This may present a dilemma. In prolonged cases, severe damage can take place, altering kidney-centered parameters and affecting growth and development, as well as predisposing the person to an earlier development of kidney failure. However, in a disease state of shorter duration, a developing kidney has a greater chance of complete recovery if recognized and treated early.

A treatment regimen that considers the type of kidney disease, precipitating etiology, duration, and expected changes in the pathophysiology of the disease must be developed. This may consist of dietary modifications, allergen identification, and control of hypertension and diabetes if present. The goal of treatment is to relieve the perturbing insult on the kidney and allow healing and restoration of normal function to occur. Therefore periodic follow-up visits are needed once the initial insult has been eliminated or has reached equilibrium.

Education of the patient as to the possible course of the disease, the patient's susceptibility to it, and the significance of prevention to maintain the disease or the recovered level of health is important. A periodic routine urinalysis is beneficial for screening, especially after any febrile or respiratory illness.

Dialysis

Patients on renal dialysis present additional challenges for the clinician because each requires different levels of monitoring and follow-up. Hemodialysis is commonly performed 3 times per week, requiring the patient to be connected to a dialyzer that cleans the blood. This procedure takes between 3 and 5 hours and requires frequent blood testing to monitor electrolyte and protein balance. Peritoneal dialysis,

on the other hand, is carried out at home by the patient and is achieved through continuous ambulatory peritoneal dialysis (CAPD) or continuous cycling peritoneal dialysis (CCPD).

From a holistic perspective, it is much easier for the clinician to treat patients on CAPD and CCPD than hemodialysis simply because the mechanism of dialysis CAPD and CCPD works with the body's alternative filtering mechanism, the omentum, to achieve dialysis rather than filtering the blood, which necessitates a rebalancing of blood chemistry.

However, both methods increase the likelihood of sepsis due to contamination of fluids. Therapies to prevent sepsis seem to work better with CAPD and CCPD than hemodialysis. Additionally, the balancing of blood chemistry and working with the patient's dietary requirements are also easier to address.[47]

NUTRITIONAL CONSIDERATIONS IN ACUTE AND CHRONIC RENAL FAILURE

The goals of nutritional therapy in ESRD are to maintain the body's chemical composition as close to that of normal to preserve protein stores until renal function is normalized. Nutritional therapy in acute renal failure (ARF) is complicated because of the rapid and fluctuating progression of the disease, in contrast to ESRD, which is slower and more predictable.

Diets with high-quality protein and high carbohydrate (2200 calories) intake, with an excess of 500 mL of water above the daily output, are desired. Intake of water, electrolytes, and minerals must be closely monitored, especially in patients undergoing dialysis, to avoid fluid overload while minimizing abnormal concentrations of these substances. Protein restriction must occur to decrease the accumulation of nitrogenous wastes as well as other inorganic ions, such as sulfates and phosphates, which result from protein metabolism. A sufficient intake of calories and vitamins is needed to minimize catabolism of protein stores. High-carbohydrate diets in patients with renal failure were found to reduce endogenous protein catabolism, decreasing the BUN.

Water and Mineral Balance

Patients with ARF often show greater impairment of water, mineral, and electrolyte excretion than do patients with ESRD, even though the creatinine clearances may be similar. This is due to an inability of the body to adjust to the rapid changes occurring with ARF as opposed to the slower rate of change with ESRD. Water intake may need to be negative to balance the serum ion concentration.

Water loss must account for the patient's temperature, any surface loss through burns or open wounds, the rate of ventilation, and the humidity of the air. In afebrile patients without burns, fluid losses of 400 to 600 mL/day are seen. Water loss can be monitored by measuring weight changes and the differences between fluid intake and output, as well as calculation from body composition analysis.

Edema and hypernatremia indicate an excess of salt and water, whereas postural hypotension suggests volume loss. In patients with edema, hypernatremia requires water and salt restriction until the serum sodium concentration is normalized and the edema is reduced. In patients with postural hypotension, careful use of salt and/or blood or colloid is needed to correct the volume deficit. Hyperkalemia usually requires dialysis to remove potassium because it is primarily due to renal impairment.

Dietary Protein Requirements

Patients with ESRD are in neutral or positive nitrogen balance with ingestion of 40 g/day of high-quality protein but can go into negative balance with only 20 g/day. It has been shown that 0.5 to 0.8 gm/kg/day of protein is needed to maintain a neutral nitrogen balance and that higher amounts are needed with patients on dialysis. Patients on hemodialysis need higher protein amounts due to loss from the procedure. This also includes peritoneal dialysis. One g/kg/day is needed, and if the person is restricted to less, he or she will go into negative nitrogen balance.

Essential amino acids (AAs) need to be part of the regimen because a person lacking only one of the essentials AAs will develop a negative nitrogen balance as the rate of protein synthesis decreases in the absence of the amino acid. Branched-chain AAs have been shown to increase muscle protein synthesis while decreasing muscle degradation. Branched-chain AAs, which include leucine, isoleucine, and valine, showed a decline in degradation, especially leucine, which, by itself, can decrease catabolism. Protein-sparing effects were noted not only during the administration of the amino acid solution but up to a week after administration.

General Principles of Nutrient Administration

1. Adequate calories and nitrogen should be administered with a minimal amount of water.
2. High-quality protein should be administered in sufficient amounts to achieve neutral nitrogen balance but not so high as to increase urea nitrogen levels.
3. Sodium intake should be adjusted to achieve sodium output unless edema is present. If edema is present, then sodium should be restricted to a quantity less than the daily excretion. Potassium, calcium, magnesium, and phosphate should be monitored to make sure adequate serum concentrations are maintained.
4. Patients should be encouraged to eat, and the minimal dietary requirements for renal insufficiency should be followed unless there is a catabolic illness requiring a greater intake of protein.

Nutritional Therapies for Chronic Kidney Disease

There are a considerable number of natural therapies that can be used for patients with CKD, not only to support kidney function and decrease rates of decline but also to support the other emunctories that must compensate for decreased kidney function. Therefore liver and skin support for detoxification and elimination are important to address as part of a comprehensive CKD program. As with all natural therapies, individualization of treatment plans is important because patients with CKD will present with their own unique presentations due to the stressors of the disease process.

Most of the causes of kidney damage can be controlled by helping people make better choices. The key environmental nephrotoxic agents include cadmium, mercury, fluorinated hydrocarbons, and glyphosate. Decreasing exposure is primarily accomplished by modifying lifestyle choices. This includes eliminating or significantly reducing consumption of conventionally grown foods (e.g., soybeans), eliminating tobacco smoking, avoiding genetically modified organism (GMO) foods, eliminating or reducing consumption of large fish (e.g., tuna), avoiding nonstick coatings on pots and pans, and avoiding clothing that is waterproof but breathable. Some of the most immediate benefits can be seen by decreasing toxins of choice, such as excessive dietary salt, excessive dietary phosphates, and drugs such as NSAIDs. Organic, mostly plant-based foods should be consumed when possible. Eating organic foods has been shown to measurably decrease POP levels within 3 days.[48]

Vitamin C

Vitamin C supplementation in patients with CKD, especially those on dialysis, has been reviewed in several studies looking at its antioxidant effects for lipid peroxidation, enhancement of cellular function, and

its role in mobilizing iron to form hemoglobin. Because there is a high turnover of RBCs in patients with CKD, iron stores will increase due to chronic inflammation and loss from dialysis. Vitamin C plays a key role in mobilizing iron for hemoglobin formation. However, in CKD, an increase in oxidation may occur because of ascorbate, causing additional stress to already-uremic patients. Depending on the study, levels of 60 to 300 mg per day or 1 to 1½ g per week are recommended above intake from food sources.[49,50]

Vitamin D

Patients with CKD show reduced levels of 1-α hydroxylase, the enzyme that converts calcifediol/25-hydroxyvitamin D (25[OH]D) to its more active form, calcitriol/1,25-dihydroxyvitamin D ($1,25[OH]_2D$). Because of this, patients with CKD are most often supplemented with a vitamin D replacement of the active form of 1,25-dihydroxvitamin D.[51] Because extra-renal 1-α hydroxylation does occur, some nephrologists recommend supplementing with the unactivated form of vitamin D, allowing the extra-renal sites to regulate calcium and phosphorus balance.[52]

The half-life of calcifediol/25-hydroxyvitamin D (25[OH]D) is 2 to 3 weeks except in renal failure, where it will last up to 2 to 3 times that. The half-life of calcitriol/1,25-dihydroxyvitamin D ($1,25[OH]_2D$ is 3 to 6 hours, but the pharmacological activity can last 3 to 6 days. When administering vitamin D, the serum calcium and phosphorus levels should be checked monthly to adjust dietary intake because of changes in kidney production as well as supplementation. In dialysis patients, they should be checked weekly.[51,53]

Vitamin E

Vitamin E has been shown to be an excellent protector and stabilizer of cell membranes, especially in RBCs and lung tissue. This is because of its antioxidant properties, anti-inflammatory, and anti–platelet aggregation effects. Vitamin E was shown in one study to lower the protein/creatinine ratios while somewhat increasing the mean GFR.[54,55] Asymmetrical dimethylarginine (ADMA), an inhibitor of endothelial nitric oxide synthase, is elevated in CKD and is considered a risk factor for the development of arteriosclerosis. A study evaluating the effects of ADMA concluded that vitamin E supplementation increased the bioavailability of nitric oxide, lowering the risk of arteriosclerosis.[56]

Fish Oil

Consumption of fish, either in the diet or supplemented fish oils, shows a reduction in the development of CKD.[57] Fish oil supports kidney function through anti-inflammatory effects and the regulation of blood pressure.[55] Fish oil has also been found to slow the progress of kidney disease in patients with IgA nephropathy. Fish oils have also been found to protect renal function in patients undergoing Cyclosporin therapy for psoriasis as well as with renal transplant.[58,59]

Flax Oil

Flax oil, a source of alpha-linoleic acid, has been found to be beneficial because it reduces renal injury in experimental polycystic kidney disease by decreasing associated interstitial nephritis.[60] This is due in large part to its anti-inflammatory properties and its ability to alter the renal content of polyunsaturated fatty acids, which promotes the formation of less inflammatory classes of renal prostanoids.[61]

N-Acetylcysteine

N-Acetylcysteine (NAC) is believed to promote the production of glutathione, an antioxidant that prevents damage to important cellular components caused by free radicals, lipid peroxidation, and heavy metals. NAC is used for the treatment of acetaminophen toxicity as well as a mucolytic in patients with cystic fibrosis and chronic obstructive pulmonary disease. NAC also provides protection in patients with CKD by increasing glutathione production and reducing exposure to acetaminophen and contrast dyes.[62,63] Additionally, NAC has been shown, along with the iron chelator deferoxamine and *Ginkgo biloba* extract, to protect against cisplatin lipid peroxidation, with NAC and deferoxamine having the greatest effect.[64]

Alpha-Lipoic Acid

Alpha-lipoic acid (ALP) has been shown to attenuate the effects of other antioxidants, such as vitamins C and E, especially in patients with diabetic nephropathy. Additionally, ALP increased renal cortical glutathione levels in patients with diabetes.[65] Another study looked at the effects of ALP on doxorubicin-induced renal damage and found that there was greater mitigation of oxidative stress, inflammation, and apoptosis parameters with pretreatment with ALP. These studies suggest that ALP, used in conjunction with other antioxidants, provides additional protection against oxidative stress in CKD.[66]

Carnitine

Carnitine supplementation slows the rate of kidney function loss while improving skeletal and cardiac muscle function as well as decreasing anemia, all found in CKD patients. Carnitine slows the rate of kidney function loss, and its deficiency is a common occurrence in dialysis patients, where supplementation has been shown to decrease hospital use in this group.[67,68]

Curcumin

Curcuma longa is a potent antioxidant that protects against protein loss, albuminuria, and hyperlipidemia as well as decreasing kidney damage due to free-radical formation. Additionally, it was found to increase glutathione and glutathione peroxidase activity while eliminating kidney microsomal and mitochondrial lipid peroxidation.[69,70]

Glandulars

Dietary use of renal protomorphogen (kidney tissue extract) helps decrease circulating immune-complex damage in autoimmune disease as well as provide nutrients and growth factors for renal regeneration. Many cultures use organ-specific nutrients when treating specific disease conditions like CKD, as well as to support normal growth and development.

Homeopathy

Homeopathic prescriptions for patients with CKD have, in many cases, slowed, stopped, or reversed the progress of the disease. This is particularly seen in patients with acute kidney failure whose course is often rapid and severe, requiring lesional, fundamental, and constitutional prescriptions. In patients with CKD, prescriptions are more often constitutional or miasmic due to the slowly evolving nature of the disease. Often used in conjunction with other natural therapies, a wide variety of homeopathic medicines are used to direct the healing process or with dialysis patients to reduce the risk of sepsis and toxemia.[71]

Botanical Medicines

Botanical medicines, such as *Aconite napellis, Arctostaphylos uva ursi, Atropa belladonna, Eupatorium purpureum, Juniperus officinalis, Galium aparine, Rheum palmatum, Salviae militorrhizae,* and *Solidago odora,* are commonly used for CKD. Botanical medicines work differently than drugs in that they contain several different constituents that affect a variety of parameters of kidney function.[72–74]

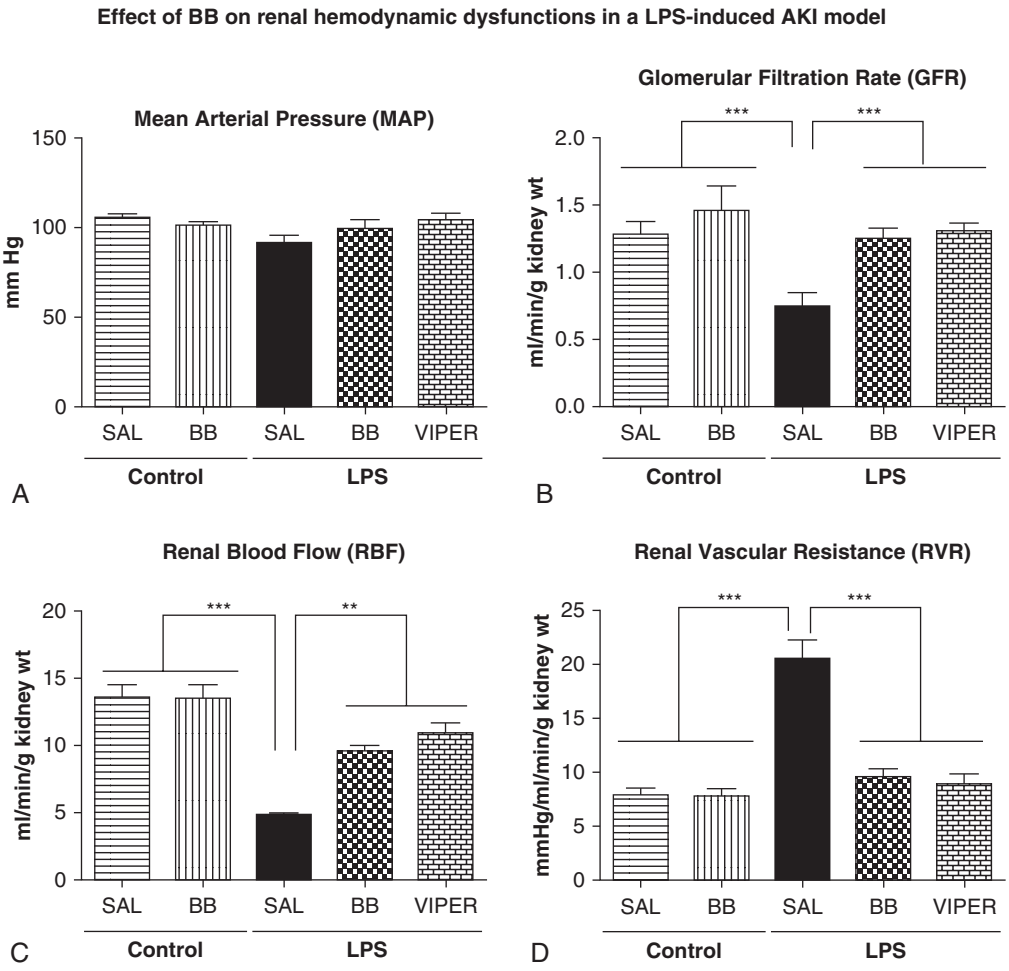


Fig. 190.4 Blueberries protect the kidneys from gut-derived toxins.

Salvia Militorrhizae

As an example, *Salvia militorrhizae* increases blood flow to the kidney by decreasing platelet adhesion, enhances urea and creatinine clearance, stabilizes the glomerular basement membrane by enhancing electrical charge, provides antioxidant and free-radical neutralization, and decreases lipid peroxidation due to the presence of magnesium lithospermate B. It also affects blood pressure by enhancing prostaglandin E2 for vasodilation while limiting the effects of thromboxane A2 that contribute to vasoconstriction.[75]

Hydrastis, Phytolacca, Bryonia, and Baptisia

Herbal medicines such as *Echinacea, Hydrastis, Phytolacca, Bryonia,* and *Baptisia,* when used in combination, reduce the risk of the development of sepsis in dialysis patients. The combination is taken prophylactically and at an increased dosage at the first signs of developing sepsis. The formula is often used in conjunction with homeopathic *Pyrogenum* and/or *Echinacea* and has been found to quickly restore normal homeostasis.[76]

Gotu kola

Traditional Chinese medicine has used *Gotu kola* to treat kidney diseases for centuries. Asiaticoside has been shown to improve microcirculation and reverse fibrosis in humans with varicose veins. Its direct benefits for the kidneys have only been shown in animals but are encouraging. In rats, *G. kola* showed a protective effect for Adriamycin-induced nephropathy, resulting in dramatically improved kidney function.[77] Another study combined *G. kola* in conjunction with naringenin and showed decreased fibrosis formation in the kidneys.[78]

Dark Chocolate

Dark chocolate consumption improves oxygenation of the kidneys in all individuals. The benefit is directly proportional to catechin levels.[79] Animal research has shown that catechins also protect the kidneys from oxidative stress from toxic drugs like cyclosporine.[80]

Blueberry

Blueberry anthocyanins specifically protect the kidneys from bowel-derived endotoxins. Fig. 190.4 shows how blueberries increase the GFR in normal kidneys (but the increase is not statistically significant) and completely protect the kidneys from the dramatic lowering of GFR caused by gut toxins in those with impaired function.[81]

Zingiber officinale

There is ample animal research showing that *Zingiber officinale* not only improves kidney function but is especially beneficial in protecting the kidney from cadmium. The primary mechanism of protection appears to be ginger's ability to decrease inflammation and oxidative damage to kidney tissue exposed to a variety of toxins. The anti-inflammatory benefits of ginger in the kidney result from its antioxidant properties and the epigenetic downregulation of proinflammatory genes.[82] Several animal studies have shown that ginger can protect the kidneys from cadmium. One study found the anti-inflammatory effects

Effect of administration of polyphenols from ginger on kidney function parameters of normal and streptozotocin-diabetic rats.

Group	Kidney function test	
	Urea (mg/dL)	Creatinine (mg/dL)
Control	14.82 ± 1.99[a]	1.72 ± 0.26[a]
Diabetic untreated	50.61 ± 6.82[b]	2.52 ± 0.53[a]
Diabetic + Free	22.63 ± 2.09[c]	2.01 ± 0.29[a]
Diabetic + Bound	27.05 ± 2.12[c]	2.83 ± 0.71[a]
Diabetic + Glibenclamide	35.82 ± 3.87[c]	2.11 ± 0.23[a]

Values are mean ± S.E.M. of 8 rats per group. Test values down the vertical columns carrying different superscripts are significantly different ($p < 0.05$).

Fig. 190.5 Ginger partially restores kidney function in diabetic rats.

of ginger to be strong enough to prevent most of the kidney damage from cadmium.[83] Another found almost no histological kidney damage when ginger was fed along with cadmium.[84] Animal research has also shown kidney protection from alcohol, malathion, carbon tetrachloride, chromates, fructose, gentamycin, ischemia, lead, and cancer drugs.[85–87] Fig. 190.5 shows restoration of kidney function in rats that already had diabetes.[87]

Herbal medicines have also been found to contribute to the development of CKD when used incorrectly, misidentified, or contaminated. Consulting a botanical material medica before prescribing for CKD is highly recommended.[88]

THERAPEUTIC APPROACH

There is no substitute for vigorously avoiding all causes of kidney damage, especially environmental toxins and NSAIDs.

Supplements

Vitamin C: 500 mg/d
Vitamin E: 1000 IU mixed tocopherols/d
Fish oil: 3 g/d
NAC: 500 mg/d

Botanical Medicines

G. kola extract: 30 to 90 mg/d
Dark chocolate: to tolerance
Blueberry extract: 1000 mg/d

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Guyton A. *Textbook of Medical Physiology. Chapters 34-38*. Philadelphia: W.B. Saunders Co; 1981.
2. Valtin H, Schafer J. *Renal Function*. 3rd ed. Boston: Little, Brown & Company; 1983.
3. United States Renal Data System. Excerpts from the 2000 U.S. Renal data system annual report: atlas of end stage renal disease in the united states. *Am J Kidney Dis*. 2000;36:S1–S279.
4. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: third National Health and Nutrition Examination Survey. *Am J Kidney Dis*. 2003;41:1–12.
5. Centers for Disease Control and Prevention. Chronic kidney disease surveillance system website. http://www.cdc.gov/ckd. Accessed March 9, 2017.
6. US Renal Data System. *USRDS 2005 Annual Data Report: Atlas of End-Stage Renal Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2004. Available at: http://www.usrds.org/atlas.htm. Accessed January 23, 2006.
7. National Institute of Diabetes and Digestive and Kidney Diseases. Web site https://www.niddk.nih.gov. Accessed November 20, 2019.
8. United States Renal Data System. Web site http://www.usrds.org. Accessed November 20, 2019.
9. National Institute of Diabetes and Digestive and Kidney Diseases. Kidney disease in children. Web site niddk.nih.gov/health-information/kidney-disease/children. Accessed November 20, 2019.
10. Zeff JL, Snider P, Myers S. A hierarchy of healing: the therapeutic order. The Unifying Theory of Naturopathic Medicine. In: JE Pizzorno, M Murray, *Textbook of Natural Medicine*. 3rd ed. St. Louis, MO: Churchill Livingston, Elsevier.
11. Peralta CA, Katz R, DeBoer I, et al. Racial and ethnic differences in kidney function decline among persons without chronic kidney disease. *J Am Soc Nephrol*. 2003;14(11):2902–2907.
12. Hsu CY, Lin F, Vittinghoff E, Shlipak MG. Racial differences in the progression from chronic renal insufficiency to end-stage renal disease in the United States. *Clin J Am Soc Nephrol*. 2014;9(7):1190–1198. https://doi.org/10.2215/CJN.09320913. Epub 2014 May 15.
13. Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J Int Med. November*. 2010;268(5):456–467.
14. Rahman M, Yang W, Akkina S, et al. CRIC study investigators. Relation of serum lipids and lipoproteins with progression of CKD: the CRIC study. *Clin J Am Soc Nephrol*. 2014;9(7):1190–1198. https://doi.org/10.2215/CJN.09320913. Epub 2014 May 15.
15. Jafar TH, Stark PC, Schmid CH, Landa M, AIPRD Study Group, et al. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Ann Intern Med*. 2003;139(4):244–252.
16. Manjunath G, Tighiouart H, Ibrahim H, et al. Level of kidney function as a risk factor for atherosclerotic cardiovascular outcomes in the community. *J Am Coll Cardiol*. 2003;41:47–55.
17. Baba M, Shimbo T, Horio M, et al. *Longitudinal Study of the Decline in Renal Function in Healthy Subjects*; 2015. https://doi.org/10.1371/journal.pone.0129036. Published: June 10, 2015.
18. Nicholas NR. A review of social isolation an important but underassessed condition in older adults. *J Prim Prev*. 2012;33(2–3):137–152.
19. Alhawary SY, Al-Abdallat ME, Alamro SA, et al. Frequency of blood groups among a sample of patients with renal failure at royal medical services royal medical services, Jordan the University of Jordan. *Jordan Euro Sci Journ*. 2015;11(33).
20. Coppo R, Amore A, Roccatello D. Dietary antigens and primary immunoglobulin A nephropathy. *J Am Soc Nephrol*. 1992;2:S173–S180.
21. *Personal communication*. Linda Frassetto, MD
22. Powles J, Fahimi S, Micha R, et al. Global, regional and national sodium intakes in 1990 and 2010: a systemic analysis of 24h urinary sodium excretion and dietary surveys worldwide. *BMJ Open*. 2013;3(12):e003733.
23. Sharma AM, Distler A. Acid-base abnormalities in hypertension. *Am J Med Sci*. 1994;307(suppl 1):S112–S115.
24. Sharma AM, Kribben A, Schattenfroh S, Cetto C, Distler A. Salt sensitivity in humans is associated with abnormal acid-base regulation. *Hypertension*. 1990;16(4):407–413.
25. Adeva MM, Souto G. Diet-induced metabolic acidosis. *Clin Nutr*. 2011;30(4):416–421.
26. Voormolen N, Noordzij M, Grootendorst DC, et al. High plasma phosphate as a risk factor for decline in renal function and mortality in pre-dialysis patients. *Nephrol Dial Transplant*. 2007;22:2909–2916.
27. O'Seaghdha CM, Hwang SJ, Muntner P, Melamed ML, Fox CS. Serum phosphorous predicts incident chronic kidney disease and end-stage renal disease. *Nephrol Dial Transplant*. 2011;26(9):2885–2890.
28. Sim JJ, Bhandari SK, Smith N, et al. Phosphorous and risk of renal failure in subjects with normal renal function. *Am J Med*. 2013;126(4):311–318. PubMed PMID: 23375678.
29. Satarug S, Vesey DA, Gobe GC. Kidney cadmium toxicity, diabetes, and high blood pressure: the perfect storm. *Tohoku J Exp Med*. 2017;241(1):65–87. PubMed PMID: 28132967.
30. Bridges CC, Joshee L, Zalups RK. Aging and the disposition and toxicity of mercury in rats. *Exp Gerontol*. 2014;53:31–39. PubMed PMID: 24548775.
31. Atchison WD, Hare MF. Mechanisms of methylmercury-induced neurotoxicity. *FASEB J*. 1994;8(9):622–629. PubMed PMID: 7516300.
32. Groves CE, Lock EA, Schnellmann RG. Role of lipid peroxidation in renal proximal tubule cell death induced by haloalkene cysteine conjugates. *Toxicol Appl Pharmacol*. 1991;107(1):54–62.
33. Seralini GE, Clair E, Mesnage R, et al. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environ Sci Eur*. 2014;26(1):14. PubMed PMID: 27752412.
34. Jayasumana C, Paranagama P, Agampodi S, et al. Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. *Environ Health*. 2015;14:6.
35. United States Environmental Protection Agency. Drinking water contaminants - standards and regulation. Web site http://water.epa.gov/drink/contaminants/basicinformation/glyphosate.cfm#four. Accessed November 18, 2015.
36. Pan Y, Zhang L, Wang F, Li X, Wang H. China National Survey of Chronic Kidney Disease Working Group. Status of non-steroidal anti-inflammatory drugs use and its association with chronic kidney disease: a cross-sectional survey in China. *Nephrology (Carlton)*. 2014;19(10):655–660. PubMed PMID: 25196389.
37. Gooch K, Culleton BF, Manns BJ, Zhang J, Alfonso H, et al. NSAID use and progression of chronic kidney disease. *Am J Med*. 2007;120(3):280.e1–7. PubMed PMID: 17349452.
38. Ungprasert P, Cheungpasitporn W, Crowson CS, Matteson EL. Individual non-steroidal anti-inflammatory drugs and risk of acute kidney injury: a systematic review and meta-analysis of observational studies. *Eur J Intern Med*. 2015;26(4):285–291. PubMed PMID: 25862494.
39. De Broe ME, Elseviers MM. Over-the-counter analgesic use. *J Am Soc Nephrol*. 2009;20(10):2098–2103.
40. Greenberg A, Cheung A, Coffman T, Falk R, Jennette J, eds. *Primer on Kidney Diseases*. San Diego: National Kidney Foundation Academic Press; 1994.
41. BERNARDORODRIGUEZ-ITURB Principal discussant: epidemic poststreptococal glomerulonephritis. *Kidney Int*. 1984;5:129—136.
42. National Institute of Diabetes and Digestive and Kidney Diseases. Nephrotic syndrome in adults. Web site https://www.niddk.nih.gov/health-information/kidney-disease/nephrotic-syndrome-adults. Accessed November 20, 2019.
43. National Institute of Diabetes and Digestive and Kidney Diseases. Glomerular diseases. Web site https://www.niddk.nih.gov/health-information/kidney-disease/glomerular-diseases. Accessed November 20, 2019.
44. Johnson CA, Levey AS, Coresh J, Levin A, Lau J, Eknoyan G. Clinical practice guidelines for chronic kidney disease in adults: Part II. Glomerular filtration rate, proteinuria, and other markers. *Am Fam Physician*. 2004;70(6):1091–1097.
45. National Kidney Foundation Practice Guidelines for Chronic Kidney Disease. *Annals of Internal Medicine*. American College of Physicians 7/21/17
46. Hajjar ER, Cafiero AC, Hanlon JT. Polypharmacy in elderly patients. *Am J Geriatr Pharmacother*. 2007;5(4):345–351. https://doi.org/10.1016/j.amjopharm.2007.12.002.

47. *Diet in Chronic Kidney Disease - Haemodialysis: How Does It Work?* http://www.diet-in-chronic-kidney-disease.com/en/108.htm. Accessed November 20, 2019.
48. Curl CL, et al. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Env Health Perspect*. 2003;111:377–382.
49. Deicher R, Hörl WH. Vitamin C in chronic kidney disease and hemodialysis patients. *Kidney Blood Press Res*. 2003;26(2):100–106.
50. Handelman GJ. New insight on vitamin C in patients with chronic kidney disease. *J Ren Nutr*. 2011;21(1):110–112. https://doi.org/10.1053/j.jrn.2010.11.003.
51. Melamed ML, Thadhani RI. Vitamin D therapy in chronic kidney disease and end stage renal disease. *Clin J Am Soc Nephrol*. 2012;7(2):358–365.
52. Slatopolsky E, Moe S. 50 Years of research and discovery in chronic kidney disease and mineral & bone disorder: the central role of phosphate. *Kidney Int*. 2011;79:S1–S2.
53. Dusso AS. Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25- dihydroxyvitamin D, and VDR activation. *Kidney Int Suppl. (2011)*. 2011;1(4):136–141.
54. Chan J, Mahan J, Trachman H, et al. Vitamin E therapy in IgA nephropathy: a double-blind, placebo-controlled study. *Periatr Nephrol*. 2003;18:1015–1019.
55. Gaby A. *Nutritional Medicine*. Concord, NH: Fitz Perlberg Publishing; 2011:772.
56. Saran R, Novak J, Desai A, et al. Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. *Nephrol Dial Transplant. November 1*. 2003;18(11):2415–2420.
57. Gopinath B, Harris DC, Flood VM, Burlutsky G, Mitchell P. Consumption of long-chain n-3 PUFA, alpha-linolenic acid and fish is associated with the prevalence of chronic kidney disease. *Br J Nutr*. 2011;105:1361–1368.
58. Stoof et al. "Does fish oil protect renal function in Cyclosporin-treated psoriasis patients?" *J Int Med*. 89;226:437–434.
59. Van der Heide JJH, Bilo HJG, Donker JM, et al. Effects of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. *N Engl J Med*. 1993;329:769–773.
60. Ogborn MR, Nitschmann E, Weiler H, Leswick D. Flaxseed ameliorates interstitial nephritis in rat poly- cystic kidney disease. *Bankovic-Calic N Kidney Int*. 1999;55(2). 4 7–23.
61. Ogborn MR, Nitschmann E, Bankovic-Calic N, Weiler HA, Aukema H. Dietary flax oil reduces renal injury, oxidized LDL content, and tissue n-6/n-3 FA ratio in experimental polycystic kidney disease. *Lipids*. 2002;37:059–065.
62. Lauterburg BH, Corcoran GB, Mitchell JR. Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in Vivo. *J Clin Invest*. 1983;71(4):980–991.
63. Alonso A, Lau J, Jaber BL, et al. Prevention of radiocontrast nephropathy with N-acetylcysteine in patients with chronic kidney disease: a meta-analysis of randomized, controlled trials. *Am J Kidney Dis*. 2004;43:1–9.
64. Inselmann, Blohmer, Kottny, et al. Modification of cisplatin-induced renal p-Aminohippurate uptake alteration and lipid peroxidation by thiols, *Ginkgo biloba* extract, deferoxamine and Torbafylline. *Nephron*. 995;70:425–429
65. Melhem MF, Craven PA, DeRubertis FR. Effects of dietary supplementation of alpha-lipoic acid on early glomerular injury in diabetes mellitus. *J Am Soc Nephrol*. 2001;12:124–133.
66. El-Sayed EM, Mansour AM, El-Sawy WS. Alpha lipoic acid prevents doxorubicin-induced nephrotoxicity by mitigation of oxidative stress, inflammation and apoptosis in rats. *J Biochem Mol Toxicol*. 2017;31:e21940.
67. Kazmi WH, Obrador GT, Sternberg M, et al. Carnitine therapy is associated with decreased hospital utilization among hemodialysis patients. *Am J Nephrol*. 2005;25(2):06–5. Epub 2005.
68. M1 C, Benatti P, Mancinelli A, et al. Carnitine replacement in end-stage renal disease and hemodialysis. *Ann N Y Acad Sci*. 2004;1033:52–66.
69. Narayanan V, Punithavathi D, Arumugam V. Curcumin prevents adriamycin nephrotoxicity in rats. *Br J Pharmacol*. 2000;129(2):231–234. PMC. Web. Nov 15. 2017.
70. Ghosh SS, Gehr TWB, Ghosh S. Curcumin and chronic kidney disease (CKD): major Mode of action through stimulating endogenous Intestinal alkaline Phosphatase. *Molecules*. 2014;19:20139–20156.
71. Boericke W. *Materia Medica with Repertory*. 9th ed. Philadelphia, PA: Boericke & Runyon; 1927.
72. Felter-Lloyd. *King's American Dispensatroy. Sandy, Oregon*. Eclectic Medical Publications; 1997.
73. Felter H. *The Eclectic Materia Medica, Pharmacology and Therapeutics*. Portland, Oregon: Eclectic Medical Publications; 1983.
74. Ellingwood F. *American Materia Medica, Therapeutics and Pharmacognosy*. Portland, Oregon: Eclectic Medical Publications; 1983.
75. Wu XJ, Wang YP, Wang W, Sun WK, Xu YM, Xuan LJ. Free radical scavenging and inhibition of lipid peroxidation by magnesium lithospermate B. *Acta Pharmacol Sinica*. 2000;21(9):855–858.
76. Scudder J. *Specific Diagnosis A Study of Disease*. Portland, OR: Eclectic Medical Publications; 1983.
77. Wang Z, Liu J, Sun W. Effects of asiaticoside on levels of podocyte cytoskeletal proteins and renal slit diaphragm proteins in adriamycin-induced rat nephropathy. *Life Sci*. 2013;93(8):352–358.
78. Meng XM, Zhang Y, Huang XR, et al. Treatment of renal fibrosis by rebalancing TGF-β/Smad signaling with the combination of asiatic acid and naringenin. *Oncotarget*. 2015. https://doi.org/10.18632/oncotarget.6100. [Epub ahead of print].
79. Pruijm M, Hofmann L, Charollais-Thoenig J, et al. Effect of dark chocolate on renal tissue oxygenation as measured by BOLD-MRI in healthy volunteers. *Clin Nephrol*. 2013;80(3):211–217.
80. Al-Malki AL, Moselhy SS. The protective effect of epicatechin against oxidative stress and nephrotoxicity in rats induced by cyclosporine. *Hum Exp Toxicol*. 2011;30(2):145–151.
81. Nair AR, Masson GS, Ebenezer PJ, et al. Role of TLR4 in lipopolysaccharide-induced acute kidney injury: protection by blueberry. *Free Radic Biol Med*. 2014;71:16–25.
82. Kim MK, Chung SW, Kim DH, et al. Modulation of age-related NF-kappaB activation by dietary zingerone via MAPK pathway. *Exp Gerontol*. 2010;45(6):419–426.
83. Onwuka FC, Erhabor O, Eteng MU, Umoh IB. Protective effects of ginger toward cadmium-induced testes and kidney lipid peroxidation and hematological impairment in albino rats. *J Med Food*. 2011;14(7–8):817–821.
84. Baiomy AA, Mansour AA. Genetic and histopathological responses to cadmium toxicity in Rabbit's kidney and liver: protection by ginger *(Zingiber officinale)*. *Biol Trace Elem Res*. 2015. [Epub ahead of print].
85. Shanmugam KR, Ramakrishna CH, Mallikarjuna K, Reddy KS. Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats. *Indian J Exp Biol*. 2010;48(2):143–149.
86. Baiomy AA, Attia HF, Soliman MM, Makrum O. Protective effect of ginger and zinc chloride mixture on the liver and kidney alterations induced by malathion toxicity. *Int J Immunopathol Pharmacol*. 2015;28(1):122–128.
87. Kazeem MI, Akanji MA, Yakubu MT. Amelioration of pancreatic and renal derangements in streptozotocin-induced diabetic rats by polyphenol extracts of Ginger *(Zingiber officinale)* rhizome. *Pathophysiology*. 2015; pii: S09284680(15)30009-2. https://doi.org/10.1016/j.pathophys.2015.08.004. [Epub ahead of print].
88. JHA V. Herbal medicines and chronic kidney disease. *Nephrology*. 2010;15:10–17. https://doi.org/10.1111/j.1440-1797.2010.01305.

191

Kidney Stones

Geovanni Espinosa, ND, LAc, IFMCP, CNS, and
Ralph Esposito, ND, LAc

OUTLINE

DIAGNOSTIC SUMMARY

- Usually asymptomatic
- Diagnosed adventitiously or from acute symptoms if urinary tract obstruction
- Excruciating, intermittent, radiating pain to the groin area originating in the flank or kidney
- Nausea, vomiting, and abdominal distention
- Chills, fever, and urinary frequency if infection present

GENERAL CONSIDERATIONS

Stone formation in the urinary tract has been recognized for thousands of years; during the past few decades, however, both the pattern and incidence of the disease have changed markedly. In the past, stone formation occurred almost exclusively in the bladder, but today most stones form in the upper urinary tract (Fig. 191.1). Males have a 3:1 ratio in the formation of kidney stones compared with females except in the sixth decade, where the incidence falls in men but rises in women—a trend toward gender equivalence.[1]

Once a kidney stone does form, there is a 50% chance of recurrence within 5 to 7 years if there is no treatment. Kidney stones are not limited to any cultural, geographical, or racial groups. In the United States, 1 of every 11 Americans is affected by kidney stones, with the emergency department (ED) being the most common resource for patients. Of these, 11% return within the first 30 days after an ED visit.[2] The incidence has been steadily growing, paralleling the rise in other diseases associated with the so-called Western diet—ischemic heart disease, cholelithiasis, hypertension, and diabetes.

In the western hemisphere, over 80% of kidney stones are usually composed of calcium salts, uric acid (5%–8%), or struvite (10%–15%). Molecular research is beginning to link certain mutations in the genes responsible for handling renal chloride, which can lead to hypercalciuria. Other identified genetic changes are being linked to excess urinary excretions of oxalate, cystine, and uric acid.[3] A recent systematic review found that 20 genes and more than 42 single-nucleotide polymorphisms (SNPs) may relate to stone matrix, calcium and phosphate regulation, inflammation, and oxidative stress, all of which contribute to stone formation. The complexities of genomics are not yet completely solidified; thus suggesting that the cause of stones is primarily related to an SNP is premature. However, this still provides us with a deeper insight as further research emerges.[4]

The incidence of renal stones varies geographically, reflecting differences in environmental factors, diet, and components of drinking water. Human urine is supersaturated with respect to calcium oxalate, uric acid, and phosphates. These substances normally remain in solution because of pH control and the secretion of inhibitors of crystal growth.

The following primary and secondary metabolic diseases cause kidney stones and must be ruled out early in the clinical process:

- Hyperparathyroidism
- Cystinuria
- Vitamin D excess
- Milk-alkali syndrome
- Destructive bone disease
- Primary oxaluria
- Cushing syndrome
- Sarcoidosis
- Acid-forming diet

Evolving research is showing that acid-forming diets (i.e., diets whose constituents require a metabolic response to neutralize and increase excretion of acids) appear to underlie many of the dietary factors—especially salt and foods with sulfur-containing amino acids—and have been shown to play a key role in the formation of both calcium and urate stones. This finding also helps explain the protective effects of a diet high in fruits and vegetables.

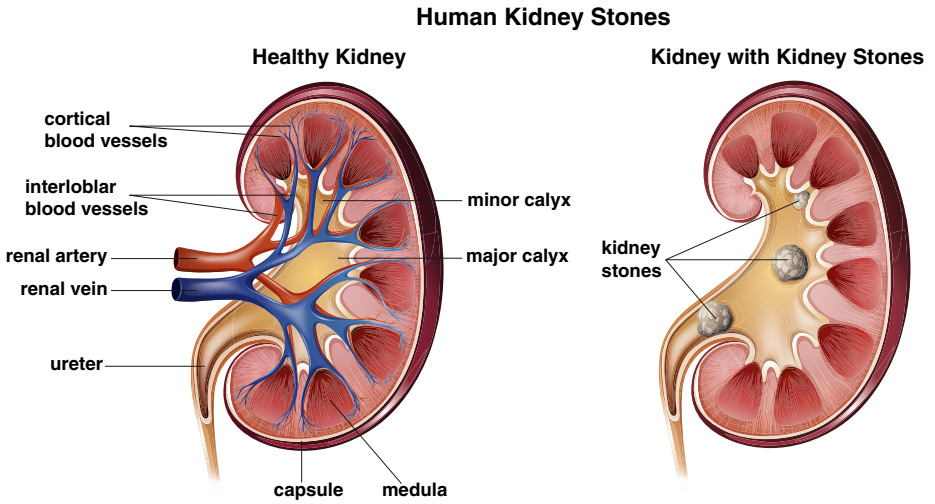


Fig. 191.1 Kidney stone. (From https://www.istockphoto.com/vector/human-anatomy-diagram-with-kidney-stones-gm922029526-253154531.)

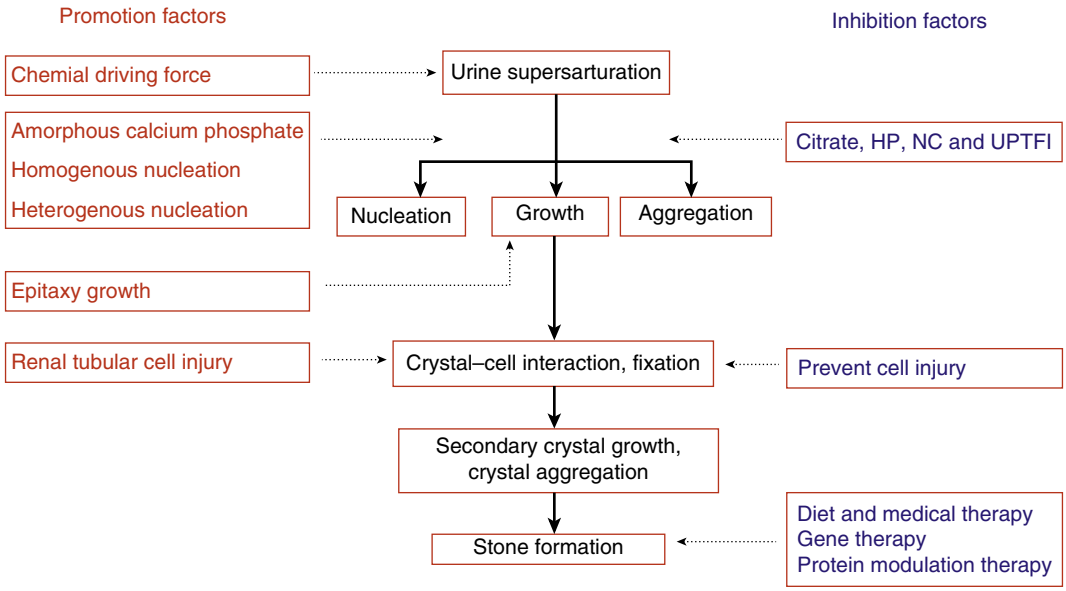


Fig. 191.2 Schematic diagram for the formation and inhibition mechanism of calcium-oxalate renal stone formation. (From Inhibition of urinary macromolecule heparin on aggregation of nano-COM and nano-COD crystals [Scientific Figure on ResearchGate]. https://www.researchgate.net/Schematic-diagram-for-the-formation-and-inhibition-mechanism-of-calcium-oxalate-renal_fig1_271332535.)

DIAGNOSTIC CONSIDERATIONS

Stone Formation

Conditions favoring stone formation can be divided into two groups: factors increasing the concentration of stone crystalloids and factors favoring stone formation at normal urinary concentrations (Fig. 191.2). The first group includes a reduction in urine volume (dehydration) and an increased rate of excretion of stone constituents. The second group is related to urinary stasis, pH changes, foreign bodies, and reduction of normal substances that solubilize stone constituents. See Tables 191.1 and 191.2 for an outline of the diagnostic possibilities.

THERAPEUTIC CONSIDERATIONS

Stone Composition

Diagnosing the type of kidney stone is critical to identifying the appropriate therapy. Careful evaluation of the following criteria usually determines the composition of the stone if one is not available for chemical analysis:

- Diet
- Underlying metabolic or disease factors
- Serum and urinary calcium, uric acid, creatinine, and electrolyte levels
- Urinalysis
- Urine culture

Table 191.3 summarizes the findings in the major types of kidney stones.

Dietary Factors

Calcium-containing stones are composed of calcium oxalate, calcium oxalate mixed with calcium phosphate, or very rarely calcium phosphate alone. The high incidence of calcium-containing stones in affluent societies is directly associated with the following dietary patterns:

- Low amounts of fiber[5]
- Intake of highly refined carbohydrates[6,7]

TABLE 191.1 Causes of Excessive Excretion of Relatively Insoluble Urinary Constituents

Constituent	Cause of Excess Excretion	Laboratory Findings
Calcium	(>250 mg/day excreted)	Low serum PO_4
	Absorptive hypercalciuria	30%–40% of all stone formers
	Renal hypercalciuria (renal tubular acidosis)	High serum parathyroid hormone
	Primary hyperparathyroidism	High urinary cyclic AMP
	Hyperthyroidism	High serum calcium
	High vitamin D intake	High $1,25(OH)_2D_3$
	Excess intake of milk and alkali	Low serum phosphate
	Aluminum salt intake	High $1,25(OH)_2D_3$
	Destructive bone disease	
	Sarcoidosis	
	Prolonged immobility	
	Methoxyflurane anesthesia	
Oxalate	Familial oxaluria	Rare
	Ileal disease, resection, or bypass	Vitamin B_6 deficiency or abnormal oxalate metabolism
	Steatorrhea	
	High oxalate intake	
	Ethylene glycol poisoning	
	Vitamin C excess (extremely unlikely)	
Uric acid	(<750 mg/day excreted)	Rapid cell destruction
	Gout	
	Idiopathic hyperuricosuria	
	Excess purine intake	
	Anticancer drugs	
	Myeloproliferative disease	
Cystine	Hereditary cystinuria	

TABLE 191.2 Physical Changes in Urine and the Kidneys

Condition	Possible Cause
Increased concentration	Dehydration
	Stasis
	Obstruction
	Foreign-body concretions
Urinary pH	Low—uric acid, cystine
	High—calcium oxalate and PO_4
Infection	*Proteus*—struvite
Uricosuria	Crystals of uric acid initiate precipitation of calcium oxalate from solution
Nuclei for stone formation	Cells, bacteria, blood clots, etc., initiate precipitation
	Sponge kidney
Deformities of kidney	Horseshoe kidney
	Caliceal obstruction or defect

AMP, Adenosine monophosphate

- High alcohol consumption[8]
- Large amounts of animal protein[8,9]
- High intake of fat[10]
- High intake of soft drinks[11]
- High intake of fructose[12]

The classification of most stones as either "idiopathic" or "hypercalciuria" reflects an ignorance of the dietary factors that induce hyperuricemia, hypercalciuria, and stone formation. The cumulative effect of these dietary factors is undoubtedly the reason for the rising incidence of kidney stones.

As a group, vegetarians have a lower risk of stone development. Studies have shown that even among meat eaters, those who ate higher amounts of fresh fruits and vegetables had a lower incidence of stones.[13] Bran supplementation, as well as the simple change from white to whole-wheat bread, has resulted in lower urinary calcium levels.[5]

Dietary factors may also play a role in acidifying or alkalinizing urine. Depending on the type of stone, this ability to alter urinary pH may help treat and prevent stones.[14] In one study, 12 healthy men were given a standardized diet plus cranberry, black currant, or plum juice. These subjects then provided 24-hour urine collections for evaluation. The researchers found that cranberry juice decreased the urinary pH and significantly increased the excretion of oxalic acid and the relative supersaturation for uric acid. Black currant juice increased urinary pH, excretion of citric acid, and loss of oxalic acid. Plum juice effected no change.[15] The researchers concluded that black currant juice could support the treatment and prevention of uric acid stones through its alkalizing effect. Conversely, because cranberry juice acidifies urine, it could be useful in the treatment of brushite and struvite stones as well as for urinary tract infections.[16]

Increased fluid intake has been recognized as one of the main approaches to decrease urine supersaturation. An increase in the urine volume results in a decrease in stone prevalence. Numerous randomized controlled trials (RCTs) have found that consumption of more than 2 L/day of water[17] or increased fluid intake to achieve a urine output of more than 2.5 L/day[18] lowers the long-term risk of kidney stone recurrence by approximately 60% versus no treatment.

One trial, conducted in stone-forming men with a baseline soft drink consumption of more than 160 mL/day, reported a reduction in self-reported physician-confirmed episodes of renal colic in those randomized and advised to abstain from soft drink intake versus no intervention for 3 years. Total fluid intake was similar in both groups.[11]

TABLE 191.3 Chemical and Physical Characteristics of Urinary Stones

Composition	Crystal Name	Frequency (%)	X-Ray Appearance	Urine Characteristics	Crystal Characteristics
Calcium oxalate	Whewellite	30–35	Opaque	Nonspecific	Small, hempseed- or mulberry-shaped, brown or black
Calcium oxalate + calcium phosphate		30–35	Opaque	pH > 5.5	Small, hempseed- or mulberry-shaped, brown or black
Calcium phosphate	Apatite	6–8	Opaque	pH > 5.5	Staghorn configuration, light in color
Magnesium ammonium phosphate	Struvite triple phosphate	15–20	Opaque	pH > 6.2 Infection	Staghorn configuration, light in color
Uric acid		6–10	Translucent	pH < 6.0	Ellipsoid, tan or red-brown
Cystine		2–3	Opaque	pH < 7.2	Multiple, faceted, maple syrup color

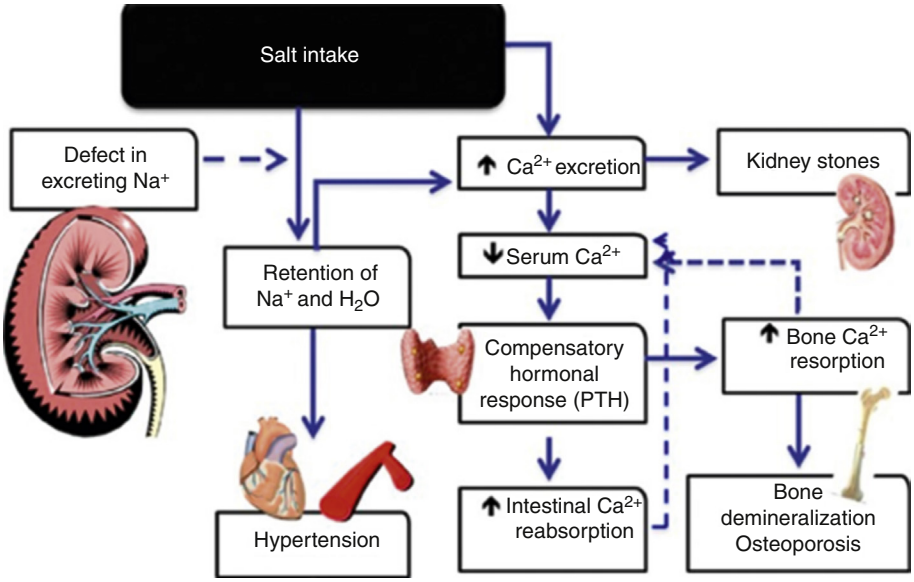


Fig. 191.3 Schematic representation of the effects of salt intake on calcium metabolism and its implications for kidney stone formation and bone health. (From Cappuccio FP. Cardiovascular and other effects of salt consumption. *Kidney Int Suppl.* 2013;3[4]: 312–315. PubMed PMID: 25019010. https://openi.nlm.nih.gov/detailedresult.php?img=PMC4089690_kisup201365f1&req=4.)

DIET

In one study, high levels of dietary calcium in men younger than age 60 were associated with decreased stone formation, but there was no effect in men older than age 60, nor was a supplemental dietary intake of more than 500 mg of calcium associated with an increased risk of stones.[19]

The specific type of stone was not elucidated in this study, and it was assumed that the majority of kidney stones reported by the cohort population consisted predominantly of calcium oxalate, as in the general population. The cause of this age-specific difference is unclear. Dietary calcium may bind to dietary oxalate in the intestine, thereby reducing oxalate absorption and the subsequent concentration of urinary oxalate. Both vitamin D deficiency and a diminished ability to absorb dietary calcium are more prevalent in older people.[19]

Sodium and Salt

Regarding dietary salt content, observational studies suggested a strong relation between sodium consumption and hypercalciuria, but more recent evidence has challenged this idea. High sodium intake is known to reduce renal tubular reabsorption of calcium, thereby increasing the amount of calcium excreted in the urine (Fig. 191.3).[20] Also, a high-sodium diet is known to increase urine pH and has been proposed to reduce urine citrate.[21] Other studies show a positive association between urinary sodium and calcium excretion and suggest that stone formers may be more sensitive to the calciuric effect of sodium.[22]

Nouvenne et al.[23] found that when patients with idiopathic calcium stones were treated with sodium restriction (60 mmol/day) and high fluid intake, a reduction of 100 mmol of urinary sodium was accompanied by a reduction of 64 mg/day in urinary calcium, with 30% of patients achieving normal urine calcium.

In contrast, other data, specifically in patients with hypocitraturia (not hypercalciuria), demonstrated that dietary sodium supplementation resulted in increased voided volume and decreased calcium-oxalate supersaturation.[24] This must be carefully examined because these patients were followed for only a short period of time and were already using pharmacological interventions for stone

disease. Although results continue to be conflicting, reduced dietary sodium is a general recommendation given to most patients with a history of kidney stones.

Fructose

Sports drinks have become increasingly popular within the past decade. The content of citrate in such beverages would be expected to increase urine citrate excretion and urine pH, providing protection against both calcium and uric acid stones. However, the purposely high sodium content of sports drinks, promoted as useful for "rehydration" in athletes, might be associated with increases in urine calcium excretion. In addition, the ingestion of significant amounts of sucrose and fructose in these drinks could also be associated with increases in calcium excretion.[7] Fructose, a component of corn syrup frequently added to sports drinks and sodas as a sweetener, would also be an undesirable way for most stone formers to increase urine volume, given its recent links to hyperuricemia, metabolic syndrome, and stones.[25,26]

Citrate is an important inhibitor of the aggregation and growth of calcium-oxalate and calcium-phosphate crystals. In the past, dietary interventions to increase citrate have included lemonade and orange juice.[27,28] The results with lemonade are conflicting, with one study showing an increase in urine volume but not in urine citrate.[29] Another citrus juice, grapefruit, may prove to be effective in increasing urinary citrate levels and reducing the BONN risk index score, suggesting an overall protective effect on crystallization. Although the urinary oxalate levels may be increased, crystallization is decreased and offset by increased citrate.[30–32]

Regular consumption of grapefruit juice should be avoided, however. In one large-scale study, women drinking 8 oz of grapefruit juice daily increased their risk of stones by 44%.

Hyperoxaluria is a metabolic risk factor for stone disease. Dietary oxalate may contribute as much as 80% of the urine oxalate.[33,34] Therefore a low-oxalate diet may provide benefit for patients with hyperoxaluria. In addition, adequate dietary calcium to minimize intestinal oxalate absorption has been shown to be beneficial. Borghi et al.,[35] in a randomized trial in men with recurrent calcium-oxalate stones, achieved a significant reduction in oxalate excretion and the incidence of recurrent stones for patients on a normal-calcium (1200 mg/day), low-animal-protein, low-salt diet compared with a low-calcium diet (400 mg/day).

Dietary Recommendations for Patients With High Urine Oxalate

Low-Oxalate Diet

A low-oxalate diet is a common prescription for recurrent calcium-oxalate kidney stones. The ultimate goal is to reduce the level of oxalic acid being excreted in the urine. It appears that people with recurrent kidney stones have a tendency to absorb higher levels of dietary oxalates, up to 50%,[33] compared with normal subjects not prone to kidney stones, who absorb only 3% to 8% of dietary oxalate. A low-oxalate diet is usually defined as less than 50 mg of oxalate per day, so foods in the high- and moderate-oxalate categories have to be curtailed. Box 191.1 provides an estimate of the oxalate content of food, which is highly variable. The level of oxalate in a particular food in published reports can vary twofold to fifteenfold. Differences in climate, soil quality, state of ripeness, or even which part of the plant is analyzed will also affect the value.

Although the role of oxalates in the development and risk of kidney stones is unclear, with some research showing only a modest correlation, clinically it would be prudent for stone formers to reduce intake. However, the stringency of following a low-oxalate diet must be considered and tailored to the patient and his or her potential comorbidities.[36]

Weight and Carbohydrate Metabolism

Weight control and the correction of carbohydrate metabolism are important because excess weight and insulin insensitivity lead to hypercalciuria and are high-risk factors for stone formation.[37,38] After glucose ingestion, there is a rise in urinary calcium, and phosphate reabsorption decreases. This leads to a low plasma phosphate concentration, which stimulates the production of 1,25-dihydroxycholecalciferol and results in increased intestinal absorption of calcium; concurrently; it also causes hypercalciuria. The ingestion of sucrose and other simple sugars causes an exaggerated rise in the urinary calcium-oxalate content in approximately 70% of people with recurrent kidney stones.[39]

Gut Flora

Studies are beginning to find a correlation between the proper balance of intestinal flora and a lower risk of oxalate kidney stones. Oxalate homeostasis depends in part on the intestinal anaerobic bacterium *Oxalobacter formigenes.* It now appears that *O. formigenes* contributes to oxalic acid homeostasis and that its absence may predispose individuals to idiopathic calcium-oxalate kidney stone disease.[39] This bacterium is found primarily in the colon, and its loss through prolonged, widespread use of antibiotics is associated with an increased risk of hyperoxaluria and calcium-oxalate stone formation.[40] Studies in animals and human volunteers have indicated that, when administered therapeutically, *O. formigenes* can repopulate the gut and reduce the urinary oxalate concentration after an oxalate load, hence reducing the incidence of calcium-oxalate kidney stone formation.[41] More recent human studies have developed showing an inverse correlation with *O. formigenes* colonization and recurrent nephrolithiasis, inflammatory bowel disease, and idiopathic calcium nephrolithiasis, thus suggesting this unique commensal may confer some protection against future kidney stone disease.[42]

Nutrients

Magnesium and Vitamin B_6

A magnesium-deficient diet accelerates the deposition of calcium in renal tubules in rats.[43] Magnesium has been shown to increase the solubility of calcium oxalate and inhibit the precipitation of both calcium phosphate and calcium oxalate.[44–46] A low ratio of urinary magnesium to calcium is an independent risk factor for stone formation.[47] Supplemental magnesium alone has been shown to be effective in preventing recurrences of kidney stones.

When magnesium is used in conjunction with vitamin B_6, an even greater effect is noted.[48,49] Pyridoxine is known to reduce the endogenous production and urinary excretion of oxalates,[50,51] and patients with recurrent oxalate stones have abnormal activation levels of erythrocyte glutamate pyruvate transaminase, erythrocyte glutamic-oxaloacetic transaminase (EGOT), urinary glutamic-pyruvic acid transaminase, and urinary glutamic-oxaloacetic transaminase, indicating clinical insufficiency of vitamin B_6 as a cofactor and impaired glutamic acid synthesis. These levels return to normal after 3 months of treatment.[51] Induced pyridoxine deficiency in rats has been shown to produce oxaluria and calcium-oxalate lithiasis (which is prevented by magnesium supplementation).[50]

Several studies have confirmed the benefits of vitamin B_6 as a sole agent in reducing urine oxalate, albeit with a small population size. Doses between 200 to 500 mg have been shown to normalized hyperoxaluria in those with calcium-oxalate stones. However, other studies have questioned the benefit of B_6 as a sole agent, with a large review

BOX 191.1 Oxalate Content of Select Foods

Vegetables

High Oxalate, >10 mg per Serving

Beets—greens or root[a]
Celery
Collards
Dandelion greens
Eggplant
Escarole
Green beans
Kale
Leeks
Okra[b]
Parsley
Parsnips
Peppers, green
Potatoes
Pumpkin
Spinach[a]
Squash, yellow summer
Sweet potatoes
Swiss chard[a]
Tomato sauce, canned
Turnip greens
Watercress

Moderate Oxalate, <10 mg per Serving

Asparagus
Artichokes
Broccoli
Brussels sprouts
Carrots
Cucumber
Garlic
Lettuce
Mushrooms
Mustard greens
Onions
Peppers, green
Potato chips
Potato salad (¼ cup)
Pumpkin
Radishes
Snow peas
Tomato, fresh[c]
Tomato sauce, canned (¼ cup)

Low Oxalate, 2–5 mg per Serving

Acorn squash
Arugula
Ketchup (1 tbsp)
Onions
Pepper, red
Zucchini squash

Fruits

High Oxalate, >10 mg per Serving

Concord grapes
Figs, dried[b]
Kiwi
Lemon peel
Lime peel
Orange peel
Rhubarb[a]

Moderate Oxalate, <10 mg per Serving

Apples
Apricots
Berries (¼ cup)
Blackberries
Blueberries
Cherries, red sour
Cranberries, dried
Currants, black
Oranges
Peaches
Pears
Pineapple
Plums
Prunes, Italian
Red raspberries
Tangerines

Low Oxalate, 2–5 mg per Serving

Apples, peeled
Avocado
Cantaloupe
Cherries, bing
Cranberries
Grapes
Lemon juice (1 cup)
Lemons
Lime juice (1 cup)
Raisins

Grains

High Oxalate, >10 mg per Serving

Bread, whole wheat
Buckwheat[c]
Oatmeal
Popcorn
Spelt
Stone-ground flour
Wheat bran
Wheat germ
Whole-wheat flour

Moderate Oxalate, <10 mg per Serving

Bagel (1 medium)
Barley, cooked
Bread, white (2 slices)
Corn
Corn tortilla (1 moderate)
Cornbread
Cornmeal, yellow (1 cup dry)
Cornstarch (¼ cup)
Pasta
Rice, brown
Spaghetti
Wheat, white flour

Continued

BOX 191.1 Oxalate Content of Select Foods—cont'd

Low Oxalate, 2–5 mg per Serving
Rice, white
Rice, wild
Rye bread

Legumes
High Oxalate, >10 mg per Serving
Garbanzo beans
Lentils
Soy and all soy products

Moderate Oxalate, <10 mg per Serving
Lima beans
Split peas

Low Oxalate, 2–5 mg per Serving
Peas, green

Nuts and Seeds
High Oxalate, >10 mg per Serving
Almonds
Brazil nuts
Hazelnuts
Peanuts
Peanut butter
Pecans
Sesame seeds
Sunflower seeds

Moderate Oxalate, <10 mg per Serving
Cashews
Flaxseed
Walnuts

Low Oxalate, 2–5 mg per Serving
Coconut

Herbs
Moderate Oxalate, <10 mg per Serving
Cinnamon, ground (11/2 tsp)
Ginger, powdered (1 tbsp)
Pepper, black (1 tsp per day)
Thyme, dried (1 tsp)

Low Oxalate, 2–5 mg per Serving
Basil, fresh (1 tbsp)
Dill (1 tbsp)
Ginger, raw, sliced (1 tsp)
Malt, powder (1 tbsp)
Mustard, Dijon (1/2 cup)
Nutmeg (1 tbsp)
Pepper (1 tsp)

Miscellaneous
High Oxalate, >10 mg per Serving
Beer
Chocolate
Cocoa
Soy sauce (1 tbsp)
Tea, black
Tea, green

Moderate Oxalate, <10 mg per Serving
Coffee
Red wine
Sardines
Tea, rosehip

Low Oxalate, 2–5 mg per Serving
Beef
Chicken
Corned beef, canned
Eggs
Fish, haddock, plaice, and flounder
Ham
Hamburger
Lamb
Pork
Turkey
Venison

[a]Greater than 200 mg per serving.
[b]Greater than 50 mg per serving.
[c]Greater than 100 mg per serving.

showing no risk association with vitamin B_6 and kidney stone incident.[52] In this large review, the cohorts with the highest vitamin B_6 intake also had the highest use of calcium supplementation, which was not accounted for in the risk incidence of nephrolithiasis. Calcium may have provided a protective effect and altered the risk association.

Glutamic Acid

Depressed levels of glutamic acid (from vitamin B_6 deficiency or for other reasons) are significant in the formation of kidney stones because a higher concentration of glutamic acid in the urine induces precipitation of calcium oxalate. Glutamic acid supplementation in rats significantly reduces the incidence of calculi, and it may do so in humans as well.[53] Supplementation with glutamic acid may, however, be superfluous if adequate vitamin B_6 levels are attained.

Calcium

Although most doctors still tell their patients with kidney stones to avoid calcium supplements, this advice is mostly outdated. A strong body of evidence has emerged refuting the general trend to restrict calcium supplementation in patients with kidney stones.

One study has even shown that calcium supplementation reduces oxalate excretion.[54] This study measured urinary oxalate excretion after calcium supplementation and the administration of oxalic acid. Calcium was given in the form of calcium carbonate or calcium citrate malate at a dose of 300 mg elemental calcium daily. Compared with baseline assessment, calcium supplementation significantly reduced oxalate absorption and excretion.

Another study evaluated calcium intake and the dietary profiles in 37 outpatients with kidney stones and 45 control subjects. Dietary calcium, assessed from 4-day dietary records, was significantly lower for patients with urinary calculi.[55] A third study further supporting the benefits of calcium intake is a 5-year randomized trial comparing the effect of two diets in 120 men with recurrent calcium-oxalate stones and hypercalciuria.[56] Upon completion, the men consuming the normal-calcium diet experienced a 51% reduction in the risk of stone recurrence. During follow-up, urinary calcium levels dropped

significantly in both groups, but urinary oxalate was reduced in the normal-calcium-intake group and increased in the low-calcium-intake group. Finally, one large prospective epidemiological investigation also reported that kidney stones were more likely to manifest in individuals with the lowest daily calcium intakes (516 mg) than in those with the highest daily calcium intakes (1326 mg).[57]

These studies clearly demonstrate that calcium supplementation (300–1000 mg daily) may prove to be an effective preventive measure against calcium-oxalate kidney stones.

One large trial, however, of more than 36,000 postmenopausal women with no history of kidney stones showed, compared with placebo, a 17% increased risk of self-reported urinary tract stones in women who consumed 1000 mg of calcium carbonate plus 400 IU of vitamin D daily. For postmenopausal women, the implications of calcium carbonate ingestion may differ from those for younger women and men, for unknown reasons. Calcium absorbability comes into question in this population of women, particularly when the elemental form used is carbonate.[58] This study contradicts other data indicating that vitamin D supplementation can be provided to postmenopausal women who are not stone formers and who are vitamin D–insufficient without fear of an increased risk of kidney stones.[59]

Citrate

Low urinary citrate excretion is known to pose a risk for nephrolithiasis.[60] Although urinary calcium rises in patients consuming calcium citrate, some of citrate's effects inhibit the formation of kidney stones. Specifically, citrate has the ability to reduce urinary saturation of calcium oxalate and calcium phosphate and to retard the nucleation and crystal growth of calcium salts. The use of potassium or sodium citrate in the treatment of recurrent urinary saturation of calcium oxalate has been shown to be effective in clinical studies, ending stone formation in nearly 90% of the subjects.[61] For example, in one study, 31 mmol/day of potassium citrate provided to recurrent stone formers who were also hypocitraturic resulted in a drop of stone formation from 0.7 to 0.13 per year.[62]

Another 3-year, double-blind study of 57 people with a history of calcium stones and low urinary citrate levels revealed that those given potassium citrate developed fewer kidney stones than they had previously. In comparison, the group given a placebo had no change in their rate of stone formation.[63]

However, rather than potassium or sodium citrate, magnesium citrate appears to offer the greatest benefit.

Magnesium citrate slows the growth rate of brushite crystals, nucleation rate, and supersaturation of urine. In addition, because magnesium competes with calcium in binding oxalates in both the gut and urine, the ratio of magnesium to calcium in the urine is used in estimating stone risk.[64]

Vitamin K

The urinary glycoprotein, a powerful inhibitor of calcium-oxalate monohydrate growth, requires posttranscriptional carboxylation of glutamic acid to form gamma-carboxyglutamic acid. Vitamin K is an essential factor for this carboxylation to occur.[65] Impairment of glutamic acid formation or a vitamin K deficiency reduces the formation of this glycoprotein. The presence of vitamin K in green leafy vegetables may contribute to the lower incidence of kidney stones among vegetarians.[66]

Uric Acid Metabolism

The level of dietary purine consumption is linearly related to the rate of urinary uric acid excretion.[67] This relationship is important because hyperuricosuria is a causative factor in recurrent calcium-oxalate stones. High levels of supplemental folic acid promote purine-scavenging and xanthine oxidase inhibition, resulting in decreased excretion of uric acid (see Chapter 174). Higher urine pH also helps with uric acid solubility[68]; however, manipulation of urine pH is a complex task because it will be affected not only by diet and hydration status but also by weight and body mass index (BMI). Higher BMIs have been associated with lower urine pH.[69,70] Including a weight-reduction goal when appropriate may benefit patients with uric acid stones or low urine pH, although the long-term benefits are unknown.

Botanical Medicines

There are limited clinical trials with the use of medicinal herbs for kidney stones, and those that exist are poorly designed. We focus briefly on the traditional in vitro and in vivo use of a number of botanicals.

Anthraquinones isolated from the *Rubia, Cassia,* and *Aloe* species bind calcium and significantly reduce the growth rate of urinary crystals when used in oral doses lower than the laxative dose.[71,72] *Rubia tinctura, Rumex, Rheum, Polygonum aviculare, Aloe,*[73] *Senna, Rhamnus alnus,* and *Mitchella repens* are sources of these anthraquinones and may be used to prevent stone formation; during acute attacks, they may help reduce the size of the stone.

The furanocoumarin-containing herb *Ammi visnaga* has been shown to be unusually effective in relaxing the ureter and allowing the stone to pass.[74] This action is due to its calcium channel–blocking capabilities, which act primarily on the ureters. Atropine and papaverine have similar but less active smooth muscle–relaxing effects. *Peucedanum, Leptotania, Ruta graveolens,* and *Hydrangea* contain similar furanocoumarins that also promote smooth muscle relaxation, and all of these herbs have historical uses in the treatment of kidney stones.

Eupatorium purpureum (gravel root), as its name implies, has traditionally been used for treating urinary stones ("gravel"). The Eclectics, physicians who employed natural therapies at the end of the 19th and beginning of the 20th centuries, considered it as mildly astringent, a stimulant, and a tonic with a specific action on the urinary tract.[66] It was said to have the power to dissolve concretions.

One of several reasons why kidney stones are painful when they are passed is because they produce spasm in the ureters. Spasmolytic herbs have been clinically effective for controlling ureteral spasm in such cases, whereas diuretic herbs are also used to push sufficiently small stones through the ureters more slowly and tolerably. Currently, the use of these herbs is more dependent on tradition than on scientific data.

Some of the more popular spasmolytic herbs that have been reliably effective in this realm include *Ammi visnaga* (khella) fruit, which is also strong enough to use for angina pectoris and acute asthma, and *Lobelia, Piscidia piscipula* (Jamaican dogwood) bark, *gelsemium,* and western pasque flower. *Hyoscyamus niger* (henbane) herb is also useful; it is an even stronger anticholinergic antispasmodic—like belladonna but with an affinity for the genitourinary tract.[75]

In two clinical trials, participants with a history of nephrolithiasis and with radiographic stones present at baseline were randomized to *Orthosiphon grandiflorus* extract 2.5 g in tea twice daily or to sodium-potassium citrate 5 to 10 g three times daily for 18 months.[24] Serial ultrasound was used to measure mean annualized reduction in stone diameter at 18 months. The two treatments were equally effective at reducing stone size over a year's time, with far fewer adverse effects in the tea group.[76]

Phyllanthus niruri has been shown to inhibit calcium-oxalate crystallization in the test tube. Although reportedly diuretic in rodent and human studies,[77,78] low-dose studies find that it is not significantly diuretic in rats, although it still significantly dissolves stones.[78]

In one clinical trial, 69 subjects were randomized to extract capsules 450 mg of *Phyllanthus* three times daily or to placebo for 3 months.[79] Among those randomized to receive *P. niruri,* there was a reduction of hypercalciuria in those patients who had this problem initially, but no significant effect on calculus elimination or calculus size was observed.

Lifestyle

Sleep Position

Even though the etiology of recurrent unilateral kidney stones is unclear, patients with recurrent renal calculi most often present with calculi on the same side. Data from the literature support the notion that sleep posture may contribute to alterations of renal hemodynamics. Researchers studied 110 patients who suffered from kidney stones confined to one side of their bodies. Of these patients, 93 consistently favored sleeping with one side in a dependent position. Further study showed that the side of the stone was identical to the dependent sleep side in 76%, with positive predictive values of right-side-down and left-side-down sleep postures for ipsilateral stone formation at 82% and 70%, respectively.[80]

Stress

One case-control study of 200 patients with symptomatic kidney stones and 200 matched controls demonstrated a statistically significantly higher rate of kidney stone formation in people who had more stressful life events. Such events were defined as those that the subjects perceived as highly stressful by inflicting an intense emotional effect on them, with apprehension and distress, for at least 1 week.[81] It is hypothesized that stressful events may encourage the loss of litholytic urinary constituents such as magnesium and citrate.[82] The sympathetic "fight-or-flight" response may increase vasopressin release, thus contributing to a more hypertonic urine.[83] Furthermore, the stress response results in a stimulation of the hypothalamic–pituitary axis, resulting in increased release of not only vasopressin but also adrenocorticotropic hormone (ACTH). This stress response and increased ACTH can directly increase parathyroid hormone, resulting in hypercalcemia and subsequent hypercalciuria.[84] Furthermore, the stress adaptation response via the hypothalamic–pituitary–adrenal axis may chronically increase cortisol secretion, which has a similar physiological effect as vasopressin on the renal tubules, thus further contributing to hypertonic urine.

Miscellaneous

Toxin Exposure

Hair mineral analysis may be of value because many heavy metals (mercury, gold, uranium, and cadmium) are nephrotoxic. Cadmium, in particular, has been shown to greatly increase the incidence of kidney stones. A prospective study of coppersmiths showed a 40% incidence of kidney stones, which correlated with elevations of serum cadmium concentration.[85] Additional studies analyzed populations living in cadmium-contaminated environments and found that higher urinary calcium excretion correlated with higher urinary cadmium levels and an increased risk of urinary stone disease.[86,87]

Polyaromatic hydrocarbons (PAHs) constitute a group of chemicals generated from vehicle exhaust, asphalt, coal tar, wildfires, agricultural burning, soil, charbroiled foods, and tobacco smoke. Data from the U.S. National Health and Nutrition Examination Surveys (NHANES III), 2011–2012 examined the potential link between urinary PAH and kidney stones.[88] In 5560 American adults aged 20 to 80, urinary 2-hydroxyfluorene (odds ratio [OR] 1.35, 95% confidence interval [CI]; 1,02–1.78), 3-hydroxyfluorene (OR = 1.35, 95% CI; 1.07–1.70), 1-hydroxyphenanthrene (OR = 1.48, 95% CI; 1.08–2.03), 1-hydroxypyrene (OR = 1.36, 95% CI; 1.05–1.77), and 2-hydroxynaphthalene (OR = 1.25, 95% CI; 1.00–1.58) were significantly associated with kidney stones.

Interestingly, researchers examining data from NHANES III found that individuals with the highest dietary zinc intake (>15 mg/day) were associated with a significantly increased risk of kidney stone disease compared with those with lower dietary zinc intake (<7 mg/day).[89]

Vitamin C and the Risk of Oxalate Stones

Vitamin C is often cited in the medical literature as a potential factor in the development of calcium-oxalate kidney stones. Numerous studies have now demonstrated that in persons not undergoing hemodialysis or suffering from recurrent kidney stones, severe kidney disease, or gout, high-dose vitamin C therapy does not cause kidney stones. Vitamin C ingestion of up to 10 g/day has not had any effect on urinary oxalate levels.[90,91]

On the contrary, several human trials consistently indicate that a large percentage of individuals—regardless of kidney stone history, age, gender, and race—ingesting 1000 to 2000 mg of ascorbic acid for only short periods experience hyperoxaluria to some degree.

A more recent clinical study concurs with previous studies in demonstrating mean increases in total oxalate excretion with ascorbic acid supplementation.[91]

Another recent double-blind, crossover RCT of 50 healthy adults compared vitamin C with metabolites (Ester-C) to ascorbic acid. Only 41% of subjects on vitamin C with metabolites experienced an increase in 24-hour oxalate, which is 25% fewer compared with ascorbic acid. This implies that if one needs to consume vitamin C, Ester-C may be most beneficial for stone formers; however, the beneficial effects of Ester-C are still controversial.[92]

Inositol Hexaphosphate

Inositol hexaphosphate (also known as phytic acid or IP6), a naturally occurring compound found in whole grains, cereals, legumes, seeds, and nuts, is known for its antineoplastic activity. One trial showed that the use of 120 mg per day of IP-6 significantly reduced, within in 15 days, the formation of calcium-oxalate crystals in the urine of people with a history of kidney stones.[93] One literature review reported several human studies demonstrating the effectiveness of phytic acid against four types of kidney stones.[94]

THERAPEUTIC APPROACH

Effective treatment of kidney stones requires accurate differentiation between the various stone types and recognition and control of any underlying metabolic diseases or structural abnormalities of the urinary tract. Tables 191.1 and 191.2 list diagnosable causes of kidney stones.

Prevention of recurrence is the therapeutic aim in the treatment of kidney stones. Because dietary management is effective, inexpensive, and free of side effects, it is the treatment of choice. The specific treatment is determined by the type of stone and may include the following:

- Reducing urinary calcium
- Reducing purine intake
- Avoiding foods high in oxalate
- Increasing intake of foods with a high magnesium/calcium ratio
- Increasing intake of vitamin K–rich foods

For all types of stones, increasing urine flow to dilute the urine is vital. Enough fluids should be consumed to produce urine with a specific gravity lower than 1.015 and urinary volume of at least 2000 mL daily.

As far as lifestyle changes are concerned, if stones occur on one side only, it may be helpful to avoid sleeping on that side. Stress-reduction techniques and counseling should be considered for anyone with stones and a history of significant stress.

Acute Obstruction

Surgical removal or lithotripsy may be necessary; the following agents may also be used:

- *Ammi visnaga* extract (12% khellin content): 250 mg three times a day
- *Rubia tinctura, Rumex crispus,* or *A. vera* at doses lower than used for laxative effect

Calcium Stones

Diet

- Increase intake of fiber, complex carbohydrates, and green leafy vegetables.
- Decrease intake of simple carbohydrates and purines (meat, fish, poultry, yeast).
- Increase consumption of foods with high magnesium/calcium ratios (barley, bran, corn, buckwheat, rye, soy, oats, brown rice, avocados, bananas, cashews, coconut, peanuts, sesame seeds, lima beans, potatoes).
- If the stones are oxalate, reduce consumption of oxalate-containing foods (black tea, cocoa, spinach, beet leaves, rhubarb, parsley, cranberries, nuts).
- Limit consumption of dairy products.

Supplements

- Vitamin B_6: 25 mg/day
- Vitamin K: 2 mg/day
- Magnesium: 600 mg/day
- Calcium: 300 to 1000 mg/day
- IP6: 120 mg/day

Botanicals

Use any of the following agents in a dose less than that used for laxative effect:

- *R. tinctura*
- *R. crispus*
- *A. vera*

Miscellaneous

Avoid aluminum compounds and alkalis.

Uric Acid Stones

Diet

- Decrease consumption of purines (see list given for calcium stones).
- Ensure adequate fluid intake and a high alkali load, with plenty of fruits and vegetables.

Supplements

Folic acid: 5 mg/day

Miscellaneous

Alkalinize urine: citrate, bicarbonate, black currant juice.

Magnesium–Ammonium–Phosphate Stones

Miscellaneous

- Eradicate infection (see Chapter 163).
- Acidify urine with ammonium chloride (100–200 mg three times a day).

Cystine Stones

Diet

Avoid methionine-rich foods (soy, wheat, dairy products except whole milk, fish, meat, lima beans, garbanzo beans, and mushrooms; all nuts except coconut, hazelnut, and sunflower seeds).

Miscellaneous

Alkalinize urine: optimal pH is 7.5 to 8.

Brushite and Struvite Stones

Acidify urine with cranberry juice.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Heller HJ, Sakhaee K, Moe OW, Pak CYC. Etiological role of estrogen status in renal stone formation. *J Urol*. 2002;168(5):1923–1927. https://doi.org/10.1097/01.ju.0000033907.21910.be.
2. Scales CD, Smith AC, Hanley JM, Saigal CS. Urologic diseases in America Project CS, Project UD in A. Prevalence of kidney stones in the United States. *Eur Urol*. 2012;62(1):160–165. https://doi.org/10.1016/j.eururo.2012.03.052.
3. Langman CB. The molecular basis of kidney stones. *Curr Opin Pediatr*. 2004;16(2):188–193.
4. Taguchi K, Yasui T, Milliner DS, Hoppe B, Chi T. Genetic risk factors for idiopathic urolithiasis: a systematic review of the literature and causal network analysis. *Eur Urol Focus*. 2017;3(1):72–81. https://doi.org/10.1016/j.euf.2017.04.010.
5. Shah PJ, Williams G, Green NA. Idiopathic hypercalciuria: its control with unprocessed bran. *Br J Urol*. 1980;52:426–429.
6. Thom JA, Morris JE, Bishop A, Blacklock NJ. *The Influence of Refined Carbohydrate on Urinary Calcium Excretion*. Vol. 50. 1978. https://doi.org/10.1111/j.1464-410x.1978.tb06191.x.
7. Lemann J, Piering WF, Lennon EJ, Kelly OA, Brock J. Possible role of carbohydrate-induced Calciuria in calcium oxalate kidney-stone formation. *N Engl J Med*. 1969;280(5):232–237. https://doi.org/10.1056/NEJM196901302800502.
8. Zechner O, Latal D, Pflüger H, Scheiber V. Nutritional risk factors in urinary stone disease. *J Urol*. 1981;125:51–54.
9. Robertson WG, Peacock M, Marshall DH. Prevalence of urinary stone disease in vegetarians. *Eur Urol*. 1982;8:334–339.
10. Griffith HM, O'Shea B, Kevany JP, McCormick JS. A control study of dietary factors in renal stone formation. *Br J Urol*. 1981;53:416–420.
11. Shuster J, Jenkins A, Logan C, et al. *Soft Drink Consumption and Urinary Stone Recurrence: A Randomized Prevention Trial*. Vol. 45. 1992. https://doi.org/10.1016/0895-4356(92)90074-W.
12. De SK, Liu X, Monga M. Changing trends in the American diet and the rising prevalence of kidney stones. *Urology*. 2014;84(5):1030–1033. https://doi.org/10.1016/j.urology.2014.06.037.
13. Siener R, Hesse A. The effect of a vegetarian and different omnivorous diets on urinary risk factors for uric acid stone formation. *Eur J Nutr*. 2003;42:332–337. https://doi.org/10.1007/s00394-003-0428-0.
14. Rose GA, Westbury EJ. The influence of calcium content of water, intake of vegetables and fruit and of other food factors upon the incidence of renal calculi. *Urol Res*. 1975;3:61–66.
15. Kessler T, Jansen B, Hesse A. Effect of blackcurrant-, cranberry- and plum juice consumption on risk factors associated with kidney stone formation. *Eur J Clin Nutr*. 2002;56:1020–1023. https://doi.org/10.1038/sj.ejcn.1601442.
16. Frassetto L, Kohlstadt I. Treatment and prevention of kidney stones: an update. *Am Fam Physician*. 2011;84(11):1234–1242.
17. Borghi L, Meschi T, Amato F, Briganti A, Novarini A, Giannini A. Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol*. 1996;155:839–843. https://doi.org/10.1016/S0022-5347(01)66321-3.
18. Sarica K, Inal Y, Erturhan S, Yağci F. The effect of calcium channel blockers on stone regrowth and recurrence after shock wave lithotripsy. *Urol Res*. 2006;34:184–189. https://doi.org/10.1007/s00240-006-0040-x.
19. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr*. 2003;89(5):552–572. https://doi.org/10.1079/BJN2003837.
20. Goldfarb S. Dietary factors in the pathogenesis and prophylaxis of calcium nephrolithiasis. *Kidney Int*. 1988;34(4):544–555.
21. Sakhaee K, Harvey JA, Padalino PK, Whitson P, Pak CY. The potential role of salt abuse on the risk for kidney stone formation. *J Urol*. 1993;150(2 Pt 1):310–312.
22. Seeger H, Kaelin A, Ferraro PM, et al. Changes in urinary risk profile after short-term low sodium and low calcium diet in recurrent Swiss kidney stone formers. *BMC Nephrol*. 2017;18(1):349. https://doi.org/10.1186/s12882-017-0755-7.
23. Nouvenne A, Meschi T, Prati B, et al. Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial. *Am J Clin Nutr*. 2010;91:565–570. https://doi.org/10.3945/ajcn.2009.28614.
24. Stoller ML, Chi T, Eisner BH, Shami G, Gentle DL. Changes in urinary stone risk factors in hypocitraturic calcium oxalate stone formers treated with dietary sodium supplementation. *J Urol*. 2009;181:1140–1144. https://doi.org/10.1016/j.juro.2008.11.020.
25. Taylor EN, Curhan GC. Fructose consumption and the risk of kidney stones. *Kidney Int*. 2008;73:207–212. https://doi.org/10.1038/sj.ki.5002588.
26. Nakagawa T, Hu H, Zharikov S, et al. A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol*. 2006;290:F625–F631. https://doi.org/10.1152/ajprenal.00140.2005.
27. Seltzer MA, Low RK, McDonald M, Shami GS, Stoller ML. Dietary manipulation with lemonade to treat hypocitraturic calcium nephrolithiasis. *J Urol*. 1996;156(3):907–909.
28. Wabner CL, Pak CY. Effect of orange juice consumption on urinary stone risk factors. *J Urol*. 1993;149(6):1405–1408.
29. Koff SG, Paquette EL, Cullen J, Gancarczyk KK, Tucciarone PR, Schenkman NS. Comparison between lemonade and potassium citrate and impact on urine pH and 24-hour urine parameters in patients with kidney stone formation. *Urology*. 2007;69(6):1013–1016. https://doi.org/10.1016/j.urology.2007.02.008.
30. Hönow R, Laube N, Schneider A, Kessler T, Hesse A. Influence of grapefruit-, orange- and apple-juice consumption on urinary variables and risk of crystallization. *Br J Nutr*. 2003;90(2):295–300.
31. Rahman F, Birowo P, Widyahening IS, Rasyid N. Effect of citrus-based products on urine profile: a systematic review and meta-analysis. *F1000Research*. 2017;6:220. https://doi.org/10.12688/f1000research.10976.1.
32. Goldfarb DS, Asplin JR. Effect of grapefruit juice on urinary lithogenicity. *J Urol*. 2001;166(1):263–267.
33. Holmes RP, Goodman HO, Assimos DG. Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int*. 2001;59(1):270–276. https://doi.org/10.1046/j.1523-1755.2001.00488.x.
34. Assimos DG, Holmes RP. Role of diet in the therapy of urolithiasis. *Urol Clin North Am*. 2000;27(2):255–268.
35. Borghi L, Schianchi T, Meschi T, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med*. 2002;346(2):77–84. https://doi.org/10.1056/NEJMoa010369.
36. Taylor EN, Curhan GC. Oxalate intake and the risk for nephrolithiasis. *J Am Soc Nephrol*. 2007;18(7):2198–2204. https://doi.org/10.1681/ASN.2007020219.
37. Ulmann A, Aubert J, Bourdeau A, Cheynel C, Bader C. Effects of weight and glucose ingestion on urinary calcium and phosphate excretion: implications for calcium urolithiasis. *J Clin Endocrinol Metab*. 1982;54:1063–1068.
38. Rao PN, Gordon C, Davies D, Blacklock NJ. Are stone formers maladapted to refined carbohydrates? *Br J Urol*. 1982;54(6):575–577.
39. Blacklock NJ. Sucrose and idiopathic renal stone. *Nutr Heal (Berkhamsted, Hertfordshire)*. 1987;5:9–17. https://doi.org/10.1177/026010608700500203.
40. Sidhu H, Hoppe B, Hesse A, et al. Absence of Oxalobacter formigenes in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet (London, England)*. 1998;352(9133):1026–1029. https://doi.org/10.1016/S0140-6736(98)03038-4.
41. Stewart CS, Duncan SH, Cave DR. Oxalobacter formigenes and its role in oxalate metabolism in the human gut. *FEMS Microbiol Lett*. 2004;230(1):1–7.
42. Mehta M, Goldfarb DS, Nazzal L. The role of the microbiome in kidney stone formation. *Int J Surg*. 2016;36(Pt D):607–612. https://doi.org/10.1016/j.ijsu.2016.11.024.
43. Rushton HG, Spector M. Effects of magnesium deficiency on intratubular calcium oxalate formation and crystalluria in hyperoxaluric rats. *J Urol*. 1982;127:598–604.
44. Wunderlich W. Aspects of the influence of magnesium ions on the formation of calcium oxalate. *Urol Res*. 1981;9:157–161. https://doi.org/10.1007/BF00264820.
45. Hallson PC, Rose GA, Sulaiman S. Magnesium reduces calcium oxalate crystal formation in human whole urine. *Clin Sci*. 1982;62:17–19.

46. Johansson G, Backman U, Danielson BG, Ljunghall S, Wikström B. Magnesium metabolism in renal stone disease. *Invest Urol*. 1980;18:93–96.
47. Prien E, Gershoff S. Magnesium oxide-pyridoxine therapy for recurrent calcium oxalate calculi. *J Urol*. 1974;112:509–512.
48. Gershoff SN, Prien EL. Effect of daily MgO and vitamin B6 administration to patients with recurring calcium oxalate kidney stones. *Am J Clin Nutr*. 1967;20(5):393–399.
49. Will EJ, Bijvoet OL. Primary Oxalosis: clinical and Biochemical response to high-dose pyridoxine therapy. *Metabol Clin Exp*. 1979;28:542–548.
50. Lyon ES, Borden TA, Ellis JE, Vermeulen CW. Calcium oxalate lithiasis produced by pyridoxine deficiency and inhibition with high magnesium diets. *Invest Urol*. 1966;4(2):133–142.
51. Murthy MS, Farooqui S, Talwar HS, et al. Effect of pyridoxine supplementation on recurrent stone formers. *Int J Clin Pharmacol Ther Toxicol*. 1982;20(9):434–437.
52. Ferraro PM, Taylor EN, Gambaro G, Curhan GC. Vitamin B6 intake and the risk of incident kidney stones. *Urolithiasis*. 2017. https://doi.org/10.1007/s00240-017-0999-5.
53. Azoury R, Garti N, Perlberg S, Sarig S. May enzyme activity in urine play a role in kidney stone formation? *Urol Res*. 1982;10(4):185–189.
54. Liebman M, Chai W. Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *Am J Clin Nutr*. 1997;65:1453–1459. https://doi.org/10.1097/00005392-200005000-00052.
55. Pizzato AC, Barros EJG. Dietary calcium intake among patients with urinary calculi. *Nutr Res*. 2014;23(12):1651–1660. https://doi.org/10.1016/j.nutres.2003.09.001.
56. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med*. 1993;328:833–838. https://doi.org/10.1097/00019616-199309000-00015.
57. Lemann J, Pleuss JA, Worcester EM, Hornick L, Schrab D, Hoffmann RG. Urinary oxalate excretion increases with body size and decreases with increasing dietary calcium intake among healthy adults. *Kidney Int*. 1996;49:200–208. https://doi.org/10.1097/00005392-199802001-00030.
58. Wallace RB, Wactawski-Wende J, O'Sullivan MJ, et al. Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements. *Am J Clin Nutr*. 2011;94(1):270–277. https://doi.org/10.3945/ajcn.110.003350.
59. Penniston KL, Jones AN, Nakada SY, Hansen KE. Vitamin D repletion does not alter urinary calcium excretion in healthy postmenopausal women. *BJU Int*. 2009;104(10):1512–1516. https://doi.org/10.1111/j.1464-410X.2009.08559.x.
60. Usui Y, Matsuzaki S, Matsushita K, Shima M. Urinary citrate in kidney stone disease. *Tokai J Exp Clin Med*. 2003;28:65–70.
61. Pak CYC, Fuller C. Idiopathic hypocitraturic calcium-oxalate nephrolithiasis successfully treated with potassium citrate. *Ann Intern Med*. 1986;104:32–37.
62. Whalley NA, Meyers AM, Martins M, Margolius LP. Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia. *Br J Urol*. 1996;78:10–14.
63. Barcelo P, Wuhl O, Servitge E, Rousaud A, Pak CY. Randomized double-blind study of potassium citrate in idiopathic hypocitraturic calcium nephrolithiasis. *J Urol*. 1993;150(6):1761–1764.
64. Massey L. Magnesium therapy for nephrolithiasis. *Magnes Res*. 2005;18:123–126.
65. Nakagawa Y, Margolis HC, Yokoyama S, Kézdy FJ, Kaiser ET, Coe FL. Purification and characterization of a calcium oxalate monohydrate crystal growth inhibitor from human kidney tissue culture medium. *J Biol Chem*. 1981;256(8):3936–3944.
66. Dharmsathaphorn K, Freeman DH, Binder HJ, Dobbins JW. Increased risk of nephrolithiasis in patients with steatorrhea. *Dig Dis Sci*. 1982;27:401–405. https://doi.org/10.1007/BF01295647.
67. Coe F, Moran E, Kavalich A. The contribution of dietary purine over-consumption to hyperpuricosuria in calcium oxalate stone formers. *J Chronic Dis*. 1976;29. 793-300.
68. Coe FL, Strauss AL, Tembe V, Le Dun S. Uric acid saturation in calcium nephrolithiasis. *Kidney Int*. 1980;17(5):662–668.
69. Maalouf NM, Sakhaee K, Parks JH, Coe FL, Adams-Huet B, Pak CYC. Association of urinary pH with body weight in nephrolithiasis. *Kidney Int*. 2004;65(4):1422–1425. https://doi.org/10.1111/j.1523-1755.2004.00522.x.
70. Li W-M, Chou Y-H, Li C-C, et al. Association of body mass index and urine pH in patients with urolithiasis. *Urol Res*. 2009;37(4):193–196. https://doi.org/10.1007/s00240-009-0194-4.
71. Anton R, Haag-Berrurier M. Therapeutic use of natural anthraquinone for other than laxative actions. *Pharmacology*. 1980;20(suppl 1):104–112.
72. Berg W, Hesse A, Hensel K, Unger G, Hartmann U, Schneider HJ. [Influence of anthraquinones on the formation of urinary calculi in experimental animals (author's transl)]. *Urologe A*. 1976;15(4):188–191.
73. Riley K. The biological efficacy of aloes. *J John Bastyr Coll Nat Med*. 1981;2:18–27.
74. Samaan K. The pharmacological basis of drug treatment of spasm of the ureter or bladder and of ureteral stone. *Br J Urol*. 1933;5:213–224.
75. Heron S, Yarnell E. Recurrent kidney stones: a naturopathic approach. *Altern Complement Ther*. 1998;4:60–67.
76. Premgamone A, Sriboonlue P, Disatapornjaroen W, Maskasem S, Sinsupan N, Apinives C. A long-term study on the efficacy of a herbal plant, Orthosiphon grandiflorus, and sodium potassium citrate in renal calculi treatment. *Southeast Asian J Trop Med Public Health*. 2001;32(3):654–660.
77. Laranja SM, Bergamaschi CM, Schor N. Evaluation of acute administration of natural products with potential diuretic effects, in humans. *Mem Inst Oswaldo Cruz*. 1991;86(suppl 2):237–240.
78. Freitas AM, Schor N, Boim MA. The effect of Phyllanthus niruri on urinary inhibitors of calcium oxalate crystallization and other factors associated with renal stone formation. *BJU Int*. 2002;89(9):829–834.
79. Nishiura JL, Campos AH, Boim MA, Heilberg IP, Schor N. Phyllanthus niruri normalizes elevated urinary calcium levels in calcium stone forming (CSF) patients. *Urol Res*. 2004;32(5):362–366. https://doi.org/10.1007/s00240-004-0432-8.
80. Shekarriz B, Lu HF, Stoller ML. Correlation of unilateral urolithiasis with sleep posture. *J Urol*. 2001;165(4):1085–1087.
81. Najem GR, Seebode JJ, Samady AJ, Feuerman M, Friedman L. Stressful life events and risk of symptomatic kidney stones. *Int J Epidemiol*. 1997;26(5):1017–1023.
82. Brundig P, Berg W, Schneider HJ. [Stress and risk of urolith formation. II. The influence of stress on litholytic urinary substances (author's transl)]. *Urol Int*. 1981;36(4):265–273.
83. Walters DC. Stress as a principal cause of calcium oxalate urolithiasis. *Int Urol Nephrol*. 1986;18(3):271–275.
84. Kohri K, Kataoka K, Iguchi M, Yachiku S, Kurita T, Sonoda T. Relation between parathyroid hormone and adrenocorticotropic hormone in primary hyperparathyroidism. *Urol Int*. 1984;39(1):1–4.
85. Scott R, Cunningham C, McLelland A, Fell GS, Fitzgerald-Finch OP, McKellar N. The importance of cadmium as a factor in calcified upper urinary tract stone disease—a prospective 7-year study. *Br J Urol*. 1982;54:584–589.
86. Kaewnate Y, Niyomtam S, Tangvarasittichai O, Meemark S, Pingmuangkaew P, Tangvarasittichai S. Association of elevated urinary cadmium with urinary stone, hypercalciuria and renal tubular Dysfunction in the population of cadmium-contaminated area. *Bull Environ Contam Toxicol*. 2012;89(6):1120–1124. https://doi.org/10.1007/s00128-012-0856-8.
87. Swaddiwudhipong W, Mahasakpan P, Limpatanachote P, Krintratun S. An association between urinary cadmium and urinary stone disease in persons living in cadmium-contaminated villages in northwestern Thailand: a population study. *Environ Res*. 2011;111(4):579–583. https://doi.org/10.1016/j.envres.2011.01.007.
88. Shiue I. Urinary polyaromatic hydrocarbons are associated with adult celiac disease and kidney stones: USA NHANES, 2011-2012. *Environ Poll Res Int*. 2016;23:3971–3977. PubMed PMID:26728287.
89. Tang J, McFann K, Chonchol M. Dietary zinc intake and kidney stone formation: evaluation of NHANES III. *Am J Nephrol*. 2012;36(6):549–553. PubMed PMID:23221031.
90. Rivers JM. Safety of high-level vitamin C ingestion. *Int J Vitam Nutr Res Suppl*. 1989;30:95–102. https://doi.org/10.1111/j.1749-6632.1987.tb23780.x.

91. Massey LK, Liebman M, Kynast-Gales SA. *Ascorbate Increases Human Oxaluria and Kidney Stone Risk*. Vol. 135. 2005.
92. Moyad MA, Combs MA, Crowley DC, et al. Vitamin C with metabolites reduce oxalate levels compared to ascorbic acid: a Preliminary and Novel clinical Urologic finding. *Urol Nurs*. 2009;29(2):95–102.
93. Grases F, Costa-Bauzá A. Phytate (IP6) is a powerful agent for preventing calcifications in biological fluids: usefulness in renal lithiasis treatment. In: *Anticancer Research*. Vol. 19. 1999:3717–3722.
94. Kumar V, Sinha AK, Makkar HPS, Becker K. Dietary roles of phytate and phytase in human nutrition: a review. *Food Chem*. 2010;120:945–959. https://doi.org/10.1016/j.foodchem.2009.11.052.

Leukoplakia

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Adherent white patch or plaque appearing anywhere on the oral mucosa
- Asymptomatic until ulceration, fissuring, or malignant transformation occurs
- Diagnosis confirmed by biopsy

GENERAL CONSIDERATIONS

Leukoplakia is a clinical term signifying a white, plaque-like lesion occurring anywhere on the oral mucosa. It is generally a reaction to irritation, such as cigarette smoking or tobacco or areca (betel) nut chewing, as well as an early sign of human immunodeficiency virus (HIV) infection. It appears most commonly in men 50 to 70 years of age. In 90% of cases, leukoplakia represents epithelial hyperkeratosis and hyperplasia.[1] In the remaining 10%, there is also epithelial dysplasia; these lesions are considered precancerous.

Prompt workup and effective treatment of these lesions can reduce the risk of malignant transformation and provide early diagnosis of invasive cancer. A biopsy should be performed of any persistent or suspicious leukoplakia, and appropriate therapy should be provided based on histological features. Benign lesions can be observed or treated with oral or topical therapy, and dysplastic lesions should be excised. The risk of malignant transformation remains even after treatment, and patients should be followed closely. Oral squamous cell carcinoma is an aggressive malignancy that most often results from malignant evolution of oral leukoplakia. These tumors are primarily treated with surgical resection and adjuvant radiation or chemoradiation.[2]

Oral cancer is among the most common malignant neoplasms, with nearly 50,000 new cases and 12,000 deaths reported in the United States alone each year. Survival rates for patients undergoing surgery and chemoradiation have not significantly changed in the past few decades. Success in preventing death due to oral cancer is achieved through the prevention of oral cancer. Abstinence from tobacco and a higher intake of antioxidant nutrients are the primary preventive measures. However, the risk of oral squamous cell carcinoma remains elevated even after alcohol and tobacco cessation and may take up to 20 years to reach baseline levels.[3]

Areca (betel) nut chewing is practiced by 10% to 20% of the world's population and is a major cause of oral cancer in the Asia-Pacific region. The betel nut, from the *Areca catechu* palm tree, when chewed, is considered the fourth most commonly used addictive substance in the world after tobacco, alcohol, and caffeine. The highest incidence of oral cancers is found in Melanesia, including Papua New Guinea and the Solomon Islands, and in Guam, where the oral cancer mortality rate among native Chamorro people is six times higher than that in the United States.[4]

Significantly increased lipid peroxidation and decreased antioxidant levels have been observed in the plasma of patients with leukoplakia. Glutathione deficiency is thought to play a critical role in malignant transformation.[5]

It has recently been demonstrated that CD8+ and CD163+ tumor-infiltrating cells and the inhibitory checkpoint programmed cell death ligand 1 (PD-L1) are overexpressed in oral squamous cell carcinoma and oral leukoplakia, thus evading host immune response. This finding suggests a possible therapeutic benefit to checkpoint-inhibiting agents.[6]

Serum ceruloplasmin is also significantly increased in oral leukoplakia and oral carcinoma. Copper chelating agents may similarly be potentially therapeutic in both conditions.[7] Environmental nickel exposure is correlated with an increased risk of oral carcinoma in patients with oral leukoplakia as well.[8]

THERAPEUTIC CONSIDERATIONS

The treatment of leukoplakia involves the removal of all irritants. Electrodesiccation, cryosurgery, and proteolytic enzymes have not given predictably favorable results.[9] Photodynamic therapy, vitamin A, beta-carotene, and lycopene have shown clinical resolution rates higher than 50%.[10]

VITAMIN A AND BETA-CAROTENE

Historically, vitamin A supplementation and, more recently, beta-carotene supplementation have been clinically effective in the treatment of leukoplakia.[11–16] Stich et al.,[15,16] using the micronucleus test

to monitor efficacy, were among the first to evaluate vitamin A and beta-carotene for leukoplakia. The micronucleus test is a useful indicator of the neoplastic tendency of epithelial cells because it provides immediate information about genotoxic damage. Micronuclei are formed during chromatid or chromosomal breakage, with the rate of formation tied closely to carcinogenesis in the oral cavity. This rate is a good predictor of cancer, which typically takes years or decades to generate clinically recognizable signs. On the basis of the results of the micronucleus test, Stich et al. found that these two dietary factors, particularly beta-carotene, were effective in decreasing the mean proportion of cells with micronuclei on the buccal mucosa in those who chew Asian betel nut and tobacco.[15,16] The subjects continued to chew betel nut and tobacco during the study.

The epidemiological and experimental data documenting the protective effect of carotenoids and retinoids against epithelial cancers are overwhelming. The inverse relationship between serum retinol and carotene levels and cancer incidence holds true for oral carcinomas as well.[17,18]

Dosages used in clinical studies testing vitamin A in the treatment of leukoplakia generally were fairly high (i.e., 150,000–900,000 IU/day) but extremely effective.[11–14] Because beta-carotene appears to be as effective as retinol in decreasing the levels of micronuclei and has a much higher therapeutic index, beta-carotene should probably be the treatment of choice for this condition. (Note: Vulvar leukoplakia is also responsive to retinol and could therefore respond to beta-carotene as well.)[19,20] However, the only head-to-head comparison study of the two supplements did find an advantage for retinol (see Table 192.1). In this study, 160 men and women with leukoplakia were randomly assigned to receive oral vitamin A (retinyl acetate 300,000 IU/week for 12 months; $n = 50$), oral beta-carotene (360 mg/week for 12 months; $n = 55$), or placebo ($n = 55$).[21] The complete regression rates were 10% in the placebo arm, 52% with vitamin A, and 33% with beta-carotene. Homogeneous leukoplakias and smaller lesions responded better than nonhomogeneous and larger lesions. No major toxicities were observed even in the group given prolonged vitamin A supplementation.

LYCOPENE

A randomized controlled trial of the carotenoid lycopene conducted over 5 months found no significant difference in the clinical response of subjects taking 8 mg lycopene orally twice daily compared with those taking 4 mg twice daily. Both groups had significantly greater responses than those in the control group. Histological assessment was significantly better in the group taking 8 mg of lycopene.[22]

OTHER ANTIOXIDANTS

Antioxidants in addition to beta-carotene may be helpful in treating leukoplakia. For example, studies have shown alpha-tocopherol (400 IU twice daily for 24 weeks) to produce a 65% response rate.[23] However, it makes the most sense to use a combination of antioxidant nutrients (including using mixed tocopherols rather than just one of the eight isomers), given their beneficial interactions and their limitations as monotherapy. Preliminary results using a combination of vitamin C (1000 mg/day), beta-carotene (30 mg/day), and vitamin E (400 IU/day) are encouraging.[24]

TABLE 192.1 Beta-Carotene Trials

Study	Dosage	Response Rate (%)
Stich et al.[15]	180 mg/week	15
Stich et al.[16]	180 mg/week	27
Garewal et al.[27]	30 mg/day	71
Brandt et al.[28]	90 mg/day	60
Toma et al.[29]	90 mg/day	44
Malaker et al.[30]	30 mg/day	50
Garewal et al.[31]	60 mg/day	56
Sankaranarayanan et al.[21]	360 mg/week	33

CURCUMIN

Curcumin has recently shown promise in vitro as a chemopreventive agent for leukoplakia.[25]

ANTHOCYANINS

Black raspberry extract has demonstrated reduction of DNA damage and mutagenesis in cell studies of oral leukoplakia, supporting a role for this anthocyanin in the inhibition of oral carcinogenesis.[26]

THERAPEUTIC APPROACH

Because leukoplakia is due to a combination of excessive carcinogenic irritation in the context of marginal or low levels of vitamin A, the approach is simple: eliminate all sources of irritation and establish optimal vitamin A, beta-carotene, and antioxidant levels. Particularly significant irritation results from the smoking or chewing of tobacco, the chewing of betel nut, and exposure to ultraviolet light.

SUPPLEMENTS

- Vitamin A: 25,000 IU/day
- Beta-carotene: 30 to 90 mg/day
- Lycopene 8 mg twice daily
- Vitamin C: 1000 to 3000 mg/day
- Vitamin E (mixed tocopherols): 400 IU/day
- Curcumin: 500 mg twice daily
- Black raspberry extract: 500 mg daily

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. van der Waal I, Axell T. Oral leukoplakia: a proposal for uniform reporting. *Oral Oncol*. 2002;38:521–526.
2. Bewley AF, Farwell DG. Oral leukoplakia and oral cavity squamous cell carcinoma. *Clin Dermatol*. 2017;35(5):461–467.
3. Marron M, Boffetta P, Zhang ZF, et al. Cessation of alcohol drinking, tobacco smoking and the reversal of head and neck cancer risk. *Int J Epidemiol*. 2010;39(1):182–196.
4. Hernandez BY, Zhu X, Goodman MT, et al. Betel nut chewing, oral premalignant lesions, and the oral microbiome. *Plos One*. 2017. https://doi.org/10.1371/journal/pone.0172196 Feb 22.
5. Srivastava KC, Srivastava D. Analysis of plasma lipid peroxidation and antioxidant enzymes status in patients of oral leukoplakia: a case control study. *Dent Res J*. 2014;11(2):180–186.
6. Stasikowska-Kanicka O, Wągrowska-Danilewicz M, Danilewicz M. CD8+ and CD163+ infiltrating cells and PD-L1 immunoexpression in oral leukoplakia and oral carcinoma. *APMIS*. 2018;126(9):732–738.
7. Shah PH, Venkatesh R, More CB. Determination of role of ceruloplasmin in oral potentially malignant disorders and oral malignancy-A cross-sectional study. *Oral Dis*. 2017;23(8):1066–1071.
8. Tsai KY, Su CC, Chiang CT, et al. Environmental heavy metal as a potential risk factor for the progression of oral potentially malignant disorders in central Taiwan. *Cancer Epidemiol*. 2017;47:118–124.
9. Krupp M, Chatton M. *Current Medical Diagnosis and Treatment*. Los Altos, CA: Lange Medical Publications; 1984.
10. Ribeiro AS, Salles PR, da Silva TA, et al. A review of the nonsurgical treatment of oral leukoplakia. *Int J Dent*. 2010;2010 186018. Epub 2010 Feb 23.
11. Johnson JE, Ringsdorf Jr WM, Cheraskin E. Relationship of vitamin A and oral leukoplakia. *Arch Dermatol*. 1963;88:607–612.
12. Mulay DN, Urbach F. Local therapy of oral leukoplakia with vitamin A. *AMA Arch Derm*. 1958;78:637–638.
13. Smith JF. Clinical evaluation of massive buccal vitamin A dosage in oral hyperkeratosis. *Oral Surg Oral Med Oral Pathol*. 1962;15:282–292.
14. Zegarelli E, Kutscher A, Stevers H. Keratotic lesions of the oral mucosa membranes treated with high dosage topical and systemic vitamin A. *N Y State Dent J*. 1959;25:244–252.
15. Stich HF, Rosin MP, Vallejera MO. Reduction with vitamin A and beta-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betel nut and tobacco chewers. *Lancet*. 1984;1:1204–1206.
16. Stich HF, Stich W, Rosin MP, et al. Use of the micronucleus test to monitor the effect of vitamin A, beta-carotene and canthaxanthin on the buccal mucosa of betel nut/tobacco chewers. *Int J Cancer*. 1984;34:745–750.
17. Garewal HS, Schantz S. Emerging role of beta-carotene and antioxidant nutrients in prevention of oral cancer. *Arch Otolaryngol Head Neck Surg*. 1995;121:141–144.
18. Ibrahim K, Jafarey NA, Zuberi SJ. Plasma vitamin "A" and carotene levels in squamous cell carcinoma of oral cavity and oropharynx. *Clin Oncol*. 1977;3:203–207.
19. Hyams M, Gallagher P. Vitamin A therapy in the treatment of vulvar leukoplakia. *Am J Obstet Gynecol*. 1950;59:1346–1354.
20. Hunt E. Vitamin A in leukoplakia. *Lancet*. 1947;2:141.
21. Sankaranarayanan R, Mathew B, Varghese C, et al. Chemoprevention of oral leukoplakia with vitamin A and beta carotene: an assessment. *Oral Oncol*. 1997;33:231–236.
22. Singh M, Krishanappa R, Bagewadi A, et al. Efficacy of oral lycopene in the treatment of oral leukoplakia. *Oral Oncol*. 2004;40(6):591–596.
23. Benner SE, Winn RJ, Lippman SM, et al. Regression of oral leukoplakia with alpha-tocopherol. *J Natl Cancer Inst*. 1993;85:44–47.
24. Kaugars GE, Silverman Jr S, Lovas LG, et al. Use of antioxidant supplements in the treatment of human oral leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1996;81:5–14.
25. Chakravarti N, Kadar H, Yoon DJ, et al. Differential inhibition of protein translation machinery by curcumin in normal, immortalized, and malignant oral epithelial cells. *Cancer Prev Res (Phila Pa)*. 2010;3(3):331–338.
26. Guttenplan JB, Chen KM, Sun YW, et al. Effects of black raspberry extract and berry compounds on repair of DNA damage and mutagenesis induced by chemical and physical agents in human oral leukoplakia and rat oral fibroblasts. *Chem Res Toxicol*. 2017;30(12):2159–2164.
27. Garewal HS, Meyskens Jr FL, Killen D, et al. Response of oral leukoplakia to beta-carotene. *J Clin Oncol*. 1990;8:1715–1720.
28. Brandt R, et al. Response of oral lesions with the use of antioxidant vitamins and beta-carotene supplements. In: Laidlaw S, Swendseid M, eds. *Vitamins and Cancer Prevention*. Baton Rouge: Louisiana State Press; 1991.
29. Toma S, Benso S, Albanese E, et al. Treatment of oral leukoplakia with beta-carotene. *Oncology*. 1992;49:77–81.
30. Malaker K, Anderson BJ, Beecroft WA, et al. Management of oral mucosal dysplasia with beta-carotene and retinoic acid: a pilot crossover study. *Cancer Detect Prev*. 1991;15:335–340.
31. Garewal HS. Beta-carotene in oral leukoplakia. *Proc Am Soc Clin Oncol*. 1992;11:141.

Lichen Planus

John Nowicki, ND, and *Sam Russo**, *ND, LAc, RMSK*

OUTLINE

DIAGNOSTIC SUMMARY

- Lichen planus is an inflammatory, pruritic disease of the skin and mucous membranes described by the "six P's": pruritic, polygonal, planar (flat-topped), purple papules, and plaques. Intense pruritus is commonly the primary complaint, although lesions can present without pruritus.
- Skin lesions are flat-topped, violaceous to purple, polygonal or oval papules 1 to 10 mm wide with sharply defined edges and shiny. Lesions may be grouped, linear (Koebner phenomenon), annular, or disseminated when generalized. White lines (Wickham striae) may be present. A handheld lens and application of a clear oil over a lesion intensify the visibility of the striae. In dark-skinned patients, postinflammatory hyperpigmentation commonly occurs. Skin lesions may occur anywhere, with a predilection for the trunk and flexor surfaces, particularly the wrists as well as the lumbar region, eyelids, shins, scalp, glans penis, and mouth.
- Oral lesions appear inflamed, with leukohyperkeratosis, manifesting as reticulated white puncta or papules and lines in a lacelike pattern. Plaques may form and can become hyperkeratotic. Erosions with blisters may occur. Both buccal mucosae are usually affected, although the tongue, lips, and gingivae may be as well. Skin lesions occur in 10% to 60% of cases of oral lichen planus. Biopsy should be performed if there is doubt about the diagnosis or if blisters, plaques, or erosions occur in oral lichen planus.
- Other locations include (1) the genitalia, as papular, annular, or erosive lesions on the penis, scrotum, labia majora, labia minora, and vagina; (2) the scalp, with atrophic skin and scarring alopecia; and (3) the nails, with destruction of the nail fold and bed and with longitudinal splintering.

GENERAL CONSIDERATIONS

Lichen planus (LP) is an immune-mediated inflammatory disease primarily affecting the skin and mucous membranes, including the oral, vulvovaginal, esophageal, laryngeal, and conjunctival mucosa. Variants are based on the morphology of the lesions and the site of involvement and include the following:

- Hypertrophic: Large, thick plaques on the feet and shins; more common in African American males. Hypertrophic lesions may become hyperkeratotic.
- Follicular: Individual keratotic-follicular papules and plaques that lead to cicatricial alopecia. Graham Little syndrome is the complex of spinous follicular lesions, any lichen planus, and cicatricial alopecia.
- Vesicular: Development of vesicular or bullous lesions within or independent of lesions. In the latter, direct immunofluorescence is consistent with bullous pemphigoid, and patients have bullous pemphigoid immunoglobulin (Ig) G autoantibodies.
- Actinicus: Papular lesions in sun-exposed areas, especially the dorsa of the hands and arms.
- Ulcerative: Therapy-resistant ulcers, particularly on the soles, requiring skin grafting.

Despite these numerous clinical attributes, histological findings remain characteristic in the variants, ensuring the correct diagnosis. One of the primary histopathological features of LP is the vacuolar degeneration of the keratinocytes in the basal layer.[1]

Although the etiology is often unknown, trigger factors such as drugs, metals, or infection (hepatitis C virus) resulting in the alteration of cell-mediated immunity appear to play a role. Human leukocyte antigen–associated genetic susceptibility explains a predisposition in some patients. The immune system plays a role, as evidenced by two findings: first, immunoglobulins (primarily IgM; possibly also IgA and IgG) and complement (C3) are found at the dermal-epidermal junction in 95% of lichen planus lesions; second, lesions are associated with certain pathological immune conditions, such as autoimmune diseases, graft-versus-host disease reactions, dermatomyositis, viral infections, and as cutaneous manifestations of malignant lymphoma. The primary immune factor involved appears to be T-cell–mediated processes, but the responsible antigen remains unidentified.[2] In early lesions, activated helper T (T_H) cells target basal cells, which may have antigenic alterations. Suppressor T (T_s) cells predominate in older lesions.

Epidemiological studies have demonstrated a higher prevalence of hepatitis C virus infection in patients with lichen planus than in the normal population. Most studies show that the lesions are the result of

* Previous edition contributor

the host immune response to the viral components and not the result of the direct action of the virus.[3] However, the mechanisms involved have not been clearly elucidated, and a pathogenic relationship has yet to be established.

Incidence, Onset, and Course

Although not common, lichen planus occurs in 6 per 1000 patients seen by dermatologists, with an annual prevalence of 4.4 per 1000 people in the United States. However, oral lichen planus may be the most common cause of white lesions in the mouth, occurring in 0.5% to 1% of dental patients. Women tend to be affected more than men, and the age at onset is 30 to 60 years.

Onset can be acute or insidious, with lesions remaining for months to years. Diagnosis is commonly made via history, physical examination, and biopsy if necessary. The course of oral lichen planus may be chronic, lasting from months to years. Approximately two thirds of patients experience spontaneous resolution within 1 year. Recurrence is uncommon in both forms, being seen in less than 20% of patients. Patients with mucous membrane involvement usually have a more prolonged course, possibly lasting months to decades, although 50% experience remission within 2 years. The incidence of oral squamous cell carcinoma in individuals with oral lichen planus is 5% higher than in those with lesions in other locations. A significant increase of malignant transformation risk has been noted among smokers (odds ratio [OR] = 2, 95% confidence interval [CI]; 1.25–3.22), alcoholics (OR = 3.52, 95% CI; 1.54–8.03), and HCV-infected patients (OR = 5, 95% CI; 1.56–16.07), compared with patients without these risk factors.[4] The list of possible causative medications is long. The most common are gold, antimalarial agents, penicillamine, thiazide diuretics, beta blockers, nonsteroidal anti-inflammatory drugs (NSAIDs), quinidine, and angiotensin-converting enzyme (ACE) inhibitors.[5] The interval between administration of the offending agent and the development of lichenoid drug eruptions is usually a few months, ranging from 10 days to several years.[5]

THERAPEUTIC CONSIDERATIONS

Dental Amalgams

Dental amalgams appear to play a role in the etiology of oral lichen planus. In one study, 161 patients with chronic lichenoid reactions (142) or oral lichen planus (19) underwent replacement of mercury amalgam fillings with alternative substances. Five patients served as controls, receiving no restoration. Evaluation 6 to 12 months after restoration showed that 95% of the patients with chronic lichenoid reactions had marked improvement or complete restoration of normal mucosa. The effect was more apparent for those with gold crowns than those with CM (palladium-based) crowns. In the oral lichen planus group, those areas of lesions in contact with amalgam showed an improvement in 63% of patients, but lesions at other sites were not affected.[6] A second study found that if gold crowns are already present, patients should undergo patch testing for gold allergy; removal of the crowns should be considered in those with positive results.[7] The former study downplays the significance of patch testing for mercury because many patch-negative patients still responded to amalgam removal. However, in cases of positive patch-test reactions to mercury compounds, partial or complete replacement of amalgam fillings will lead to a significant improvement in nearly all patients.[8,9]

Photodynamic Therapy

Phototherapy, especially with narrow-band ultraviolet B (NB-UVB), is effective, but caution must be taken because of the risk of keobnerization (i.e., injury to the skin can cause further formation).[10] A retrospective analysis of 50 patients with generalized cutaneous lichen planus treated by broad-band ($n = 7$) or narrow-band ($n = 43$) ultraviolet B radiation showed a complete response in 70%; 85% of these responders were still in remission after a median of 34.7 months.[11] Another report involves a 64-year-old man with lichen planus of the penis that did not respond to steroids.[12] In this case, photodynamic therapy was preceded (4 hours before treatment) by application of 5-aminolevulinic acid (20%) ointment. The area was irradiated with a Paterson-Whitehurst lamp (Paterson Institute, Christie Hospital, Manchester, United Kingdom) at a center wavelength of 630 nm and a rectangular passband of 27 nm. The dose was gradually increased to 50 J/cm^2 at a fluence rate of 55 mW/cm^2 to prevent edema and phimosis. Treatment was repeated after 6 weeks. The lesions resolved completely after another 4 weeks, with no recurrence at 6 months. Photodynamic therapy with medium-dose ultraviolet A-1 has successfully treated ulcerative lichen planus of the feet in another case.[13]

Supplements and Botanical Medicines

Vitamin Deficiencies

Correction of low levels of vitamins B_1 and B_6 in patients with oral lichen planus (OLP) resulted in both clinical and subjective improvement in the majority treated but did not produce complete remission of the lesions.[14]

In 77 patients with lichen planus (cutaneous lichen planus [CLP], 49 cases; OLP, 28 cases), compared with the control group (19.82 mg/dL), the level of ascorbic acid was significantly lower, both in patients with CLP (8.47 mg/dL, p = 0.001) and in those with OLP (8.04 mg/dL, p = 0.001).[15] In patients with lichen planus, it was found that the deficiency of ascorbic acid increases with age. In addition, the urinary concentrations of ascorbic acid were significantly lower in patients with lichen planus associated with infections, compared with patients with lichen planus without infections.

An additional study found that patients with OLP had a significantly higher frequency of deficiencies in hemoglobin (men < 13 g/dL, women < 12 g/dL), iron (<60 μg/dL), and/or vitamin B_{12} (< 200 pg/mL) and abnormally elevated blood homocysteine levels than healthy control participants.[16]

Vitamin A and Beta-Carotene

Six patients with hypertrophic lichen planus of the palms or soles used topical retinoic acid (0.1% in petroleum jelly base) three times daily for 3 weeks. This frequency caused irritation, so application was switched to two to three times every other day. Regression occurred after 2 to 3 weeks. The duration of treatment was unspecified in the study report. Two cases showed slight recurrences after 3 to 4 months.[17]

A second study compared topical retinoic acid 0.05% (RA) and fluocinolone acetonide 0.1% (FA) applied four times daily. Thirty-three patients with varying stages of OLP were alternately given RA (15) or FA (18); 56% of patients receiving FA improved, but only 13% of patients receiving RA improved. The extent of change was greater in the FA group as well.[18] Small doses of vitamin A acid locally applied produced improvement or regression in hypertrophic lichen planus of the palms or soles in a small number of patients.[19] Retinaldehyde gel 0.1% showed good clinical efficacy in 17 patients with OLP, resulting in 6% disappearance and 82% improvement of lesions, confirmed by the downregulation of filaggrin and CK-10 as markers of terminal differentiation.[20] In a trial of seven patients with lichen planus of either the penis or anal region, application of 0.1% retinoic acid in Orabase overnight and 10 mg (33,330 IU) of oral retinoic acid three to four times daily for 3 days to 3 weeks resulted in improvement or resolution in all cases.[21]

In another study, 18 patients with mucosal dysplasia (10 leukoplakia, 4 lichen planus, 3 erythroplasia, 1 leukokeratosis) were evaluated for serum vitamin A, beta-carotene, and levels of cis-retinoic acid,

which were low normal. All patients received beta-carotene 30 mg four times daily. Disease worsened in one patient, remained stable in nine, and improved in eight, with eventual complete resolution in six of those with improvement. Patients with stable disease were then treated with isotretinoin (Accutane 10 mg three times daily) in conjunction with beta-carotene; three showed improvement. The response to treatment was much higher in smokers (30 packs per year or more) than in nonsmokers.[22]

Synthetic Retinoids

After topical therapy with 0.1% 13-cis-retinoic acid for 8 weeks, restoration of physiological cell morphology was observed in a small group of patients with OLP.[23] Both 0.05% and 0.18% isotretinoin also appear to be effective in significantly improving atrophic-erosive OLP.[24]

A prospective, open-label, single-arm pilot study was performed to assess the efficacy and safety of oral alitretinoin taken at 30 mg once daily for up to 24 weeks in the treatment of severe OLP refractory to standard topical therapy.[25] At the end of treatment, 40% of patients had a >50% reduction in disease severity as measured by the Escudier severity score. Therapy was well tolerated. Adverse events were mild and included headache, mucocutaneous dryness, musculoskeletal pain, increased thyroid-stimulating hormone, and dyslipidemia.

Glycyrrhizin

Seventeen patients with OLP and hepatitis C were divided into two groups; nine received intravenous glycyrrhizin, and eight were given information on dental hygiene. The treatment group received 40 mL of a 0.2% solution of glycyrrhizin daily for 4 consecutive weeks. Six of the nine treated patients demonstrated clinical improvement. The patients who responded tended to have only mildly raised serum alanine transaminase (ALT) and aspartate transaminase (AST).[26]

Aloe Vera

Thirty-four women with vulval lichen planus, with erosive and ulcerative lesions in 83% and 17%, respectively, were randomized into two groups to receive either topical aloe gel or placebo. Of 17 patients treated with *Aloe vera,* 14 (82%) improved clinically by at least 50% after 8 weeks of treatment, whereas 1 (5%) of 17 placebo-treated patients had a similar response ($P < 0.001$). Neither group experienced side effects.[27]

Hyaluronic Acid

A total of 124 patients with erosive OLP participated in a randomized, placebo-controlled, double-blind trial to evaluate the efficacy of a topical 0.2% hyaluronic acid (HA) preparation for 28 days. Application of topical HA produced a significant reduction ($P < 0.05$) in soreness scores compared with placebo for up to 4 hours postapplication; there was also a significant reduction ($P < 0.05$) in the size of the erosive/ulcerated area after 28 days of treatment compared with baseline. Very frequent applications should be considered to obtain a more significant clinical benefit.[28]

Purslane

Twenty patients with OLP received 235 mg of purslane per day for 3 months, and 17 received a placebo. Approximately 83% of the purslane patients responded with partial or complete clinical improvement, compared with partial improvement in 17% of the placebo group. According to visual analog scale (VAS) scores, a partial or complete response was observed in all purslane-treated patients, whereas 71% of the controls demonstrated partial responses ($P < 0.001$).[29]

THERAPEUTIC APPROACH

If the lesions are oral and the patient has significant metal-based fillings, heavy-metal detoxification and replacement of the metal fillings should be considered.

Correction of vitamin and mineral deficiencies may aid in treatment benefit.

Topical Treatments

Apply four times a day:

- Vitamin A (0.1% solution): May cause inflammation and mildly increase pain temporarily.
- Hyaluronic acid gel
- *A. vera* gel
- Glycyrrhizin: topical application

Oral Treatments

- Vitamin A: 33,330 IU three to four times a day for 3 days to 3 weeks while monitoring liver enzymes or beta-carotene 30 mg four times a day
- Purslane: 235 mg dried herb per day

Intravenous Treatments

- Glycyrrhizin: 40 mL of a 0.2% solution a day for 4 consecutive weeks

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wagner G, Rose C, Sachse MM. Clinical variants of lichen planus. *J Dtsch Dermatol Ges*. 2013;11(4):309–319. PubMed PMID: 23320493.
2. Gorouhi F, Davari P, Fazel N. Cutaneous and mucosal lichen planus: a comprehensive review of clinical subtypes, risk factors, diagnosis, and prognosis. *Scientific World Journal*. 2014: 742826. PubMed PMID: 24672362.
3. Georgescu SR, Tampa M, Mitran MI, et al. Potential pathogenic mechanisms involved in the association between lichen planus and hepatitis C virus infection. *Exp Ther Med*. 2019;17(2):1045–1051. PubMed PMID: 30679972.
4. Aghbari SMH, Abushouk AI, Attia A, et al. Malignant transformation of oral lichen planus and oral lichenoid lesions: a meta-analysis of 20095 patient data. *Oral Oncology*. 2017;68:92–102. PubMed PMID: 28438300.
5. Ellgehausen P, Elsner P, Burg G. Drug-induced lichen planus. *Clin Dermatol*. 1998;16:325–332.
6. Bratel J, Hakeberg M, Jontell M. Effect of replacement of dental amalgam on oral lichenoid reactions. *J Dent*. 1996;24:41–45.
7. Izumi AK. Allergic contact gingivostomatitis due to gold. *Arch Dermatol Res*. 1982;272:387–391.
8. Laeijendecker Ronald MD, Dekker Sybren K, Burger Piet M, et al. Oral lichen planus and allergy to dental amalgam restorations. *Arch Dermatol*. 2004;140:1434–1438.
9. Wong L, Freeman S. Oral lichenoid lesions (OLL) and mercury in amalgam fillings. *Contact Dermatitis*. 2003;48(2):74–79.
10. Puza C, Cardones AR. Concepts and controversies in the treatment of cutaneous lichen planus. *G Ital Dermatol Venereol*. 2017;152(6):607–614. PubMed PMID: 28895668.
11. Pavlotsky F, Nathansohn N, Kriger G, et al. Ultraviolet-B treatment for cutaneous lichen planus: our experience with 50 patients. *Photodermatol Photoimmunol Photomed*. 2008;24(2):83–86.
12. Sehgal VN, Abraham GJ, Malik GB. Griseofulvin therapy in lichen planus. A double-blind controlled trial. *Br J Dermatol*. 1972;87:383–385.
13. Mansura A, Alkalay R, Slodownik D, et al. Ultraviolet A-1 as a treatment for ulcerative lichen planus of the feet. *Photodermatol Photoimmunol Photomed*. 2006;22(3):164–165.
14. Jolly M, Nobile S. Vitamin status of patients with oral lichen planus. *Aust Dent J*. 1977;22(6):446–450.
15. Nicolae I, Mitran CI, Mitran MI, et al. Ascorbic acid deficiency in patients with lichen planus. *J Immunoassay Immunochem*. 2017;38(4):430–437. PubMed PMID: 28422565.
16. Chen HM, Wang YP, Chang JY, et al. Significant association of deficiencies of hemoglobin, iron, folic acid, and vitamin B12 and high homocysteine level with oral lichen planus. *J Formos Med Assoc*. 2015;114(2):124–129. PubMed PMID: 25557154.
17. Gunther S. Topical administration of vitamin A acid (retinoic acid) in palmar keratoses: callosities, hyperkeratotic eczema, hypertrophic lichen planus, pityriasis rubra pilaris. *Dermatologica*. 1972;145:344–347.
18. Buajeeb W, Kraivaphan P, Pobrurksa C. Efficacy of topical retinoic acid compared with topical fluocinolone acetonide in the treatment of oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1997;83:21–25.
19. Günther S. Local vitamin A acid therapy in palmar/plantar hyperkeratoses (authors transl). *Z Orthop Ihre Grenzgeb German*. 1975;113(5):916–919.
20. Boisnic S, Licu D, Ben Slama L, et al. Topical retinaldehyde treatment in oral lichen planus and leukoplakia. *Int J Tissue React*. 2002;24(4):123–130.
21. Günther S. Effect of retinoic acid in lichen planus of the genitalia and perianal region. *Br J Vener Dis*. 1973;49(6):553–554.
22. Malaker K, Anderson BJ, Beecroft WA, et al. Management of oral mucosal dysplasia with beta-carotene retinoic acid: a pilot cross-over study. *Cancer Detect Prev*. 1991;15:335–340.
23. Mastrangelo F, Dolci M, Stuppìa L, et al. SEM analysis of oral lichen planus before and after treatment with 13 cis-retinoic acid. *Int J Immunopathol Pharmacol*. 2007;20(suppl 1):75–79.
24. Scardina GA, Messina P, Carini F, et al. A randomized trial assessing the effectiveness of different concentrations of isotretinoin in the management of lichen planus. *Int J Oral Maxillofac Surg*. 2006;35(1):67–71.
25. Kunz M, Urosevic-Maiwald M, Goldinger SM, et al. Efficacy and safety of oral alitretinoin in severe oral lichen planus—results of a prospective pilot study. *J Eur Acad Dermatol Venereol*. 2016;30(2):293–298. PubMed PMID: 26507685.
26. Da Nagao Y, Sata M, Suzuki H, et al. Effectiveness of glycyrrhizin for oral lichen planus in patients with chronic HCV infection. *J Gastroenterol*. 1996;31:691–695.
27. Rajar UD, Majeed R, Parveen N, et al. Efficacy of aloe vera gel in the treatment of vulval lichen planus. *J Coll Physicians Surg Pak*. 2008;18(10):612–614.
28. Nolan A, Badminton J, Maguire J, et al. The efficacy of topical hyaluronic acid in the management of oral lichen planus. *J Oral Pathol Med*. 2009;38(3):299–303.
29. Agha-Hosseini F, Borhan-Mojabi K, Monsef-Esfahani HR, et al. Efficacy of purslane in the treatment of oral lichen planus. *Phytother Res*. 2010;24(2):240–244.

194

Lower Urinary Tract Symptoms, Overactive Bladder Syndrome, and Urinary Incontinence (LUTS, OAB, and UI)

Tracey Seipel, FANPA, ABC, and *Alexander G. Schauss, PhD*

OUTLINE

DIAGNOSTIC SUMMARY

The diagnostic summary data are modified from the International Continence Society and the National Society for Continence.[1,2]

LOWER URINARY TRACT SYMPTOMS

Lower urinary tract symptoms (LUTS) are categorized according to the three stages of the bladder cycle:

Storage/Filling Symptoms
- Frequency (more than 8 micturitions in a 24-hour period)[3,6]
- Urgency[3,6]
- Nocturia (more than once per night)[3,6]
- Urgency incontinence[3]
- Stress incontinence
- Nocturnal enuresis/incontinence
- Bladder/urethral pain
- Absent or impaired sensation

Emptying/Voiding Symptoms
- Hesitancy[6]
- Straining to void
- Poor stream[4]
- Dysuria[6]
- Intermittency
- Terminal dribble

Post-Voiding Symptoms
- Postmicturition dribble
- Feeling of incomplete emptying[6]
- Postmicturition incontinence

Overactive Bladder

Urinary urgency with or without urinary frequency, nocturia, and urgency urinary incontinence and in the absence of urinary tract infection or other obvious pathology.

Urinary Incontinence

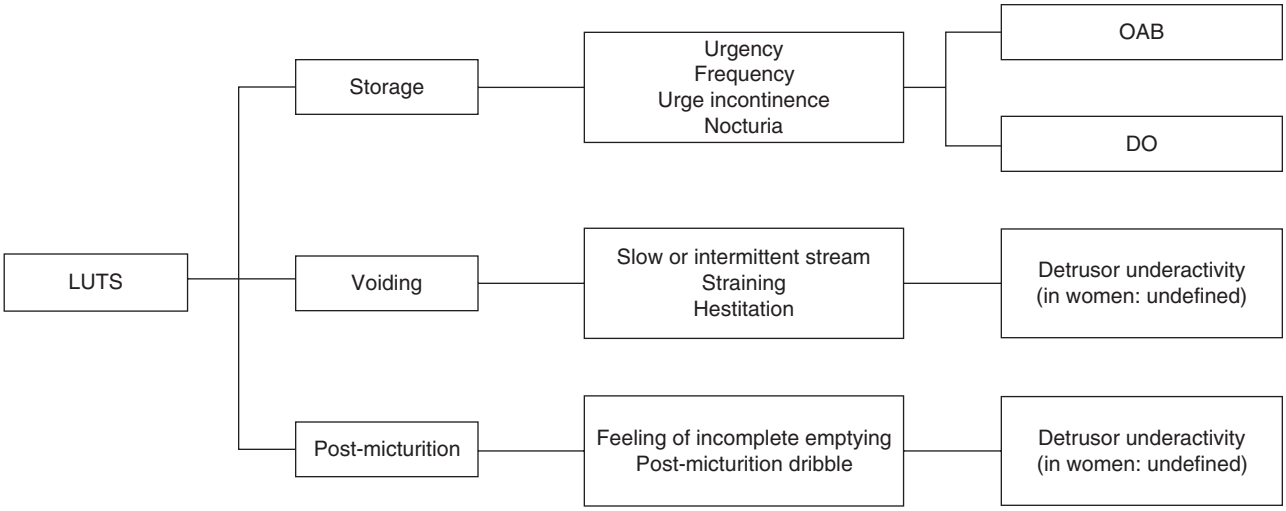
Stress Urinary Incontinence

Involuntary urine loss with stress urinary incontinence (SUI) occurs without warning when pressure is exerted on the pelvic floor muscles, for example, with coughing, laughing, jumping, and sneezing or with heavy lifting.

Urgency Urinary Incontinence

Urine loss with urgency urinary incontinence (UUI) occurs with warning when the individual has an urgency sensation of the need to urinate and cannot reach the restroom in time.

Mixed urinary incontinence (MUI) is a combination of stress and urge incontinence. Overflow incontinence refers to leakage that occurs when the quantity of urine produced exceeds the bladder's holding



Distribution for LUTS as assessed in the EpiLUTS study indicating overlapping or nonoverlapping symptoms. Storage LUTS include the following symptoms: micturition frequency, nocturia, urinary urgency, and urinary incontinence. Voiding LUTS include slow or weak stream, hesitancy, and terminal dribble. Post-micturition LUTS include sensation of incomplete emptying and post-micturition dribble (n = 15 861 women)

NO LUTS	25.3%
Voiding only	5.2%
Storage only	22.4%
Post-micturition only	0.9%
Voiding and storage	14.8%
Voiding and post-micturition	2.0%
Storage and post-micturition	3.6%
Voiding storage and post-micturition	26.0%

OAB – overactive bladder, DO – detrusor overactivity

Fig. 194.1 Lower urinary tract symptoms (LUTS) by type, key symptoms, underlying pathophysiology, and relation to disease entity. (Adapted from Perabo FGE. Drug development for LUTS – the challenge for industry. Drug Discov Today Ther Strat. 2012;9:E5-E14; Sexton CC, Coyne KS, Kopp ZS, et al. The overlap of storage, voiding and postmicturition symptoms and implications for treatment seeking in the USA, UK and Sweden: EpiLUTS. BJU Int 2009;103 (Suppl 3):12–23.)

capacity. Enuresis is overnight incontinence or bedwetting. Other, less common forms of urinary incontinence include continual incontinence and situational incontinence.

GENERAL CONSIDERATIONS

From the mid-1990s, the term *lower urinary tract symptoms* (LUTS) was increasingly used for women and replaced with the term *prostatism* in men. In 2002 the International Continence Society (ICS) categorized the term *overactive bladder syndrome* or *overactive bladder* (OAB) to cover the increasingly more prevalent symptom picture of urinary urgency, with or without day frequency, nocturia, and urinary incontinence (Fig. 194.1). In the United States, the 2018 International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) codes used to indicate a diagnosis for reimbursement purposes are as follows: R32 for unspecified urinary incontinence; N39.3 and N39.4 for stress incontinence and stress incontinence and other specific urinary incontinence, respectively; N39.44 for nocturnal enuresis; R35.0 for urinary frequency (micturition); F98.0) for nonorganic enuresis; and R39.81 for functional urinary incontinence, as well as urinary incontinence associated with cognitive impairment. Other codes for symptoms and signs involving the genitourinary system in ICD-10-CM are found between R30 and R39.

OAB with UUI is also called OAB wet, and OAB without UUI is also called OAB dry. With time, OAB dry tends to progress to OAB wet. OAB and UUI are considered to be due to overactivity or excessive neural stimulation of the detrusor muscle. This excessive contraction of the detrusor muscle results in the sensation of needing to void. Detrusor overactivity can be determined through urodynamic testing.

SUI occurs when the pelvic floor muscles that support the organs and structures of the lower abdomen are weakened and fail to support the internal and external urethral sphincters. The most common causes of stress incontinence among women are pregnancies involving multiple vaginal deliveries, during which time the sphincter and pelvic muscles are stretched and weakened.

OAB and urinary incontinence (UI) are psychologically distressing conditions that negatively affect physical and emotional status and health-related quality of life (HR-QOL) in those affected, resulting in greatly increased healthcare costs.[7–11] Millions of individuals worldwide are affected by UI, yet the associated embarrassment results in the condition being underreported and underdiagnosed.

The prevalence of UI can be challenging to determine. Current estimates suggest it may be as high as one in five people with UI and 15% with OAB.[11] The National Association for Continence estimates that 33 million Americans suffer from OAB, with 12.2 million experiencing concomitant urgency incontinence.[2]

The 2002 EPIC study included five Western countries (i.e., Canada, Germany, Italy, Sweden, and the United Kingdom) and demonstrated an average OAB prevalence of 11.8% (11% men and 13% women) and an

UI of 9.4%.[10] Women are more likely to experience UI, predominantly SUI, whereas men more commonly experience UUI.[12] Data analyzed on 17,580 adults surveyed in the 2001 and 2008 cycles of the National Health and Nutrition Examination Survey (NHANES) found that the prevalence of UI was 51.1% in women and 13.9% in men, with increasing prevalence rates observed for both men and women from the first survey to the second survey.[13] In a 2010 prevalence study of incontinence in Australia, 4.2 million (19%) Australians aged 15 years and over had urinary incontinence, and 1.3 million had fecal incontinence, with an economic burden of AUS $42.9 billion, or approximately AUS $9014 per person.[14] The prevalence rate jumps much higher in the residential aged care community, where 70.9% were found to have urinary or fecal incontinence (or both), with the prevalence projected to rise to 250,000 by 2030.[14]

A 2009 population-based survey conducted to estimate the prevalence rate of UI in China found the prevalence rate to be 22.1%, with SUI at 12.9%, UUI at 1.7%, and MUI at 7.5%).[15] The authors of the China study concluded that UI is a common disorder in Chinese women owing to numerous risk factors besides pregnancy. In a cross-sectional survey that included 19,024 women, the overall prevalence rate of UI was 30.9%, with estimates of SUI, UUI, and MUI being 18.9%, 2.6%, and 9.4%, and a corresponding proportional distribution of 61%, 8%, and 31%, respectively.[16] The study found that only 25% of women reported consulting health practitioners about this issue. In a randomly sampled prevalence survey performed in Fuzhou, Fujian Province, China, 19.0% of women were found to have UI, with the prevalence of SUI, UUI, and MUI at 16.6%, 10.0%, and 7.7%, respectively.[17] Prevalence increased significantly with age. It is not surprising that the prevalence of UI and OAB syndrome is lower in Occidental women, because women in China have given birth to fewer children in their lifetime.[17]

Many individuals with LUTS do not tell their doctor or even their partner about their condition.[8] Women often view UI as a "normal" part of being female.[18] Doctors do not often question about patients' bladder control; 50% to 75% of incontinent community-dwelling patients never describe their symptoms to physicians.[7,19] In a 2017 review paper on medical/surgical options and complications for women affected by UI, although 10% to 20% of women and up to 77% of those residing in nursing homes have UI, only 25% were found to seek or receive treatment for the condition.[20]

Individuals affected by UI tend to modify their lifestyle to cope, wearing adult pads/diapers/absorbent underwear and traveling no more than 1 hour at a time in case of the need to change the pad/diaper or find a restroom. They tend to stop socializing and exercising for fear of having an accident in public, which negatively affects mental and cardiovascular health. They have increased issues with intimacy. Those affected often experience interrupted sleep and resultant daytime fatigue because of frequent nocturia. They typically stop visiting and staying overnight with family or friends for fear of having an accident or disrupting people who may be asleep in the household. With time, sufferers can become reclusive and isolated and have a significant reduction in quality of life. Urinary incontinence is a leading cause of elderly admittance to nursing care.

The etiology of male LUTS is multifactorial, with BPH being a common cause. However, in men, bladder dysfunction can occur independently of the prostate (Fig. 194.2). Less than 50% of cases of bladder dysfunction in men have urodynamically proven bladder outlet obstruction that may be attributed to BPH or other obstructive causes.[9,21] A differential diagnosis of non–prostate-related OAB needs to be ruled out when assessing BPH symptoms.

For men, the occurrence of LUTS, OAB, and UI increases with age. Research shows that 11% of men over the age of 40 have experienced a UI episode in the past year. For men over the age of 60 years, daily UI may be as high as 11%, and in men over the age of 80 years, daily UI is 32%.[22]

For women, UI affects approximately 25% at reproductive age, 50% postmenopause, and 50% to 75% of women in nursing homes.[8,23]

LUTS negatively affect HR-QOL and are associated with high healthcare costs.[9,10,24] OAB and UI are highly prevalent and progress over time, and the severity of UI symptoms increases over time. The number of people affected by poor bladder control is comparable to the number affected by arthritis.

In summary, the problem is widespread and affects people of all ages, including children and young adults. The National Association for Continence (NAFC) estimates that 25 million adult Americans experience transient or chronic UI, increasing to more than 50 million Americans when including OAB sufferers.[2]

Causes/Contributing Factors

OAB: overactivity of the detrusor (bladder) muscle
SUI: weakened pelvic floor (supporting) muscles

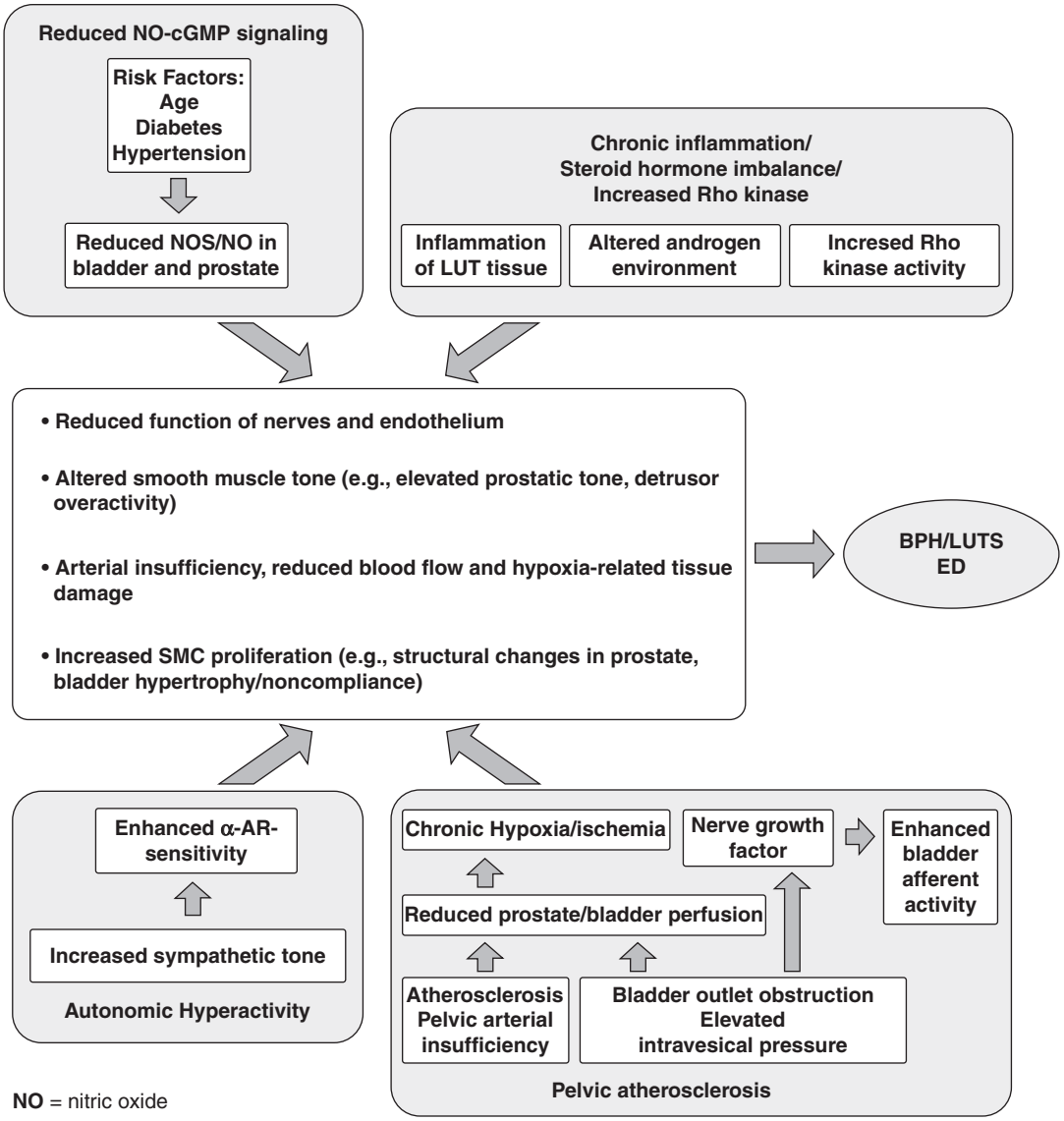
Considerations (Causes, Contributing/Interacting Factors)

- Childbirth (40% of women suffer some form of leaking urine for many months after bearing children)[25]
- High body mass index (places strain on the pelvic floor)
- Constipation and other bowel symptoms (places strain on the pelvic floor)
- History of surgery such as hysterectomy, prostatectomy, or prolapse repair[26]
- Increasing age
- BPH (can lead to secondary OAB due to the strain on the bladder)
- Diabetes mellitus (greater risk of developing OAB)
- Stroke
- Multiple sclerosis
- Alzheimer's disease
- Parkinson's disease
- Poor bladder habits: urinating "just in case" before the bladder has sufficiently filled teaches the bladder that it can only hold a smaller volume of urine and needs to empty before completely necessary.
- Waiting too long to urinate can also interfere with the natural bladder-emptying signals.
- Spinal cord injury
- Medications (antihypertensives, heart medications, diuretics, muscle relaxants, antihistamines, sedatives, and antidepressants)
- Bladder abnormalities, such as tumors or bladder stones
- Declining cognitive function due to aging, which may make it more difficult for the bladder to understand the signals it receives from the brain
- Poor mobility (can lead to urinary urgency)
- High alcohol intake—more than 7 standard drinks/week
- Age-related changes in the lower urinary tract
- Urinary tract infection
- Bladder cancer
- Declining mental health and depression

Addressing the underlying disease is the most effective treatment; however, the cause of OAB and/or UI often cannot be determined.

Chemical irritants, including bacterial lipopolysaccharide (LPS), acid, turpentine, mustard oil, croton oil, and acrolein, directly damage the urothelium and other cells of the bladder, resulting in various degrees of erosion of the mucosa, edema, hemorrhage, and leukocyte infiltration of the bladder wall.[27] Although the cause of chronic bladder pain is yet unknown, it is plausible that irritants may contribute to the etiology.

The elderly may have combined difficulty with bladder-storage problems and bladder emptying. Incomplete emptying may exacerbate urgency, and OAB may require specialist assessment.



NO = nitric oxide
cGMP = cyclic guanosine monophosphate
NOS = nitric oxide synthase
Rho kinases = (ROCKs) are serine/threonine kinases that are important in fundamental processes of cell migration, cell proliferation and cell survival.
SMC = smooth muscle cell
AR = adrenergic receptor

Fig. 194.2 Potential pathophysiological pathways leading to lower urinary tract symptoms (LUTS) in men. (From: Kirby M, Chapple C, Jackson G, Eardley I, Edwards D, Hackett G, Ralph D, Rees J, Speakman M, Spinks J, Wylie K. Erectile dysfunction and lower urinary tract symptoms: a consensus on the importance of co-diagnosis. *Int J Clin Pract.* 2013;67[7]:606–618. PubMed PMID: 23617950. [Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC3748789_ijcp0067-0606-f2&query=lower+urinary+tract+symptoms&req=4&npos=4 (accessed 14 Sept 2018)].)

DIAGNOSIS

Due to the underreporting of bladder symptoms, it is important that questions about bladder control, frequency, urgency, and nocturia are included in the initial naturopathic consultation. A thorough history needs to be taken to identify possible causes of LUTS and associated comorbidities, including medications that may contribute to hyperactivity of the bladder.

LUTS Questionnaires

Refer to the LUTS chart at the start of this chapter, undertake a differential diagnosis, and then provide a referral if required.

A bladder symptom diary can capture fluid intake and urinary frequency, urinary urgency, incontinence, nocturia, and enuresis; assist in diagnosis; and serve as a record to gauge improvement with treatment (see Fig. 194.3).

Validated quality-of-life questionnaires for overactive bladder are available and are useful to track improvement with treatment (e.g., Overactive Bladder Short Form [OAB-SF], Urinary Distress Inventory [UDI], and Incontinence Impact Questionnaire [IIQ] questionnaires; see Figs. 194.4–194.6).

Referral to a urinary specialist may be required to complete the physical examination of the abdomen and external genitalia and digital

Bladder Symptom Diary (Frequency and Urgency)

Start Date: _____

Name: _____

Total number of episodes: Please place amount/number/tally marks as each symptom occurs

SYMPTOM	NUMBER (per day 1)	NUMBER (per day 2)	NUMBER (per day 3)
# Times urinating in day			
Average volume/voiding			
# Times get out of bed to urinate			
Night time bladder accidents			
Urge incontinence			
Stress incontinence			
Other incontinence			

Day 3 -

Additional Notes / Comments:

_____

_____

_____

Fig. 194.3 Bladder symptom diary.

rectal examination. Referral may also be necessary if the symptoms are complicated by recurrent or persistent urinary tract infection, urinary retention, renal impairment that is suspected to be caused by lower urinary tract dysfunction, or suspected urological cancer or for cystoscopy or imaging.

Although urodynamic studies for determining the underlying pathophysiology of LUTS are standard, they have not advanced in decades and are limited due to their invasiveness. Research showing no change in outcome whether performing or not performing the invasive urodynamic testing before treatment calls into question the

OAB-SF Questionnaire

6-Item Symptom Bother Scale Short Form

During the past 4 weeks how bothered were you by…	Not at all	A little bit	Some-what	Quite a bit	A great deal	A very great deal
1. An uncomfortable urge to urinate?	1	2	3	4	5	6
2. A sudden urge to urinate with little or no warning?	1	2	3	4	5	6
3. Accidental loss of small amounts of urine?	1	2	3	4	5	6
4. Night-time urination?	1	2	3	4	5	6
5. Waking up at night because you had to urinate?	1	2	3	4	5	6
6. Urine loss associated with a strong desire to urinate?	1	2	3	4	5	6

OAB-SF Questionnaire

12-Item Symptom Bother Scale Short Form

During the past 4 weeks, how often have your bladder symptoms...	Not at all	A little bit	Some-what	Quite a bit	A great deal	A very great deal
7. Caused you to plan “escape routes” to restrooms in public places?	1	2	3	4	5	6
8. Made you feel like there is something wrong with you?	1	2	3	4	5	6
9. Interfered with your ability to get a good night’s rest?	1	2	3	4	5	6
10. Caused you to decrease your physical activities (exercising, sports, etc.)?	1	2	3	4	5	6
11. Made you avoid activities away from restrooms (walks, running, hiking)?	1	2	3	4	5	6
12. Made you frustrated or annoyed about the amount of time you spend in the restroom?	1	2	3	4	5	6
13. Awakened you during sleep?	1	2	3	4	5	6
14. Made you uncomfortable while travelling with others because of needing to stop for a restroom?	1	2	3	4	5	6
15. Affected your relationships with family and friends?	1	2	3	4	5	6
16. Caused you embarrassment?	1	2	3	4	5	6
17. Interfered with getting the amount of sleep you needed?	1	2	3	4	5	6
18. Caused you to have problems with your partner or spouse?	1	2	3	4	5	6
19. Caused you to locate the closest restroom as soon as you arrive at a place you have never been?	1	2	3	4	5	6

Fig. 194.4 Overactive Bladder–Short Form (OAB-SF) questionnaire. (Coyne K, Revicke D, Hunt T, Corey R, Stewart W, Bentkover J, et al. Psychometric validation of an overactive bladder symptom and health-related quality of life questionnaire: the OAB-q. Qual Life Res. 2002;11(6):563–74. Matza L, Thompson C, Krasnow J, Brewster-Jordan J, Zyczynski T, Coyne K. Test-retest reliability of four questionnaires for patients with overactive bladder: the overactive bladder questionnaire (OAB-q), patient perception of bladder condition (PPBC), urgency questionnaire (UQ), and the primary OAB symptom questionnaire (POSQ). Neurourol Urodyn. 2005;24(3):215–25.)

Incontinence Impact Questionnaire (IIQ)

Has urine leakage affected the following:

	Not at all	Slightly	Moderately	Greatly
1. Household chores	1	2	3	4
2. Physical recreation	1	2	3	4
3. Entertainment activities	1	2	3	4
4. Travel >30 minutes away from home	1	2	3	4
5. Social activities	1	2	3	4
6. Emotional health (nervousness, depression etc.)	1	2	3	4
7. Feeling frustrated	1	2	3	4

Fig. 194.5 Incontinence Impact Questionnaire (IIQ). (Robinson J, Shea J. Development and testing of a measure of health-related quality of life for men with urinary incontinence. J Am Geriatr Soc. 2002;50(5):335–45.)

Urogenital Distress Inventory

Do you experience, and if so, how much are you bothered by the following:

	Not at all	Slightly	Moderately	Greatly
1. Frequent urination	1	2	3	4
2. Leakage related to feeling of urgency	1	2	3	4
3. Leakage related to activity, coughing, sneezing	1	2	3	4
4. Small amounts of leakage (drops)	1	2	3	4
5. Difficulty emptying bladder	1	2	3	4
6. Pain or discomfort in lower abdominal or genital area	1	2	3	4

Fig. 194.6 Urinary Distress Inventory (UDI) questionnaire. (Robinson J, Shea J. Development and testing of a measure of health-related quality of life for men with urinary incontinence. J Am Geriatr Soc. 2002;50(5):335–45.)

treatment-outcome benefits of using these procedures. Emerging noninvasive diagnostic tools include imaging, external condom catheter method, penile cuff test, and near-infrared spectroscopy.[28]

DIFFERENTIAL DIAGNOSIS

- Benign prostatic hyperplasia (BPH):
  - Use of the International Prostate Symptom Score (IPSS) assists
  - Specialist assessment may be required
  - Prostate-specific antigen (bound and unbound) may be requested
- Interstitial/nonbacterial cystitis
- Urinary tract infection (UTI)
- Diabetes mellitus
- Stroke
- Parkinson's disease
- Multiple sclerosis
- Bladder or renal stones
- Diabetes insipidus
- Renal impairment:
  - Check urinalysis dipstick for blood, glucose, protein, leukocytes, and nitrites; pathology for serum creatinine and glomerular filtration rate (GFR).
- Nocturnal polyuria syndrome
- Bladder cancer
- Bladder polyps

THERAPEUTIC CONSIDERATIONS

Pharmaceutical

Anticholinergic/Antimuscarinic and Beta 3-Adrenergic Agonists

Acetylcholine is the primary excitatory neurotransmitter involved in bladder emptying. The two medication classes commonly used are anticholinergic/antimuscarinic (e.g., oxybutynin, tolterodine, solifenacin) and beta 3-adrenergic agonists (e.g., mirabegron). These medications reduce symptoms of OAB and UI with variable outcomes.[29,30] A 2017 study found that prescribing of medications with anticholinergic properties to nursing home residents is common, despite "an association with an increased risk of falls, delirium, and other [adverse] outcomes."[31]

A systematic literature review of randomized controlled trials between 1966 and 2011 evaluating fesoterodine, tolterodine, oxybutynin, solifenacin, and trospium concluded that approximately 13% of participants achieved continence, and approximately 6% of participants discontinued treatment due to the severity of adverse effects (e.g., dry mouth, constipation, blurry vision, drowsiness, and cognitive issues such as confusion and memory loss).[32] Improved quality of life was limited.

The newer medications, such as darifenacin, solifenacin, and trospium, are more targeted for bladder tissue, whereas transdermal delivery systems still produce unwanted anticholinergic effects in tissues other than the bladder.[32]

Significantly, a growing body of research shows a positive association between anticholinergic/antimuscarinic medications and memory

loss that was originally thought to be temporal in nature. One reported study in *JAMA* highlighted the negative effect of these medications on dementia. A 2018 *BMJ* study that analyzed 40,770 patients aged 65 to 99 with a diagnosis of dementia between April 2006 and July 2015, compared with 283,933 controls without dementia, found that the use of anticholinergics is associated with impaired cognition in the short term and in the long term was linked to increased risk of dementia incidence, with associations persisting up to 20 years after exposure.[33,34]

Mirabegron, a drug used to treat OAB, is a beta-3 adrenergic agonist that activates the beta-3 adrenergic receptor in the detrusor muscle in the bladder, which can lead to muscle relaxation and increased bladder capacity.[35] Postsurveillance data show side effects, including elevated blood pressure (hypertension) in more than 10% of individuals, and in 1% to 10% of users, side effects include nasopharyngitis, UTI, dry mouth, joint pain, cystitis, back pain, upper respiratory tract infections, sinusitis, headache, dizziness, diarrhea and constipation, tachycardia, and urinary retention.[36] The overall incidence of drug-related adverse events reported in a randomized clinical trial of mirabegron was 15.8%.[37]

Botox

Sacral nerve stimulation and OnabotulinumtoxinA (Botox, Dysport, Xeomin) are options prescribed by some physicians for UI and OAB. The rationale proposed for the use of OnabotulinumtoxinA involves the drug's ability to inhibit the calcium-mediated release of acetylcholine vesicles at the presynaptic neuromuscular junction in peripheral nerve endings, resulting in temporary flaccid paralysis of the bladder muscle.[38] Botox is the most commonly known drug of the seven antigenically distinct forms of botulinum toxin.[39] In one study, 35 patients (29 women; 6 men) with symptoms of bladder frequency, urgency, and/or urge incontinence in whom previous anticholinergic therapy had failed were administered 300 U of botulinum-A toxin injected transurethrally at 30 sites within the bladder.[40] Of participants, 60% experienced improvement in UDI and IIQ scores and a slight to complete improvement in bladder symptoms by 3 weeks that lasted for up to 6 months. Thereafter, a repeat of the procedure would be needed, and as often as needed, in the hope of a lasting resolution. Mild hematuria, pelvic pain, and dysuria were experienced in 20% of participants. Urinary retention, UTI, hematuria, insomnia, and fatigue following Botox injections are the most common serious side effects reported within the initial 12 weeks after injections are administered.

Surgery

More invasive therapies include surgery with augmentation cystoplasty or detrusor myectomy (removing part or all of the outer muscle layer surrounding the bladder). Detrusor myectomy has been used for years for bladder augmentation in treating refractory detrusor overactivity.[41] This procedure may improve the compliance of the bladder to limit or prevent upper tract damage due to high bladder pressures. In a 1-year postsurgical follow-up of detrusor myectomy for detrusor overactivity, 17 of 27 patients studied experience an overall improvement, with those with idiopathic instability (12 of 17) experiencing a higher rate of success.[42] In a 2-year follow-up study of detrusor myectomy, it was found to be successful in nearly 80% of patients with idiopathic detrusor overactivity in a review of 30 consecutive patients, although "detrusor contractility is affected in most and almost half of the patients required clean intermittent self-catheterization afterward," with 45% of patients needing to start clean intermittent self-catheterization after surgery.[43]

Sling procedures, bulking injections (such as certain collagens), and other surgical procedures support or move the bladder to improve continence. Selective bladder denervation to treat OAB syndrome using radiofrequency ablation is also an option,[44] and the tension-free vaginal tape procedure in women with stress UI can be performed as yet another option, although "the possibility of persistent overactive bladder symptoms" following treatment should be considered.[45]

Physical Therapy

Pelvic Muscle Rehabilitation

Pelvic floor muscle or Kegel exercises (see Box 194.1). Arnold Kegel first advocated pelvic floor muscle strengthening and retraining to treat SUI. Kegel's program has shown some promise.[46,47] Regular, daily exercising of pelvic floor muscles can improve, and even prevent, SUI and MUI. This is particularly helpful for younger women.[48] A specialist physiotherapist or physical therapist can help ensure Kegel exercises are being performed correctly.

Research supports the effectiveness of home-based Kegel exercises, with no supervision, for SUI and MUI. In one study, 72 women with urodynamically proven UI were divided into SUI and MUI groups.[49] The women undertook Kegel exercise, consisting of 10 repetitions of 10 sets of contractions/day, for at least 8 weeks. There were statistically significant improvements in both IIQ and UDI scores, with better results in the SUI group than in the MUI group.

Vaginal weight training. Small weights are held within the vagina by tightening the vaginal muscles. This exercise should be performed for 15 minutes, twice daily, for 4 to 6 weeks before effectiveness can be assessed.

Biofeedback

Used in conjunction with Kegel exercises, biofeedback using sensors to detect if the muscles are contracting helps the individual gain awareness and control of the pelvic floor muscles.

Pelvic Floor Electrical and Magnetic Stimulation

In this type of therapy, mild electrical pulses stimulate muscle contractions. This stimulation is best used in conjunction with Kegel exercises.

Posture

General good posture helps to support the inner and outer abdominal muscles to position correctly and, thereby, also the pelvic floor muscles. During urination, changes in posture can help with more complete bladder emptying. After urination feels complete, structurally (with a straight back), bend forward after urination to move the position of bladder so that it is more likely to be above the urethral sphincter. This may help to eliminate any residual urine volume.

Neural Reflex for Overactive Bladder

Plantar flexion (or pressing the toes into the ground) stimulates the tibial nerve and setting off a somatovesical neural reflex to the bladder, reducing the symptom of urinary urgency.

Transcutaneous Tibial Nerve Stimulation

Transcutaneous tibial nerve stimulation can result in improvement in symptoms of OAB and UI.[50]

Urge-Suppression Techniques

- Stop and sit down
- Apply pressure to the perineum either manually or by crossing the legs
- Practice breathing from the diaphragm
- Raise the pelvic floor muscles.
- Distract mentally (e.g., by thinking of a "happy," peaceful place or counting backward from 50.

Behavioral Therapy

Bladder Retraining

Bladder retraining (see Box 194.2) teaches the bladder to adequately fill and then empty. It involves modifying habits of urinating too much, "just in case," and teaches people to resist the urge to void and, over time, extend the timeframe between voids. A schedule for voiding is developed to allow the person to resist the urge and develop more control over the urge. The mechanisms to explain how bladder training works, when it does, are not fully understood. However, the behavioral interventions that have been reported to have some benefit in managing urinary symptoms to promote bladder health include habit (lifestyle) changes and training techniques. The former is discussed in the section on lifestyle.

Behavioral interventions using training techniques to manage symptoms would include urgency control methods that incorporate deep breathing and mental tasks that try to delay or ignore urgency, bladder training that progressively increases the intervals between voidings and timing to urination, pelvic floor muscle training, and delayed voiding through progressive increases between the onset of urgency and voiding.

Although a 1999 study reported in *Neurourology Urodynamics* failed to find any objective benefit of behavioral therapy and bladder training or pelvic floor muscle training by itself, or a combination training program of both, on urodynamic parameters in women with UI, subjective reports by participants indicated that some improvement occurred immediately after treatment.[51,52]

Botanical Medicines

Crateva nurvala/Crateva magna (Varuna, Three-Leaf Caper)

Crateva is documented in traditional Ayurvedic herbal texts and in research as effective for improving and normalizing the tone of the bladder wall (as assessed by cystometric studies), beneficial for neurogenic bladder and postprostatectomic atony of the bladder.[53–58] Crateva can benefit men with hypotonic bladder due to BPH and decreases residual urine volume. Research with dogs shows that *Crateva* increases the tone of both smooth and skeletal muscle in vitro. Forty days of treatment with *Crateva* resulted in hypertonic curves of the urinary bladder compared with initial curves.[53,58]

Equisetum arvense (Horsetail)

Western herbal medicine traditionally recommends *Equisetum arvense* as a genito-urinary astringent, likely due to the silica content, for UI and enuresis in children.[58,59] *Equisetum* has also been shown to have anti-inflammatory, antibacterial, and antilithogenic effects.[60,61] Note that *Equisteum* extracted with alcohol or heat will destroy the naturally occurring thiaminase.

Crateva and *Equisetum* Combined

A randomized controlled trial with 120 male and female participants using a proprietary blend of *C. nurvala* (equivalent to 6000 mg dry bark) and *E. arvense* (equivalent to 3000 mg dry herb) resulted in statistically significant reductions in day frequency and UI and improved quality of life within 2 months of treatment; however, dropout was high (23%).[62] There were no anticholinergic side effects.

Lindera aggregata/L strychnifolia (Japanese Evergreen Spicebush, Benzoin, Wuyao)

Lindera is documented for use with frequent urination and UI due to cold from a deficient bladder.[63] It promotes the movement of chi or energy and blood and disperses cold, especially in the lower abdomen.

Urox (*Crateva, Equisetum,* and *Lindera*)

Urox is a proprietary blend of concentrated extracts of *Crateva, Equisetum,* and *Lindera.* An 8-week randomized controlled trial with 150 male and female participants showed that 840 mg Urox daily resulted in significant reductions in day frequency, nocturia, urinary urgency, and UI.[64]

Results showed that by 8 weeks, 60% of participants had returned to normal day frequency (8 or fewer micturitions/day); nocturia incidence halved, with 24% of participants experiencing normal nighttime urination; 20% were free from urinary urgency; and 23% had resolution of their UI (both SUI and UUI). A corresponding reduction in adult diaper/pad usage occurred for 75% of study participants. The correlated benefits of less interrupted sleep were also considered clinically relevant. There were significant improvements in HR-QOL. No anticholinergic/antimuscarinic side effects were observed, and no side effects resulted in withdrawal from the study.

Urox produces faster and better results for OAB and UI than any other herbal assessed in randomized controlled research. Urox is the only herbal shown to produce statistically significant reductions in both SUI and UUI in both men and women.

Pumpkin Seed (*Cucurbita pepo*) and Soy

Pumpkin seeds contain zinc and act as 5-alpha-reductase inhibitors to assist with bladder symptoms associated with BPH and prostate health. Soy is typically recommended for hormone-balancing effects.

A 12-week randomized controlled trial in 120 women with LUTS of OAB participated in this study to assess the effectiveness of Cucuflavone (97.5% pumpkin seed extract, EFLA 940 and 12.5% soy germ, SOYLIFE 40 combination).[65] The daily dosage equated to 875 mg pumpkin seed extract and 125 mg soy germ extract for 12 weeks. Urinary frequency reached the normal range by 12 weeks, nocturia was reduced by 31%, and urinary urgency was reduced by 31%. There was a 30% dropout rate, which might have been due to the length of time for symptoms to improve. There was no statistically significant improvement in UI symptoms.

Angelica archangelica (Garden Angelica, Wild Celery)

Angelica archangelica is recommended for bladder control and has a large usage in Europe. An 8-week randomized trial with men ($n = 69$) using two tablets daily of *A. archangelica* (Sagapro) was assessed for its effects on nocturnal bladder symptoms.[66] There was no significant reduction in overall nocturia, although subgroup analysis of only those with a decreased bladder capacity reached significance. Men with BPH were included, which may explain some of the variations in results.

Vaccinium macrocarpon (Cranberry)

Cranberry is typically recommended for UTI. A randomized controlled trial in 69 male participants assessed the effectiveness of 1500 mg whole cranberry *(Vaccinium macrocarpon)* fruit powder for 6 months in men with both LUTS and nonbacterial prostatitis.[67] These men did not fit the typical picture of OAB or UI, although results showed a statistically significant reduction for urinary urgency at 3 and 6 months and for frequency and nocturia at 6 months.

Other Herbals

Agathosma betulina (buchu)
Zea mays (cornsilk)
Galium aparine (cleavers)
Agrimonia eupatoria (agrimony)
Cinnamomum cassia (cinnamon)
Rhus aromatica (fragrant sumach)
Humulus lupus (hops)
Ephedra viridis (Mormon tea)
Chimaphila umbellate (Pipsissewa)

Chinese and Japanese Herbal Medicine

Hachi-mi-jio-gan. Hachi-mi-jio-gan extract (Harncare; HE) contains the ancient traditional Chinese medicine (TCM) herbal mixture Ba-Wei-Die-Huang-Wan. The daily dose contains 4.4 g of the eight-ingredient formula with concentrated extracts equivalent to the following:

- *Rehmannia glutinosa* root 5 g
- *Cornus* fruit 3 g
- *Dioscorea* rhizome 3 g
- *Alismatis* rhizome 3 g
- *Moutan* bark 3 g
- Cinnamon bark 1 g
- Hoelen *(Poria cocus)* 3 g
- Heat-processed lateral root of aconite 1 g

Gosha-jinki-gan. Gosha-jinki-gan (GJG) contains the same type and amount of concentrated extracts as Hachi-mi-jio-gan, with the addition of two concentrated extracts equivalent to Achyranthis Radix (3 g) and Plantaginis Semen (3 g).

An observational study assessed the effectiveness of 7.5 g/day GJG for 8 weeks in 44 Japanese women diagnosed with OAB. Urinary frequency, nocturia, International Prostate Symptom Score (IPSS) index scores, and HR-QOL index scores were all significantly reduced, with adverse reactions observed in 9%.[68]

Rat studies have confirmed that GJG partially reduces the tachykinins and TRPV1 and P2X3 purine receptors (receptors responsible for hypersensitivity) in the bladder without destroying the nerve fibers.[69] GJG has an inhibitory effect on the bladder and maintains the balance of the sympathetic and parasympathetic nervous systems.[70]

A study with 45 female rats assessing the mechanism of Harncare showed that it reduced the bladder expression of tachykinins and P2X3 and TRPV1 receptors and inhibited adenosine triphosphate (ATP)–induced detrusor overactivity, which may explain part of its beneficial effects on LUTS.[71]

Further rat studies showed that treatment with Harncare for 4 weeks significantly inhibited, in a dose-dependent manner, the acetylcholine-induced contraction of isolated rat bladder strips, with a 7-times-greater affinity for purinergic receptors than for muscarinic and 1,4-DHP receptors, with little or no effect on the level and activity of hepatic CYP, which would indicate minimal pharmacokinetic interaction with drugs.[72]

Sixty male participants with LUTS and cold sensitivity and who were unresponsive to alpha-1 blockers or antimuscarinic drugs were given either a mixture of Harncare or GJG for 12 weeks in addition to alpha-1 adrenergic receptor blockers or antimuscarinic drugs as add-on therapy. The IPSS and HR-QOL scores, Benign Prostatic Hyperplasia Impact Index score, and the number of nocturnal voids were statistically much improved, with no change in maximal urinary flow rate and postvoid residual urine. Mild adverse reactions were observed in 8.3% of patients.[73]

Both Harncare and GJG mixtures seem safe and effective potential therapeutic alternatives in patients with LUTS and cold sensitivity unresponsive to alpha-1 blockers or antimuscarinic drugs.

Alpinia oxyphylla

Alpinia oxyphylla (Zingiberaceae) is a Chinese medicinal herb, and the fruit is commonly used in TCM for the treatment of LUTS of OAB. In vitro testing of 95% ethanol extract of the capsular fruits exhibited significant dose-dependent antimuscarinic activity against carbachol-induced contraction of the rat detrusor muscle.[74]

Dietary/Nutritional

Minerals play an important role in connective tissue structural integrity and neuromuscular signaling.

Magnesium at therapeutic doses can be of benefit in OAB, as may calcium. Forty women who had sensory urgency or detrusor instability participated in a randomized, double-blind, placebo-controlled study to compare the effect of magnesium hydroxide or placebo on symptoms, frequency/volume changes, and cystometric results. Magnesium was well tolerated, and 11 of 20 subjects in the magnesium group reported subjective improvements, compared with only 5 taking placebo, yet there was no statistically significant improvement in pre- and posttreatment urodynamic parameters.[75] A better-absorbed form of magnesium, such as chelate, orotate, or diglycinate, is recommended. Regarding whether magnesium and calcium serum levels are different in women with detrusor overactivity or urodynamic stress incontinence, investigation of blood levels determined there were no differences.[76]

Diet

Bladder stimulants include diuretics such as caffeine and alcohol, and bladder irritants include certain acidic fruits, spices, and tomato-based products. When 10 studies, including 2 randomized controlled trials, were analyzed to examine the role of the diet in OAB, the analysis found that "diet seems to have an impact on OAB, particularly caffeine consumption, which increases storage symptoms."[77] Similar results were found in a study of caffeine, alcohol, and carbonated drinks, which concluded that "in addition to fluid intake, there is some evidence to support a role of caffeine, alcohol, and carbonated beverages in the pathogenesis of OAB and lower urinary tract dysfunction."[78]

Hence, caffeine has been the subject of studies on its effects on UI in women. In a study conducted in the United States of women who participated in the 2005 to 2006 and 2007 to 2008 NHANES, a cross-sectional nationally representative survey, the U.S. prevalence for any UI was 41.0%, and the prevalence was 16.5% for moderate/severe UI, with SUI being the most common UI type (36.6%). The women surveyed consumed a mean caffeine intake of 126 mg a day. Caffeine intake equal to or exceeding 204 mg a day was associated with any UI, but not moderate/severe UI, after controlling for multiple factors.[79] An 8-oz cup of brewed coffee can contain from 95 to 165 mg of caffeine per cup, whereas a 12-oz serving of a carbonated cola soda can contain as much as 46 mg of caffeine. An 8-oz energy drink can contain from 27 to 164 mg of caffeine, and an "energy shot" can contain 40 to 100 mg per ounce.[80] By comparison, in a survey of men aged 20 years or older, a significant association was found between caffeine intake and moderate to severe UI. Men with a caffeine intake at the upper 75th percentile (234 mg or more a day) and 90th percentile (392 mg or more per day) were significantly more likely to experience moderate to severe UI even after adjusting for prostate condition.[81] These findings support the need to reduce caffeine intake in men with UI.

Nevertheless, quite the opposite conclusion was reported after a meta-analysis of observational studies examining the association between coffee and caffeine intake and the risk of UI. The meta-analysis found "no evidence for an association between coffee/caffeine consumption and the risk of UI."[82] Similarly, a prospective cohort study in 21,564 women with moderate UI enrolled in the Nurses' Health Study II did not find an association between the risk of UI progression over 2 years among women with moderate incontinence, even when comparing women consuming 450 mg or more to those consuming 150 mg or less of caffeine per day.[83] The study did not examine the acute effects of caffeine on UI.

Potential foods known to be bladder irritants include:

- Coffee, tea, and chocolate (containing caffeine, a diuretic)
- Alcohol (diuretic)

- Carbonated drinks
- Artificial sweeteners (e.g., aspartame [NutraSweet], saccharin), corn sweeteners, fructose
- Acidic fruits and juices, including lemon, lime, grapefruit, and orange
- Spicy foods (e.g., chili)
- Watermelon
- Tomato-based products (e.g., tomato juice)

It is recommended to remove them from the diet and then, after 2 weeks, slowly and individually reintroduce each food to determine whether it is contributing to bladder symptoms. Additional common foods that have been reported to be bladder irritants include apples and apple juice, cantaloupe, citrus fruits, cranberries and cranberry juice, grapes, guava, cow's milk and its by-products (cheese, cottage cheese, ice cream, yogurt), peaches, pineapples, plums, strawberries, sugar (sucrose), and vinegar.[84,85] This list of possible bladder irritants includes foods that may or may not affect a patient's bladder symptoms.

Fluid

With age, the thirst reflex reduces. Many individuals with OAB and UI restrict their water intake to help manage their condition. It is important to maintain a fluid intake of 1.5 to 2 L/day and manage bladder control through healthy bladder habits and supplements. Dehydration may cause additional strain on the kidneys and bladder.

Medications That Increase Urgency

There are medications that can increase the frequency or urgency to urinate. Among them are antihypertensives and medications to reduce muscular pain (relaxants). Among the medications to treat hypertension are diuretics such as hydrochlorothiazide and furosemide; alpha-antagonists, including doxazosin maleate and prazosin; and angiotensin-converting enzyme (ACE) inhibitors such as captopril and enalapril maleate.

Lifestyle

Habit changes involve lifestyle modifications. Modifications include changes in diet, fluid intake, and food and beverage choices; weight management; and smoking cessation. Lifestyle changes can also include timed voiding using a bladder diary.

Increasing age is a risk factor for OAB and UI; however, most risk factors are modifiable, including the following:

- Excess weight; obesity[86]
- Lack of physical activity[86]
- Smoking[86]
- Stress[2]
- Heavy alcohol intake (more than 7 standard drinks per week)[2]
- Caffeine, a dietary diuretic that can increase urinary frequency
- Constipation (pressure and bearing down)
- Vertebral misalignment
- Medications (antihypertensives, heart medications, diuretics, muscle relaxants, antihistamines, sedatives, and antidepressants)

Elderly individuals may have combined difficulty with bladder storage problems and bladder emptying. Incomplete emptying may exacerbate urgency and OAB and may require specialist assessment.

Erectile dysfunction is correlated with LUTS, but no evidence has been produced that shows a causal relationship. Preclinical evidence that both develop due to several common pathophysiological mechanisms has been proposed.[87]

It is important for practitioners to discuss sexual function in male patients presenting for LUTS because epidemiological evidence, based on well-powered multivariate analyses, provides compelling evidence of an association between LUTS and erectile dysfunction in men. This is even more important to discuss with the patient because men with LUTS have an increased risk of all-cause mortality, particularly for older males with nocturia.[88]

Other Holistic Treatments

Hypnotherapy

Hypnotherapy may be of benefit based on the early work of Freeman and Baxby reported in 1982.[89] In their study, 50 incontinent women with detrusor instability completed 12 sessions of hypnotherapy over a 1-month period. Thereafter, the patients continued the treatment using a prerecorded cassette tape and were followed up for at least 6 months. Of the 50 patients, 29 claimed to be entirely symptom-free, 6 showed no objective improvement, and 14 reported some improvement. Well-controlled clinical studies of the efficacy of hypnotherapy are lacking, although case histories continue to appear in the literature attesting to its benefit.[90]

Physiotherapy

Specialist physiotherapists can correctly instruct for Kegel/pelvic floor exercises and posture during urination and can assist with any structural contributing factors. A multicenter, randomized clinical trial compared physiotherapy to midurethral-sling surgery in women with SUI.[91] In the study, 230 women were randomly assigned to the physiotherapy group and an equal number to the surgery group. Forty-nine percent of the women in the physiotherapy group and 11.2% of the women in the surgery group crossed over to the other treatment. Using an intention-to-treat analysis that analyzed the outcome for all participants (including those who dropped out of the study before completing the assigned group), "subject improvement was reported by 90.8% of women in the surgery group and 64.4% of women in the physiotherapy group" after 1 year. The rates of subjective resolution were 85.2% in the surgery group and 53.4% in the physiotherapy group, and the rates of an objective "cure" were 76.5% and 58.8%, respectively. These results are associated with the broad variation in the rates of success for physiotherapy reported in the literature, which vary from 53% to 97% when measuring subjective success and from 5% to 49% when measured by objective means.[92]

Acupuncture

To assess the effectiveness of acupuncture for SUI in adults, a review was performed, and the results of randomized and quasi-randomized controlled trials were analyzed.[93] The analysis of the studies selected for inclusion in the review was not conclusive as to whether acupuncture in adults with SUI was more effective than medications or other treatment options.

Another approach taken to assess the efficacy of acupuncture was undertaken by experts in acupuncture familiar with the ancient literature in the 4th edition of the *Canon of Chinese Medicine* that discusses the use of acupuncture for urinary incontinence. This led to the reviewers identifying 356 articles reporting on the use of this treatment for UI involving 41 acupoints. The results of this group analysis showed that "urinary incontinence belongs to external genitalia diseases, which should be treated from yin, indicating more yin-meridians be used and special acupoints be focused on."[94] A randomized, multicenter clinical trial to determine the efficacy of electroacupuncture in the treatment of SUI in women, compared with pelvic floor muscle training, is underway in China, but the results are unknown at this time. A description of the study is published.[95]

A randomized controlled trial involved 240 women with OAB using weekly acupuncture compared with daily oral tolterodine tartrate 2 mg twice daily, over 4 weeks. Both groups showed statistically significant decreases in urinary urgency, UI, urinary frequency, and nocturia and an increase in volume voided per micturition. There was no difference in the changes in OAB symptoms between groups.[74]

Chiropractic

Vertebral misalignment can contribute to poor bladder control, and if appropriate, chiropractic treatment can be beneficial. Current observations by chiropractors indicate that some patients with SUI may have imbalances in several lumbopelvic muscles that inhibit the pelvic floor muscles and joints, leading to incontinence.[96] Comorbidities between incontinence, lumbopelvic pain, and breathing difficulties have also been suggested. [96,97]

A retrospective case-series study using chiropractic management of UI that included 21 women, 13 to 90 years of age, with UI found that 13 patients had lumbosacral dysfunction. Chiropractic manipulative (high-velocity, low-amplitude manipulation; Cox flexion distraction manipulation; and/or use of percussion instrument treatment for myofascial trigger points) and soft tissue treatments were used to address soft tissue and articular dysfunctions. UI symptoms resolved in 10 patients, 7 improved, and 4 showed only slight improvement.[98]

Case studies reporting on the resolution of various bladder-related disorders abound in the literature. In one case, a 13-year-old female developed unpredictable UI and right hip pain immediately after emergency open appendectomy surgery. The UI persisted for 10 months, forcing the patient to wear a pad throughout the day and night. A combination of chiropractic therapy and applied kinesiology methods was used to diagnose and treat the patient. Muscle impairments were identified in the lumbar spine and pelvis, which, upon treatment, resulted in rapid resolution of UI and hip pain. A 6-year follow-up confirmed continued resolution.[99]

Therapeutic Approach

A key aspect of the therapeutic approach for patients suffering any of the forms of LUTS, OAB, and UI is fully exploring and eliminating as many causative, irritant, or contributing factors as possible. Eliminate or find alternatives for any drug that may aggravate.

Weight should be achieved.

Diet

Reduce bladder stimulants, including diuretics such as caffeine and alcohol, and bladder irritants, such as certain acidic fruits, spices, and tomato-based products, to determine whether there is an improvement in symptoms. Gradually reintroduce foods to determine which are problematic. Ensure adequate fluid intake.

Nutritional Supplements

Magnesium citrate: 250 mg bid

Botanical Medicines

C. *nurvala:* dose should be equivalent to 6000 mg dry bark daily
Urox (*Crateva, Equisetum,* and *Lindera* blend) 840 mg daily

BOX 194.1 Kegel Exercises

Identify the muscles by stopping urination midstream. Then, when not urinating, contract/tighten these muscles for 2 to 3 seconds and then release for 2 to 3 seconds. These exercises can be done when sitting, standing, or lying down.

Slowly increase the duration of the contraction up to 10 seconds. Ideally, build up to performing three sets of 10 contractions (10-second duration each) three times a day. It may take up to 8 weeks to see benefits.

If in doubt regarding the correct muscles to contract, return to squeezing the lower urinary muscles as required to stop urination midstream.

BOX 194.2 Bladder Retraining

A bladder symptom diary (See Fig. 194.3) is kept to determine the voiding pattern throughout the day and evening.

The aim of the bladder training/retraining program is to extend the timeframe between voiding by 15 min per week, gradually lengthening the time between toilet visits so that the person can hold urine in the bladder for up to 4 hours.

It is important to gradually increase the intervals between micturitions and to record the timing and voiding volume of each micturition. If a relapse occurs, remain positive and manage the relapse. Then forge forward with increasing voiding-interval timeframes and, ideally, volume-holding capacity.

Management techniques such as relaxation, meditation, and so forth can assist. It is important to "teach" the bladder that it can hold urine for longer periods of time.

In the event of an accident, breaking the program is fine, but then return to the schedule with the aim of expanding the voiding-interval timeframe.

Pumpkin seed extract (875 mg) and soy extract (125 mg) daily
Cranberry whole fruit powder 1500 mg daily

Physical Medicine

Kegel exercises (Box 194.1)
Acupuncture as appropriate
Chiropractic as appropriate

Behavioral Therapy

Bladder retraining (Box 194.2)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsetn U. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the international continence society. *Urology*. 2003;61(1):37–49. PMID: 12559262.
2. Conditions A-Z. *National Association for Continence*; 2018. Available from: http://www.nafc.org/conditions. Accessed 12 January 2018.
3. Overactive bladder (OAB)
4. Benign prostatic hyperplasia (BPH)
5. Kaplan D, Culligan PJ, Sand PK. Involuntary urine loss in women: help for a hidden problem. *Patient Care*. 1998;32(20):141–142.
6. Bartoli S, Aguzzi G, Tarricone R. Impact on quality of life of urinary incontinence and overactive bladder: a systematic literature review. *Urology*. 2010;75(3):491–500. PMID: 19962738.
7. Abrams P, Kelleher CJ, Kerr LA, Rogers RG. Overactive bladder significantly affects quality of life. *Am J Manag Care*. 2000;6(suppl 11):S580–S590. PMID: 11183901.
8. Irwin DE, Mungapen L, Milsom I, Kopp Z, Reeves P, Kelleher C. The economic impact of overactive bladder syndrome in six Western countries. *BJU International*. 2009;103(2):202–209. PMID: 19278532.
9. Who's at risk? Continence Foundation of Australia. Available from: http://www.continence.org.au/pages/the-facts.html. Accessed 12 January 2018.
10. Coyne KS, Sexton CC, Thompson CL, et al. The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: results from the Epidemiology of LUTS (EpiLUTS) study. *BJU International*. 2009;104(3):352–360. PMID: 19281467.
11. Markland AD, Richter HE, Fwu C-W, Eggers P, Kusek JW. Prevalence and trends of urinary incontinence in adults in the United States, 2001 to 2008. *J Urol*. 2011;186(2):589–593.
12. Continence Foundation of Australia. Available from: https://www.continence.org.au/pages/prevalence-and-economic-impact-of-incontinence-in-australia-deloitte-access-economics-2010.html. Accessed 31 July 2018.
13. Ge J, Yang P, Zhang Y, Li X, Wang Q, Lu Y. Prevalence and risk factors of urinary incontinence in Chinese women: a population-based study. *Asia Pac J Public Health*. 2015;27(2):NP1118–N1131. PMID: 22186396.
14. Zhu L, Lang J, Liu C, Han S, Huang J, Li X. The epidemiological study of women with urinary incontinence and risk factors for stress urinary incontinence in China. *Menopause*. 2009;16(4):831–836. PMID: 19240656.
15. Song Y-F, Zhang W-J, Song J, Xu B. Prevalence and risk factors of urinary incontinence in Fuzhou Chinese women. *Chinese Med J*. 2005;118(11):887–892. PMID: 15978188.
16. Peake S, Manderson L, Potts H. "Part and parcel of being a woman": female urinary incontinence and constructions of control. *Med Anthropol Quart*. 1999;13(3):267–285. PMID: 10509310.
17. Benner JS, Becker R, Fanning K, et al. Bother related to bladder control and health care seeking behavior in adults in the United States. *J Urol*. 2009;181(6):2591–2598. PMID: 19375096.
18. Lukacz ES, Santiago-Lastra Y, Albo ME, Brubaker L. Urinary incontinence in women: a review. *JAMA*. 2017;318(16):1592–1604. PMID: 29067433.
19. Liao C-H, Kuo H-C. Measurement of international prostate symptom score subscores in male lower urinary tract symptoms. *Incontinence Pelvic Floor Dysfunct*. 2010;4:39–43.
20. Shamliyan TA, Wyman JF, Ping R, Wilt TJ, Kane RL. Male urinary incontinence: prevalence, risk factors, and preventive interventions. *Rev Urol*. 2009;11(3):145. PMID: 19918340.
21. Chutka DS, Fleming KC, Evans MP, Evans JM, Andrews KL, eds. *Urinary Incontinence in the Elderly Population*. Elsevier: Mayo Clinic Proceedings; 1996;71(1):93–101. PMID: 8538240.
22. Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. *Eur Urol*. 2006;50(6):1306–1315. PMID: 17049716.
23. Rane A. Incontinence in women. Don't suffer it. *Aus Family Physician*. 1999;28(6):584–586. PMID: 10399392.
24. Chiarelli P, Brown WJ. Leaking urine in Australian women: prevalence and associated conditions. *Women Health*. 1999;29(1):1–13. PMID: 10427637.
25. Bjorling DE, Wang ZY, Bushman W. Models of inflammation of the lower urinary tract. *Neurol Urodynamics*. 2011;30(5):673–682. PMID: 21661012.
26. Gravas S, Kyriazis I, Klausner AP. Lower urinary tract symptoms including bladder outlet obstruction: what's new in diagnostics? *Eur Urol Focus*. 2018. PMID: 29665998.
27. Herschorn S, Jones JS, Oelke M, MacDiarmid S, Wang JT, Guan Z. Efficacy and tolerability of fesoterodine in men with overactive bladder: a pooled analysis of 2 phase III studies. *Urology*. 2010;75(5):1149–1155. PMID: 19914702.
28. Madhuvrata P, Cody JD, Ellis G, Herbison GP, Hay-Smith E. *Which Anticholinergic Drug for Overactive Bladder Symptoms in Adults*. 2012;1:CD005429. PMID: 22258963.
29. Niznik J, Zhao X, Jiang T, et al. Anticholinergic prescribing in medicare part D beneficiaries residing in nursing homes: results from a retrospective cross-sectional analysis of medicare data. *Drugs Aging*. 2017;34(12):925–939. PMID: 29214512.
30. Shamliyan T, Wyman JF, Ramakrishnan R, Sainfort F, Kane RL. Benefits and harms of pharmacologic treatment for urinary incontinence in women: a systematic review. *Ann Inter Med*. 2012;156(12):861–874. PMID: 22711079.
31. Gray SL, Anderson ML, Dublin S, et al. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Inter Med*. 2015;175(3):401–407. PMID: 25621434.
32. Richardson K, Fox C, Maidment I, et al. Anticholinergic drugs and risk of dementia: case-control study. *BMJ*. 2018;361:k1315. PMID: 29695481.
33. Sacco E, Bientinesi R, Tienforti D, et al. Discovery history and clinical development of mirabssssegron for the treatment of overactive bladder and urinary incontinence. *Exp Opin Drug Discovery*. 2014;9(4):433–448. PMID: 24559030.
34. NICE guidance. Mirabegron for treating symptoms of overactive bladder. National Institute for Health and Care Excellence: clinical guidelines London; 2013. Available from: https://www.nice.org.uk/guidance/ta290. Accessed 30 June 2018.
35. Kuo HC, Lee KS, Na Y, et al. Results of a randomized, double–blind, parallel–group, placebo–and active–controlled, multicenter study of mirabegron, a β3–adrenoceptor agonist, in patients with overactive bladder in Asia. *Neurourol Urodynamics*. 2015;34(7):685–692. PMID: 25130281.
36. Dolly J. *General Properties and Cellular Mechanisms of Neurotoxins. Therapy with Botulinum Toxin*. New York: Marcel Dekker; 1994.
37. Orasanu B, Mahajan ST. The use of botulinum toxin for the treatment of overactive bladder syndrome. *Indian J Urol*. 2013;29(1):2. PMID: 23671356.
38. Rapp DE, Lucioni A, Katz EE, O'Connor RC, Gerber GS, Bales GT. Use of botulinum-A toxin for the treatment of refractory overactive bladder symptoms: an initial experience. *Urology*. 2004;63(6):1071–1075.
39. Aslam MZ, Agarwal M. Detrusor myectomy: long-term functional outcomes. *Int J Urol*. 2012;19(12):1099–1102. PMID: 22852876.
40. Swami KS, Feneley RC, Hammonds JC, Abrams P. Detrusor myectomy for detrusor overactivity: a minimum 1-year follow-up. *Br J Urol*. 1998;81(1):68–72. PMID: 9467479.
41. Kumar SP, Abrams PH. Detrusor myectomy: long-term results with a minimum follow-up of 2 years. *BJU Int*. 2005;96(3):341–344. PMID: 9292244.
42. Fugett 2nd J, Phillips L, Tobin E, et al. Selective bladder denervation for overactive bladder (OAB) syndrome: from concept to healing outcomes using the ovine model. *Neurourol Urodyn*. 2018;37(7):2097–2105. PMID: 16042727.
43. Choe JH, Choo MS, Lee KS. The impact of tension-free vaginal tape on overactive bladder symptoms in women with stress urinary incontinence: significance of detrusor overactivity. *J Urol*. 2008;179(1):214–219. PMID: 18001792.
44. Van Zak DB. Noninvasive feedback of external pubococcegii muscle activity as a treatment for urinary incontinence. *Int J Psychosom*. 1993;40(1-4). PMID: 8070987.
45. Ferguson LK, McKey LP, Bishop RK, Kloen BP, Verheul CJ, Dougherty CM. Stress urinary incontinence: effect of pelvic muscle Exercise. *Obstetr Gynecol*. 1990;75(4):671–675. PMID: 2314786.

46. Eslami M. Evaluation and Management of Incontinence in females. Proceedings of UCLA Health Care Fall. 2001. http://www.med.ucla.edu/modules/xfsection/article.php?articleid=93. Accessed 3 November 2016.
47. Cavkaytar S, Kokanali MK, Topcu HO, Aksakal OS, Doganay M. Effect of home-based Kegel exercises on quality of life in women with stress and mixed urinary incontinence. *J Obstetr Gynaecol*. 2015;35(4):407–410. PMID: 25264854.
48. Bykoviene L, Kubilius R, Aniuliene R, Bartuseviciene E, Bartusevicius A. Pelvic floor muscle training with or without tibial nerve stimulation and lifestyle changes have comparable effects on the overactive bladder. A randomized clinical trial. *Urol J*. 2018;15(4):186–192. PMID: 29781068.
49. Elser DM, Wyman JF, McClish DK, Robinson D, Fantl JA, Bump RC. The effect of bladder training, pelvic floor muscle training, or combination training on urodynamic parameters in women with urinary incontinence. Continence Program for Women Research Group. *Neurourol Urodyn*. 1999;18(5):427–436. PMID: 10494113.
50. Waterfield A. Bladder training plus pelvic muscle exercises reduced urinary incontinence in women immediately after treatment. *Evidence Based Nursing*. 1999;2(3):85. https//www.doi.org/10.1136/ebn.2.3.85.
51. Deshpande PJ, Sahu M, Kumar P. Crataeva nurvala Hook and Forst (Varuna)—the Ayurvedic drug of choice in urinary disorders. *Indian J Med Res*. 1982;76(Suppl):46–53. PMID: 7185756.
52. Indian Herbal Pharmacopeia Revised New Edition. 2002:153–160.
53. Nadkarni KM. Indian Materia Medica. Prakashan: Bombay Popular. 1976:387–388.
54. Prabhakar YS and Suresh Kumar D. Crataeva nurvala: an Ayurvedic remedy for urological disorders. *Br J Phytotherapy*. 1997;4(3):103–9.
55. Das PK RR, Lal R, Tripathi RM, Ram AK, Biswas M. Anti-inflammatory and anti-arthritic activity of Varuna. *Indian J Med Res*. 1974;9:49–56.
56. Bone K. Clinical applications of Ayurvedic and Chinese herbs; monographs for the western herbal practitioner. Warwick, Queensland, Australia: Phytotherapy Press; 1997.
57. BHP Association Scientific Committee: British Herbal Pharmacopeia. Lane House, Cowling, Nr. Keighley, West Yorks, UK: British Herbal Association; 1983:83–84.
58. Grases F, Melero G, Costa-Bauza A, Prieto R, March JG. Urolithiasis and phytotherapy. *Int Urol Nephrol*. 1994;26(5):507–511. PMID: 7860196.
59. Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. *Biosci Biotechnol Biochem*. 1999;63(10):1787–1790. PMID: 10671036.
60. Schauss AG, Spiller G, Chaves S, Gawlicka AK. Effectiveness of UroLogic in reducing the symptoms of overactive bladder and urinary incontinence. *FASEB J*. 2006;20(5):102.
61. Bensky D, Gamble A. *Chinese Herbal: Materia Medica*. Seattle, USA: Eastland Press Inc.; 1993:238–239.
62. Schoendorfer N, Sharp N, Seipel T, Schauss AG, Ahuja KDK. Urox containing concentrated extracts of Crataeva nurvala stem bark, Equisetum arvense stem and Lindera aggregata root, in the treatment of symptoms of overactive bladder and urinary incontinence: a phase 2, randomised, double-blind placebo controlled trial. *BMC Complemen Alter Med*. 2018;18(1):42. PMID: 29385990.
63. Shim B, Jeong H, Lee S, Hwang S, Moon B, Storni C. A randomized double-blind placebo-controlled clinical trial of a product containing pumpkin seed extract and soy germ extract to improve overactive bladder-related voiding dysfunction and quality of life. *J Functional Foods*. 2014;8(1):111–117. https//www.doi.org/10.1016/j.jff.2014.03.010.
64. Sigurdsson S, Geirsson G, Gudmundsdottir H, Egilsdottir PB, Gudbjarnason S. A parallel, randomized, double-blind, placebo-controlled study to investigate the effect of SagaPro on nocturia in men. *Scand J Urol*. 2013;47(1):26–32. PMID: 23323790.
65. Vidlar A, Vostalova J, Ulrichova J, et al. The effectiveness of dried cranberries (Vaccinium macrocarpon) in men with lower urinary tract symptoms. *Br J Nutr*. 2010;104(8):1181–1189. PMID: 20804630.
66. Kajiwara M, Mutaguchi K. Clinical efficacy and tolerability of gosha-jinki-gan, Japanese traditional herbal medicine, in females with overactive bladder. *Hinyokika Kiyo Acta Urologica Japonica*. 2008;54(2):95–99. PMID: 18323165.
67. Imamura T, Ishizuka O, Aizawa N, et al. Gosha-jinki-gan reduces transmitter proteins and sensory receptors associated with C fiber activation induced by acetic acid in rat urinary bladder. *Neurourol Urodyn*. 2008;27(8):832–837. PMID: 18551569.
68. Nishijima S, Sugaya K, Miyazato M, Ogawa Y. Effect of Gosha-jinki-gan, a blended herbal medicine, on bladder activity in rats. *J Urol*. 2007;177(2):762–765. PMID: 17222677.
69. Imamura T, Ishizuka O, Zhong C, et al. An extract (THC-002) of Ba-Wei-Die-Huang-Wan inhibits expression of tachykinins, and P2X3 and TRPV1 receptors, and inhibits ATP-induced detrusor overactivity in spontaneously hypertensive rats. *Neurourol Urodyn*. 2009;28(6):529–534. PMID: 19283865.
70. Ito Y, Seki M, Nishioka Y, et al. Pharmacological effects of Hachi-mi-jio-gan extract (Harncare) on the contractile response and on pharmacologically relevant receptors in the rat bladder. *Yakugaku Zasshi*. 2009;129(8):957–964. PMID: 19652502.
71. Yagi H, Sato R, Nishio K, Arai G, Soh S, Okada H. Clinical efficacy and tolerability of two Japanese traditional herbal medicines, Hachimi-jio-gan and Gosha-jinki-gan, for lower urinary tract symptoms with cold sensitivity. *J Traditional Complement Med*. 2015;5(4):258–261. PMID: 26587398.
72. Yuan Y, Tan YF, Xu P, et al. Izalpinin from fruits of Alpinia oxyphylla with antagonistic activity against the rat bladder contractility. *Afr J Tradit Complement Altern Med*. 2014;11(4):120–125. PMID: 25392590.
73. Gordon D, Groutz A, Ascher-Landsberg J, Lessing JB, David MP, Razz O. Double-blind, placebo-controlled study of magnesium hydroxide for treatment of sensory urgency and detrusor instability: preliminary results. *Br J Obstetr Gynaecol*. 1998;105(6):667–669. PMID: 9647159.
74. Bano F, Barrington JW. Serum calcium and magnesium levels in women with detrusor overactivity. *J Obstetr Gynaecol*. 2005;25(4):371–373. PMID: 160912323.
75. Turmel N, Hentzen C, Chesnel C, Charlannes A, Le Breton F, Amarenco G. [Food, diet and dietetic in treatment of urinary tract dysfunctions. A review. *Prog Urol*. 2017;27(7):395–401. PMID: 28576427.
76. Robinson D, Hanna-Mitchell A, Rantell A, Thiagamoorthy G, Cardozo L. Are we justified in suggesting change to caffeine, alcohol, and carbonated drink intake in lower urinary tract disease? Report from the ICI-RS 2015. *Neurourol Urodyn*. 2017;36(4):876–881. PMID: 28444711.
77. Gleason JL, Richter HE, Redden DT, Goode PS, Burgio KL, Markland AD. Caffeine and urinary incontinence in US women. *Int Urogynecol J*. 2013;24(2):295–302. PMID: 22699886.
78. Mayo Clinic. Available from: https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/caffeine/art-20049372. Accessed 4 August 2018.
79. Davis NJ, Vaughan CP, Johnson 2nd TM, et al. Caffeine intake and its association with urinary incontinence in United States men: results from National Health and Nutrition Examination Surveys 2005-2006 and 2007-2008. *J Urol*. 2013;189(6):2170–2174. PMID: 23276513.
80. Sun S, Liu D, Jiao Z. Coffee and caffeine intake and risk of urinary incontinence: a meta-analysis of observational studies. *BMC Urology*. 2016;16(1):61. PMID: 27716171.
81. Townsend MK, Resnick NM, Grodstein F. Caffeine intake and risk of urinary incontinence progression among women. Obstetrics and Gynecology. 2012;119(5):950–957. PMID: 22525905.
82. Chalker R, Whitmore KE. *Overcoming Bladder Disorders*. New York: Harper Collins; 1990.
83. The John Hopkins Women's Centre for Pelvic Health. Available from: https://www.hopkinsmedicine.org/johns_hopkins_bayview/_docs/medical_services/gynecology_obstetrics/bladder_irritants.pdf. Accessed 3 August 2018.
84. Burgio KL, Newman DK, Rosenberg MT, Sampselle C. Impact of behaviour and lifestyle on bladder health. *Int J Clin Pract*. 2013;67(6):495–504. PMID: 23679903.
85. Kirby M, Chapple C, Jackson G, et al. Erectile dysfunction and lower urinary tract symptoms: a consensus on the importance of co-diagnosis. *Int J Clin Pract*. 2013;67(7):606–618. PMID: 23617950.
86. Lightner DJ, Krambeck AE, Jacobson DJ, et al. Nocturia is associated with an increased risk of coronary heart disease and death. *BJU Int*. 2012;110(6):848–853. PMID: 22233166.
87. Freeman RM, Baxby K. Hypnotherapy for incontinence caused by the unstable detrusor. *Br Med J (Clinical Research Ed)*. 1982;284(6332):1831–1834. PMID: 6805716.

88. Baram DA. Hypnosis in reproductive health care: a review and case reports. *Birth (Berkeley, Calif)*. 1995;22(1):37–42. PMID: 7741949.
89. Labrie J, Berghmans BL, Fischer K, et al. Surgery versus physiotherapy for stress urinary incontinence. *N Eng J Med*. 2013;369(12):1124–1133. PMID: 24047061.
90. Imamura M, Abrams P, Bain C, et al. Systematic review and economic modelling of the effectiveness and cost-effectiveness of non-surgical treatments for women with stress urinary incontinence. *Health Technol Assess*. 2010;14(40):1. PMID: 20738930.
91. Wang Y, Zhishun L, Peng W, Zhao J, Liu B. Acupuncture for stress urinary incontinence in adults. *Cochrane Database Sys Rev*. 2013;7. PMID: 23818069.
92. Zhang W, Tan Z, Cao J, et al. [Acupoints selection rules analysis of ancient acupuncture for urinary incontinence based on data mining technology]. *Zhongguo Zhen Jiu*. 2015;35(12):1299–1303. PMID: 26964186.
93. Su T, Zhou J, Liu Z, et al. The efficacy of electroacupuncture for the treatment of simple female stress urinary incontinence - comparison with pelvic floor muscle training: study protocol for a multicenter randomized controlled trial. *Trials*. 2015;16(1). PMID: 25887231.
94. Smith MD, Russell A, Hodges PW. Is there a relationship between parity, pregnancy, back pain and incontinence? *Int Urogynecol J Pelvic Floor Dysfunction*. 2008;19(2):205–211. PMID: 17665083.
95. Pool-Goudzwaard AL, Slieker ten Hove MC, Vierhout ME, et al. Relations between pregnancy-related low back pain, pelvic floor activity and pelvic floor dysfunction. *Int Urogynecol J Pelvic Floor Dysfunction*. 2005;16(6):468–474. PMID: 15803285.
96. Cuthbert SC, Rosner AL. Conservative chiropractic management of urinary incontinence using applied kinesiology: a retrospective case-series report. *J Chiropract Med*. 2012;11(1):49–57. PMID: 22942842.
97. Cuthbert SC, Rosner AL. Conservative management of post-surgical urinary incontinence in an adolescent using applied kinesiology: a case report. *Alter Med Rev*. 2011;16(2):164–171. PMID: 21649458.

Macular Degeneration

John Nowicki, ND, and *Michael T. Murray, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Progressive visual loss occurs due to degeneration of the macula.
- Ophthalmological examination may show spots of pigment near the macula and blurring of the macular borders.

GENERAL CONSIDERATIONS

The *macula,* the area of the retina where most images focus, is the portion of the eye responsible for fine vision. Degeneration of the macula is the leading cause of severe visual loss in the United States and Europe in persons 55 years and older and is second only to cataracts as a cause of decreased vision in persons older than 65 years. It is estimated that more than 150,000 Americans are legally blind from age-related macular degeneration (ARMD), with 20,000 new cases occurring each year.[1,2]

Types of Macular Degeneration

The two most common types of ARMD are the atrophic (dry) form, by far the more common, and the neovascular (wet) form. In either form, patients may experience blurred vision. The patient may note that straight objects appear distorted or bent; the presence of a dark spot near or around the center of the visual field; and, while reading, that parts of words are missing.

Dry Age-Related Macular Degeneration

Between 80% and 95% of people with ARMD have the dry form of the disease.

The primary lesions are atrophic changes in the retinal pigmented epithelium (RPE), which constitutes the innermost layer of the retina. Beginning in early life and continuing throughout life, cells of the RPE gradually accumulate sacs of cellular debris (lipofuscin). The lipofuscin bodies are either remnants of incompletely degraded abnormal molecules from damaged RPE cells or derivatives of phagocytized rod and cone membranes. Progressive engorgement of the RPE cells with lipofuscin is associated with the extrusion of tissue components like hyaline, sialomucin, and cerebroside. The hallmark excrescences beneath the RPE apparent on ophthalmoscopic examination are referred to as drusen. The formation of drusen is a result of free-radical damage similar to the type of damage that induces cataracts (see Chapter 219). However, decreases in nutrient, blood, and oxygen supply to the retina are the harbinger of the development of drusen and subsequent macular degeneration.

The presence of complement and other inflammatory mediators in drusen, along with the identification of ARMD-susceptibility genes encoding complement factors, supports the concept that local inflammation and immune-mediated processes play a key role in ARMD pathogenesis. This may be further accelerated through systemic immune activation, and increased levels of C-reactive protein (CRP) have been associated with a higher risk of ARMD. Aside from being an inflammatory risk marker, CRP may also play a role in the progression of the disease because it has been identified in drusen.[3] Oxidative stress and inflammation in the microenvironment of the local circulation certainly contribute to the disease process. However, conditions such as ARMD may also be indicative of a more systemic disease process.

Although identified nearly three decades ago, reticular psuedodrusen (RPD) have been recently recognized as a distinctive phenotype and are strongly associated with late ARMD. Unlike drusen, RPD are located in the subretinal space. Identification is not straightforward at fundus examination, and at least two different imaging modalities (i.e., spectral-domain optical coherence tomography [SD-OCT], infrared [IR] reflectivity, multicolor imaging) should be considered for more accurate diagnosis.[4] Although ARMD and RPD share several nonocular risk factors, such as older age, female sex, current smoking, and high body mass index (BMI), other reported risk factors for RPD included less education, B–vitamin complex use, history of steroid eye drop use, and glaucoma.[5]

The disease progresses slowly, and only central vision is lost; peripheral vision remains intact. Although it is rare for anyone to become blind from dry ARMD, in the late stages of ARMD, there is excessive recruitment of scar tissue that leads to irreversible destruction of photoreceptors. Currently, there is no standard medical treatment for this common form of ARMD, although the use of nutritional supplements designed to address the underlying oxidative damage is becoming the "unofficial" standard of care.

Wet Age-Related Macular Degeneration

Wet ARMD is also known as the neovascular form or advanced ARMD. It affects 5% to 20% of persons with ARMD. Wet ARMD is characterized by the growth of abnormal blood vessels. A common early symptom of wet ARMD is straight lines that appear wavy. Because the disease can rapidly progress to a point at which surgery cannot be used, treatment should begin as soon as possible.

Wet ARMD can be treated quite effectively in the early stages with laser photocoagulation therapy. Photodynamic therapy using photosensitive drugs (verteporfin) and a low-powered laser or low-dose radiation therapy are also alternatives.[6,7] Antiangiogenics or anti–vascular endothelial growth factor (anti-VEGF) agents are also used. These drugs can cause regression of the abnormal blood vessels and improvement of vision when injected directly into the vitreous humor of the eye. The injections have to be repeated on a monthly or bimonthly basis. Examples of these agents include ranibizumab (Lucentis), bevacizumab (Avastin), and pegaptanib (Macugen).[1,2]

THERAPEUTIC CONSIDERATIONS

The primary treatment goal of the dry form and prevention of the wet form of ARMD involve the use of antioxidants and natural substances that correct the underlying pathophysiology—free-radical damage and poor oxygenation of the macula. This can be accomplished by reducing the risk factors for ARMD, focusing on preventive factors against atherosclerosis, increasing dietary intake of fresh fruits and vegetables, and supplementing with nutritional and botanical antioxidants.

In particular, tobacco smoking greatly increases the risk of ARMD.[8] Smoking a pack of cigarettes a day for any significant length of time increases the risk for ARMD by two to three times compared with someone who has never smoked.[8,9] This risk does not return to the control rate until a person has stopped smoking for 15 years.[9]

There is also a strong genetic component to consider. Although a number of genetic markers have been identified, a family history may be the most convenient screening method. The lifetime risk of developing late-stage macular degeneration is 50% for people who have a relative with macular degeneration versus 12% for people who do not, a fourfold higher risk. Although genomic family scans of 364 families have revealed a region of chromosome 10 as possibly containing an association with ARMD,[10] diversity in phenotype and the late onset of disease complicate the feasibility of linkage studies. Interestingly, a higher birth weight and a lower ratio of head circumference to birth weight are associated with significantly higher risk.[11]

Reducing and Preventing Atherosclerosis

Although atherosclerosis is now a well-accepted risk factor for macular degeneration, this association was unconfirmed until 1995.[12] In the confirmation study, 104 subjects with macular degeneration and 1324 subjects without macular degeneration were evaluated for atherosclerosis via ultrasonographic determination of the intimal-medial thickness, assessed for the presence of atherosclerotic plaque in the carotid arteries, and measured for the ankle/arm systolic blood pressure ratio (an indicator of peripheral atherosclerosis). The results indicated that in subjects younger than 85 years, plaques in the carotid bifurcation were associated with a 4.7-times-higher prevalence of macular degeneration. Lower-extremity atherosclerosis was associated with a 2.5-times-greater risk. These results indicated that measures designed to reduce the risk of atherosclerosis are of great significance in the prevention and treatment of macular degeneration (see Chapter 149 for a comprehensive program for prevention and reversal).

Diet

A diet rich in fruits and vegetables is associated with a lower risk for ARMD. This protection is the result of a greater intake of antioxidant vitamins and minerals.[13–15] However, various food components, such as flavonoids and the non–provitamin A carotenes lutein, zeaxanthin, and lycopene, may be more significant in protecting against ARMD than traditional nutritional antioxidants (e.g., vitamins C and E, zinc, and selenium). The macula, especially its central portion, the fovea, owes its yellow color to its high concentration of lutein and zeaxanthin. These yellow carotenoids function in preventing oxidative damage to the area of the retina responsible for fine vision and have a central role in protecting against the development of macular degeneration.[16,17]

The carotene lycopene, a component of tomatoes and other red fruits and vegetables, is also protective. In one study, individuals with lycopene content in the lowest quintile were twice as likely to have ARMD.[15] In addition, moderate wine consumption is associated with a decreased risk of ARMD.[18] Red wine contains anthocyanins, which are powerful antioxidants and likely responsible for the protective effect. It is important to note that beer consumption increased the accumulation of drusen and the risk of exudative macular disease and therefore should be avoided.[19]

Similar to atherosclerosis, the type of dietary fat appears to play a role in ARMD. A cohort study of 261 individuals with early or intermediate stages of ARMD revealed a twofold increased risk of progression with a high intake of animal fats and processed baked goods. In contrast, increased intake of fish and nuts was associated with a lower risk of ARMD progression.[20] The intake of long-chain omega-3 fatty acids reduces the incidence of ARMD and was shown to be inversely associated with the 12-year progression to ARMD.[21–23]

Nutritional Supplements

In addition to recommending a diet high in antioxidants, supplementation with nutritional antioxidants (e.g., vitamin C, selenium, beta-carotene, and vitamin E) is certainly important in the treatment and prevention of macular degeneration. The Age-Related Eye Disease Study (AREDS) research group confirmed that a combination of these nutrients will likely produce better results than any single nutrient alone.[24] The specific daily amounts of antioxidants and zinc used by the study researchers were 500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene (often labeled as equivalent to 25,000 IU of vitamin A), 80 mg of zinc as zinc oxide, and 2 mg of copper as cupric oxide. It was further shown that a deficiency in any one of these antioxidants alone does not account for the impaired antioxidant status in ARMD. Instead, the lower antioxidant status reflects decreases in a combination of nutrients.[10] The Age-Related Eye Disease Study 2 assessed the value of substituting lutein/zeaxanthin in the AREDS formulation and found that participants taking lutein/zeaxanthin had a slightly lower progression rate to late ARMD than participants not taking lutein/zeaxanthin.[25] In fact, the totality of evidence on beneficial and adverse effects suggests that lutein/zeaxanthin could be more appropriate than beta-carotene in the AREDS-type supplements. A 2017 Cochrane review assessed the effects of antioxidant vitamin or mineral supplementation on the progression of ARMD in people with AMD.[26] The authors included 19 randomized controlled trials (RCTs) conducted in the United States, Europe, China, and Australia. Compared with placebo or no intervention, it was concluded that individuals with ARMD may experience some delay in progression of the disease with the use of antioxidant vitamin or mineral supplementation (alone or in combination).

In addition to the AREDS research, several studies using various commercially available broad-based antioxidant formulas have shown promising results. For example, a 1.5-year study demonstrated that the

progression of dry ARMD could be halted (but not reversed) with a broad-spectrum 14-component antioxidant capsule (Ocuguard).[27] A retrospective study of a nutritional supplement called ICAPS Plus (which contains beta-carotene, vitamins C and E, zinc, copper, manganese, selenium, and riboflavin) compared 38 patients who used the preparation regularly with 37 patients who used only one bottle and who served as controls.[28] Of the treated patients, 15 showed improvement in their vision by one line or more on a vision acuity chart, compared with only 6 in the control group. In addition, 3 of the 38 in the treatment group lost one line or more of vision, compared with 13 in the control group. In a second prospective blinded clinical trial reported in the same review, vision and contrast sensitivity were evaluated. After 6 months, visual acuity was the same or better in 36 of 61 controls compared with 168 of 192 treated patients.

In a randomized, double-blind, placebo-controlled trial, 5442 female health care professionals 40 years of age and older, with preexisting cardiovascular disease or three or more cardiovascular disease risk factors, randomly received a combination of folic acid (2.5 mg/day), pyridoxine hydrochloride (50 mg/day), and cyanocobalamin (1 mg/day) or placebo.[29] After an average of 7.3 years of treatment and follow-up, there were 55 cases of ARMD in the combination treatment group and 82 in the placebo group. For visually significant ARMD, there were 26 cases in the combination treatment group and 44 in the placebo group. These results indicate a 34% and 41% reduced relative risk, respectively.

Xanthophylls (Lutein, Zeaxanthin)

Several studies have confirmed the benefit of supplementing the diet with xanthophylls (e.g., lutein, zeaxanthin). A meta-analysis of 20 RCTs involving 938 patients with ARMD and 826 healthy subjects revealed a dose–response relationship between supplementation with lutein, zeaxanthin, and meso-zeaxanthin and improved macular pigment optical density (MPOD) both in patients with ARMD and healthy subjects.[30] A 12-month double-blind study, the Lutein Antioxidant Supplementation Trial, sought to determine whether nutritional supplementation with lutein or lutein together with antioxidants, vitamins, and minerals improved visual function and symptoms in atrophic ARMD.[31] Patients receiving lutein (10 mg) alone or in combination with a broad-spectrum vitamin and mineral supplement showed improvements in mean eye macular pigment optical density, Snellen-equivalent visual acuity, and contrast sensitivity. Patients who received the placebo, however, showed no significant changes in any of the measured findings.

A prospective randomized interventional study evaluated if the addition of epilutein would augment MPOD in 40 patients with ARMD.[32] Subjects were divided into two groups. Group 1 received daily oral administration of 8 mg epilutein in combination with 2 mg lutein, whereas group 2 received 10 mg of lutein for 2 months. In patients with early-stage ARMD, the administration of lutein in combination with epilutein was associated with an increase MPOD compared with the administration of lutein alone.

In a study using a comprehensive formula, 27 patients with nonadvanced ARMD and visual acuity at or equal to 0.2 logarithm of the minimum angle of resolution were enrolled and randomly divided into two age-similar groups.[33] Fifteen patients added an oral supplement containing vitamin C (180 mg), vitamin E (30 mg), zinc (22.5 mg), copper (1 mg), lutein (10 mg), zeaxanthin (1 mg), and astaxanthin (4 mg) daily for 12 months, whereas 12 patients had no dietary supplementation during the same period. Based on multifocal electroretinogram response-amplitude densities and visual acuity assessments, the results clearly indicate that early-stage ARMD can respond positively to supplementation with carotenoids and antioxidants.

See Chapter 57 for more information on lutein, zeaxanthin, and astaxanthin.

Zinc

Zinc plays an essential role in the metabolism of the retina, and the elderly are at high risk for zinc deficiency. In addition to the studies with a combination of nutrients, a 2-year prospective, randomized, double-blind, placebo-controlled trial involving 151 subjects with dry ARMD demonstrated that the group taking 200 mg/day of zinc sulfate (approximately 80 mg of elemental zinc) had significantly less visual loss than the placebo group.[34] This initial report was one of the key prompts of the AREDS group to further evaluate whether antioxidant or zinc supplements delay age-related macular degeneration.

In another study using a zinc-monocysteine (ZMC) supplement, 40 subjects with ARMD were randomized to receive either ZMC 25 mg or placebo twice daily for 6 months.[35] At the end of the study, the ZMC group showed improved visual acuity and contrast sensitivity. Macular light-flash recovery time shortened in the ZMC group at 3 months by 2.1 to 3.6 s and at 6 months by 7.2 to 7.4 s. No improvement occurred in the placebo group. ZMC was well tolerated, with a gastrointestinal irritation rate of less than 2%.

It should be noted that a double-blind study in 112 patients with wet ARMD failed to show any benefit with zinc supplementation.[36]

Flavonoid-Rich Extracts

Flavonoid-rich extracts of bilberry *(Vaccinium myrtillus), Ginkgo biloba,* or grape seed *(Vitis vinifera)* offer significant benefits in the prevention and treatment of ARMD. In addition to possessing excellent antioxidant activity, all three extracts have been shown to exert positive effects on retinal blood flow and function. Clinical studies in humans have demonstrated that all three are also capable of halting the progressive visual loss of dry ARMD and possibly even improving visual function.[37–40] Of the three extracts, bilberry extracts standardized to contain 25% anthocyanidins appear to be the most beneficial. Studies of animals with visible light-induced retinal degeneration demonstrate that bilberry anthocyanin extracts at appropriate dosages significantly inhibited retinal dysfunction; increased the levels of superoxide dismutase, glutathione peroxidase, and catalase; suppressed lipid peroxidation and proinflammatory cytokines; and attenuated the changes caused by light to certain apoptotic proteins.[41] The anthocyanosides of *V. myrtillus* have a strong affinity for the retinal pigmented epithelium that constitutes the optical or functional portion of the retina, reinforcing the collagen structures of the retina and preventing free-radical damage. Extracts of anthocyanins from blueberries inhibit the induction and progression of ARMD through antioxidant mechanisms (e.g., decreasing levels of malondialdehyde and increasing the levels of superoxide dismutase, catalase, and glutathione peroxidase) as well as decreasing the levels of vascular-endothelial cell-growth factor in human retinal epithelial cells.[42] In addition, proanthocyanidins of Japanese horse chestnut seed shells *(Aesculus turbinata)* also inhibit oxidative stress and apoptosis, resulting in a protective effect on the retina from diseases such as ARMD.[43] Because the retinal pigmented epithelium is the portion of the eye affected in ARMD, anthocyanosides appear to be ideal therapeutic agents for the disorder.

G. biloba extract with a 24% ginkgo flavoglycoside content is perhaps a better choice if the patient is also showing signs of cerebrovascular insufficiency, and grape seed extract may be the most useful in the patient with significant photophobia or poor night vision.

Omega-3 Polyunsaturated Fatty Acids

Numerous epidemiological studies have demonstrated that omega-3 polyunsaturated fatty acids (PUFAs) are associated with a decreased

risk of ARMD. Omega-3 PUFAs have structural, functional, and neuroprotective roles and may favor the retinal accumulation of lutein and zeaxanthin and thus increase MPOD. A double-blind, placebo-controlled, prospective randomized clinical trial performed in 120 patients examined the associations of MPOD with plasma omega-3 PUFAs in subjects with a family history of ARMD.[44] The subjects included in the study were aged 40 to 70, had at least one parent treated for ARMD, had no major eye conditions, and had no use of supplements containing lutein or zeaxanthin the preceding year. After multivariate adjustment, high MPOD was significantly associated with higher plasma levels of omega-3 docosapentanoic acid. However, a Cochrane review found that omega-3 long-chain PUFA supplementation in people with ARMD for periods up to 5 years does not reduce the risk of progression to advanced ARMD or the development of moderate to severe visual loss.[45]

Exercise

Although exercise may not play a role in reversing or directly preventing ARMD, patients with visual impairment should consider strength and balance training to minimize the risk of falling.[21]

THERAPEUTIC APPROACH

As with most diseases, prevention or treatment of ARMD at an early stage is most effective. The treatment of the wet form is laser photocoagulation soon after diagnosis. Because free-radical damage and a lack of blood and oxygen supply to the macula appear to be the primary causes of macular degeneration, the consumption of antioxidant supplements and promotion of retinal blood flow are the keys to effective treatment.

The use of nutritional supplementation in ARMD has undergone extensive cost–benefit analysis. Compared with no therapy, antioxidant therapy yielded a cost-effectiveness ratio of $21,387 per quality-adjusted life-year (i.e., it lowered medical costs by $21,387 per quality-adjusted life-year gained) and lowered the percentage of patients with ARMD who developed visual impairment in the better-seeing eye from 7.0% to 5.6%.[46]

Diet

Foods to avoid in ARMD:

- Fried and grilled foods and other sources of free radicals
- Animal fat
- Processed baked goods
- Beer

Foods to add to the diet of a patient with ARMD:

- Yellow vegetables, green vegetables, tomato products (carotenes)
- Flavonoid-rich berries (blueberries, blackberries, cherries, etc.)
- Fresh fruits and vegetables, nuts, and fish
- Moderate wine consumption (advisable in those without contraindications to alcoholic beverages)

Supplements

- Vitamin C: 500 to 1000 mg/day
- Vitamin E (mixed tocopherols): 200 to 400 IU/day
- Selenium: 200 to 400 mcg/day
- Folic acid: 800 mcg/day
- Pyridoxine hydrochloride: 50 mg/day
- Vitamin B_{12}: 800 mcg/day
- Lutein: 10 to 15 mg/day
- Zeaxanthin: 1 to 2 mg/day
- Astaxanthin: 4 to 6 mg/day

Botanical Medicines

One of the following extracts may be selected:

- *G. biloba* extract (24% ginkgo heterosides): 120 to 240 mg/day
- *V. myrtillus* (bilberry) extract (25% anthocyanidin content): 120 to 240 mg/day
- Grape seed extract (95% procyanidolic content): 150 to 300 mg/day

Exercise

Balance training and strength exercises are recommended, especially for patients who are visually impaired.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. de Jong PT. Age-related macular degeneration. *N Engl J Med*. 2006;355(14):1474–1485.
2. Kaufman SR. Developments in age-related macular degeneration: diagnosis and treatment. *Geriatrics*. 2009;64(3):16–19.
3. Molins B, Romero-Vazquez S, Fuentes-Prior P, Adan A, Dick AD. C-reactive protein as a therapeutic target in age-related macular degeneration. *Front Immunol*. 2018;9:808. PubMed PMID: 29725335.
4. Sivaprasad S, Bird A, Nitiahpapand R, et al. Perspectives on reticular pseudodrusen in age-related macular degeneration. *Survey Ophthalmol*. 2016;61(5):521–537. PubMed PMID: 26994868.
5. Rabiolo A, Sacconi R, Cicinelli MV, Querques L, Bandello F, Querques G. Spotlight on reticular psuedodrusen. *Clin Ophthalmol (Auckland, N.Z.)*. 2017;11:1707–1718. PubMed PMID: 29033536.
6. Meads C, Salas C, Roberts T, et al. Clinical effectiveness and cost-utility of photodynamic therapy for wet age-related macular degeneration: a systematic review and economic evaluation. *Health Technol Assess*. 2003;7:1–98.
7. Treatment of Age-related Macular Degeneration with Low-dose Radiation Therapy Study Group. The results of randomized controlled trial of low-dose radiation for wet-type age-related macular degeneration on a 1 year term basis. *Jpn J Ophthalmol*. 2003;47:626–627.
8. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol*. 2010:10. http://dx-.doi.org/10.1186/1471-2415-10-31.
9. Seddon JM, Willett WC, Speizer FE. A prospective study of cigarette smoking and age-related macular degeneration in women. *JAMA*. 1996;276:1141–1146.
10. Chung M, Lotery AJ. Genetics update of macular diseases. *Ophthalmol Clin North Am*. 2002;15:459–465.
11. Hall NF, Gale CR, Syddall H, et al. Relation between size at birth and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2002;43:3641–3645.
12. Vinderling JR, Dielemans I, Bots ML. Age-related macular degeneration is associated with atherosclerosis. The Rotterdam study. *Am J Epidemiol*. 1995;142:404–409.
13. Antioxidant status and neovascular age-related macular degeneration. Eye Disease Case-Control Study Group. *Arch Ophthalmol*. 1993;111:104–109.
14. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr*. 1995;62:1448S–1461S.
15. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol*. 1995;113:1518–1523.
16. Landrum JT, Bone RA, Kilburn MD. The macular pigment: a possible role in protection from age-related macular degeneration. *Adv Pharmacol*. 1997;38:537–556.
17. Carpentier S, Knaus M, Suh M. Associations between lutein, zeaxanthin, and age-related macular degeneration: an overview. *Crit Rev Food Sci Nutr*. 2009;49(4):313–326.
18. Obisesan TO, Hirsch R, Kosoko O, et al. Moderate wine consumption is associated with decreased odds of developing age-related macular degeneration in NHANES-1. *J Am Geriatr Soc*. 1998;46:1–7.
19. Ritter LL, Klein R, Klein BE, et al. Alcohol use and age-related maculopathy in the beaver dam eye study. *Am J Ophthalmol*. 1995;120:190–196.
20. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. *Arch Ophthalmol*. 2003;121:1728–1737.
21. Singh MA. Exercise to prevent and treat functional disability. *Clin Geriatr Med*. 2002;18:431–462.
22. SanGiovanni JP, Chew EY, Agrón E, et al. The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS report no. 23. *Arch Ophthalmol*. 2008;126(9):1274–1279.
23. SanGiovanni JP, Agrón E, Clemons TE, et al. Omega-3 long-chain polyunsaturated fatty acid intake inversely associated with 12-year progression to advanced age-related macular degeneration. *Arch Ophthalmol*. 2009;127(1):110–112.
24. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss. AREDS Research Group. *Arch Ophthalmol*. 2001;119:1417–1436.
25. Age-Related Eye Disease Study 2 (AREDS) Research Group. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No.3. *JAMA Opthalmology*. 2014;132(2):142–149. PubMed PMID: 24310343.
26. Evans JR, Lawrenson JG. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. *Cochrane Database Syst Rev*. 2017;7:CD000254. PubMed PMID: 28756618.
27. Richer S. Multicenter ophthalmic and nutritional age-related macular degeneration study. Part 1: design, subjects and procedures and Part 2: antioxidant intervention and conclusions. *J Am Optom Assoc*. 1996;67:12–49.
28. Bartlett H, Eperjesi F. Age-related macular degeneration and nutritional supplementation: a review of randomised controlled trials. *Ophthalmic Physiol Opt*. 2003;23:383–399.
29. Christen WG, Glynn RJ, Chew EY, et al. Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the women's antioxidant and folic acid cardiovascular study. *Arch Intern Med*. 2009;169(4):335–341.
30. Ma L, Liu R, Du JH, Liu T, Wu SS, Liu XH. Lutein, zeaxanthin and meso-zeaxanthin supplementation associated with macular pigment optical density. *Nutrients*. 2016;8(7). PubMed PMID: 27420092.
31. Richer S, Stiles W, Statkute L, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*. 2004;75:216–230.
32. Forte R, Panzella L, Cesarano I, Cennamo G, Eidenberger T, Napolitano A. Epilutein for early-stage age-related macular degeneration: a randomized and prospective study. *Ophthalmic Res*. 2017;58(4):231–241. PubMed PMID: 28957818.
33. Parisi V, Tedeschi M, Gallinaro G, et al. Carotenoids and antioxidants in age-related maculopathy Italian study: multifocal electroretinogram modifications after 1 year. *Ophthalmol*. 2008;115(2):324–333. e2. Epub 2007 Aug 22.
34. Newsome DA, Swartz M, Leone NC, et al. Oral zinc in macular degeneration. *Arch Ophthalmol*. 1988;106:192–198.
35. Newsome DA. A randomized, prospective, placebo-controlled clinical trial of a novel zinc-monocysteine compound in age-related macular degeneration. *Curr Eye Res*. 2008;33(7):591–598.
36. Stur M, Tittle M, Reitner A, et al. Oral zinc and the second eye in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1225–1235.
37. Scharrer A, Ober M. Anthocyanosides in the treatment of retinopathies. *Klin Monatsbl Augenheilkd*. 1981;178:386–389.
38. Caselli L. Clinical and electroretinographic study on the activity of anthocyanosides. *Arch Med Int*. 1985;37:29–35.
39. Lebuisson DA, Leroy L, Rigal G. Treatment of senile macular degeneration with Ginkgo biloba extract: a preliminary double-blind, drug vs. placebo study. *Presse Med*. 1986;15:1556–1558.
40. Corbe C, Boisin JP, Siou A. Light vision and chorioretinal circulation: study of the effect of procyanidolic oligomers (Endotelon). *J Fr Ophthalmol*. 1988;11:453–460.
41. Wang Y, Zhao L, Lu F, Yang X, Deng Q, Huang F. Retinoprotective effects of bilberry anthocyanins via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms in a visible light-induced retinal degeneration model in pigmented rabbits. *Mol*. 2015;20(12):22395–22410. PubMed PMID: 26694327.
42. Huang WY, Wu H, Li DJ, et al. Protective effects of blueberry anthocyanins against H2O2-induced oxidative injuries in human retinal pigment epithelial cells. *J Agri Food Chem*. 2018;66(7):1638–1648. PubMed PMID: 29393642.
43. Ishihara T, Kaidzu S, Kimura H, Koyama Y, Matsuoka Y, Ohira A. Protective effect of highly polymeric A-type proanthocyanidins from seed shells of Japanese horse chestnut (Aesculus turbinate BLUME) against light-induced oxidative damage in rat retina. *Nutrients*. 2018;10(5). PubMed PMID: 29748512.

44. Merle BMJ, Buaud B, Korobelnik JF, et al. Plasma long-chain omega-3 polyunsaturated fatty acids and macular pigment in subjects with family history of age-related macular degeneration: the limpia study. *Acta Ophthalmologica*. 2017;95(8):e763–e769. PubMed PMID: 28271618.
45. Lawrenson JG, Evans JR. Omega 3 fatty acids for preventing or slowing the progression of age-related macular degeneration. *Cochrane Database Sys Rev*. 2015;9(4):CD010015. PubMed PMID: 25856365.
46. Rein DB, Saaddine JB, Wittenborn JS, et al. Cost-effectiveness of vitamin therapy for age-related macular degeneration. *Ophthalmology*. 2007;114(7):1319–1326.

Menopause

Tori Hudson, ND,* and *Joseph Katzinger, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Last spontaneous menstrual period occurred 12 months prior.
- Average age at onset is 51 years.
- Symptoms can but do not necessarily include hot flashes, night sweats, palpitations, headaches, insomnia, mood swings, anxiety, vaginal dryness, urinary incontinence, rheumatism, fatigue, hair thinning, skin dryness, acne, facial hair, low libido, bladder infections, vaginal infections, nausea, mild cognitive changes, and irregular bleeding with perimenopause.

The baseline evaluation of the menopausal woman should consist of the following assessments:

- Detailed personal medical history, including an understanding of the chief complaint, review of other systems, medical history, past and present medications, family history, dietary habits and history, exercise habits and history, social history, and occupation
- General physical examination
- Breast examination
- Pelvic examination
- Laboratory tests to consider: complete blood count, blood chemistry panel, lipid panel with subfractions, thyroid function panel, follicle-stimulating hormone (FSH), homocysteine, C-reactive protein, and estrogen metabolites
- Screening mammography
- Bone density testing
- Cervical cytology (Pap smear or liquid-based technologies)
- Electrocardiogram
- Colonoscopy

Some of these tests are routinely performed either initially or on a yearly basis. The indications and frequency for other tests are determined by individual health history, disease risks, current health problems, and family history.

*Previous edition contributor

INTRODUCTION

Menopause denotes the cessation of menstruation in a woman, which usually occurs when she reaches the age of 51 years. Twelve months without a spontaneous period is the commonly accepted rule for diagnosing menopause. The time before menopause is referred to as the *menopausal transition,* whereas the time after menopause is referred to as *postmenopausal.* Per the 2012 Staging of Reproductive Aging Workshop (STRAW) classifications, *perimenopause* includes the menopausal transition as well as the first year following the final menstrual period.[1] During the perimenopausal period, women ovulate irregularly, infrequently, or not at all; therefore, they begin to experience changes in the menstrual cycle with or without other symptoms.

With the prolongation of life expectancy, the menopausal and postmenopausal periods are becoming more significant in a woman's life. In fact, today's average woman can expect to live at least one-third or more of her life in the postmenopausal stage.

In 2015, nearly 50% of the women in the United States were postmenopausal, and by 2025, the number of postmenopausal women globally will reach 1.1 billion. Between 1990 and 2020, the menopausal population in the country will double. This dramatic rise in the number of menopausal women is changing the way health care providers work with women and changing medicine itself. At no other time in history have so many individuals been dealing with the same set of health issues. Now more than ever, clinicians have options for the management of menopause.

Current conventional medical treatment of menopause primarily involves short-term (1–4 years) hormonal therapy (HT) for the primary indication of moderate to severe vasomotor symptoms, using estrogen with or without a progestogen. The obvious question is, "Is hormone replacement therapy necessary?" The goal of this chapter is to answer this question and offer an approach to perimenopause and menopause that provides many different options.

GENERAL CONSIDERATIONS

The goals of an integrative medicine approach to menopause are to provide relief from common menopausal symptoms and to prevent and/or treat osteoporosis, cardiovascular disease, and other diseases of aging while minimizing the risks of breast cancer, coronary heart disease (CHD), venous thromboembolism, strokes, and gallbladder disease. The evaluation process reveals a woman's perimenopausal/menopausal symptoms, other acute and chronic health problems, health habits, mental/emotional stressors, and risks for future diseases.

One of the most complicated and difficult health care decisions that menopausal women face today is whether to use HT. Women look to their health care providers for definitive answers to these questions. Practitioners are faced with an even greater challenge: evaluating the benefits and risks of conventional hormone therapy (cHT) and natural hormone therapy (nHT) for an individual patient.

In the year 2000, the peak of HT usage, some 46 million prescriptions for conjugated estrogen (Premarin) were written in the United States. An additional 22.3 million prescriptions were written for the same preparation with medroxyprogesterone (Prempro). Almost one in three women given a prescription for cHT never has it filled. Of those who do start cHT, the majority discontinue its use shortly after starting therapy. And after the initial results of the Women's Health Initiative (WHI) were published, most women opted to discontinue or avoid starting HT—in 1999, 13.5% of women age 45 to 64 were taking HT (23.2% of 57-year-old women), but this declined dramatically to 2.7% in the year 2010.[2] Reasons given for discontinuing the drug include uterine bleeding; side effects, including mood changes, breast tenderness, bloating, and weight gain; a fear of breast cancer; and not understanding or believing in the need for its long-term use. Lack of compliance can also be attributed to inadequate education of many healthcare practitioners regarding the needs of menopausal women, both gynecological and nongynecological. Many practitioners also have a limited understanding of the many therapeutic options for menopausal symptoms and of the more global issues of menopause. In addition, holistic menopause practitioners who use both cHT and nHT hold that compliance improves with the use of nHT and that many side effects disappear or improve when patients switch from cHT to nHT.

Sadly, many menopausal women have a limited understanding of the long-term health risks associated with this change in hormone status, and very few women believe that they are well informed about the benefits and risks of cHT or nHT. Each woman wants to know whether HT is right for her—how she may benefit, how long she will have to take the drug to receive those benefits, and what the short-term side effects and potential long-term negative effects are.

Scientific evidence regarding the use of postmenopausal HT comes in many shapes: observational studies, large-scale randomized trials, small clinical trials, biological plausibility, in vitro studies, and animal studies. Other factors involved in the hormone conundrum are theoretical questions, areas of scientific uncertainty, popular consumer opinions and fears, and history. Definitive evidence for the benefits and risks of HT has been clarified by the two Heart and Estrogen/Progestin Replacement Studies (HERS I and HERS II), the Women's Health Initiative (WHI), and the Early versus Late Intervention Trial with Estradiol (ELITE). Another large-scale trial conducted in 14 countries, the Women's International Study of Long Duration Oestrogen after Menopause (WISDOM), was discontinued in November 2004 for scientific and practical reasons. Because WISDOM was to be completed after the publication of WHI results, "there were no safety concerns for the 5700 women enrolled in the study. However, the trial was not expected to provide substantial evidence that would have an impact on clinical practice decisions in the next 10 years."[3]

The North American Menopause Society (NAMS) has a very comprehensive position statement, last updated in 2017.[4,5] In summary, this document says that although there are many benefits (with regard to vasomotor symptoms, urogenital atrophy, urinary health, osteoporosis, and reduction in the onset of type 2 diabetes mellitus), there are also slight statistical risks for some women (of stroke, venous thromboembolism, dementia, and coronary heart disease). What has emerged from this position statement, as well as others, is that *the timing of HT initiation may be very important,* the so-called "hormone-timing" or "timing" hypothesis. Specifically, younger women (those <59) may have a very favorable risk–benefit profile with HT use, including a potential reduction in mortality and cardiovascular risk, whereas women aged 60+ may see an increase in risk. Thus the NAMS current position concludes, "For women aged younger than 60 years or who are within 10 years of menopause onset and have no contraindications, the benefit-risk ratio appears favorable for treatment of bothersome VMS (vasomotor symptoms) and for those at elevated risk of bone loss or fracture. Longer duration may be more favorable for ET (estrogen therapy) than for EPT (estrogen-progestogen therapy), based on the WHI RCTs." Indeed, the ELITE trial found that oral estradiol therapy was associated with less progression of subclinical atherosclerosis (determined by carotid artery intima-media thickness) than placebo *when initiated within 6 years after menopause,* but not when initiated 10 or more years after.[6]

The bottom line is that women and their health care practitioners are faced with trying to make the best decisions possible on the basis of what we do know, what we do not know, and what we are still uncertain about.

Benefits

Symptoms

The decline of endogenous estrogen leads to multiple tissue and organ changes and problems. In addition to the reproductive and urinary tracts, estrogen-sensitive tissues include the skin, bone, vascular lining, the teeth and gums, the eyes, the brain, and the central nervous system.

Some 50% to 80% of women in the United States report menopause-related hot flashes, night sweats, vaginal dryness, insomnia, mood swings, and depression. During the first 5 to 10 years of menopause, vulvovaginal symptoms begin to appear. Later, as the other mucous membranes of the urogenital tract become affected, rates of incontinence and infection rise. Vasomotor symptoms persisted an average of 7.4 years in the Study of Women's Health Across the Nation and have subsequently been found to be associated with cardiovascular, bone, and cognitive risks.[7–9]

There is strong evidence from randomized clinical trials that estrogen therapy is very effective in controlling vasomotor and genitourinary symptoms. Vaginal estrogen is as efficacious as oral or transdermal estrogen for genitourinary symptoms and may be an advantageous method because of the local delivery and effect, and even at a low dose (e.g., 7.5 ug vaginal ring), it appears to be effective for managing symptoms of vulvovaginal atrophy.[10] Lower doses of HT (oral conjugated equine estrogens [CEE] 0.3 mg; oral 17b-estradiol ≤0.5 mg; or estradiol patch 0.025 mg) may take 6 to 8 weeks to provide symptom relief but may also lower the incidence of adverse effects, such as breast tenderness or unscheduled vaginal bleeding.[5]

Sex steroids also affect sleep, libido, cognitive function, motor coordination, and pain sensitivity. Data are mixed regarding the role of menopause in depression and mood swings; however, depression and mood disorders appear to be more common or at least exacerbated in perimenopause or early menopause compared with the reproductive period, and HT benefits many women. Although a 2017 systematic review found no benefit of bioidentical hormone therapy for

menopausal-related depression, longer exposure to exogenous estrogen does appear to have a protective effect, and it may be that estradiol (either as bioidentical or conventional) has an antidepressant effect in perimenopausal but not postmenopausal depressed women, although this is not clearly established.[11–13]

The NAMS and its advisory panel published a post-WHI report and recommendations on postmenopausal HT. One of its basic recommendations is that the treatment of menopausal symptoms (especially vasomotor and urogenital) remains the primary indication for HT or estrogen replacement therapy (ERT). The NAMS 2017 position paper also cites the U.S. Food and Drug Administration (FDA) approval of HT for premature hypoestrogenism as well as for genitourinary symptoms.

Osteoporosis

Estrogen is known to inhibit the age-related bone loss that occurs in most menopausal women, and both observational and controlled trials are in agreement about the benefit of HT for the prevention and treatment of osteoporosis. Observational studies have indicated that the use of estrogen reduces the risk of vertebral fracture by approximately 50% and the risk of hip fracture by 25% to 30%.[14] A systematic review and meta-analysis of randomized controlled trials published in 2016 found that the overall relative risk of HT was 0.74 for total fractures, 0.72 for hip fractures, and 0.63 for vertebral fractures. Again, the benefit was more apparent among women younger than 60, and there was a greater decrease in fracture risk with estradiol compared with conjugated equine estrogens.[15] Estrogen had been considered the therapeutic agent of choice for both the prevention and treatment of postmenopausal osteoporosis in women for many years—until the WHI results, which shifted the perception of benefit versus risk. Estrogen has been the most studied agent for prevention and has been shown to decrease bone resorption, prevent osteoporosis, and reduce fractures. Nevertheless, the FDA has removed estrogen from the list of agents approved as effective in treating women who already have osteoporosis (although in 2013 it approved Duavee, a combination of conjugated estrogens with bazedoxifene, for use in postmenopausal women with an intact uterus for the prevention of osteoporosis and for the treatment of moderate to severe vasomotor symptoms).[16] The decision to remove estrogen from the approved list had nothing to do with any new discovery of a decrease in effectiveness for this indication; rather, it arose as an issue of fairness in new standards that have been applied to approving other drugs for osteoporosis treatment, such as the bisphosphonates (risedronate and alendronate). These agents have involved prospective fracture reduction studies on women with low bone mass and/or fracture and were not associated with the same perception of adverse effects demonstrated in the WHI.[17]

That being said, the WHI was a large clinical trial in which 16,608 postmenopausal women 50 to 79 years of age were studied at 40 U.S. clinical centers.[18] The regimen used was continuous combined estrogen–progestogen therapy (CCEPT) (e.g., Prempro). Women in the CCEPT group experienced lower rates of hip fracture (10 per 10,000 person-years vs. 15 per 10,000 person-years in the placebo group, a 34% lower relative risk) and vertebral fracture (9 events per 10,000 women annually vs. 15 events per 10,000 women annually for placebo, also a 34% lower relative risk). Statistically significant fracture reductions were also seen in other osteoporotic fractures (23%) and total fractures (24%).

The different estrogen agents approved for the prevention of osteoporosis and their antiresorptive effects are as follows (with dose-dependent benefits):

- Oral micronized estradiol, 1.0 mg/day
- Conjugated equine estrogens (CEE), 0.625 mg/day
- Ethinyl estradiol, 5 mcg/day
- Transdermal estradiol, 50 mcg/day
- Esterified estrogen, 0.3 mg/day

It is important to keep in mind that some women derive benefits from these doses, some will need higher doses, and others will have adequate bone protection with lower doses. Dual-energy x-ray absorptiometry (DEXA) scans provide the most reliable objective information on the status of bone mineral density.

Colorectal Cancer

Accumulated observational studies suggested that the use of ERT reduced the risk for colorectal cancer as well as the risk of dying from colon cancer.[19] Although not all study findings are consistent, some studies have shown that the risk of developing fatal colon cancer was reduced by 33%[20]; others show as little as an 8% reduction in risk.[21] In the WHI intervention phase, women who received combined estrogen and progestin versus placebo were 38% less likely to develop colorectal cancer; however, further analysis of the WHI data, postintervention data, and analysis of the HERS trial found no strong evidence of a protective effect of CEE alone or CEE and medroxyprogesterone acetate (MPA) on the risk of colorectal cancer.[22]

Risks

Endometrial Cancer

Unopposed estrogen administration is associated with an increase in the risk of endometrial cancer by a factor of 2.3, although this greatly increases to 9.5 among women using unopposed estrogen for more than 10 years, and the risk remains elevated at least 5 years after cessation of estrogen. Vaginal estrogen does not substantially lower this risk.[23] This results in an excess of 46 cases per 10,000 women for women who use unopposed estrogen for at least 10 years.[24] Adding a proved dose and delivery method of a progestogen (progestins or progesterone), which opposes the effects of estrogen on the endometrium, reduces the risk to a minimum and is essential in preventing endometrial hyperplasia and endometrial cancer.

Support for the use of natural hormones, particularly oral micronized progesterone (OMP), came in the form of the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial and its study of the effects of HT on endometrial histology in postmenopausal women.[25] This trial confirmed that daily CEE, 0.625 mg, enhanced the development of endometrial hyperplasia and that combining CEE with cyclic or continuous MPA protected the endometrium from hyperplastic changes. What was new was that the trial also concluded that cyclic OMP (200 mg daily for 12 days per month) also protected the endometrium from the hyperplastic changes associated with estrogen-only therapy. Thus the PEPI was the first clinical trial proving that OMP was as appropriate as MPA for use in the protection of the endometrium. Compounded OMP was already available, but the product Prometrium came on to the market as a result of that study. Unfortunately, data from the E3N (a large French cohort study) suggest that 100 mg OMP used continuously or 200 mg used sequentially may not be adequate; an overall hazard ratio of 1.80 for endometrial cancer was associated with its use (but other progesterone derivatives were not associated with an elevated risk). This increase in risk with OMP compared with other progesterone derivatives was also observed in the European Prospective Investigation into Cancer and Nutrition (a cohort of over 115,000 postmenopausal women), which found an overall hazard ratio of 1.53 with OMP use, compared with 0.84 with other progesterone derivatives. Although other trials have not observed this increased risk with OMP, and a 2016 international panel of experts approved this dosing, this issue needs further evaluation.[23,26–28]

Venous Thromboembolism

Venous thromboembolism (VTE) is an almost expected complication of the postmenopausal use of exogenous hormones. Observational studies have demonstrated that in postmenopausal women who use ERT, the risk of VTE is increased by a factor of 2 to 3.5. These observations are consistent with the data found in HERS I (discussed later). The investigators reported that the risk of thromboembolic events was increased by a factor of 2.7 in the women receiving the estrogen-progestin therapy.[20] HERS II showed that the relative risk (RR) of VTE increased 108% (RR 2.08), and the absolute risk was 0.59% in the HT group compared with 0.29% in the placebo group (59 events vs. 28 events annually per 10,000 women, respectively).[29]

The WHI reported similar results. The relative risk was 2.11 in the HT group, a 111% increased risk. The absolute risk was 34 events in the HT group compared with 16 events in the placebo group per 10,000 women.[20] Owing to the uncommon presence of idiopathic venous thromboembolism in women above 50 years of age, the absolute risk associated with postmenopausal HT is pretty small. For women who are already at risk for thromboembolism or who are older, the absolute risks of HT will be higher. For example, women in the CEE and MPA arm of the WHI aged 50 to 59 had a 2.27 relative risk for VTE, whereas it was 4.28 among those age 60 to 69 and 7.46 among women age 70 to 79.[30] Although no randomized trials have confirmed it, observational studies and clinical findings strongly suggest that the increase in risk with oral ERT may be avoided with transdermal therapy, as found in the Estrogen Thromboembolism Risk (ESTHER) case-control study and two recent nested case-control studies.[31,32]

Coronary Heart Disease

For the past 30 years, practitioners have been advising menopausal women that HT reduces the risk of coronary heart disease (CHD). More than 40 observational studies in the past three decades have suggested that this risk is 35% to 50% lower among women who take estrogen than in women who do not.[33,34] These observational data have been reinforced by additional research demonstrating some of the impact of estrogen on individual factors known to be associated with CHD, thereby also establishing a biologically plausible mechanism for the association of HT and reduced CHD. Randomized trials have shown that estrogen therapy reduces plasma levels of low-density-lipoprotein cholesterol (LDL-C) by 10% to 14% and raises plasma levels of high-density-lipoprotein cholesterol (HDL-C) by 7% to 8%.[20,35] Estrogen has also been shown to reduce Lp(a) lipoprotein, inhibit oxidation of LDL-C, improve endothelial vascular function, reduce fibrinogen, and reduce thickening in the arterial walls.

However, estrogen may have detrimental effects on cardiovascular risk markers, such as by raising triglyceride levels, increasing clotting, and raising levels of C-reactive protein. These detrimental effects of estrogen may override the beneficial effects of estrogen on cardiovascular function and may explain the surprising results of randomized, placebo-controlled trials indicating that HT did not reduce the overall rate of CHD. In HERS II and the WHI, it actually increased the rate of CHD. These new data from randomized trials, compared with the 30 years of observational data and the biologically plausible mechanisms, have rendered recommendations to postmenopausal women about HT more complex and challenging. Both patients and practitioners have questions with no clear-cut answers, and concerns about the benefits and risks of HT are more difficult to sort out.

Heart disease is the leading cause of morbidity and mortality in women, and an understanding of the current state of the evidence and the potential for the cardioprotective effects of HT, as well as the negative cardiovascular effects of HT, is of vital importance to women's health. In this discussion, however, one must not forget the potential cardioprotective effects of nutrition, exercise, stress management, and select nutrients such as niacin, magnesium, and fish oils.

The basic problem with HT and CHD is that the later randomized trials of estrogen among women with preexisting CHD have not confirmed the 40 observational studies of the last 30 years. The first large-scale randomized, double-blind, placebo-controlled trial of HT (a combination of CEEs [Premarin] and CEEs with medroxyprogesterone acetate [Provera]) for the secondary prevention of CHD was HERS I.[20] In a total of 2763 women with established CHD studied at 20 clinical centers throughout the United States, the death rates from coronary causes and nonfatal myocardial infarctions in the hormone group and the placebo group were similar. Perhaps more worrisome was the 50% increase in the risk of CHD events (thromboembolism) during the first year in the women receiving HT. The HERS I data prompted a barrage of questions, as follows:

- Are the HERS data simply wrong?
- Does one clinical trial outweigh the vast array of observational studies?
- Is the pattern of early risk real, and if so, what caused it?
- Is the early risk related to some other factor present in a subgroup of women but not others?
- Were the results related to the hormone regimen used?
- Would the results be different with a natural estradiol and/or a natural progesterone?
- Did the MPA counteract the potential beneficial effects of estrogen?
- Do these HERS I results for secondary prevention have implications for questioning HT's role in primary prevention?

A brief attempt at some answers was made after HERS I: The early risk for increased CHD events in the hormone-treated group has been a pattern seen in subsequent studies—the Puget Sound Group Health Cooperative of Seattle,[36] the Nurses' Health Study,[37] and the early data from WHI.[38] An early risk for blood clots in the legs and lungs, heart attack, and stroke appears to be a real phenomenon in postmenopausal women who begin HT. Two hypotheses to explain this finding are that estrogen has a prothrombotic effect and a proinflammatory effect on the vascular endothelium.

There indeed may be some subgroup of women with a predisposition for the prothrombotic and/or proinflammatory effects of estrogen—for example, women with hyperinsulinemia, hypertension, elevated homocysteine levels, increased C-reactive protein and Lp(a) lipoprotein levels, obesity, and/or elevated LDL-C. Interestingly, in the HERS I trial, differences were observed between hormone-treated women who also took lipid-lowering therapy (statins) and hormone-treated women who did not. The rate of CHD events after 1 year was much lower in the HT-plus-statin group than in those who did not use statins. The same was true for the risk of venous thromboembolic events.

Another controlled trial attempted to address the question of whether estrogen inhibits atherosclerosis. In the placebo-controlled Estrogen Replacement and Atherosclerosis (ERA) trial, neither estrogen alone nor estrogen plus the progestin[39] affected the progression of coronary atherosclerosis. Plasma levels of LDL-C were reduced by 9.4% in the unopposed estrogen group and by 16.5% in the estrogen-plus-MPA group. Both groups had significant increases in HDL-C (18.8% and 14.2%, respectively) compared with the placebo group. The two estrogen groups had a rise in triglyceride levels (6.1% and 10.1%, respectively), but these changes were not significantly different from the levels in the placebo group. The ERA trial provided the first anatomical end point (angiographically determined effects on coronary arteries) in women who had known CAD. The ERA trial supported the overall null effect found by the HERS I trial and also showed that the MPA did not cancel out the beneficial effects of estrogen, as

was suspected in the HERS I trial. These data and other study results indicate that HT does not have a significant impact on the progression of atherosclerosis in women with established heart disease.

Preliminary data from the Papworth Hormone-Replacement Therapy Atherosclerosis Study (PHASE), a small clinical trial in 225 postmenopausal women with angiographically proved CAD, showed no cardiovascular benefits of HT and also possibly a slight increase in the rates of cardiovascular events during the first 2 years of HT.[40] This trial evaluated transdermal natural estradiol alone or with norethindrone, a different progestin.

An interim analysis of the WHI data also suggested that there had been a slight increase in the number of heart attacks, strokes, and thromboembolic events during the first 1 or 2 years in postmenopausal women undergoing HT.[41]

One clinical trial looked at estrogen use in postmenopausal women without clinical coronary disease. The Estrogen in the Prevention of Atherosclerosis Trial (EPAT) was designed to determine whether unopposed natural estradiol reduces the progression of subclinical atherosclerosis.[42] Postmenopausal women with high cholesterol levels but no preexisting cardiovascular disease, diabetes, or smoking history received either oral micronized natural estradiol or placebo and also lipid-lowering medication if serum LDL-C exceeded 160 mg/dL. The women were monitored for 2 years. The investigators evaluated the rate of change in carotid artery intimal-medial thickness. Women in the placebo group had an expected progression in the thickening of the carotid arteries over the 2 years. The women taking estradiol had a small amount of regression of the thickness of the carotid arteries. In women not receiving lipid-lowering therapy, the placebo group had a greater progression, and the estradiol group had no progression of intimal-medial thickness—a dramatic difference. Curiously, in the women who received lipid-lowering drugs, there was no difference in the rate of progression between the women receiving estrogen and those receiving placebo.

On the basis of data from these randomized clinical trials conducted before HERS II and WHI, HT did not appear to reduce the risk of cardiovascular events in postmenopausal women who already had CHD. Then along came the world-shifting news in July 2002, when the results of HERS II and the WHI were published less than a week apart.

HERS II investigated the effects of 0.625 mg of CEE plus 2.5 mg of MPA on the prevention of CHD in older U.S. women (average age 71 years) with preexisting CHD.[29] This study, a continuation of HERS I, was intended to determine whether a trend toward prevention of heart disease would appear if the study were continued longer. Once again, though, the results were not positive for women with heart disease. In HERS II there were no significant decreases in the rates of primary CHD events (or strokes or clots) among women assigned to the HT treatment group who had already had CHD before starting the HT regimen (RR, 1% decreased risk and absolute risk of 3.66% for HT vs. 3.68% for placebo). The investigators concluded that "postmenopausal hormone replacement therapy should not be used to reduce the risk of CHD events in women who already have CHD."[29]

A few days later, the results of the landmark WHI study were published.[18] The WHI investigated the effect of the most common HT regimen in the United States (0.625 mg Premarin plus 2.5 mg Provera, or Prempro) on the incidence of heart disease, breast and colorectal cancers, and fractures in postmenopausal women. The cardiovascular research was intended to investigate the effect of this HT regimen on the prevention of CHD in healthy postmenopausal U.S. women (aged 50–79 years) who do not have CHD. After a mean of 5.2 years of follow-up, the trial was stopped earlier than planned for the women using combined HT (mostly owing to harm from breast cancer incidence). In the WHI, there was a significantly higher risk of CHD annually per 10,000 women: a 29% increase in the relative risk and 37 more events in the HT group compared with 30 events in the placebo group. These findings, showing that estrogen plus progestin does not confer benefit in preventing CHD among women with a uterus, concurs with the HERS findings among women with clinical CHD as well as those of the ERA trial and others. The WHI results extend the findings of the earlier trials to include a wider range of women.

More recent analyses also suggest that the "timing hypothesis" may play a central role in determining risk and currently is the best explanation for the relationship between cardiovascular risk and HT. As mentioned previously, initial data from the WHI suggested an increase in CHD risk, but the WHI was a somewhat older population (mean age 63). Women aged 50 to 59 in the WHI receiving CEE appear to have had a protective benefit on CHD, as well as a 30% statistically significant reduction in overall mortality.[43] A 13-year follow-up of the WHI and a meta-analysis of trials (including the WHI) came to the same conclusion: When given to women near menopause, HT appears to have a cardioprotective effect, whereas it may increase the risk when given to women 60 years or older.[44,45] This timing hypothesis was also supported by data from the ELITE trial, which found oral estradiol to be associated with less progression of coronary atherosclerosis (measured by CIMT) when initiated within 6 years of menopause but not when given 10 years or more after menopause.[6]

Stroke

In the WHI, there was no excess risk of stroke in the estrogen-plus-progestin group in the first year, but such a risk did appear in the second year, and it persisted.[46] The mechanism does not seem to be related to an increase in blood pressure. The WHI findings were consistent but more dramatic than those of the HERS, which reported a nonsignificant 23% increase in the treatment group.[29] The WHI results were also more extreme than those of the Women's Estrogen for Stroke Trial (WEST) of estradiol (without progestin) in women with a history of a prior stroke. Overall, the WEST trial found no effect of estrogen on recurrent strokes but some rise in rates in the first 6 months.[47] Some might criticize the WHI statistics because they include older women. However, there was no indication that excess strokes were more likely to occur in older women, in women with prior stroke, through differences in race/ethnicity, or in women with high blood pressure. It is apparent that estrogen plus a progestin increases the risk of stroke in women who have been judged to be healthy. The estrogen-only arm of the WHI showed a slight increase in stroke for women in the youngest age group at study entry.[48] The ERT arm was quite different from the HT arm. A 2015 Cochrane review of 19 randomized controlled trials found that although there was an increase in stroke risk with HT therapy, this appeared to be limited to women who started treatment 10 or more years after menopause.[49]

One of the most recent studies on estrogen leads us to the most provocative question: Do different kinds of estrogens have different effects? In this study, the risk of VTE was compared among those who used esterified estrogen, conjugated equine estrogen, and no estrogen at all.[50] The findings concluded that CEE but not esterified estrogen was associated with venous thrombotic risk. Transdermal estrogen may also have an advantage on stroke risk compared with oral ET.[51]

Gallstones

The risk of gallstones or cholecystectomy appears to be raised by a factor of 2 to 3 in postmenopausal women who are taking ERT. This finding has been reported by several large observational studies. In HERS I, the women taking estrogen plus progestin were at a 38% higher risk of gallbladder disease than women who were taking a placebo.[20] HERS II found similar results, reporting a higher incidence of biliary tract

surgery, with a 48% increased relative risk for those in the HT group and an absolute risk of 191 versus 129 events annually per 10,000 women.[52] A 2017 Cochrane review that was heavily weighted by the WHI and HERS trials found that the absolute risk for gallbladder disease after 5.6 years of HT use increased from 27 per 1000 with placebo to between 38 and 60 per 1000 with HT.[53] It has been suggested by observational trials but not proven by randomized trials that transdermal estrogen may help to reduce this increase in risk.[54]

Areas of Uncertainty

Breast cancer. For many women, fears about breast cancer and a higher risk of breast cancer from HT dominate the decision-making process about menopause management. Clinicians must be educated about this topic and must be prepared to summarize the results of relevant research in order to counsel each woman about the benefits and risks in her particular situation.

It is true that there are unanswered questions about the long-term safety of HT, especially regarding the risk of breast cancer. What is often forgotten is that the clinical trial data on ERT span the last 100 years. No other pharmacological agent has been as thoroughly studied as estrogen. A half-century of research preceded the early 1940s, when HT became commercially available. Even though patient fears are high and alternative practitioners are particularly suspicious and skeptical about its safety, no data clearly and consistently demonstrate a higher risk of breast cancer associated with HT. Nevertheless, many patients and practitioners continue to be certain that taking HT will cause breast cancer.

Close to 60 observational studies of the association between HT and breast cancer have been published during the past 25 years, and no definitive answer exists because of inconsistency in the results of those studies. One analysis (before the WHI) of the evidence in those studies found that more than half of them reported either no difference in risk or a decrease in the risk of breast cancer with ERT/HT use.[55] The remainder of the studies reported only slight rises in breast cancer risk. Another group of researchers attempted to reanalyze more than 90% of the published data on breast cancer and HT use.[56] They reported that postmenopausal women who had ever used HT had a small but statistically significant increase in the risk for breast cancer compared with women who had never used HT. In women who were currently using HT or had recently used it, the relative risk rose by a factor of 1.02 to 1.04 for each year of use. After HT had been discontinued for 5 years, no significant excess risk remained. Also, breast cancers diagnosed in women who had used HT tended to be less advanced and were more localized.

Later reports from observational studies are also inconsistent. The report from the National Cancer Institute's Breast Cancer Detection Demonstration Project was published in January 2000.[57] These findings showed that the risk associated with recent HT use (both current use and use within the previous 4 years) was twice that associated with ERT. However, the relative risk for recent use of ERT was not statistically significant, and the difference between risks with ERT and HT was not tested for statistical significance. Right after that report was published, another group of investigators published and reported higher risk estimates for sequential HT regimens for 5 years or more of use than for continuous combined HT, although the difference was not statistically significant.[58] In contrast to previous reports, this study found no difference in risk between current and past users.

At the end of 2000, the Nurses' Health Study published its estimates of breast cancer risk associated with HT in postmenopausal women.[59] The results were expressed as percentage increases in the cumulative risk of breast cancer and were frightening to many: The use of estrogen alone for 10 years was found to lead to a 23% increase by age 70 years, and the use of estrogen plus progestin for 10 years was found to lead to a 67% increase by age 70 years. It is important for clinicians to realize (1) that the result is not an actual mathematical conclusion but the conclusion of the model—the consequences of a small difference in risk at the beginning that then is magnified as the math is carried out into the future—and (2) that risk estimates represent a projection, not an actual measurement.

One of the most disturbing reviews was published in 1998. Colditz[60] summarized the evidence that endogenous estrogen and ERT not only increase the risk but are causally related to breast cancer. In his review of hormones and breast cancer, he included reports on cell proliferation and endogenous hormone levels as well as epidemiological studies of the relationship between the use of postmenopausal hormones and the risk of breast cancer. He found evidence of a causal relationship between female hormones and breast cancer based on consistency, dose–response pattern, biological plausibility, temporality, strength of association, and coherence. He stated that the magnitude of the increase in breast cancer risk per year of hormone use is comparable with that associated with delaying menopause by a year. The fact that women who menstruate longer have a higher risk of breast cancer also provides support for a biological mechanism for the relationship between the use of exogenous hormones and increased risk. Colditz concluded that existing evidence supports a causal relationship between the use of estrogens and progestins, levels of endogenous estrogens, and breast cancer incidence in postmenopausal women.

The WHI was the first randomized controlled trial confirming that conjugated equine estrogens combined with progestins do increase the incidence of breast cancer and the first to quantify the increase. The 26% higher risk of breast cancer occurred after about 4 years and translated to 8 more cases annually per 10,000 women.[18] This is consistent with other epidemiological data. The study was discontinued primarily because of the breast cancer incidence, which crossed the monitoring boundary for safety.

It is important to note that among women who received estrogen only (CEE) for a median of 5.9 years in the WHI, there was a decrease in invasive breast cancer incidence and breast cancer mortality over the median 11.8 years of follow-up.[61] Unopposed estrogen was also found not to increase breast cancer risk in the Caroline Breast Cancer Study.[62] Although estrogen should not be recommended as a preventative treatment for women without a uterus, they may be reassured regarding the effects of estrogen on breast cancer risk.

At the other end of the pendulum, investigations that have found no increased risk of breast cancer with HT or ERT use receive much less attention. The Iowa Women's Health Study is prospectively following a cohort of women who were selected in 1985.[63] After 6 years of monitoring, a statistically significant increase in the risk of breast cancer could not be detected in women who either had ever used or were currently using HT. Another report through 8 years of follow-up examined whether postmenopausal HT raised the risks for breast cancer and death from cancer in women with a family history of breast cancer.[64] There was no significant increase in the rate of breast cancer even in women who had a family history of breast cancer and had been using HT longer than 5 years. These results are consistent with those of other reports that there is no additional risk in using HT/ERT for women who have a first-degree relative with breast cancer.

The latest analysis from the Iowa Women's Health Study, an 11-year follow-up, showed an association between women who had ever used postmenopausal HT and the risk of breast cancers that were more localized and had a better prognosis.[65] The researchers did not find an increased risk of invasive ductal or lobular carcinoma in women who had used HT for either less than or more than 5 years. A slight increase in risk was observed in current users and users for less than

5 years; current users with more than 5 years of use had no increase in risk. These results are the opposite of those seen in the Nurses' Health Study—that women who use HT for more than 5 years have a higher risk.[66] Two other studies, the Carolina Breast Cancer Study[67] and analyses from the National Health and Nutrition Examination Survey (NHANES),[68] found no increase in risk with postmenopausal hormones. These later studies perpetuate the inconsistency in research on this issue that has been seen in the last 25 years. This pattern provides some logic to the point of view that if there is an increased risk of breast cancer associated with ERT/HT, the risk must be small, because by now, after so many years, we would have seen more consistency in the data, and the size of the risk estimates would be large rather than slight.

It is to be hoped that, in consultations with patients, clinicians can offer a balanced view, reassuring them that no studies find an increased risk of breast cancer with the short-term use of HT and that the conflicting, inconsistent results of more than 60 studies show that if there is an increased risk with long-term use, it is a small one. We can evaluate the benefits and risks for each patient and make our recommendations on a short-term basis, which can help each individual to make decisions that are not necessarily permanent. Research in this area of medicine is prolific, and if we keep up to date on the latest findings, we can inform our patients so that they can make informed, timely choices about their health care.

Cognitive function. Observational studies have suggested that there is a relationship between endogenous estrogen exposure and cognition.[69] A number of other observational reports have demonstrated that HT use may prevent or delay the onset or progression of Alzheimer's disease, but additional observational results have been conflicting.[70] A meta-analysis and systematic review was conducted in March of 2001 in which 29 studies were rated.[71] In women who were symptomatic from menopause, postmenopausal use of estrogen improved verbal memory, vigilance, reasoning, and motor speed. There were no consistent effects on visual recall, working memory, complex attention, mental tracking, mental status, or verbal functions. Estrogen did not appear to enhance asymptomatic women's performance consistently on formal cognitive testing. The meta-analysis did suggest that HT was associated with a lower risk of dementia, but the reviewers acknowledged that the studies analyzed had important methodology problems and that the information was inadequate to allow a proper assessment of the effects of various estrogen preparations or doses, progestin use, and duration of use.

Studies in animals and the laboratory suggest plausible mechanisms for the potential of estrogen and the prevention and/or treatment of Alzheimer's disease. Estrogen increases dendritic spine growth, axonal elongation, synapse formation, and neuronal survival. It also influences several neurotransmitters, including acetylcholine; modulates nerve growth factor; increases apolipoprotein E; enhances blood flow; serves as an antioxidant; and enhances the uptake and metabolism of glucose. All of these effects could possibly inhibit the neurotoxicity of beta-amyloid and the damaging effects of free radicals, moderate the inflammatory events involved in plaque formation in the brain, enhance cerebral blood flow, and facilitate neuronal repair after brain injury.

Results of randomized trials of ERT and Alzheimer's disease and the potential of estrogen for the treatment of this disorder have not been impressive in terms of benefits. In one study, estrogen replacement for 1 year did not slow disease progression or improve global, cognitive, or functional outcomes in women with mild to moderate Alzheimer's disease.[72] In another, participants at 10 of the 20 HERS centers were enrolled in the cognitive function substudy. The mean age of participants at the time of cognitive function testing was 71 ± 6 years. Among these older women with coronary artery disease (CAD), 4 years of treatment with conjugated equine estrogens plus progestins did not result in better cognitive function as measured on six different standardized tests.[73]

In a later prospective study of dementia, prior use of HT was associated with reduced risk of Alzheimer's disease, but the duration of use very specifically affected the benefit; there was no apparent benefit from the current use of HT unless the use exceeded 10 years.[74] More recent data suggest that the "timing hypothesis" may be relevant to cognitive function as well. For example, a 2017 Cochrane review found that women over 65 taking continuous combined HT showed an increase in the incidence of dementia (from 9 per 1000 to 11–30 per 1000).[53] However, the results from a subset of the Kronos Early Estrogen Prevention Study (KEEPS) data suggest no effect on cognitive function among recently postmenopausal women.[75] The timing hypothesis was not confirmed in a trial looking for just this effect, however. In a randomized trial with a duration of 57 months, no effect on cognitive function was observed, and no difference was observed when HT was initiated within 6 years of menopause or after 10+ years postmenopause.[76]

Ovarian cancer. Evidence concerning a possible positive association between HT use and ovarian cancer risk is less consistent and of lesser significance than that for endometrial and breast cancers. Most of the data show a weak positive association. A large prospective cohort (observational) U.S. study of 211,581 postmenopausal women treated for longer than 10 years with conventional HT found an association with an increased risk of ovarian cancer.[77] No distinction was made regarding the type or regimen of HT or whether a progestogen was added to the ERT. Participants had no history of cancer, hysterectomy, or ovarian surgery. The study, monitoring women from 1982 to 1996, showed that women who were using HT at study entry had higher death rates from ovarian cancer than women who had never used HT. The risk was slightly but not significantly higher among former estrogen users. Women who used HT at baseline and for 10 years or more had a relative risk of 2.20, and former users with at least 10 years of use had a relative risk of 1.59. The annual age-adjusted cancer death rates from ovarian cancer per 100,000 women were 64.4 for baseline HT users with 10 or more years of use, 38.3 for former users with 10 or more years of use, and 26.4 for women who had never used HT.

In this observational study, as in the Nurses' Health Study, the lack of information is almost more disturbing than the actual information. We have no data as to the type, dose, or combination of estrogen and progestogen used by the participants. As a result, many assumptions were made that influenced data analysis and the effect on relative risk. We know that the way in which hormones have been prescribed has changed from 1982 through 1996 and since then. In the early 1980s, most women were prescribed unopposed daily estrogen. During the 1980s, sequential estrogen plus progestogen therapy was introduced to eliminate the increased risk of endometrial cancer. Most women began to take 7 to 10 days of progestogen per month along with their estrogen. In the 1990s, common prescribing habits involved continuous and combined estrogen/progestogen regimens and lower doses of both hormones. Whether sequential or cyclic, progestogen is generally prescribed for at least 12 days per month. The number of different estrogens and progestogens has also significantly expanded and changed since the early 1980s and even in the last few years.

The investigators also reported on relative risk. Given the low incidence of ovarian cancer, even if there is a significant increase in relative risk, it may not have much of an impact on absolute risk. Although this study shows a doubling of the relative risk, the incidence of ovarian cancer mortality in postmenopausal women is extremely low, at 1.6%.

It is also interesting to remember that 7 years or more of oral contraceptive use in reproductive-age women actually lowers the incidence of ovarian cancer.

A large, prospective study reported a significant twofold-higher risk of ovarian cancer among long-term users of HT and ERT.[78] A total of 44,241 postmenopausal women were selected from the Breast Cancer Detection Demonstration Project (BCDDP); 329 women who experienced ovarian cancer were identified. Women who used estrogen-only replacement therapy, especially for more than 10 years, were at significantly higher risk of ovarian cancer, with a relative risk of 1.8; women who used estrogen only for 20 or more years had a relative risk of 3.2. The good news was that women who used short-term combination replacement therapy were not at increased risk.

In 2015 a meta-analysis of 52 epidemiological studies was published in the *Lancet.* An increase in risk was observed even among women using HT for less than 5 years, as well as among those taking estrogen only or estrogen and a progestogen. The absolute risk for women taking HT for about 5 years and starting around age 50 was 1 additional case per 1000 users and one additional ovarian cancer death per 1700 users.[79]

Focus on estrogen only. The WHI originally had three components: HT, a low-fat dietary modification, and supplementation with calcium and vitamin D. In addition, many of the women who did not qualify for the active treatments of the WHI became part of an observational group that was studied. Both arms of the placebo-controlled HT component (estrogen plus progestin [HT] and estrogen only [ERT]) were terminated before the planned ending date. The ERT arm of the WHI was halted on March 1, 2004, after 6.8 years of follow-up and less than a year before the planned closing date.[48] The ERT arm was quite different from the HT arm in that the risks in the ERT arm did not exceed the benefits. The study showed a slightly increased risk of stroke, a decreased risk of hip fracture, a lack of increase in the risk of breast cancer, and a possible reduction in breast cancer risk and no effect on the incidence of CHD.

The key clinically relevant issues from the WHI ERT study are as follows:

- CHD was reduced by almost half in the youngest age group (the most common age group of women who initiate ERT).
- The risk of stroke increased only slightly for women in the youngest study-entrance age group.
- The risk of VTE was increased in all three age groups, but the degree of risk increased with age at study entry.
- The risk of invasive breast cancer was reduced in all three age groups.
- The risk of colorectal cancer was decreased in the two younger groups, especially in women aged 50 to 59; it was increased in women aged 70 and older.
- The overall reduction in risk of fracture was most evident in younger women.
- ERT was associated with a lower death rate in the group that was the youngest at study entrance.
- The middle age group had about the same death rate in both the ERT and placebo groups, and there was a slightly higher death rate in the oldest age group.

What the WHI ERT study basically showed is that ERT, using CEE, is safe for most menopausal women who do not have a uterus. ERT reduced the risk of CHD in those women who started ERT near the age of menopause; it decreased the risk of fractures; it did not increase the risk of breast cancer; the risk of colorectal cancer was increased in women who started therapy at age 70 or older; and it was associated with a slight increase in the risk of stroke and VTE, but there was no increase in death rates.

It would appear that the use of ERT alone by women without a uterus is safer than using estrogen plus a progestin.

Natural hormones. It is important to understand that most of the research on hormone regimens is on CEEs and MPA. However, that is not exclusively the case, and certainly some of the cardiovascular research has equally implicated 17-beta-estradiol (bioidentical estradiol). Nonetheless, in the most damaging data to date, from HERS I and II and the WHI, the hormones used were the equine estrogens and progestin. So, what we definitely know to be negative effects are associated with those regimens, and we do not, in fact, know whether the data can be applied to other regimens. It would be naive to dismiss the data and not to admit the possibility that the results would be the same. When hormones are determined to be appropriate or even necessary, however, it would at the very least seem logical to use the hormones we know not to be those with adverse effects.

Natural compounded estrogens and natural compounded progesterone, as well as natural testosterone and dehydroepiandrosterone (DHEA), such as those used by compounding natural pharmacies and the natural progesterone creams sold over the counter, are distinct and different in several critical ways from CEEs, conjugated plant estrogens, synthetic estrogens, and synthetic progestins. Natural hormones are made from either beta-sitosterol extracted from the soybean or from diosgenin extracted from Mexican wild yam root. Those compounds are then made into the desired hormone and are biochemically identical to the hormone the body produces. By definition, a natural hormone is plant derived and bioidentical. The natural compounded estrogens are either estriol, estrone, or estradiol. Estriol is particularly unique because it has approximately one-fourth the potency of estradiol and estrone. Natural compounded estrogens are generally used in lower doses owing to the combined effect of the weaker estriol along with the estradiol and/or estrone. These natural estrogens are thought to be metabolized significantly differently by the body, have shorter half-lives, can be used in customized dosing regimens and potencies to fit each individual woman and clinical situation, and can be adjusted to be stronger or weaker in small units to taper someone off or onto hormones.

There is another distinction worth making; hormones may be bioidentical, that is, have the same molecular structure as endogenously produced hormones, and manufactured in standardized dosages by drug companies, for example, Vivelle (estradiol) and Prometrium (micronized progesterone). These are distinct from compounded bioidentical hormones, which also optimize dosages, currently do not have FDA approval, and are discouraged by most conventional organizations.[80]

CEEs, those that were used in the WHI study, are quite different. In the 1970s it was believed that Premarin consisted only of 10 estrogens: 17-β-estradiol, 17-β-dihydroequilin, 17-β-dihydroequilenin, 17-α-dihydroequilin, 17-α-estradiol, estrone, equilin, 17-α-dihydroequilenin, Δ-8-9-dehydroestrone sulfate, and equilenin. Since then, advancements in technology have shown that the original 10 estrogens make up less than 40% of the hormonal content of Premarin. Through the use of modern analytic techniques, more than 200 individual components have been identified, including androgens and progestins.[81] The composition of Premarin is complex, and different estrogens have various effects in different tissues. Herein may lie an explanation for problems with conjugated equine estrogens versus natural bioidentical estradiol, estrone, and estriol.

The steroid hormones, including the sex steroids produced by the ovaries, represent a subclass of lipids that share a four-ring steroid structure. The native compound from which all the sex steroids are derived is cholestane, the parent of cholesterol. When nutritional states of the individual and cell are normal, the principal precursor of steroid

production is cholesterol in the plasma. The cholesterol enters the cells through a lipoprotein receptor system. The activity of the enzymes within the cells of that tissue determines the particular classes of steroids produced. Steroids, either endogenous or exogenous, enter the cells via passive diffusion. The tissues responsive to steroids have very specific intracellular receptors, each with a high affinity for its particular steroid. The primary action of the steroids is the binding of a steroid hormone to a receptor and the interactions of this receptor-hormone complex with the components of the cell. When the steroid binds, the steroid-receptor complex becomes activated and binds with very specific regions within the steroid-responsive regions of the genes. Most of the effects of steroids on responsive cells are mediated through the activation of very specific genes. The hypothesis is that a nonbioidentical hormone may act like a constant and indiscriminant environmental toxin to the genetic material within the cell because, even though it can bind to the receptor-hormone complex, it is a foreign substance.

Consider CEEs, which consist of more than 200 substances mostly foreign to a human. These substances, once ingested, are a part of that complex and therefore are activating those genes within the cell. Thus there is a profound distinction between a hormone that is bioidentical to human hormones and one that is not. Besides the effect on the genes themselves, bioidentical hormones and nonbioidentical hormones may very well leave different metabolic footprints on the rest of the body, with different metabolic consequences. They may be directly cytotoxic to estrogen-sensitive tissues, alter binding of other hormones to those receptors, or change the liver's metabolism of carcinogens. It is because of this distinction and the potential difference in metabolic consequences—as well as the shorter half-life of natural hormones—that naturopathic physicians prefer bioidentical natural hormones almost exclusively for the treatment of symptomatic menopause when hormones are required.

The distinctions between synthetic progestins and progesterone are even clearer. Natural progesterone has been studied and shown to have less adverse effects on the cardiovascular system than synthetic progestins such as MPA. Specifically, natural progesterone lowers HDL-C significantly less than MPA, is less atherogenic, and does not cause coronary artery spasm, whereas MPA does.

Natural estradiol may not be without concerns about its effects on the cardiovascular system and breast. However, it is typically used in a half-strength dose (0.5 mg total daily) because it is combined with the significantly weaker estriol. Estriol has been shown to have some ability to act as an antiestrogen in the breast and no significant effect on the cardiovascular system.

Further consideration of a natural hormone approach and a more holistic approach to menopause would lead one to use the hormones in combination with other strategies known to reduce the risk of breast cancer and heart disease. For example, soy, flaxseeds, cabbage family foods, and supplements can promote the metabolism of estrogens to their anticarcinogenic breakdown products. Diets designed to help prevent breast cancer and heart disease can also be emphasized, along with nutritional and botanical supplements to be considered, such as vitamins E and C, the carotenes, soy, coenzyme Q_{10}, green tea, and garlic.

Women who are using CEE and MPA should consider other natural estrogen and natural progesterone regimens or nonhormonal menopause management. Women who are using natural estrogens and natural progesterone should consider a reevaluation of the hormone regimen to establish the lowest dose for achieving the benefits and minimizing the risks. Regular, at least annual, consideration should be given to the duration of use on an individual basis. Women who do not have a uterus and are being treated with any hormone regimen should take estrogen alone. Neither synthetic MPA nor natural progesterone is needed.

Additional herbal and nutritional supplements may be considered as well. Studies on black cohosh, red clover, soy, maca, bioflavonoids, and kava have all shown proved scientific efficacy in the treatment of menopausal symptoms. The majority of women require only these nonhormonal supplements for symptom relief and never need any kind of HT. Other women may be able to lower their dose of cHT or natural hormones by using them in combination with the herbal and nutritional supplements.

Guidelines on the use of the different forms of HT are given following the discussion of nonhormonal approaches.

The Major Symptoms of Menopause

Common complaints of perimenopause and menopause are as follows:

- Irregular bleeding (in perimenopause)
- Hot flashes (or night sweats)
- Vulvovaginal thinning, dryness, dyspareunia, and burning (known as atrophic changes)
- Bladder frequency/urgency/leakage
- Mood changes
- Cognitive changes
- Body aches
- Sleep disturbances

Other symptoms can include fatigue, sexual dysfunction, hair thinning, the appearance of facial hair, headaches, voice impairment, dry skin, and joint pains.

TREATMENT OVERVIEW

The goal of a natural and integrative approach to menopause is to recognize that there are many options for symptom management, disease prevention, and disease treatment. One might categorize these in the following way:

1. Diet, exercise, and stress management
2. Nutritional supplementation
3. Botanical medicines
4. Compounded bioidentical HT (cbHT)
5. Bioidentical HT (bHT) (FDA-approved prescription items)
6. Nonbioidentical HT (HT) (FDA-approved prescription items)
7. Condition-specific nonestrogen pharmaceuticals (for either symptom relief or disease prevention/treatment)

There are three fundamental goals in treatment: relief of symptoms, prevention of disease, and treatment of disease. Each woman is assessed individually to determine the scope and severity of her symptoms and is evaluated subjectively and objectively as to her risks of osteoporosis, heart disease, breast cancer, Alzheimer's disease, type 2 diabetes, and colorectal cancer as well as other chronic health problems.

Diet, exercise, lifestyle, and/or nutritional supplements and botanical therapies will be effective for the management of menopausal symptoms in the majority of women. When these are not adequate, HT or other medications can be recommended.

Diet, Exercise, Toxin Avoidance, and Stress Management

Nutrition

Nutrition plays a fundamental role in integrative medicine. Although dietary advice should be individualized, common themes include a diet rich in whole, "natural," and unprocessed foods, with an emphasis on vegetables, whole grains, beans, seeds, nuts, fruits, lean low-fat proteins, and healthy fats, such as olive oil; to be minimized are saturated fats and fried foods, simple carbohydrates, alcohol, sugar, and salt. For example, a systematic review of the Mediterranean diet has shown it to be associated with lower cancer mortality, including a reduction in

breast cancer incidence.[82] This is supported by the results from a randomized, single-blind controlled Spanish trial that enrolled over 4000 women aged 60 to 80, who were advised to reduce fat intake (control) and follow a Mediterranean diet supplemented with olive oil or a Mediterranean diet supplemented with nuts. They observed a 68% reduction in breast cancer with the diet plus olive oil group and a 41% reduction in the diet plus nuts group.[83] Similarly, a Mediterranean diet was inversely associated with vasomotor menopausal symptoms in a prospective cohort study following over 6000 Australian women.[84] Some specific foods, such as soy and flax, have been studied for their beneficial effects on menopause-related symptoms.

Soy. Soy foods may be useful in menopause primarily for their potential benefits in moderating hot flashes, slowing bone loss, improving the lipid profile and blood pressure, and lowering the risk for CAD. There are conflicting studies on soy for hot flashes, some showing effect and others not, making it difficult to draw any definitive conclusion. Two systematic reviews of isoflavones (from soy and red clover [*Trifolium pratense*]) and menopausal symptoms and the consensus opinion from the North American Menopause Society offer a good summary of the research.[85] The first systematic review evaluated the literature of randomized controlled clinical trials on soy and perimenopausal symptoms.[85] Only 4 out of 10 trials showed benefit. In the second systematic review, 25 trials of soy and red clover isoflavones involving approximately 2300 women met the study criteria, the results were mainly positive but not consistent.[86]

A consensus opinion of the NAMS[85–87] reports many diverse areas of the impact of soy. The evidence for isoflavones and hot flashes showed mixed results, but they appear to be modestly effective in relieving menopausal symptoms; supplements that provide higher amounts of genistein or an increase in S-equol may be more beneficial. In addition, the NAMS concludes that soy food consumption is associated with a lower risk of breast and endometrial cancer in observational studies. The NAMS also reports its opinion that the efficacy of soy on bone health has yet to be adequately proved. Cardiovascular benefits are still evolving, but the effect of soy does appear to lessen the arterial stiffness, yet the evidence is mixed on the lipid values in postmenopausal women. Preliminary evidence of the cognitive benefit from soy isoflavones seems to point in the direction that younger postmenopausal women derive more benefit within the first few years of menopause rather than older postmenopausal women. Another study has shed some light on why the research on soy isoflavones and vasomotor symptoms is so contradictory. This randomized, double-blind, placebo-controlled clinical trial comprising 96 menopausal women was conducted over 6 months.[88]

In this study, 66 women were given 135 mg of soy isoflavones, and 30 women were given a placebo. After 1 week, the women in the treatment group were tested and further divided into two subgroups, equol-producing (EP) and non–equol-producing (non-EP), according to peak levels of equol in their urine. The women in both of these subgroups were then given 3 g of soy germ extract powder twice a day, totaling 135 mg of isoflavones daily, for 6 months. Menopausal symptoms were evaluated using a modified Kupperman Index measuring 17 items (hot flashes, excessive sweating, coldness of the extremities, shortness of breath, numbness of the extremities, paresthesias of extremities, insomnia, easy awakening, excitability, nervousness, melancholia, vertigo, weakness, arthralgia or myalgia, headaches, palpitations, and formication). Symptoms were scored as none, mild, moderate, or severe. Compared with the placebo group, symptoms of hot flashes and excessive sweating were significantly reduced after 3 months, and weakness, palpitations, limb paresthesias, and total symptoms significantly decreased after 6 months ($P < 0.05$), but only in the EP group. At 3 months, total scores had decreased by 66% in the EP group, 54% in the non-EP group, and 59% in the placebo group. At 6 months, symptom scores had decreased by 84% in the EP group, 58% in the non-EP group, and 66% in the placebo group. Studies that may have had a higher percentage of women who were equol producers have been previously suspected to be the determining factor in the effectiveness of soy isoflavones, but the current study seems to be the first to demonstrate more clearly that a woman's ability to produce equol determines her response to soy isoflavone supplementation. Daidzein and genistein are the two most significant phytoestrogens in soy. Daidzein is converted to equol, a metabolite of daidzein, by bacterial flora in the gut. For clinicians, part of the task in treating menopausal women with soy may be to test for equol production before treatment and/or improve their gut flora so that they can more easily transform soy isoflavones to equol.

In 2015 a model-based meta-analysis of 16 studies was published that found a maximal percentage decrease in menopausal hot flashes of 25.2% (after eliminating placebo effect), which accounted for 57% of the maximal effect of estradiol. Additionally, the study found that to achieve 50% of soy's maximum effect, a 13-week period is needed, much longer than estradiol's 3-week period for 50% effect. This may also partly explain the lack of effect observed in some short-term studies.[89]

In reviewing traditional Asian diets, the average adult daily intake of soy isoflavones is somewhere between 50 and 150 mg per day. The isoflavone content of soy foods varies with the form (Table 196.1).

Flaxseed. Another significant dietary source of phytoestrogens is flaxseed *(Linum usitatissimum)*. Flaxseed contains the lignans matairesinol and secoisolariciresinol, which are known to have estrogenic activity, as well as other lignans that are modified by intestinal bacteria to form estrogenic compounds. Lignans are absorbed in the circulation and have both estrogenic and antiestrogenic activity.[90]

Only a small amount of research has been done in the area of flaxseed and hot flashes. One small but encouraging study, in 2007, showed that women who consumed 2 tbsp of flaxseed twice daily halved their number of hot flashes within 6 weeks and reduced the intensity of their hot flashes by 57%.[91] A 2014 systematic review also

TABLE 196.1 Isoflavone Content of Soy Foods

Soy Food	Amount	Isoflavones (mg)
Textured soy protein granules	¼ cup	62
Roasted soy nuts	¼ cup	60
Tofu, low-fat and regular	½ cup	35
Tempeh	½ cup	35
Soy beverage powders	1–2 scoops	25–90 (varies with product)
Regular soy milk	1 cup	30
Low-fat soy milk	1 cup	20
Roasted soy butter	2 tbsp	17
Cooked soybeans	½ cup	150
Soy isoflavone pills	Varies with the manufacturer; read label	Varies with the manufacturer; read label
Fermented soy isoflavone pills		Will contain lower amount of isoflavones but may be better absorbed

found flax consumption to be associated with a reduced risk of breast cancer as well as a possible reduction in mortality risk among women with breast cancer.[92]

Exercise

The benefits of exercise for peri- and postmenopausal women are numerous (Box 196.1). Women can achieve substantial reductions in cardiovascular disease[93] and breast cancer risk,[94] increases in bone density,[95] and reductions in body fat and body mass index while also experiencing an improved sense of well-being.[96]

However, whether exercise can reduce hot flashes during menopause is not clear. Moderate exercise may be beneficial for hot flashes; more vigorous exercise may actually exacerbate them.

Toxin Avoidance

Often overlooked is the impact of environmental pollutants on menopause and related conditions, yet it should be self-evident that endocrine-disrupting chemicals would affect hormonal activity. For example, Fig. 196.1 highlights the toxins likely to affect female reproductive pathways.

Endocrine-disrupting chemicals. In an analysis of NHANES data, at least 15 endocrine-disrupting chemicals (EDCs) were found to be associated with earlier menopause, with women with higher serum levels reaching menopause roughly 2 to 4 years earlier than women with lower levels. Additionally, women exposed to EDCs were up to 6 times more likely to be menopausal than nonexposed women. The list of EDCs associated with earlier menopause included nine polychlorinated biphenyl (PCBs), three pesticides, a furan, and two phthalates.[97]

Polychlorinated biphenyls. Some PCB compounds, such as PCB138, have also been associated with an increase of over threefold in the risk for breast cancer among women with the highest levels.[98]

Phthalates and bisphenol A. Another recent review found that EDCs, including phthalates and bisphenol A, were likely causes of premature ovarian insufficiency.[99] Phthalates specifically have been

BOX 196.1 Health Benefits of Regular Exercise in Menopause

Relief from hot flashes
Decreased bone loss
Improved heart function
Improved circulation
Reduced blood pressure
Decreased blood cholesterol levels
Improved ability to deal with stress
Improved oxygen and nutrient utilization in all tissues
Increased self-esteem, mood, and frame of mind
Increased endurance and energy level

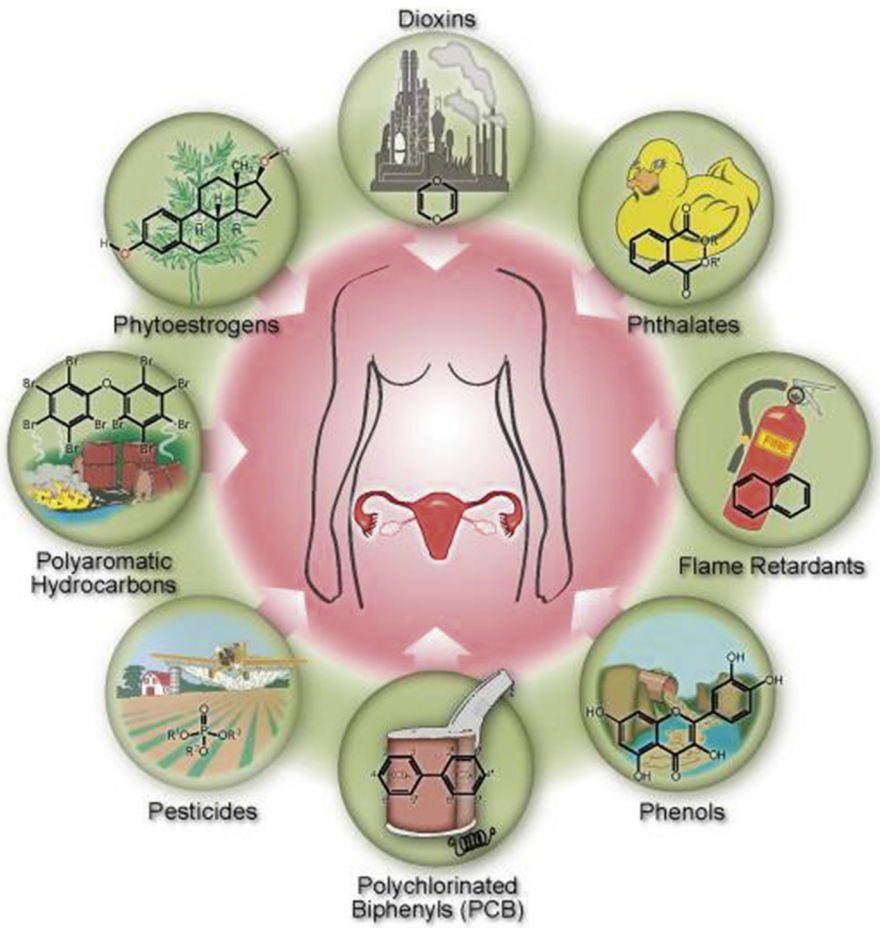


Fig. 196.1 Environmental toxins likely to affect female reproductive health. (From Grindler NM, Allsworth JE, Macones GA, et al. Persistent organic pollutants and early menopause in U.S. women. *PLoS One.* 2015 Jan 28;10[1]:e0116057. Reproduced from an open-access article distributed under the terms of the Creative Commons Public Domain declaration.)

linked to vasomotor symptoms; women with the highest urinary levels of phthalate metabolites (from personal care products) were about 45% more likely to have ever experienced hot flashes, to have had hot flashes in the past 30 days, and to have more frequent hot flashes.[100]

Lead. Data from NHANES have also shown that lead body burden, assessed via blood lead levels, was also associated with earlier menopause, with an increased likelihood of over fourfold for being menopausal among women with the highest lead exposure.[101]

A complete treatment plan must include education about the role of these toxins in menopausal status and symptoms, as well as clear advice for reducing relevant exposure (e.g., avoiding personal care products with phthalates, etc.).

Stress Management

Perimenopause/menopause can be a stressful time in a woman's life. Surprisingly, studies of women in midlife suggest that depression, as defined by the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-V),* is no more common during menopause than at any other time of life.[102,103] Most studies indicate that the perimenopausal transition is associated with depressive symptoms and that women experience mood changes but fail to meet the DSM-IV criteria for the diagnosis of depression.[104–106]

A woman's ability to manage stress can be enhanced with self-care tools that may include meditation, yoga, breathing exercises, being out in nature, and getting regular exercise.

Dietary Supplements

There are a number of nutritional supplements that may be beneficial for hot flashes and night sweats. Most women should probably take a good multivitamin—one that provides 70% to 100% of the recommended daily allowances of vitamins and minerals. In addition, there are numerous supplements for other individual symptoms commonly experienced during menopause, such as insomnia, depression, and anxiety. These supplements include melatonin, L-tryptophan, and 5-HTP for insomnia; vitamins B_6 and B_{12}, folic acid, and L-tyrosine for depression; and gamma-aminobutyric acid (GABA) and L-theanine for anxiety.

Hesperidin, Vitamin C, and Procyanidolic Oligomers

Combined with vitamin C, hesperidin and other citrus flavonoids may be effective in relieving hot flashes. In one clinical study, 94 women suffering from hot flashes were given a formula containing 900 mg of hesperidin, 300 mg of hesperidin methyl chalcone (another citrus flavonoid), and 1200 mg of vitamin C daily.[107] At the end of 1 month, symptoms of hot flashes were relieved in 53% of the patients and reduced in 34%. Improvements in nocturnal leg cramps, nosebleeds, and easy bruising were also noted. The only side effect was a slightly offensive body odor with a tendency for the perspiration to discolor the clothing. Dietary vitamin C has also been positively associated with bone mineral density among postmenopausal women.[108]

Perhaps more useful than hesperidin are preparations containing procyanidolic oligomers (PCOs); see Chapter 209. In a double-blind study, a group of perimenopausal Taiwanese women 45 to 55 years of age were given either placebo or 100 mg of PCOs (as Pycnogenol) twice daily for 6 months.[109] Of the total, 155 women received the Pycnogenol, and 75 women received the placebo. The 36-item Women's Health Questionnaire was used to evaluate the climacteric symptoms at baseline and at 1, 3, and 6 months. Blood pressure decreased similarly in both groups. HDL-C increased and LDL-C decreased significantly from baseline with Pycnogenol, but no significant differences were seen in HDL-C between the two groups. However, LDL-C was more significantly reduced in the Pycnogenol group. Perimenopausal symptoms of depression, vasomotor symptoms, memory, anxiety, sexual function, and sleep all improved significantly ($P < 0.001$) in both severity and frequency with Pycnogenol as soon as 1 month after starting the treatment. Most symptoms also improved with placebo but not significantly. In two more recent small trials, Pycnogenol supplementation was also found to reduce common symptoms of menopause (including hot flushes, night sweats, mood swings, etc.), as well as cardiovascular risk factors such as C-reactive protein (CRP) and homocysteine.[110,111]

Gamma-Oryzanol

Gamma-oryzanol (ferulic acid) is a growth-promoting substance found in grains and isolated from rice bran oil. In the treatment of hot flashes, its primary action is to enhance pituitary function and promote endorphin release by the hypothalamus. Gamma-oryzanol was first shown to be effective in the treatment of menopausal symptoms, including hot flashes, in the early 1960s.[112] Subsequent studies have further documented its effectiveness.[113]

In one of the earlier studies, 8 menopausal women and 13 women whose ovaries had been surgically removed were given 300 mg/day of gamma-oryzanol. At the end of the 38-day trial, more than 67% of the women had a 50% or greater reduction in menopausal symptoms.[112] In a later study, the benefit of a 300-mg/day dose of gamma-oryzanol was even more effective, in that 85% of the subjects reported improvement in their menopausal symptoms.[113]

Gamma-oryzanol is an extremely safe natural substance. No significant side effects have been produced in experimental and clinical studies. In addition to being helpful in improving the symptoms of menopause, gamma-oryzanol has also been shown to be quite effective in lowering blood cholesterol and triglyceride levels.[114]

Fish Oils

A study on fish oil supplementation and hot flashes has afforded clinicians with another option in treating hot flashes and night sweats. Women in this study were between 40 and 55 years of age and had moderate to severe psychological distress, defined as a score of 72 or greater on the Psychological General Well-Being Schedule. Only women with hot flashes were included in the current analysis.[115] In total, 120 women were randomly assigned to an ethyl-eicosapentaenoic acid (E-EPA) plus omega-3 fatty acid supplementation or placebo for 8 weeks. The E-EPA supplementation was a 500-mg capsule taken three times daily, with each capsule containing 350 mg of eicosapentaenoic acid (EPA) and 50 mg of docosahexaenoic acid (DHA). The baseline level of hot flashes was an average of 2.8 per day. After 8 weeks, the frequency of hot flashes decreased by a mean of 1.58 per day in the E-EPA group and by only 0.50 per day in the placebo group. There was a significant reduction of 55% in the frequency of hot flashes in the E-EPA group compared with only a 25% decrease in the placebo group. There was also a greater responder rate in the E-EPA group of 58.5%, versus 34.4% in the placebo group. However, there were no differences in the severity of hot flashes or in quality-of-life scores between the two groups.

In another fish oil–hot flash study, 20 perimenopausal or postmenopausal women received 8 weeks of fish oil (840 mg EPA and 375 mg DHA). Hot flashes were monitored using diaries and the hot flash–related daily interference scale. The response rate was 70% (a Montgomery-Åsberg Depression Rating Scale [MADRS] score decrease of 50% or more; the remission rate was 45%). Responders had significantly lower pretreatment DHA levels than nonresponders did, with a pretreatment score of 4.3 and posttreatment score of 1.8 for daytime hot flashes and a pretreatment score of 4.6 and posttreatment score of 0.7 for night-time hot flashes.[116]

A 2018 systematic review and meta-analysis found that although fish oil may reduce the severity and frequency of night sweats, no other benefit on vasomotor symptoms was observed. The lack of controlled trials, however, prevents any firm conclusions.[117]

Vitamin E

In the late 1940s, several clinical studies found vitamin E to be effective compared with placebo in relieving hot flashes and menopausal vaginal complaints.[118,119] Unfortunately there have been no further clinical investigations, aside from one small randomized trial in 2007, which found reductions in both hot flash severity and frequency.[120] In one of the earlier studies, vitamin E supplementation was shown to improve not only the symptoms but also the blood supply to the vaginal wall when taken for at least 4 weeks.[118] A follow-up study published in 1949 demonstrated that vitamin E (400 IU/day) was effective in about 50% of postmenopausal women with atrophic vaginitis.[119]

Vitamin E oil, creams, ointments, or suppositories can be used topically to provide symptomatic relief of atrophic vaginitis. Vitamin E may be effective in relieving the dryness and irritation of atrophic vaginitis as well as other forms of vaginitis.[107]

Botanical Medicines

Many plants have been shown to exert a tonic effect on the female glandular system. As a class, these botanicals are often referred to as "uterine tonics." Much of their effect is thought to result from phytoestrogens in the plants as well as the plants' ability to improve blood flow to the female organs. Some of the botanicals used in menopausal women work to nourish and tone the female glandular and organ system rather than exerting a druglike effect. This nonspecific mode of action makes many botanicals useful in a broad range of female conditions. Other plants are used for specific symptoms, such as valerian for insomnia or chaste tree for dysfunctional uterine bleeding. Their mechanisms of action do have pharmaceutical effects, whether it be a sedative effect in the case of valerian or increasing luteinizing hormone and an indirect progesterone-like effect in the case of chaste tree. Mechanisms of action of other botanicals have so far eluded us.

Phytoestrogens are found in many medicinal herbs with a historical use in conditions that are now treated by estrogens. Phytoestrogen-containing herbs offer significant advantages over the use of estrogens in the treatment of menopausal symptoms. Although both synthetic and natural estrogens may pose significant health risks, phytoestrogens have not been associated with these side effects. In fact, epidemiological data and experimental studies in animals have demonstrated that phytoestrogens are extremely effective in inhibiting mammary tumors, not only because they occupy estrogen receptors but also through other unrelated anticancer mechanisms.[121,122]

Plants manufacture thousands of chemical compounds that are vital to the health and function of the plant. Those chemical compounds, generally known as micronutrients, are consumed in the diet by humans whenever the plants are eaten. One of these classes of chemical compounds manufactured by plants comprises the phytoestrogens. More than 300 plants contain phytoestrogen compounds. They compose a large part of our diet and are found in medicinal plants as well. There are several subclassifications of phytoestrogens; the partial list in Table 196.2 may be helpful.

Phytoestrogens in medicinal herbs are capable of exerting estrogenic effects, although their activity is at most only 2% as strong as that of estrogen.[123] Isoflavones have a structure similar to that of endogenous steroidal sex hormones. They have the ability to bind to estrogen receptors on human cells; in women, they have a preference for binding to the beta form of the estrogen receptor. As a result, they preferentially express estrogenic effects in the central nervous system, blood vessels, bone, and skin without causing stimulation of the breast or uterus.[124]

TABLE 196.2 Sources of Phytoestrogens

Phytoestrogen	Sources
Lignans	Vegetables, fruits, nuts, cereals, spices, seeds—especially flaxseeds
Isoflavones	Spinach, fruits, clovers, peas, beans—especially soy
Flavones	Beans, green vegetables, fruits, nuts
Chalcones	Licorice root
Diterpenoids	Coffee
Triterpenoids	Licorice root, hops
Coumarins	Cabbage, peas, spinach, licorice, clover
Acyclics	Hops

In light of often confusing and contradictory research on menopausal HT, with slight risks as well as benefits, even more women are looking for safe and effective botanical alternatives for symptom relief. Botanical alternatives for menopausal symptoms are increasingly popular despite limited research to demonstrate efficacy. Many women are determined to use nonhormonal therapies, bioidentical hormones, or lower-dose hormones in combination with botanicals in order to create a risk/benefit ratio they feel comfortable with.

Black Cohosh *(Cimicifuga racemosa)*

In the last 30 years, black cohosh has emerged as the most studied of the herbal alternatives to hormone replacement therapy for menopause symptoms (see Chapter 66 for a complete description). Despite some studies demonstrating no benefit of black cohosh, the collective findings in black cohosh studies and long-term clinical anecdotal evidence on black cohosh indicate that it is most effective for menopause symptoms of daytime or nighttime hot flashes, mood swings, sleep disorders, and body aches.[125]

In one of the largest studies, 629 women with menopausal complaints were seen by 131 general practitioners.[126] In this study, as early as 4 weeks after beginning the therapy, a clear improvement in the menopausal ailments was seen in approximately 80% of the women. After 6 weeks, complete disappearance of symptoms occurred in approximately 50%.

Some recent studies have used black cohosh in combination with other botanical extracts. For example, in a trial of black cohosh combined with St. John's wort, healthy perimenopausal women with typical climacteric symptoms and not on HT for at least the previous 3 months were given a 264-mg tablet containing 0.364 mL of extract from black cohosh, equivalent to 1 mg terpene glycosides, and 84 mg of St. John's wort extract with 0.25 mg hypericin.[127] Forty-two women in the treatment group and 35 women in the placebo group completed the study. Mean Kupperman Index scores at 4 and 12 weeks were significantly lower in the treatment group ($P \leq 0.002$). At the end of the study, the average decrease in the Kupperman Index was 20 points in the treatment group and only 8.2 points in the placebo group ($P < 0.001$). Vaginal dryness and low libido were two symptoms that did not improve, but the average hot flash score was significantly lower in the black cohosh/St. John's wort group.

In 2007 a clinical trial of a combination of black cohosh, red clover, soy, chaste tree, valerian, and vitamin E resulted in a significant lowering of the mean score value of the Kupperman Index after 4 and 6 months, yet it was found equal to placebo after 2 months.[128] This multicenter, randomized, double-blind, placebo-controlled trial included 125 symptomatic postmenopausal women 45 to 65 years of age. The

supplement tested contained 72 mg of total isoflavones, with 60 mg of soy isoflavones and 12 mg of red cover isoflavones, in combination with the following: 40 mg of black cohosh extract, 30 mg of chaste-tree extract, 250 mg of valerian extract, and 121 mg of vitamin E. After 2 months, the reduction in mean score value of the Kupperman Index was the same in the placebo and treatment groups. At months 4 and 6, the Kupperman Index was significantly lower in the treatment group (4 months: 13.6 score for placebo and 11.1 for treatment; 6 months: 12.2 for placebo and 9.6 for treatment). Secondarily, lipids were evaluated. No difference was seen in total cholesterol or HDL-C, but there was a nonsignificant decrease ($P = 0.0608$) in LDL-C and a statistically significant reduction in triglycerides ($P = 0.0151$) in the herbal treatment group. The study investigators' and patients' Clinical Global Impression scores for the treatment group were superior compared with placebo. Both herbal and placebo groups tolerated the treatment well, but a few individuals had mild and temporary side effects.

Also in 2007, black cohosh was studied for its effects on lipids, fibrinogen, glucose, and insulin. In total, 351 perimenopausal or postmenopausal women aged 45 to 55 were randomized to a 3-month double-blind trial of either (1) black cohosh extract at 160 mg/day; (2) a multibotanical formula containing black cohosh, alfalfa, chaste-tree berry, dong quai, false unicorn, licorice root, oats, pomegranate, Siberian ginseng, and boron; (3) a multibotanical formula administered with boron and a soy diet with dietary counseling; (4) 0.625 mg of conjugated equine estrogen with or without 2.5 mg of medroxyprogesterone acetate (women with a uterus received both medications, whereas women without a uterus received the estrogen only); or (5) a placebo.[129] Although the primary objective of the study was to determine the effects on hot flashes, the secondary measures included the effects on lipids, fibrinogen, glucose, and insulin. Study participants were women who were experiencing hot flashes, mostly white women with college degrees. The average total and LDL-C levels were borderline high (219 and 146 mg/dL); HDL-C was medium at 53 mg/dL, and triglycerides were normal at 107 mg/dL. At the 3-month mark, there were no statistically significant treatment effects on total cholesterol, LDL-C, HDL-C, triglycerides, fibrinogen, glucose, or insulin among women who had any of the three herbal regimens (all three of which contained black cohosh). The evidence from this randomized controlled trial of perimenopausal and postmenopausal women does not support any short-term benefit or adverse effects of black cohosh on lipids, fibrinogen, glucose, or insulin when used alone, in combination with other botanicals, or in conjunction with soy consumption. This is reassuring in that black cohosh does not appear to affect any thrombotic biomarkers, as does oral estrogen therapy (OET), nor does it increase triglycerides, as seen with OET.

A 2014 systematic review also found that there was no association between black cohosh and breast cancer risk (with two studies reporting a significant decrease in risk).[130]

Hops *(Humulus lupulus)*

Hops have shown positive effects for mood issues, such as anxiety and restlessness, and for sleep disruptions in menopausal women. In one randomized, double-blind, placebo-controlled study, 67 menopausal women were given either a placebo or 120 mg or 300 mg of standardized hop extract (providing 100 mcg and 250 mcg of 8-prenylnaringenin, respectively) for 12 weeks.[131] At 6 weeks, the 120-mg dose was significantly superior to placebo, but not at 12 weeks. Even so, there was a more rapid decrease in menopausal symptoms scored for both doses of hop extract, especially the hot flash score. The higher dose was not any better than the lower one.

In 2016 another randomized controlled trial found improvement in the Greene Climacteric Scale as well as a reduction in the number of hot flashes, using 500 mg powdered flowering hops (corymb).[132]

Another double-blind study in 36 menopausal women indicated significant reductions in the Kupperman Index and the visual analog scale (VAS) for the hops group and a marginal reduction in symptoms for the menopause rating scale (MRS) after 16 weeks.[133]

Maca *(Maca peruvianum)*

Research on perimenopausal and menopausal women using a proprietary maca product (Maca-GO) found that, unlike hormone replacement therapy (HT) and phytoestrogenic botanicals, maca can increase the body's production of estrogen—versus simply adding estrogen replacement to the body—and lower levels of cortisol and adrenocorticotropic hormone.[134] What makes this especially interesting is that, as evidenced by other research conducted on the composition of various powdered preparations of maca root, it appears that the herb does not contain plant estrogen or hormones.[135–138] It has been suggested that maca's therapeutic actions rely on plant sterols stimulating the hypothalamus, pituitary, adrenal, and ovarian glands and therefore also affecting the thyroid and pineal glands. Thus it has effects in improving sleep, mood, fertility, energy, and hot flashes. As such, maca tends to treat menopausal symptoms as a whole rather than only one specific symptom of menopause (such as hot flashes).

In one double-blind, randomized 4-month study of 20 early-postmenopausal women, patients were given either placebo or two 500-mg capsules of Maca-GO twice daily for a total of 2 g/day.[134] Menopausal symptoms were assessed according to Greene's Score and the Kupperman Index. After 2 months, this maca product stimulated estradiol production and suppressed FSH, T3, adrenocorticotropic hormone, and cortisol. It also had a small effect on increasing bone density and alleviated numerous menopausal symptoms, including hot flashes, insomnia, depression, nervousness, and diminished concentration.[19]

A small randomized, double-blind, placebo-controlled crossover trial comprising 14 postmenopausal women was completed using 3.5 g of powdered maca *(Lepidium meyenii)* for 6 weeks and matching placebo for 6 weeks.[139] Measurements of estradiol, FSH, luteinizing hormone (LH), and sex hormone–binding globulin (SHBG) were taken at baseline and then at weeks 6 and 12. The Greene Climacteric Scale was used to assess the severity of menopause symptoms. Serum concentrations of estradiol, FSH, LH, and SHBG were similar in both groups. The Greene Climacteric Scale revealed a significant reduction in psychological symptoms, including anxiety, depression, and sexual dysfunction, after maca consumption compared with baseline and placebo. These findings were independent of androgenic or alpha-estrogenic activity present in the maca, using assays to measure hormone-dependent activity.

Red Clover *(Trifolium praetense)*

Red clover, a member of the legume family, has been used worldwide as a source of hay for cattle, horses, and sheep and used by humans in the past as a source of protein in the leaves and young sprouts. Historically it has also been recognized as a medicinal plant for humans and, more recently, as a menopausal herb. The principal substances of red clover are the flavonoid glycosides, coumestans, volatile oils, L-dopa, caffeic acid conjugates, polysaccharides, and some miscellaneous resins, fatty acids, hydrocarbons, alcohols, chlorophylls, minerals, and vitamins.

At least six clinical trials have been conducted on the effect of red clover isoflavones on vasomotor symptoms; three showed benefit and three did not. The first two published studies on red clover and vasomotor symptoms showed no statistically significant difference between the red clover standardized extract and placebo during a 3-month period, although both groups did improve.[140,141] It was suggested that the negative results of these studies were due to inadequate controls

and that women in the control group were in fact obtaining meaningful amounts of phytoestrogens in their diets. Two other studies using 40 mg of standardized red clover extract produced a 75% reduction in hot flashes after 16 weeks in 30 women. The difference between placebo and red clover isoflavones was statistically significant ($P < 0.001$).[142] A similar study evaluating 40 mg of red clover standardized isoflavones for 2 months in 23 postmenopausal women found that the red clover users had a 54% reduction in hot flashes versus 30% in the placebo group.[143] Two more recent studies continue the contradictions. In 2002 the use of 80 mg of isoflavones daily resulted in a significant reduction in hot flashes compared with baseline.[144] Another study, called the ICE study, compared two different doses of red clover isoflavones—82 mg and 57 mg per day—with placebo for 12 weeks. The reductions in the mean daily hot flash count at 12 weeks were similar for both treated groups as well as the placebo group.[145]

Other intriguing effects of red clover reported by these studies are as follows: no endometrial thickening and an increase in HDL-C, as well as no abnormalities in liver function parameters, complete blood count, or estradiol determination. Last, a published study showed that red clover isoflavones may reduce the risk of coronary vascular disease by increasing arterial elasticity by 23%.[146,147] A 2016 systematic review and meta-analysis found that red clover was associated with a lower frequency of hot flashes and vaginal dryness, and objective markers of vaginal atrophy were also improved.[148]

Siberian Rhubarb *(Rheum rhaponticum)*

A special extract, ERr 731, from the roots of rhapontic rhubarb has been in wide use in Germany since 1993, specifically for the treatment of menopausal symptoms. This medicinal species of rhubarb is not the same species as the garden rhubarb used for food. Other rhubarb species included *Rheum officinale, Rheum palmatum,* and *Rheum polygonatum;* these have been traditionally used as laxatives owing to their content of anthraquinone glycosides such as emodin and aloe-emodin. The *R. rhaponticum* used in clinical studies and in the extract form of Err 731 does not contain any emodin or aloe-emodin and thus has no laxative effects.

A standardized extract, ERr 731, from the roots of *R. rhaponticum,* also known as Siberian rhubarb, was studied in a 12-week randomized, double-blind, placebo-controlled clinical trial in 109 perimenopausal women with climacteric complaints. One tablet (250 mg) containing 4 mg of dry extract was used in the treatment group ($n = 54$), whereas the control group ($n = 55$) received a placebo. The primary outcome was the change in the Menopause Rating Scale II (MRS II). After 12 weeks, the MRS II total score and each MRS II symptom significantly decreased in the rhubarb extract group compared with the placebo group ($P < 0.0001$). The overall menopause quality-of-life score was also significantly better in the treatment group compared with placebo. No adverse events were observed.[149]

Another randomized, double-blind, placebo-controlled clinical trial of the standardized extract of *R. rhaponticum* was conducted in 109 perimenopausal women with menopausal symptoms, including anxiety. One tablet containing either 250 mg of ERr 731 (containing 4 mg of *R. rhaponticum* dry extract) or placebo was given for 12 weeks. After only 4 weeks of treatment, the Hamilton Anxiety Scale total score and anxiety score for ERr 731 group decreased significantly. This was maintained after the 4 weeks and was even more significant after 12 weeks of treatment. Anxiety decreased from moderate or severe to slight in 33 of 39 ERr 731 women. Quality-of-life issues also increased in the ERr 731 group far more significantly than in the placebo group, by 22.4 points versus 7.6 points.[150]

In an observational study, 363 menopausal women with menopausal symptoms were given one 4-mg tablet of ERr 731 for 6 months. The MRS was used to evaluate symptoms, and a change in the MRS was the primary outcome. After 6 months of treatment, 252 women completed the study. At that point, there was a significant decrease in the total MRS score from an average of 14.7 points at baseline to 6.9 points ($P < 0.0001$)—a decrease of 7.8 points. The most pronounced improvement occurred within the first 3 months of treatment in those women who were the most symptomatic at baseline, with a score equal to or greater than 18 points. The most significant improvement was for symptoms of hot flashes, irritability, sleep problems, depressive mood, and physical/mental exhaustion.[151]

St. John's Wort *(Hypericum perforatum)*

Research on St. John's wort extract has focused mainly on mild to moderate depression (see Chapter 88), and several studies have also considered menopausal symptoms. In a study published in 2010, a total of 100 women with an average age of 50 participated in a randomized, double-blind, placebo-controlled clinical trial comparing St. John's wort with placebo in women with perimenopausal/menopausal hot flashes.[152] Fifty women received 20 drops three times daily of St. John's wort extract (Hypiran) containing hypericin 0.2 mg/mL, and 50 women received a placebo of distilled water. The study duration was 2 months. Clinical examinations and interviews were performed at baseline, 4 weeks, and 8 weeks. Treatment effectiveness was measured by the Blagg–Kupperman Index. Evaluation of the frequency, duration, and severity of hot flashes was the main objective of the study. Forty-five women in the treatment group and 43 in the control group completed the study.

In women taking St. John's wort, the frequency of hot flashes began to decline during the first and second months, but more improvement was shown during the second month. There was no statistical change in hot flash frequency during the first month of placebo but some improvement during the second month. Women who used St. John's wort showed more improvement in frequency than did those on placebo. The decline in the duration of hot flashes was statistically significant at week 8, and the decline was much more evident in the St. John's wort arm. The severity of hot flashes was relieved by St. John's wort during the 2 months of treatment and was more significant in the second month. Women in the placebo group did not show any significant decrease in the severity of hot flashes during the first month. They did have some improvement during the second month, but not as much as those in the St. John's wort group.

Another double-blind, randomized clinical trial studied the effect of St. John's wort extract compared with placebo on symptoms and quality of life of 47 symptomatic perimenopausal women aged 40 to 65 with three or more hot flashes per day.[153] Women were randomly assigned to receive a St. John's wort extract (900 mg three times daily) or a placebo. After 12 weeks of treatment, a nonsignificant difference in favor of the St. John's wort group was observed on the daily hot flash frequency and the hot flash severity scores. After 3 months of treatment, women in the St. John's wort group reported significantly better quality-of-life scores and significantly fewer sleep problems compared with those on placebo.

A drug-monitoring study conducted in women with menopausal symptoms using 900 mg of St. John's wort for 12 weeks found that about three-quarters of the women experienced improvement in both the self-rating scale and the physician rating; they also improved significantly in psychological and psychosomatic symptoms as well as a feeling of sexual well-being.[154]

Several double-blind studies have used a combination of St. John's wort and black cohosh extract. These studies have been described previously.

Although it appears to be common for practitioners to use chaste tree *(Vitex agnus castus)* in perimenopause and menopausal women

for symptom relief, there have been no mono-ingredient studies on this plant for these symptoms. Vitex has been shown to be helpful when combined with black cohosh. The most recent menopause-related study using Vitex was in combination with St. John's wort. In this double-blind, randomized, placebo-controlled 16-week trial of late-perimenopausal or postmenopausal women who reported hot flashes and other menopause symptoms, the herbal combination showed no significant difference from that of placebo.[155]

Kava *(Piper methysticum)*

Kava has most often been associated with analgesic, sedative, anxiolytic, muscle relaxant, and anticonvulsant effects. It is not typically thought of as an herb for menopause; however, anxiety, irritability, tension, nervousness, and sleep disruption are common perimenopausal and menopausal symptoms for which kava can offer some help. Several controlled trials have investigated the value of kava for menopausal symptoms.[156–158] The main results noted were a significant reduction in anxiety and depression. For more information on the pharmacology of kava, see Chapter 103.

Ginseng *(Panax ginseng)*

Panax ginseng, also known as Korean or Chinese ginseng, contains at least 13 different triterpenoid saponins, collectively known as ginsenosides. Whether it involves reducing mental or physical fatigue,[159–162] enhancing the ability to cope with various physical and mental stressors by supporting the adrenal glands,[163] or treating the atrophic vaginal changes due to lack of estrogen,[164] ginseng is a valuable tool for many menopausal women. In one trial, a standardized extract of *P. ginseng* was studied in 384 postmenopausal women.[165]

In 2016 a systematic review of ginseng was published, indicating evidence of improvement in total hot flash score, as well as sexual function and arousal. No effect was consistently found for hot flash frequency, hormone levels, or endometrial thickness.[166]

Depression and well-being showed a statistically significant improvement with ginseng. In another randomized controlled trial, 1 month of *P. ginseng* increased energy and decreased insomnia and depression.[167] These results indicate that *P. ginseng* can significantly improve the general sense of well-being of women going through menopause.

Kudzu *(Pueraria mirifica)*

Pueraria mirifica was examined for its effect on vaginal symptoms, vaginal health index, vaginal pH, and vaginal cytology in postmenopausal women.[168] In this randomized, double-blind, placebo-controlled study, 51 women were given either 20, 30, or 50 mg of *Pueraria mirifica* or placebo daily for 24 weeks. After 12 weeks of treatment, significant improvements in vaginal symptoms were seen, and they were maintained over the study period. The mean vaginal dryness symptoms decreased at all the herbal doses, but the results were not significantly different from those seen in the placebo group. The frequency of dyspareunia decreased from 56.9% to 39.2% in the study group, whereas it did not change in the placebo group. The changes in the vaginal health index (scoring vaginal appearance with regard to moisture, fluid volume, elasticity, epithelial integrity, and pH on a scale of 1, poorest, to 5, best) were significantly improved in the herbal group, as noted in weeks 12 and 24. Before treatment, the mean vaginal pH was 8.41 in the treated group; after 12 and 24 weeks, the mean pH was 5.52 and 5.83, respectively. After 12 weeks of treatment, most measures of vaginal health in the treated group were significantly higher than in the placebo group.

After 12 and 24 weeks of treatment, the maturation value and maturation index were also significantly higher in the study group than in the placebo group. In essence, *P. mirifica* improved the parabasal:intermediate:superficial cells ratio, which is what occurs when vaginal estrogen is used. There were no statistically significant changes in endometrial thickness and no significant difference in adverse effects between the treatment and placebo groups.

A 12-week prospective and randomized trial published in 2017 found that although *P. mirifica* gel was not as effective as CEE for improving signs of vaginal atrophy, it did improve the vaginal maturation index significantly, with no significant difference in symptom scores between the two groups.[169]

Dong Quai *(Angelica sinensis)*

By far the most popular use of *Angelica* species has been the use of *Angelica sinensis* in the treatment of menopausal complaints. Although a double-blind, placebo-controlled study showed no significant benefit, the preparation used (a dried aqueous extract) was clearly lacking some of the important volatile compounds, although it was standardized for ferulic acid content.[170] In addition, the traditional use of *Angelica* has been in combination with other plants. A study conducted in China showed that a combination of *A. sinensis, Paeonia lactiflora, Ligusticum monnieri, Atractylodes chinensis, Sclerotium poriae,* and *Alisma orientalis* was effective in roughly 70% of women experiencing menopausal symptoms.[171] Although not a double-blind study, this study shows promise for using *Angelica* in the management of menopausal symptoms in combination with other compounds. Also, in a double-blind study, the combination of 100 mg *Angelica,* 60 mg soy isoflavones, and 50 mg of black cohosh extract significantly reduced menstrual migraines.[172]

Interestingly, in an in vitro study with human bone, the aqueous extract of *A. sinensis* was found to directly stimulate proliferation, alkaline phosphatase activity, protein secretion, and type I collagen synthesis in a dose-dependent manner.[173] These results indicate the *A. sinensis* may have an effect on preventing age-related bone loss.

Valerian

A double-blind study on the impact of valerian for sleep quality in postmenopausal women who were experiencing insomnia concluded that valerian may be helpful. The postmenopausal women studied were generally healthy women 50 to 60 years of age who were menopausal for at least 1 year, were not using a hormone therapy, and were experiencing insomnia as evaluated by the Pittsburgh Sleep Quality Index (PSQI). One group of women was given capsules containing 530 mg of concentrated valerian extract twice per day, and the other group was given a placebo twice per day, for 4 weeks. A statistically significant change was reported in the quality of sleep in the valerian group compared with the placebo group. The average score on the sleep scale before valerian was 9.8, and after valerian, it was 6.02. The placebo group had an initial average sleep scale score of 11.1 and a score of 9.4 after placebo. Overall, 30% of the women taking valerian and 4% taking placebo reported an improvement in their sleep quality.[174]

Interestingly, although valerian is typically used to improve sleep, a 2018 triple-blind randomized trial also found it to be effective for reducing hot flash frequency when given at 530 mg, twice per day.[175]

Conventional Medications

Nonhormonal options for hot flashes include clonidine, usually 0.1 to 0.2 mg daily at bedtime. The neuroleptic Neurontin is administered at 300 mg three times daily. Venlafaxine and paroxetine have shown a reduction in hot flashes in studies using 37.5 to 75 mg and 10 to 20 mg daily, respectively. However, selective serotonin reuptake inhibitors and selective norepinephrine reuptake inhibitors have occasionally been shown to cause vasomotor symptoms in both men and women.

Bellergal, an ergot and belladonna combination, was used for many years for vasomotor symptoms but is now available only from compounding pharmacies. There are no studies, and empirical reports show mixed results.

HORMONE THERAPY

Recommending estrogen/progestogen HT, regardless of its source or makeup, requires weighing the benefits and risks of short- and long-term treatment. These benefits and risks continually change for any individual woman as she ages, as her menopause-related symptom picture and her general health change. Risk factors are related to baseline diseases and the risks they incur, age, age at menopause, and cause of menopause (surgical, physiological, medication induced), as well as the prior use of HT, including route of administration and response or medical problems that emerged during HT treatment.

It is easy for practitioners to be confused by the last 60 years of research on HT, but understanding the benefits and risks of HT is critical to sound clinical decision making and the education of patients. Any HT, whether bioidentical or nonbioidentical, should be used at the lowest dose, for the shortest duration, and in the safest way possible. Each type of estrogen and progestogen, route of administration, timing of initiation, and duration of use will have distinct benefits and adverse effects.

The NAMS has strongly recommended that new uniform and consistent terminology be adopted in describing HT in menopause management. The NAMS menopausal HT terminology is as follows[4]:

- EPT: combined estrogen-progestogen therapy (form of estrogen and progestogen is not specific)
- ET: estrogen therapy (the form of estrogen is not specific)
- HT: hormone therapy (encompassing ET and EPT; the form of estrogen and progestogen is not specific)
- Local therapy: vaginal ET administration that does not result in clinically significant systemic absorption (the form of estrogen is not specific)
- Progestogen: encompassing both progesterone and progestin
- Systemic therapy: HT administration that results in absorption in the blood high enough to provide clinically significant effects
- Timing of HT initiation: length of time after menopause occurs when HT is initiated

Additional terms that can be helpful in making distinctions between hormone regimens (not NAMS terminology) are as follows:

- cHT: conventional HT (FDA-approved prescription items)
- cET: conventional estrogen therapy (FDA-approved prescription items)
- bHT: bioidentical HT (FDA-approved prescription items)
- bET: bioidentical estrogen therapy (FDA-approved prescription items)
- cbHT: compounded bioidentical HT (from a compounding pharmacy)
- cbET: compounded bioidentical estrogen therapy (from a compounding pharmacy)

Bioidentical or Natural Hormones

One of the greatest areas of confusion in menopausal management today is the subject of bioidentical or natural hormones. Bioidentical estrogens require a prescription and are available from regular pharmacies or as nonpatented forms prepared by compounding pharmacies. The advantages of conventional pharmaceutical HT include years of scientific study and the assurance of standardization. Insurance coverage generally pays for pharmaceutical hormone prescriptions but does not always pay for compounded hormones. Pharmaceutical preparations are limited in dose forms and combinations; they also contain additives, binders, adhesives, and/or preservatives. Occasionally these substances can cause side effects, including skin reactions, headaches, and digestive problems.

The use of compounded forms of hormones has three distinct inarguable advantages in that there is a greater array of dosing, hormone combinations, and delivery options. Customized doses of a particular hormone are available that pharmaceutical companies do not make. Compounded hormones also are available in capsules, sublingual lozenges or pellets, creams, gels, vaginal creams/gels or tablets, nasal sprays, injections, and subcutaneous implanted pellets. In addition, any combination of estradiol, estriol, estrone, progesterone, testosterone, and DHEA can be formulated in a compounded hormone prescription to best meet the individual needs of the patient.

Bioidentical Estrogens

Some would argue that the advantage of conventional pharmaceutical company HT is that it has undergone years of scientific study. Although this is true, there has been little effort to make distinctions between different kinds of estrogens in particular. To a lesser degree but still true, there has not been much effort to distinguish between the various progestins (synthetic) and bioidentical progesterone. Whereas many alternative practitioners point to the superior safety profile of bioidentical estrogens over nonbioidentical estrogens, it cannot be stated with science-based facts that an equivalent dose of bioidentical estradiol is innately safer or better than the synthetic form or CEE. Whereas logic and common sense might lead us to assert that a bioidentical estrogen is more akin to the body's enzymatic pathways, bowel flora, and general physiology, thus leading to enhanced safety, we have yet to adequately prove this. Indeed, a retrospective analysis of previous studies published in 2018 suggested that the breast cancer reduction benefit with CEE alone is greater than with bioidentical estrogen alone.[176] For now, it remains in the realm of hypotheticals and philosophical issues.

The ability to individualize prescriptions with compounded bioidentical hormones can provide endless customized options with the ability to be flexible in dosing and delivery according to the needs of each woman. If for no other reason, this is compelling enough reason to prescribe compounded bioidentical hormones. The combination of estriol with estradiol and the use of bioidentical progesterone and testosterone with the estrogens provides the maximum potential benefit and a more individualized approach for each patient. The hope is that there is less risk that nonbioidentical hormones in the case of combining the weaker estriol with estradiol/estrone.

Estriol seems to be helpful in treating many of the symptoms of menopause, such as hot flashes. Vaginal estriol for vaginal dryness is considered a potentially preferred form of vaginal estrogen owing to the dominance of estriol receptors in the vagina and vulva.

Alternative practitioners often use estriol to treat menopausal symptoms because it is thought to have a better safety profile than estradiol and estrone. Estriol is about one fourth as potent as estradiol.[177]

Estriol can be taken orally in capsules or tablets and intravaginally as a cream. Vaginal estriol creams and suppositories have been shown to restore normal vaginal cytology[178] and to decrease the incidence of bladder infections.[179]

These creams most likely work by restoring the vaginal flora; improving vaginal and bladder health; and increasing the lubrication, elasticity, and thickness of the vaginal epithelium. A common prescription is 1 mg of estriol per gram of cream inserted vaginally daily for 2 weeks and then twice a week for maintenance.

A popular practice for prescribing compounded bioidentical estrogens is to combine the lower dose and perhaps potentially safer effects of estriol with small doses of estradiol and estrone. Currently, those

who prescribe a triple-estrogen compound typically use a formula composed of 80% estriol, 10% estradiol, and 10% estrone. Progesterone is added to the formula at a minimum of 100 mg/day to protect the uterus from the potential effects of the estrogen in thickening the lining of the endometrium. Use of estrogen only, without the proper dose of progesterone, in women with a uterus might put them at risk for endometrial hyperplasia or even endometrial (uterine) cancer.

A biestrogen formulation, with estriol and estradiol, is increasingly popular because of concerns that estrone may be associated with more carcinogenic estrogen metabolites, which are associated with an increase in the risk of breast cancer. (Recommended dose ratios for both triestrogen and biestrogen formulations are discussed later.)

Progesterone

Progesterone is available with a prescription as oral capsules, sublingual drops, sublingual pellets, lozenges, transvaginal or rectal suppositories, and by injection. Progesterone is also available over the counter as a cream. Progesterone is added to a compounded biestrogen or triestrogen formulas at a minimum of 100 mg/day to protect the uterus.

For women with a uterus, a progestogen must be added to any estrogen preparation to prevent endometrial hyperplasia and uterine cancer. *Progesterone* is a natural hormone made by the ovaries, and its main function is to support pregnancy. *Progestin* is the term applied to the synthetic derivatives, which differ in biochemical structure from progesterone. Progestins used in conventional HT and birth control pills often account for the side effects that patients experience, such as irritability, depression, bloating, and mood swings. Progestins tend to cause water retention, can affect brain chemistry, and alter other steroid pathways. *Progestogen* is the term applied to any substance possessing progesterone qualities. It can refer to progesterone or progestin.

The advantages of bioidentical progesterone over progestins are better validated than are the advantages of estrogens, although, as mentioned previously, an increase in risk for endometrial cancer with oral micronized progesterone but not synthetic progestins in both the French and European studies is concerning.

Bioidentical progesterone minimizes the side effects associated with progestogens and has a more favorable effect on lipid profiles and cardiovascular function.[180]

In some women, insomnia, fatigue, anxiety, and mood swings may be more responsive to progesterone than estrogen.

Progesterone Cream

Bioidentical progesterone by itself can also be used very effectively in perimenopause. Problems that can be addressed include regulation of the menstrual cycle, hot flashes, night sweats, mood swings, sleep disruption, and premenstrual symptoms.

A transdermal progesterone cream was studied for its ability to control vasomotor symptoms (hot flashes) and in order to evaluate its ability to prevent bone loss. In this study, 102 healthy women within 5 years of menopause were randomly assigned to receive either transdermal progesterone cream or a placebo.[181] Subjects were instructed to apply a 1/4 tsp of cream (this amount contained 20 mg progesterone or placebo) to the skin daily. Each also received a multivitamin and 1200 mg of calcium. Measurements included medical history, physical examination, DEXA scanning of the hip and spine, measurements of thyroid-stimulating hormone (TSH) and FSH, a lipids profile (cholesterol, etc.), and a regular blood chemistry profile. The women kept weekly symptom diaries and were seen every 4 months for 1 year. Bone density scanning and blood chemistry profiles were obtained again at the end of 1 year.

Before the initiation of the study, 30 of the 43 (69%) in the treatment group and 26 of the 47 (55%) in the placebo group had hot flashes. Twenty-five of 30 (83%) women in the treatment group experienced improvement or resolution of the hot flashes, and 5 of 26 (19%) placebo subjects showed improvement or resolution. The numbers of women who showed a gain in bone mineral density did not differ in the two groups.

The latest randomized clinical trial compared the effect of a transdermal natural progesterone cream (32 mg/day) with a placebo cream. Eighty postmenopausal women in Australia were randomly assigned to each group. They were evaluated using the Greene Climacteric Scale and the Menopause Quality of Life Questionnaire; serum lipid levels and bone markers were also monitored over 12 weeks. No detectable change was seen in vasomotor symptoms, moods, libido, serum lipid levels, or metabolic markers of bone turnover. There was a slight elevation of blood levels of progesterone. The researchers concluded that the 32 mg of transdermal progesterone was not sufficiently absorbed into the bloodstream to achieve biological effects.[182]

Testosterone

The majority of women treated with estrogen replacement have a resolution of their menopausal symptoms. For those who do not, and especially for those complaining of a loss of libido, estrogen with testosterone may be beneficial.

One study of early postmenopausal women (both natural and surgical) who were switched from estrogen alone to estrogen/testosterone therapy found that overall symptom relief was superior to estrogen-only therapy. Sex drive and satisfaction both increased.[183] A double-blind study of women dissatisfied with their HT regimens showed that sexual desire, satisfaction, and frequency of sexual activity were increased when they used the estrogen/testosterone combination.[184]

Other studies have shown that the combination of 1.25 mg of esterified estrogen and 2.5 mg of methyltestosterone given daily for 2 years after surgical menopause significantly reduced the intensity of hot flashes and vaginal dryness in 81% and 73% of women, respectively.[185]

A trial comprising 814 postmenopausal women with hypoactive sexual desire revealed that a 300-g testosterone patch improved the frequency of satisfying sexual episodes and decreased their distress.[186]

In this study, the women were not treated with estrogen or progesterone. Three excess cases of breast cancer were detected but were not statistically significant. Formulations of CEE and methyltestosterone combined either 0.625 or 1.25 mg of CEE with 5 mg of methyltestosterone. Other preparations come as either 1.25 or 0.625 esterified estrogens combined with 2.5 or 1.25 mg of methyltestosterone, respectively. At present, bioidentical testosterone can be obtained only from a compounding pharmacy, where 4 to 6 mg of bioidentical testosterone is generally formulated alone or together with the biestrogen or triestrogen formulation. Testosterone cream applied to the genital region can be used as an alternative delivery method. Common prescriptions are anywhere from 1 to 10 mg/g of cream. The cream is applied to the external genitalia just before sexual activity to enhance sensitivity to touch and orgasm. Such use should not occur more than twice a week to avert local testosterone side effects, such as clitoral enlargement. The NAMS concluded that "Postmenopausal women with decreased sexual desire who have no cause other than being postmenopausal, may be candidates for testosterone treatment." Other causes of low libido should be ruled out, and laboratory testing of testosterone levels should be used to monitor for supraphysiological levels before and during therapy. Testosterone therapy is contraindicated in women with breast or uterine cancer and in those with cardiovascular or liver disease. Testosterone should be given at the lowest dose for the shortest time that meets treatment objectives.

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is a bioidentical hormone and another of the androgens. It is the most abundant circulating steroid in

humans. DHEA is called a precursor hormone because it is produced in large amounts in the body and because other steroid hormones are made from DHEA, including estrogen and testosterone.

Many claims have been made about DHEA's effect on the immune system, and its antiaging properties are said to include better memory, less fatigue, more energy, relaxation, and increased ability to handle stress. It is also touted as having positive effects on bone density and helping to prevent cancer and cardiovascular disease. However, the exact effect DHEA has on the body's cells is unclear. Observationally, DHEA may increase energy, improve stress response, improve muscle mass, and occasionally increase libido. However, in the DAWN trial, DHEA supplementation of 50 mg/day did not improve well-being or cognitive performance in healthy older adults.[187] In a 1-year study with 10 mg/day of DHEA alone or in combination with HT in postmenopausal women, DHEA was able to restore the androgenic milieu and had a positive effect on estrogenic tone in postmenopausal women.[188] (There also appears to be an insulin-sensitizing effect of DHEA, reducing abdominal visceral fat, lowering insulin level, and producing a significant increase in insulin sensitivity.)[189]

Perhaps the most intriguing use of DHEA in menopausal women is for vaginal atrophy and/or sexual dysfunction. One study used three doses of DHEA ovules (0.25%, or 3.25 mg DHEA; 0.5%, or 6.5 mg DHEA; and 1.0%, or 13 mg DHEA) in 54 women; this was applied daily intravaginally for 12 weeks.[190] All three doses induced a significant beneficial change in vaginal epithelial cells, pH, and bothersome symptoms at 2 weeks. These benefits were accomplished with no effect on endometrial histology and no or minimal effects on serum estrogens and androgens and their metabolites. Another intravaginal DHEA study was a prospective, randomized, double-blind, placebo-controlled trial that evaluated the effect of daily local intravaginal DHEA ovules for 12 weeks in postmenopausal women.[191] The main assessment criteria were sexual dysfunction parameters of libido, arousal, orgasm, and dyspareunia in postmenopausal women who had vaginal atrophy. In this study, 218 postmenopausal women were randomized to receive a daily ovule of either no DHEA, 3.25 mg DHEA, 6.5 mg DHEA, or 13 mg of DHEA. At 12 weeks, compared with placebo, the 13-mg ovule showed a 68% improvement in the abbreviated sexual function arousal/sensation domain, in the arousal/lubrication domain by 39%, in orgasm by 75%, and in dryness during intercourse by 57%. DHEA also fared better than placebo in the desire domain of menopause-specific quality of life by 49% to 23%.

Subsequent randomized trials with intravaginal DHEA have supported the clinical benefits for dyspareunia, vaginal dryness, and vulvovaginal atrophy.[192]

Nonbioidentical Hormones

Nonbioidentical conventional HT includes estrogens manufactured by a pharmaceutical company that are not identical in chemical structure to an endogenous hormone. As examples, conjugated equine estrogens are derived from the urine of pregnant mares. Esterified estrogens are in part estrone sulfate and in part equilin sulfates. Progestins are synthetic substances.

Oral capsules containing 1 mg of bioidentical hormones are equivalent to 0.625 mg of CEE. Estrogen patches that contain 0.05 mg of bioidentical estradiol are considered to be equivalent to 0.625 mg CEE (Premarin) or 0.625 mg of esterified estrogens and equivalent to 1 mg of oral bioidentical estradiol. Current products are available through the NAMS.

Recommended dose ratios for triestrogen and biestrogen formulations with progesterone are as follows:

- A triestrogen formulation is considered comparable with 0.625 mg Premarin/2.5 mg Provera = estriol 1 mg/estradiol 0.125 mg/estrone 0.125 mg/Prog 50 mg 1 cap twice a day.
- Or estriol 2 mg/estradiol 0.25 mg/estrone 0.25 mg/Prog 100 mg 1 cap a day

  A biestrogen formulation is considered comparable with:
- 0.625 mg Premarin/2.5 mg Provera = estriol 1 mg/estradiol 0.250 mg/Prog 50 mg, 1 cap twice a day, or estriol 2 mg/estradiol 0.50 mg/Prog 100 mg, 1 cap a day
- Vaginal estriol: estriol cream 1 mg/g; insert 1 g nightly for 2 weeks then a maintenance of twice a week.

Perimenopause–Menopause Evaluation

The onset of perimenopause–menopause is an important time for a comprehensive health and lifestyle evaluation. A comprehensive medical history and complete physical examination are essential before initiating menopausal HT of any kind. Assessment of risk factors for stroke, CHD, venous thrombotic embolism, osteoporosis, diabetes, and breast/ovarian/uterine cancer is highly recommended. DEXA testing, lipid profiles, fasting glucose, and mammography should be performed according to national guidelines, age, and medical judgment. Other selected tests depend on age, symptoms, and other medical problems.

There is no one test for menopause. Tests to determine ovarian function are not routinely done because the diagnosis of perimenopause or menopause can largely be made based on the medical history. Practitioners can use hormone testing on an individual basis, mostly to differentiate menopause from thyroid problems, abnormal causes of a lack of menses such as elevated prolactin levels, or premature ovarian failure (premature menopause). The FSH test is not as accurate as one would like in a perimenopausal woman. The difficulty with FSH tests is that they can fluctuate immensely during perimenopause. What a woman's FSH is on any given day is not meaningful in diagnosing her symptoms as being due to menopausal changes or not. In a woman who is still having menses, especially irregular/random cycles, as is often the case in perimenopause, the FSH fluctuates unpredictably; it can easily be within normal range on a certain day and elevated on another. In fact, FSH tests are frequently normal in perimenopausal women, and a physician with lack of experience in menopausal/midlife patients may mistakenly attribute symptoms to depression, anxiety, or unknowns rather than to perimenopause. There are two regular scenarios to consider for ordering an FSH: (1) If a woman is suspected to be perimenopausal or menopausal and is using contraception, the FSH can be useful in determining whether she still needs contraception. An FSH above 30 mIU/mL that is also above 30 mIU/mL when repeated 1 month later would justify a diagnosis of menopause. It is important to continue the contraception during that 1-month period until this is determined. (2) If a woman is reporting irregular menses, irritability, fatigue, insomnia, and so on, an FSH and a TSH test will be helpful in sorting out her problems.

There is a popular notion, especially among alternative health care providers, that saliva or serum testing or urinary hormone testing can be done to determine hormone management. One major problem with this thinking is that there is little or no value in these tests in a perimenopausal woman for the purpose of diagnosing her hormone levels. What her levels are on the hour/day(s) of the test is the only information that is gleaned, but this is not diagnostic of perimenopause, and as mentioned, it is highly variable. There is no scientific evidence to support claims of increased efficacy, enhanced safety, or need for testing in order to determine the dosing of the hormonal prescriptions. In particular, there are numerous problems with saliva testing of estrogen and progesterone: (1) There is only a very small amount of these hormones in the saliva; (2) there are high false-positive elevations in those already taking a sublingual hormone; (3) there may be contamination of the saliva collection tube if a patient is already using

topical hormone creams/gels; (4) there is little proficiency testing; (5) varying technologies yield broad differences in results; (6) technical challenges are not adequately addressed by all laboratories conducting these tests; (7) there is a lack of scientifically proved accuracy; and (8) there are interfering components, such as food, beverages, medications, and chewing gum.

Salivary testing of cortisol and DHEA levels holds more promise because these do not fluctuate so much from day to day, we have a known daily rhythm of cortisol production, and there are naturally higher amounts of these in the saliva than of the hormones estrogen and progesterone.

Serum testing of estrogen, progesterone, and testosterone has proven to be more accurate with standardized methods of measuring. However, it is not often that even these are necessary tests in perimenopausal and menopausal women. For one, the reference ranges are often wide, and with testosterone in particular, we are not even sure what the normal reference range of testosterone is for women. Again, in a perimenopausal woman, estrogen levels in particular fluctuate from day to day and therefore are not valuable in diagnosis or management. In a postmenopausal woman, estrogen and progesterone levels are predictably low, as they are supposed to be.

Although saliva estrogen/progesterone testing has yet to prove its accuracy or efficacy, even if it were accurate, neither salivary nor serum hormone testing is necessary or even helpful for the perimenopausal woman because it is difficult to draw conclusions from test results when the hormones are in such a fluctuating state. There are so many peaks and valleys and erratic hormone activity that testing offers little value in most situations. For the women taking HT, it is tempting to think that the blood or saliva could be tested to determine dose. This is a popular recommendation in some consumer menopause books. However, there is no mathematical grid or equation comparing values of estrogen or progesterone or testosterone levels in the blood or saliva and how that would equate with a certain dose of the comparable hormone. There are reference ranges for these hormones, but we do not know exactly what dose to give in order to keep a patient within the reference range. Women absorb and metabolize hormones differently. The form of the hormones and the delivery method—oral, transdermal, sublingual, injection—are also different from woman to woman. In very occasional and selective cases, serum testing may be a helpful guide, but these are generally cases in which a woman is on HT and not doing well, and despite our best efforts with a good medical history and adjusting the dose, she still does not feel well. The majority of the time, it requires the practitioner's experience and menopause expertise and time to listen to the patient to know what dose to prescribe and what dosing adjustments and forms and deliveries of hormones may work best. Even if testing is done, it basically comes down to good clinical judgment and the willingness of the woman and her practitioner to try something else.

Urinary testing of estrogen metabolites can be considered in evaluating a woman's risk of health problems that may be associated with higher or lower levels of certain estrogen metabolites. Although such data are limited, the use of estrogen metabolism testing to try to gain some insight as to a patient's risk of cervical and breast cancer in particular or how to decrease the recurrence of these diseases is a preventive medicine practice that is hard to argue with. A complete discussion of the metabolites of the various forms of estrogen, progesterones, DHEA, and testosterone is beyond the scope of this chapter. As an example, the metabolites of one estrogen, estrone, are known to play both oncogenic and antioncogenic roles. Estrone's oncogenic metabolites, 4-hydroxyestrone (4-OH estrone), considered the most carcinogenic estrogen metabolite, and 16α-OH estrone (needed in small amounts because of its bone-building actions), are produced by Phase I metabolism. Estrone's protective metabolites (2-hydroxyestrone [2-OH estrone], 2-methoxyestrone [2-CH3O-estrone], and estriol [E3]) are produced in Phase II metabolism. The primary value of these tests is that they help the clinician to use nutrients, botanicals, and lifestyle modification to facilitate the optimal metabolism of these hormones through the pathways that lead to the potential for the reduction of breast and cervical cancers.

Practitioners should be encouraged to rethink the use of salivary and serum estrogen/progesterone testing in the diagnosis and management of perimenopausal/menopausal symptoms. In the perimenopausal woman, these hormones fluctuate within a day and from day to day. In the postmenopausal woman not on hormones, I would also assert that this is a poor use of a woman's funds. Her estrogen and progesterone levels are low. She is a postmenopausal woman, and Mother Nature had this in mind. It is rarely necessary to test the estrogen levels of a postmenopausal woman on HT for prescription management; however, there are times when testing may help to determine absorption/delivery and dosing issues when there is a lack of response or an adverse response to customary prescriptions. For select and very difficult cases, it is likely more useful to use the testing of neurotransmitters, amino acids, and nutrients while ensuring that due diligence has been done with regard to the basics of history, physical examination, and conventional testing.

THERAPEUTIC APPROACH

Menopause is a normal and natural part of aging, and each woman experiences it in her own way. However, premature, surgical, or medication-induced menopause is not normal and should be addressed with individual consideration as to the benefits and risks of each therapy. Using natural therapies, HT, other pharmaceuticals, or some combination of each is a personal decision for each woman. Our views of menopause and aging and our concerns about long-term health problems evolve over time. Balance is necessary, and the overmedicalization of menopause is inappropriate. The integrative provider can remind women that menopause can be a time of positive, life-changing insights, empowerment, and personal growth.

Many natural measures can help alleviate the most common symptoms of menopause. In most cases, HT either is not necessary or is needed for only 1 to 4 years. However, in women at high risk for osteoporosis and those who have already experienced significant bone loss and also have menopausal symptoms or do not tolerate osteoporosis medications, HT may be indicated. For women who have menopausal symptoms that they are not tolerating well, bHT, cbHT, or cHT can be used, with periodic attempts at reducing or discontinuing the hormones.

DIET

Consumption of foods that may provide modest symptom relief, such as soy foods, legumes in general, and flaxseeds, should be increased. A whole-foods, Mediterranean-type diet that addresses the prevention or management of cardiovascular disease, diabetes, and osteoporosis should also be attended to.

SUPPLEMENTS

- Vitamin E (mixed tocopherols): 800 IU/day until symptoms have improved, then 400 IU/day
- Hesperidin: 900 mg/day; or PCOs from grape seed or pine bark: 200 mg/day
- Vitamin C: 1200 mg/day
- Consider gamma-oryzanol: 300 mg/day

BOTANICAL MEDICINES

Choose one or more of the following for general symptom relief:

- Standardized extract of black cohosh: 40 mg to 80 mg/day
- Gelatinized maca extract: 1000 mg twice a day or dose equivalent to 3500 dried powdered maca root a day
- Red clover extract: 40 to 80 mg/day

Choose one of the following if symptoms of anxiety or depression are significant (can be used with the previous list):

- Hops extract standardized for 8-prenylnaringenin: 120 to 300 mg/day
- Kava extract: 45 to 80 mg of kavalactones three times a day
- St. John's wort extract standardized to 0.3% hypericin: 900 to 1800 mg/day

Choose the following if symptoms of vaginal atrophy do not respond after 2 months of treatment with other botanical(s):

- Kudzu: dried powdered root 20 to 50 mg a day

Choose the following if symptoms of menopausal migraine are significant:

- 100 mg *A. sinensis* extract, 60 mg soy isoflavones, and 50 mg of black cohosh extract a day

If symptoms do not sufficiently improve, consider HT.

LIFESTYLE

Facilitate a regular exercise program, at least 30 minutes four times a week. For weight loss in midlife women, it is likely that 60 minutes of exercise every day will be needed to combat insulin resistance and achieve successful weight loss.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Davis SR, Lambrinoudaki I, Lumsden M, et al. *Menopause. Nat Rev Dis Primers.* 2015;1:15004. PMID: 27188659.
2. Jewett PI, Gangnon RE, Trentham-Dietz A, et al. Trends of postmenopausal estrogen plus progestin prevalence in the United States between 1970 and 2010. *Obstet Gynecol.* 2014;124(4):727–733. PMID: 25198271.
3. United Kingdom Medical Research Council. Discontinuation of WISDOM announced. Available online at http//www.obgyn.net/ displayarticle.asp?page=/news/NAMS_110702. Accessed June 18 2005.
4. Position Statement. Estrogen and progestogen use in postmenopausal women: 2010 position statement of the North American Menopause Society. *Menopause.* 2010;17(2):242–255.
5. The NAMS 2017 Hormone Therapy Position Statement Advisory Panel. The 2017 hormone therapy position statement of the North American Menopause Society. *Menopause.* 2017;24(7):728–753. PMID: 28650869.
6. Hodis HN, Mack WJ, Henderson VW, et al. Vascular effects of early versus late postmenopausal treatment with estradiol. *N Engl J Med.* 2016;374(13):1221–1231. PMID: 27028912.
7. Avis NE, Crawford SL, Greendale G, et al. Duration of menopausal vasomotor symptoms over the menopause transition. *JAMA Intern Med.* 2015;175(4):531–539. PMID: 25686030.
8. Thurston RC, Chang Y, Barinas-Mitchell E, et al. Menopausal hot flashes and carotid intima media thickness among midlife women. *Stroke.* 2016;47(12):2910–2915. PMID: 27834746.
9. Biglia N, Cagnacci A, Gambacciani M, et al. Vasomotor symptoms in menopause: a biomarker of cardiovascular disease risk and other chronic diseases? *Climacteric.* 2017;20(4):306–312. PMID: 28453310.
10. Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. *Climacteric.* 2015;18(2):121–134. PMID: 25327484.
11. Whedon JM, KizhakkeVeettil A, Rugo NA, et al. Bioidentical estrogen for menopausal depressive symptoms: a systematic review and meta-analysis. *J Womens Health (Larchmt).* 2017;26(1):18–28. PMID: 27603786.
12. Georgakis MK, Thomopoulos TP, Diamantaras AA. Association of age at menopause and duration of reproductive period with depression after menopause: a systematic review and meta-analysis. *JAMA Psychiatry.* 2016;73(2):139–149. PMID: 26747373.
13. Rubinow DR, Johnson SL, Schmidt PJ, et al. Efficacy of estradiol in perimenopausal depression: so much promise and so few answers. *Depress Anxiety.* 2015;32(8):539–549. PMID: 26130315.
14. Barrett-Connor E. Hormone replacement therapy. *BMJ.* 1998;317:457–461.
15. Zhu L1, Jiang X, Sun Y, Shu W. Effect of hormone therapy on the risk of bone fractures: a systematic review and meta-analysis of randomized controlled trials. *Menopause.* 2016;23(4):461–470. PMID: 26529613.
16. Palacios S, Currie H, Mikkola TS, et al. Perspective on prescribing conjugated estrogens/bazedoxifene for estrogen-deficiency symptoms of menopause: a practical guide. *Maturitas.* 2015;80(4):435–440. PMID: 25684082.
17. Torgerson DJ, Bell-Syer SE. Hormone replacement therapy and prevention of nonvertebral fractures: a meta-analysis of randomized trials. *JAMA.* 2001;285:2891–2897.
18. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288:321–333.
19. Nanda K, Bastian LA, Hasselblad V, et al. Hormone replacement therapy and the risk of colorectal cancer: a meta-analysis. *Obstet Gynecol.* 1999;93:880–888.
20. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA.* 1998;280:605–613.
21. MacLennan SC, MacLennan AH, Ryan P. Colorectal cancer and oestrogen replacement therapy: a meta-analysis of epidemiological studies. *Med J Aust.* 1995;162:491–493.
22. US Preventive Services Task Force. Hormone therapy for the primary prevention of chronic conditions in postmenopausal women: US preventive Services task Force recommendation statement. *JAMA.* 2017;318(22):2224–2233. PMID: 29234814.
23. Sjögren LL, Mørch LS, Løkkegaard E. Hormone replacement therapy and the risk of endometrial cancer: a systematic review. *Maturitas.* 2016;91:25–35. PMID: 27451318.
24. Grady D, Gebretsadik T, Kerlikowske K, et al. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet Gynecol.* 1995;85:304–313.
25. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Writing Group for the PEPI Trial. *JAMA.* 1996;275:370–375.
26. Fournier A, Dossus L, Mesrine S, et al. Risks of endometrial cancer associated with different hormone replacement therapies in the E3N cohort, 1992-2008. *Am J Epidemiol.* 2014;180(5):508–517. PMID: 25008104.
27. Allen NE, Tsilidis KK, Key TJ, et al. Menopausal hormone therapy and risk of endometrial carcinoma among postmenopausal women in the European Prospective Investigation into Cancer and Nutrition. *Am J Epidemiol.* 2010;172(12):1394–1403. PMID: 20961969.
28. Stute P, Neulen J, Wildt L. The impact of micronized progesterone on the endometrium: a systematic review. *Climacteric.* 2016;19(4):316–328. PMID: 27277331.
29. Grady D, Herrington D, Bittner V, et al. Cardiovascular disease outcomes during 6.8 years of hormone therapy: heart and Estrogen/progestin Replacement Study follow-up (HERS II). *JAMA.* 2002;288:49–57.
30. Cushman M, Kuller LH, Prentice R, et al. Estrogen plus progestin and risk of venous thrombosis. *JAMA.* 2004;292:1573–1580. PMID: 15467059.
31. Scarabin P-V, Oger E, Plu-Bureau G. On behalf of the EStrogen and THromboEmbolism Risk (ESTHER) Study Group. Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet.* 2003;362:428–432. PMID: 12927428.
32. Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD databases. *BMJ.* 2019;364:k4810. PMID: 30626577.
33. Barrett-Connor E, Grady D. Hormone replacement therapy, heart disease, and other considerations. *Annu Rev Public Health.* 1998;19:55–72.
34. Grodstein F, Manson JE, Colditz GA, et al. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann Intern Med.* 2000;133:933–941.
35. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA.* 1995;273:199–208 (erratum, *JAMA* 1995;274:1676). The Writing Group for the PEPI trial.
36. Heckbert SR, Weiss NS, Koepsell TD, et al. Duration of estrogen replacement therapy in relation to the risk of incident myocardial infarction in postmenopausal women. *Arch Intern Med.* 1997;157:1330–1336.
37. Grodstein F, Manson JE, Stampfer MJ. Postmenopausal hormone use and secondary prevention of coronary events in the Nurses' Health Study: a prospective, observational study. *Ann Intern Med.* 2001;135:1–8.
38. The Women's Health Initiative Study Group. Design of the women's health initiative clinical trial and observational study. *Control Clin Trials.* 1998;19:61–109.
39. Herrington DM, Reboussin DM, Brosnihan KB, et al. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med.* 2000;343:522–529.
40. Clarke S, Kelleher J, Lloyd-Jones H, et al. Transdermal hormone replacement therapy for secondary prevention of coronary artery disease in postmenopausal women [abstract]. *Eur Heart J.* 2000;21(suppl):212.
41. Lenfant C. *Preliminary Trends in the Women's Health Initiative.* Bethesda, MD: National Heart, Lung, and Blood Institute Communications Office; 2000.
42. Hodis H, Mack W, Lobo R, et al. Estrogen in the prevention of atherosclerosis: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med.* 2001;135:939–953.
43. Rossouw JE, et al. Postmenopausal hormone therapy and cardiovascular disease by age and years since menopause. *JAMA.* 2007;297:1465–1477. PMID: 17405972.

44. Manson JE, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA*. 2013;310:1353–1368. PMID: 24084921.
45. Salpeter SR, Walsh JM, Greyber E, et al. Brief report: coronary heart disease events associated with hormone therapy in younger and older women. A meta-analysis. *J Gen Intern Med*. 2006;21:363–366. PMID: 16686814.
46. Wassertheil-Smoller S, Hendrix S, Limacher M, et al. Effect of estrogen plus progestine on stroke in postmenopausal women. *JAMA*. 2003;289:2673–2684.
47. Viscoli CM, Brass LM, Kernan WN, et al. A clinical trial of estrogen-replacement therapy after ischemic stroke. *N Engl J Med*. 2001;345:1243–1249.
48. Anderson G, Limacher M, Assaf A, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA*. 2004;291:1701–1712.
49. Boardman HM, Hartley L, Eisinga A, et al. Hormone therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev*. 2015;(3):CD002229. PMID: 25754617.
50. Smith N, Heckbert S, Lemaitre R, et al. Esterified estrogens and conjugated equine estrogens and the risk of venous thrombosis. *JAMA*. 2004;292:1581–1587.
51. Bezwada P, Shaikh A, Misra D. The effect of transdermal estrogen patch Use on cardiovascular outcomes: a systematic review. *J Womens Health (Larchmt)*. 2017;26(12):1319–1325. PMID: 28622476.
52. Hulley S, Furberg C, Barrett-Connor E, HERS Research Group, et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: heart and Estrogen/Progestin Replacement Study follow-up (HERS II). *JAMA*. 2002;288:58–66.
53. Marjoribanks J, Farquhar C, Roberts H, et al. Long-term hormone therapy for perimenopausal and postmenopausal women. *Cochrane Database Syst Rev*. 2017;1:CD004143. PMID: 28093732.
54. Liu B1, Beral V, Balkwill A, et al. Gallbladder disease and use of transdermal versus oral hormone replacement therapy in postmenopausal women: prospective cohort study. *BMJ*. 2008;337:a386. PMID: 18617493.
55. Bush TL, Whiteman M, Flaws JA. Hormone replacement therapy and breast cancer: a qualitative review. *Obstet Gynecol*. 2001;98:498–508.
56. Breast cancer and hormone replacement therapy. collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet*. 1997;350:1047–1059.
57. Schairer C, Lubin J, Troisi R, et al. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. *JAMA*. 2000;283:485–491.
58. Ross RK, Paganini-Hill A, Wan PC, et al. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. *J Natl Cancer Inst*. 2000;92:328–332.
59. Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: data from the Nurses' Health Study. *Am J Epidemiol*. 2000;152:950–964.
60. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J Natl Cancer Inst*. 1998;90:814–823.
61. Anderson GL, Chlebowski RT, Aragaki AK, et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol*. 2012;13(5):476–486. PMID: 22401913.
62. DeBono NL, Robinson WR, Lund JL, et al. Race, menopausal hormone therapy, and invasive breast cancer in the Carolina breast cancer study. *J Womens Health (Larchmt)*. 2018;27(3):377–386. PMID: 28570827.
63. Folsom AR, Mink PJ, Sellers TA, et al. Hormonal replacement therapy and morbidity and mortality in a prospective study of postmenopausal women. *Am J Public Health*. 1995;85:1128–1132.
64. Sellers T, Mink P, Cerhan J, et al. The role of hormone replacement therapy in the risk for breast cancer and total mortality in women with a family history of breast cancer. *Ann Intern Med*. 1997;127:973–980.
65. Gapstur SM, Morrow M, Sellers TA. Hormone replacement therapy and risk of breast cancer with a favorable histology: results of the Iowa Women's Health Study. *JAMA*. 1999;28:2091–2097.
66. Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N Engl J Med*. 1995;332:1589–1593.
67. Moorman PG, Kuwabara H, Millikan RC, et al. Menopausal hormones and breast cancer in a biracial population. *Am J Public Health*. 2000;90:966–971.
68. Lando JF, Heck KE, Brett KM. Hormone replacement therapy and breast cancer risk in a nationally representative cohort. *Am J Prev Med*. 1999;17:176–180.
69. Smith CA, McCleary CA, Murdock GA, et al. Lifelong estrogen exposure and cognitive performance in elderly women. *Brain Cogn*. 1999;39:203–218.
70. Grodstein F, Chen J, Pollen DA, et al. Postmenopausal hormone therapy and cognitive function in healthy older women. *J Am Geriatr Soc*. 2000;48:746–752.
71. LeBlanc ES, Janowsky J, Chan BK, et al. Hormone replacement therapy and cognition: systematic review and meta-analysis. *JAMA*. 2001;285:1489–1499.
72. Mulnard RA, Cotman CW, Kawas C, et al. Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. *JAMA*. 2000;283:1007–1015.
73. Grady D, Yaffe K, Kristof M, et al. Effect of postmenopausal hormone therapy on cognitive function: the heart and estrogen/progestin replacement study. *Am J Med*. 2002;113:543–548.
74. Zandi PP, Carlson MC, Plassman BL, et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. *JAMA*. 2002;288:2123–2129.
75. Gleason CE, Dowling NM, Wharton W, et al. Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-cognitive and affective study. *PLoS Med*. 2015;12(6):e1001833; discussion e1001833. PMID: 26035291.
76. Henderson VW, St John JA, Hodis HN, et al. Cognitive effects of estradiol after menopause: a randomized trial of the timing hypothesis. *Neurology*. 2016;87(7):699–708. PMID: 27421538.
77. Rodriguez C, Patel AV, Calle EE, et al. Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of U.S. women. *JAMA*. 2001;285:1460–1465.
78. Lacey JV, Mink Jr PJ, Lubin JH, et al. Menopausal hormone replacement therapy and risk of ovarian cancer. *JAM*. 2002;288:334–341.
79. Collaborative Group On Epidemiological Studies Of Ovarian Cancer, et al. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. *Lancet*. 2015;385(9980):1835–1842. PMID: 25684585.
80. Santoro N, Braunstein GD, Butts CL, et al. Compounded bioidentical hormones in endocrinology practice: an Endocrine Society scientific statement. *J Clin Endocrinol Metab*. 2016;101(4):1318–1343. PMID: 27032319.
81. Klein R. The composition of Premarin. *Int J Fertil Womens Med*. 1998;43:223.
82. Schwingshackl L, Schwedhelm C, Galbete C, et al. Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis. *Nutrients*. 2017;9(10):E1063. PMID: 28954418.
83. Toledo E, Salas-Salvadó J, Donat-Vargas C, et al. Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. *JAMA Intern Med*. 2015;175(11):1752–1760. PMID: 26365989.
84. Herber-Gast GC, Mishra GD. Fruit, Mediterranean-style, and high-fat and -sugar diets are associated with the risk of night sweats and hot flushes in midlife: results from a prospective cohort study. *Am J Clin Nutr*. 2013;97(5):1092–1099. PMID: 23553160.
85. Huntley A, Ernst E. Soy for the treatment of perimenopausal symptoms: a systematic review. *Maturitas*. 2004;47:1–9.
86. Krebs E, Ensrud K, MacDonald R, et al. Phytoestrogens for treatment of menopausal symptoms: a systematic review. *Obstet Gynecol*. 2004;104:824–836.

87. North American Menopause Society. 2011 Isoflavones Report. The role of soy isoflavones in menopausal health: report of The North American Menopause Society/Wulf H. *Menopause*. 2011;18(7):732–753. Utian Translational Science Symptosium in Chicago, IL (October 2010).
88. Jou HJ, Wu SS, Change FW, et al. Effect of intestinal production of equol on menopausal symptoms in women treated with soy isoflavones. *Intl J Gynecology and Obstetrics*. 2008;102(1):44–49.
89. Li L, Lv Y, Xu L, Zheng Q. Quantitative efficacy of soy isoflavones on menopausal hot flashes. *Br J Clin Pharmacol*. 2015;79(4):593–604. PMID: 25316502.
90. Thompson L, Robb P, Serraino M, et al. Mammalian lignan production from various foods. *Nutr and Canc*. 1991;16(1):43–52.
91. Pruthi SL, Thompson PJ, Novotny DL, et al. Pilot evaluation of flaxseed for the management of hot flashes. *J Soc Integ Oncol*. 2007;5(3): 106–112.
92. Flower G, Fritz H, Balneaves LG, et al. Flax and breast cancer: a systematic review. *Integr Cancer Ther*. 2014;13(3):181–192. PMID: 24013641.
93. Manson J, Greenland P, LaCroix AZ, et al. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. *N Engl J Med*. 2002;347:716–725.
94. McTiernan A, Kooperberg C, White E, et al. Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. *JAMA*. 2003;290:1331–1336.
95. Kemmler W, Engelke K, Weineck J, et al. The Erlangen fitness osteoporosis prevention study: a controlled exercise trial in early postmenopausal women with low bone density-first year results. *Arch Phys Med Rehabil*. 2003;84:673–682.
96. Fitzpatrick L, Santen R. Hot flashes: the old and the new, what is really true? *Mayo Clinic Proc*. 2002;77:1155–1158.
97. Grindler NM, Allsworth JE, Macones GA, et al. Persistent organic pollutants and early menopause in U.S. women. *PLoS One*. 2015;10(1):e0116057. PMID: 25629726.
98. Morgan M, Deoraj A, Felty Q, et al. Environmental estrogen-like endocrine disrupting chemicals and breast cancer. *Mol Cell Endocrinol*. 2017;457:89–102. PMID: 27717745.
99. Vabre P, Gatimel N, Moreau J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. *Environ Health*. 2017;16(1):37. PMID: 28388912.
100. Ziv-Gal A, Gallicchio L, Chiang C, et al. Phthalate metabolite levels and menopausal hot flashes in midlife women. *Reprod Toxicol*. 2016;60:76–81. PMID: 26867866.
101. Mendola P, Brett K, Dibari JN, et al. Menopause and lead body burden among US women aged 45-55, NHANES 1999-2010. *Environ Res*. 2013;121:110–113. PMID: 23352036.
102. Freeman E, Sammel M, Liu L, et al. Hormones and menopausal status as predictors of depression in women in transition to menopause. *Arch Gen Psych*. 2004;61:62–70.
103. Schmidt P, Haw N, Rubinow D. A longitudinal evaluation of the relationship between reproductive status and mood in perimenopausal women. *Am J Psychiatry*. 2004;161:2238–2244.
104. Avis N, Brambilla D, McKinlay S, et al. A longitudinal analysis of the association between menopause and depression: results from the Massachusetts Women's Health Study. *Ann Epidemiol*. 1994;4:214–220.
105. Hunter M. Psychological and somatic experience of the menopause: a prospective study. *Psychosom Med*. 1990;52:357–367.
106. Kaufert P, Gilbert P, Tate R. The Manitoba Project: a re-examination of the link between menopause and depressive. *Maturitas*. 1992;14:143–155.
107. Wilson RA. *Feminine forever*. New York: Evans.
108. Kim YA, Kim KM, Lim S, et al. Favorable effect of dietary vitamin C on bone mineral density in postmenopausal women. (KNHANES IV, 2009): discrepancies regarding skeletal sites, age, and vitamin D status. *Osteoporos Int*. 2015;26(9):2329–2337. PMID: 25906241.
109. Yang H-M, Liao MF, Zhu S-Y, et al. A randomised, double-blind, placebo-controlled trial on the effect of Pycnogenol on the climacteric syndrome in perimenopausal women. *Acta Obstetricia et Gynecologica*. 2007;86:978–985.
110. Luzzi R, Belcaro G, Hosoi M, et al. Normalization of cardiovascular risk factors in peri-menopausal women with Pycnogenol®. *Minerva Ginecol*. 2017;69(1):29–34. PMID: 28116886.
111. Errichi S, Bottari A, Belcaro G, et al. *Supplementation with Pycnogenol® Improves Signs and Symptoms of Menopausal Transition*. PMID: 22108479.
112. Murase Y, Iishima H. Clinical studies of oral administration of gamma-oryzanol on climacteric complaints and its syndrome. *Obstet Gynecol Prac*. 1963;12:147–149.
113. Ishihara M. Effect of gamma-oryzanol on serum lipid peroxide levels and climacteric disturbances. *Asia Oceania J Obstet Gynecol*. 1984;10:317–323.
114. Yoshino G, Kazumi T, Amano M, et al. Effects of gamma-oryzanol on hyperlipidemic subjects. *Curr Ther Res Clin Exp*. 1989;45:543–552.
115. Lucas M, Asselin G, Merette C, et al. Effects of ethyl-eicosapentaenoic acid omega-3 fatty acid supplementation on hot flashes and quality of life among middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. *Menopause*. 2009;16(2):357–366.
116. Freeman M, Hibbeln J, Silver M, et al. Omega-3 fatty acids for major depressive disorder associated with the menopausal transition: a preliminary open trial. *Menopause*. 2011;18(3): 279–71.
117. Christy CJ. Vitamin E in menopause. *Am J Obstet Gynecol*. 1945;50:84–87.
118. Mohammady M, Janani L, Jahanfar S, et al. Effect of omega-3 supplements on vasomotor symptoms in menopausal women: a systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2018;228:295–302. PMID: 30056356.
119. McLaren HC. Vitamin E in the menopause. *Br Med J*. 1949;2:1378–1381.
120. Ziaei S, Kazemnejad A, Zareai M. The effect of vitamin E on hot flashes in menopausal women. *Gynecol Obstet Invest*. 2007;64(4):204–207. PMID: 17664882.
121. Aldercreutz H, Mazur W. Phyto-estrogens and Western diseases. *Ann Med*. 1997;29:95–120.
122. Rose DP. Dietary fiber, phytoestrogens, and breast cancer. *Nutrition*. 1992;8:47–51.
123. Elghamry MI, Shihata IM. Biological activity of phytoestrogens. 3. Experimental studies on some biological properties of beta-sitosterol. *Planta Med*. 1966;14:352–357.
124. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*. 1997;138:863–870.
125. Borrelli F, Ernst E. *Cimicifuga racemosa*: a systematic review of its clinical efficacy. *Eur J Clin Pharmacol*. 2002;58:235–241.
126. Stolze H. An alternative to treat menopausal complaints. *Gyne*. 1982;3:14–16.
127. Chung D, Kim H, Park K, et al. Black cohosh and St. John's wort (GYNOPlus) for climacteric symptoms. *Yonsei Med J*. 2007;48(2):289–294.
128. Cancellieri F, De Leo V, Genazzani A, et al. Efficacy on menopausal neurovegetative symptoms and some plasma lipids blood levels of an herbal product containing isoflavones and other plant extracts. *Maturitas*. 2007;56:249–256.
129. Reed S, Newton K, LaCroix A, et al. Vaginal, endometrial, and reproductive hormone findings: randomized, placebo-controlled trial of black cohosh, multibotanical herbs, and dietary soy for vasomotor symptoms: the Herbal Alternaties for Menopause (HALT) Study. *Menopause*. 2008;15(1):51–58.
130. Fritz H, Seely D, McGowan J, et al. Black cohosh and breast cancer: a systematic review. *Integr Cancer Ther*. 2014;13(1):12–29. PMID: 23439657.
131. Heyerick A, Vervarcke S, Depypere H, et al. A first prospective, randomized, double-blind, placebo-controlled study on the use of a standardized hop extract to alleviate menopausal discomforts. *Maturitas*. 2006;54:164–175.
132. Aghamiri V, Mirghafourvand M, Mohammad-Alizadeh-Charandabi S3, et al. The effect of Hop (*Humulus lupulus* L.) on early menopausal symptoms and hot flashes: a randomized placebo-controlled trial. *Complement Ther Clin Pract*. 2016;23:130–135. PMID: 25982391.
133. Erkkola R, Vervarcke S, Vansteelandt S, et al. A randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a stan-

dardized hop extract to alleviate menopausal discomforts. *Phytomedicine*. 2010;17:389–396.
134. Meissner H, Mscisz A, Reich-Bilinska R, et al. Hormone-balancing effect of pre-gelatinized organic maca (*Lepidium peruvianum* Chacon): (III) Clinical response of early-postmenopausal women to Maca in a double blind, randomized, placebo-controlled, crossover configuration, outpatient study. *Int J Biomedical Science*. 2006;2(4):375–394.
135. Dini A, Migliuolo G, Rastrelli L, et al. Chemical composition of *Lepidium meyenii*. *Food Chem*. 1994;49:347.
136. Fahey J, Zalcmann A, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*. 2001;56:5–51.
137. Ganzera M, Zhao J, Muhammad I, et al. Chemical profiling and standardization of *Lepidium meyenii* (Maca) by reversed phase high performance liquid chromatography. *Chem Pharm Bull*. 2002;50:988–991.
138. Li G, Ammermann U, Quiros C. Glucosinolate contents in Maca (*Lepidium peruvianum* Chacon) seeds, sprouts, mature plants and several derived commercial products. *Econ Bot*. 2001;55(20):255–262.
139. Brooks N, Wilcox G, Walker K, et al. Beneficial effects of *Lepidium meyenii* (Maca) on psychological symptoms and measures of sexual dysfunction in postmenopausal women are not related to estrogen or androgen content. *Menopause*. 2008;15(6):1157–1162.
140. Baber RJ, Templeman C, Morton T, et al. Randomized placebo-controlled trial of an isoflavone supplement and menopausal symptoms in women. *Climacteric*. 1999;2:85–92.
141. Knight D, Howes J, Eden J. The effect of Promensil, an isoflavone extract, on menopausal symptoms. *Climacteric*. 1999;2:79–84.
142. Jeri A, deRomana C. The effect of isoflavone phytoestrogens in relieving hot flushes in Peruvian post-menopausal women. In: *Proceedings of the 9th International Menopause Society World Congress on Menopause*. Yokohama, Japan.
143. Nachtigall L, La Grega L, Lee W, et al. The effects of isoflavones derived from red clover on vasomotor symptoms and endometrial thickness. In: *Proceedings of the 9th International Menopause Society World Congress on the Menopause*. Yokohama, Japan.
144. van de Weijer P, Barentsen R. Isoflavones from red clover Promensil significantly reduce menopausal hot flush symptoms compared with placebo. *Maturitas*. 2002;42:187–193.
145. Tice J, Ettinger B, Ensrud K, et al. Phytoestrogen supplements for the treatment of hot flashes: the isoflavone clover extract (ICE) study. *JAMA*. 2003;290:207–214.
146. Nestel PJ, Pomeroy S, Kay S, et al. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. *J Clin Endocrinol Metab*. 1999;84:895–898.
147. Thompson Coon J, Pittler M, Ernst E. Trifolium pretense isoflavones in the treatment of menopausal hot flushes: a systematic review and meta-analysis. *Phytomedicine*. 2007;14:153–159.
148. Ghazanfarpour M1, Sadeghi R2, Roudsari RL, et al. Red clover for treatment of hot flashes and menopausal symptoms: a systematic review and meta-analysis. *J Obstet Gynaecol*. 2016;36(3):301–311. PMID: 26471215.
149. Hewger M, Ventskiovskiy B, Borzenko I, et al. Efficacy and safety of a special extract of *Rheum rhaponticum* (Err 731) in perimenopausal women with climacteric complaints: a 12 week randomized, double-blind, placebo-controlled trial. *Menopause. J NAMS*. 2006;13(5):744–759.
150. Kaszkin-Bettag, Ventskovskiy B, Kravchenko A, et al. The special extract ERr 731 of the roots of *Rheum rhaponticum* decreases anxiety and improves health state and general well-being in perimenopausal women. *Menopause*. 2007;14(2):270–283.
151. Kaszkin-Bettag M, Beck S, Richardson A, et al. Efficacy of the special extract ERr731 from Rhapontic rhubarb for menopausal complaints: a 6-month open observational study. *Alternative Therapies*. 2008;14(6):32–38.
152. Abdali K, Khajehei M, Tabatabaee R. Effect of St. John's wort on severity, frequency, and duration of hot flashes in premenopausal, perimenopausal and postmenopausal women: a randomized, double-blind, placebo-controlled study. *Menopause*. 2010;17(2):326–331.
153. Al-Akoum M, Maunsell E, Verreault R, et al. Effects of *Hypericum perforatum* (St. John's wort) on hot flashes and quality of life in perimenopausal women: a randomized pilot trial. *Menopause*. 2009;16(2):307–314.
154. Grube B, Walper A, Whatley D. St. John's wort extract: efficacy for menopausal symptoms of psychological origin. *Adv Ther*. 1999;16:177–186.
155. van Die D, Burger H, Bone K, et al. *Hypericum perforatum* with *Vitex agnus-castus* in menopausal symptoms: a randomized, controlled trial. *Menopause*. 2009;16(1):156–163.
156. Warnecke G. Psychomatic dysfunction in the female climacteric: clinical effectiveness and tolerance of kava extract WS 1490 (in German). *Fortschr Med*. 1991;109:119–122.
157. De Leo V, L Marca A, Lanzetta D, et al. Valutazione dell-associazione di estratto do Kava-Kava e terpie ormoale sostitutiva nel tratta mento d'ansia in postmenpausa. *Minerva Ginecol*. 2000;52:263–267.
158. Cagnacci A, Arangino S, Rensi, et al. Kava-Kava administration reduces anxiety in perimenopausal women. *Maturitas*. 2003;44:103–109.
159. Hikino H. Traditional remedies and modern assessment: the case of ginseng. In: Wijesekera R, ed. *The Medicinal Plant Industry*. Boca Raton, FL: CRC Press; 1991:149–166.
160. Shibata S, Tanaka O, Shoji J, et al. Chemistry and pharmacology of *Panax*. In: *Economic and Medicinal Plant Research*. Vol. 1. London: Academic Press; 1985.
161. Hallstrom C, Fulder S, Carruthers M. Effect of ginseng on the performance of nurses on night duty. *Comp Med East West*. 1982;6:277–282.
162. D'Angelo L, Grimaldi R, Caravaggi M, et al. A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. *J Ethnopharmacol*. 1986;16:15–22.
163. Bombardelli E, Cirstoni A, Lietti A. The effect of acute and chronic *Panax ginseng* saponins treatment on adrenal function; biochemical and pharmacological. In: *Proceedings of the 3rd International Ginseng Symposium*. Korean Ginseng Research Institute; 1980:9–16.
164. Punnonen R, Lukola A. Oestrogen-like effect of ginseng. *Br Med J*. 1980;281:1110.
165. Wiklund I, Mattsson L, Lindgren R, et al. Effects of a standardized ginseng on the quality of life and physiological parameters in symptomatic postmenopasual women: a double-blind, placebo-controlled trial. *Int J Clin Pharm Res*. 1999;XIX:89–99.
166. Lee HW, Choi J, et al. Ginseng for managing menopausal woman's health: a systematic review of double-blind, randomized, placebo-controlled trials. *Medicine (Baltimore)*. 2016;95(38):e4914. PMID: 27661038.
167. Tode T, Kikuchi Y, Hirata J, et al. Effect of Korean red ginseng on psychological functions in patients with severe climacteric syndromes. *Int J Gynaecol Obstet*. 1999;67:169–174.
168. Manonai J, Chittacharoen A, Theppisai U, et al. Effect of *Pueraria mirifica* on vaginal health. *Menopause*. 2007;14(5):919–924.
169. Suwanvesh N, Manonai J, Sophonsritsuk A, et al. Comparison of *Pueraria mirifica* gel and conjugated equine estrogen cream effects on vaginal health in postmenopausal women. *Menopause*. 2017;24(2):210–215. PMID: 27749740.
170. Hirata JD, Swiersz LM, Zell B, et al. Does dong quai have estrogenic effects in postmenopausal women? A double-blind, placebo-controlled trial. *Fertil Steril*. 1997;68:981–986.
171. Chang HM, But PPH, eds. *Pharmacology and Applications of Chinese Materia Medica*. Singapore: World Scientific; 1987:489–505.
172. Burke BE, Olson RD, Cusack BJ. Randomized, controlled trial of phytoestrogen in the prophylactic treatment of menstrual migraine. *Biomed Pharmacother*. 2002;56:283–288.
173. Yang Q, Populo SM, Zhang J, et al. Effect of Angelica sinensis on the proliferation of human bone cells. *Clin Chim Acta*. 2002;324:89–97.
174. Taavoni S, Ekbatani N, Kashaniyan M, et al. Effect of valerian on sleep quality in postmenopausal women: a randomized placebo-controlled clinical trial. *Menopause*. 2011;18(9):951–955.
175. Jenabi E, Shobeiri F, Hazavehei SMM, et al. The effect of Valerian on the severity and frequency of hot flashes: a triple-blind randomized clinical trial. *Women Health*. 2018;58(3):297–304. PMID: 28278010.

176. Zeng Z, Jiang X, Li X, et al. Conjugated equine estrogen and medroxyprogesterone acetate are associated with decreased risk of breast cancer relative to bioidentical hormone therapy and controls. *PLoS One*. 2018;13(5):e0197064. PMID: 29768475.
177. Head K. Estriol: safety and efficacy. *Alt Med Rev*. 1998;3(2):101–113.
178. Hustin J, Van den Eynde J. Cytological evaluation of the effect of various estrogens given in postmenopause. *Acta Cytol*. 1977;21:225–228.
179. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with urinary tract infections. *N Engl J Med*. 1993;329:753–756.
180. Hermsmeyer K, Thompson T, Pohost G, et al. Cardiovascular effects of medroxyprogesterone acetate and progesterone: a case of mistake identity? *Nat Clin Pract Cardiovas Med*. 2008;5(7):387–395.
181. Leonetti HB, Longo S, Anasti JN. Transdermal progesterone cream for vasomotor symptoms and postmenopausal bone loss. *Obstet Gynecol*. 1999;94:225–228.
182. Wren BG, Champion SM, Willetts K, et al. Transdermal progesterone and its effect on vasomotor symptoms, blood lipid levels, bone metabolic markers, moods, and quality of life for postmenopausal women. *Menopause*. 2003;10:13–18.
183. Dobay B, Balos R, Willard N. *Improved Menopausal Symptom Relief with Estrogen-Androgen Therapy*. Chicago, IL: Presented at the Annual Conference of the North American Menopause Society; 1996.
184. Sarrel P, Dobay B, Wiita B. Sexual behavior and neuroendocrine responses to estrogen and estrogen-androgen in postmenopausal women dissatisfied with estrogen-only therapy. *J Reprod Med*. 1998;43(10):847–856.
185. Watts N, Notelovitz M, Timmons M, et al. Comparison of oral estrogens and estrogens plus androgen on bone mineral density, menopausal symptoms, and lipid-lipoprotein profiles in surgical menopause. *Obstet Gynecol*. 1995;85:529–537.
186. Davis SR, Moreau M, Kroll R, et al. APHRODITE study Team testosterone for low libido in postmenopausal women not taking estrogen. *NEJM*. 2008;359(19):2005–2017.
187. Kritz-Silverstein D, von Muhlen D, Laughlin G, et al. Effects of dehydroepiandrosterone supplementation on cognitive function and quality of life: the DHEA and Well-Ness (DAWN) trial. *J Am Geriatr Soc*. 2008;56:1292–1298.
188. Pluchino N, Ninni F, Stomati M, et al. One-year therapy with 10 mg/day DHEA alone or in combination with HT in postmenopausal women: effects on hormonal milieu. *Maturita*. 2008;59:293–303.
189. Villareal D, Holloszy J. Efect of DHEA on abdominal fat and insulin action in elderly women and men. *JAMA*. 2004;292:2243–2248.
190. Labrie F, Archer D, Bouchard C, et al. Intravgaginal dehydrepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy. *Menopause*. 2009;16(5):907–922.
191. Labrie F, Archer D, Bouchard C, et al. Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women. *Menopause*. 2009;16:923–931.
192. Labrie F, Archer DF, Koltun W, et al. Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause*. 2016;23(3):243–256. PMID: 26731686.

197

Menorrhagia

Angela Sadlon, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Excessive menstrual bleeding occurring at regular cyclic intervals (cycles are usually but not necessarily of normal length) that interferes with the woman's physical, social, emotional, and/or material quality of life.[1,2] In the recent past, this definition included a quantity of blood loss: blood loss greater than 80 mL per cycle.[2]

In 2013 the American College of Obstetricians and Gynecologists supported the use of the acronym PALM-COEIN to determine the etiology of abnormal menstrual bleeding (which includes menorrhagia).[3] The acronym distinguishes structural from nonstructural causes.

Structural causes: **P**olyp, **A**denomyosis, **L**eiomyoma (submucosal or other), **M**alignancy and hyperplasia.

Nonstructural causes: **C**oagulopathy, **O**vulatory dysfunction, **E**ndometrial, **I**atrogenic, **N**ot yet classified.

According to the article, up to 13% of women with menorrhagia have some variant of von Willebrand disease, and up to 20% have an underlying coagulation disorder.[3] It is important to ask about epistaxis (1–2 times monthly), frequent gingival bleeding, bruisability (1–2 times monthly), and a family history of bleeding symptoms.[3]

The following tests and procedures are performed for diagnosis on an as-needed basis: pelvic examination, pelvic ultrasound and/or pelvic sonohistogram, cervical cytology, sexually transmitted infection testing, thyroid function studies, pregnancy test, complete blood count (CBC), ferritin measurement, liver function or coagulation studies, measurement of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and hysteroscopy.

GENERAL CONSIDERATIONS

There are many types of abnormal uterine bleeding. This chapter focuses on menorrhagia (regular/normal intervals with excessive flow and duration). Other patterns of abnormal bleeding are oligomenorrhea (interval greater than 35 days), polymenorrhea (interval less than 21 days), metrorrhagia (irregular/frequent intervals with excessive flow and duration), menometrorrhagia (prolonged heavy bleeding at irregular intervals), and intermenstrual bleeding (variable amounts occurring between regular menses).

The normal menstrual cycle is defined as 28 days (±7 days) in length and 4 days (±4 days) in duration, with a blood loss of 40 mL (±20 mL).[4]

The complaint of menorrhagia is largely subjective, because an objective measurement of blood loss is rarely made. Furthermore, there is a poor correlation between measured blood loss and a patient's assessment of her bleeding (discussed in more detail later).[4]

Etiology

As with any disease, proper determination of the cause is essential for effective treatment. The appropriate methodology for ruling out pathological causes is beyond the scope of this chapter and can be found in any good text on gynecology (Table 197.1).[5] It is important to be aware of the scope of causes so that one does not just assume

TABLE 197.1 Pathological Causes of Menorrhagia

Cause	Possible Etiology
Anovulation	Excessive estrogen Failure of midcycle surge of luteinizing hormone Hypothyroidism Hyperprolactinemia Polycystic ovary disease
Intrauterine structural defects	Fibroids Polyps Cancer Ectopic pregnancy Intrauterine devices
Bleeding disorders	See Table 197.2

Data from *The Merck Manual*.[5]

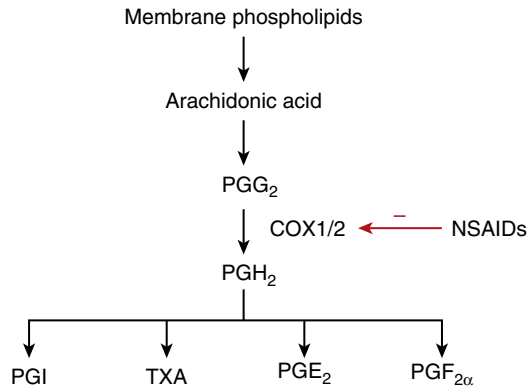


Fig. 197.1 Synthesis and signaling of prostaglandins. *PG,* Prostaglandin; *PGI,* prostacyclin; *TXA,* thromboxane. (From Maybin JA, Critchley HO. Medical management of heavy menstrual bleeding. *Womens Health* [Lond]. 2016;12[1]:27–34. https://doi.org/10.2217/whe.15.100. [Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC4728737_emss-66793-f0002&query=menorrhagia&req=4&npos=246 (accessed Sept 13, 2017)]

that the problem is "dysfunctional uterine bleeding" (DUB)—defined as abnormal uterine bleeding without any demonstrable organic cause.

Abnormalities in Prostaglandin Metabolism

The etiology of structural menorrhagia is currently believed to involve abnormalities in the biochemical processes of the endometrium that control the supply of arachidonic acid for prostaglandin synthesis.[6,7] Menorrhagic endometrium incorporates arachidonic acid into neutral lipids to a much greater extent than normal, whereas its incorporation into phospholipids is lower. The greater arachidonic acid release during menstruation results in the higher production of series 2 prostaglandins, which are thought to be the major factor both in the excessive bleeding seen at menstruation and in the symptoms of dysmenorrhea (Fig. 197.1). The excessive bleeding during the first 3 days appears to be due to the vasodilatory properties of prostaglandins (PGs) E_2 and PGI_2 and the antiaggregating activity of PGI_2, whereas the pain of dysmenorrhea is due to the overproduction of PGF_{2a}.

Thyroid Abnormalities

The association of overt hypothyroidism or hyperthyroidism with menstrual disturbances is well known.[5,8] However, even minimal thyroid dysfunction, particularly minimal subclinical insufficiency as determined by testing the thyroid-stimulating hormone (TSH), may be responsible for menorrhagia and other menstrual disturbances.[9] Patients with minimal thyroid insufficiency and menorrhagia have shown dramatic responses to thyroxine.[9] It has been recommended that patients with long-standing menstrual dysfunction (who have no obvious uterine disease) should be considered for TSH testing. This approach is preferable to the empiric use of thyroid hormone.

Estimating Menstrual Blood Loss

Physicians often believe that they can assess menstrual blood loss by asking the patient to estimate the number of pads or tampons used during each period and the duration of the period. However, studies have demonstrated that there is no correlation between measured blood loss and these assessments.[10,11] A woman's assessment of her blood loss is extremely subjective, as demonstrated by one study finding that 40% of women with a menstrual blood loss exceeding 80 mL considered their periods only moderately heavy or scanty, whereas 14% of those with a measured loss of less than 20 mL judged their periods to be heavy.[11]

Serum ferritin levels may be the best indicator of excessive menstrual blood loss, but their determination may not be practical in terms of immediate need to know. Despite inaccuracies of patient reporting, it is important for the clinician to make an attempt at understanding the quantity of blood loss. Excessive blood loss should be a concern if a woman is bleeding longer than 7 straight days or more frequently than every 21 days and is changing a pad or tampon every hour for more than half a day. Women who are changing a pad and/or tampon every half hour or even more often require urgent, perhaps emergency attention. Symptoms of light-headedness, dizziness, fainting, or the like are cause for immediate concern. Any amount of bleeding in a postmenopausal woman is considered abnormal, unless it clearly results from withdrawal of exogenous hormones.[5]

THERAPEUTIC CONSIDERATIONS

Conventional Medicine

The first line of therapy for menorrhagia in conventional medicine is hormonal (progestin intrauterine device [IUD], combined hormonal contraceptives, cyclic oral progestogens, progesterone only, danazol, or ulipristal acetate) and/or hemostatic therapies (tranexamic acid or 1-deamino-8-arginine).[1] Only when those therapies are ineffective are more aggressive and invasive therapies performed, such as endometrial ablation, dilation and curettage (D&C), polypectomy, myomectomy, uterine artery embolization, and hysterectomy.[1,3] Although the need for hysterectomy is recognized in select circumstances, most cases of menorrhagia can be treated with nonsurgical therapies, including botanicals, nutritional interventions, and hormonal and pharmaceutical therapies.

Psychological Considerations

Stress was shown to affect bleeding patterns directly by influencing the hypothalamic–pituitary–adrenal–ovarian axis, as shown in an animal study.[12] However, there are conflicting clinical trials demonstrating the significance of stress and menorrhagia.[13,14]

Nutrition

Iron Deficiency

A menstrual blood loss exceeding 60 mL per period is associated with negative iron balance in most cases.[15] Although menstrual blood loss is well recognized as a major cause of iron-deficiency anemia in fertile women, it is not so well known that chronic iron deficiency can be a cause of menorrhagia. Taymor et al. have made such a suggestion on the basis of several observations[16]:

- Response to iron supplementation alone in 74 of 83 patients (in whom organic disease had been excluded)
- High rate of organic disease (fibroids, polyps, adenomyosis, etc.) in the patients with no response to iron supplementation
- Associated rise in serum iron levels in 44 of 57 patients
- Decreased response to iron therapy when initial serum iron levels were high
- Correlation of menorrhagia with depleted tissue iron stores (bone marrow) irrespective of serum iron level
- A significant double-blind, placebo-controlled study displaying improvement in 75% of those given iron supplementation compared with 32.5% of those given the placebo

Hematological screening and serum ferritin determination (the first parameter to indicate decreased iron levels) should be performed for patients complaining of menorrhagia. In one study, women who were menorrhagic (according to subjective information) displayed

significantly lower serum ferritin levels than controls, but hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin were not significantly different between the two groups.[17] (The investigators in this study erroneously stated that such women do not require prophylactic iron supplementation because no hematological abnormalities appeared despite significantly reduced iron stores.)

Iron supplementation, at a daily dose of 100 mg of elemental iron, has been recommended as a prophylactic therapy by several researchers because chronic iron deficiency appears to promote menorrhagia, and iron-containing enzymes are depleted before hematological changes are observed.[18] A decreased serum ferritin level is a good indication of the need for iron supplementation.

Vitamin A

In one study, serum retinol levels were found to be significantly lower in women with menorrhagia than in healthy controls.[19] In this study, vitamin A was used as a treatment in 40 women diagnosed as having menorrhagia due to several different causes. In the group that received 60,000 IU of vitamin A for 35 days, menstruation returned to normal in 23 patients (57.5%) and was reduced in 14 (35%).[19] Overall, the vitamin A was ineffective in only 3 of the 40 women (7.5%), and 92.5% of the 37 women had either complete relief or significant improvement.[19] Although potentially effective, this therapy should not be used in women with the potential of pregnancy.

Vitamin C and Bioflavonoids

Capillary fragility is believed to play a role in many cases of menorrhagia. Supplementation with vitamin C (200 mg three times daily) and bioflavonoids (dose not specified) was shown to reduce menorrhagia in 14 of 16 patients.[20] One of the patients with no response had endometriosis, and the other had metrorrhagia. Bioflavonoids, like vitamin C, can help strengthen the walls of capillaries. Bioflavonoids may also reduce heavy bleeding through their anti-inflammatory effect. A natural anti-inflammatory such as a bioflavonoid may be used to reduce heavy bleeding, just as conventional medicine uses nonsteroidal anti-inflammatory agents.

Because vitamin C is known to significantly increase iron absorption, its therapeutic effect could also be due to enhanced iron absorption.

Vitamin E

One group of investigators has suggested that free radicals have a causative role in endometrial bleeding, particularly in the presence of an intrauterine device.[21] Vitamin E supplementation (100 IU every 2 days) resulted in improvement in all patients by the end of 10 weeks.[22] Although vitamin E may have produced its effects via its antioxidant activity, it is equally plausible that the vitamin affected prostaglandin metabolism in a manner that reduced bleeding.

Vitamin K and Chlorophyll

Although bleeding time and prothrombin levels in women with menorrhagia are typically normal, the use of vitamin K (historically in the form of crude preparations of chlorophyll) has clinical and limited research support.[23] Also, some women are found to have an inherited or acquired bleeding disorder. Table 197.2 lists some causes of acquired hemorrhagic disorders.[23]

Essential Fatty Acids

Menorrhagia is associated with the increased availability of arachidonic acid in the uterus.[24] It now appears that the majority of tissue arachidonic acid is derived from the diet. It is therefore possible that by reducing the intake of animal products and/or increasing the intake of linoleic, linolenic, and dihomo-gamma-linolenic acid, blood loss could be curtailed by decreasing the availability of arachidonic acid. Consuming larger proportions of fish, nuts, and seeds can have an effect over time in altering the production of arachidonic acid. The use of fish oils, flax oil, and other seed oils as supplements may produce this favorable effect more quickly.

TABLE 197.2 Acquired Generalized Hemorrhagic Disorders

Factor	Possible Cause
Deficiency of vitamin K	Low intake, impaired absorption, antimicrobial inhibition of gut flora that synthesize vitamin K
Drug-induced hemorrhage	Heparin, warfarin
Dysproteinemias	Myeloma, macroglobinemia
Disseminated intravascular coagulation	
Severe hepatic disease	
Circulating inhibitors of coagulation	
Primary fibrinolysis	

Data from Gubner.[23]

Vitamin B Complex

There may be a correlation between a nutritional deficiency of B vitamins and menorrhagia. It has been shown that in vitamin B complex deficiency, the liver loses its ability to inactivate estrogen. Some cases of menorrhagia are due to an excess estrogen effect on the endometrium. Therefore supplementing with a complex of B vitamins may normalize estrogen metabolism. A study conducted in the 1940s in a series of consecutive cases showed that a B-complex preparation led to "prompt" improvement in both menorrhagia and metrorrhagia.[25] The preparations used were thiamin 3 to 9 mg, riboflavin 4.5 to 9 mg, and niacin up to 60 mg.[25]

IUDs can cause menorrhagia, especially within the first few months of insertion. A double-blind, randomized, controlled trial was conducted on 126 women with increased bleeding occurring within 1 month after insertion of a copper IUD (TCu380A). The participants were given a placebo (dried starch) or 100 mg of thiamin (vitamin B_1) for 3 months. Symptoms were tracked using the Higham scale. Benefit was seen after 1 month of vitamin B_1 usage and continued for the next 2 months. Improvement in length of menstrual bleeding, number of sanitary pads, and spotting were seen in the therapy group.[26] This quantity of thiamine can easily be achieved in one to two B-complex capsules from a physician-grade nutraceutical company. If no benefit is shown, the patient may not be B-vitamin deficient.

Botanical Medicines

Zingiber Officinale (Ginger)

Ginger has been shown to inhibit prostaglandin synthetase, the enzyme believed to be related to the altered prostaglandin-2 ratio associated with excessive menstrual loss.[27] The inhibition of prostaglandin and leukotriene formation could explain the traditional use of ginger as an anti-inflammatory agent.

A placebo-controlled, randomized clinical study published in 2014 showed benefit of the use of ginger for heavy menstrual bleeding. The intervention group (n = 46) received 250 mg of ginger (dried rhizome, crushed and powdered) three times daily for 4 days (starting from the day before menses until the third day of menses) per month for 3 months. The bleeding amount was measured using

the Pictorial Blood Assessment Chart. The percentage decrease in hemorrhage was 46.6% in the ginger group and 2.1% in the placebo group.[28]

Vitex Agnus Castus (Chaste Tree)

Chaste tree is probably the best-known herb in all of Europe for the treatment of hormonal imbalances and abnormal bleeding in women. Since at least the time of the ancient Greeks, chaste tree has been used for the full scope of menstrual disorders, including heavy menses. It is mainly the seeds that are used for medicine in Europe and the United States. Chaste tree acts on the hypothalamus and pituitary glands. It increases LH production and mildly inhibits the release of FSH. The result is a shift in the ratio of estrogen and progesterone that effectively becomes a progesterone-like action. Chaste tree has been studied and been shown to effect improvements in amenorrhea, polymenorrhea, oligomenorrhea, and menorrhagia.[29–31]

In a randomized, double-blinded, clinical controlled trial of women with IUD-induced bleeding, 42 women received Vitex (dosage not given), and the other 42 women received mefenamic acid (250 mg three times daily) for 8 days, starting with the first day of menses. The Vitex had slow improvement until the fourth month, where its efficacy was statistically similar to mefenamic acid, at 47.6% and 52% bleeding improvement, respectively.[32]

Chaste tree is the most important herb for normalizing the menstrual flow, but it is not a fast-acting herb. Its effects may not be known for 3 or 4 months.

Pomegranate Flower

The Pomegranate tree, *Punica granatum* L., is found throughout India, Iran, and the Mediterranean regions, but over time, it has been cultivated elsewhere in the world. All parts of the tree have medicinal value, with the fruit being the most well known. The flower was shown to improve insulin sensitivity and lower glucose levels (in a rat study) and improve atherosclerosis and lipid profiles.[33] Pomegranate has research showing benefit on bleeding disorders, such as excessive menstruation and gingivitis.[34–36] In a randomized, double-blind, clinical trial on idiopathic heavy menstrual bleeding, *P. granatum* flower was shown to be as effective as tranexamic acid. This was a small study, with each group having 38 women who finished the 3-month study. The dosages per cycle were as follows: tranexamic acid 500 mg every 6 hours for the first 5 days of the menstruation and 500 mg *P. granatum* every 6 hours for the first 5 days of menstruation. Improvements in length of bleeding and quantity of blood loss were significant and comparable in both groups.[34]

Myrtle Berry

Myrtus communis L. *(Myrtaceae),* myrtle berry, is native to the Mediterranean and Middle East. Its vast actions include anti-inflammatory, antimicrobial, antidiarrheal, antidiabetic, antispasmodic, vasodilator, antiulcer, antioxidant, anticancer, anxiolytic, and sedative-hypnotic effects.[37] The whole plant, when analyzed, contained the following chemical components: flavonoids, tannins, phenolic acids, glycosides, and terpenoids.[37]

In 2014 a 3-month, randomized, double-blind, placebo-controlled pilot study was conducted on 30 women with abnormal uterine bleeding. The therapy group received 5 mL of myrtle berry syrup 30 minutes after each meal, three times daily (15 mL/d) for 7 days, starting on day 1 of menstruation. The treatment group saw a significant reduction in menstrual duration (10.6 days to 8.2 days) and blood loss versus the placebo group. The treatment group also experienced significant beneficial changes in quality-of-life scores.[38]

Traditional Astringent Herbs

Astringent herbs are used to reduce blood loss from the reproductive tract as well as the gastrointestinal tract, respiratory tract, and skin. The astringents most effective in uterine blood loss are often those that are high in tannins, although the tannins are most likely not the only constituent responsible. The following eight herbs are major astringent and hemostatic agents used in chronic and acute menorrhagia. These herbs are usually used in combination formulations for weeks or months before they produce results.

- Yarrow *(Achillea millefolium)*
- Ladies' mantle *(Alchemilla vulgaris)*
- Cranesbill *(Geranium maculatum)*
- Beth root *(Trillium erectum)*
- Greater periwinkle *(Vinca major)*
- Horsetail *(Equisetum arvense)*
- Goldenseal *(Hydrastis canadensis)*
- Shepherd's purse *(Capsella bursa pastoris)*

Shepherd's purse has a long history of use in the management of obstetrical and gynecological hemorrhage.[39] Intravenous and intramuscular injections of this herb have been found to be effective (in uncontrolled studies) in menorrhagia owing to functional abnormalities and fibroids.[40,41] The hemostatic action of shepherd's purse is believed to be due to its high concentration of oxalic and dicarboxylic acids.

Traditional Uterine Tonics

Uterine tonics have enjoyed a long history of use in traditional herbal medicine for easing menstrual flow. Traditional and empiric views of uterine tonics hold that if the uterus is hypotonic, there may be heavy bleeding, and that improving uterine tone tends to normalize and regulate menstrual bleeding. Uterine tonics or amphoterics that regulate tone and potentially reduce bleeding are as follows:

- Blue cohosh *(Caulophyllum thalictroides)*
- Helonia *(Chamaelirium luteum)*
- Squaw vine *(Mitchella repens)*
- Raspberry leaves *(Rubus idaeus)*
- Life root *(Senecio aureus)*

Astringent and uterine tonic herbs can be used in combination formulations or for weeks to months as teas, liquid extracts, or powders in capsules. These herbs must usually be taken in combination formulations for weeks or months to produce results.

Traditional Herbs for Semiacute and Acute Blood Loss When the Patient is Stable

The traditional herbs listed here have dose-specific toxicities and should be used only after reference to a botanical reference text to ensure proper dosing. Cinnamon essential oil should be used at 1 to 5 drops every 3 to 4 hours. The other herbs should not exceed 20 drops every 2 hours or 1 capsule every 4 hours.

- Cinnamon essential oil *(Cinnamomum verum)*
- Life root *(S. aureus)*
- Canadian fleabane *(Erigeron canadensis)*
- Greater periwinkle *(V. major)*
- Shepherd's purse *(C. bursa pastorus)*
- Yarrow *(A. millefolium)*
- Savin *(Sabina officinalis)*
- Beth root *(T. erectum)*

These botanicals should be used as the sole treatment only in women who are stable. In unstable patients, they should be used only as adjuncts to intravenous estrogens or other pharmaceutical or surgical interventions. They can be used in patients with chronic menorrhagia who are stable and for semiacute blood loss or acute blood loss if a patient shows no signs of instability and improves within 12 to 24 hours.

Bioidentical Hormones

Estrogens and progestogens, including bioidentical estradiol and progesterone, can be extremely effective in managing menorrhagia, in the same way as conjugated equine estrogens (CEEs) or synthetic estrogens and progestins are in conventional medicine. For control of an acute bleeding episode, the use of bioidentical estradiol should be just as effective as one of the dosing regimens of CEE. These hormones are prescription items and should be administered by a practitioner qualified to use them. Cyclic bioidentical progesterone can be used to correct recurring menorrhagia, and a short course of oral bioidentical progesterone can be used in many cases of acute menorrhagia. Bioidentical progesterone creams will not have as significant an effect as the higher-dose pills or oral micronized natural progesterone.

THERAPEUTIC APPROACH

Patients who are unstable—as evidenced by hypotension, dizziness, loss of consciousness, chills or fever, or passage of large amounts of tissue—require transfer to a hospital for intravenous estrogens, D&C, and/or hysterectomy or uterine ablation.

The first step in treating a woman with menorrhagia is to control the cause. When the excessive bleeding has been determined to be related to prothrombin time, hematological status, or thyroid function, such abnormalities can be corrected. Mechanical causes of menorrhagia may be managed without removal of the cause, such as an endometrial polyp or uterine fibroid. But if no improvement occurs, conventional treatment, including surgery, may be necessary. Endometrial hyperplasia requires definitive and proven progesterone or progestin treatment with biopsy-proven improvement. Endometrial cancer requires a hysterectomy. Infections of the uterus must be treated appropriately. Ectopic pregnancy with or without bleeding necessitates immediate conventional intervention. In cases of chronic menorrhagia or episodic acute blood loss that is effectively managed, a CBC and serum ferritin measurement can be used to help monitor the patient's anemia status.

Diet

The diet should be relatively low in sources of arachidonic acid (animal fats), high in fish oils (docosahexaenoic and eicosapentaenoic acids), and high in linolenic and linoleic acids (vegetable oil sources). Green leafy vegetables and other sources of vitamin K should be eaten freely. Fruits, vegetables, and spices with anti-inflammatory effects include garlic, onions, cumin, pineapple, and citrus.

Supplements

- High-potency multiple vitamin and mineral formula
- Vitamin C: 1 g three times per day with meals
- Vitamin A (retinol) 60,000 IU (18,000 mcg) daily for 35 days. Avoid pregnancy with this treatment
- Bioflavonoids (mixed from citrus): 1000 mg/day
- Chlorophyll: 25 mg/day (use a crude form) or 1 mg vitamin K_1

If low serum ferritin is confirmed:

- Iron: 30 mg, bound to either pyrophosphate, succinate, glycinate, or fumarate, twice a day between meals (if this recommendation results in abdominal discomfort, take 30 mg with meals three times a day)

Botanical Medicines

For chronic recurring menorrhagia:

- *V. agnus castus (chaste berry):* The usual dosage of chaste berry extract (often standardized to contain 0.5 % agnuside) in tablet or capsule form is 175 to 225 mg/day. If using the liquid extract, the typical dosage is 2 to 4 mL (approximately ½–1 tsp)/day.

For semiacute cases, the following botanicals should be given:

- Ginger 250 mg (powdered rhizome) three times daily for 4 days. Start the day before menses and continue until the third day of menses.
- Botanical tincture of equal parts of each herb (20–30 drops every 2–3 hours): yarrow, greater periwinkle, shepherd's purse, life root
- Cinnamon oil: 1 to 5 drops every 3 to 4 hours

Bioidentical Hormones

- Oral micronized progesterone: 100 mg twice a day given in the luteal phase for 12 days per month for recurring menorrhagia; 200 to 400 mg per day for 7 to 12 days may be used for semiacute blood loss, followed by a cyclic hormone product for 21 days on and 7 days off.
- Oral micronized progesterone for semiacute blood loss: 200 to 400 mg per day for 7 to 12 days, followed by a cyclic hormone program for 21 days on and 7 days off.
- Progesterone cream (a product that contains at least 400 mg of progesterone per ounce): ¼ to ½ tsp twice a day for 12 days per month during the luteal phase for cases of mild recurring menorrhagia.
- Bioidentical estradiol: High-dose regimen for acute bleeding is as follows: 4 mg estradiol every 4 hours for 24 hours, then a single daily dose of 1 mg for 7 to 10 days, followed by oral micronized progesterone 200 mg before bed for 7 to 12 days.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Davies J, Kadir RA. Heavy menstrual bleeding: an update on management. *Thromb Res*. 2017;151(suppl 1):S70–S77.
2. Excellence NIfHaC. *Heavy menstrual bleeding: assessment and management*; 2007. Available at nice.org.uk/guidance/cg44. Accessed January 1, 2018.
3. Gynecologists ACoOa. ACOG committee opinion no. 557: Management of acute abnormal uterine bleeding in nonpregnant reproductive-aged women. *Obstet Gynecol*. 2013;121(4):891–896.
4. Hapangama DK, Bulmer JN. Pathophysiology of heavy menstrual bleeding. *Womens Health (Lond)*. 2016;12(1):3–13.
5. *The Merck Manual of Diagnosis and Therapy*. 19th ed. Whitehouse Station, NJ: Merck Sharp & Dohme Corp.; 2011.
6. Downing I, Hutchon DJ, Poyser NL. Uptake of [3H]-arachidonic acid by human endometrium. Differences between normal and menorrhagic tissue. *Prostaglandins*. 1983;26(1):55–69.
7. Stott PC. The outcome of menorrhagia: a retrospective case control study. *J R Coll Gen Pract*. 1983;33(256):715–720.
8. Munro MG. Practical aspects of the two FIGO systems for management of abnormal uterine bleeding in the reproductive years. *Best Pract Res Clin Obstet Gynaecol*. 2017;40:3–22.
9. Stoffer SS. Menstrual disorders and mild thyroid insufficiency: intriguing cases suggesting an association. *Postgrad Med*. 1982;72(2):75–77, 80–72.
10. Chimbira TH, Anderson AB, Turnbull A. Relation between measured menstrual blood loss and patient's subjective assessment of loss, duration of bleeding, number of sanitary towels used, uterine weight and endometrial surface area. *Br J Obstet Gynaecol*. 1980;87(7):603–609.
11. Hallberg L, Högdahl AM, Nilsson L, Rybo G. Menstrual blood loss—a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol Scand*. 1966;45(3):320–351.
12. Kala M, Nivsarkar M. Role of cortisol and superoxide dismutase in psychological stress induced anovulation. *Gen Comp Endocrinol*. 2016;225:117–124.
13. Nagma S, Kapoor G, Bharti R, Batra A, Aggarwal A, Sablok A. To evaluate the effect of perceived stress on menstrual function. *J Clin Diagn Res*. 2015;9(3):QC01–QC03.
14. Ekpenyong CE, Davis KJ, Akpan UP, Daniel NE. Academic stress and menstrual disorders among female undergraduates in Uyo, South Eastern Nigeria—the need for health education. *Niger J Physiol Sci*. 2011;26(2):193–198.
15. Arvidsson B, Ekenved G, Rybo G, Sölvell L. Iron prophylaxis in menorrhagia. *Acta Obstet Gynecol Scand*. 1981;60(2):157–160.
16. Taymor ML, Sturgis SH, Yahia C. The etiological role of chronic iron deficiency in production of menorrhagia. *JAMA*. 1964;(187):323–327.
17. Lewis GJ. Do women with menorrhagia need iron? *Br Med J (Clin Res Ed)*. 1982;284(6323):1158.
18. Ando K, Morita S, Higashi T, et al. Health-related quality of life among Japanese women with iron-deficiency anemia. *Qual Life Res*. 2006;15(10):1559–1563.
19. Lithgow DM, Politzer WM. Vitamin A in the treatment of menorrhagia. *S Afr Med J*. 1977;51(7):191–193.
20. Cohen JD, Rubin HW. Functional menorrhagia: treatment with bioflavonoids and vitamin C. *Curr Ther Res Clin Exp*. 1960;2:539–542.
21. Dasgupta PR, Dutta S, Banerjee P, Majumdar S. Vitamin E (alpha tocopherol) in the management of menorrhagia associated with the use of intrauterine contraceptive devices (IUCD). *Int J Fertil*. 1983;28(1):55–56.
22. Stone KJ, Willis AL, Hart WM, Kirtland SJ, Kernoff PB, McNicol GP. The metabolism of dihomo-gamma-linolenic acid in man. *Lipids*. 1979;14(2):174–180.
23. Gubner R, Ungerleider H. Vitamin K therapy in menorrhagia. *South Med J*. 1944;37:556–558.
24. Kelly RW, Lumsden MA, Abel MH, Baird DT. The relationship between menstrual blood loss and prostaglandin production in the human: evidence for increased availability of arachidonic acid in women suffering from menorrhagia. *Prostaglandins Leukot Med*. 1984;16(1):69–78.
25. Biskind M. Nutritional deficiency in the etiology of menorrhagia, metrorrhagia, cystic mastitis, and premenstrual tension; treatment with vitamin B complex. *J Clin Endocrinol*. 1943;(3):227–234.
26. Jafari A, Abedi P, Sayahi M, Torkashvand R. The effect of vitamin B1 on bleeding and spotting in women using an intrauterine device: a double-blind randomised controlled trial. *Eur J Contracept Reprod Health Care*. 2014;19(3):180–186.
27. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (Zingiber officinale). *J Ethnopharmacol*. 1989;27(1-2):129–140.
28. Kashefi F, Khajehei M, Alavinia M, Golmakani E, Asili J. Effect of ginger (Zingiber officinale) on heavy menstrual bleeding: a placebo-controlled, randomized clinical trial. *Phytother Res*. 2015;29(1):114–119.
29. Probst V, Roth OA. [A plant extract with hormonic effects]. *Dtsch Med Wochenschr*. 1954;79(35):1271–1274.
30. Bleier W. [Use of plant substances in the therapy of menstruation & bleeding disorders & other endocrine diseases of women]. *Zentralbl Gynakol*. 1959;81(18):701–709.
31. Milewicz A, Gejdel E, Sworen H, et al. [Vitex agnus castus extract in the treatment of luteal phase defects due to latent hyperprolactinemia. Results of a randomized placebo-controlled double-blind study]. *Arzneimittelforschung*. 1993;43(7):752–756.
32. Yavarikia P, Shahnazi M, Hadavand Mirzaie S, Javadzadeh Y, Lutfi R. Comparing the effect of mefenamic acid and vitex agnus on intrauterine device induced bleeding. *J Caring Sci*. 2013;2(3):245–254.
33. Jurenka JS. Therapeutic applications of pomegranate (Punica granatum L.): a review. *Altern Med Rev*. 2008;13(2):128–144.
34. Goshtasebi A, Mazari Z, Behboudi Gandevani S, Naseri M. Anti-hemorrhagic activity of Punica granatum L. flower (Persian Golnar) against heavy menstrual bleeding of endometrial origin: a double-blind, randomized controlled trial. *Med J Islam Repub Iran*. 2015;29:199.
35. Batista AL, Lins RD, de Souza Coelho R, do Nascimento Barbosa D, Moura Belém N, Alves Celestino FJ. Clinical efficacy analysis of the mouth rinsing with pomegranate and chamomile plant extracts in the gingival bleeding reduction. *Complement Ther Clin Pract*. 2014;20(1):93–98.
36. Sedigh-Rahimabadi M, Fani M, Rostami-Chijan M, Zarshenas MM, Shams M. A traditional mouthwash (Punica granatum var pleniflora) for controlling gingivitis of diabetic patients: a double-blind bandomized controlled clinical trial. *J Evid Based Complementary Altern Med*. 2017;22(1):59–67.
37. Sisay M, Gashaw T. Ethnobotanical, ethnopharmacological, and phytochemical studies of myrtus communis linn: a popular herb in unani system of medicine. *J Evid Based Complementary Altern Med*. 2017;22(4):1035–1043.
38. Qaraaty M, Kamali SH, Dabaghian FH, et al. Effect of myrtle fruit syrup on abnormal uterine bleeding: a randomized double-blind, placebo-controlled pilot study. *Daru*. 2014;22:45.
39. Ghalandari S, Kariman N, Sheikhan Z, Mojab F, Mirzaei M, Shahrahmani H. Effect of hydroalcoholic extract of capsella bursa pastoris on early postpartum hemorrhage: a clinical trial study. *J Altern Complement Med*. 2017;23(10):794–799.
40. Schumann E. Newer concepts of blood coagulation and control of hemorrhage. *Am J Obstet Gynecol*. 1939;(38):1002–1007.
41. Steinberg A, Segal HI, Parris HM. Role of oxalic acid and certain related dicarboxylic acids in the control of hemorrhage. *Ann Otol Rhinol Laryngol*. 1940;(49):1008–1021.

Migraine Headache

Nancy Sudak, MD,* and *Joseph Katzinger, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Recurrent paroxysmal attacks of headache occur.
- Headaches are typically pounding and unilateral but may become generalized.
- Attacks may be preceded by psychological or visual disturbances (auras); accompanied by anorexia, nausea, and gastrointestinal upset; and followed by drowsiness.
- Physical examination typically reveals no focal neurological deficit.

GENERAL CONSIDERATIONS

The lifetime prevalence of migraine headache is at least 18%.[1] Women are more frequently affected than men, and more than half of affected patients have a family history of migraine. The prevalence is highest in North America and lowest in Africa and Asia.[2] Although many migraines come on spontaneously, 10% to 20% of migraineurs may have warning symptoms (auras) before the onset of pain.[3] Typical auras last a few minutes and may include the following:

- Blurring or bright spots of vision
- Anxiety
- Fatigue
- Disturbed thinking
- Unilateral peripheral numbness or tingling

The pain of migraine headache results from sensory responses distinct from brain tissue because the brain itself is insensate. Cephalic pain arises from the meninges, large cranial vessels, proximal intracranial vessels, and the scalp vasculature and muscles when stretched or tensed.

Pathophysiology

The sequence of events producing migraine headache is unclear, but migraine headache is no longer believed to be a primary vascular event.[1,4,5] Considerable evidence supports an association between migraine headache and vasomotor instability, but vasomotor changes are now believed to be epiphenomena, and a singular pathophysiological mechanism has not been identified. Despite the awareness of the multiple pathways involved in migraine headache, a clear understanding of its pathogenesis is, at present, not within reach. However, many of the key elements involved are worth discussing because they relate to prevention and treatment. Fig. 198.1 provides an overview of how the various anomalies documented among migraineurs may interact.

Vasomotor Activity

Migraineurs experience heightened central nervous system (CNS) activity, which appears to be mediated by the trigeminovascular system. Stimulation of the trigeminal ganglion results in the release of vasoactive neuropeptides, including substance P, calcitonin gene–related peptide, and neurokinin A. The release of these neuropeptides results in the process of neurogenic inflammation. The two main components of this sterile inflammatory response are vasodilation and plasma protein extravasation.

It has been clinically observed that superficial temporal vessels are visibly dilated during migraine, and local compression of these vessels or the carotid artery temporarily relieves migraine pain.[6] However, other types of extracranial vasodilation (e.g., heat- or exercise-induced) are not associated with migraine. Despite the extracranial vasodilation, the patient appears pale during the headache, suggesting constriction of the small vessels. This observation is supported by the presence of a lower skin temperature on the affected side. The clinical manifestations of focal or diffuse cerebral or brainstem dysfunction have been attributed to intracranial vasoconstriction. Some studies measuring cerebral blood flow have demonstrated a reduction in flow, sometimes to very low and critical levels, during the prodromal stage. The aura of migraine is apparently caused by cortical spreading depression (CSD),[7–10] a process that produces a transient depression of spontaneous and evoked neuronal activity. During this time, the brain fails to maintain normal ionic homeostasis and efflux of excitatory amino acids from neurons.[7] CSD may represent a significant link to the decrease in cerebral blood flow observed during a migraine aura.[11,12] This stage may be followed by a phase of increased blood flow, which can persist for more than 48 hours.

*Previous edition contributor

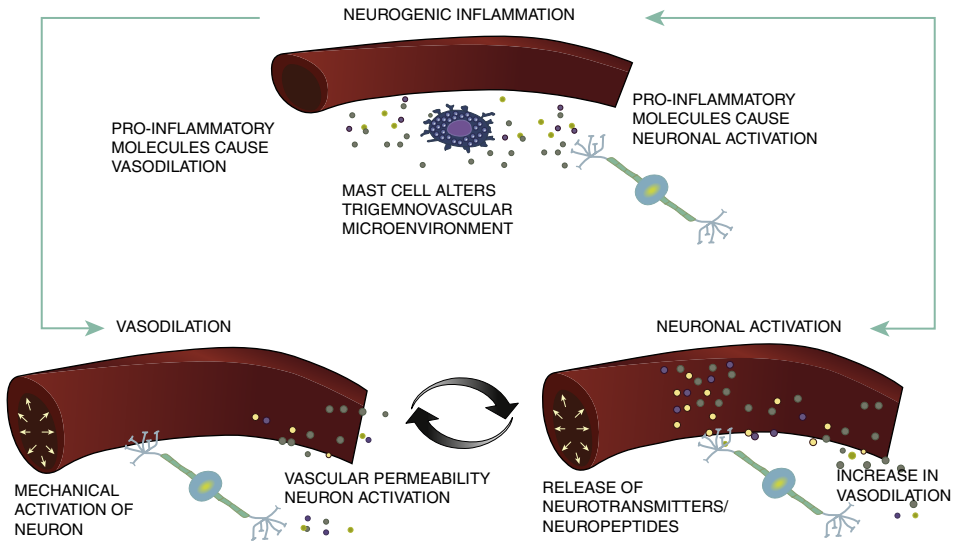


Fig. 198.1 Model of vascular-neural coupling. Mast cell activation and degranulation alter the trigeminovascular microenvironment via the release of inflammatory molecules. Inflammatory molecules can mediate the vasodilation of nearby vessels and cause nociceptor activation. These vessels can activate trigeminal neurons mechanically or by the release of inflammatory mediators due to increased vascular permeability, causing a positive feedback loop. (From Mason BN, Russo AF. Vascular contributions to migraine: time to revisit? *Front Cell Neurosci.* 2018 Aug 3;12:233. PMID: 30127722. Reproduced from an open-access article distributed under the terms of the Creative Commons Attribution License [CC BY].)

Increasing evidence suggests that CSD may play a role in migraine pathophysiology and overall cerebral hyperexcitability. In addition, triggering CSD can require activation of the *N*-methyl-D-aspartate (NMDA) receptor, which augments depolarization.[13] In one study, CSD was prevented in the presence of ifenprodil, a selective NMDA receptor antagonist.[14] NMDA receptor antagonist therapy is suggested to be a potentially novel therapeutic approach.[15] The anticonvulsant topiramate has become a first-line treatment for migraine; its efficacy as a gamma-aminobutyric acid (GABA) agonist and glutamate antagonist supports the concept of excessive cerebral excitability as related to NMDA-receptor activation.[16] Calcitonin gene–related peptide (CGRP), among the most potent vasodilators known, is found to be elevated after migraine. CGRP antagonists have been demonstrated to be effective in relieving migraine pain without causing vasoconstriction and are being investigated for their therapeutic efficacy and safety.[17]

Evidence indicates that patients with migraine have a functional abnormality of vasomotor control. Migraineurs suffer from syncope and orthostatic intolerance more often than patients who do not have migraine (who are without clear interictal signs of autonomic nervous system dysfunction)[18]; they also seem to be abnormally sensitive to the vasodilatory effects of physical and chemical agents.

Migraine also has complex relations with disorders of the cerebrovascular system, the cardiovascular system, and the heart. Right-to-left shunt, patent foramen ovale, and mitral valve prolapse are all associated with a higher prevalence of migraine headache. Migraine with aura has also been shown to be associated with an increase in cardiovascular risk factors (ratio of total cholesterol to high-density-lipoprotein cholesterol >5, hypertension, and history of early-onset coronary heart disease or stroke), as well as an increase in both all-cause and cardiovascular mortality.[19,20] Although the risk for stroke is highest among young women who have migraine with aura, particularly if they smoke or use oral contraceptives, studies suggest that male and female migraineurs are at increased risk for ischemic stroke regardless of age.[21] It is additionally worth noting that migraine and epilepsy are comorbid disorders that share common physiological mechanisms and treatments.[22]

Platelets and Serotonin

Serotonin is normally stored in platelets and released by platelet aggregation and in response to various stimuli, such as catecholamines. There is no difference in total serotonin content between normal platelets and the platelets of patients with migraine. However, the quantity of serotonin released by the platelets of the patient with migraine in response to serotonin stimulation, although normal or even subnormal immediately after an attack, becomes progressively higher as the next attack approaches.[23] The rise of plasma serotonin may not be causative in migraine but could represent a self-defense mechanism.[7] Platelet activation has been observed in electron micrographs during migraine, which may be a result of the neurogenic inflammation.[8] A genetic predisposition to altered serotonin functionality in migraine has also emerged; for example, a polymorphism with a variable number of tandem repeats in a region of the serotonin transporter gene (SERT) has been implicated. The variant with more repeats and greater expression has been found by meta-analysis to increase the risk for migraine by 34% to 55% among heterozygotes and homozygotes, respectively.[24] Furthermore, SERT proteins expressed by platelets are identical to those expressed in the brain. It is worth noting, on the other hand, that platelet deactivating agents—such as aspirin, feverfew, and essential fatty acids—appear to be effective in migraine prevention. Biochemical studies have shown that serotonin is a stress-response system that involves increased turnover during both acute and longer-term stress.[25]

The connection between serotonin and headaches began in the 1960s when researchers found that there was an increase in the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the urine during a migraine.[26] Initially, serotonin excess was implicated; however, newer information indicates that the factor responsible for the increase in 5-HIAA is more likely the increased breakdown of serotonin as a result of the increased activity of monoamine oxidase (MAO).[27,28] Because migraineurs appear to have low levels of serotonin in their tissues, researchers began to refer to migraine as a "low-serotonin syndrome."[28] Low serotonin levels are thought to lead to a decrease in

the pain threshold in patients with chronic headaches. This contention is strongly supported by over 35 years of research, including positive clinical results in double-blind studies with the serotonin precursor 5-hydroxytryptophan (5-HTP). Current understanding indicates that a low-serotonin state facilitates activation of the trigeminovascular nociceptive pathway, as induced by CSD.[29] For more information on the clinical studies with 5-HTP in migraine headaches, see Chapter 87.

The triptan family of drugs includes the $5HT_{1b}$ and $5HT_{1d}$ receptor agonists. These agents constrict blood vessels, block neurogenic inflammation and neural peptide release, and may also inhibit neuronal activity within the trigeminovascular system.[30–32] The safety of these agents has been questioned because the triptans activate serotonin receptors on cerebral vessels and, to a lesser extent, coronary arteries. The chest pain that is reported by 3% to 5% of patients taking oral triptans has generally not been associated with electrocardiographic changes and does not appear to be due to cardiac ischemia.[33] However, triptans should be avoided in patients with ischemic heart disease, uncontrolled hypertension, or cerebrovascular disease.[1] It is generally advisable to avoid the use of triptans, ergotamine-based drugs, and selective serotonin reuptake inhibitors (SSRIs) simultaneously, although the risk of serotonin syndrome appears to be low.[33] Extreme caution is advised in considering the use of an SSRI along with 5-HTP. Although SSRIs are sometimes used for migraine prophylaxis, the scientific effect is unknown, the quality of evidence is poor, and the impression of clinical effect is low.[25,34,35]

Neuronal Disorder

It has been suggested that the trigeminovascular neurons, which innervate the pial arteries, release calcitonin gene–related peptide and the peptide substance P, either directly from various cellular signals or secondarily to CNS activation.[36] Substance P is a key pain mediator, and its release into the arteries is associated with vasodilation, mast cell degranulation, and increased vascular permeability. It is believed that the endothelial cells of the arteries respond to substance P and CGRP by releasing vasoactive substances such as arachidonic acid metabolites, purine compounds, or molecules containing carbonyl groups. This theory suggests that functional changes within the noradrenergic system constitute the threshold for migraine activation, and it is through modulation of sympathetic activity that potentiators exert their effects.[36] Chronic stress is a chief potentiator in this model.

Unified Hypothesis

The mechanism of migraine can be described as a three-stage process: initiation, prodrome, and headache. Although a particular stressor may be associated with the onset of a specific attack, it appears that the initiation is dependent on the accumulation over time of several stressors. These stressors ultimately affect serotonin metabolism (Box 198.1). Once a critical threshold of susceptibility is reached, a cascade of events is initiated. This susceptibility is probably based on a combination of decreased tissue serotonin levels; platelet modulation; alteration in the responsiveness of key cerebrovascular end organs; increased sensitivity of the intrinsic noradrenergic system of the brain; and the accumulation of histamine, arachidonic acid metabolites, or other mediators of inflammation. The platelet changes include increased adhesiveness, enhanced tendency to release serotonin, and increased levels of arachidonic acid in the membranes. Once platelets are stimulated to secrete serotonin, sequelae, including platelet aggregation, vasospasm, and inflammatory processes, lead to local cerebral ischemia. This is followed by rebound vasodilation and the release of peptide substance P and other mediators of pain. These events are summarized in Fig 198.2.

BOX 198.1 Factors That Can Trigger Migraine Headaches

- Low serotonin levels
  - Genetics
  - Shunting of tryptophan into other pathways
- Foods
  - Food allergies
  - Histamine-releasing foods
  - Histamine-containing foods
- Alcohol, especially red wine
- Chemicals
  - Nitrates
  - Monosodium glutamate (MSG)
  - Nitroglycerin
- Withdrawal from caffeine or other drugs that constrict blood vessels
- Stress
- Emotional changes, especially letdown after stress, and intense emotions such as anger
- Hormonal changes (e.g., menstruation, ovulation, birth control pills)
- Too little or too much sleep
- Exhaustion
- Poor posture
- Muscle tension
- Weather changes (e.g., changes in barometric pressure, exposure to sun, increased temperature may exacerbate ambient air pollution)
- Glare or eyestrain

THERAPEUTIC CONSIDERATIONS

Conventional Treatments

Pharmacological treatment of migraine headache tends to be inadequate because it fails to address the underlying cause. Modern first-line pharmaceuticals for prophylaxis in adults include propranolol, valproic acid, and topiramate, but a host of other drugs can be helpful for migraine prevention. In 2018 the first CGRP-related therapies were approved, including Erenumab, a CGRP inhibitor. The initial results are encouraging in terms of both efficacy and safety, but long-term data are needed because these therapies carry a potential risk for neovascularization-related adverse effects, such as delay in recovery from ischemia and the healing of wounds and ulcers.[37,38] Despite their effectiveness, some preventive drugs can exacerbate other conditions. Flunarizine (unavailable in the United States), valproate, and amitriptyline, for example, should be used with caution or avoided in obese patients because they tend to cause weight gain. Amitriptyline and pizotifen can worsen glaucoma and urinary retention. Beta blockers can exacerbate asthma and diabetes, and topiramate can enhance the risk of renal calculi.[39] Most guidelines from European countries include a nondrug treatment section, which also includes behavioral and psychological techniques.[40] Medications are considered efficacious if they reduce migraine frequency by at least 50%.[41] This is an important consideration where nonpharmacological treatment options are concerned. The first step in treating migraine headache is to identify the precipitating factor or factors. Although food intolerance/allergy is important, many other factors must be considered as either primary causes or contributors to the migraine process. In particular, it is very important to assess the role that headache medications may be playing, particularly in chronic headaches.

Drug Reactions

Medication-overuse headache (MOH), also known as analgesic rebound headache, drug-induced headache, and medication-misuse

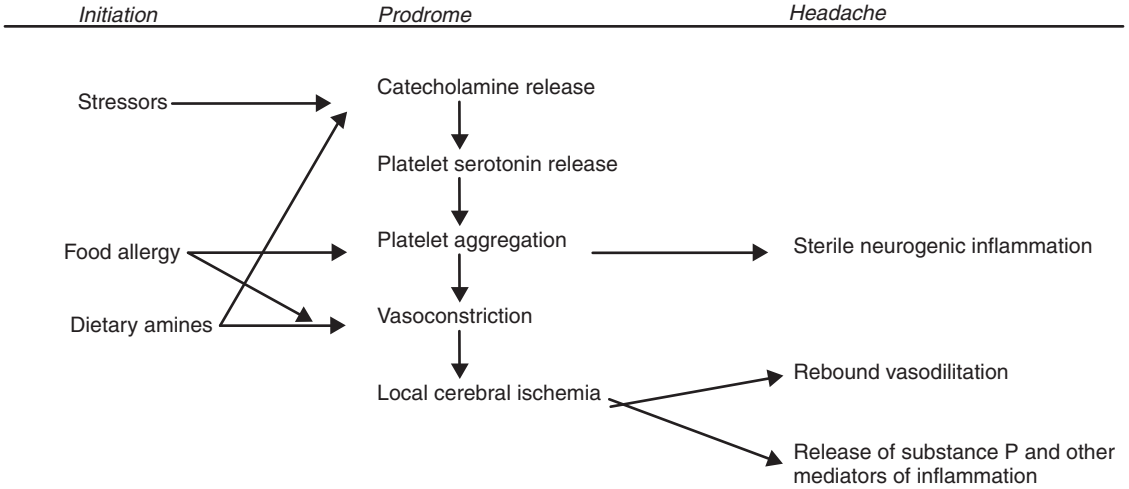


Fig. 198.2 Suggested pathogenesis of migraine headache.

headache, deserves mention because it frequently precludes the successful treatment of the underlying primary headache disorder, most often migraine or tension-type headache. In some cases, MOH may be associated with the development or maintenance of a chronic daily headache syndrome.[42] After successful MOH treatment, preventive medications for the underlying primary headache disorder have a greater chance of success. Patients who were able to discontinue their daily use of symptom-modifying medications experienced improvement in their chronic headaches in addition to greater efficacy of their prophylactic medications.[43] All drugs used for symptomatic relief on a regular basis can produce MOH, although the precise mechanism of action is unknown.[44] Central sensitization, changes in trigeminal pain processing, receptor downregulation, and biobehavioral factors have all been proposed as possibly leading to MOH. Drugs that carry the highest potential for causing MOH include opioids, butalbital-containing analgesics, and aspirin/acetaminophen/caffeine combinations. Triptans carry intermediate to high potential for causing MOH when used frequently, and nonsteroidal anti-inflammatory drugs (NSAIDs) carry the lowest risk.[45] Ergotamine can cause MOH in addition to other symptoms when taken regularly. Regular use of ergotamine in migraine can result in a dependency cycle in which headaches escalate in severity on cessation of the medication. Ergotamine withdrawal predictably causes debilitating headaches, often accompanied by nausea and vomiting, generally resulting in an improved status by 72 hours. Ginger preparations can be helpful during ergotamine withdrawal.

MOH should be suspected in any patient with chronic headaches who is taking large or regular quantities of analgesics and who is experiencing daily predictable headache. Any medication used regularly for headache pain could be a causative agent and hence should be eliminated (opiates, barbiturates, and benzodiazepines must be withdrawn slowly, but NSAIDs, triptans, ergot alkaloids, and acetaminophen may be stopped abruptly). Although the logic may seem counterintuitive to patients, they may be pleasantly surprised by a sizable improvement in outcome. For those having trouble tolerating the transition, acupuncture may be offered. Agents that are sometimes used during the transition when medications are unavoidable include metoclopramide, dihydroergotamine mesylate, naproxen sodium, and prednisone.

Diet

Food Allergy/Intolerance

Food allergy/intolerance can play an important role in many cases of migraine headache. Double-blind placebo-controlled studies have demonstrated that the detection and removal of antigenic/intolerable foods will eliminate or greatly reduce migraine symptoms in the majority of patients. What is unclear is the percentage of migraine patients for whom food control is the most important factor. Table 198.1 summarizes the results of several clinical studies. Success ranges from 30% to 93%, with the majority of studies showing a remarkably high degree of success.[46–53]

TABLE 198.1 Food Allergy/Intolerance and Migraine Headache

Study	Percentage Responding	Method
Mansfield et al.[46]	30	Elimination
Carter et al.[47]	93	Oligoantigenic diet
Hughes et al.[48]	80	Fasting, rotation, elimination
Egger et al.[49]	93	Elimination
Monro et al.[50]	70	RAST,[a] elimination, sodium
Grant[51]	85	Elimination

[a]Radioallergosorbent test (blood test for immunoglobulin E [IgE]–mediated allergy).

A plausible explanation for the large difference between the results of Mansfield et al.[46] and the others[47–53] is that the Mansfield design was carefully selected for food allergy only, whereas the others included food intolerance. These studies found the incidence of food allergy to be similar among the major types of migraine. Few studies have been performed since the 1980s, when much of the groundwork regarding the relationship between food intolerance and migraine was laid. The foods most commonly found to induce migraine headaches are listed in Table 198.2.

The mechanism by which food allergy/intolerance induces a migraine attack is still unknown. Several theories have been proposed:

- Idiopathic response to a pharmacologically active substance, such as tyramine
- Monoamine oxidase deficiency
- Platelet phenolsulfotransferase deficiency; immunologically mediated food allergy
- Platelet abnormalities

Egger et al.[49] suggested that migraine headache may occur through chronic activation of the nonspecific responsiveness of cerebrovascular end organs as a result of long-term antigenic stimulation. This mechanism would be analogous to the bronchiolar response in asthma

TABLE 198.2 Foods That Most Commonly Induce Migraine Headaches

Food	Egger et al.[49] (%)	Hughes et al.[48] (%)	Monro et al.[50] (%)
Cow's milk	67	57	65
Wheat	52	43	57
Chocolate	55	57	26
Egg	60	24	22
Orange	52	—	13
Benzoic acid	35	—	—
Cheese	32	—	—
Tomato	32	14	—
Tartrazine	30	—	—
Rye	30	—	—
Rice	—	—	30
Fish	22	29 (shell)	17
Grapes	12	33	—
Onion	—	24	—
Soy	17	24	—
Pork	22	—	17
Peanuts	12	29	—
Alcohol	—	29	9
Monosodium glutamate	—	19	—
Walnuts	—	19	—
Beef	20	14	—
Tea	17	—	17
Coffee	15	19	17
Nuts	12	19 (cashew)	17
Goat's milk	15	14	—
Corn	20	9	—
Oats	15	—	—
Cane sugar	7	19	—
Yeast	12	14	—
Apple	12	—	—
Peach	12	—	—
Potato	12	—	—
Chicken	7	14	—
Banana	7	—	—
Strawberry	7	—	—
Melon	7	—	—
Carrot	7	—	—

to exercise or cold after antigen contact. Allergic reactions to foods are known to cause platelet degranulation, with resultant serotonin release.[52]

In children, common migraine triggers include cheese, chocolate, citrus fruits, hot dogs, monosodium glutamate, aspartame, fatty foods, ice cream, and caffeine withdrawal. Among adolescents, alcoholic drinks, especially red wine and beer, may also be a trigger. Tyramine, phenylethylamine, histamine, nitrites, and sulfites are involved in the mechanism of food intolerance headache, whereas immunoglobulin E-mediated food allergy is an infrequent cause.[54]

Several methods may be used to detect food allergies; most of these are described in Chapter 14. Laboratory testing can be convenient, but one major drawback of food intolerance testing with immunoglobulin G (IgG) antibodies can be a lack of reproducible findings with split blood samples among various laboratories. However, a study published in Mexico in 2007 found that when migraineurs removed foods to which they had mounted IgG responses, they experienced a robust improvement in their migraines, with 43 of 65 patients showing complete remission after 1 month of specific food elimination.[55] In 2010 a double-blind, randomized, crossover trial found a statistically significant reduction in headache days and migraine attacks after following an IgG-based elimination diet. Interestingly, the average number of foods with a high titer was 24 out of 266 tested.[56] Patients with both migraine and irritable bowel syndrome (IBS) following an IgG-guided elimination diet also found significant improvement for both conditions when randomized and double-blinded.[57]

Elimination diets/challenge testing are reliable and experiential methods for detecting food intolerance, but they also have limitations: some foods evoke a delayed response, so it may require several days of repeated challenge to elicit recognizable symptoms. Food triggers can also be inconsistent. The ingestion of large amounts of several foods may be necessary to detect those that are marginally reactive. The recommended procedure for the diagnosis and management of food allergy/intolerance is described in the section "Therapeutic Approach."

BOX 198.2 Factors Involved With Histamine-Induced Headaches

Histamine levels increased by:
- Histamine in alcoholic beverages (particularly red wine)
- Histamine in food
- Histamine-releasing foods
- Food allergy
- Vitamin B_6 deficiency

Histamine breakdown inhibited by:
- Vitamin B_6 antagonists
  - Alcohol
  - Drugs
  - Food additives (e.g., yellow dye #5, monosodium glutamate)
- Vitamin C deficiency

Histamine release prevented by:
- Disodium cromoglycate
- Quercetin
- Antioxidants (e.g., vitamin C, vitamin E, selenium, etc.)

Histamine breakdown promoted by:
- Vitamin B_6
- Vitamin C

Dietary Amines

Foods such as chocolate, aged cheese, pickled herring, sausages, sour cream, fermented foods, beer, and wine may precipitate migraine attacks because they contain histamine and/or other vasoactive compounds such as phenylethylamine that have a vasodilatory effect (Box 198.2).[53,58,59] Tyramine is a component of many of these foods and has long been regarded as an important migraine trigger, but controlled studies have yielded conflicting findings.[60] Red wine is more likely than white wine to trigger a headache because it contains 20 to 200 times the amount of histamine and stimulates the release of vasoactive compounds by platelets.[23,58,61]

Red wine is additionally much higher in flavonoids, the antioxidant components shown to help prevent heart disease. These compounds can also inhibit the enzyme phenol sulfotransferase, which normally breaks down serotonin and other vasoactive amines in platelets. Many migraineurs have been found to have significantly lower levels of this enzyme,[60] although the implications are confusing. Because red wine contains substances that are potent inhibitors of this enzyme, it often triggers migraines in these individuals, especially if consumed along with foods high in high vasoactive amine content, like cheese or

chocolate. A standard treatment for histamine-induced headaches is to use a histamine-free diet along with vitamin B_6 supplementation.[61,62]

The activity of the enzyme diamine oxidase, which breaks down histamine in the lining of the small intestine before it is absorbed into the circulation, appears to play a key role in determining reactivity to dietary histamine. Individuals sensitive to dietary histamine have lower levels (about 50%) of this enzyme in their tissues compared with control subjects.[58] Diamine oxidase is a vitamin B_6–dependent enzyme. Not surprisingly, compounds that antagonize vitamin B_6 also inhibit diamine oxidase.[58] These inhibitory factors include food coloring agents (specifically the hydrazine dyes, such as FD&C yellow #5), some drugs (isoniazid, hydralazine, dopamine, and penicillamine), oral contraceptives, alcohol, and excessive protein intake.

Vitamin B_6 supplementation (usually 1 mg/kg) has been shown to improve histamine tolerance, presumably by increasing diamine oxidase activity.[58,62] Women have lower levels of diamine oxidase, which may explain the higher incidence of histamine-induced headaches among them. Women are also much more frequently unable to tolerate red wine. Interestingly, the level of diamine oxidase in a woman increases by over 500 times during pregnancy.[63,64] It is very common for women with histamine-induced headaches to experience complete remission of their headaches during pregnancy.

Miscellaneous Diet-Related Triggers

Hypoglycemia can be a trigger for migraine headache[65,66] and is modifiable by dietary manipulation. Glycemic dysregulation is common in the setting of the standard American diet, given a tendency to consume unregulated quantities of high-glycemic-index carbohydrates. Hypoglycemia may be a result of inappropriate carbohydrate intake, especially when insulin levels become elevated. In 2018 a randomized trial found that a low-glycemic-index diet had a similar effectiveness to medications for migraine prophylaxis in terms of migraine frequency reduction, although a longer period was required for an effect (90 days vs. 30 days).[67] Excessive sodium intake may also represent a migraine trigger,[68] perhaps because of increasing angiotensin levels in response to sodium ingestion. In a randomized trial, a low-lipid diet (<20% of energy from lipids) has also proven effective for reducing the number and severity of headaches over a 1-year period compared with a typical lipid diet. It is speculated that a higher-lipid diet may trigger a greater degree of platelet aggregation and, consequently, an increase in serotonin release.[69]

For individuals who are lactose-intolerant, avoidance of dairy products may afford improvement in the experience of migraine headache.[70] Aspartame, a common sweetener, may be associated with an increased likelihood of migraine incidence[71,72] and is easily avoided. Sucralose has become another commonly used artificial sweetener and may be a migraine trigger.[73] Monosodium glutamate is a well-recognized migraine trigger that can directly activate the NMDA receptor.

Helicobacter pylori

Gasbarrini et al.[74] demonstrated that migraine headaches improved dramatically in 100% of patients for whom *Helicobacter pylori* was identified and treated through standard measures. Further research has shown that the prevalence of *H. pylori* infection is significantly higher in migraineurs without aura compared with controls, indicating that *H. pylori* infection is a probable independent environmental risk factor for migraine without aura, especially in patients who are not genetically or hormonally susceptible.[75] It appears that incidental *H. pylori* positivity is more relevant in migraineurs compared with controls because a high percentage experienced relief from migraines when the infection was eradicated.[76]

Nutritional Supplements

5-Hydroxytryptophan (5-HTP)

5-HTP, the molecular intermediate between tryptophan and serotonin, is readily absorbed when taken orally and easily crosses the blood–brain barrier. In addition to augmenting serotonin levels, it may also increase endorphin levels. The use of 5-HTP in the prevention of migraine headache offers considerable advantages over drug therapy. Although a number of drugs have been shown to be useful in the prevention of migraine headache, all of the currently used drugs carry with them a risk of significant adverse effects. 5-HTP is at least as effective as other pharmacological agents used in the prevention of migraine headaches and is safer and better tolerated. Although some studies have employed a dosage of 600 mg/day or more,[77] 5-HTP can be effective at a dosage as low as 200 mg/day.[78] The clinical studies with 5-HTP in migraine headaches are discussed in Chapter 87.

Essential Fatty Acids

The role of essential fatty acids (EFAs) in the pathogenesis of migraine may be quite important but does not appear to have received much research attention. Considering the significance of platelet aggregation and arachidonic acid metabolites in the mediation of the events leading to the cerebral ischemia of migraine, manipulation of dietary EFAs may be very useful. The therapeutic efficacy of EFA manipulation was demonstrated in a 2013 clinical trial; a high–omega-3 and low–omega-6 diet (H3-L6) significantly reduced pain and increased quality of life among participants with chronic headache (97% of whom had migraine or migraine-like symptoms). This was more effective than lowering only the omega-6 component of the diet, with a proposed mechanism of action mediated via antinociceptive omega-3–derived mediators, including resolvins D2 and E1, known to stimulate the resolution of inflammation.[79]

It has been demonstrated that reducing the consumption of animal fats and increasing the consumption of fish will significantly modify platelet and membrane EFA ratios and decrease platelet aggregation.[80–82] It is also noteworthy that 60% of the brain is composed of lipids; therefore appropriate consumption of the essential fats is mandatory for proper neurological function. In two small placebo-controlled studies,[83,84] omega-3 fatty acids performed significantly better than placebo in reducing headache frequency and intensity. A small, randomized, double-blind crossover study of 27 adolescents with migraine revealed marked improvement in migraine headache frequency, duration, and severity with fish oil, but patients were noted to respond equally well to olive oil supplements, the chosen placebo for this study.[85] In 2018 a systematic review and meta-analysis of randomized controlled trials was published; this analysis did not find benefit for reducing headache frequency, but it did find a significant reduction of approximately 3.5 hours in migraine duration.[86] The proposed mechanisms of action of the omega-3 fatty acids include reduced platelet serotonin release, modulation of prostaglandin synthesis, and diminution of cerebral vasospasm.

Bic et al. proposed that free fatty acids are an important underlying factor in the development of migraine headache because elevated blood lipids and free fatty acids are associated with increased platelet aggregability, decreased 5-HT, and increased prostaglandin levels, all of which may play a role in migraine.[87] They list biological states that produce increased free fatty acids and lipids, including high dietary fat intake, obesity, insulin resistance, vigorous exercise, hunger, consumption of alcohol and caffeine, oral contraceptive use, tobacco abuse, and stress. These variables may be manipulated successfully through lifestyle practices, diet, and pharmacological measures.

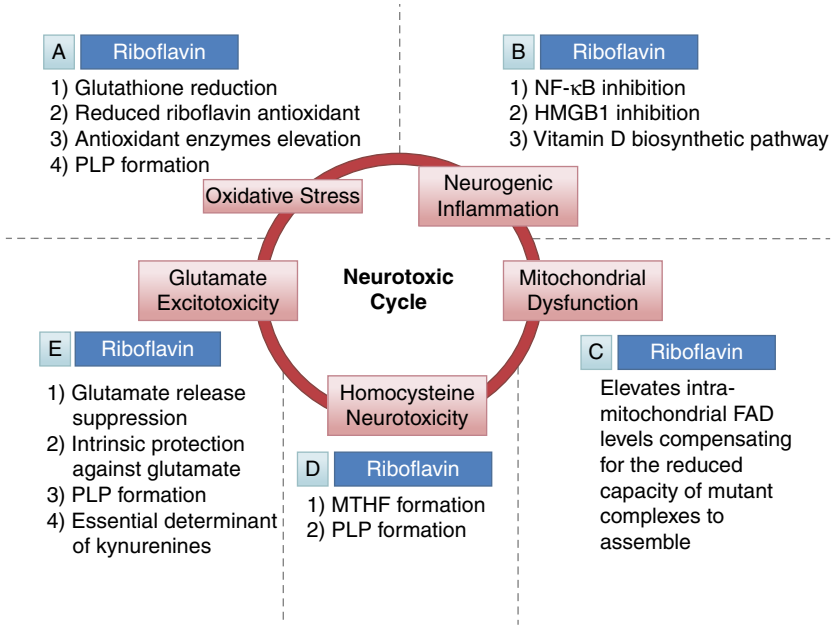


Fig. 198.3 Riboflavin mechanisms of action. Riboflavin protects against neurotoxicity by ameliorating oxidative stress, mitochondrial dysfunction, neurogenic inflammation, glutamate excitotoxicity, and homocysteine neurotoxicity. Oxidative stress, mitochondrial dysfunction, neurogenic inflammation, glutamate excitotoxicity, and homocysteine neurotoxicity are involved in neurodegeneration and neurotoxicity. Also, those neurotoxic factors have the ability to cause each other, leading to the formation of a neurotoxic cycle. Riboflavin is capable of attacking this proposed neurotoxic cycle via multiple neuroprotective mechanisms that tackle different neurotoxic factors in this cycle. (From Marashly ET, Bohlega SA. Riboflavin has neuroprotective potential: focus on Parkinson's disease and migraine. *Front Neurol*. 2017 Jul 20;8:333. Reproduced from an open-access article distributed under the terms of the Creative Commons Attribution License [CC BY].)

Riboflavin (Vitamin B_2)

A deficit of mitochondrial energy metabolism may play a role in migraine pathogenesis.[88] Riboflavin (vitamin B_2) is a precursor of flavin mononucleotide and flavin adenine dinucleotide, which are required for the activity of flavoenzymes involved in the electron transport chain. A randomized, placebo-controlled, double-blind study demonstrated that a daily oral dose of 400 mg of riboflavin was superior to placebo for migraine prophylaxis. The effect began at 1 month, with maximal effect after 3 months of therapy. At that point, 59% of those taking riboflavin were considered responders versus only 15% of those taking a placebo.[89] The hypothesis that vitamin B_2 benefits migraine by increasing complex I and II activity in mitochondrial energy metabolism can be tested by nuclear magnetic resonance spectroscopy.[89,90] Vitamin B_2 appears to improve mitochondrial function without changing neuronal excitability, which could explain the excellent tolerance and lack of CNS adverse effects. In addition to improving mitochondrial function, riboflavin has other mechanisms for reducing neurotoxicity, including protection against glutamate excitotoxicity and neurogenic inflammation (Fig. 198.3). Diarrhea and polyuria may be associated with the administration of high-dose vitamin B_2.[74] It is notable that riboflavin is well tolerated, is less expensive than typical migraine-targeted medications, and may provide protection from oxidative toxicity in the brain, making it an excellent therapeutic option.[91]

In a study of riboflavin prophylaxis for pediatric and adolescent migraine, 200 mg/day was an adequate dose, and although it took 4 months to achieve optimal results, nearly 70% reported a 50% or greater reduction in migraine frequency as well as a decrease in intensity.[92] Another study suggested a lack of efficacy for riboflavin in children, but the inclusion and exclusion criteria were acknowledged to be very strict.[93] It has also been found that 100 mg per day may be inadequate, whereas 200 mg per day effectively reduces headache frequency among children.[94]

An intriguing study was done in 2000 that compared riboflavin with beta-blocker therapy. Although both groups had a sizeable number of "responders" (53% and 55% had a reduction in frequency of more than 50%), they appeared to work via different mechanisms, as the beta-blocker group had reduced intensity of auditory-invoked cortical potentials that was directly proportional to the clinical response. Given that riboflavin did not alter cortical potentials, it suggests that these therapies may have value when used together or as viable alternatives to nonresponders to either therapy.[95] Lastly, mitochondrial DNA mutations may influence the clinical effect of riboflavin. In a trial comparing those with haplogroup H versus non-H, 44.8% compared with 77% responded to riboflavin supplementation, further supporting the hypothesis that riboflavin helps correct underlying mitochondrial pathology.[96]

Other B Vitamins

Other B vitamins, including folic acid, may also be instrumental in aborting or preventing migraine headache.[97] There may also be a genetic influence on the effectiveness of other B vitamins as well. For example, elevated homocysteine levels have been associated with a two- to nearly sixfold increase in the risk for migraine with aura, in part determined by polymorphisms in the methylenetetrahydrofolate reductase *(MTHFR)* gene, and dietary folate has been found to be inversely related to migraine disability, also influenced by MTHFR genotype (Fig. 198.4 shows the influence of B vitamins on homocysteine toxicity).[98,99] Several randomized trials have now been published documenting both a reduction in homocysteine (in response to B_6, B_{12}, and folic acid supplementation) as well as reductions in migraine frequency and severity, in part influenced by MTHFR and methionine synthase reductase (MTRR) polymorphisms. Given that those with the underactive MTHFR genotype are less responsive, this suggests that a

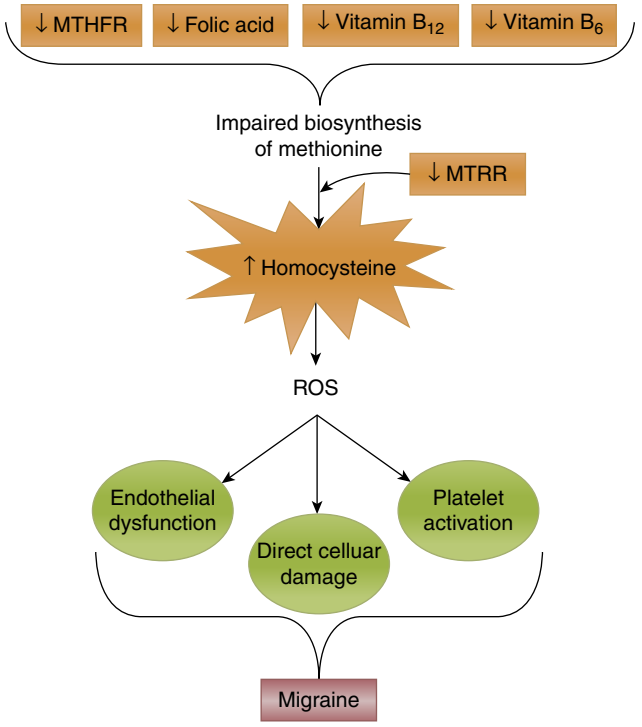


Fig. 198.4 Schematic representation depicting the role of vitamins B_6, B_{12}, and folic acid in migraine pathophysiology. *MTRR* (or *MSR*), methionine synthase reductase; *MTHFR,* methylenetetrahydrofolate reductase; *ROS,* reactive oxygen species. (From Shaik MM, Gan SH. Vitamin supplementation as possible prophylactic treatment against migraine with aura and menstrual migraine. *Biomed Res Int.* 2015;2015:469–529. PMID: 25815319.)

higher dose of these B vitamins may be warranted for these patients and/or the use of activated versions of these vitamins.[100,101] Open-label trials of vitamin B_{12} as intranasal hydroxocobalamin given alone as well as folic acid supplemented at 2 mg to 5 mg per day have also had promising results.[102,103]

Coenzyme Q_{10}

Coenzyme Q_{10} (CoQ_{10}) is a critical component of the electron transport chain and functions as an important antioxidant. Evidence supports the administration of CoQ_{10} in reducing the frequency of migraines by 61%.[104] After 3 months of receiving 150 mg of CoQ_{10} at breakfast, the average number of headache days decreased from seven to three per month in 31 adults who completed an open-label study. A double-blind, randomized study of 42 adult patients, using 100 mg of water-soluble CoQ_{10} three times daily, revealed similar results.[105] More recently, CoQ_{10} was given at 400 mg per day to women with episodic migraine in a randomized and double-blinded trial. This high dose not only significantly improved the severity, frequency, and duration of migraine, but it also reduced CGRP and tumor necrosis factor-α (TNF-α) significantly (although not interleukin-6 [IL-6] or interleukin-10 [IL-10]), suggesting another possible mechanism of action.[106]

CoQ_{10} deficiency appears to be common in the pediatric and adolescent populations and can be an important therapeutic consideration in these age groups.[107] Like riboflavin, CoQ_{10} is well tolerated (although expensive), with little risk of toxicity. It is ideal for women of childbearing age and for children because of its excellent tolerability. Liquid gel formulations should be favored for their high bioavailability. CoQ_{10} must be used with caution in patients who also take warfarin because it may antagonize the anticoagulation effects of warfarin. It is also noteworthy that many medications can interfere with CoQ_{10} activity, including statins, beta blockers, and certain antidepressants and antipsychotics.[108]

Magnesium

The high frequency of magnesium deficiency is well established. Magnesium is the least abundant serum electrolyte and the second most abundant intracellular cation; it is integral to the metabolism of calcium, potassium, phosphorus, zinc, copper, iron, sodium, lead, calcium, hydrochloric acid, acetylcholine, nitric oxide, and many enzymes. Magnesium absorption depends on dietary adequacy, selenium, parathyroid hormone, and vitamins B_6 and D. Absorption is hindered by excess fat. Magnesium levels are depleted by a multitude of common factors, including stress, excess alcohol intake, high estrogen levels, low progesterone, dietary excesses, certain drugs, hyperthyroidism, and hyperparathyroidism. Inadequate dietary intake of magnesium is likely in 75% of the U.S. population,[109] and it is believed to be the most common micronutrient deficiency, manifested by a diverse range of associated pathologies.[110] Physiological and psychological stress results in magnesium depletion, and both acute and chronic stress are associated with increased episodes of migraine.

Substantial documentation linking low magnesium levels to both migraine and tension headaches exists in the medical literature.[111–113] Low brain and tissue magnesium concentrations have been found in patients with migraine, indicating a need for supplementation. Among magnesium's central functions are to maintain vascular tone and prevent neuronal hyperexcitation.[111–114] Cerebrospinal fluid levels of magnesium have been found to be low in migraine, and low magnesium levels can predispose to several physiological dysfunctions known to be prominent in migraine. These include induction of cerebral vasospasm; potentiation of the contractile response of blood vessels to vasoactive substances such as serotonin; enhancement of the sensitivity of NMDA receptors to glutamate; and an increase in platelet aggregation, leading to serotonin release.[115] Magnesium deficiency may play an important role in the pathogenesis of migraine by promoting CSD.[73]

Two notable double-blind studies have provided conflicting results on the prevention of migraines in people prone to recurrent migraine headaches. In the first study, 250 mg of magnesium or placebo was given twice daily to 69 patients (35 received magnesium, and 34 received placebo) for 12 weeks.[116] The number of responders was 10 in each group (28.6% with magnesium and 29.4% with placebo). There was no benefit with magnesium compared with placebo with respect to migraine frequency or intensity. In the other double-blind study, patients experiencing recurrent migraines were given either 600 mg of oral magnesium daily for 12 weeks or placebo.[115,117] By the ninth week, the frequency of attacks was reduced by 41.6% in the magnesium group, compared with only 15.8% in the placebo group. Drug consumption for symptomatic treatment per patient also decreased significantly in the magnesium group. In a study that sought to determine magnesium efficacy specifically in migraineurs without aura, 600 mg of magnesium citrate daily produced a statistically significant improvement in outcome compared with placebo and also produced an improvement in cortical blood flow.[118]

It appears that magnesium supplementation may specifically be effective in those individuals with low tissue or low ionized levels of magnesium. Low tissue levels of magnesium are common in patients with migraine, but most cases go unnoticed because physicians generally rely on serum magnesium levels to assess magnesium status. Because most of the body's magnesium is intracellular, serum levels are unreliable indicators. A low magnesium level in the serum reflects late-stage deficiency. More sensitive tests of magnesium status include erythrocyte magnesium levels or ionized magnesium, the

most physiologically active form. This latter indicator has been found to indicate magnesium deficiency much more commonly during menstrual migraines than at other times, suggesting a potential therapeutic value of magnesium for these headaches.[119]

Hypothetically, patients with an acute migraine episode and low serum levels (<0.54 mmol/L) of ionized magnesium are more likely to respond to an intravenous infusion of magnesium sulfate ($MgSO_4$) than patients with higher levels of ionized magnesium in serum; this hypothesis has been tested.[120,121] Serum samples to determine levels of ionized magnesium were drawn immediately before infusion of 1 g of $MgSO_4$ in 40 patients with acute migraine headache. Pain reduction of 50% or more, as measured on a verbal pain intensity scale of 1 to 10, occurred within 15 minutes of infusion in 35 patients. In 21 patients, at least this degree of improvement or complete relief persisted for 24 hours or more. Pain relief lasted at least 24 hours in 18 of 21 patients (86%) with serum ionized magnesium levels below 0.54 mmol/L and in 3 of 19 patients (16%) with ionized magnesium levels at or above 0.54 mmol/L. The average ionized magnesium level in patients with relief lasting for at least 24 hours was initially significantly lower than that in patients with no relief or only fleeting relief.

Intravenous magnesium has been shown to be an extremely effective treatment in some cases of acute migraine, tension, and cluster headaches. A dosage of 1 to 3 g of intravenous magnesium (over a 10-minute period) generally results in a nearly 90% success rate in patients with low ionized magnesium levels.[120–123] Patients treated with $MgSO_4$ typically observe complete elimination of migraine-associated symptoms such as nausea, photosensitivity, and phonosensitivity. Adverse effects are rare, with the exception of a brief flushed feeling. Indeed, a 2016 meta-analysis of 21 randomized trials found that intravenous magnesium relieved acute migraines within 15 to 45 minutes, 2 hours, and 24 hours after infusion (odds ratios [ORs] = 0.23, 0.20, and 0.25, respectively). Additionally, oral magnesium significantly alleviated the frequency and intensity of migraine (ORs = 0.20 and 0.27), highlighting the important role of this mineral for both acute treatment and prophylaxis.[124]

Another possible benefit of magnesium in migraineurs may be its ability to improve mitral valve prolapse, which is linked to migraines because it causes mechanical damage to platelets, promoting their release of vasoactive substances like histamine, platelet-activating factor, and serotonin. Because research indicates that 85% of patients with mitral valve prolapse have chronic magnesium deficiency, magnesium supplementation is advisable.[125] This recommendation is further supported by several studies demonstrating that oral magnesium supplementation improves mitral valve prolapse.

Supplementation is readily achieved by using magnesium bound to glycine, citrate, malate, aspartate, or another Krebs cycle intermediate (caution is advised relative to the possible neuroexcitatory effects of aspartate). Typical dosage ranges are 200 to 800 mg/day, depending on bowel tolerance. Magnesium sulfates, oxides, and hydroxides are less predictably absorbed and may produce adverse gastrointestinal effects, but they may also be effective. The coadministration of vitamin B_6 is ideal because it facilitates magnesium transport into cells. Caution is advised in administering magnesium to patients with renal insufficiency.

Lipoic acid

Lipoic acid improves mitochondrial function, has antioxidant effects, and reduces insulin resistance. For these reasons it may be particularly useful for migraineurs with insulin resistance, as demonstrated in an open-label trial using 400 mg lipoic acid b.i.d. for 6 months, at which point nearly 70% of participants had a frequency reduction of at least 50%.[126]A previously conducted randomized trial among migraineurs with and without aura suggested efficacy for improving headache severity and frequency, but it was underpowered.[127]

Botanical Medicines

Botanical medicines have a lengthy history of use as traditional remedies for migraine headache. Although many botanicals have been used, feverfew *(Tanacetum parthenium),* ginger *(Zingiber officinalis),* and butterbur *(Petasides hybridus)* tend to be widely used for migraine prophylaxis and treatment.

Tanacetum parthenium

Perhaps the most popular preventive treatment of migraine headaches is the herb feverfew. Scientific interest in feverfew began when a 1983 survey found that 70% of 270 migraine sufferers who had eaten feverfew daily for prolonged periods found that the herb decreased the frequency and/or intensity of their attacks.[128] Many of these patients had been unresponsive to routine medications. This survey prompted several clinical investigations that are supportive of the therapeutic and preventive effects of feverfew in the treatment of migraine frequency and intensity.[128–131] However, at least three crossover randomized controlled trials found no benefit with feverfew, although each study had limitations.[132] Noting that the stability of feverfew can be problematic, a randomized, double-blind, multicenter, placebo-controlled study confirmed the efficacy of the carbon dioxide–based feverfew extract MIG-99 in migraine prophylaxis, using only 6.25 mg three times daily for up to 4 months.[133]

Feverfew has been suggested to inhibit serotonin release, inhibit prostaglandin synthesis and platelet aggregation, inhibit polymorphonuclear leukocyte degranulation and phagocytosis of neutrophils, inhibit mast cell release of histamine, promote cytotoxic activity against human tumor cells, and possess both antimicrobial activity and antithrombotic potential.[134] In vitro studies have demonstrated that feverfew extract inhibits serotonin release from platelets.[129,135] The active constituent remains incompletely appreciated; the plant is rich in sesquiterpene lactones, the principal one being parthenolide. Given the diversity of feverfew's pharmacological activity, parthenolide may not be the sole active principle. Feverfew should be avoided or used with caution in patients on anticoagulants because of its platelet-inhibitory effects.[105,107] Feverfew is generally well tolerated, but oral ulceration (especially for those who chew feverfew leaves) and gastrointestinal symptoms are the most common adverse events; these effects are mild and reversible with discontinuation. Because feverfew is in the same *(Asteraceae)* family as ragweed and chamomile, individuals with such allergies should avoid feverfew as well. Feverfew is not known to be safe in pregnancy and lactation and should be slowly tapered when discontinued.

Zingiber officinalis

The common spice ginger root has been shown to exert significant effects in suppressing inflammation and platelet aggregation.[108–110] With respect to migraine headache, there is much anecdotal information and speculation about its usefulness based on its known properties. In recent years, several trials have been published demonstrating ginger's effectiveness for acute headache relief. In a double-blinded and randomized trial, 100 participants with acute migraine without aura were given either ginger powder (250 mg ginger rhizome) or sumatriptan; the treatments had similar efficacy for relief, but ginger had a better side-effect profile, with only 4% reporting clinical side effects (dyspepsia), compared with 20% of those taking sumatriptan reporting effects, including dizziness, a sedative effect, vertigo, and heartburn.[136] Oral ginger (400 mg of a 5% extract) also was shown to be superior to placebo in terms of improving functional status, pain,

and migraine symptoms when added to intravenous (IV) ketoprofen in a randomized, double-blinded trial conducted in an emergency room setting.[137]

Petasides hybridus

Butterbur *(P. hybridus)* has been identified as an effective herbal therapy for migraine; its recorded use dates back at least 900 years. In the Middle Ages, it was used to treat fever and bubonic plague, and in the 17th century, it was an oral treatment for cough and asthma and a topical treatment for skin wounds. It has vascular wall antispasmodic properties and an affinity for cerebral vessels; it also inhibits leukotriene synthesis and lipoxygenase activity.[138] Leukotriene synthesis is apparently inhibited by the active principles petasine and isopetasine, which also have a high affinity for blood vessels and produce vasodilation.[139] In a randomized, placebo-controlled, double-blind study of 60 patients,[139] migraine frequency was decreased by 60% without adverse events (and dysmenorrhea incidentally improved markedly in 8 patients). It is administered prophylactically. Supplementation should be carried out daily for 4 to 6 months and tapered until migraine incidence begins to increase. Butterbur is generally well tolerated, but diarrhea has been reported in some individuals. No drug interactions have been identified, but it is not known to be safe during pregnancy or lactation. The plant's pyrrolizidine alkaloids are thought to be hepatotoxic and carcinogenic; for that reason, only extracts that have removed these specific alkaloids should be used.[138] The typical adult dosage ranges from 50 to 100 mg twice daily with meals, but 75 mg twice daily has performed better than 50 mg twice daily in some studies.[140] Butterbur has diverse properties and could be especially helpful for migraineurs who also experience dysmenorrhea and/or allergic rhinitis.[141] In one study, its efficacy was demonstrated in children and adolescents between the ages of 6 and 17 years.[142] Although no allergic reactions have been reported, patients who are hypersensitive to the *Asteraceae* family (e.g., ragweed, marigolds, daisies, chrysanthemums) should exercise caution because of a potential risk of cross-reactivity.

Hormones

Melatonin

Given the serotonergic involvement in migraine, it is logical to consider melatonin's function as well. Melatonin is the product of CNS serotonin, which is a downstream metabolite of dietary tryptophan. Nearly 90% of serotonin is produced in the walls of the gastrointestinal tract, stored in platelets, and distributed to the rest of the body except the CNS (serotonin cannot cross the blood–brain barrier).[143] Tryptophan and 5-hydroxytryptophan can cross the blood–brain barrier to be converted into serotonin in the pineal gland, which contains 90% of the CNS serotonin and most of the melatonin.[143] The pineal gland serves as an important interface between the environment and the CNS; as such, it may play a role in triggering a variety of external and internal effects such as reactions to certain foods, toxins/odors, flickering lights, sleep deprivation, travel through time zones, menses, and so on. Decreased levels of plasma and urinary melatonin have been observed in patients with migraine.[144–146] A deficiency of melatonin appears to produce excessive stimulation of the trigeminovascular system.[143,147] Melatonin therapy is successful in some individuals, particularly those with delayed-sleep-phase syndrome,[148] and may resynchronize circadian rhythms to lifestyle, subsequently relieving triggers and symptoms.[144] In patients with migraine, the brain does not seem to tolerate the peaks and troughs of life well. Regular sleep, regular meals, exercise, avoidance of both excessive stimulation and relaxation, and evasion of dietary triggers can all help reduce activation. Peres has studied the role of melatonin in migraine extensively and demonstrated in an open-label trial that a 3-mg dose of melatonin was effective in migraine prevention in 25 of 32 individuals with migraine by over 50%. Eight patients experienced a 100% reduction in migraines.[149] Other open-label trials have found benefit with melatonin supplementation at doses up to 4 mg, although the one published randomized trial using 2 mg melatonin did not show benefit.[150,151]

Although the mechanism of action of melatonin is not understood, melatonin is known to possess the following properties as related to migraine: anti-inflammatory activity; prevention of translocation of nuclear factor kappa B to the nucleus; free radical scavenging; inhibition of CGRP-induced vasodilatation; increased inhibitory neurotransmitter activity; direct analgesic effects; inhibition of adhesion molecules; inhibition of nitric oxide synthase; and hypnotic effect from GABAergic upregulation.[152,153]

Sex Steroid Hormones

Studies have determined that the onset of migraine rises with menarche.[154] Episodes are linked to menstruation in 60% of female migraineurs, a condition described as menstrual migraine.[155] Menstrual migraine is particularly significant because many women report their menstrual migraines to be more disabling, longer-lasting, and less responsive to traditional abortive and preventive treatment.[156] Such headaches usually improve with pregnancy, perhaps owing to sustained estrogen levels.[157] None of the "21/7" contraceptive regimens benefits menstrual migraine, but skipping the placebo pills can prevent migraine in some patients.[158] Migraine incidence tends to decrease with advancing age but may either regress or worsen during perimenopause. It has been noted that headaches occur during and after the simultaneous decline of both estrogen and progesterone. There is no uniform agreement over the relation between hormone levels and migraine, although falling estrogen levels have been noted to be associated with menstrual migraine. DeLignieres et al. used percutaneous estradiol gel perimenstrually, with significant headache reduction.[159] In another study, when percutaneous estradiol was used during the luteal phase, postdosing rebound migraines were experienced.[160] Plasma taken from menstruating women and reinfused at another point of the menstrual cycle will reproduce menstrual symptoms, including headache; this phenomenon may be indicative of the presence of a prostaglandin-generating factor in plasma.[155] Transdermal progesterone therapy has been anecdotally successful in preventing migraine for some patients, although published studies are lacking. Hysterectomy or oophorectomy has not been demonstrated to be helpful.[155]

Although estrogen therapy can be effective for menstrual migraine, it is important to remember that migraineurs (especially those with aura) are at higher risk for ischemic strokes; using estrogen replacement therapy in these women, particularly in the setting of coagulopathy or other factors that promote coagulation, could be risky.[158] Additional medications or supplements used daily for a portion of the luteal phase have also successfully prevented menstrual migraine; these include naproxen, magnesium, and triptans.[161]

Physical Medicine and Relaxation Therapies

Manual Medicine

Purveyors of manual medicine seem to achieve success in reducing headache through various techniques such as spinal manipulation, massage, myofascial release, and craniosacral therapy. Manual medicine practitioners frequently identify loss of mobility in the cervical and thoracic spine in migraineurs. Although many forms of physical medicine seem helpful in shortening the duration and intensity of an episode of migraine,[162] literature support has been somewhat sparse with regard to manipulation as a modality to prevent recurrent migraine episodes. However, a randomized controlled trial of

chiropractic spinal manipulation performed in the year 2000 revealed a significant improvement in migraine frequency, duration, disability, and medication use in 83 treatment-group participants.[163] In another study of 218 patients, spinal manipulation performed as favorably as amitriptyline for migraine prophylaxis.[164] A Cochrane review performed in 2004 suggested that spinal manipulation may have a place in migraine headache prophylaxis.[165] Tension headache may also respond favorably to these techniques because of the structural component involved in muscular tension. The incidence of migraine in patients with temporomandibular joint (TMJ) dysfunction is similar to that in the general population, whereas the incidence of tension headache in patients with TMJ dysfunction is much higher than in the general population.[166] Physical therapy for migraine tends to be most effective when it is combined with other treatments, such as thermal biofeedback, relaxation training, and exercise.[167]

Acupuncture

Sufficient evidence exists to support the use of acupuncture to relieve migraine pain.[168–174] Interestingly, the mechanism of relief is not clearly endorphin mediated. One study found that the injection of saline or naloxone did not affect the efficacy of the therapy,[169] and another found that whereas acupuncture increased endorphin levels in controls, the low levels of serum endorphins found in migraine patients did not rise with treatment.[175] The mechanism of action may instead involve normalization of serotonin levels. One study found that acupuncture was effective in relieving pain when it normalized serotonin levels but was ineffective in relieving pain and in raising serotonin levels in patients with very low levels of serotonin.[176]

Although limitations in experimental design make interpretation difficult, acupuncture appears to be successful in reducing the frequency of migraine episodes, generally by around 50%. Patients may be blinded with "sham" acupuncture treatments, but practitioners cannot be blinded. Interestingly, clinical outcomes between true and sham acupuncture were similar in two studies.[177,178] Other studies and systematic reviews[179–182] shed a favorable light on acupuncture in spite of the difficulties posed by study design.

Biofeedback and Relaxation Therapy

Today, the physiological effect of emotional stress is commonly accepted. Stress is and always has been a part of life. The objective must be to find ways to reduce stressful circumstances when possible and to learn techniques to cope with unavoidable stressors more effectively. Practitioners who take the time to ask patients to describe their emotional stressors typically find that they have deeply enhanced the therapeutic relationship simply by sending a message of caring. They also validate the effects of stress by talking to patients about their lives rather than dismissing symptoms as "just stress." Patients must be taught that stress management is a critical component of each individual's daily maintenance needs and not just an occasional luxury. It is also sometimes helpful to ask patients why they think they are experiencing their particular condition. Their comments can be extremely elucidating, often allowing for a breakthrough in therapy.

Traditional peripheral biofeedback has produced grade A evidence for effectively treating migraines.[183] Thermal biofeedback uses the temperature of the hands to help the individual learn that inducing the relaxation response will raise hand temperature and facilitate other positive physiological changes in the body. Learning how to take more active control over the body may reduce headache frequency and severity. The effectiveness of biofeedback and relaxation training in reducing the frequency and severity of migraine headaches has been the subject of dozens of clinical studies,[180] revealing that these techniques can be as effective as beta blockers for headache prevention but without the adverse effects. A recent article concluded that biofeedback is costly and no more effective than employing simpler relaxation techniques in migraine and tension headache.[184] Other relevant modalities to consider include cognitive-behavioral therapy, neurolinguistic programming, hypnotherapy, transcutaneous electrical nerve stimulation (TENS), electromyographic (EMG) biofeedback, massage, and laser therapy.[185,186] In particular, there is grade A evidence for relaxation training, thermal biofeedback combined with relaxation training, EMG biofeedback, and cognitive-behavioral therapy for migraine prophylaxis.[187] Neurofeedback, a technique that uses electroencephalographic feedback to teach patients how to alter brainwave activity, was effective in an open-label trial of 37 patients, with efficacy sustained on average for 14.5 months after discontinuation of treatment.[183] Noninvasive vagus nerve stimulation may also be of value; in a trial comparing active therapy with sham treatment, vagus nerve stimulation appeared to reduce the number of headache days, but only after 6 months of use (no benefit was seen at 2 months). Sham trials also suggest it may not only prevent headaches but also may aid in the treatment of acute migraines. In animal studies, it has been shown to stop CSD, the phenomenon that underlies aura.[188–190] Given the safety and efficacy of these techniques, strong consideration should be given to including them routinely in the care of patients with migraine headaches.[191]

Exercise should not be overlooked as a helpful modality in migraine headache. Thirty-six patients with migraine who exercised for 30 minutes three times a week over 6 weeks experienced significant improvement in headache parameters. Preexercise β-endorphin levels in these individuals were inversely proportional to the degree of improvement in their postexercise headache parameters.[192]

Finally, the role of education in the setting of migraine is paramount to the delivery of quality care. In an open-label prospective study involving a total of 284 migraineurs, 46% of subjects reported a 50% or greater reduction in headache frequency when they were provided with educational materials that included general information about migraine headache, a headache diary, instructions on how to do range-of-motion stretching exercises, and biofeedback tapes.[193] Subjects who tended to worry about impending migraines experienced a significant degree of benefit from the program. Patients reported higher self-efficacy and coping skills after exposure to the educational program.

THERAPEUTIC APPROACH

Migraine headache is a multifaceted condition and should be understood as a symptom rather than a disease. Symptoms are often debilitating, frequently interfering significantly with an individual's quality of life. A holistic, multidisciplinary approach is necessary for a satisfactory outcome. The challenge for the clinician is to determine which of many factors are responsible for each patient's migraine process. Identification and avoidance of precipitating factors are important in reducing the frequency of headaches. Avoidance of initiators is particularly significant considering that they are cumulative in effect.

Owing to the high incidence (80%–90%) of food allergy/intolerance in patients with migraine headache, diagnosis and management may begin with at least a 1-week trial (depending on migraine headache frequency) of careful avoidance of all foods to which the patient may be allergic or intolerant. This may be accomplished through supervised modified fasting or the use of elimination diets. All other possible allergens (e.g., vitamins, unnecessary drugs, herbs, etc.) should also be avoided. During this procedure, food-sensitive patients are likely to exhibit a significant exacerbation of symptoms early in the week, followed by almost total relief by the end of the fast/modified diet. This sequence has to do with the addictive characteristic of the

reactive foods. Once the patient is symptom-free, one new food is reintroduced (and eaten several times) each day while symptoms are carefully recorded. Some authors recommend reintroduction on a 4-day cycle. Suspected foods (symptom onset ranges from 20 minutes to 2 weeks) are eliminated, and apparently safe foods are rotated throughout a 4-day cycle (see Chapter 46). Once a symptom-free period of at least 6 months has been established, the 4-day rotation diet should no longer be necessary.

An oligoantigenic diet, in which common food triggers are eliminated, may also be used successfully by some patients, but a longer trial (4–8 weeks) is typically necessary, depending on the frequency of episodes. Foods that are commonly eliminated during this period include dairy products, gluten-containing grains, eggs, corn, chocolate, peanuts, coffee, black tea, soft drinks, alcoholic beverages, and all processed foods. Some clinicians employ food allergy immunoglobulin testing, with mixed results.

It is worth noting that the cause of migraine headache is often not a simple matter of food intolerance. Aberrations in digestive function and detoxification may also complicate the picture. Metabolic waste products of pathogenic organisms may produce distant symptoms such as headache, and foundational care for patients with migraine headache should include correcting intestinal dysbiosis if identified clinically or in the laboratory through stool culture, organic acids analysis, or intestinal permeability assessment.

Toxic overload or suboptimal function of detoxification enzymes may theoretically trigger headaches and should be addressed if suspected.[194] Susceptibility to toxicity is produced by excessive environmental exposures, genetic polymorphisms in detoxification enzyme production, and depletion of nutrient cofactors catalyzing Phase I and/or Phase II detoxification reactions.[195]Although very few studies have looked explicitly for a role of environmental factors in migraine, a few have been published that suggest environment is a factor. A time-stratified case-crossover study of nearly 20,000 patients found that short-term exposure to higher concentrations of $PM_{2.5}$, PM_{10}, NO_2, O_3, and CO immediately increased the risk of emergency department visits for migraine. NO_2 had the strongest association, and all were exacerbated by higher temperatures. A similar effect on migraine emergency room visits in Canada was found, with associations between SO_2, NO_2, and $PM_{2.5}$.[196–198]

Diet

As discussed previously, all food allergens must be eliminated, and a 4-day rotation diet should be used until the patient is symptom-free for at least 6 months. Foods containing vasoactive amines should initially be eliminated. After symptoms have been controlled, such foods can be carefully reintroduced. The primary foods to eliminate initially are alcoholic beverages, cheese, chocolate, citrus fruits, and shellfish. The diet should be low in sources of arachidonic acid (land-animal fats) and high in foods that inhibit platelet aggregation (e.g., vegetable oils, fish oils, garlic, and onion).

Supplements

- Magnesium: 250 to 800 mg a day in divided doses, titrated to symptoms and bowel tolerance (citrate form may be best, but research is unclear)
- Vitamin B_6: 50 to 75 mg a day balanced with B complex (if no response, try P-5-P)
- 5-HTP: 100 to 200 mg once or twice a day
- Vitamin B_2 (riboflavin): 400 mg once a day balanced with B complex
- Folate: 2 to 5 mg per day; 5-MTHF may be more effective, particularly if MTHFR C677T TT genotype
- CoQ_{10}: 150 mg once a day
- Lipoic acid: 400 mg twice a day, especially if insulin resistance present

Hormonal Therapies

- Trial of melatonin 0.3 to 3 mg at bedtime
- Trial of estradiol, carefully individualized to the clinical picture

Botanical Medicines

- *T. parthenium* (feverfew): 0.25 to 0.5 mg parthenolide twice a day
- *Z. officinalis* (ginger):
  - Fresh ginger: approximately 10 g/day (a 6-mm slice)
  - Dried ginger: 500 mg four times a day
  - Extract standardized to contain 20% of gingerol and shogaol: 100 to 200 mg three times a day for prevention and 200 mg every 2 hours (up to six times a day) in the treatment of an acute migraine
- *P. hybridus* (butterbur): 75 mg two times a day with meals

Physical Medicine and Relaxation Therapies

- TENS to control secondary muscle spasm
- Acupuncture to balance meridians
- Biofeedback
- Guided imagery
- Vagus nerve stimulation

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Goadsby P, Lipton R, Ferrari M. Migraine: current understanding and treatment. *N Engl J Med*. 2002;346(4):257–270.
2. Brixner D. Prevalence and burden of migraine and the impact on managed care. *Managed Care*. 2007:2–3.
3. Izzati-Zade K. The role of serotonin in the pathogenesis and clinical presentation of migraine attacks. *Neurosci Behav Physiol*. 2008;38(5):501–505.
4. Rose FC. The pathogenesis of a migraine attack. *TINS*. 1983;6:247–248.
5. Shinhoj E. Hemodynamic studies within the brain during migraine. *Arch Neurol*. 1979;29:257–266.
6. Blacklow RS. *Macbryde's Signs and Symptoms*. 6th ed. New York, NY: JB Lippincott; 1983.
7. Ferrari MD. Migraine. *Lancet*. 1998;351:1043–1051.
8. Silberstein SD. Advances in understanding the pathophysiology of headache. *Neurology*. 1992;42(suppl 2):6–10.
9. Simkins RT, Tepley N, Barkley GL, et al. Spontaneous neuromagnetic fields in migraine: possible link to spreading cortical depression. *Neurology*. 1989;39(suppl 1):325.
10. Goadsby P. How do the currently used prophylactic agents work in migraine? *Cephalalgia*. 1997;17:85–92.
11. Olesen J, Edvinsson L., eds. *Basic Mechanisms of Headache*. Amsterdam: Elsevier; 1988;301–312.
12. Lauritzen M, SkyhØj Olsen T, Lassen NA, et al. Changes in regional cerebral blood flow during the course of classic migraine attacks. *Ann Neurol*. 1983;13:633–641.
13. Eikermann-Haerter K, Ayata C. Cortical spreading depression and migraine. *Curr Neurol Neurosci Rep*. 2010;10:167–173.
14. Salomone S, Caraci F, Capasso A. Migraine: an overview. *Open Neurol J*. 2009;3:64–71.
15. Spengos K, Theleritis C, Paparrigopoulos T. Memantine and NMDA antagonism for chronic migraine: a potentially novel therapeutic approach? *Headache*. 2008;48(2):284–286.
16. White HS. Molecular pharmacology of topiramate: managing seizures and preventing migraine. *Headache*. 2005;45:S48–S56.
17. Rapoport A. Antimigraine drugs: new frontiers. *Neurol Sci*. 2009;30:S49–S54.
18. Thijs RD, Kruit MC, van Buchem MA. Syncope in migraine: the population-based CAMERA study. *Neurology*. 2006;66(7):1034–1037.
19. Adelborg K, Szépligeti SK, Holland-Bill L, et al. Migraine and risk of cardiovascular diseases: Danish population based matched cohort study. *BMJ*. 2018;360:k96. PubMed PMID: 2938618.
20. Gudmundsson LS, Scher AI, Aspelund T, et al. Migraine with aura and risk of cardiovascular and all cause mortality in men and women: prospective cohort study. *BMJ*. 2010;341:c3966. PMID: 20736269.
21. Schwedt T. The migraine association with cardiac anomalies, cardiovascular disease, and stroke. *Neurol Clin*. 2009;27(2):513–523.
22. Rogawski M. Common pathophysiologic mechanisms in migraine and epilepsy. *Arch Neurol*. 2008;65(6):709–714.
23. Hanington E. The platelet and migraine. *Headache*. 1986;26:411–415.
24. Liu H, Liu M, Wang Y, et al. Association of 5-HTT gene polymorphisms with migraine: a systematic review and meta-analysis. *J Neurol Sci*. 2011;305(1-2):57–66. PMID: 21450309.
25. Panconesi A. Serotonin and migraine: a reconsideration of the central theory. *J Headache Pain*. 2008;9(5):267–276.
26. Ferrari MD, Odink J, Tapparelli C, et al. Serotonin metabolism in migraine. *Neurology*. 1989;33:1239–1242.
27. Fioroni L, Andrea GD, Alecci M, et al. Platelet serotonin pathway in menstrual migraine. *Cephalalgia*. 1996;16:427–430.
28. Sicuteri F. Migraine, a central biochemical dysnociception. *Headache*. 1986;16:145–149.
29. Hamel E. Serotonin and migraine: biology and clinical implications. *Cephalalgia*. 2007;27(11):1293–1300.
30. Moskowitz MA, Cutrer FM. Sumatriptan: a receptor-targeted treatment for migraine. *Annu Rev Med*. 1993;44:145–154.
31. Silberstein SD, Goadsby PJ, Lipton RB. Management of migraine: an algorithmic approach. *Neurology*. 2000;55(suppl 2):S46–S52.
32. Humphrey PPA, Fenuik W, Perren MJ, et al. Serotonin and migraine. *Ann N Y Acad Sci*. 1990;600:587–598.
33. Jamieson DG. The safety of triptans in the treatment of patients with migraine. *Am J Med*. 2002;112:135–140.
34. Silberstein SD. Practice parameter: evidence based guidelines for migraine headache (an evidence-based review). *Neurology*. 2000;55:754–762.
35. Saper JR, Silberstein SD, Lake AE, et al. Double-blind trial of fluoxetine: chronic daily headache and migraine. *Headache*. 1994;34:497–502.
36. Welch KMA. Migraine. a biobehavioral disorder. *Arch Neurol*. 1987;44:323–327.
37. Tepper S, Ashina M, Reuter U, et al. Safety and efficacy of Erenumab for preventive treatment of chronic migraine: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*. 2017;16(6):425–434. PMID: 28460892.
38. Majima M, Ito Y, Hosono K, et al. CGRP/CGRP receptor antibodies: potential adverse effects due to blockade of neovascularization? *Trends Pharmacol Sci*. 2019;40(1):11–21. PMID: 30502971.
39. Moschiano F, D'Amico D, Bussone G. Migraine prophylaxis: key points for the practising clinician. *Neurol Sci*. 2009;30(suppl 1):S33–S37.
40. Antonaci F, Dumitrache C, De Cillis I, et al. A review of current European treatment guidelines for migraine. *J Headache Pain*. 2010;11(1):13–19.
41. Schurks M, Diener HC, Goadsby P. Update on the prophylaxis of migraine. *Curr Treat Options Neurol*. 2008;10(1):20–29.
42. Bigal ME, Rapoport AM, Sheftell FD, et al. Transformed migraine and medication overuse in a tertiary headache centre: clinical characteristics and treatment outcomes. *Cephalalgia*. 2004;24(6):483–490.
43. Mathew NT, Kurman R, Perez F. Drug induced refractory headache: clinical features and management. *Headache*. 1990;30(10):634–638.
44. Diener HC, Limmroth V. Medication-overuse headache: a worldwide problem. *Lancet Neurol*. 2004;3(8):475–483.
45. Garza I, Schwedt T. Medication overuse headache: etiology, clinical features, and diagnosis. http://www.uptodate.com/online/content/topic.do?topicKey=headache/15130&selectedTitle=1~35&source=search_result. Accessed June 16, 2010.
46. Mansfield LE, Vaughan TR, Waller ST, et al. Food allergy and adult migraine: double-blind and mediator confirmation of an allergic etiology. *Ann Allergy*. 1985;55:126–129.
47. Carter CM, Egger J, Soothill JF. A dietary management of severe childhood migraine. *Hum Nutr Appl Nutr*. 1985;39A:294–303.
48. Hughes EC, Gott PS, Weinstein RC, et al. Migraine: a diagnostic test for etiology of food sensitivity by a nutritionally supported fast and confirmed by long-term report. *Ann Allergy*. 1985;55:28–32.
49. Egger J, Carter CM, Wilson J, et al. Is migraine food allergy? *Lancet*. 1983;2:865–869.
50. Monro J, Brostoff J, Carini C, et al. Food allergy in migraine. *Lancet*. 1980;2:1–4.
51. Grant ECG. Food allergies and migraine. *Lancet*. 1979;1:966–969.
52. Little CH, Stewart AG, Fennessy MR. Platelet serotonin release in rheumatoid arthritis as studied in food intolerant patients. *Lancet*. 1983;2:297–299.
53. Peatfield RC. Relationship between food, wine, and beer-precipitated headaches. *Headache*. 1995;35:355–357.
54. Millichap JG, Yee M. The diet factor in pediatric and adolescent migraine. *Pediatr Neurol*. 2003;28(1):9–15.
55. Hernandez CMA, Pinto ME, Montiel HLH. Food allergy mediated by IgG antibodies associated with migraine in adults. *Rev Alerg Mex*. 2007;54(5):162–168.
56. Alpay K1, Ertas M, Orhan EK, et al. Diet restriction in migraine, based on IgG against foods: a clinical double-blind, randomised, cross-over trial. *Cephalalgia*. 2010;30(7):829–837. PMID: 20647174.
57. Aydinlar EI1, Dikmen PY, Tiftikci A, et al. IgG-based elimination diet in migraine plus irritable bowel syndrome. *Headache*. 2013;53(3):514–525. PMID: 23216231.
58. Jarisch R, Wantke F. Wine and headache. *Int Arch Allergy Immunol*. 1996;110:7–12.

59. Wantke F, Gotz M, Jarisch R. Histamine free diet: treatment of choice for histamine induced food intolerance and supporting treatment for chronic headaches. *Clin Exp Allergy*. 1993;23:982–985.
60. Vaughan T. The role of food in the pathogenesis of migraine headache. *Clin Rev in Allergy*. 1994:167–180.
61. Jarman J, Glover V, Sandler M. Release of (14C)5-hydroxytryptamine from human platelets by red wine. *Life Sci*. 1991;48:2297–2300.
62. Martner Hewes PM, Hunt IF, Murphy NJ, et al. Vitamin B_6 nutriture and plasma diamine oxidase activity in pregnant Hispanic teenagers. *Am J Clin Nutr*. 1988;44:907–913.
63. Sabbah A, Heulin MG, Drouet M, et al. Antihistaminic or anti-degranulating activity of pregnancy serum. *Allergy Immunol (Paris)*. 1988;20(6):236–240.
64. Lindberg S. 14C-histamine elimination from blood of pregnant and non-pregnant women with special reference to the uterus. *Acta Obst Gynecol Scand*. 1963;62:1–25.
65. Wilkinson Jr CF. Recurrent migrainoid headaches associated with spontaneous hypoglycemia. *Am J Med Sci*. 1949;218:209–212.
66. Dexter J, Roberts J, Byer A. The five hour glucose tolerance test and effect of low sucrose diet in migraine. *Headache*. 1978;18:91–94.
67. Evcili G, Utku U1, Öğün MN, et al. Early and long period follow-up results of low glycemic index diet for migraine prophylaxis. *Agri*. 2018;30(1):8–11. PMID: 29450870.
68. Brainard JB. Angiotensin and aldosterone elevation in salt-induced migraine. *Headache*. 1981;21:222–226.
69. Ferrara LA, Pacioni D, Di Fronzo V, et al. Low-lipid diet reduces frequency and severity of acute migraine attacks. *Nutr Metab Cardiovasc Dis*. 2015;25(4):370–375. PMID: 25698152.
70. Ratner D, Shoshani E, Dubnov B. Milk protein-free diet for nonseasonal asthma and migraine in lactase-deficient patients. *Isr J Med Sci*. 1983;19:806–809.
71. Koehler SM, Glaros A. The effect of aspartame on migraine headache. *Headache*. 1988;28:10–13.
72. Blumenthal HR, Vance DA. Chewing gum headaches. *Headache*. 1997;37(10):665–666.
73. Bigal ME, Krymchantowski AV. Migraine triggered by sucralose-a case report. *Headache*. 2006;46(3):515–517.
74. Gasbarrini A, De Luca A, Fiore G, et al. Beneficial effects of *Helicobacter pylori* eradication on migraine. *Hepatogastroenterology*. 1998;45(21):465–470.
75. Yiannopoulou KG, Efthymiou A, Karydakis K, et al. Helicobacter pylori infection as an environmental risk factor for migraine without aura. *J Headache Pain*. 2007;8(6):329–333.
76. Tunca A, Türkay C, Tekin O, et al. Is *Helicobacter pylori* infection a risk factor for migraine? A case-control study. *Acta Neurol Belg*. 2004;104(4):161–164.
77. Boiardi A, Crenna P, Merati B, et al. 5-OH-Tryptophane in migraine: clinical and neurophysiological considerations. *J Neurology*. 1981;225(1):41–46.
78. Bono G, Criscuoli M, Martignoni E, et al. Serotonin precursors in migraine prophylaxis. In: Adv Neurol. 33:357–363.
79. Ramsden CE1, Faurot KR, Zamora D, et al. Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: a randomized trial. *Pain*. 2013;154(11):2441–2451. PMID: 23886520.
80. Gerrard JM, White JG, Krivit W. Labile aggregation stimulating substance, free fatty acids and platelet aggregation. *J Lab Clin Med*. 1976:73–82.
81. Sanders TAB, Roshanai F. The influence of different types of omega-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clin Sci*. 1983;64:91–99.
82. Woodcock BE, Smith E, Lambert WH, et al. Beneficial effect of fish oil on blood viscosity in peripheral vascular disease. *Br Med J*. 1984;288:592–594.
83. McCarren T, Hitzemann R, Smith R, et al. Amelioration of severe migraine by fish oil (w-3) fatty acids. *Am J Clin Nutr*. 1985;41(4):874.
84. Glueck CJ, McCarren T, Hitzemann R, et al. Amelioration of severe migraine with omega-3 fatty acids: a double-blind, placebo-controlled clinical trial. *Am J Clin Nutr*. 1986;43(4):710.
85. Harel Z, Generoso G, Riggs S, et al. Supplementation with omega-3 polyunsaturated fatty acids in the management of recurrent migraines in adolescents. *J Adolescent Health*. 2002;31:154–161.
86. Maghsoumi-Norouzabad L, Mansoori A, Abed R, et al. Effects of omega-3 fatty acids on the frequency, severity, and duration of migraine attacks: a systematic review and meta-analysis of randomized controlled trials. *Nutr Neurosci*. 2018;21(9):614–623. PMID: 28665211.
87. Bic Z, Blix GG, Hopp HP, et al. In search of the ideal treatment for migraine headache. *Medical Hypoth*. 1998;50:1–7.
88. Montagna P, Sacquegna T, Cortelli P, et al. Migraine as a defect of brain oxidative metabolism: a hypothesis. *J Neurol*. 1989;236:124–125.
89. Schoenen J, Jacquy J, Lenaerts M. Effectiveness of high dose riboflavin in migraine prophylaxis: a randomized controlled trial. *Neurology*. 1998;50:466–470.
90. Welch KMA, Levine SR, D'Andrea G, et al. Preliminary observations on brain energy metabolism in migraine studied by in vivo phosphorus 31 NMR spectroscopy. *Neurology*. 1989;39:538–541.
91. Schoenen J, Lenaerts M, Bastings E. High-dose riboflavin as a prophylactic treatment of migraine: results of an open pilot study. *Cephalalgia*. 1994;14:328–329.
92. Condo M, Posar A, Arbizzani A, et al. Riboflavin prophylaxis in pediatric and adolescent migraine. *J Headache Pain*. 2009;10(5):361–365.
93. MacLennan SC, Wade FM, Forrest KM, et al. High dose riboflavin for migraine prophylaxis in children: a double blind, randomized, placebo-controlled trial. *J Child Neurology*. 2008;23(11):1300–1304.
94. Talebian A, Soltani B, Banafshe HR, et al. Prophylactic effect of riboflavin on pediatric migraine: a randomized, double-blind, placebo-controlled trial. *Electron Physician*. 2018;10(2):6279–6285. PMID: 29629048.
95. Sándor PS, Afra J, Ambrosini A, et al. Prophylactic treatment of migraine with beta-blockers and riboflavin: differential effects on the intensity dependence of auditory evoked cortical potentials. *Headache*. 2000;40(1):30–35. PMID: 10759900.
96. Di Lorenzo C, Pierelli F, Coppola G, et al. Mitochondrial DNA haplogroups influence the therapeutic response to riboflavin in migraineurs. *Neurology*. 2009;72(18):1588–1594. PMID: 19414726.
97. Kopjas TL. The use of folic acid in vascular headache of the migraine type. *Headache*. 1969:167–170.
98. Oterino A, Toriello M, Valle N, et al. The relationship between homocysteine and genes of folate-related enzymes in migraine patients. *Headache*. 2010;50(1):99–168. PMID: 19619240.
99. Menon S1, Lea RA, Ingle S, et al. Effects of dietary folate intake on migraine disability and frequency. *Headache*. 2015;55(2):301–309. PMID: 25598270.
100. Menon S1, Lea RA, Roy B, et al. Genotypes of the MTHFR C677T and MTRR A66G genes act independently to reduce migraine disability in response to vitamin supplementation. *Pharmacogenet Genomics*. 2012;22(10):741–749. PMID: 22926161.
101. Lea R, Colson N, Quinlan S, et al. The effects of vitamin supplementation and MTHFR (C677T) genotype on homocysteine-lowering and migraine disability. *Pharmacogenet Genomics*. 2009;19(6):422–428. PMID: 19384265.
102. Di Rosa G, Attinà S, Spanò M, et al. Efficacy of folic acid in children with migraine, hyperhomocysteinemia and MTHFR polymorphisms. *Headache*. 2007;47(9):1342–1344. PMID: 17927652.
103. van der Kuy PH1, Merkus FW, Lohman JJ, et al. Hydroxocobalamin, a nitric oxide scavenger, in the prophylaxis of migraine: an open, pilot study. *Cephalalgia*. 2002;22(7):513–519. PMID: 12230592.
104. Rozen TD, Oshinsky ML, Gebeline CA, et al. Open label trial of coenzyme Q10 as a migraine preventive. *Cephalalgia*. 2002;22(2):137–141.
105. Sandor PS, Di Clemente L, Coppola G, et al. Efficacy of coenzyme Q10 in migraine prophylaxis: a randomized controlled trial. *Neurology*. 2005;64(4):713–715.
106. Dahri M, Tarighat-Esfanjani A, Asghari-Jafarabadi M, et al. Oral coenzyme Q10 supplementation in patients with migraine: effects on clinical features and inflammatory markers. *Nutr Neurosci*. 2018:1–9. PMID: 29298622.

107. Hershey AD, Powers SW, Vockell AL, et al. Coenzyme Q10 deficiency and response to supplementation in pediatric and adolescent migraine. *Headache*. 2007;47(1):73–80.
108. Marcolina S. Nonpharmacologic management of migraines. *Alternative Medicine Alert*. 2007:61–72.
109. Altura BM, Brodsky MA, Elin RJ, et al. Magnesium: growing in clinical importance. *Patient Care*. 1994;15:130–150.
110. Johnson S. The multifaceted and widespread pathology of magnesium deficiency. *Med Hypotheses*. 2001;56(2):163–170.
111. Swanson DR. Migraine and magnesium: eleven neglected connections. *Perspect Biol Med*. 1988;31:526–557.
112. Ramadan NM, Halvorson H, Vande-Linde A, et al. Low brain magnesium in migraine. *Headache*. 1989;29:590–593.
113. Gallai V, Sarchielli P, Morucci P, et al. Magnesium content of mononuclear blood cells in migraine patients. *Headache*. 1994;34:160–165.
114. Mazzotta G, Sarchielli P, Alberti A, et al. Electromyographical ischemic test and intracellular and extracellular magnesium concentration in migraine and tension-type headache patients. *Headache*. 1996;36:357–361.
115. Peikert A, Wilimzig C, Köhne-Volland R. Prophylaxis of migraine with oral magnesium: results from a prospective, multi-center, placebo-controlled and double-blind randomized study. *Cephalalgia*. 1996;16(4):257–263.
116. Pfaffenrath V, Wessely P, Meyer C. Magnesium in the prophylaxis of migraine: a double-blind placebo-controlled study. *Cephalalgia*. 1996;16:436–440.
117. Lindberg JS, Zobitz MM, Poindexter JR, et al. Magnesium bioavailability from magnesium citrate and magnesium oxide. *J Am Coll Nutr*. 1990;9:48–55.
118. Koseoglu E, Talaslioglu A, Gönül AS, et al. The effects of magnesium prophylaxis in migraine without aura. *Magnesium Research*. 2008;21(2):101–108.
119. Mauskop A1, Altura BT, Altura BM. Serum ionized magnesium levels and serum ionized calcium/ionized magnesium ratios in women with menstrual migraine. *Headache*. 2002;42(4):242–248. PMID: 12010379.
120. Mauskop A, Altura BT, Cracco RQ, et al. Intravenous magnesium sulphate relieves migraine attacks in patients with low serum ionized magnesium levels: a pilot study. *Clin Sci*. 1995;89:633–636.
121. Mauskop A, Altura BM. Role of magnesium in the pathogenesis and treatment of migraines. *Clin Neuroscience*. 1998;5:24–27.
122. Mauskop A, Altura BT, Cracco RQ, et al. Intravenous magnesium sulfate rapidly alleviates headaches of various types. *Headache*. 1996;36:154–160.
123. Mauskop A, Altura BT, Cracco RQ, et al. Intravenous magnesium sulfate relieves cluster headaches in patients with low serum ionized magnesium levels. *Headache*. 1995;35:597–600.
124. Chiu HY, Yeh TH, Huang YC, et al. Effects of intravenous and oral magnesium on reducing migraine: a meta-analysis of randomized controlled trials. *Pain Physician*. 2016;19(1):E97–E112. PMID: 26752497.
125. Galland LD, Baker SM, McLellan RK. Magnesium deficiency in the pathogenesis of mitral valve prolapse. *Magnesium*. 1986;5:165–174.
126. Cavestro C, Bedogni G, Molinari F, et al. Alpha-lipoic acid shows promise to improve migraine in patients with insulin resistance: a 6-month exploratory study. *J Med Food*. 2018;21(3):269–273. PMID: 28976801.
127. Magis D, Ambrosini A, Sándor P, et al. A randomized double-blind placebo-controlled trial of thioctic acid in migraine prophylaxis. *Headache*. 2007;47(1):52–57. PMID: 17355494.
128. Johnson ES, Kaddam NP, Hylands DM, et al. Efficacy of feverfew as prophylactic treatment of migraine. *Br Med J*. 1985;291:569–573.
129. Murphy JJ, Heptinstall S, Mitchell JRA. Randomized double-blind placebo-controlled trial of feverfew in migraine prevention. *Lancet*. 1988;2:189–192.
130. Barsby RWJ, Salan U, Knight BW, et al. Feverfew and vascular smooth muscle: extracts from fresh and dried plants show opposing pharmacological profiles, dependent upon sesquiterpene lactone content. *Planta Medica*. 1993;59:20–25.
131. Heptinstall S, Awang DV, Dawson BA, et al. Parthenolide content and bioactivity of feverfew (*Tanacetum parthenium* [L.] Schultz-Bip.): estimation of commercial and authenticated feverfew products. *J Pharm Pharmacol*. 1992;44:391–395.
132. Ernst E, Pittler MH. The efficacy and safety of feverfew (*Tanacetum parthenium* L.): an update of a systematic review. *Public Health Nutrition*. 3(4A):509–514.
133. Diener HC, Pfaffenrath V, Schnitker J, et al. Efficacy and safety of 6.25 mg t.i.d. feverfew CO2-extract (MIG-99) in migraine prevention: a randomized, double-blind, multicentre, placebo-controlled study. *Cephalalgia*. 2005;25(11):1031–1041.
134. Miller LG. Herbal medicinals: selected clinical considerations focusing on known or potential drug-herb interactions. *Arch Int Med*. 1998;158(9):2200–2211.
135. Heptinstall S, White A, Williamson L, et al. Extracts of feverfew inhibit granule secretion in blood platelets and polymorphonuclear leucocytes. *Lancet*. 1985:1071–1074.
136. Maghbooli M, Golipour F, Moghimi Esfandabadi A, et al. Comparison between the efficacy of ginger and sumatriptan in the ablative treatment of the common migraine. *Phytother Res*. 2014;28(3):412–415. PMID: 23657930.
137. Martins LB, Rodrigues AMDS, Rodrigues DF, et al. Double-blind placebo-controlled randomized clinical trial of ginger (*Zingiber officinale* Rosc.) addition in migraine acute treatment. *Cephalalgia*. 2018: 333102418776016. PMID: 29768938.
138. Epstein MT, Hockaday JM, Hockaday TD. *Petasites hybridus* (butterbur). *Alt Med Rev*. 2001;6(2):207–209.
139. Grossman M, Schmidramsl H. An extract of *Petasites hybridus* is effective in the prophylaxis of migraine. *Int J Clin Pharm*. 2000;38:430–435.
140. Sutherland A, Sweet BV. Butterbur: an alternative therapy for migraine prevention. *Am J Health Syst Pharm*. 2010;67(9):705–711.
141. Schapowal AP. Randomised controlled trial of butterbur and cetirizine for treating seasonal allergic rhinitis. *BMJ*. 2002:144–146.
142. Pothmann R, Danesch U. Migraine prevention in children and adolescents: results of an open study with a special butterbur root extract. *Headache*. 2005;45(3):196–203.
143. JU Toglia. Melatonin: a significant contributor to the pathogenesis of migraine. *Med Hypotheses*. 2001;57(4):432–434.
144. Gagnier J. The therapeutic potential of melatonin in migraines and other headache types. *Altern Med Rev*. 2001;6(4):383–389.
145. Claustrat B, Loisy C, Brun J, et al. Nocturnal plasma melatonin levels in migraine: a preliminary report. *Headache*. 1989;29(4):242–244.
146. Brun J, Claustrat B, Saddier P, et al. Nocturnal melatonin secretion is decreased in patients with migraine without aura associated with menses. *Cephalalgia*. 1995;15:136–139.
147. Anton-Tay F, Chou C, Anton S, et al. Brain serotonin concentration following intraperitoneal administration of melatonin. *Science*. 1968;162:277–278.
148. Nagtegaal JE, Smits MG, Swart AC, et al. Melatonin-responsive headache in delayed sleep phase syndrome: preliminary observations. *Headache*. 1998;38:303–307.
149. Peres JFP, Zukerman E, da Cunha Tanuri F, et al. Melatonin, 3 mg, is effective for migraine prevention. *Neurology*. 2004;63(4):757.
150. Alstadhaug KB, Odeh F, Salvesen R, et al. Prophylaxis of migraine with melatonin: a randomized controlled trial. *Neurology*. 2010;75:1527–1532. PMID: 20975054.
151. Bougea A, Spantideas N, Lyras V, et al. Melatonin 4 mg as prophylactic therapy for primary headaches: a pilot study. *Funct Neurol*. 2016;31(1):33–37. PMID: 27027892.
152. Peres M. Melatonin, the pineal gland and their implications for headache disorders. *Cephalalgia*. 2005:403–411.
153. Vogler B, Rapoport AM, Tepper SJ, et al. Role of melatonin in the pathophysiology of migraine: implications for treatment. *CNS Drugs*. 2006;20(5):343–350.
154. Epstein MT, Hockaday JM, Hockaday TD. Migraine and reproductive hormones through the menstrual cycle. *Lancet*. 1975;1:543–548.
155. Silberstein SD. The role of sex hormones in headache. *Neurology*. 1992;42(supp 2):S37–S42.
156. Calhoun AH, Hutchinson S. Hormonal therapies for menstrual migraine. *Curr Pain Headache Rep*. 2009;13(5):381–385.

157. Silberstein SC, Merriam GR. Estrogens, progestins, and headache. *Neurol*. 1991;41:786–793.
158. MacGregor E. Estrogen replacement and migraine. *Maturitas*. 2009:51–55.
159. DeLignieres B, Vincens M, Mauvais-Jarvis P, et al. Prevention of menstrual migraine by percutaneous oestradiol. *Br Med J*. 1986;293:1540.
160. MacGregor EA, Frith A, Ellis J, et al. Prevention of menstrual attacks of migraine: a double-blind placebo-controlled crossover study. *Neurology*. 2006;67(12):2159–2163.
161. Silberstein SD, Goldberg J. Menstrually related migraine: breaking the cycle in your clinical practice. *J Reprod Med*. 2007;52(10):888–895.
162. Parker GB, Tupling H, Pryor DS. A controlled trial of cervical manipulation for migraine. *Aust NZ J Med*. 1978;8(6):589–593.
163. Tuchin PJ, Pollard H, Bonello R. A randomized controlled trial of chiropractic spinal manipulative therapy for migraine. *J Manip Phys Ther*. 2000;23(2):91–95.
164. Nelson CF, Bronfort G, Evans R, et al. The efficacy of spinal manipulation, amitriptyline and the combination of both therapies for the prophylaxis of migraine headache. *J Manip Physiol Ther*. 1998;21:511–519.
165. Bronfort G, Nilsson N, Haas M, et al. Non-invasive physical treatments for chronic/recurrent headache. *Cochrane Database Syst Rev*. 2004;3:CD001878.
166. Watts PG, Peet KM, Juniper RP. Migraine and the temporomandibular joint: the final answer? *Br Dent J*. 1986;161:170–173.
167. Biondi D. Physical treatments for headache: a structured review. *Headache*. 2005;45:738–746.
168. Melchant D, Linde K, Fischer P, et al. Acupuncture for recurrent headache: a systematic review of randomized controlled trials. *Cephalalgia*. 1999;19(suppl):779–786.
169. Lenhard L, Waite PM. Acupuncture in the prophylactic treatment of migraine headache. Pilot study. *NZ Med J*. 1983;96:663–666.
170. Pickett H, Blackwell JC. Acupuncture for migraine headaches. *Am Family Physician*. 2010;81(8):1036–1037.
171. Vickers AJ, Rees RW, Zollman CE, et al. Acupuncture for chronic headache in primary care: large, pragmatic, randomised trial. *BMJ*. 2004;328(7442):744.
172. Allais G, De Lorenzo C, Quirico PE. Acupuncture in the prophylactic treatment of migraine without aura: a comparison with flunarizine. *Headache*. 2002;42:855–861.
173. Haigh C. Acupuncture reduced frequency and pain intensity of primary migraine or tension-type headaches. *Evid Based Nurs*. 2009;12(2):47.
174. Schiapparelli P, Allais G, Castagnoli Gabellari I, et al. Non-pharmacological approach to migraine prophylaxis: part II. *Neurol Sci*. 2010;31(suppl 1):S137–S139.
175. Facchinetti F, Nappi G, Savoldi F, et al. Primary headaches: reduced circulating beta-lipotropin and beta-endorphin levels with impaired reactivity to acupuncture. *Cephalalgia*. 1985;1:195–201.
176. Markelova VF, Vesnina VA, Malygina SI, et al. Changes in blood serotonin levels in patients with migraine headaches before and after a course of reflexotherapy. *Zh Nevropatol Psikhiatr*. 1984;84:1313–1316.
177. Diener HC, Kronfeld K, Boewing G, et al. Efficacy of acupuncture for the prophylaxis of migraine: a multicenter randomized controlled clinical trial. *Lancet Neurol*. 2006;5:310–316.
178. Linde K, Streng A, Jürgens S, et al. Acupuncture for patients with migraine: a randomized controlled trial. *JAMA*. 2005;293(17):2118–2125.
179. Baischer W. Acupuncture in migraine: long-term outcome and predicting factors. *Headache*. 1995;35:472–474.
180. Holroyd KA, Penzien DB. Pharmacological versus non-pharmacological prophylaxis of recurrent migraine headache: a meta-analytic review of clinical trials. *Pain*. 1990;42:1–13.
181. Manias P, Tagaris G, Karageorgiou K. Acupuncture in headache: a critical review. *Clin J Pain*. 2000;16:334–339.
182. Vincent CA. A controlled trial of the treatment of migraine by acupuncture. *Clin J Pain*. 1989;5:305–312.
183. Stokes DA, Lappin MS. Neurofeedback and biofeedback with 37 migraineurs: a clinical outcome study. *Behav Brain Funct*. 2010;6:9.
184. Mullally WJ, Hall K, Goldstein R. Efficacy of biofeedback in the treatment of migraine and tension type headaches. *Pain Physician*. 2009;12(6):1005–1011.
185. Allais G, De Lorenzo C, Quirico PE, et al. Non-pharmacological approaches to chronic headaches: transcutaneous electrical nerve stimulation, laser therapy and acupuncture in transformed migraine treatment. *Neurol Sci*. 2003;24:S138–S142.
186. Becker WJ, Sauro KM. Recent studies on stress management-related treatments for migraine. *Headache*. 2009;49(9):1387–1390.
187. Campbell, J. & Penzien, Donald. Evidenced-based guidelines for migraine headache: behavioral and physical treatments. U.S. Headache Consortium. https://www.researchgate.net/publication/254479333_Evidenced-Based_Guidelines_For_Migraine_Headache_Behavioral_and_Physical_Treatments. Published 2000. Accessed August 9, 2019.
188. Silberstein SD, Calhoun AH, Lipton RB, et al. Chronic migraine headache prevention with noninvasive vagus nerve stimulation: the event study. *Neurology*. 2016;87(5):529–538. PMID: 27412146.
189. Martelletti P, Barbanti P, Grazzi L, et al. Consistent effects of non-invasive vagus nerve stimulation (nVNS) for the acute treatment of migraine: additional findings from the randomized, sham-controlled, double-blind PRESTO trial. *J Headache Pain*. 2018;19(1):101. PMID: 30382909.
190. Chen SP, Ay I, de Morais AL, et al. Vagus nerve stimulation inhibits cortical spreading depression. *Pain*. 2016;157(4):797–805. PMID: 26645547.
191. Grazzi L, Andrasik F. Non-pharmacological approaches in migraine prophylaxis: behavioral medicine. *Neurol Sci*. 2010;31(suppl 1):S133–S135.
192. Koseoglu E, Akboyraz A, Soyuer A, et al. Aerobic exercise and plasma beta endorphin levels in patients with migrainous headache without aura. *Cephalalgia*. 2003;23(10):972–976.
193. Smith TR, Nicholson RA, Banks JW. Migraine education improves quality of life in a primary care setting. *Headache*. 2010;50(4):600–612.
194. Liska DJ, Rountree R. The role of detoxification in the prevention of chronic degenerative diseases. *Applied Nutr Sci Reports*. 2002;650:1–8.
195. Ioannides C. Effect of diet and nutrition on the expression of cytochromes P450. *Xenobiotica*. 1999;29(2):109–154.
196. Lee H, Myung W, Cheong HK, et al. Ambient air pollution exposure and risk of migraine: synergistic effect with high temperature. *Environ Int*. 2018;121(Pt 1):383–391. PMID: 30245361.
197. Szyszkowicz M, Rowe BH, Kaplan GG, et al. Ambient sulphur dioxide exposure and emergency department visits for migraine in Vancouver, Canada. *Int J Occup Med Environ Health*. 2009;22(1):7–12. PMID: 19329386.
198. Szyszkowicz M, Kaplan GG, Grafstein E, et al. Emergency department visits for migraine and headache: a multi-city study. *Int J Occup Med Environ Health*. 2009;22(3):235–242. PMID: 19819836.

199

Multiple Sclerosis

Michael Alexander Lane, MD, and *Vijayshree Yadav, MD, MCR, FAAN*

OUTLINE

DIAGNOSTIC SUMMARY

- Episodic neurological symptoms, depending on the parts of the central nervous system (CNS) affected
- Typical onset in adults between ages 20 and 55 years
- Symptomatology not consistent with a single neurological lesion
- Diagnosis made based on clinical symptoms, magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) analysis

GENERAL CONSIDERATIONS

Multiple sclerosis (MS) is a disabling disease of the CNS that commonly affects young and middle-aged adults.[1] Physicians have recognized MS since the mid-19th century, and although there were initially no management options, there has been significant therapeutic advancement since the 1990s with multiple effective disease-modifying therapies (DMTs) now available to treat MS.

In MS, the dysregulated immune system attacks the protective sheath (myelin) that covers nerve fibers in multiple locations through the CNS, referred to as areas of demyelination. MS pathology primarily consists of these multifocal areas of demyelination (plaques) in the brain, spinal cord, and optic nerves. In addition to the areas of demyelination, significant axonal damage affecting the CNS can also occur in severe forms of MS. Frequently, the nerves are permanently damaged or deteriorate concurrently with damage to myelin. Inflammatory cells composed predominantly of macrophages and lymphocytes are present when there is active demyelination within MS plaques, indicating that MS is an inflammatory disease.

MRI provides a means of visualizing MS lesions within the brain and spinal cord (Fig. 199.1). MS signs and symptoms depend on the parts of the CNS affected, and patients have varying levels of permanent disability depending on the degree of inflammation and resultant damage. Clinically, MS can cause a variety of neurological problems, depending primarily on the location and severity of MS plaques (Table 199.1). Many MS symptoms, such as fatigue, cognitive impairment, and heat sensitivity, however, are not easily localized anatomically and are not well understood. In about 85% of cases, MS starts with a relapsing-remitting course.[2] Patients experience relapses or attacks of MS during which they develop a new neurological problem or worsening of preexisting symptoms. Relapses develop over a few days or weeks, followed by a period of improvement and stability, and typically last for more than 48 hours. In between relapses, patients are in remission and clinically stable, although they may have residual permanent neurological signs and symptoms from previous relapses. Relapses that cause symptoms represent only the "tip of the iceberg" of disease activity at this stage of the illness. Serial MRI studies in patients with MS have disclosed that new, asymptomatic MS lesions appear within the brain 5 to 10 times more commonly than symptomatic lesions, causing permanent damage that contributes to the overall MS disease burden.[3]

According to the natural history data, about 50% of patients with relapsing-remitting MS (RRMS) enter a progressive phase of the disease 10 to 15 years after onset. This progressive phase, called secondary progressive MS (SPMS), is characterized by a steady worsening of signs and symptoms. Patients with SPMS may or may not continue to have relapses. About 15% of MS patients have progressive worsening from the onset of their illness, a form of MS referred to as primary progressive MS (PPMS). Although MS is rarely fatal, it is often disabling. The natural history of MS suggested relatively quick progression of the disease from onset to having ambulation difficulty and walking with a cane occurring over a median of approximately 15 years. More recent studies report a significantly longer time to reaching this disability milestone, with a median time from onset to cane of 30 years.[4,5] Early studies of PPMS also reported short median time from disease onset to cane of less than 10 years. However, more recent studies show the median time of progression to be closer to 15 years. The widespread

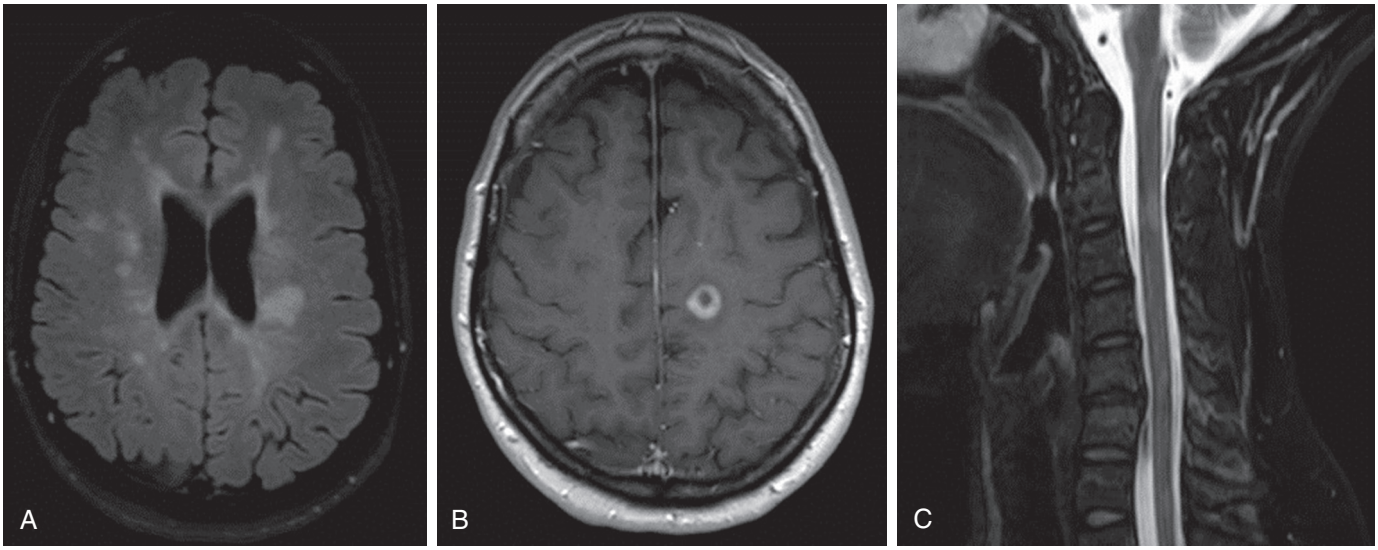


Fig. 199.1 Magnetic resonance imaging (MRI) of the brain and cervical spine of a patient with multiple sclerosis (MS). A, Axial T2 MRI brain with large areas of increased signal intensity adjacent to the lateral ventricles in the classic distribution typical for MS. B, Axial T1 MRI brain after gadolinium contrast agent with a ring-enhancing lesion in left centrum semiovale. C, Multiple cervical spine lesions, T2 sequence with increased signal intensity at multiple levels.

TABLE 199.1 Symptoms of Multiple Sclerosis and Their Localization

Location of Lesion	Symptoms
Cortical	Cognitive problems
Optic nerve	Vision loss
Brainstem	Diplopia
	Facial sensory loss
	Facial weakness
	Respiratory irregularities
Cerebellum	Imbalance
	Incoordination
	Vertigo
Spinal cord	Numbness, paresthesias, and/or weakness from level down
	Stiffness in limbs
	Constipation
	Urinary urgency, retention, incontinence

availability and use of immune-modulating medications for relapsing MS likely have played a role in the improved long-term MS prognosis.[6]

Up to 15% of patients with MS never develop any overt permanent disability. Most patients, however, develop varying degrees of permanent neurological disability. Although it can be difficult to predict which patients will progress and which patients will have benign MS, several prognostic factors for unfavorable clinical outcomes have been identified. Some of these factors include older age at onset; initial symptoms involving motor function; higher initial clinical activity, including high relapse rate; and increased disease progression in the first 5 years. Smoking tobacco and low serum vitamin D levels have also been determined to be predictors of poorer long-term outcome.

Epidemiology

It is estimated that MS affects approximately 2.5 million people worldwide and 400,000 people in the United States.[6] MS affects about 1 out of 1000 persons in the United States, Canada, and northern Europe. MS typically begins between the ages of 20 and 40 but may occur at any age. Women are more commonly affected than men, with about 60% of cases being female. A strong racial influence on the risk of developing MS exists: it is most common among individuals of white and Caucasian backgrounds, particularly those of northern European descent.[7,8] Typical MS is rare among Asians and black Africans but is relatively common among black Americans. The racial predilection of MS is one piece of evidence indicating the strong influence of genes on the risk of developing MS. In addition to racial influences, there is also an interesting geographical distribution of the disease. Areas with the highest prevalence are located in higher latitudes, in both the northern and southern hemispheres.[9,10] These high-risk areas include the northern United States, Canada, Great Britain, Scandinavia, northern Europe, New Zealand, and Tasmania.[11,12]

Pathogenesis

Experts agree that MS results from an acquired immune dysregulation and aberrant immune activation, leading to inflammatory processes driven by macrophages and B and T lymphocytes in the CNS that result in demyelination and axonal damage. Activated T cells produce proinflammatory cytokines, which additionally contribute to tissue damage. Increased levels of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interferon (IFN)-γ, and interleukin (IL)-2, have been found in the peripheral blood, CSF, and brain lesions of patients with MS.[13–18] Numerous reports document the association between these cytokines and disease activity, thus implicating them as mediators of the immunopathogenesis of MS.[13,15–17] Anti-inflammatory cytokines IL-4, IL-5, IL-10, and IL-13 and transforming growth factor beta-1 (TGF-β1)[19] have down-regulatory effects on the immune system and are thought to be beneficial in MS.[20] Besides the role of macrophages, lymphocytes, and cytokines, other biomarkers, such as enzymes, can play an important role in MS pathogenesis. Matrix metallopeptidase-9 (MMP-9) is a group of enzymes responsible for the migration of immune cells to sites of inflammation as well as remodeling extracellular matrix, basement membrane, and other tissues in the body by breaking down collagen components in these tissues. MMP-9s are thought to play a significant role in the transmigration of inflammatory cells into the CNS by aiding in the disruption of the blood–brain barrier.[21,22] Several studies have shown higher serum levels of MMP-9 in patients with MS.[23–25]

MS pathology is defined as having three different categories of acute white-matter demyelination (The figure discussed in this section is under Reference 1 and could not be included due to copyright concerns.).[26] The most common types (patterns I and II) show a background of mononuclear phagocytes with perivascular and parenchymal T-cell infiltration. Pattern II is also characterized by immunoglobulin and complement deposition of antimyelin antibodies that can damage myelin either by initiating complement-mediated demyelination or assisting the phagocytosis of myelin by macrophages. Pattern III is seen in approximately 25% of biopsied active lesions and consists of oligodendrocyte apoptosis, and these lesions are similar to viral, toxic, and ischemic processes suggesting a "dying-back" phenomenon. This model of the acute MS lesion appears consistent with the most prevalent theory about MS, namely, that it is an immune-mediated disease. Less understood is what happens chronically after the acute phase: whether there is persistent myelin degeneration (smoldering), inflammation resolves without inflammation (chronic inactive), or if lesions are invested with a thin myelin sheath (remyelinated).

Risk Factors

Genes

Substantial evidence indicates that genetic and racial background influence the risk of developing MS.[27,28] Caucasian and African American populations appear to be at a much higher risk of developing MS than Asians. Having a first-order relative (parent or sibling) with MS increases the risk of developing the disease by five- to twentyfold. Perhaps the most compelling evidence of the genetic influence on the risk of developing MS comes from studies of twins where at least one twin has MS.[29] Among nonidentical twins, the chances of the second twin having MS are 1% to 2%, which is similar to the risk of nontwin sibling pairs. Among identical twins, the chance of the second twin having MS is only 25%, indicating the strong influence of environmental factors besides the genetic background. Although the cause of MS remains unknown, more than 100 genes are thought to affect the risk of developing MS.[30] The human leukocyte antigen (HLA) genes are thought to have the strongest association with MS risk, with the most evidence for HLA-DRB1.[31]

Infections

Considerable interest lies in identifying the target(s) of the pernicious inflammatory response in MS.[29] Viruses and microbial agents have been postulated to be associated with an increased risk of developing MS. For decades there has been interest in the possibility that one or more viruses or other microbial infections might cause MS. This interest stems from the inflammatory nature of MS and the apparent influence of the environment on the risk of developing MS, both suggesting the possibility of an infectious etiology. In addition, a spontaneous inflammatory demyelinating disease, called Japanese macaque encephalomyelitis (JME), has been observed in a colony of Japanese macaques at the Oregon National Primate Research Center. This disease appears remarkably similar to MS, with similar MRI white-matter lesions, clinical findings, and pathological findings. The JME white-matter lesions were cultured and revealed a previously undescribed herpesvirus.[32]

Although various infectious agents have been reported to be associated with MS—including measles, Epstein–Barr virus, distemper virus, coronavirus, retrovirus, herpes simplex, human herpesvirus-6, and *Chlamydia pneumoniae*—there is no convincing evidence at present of a linkage between any infectious agent and MS.[33] The lack of evidence does not exclude the possibility that an infectious agent causes MS, but currently, there is no widely accepted evidence associating MS with any specific virus or other microbe.

Environment

Geographical and seasonal influences. The association with latitude and MS prevalence has shown mixed results and is confounded by factors that include migration, lifestyle habits, and biological and social influences. In general, there is an increased prevalence of MS in northern latitudes (high risk) and a decreased prevalence in southern latitudes (low risk). People who move from a low-risk to a high-risk area before age 15 acquire a higher risk of developing MS, whereas those who make the same move after adolescence retain a lower risk.[12,34] These observations suggest that an environmental exposure in the first two decades of life can influence the risk of developing MS. They also suggest that early sunlight exposure, which is correlated to serum vitamin D levels, may influence the risk for developing MS.[35]

Although not consistent to all geographical areas, there are several European epidemiological studies showing an association between season of birth and the risk of developing MS.[36–38] These studies indicate that there appears to be a lower risk for MS for births occurring after summer and a higher risk for MS for births occurring after winter. The authors reporting these findings suggest that maternal levels of vitamin D during the third trimester of pregnancy may influence the risk for MS, with a lower risk when maternal vitamin D levels are high (summer months) and a higher risk when maternal vitamin D levels are low (winter months). Taken together, there is some evidence to suggest that sunlight exposure and vitamin D levels at a young age may have an influential role in the risk of developing MS.

Diet. Diet has been investigated as a risk factor for acquiring MS. An association was first suspected based on the observation that inland farming communities in Norway had a higher incidence of MS than areas near the coastline. It was discovered that the diets of the farmers were much higher in animal and dairy products than the diets of the coastal dwellers, whose diet was enriched by cold-water fish.[39] Additional studies have since correlated consumption of animal fat, animal protein, and meat from nonmarine mammals with the risk for MS.[40] Individual dietary components may also affect MS pathogenesis. One example is the discovery of molecular mimicry between myelin and some dietary proteins, such as butyrophilin protein in cow's milk, inducing antibodies targeted against myelin oligodendrocyte glycoprotein (MOG). High salt intake was recently postulated as a risk factor for MS development and to have an association with increased relapses in people with MS. However, this association has not been validated.[41]

An epidemiological review evaluating the relationship between diet and MS from 1952 through 1995 suggested that the risk of MS is significantly correlated with the following parameters: consumption of animal fat, animal protein, and meat from nonmarine mammals.[40] However, a large prospective cohort study using data from the Nurses' Health Study and Nurses' Health Study II found no evidence linking the risk of MS with the intake of saturated fats. The authors did note, however, that intake of linolenic acid, an omega-3 fatty acid (O3FA), but not fish oils or docosahexaenoic acid, was associated with a trend toward a lower risk for MS.[42]

Using data from the same cohorts, this same group also found no relationship between intake of fruits and vegetables and the risk of MS.[43] A case-control study in Canada assessing the relationship between nutritional factors and the risk of MS in 197 incident cases of MS and 202 frequency-matched controls found a positive association between animal fat intake and the risk of MS.[44]

Recent data highlight that vascular disease risk factors such as obesity, hypertension, hyperlipidemia, heart disease, and diabetes can deleteriously affect MS progression. The presence of obesity as a child and in early adulthood has been associated with a greater risk of developing MS.[41] Because most of the vascular disease risk factors, including

obesity, are influenced by dietary habits, these studies suggest that there likely is an influence of diet on the risk of developing MS.

Gut microbiome. The gut microbiome is an ecosystem of commensal, symbiotic, and pathogenic microorganisms that outnumber their host's genes by more than 100 times.[45] The gut microbiome and its relationship with health and disease have been subject to extensive research, and the gut microbiome has been shown to be involved in maintaining human metabolism, nutrition, physiology, immune function, and mental health. The gut–brain axis is a bidirectional neurohumoral communication system that integrates neural, hormonal, and immunological signaling between the host gut and brain activities.[46] Commensal, probiotic, and pathogenic bacteria in the gastrointestinal tract can activate neural pathways and CNS signaling systems and can influence the development of anxiety and depression.[47] The bidirectional relationship is reflected in the observation that stress can influence the integrity of the gut epithelium and can alter peristalsis, secretions, and mucin production, promoting changes in microbial composition.[45] The concept of a gut–brain axis suggests that modulation of the gut microbiota may be a feasible approach to the management of CNS disorders with significantly less toxicity than many of the pharmaceuticals used currently.

The gut microbiome has been implicated in the induction of autoimmune conditions in human diseases and experimental animal models, including inflammatory bowel disease, ankylosing spondylitis, uveitis, rheumatoid arthritis, type 1 diabetes, and experimental autoimmune encephalomyelitis (EAE).[48–50] Current methodologies investigating gut microbial diversity, such as next-generation, 16S rRNA gene-sequencing technologies, suggest a "dysbiotic" gut microbiota in both adult and pediatric subjects with MS.[51–53] An alteration in gut microbiota has also been apparent in subjects with MS on DMTs such as glatiramer acetate and interferons.[53] Additionally, alterations in gut microbiota appear to be associated with relapse risk in pediatric and adult MS subjects.[51,53] The current, but limited, knowledge of gut microbiome changes in MS are intriguing, and it remains unclear if alterations in the gut microbiome precede or are a consequence of MS pathogenesis. University of California–San Francisco (UCSF) researchers suggested a causal relationship of gut microbiota and MS in a study where transplanting microbiota from MS subjects was found to exacerbate symptoms in mice with EAE.[54,55] The same group evaluated 34 sets of twins discordant for MS, revealing that transplanting gut microbiota from the MS-affected twins into transgenic mice induced CNS-specific autoimmunity at a higher incidence than microbiota taken from the healthy twins.[56] Although human and mouse microbiota are not directly comparable, the biological pathways represented by MS-associated bacterial taxa largely overlap.

Many factors, both nonmodifiable and modifiable, can influence the composition of an individual's gut microbiome. These include genotype, age, sex, disease modifying therapies (DMTs) for MS such as interferon and glatiramer acetate, antibiotics, and diet. An animal model of the gut microbiome suggests that diet can rapidly influence the gut microbiome, with changes occurring within a single day of diet change from a low-fat, plant-polysaccharide–rich diet to a high-fat, high-sugar diet.[57,58] Analysis of the fecal microbiome of a high-fiber diet compared with a Western diet revealed underrepresentation of Enterobacteriaceae (*Shigella* and *Escherichia*) with an abundance of bacteria from the genus *Prevotella* and *Xylanibacter,* known to contain a set of bacterial genes for cellulose and xylan hydrolysis, useful in digestion in a predominantly plant-based diet.[58,59] The Enterobacteriaceae family members associated with a Western diet have been shown to exacerbate small intestinal inflammation, whereas the bacteria associated with high-fiber diets, including *Prevotella* and *Xylanibacter,* are associated with a protective role against gut inflammation.

Toxins and toxicants. All environmental pollutants have demonstrated prooxidant activity, with the most toxic compounds causing the most oxidative damage. These toxicants also typically deplete the level of reduced glutathione in the brain tissue and inhibit the function of the antioxidant enzymes. Both reactive oxygen and reactive nitrogen species (ROS and RNS) can directly oxidize and damage DNA, protein, and lipids in the brain, leading to neurodegeneration. Neurons are directly affected by this oxidative stress, as are the glial cells. Oxidative stress activates the glial cells, leading to an increased production and release of the proinflammatory chemicals interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10); gamma-interferon (IFN-γ); and TNF-α.

The resulting neuroinflammation is a key component in the pathobiology of multiple sclerosis.[60] Activated glial cells and mast cells both appear to be responsible for the release of the proinflammatory chemical soup that fuels neuroinflammation. Glial cell activation has been implicated in the pathogenesis of epilepsy, Alzheimer's and Parkinson's diseases, MS, motor neuron diseases (amyotrophic lateral sclerosis [ALS]), stroke, and mood disorders.[61] Neuroinflammation is triggered by traumatic brain injury, endotoxicity (circulating levels of lipopolysaccharides), elevated blood sugar (glycation end products), stress, and a host of environmental toxicants, including air pollutants, heavy metals, and organophosphate pesticides.[62]

In a case-control study of children with MS having a father who worked in a gardening-related occupation (odds ratio [OR] = 2.18, 95% confidence interval [CI]: 1.14–4.16) or any household use of pesticide-related products (OR = 1.73, 95% CI; 1.06–2.81) were both associated with an increased risk of developing pediatric MS.[63] An Italian case-control study indicated that occupational solvent exposures could be related to the risk of MS, as both shoe/leather workers and mechanical manufacturing industry workers were found to have a twofold increase in the odds of developing MS.[64]

Summary of Risk Factors

Although there is general agreement that MS is an immune-mediated disease, why people develop MS remains uncertain. Epidemiological studies suggest a complex relationship between genetic and environmental factors that can influence both the risk of acquiring MS and disease progression in MS.

DIAGNOSTIC CONSIDERATIONS

MS remains a clinical diagnosis.[1] No single test (e.g., blood test, MRI examination, CSF study) is adequate to diagnose MS. The diagnosis relies on a knowledgeable physician taking a detailed history, performing a neurological examination, conducting various tests, and then making a diagnosis on the basis of all the data. Among the various practitioners, trained and experienced neurologists usually accurately diagnose MS. Even among neurologists, the rate of misdiagnosis of MS can be high because there are many mimics that appear similar to MS.[65] The diagnosis of MS rests on the *objective* demonstration of two or more areas of demyelination in the CNS that have occurred more than one time, and a diagnosis cannot be based only on a patient's symptoms.

Means of "objective" demonstration of areas of demyelination include the neurological examination; MRI of the brain and spinal cord; CSF examination; and electrophysiological tests (called evoked potentials) that assess visual, auditory, and somatosensory pathways. Evaluation of the CSF in a patient with suspected MS includes the presence of immunoglobulin G (IgG) production within the CNS, as seen by qualitative (oligoclonal bands) and quantitative (total IgG,

TABLE 199.2 Differential Diagnosis for Multiple Sclerosis

Infections of the central nerous system (CNS)	Syphilis
	Progressive multifocal leukoencephalopathy (PML)
	Lyme disease
	Human immunodeficiency virus (HIV)
	Human T-cell lymphotropic virus-1 (HTLV-1)
Inflammatory disorders of the CNS	Sjogren's syndrome
	Vasculitis
	Systemic lupus erythematosus (SLE)
	Neurosarcoidosis
	Behçet's disease
Genetic disorders	Hereditary myelopathies
	Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
	Leukodystrophies
	Hereditary cerebellar degeneration
	Mitochondrial disease
Brain tumors	Metastases
	Lymphoma
Deficiencies	Copper deficiency
	Vitamin B_{12} deficiency
Structural damage in brain or spinal cord	Cervical spondylosis
	Herniated disc
	Chiari's malformation
Other non-MS demyelinating disorders	Neuromyelitis optica (NMO)
	Acute disseminated encephalomyelitis (ADEM)

IgG index, and IgG synthesis rate) IgG changes. Excluding other disorders that can masquerade as MS is critical; these include unusual causes of stroke; nutritional deficiencies; genetic leukodystrophies or leukoencephalopathies; cancer; spinal cord compression from tumors, herniated disks, or spinal canal stenosis; vascular malformations of the spinal cord; infectious etiologies; and various other inflammatory disorders of the CNS (see Table 199.2 for a list). Although MS was formerly difficult to diagnose, MRI scanning has significantly improved the ability to diagnose MS in its early stages because it allows imaging of areas of demyelination in the brain and spinal cord and also helps exclude the presence of other diseases that might explain the patient's symptoms. The criteria used to diagnose MS are summarized in the revised 2017 McDonald criteria table (Table 199.3).

THERAPEUTIC CONSIDERATIONS

Disease Modifying Therapies (DMTs)

The conventional approach to treating MS includes the use of medications to control disease activity and some symptoms and rehabilitation interventions to alleviate symptoms resulting from damage to the CNS. Medications that help decrease MS disease activity are referred to as disease-modifying agents, or DMTs. (See Table 199.4 for a complete list.) Most of the DMTs are approved by the U.S. Food and Drug Administration (FDA) for relapsing forms of MS. These DMTs can broadly be broken down into route of delivery, including injectable medications, oral medications, and infusions. Compared with a placebo, these medications decrease the relapse rate by 30% to 67%, decrease new lesion formation in the brain as detected by MRI, and decrease the risk of developing a permanent neurological disability.

TABLE 199.3 2017 Revised McDonald MS Diagnostic Criteria

Clinical Presentation (Attacks)	Lesions (Objective Clinical Evidence or MRI)	Additional Data Needed to Make MS Diagnosis
≥2	≥2	None. Dissemination in space (DIS) and dissemination in time (DIT) have been met
≥2	1	One of these criteria: • DIS: additional clinical attack implicating different CNS site • DIS: ≥1 MS-typical T2 lesions in ≥2 areas of CNS: periventricular, juxtacortical/cortical, infratentorial or spinal cord
1	≥2	One of these criteria: • DIT: additional clinical attack • DIT: simultaneous presence of both enhancing and nonenhancing MS-typical MRI lesion • DIT: new lesion or enhancing MRI lesion compared with baseline scan • CSF-specific oligoclonal bands
1	1	DIS: additional clinical attack implicating a different CNS site or by ≥1 MRI -typical lesion in ≥2 areas of CNS AND one of these criteria: • DIT: additional clinical attack or by MRI • DIT: simultaneous presence of both enhancing and nonenhancing MS-typical MRI lesion • DIT: new lesion or enhancing MRI lesion compared with baseline scan • CSF-specific oligoclonal bands
0 (progression from onset)		1 year of disability progression (retrospective or prospective) AND two of these criteria: • ≥1 symptomatic or asymptomatic MS-typical lesion • ≥2 spinal cord lesions • CSF-specific oligoclonal bands

CFS, Cerebrospinal fluid; *CNS,* central nervous system; *MRI,* magnetic resonance imaging; *MS,* multiple sclerosis.
If the 2017 McDonald criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is MS. If MS is suspected by virtue of a clinically isolated syndrome but the 2017 McDonald criteria are not completely met, the diagnosis is possible MS. If another diagnosis arises during the evaluation that better explains the clinical presentation, the diagnosis is not multiple sclerosis.

TABLE 199.4 Disease Modifying Therapies for Multiple Sclerosis

	Drug	FDA Approval	Dosing	AEs	FDA Warnings/Monitoring
Injectable	Interferon beta-1a (Avonex, Rebif, Plegridy: pegylated interferon)	Avonex: RMS, CIS 1996 Rebif: RMS 2002 Plegridy: RMS 2014	Avonex: 30 mcg IM once weekly Rebif: 22 mcg or 44 mcg SQ three times a week Plegridy: 125 mcg SQ every 14 days	Flu-like symptoms (chills, fever, muscle pain, fatigue, weakness) after injection, headache, injection-site reactions (swelling redness, pain)	• Monitor closely if history of depression, seizures, cardiac problems • Rare allergic reactions **Monitoring:** • Baseline and periodic LFTs • Periodic CBC due to lowering of WBCs, RBCs, platelets
	Interferon beta-1b (Betaseron or Extavia)	Betaseron: RMS 1993 Extavia: RMS, CIS 2009	0.25 mg SQ every other day	Flu-like symptoms after injection, headache, injection-site reactions (swelling redness, pain), injection-site skin breakdown	• Monitor closely if history of depression, seizures, cardiac problems • Rare allergic reactions • Possible skin damage and infection **Monitoring:** • Baseline and periodic LFTs • Periodic CBC due to lowering of WBCs, RBCs, platelets
	Glatiramer acetate (Copaxone, generic)	RMS 1996	20 mg SQ every day, or 40 mg subcutaneously three times per week	Injection-site reactions (redness, pain, swelling), flushing, shortness of breath, rash, chest pain	• ~16% postinjection reaction, including flushing, chest pain, palpitations, anxiety, shortness of breath, constriction of the throat, and transient skin eruptions; resolves after 15 min • Lipoatrophy (skin depressions) at injection sites, skin damage • Careful rotation of injection sites **Monitoring** • No screening labs required
	Daclizumab (Zinbryta)	RMS 2016 **Withdrawn from market March 2, 2018**	150 mg SQ once a month	Colds, upper respiratory tract infections, rash, flu, rash, throat pain, bronchitis, eczema, depression, swollen lymph nodes	• Risk for severe liver injury • Immune-mediated disorders **Monitoring** • Baseline LFTs and monthly during treatment up to 6 months after last dose
Oral	Teriflunomide (Aubagio)	RMS 2012	7- or 14-mg pill once daily	Headache, hair thinning, diarrhea, nausea, abnormal liver tests	• Can cause major birth defects up to 2 years after discontinuation **Monitoring** • Baseline LFTs and monthly during first 6 mo, then routinely • CBC at baseline and routinely due to lowering WBCs and increased risk for infection • Screen for TB • Renal function tests routinely
	Fingolimod (Gilenya)	RMS 2010	0.5-mg capsule once daily	Headache, flu, diarrhea, back pain, liver enzyme elevations, sinusitis, abdominal pain, pain in extremities and cough	• Risk of macular edema • Lowers heart rate • Cryptococcal meningitis **Monitoring** • Vision test before and 3 months after starting • Requires a first-dose observation, ECG before first dose • CBC at baseline and routinely; consider discontinuation if ALC <200 • Increased risk for PML • VZV (chickenpox/shingles) antibody, if negative—recommend VZV vaccination 6 wk before start
	Dimethyl fumarate (Tecfidera)	RMS 2013	120-mg capsule taken twice daily for 1 week, followed by 240-mg capsule taken twice daily thereafter	Flushing (sensation of heat or itching and a blush on the skin), gastrointestinal issues (nausea, diarrhea, abdominal pain)	• Can cause severe allergic reactions **Monitoring** • CBC at baseline and routinely, at least 6 months initially; consider discontinuation if ALC <600 • Some cases of PML

Continued

TABLE 199.4 Disease Modifying Therapies for Multiple Sclerosis—cont'd

	Drug	FDA Approval	Dosing	AEs	FDA Warnings/Monitoring
Infusion	Natalizumab (Tysabri)	RMS 2006	300 mg IV once every 28 days	Headache, fatigue, joint pain, chest discomfort, urinary tract infection, lower respiratory tract infection, gastroenteritis, vaginitis, depression, pain in extremity, abdominal discomfort, diarrhea, and rash.	• Increases risk of PML • Liver damage risk • Anaphylaxis and other allergic reactions • Risk of infections, meningitis, and encephalitis **Monitoring** • JCV antibody at least every 6 mo • Baseline and periodic LFTs
	Alemtuzumab (Lemtrada)	RMS (if inadequate response to ≥2 DMTs)	12 mg IV per day for 5 consecutive days, followed by 12 mg per day on 3 consecutive days 1 year later	Rash, headache, fever, nasal congestion, nausea, urinary tract infection, fatigue, insomnia, upper respiratory tract infection, herpes viral infections, hives, itching, thyroid gland disorders, fungal infection, pain in joints, extremities and back, diarrhea, vomiting, flushing Infusion reactions (including nausea, hives, itching, insomnia, chills, flushing, fatigue, shortness of breath, changes in the sense of taste, indigestion, dizziness, pain) are also common while the medication is being administered and for 24 hours or more after the infusion is over	• Serious, sometimes fatal autoimmune conditions (ITP, autoimmune renal disease) • Risk of infusion reactions, steroids given before and during first 3 days after infusion • Increases risk of malignancies (thyroid cancer, melanoma, blood cancers) • Skin testing before and yearly • Thyroid disorders • No live vaccinations **Monitoring** • CBC, kidney tests, UA before and every month until 48 months after last infusion • Thyroid testing every 3 months until 48 months after last infusion • VZV antibody, may require vaccination 6 wk before start
	Mitoxantrone (Novantrone)	RMS, SPMS 2000	12 mg/m^2 IV every 3 months Lifetime cumulative dose limit of approximately 8–12 doses over 2–3 years (140 mg/m^2)	Nausea, hair loss, menstrual change, upper respiratory infection, urinary tract infection, mouth sores, irregular heartbeat, diarrhea, constipation, back pain, sinusitis, headache, blue-green urine	• AML, can be fatal • Risk of heart damage • Contraindicated if preexisting heart problems, liver disease, and certain blood disorders **Monitoring** • Tests of heart function before each dose and periodically after treatment has ended • Baseline and periodic LFTs
	Ocrelizumab (Ocrevus)	RMS, PPMS 2017	600 mg every 6 months (first dose: 300 mg IV on day 1 and 300 mg IV 2 weeks later)	Infusion reactions (most commonly itchy skin, rash, throat irritation, flushed face or fever, headache), which in rare instances may be life threatening; increased risk of infections, including respiratory tract infections and herpes infections; possible increase in malignancies, including breast cancer	• Infusion reactions • Chronic hepatitis B reactivation • Increases risk of infection • Vaccinations 6 wk before infusion **Monitoring** • Hepatitis B antibody screening • An increased risk of breast cancer may exist; follow standard breast cancer screening guidelines

AE, Adverse effect; *ALC,* absolute lymphocyte count; *AML,* acute myeloid leukemia; *CBC,* complete blood count; *CIS,* clinically isolated syndrome; *ECG,* electrocardiogram; *FDA,* U.S. Food and Drug Administration; *IM,* intramuscular; *ITP,* immune thrombocytopenia; *IV,* intravenous; *JCV,* John Cunningham virus; *LFT,* liver function tests; *MS,* multiple sclerosis; *PML,* progressive multifocal leukoencephalopathy; *PPMS,* primary progressive multiple sclerosis; *RBC,* red blood cell; *RMS,* relapsing multiple sclerosis; *SPMS,* secondary progressive multiple sclerosis; *SQ,* subcutaneous; *TB,* tuberculosis; *UA,* urinalysis; *VZV,* varicella zoster virus; *WBC,* white blood cell.

The injectable medications form the platform therapies and include human recombinant interferon-β (Avonex, Betaseron, Rebif, and Plegridy) and glatiramer acetate (Copaxone, Glatopa). Interferons (IFNs) are cytokines that mediate antiviral, antiproliferative, and immunomodulatory processes. There are three major forms of IFNs: alpha (α), beta (β), and gamma (γ), which have some overlaps in function yet are distinct, with IFN-β showing benefit in the management of MS. Although the precise mechanism by which IFN-β works in MS is not certain, the immunomodulatory effects proposed to occur include inhibition of proinflammatory T-cell activation and proliferation, destruction of autoreactive T cells, cytokine modulation, and prevention of migration across the blood–brain barrier.[19,66] IFN-β therapy has been found to decrease annualized relapse rates by 27% to 36% and reduce disability progression by 30% to 38% in various clinical trials. Glatiramer acetate is a random polymer of four amino acids that stimulates protective T cells and is the safest option of the DMTs. In multiple randomized trials, glatiramer was shown to reduce the relapse rate by 28% and reduce disability accumulation (risk ratio of 0.6) compared with placebo.[67] Glatiramer acetate and IFN-β therapies are frequently used as first-line therapy for MS due to an excellent safety profile. Another injectable, daclizumab (Zynbryta), has been taken off the market due to hepatitis risk.

The oral medications include three medications, fingolimod (Gilenya), teriflunomide (Aubagio), and dimethyl fumarate (Tecfidera). The mechanism of action of fingolimod involves the reduction of CNS inflammation and axonal damage by retaining lymphocytes in the lymph nodes so that fewer are able to enter the CNS.[68,69] Fingolimod has been shown to reduce relapse rates by 50%, decrease the rate of disease progression, and decrease disease activity (MRI lesions).[68,69]

Teriflunomide prevents the division of active immune cells, including T and B lymphocytes.[19,70] Teriflunomide has been shown to reduce relapse rates by 36% compared with placebo and to decrease the rate of brain atrophy as assessed by MRI. Teriflunomide remains in the bloodstream for up to 2 years after discontinuation and is associated with fetal complications in pregnancy when taken by either males or females.[19]

Dimethyl fumarate is based on a German psoriasis treatment that may enhance Th2 cellular response through the activation of intracellular nuclear pathways.[71] Dimethyl fumarate affects transcription pathways that change the balance of T helper cell profiles, causing immunosuppression. Dimethyl fumarate decreases the absolute relapse risk by 56% compared with placebo, decreases the number of new lesions on MRI, and decreases the rate of confirmed disability progression.[71]

The FDA approved-infusions include natalizumab (Tysabri), alemtuzumab (Lemtrada), and ocrelizumab (Ocrevus). Natalizumab is a monoclonal antibody against α-4 integrin that prevents inflammatory cells from entering the CNS and has been shown to decrease the annualized relapse rate by 68% and reduce disease activity (new or enlarging MRI lesions) by 83% over 2 years compared with placebo.[72,73] The main risk with natalizumab is the possibility of developing progressive multifocal leukoencephalopathy (PML), which is associated with exposure to John Cunningham virus (JCV). Natalizumab was taken off the market shortly after its release due to the development of PML before reintroduction with recommendations to monitor JCV antibody, which correlates with risk of PML development.[19]

Alemtuzumab is a monoclonal antibody to CD-52 that is present on most immune cells in the body and was approved by the FDA in 2014 for relapsing MS. Alemtuzumab is given as two annual infusions and has been shown to reduce relapses by 55% compared with the use of IFN-β-1a.[74]

Ocrelizumab is a monoclonal antibody directed at B-lymphocytes and was approved by the FDA in 2017 for both primary progressive MS and relapsing forms of MS. Ocrelizumab has been shown to reduce annual relapses by 50% compared with IFN-β-1a, with a 95% reduction in new active MRI lesions compared with IFN-β-1a.[74] Ocrelizumab, in addition to being approved for the management of relapsing MS, has been shown to be effective in decreasing the accumulation of disability in patients with primary progressive MS and is the only FDA-approved drug for this indication. Ocrelizumab reduced confirmed disability progression by 24% compared with a placebo in those with primary progressive MS.[74]

Although conventional DMTs are able to reduce disease activity in relapsing forms of MS, they have limitations, which include only a modest effect on prolonging time to disability, rising costs (average cost is more than $60,000 per year), and side effects (e.g., injection-site reactions and neutralizing antibodies for IFN-β; bradycardia and arrhythmia for fingolimod; birth defects with teriflunomide; flushing and diarrhea with dimethyl fumarate).

Corticosteroids, such as methylprednisolone, given in high doses can decrease the duration of relapses of MS but do not affect the degree of eventual recovery after relapses.[75] A number of different medications are useful for treating various symptoms of MS, such as fatigue, bladder dysfunction, and spasticity, but these medications do not reverse the damage that has already occurred or decrease disease activity. Given these considerations, an effort to identify natural therapies that have benefit for people with MS is warranted.

Natural Medicine Therapeutic Considerations

From a natural medicine standpoint, there are four major approaches to treating MS:

- Diet
- Nutritional supplements
- Exercise
- Stress management

Including all four provides the most comprehensive natural medicine treatment plan, and it is recommended that these should be used in combination with appropriate conventional therapies.

Diet

Diet is one way to influence general health, which is important to maintain in people with a chronic disease such as MS. There is no panacea diet for MS. However, various health factors, particularly vascular disease risk factors such as hypertension, hyperlipidemia, salt intake, diabetes, and obesity, can contribute to MS disability progression.[41] These vascular risk factors are readily influenced by diet and can be modulated with intervention.

Low-Saturated-Fat Diets

Swank diet. The Swank diet is one of the oldest and most well known dietary interventions used by people with MS. Dr. Roy Swank reported that a diet low in saturated fats maintained over a long period of time tends to slow disease progression, reduce the number of attacks, and decrease mortality.[76,77] Swank began treating patients with his low-fat diet in 1948. The approach to using a low-fat diet supplemented with cod liver oil is based on epidemiological studies that found a decreased incidence of MS in populations that had a low consumption of animal fats with a high consumption of cold-water fish.

Based on current knowledge of the pathogenesis of MS, the rationale of using the Swank diet or other diets low in saturated fats in patients with MS relates to the general health benefits of such a diet and the anti-inflammatory and perhaps neuron membrane–stabilizing effects

of a diet enriched with O3FAs. Although the consumption of red meat is significantly restricted on the Swank diet, fish appears to be particularly indicated because of its excellent protein content and, perhaps more importantly, its high content of O3FAs. Cold-water fish such as mackerel, salmon, and herring are rich in O3FAs, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). As reviewed in the "Nutritional Supplements" section, O3FAs have anti-inflammatory effects that may be of benefit in MS. In addition, because optimal neuronal functioning depends on cell membrane fluidity, which in turn depends on lipid composition, optimal essential fatty acid (EFA) levels may be important in exerting neuroprotective effects by maintaining healthy neuronal functioning.[77,78] Decreasing animal fats and increasing O3FAs in the diet thus may improve neuronal function by modulating neuronal lipid composition.

Since Dr. Swank's observational studies, two pilot studies evaluating diet in MS have been conducted. An open-label study evaluated the effects of a diet low in saturated fats combined with fish-oil supplementation and vitamin B complex and vitamin C in newly diagnosed RRMS.[79] Beside dietary modifications, subjects were advised to reduce their intake of sugar, coffee, tea, and alcohol and to stop smoking. Diet was monitored over 2 years by a 4-day dietary record at the end of each year, and plasma fatty acid levels were monitored at baseline, year 1, and year 2.

A significant increase occurred in the intake of fish, and a reduction in food items containing saturated fats occurred after 2 years. A significant increase occurred in plasma levels of O3FAs, and a significant decrease occurred in plasma omega-6 fatty acids (O6FAs). Over the 2 years of the study, patients had significant reductions in both relapse rates and disability. This study, however, had significant limitations because there was no comparison group.

Although caution is warranted in interpreting the results of this small open-label study, its outcome does concur with Dr. Swank's long-term studies on diet and fish-oil supplementation for people with MS.

A partially blinded, randomized controlled study evaluated the effect of low-fat dietary intervention with O3FA supplementation in 31 subjects with RRMS.[80] The intervention lasted for 12 months, and the primary outcome was quality of life, evaluated using the Short Form Health Survey Questionnaire (SF-36). Subjects were randomized into one of two groups: the low-fat diet/fish-oil group and the diet/olive-oil group. The low-fat diet/fish-oil group followed a diet that did not exceed 15% of saturated fats (percentage of total daily calorie intake) plus fish-oil capsules (daily doses of EPA 1.98 g and DHA 1.32 g). The diet/olive-oil group followed the American Heart Association's Step I diet, 30% saturated fats (percentage of total daily calorie intake) plus olive-oil capsules (6 capsules of 1 g of olive oil per day). The subjects were followed for an average of 11 ± 2.9 months, and the low-fat diet/fish-oil group maintained better quality-of-life scores for physical well-being (although not statistically significant) than the group using olive-oil supplementation. The scores on the mental health component were similar in the two intervention groups. At the 6-month time point, the olive-oil group reported an improvement in fatigue compared with the fish-oil group ($P = 0.035$), which continued for 12 months. For both intervention groups, relapse rates were reduced compared with the year before entering the study. This study suggests that a diet low in saturated fats with fish-oil supplementation might promote better physical and mental health for people with MS. Because all subjects improved after 12 months, the study also suggests that diet modification in addition to supplementation with a "good oil" (fish oil or olive oil) may be beneficial in people with MS.

The epidemiological studies suggesting a link between a high-fat diet and an increased risk of MS coupled with the results from the three low-fat-diet studies reviewed show a consistency in identifying a low-fat diet as a therapy that may benefit people with MS. A person with MS following a low-fat diet will gain general health benefits.

McDougall diet. The McDougall diet is a very low-fat, strictly plant-based diet that is based mainly on complex carbohydrates as the main source of energy. This diet is based on starch, with 10% of calories derived from fat, 14% from protein, and 76% from carbohydrates. Animal-derived products, dairy products, and oils are restricted from the McDougall diet.

Paleolithic diet. The Paleolithic (Paleo) diet is based on the idea that humans are better equipped to handle the diet consumed by their Paleolithic ancestors. The Paleo diet is a component of the Wahls protocol for MS management developed by Dr. Terry Wahls. The diet consists of nondomesticated lean meats and plant-based foods except fruits, nuts, roots, and legumes. The ratio of saturated to polyunsaturated fatty acids is 1.4 to 2.0:1. The diet consists of three cups of green leafy vegetables, three cups of sulfur-rich vegetables, and three cups of intensely colored vegetables daily. In addition, two tablespoons of O3FAs and 4 oz or more each of animal protein and plant protein is to be consumed daily. No more than two servings per week of gluten-free grains/starchy foods are recommended, and gluten, dairy, and eggs are prohibited.

Small open-label studies have been conducted in subjects with secondary progressive MS with a multimodal intervention consisting of diet, massage, acupuncture, meditation, and implementation of the Paleo diet. These limited studies showed the diet was well tolerated, and although adherence to the diet was not consistent, participants had improved fatigue. The fatigue changes were not clinically significant, and the results were further diluted by the fact that additional interventions were employed rather than just the diet. The Paleo diet also has the added risk of nutritional deficiencies, including folic acid, vitamin B_1, and vitamin B_6, from the decreased intake of fortified cereals, as well as calcium and vitamin D deficiency from the lack of dairy intake.[41]

Caloric Restriction

Diet can lead to inflammation via oxidative stress based on the type and amount of food that is consumed. Increases in caloric intake and glycemic load, and a high intake of saturated fat, trans fat, or O6FAs can lead to postprandial inflammation. Inflammation is decreased by the consumption of polyphenols, O3FAs, caloric restriction, and exercise. Caloric restriction has shown beneficial effects on disease activity in mouse models of MS.

A pilot study evaluating the effect of restricting the diet to 1700 to 1800 kcal was performed in 33 subjects with RRMS and 10 subjects with PPMS. Although there were no differences in neurological examination results in the follow-up period of 6 months, there was a 59% reduction of activated MMP-9 in subjects with PPMS and 51% reduction in subjects with RRMS.[81] In another study, 60 subjects with RRMS were randomized to one of three parallel groups: one group that continued a usual diet, one group with a usual diet enhanced by an initial 7-day fasting episode, and one group with ketogenic diet over the course of 6 months. Subjects were asked to complete the Multiple Sclerosis Quality of Life-54 Questionnaire (MS-54). The subjects who were on the ketogenic diet and those on the initial fasting diet showed an improvement in the MS-54.[82]

Nutritional Supplements

Essential fatty acids. O3FAs are found in both plant and animal forms, with alpha-linolenic acid (ALA) being found in plant foods and EPA and DHA being found in marine foods. O6FAs include linoleic acid, which is found in vegetable oils, nuts, and seeds, and arachidonic

acid, which is found in meat and eggs. The body can synthesize most of the fats needed from the diet. There are two essential fatty acids (EFAs) that cannot be synthesized in the body and must be obtained from food: ALA (an O3FA) and linoleic acid (an O6FA). Omega-9 fatty acids (O9FAs) are monounsaturated fats, are made in the body, and are considered nonessential.

Omega-3 fatty acids. There have been multiple studies evaluating O3FAs in RRMS. One open-label study in RRMS patients ($n = 10$) showed a significant decrease in MMP-9 levels secreted from unstimulated immune cells after supplementing with fish-oil concentrate at 8 g/day (containing 2.9 g EPA and 1.9 g DHA) for 3 months. All subjects showed a decrease in MMP-9 levels whether or not they were on MS disease-modifying medication.[83]

O3FAs have been shown to have immunomodulatory effects. In vitro, animal, and ex vivo human studies have reported a decrease in mRNA and protein levels of a number of cytokines, including TNF-α, IFN-γ, IL-1, IL-2, and VCAM-1. One published study documented the effects of supplementation with fish oils enriched with the O3FAs EPA and DHA on cytokine secretion in MS.[84] In this study, levels of IL-1-β, TNF-α, IL-2, IFN-γ, prostaglandin E2 (PGE2), and LTB4 secreted from unstimulated and stimulated immune cells in people with MS and healthy controls were evaluated. Twenty subjects with MS and 15 age-matched healthy controls were supplemented with 6 g/day of fish oil containing 3 g EPA and 1.8 g DHA for 6 months. All subjects with MS had a stable course of MS for at least 3 months before enrollment, had not modified their diet as a consequence of developing MS, and were not on any DMTs.

Outcome measures compared baseline levels of the cytokines mentioned to levels after 3 and 6 months of supplementation in both subjects with MS and healthy controls. After 3 and 6 months of fish-oil supplementation, there was a significant decrease in the levels of soluble IL-1β ($P = 0.03$), TNF-α ($P = 0.02$), IL-2 ($P = 0.002$), and IFN-γ ($P = 0.01$) in the unstimulated peripheral blood mononuclear cells (PBMCs) of both groups. A significant difference was observed after 3 and 6 months of supplementation in the levels of soluble IL-1β ($P = 0.01$), TNF-α ($P = 0.02$), IL-2 ($P = 0.003$), and IFN-γ ($P = 0.005$) from stimulated immune cells of both groups. Cytokine levels returned to baseline values after a 3-month washout period.

One study has examined the use of O3FA supplementation in MS.[85] This was a large ($n = 312$) double-blind, placebo-controlled trial in which patients with MS were randomized to receive either 20 capsules of fish oil per day or olive oil containing 72% oleic acid for 2 years. The total daily dose of EPA was 1.71 g, and the total daily dose of DHA was 1.41 g. This study reported a trend in improvement in the O3FA–treated subjects compared with controls; however, these results did not achieve statistical significance ($P = 0.07$). There are some criticisms of the study design, including that both groups in the study were advised to follow a diet low in animal fats and high in polyunsaturated fatty acids. Importantly, both groups developed changes in serum fatty acid content over the 2 years of the study. The lack of a comparison of fish-oil supplementation with a placebo in patients who did not have other dietary modifications may have affected the ability of the study to detect a statistically significant therapeutic benefit of O3FA supplementation.

Omega-6 fatty acids. There is some experimental basis for considering supplementation with O6FAs. Two studies in an animal model of MS reported that supplementation with linoleic acid, which is rich in O6FAs, decreased the severity of disease[86] and reduced inflammation in the CNS.[87]

O6FA (linoleic acid) supplementation for the treatment of MS has been investigated in at least three double-blind clinical trials.[88–90] O6FA spread (11–23 g/day linoleic acid) was provided in comparison with oleic acid (control). Disability progression at 24 months was provided from two trials encompassing 144 patients and did not show a difference. Two additional studies evaluated relapse risk in 132 subjects with RRMS and showed a small decrease in relapse rate at 24 months (weighted mean difference [WMD] of 0.79, CI 0.63–1.00, $p = 0.05$). Linoleic acid (2.9–3.4 g/day) did not show a significant decrease in the rate of progression in 65 subjects with progressive MS.[19]

Evening primrose oil, which is rich in the O6FA gamma-linoleic acid, is commonly used by patients with MS. Gamma-linolenic acid might be more effective than linoleic acid because of its easier incorporation into brain lipids and its possibly greater effect on immune function.[91] However, evening primrose oil contains low levels of gamma-linolenic acid, and the product is relatively expensive. Large and prohibitively expensive amounts of evening primrose oil would have to be used to obtain adequate supplementation. In addition, a single pilot trial of evening primrose oil in MS failed to demonstrate any benefit.[88] Despite its common use by patients with MS, supplementation with evening primrose oil is not recommended.[92] The data to support O3FA or O6FA supplementation are lacking, and although there may be no harm in the use of EFAs, there is no major effect on disease progression in MS.

Vitamin D. Epidemiological studies have found that low vitamin D intake and low serum levels of vitamin D may increase the risk of MS.[93,94]A retrospective study of serum levels of vitamin D in people with MS ($n = 199$) found 84% of them to be deficient.[95] Studies of vitamin D in animal models of MS have shown that vitamin D has the ability to decrease immune cell–mediated inflammation and prevent disease.[96,97] MS studies in animal models suggest that vitamin D may have a beneficial role in MS by affecting the ability of inflammatory cells to enter the CNS.[98]

Numerous human studies have evaluated both vitamin D supplementation and associations between serum vitamin D levels and biomarkers of MS disease progression. One open-label study ($N = 16$ subjects with MS) using oral calcitriol at a target dose of 2.5 mcg/dL found the intervention safe and tolerable up to a year of supplementation.[99] Another study examined the seasonal variation in the serum levels of vitamin D in people with MS ($n = 103$) and healthy controls ($n = 110$) and found these levels to be significantly higher in the summer compared with the winter in both cohorts.[99] This study also observed that higher circulating levels of vitamin D in women were correlated with lower MS-related disability. A pilot study evaluating 29 subjects with RRMS found a positive correlation between serum vitamin D levels and levels of the anti-inflammatory cytokine IL-4.[100] In another 1-year prospective study, vitamin D supplementation and increases in serum vitamin D concentrations resulted in a significant decrease in annual relapses in MS subjects. This was observed in subjects who had vitamin D levels below 50 nmol/L.[19]

Thus emerging evidence from both animal studies and human studies suggests that vitamin D may have potential beneficial effects in MS. Given that 30% to 50% of the general population may be deficient in vitamin D,[101] experts believe that serum levels of vitamin D should be evaluated to assess and treat vitamin D deficiency in MS. Experts recommend vitamin D supplementation to target a serum level of (40–60 ng/mL or 75–150 nmol/L).[19]

Lipoic acid. Oral lipoic acid (LA) is an over-the-counter supplement that has been investigated as an antioxidant, anti-inflammatory, and neuroprotective agent in MS. LA and its reduced form, dihydrolipoic acid (DHLA), are potent antioxidants with multiple modes of action. LA/DHLA can regenerate other antioxidants, such as glutathione, vitamin C, and vitamin E; serve as an ROS scavenger; repair oxidative damage; and chelate metallic ions involved in oxidative injury. DHLA acts by restoring reduced levels of other antioxidants, such as glutathione,

and by repairing oxidative damage.[102–104] LA is absorbed from the diet and synthesized de novo; it readily converts intracellularly to DHLA. Both LA and DHLA are present in both extracellular and intracellular environments.[105] In an animal model of MS, LA has been shown to suppress the development of disease by preventing inflammatory T cells from entering the CNS.[106–108] The immunomodulatory effects of LA include inhibition of T-cell production of MMP-9, inhibition of the expression of the adhesion molecules ICAM-1 and VCAM-1, and stimulation of cAMP by the prostaglandin receptors EP2 and EP4.[106,107,109]

One small double-blind, placebo-controlled study evaluated the optimal dosing of oral LA in RRMS.[110] In this study, 37 subjects were randomized to one of four groups: (1) placebo, (2) LA 600 mg twice a day, (3) LA 1200 mg once a day, and (4) LA 1200 mg twice a day. The study found that LA given at 600 mg twice a day was barely measurable in serum, whereas LA given at the dose of 1200 mg once daily showed significantly higher serum levels. The study also found an association between higher LA serum levels and lower MMP-9 levels and higher LA serum levels with lower soluble ICAM-1 levels. The investigators concluded that oral LA between 600 and 1200 mg can be measured in serum and that higher serum levels of LA are associated with an increased immunomodulatory activity that may benefit people with MS.

A pilot double-blind, placebo-controlled study of daily oral 1200 mg LA in 51 patients with SPMS revealed a decreased annualized rate of brain volume over the course of 2 years in the LA-treated subjects.[111] There was also a trend of improved 25-foot walk time in the group that received LA. Larger studies are under way to further investigate the association of LA and whole-brain atrophy in progressive MS.

Biotin. Biotin, also known as vitamin H or vitamin B_7, is a water-soluble B vitamin that is taken orally and is known to have multiple functions in energy metabolism and fatty acid synthesis. It is known to participate in myelin synthesis by activating the enzyme acetyl-CoA carboxylase. High-dose biotin (300–600 mg daily) has been studied in both primary and secondary progressive MS. An initial pilot study of 23 patients with progressive MS was performed, and 91.3% of the patients had an improvement in clinical measurements.[112]

Patients with MS with a progressive decline in disability score over the previous 2 years were randomized to either placebo or a formulation termed MD10003, a highly concentrated oral form of biotin.[113] MD10003 was given at 100 mg three times daily over a 12-month period, and 12.6% of treated patients had an improvement in disability score by 1 point on the Expanded Disability Status Scale (EDSS), versus none of the placebo group.

Although these studies have revealed promising results, there have been negative results associated with biotin. A study completed in Italy including 41 patients with progressive MS treated with high-dose biotin resulted in a significant increase in relapse rate.[114] Many of these patients had PPMS and had never experienced an MS relapse before. High-dose biotin has also been shown to interfere with various laboratory tests, resulting in a number of adverse events, including at least one death.[115] Some of the laboratory values affected include measures of cardiac injury and thyroid function.[116]

Botanical Medicines

Ginkgo biloba

Cognitive impairment affects up to 40% to 50% of people with MS,[117,118] and there are currently no effective symptomatic therapies for cognitive dysfunction in MS. *Ginkgo biloba* has been evaluated for cognitive impairment in Alzheimer's disease, with mixed findings. A recent meta-analysis of these studies reports that *G. biloba* is safe, but the benefits for cognitive impairment and dementia are not predictable.[119]

There is one randomized, placebo-controlled pilot study evaluating the effects of a standardized *G. biloba* extract on cognitive performance in 43 subjects with MS.[120] Subjects were randomized to receive 120 mg of *G. biloba* twice a day or placebo for 12 weeks. The outcomes of the study included several neuropsychological tests, including the Stroop test, which is a measure of attention and executive function. Subjects receiving *G. biloba* showed significantly improved performance on the Stroop test as well as improvement in subjective reports of cognitive deficits compared with the placebo group. This pilot study also showed that *G. biloba* extract was safe and well tolerated. Although the studies evaluating *G. biloba* in people with dementia have shown mixed results, this supplement has been shown to be safe and well tolerated in many clinical trials. The pilot study in MS also demonstrated that *G. biloba* standardized extract given at 120 mg twice a day for 12 weeks was safe in people with MS. Given its safety profile and limited evidence on improving attention and executive function in MS, *G. biloba* standardized extract may benefit MS patients with cognitive impairment.

Cannabinoids

Cannabinoids are a group of compounds found in the plant cannabis, also known as marijuana. The major psychoactive constituent in cannabis is delta-9-tetrahydrocannabinol (THC). THC binds to cannabinoid receptors (CB) in the CNS and acts as a partial agonist to both CB1 and CB2 receptors. Cannabidiol (CBD) is a nonpsychoactive constituent in cannabis and the major constituent in the plant. It is thought to decrease the clearance of THC by affecting liver metabolism. It binds to both CB1 and CB2 receptors in the CNS, with a higher affinity to the CB2 receptor. Cannabinoids can be delivered orally (e.g., cannabis extract, synthetic THC), through mucosa (e.g., cannabis extract oral spray, nabiximols [Sativex]), and smoked.

In a review of nine controlled studies evaluating a combination of THC and CBD (Marinol) for spasticity in MS, it was found that THC–CBD was well tolerated and improved patient self-reports of spasticity, although objective measures for spasticity such as the Ashworth score did not show significant improvement compared with a placebo.[121] The authors report that side effects were mild in both the treatment and placebo groups. The authors concluded that there was a significant improvement in patient-reported spasticity with the combination of THC–CBD and that THC–CBD was well tolerated in MS. Unlike the dietary supplements discussed, Marinol is a controlled substance and requires a prescription in the United States.

OTHER CONSIDERATIONS

Exercise

In the past, MS patients were often advised not to exercise because increased body temperature and nerve fiber fatigue resulting from exercise were thought to induce transient symptomatic worsening and provide no long-term benefit. However, research has since shown that regular exercise is beneficial for people with MS.[122–128] Four studies and two meta-analyses reported that compared with an MS nonexercise group, the MS exercise group demonstrated improvement in the subjects' reports of fatigue, quality of life, well-being, and walking ability.[123,124,127,129] A systemic review of 26 studies on the effects of exercise, physical activity, and physical fitness on cognitive-performance outcomes in people with MS suggested beneficial effects of physical fitness, physical activity, and regular exercise on cognitive performance in people with MS.[130] In addition to direct effects on MS, regular exercise provides benefits in multiple facets of health, including cardiovascular risk; metabolic functioning, including blood sugar maintenance; mental health and mood; bone

mineralization; and reduced risk for falls.[132] MS represents only one aspect of a person's health, and balancing other factors by maintaining a healthy lifestyle is crucial. In summary, evidence indicates that regular exercise is beneficial in MS and should be part of any natural medicine approach to treating MS.

Stress and Multiple Sclerosis

Patients with MS often report that stress worsens their MS symptoms and consequently triggers an exacerbation. In a review of the scientific literature reporting associations between psychological stress and worsening of MS symptoms, an expert panel concluded that there was a possible relationship between antecedent stress and either MS onset or exacerbations.[133] A prospective longitudinal study of patients with MS designed to examine the relationship between stressful life events, psychological stress, and disease activity as measured by MRI found that increased conflicts and disruptions in routine were followed by an increased risk of developing new brain lesions 8 weeks later.[134] Perceived stress in patients with MS has been associated with MS exacerbations in a number of studies.[135]

Given the emerging evidence that antecedent stressors may contribute to the development of new lesions in MS and that perceived stress is associated with MS exacerbations, therapies that reduce stress are highly recommended in MS.

Mind–Body

There are very few scientific studies evaluating mind–body interventions such as yoga, meditation, and prayer in MS.[136] Mind–body techniques using meditation, yoga, and slowed breathing to reduce stress in cancer patients have shown that these therapies are effective in decreasing stress, improving quality of life, and improving sleep.[137]

Stress Management Therapy

A randomized controlled study investigated the effect of stress management therapy (SMT) in MRI outcomes in people with MS.[130] Experienced therapists administered 16 individual 50-minute SMT sessions over a 24-week period followed by a 24-week period of observation. SMT first consisted of six sessions focused on relaxation, teaching problem-solving skills, cognitive restructuring, and enhancement of social support. Participants were then able to tailor their treatment using option modules, including management of cognitive problems, communication, assertiveness, fatigue management, anxiety reduction, management of sexual dysfunction, and management of insomnia. MRIs were obtained at 8-week intervals during the 24-week active-treatment period. Although the study was underpowered and enrolled fewer patients than originally planned, significantly more patients who underwent SMT (76.8%) were free of lesions versus the controls (54.7%).

Tai Chi

There are two reported pilot studies evaluating the use of Tai Chi in people with MS.[125,126] To evaluate the effects of training in the principles of "mindfulness of movement" from tai chi/qi gong in MS, 16 patients with secondary progressive MS were divided into 8 matched pairs. Each pair was randomized into a mindfulness group or a usual-care group (i.e., standard medical care for MS). Although there was no difference between groups in measures of balance, there was a significant improvement in the mindfulness group in self-reported measures of MS-related symptoms.[125] In a nonrandomized uncontrolled pilot study, 19 people with MS underwent tai chi training twice weekly for 8 weeks. Outcomes measures compared pretraining with posttraining scores. Posttraining outcomes demonstrated an improvement in walking speed, hamstring flexibility, and subjects' reports of well-being and quality of life.[126]

Yoga

There has been one randomized controlled study evaluating yoga in MS.[138] Sixty-nine subjects were randomized to one of three groups: (1) wait-list control ($n = 22$), (2) exercise ($n = 26$), and (3) yoga ($n = 21$). Those in the yoga group showed significant improvements in quality of life and physical measures compared with those randomized to the exercise or wait-list control group.

Mindfulness-Based Intervention

There has been one randomized study evaluating a mindfulness-based intervention (MBI) in MS.[139] One hundred and fifty subjects were randomized to an MBI ($n = 76$) or usual care ($n = 74$), with an intervention period of 8 weeks and a 6-month postintervention follow-up. Subjects randomized to MBI underwent training that included a 2½ hour session, once a week, for 8 weeks and one 7-hour session on Saturdays (Jon Kabat-Zinn's Mindfulness-Based Stress Reduction Program).[140] Subjects receiving usual care were offered MBI training after completing outcome assessments at the time equivalent of the MBI intervention and at 6 months postintervention. Compared with the usual-care group, subjects randomized to MBI showed significant improvements in quality of life, fatigue, anxiety, and depression. Mind–body interventions show promise of benefit in MS and offer a nonpharmacological therapy that can be effective in reducing stress, improving fatigue, and improving quality of life in MS.

THERAPEUTIC APPROACH

We believe that a natural medicine approach to MS management should be personalized for each individual, including dietary modification, nutritional supplementation, incorporation of exercise, and stress reduction techniques, complementary to the conventional medical approach including the use of DMT. Although the data supporting the benefits of each of these individual complementary therapies in MS management are limited, natural medicine approaches when used in conjunction with the approved MS DMTs have the potential to improve the general health of patients and may provide specific benefit in helping control the disease and improve symptoms.

Diet

Although there is no one diet for MS that has a proven efficacy, a few diets, such as the Swank diet (low saturated fat, with 15 g/day or less of saturated fat intake; unsaturated fat intake of a minimum of 20 g/day and maximum of 50 g/day; other details as described earlier), McDougall diet (very low-fat, strictly plant-based diet primarily based on starch, with ~10% of calories to be derived from fat, 14% from protein, and 76% from carbohydrates), and modified Paleo diet (nondomesticated, lean meats and plant-based foods except fruits, nuts, roots, and legumes; three cups each of green leafy, sulfur-rich, and intensely colored vegetables daily, two tablespoons of O3FAs, 4 oz or more each of animal and plant protein to be consumed daily) have some limited evidence of benefit with fatigue, quality of life, and possibly mortality (long-term Swank diet studies) in MS. The common theme of all of these diets is that patients with MS should limit processed foods and consume fresh, high-quality, whole foods. The key difference between the McDougall diet and the modified Paleo diet is the consumption of animal food, which is an area of unresolved controversy.

Nutritional Supplements

Various oral supplements have been studied in MS, including fish oils, vitamin C, vitamin D, biotin, lipoic acid, and *G. biloba*. Except for the stronger data showing possible beneficial effects of vitamin D supplementation, other supplements have not yet shown convincing benefit in MS management. Biotin supplementation remains currently under investigation, and because of its potential to cause interference with certain blood-based laboratory tests, careful use in clinical practice is warranted. An antioxidant, lipoic acid has convincing potential to decrease the rate of brain-volume loss in those with SPMS, and hence its supplementation may be considered. Our recommendation for vitamin D_3 and lipoic acid supplementation is as follows:

- Vitamin D_3: 2000 to 8000 Units/day with the goal of achieving blood levels at an ideal range of 40 to 60 ng/mL 25-hydroxy D3 (supplementing with vitamin D requires attention to vitamins A and K2).
- Consider lipoic acid 1200 mg daily.

Exercise

The type and amount of exercise should be tailored to the patient. Mild to moderate exercise for at least 30 minutes three times a week is recommended for most people with MS. Types of exercise recommended for MS can include walking, stretching, bicycling, low-impact aerobics, stationary cycling, swimming or water aerobics, yoga, and tai chi. Strategies to prevent overheating can include the use of air-conditioning and a cooling vest that can prevent temporary worsening of MS symptoms.

Stress Reduction

As with exercise, the therapies used for stress should also be tailored to the individual person with MS. Broadly, key stress-reduction therapies recommended for MS are yoga, exercise, meditation, deep breathing or breathing exercises, and prayer.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. *N Engl J Med*. 2018;378:169–180.
2. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National multiple sclerosis society (USA) advisory committee on clinical trials of new agents in multiple sclerosis. *Neurology*. 1996;46:907–911.
3. Frank JA, Stone LA, Smith ME, et al. Serial contrast-enhanced magnetic resonance imaging in patients with early relapsing-remitting multiple sclerosis: implications for treatment trials. *Annals Neurol*. 1994;36 (suppl):S86–S90.
4. Debouverie M, Louis S, Pittion-Vouyovitch S, et al. Multiple sclerosis with a progressive course from onset in Lorraine-Eastern France. *J Neurol*. 2007;254:1370–1375.
5. Andersson PB, Waubant E, Gee L, Goodkin DE. Multiple sclerosis that is progressive from the time of onset: clinical characteristics and progression of disability. *Archiv Neurol*. 1999;56:1138–1142.
6. Tremlett H, Zhao Y. Rieckmann P, Hutchinson M. New perspectives in the natural history of multiple sclerosis. *Neurology*. 2010;74(24):2004–2015.
7. Weinshenker BG. Epidemiology of multiple sclerosis. *Neurol Clin*. 1996;14:291–308.
8. Hogancamp WE, Rodriguez M, Weinshenker BG. The epidemiology of multiple sclerosis. *Mayo Clin Proc*. 1997;72:871–878.
9. Ebers GC, Sadovnick AD. The geographic distribution of multiple sclerosis: a review. *Neuroepidemiology*. 1993;12:1–5.
10. Sadovnick AD, Ebers GC. Epidemiology of multiple sclerosis: a critical overview. *Can J Neurol Sci*. 1993;20:17–29.
11. Ellison GW, Visscher BR, Graves MC, Fahey JL. Multiple sclerosis. *Ann Intern Med*. 1984;101:514–526.
12. Love S. Demyelinating diseases. *Journal of Clinical Pathology*. 2006;59(11):1151–1159.
13. Sharief MK, Hentges R. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. *N Engl J Med*. 1991;325:467–472.
14. Trotter JL, Collins KG, van der Veen RC. Serum cytokine levels in chronic progressive multiple sclerosis: interleukin-2 levels parallel tumor necrosis factor-alpha levels. *J Neuroimmunol*. 1991;33:29–36.
15. Hartung HP, Archelos JJ, Zielasek J, et al. Circulating adhesion molecules and inflammatory mediators in demyelination: a review. *Neurology*. 1995;45:S22–S32.
16. Hauser SL, Doolittle TH, Lincoln R, et al. Cytokine accumulations in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. *Neurology*. 1990;40:1735–1739.
17. Rudick RA, Ransohoff RM. Cytokine secretion by multiple sclerosis monocytes. Relationship to disease activity. *Archiv Neurol*. 1992;49:265–270.
18. Hofman FM, Hinton DR, Johnson K, Merrill JE. Tumor necrosis factor identified in multiple sclerosis brain. *J Exp Med*. 1989;170:607–612.
19. Confavreux C, O'Connor P, Comi G, et al. Oral teriflunomide for patients with relapsing multiple sclerosis (TOWER): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol*. 2014;13: 247–256.
20. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol*. 1998;16:137–161.
21. Leppert D, Waubant E, Galardy R, et al. T cell gelatinases mediate basement membrane transmigration in vitro. *J Immunol*. 1995;154: 4379–4389.
22. Leppert D, Lindberg RL, Kappos L, Leib SL. Matrix metalloproteinases: multifunctional effectors of inflammation in multiple sclerosis and bacterial meningitis. *Brain Res Brain Res Rev*. 2001;36:249–257.
23. Trojano M, Avolio C, Liuzzi GM, et al. Changes of serum sICAM-1 and MMP-9 induced by rIFNbeta-1b treatment in relapsing-remitting MS. *Neurology*. 1999;53:1402–1408.
24. Waubant E, Goodkin DE, Gee L, et al. Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. *Neurology*. 1999;53:1397–1401.
25. Lee MA, Palace J, Stabler G, et al. Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis. A longitudinal clinical and MRI study. *Brain J Neurol*. 1999;122(Pt 2):191–197.
26. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. *Brain Pathol*. 2007;17:210–218.
27. Dyment DA, Sadovnick AD, Ebers GC. Genetics of multiple sclerosis. *Hum Mol Genet*. 1997;6:1693–1698.
28. Sadovnick AD, Dyment D, Ebers GC. Genetic epidemiology of multiple sclerosis. *Epidemiol Rev*. 1997;19:99–106.
29. James WH. Review of the contribution of twin studies in the search for non-genetic causes of multiple sclerosis. *Neuroepidemiology*. 1996;15:132–141.
30. Baranzini SE, Oksenberg JR. The genetics of multiple sclerosis: from 0 to 200 in 50 years. *Trends Genet*. 2017;33:960–970.
31. Didonna A, Oksenberg JR. The genetics of multiple sclerosis. In: Zagon IS, McLaughlin PJ, eds. *Multiple Sclerosis: Perspectives in Treatment and Pathogenesis*. Brisbane, AU: Codon Publications; 2017.
32. Axthelm MK, Bourdette DN, Marracci GH, et al. Japanese macaque encephalomyelitis: a spontaneous multiple sclerosis-like disease in a nonhuman primate. *Ann Neurol*. 2011;70:362–373.
33. Gilden DH, Devlin ME, Burgoon MP, Owens GP. The search for virus in multiple sclerosis brain. *Mult Scler*. 1996;2:179–183.
34. Gale CR, Martyn CN. Migrant studies in multiple sclerosis. *Prog Neurobiol*. 1995;47:425–448.
35. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: noninfectious factors. *Ann Neurol*. 2007;61:504–513.
36. Salemi G, Ragonese P, Aridon P, et al. Is season of birth associated with multiple sclerosis? *Acta Neurol Scand*. 2000;101:381–383.
37. Fernandes de Abreu DA, Babron MC, Rebeix I, et al. Season of birth and not vitamin D receptor promoter polymorphisms is a risk factor for multiple sclerosis. *Mult Scler*. 2009;15:1146–1152.
38. Templer DI, Trent NH, Spencer DA, et al. Season of birth in multiple sclerosis. *Acta Neurol Scand*. 1992;85:107–109.
39. Swank RL, Lerstad O, Strom A, Backer J. Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. *N Engl J Med*. 1952;246:722–728.
40. Lauer K. Diet and multiple sclerosis. *Neurology*. 1997;49:S55–S61.
41. Altowaijri G, Fryman A, Yadav V. Dietary interventions and multiple sclerosis. *Curr Neurol Neurosci Rep*. 2017;17:28.
42. Zhang SM, Willett WC, Hernan MA, et al. Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women. *Am J Epidemiol*. 2000;152:1056–1064.
43. Zhang SM, Hernan MA, Olek MJ, et al. Intakes of carotenoids, vitamin C, and vitamin E and MS risk among two large cohorts of women. *Neurology*. 2001;57:75–80.
44. Ghadirian P, Jain M, Ducic S, et al. Nutritional factors in the aetiology of multiple sclerosis: a case-control study in Montreal, Canada. *Int J Epidemiol*. 1998;27:845–852.
45. Bull MJ, Plummer NT. Part 1: The human gut microbiome in health and disease. *Integr Med (Encinitas)*. 2014;13:17–22.
46. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol*. 2012;10:735–742.
47. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci*. 2013;36:305–312.
48. Haberman Y, Tickle TL, Dexheimer PJ, et al. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *J Clin Invest*. 2014;124:3617–3633.
49. Dunne JL, Triplett EW, Gevers D, et al. The intestinal microbiome in type 1 diabetes. *Clin Exp Immunol*. 2014;177:30–37.
50. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2011;108(suppl 1): 4615–4622.

51. Tremlett H, Fadrosh DW, Faruqi AA, et al. Gut microbiota composition and relapse risk in pediatric MS: a pilot study. *J Neurol Sci*. 2016;363:153–157.
52. Tremlett H, Fadrosh DW, Faruqi AA, et al. Associations between the gut microbiota and host immune markers in pediatric multiple sclerosis and controls. *BMC Neurol*. 2016;16:182.
53. Jangi S, Gandhi R, Cox LM, et al. Alterations of the human gut microbiome in multiple sclerosis. *Nat Commun*. 2016;7:12015.
54. Cekanaviciute E, Yoo BB, Runia TF, et al. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci U S A*. 2017;114:10713–10718.
55. Correction for Cekanaviciute, Yoo BB, Runia TF, et al. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci U S A*. 2017;114:E8943.
56. Berer K, Gerdes LA, Cekanaviciute E, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci U S A*. 2017;114:10719–10724.
57. Turnbaugh PJ, Ridaura VK, Faith JJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med*. 2009;1:6ra14.
58. Zhang M, Chekan JR, Dodd D, et al. Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proc Natl Acad Sci U S A*. 2014;111:E3708–E3717.
59. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A*. 2010;107:14691–14696.
60. Nguyen LT, Ramanathan M, Weinstock-Guttman B, et al. Sex differences in in vitro pro-inflammatory cytokine production from peripheral blood of multiple sclerosis patients. *J Neurol Sci*. 2003;209(1-2):93–99. PubMed PMID: 12686409.
61. Skaper SD, Facci L, Giusti P. Neuroinflammation, microglia and mast cells in the pathophysiology of neurocognitive disorders: a review. *CNS Neurol Disord Drug Targets*. 2014;13(10):1654–1666. PubMed PMID: 25470401.
62. Crinnion WJ, Pizzorno JE. *Clinical Environmental Medicine: Identification and Natural Treatment of Diseases Caused by Common pollutants*. St. Louis, Missouri: Elsevier; 2019.
63. Graves JS, Chitnis T, Weinstock-Guttman B, et al. Network of pediatric multiple sclerosis centers. Maternal and perinatal exposures are associated with risk for pediatric-onset multiple sclerosis. *Pediatrics*. 2017;139(4):e20162838. PubMed PMID: 28562303.
64. Oddone E, Scaburri A, Modonesi C, et al. Multiple sclerosis and occupational exposures: results of an explorative study. *G Ital Med Lav Ergon*. 2013;35(3):133–137. PubMed PMID: 24734319.
65. Solomon AJ, Bourdette DN, Cross AH, et al. The contemporary spectrum of multiple sclerosis misdiagnosis: a multicenter study. *Neurology*. 2016;87:1393–1399.
66. Dhib-Jalbut S, Marks S. Interferon-beta mechanisms of action in multiple sclerosis. *Neurology*. 2010;74(suppl 1):S17–S24.
67. Dhib-Jalbut S. Glatiramer acetate (Copaxone) therapy for multiple sclerosis. *Pharmacol Ther*. 2003;98:245–255.
68. O'Connor P, Comi G, Montalban X, et al. Oral fingolimod (FTY720) in multiple sclerosis: two-year results of a phase II extension study. *Neurology*. 2009;72:73–79.
69. Kappos L, Radue EW, O'Connor P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med*. 2010;362:387–401.
70. Kappos L, De Stefano N, Freedman MS, et al. Inclusion of brain volume loss in a revised measure of "no evidence of disease activity" (NEDA-4) in relapsing-remitting multiple sclerosis. *Mult Scler*. 2016;22:1297–1305.
71. Gold R, Giovannoni G, Phillips JT, et al. Efficacy and safety of delayed-release dimethyl fumarate in patients newly diagnosed with relapsing-remitting multiple sclerosis (RRMS). *Mult Scler*. 2015;21:57–66.
72. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*. 2006;354:899–910.
73. Miller DH, Soon D, Fernando KT, et al. MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. *Neurology*. 2007;68:1390–1401.
74. McGuire VA, Ruiz-Zorrilla Diez T, Emmerich CH, et al. Dimethyl fumarate blocks pro-inflammatory cytokine production via inhibition of TLR induced M1 and K63 ubiquitin chain formation. *Sci Rep*. 2016;6:31159.
75. Kinkel R. Methylprednisolone. In: Rudick R, Goodkin D, eds. *Multiple Sclerosis Therapeutics*. London: Martin Dunitz; 1999:349–370.
76. Swank RL. Multiple sclerosis: twenty years on low fat diet. *Archiv Neurol*. 1970;23:460–474.
77. Swank RL, Dugan BB. Effect of low saturated fat diet in early and late cases of multiple sclerosis. *Lancet*. 1990;336:37–39.
78. Youdim KA, Martin A, Joseph JA. Essential fatty acids and the brain: possible health implications. *Int J Dev Neurosci*. 2000;18:383–399.
79. Nordvik I, Myhr KM, Nyland H, Bjerve KS. Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients. *Acta Neurol Scand*. 2000;102:143–149.
80. Weinstock-Guttman B, Baier M, Park Y, et al. Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients. *Prostaglandins Leukot Essent Fatty Acids*. 2005;73:397–404.
81. Choi IY, Piccio L, Childress P, et al. A diet mimicking fasting promotes regeneration and reduces autoimmunity and multiple sclerosis symptoms. *Cell Rep*. 2016;15:2136–2146.
82. Bock M, Michalsen A, Paul F. Ketogenic diet and prolonged fasting improve health related quality of life and blood lipid profile in multiple sclerosis—a randomized controlled trial. Paper presented at ECTRIMS 2015, Barcelona, Spain, 2015.
83. Shinto L, Marracci G, Baldauf-Wagner S, et al. Omega-3 fatty acid supplementation decreases matrix metalloproteinase-9 production in relapsing-remitting multiple sclerosis. *Prostaglandins Leukot Essent Fatty Acids*. 2009;80:131–136.
84. Gallai V, Sarchielli P, Trequattrini A, et al. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. *J Neuroimmunol*. 1995;56:143–153.
85. Bates D, Cartlidge NE, French JM, et al. A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 1989;52:18–22.
86. Meade CJ, Mertin J, Sheena J, Hunt R. Reduction by linoleic acid of the severity of experimental allergic encephalomyelitis in the guinea pig. *J Neurol Sci*. 1978;35:291–308.
87. Harbige LS, Layward L, Morris-Downes MM, et al. The protective effects of omega-6 fatty acids in experimental autoimmune encephalomyelitis (EAE) in relation to transforming growth factor-beta 1 (TGF-beta1) up-regulation and increased prostaglandin E2 (PGE2) production. *Clin Exp Immunol*. 2000;122:445–452.
88. Bates D, Fawcett PR, Shaw DA, Weightman D. Polyunsaturated fatty acids in treatment of acute remitting multiple sclerosis. *Br Med J*. 1978;2:1390–1391.
89. Millar JH, Zilkha KJ, Langman MJ, et al. Double-blind trial of linoleate supplementation of the diet in multiple sclerosis. *Br Med J*. 1973;1:765–768.
90. Paty DW, Cousin HK, Read S, Adlakha K. Linoleic acid in multiple sclerosis: failure to show any therapeutic benefit. *Acta Neurol Scand*. 1978;58:53–58.
91. Horrobin DF. Multiple sclerosis: the rational basis for treatment with colchicine and evening primrose oil. *Med Hypotheses*. 1979;5:365–378.
92. Farinotti M, Vacchi L, Simi S, et al. Dietary interventions for multiple sclerosis. *Cochrane Database Syst Rev*. 2012;12:CD004192.
93. Munger KL, Levin LI, Hollis BW, et al. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA*. 2006;296:2832–2838.
94. Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology*. 2004;62:60–65.

95. Hiremath GS, Cettomai D, Baynes M, et al. Vitamin D status and effect of low-dose cholecalciferol and high-dose ergocalciferol supplementation in multiple sclerosis. *Mult Scler*. 2009;15:735–740.
96. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest*. 1991;87:1103–1107.
97. Cantorna MT, Humpal-Winter J, DeLuca HF. Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. *J Nutr*. 1999;129:1966–1971.
98. Nashold FE, Miller DJ, Hayes CE. 1,25-dihydroxyvitamin D3 treatment decreases macrophage accumulation in the CNS of mice with experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2000;103:171–179.
99. Wingerchuk DM, Lesaux J, Rice GP, et al. A pilot study of oral calcitriol (1,25-dihydroxyvitamin D3) for relapsing-remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2005;76:1294–1296.
100. Smolders J, Thewissen M, Peelen E, et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. *PLoS One*. 2009;4:e6635.
101. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
102. Packer L, Roy S, Sen CK. Alpha-lipoic acid: a metabolic antioxidant and potential redox modulator of transcription. *Adv Pharmacol*. 1997;38:79–101.
103. Moini H, Packer L, Saris NE. Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoic acid. *Toxicol Appl Pharmacol*. 2002;182:84–90.
104. Hagen TM, Liu J, Lykkesfeldt J, et al. Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc Natl Acad Sci U S A*. 2002;99:1870–1875.
105. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol*. 1997;29:315–331.
106. Marracci GH, Jones RE, McKeon GP, Bourdette DN. Alpha lipoic acid inhibits T cell migration into the spinal cord and suppresses and treats experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2002;131:104–114.
107. Morini M, Roccatagliata L, Dell'Eva R, et al. Alpha-lipoic acid is effective in prevention and treatment of experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2004;148:146–153.
108. Schreibelt G, Musters RJ, Reijerkerk A, et al. Lipoic acid affects cellular migration into the central nervous system and stabilizes blood-brain barrier integrity. *J Immunol*. 2006;177:2630–2637.
109. Marracci GH, McKeon GP, Marquardt WE, et al. Alpha lipoic acid inhibits human T-cell migration: implications for multiple sclerosis. *J Neurosci Res*. 2004;78:362–370.
110. Yadav V, Marracci G, Lovera J, et al. Lipoic acid in multiple sclerosis: a pilot study. *Mult Scler*. 2005;11:159–165.
111. Spain R, Powers K, Murchison C, et al. Lipoic acid in secondary progressive MS: a randomized controlled pilot trial. *Neurol Neuroimmunol Neuroinflamm*. 2017;4:e374.
112. Sedel F, Papeix C, Bellanger A, et al. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. *Mult Scler Relat Disord*. 2015;4:159–169.
113. Tourbah A, Lebrun-Frenay C, Edan G, et al. MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: a randomised, double-blind, placebo-controlled study. *Mult Scler*. 2016;22:1719–1731.
114. Boyle AM. High-dose biotin increases inflammation and relapse in PPMS. Paper presented at European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) 2017, 2017.
115. U.S. Food and Drug Administration. The FDA warns that biotin may interfere with lab tests: FDA safety communication. https://www.fda.gov/medical-devices/safety-communications/fda-warns-biotin-may-interfere-lab-tests-fda-safety-communication. Published November 28, 2017. Accessed 28 Dec 2018.
116. Minkovsky A, Lee MN, Dowlatshahi M, et al. High-dose biotin treatment for secondary progressive multiple sclerosis may interfere with thyroid assays. *AACE Clin Case Rep*. 2016;2:e370–e373.
117. Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurology*. 1991;41:685–691.
118. Amato MP, Ponziani G, Siracusa G, Sorbi S. Cognitive dysfunction in early-onset multiple sclerosis: a reappraisal after 10 years. *Archiv Neurol*. 2001;58:1602–1606.
119. Birks J, Grimley Evans J. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database Syst Rev*. 2009:CD003120.
120. Lovera J, Bagert B, Smoot K, et al. Ginkgo biloba for the improvement of cognitive performance in multiple sclerosis: a randomized, placebo-controlled trial. *Mult Scler*. 2007;13:376–385.
121. Lakhan SE, Rowland M. Whole plant cannabis extracts in the treatment of spasticity in multiple sclerosis: a systematic review. *BMC Neurol*. 2009;9:59.
122. Petajan JH, White AT. Recommendations for physical activity in patients with multiple sclerosis. *Sports Med*. 1999;27:179–191.
123. Sutherland G, Andersen MB. Exercise and multiple sclerosis: physiological, psychological, and quality of life issues. *J Sports Med Phys Fitness*. 2001;41:421–432.
124. Mostert S, Kesselring J. Effects of a short-term exercise training program on aerobic fitness, fatigue, health perception and activity level of subjects with multiple sclerosis. *Mult Scler*. 2002;8:161–168.
125. Mills N, Allen J. Mindfulness of movement as a coping strategy in multiple sclerosis. A pilot study. *Gen Hosp Psychiatry*. 2000;22:425–431.
126. Husted C, Pham L, Hekking A, Niederman R. Improving quality of life for people with chronic conditions: the example of t'ai chi and multiple sclerosis. *Altern Ther Health Med*. 1999;5:70–74.
127. Di Fabio RP, Soderberg J, Choi T, et al. Extended outpatient rehabilitation: its influence on symptom frequency, fatigue, and functional status for persons with progressive multiple sclerosis. *Arch Phys Med Rehabil*. 1998;79:141–146.
128. Gehlsen GM, Grigsby SA, Winant DM. Effects of an aquatic fitness program on the muscular strength and endurance of patients with multiple sclerosis. *Phys Ther*. 1984;64:653–657.
129. Snook EM, Motl RW. Effect of exercise training on walking mobility in multiple sclerosis: a meta-analysis. *Neurorehabil Neural Repair*. 2009;23:108–116.
130. Motl RW, Gosney JL. Effect of exercise training on quality of life in multiple sclerosis: a meta-analysis. *Mult Scler*. 2008;14:129–135.
131. Sandroff BM, Motl RW, Scudder MR, DeLuca J. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. *Neuropsychol Rev*. 2016;26:271–294.
132. Ruegsegger GN, Booth FW. Health Benefits of Exercise. *Cold Spring Harbor Perspectives in Medicine*. 2018;8(7):a029694. https://doi.org/10.1101/cshperspect.a029694.
133. Goodin DS, Ebers GC, Johnson KP, et al. The relationship of MS to physical trauma and psychological stress: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*. 1999;52:1737–1745.
134. Mohr DC, Goodkin DE, Bacchetti P, et al. Psychological stress and the subsequent appearance of new brain MRI lesions in MS. *Neurology*. 2000;55:55–61.
135. Mohr DC. Stress and multiple sclerosis. *J Neurol*. 2007;254(suppl 2):II65–II68.
136. Wahbeh H, Elsas SM, Oken BS. Mind-body interventions: applications in neurology. *Neurology*. 2008;70:2321–2328.
137. Smith JE, Richardson J, Hoffman C, Pilkington K. Mindfulness-based stress reduction as supportive therapy in cancer care: systematic review. *J Adv Nurs*. 2005;52:315–327.
138. Oken BS, Kishiyama S, Zajdel D, et al. Randomized controlled trial of yoga and exercise in multiple sclerosis. *Neurology*. 2004;62:2058–2064.
139. Grossman I, Miller A. Multiple sclerosis pharmacogenetics: personalized approach towards tailored therapeutics. *EPMA J*. 2010;1:317–327.
140. Kabat-Zinn J. *Full Catastrophe Living: Using the Wisdom of Body and Mind to Face Stress, Pain, and Illness*. New York: Bantam; 1990.

Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis[91]

John Nowicki, ND, and *Joseph E. Pizzorno, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Somewhat enlarged liver on examination
- Possible presentation of fatigue
- Liver enzymes may be elevated (especially alanine transaminase [ALT]), but not reliable
- Liver ultrasound
- Biopsy to confirm diagnosis

GENERAL CONSIDERATIONS

The liver metabolizes many potentially harmful environmental contaminants and facilitates the excretion of these contaminants from the body. Eighty percent of the liver is made up of hepatocytes, which play a critical role in the metabolism of amino acid and ammonia, biochemical oxidation reactions, and detoxification of a variety of drugs, vitamins, hormones, and environmental toxicants. Kupffer cells constitute most of the tissue macrophages present in the body and play a protective role against gut-derived bacterial endotoxins and microbial debris. Upon activation, Kupffer cells release cytokines, prostanoids, nitric oxide, and reactive oxygen species (ROS), which cause inflammation and can influence the noxiousness of environmental toxicants.[1]

Hepatotoxicity is the most common organ injury due to occupational and environmental chemical exposures. Variations in genetics, dietary factors, and nutrient cofactors all affect an individual's ability to metabolize chemicals effectively and efficiently. A variety of toxins and toxicants can cause liver dysfunction because of the central role this organ plays in xenobiotic metabolism. Industrial toxicants and drugs have been associated with the development of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).[2]

NAFLD is characterized by fatty infiltration of the liver, primarily by triglycerides, and insulin resistance in the absence of chronic alcohol consumption. NAFLD has been described as the hepatic component of metabolic syndrome.[3] The diagnosis of NASH is established by the presence of lobular inflammation and cell injury (and in some cases progressive fibrosis) in addition to hepatocellular fat accumulation.[4] Pathophysiology involves insulin resistance, which causes steatosis, and oxidative stress, which produces lipid peroxidation and activates inflammatory cytokines, resulting in NASH.[5]

Using ultrasonographic data from National Health and Nutritional Examination Survey (NHANES) III, prevalence estimates found the age-adjusted incidence of hepatic steatosis and NAFLD in the U.S. population to be 21.4% and 19.0%, respectively.[6] This equates to approximately 32.5 million adults with hepatic steatosis and 28.8 million adults with NAFLD. More recent analysis has found the prevalence of NAFLD to be 30.0% nationwide, making NAFLD the most common chronic liver disease in the United States.[7] Defining fatty liver as a fatty liver index score of ≥30 shows that the prevalence of NAFLD has increased from 18% in 1988 to 1991, to 29% in 1999 to 2000, to 31% in 2011 to 2012.[8] The prevalence of NAFLD increases significantly to 80% to 90% in obese adults, 90% in patients with hyperlipidemia, and 30% to 50% in patients with diabetes and parallels the prevalence of metabolic syndrome, insulin resistance, type 2 diabetes, and central obesity.[9,10] Having metabolic syndrome is an independent risk factor for NAFLD, and those with metabolic syndrome have a 2.37-fold risk of NAFLD compared with those without metabolic syndrome.[11] Alarmingly, NAFLD is emerging as a common pediatric disease, affecting approximately 3% to 9% of all children in the United States and up to 50% of obese children.[12]

TABLE 200.1 Prevalence of NAFLD in the United States, NHANES III, 1988 to 1994

Sex by Age	Non-Hispanic White	Non-Hispanic Black	Mexican American	Total
	Prevalence	Prevalence	Prevalence	Prevalence
Men				
<30 years	8.3	10.9	15.6	9.9
30–40 years	15.9	12.5	25.7	16.1
40–50 years	22.2	17.1	36.2	22.3
50–60 years	28.0	17.4	41.4	29.3
≥60 years	28.1	22.6	33.4	27.6
Women				
<30 years	9.5	12.0	16.5	10.6
30–40 years	11.1	10.4	23.2	12.5
40–50 years	15.0	14.3	34.7	16.1
50–60 years	20.5	21.9	35.7	21.6
≥60 years	25.7	23.9	34.4	25.4

NAFLD, Nonalcoholic fatty liver disease; NHANES III, National Health and Nutritional Examination Survey.
From Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: The Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Epidemiol.* 2013;*178*(1):
38–45.

The prevalence of NASH has been difficult to establish because a liver biopsy is required for diagnosis. In 1990 an autopsy-based cross-sectional study reported the overall prevalence of NASH in adults in North America to be 18.5% in obese and 2.7% in nonobese individuals.[13] Since that time, the rates of diabetes and obesity have steadily increased, along with the quantity and concentration of toxic compounds in the environment.[14–16] More recent evidence estimates the prevalence of NASH to be 12.2%, and among patients with a positive ultrasound for fatty liver, the prevalence of NASH increases to 29.9%.[17]

NAFLD and NASH disproportionally affect Mexican Americans, men, older adults, and people with diabetes and obesity (Table 200.1). However, when Mexican American and Caucasian subjects are well matched for clinical parameters, particularly for the degree of obesity, there are no significant differences in the severity of insulin resistance or steatohepatitis.[18] Aging of the population and the increasing prevalence of diabetes and obesity are expected to contribute to an increase in the overall burden of liver disease in the United States. Although liver-related deaths are increased by NAFLD, cardiovascular mortality is the leading cause of death,[19] and NAFLD is an independent risk factor for cardiovascular disease.[20]

TOXICITY—MECHANISMS OF ACTION

Excess intracellular fatty acids (FAs), oxidative stress, insulin resistance with a decreased adiponectin-leptin ratio, adenosine triphosphate (ATP) depletion, metabolic endotoxemia, and mitochondrial dysfunction are all important causes of hepatocellular injury in the steatotic liver.[21] It is likely these factors are not mutually exclusive and act in a more coordinated manner. For example, mitochondrial structural defects lead to increased FA beta-oxidation, leading to abnormal cytokine production and insulin resistance, which are crucial pathophysiological factors in NASH.[22,23] Interleukin (IL)-6 levels are increased in NAFLD but are significantly higher in those with steatohepatitis as opposed to simple fatty liver.[24] Accumulation of intrahepatic FAs can promote the formation of reactive oxygen intermediates, which in turn can impair liver function directly or indirectly by perpetuating the inflammatory response.[25] Serum-free FAs have direct hepatotoxicity through the induction of an endoplasmic reticulum stress response and subsequent activation of the mitochondrial pathway of cell death.[26]

NAFLD is associated with decreased cellular glutathione (GSH), the major endogenous antioxidant produced in the body.[27] In addition, cytochrome P450 2E1 (CYP2E1) has emerged as an important cause of ROS overproduction, and higher hepatic CYP2E1 expression and activity have been observed in the context of obesity and NAFLD.[28] The higher levels of CYP2E1 in NAFLD may aggravate liver injury from xenobiotic compounds through the generation of harmful reactive metabolites.

High-Fructose Corn Syrup

Several studies have shown high-fructose corn syrup (HFCS) to be a contributing factor to energy overconsumption, weight gain, and the rise in the prevalence of obesity.[29–31] The fructose in sugar-sweetened beverages promotes insulin resistance.[32] HFCS also promotes dyslipidemia,[33] increases visceral fat deposits, and increases hepatic *de novo* lipogenesis.[34] In patients with NAFLD, *de novo* synthesis of FAs from glucose and fructose is dysregulated, leading to an increase in plasma free fatty acids (FFAs) and a subsequent increase in the liver triglyceride content.[35] Fructose also provokes a hepatic stress response involving activation of c-Jun N-terminal kinases (JNK) and subsequent reduced hepatic insulin signaling.[36]

In a small-scale study, it was observed that consumption of fructose in 49 patients with NAFLD was two- to threefold higher than in 24 control subjects, and hepatic mRNA expression of fructokinase and FA synthase was increased in patients with NAFLD.[37] Another study showed that 80% of patients (25 out of 31) with NAFLD consumed an excessive amount of soft drinks, totaling more than 50 g of added sugar per day.[38] In patients with NAFLD, fructose consumption was linked with lower hepatic fat content but increased hepatic fibrosis, suggesting that fructose may enhance liver inflammation.[39]

Obesity

The release of FAs from dysfunctional and insulin-resistant adipocytes results in lipotoxicity caused by the accumulation of triglyceride-derived toxic metabolites in the liver.[40] Excess adiposity is associated with increased proinflammatory cytokines, oxidative stress, and an exaggerated inflammatory response to endotoxin administration.[41]

Gut-Derived Endotoxins

Dysfunctional gut microbiota may play a role in the development of NASH and activate inflammation and endoplasmic reticulum stress.[42] Alterations in gut microbiota, increased intestinal permeability, and metabolic endotoxemia create a low-grade inflammatory state that contributes to the development of obesity and associated NAFLD (Fig. 200.1).[43]

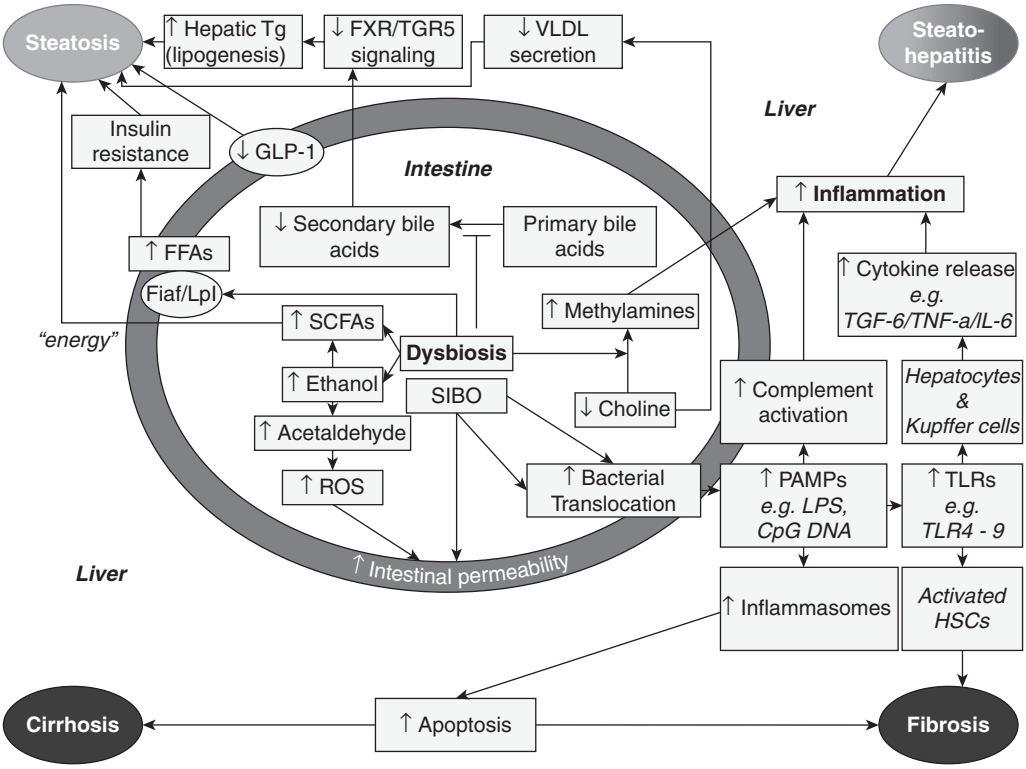


Fig. 200.1 Mechanisms by which gut bacteria affect the hallmarks of nonalcoholic fatty liver disease. *FFAs,* Free fatty acids; *Fiaf,* fasting-induced adipocyte factor; *FXR/TGR5,* farnesoid X receptor/*G* protein-coupled bile acid receptor 1; *GLP-1,* glucagon-like peptide-1; *HSCs,* hepatic stellate cells; *IL-6,* interleukin-6; *Lpl,* lipoprotein lipase; *LPS,* lipopolysaccharide; *PAMPs,* pathogen-associated molecular patterns; *ROS,* reactive oxygen species; *SCFAs,* short-chain fatty acids; *SIBO,* small intestinal bacterial overgrowth; *Tg,* triglyceride; *TGF-β,* transforming growth factor-β; *TLR,* toll-like receptor; *TNF-α,* tumor necrosis factor-α; VLDL, very low-density lipoprotein. (From van Best N, Jansen PL, Rensen SS. The gut microbiota of nonalcoholic fatty liver disease: current methods and their interpretation. *Hepatol Int.* 2015;9[3]:406–415.)

Air Particulate Matter

Toll-like receptor (TLR) activation of Kupffer cells, resident hepatic macrophages, and proinflammatory cytokine production have all been shown to play a role in the progression of NAFLD.[44] Animal studies suggest airborne pollutants may play a role in the pathogenesis of NAFLD. Mice exposed to diesel exhaust particles at 50 μg/kg body weight developed inflammation and oxidative DNA damage in the liver without systemic inflammation.[45] Obese, diabetic mice had increased levels of aspartate transaminase (AST) and ALT, enhanced steatosis, and elevated markers of oxidative stress after pulmonary exposure to diesel exhaust particles.[46] Researchers have postulated that inhaled fine particulate matter (PM) may aggravate NAFLD by crossing the alveolar membranes and entering the circulation, where it accumulates in hepatic Kupffer cells and triggers toll-like receptor (TLR) 4–dependent activation of cytokine release, which leads to inflammation and hepatic stellate cell collagen synthesis.[47]

Chemicals

Chloroalkenes

Vinyl chloride (VC) is metabolized by CYP2E1, forming chloroethylene oxide, a highly reactive genotoxic epoxide.[48] Occupational exposure to VC has been associated with steatohepatitis in lean Brazilian petrochemical workers,[49] and ultrasound studies demonstrated hepatomegaly, steatosis, and fibrosis in VC workers.[50]

A study of 29 dry-cleaner workers exposed to 16 PPM of perchloroethylene (PCE), far less than the permissible exposure limit of 100 PPM in the United States, showed they had fatty infiltration, with mostly normal serum aminotransferase levels.[51]

Volatile Organic Compounds

Exposure to volatile organic compounds (VOCs), such as toluene, benzene, styrene, and xylene, have been associated with NASH with both normal liver enzymes and abnormal liver enzymes.[52] Seventy-five percent of household painters with VOC exposures and abnormal liver enzymes had fatty liver on biopsy,[53] and 100% of toluene-exposed printers with persistent mild liver enzyme elevation had hepatic steatosis.[54]

Persistent Organic Pollutants

Persistent organic pollutants (POPs) are lipophilic in nature and can easily cross the biological membranes and accumulate in fatty tissues. Multiple animal studies show that exposure to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) produces toxic manifestations in the liver, including lipid accumulation, hepatocellular hypertrophy inflammatory cell infiltration, and hyperplasia,[55] as well as an increase in total hepatic FAs, triglycerides, and serum ALT levels.[56] Polychlorinated biphenyl (PCB) 153 exposure causes NAFLD with hepatic antioxidant depletion.[57]

Toxic Metals

The presence of toxic heavy metals (arsenic, mercury, cadmium, chromium, copper, nickel, lead, and zinc) has been shown to be a significant risk factor for fatty liver disease in men (odds ratio [OR] 1.83, 95% confidence interval [CI]; 1.161–2.899, $p = 0.009$), especially with a body mass index (BMI) of less than 24 kg/m^2.[58]

Cadmium

Low urinary levels of cadmium (0.65–0.83 ug/g) are associated with NAFLD (OR 2.21) and NASH (OR 1.30).[59]

Mercury

Elevations of serum ALT, indicating NAFLD, are associated with blood mercury levels. In the NHANES 2003 to 2004 cohort, individuals with blood mercury levels in the second quartile (25th to 50th percentile) were twice as likely to have elevated ALT.[60] Data collected from the Korea National Health and Nutrition Examination Survey (KNHANES) showed that increasing blood mercury levels were associated with increases in AST, ALT, and elevated gamma-glutamyl transferase (GGTP; >56 IU/L).[61,62] The primary mechanism of mercury hepatotoxicity may be related to the poisoning of cysteine-containing proteins and GSH depletion.[63]

DIAGNOSTIC CONSIDERATIONS

The gold standard for diagnosing and staging NAFLD is histology via biopsy of the liver.[64] However, the risks and costs of liver biopsy have prompted researchers to seek noninvasive methods to diagnose and stage NAFLD. Cytokeratin CK-18 is a serum marker of NASH that has been the most validated. CK-18 fragments stem from apoptosis of hepatocytes and can be measured in plasma. The utility of CK-18 fragments has been validated in a multicenter study, which demonstrated that for every 50-U/L increase in the plasma level of CK-18, the likelihood of having NASH increased by 74% (OR: 1.74; 95% CI, 1.31–2.31).[65]

Conventional biomarkers of hepatotoxicity include serum ALT, AST, alkaline phosphatase (ALP), total bilirubin, gamma-glutamyltransferase (GGT), and albumin. Research indicates that several liver enzymes increase in proportionate response to the load of specific classes of toxins.

Alkaline Phosphatase

ALP is a hydrolase enzyme responsible for dephosphorylation. It is present in higher concentration in the liver, kidney, and bone. Chronic exposure of pesticides in agricultural workers was found to be associated with significantly higher activities of ALP compared with controls, and the number of years exposed to pesticides predicted higher activities of ALP.[66]

Bilirubin

Bilirubin levels increase in proportion to the level of various PCBs, which is significant because bilirubin is considered the best prognostic measure of chronic liver dysfunction.[67,68] Direct bilirubin is inversely associated with NAFLD, with a significant dose-response relationship ($p = <0.05$), and serves as a protective biomarker, likely based on the endogenous antioxidant and cytoprotectant properties of bilirubin.[69]

Transaminase Enzymes—ALT and AST

ALT is a transaminase enzyme that catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate. For men aged 18 to 20, ALT values >37 IU/L are considered elevated, whereas the cutoff for men over the age of 21 is >48 IU/L. For women aged 18 to 20, ALT values >30 IU/L are considered elevated, and ALT values >31 IU/L are considered elevated for women over the age of 21.

Unexplained elevations in ALT level have been used to signify the presence of NAFLD. Data from the NHANES III suggests that the prevalence of unexplained elevations in ALT level is 7% in individuals with metabolic syndrome and 3.5% in those without metabolic syndrome.[70] However, many patients with NAFLD have normal ALT levels, and the data likely underestimate the actual frequency of NAFLD.[71]

ALT increases in a dose-dependent manner with the body load of blood cadmium, lead, mercury, and PCBs within and above the normal range.[72] Exposure to polycyclic aromatic hydrocarbons causes elevations in AST and ALT.[73] When serum log-perfluorooctanoic acid (PFOA; a perfluorinated chemical) increases by 1 unit, serum ALT increases by 1.86 units (95% CI, 1.24–2.48; $p = 0.005$).[74]

The AST level may occasionally be higher than the ALT level, especially in the presence of cirrhosis, but the AST/ALT ratio is rarely greater than 2.[75] Among patients who have NAFLD without advanced fibrosis, the AST/ALT ratio is typically less than 1, but it tends to reverse as the degree of fibrosis progresses to cirrhosis.[76]

Gamma-Glutamyltransferase

Elevations of GGT directly correlate with alcohol consumption and toxic metal load (cadmium and lead).[77,78] Serum GGT, within its reference range, is also associated with organochlorine pesticides and polycyclic aromatic hydrocarbons.[79] GGT elevates by exposure to other chemicals, especially POPs and several prescription drugs. Workers with a history of alcohol consumption and high exposure to TCDD were found to have a statistically significant elevated risk for out-of-range GGT compared with referents.[80] GGT has been shown to be a surrogate marker of NAFLD and may be a simple and reliable marker of visceral and hepatic fat deposition and hepatic steatosis.[81,82] GGT shows a significant positive association with Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) in subjects with NAFLD, even after waist circumference and hemoglobin (Hb) A_{1c} are adjusted.[83]

THERAPEUTIC CONSIDERATIONS

Managing comorbidities—obesity, diabetes, and hyperlipidemia—with diet and lifestyle modifications is at the forefront of treatment. Weight loss improves liver aminotransferase levels and hepatic steatosis in proportion to the total amount of weight loss. Thirty-one obese subjects were randomized to 48 weeks of lifestyle intervention (200 minutes a week of moderate physical activity) versus standard dietary counseling alone. Participants who lost ≥7% of weight compared with those who lost <7% had significant improvements in steatosis, lobular inflammation, and ballooning injury.[84] The addition of flaxseed oil may enhance these lifestyle modifications.[85]

Antioxidants, such as vitamin E and vitamin C, have shown promise. A double-blind, randomized, placebo-controlled study demonstrated that a combination of vitamin C (1000 mg/day × 6 months) and vitamin E (1000 IU/day × 6 months) resulted in statistically significant improvement in fibrosis score.[86] Treatment with silymarin plus vitamin E along with diet and lifestyle modifications reduces GGT levels and decreases noninvasive NAFLD index scores.[87] Ginger has been hypothesized to prevent NAFLD via several mechanisms, including sensitizing insulin effects, downregulating proinflammatory cytokines, exerting antioxidant and antidyslipidemic effects, and reducing hepatic triglyceride content.[88]

Lipotropic agents such as choline, methionine, betaine, folate, and vitamin B_{12} help promote the export of fat from the liver and may be helpful in a variety of liver conditions, including chemical-induced liver disease. Supplementation with chlorella decreases toxic metals and metabolites by directly preventing the absorption of toxins, increasing stool and urinary excretion of metals, and preventing enterohepatic recirculation of toxins.[89]

Bile acid therapy with ursodeoxycholic acid may be beneficial by reducing bile acid cytotoxicity and protecting hepatocytes against bile acid–induced apoptosis.[90]

THERAPEUTIC APPROACH

There are few examples of diseases more clearly due to diet, lifestyle choices, and environmental toxins than NAFLD and NASH. Although

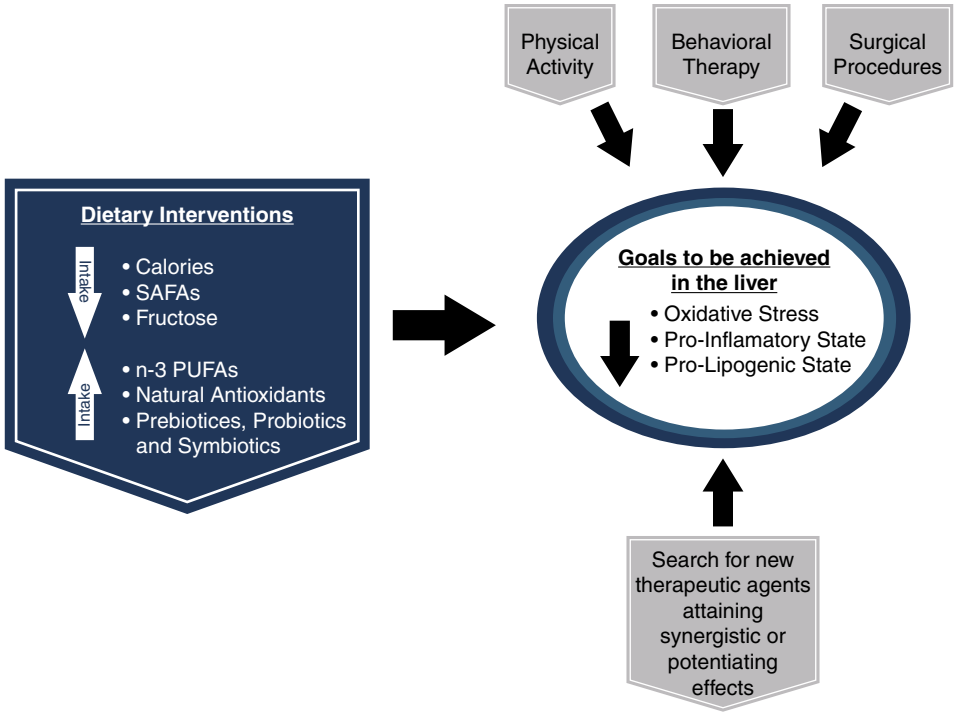


Fig. 200.2 Interventions for the prevention and treatment of nonalcoholic fatty liver disease (NAFLD). *n-3 PUFAs,* n-3 polyunsaturated fatty acids; *SAFAs,* saturated fatty acids. (From Hernandez-Rodas MC, Valenzuela R, Videla LA. Relevant aspects of nutritional and dietary interventions in non-alcoholic fatty liver disease. *Int J Molecular Sci.* 2015;16[10]:25168–25198. PubMed PMID: 26512643.)

several natural health products are helpful, little benefit will be achieved without addressing the fundamental issues of excessive consumption of high-fructose corn syrup, excessive weight, and a toxic gut (Fig. 200.2). During the recovery stage, patients must be as careful as possible to avoid every identifiable source of environmental metals and chemicals.

Supplements

- Vitamin E (mixed tocopherols): 1000 IU/d
- Vitamin C: 1000 mg bid
- Lipotropic formula (choline, methionine, betaine a B-complex, etc.): Dosage according to formula

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. *Liver Int*. 2006;26(10):1175–1186. PubMed PMID:PubMed PMID: 17105582.
2. Cotrim HP, Andrade ZA, Parana R, et al. Nonalcoholic steatohepatitis: a toxic liver disease in industrial workers. *Liver*. 1999;19(4):299–304. PubMed PMID: 10459628.
3. Akbar DH, Kawther AH. Nonalcoholic fatty liver disease and metabolic syndrome: what we know and what we don't know. *Med Sci Monitor*. 2006;12(1):RA23–RA26. PubMed PMID: 16369477.
4. Neuschwander-Tetri BA. Evolving pathophysiological concepts in nonalcoholic steatohepatitis. *Curr Gastroenterol Rep*. 2002;4(1):31–36. PubMed PMID: 11825539.
5. McCullough AJ. Update on nonalcoholic fatty liver disease. *J Clin Gastroenterol*. 2002;34(3):255–262. PubMed PMID: 11873108.
6. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol*. 2013;178(1):38–45. PubMed PMID: 23703888.
7. Le MH, Devaki P, Ha NB, et al. Prevalence of nonalcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. *PLoS ONE*. 2017;12(3):e0173499. PubMed PMID: 28346543.
8. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther*. 2015;41(1):65–76. PubMed PMID: 25376360.
9. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol*. 2013;10(11):686–690. PubMed PMID: 24042449.
10. Bellentani S, Scaglioni F, Marino M, et al. Epidemiology of nonalcoholic fatty liver disease. *Digestive Diseases*. 2010;28(1):155–161. PubMed PMID: 20460905.
11. Tsai CH, Li TC, Lin CC. Metabolic syndrome as a risk factor for nonalcoholic fatty liver disease. *Southern Med J*. 2008;101(9):900–905. PubMed PMID: 18708987.
12. Papandreou D, Ruosso I, Mavromichalis I. Update on nonalcoholic fatty liver disease in children. *Clin Nutri Off J Eur Soc Parenteral Enteral Nutri*. 2007;26(4):409–415. PubMed PMID: 17449148.
13. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology*. 1990;12(5):1106–1110. PubMed PMID: 2227807.
14. Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA*. 2006;295(13):1549–1555. PubMed PMID: 16595758.
15. Carpenter DO. Environmental contaminants as risk factors for developing diabetes. *Rev Environ Health*. 2008;23(1):59–74. PubMed PMID: 18557598.
16. Lee DH, Lee IK, Jin SH, et al. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. *Diabetes Care*. 2007;30(3):622–628. PubMed PMID: 17327331.
17. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology*. 2011;140(1):124–131. PubMed PMID: 20858492.
18. Lomonaco R, Ortiz-Lopez C, Orsak B, et al. Role of ethnicity in overweight and obese patients with nonalcoholic steatohepatitis. *Hepatology*. 2011;54(3):837–845. PubMed PMID: 21674556.
19. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology*. 2005;129(1):113–121. PubMed PMID: 16012941.
20. Bhatia LS, Curzen NP, Calder PC, et al. Nonalcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J*. 2012;33(10):1190–1200. PubMed PMID: 22408036.
21. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology*. 2003;37(5):1202–1219. PubMed PMID: 12717402.
22. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*. 2001;120(5):1183–1192. PubMed PMID: 11266382.
23. Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: cytokine-adipokine interplay and regulation of insulin resistance. *Gastroenterology*. 2006;131(3):934–945. PubMed PMID: 16952562.
24. Wieckowska A, Papouchado BG, Li Z, et al. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2008;103(6):1372–1379. PubMed PMID: 18510618.
25. Gentile CL, Pagliassotti MJ. The role of fatty acids in the development and progression of nonalcoholic fatty liver disease. *J Nutri Biochem*. 2008;19(9):567–576. PubMed PMID: 18430557.
26. Ibrahim SH, Kohli R, Gores GJ. Mechanisms of lipotoxicity in NAFLD and clinical implications. *J Pediatr Gastroenterol Nutri*. 2011;53(2):131–140. PubMed PMID: 21629127.
27. Merrell MD, Cherrington NJ. Drug metabolism alterations in nonalcoholic fatty liver disease. *Drug Metabol Rev*. 2011;43(3):317–334. PubMed PMID: 21612324.
28. Aubert J, Begriche K, Knockaert L, et al. Increased expression of cytochrome P450 2E1 in nonalcoholic fatty liver disease: mechanisms and pathophysiological role. *Clin Res Hepatol Gastroenterol*. 2011;35(10):630–637. PubMed PMID: 21664213.
29. Ludwig DS, Peterson KE, Gortmaker SL. Relation between consumption of sugar sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet*. 2001;357:505–508. PubMed PMID: 11229668.
30. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutri*. 2004;79:537–543. PubMed PMID: 15051594.
31. Elliot SS, Keim NL, Stern JS, et al. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutri*. 2002;76:911–922. PubMed PMID: 12399260.
32. Stanhope KL. Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome. *Annual Rev Med*. 2012;63:329–343.
33. Mock K, Sundus L, Benedito VA, et al. High-fructose corn syrup-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation. *J Nutri Biochem*. 2017;39:32–39.
34. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Investig*. 2009;119(5):1322–1334.
35. Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Investig*. 2005;115(5):1343–1351. PubMed PMID: 15864352.
36. Basaranoglu M, Basaranoglu G, Sabunco T, et al. Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol*. 2013;19(8):1166–1172. PubMed PMID: 23482247.
37. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for nonalcoholic fatty liver disease. *J Hepatol*. 2008;48(6):993–999. PubMed PMID: 18395287.
38. Assy N, Nasser G, Kamayse I, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol*. 2008;22(10):811–816. PubMed PMID: 18925303.
39. Abdelmalek MF, Suzuki A, Guy C, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology*. 2010;51(6):1961–1971. PubMed PMID: 20301112.
40. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology*. 2012;142(4):711–725. PubMed PMID: 22326434.
41. Yang SQ, Lin HZ, Lane MD, et al. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci U S A*. 1997;94(6):2557–2562. PubMed PMID: 9122234.
42. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;52(5):1836–1846. PubMed PMID: 21038418.
43. Frazier TH, DiBaise JK, McClain CJ. Gut microbiota, intestinal permeability, obesity-induced inflammation, and liver injury. *JPEN J Parenteral Enteral Nutri*. 2011;35(suppl 5):14S–20S. PubMed PMID: 21807932.

44. Isogawa M, Robek MD, Furuichi Y, Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol*. 2005;79(11):7269–7272. PubMed PMID: 15890966.
45. Folkmann JK, Risom L, Hansen CS, et al. Oxidatively damaged DNA and inflammation in the liver of dyslipidemic ApoE-/-mice exposed to diesel exhaust particles. *Toxicology*. 2007;237(1–3):134–144. PubMed PMID: 17602821.
46. Tomaru M, Takano H, Inoue K, et al. Pulmonary exposure to diesel exhaust particles enhances fatty change of the liver in obese diabetic mice. *Int J Molecul Med*. 2007;19(1):17–22. PubMed PMID: 17143543.
47. Tan HH, Fiel MI, Sun Q, et al. Kupffer cell activation by ambient air particulate matter exposure may exacerbate nonalcoholic fatty liver disease. *J Immunotoxicol*. 2009;6(4):266–275. PubMed PMID: 19908945.
48. Huang CY, Huang KL, Cheng TJ, et al. The GST T1 and CYP2E1 genotypes are possible factors causing vinyl chloride induced abnormal liver function. *Archiv Toxicol*. 1997;71(8):482–488. PubMed PMID: 9248625.
49. Cotrim HP, De Freitas LA, Freitas C, et al. Clinical and histopathological features of NASH in workers exposed to chemicals with or without associated metabolic conditions. *Liver Int*. 2004;24(2):131–135. PubMed PMID: 15078477.
50. Hsiao TJ, Wang JD, Yang PM, et al. Liver fibrosis in asymptomatic polyvinyl chloride workers. *J Occup Environ Med*. 2004;46(9):962–966. PubMed PMID: 15354062.
51. Brodkin CA, Daniell W, Checkoway H, et al. Hepatic ultrasonic changes in workers exposed to perchloroethylene. *Occup Environ Med*. 1995;52(10):679–685. PubMed PMID: 7489059.
52. Brautbar N, Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. *Int J Hyg Environ Health*. 2002;205(6):479–491. PubMed PMID: 12455270.
53. Dossing M, Arlien-Soborg P, Milling Petersen L, Ranek L. Liver damage associated with occupational exposure to organic solvents in house painters. *Eur J Clin Investig*. 1983;13(2):151–157. PubMed PMID: 6409638.
54. Guzelian PS, Mills S, Fallon HJ. Liver structure and function in print workers exposed to toluene. *J Occup Med*. 1988;30(10):791–796. PubMed PMID: 3230426.
55. Kopec AK, D'Souza ML, Mets BD, et al. Non-additive hepatic gene expression elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) co-treatment in C57BL/6 mice. *Toxicol Appl Pharmacol*. 2011;256(2):154–167. PubMed PMID: 21851831.
56. Boverhof DR, Burgoon LD, Tashiro C, et al. Comparative toxicogenomic analysis of the hepatotoxic effects of TCDD in Sprague Dawley rats and C57BL/6 mice. *Toxicol Sci*. 2006;94(2):398–416. PubMed PMID: 16960034.
57. Shi X, Wahlang B, Wei X, et al. Metabolomic analysis of the effects of polychlorinated biphenyls in nonalcoholic fatty liver disease. *J Proteome Res*. 2012;11(7):3805–3815. PubMed PMID: 22686559.
58. Lin YC, Lian IB, Kor CT, et al. Association between soil heavy metals and fatty liver disease in men in Taiwan: a cross-sectional study. *BMJ Open*. 2017;7(1):e014215. PubMed PMID: 28115335.
59. Hyder O, Chung M, Cosgrove D, et al. Cadmium exposure and liver disease among US adults. *J Gastrointestinal Surg Off J Soc Surg Aliment Tract*. 2013;17(7):1265–1273. PubMed PMID: 23636881.
60. Lin YS, Ginsberg G, Caffrey JL, et al. Association of body burden of mercury with liver function test status in the U.S. population. *Environ Int*. 2014;70:88–94. PubMed PMID: 24908642.
61. Lee H, Kim Y, Sim CS, et al. Associations between blood mercury levels and subclinical changes in liver enzymes among South Korean general adults: analysis of 2008-2012 Korean National Health and Nutrition Examination Survey data. *Environ Res*. 2014;130:14–19. PubMed PMID: 24525240.
62. Seo MS, Lee HR, Shim JY, et al. Relationship between blood mercury concentrations and serum γ-glutamyltranspeptidase level in Korean adults using data from the 2010 Korean National Health and Nutrition Examination Survey. *Clinica Chimica Acta*. 2014;430:160–163. PubMed PMID: 24508988.
63. Lin TH, Huang YL, Huang SF. Lipid peroxidation in liver of rats administered with methyl mercuric chloride. *Biol Trace Element Res*. 1996;54(1):33–41. PubMed PMID: 8862759.
64. Sanyal AJ. American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology*. 2002;123(5):1705–1725. PubMed PMID: 12404245.
65. Feldstein AE, Wieckowska A, Lopez AR, et al. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology*. 2009;50(4):1072–1078. PubMed PMID: 19585618.
66. Araoud M, Neffeti F, Douki W, et al. Adverse effects of pesticides on biochemical and haematological parameters in Tunisian agricultural workers. *J Exposure Sci Environ Epidemiol*. 2012;22(3):243–247. PubMed PMID: 22377683.
67. Dufour DR, Lott JA, Nolte FS, et al. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clin Chem*. 2000;46(12):2050–2068. PubMed PMID: 11106350.
68. Kumar J, Lind L, Salihovic S, et al. Persistent organic pollutants and liver dysfunction biomarkers in a population-based human sample of men and women. *Environ Res*. 2014;134:251–256. PubMed PMID: 25173059.
69. Tian J, Zhong R, Liu C, et al. Association between bilirubin and risk of nonalcoholic fatty liver disease based on a prospective cohort study. *Sci Rep*. 2016;6:310006. PubMed PMID: 27484402.
70. Liangpunsakul S, Chalasani N. Unexplained elevations in alanine aminotransferase in individuals with the metabolic syndrome: results from the third National Health and Nutrition Survey (NHANES III). *Am J Med Sci*. 2005;329(3):111–116. PubMed PMID: 15767815.
71. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology*. 2003;37(6):1286–1292. PubMed PMID: 12774006.
72. Cave M, Appana S, Patel M, et al. Polychlorinated biphenyls, lead, and mercury are associated with liver disease in American adults: NHANES 2003-2004. *Environ Health Perspect*. 2010;118(12):1735–1742. PubMed PMID: 21126940.
73. Min YS, Lim HS, Kim H. Biomarkers for polycyclic aromatic hydrocarbons and serum liver enzymes. *Am J Industr Med*. 2015;58(7):764–772. PubMed PMID: 25940037.
74. Lin CY, Lin LY, Chiang CK, et al. Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. *Am J Gastroenterol*. 2010;105(6):1354–1363. PubMed PMID: 20010922.
75. Bacon BR, Farahvash MJ, Janney CG, et al. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology*. 1994;107(4):1103–1109. PubMed PMID: 7523217.
76. Angulo P, Keach JC, Batts KP, et al. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology*. 1999;30(6):1356–1362. PubMed PMID: 10573511.
77. Nagaya T, Yoshida H, Takahashi H, et al. Dose-response relationships between drinking and serum tests in Japanese men aged 40–59 years. *Alcohol*. 1999;17(2):133–138. PubMed PMID: 10064381.
78. Lee DH, Lim JS, Song K, et al. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress-related markers in the U.S. population: results from the third National Health and Nutrition Examination Survey. *Environ Health Perspect*. 2006;114(3):350–354. PubMed PMID: 16507456.
79. Lee DH, Jacobs DR. Is serum gamma-glutamyltransferase an exposure marker of xenobiotics? Empirical evidence with polycyclic aromatic hydrocarbon. *Clin Chem Laboratory Med*. 2009;47(7):860–862. PubMed PMID: 19575547.
80. Calvert GM, Hornung RW, Sweeney MH, et al. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. *JAMA*. 1992;267(16):2209–2214. PubMed PMID: 1348289.
81. Angulo P. Nonalcoholic fatty liver disease. *Rev Gastroenterol Mexico*. 2005;70(suppl 3):52–56. PubMed PMID: 17471859.
82. Karp DR, Shimooku K, Lipsky PE. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. *J Biol Chem*. 2001;276(6):3798–3804. PubMed PMID: 11080500.
83. Hossain IA, Rahman Shah MM, Rahman MK, et al. Gamma glutamyl transferase is an independent determinant for the association of insulin resistance with nonalcoholic fatty liver disease in Bangladeshi adults:

association of GGT and HOMA-IR with NAFLD. *Diabetes Metab Syndr*. 2016;10(1 suppl 1):S25–S29. PubMed PMID: 26482965.
84. Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology*. 2010;51(1):121–129. PubMed PMID: 19827166.
85. Yari Z, Rahimlou M, Eslamparast T, et al. Flaxseed supplementation in nonalcoholic fatty liver disease: a pilot randomized, open labeled, controlled study. *Int J Food Sci Nutri*. 2016;67(4):461–469. PubMed PMID: 26983396.
86. Harrison SA, Torgerson S, Hayashi P, et al. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol*. 2003;98(11):2485–2490. PubMed PMID: 14638353.
87. Aller R, Izaola O, Gómez S, et al. Effect of silymarin plus vitamin E in patients with nonalcoholic fatty liver disease. A randomized clinical pilot study. *Eur Rev Med Pharmacol Sci*. 2015;19(16):3118–3124. PubMed PMID: 26367736.
88. Sahebkar A. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. *World J Gastroenterol*. 2011;17(2):271–272. PubMed PMID: 21246004.
89. Uchikawa T, Kumamoto Y, Maruyama I, et al. Enhanced elimination of tissue methylmercury in *Parachlorella beijerinckii*-fed mice. *J Toxicol Sci*. 2011;36(1):121–126. PubMed PMID: 21297350.
90. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology*. 2002;36(3):525–531. PubMed PMID: 12198643
91. Adapted (with permission) from Crinnion and Pizzorno, Clinical Environmental Medicine, Elsevier 2018.

Obesity

Joseph Katzinger, ND, and *Michael T. Murray, ND*

OUTLINE

DIAGNOSTIC SUMMARY

Obesity is defined as the following:

- Having a body-mass index greater than 30
- Having a body-fat percentage greater than 30% for women and 25% for men

GENERAL CONSIDERATIONS

Obesity is a major contributor to mortality and morbidity, having surpassed smoking as the number one cause of premature death in the United States.[1,2] The simplest definition of obesity is an excessive amount of body fat. It must be distinguished from overweight, which refers to an excess of body weight relative to height. A muscular athlete may be overweight yet have a low percentage of body fat. With this in mind, it is obvious that using body weight alone as an index of obesity is not entirely accurate. Nonetheless, a simple measure known as the body-mass index (BMI) is now the accepted standard for classifying individuals with regard to their body composition (Table 201.1). BMI generally correlates well with a person's total body fat; however, several studies indicate that although it may be fairly specific, it has a sensitivity of approximately 50% and thus underestimates the true prevalence of excess adiposity.[3,4] The BMI is calculated as follows:

$$BMI = (lb \times 703)/in^2 (lb = pounds;\ in = inches)$$

$$Metric: BMI = kg/m^2 (kg = kilograms;\ m = height\ in\ meters)$$

Despite the promotion of the BMI within the medical community as the definitive measure of adiposity, height and weight indices are still popular measurements made in this regard. The indices in widest use are the tables of desirable weight for height provided by the Metropolitan Life Insurance Company. However, the Metropolitan tables are often criticized for three major shortcomings:

- The stated weight ranges merely reflect the weights of those with the lowest mortality of insured persons, which may not reflect the U.S. population.
- The weight ranges for the lowest mortality do not necessarily reflect optimal healthy weight for height.
- The standard values make it difficult to assess the degree of obesity (e.g., a person within the proper weight range may have excess body fat and lower-than-optimal lean body mass, or an individual with increased muscular development may be "overweight" despite having a low percentage of body fat). Again, it is important to recognize that weight alone is a poor reflector of body-fat composition.

PREVALENCE

The prevalence of obesity has reached epidemic proportions and has steadily increased over the years among both genders, all ages, all racial/ethnic groups, and all educational levels. From 1960 to 2008, the prevalence of overweight (BMI between 25 and 30) increased from 31.5% to 34% among U.S. adults ages 20 to 74. The prevalence of obesity (BMI >30) during this same time period more than doubled, from 13.3% to 34%, with most of this rise occurring in the past 20 years. The obesity trend among adults has continued, with an estimated 39.8% prevalence among all adults over 20 in 2015 to 2016.[5] From 1960 to 2008, the prevalence of extreme obesity (BMI >40) increased from 0.8% to 5.7%. Childhood and adolescent obesity have also increased dramatically. Results from the 2007 to 2008 National Health and Nutrition Examination Survey (NHANES), using measured heights and weights,

indicated that an estimated 16.9% of children and adolescents ages 2 to 19 years were obese, which increased to 18.5% per the 2015 to 2016 NHANES analysis. Given the health challenges associated with obesity, as shown in Box 201.1, the significance of these increases is staggering.[6–8]

Obese individuals have an average of a 5- to 7-year shorter life expectancy compared with normal-weight individuals (BMI 20–25), with a greater relative risk for mortality associated with a greater degree of obesity.[1,9] Most of the increased risk for mortality is due to cardiovascular causes; obesity carries with it a tremendous risk for type 2 diabetes, elevated cholesterol levels, high blood pressure, and other risk factors for atherosclerosis. In 2009 the estimated annual medical spending due to overweight and obesity was estimated to be $147 billion.[10] The economic cost attributed to obesity was nearly $316 billion in 2010. It is expected to grow to $861 billion by 2030. As shown in Fig. 201.1, the adjusted annual incremental cost for a normal-weight normoglycemic adult was estimated to be nearly $336, compared with $4649 for a diabetic with class III obesity, a nearly 14-fold increase in cost. Weight was shown to increase health care spending for each glycemic stage.[11]

TABLE 201.1 Classification of Body-Mass Index

Underweight	**<18.5**
Normal	18.5–24.9
Overweight	25–29.9
Obesity	30–39.9
Extreme obesity	>40

DETERMINATION OF BODY COMPOSITION

The importance of determining body-fat composition and classifying obesity accurately is hard to overstate because it offers valuable monitoring, prognostic, and therapeutic information. In terms of body-fat percentage, obesity is defined as greater than 30% body fat for women and 25% body fat for men. Because direct analysis of body composition cannot be performed on live subjects, indirect methods must be employed, such as those listed in Box 201.2.

Visual Observation

Superficial visual observation is often all that is required for a qualitative analysis of obesity. One popular way of classifying body types is somatotyping—a physical, anthropological classification of physique based on body size and proportion:

- The endomorph has a relatively large body and short arms and legs.
- The mesomorph has a large, muscular chest that dominates the abdomen and has prominent bony joints.
- The ectomorph has a relatively small frame (a slender, delicate bone structure) and long arms and legs.

The endomorph is at greatest risk for developing obesity, the mesomorph is at moderate risk, and the ectomorph is extremely unlikely to develop obesity. Indeed, somatotyping also has some predictive value for metabolic syndrome because ectomorphy is more predictive of a favorable metabolic profile, even among obese women.[12]

BOX 201.1 Morbidity Associated With an Increased Risk Due to Obesity

Cardiovascular
- Angina
- Atherosclerosis
- Congestive heart failure
- Deep vein thrombosis
- Heart attack
- High blood pressure
- High cholesterol levels
- Pulmonary embolism
- Stroke

Dermatology
- Cellulitis
- Hirsutism
- Intertrigo
- Lymphedema
- Stretch marks

Endocrinology and Reproduction
- Complications during pregnancy
- Diabetes mellitus
- Infertility
- Menstrual disorders
- Intrauterine fetal death
- Polycystic ovary syndrome

Gastrointestinal
- Gastroesophageal reflux disease
- Cholelithiasis
- Fatty liver disease

Neurology
- Carpal tunnel syndrome
- Dementia
- Idiopathic intracranial hypertension
- Migraine headaches
- Multiple sclerosis

Oncology
- Cancer

Psychiatry
- Depression
- Social stigmatization

Respiratory
- Asthma
- Obstructive sleep apnea

Rheumatology and Orthopedics
- Chronic low back pain
- Gout
- Osteoarthritis

Urology and Nephrology
- Chronic renal failure
- Erectile dysfunction
- Hypogonadism
- Urinary incontinence

The distribution of body fat is also important in the classification of obesity. Two basic distribution patterns exist: gynecoid and android, or female- and male-patterned obesity. These types are discussed in greater detail under "Types of Obesity."

Skinfold Thickness

The amount of total body fat can be estimated by measuring the thickness of the subcutaneous fat (skinfold or fatfold thickness). Skinfold thickness is measured with skinfold calipers at several sites on the body to improve accuracy. The most common measurement sites are the triceps, biceps, subscapular, and suprailiac skinfolds.

Although skinfold thickness measurements are easy to obtain and are generally accurate in estimating body-fat percentage, their limitations include the inability to control intersubject and intrasubject variations in skinfold compressibility, the inability to palpate the fat–muscle interface, and the impossibility of obtaining interpretable measurements on very obese individuals. Additionally, interobserver

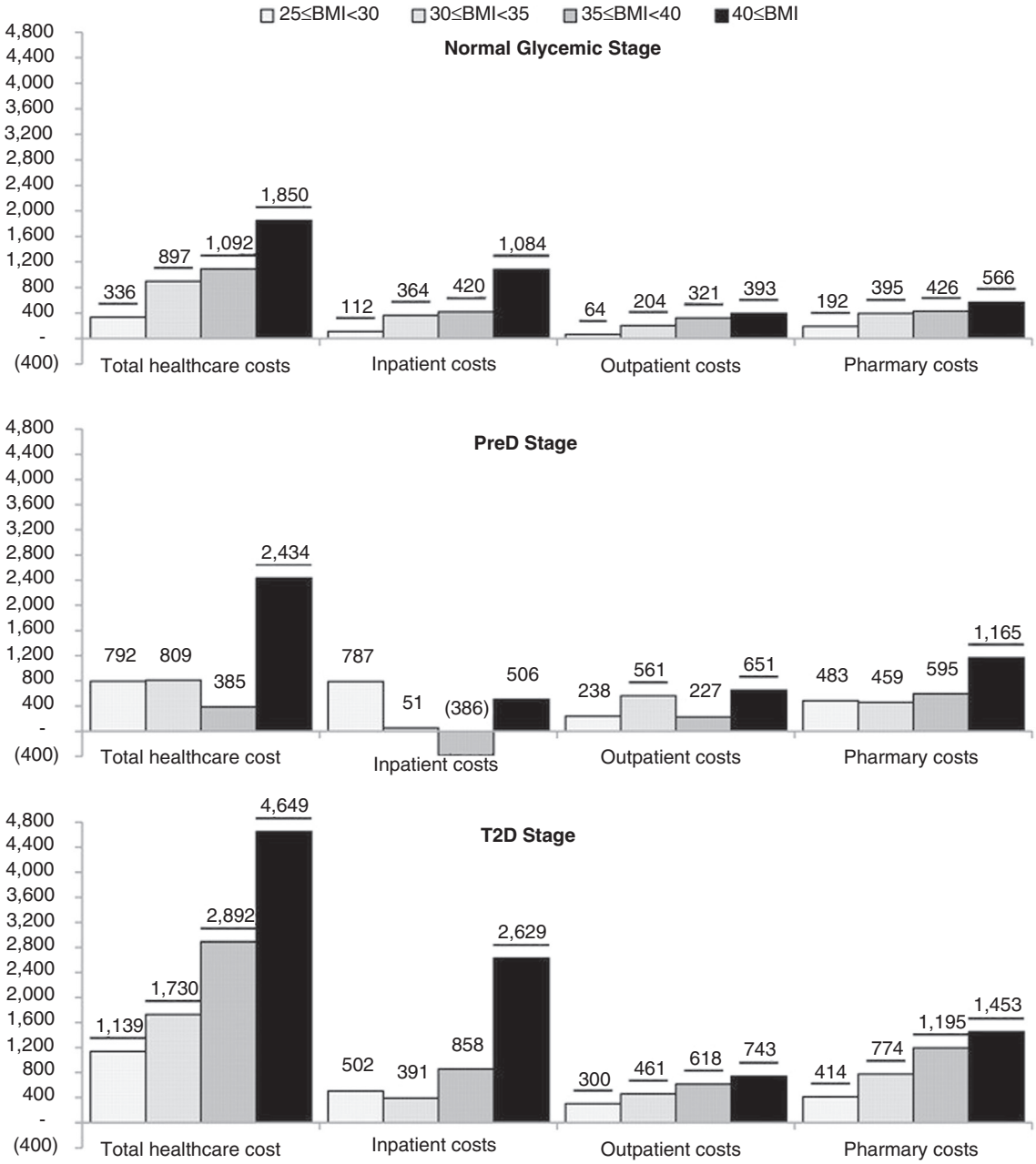


Fig. 201.1 Adjusted incremental annualized costs, relative to 18.5 ≤ BMI < 25, in each glycemic stage. Incremental costs were adjusted for age, sex, race, smoking status, employment status, insurance type, and year starting the stage. Underscored incremental costs had $p < 0.05$ compared with normal BMI, based on generalized linear regression model with log-link and gamma distribution. *BMI,* Body-mass index; *PreD,* prediabetes, *T2D,* type 2 diabetes. (From Li Q, Blume SW, Huang JC, et al. The economic burden of obesity by glycemic stage in the United States. *Pharmacoeconomics.* 2015 Jul;33[7]:735–748. PMID: 25564434. Reproduced from an open access article, distributed under the terms of the Creative Commons Attribution Noncommercial License.)

BOX 201.2 Indirect Methods of Analyzing Body-Fat Composition

- Visual observation (somatotypes)
- Anthropometric measurements
- Height and weight
- Circumferences and diameters
- Skinfold thickness
- Isotope or chemical dilution
- Body water
- Body potassium
- Body fat
- Body density and body volume
- Conductivity
- Total-body electrical conductivity
- Bioelectric impedance
- Neutron activation
- Imaging techniques
- Ultrasound
- Computer tomography
- Nuclear magnetic resonance
- Nuclear magnetic spectroscopy

variability, as well as the use of different types of skinfold calipers, may contribute to measurement errors.

However, for most clinical purposes, skinfold measurements provide the easiest and least expensive method for estimating body-fat percentage. For more precise estimations, other methods (e.g., bioelectrical impedance, ultrasound, total body electrical conductivity, and hydrostatic weighing) offer significant advantages.

Body Density

Measurement of body density provides a quantitative technique for measuring body fat. Density is determined from the specific gravity, which is calculated by measuring the different weights of the body in and out of water. In this procedure, individuals are weighed underwater and out of water, taking into account the residual volume of the lungs. This information is used to fractionate the body into its fat and nonfat components because fat is lighter than water and other tissues are heavier than water. The method is relatively simple if appropriate facilities are available. The major limitation of hydrostatic weighing is that it requires considerable cooperation from the subject, who must exhale completely and then submerge totally underwater up to 10 times, making the method impossible to use with elderly, ill, or hospitalized patients.

With the advent of more sophisticated body-composition analyzers, the hydrostatic weighing procedure has generally fallen out of favor, although many experts still consider it to be the gold standard of body-composition determination. However, dual-energy x-ray absorptiometry (DEXA) is fast becoming the new gold standard because it provides greater precision with only one measurement and can show exactly where fat is distributed throughout the body. DEXA is based on a three-compartment model that divides the body into total body mineral, fat-free soft (lean) mass, and fat tissue mass. DEXA uses a whole-body scanner that has two low-dose x-ray beams from different sources reading bone and soft tissue mass simultaneously. The sources are mounted beneath the table with a detector overhead. The scanner passes across a person's reclining body, with data collected at 0.5-cm intervals. A scan takes between 10 and 20 minutes.

TABLE 201.2 Body-Fat Rating Chart for Use With a Body-Fat Measuring Scale

age	Risky	Excellent	Good	Fair	Poor
Male					
19–24	<6%	10.8%	14.9%	19%	23.3%
25–29	<6%	12.8%	16.5%	20.3%	24.4%
30–34	<6%	14.5%	18%	21.5%	25.2%
35–39	<6%	16.1%	19.4%	22.6%	26.1%
40–44	<6%	17.5%	20.5%	23.6%	26.9%
45–49	<6%	18.6%	21.5%	24.5%	27.6%
50–54	<6%	19.8%	22.7%	25.6%	28.7%
55–59	<6%	20.2%	23.2%	26.2%	29.3%
60	<6%	20.3%	23.5%	26.7%	29.8%
Female					
19–24	<9%	18.9%	22.1%	25.0%	29.6%
25–29	<9%	18.9%	22%	25.4%	29.8%
30–34	<9%	19.7%	22.7%	26.4%	30.5%
35–39	<9%	21%	24%	27.7%	31.5%
40–44	<9%	22.6%	25.6%	29.3%	32.8%
45–49	<9%	24.3%	27.3%	30.9%	34.1%
50–54	<9%	26.6%	29.7%	33.1%	36.2%
55–59	<9%	27.4%	30.7%	34%	37.3%
60	<9%	27.6%	31%	34.4%	38%

Bioelectric Impedance

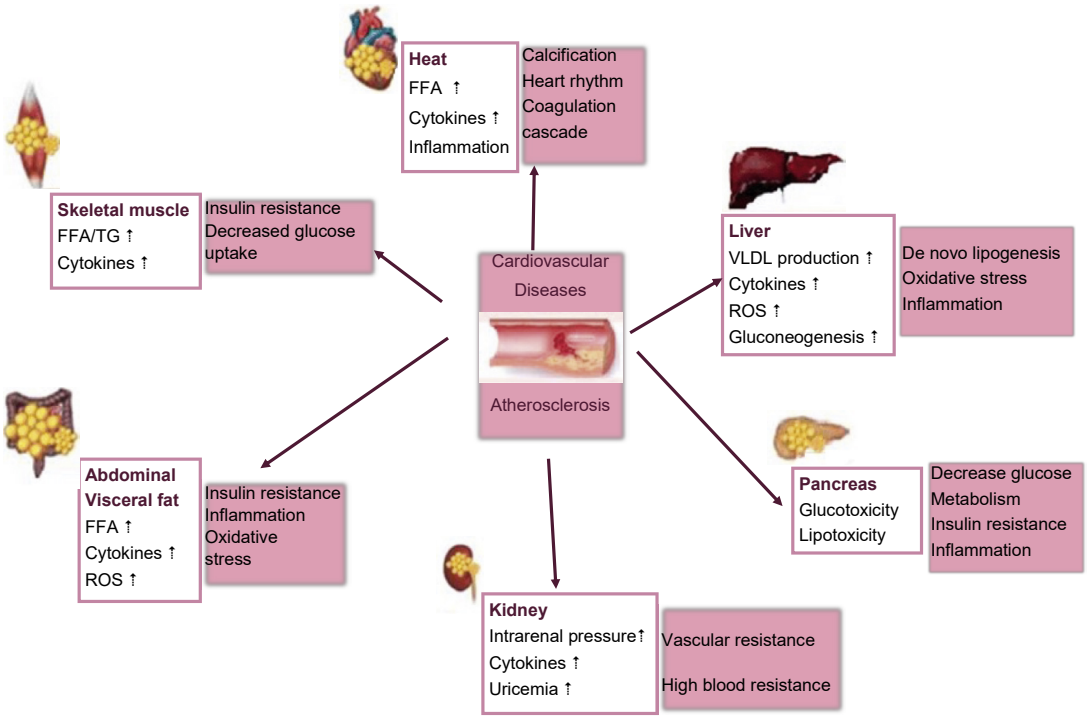
The bioelectrical impedance method for determining body composition is based on measuring the conduction of an applied electrical current through body tissues. In biological structures, the application of a constant, low-level alternating current results in a frequency-dependent impedance to the flow of the current, according to the type of tissue. Intracellular and extracellular fluids behave as electrical conductors, whereas the cell membranes act as electrical condensers. At low frequencies, such as 1 kHz, the current mainly passes through the extracellular fluids, whereas at higher frequencies, such as 500 to 800 kHz, it penetrates the intracellular and extracellular fluids. Thus body fluids and electrolytes function as electrical conductors, whereas cell membranes behave as capacitors.

Because fat-free mass has a much greater conductivity than does fat, there is a strong relationship between conductance and lean body mass. Body composition analysis, as determined by bioelectrical impedance, is a safe, noninvasive procedure that provides rapid measurements. Home scales equipped with bioelectrical impedance units to assess body-fat percentage and weight are now available. These scales typically cost between $50 and $200 (Table 201.2).

TYPES OF OBESITY

Obesity is divided into several categories on the basis of the size and number of fat cells and also on how the fat is distributed in the body (e.g., in the abdomen vs. the hips).

In hyperplastic obesity, there are increased numbers of fat cells throughout the body. The number of fat cells that a person has depends primarily on the diet of the mother while the individual was still in the womb as well as on early infant nutrition. An excess of calories during these early stages of development can lead to the formation of an increased number of fat cells for the rest of that individual's life. Because it is harder to develop new fat cells in adulthood, hyperplastic obesity



Mechanisms of various ectopic fats related with cardiovascular diseases

Fig. 201.2 Mechanisms of various ectopic fats related to cardiovascular disease. (From Gruzdeva O, Borodkina D, Uchasova E, et al. Localization of fat depots and cardiovascular risk. *Lipids Health Dis.* 2018 Sep 15;17[1]:218. PMID: 30219068. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

usually begins in childhood. Fortunately, this type of obesity tends to be associated with fewer serious health effects than other types of obesity.

Hypertrophic obesity is characterized by an increase in the size of each fat cell and is linked to diabetes, heart disease, high blood pressure, and other serious disturbances of metabolism.[8] With hypertrophic obesity, the fat distribution is usually around the waist. This type of distribution is referred to as male-patterned or "android" because it is typically seen in the obese male. If the waist is larger than the hips, a person is said to have android obesity. If the hips are larger, then a person has female-patterned or "gynecoid" obesity. Both of these patterns are associated with an increase in visceral and ectopic fat deposits, with organ damage as a result (Fig. 201.2). There appear to be both genetic and epigenetic influences on the capacity of subcutaneous adipose tissue to accommodate excess fat; once this capacity is exceeded, fat deposits in intra-abdominal/visceral areas, and these dysfunctional adipose deposits produce a variety of toxic lipid intermediates associated with inflammation, cardiovascular risk, and insulin resistance.[13]

The waist-to-hip ratio is determined by measuring the circumference of the waist about ½ in. above the navel and measuring the circumference of the hips at the greatest protrusion of the buttocks. The waist circumference is then divided by the hip circumference. A waist-to-hip ratio above 1 for men and above 0.8 for women is associated with metabolic syndrome (also known as syndrome X) and increases the risk of developing type 2 diabetes, high blood pressure, coronary heart disease, stroke, and gout.

Finally, in hyperplastic-hypertrophic obesity, there is an increase in both the number and size of fat cells.

CAUSES OF OBESITY

Although there may or may not be a specific "obesity gene," the tendency to be overweight is definitely inherited. Genome-wide association studies (GWASs) have already found 127 susceptibility-gene sites within the human genome, such as the *FTO* and *SLC6A14* genes, with the former now considered to be the most significant candidate gene contributing to obesity in both children and adults.[14] Nonetheless, even high-risk individuals can avoid obesity, indicating that dietary and lifestyle factors (primarily little or no physical activity) are chiefly responsible for obesity. It is worth noting, for example, that Americans are estimated to spend the majority of their waking hours in sedentary activity, averaging 7.2 to 9.5 hours per day.[15] However, in looking at possible causes beyond diet and lifestyle, researchers have focused on both psychological and physiological factors.

Psychological Factors

In the past, psychological factors were thought to be largely responsible for obesity. An early popular theory proposed that overweight individuals were insensitive to internal signals for hunger and satiety while simultaneously being extremely sensitive to external stimuli (sight, smell, and taste) that could increase the appetite. One source of external stimuli that has definitely been shown to be associated with obesity is watching television.

Watching television has been demonstrated to be linked to the onset of obesity, and there is a dose-related effect. Increased television viewing and decreased physical activity are thought to be primary causes of the growing prevalence of obesity among children in the United States. Television viewing in childhood and adolescence is associated not only with being overweight but also with poor fitness and the presence of obesity, smoking, and elevated cholesterol levels in adulthood, indicating that excessive television viewing has long-lasting adverse effects on health.[16] We now have preliminary data showing that interventions that target sedentary activity among children as young as 4 to 7, particularly to reduce television watching (and screen-time in general), do lower BMI, partly mediated by reduced energy intake.[17]

In addition to leading to childhood obesity, television viewing also contributes to overweight in adults. In one study, 50,277 women with BMIs below 30 completed questions on physical activity and sedentary

behaviors at baseline. During 6 years of follow-up, 3757 (7.5%) of these women became obese (their BMIs were at or above 30), and 1515 new cases of type 2 diabetes occurred. Time spent watching television was positively associated with the risk of obesity and type 2 diabetes. Each 2-hour-per-day increment in television watching was associated with a 23% increase in obesity and a 14% increase in the risk of diabetes. In contrast, each 2-hour-per-day increment in sitting at work was associated with a 5% increase in obesity and a 7% increase in diabetes.[18] In 2018 a meta-analysis of 34 prospective studies found positive associations between sedentary behavior and all-cause, cardiovascular, and cancer mortality, as well as incident type 2 diabetes, with even stronger associations for television viewing specifically (all adjusted for physical activity; Fig. 201.3).

Although watching television fits nicely with the psychological theory (increased sensitivity to external cues), several physiological effects of watching television promote obesity, such as reducing physical activity, interfering with sleep, encouraging increased energy consumption, and the actual lowering of resting (basal) metabolic rate to a level similar to that experienced during trance-like states. These factors clearly support the physiological view.

Physiological Factors

Although the psychological theories primarily propose that obese individuals have a decreased sensitivity to internal cues of hunger and satisfaction, an emerging theory of obesity states almost the opposite—that obese individuals appear to be extremely sensitive to specific internal cues.[7] Unfortunately, these cues relate to dysfunctional appetite control due to a combination of genetic, dietary, and lifestyle factors. At the center of this dysfunction, in many cases, is resistance to the hormone insulin as a result of a conditioned reaction to a high-glycemic diet. The development, progression, and maintenance of obesity constitute a vicious positive-feedback cycle consisting of insulin resistance, central adiposity, alterations in adipokine secretion by adipocytes and gut-derived hormones, impaired diet-induced thermogenesis, and low brain serotonin levels. All of these factors are interrelated and support the theory that obesity is primarily an adaptive physiological response that is out of control. Failure to address these underlying areas and provide proper psychological support results in only temporary weight loss at best.

Body weight is closely tied to what is referred to as the set point—the weight that a body tries to maintain by regulating the amount of food and calories consumed. Research with animals and humans has found that each person has a programmed set-point weight. It has been postulated that individual fat cells control this set point: when the enlarged fat cells in obese individuals become smaller, they either send powerful messages to the brain to eat or they block the action of appetite-suppressing compounds like leptin.

The existence of this set point helps to explain why most diets do not work. Although the obese individual can fight off the impulse to eat for a time, eventually the signals become too strong to ignore. The result is rebound overeating, with individuals often exceeding their previous weight. In addition, their set point is now set at a higher level, making it even more difficult to lose weight. This has been termed the "ratchet effect" and "yo-yo dieting."

The key to overcoming the fat cells' set point appears to be increasing the sensitivity of the fat cells to insulin. This sensitivity apparently can be improved, and the set point lowered, by exercise, a specially designed diet, and several nutritional supplements (discussed later). The set-point theory suggests that a diet that does not improve insulin sensitivity will most likely fail to provide long-term results.

When fat cells, particularly those around the abdomen, become full of fat, they secrete a number of biological products (e.g., resistin, leptin, tumor necrosis factor, free fatty acids) that dampen the effect of insulin, impair glucose utilization in skeletal muscle, and promote glucose production by the liver. Also important is that as the number and size of fat cells increase, they lead to a reduction in the secretion of compounds that promote insulin action, including a novel protein produced by fat cells known as adiponectin. Adiponectin is not only associated with improved insulin sensitivity but also has anti-inflammatory activity, lowers triglycerides, and blocks the development of atherosclerosis. The net effect of all of these actions by fat cells is that they severely stress the mechanisms governing blood sugar control and also lead to the development of the major complication of diabetes—atherosclerosis. Because of all of these newly discovered hormones secreted by fat cells, many experts now consider the adipose tissue a member of the endocrine system.[19,20]

Adipokines and Gut-Derived Hormonal Alterations

It could be argued that obese individuals are more sensitive to internal signals to eat. Appetite reflects a complex system that has evolved to help humans deal with food shortages. Therefore it is extremely biased toward weight gain. It makes sense that the people who survived famines were those who were more adept at storing fat than burning it. So there is a built-in tendency in many people to overeat even though food is readily available in developed countries.

To combat the tendency to eat more than is required, it is important to accentuate the normal physiological processes that curb the appetite. An elaborate system exists that is supposed to tell the hypothalamus when the body requires more food as well as when enough food has been consumed. In addition, adipokines like leptin, a strong signal of appetite control, actually originate from the gastrointestinal tract. In addition to nerve signals feeding back to the central nervous system is a growing list of gut-derived hormones and peptides, such as neuropeptide Y and the analogs ghrelin and cholecystokinin.[21] For example, peptide YY 3-36 (or PYY for short) dramatically reduced appetite in both obese and normal-weight individuals.[22] The subjects consumed about 30% less at an "all-you-can-eat" buffet after receiving an infusion of this hormone than they did when they were given only saline solution. The subjects also ate significantly less over the next 24 hours.

Unlike PYY, the stomach-derived hormone ghrelin increases appetite. Ghrelin levels are highest when the stomach is empty and during calorie restriction. Obese individuals tend to have elevated ghrelin levels to begin with, and when they try to lose weight, their ghrelin levels increase. Part of the success of gastroplasty in producing permanent weight loss is thought to be due to significantly reduced ghrelin levels. For example, a diet-induced weight loss of 17% of initial body weight was associated with a 24% increase in the area under the curve (AUC) for the 24-hour ghrelin profile. However, despite a 36% weight loss after gastric bypass, the AUC for the ghrelin profile in the gastric-bypass group was 77% lower than in normal-weight controls and 72% lower than in matched obese controls.[23]

Although it is possible to use various appetite regulators as therapeutic agents in human obesity, preliminary studies seem to indicate that in humans, compensatory actions may negate the effect. The perfect drug or natural product to affect appetite must be able to increase insulin sensitivity and produce a targeted effect of reducing factors that increase appetite while simultaneously increasing factors that decrease appetite. Highly viscous dietary fiber may prove useful in this application.

Gut-Derived Appetite Regulators

The main hormones inhibiting food intake are cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), oxyntomodulin, and PYY, whereas hormonal stimulators of appetite include ghrelin and orexin A.[21] It could be strongly argued that secretion of these regulators, as well as overall enteroendocrine cell function, is regulated by the presence or absence of highly viscous dietary fiber, especially because the main

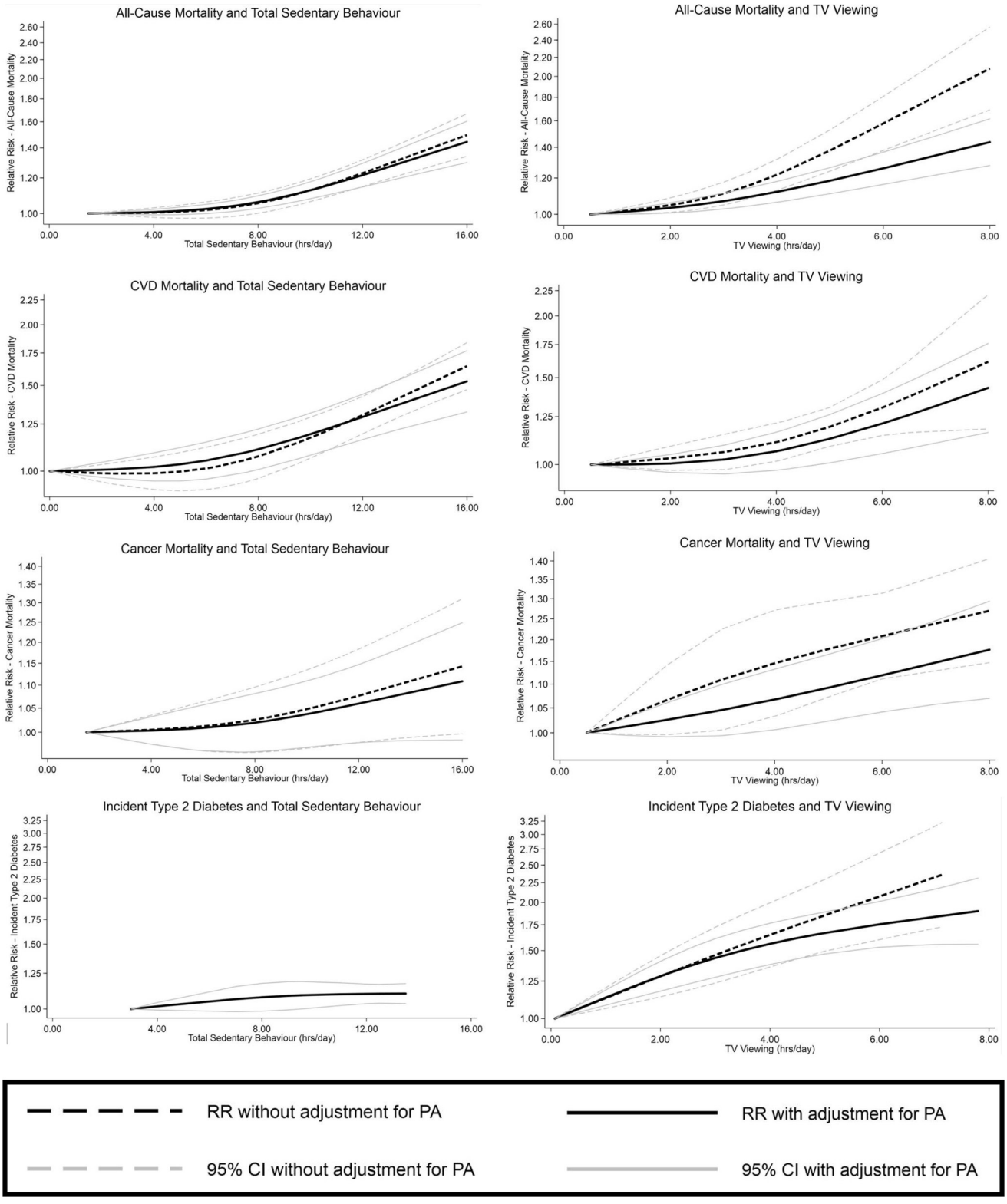


Fig. 201.3 Nonlinear associations between sedentary behavior and health outcomes presented with and without physical activity (PA) adjustment. (From Patterson R, McNamara E, Tainio M, et al. Sedentary behaviour and risk of all-cause, cardiovascular and cancer mortality, and incident type 2 diabetes: a systematic review and dose response meta-analysis. *Eur J Epidemiol.* 2018 Sep;33[9]:811–829. PMID: 29589226. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

targets for these neurotransmitters are vagal afferent neurons and the appetite-inhibiting effects of CCK are enhanced by the mechanical effects of dietary fiber (gastric distention). Brief descriptions of these gut-derived appetite regulators follow.

Cholecystokinin. The role of CCK as a regulator of protein and fat digestion in the upper small intestine has been recognized for several decades. CCK determines the capacity for digestion by controlling gastric emptying as well as the delivery of enzymes from the pancreas. The inhibition of appetite by CCK has been demonstrated in human studies.[21,24] The reduction of food intake by low-dose exogenous CCK is enhanced with moderate gastric distention, implying synergistic interactions between CCK and the stimulation of gastric mechanoreceptors. Not surprisingly, viscous dietary fiber has been shown to increase the secretion of CCK.[25,26]

Glucagon-like Peptide-1. The distal intestinal glucagon-like peptide-1 (GLP-1) is derived from different regions of the glucagon precursor. Infusion of GLP-1 inhibits food intake, decreases sensations of hunger, and inhibits plasma ghrelin (discussed further later in the chapter). The secretion of GLP-1 is clearly influenced by food intake, but the specific food components, as well as the role of dietary fiber, have not yet been determined.[27]

Oxyntomodulin. Oxyntomodulin (OXM) is released from the gut postprandially in proportion to food volume and energy content. Circulating levels of OXM are elevated in several conditions associated with anorexia. Injection of OXM reduces food intake, presumably by suppressing ghrelin. Elevated levels of endogenous OXM associated with disorders of the gastrointestinal tract may contribute to anorexia.[28]

Peptide YY. Peptide YY (PYY) is secreted by enteroendocrine cells of the ileum and colon, and a related compound pancreatic polypeptide is secreted by the pancreas.[29] In obese subjects, basal and postprandial plasma concentrations of PYY are reduced compared with normal subjects. Administration of PYY inhibits food intake, with effects lasting up to 24 hours after a 2-hour infusion, suggesting that PYY exerts significant long-term appetite-suppressing action.[30] Like CCK, viscous dietary fiber likely raises PYY.[31]

Ghrelin. Ghrelin is an interesting peptide that originates from X-cells in the stomach lining. This compound makes the stomach rumble, and it is a powerful appetite stimulator.[32] Not surprisingly, plasma concentrations of ghrelin are depressed in patients with anorexia nervosa and elevated in obesity. Concentrations of ghrelin decrease on feeding, especially with meals containing dietary fiber. However, because this effect is mediated by insulin, resistance to insulin is associated with higher ghrelin levels.[33]

Orexin A. The peptide orexin A has been most intensively studied in the hypothalamus, but it also occurs in enteric neurons and in gut endocrine cells, particularly enterochromaffin cells. Orexin A is a stimulator of appetite and is thought to inhibit CCK-stimulated excitation of vagal afferent fibers (thereby negating the appetite-suppressing effect of CCK). Plasma concentrations of orexin A increase with fasting and may contribute to overeating during or immediately after a meal by suppressing satiety signaling by CCK.[21]

Diet-Induced Thermogenesis

Another physiological difference between obese and thin people is how much of the food consumed is converted immediately to heat. This process is known as diet-induced thermogenesis (heat production). Researchers have found that a meal may stimulate up to a 40% increase in diet-induced thermogenesis in lean individuals. In contrast, overweight individuals often display only a 10% or less increase.[34] In overweight individuals, the food energy is stored instead of being converted to heat, as it is in lean individuals.

A major factor for the decreased thermogenesis in overweight people is, once again, insulin insensitivity.[35] Therefore, the enhancement of insulin sensitivity may go a long way toward reestablishing normal thermogenesis as well as resetting the set point in overweight individuals.

Researchers have also shown that even after weight loss has been achieved, individuals predisposed to obesity still have decreased diet-induced thermogenesis compared with lean individuals.[36] Therefore it is important to continue to support insulin sensitivity and proper metabolism indefinitely if weight loss is to be maintained.

In addition to insulin insensitivity and reduced sympathetic nervous system activity, another factor determines diet-induced thermogenesis: the amount of brown fat. Most fat in the body is "white fat," consisting of an energy reserve containing triglycerides stored in a single compartment. Tissue composed of white fat looks white or pale yellow. Brown fat cells contain multiple fat-storage compartments. The triglycerides are localized in smaller droplets surrounding numerous mitochondria. An extensive blood vessel network and the density of the mitochondria give the tissue its brown appearance as well as its increased capacity to metabolize fatty acids.[37]

Brown fat does not metabolize fatty acids to adenosine triphosphate (ATP) as efficiently as other tissues of the body, including white fat, because the mitochondrial brown fat uncoupling protein 1 (UCP1), found in brown fat only, uncouples the mitochondrial membrane potential, dissipating energy as heat rather than as ATP production.[38] Brown-fat activity is stimulated by cold exposure and plays a major role in diet-induced thermogenesis (Fig. 201.4). Additionally, activation of brown fat appears to improve insulin sensitivity, and cold acclimation protocols not only activate brown fat but also increase resting energy expenditure, whole-body glucose disposal, and insulin sensitivity, but only among individuals with detectable brown-fat deposits.[39] Therapies to increase the proportion and activation of brown fat may prove to be valuable in the treatment of both obesity and diabetes.

Some theories suggest that lean people have a higher ratio of brown to white fat than overweight individuals. Evidence supports this theory. The amount of brown fat in modern humans is extremely small (estimates are 0.5%–5% of total body weight), but because of its profound effect on diet-induced thermogenesis, as little as 1 oz of brown fat (0.1% of body weight) could make the difference between maintaining body weight or putting on an extra 10 lb/year.[37]

Lean individuals also tend to respond differently to excess calories than those who are overweight. In one experiment, lean individuals were overfed to increase their weight. In order to maintain the excess weight, they had to increase their caloric intake by 50% over their previous intake.[40] The opposite appears to be the case in overweight and formerly overweight individuals. In addition to requiring fewer calories to gain and maintain their weight, studies have shown that in order to maintain a reduced weight, formerly obese persons must restrict their food intake to approximately 25% less than a lean person of similar weight and body size.[41]

Individuals predisposed to obesity because of decreased diet-induced thermogenesis have been shown to be extremely sensitive to marked weight gain when consuming a high-fat diet compared with lean individuals.[42] These individuals are not only more sensitive to the weight gain–promoting effects of a high-fat diet but also tend to consume much more dietary fat than lean individuals and to exercise less. Additionally, the combination of increasing exercise and decreasing caloric intake, at least among women, has been associated with a decrease in brown-fat activity rather than an increase, providing one further reason why weight loss becomes increasingly difficult over time.[43]

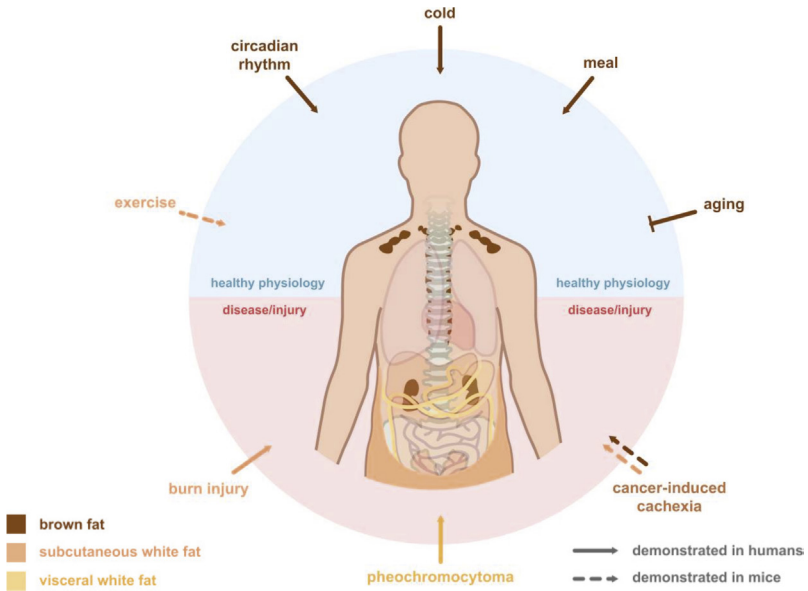


Fig. 201.4 Regulation of human brown fat and induction of white-fat browning. Several physiological conditions have been demonstrated to influence brown-fat activity in humans. When present in human adults, cold activates the brown-fat depots, which mainly are present at the cervical, supraclavicular, and paravertebral areas and sometimes also in the perirenal region. Brown fat is also induced in response to a meal and is activated in a circadian manner in mice and seems to have a biorhythm with an opposing pattern to plasma glucose in humans. Aging is negatively associated with the appearance of active brown fat. In terms of browning of white-fat depots, physical exercise has been shown in mice to induce browning of subcutaneous inguinal fat; however, this has not been reproducible in subcutaneous fat in humans. (From Scheele C, Nielsen S. Metabolic regulation and the anti-obesity perspectives of human brown fat. *Redox Biol.* 2017 Aug;12:770–775. PMID: 28431377. Reproduced from an open access article under the CC BY-NC-ND license [http://creativecommons.org/licenses/by-nc-nd/4.0/].)

The Low-Serotonin Theory

A considerable body of evidence demonstrates that brain serotonin plays a major role in influencing eating behavior. Initial studies showed that when animals and humans are fed diets deficient in tryptophan, appetite is significantly increased, resulting in the binge eating of carbohydrates.[44,45] The diet low in tryptophan leads to low brain serotonin levels, a condition the brain interprets as starvation, so the appetite control centers are stimulated to prefer carbohydrates. Feeding animals or humans a carbohydrate meal leads to increased tryptophan delivery to the brain, resulting in the elevated manufacture of serotonin. This scenario has led to the idea that low serotonin levels lead to "carbohydrate cravings" and play a major role in the development of obesity.

Furthermore, it has been demonstrated that concentrations of tryptophan in the bloodstream and subsequent brain serotonin levels plummet with dieting.[46] In response to severe drops in serotonin levels, the brain simply puts out such a strong message to eat that it cannot be ignored. This explains why most diets do not work.

Cravings for carbohydrates due to low serotonin levels can be mild or severe. They may range in severity from the desire to nibble on a piece of bread or a cookie to uncontrollable binging. At the upper end of the spectrum of carbohydrate addiction is bulimia, a potentially serious eating disorder characterized by binge eating and purging of the food through forced vomiting or the use of laxatives. The medical consequences of bulimia can be severe (e.g., rupture of the stomach, erosion of the dental enamel, and cardiac disturbances due to loss of potassium).

Environmental Toxins

Environmental pollutants and exposure to everyday chemicals have also emerged as playing a significant role in the diabetes and obesity epidemic, one that has largely been overlooked. Multiple lines of evidence point to toxicants commonly found in the environment—named "obesogens" by researchers—with multiple mechanisms of action. Examples include increasing both the number and size of fat cells, impairing insulin sensitivity, and influencing hormones involved in appetite and satiety (Fig. 201.5). Not only do these toxins pose a risk for obesity, but their long-term storage in adipose cells may be a risk factor for numerous chronic conditions typically associated with obesity.

For example, in an analysis of the 1999 to 2002 NHANES data set, six persistent organic pollutants (POPs; two polychlorinated dibenzodioxins [PCDDs], one polychlorinated biphenyl [PCB], and three metabolites of organochlorine pesticides) carried an adjusted odds ratio for diabetes as high as 11.8 (oxychlordane) when comparing the highest to lowest individual toxin exposure levels. When participants were classified by their combined exposure to all six POPs, the prevalence of diabetes rose to 40-fold higher in the highest exposure group. The risk of diabetes associated with high exposure was stronger as BMI increased and was maintained at all BMI levels. However, in people

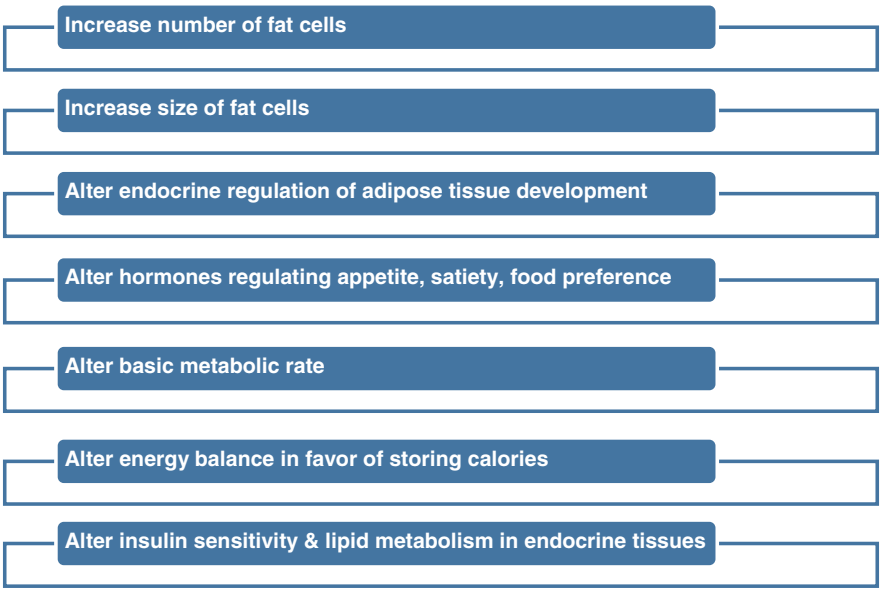


Fig. 201.5 Mechanisms of obesogenic endocrine disruptors. (From Darbre PD. Endocrine disruptors and obesity. *Curr Obes Rep.* 2017 Mar;6[1]:18–27. PMID: 28205155. Reproduced under the terms of the Creative Commons Attribution 4.0 International License [http://creativecommons.org/licenses/by/4.0/].)

with low toxin exposure, the typical link between obesity and diabetes was not observed. Similarly, diabetes was quite rare among those with low exposure, even in the highest BMI category, and similar (but not identical) trends have been observed in Finland and Spain (see Fig. 201.6).[47]

This strongly suggests that these toxins may be playing an active role in the metabolic dysfunction associated with obesity and diabetes, and adipose tissue is a storage depot for these metabolic toxins (rather than an independent toxin itself). The release of these toxins during weight loss may contribute to some of the adverse effects often reported, including impaired energy metabolism and subsequent weight regain.[48] Indeed, weight loss in those with a high POP burden may present a hazard because POP levels are able to successfully explain the "obesity paradox"—that is, individuals with an elevated BMI have a paradoxically lower mortality risk in some studies. Analysis of NHANES data found that this paradox does not exist if POP levels are low, and that adiposity may have a protective effect for those with high exposure, by pulling lipophilic toxins out of circulation and thus diluting their impact on physiological function.[49] It is also worth noting that individuals with an elevated BMI may have lower serum levels of these toxins than those with a lower BMI, even if they have a higher total body burden, because toxins are pulled into the adipose tissue and out of circulation, misleading the practitioner into suspecting a low toxin load.

THERAPEUTIC CONSIDERATIONS

Over the long term, the control of obesity is one of the greatest clinical challenges. Few people want to be overweight, most express a strong desire to lose weight, yet only 5% of obese individuals can attain and maintain "normal" body weight for a year or more, whereas 66% of those just a few pounds or so overweight are able to do the same.

The successful program for obesity is consistent with the basic foundations of good health—a positive mental attitude, a healthy lifestyle (regular exercise being especially important), a health-promoting diet, and supplementary measures. All of these components are interrelated, creating a situation where no single component is more important than any other. Improvement in one facet may be enough to result in some positive changes, but incorporating all components yields the greatest results.

Literally hundreds of diets and diet programs claim to be the answer to the problem of obesity and represent a multibillion-dollar industry. Dieters are constantly bombarded with new reports of yet another "wonder" diet. However, the basic equation for losing weight never changes. In order for an individual to lose weight, energy intake must be less than energy expenditure. This goal can be achieved by decreasing caloric intake or by increasing the rate at which calories are metabolized.

To lose 1 lb, a person must consume 3500 fewer calories than he or she expends. The loss of 1 lb each week requires a negative caloric balance of 500 calories/day. This can be achieved by decreasing the amount of calories ingested or by exercise. Reducing a person's caloric intake by 500 calories is often difficult, as is increasing metabolism by an additional 500 calories/day by exercise (accomplished by a 45-minute jog, playing tennis for an hour, or a brisk walk for 1.25 hours). The most sensible approach to weight loss is to both decrease caloric intake and increase energy expenditure through exercise.

Most individuals begin to lose weight if they decrease their caloric intake below 1500 calories/day and exercise for 15 to 20 minutes three to four times a week. Starvation and crash diets usually result in rapid weight loss (largely muscle and water) but cause rebound weight gain. The most successful approach to weight loss is gradual weight reduction (0.5–1 lb/week) by adopting long-standing dietary and lifestyle habits that promote health and the attainment and maintenance of ideal body weight. Exercise is critical to maintaining muscle mass and bone mineral density and preventing the accumulation of visceral fat both during active weight loss as well as after weight loss has been achieved.[50,51]

Although many obese individuals may require the loss of considerable weight to achieve their long-term goals, it is important to stress that even modest reductions in body weight can produce significant health benefits. For example, a 5% to 10% reduction in weight is accompanied by clinically meaningful improvements in cholesterol, blood pressure, blood glucose, and other health indices. In 2017 a systematic review and meta-analysis of 54 randomized controlled trials published in *BMJ* found that weight-loss interventions (which

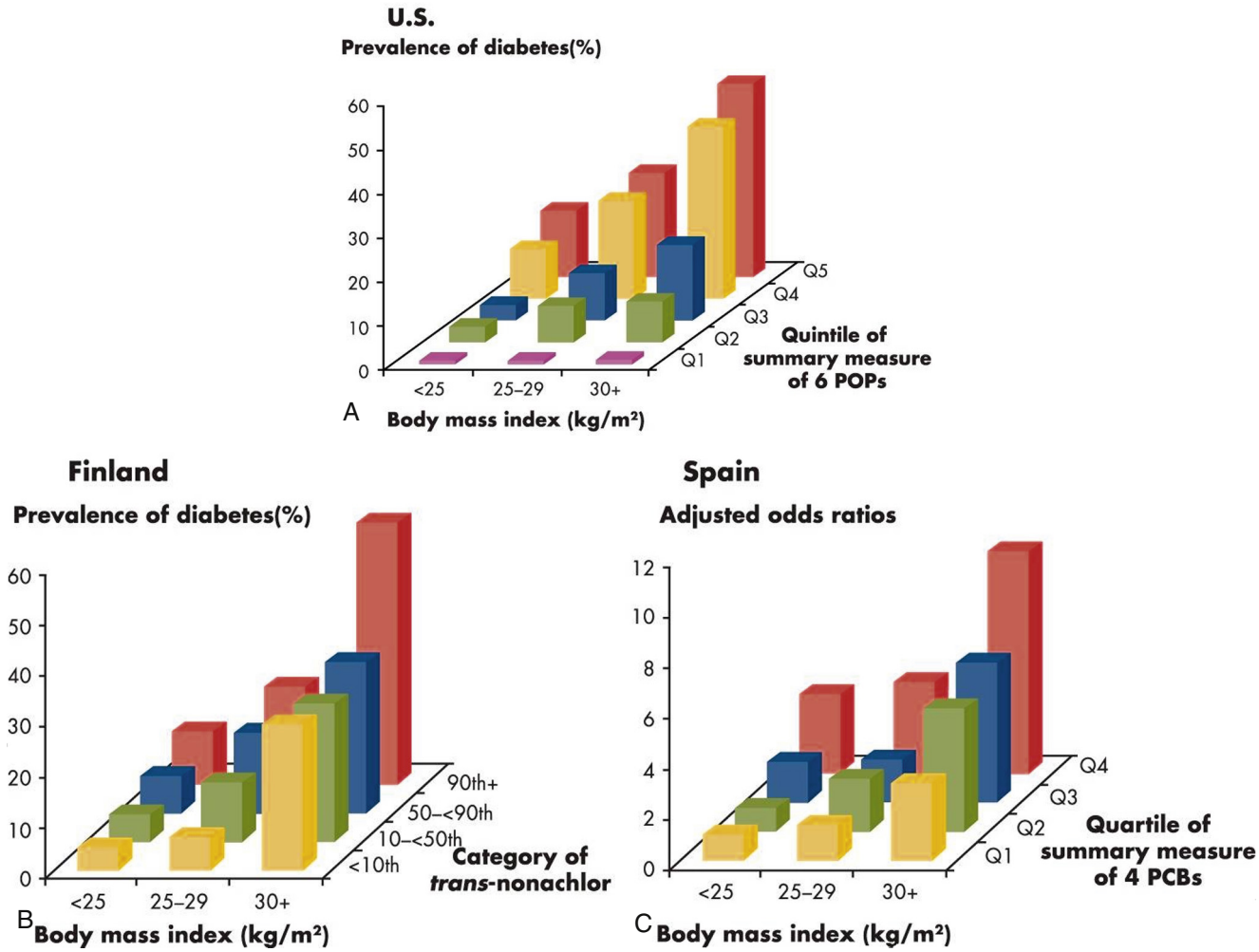


Fig. 201.6 Interaction between body-mass index (BMI) and persistent organic pollutants (POPs) in estimating the prevalence of type 2 diabetes (T2D). (From Lee DH, Porta M, Jacobs DR Jr, et al. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. *Endocr Rev.* 2014 Aug;35[4]:557–601. PMID: 24483949.)

were primarily low fat) decreased all-cause mortality by nearly 20%, supported by high-quality evidence. Lesser-quality evidence also supported reductions in cardiovascular and cancer mortality.[52]

Behavioral Therapy

Although clinical studies indicate that behavioral approaches to the management of obesity are often successful in achieving clinically significant weight loss, the lost weight is generally regained. The great majority of patients return to their pretreatment weight within 3 years. In order to provide the best insights on effective interventions, it is important to examine the psychological characteristics of people who have lost significant amounts of weight and regain only minimal weight.[53] Three main behavioral characteristics have been identified in individuals who avoid regaining significant weight:

- High levels of physical activity (approximately 1 hour daily)
- Eating a low-calorie, low-fat, low-glycemic diet
- Eating breakfast regularly
- Self-monitoring weight
- Maintaining a consistent eating pattern across weekdays and weekends
- Avoiding depression

These characteristics are intertwined within a whole host of additional factors that provide the necessary leverage for successful weight loss. For example, most patients presenting for treatment of obesity want to lose 20% to 30% of their initial body weight. Because only modest weight loss is generally achieved, many patients quickly lose the motivation and determination to keep the weight off. However, if there is a significant boost to self-esteem and self-confidence, as well as improved appearance, the feeling of being more attractive, and being able to wear more fashionable clothing, it can provide a tremendous impetus for continued weight loss until the initial goals are achieved or weight loss is maintained.

It is important to remember that the majority of people want to lose weight for the sake of changes in their physical appearance, not for the health benefits. Although there is widespread awareness that being overweight is associated with increased health risks, the reality is that relatively few patients give this as their reason for seeking treatment. Identifying the primary goals of weight loss in patients is a key step in helping them achieve success.

Dietary Strategies

The dietary strategy that we recommend for obesity is the same as that given in Chapter 44. The principles and goals detailed there reinforce

some of the key goals of weight loss and the maintenance of ideal body weight. The importance of adequate protein consumption should be stressed. We recommend 2.0 g of protein per 2 lb body weight unless a patient is showing signs of renal failure.

In 2017 an analysis of the Diabetes Prevention Program (DPP) was published, in which approximately 3000 individuals were randomized to either placebo, metformin, or intensive lifestyle intervention (a low-calorie, low-fat diet combined with at least 150 minutes moderate exercise per week) for 1 year. In all three groups, higher carbohydrate intake at baseline was associated with lower body weight. Furthermore, at the conclusion of the trial, increasing carbohydrate intake (in the context of calorie reduction) was strongly associated with weight loss in the lifestyle group (and to a lesser degree in the placebo group). A greater intake of dietary fiber was also strongly associated with weight loss in all three groups, leading the authors to conclude that "in the context of overall calorie reduction, a dietary shift toward greater carbohydrate and lower fat intake, specifically increasing the intake of dietary fiber, fruits, and vegetables, promotes weight loss in individuals at high risk of developing type 2 diabetes." Note that the carbohydrates as reported in this study are not simple sugars but primarily from food sources.[54]

The importance of higher protein consumption was demonstrated in the Diogenes study. The name *Diogenes* is an acronym standing for "Diet, Obesity and Genes." This 5-year program involved a consortium of 29 world-class centers in diet and health studies, epidemiology, dietary genomics, and food technology across Europe. Over 700 overweight adults from eight European countries who had lost at least 8% of their initial body weight with an 800-calorie diet were randomly assigned to one of five ad libitum diets to prevent weight regain over a 26-week period: a low-protein and low-glycemic-index diet, a low-protein and high-glycemic-index diet, a high-protein and low-glycemic-index diet, a high-protein and high-glycemic-index diet, or a control diet. The dropout rate was the highest in the group assigned to both a low protein and a high glycemic index diet (37.4%, compared with 26.4% in those assigned to either of the high protein diet groups and 25.6% in those assigned to either of the low glycemic index groups). The mean initial weight loss with the low-calorie diet was 11 kg. In the analysis of participants who completed the study, only the low-protein and high-glycemic-index diet was associated with subsequent significant weight regain (1.67 kg). In an intention-to-treat analysis, the weight regain was 0.93 kg less in the groups assigned to a high-protein diet than in those assigned to a low-protein diet and 0.95 kg less in the groups assigned to a low-glycemic-index diet than in those assigned to a high-glycemic-index diet. The groups did not differ significantly with respect to diet-related adverse events.[55]

Water

It is well established that water consumption can acutely reduce caloric intake at mealtimes, especially among middle-aged and older adults. In the most recent study, 48 adults aged 55 to 75 years with a BMI between 25 and 40 were assigned to one of two groups: (1) a hypocaloric diet with 500 mL water before each daily meal (water group) or (2) a hypocaloric diet alone (nonwater group). Weight loss was approximately 2 kg greater in the water group than in the nonwater group, and the water group showed a 44% greater decline in weight over the 12 weeks than the nonwater group. Thus, when combined with a hypocaloric diet, consuming 500 mL water before each main meal leads to greater weight loss than a hypocaloric diet alone in middle-aged and older adults.[56]

The Atkins Diet

Although literally hundreds of fad diets have been promoted over the years, we would be remiss if we did not mention the most famous weight-loss diet of all time—the "Atkins Diet." This high-protein, high-fat, low-carbohydrate diet was developed by Robert Atkins during the 1960s. In the early 1990s, Atkins brought his diet back into the nutrition spotlight with the publication of his best-selling book *Dr. Atkins' New Diet Revolution.* An estimated 50 million people or more worldwide have tried the Atkins Diet, which emphasizes the consumption of protein and fat. Individuals following the Atkins Diet are permitted to eat unlimited amounts of all meats, poultry, fish, eggs, and most cheeses.

The Atkins Diet is divided into four phases: induction, ongoing weight loss, premaintenance, and maintenance. During the induction phase (the first 14 days of the diet), carbohydrate intake is limited to no more than 20 g per day. No fruit, bread, grains, starchy vegetables, or dairy products except cheese, cream, and butter are allowed during this phase. During the ongoing weight-loss phase, dieters experiment with various levels of carbohydrate consumption until they determine the most liberal level of carbohydrate intake that allows them to continue to lose weight. Dieters are encouraged to maintain this level of carbohydrate intake until their weight loss goals are met. Then, during the premaintenance and maintenance phases, dieters determine the level of carbohydrate consumption that allows them to maintain their weight. To avoid regaining weight, dieters must stick to this level of carbohydrate consumption, perhaps for the rest of their lives.

Although we agree with the underlying principle of the Atkins Diet, that diets high in sugar and refined carbohydrates cause weight gain and ultimately lead to obesity, we disagree with the more extreme interpretations of application. One of the big reasons why the Atkins Diet is so attractive to dieters who have tried unsuccessfully to lose weight on low-fat, low-calorie diets is that while on the Atkins Diet, they can eat as many calories as desired from protein and fat as long as carbohydrate consumption is restricted. Therefore many Atkins dieters are spared the feelings of hunger and deprivation that accompany other weight-loss regimens. However, we do not agree that such an incomplete diet is conducive to long-term health. In addition, the lack of differentiation between healthy and unhealthy high-protein/high-fat foods, in the long run, is not an effective approach to eating. This likely helps explain the limited benefits found, as discussed herein.

Despite its enormous popularity, the Atkins program was not evaluated in a proper clinical trial until 2003. In this initial study, although people following the Atkins Diet did experience initial weight loss—although likely as a result of water loss rather than true fat loss—in the long run, they gained it all back, plus more. In the study, 63 obese men and women were randomly assigned to the Atkins Diet or a low-calorie, high-carbohydrate, low-fat diet. Professional contact was minimal, so as to replicate the approach used by most dieters. Although subjects on the Atkins Diet had lost more weight than subjects on the conventional diet at 6 months, the difference at 12 months was not significant. Adherence was poor, and attrition was high in both groups.[57]

Since this initial clinical evaluation, other studies have shown similar results. For example, in one study of 34 adults with impaired glucose tolerance, 12 weeks of a low-fat (18% of total calories), high–complex carbohydrate (more than 62% of total calories) diet alone (High-CHO) or paired with an aerobic exercise training program (High-CHO + Ex) was compared with the effects of an Atkin's-style diet (41% fat, 14% protein, 45% carbohydrate of total calories; the control group). Fiber intake averaged 58 to 61 g/day in the two high-carbohydrate groups versus 18.5 g/day in the control group. In the High-CHO + Ex group, participants engaged in aerobic exercise for 45 minutes a day, 4 days per week, at 80% of peak oxygen consumption. All participants were instructed to consume food ad libitum. Although caloric intake was similar in all three groups, both High-CHO groups (with and without exercise) lost more weight (mean loss 10.5 lb with exercise and 7

lb without exercise than the control group (mean loss: a fraction of 1 lb). Similarly, a higher percentage of body fat was lost by the High-CHO + Ex (3.5%) group and the High-CHO without exercise group (2.2%) than by controls (0.2% increase in body fat). Thigh fat area also decreased significantly in both of the High-CHO groups but not in the control group. The resting metabolic rate and rate of fat oxidation were not decreased in the High-CHO or control groups.[58]

In another study, 132 obese adults (BMI >35), of whom 83% had type 2 diabetes or metabolic syndrome, were counseled to consume either an Atkins-like diet limited to less than 30 g of carbohydrates per day or to a diet restricted by 500 kcal per day with less than 30% of these calories coming from fat. Although initially the Atkins Diet did promote weight loss during the first 6-month period, this effect began to disappear during the second 6-month period. At 12 months, the difference between average weight loss in the two groups was no longer statistically significant (11 lb in the Atkins group vs. 7 lb in the low-fat group), although changes in triglyceride levels favored the Atkins Diet (−57 mg/dL vs. +4 mg/dL), as did reductions in hemoglobin A_{1c} (−0.7 vs. −0.1%) in the patients with type 2 diabetes.[59]

Another study worth commenting on was funded by the Atkins Foundation. In this study, 120 overweight but otherwise healthy adult subjects with elevated lipid levels followed either the Atkins Diet or a diet containing fewer than 30% calories from fat, 10% or fewer calories from saturated fat, less than 300 mg cholesterol, and a deficit of 500 to 1000 calories. At 24 weeks, the Atkins group had lost a mean of 26 lb versus a mean loss of 14 lb in the reduced-fat group. Triglyceride levels fell more in the Atkins group (−74 mg/dL) than in the restricted-fat group (+28 mg/dL) as well, and high-density-lipoprotein cholesterol (HDL-C) levels increased in the Atkins group (by 5.5 mg/dL) and decreased in the low-fat group (by 1.6 mg/dL). The main criticism of this study was that the "low-fat" group received almost 30% of their caloric intake from fat, and the dietitians administering the dietary recommendations made no clear attempt to significantly restrict sugar and refined carbohydrates. Thus the control diet with which the Atkins Diet was compared was significantly less than ideal.[60]

In 2014 a systematic review and meta-analysis of low-carbohydrate diets (vs. isoenergetic balanced diets) found little to no difference in weight loss between the two diet types at both 3 to 6 months and 1 to 2 years. Additionally, this analysis of 19 trials and over 3000 participants found little or no difference in cardiovascular risk factors among overweight/obese participants at 2 years, follow-up, including lipids, blood pressure, and fasting glucose, both among diabetics and nondiabetics.[61]

The findings from these clinical trials indicate that although adhering strictly to the Atkins Diet (dramatically reducing carbohydrate intake while allowing free access to high-fat and high-protein foods) sometimes leads to more weight loss in the first 6 months, eating a more healthful diet, as described in Chapter 44, is associated with equal efficacy in the long run and is considerably more health-promoting. In some but not all trials, the low-carbohydrate diet was associated with a greater improvement in some risk factors, but this benefit was not maintained. On the basis of the current evidence, we do not recommend the Atkins Diet. Furthermore, because the high protein content of the Atkins Diet places stress on the liver and kidneys, we do not recommend it for anyone with impaired liver or kidney function.

Natural Weight-Loss Aids

Several natural weight loss aids can be useful in helping either to reduce appetite or enhance metabolism. In decreasing order of efficacy, we rate these items as follows:

- Meal-replacement formulas
- Fiber supplements
- Chromium
- Medium-chain triglycerides
- Hydroxycitrate
- 5-hydroxytryptophan (5-HTP)

Meal-Replacement Formulas

Meal-replacement formulas are a popular weight-loss strategy, and their effectiveness has been confirmed in several clinical trials.[62–65] In these studies, dietary compliance and convenience were viewed more favorably by participants who consumed meal-replacement (MR) formulas than by those in a conventional weight-loss program. Ideally, these formulations should provide high-quality nutrition; they should be high in protein and low in glycemic load and high in viscous, soluble fiber. A protein target of 2.2 g protein/kg lean body mass (LBM) per day produces greater fat loss than 1.1 g protein/kg LBM per day.[66]

Medifast is a popular physician-supervised weight-loss program that relies heavily on MR formulas. In one 40-week randomized controlled clinical trial that included 90 obese adults with a BMI between 30 and 50, subjects were randomly assigned to one of two weight-loss programs for 16 weeks and then followed for a 24-week period of weight maintenance. The dietary interventions consisted of an MR program (Medifast) or a self-selected isocaloric food-based (FB) meal plan. The Medifast plan included 5 MRs (90–110 kcal/each), 5 to 7 oz lean protein, 1½ cups of nonstarchy vegetables, and up to two fat servings daily (providing 800–1000 kcal). The MRs used in this study were low in fat, low in glycemic index (GI), and low in sugar, providing a balanced ratio of carbohydrates to proteins; they were based on either soy and/or whey protein. The food-based plan included 3 oz of grains, 1 cup of vegetables, 1 cup of fruit, 2 cups of milk, 5 to 7 oz of lean protein, and 3 tsp of fat daily (providing about 1000 kcal/day). The FB group was also instructed to take a multivitamin and additional calcium to ensure that micronutrient needs were met. Weight loss at 16 weeks was significantly better in the Medifast group (MD) versus the FB group, 12.3% versus 6.9%, respectively. Significantly more of the MD participants lost 5% or more of their initial weight at week 16 (93% vs. 55%) and week 40 (62% vs. 30%). Significant improvements in body composition were also observed in MD participants compared with those in the FB group at weeks 16 and 40. At week 40, a mean body-fat percentage decrease in the MD group was 2.9%, whereas the FB group decreased by 1.8%. Moreover, lean muscle mass as a percentage of total weight was significantly increased by 4.5% from baseline to week 40 in the MD group, whereas the FB group did not experience any significant change. Blood pressure also dropped. At week 40, systolic blood pressure had dropped by 6.0 mm Hg (4.5%) in the MD group and 8.3 mm Hg in the FB group. Diastolic blood pressure was decreased by 5.5 mm Hg (6.2%) in the MD group and 0.9 mm Hg (0.45%) in the FB group. One advantage for the FB group was in the reduction of C-reactive protein (CRP). At week 40, CRP levels in the FB group decreased by a mean of 4.1 (30.1%), whereas in the MD group they decreased by a mean of 3.1 (40.7%).[67]

Fiber Supplements

A tremendous amount of clinical evidence indicates that increasing the amount of dietary fiber promotes weight loss. The best supplemental fiber sources for weight loss are PGX (see following discussion), glucomannan, gum karaya, psyllium, chitin, guar gum, and pectin because they are viscous, water-soluble fibers. When taken with water before meals, these fiber sources bind to the water in the stomach to form a gelatinous mass that induces a sense of satiety. As a result, individuals are less likely to overeat.

The benefits of fiber go well beyond this mechanical effect, however. Fiber supplements have been shown to enhance blood sugar

TABLE 201.3 Clinical Studies With Dietary Fiber Supplements

Fiber	No. of Subjects	Length of Study	Dosage (g/Day)	Calorie Restriction	Average Weight Loss (Fiber, lb)	Average Weight Loss (Placebo Group)	Reference
Guar	9	2 mo	20	None	9.4	No placebo group	51
Guar	7	1 yr	20	None	61.9	No placebo group	52
Guar	21	2.5 mo	20	None	15.6	No placebo group	53
Guar	33	2.5 mo	15	None	5.5	0.9 lb in placebo group	54
Glucomannan	20	2 mo	3	None	5.5	Weight gain of 1.5 lb	55
Glucomannan	20	2 mo	3	None	8.14	0.44 lb in placebo	56
Citrus pectin	14	4 wk	5.56	Yes	12.8	No placebo group	57
Mixture A	60	12 wk	5	Yes	18.7	14.7 lb in placebo group	58
Mixture A	89	11 wk	10	Yes	13.9	9.2 lb in placebo group	59
Mixture B	45	3 mo	7	Yes	13.6	9 lb in placebo group	60
Mixture B	97	3 mo	7	Yes	10.8	7.3 lb in placebo group	61
Mixture B	52	6 mo	7	Yes	12.1	6.1 lb in placebo group	62

Mixture A = 80% fiber from grains, 20% fiber from citrus; mixture B = 90% insoluble and 10% soluble fiber from beet, barley, and citrus.

control, decrease insulin levels, and reduce the number of calories absorbed by the body. In some of the clinical studies demonstrating weight loss, fiber supplements were shown to reduce the number of calories absorbed by 30 to 180 per day. Although modest, this reduction in calories would, over the course of a year, result in a 3- to 18-lb weight loss.[68,69]

Several studies have used guar gum, a water-soluble fiber obtained from the Indian cluster bean *(Cyamopsis tetragonoloba)*, with good results (Table 201.3).[70–81] In one study, nine women weighing between 160 and 242 lb were given 10 g of guar gum immediately before lunch and dinner. They were told not to consciously alter their eating habits. After 2 months, the women reported an average weight loss of 9.4 lb. Reductions were also noted for cholesterol and triglyceride levels.[70]

Studies with soluble fiber in the treatment of elevated cholesterol levels have shown a dose-dependent effect.[68,69] Dietary fiber supplements appear to exert a dose-dependent effect in weight-loss studies as well. Therefore, to achieve the greatest benefit, the dosage should be as high as possible. Because water-soluble fibers are fermented by intestinal bacteria, a great deal of gas can be produced, leading to increased flatulence and abdominal discomfort. Patients should be advised to start out with a dosage between 1 and 2 g before meals and at bedtime and to gradually increase the dosage to 5 g.

Several studies have used PGX (PolyGlycopleX), a novel natural polysaccharide matrix composed of three natural compounds (glucomannan, alginate, and xanthan gum) combined in a proprietary process that leads them to coalesce and form an entirely new matrix that is the most viscous fiber known to date.[82,83] Detailed clinical studies have now shown PGX to exert the following benefits:

- Reduce appetite and promote effective weight loss[84,85]
- Increase the level of compounds that block the appetite and promote satiety[86]
- Decrease the level of compounds that stimulate overeating[84]
- Reduce postprandial (after-meal) and delayed (second meal) postprandial blood glucose levels when added to or taken with foods[87,88]
- Reduce the glycemic index of any food or beverage by 15% to 70%[87,88]
- Increase insulin sensitivity and decrease blood insulin levels[89]
- Stabilize blood sugar control in the overweight and obese individuals
- Lower blood cholesterol and triglycerides[84,85]

A 2017 three-arm, 12-week clinical study using rice bran as the control showed that the granule form of PGX was the most effective in terms of weight loss, decreased waist size, and interestingly, reduced number of meals a day.[90]

PGX is available in a soft gelatin capsule, as granules to be added into food and beverages, and in a meal-replacement drink mix. The typical dosage is 2.5 to 5 g before each meal. PGX has an excellent safety profile.[91] As with other sources of dietary fiber, to avoid minor side effects—such as increased gas, bloating, loose stools, or constipation—it is best to start with small amounts and then gradually increase the dosage within a few days to a week. Clinical experience and studies suggest the granules are less expensive, are more effective, and achieve better patient compliance.

Chromium

One of the key goals for enhancing weight loss is to increase the sensitivity of body cells to insulin. Chromium plays a key role in cellular sensitivity to insulin. In some clinical studies in individuals with diabetes, supplementing the diet with chromium has been shown to decrease fasting glucose levels, improve glucose tolerance, lower insulin levels, and decrease total cholesterol and triglyceride levels while also increasing the levels of HDL-C (see Chapter 165). Chromium supplementation has been demonstrated to lower body weight yet increase lean body mass, presumably as a result of increased insulin sensitivity. A meta-analysis of 11 randomized trials found that chromium supplementation generated statistically significant reductions in body weight, although the effects were small, indicating that chromium alone is unlikely to produce meaningful results but should be combined with other therapies.[92] In one study, patients were given chromium bound to picolinic acid (chromium picolinate) in one of three doses daily for 2.5 months: placebo, 200 mcg, or 400 mcg.[93] Patients taking the 200- and 400-mcg doses lost an average of 4.2 lb of fat. The group taking the placebo lost only 0.4 lb. Even more impressive was the fact that the chromium groups gained more muscle (1.4 vs. 0.2 lb) than those taking the placebo. The results were most striking in elderly subjects and in men. The men taking chromium picolinate lost more than seven times the amount of body fat as those taking the placebo (7.7 vs. 1 lb). However, an increase in lean body weight percentage with chromium supplementation may be limited to men; two clinical trials in women involved in an exercise program did not show any significant changes in body composition with chromium supplementation.[94,95] The 400-mcg dosage is more effective than the 200-mcg dosage (Table 201.4). Several

TABLE 201.4 Effects of 200 Versus 400 mcg/day of Chromium Picolinate for 2.5 Months

Dosage	Fat Loss	Muscle Gain	Total Weight Loss
200 mcg chromium picolinate	–3.3 lb	+1.5 lb	–1.8 lb
400 mcg chromium picolinate	–4.6 lb	+1.1 lb	–3.5 lb

recent studies with chromium picolinate at dosages of 1000 mcg/day did not show any benefits, indicating that the effect of chromium is probably limited to restoring and maintaining optimal tissue concentrations of chromium versus any pharmacological effect.[96,97] Higher dosages are not likely to show any greater effect than 400 mcg daily.

Medium-Chain Triglycerides

Medium-chain triglycerides (MCTs) are saturated fats (extracted from coconut oil) that range in length from 6 to 12 carbon chains. MCTs are used by the body differently from the long-chain triglycerides (LCTs), which are the most abundant fats found in nature. LCTs are the storage fats for both humans and plants and range in length from 18 to 24 carbons. This difference in length makes a substantial difference in how MCTs and LCTs are metabolized. Unlike regular fats, MCTs appear to promote weight loss rather than weight gain.

MCTs may promote weight loss by increasing thermogenesis and energy expenditure.[98,99] In contrast, the LCTs are usually stored in the fat deposits, and because their energy is conserved, a high-fat diet tends to decrease the metabolic rate. In one study, the thermogenic effect of a high-calorie diet containing 40% fat as MCTs was compared with one containing 40% fat as LCTs. The thermogenic effect (calories wasted 6 hours after a meal) of the MCTs was almost twice as high as that of the LCTs—120 versus 66 calories. The researchers concluded that the excess energy provided by fats in the form of MCTs would not be efficiently stored as fat but rather wasted as heat.[101] A second study demonstrated that MCT oil given over a 6-day period can increase diet-induced thermogenesis by 50%.[102]

In another study, researchers compared single meals of 400 calories composed entirely of MCTs or LTCs.[103] The thermic effect of MCTs over 6 hours was three times greater than that of LCTs. In addition, whereas the LCTs elevated blood fat levels by 68%, MCTs had no effect on the blood fat level. Researchers concluded that the substitution of MCTs for LCTs would produce weight loss as long as the caloric level remained the same.

In one double-blind study, 101 hypertriacylglycerolemic subjects were randomly assigned to ingest 25 to 30 g/day of MCT or LCT oil as the only cooking oil for 8 consecutive weeks. Compared with subjects having BMIs between 24 and 28 in the LCT group, corresponding subjects in the MCT group showed significantly greater decreases in body weight, BMI, body fat, waist circumference (WC), ratio of WC to hip circumference (HC), total fat area and subcutaneous fat area in the abdomen, as well as blood triglycerides and low-density-lipoprotein cholesterol (LDL-C) levels at week 8.[104] A 2015 meta-analysis of 13 trials found that compared with LCTs, MCTs reduced weight (by approximately 1 lb) and were associated with other improvements in body composition, including lower waist and hip circumference and total body and visceral fat (although pointing out that larger trials from independent funding sources are needed).[105]

In order to gain the benefit from MCTs, a diet must remain low in LCTs. MCTs can be used as oils for salad dressing, bread spreads, or simply taken as supplements. A good dosage recommendation for MCTs is 1 to 2 tbsp/day.

Patients with diabetes and individuals with liver disease should be monitored closely when using MCTs because they may develop ketoacidosis.

Hydroxycitrate

Hydroxycitrate (HCA) is a natural substance isolated from the fruit of the Malabar tamarind *(Garcinia cambogia)*. This is a yellowish fruit about the size of an orange with a thin skin and deep furrows, similar to an acorn squash. It is native to southern India, where it is dried and used extensively in curries. The dried fruit contains about 30% hydroxycitric acid.

HCA has been shown to be a powerful lipogenic inhibitor in animals.[106,107] Whether or not it demonstrates this effect in humans has not yet been proven. The weight-loss–promoting effects in animals are perhaps best exemplified in a study that shows HCA producing a "significant reduction in food intake, and body weight gain" in rats.[108] HCA may not only be a powerful inhibitor of fat production but also an appetite suppressant. It is critical, when using an HCA formula, that a low-fat diet be maintained because HCA only inhibits the conversion of carbohydrates into fat.

By itself, HCA may offer a safe, natural aid for weight loss when taken at a dosage of 1500 mg three times daily. In two clinical trials, a total of 90 moderately obese subjects (ages 21–50, BMI >26) were randomly divided into three groups. Group A was administered HCA 4667 mg; group B was administered a combination of HCA 4667 mg, niacin-bound chromium 4 mg, and *Gymnema sylvestre* extract 400 mg; and group C was given a placebo daily in three equally divided doses 30 to 60 minutes before meals. All subjects were provided with a diet of 2000 kcal/day and participated in a supervised walking program for 30 min/day, 5 days/week. Eighty-two subjects completed the study. At the end of 8 weeks, both body weight and BMI decreased by 5.4% in group A; LDL-C and triglycerides were reduced by 12.9% and 6.9%, respectively; HDL-C levels increased by 8.9%; serum leptin levels decreased by 38%; serotonin levels increased by 44.5%; and urinary excretion of fat metabolites increased by 32% to 109%. Group B demonstrated similar beneficial changes, but generally to a greater extent. Specifically, group B reduced body weight and BMI by 7.8% and 7.9%, respectively; food intake was reduced by 14.1%; total cholesterol, LDL-C, and triglyceride levels were reduced by 9.1%, 17.9%, and 18.1%, respectively; and HDL-C and serotonin levels increased by 20.7% and 50%, respectively. Serum leptin levels decreased by 40.5%, and enhanced excretion of urinary fat metabolites increased by 146% to 281%. No significant adverse effects were observed in either trial.[109] Close attention to product quality may be particularly important because a number of safety concerns have emerged, specifically for hepatotoxicity. These appear to be limited to specific products containing numerous ingredients in addition to HCA (*Hydroxycut* has been named by the U.S. Food and Drug Administration [FDA] as a possible hepatotoxin). Furthermore, some concerns have been raised about the potential for elevated serotonin levels, so caution should be used in those patients taking serotonin-elevating medications.[110]

5-Hydroxytryptophan

More than three decades ago, researchers demonstrated that the administration of 5-HTP to rats genetically bred to overeat and be obese resulted in a significant reduction in food intake. Further research revealed that these rats had decreased activity of tryptophan hydroxylase, which converts tryptophan to 5-HTP, itself subsequently converted to serotonin. In other words, these rats were fat as a result of a genetically determined low level of activity of the enzyme that starts the manufacture of serotonin from tryptophan. Hence, these rats never got the message to stop eating until they had consumed far greater

amounts of food than normal rats. Circumstantial evidence indicates that many humans are also genetically predisposed to obesity, a predisposition that may involve the same mechanism as that in rats genetically predisposed to obesity (i.e., decreased conversion of tryptophan to 5-HTP and, as a result, decreased serotonin levels). By providing preformed 5-HTP, this genetic defect is bypassed, and more serotonin is manufactured. (For a complete discussion of this interesting nutrient, see Chapter 87.)

The early animal studies with 5-HTP as a weight-loss aid have been followed by a series of three human clinical studies in overweight women.[111–113] The first study showed that 5-HTP was able to reduce caloric intake and promote weight loss despite the fact that the women made no conscious effort to lose weight.[111] The average amount of weight lost during the 5-week period of 5-HTP supplementation was a little more than 3 lb.

The second study sought to determine whether 5-HTP helped overweight individuals adhere to dietary recommendations.[111] This 12-week study was divided into two 6-week periods. For the first 6 weeks, there were no dietary recommendations, and for the second 6 weeks, the women were placed on a 1200-calorie diet. As shown in Table 201.5, the women taking the placebo lost 2.28 lb, whereas the women taking the 5-HTP lost 10.34 lb.

TABLE 201.5 Impact of 5-HTP on Weight Loss

	Placebo	5-HTP Group
Weight (lb)		
Baseline	207.68	229.46
After 6 weeks	206.58	225.94
After 12 weeks	205.4	219.12
Total Weight Loss (lb)		
After 6 weeks	1.1	3.52
After 12 weeks	2.28	10.34

As in the previous study, 5-HTP appeared to promote weight loss by promoting satiety, leading to the consumption of fewer calories at meals. All the women taking 5-HTP reported early satiety.

The third study with 5-HTP was similar to the second study: for the first 6 weeks, there were no dietary restrictions, and for the second 6 weeks, the women were placed on a diet of 1200 calories per day.[113] The group receiving the 5-HTP lost an average of 4.39 lb after the first 6 weeks and an average of 11.63 lb after 12 weeks. In comparison, the placebo group lost an average of only 0.62 lb after the first 6 weeks and 1.87 lb after 12 weeks. The lack of weight loss during the second 6-week period in the placebo group obviously reflects the fact that the women had difficulty adhering to the diet.

Early satiety was reported by 100% of the subjects during the first 6-week period. During the second 6-week period, even with severe caloric restriction, 90% of the women taking 5-HTP reported early satiety. Many of the women receiving the 5-HTP (300 mg three times daily) reported mild nausea during the first 6 weeks of therapy. However, the symptom was never severe enough for any of the women to drop out of the study. No other side effects were reported.

Detox Support

Given the large role toxins appear to play in obesity and the metabolic and mitochondrial dysfunction associated with it, as well as the increased toxin release into the bloodstream during weight loss, providing support for detoxification pathways during weight loss seems warranted (see Chapter 35, Environmental Medicine, for more detailed discussion). Support for glutathione conjugation appears particularly important because glutathione is known to be depleted by POP exposure and is necessary for the conjugation and elimination of many POPs, including PCBs and organochlorine pesticides.[114] Indeed, serum γ-glutamyltransferase (GGT) levels have been suggested as one possible biomarker for POP exposure and have been shown to modulate the influence of BMI on impaired fasting glucose (IFG). Data from over 6000 participants in the Fifth Korean National Health and Nutrition Examination Survey found that those with a BMI over 25 had approximately a 1.5-fold increase in the prevalence of IFG if they were in the lowest tertile of GGT. However, in the highest tertile of GGT, that risk jumped to approximately fivefold (see Fig. 201.7).

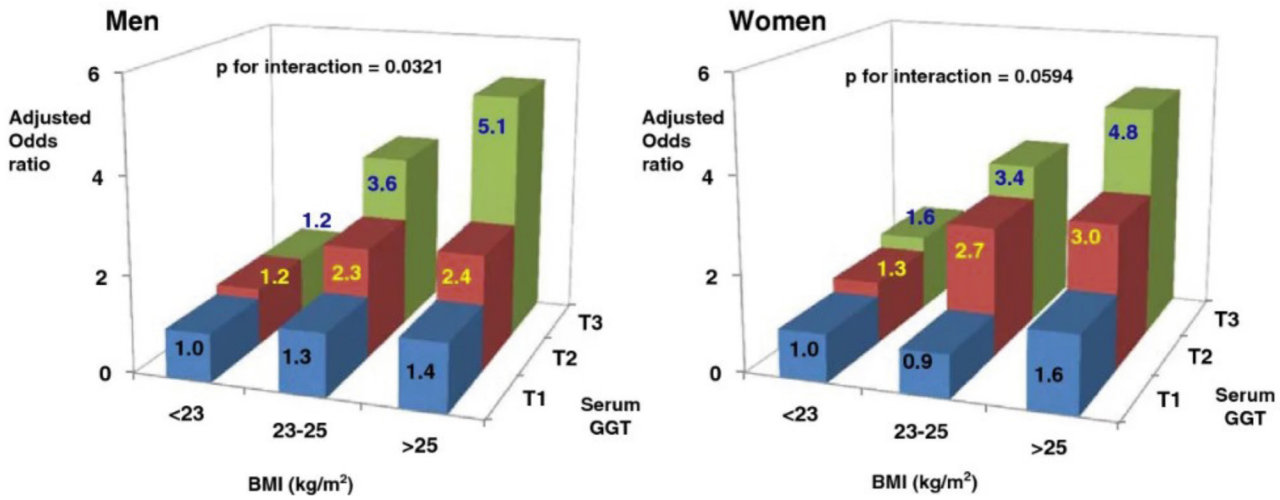


Fig. 201.7 Adjusted odds ratios by tertiles (T) of serum γ-glutamyltransferase (GGT) and category of body-mass index (BMI). BMI was classified into three categories (<23, 23–25, and >25 kg/m^2), and serum GGT was categorized into gender-specific tertiles. T1: first tertile; T2: second tertile; T3: third tertile. (From Hong NS, Kim JG, Lee YM, et al. Different associations between obesity and impaired fasting glucose depending on serum gamma-glutamyltransferase levels within normal range: a cross-sectional study. *BMC Endocr Disord*. 2014 Jul 12;14:57. PMID: 25015117). Reproduced from an Open Access article distributed under the terms of the Creative Commons Attribution License [http://creativecommons.org/licenses/by/4.0].)

In addition to supporting glutathione production, enhancing toxin elimination should also be emphasized. This may be done with the use of supplemental fiber to prevent enterohepatic recirculation of these toxins, and emerging evidence suggests it may also be done with sauna therapy. A number of compounds, including PCBs and phthalates, have been shown to be released into the sweat, and the amount of sweat loss during sauna therapy may be directly related to BMI.[115–116]

THERAPEUTIC APPROACH

A successful program for weight loss must be consistent with the four cornerstones of good health:

- Proper diet
- Adequate exercise
- A positive mental attitude
- The right support for the body through natural measures

All of these components are critical and interrelated.

Diet

The recommendations given in Chapter 44 should be followed. Adequate protein is very important, 2 g per kg of body weight. Emphasis should be placed on calorie reduction, with the intake of healthy complex carbohydrates—especially those rich in fiber—positively associated with weight loss over time.

Psychological Support

Referral to counseling should be considered. Overweight individuals tend to suffer heavy assaults on their self-esteem and self-image.

Lifestyle

Exercise is absolutely critical to an effective weight-loss program. Recommendations are given in Chapter 36.

Supplements:

- Viscous, soluble fiber (e.g., PGX): 2.5 to 5 g before meals
- 5-HTP: begun at 50 to 100 mg 20 minutes before meals for 2 weeks and then doubled (to a maximum of 300 mg) if weight loss is less than 1 lb/week. Higher dosages of 5-HTP (e.g., 300 mg) are associated with nausea, but this symptom disappears after 6 weeks of use.
- Chromium: 200 to 400 mcg/day
- Medium-chain triglycerides: 1 to 2 tbsp incorporated into the diet
- Glutathione support: N-acetylcysteine 600 mg twice per day

Botanical Medicine

- Hydroxycitrate (from *G. cambogia*): 1500 mg three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jia H, Lubetkin EI. Trends in quality-adjusted life-years lost contributed by smoking and obesity. *Am J Prev Med*. 2010;38(2):138–144.
2. Jia H, Lubetkin EI. Obesity-related quality-adjusted life years lost in the U.S. from 1993 to 2008. *Am J Prev Med*. 2010;39(3):220–227.
3. Gába A, Přidalová, M. Diagnostic performance of body mass index to identify adiposity in women. *Eur J Clin Nutr*. 2016;70(8):898–903. PMID: 26669574.
4. Okorodudu DO, Jumean MF, Montori VM, et al. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta-analysis. *Int J Obes (Lond)*. 2010;34(5):791–799. PMID: 20125098.
5. Hales CM, Carroll MD, Fryar CD, et al. Prevalence of Obesity Among Adults and Youth: United States, 2015-2016. *NCHS Data Brief*. 2017;(288):1–8. PMID: 29155689.
6. Centers for Disease Control. Vital signs: state-specific obesity prevalence among adults – United States, 2009. *MMWR Morb Mortal Wkly Rep*. 2010;59:951–955.
7. Flegal KM, Carroll MD, Ogden CL, et al. Prevalence and trends in obesity among U.S. adults, 1999–2008. *JAMA*. 2010;303(3):235–241.
8. Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of high body mass index in U.S. children and adolescents, 2007-2008. *JAMA*. 2010;303(3):242–249.
9. Berrington de Gonzalez A, Hartge P, Cerhan JR, et al. Body-mass index and mortality among 1.46 million white adults. *N Engl J Med. Dec*. 2010;363(23):2211–2219.
10. Finkelstein EA, Trogdon JG, Cohen JW, et al. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Affairs*. 2009;5:W822–W831.
11. Li Q, Blume SW, Huang JC, et al. The Economic Burden of Obesity by Glycemic Stage in the United States. *Pharmacoeconomics*. 2015;33(7):735–748. PMID: 25564434.
12. Galić BS, Pavlica T, Udicki M, et al. Somatotype characteristics of normal-weight and obese women among different metabolic subtypes. *Arch Endocrinol Metab*. 2016;60(1):60–65. PMID: 26909484.
13. Hammarstedt A, Gogg S, Hedjazifar S, et al. Impaired adipogenesis and dysfunctional adipose tissue in human hypertrophic obesity. *Physiol Rev*. 2018;98(4):1911–1941. PMID: 30067159.
14. Singh RK, Kumar P, Mahalingam K. Molecular genetics of human obesity: a comprehensive review. *C R Biol*. 2017;340(2):87–108. PMID: 28089486.
15. Matthews CE, Chen KY, Freedson PS, et al. Amount of time spent in sedentary behaviors in the United States, 2003-2004. *Am J Epidemiol*. 2008;167(7):875–881. PMID: 18303006.
16. Hancox RJ, Milne BJ, Poulton R. Association between child and adolescent television viewing and adult health: a longitudinal birth cohort study. *Lancet*. 2004;364:257–262.
17. Epstein LH, Roemmich JN, Robinson JL, et al. A randomized trial of the effects of reducing television viewing and computer use on body mass index in young children. *Arch Pediatr Adolesc Med*. 2008;162(3):239–245. PMID: 18316661.
18. Hu FB, Li TY, Colditz GA, et al. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA*. 2003;289:1785–1791.
19. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes*. 2004;53(suppl 1):S143–S151.
20. Guilherme A, Virbasius JV, Puri V, et al. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol*. 2008;9(5):367–377.
21. Small CJ, Bloom SR. Gut hormones and the control of appetite. *Trends Endocrinol Metab*. 2004;15:259–263.
22. Batterham RL, Cohen MA, Ellis SM, et al. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med*. 2003;349:941–948.
23. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med*. 2002;346:1623–1630.
24. Burton-Freeman B, Davis PA, Schneeman BO. Plasma cholecystokinin is associated with subjective measures of satiety in women. *Am J Clin Nutr*. 2002;76:659–667.
25. Bourdon I, Olson B, Backus R, et al. Beans, as a source of dietary fiber, increase cholecystokinin and apolipoprotein b48 response to test meals in men. *J Nutr*. 2001;131:1485–1490.
26. Heini AF, Lara-Castro C, Schneider H, et al. Effect of hydrolyzed guar fiber on fasting and postprandial satiety and satiety hormones: a double-blind, placebo-controlled trial during controlled weight loss. *Int J Obes Relat Metab Disord*. 1998;22:906–909.
27. Naslund E, Barkeling B, King N, et al. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord*. 1999;23:304–311.
28. Cohen MA, Ellis SM, Le Roux CW, et al. Oxyntomodulin suppresses appetite and reduces food intake in humans. *J Clin Endocrinol Metab*. 2003;88:4696–4701.
29. Batterham RL, Le Roux CW, Cohen MA, et al. Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab*. 2003;88:3989–3992.
30. Batterham RL, Cohen MA, Ellis SM, et al. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med*. 2003;349:941–948.
31. Reimer RA, Pelletier X, Carabin IG, et al. Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur J Clin Nutr*. 2010;64(10):1186–1191. PMID: 20664618.
32. Cummings DE, Frayo RS, Marmonier C, et al. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab*. 2004;287:E297–E304.
33. Anderwald C, Brabant G, Bernroider E, et al. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. *Diabetes*. 2003;52:1792–1798.
34. Laville M, Cornu C, Normand S, et al. Decreased glucose-induced thermogenesis at the onset of obesity. *Am J Clin Nutr*. 1993;57:851–856.
35. Ravussin E, Acheson KJ, Vernet O, et al. Evidence that insulin resistance is responsible for the decreased thermic effect of glucose in human obesity. *J Clin Invest*. 1985;76:1268–1273.
36. Nelson KM, Weinsier RL, James LD, et al. Effect of weight reduction on resting energy expenditure, substrate utilization, and the thermic effect of food in moderately obese women. *Am J Clin Nutr*. 1992;55:924–933.
37. Schulz LO. Brown adipose tissue: regulation of thermogenesis and implications for obesity. *J Am Diet Assoc*. 1987;87:761–764.
38. Scheele C, Nielsen S. Metabolic regulation and the anti-obesity perspectives of human brown fat. *Redox Biol*. 2017;12:770–775. PMID: 28431377.
39. Chondronikola M, Volpi E, Børsheim E, et al. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes*. 2014;63(12):4089–4099. PMID: 25056438.
40. Sims EA, Danforth Jr E, Horton ES, et al. Endocrine and metabolic effects of experimental obesity in man. *Recent Prog Horm Res*. 1973;29:457–496.
41. Leibel RL, Hirsch J. Diminished energy requirements in reduced obese patients. *Metabolism*. 1984;33:164–170.
42. Eck LH, Klesges RC, Hanson CL, et al. Children at familial risk for obesity: an examination of dietary intake, physical activity, and weight status. *Int J Obes Relat Metab Disord*. 1992;16:71–78.
43. Nakhuda A, Josse AR, Gburcik V, et al. Biomarkers of browning of white adipose tissue and their regulation during exercise- and diet-induced weight loss. *Am J Clin Nutr*. 2016;104(3):557–565. PMID: 27488235.
44. Wurtman RJ, Wurtman JJ. Brain serotonin, carbohydrate-craving, obesity and depression. *Adv Exp Med Biol*. 1996;398:35–41.
45. Wurtman J, Suffes S. *The Serotonin Solution*. New York: Fawcett Columbine.
46. Goodwin GM, Cowen PJ, Fairburn CG, et al. Plasma concentrations of tryptophan and dieting. *BMJ*. 1990;300:1499–1500.

47. Lee DH, Porta M, Jacobs Jr DR, et al. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. *Endocr Rev*. 2014;35(4):557–601. PMID: 24483949.
48. Cheikh Rouhou M, Karelis AD, St-Pierre DH, et al. Adverse effects of weight loss: Are persistent organic pollutants a potential culprit? *Diabetes Metab*. 2016;42(4):215–223. PMID: 27321206.
49. Hong NS, Kim KS, Lee IK, et al. The association between obesity and mortality in the elderly differs by serum concentrations of persistent organic pollutants: a possible explanation for the obesity paradox. *Int J Obes (Lond)*. 2012;36(9):1170–1175. PMID: 21946706.
50. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med*. 2011;364(13):1218–1229.
51. Hunter GR, Brock DW, Byrne NM, et al. Exercise training prevents regain of visceral fat for 1 year following weight loss. *Obesity*. 2010;18(4):690–695.
52. Ma C, Avenell A, Bolland M, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ*. 2017;359:j4849. PMID: 29138133.
53. Wing RR, Phelan S. Long-term weight loss maintenance. *Am J Clin Nutr*. 2005;82(suppl 1):S222–S225.
54. Sylvetsky AC, Edelstein SL, Walford G, et al. A high-carbohydrate, high-fiber, low-fat diet results in weight loss among adults at high risk of type 2 diabetes. *J Nutr*. 2017;147(11):2060–2066. PMID: 28954840.
55. Larsen TM, Dalskov SM van Baak M, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med*. 2010;363(22):2102–2113.
56. Dennis EA, Dengo AL, Comber DL, et al. Water consumption increases weight loss during a hypocaloric diet intervention in middle-aged and older adults. *Obesity*. 2010;18(2):300–307.
57. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*. 2003;348:2082–2090.
58. Hays NP, Starling RD, Liu X, et al. Effects of an ad libitum low-fat, high-carbohydrate diet on body weight, body composition, and fat distribution in older men and women. *Arch Intern Med*. 2004;164:210–217.
59. Stern L, Iqbal N, Seshadri P, et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Intern Med*. 2004;140:769–777.
60. Yancy Jr WS, Olsen MK, Guyton JR, et al. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia. *Ann Intern Med*. 2004;140:769–777.
61. Naude CE, Schoonees A, Senekal M, et al. Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. *PLoS One*. 2014;9(7):e100652. PMID: 25007189.
62. Noakes M, Foster PR, Keogh JB, et al. Meal replacements are as effective as structured weight-loss diets for treating obesity in adults with features of metabolic syndrome. *J Nutr*. 2004;134:1894–1899.
63. Ashley JM, St Jeor ST, Perumean-Chaney S, et al. Meal replacements in weight intervention. *Obes Res*. 2001;9(suppl 4):S312–S320.
64. Ditschuneit HH, Flechtner-Mors M. Value of structured meals for weight management: risk factors and long-term weight maintenance. *Obes Res*. 2001;9(suppl 4):S284–S289.
65. Allison DB, Gadbury G, Schwartz LG, et al. A novel soy-based meal replacement formula for weight loss among obese individuals: a randomized controlled clinical trial. *Eur J Clin Nutr*. 2003;57:514–522.
66. Treyzon L, Chen S, Hong K, et al. A controlled trial of protein enrichment of meal replacements for weight reduction with retention of lean body mass. *Nutr J*. 2008;7:23.
67. Davis LM, Coleman C, Kiel J, et al. Efficacy of a meal replacement diet plan compared to a food-based diet plan after a period of weight loss and weight maintenance: a randomized controlled trial. *Nutr J*. 2010;9:11.
68. Howarth NC, Saltzman E, Roberts SB. Dietary fiber and weight regulation. *Nutr Rev*. 2001;59:129–139.
69. Spiller GA. *Dietary Fiber in Health and Nutrition*. Boca Raton, FL: CRC Press.
70. Krotkiewski M. Effect of guar on body weight, hunger ratings and metabolism in obese subjects. *Br J Nutr*. 1984;52:97–105.
71. Krotkiewski M, Smith U. Dietary fibre in obesity. In: eds: Leeds AR, Avenell A. *Dietary Fiber Perspectives: Reviews and Bibliography*. London: John Libbey; 61–66.
72. Krotkiewski M. Effect of guar gum on body-weight, hunger ratings and metabolism in obese subjects. *Br J Nutr*. 1984;52:97–105.
73. Anonymous. Better than oat bran. *Sci News*. 1994;145:28.
74. Walsh DE, Yaghoubian V, Behforooz A. Effect of glucomannan on obese patients: a clinical study. *Int J Obes*. 1984;8:289–293.
75. Biancardi G, Palmiero L, Ghirardi PE. Glucomannan in the treatment of overweight patients with osteoarthrosis. *Curr Ther Res*. 1989;46:908–912.
76. El-Shebini SM, Hanna LM, Topouzada ST, et al. The role of pectin as a slimming agent. *J Clin Biochem Nutr*. 1988;4:255–262.
77. Solum TT, Ryttig KR, Solum E, et al. The influence of a high-fibre diet on body weight, serum lipids and blood pressure in slightly overweight persons: a randomized, double-blind, placebo-controlled investigation with diet and fibre tablets (DumoVital). *Int J Obes*. 1987;11(suppl 1):67–71.
78. Ryttig KR, Larsen S, Haegh L. Treatment of slightly to moderately overweight persons: a double-blind placebo-controlled study with diet and fibre tablets (DumoVital). *Tidsskr Nor Laegeforen*. 1984;104:989–991.
79. Rossner S, von Zwigbergk D, Ohlin A, et al. Weight reduction with dietary fibre supplements: results of two double-blind studies. *Acta Med Scand*. 1987;222:83–88.
80. Ryttig KR, Tellnes G, Haegh L, et al. A dietary fibre supplement and weight maintenance after weight reduction: a randomized, double-blind, placebo-controlled long-term trial. *Int J Obes*. 1989;13:165–769.
81. Rigaud D, Ryttig KR, Leeds AR, et al. Mild overweight treated with energy restriction and a dietary fiber supplement: a 6-month randomized, double-blind, placebo-controlled trial. *Int J Obesity*. 1990;14:763–771.
82. Abdelhameed AS, Ang S, Morris GA, et al. An analytical ultracentrifuge study on ternary mixtures of konjac Glucomannan supplemented with sodium alginate and xanthan gum. *Carbohydrate Polymers*. 2010;81:141–148.
83. Harding SE, Smith IH, Lawson CJ, et al. Studies on macromolecular interactions in ternary mixtures of konjac glucomannan, xanthan gum and sodium alginate. *J Carbpol*. 2010;10:1016–1020.
84. Lyon MR, Reichert RG. The effect of a novel polysaccharide blend (PGX™ granules) on weight loss and other laboratory parameters in mild to moderately obese adults: an observational retrospective clinical analysis. *Altern Med Rev*. 2010;15(1):68–75.
85. Lyon M, Wood S, Pelletier X, et al. Effects of a 3-month supplementation with a novel soluble highly viscous polysaccharide on anthropometry and blood lipids in nondieting overweight or obese adults. *J Hum Nutr Diet*. 2011;24(4):351–359.
86. Reimer RA, Pelletier X, Carabin IG. Increased plasma PYY levels following supplementation with the functional fiber PolyGlycopleX in healthy adults. *Eur J Clin Nutr*. 2010;64(10):1186–1191.
87. Brand-Miller JC, Atkinson FS, Gahler RJ, et al. Effects of PGX, a novel functional fibre, on acute and delayed postprandial glycaemia. *Eur J Clin Nutr*. 2010;64(12):1488–1493.
88. Jenkins AL, Kacinik V, Lyon MR, et al. Reduction of postprandial glycemia by the novel viscous polysaccharide PGX in a dose-dependent manner, independent of food form. *J Am Coll Nutr*. 2010;29(2):92–98.
89. Vuksan V, Lyon M, Breitman P, et al. 3-Week consumption of a highly viscous dietary fibre blend results in improvements in insulin sensitivity and reductions in body fat: results of a double blind, placebo controlled trial. Presented at the 64th Annual Meeting of the American Diabetes Association: Orlando, FL.
90. Solah VA, Kerr DA, Hunt WJ, et al. Effect of fibre supplementation on body weight and composition, frequency of eating and dietary choice in overweight individuals. *Nutrients*. 2017;9(2). PMID: 28212353.
91. Carabin IG, Lyon MR, Wood S, et al. Supplementation of the diet with the functional fiber PolyGlycoplex is well tolerated by healthy subjects in a clinical trial. *Nutr J*. 2009;8:9.
92. Onakpoya I, Posadzki P, Ernst E. Chromium supplementation in overweight and obesity: a systematic review and meta-analysis of randomized clinical trials. *Obes Rev*. 2013;14(6):496–507. PMID: 2349591.

93. Evans GW. Chromium picolinate is an efficacious and safe supplement. *Int J Sport Nutr*. 1993;3:117–122.
94. Campbell WW, Joseph LJ, Anderson RA, et al. Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women. *Int J Sport Nutr Exerc Metab*. 2002;12:125–135.
95. Volpe SL, Huang HW, Larpadisorn K, et al. Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. *J Am Coll Nutr*. 2001;20:293–306.
96. Yazaki Y, Faridi Z, Ma Y, et al. A pilot study of chromium picolinate for weight loss. *J Altern Complement Med*. 2010;16(3):291–299.
97. Iqbal N, Cardillo S, Volger S, et al. Chromium picolinate does not improve key features of metabolic syndrome in obese nondiabetic adults. *Metab Syndr Relat Disord*. 2009;7(2):143–150.
98. Baba N, Bracco EF, Hashim SA. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. *Am J Clin Nutr*. 1982;35:678–682.
99. St-Onge MP, Jones PJ. Greater rise in fat oxidation with medium-chain triglyceride consumption relative to long-chain triglyceride is associated with lower initial body weight and greater loss of subcutaneous adipose tissue. *Int J Obes Relat Metab Disord*. 2003;27:1565–1571.
100. St-Onge MP, Ross R, Parsons WD, et al. Medium-chain triglycerides increase energy expenditure and decrease adiposity in overweight men. *Obes Res*. 2003;11:395–402.
101. Seaton TB, Welle SL, Warenko MK, et al. Thermic effect of medium-chain and long-chain triglycerides in man. *Am J Clin Nutr*. 1986;44:630–634.
102. Hill JO, Peters JC, Yang D, et al. Thermogenesis in humans during overfeeding with medium-chain triglycerides. *Metabolism*. 1989;38:641–648.
103. Zhang Y, Liu Y, Wang J, et al. Medium- and long-chain triacylglycerols reduce body fat and blood triacylglycerols in hypertriacylglycerolemic, overweight but not obese, Chinese individuals. *Lipids*. 2010;45(6):501–510.
104. Mumme K, Stonehouse W. Effects of medium-chain triglycerides on weight loss and body composition: a meta-analysis of randomized controlled trials. *J Acad Nutr Diet*. 2015;115(2):249–263. PMID: 25636220.
105. Chee H, Romsos DR, Leveille GA. Influence of (-)-hydroxycitrate on lipogenesis in chickens and rats. *J Nutr*. 1977;107:112–119.
106. Sullivan AC, Triscari J, Hamilton JG, et al. Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. *I. Lipogenesis. Lipids*. 1974;9:121–128.
107. Rao RN, Sakariah KK. Lipid-lowering and antiobesity effect of (-)-hydroxycitric acid. *Nutr Res*. 1988;8:209–212.
108. Preuss HG, Garis RI, Bramble JD, et al. Efficacy of a novel calcium/potassium salt of (-)-hydroxycitric acid in weight control. *Int J Clin Pharmacol Res*. 2005;25(3):133–144.
109. Semwal RB, Semwal DK, Vermaak I, et al. A comprehensive scientific overview of Garcinia cambogia. *Fitoterapia*. 2015;102:134–148. PMID: 25732350.
110. Ceci F, Cangiano C, Cairella M, et al. The effects of oral 5-hydroxytryptophan administration on feeding behavior in obese adult female subjects. *J Neural Transm*. 1989;76:109–117.
111. Ceci F, Cangiano C, Cairella M, et al. Effects of 5-hydroxytryptophan on eating behavior and adherence to dietary prescriptions in obese adult subjects. *Adv Exp Med Biol*. 1991;294:591–593.
112. Cangiano C, Ceci F, Cascino A, et al. Eating behavior and adherence to dietary prescriptions in obese adult subjects treated with 5-hydroxytryptophan. *Am J Clin Nutr*. 1992;56:863–867.
113. Lee DH, Jacobs DR Jr. Hormesis and public health: can glutathione depletion and mitochondrial dysfunction due to very low-dose chronic exposure to persistent organic pollutants be mitigated? *J Epidemiol Community Health*. 2015;69(3):294–300. PMID: 25271248.
114. Genuis SJ, Beesoon S, Lobo R, et al. Human elimination of phthalate compounds: blood, urine, and sweat (BUS) study. *ScientificWorldJournal*. 2012;2012:615068. PMID: 23213291.
115. Genuis SJ, Beesoon S, Birkholz D. Biomonitoring and Elimination of Perfluorinated Compounds and Polychlorinated Biphenyls through Perspiration: Blood, Urine, and Sweat Study. *ISRN Toxicol*. 2013:483832. PMID: 24083032.
116. Podstawski R, Boraczyński M, Boraczyński T, et al. Sauna-induced body mass loss in young sedentary women and men. *ScientificWorldJournal*. 2014;2014:307421. PMID: 25614882.

202

Osteoarthritis

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Clinical symptoms: mild early-morning stiffness, stiffness after periods of rest, pain that worsens on joint use, and loss of joint function
- Clinical signs: local tenderness, soft tissue swelling, joint crepitus, bony swelling, restricted mobility, Heberden's (proximal interphalangeal joints) or the less common Bouchard's (distal interphalangeal joints) nodes or both, and other signs of degenerative loss of articular cartilage
- X-ray findings: narrowed joint spaces, osteophytes, increased density of subchondral bone, subchondral sclerosis, bony cysts, soft tissue swelling, and periarticular swelling

GENERAL CONSIDERATIONS

Osteoarthritis (OA), or degenerative joint disease, is characterized by joint degeneration, loss of cartilage, and alterations of the subchondral bone. It is by far the most common form of arthritis. It is estimated that more than 40 million Americans have OA, and OA is believed to be responsible for 25% of all office visits to primary care physicians. Although 80% of persons above 50 years of age will have x-ray evidence of significant OA, only 60% will experience symptoms. Men and women are equally affected, but symptoms occur earlier and appear to be more severe in women. OA incidence increases dramatically with age and body-mass index (especially OA of the knee).[1,2] Table 202.1 lists some of the diseases that are now thought to be OA of specific joints.

The weight-bearing joints and peripheral and axial articulations are the joints principally affected by the degenerative changes associated with OA. Much destruction of hyaline cartilage occurs, followed by hardening and the formation of large bone spurs (calcified osteophytes) in the joint margins. Pain, deformity, and limitation of joint motion result from this degeneration. Inflammation is usually minimal.

There is considerable evidence that endocrine factors may initiate or accelerate the development of arthritis because virtually all hormones act, directly or indirectly, on connective tissue cells: fibroblasts, osteoblasts, osteoclasts, and chondrocytes (Fig. 202.1). Patients with hypothyroidism have been shown to have an increased risk of osteoarthritis because thyroid hormones are necessary for the maturation of chondrocytes.[3] The higher prevalence (2–10 times higher) of arthritis in women suggests that estrogens may play a role. In addition, menopause coincides with the appearance of several arthritic conditions, including OA and rheumatoid arthritis (RA).

OA is divided into two categories, primary and secondary. In primary OA, the degenerative "wear-and-tear" process occurs after the fifth and sixth decades, with no apparent predisposing abnormalities. The cumulative effects of decades of use lead to degenerative changes by stressing the collagen matrix of the cartilage. Damage to the cartilage results in the release of enzymes that destroy collagen components. With aging, the ability to restore and synthesize normal collagen structures decreases.

Secondary OA is associated with some predisposing factors that can be responsible for the degenerative changes. Such factors include the following:

- Congenital abnormalities in joint structure or function (e.g., hypermobility and abnormally shaped joint surfaces)

TABLE 202.1 Diseases Thought to Be Osteoarthritis of Specific Joints

Joint	Disease
Hands	Heberden's and Bouchard's nodes
Hip	Malum coxae senilis
Temporomandibular joint	Costen's syndrome
Knee	Chondromalacia patellae
Spine	Ankylosing hyperostosis (interstitial skeletal hyperostosis)

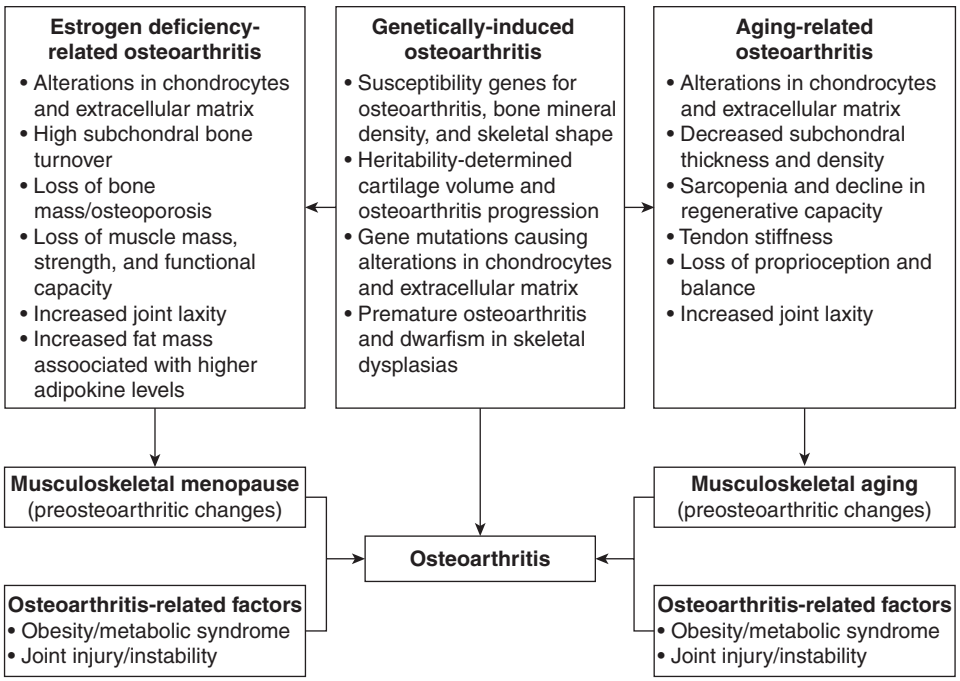


Fig. 202.1 Three subsets of osteoarthritis with distinct etiological, clinical, and therapeutic characteristics. (From Bruyère O, Cooper C, Arden N, et al. Can we identify patients with high risk of osteoarthritis progression who will respond to treatment? A focus on epidemiology and phenotype of osteoarthritis. *Drugs Aging.* 2015;32[3]:179–187. PubMed PMID: 25701074.)

BOX 202.1 Multifactorial Etiology of Osteoarthritis

- Hypermobility/joint instability
- Age-related changes in collagen matrix repair mechanisms
- Hormonal and gender factors
- Altered biochemistry
- Genetic predisposition
- Inflammation
- Fractures and mechanical damage
- Inflammatory joint disease
- Acromegaly
- Others

- Trauma (e.g., obesity, fractures along joint surfaces, surgery, excessive playing of basketball at an older age)
- Crystal deposition
- Presence of abnormal cartilage
- Previous inflammatory disease of the joint (e.g., rheumatoid arthritis, gout, septic arthritis)

Box 202.1 summarizes the many factors involved in the pathogenesis of OA.

One of the most interesting clinical features of OA is the lack of correlation between its severity as determined by a radiograph and the degree of pain. In some cases, the joint appears normal, with little if any joint-space narrowing, yet the pain can be excruciating. Conversely, there are cases in which there is tremendous deformity yet little, if any, pain. In fact, about 40% of individuals with the worst x-ray classification for OA are pain-free.[4] The exact cause of the pain in OA is still not well defined, but there are numerous potential causes (Box 202.2). Depression and anxiety appear to increase the experience of the pain of OA.

BOX 202.2 Potential Causes of Pain in Osteoarthritis

Bone
- Periosteal elevation by osteophytes
- Trabecular microfractures
- Pressure on subchondral bone
- Hypertension in bone marrow

Articular Misalignment or Surface Damage
- Synovial inflammation
- Pinching of synovial villi
- Joint-capsule distention

Periarticular Inflammation
- Ligament damage
- Muscle spasm
- Bursitis

Psychological Factors
- Anxiety
- Depression
- Lack of social support
- Secondary gain

Physical Demands
- Occupational trauma
- Obesity
- Neuromuscular integrity
- Protective reflexes
- Muscle weakness

BOX 202.3 Important Causes of the Misdiagnosis of Osteoarthritis

The source of the pain is not osteoarthritis (OA), but one of the following:
- Arthritis of other origin
- Pathological changes of the adjacent bone (e.g., tumor, osteomyelitis, metabolic bone disease)
- Mechanical injuries
- Pathological fractures
- Referred pain of neuritis, neuropathy, or radiculopathy
- Other neurological disorders causing stiffness of the joints

The source of the pain is OA, but not at the joint suspected:
- OA of the hip, pain localized to the knee
- OA of the cervical spine, causing pain in the shoulder
- OA of the lumbar spine, causing pain in the hip, knee, or ankle
- OA of the shoulder, causing pain in the elbow

The source of pain is caused by secondary soft tissue alterations of OA:
- Tendinitis or ligamentitis (especially of the knee)
- Enthesopathy or tendinopathy due to joint contracture
- Bursitis

Misinterpretation of deformity:
- Pseudohypertrophic osteoarthropathy
- Psoriatic arthritis
- Flexion contracture of the joints
- Mucopolysaccharidoses
- Neurogenic arthropathies
- Calcium pyrophosphate dihydrate crystal-deposition disease
- Genu varum and valgum

Misinterpretation of x-ray films:
- Arthritis with previous OA changes
- Initial stage of osteoarthritis; radiograph may be normal
- Flexion contracture; can cause a virtual loss of joint-space width
- Neurogenic and metabolic arthropathies

DIAGNOSIS

The onset of OA can be subtle. Morning joint stiffness is often the first symptom. As the disease progresses, pain on motion of the involved joint is worsened by prolonged activity and relieved by rest. There are usually no obvious signs of inflammation.

The specific clinical picture varies with the joint involved. Disease of the hands leads to pain and limitation of use. Knee involvement produces pain, swelling, and instability. OA of the hip causes local pain and a limp. Spinal OA (which is common) may result in compression of nerves and blood vessels, causing pain and vascular insufficiency.

The classic presentation of OA is easy to distinguish from other arthritides, especially RA, which is usually associated with much more inflammation of surrounding soft tissues. After a detailed medical history and physical examination, the best diagnostic tool to confirm the diagnosis of OA is a radiograph of the suspected joint. The classic finding in joints affected with OA is joint-space narrowing, loss of cartilage, and the presence of bone spurs (osteophytes). Box 202.3 lists important causes of the misdiagnosis of OA.

THERAPEUTIC CONSIDERATIONS

Data collected from the earliest lesions to the most advanced stages of clinical OA suggest that the cellular and tissue response is purposeful and aimed at repair of the anatomical defects. The process contributing to OA appears to be arrestable and sometimes reversible. The key factor in OA is increased catabolism in the extracellular matrix (ECM) of the articular cartilage. Cellular senescence due to aging, increased expression of inflammatory mediators, oxidative stress, and nutrient deficiency are all important contributors to OA development. The major therapeutic goal is to prevent this increased catabolism of the ECM and to ultimately enhance its repair as well as that of the articular connective tissue cells.[5,6]

Several studies have attempted to determine the "natural course" of OA.[6,7] One group of researchers studied the course of OA of the hip over a 10-year period. All subjects had pseudocystic changes suggestive of advanced OA, yet the researchers reported marked clinical improvement and radiological recovery of the joint space in 14 of 31 hips.[7] The authors purposely applied no therapy and regarded their results as reflecting the natural course of the disease. These results, as well as others, raise the serious concern that some medical interventions may promote disease progression.

Environmental Toxins

Bone tissue is highly sensitive to many types of toxic substances. For example, persistent organic pollutants (POPs) stored in adipose tissue can be related to differentiation, metabolism, and function in adipose tissue and may therefore be involved in the relation between obesity and OA.[8] In women, dioxin-like polychlorinated biphenyls (PCBs), nondioxin-like PCBs, and organochlorine (OC) pesticides are positively associated with arthritis.[9] Animal studies show 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits tibial growth, and 3,3',4,4',5-pentachlorobiphenyl (PCB-126) impairs mineralization and reduces the collagen content in ovariectomized rats.[10]

Lead has complex effects on bone cell function, including altering the plasma levels of calciotropic hormones, perturbing calcium-mediated and other sensitive signal transduction pathways, uncoupling osteoblasts and osteoclasts from normal paracrine control by interfering with hormone processes, and biochemically inhibiting enzymes resulting in altered cellular energetics. In lead-intoxicated children, blood levels of 1,25-dihydroxyvitamin D3 are reduced to levels comparable to those of patients with metabolic bone disease.[11]

Conventional Pharmacological Treatment

Nonsteroidal anti-inflammatory drugs (NSAIDs) have become the main treatment of OA in conventional medicine. Although these drugs provide short-term symptomatic relief, they may increase the rate of degeneration of the joint cartilage. Experimental studies have shown that aspirin and other NSAIDs inhibit collagen matrix synthesis and accelerate the destruction of cartilage.[12] Some retrospective clinical studies have shown that NSAID use is associated with acceleration of OA and increased joint destruction.[13–16] Simply stated, NSAIDs appear to suppress the symptoms but accelerate the progression of OA. Furthermore, a patient is unlikely to die of OA, but NSAID use is associated with significant risk for mortality. With older NSAIDs, the risk is primarily related to gastrointestinal bleeding, whereas the newer cyclooxygenase-2 (COX-2) inhibitors celecoxib (Celebrex) and rofecoxib (Vioxx) are associated with an increase in cardiovascular events.

Physical Therapy

Skewed musculoskeletal dynamics play a role in abnormally stressing involved joints. Despite this relatively simple concept, it is only recently that this idea has been researched. An 18-month study of 230 patients with tibiofemoral osteophytes and at least some difficulty with knee-requiring activity revealed conclusively that patients with varus alignment had a fourfold increased risk of medial OA progression.[17] Similarly, those with valgus alignment showed almost five times the risk of lateral OA progression. Not surprisingly, these researchers learned that the decline was greater for those with more extensive

BOX 202.4 Nonpharmacological Approaches to Pain in Osteoarthritis

- Acupuncture
- Psychological aids
- Exercise
- Physical therapy
- Ultrasound
- Transcutaneous nerve stimulation
- Laser therapy
- Diathermy
- Thermal baths
- Massage
- Weight loss

misalignments. This clearly documents the importance of considering musculoskeletal dynamics in evaluating any subject presenting with OA. Those with misalignments may have to consider manual manipulation therapies, orthotics, and extremity adjustments, along with techniques such as regular massage therapy, to relax hypertonic muscles. From a prevention standpoint, assessing and treating younger individuals may also decrease the risk of OA later in life.

In addition to addressing alignment issues, various physical therapy modalities (e.g., exercise, heat, cold, diathermy, ultrasound) are often beneficial in improving joint mobility and reducing pain in OA, especially when administered regularly (Box 202.4). Much of the benefit of physical therapy is thought to be due to achieving proper hydration within the joint capsule.

Clinical and experimental studies seem to indicate that short-wave diathermy may be of the greatest benefit.[18–20] Combining short-wave diathermy therapy with periodic ice massage, rest, and appropriate exercises appears to be the most effective approach. Ultrasound and laser therapy have also been shown to be helpful.[21,22]

The best exercises are isometrics and swimming. These types of exercises increase circulation to the joint and strengthen surrounding muscles without placing excess strain on joints. Increasing quadriceps strength has been shown to improve the clinical features and reduce pain in OA of the knee.[23] Walking programs help improve functional status and relieve pain in patients with OA of the knee.[24] Patient-specific physical therapies may be useful as well. For example, four older adults with hand OA benefited from keyboard playing for 20 minutes a day, 4 days a week.[25]

Dietary and Exercise Considerations

Dietary therapy primarily involves the achievement of normal body weight and improved insulin sensitivity because excess weight means increased stress on weight-bearing joints affected with OA. Obesity is a major risk factor for OA of the knee, and there is also considerable evidence linking OA to metabolic syndrome because of the negative effects that insulin resistance, inflammatory adipokines, and other features of this syndrome have on inflammation and joint structures.[26] Insulin stimulates chondrocytes to increase the synthesis and assembly of proteoglycans. Because the most prominent early change seen in the articular cartilage in OA is a decrease in both proteoglycan content and state of aggregation, insulin insensitivity or deficiency predisposes to OA.

Weight reduction, possibly due to a combination of mechanical and physiological factors, reduces the risk for OA and has also been shown to reduce pain and improve cartilage function in existing OA, especially when combined with exercise.[2,27–30] Lack of exercise decreases the hydration of the joint cartilages and retards the diffusion of nutrients into the affected area. When OA pain develops, sufferers often tend to reduce activity, which in turn decreases muscle strength. Greater muscle weakness increases joint wear, and inactivity can lead to weight gain, which can exacerbate OA, causing this cycle to repeat. In addition, patients with diabetes and cardiovascular concerns may also increase their risks for these illnesses as exercise diminishes. Weight loss and exercise have independently been effectively used to decrease the causative factors of OA and produce clinical improvement, but the best results are achieved by a combined approach. One study involved 252 obese elderly patients with a body-mass index of greater than 28 and radiographically confirmed OA who were randomized into healthy-lifestyle (control), diet-only, exercise-only, and diet-plus-exercise groups.[31] The exercise program involved hour-long sessions focusing on aerobics and resistance training three times a week. The goal of the dietary interventions was an average weight loss of 5% during the 18-month period. The most benefit was demonstrated in the diet-plus-exercise group. Compared with control patients and the diet-only group, subjects in the diet-plus-exercise group experienced a significant improvement in self-reported physical function, 6-minute walking distance, stair-climb times, and knee pain scores. Improvements in the exercise-only group were limited to the 6-minute walk distance.

GENERAL DIETARY GUIDELINES

In general, the principles detailed in Chapter 44 are appropriate for OA. Otherwise, the "Mediterranean diet" seems prudent. Typically, this diet comprises abundant plant foods (including fruits, vegetables, whole-grain cereals, beans, nuts, and seeds); minimally processed seasonally fresh and locally grown foods; fish and poultry; olive oil as the main source of lipid; and dairy products, red meat, and wine in low to moderate amounts. Thus the diet is rich in long-chain omega-3 polyunsaturated fatty acids and oleic acid (omega-9 monounsaturated), antioxidant nutrients, and unrefined carbohydrates. A systematic review of the scientific literature indicated the Mediterranean diet (MD) was associated with a lower prevalence of OA.[31] Biomarkers of inflammation and cartilage degradation related to OA were also analyzed, and there was decreased interleukin1-α in the MD group.

One popular dietary practice in the treatment of OA, conceived by Childers, a horticulturist, is the elimination of foods from the *Solanaceae* family (nightshade family). Childers arrived at this method after finding that this simple dietary elimination cured his own OA.[32] Childers developed a theory that genetically susceptible individuals might develop arthritis and other complaints from long-term low-level consumption of the *Solanum* alkaloids found in tomatoes, potatoes, eggplant, peppers, and tobacco. Presumably, these alkaloids inhibit normal collagen repair or promote inflammatory degeneration of the joints. Although yet unproven, it seems that this diet has been of benefit to some individuals.

Nutritional Supplements

Because Americans may spend more on natural remedies for OA than for any other medical condition, the practitioner should be well informed about the clinical effectiveness and safety of the various natural options on the marketplace.

Glucosamine Sulfate

Glucosamine sulfate has emerged as the most popular nutritional approach to OA. It is a simple molecule composed of glucose and an amine. Its main physiological effect on joints is to stimulate the manufacture of glycosaminoglycans (GAGs). Glucosamine also promotes the incorporation of sulfur into cartilage. It appears that as some

people age, they lose the ability to manufacture sufficient levels of glucosamine. The result is that cartilage loses its gel-like nature and ability to act as a shock absorber. The inability to manufacture glucosamine may be the major factor leading to OA. Extensive preclinical and clinical research, including long-term double-blind studies, supports a rationale and role for glucosamine as a major consideration in the treatment of OA. This research and the clinical benefits of glucosamine sulfate in the treatment of OA are fully described in Chapter 83.

Chondroitin Sulfate

Chondroitin sulfate, as well as shark cartilage, bovine cartilage extracts, and sea cucumber, contains a mixture of intact or partially hydrolyzed GAGs of molecular weights ranging from 14,000 to more than 30,000. Chondroitin sulfate (CS) is composed of repeating units of derivatives of glucosamine sulfate with attached sugar molecules. Although the absorption rate of glucosamine sulfate is 90% to 98%, the absorption of intact chondroitin sulfate is estimated to be anywhere from 0% to 13%.[33,34] The difference in absorption is largely due to the difference in size. Chondroitin sulfate is at least 50 to 300 times larger than glucosamine sulfate, too large to pass the normal intact intestinal barrier. If chondroitin sulfate molecules were absorbed intact or partially digested, they would still be unlikely to produce any significant benefit because the chondroitin sulfate molecules are too large to be delivered to cartilage cells. Furthermore, in patients with OA, levels of chondroitin sulfate in the synovial tissues are typically elevated.[35] These absorption problems suggest that any direct effect of these compounds in OA is highly unlikely. However, conflicting evidence supports the notion that exogenous chondroitin sulfate is indeed absorbed as a high-molecular-weight polysaccharide together with derivatives originating from a partial depolymerization, desulfation, or both.[36–38]

Any clinical benefit from chondroitin sulfate is most likely due to the absorption of sulfur or smaller GAG molecules broken down by the digestive tract. However, even this is controversial, because in one human study, 1 g of chondroitin sulfate failed to increase serum GAG concentration at all, based on a highly sensitive measure of intact or depolymerized GAG absorption. These results prompted the researchers to conclude: "We suggest that chondroprotection by orally administered chondroitin sulfate is a biologically and pharmacologically unfounded theory."[33]

Pooled literature on the biochemistry of chondroitin sulfate offers enough information to assert that neither intact nor polymerized chondroitin sulfate is absorbed by the mammalian gastrointestinal tract. Therefore no direct action of orally administered chondroitin sulfate on cartilage or chondrocytes is possible.

Despite the unlikeliness of any direct action, two published meta-analyses indicate that chondroitin may be superior to placebo in reducing the pain of OA. One of these analyses may have been exaggerated by publication bias related to the manufacturer's sponsorship. The second meta-analysis did find chondroitin to be superior to placebo in reducing the painful symptoms of OA, although these authors also called for trials of longer duration. Finally, one study using 800 mg of chondroitin sulfate for two separate periods of 3 months over 1 year did show decreased pain and improved knee function as well as a decrease in joint-space narrowing over placebo.[39] This study reveals that even intermittent use of chondroitin may be effective. Another study seemed to point to the requirement of continuous long-term therapy as producing the best results. Patients with painful osteoarthritis of the knee were treated with oral chondroitin sulfate at a dose of either 260 mg/d (low-dose group, control group) or 1560 mg/d (high-dose group). Symptoms were evaluated by the Lequesne's index and visual analog scale for pain. In a subgroup of 73 patients with severe symptoms (Lequesne's index ≥8), the chondroitin sulfate dose of 1560 mg/d improved pain faster after 6 and 9 months of therapy compared with the low dosage.[40]

The other clinical studies that have been done with orally administered chondroitin sulfate demonstrate that it is less effective than glucosamine sulfate.[41–45] Furthermore, there is no evidence that using both glucosamine and chondroitin together is more effective than either alone.[46] In general, the more impressive results have been achieved with glucosamine sulfate. Nevertheless, given the safety record of chondroitin and the evidence of modifying joint-space pathology, chondroitin is a reasonable addition to the glucosamine regimen of a patient with osteoarthritis. In addition, chondroitin sulfate may be acting in some indirect way in improving joint health.

Hyaluronic Acid

Hyaluronic acid (HA) is an important GAG in joints, providing a structural framework and supporting the ability of cartilage to hold water. A type of connective tissue, it is the actual "glue" that holds the body together. By the time most people reach the age of 70, the HA content of the body has dropped by 80% from when they were 40, predisposing them to the loss of connective tissue integrity, particularly of the skin and joints. Weekly injections of HA (Synvisc, Hyalgan, Supartz, etc.) into joints affected by OA (viscosupplementation) has been shown to be an effective treatment for OA, with beneficial effects on pain, function, and patient global assessment. Such results have been seen at different postinjection periods but especially at the 5- to 13-week postinjection period.

Oral supplementation with HA has been shown to be a viable method of producing comparable results to intraarticular treatment.[47–50] HA supplements feature HA derived from rooster combs, cow eyes, or bacterial fermentation.

In the first double-blind study conducted with oral HA in OA, 20 patients with knee OA were given either HA (80 mg/day) or placebo for 8 weeks.[51] Compared with the placebo group, pain scores were significantly improved in the HA group. In another study, 60 patients with OA were randomized equally to receive either HA 200 mg, HA 100 mg, or placebo for 8 weeks.[47] After conducting a stratification analysis on the subjects with more severe pain (i.e., those with a Western Ontario and McMaster Universities Arthritis Index [WOMAC] pain score >10), significant reductions in pain scores and total symptom scores were seen with the 200-mg dose but not with the 100-mg dose.

Natural Eggshell Membrane

Natural eggshell membrane (NEM) provides GAGs, CS, and HA. NEM supports the body's production of type II collagen, which is the main component of the cartilage that covers bones, particularly weight-bearing joints such as the knees and hips. NEM also boosts the production of GAGs.

The clinical research on NEM began with a pilot study published in June 2009; patients with mild to moderate joint pain received 500 mg NEM daily.[52] After just 7 days, participants had an average 26% improvement in pain and a 28% improvement in flexibility. After 30 days, they had an average 73% reduction in pain, with 45% of participants reporting they were completely pain-free. They also had a 44% improvement in flexibility and 76% less pain through their range of motion (ROM). No adverse side effects were reported.

To further support the safety and efficacy of NEM in osteoarthritis, a double-blind, placebo-controlled clinical trial was conducted involving 67 patients with OA of the knee.[53] They received either 500 mg of NEM daily or a placebo. After only 10 days, those receiving NEM supplements had a 12% reduction in pain and 17% less stiffness. At the end of the 60-day trial, those receiving NEM had an average pain reduction of 15% and had 31% less stiffness. One third of the patients

receiving NEM experienced a reduction in pain of 40% or greater, and more than half had at least 50% less stiffness. No adverse side effects were reported.

Additionally, 11 (single-arm trial) and 28 (double-arm trial) patients received oral NEM 500 mg once daily for 4 weeks.[54] In the single-arm trial, supplementation with NEM produced a significant treatment response at 7 days for flexibility (27.8% increase) and at 30 days for general pain (72.5% reduction), flexibility (43.7% increase), and ROM-associated pain (75.9% reduction). In the double-arm trial, supplementation with NEM produced a significant treatment response for pain at 7 days for both treatment arms (X: 18.4%, Y: 31.3% reduction). There was no clinically meaningful difference between treatment arms at 7 days, so the Y arm crossed over to the X formulation for the remainder of the study. The significant treatment response continued through 30 days for pain (30.2% reduction). There were no adverse events reported.

Niacinamide

In the 1940s and 1950s, William Kaufman, and later Abram Hoffer, reported good clinical results in the treatment of hundreds of patients with RA and OA using high-dose niacinamide (i.e., 900–4000 mg/day in divided doses).[55] Kaufman documented improvements in joint function, ROM, and increased muscle strength and endurance as well as a reduction in the sedimentation rate. Most patients achieved noticeable benefits within 1 to 3 months, with peak benefits noted between 1 and 3 years of continuous use.

These clinical results were evaluated in a well-designed double-blind, placebo-controlled trial.[56] Seventy-two patients with OA were randomized for treatment with niacinamide (3000 mg daily in six divided doses) or placebo for 12 weeks. Outcome measures included global arthritis impact and pain, joint ROM and flexibility, erythrocyte sedimentation rate (ESR), complete blood count, liver function tests, serum cholesterol, serum uric acid, and fasting blood sugar. The researchers found that niacinamide produced a 29% improvement in global arthritis impact compared with a 10% worsening in the placebo group. Pain levels did not change, but those on niacinamide reduced their NSAID use. Niacinamide supplementation reduced the ESR by 22% and increased joint mobility by 4.5 degrees over controls (8 degrees vs. 3.5 degrees); otherwise, there were no other changes in blood chemistry. Side effects, primarily mild gastrointestinal complaints, were more common in the niacinamide group but could be effectively managed by recommending that the pills be taken with food or fluids.

Niacinamide at this high dose can result in significant side effects (e.g., glucose intolerance, liver damage) and therefore requires strict supervision.

S-Adenosylmethionine

S-Adenosylmethionine (SAMe) is an important physiological agent formed in the body by combining the essential amino acid methionine with adenosine triphosphate (ATP). A deficiency of SAMe in the joint tissue, just like a deficiency of glucosamine, leads to loss of the gel-like nature and shock-absorbing qualities of cartilage. A meta-analysis of 11 studies reports the benefits of SAMe in the treatment of OA by reducing pain and improving functional limitations.[57]

SAMe has been shown to be important in the manufacture of cartilage components.[58] In one double-blind study, supplemental SAMe increased cartilage formation as determined by magnetic resonance imaging in 14 patients with OA of the hands.[59] In addition to this effect, SAMe has also demonstrated some mild pain-relieving and anti-inflammatory effects in animal studies.

In total, 21,524 patients with OA have been treated with SAMe in published clinical trials. In double-blind trials, SAMe has demonstrated similar reductions in pain scores and clinical symptoms to NSAIDs like ibuprofen, indomethacin, naproxen, and piroxicam. All of these studies indicate that SAMe offers significant advantages over NSAIDs. Although the drugs are associated with a significant risk of toxicity, side effects, and actual promotion of the disease process in OA, SAMe offers similar benefits with minimal risk or side effects. Side effects include occasional gastrointestinal disturbances, mainly diarrhea.[37] For more information, see Chapter 110.

Vitamin C

Results from the Framingham Osteoarthritis Cohort Study indicate that a high intake of antioxidant nutrients, especially vitamin C, may reduce the risk of cartilage loss and disease progression in people with OA.[36] A threefold reduction in the risk of OA progression was found in the middle and highest tertiles of vitamin C intake. These results highlight the importance of a diet rich in plant-based antioxidant nutrients protecting against chronic degenerative diseases, including OA.

Deficient intake of vitamin C is common in the elderly, resulting in altered collagen synthesis and compromised connective tissue repair.[60] Several in vitro studies have demonstrated that vitamin C has an anabolic effect on cartilage.[61,62] Research has confirmed the importance, indeed necessity, for an excess of ascorbic acid in human chondrocyte protein synthesis.[63] One in vivo study of experimental OA in guinea pigs found that cartilage erosion was much less, and the overall histological and biochemical changes in and around the OA joint much milder, in animals kept on high doses of vitamin C.[60] A human study showed that a history of self-reported vitamin C supplementation indicated an effect in halting the progression of OA.[64]

Vitamins C and E appear to possess synergistic effects.[60] The researchers concluded: "Thus, both vitamins E and C appear to enhance the stability of sulfated proteoglycans in the complex structure comprising articular cartilage. Judicious use of these vitamins in the treatment of OA, either alone or in combination with other therapeutic means, may thus be of great benefit to the patient population by retarding the erosion of cartilage."

Vitamin D

Vitamin D has a range of effects on cell types within osteoarthritis-affected joints acting through the vitamin D receptor and thus altering gene expression (Fig. 202.2). Several studies have shown that low serum levels of vitamin D_3 appear to be associated with an increased risk for the progression of OA, especially in people under 60 years of age.[65,66] Four clinical studies have been conducted with vitamin D_3 supplementation in patients with OA.[67] In a meta-analysis, these studies were judged to be of high quality. Table 202.2 shows the main features of these studies. The results indicated that vitamin D supplementation had a statistically significant but small-to-moderate effect on pain control in patients with knee OA. However, no effects were observed for the change in tibial cartilage volume or joint-space width. There was no difference seen between patients who had sufficient or insufficient serum 25(OH)D levels at baseline.

Pantothenic Acid

An acute deficiency of pantothenic acid in the rat causes a pronounced failure of cartilage growth and eventually produces lesions similar to OA. Clinical improvement in OA symptomatology with the administration of 12.5 mg of pantothenic acid has been reported.[68,69] Results often did not appear for 7 to 14 days. However, a larger double-blind study in patients with primarily RA displayed no significant benefit from the administration of 500 mg of pantothenic acid.[70]

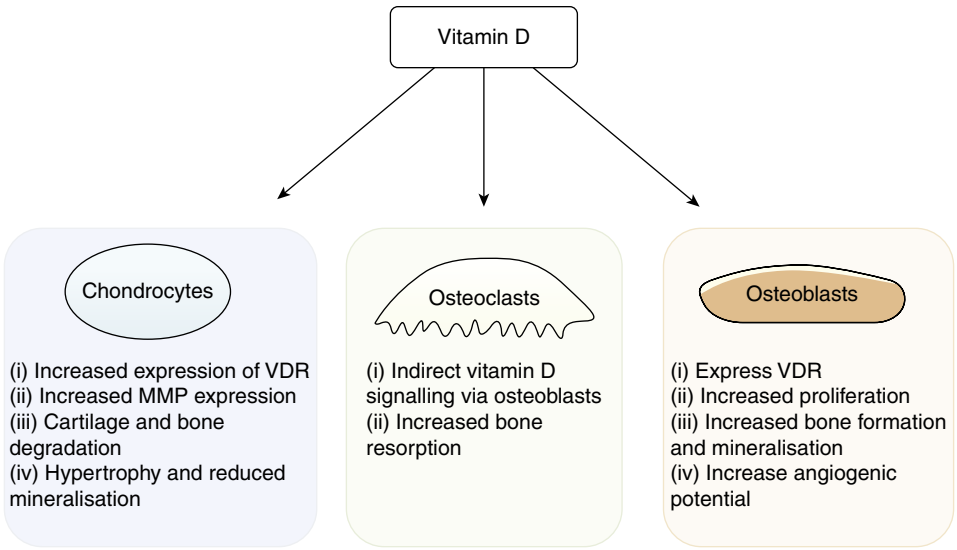


Fig. 202.2 The effects of vitamin D on several different cell types in osteoarthritis, including chondrocytes, osteoclasts, and osteoblasts. *MMP,* matrix metalloproteinases; *VDR,* vitamin D receptor. (From Mabey T, Honsawek S. Role of vitamin D in osteoarthritis: molecular, cellular, and clinical perspectives. *Int J Endocrinol.* 2015;383918. PubMed PMID: 26229532.)

TABLE 202.2 Main Characteristics of the Included Studies on Vitamin D Supplementation and Progression of Knee OA[67]

Authors	Year	Patients	Dose	Follow-up	Sample Size	Outcomes of Interest
McAlindon[110]	2013	>45 years old, symptomatic knee OA	Cholecalciferol—the initial dose was 2000 IU/day, with subsequent adjustment in 2000-IU increments at 4, 8, and 12 months	2 years	146	WOMAC pain score, MRI cartilage volume, joint-space width
Sanghi[111]	2013	>50 years old, symptomatic knee OA	Cholecalciferol—60,000 IU/day for 10 days followed by 60,000 IU/month	1 year	103	WOMAC pain score
Arden[112]	2016	>50 years old, symptomatic knee OA	800 IU of oral cholecalciferol daily	3 years	468	WOMAC pain score, joint-space width
Jin[113]	2016	50–79 years old, symptomatic knee OA	Cholecalciferol—participants in the treatment group were given a monthly capsule of 50,000 IU for 24 months	2 years	413	WOMAC pain score, MRI cartilage volume

MRI, Magnetic resonance imaging; *OA,* osteoarthritis; *WOMAC,* Western Ontario and McMaster Universities Arthritis Index.

Vitamins A and E, Pyridoxine, Zinc, Copper, and Boron

Vitamins A and E, pyridoxine, zinc, copper, and boron are required for the synthesis of collagen and the maintenance of normal cartilage structures. A deficiency of any one of these would allow accelerated joint degeneration. In addition, supplementation at appropriate levels may promote cartilage repair and synthesis. For example, boron supplementation has been used in the treatment of OA in Germany since the mid-1970s. This use was recently evaluated in a small double-blind clinical study and an open trial. In the double-blind study, of the patients given 6 mg of boron (as sodium tetraborate decahydrate), 71% improved, compared with only 10% in the placebo group.[71] In the open trial, boron supplementation (6–9 mg daily) produced effective relief in 90% of arthritis patients, including patients with OA, juvenile arthritis, and RA.[72] The preliminary indication is that boron supplements are of value in arthritis, with many patients with OA experiencing complete resolution.

Vitamin K

Studies have shown that low vitamin K status is associated with OA.[73,74] In one study looking at dietary factors in OA, only vitamin K intake was shown to be inversely associated with the prevalence of radiographic knee OA.[73] The presence of joint-space narrowing of the knee was also inversely associated with vitamin K intake. The prevalence of radiographic knee OA for each dietary vitamin K intake quartile decreased with the increased intake.[73] Vitamin K may have a protective role against knee OA and might lead to a disease-modifying treatment. An ancillary study to a randomized controlled trial assessed the effects of phylloquinone supplementation (vitamin K arm) versus placebo on bone loss and vascular calcification among older adults regardless of their vitamin K status. The parent study was a 3-year double-blind controlled trial. Participants were randomized and stratified by gender to either the treatment group (which received 500 mcg of phylloquinone as part of a single daily effervescent multivitamin formulation) or the comparator group (which received an identical multivitamin formulation without phylloquinone) (Hermes Arzneimittel GMBH, Munich, Germany), hereafter referred to as the placebo group. All study participants also received elemental calcium (600 mg/day) and vitamin D (400 IU/day) as separate tablets. Although there were no effects of vitamin K for radiographic OA outcomes, those with

insufficient vitamin K at baseline who attained sufficient concentrations at follow-up had 47% less joint-space narrowing.[74] Foods rich in vitamin K include green tea, kale, turnip greens, spinach, and other green leafy vegetables.

Botanical Medicines

Historically, many herbs have been used in the treatment of OA. When inflammation is present, those botanicals and nutritional factors possessing anti-inflammatory activity can often be quite helpful. The research on curcumin, bromelain, and ginger is discussed in Chapters 73, 59, and 130, respectively, to avoid duplication. Curcumin appears particularly helpful.

Boswellia Serrata

Boswellia serrata, a large branching tree native to India, yields an exudative gum resin known as salai guggul, which has been used for centuries, including use in arthritic conditions. Newer preparations concentrated for the active components (boswellic acids) are showing significant clinical results. Initially, boswellic acid extracts demonstrated antiarthritic effects in various animal models. Among several mechanisms of action are inhibition of inflammatory mediators, prevention of decreased GAG synthesis, and improved blood supply to joint tissues.[75] Clinical studies using herbal formulas with *Boswellia* have yielded good results in OA of the knee, with patients experiencing decreased knee pain and swelling and increased knee flexion and walking distance.[76–79] Corroborating the improvements in pain scores and joint function with *Boswellia* were significant reductions in synovial fluid matrix metalloproteinase-3, indicating improved stability of the collagen matrix.[77,78] The standard dosage for boswellic acids in arthritis is 400 mg three times daily. No side effects due to boswellic acids have been reported.

Procyanidolic Oligomers

Two double-blind studies have been conducted with Pycnogenol in OA. In the first study, Pycnogenol (100 mg) or placebo was given daily for 3 months to 156 patients with OA.[80] The global score on the WOMAC questionnaire for OA decreased by 56% in the treatment group versus 9.6% in the placebo group. Walking distance in the treadmill test was prolonged from 68 m at the start to 198 m in the Pycnogenol group compared with an increase from 65 to 88 m in the placebo group. The use of drugs decreased by 58% in the Pycnogenol group versus 1% under placebo. Foot edema decreased in 79% of Pycnogenol patients versus 1% in controls. Similar results were seen in a second study when Pycnogenol was given at a dose of 100 mg daily.[81]

Limbrel (Flavocoxid)

Limbrel (Flavocoxid) is a proprietary mixture of the two flavonoid molecules baicalin and catechin. It is being promoted as possessing balanced inhibition of cyclooxygenases 1 and 2 and 5-lipoxygenase without the associated cardiovascular, renal, or gastrointestinal side effects of standard NSAIDs. Double-blind studies have shown results comparable with those with naproxen in relieving pain due to OA, with both medications being given at a dose of 500 mg twice daily.[82–84] Flavocoxid does not increase bleeding time, inhibit platelet aggregation, or inhibit or potentiate the anticoagulant effect of warfarin.[85]

Harpagophytum Procumbens (Devil's Claw)

Harpagophytum procumbens is a South African plant that grows in regions bordering the Kalahari. Secondary tuberous roots are used to prepare powders or extracts usually standardized for harpagoside, the principal active compound. A systematic review of the clinical efficacy of harpagophytum for OA concluded that products providing less than 30 mg of harpagoside per day in the treatment of knee and hip OA were of little use, whereas dosages providing 60 mg of harpagoside daily showed moderate evidence of efficacy in the treatment of spine, hip, and knee OA.[86] Some of the individual studies showed a significant benefit. For example, in a 2-month double-blind study on spine and knee OA, 670 mg of powder three times a day was more efficient than placebo in reducing pain scores.[87] In a 4-month double-blind study on hip and knee OA, 2.6 g of powder/day was equal in efficacy to 200 mg of diacerein per day in improving pain scores, but it was better tolerated.[88] In a review of 28 clinical trials of devil's claw extracts, adverse events occurred at a rate of about 3% and did not exceed the rate of those experienced with placebo.[89] Long-term use appears to be safe and without toxicity.

Yucca

A double-blind clinical trial found that a saponin extract of yucca demonstrated a positive therapeutic effect.[90] Results were of gradual onset, and no direct joint effects of the yucca saponin were noted. The researchers suggested that the clinical improvement was due to indirect effects on the gastrointestinal flora. This is an interesting suggestion because bacterial lipopolysaccharides (endotoxins) have been shown to depress the biosynthesis of proteoglycans.[91] It is entirely possible that yucca decreases the absorption of bacterial endotoxins and thus reduces this inhibition of proteoglycan synthesis. If this is the mechanism of action, then other saponin-containing herbs and other ways of reducing the endotoxin load may be useful.

Additional Therapeutic Considerations

Topical Analgesics

The mainstay of natural topical preparations for OA is those containing either menthol-related compounds (e.g., one popular combination contains camphor 4%, menthol 10%, and methyl salicylate 30%) and/or capsaicin (typically creams containing 0.075%). These time-tested topical analgesics can often provide significant relief in OA, although each has compliance issues. Alternatives are products containing Celadrin—a mixture of cetylated fatty acids (Celadrin) with cetyl myristoleate, cetyl myristate, cetyl palmitoleate, cetyl laureate, cetyl palmitate, and cetyl oleate. Celadrin has been shown to affect several key factors that contribute to inflammation. Its main action appears to be its ability to enhance the health and integrity of cell membranes. It therefore halts the production of inflammatory compounds known as prostaglandins. It also reduces the production of the negative immune factors like interleukin (IL)-6, which play a central role in inflammation. Studies have assessed both the oral and topical use of Celadrin. In a study with oral Celadrin, 64 patients with chronic OA of the knee were evaluated at baseline and at 30 and 68 days after consuming either placebo or Celadrin. Results indicated that compared with placebo, Celadrin improves knee range of motion (patients treated with Celadrin exhibited a significant 10.1-degree increase in knee flexion and overall joint function compared with patients given placebo).[92]

The effect of the Celadrin cream was studied in OA of the knees. Forty patients were randomly assigned to receive either the Celadrin cream or a placebo. Patients were tested on three occasions: (1) at baseline, (2) 30 minutes after initial treatment, and (3) after 30-day treatment of cream application twice a day. Assessments included knee ROM, timed "up-and-go" from a chair and stair climbing, and two other functional tests. For stair-climbing ability and the up-and-go test, significant decreases in time were observed 30 minutes after the first administration and after 1 month of use only in the Celadrin group. Likewise, the ROM of the knees increased with Celadrin both 30 minutes after the initial application and after 1 month's use. In contrast, no difference was observed in the placebo group. The other functional tests also clearly demonstrated improvements with Celadrin, whereas the placebo failed to produce results.[93]

In another study with topical Celadrin in patients with knee OA, patients were assessed by having them stand on a special platform for 20 and 40 seconds to measure static postural stability (the ability to stand comfortably in one place for a period of time). Again, only those subjects using the Celadrin cream demonstrated improvements.[94]

One of the key points regarding Celadrin is that unlike many other natural approaches, it produces almost immediate results.

Proteolytic Enzymes

Preparations of proteolytic enzymes (e.g., pancreatic proteases chymotrypsin and trypsin, bromelain, and fungal proteases) have been found useful in OA as well as a wide range of other inflammatory conditions (see Chapters 95 and 100 for more information). Several studies have used tablets containing a combination of bromelain 90 mg, trypsin 48 mg, and rutin 100 mg (Phlogenzym) at a dosage of 2 tablets three times daily or 3 tablets twice daily on an empty stomach. Two of these studies, both double-blinded, showed that this combination at the recommended dosage level produced clinical results in reducing pain scores due to OA on par with the drug diclofenac (100 mg daily).[95,96] However, another double-blind study with bromelain alone (800 mg daily) produced no significant effects in OA.[97]

Acupuncture

Traditional Chinese medicine typically views OA as a "bi" or pain syndrome, with pathogenic etiologies stemming from coldness, dampness, heat, and wind. Each one of these types typically has a separate clinical picture and requires the use of specific acupuncture points. Standard Western medical systems have been slow to adopt acupuncture practice as standard adjunctive care despite mounting research evidence supporting the safety and efficacy of acupuncture in reducing pain from OA.[98–100]

For example, several studies have focused on knee OA. One observational study of 563 patients (88% female) with chronic knee OA found that 75% of the participants achieved a reduction in pain of 45% or more with acupuncture.[101] An open randomized controlled trial evaluated the effect of acupuncture with and without adjunctive conventional medications.[102] In this study, 30 patients with symptomatic knee OA were randomized to one of three treatment groups. Those patients given acupuncture only or acupuncture combined with conventional medications achieved a highly significant improvement in pain. The acupuncture-free group experienced no change until after a course of acupuncture later in the study, when their improvement became significant. Similarly, significant positive changes were seen with pain and stiffness scores. These benefits were maintained during the month after the acupuncture course.

Traditional Chinese medicine considers an etiology of pain as "stuck qi." In this case, the addition of electrical energy to the area can reestablish qi flow, thus alleviating pain. One single-blinded, randomized controlled study examined the effectiveness of electroacupuncture and transcutaneous electrical nerve stimulation (TENS) to alleviate OA-induced knee pain.[103] In this research, 24 subjects (23 women and 1 man) with a mean age of 85 years were given electroacupuncture, TENS, or no treatment. Subjects in the electroacupuncture group received low-frequency (2-Hz) treatment at two local acupuncture points (ST-35, Dubi and Neixiyan) of the painful knee for 20 minutes. Subjects in the TENS group received low-frequency TENS treatment of 2 Hz and pulse width of 200 ms at the same acupuncture points for 20 minutes. In both treatment groups, electrical treatment was carried out for a total of eight sessions in 2 weeks. The other control subjects received OA knee care and education only. After eight sessions, there was a significant reduction of knee pain in both the electroacupuncture and TENS groups, with prolonged analgesic effect at a 2-week follow-up evaluation. Electroacupuncture also gained an advantage by lowering scores for the timed up-and-go test, which TENS did not achieve.

Electroacupuncture has also been tested in a head-to-head 186-patient randomized, single-blind, placebo-controlled trial against the NSAID diclofenac.[104] For these knee patients, the improvement of OA symptoms in most outcome parameters was greatest in the electroacupuncture group. Unlike the diclofenac and placebo groups, most of the patients rated their results with electroacupuncture as "much better," and substantially better pain management and functionality were obtained in the electroacupuncture group as well.

In considering an acupuncture regimen, the researchers of one randomized controlled trial for patients with OA of the knee concluded that acupuncture is most effective when it is employed early in the treatment plan. Additionally, on the basis of patient evaluations, it seems that, to avoid a rebound effect, treatments should be administered with a tapering, methodical decrease in frequency once the acute treatment period is completed.[105] Because it has been reported that the density of peripheral nerve endings in the skin or muscles is much greater in the acupuncture points compared with areas beyond these points, it is possible that an abrupt lack of stimulation in these areas may untowardly affect neurotransmitter release, thus contributing to a rebound effect.

Magnetic Therapies

Magnetic therapy has been used in the treatment of a wide variety of chronic pain syndromes.[106] Magnetic fields may have the ability to stimulate chondrocyte proliferation and increase the synthesis of proteoglycans. A number of studies clearly support magnetic therapy when used for knee OA.[107,108] One is a multicenter, double-blind, randomized, placebo-controlled trial that enrolled 75 patients with OA of the knee who had previously been unable to obtain acceptable results using conventional treatments.[107] Using low-frequency pulsed fields, improvements in the level of pain, functionality, and physician global evaluation of patients' condition were notable. Mean morning stiffness also decreased by 20 minutes in the group using magnetic therapy while increasing by 2 minutes in the placebo group. A second placebo-controlled, randomized, double-blind clinical study of 176 patients with osteoarthritic knees also showed significant results using low-amplitude and low-frequency fields.[109] The reduction in pain after a treatment session was significantly greater in the magnet-on group (46%) compared with the magnet-off group (8%). A smaller, 29-subject study of knee OA used either high-strength magnetic or placebo knee-sleeve treatment for 4 hours in a monitored setting and self-treatment 6 hours daily for 6 weeks.[108] This study demonstrated a significant decrease in pain scores in the active group and only a minimal improvement in the placebo group at 4 hours of treatment but no significant differences at 6 weeks. Magnetic therapies may be a useful treatment for OA.

Relaxation Techniques

Relaxation techniques such as meditation, deep breathing, and guided imagery have been used for many types of pain conditions. A study of 66 elderly people suffering from chronic OA pain evaluated the effect of daily music listening on pain levels from OA.[110] Differences in perceptions of pain were measured over 14 days in an experimental group that listened to Mozart selections for 20 minutes daily and a control group that sat quietly for 20 minutes daily. Those who listened to music had less pain compared with those who sat quietly and did not listen to music. The amount of pain perceived by the Mozart group also decreased incrementally over the 14-day study period.

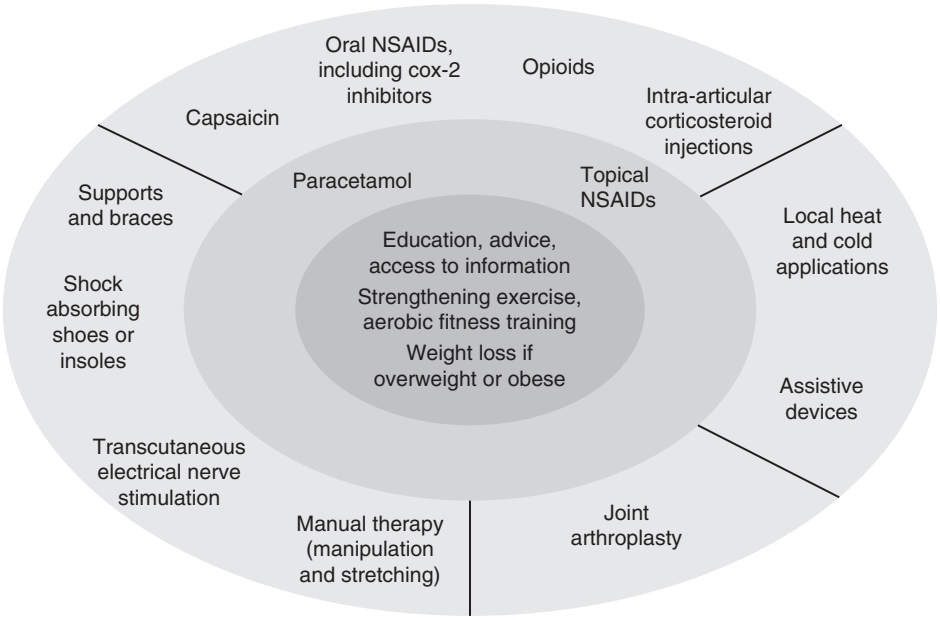


Fig. 202.3 National Institute for Health and Clinical Excellence (NICE) osteoarthritis treatment recommendations. (From: Dziedzic KS, Healey EL, et al. Implementing the NICE osteoarthritis guidelines: a mixed methods study and cluster randomized trial of a model osteoarthritis consultation in primary care—the Management of OsteoArthritis In Consultations [MOSAICS] study protocol. *Implement. Sci.* 2014;9:95. PubMed PMID: 25209897.)

THERAPEUTIC APPROACH

Although there appear to be several worthwhile approaches used to control OA through natural therapies, a direct clinical study of a comprehensive, integrated program has yet to be conducted. The therapeutic approach recommended here is based on reducing joint stress and trauma, promoting collagen repair mechanisms, and eliminating foods and other factors that may inhibit normal collagen repair (Fig. 202.3). All diseases or predisposing factors (see Table 202.1) should, of course, be controlled. NSAIDs such as aspirin should be avoided as much as possible. If NSAIDs must be used, deglycyrrhizinated *G. glabra* should be used to help protect the gastrointestinal tract from their damaging effects, and the use of NSAIDs should be discontinued as soon as possible (see Chapter 85 for more information).

Diet

The achievement of ideal body weight is the primary dietary goal. All simple, processed, concentrated carbohydrates must be avoided; complex-carbohydrate, high-fiber foods should be emphasized; and fats should be kept to a minimum. Plants of the *Solanaceae* family should be eliminated (tomatoes, potatoes, eggplant, peppers, and tobacco). Liberal consumption of flavonoid-rich berries or extracts is also important.

Supplements

- Glucosamine sulfate: 1500 mg/day
- Niacinamide: 500 mg six times/day (under strict supervision—liver enzymes must be regularly assayed)
- Vitamin A: 5000 IU/day
- Vitamin C: 1000 to 3000 mg/day
- Vitamin B_6: 50 mg/day
- Vitamin K: 0.5 mg/day
- Pantothenic acid: 12.5 mg/day
- SAMe: 400 mg three times a day
- Zinc: 45 mg/day
- Copper: 1 mg/day
- Boron: 6 mg/day

Botanical Medicines

- Curcumin, see Chapter 73, *Curcuma longa*
- Ginger, see Chapter 130, *Zingiber officinale*
- Bromelain, see Chapter 59
- *Boswellia serrata:* equivalent to 400 mg boswellic acids three times a day
- Procyanidolic oligomers from either pine bark (Pycnogenol) or grape seed extract: 100 to 300 mg a day
- Limbrel (Flavocoxid): 500 mg twice a day
- *H. procumbens:* Dosage should provide a minimum of 60 mg harpagoside a day.
  - Dried root powder (tablet or capsule): 2000 mg three times a day
  - Fluid extract (1:1): 2 mL three times a day
  - Dry powdered extract (standardized to contain 2.5% harpagoside): 750 to 1000 mg three times a day
- Yucca leaves: 2 to 4 g three times a day

Topical Application

Choose one of the following:

- Cetylated fatty acid cream (Celadrin) can be applied to affected areas twice a day.
- Menthol preparations can be applied to affected areas twice a day.
- Capsaicin preparations can be applied to affected areas twice a day.

Physical Therapy and Exercise

Physical activity that induces physiological or traumatic strain, such as occupational or recreational overuse of a joint, must be avoided. Normalization of posture and orthopedic correction of structural abnormalities should be used to limit joint strain. Daily nontraumatic exercise (isometrics, walking, and swimming) is important but should be carefully monitored. Short-wave diathermy, hydrotherapy,

and other physical therapy modalities that improve joint perfusion are indicated.

Acupuncture

Acupuncture can be administered two to four times weekly until acute symptoms resolve. Then the frequency of treatments should be gradually decreased. Maintenance treatments once every 2 weeks to once a month may help prevent recurrence.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. *Clin Geriatr Med*. 2010;26(3):355–369.
2. Grotle M, Hagen KB, Natvig B, et al. Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. *BMC Musculoskelet Disord*. 2008;9:132.
3. Askari A, Ehrampoush E, Homayounfar R, et al. Serum insulin in pathogenesis and treatment of osteoarthritis. *Med Hypotheses*. 2017;99:45–46. PubMed PMID: 28110696.
4. Summers MN, Haley WE, Reveille JD, et al. Radiographic assessment and psychologic variables as predictors of pain and functional impairment in osteoarthritis of the knee or hip. *Arthritis Rheum*. 1988;31:204–209.
5. Heinegård D, Saxne T. The role of the cartilage matrix in osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):50–57.
6. Bland JH, Cooper SM. Osteoarthritis: a review of the cell biology involved and evidence for reversibility. Management rationally related to known genesis and pathophysiology. *Sem Arthr Rheum*. 1984;14:106–133.
7. Perry GH, Smith MJ, Whiteside CG. Spontaneous recovery of the hip joint space in degenerative hip disease. *Ann Rheum Dis*. 1972;31:440–448.
8. Mullerova D, Kopecky J. White adipose tissue: storage and effector site for environmental pollutants. *Physiol Res*. 2007;56(4):375–381. PubMed PMID: 16925464.
9. Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. *Environ Health Perspect*. 2007;115(6):883–888. PubMed PMID: 17589595.
10. Lind PM, Eriksen EF, Sahlin L, et al. Effects of the antiestrogenic environmental pollutant 3,3',4,4',5-pentachlorobiphenyl (PCB #126) in rat bone and uterus: diverging effects in ovariectomized and intact animals. *Toxicol Appl Pharmacol*. 1999;154(3):236–244. PubMed PMID: 9931283.
11. Rosen JF, Chesney RW, Hamstra A, et al. Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. *N Engl J Med*. 1980;302(20):1128–1131. PubMed PMID: 7366636.
12. Shield MJ. Antiinflammatory drugs and their effects on cartilage synthesis and renal function. *Eur J Rheumatol Inflam*. 1993;13:7–16.
13. Brooks PM, Potter SR, Buchanan WW. NSAID and osteoarthritis: help or hindrance? *J Rheumatol*. 1982;9:3–5.
14. Newman NM, Ling RS. Acetabular bone destruction related to nonsteroidal antiinflammatory drugs. *Lancet*. 1985;2:11–13.
15. Solomon L. Drug induced arthropathy and necrosis of the femoral head. *J Bone Joint Surg*. 1973;55:246–261.
16. Ronningen H, Langeland N. Indomethacin treatment in osteoarthritis of the hip joint. *Acta Orthop Scand*. 1979;50:169–174.
17. Sharma L, Song J, Felson DT, et al. The role of knee alignment in disease progression and functional decline in knee osteoarthritis. *JAMA*. 2001;286:792.
18. Wright V. Treatment of osteoarthritis of the knees. *Ann Rheum Dis*. 1964;23:389–391.
19. Clarke GR, Willis LA, Stenners L, et al. Evaluation of physiotherapy in the treatment of osteoarthrosis of the knee. *Rheumatol Rehabil*. 1974;13:190–197.
20. Vanharanta H. Effect of short-wave diathermy on mobility and radiological stage of the knee in the development of experimental osteoarthritis. *Am J Phys Med*. 1982;61:59–65.
21. Zhou XY, Zhang XX, Yu GY, et al. Effects of low-intensity pulsed ultrasound on knee osteoarthritis: a meta-analysis of randomized clinical trials. *Biomed Res Int*. 2018;7469197.
22. Stelian J, Gil I, Habot B, et al. Improvement of pain and disability in elderly patients with degenerative osteoarthritis of the knee treated with narrow-band light therapy. *J Am Geriatr Soc*. 1992;40:23–26.
23. Fisher NM, Pendergast DR, Gresham GE. Muscle rehabilitation: its effects on muscular and functional performance of patients with knee osteoarthritis. *Arch Phys Med Rehabil*. 1991;72:367–374.
24. Kovar PA, Allegrante JP, MacKenzie CR, et al. Supervised fitness walking in patients with osteoarthritis of the knee: a randomized controlled trial. *Ann Intern Med*. 1992;116:529–534.
25. Zelazny CM. Therapeutic instrumental music playing in hand rehabilitation for older adults with osteoarthritis: four case studies. *J Music Ther*. 2001;38:97–113.
26. Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol*. 2010;22(5):512–519.
27. Chu IJH, Lim AYT, Ng CLW. Effects of meaningful weight loss beyond symptomatic relief in adults with knee osteoarthritis and obesity: a systematic review and meta-analysis. *Obes Rev*. 2018. https://doi.org/10.1111/obr.12726.
28. Huang MH, Chen CH, Chen TW, et al. The effects of weight reduction on the rehabilitation of patients with knee osteoarthritis and obesity. *Arthritis Care Res*. 2000;13:398–405.
29. Messier SP, Loeser RF, Miller GD, et al. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the arthritis, diet, and activity promotion trial. *Arthritis Rheum*. 2004;50:1501–1510.
30. Richette P, Poitou C, Garnero P, et al. Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. *Ann Rheum Dis*. 2011;70(1):139–144.
31. Morales-Ivorra I, Romera-Baures M, Roman-Viñas B, et al. Osteoarthritis and the Mediterranean diet: a systematic review. *Nutrients*. 2018;10(8);pii:E1030. https://doi.org/10.3390/nu10081030.
32. Childers NF, Russo GM. *The Nightshades and Health*. Somerville, NJ: Horticulture Publications, Somerset Press; 1977.
33. Morelli V, Naquin C, Weaver V. Alternative therapies for traditional disease states: osteoarthritis. *Am Fam Physician*. 2003;67:339–344.
34. Baici A, Horler D, Moser B, et al. Analysis of glycosaminoglycans in human sera after oral administration of chondroitin sulfate. *Rheumatol Int*. 1992;12:81–88.
35. Conte A, Volpi N, Palmieri L, et al. Biochemical and pharmacokinetic aspects of oral treatment with chondroitin sulfate. *Arzneimittelforschung*. 1995;45:918–925.
36. Shinmei M, Kobayashi T, Yoshihara Y, et al. Significance of the levels of carboxy terminal type II procollagen peptide, chondroitin sulfate isomers, tissue inhibitor of metalloproteinases, and metalloproteinases in osteoarthritis joint fluid. *J Rheumatol Suppl*. 1995;43:78–81.
37. Volpi N. Oral bioavailability of chondroitin sulfate (Condrosulf) and its constituents in healthy male volunteers. *Osteoarthritis Cartilage*. 2002;10:768–777.
38. Conte A, de Bernardi M, Palmieri L, et al. Metabolic fate of exogenous chondroitin sulfate in man. *Arzneimittelforschung*. 1991;41:768–772.
39. Baici A, Wagenhauser FJ. Bioavailability of oral chondroitin sulfate. *Rheumatology Int*. 1993;13:41–43.
40. Uebelhart D, Malaise M, Marcolongo R, et al. Intermittent treatment of knee osteoarthritis with oral chondroitin sulfate: a one-year, randomized, double-blind, multicenter study versus placebo. *Osteoarthritis Cartilage*. 2004;12:269–276.
41. Morita M, Yamada K, Date H, et al. Efficacy of chondroitin sulfate for painful knee osteoarthritis: a one-year, randomized, double-blind, multicenter clinical study in Japan. *Biol Pharm Bull*. 2018;41(2):163–171.
42. L'Hirondel JL. Double-blind clinical study with oral administration of chondroitin sulfate versus placebo in tibiofemoral gonarthrosis. *Litera Rheumatologica*. 1992;14:77–82.
43. Conrozier T, Vignon E. The effect of chondroitin sulfate treatment in coxarthritis: a double-blind placebo study. *Litera Rheumatologica*. 1992;14:69–75.
44. Morreale P, Manopulo R, Galati M, et al. Comparison of the antiinflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. *J Rheumatol*. 1996;23:1385–1391.
45. Mazières B, Hucher M, Zaïm M, et al. Effect of chondroitin sulphate in symptomatic knee osteoarthritis: a multicentre, randomised, double-blind, placebo-controlled study. *Ann Rheum Dis*. 2007;66(5):639–645.
46. Kahan A, Uebelhart D, De Vathaire F, et al. Long-term effects of chondroitins 4 and 6 sulfate on knee osteoarthritis: the study on osteoarthritis

progression prevention, a two-year, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 2009;60(2):524–533.
47. Ricci M, Micheloni GM, Berti M, et al. Clinical comparison of oral administration and viscosupplementation of hyaluronic acid (HA) in early knee osteoarthritis. *Musculoskelet Surg*. 2017;101(1):45–49.
48. Nelson FR, Zvirbulis RA, Zonca B, et al. The effects of an oral preparation containing hyaluronic acid (Oralvisc®) on obese knee osteoarthritis patients determined by pain, function, bradykinin, leptin, inflammatory cytokines, and heavy water analyses. *Rheumatol Int*. 2015;35(1):43–52.
49. Kalman DS, Heimer M, Valdeon A, et al. Effect of a natural extract of chicken combs with a high content of hyaluronic acid (Hyal-Joint) on pain relief and quality of life in subjects with knee osteoarthritis: a pilot randomized double-blind placebo-controlled trial. *Nutr J*. 2008;7:3.
50. Sato T, Iwaso H. An effectiveness study of hyaluronic acid (Hyabest J) in the treatment of osteoarthritis of the knee on the patients in the United States. *New Rem Clin*. 2009;58(3):551–558.
51. Kelly GS. The role of glucosamine sulfate and chondroitin sulfates in the treatment of degenerative joint disease. *Altern Med Rev*. 1998;3:27–39.
52. Ruff KJ, DeVore DP, Leu MD, Robinson MA. Eggshell membrane: a possible new natural therapeutic for joint and connective tissue disorders. Results from two open-label human clinical studies. *Clin Interv Aging*. 2009;4:235–240.
53. Ruff KJ, Winkler A, Jackson RW, DeVore DP, Ritz BW. Eggshell membrane in the treatment of pain and stiffness from osteoarthritis of the knee: a randomized, multicenter, double-blind, placebo-controlled clinical study. *Clin Rheumatol*. 2009;28(8):907–914.
54. Ruff KJ, Morrison D, Duncan SA, et al. Beneficial effects of natural eggshell membrane versus placebo in exercise-induced joint pain, stiffness, and cartilage turnover in healthy, postmenopausal women. *Clin Interv Aging*. 2018;13:285–295.
55. Hoffer A. Treatment of arthritis by nicotinic acid and nicotinamide. *Can Med Assoc J*. 1959;81:235–238.
56. Jonas WB, Rapoza CP, Blair WF. The effect of niacinamide on osteoarthritis: a pilot study. *Inflamm Res*. 1996;45:330–334.
57. Soeken KL, Lee WL, Bausell RB, et al. Safety and efficacy of S-adenosylmethionine (SAMe) for osteoarthritis. *J Fam Pract*. 2002;51:425–430.
58. Harmand MF, Vilamitjana J, Maloche E, et al. Effects of S-adenosylmethionine on human articular chondrocyte differentiation: an in vitro study. *Am J Med*. 1987;83:48–54.
59. Konig H, Stahl H, Sieper J, et al. Magnetic resonance tomography of finger polyarthritis: morphology and cartilage signals after ademetionine therapy. *Aktuelle Radiol*. 1995;5:36–40.
60. Schwartz ER. The modulation of osteoarthritic development by vitamins C and E. *Int J Vitam Nutr Res Suppl*. 1984;26:141–146.
61. Bates CJ. Proline and hydroxyproline excretion and vitamin C status in elderly human subjects. *Clin Sci Mol Med*. 1977;52:535–543.
62. Prins AP, Lipman JM, McDevitt CA, et al. Effect of purified growth factors on rabbit articular chondrocytes in monolayer culture. II. Sulfated proteoglycan synthesis. *Arthr Rheum*. 1982;25:1228–1238.
63. Krystal G, Morris GM, Sokoloff L. Stimulation of DNA synthesis by ascorbate in cultures of articular chondrocytes. *Arth Rheum*. 1982;25:318–325.
64. Peregoy J, Wilder FV. The effects of vitamin C supplementation on incident and progressive knee osteoarthritis: a longitudinal study. *Public Health Nutr*. 2011;14(4):709–715.
65. McAlindon TE, Felson DT, Zhang Y, et al. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knees among participants in the Framingham Study. *Ann Intern Med*. 1996;125:353–359.
66. Nawabi DH, Chin KF, Keen RW, et al. Vitamin D deficiency in patients with osteoarthritis undergoing total hip replacement: a cause for concern? *J Bone Joint Surg Br*. 2010;92(4):496–499.
67. Diao N, Yang B, Yu F. Effect of vitamin D supplementation on knee osteoarthritis: a systematic review and meta-analysis of randomized clinical trials. *Clin Biochem*. 2017;50(18):1312–1316.
68. Anand JC. Osteoarthritis and pantothenic acid. *J Coll Gen Pract*. 1963;5:136–137.
69. Anand JC. Osteoarthritis and pantothenic acid. *Lancet*. 1963;41:1168.
70. Calcium pantothenate in arthritis conditions. A report from the general practitioner research group. *Practitioner*. 1980;224:208–211.
71. Travers RL, Rennie GC, Newnham RE. Boron and arthritis. The results of a double-blind pilot study. *J Nutr Med*. 1990;1:127–132.
72. Newnham RE. Arthritis or skeletal fluorosis and boron. *Int Clin Nutr Rev*. 1991;11:68–70.
73. Oka H, Akune T, Muraki S, et al. Association of low dietary vitamin K intake with radiographic knee osteoarthritis in the Japanese elderly population: dietary survey in a population-based cohort of the ROAD study. *J Orthop Sci*. 2009;14(6):687–692.
74. Neogi T, Booth SL, Zhang YQ, et al. Low vitamin K status is associated with osteoarthritis in the hand and knee. *Arthritis Rheum*. 2006;54(4):1255–1261.
75. Singh GB, Atal CK. Pharmacology of an extract of salai guggal ex-*Boswellia serrata*, a new non-steroidal anti-inflammatory agent. *Agents Action*. 1986;18:407–412.
76. Kulkani RR, Patki PS, Jog VP, et al. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. *J Ethnopharmacol*. 1991;33:91–95.
77. Sengupta K, Alluri KV, Satish AR, et al. A double blind, randomized, placebo controlled study of the efficacy and safety of 5-Loxin for treatment of osteoarthritis of the knee. *Arthritis Res Ther*. 2008;10(4):R85.
78. Sengupta K, Krishnaraju AV, Vishal AA, et al. Comparative efficacy and tolerability of 5-Loxin and Aflapin, against osteoarthritis of the knee: a double blind, randomized, placebo controlled clinical study. *Int J Med Sci*. 2010;7(6):366–377.
79. Kimmatkar N, Thawani V, Hingorani L, et al. Efficacy and tolerability of *Boswellia serrata* extract in treatment of osteoarthritis of knee: a randomized double blind placebo controlled trial. *Phytomedicine*. 2003;10(1):3–7.
80. Belcaro G, Cesarone MR, Errichi S, et al. Treatment of osteoarthritis with Pycnogenol. The SVOS (San Valentino Osteo-arthrosis Study). Evaluation of signs, symptoms, physical performance and vascular aspects. *Phytother Res*. 2008;22(4):518–523.
81. Cisár P, Jány R, Waczulíková I, et al. Effect of pine bark extract (Pycnogenol) on symptoms of knee osteoarthritis. *Phytother Res*. 2008;22(8):1087–1092.
82. Levy R, Khokhlov A, Kopenkin S, et al. Efficacy and safety of flavocoxid compared with naproxen in subjects with osteoarthritis of the knee: a subset analysis. *Adv Ther*. 2010;27(12):953–962.
83. Levy RM, Khokhlov A, Kopenkin S, et al. Efficacy and safety of flavocoxid, a novel therapeutic, compared with naproxen: a randomized multicenter controlled trial in subjects with osteoarthritis of the knee. *Adv Ther*. 2010;27(10):731–742.
84. Levy RM, Saikovsky R, Shmidt E, et al. Flavocoxid is as effective as naproxen for managing the signs and symptoms of osteoarthritis of the knee in humans: a short-term randomized, double-blind pilot study. *Nutr Res*. 2009;29(5):298–304.
85. Pillai L, Levy RM, Yimam M, et al. Flavocoxid, an antiinflammatory agent of botanical origin, does not affect coagulation or interact with anticoagulation therapies. *Adv Ther*. 2010;27(6):400–411.
86. Gagnier JJ, Chrubasik S, Manheimer E. *Harpagophytum procumbens* for osteoarthritis and low back pain: a systematic review. *BMC Complement Altern Med*. 2004;4:13–23.
87. Lecomte A, Costa JP. *Harpagophytum* and osteoarthritis: a double-blind placebo-controlled trial. *37 2 Le Magazine*. 1992;15:27–30.
88. Chantre P, Cappelaere A, Leblan D, et al. Efficacy and tolerance of *Harpagophytum procumbens* versus diacerhein in treatment of osteoarthritis. *Phytomedicine*. 2000;7:177–183.
89. Vlachojannis J, Roufogalis BD, Chrubasik S. Systematic review on the safety of *Harpagophytum* preparations for osteoarthritic and low back pain. *Phytother Res*. 2008;22:149–152.
90. Bingham R, Bellew BA, Bellew JG. Yucca plant saponin in the management of arthritis. *J Appl Nutr*. 1975;27:45–50.

91. Morales TI, Wahl LM, Hascall VC. The effect of bacterial lipopolysaccharides on the biosynthesis and release of proteoglycans from calf articular cartilage cultures. *J Biol Chem*. 1984;259:6720–6729.
92. Hesslink Jr R, Armstrong 3rd D, Nagendran MV, et al. Cetylated fatty acids improve knee function in patients with osteoarthritis. *J Rheumatol*. 2002;29(8):1708–1712.
93. Kraemer WJ, Ratamess NA, Anderson JM, et al. Effect of a cetylated fatty acid topical cream on functional mobility and quality of life of patients with osteoarthritis. *J Rheumatol*. 2004;31(4):767–774.
94. Kraemer WJ, Ratamess NA, Maresh CM, et al. Effects of treatment with a cetylated fatty acid topical cream on static postural stability and plantar pressure distribution in patients with knee osteoarthritis. *J Strength Cond Res*. 2005;19(1):115–121.
95. Tilwe GH, Beria S, Turakhia NH, et al. Efficacy and tolerability of oral enzyme therapy as compared to diclofenac in active osteoarthrosis of knee joint: an open randomized controlled clinical trial. *J Assoc Physicians India*. 2001;49:617–621.
96. Klein G, Kullich W, Schnitker J, et al. Efficacy and tolerance of an oral enzyme combination in painful osteoarthritis of the hip: a double-blind, randomised study comparing oral enzymes with non-steroidal antiinflammatory drugs. *Clin Exp Rheumatol*. 2006;24(1):25–23.
97. Brien S, Lewith G, Walker AF, et al. Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study. *QJM*. 2006;99(12):841–850.
98. Soeken KL. Selected CAM therapies for arthritis-related pain: the evidence from systematic reviews. *Clin J Pain*. 2004;20:13–18.
99. Ferrandez Infante A, Garcia Olmos L, Gonzalez Gamarra A, et al. Effectiveness of acupuncture in the treatment of pain from osteoarthritis of the knee. *Aten Primaria*. 2002;30:602–608.
100. Vas J, Perea-Milla E, Mendez C. Acupuncture and moxibustion as an adjunctive treatment for osteoarthritis of the knee: a large case series. *Acupunct Med*. 2004;22:23–28.
101. Ng MM, Leung MC, Poon DM. The effects of electro-acupuncture and transcutaneous electrical nerve stimulation on patients with painful osteoarthritic knees: a randomized controlled trial with follow-up evaluation. *J Altern Complement Med*. 2003;9:641–649.
102. Tukmachi E, Jubb R, Dempsey E, et al. The effect of acupuncture on the symptoms of knee osteoarthritis: an open randomised controlled study. *Acupunct Med*. 2004;22:14–22.
103. Sangdee C, Teekachunhatean S, Sananpanich K. Electroacupuncture versus diclofenac in symptomatic treatment of osteoarthritis of the knee: a randomized controlled trial. *BMC Complement Altern Med*. 2002;2:3.
104. Singh BB, Berman BM, Hadhazy V, et al. Clinical decisions in the use of acupuncture as an adjunctive therapy for osteoarthritis of the knee. *Altern Ther Health Med*. 2001;7:58–65.
105. Li AH, Zhang JM, Xie YK. Human acupuncture points mapped in rats are associated with excitable muscle/skin-nerve complexes with enriched nerve endings. *Brain Res*. 2004;1012:154–159.
106. Panagos A, Jensen M, Cardenas DD. Treatment of myofascial shoulder pain in the spinal cord injured population using static magnetic fields: a case series. *J Spinal Cord Med*. 2004;27:138–142.
107. Pipitone N, Scott DL. Magnetic pulse treatment for knee osteoarthritis: a randomised, double-blind, placebo-controlled study. *Curr Med Res Opin*. 2001;17:190–196.
108. Wolsko PM, Eisenberg DM, Simon LS, et al. Double-blind placebo-controlled trial of static magnets for the treatment of osteoarthritis of the knee: results of a pilot study. *Altern Ther Health Med*. 2004;10:36–43.
109. Jacobson JI, Gorman R, Yamanashi WS, et al. Low-amplitude, extremely low frequency magnetic fields for the treatment of osteoarthritic knees: a double-blind clinical study. *Altern Ther Health Med*. 2001;7(54–64):66–69.
110. Driban J, Price L, Lo, G, et al. Evaluation of bone marrow lesion volume as a knee osteoarthritis biomarker - longitudinal relationships with pain and structural changes: data from the Osteoarthritis Initiative. *Arthritis Res Ther*. 2013;15(5):R112.
111. Sanghi D, Mishra A, Sharma A, et al. Does vitamin D improve Osteoarthritis of the knee: a randomized controlled pilot trial. *Clin Orthop Relat Res®*. 2013;471(11):3556–3562. https://doi:10.1007/s11999-013-3201-6.
112. Arden N, Cro S, Sheard S, et al. The effect of vitamin D supplementation on knee osteoarthritis, the VIDEO study: a randomised controlled trial. *Osteoarthr Cartil*. 2016;24(11):1858–1866. https://doi:10.1016/j.joca.2016.05.020.
113. Wang X, Blizzard L, Jin X, et al. Quantitative assessment of knee effusion-synovitis in older adults: association with knee structural abnormalities. *Arthritis Rheumatol*. 2016;68(4):837–844. https://doi:10.1002/art.39526.

Osteoporosis

Lara Pizzorno, MAR, MA, LMT

OUTLINE

DIAGNOSTIC SUMMARY

- Usually asymptomatic until fracture, kyphosis, and/or severe backache occurs
- Most common in postmenopausal white women
- Spontaneous fractures of the hip and vertebra
- Excess decrease in height
- Defined as a T-score at or below a bone mineral density of −2.5 standard deviations below that of a young normal adult

GENERAL CONSIDERATIONS

Osteoporosis Defined

Osteoporosis, literally "porous bone," is defined by the World Health Organization (WHO) consensus report as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk."[1]

PATHOPHYSIOLOGY

Constant, balanced bone remodeling is essential for the maintenance of a healthy skeleton throughout life. A balance between the activities of osteoclasts, which resorb old and/or damaged bone, and the bone-rebuilding activity of the osteoblasts, which form new bone, is the key to bone health. The remodeling actions of both osteoclasts and osteoblasts are required for the maintenance of a healthy skeleton. When osteoclast activity is upregulated—as it is by numerous factors discussed under "Therapeutic Considerations/Underlying Causes"—bone resorption outpaces bone formation promoting osteoporosis. However, when osteoclast activity is prevented—as it is by the bisphosphonates and denosumab (the antiresorptive drugs)—old and damaged bone accumulates. Although the bones are initially denser on x-ray, the bone quality is poor, and decreased risk of fracture is transient. When the nutrients osteoblasts require to lay down new bone—discussed under "Therapeutic Considerations"—are not adequately provided by the diet or supplements or are not effectively absorbed, osteoblasts' ability to create new bone is significantly impaired, once again promoting osteoporosis.

Remodeling begins in response to proinflammatory cellular signaling that activates the receptor activator of nuclear factor-kappa-B ligand (RANKL) or by an insufficiency of calcium in circulation, which triggers the secretion of parathyroid hormone. In either case, the production and activity of osteoclasts are stimulated. Once osteoclasts' resorbing actions are completed (typically in ~2 weeks), the body switches gears, readying osteoblasts to take over (which takes ~2 more weeks), after which, new bone formation (which takes ~13 weeks) begins. Together, the osteoclasts and osteoblasts that work in tandem in any particular region of the bone surface to accomplish

healthy remodeling are called the basic multicellular unit (BMU). The average lifespan of a BMU osteoclast–osteoblast team is called the remodeling period. When all systems are functioning healthfully, a full cycle of both aspects of the remodeling process is completed in 3 to 6 months.[2] Unfortunately, many factors in the modern Western lifestyle can disrupt this healthy balance by increasing osteoclast activation and/or hampering osteoblasts' ability to produce new bone. Because it takes far longer to build than to resorb bone, in either instance, bone removal outpaces rebuilding, leading to osteopenia and osteoporosis.

Prevention and Reversal

The solution is neither to inhibit all osteoclast activity (as do the bisphosphonates and denosumab) nor to artificially boost osteoblast activity (as do teriparatide and abaloparatide) but to restore balanced bone remodeling. This is done by (1) identifying, in each individual person, the specific factors promoting excessive osteoclast activation and eliminating or at least ameliorating them, and (2) ensuring the nutrient needs of the unique individual (not just those of the mythical "average" person) are met so that osteoblasts are provided with an optimal supply of all the nutrients required to build new bone.

DIAGNOSIS

In both women and men, the importance of clinician assessment of risk cannot be overstated, in part because of the following two problems with the standard criterion for the diagnosis of osteoporosis (i.e., a dual-energy x-ray absorptiometry [DXA] T-score of≤−2.5 at the lumbar spine, femur neck, or total hip): (1) most fractures in older adults occur in individuals who do not have osteoporosis by bone mineral density (BMD) criteria, and (2) BMD norms have been calculated using data for young non-Hispanic white females, and race/ethnicity significantly affects the risk for osteoporosis.[3,4]

Recognizing that most fractures occur in individuals whose BMD indicates osteopenia, not osteoporosis, the most recent position statement from the National Bone Health Alliance Working Group has proposed that postmenopausal women and men aged 50 years or older should be diagnosed with osteoporosis if they have a "demonstrable elevated risk" for future fractures. ***Demonstrable elevated risk*** includes a T-score of less than or equal to −2.5 at the spine or hip or: (1) having experienced a hip fracture with or without BMD testing; (2) having osteopenia by BMD testing and having sustained a vertebral, proximal humeral, pelvic, or, in some cases, distal forearm fracture; or (3) having elevated risk based on the WHO Fracture Risk Algorithm, FRAX (i.e., FRAX scores with ≥3% [hip] or 20% [major] 10-year fracture risk). According to the National Bone Health Alliance Working Group, prior low trauma fracture is suggestive of the highest risk for future fracture, and a low-trauma clinical vertebral fracture, proximal humerus fracture, or pelvic fracture is diagnostic of osteoporosis in a person with osteopenia. (4) The incidental finding of a vertebral fracture on a radiograph is also considered diagnostic of osteoporosis if the clinician has a reason to believe that it is likely to have been caused by reduced bone strength due to lower bone mass.

Dual-Energy X-Ray Absorptiometry

In the United States, the standard criterion for the diagnosis of osteoporosis in postmenopausal women and older men is a bone mineral density test (DXA) T-score of≤−2.5 at the lumbar spine, femur neck, or total hip.[3] Clinical practice guidelines universally recommend BMD screening (DXA) to identify osteoporosis, but not until women are 65 years and older and men are 70 years and older. Risk assessment is recommended to determine whether earlier DXA screening is warranted, and clearly, there are other ways to identify individuals at high fracture risk.

Bindex

The tool most recently approved by the U.S. Food and Drug Administration (FDA) is Bindex, a point-of-care device that measures the cortical bone thickness of the tibia; calculates the Density Index, which estimates hip BMD as measured with DXA; and detects osteoporosis with 90% sensitivity and specificity.[5] A Category III Current Procedural Terminology (CPT) code was issued for Bindex measurement in January 2018 that became effective July 1, 2018: 0508T—pulse-echo ultrasound bone density measurement resulting in indicator of axial bone mineral density, tibia[6]

In addition to DXA and Bindex, clinicians can diagnose osteoporosis using the occurrence of low-trauma fractures, fracture risk algorithms (e.g., FRAX), biochemical markers of bone turnover, and clinician judgment of risk based on the presence of underlying causes of bone loss (discussed under "Therapeutic Considerations").

Fracture Risk Algorithms

The **fracture risk assessment tool** may also be used to diagnose osteoporosis. FRAX is a WHO-sponsored, country-specific fracture risk assessment tool that combines BMD at the femoral neck (or total hip) with a group of well-validated and weighted clinical risk factors for fracture that are largely independent of BMD. Its use in the United States allows the assessment of fracture risk in both genders and four ethnic groups and is recommended primarily for individuals with a BMD finding of osteopenia. If the FRAX 10-year probability of hip fracture is ≥3% or the 10-year probability of major osteoporotic fracture is ≥20%, a diagnosis of osteoporosis can be made.[3]

Biochemical Markers of Bone Turnover

Biochemical markers of bone turnover (BTMs) can be used to easily and noninvasively assess skeletal turnover. BMD measurement does not assess all risk factors for fracture. Serum and urinary BTMs may:

- Predict risk of fracture independently of bone density in patients
- Predict rapidity of bone loss in patients
- Predict extent of fracture risk reduction when repeated after 3 to 6 months of treatment
- Predict magnitude of BMD increases with treatment

The most reliable markers of bone turnover are the serum rather than urinary forms of markers of bone formation and resorption. Serum bone formation markers include total osteocalcin (OC), a noncollagenous protein hormone produced by osteoblasts that binds calcium when activated by vitamin K_2; the bone isoenzyme of alkaline phosphatase (BAP), also produced by osteoblasts and involved in bone mineralization; and the N-terminal propeptide of type I collagen (PINP),which is cleaved from type I collagen during bone formation and released into the circulation, where its concentration is directly proportional to the amount of new collagen produced by osteoblasts.[3] The most accurate and reliable bone resorption markers include serum C-terminal crosslinking telopeptides of type I collagen (sCTX), which are proteolytic fragments of bone collagen matrix, and the 5b isoenzyme of tartrate-resistant acid phosphatase (TRAP-5B), an enzyme produced by osteoclasts.

For postmenopausal women, negative and significant correlations are frequently reported between BTMs and lumbar BMD, especially for the bone formation markers, BAP and OC, and for the bone resorption marker sCTX. In premenopausal women, bone formation markers are rarely significantly correlated with BMD. Trends seem more marked with bone alkaline phosphatase (ALP), osteocalcin (OC), and sCTX, for which high levels indicate screening for osteoporosis, particularly when seen in conjunction with vertebral fracture or high bone remodeling diseases, such as hyperparathyroidism or endogenous hypercortisolism, or in anyone who has taken glucocorticoids for 3 months or longer.

A high level of OC, bone ALP, or sCTX has been seen with prevalent vertebral fractures. Bone formation (ALP) and resorption (sCTX) levels have consistently been found to be higher in osteoporotic patients compared with controls. The most relevant BTM data reported in endocrine diseases associated with osteoporosis are high levels of bone ALP in primary hyperparathyroidism and low levels of OC in endogenous hypercortisolism.

As noted, serum rather than urine testing is preferable. sCTX is an instructive example as it has several advantages over urinary markers of type I collagen degradation that also apply to other BTMs: sCTX does not require the measurement of urinary creatinine, which is used to normalize varying urine excretion rates; it is widely available for any clinical laboratory; reference ranges established in large and well-documented populations of healthy premenopausal and postmenopausal women are available, although normal ranges in men are more limited; and sCTX has both a short-term and long-term within–subject variability that is approximately half that of urinary markers (around 10%–15% compared with around 20%–30%). However, this low within-subject variability is only obtained when sCTX is measured in fasting subjects because food intake markedly decreases CTX levels and increases its variability.[7–9]

M81.0 is the billable/specific *International Classification of Diseases,* Tenth Revision, Clinical Modification (ICD-10-CM) code that can be used to indicate a diagnosis of age-related osteoporosis *without current pathological fracture* for reimbursement purposes. The 2018 edition of the ICD-10-CM M81.0 became effective on October 1, 2017.[10] *M80** is the ICD-10-CM code for a pathological bone fracture due to the low bone mass and microarchitectural deterioration characteristic of osteoporosis.[11]

PREVALENCE AND RISK FACTORS

In women, the prevalence of osteoporosis at the femoral neck or lumbar spine ranges from 6.8% in those aged 50 to 59 to 34.9% in those aged 80 and older. Among U.S. women aged 65 and older, approximately 25.1% have osteoporosis (BMD T-score of≤−2.5), and 52.3% have low bone mass (BMD T-score between −1.0 and −2.5) calculated using BMD norms for young non-Hispanic white females, age-adjusted to the 2010 Census Bureau estimates. A meta-analysis of data from 12 cohort studies including 29,082 women reported that at the age of 65 years, a woman's risk ratio for hip fractures increased by 2.88 for each standard deviation (SD) decrease in BMD, and the risk of other osteoporotic fractures increased by 1.38 per SD decrease in BMD.[10] Data from the Study of Osteoporotic Fractures and Women's Health Initiative shows that postmenopausal women aged 50 and older with baseline T scores between −2.49 and −2.00 are likely to rapidly progress to osteoporosis, whereas postmenopausal women with baseline T scores of greater than −1.50 transition to osteoporosis much more slowly.[10]

In men, according to estimates from the National Health and Nutrition Examination Survey (NHANES) 2005–2008, the prevalence of osteoporosis at the femoral neck or lumbar spine ranges from 3.4% in those aged 50 to 59 to 10.9% in men aged 80 and older. Among U.S. men aged 65 and older, approximately 5.7% have osteoporosis, and 44.2 % have low bone mass.[10] Despite lower overall fracture incidence, men have greater morbidity and mortality associated with hip fracture compared with women. Although the relationship between BMD and fracture risk is similar in both sexes, gender differences in bone size and geometry cause men's bones to fracture at a higher mean BMD than women's.[4] Men are far more likely than women to die within 1 year after a hip fracture. The mortality rate in men within 1 year of hip fracture is 37.5%, which is 51% higher than in women.[12] Almost one third of men with hip fractures who survive have subsequent osteoporotic fractures during their remaining lifetime. After a hip fracture, most new fractures occur in relatively younger men within 5 years, whereas most aged 75 and older die before experiencing a new fracture. No standard BMD screening schedule exists for men, but because of these grim statistics, the American College of Physicians recommends that osteoporosis risk factors be evaluated in men aged 50 to 69 to determine whether DXA testing should be considered.[3]

Regardless of Ethnicity, Risk for Osteoporosis Is High

Although African Americans are the ethnic group at the lowest risk, osteoporosis is projected to occur in 53% of African American women and 24% of African American men.[13] Among Caucasians, the risk for low bone mass/osteoporosis is even higher; it is currently projected to develop in three out of four (77%) women and close to one of two (43%) men. Asian American women are at similar risk for osteoporosis as Caucasians. They share many of the risk factors that apply to Caucasian women, plus they tend to be slender and small-boned, and ~90% are lactose intolerant and avoid dairy products. Currently, vertebral or spinal fracture occurrence is high among Asian American women, whereas hip fractures tend to be fewer due to differences in hip geometry and microstructural skeletal organization. However, Asians are not immune to hip fracture; the incidence of osteoporotic hip fractures increased by 300% in Hong Kong from the 1960s to 1990s, and more than 50% of all osteoporotic hip fractures are projected to occur in Asia by the year 2050.[14-16] Mexican American women in the United States have an estimated prevalence of hip osteoporosis of 14%, compared with 17% of Caucasian and 6% of Black American women.[17] However, from 2005 to 2025, a 175% increase in the incidence of osteoporotic fractures is projected to occur in Hispanic Americans, meaning that 86% of Hispanic American women and 53% of Hispanic American men will be at increased risk of osteoporotic fractures by 2025.[13]

Risk Factors Indicating the Need for DXA Screening

All professional societies recommend DXA screening for women aged 65 years and older. For women younger than 65 years, risk factors suggestive of the need for DXA testing vary somewhat among authorities.

The *American Association of Clinical Endocrinologists* recommends that DXA testing be offered to all postmenopausal women between the age of 40 and 65 years with a history of fracture without major trauma, those with osteopenia identified radiographically, those starting or taking glucocorticoids for 3 months or longer, and perimenopausal women if they have low body weight (<127 pounds or bone mass index <20 kg/m^2) along with a history of ever having received long-term glucocorticoid therapy, a family history of an osteoporotic fracture, early menopause, current smoking, or excessive consumption of alcohol.

The *American College of Obstetricians and Gynecologists* recommends that women younger than 65 should receive DXA screening if they have a medical history of fragility fracture, a body weight of <127 lb, a medical cause of bone loss (medication or disease), are a current smoker, or have alcoholism or rheumatoid arthritis.

The list of risk factors of the *American College of Radiology* (ACR) is perhaps the most extensive. The ACR recommends testing those younger than 65 years with risk factors, including estrogen deficiency; a history of maternal hip fracture over the age of 50 years; weight <127 lb; >1 year of amenorrhea before the age of 42 years; and women aged 50 years and older who experience a wrist, hip, spine, or proximal humerus fracture with minimal or no trauma. Other risk factors

indicating the need for DXA include loss of height, thoracic kyphosis, radiological evidence of low bone mass or compression fracture, and >3 months of glucocorticoid therapy or other medications associated with bone loss (aromatase inhibitors, chronic heparin, etc.). Metabolic or medical conditions that affect bone loss in the ACR's list of risk factors include chronic renal failure; rheumatoid arthritis (RA) and other inflammatory arthritides; eating disorders, including anorexia nervosa and bulimia; organ transplantation; prolonged immobilization; and gastric bypass surgery. They also include endocrine disorders that affect BMD (e.g., hyperparathyroidism, hyper- or hypothyroidism) and conditions associated with secondary osteoporosis, such as malabsorption, malnutrition, osteomalacia, vitamin D deficiency, endometriosis, acromegaly, chronic alcoholism, cirrhosis, and multiple myeloma.

The ACR's list includes most of the previous factors and adds that women younger than 65 are at risk if they are postmenopausal women discontinuing estrogen replacement (hormone replacement therapy [HRT]).

The *International Society for Clinical Densitometry* adds those women with a fragility history of hip fracture in a parent, current smokers, those with rheumatoid arthritis, and those with alcohol intake of more than 2 units per day (one unit is 12 oz of beer, 4 oz of wine, or 1 oz of liquor).

The *National Osteoporosis Foundation* provides a list of risk factors in its most current (2014) Clinician's Guide.[8]

Conditions, Diseases, and Medications That Cause or Contribute to Osteoporosis and Fractures

Additional risk factors that should be considered but have not yet been included in any of the previously discussed lists appear in italics in the following lists and have been added by this author. Many diseases have been shown to increase the risk of bone loss. A full discussion is beyond the scope of this chapter. We only cover nonalcoholic fatty liver disease (NAFLD) here because its impact on bone is not commonly recognized and because it has become surprisingly prevalent.

Nonalcoholic Fatty Liver Disease

NAFLD causes bone loss by disrupting the body's ability to convert D3 into 25(OH)D. NAFLD is increasingly common; it's now the most rapidly increasing and frequently seen chronic liver disease, affecting about one out of three people in the Western world. Following menopause, the risk for NAFLD rises significantly; in more than half (55%) of women over age 60, liver function is compromised by NAFLD.[18] Approximately 50% of patients with chronic liver disease have osteoporosis.[19] The primary cause of NAFLD is insulin resistance, which itself is caused by diets rich in proinflammatory fats and/or refined carbohydrates (i.e., the standard American diet).[20]

Lifestyle Factors

- Alcohol abuse
- Excessive thinness
- Smoking (active or passive)
- Inadequate physical activity
- Frequent falling
- Immobilization

Nutritional Factors

- Vitamin D insufficiency
- Excess vitamin A
- *Vitamin A insufficiency*
- Low calcium intake
- High salt intake
- *Inadequate protein intake*[21-23]
- *Vitamin K_2 insufficiency*
- *Magnesium insufficiency*
- *Trace mineral insufficiencies*
- *Excessive intake of phosphate food additives*[24]
- *Hydrochloric acid insufficiency*

Environmental Factors

- Heavy metals—lead, mercury, and especially cadmium

Genetic Diseases

- Cystic fibrosis
- Glycogen storage diseases
- Ehlers–Danlos (genetic defects in connective tissue)
- Gaucher disease
- Hemochromatosis
- Homocystinuria
- Hypophosphatasia
- Marfan syndrome (genetic defects in connective tissue)
- Menkes steely hair syndrome
- Osteogenesis imperfecta
- Parental history of hip fracture
- Porphyria
- Riley–Day syndrome

Single-Nucleotide Polymorphisms[25]

Bone Formation

- *COL1A1* (collagen type 1 alpha; encodes instructions for the production of type 1 collagen)
- *GSTT1 and GSTM1* (glutathione S-transferases; detoxification, oxidative stress)
- *MTHFR* (converts 5,19-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cofactor for conversion of homocysteine to methionine; also involved in major signaling pathways in osteoblasts affected by DNA methylation and in sclerostin [*SOST*] inhibition)
- *IGF-1* (insulin growth factor-1; stimulates bone formation, recruits preosteoblasts)
- *BMP4* (bone morphogenetic protein; bone and cartilage development and repair)
- *LRP5* (low density lipoprotein receptor–related protein 5; regulates osteoblast proliferation and bone formation)
- *RunX2* (osteoblast differentiation)
- *CYP17* (encodes 17α-hydroxylase and 17,20-lyase involved in steroid hormone biosynthesis)[26]
- *ESR1* (estrogen receptor 1; estrogen has direct effects on osteocytes, osteoblasts, and osteoclasts and inhibitory effects by blocking the activation of osteoclasts either directly or via osteoblasts, by suppressing the production of bone resorbing cytokines from osteoblasts, and in T cells)[26]

Bone Resorption

- *CYP1A2* (detoxification of xenobiotics, caffeine)
- *MTHFR*
- *COMT* (catechol-o-methyl transferase; inactivates circulating catechol-estrogens and the catecholamine neurotransmitters dopamine, epinephrine, and norepinephrine)[26]
- *BMP2*
- *SOST* (sclerostin; inhibits bone formation)
- *GSTM1*

Inflammation

- *Interleukin 6 (IL-6)/IL-6 receptor* (stimulates osteoclasts)
- *C-reactive protein (CRP)*
- *Tumor necrosis factor-alpha* (TNF-α; induces reactive oxygen species [ROS], activates nuclear factor kappa B [NF-κB], RANKL, osteoclasts)
- *APOE* (APOE4 associated with reduced fat-soluble vitamin absorption; APOE2 with impaired delivery of K2)
- *FADS1* (fatty acid desaturase 1) polymorphisms (affect dietary eicosapentaenoic acid [EPA]/ docosahexaenoic acid [DHA] dose required to achieve the same circulating levels of EPA/DHA)[27]
- *GPx1* (glutathione peroxidase 1)[28]

Vitamin D Single-Nucleotide Polymorphisms

- *DHCR7* (encodes 7 dehydrocholesterol reductase, converts 7-dehydrocholesterol to cholesterol for production of vitamin D from sun exposure)
- *GC* (encodes GC-globulin, major carrier protein of 25[OH]D in circulation)
- *CYP2R1* (converts vitamin D into the active ligand for the vitamin D receptor [VDR])
- *CYP24A1* (regulates level of vitamin D)
- *CYP27A1* (conversion D3 to 25[OH]D)
- *CYP27B1* (regulates level 1,25-D)
- *VDR Fokl*
- *VDR Bsml*
- *VDR Taql*
- *VDR Apal*
- VDR Cdx2

Vitamin K Single-Nucleotide Polymorphisms

- APOE
- CCGX (enzyme that activates all the vitamin K–dependent proteins)
- VKOR (enzyme involved in vitamin K recycling)

Vitamin A Single-Nucleotide Polymorphism

- *BCMO1* (single-nucleotide polymorphisms [SNPs] in beta-carotene 15,15′-monooxygenase)

Vitamin B_6 Single-Nucleotide Polymorphism

- *PNPO* (pyridoxine 5′-phosphate oxidase, delivery of magnesium into cells)

Calciotropic and Sex Hormone Single-Nucleotide Polymorphisms

- *PTH/PTHR* (parathyroid hormone and its receptor; calcium homeostasis, vitamin D synthesis, regulation osteoblast, osteoclast activity)
- *CT/CTR* (calcitonin and its receptor; increases osteoblast activity, prevents calcium and phosphorus loss)
- *AR* (androgen receptor; regulates osteoblast function)
- *CYP19A1* (aromatase; catalyzes androgen conversion to estrogens)
- *CaSR* (calcium-sensing receptor; regulates calcium homeostasis at parathyroid, kidney, bowel, and bone level)
- *GR* (glucocorticoid receptor; inhibits bone formation, suppresses calcium absorption)

Hypogonadal States

- Androgen insensitivity
- Anorexia nervosa
- Athletic amenorrhea
- *Andropause (men)*
- *Menopause (women)*
- Hyperprolactinemia
- Panhypopituitarism
- Premature menopause (<40 years)
- Turner's and Klinefelter's syndromes

Endocrine Disorders

- Central obesity
- Cushing's syndrome
- Diabetes mellitus (types 1 and 2)
- Hyperparathyroidism
- *Insulin resistance*[29]
- Thyrotoxicosis (*hyperthyroidism)*
- Hypothyroidism

Gastrointestinal Disorders

- Celiac disease
- Gastric bypass
- *Gastrectomy*[30]
- Gastrointestinal surgery
- Inflammatory bowel disease
- Malabsorption
- Pancreatic disease
- Primary biliary cirrhosis

Hematological Disorders

- *Anemia*[31]
- Hemophilia
- Leukemia and lymphomas
- Monoclonal gammopathies
- Multiple myeloma
- Sickle cell disease
- Systemic mastocytosis
- Thalassemia

Rheumatic and Autoimmune Diseases

- Ankylosing spondylitis
- Other rheumatic and autoimmune diseases
- Rheumatoid arthritis
- Systemic lupus
- *Fibromyalgia*

Neurological and Musculoskeletal Risk Factors

- Epilepsy
- Multiple sclerosis
- Muscular dystrophy
- Parkinson's disease
- Spinal cord injury
- Stroke

Miscellaneous Conditions and Diseases

- *Atrial fibrillation*[32]
- AIDS/HIV
- Amyloidosis
- *Atopic dermatitis*[33]
- *Atherosclerosis*
- *Cerebral artery disease*[34]
- Chronic metabolic acidosis
- *Chronic kidney disease, chronic nephrolithiasis*[35]
- *Chronic liver disease (NAFLD, NASH, hepatitis)*[18,19]

- Chronic obstructive lung disease
- *Crohn's disease*[36]
- Congestive heart failure
- Depression
- End-stage renal disease
- *Kidney transplant*[37]
- *Herpes zoster*[38]
- *Homocysteine levels, elevated*[39]
- Hypercalciuria
- *Hypertension*[34]
- Idiopathic scoliosis
- *Nonalcoholic fatty liver disease*
- Posttransplant bone disease
- *Sarcopenia*[40]
- Sarcoidosis
- Weight loss

Medications

- Aluminum (in antacids)
- *Antacids, H2 blockers*
- Anticoagulants (*warfarin*, heparin)
- Anticonvulsants
- Antidepressants (selective serotonin reuptake inhibitors [SSRIs], monoamine oxidase inhibitors [MAOIs], atypical antipsychotics)
- Aromatase inhibitors
- Barbiturates
- *Benzodiazepines*
- *Calcineurin inhibitors*
- Cancer chemotherapeutic drugs
- Depo-medroxyprogesterone (premenopausal contraception)
- Glucocorticoids (≥5 mg/d prednisone or equivalent for ≥3 months)
- Gonadotropin-releasing hormone (GnRH) agonists
- *Hormonal contraceptives (oral contraceptives, IUDs, injectables)*
- Lithium cyclosporine A tacrolimus
- Loop diuretics
- Methotrexate
- *Nonsteroidal anti-inflammatory drugs (NSAIDs; with the exception of aspirin)*
- *Opioid pain medications (morphine, codeine, hydrocodone, oxycodone)*
- Parental nutrition
- Proton pump inhibitors
- Selective serotonin reuptake inhibitors
- Tamoxifen (premenopausal use)
- Thiazolidinediones (glitazones such as Actos and Avandia)
- Thyroid hormones (in excess)[8]

Risk Factors for Men

With the exception of menopause, men share all the previous risk factors noted for women. ***In men, common significant risk factors include the following***[10]:

- Age >70 years
- Low body weight (body mass index <20–25 kg/m^2)
- Weight loss
- Physical inactivity
- Previous fragility fracture
- Corticosteroid use
- Androgen deprivation medications
- *Erectile dysfunction*[41,42]
- *Low testosterone*[43-45]
- *5-Alpha reductase inhibitor therapy (used for male pattern baldness)*[46]
- *Excessive alcohol consumption*
- *Smoking*[47]
- *Hypertension*[48]
- *Aortic calcification*[49,50]
- *Coronary artery disease*[51,52]
- *Sarcopenia*[40]
- *Cadmium exposure*[53]

CAUSES OF BONE LOSS

Anything that fuels chronic low-grade inflammation will trigger the cellular pathways that activate osteoclasts and/or hamper the ability of osteoblasts to produce new bone, promoting bone loss. Referred to as "secondary causes of osteoporosis" in the medical literature, inflammation dysfunction is an important underlying contributor to bone loss. Chronically upregulated inflammation combined with many other causes discussed in this section clearly explain the osteoporosis epidemic. Because treatment of the cause is foundational to naturopathic medicine, their control is critical for long-term successful care. What is surprising is the number and diversity of causes that must be considered.

Standard American Diet

Heavily reliant upon highly processed foods and loaded with refined sugars and carbohydrates, sodium, proinflammatory fats (saturated fat, arachidonic acid, trans fats), excessive amounts of protein, and a mix of inflammation-provoking chemicals (e.g., phosphates and other food additives, pesticide residues), the standard American diet (SAD) is a recipe for bone loss.[54]

Sodium

The SAD's high sodium content causes calcium to be lost in the urine, which, in turn, stimulates bone resorption. Sodium and calcium compete for reabsorption in the kidneys. For every 100 mmol of sodium excreted in urine, 1 mmol of calcium is also lost. The recommended upper limit for sodium consumption for adults 19 years and older in the United States is 100 mmol, which equals 2300 mg or 1 teaspoon of salt per day.[55] The average daily sodium intake for Americans aged 2 years and older, derived primarily from processed and restaurant foods, is 3436 mg.[56] Postmenopausal women lose more calcium relative to sodium than premenopausal women or men, and unlike either group, they are unable to compensate by absorbing additional calcium.[57,58] The difference in the amount of calcium that will be lost in urine from a woman consuming 50 mmol of sodium (50 mmol of sodium = 1150 mg sodium = ½ teaspoon of salt) and one ingesting 150 mmol (150 mmol = 3450 mg sodium = ~1.5 teaspoons of salt) amounts to 1 mmol (40.1 mg) more calcium lost per day. To put the harmful effects of this into "real-life" perspective: 1 mmol/d is the average negative calcium balance in the postmenopausal population (i.e., the amount of calcium the average postmenopausal woman loses daily from her total bone mass).[57]

Excessive Protein

Calcium-balance studies have confirmed that calcium excretion in urine increases with high-protein diets: 0.25 mmol of calcium is lost for every 10 g of protein consumed. However, the increased incidence of fractures seen in subjects with high protein intake occurs mostly in individuals consuming nondairy animal protein. Why? Because the high concentration of calcium in dairy foods helps offset its protein-induced loss.[54] Several studies have indicated that "protein and calcium act synergistically on bone—*if* both are present in adequate quantities

in the diet," and that only when calcium intake is low does calcium loss caused by animal protein become a problem.[59-64] When calcium intake is insufficient, high intake of animal protein can almost triple the risk for hip fracture. Data collected on 1752 men and 1972 women who participated in the Framingham offspring study showed those with calcium intakes of less than 800 mg/d, who were also consuming the most animal protein, had 2.8 times the risk of hip fracture compared with those consuming the least animal protein. In contrast, those consuming at least 800 mg/d of calcium, and also consuming the most animal protein, had an 85% **reduced** risk of hip fracture.[65]

Refined Sugars and Carbohydrates

The average American consumes 125 g of sucrose (table sugar, produced from sugar cane and sugar beets) and 50 g of high-fructose corn syrup daily. The SAD's sugar-laden processed foods cause insulin secretion to spike with virtually every meal and snack, and hyperinsulinemia promotes osteoporosis as well as metabolic syndrome, type 2 diabetes, and cardiovascular disease. One of the ways in which chronically elevated insulin promotes bone loss is by inhibiting the kidney's ability to reabsorb calcium, so more is lost in the urine.[66] High-fructose corn syrup (which is composed of both fructose and glucose in approximately equal parts) is now the predominant sweetener used in processed foods and beverages. Thus increased consumption of these items results in increased consumption of glucose. In studies using human cells, high-dose glucose consumption has been shown to promote bone loss by inhibiting osteoblast proliferation. In human studies, providing male subjects 100 g of glucose increased the amount of calcium lost in their urine by ~30% and increased the amount of magnesium lost by ~40%; 100 g of glucose is present in a can of soda or a typical serving of fruit juice or in virtually any processed food made from refined flour, corn, or potatoes.[67] Researchers looked at what happened to calcium excretion when they varied the amount of refined carbohydrate in an otherwise standardized diet in 18 normal subjects. The amount of calcium in the urine varies throughout the day, but increasing the refined carbohydrate content of the diet in this study caused a significant increase in the number of single urines with a calcium concentration above 9 mmol.[68] To put this in perspective, the normal amount of calcium that should be found in an entire day's (24-hour) urine collection is ~15 mmol.[69]

Metabolic Acidosis

Several components of the SAD (especially salt and sulfur-containing amino acids) cause chronic low-grade metabolic acidosis, which not only results in calcium being withdrawn from bone to restore a more alkaline state but also ramps up osteoclast activity.[70]

Phosphate Additives

Increasing consumption of processed foods in the United States over the past decades has resulted in phosphorus consumption rising to a level that far exceeds the human requirement for this mineral and promotes bone loss.[71] When the calcium-to-phosphorus dietary intake ratio is less than 1:1, secretion of parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) is triggered. Chronic elevation of either PTH or FGF-23 causes bone loss. A number of animal studies have shown that high dietary phosphorus relative to calcium induces secondary hyperparathyroidism, bone resorption, lower peak bone mass, and fragile bones in young as well as old animals. Several acute studies in healthy adult humans have shown that, even in healthy subjects, oral phosphate loading increases levels of markers of bone and cardiovascular disease. The PTH + FGF-23 combo, whose harmful effects were initially identified in patients with chronic kidney disease, is now known to cause bone loss and adverse cardiovascular effects, including arterial calcification, endothelial dysfunction, and left ventricular hypertrophy, in the general "healthy" population.[72] Phosphorus is a processing aid with many different functions in processed foods. In any average person's refrigerator or pantry, the majority of food products consumed for breakfast, lunch, dinner, and snacks contain phosphorus in at least one, and often a number of, added ingredients. Although the amount of phosphorus in each item may be low, the total daily load of phosphorus adds up. Unlike phosphorus in whole foods, where it is bound to protein and other nutrients and thus is far more slowly and less efficiently absorbed, phosphorus in food additives is rapidly and almost completely absorbed. Colas are especially rich in phosphoric acid, a commonly used inorganic phosphate additive in products ranging from all carbonated cola beverages to commercial salad dressings; phosphoric acid has an "absorption efficiency approaching 100%."[73,74]

Saturated Fats

Saturated fats in meat and dairy products from organically raised, pastured animals contain significant amounts of a bone-protective fatty acid, conjugated linolenic acid (CLA). Over the last 20 years, CLA has drawn significant attention for improving bone mass; reducing body fat, cardiovascular diseases, and cancer; and beneficially modulating immune and inflammatory responses.[75] In contrast, meat and dairy products derived from animals fed the animal version of the SAD (50% hay and silage and 50% grain) are high in proinflammatory omega-6 fats and low in CLA. Meat and dairy products from grass-fed animals contain 300% to 500% more CLA than products from cattle fed the conventional diet.[76-78]

Polyunsaturated Fats

The SAD delivers high levels of omega-6s and very low amounts of omega-3s, typically a ratio of 20:1. This imbalance in essential fatty acids destroys bone through several inflammatory mechanisms, all of which combine to increase the production and activity of osteoclasts and inhibit the production and activity of osteoblasts.[79] The omega-3 fats are metabolized into anti-inflammatory prostaglandins (the PGE3 series) and are further converted into the resolvins and protections or specialized proresolving mediators (SPMs), all of which decrease inflammation and the production and activity of osteoclasts.[80,81] One of the most nefarious ways in which excessive consumption of omega-6s promotes bone loss is by causing mesenchymal stem cells (MSCs) to develop into adipocytes rather than osteoblasts.[82] Many studies have now shown that the anti-inflammatory effects of the omega-3s lower osteoclast activity and reduce bone resorption. A reduction in the ratio of omega-6 to omega-3 decreases the risk of not only osteoporosis but also obesity, cardiovascular diseases, cancers, rheumatoid arthritis, kidney disease, and virtually every other disease affected by chronic inflammation. The omega-6s are not the enemy when in balance with the omega-3s. As demonstrated specifically in elders in the Framingham Osteoporosis Study, which evaluated 623 individuals whose average age was 75 years, omega-6 fatty acids (arachidonic acid [AA]) and omega-3s (DHA and EPA) protect hip bone health—*if* intake of omega-3s (EPA and DHA) is high enough to produce a ratio of no more than 4:1 omega-6:omega-3.[81-85]

Trans Fats

Trans fats are hidden in many processed foods, including chips, cookies, cakes, pies, pastries, donuts, fried foods, artificial creamers and whipped cream, and French fries. Some frozen pizza brands contain up to 5 g of trans fats per serving; microwave popcorn contains up to 7 g per serving.[86] They cause bone loss by upregulating inflammation.

Monounsaturated Fats

As noted, the SAD is high in omega-6–rich vegetable oils and low in monounsaturated fats as well as omega-3s. Several studies have found positive associations between diets in which olive oil was the primary source of added fat and bone density, and other research showed that a higher ratio of monounsaturated fat in the diet was associated with a reduced risk of fracture in the elderly.[87]

Gluten

In 460 BC, Hippocrates, the father of Western medicine, stated, "All disease begins in the gut," a foundational concept still taught in naturopathic medical schools. In 2010 AD, Dr. Alessio Fasano translated this medical insight into 21st-century idiom, saying: "The gut is not like Las Vegas. What happens in the gut does not stay in the gut."[88] Fasano was alluding to the fact that gluten, the major protein in wheat, disrupts gut function, not only in those with celiac disease but also in approximately 80% of humans, causing chronic inflammation that promotes numerous diseases, including osteoporosis. Fasano's research team discovered that the tight junctions do not, as was considered gospel until very recently, form an impenetrable, static barrier wall. Instead, these so-called "tight junctions" are actually doors that can be opened by zonulin, a protein that intestinal cells produce as a defensive mechanism. To date, only three agents have been found that trigger zonulin's release: *Vibrio cholerae* (specifically, its toxin), pathogenic intestinal bacteria,[89] and gluten—more accurately, a polypeptide in gluten called gliadin and more specifically, one of the indigestible peptide fractions within gliadin called α-gliadin. Exposure to α-gliadin increases risk of chronic inflammation in ~80% of humans; only 80% because 20% do not produce zonulin. However, in addition to zonulin, gluten's peptides contain at least 50 toxic sections (epitopes) that exert cytotoxic, immunomodulatory, and gut-permeating activities. Gluten causes a leaky gut, with widespread harmful ramifications for bone remodeling. When gluten is consumed at most every meal and snack day in and day out, as is the case in the SAD, intestinal permeability is constant. This allows undigested food components and inflammatory microorganisms to gain access to the rest of the body. Why are we suddenly seeing a dramatic increase in gluten sensitivity? Because wheat has been significantly "hybridized" and "deamidated" over the last 50 years.[90] Hybridization creates a new protein by combining different strains of wheat, altering gluten's protein sequence by as much as 5%. The result is a "new"—and to the food industry—"improved" wheat.[91,92] Deamidation, used extensively in the processed food industry, uses acids or enzymes to render gluten, which is normally only soluble in alcohol, water soluble. This allows it to be more easily mixed with other food products but also has been shown to create substances that generate a severe immune response in humans.[93-95] Any individual with osteopenia or osteoporosis should be evaluated for a gluten-related disorder as a contributing factor. Tests should be run for celiac disease and wheat allergy. If both are negative, a gluten-free diet trial should be run to evaluate for gluten sensitivity. If gluten sensitivity exists, beneficial responses will be seen within a matter of weeks, not months as is often the case for symptoms to lessen in someone with celiac disease.

Symptoms suggestive of gluten-sensitivity include the following:

- Osteopenia, osteoporosis
- Dermatitis (eczema or skin rash)
- Bone, joint or muscle pain (e.g., rheumatoid arthritis)
- Symptoms similar to those of irritable bowel syndrome (IBS) symptoms (abdominal pain, bloating, bowel habit abnormalities—either diarrhea or constipation)
- Weight loss
- Leg or arm numbness
- Muscle cramps
- Anemia
- "Foggy mind," headache, fatigue
- Depression[96-100]

Commonly Prescription and Over-the-Counter Drugs

Aromatase Inhibitors

Use: Estrogen-receptor-positive breast and ovarian cancer, prostate cancer. *Commonly prescribed examples:* Exemestane (Aromasin), an irreversible steroidal inhibitor, forms a permanent bond with the aromatase enzyme, deactivating it. Anastrozole (Arimidex) and letrozole (Femara) are nonsteroidal inhibitors that inhibit estrogen synthesis by outcompeting androgens to bind with aromatase. *Bone-destructive mechanism:* Aromatase inhibitors thwart the activity of aromatase, the enzyme that converts androgens into estrogens. The loss of estrogen causes bone loss. Letrozole and anastrozole increase bone turnover, decrease BMD, and increase the relative risk of vertebral and nonvertebral fractures by 40%, compared with tamoxifen. Bone loss with increased risk of fragility fractures also occurs in women receiving exemestane. Only partial recovery of BMD occurs following withdrawal of aromatase inhibitors.[101]

Gonadotropin-Releasing Hormone Agonists

Use: Management of endometriosis and breast cancer in premenopausal women; prostate cancer in men. *Commonly prescribed examples:* Leuprolide (Lupron, Eligard), buserelin (Suprefact, Suprecor), deslorelin (Suprelorin, Ovuplant). *Bone-destructive mechanisms:* Suppress secretion of gonadotropin, which is required for follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production, which is required for the ovaries' production of estrogen and progesterone in women and for the production of testosterone and its conversion into estrogen (estradiol) in men. Thus GnRH agonists suppress estrogen production, causing bone loss: in women, a decrease in BMD of about 6% per year. In men, GnRH agonists are often given along with aromatase inhibitors to maximize androgen deprivation. BMD at the hip, wrist, and lumbar spine decreases by 2% to 5% after 12 months of androgen deprivation therapy. The relative risk of vertebral and hip fractures increases by 40% to 50%. Men also experience a loss of lean body mass, an increase in fat mass, and impaired muscular strength, all of which contribute to increased risk of fractures.[101,102]

Anticonvulsants

Use: To manage epilepsy, bipolar disorder, neuropathic pain. *Commonly prescribed examples:* Dilantin, Luminal, Depacon. *Bone-destructive mechanisms:* Interfere with vitamin D absorption and metabolism, cause vitamin D and calcium deficiency, may cause deficiency of folate and/or vitamin B_6, also reduce blood levels of vitamin K.[103]

Benzodiazepines

Use: To manage epilepsy, anxiety, insomnia, depression, schizophrenia, restless leg syndrome. *Commonly prescribed examples:* Valium, Halcion, Xanax, Librium (far too many to list here). *Bone-destructive mechanism:* Bind to dopamine receptors, causing chronic elevation of prolactin and suppressing the activity of the hypothalamic–pituitary–gonadal (HPA) axis. Because function of the HPA axis is required for the production of estrogen and progesterone in women and testosterone in men, this disruption promotes bone loss.[104-106]

Antidepressants

Use: Manage symptoms of depression, anxiety disorders, and some personality disorders (e.g., obsessive-compulsive disorder, eating disorders, premature ejaculation). MAOIs are also used to manage Parkinson's disease. Atypical antipsychotics are used to manage schizophrenia, bipolar disorder, and autism. *Commonly prescribed examples:* SSRIs (e.g., Prozac, Zoloft, Paxil), MAOIs (e.g., selegiline [Emsam, Deprenyl]), atypical antipsychotics (e.g., olanzapine [Zyprexa], risperidone [Risperdal], blonanserin [Lonasen]). *Bone-destructive mechanism:* Inhibit dopamine production and neurotransmission, causing chronic elevation of prolactin, which disrupts the activity of the HPA axis and the production of sex hormones.[107-109]

Insulin-Sensitizing Medications

Use: Manage type 2 diabetes *Commonly prescribed examples:* Thiazolidinediones (a.k.a. glitazones, e.g., Avandia, Actos).*Bone-destructive mechanisms*: Trigger MSCs to develop into adipocytes rather than osteoblasts, chondrocytes, or myocytes, thus decreasing bone formation and increasing the formation of visceral adipose tissue (VAT; belly fat). Excess VAT is linked to abdominal obesity, insulin resistance, type 2 diabetes, and other inflammatory diseases.[110] The glitazones (rosiglitazone and pioglitazone) are selective agonists of peroxisome proliferator-activated receptor-γ (PPAR-γ), whose activation of in mesenchymal cells leads to increased adipocyte differentiation and decreased osteoblast differentiation. The thiazolidinediones also decrease the expression of insulin-like growth factor-I (IGF-1), a growth factor that promotes bone formation. Plus, thiazolidinediones promote osteoclast development and bone resorption. Long-term treatment with thiazolidinediones increases the risk of fractures by up to fourfold in postmenopausal women and in men. Risk correlates with the duration of treatment and is significant within 12 to 18 months.[101]

Opioid Pain Medications

Use: Manage chronic pain. *Commonly prescribed examples:* morphine (sold under more than 100 trade names), codeine (sold under dozens of trade names, often combined with aspirin, acetaminophen, or ibuprofen), hydrocodone (Lortab, Norco, Vicodin), oxycodone, methadone, tramadol. *Bone-destructive mechanisms:* Disrupt normal HPA regulation of hormone production. Increase the production of prolactin. Inhibit the production of estrogen and dehydroepiandrosterone (DHEA). Increase production of thyroid-stimulating hormone (TSH), which directly suppresses bone remodeling. Lower testosterone production in men.[111] The use of opioids as a long-term treatment for chronic pain has increased so dramatically that opioid-induced deficiency of androgens (DHEA and testosterone) has been given its own acronym in the medical literature: OPIAD.[112]

Glucocorticoid Medications

Use: Manage allergies, asthma, autoimmune diseases. *Commonly prescribed examples:* Prednisone, Prednisolone, Kenalog, Dexamethasone. *Bone-destructive mechanisms:* Glucocorticoids suppress osteoblast activity and hence bone formation while, at the same time, osteoclast numbers are either unchanged or slightly increased. Glucocorticoids induce production of caspase 3 and other proteins central to cellular apoptosis, causing apoptosis in both osteoblasts and osteocytes. Because osteocytes play a central role in skeletal sensing of the need for bone repair and in bone repair, this results in weakening of bone (within 6 months) even when glucocorticoids are used at very low doses. Glucocorticoids change the balance between receptor activator for NF-κB ligand (RANKL) and osteoprotegerin (OPG). RANKL, which is generated by osteoblasts and osteocytes, is a key regulator of osteoclast recruitment, activation, and survival. Osteoblasts and osteocytes also produce OPG, a decoy receptor for RANKL that inhibits its actions on osteoclasts. The balance between RANKL and OPG is a central determinant of more (RANKL) or less (OPG) bone resorption; glucocorticoids tip this balance in favor of RANKL. Glucocorticoids also cause an increase in the production of macrophage colony-stimulating factor, a proinflammatory cytokine that triggers the production of more proinflammatory cytokines, promoting the generation of osteoclasts. Glucocorticoids also directly prolong the life span of mature osteoclasts. Glucocorticoids inhibit Wnt protein expression in mature osteoblasts, which results in MSCs becoming adipocytes instead of osteoblasts. Glucocorticoids deplete vitamin D_3. BMD drops 6% to 12% within the first year of glucocorticoid use, and approximately 3% per year afterward. Fracture risk escalates up to 75% within the first 3 months (Fig. 203.1).[113,114]

Calcineurin Inhibitors

Use: As immune system suppressants given in combination with glucocorticoids in patients undergoing organ transplantation to help prevent organ rejection. *Commonly used examples:* Cyclosporine and tacrolimus. *Bone-destructive mechanisms:* Disrupt vitamin D metabolism and calcium absorption and cause secondary hyperparathyroidism.[101]

Antacids, H2 Blockers, Proton Pump Inhibitors

Use: Manage symptoms of indigestion, heartburn, gastroesophageal reflux disease (GERD). *Commonly prescribed examples:* Antacids include Maalox, Mylanta, Rolaids, Tums, Alka Seltzer, and Milk of Magnesia. H2 blockers include Tagamet, Zantac, Pepcid, and Axid. Proton pump inhibitors include Prilosec, Prevacid, and Nexium. *Bone-destructive mechanisms:* Stomach acid is required for the digestion of food. Many vitamins (particularly B_{12}, whose absorption requires both HCl and intrinsic factor) and minerals required for healthy bones, including calcium, are not solubilized without stomach acid. Antacids neutralize stomach acid after its secretion. H2 blockers block the activity of histamine-producing cells in the stomach lining, whose release of histamine signals parietal cells to secrete HCl, thus preventing HCl production. Proton pump inhibitors (PPIs) block the proton pump inside parietal cells in the stomach lining, which produce and secrete HCl and intrinsic factor. PPIs are the most potent of the acid-blockers; just one pill can reduce stomach acid secretion by 90% to 95% for 24 hours. PPIs are strongly associated with significantly increased fracture risk.[115-119]

Loop Diuretics

Use: Manage high blood pressure, heart failure, liver cirrhosis, and certain kidney diseases. *Commonly prescribed example:* Furosemide (Lasix). *Bone-destructive mechanisms:* Directly increase the kidney's elimination of calcium, reducing its blood levels, triggering PTH secretion and the withdrawal of calcium from bone. Loop diuretics also increase sodium loss, sometimes to the point of causing hyponatremia. Approximately one third of total body sodium resides in the bone, with 40% of bone sodium content being exchangeable with sodium in the bloodstream. A moderate but persistent loss of both bone sodium and calcium either indirectly, via an increased renal excretion caused by loop diuretics, or directly, by hyponatremia-induced bone resorption, negatively affects bone strength and fracture risk. Hyponatremia also increases bone resorption by

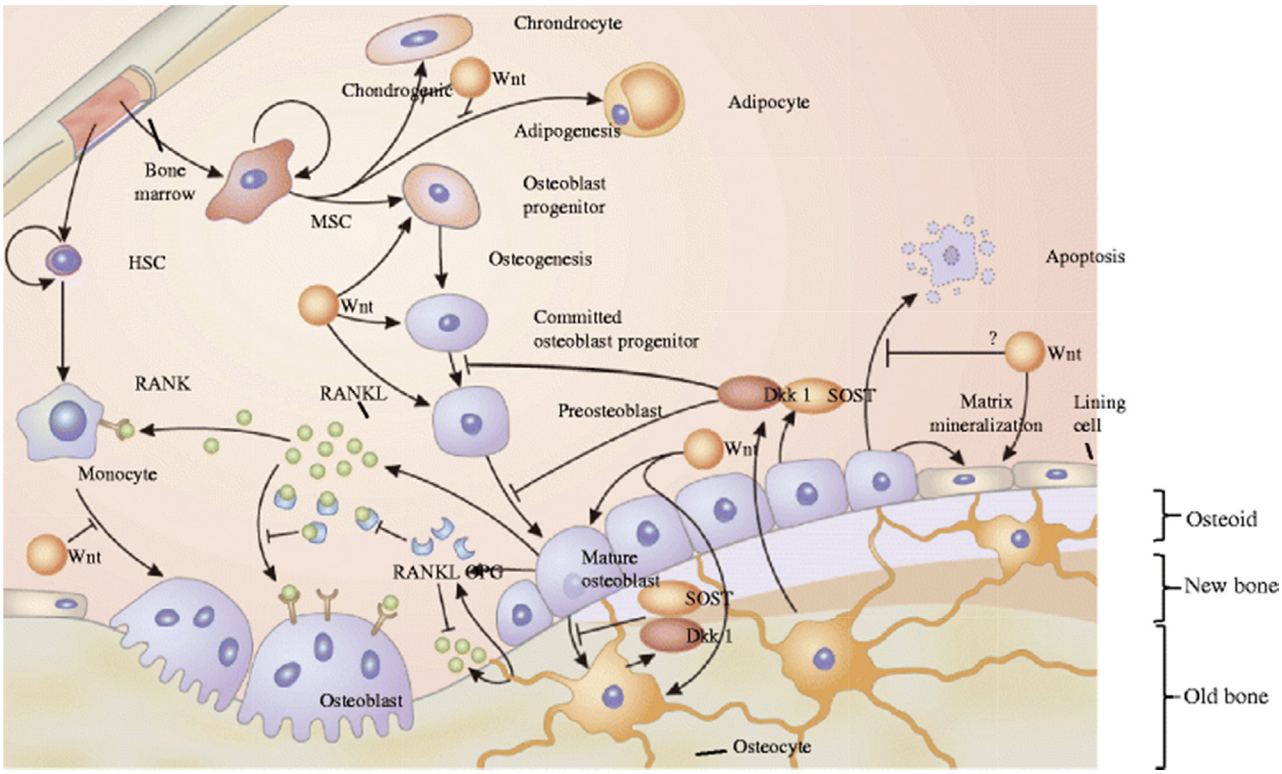


Fig. 203.1 Wnt pathway. Baron, R., Kneissel, M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 19, 179 -192 (2013) https://doi.org/10.1038/nm.3074.

increasing both osteoclast formation and activity. Add to this the depletion in blood volume caused by all forms of diuretics, which increases the likelihood of postural hypotension (dizziness when sitting up or rising into standing position), and you have a perfect setup for a fracture-causing fall. Loop diuretics may also deplete magnesium.[114,120-122]

Anticoagulants (Coumarins, Heparin)

Use: Coumarins prevent excessive blood clot formation; heparin prevents deep vein thrombosis and pulmonary embolisms and is the most frequently used anticoagulant in hemodialysis for patients with chronic kidney disease. *Commonly prescribed examples:* Warfarin (Coumadin), heparin, unfractionated heparin, low-molecular-weight heparins. *Bone-destructive mechanisms:* Warfarin prevents vitamin K recycling and therefore the activation of osteocalcin, a vitamin K–dependent protein (VKDP) that ushers calcium into bone, and the activation of matrix Gla protein, a VKDP that inhibits calcium deposition in soft tissues (e.g., blood vessels, heart, kidneys, breast, brain). Heparin does not affect cortical bone but rapidly decreases trabecular bone volume by disrupting vitamin D metabolism and inducing secondary hyperparathyroidism. The result is a significant drop in osteoblasts' production and activity and in osteoid surface in the bone, accompanied by a large increase in osteoclasts and bone resorption.[114,123] Low-molecular-weight heparin may be less destructive to bone than other forms of heparin, but not enough clinical trials have been run to determine whether this is the case.[124]

Thyroid Hormone Replacement Medications

Use: Manage hypothyroidism. *Commonly prescribed examples:* Armour thyroid, levothyroxine, Levoxyl, Synthroid, Unithroid. *Bone-destructive mechanism:* In 25% of patients, doses of thyroxine prescribed are slightly higher than needed or become so over time.[101] A dose of thyroxine (LT4) even slightly in excess of need causes suppression of TSH. TSH directly protects bone by inhibiting the production of osteoclasts.[125] In addition, low TSH may indicate hyperthyroidism, which significantly increases bone turnover and reduces BMD. Although bone formation and bone resorption both increase, bone resorption outpaces bone formation, resulting in bone loss. TSH should be monitored regularly, and the LT4 dosage adjusted according to results, in anyone on long-term LT4 replacement therapy.[114,126]

Hormonal Contraceptives: Birth Control Pills, Intrauterine Devices, Injectable Depo-Provera

Use: Prevent pregnancy. *Commonly prescribed examples:* Oral contraceptives contain either a combination of patented versions of "estrogen" and progesterone (a "progestin") or a progestin only. Commonly prescribed combination "estrogen" and progestin pills include Apri, Yaz, Yasmin, Ortho-Novum, Ogestrel. Commonly prescribed progestin-only pills include Camila, Errin, Heather, Jolivette, Nora-BE.[56] *Depo-Provera* (medroxyprogesterone) is a long-acting progestin-only contraceptive provided via injection every 3 months. *Intrauterine devices (IUDs)* that dispense the progestin levonorgestrel (e.g., Mirena) are another often-prescribed option. Mirena remains effective (and is left in place) for 5 years. *Bone-destructive mechanisms: Oral contraceptives* lower blood levels of vitamin B_6 and vitamin B_{12}, causing levels of homocysteine to rise. High levels of homocysteine are associated with both cardiovascular disease and osteoporosis, in particular, hip fracture. Homocysteine inhibits lysyl-oxidase, which plays an important role in collagen crosslink formation, so inhibition results in the production of a weakened bone matrix. Homocysteine causes the balance between RANKL and OPG to shift in favor of RANKL, promoting osteoclast generation and activity. Elevated levels of homocysteine also increase oxidative stress, another key instigator of osteoclast production and activity.[127,128] Birth con-

trol pills, whether they contain only "estrogen" or combine the "estrogen" with a progestin, work by inhibiting follicular development and preventing ovulation. Thus both types of birth control pills prevent ovulation and, therefore, the formation of the corpus luteum, which produces progesterone, which is required for the development of osteoblasts.[129-136] In the Canadian Multicentre Osteoporosis Study, oral contraceptive users had BMD scores 2.3% to 3.7% lower than those of women who had never used oral contraceptives.[137] The progestin-only contraceptive Depo-Provera (medroxyprogesterone) is the most widely used contraceptive worldwide. Because of its common use in younger women and documentation that its administration may be associated with loss of BMD, the FDA has attached a black box warning to its label.[138] The progestin-only contraceptive Mirena, now being used in women as young as 14 years of age, is an IUD that dispenses levonorgestrel and not only prevents ovulation but also typically causes amenorrhea. A recent meta-analysis estimated a BMD increase of 0.5% per year in women with normal ovulation but a decrease in BMD of 0.7% per year in young women with ovulatory disturbances (anovulation or short luteal phase).[139]

Environmental Toxins

Lead

Since January 1, 1996, when the U.S. Clean Air Act banned the sale of leaded fuel for use in on-road vehicles, lead levels have dropped significantly in the population. However, virtually everyone older than 50 was exposed to leaded gasoline during their childhood and teenage years when growing bones were most vulnerable. Now, as these individuals enter menopause or andropause, and their rate of bone loss increases, the lead that was safely sequestered is being released—and greatly contributing to postmenopausal and andropausal bone loss.[140-142] Furthermore, although public health measures removed lead from gasoline (except aviation fuel), lead continues to contaminate many U.S. homes. In 2006 an estimated 22% of U.S. homes—23.2 million of them—still contained lead-based paint. Paint deteriorates, flakes, and becomes lead-laden dust in the air and on the floors, carpets, and so forth in our homes. Data from the 2007–2010 NHANES revealed that 535,000 young children could have unsafe blood lead levels (at or above 5 μg/dL).[143] A recent analysis indicated that the United States loses $50.9 billion in economic activity each year because of IQ points lost to lead exposure.[144] More than 90% of the lead is sequestered in bones. But bone is constantly being remodeled, and in the process of bone resorption, calcium is not the only mineral released into the bloodstream. The lead that was quarantined in bones is released into the bloodstream along with calcium. Clear associations have now been found between increased bone levels of lead and osteoporosis,[145] kidney disease, cataracts, and high blood pressure. Current research has led to strong recommendations to further lower the presently allowed blood lead level to minimize chronic cumulative lead toxicity.[140,142] Lead decreases osteoblast formation while not affecting osteoclast production and activity, and it also damages the kidneys, impairing their ability to convert 25(OH)D to 1,25-D.[140-142] Lead blocks the production of transcription factor-2 (Cbfa-1/Runx-2), a transcription factor involved in MSCs' maturation into osteoblasts rather than adipocytes. Lead increases the production of sclerostin, a bone morphogenetic protein agonist produced by osteocytes, and peroxisome proliferator-activated receptor-γ (PPAR-γ), whose activation in MSCs causes their differentiation into adipocytes. The combination of these factors—Cbfa-1/Runx-2, sclerostin, and PPARγ—is called the Wnt/β-catenin pathway (see Fig. 203.1) When Cbfa-1/Runx-2 is not inhibited, and sclerostin and PPAR-γ are, this pathway builds bone. Lead distorts the healthful balance among the bone-remodeling actions of the Wnt/β-catenin pathway. Lead also interferes with heme synthesis, calcium-dependent enzyme systems, and hormone metabolism (estrogen, progesterone, testosterone, cortisol, growth hormone—all of which affect bone) and causes nerve degeneration, impairing muscle contractions that trigger bone formation. In premenopausal women, elevated levels of uric acid, one of lead's adverse effects on the kidneys, correlate with lack of ovulation and therefore inhibition of progesterone production. Lack of progesterone inhibits osteoblast production. Lack of ovulation results in infertility.[146] Bone lead levels can be evaluated by means of K-x-ray fluorescence, a technique developed in the last 20 years that measures the lead content in the cortex of the tibia and the patella. One caveat: K-x-ray fluorescence testing may overestimate lead levels in individuals having low bone density and underestimate lead levels in individuals with higher bone density. It is therefore important to ensure the radiologist interpreting your patient's films is aware of these issues and knows how to accommodate for them.[147,148]

Cadmium

Cadmium accumulates in kidneys and bone and has a biological half-life of 10 to 30 years.[149,150] The urinary cadmium level considered "safe" by the U.S. Occupational Safety and Health Administration (OSHA) is 3 mcg/g creatinine.[151] However, according to the latest research, a "safe" level of urinary cadmium appears to be no more than 0.05 mcg/g creatinine, preferably less. A urine cadmium level of even 0.5 mcg/g creatinine may still have adverse effects on bone; in the studies, the level of urinary cadmium shown to cause bone loss has continued to be lower. In 1999 a study in Belgium of populations living in the vicinity of zinc smelters found that urinary cadmium excretion of just 1 mcg/g creatinine was associated with a 73% increased risk of bone fractures in women and a 60% increased risk of height loss in men. This amount—1 mcg/g creatinine—is one third the U.S. OSHA "safe" level. Other studies conducted on populations in cadmium-contaminated areas in China and Sweden have produced similar findings. In China, the osteoporosis prevalence in women aged 50 or older who lived near a smelter increased from 34% in the control group to 51.9% in heavily polluted areas. Early kidney damage, osteoporosis and a threefold increased risk of fractures were also found in a study of more than 1000 people living in the vicinity of a nickel cadmium battery plant in southern Sweden. In these studies, urinary cadmium levels of 3 nmol/mmol creatinine (equivalent to 2.98 mcg/g creatinine, i.e., the U.S. OSHA supposedly "safe" level) caused osteoporosis. In another a population-based study of 2688 women in Sweden, in comparison with women whose urinary cadmium level was less than 0.50 mcg/g creatinine, those with urinary cadmium levels of 0.75 or greater mcg/g creatinine had a 245% increased likelihood of osteoporosis at the femoral neck and a 197% increased likelihood of osteoporosis of the lumbar spine. Among women who had higher urine levels of cadmium, despite the fact that they had never smoked, the situation was surprisingly worse. The likelihood of osteoporosis among never-smokers who, nonetheless, had high urinary levels of cadmium was increased by 347% for osteoporosis at the femoral neck and by 326% for osteoporosis at the lumbar spine. The authors of this paper concluded that even among women who have never smoked, possible cadmium toxicity is of much greater concern than has been previously thought. And these never-smokers developed osteoporosis with urinary cadmium levels of just 0.75 mcg/g, far less than the U.S. OSHA "safe" level of 3 mcg/g creatinine. Data from a number of recent studies now indicate that urinary excretion of cadmium greater than 0.05 mcg/g creatinine damages the kidneys and promotes bone loss, fragility, and fractures.[152] When researchers drew data from two NHANES studies

(1988–1994 and 1999–2004) involving 4258 women aged 50 or older, women with urinary cadmium levels between 0.50 and 1.00 mcg/g creatinine had a 43% greater risk for hip-BMD–defined osteoporosis compared with those with urinary cadmium levels lower than or equal to 0.50 mcg/g. Once again, smokers did not show a statistically increased risk. This is not to say that smokers' bones are not at increased risk of cadmium-caused osteoporosis. Cigarette smoke delivers cadmium, which is well absorbed from the soil by tobacco plants, to the lungs, where it is much better absorbed than the cadmium ingested in food. Unfortunately, the high-phosphate fertilizers used in conventional agriculture deposit significant amounts of cadmium in the soil, with the amount depending on where the phosphates were mined. The phosphate fertilizers used on conventionally grown crops can contain cadmium in amounts up to 300 mg/kg.[153] The U.S. FDA's Total Diet Study update (2007) reported a 26% increase in dietary cadmium exposure from 1990 through 2003. Cadmium intake, which occurs primarily from consumption of phosphate-fertilized food, rose from 8.81 to 11.06 mcg per person per day. This translates to 21% of the supposedly "safe" tolerable intake of cadmium for a week.[154]According to NHANES III data covering the years from 1988 to 2004, 73% of U.S. women 50 years of age or older are estimated to have cadmium body burdens greater than 0.50 mcg/g creatinine, a level that has repeatedly been found to significantly increase risk for osteoporosis. These data and the results of the most recent studies suggest that 21% of osteoporosis prevalence among women aged 50 years or older may be attributable to cadmium.[154]

What these studies tell us is that (1) U.S. women are at increased risk for osteoporosis at urinary cadmium levels way below OSHA's "safety" standard of 3 mcg/g creatinine[151,154] and (2) dietary cadmium in conventionally grown foods, rather than tobacco, is the primary source of cadmium-related osteoporosis risk in the U.S. female population aged 50 years or older. Comparable research has not yet been done on men, but this author predicts it will reveal that dietary cadmium is a significant risk factor for osteoporosis in aging men as well.[155] Cadmium disrupts parathyroid hormone's signaling to kidney cells to activate vitamin D, inhibits the enzymes in the kidney that convert 25(OH) D to 1,25-$(OH)_2D_3$, and damages the renal tubules, causing hypercalciuria. Cadmium also inhibits the activity of bone-specific alkaline phosphatase, diminishes osteoblasts' ability to mineralize bone and produce collagen, further diminishes bone's collagen content by stimulating osteoclast formation and activity, and activates gene expression of "toxic response" pathways in bone cells, further stimulating osteoclastic bone resorption.

Mercury

Mercury is ubiquitous in the environment, but the most common sources of exposure are dental amalgam fillings and fish, with lesser amounts coming from air, water, vaccinations, and cosmetics. Mercury compounds, such as those found in fish, vaccinations, and cosmetics, are water soluble, with a bioavailability of 7% to 15% after ingestion. Upon entering the body, mercury compounds accumulate mainly in the kidneys, causing damage and disrupting, among other essential kidney functions, the activation of vitamin D. Possibly by preventing the formation of 1,25-D, mercury causes hypocalcemia and calcium's withdrawal from bone.[156] Human exposure to elemental mercury, from dental amalgams (so-called "silver" fillings, which are typically 55% mercury), for example, is mainly by inhalation, which is followed by rapid absorption and distribution to all major organs. The primary target organs of elemental mercury are the brain and the kidneys. Elemental mercury is lipid (fat) soluble and can cross the blood–brain barrier, whereas inorganic mercury compounds are not lipid soluble, which is why inorganic mercury preferentially accumulates in the kidneys.[156] Elemental mercury also enters the brain from the nasal cavity through the olfactory pathway; for this reason, it is essential that the removal of dental amalgams be done by an ecologically trained dentist who knows how to minimize the release of mercury during extraction.[157,158] Emerging research is showing that mercury poisoning plays a significant role in high blood pressure, cardiovascular disease, stroke, mitochondrial dysfunction, and oxidative stress.[159] All of these conditions promote chronic inflammation, which promotes excessive activation of osteoclasts, excessive bone loss, and osteoporosis.

Fluoride

The primary sources of exposure to fluoride are diet (food and water) and fluoride-containing dental products (e.g., toothpaste). Fluoride is found in higher concentrations in soft, alkaline, and calcium-deficient waters, and because the fluoride compounds that occur naturally in drinking water are almost totally bioavailable (90%), they are virtually all absorbed from the gastrointestinal tract.[160,161] Along with fluoridation of community drinking water to prevent dental caries has come an increase in dental fluorosis. In its mild forms, fluorosis appears as tiny white streaks or specks in the tooth enamel. In its severe form, pitting and brown discolorations, which are permanent and can darken over time, disfigure the teeth. As of 2005, 23% of persons in the United States aged 6 to 39 years had mild or more severe dental fluorosis.[161] Fluoride activates both osteoblasts and osteoclasts. Although fluoride may increase bone mass, the newly formed bone lacks normal structure and strength. Under a microscope, the "crystallization pattern" of bone from fluoride-treated animals and humans reveals itself to be abnormal. In trabecular bone, fluoride increases bone volume and thickness but does not increase bone structure, reducing bone quality despite the increase in mass. After ingestion, fluorine goes first to the liver, but it is such a powerful oxidizer—the strongest oxidizer currently known—that it impairs the liver's attempts to prepare it for excretion and passes into the bloodstream, from which it is rapidly distributed to all tissues, including bones.[162] Once inside bone, fluorine overwhelms bone cells' ability to produce glutathione, and when defenses have been eliminated, it destroys osteoblasts and causes inflammation, triggering increased osteoclast production and activity.[163,164] Fluorine accumulates in bone, binding to and blocking the activity of bone-specific alkaline phosphatase, an enzyme involved in the production of osteoblasts. Fluorine also reduces levels of copper, zinc, manganese, and other trace minerals required by other enzymes involved in osteoblast activity.[165] Bones are largely composed of calcium compounds, up to 50% of which is hydroxyapatite. Fluorine converts hydroxyapatite to fluorapatite. This changes bones' crystalline structure, delays further mineralization with calcium, and causes a reduction in bones' mechanical strength properties.[161] Hydrogen fluoride reacts with calcium to form an insoluble salt, CaF_2. This salt must be eliminated by the kidneys, and as it goes, it carries out some calcium from the bone matrix.[166] Increased fluoride intake has been repeatedly shown to increase blood levels of parathyroid hormone and to cause hyperparathyroidism and increased bone resorption.

Pesticides

Dietary exposure to pesticide residues, even to very low doses of pesticide mixtures, is now recognized to be damaging to human health. Several animal studies, using models that duplicate human dietary exposure, have recently looked at the effects of pesticides both singly

and when combined (as they are in real life) in doses corresponding to what the U.S. Environmental Protection Agency (EPA) says are "Acceptable Daily Intakes" (ADIs). (ADIs are also referred to as NOAELs, the acronym for "no observed adverse effect levels.") When pesticides were approved for use on food crops, they were evaluated singly. We now know that when combined, their toxic effects are synergistic. A study in which female mice were exposed to pesticides while pregnant and while breastfeeding their young found that cell signaling was disrupted in offspring bone marrow cells, adversely affecting lymphocyte and red blood cell production. Hypoxia causes inflammation. Lymphocytes eliminate pathogens, toxins, and cellular debris, all of which cause inflammation. Chronic inflammation triggers excessive activation of osteoclasts and bone loss. Endosulfan, a widely used pesticide evaluated in this study, is a well-known pro-oxidant that activates NF-κB and JNK, which trigger the production of other proinflammatory mediators (i.e., TNF-α, IL-6), which then restimulate the original compounds (JNK and NF-κB) that initiate inflammation. The end result is a vicious feed-forward cycle of chronic inflammation. Subclinical chronic inflammation has been recognized as a driving factor in metabolic syndrome, which is a driving factor in type 2 diabetes, cardiovascular disease, and osteoporosis.[167]

Exposure to persistent organochlorine compounds is strongly associated with an increased incidence of kidney disease, diabetes, and cardiovascular disease, all conditions in which low-grade, chronic inflammation plays a prominent role, and each of which is strongly associated with an increased risk of osteoporosis.[168-176]

Excessive Alcohol Consumption

More than one drink (1 ounce of ethanol) per day for women or two drinks per day for men will decrease bone formation via a number of mechanisms. Excessive alcohol intake decreases the secretion of leptin, which has recently been found drive MSC differentiation into osteoblasts; suppresses the activity of IGF-1, an agent of growth hormone, which is an important regulator of bone growth and remodeling; damages the liver, thus impeding the conversion of D3 to 25(OH)D; lowers blood levels of estradiol; causes osteocytes to commit apoptosis; suppresses the bone-building Wnt pathway and increases the activity of its antagonist, DKK1; and lastly, high levels of acetaldehyde, the main metabolite of ethanol, directly inhibit osteoblast proliferation and activity. A small amount of alcohol, however, regardless of the type consumed, has beneficial effects on bone, causing blood levels of several markers of bone resorption (osteocalcin, CTx, and NTx) to drop. Plus, an increase in estrogen level has been observed in women drinking small amounts (no more than 10 g/d) of alcohol and in men with light alcohol consumption.[177]

Nutrient Insufficiencies

The SAD is also sorely lacking in the foods (e.g., leafy greens and other vegetables, whole grains, legumes, nuts, and seeds) rich in the vitamins and minerals that bones must have to rebuild. Americans' intake falls short for every one of the key bone-building nutrients (Table 203.1) The role of deficiencies of each nutrient is discussed fully under "Therapeutic Considerations."

THERAPEUTIC CONSIDERATIONS

Pharmacological Therapy Is Rarely an Effective Long-Term Solution

An inadequate supply of the nutrients essential for healthy bones and an overabundance of harmful factors that mitigate against healthy bone remodeling are the real causes of the osteoporosis epidemic. The drugs currently available to treat osteoporosis, none of which remedies its true causes, offer, at best, only short-term postponement of excessive bone loss and, at worst, cause serious adverse effects, including the fractures they are prescribed to prevent (Table 203.2).

TABLE 203.1 Essential Nutrients for Healthy Bones

Major Minerals
- Calcium
- Magnesium

Trace Minerals
- Boron
- Zinc
- Copper
- Manganese
- Silicon
- Selenium
- Iodine
- Strontium citrate

Vitamins
- Vitamin K_1
- Vitamin K_2
- Vitamin A
- B vitamins: B_6, B_{12}, folate, riboflavin, niacin
- Vitamin C
- Vitamin E

Fats
- Essential fatty acids: omega-3s eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)
- Organic, cold-pressed olive, canola, or coconut oil or organic butter from pastured cows, to enable absorption of the fat-soluble vitamins (e.g., vitamins D_3, K_2, E, and A, all of which require fat for absorption)

TABLE 203.2 Commonly Prescribed Drugs for Osteoporosis
- Pamidronate (APD, Aredia)
- Neridronate (Nerixia)
- Olpadronate
- Alendronate (Fosamax)
- Ibandronate (Boniva)
- Risedronate (Actonel)
- Zoledronate (Zometa, Aclasta)

Pharmacological Therapy Adverse Effects

The two most common significant adverse drug reactions (ADRs) caused by the antiresorptives are atypical femur fractures and osteonecrosis of the jaw.[178]

Epidemiological studies have found that women taking alendronate over extended periods are 12.5 times more likely to suffer a femur fracture than women not taking the bisphosphonate: "Current epidemiologic evidence suggests that long-term bisphosphonate use (>5 years) may be an important risk factor for atypical fractures."[179,180]

Osteonecrosis of the jaw (*ONJ;* also now referred to in the medical literature as bisphosphonate-related ONJ *[BRONJ]*. antiresorptive

agent-induced ONJ *[ARONJ]*, or the all-inclusive term drug-induced ONJ *[DRONJ]*). Preventing osteoclasts from playing their required roles in normal bone remodeling, which is what the bisphosphonates and denosumab do, results in the accumulation of dying bone in the jaw and its eventual exposure to the inside of the mouth, along with greatly increased risk of oral infection—precisely what is seen in individuals taking bisphosphonates or denosumab who develop ONJ.[181] Since 2001, thousands of case reports have been published of patients who developed ONJ after taking a bisphosphonate (often alendronate BECAUSE it is the most commonly prescribed but also quite frequently zoledronic acid) to prevent or treat bone loss. Just one of the papers reporting bisphosphonate-induced ONJ, published in 2010, was entitled "Bisphosphonate-Induced Osteonecrosis of the Jaw: A Review of 2400 Patient Cases."[182] The route of administration (oral or IV) of a bisphosphonate and the duration of treatment affects the projected time to onset of ONJ. The average time to ONJ onset is 3 years for intravenous (IV) bisphosphonates and 5 years for oral bisphosphonates.[2] However, the risk for ONJ has been found to increase fourfold after 2 years with both IV and oral bisphosphonates. A study published in 2012 in the *Journal of Evidence-Based Dental Practice* involved 191 patients (>40 years of age) with ONJ drawn from dental practices in three practice-based research networks. The risk of ONJ was greater with IV bisphosphonates, for which the odds ratio was 299.5, than oral bisphosphonates, for which the odds ratio of 9.8 was still quite high.[183] Denosumab is not less likely to cause ONJ. When denosumab was compared head to head with the IV bisphosphonate zoledronic acid, in a large group of patients, ONJ occurred in more patients treated with denosumab (20, or 2%, of 1026) than those treated with zoledronic acid (14, or 1.4%, of 1020 patients).[184]

Other potential adverse effects of the bisphosphonates and denosumab include the following:

- Atrial fibrillation[185-189]
- Erosions and ulcerations of the esophagus (throat) and severe damage to the lining of the gastrointestinal tract[190]
- Esophageal cancer[191]
- Influenza-like illness[192-194]
- Myalgia[195]
- Deterioration of kidney function and kidney failure[196,197]
- Symptomatic hypocalcemia[198-200]
- Conjunctivitis, uveitis, and scleritis[201-203]
- Inflammatory jaw diseases in addition to ONJ, including osteomyelitis, osteitis, periostitis, and sequestrum[204]

Denosumab causes all the adverse effects seen with the bisphosphonates, plus infections requiring hospitalization, including eczema, cellulitis, pneumonia, urinary tract infection, pyelonephritis, diverticulitis, appendicitis, and sepsis.[205-208]

The antiresorptives' lack of efficacy is demonstrated by the number needed to treat (NNT) to prevent one fracture. Table 203.3 provides data collected by Curtis et al. (2008) on the vertebral and hip fracture efficacy of the oral bisphosphonates, specifically on the number of women who must adhere to treatment for 1 year to prevent one fracture of the type specified.[209] The NNT data are unimpressive, and when the incidence of ADRs is considered, these drugs appear useful only for very limited indications, as described in the following discussion.

All patients = osteopenic + osteoporotic. (The majority of osteoporotic fractures happen in individuals with BMD T-scores in the osteopenic range (−2.5< T-score <−1).[210]

A review published in the journal *Drugs* in 2011 included an evaluation of the NNTs for denosumab. The researchers reviewed placebo-controlled, randomized, double-blind, pivotal Phase III trials employed as part of the regulatory process in order to calculate NNTs for denosumab for 3 years to prevent one hip fracture. After 3 years, postmenopausal osteoporotic women receiving denosumab had "a slightly reduced risk of hip fracture with a cumulative incidence of 0.7% in the denosumab group versus 1% in the placebo group" (i.e., 3 out of 1000 women benefitted), giving an NNT of 334 (i.e., 334 women had to adhere to treatment with denosumab for 3 years to prevent one hip fracture).[211]

TABLE 203.3 Number Needed to Treat for 1 Year to Prevent One Fracture

	WOMEN			
Fracture Type	**45–54 years**	**55–64 years**	**65–71 years**	**72–78 years**
Hip, all patients	6058	4406	691	176
Hip, with osteoporosis	5238	3601	567	107
Wrist, all patients	1008	840	n/a	n/a
Wrist, with osteoporosis	863	704	n/a	n/a
Vertebral, all patients	2687	2121	629	221
Vertebral, with osteoporosis	906	647	449	155

n/a = not applicable because relative risk estimates have shown no protective effect.

Created using data provided in Curtis JR, Westfall AO, Cheng H, et al. Benefit of adherence with bisphosphonates depends on age and fracture type: Results from an analysis of 101,038 new bisphosphonate users. *J Bone Miner Res*. 2008 Sep;23(9):1435–1441. https://doi.org/10.1359/jbmr.080418.

Not surprisingly, given their numerous adverse effects, long-term adherence to the oral bisphosphonates is quite poor. Even in persons with full-blown osteoporosis who have already suffered an osteoporotic fracture, more than half of such patients prescribed these drugs discontinue bisphosphonate therapy within 1 year. In their study, tracking 101,038 new bisphosphonate users for 3 years, Curtis et al. found that the proportion of persons with high adherence at 1, 2, and 3 years was 44%, 39%, and 35%, respectively.[209]

The anabolic drug approved for the management of osteoporosis is teriparatide, a fragment of human PTH including the amino acid sequence 1-34. If the amount of PTH secreted is chronically elevated, hyperparathyroidism results, causing bone loss. If PTH is elevated intermittently, the effect of once-daily injections of teriparatide, more osteoblast than osteoclast stimulation occurs, producing a net effect of increased BMD. Unfortunately, even chronic intermittent elevation of PTH by the standard dose of teriparatide (20 mcg/d) has been found to cause a number of undesirable side effects, including not only nausea, leg cramps, and dizziness but also hypercalcemia, hypercalciuria, hyperuricemia, hypomagnesia, and myopathy.[213-216] Of more concern are the chronically elevated cortisol levels seen and the potential for long-term increased risk of osteosarcoma. Chronic elevation in cortisol levels is not a surprising outcome because teriparatide causes a spike in PTH levels, and PTH increases the adrenal glands' secretion

of cortisol. The issue specific to bone is that cortisol, when continuously elevated, kills osteocytes.[217,218] Osteosarcoma occurred in 45% of the animals in the studies conducted to test the teriparatide's safety and efficacy and resulted in its carrying an FDA black box warning of this potential outcome.[219,220] Although teriparatide advocates claim human development of osteosarcoma is "unlikely," physicians and researchers have voiced concerns about the long-term consequences of teriparatide's use, even when restricted to short-term use in younger patients with glucocorticoid-induced osteoporosis.[221] Teriparatide may also increase the risk of cognitive decline and Alzheimer's disease. In a 10-year longitudinal prospective study, 514 individuals, ranging in age from 75 to 85 years, were assessed using the Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR) at baseline and at intervals of 1, 5, and 10 years. Elevated PTH indicated a 220% increased risk of an at least 4-point decrease in MMSE and a 300% risk of an increase in CDR class within the first year of follow-up. The risk remained significantly elevated even after controlling for age, gender, baseline cognition, serum Ca2+, creatinine, and APOE4.[222]

The connection is that sustained elevated levels of PTH in the brain increase the risk of calcium overloading, which leads to impaired blood flow in the brain and brain degeneration.[223-225] Interestingly, when PTH activity is excessive, the human body tries to inhibit PTH production in two ways: by increasing blood levels of calcium and decreasing blood levels of magnesium. As noted previously, both hypercalcemia and hypomagnesia are seen in response to teriparatide.

After 3 to 5 years maximum, a "drug holiday" is recommended from treatment with the antiresorptives. The use of teriparatide is currently approved for no more than 24 months; studies indicate that the drug's ability to stimulate bone formation begins to wane after 6 to 12 months, and as soon as it is discontinued, significant bone loss ensues almost immediately.[226,227] Because the bones continue to remodel throughout a person's life, any effective bone-building regimen must be one that can be followed long term, and the only protocol that meets these requirements is to identify and avoid as many of the numerous factors present in our modern lifestyle that cause excessive osteoclast activation as possible while providing an adequate supply of all the nutrients bones require to rebuild via diet and supplements, along with weight-bearing exercise.

Minerals

Calcium

The average dietary intake of calcium in the United States today is 600 to 700 mg, far short of the 1200 to 1500 mg/d intake recommended.[228-230,646] Our ability to absorb calcium decreases with age. By age 65, in both men and women, calcium absorption is only 50% of adolescent absorption levels.[231-233] Effective absorption and healthful use of calcium require both vitamin D_3 and vitamin K_2. Without adequate vitamin D, only 10% to 15% of dietary calcium is absorbed.[234] Vitamin D, although essential for active absorption of calcium, does nothing to regulate what happens to that calcium once inside the body. This is the job of vitamin K–dependent proteins (VKDPs) activated by vitamin K_2: osteocalcin, which is responsible for delivering calcium to bones, and matrix Gla protein, which is responsible for preventing calcium deposition in soft tissues, such as arteries and kidneys.[235-244] Calcium supplements to consider include calcium carbonate, calcium citrate, and algae-derived calcium (AlgaeCal Plus). Supplements providing calcium alone or calcium plus vitamin D help slow the rate at which bone is lost, but that's all.[230,245] Almost all calcium supplements that have been shown effective in clinical studies include multiple supportive nutrients, not just calcium. For example, AlgaeCal, as can be seen in Figs. 203.2, 203.3, and 203.4, has been shown effective in several clinical studies, likely because it naturally contains not just calcium but also ~70 trace minerals (which are lacking in the modern food supply and are essential for bone health) along with vitamin D_3, K_2 (as MK-7), vitamin C, and additional magnesium and boron.[246-250]

Calcium bioavailability does not differ significantly among calcium supplements when taken with food, except in those who have had gastric bypass surgery or are taking acid-blocking medications. These individuals should use calcium citrate.[251-253] Hydroxyapatite is an expensive form of calcium marketed as ready-made to be utilized in bone; however, the science is not supportive of this claim. When calcium is consumed, before absorption, it will be disassociated during digestion from any compound to which it has been bound. Calcium will be released from the phosphorus in hydroxyapatite, as it will from the citrate in calcium citrate or carbonate in calcium carbonate. In comparison studies, hydroxyapatite has not been shown to produce better results than calcium carbonate or citrate.[254] Furthermore, adding to the load of phosphorus from phosphate additives (discussed previously) is not recommended.[255] Calcium absorption is optimized with sufficient vitamin D_3 to maintain a 25(OH)D level of 50 to 80 ng/mL—consuming foods containing 500 to 700 mg of calcium, along with supplemental calcium taken in a dose of ~350 mg twice daily. When more than 500 mg of calcium is consumed at one time, the additional calcium will be excreted, serving a useful function to avoid short-term overload but not furthering bone formation.[252,256-259] Calcium supplementation does not increase the risk of heart attack or kidney stones when calcium intake is balanced with adequate supplies of K_2 as well as D_3.[260,261] Supplemental calcium may cause constipation when supplies of magnesium are insufficient. Supplemental magnesium will be best absorbed taken with pyridoxal-5-phosphate (P5P), the activated form of vitamin B_6. Dairy products do not increase fracture risk, with one possible exception: lactose-containing cow's milk due to the proinflammatory galactose derived from lactose during digestion. The consumption of lactose-free cow's milk avoids this issue.[262] The Upper Limit (UL) recommended for calcium is 2500 mg/d for men and women aged 19 to 51 and 2000 mg/d for men and women age 52 and older.

Magnesium

Of the magnesium in the human body, 60% is found in bone. Magnesium is a key constituent of the bone matrix and is required for osteoblast production, development, and activity. PTH signaling, which increases the kidneys' resorption of calcium and the production of 1,25-D, also cannot occur without magnesium.[263,264] Just supplementing postmenopausal women with low PTH with magnesium (thus restoring calcium absorption) has been shown to improve BMD and reverse osteoporosis.[265,266] Magnesium insufficiency results in increased production of peroxynitrite, superoxide, the PGE2 series (proinflammatory) prostaglandins, substance P, C-reactive protein, TNF-α, and RANKL, all of which promote osteoclast differentiation and activity.[267-270] Americans of all ages consume less magnesium than their respective Estimated Average Requirement (EAR), which is the amount thought to be "adequate," not optimal, for 50% of the population; 55% of women and 58% of men aged 51 to 70 years, and 70% of women and 80% of men over age 70 are not meeting the EAR. Stress and a number of commonly prescribed drugs (oral contraceptives, gentamycin, amphotericin, hydrochlorothiazide, furosemide, and proton pump inhibitors) increase the risk of magnesium deficiency.[271-278] The recommended balance between calcium and magnesium is 2:1; thus, a daily intake of 1200 mg of calcium requires 600 mg of magnesium.[279,280] Magnesium oxide, which is 60% elemental magnesium compared with magnesium citrate, which is only 11% elemental magnesium, is preferable and should be consumed with

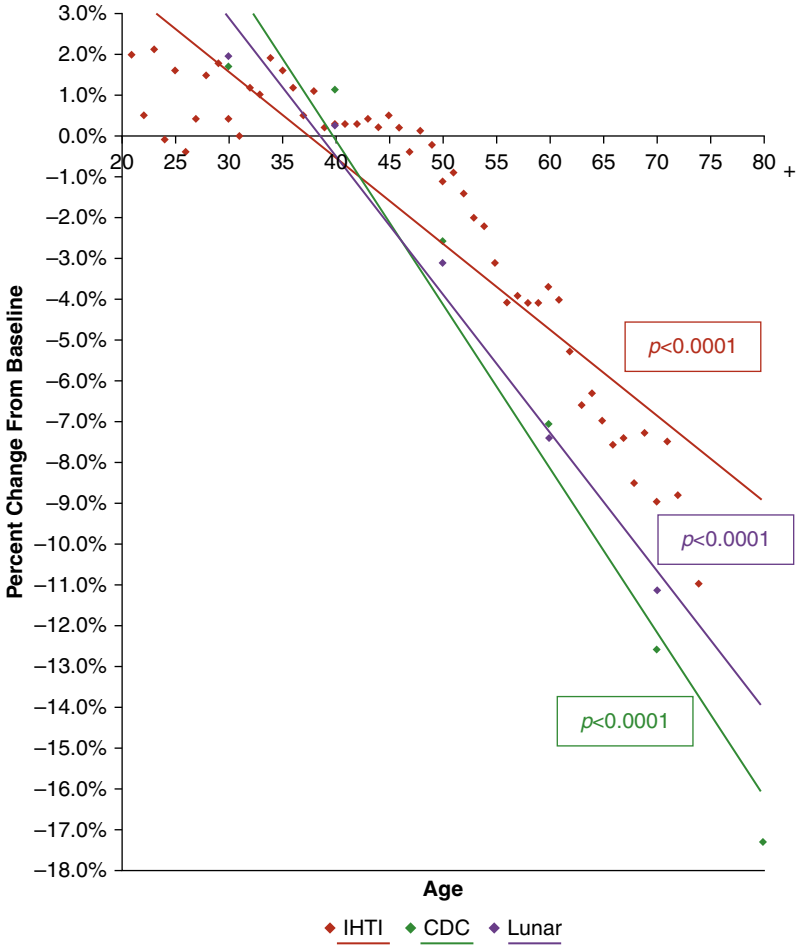


Fig. 203.2 Trendline analysis of annualized changes in bone mineral density (BMD) for women aged 20 to 80C from the Integrative Health Technology's (IHTI's) longitudinal database (*N* = 16,289), the Center for Disease Control and Prevention's database (*N* = 8283), and GE Lunar's database (*N* = 1313).

food to ensure stomach acid availability.[281-285] Magnesium is best absorbed and utilized when taken in divided doses over the course of the day along with P5P.[286-289]

Boron

Boron improves calcium retention[290] and boosts osteoblast production and activity,[291-293] largely by increasing the production of the key proteins involved in the bone mineralization process, including collagen type I (COL I), osteopontin (OPN), bone sialoprotein (BSP), and osteocalcin (OCN),[293] bone morphogenetic protein,[294-296] and Runt-related transcription factor 2 (Runx2). Boron supplementation also maximizes estrogen's effect of increasing calcium and magnesium absorption into bone, optimizing the benefit to be derived from waning estrogen during and after the postmenopausal transition.[290,297]

Boron may be especially helpful as men age because it increases the amount of free testosterone in circulation.[298-302] In addition, boron increases the amount of time vitamin D [25(OH)D] remains in circulation.[303-306]

Boron improves magnesium absorption and lowers CRP[307] and TNF-α.[298,308,309] Governmental health authorities have not provided us with an EAR, Recommended Daily Intake (RDI), or Recommended Daily Allowance (RDA) for boron, but we do have a recommendation for boron's Tolerable Upper Intake Level (UL). For adults 18 years or older, the UL is 20 mg per day.[310] This is reassuring because the research indicates that 3 to 6 mg/d will meet our needs; however, a supplement is typically required. In boron-rich areas of the world, such as Turkey, daily boron intake averages 12.6 mg with no adverse effects, a good indication of boron's safety.[311] In the United States, however, soils are not boron-rich, and the diet provides far less than 3 mg/d. In 1998 boron researchers Rainey et al. estimated that the average daily intake of boron in the United States was 1.17 mg a day for men and 0.96 mg per day for women. And people were eating more fruits and vegetables then.[312] Food Processor data from 1996 that has been widely quoted reporting the boron-content of foods were found to have greatly overestimated the actual amounts of boron present. Unless your patients are consuming 6 oz of raisins, 6 avocados, or 18 oz of peanut butter daily, a supplement is needed to provide at least 3 mg/d.[313]

Zinc

Zinc plays a critical role in collagen formation, promotes osteoblast proliferation, and is required for their production of the bone matrix and its calcification. Zinc's role in bone is both structural, because bone mineral is composed of hydroxyapatite crystals, which contain zinc, and formative, because zinc is required for osteoblasts' activity; plus, zinc promotes bone mineralization through its role as a cofactor for the bone-building enzyme bone alkaline phosphatase. Zinc also inhibits osteoclasts' bone-resorbing activity, lessens inflammation by acting as an antioxidant, prevents age-associated hyperparathyroidism, is required for the production of 1,25-D, and protects against the absorption of toxic heavy metals.[314-323] The RDI for zinc is 11 mg/d for

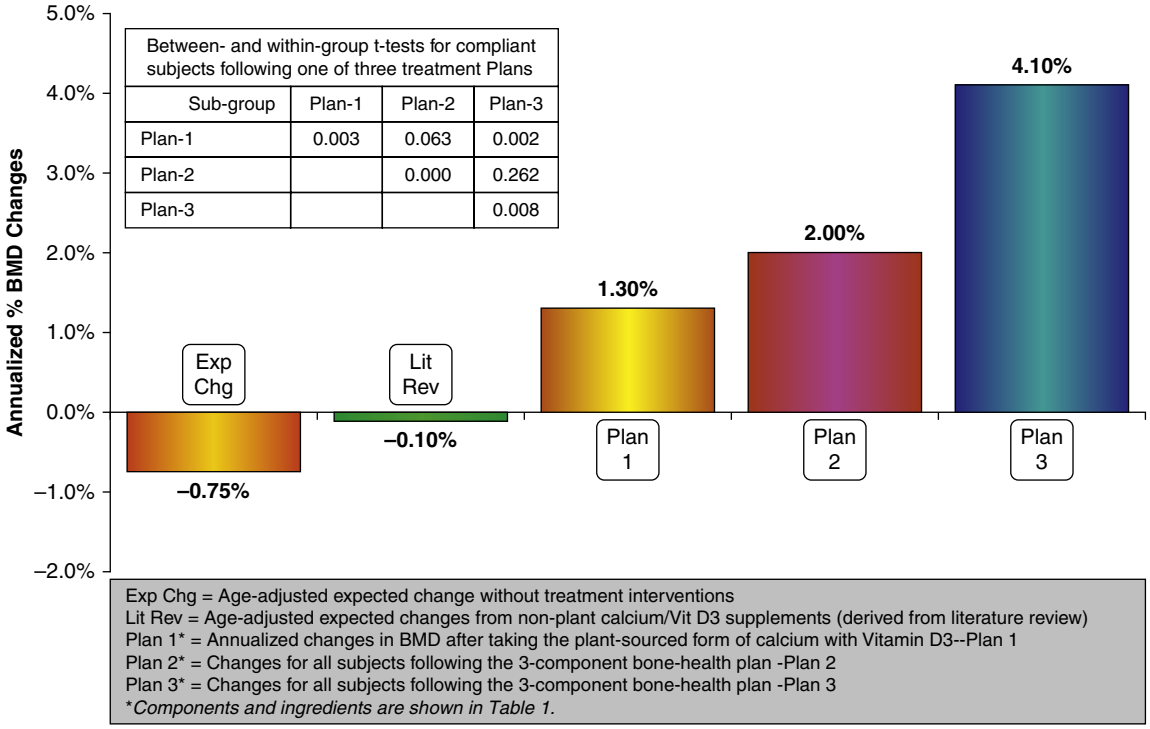


Fig. 203.3 Within- and between-groups comparisons of annualized changes in bone mineral density (BMD) in women over 40 who followed one of three different bone-health treatment plans and who were rated in the upper 50th percentile of protocol compliance. *P* levels on the diagonal represent changes from baseline using within-group repeated measures *t*-tests. (Kaats GR, Preuss HG, Croft HA, Keith SC, Keith PL. A Comparative Effectiveness Study of Bone Density Changes in Women Over 40 Following Three Bone Health Plans Containing Variations of the Same Novel Plant-sourced Calcium. *Int J Med Sci.* 2011;8(3):180–191; doi:10.7150/ijms.8.180. Available from. http://www.medsci.org/v08p0180.htm.)

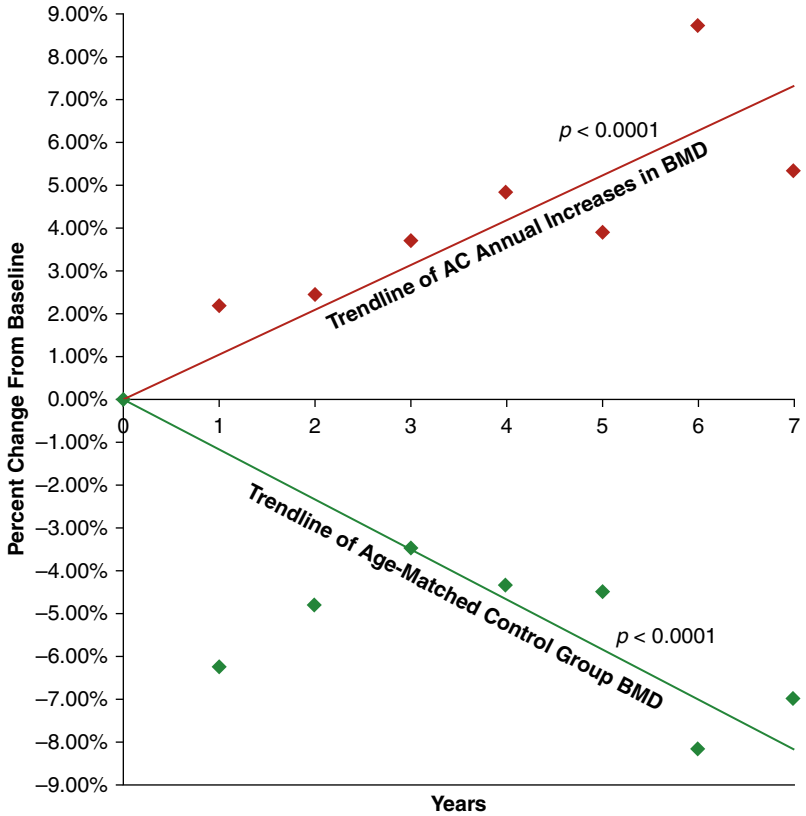


Fig. 203.4 Trendline comparisons between consumers taking AC and an age-matched control group.

men and 8 mg/d for women (if pregnant, 11 mg/d; if breastfeeding, 12 mg/d). The current research shows that 15 mg is required to increase bone density. Because zinc's UL is 40 mg per day, 15 to 20 mg/d should be safe. NHANES III reported that >6% to 7% of Americans were at risk of zinc deficiency. Among women aged 50 or older and men aged 60 or older—those with the greatest age-related risk of osteoporosis—almost none consumed the RDI for zinc.[314,324,325] Long-term consumption of zinc in amounts greater than 40 mg/d can cause a copper deficiency. Intake of zinc:copper should be 15:1.[326,327]

Copper

Copper is a cofactor required by a wide array of enzymes, including two powerful antioxidants, ceruloplasmin and superoxide dismutase, plus enzymes involved in the formation of collagen and connective tissue and tooth and bone mineralization. In humans, copper deficiency is known to promote poor bone quality, periodontal disease, and osteoporosis.[328] The main adverse effect attributed to copper deficiency is that lysyl oxidase, an enzyme whose activity is essential for crosslinking between collagen and elastin that requires copper as its cofactor, stops working. This quickly results in greatly reduced strength in the bone matrix. Copper also directly inhibits osteoclasts' bone-resorbing activities.[315,322]According to national surveys, the average dietary intake of copper in the United States is 1.0 to 1.1 mg per day for adult women and 1.2 to 1.6 mg per day for adult men. Intake of copper should be balanced with zinc intake and not exceed 4 mg/d. Consumed in excess or out of balance with zinc, copper can increase the production of free radicals, causing oxidative stress, damage to lipids, and bone loss.[315,327]

Manganese

Manganese is critical for bone because it's required for the production of chondroitin sulfate, which plays a key role in the first steps in bone formation, and also because it is the cofactor for mitochondrial superoxide dismutase (MnSOD), which is so essential for life that animals genetically modified to lack MnSOD die almost immediately.[329-331] In humans, manganese insufficiency promotes low bone mineral density and osteoporosis, and low MnSOD activity increases the risk for osteoporosis—and cancer, diabetes, cardiovascular disease, and Alzheimer's disease. Osteoblasts build bone using enzymes that require manganese. Manganese is also required for the production of cholesterol, the precursor of all our hormones, for thyroid hormone production. Low manganese levels are frequently found in patients with hypothyroidism, and hypothyroidism promotes bone loss.[315,332-336] Human studies have found that dietary intake of 3 mg/d was not enough to maintain positive balance (i.e., prevent a decline in the body's manganese levels); in one study, slightly more than 5 mg per day was needed. The research indicates that manganese intake of 3 to 5 mg per day should be recommended. The UL has been set at more than twice that—11 mg per day, and no ADRs (adverse effects) have been reported below this level of intake.[337]

Silicon

Silicon increases the synthesis of type I collagen, the production of osteoblasts, bone-specific alkaline phosphatase, osteocalcin, and the extracellular matrix, and it improves calcium's incorporation into bone.[338] Not surprisingly, in vitro and in vivo (animal and human) studies of silicon-containing implants and ceramics show that these implants bond far more effectively to bone than their non–silicon-containing counterparts.[339] In animal models of menopause, silicon has improved calcium–magnesium imbalances. What typically happens with menopause is that blood levels of calcium increase, a sign that calcium is being withdrawn from bone, whereas those of magnesium decrease, increasing the risk of high blood pressure. Bioavailable silicon lowers blood levels of calcium and increases those of magnesium.[340] In two large epidemiological studies conducted in the United States, the original Framingham Study and the Framingham Offspring study, consumption of *at least* 40 mg/d of silicon was associated with greater BMD: a difference of as much as 10% more BMD was seen between those individuals with the highest (>40 mg/d) and lowest (<14 mg/d) intakes of silicon. In these studies, men's silicon intake was significantly greater than women's, averaging 30 mg/d in the original Framingham Study and 33 mg/d in the Framingham Offspring study, whereas women averaged 24 mg/d in the original Framingham study and 25 mg/d in the Framingham Offspring study. Men's greater silicon intake was explained by their proclivity for beer.[338,339,341] A study that focused specifically on whether supplementing with silicon could increase BMD in postmenopausal women with osteoporosis found that it did—and it significantly outperformed a bisphosphonate.[342] Another study of silicon supplementation in postmenopausal women with osteoporosis evaluated silicon's effect on trabecular bone. Study participants were divided into three groups: a control group, a group given an injection containing 16.5 mg/wk for 4 months, and a third group receiving an oral silicon supplement providing 27.5 mg/wk for 3 months. The women consumed their normal diets, and no supplemental calcium or vitamin D was given. Both groups receiving supplemental silicon had significant increases in trabecular bone volume compared with the control group.[343,344] Silicon's effects have also been studied in osteopenic women who were given either 3, 6, or 12 mg/d and compared with controls who did not receive a silicon supplement. All four groups received calcium and vitamin D supplementation, but no other forms of treatment. After 1 year, the control group showed a decrease in femoral bone density, whereas the groups given silicon maintained but did not further improve bone density.[345] However, because consumption of *at least* 40 mg per day is the amount that was found to improve BMD in the Framingham studies, the doses of silicon provided in this research were far too low, even for the group receiving the highest dose, which was just 12 mg/d. Other research suggests that estrogen boosts silicon's utilization. In the Framingham studies noted previously, higher silicon intake correlated with increased BMD for men, premenopausal women, and postmenopausal women on HRT, but not those not on HRT. A later study conducted in Aberdeen, Scotland, found that higher silicon intake had a highly beneficial effect on femoral BMD but only in women who were "estrogen-replete," that is, were premenopausal or, if postmenopausal, on HRT. Among these women, femoral BMD was an average of 2% higher in those consuming the most silicon (an average of 31.5 mg/d) compared with those consuming the least (an average of 16.5 mg/d).[346] Several factors lessen the ability to absorb silicon, including a vegetarian or vegan diet high in phytates, the age-associated decrease in stomach acid production, and hypothyroidism, which decreases our ability to metabolize silica and absorb silicon.[339] Mineral water and beer are, by far, the richest dietary sources of bioavailable silicon. Following these, the silicon in whole grains and grain products (breakfast cereals, breads, rice, and pasta) is moderately well absorbed, whereas even less is absorbed from green beans and fruits. Bananas, despite their relatively high silicon content, provide virtually no usable silicon because the highly polymerized form found in bananas—and in colloidal silica supplements—is not soluble.[347,348] Silicon is primarily found in nature as silica (silicon dioxide, SiO_2), an inorganic compound that is insoluble in water and that the human body is unable to convert to a soluble, bioavailable form. Certain marine algae, such as *algas calcareus,* from which *AlgaeCal* is produced, dissolve inorganic silica and convert it to orthosilicic acid, silicon's water-soluble, bioavailable form. Independent laboratory analysis of AlgaeCal powder indicates that the daily 4-capsule serving of AlgaeCal Plus provides 24.5 mg of orthosilicic acid (OSA). The

next best supplemental option is BioSil, which is OSA concentrated from liquids and then stabilized by being combined with choline. One capsule of BioSil provides 5 mg of OSA and 100 mg of choline. The recommended dose is 2 capsules per day.[349] Silicon is present in some antacids, for example, magnesium trisilicate. Unfortunately, the silicon in antacids is silicate, a highly polymerized form that is very poorly absorbed.[350] Water-soluble forms of silicon are absorbed in the intestinal tract, with excess amounts eliminated by the kidneys within 4 to 8 hours, so it is highly unlikely that silicon might accumulate to excessive levels in healthy individuals. Individuals with chronic kidney disease who are on dialysis might accumulate silicon because renal failure impairs its excretion. However, even though blood levels of silicon up to 10 times normal have been reported in patients with renal failure, no adverse effects have been seen, even at these levels.[339]

Selenium

Several large human studies have confirmed that an insufficient supply of selenium adversely changes bone metabolism, promoting bone loss.[351,352] Blood levels of selenium are inversely related to the rate of bone turnover in both men and women: low selenium results in increased bone resorption and loss of BMD, whereas adequate selenium correlates with lessened bone resorption and increased BMD.[353,354] Selenium is essential for bone health for two reasons: (1) As the required cofactor for the selenoenzymes, selenium plays a key role in an array of antioxidant defenses that both lessen the production and activation of bone-resorbing osteoclasts and increase the proliferation and activity of bone-building osteoblasts.[28,354-356] (2) Healthy thyroid hormone function is dependent on selenium because this trace mineral is a required cofactor for the deiodinase enzymes, which activate and deactivate the thyroid hormones, according to need. Both hypo- and hyperthyroidism cause bone loss.[353,357-359] Ensuring optimal amounts of selenium are present to promote selenoenzyme activity is especially important for individuals whose genetic inheritance includes an SNP that results in the production of a slow version of glutathione peroxidase 1 (GPx1). Carriers of this SNP have increased levels of bone turnover markers and are at increased risk of low BMD. SNPs that result in the production of slow versions of other selenoenzymes are also associated with increased inflammatory signaling and increased risk of osteoarthritis.[28] Selenium's importance for bone health is vividly illustrated by a recent case-control study that investigated the link between antioxidant intake and the risk of osteoporotic hip fracture and whether this link was modified by cigarette smoking, which is well known to greatly increase the risk for hip fracture. Ever-smokers were divided into five quintiles according to their selenium intake. Those in the highest quintile had a 73% lower risk of hip fracture compared with those in the lowest quintile.[354] The RDI for selenium is 55 mcg/d. The UL is 400 mcg/d. The NOAEL is 800 mcg/d. The dosage used in clinical trials, provided by most supplements containing selenium and recommended here for optimal health, is 200 mcg/d.[360] The most accurate way to determine whether supplemental selenium should be considered is a laboratory test to check glutathione peroxidase activity. A number of labs now offer this test, including Genova Diagnostics (see https://www.gdx.net/product/oxidative-stress-analysis-2-test-blood) and the Mayo Clinic Laboratories (see http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9765). Selenium is a "Goldilocks" nutrient: either too little or too much is harmful. Insufficient selenium promotes free-radical damage, chronic inflammation, increased osteoclast production and activity, decreased osteoblast production and activity, and impaired thyroid function—all of which cause bone loss. Epidemiological studies have suggested that low selenium status increases the risk of colorectal, prostate, lung, bladder, skin, esophageal, and gastric cancers and male infertility. However, if regularly consumed in amounts greater than the NOAEL (800 mcg/d), selenium can be toxic.[361] Early indicators of toxicity are a garlic odor on the breath and a metallic taste in the mouth. The most common clinical signs of chronic excessive selenium intake are hair loss, white spots on the nails, nail brittleness, and nail loss. Excessive consumption of selenium during pregnancy may cause birth defects. Choose a selenium supplement that contains selenomethionine and/or selenocysteine, the organic forms of selenium, and delivers no more than 200 mcg of selenium per daily dose. On days when selenium-rich foods are consumed, particularly Brazil nuts (so rich in selenium that no more than 2 Brazil nuts/d should be eaten),[362] supplemental selenium should not be taken.

Iodine

Iodine is required for the production of thyroid hormones and is therefore an essential element for bone mineralization. Depending on iodine's availability, the thyroid gland can enhance or limit its use for thyroid hormone production. When compensation fails, as in severely iodine-deficient populations, hypothyroidism and developmental brain damage are the dominating disorders. In less severe iodine deficiency, the normal thyroid gland can adapt to keep thyroid hormone production within the normal range. Prolonged thyroid hyperactivity leads to thyroid growth along with increased risk during follicular cell proliferation of mutations producing multifocal autonomous growth and function. In populations with mild to moderate iodine deficiency, such multifocal autonomous thyroid activity is a common cause of hyperthyroidism in elders, and the prevalence of thyroid enlargement and nodularity is high. The average serum TSH tends to decrease with age in such populations as a result of the high frequency of autonomous thyroid hormone production. On the other hand, hypothyroidism is more prevalent in populations with a high iodine intake. Many thyroid processes are inhibited when iodine intake becomes high, and the frequency of apoptosis of follicular cells increases. Abnormal inhibition of thyroid function by high levels of iodine is especially common in people affected by thyroid autoimmunity (Hashimoto's thyroiditis). In populations with high iodine intake, the average serum TSH tends to increase with age. This phenomenon is especially pronounced in Caucasian populations, in which a genetically determined high tendency to thyroid autoimmunity is surprisingly common. A small tendency to higher serum TSH may be observed when iodine intake is brought from mildly deficient to adequate, but at present, no evidence suggests that slightly elevated serum TSH in elderly people leads to an increase in morbidity and mortality.[363] In fact, in older people, higher TSH and lower free T4 (FT4) concentrations within the euthyroid range (see following discussion) have been recently shown to be associated with a lower risk of multiple adverse events, including mortality.[364]

In relation to bone, specifically, a cross-sectional study of 756 healthy Korean women aged 65 and older with normal thyroid function found that low-normal serum TSH levels were associated with more than double the risk for osteoporosis of the lumbar spine.[365] Other research comparing the body level of iodine in 132 postmenopausal women, 34 of whom were healthy and served as controls, 38 with osteopenia, and 60 with osteoporosis, found urinary iodine levels of 216.1 ± 125.2 in the control group, 154.6 ± 76.6 in the osteopenic group, and 137.5 ± 64.9 in the osteoporosis group, differences that were maintained after adjustment for body mass index. The urinary iodine level accurately correlated with the T-score for the lumbar spine and was significantly associated with total T-score.[366] The conclusions drawn by these researchers: the level of serum TSH has a direct association with osteoporosis, and hypothyroidism may lead to postmenopausal osteoporosis.

Hyperthyroidism also leads to a loss of BMD because it results in accelerated remodeling and a decrease in the volume of trabecular bone. Subclinical hyperthyroidism in postmenopausal women adversely affects bone mineralization and has been shown to result in significantly lower BMD in the femur, neck, and radius. When women with subclinical hyperthyroidism were compared with a control group, the decline in BMD inversely correlated with FT4 levels.[367-369]

The right amount of iodine is extremely important for bone health; the question is: How much is the right amount for the individual patient? The iodine RDI for adults of 150 mcg/d (220 mcg/d for pregnant women), although sufficient to prevent goiter, may be inadequate for the promotion of optimal bone health in older adults. Despite the widely held assumption that Americans are iodine sufficient due to the availability of iodized salt, the U.S. population is at substantial risk for iodine insufficiency. Iodine intake has been decreasing since the early 1970s as a result of changes in Americans' food and dietary habits. The addition of salt to home-cooked meals is now discouraged; iodized salt is rarely used in restaurant and processed foods; and the iodized salt sold for home use has been found to provide far less iodine than the amount listed on the label. Iodine is no longer used in the dairy industry or in the production of bread, and in addition, the widespread dispersal of perchlorate, nitrate, and thiocyanate (competitive inhibitors of iodide uptake) in the environment blocks absorption of the iodine Americans consume.[370] Given these considerations, intake of 200 mcg to as much as 1 mg/d, an amount less than the UL of 1.1 mg/d and far less than the 3 to 6 mg/d commonly consumed in Japan, occurs without increased incidence of autoimmune thyroiditis or hypothyroidism. Larger amounts than currently officially recommended may be necessary to support not only thyroid hormone production but also iodine's important antioxidant functions in the breast and other tissues in which this trace mineral is concentrated.[370] Nonetheless, the consensus among recently published epidemiological studies is that the optimal level of iodine intake to prevent disease is within a relatively narrow range around the RDI. Population iodine intake below 150 mcg/d is clearly associated with a high incidence and prevalence of goiter and hyperthyroidism caused by thyroid autonomy, and occurrence increases with age. Population iodine intake above 150 mcg/d is associated with a higher level of serum TSH and increased incidence of hypothyroidism in the population, particularly in individuals with some degree of thyroid autoimmunity, which is reflected in the presence of antithyroid antibodies (i.e., antithyroid peroxidase antibodies [anti-TPO antibodies]) and leads to Hashimoto's thyroiditis.[363] Those with a Caucasian genetic background have a high tendency to such thyroid autoimmunity, which is now thought to affect as many as half of elderly women.[371] The reference intervals for thyroid hormones are as follows: TSH 0.5 to 4.4 mIU/L; FT4 10 to 20 pmol/L; and free T3 (FT3) 1.1 to 2.4 nmol/L.[372] In sum, regular monitoring and adjusting of each patient's iodine intake to support levels of thyroid hormones within these reference ranges is essential. Lastly, proper functioning of the thyroid gland also requires sufficiency of a number of other minerals in addition to iodine (i.e., selenium, iron, zinc, copper, and calcium).[371,373]

Strontium Citrate

Of the total amount of strontium in the body, 99% is localized in bone, a trait it shares with calcium. Strontium both lowers the rate of bone resorption and promotes the rate at which new bone is built.[374,375,647]

Natural stable strontium salts (e.g., strontium citrate) are safe (at the dosages noted herein and when twice as much calcium is consumed as strontium) and highly beneficial. In more than 100 years of research and data collection on strontium, strontium itself has never been found to cause any harmful effects, except in one province in Turkey where some children developed rickets because strontium intake was very high and calcium intake was deficient. The treatment prescribed was calcium supplements. Supplemental strontium may not be advisable in patients with chronic kidney disease (CKD) on dialysis because dialysis fluid is often high in strontium, and the ability of patients with CKD to clear strontium (and everything else requiring elimination in urine) is compromised. The drug strontium ranelate is not safe and should not be used. Ranelic acid was claimed to be inert, and all of it was said to disassociate from strontium in the digestive tract and to be quickly eliminated from the body. Research has now shown that none of these assertions was correct.[376] Many studies have confirmed that the strontium ions in strontium ranelate, not ranelic acid, are doing the bone-beneficial work. The many adverse side effects (ADRs) attributed to strontium, ranging from commonly seen ADRs like nausea and skin irritation to potentially deadly effects, such as venous thromboembolism (VTE) and myocardial infarction (MI), and serious autoimmune reactions (e.g., drug rash with eosinophilia and systemic symptoms [DRESS], Stevens–Johnson syndrome [SJS], and toxic epidermal necrolysis [TEN]), are caused by immune reactions to ranelic acid (the ranelate anion in strontium ranelate), not strontium. The use of strontium ranelate has never been approved in the United States and is now significantly restricted in Europe, whereas strontium citrate is freely available.[377-379] Note that the bisphosphonates and denosumab may also cause the cutaneous ADRs seen with strontium ranelate: "Review of available evidence shows that cutaneous adverse reactions occur with all commonly used antiosteoporotic agents. Notably, there are reports of SJS and TEN for the bisphosphonates as well as of DRESS and TEN for strontium ranelate."[380,381] Strontium and calcium compete for absorption. These minerals share a common carrier system in the intestinal wall, which preferentially transports calcium rather than strontium into the circulation.[382] Strontium's active intestinal absorption, like calcium's, requires vitamin D, and just as with calcium, the ability to absorb strontium decreases with age. Like calcium, strontium is a bone-seeking mineral and incorporates into both trabecular and cortical bone. Strontium, however, deposits almost entirely into new trabecular bone, with one strontium ion substituting for *less* than 1 calcium ion out of 10.[374] Strontium improves healthy bone mineralization: apatite crystals containing strontium are more stable and show more regular shapes.[383] Strontium is almost twice as large as calcium, and its larger mass affects DXA readings. Available data indicate that approximately 50% of the increase seen in BMD over 3 years of treatment with strontium is due to strontium's larger mass, which also means that 50% of the increase seen in BMD is accurate. Strontium's larger mass than calcium also affects how quickly strontium is eliminated from the body. Both calcium and strontium are primarily excreted by the kidneys, but strontium's greater mass makes its reabsorption more difficult, so three times as much strontium is excreted in urine compared with calcium.[374,382,384] Even the tiny number of strontium-for-calcium ions exchanged in bones—one strontium ion replacing *less* than 1 calcium ion out of 10—has a major beneficial impact on bones' health, strength, and flexibility. Strontium's beneficial effects are not due to its replacing calcium in bone. They are the result of strontium's bone-building effects on a very wide range of key molecules involved in bone remodeling. Strontium increases our ability to absorb and deposit calcium in our bones[374,382,385]; regulates RANKL and OPG activity[386,387]; activates Wnt signaling, which plays a crucial role in osteoblast production and bone formation; boosts the rate at which osteoblasts differentiate and their survival,[386] preventing the increased rate of bone turnover induced by ovariectomy in animal studies (the model for human menopause)[374,388]; improves mineralization and hydroxyapatite formation[386]; increases the number of bone-forming

sites (osteoblast and osteoid surfaces)[386]; improves bones' mechanical resistance to fracture[370,383,389]; inhibits osteoclast production and activity[386]; increases the differentiation of MSCs into osteoblasts[386]; increases the maturation rate of these preosteoblasts and the activity of mature osteoblasts; increases the rate at which osteoblasts lay down minerals to form new bone; and increases the production of osteocalcin.[386] If osteopenia or osteoporosis is present, the standard 680-mg/d dose of strontium citrate is recommended. To maintain skeletal health long term, a dose of 340 mg/d is likely to be adequate.[390,391] Just as with calcium, a lower dose of strontium (340 mg) taken twice daily may be more effectively absorbed, but because bone remodeling is thought to ramp up at night, if 680 mg/d is taken once daily, it should be taken at night.[384,392,393] Calcium-containing foods and supplements should be avoided for at least 2 hours before or after taking strontium, as should medications that interfere with strontium absorption (e.g., calcium-containing antacids, oral tetracyclines, quinolone antibiotics, antacids that contain aluminum hydroxide or magnesium hydroxide, and chelating agents, such as DMSA). Human ability to absorb strontium is optimized by the preceding plus (1) ensuring intake of vitamin D_3 is adequate and (2) minimizing consumption of processed foods because all contain phosphate additives, which interfere with strontium absorption.[382]

Vitamins

Vitamin D

Vitamin D is required for the active absorption of calcium. Without sufficient vitamin D, only 10% to 15% of the calcium consumed will be absorbed. Liver and kidney health affect our ability to utilize vitamin D (D_3 or D_2),[234] which must be converted to 25(OH)D in the liver and then to its hormonal form of 1,25-D in the kidneys to enable calcium absorption.[394-398] Impaired conversion of vitamin D to 25(OH)D is surprisingly common due to NAFLD. Compared with premenopausal women, postmenopausal women have a 205% greater risk for NAFLD.[399] When evaluating patients for NAFLD, bear in mind that recent findings have shown that individuals with normal alanine aminotransferase (ALT) levels may still have NAFLD. Proton nuclear magnetic resonance (NMR) spectroscopy and ultrasonography, two noninvasive methods of diagnosing NAFLD that provide more accurate results than ALT levels alone, are now recommended.[400,401] Daily dosing is far more effective due to the differing amounts of time that the three forms of vitamin D (D_3, 25[OH]D) and 1,25-D) remain available for use within the body. Vitamin D_3 consumed in a bolus dose that is not used within 12 to 24 hours is excreted in bile, feces, and urine. In addition, D_3 plays a number of key roles that 25(OH)D cannot and therefore must be supplied daily.[402,403] The only way to sustain needed amounts of vitamin D in the bloodstream is by either daily D_3 supplementation and/or daily sunlight exposure (at the right ultraviolet [UV] wavelength). D_2 is not nearly as effective as D_3 in raising or maintaining 25(OH)D status.[404-409] Some experts recommend a blood level of 25(OH)D of 40 to 60 ng/mL; others think it should be 50 to 80 ng/mL. The Institute of Medicine (IOM) thinks 20 ng/mL is adequate, but no other authorities agree with this.[234,410-413] To reduce cancer risk, studies indicate that a 25(OH)D status of 60 to 80 ng/mL may be needed.[414] The lowest dose of vitamin D now recommended by the Endocrine Society for healthy adults, whose blood levels of vitamin D are at least 30 ng/mL, is 1500 to 2000 IU/d. The Vitamin D Council recommends 5000 IU/d for 3 months, then testing blood levels of 25(OH)D.[415] A high body weight increases the amount of vitamin D needed.[416,417] Individuals with sarcopenia (i.e., those who are overfat) may also require higher doses of vitamin D, as may those whose genetic inheritance includes the vitamin D–related SNPs discussed previously under "Risk Factors."[418] A number of other factors also affect vitamin D needs, including latitude (north of 35° latitude, especially above the 37th parallel, increases risk of vitamin D deficiency), typical amount of sun exposure, use of sunscreen, age (older adults require more),[419] skin color (individuals of African heritage tend to produce less D_3 from sun exposure and have lower levels of 25(OH)D but also tend to have a more effective vitamin D–binding protein and thus higher BMD than Caucasians),[420-422] diet, and ability to absorb fats (vitamin D is fat soluble).[423,424]

Vitamin D is nowhere near as toxic as once thought.[425-427] A few uncommon health conditions may cause hypercalcemia when taking vitamin D: sarcoidosis, Williams–Beuren syndrome, some lymphomas, and a genetic disorder resulting in the production of an inactive 1,25-D, 24-hydroxylase (or CYP24A1), the enzyme that prepares the active form of vitamin D, 1,25-D for elimination. If not suffering from one of these uncommon health conditions, extremely high doses of vitamin D (e.g., 50,000–100,000 IU daily) would need to be taken for months for vitamin D levels to become toxic. The Endocrine Society's practice guidelines for vitamin D supplementation state that vitamin D intoxication (hypercalcemia) is usually not observed until blood levels of 25(OH)D are higher than 150 ng/mL. However, it's best to err on the side of safety and keep 25(OH)D levels below 100 ng/mL, which is thought to be the uppermost range of normal.

Vitamins K_1 and K_2

In addition to involvement in blood clotting, vitamin K_1 (phylloquinone) exerts significant anti-inflammatory and thus bone-protective effects. K_1 is the required cofactor for enzymes involved in blood clot formation, for which reason, any K_1 consumed will first be triaged to activate these proteins. Only when short-term survival needs are met will any remaining K_1 be converted into K_2 and used to activate the vitamin K–dependent proteins (VKDPs) involved in calcium regulation: osteocalcin and matrix Gla protein.[428-431] To promote bone health, K_1 intake should be at least 1000 mcg/d, which can easily be provided by a diet that includes lots of leafy greens, such as kale, spinach, Swiss chard, collards, parsley, and broccoli.[432,433] K_1 is also present in plant oils, especially unhydrogenated soybean and canola oils. Partially hydrogenated oils and processed foods contain an abnormal form of K_1, called dihydrophylloquinone, which is neither absorbed nor utilized well, even to produce clotting factors, and is not converted into K_2.[434,435]

Healthful absorption and use of calcium require both vitamin D and vitamin K_2. Reviews claiming that calcium, with or without vitamin D, increases risk for heart attack, does not prevent falls, or does not lessen the risk of fracture ignore this fact of human physiology. In none of the studies cited in any of these articles is vitamin K_2 even considered.[436,437] Insufficiency of K_2 increases the risk of heart attacks, osteoporosis, and fracture.[438] In quantities sufficient for bone health, vitamin K_2 is only found in one—unpalatable in the Western diet—food, a Japanese fermented soybean product called natto. A few cheeses fermented by K_2-producing bacteria provide tiny amounts of K_2: English blue cheese, Swiss Emmental, and Norwegian Jarlsberg. K_2 is also present, but in insignificant amounts, in liver, meat, and egg yolks.[439-441] Considering nutrient levels in the foods commonly eaten, to meet bones' needs for vitamin K_2, a supplement is required.[442] For most, the supplemental form of K_2 that is, by far, the most helpful is MK-7, although for a minority of people, MK-4 may be better. MK-4 is structurally almost identical to vitamin K_1. Both are treated the same way in the body. After clotting needs have been met in the liver, MK-4 and K1 are put into triglycerides before being sent into the bloodstream and are cleared from the body within 6 to 8 hours. MK-7 is preferentially used to activate VKDPs outside the liver (e.g., osteocalcin, matrix Gla protein) and is absorbed

into low-density lipoprotein cholesterol (LDL-C) before being sent into the bloodstream. LDL-C circulates in the bloodstream for several days before it is cleared, giving MK-7 a much longer half-life than K_1 and MK-4. The half-life of K_1 and MK-4 is 6 to 8 hours, whereas the half-life of MK-7 is 96 hours or 4 days. When MK-7 is consumed daily, a reserve of K_2 is produced that enables continuous activation of VKDPs. MK-7's much longer availability explains why a very small dose—as little as 100 micrograms per day—may be all that is needed.[443,444] The dose of MK-4 used in the research showing benefit for people with osteopenia or osteoporosis is 15 mg t.i.d.—a dose that could not possibly be consumed from the diet. Nonetheless, a small percentage of individuals do better on MK-4: the few who have inherited infrequently seen genetic variations that enable them to retain and recycle vitamin K far longer than "average." The majority (85%–95%) will find that vitamin K_2 in its MK-7 form at a dose of 100 to 180 mcg/d per day is adequate. Those individuals whose genetic inheritance includes SNPs in *APOE, VKOR, CCGX,* or *CYP4F2* that result in their clearing vitamin K more quickly than "average" will definitely need the MK-7 form and will benefit from a larger daily dose than the 100 to 180 mcg/d per day sufficient for the needs of the "average" person. Those among the small number of individuals whose genetic inheritance enables them to retain vitamin K around longer than "average" may find that less MK-7 (45 micrograms per day or every other day) or the MK-4 in a dose of 15 mg bid is effective.[445-452] K_2 intake must be in balance with that of D_3, which increases both the body's ability to absorb calcium and its production of the VKDPs that regulate calcium's use. If D_3 needs are met with 1000 to 2000 IU/d, then K_2 needs are likely to be 100 to 180 mcg/d; D_3 intake of 3000 to 4000 IU/d may require 200 to 300 mcg/d of MK-7; D_3 intake of 5000 IU/d or more may require 360 mcg/d of MK-7, which is still an extremely safe dose. Vitamin K is so safe that the IOM has set no Upper Tolerable Limit for any form of vitamin K for those not taking blood thinners whose mechanisms of action is impairment of vitamin K metabolism.[453-456]

Two laboratory tests (uncarboxylated osteocalcin [unOC] and uncarboxylated matrix Gla protein [unMGP]) can be used to verify if vitamin K needs are met. The only safety issue is in patients taking warfarin, who must avoid all vitamin K_2 supplements. Vitamin K_2 can, however, be taken along with two newer types of anticoagulant drugs, the Xa inhibitors and the direct thrombin inhibitors. Because warfarin causes vascular calcification and bone loss, switching to one of these newer drugs is recommended.[101,457-470]

Vitamin A

Vitamin A has been accused of promoting bone loss. However, this only happens when not in balance with vitamin D. Human clinical studies consistently show that vitamin A in balance with vitamin D is highly beneficial, not harmful, to bone. A high intake of vitamin A combined with a low intake of vitamin D is what favors a decrease in BMD and an increase in risk for fracture. On the other hand, a high intake of vitamin D combined low intake of vitamin A promotes hypercalcemia and soft tissue calcification.[471-481] Vitamin D requires vitamin A in order to effectively bind to cellular DNA, so as vitamin D increase, so does the body's need for vitamin A. The joining together of vitamin A and vitamin D to form a heterodimer that binds to DNA is what fully triggers genes to initiate the production of a very large number of proteins and enzyme precursors, including, for example, osteocalcin and matrix Gla protein, and a lack of either vitamin D or vitamin A will compromise their production. Even if vitamin K_2 is present in adequate amounts, its ability to help build bone will be impaired if either vitamin D or vitamin A is lacking because far less osteocalcin and matrix Gla protein will be available.[481]

Vitamin D increases the production of matrix Gla protein, whereas vitamin A slightly decreases its production. The end result is that enough, but not too much, matrix Gla protein is produced. This is highly important for bone health because matrix Gla protein and osteocalcin compete for carboxylation by the available vitamin K_2. For the calcium whose absorption is boosted by vitamin D to be delivered to bone, sufficient K_2 must be free to carboxylate osteocalcin. Plus, enough K_2 must be available to carboxylate matrix Gla protein to prevent calcium from depositing in the vasculature or kidneys. If too much matrix Gla protein is produced with the "help" of vitamin D unopposed by vitamin A, insufficient K_2 will be available to activate it, and not only will calcium not be delivered to bone (because osteocalcin will not be carboxylated either), but also inactive matrix Gla protein will accumulate in blood vessels and kidneys and promote unhealthy calcification in these tissues along with bone loss. By slightly lessening the production of matrix Gla protein, vitamin A ensures sufficient K_2 is available to activate osteocalcin, so the calcium that vitamin D helps us absorb goes into bone, not blood vessels or kidneys. When balanced by vitamin D, vitamin A activates osteoclasts just enough to remove old or compromised bone, initiating the remodeling process. Vitamin A also balances the calcium-absorbing actions of vitamin D by increasing the urinary excretion of excess calcium, both the calcium liberated from soft tissues by matrix Gla protein (activated by vitamin K_2) and the calcium released from bone by osteoclasts. For these reasons, high doses of unopposed vitamin D may cause a functional vitamin A deficiency and promote calcification of soft tissues and the formation of calcium oxalate kidney stones.[241,243,482-497] When the vitamin D–vitamin A heterodimer is present, even a defective VDR performs significantly better. Because the genetic inheritance of 34% to 44% of Caucasians and 24% to 36% of African Americans includes a faulty VDR, vitamin A's importance for bone health in a large percentage of the population cannot be overstated.[498-501] Vitamin A is required for immune tolerance, without which, the immune system becomes hyperactive, promoting the development of autoimmune diseases (e.g., allergies, asthma, rheumatoid arthritis, Hashimoto's and Graves thyroiditis, inflammatory bowel diseases, multiple sclerosis, etc.) and bone loss. A hyperactive immune system, constantly producing highly inflammatory agents to destroy what it misperceives as threats, creates a state of systemic chronic inflammation that excessively activates osteoclasts, causing bone loss.[502-507,645] Beta-carotene does not provide balance for the actions of vitamin D, prevent vitamin D toxicity, or help optimize vitamin D's healthful effects. Most humans do a very poor job of converting beta-carotene into vitamin A. The enzyme 15,15′-monooxygenase (BCMO1) is responsible for converting beta-carotene to vitamin A. Six very common SNPs result in the production of a slow to almost completely ineffective BCMO1. Virtually everyone has inherited at least one of these six SNPs, but even the few who have inherited the SNPs that produce a BCMO1 enzyme able to make the conversion will only convert a small portion of the beta-carotene they consume into vitamin A.[508-511] In addition, vitamin A insufficiency is widespread for a number of other factors. About 70% to 90% of dietary vitamin A is absorbed, but even under optimal circumstances, only 3% or less of the beta-carotene or other carotenoids are absorbed.[512] Despite being a fat-soluble nutrient, under normal conditions, 5% of vitamin A stores are lost daily. Large variations in the concentration of provitamin A carotenoids in foods are typical—even in the same type of food—due to varietal differences, stage of maturity when harvested, climate conditions, and processing and/or cooking.[513] Cofactors required for the conversion of beta-carotene to vitamin A may not be available. The enzyme BMCO1, which converts beta-carotene to vitamin A, does so as one of three steps, which require bile acids and iron. Furthermore, the form of vitamin A produced from beta-carotene, retinal, has to

be further metabolized by several other enzymes to be converted into retinol. These enzymes require riboflavin, niacin, and zinc as cofactors.[512] Bile flow is compromised by acute and chronic liver diseases, including NAFLD, which is now estimated to be present in as many as 46% of the U.S. population and 35% of the population worldwide.[514] A number of drugs deplete vitamin A, including aluminum-containing antacids; cholesterol-lowering agents, including bile acid sequestrants, such as cholestyramine (e.g., Questran, Questran Light, Cholybar, Olestyr) and colestipol (e.g., Colestid, Cholestabyl), which reduce fat absorption and thus the absorption of vitamin A and other fat-soluble nutrients (e.g., vitamin D and vitamin K); colchine, which is used to treat gout and impairs vitamin A absorption by blocking the release of retinol-binding protein; and sucralfate (e.g., Carafate, Sulcrate), which is used to treat duodenal ulcers and GERD and decreases the absorption of all the fat-soluble nutrients[515]; fat malabsorption, food intolerances, hypochlorhydria, and exposure to liver toxins (e.g., carbon tetrachloride, which forms when surfactants are mixed with chlorine bleach, impairs vitamin A metabolism)[516]; and lastly, supplementation with increasingly high doses of vitamin D without either vitamin A or K_2. Intake of vitamin D and vitamin A should be approximately equivalent.[479-481,483-485,517,518] Vitamin D increases production of the VKDPs, so the need for a higher intake of vitamin D_3 indicates the need for a higher intake of vitamin K_2, as well as sufficient vitamin A to maintain balance with D_3. In those who require 1000 to 2000 IU of vitamin D_3 per day, 1000 to 2000 IU of vitamin A and 100 to 120 micrograms of vitamin K_2 in the form of MK-7 per day should maintain balance. Those who require 5000 IU of vitamin D_3 per day will need ~5000 IU of vitamin A and 180 to 200 mcg/d of MK-7. Anyone requiring 10,000 IU of D_3/d and taking a comparable amount (10,000 IU) of vitamin A should be getting 360 mcg/d of MK-7, which is the K_2 dosage being used in the current research involving postmenopausal women with osteoporosis and men and women with coronary artery disease and CKD. No adverse events have been reported in any of the studies.[453] When taken in balance with vitamin D_3, and not consumed in excessive amounts (excess in adults = greater than 10,000 IU per day total from food and supplements combined), vitamin A is safe, exerts many beneficial effects on our bones, and promotes immune tolerance. If not balanced by comparable intake of vitamin D_3, vitamin A intake greater than 3000 IU/d may increase loss of BMD and risk of fracture in older people. The UL for vitamin A in adults (19 years and older) is 10,000 IU/d. Although 10,000 IU is also the UL given for vitamin A for pregnant or breastfeeding women, err on the side of caution. A safer UL may be 5000 IU/d, total, from both food and supplements. However, most if not all of the vitamin A safety studies have been done in the context of vitamin D deficiency. An adequate supply of vitamin A is especially important for pregnant women for normal fetal development and for women who are breastfeeding for healthy postnatal development. The adverse effects of vitamin A deficiency during pregnancy cannot be compensated for by postnatal supplementation. Synthetic retinoids (i.e., the acne and antiwrinkle drug isotretinoin) should be avoided; its potential adverse effects far outweigh any benefit.[519,520]

B Vitamins: B_6, B_{12}, Folate, Riboflavin, Niacin

The B vitamins work together to optimize methylation, prevent elevated levels of homocysteine, and promote the production of SAMe and glutathione. In their active forms, the B vitamins also play an essential role in the production and recycling of thyroid hormones, conversion of alpha-linolenic acid (ALA) and EPA to DHA, recycling glutathione and vitamin K, absorption of magnesium, and production of endothelial nitric oxide. Because of the high-incidence SNPs resulting in slower versions of the enzymes involved in B vitamin activation, a full-spectrum B complex providing the B vitamins in their active forms is recommended (i.e., pyridoxal-5-phosphate, methylcobalamin, methyltetrehydrafolate, flavin mononucleotide, and/or flavin adenine dinucleotide).[521-533,644]

Vitamin C

By directly disarming both ROS and reactive nitrogen species (RNS), vitamin C lessens free-radical damage, protecting against the development of excessive osteoclast activation caused by chronic inflammation. In addition, vitamin C recycles other antioxidants, including vitamin E and glutathione, further increasing our ability to disarm free radicals.[534,535] With aging, not only does the ability to produce glutathione decline, but also the needs for it increase, particularly in women when the loss of estrogen production that occurs with menopause results in an increased amount of both ROS and RNS, increased production of osteoclasts, and increased bone resorption. In animal studies, this entire scenario is completely reversed by supplementing laboratory rats with vitamin C, which increases glutathione concentrations.[536-541] Vitamin C's antioxidant activities also inhibit the production of other proinflammatory compounds, including the highly inflammatory signaling molecules of the prostaglandin E2 series.[542] Vitamin C improves blood sugar control. A review of 22 studies including 937 participants found that supplemental vitamin C (dose per day averaged 1000 mg but ranged from 500 to as much as 6000 mg/d) significantly improved blood sugar control and lowered blood sugar levels. Vitamin C was found to lower blood levels of glucose, insulin, and hemoglobin A_{1c}—all of which have been shown to inversely correlate with bone density.[543] Vitamin C is the cofactor for the dioxygenase enzymes, which are required for collagen biosynthesis. Because more than 90% of the protein in the bone matrix is collagen, vitamin C is an essential nutrient for healthy bone.[544,545] Vitamin C stimulates MSC production and then triggers the expression of genes involved in MSC differentiation into osteoblasts and chondrocytes instead of lipocytes.[546-549] Vitamin C also triggers the expression of genes in osteoblasts that are involved in the formation of trabecular bone and promotes gene signaling in mature osteoblasts that results in their increased activity and longevity.[549] Vitamin C is especially important for smokers and those exposed to secondhand smoke because it greatly increases the amount of nicotine required to inhibit osteoblast development and production of a number of proteins involved in bone formation.[550] Vitamin C, particularly supplemental vitamin C, lowers risk of hip and other osteoporotic fractures. Framingham data showed that those in the highest tertile of supplemental vitamin C intake (average intake of 260 mg/d) had a 69% lower risk of hip fracture.[545] The dietary reference intakes for vitamin C are 75 mg/d for adult women and 90 mg/d for adult men. An additional 35 mg/d is recommended for smokers because many toxic compounds in cigarette smoke rapidly deplete vitamin C. During pregnancy, the RDI is increased to 85 mg/d and further increased to 120 mg/d when breastfeeding.[551] These RDIs are grossly inadequate. Vitamin C is used up far more rapidly not just in acute illness but also in chronic inflammation, which affects the majority of Americans over age 50 and plays a major role in osteoporosis.[534,552,553] If we consider an evolutionary perspective for guidance, our prehistoric ancestors are thought to have consumed 2.3 g/d or more of vitamin C.[554] Many of today's leading clinicians believe doses ranging from 1000 to 2000 mg/d are optimal for most people. And this level of vitamin C intake is quite safe: the UL (tolerable upper limit) for vitamin C is 3000 mg/d, and the research suggests that intakes up to 4000 mg/d are well tolerated in the general population (per conversation with Dr. Joseph Pizzorno, based upon his 40+ years of experience in treating many thousands of patients; in writing *The Textbook of Natural Medicine,* now in its 5th edition; as editor of the PubMed listed journal, *Integrative Medicine: A Clinician's Journal,* and in daily review of breaking research).[555] Our needs for

vitamin C increase with age, in part because of more inflammation but also because aging liver cells produce fewer of the proteins that help transport vitamin C around the body (the sodium-dependent vitamin C transporters or SVCTs) than do the liver cells of younger individuals. In animal studies, production of a key type of SVCT declines 45% with age.[556,557] In addition, polymorphisms in SNPs rs33972313 in the *SLC23A1* gene (either GA or AA) result in the production of less effective SCVTs, increasing vitamin C needs.[558,559] Ascorbic acid is the least expensive form of supplemental vitamin C and is well tolerated by most individuals. Even those who are sensitive to this very weak "acid" are unlikely to experience gas or loose stools when taking three to six smaller doses of ~200 mg throughout the day. Furthermore, the fractional absorption of vitamin C is 100% at a dose of 200 mg but decreases as the dose increases. When 1250 mg is taken all at once, less than 50% is absorbed.[560]

No other safety issues exist. Vitamin C does not promote oxalate and kidney stone formation. The finding of high levels of oxalates in the urine with consumption of large doses of vitamin C has been revealed to be a laboratory artifact, the result of a method that converts vitamin C in the test sample to oxalate during analysis of the urine. In other words, vitamin C converts to oxalate in the laboratory collection bottle (i.e., after it has left the body). Vitamin C's effects while in the body all counteract stone formation. Vitamin C is not a prooxidant[561,562] and does not excessively increase iron absorption, cause rebound scurvy,[563] lower levels of B_{12},[564] or increase uric acid production and risk of gout.[565,566] Vitamin C should not be taken for at least 2 hours before or after taking propranolol. Taking 2 g of vitam71in C 30 minutes before taking propranolol (a beta-blocker) was found to reduce its bioavailability.[567] Also, do not take vitamin C at the same time as tetracycline. Administration of 500 mg of vitamin C along with 250 mg of tetracycline was found to increase the blood level of the drug by 3- to 15-fold after 2 hours.[567] Lastly, large doses of vitamin C may increase the dosage of warfarin required. Only two case reports have suggested this possibility, but err on the side of caution. For the best absorption of vitamin C, smaller doses (250–500 mg) should be used several times a day anyway, and this amount has never been suggested to interfere with warfarin dosage.[567]

Vitamin E

Vitamin E is a family of 8 isomers, not just α-tocopherol. Most vitamin E supplements contain only α-tocopherol; many contain dL-α-tocopherol, a synthetic (less expensive, racemic) version of α-tocopherol, which is less biologically active than natural (RRR) α-tocopherol.[568] For numerous reasons, the vitamin E supplement chosen to support bone health should contain all eight natural vitamin E isomers (i.e., "mixed tocopherols and tocotrienols"). High-dose α-tocopherol alone is not only less effective but can increase inflammation and promote bone loss: High dose α-tocopherol suppresses blood levels of γ-tocopherol, the vitamin E isomer most abundant in vitamin E–rich foods, by 30% to 70%.[569,570] Vitamin E–rich foods contain five times as much γ-tocopherol as α-tocopherol, and γ-tocopherol is a more potent anti-inflammatory agent than α-tocopherol. Mammals must neutralize two basic types of free radicals: ROS and RNS. α-Tocopherol can disarm ROS, but γ-tocopherol is required to defuse RNS. γ-Tocopherol is a much more potent inhibitor than α-tocopherol of two key enzymes responsible for inflammation, cyclooxygenase (COX) and lipoxygenase (LOX). γ-Tocopherol increases levels of an anti-inflammatory signaling molecule called PPARγ more than twice as effectively as α-tocopherol. γ-tocopherol, but not α-tocopherol, lowers levels of CRP, a key marker of inflammation associated with cardiovascular disease and bone loss. Supplementation with α-tocopherol alone may dysregulate key detoxification enzymes and immune function, further promoting inflammation. α-Tocopherol becomes a pro-oxidant if not accompanied by other antioxidants to recycle it. α-Tocopherol alone stimulates osteoclast activity and bone resorption. α-Tocopherol alone decreases blood levels of bone specific alkaline phosphatase and interferes with the physiological function of vitamin K, increasing the rate at which vitamin K is excreted and inhibiting its activation of osteocalcin and matrix Gla protein.[571-579] Human clinical trials show that mixed tocopherols are more effective than any single tocopherol against oxidative stress and inflammation.[580-582] Natural vitamin E (mixed tocopherols and tocotrienols) promotes healthy bones by neutralizing both ROS and RNS free radicals and lessening inflammation and by increasing activation of anti-inflammatory PPARs, a family of proteins that control when genes are switched on or off and inhibit key inflammatory signaling cascades, including the NFκB pathway, the pathway that is the target of denosumab (Prolia, Xgeva). Vitamin E also inhibits this pathway, but unlike denosumab, it has no adverse effects.[571,572,583] Natural vitamin E (mixed tocopherols and tocotrienols) has been shown to increase BMD and to lower the risk of sarcopenia and dementia as well as osteoporosis.[584-589] Animal studies suggest natural vitamin E may be especially helpful for men with osteoporosis.[590] The RDI only covers α-tocopherol and is far too low: RDA = 15 mg (or 22 IU of natural α-tocopherol or 16 IU of synthetic dL-α-tocopherol). The tolerable UL was set at 1000 mg/d for either natural (RRR) or synthetic (racemic) supplemental α-tocopherol.[534,591-593] The most current papers on vitamin E argue that the scientific evidence is strong enough to recommend daily intakes of at least 150 IU of α-tocopherol per day.[594] A solid body of peer-reviewed medical literature has demonstrated that vitamin E is safe in amounts up to 1600 IU/d.[555,595] Individuals not regularly consuming vitamin E–rich foods are likely to be deficient. In several studies, more than 40% of elderly individuals (aged 65–98 years) were not even consuming the RDI. Data show that elderly humans (age 65 and older) whose diets provide more than five times the RDI of vitamin E (a dose of mixed tocopherols and tocotrienols of ~550 IU/d) had significantly better immune responses and increased resistance to infectious diseases compared with nonsupplemented controls.[592] To support the health of both bones and immune system, 400 to 800 IU of mixed tocopherols and tocotrienols should be consumed daily. The vitamin E supplement you recommend should provide the full complement of mixed tocopherols and tocotrienols with a 5:1 ratio of γ-tocopherol to α-tocopherol. Needs for γ-tocopherol are increased in individuals taking a nitrogen-bisphosphonate (e.g., Fosamax, Boniva) because these drugs greatly depress levels of both γ-tocopherol and coenzyme Q_{10} (CoQ_{10}).[596] A solid body of peer-reviewed medical literature has demonstrated that natural vitamin E (mixed tocopherols and tocotrienols) is safe in amounts up to 1600 IU/d.[555]

Omega-3 Essential Fatty Acids

ALA, EPA, and DHA lower osteoclast-activating chronic inflammation because they are metabolized by the same enzymes as the potentially proinflammatory omega-6 essential fatty acid, arachidonic acid. In addition, the omega-3s promote bone formation by signaling that directs MSCs to develop into osteoblasts rather than adipocytes. In young individuals, inflammation is interpreted by MSCs as a signal to produce more osteoblasts and more bone.[82,83] With aging, if the supply of omega-3s is out of balance with that of omega-6s, MSCs preferentially develop into adipocytes, promoting sarcopenia.[597,598] When sufficient EPA/DHA is consumed daily to result in a ratio of omega-6:omega-3 of no greater than 4:1, metabolism shifts to one that favors an anti-inflammatory response, prevents chronic low-grade inflammation, quickly resolves acute inflammation, puts a damper on the production of osteoclasts, and encourages MSCs to become osteoblasts rather than adipocytes. The ratio of omega-6:omega-3 typically seen in the modern Western diet is

20:1 and promotes inflammation and sarcopenia. Supplemental EPA/DHA is recommended because even if intake of ALA-rich plant foods is high, humans are able to convert only a very small amount of the ALA in plant foods into EPA and DHA, a conversion that is further reduced by 40% to 50% when the diet is rich in omega-6-oils, as is the SAD. In actuality, less than 0.1% to 0.2% of the ALA consumed is converted into arachidonic acid (AA).[599-604] Men do an especially poor job of converting ALA to EPA/DHA, producing far less than women, who are more capable of producing EPA/DHA from ALA because DHA is essential for fetal brain development and brain function.[605-607] Cold, deep-water, wild-caught fish are the best dietary sources of EPA/DHA, but at least two servings of fish every day would need to be consumed to ingest the 2.6 g/d of EPA/DHA needed for healthy bones. If the wild-caught fish is large, it may contain unsafe levels of bone-destructive mercury, which accumulates in fish in a form that is water soluble and highly bioavailable.[156,608] Mercury deposits in the kidneys, promoting bone loss because the kidneys are the primary location in which 25(OH)D is converted to 1,25-D. Farmed fish are not a good source of omega-3s because their feed is high in omega-6 oils, resulting in their flesh containing high levels of AA. For all these reasons, supplemental EPA/DHA is recommended. To attain a ratio of omega-6:omega-3 of at least 4:1, preferably lower (2:1),[83,609-615] the consumption of 1 g of omega-3 for every 4 g of omega-6 in the diet is required. The "average" daily intake of omega-6 in the United States is 20 g/d, whereas the average intake of EPA and DHA, from both food and supplements, is barely 1 g/d of EPA/DHA, so an additional 3 to 4 g/d of EPA/DHA is needed to support omega-3's beneficial effects on bone, and APOE4 carriers are[616-620] likely to require even higher doses to receive benefit.[621,622] Omega-3s in their natural triglyceride form are significantly more effectively absorbed (EPA 3.4-fold better and DHA 2.7-fold better) and utilized than ethyl esters (EEs) or synthetic triglycerides (rTG).[623-625]

Exercise

The skeletal and the muscular systems are tightly intertwined: the strongest mechanical forces applied to bones are those created by muscle contractions, which condition bone density, strength, and microarchitecture. Not surprisingly, sarcopenia decreases bone strength; fortunately, it's very responsive to progressive resistance training, especially in elders. Improvements in strength up to 200% are not unusual in just a few months, although the increase in muscle mass is usually only ~10% in older adults.[626] By stressing muscles, which stress bones, exercise activates osteocytes, long-lived cells—their average half-life is 25 years—that form a signaling network in bone that, similar to the nervous system, transmits signals that direct and properly balance the activities of osteoblasts and osteoclasts. Osteocytes tend to die off with aging, especially if they are not stimulated by mechanotransduction (i.e., exercise).[627] Studies of limb loading in animals show that although mechanical stress results in very small gains in total bone, these gains occur where bone is subjected to the most strain (i.e., where bone is most likely to fracture). The end result is a greater than 100-fold increase in "fatigue resistance" (the highest stress that the bone material can withstand for a given number of cycles without breaking), despite a much smaller absolute gain in bone mass. In addition, in response to exercise, osteocyte signaling increases the pool of MSCs, halts the secretion of sclerostin, and increases that of proteins involved in Wnt signaling. Sclerostin inhibits the development of osteoblasts and bone formation. Wnt signaling promotes the production of both osteoblasts and myocytes.[628,629]

How important is this? The newest pharmaceutical agent in the pipeline, Romosozumab, was created to be a patentable antibody to sclerostin. Its ADRs already include increased risk of vascular calcification, stroke, and "cardiovascular "events" (e.g., myocardial infarction), along with a potentially increased risk of cancer.[630] By improving muscle strength and balance and combating sarcopenia, regular exercise, particularly when vitamin D levels are adequate, also lowers the risk of falls, which greatly increase the risk of fragility fractures.[631-633] One reason for this is that muscle fiber membranes are richly supplied with vitamin D receptors, whose exposure to vitamin D triggers muscle protein synthesis. The IOM still claims the evidence is not sufficient for vitamin D's importance in preventing sarcopenia; in Europe, however, a panel of experts determined vitamin D level of *at least* 30 to 44 ng/mL is necessary for musculoskeletal health.[626] Exercise increases bone diameter throughout the life span, further diminishing fracture risk by mechanically counteracting the age-associated thinning of bones and increases in bone porosity.[632] Prospective and case-control studies show that, compared with sedentary individuals, those who engage in regular, vigorous physical activity are 20% to 40% less likely to suffer a hip fracture.[634] The Nurses' Health Study found that active women with a minimum of 24 MET-hours per week had a 55% lower risk of hip fracture compared with sedentary women getting less than 3.[635] Although well-designed, carefully supervised exercise trials have failed to show any absolute gain in bone mass in dedicated exercisers, a number have reported a halting of further age-related bone loss.[636-639] The deciding factor that determines whether or not exercise halts bone loss appears to be calcium. Substantial evidence shows that individuals who consume low levels of calcium have lower bone mass (and greater risk of fracture) than age-matched individuals who consume adequate or higher levels of calcium. These findings have been documented in adolescents and young as well as older women. In addition, it's thought that physically active people may need more calcium to support the increased bone formation potentially stimulated by physical activity. This increased need has been suggested as the reason why the research on exercise and bone formation shows such inconsistent results. The lack of bone building in response to exercise is thought to be due to the higher levels of calcium needed were not available.[640] In the research overall, in exercise studies in which calcium intakes were less than 1000 mg/d, bone formation has been negligible.[641] To effectively promote bone health, exercise should occur a minimum of 3 times a week for 1 hour; be dynamic; involve some impact; exceed a threshold intensity, at least 70% but preferably 85% of maximal capacity; exceed a threshold strain frequency; involve short bouts of intense exercise with less intense recovery periods; impose an unusual loading pattern on the bones; and be combined with optimal nutritional support: protein, calcium, D_3, K_2, magnesium, boron, and other trace minerals. For those individuals with osteoporosis, impact-producing exercises and those that involve spinal flexion should be avoided to prevent injuries.[636]

A warning: hormonal contraceptives prevent normal estrogen and progesterone production, thus preventing normal bone mass accretion in young women. Suppression of normal age-related increases in BMD have been documented in oral contraceptive users who completed a 2-year exercise program, and the age at which oral contraceptive use was initiated is the most significant predictor of low spinal bone mass in female endurance athletes.[642,643] These findings have serious implications for the ability of young women—even those who exercise regularly—to achieve the peak bone mass they must have to avoid the early onset of osteoporosis.[636]

THERAPEUTIC APPROACH

Diet

A whole-foods, Mediterranean-type diet is optimal for strong bones (and for virtually all other aspects of health). The diet should include organically grown plant foods whenever possible (vegetables, fruits, legumes, nuts, and seeds), wild-caught fish, free-range eggs, and dairy and meat products from pastured animals and extra-virgin olive oil

(EVOO) as the primary vegetable oil. Minimize consumption of processed foods to lessen the intake of phosphate additives, pesticide residues, nutrient-depleted highly refined carbohydrates, cadmium, excessive omega-6 fatty acids, and sodium. Ensure protein intake of 1.8 g/kg. Consider minimizing intake or recommending the avoidance of gluten if gluten sensitivity is suspected.

Lifestyle Factors

Minimize exposure to environmental toxins.
If smoking, quit, and minimize exposure to secondhand smoke.
Limit alcohol consumption to no more than 2 units/d for women, 4 units/d for men (1 unit is 12 oz of beer, 4 oz of wine, or 1 oz of liquor).
Coffee, 3 to 4 cups/d, is beneficial with adequate calcium intake (1200–1500 mg/d).
Decaf is recommended for those with *CYP1A2* SNPs that result in slower detoxification of caffeine.
Minimize drug use.
Monitor thyroid hormone levels at least annually and adjust T3/T4 dosages as indicated.
Switch to less harmful pharmaceutical agents when use is necessary.
Engage in weight-bearing exercise for a minimum of 3 hours/week.
Encourage movement! Ideally, some type of weight-bearing exercise should be undertaken for 1 hour each day.
Individuals with osteoporosis should avoid exercises that produce a forward-rounding curvature of the neck and thoracic vertebrae because this may promote "cervical wedging."
Consider heavy metals testing: lead, mercury, cadmium.

Genetic Susceptibility

Evaluate key SNPs and adjust nutrient recommendations accordingly.

Evaluate and Treat for Comorbidities

Evaluate for and treat cardiovascular disease (CVD), metabolic syndrome (MetS), diabetes, CKD, NAFLD, hyper-/hypothyroidism, obesity (gastric bypass), sarcopenia, GERD, IBS, inflammatory bowel disease (IBD), rheumatoid arthritis, multiple sclerosis, celiac disease, gluten sensitivity, and eating disorders.

Nutritional Supplements

Calcium: 1200 to 1500 mg/d
Magnesium: 600 to 750 mg/d
Trace minerals: zinc/copper, 15/1 mg/d; manganese, 5 mg/d; silicon, 40 mg/d; selenium, 200 mcg/d; boron, 3 to 6 mg/d; iodine, 1 mg/d
Vitamin D_3: 2000 to 5000 IU or higher, depending on 25(OH)D level
Vitamin A: should be approximately equal to D_3 intake
Vitamin K_1: 1000 mcg/d
Vitamin K_2 (MK-7): 100 to 400 mcg/d, depending on D_3 needs
Vitamin C: 1000 to 3000 mg/d
B vitamins: A full-spectrum B complex providing B_6, B_{12}, folate, and riboflavin in their activated forms (i.e., pyridoxal-5-phosphate, methylcobalamin, methyltetrehydrafolate, flavin mononucleotide, and/or flavin adenine dinucleotide)
Mixed tocopherols and tocotrienols providing gamma-tocopherol:alpha-tocopherol ratio of 5:1: 400 to 800 IU/d
EPA/DHA: an amount sufficient to produce a ratio of n-6:n-3 of no more than 4:1, typically 3 to 4 g/d
Consider strontium citrate: 340 to 680 mg/d
Consider BHRT: bio-identical Bi-est, Progesterone, DHEA; dosage determined and compounded for the individual per results of 24-hour urine comprehensive hormone testing

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Thomsen K, Jepsen DB, Matzen L, et al. Is calcaneal quantitative ultrasound useful as a prescreen stratification tool for osteoporosis? *Osteoporos Int*. 2015;26(5):1459–1475. https://doi.org/10.1007/s00198-014-3012-y. Epub 2015 Jan 30. PMID: 25634771.
2. Das S, Crockett JC. Osteoporosis - a current view of pharmacological prevention and treatment. *Drug Des Devel Ther*. 2013;7:435–448. https://doi.org/10.2147/DDDT.S31504. Print 2013. PMID: 23807838.
3. Siris ES, Adler R, Bilezikian J, et al. The clinical diagnosis of osteoporosis: a position statement from the national bone health Alliance working group. *Osteoporos Int*. 2014;25(5):1439–1443. https://doi.org/10.1007/s00198-014-2655-z. Epub 2014 Feb 28. PMID: 24577348 PMCID: PMC3988515.
4. Broussard DL, Magnus JH. Risk assessment and screening for low bone mineral density in a multi-ethnic population of women and men: does one approach fit all? *Osteoporos Int*. 2004;15(5):349–360. Epub 2003 Dec 16. PMID: 14676991.
5. Karjalainen JP, Riekkinen O, Kröger H. Pulse-echo ultrasound method for detection of post-menopausal women with osteoporotic BMD. *Osteoporos Int*. 2018;29(5):1193–1199. https://doi.org/10.1007/s00198-018-4408-x. Epub 2018 Feb 19. PMID: 29460101.
6. CPT® Category III Codes. American Medical Association. https://www.ama-assn.org/sites/ama-assn.org/files/corp/media-browser/public/cpt/cpt-category3-codes-descriptors.pdf. Accessed 5 February 2020.
7. Morris HA, Eastell R, Jorgensen NR, et al. IFCC-IOF Working Group for Standardisation of Bone Marker Assays (WG-BMA). Clinical usefulness of bone turnover marker concentrations in osteoporosis. *Clin Chim Acta*. 2017;467:34–41. https://doi.org/10.1016/j.cca.2016.06.036. Epub 2016 Jun 30. PMID: 27374301.
8. Cosman F, de Beur SJ, LeBoff MS, et al. National osteoporosis foundation. clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int*. 2014;25(10):2359–2381. https://doi.org/10.1007/s00198-014-2794-2. Epub 2014 Aug 15 PMID: 25182228 PMCID: PMC4176573.
9. Biver E, Chopin F, Coiffier G, et al. Bone turnover markers for osteoporotic status assessment? A systematic review of their diagnosis value at baseline in osteoporosis. *Joint Bone Spine*. 2012;79(1):20–25. https://doi.org/10.1016/j.jbspin.2011.05.003. Epub 2011 Jul 2 PMID: 21724445.
10. Gourlay ML, Overman RA, Ensrud KE. Bone density screening and re-screening in postmenopausal women and older men. *Curr Osteoporos Rep*. 2015;13(6):390–398. https://doi.org/10.1007/s11914-015-0289-5. PMID: 26408154 PMCID: PMC4623874.
11. Broussard DL, Magnus JH. Risk assessment and screening for low bone mineral density in a multi-ethnic population of women and men: does one approach fit all? *Osteoporos Int*. 2004;15(5):349–360. Epub 2003 Dec 16 PMID: 14676991.
12. Jain S, Bilori B, Gupta A, et al. Are men at high risk for osteoporosis underscreened? a quality improvement project. *Perm J*. 2016;20(1):60–64. https://doi.org/10.7812/TPP/14-190. PMID: 26824964.
13. Burge R, Dawson-Hughes B, Solomon DH, et al. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007;22(3):465–475. PMID: 17144789.
14. Osteoporosis and Asian American Women. NIH Osteoporosis and Related Bone Diseases National Resource Center. https://www.bones.nih.gov/health-info/bone/osteoporosis/background/asian-american-women. Accessed 5 February 2020.
15. Cong E, Walker MD. The Chinese skeleton: insights into microstructure that help to explain the epidemiology of fracture. *Bone Res*. 2014;2:14009. https://doi.org/10.1038/boneres.2014.9. eCollection 2014 PMID: 26273521 PMCID: PMC4472143.
16. Facts and statistics. International Osteoporosis Foundation. https://www.iofbonehealth.org/facts-statistics#category-25. Accessed 5 February 2020.
17. Bone Health and Osteoporosis: A Report of the Surgeon General. https://www.ncbi.nlm.nih.gov/books/NBK45515/#ch4.s5. Accessed 23 May 2018.
18. Nakchbandi IA, van der Merwe SW. Current understanding of osteoporosis associated with liver disease. *Nat Rev Gastroenterol Hepatol*. 2009;6(11):660–670. https://doi.org/10.1038/nrgastro.2009.166. PMID: 19881518.
19. Luxon BA. Bone disorders in chronic liver diseases. *Curr Gastroenterol Rep*. 2011;13(1):40–48. https://doi.org/10.1007/s11894-010-0166-4. PMID: 21104208.
20. Asrih M, Jornayvaz FR. Diets and nonalcoholic fatty liver disease: the good and the bad. *Clin Nutr*. 2013pii: S0261-5614(13)00304-X. https://doi.org/10.1016/j.clnu.2013.11.003. Epub ahead of print. PMID: 24262589.
21. Bonjour JP. The dietary protein, IGF-I, skeletal health axis. *Horm Mol Biol Clin Investig*. 2016;28(1):39–53. https://doi.org/10.1515/hmbci-2016-0003. DOI: 10.1515/hmbci-2016-0003. PMID: 26985688.
22. Courtney-Martin G, Ball RO, Pencharz PB, et al. Protein requirements during aging. *Nutrients*. 2016;8(8):E492. https://doi.org/10.3390/nu8080492. PMID: 2752927.
23. Phillips SM. Nutrition in the elderly: a recommendation for more (evenly distributed) protein?. *Am J Clin Nutr*. 2017:ajcn159863. https://doi.org/10.3945/ajcn.117.159863. [Epub ahead of print] PMID: 28592608.
24. Pizzorno L. Canaries in the phosphate-toxicity coal mines. *Integr Med (Encinitas)*. 2014;13(6):24–32. Review PMID: 26770122.
25. Allen-Evenson S, Archibald A. *No Bones about It! AIHM, Integrative Healthcare Symposium Annual Conference.* New York Hilton, Midtown. 2018. OR if not in this reference, in italics with reference following.
26. Gonçalves CG, Almeida BC, Camargo-Kosugi CM, et al. Polymorphisms in CYP17, COMT, and ESR1 genes in women after menopause and association with bone mineral density. *Genet Mol Res*. 2015;14(4):15802–15810. https://doi.org/10.4238/2015.December.1.32. PMID: 26634548.
27. Juan J, Huang H, Jiang X, et al. Joint effects of fatty acid desaturase 1 polymorphisms and dietary polyunsaturated fatty acid intake on circulating fatty acid proportions. *Am J Clin Nutr*. 2018;107(5):826–833. https://doi.org/10.1093/ajcn/nqy025. PMID: 29722844.
28. Zeng H, Cao JJ, Combs Jr GF. Selenium in bone health: roles in antioxidant protection and cell proliferation. *Nutrients*. 2013;5(1):97–110. https://doi.org/10.3390/nu5010097. PMID: 23306191.
29. Verroken C, Zmierczak HG, Goemaere S, et al. Insulin resistance is associated with smaller cortical bone size in nondiabetic men at the age of peak bone mass. *J Clin Endocrinol Metab*. 2017;102(6):1807–1815. https://doi.org/10.1210/jc.2016-3609. PMID: 28001453.
30. Krela-Kaźmierczak I, Szymczak A, Łykowska-Szuber L, et al. Osteoporosis in gastrointestinal diseases. *Adv Clin Exp Med*. 2016;25(1):185–190. https://doi.org/10.17219/acem/33746. PMID: 26935513.
31. Taxel P, Kenny A. Differential diagnosis and secondary causes of osteoporosis. *Clin Cornerstone*. 2000;2(6):11–21. PMID: 10938988.
32. Wong CX, Gan SW, Lee SW, et al. Atrial fibrillation and risk of hip fracture: a population-based analysis of 113,600 individuals. *Int J Cardiol*. 2017;243:229–232. https://doi.org/10.1016/j.ijcard.2017.05.012. Epub 2017 May 5. PMID: 2852898.
33. Wu CY, Lu YY, Lu CC, et al. Osteoporosis in adult patients with atopic dermatitis: a nationwide population-based study. *PLoS One*. 2017;12(2):e0171667. https://doi.org/10.1371/journal.pone.0171667. eCollection 2017 PMID: 28207767.
34. Jin ES, Jeong JH, Lee B, et al. Association factor analysis between osteoporosis with cerebral artery disease: the STROBE study. *Medicine (Baltimore)*. 2017;96(9):e6164. https://doi.org/10.1097/MD.0000000000006164. PMID: 28248870.
35. Taylor EN, Feskanich D, Paik JM, et al. Nephrolithiasis and risk of incident bone fracture. *J Urol*. 2016;195(5):1482–1486. https://doi.org/10.1016/j.juro.2015.12.069. Epub 2015 Dec 18 PMID: 26707509.
36. Schneider SM, Al-Jaouni R, Filippi J, et al. Sarcopenia is prevalent in patients with Crohn's disease in clinical remission. *Inflamm Bowel Dis*. 2008;14(11):1562–1568. https://doi.org/10.1002/ibd.20504. PMID: 18478564.
37. Alis G, Alis M, Erturk T, et al. Evaluation of bone disease in kidney transplant recipients. *Transplant Proc*. 2017;49(3):509–511. https://doi.org/10.1016/j.transproceed.2017.01.040. PMID: 28340823.

38. Wu CH, Chai CY, Tung YC, et al. Herpes zoster as a risk factor for osteoporosis: a 15-year nationwide population-based study. *Medicine (Baltimore)*. 2016;95(25):e3943. https://doi.org/10.1097/MD.0000000000003943. PMID: 27336887.
39. Ebesunun MO, Umahoin KO, Alonge TO, et al. Plasma homocysteine, B vitamins and bone mineral density in osteoporosis: a possible risk for bone fracture. *Afr J Med Med Sci*. 2014;43(1):41–47. PMID: 25335377.
40. Cauley JA. Osteoporosis: fracture epidemiology update 2016. *Curr Opin Rheumatol*. 2017;29(2):150–156. https://doi.org/10.1097/BOR.0000000000000365. PMID: 28072591.
41. Wu CH, Lu YY, Chai CY, et al. Increased risk of osteoporosis in patients with erectile dysfunction: a nationwide population-based cohort study. *Medicine (Baltimore)*. 2016;95(26):e4024. https://doi.org/10.1097/MD.0000000000004024. PMID: 27368024.
42. Wu CH, Tung YC, Lin TK, et al. Hip fracture in people with erectile dysfunction: a nationwide population-based cohort study. *PLoS One*. 2016;11(4):e0153467. https://doi.org/10.1371/journal.pone.0153467. eCollection 2016 PMID: 27078254.
43. Kenny AM, Prestwood KM, Marcello KM, et al. Determinants of bone density in healthy older men with low testosterone levels. *J Gerontol A Biol Sci Med Sci*. 2000;55(9):M492–M497. PMID: 10995046.
44. Bobjer J, Bogefors K, Isaksson S, et al. High prevalence of hypogonadism and associated impaired metabolic and bone mineral status in subfertile men. *Clin Endocrinol (Oxf)*. 2016;85(2):189–195. https://doi.org/10.1111/cen.13038. Epub 2016 Feb 29 PMID: 26857217.
45. Golds G, Houdek D, Arnason T. Male hypogonadism and osteoporosis: the effects, clinical consequences, and treatment of testosterone deficiency in bone health. *Int J Endocrinol*. 2017:4602129. https://doi.org/10.1155/2017/4602129. Epub 2017 Mar 16 PMID: 28408926.
46. Arias-Santiago S, Camacho-Martínez FM. Adverse effects of 5-alpha reductase inhibitor therapy in men with androgenetic alopecia: is there cause for concern?. *Actas Dermosifiliogr*. 2016;107(9):709–711. https://doi.org/10.1016/j.ad.2016.06.006. Epub 2016 Jul 18. [Article in English, Spanish] PMID: 27436801.
47. Pompe E, Bartstra J, Verhaar HJ, et al. Bone density loss on computed tomography at 3-year follow-up in current compared to former male smokers. *Eur J Radiol*. 2017;89:177–181. https://doi.org/10.1016/j.ejrad.2017.02.011. Epub 2017 Feb 9 PMID: 28267536.
48. Li XS, He H, Zhao YL, et al. Bone mineral density is negatively associated with arterial stiffness in men with hypertension. *J Clin Hypertens (Greenwich)*. 2016;18(11):1106–1111. https://doi.org/10.1111/jch.12848. Epub 2016 Jun 3 PMID: 27255660.
49. Estublier C, Chapurlat R, Szulc P. Association of severe disc degeneration with all-cause mortality and abdominal aortic calcification assessed prospectively in older men: findings of a single-center prospective study of osteoporosis in men. *Arthritis Rheumatol*. 2015;67(5):1295–1304. https://doi.org/10.1002/art.39055. PMID: 25772505.
50. Szulc P, Blackwell T, Schousboe JT, et al. High hip fracture risk in men with severe aortic calcification: MrOS study. *J Bone Miner Res*. 2014;29(4):968–975. https://doi.org/10.1002/jbmr.2085. PMID: 23983224.
51. Alan B, Akpolat V, Aktan A, et al. Relationship between osteopenic syndrome and severity of coronary artery disease detected with coronary angiography and Gensini score in men. *Clin Interv Aging*. 2016;11:377–382. https://doi.org/10.2147/CIA.S104036. eCollection 2016 PMID: 27069361.
52. Yesil Y, Ulger Z, Halil M, et al. Coexistence of osteoporosis (OP) and coronary artery disease (CAD) in the elderly: it is not just a by chance event. *Arch Gerontol Geriatr*. 2012;54(3):473–476. https://doi.org/10.1016/j.archger.2011.06.007. Epub 2011 Jul 1 PMID: 21723624.
53. Wallin M, Barregard L, Sallsten G, et al. Low-Level cadmium exposure is associated with decreased bone mineral density and increased risk of incident fractures in elderly men: the MrOS Sweden Study. *J Bone Miner Res*. 2016;31(4):732–741. https://doi.org/10.1002/jbmr. 2743. Epub 2015 Dec 6 PMID: 26572678.
54. Emkey RD, Emkey GR. Calcium metabolism and correcting calcium deficiencies. *Endocrinol Metab Clin North Am*. 2012;41(3):527–556. https://doi.org/10.1016/j.ecl.2012.04.019. Epub 2012 May 30 PMID: 22877428.
55. Get the facts: sodium and the dietary guidelines. https://www.cdc.gov/salt/pdfs/sodium_dietary_guidelines.pdf. Accessed 5 February 2020.
56. Types of birth control pills (oral contraceptives). Drugs.com. https://www.drugs.com/article/birth-control-pill.html. Accessed 5 February 2020.
57. Nordin BE, Need AG, Morris HA, et al. The nature and significance of the relationship between urinary sodium and urinary calcium in women. *J Nutr*. 1993;123(9):1615–1622. PMID: 8360790.
58. Breslau NA, McGuire JL, Zerwekh JE, et al. The role of dietary sodium on renal excretion and intestinal absorption of calcium on vitamin D metabolism. *J Clin Endocrinol Metab*. 1982;55(2):369–373. PMID:6896338.
59. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr*. 2002;75(4):773–779. PMID: 11916767.
60. Heaney RP. Protein and calcium: antagonists or synergists? Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr*. 2002;75(4):609–610. PMID: 11916747.
61. Heaney RP, Layman DK. Amount and type of protein influences bone health. *Am J Clin Nutr*. 2008;87(5):1567S–1570S. PMID: 18469289.
62. Martínez-Ramírez MJ, Delgado-Martínez AD, Ruiz-Bailén M, et al. Protein intake and fracture risk in elderly people: a case-control study. *Clin Nutr*. 2012;31(3):391–395. https://doi.org/10.1016/j.clnu.2011.11.016. Epub 2011 Dec 17 PMID: 22182947.
63. Mangano KM, Sahni S, Kerstetter JE. Dietary protein is beneficial to bone health under conditions of adequate calcium intake: an update on clinical research. *Curr Opin Clin Nutr Metab Care*. 2014;17(1):69–74. https://doi.org/10.1097/MCO.0000000000000013. PMID: 24316688.
64. Sahni S, Mangano KM, Tucker KL, et al. Protective association of milk intake on the risk of hip fracture: results from the Framingham Original Cohort. *J Bone Miner Res*. 2014;29(8):1756–1762. https://doi.org/10.1002/jbmr. 2219 PMID: 24760749.
65. Sahni S, Cupples LA, McLean RR, et al. Protective effect of high protein and calcium intake on the risk of hip fracture in the Framingham offspring cohort. *J Bone Miner Res*. 2010;25(12):2770–2776. https://doi.org/10.1002/jbmr.194. Epub 2010 Jul 26 PMID: 20662074.
66. Tsanzi E, Fitch CW, Tou JC. Effect of consuming different caloric sweeteners on bone health and possible mechanisms. *Nutr Rev*. 2008;66(6):301–309. https://doi.org/10.1111/j.1753-4887.2008.00037.x. PMID: 18522618.
67. Lemann Jr J, Lennon EJ, Piering WR, et al. Evidence that glucose ingestion inhibits net renal tubular reabsorption of calcium and magnesium in man. *J Lab Clin Med*. 1970;75(4):578–585. PMID: 5444345.
68. Thom JA, Morris JE, Bishop A, et al. The influence of refined carbohydrate on urinary calcium excretion. *Br J Urol*. 1978;50(7):459–464. PMID: 572722.
69. Urine Calcium: Laboratory Measurement and Clinical Utility http://www.medscape.com/viewarticle/732914. Accessed 24 February 2014.
70. Pizzorno J, Frassetto LA, Katzinger J. Diet-induced acidosis: is it real and clinically relevant? *Br J Nutr*. 2010;103(8):1185–1194. https://doi.org/10.1017/S0007114509993047. Epub 2009 Dec 15 PMID: 20003625.
71. Uribarri J, Calvo MS. Dietary phosphorus intake and health. *Am J Clin Nutr*. 2013. Epub ahead of print PMID: 24381089.
72. Calvo MS, Moshfegh AJ, Tucker KL. Assessing the health impact of phosphorus in the food supply: issues and considerations. *Adv Nutr*. 2014;5(1):104–113. https://doi.org/10.3945/an.113.004861. PMID: 24425729.
73. Uribarri J, Calvo MS. Dietary phosphorus excess: a risk factor in chronic bone, kidney, and cardiovascular disease? *Adv Nutr*. 2013;4(5):542–544. https://doi.org/10.3945/an.113.004234. PMID: 24038251.
74. Calvo MS, Tucker KL. Is phosphorus intake that exceeds dietary requirements a risk factor in bone health? *Ann N Y Acad Sci*. 2013;1301(1):29–35. https://doi.org/10.1111/nyas.12300. PMID: 24472074.
75. Dilzer A, Park Y. Implication of conjugated linoleic acid (CLA) in human health. *Crit Rev Food Sci Nutr*. 2012;52(6):488–513. https://doi.org/10.1080/10408398.2010.501409. PMID: 22452730.
76. Lawson RE, Moss AR, Givens DI.The role of dairy products in supplying conjugated linoleic acid to man's diet: a review. *Nutr Res Rev*. 2001;14(1):153–172. https://doi.org/10.1079/NRR200121. PMID: 19087420.

77. Benbrook CM, Butler G, Latif MA, et al. Organic production enhances milk nutritional quality by shifting Fatty Acid composition: a United States-wide, 18-month study. *PLoS One*. 2013;8(12):e82429. https://doi.org/10.1371/journal.pone.0082429. eCollection 2013 PMID: 24349282.
78. Dhiman TR, Nam SH, Ure AL. Factors affecting conjugated linoleic acid content in milk and meat. *Crit Rev Food Sci Nutr*. 2005;45(6):463–482. PMID: 16183568.
79. Kruger MC, Coetzee M, Haag M, et al. Long-chain polyunsaturated fatty acids: selected mechanisms of action on bone. *Prog Lipid Res*. 2010;49(4):438–449. https://doi.org/10.1016/j.plipres.2010.06.002. Epub 2010 Jun 17 PMID: 20600307.
80. Yuan J, Akiyama M, Nakahama K, et al. The effects of polyunsaturated fatty acids and their metabolites on osteoclastogenesis in vitro. *Prostaglandins Other Lipid Mediat*. 2010;92(1–4):85–90. https://doi.org/10.1016/j.prostaglandins.2010.04.001. Epub 2010 Apr 13 PMID: 20394833.
81. Kajarabille N, Díaz-Castro J, Hijano S, et al. A new insight to bone turnover: role of ω-3 polyunsaturated fatty acids. *Sci World J*. 2013:589641. https://doi.org/10.1155/2013/589641. PMID: 24302863.
82. Casado-Díaz A, Santiago-Mora R, Dorado G, et al. The omega-6 arachidonic fatty acid, but not the omega-3 fatty acids, inhibits osteoblastogenesis and induces adipogenesis of human mesenchymal stem cells: potential implication in osteoporosis. *Osteoporos Int*. 2013;24(5):1647–1661. https://doi.org/10.1007/s00198-012-2138-z. Epub 2012 Oct 27 PMID: 23104199.
83. Kelly OJ, Gilman JC, Kim Y, et al. Long-chain polyunsaturated fatty acids may mutually benefit both obesity and osteoporosis. *Nutr Res*. 2013;33(7):521–533. https://doi.org/10.1016/j.nutres.2013.04.012. Epub 2013 Jun 10 PMID: 23827126.
84. Lee SM, An WS. Cardioprotective effects of ω -3 PUFAs in chronic kidney disease. *Biomed Res Int*. 2013;2013:712949. https://doi.org/10.1155/2013/712949. Epub 2013 Apr 4 PMID: 23653897.
85. Guebre-Egziabher F, Debard C, Drai J, et al. Differential dose effect of fish oil on inflammation and adipose tissue gene expression in chronic kidney disease patients. *Nutrition*. 2013;29(5):730–736. https://doi.org/10.1016/j.nut.2012.10.011. Epub 2013 Jan 30 PMID: 23375525.
86. Dietz WH, Scanlon KS. Eliminating the use of partially hydrogenated oil in food production and preparation. *JAMA*. 2012;308(2):143–144. https://doi.org/10.1001/jama.2012.7379. PMID: 22782414.
87. Kontogianni MD, Melistas L, Yannakoulia M, et al. Association between dietary patterns and indices of bone mass in a sample of Mediterranean women. *Nutrition*. 2009;25(2):165–171. https://doi.org/10.1016/j.nut.2008.07.019. Epub 2008 Oct 11 PMID: 18849146.
88. Fasano A. *Linus Pauling Award Acceptance Speech*. Houston, TX: IFM Symposium; 2012.
89. El Asmar R, Panigrahi P, Bamford P, et al. Host-dependent activation of the zonulin system is involved in the impairment of the gut barrier function following bacterial colonization. *Gastroenterology*. 2002;123(5):1607–1615. PMID: 12404235.
90. Kharrazian D. *Why Isn't My Brain Working? Elephant Press*; 2013:128. Carlsbad, CA.
91. Yuan Z, Liu D, Citations: Zhang L, et al. Mitotic illegitimate recombination is a mechanism for novel changes in high-molecular-weight glutenin subunits in wheat-rye hybrids. *PLoS One*. 2011;6(8):e23511. https://doi.org/10.1371/journal.pone.0023511. Epub 2011 Aug 22 PMID: 21887262.
92. Molnár-Láng M, Kruppa K, Cseh A, et al. Identification and phenotypic description of new wheat: six-rowed winter barley disomic additions. *Genome*. 2012;55(4):302–311. https://doi.org/10.1139/G2012-013. Epub 2012 Mar 23 PMID: 22439846.
93. Leduc V, Moneret-Vautrin DA, Guerin L, et al. Anaphylaxis to wheat isolates: immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. *J Allergy Clin Immunol*. 2003;111(4):897–899. PMID: 12704375.
94. Battais F, Richard C, Jacquenet S, et al. Wheat grain allergies: an update on wheat allergens. *Eur Ann Allergy Clin Immunol*. 2008;40(3):67–76. PMID: 19334370.
95. Denery-Papini S, Bodinier M, Larré C, et al. Allergy to deamidated gluten in patients tolerant to wheat: specific epitopes linked to deamidation. *Allergy*. 2012;67(8):1023–1032. https://doi.org/10.1111/j.1398-9995.2012.02860.x. Epub 2012 Jun 28 PMID: 22737987.
96. Sapone A, Lammers KM, Fasano A, et al. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med*. 2011;9:23. https://doi.org/10.1186/1741-7015-9-23. PMID: 21392369.
97. Sapone A, Bai JC, Fasano A, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med*. 2012;10:13. https://doi.org/10.1186/1741-7015-10-13. Review PMID: 22313950.
98. Catassi C, Sapone A, Fasano A, et al. Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders. *Nutrients*. 2013;5(10):3839–3853. https://doi.org/10.3390/nu5103839. PMID: 24077239.
99. Elkan AC, Sjöberg B, Kolsrud B, et al. Gluten-free vegan diet induces decreased LDL and oxidized LDL levels and raised atheroprotective natural antibodies against phosphorylcholine in patients with rheumatoid arthritis: a randomized study. *Arthritis Res Ther*. 2008;10(2):R34. https://doi.org/10.1186/ar2388. Epub 2008 Mar 18 PMID: 18348715.
100. Hafström I, Ringertz B, Spångberg A, et al. A vegan diet free of gluten improves the signs and symptoms of rheumatoid arthritis: the effects on arthritis correlate with a reduction in antibodies to food antigens. *Rheumatology (Oxford)*. 2001;40(10):1175–1179. PMID: 11600749.
101. Mazziotti G, Canalis E, Giustina A. Drug-induced osteoporosis: mechanisms and clinical implications. *Am J Med*. 2010;123(10):877–884. https://doi.org/10.1016/j.amjmed.2010.02.028. Review PMID: 20920685.
102. Ziaran S, Goncalves FM, Sn JB. Complex metabolic and skeletal changes in men taking long-term androgen deprivation therapy. *Clin Genitourin Cancer*. 2013;11(1):33–38. https://doi.org/10.1016/j.clgc.2012.08.005. Epub 2012 Sep 19 PMID: 23000203.
103. Beerhorst K, van der Kruijs SJ, Verschuure P, et al. Bone disease during chronic antiepileptic drug therapy: general versus specific risk factors. *J Neurol Sci*. 2013;331(1–2):19–25. https://doi.org/10.1016/j.jns.2013.05.005. Epub 2013 May 21 PMID: 23706474.
104. Luz Rentero M, Carbonell C, Casillas M, et al. Risk factors for osteoporosis and fractures in postmenopausal women between 50 and 65 years of age in a primary care setting in Spain: a questionnaire. *Open Rheumatol J*. 2008;2:58–63. PMID: 19088873.
105. Bolton JM, Targownik LE, Leung S, et al. Risk of low bone mineral density associated with psychotropic medications and mental disorders in postmenopausal women. *J Clin Psychopharmacol*. 2011;31:56–60. PMID: 21192144.
106. Bolton JM, Metge C, Lix L, et al. Fracture risk from psychotropic medications: a population-based analysis. *J Clin Psychopharmacol*. 2008;28(4):384–391. PMID: 18626264.
107. Damsa C, Bumb A, Bianchi-Demicheli F, et al. "Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. *J Clin Psychiatry*. 2004;65(8):1064–1068.
108. Koulu M, Scheinin M, Kaarttinen A, et al. Inhibition of monoamine oxidase by moclobemide: effects on monoamine metabolism and secretion of anterior pituitary hormones and cortisol in healthy volunteers. *Br J Clin Pharmacol*. 1989;27(2):243–255. PMID: 2469451.
109. Bushe C, Shaw M. Prevalence of hyperprolactinaemia in a naturalistic cohort of schizophrenia and bipolar outpatients during treatment with typical and atypical antipsychotics. *J Psychopharmacol*. 2007;21(7):768–773. Epub 2007 Jul 2 PMID: 17606473.
110. Antonopoulou M, Bahtiyar G, Banerji MA, et al. Diabetes and bone health. *Maturitas*. 2013;76(3):253–259. https://doi.org/10.1016/j.maturitas.2013.04.004. Epub 2013 Apr 28 PMID: 23628280.
111. Daniel HW. Opioid endocrinopathy in women consuming prescribed sustained-action opioids for control of nonmalignant pain. *J Pain*. 2008;9(1):28–36. Epub 2007 Nov 1 PMID: 17936076.
112. Smith HS, Elliott JA. Opioid-induced androgen deficiency (OPIAD). *Pain Physician*. 2012;15(suppl 3):ES145–E156. PMID: 22786453.
113. Seibel MJ, Cooper MS, Zhou H. Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives. *Lancet Diabetes Endocrinol*. 2013;1(1):59–70. https://doi.org/10.1016/S2213-8587(13)70045-7. Epub 2013 Jun 28 PMID: 24622268.
114. Davidge Pitts CJ, Kearns AE. Update on medications with adverse skeletal effects. *Mayo Clin Proc*. 2011;86(4):338–343; quiz 343. https://doi.org/10.4065/mcp.2010.0636. Epub 2011 Mar 9 PMID: 21389249.

115. Corley DA, Kubo A, Zhao W, et al. Proton pump inhibitors and histamine-2 receptor antagonists are associated with hip fractures among at-risk patients. *Gastroenterology*. 2010;139(1):93–101. https://doi.org/10.1053/j.gastro.2010.03.055. Epub 2010 Mar 27 PMID: 20353792.
116. Eom CS, Park SM, Myung SK, et al. Use of acid-suppressive drugs and risk of fracture: a meta-analysis of observational studies. *Ann Fam Med*. 2011;9(3):257–267. https://doi.org/10.1370/afm. 1243 PMID: 21555754.
117. Kwok CS, Yeong JK, Loke YK. Meta-analysis: risk of fractures with acid-suppressing medication. *Bone*. 2011;48(4):768–776. https://doi.org/10.1016/j.bone.2010.12.015. Epub 2010 Dec 23 PMID: 21185417.
118. Vestergaard P, Rejnmark L, Mosekilde L. Proton pump inhibitors, histamine H2 receptor antagonists, and other antacid medications and the risk of fracture. *Calcif Tissue Int*. 2006;79(2):76–83. Epub 2006 Aug 15 PMID: 16927047.
119. Kann PH, Hadji P, Bergmann RS. Pharmacogenic osteoporosis beyond cortisone : proton pump inhibitors, glitazones and diuretics. [Article in German] *Z Rheumatol*. 2014. Epub ahead of print PMID: 24728601.
120. Ilić K, Obradović N, Vujasinović-Stupar N. The relationship among hypertension, antihypertensive medications, and osteoporosis: a narrative review. *Calcif Tissue Int*. 2013;92(3):217–227. https://doi.org/10.1007/s00223-012-9671-9. Epub 2012 Nov 29 PMID: 23192372.
121. Ghosh M, Majumdar SR. Antihypertensive medications, bone mineral density, and fractures: a review of old cardiac drugs that provides new insights into osteoporosis. *Endocrine*. 2014. Epub ahead of print PMID: 24504763.
122. Arampatzis S, Gaetcke LM, Funk GC, et al. Diuretic-induced hyponatremia and osteoporotic fractures in patients admitted to the emergency department. *Maturitas*. 2013;75(1):81–86. https://doi.org/10.1016/j.maturitas.2013.02.007. Epub 2013 Mar 13. PMID: 23489552.
123. Meng Y, Zhang H, Li Y, et al. Effects of unfractionated heparin on renal osteodystrophy and vascular calcification in chronic kidney disease rats. *Bone*. 2014;58:168–176. https://doi.org/10.1016/j.bone.2013.10.010. Epub 2013 Oct 18 PMID: 24145307.
124. Lefkou E, Khamashta M, Hampson G, et al. Review: low-molecular-weight heparin-induced osteoporosis and osteoporotic fractures: a myth or an existing entity? *Lupus*. 2010;19(1):3–12. https://doi.org/10.1177/0961203309353171. Epub 2009 Nov 24 PMID: 19934178.
125. Ma R, Morshed S, Latif R, et al. The influence of thyroid-stimulating hormone and thyroid-stimulating hormone receptor antibodies on osteoclastogenesis. *Thyroid*. 2011;21(8):897–906. https://doi.org/10.1089/thy.2010.0457. Epub 2011 Jul 11 PMID: 21745106.
126. Christy AL, D'Souza V, Babu RP, et al. Utility of c-terminal telopeptide in evaluating levothyroxine replacement therapy-induced bone loss. *Biomark Insights*. 2014;9:1–6. https://doi.org/10.4137/BMI.S13965. eCollection 2014 PMID: 24634578.
127. Lussana F, Zighetti ML, Bucciarelli P, et al. Blood levels of homocysteine, folate, vitamin B6 and B12 in women using oral contraceptives compared to non-users. *Thromb Res*. 2003;112(1–2):37–41. PMID: 15013271.
128. Herrmann W, Kirsch SH, Kruse V, et al. One year B and D vitamins supplementation improves metabolic bone markers. *Clin Chem Lab Med*. 2013;51(3):639–647. https://doi.org/10.1515/cclm-2012-0599. PMID: 23183751.
129. Cauley JA, Robbins J, Chen Z, et al. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. *JAMA*. 2003;290(13):1729–1738. PMID: 14519707.
130. Sitruk-Ware R. New progestogens: a review of their effects in perimenopausal and postmenopausal women. *Drugs Aging*. 2004;21(13):865–883. PMID: 15493951.
131. Freeman S, Shulman LP. Considerations for the use of progestin-only contraceptives. *J Am Acad Nurse Pract*. 2010;22(2):81–91. PMID: 20132366.
132. Quinkler M, Kaur K, Hewison M, et al. Progesterone is extensively metabolized in osteoblasts: implications for progesterone action on bone. *Horm Metab Res*. 2008;40(10):679–684. Epub 2008 Jun 6 PMID: 18537080.
133. Liang M, Liao EY, Xu X, et al. Effects of progesterone and 18-methyl levonorgestrel on osteoblastic cells. *Endocr Res*. 2003;29(4):483–501. PMID: 14682477.
134. Kaunitz AM, Arias R, McClung M. Bone density recovery after depot medroxyprogesterone acetate injectable contraception use. *Contraception*. 2008;77(2):67–76. PMID: 18226668.
135. Isley MM, Kaunitz AM. Update on hormonal contraception and bone density. *Rev Endocr Metab Disord*. 2011;12(2):93–106.
136. Seifert-Klauss V, Schmidmayr M, Hobmaier E, Wimmer T. Progesterone and bone: a closer link than previously realized. *Climacteric*. 2012;15(suppl 1):26–31.
137. Prior JC, Kirkland SA, Joseph L, et al. Oral contraceptive use and bone mineral density in premenopausal women: cross-sectional, population-based data from the Canadian Multicentre Osteoporosis Study. *CMAJ*. 2001;165(8):1023–1029. PMID: 11699697.
138. Miller PD. *Endocrinol Metab Clin North Am*. 2012;41(3):613–628. https://doi.org/10.1016/j.ecl.2012.05.005. Epub 2012 Jul 12. Review. Unrecognized and unappreciated secondary causes of osteoporosis. PMID: 22877432.
139. Bahamondes MV, Monteiro I, Castro S, Espejo-Arce X, Bahamondes L. Prospective study of the forearm bone mineral density of long- term users of the levonorgestrel-releasing intrauterine system. *Hum Reprod*. 2010;25(5):1158–1164. PMID: 20185512.
140. Rosin A. The long-term consequences of exposure to lead. *Isr Med Assoc J*. 2009;11(11):689–694. PMID: 20108558.
141. Beier EE, Maher JR, Sheu TJ, et al. Heavy metal lead exposure, osteoporotic-like phenotype in an animal model, and depression of Wnt signaling. *Environ Health Perspect*. 2013;121(1):97–104. https://doi.org/10.1289/ehp.1205374. Epub 2012 Oct 19 PMID: 23086611.
142. Chang WJ, Joe KT, Park HY. Jeong JD1, Lee DH.The relationship of liver function tests to mixed exposure to lead and organic solvents. *Ann Occup Environ Med*. 2013;25(1):5. https://doi.org/10.1186/2052-4374-25-5. PMID: 24472152.
143. Kessler R. Lead-based decorative paints: where are they still sold—and why? *Environ Health Perspect*; DOI:10.1289/ehp.122–A96, http://ehp.niehs.nih.gov/122-a96/#r52. Accessed 2 April 2014.
144. Trasande L, Liu Y. Reducing the staggering costs of environmental disease in children, estimated at $76.6 billion in 2008. *Health Aff*. 2011;30(5):863–870. https://doi.org/10.1377/hlthaff.2010.1239.
145. Khalil N, Cauley JA, Wilson JW, et al. Relationship of blood lead levels to incident nonspine fractures and falls in older women: the study of osteoporotic fractures. *J Bone Miner Res*. 2008;23(9):1417–1425. https://doi.org/10.1359/jbmr.080404. PMID: 18410230.
146. Mumford SL, Dasharathy SS, Pollack AZ, et al. Serum uric acid in relation to endogenous reproductive hormones during the menstrual cycle: findings from the BioCycle study. *Hum Reprod*. 2013;28(7):1853–1862. https://doi.org/10.1093/humrep/det085. Epub 2013 Apr 5 PMID: 23562957.
147. Lodwick CJ, Lodwick JC, Spitz HB. The influence of bone-density on in vivo K x-ray fluorescence bone-lead measurements. *Health Phys*. 2011;100(5):502–507. https://doi.org/10.1097/HP.0b013e3181f725af. PMID: 21451320.
148. *TOXICOLOGICAL PROFILE FOR LEAD X-ray fluorescence techniques (XRF) can be used for the determination of lead concentration in bones.* www.atsdr.cdc.gov/toxprofiles/tp13-c2.pdf. Accessed 5 February 2020.
149. Jin T, Nordberg G, Ye T, et al. Osteoporosis and renal dysfunction in a general population exposed to cadmium in China. *Environ Res*. 2004;96(3):353–359. PMID: 15364604.
150. Kazantzis G. Cadmium, osteoporosis and calcium metabolism. *Biometals*. 2004;17(5):493–498. PMID: 15688852.
151. 1910.1027 App A - substance safety data sheet - cadmium. United States Department of Labor: Occupational Safety and Health Administration. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10036. Accessed 5 February 2020.
152. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. *Cien Saude Colet*. 2011;16(5):2587–2602. PMID: 21655733 Republished from Environ health Perspect. 2010 Feb;118(2):182-90.
153. Julin B, Wolk A, Johansson JE, et al. Dietary cadmium exposure and prostate cancer incidence: a population-based prospective cohort study. *Br J Cancer*. 2012;107(5):895–900. https://doi.org/10.1038/bjc.2012.311. Epub 2012 Jul 31. PMID: 22850555.

154. Gallagher CM, Kovach JS, Meliker JR. Urinary cadmium and osteoporosis in U.S. Women >or= 50 years of age: NHANES 1988-1994 and 1999-2004. *Environ Health Perspect*. 2008;116(10):1338–1343. https://doi.org/10.1289/ehp.11452. Epub 2008 Jun 13 PMID: 18941575.
155. Engström A, Michaëlsson K, Vahter M, et al. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone*. 2012;50(6):1372–1378. https://doi.org/10.1016/j.bone.2012.03.018. Epub 2012 Mar 24 PMID: 22465267.
156. Park JD, Zheng W. Human exposure and health effects of inorganic and elemental mercury. *J Prev Med Public Health*. 2012;45(6):344–352. https://doi.org/10.3961/jpmph.2012.45.6.344. Epub 2012 Nov 29 PMID: 23230464.
157. Mutter J, Naumann J, Schneider R, et al. Mercury and autism: accelerating evidence? *Neuro Endocrinol Lett*. 2005;26(5):439–446. PMID: 16264412.
158. Mutter J, Curth A, Naumann J, et al. Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism. *J Alzheimers Dis*. 2010;22(2):357–374. https://doi.org/10.3233/JAD-2010-100705. PMID: 20847438.
159. Pizzorno J, The Path Ahead: Clinical Experience in Decreasing Mercury Load, August, 2011-VOL. 10, NO. 4 http://www.imjournal.com/index.cfm/fuseaction/archives.display&action=download&id=8802. Accessed 12 April 2014. (however, IMCJ requires subscription for access, so link may not work)
160. Jha SK, Mishra VK, Sharma DK, et al. Fluoride in the environment and its metabolism in humans. *Rev Environ Contam Toxicol*. 2011;211:121–142. PMID: 21287392.
161. Everett ET. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. *J Dent Res*. 2011;90(5):552–560. Epub 2010 Oct 6 PMID: 20929720.
162. Whitford GM. Intake and metabolism of fluoride. *Adv Dent Res*. 1994;8(1):5–14. PMID: 7993560.
163. Bergandi L, Aina V, Malavasi G, et al. The toxic effect of fluoride on MG-63 osteoblast cells is also dependent on the production of nitric oxide. *Chem Biol Interact*. 2011;190(2–3):179–186. Epub 2011 Feb 15 PMID: 21329685.
164. Bergandi L, Aina V, Garetto S, et al. Fluoride-containing bioactive glasses inhibit pentose phosphate oxidative pathway and glucose 6-phosphate dehydrogenase activity in human osteoblasts. *Chem Biol Interact*. 2010;183(3):405–415. Epub 2009 Nov 27 PMID: 19945446.
165. Song YE, Tan H, Liu KJ, et al. Effect of fluoride exposure on bone metabolism indicators ALP, BALP, and BGP. *Environ Health Prev Med*. 2011;16(3):158–163. Epub 2010 Oct 2 PMID: 21431799.
166. DePaula CA, Pan Y, Guzelsu N. Uniform partial dissolution of bone mineral by using fluoride and phosphate ions combination. *Connect Tissue Res*. 2008;49(5):328–342. PMID: 18991086.
167. Demur C, Métais B, Canlet C, et al. Dietary exposure to a low dose of pesticides alone or as a mixture: the biological metabolic fingerprint and impact on hematopoiesis. *Toxicology*. 2013;308:74–87. https://doi.org/10.1016/j.tox.2013.03.004. Epub 2013 Mar 22 PMID: 23528616.
168. Montagnani A, Gonnelli S. Antidiabetic therapy effects on bone metabolism and fracture risk. *Diabetes Obes Metab*. 2013;15(9):784–791. https://doi.org/10.1111/dom.12077. Epub 2013 Feb 24 PMID: 23368527.
169. Montagnani A, Gonnelli S, Alessandri M, et al. Osteoporosis and risk of fracture in patients with diabetes: an update. *Aging Clin Exp Res*. 2011;23(2):84–90. PMID: 21743287.
170. Schwartz AV, Sellmeyer DE. Diabetes, fracture, and bone fragility. *Curr Osteoporos Rep*. 2007;5(3):105–111. PMID: 17925191.
171. Yamamoto M. Secondary osteoporosis or secondary contributors to bone loss in fracture. Bone metabolic disorders in patients with diabetes mellitus. Article in Japanese *Clin Calcium*. 2013;23(9):1327–1335. CliCa130913271335 PMID: 23999370.
172. Varenna M, Manara M, Galli L, et al. The association between osteoporosis and hypertension: the role of a low dairy intake. *Calcif Tissue Int*. 2013;93(1):86–92. https://doi.org/10.1007/s00223-013-9731-9. Epub 2013 May 8 PMID: 23652773.
173. Fehérvári M, Sarkadi H, Krepuska M, et al. Bone mineral density is associated with site-specific atherosclerosis in patients with severe peripheral artery disease. *Calcif Tissue Int*. 2013;93(1):55–61. https://doi.org/10.1007/s00223-013-9727-5. Epub 2013 Apr 6 PMID: 23564349.
174. Fehérvári M, Krepuska M, Csobay-Novák C, et al. Prevalence of osteoporosis in patients with severe peripheral artery disease. *Orv Hetil*. 2013;154(10):369–375. https://doi.org/10.1556/OH. Article in Hungarian, 2013.29532 PMID: 23461977.
175. Palacios S, Neyro JL, Puertas JC, et al. Clinical profile of Spanish postmenopausal women with a diagnosis of osteoporosis and risk factors for endometrial pathology, breast cancer, and cardiovascular disease. *Menopause*. 2013;20(8):852–859. https://doi.org/10.1097/GME.0b013e318280a2f6. PMID: 23481116.
176. Tankó LB, Christiansen C, Cox DA, et al. Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res*. 2005;20(11):1912–1920. Epub 2005 Jul 18 PMID: 16234963.
177. Maurel DB, Boisseau N, Benhamou CL, et al. Alcohol and bone: review of dose effects and mechanisms. *Osteoporos Int*. 2012;23(1):1–16. https://doi.org/10.1007/s00198-011-1787-7. Epub 2011 Sep 17 PMID: 21927919.
178. Warriner AH, Saag KG. Osteoporosis diagnosis and medical treatment. *Orthop Clin North Am*. 2013;44(2):125–135. https://doi.org/10.1016/j.ocl.2013.01.005. Epub 2013 Feb 26 PMID: 23544819.
179. Donnelly E, Saleh A, Unnanuntana A, et al. Atypical femoral fractures: epidemiology, etiology, and patient management. *Curr Opin Support Palliat Care*. 2012;6(3):348–354. https://doi.org/10.1097/SPC.0b013e3283552d7d. PMID: 22643705.
180. Ashcraft & Gerel, LLP. (2020). Our Practice Areas | Ashcraft & Gerel. [online] Available at: https://www.ashcraftandgerel.com/practice-areas/dangerioupharmaceuticals-and-medical-devices/fosamax-zometa-and-aredia. Accessed 16 December 2013.
181. Iglesias JE, Salum FG, Figueiredo MA, et al. Important aspects concerning alendronate-related osteonecrosis of the jaws: a literature review. *Gerodontology*. 2013. https://doi.org/10.1111/ger.12093. Epub ahead of print PMID: 24256517.
182. Filleul O, Crompot E, Saussez S. Bisphosphonate-induced osteonecrosis of the jaw: a review of 2,400 patient cases. *J Cancer Res Clin Oncol*. 2010;136(8):1117–1124. https://doi.org/10.1007/s00432-010-0907-7. Epub 2010 May 28 PMID: 20508948.
183. Akintoye SO, Hersh EV. Risks for jaw osteonecrosis drastically increases after 2 years of bisphosphonate therapy. *J Evid Based Dent Pract*. 2012;12(suppl 3):251–253. https://doi.org/10.1016/S1532-3382(12)70048-9. PMID: 23253854.
184. Saad F, Brown JE, Van Poznak C, et al. Incidence, risk factors, and outcomes of osteonecrosis of the jaw: integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. *Ann Oncol*. 2012;23(5):1341–1347. https://doi.org/10.1093/annonc/mdr435. Epub 2011 Oct 10 PMID: 21986094.
185. Mak A, Cheung MW, Ho RC, et al. Bisphosphonates and atrial fibrillation: Bayesian meta-analyses of randomized controlled trials and observational studies. *BMC Musculoskelet Disord*. 2009;10:113. https://doi.org/10.1186/1471-2474-10-113. PMID: 19772579.
186. Howard PA, Barnes BJ, Vacek JL, et al. Impact of bisphosphonates on the risk of atrial fibrillation. *Am J Cardiovasc Drugs*. 2010;10(6):359–367. https://doi.org/10.2165/11584720-000000000-00000. PMID: 21090829.
187. [No authors listed] Bisphosphonates and atrial fibrillation. Clinical trial data suggest possible link. *Prescrire Int*. 2011;20(115):96–97. PMID: 21648212.
188. Cummings SR, Schwartz AV, Black DM. Alendronate and atrial fibrillation. *N Engl J Med*. 2007;356(18):1895–1896. PMID: 17476024.
189. Black DM, Delmas PD, Eastell R, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N Engl J Med*. 2007;356:1809–1822. PMID: 17476007.
190. Woo C, Gao G, Wade S, et al. Gastrointestinal side effects in postmenopausal women using osteoporosis therapy: 1-year findings in the POSSIBLE US study. *Curr Med Res Opin*. 2010;26(4):1003–1009. https://doi.org/10.1185/03007991003633603. PMID: 20201623.
191. Green J, Czanner G, Reeves G, et al. Oral bisphosphonates and risk of cancer of oesophagus, stomach, and colorectum: case-control analysis within a UK primary care cohort. *BMJ*. 2010;341:c4444. https://doi.org/10.1136/bmj.c4444. PMID: 20813820.

192. Body JJ. Dosing regimens and main adverse events of bisphosphonates. *Semin Oncol*. 2001;28(4 suppl 11):49–53. PMID: 11544576.
193. [No authors listed] Ibandronic acid. New drug. Osteoporosis: one tablet a month, but risk of a 'flu-like' syndrome. *Prescrire Int*. 2008;17(93):11. PMID: 18354857.
194. Olson K, Van Poznak C. Significance and impact of bisphosphonate-induced acute phase responses. *J Oncol Pharm Pract*. 2007;13(4):223–229. PMID: 18045781.
195. Medsafe, New Zealand, Fosamax Data Sheet, http://www.medsafe.govt.nz/profs/Datasheet/f/Fosamaxtab.pdf. Accessed 20 December 2013.
196. Diel IJ, Bergner R, Grötz KA. Adverse effects of bisphosphonates: current issues. *J Support Oncol*. 2007;5(10):475–482. PMID: 18240669.
197. Hirschberg R. Renal complications from bisphosphonate treatment. *Curr Opin Support Palliat Care*. 2012;6(3):342–347. https://doi.org/10.1097/SPC.0b013e328356062e. PMID: 22710581.
198. Diab DL, Watts NB. Denosumab in osteoporosis. *Expert Opin Drug Saf*. 2013. Epub ahead of print.
199. Tsourdi E, Rachner TD, Gruber M, et al. Seizures associated with zoledronic acid for osteoporosis. *J Clin Endocrinol Metab*. 2011;96(7):1955–1959. https://doi.org/10.1210/jc.2011-0418. Epub 2011 Apr 20 PMID: 21508139.
200. Ho JW. Bisphosphonate stimulation of osteoblasts and osteoblastic metastasis as a mechanism of hypocalcaemia. *Med Hypotheses*. 2012;78(3):377–379. https://doi.org/10.1016/j.mehy.2011.12.002. Epub 2011 Dec 27 PMID: 22206806.
201. Etminan M, Forooghian F, Maberley D. Inflammatory ocular adverse events with the use of oral bisphosphonates: a retrospective cohort study. *CMAJ*. 2012;184(8):E431–E434. https://doi.org/10.1503/cmaj.111752. Epub 2012 Apr 2 PMID: 22470169.
202. McKague M, Jorgenson D, Buxton KA. Ocular side effects of bisphosphonates: a case report and literature review. *Can Fam Physician*. 2010;56(10):1015–1017. PMID: 20944044.
203. Moorthy RS, London NJ, Garg SJ, et al. Drug-induced uveitis. *Curr Opin Ophthalmol*. 2013;24(6):589–597. https://doi.org/10.1097/01.icu.0000434534.32063.5c. PMID: 24100371.
204. Vestergaard P, Schwartz K, Rejnmark L, et al. Oral bisphosphonate use increases the risk for inflammatory jaw disease: a cohort study. *J Oral Maxillofac Surg*. 2012;70(4):821–829. https://doi.org/10.1016/j.joms.2011.02.093. Epub 2011 Jul 20 PMID: 21764202.
205. Reid IR, Cornish J. Epidemiology and pathogenesis of osteonecrosis of the jaw. *Nature Reviews Rheumatology*. 2012;8(2012):90–96. https://doi.org/10.1038/nrrheum.2011.181. http://www.nature.com/nrrheum/journal/v8/n2/full/nrrheum.2011.181.html. Accessed 5 February 2020.
206. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med*. 2006;354(8):821–831. PMID: 16495394.
207. Bridgeman MB, Pathak R. Denosumab for the reduction of bone loss in postmenopausal osteoporosis: a review. *Clin Ther*. 2011;33(11):1547–1559. https://doi.org/10.1016/j.clinthera.2011.10.008. PMID: 22108301.
208. Watts NB, Roux C, Modlin JF, et al. Infections in postmenopausal women with osteoporosis treated with denosumab or placebo: coincidence or causal association? *Osteoporos Int*. 2012;23(1):327–337. https://doi.org/10.1007/s00198-011-1755-2. Epub 2011 Sep 3 PMID: 21892677.
209. Curtis JR, Westfall AO, Cheng H, et al. Benefit of adherence with bisphosphonates depends on age and fracture type: results from an analysis of 101,038 new bisphosphonate users. *J Bone Miner Res*. 2008;23(9):1435–1441. https://doi.org/10.1359/jbmr.080418. PMID: 18442318.
210. Eriksen EF. Treatment of osteopenia. *Rev Endocr Metab Disord*. 2012;13(3):209–223. https://doi.org/10.1007/s11154-011-9187-z. PMID: 21710179.
211. Reginster JY. Antifracture efficacy of currently available therapies for postmenopausal osteoporosis. *Drugs*. 2011;71(1):65–78. https://doi.org/10.2165/11587570-000000000-00000. PMID: 21175240.
213. Bunyaratavej N. Experience of application: blood screening and bone turnover markers for prevention of unwanted effects and early outcomes of teriparatide. *J Med Assoc Thai*. 2011;94(suppl 5):S31–S34. PMID: 22338923.
214. Italian Society of Osteoporosis. Mineral Metabolism and Skeletal Diseases (SIOMMMS); Italian Society of Rheumatology (SIR). Safety profile of drugs used in the treatment of osteoporosis: a systematical review of the literature. *Reumatismo*. 2013;65(4):143–166. https://doi.org/10.4081/reumatismo.2013.143. PMID: 24192561.
215. Kakaria PJ, Nashel DJ, Nylen ES. Debilitating muscle cramps after teriparatide therapy. *Ann Intern Med*. 2005;142(4):310. PMID: 15710971.
216. Luigetti M, Capone F, Monforte M, et al. Muscle cramps and weakness after teriparatide therapy: a new drug-induced myopathy? *Muscle Nerve*. 2013;47(4):615. https://doi.org/10.1002/mus.23661. Epub 2013 Jan 16. PMID: 23322619.
217. Morelli V, Masserini B, Salcuni AS, et al. Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects. *Clin Endocrinol (Oxf)*. 2010;73(2):161–166. https://doi.org/10.1111/j.1365-2265.2010.03794.x. Epub 2010 Feb 23 PMID: 20184600.
218. De Leo M, Cozzolino A, Colao A, et al. Subclinical Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab*. 2012;26(4):497–505. https://doi.org/10.1016/j.beem.2012.02.001. Epub 2012 May 22 PMID: 22863391.
219. Marcus R. Present at the beginning: a personal reminiscence on the history of teriparatide. *Osteoporos Int*. 2011;22(8):2241–2248. https://doi.org/10.1007/s00198-011-1598-x. Epub 2011 Mar 31 PMID: 21452026.
220. Subbiah V, Madsen VS, Raymond AK, et al. Of mice and men: divergent risks of teriparatide-induced osteosarcoma. *Osteoporos Int*. 2010;21(6):1041–1045. https://doi.org/10.1007/s00198-009-1004-0. Epub 2009 Jul 14 PMID: 19597911.
221. Tastekin N, Zateri C. Probable osteosarcoma risk after prolonged teriparatide treatment: comment on the article by Saag et al. *Arthritis Rheum*. 2010; 62(6):1837; author reply 1837–1838. https://doi.org/10.1002/art.27427. PMID: PMID: 20191586.
222. Björkman MP, Sorva AJ, Tilvis RS. Does elevated parathyroid hormone concentration predict cognitive decline in older people? *Aging Clin Exp Res*. 2010;22(2):164–169. https://doi.org/10.3275/6626. Epub 2009 Nov 17 PMID: 19934619.
223. Braverman ER, Chen TJ, Chen AL, et al. Age-related increases in parathyroid hormone may be antecedent to both osteoporosis and dementia. *BMC Endocr Disord*. 2009;9:21. https://doi.org/10.1186/1472-6823-9-21. PMID: 19825157.
224. Fernández-Soto ML, González-Jiménez A, Chamorro-Fernández M, et al. Clinical and hormonal variables related to bone mass loss in anorexia nervosa patients. *Vitam Horm*. 2013;92:259–269. https://doi.org/10.1016/B978-0-12-410473-0.00010-6. PMID: 23601428.
225. Lawson EA, Donoho D, Miller KK, et al. Hypercortisolemia is associated with severity of bone loss and depression in hypothalamic amenorrhea and anorexia nervosa. *J Clin Endocrinol Metab*. 2009;94(12):4710–4716. https://doi.org/10.1210/jc.2009-1046. Epub 2009 Oct 16 PMID: 19837921.
226. Yu EW, Neer RM, Lee H, et al. Time-dependent changes in skeletal response to teriparatide: escalating vs. constant dose teriparatide (PTH 1-34) in osteoporotic women. *Bone*. 2011;48(4):713–719. https://doi.org/10.1016/j.bone.2010.11.012. Epub 2010 Nov 24 PMID: 21111078.
227. Uihlein AV, Leder BZ. Anabolic therapies for osteoporosis. *Endocrinol Metab Clin North Am*. 2012;41(3):507–525. https://doi.org/10.1016/j.ecl.2012.05.002. Epub 2012 Jun 20 PMID: 22877427.
228. Hoy MK, Goldman JD. *Calcium Intake of the U.S. Population / What We Eat in America*, NHANES 2009-2010, USDA; http://www.ars.usda.gov/SP2UserFiles/Place/80400530/pdf/DBrief/13_calcium_intake_0910.pdf. Accessed 10 December 2015.
229. Data from Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press; . 2010. Reviewed August, 2011.
230. Nordin BE. The effect of calcium supplementation on bone loss in 32 controlled trials in postmenopausal women. *Osteoporos Int*. 2009;20(12):2135–2143. https://doi.org/10.1007/s00198-009-0926-x. Epub 2009 May 21 PMID: 19459026.
231. Weaver CM. Age related calcium requirements due to changes in absorption and utilization. *J Nutr*. 1994;124(suppl 8):1418S–1425S. PMID: 8064395.
232. Heaney RP, Recker RR, Stegman MR, et al. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. *J Bone Miner Res*. 1989;4(4):469–475. PMID: 2816496.

233. Heaney RP, Gallagher JC, Johnston CC, et al. Calcium nutrition and bone health in the elderly. *Am J Clin Nutr*. 1982;36(suppl 5):986–1013. PMID: 6765074.
234. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911–1930. https://doi.org/10.1210/jc.2011-0385. Epub 2011 Jun 6 PMID: 21646368.
235. Pizzorno, L. *Vitamin K, Longevity Medicine Review,* 2009, http://www.lmreview.com/articles/view/vitamin-k/. Accessed 5 February 2020.
236. Pizzorno, L. Vitamin K2, but not vitamin K1, is helpful for bone density. *Longevity Medicine Review* 2009, http://www.lmreview.com/articles/view/vitamin-k2-but-not-vitamin-k1-is-helpfulfor-bone-density/. Accessed 5 February 2020.
237. Pizzorno, L. *Vitamin D and Vitamin K team up to lower CVD risk: Part I*, Longevity Medicine Review 2009, http://www.lmreview.com/articles/view/vitamin-d-and-vitamin-k-team-up-to-lower-cvdrisk-part-1/. Accessed 5 February 2020.
238. Pizzorno, L. *Vitamin D and Vitamin K team up to lower CVD risk: Part 2*, Longevity Medicine Review 2009, http://www.lmreview.com/articles/view/vitamin-d-and-vitamin-k-team-up-to-lowercvd-risk-part-2/. Accessed 5 February 2020.
239. Pizzorno L. Vitamin K2: optimal levels essential for the prevention of age-associated chronic disease. *Longevity Medicine Review*. 2011. http://www.lmreview.com/articles/view/Vitamin-K2-Essential-for-Prevention-of-Age-Associated-Chronic-Disease/. Accessed 5 February 2020.
240. Wallace RB, Wactawski-Wende J, O'Sullivan MJ, et al. Urinary tract stone occurrence in the Women's Health Initiative randomized clinical trial of calcium and vitamin D supplements. *Am J Clin Nutr*. 2011. Epub ahead of print PMID: 21525191.
241. Masterjohn C. Vitamin D toxicity redefined: vitamin K and the molecular mechanism. *Med Hypotheses*. 2007;68(5):1026–1034. PMID: 17145139.
242. Adams J, Pepping J. Vitamin K in the treatment and prevention of osteoporosis and arterial calcification. *Am J Health Syst Pharm*. 2005;62(15):1574–1581. PMID: 16030366.
243. Price PA, Faus SA, Williamson MK. Warfarin-induced artery calcification is accelerated by growth and vitamin D. *Arterioscler Thromb Vasc Biol*. 2000;20(2):317–327. PMID: 10669626.
244. Spronk HM, Soute BA, Schurgers LJ, et al. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. *J Vasc Res*. 2003;40(6):531–537. PMID: 14654717.
245. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet*. 2007;370(9588):657–666. PMID: 17720017.
246. Adluri RS, Zhan L, Bagchi M, et al. Comparative effects of a novel plant-based calcium supplement with two common calcium salts on proliferation and mineralization in human osteoblast cells. *Mol Cell Biochem*. 2010;340(1–2):73–80. https://doi.org/10.1007/s11010-010-0402-0. Epub 2010 Mar 7 PMID: 20213262.
247. Michalek JE, Preuss HG, Croft HA, et al. Changes in total body bone mineral density following a common bone health plan with two versions of a unique bone health supplement: a comparative effectiveness research study. *Nutr J*. 2011;10:32. PMID: 21492428.
248. Kaats GR, Preuss HG, Croft HA, et al. A comparative effectiveness study of bone density changes in women over 40 following three bone health plans containing variations of the same novel plant-sourced calcium. *Int J Med Sci*. 2011;8(3):180–191. PMID: 21448303.
249. Kaats GR, et al. A 7-year longitudinal trial of the safety and efficacy of a vitamin/mineral enhanced plant-sourced calcium supplement. *J Am Coll Nutr*. 2016:1–9. PMID: 26885697.
250. Thomas D. The mineral depletion of foods available to us as a nation (1940-2002)—a review of the 6th Edition of McCance and Widdowson. *Nutr Health*. 2007;19(1–2):21–55. PMID: 18309763.
251. Schafer AL, Weaver CM, Black DM, et al. Intestinal calcium absorption decreases dramatically after gastric bypass surgery despite Optimization of vitamin D status. *J Bone Miner Res*. 2015;30(8):1377–1385. https://doi.org/10.1002/jbmr. 2467. Epub 2015 May 21 PMID: 25640580.
252. Straub DA. Calcium supplementation in clinical practice: a review of forms, doses, and indications. *Nutr Clin Pract*. 2007;22(3):286–296. PMID: 17507729.
253. Reinwald S, Weaver CM, Kester JJ. The health benefits of calcium citrate malate: a review of the supporting science. *Adv Food Nutr Res*. 2008;54:219–346. https://doi.org/10.1016/S1043-4526(07)00006-X. PMID: 18291308.
254. Bristow SM, Gamble GD, Stewart A, et al. Acute and 3-month effects of microcrystalline hydroxyapatite, calcium citrate and calcium carbonate on serum calcium and markers of bone turnover: a randomised controlled trial in postmenopausal women. *Br J Nutr*. 2014;112(10):1611–1620. https://doi.org/10.1017/S0007114514002785. Epub 2014 Oct 2 PMID: 25274192.
255. Pizzorno L. Canaries in the phosphate-toxicity coal mines. *Integr Med (Encinitas)*. 2014;13(6):24–32. PMCID: PMC4566440 PMID: 26770122.
256. Ju J, Kwak Y, Hao X, et al. Inhibitory effects of calcium against intestinal cancer in human colon cancer cells and Apc(Min/+) mice. *Nutr Res Pract*. 2012vol. 6(5):396–404. https://doi.org/10.4162/nrp.2012.6.5.396. Epub 2012 Oct 31 PMID: 23198018.
257. Heaney RP, Dowell MS, Barger-Lux MJ. Absorption of calcium as the carbonate and citrate salts, with some observations on method. *Osteoporos Int*. 1999;9(1):19–23. PMID: 10367025.
258. Heaney RP. Factors influencing the measurement of bioavailability, taking calcium as a model. *J Nutr*. 2001; 131(suppl 4):1344S–8S. PMID: PMID: 11285351; Weaver C. "Bioavailability of calcium." NIH consensus development Conference on optimal calcium intake: NIH consensus development Conference, June 6-8, 1994
259. Auditorium M. Warren grant Magnuson clinical center, *National Institutes of Health*. Bethesda: Maryland. The Institutes; 1994.
260. Wang L, Manson JE, Sesso HD. Calcium intake and risk of cardiovascular disease: a review of prospective studies and randomized clinical trials. *Am J Cardiovasc Drugs*. 2012;12(2):105–116. https://doi.org/10.2165/11595400-000000000-00000. PMID: 22283597.
261. Paik JM, Curhan GC, Sun Q, et al. Calcium supplement intake and risk of cardiovascular disease in women. *Osteoporos Int*. 2014;25(8):2047–2056. https://doi.org/10.1007/s00198-014-2732-3. Epub 2014 May 7 PMID: 24803331.
262. Michaëlsson K, Wolk A, Langenskiöld S, et al. Milk intake and risk of mortality and fractures in women and men: cohort studies. *BMJ*. 2014;349:g6015. https://doi.org/10.1136/bmj.g6015. PMID: 25352269.
263. Rude RK, Gruber HE. Magnesium deficiency and osteoporosis: animal and human observations. *J Nutr Biochem*. 2004;15(12):710–716. PMID: 15607643.
264. Castiglioni S, Cazzaniga A, Albisetti W, et al. Magnesium and osteoporosis: current state of knowledge and future research directions. *Nutrients*. 2013;5(8):3022–3033. https://doi.org/10.3390/nu5083022. PMID: 23912329.
265. Kanazawa I, Yamamoto M, Yamaguchi T, et al. A case of magnesium deficiency associated with insufficient parathyroid hormone action and severe osteoporosis. *Endocr J*. 2007;54(6):935–940. Epub 2007 Nov 30 PMID: 18048993.
266. Hoorn EJ, Zietse R. Disorders of calcium and magnesium balance: a physiology-based approach. *Pediatr Nephrol*. 2013;28(8):1195–1206. https://doi.org/10.1007/s00467-012-2350-2. Epub 2012 Nov 10 PMID: 23142866.
267. Zofková I, Nemcikova P, Matucha P. Trace elements and bone health. *Clin Chem Lab Med*. 2013;51(8):1555–1561. https://doi.org/10.1515/cclm-2012-0868. PMID: 23509220.
268. Rosanoff A, Weaver CM, Rude RK. Suboptimal magnesium status in the United States: are the health consequences underestimated? *Nutr Rev*. 2012;70(3):153–164. https://doi.org/10.1111/j.1753-4887.2011.00465.x. Epub 2012 Feb 15 PMID: 22364157.
269. Hoorn EJ, Zietse R. Disorders of calcium and magnesium balance: a physiology-based approach. *Pediatr Nephrol*. 2013;28(8):1195–1206. https://doi.org/10.1007/s00467-012-2350-2. Epub 2012 Nov 10. PMID: 2314286.
270. Rude RK, Singer FR, Gruber HE. Skeletal and hormonal effects of magnesium deficiency. *J Am Coll Nutr*. 2009;28(2):131–141. PMID: 19828898.

271. Takase B, Akima T, Uehata A, et al. Effect of chronic stress and sleep deprivation on both flow-mediated dilation in the brachial artery and the intracellular magnesium level in humans. *Clin Cardiol*. 2004;27(4):223–227. PMID: 15119699.
272. Palmery M, Saraceno A, Vaiarelli A, et al. Oral contraceptives and changes in nutritional requirements. *Eur Rev Med Pharmacol Sci*. 2013;17(13):1804–1813. PMID: 23852908.
273. Elliott C, Newman N, Madan A. Gentamicin effects on urinary electrolyte excretion in healthy subjects. *Clin Pharmacol Ther*. 2000;67(1):16–21. PMID: 10668849.
274. Sabra R, Branch RA. Amphotericin B nephrotoxicity. *Drug Saf*. 1990;5(2):94–108. PMID: 2182052.
275. Ribeiro MC, Avila DS, Barbosa NB, et al. Hydrochlorothiazide and high-fat diets reduce plasma magnesium levels and increase hepatic oxidative stress in rats. *Magnes Res*. 2013;26(1):32–40. https://doi.org/10.1684/mrh.2013.0334. PMID: 23657239.
276. Scalco R, Premaor MO, Fröehlich PE. High prevalence of hypovitaminosis D and secondary hyperparathyroidism in elders living in nonprofit homes in South Brazil. *Endocrine*. 2008;33(1):95–100. https://doi.org/10.1007/s12020-008-9061-2. Epub 2008 Apr 10 PMID: 18401764.
277. Spasov AA, Ozerov AA, Iezhitsa IN, et al. Correction of furosemide-induced magnesium deficiency with different stereoisomers of organic magnesium salts: a comparative study. *Bull Exp Biol Med*. 2011;151(3):333–335. PMID: 22451880.
278. Luk CP, Parsons R, Lee YP, et al. Proton pump inhibitor-associated hypomagnesemia: what do FDA data tell us? *Ann Pharmacother*. 2013;47(6):773–780. https://doi.org/10.1345/aph.1R556. Epub 2013 Apr 30 PMID: 23632281.
279. Rosanoff A, Dai Q, Shapses SA. Essential nutrient interactions: does low or Suboptimal magnesium status Interact with vitamin D and/or calcium status? *Adv Nutr*. 2016;7(1):25–43. https://doi.org/10.3945/an.115.008631. Print 2016 Jan PMID: 26773013.
280. Dai Q, Shu XO, Deng X, et al. Modifying effect of calcium/magnesium intake ratio and mortality: a population-based cohort study. *BMJ Open*. 2013;3(2):e002111. https://doi.org/10.1136/bmjopen-2012-002111. Print 2013 PMID: 23430595. PMCID: PMC3585973.
281. *Molecular weights*: https://pubchem.ncbi.nlm.nih.gov/compound/magnesium_oxide. Accessed 5 February 2020. For the other magnesium salts, replace oxide with the name of salt desired.
282. Ranade VV, Somberg JC. Bioavailability and pharmacokinetics of magnesium after administration of magnesium salts to humans. *Am J Ther*. 2001;8(5):345–357. PMID: 11550076.
283. Firoz M, Graber M. Bioavailability of US commercial magnesium preparations. *Magnes Res*. 2001;14(4):257–262. PMID: 11794633.
284. Altura BT, Wilimzig C, Trnovec T, et al. Comparative effects of a Mg-enriched diet and different orally administered magnesium oxide preparations on ionized Mg, Mg metabolism and electrolytes in serum of human volunteers. *J Am Coll Nutr*. 1994;13(5):447–454. PMID: 7836622.
285. Coudray C, Rambeau M, Feillet-Coudray C, et al. Study of magnesium bioavailability from ten organic and inorganic Mg salts in Mg-depleted rats using a stable isotope approach. *Magnes Res*. 2005;18(4):215–223. PMID: 16548135.
286. di Salvo ML, Safo MK, Contestabile R. Biomedical aspects of pyridoxal 5'-phosphate availability. *Front Biosci (Elite Ed)*. 2012;4:897–913. PMID: 22201923.
287. Sakakeeny L, Roubenoff R, Obin M, et al. Plasma pyridoxal-5-phosphate is inversely associated with systemic markers of inflammation in a population of U.S. adults. *J Nutr*. 2012;142(7):1280–1285. https://doi.org/10.3945/jn.111.153056. Epub 2012 May 23 PMID: 22623384.
288. Spasov AA, Lezgitsa IN, Kharitonova MV, et al. Effect of some organic and inorganic magnesium salts on lipoprotein state in rats fed with magnesium-deficient diet. *Eksp Klin Farmakol*. 2008;71(4):35–40. Article in Russian PMID: 18819439.
289. Spasov AA, Petrov VI, Iezhitsa IN, et al. Comparative study of magnesium salts bioavailability in rats fed a magnesium-deficient diet. *Vestn Ross Akad Med Nauk*. 2010;(2):29–37. Article in Russian PMID: 20364677.
290. Nielsen F, Hunt C, Mullen L, et al. 1987. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J*. 1987;1(5):394–397. PMID: 3678698.
291. Nielsen FH. Is boron nutritionally relevant?. *Nutr Rev*. 2008;66(4):183–191. https://doi.org/10.1111/j.1753-4887.2008.00023.x. Review PMID: 18366532.
292. Nielsen FH, Stoecker BJ. Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone. *J Trace Elem Med Biol*. 2009;23(3):195–203. https://doi.org/10.1016/j.jtemb.2009.03.003. Epub 2009 May 8 PMID: 19486829.
293. Hakki SS, Bozkurt BS, Hakki EE. Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1). *J Trace Elem Med Biol*. 2010;24(4):243–250. https://doi.org/10.1016/j.jtemb.2010.03.003. Epub 2010 Aug 3 PMID: 20685097.
294. Rosen V, Wozney JM. Bone morphogenetic proteins. In: Bilezikian JP, Raisz LG, Rodan GA, eds. *Principles of Bone Biology*. Vol. 2. second edition 20022002:919–928. [Chapter 50].
295. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors*. 2004;22(4):233–241. Review PMID: 15621726.
296. Martinovic S, Borovecki F, Miljavac V, et al. Requirement of a bone morphogenetic protein for the maintenance and stimulation of osteoblast differentiation. *Arch Histol Cytol*. 2006;69(1):23–36. PMID: 16609267.
297. Beattie JH, Peace HS. The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women. *Br J Nutr*. 1993;69(3):871–884. PMID: 8329361.
298. Naghii MR, Mofid M, Asgari AR, et al. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. *J Trace Elem Med Biol*. 2011;25(1):54–58. https://doi.org/10.1016/j.jtemb.2010.10.001. Epub 2010 Dec 3 PMID: 21129941.
299. Hunt CD, Mullen LM, et al. Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J*. 1987;1(5):394–397. PMID: 3678698.
300. Morgentaler A. *Testosterone for Life*. New York: McGraw Hill; 2009:65.
301. Chueh KS, Huang SP, Lee YC, et al. The comparison of the aging male symptoms (AMS) scale and androgen deficiency in the aging male (ADAM) questionnaire to detect androgen deficiency in middle-aged men. *J Androl*. 2012;33(5):817–823. Epub 2012 Jan 12 PMID: 22240559.
302. Pizzorno L. Nothing boring about boron. *Integr Med (Encinitas)*. 2015;14(4):35–48. PMID: 26770156.
303. Nielsen FH, Mullen LM, Gallegher SK. Effect of boron depletion and repletion on blood indicators of calcium status in humans fed a magnesium-low diet. *J Trace Elem Exp Med*. 1990;3:45–54. Available at: USDA National Agricultural Library, http://handle.nal.usda.gov/10113/49316. Accessed 2 January 2015.
304. Miljkovic D, Scorei RI, Cimpoiaşu VM, et al. Calcium fructoborate: plant-based dietary boron for human nutrition. *J Diet Suppl*. 2009;6(3):211–226. https://doi.org/10.1080/19390210903070772. PMID: 22435474.
305. Miljkovic D, Miljkovic N, McCarty MF. Up-regulatory impact of boron on vitamin D function—does it reflect inhibition of 24-hydroxylase? *Med Hypotheses*. 2004;63(6):1054–1056. PMID: 15504575.
306. Henry HL. The 25(OH)D(3)/1alpha,25(OH)(2)D(3)-24R-hydroxylase: a catabolic or biosynthetic enzyme? *Steroids*. 2001;66(3–5):391–398. PMID: 11179748.
307. Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys*. 2012;523(1):9–18. https://doi.org/10.1016/j.abb.2011.11.003. Epub 2011 Nov 12 PMID: 22100522.
308. Nielsen FH. The emergence of boron as nutritionally important throughout the life cycle. *Nutrition*. 2000;16(7–8):512–514. Review PMID: 10906539.
309. Mitrut P, Petrisor I, et al. A double-blind, placebo-controlled pilot study to evaluate the effect of calcium fructoborate on systemic inflammation and dyslipidemia markers for middle-aged people with primary osteoarthritis. *Biol Trace Elem Res*. 2011;144(1–3):253–263. https://doi.org/10.1007/s12011-011-9083-0. Epub 2011 May 24 PMID: 21607703.

310. Food and Nutrition Board. *Standing Committee on the Scientific Evaluation of Dietary Reference Intake, Institute of Medicine, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academy Press; 2002.
311. Başaran N, Duydu Y, Bolt HMJ. Reproductive toxicity in boron exposed workers in Bandirma. *Turkey. Trace Elem Med Biol*. 2012;26(2–3):165–167. https://doi.org/10.1016/j.jtemb.2012.04.013. Epub 2012 May 8 PMID: 22575543.
312. Rainey C, Nyquist L. Multicountry estimation of dietary boron intake. *Biol Trace Elem Res*. 1998;66(1–3):79–86. PMID: 10050910.
313. Meacham S, et al. Boron in human health: evidence for dietary recommendations and public policies. *Open Mineral Processing Journal*. 2010;3(1): (2010): 36–53.
314. King JC, Brown KH, Gibson RS, et al. Biomarkers of nutrition for development (BOND)-Zinc review. *J Nutr*. 2016:jn220079. Epub ahead of print PMID: 26962190.
315. Zofkova I, Davis M, Blahos J. Trace elements have beneficial, as well as detrimental effects on bone homeostasis. *Physiol Res*. 2017;66(3):391–402. Epub 2017 Feb 28 PMID: 28248532.
316. Meunier N, O'Connor JM, Maiani G, et al. Importance of zinc in the elderly: the ZENITH study. *Eur J Clin Nutr*. 2005;59(suppl 2):S1–S4. PMID: 16254574.
317. Prasad A. Impact of discovery of human zinc deficiency on health. *Journal of Trace Elements in Medicine and Biology*. 2014;28(28):357–363. https://doi.org/10.1016/j.jtemb.2014.09.002.
318. Hosea HJ, Taylor CG, Wood T, et al. Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. *Exp Biol Med (Maywood)*. 2004;229(4):303–311. PMID: 15044713.
319. Jamieson JA, Taylor CG, Weiler HA. Marginal zinc deficiency exacerbates bone lead accumulation and high dietary zinc attenuates lead accumulation at the expense of bone density in growing rats. *Toxicol Sci*. 2006;92(1):286–294. Epub 2006 Apr 19 PMID: 16624848.
320. Prasad A. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol*. 2014;28(2014):364–371. https://doi.org/10.1016/j.jtemb.2014.07.019.
321. Lowe NM, Lowe NM, Fraser WD, et al. Is there a potential therapeutic value of copper and zinc for osteoporosis? *Proc Nutr Soc*. 2002;61(2). 181-5. Proc Nutr Soc. 2002 May;61(2):181–185 PMID: 12133199.
322. Dermience M, Lognay G, Mathieu F, et al. Effects of thirty elements on bone metabolism. *J Trace Elem Med Biol*. 2015;32:86–106. https://doi.org/10.1016/j.jtemb.2015.06.005. Epub 2015 Jun 26 PMID: 26302917.
323. Mahdavi-Roshan M, Ebrahimi M, Ebrahimi A. Copper, magnesium, zinc and calcium status in osteopenic and osteoporotic post-menopausal women. *Clin Cases Miner Bone Metab*. 2015;12(1):18–21. https://doi.org/10.11138/ccmbm/2015.12.1.018. PMID: 26136790.
324. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol*. 2006;20(1):3–18. Epub 2006 Feb 21 PMID: 16632171.
325. Sandstead HH, Freeland-Graves JH. Dietary phytate, zinc and hidden zinc deficiency. *J Trace Elem Med Biol*. 2014;28(2014):414–417. https://doi.org/10.1016/j.jtemb.2014.08.011.
326. Gaby A. *Nutritional Medicine*. 2nd ed. Concord, NH: Fritz Perlberg Publishing; 2017:164.
327. Pierdomenico SD, Costantini F, et al. Copper/zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. *Free Radic Biol Med*. 1998;25(6):676–681. PMID: 9801067.
328. Sierpinska T, Konstantynowicz J, Orywal K, et al. Copper deficit as a potential pathogenic factor of reduced bone mineral density and severe tooth wear. *Osteoporos Int*. 2014;25(2):447–454. PMID: 23797848.
329. Jing Y, Jing J, Ye L, et al. Chondrogenesis and osteogenesis are one continuous developmental and lineage defined biological process. *Sci Rep*. 2017;7(1):10020. https://doi.org/10.1038/s41598-017-10048-z. PMID: 28855706.
330. Saltman PD, Strause LG. The role of trace minerals in osteoporosis. *J Am Coll Nutr*. 1993;12(4):384–389. PMID: 8409100.
331. Landete-Castillejos T, Molina-Quilez I, Estevez JA, et al. Alternative hypothesis for the origin of osteoporosis: the role of Mn. *Front Biosci (Elite Ed)*. 2012;4: 1385–1390 PMID: 22201963.
332. Bresciani G, da Cruz IB, González-Gallego J. Manganese superoxide dismutase and oxidative stress modulation. *Adv Clin Chem*. 2015;68:87–130. https://doi.org/10.1016/bs.acc.2014.11.001. Epub 2015 Jan 7 PMID: 25858870.
333. Linares GR, Xing W, Govoni KE, et al. Glutaredoxin 5 regulates osteoblast apoptosis by protecting against oxidative stress. *Bone*. 2009;44(5):795–804. https://doi.org/10.1016/j.bone.2009.01.003. Epub 2009 Jan 14 PMID: 19442627.
334. Li Y, Yu C, Shen G, et al. Sirt3-MnSOD axis represses nicotine-induced mitochondrial oxidative stress and mtDNA damage in osteoblasts. *Acta Biochim Biophys Sin (Shanghai)*. 2015;47(4): 306–312. https://doi.org/10.1093/abbs/gmv013. Epub 2015 Mar 10 PMID: 25757953.
335. Watts DL. The nutritional relationships of manganese. *J Orthomol Med*. 1990: 5(No. 4), 1990. http://orthomolecular.org/library/jom/1990/toc4.shtml. Accessed 5 February 2020.
336. Zofková I, Nemcikova P, Matucha P. Trace elements and bone health. *Clin Chem Lab Med*. 2013;51(8): 1555–1561. https://doi.org/10.1515/cclm-2012-0868. PMID: 23509220.
337. Freeland-Graves J, Mousa T, Sanjeevi N, Chapter 2, nutritional requirements for manganese, in Manganese in Health and Disease, ed. Costa LG, Aschner M. *Royal society of Chemistry, Nov 27, 2014*. https://books.google.com/books?hl=en&lr=&id=zGooDwAAQBAJ&oi=fnd&pg=PR9&dq=Manganese+in+health+and+disease,+ed.+Costa+LG,+Aschner+M&ots=a5gtgoVC1c&sig=7L4hKLc1-_g0dy8–7mSRCRYu_b8#v=onepage&q=Manganese%20in%20health%20and%20disease%2C%20ed.%20Costa%20LG%2C%20Aschner%20M&f=false. Accessed 5 February 2020.
338. Price CT, Koval KJ, Langford JR. Silicon: a review of its potential role in the prevention and treatment of postmenopausal osteoporosis. *Int J Endocrinol*. 2013;2013:316783. https://doi.org/10.1155/2013/316783. Epub 2013 May 15 PMID: 23762049.
339. Jugdaohsingh R. Silicon and bone health. *J Nutr Health Aging*. 2007;11(2):99–110. PMID: 17435952.
340. Charnot Y, Pérès G. Study of the endocrine control of silicon metabolism. Article in French *Ann Endocrinol (Paris)*. 1971;32(3):397–402. PMID: 5114904.
341. Jugdaohsingh R, Tucker KL, Qiao N, et al. Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the Framingham Offspring cohort. *J Bone Miner Res*. 2004;19(2):297–307. Epub 2003 Dec 16 PMID: 14969400.
342. Eisinger J, Clairet D. Effects of silicon, fluoride, etidronate and magnesium on bone mineral density: a retrospective study. *Magnes Res*. 1993;6(3): 247–249. PMID: 8292498.
343. Schiano A, Eisinger F, Detolle P, et al. Silicon, bone tissue and immunity. Article in French *Rev Rhum Mal Osteoartic*. 1979;46(7–9):483–486. PMID: 504950.
344. Jugdaohsingh R. Silicon and bone health. *J Nutr Health Aging*. 2007;11(2):99–110. Review PMID: 17435952.
345. Spector TD, Calomme MR, Anderson SH, et al. Choline-stabilized orthosilicic acid supplementation as an adjunct to calcium/vitamin D3 stimulates markers of bone formation in osteopenic females: a randomized, placebo-controlled trial. *BMC Musculoskelet Disord*. 2008;9:85. https://doi.org/10.1186/1471-2474-9-85. PMID: 18547426.
346. Macdonald HM, Hardcastle AC, Jugdaohsingh R, et al. Dietary silicon interacts with oestrogen to influence bone health: evidence from the Aberdeen Prospective Osteoporosis Screening Study. *Bone*. 2012;50(3):681–687. https://doi.org/10.1016/j.bone.2011.11.020. Epub 2011 Dec 7 PMID: 22173054.
347. Jugdaohsingh R, Anderson SH, Tucker KL, et al. Dietary silicon intake and absorption. *Am J Clin Nutr*. 2002;75(5):887–893. PMID: 11976163.
348. Sripanyakorn S, Jugdaohsingh R, Dissayabutr W, et al. The comparative absorption of silicon from different foods and food supplements. *Br J Nutr*. 2009;102(6):825–834. https://doi.org/10.1017/S0007114509311757. Epub 2009 Apr 9 PMID: 19356271.
349. ch-OSA® (choline-stabilized orthosilicic acid). http://www.derma.ee/files/Biosil%20monograaf.pdf. Accessed 5 February 2020.

350. Araújo LA, Addor F, Campos PM. Use of silicon for skin and hair care: an approach of chemical forms available and efficacy. *An Bras Dermatol*. 2016;91(3):331–335. https://doi.org/10.1590/abd1806-4841.20163986. PMID: 27438201.
351. Jakob F, Becker K, Paar E, et al. Expression and regulation of thioredoxin reductases and other selenoproteins in bone. *Methods Enzymol*. 2002;347:168–179. PMID: 11898403.
352. Hoeg A, Gogakos A, Murphy E, et al. Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women. *J Clin Endocrinol Metab*. 2012;97(11):4061–4070. https://doi.org/10.1210/jc.2012-2121. Epub 2012 Aug 17. 22904175.
353. Beukhof CM, Medici M, van den Beld AW, et al. Selenium status is positively associated with bone mineral density in healthy aging European men. *PLoS One*. 2016;11(4):e0152748. https://doi.org/10.1371/journal.pone.0152748. eCollection 2016 PMID: 27055238.
354. Zhang Z, Zhang J, Xiao J. Selenoproteins and selenium status in bone physiology and pathology. *Biochim Biophys Acta*. 2014;1840(11):3246–3256. https://doi.org/10.1016/j.bbagen.2014.08.001. Epub 2014 Aug 10 PMID: 25116856.
355. Steinbrenner H, Speckmann B, Klotz LO. Selenoproteins: antioxidant selenoenzymes and beyond. *Arch Biochem Biophys*. 2016;595:113–119. https://doi.org/10.1016/j.abb.2015.06.024. PMID: 27095226.
356. Ebert R, Ulmer M, Zeck S, et al. Selenium supplementation restores the antioxidative capacity and prevents cell damage in bone marrow stromal cells in vitro. *Stem Cells*. 2006;24(5):1226–1235. Epub 2006 Jan 19 PMID: 16424399.
357. Williams GR. Actions of thyroid hormones in bone. *Endokrynol Pol*. 2009;60(5):380–388. PMID: 19885809.
358. Zaidi M, Davies TF, Zallone A, Blair HC, et al. Thyroid-stimulating hormone, thyroid hormones, and bone loss. *Curr Osteoporos Rep*. 2009;7(2):47–52. PMID: 19631028.
359. Yoshihara A, Yoshimura Noh J, Mukasa K, et al. The characteristics of osteoporotic patients in Graves' disease patients newly diagnosed after menopause: a prospective observational study. *Endocr J*. 2016;63(12):1113–1122. https://doi.org/10.1507/endocrj.EJ16-0261. Epub 2016 Sep 7 PMID: 27600197.
360. National Institutes of Health. Office of dietary supplements. Selenium, dietary supplement fact sheet, health professionals, https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/. Accessed 3 August 2017.
361. Gaby A. "*Selenium*" *in Nutritional Medicine*. 2nd ed. Concord, NH: Fritz Perlburg Publishing; 2017:176–179.
362. Murray MT, Pizzorno JE, Pizzorno L. *Encyclopedia of Healing Foods*. NY: Atria Books; 2005:413–414.
363. Laurberg P, Cerqueira C, Ovesen L, et al. Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab*. 2010;24(1):13–27. https://doi.org/10.1016/j.beem.2009.08.013. PMID: 20172467.
364. Cappola AR, Arnold AM, Wulczyn K, et al. Thyroid function in the euthyroid range and adverse outcomes in older adults. *J Clin Endocrinol Metab*. 2015;100(3):1088–1096. https://doi.org/10.1210/jc.2014-3586. Epub 2014 Dec 16 PMID: 25514105 PMCID: PMC4333030. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4333030/. Accessed 5 February 2020.
365. Noh HM, Park YS, Lee J, et al. A cross-sectional study to examine the correlation between serum TSH levels and the osteoporosis of the lumbar spine in healthy women with normal thyroid function. *Osteoporos Int*. 2015;26(3):997–1003. https://doi.org/10.1007/s00198-014-2906-z. Epub 2014 Sep 25 PMID: 25253541.
366. Arslanca T, Korkmaz V, Arslanca SB, et al. Body iodine status in women with postmenopausal osteoporosis. *Menopause*. 2018;25(3):320–323. https://doi.org/10.1097/GME.0000000000000987. PMID: 28953213.
367. Acar B, Ozay AC, Ozay OE, et al. Evaluation of thyroid function status among postmenopausal; women with and without osteoporosis. *Int J Gynaecol Obstet*. 2016;134(1):53–57. https://doi.org/10.1016/j.ijgo.2015.11.025. Epub 2016 Apr 13 PMID: 27177516.
368. Zhang J, Lazar MA. The mechanism of action of thyroid hormones. *Annu Rev Physiol*. 2000;62:439–466. PMID: 10845098.
369. Földes J, Tarján G, Szathmari M, et al. Bone mineral density in patients with endogenous subclinical hyperthyroidism: is this thyroid status a risk factor for osteoporosis? *Clin Endocrinol (Oxf)*. 1993;39(5):521–527. PMID: 8252739.
370. Pizzorno L. Iodine: the next vitamin D? Parts 1&2, *Longevity Medicine Review*, 2009, http://www.lmreview.com/articles/view/iodine-the-next-vitamin-d-part-I/, http://www.lmreview.com/articles/view/iodine-the-next-vitamin-d-part-II/. Accessed 5 February 2020.
371. Stuss M, Michalska-Kasiczak M, Sewerynek E. The role of selenium in thyroid gland pathophysiology. *Endokrynol Pol*. 2017;68(4):440–465. https://doi.org/10.5603/EP. 2017.0051 PMID: 28819948.
372. Barth JH, Spencer JD, Goodall SR, et al. Reference intervals for thyroid hormones on Advia Centaur derived from three reference populations and a review of the literature. *Ann Clin Biochem*. 2016;53(Pt 3):385–389. https://doi.org/10.1177/0004563216636897. PMID: 27048695.
373. Köhrle J. Selenium and the thyroid. *Curr Opin Endocrinol Diabetes Obes*. 2015;22(5):392–401. https://doi.org/10.1097/MED.0000000000000190. PMID: 26313901.
374. Pors Nielsen S. The biological role of strontium. *Bone*. 2004;35(3):583–588. https://doi.org/10.1016/j.bone.2004.04.026. PMID: 15336592.
375. Genuis SJ, Schwalfenberg GK. Picking a bone with contemporary osteoporosis management: nutrient strategies to enhance skeletal integrity. *Clin Nutr*. 2007;26(2):193–207. https://doi.org/10.1016/j.clnu.2006.08.004. Epub 2006 Oct 13 PMID: 17046114.
376. Yamaguchi M, Weitzmann MN. The intact strontium ranelate complex stimulates osteoblastogenesis and suppresses osteoclastogenesis by antagonizing NF-κB activation. *Mol Cell Biochem*. 2012;359(1–2):399–407. https://doi.org/10.1007/s11010-011-1034-8. Epub 2011 Aug 27 PMID: 21874315.
377. Rossini M, Adami G, Adami S, et al. Safety issues and adverse reactions with osteoporosis management. *Expert Opin Drug Saf*. 2016;15(3):321–332. https://doi.org/10.1517/14740338.2016.1136287. Epub 2016 Jan 14 PMID: 26699669.
378. [No authors listed]. Strontium still authorised despite an unfavourable opinion of the European Pharmacovigilance committee. *Prescrire Int*. 2015;24(156):15. PMID: 25729823.
379. Bolland MJ, Grey A. A comparison of adverse event and fracture efficacy data for strontium ranelate in regulatory documents and the publication record. *BMJ Open*. 2014;4(10):e005787. https://doi.org/10.1136/bmjopen-2014-005787. PMID: 25293384. PMCID: PMC4187454.
380. Musette P, Brandi ML, Cacoub P, et al. Treatment of osteoporosis: recognizing and managing cutaneous adverse reactions and drug-induced hypersensitivity. *Osteoporos Int*. 2010;21(5):723–732. https://doi.org/10.1007/s00198-009-1097-5. Epub 2009 Nov 17 PMID: 19921087.
381. Heard LK, Richardson VN. A case of autoinflammatory skin and bone disease flared by a change in osteoporosis management. *JAAD Case Rep*. 2017;3(2). https://doi.org/10.1016/j.jdcr.2017.01.004. http://www.sciencedirect.com/science/article/pii/S2352512617300073. Accessed 5 February 2020.
382. Marie PJ, Ammann P, Boivin G, et al. Mechanisms of action and therapeutic potential of strontium in bone. *Calcif Tissue Int*. 2001;69(3):121–129. PMID: 11683526.
383. Dahl SG, Allain P, Marie PJ, et al. Incorporation and distribution of strontium in bone. crystal surface of the bone matrix *Bone*. 2001;28(4):446–453. PMID: 11336927.
384. Wohl GR, Chettle DR, Pejović-Milić A, et al. Accumulation of bone strontium measured by in vivo XRF in rats supplemented with strontium citrate and strontium ranelate. *Bone*. 2013;52(1):63–69. https://doi.org/10.1016/j.bone.2012.09.002. Epub 2012 Sep 17 PMID: 22995463.
385. Shorr E, Carter AC. The usefulness of strontium as an adjuvant to calcium in the mineralization of the skeleton in man. *Bull Hosp Joint Dis*. 1952;13(1):59–66. PMID: 14935450.
386. Saidak Z, Marie PJ. Strontium signaling: molecular mechanisms and therapeutic implications in osteoporosis. *Pharmacol Ther*.

2012;136(2):216–226. https://doi.org/10.1016/j.pharmthera.2012.07.009. Epub 2012 Jul 20 PMID: 22820094.
387. Reginster JY, Bruyere O, Collette J. Strontium ranelate treatment increases osteoprotegerin serum levels in postmenopausal osteoporotic women. *Bone*. 2012;50(5):1201–1202; author reply 1203–1204. doi: 10.1016/j.bone.2011.12.024. Epub 2012 Jan 14 PMID: 22270053.
388. Skoryna SC. Effects of oral supplementation with stable strontium. *Can Med Assoc J*. 1981;125(7):703–712. PMID: 6120036.
389. Riedel C, Zimmermann EA, Zustin J, et al. The incorporation of fluoride and strontium in hydroxyapatite affects the composition, structure, and mechanical properties of human cortical bone. *J Biomed Mater Res A*. 2017;105(2):433–442. https://doi.org/10.1002/jbm.a.35917. Epub 2016 Oct 21 PMID: 27684387.
390. Moise H, Chettle DR, Pejović-Milić A. Monitoring bone strontium intake in osteoporotic females self-supplementing with strontium citrate with a novel in-vivo X-ray fluorescence based diagnostic tool. *Bone*. 2014;61:48–54. https://doi.org/10.1016/j.bone.2014.01.002. Epub 2014 Jan 14 PMID: 24434614.
391. Moise H, Chettle DR, Pejović-Milić A. Modeling elemental strontium in human bone based on in vivo x-ray fluorescence measurements in osteoporotic females self-supplementing with strontium citrate. *Physiol Meas*. 2016;37(3):429–441. https://doi.org/10.1088/0967-3334/37/3/429. Epub 2016 Feb 24 PMID: 26910208.
392. Manette C, Collette J, Sarlet N, et al. Comprehensive therapy in osteoporosis using a single drug: from ADFR to strontium ranelate. *Curr Med Chem*. 2006;13(13):1585–1590. PMID: 16787205.
393. Deeks ED, Dhillon S. Strontium ranelate: a review of its use in the treatment of postmenopausal osteoporosis. *Drugs*. 2010;70(6):733–759. https://doi.org/10.2165/10481900-000000000-00000. PMID: 20394457.
394. Frith J, Day CP, Henderson E. Non-alcoholic fatty liver disease in older people. *Gerontology*. 2009;55(6):607–613. https://doi.org/10.1159/000235677. Epub 2009 Aug 19 PMID: 19690397.
395. Frassetto LA, Morris Jr RC, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am J Physiol*. 1996;271(6 Pt 2):F1114–F1122. PMID: 8997384.
396. George J, Ganesh HK, Acharya S, et al. Bone mineral density and disorders of mineral metabolism in chronic liver disease. *World J Gastroenterol*. 2009;15(28):3516–3522. PMID: 19630107.
397. Okano T. [The role of the liver in vitamin D metabolism]. Article in Japanese *Clin Calcium*. 2015;25(11):1613–1618. https://doi.org/CliCa151116131618. PMID: 26503864.
398. Frith J, Newton JL. Liver disease in older women. *Maturitas*. 2010;65(3):210–214. https://doi.org/10.1016/j.maturitas.2009.11.010. Epub 2009 Dec 3 PMID: 19962256.
399. Ninkovic M, Love SA, Tom B, et al. High prevalence of osteoporosis in patients with chronic liver disease prior to liver transplantation. *Calcif Tissue Int*. 2001;69(6):321–326. PMID: 11800228.
400. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther*. 2011;34(3):274–285. https://doi.org/10.1111/j.1365-2036.2011.04724.x. Epub 2011 May 30 PMID: 21623852.
401. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. *Gastroenterology*. 2002;122(6):1649–1657. PMID: 12016429.
402. Lips P, Binkley N, Pfeifer M, et al. Once-weekly dose of 8400 IU vitamin D(3) compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *Am J Clin Nutr*. 2010;91(4):985–991. https://doi.org/10.3945/ajcn.2009.28113. Epub 2010 Feb 3 PMID: 20130093. (This reference shows once-weekly bolus dosing ineffective.)
403. Lips P, Bouillon R, van Schoor NM, et al. Reducing fracture risk with calcium and vitamin D. *Clin Endocrinol (Oxf)*. 2010;73(3):277–285. https://doi.org/10.1111/j.1365-2265.2009.03701.x. PMID: 20796001. (Same lead author; this one shows daily dosing, effective.)
404. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr*. 2006;84(4):694–697. PMID: 17023693.
405. Heaney RP, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D3 is more potent than vitamin D2 in humans. *J Clin Endocrinol Metab*. 2010. Epub ahead of print PMID: 21177785.
406. Logan VF, Gray AR, Peddie MC, et al. Long term vitamin D3 supplementation is more effective than vitamin D2 in maintaining serum 25-hydroxyvitamin D status over the winter months. *Br J Nutr*. 2013;109(6):1082–1088. https://doi.org/10.1017/S0007114512002851. Epub 2012 Jul 11 PMID: 23168298.
407. Trang HM, Cole DE, Rubin LA, et al. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am J Clin Nutr*. 1998;68(4):854–858. PMID: 9771862.
408. Tripkovic L, Lambert H, Hart K, et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am J Clin Nutr*. 2012;95(6):1357–1364. https://doi.org/10.3945/ajcn.111.031070. Epub 2012 May 2 PMID: 22552031.
409. Chowdhury R, Kunutsor S, Vitezova A, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ*. 2014;348:g1903. https://doi.org/10.1136/bmj.g1903. PMID: 24690623.
410. Maxmen A. Nutrition advice: the vitamin D-lemma. *Nature*. 2011;475:23–25. https://doi.org/10.1038/475023a. PMID: 21734684.
411. Holick MF. Evidence-based D-bate on health benefits of vitamin D revisited. *Dermatoendocrinol*. 2012;4(2):183–190. https://doi.org/10.4161/derm.20015. PMID: 22928075.
412. Vieth R. What is the optimal vitamin D status for health? *Prog Biophys Mol Biol*. 2006;92(1):26–32. PMID: 16766239.
413. Veugelers PJ, Pham TM, Ekwaru JP. Optimal vitamin D supplementation doses that minimize the risk for both low and high serum 25-hydroxyvitamin D concentrations in the general population. *Nutrients*. 2015;7(12):10189–10208. https://doi.org/10.3390/nu7125527. PMID: 26690210.
414. Garland CF, French CB, Baggerly LL, Heaney RP. Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. *Anticancer Res*. 2011;31(2):607–611. PMID: 21378345.
415. vitamindcouncil.org. (2018). [online] Available at: https://www.vitamindcouncil.org/how-much-vitamin-d-is-needed-to-achieve-optimal-levels/. Accessed 6 July 2018.
416. Zittermann A, Ernst JB, Gummert JF, et al. Vitamin D supplementation, body weight and human serum 25-hydroxyvitamin D response: a systematic review. *Eur J Nutr*. 2014;53(2):367–374. https://doi.org/10.1007/s00394-013-0634-3. Epub 2013 Dec 1. Review PMID: 24292820.
417. Ekwaru JP, Zwicker JD, Holick MF, et al. The importance of body weight for the dose response relationship of oral vitamin D supplementation and serum 25-hydroxyvitamin D in healthy volunteers. *PLoS One*. 2014;9(11):e111265. https://doi.org/10.1371/journal.pone.0111265. eCollection 2014 PMID: 25372709.
418. Pereira-Santos M, Costa PR, Assis AM, et al. Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obes Rev*. 2015;16(4):341–349. https://doi.org/10.1111/obr.12239. Epub 2015 Feb 17 PMID: 25688659.
419. Whiting SJ, Calvo MS. Correcting poor vitamin D status: do older adults need higher repletion doses of vitamin D3 than younger adults? *Mol Nutr Food Res*. 2010;54(8):1077–1084. https://doi.org/10.1002/mnfr.200900536. PMID: 20440693.
420. Holick MF. Bioavailability of vitamin D and its metabolites in black and white adults. *N Engl J Med*. 2013;369(21):2047–2048. https://doi.org/10.1056/NEJMe1312291. PMID: 24256384.
421. Powe CE, Evans MK, Wenger J, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med*. 2013;369(21):1991–2000. https://doi.org/10.1056/NEJMoa1306357. PMID: 24256378.
422. Aloia J, Mikhail M, Dhaliwal R, et al. Free 25(OH)D and the vitamin D paradox in African Americans. *J Clin Endocrinol Metab*. 2015;100(9):3356–3363. Epub 2015 Jul 10 PMID: 26161453.
423. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. *Am J Clin Nutr*. 1995;61(3 suppl l):638S–645S. PMID: 7879731.

424. Holick MF, Chen TC, Lu Z, Sauter E. Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res*. 2007;22(suppl 2):V28–V33. https://doi.org/10.1359/jbmr.07s211. PMID: 18290718.
425. Dudenkov DV, Yawn BP, Oberhelman SS, et al. Changing incidence of serum 25-hydroxyvitamin D Values above 50 ng/mL: a 10-year population-based study. *Mayo Clin Proc*. 2015;90(5):577–586. PMID: 25939935.
426. Holick MF. Vitamin D is not as toxic as was once thought: a Historical and an up-to-date perspective. *Mayo Clin Proc*. 2015;90(5):561–564. https://doi.org/10.1016/j.mayocp.2015.03.015. PMID: 25939933.
427. Vitamin D. Toxicity Rare in people who take supplements. *Mayo Clinic Researchers Report*. Mayo Clinic News Release, 2015.
428. McCann JC, Ames BN. Vitamin K, an example of triage theory: Is micronutrient inadequacy linked to diseases of aging? *Am J Clin Nutr*. 2009;90(4):889–907. https://doi.org/10.3945/ajcn.2009.27930. Epub 2009 Aug 19 PMID: 19692494.
429. Shea MK, Dallal GE, Dawson-Hughes B, et al. Vitamin K, circulating cytokines, and bone mineral density in older men and women. *Am J Clin Nutr*. 2008;88(2):356–363. PMID: 18689371.
430. Flore R, Ponziani FR, Di Rienzo TA, et al. Something more to say about calcium homeostasis: the role of vitamin K2 in vascular calcification and osteoporosis. *Eur Rev Med Pharmacol Sci*. 2013;17(18):2433–2440. PMID: 24089220.
431. Shea MK, Booth SL, Massaro JM, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol*. 2008;167(3):313–320. PMID: 18006902.
432. Kaneki M, Hosoi T, Ouchi Y, et al. Pleiotropic actions of vitamin K: protector of bone health and beyond? *Nutrition*. 2006;22(7–8):845–852. PMID: 16815498.
433. Iwamoto J, Sato Y, Takeda T, et al. High-dose vitamin K supplementation reduces fracture incidence in postmenopausal women: a review of the literature. *Nutr Res*. 2009;29(4):221–228. https://doi.org/10.1016/j.nutres.2009.03.012. PMID: 19410972.
434. Jacques PF, Hannan MT, et al. Dihydrophylloquinone intake is associated with low bone mineral density in men and women. *Am J Clin Nutr*. 2007;86(2):504–508. PMID: 17684225.
435. Booth SL, Lichtenstein AH, O'Brien-Morse M, et al. Effects of a hydrogenated form of vitamin K on bone formation and resorption. *Am J Clin Nutr*. 2001;74(6):783–790. PMID: 11722960.
436. Bolland MJ, Avenell A, Baron JA, et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ*. 2010;341:c3691. https://doi.org/10.1136/bmj.c3691. PMID: 20671013.
437. Bolland MJ, Grey A, Avenell A, et al. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ*. 2011;342:d2040. https://doi.org/10.1136/bmj.d2040. PMID: 21505219.
438. Vossen LM, Schurgers LJ, van Varik BJ, et al. Menaquinone-7 supplementation to reduce vascular calcification in patients with coronary artery disease: rationale and study protocol (VitaK-CAC trial). *Nutrients*. 2015;7(11):8905–8915. https://doi.org/10.3390/nu7115443. PMID: 26516910.
439. Manoury E, Jourdon K, Boyaval P, Fourcassié P. Quantitative measurement of vitamin K2 (menaquinones) in various fermented dairy products using a reliable high-performance liquid chromatography method. *J Dairy Sci*. 2013;96(3):1335–1346. https://doi.org/10.3168/jds.2012-5494. Epub 2013 Jan 17 PMID: 23332840.
440. Hojo K, Watanabe R, Mori T, Taketomo N. Quantitative measurement of tetrahydromenaquinone-9 in cheese fermented by propionibacteria. *J Dairy Sci*. 2007;90(9):4078–4083. PMID: 17699024.
441. Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis*. 2000;30(6):298–307. PMID: 11356998.
442. Elder SJ, Haytowitz DB, Howe J, Peterson JW, Booth SL. Vitamin k contents of meat, dairy, and fast food in the u.s. Diet. *J Agric Food Chem*. 2006;54(2):463–467. PMID: 16417305.
443. Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. *Biochim Biophys Acta*. 2002;1570(1):27–32. PMID: 11960685.
444. Schurgers LJ, Teunissen KJ, Hamulyák K, et al. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood*. 2007;109(8):3279–3283. Epub 2006 Dec 7 PMID: 17158229.
445. Shearer MJ, Fu X, Booth SL. Vitamin K nutrition, metabolism, and requirements: current concepts and future research. *Adv Nutr*. 2012;3(2):182–195. https://doi.org/10.3945/an.111.001800. PMID: 22516726.
446. Pilkey RM, Morton AR, Boffa MB, et al. Subclinical vitamin K deficiency in hemodialysis patients. *Am J Kidney Dis*. 2007;49(3):432–439. PMID: 17336705.
447. Kaneki M. Genomic approaches to bone and joint diseases. new insights into molecular mechanisms underlying protective effects of vitamin K on bone health. Article in Japanese *Clin Calcium*. 2008;18(2):224–232. CliCa0802224232 PMID: 18245893.
448. Jeenduang N, Porntadavity S, Wanmasae S. Combined PCSK9 and APOE polymorphisms are genetic risk factors associated with elevated plasma lipid levels in a Thai population. *Lipids*. 2015;50(6):543–553. https://doi.org/10.1007/s11745-015-4017-9. Epub 2015 Apr 22 PMID: 25899039.
449. Kohnke H, Sörlin K, Granath G, Wadelius M. Warfarin dose related to apolipoprotein E (APOE) genotype. *Eur J Clin Pharmacol*. 2005;61(5–6):381–388. Epub 2005 Jun 11 PMID: 15952022.
450. Puehringer H, Loreth RM, Klose G, et al. VKORC1 -1639G>A and CYP2C9*3 are the major genetic predictors of phenprocoumon dose requirement. *Eur J Clin Pharmacol*. 2010;66(6):591–598. https://doi.org/10.1007/s00228-010-0809-2. Epub 2010 Apr 8 PMID: 20376629.
451. Haraikawa M, Tsugawa N, Sogabe N, et al. Effects of gamma-glutamyl carboxylase gene polymorphism (R325Q) on the association between dietary vitamin K intake and gamma-carboxylation of osteocalcin in young adults. *Asia Pac J Clin Nutr*. 2013;22(4):646–654. https://doi.org/10.6133/apjcn.2013.22.4.01. PMID: 24231026.
452. Sogabe N, Tsugawa N, Maruyama R, et al. Nutritional effects of gamma-glutamyl carboxylase gene polymorphism on the correlation between the vitamin K status and gamma-carboxylation of osteocalcin in young males. *J Nutr Sci Vitaminol (Tokyo)*. 2007;53(5):419–425. PMID: 18079608.
453. Caluwé R, Vandecasteele S, Van Vlem B, Vermeer C, De Vriese AS. Vitamin K2 supplementation in haemodialysis patients: a randomized dose-finding study. *Nephrol Dial Transplant*. 2014;29(7):1385–1390. https://doi.org/10.1093/ndt/gft464. Epub 2013 Nov 26 PMID: 24285428.
454. Yamaguchi M. Regulatory mechanism of food factors in bone metabolism and prevention of osteoporosis. *Yakugaku Zasshi*. 2006;126(11):1117–1137. PMID: 17077614.
455. Hamidi MS, Gajic-Veljanoski O, Cheung AM. Vitamin K and bone health. *J Clin Densitom*. 2013;16(4):409–413. https://doi.org/10.1016/j.jocd.2013.08.017. Epub 2013 Oct 3 PMID: 24090644.
456. Knapen MH, Drummen NE, Smit E, Vermeer C, Theuwissen E. Three-year low-dose menaquinone-7 supplementation helps decrease bone loss in healthy postmenopausal women. *Osteoporos Int*. 2013;24(9):2499–2507. https://doi.org/10.1007/s00198-013-2325-6. Epub 2013 Mar 23 PMID: 23525894.
457. Poterucha TJ, Goldhaber SZ. Warfarin and vascular calcification. *Am J Med*. 2015. pii: S0002-9343(15)30031-0. https://doi.org/10.1016/j.amjmed.2015.11.032. Epub ahead of print PMID: 26714212.
458. Han KH, O'Neill WC. Increased peripheral arterial calcification in patients receiving warfarin. *J Am Heart Assoc*. 2016;5(1):e002665. https://doi.org/10.1161/JAHA.115.002665. PMID: 26811161.
459. Tantisattamo E, Han KH, O'Neill WC. Increased vascular calcification in patients receiving warfarin. *Arterioscler Thromb Vasc Biol*. 2015;35(1):237–242. https://doi.org/10.1161/ATVBAHA.114.304392. Epub 2014 Oct 16 PMID: 25324574.
460. Namba S, Yamaoka-Tojo M, Hashikata T, et al. Long-term warfarin therapy and biomarkers for osteoporosis and atherosclerosis. *BBA Clin*. 2015;4:76–80. https://doi.org/10.1016/j.bbacli.2015.08.002. eCollection 2015 PMID: 26674156.
461. Pearson DA. Bone health and osteoporosis: the role of vitamin K and potential antagonism by anticoagulants. *Nutr Clin Pract*. 2007;22(5):517–544. PMID: 17906277.

462. Sugiyama T, Takaki T, Sakanaka K, et al. Warfarin-induced impairment of cortical bone material quality and compensatory adaptation of cortical bone structure to mechanical stimuli. *J Endocrinol*. 2007;194(1):213–222. PMID: 17592035.
463. Rejnmark L, Vestergaard P, Mosekilde L. Fracture risk in users of oral anticoagulants: a nationwide case-control study. *Int J Cardiol*. 2007;118(3):338–344. Epub 2006 Oct 18 PMID: 17055083.
464. Sugiyama T, Kugimiya F, Kono S, Kim YT, et al. Warfarin use and fracture risk: an evidence-based mechanistic insight. *Osteoporos Int*. 2015;26(3):1231–1232. https://doi.org/10.1007/s00198-014-2912-1. Epub 2014 Oct 10 PMID: 25300528.
465. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. *Clin J Am Soc Nephrol*. 2008;3(5):1504–1510. https://doi.org/10.2215/CJN.00770208. Epub 2008 May 21 PMID: 18495950.
466. Cozzolino M, Brandenburg V. Warfarin: to use or not to use in chronic kidney disease patients? *J Nephrol*. 2010;23(6):648–652. PMID: 20349408.
467. Tufano A, Coppola A, Contaldi P, et al. Oral anticoagulant drugs and the risk of osteoporosis: new anticoagulants better than old? *Semin Thromb Hemost*. 2015;41(4):382–388. https://doi.org/10.1055/s-0034-1543999. Epub 2015 Feb 19 PMID: 25703521.
468. Roca B, Roca M. The new oral anticoagulants: reasonable alternatives to warfarin. *Cleve Clin J Med*. 2015;82(12):847–854. https://doi.org/10.3949/ccjm.82a.14052. PMID: 26651894.
469. Siegal DM, Curnutte JT, Connolly SJ, et al. Andexanet Alfa for the reversal of factor Xa inhibitor activity. *N Engl J Med*. 2015;373(25):2413–2424. https://doi.org/10.1056/NEJMoa1510991. Epub 2015 Nov 11 PMID: 26559317.
470. O'Malley PA. The Antidote is Finally here! Idarucizumab, A specific reversal agent for the anticoagulant effects of Dabigatran. *Clin Nurse Spec*. 2016;30(2):81–83. https://doi.org/10.1097/NUR.0000000000000192. PMID: 26848896.
471. Joo NS, Yang SW, Song BC, et al. Vitamin A intake, serum vitamin D and bone mineral density: analysis of the Korea national health and nutrition Examination survey (KNHANES, 2008-2011). *Nutrients*. 2015;7(3):1716–1727. https://doi.org/10.3390/nu7031716. PMID: 25763530.
472. Mata-Granados JM, Luque de Castro MD, Quesada Gomez JM. Inappropriate serum levels of retinol, alpha-tocopherol, 25 hydroxyvitamin D3 and 24,25 dihydroxyvitamin D3 levels in healthy Spanish adults: simultaneous assessment by HPLC. *Clin Biochem*. 2008;41(9):676–680. https://doi.org/10.1016/j.clinbiochem.2008.02.003. Epub 2008 Feb 15 PMID: 18313404.
473. Johansson S, Melhus H. Vitamin A antagonizes calcium response to vitamin D in man. *J Bone Miner Res*. 2001;16(10):1899–1905. PMID: 11585356.
474. Michaëlsson K, Lithell H, Vessby B, et al. Serum retinol levels and the risk of fracture. *N Engl J Med*. 2003;348(4):287–294. https://doi.org/10.1056/NEJMoa021171. PMID: 12540641.
475. Boucher BJ. Serum retinol levels and fracture risk. *N Engl J Med*. 2003;348(19):1927–1928; author reply 1927-8. Comment on hypervitaminosis A and fractures. https://doi.org/10.1056/NEJM200305083481917. PMID: 12736290.
476. De França NA, Camargo MB, Lazaretti-Castro M, et al. Antioxidant intake and bone status in a cross-sectional study of Brazilian women with osteoporosis. *Nutr Health*. 2013;22(2):133–142. https://doi.org/10.1177/0260106014563445. Epub 2015 Jan 14 PMID: 25593111.
477. Mata-Granados JM, Cuenca-Acevedo R, Luque de Castro MD, et al. Vitamin D deficiency and high serum levels of vitamin A increase the risk of osteoporosis evaluated by Quantitative Ultrasound Measurements (QUS) in postmenopausal Spanish women. *Clin Biochem*. 2010;43(13–14):1064–1068. https://doi.org/10.1016/j.clinbiochem.2010.06.001. Epub 2010 Jun 17 PMID: 20599880.
478. Mata-Granados JM, Cuenca-Acevedo JR, Luque de Castro MD, et al. Vitamin D insufficiency together with high serum levels of vitamin A increases the risk for osteoporosis in postmenopausal women. *Arch Osteoporos*. 2013;8:124. https://doi.org/10.1007/s11657-013-0124-5. Epub 2013 Feb 16 PMID: 23417776.
479. Caire-Juvera G, Ritenbaugh C, Wactawski-Wende J, et al. Vitamin A and retinol intakes and the risk of fractures among participants of the Women's Health Initiative Observational Study. *Am J Clin Nutr*. 2009;89(1):323–330. https://doi.org/10.3945/ajcn.2008.26451. Epub 2008 Dec 3 PMID: 19056568.
480. Conaway HH, Henning P, Lerner UH. Vitamin a metabolism, action, and role in skeletal homeostasis. *Endocr Rev*. 2013;34(6):766–797. https://doi.org/10.1210/er.2012-1071. Epub 2013 May 29 PMID: 23720297.
481. Rhéume-Bleue K. *Vitamin K and the Calcium Paradox*. Mississauga, Ontario: John Wiley & Sons; 2012:195.
482. Morgan AF, Kimmel L, Hawkins NC. A comparison of the hypervitaminoses induced by irradiated ergosterol and fish liver oil concentrates,. *J Biol Chem*. 1937;120(1):85–102.
483. Clark I, Bassett CAL. The amelioration of hypervitaminosis D in rats with vitamin A. *J Exp Med*. 1962;115:147–156.
484. Callari D, Garra ML, Billitteri A. Retinoic acid action on D3 hypervitaminosis. *Boll Soc Ital Biol Sper*. 1986;(6):835–841. LXII.
485. Metz AL, Walser MM, Olson WG. The interaction of dietary vitamin A and vitamin D related to skeletal development in the Turkey Poult. *J Nutr*. 1985;115:929–935.
486. Fu X, Wang XD, Mernitz H, et al. 9-Cis retinoic acid reduces 1alpha,25-dihydroxycholecalciferol-induced renal calcification by altering vitamin K-dependent gamma-carboxylation of matrix gamma-carboxyglutamic acid protein in A/J male mice. *J Nutr*. 2008;138(12):2337–2341. https://doi.org/10.3945/jn.108.093724. PMID: 19022954.
487. Cancela ML, Price PA. Retinoic acid induces matrix Gla protein gene expression in human cells. *Endocrinology*. 1992;130(1):102–108. PMID: 1727694.
488. Farzaneh-Far A, Weissberg PL, Proudfoot D, et al. Transcriptional regulation of matrix gla protein. *Z Kardiol*. 2001;90(suppl 3):38–42. PMID: 11374031.
489. Proudfoot D, Shanahan CM. Molecular mechanisms mediating vascular calcification: role of matrix Gla protein. *Nephrology (Carlton)*. 2006;11(5):455–461. PMID: 17014561.
490. Westenfeld R, Krueger T, Schlieper G, et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis*. 2012;59(2):186–195. https://doi.org/10.1053/j.ajkd.2011.10.041.
491. Fusaro M, D'Angelo A, Gallieni M. Consequences of vitamin K2 deficiency in hemodialysis patients. *Am J Kidney Dis*. 2012;60(1):169. https://doi.org/10.1053/j.ajkd.2012.03.021. PMID: 22709599.
492. Sakly R, Achour A, Zouaghi H. Antilithogenic and litholytic action of vitamin A vis-a-vis experimental calculi in rats. *Ann Urol (Paris)*. 1994;28(3):128–131. PMID: 8031018.
493. Grases F, Garcia-Gonzalez R, Genestar C, et al. Vitamin A and urolithiasis. *Clin Chim Acta*. 1998;269(2):147–157. PMID: 9526674.
494. Bardaoui M, Sakly R, Neffati F, et al. Effect of vitamin A supplemented diet on calcium oxalate renal stone formation in rats. *Exp Toxicol Pathol*. 2010;62(5):573–576. https://doi.org/10.1016/j.etp.2009.08.005. Epub 2009 Sep 18 PMID: 19766470.
495. Wagner J. Potential role of retinoids in the therapy of renal disease. *Nephrol Dial Transplant*. 2001;16(3):441–444. PMID: 11239012(Comment in Vitamin A in the treatment of renal disease. Nephrol Dial Transplant. 2001).
496. Sakly R, Fekih M, Ben Amor A, et al. Possible role of vitamin A and E deficiency in human idiopathic lithiasis. [Article in French] *Ann Urol (Paris)*. 2003;37(4):217–219. PMID: 12951718.
497. Barber T, Esteban-Pretel G, Marín MP, et al. Vitamin A deficiency and alterations in the extracellular matrix. *Nutrients*. 2014;6(11):4984–5017. https://doi.org/10.3390/nu6114984. PMID: 25389900.
498. Uitterlinden AG, Fang Y, Van Meurs JB, et al. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004;338(2):143–156. PMID: 15315818.
499. Sanchez-Martinez R, Castillo A, Steinmeyer A, et al. The retinoid X receptor ligand restores defective signaling by the vitamin D receptor. *EMBO Rep*. 2006;7(10):1030–1034. PMID: 16936639.
500. Bettoun DJ, Burris TP, Houck KA, et al. Retinoid X receptor is a nonsilent major contributor to vitamin D receptor-mediated transcriptional activation. *Mol Endocrinol*. 2003;17(11):2320–2328. PMID: 12893883.
501. *FAT-SOLUBLE VITAMINS* AND NONNUTRIENTS *Chapter 9 in Nutrient Metabolism*. 497 © 2015 Elsevier Ltd. All rights reserved.

502. Pizzorno L. Vitamin A: tolerance extends longevity. Longevity Medicine Review. 2009 http://www.lmreview.com/articles/view/vitamin-a-tolerance-extends-longevity/. Accessed 5 February 2020.
503. Pino-Lagos K, Benson MJ, Noelle RJ. Retinoic acid in the immune system. *Ann N Y Acad Sci*. 2008;1143:170–187. PMID: 19076350.
504. Lu LF, Rudensky A. Molecular orchestration of differentiation and function of regulatory T cells. *Genes Dev*. 2009;23(11):1270–1282. PMID: 19487568.
505. Strober W. Vitamin A rewrites the ABCs of oral tolerance. *Mucosal Immunol*. 2008;1(2):92–95. PMID: 19079166.
506. Pizzorno JE, Murray JT. *Textbook of Natural Medicine,* 4th ed. Vitamin A. St. Louis, MO: Elsevier; 2013:1095–1101 (Chapter 135).
507. Chiu H, Fischman D, Hammerling U. Vitamin A depletion causes oxidative stress, mitochondrial dysfunction, and PARP-1-dependent energy deprivation. *FASEB J*. 2008;22:3878–3887.
508. Leung WC, Hessel S, Méplan C, et al. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. *FASEB J*. 2009;23(4):1041–1053. PMID: 19103647.
509. Lietz G, Oxley A, Leung W, et al. Single nucleotide polymorphisms upstream from the β-carotene 15,15'-monoxygenase gene influence provitamin A conversion efficiency in female volunteers. *J Nutr*. 2012;142(1). https://doi.org/10.3945/jn.111.140756. 161S-5S. Epub 2011 Nov 23 PMID: 22113863.
510. Scott KJ, Rodriquez-Amaya D. Pro-vitamin A carotenoid conversion factors: retinol equivalents – fact or fiction? *Food Chemistry*. 2000;69: 127. https://doi.org/10.1016/S0308-8146(99)00256-3.
511. Pizzorno L. Common genetic variants and other host-related factors greatly increase susceptibility to vitamin A deficiency. *Longevity Medicine Review*. 2010. http://www.lmreview.com/articles/view/common-genetic-variants-and-other-host-related-factors-greatly-increase-susceptibility-to-vitamin-a-deficiency/. Accessed 5 February 2020.
512. Kohlmeier RH. *"Vitamin A," in "Fat Soluble Vitamins and Non-nutrients," Nutrient Metabolism.* Elsevier: London: 464–478.
513. Patrick L. Beta-carotene: the controversy continues. *Altern Med Rev*. 2000;5(6):530–545. PMID: 11134976.
514. Sheth S, Chopra S. Epidemiology. clinical features and diagnosis of nonalcoholic fatty liver disease, up to Date, @ http://www.uptodate.com/contents/epidemiology-clinical-features-and-diagnosis-of-nonalcoholic-fatty-liver-disease-in-adults. Accessed 6 August 2016.
515. Stargrove M, Treasure J, McKee D. *Herb, Nutrient, and D. Interactions: Clinical Implications and Therapeutic Strategies*. St Louis, MO: Mosby: Elsevier; 2008:235–236.
516. Odabasi M. Halogenated volatile organic compounds from the use of chlorine-bleach-containing household products. *Environ Sci Technol*. 2008;42(5):1445–1451. PMID: 18441786.
517. Whitney E, Rolfes S. *Chapter 11, "The Fat-Soluble Vitamins," in Understanding Nutrition*. Belmont, CA: Wadsworth; 2008:369–389.
518. K.M. Chapter 9, Fat-Soluble Vitamins and Nonnutrients, Vitamin A, in *Nutrient Metabolism*, 2nd ed. Elsevier: London; 2015: 486–500
519. Rutkowski M, Grzegorczyk K. Adverse effects of antioxidative vitamins. *Int J Occup Med Environ Health*. 2012;25(2):105–121. https://doi.org/10.2478/S13382-012-0022-x. Epub 2012 Apr 19 PMID: 22528540.
520. Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr*. 2006;83(2):191–201. PMID: 16469975.
521. Morris MS, Feigerlova E, Demarquet L, Guéant JL. One carbon metabolism and bone homeostasis and remodeling: a review of experimental research and population studies. *Biochimie*. 2016;126:115–123. https://doi.org/10.1016/j.biochi.2016.04.009. Epub 2016 Apr 13 PMID: 27086080.
522. Jacques PF, Selhub J. Relation between homocysteine and B-vitamin status indicators and bone mineral density in older Americans. *Bone*. 2005;37(2):234–242. PMID: 15950558.
523. Vaes BL, Lute C, Blom HJ, et al. Vitamin B(12) deficiency stimulates osteoclastogenesis via increased homocysteine and methylmalonic acid. *Calcif Tissue Int*. 2009;84(5):413–422. https://doi.org/10.1007/s00223-009-9244-8. Epub 2009 Apr 12 PMID: 19363664.
524. Vacek TP, Kalani A, Voor MJ, et al. The role of homocysteine in bone remodeling. *Clin Chem Lab Med*. 2013;51(3):579–590. https://doi.org/10.1515/cclm-2012-0605. PMID: 23449525.
525. Herrmann M, Tami A, Wildemann B, et al. Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone. *Bone*. 2009;44(3):467–475. https://doi.org/10.1016/j.bone.2008.10.051. Epub 2008 Nov 12 PMID: 19056526.
526. Petramala L, Acca M, Francucci CM, et al. Hyperhomocysteinemia: a biochemical link between bone and cardiovascular system diseases? *J Endocrinol Invest*. 2009;32(suppl 4):10–14. PMID: 19724160.
527. McLean RR, Jacques PF, Selhub J, et al. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med*. 2004;350(20):2042–2049. PMID: 15141042.
528. Boylan LM, Spallholz JE. In vitro evidence for a relationship between magnesium and vitamin B-6. *Magnes Res*. 1990;3(2):79–85. PMID: 2133627.
529. Mujica-Coopman MF, Franco-Sena AB, Farias DR, et al. Vitamin B-6 status in Unsupplemented pregnant women is associated positively with serum docosahexaenoic acid and inversely with the n-6-to-n-3 fatty acid ratio. *J Nutr*. 2016:jn239483. https://doi.org/10.3945/jn.116.239483. Epub ahead of print PMID: 28031376.
530. Dalto DB, Matte JJ. Pyridoxine (vitamin B_6) and the glutathione peroxidase system; a link between one-carbon metabolism and Antioxidation. *Nutrients*. 2017;9(3):E189. https://doi.org/10.3390/nu9030189. PMID: 28245568. https://www.ncbi.nlm.nih.gov/pubmed/28245568. PMCID: PMC5372852. Accessed 5 February 2020.
531. Williams GR, Bassett JHD. Thyroid diseases and bone health. *J Endocrinol Invest*. 2017. https://doi.org/10.1007/s40618-017-0753-4. Epub ahead of print PMID: 28853052.
532. Marenzana M, Arnett TR. The key role of the blood supply to bone. *Bone Res*. 2013;1(3):203–215. https://doi.org/10.4248/BR201303001. eCollection 2013 Sep PMID: 26273504.
533. Evans DM, Ralston SH. Nitric oxide and bone. *J Bone Miner Res*. 1996;11(3):300–305. PMID: 8852940.
534. Calder PC, Albers R, Antoine JM, et al. Inflammatory disease processes and interactions with nutrition. *Br J Nutr*. 2009;101(suppl 1):S1–S45. https://doi.org/10.1017/S0007114509377867. PMID: 19586558.
535. Jan AT, Azam M, Siddiqui K, et al. Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. *Int J Mol Sci*. 2015;16(12):29592–29630. https://doi.org/10.3390/ijms161226183. PMID: 26690422.
536. Sekhar RV, Patel SG, Guthikonda AP, et al. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. *Am J Clin Nutr*. 2011;94(3):847–853. https://doi.org/10.3945/ajcn.110.003483. Epub 2011 Jul 27 PMID: 21795440.
537. McCarty MF, DiNicolantonio JJ. An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly. *Age (Dordr)*. 2015;37(5):96. https://doi.org/10.1007/s11357-015-9823-8. Epub 2015 Sep 11 PMID: 26362762. PMCID: PMC5005830.
538. Bellanti F, Matteo M, Rollo T, et al. Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Redox Biol*. 2013;1:340–346. https://doi.org/10.1016/j.redox.2013.05.003. eCollection 2013 PMID: 24024169. PMCID: PMC3757703.
539. Unfer TC, Figueiredo CG, Zanchi MM, et al. Estrogen plus progestin increase superoxide dismutase and total antioxidant capacity in postmenopausal women. *Climacteric*. 2015;18(3):379–388. https://doi.org/10.3109/13697137.2014.964669. Epub 2014 Nov 12 PMID: 25236970.
540. Priyanka HP, Sharma U, Gopinath S, et al. Menstrual cycle and reproductive aging alters immune reactivity, NGF expression, antioxidant enzyme activities, and intracellular signaling pathways in the peripheral blood mononuclear cells of healthy women. *Brain Behav Immun*. 2013;32:131–143. https://doi.org/10.1016/j.bbi.2013.03.008. Epub 2013 Mar 27 PMID: 23542336.
541. Lean JM, Davies JT, Fuller K, et al. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest*. 2003;112(6):915–923. PMID: 12975476. PMCID: PMC193670.

542. Candelario-Jalil E, Akundi RS, Bhatia HS, et al. Ascorbic acid enhances the inhibitory effect of aspirin on neuronal cyclooxygenase-2-mediated prostaglandin E2 production. *J Neuroimmunol*. 2006;174(1–2):39–51. Epub 2006 Mar 10 PMID: 16529823.
543. Ashor AW, Werner AD, Lara J, et al. Effects of vitamin C supplementation on glycaemic control: a systematic review and meta-analysis of randomised controlled trials. *Eur J Clin Nutr*. 2017. https://doi.org/10.1038/ejcn.2017.24. Epub ahead of print PMID: 28294172.
544. De Tullio MC. Beyond the antioxidant: the double life of vitamin C. *Subcell Biochem*. 2012;56:49–65. https://doi.org/10.1007/978-94-007-2199-9_4. PMID: 22116694.
545. Sahni S, Marian T, Blumberg J, et al. Protective effect of total and supplemental vitamin C intake on the risk of hip fracture - a 17-year follow-up from the Framingham Osteoporosis Study. *Osteoporos Int*. 2009;20(11):1853–1861. https://doi.org/10.1007/s00198-009-0897-y. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2766028/pdf/nihms122048.pdf. Accessed 5 February 2020.
546. Bae SH, Ryu H, Rhee KJ, et al. L-ascorbic acid 2-phosphate and fibroblast growth factor-2 treatment maintains differentiation potential in bone marrow-derived mesenchymal stem cells through expression of hepatocyte growth factor. *Growth Factors*. 2015;33(2):71–78. https://doi.org/10.3109/08977194.2015.1013628. Epub 2015 Feb 25 PMID: 25714612.
547. Wei F, Qu C, Song T, et al. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. *J Cell Physiol*. 2012;227(9):3216–3224. https://doi.org/10.1002/jcp.24012. PMID: 22105792.
548. Yan Y, Zeng W, Song S, et al. Vitamin C induces periodontal ligament progenitor cell differentiation via activation of ERK pathway mediated by PELP1. *Protein Cell*. 2013;4(8):620–627. https://doi.org/10.1007/s13238-013-3030-0. Epub 2013 Jul 8 PMID: 23836152.
549. Aghajanian P, Hall S, Wongworawat MD, et al. The roles and mechanisms of actions of vitamin C in bone: new developments. *J Bone Miner Res*. 2015;30(11):1945–1955. https://doi.org/10.1002/jbmr.2709. Epub 2015 Oct 7 PMID: 26358868.
550. Shen Y, Liu HX, Ying XZ, et al. Dose-dependent effects of nicotine on proliferation and differentiation of human bone marrow stromal cells and the antagonistic action of vitamin C. *J Cell Biochem*. 2013;114(8):1720–1728. https://doi.org/10.1002/jcb.24512. PMID: 23386463.
551. Food and Nutrition Board IOM. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington; 2000.
552. Nieves JW. Skeletal effects of nutrients and nutraceuticals, beyond calcium and vitamin D. *Osteoporos Int*. 2013;24(3):771–786. https://doi.org/10.1007/s00198-012-2214-4. Epub 2012 Nov 14 PMID: 23152094.
553. Hart A, Cota A, Makhdom A, et al. The role of vitamin C in Orthopedic trauma and bone health. *Am J Orthop (Belle Mead NJ)*. 2015;44(7):306–311. PMID: 26161758.
554. Gaby A. *Vitamin C, in Nutritional Medicine*. 2nd ed. Concord, NH: Fritz Perlberg Publishing; 2017:105.
555. Hathcock JN, Azzi A, Blumberg J, et al. Vitamins E and C are safe across a broad range of intakes. *Am J Clin Nutr*. 2005;81(4):736–745. PMID: 15817846.
556. Michels AJ, Joisher N, Hagen TM. Age-related decline of sodium-dependent ascorbic acid transport in isolated rat hepatocytes. *Arch Biochem Biophys*. 2003;410(1):112–120. PMID: 12559983.
557. Lykkesfeldt J, Moos T. Age-dependent change in Vitamin C status: a phenomenon of maturation rather than of ageing. *Mech Ageing Dev*. 2005;126(8):892–898. Epub 2005 Apr 25 PMID: 15992612.
558. Timpson NJ, Forouhi NG, Brion MJ, et al. Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): evidence from 5 independent studies with >15,000 participants. *Am J Clin Nutr*. 2010;92(2):375–382. https://doi.org/10.3945/ajcn.2010.29438. Epub 2010 Jun 2 PMID: 20519558.
559. Bürzle M, Suzuki Y, Ackermann D, et al. The sodium-dependent ascorbic acid transporter family SLC23. *Mol Aspects Med*. 2013;34(2–3):436–454. https://doi.org/10.1016/j.mam.2012.12.002. PMID: 23506882.
560. Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci U S A*. 1996;93(8):3704–3709. PMID: 8623000, PMCID: PMC39676.
561. Mason SA, Baptista R, Della Gatta PA, et al. High-dose vitamin C supplementation increases skeletal muscle vitamin C concentration and SVCT2 transporter expression but does not alter redox status in healthy males. *Free Radic Biol Med*. 2014;77:130–138. https://doi.org/10.1016/j.freeradbiomed.2014.09.013. Epub 2014 Sep 19 PMID: 25242204.
562. Mason SA, Della Gatta PA, Snow RJ, et al. Ascorbic acid supplementation improves skeletal muscle oxidative stress and insulin sensitivity in people with type 2 diabetes: findings of a randomized controlled study. *Free Radic Biol Med*. 2016;93:227–238. https://doi.org/10.1016/j.freeradbiomed.2016.01.006. Epub 2016 Jan 13 PMID: 26774673.
563. Gaby A, *"Vitamin C" in Nutritional Medicine*, 2nd ed: 107–108.
564. Gaby A, *Nutritional Medicine*, 2nd ed. (Chapter 213): Kidney Stones: 843
565. Beyl Jr RN, Hughes L, Morgan S. Update on importance of diet in gout. *Am J Med*. 2016;129(11):1153–1158. https://doi.org/10.1016/j.amjmed.2016.06.040. Epub 2016 Jul 22 PMID: 27452679.
566. Gao X, Curhan G, Forman JP, et al. Vitamin C intake and serum uric acid concentration in men. *J Rheumatol*. 2008;35(9):1853–1858. Epub 2008 May 1 PMID: 18464304.
567. Gaby A, *"Vitamin C" in Nutritional Medicine*, 2nd: 109.
568. Sylvester PW. Vitamin E and apoptosis. *Vitam Horm*. 2007;76:329–356. PMID: 17628180.
569. Miller 3rd ER, Pastor-Barriuso R, Dalal D, et al. Meta-Analysis: high-dosage Vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005;142(1):37–46. PMID: 15537682.
570. Wolf G. How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. *Nutr Rev*. 2006;64(6):295–299. PMID: 16808116.
571. Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of a- and γ-tocopherol. *Mol Aspects Med*. 2007;28(5–6):668–691. Epub 2007 Jan 11 PMID: 17316780.
572. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med*. 2014;72:76–90. https://doi.org/10.1016/j.freeradbiomed.2014.03.035. Epub 2014 Apr 3 PMID: 24704972.
573. Koh JM, Khang YH, Jung CH, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int*. 2005;16(10):1263–1271. Epub 2005 Feb 9 PMID: 15702263.
574. Kim BJ, Yu YM, Kim EN, et al. Relationship between serum hsCRP concentration and biochemical bone turnover markers in healthy pre- and postmenopausal women. *Clin Endocrinol (Oxf)*. 2007;67(1):152–158. Epub 2007 Apr 27 PMID: 17466002.
575. Ochi H, Takeda S. The two sides of vitamin E supplementation. *Gerontology*. 2015;61(4):319–326. https://doi.org/10.1159/000366419. Epub 2014 Nov 22 PMID: 25428288.
576. Hamidi MS, Corey PN, Cheung AM. Effects of vitamin E on bone turnover markers among US postmenopausal women. *J Bone Miner Res*. 2012;27(6):1368–1380. https://doi.org/10.1002/jbmr. 1566 PMID: 22308007.
577. Traber MG. Vitamin E and K interactions—a 50-year-old problem. *Nutr Rev*. 2008;66(11):624–629. https://doi.org/10.1111/j.1753-4887.2008.00123.x. PMID: 19019024.
578. Bender DA. Vitamin E: Tocopherols and Tocotrienols. In: Bender DA, ed. *Nutritional Biochemistry of the Vitamins*. New York: Cambridge University Press; 2003:109–130.
579. Shearer MJ, Newman P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. *J Lipid Res*. 2014;55(3):345–362. https://doi.org/10.1194/jlr.R045559. Epub 2014 Jan 31 PMID: 24489112.
580. Devaraj S, Leonard S, Traber M, et al. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med*. 2008;44(6):1203–1208. PMID: 18191645.
581. Kline K, Lawson K, Yu W, et al. Vitamin E and cancer. *Vitam Horm*. 2007;76:435–461. Review PMID: 17628185.
582. Munteanu A, Zingg JM. Cellular, molecular and clinical aspects of vitamin E on atherosclerosis prevention. *Mol Aspects Med*. 2007;28(5–6):538–590. Review PMID: 17825403.

583. Hanley DA, Adachi JD, Bell A, et al. Denosumab: mechanism of action and clinical outcomes. *Int J Clin Pract*. 2012;66(12):1139–1146. https://doi.org/10.1111/ijcp.12022. Epub 2012 Sep 12 PMID: 22967310.
584. Shi WQ, Liu J, Cao Y, et al. Association of dietary and serum vitamin E with bone mineral density in middle-aged and elderly Chinese adults: a cross-sectional study. *Br J Nutr*. 2016;115(1):113–120. https://doi.org/10.1017/S0007114515004134. Epub 2015 Oct 28 PMID: 26507315.
585. Peroni G, et al. Focus on pivotal role of dietary intake (diet and supplement) and blood levels of tocopherols and tocotrienols in obtaining successful aging. *Int J Mol Sci*. 2015;16(10):23227–23249. https://doi.org/10.3390/ijms161023227. PMID: 26404241.
586. Melhus H, Michaelsson K, Holmberg L, et al. Smoking, anti-oxidant vitamins, and the risk of hip fracture. *J Bone Miner Res*. 1999;14(1):129–135. PMID: 9893075.
587. Guralp O. Effects of vitamin E on bone remodeling in perimenopausal women: mini review. *Maturitas*. 2014;79(4):476–480. https://doi.org/10.1016/j.maturitas.2014.08.012. Epub 2014 Sep 6 PMID: 25248856.
588. Michaëlsson K, Wolk A, Byberg L, et al. Intake and serum concentrations of α-tocopherol in relation to fractures in elderly women and men: 2cohort studies. *Am J Clin Nutr*. 2014;99(1):107–114. https://doi.org/10.3945/ajcn.113.064691. PMID: 24225359.
589. Mata-Granados JM, Cuenca-Acebedo R, Luque de Castro MD, et al. Lower vitamin E serum levels are associated with osteoporosis in early postmenopausal women: a cross-sectional study. *J Bone Miner Metab*. 2013;31(4):455–460. https://doi.org/10.1007/s00774-013-0432-2. Epub 2013 Mar 28 PMID: 23536191.
590. Muhammad N, Luke DA, Shuid AN, et al. Tocotrienol supplementation in postmenopausal osteoporosis: evidence from a laboratory study. *Clinics (Sao Paulo)*. 2013;68(10):1338–1343. https://doi.org/10.6061/clinics/2013(10)08. PMID: 24212841.
591. Food and Nutrition Board. Institute of medicine. Vitamin E. In: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington: National Academy Press; 2000:186–283.
592. Pekmezci D. Vitamin E and immunity. *Vitam Horm*. 2011;86:179–215. https://doi.org/10.1016/B978-0-12-386960-9.00008-3. PMID: 21419272.
593. Serafini M. Dietary vitamin E and T cell-mediated function in the elderly: effectiveness and mechanism of action. *Int J Dev Neurosci*. 2000;18(4–5):401–410. Review PMID: 10817923.
594. Weber P, Bendich A, Machlin LJ. Vitamin E and human health: rationale for determining recommended intake levels. *Nutrition*. 1997;13(5):450–460. PMID: 9225339.
595. Meydani SN, Meydani M, Blumberg JB, et al. Assessment of the safety of supplementation with different amounts of vitamin E in healthy older adults. *Am J Clin Nutr*. 1998;68(2):311–318. PMID: 9701188.
596. Kalyan S, Huebbe P, Esatbeyoglu T, et al. Nitrogen-bisphosphonate therapy is linked to compromised coenzyme Q10 and vitamin E status in postmenopausal women. *J Clin Endocrinol Metab*. 2014;99(4):1307–1313. https://doi.org/10.1210/jc.2013-3648. Epub 2014 Jan 13 PMID: 24423355.
597. Thanabalasundaram G, Arumalla N, Tailor HD, et al. Regulation of differentiation of mesenchymal stem cells into musculoskeletal cells. *Curr Stem Cell Res Ther*. 2012;7(2):95–102. PMID: 22023628.
598. Laschober GT, Brunauer R, Jamnig A, et al. Age-specific changes of mesenchymal stem cells are paralleled by upregulation of CD106 expression as a response to an inflammatory environment. *Rejuvenation Res*. 2011;14(2):119–131. https://doi.org/10.1089/rej.2010.1077. Epub 2011 Jan 8 PMID: 21214384.
599. Whelan J. The health implications of changing linoleic acid intakes. *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3–5):165–167. https://doi.org/10.1016/j.plefa.2008.09.013. Epub 2008 Nov 5 PMID: 18990554.
600. Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Sci Nutr*. 2014;2(5):443–463. https://doi.org/10.1002/fsn3.121. Epub 2014 Jun 29 PMID: 25473503.
601. Adam O, Tesche A, Wolfram G. Impact of linoleic acid intake on arachidonic acid formation and eicosanoid biosynthesis in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2008;79(3–5):177–181. https://doi.org/10.1016/j.plefa.2008.09.007. Epub 2008 Oct 29 PMID: 18973995.
602. Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metab*. 2007;32(4):619–634. PMID: 17622276.
603. Brenna JT, Salem Jr N, Sinclair AJ, et al. alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2009;80(2–3):85–91. https://doi.org/10.1016/j.plefa.2009.01.004. Epub 2009 Mar 9 PMID: 19269799.
604. Poulsen RC, Moughan PJ, Kruger MC. Long-chain polyunsaturated fatty acids and the regulation of bone metabolism. *Exp Biol Med (Maywood)*. 2007;232(10):1275–1288. PMID: 17959840.
605. Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care*. 2004;7(2):137–144. PMID: 15075703.
606. Linus Pauling Institute Essential Fatty Acids http://lpi.oregonstate.edu/mic/other-nutrients/essential-fatty-acids#reference6. Accessed 5 February 2020.
607. Giltay EJ, Gooren LJ, Toorians AW, et al. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr*. 2004;80(5):1167–1174. PMID: 15531662.
608. Weaver KL, Ivester P, Chilton JA, et al. The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. *J Am Diet Assoc*. 2008;108(7):1178–1185. https://doi.org/10.1016/j.jada.2008.04.023. PMID: 18589026.
609. Simopoulos AP. Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications. *World Rev Nutr Diet*. 2009;100:1–21. https://doi.org/10.1159/000235706. Epub 2009 Aug 17 PMID: 19696523.
610. Kruger MC, Coetzer H, de Winter R, et al. Eicosapentaenoic acid and docosahexaenoic acid supplementation increases calcium balance. *Nutr Res*. 1995;15(2):211–219. https://doi.org/10.1016/0271-5317(95)92587-A. http://www.sciencedirect.com/science/article/pii/027153179592587A?np=y. Accessed 5 February 2020.
611. Kruger MC, Schollum LM. Is docosahexaenoic acid more effective than eicosapentaenoic acid for increasing calcium bioavailability? *Prostaglandins Leukot Essent Fatty Acids*. 2005;73(5):327–334. PMID: 16154334.
612. Järvinen R, Tuppurainen M, Erkkilä AT, et al. Associations of dietary polyunsaturated fatty acids with bone mineral density in elderly women. *Eur J Clin Nutr*. 2012;66(4):496–503. https://doi.org/10.1038/ejcn.2011.188. Epub 2011 Nov 23 PMID: 22113249.
613. Farina EK, Kiel DP, Roubenoff R, et al. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. *Am J Clin Nutr*. 2011;93(5):1142–1151. https://doi.org/10.3945/ajcn.110.005926. Epub 2011 Mar 2 PMID: 21367955.
614. Gómez Candela C, Bermejo López LM, Loria Kohen V. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: nutritional recommendations. *Nutr Hosp*. 2011;26(2):323–329. https://doi.org/10.1590/S0212-16112011000200013. PMID: 21666970.
615. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)*. 2008;233(6):674–688. https://doi.org/10.3181/0711-MR-311. Epub 2008 Apr 11 PMID: 18408140.
616. Papanikolaou Y, Brooks J, Reider C, et al. U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003-2008. *Nutr J*. 2014;13:31. https://doi.org/10.1186/1475-2891-13-31. PMID: 24694001.
617. Flock MR, Harris WS, Kris-Etherton PM. Long-chain omega-3 fatty acids: time to establish a dietary reference intake. *Nutr Rev*. 2013;71(10):692–707. https://doi.org/10.1111/nure.12071. Epub 2013 Oct 3 PMID: 24117792.
618. US Food and Drug Administration. *Letter Regarding Dietary Supplement Health Claim for omega-3 Fatty Acids and Coronary Heart Disease*. Washington, DC: USDA; 2000 European food safety authority; 2000. Docket No. 91N-0103.

619. Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA). docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA J*. 2012;10:2815–2882.
620. Froyland L, Bentsen H, Graff IE, et al. Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods. *Oslo: Steering Committee of the Norwegian Scientific Committee for Food Safety*. 2011.
621. Phillips MC. Apolipoprotein E isoforms and lipoprotein metabolism. *IUBMB Life*. 2014;66(9):616–623. https://doi.org/10.1002/iub1314. PMID: 25328986.
622. Chouinard-Watkins R, Plourde M. Fatty acid metabolism in carriers of apolipoprotein E epsilon 4 allele: is it contributing to higher risk of cognitive decline and coronary heart disease? *Nutrients*. 2014;6(10):4452–4471. https://doi.org/10.3390/nu6104452. PMID: 25333200.
623. Opperman M, Benade S. Analysis of the omega-3 fatty acid content of South African fish oil supplements: a follow-up study. *Cardiovasc J Afr*. 2013;24(8):297–302. https://doi.org/10.5830/CVJA-2013-074. PMID: 24240381.
624. Dyerberg J, Madsen P, Møller JM, et al. Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukot Essent Fatty Acids*. 2010;83(3):137–141. https://doi.org/10.1016/j.plefa.2010.06.007. PMID: 20638827.
625. Offman E, Davidson M, Abu-Rashid M, et al. Systemic bioavailability and dose Proportionality of omega-3 administered in free fatty acid form compared with ethyl ester form: results of a phase 1 study in healthy volunteers. *Eur J Drug Metab Pharmacokinet*. 2017. https://doi.org/10.1007/s13318-016-0398-2. Epub ahead of print PMID: 28116646.
626. Cederholm T, Cruz-Jentoft AJ, Maggi S. Sarcopenia and fragility fractures. *Eur J Phys Rehabil Med*. 2013;49(1):111–117. PMID: 23575205.
627. Hughes JM, Petit MA. Biological underpinnings of Frost's mechanostat thresholds: the important role of osteocytes. *J Musculoskelet Neuronal Interact*. 2010;10(2):128–135. PMID: 20516629.
628. Schwab P, Scalapino K. Exercise for bone health: rationale and prescription. *Curr Opin Rheumatol*. 2011;23(2):137–141. https://doi.org/10.1097/BOR.0b013e3283434501. PMID: 21178629.
629. Rudnicki MA, Williams BO. Wnt signaling in bone and muscle. *Bone*. 2015;80:60–66. https://doi.org/10.1016/j.bone.2015.02.009. PMID: 26453496. PMCID: PMC4600531.
630. McClung MR, Brown JP, Diez-Perez A, et al. Effects of 24 months of treatment with romosozumab followed by 12 months of denosumab or placebo in postmenopausal women with low bone mineral density: a randomized, double-blind, phase 2, parallel group study. *J Bone Miner Res*. 2018. https://doi.org/10.1002/jbmr. 3452. [Epub ahead of print] PMID: 29694685.
631. Moayyeri A. The association between physical activity and osteoporotic fractures: a review of the evidence and implications for future research. *Ann Epidemiol*. 2008;18(11):827–835. https://doi.org/10.1016/j.annepidem.2008.08.007. Epub 2008 Sep 21 PMID: 18809340.
632. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women: interaction of mechanical, hormonal and dietary factors. *Sports Med*. 2005;35(9):779–830. PMID: 16138787.
633. Martone AM, Lattanzio F, Abbatecola AM, et al. Treating sarcopenia in older and oldest old. *Curr Pharm Des*. 2015;21(13):1715–1722. PMID: 25633117.
634. Gregg EW, Pereira MA, Caspersen CJ. Physical activity, falls, and fractures among older adults: a review of the epidemiologic evidence. *J Am Geriatr Soc*. 2000;48(8):883–893. PMID: 10968291.
635. Feskanich D, Willett W, Colditz G. Walking and leisure-time activity and risk of hip fracture in postmenopausal women. *JAMA*. 2002;288:2300–2306.
636. Optimizing bone health: *Impact of nutrition, exercise and hormones Sports Science Exchange* 2001, https://www.gssiweb.org/en/sports-science-exchange/Article/sse-82-optimizing-bone-health-impact-of-nutrition-exercise-and-hormones. Accessed 5 February 2020.
637. Martyn-St James M, Carroll S. Meta-analysis of walking for preservation of bone mineral density in postmenopausal women. *Bone*. 2008;43(3):521–531. https://doi.org/10.1016/j.bone.2008.05.012. Epub 2008 May 26 PMID: 18602880.
638. Howe TE, Shea B, Dawson LJ, et al. Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev*. 2011;(7):CD000333. https://doi.org/10.1002/14651858.CD000333.pub2. PMID: 21735380.
639. Schmitt NM, Schmitt J, Dören M. The role of physical activity in the prevention of osteoporosis in postmenopausal women—an update. *Maturitas*. 2009;63(1):34–38. https://doi.org/10.1016/j.maturitas.2009.03.002. Epub 2009 Apr 7 PMID: 19356867.
640. Weaver CM. Calcium requirements of physically active people. *Am J Clin Nutr*. 2000;72(2 suppl l): 579S– 5784S PMID: 10919963.
641. Nguyen TV, Conter JR, Eisman JA. Osteoporosis in elderly men and women: effects of dietary calcium, physical activity, and body mass index. *J Bone Miner Res*. 2000;15:322–331. PMID: 10703935.
642. Burr DB, Yoshikawa T, Teegarden D, et al. Exercise and oral contraceptive use suppress the normal age-related increase in bone mass and strength of the femoral neck in women 18-31 years of age. *Bone*. 2000;27(6):855–863. PMID: 11113398.
643. Hartard M, Kleinmond C, Kirchbichler A, et al. Age at first oral contraceptive use as a major determinant of vertebral bone mass in female endurance athletes. *Bone*. 2004;35(4):836–841. PMID: 15454090.
644. Gaby A. "*Vitamin B6,*" *Chapter 18, Nutritional Medicine*. 2nd ed; 2017:89. https://www.amazon.com/Nutritional-Medicine-Second-Alan-Gaby/dp/1532322097. Accessed 5 February 2020.
645. Ertesvåg A, Naderi S, Blomhoff HK. Regulation B cell proliferation and differentiation by retinoic acid. Semin Immunol. 2009;21(1):36 -41. PMID: 18703353.
646. Calcium. *Australian Government National Health and Medical Research Council*. https://www.nrv.gov.au/nutrients/calcium. Accessed 5 February 2020.
647. Essential chemical elements for humans. Wikipedia: The Free Encyclopedia. https://en.wikipedia.org/wiki/Mineral_(nutrient)#Essential_chemical_elements_ for_humans. Accessed 5 February 2020.

Otitis Media

Kristaps Paddock, ND

OUTLINE

DIAGNOSTIC SUMMARY

Acute otitis media is characterized by the following:

- Earache or irritability
- History of recent upper respiratory infection or allergy
- Red, opaque, bulging eardrum with loss of the normal features
- Fever and chills

Chronic or serous otitis media is characterized by the following:

- Painless hearing loss
- Dull, immobile tympanic membrane

GENERAL CONSIDERATIONS

Otitis media occurs as a result of inflammation, swelling, or infection of the middle ear. Otitis media is of two types: chronic and acute.

Acute otitis media (AOM) is usually preceded by an upper respiratory infection or allergy. The organisms most commonly cultured from middle ear fluid during acute otitis media include *Streptococcus pneumoniae* (40%–50%), *Haemophilus influenzae* (30%–40%), and *Moraxella catarrhalis* (10%–15%). Chronic otitis media is also known as serous, secretory, or nonsuppurative otitis media (i.e., otitis media no effusion [NOE]); chronic otitis media with effusion (OME); and "glue ear," which involves a constant swelling of the middle ear (Fig. 204.1).

Acute otitis media affects two thirds of American children by 2 years of age, and chronic otitis media affects two thirds of children younger than the age of 6.[1] Otitis media is the most common diagnosis in children and is the leading cause of all visits to pediatricians. It is the main reason for antibiotic and surgical interventions in kids. Children diagnosed with otitis media during infancy are also at greater risk for developing late-onset atopy, such as allergic eczema and asthma during the school-age years. The more frequent the otitis, the stronger these associations.[2] A conservative estimate is that approximately $4 billion to $8 billion is spent annually on the medical and surgical treatment of earache in the United States.

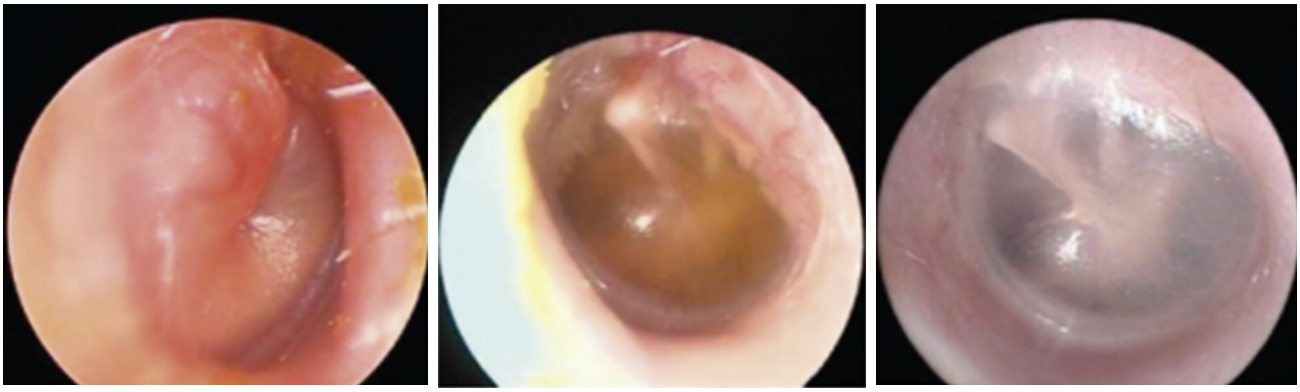
Standard Medical Treatment

The standard medical approach to otitis media in children is to administer antibiotics, analgesics (e.g., acetaminophen), and/or antihistamines. If the ear infection is long-standing and unresponsive to the drugs, surgery is performed. The surgery involves the placement of a tiny plastic myringotomy tube through the eardrum to facilitate the normal drainage of fluid into the throat via the eustachian tube. It is not a curative procedure because children with myringotomy tubes in their ears are more likely to have further problems with otitis media.

Myringotomies are currently being performed in nearly 1 million American children each year. It appears that the unnecessary surgery of the past, the tonsillectomy, has been replaced by this procedure. In fact, there is a direct correlation between the decline of the tonsillectomy and the rise of the myringotomy. More than 2 million myringotomy tubes are inserted into children's ears each year. In addition, there are also 600,000 tonsillectomies and adenoidectomies. These surgeries are unnecessary for most children.

A 1994 evaluation of the appropriateness of myringotomy tubes in children younger than 16 years of age in the United States found that only 42% were judged to have been appropriate.[3] These results mean that several hundred thousand children are subjected to a procedure that will do them little good and possibly significant harm.

Several well-designed studies have demonstrated no significant differences in the clinical course of acute otitis media when conventional treatments were compared with placebo. Specifically, no differences were found between nonantibiotic treatment, ear tubes, ear tubes with antibiotics, and antibiotics alone.[4–8] Interestingly, in some studies, children not receiving antibiotics did have fewer recurrences than those receiving antibiotics. This reduced recurrence rate is undoubtedly a



(a) AOM (b) OME (c) NOE

Fig. 204.1 Sample images from the three diagnostic categories of otitis media. (From Kuruvilla A, Shaikh N, Hobeman A, Kovacevic J. (2013). Automated diagnosis of otitis media: vocabulary and grammar. *Int J Biomed Imag.* 2013:327515. PubMed PMID: 23997759. (Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC3749602_IJBI2013-327515.001&query=otitis+media&req=4&npos=11 [accessed Sept 23, 2018]).

reflection of the suppressive effects antibiotics have on the immune system while also disturbing the normal flora of the upper respiratory tract.[9]

Because most children with acute otitis media (70%–90%) have spontaneous resolution within 7 to 14 days, antibiotics should not be the initial treatment routinely prescribed for all children.[8,10] Extensive reviews of the scientific literature on the value of antibiotics in the treatment of otitis media over the past 30 years have led to the following conclusions:

- The benefit of routine antimicrobial use for otitis media, judged by either short- or long-term outcomes, is unproven.
- Existing research offers no compelling evidence that children with acute otitis media routinely given antimicrobials have a shorter duration of symptoms, fewer recurrences, or better long-term outcomes than those who do not receive them.
- Although the prevention of mastoiditis and meningitis is a rationale for antimicrobial treatment, little evidence exists that routine treatment is effective for this purpose.
- Antimicrobials did not improve outcome at 2 months, and no differences in rates of recovery were found for either antimicrobial type or duration.

Although these results have been accepted by some U.S. pediatricians, others still rely heavily on antibiotics to treat otitis media. Instead of antibiotics, the recommendation from this group of experts was to use analgesics (e.g., acetaminophen) and for the caregiver to keep a close watch. Results of clinical trials have shown that more than 80% of children with acute otitis media respond to a placebo within 48 hours. Although analgesics may be of use to limit the child's pain, they have their own toxicity profile. Therefore it is recommended that other proven pain-relieving options like botanical eardrops be used as a replacement analgesic (see later discussion).

Risks of antibiotics include allergic reactions, gastric upset, accelerated bacterial resistance, and unfavorable changes in nasopharyngeal bacterial flora. Antibiotics not only fail to eradicate the organisms but can induce middle-ear superinfection. Moreover, antibiotic prescribing can increase the rate of return office visits and the likelihood of seeking medical care for future illness.[11] Additionally, studies on concomitant antibiotic and steroid treatment have revealed poor results on long-term efficacy for chronic otitis media.[12]

The position of the American Academy of Otolaryngology–Head and Neck Surgery states that there is no evidence indicating that systemic antibiotics alone can improve treatment outcome and that these agents should not be used save the situation of an underlying systemic infection.[13] According to many experts as well as the World Health Organization, we are coming dangerously close to arriving at a "postantibiotic era" in which many infectious diseases will once again become almost impossible to treat because of an overreliance on antibiotics (see Chapter 136 for further information).[14]

Otitis media is typically a self-limited disease. High rates of spontaneous resolution have been well documented in the medical literature. Three meta-analyses independently found that approximately 80% of children with acute otitis media had spontaneous clinical relief within 2 to 14 days. Some studies of children younger than 2 years of age do suggest a lower spontaneous resolution of about 30% after a few days.[11]

The risks and failure with antibiotics, when coupled with the high rate of spontaneous resolution and the high recurrence rate of otitis media following the insertion of ear tubes, suggest that conservative (nonantibiotic, nonsurgical) treatment alone may reduce the incidence of otitis media and decrease the associated yearly financial costs. To examine this concept, the authors of one study gave the caregivers of children with nonsevere acute otitis media a "safety prescription" of antibiotics to be filled only if there was no improvement within 2 days. This method of "wait and see" reduced median antibiotic prescriptions by 31%, compared with 12% in a control practice.[15]

Children with otitis media must be evaluated individually, and appropriate follow-up, including physician–family communication, should be devised and assured before a decision not to use these procedures is made. Special circumstances in the interest of preventing hearing loss–induced developmental delays would suggest a more appropriate use of ear tubes.

Finally, pneumococcal and viral vaccines have been designed but have also shown little benefit, probably due to the multifactorial nature of this condition.[11] Given the inherent risks and complications, vaccinations do not appear to be warranted at this time.

Causes

The primary risk factors for otitis media include the following[1]:

- Day-care attendance
- Wood-burning stoves
- Parental smoking or exposure to other secondhand smoke
- Food allergies
- Not being breastfed

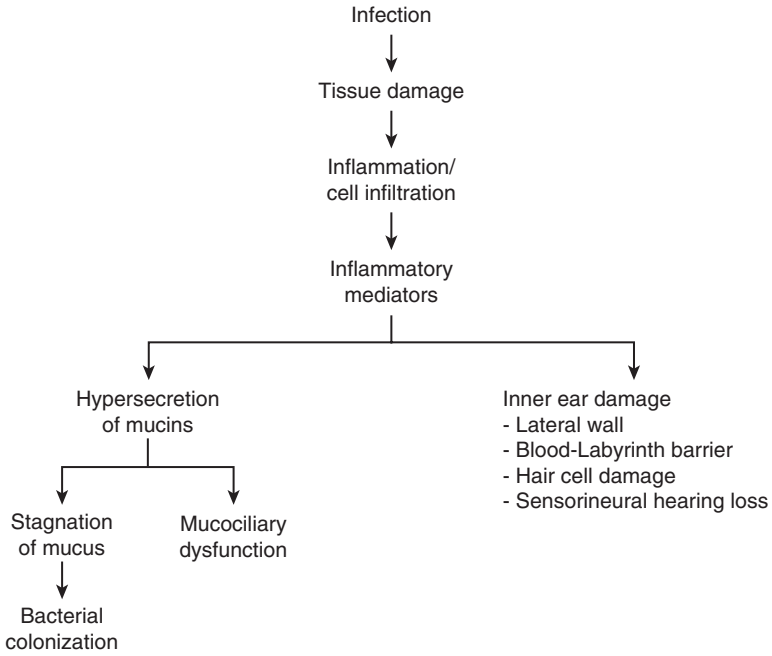


Fig. 204.2 Pathological effects of inflammatory mediators in otitis media. (From Juhn SK, Jung MK, Hoffman MD, Drew BR, Preciado DA, Suasen NJ, Jung TT, Kim BH, Park SY, Lin J, Ondrey FG, Mains DR, Huang T. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. *Clin Exp Otorhinolar.* 2008;1[3]:117–138. PubMed PMID: 19434244. (Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC2671742_ceo-1-117-g003&query=otitis+media&req=4&npos=59 [accessed Sept 23, 2018]).

- Pacifier use[11]
- Previous antibiotic use
- Winter season
- Underlying rhinitis, cleft palate, and Down syndrome

These factors share a common mechanism: abnormal eustachian tube function, which appears to be the underlying cause in virtually all cases of otitis media. The eustachian tube regulates gas pressure in the middle ear, protects the middle ear from nose and throat secretions and bacteria, and clears fluids from the middle ear. Swallowing causes active opening of the eustachian tube owing to the action of the surrounding muscles. Infants and small children are particularly susceptible to eustachian tube problems because the tube is smaller in diameter and more horizontal.

Obstruction of the eustachian tube leads first to fluid buildup and then, if the bacteria present are pathogenic and the immune system is impaired, to bacterial infection (Fig. 204.2). Obstruction results from collapse of the tube (due to weak tissues holding the tube in place, an abnormal opening mechanism, or both), blockage with mucus in response to allergy or irritation, swelling of the mucous membrane, or infection.

Otitis media may also have a genetic component. In twin studies, monozygotic twins tend to have a higher concordance rate in otitis media histories than dizygotic twins.[11] Although immunoglobulin markers[16] and human leukocyte antigen (HLA)[17] have also been a focus of research, no specific genetic associations have been elucidated thus far. Research has also shown that children with blood type A have about a 50% higher rate of infection and are susceptible to more severe and repeated bouts of ear infection. Children whose mothers are blood type A who have ear infections before 1 year of age have an astounding 2677% greater risk of having recurrent ear infections.[18]

Several studies have also linked otitis media with childhood obesity. This correlation has been shown for several childhood age groups and occurs independently of socioeconomic or other clinical factors.[19–22] However, these studies demonstrate association rather than causation, and it is not currently clear that obesity is a risk factor for developing otitis media. One study posited that otitis media may be a factor in the development of obesity; the proposed mechanism of action is that otitis media may cause changes in taste function, specifically reduction in the ability to taste sweet and salty foods, and that these changes are associated with the development of obesity.[23] Given the prevalence of obesity in the United States, special consideration should be given to the successful treatment of otitis media, along with other standard dietary recommendations regarding obesity.

Contemporary pesticides, such as triazines or organophosphates, possess immunotoxic properties. One study aimed to determine whether prenatal environmental exposure to these current-use pesticides was associated with otitis media during the first 2 years of life among children from the PELAGIE mother–child cohort.[24] Children whose mothers reported an organic diet during pregnancy had a reduced risk of otitis media, and the presence in maternal urine of dealkylated triazine metabolites was positively associated with recurrent otitis media. In addition, passive smoking poses a significant risk for the development of otitis media, with the child's hazard of otitis media during the first 4 years of life being associated with mother's current smoking habits (hazard ratio [HR] = 2.47, 95% confidence interval [CI]; 1,45–4.21), mother's previous smoking habits (HR = 2.00, 95% CI; 1.19–3.36), and number of smokers in the home (HR = 1.17, 95% CI, 1.05–1.31).[25]

THERAPEUTIC CONSIDERATIONS

The primary treatment goals are to ensure patency of the eustachian tubes and to promote drainage by identifying and addressing causative factors. Supporting the immune system is also

important. The recommendations that follow should be used along with the recommendations for immune support provided in Chapter 136.

Bottle-Feeding

Recurrent ear infection is strongly associated with early bottle-feeding, whereas breastfeeding (for a minimum of 3 months) has a protective effect.[26,27] Whether this is due to cow's milk allergy or to the protective effect of human milk against infection has not yet been conclusively determined. It is likely a combination of the two. In addition, bottle-feeding while a child is lying on his or her back (bottle-propping) leads to regurgitation of the bottle's contents into the middle ear and should be avoided.

Because of its high antibody content, which helps to inhibit infectious agents, human milk offers protection from viral (e.g., respiratory syncytial virus, rhinovirus, or influenza A) or bacterial (e.g., *S. pneumoniae, M. catarrhalis,* or *H. influenzae*) agents.[28] The thymus gland of breastfed infants is also roughly 20 times larger than that of formula-fed infants.[29]

Food Allergy

The role of allergy as the major cause of chronic otitis media has been firmly established in the research literature.[30–35] Most studies show that 85% to 93% of children with chronic otitis media have allergies: 16% to inhalants only, 14% to food only, and 70% to both.

Prolonged breastfeeding may prevent otitis media by the avoidance of food allergies, particularly if the mother avoids sensitizing foods (i.e., those to which she is allergic) during pregnancy and lactation. In addition to breastfeeding, the exclusion or limited consumption of the foods to which children are most commonly allergic (e.g., wheat, egg, peanuts, corn, citrus, chocolate, and dairy) is also valuable, particularly during the first 9 months.

Because a child's digestive tract is quite permeable to food antigens (especially during the first 3 months), careful control of eating patterns (e.g., no frequent repetitions of any food, avoidance of the common allergenic foods, and the introduction of new foods in a controlled manner by introducing one new food at a time and carefully watching for a reaction) will reduce and prevent the development of food allergies.

An allergic reaction causes blockage of the eustachian tube by two mechanisms: (1) inflammatory swelling of the mucous membranes lining the tube; and (2) inflammatory swelling of the nose, causing the "Toynbee phenomenon" (swallowing when both mouth and nose are closed, forcing air and secretions into the middle ear). The middle and inner ear are immunologically responsive, and this includes responsiveness to food hypersensitivities.[36] In chronic earaches, an allergic cause should always be considered, and the offending allergens should be determined and avoided.

One illustrative study of 153 children with earaches demonstrated that 93.3% of the children were allergic to foods, inhalants, or both.[30] When 119 of these children were treated with serial dilution titration therapy for inhalant sensitivities and an elimination diet for food allergens, 92% had improvement after 12 months. This outcome compares favorably with that of the surgically treated control group (ear tubes and, as indicated, removal of the tonsils and adenoids), which showed only a 52% response.

In another study, a total of 104 children ranging in age from 1.5 to 9 years with recurrent otitis media were evaluated for food allergy by means of skin-prick testing, specific immunoglobulin E (IgE) tests, and food challenges.[35] The results indicated a statistically significant association between food allergy and recurrent otitis media in 81 of the 104 patients (78%). The elimination diet led to a significant amelioration of chronic otitis media in 70 of 81 patients (86%) as assessed by detailed clinical evaluation. The challenge diet with the suspected offending foods provoked a recurrence of serous otitis media in 94% of patients.

TABLE 204.1 Food Allergy in Children With Chronic Otitis Media

Food	No. of Patients	Percentage of Patients
Cow's milk	31	38
Wheat	27	33
Egg white	20	25
Peanuts	16	20
Soy	14	17
Corn	12	15
Tomatoes	4	5
Chicken	4	5
Apple	3	4

TABLE 204.2 The Frequency Distribution According to Number of Food Allergens

No. of Food Allergens	Patients/Total	Percentage
1	11/81	13.6
2–4	66/81	81.5
5–7	3/81	3.7
8–10	1/81	1.3

The frequency distribution of allergies to individual foods is listed in Table 204.1, and the frequency distribution according to number of food allergens is listed in Table 204.2.

Reflux

Studies strongly suggest a link between gastroesophageal reflux disease (GERD) in children with otitis media, as well as adults.[37,38] Research demonstrates significant improvements in disease burden from otitis media and hearing loss with acid-blocking drug therapy. In one study, 47 patients 6 months to 7 years of age, average age 18.5 months, were prescribed a 15-mg dose of lansoprazole in the morning and, depending on age, ranitidine (4 mg/kg per day) or nizatidine (5 mg/kg per day) at bedtime.[39] The Pediatric Reflux Finding Score, which is used to assess the severity of extraesophageal reflux via fiberoptic laryngoscopy, showed significant improvements at visits 2 and 3. Of note, although there were improvements, there was also a high dropout rate in this study, possibly owing to medication side effects.

Helicobacter pylori, a bacterium whose overgrowth causes stomach ulceration, has also been identified in effusion samples taken from patients suffering from otitis media. The presence of *H. pylori* in effusion is associated with a diagnosis of GERD. It has been postulated that this bacterium, introduced to the inner ear by reflux, may be linked to chronic otitis media, but this has not been clearly demonstrated.[40,41] Pepsin and pepsinogen have also been identified in middle ear effusion and have been postulated to have been introduced by reflux, causing otitis media.[42]

From a naturopathic standpoint, the idea that digestive function plays a role in otitis media is important. Although the use of the medications mentioned previously is to be considered with caution, the use of treatments such as food allergy avoidance, a focus on healthful foods, and measures to support digestive health are likely to produce similar positive effects.

Thymus Gland Extract

The thymus gland secretes a family of hormones that act on white blood cells to ensure their proper development and function. Studies with calf thymus extracts given orally have demonstrated impressive clinical results in various clinical conditions in children.[43–45] Specifically, thymus extracts have been shown to improve immune function, decrease children's food allergies, and improve a child's resistance to chronic respiratory infections. For more information, see Chapter 136.

Naturopathic Ear Drops

Even though most cases of otitis media resolve spontaneously, otalgia can often persist during the period of improvement, sometimes motivating anxious parents to request unnecessary antibiotic therapy. Naturopathic botanical ear drops have been shown to be as effective as either antibiotic and anesthetic drops and offer a much less toxic approach to pain management.[46,47]

In a double-blind outpatient trial, one group from Israel studied 171 children ages 5 to 18 who were randomly assigned to receive treatment with naturopathic herbal extract ear drops (NHEDs) or anesthetic ear drops (amethocaine and phenazone) with or without amoxicillin (80 mg/kg/day).[46] The NHED was a combination of *Calendula officinalis* flowers (marigold, 28%), *Hypericum perforatum* complete herb (St. John's wort, 30%), and *Verbascum thapsus* flowers (mullein, 25%), with the essential oils *Allium sativum* (garlic, 0.05%) (10%), *Lavendula officinalis* (lavender, 5%), and tocopherol acetate oil (vitamin E, 2%). Dosage was 5 drops three times daily. All groups had a statistically significant improvement in ear pain over the course of the 3 days, with a 95.9% reduction in the NHED-alone group. The NHED plus antibiotics produced a 90.9% diminution in pain. The anesthetic alone and anesthetic with antibiotics produced 84.7% and 77.8% reductions, respectively.

Considering the positive outcomes in this study and significantly lower toxicity profile than those of conventional antibiotic or anesthetic medications, naturopathic eardrops may be the superior choice for children with ear pain–associated otalgia. As a side note, there are numerous other botanical anodynes not employed in this formula that would be worthy of study as well.

Echinacea

Echinachea is well known to treat upper respiratory infection, and in vitro studies of *Echinacea* suggest a stimulatory effect of *Echinacea purpurea* preparations on various components of the cytokine cascade.[48] A few evaluations in adults show improvement in cold severity and duration,[49] whereas other studies do not show benefit.[50] One analysis suggests that *Echinacea* shows no benefit and is associated with a borderline increased risk of infection compared with a placebo. Despite randomization, this study did have notable disparities among treatment groups regarding frequency of confounding factors, including frequency of otitis media before study entry and day-care attendance. The children on *Echinacea* in this study's sham manipulation group had double the incidence of upper respiratory infections in the previous year compared with the placebo arm and were 50% more likely to be in day care. These confounding factors are so significant that the results should be scrutinized. As a monotherapy, *Echinacea's* efficacy is unclear. Given this botanical's long history of benefit, it is likely that it is a helpful ally in combination with other natural treatments.

Xylitol

Xylitol is a commonly used sweetener known mostly for its anticariogenic properties. It is a sugar alcohol derived mainly from birch and other hardwood trees and has demonstrated inhibition of *S. pneumoniae.* Two randomized trials illustrated xylitol's ability to reduce acute otitis media incidence by 40%. In one study of 306 day-care children with recurrent acute otitis media, 157 children were given xylitol (8.4 g/day) chewing gum, and 149 children were given a sucrose control gum.[51] During the 2 months of the trial, at least one event of acute otitis media was experienced by 20.8% of the children who received sucrose compared with only 12.1% of those receiving the chewing gum containing xylitol. Significantly fewer antimicrobials were prescribed among those receiving xylitol.

In a second randomized, controlled, blinded trial, 857 healthy children were randomized to one of five treatment groups to receive control syrup, xylitol syrup, control chewing gum, xylitol gum, or xylitol lozenges for 3 months.[52] The daily dose of xylitol varied from 8.4 g (chewing gum) to 10 g (syrup). Although at least one event of otitis was experienced by 41% of the 165 children who received control syrup, only 29% of the 159 children receiving xylitol syrup were affected. Likewise, compared with control subjects, the occurrence of otitis decreased by 40% in the children who received xylitol chewing gum and 20% in the lozenge group. Thus the occurrence of acute otitis media during the follow-up period was significantly lower in those who received xylitol syrup or gum, and these children required antimicrobials less often than did controls.

Xylitol has been criticized as a practical method because studies suggest dosing needs to be four to five times per day, which may decrease compliance and therefore overall effectiveness. Although two children in the xylitol group experienced diarrhea, no other untoward effects were reported for this safe and useful treatment.

Sinupret

Sinupret is an herbal combination originating in Germany that consists of sorrel (aerial) *(Rumex acetosa)* 36 mg, European elder (flower) *(Sambucus nigra)* 36 mg, Cowslip (flower with calyx) *(Primula veris)* 36 mg, European vervain (aerial) *(Verbena officinalis)* 36 mg, and gentian (root; *Gentiana lutea)* 12 mg.

One study of 64 children evaluated 32 children using amoxicillin alone and 32 using amoxicillin plus Sinupret.[53] The Sinupret seemed to have more effective antibiotic properties and reduced the frequency of complications. A second integrative study of 40 children with a mean age of 4.5 years found similar results.[54] This herbal combination may be a reasonable choice for children already started on antibiotic medication.

Vitamin A

Vitamin A plays a role in the prevention of oxidative tissue damage, immune system function, and mucous membrane integrity. Although it has not been studied in children, animal studies have shown that pretreatment with vitamin A before *S. pneumoniae* inoculation conferred protection by increasing antioxidant enzyme activity and reducing the formation of malondialdehyde and nitric oxide.[55]

Vitamin D

Vitamin D deficiency has long been associated with a variety of health conditions and has drawn attention for its role in immune system regulation, having been studied for a number of immune-related conditions. Several studies have found vitamin D levels to be low in patients suffering from both acute and chronic otitis media, compared with controls, suggesting a potential role for vitamin D supplementation in the prevention and treatment of otitis media.[56–58] Furthermore, a double-blind, placebo-controlled study of 116 children with a history of recurrent acute otitis media found that treating vitamin D deficiency with oral administration of vitamin D at a level of 1000 IU per day reduced the risk of acute otitis media.[59] The study also found that the likelihood of acute otitis media was lowest in those children who achieved serum vitamin D levels of at least 30 ng/mL.

Probiotics

Probiotics of various species and strains have been studied for their potential role in preventing and treating otitis media. These bacteria may have a role in immune regulation at mucosal membranes and may play a role in colony-forming activity in the upper respiratory tract.

Bifidobacterium animalis subspecies *lactis* BB-12 has been studied in the prevention of upper respiratory tract infections and acute otitis media. One study reported that supplementation of infants with this strain of bacteria lowers the risk of upper respiratory tract infection but does not appear to lower the risk of otitis media.[60,61] Upper respiratory tract infections may cause otitis media, especially when these infections become chronic, and there may be a role for this bacteria in prevention. However, further research is needed to confirm this.

In vitro studies of *Lactobacillus rhamnosus* GG have shown that it can inhibit adherence of *S. pneumoniae* to human epithelial cells and therefore may have a role to play in the prevention of otitis media.[62] Further, supplementation with *L. rhamnosus* GG has been shown to result in colonization of adenoid tissue and the middle ear. However, those studies that demonstrated colonization also failed to display differences in symptoms or inhibition of pathogenic bacteria and viruses.[63,64] A meta-analysis of four randomized controlled trials involving 1805 participants found that *L. rhamnosus* GG administration was associated with reduced incidence of acute otitis media, upper respiratory infections, and antibiotic treatments.[65] The variance in these studies warrants further research.

Streptococcus salivarius K12 has been shown by several studies of school-age children to reduce the incidence of acute otitis media and streptococcal pharyngitis. One study of 222 children in their first year of kindergarten found that *S. salivarius* K12 significantly reduced episodes of acute otitis media during a 6-month treatment period and a 3-month follow-up period.[66] Another study of 133 school-aged children found that *S. salivarius* K12 reduced the incidence of pharyngotonsillitis by 90% and the incidence of acute otitis media by 70%.[67] A study of a related strain, *S. salivarius 24SMB,* administered with *Streptococcus oralis* 89a, showed that it reduced the incidence of acute otitis media as well.[68]

Humidifiers

Humidifiers are popular treatments for otitis media and upper respiratory tract infections in children. According to a 1994 study that evaluated the role of low humidity in this disorder, this may be justified.[69] Using a rat model, the study examined the effect of low humidity on the middle ear. Twenty-three rats were housed for 5 days in a low-humidity environment (10%–12% relative humidity), and 23 control rats were housed at 50% to 55% relative humidity. Microscopic ear examinations were graded for otitis media before testing and on test days 3 and 5. The lining of the middle ears and eustachian tubes were examined by biopsy. Significantly more effusions were observed in the low-humidity group on both days 3 and 5, but biopsy results were similar in both groups. Possible explanations are that low humidity may induce nasal swelling and reduce ventilation of the eustachian tube or that it may dry the eustachian tube lining, which could lead to an inability to clear fluid as well as to increased secretions. The mast cells residing in the lining of the eustachian tubes may also come into play by releasing histamine and producing swelling.

Although preliminary, this research indicates that increasing humidity with the help of a humidifier may be an important goal in the treatment of otitis media with effusion.

Osteopathic Manipulation

One case study reports the benefits of osteopathic manipulation to decrease the frequency of otitis media in children with recurrent disease.[70] However, another randomized controlled trial using up to five osteopathic manipulative treatments over a 3-month period did not show benefit.[71] The osteopathic approach acknowledges abnormal structural dynamics, which can contribute to change in function. These dynamics may be modulated with cranial manipulative work.[72,73] In young patients with structural dysfunction, this may be a beneficial treatment in conjunction with other therapies suggested in this chapter.

Homeopathy

One randomized, double-blind, placebo-controlled pilot study was conducted in a private pediatric practice with 75 children aged 18 months to 6 years.[74] All children had middle ear effusion and ear pain, fever, or both for no more than 36 hours. Each child received either an individualized homeopathic medicine or a placebo three times daily by mouth for 5 days or until symptoms subsided. Results demonstrated a statistically significant decrease in symptoms using homeopathy over placebo and nonstatistically significant fewer treatment failures in the group receiving homeopathy.

Similarly, a second study found excellent results regarding the homeopathic pain-reduction rate; that is, pain was reduced by almost 2½ times over placebo controls, with 39% of the patients improving after 6 hours and another 33% after 12 hours. A follow-up study using a different homeopathic agent was given if results were not seen in the first 6 hours.[75] A German prospective study has also found comparable results in evaluating homeopathic treatment versus conventional therapies.[76]

More clinical trials are necessary to elucidate the optimal uses of homeopathy. In the meantime, homeopathy is proving to be an effective and extremely safe option to consider in the treatment of otitis media.

THERAPEUTIC APPROACH

The key factor in the natural approach to chronic otitis media in children appears to be the recognition and elimination of allergies, particularly food allergies, as well as support for the immune system and digestive function.

Diet

Because it is usually not possible to determine the exact allergen during an acute attack, the most common allergic foods should be eliminated from the diet. These include:

- Milk and other dairy products
- Eggs
- Wheat
- Corn
- Oranges
- Peanuts
- Chocolate

Concentrated simple carbohydrates (e.g., sugar, honey, dried fruit, concentrated fruit juice) should also be eliminated because they inhibit the immune system. These simple dietary recommendations bring relief to most children in a matter of days.

Nutritional Supplements

- Children's multivitamin and mineral formula
- Vitamin A: 50,000 IU/day for up to 2 days in children below 6 years of age and 4 days in children above age 6
- Beta-carotene (natural mixed carotenoids): age in years × 20,000 IU/day (up to 200,000 IU/day)
- Vitamin C: age in years × 50 mg every 2 hours

- Bioflavonoids: age in years × 50 mg every 2 hours
- Zinc: age in years × 2.5 mg a day (up to 30 mg)
- Thymus extract: the equivalent of 120 mg pure polypeptides with molecular weights less than 10,000 or roughly 500 mg of the crude polypeptide fraction a day

Botanical Medicines

- *Echinacea* species: One half the adult dosage is appropriate for children younger than 6 years of age. The full adult dosage is appropriate for children above age 6 (*Echinacea* is safe for children). The following doses can be given up to three times a day:
  - Dried root (or as tea): 0.5 to 1 g
  - Freeze-dried plant: 325 to 650 mg
  - Juice of aerial portion of *Echinacea purpurea* stabilized in 22% ethanol: 2 to 3 mL
  - Tincture (1:5): 2 to 4 mL
  - Fluid extract (1:1): 2 to 4 mL
  - Solid (dry powdered) extract (6.5:1 or 3.5% echinacoside): 150 to 300 mg
- Xylitol: approximately 8 g/day as either chewing gum chewed throughout the day or 10 g of the syrup per day in divided doses
- Naturopathic eardrop formula: five drops in the affected ear three times daily
- Sinupret: as adjunctive treatment when antibiotics are employed
- Consider cranial manipulative work

Physical Medicine

Local application of heat is often helpful in reducing discomfort. It can take the form of a hot pack or the application of warm oil (especially mullein oil). These treatments help to reduce pressure in the middle ear and promote fluid drainage.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Daly KA. Epidemiology of otitis media. *Otolaryngol Clin North Am*. 1991;24:775–786.
2. MacIntyre EA, Chen CM, Herbarth O, et al. Early-life otitis media and incident atopic disease at school age in a birth cohort. *Pediatr Infect Dis J*. 2010 ;29(12):e96–e99.
3. Kleinman LC, Kosecoff J, Dubois RW, et al. The medical appropriateness of tympanostomy tubes proposed for children younger than 16 years in the United States. *JAMA*. 1994;271:1250–1255.
4. Bluestone CD. Otitis media in children: to treat or not to treat? *N Engl J Med*. 1982;306:1399–1404.
5. van Buchem FL, Dunk JH, van't Hof MA. Therapy of acute otitis media: myringotomy, antibiotics, or neither? *Lancet*. 1981;2:883–887.
6. Williams RL, Chalmers TC, Stange KC, et al. Use of antibiotics in preventing recurrent acute otitis media and in treating otitis media with effusion: a meta-analytic attempt to resolve the brouhaha. *JAMA*. 1993;270:1344–1351.
7. Rosenfeld RM, Vertrees JE, Carr J, et al. Clinical efficacy of antimicrobial drugs for acute otitis media: metaanalysis of 5400 children from thirty-three randomized trials. *J Pediatr*. 1994;124:355–367.
8. Froom J, Culpepper L, Jacobs M, et al. Antimicrobials for acute otitis media? A review from the International Primary Care Network. *Br Med J*. 1997;315:98–102.
9. Del Castillo F, Baquero-Artigao F, Garcia-Perea A. Influence of recent antibiotic therapy on antimicrobial resistance of *Streptococcus pneumoniae* in children with acute otitis media in Spain. *Pediatr Infect Dis J*. 1998;17: 94–97.
10. Scottish Intercollegiate Guidelines Network. *Diagnosis and management of childhood otitis media in primary care: a national clinical guideline.* http://www.sign.ac.uk/pdf/sign66.pdf. Accessed February 13, 2011.
11. Rovers MM, Schilder AG, Zielhuis GA, et al. Otitis media. *Lancet*. 2004;363:465–473.
12. Mandel EM, Casselbrant ML, Rockette HE, et al. Systemic steroid for chronic otitis media with effusion in children. *Pediatrics*. 2002;110:1071–1080.
13. Hannley MT, Denneny III JC, Holzer SS. Use of ototopical antibiotics in treating three common ear diseases. *Otolaryngol Head Neck Surg*. 2000;122:934–940.
14. Woodhead M. Antibiotic resistance. *Br J Hosp Med*. 1996;56:314–315.
15. Cates C. An evidence based approach to reducing antibiotic use in children with acute otitis media: controlled before and after study. *BMJ*. 1999;318:715–716.
16. Kelly KM. Recurrent otitis media: genetic immunoglobulin markers in children and their parents. *Int J Pediatr Otorhinolaryngol*. 1993;25:279–280.
17. Kalm O, Johnson U, Prellner K. HLA frequency in patients with chronic secretory otitis media. *Int J Pediatr Otorhinolaryngol*. 1994;30:151–157.
18. Gannon MM, Jagger C, Haggard MP. Maternal blood group in otitis media with effusion. *Clin Otolaryngol*. 1994;19:327–331.
19. Kim SH, Park DC, Byun JY, Park MS, Cha CI, Yeo SG. The relationship between overweight and otitis media with effusion in children. *Int J Obes (Lond)*. 2011;35(2):279–282.
20. Nelson HM, Daly KA, Davey CS, Himes JH, Synder DJ, Bartoshuk LM. Otitis media and associations with overweight status in toddlers. *Physiol Behav*. 2011;28(5):511–517.
21. Kuhle S, Kirk SF, Ohinmaa A, Urschitz MS, Veugelers PJ. The association between childhood overweight and obesity and otitis media. *Pediatr Obes*. 2012;7(2):151–157.
22. Kaya S, Selimoglu E, Cureoglu S, Selimoglu MA. Relationship between chronic otitis media with effusion and overweight or obesity in children. *J Laryngol Otol*. 2017;131(10):866–870.
23. Shin IH, Park DC, Kwon C, Yeo SG. Changes in taste function related to obesity and chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg*. 2011;137(3):242–246.
24. Buscali C, Chevrier C, Serrano T, et al. Prenatal pesticide exposure and otitis media during early childhood in the PELAGIE mother-child cohort. *Occup Environ Med*. 2015;72(12):837–844. PubMed PMID: 26347056.
25. Jensen RG, Koch A, Homoe P, Bjerregaard P. Tobacco smoke increases the risk of otitis media among Greenlandic Inuit children while exposure to organochlorines remain insignificant. *Environ Int*. 54:112–118. PubMed PMID: 23434818.
26. Saarinen UM. Prolonged breast feeding as prophylaxis for recurrent otitis media. *Acta Ped Scand*. 1982;71:567–571.
27. Uhari M, Mäntysaari K, Niemelä M. A meta-analytic review of the risk factors for acute otitis media. *Clin Infect Dis*. 1996;22:1079–1083.
28. Breast Editor. feeding prevents otitis media. *Nutr Rev*. 1983;41:241–242.
29. Hasselbalch H, Jeppesen DL, Engelmann MD, et al. Decreased thymus size in formula-fed infants compared with breastfed infants. *Acta Pediatr*. 1996;85:1029–1032.
30. McMahan JT, Calenoff E, Croft DJ, et al. Chronic otitis media with effusion and allergy: modified RAST analysis of 119 cases. *Otol Head Neck Surg*. 1981;89:427–431.
31. Viscomi GJ. Allergic secretory otitis media: an approach to management. *Laryngoscope*. 1975;85:751–758.
32. Van Cauwenberge PB. The role of allergy in otitis media with effusion. *Ther Umschau*. 1982;39:1011–1016.
33. Bellionin P, Cantani A, Salvinelli F. Allergy: a leading role in otitis media with effusion. *Allergol Immunol*. 1987;15:205–208.
34. Hurst DS. Association of otitis media with effusion and allergy as demonstrated by intradermal skin testing and eosinophil protein levels in both middle ear effusions and mucosal biopsies. *Laryngoscope*. 1996;106:1128–1137.
35. Nsouli TM, Nsouli SM, Linde RE, et al. Role of food allergy in serous otitis media. *Ann Allergy*. 1994;73:215–219.
36. Ramakrishnan JB. The role of food allergy in otolaryngology disorders. *Curr Opin Otolaryngol Head Neck Surg*. 2010;18(3):195–199.
37. Miura MS, Mascaro M, Rosenfeld RM. Association between otitis media and gastroesophageal reflux: a systematic review. *Otolaryngol Head Neck Surg*. 201;146(3):345–352.
38. Sone M, Kato T, Suzuki Y, et al. Relevance and characteristics of gastroesophageal reflux in adult patients with otitis media with effusion. *Auris Nasus Larynx*. 2011;38(2):203–207.
39. McCoul Edward D, Goldstein Nira A, Bernard Koliskor, et al. A prospective study of the effect of gastroesophageal reflux disease treatment on children with otitis media. *Arch Otolaryngol Head Neck Surg*. 2011;137(1): 35–41.
40. Park CW, Chung JH, Min HJ, et al. *Helicobacter pylori* in middle ear of children with otitis media with effusion. *Chin Med J (Engl)*. 2011;124(24):4275–4278.
41. Boronat-Echeverria N, Aguirre-Mariscal H, Carmolinga-Ponce M, et al. *Helicobacter pylori* detection and clinical symptomatology of gastroesophageal reflux disease in pediatric patients with otitis media with effusion. *Int J Pedatr Otorhinolargyngol*. 2016;87:126–129.
42. Luo HN, Yang QM, Sheng Y, et al. Role of pepsin and pepsinogen: linking laryngopharyngeal reflux with otitis media with effusion in children. *Laryngoscope*. 2014;124(7):E294–E300.
43. Fiocchi A, Borella E, Riva E, et al. A double-blind clinical trial for the evaluation of the therapeutical effectiveness of a calf thymus derivative (Thymomodulin) in children with recurrent respiratory infections. *Thymus*. 1986;8:831–839.
44. Genova R, Guerra A. Thymomodulin in management of food allergy in children. *Int J Tissue React*. 1986;8:239–242.
45. Cazzola P, Mazzanti P, Bossi G. In vivo modulating effect of a calf thymus acid lysate on human T lymphocyte subsets and CD4+/CD8+ ratio in the course of different diseases. *Curr Ther Res*. 1987;42:1011–1017.
46. Sarrell EM, Cohen HA, Kahan E. Naturopathic treatment for ear pain in children. *Pediatrics*. 2003;111:574–579.
47. Sarrell EM, Mandelberg A, Cohen HA. Efficacy of naturopathic extracts in the management of ear pain associated with acute otitis media. *Arch Pediatr Adolesc Med*. 2001;155:796–799.
48. Burger RA, Torres AR, Warren RP, et al. Echinacea-induced cytokine production by human macrophages. *Int J Immunopharmacol*. 1997;19:371–379.
49. Melchart P, Walther E, Linde K, et al. Echinacea root extracts for the prevention of upper respiratory tract infections. *Archiv Fam Med*. 1998;7:541–545.

50. Taylor JA, Weber W, Standish L, et al. Efficacy and safety of echinacea in treating upper respiratory tract infections in children: a randomized controlled trial. *JAMA*. 2003;290:2824–2830.
51. Uhari M, Kontiokari T, Koskela M, et al. Xylitol chewing gum in prevention of acute otitis media: double blind randomised trial. *BMJ*. 1996;313:1180–1184.
52. Uhari M, Kontiokari T, Niemela M. A novel use of xylitol sugar in preventing acute otitis media. *Pediatrics*. 1998;102:879–884.
53. Gotsadze K, Gogatishvili R. Usage of medicines inducing secretolisys in treatment of acute otitis media in children. *Georgian Med News*. 2005;(122):21–25.
54. Subbotina MV, Kunitsina MN, Buksha IA, et al. The use of Sinupret in the combined treatment of acute otitis media in children. *Vestn Otorinolaringol*. 2009;2:43–45.
55. Aladag I, Guven M, Eyibilen A, et al. Efficacy of vitamin A in experimentally induced acute otitis media. *Int J Pediatr Otorhinolaryngol*. 2007;71(4):623–628.
56. Cayir A, Turan MI, Ozkan O, Cayir Y. Vitamin D levels in children diagnosed with acute otiti media. *J Pak Med Assoc*. 2014;64(11):1274–1277.
57. Walker RE, Bartley J, Camargo Jr CA, Flint D, Thrompson JMD, Mitchell EA. Higher serum 25(OH)D concentration is associated with lower risk of chronic otitis media with effusion: a case-control study. *Acta Paediatr*. 2017;106(9):1487–1492.
58. Akcan FA, Dundar Y, Akcan HB, et al. Clinical role of vitamin D in prognosis of otitis media with effusion. *Int J Pediatr Otorhinolaryngol*. 2018;105:1–5.
59. Marchisio P, Consonni D, Baggi E, et al. Vitamin D supplementation reduces the risk of acute otitis media in otitis-prone children. *Pediatr Infect Dis J*. 2013;32(10):1055–1060.
60. Taipale T, Pienihakkinen K, Isolauri E, et al. *Bifidobacterium animalis* subsp. *lactis* BB-12 in reducing the risk of infections in infancy. *Br J Nutr*. 2011;105(3):409–416.
61. Taipale TJ, Pienihakkinen K, Isolauri E, Jokela JT, Soderling EM. *Bifidobacterium animalis* subsp. *lactis* BB-12 in reducing the risk of infections in early childhood. *Pediatr Res*. 2016;79(1-1):65–69.
62. Wong SS, Quan Toh Z, Dunne EM, et al. Inhibition of *Streptococcus pneumoniae* adherence to human epithelial cells in vitro by the probiotic *Lactobacillus rhamnosus* GG. *BMC Res Notes*. 2013;5:135.
63. Tapiovaara L, Lehtoranta L, Swanljung E, et al. *Lactobacillus rhamnosus* GG in the middle ear after randomized, double-blind placebo-controlled oral administration. *Int J Pediatr Otorhinolaryngol*. 2014;78(10):1637–1641.
64. Swanljung E, Tapiovaara L, Lehtoranta L, et al. *Lactobacillus rhamnosus* GG in adenoid tissue. Double-blind, placebo-controlled, randomized clinical trial. *Acta Otolaryngol*. 2015;135(8):824–830.
65. Liu S, Hu P, Du X, Zhou T, Pei X. *Lactobacillus rhamnosus* GG supplementation for preventing respiratory infections in children: a meta-analysis of randomized, placebo-controlled trials. *Indian Pediatr*. 2013;50(4):377–381.
66. DiPierro F, Colombo M, Giuliani MG, et al. Effect of administration of *Streptococcus salivarius* K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children. *Eur Rev Med Pharmacol Sci*. 2016;20(21):4601–4606.
67. Di Pierro F, Risso P, Poggi E, et al. Use of *Streptococcus salivarius* K12 to reduce the incidence of pharyngo-tonsillitis and acute otitis media in children: a retrospective analysis in non-recurrent pediatric subjects. *Minerva Pediatr*. 2018;70(3):24–245.
68. LaMantia I, Varricchio A, Ciprandi G. Bacteriotherapy with Streptococcus salivarius 24SMB and Streptococcus oralis 89a nasal spray for preventing recurrent acute otitis media in children: a real life clinical experience. *Int J Gen Med*. 2107;19:171–175.
69. Lovejoy HM, McGuirt WF, Ayres PH, et al. Effects of low humidity on the rat middle ear. *Laryngoscope*. 1994;104:1055–1058.
70. Mills MV, Henley CE, Barnes LL, et al. The use of osteopathic manipulative treatment as adjuvant therapy in children with recurrent acute otitis media. *Arch Pediatr Adolesc Med*. 2003;157(9):861–866.
71. Wahl RA, Aldous MB, Worden KA, et al. *Echinacea purpurea* and osteopathic manipulative treatment in children with recurrent otitis media: a randomized controlled trial. *BMC Complement Altern Med*. 2008;8:56.
72. Mills MV, Henley CE, Barnes LL, et al. The use of osteopathic manipulative treatment as adjuvant therapy in children with recurrent acute otitis media. *Arch Pediatr Adolesc Med*. 2003;157(9):861–866.
73. Galbreath WO. Acute otitis media: including its postural and manipulative treatment. *J Am Osteopathic Ass*. 1929:377–379.
74. Jacobs J, Springer DA, Crothers D. Homeopathic treatment of acute otitis media in children: a preliminary randomized placebo-controlled trial. *Pediatr Infect Dis J*. 2001;20:177–183.
75. Frei H, Thurneysen A. Homeopathy in acute otitis media in children: treatment effect or spontaneous resolution? *Br Homeopath J*. 2001;90:180–182.
76. Friese KH, Kruse S, Moeller H. Acute otitis media in children: comparison between conventional and homeopathic therapy. *HNO*. 1996;44:462–466.

205

Parkinson's Disease

Robert Kachko, ND, LAc, and *Pina LoGiudice, ND, LAc*

OUTLINE

DIAGNOSTIC SUMMARY

Motor symptoms are considered cardinal in Parkinson's disease (PD)[1]:

- Tremor—The most common parkinsonian feature is a resting tremor, maximal when the limb is at rest and diminishing with voluntary movement and sleep. The tremor affects the most distal part of the extremity to a greater extent; it is typically unilateral at onset, becoming bilateral as the disease progresses. Approximately 30% of PD sufferers do not have tremor at disease onset, yet most develop it as the disease progresses.
- Joint stiffness and increased muscle tone—Combined with a resting tremor, these symptoms produce a ratchety, "cogwheel rigidity" when the limb is passively moved.
- Slow movement (bradykinesia) or inability to move (akinesia)—This produces difficulties not only with the execution of a movement but also with the planning and initiation of movement. The performance of sequential and simultaneous movements is also hindered. For many, bradykinesia is the most disabling symptom in the early stages of PD.[2]
- Other common motor symptoms[1]:
  - Postural instability is typical in the late stages of PD, leading to impaired balance and frequent falls. It is often absent in the initial stages of the disease, especially in younger patients.
  - "Pill-rolling" motion of the thumb and forefinger
  - Stooped posture, progressively shortened and accelerated steps that get progressively faster and may end up in a fall ("festinating gait")
  - Reduced or fixed facial expressions ("masked face"); low-volume or monotone voice or both
  - Small handwriting (micrographia) that decreases in size toward the end of a writing sample
- Gastrointestinal symptoms—Constipation is often one of the earliest symptoms, with difficulty swallowing (dysphagia) common later in the disease course.
- Neuropsychiatric symptoms:
  - Cognitive disturbances are being increasingly recognized as symptoms of PD. Although they can occur early in PD, cognitive deficits progress with the course of the disease, leading to dementia in about 80% of cases[3,4] by the 10th year. The most common cognitive deficits include the inability to make decisions and adapt to new environments, poor problem solving, fluctuations in attention, slowed cognitive speed, and memory problems—specifically in recalling learned information, with an important improvement when cues are given.
  - Alterations in behavior and mood, including depression, apathy, and anxiety, accompany PD. Poor impulse control can result in behaviors such as punding (complex, prolonged, purposeless, and stereotyped behavior), binge eating, craving, hypersexuality, or pathological gambling. These are likely related to a dopamine dysregulation syndrome often associated with the medications for PD.[1]
  - Psychotic symptoms, such as hallucinations or delusions, can be common in later PD.[1]

GENERAL CONSIDERATIONS

Epidemiology

First described by James Parkinson in 1817, PD occurs in approximately 0.3% of the general population and about 1% of the population older than 60 years of age in industrialized countries.[2,5] There is evidence of a disease entity described as "kampavata" (*kampa:* shaking; *vata:* lack of muscle movement) in Ayurveda as far back as 4500 years ago.[6]

This progressive neurological disorder results from a deterioration of neurons in the substantia nigra of the basal ganglia. This creates a shortage of the neurotransmitter dopamine, causing the impairments that characterize the disease via disruptions in the connections to the

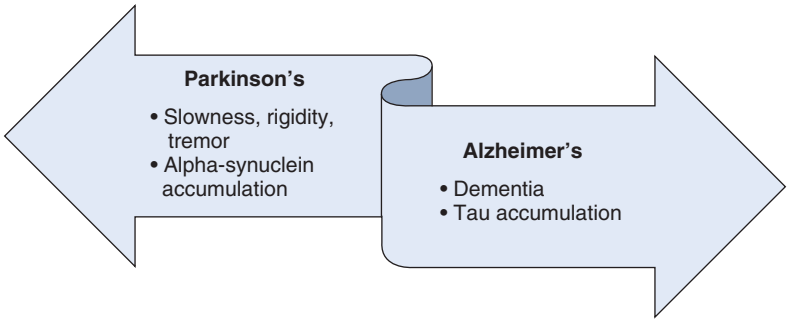


Fig. 205.1 Characteristics of Parkinson's disease and Alzheimer's disease.

motor cortex and thalamus. People with PD often experience limb tremor, muscle rigidity, difficulty walking, and problems with balance and coordination. According to the Parkinson's Disease Foundation, PD affects more than 10 million people worldwide and at least 1 million in the United States.[5] About 60,000 new cases are reported annually in the United States. These figures are expected to increase as the average age of the population increases. The disorder appears to be 1.5 times more common in men than in women.

The prevalence and incidence of PD rise with age, with the average age at onset being approximately 60 years. Because PD is a disease of the aged, disease rates are low in people below age 40, although 4% of PD diagnoses occur before age 50. PD is found in many geographical regions of the world, and rates vary from country to country.

Pathophysiology

As with most neurodegenerative diseases, oxidative and nitric oxide stress, inflammation, and mitochondrial dysfunction are the primary contributors to cell death. A decrease in glutathione concentrations, the brain's primary antioxidant, is the earliest reported biochemical event to occur in the parkinsonian substantia nigra. Mitochondrial dysfunction has been implicated ever since it was observed that an inhibitor of complex I of the electron transport chain can induce parkinsonism. Recent research indicates that several PD-associated genes interface with pathways regulating mitochondrial function, morphology, and dynamics. Sporadic and familial PD may converge at the level of mitochondrial integrity.[7]

The primary pathology of PD is the degeneration of brain cells, or neurons, in the substantia nigra pars compacta of the brain. These neurons produce dopamine, a critical signaling molecule. The loss of dopamine results in a profound and multifaceted disruption of normal information flow through the basal ganglia. Dopamine loss leads to an increased inhibitory influence on D2 receptors, which influences gamma-aminobutyric acid (GABA) signaling in the subthalamic nucleus. It is estimated that more than 60% to 80% (550,000 neurons in the substantia nigra at normal levels reduced to approximately 100,000) of the substantia nigra has been lost by the time the diagnosis of PD is made.[8,9] There is an early period of compensation in which the increased synthesis of dopamine (via increased tyrosine hydroxylase activity) and proliferation of D2 receptors masks clinical symptoms.

Several neurodegenerative disorders have excessive protein deposition as a common pathophysiological mechanism; the primary differences are the location of deposition and/or the protein(s) that accumulate (Fig. 205.1). Pathologically, Lewy bodies are considered the hallmark of classic PD. Found in the substantia nigra, Lewy bodies are abnormal intracytoplasmic accumulations of α-synuclein, a normal cellular protein that has been marked for degradation by the ubiquitin complex but appears to have escaped the normal degradation process. The presence of Lewy bodies cannot be used as a diagnostic criterion because they can be seen only at autopsy. Autopsies have uncovered Lewy bodies in as many as 10% of older persons without a diagnosis of PD, and there is evidence that Lewy bodies are actually neuroprotective as opposed to directly neurotoxic.[10,11] Unfortunately, symptoms do not occur until the nervous system's "reserve" has been exhausted and significant nervous system damage has already occurred.

Substantia Nigra Cell Death

Apoptosis accounts for much of the pathology seen in PD as well as in diseases like Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (Lou Gehrig's disease), which are marked by the loss of brain neurons. Elevated apoptosis (at a rate of four times higher in PD compared with normal controls) in these neurological diseases seems to be related to a lack of production of nerve growth factor and to free-radical damage. A combination of such factors could likely cause many cells to destroy themselves. Manipulation of this process of apoptosis may help in treating these neurological diseases. In fact, studies in animal models imply that long-term delivery of nerve growth factors could protect against programmed cell death in these conditions.[12–14] Until the mechanisms of apoptosis are elucidated, some natural therapeutics that may decrease programmed cell death, such as melatonin therapy, may be of benefit in PD (see further discussion of melatonin later in the chapter).

Mitochondrial Dysfunction and Inflammation

Decreased activity of substantia nigra mitochondrial complex I has been estimated in 32% to 38% of patients with nonfamilial PD.[15,16] In addition, reduced mitochondrial membrane activity (with opening of mitochondrial permeability transition pores [PTPs]) and adenosine triphosphate (ATP) production have been found in skin fibroblasts of patients with PD with certain concomitant genetic mutations.[17] Patients with PD also have been shown to have elevated levels of the proinflammatory cytokines interleukin (IL)-1 beta, tumor necrosis factor (TNF)-alpha, and interferon-gamma.[18,19]

Oxidative Stress and Glutathione Deficiency

Biochemical changes, including increased levels of neurotoxic metals, the inhibition of mitochondrial complex I activity, and depleted glutathione levels occurring in the substantia nigra,[20] all suggest that oxidative stress is present and pathologically involved in patients with PD.[21] Using healthy patients as a control, studies in patients dying from PD have observed 40% reduced glutathione levels in these patients, whereas oxidized glutathione was insignificantly marginally elevated.[22] Depletion of glutathione levels in the brain may be an early component of the disease process because these suboptimal levels have also been found to occur in presymptomatic PD, also known as incidental Lewy body disease.[23]

Some researchers are finding that this glutathione deficiency may be a common denominator in all parkinsonian conditions associated with nigral damage.[20] Although not completely elucidated, it is known that

glutathione exhibits several functions in the brain by acting as an antioxidant and a redox regulator. Glutathione depletion has been shown to affect mitochondrial function, probably via selective inhibition of mitochondrial complex I activity. Oxidative damage due to glutathione depletion may also encourage the aggregation of defective proteins, leading to cell death of nigral-striatal dopaminergic neurons.[21]

Glutathione depletion may enhance the susceptibility of substantia nigra to destruction by endogenous or exogenous toxins. Restoring antioxidant levels within the brain may be a valuable therapeutic strategy for PD.[20]

Environmental Exposures

Epidemiological studies and experimental animal models have identified an association between PD and the following environmental risk factors: rural residence; farming; well-water drinking; exposure to pesticides (including organochlorines, carbamates, paraquat, maneb, rotenone, and diethyldithiocarbamate); and long-term occupational exposure to copper, iron, lead, and manganese.[24] Additionally, it is possible that the pesticides and metals act synergistically with other exposures to increase the risk of PD.[25–28] Another example of environmental exposure is iatrogenic parkinsonism caused by exposure to central dopamine antagonists. The literature for these associations is provided in the following subsections.

MPTP

The first hint that PD may be related to an environmental toxin came from a report based on a series of patients who developed parkinsonism after exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant found in synthetic heroin.[29] It was found that MPTP can freely cross the blood–brain barrier, is selectively taken up by dopaminergic cells, disrupts calcium homeostasis, induces endoplasmic reticulum stress, and inhibits mitochondrial complex 1 function in the respiratory chain, an established factor in the PD pathophysiology.[30,31] MPTP is the only environmental agent that has been directly linked to the development of levodopa-responsive parkinsonism, a form that is clinically indistinguishable from PD.[1]

"Natural" Pesticides (Rotenone)

Rats exposed to the "organic" pesticide rotenone develop parkinsonian symptoms as well as changes in the brain resembling those seen in PD in humans. Mimicking the effect of MPTP, rotenone works by inhibiting complex I of the mitochondrial respiratory chain. It was observed that chronic, systemic inhibition of mitochondrial complex I by the lipophilic pesticide rotenone can cause highly selective nigrostriatal dopaminergic degeneration that is associated behaviorally with hypokinesia and rigidity. Additionally, nigral neurons in rotenone-treated rats accumulate fibrillar cytoplasmic inclusions that contain ubiquitin and α-synuclein.[32] These inclusions are the major constituent of intracellular protein inclusions forming the Lewy bodies and Lewy neuritis in dopaminergic neurons of the substantia nigra. Other studies have also shown that chronic administration of rotenone over a long period can increase nitric oxide and lipid peroxidation products in the brain cortex and striatum in rats, which then clearly mimic parkinsonian behavioral symptoms such as akinesia and rigidity.[33] Interestingly, rotenone is a natural pesticide and until very recently was allowed to be used in organic produce.

Synthetic Pesticides (2,4-dichlorophenoxyacetic acid [2,4-D], Paraquat, Maneb, and Permethrin)

A 2009 case-controlled multicenter study analyzed the effect of occupation on PD risk. Occupational pesticide exposure emerged as the most consistent etiological association with parkinsonism. In experimental settings, the three specific pesticides identified (2,4-dichlorophenoxyacetic acid, paraquat, and permethrin) all have effects on dopaminergic neurons. This convergence of epidemiological and laboratory data from experimental models of PD lends credence to a causative role of certain pesticides in the neurodegenerative process.[34]

Maneb and paraquat, two widely used pesticides, have been associated with PD. Researchers studied pesticide exposure in California's Central Valley and found that when people were exposed to both maneb and paraquat within 500 m of their homes, their likelihood of being diagnosed with PD was substantially increased compared with those without exposure. The majority of the risk for being diagnosed with PD was attributed to exposure between 1974 and 1989—and all those so exposed were then children, teenagers, or young adults. This group of people, who had younger-onset PD (meaning that they were diagnosed before the age of 60), were four to six times more likely to develop PD if they were exposed to both pesticides.[35]

Another 2009 study done in five countries in Europe also explored this association; 959 prevalent cases of parkinsonism and 1989 controls were questioned regarding lifetime occupational and hobby exposure to solvents, pesticides, iron, copper, and manganese.[34] Pesticide exposure and increased prevalence of PD suggest a causative role.

Repeated traumatic loss of consciousness was also associated with increased risk.

Solvents

Some case-control studies and case reports have shown an association between solvent exposure and parkinsonism.[36] Records from a major United Kingdom engineering company were culled to explore the relationship of metal and solvent exposure and PD. The results of this survey demonstrated a significant exposure–response relationship for solvents and almost a 400% increase in risk for employees exposed for 30 years or more. This study suggests that sustained cumulative exposure is probably significant because there was no evidence of an overall increase in risk for those exposed for shorter durations. The authors noted that possibly the more heavily affected workers experienced exposure before the introduction of stricter industrial environmental controls later in the 20th century or exposure to older solvents such as trichloroethylene, 1,1,1-trichloroethane, carbon tetrachloride, kerosene, white spirit, and acetone, which were more widely used three or more decades ago and may have played a greater etiological role.[37]

Although the study mentioned previously showed no increased risk of PD in relation to metal exposure, occupational exposure to specific metals—especially manganese, copper, lead, iron, mercury, zinc, and aluminum—appears, based on epidemiological studies, to be a risk factor for PD.[38] Elevated levels of several of these metals have also been reported in the substantia nigra of PD subjects. Like pesticides, several divalent and trivalent metal ions are also found to contribute to accelerations in the rate of α-synuclein fibril formation. In one study, aluminum contributed to fibril formation, along with the chloride salts of copper^{2+}, iron^{3+}, cobalt$^{3+,}$ and manganese^{2+}.[38] Chronic mercury inhalation has also been linked to cortical and cerebellar atrophy, dementia, Parkinson's disease, and ataxia of the lower limbs.[39]

Manganese

Manganese toxicity is different than PD, but the two present very similarly. With toxicity, manganese-induced damage is found in the substantia nigra, globus pallidus, and caudate nucleus with depletion of dopamine and serotonin levels and has been linked to psychiatric changes followed by impaired motor activity with muscle rigidity and tremors.[24]

Welders, who are commonly exposed to substantial amounts of manganese, do not have a higher incidence of PD, but they do appear to

develop the disease approximately 11 years before most other patients with PD do. Other sources of manganese include well water and industrial waste. Unfortunately, it is not routine to assess manganese levels in patients presumed to have PD, and little research has been done on chronic, low-level exposure to ambient manganese (i.e., from industrial waste.) As imaging techniques improve and become more readily available, it will be easier to differentiate manganism from PD.

Iron

Elemental iron has been shown to be elevated by about 50% in the substantia nigra in patients with PD compared with controls.[40] Iron accumulation has been related to neurological disorders such as Alzheimer's disease, PD, type I neurodegeneration with brain iron accumulation, and others. Increased levels of iron as well as accompanying lipid peroxidation combined with a decreased level of glutathione and superoxide dismutase activity are present in the substantia nigra of patients with PD.[21,41] Although it is unclear whether the accumulation of iron in the brain is primary or secondary to the development of neurodegenerative disorders,[42] one animal study has shown that unilateral injection of $FeCl_3$ into the substantia nigra of adult rats resulted in a 95% decrease of striatal dopamine, which impaired dopamine-related behavioral responses. This supports the assumption that iron may trigger the dopaminergic neurodegeneration of PD.[43]

Detoxification Dysfunction and Genetics

The role of xenobiotic metabolizing enzymes (XMEs) in the disease etiology of PD has been under investigation by numerous researchers for the past several decades. The association of a number of defects in both Phase I and Phase II reactions with PD and motor neuron disease has been extensively studied. It has been demonstrated by in vivo or in vitro methods and by the functional genomics of XMEs and neurodegenerative diseases that PD and motor neuron disease (MND) can be associated with problems in xenobiotic metabolism; moreover, it appears that 38% to 39% of patients with MND/PD have a defect in the S-oxidation of the mucoactive drug carbocysteine by an unknown cytosolic oxidase. This implies that in a significant number of patients with PD, there may be an underlying dysfunction in the body's ability to detoxify.[44]

This dysfunction theoretically could lead to an increased vulnerability to neurotoxins after exposure to sulfur-containing substances.[45] Connections with Alzheimer's disease and other motor neuron diseases have also been made.[46]

First-degree relatives of patients with PD are 2.3 times more likely to develop the disease than first-degree relatives of controls, although genetics may play a larger role in those diagnosed before the age of 50.[47,48] Certainly, genetic determination is but one factor and must be looked at in light of the strong support that has been found for the roles of nutritional and environmental factors, as discussed elsewhere in this chapter.[49] Mutations in the gene on chromosome 4q21.3-q22 that codes for alpha-synuclein (*SNCA* gene) have been implicated in multiple synucleinopathies.[50] Other genes to consider include Glucocerebrosidase *(GBA), LRRK2, PARK2, PINK1,* and *DJ-1,* although routine genetic testing for PD is not yet indicated.

DIAGNOSTIC CONSIDERATIONS

No diagnostic test can clearly identify PD. It is usually diagnosed by a neurologist who can evaluate symptoms and their severity. Often a proper diagnosis will depend on a thorough neurological examination. As a minimum, bradykinesia with either tremor or rigidity must be present to diagnose PD.[51] Patients with accurately diagnosed PD should see a beneficial response to dopaminergic therapy. Lack of response to high-dose (1000–1500 mg) levodopa daily for at least 2 months makes PD highly unlikely. Postural instability tends to develop later in the course of the disease, and early instability likely points to another cause of parkinsonism. Brain imaging (magnetic resonance imaging [MRI]) and blood and urine tests are not necessary to diagnose PD but can help rule out other conditions in the differential diagnosis. The prevalence of dementia in PD is approximately 40%, and Parkinson's disease dementia (PDD) accounts for 3.6% of all cases of dementia. PDD and dementia with Lewy bodies may represent different clinical manifestations of the same disease.[52–54]

THERAPEUTIC CONSIDERATIONS

Naturopathic philosophy prioritizes the prevention of disease as the primary therapeutic strategy. This is especially difficult in PD, where the degenerative process begins about a decade before the symptoms of the disease are diagnosed. The slow, insidious nature of PD makes prevention and even early detection especially difficult. Conventionally, few efforts have been made to identify susceptible individuals, most likely because there are no established conventional neuroprotective agents; an individual identified as having a high likelihood of developing PD would be left to wait until symptoms appeared. Naturopathically, there are dozens of lifestyle modifications and nutraceuticals with preliminary data supporting their role in neuroprotection. With tools in hand, identifying those at risk for developing PD becomes practical and pressing. Immediate family members of those with PD are at increased risk, and symptoms such as restless legs syndrome, loss of smell, and constipation often precede the onset of tremor by several years; individuals with these symptoms are encouraged to consider the therapeutic strategies described in the following discussion.

Conventional Medicine

Unfortunately, no conventional therapy has been shown to modify the progressive pathology of degeneration in PD.[55] However, PD symptoms are somewhat treatable. For decades, the drug levodopa, commonly known as L-dopa, has been the mainstay of PD treatment. It is synthesized by the enzyme tyrosine hydroxylase from the food-derived aromatic amino acid tyrosine. Modern treatment combines L-dopa with a peripheral decarboxylase enzyme inhibitor (Carbidopa) to minimize conversion of L-dopa to dopamine outside the nervous system.[41]

Although initially effective in the early stages of the disease, L-dopa provides only symptomatic relief, without altering disease progression, and it loses efficacy with time.[2] The side effects of L-dopa can include motor complications, particularly fluctuations and dyskinesias, as well as nausea, vomiting, orthostatic hypotension, sedation, hallucinations, and delusions; moreover, L-dopa contributes to decreased slow-wave peristaltic activity,[56] which may contribute to inadequate digestive function. It may also contribute to subtle detrimental effects on cognitive function[57] and may have a wide array of other psychiatric manifestations, such as propensity to gamble.[58] Responses to the drug may become more erratic over time. For these reasons, newer drugs are now also used either alone or in combination with L-dopa. Proper concomitant naturopathic care can drastically reduce the side effects of L-dopa. It is important to emphasize that in spite of the side effects, this drug is excellent for controlling symptoms and must be considered as part of the overall treatment plan.

After being virtually abandoned for 20 years, another allopathic treatment that is gaining more prominence is deep brain stimulation (DBS). DBS involves implanting a brain stimulator, a device similar to a heart pacemaker, in a certain area of the brain. The desired effect is to decrease the overactivity of the excitatory glutamatergic subthalamus–internal pallidum pathway caused by the loss of dopaminergic neurons

within the substantia nigra. How DBS works is not well known, but it has been hypothesized that the stimulatory effect may modulate the neuronal activity and thus avoid disease-related abnormal neuronal discharges. Potential candidates for DBS are selected according to strict criteria. For some people, DBS may control symptoms so well that medications can be greatly reduced.[59]

Diet

Healthy dietary habits might be useful to treat and prevent PD. One large case-control study[60,61] revealed that patients with PD tended to consume fewer raw vegetables, less alcohol and coffee, and more meat than control subjects. Patients with PD also reported higher carbohydrate consumption and equivalent intakes of protein and fat. Other researchers have shown increased consumption of animal-source fat in patients with PD.[62–64] Although researchers need to learn more about how food choices relate to PD, a higher intake of vegetables and a low intake of fat seem to be reasonable choices in helping prevent and possibly treat neurodegeneration. In a review of dietary factors that affect PD progression, foods associated with more rapid PD progression include canned fruits and vegetables, diet and nondiet soda, fried foods, beef, ice cream, yogurt, and cheese. Foods associated with a reduced rate of progression resemble the classic Mediterranean diet and include fresh vegetables, fresh fruit, nuts and seeds, nonfried fish, olive oil, wine, coconut oil, fresh herbs, and spices.[65] Coffee and caffeine intake have been shown to lower the risk of PD in several meta-analyses.[66–68] The ketogenic diet may have some theoretical utility but requires further study.

Food Sources of L-Dopa

Interestingly, certain foods are a good source of natural L-dopa. Anecdotal reports have demonstrated that patients with PD show improved symptom control when they are consuming meals of broad beans. In some cases, the response to *Vicia faba* (fava beans) may be even greater than to conventional L-dopa medication. Fava beans are a good source of L-dopa: a 100-g serving of *V. faba* pods contains about 250 mg of L-dopa, equivalent to the L-dopa content of one of the standard pharmaceutical formulations.[41] Until more is known about how to use fava beans as an L-dopa source, unsupervised replacement or coadministration of L-dopa with fava beans is not recommended.

Calorie Restriction

Overeating is a major modifiable risk factor for several age-related diseases, including PD. Calorie restriction (CR) is usually defined as 10% to 25% less caloric intake than the average Western diet. Throughout history, the majority of the world's population has struggled to obtain food. The *ability* to overeat is a relatively new phenomenon, and accordingly, the amount of food one should eat has only recently become a subject of study. In a primate model of PD, a CR diet (30% reduced) decreased the progression of PD. Animals that had been fed the reduced-intake diet had more dopamine in their brains and more glial-cell-line–derived neurotrophic factor (GDNF), a small protein that promotes the survival of neurons. GDNF's most prominent feature is its ability to support the survival of dopaminergic and motoneurons. The results suggest that CR extends the life span and increases the resistance of the brain to insults that involve metabolic compromise and excitotoxicity.[69] As opposed to the attempts at augmenting antioxidant levels using exogenously supplied antioxidants, which have been largely unsuccessful, one of the proposed mechanisms of CR is enhanced production of antioxidants via intrinsic pathways.[70] Given what is known about PD and CR, this theory is highly plausible and warrants further investigation.

Low-Protein Diet

For those patients taking L-dopa, a low-protein diet may be useful. One double-blind study compared a low-protein intake of 50 g/day for men and 40 g/day for women with a high-protein intake of 80 g/day for men and 70 g/day for women. By the end of the trial, total performance scores were significantly improved in the treatment group given lower protein intakes. Additionally, tremor, hand agility, and mobility in the low-protein groups also improved.[71]

Absorption of L-dopa is delayed or diminished by amino acids in protein meals.[57] It has been shown that the modification of meal patterns so that most of the protein intake occurs in the evening also improved symptoms.[71] It is therefore recommended that patients on L-dopa take their medication with a high-carbohydrate meal and delay protein intake until the final meal of the day so as to optimize the therapeutic efficacy of the medication.[41]

Supplements

Given the abundance of data suggesting that an excessive free-radical burden contributes to PD, it is logical to consider antioxidant augmentation as a therapeutic target. Unfortunately, the PD–antioxidant research has been disappointing. One large, well-designed study attempted to use high-dose vitamin E as a treatment, with no apparent improvement in symptoms or disease progression.[72] A prospective study evaluated 76,890 women for 14 years and 47,331 men for 12 years. All the participants were health care professionals, mostly doctors and nurses. Every 2 to 4 years, they filled out detailed surveys about their diets, including their vitamin intake from both foods and pills. A total of 371 people developed PD during the study. The investigators found that neither vitamin C nor carotenoid intake lowered the risk. The results were the same for the use of vitamin E supplements. This study seems to support the idea that vitamin E supplementation does not help protect against PD. However, those participants who ate vitamin E–rich foods as parts of their diets curiously developed the fewest cases of PD.[73] Co-supplementation of 1000 mg omega-3 fatty acids, flaxseed oil, and 400 IU vitamin E for 12 weeks has led to improved metabolic status in patients with PD. Patients had reductions in high-sensitivity C-reactive protein (hs-CRP) and increases in glutathione concentrations as well as total antioxidant capacity.[74]

Glutathione

Given the importance of glutathione as a powerful brain tissue antioxidant, effective repletion should be a therapeutic priority. Combined intravenous and oral liposomal glutathione replacement is safe, is well tolerated, and may offer a therapeutic benefit.[71] Intranasal glutathione has been shown to be safe up to 600 mg per day but has not been shown to be superior to placebo after a 3-month intervention.[75] *N*-acetylcysteine and α-lipoic acid are glutathione precursors that may also be of use. As a systemic antioxidant, glutathione's ongoing repletion may help ameliorate PD-related damage to the heart, liver, muscles, and other organs as well. It is also recommended to take other antioxidants for synergistic support. Especially noteworthy is high-dose vitamin C, which provides antioxidant-reducing equivalents known to conserve glutathione.[71]

Vitamin D

Vitamin D, a fat-soluble prohormone, has been linked to maintaining physiological function as well as preventing diseases such as bone, cardiovascular, autoimmune, and neurological disorders. Endogenous synthesis occurs in the skin with exposure to ultraviolet-B radiation, primarily from sunlight. Diet, such as fortified foods and certain fish, can also provide minor amounts of vitamin D. Deficiency can result from inadequate sun exposure, metabolic or absorption disorders, and other genetically influenced factors.

Patients with chronic neurodegenerative disease are at increased risk for vitamin D insufficiency due to advanced age, obesity, and decreased sun exposure. One study demonstrated that patients with PD were more likely to exhibit vitamin D insufficiency than those with Alzheimer's disease, a related neurodegenerative disorder. Vitamin D regulates multiple cellular processes known to be abnormal in PD, including cellular differentiation, proliferation, and apoptosis. The researchers of this study suggest that patients with PD should be checked more regularly for low 25-hydroxyvitamin D levels as a strategy for minimizing further complications of the disease.[76] Polymorphisms in the Vitamin D Receptor FokI gene are associated with an increased risk of PD.[77] There is currently no consensus on vitamin D deficiency and substantially increased risk of PD.

Coenzyme Q_{10}

Coenzyme Q_{10} (CoQ_{10}) is also known as ubiquinone; its primary biochemical action is as a cofactor in the electron transport chain, the series of redox reactions involved in the synthesis of ATP. CoQ_{10} is best known for its role in treating cardiovascular disease (CVD), and CVD is a well-established comorbidity in PD. Preliminary data suggest that CoQ_{10} is one of the most promising neuroprotective agents for PD, although a recent meta-analysis failed to show the superiority of CoQ10 supplementation over a placebo.[78]

Nonetheless, a randomized, placebo-controlled, double-blind clinical pilot study suggested that high-dose CoQ_{10} may also help slow symptom progression in early PD. In this study, 80 unmedicated patients with early-stage disease were randomly given CoQ_{10} at dosages of 300, 600, or 1200 mg/day or a placebo. Using the Unified Parkinson's Disease Rating Scale (UPDRS), they were followed up for 16 months or until disability requiring treatment with L-dopa had developed. It was found that patients on CoQ_{10} fared significantly better than their placebo counterparts, with those taking 1200 mg showing the greatest results.[79] A second placebo-controlled, double-blind experiment used 360 mg of CoQ_{10} for 4 weeks in 28 treated and stable patients with PD. CoQ_{10} supplementation provided a significant although mild symptomatic benefit in PD symptoms and significantly better improvement in visual defects compared with placebo.[80]

High doses of CoQ_{10} have proven to be safe in the short term. In a 2-week open-label trial, 17 patients with PD received an increasing dosage of CoQ_{10} (1200, 1800, 2400, and 3000 mg/day, respectively) with an unvarying dosage of 1200 IU/day of the α-tocopherol form of vitamin E. The plasma level of CoQ_{10} was measured at each dosage. Thirteen of the subjects were increased to the 3000-mg dose, with no CoQ_{10}-related side effects. One patient became orthostatic, and one was dyspeptic, but these conditions were unrelated to the CoQ_{10}. Looking at the blood, plasma levels of ubiquinone reached a plateau at the 2400-mg/day dosage. This study was not long enough to assess any clinical effect on the disease itself,[81] but a follow-up study is under way.

Melatonin

Melatonin is a hormone manufactured from serotonin and is secreted by the pineal gland. Known as a powerful antioxidant, melatonin is an established treatment option for jet lag and various sleep disorders. Although studies show that melatonin does not induce any increase in cerebrovascular blood flow,[82] evidence supports the role of melatonin as a protector of neuronal cells, most likely by supporting mitochondrial function and preventing apoptosis.

As an antioxidant, melatonin can directly scavenge oxidants produced during normal metabolism, and it indirectly promotes the activity of antioxidant enzymes such as superoxide dismutase and catalase. Second, melatonin increases the activity and the expression of electron transport chain complexes during physiological and pathological situations. As a result, melatonin increases ATP production and promotes glutathione homeostasis. It has also been postulated that melatonin may interact with the mitochondrial genome to enhance the production of proteins.[83] Tissue culture models of PD using low doses of 6-hydroxydopamine to induce apoptosis of undifferentiated and neuronal rat adrenal pheochromocytoma cells have also shown melatonin to prevent apoptosis in these models.[84]

It is theoretically possible that melatonin exacerbates the symptoms of PD because of its putative interference with dopamine release.[85] But the larger body of literature agrees that PD is probably due to multiple issues of compromised mitochondrial activity in the substantia nigra,[86] loss of glutathione,[87] oxidative damage, and increased apoptotic events. Reasonably, melatonin supplementation may yield a direct benefit. Clinical studies are necessary to evaluate the effectiveness of melatonin in PD.

Reduced Nicotinamide Adenine Dinucleotide

Found useful in treating animal models of PD,[88] the coenzyme nicotinamide adenine dinucleotide (NADH) is known to enhance endogenous dopamine production by supplying reducing equivalents to the rate-limiting, tyrosine hydroxylase–catalyzed step of dopamine synthesis in both tissue culture and human evaluations.[89] A positive effect has been found using NADH both intravenously and intramuscularly in 34 patients with PD in an open-label trial. In this trial, each patient experienced a beneficial clinical effect: 21 showed a "very good" (better than 30%) improvement of disability, and 13 patients showed a "moderate" (up to 30%) improvement. The effect of NADH was dependent on both the dose given and the severity of disease. The optimal therapeutic range for NADH was 25 to 50 mg per day. Intravenous administration seemed to work better than intramuscular injection. The presence of homovanillic acid in the urine was significantly increased in all patients. The presence of this metabolite indicates stimulation of endogenous L-dopa biosynthesis.[90] A second study of 15 patients prospectively investigated the administration of one 10-mg treatment over 30 minutes a day for 7 days. These patients were also taking L-dopa medications. The patients' UPDRS scores improved, and significant increases in plasma L-dopa were observed.[91] Nevertheless, because of the lack of sufficient studies and theoretical considerations for underlying NADH disposal, many in the medical community do not currently recommend the widespread use of NADH for PD.[89] As with many newer therapies, more rigorous studies are necessary to confirm the benefit and elucidate any side-effect risk.

B Vitamins

Higher dietary intake of vitamin B_6 has been shown to have a protective effect in the development of PD. Patients diagnosed with PD also have lower vitamin B_{12} levels than controls.[92] Dietary intake of folate has not been observed to be consequential in the development of PD, although hyperhomocysteinemia occurs in 10% to 30% of patients with PD.[93]

Creatine

Creatine is an important player in brain homeostasis and acts as a temporal and spatial buffer for cytosolic and mitochondrial pools of the cellular energy currency, ATP, and its precursor adenosine diphosphate (ADP).[94] Well known mostly as a body-building supplement, oral creatine monohydrate has also been shown to enhance memory and is being studied for the treatment of neurological, neuromuscular, and atherosclerotic disease.[88]

Adhihetty et al.[95] have written an excellent review article on the neuroprotective potential of creatine to combat cellular energy impairment in neurodegenerative disease.

Botanical Medicines

Camellia sinensis (Green Tea)

Epidemiological studies consistently demonstrate that the consumption of green and black tea offers protection against the development of PD. As a good source of polyphenols, green tea may play a role in preventing and treating the oxidative stress prevalent in several neurodegenerative disorders. The biological properties of green tea polyphenols reported in the literature include significant penetration of the blood–brain barrier, antioxidant actions, free-radical scavenging, iron-chelating properties, inhibition of (3)H-dopamine and (3)H-methyl-4-phenylpyridine uptake, reduction of catechol-*O*-methyltransferase activity, activation of protein kinase C or the extracellular signal-regulated kinase signal pathway, and modulation of the cell survival/cell cycle gene.[96,97] Green tea polyphenols have demonstrated neuroprotective activity in cell cultures and animal models, including the prevention of neurotoxin-induced cell injury.[96,98] Green tea polyphenols, such as (-)-epigallocatechin-3-gallate, are now being considered as therapeutic agents aimed at altering brain-aging processes and serving as possible neuroprotective agents.[99,100]

Ginkgo biloba

Ginkgo biloba extract (GBE) exerts profound, widespread tissue effects, including membrane-stabilizing, antioxidant, and free-radical–scavenging effects. GBE also enhances the utilization of oxygen and glucose. GBE is an extremely effective inhibitor of lipid peroxidation of cellular membranes. Although there are no clinical studies in patients with PD, GBE is well researched for its beneficial effects in Alzheimer's disease[101,102] and has been shown useful in animal models of PD.[101,103] GBE exhibits a protective effect on the PD models both in vivo and in vitro. Antioxidation and antiapoptosis are proposed to be involved in the mechanisms underlying the neuroprotective effect of GBE.[103]

Mucuna puriens (Velvet Bean)

The seed powder of the velvet bean has long been used in traditional Ayurvedic medicine for diseases, including PD. It is a rich natural source of L-dopa, but other components also contribute to its medicinal actions. An extract of velvet bean was studied in 60 patients with PD (before treatment, 26 patients were given Sinemet with the extract, and the remaining 34 were not given any medication).[104] Statistically significant reductions in symptom scores were seen from the beginning to the end of the 12-week study. The amount used in the study was 7.5 g of the extract dissolved in water 3 to 6 times daily. In another study, eight patients with PD were challenged with single doses of 200-/50-mg L-dopa/carbidopa (LD/CD) and 15 and 30 g of velvet bean preparation in a randomized order at weekly intervals.[105] Compared with standard LD/CD, the 30-g velvet bean preparation led to a considerably faster onset of effect (34.6 vs. 68.5 minutes), reflected in shorter times to peak L-dopa concentrations in the blood, and fewer side effects. The researchers felt that the velvet bean might possess advantages over conventional L-dopa preparations. This conclusion has been confirmed in various animal models.[106,107] In a small pilot trial comparing velvet bean to LD/CD administration, velvet bean had lower tolerability (gastrointestinal symptoms or worsening motor performance) but performed as well as LD/CD in those who were able to tolerate it.[108] Individuals taking medications like Sinemet and L-dopa should be aware that velvet bean consumption may lead to too-high L-dopa levels.

Piper methysticum (Kava-Kava)—Caution

Although no side effects have been reported using standardized kava extracts at recommended levels in clinical studies, there have been isolated reports of kava causing an onset of parkinsonian symptoms.[109] Additionally, several case reports have indicated that kava may interfere with dopamine and worsen PD.[110,111] Until this issue is resolved, kava extract should not be used in patients with PD or those considered genetically susceptible.

Other Therapeutic Considerations

Smoking

A number of epidemiological studies demonstrate that smoking is associated with a lower incidence[112] and delayed onset[113] of PD. Although it is unclear which mechanisms account for this effect, it is postulated that nicotine may enhance the striatal stimulation of dopaminergic neurons, which are selectively damaged in PD. Because the risk:benefit ratio of smoking is high, it is not advised that smoking be suggested as a reasonable preventive measure.

Estrogen

Increasing evidence suggests that estrogens may modulate the activity of dopamine,[114,115] may act as an antiapoptotic agent,[116] and could affect the neuronal pathways affected in PD.[117] Animal studies have demonstrated that estrogens influence the synthesis, release, and metabolism of dopamine and may actually modulate the expression and function of dopamine receptors. Some clinical studies have also suggested that parkinsonian symptoms may worsen after menopause and that hormone replacement therapy can be protective,[118,119] whereas others hypothesize that decreases in circulating estrogen may improve PD.[114]

The conflicting findings suggest that several variables, including age, estrogen dose and formulation, and timing and length of dosing period, may determine whether benefits are seen and what their nature might be.[117] At this time, it may be best for clinicians to pay close attention to correlations between menstrual patterns and symptomatic disease to make the best patient-specific choices with regard to hormone replacement.

Exercise

Moderate to vigorous physical activity and exercise is associated with a reduced risk for developing PD.[120–122] As part of interdisciplinary treatment, and when approved by a physician, yoga may offer therapeutic benefit.[123] Low-intensity progressive cycling exercise delivered over 16 sessions spanning 2 months improved motor function in PD, particularly akinesia.[124] Larger, well-designed clinical trials are necessary before targeted physiotherapeutic recommendations can be made for patients with PD. Until that time, physical activity should be encouraged within the confines of approved and tolerable motor activities.

Homeopathy

No clinical studies are available to support the use of homeopathy in PD, although anecdotal success stories are known. Some of the remedies and the symptoms they are said to relieve include the following:

- *Agaricus muscarius:* crawling sensations, vertigo with impulse to fall backward, symptoms worse in cold weather
- *Antimonium crudum:* parkinsonian movements associated with gastric symptoms; desire for sour foods that do not sit well in the digestive tract; a thickly white-coated tongue; stubbornness; anxiousness; a general feeling of disgust with worse symptomatology caused by heat, wine, or moonlight
- *Argentum nitricum:* tremulousness

Acupuncture and *Tui Na*

In traditional Chinese medicine, pathogenic wind is considered the main agent responsible for the symptoms of PD. The treatment strategy is thus

to calm this wind and tranquilize the mind. In more conventional terms, it is hypothesized that acupuncture may increase levels of dopamine in the brain and augment the excitability of the dopamine neurons.[125] Animal studies have shown neuroprotective effects of acupuncture on the nigrostriatal system.[126,127] Acupuncture and *tui na,* which is a Chinese therapeutic massage, have both improved clinical disease symptoms and signs and possibly delayed disease progression.[128,129] In one study, 20 patients with PD were treated twice a week with acupuncture. Standard scales, including the UPDRS and Hoehn and Yahr staging, were used. In addition, quantitative motor tests, including timed evaluations of arm pronation supination movements, finger dexterity, finger movements between two fixed measured points, and the stand-walk-sit test, as well as a patient questionnaire designed for the study were employed. Objective improvements were noted in the sleep and rest categories, with no other obvious improvements. However, on the patient questionnaire, 85% of patients reported subjective improvement of individual symptoms, including tremor, walking, handwriting, slowness, pain, sleep, depression, and anxiety.[130] Another study of 26 patients found improvements in auditory-evoked brainstem potential examinations.[118] A 2017 systemic review and meta-analysis concluded that treatment with combined acupuncture (including electroacupuncture) and conventional medication (CM) showed significant improvements in the total UPDRS and Webster scales compared with those treated using CM alone.[131]

Acupuncture treatments are considered safe and well tolerated[130] and should be considered as a safe means to improve sleep and patient perception of parkinsonian symptoms. More studies are necessary to confirm objective improvements in clinical presentations.

THERAPEUTIC APPROACH

Diagnosis

Besides standard neurological rating scales and imaging, serum iron, ferritin, and total iron binding may be useful to look for iron overload. Blood levels of homocysteine may also uncover hyperhomocysteinemia, a potential facilitator of disease progression. A careful history and testing should evaluate for heavy metal and pesticide exposures.

Dietary Recommendations

- The diet should be low in animal fats and high in fiber, specifically legumes and vegetables.
- Antioxidant-rich foods are recommended, including nuts and seeds, green leafy vegetables (bok choy, chard, etc.), beans, spices (turmeric, clove, cinnamon), coffee, and chocolate.
- Pesticides should be avoided by eating organic when possible.
- To maintain bowel health and facilitate the detoxification processes of the liver, high-sulfur-containing foods like garlic, onions, and eggs, as well as water-soluble fibers such as guar gum, oat bran, pectin, and psyllium seed, are recommended.
- For patients taking levodopa, lower protein intake is recommended (50 g/day for men and 40 g/day for women). In an effort to optimize the medication's therapeutic efficacy, such patients should take their medications 30 to 45 minutes before protein-containing meals.
- Because of constant movement, weight loss may be an issue with some patients; therefore caloric intake may need to be adjusted to specific patient needs.

Lifestyle Recommendations

Patients with PD should be advised to do the following:

- Avoid cooking in aluminum pots.
- Exercise regularly—tandem and recumbent bikes are especially well suited for individuals with PD.
- Hold on to banisters and sit in chairs with higher arms.

Nutritional Supplementation

- Iron and manganese supplements should be avoided.
- High-potency multiple vitamin and mineral formula (without iron)
- Vitamin D_3: 2000 to 4000 IU a day (ideally, measure blood levels and adjust dose accordingly)
- Fish oils: 1000 to 3000 mg eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) a day
- Exogenous glutathione should be administered; however, the best delivery system has yet to be elucidated. Intranasal glutathione has been anecdotally reported to be effective. N-acetyl cysteine (500 mg twice a day) and ketogenic diets have been shown to boost brain glutathione levels.
- Vitamins B_6 and B_{12}, as well as folic acid, should be provided in doses sufficient to maintain homocysteine levels below 10. If homocysteine levels do not drop adequately, betaine may be added to the regimen.
- NADH (trade name: Enada): 10 to 20 mg a day
- CoQ10: Estimated daily dose to achieve target levels in PD based upon available forms (for best results, take in divided dosages):
  - Ubiquinone powder in hard gelatin capsule: 1200 to 2400 mg in divided doses
  - Ubiquinone suspended in oil in soft gelatin capsule w/rice bran oil: 600 to 1200 mg in divided doses
  - Ubiquinone solubilized (e.g., Q-gel) in soft gelatin capsule: 300 to 400 mg in divided doses
  - Ubiquinone nanonized in soft or hard gelatin capsule: 250 to 400 mg in divided doses
  - Ubiquinone emulsified with soy peptide (BioQ10 SA) in soft or hard gelatin capsule: 200 to 400 mg in divided doses
  - Ubiquinol in soft gel capsule: 200 to 400 mg in divided doses

Botanical Medicines

- Green tea: Three cups a day may be taken, or about 3 g of soluble components providing roughly 240 to 320 mg of polyphenols. For a green tea extract standardized for 80% total polyphenol and 55% epigallocatechin gallate content, this would mean a daily dose of 300 to 400 mg.
- *Mucuna puriens:* Dose equivalent to 30 g dried powdered seed

Acupuncture

Treatments involving needling and *tui na* massage according to traditional Chinese medicine are recommended.

CONCLUSION

Naturopathic medicine holds tremendous potential for the prevention and treatment of PD. Unlike conventional therapies that treat only symptoms and have notable side-effect profiles, naturopathic strategies attempt to optimize function as well as to reduce free-radical production and exposure to neurotoxins. It is ideal for patients that the conventional and naturopathic strategies are not at odds with one another; rather, they complement each other well.

Randomized controlled trials are ill-suited study designs for diet and lifestyle interventions and even less optimal for diseases like PD, where the disease is not diagnosed until late in the course. In the meantime, dozens of epidemiological studies suggest that the diet and lifestyle choices we make over the course of our lives offer some protection against the development of this disease. It is logical and prudent to incorporate foods, activities, and supplements that may have neuroprotective potential; this is especially true for those who are at high

risk of developing PD, have symptoms associated with PD, or have been recently diagnosed. Whereas neuronal regeneration is considered virtually impossible, neuroprotective strategies are likely to be elucidated in the near future. As with all therapies, individuals are advised to explore the risks and benefits with their providers and initiate the strategies that are most appropriate for each situation.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry*. 2008;79(4):368–376. PMID 18344392. https://doi.org/10.1136/jnnp.2007.131045.
2. Samii A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet*. 2004;363:1783–1793.
3. Caballol N, Martí MJ, Tolosa E. Cognitive dysfunction and dementia in Parkinson disease. *Mov Disord*. 2007;17(suppl 22):S358–S366.
4. Leverenz JB, Quinn JF, Zabetian C, et al. Cognitive impairment and dementia in patients with Parkinson's disease. *Curr Top Med Chem*. 2009;9(10):903–912.
5. Parkinson's Disease Foundation. Statistics. https://parkinson.org/Understanding-Parkinsons/Statistics?_ga=2.81540638.865534588.1558044976-141936956.1558044976. Accessed 23 Oct 2019.
6. Manyam BV. Paralysis agitans and levodopa in "Ayurveda": ancient Indian medical treatise. *Mov Disord*. 1990;5:47.
7. Winklhofer KF, Haass C. Mitochondrial dysfunction in Parkinson's disease. *Biochim Biophys Acta*. 2010;1802(1):29–44.
8. Pakkenberg B, Møller A, Gundersen HJ, et al. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J Neurol Neurosurg Psychiatry*. 1991;54:30.
9. Hornykiewicz O. The discovery of dopamine deficiency in the parkinsonian brain. *J Neural Transm Suppl*. 2006;9–15.
10. Wakabayashi K, Tanji K, Mori F, Takahashi H. The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of alpha-synuclein aggregates. *Neuropathology*. 2007;27:494.
11. Bodner RA, Outeiro TF, Altmann S, et al. Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. *Proc Natl Acad Sci U S A*. 2006;103:4246.
12. National Institute of Environmental Health Sciences. *Fourteenth Annual Report on Carcinogens.* Triangle Park, NC: National Institute of Environmental Health Sciences; 2016.
13. Martin SJ, Green DR. Protease activation during apoptosis: death by a thousand cuts? *Cell*. 1995;82:349–352.
14. Schreck R, Meier B, Mannel DN, et al. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med*. 1992;175:1181–1194.
15. Schapira AH, Cooper JM, Dexter D, et al. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet*. 1989;1:1269.
16. Greenamyre JT, Hastings TG, Biomedicine. Parkinson's—divergent causes, convergent mechanisms. *Science*. 2004;304:1120.
17. Mortiboys H, Johansen KK, Aasly JO, Bandmann O. Mitochondrial impairment in patients with Parkinson disease with the G2019S mutation in LRRK2. *Neurology*. 2010;75:2017.
18. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol*. 2009;8:382.
19. Hirsch EC, Jenner P, Przedborski S. Pathogenesis of Parkinson's disease. *Mov Disord*. 2013;28:24.
20. Fitzmaurice PS, Ang L, Guttman M, et al. Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. *Mov Disord*. 2003;18:969–976.
21. Bharath S, Hsu M, Kaur D, et al. Glutathione, iron and Parkinson's disease. *Biochem Pharmacol*. 2002;64:1037–1048.
22. Sian J, Dexter DT, Lees AJ, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann Neurol*. 1994;36:348–355.
23. Owen AD, Schapira AH, Jenner P, et al. Oxidative stress and Parkinson's disease. *Ann N Y Acad Sci*. 1996;786:217–223.
24. Olanow CW. Manganese-induced parkinsonism and Parkinson's disease. *Ann N Y Acad Sci*. 2004;1012:209–223.
25. Di Monte DA. The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? *Lancet Neurol*. 2003;2:531–538.
26. Logroscino G. The role of early life environmental risk factors in Parkinson disease: what is the evidence? *Environ Health Perspect*. 2005;113:1234–1238.
27. Landrigan PJ, Sonawane B, Butler RN, et al. Early environmental origins of neurodegenerative disease in later life. *Environ Health Perspect*. 2005;113:1230–1233.
28. Siderowf A, Stern M. Update on Parkinson disease. *Ann Intern Med*. 2003;138:651–658.
29. Snyder SH, D'Amato RJ. Predicting Parkinson's disease. *Nature*. 1985;317:198–199.
30. Calne DB, Langston JW, Martin WR, et al. Positron emission tomography after MPTP: observations relating to the cause of Parkinson's disease. *Nature*. 1985;317:246–248.
31. Selvaraj S, Sun Y, Watt JA, et al. Neurotoxin-induced ER stress in mouse dopaminergic neurons involves downregulation of TRPC1 and inhibition of AKT/mTOR signaling. *J Clin Invest*. 2012;122:1354.
32. Betarbet R, Sherer TB, MacKenzie G, et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci*. 2000;3:1301–1306.
33. Bashkatova V, Alam M, Vanin A, et al. Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. *Exp Neurol*. 2004;186:235–241.
34. Tanner CM, Ross GW, Jewell SA, et al. Occupation and risk of parkinsonism: a multicenter case-control study. *Arch Neurol*. 2009;66(9):1106–1113.
35. Costello S, Cockburn M, Bronstein J, et al. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am J Epidemiol*. 2009;169(8):919–926.
36. Vanacore N, Gasparini M, Brusa L, et al. A possible association between exposure to n-hexane and parkinsonism. *Neurol Sci*. 2000;21:49–52.
37. McDonnell L, Maginnis C, Lewis S, et al. Occupational exposure to solvents and metals and Parkinson's disease. *Neurology*. 2003;61:716–717.
38. Uversky VN, Li J, Fink AL. Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein: a possible molecular NK between Parkinson's disease and heavy metal exposure. *J Biol Chem*. 2001;276:44284–44296.
39. Miller K, Ochudlo S, Opala G, et al. Parkinsonism in chronic occupational metallic mercury intoxication. *Neurol Neurochir Pol*. 2003;37(suppl 5):31–38.
40. Oakley AE, Collingwood JF, Dobson J, et al. Individual dopaminergic neurons show raised iron levels in Parkinson disease. *Neurology*. 2007;68:1820.
41. Caspi O, Thomson C. Parkinson's disease: "don't become your disease!" *Integr Med*. 1999;2:37–42.
42. Sadrzadeh SM, Saffari Y. Iron and brain disorders. *Am J Clin Pathol*. 2004;121(suppl):S64–S70.
43. Youdim MB, Ben-Shachar D, Riederer P. Iron in brain function and dysfunction with emphasis on Parkinson's disease. *Eur Neurol*. 1991;31(suppl 1):34–40.
44. Steventon GB, Sturman S, Waring RH, et al. A review of xenobiotic metabolism enzymes in Parkinson's disease and motor neuron disease. *Drug Metabol Drug Interact*. 2001;18(2):79–98.
45. Heafield MT, Fearn S, Steventon GB, et al. Plasma cysteine and sulphate levels in patients with motor neurone, Parkinson's and Alzheimer's disease. *Neurosci Lett*. 1990;110:216–220.
46. Steventon GB, Heafield MT, Sturman S, et al. Xenobiotic metabolism in Alzheimer's disease. *Neurology*. 1990;40:1095–1098.
47. Marder K, Tang MX, Mejia H, et al. Risk of Parkinson's disease among first-degree relatives: a community-based study. *Neurology*. 1996;47:155.
48. Tanner CM, Ottman R, Goldman SM, et al. Parkinson disease in twins: an etiologic study. *JAMA*. 1999;281:341.
49. Wu JM. Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers. *J Natl Cancer Inst*. 1991;83:963.
50. Maries E, Dass B, Collier TJ, et al. The role of alpha-synuclein in Parkinson's disease: insights from animal models. *Nat Rev Neurosci*. 2003;4:727.
51. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*. 2015;30:1591.
52. Svenningsson P, Westman E, Ballard C, Aarsland D. Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment. *Lancet Neurol*. 2012;11:697.

53. Cummings JL. Intellectual impairment in Parkinson's disease: clinical, pathologic, and biochemical correlates. *J Geriatr Psychiatry Neurol*. 1988;1:24.
54. Aarsland D, Zaccai J, Brayne C. A systematic review of prevalence studies of dementia in Parkinson's disease. *Mov Disord*. 2005;20:1255.
55. Schapira AH. Progress in Parkinson's disease. *Neurology*. 2003;61:S1–S3.
56. Jankovic J. Levodopa strengths and weaknesses. *Neurology*. 2002;58(4 suppl 1):S19–S32.
57. Lu CL, Shan DE, Chen CY, et al. Impaired gastric myoelectrical activity in patients with Parkinson's disease and effect of levodopa treatment. *Dig Dis Sci*. 2004;49:744–749.
58. Feigin A, Ghilardi MF, Carbon M, et al. Effects of levodopa on motor sequence learning in Parkinson's disease. *Neurology*. 2003;60:1744–1749.
59. Driver-Dunckley E, Samanta J, Stacy M. Pathological gambling associated with dopamine agonist therapy in Parkinson's disease. *Neurology*. 2003;61:422–423.
60. Fraix V, Pollak P, Chabardes S, et al. Deep brain stimulation. *Rev Neurol (Paris)*. 2004;160(5 Pt 1):511–521.
61. Hellenbrand W, Seidler A, Boeing H, et al. Diet and Parkinson's disease. I: a possible role for the past intake of specific foods and food groups. Results from a self-administered food-frequency questionnaire in a case-control study. *Neurology*. 1996;47:636–643.
62. Hellenbrand W, Boeing H, Robra BP, et al. Diet and Parkinson's disease. II: a possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case-control study. *Neurology*. 1996;47:644–650.
63. Logroscino G, Mayeux R. Diet and Parkinson's disease. *Neurology*. 1997;49:310–311.
64. Logroscino G, Marder K, Cote L, et al. Dietary lipids and antioxidants in PD: a population-based, case-control study. *Ann Neurol*. 1996;39:89–94.
65. Mischley LK, Lau RC, Bennett RD. Role of diet and nutritional supplements in Parkinson's disease progression. *Oxidative Med Cellul Longevity*. 2017;2017:6405278. https://doi.org/10.1155/2017/6405278.
66. Costa J, Lunet N, Santos C, et al. Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. *J Alzheimers Dis*. 2010;20(suppl 1):S221.
67. Palacios N, Gao X, McCullough ML, et al. Caffeine and risk of Parkinson's disease in a large cohort of men and women. *Mov Disord*. 2012;27:1276.
68. Liu R, Guo X, Park Y, et al. Caffeine intake, smoking, and risk of Parkinson disease in men and women. *Am J Epidemiol*. 2012;175:1200.
69. Maswood N, Young J, Tilmont E, et al. Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of PD. *Proc Natl Acad Sci USA*. 2004;101(52):18171–18176.
70. Hardingham GE, Lipton SA. Regulation of neuronal oxidative and nitrosative stress by endogenous protective pathways and disease processes. *Antioxid Redox Signal*. 2011;14(8):1421–1424.
71. Kidd PM. Parkinson's disease as multifactorial oxidative neurodegeneration: implications for integrative management. *Altern Med Rev*. 2000;5:502–529.
72. LeWitt PA. Deprenyl's effect at slowing progression of Parkinsonian disability: the DATATOP study. The parkinson study group. *Acta Neurol Scand Suppl*. 1991;136:79–86.
73. Zhang SM, Hernán MA, Chen H, et al. Intakes of vitamins E and C, carotenoids, vitamin supplements, and PD risk. *Neurology*. 2002;59:1161–1169.
74. Taghizadeh M. The effects of omega-3 fatty acids and vitamin E co-supplementation on clinical and metabolic status in patients with Parkinson's disease: a randomized, double-blind, placebo-controlled trial. *Neurochem Int*. 2017;9(108):183–189.
75. Mischley LK, Lau RC, Shankland EG, et al. Phase IIb study of intranasal glutathione in Parkinson's disease. *J Parkinson's Dis*. 2017;7(2):289–299. https://doi.org/10.3233/JPD-161040.
76. Evatt ML, Delong MR, Khazai N, et al. Prevalence of vitamin D insufficiency in patients with Parkinson disease and Alzheimer disease. *Arch Neurol*. 2008;65(10):1348–1352.
77. Gatto NM, Paul KC, Sinsheimer JS, et al. Vitamin D receptor gene polymorphisms and cognitive decline in Parkinson's disease. *J Neurol Sci*. 2016;370:100–106. https://doi.org/10.1016/j.jns.2016.09.013.
78. Zhu Z. The efficacy and safety of coenzyme Q10 in Parkinson's disease: a meta-analysis of randomized controlled trials. *Neurol Sci*. 2017;38:215–224.
79. Shults CW, Oakes D, Kieburtz K, et al. Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Arch Neurol*. 2002;59:1541–1550.
80. Muller T, Buttner T, Gholipour AF, et al. Coenzyme Q10 supplementation provides mild symptomatic benefit in patients with Parkinson's disease. *Neurosci Lett*. 2003;341:201–204.
81. Shults CW, Flint Beal M, Song D, et al. Pilot trial of high dosages of coenzyme Q10 in patients with Parkinson's disease. *Exp Neurol*. 2004;188:491–494.
82. van der Helm-van Mil AH, van Someren EJ, van den Boom R, et al. No influence of melatonin on cerebral blood flow in humans. *J Clin Endocrinol Metab*. 2003;88:5989–5994.
83. Leon J, Acuña-Castroviejo D, Sainz RM, et al. Melatonin and mitochondrial function. *Life Sci*. 2004;75:765–790.
84. Mayo JC, Sainz RM, Uria H, et al. Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. *J Pineal Res*. 1998;24:179–192.
85. Zisapel N. Melatonin-dopamine interactions: from basic neurochemistry to a clinical setting. *Cell Mol Neurobiol*. 2001;21:605–616.
86. Hattori N, Tanaka M, Ozawa T, et al. Immunohistochemical studies on complexes I, II, III, and IV of mitochondria in Parkinson's disease. *Ann Neurol*. 1991;30:563–571.
87. Schapira AH, Cooper JM, Dexter D, et al. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet*. 1989;1:1269.
88. Beal MF. Bioenergetic approaches for neuroprotection in Parkinson's disease. *Ann Neurol*. 2003;53(suppl 3):S39–S47; discussion S47-S48.
89. Swerdlow RH. Is NADH effective in the treatment of Parkinson's disease? *Drugs Aging*. 1998;13:263–268.
90. Birkmayer W, Birkmayer GJ. Nicotinamidadenindinucleotide (NADH): the new approach in the therapy of Parkinson's disease. *Ann Clin Lab Sci*. 1989;19:38–43.
91. Kuhn W, Muller T, Winkel R, et al. Parenteral application of NADH in Parkinson's disease: clinical improvement partially due to stimulation of endogenous levodopa biosynthesis. *J Neural Transm*. 1996;103:1187–1193.
92. Shen L. Associations between B vitamins and Parkinson's disease. *Nutrients*. 2015;7(9):7197–7208. https://doi.org/10.3390/nu7095333.
93. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry*. 2008;79:368–376.
94. Rae C, Digney AL, McEwan SR, et al. Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial. *Proc R Soc Lond B Biol Sci*. 2003;270:2147–2150.
95. Adhihetty PJ, Beal MF. Creatine and its potential therapeutic value for targeting cellular energy impairment in neurodegenerative diseases. *Neuromulecular Med*. 2008;10(4):275–290.
96. Pan T, Jankovic J, Le W. Potential therapeutic properties of green tea polyphenols in Parkinson's disease. *Drugs Aging*. 2003;20:711–721.
97. Levites Y, Weinreb O, Maor G, et al. Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. *J Neurochem*. 2001;78:1073–1082.
98. Choi JY, Park CS, Kim DJ, et al. Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. *Neurotoxicology*. 2002;23:367–374.
99. Weinreb O, Mandel S, Amit T, et al. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutr Biochem*. 2004;15:506–516.
100. Hang L, Basil AH, Lim KL. Nutraceuticals in Parkinson's disease. *Neuromol Med*. 2016;18:306–321. https://doi.org/10.1007/s12017-016-8398-6.
101. Gessner B, Voelp A, Klasser M. Study of the long-term action of a *Ginkgo biloba* extract on vigilance and mental performance as determined by means of quantitative pharmaco-EEG and psychometric measurements. *Arzneimittelforschung*. 1985;35:1459–1465.

102. Le Bars PL, Katz MM, Berman N, et al. A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. *JAMA*. 1997;278:1327–1332.
103. Yang SF, Wu Q, Sun AS, et al. Protective effect and mechanism of *Ginkgo biloba* leaf extracts for Parkinson disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Acta Pharmacol Sin*. 2001;22:1089–1093.
104. HP-200 in Parkinson's disease Study Group. An alternative medicine treatment for Parkinson's disease: results of a multicenter clinical trial. *J Altern Complement Med*. 1995;1:249–255.
105. Katzenschlager R, Evans A, Manson A, et al. *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. *J Neurol Neurosurg Psychiatry*. 2004;75(12):1672–1677.
106. Lieu CA, Kunselman AR, Manyam BV, et al. A water extract of *Mucuna pruriens* provides long-term amelioration of parkinsonism with reduced risk for dyskinesias. *Parkinsonism Relat Disord*. 2010;16(7):458–465.
107. Kasture S, Pontis S, Pinna A, et al. Assessment of symptomatic and neuroprotective efficacy of *Mucuna pruriens* seed extract in rodent model of Parkinson's disease. *Neurotox Res*. 2009;15(2):111–122.
108. Cilia R. Daily intake of *Mucuna pruriens* in advanced Parkinson's disease: a 16-week, noninferiority, randomized, crossover, pilot study. *Parkinsonism Relat Disorders*. 2018;49:60–66.
109. Meseguer E, Taboada R, Sanchez V, et al. Life-threatening parkinsonism induced by kava-kava. *Mov Disord*. 2002;17:195–196.
110. Schelosky L, Raffauf C, Jendroska K, et al. Kava and dopamine antagonism. *J Neurol Neurosurg Psychiatry*. 1995;58:639–640.
111. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: a systematic review. *Drugs*. 2001;61:2163–2175.
112. Quik M. Smoking, nicotine and Parkinson's disease. *Trends Neurosci*. 2004;27:561–568.
113. Tzourio C, Rocca WA, Breteler MM, et al. Smoking and Parkinson's disease. An age-dependent risk effect? The Europarkinson study group. *Neurology*. 1997;49:1267–1272.
114. Session DR, Pearlstone MM, Jewelewicz R, et al. Estrogens and Parkinson's disease. *Med Hypotheses*. 1994;42:280–282.
115. Kompoliti K. Estrogen and movement disorders. *Clin Neuropharmacol*. 1999;22:318–326.
116. Saunders-Pullman R. Estrogens and Parkinson disease: neuroprotective, symptomatic, neither, or both? *Endocrine*. 2003;21:81–87.
117. Shulman LM. Is there a connection between estrogen and Parkinson's disease? *Parkinsonism Relat Disord*. 2002;8:289–295.
118. Marder K, Tang MX, Alfaro B, et al. Postmenopausal estrogen use and Parkinson's disease with and without dementia. *Neurology*. 1998;50:1141–1143.
119. Saunders-Pullman R, Gordon-Elliott J, Parides M, et al. The effect of estrogen replacement on early Parkinson's disease. *Neurology*. 1999;52:1417–1421.
120. Logroscino G, Sesso HD, Paffenbarger Jr RS, Lee IM. Physical activity and risk of Parkinson's disease: a prospective cohort study. *J Neurol Neurosurg Psychiatry*. 2006;77:1318.
121. Xu Q, Park Y, Huang X, et al. Physical activities and future risk of Parkinson disease. *Neurology*. 2010;75:341.
122. Ahlskog JE. Does vigorous exercise have a neuroprotective effect in Parkinson disease? *Neurology*. 2011;77:288.
123. Hawkins B. Perceived activities and participation outcomes of a yoga intervention for individuals with Parkinson's disease: a mixed methods study. *Int J Yoga Ther*. 2018;28(1):51–61.
124. Chang HC, Lu CS, Chiou WD, Chen CC, Weng YH, Chang Y-J. An 8-week low-intensity progressive cycling training improves motor functions in patients with early-stage Parkinson's disease. *J Clin Neurol (Seoul, Korea)*. 2018;14(2):225–233. https://doi.org/10.3988/jcn.2018.14.2.225.
125. Wang L, He C, Liu Y, et al. Effect of acupuncture on the auditory evoked brain stem potential in Parkinson's disease. *J Tradit Chin Med*. 2002;22:15–17.
126. Zhu W, Xi G, Ju J. Effect of acupuncture and Chinese medicine treatment on brain dopamine level of MPTP-lesioned C57BL mice. *Zhen Ci Yan Jiu*. 1996;21:46–49.
127. Park HJ, Lim S, Joo WS, et al. Acupuncture prevents 6-hydroxydopamine-induced neuronal death in the nigrostriatal dopaminergic system in the rat Parkinson's disease model. *Exp Neurol*. 2003;180:93–98.
128. Zhuang X, Wang L. Acupuncture treatment of Parkinson's disease: a report of 29 cases. *J Tradit Chin Med*. 2000;20:265–267.
129. Walton-Hadlock J. Primary Parkinson's disease: the use of *tui na* and acupuncture in accord with an evolving hypothesis of its cause from the perspective of Chinese traditional medicine—part 2. *Am J Acupunct*. 1999;27:31–49.
130. Shulman LM, Wen X, Weiner WJ, et al. Acupuncture therapy for the symptoms of Parkinson's disease. *Mov Disord*. 2002;17:799–802.
131. Noh H. Effectiveness and safety of acupuncture in the treatment of Parkinson's disease: a systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med*. 2017;34:86–103.

Pelvic Inflammatory Disease

Joseph Katzinger, ND and *Tori Hudson**, *ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Dyspareunia
- Mucopurulent cervical discharge
- Pelvic pain; bilateral adnexal tenderness, uterine compression tenderness on bimanual examination
- Palpable adnexal mass
- Elevated temperature (above 101°F)
- Cervical motion tenderness
- White blood cell count (WBC) 20,000/μL, with marked leukocytosis, elevated sedimentation rate, or both. Note that the Centers for Disease Control and Prevention (CDC) now also considers an elevated C-reactive protein as supportive of a pelvic inflammatory disease diagnosis.
- Presence of abundant numbers of WBC on saline microscopy of vaginal fluid
- *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) most common, followed by *Ureaplasma urealyticum, Mycoplasma hominis, Streptococcus* species, *Escherichia coli, Haemophilus influenzae, Peptostreptococcus,* and *Peptococcus*
- Transvaginal ultrasound shows thickened fluid-filled tubes or tubo-ovarian mass.
- Acute and chronic endometritis on endometrial biopsy
- Laparoscopy—the gold standard

GENERAL CONSIDERATIONS

Pelvic inflammatory disease (PID) is a categorical name for a range of pelvic infections and inflammations. The CDC defines PID by the presence of abdominal, adnexal, or cervical motion tenderness in the absence of another definable cause of the patient's symptoms but recognizes that requiring all three clinical criteria results in insufficient diagnostic sensitivity.[1] The diagnosis does not require the presence of elevated WBC or erythrocyte sedimentation rate (ESR) or fever.

PID causes women to make an estimated 2.5 million outpatient visits to health care providers each year. The lifetime risk in the United States has been estimated to be 4.4% but as high as 10% in women previously diagnosed with a sexually transmitted infection.[2] One fourth of women diagnosed with PID will suffer serious long-term sequelae, and all are at risk for recurrence.[3]

The social and physical costs of PID are also significant: the risk of ectopic pregnancy increases sixfold after a single episode of PID, and there is an estimated 13% to 18% risk of infertility after one infection and a 70% risk after three infections.[4] The Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) study, which treated and followed over 800 urban American women diagnosed with PID over a 3-year period, found that over 40% had chronic pelvic pain, and 20% had recurrent PID.[5] For these reasons as well as costs, prevention and aggressive full-spectrum care of the acute phase are vital.

Etiology

Several organisms (listed in Table 206.1[6]) are known to be implicated in the etiology of PID, but GC and CT are the most common. More recent data, however, suggest that the proportion of cases due to GC and/or CT may be declining; a retrospective study found that less than 20% of acute PID cases had laboratory-confirmed GC or CT.[7] Because of the complexities and inconsistencies involved in sampling and laboratory verification, it is difficult to make definitive statements about the causal agents in PID.

Asymptomatic chlamydial infections are an important cause of PID. More than half a million new infections with GC were reported to the CDC in 2017.[8] In this same year, nearly 2 million CT infections were reported to the CDC, making it the most common notifiable disease in the U.S., and the most common STD.[9] The true incidence of

* Previous edition contributor

TABLE 206.1 Microorganisms Involved in Pelvic Inflammatory Disease

Organism	Incidence (%)
Neisseria gonorrheae	40–60
Chlamydia trachomatis	30
Mycoplasma hominis	8
Ureaplasma urealyticum	4

Data from Karchmer AW. Sexually transmitted diseases. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American, 1996:7:XXII-10-13.

both infections is probably higher because not all states require reporting, and both infections may go undiagnosed and unreported. A higher proportion of PID has been ascribed to CT than to GC.[10]

Testing methods for GC and CT include cell culture, direct fluorescent antibody (DFA) testing, enzyme immunoassay (EIA) antigen-detection technique, nucleic acid hybridization tests (DNA probes), nucleic acid amplification (polymerase chain reaction [PCR] or ligase chain reaction [LCR]), and the ThinPrep Pap Collection system. The CDC recommendations are that all sexually active adolescents undergo routine screening for CT during annual pelvic examinations. The CDC also recommends that routine screening of asymptomatic women between 20 and 24 years of age should be considered, particularly if they have a new male sex partner, more than one male sex partner, or do not use barrier contraception.

Neisseria Gonorrhea

The incidence of GC in the United States increased by a factor of 2.7 from 1960 to 1980.[11] Although it reached a historic low in 2009, by 2016/2017 its incidence had increased by more than 75%. The infection has reached pandemic proportions in the United States, where it is estimated that for each reported case, four cases go unreported.[12] Gonococci have been recovered from the urethra of 2.2% of sexually active servicemen who had minimal or no symptoms and from 40% of asymptomatic males who had contact with symptomatic women.[13] In addition, an estimated 80% of the men and women exposed to *Gonococcus* will develop infections.[14]

For a seemingly delicate and fastidious species, *Neisseria* has impressive infective abilities, preferring human columnar and transitional epithelia. In less than 1 hour after intercourse, *Gonococcus* can establish itself on the urethral mucosa, where it successfully resists the flow of urine.[15] Favored sites in the lower female genital tract are Bartholin's and Skene's glands, the urethra, and the endocervical canal.[12] Direct spreading can occur from the endocervix across the endometrial surface to the tubal mucosa, or migration can occur through subendothelial vascular and lymphatic channels.[4] Perhaps the most common method of spreading, however, is by vector: GC attached to spermatozoa are physically carried to the fallopian tubes.[12] Primary pathogens can also enter the upper tract from retrograde menstruation or uterine contractions during intercourse.[16]

In the acute state, the gonococci and polymorphonuclear lymphocytes accumulate in the subepithelial connective tissue, resulting in patchy destruction of the overlying mucosa.[15,17] The consequent thinning of the mucosal lining is thought to facilitate the penetration of GC into deeper tissue.[15] It is probably for this reason that gonococci are reported to survive only a short time in the fallopian tubes.[18] However, they may be not only surviving but thriving. The descent of the microbe beyond the surfaces being examined makes detection difficult.[18] It has also recently been speculated that its ability to persist, despite stimulating a robust inflammatory response involving the recruitment and activation of neutrophils, is precisely how it damages the host epithelium. It is the sustained inflammation and neutrophil influx responsible for tissue damage, which continues due to GCs surprising resistance.[19]

Concomitant infections are known to occur with GC.[20] Some researchers have even proposed that GC's primary role appears to be paving the way for secondary invaders from normal vaginal flora, allowing access to the upper tract.[21] The associated infection will frequently be CT, but a superinfection can also be present, in which case one will find that anaerobic bacteria have colonized as well.[20]

Chlamydia Trachomatis

In the United States it was once estimated that 20% to 30% of PID cases were caused by CT, and although this rate may have declined in recent years, it is still the leading cause of PID.[6,22] Further, one study found that acute chlamydial PID may be subclinical or silent in 66% to 75% of the cases.[23] Laboratory diagnosis of chlamydial infection is difficult, which, combined with CT's propensity to be asymptomatic, renders a thorough assessment of the scope of these infections nearly impossible. Much like GC, CT eludes both innate and adaptive immune responses. When under assault by the immune system, the intracellular form of CT (known as the reticulate body) enters a persistent nonreplicative state and may return to its infectious extracellular form (the elementary body) when conditions improve.[24]

A 5-year study conducted in urban Sweden showed that despite a decrease in the incidence of gonococcal PID, the total number of cases of acute PID was unchanged or even increased in the last year of the study.[25] Another European study, conducted in 1977, found that 62% of women with acute salpingitis had elevated (titer ≥1:64) chlamydial immunoglobulin G (IgG) antibodies.[26] However, most other studies completed to date show much lower percentages. These numbers will no doubt change as diagnostic technology improves and the clinical presentation of CT is more widely understood. Currently, the CDC recommends the use of nucleic acid amplification tests for both screening and diagnosis. They are estimated to have a sensitivity greater than 90% and a specificity greater than 90%.[27]

Anaerobic Infections

Anaerobes are the organisms most commonly isolated from the fallopian tubes or cul-de-sacs of patients with PID.[28] Anaerobic bacteria are probably not the chief causative agents, but rather opportunists, establishing themselves in unsuccessfully defended tissues.

Anaerobic infections are commonly found in immunocompromised hosts and are generally of endogenous origin.[29] The cervix and vagina of a normal, healthy woman contain both anaerobic and aerobic bacteria.[30] Anaerobic infections establish themselves more often in older patients and in women with a history of prior PID.[11]

Other Organisms

Facultative aerobic organisms found in tuboperitoneal fluids from women with salpingitis have included coliforms, *H. influenzae, Streptococcus* species, and *Mycoplasma genitalium,* and *M. hominis.*[31]

M. hominis has not been demonstrated as a sole etiological agent, but rather seems to be a common contributor to the polymicrobial milieu that is often discovered in PID. One study found *M. hominis* in cervical cultures from 81% of women patients with GC and 64% of those without GC.[32] Similarly, *M. genitalium* has not conclusively proven to be causative, but a recent meta-analysis found over a twofold risk for PID associated with infection by this sexually transmitted organism, suggesting it may play a more significant role than previously believed.[33]

Complications

There are serious physical consequences for women who have had PID. It has been estimated that in the postinfection state, one of four

women suffers from one or more sequelae such as abdominal pain, infertility, or ectopic pregnancy.[11] Dyspareunia is a symptom that is often not investigated but, when relevant questions are asked, is frequently found in the post-PID sufferer.

Death from salpingitis is rare and is generally due to rupture of the tubo-ovarian abscess with subsequent peritonitis. A mortality rate of 5.2% to 5.9% has been calculated for tubo-ovarian abscesses; before 1950, mortality was 80% to 100%.[34,35] Better diagnostic understanding of this complication, treatment with antibiotics, and prompt surgical intervention have phenomenally improved both morbidity and mortality statistics.

Fitz–Hugh–Curtis syndrome is a rare perihepatitis complicating the primary condition of PID. Characteristic violin-string adhesions attach the liver to the abdominal wall.[29,36] These adhesions are due to local peritonitis involving the anterior liver surface and the adjacent abdominal wall.[37] Historically, GC was thought to be the main contributor to this syndrome, but CT is now found more frequently.[29,30]

Infertility is a serious concern. Once a woman has had PID, she is at risk for additional attacks. This is in part because after the fallopian tubes have been damaged by the infectious process, normal defense mechanisms are impaired. Reinfection has been found to be the most important cause of infertility after PID.[38] One study comparing the rate of nonsurgical infertility in 1973 with that of 1976 noted a 45% increase. This translates to 122,000 infertile couples per year.[39] This increased incidence is consistent with the concurrent epidemic of sexually transmitted disease (STD)–associated PID.

An alarming statistical analysis has shown that for every 1000 girls born in 1950, a total of 138 had one or more bouts of PID by age 30, 26 were infertile because of the PID, and 9 had surgery for ectopic pregnancy.[11] It was once postulated that one of every two women reaching reproductive age would have an episode of salpingitis.[30] While the incidence has declined over the last two decades, a recent study conducted in England found that over 16% of women between the ages of 35 to 44 had at least one previous episode of salpingitis, suggesting that this remains quite a common pathology.[40]

Ectopic pregnancy is a severe sequelae, and any woman with a history of PID faces a twofold to tenfold increased risk.[11,41] Before a standardized approach and treatment with effective antibiotics were implemented, nearly 10% of women with salpingitis had a subsequent ectopic pregnancy, and in the 1970s ectopic pregnancies were the leading cause of maternal death in nonwhite women.[39] Even with modern treatments, 18% of women treated for PID reported infertility after 3 years of follow-up, and 0.6% of them had an ectopic pregnancy. Increased episodes as well as a delay in appropriate treatment have both been associated with poorer outcomes.[42]

Risk Factors

In addition to the obvious factor of sexual contact, the main risk factors are age, use or history of use of an intrauterine device (IUD), and previous history of PID. An earlier "sexual debut" puts a young woman in a high-risk group for PID, especially when there are multiple sex partners. The risk in sexually active 15-year-olds is 1 in 8, whereas in the average 24-year-old, it is 1 in 80.[11] One interesting hypothesis for these data is that the cervical mucus in the younger woman may be estrogen-dominated, creating an environment that is more accessible to pathogens.[31] Women with multiple partners have a 4.6-fold greater risk than women in monogamous relationships.[30]

A woman may face an increased risk of PID if she uses an IUD, although this may be insignificant with current IUDs. Oral contraceptive (OC) users are somewhat less likely to have GC; on the other hand, they are at increased risk for chlamydial invasion. Birth control remains a potent issue, with barrier methods being the techniques of choice because of their decreased PID risk. It should also be noted that one author included his own clinical observation that women in his practice who had vasectomized men as partners only seldom developed PID.[43]

The last risk factor to be considered is iatrogenic. This occurs when invasive procedures have introduced pathogens or disturbed the tract flora in some other way and induced PID. Among these procedures are the following:

- Cervical dilation
- Abortion
- Curettage
- Tubal insufflation
- Hysterosalpingography
- Insertion of an IUD

A hospital in Lund, Sweden, reported that 15% of its PID cases were iatrogenic. This indicates that PID may not be strictly an STD.[29]

Pathogen Access to the Upper Female Tract

The route by which the pathogens gain access to the upper female tract has only recently been explored. Menstruation, sperm, and trichomonads have all been shown to be important in the transportation of pathogens into the salpinx.

Often the onset of menses corresponds with the onset of an episode of PID. Infections occurring around the menses tend to be GC rather than CT, a clinical curiosity that may ultimately shed light on the etiology. One hypothesis is that menstrual regurgitation assists the inflammatory response by carrying sloughed endometrial epithelium, which may have attached GC or intracellular CT. These organisms can then proliferate in the tubal epithelium or on peritoneal surfaces.[16,44]

Human sperm has proved to be an interesting and multifaceted variable in the precipitation of PID. Some of the research targets bacteriospermia as a cause of infertility in men, findings that are clearly relevant to PID. Increasingly, STD research is noting the incidence of asymptomatic male carriers.[12,13,43,45–48] A large population study discovered that 66% to 75% of the men who tested positive for GC were asymptomatic.[13] It is also important to note that although a Gram stain of urethral secretions has fairly high sensitivity and specificity for GC diagnosis among *symptomatic* men, a negative Gram stain is not sufficient to rule out infection among asymptomatic men.[49]

Designed to travel during intercourse, sperm also serve as effective vectors. Researchers took a look at this most basic interaction between a man and a woman by means of a laboratory experiment. They introduced organisms into capillary tubes containing cervical mucus, either alone or with added spermatozoa, and observed microbial motility. Cervical mucus had already been considered an effective mechanical and immunological barrier between the abundant flora of the vagina and the upper tract, and the test results confirmed this idea. However, they also demonstrated that organisms attached to sperm could easily traverse the length of the mucus column. This may be particularly important during menses, because sperm migration has been observed through menstrual plasma but not during the luteal phase or through the cervical mucus of pregnancy.

Electron microscopy has produced amazing photographic evidence of organisms attached to sperm.[15,50] The mechanism observed with piliated GC is that pili twist together, with the tails of the spermatozoa in a rope-in-a-spiderweb arrangement around the bacteria. Sperm have also been found to be intimately associated with cytomegalovirus, *Toxoplasma, Ureaplasma urealyticum,*[44] and CT.[51]

Motile trichomonads serve as another transporter of PID. They can ascend from the vagina to the fallopian tubes, carrying additional invaders. In fact, it has been observed that trichomonads are never isolated from humans when heavy bacterial contamination is absent.[44]

DIAGNOSIS

Pelvic or lower abdominal pain is the most dependable symptom of PID; unfortunately, however, it is not specific. Rebound tenderness is not reliably reported; cervical motion tenderness and adnexal tenderness are much more common. The clinical picture of the various types of PID can easily mislead. In a large study, the clinical diagnosis of PID was confirmed at laparoscopy in only 65% of the patients. Appendicitis, hemorrhagic corpus luteum, pelvic endometriosis, ectopic pregnancy, mesenteric adenitis, and ovarian tumors accounted for 12%, whereas 23% were found to be normal.[52] Many PID patients have atypical signs and symptoms, and some have no signs or symptoms at all.[11] Table 206.2 lists those most commonly found.

The woman with GC may appear more toxic and febrile and manifest leukocytosis, whereas CT-caused PID may give her an ESR. Most episodes of gonococcal PID occur at or shortly after menses.[53] Gonococcal PID has a generally more severe clinical picture, but tissue damage and long-term sequelae can be more severe in CT. This attempt to differentiate clinical pictures becomes meaningless, of course, in the presence of mixed infections.

Any mucopurulent discharge should yield oxygen-sensitive organisms because offensive odor is considered diagnostic of anaerobic infection. Any woman who has such a discharge probably has well-developed PID, with opportunistic anaerobes following the primary invaders.[38] Box 206.1 shows the differential diagnoses of PID. Potentially lethal conditions to consider include ectopic pregnancy, tubo-ovarian abscess, ovarian cyst rupture with hemorrhage, and appendicitis.

In light of Fitz–Hugh–Curtis syndrome, symptoms from the upper right quadrant in a sexually active woman may be an indirect sign of genital infection. The pain usually has a sudden onset and can overshadow the signs and symptoms of the underlying PID.[37]

In the event of rupture of a tubo-ovarian abscess, a sudden severe exacerbation of the pain can be observed. The pain is referred to the side of the rupture and is typically followed by generalized peritonitis and collapse. Shoulder pain may be present. The pulse will likely be elevated out of proportion to the fever and is frequently as high as 170.[35] Surgery must be performed within 12 hours or mortality becomes probable.

A careful history is, as always, invaluable. The patient's risk factors and history should be assessed for likelihood of infection with a sexually transmitted organism. The patient should be questioned about any new sex partner, method of contraception, and recent medical procedures. The source, severity, and characteristics of the pelvic/abdominal pain should be evaluated. Mucopurulent cervicitis should be considered as well, and the cervix should be cultured for GC and CT, with a nucleic acid amplification test (NAAT) performed as well. In 2018 a very large retrospective study conducted in Denmark found that a negative test for chlamydia by non-NAAT methodology was associated with a 17% higher risk for PID at 12 months compared with a negative NAAT, highlighting the superior sensitivity of the NAAT.[54]

Empiric treatment of PID should be initiated in sexually active young women and other women at risk for STDs if they are experiencing pelvic or lower abdominal pain, if no cause for the symptoms other than PID can be identified, and if one or more of the following minimum criteria are present on pelvic examination:

- Cervical motion tenderness or uterine tenderness or adnexal tenderness

The following additional criteria above and beyond the minimum criteria may enhance the diagnosis of PID:

- Oral temperature above 101°F or 38.3°C
- Abnormal cervical or vaginal mucopurulent discharge, or cervical friability
- Presence of abundant numbers of WBCs on saline microscopy of vaginal secretions
- Elevated ESR
- Elevated C-reactive protein
- Laboratory documentation of cervical infection with GC or CT

The following criteria for hospitalization are suggested:

- Surgical emergencies (e.g., appendicitis) cannot be excluded.
- The patient is pregnant.
- The patient does not respond clinically to oral antimicrobial therapy.
- The patient is unable to follow or tolerate an outpatient oral regimen.
- The patient has severe illness, nausea and vomiting, or high fever.
- The patient has a tubo-ovarian abscess.

THERAPEUTIC CONSIDERATIONS

Criteria for hospitalization include surgical emergency or septic-appearing patient, pregnancy, failure to respond to oral antibiotics, and suspicion of tubo-ovarian abscess. Referral is necessary if the diagnosis is uncertain or a surgical emergency threatens. Should the physician decide not to hospitalize, a regimen of antibiotic therapy combined with the supportive therapies discussed later can be tried if the patient's clinical and laboratory status can be reassessed in 48 to 72 hours. Laboratory values and objective patient criteria should direct all acute-phase treatment. The

TABLE 206.2 Common Signs and Symptoms in Acute Pelvic Inflammatory Disease

Symptom	Incidence (%)
Lower abdominal pain	90
Adnexal tenderness on palpation	90
Pain on movement of the cervix	90
Vaginal discharge	55
Adnexal mass or swelling	50
Fever or chills	40
Irregular vaginal bleeding	35
Anorexia, nausea, and vomiting	25

Data from Karchmer AW. Sexually transmitted diseases. In Dale DC, Federman DD, eds. *Scientific American Medicine*. New York: Scientific American, 1996:7:XXII-10-13.

BOX 206.1 Differential Diagnosis of Pelvic Inflammatory

- Acute appendicitis
- Acute cholecystitis
- Acute pyelonephritis
- Ectopic pregnancy
- Endometriosis
- Hemorrhagic ovarian cysts
- Intrauterine pregnancy
- Mesenteric lymphadenitis
- Ovarian cyst with torsion
- Ovarian tumor
- Pelvic thrombophlebitis
- Septic abortion

CDC leaves it to the individual practitioner to decide on the severity of the disease and the optimal treatment. Its guidelines do say that "most experts encourage hospitalization and treatment with intravenous antibiotics…. No evidence is available to suggest that adolescents have improved outcomes from hospitalization for treatment of PID, and the clinical response to outpatient treatment is similar among younger and older women. The decision to hospitalize adolescents with acute PID should be based on the same criteria used for older women."[55]

Antibiotics

PID is a complex syndrome, with inconsistent and variable presentations in different women and including a range of symptoms and etiological microorganisms. Because of this, broad-spectrum antibiotic regimens are employed that allow for some flexibility on the part of the practitioner. Regimens are tailored on the basis of clinical severity and laboratory findings, patient compliance, cost of medications, and availability of medications. Because some antibiotics cover only one organism, the CDC outpatient recommendations take into account the limited anaerobic activity of any one regimen.

Oral Treatment

Oral therapy can be considered for women with mild to moderately severe acute PID because the clinical outcomes among women treated with oral therapy are similar to those in women treated with parenteral therapy. Women who do not respond to oral therapy within 72 hours should be reevaluated to confirm the diagnosis and should be given parenteral therapy (see Box 206.2).

Fifteen percent of women with PID fail to respond to primary antimicrobial treatment, 20% have at least one recurrence, and 15% are rendered infertile.[28]

Given the polymicrobial nature of PID, the complexities of isolation, antibiotic-resistant strains of microorganisms, and the realities of recurrence rate with antibiotic use, an approach that combines immune system enhancement and nontoxic therapies concurrently with antibiotics seems sound. However, there are no evidence-based therapies using natural therapies exclusively for the treatment of PID. Antibiotics can help with the first phase of treatment but may not offer sufficient intervention for the devastation that regularly occurs in the wake of the primary infection.

BOX 206.2 Recommended CDC Oral Regimen (Last Updated 2015)

Recommended Regimen

Ceftriaxone 250 mg IM in a single dose
PLUS
Doxycycline 100 mg orally twice a day for 14 days
WITH[a] or WITHOUT
Metronidazole 500 mg orally twice a day for 14 days
OR
Cefoxitin 2 g IM in a single dose and Probenecid, 1 g orally administered concurrently in a single dose
PLUS
Doxycycline 100 mg orally twice a day for 14 days
WITH or WITHOUT
Metronidazole 500 mg orally twice a day for 14 days
OR
Other parenteral third-generation cephalosporin (e.g., ceftizoxime or cefotaxime)
PLUS
Doxycycline 100 mg orally twice a day for 14 days
WITH[a] or WITHOUT
Metronidazole 500 mg orally twice a day for 14 days

These regimens provide coverage against frequent etiological agents of PID, but the optimal choice of a cephalosporin is unclear. Cefoxitin, a second-generation cephalosporin, has better anaerobic coverage than ceftriaxone, and in combination with probenecid and doxycycline, it has been effective in short-term clinical response in women with PID. Ceftriaxone has better coverage against *N. gonorrhoeae*. The addition of metronidazole will also effectively treat bacterial vaginosis (BV), which is frequently associated with PID.

Several randomized trials have demonstrated the efficacy of parenteral regimens. Clinical experience should guide decisions regarding the transition to oral therapy, which usually can be initiated within 24 to 48 hours of clinical improvement. In women with tubo-ovarian abscesses, at least 24 hours of inpatient observation is recommended.

Recommended Parenteral Regimen

Cefotetan 2 g IV every 12 hours
PLUS
Doxycycline 100 mg orally or IV every 12 hours
OR
Cefoxitin 2 g IV every 6 hours
PLUS
Doxycycline 100 mg orally or IV every 12 hours
Clindamycin 900 mg IV every 8 hours
PLUS
Gentamicin loading dose IV or IM (2 mg/kg of body weight), followed by a maintenance dose (1.5 mg/kg) every 8 hours. Single daily dosing (3–5 mg/kg) can be substituted.

Because of the pain associated with intravenous infusion, doxycycline should be administered orally when possible. Oral and IV administration of doxycycline provide similar bioavailability. Although the use of a single daily dose of gentamicin has not been evaluated for the treatment of PID, it is efficacious in analogous situations.

When using the parenteral cefotetan or cefoxitin regimens, oral therapy with doxycycline 100 mg twice daily can be used 24 to 48 hours after clinical improvement to complete the 14 days of therapy. For the clindamycin/gentamicin regimen, oral therapy with clindamycin (450 mg orally four times daily) or doxycycline (100 mg twice daily) can be used to complete the 14 days of therapy. However, when tubo-ovarian abscess is present, clindamycin (450 mg orally four times daily) or metronidazole (500 mg twice daily) should be used to complete at least 14 days of therapy with doxycycline to provide more effective anaerobic coverage than doxycycline alone. Limited data are available to support the use of other parenteral second- or third-generation cephalosporins (e.g., ceftizoxime, cefotaxime, and ceftriaxone). In addition, these cephalosporins are less active than cefotetan or cefoxitin against anaerobic bacteria.

[a]The recommended third-generation cephalosporins are limited in the coverage of anaerobes. Therefore until it is known that extended anaerobic coverage is not important for treatment of acute PID, the addition of metronidazole to treatment regimens with third-generation cephalosporins should be considered (Source: Walker CK, Wiesenfeld HC. Antibiotic therapy for acute pelvic inflammatory disease: the 2006 CDC Sexually Transmitted Diseases Treatment Guidelines. *Clin Infect Dis* 2007;28[Supp 1]:S29–36).

Data from Centers for Disease Control and Prevention. Sexually Transmitted Diseases: Treatment Guidelines, 2015: Pelvic Inflammatory Disease. https://www.cdc.gov/std/tg2015/pid.htm, accessed 2-2019.

Physical Medicine

Diathermy

In the 1920s and 1930s, pulsed, high-frequency diathermy was first reported to be beneficial in the treatment of women with PID.[56–58]

Pulsing electric energy for a short duration (65 mcs every 1600 mcs) at high intensity achieves the desired therapeutic result without the hyperpyrexia typically associated with diathermy. Local recovery is enhanced, the reticuloendothelial system is stimulated, and gamma-globulin fractions are increased.[30] There is very little research on using this therapy, and practitioners should be very cautious in using it as a substitute for antibiotic therapy. A small study conducted in 2011 suggests that short-wave diathermy may provide pain relief to women with chronic PID, compared with sham diathermy and analgesic therapy.[59]

Sitz Baths

Traditionally, sitz baths have been an important component of the naturopathic treatment of PID. The contrast sitz bath is primarily used to increase pelvic circulation, bring an influx of macrophages to the area, and provide decongestion of the pelvic inflammatory reaction (for further discussion, see Chapter 40).

Nutritional Supplements

Vitamin C

Vitamin C may be useful in the treatment of women with PID for the following reasons:

- Its anti-inflammatory effects help to decrease tissue destruction.
- Its support of collagen tissue repair helps to prevent the spread of infection (especially important in GC infections, which can spread through the subepithelial connective tissue, resulting in disorganization of the collagen matrix[14]).
- Its fibrinolytic activity helps to prevent pelvic scarring.

Beta-Carotene

The normal ovary has a high concentration of beta-carotene. As these structures are bombarded by inflammation and the unwelcome company of aggressive microbes, it is essential to maintain optimal levels of carotene to allow for an optimal defense. Beta-carotene potentiates the beneficial effects of interferon and enhances numerous other immune functions, such as antibody levels and WBC activity.[60,61] Beta-carotene is also important as an antioxidant, helping to limit the cell damage induced by the inflammatory process (see Chapter 57 for a full discussion of this nutrient).

Bromelain

Bromelain should be considered an important component of the treatment regimen. Adnexal exudate in PID frequently suppurates to form abscesses. If tissue irritation is relieved or ameliorated during the acute stage, much of the exudate can be absorbed, and fewer adhesions will be formed.[56] Adhesions will form as the exudate lingers, the structures being overwhelmed by the inflammation. Also, after resolution, agglutination of the villous fold in the lumen of the tube may occur, resulting in scarring and tubal occlusion.[12]

Bromelain activates fibrinolysis, which can greatly diminish the enduring sequelae of the inevitable exudate. Bromelain also demonstrates antimicrobial properties, and an Italian study has shown that it penetrates the salpinx[62] (see Chapter 59 for further discussion).

Probiotics

When individuals take antibiotics, the gastrointestinal, vaginal, and bladder microflora are disrupted. This can cause side effects with antibiotic use or after such use, including diarrhea, candidal vulvovaginitis, and acute cystitis. Probiotics are generally defined as live microorganisms whose function is dependent on the ability of a strain to benefit the host when it is administered orally.

There are many species and strains that have been proved to have adhesive properties and can proliferate in these organs and thus prevent opportunistic overgrowth, as well as the side effects from antibiotics, and also restore the normal microflora. No one species or strain can be recommended here, but combination products to be considered should include at least one or more of the following: the *Lactobacillus* species *L. rhamnosus, L. plantarum, L. rheuteri,* and *L. acidophilus* and the *Bifidobacterium* species *B. bifidum, B. lactis, B. breve,* and *B. longum.* A recent in vitro–based study found that *Lactobacillus* species, especially *L. crispatus,* reduced the ability of CT to infect cervical cells. Cervical cells exposed to *L. crispatus* had a more fluid plasma membrane, with reduced exposure of polar lipids and α5β1 integrin subunits, with the latter potentially a key aspect of preventing CT infection. This same strain appears to inhibit GC infection as well.[63,64]

Botanical Medicines

Many botanicals have been shown to have antimicrobial and immune-stimulating effects. Allicin extracts from garlic, goldenseal, and Oregon grape root and echinacea should all be considered at least as part of a treatment plan and as adjuncts to antibiotic therapy.

Vaginal Depletion Packs

The use of the vaginal depletion pack has historically been a part of traditional PID treatment because it promotes the drainage of exudate from the involved tissues. (This is discussed in more detail in Appendix 14.) It may also stimulate the immune cells within the vagina to provide a first line of defense.

Hydrastis Canadensis

The immune-potentiating specific antimicrobial properties and the general antibacterial nature of goldenseal make it indispensable in the care of PID. Because *Hydrastis canadensis* is also a trophorestorative to mucous membranes, the herb should be used throughout the rehabilitation period. (*H. canadensis* is discussed in more detail in Chapter 86.)

Prevention

STD prevention is an extremely important issue for all heterosexually and bisexually active women. The woman with no history of PID should still be concerned about the significant population of asymptomatic male carriers of STDs. The choice of birth control is pivotal. Women who use barrier methods of contraception have a lower risk of PID.

Oral Contraceptives

A surprising number of articles laud the use of oral contraceptives (OCs) for their apparent inhibition of GC.[30] Burnham[28] has suggested OC use after a first episode of PID to prevent recurrence. Apparently, estrogens create a thicker cervical plug, which offers protection against gonococci.[14,16] OCs also decrease the length and volume of menstrual flow, thus decreasing the exposure of the GC to this handy culture medium.

On the other hand, OC users have a higher risk of chlamydial infections.[14,44,53,65] Progesterone can produce cervical eversion, exposing the endocervical columnar epithelium—the target tissue of CT.[53] In one of the largest studies to date, an analysis of over 50,000 married women in China found that subdermal implants were associated with a nearly 3.5-fold adjusted increase in risk for PID, whereas no other form of birth control influenced risk. Although not described in

the study, it is likely that most subdermal implants are progesterone based.[66] Estradiol has been implicated in suppressing the endocervical antibodies necessary for the resolution of CT.[53] Animal experimentation finds that estrogen-treated individuals have a higher number of infected cervical cells and a longer duration of infection.[67] Because women are probably not selectively exposed to GC versus CT, OCs are not recommended.

Intrauterine Devices

The IUD has a bad reputation in PID. This reputation is a carryover from the former Dalkon Shield, which is no longer available. Current IUDs such as the Copper T and the Mirena are generally considered safe. Indeed, a 2017 analysis of 14 studies eligible for review found that either no risk or no clinically meaningful risk for PID was associated with IUD use.[68] However, the use of an IUD is associated with a slightly increased risk of PID, especially in the first 3 weeks of use.[56] An IUD allows the colonization of bacteria on its surface while simultaneously reducing local immunological capacity.[70] If a woman with suspected PID has an IUD, it was previously recommended to be removed 12 to 24 hours after initiation of antibiotic therapy in order to prevent the spread of the infection during its removal.[11,23,28,31,37,71,72] However, in 2013 the CDC revised its guidelines and instead suggests that if a woman with an IUD is diagnosed with PID, the IUD does not need to be removed, although if no improvement occurs after 48 to 72 hours, removal should be considered.[73] A systematic review of the evidence suggests there is no benefit to removal, and it may be that women who do not remove their IUDs have shorter hospitalizations.[74]

Barrier Methods

Barrier methods of contraception are excellent choices for the prevention of PID. The condom is preferred to cervical protectors because with this method, the sperm more rarely reach the vaginal vault.

Douching

Haphazard douching is to be avoided because it disturbs the vaginal flora. All forms of douching increase the risk of PID and can cause organisms to ascend into the upper genital tract.

One study compared 100 consecutive patients hospitalized for PID with 762 controls and 119 women suspected of having PID.[75] Current douching (defined as any douching during the previous 2 months) was more common among those with PID than among those in both control groups. Among current douchers, PID was related to the frequency of douching. Those who douched three or more times per month were 3.6 times more likely to develop PID than those who douched less than once per month.

A much larger study surveyed 6984 women older than 18 years of age and found that 32% said they had douched within the previous week; 13% reported regular douching more than once a week.[76]

Intercourse During Menses

Intercourse during menses is not recommended unless a condom is used. GC risk is increased by the loss of the protective cervical mucus plug and by the prevalence of blood, a medium of choice for *Gonococcus.* The endometrium is also thought to offer local protection against bacterial invasion, and it is this layer that is being sloughed off during menses.

Smoking

When 197 women hospitalized for their first PID infection were compared with 667 controls with nongynecological conditions, it was found that cigarette smokers, compared with women who had never smoked, had an elevated risk of PID of 1.7, and former cigarette smokers had an elevated risk of 2.3. There was no dose-response relationship.[77]

A more rigorous study found similar results. This was a case-controlled, population-based study of 131 women between 18 and 40 years of age who were treated for their first episode of PID compared with 294 randomly selected patients from the same health maintenance organization. Current smokers, compared with those who had never smoked, had an increased risk of PID. Women who smoked 10 or more cigarettes a day had a higher risk than those who smoked less.[78] A subsequent study of nearly 1500 indigenous women living in Queensland found a 3.1-fold increase in risk for PID among smokers, and a 4-fold increase in risk for PID hospitalization was found among women with low serum folate levels.[79]

Education

A physician should review the signs and symptoms of PID with all sexually active women and encourage any woman to seek counsel if she appears to fit the clinical picture for PID. The diagnosis is easier to make and the recovery more rapid when treatment is instituted early.

THERAPEUTIC APPROACH

Treatment consists of two phases, both of which are important. The first therapeutic goal is to eliminate all pathogens and normalize the microflora of the adnexa. The second is to rehabilitate the damaged tissues.

Women should avoid intercourse until all signs and symptoms are resolved and their male partners have been examined and treated. In addition, all partners from up to 2 months before the illness should be examined[28] and treated if a diagnosis of PID is made. Abstinence from sexual intercourse must also accompany all forms of treatment, and retesting at 3 months is suggested for women with a previous diagnosis of GC or CT infection.

These therapies are recommended as adjuncts to appropriate antibiotic treatment and immune support, as discussed in Chapter 136.

Diet

All dietary inhibitors of immune function (sugar, alcohol, saturated fats, simple carbohydrates) should be limited during both phases of treatment.

Supplements

- Vitamin A (only if certain not pregnant): 20,000 IU/d for 2 weeks
- Beta-carotene: 100,000 IU/day for 2 or more months
- Vitamin E (mixed tocopherols): 400 IU/day for 3 months
- Vitamin C: 1000 mg four times a day for the first week of treatment and then decreased over 3 days to 500 mg three times a day
- Chlorophyll: 10 mg of fat-/oil-soluble form four times a day for 1 month
- Bromelain: 250 mg (1800 milk-clotting units [MCU]) four times a day for the first week and three times a day for 6 weeks
- Probiotics supplement: minimum 1 billion CFU per day, consider up to 24 billion CFU per day during antibiotic treatment and for 2 months thereafter

Botanical Medicines

- *H. canadensis:* 500 mg of the solid extract (4:1 or 8%–12% alkaloid content) three times a day during the acute phase; 250 mg three times a day during recovery
- Vaginal packs: daily during the acute phase until there is adequate clinical and laboratory response. After the acute phase, vaginal packs need to be used three times a week, alternating with chlorophyll douches, for 3 weeks.

Physical Medicine

- Diathermy: pulsed, high-intensity diathermy for 10 minutes over the suprapubic area, 10 minutes over the liver, and 10 minutes in the area of the left adrenal (the right being presumably stimulated with the liver)
- Sitz baths: one to two times/day throughout the acute phase. Contrast sitz baths are given in groups of three alterations of hot to cold. Two separate tubs are necessary during this process. The hot is at 105°F to 115°F, the cold at 55°F to 85°F, with the temperatures dependent on the patient's tolerance. The standard treatment is 3 minutes hot and 30 seconds cold, with this cycle being repeated three times in one sitting. The water level in the hot tub is set 1 inch higher than in the cold. Adequate draping is necessary to prevent chilling. As with all hydrotherapy treatments, one always finishes with the cold.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. 2015 sexually transmitted diseases treatment guidelines: pelvic inflammatory disease (PID). Centers for Disease Control and Prevention. https://www.cdc.gov/std/tg2015/pid.htm. Accessed February 2019.
2. Kreisel K1, Torrone E1, Bernstein K, et al. Prevalence of pelvic inflammatory disease in sexually experienced women of reproductive age - United States, 2013-2014. *MMWR Morb Mortal Wkly Rep*. 2017;66(3):80–83. PMID: 28125569.
3. Washington AE, Arno P, Brooks A. The economic cost of pelvic inflammatory disease. *JAMA*. 1986;255:1735–1738.
4. Dodson FS. The polymicrobial etiology of acute pelvic inflammatory disease and treatment regimens. *Rev Inf Dis*. 1985;7:s6996–s7002.
5. Ness RB1, Trautmann G, Richter HE, et al. Effectiveness of treatment strategies of some women with pelvic inflammatory disease: a randomized trial. *Obstet Gynecol*. 2005;106(3):573–580. PMID: 16135590.
6. Karchmer AW. Sexually transmitted diseases. In: (eds. Dale DC, Federman DD.) *Scientific American Medicine.* Scientific American: New York.
7. Burnett AM1, Anderson CP, Zwank MD. Laboratory-confirmed gonorrhea and/or chlamydia rates in clinically diagnosed pelvic inflammatory disease and cervicitis. *Am J Emerg Med*. 2012;30(7):1114–1117. PMID: 22030186.
8. Sexually transmitted disease surveillance 2017: gonorrhea. Centers for Disease Control and Prevention.https://www.cdc.gov/std/stats17/Gonorrhea.htm. Accessed February 2019.
9. Sexually transmitted disease surveillance 2017: chlamydia. Centers for Disease Control and Prevention. https://www.cdc.gov/std/stats17/chlamydia.htm. Accessed February 2019.
10. Black C. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Micr Rev*. 1997:160–184.
11. Westrom L. Incidence, prevalence and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. *Am J Ob Gyn*. 1980;138:880–892.
12. Spence M. The role of gonococcus in salpingitis. *J Repro Med*. 1977;19:31–35.
13. Handsfield H, Lipman T, Harisch J, et al. Asymptomatic gonorrhoeae in men. *N Engl J Med*. 1974;290:117–123.
14. Ringsdorf W, Cheraskin E. Vitamin C and human wound healing. *Oral Surg*. 1985:231–233.
15. Ward M, Watt P, Robertson J. The human fallopian tubes: a laboratory model for gonococcal infection. *J Inf Dis*. 1974;129:650–659.
16. Denhanm I. Pelvic inflammatory disease. *Austral Fam Phy*. 1986;15:254–256.
17. McGee ZA, Stephens D, Hoffman LH, et al. Mechanisms of mucosal invasion by pathogenic *Neisseria*. *Rev Infect Dis*. 1983;5(suppl 4):708–714.
18. Lip J, Burgoyne X. Cervical and peritoneal bacterial flora associated with salpingitis. *Ob Gyn*. 1966;28:561–563.
19. Stevens JS1, Criss AK. Pathogenesis of *Neisseria gonorrhoeae* in the female reproductive tract: neutrophilic host response, sustained infection, and clinical sequelae. *Curr Opin Hematol*. 2018;25(1):13–21. PMID: 29016383.
20. Cunningham FG, Hath J, Gilstrap L. The bacterial pathogenesis of acute pelvic inflammatory disease. *Ob Gyn*. 1978;52:161–164.
21. Chow A, Malkasian K, Marshall J, et al. The bacteriology of acute pelvic inflammatory disease. *Am J Ob Gyn*. 1974;122:876–879.
22. Thompson S, Washington E. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. *Epid Rev*. 1983;5:96–123.
23. Guderian AM, Trobough G. Residues of pelvic inflammatory disease in intrauterine device users: a result of intrauterine device or *Chlamydia trachomatis* infection? *Am J Ob Gyn*. 1986;154:497–503.
24. Witkin SS1, Minis E2, Athanasiou A, et al. *Chlamydia trachomatis:* the persistent pathogen. *Clin Vaccine Immunol*. 2017;24(10). pii: e00203-17. PMID: 28835360.
25. Forslin L, Falk V, Danielsson D. Changes in the incidence of acute gonococcal and nongonococcal salpingitis. *Br J Vener Dis*. 1978;54:247–250.
26. Trehearne JD, Ripa KT, Mardh P-A. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis*. 1979;55:26–29.
27. Papp JR, Schachter J, Gaydos C, et al. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*–2014. *MMWR Recomm Rep*. 2014;63(No. RR-02). PMID: 24622331.
28. Burnham RC. Therapy for acute pelvic inflammatory disease: a critique of recent treatment trials. *Am J Ob Gyn*. 1984;148:235–240.
29. Mardh PA. An overview of infectious agents of salpingitis, their biology and recent advances in methods of detection. *Am J Ob Gyn*. 1980;138:933–951.
30. Shafer M, Irwin C, Sweet R. Acute salpingitis in the adolescent female. *J Ped*. 1982;100:339–350.
31. Holmes K, Eschenbach D, Knapp J. Salpingitis: overview of etiology and epidemiology. *Am J Ob Gyn*. 1980;138:893–900.
32. Eschenbach D, Buchanan T, Pollock H, et al. Polymicrobial etiology of acute pelvic inflammatory disease. *Am J Ob Gyn*. 1975;122:166–177.
33. Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis*. 2015;61(3):418–426. PMID: 2590017.
34. Mickal A, Sellmann A, Beebe J. Ruptured tuboovarian abscess. *Am J Ob Gyn*. 1968;100:432–436.
35. Pedowitz P, Bloomfield R. Ruptured adnexal abscess. *Am J Ob Gyn*. 1964;88:721–729.
36. Urnes A, Stray-Pedersen B, Raknerud N. Case report: massive ascites as a complication to subclinical perihepatitis and pelvic inflammatory disease. *Acta Obstet Gyn Scand*. 1986;65:277–278.
37. Wolner-Hanssen P, Westrom L, Mardh PA. Perihepatitis and chlamydial salpingitis. *Lancet*. 1980;i:901–903.
38. Bartlett J. Anaerobic infections of the pelvis. *Clin Ob Gyn*. 1979;21:351–359.
39. Curran J. Economic consequences of pelvic inflammatory disease in the U.S. *Am J Ob Gyn*. 1980;138:848–851.
40. Price MJ, Ades AE, Welton NJ, Simms I, Horner PJ. Pelvic inflammatory disease and salpingitis: incidence of primary and repeat episodes in England. *Epidemiol Infect*. 2017;145(1):208–215.
41. den Heijer CDJ, Hoebe CJPA, Driessen JHM, et al. Chlamydia trachomatis and the risk of pelvic inflammatory disease, ectopic pregnancy, and female infertility: a retrospective Cohort Study among primary care patients. *Clin Infect Dis*. 2019;69(9):1517 –1525.
42. Brunham RC, Gottlieb, SL, Paavonen J. Pelvic inflammatory disease. *N Engl J Med*. 2015;372(21):2039–2048.
43. Toth A, O'Leary W, Ledger W. Evidence for microbial transfer by spermatozoa. *Ob Gyn*. 1982;59:556–559.
44. Kinghorn GR, Waugh MA. Oral contraceptive use and prevalence of infection with *Chlamydia trachomatis* in women. *Br J Vener Dis*. 1981;57:187–190.
45. Lang D, Kummer J. Cytomegalovirus in semen: observation in selected populations. *J Inf Dis*. 1975;132:472–473.
46. Dahlberg B. Asymptomatic bacteriospermia. *Urology*. 1976;VIII:563–566.
47. Lang D, Kummer J. Demonstration of cytomegalovirus in semen. *N Engl J Med*. 1972;287:756–758.
48. Eschenbach DA. Acute pelvic inflammatory disease: etiology, risk factors and pathogenesis. *Clin Ob Gyn*. 1976;19:147–169.
49. 2015 sexually transmitted diseases treatment guidelines: gonococcal infections. Centers for Disease Control and Prevention. https://www.cdc.gov/std/tg2015/gonorrhea.htm. Accessed February 2019.
50. Gomez C, Stenback W, James A, et al. Attachment of *Neisseria gonorrhoeae* to human sperm. *Br J Ven Dis*. 1979;55:245–255.
51. Friberg J. Chlamydia attached to spermatozoa. *J Inf Dis*. 1985;152:854.
52. Jacobson L, Westrom L. Objectivized diagnosis of acute pelvic inflammatory disease: diagnostic and prognostic value of routine laparoscopy. *Ob Gyn*. 1969;105:1088.
53. Cromer B, Heald F. Pelvic inflammatory disease associated with *Neisseria gonorrhoeae* and *Chlamydia trachomatis.* clinical correlates. *Sex Trans Dis*. 1987;14:125–129.
54. Davies B1, Turner KME2, Benfield T, et al. Pelvic inflammatory disease risk following negative results from chlamydia nucleic acid amplification tests (NAATs) versus non-NAATs in Denmark: a retrospective cohort. *PLoS Med*. 2018;15(1):e1002483. PMID: 29293503.
55. https://www.cdc.gov/std/tg2015/pid.htm. Accessed 2-2019.
56. Gellhorn G. Diathermy in gynecology. *JAMA*. 1928:1005–1008.
57. Horowitz E, Derow D, Bierman W. Temperature determination in the female pelvis during diathermy. *Am J M Sc*. 1935;189:555–556.

58. Kottke F, Gullickson G, Erickson H, et al. Study of the reactive value of long wave diathermy and microwave diathermy for heating the pelvis. *Arch Phys Med Rehab*. 1955:137–140.
59. Lamina S1, Hanif S, Gagarawa YS. Short wave diathermy in the symptomatic management of chronic pelvic inflammatory disease pain: a randomized controlled trial. *Physiother Res Int*. 2011;16(1):50–56. PMID: 20564681.
60. Rhodes J. Human interferon action: reciprocal regulation by retinoic acid and beta-carotene. *J Natl Cancer Inst*. 1983;70:833–837.
61. Alexander M, Newmark H, Miller RG. Oral beta-carotene can increase the number of OKT4? cells in human blood. *Immunol Letters*. 1985;9:221–224.
62. Luerti M, Vignali M. Influence of bromelain on penetration of antibiotics in uterus, salpinx and ovary. *Drugs Exp Clin Res*. 1978;4:45–48.
63. Parolin C1, G1 F, Foschi C, et al. Lactobacillus crispatus BC5 Interferes with *Chlamydia trachomatis* Infectivity through integrin Modulation in cervical cells. *Front Microbiol*. 2018;9:2630. PMID: 30459737.
64. Foschi C, Salvo M, Cevenini R, et al. Vaginal *Lactobacilli* Reduce *Neisseria gonorrhoeae* Viability through multiple strategies: an in vitro study. *Front Cell Infect Microbiol*. 2017;7:502. PMID: 29270390.
65. Washington AE, Gove S, Schachter J, et al. Oral contraceptives, *Chlamydia trachomatis* infection and pelvic inflammatory disease. *JAMA*. 1985;253:2446–2450.
66. Wang LY1, OuYang L1, Tong F, et al. The effect of contraceptive methods on reproductive tract infections risk: a cross-sectional study having a sample of 52,481 women. *Arch Gynecol Obstet*. 2016;294(6):1249–1256. PMID: 27538571.
67. Rank R, White H, Hough A. Effect of estradiol on Chlamydial genital infection of female Guinea pigs. *Infect Immun*. 1982;38:699–705.
68. Jatlaoui TC1, Riley HEM2, Curtis KM2. The safety of intrauterine devices among young women: a systematic review. *Contraception*. 2017;95(1):17–39. 27771475.
70. Keith L, Berger G, Edelman D, et al. On the causation of pelvic disease. *Am J Ob Gyn*. 1984;149:215–224.
71. Burkman R. Intrauterine device use and the risk of pelvic inflammatory disease. *Am J Ob Gyn*. 1980;138:861–863.
72. Eschenbach D, Harnisch J, Holmes K. Pathogenesis of acute pelvic inflammatory disease: role of contraception and other risk factors. *Am J Ob Gyn*. 1980;128:838–850.
73. CDC. U.S. Selected practice recommendations for contraceptive use, 2013: Adapted from the World Health Organization selected practice recommendations for contraceptive use, 2nd ed. *MMWR Recomm Rep*. 2013;62(No. RR-05). PMID: 23784109.
74. Tepper NK, Steenland MW, Gaffield ME, et al. Retention of intrauterine devices in women who acquire pelvic inflammatory disease: a systematic review. *Contraception*. 2013;87:655–660. PMID: 23040135.
75. Wolner-Hanssen P. Association between vaginal douching and acute pelvic inflammatory disease. *JAMA*. 1990;263:1936–1941.
76. Tucker ME. Douching raises pelvic inflammatory disease risk. *Fam Pract News*. 1996, August 15.
77. Marchbanks P, Lee NC, Peterson HB. Cigarette smoking as a risk factor for pelvic inflammatory disease. *Am J Ob Gyn*. 1990;162:639–644.
78. Scholes D. Current cigarette smoking and risk of acute pelvic inflammatory disease. *Am J Pub Health*. 1992;82:1352–1355.
79. Li M, McDermott R. Smoking, poor nutrition, and sexually transmitted infections associated with pelvic inflammatory disease in remote North Queensland Indigenous communities, 1998-2005. *BMC Womens Health*. 2015;15:31. PMID: 25887145.

Peptic Ulcer—Duodenal and Gastric

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Epigastric distress 45 to 60 minutes after meals or nocturnal pain; both relieved by food, antacids, or vomiting
- Epigastric tenderness and guarding
- Symptoms chronic and periodic
- Gastric analysis showing acid in all cases, with hypersecretion in about one half of patients with duodenal ulcers
- Ulcer crater or deformity, usually occurring at the duodenal bulb (duodenal ulcer) or pylorus (gastric ulcer), on radiography or fiberoptic examination
- Positive test for occult blood in stool

GENERAL CONSIDERATIONS

Peptic ulcer formation occurs in the stomach (gastric ulcer) and the first portion of the small intestine (duodenal ulcer) (Fig. 207.1). Duodenal ulcers are more common, with an estimated prevalence of 6% to 12% in the United States. Approximately 10% of the U.S. population has clinical evidence of duodenal ulcer at some time in their lifetime. It is four times more common in men than in women and four to five times more common than clinically evident benign gastric ulcer.

Although symptoms of a peptic ulcer may be absent or quite vague, most peptic ulcers are associated with abdominal discomfort noted 45 to 60 minutes after meals or during the night. In the typical case, the pain is described as gnawing, burning, cramp-like, aching, or "heartburn." Eating or using antacids usually results in great relief. In the elderly, the presentation of peptic ulcer disease may also be subtle and atypical compared with that in younger patients, leading to a delay in diagnosis.

Even though duodenal and gastric ulcers occur at different locations, they appear to be the result of similar mechanisms. Specifically, the development of a duodenal or gastric ulcer is due to damage to the protective factors that line the stomach and duodenum.

In the past, the focus has primarily been on the acidic secretions of the stomach as the primary cause of both gastric and duodenal ulcers. However, the focus has shifted to the bacterium *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs (NSAIDs).

Gastric acid is extremely corrosive, with a pH of 1 to 3. To protect against ulcers, the lining of the stomach and small intestine is protected by a layer of mucin. In addition, the constant renewal of intestinal cells and the secretion of factors that neutralize the acid when it comes in contact with the stomach and intestinal linings also protect against ulcer formation.

Excessive gastric acid output is rarely a factor in gastric ulcers because in these patients, gastric acid output is usually normal or reduced. In contrast, almost one half of patients with duodenal ulcers have increased gastric acid output. This increase may be due to an increase in the number of parietal cells. As a group, patients with duodenal ulcers have twice as many parietal cells as normal controls.

Even with an increase in gastric acid output, under normal circumstances, there are enough protective factors to prevent ulcer formation. However, when the integrity of these protective factors is impaired, an ulcer can form. A loss of integrity can be due to *H. pylori,* NSAIDs, alcohol, nutrient deficiency, stress, and many other factors (Fig. 207.2). Of these, *H. pylori* and NSAIDs are by far the most significant.

Several chronic diseases have also been associated with an increased risk of developing peptic ulcers. These include Crohn's disease, chronic renal failure, liver cirrhosis, cystic fibrosis, chronic obstructive pulmonary disease, systemic mastocytosis (a condition in which there are too many immune mast cells in the body), and myeloproliferative disorders (e.g., polycythemia vera, chronic myelogenous leukemia, agnogenic myeloid metaplasia, and essential thrombocythemia).[1]

Helicobacter pylori

The role of *H. pylori* in peptic ulcer disease has been extensively investigated. It has been shown that 90% to 100% of patients with duodenal ulcers, 70% with gastric ulcers, and about 50% of those above

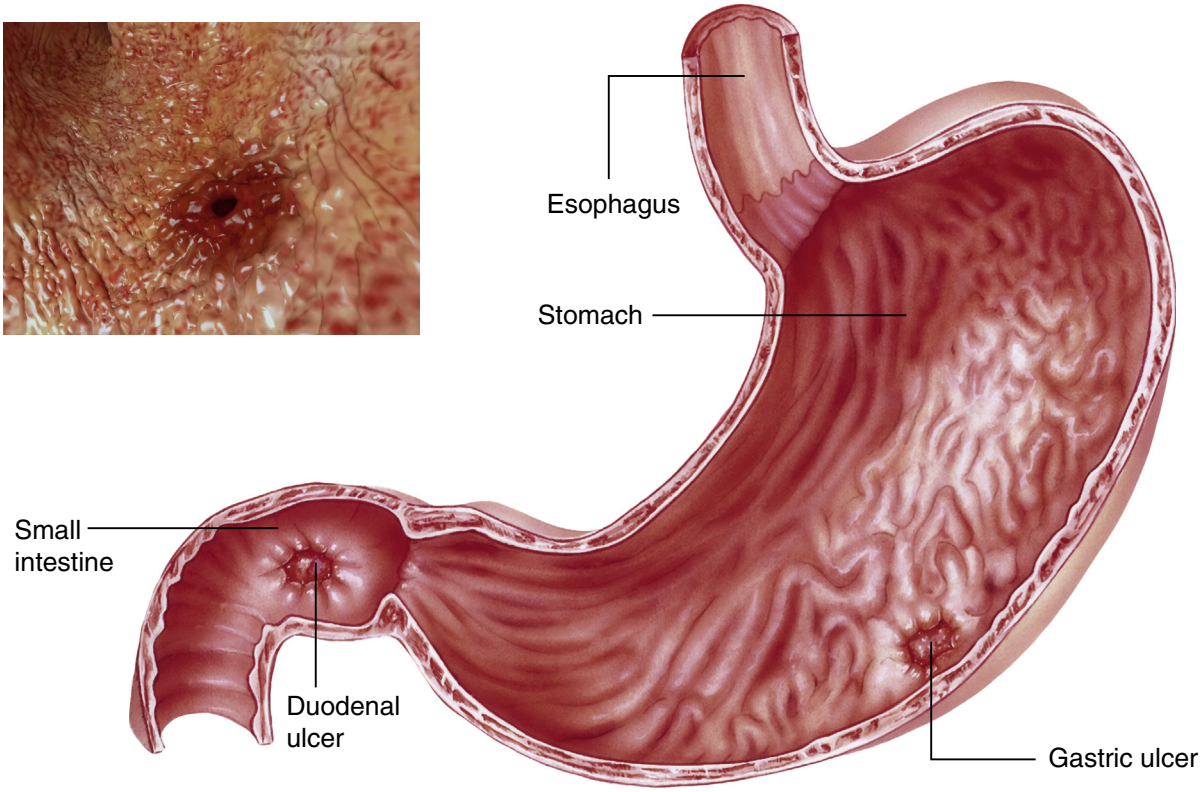


Fig. 207.1 Peptic ulcer. (From https://www.mayoclinic.org/diseases-conditions/peptic-ulcer/symptoms-causes/syc-20354223.)

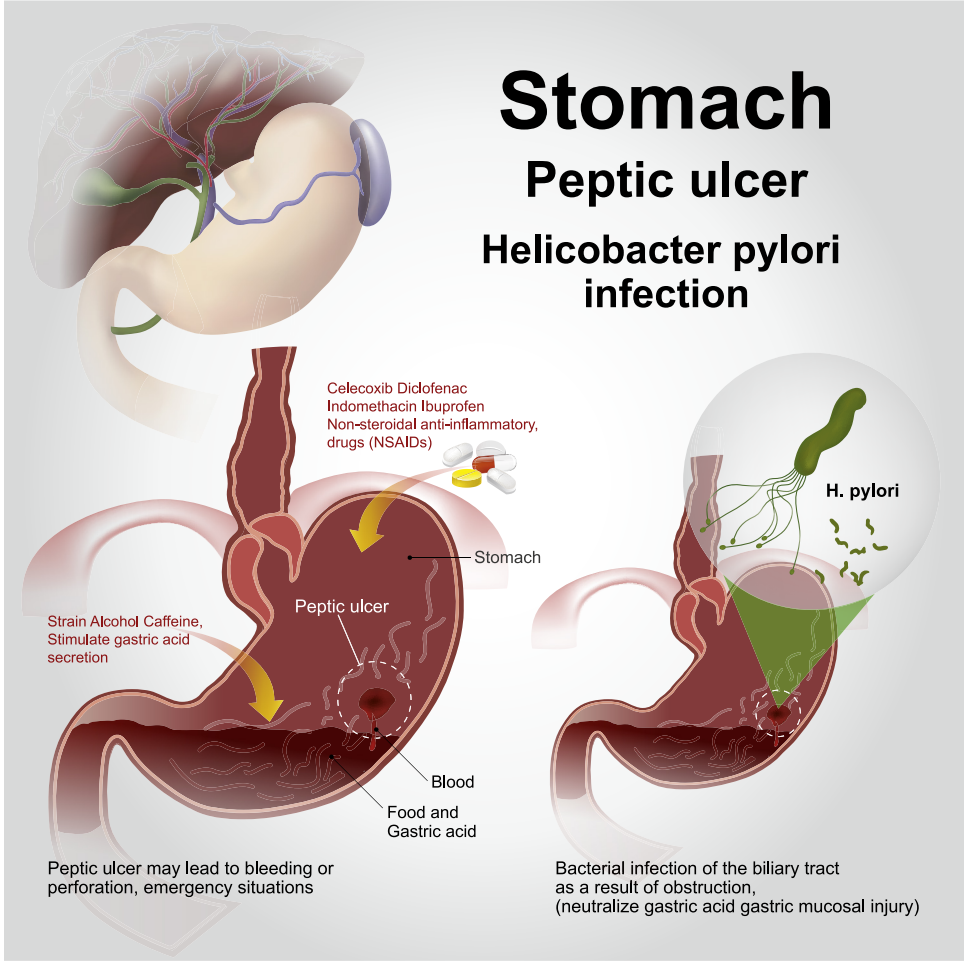


Fig. 207.2 Factors involved in peptic ulcer formation. (From https://www.istockphoto.com/vector/stomach-peptic-ulcer-gm671426088-122966903.)

50 years of age test positive for *H. pylori*.[2] However, more than 80% of *H. pylori*–infected people never actually develop an ulcer.[3] Although it is has been discovered that certain genotypic strains of *H. pylori* may confer additional cytotoxic risk for gastric pathologies,[4] conflicting information warrants further research.[5,6] The presence of *H. pylori* is determined by testing the level of antibodies to *H. pylori* in the blood or saliva, by culturing material collected during an endoscopy, and/or measuring the breath for urea.

Low gastric output and low antioxidant content in the gastrointestinal mucosa are thought to predispose individuals to *H. pylori* colonization. This increases gastric pH, thereby setting up a positive-feedback scenario and increasing the likelihood for the colonization of the stomach and duodenum with other organisms.[7]

Aspirin and Other Nonsteroidal Anti-Inflammatory Drugs

Aspirin and other NSAIDs are associated with a significant risk of peptic ulcer as well as increased gut permeability. Long-term low-dose (≤325 mg) aspirin therapy has been shown to increase the risk of major gastrointestinal (GI) bleeding, as well as a number of other adverse GI events, including excess stomach acid, gastritis, abdominal pain, epigastric burning, stomach ulcer, and duodenal ulcer.[8,9]

The combination of NSAID use and smoking is particularly harmful to the ulcer patient.[10] Most studies documenting the relative frequency of peptic ulcers as a consequence of NSAID use have focused on NSAIDs in the treatment of arthritis and headaches. Although there has been a reduction in ulcers induced by NSAIDs with the development of the selective cyclooxygenase-2 inhibitors (Rofecoxib, Celecoxib) for arthritic conditions, aspirin-induced ulcers remain an ongoing issue owing to the increased use of aspirin for protection against cardiovascular events.[10] The risk of GI bleeding due to peptic ulcers has been evaluated for aspirin at the daily doses of 300, 150, and 75 mg commonly recommended to prevent heart attacks and strokes.[11] A review of randomized controlled trials on the gastrointestinal toxicity of aspirin revealed that the pooled odds ratio for categories of GI bleeding are between 1.5 and 2.0.[12]

A study conducted at five test hospitals in England found an increased risk of GI bleeding due to peptic ulcer at all dosage levels. The dosage of 75 mg/day was associated with 40% less bleeding than 300 mg/day and 30% less bleeding than 150 mg/day. The researchers concluded: "No conventionally used prophylactic aspirin regimen seems free of the risk of peptic ulcer complications."

THERAPEUTIC CONSIDERATIONS

Individuals experiencing any symptoms of a peptic ulcer need competent medical care. Peptic ulcer complications such as hemorrhage, perforation, and obstruction represent medical emergencies that require immediate hospitalization.

The optimal treatment of a peptic ulcer involves the identification of the causative factor and its appropriate elimination.

Lifestyle Factors

Stress and Emotions

Stress is universally believed to be an important factor in the pathogenesis of peptic ulcers. However, this belief is based primarily on observation. The medical literature is controversial, and every substantial attempt to examine this assumption has been fraught with methodological errors.[13]

This is further complicated by the observation that men and women with peptic ulcers appear to have distinctly different psychological profiles. In addition, several studies have shown that the number of stressful life events is not significantly different in peptic ulcer patients compared with carefully selected, ulcer-free controls. These data suggest that it is not simply stress but, rather, the patient's response to stress that is the significant factor. A large prospective study of 4000 persons who had no history of peptic ulcer disease revealed that those who perceived more significant stress in their lives were at increased risk for developing peptic ulcers.[14] A register-based cohort study found the highest level of perceived everyday life stress had a 2.2-fold increase in the risk of either receiving triple treatment or being diagnosed with peptic ulcer during the following 33 months compared with that of the lowest-stress group (see Chapter 140 for a more complete discussion).[15]

Psychological factors are likely contributory in some patients with peptic ulcer disease. As a group, ulcer patients have been characterized as tending to repress emotions. Patients should be encouraged to discover enjoyable outlets of self-expression and emotion.

Smoking

Smoking is related to an increased frequency of peptic ulcers, a decreased response to peptic ulcer therapy, and increased mortality due to peptic ulcers. Three postulated mechanisms for this association are as follows[14]:

- Decreased pancreatic bicarbonate secretion (an important neutralizer of gastric acid)
- Increased reflux of bile salts into the stomach
- Acceleration of gastric emptying into the duodenum

Bile salts are extremely irritating to the stomach and initial portions of the duodenum. Bile salt reflux induced by smoking appears to be the most likely factor responsible for the greater incidence of peptic ulcer among smokers. In addition, cigarette smoke and its active ingredients cause mucosal cell death, inhibit cell renewal, interfere with the mucosal immune system, and decrease blood flow in the GI mucosa.[16] The psychological aspects of smoking are also important because the chronic anxiety and psychological stress associated with smoking appear to worsen ulcer activity.

Nutritional Factors

Food Allergy

Clinical and experimental evidence points to food allergy as a prime etiological factor.[17–20] The lesions of peptic ulcers and the Arthus reaction (a local inflammatory response due to deposition of immune complexes in tissues) show the same microanatomic changes.[2] In one study, 98% of patients with radiographic evidence of a peptic ulcer had coexisting lower and upper respiratory tract allergic disease.[19] In another study, 25 of 43 allergic children had x-ray–diagnosed peptic ulcers.[20] Clinically, an elimination diet has been used with great success in treating and preventing recurrent ulcers.[18,19] Food allergy is also consistent with the high recurrence rate of peptic ulcers.

Ironically, many people with peptic ulcers soothe themselves by consuming milk, a highly allergic food. Milk should be avoided on this basis alone. However, there is additional evidence suggesting that milk should be avoided by patients with peptic ulcers. For example, population studies show that the higher the milk consumption, the greater the likelihood of ulcer, and milk significantly increases the production of stomach acid.[21]

Fiber

A diet rich in fiber and low in refined sugar is associated with a reduced rate of duodenal ulcers compared with a low-fiber diet.[3] The therapeutic use of a high-fiber diet in patients with recently healed duodenal ulcers reduces the recurrence rate by one half.[22] This is probably a result of fiber's ability to delay gastric emptying of the liquid phase, counteracting the rapid movement of this phase into the duodenum, which is normally seen in ulcer patients. Although several fibers often

used to supplement the diet (e.g., pectin, guar gum, psyllium) have been shown to produce beneficial effects, a diet rich in plant foods is best.[23,24]

Cabbage

Raw cabbage juice has been well documented as having remarkable success in treating peptic ulcers.[25–27] One liter per day of the fresh juice, taken in divided doses, resulted in total ulcer healing in an average of only 10 days. Further research has shown that the high glutamine content of the juice is likely responsible for the efficacy of cabbage in treating ulcers. In a double-blind clinical study of 57 patients, 24 using 1.6 g/day of glutamine and 33 using conventional therapy (antacids, antispasmodics, diet, milk, and a bland diet), glutamine proved to be the more effective treatment.[28] One half of the glutamine patients showed complete healing (according to radiographic analysis) within 2 weeks, and 22 of the 24 showed complete relief and healing within 4 weeks. The authors postulated these results may arise from the role of glutamine in the biosynthesis of the hexosamine moiety in certain mucoproteins. This could stimulate mucin synthesis, which would benefit peptic ulcer patients.

In addition, cabbage-family isothiocyanates like sulforaphane (SF) have shown considerable activity against *H. pylori.* In one double-blind study, 48 *H. pylori*–infected patients were randomly assigned to feedings of broccoli sprouts (70 g/day; containing 420 µmol of SF precursor) for 8 weeks or to consumption of an equal weight of alfalfa sprouts (not containing SF) as placebo.[29] Intervention with broccoli sprouts but not with placebo decreased the levels of urease measured by the urea breath test and *H. pylori* stool antigen (both biomarkers of *H. pylori* colonization) and serum pepsinogens I and II (biomarkers of gastric inflammation). Values recovered to their original levels 2 months after treatment was discontinued.

Bismuth Subcitrate

Bismuth is a naturally occurring mineral that can act as an antacid; it also exerts activity against *H. pylori.* The best-known and most widely used bismuth preparation is bismuth subsalicylate (Pepto-Bismol). However, bismuth subcitrate has produced the best results against *H. pylori* and in the treatment of peptic ulcers.[28,30] In the United States, bismuth subcitrate preparations are available through compounding pharmacies (to find a compounding pharmacist in your area, call the International Academy of Compounding Pharmacists at 1-800-927-4227).

A key advantage of bismuth preparations over standard antibiotic approaches to eradicating *H. pylori* is that the bacteria are unlikely to develop resistance to bismuth. As concerns in the medical community grow regarding the failures of current regimens due to drug resistance, nonpharmacological management options are becoming required.[31]

In India for example, 80% of isolates from 259 peptic ulcer patients tested showed resistance to metronidazole.[32] Although resistance to ciprofloxacin and tetracycline was minimal (1%–4%), it is evident from recent medical history that greater resistance to these antibiotics will continue to develop. This makes bismuth a rational treatment choice to prevent the further development of recalcitrant strains. The usual dosage for bismuth subcitrate is 240 mg twice daily before meals. For bismuth subsalicylate, the dosage is 500 mg four times daily. Bismuth preparations are extremely safe when taken at prescribed dosages. Bismuth subcitrate may cause a temporary and harmless darkening of the tongue, stool, or both. Bismuth subsalicylate should not be taken by children recovering from the flu, chickenpox, or other viral infections because it may mask the nausea and vomiting associated with Reye syndrome, a rare but serious illness.

Polyphenols

Dietary polyphenols with multiple biological mechanisms of action play a pivotal part in the management of gastric and duodenal ulcers. A review study evaluated the potential effects of commonly used dietary polyphenols in the prevention and/or treatment of peptic ulcer based on cellular, preclinical, and clinical studies, along with the possible molecular and intracellular mechanisms.[33] The study confirmed that dietary polyphenols possess protective and therapeutic potential in peptic ulcer mediated by improving cytoprotection, reepithelialization, neovascularization, and angiogenesis; upregulating tissue growth factors and prostaglandins; downregulating antiangiogenic factors; enhancing endothelial nitric oxide synthase-derived NO; suppressing oxidative mucosal damage; amplifying antioxidant performance and antacid and antisecretory activity; increasing endogenous mucosal defensive agents; and blocking *H. pylori* colonization–associated gastric morphological changes and gastroduodenal inflammation and ulceration. In addition, anti-inflammatory activity due to down regulation of proinflammatory cytokines and cellular and intercellular adhesion agents, suppressing leukocyte-endothelium interaction, inhibiting nuclear signaling pathways of inflammatory process, and modulating intracellular transduction and transcription pathways have key roles in the antiulcer action of dietary polyphenols.

Flavonoids

Flavonoids are known to counteract both the production and secretion of histamine, an important factor in ulcer formation. They are generally regarded as antiallergy compounds. The use of these compounds seems particularly indicated owing to the probable allergic etiology of peptic ulcers.

Catechin, via its ability to inhibit histidine decarboxylase, offers antiulcer activity. Animal studies have demonstrated that catechin has significant antiulcer activity in various models.[34,35] In a human clinical study, oral administration (1000 mg five times daily) resulted in reduced histamine levels in the gastric tissue (determined by biopsy) of normal patients and in those with gastric and duodenal ulcers and acute gastritis.[35] It was also demonstrated that histamine levels, which significantly increase in patients with urticaria and food allergy after the local application of the antigen to the gastric mucosa, could be decreased by the prior administration of catechin.

In one study, several flavonoids were shown to inhibit *H. pylori* in a clear-cut concentration-dependent manner.[36] In addition, unlike antibiotics, the flavonoids were also shown to augment natural defense factors that prevent ulcer formation. The activity of flavone, the most potent flavonoid in the study, was shown to be similar to that of bismuth subcitrate.

Sofalcone—a synthetic derivative of sophoradin, a flavonoid found in Sophora tonkinensis, an herb used in traditional Chinese medicine—has shown good results in healing ulcers after the eradication of *H. pylori.*[37,38]

Vitamins A and E have been shown to inhibit the development of stress ulcers in rats and are important in maintaining the integrity of the mucosal barrier.[39,40] High-dose vitamin A therapy was shown to be useful in the treatment of chronic gastric ulcers in one clinical trial.[41]

Zinc increases mucin production in vitro and has been shown to have a protective effect on peptic ulcers in animals and a curative effect in humans.[42,43]

Melatonin has been shown in experimental studies to be successful in mitigating the breakdown of the gastric lining and resultant ulcer formation.[44] Because this research has focused more on hypersecretion situations, melatonin may be more applicable for duodenal ulcer conditions, where the acid secretion is generally increased.

Botanical Medicines

Glycyrrhiza glabra. Licorice has historically been regarded as an excellent medicine for peptic ulcer. However, owing to the known aldosterone-like side effects of glycyrrhizinic acid (GA), a procedure was developed to remove GA from licorice and thus to form deglycyrrhizinated licorice (DGL). The result is a very successful antiulcer agent without any known side effects (see Chapter 85).[7,45–49]

The proposed mechanism of DGL is the stimulation or acceleration of the differentiation of glandular cells as well as the formation and secretion mucus.[45] Clinical studies have demonstrated no significant differences in recurrence rates between cimetidine and DGL drug regimens, and rat and human studies have shown the efficacy of DGL in preventing aspirin-induced ulceration and gastric bleeding.[47-49]

DGL may affect *H. pylori* because DGL is composed of several flavonoids that have been shown to inhibit *H. pylori.*[36,50] In a double-blind clinical trial study, 60 patients with peptic ulcer disease and positive rapid urease test were enrolled to evaluate licorice compared with bismuth in quadruple regimen on the eradication of *H. pylori.*[51] The patients were randomly allocated into two equal groups. In the first group, licorice, amoxicillin, metronidazole, and omeprazole were prescribed, and in the second (control) group, bismuth subsalicylate, amoxicillin, metronidazole, and omeprazole were prescribed. After 4 weeks of treatment, a urea breath test was done in all patients to evaluate *H. pylori* eradication. Response to treatment was observed in 20 (67%) and 17 (57%) patients of the case and control groups, respectively ($P > 0.05$). Therefore licorice may be as effective as bismuth in *H. pylori* eradication, and in patients for whom bismuth is contraindicated, licorice may be a safe alternative.

It appears that to be effective in healing peptic ulcers, DGL must mix with saliva. DGL may promote the release of salivary compounds that stimulate the growth and regeneration of stomach and intestinal cells. DGL in capsule form has not been shown to be effective.

The standard dosage for DGL is two to four 380-mg chewable tablets between meals or 20 minutes before meals. Taking DGL after meals is associated with poor results. DGL therapy should be continued for at least 8 to 16 weeks after a full therapeutic response.

Mastic (Pistacia lentiscus). Mastic is a resin obtained from the mastic tree (*Pistacia lentiscus*). In Greece it is known as the "tears of Chios," being traditionally produced on the Greek island of Chios. Like other natural resins, it is produced in "tears" or droplets. Originally liquid, it is sun-dried into drops of hard, brittle, translucent resin. When chewed, the resin softens and becomes a bright white, opaque gum. The flavor is bitter at first, but after chewing, the gum releases a refreshing, slightly piney or cedar flavor.

People in the Mediterranean region have used mastic gum as a medicine for GI ailments for several thousand years. Studies indicate that it may have benefit in healing peptic ulcers.

In a double-blind clinical trial carried out on 38 patients with symptomatic and endoscopically proved duodenal ulcer, patients were given either mastic gum (1 g daily) or a placebo for 2 weeks.[52] Symptomatic relief was obtained in 16 (80%) patients on mastic gum and 9 (50%) patients on placebo, whereas endoscopically proved healing occurred in 14 (70%) patients on mastic gum and 4 (22%) patients on placebo.

In another study, 52 patients with *H. pylori* infection were randomized to receive either 350 mg three times daily of pure mastic gum for 14 days (group A), 1.05 g three times daily of pure mastic gum for 14 days (group B), pantoprazole 20 mg twice daily plus pure mastic gum 350 mg three times daily for 14 days (group C), or pantoprazole 20 mg twice daily plus amoxicillin 1g twice daily plus clarithromycin 500 mg twice daily for 10 days (group D).[53] Eradication of *H. pylori* was confirmed in 4 of 13 patients in group A and 5 of 13 in group B. No patient in group C achieved eradication, whereas 10 of 13 patients in group D did. These results confirm that mastic gum has some bactericidal activity on *H. pylori* in vivo but not enough to produce consistent clinical eradication.

Rheum species. In cases of active intestinal bleeding, rhubarb (*Rheum* species) preparations can be extremely effective. In one double-blind study, three kinds of alcohol-extracted rhubarb tablets were studied (*Rheum officinale Baill; Rheum palmatum* L.; *Rheum tanguticum Maxim ex Balf*).[54] Their efficacies in a group of 312 cases of bleeding gastric and duodenal ulcers were 90.7%, 93.7%, and 92.8%, respectively. The time taken for the stool occult blood to change from positive to negative was 57.1, 53.4, and 56 hours, respectively. The beneficial actions are due to the presence of astringent anthraquinones and flavonoids.

Plantain banana. In rats, the dried extract of the unripe plantain banana (*Musa sapientum* var. *paradisiaca*) has been found to have antiulcerogenic activity against various experimentally induced ulcers.[55–57] This effect appears to be similar to that of DGL (i.e., stimulation of mucosal cell growth rather than inhibition of gastric acid secretion).

Zingiber officinale. Ginger root *(Zingiber officinale)* has traditionally been used for the treatment of GI ailments such as indigestion, motion sickness, and nausea associated with pregnancy. One study used in vitro experimentation to test a methanol extract of ginger root as an agent against 19 strains of *H. pylori.* Results showed that growth was inhibited against all 19 strains.[58] Although more research is required to characterize the best form of this botanical medicine and to learn of its efficacy in actual patients, given its safety profile and cost-effectiveness, it is certainly worth a try.

Artemisia douglasiana. Growing on the western slopes of the Rockies, *Artemisia douglasiana* has been used as a folk remedy in Argentina to treat gastric ulcers and skin lesions since the late 1960s. *A. douglasiana* and its active constituent dehydroleucodine are known to act as potent antioxidants, and like DGL and plantain, they confer protection to the gastric lining via significantly enhanced secretion of mucus.[59,60]

Allium sativum. People whose diets are high in garlic and onions are well known to have a lower incidence of stomach cancer. *H. pylori* is a risk factor for stomach cancer as well as peptic ulcers, and in vitro studies have demonstrated that garlic does indeed inhibit its growth. Also noteworthy is the report that garlic can be useful for antibiotic-resistant strains.[61] The author of this paper points out that human clinical trials are necessary to verify the efficacy of this low-cost treatment option.

Probiotics

A novel approach to the reduction of *H. pylori*–associated gastric pathology is found in the administration of the probiotic bacterium *Lactobacillus rhamnosus* yoba 2012 (LRY), the generic variant of *Lactobacillus rhamnosus* GG (LGG). This gastrointestinal isolate inhibits *H. pylori* by competition for substrate and binding sites as well as the production of antimicrobial compounds such as lactic acid. In addition, it attenuates the host's *H. pylori*–induced apoptosis and inflammation responses and stimulates angiogenesis in the gastric and duodenal epithelium. Although LRY is not able to eradicate *H. pylori* completely, its cosupplementation in antibiotic eradication therapy has been shown to relieve the side effects of this therapy.[62] A systematic review of randomized controlled trials in colonized adults and children confirmed that probiotics do not eradicate *H. pylori* but maintain lower levels of this pathogen in the stomach and, in combination with

antibiotics, may increase eradication rate, decrease therapy-related adverse effects, and alleviate most disease-related clinical symptoms.[63]

THERAPEUTIC APPROACH

Peptic ulcer disease should be recognized as a heterogeneous group of disorders with a common final pathway leading to an ulcerative lesion in either the gastric or duodenal mucosa. Patients must be carefully evaluated to determine which of the previously mentioned factors is most relevant to their health problem. This is difficult, and a more general approach may be necessary.

The first step is to identify and eliminate or reduce all factors implicated in the etiology of peptic ulcers: food allergy, cigarette smoking, stress, and drugs—especially aspirin and other NSAIDs. Although short-term antibiotics may be necessary, a nonmicrobial approach for *H. pylori*–associated gastritis with long-term phytochemicals or other natural agents has been shown to be efficacious.[64] Once the causative factors have been controlled, attention should be directed at healing the ulcers, inhibiting exacerbating factors (e.g., reducing excess acid secretion if present), and promoting tissue resistance. Finally, the proper diet and lifestyle should be developed to prevent further recurrence.

Peptic ulcer complications—hemorrhage, perforation, and obstruction—represent medical emergencies that require immediate hospitalization.

Psychological Considerations

The physician should assist the patient in developing an effective stress reduction program, eliminating or controlling stressors, and designing a regular relaxation plan.

Diet

The patient should eliminate allergic food, eat foods high in dietary fiber and polyphenols and low in refined sugars, and eat various members of the cabbage family and garlic.

Supplements

- Vitamin A: 20,000 IU three times a day—short term
- Vitamin C: 500 mg twice a day
- Vitamin E: 100 IU twice a day (mixed tocopherols)
- Flavonoids: 500 mg twice a day
- Zinc: 20 mg/day
- Glutamine: 500 mg three times a day
- Bismuth subcitrate: 240 mg twice a day before meals
- Probiotics: the dosage of viable bacteria given in supplemental forms should generally be 10^9 to 10^{11} bacteria per dose.

Botanical Medicines

- Deglycyrrhizinated licorice: 380 to 760 mg three times a day 20 minutes before meals
- Mastic gum: 350 to 1000 mg three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Smoot DT, Go MF, Cryer B. Peptic ulcer disease. *Prim Care*. 2001;28:487–503.
2. Berstad K, Berstad A. *Helicobacter pylori* infection in peptic ulcer disease. *Scand J Gastroenterol*. 1993;28:561–567.
3. Misciagna G, Cisternino AM, Freudenheim J. Diet and duodenal ulcer. *Dig Liver Dis*. 2000;32:468–472.
4. Bulent K, Murat A, Esin A, et al. Association of CagA and VacA presence with ulcer and non-ulcer dyspepsia in a Turkish population. *World J Gastroenterol*. 2003;9:1580–1583.
5. Zhang Y, Liu H, Zhou K. Lack of correlation of vacA genotype, cagA gene of *Helicobacter pylori* and their expression products with various gastroduodenal diseases. *Chin Med J (Engl)*. 2001;114:703–706.
6. Faundez G, Troncoso M, Figueroa G. cagA and vacA in strains of *Helicobacter pylori* from ulcer and non-ulcerative dyspepsia patients. *BMC Gastroenterol*. 2002;2:20.
7. Sarker SA, Gyr K. Non-immunological defense mechanisms of the gut. *Gut*. 1992;33:987–993.
8. De Berardis G, Lucisano G, et al. Association of aspirin use with major bleeding in patients with and without diabetes. *JAMA*. 2012;307(21):2286–2294. PubMed PMID: 22706834.
9. Tournoij E, Peters RJ, et al. The prevalence of intolerance for low-dose acetylsalicylacid in the secondary prevention of atherothrombosis. *Eur J Vasc Endovasc Surg*. 2009;37(5):597–603. PubMed PMID: 19297216.
10. Gray GM. Peptic ulcer diseases. In: Dale DC, Federman DD, eds. *Scientific American medicine.* New York: Scientific American.
11. Weil J, Colin-Jones D, Langman M. Prophylactic aspirin and risk of peptic ulcer bleeding. *BMJ*. 1995;310:827–830.
12. Roderick PJ, Wilkes HC, Meade TW. The gastrointestinal toxicity of aspirin: an overview of randomized controlled trials. *Br J Clin Pharmacol*. 1993;35(3):219–226. PubMed PMID: 8471398.
13. Feldman EJ, Sabovich KA. Stress and peptic ulcer disease. *Gastroenterology*. 1980;78:1087–1089.
14. Anda RF, Williamson DF, Escobedo LG, et al. Self-perceived stress and the risk of peptic ulcer disease: a longitudinal study of US adults. *Arch Intern Med*. 1992;152:829–833.
15. Deding U, Ejlskov L, Grabas MP, Nielsen BJ, Torp-Pedersen C, Boggild H. Perceived stress as a risk factor for peptic ulcers: a register-based cohort study. *BMC Gastroenterol*. 2016;16(1):140. PubMed PMID: 27894275.
16. Li LF, Chan RL, Lu L, Shen J, et al. Cigarette smoking and gastrointestinal diseases: the causal relationship and underlying molecular mechanisms (review). *Int J Molecul Med*. 2014;34(2):372–380. PubMed PMID: 24859303.
17. Siegel J. Gastrointestinal ulcer: Arthus reaction! *Ann Allergy*. 1974;32:127–130.
18. Andre C, Moulinier B, Andre F, et al. Evidence for anaphylactic reactions in peptic ulcer and varioliform gastritis. *Ann Allergy*. 1983;51:325–328.
19. Siegel J. Immunologic approach to the treatment and prevention of gastrointestinal ulcers. *Ann Allergy*. 1977;38:27–41.
20. Rebhun J. Duodenal ulceration in allergic children. *Ann Allergy*. 1975;34:145–149.
21. Kumar N, Kumar A, Broor SL, et al. Effect of milk on patients with duodenal ulcers. *Br Med J*. 1986;293:666.
22. Rydning A, Berstad A, Aadland E, et al. Prophylactic effect of dietary fiber in duodenal ulcer disease. *Lancet*. 1982;2:736–739.
23. Kang JY, Tay HH, Guan R, et al. Dietary supplementation with pectin in the maintenance treatment of duodenal ulcer: a controlled study. *Scand J Gastroenterol*. 1988;23:95–99.
24. Harju E, Larmi TK. Effect of guar gum added to the diet of patients with duodenal ulcer. *JPEN J Parenter Enteral Nutr*. 1985;9:496–500.
25. Cheney G. Rapid healing of peptic ulcers in patients receiving fresh cabbage juice. *Calif Med*. 1949;70:10–14.
26. Cheney G. Anti-peptic ulcer dietary factor. *J Am Diet Assoc*. 1950;26:668–672.
27. Shive W, Snider RN, DuBilier B, et al. Glutamine in treatment of peptic ulcer: preliminary report. *Tex State J Med*. 1957;53:840–842.
28. Marshall BJ, Valenzuela JE, McCallum RW, et al. Bismuth subsalicylate suppression of *Helicobacter pylori* in nonulcer dyspepsia: a double-blind placebo-controlled trial. *Dig Dis Sci*. 1993;38:1674–1680.
29. Yanaka A, Fahey JW, Fukumoto A, et al. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in Helicobacter pylori-infected mice and humans. *Cancer Prev Res (Phila)*. 2009;2(4):353–360.
30. Kang JY, Tay HH, Wee A, et al. Effect of colloidal bismuth subcitrate on symptoms and gastric histology in non-ulcer dyspepsia: a double blind placebo controlled study. *Gut*. 1990;31:476–480.
31. Loughlin MF. Novel therapeutic targets in *Helicobacter pylori*. *Expert Opin Ther Targets*. 2003;7:725–735.
32. Thyagarajan SP, Ray P, Das BK, et al. Geographical difference in antimicrobial resistance pattern of *Helicobacter pylori* clinical isolates from Indian patients: multicentric study. *J Gastroenterol Hepatol*. 2003;18:1373–1378.
33. Farzaei MH, Abdollahi M, Rahimi R. Role of dietary polyphenols in the management of peptic ulcer. *World J Gastroenterol WJG*. 2015;21(21):6499–6517. PubMed PMID: 26074689.
34. Parmar NS, Ghosh MN. Gastric anti-ulcer activity of (+)-cyanidanol-3, a histidine decarboxylase inhibitor. *Eur J Pharmacol*. 1981;69:25–32.
35. Wendt P, Reiman H, Swoboda K, et al. The use of flavonoids as inhibitors of histidine decarboxylase in gastric disease: experimental and clinical studies. *Naunyn Schmiedebergs Arch Pharmacol*. 1980;313(suppl):238.
36. Beil W, Birkholz C, Sewing KF. Effects of flavonoids on parietal cell acid secretion, gastric mucosal prostaglandin production and Helicobacter pylori growth. *Arzneimittelforschung*. 1995;45:697–700.
37. Higuchi K, Watanabe T, Tanigawa T, et al. Sofalcone, a gastroprotective drug, promotes gastric ulcer healing following eradication therapy for Helicobacter pylori: a randomized controlled comparative trial with cimetidine, an H2-receptor antagonist. *J Gastroenterol Hepatol*. 2010 May;25(suppl 1):S155–S160.
38. Isomoto H, Furusu H, Ohnita K, et al. Sofalcone, a mucoprotective agent, increases the cure rate of Helicobacter pylori infection when combined with rabeprazole, amoxicillin and clarithromycin. *World J Gastroenterol*. 2005;11(11):1629–1633.
39. Schumpelick V, Farthmann E. Study on the protective effect of vitamin A on stress ulcer of the rat (author's transl). *Arzneimittelforschung*. 1976;26:386–388.
40. al-Moutairy AR, Tariq M. Effect of vitamin E and selenium on hypothermic restraint stress and chemically-induced ulcers. *Dig Dis Sci*. 1996;41:1165–1171.
41. Patty I, Benedek S, Deák G, et al. Cytoprotective effect of vitamin A and its clinical importance in the treatment of patients with chronic gastric ulcer. *Int J Tissue React*. 1983;5(3):301–307.
42. Oner G, Bor NM, Onuk E, et al. The role of zinc ion in the development of gastric ulcers in rats. *Eur J Pharmacol*. 1981;70:241–243.
43. Frommer DJ. The healing of gastric ulcers by zinc sulphate. *Med J Aust*. 1975;2:793–796.
44. Bandyopadhyay D, Biswas K, Bhattacharyya M, et al. Gastric toxicity and mucosal ulceration induced by oxygen-derived reactive species: protection by melatonin. *Curr Mol Med*. 2001;1:501–513.
45. van Marle J, Aarsen PN, Lind A, et al. Deglycyrrhizinised liquorice (DGL) and the renewal of rat stomach epithelium. *Eur J Pharmacol*. 1981;72:219–225.
46. Morgan AG, McAdam WA, Pacsoo C, et al. Comparison between cimitidine and Caved-S in the treatment of gastric ulceration, and subsequent maintenance therapy. *Gut*. 1982;23:545–551.
47. Tewari SN, Trembalowicz FC. Some experience with deglycyrrhizinated liquorice in the treatment of gastric and duodenal ulcers with special reference to its spasmolytic effect. *Gut*. 1968;9:48–51.
48. Balakrishnan V, Pillai MV, Raveendran PM, et al. Deglycyrrhizinated liquorice in the treatment of chronic duodenal ulcer. *J Assoc Physicians India*. 1978;26:811–814.
49. Rees WD, Rhodes J, Wright JE, et al. Effect of deglycyrrhizinated liquorice on gastric mucosal damage by aspirin. *Scand J Gastroenterol*. 1979;14:605–607.
50. Fukai T, Marumo A, Kaitou K, et al. Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sci*. 2002;71:1449–1463.
51. Momeni A, Rahimian G, Kiasi A, Amiri M, Kheiri S. Effect of licorice versus bismuth on eradication of Helicobacter pylori in patients with peptic ulcer disease. *Pharma Res*. 2014;6(4):341–344. PubMed PMID: 25276073.

52. Al-Habbal MJ, Al-Habbal Z, Huwez FU. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. *Clin Exp Pharmacol Physiol*. 1984;11(5):541–544.
53. Dabos KJ, Sfika E, Vlatta LJ, et al. The effect of mastic gum on Helicobacter pylori: a randomized pilot study. *Phytomedicine*. 2010;17(3-4):296–299.
54. Zhou H, Jiao D. 312 cases of gastric and duodenal ulcer bleeding treated with 3 kinds of alcoholic extract rhubarb tablets. *Zhong Xi Yi Jie He Za Zhi*. 1990;10:150–151. 131-132.
55. Best R, Lewis DA, Nasser N. The anti-ulcerogenic activity of the unripe plantain banana (Musa species). *Br J Pharmacol*. 1984;82:107–116.
56. Sanyal AK, Gupta KK, Chowdhury NK. Banana and experimental peptic ulcer. *J Pharm Pharmacol*. 1963;15:283–284.
57. Goel RK, Sairam K, Rao CV. Role of gastric antioxidant and anti-*Helicobacter pylori* activities in antiulcerogenic activity of plantain banana (*Musa sapientum* var. *paradisiaca*). *Indian J Exp Biol*. 2001;39:719–722.
58. Mahady GB, Pendland SL, Yun GS, et al. Ginger (*Zingiber officinale Roscoe*) and the gingerols inhibit the growth of Cag A+ strains of *Helicobacter pylori*. *Anticancer Res*. 2003;23:3699–3702.
59. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz J Med Biol Res*. 2002;35:523–534.
60. Repetto M, Maria A, Guzman J, et al. Protective effect of *Artemisia douglasiana* Besser extracts in gastric mucosal injury. *J Pharm Pharmacol*. 2003;55:551–557.
61. Sivam GP. Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr*. 2001;131:1106S–1108S.
62. Westerik N, Reid G, Sybesma W, Kort R. The probiotic Lactobacillus rhamnosus for alleviation of Helicobacter pylori-associated gastric pathology in East Africa. *Front Microbiol*. 2018;9:1873. PubMed PMID: 30154777.
63. Lu M, Yu S, Deng J, et al. Efficacy of probiotic supplementation therapy for Helicobacter pylori eradication: a meta-analysis of randomized controlled trials. *PLoS One*. 2016;11(10):e0163743. PubMed PMID: 27723762.
64. Han YM, Park JM, Jeong M, et al. Dietary, non-microbial intervention to prevent Helicobacter pylori-associated gastric diseases. *Annal Translat Med*. 2015;3(9):122. PubMed PMID: 26207250.

Periodontal Disease

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Gingivitis—inflammation of the gingiva characterized by erythema, contour changes, and bleeding
- Periodontitis—localized pain, loose teeth, demonstration of dental pockets, erythema, swelling, or suppuration; possibly also alveolar bone destruction seen on radiograph

GENERAL CONSIDERATIONS

Periodontal disease is an inclusive term used to describe an inflammatory condition of the gingiva (gingivitis) or periodontium (periodontitis) or both. Periodontal disease usually progresses from gingivitis to periodontitis. It may be a manifestation of a more systemic condition such as diabetes mellitus, collagen diseases, leukemia or other disorders of leukocyte function, anemia, or vitamin-deficiency states.[1] Periodontal disease may also contribute to systemic disease (Fig. 208.1). For example, periodontal disease has been linked to atherosclerosis via an increased level of serum C-reactive protein, a marker for inflammation and a strong risk factor for coronary artery disease.[1]

Because alveolar bone loss may be noninflammatory, the definition of periodontal disease used here excludes the processes causing only the loss of teeth (the majority of which are due to osteoporosis or endocrine imbalances). These conditions reflect systemic disease, with local factors playing only a minor role. Therefore the focus should be on treating the underlying condition rather than the "periodontal disease." In this context, noninflammatory alveolar bone loss should be viewed as a separate entity because it involves a different etiology (see Chapter 203).

The focus of this chapter is the use of nutrition and lifestyle improvement as an adjunctive therapy to aid in the control and prevention of the causes of inflammatory periodontal disease. This is a good example of a condition that is best treated with combined expertise (i.e., a dentist or periodontist and a nutritionally minded physician). Although oral hygiene is of great importance in treating and preventing periodontal disease, it is insufficient in many cases. The host defense must be normalized if the development and progression of the disease are to be controlled. To a large extent, the nutritional status of the individual determines the status of host defense factors.

Prevalence and Epidemiology

The prevalence of periodontal disease increases directly with age. The rate of periodontal disease is approximately 15% at age 10, 38% at age 20, 46% at age 35, and 54% at age 50. As a group, men have a higher prevalence and severity of periodontal disease than do women. Periodontal disease is inversely related to increasing levels of education and income. Rural inhabitants have a higher level of severity and prevalence than do their urban counterparts.[1]

Pathophysiology

Understanding the underlying pathophysiology of any disease process leads to a more effective treatment plan. In periodontal disease this involves understanding the normal host protective factors in the periodontium. Page and Schroeder[2] concluded: "Clearly bacteria are essential agents, but their presence is in itself insufficient; host factors must be involved if the disease is to develop and progress."

Factors involved in host resistance include the following:

- The environment of the gingival sulcus
- Bacterial factors
- Leukocyte function
- Complement activation
- Immunoglobulin (Ig) E and mast cell function
- Amalgam restoration
- Miscellaneous local factors
- The structure and integrity of the collagen matrix of the periodontium and gingiva

Gingival Sulcus

The gingival sulcus, a V-shaped crevice that surrounds each tooth, is bounded by the surface of the tooth on one side and the epithelium lining the free margin of the gingiva on the other. The anatomy of the

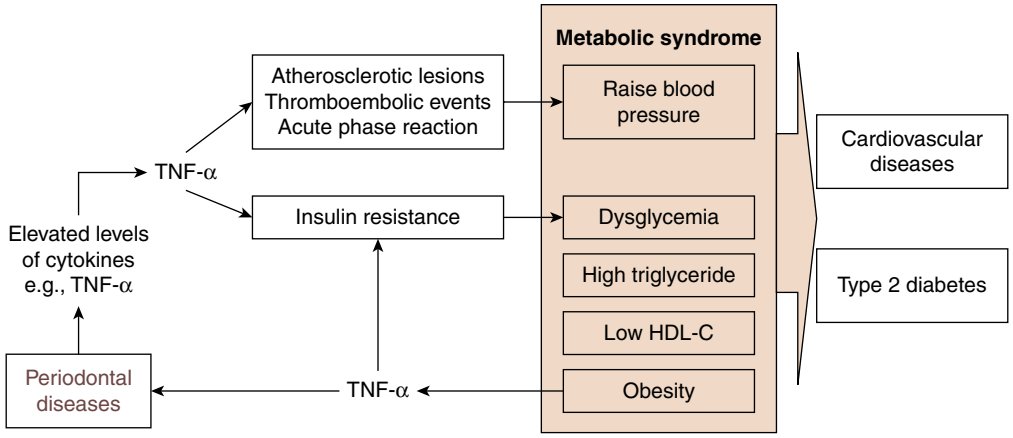


Fig. 208.1 A biological working model on the onset of destructive periodontal disease in systemic conditions. (From Khosravi R, Ka K, Huang T, Khalili S, Nguyen BH, Nicolau B, Tran SD. Tumor necrosis factor-α and interleukin-6: potential interorgan inflammatory mediators contributing to destructive periodontal disease in obesity or metabolic syndrome. *Mediat Inflamm.* 2013:728987. PubMed PMID: 24068858.)

gingival sulcus is ideal for bacterial growth because it is resistant to the washing and cleansing action of saliva. Furthermore, the gingival fluid (sulcular fluid) provides a rich nutrient source for microorganisms. The clinical determination of the depth of the gingival sulcus is an important diagnostic parameter. Patients with periodontal disease should be monitored; biannual visits to the dentist should be sufficient in most cases.

Bacterial Factors

Bacterial plaque has long been considered the etiological agent in most forms of periodontal disease. However, host defense factors are undeniably another key factor. Bacteria are known to produce and secrete numerous compounds that are quite detrimental to the status of the host's defense mechanisms. These compounds include:

- Endotoxins and exotoxins
- Free radicals and collagen-destroying enzymes
- Leukotoxins
- Bacterial antigens, waste products, and toxic compounds

Polymorphonuclear Leukocytes

Polymorphonuclear neutrophils (PMNs) constitute a first line of defense against microbial overgrowth. Defects in PMN functions are "catastrophic" to the periodontium. PMN functions are depressed in the geriatric population as well as in patients with diabetes, Crohn's disease, Chédiak–Higashi syndrome, Down syndrome, and juvenile periodontitis. These patients are at extremely high risk for developing rapidly progressing periodontal disease, as are people with transient neutropenia. Transient defects in PMN function may be responsible for the periods of quiescence and exacerbation noted in periodontal disease. In addition to serving a vital role in protecting against periodontal disease, PMNs also play a major role in tissue destruction. PMNs release numerous free radicals, collagenases, hyaluronidases, inflammatory mediators, and an osteoclast stimulator.

Macrophages and Monocytes

These leukocytes are found in increased numbers in periodontal disease. They serve to phagocytize bacteria and debris and are the primary source of prostaglandins in the diseased gingiva, releasing large quantities of enzymes believed to play a major role in collagen destruction.

Lymphocytes

The major role lymphocytes play in periodontal disease involves lymphokine production. Their role in periodontal disease is overshadowed by the roles of the other immune system components discussed, but lymphokines are involved in promoting PMN and monocyte chemotaxis, fibroblast destruction, and osteoclast activation.

Complement

The complement system is composed of at least 22 proteins and accounts for more than 10% of the total serum globulin. On activation, complement components act in a cascade fashion. Complement can be activated via the classic or alternative pathway. The complement system plays a critical role in immunological and nonspecific resistance to infection and in the pathogenesis of tissue injury. The products of complement activation regulate several events, including the release of mediators from mast cells; promotion of smooth muscle contraction; chemotaxis of PMNs, monocytes, and eosinophils; and phagocytosis by immune adherence. The net effect is an increase in gingival permeability, resulting in the increased penetration of bacteria and bacterial by-products and the initiation of a positive-feedback cycle.

Other effects of complement activation include solubilization of immune complexes, cell membrane lysis, neutralization of viruses, and the killing of bacteria. In periodontal disease, activation of complement via the alternative pathway within the periodontal pocket is possibly the major factor in tissue destruction.

Mast Cells and Immunoglobulin E

Mast cell degranulation is also a major factor in periodontal disease. Degranulation results in the release of inflammatory mediators (e.g., histamine, prostaglandins, leukotrienes, kinins, serotonin, heparin, and serine proteases). Mast cell degranulation can be initiated by IgE complexes, complement components, mechanical trauma, endotoxins, and free radicals. The finding of increased IgE concentrations in the gingivae of patients with periodontal disease suggests that allergic reactions may be a factor in the progression of the disease in some patients, especially those with atopic disease.[3]

Amalgam Restorations

Faulty dental restorations and prostheses are common causes of gingival inflammation and periodontal destruction. Overhanging margins provide an ideal location for the accumulation of plaque and the multiplication of bacteria. If the restoration is a silver amalgam filling, there may be even more involvement due to decreased activities of antioxidant enzymes. Mercury accumulation results in a depletion

of the free-radical–scavenging enzymes glutathione peroxidase, superoxide dismutase, and catalase.[4] The proteoglycans and glycosaminoglycans of the collagen matrix are particularly sensitive to free-radical damage.

Miscellaneous Local Factors

Numerous local factors favor the progression of periodontal disease. These include the following:

- Food impaction
- Unreplaced missing teeth
- Malocclusion
- Tongue thrusting
- Bruxism
- Toothbrush trauma
- Mouth breathing
- Tobacco (discussed later)

Tobacco and Alcohol

Tobacco smoking is associated with increased susceptibility to severe periodontal disease and tooth loss.[5,6] Many of the harmful effects of tobacco smoking result from free-radical damage, particularly to epithelial cells. Furthermore, smoking greatly reduces the levels of ascorbic acid, thereby potentiating its damaging effects.[7] Flavonoids have been shown to greatly reduce some of the toxic effects of smoking.[8,9]

In addition to cigarette smoking, there is a dose-dependent association between alcohol consumption and periodontal disease.[9] Presumably, the combination of smoking and drinking alcohol produces an even greater negative effect on periodontal health.

Structure and Integrity of the Collagen Matrix

The collagen matrix of the periodontal membrane serves as periosteum to the alveolar bone and enables the dissipation of the tremendous amount of pressure exerted during mastication. The status of the collagen matrix of the periodontium, specifically the extracellular proteoglycans of the gingival epithelium, determines the rate of diffusion and the permeability of inflammatory mediators, bacteria and their by-products, and destructive enzymes from the oral cavity.[10,11] Owing to the high rate of protein turnover in periodontal collagen, the integrity of the collagen matrix in this area is extremely vulnerable to atrophy when the necessary cofactors for collagen synthesis (e.g., protein; vitamins C, B_6, and A; zinc; copper) are absent or deficient. Poor wound healing is associated with increased risk for periodontal disease.[12]

The collagen of the periodontium is particularly rich in glycosaminoglycans. Heparin sulfate, dermatan sulfate, and chondroitin sulfate proteoglycan 4 are the major glycosaminoglycans present. Stabilization of collagen is the major treatment goal (see later).

Environmental Toxicants

Polymorphism of N-acetyltransferase 2, an enzyme involved in Phase II metabolism of xenobiotics, is associated with periodontitis; subjects with the slow-acetylating genotype had more severe periodontal disease.[13] Lead is a xenobiotic that has significant effects on bone metabolism and may therefore increase the risk of periodontal disease. Prevalence ratios for periodontitis when comparing individuals with blood lead levels (BLLs) >7 μg/dL with those with a BLL of <3 μg/dL were 1.70 (95% confidence interval [CI]; 1.02–2.85) for men and 3.80 (95% CI; 1.66–8.73) for women.[14] In addition, workers exposed to acid fumes or chemicals in an oil company reported a higher risk of periodontal disease.[15]

Persistent organic pollutants (POPs) can increase the risk of periodontal disease through the disturbance of the immune system. A cross-sectional study investigated associations of concentrations of serum POPs with the prevalence of periodontal disease in 1234 adults ≥20 years of age.[16] Among several POPs, organochlorine (OC) pesticides were most strongly associated with periodontal disease. Polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins also showed significant positive associations with either or both clinical attachment loss ≥4 mm and/or the presence of pocket depth ≥4 mm (i.e., definitions of periodontal disease). It was concluded that POPs, especially OC pesticides, were positively associated with periodontal disease, particularly through immunomodulation due to OC pesticide exposure.

THERAPEUTIC CONSIDERATIONS

Therapeutic goals in treating periodontal disease from a nutritional perspective include the following:

- Decreasing wound healing time (because the time span for wound healing is longer in patients who are more susceptible to periodontal disease[17])
- Improving membrane and collagen integrity
- Decreasing inflammation and free-radical damage (inflammation can induce a vicious cycle and promote periodontal disease)
- Enhancing immune status (defects in the immune system, particularly PMNs, are catastrophic to the periodontium)

In general, periodontal disease and dental caries are a sensitive alarm bell for an unhealthy diet, which predicts the future onset of the diseases of civilizations.[18] A small pilot study demonstrated how quickly following a healthier diet produces results.[19] The experimental group changed from the standard American diet to a diet low in carbohydrates and rich in omega-3 fatty acids, vitamins C and D, antioxidants, and dietary fiber for 4 weeks. Participants of the control group did not change their dietary behavior. Plaque index, gingival bleeding, probing depths, and bleeding upon probing were assessed by a dentist with a pressure-sensitive periodontal probe. Measurements were performed after 1 and 2 weeks without a dietary change (baseline), followed by a 2-week transitional period, and finally performed weekly for 4 weeks. Plaque index values did not change in either group, but all inflammatory parameters decreased in the experimental group to approximately half that of the baseline values and were significantly different compared with those of the control group.

The severity of periodontal disease is closely associated with micronutrient intake.[20] Specifically, insufficient intake of vitamin A, B_1, C, and E and iron, folate, and phosphorus is significantly associated with the severity of periodontal disease.

Vitamin C

Vitamin C (ascorbic acid) plays a major role in preventing periodontal disease, as is evident from many early experimental studies.[17,21–23] The classic symptom of gingivitis seen in scurvy illustrates the vital function of vitamin C in maintaining the integrity of membrane and collagen as well as supporting immunocompetence. Deficiency of vitamin C is associated with defective formation and maintenance of collagen, ground substance, and intercellular cement substance in mesenchymal tissue. The deficiency effects on bone include retardation or cessation of osteoid formation, impaired osteoblastic activity, and osteoporosis. Subclinical vitamin C deficiency plays a significant role in periodontal disease via these effects, which also include delayed wound healing.

Decreased vitamin C levels are also associated with increased permeability of the oral mucosa to endotoxin and bacterial by-products as well as impaired leukocyte functions (particularly regarding PMNs). The role vitamin C plays in increasing chemotaxis and phagocytosis by PMNs is best exemplified by its effect on Chédiak–Higashi syndrome.

This autosomal recessive trait is associated with compromised PMN and monocyte chemotaxis and phagocytosis, all of which are responsive to vitamin C supplementation. This syndrome is also associated with an extremely rapidly progressing periodontitis.

Vitamin C also enhances lymphoproliferative response to mitogens and increases interferon levels, antibody response, immunoglobulin levels, and the secretion of thymic hormones. Vitamin C also possesses significant antioxidant and anti-inflammatory properties and decreases wound healing time.

Vitamin D

Low vitamin D status is associated with both gingivitis and periodontal disease. In a study that analyzed data from 77,503 gingival units (teeth) in 6700 never smokers ages 13 to 90, those with the highest levels of vitamin D had a 30% decreased incidence of gingivitis.[24] This was consistent across racial or ethnic groups and was similar among men and women as well as among users and nonusers of vitamin and mineral supplements. Similarly, in periodontal disease, supplementation with vitamin D reduces bone loss, with men in the highest quintile experiencing 39% less bone loss and women experiencing 26% less.[25] Further strengthening the case for the causal role of vitamin D deficiency, a study found a much higher incidence of the TT VDR (vitamin D receptor) polymorphism and aggressive periodontitis.[26]

Low vitamin D status likely contributes to the link between periodontal disease and breast cancer in women.[27]

Sucrose

Sugar is known to significantly increase plaque accumulation while simultaneously decreasing PMN chemotaxis and phagocytosis. This inhibition of PMN function is due to osmotic effects and competition with vitamin C.

Vitamin C and glucose are known to compete for intracellular transport sites, with this intracellular transport being largely insulin dependent. (See Chapter 136 for further information on nutrient factors and immune function.)

Considering the average American consumes in excess of 175 g/day of sucrose and other refined carbohydrates, it is safe to say that most Americans have a chronically depressed immune status that puts them at increased risk for periodontal disease. The clinical reduction of refined carbohydrates is certainly advisable and seems to significantly reduce gingival inflammation.

Vitamin A

Vitamin A deficiency predisposes people to periodontal disease. Deficiency of vitamin A is associated with the following:

- Keratinizing metaplasia of the gingival epithelium
- Early karyolysis of gingival epithelial cells
- Inflammatory infiltration and degeneration
- Periodontal pocket formation
- Gingival calculus formation
- Increased susceptibility to infection
- Abnormal alveolar bone formation

Vitamin A is necessary for collagen synthesis and wound healing, maintaining the integrity of epithelial and mucosal surfaces and their secretions, and enhancing numerous immune functions. Beta-carotene may be a more advantageous supplement owing to its affinity for epithelial tissue and potent antioxidant activity (see Chapter 57, Beta-Carotene and Other Carotenoids).

Zinc and Copper

The severity of periodontal disease is positively associated with increased serum copper levels, whereas zinc levels are significantly decreased (i.e., an increased copper-to-zinc ratio).[28] This is consistent with other causes of chronic inflammation and signifies the activation of metallothionein, which increases ceruloplasmin formation while increasing zinc sequestration in response to inflammation.

The importance of zinc in treating periodontal disease cannot be overstated. In the United States, marginal zinc deficiency is widespread, particularly in the elderly. This may be a factor in the increasing prevalence of periodontal disease with age, although the geriatric population is at higher risk for the development of numerous nutrient deficiencies. The functions of zinc in the gingiva and periodontium include the following[29]:

- Stabilization of membranes
- Inhibition of calcium influxes
- Antioxidant activity
- A metallocomponent in at least 40 enzymes, including those for DNA, RNA, and collagen synthesis
- Inhibition of plaque growth
- Inhibition of mast cell degranulation
- Numerous immune activities, including increased PMN chemotaxis and phagocytosis
- Promotion of wound healing

The positive effects of zinc in established periodontal disease are also due to its action on calcium- and calmodulin-mediated processes such as mast cell degranulation, tissue damage induced by endotoxin, and increased vascular permeability. These calcium-mediated events are responsible for much of the tissue destruction seen in periodontal disease.

Regular (twice-daily) use of a mouthwash that contains a 5% zinc solution inhibits plaque growth.[29] However, lower concentrations or less frequent mouth washing are not particularly successful.

Vitamin E and Selenium

Vitamin E and selenium function synergistically in antioxidant mechanisms and potentiate each other's effects. Vitamin E alone has been demonstrated to be of considerable value in patients with severe periodontal disease. This can largely be attributed to the decreased wound healing time. The antioxidant effects of vitamin E are particularly needed if mercury amalgam is present. Mercury depletes the tissues of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase. In animal studies, this toxic effect of mercury is prevented by supplementation with vitamin E.[4] Selenium and vitamin E's antioxidant activities also deter periodontal disease because the effects of free radicals are extremely damaging to gingival proteoglycans and glycosaminoglycans.

Coenzyme Q_{10}

Ubiquinone, an essential coenzyme involved in mitochondrial oxidative phosphorylation, is also an effective antioxidant. Coenzyme Q_{10} (CoQ_{10}) is widely used in Japan for many conditions, including periodontal disease. A review of seven studies using CoQ_{10} found that 70% of the 332 patients involved responded favorably to supplementation.[30] A double-blind study comprising 56 subjects found that the supplemented group responded significantly, whereas the placebo group displayed very little change in periodontal pocket depth and tooth mobility.[31]

Flavonoids

Flavonoids are extremely effective in reducing inflammation and stabilizing collagen structures. Flavonoids affect collagen structure by:

- Decreasing membrane permeability, thereby decreasing the load of inflammatory mediators and bacterial products
- Preventing free radical damage with their potent antioxidant properties

- Inhibiting enzymatic cleavage by hyaluronidases and collagenases
- Inhibiting mast cell degranulation
- Cross-linking with collagen fibers directly

In addition, flavonoids may counteract the proinflammatory effects exerted by pathogen-associated molecular pattern (PAMP) proteins through Toll-like receptor (TLR) responses.[32] Flavonoids also exert significant antibiofilm activities against bacteria that play an essential role in the development of dental caries and periodontal disease.[33]

The more biologically active flavonoids (e.g., anthocyanidins and proanthocyanidins) are recommended because rutin has little collagen-stabilizing effect. Proanthocyanidins of grape seed extract have been reported to possess a wide range of biological properties that are useful against periodontal disease.[34] In one animal study, grape seed proanthocyanidin extract (GSE) strongly decreased the production of nitric oxide synthase (iNOS) and reactive oxygen species (ROS) by murine macrophages stimulated with lipopolysaccharides (LPSs) of periodontopathogens.[35]

The flavonoid components of *Camellia sinensis* (green tea) have demonstrated activity against gingival bacteria and also have direct anti-inflammatory effects. Epidemiological studies have shown green tea intake to protect against periodontal disease and tooth loss.[36] In a study involving hydroxypropylcellulose strips impregnated with green tea catechin, the pocket depth and the proportion of pathogenic bacteria were markedly decreased.[37] A double-blind study using soft candy infused with green tea catechin indicated that the green tea soft chews significantly reduced plaque and the degree of gingival inflammation.[38] A double-blind study also showed that chewing gum containing procyanidolic oligomers also minimizes gingival bleeding and plaque accumulation.[39]

Folic Acid

The use of folate, either topically or systemically, in double-blind studies produced significant reductions of gingival inflammation as determined by reduction in color changes, bleeding tendency, gingival exudate flow, and plaque scores.[40–44] The folate mouthwash, 0.1% folic acid, is significantly more effective than oral supplementation of either 2 or 5 mg/day, suggesting a local mechanism of action.[42,43] Folate has been demonstrated to bind plaque-derived endotoxin.[42,43] The use of folate mouthwash is particularly indicated for pregnant women and oral contraceptive users as well as for conditions associated with an exaggerated gingival inflammatory response or folate antimetabolites (e.g., phenytoin, methotrexate).

Epithelial cells of the cervix and the oral cavity appear to suffer from a similar "end-organ" deficiency of folic acid under the hormonal influences of pregnancy and oral contraceptive use. The cervical dysplasia associated with oral contraceptive use also responds to pharmacological doses of folic acid (i.e., 8–30 mg/day).[45,46] (Whitehead's work[45] was the inspiration for the use of folic acid by Pack and Thomson[42,43] in the treatment of gingivitis of pregnancy.) The sera and leukocytes of pregnant women and oral contraceptive users contain a macromolecule that binds folate, which, more than malabsorption or decreased intake, appears to be the major factor for end-organ folate deficiency.[47] The beneficial effects of folic acid are not limited to women; it also improves gingivitis and periodontitis.[44]

Botanical Medicines

Several botanical compounds have shown an ability to inhibit plaque formation. Perhaps the most popular botanical medicine is a toothpaste containing an alcoholic extract of *Sanguinaria canadensis* (bloodroot). Although the extract contains a mixture of benzophenanthridine alkaloids, sanguinarine is the primary alkaloid available in commercial toothpastes and mouth rinses. Sanguinarine demonstrates properties useful in preventing the formation of dental plaque having broad antimicrobial activity as well as anti-inflammatory properties. In vitro studies indicate that the antiplaque action of sanguinaria is due to its ability to inhibit bacterial adherence. Electron microscopical studies of bacteria exposed to sanguinarine demonstrate that bacteria aggregate and become morphologically irregular.[48] Sanguinarine appears to be less effective than chlorhexidine mouthwash, but it is effective in many cases and does have the advantage of being a natural compound versus a synthetic.[49,50]

The triterpenoid extract of *Centella asiatica* (gotu kola) is another useful botanical medicine in periodontal disease. This extract has demonstrated impressive wound healing properties (see Chapter 64). One study demonstrated that *Centella* extract was helpful in speeding up recovery after laser surgery for severe periodontal disease.

THERAPEUTIC APPROACH

No clear guidelines exist for determining which factors are most important for a given patient. Therefore a general approach is recommended. Patients who smoke should be advised to stop because continued smoking greatly decreases the success of any therapy for periodontal disease. Considering that factors that increase the risk for osteoporosis (e.g., low calcium and vitamin D, cigarette smoking) also predispose individuals to the risk for periodontal disease and tooth loss, attention should be directed to Chapter 203 in addition to the recommendations provided here.

Hygiene

Patients should visit a dentist periodically to eliminate accumulated plaque and calculus. Brushing after meals and daily flossing are necessary.

Diet

A diet high in dietary fiber may have a protective effect via increased salivary secretion.[24] Avoidance of sucrose and all refined carbohydrates is extremely important.

Supplements

- Vitamin C: 3 to 5 g/day in divided doses
- Vitamin D: 2000 to 6000 IU/day
- Vitamin E: 400 to 800 IU/day
- Beta-carotene 50,000 IU/day
- Selenium: 400 mcg/day
- Zinc: 30 mg/day; used as a mouthwash with 15 mL of a 5% solution twice a day
- Folic acid (activated form preferred): 800 mcg/day; used as a mouthwash with 15 mL of a 0.1% solution twice a day

Botanical Medicines

High-flavonoid–containing extracts such as those from bilberry *(Vaccinium myrtillus),* hawthorn (*Crataegus* species), grape seed *(Vitis vinifera),* or green tea *(Camellia sinensis)* can be used according to the dosages in the corresponding chapters. Of these extracts, grape seed extract or green tea extract (or the liberal consumption of green tea as a beverage) may offer the greatest protection. For a grape seed extract with a 95% proanthocyanidolic oligomer content or a green tea extract with a 90% polyphenol content, the dosage would be 150 to 300 mg a day. Additional recommendations include the following:

- *Sanguinaria canadensis:* toothpaste containing extract
- *Centella asiatica* triterpenoids: 30 mg twice a day of pure triterpenoids

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Deliargyris EN, Madianos PN, Kadoma W, et al. Periodontal disease in patients with acute myocardial infarction: prevalence and contribution to elevated C-reactive protein levels. *Am Heart J*. 2004;147:1005–1009.
2. Bartold PM, Wiebkin OW, Thonard JC. The effect of oxygen-derived free radicals on gingival proteoglycans and hyaluronic acid. *J Periodontal Res*. 1984;19:390–400.
3. Hyyppa T. Gingival IgE and histamine concentrations in patients with asthma and in patients with periodontitis. *J Clin Periodontol*. 1984;11:132–137.
4. Addya S, Chakravarti K, Basu A, et al. Effects of mercuric chloride on several scavenging enzymes in rat kidney and influence of vitamin E supplementation. *Acta Vitaminol Enzymol*. 1984;6:103–107.
5. Carson SJ, Burns J. Impact of smoking on tooth loss in adults. *Evid Based Dent*. 2016;17(3):73–74.
6. Kaldahl WB, Johnson GK, Patil KD, et al. Levels of cigarette consumption and response to periodontal therapy. *J Periodontol*. 1996;67:675–681.
7. Pelletier O. Smoking and vitamin C levels in humans. *Am J Clin Nutr*. 1968;21:1259–1267.
8. Prerovsky I, Hladovec J. Suppression of the desquamating effect of smoking on the human endothelium by hydroxyethylrutosides. *Blood Vessels*. 1979;16:239–240.
9. Tezal M, Grossi SG, Ho AW, et al. Alcohol consumption and periodontal disease. *J Clin Periodontol*. 2004;31:484–488.
10. Bartold PM, Wiebkin OW, Thonard JC. The active role of gingival proteoglycans in periodontal disease. *Med Hypotheses*. 1983;12:377–387.
11. Bartold PM, Wiebkin OW, Thonard JC. Proteoglycans of human gingival epithelium and connective tissue. *Biochem J*. 1983;11:119–127.
12. Abbas F, van der Velden U, Hart AA. Relation between wound healing after surgery and susceptibility to periodontal disease. *J Clin Periodontol*. 1984;11:221–229.
13. Kocher T, Sawaf H, Fanghanel J, Timm R, Meisel P. Association between bone loss in periodontal disease and polymorphism of N-acetyltransferase (NAT2). *J Clin Periodontol*. 2002;29(1):21–27. PubMed PMID:11846845.
14. Saraiva MC, Taichman RS, Braun T, Nriagu J, Eklund SA, Burt BA. Lead exposure and periodontitis in US adults. *J Periodontal Res*. 2007;42(1):45–52. PubMed PMID:17214639.
15. Amin WM, Al-Omoush SA, Hattab FN. Oral health status of workers exposed to acid fumes in phosphate and battery industries in Jordan. *Int Dent J*. 2001;51(3):169–174. PubMed PMID:11563682.
16. lee DH, Jacobs DR, Kocher T. Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. *Environ Health Perspect*. 2008;116(11):1558–1562. PubMed PMID:19057711.
17. Alvares O, Siegel I. Permeability of gingival sulcular epithelium in the development of scorbutic gingivitis. *J Oral Pathol*. 1981;10:40–48.
18. Hujoel PP1,2, Lingström P3. Nutrition, dental caries and periodontal disease: a narrative review. *J Clin Periodontol*. 2017;44(Suppl 18):S79–S84. https://doi.org/10.1111/jcpe.12672.
19. Woelber JP1, Bremer K2, Vach K3, et al. An oral health optimized diet can reduce gingival and periodontal inflammation in humans - a randomized controlled pilot study. *BMC Oral Health*. 2016;17(1):28. https://doi.org/10.1186/s12903-016-0257-1.
20. Luo PP1, Xu HS1, Chen YW2, Wu SP1. Periodontal disease severity is associated with micronutrient intake. *Aust Dent J*. 2018;63(2):193–201. https://doi.org/10.1111/adj.12606. Epub 2018 Apr 23.
21. Varela-López A, Navarro-Hortal MD, Giampieri F, et al. Nutraceuticals in periodontal health: a systematic review on the role of vitamins in periodontal health maintenance. *Molecules*. 2018;23(5). pii: E1226. https://doi.org/10.3390/molecules23051226.
22. Woolfe SN, Hume WR, Kenney EB. Ascorbic acid and periodontal disease: a review of the literature. *J West Soc Periodontol Periodontal Abstr*. 1980;28:44–56.
23. Alfano MC, Miller SA, Drummond JF. Effect of ascorbic acid deficiency on the permeability and collagen biosynthesis of oral mucosal epithelium. *Ann N Y Acad Sci*. 1975;258:253–263.
24. Dietrich T, Nunn M, Dawson-Hughes B, et al. Association between serum concentrations of 25-OH vitamin D and gingival inflammation. *AJCN*. 2005;82:575–580.
25. Miley DD, Garcia MN, Hildebolt CF, et al. Cross-sectional study of vitamin D and calcium supplementation effects on chronic periodontitis. *J Periodontol*. 2009;80(9):1433–1439.
26. Zhang L, Meng HX, Zhao HS, et al. Correlation study on polymorphisms of vitamin D receptor gene in patients with periodontitis. *Beijing Da Xue Xue Bao*. 2010;42(1):37–40 (Chinese).
27. Grant WB, Boucher BJ. Low vitamin D status likely contributes to the link between periodontal disease and breast cancer. *Breast Cancer Res Treat*. 2011;128(3):907–908.
28. Freeland JH, Cousins RJ, Schwartz R. Relationship of mineral status and intake to periodontal disease. *Am J Clin Nutr*. 1976;29:745–749.
29. Hsieh S, Hayali A, Navia J. Zinc. In: Curzon M, Cutress T, eds. *Trace Elements in Dental Disease*. Boston: John Wright PSG: 99–220.
30. Folkers K, Yamamura Y. *Biomedical and Clinical Aspects of Coenzyme Q*. Vol. 1. Amsterdam: Elsevier/North Holland Biomedical Press.
31. Folkers K, Yamamura Y. *Biomedical and Clinical Aspects of Coenzyme Q*. Vol. 3. Amsterdam: Elsevier/North Holland Biomedical Press.
32. Fernández-Rojas B1, Gutiérrez-Venegas G2. Flavonoids exert multiple periodontic benefits including anti-inflammatory, periodontal ligament-supporting, and alveolar bone-preserving effects. *Life Sci*. 2018. pii: S0024-3205(18)30475-2. https://doi.org/10.1016/j.lfs.2018.08.029. [Epub ahead of print].
33. Slobodníková L1, Fialová S2, Rendeková K3, Kováč J4, Mučaji P5. Antibiofilm activity of plant polyphenols. *Molecules*. 2016;(12):21. pii: E1717.
34. Petti S, Scully C. Polyphenols, oral health and disease: a review. *J Dent*. 2009;37(6):413–423.
35. Houde V, Grenier D, Chandad F. Protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens. *J Periodontol*. 2006;77(8):1371–1379.
36. Koyama Y, Kuriyama S, Aida J, et al. Association between green tea consumption and tooth loss: cross-sectional results from the Ohsaki Cohort 2006 Study. *Prev Med*. 2010;50(4):173–179.
37. Hirasawa M, Takada K, Makimura M, et al. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. *J Periodontal Res*. 2002;37:433–438.
38. Krahwinkel T, Willershausen B. The effect of sugar-free green tea chew candies on the degree of inflammation of the gingiva. *Eur J Med Res*. 2000;5:463–467.
39. Kimbrough C, Chun M, dela Roca G, et al. Pycnogenol chewing gum minimizes gingival bleeding and plaque formation. *Phytomedicine*. 2002;9:410–413.
40. Vogel RI, Fink RA, Schneider LC, et al. The effect of folic acid on gingival health. *J Periodontol*. 1976;47:667–668.
41. Vogel RI, Fink RA, Frank O, et al. The effect of topical application of folic acid on gingival health. *J Oral Med*. 1978;33:20–22.
42. Pack AR, Thomson ME. Effects of topical and systemic folic acid supplementation on gingivitis in pregnancy. *J Clin Periodontol*. 1980;7:402–414.
43. Thomson ME, Pack AR. Effects of extended systemic and topical folate supplementation on gingivitis of pregnancy. *J Clin Periodontal*. 1982;9:275–280.
44. Pack AR. Folate mouthwash. Effects on established gingivitis in periodontal patients. *J Clin Periodontol*. 1984;11:619–628.
45. Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium association with oral contraceptive therapy and reversal with folic acid. *J Am Med Assoc*. 1973;226:1421–1424.
46. Butterworth Jr CE, Hatch KD, Gore H, et al. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am J Clin Nutr*. 1982;35:73–82.
47. da Costa M, Rothenberg SP. Appearance of a folate binder in leukocytes and serum of women who are pregnant or taking oral contraceptives. *J Lab Clin Med*. 1974;83:207–214.
48. Godowski KC. Antimicrobial action of sanguinarine. *J Clin Dent*. 1989;1:96–101.
49. Grossman E, Meckel AH, Isaacs RL, et al. A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics, and sanguinarine on dental plaque and gingivitis. *J Periodontol*. 1989;60:435–440.
50. Benedicenti A, Galli D, Merlini A. The clinical therapy of periodontal disease, the use of potassium hydroxide and the water-alcohol extract of *Centella asiatica* in combination with laser therapy in the treatment of severe periodontal disease. *Parodontol Stomatol*. 1985;24:11–26.

209

Polycystic Ovary Syndrome (PCOS)

Leah Hechtman, MSci (RHHG), BHSc (Nat), ND

OUTLINE

DIAGNOSTIC SUMMARY

- No single test is used to diagnose polycystic ovary syndrome (PCOS). There are three different diagnostic criteria systems (Table 209.1).[1]
- In an attempt to reconcile the proposed criteria, the National Institutes of Health (NIH) sponsored an Evidence-Based Methodology PCOS Workshop in 2012. The resulting recommendations reinforced the use of the 2003 Rotterdam criteria but with the addition of a phenotypic classification system to aid in clinical classification and epidemiological research.
- Although the Rotterdam criteria are accepted, a spectrum exists clinically, ranging from asymptomatic women with polycystic ovarian morphology (PCOM) and biochemical hyperandrogenism to those with severe clinical and biochemical disorders.[2]
- The multiple phenotypic forms in PCOS, often partial, may be accompanied by the following:
  - Reproductive problems (cyclic disorders, failing ovulation leading to hypofertility, risk of ovarian hyperstimulation, unsuccessful in vitro fertilization [IVF], high incidence of spontaneous miscarriages, increased risk of pregnancy-induced hypertension and/or gestational diabetes)
  - Long-term complications (metabolic, cardiovascular or cancer) linked to the associated hyperinsulinemia, which is present in the majority of cases[1,3,4]
- The diagnostic criteria must exclude other androgen-excess diseases and ovulation dysfunctions including the following[1,5–7]:
  - Cushing's syndrome
  - Acromegaly
  - Congenital adrenal hyperplasia
  - Thyroid disorders
  - Hyperprolactinemia
  - Insufficient follicle-stimulating hormone for ovulation
  - Premature ovarian failure
  - Adrenal and ovarian androgen-secreting tumors.

GENERAL CONSIDERATIONS

PCOS is one of the most common reproductive endocrine diseases occurring among women of childbearing age,[5] with a prevalence of 6% to 10% (depending on the diagnostic criteria).[8] PCOS is linked with disturbances of reproductive, endocrine, and metabolic function.[5] Nowadays, PCOS is generally considered a complex genetic disorder with interactions between environmental factors, such as diet and obesity, and genetic susceptibility caused by single-nucleotide polymorphisms.[9]

Histopathologically, PCOS presents with an excess number of immature follicles that arrest before the preovulatory stage of development.[10] The size of the ovary is also slightly larger than the usual size of an ovary. PCOS can result in profound, long-term health consequences,[11] for example:

- Increased risk of infertility, miscarriage, and pregnancy complications
- Metabolic abnormalities that increase their risk of developing obesity, type 2 diabetes, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), gallbladder disease, and cardiovascular disease.[12–17]
- Increased prevalence of autoimmune diseases (e.g., type 1 diabetes mellitus), thyroid disease (especially Hashimoto's thyroiditis), and asthma.[18–21]

TABLE 209.1 Diagnostic Criteria of Polycystic Ovary Syndrome (PCOS)[1,5]

NIH consensus (1990)	Clinical and/or biological hyperandrogenism associated with cycle disorders, excluding all classic causes of hyperandrogenism
Rotterdam consensus (2004)	The presence of two of the following three criteria: dysovulatory or anovulatory cycles, hyperandrogenism (clinical and/or biological), and/or polycystic ovarian morphology (PCOM) on ultrasound
American Androgen Excess Society (AES) and the PCOS Association (AE-PCOS criteria) (2006)	Hyperandrogenism (clinical and/or biological) as a mandatory factor, associated either with ovulation disorders or with PCOM

- Increased risk of depression and anxiety, as well as a range of other psychiatric disorders.[22,23] The signs and symptoms of PCOS may challenge a woman's feminine identity and can affect her quality of life.[24] Mood implications are clinically relevant because treating PCOS requires the patient to implement lifestyle changes and have the self-motivation to do so.[25]
- Increased risk of osteoporosis, due to the influence on bone metabolism of the hormone imbalances found in PCOS.[26]

CAUSES OF PCOS

PCOS is affected by many factors, but its precise pathophysiology has not yet been determined. PCOS is characterized by a combination of metabolic and endocrine abnormalities and reproductive dysfunction,[5] most likely occurring as the result of both genetic and environmental factors, particularly obesity, metabolic syndrome, and diet.[1,27] Overall, PCOS involves oxidative stress with systemic inflammation, insulin resistance, and consequent hyperinsulinemia that all promote dysregulation of the ovarian thecal compartment and dysfunction of endothelial cells, resulting in hyperandrogenism, anovulation, and cardiovascular disorders.[3,4,28]

PCOS is more prevalent among women of certain ethnicities; for example, a higher prevalence has been noted among South Asian women compared with East Asian and Caucasian women.[29,30] Ethnic variations in PCOS may be associated with environmental factors, such as socioeconomic conditions and lifestyle.[31] Socioeconomic status may affect susceptibility to PCOS through association with adverse health behaviors and exposure to environmental toxins, leading to obesity and triggering hormonal reactions. Socioeconomic status might also partially explain geographical disparities in PCOS.[32]

Androgen Excess/Hyperandrogenism

Ovarian androgen excess is the core of PCOS. Hyperinsulinemia enhances the activation of the thecal steroidogenesis and contributes to impaired follicular maturation.[1] Higher circulating androgen levels can hinder the normal growth of follicles, resulting in oligo-ovulation or anovulation, which mainly manifests an abnormal menstrual cycle (usually oligomenorrhea/amenorrhea). Elevated androgen levels also can cause hirsutism, acne, female hair loss, and other clinical symptoms. Androgen formation is also affected by insulin and the insulin growth factor system, the renin–angiotensin system (RAS), adiponectin, leptin, growth hormone, and other factors.[5] Androgen can affect the growth and development of follicles, inhibit the formation of dominant follicles, and accumulate a large number of immature follicles, that is, encouraging the formation of the polycystic-like changes of the ovaries.[5]

Insulin Resistance and Insulin Action Abnormalities

PCOS is associated with insulin resistance (IR), metabolic syndrome, and type 2 diabetes.[33] IR can elevate serum insulin levels and increase the frequency of pulsatile gonadotropin-releasing hormone (GnRH) secretion, causing elevated serum LH levels and further promoting excess androgen production. A large number of studies have confirmed that patients with PCOS have varying degrees of IR and compensatory hyperinsulinemia, including ovarian IR. IR and hyperinsulinemia play an important role in the pathogenesis of PCOS,[5] increasing hyperandrogenism and long-term metabolic, cardiovascular, and oncological risks.[1]

Obesity

Obesity, especially abdominal obesity in adolescence and adulthood and weight gain in puberty, increases the risk of developing PCOS and increases the severity of certain PCOS features such as hyperandrogenism, hirsutism, infertility, psychological features, and pregnancy complications (e.g., preeclampsia and gestational diabetes).[2] Obesity is seen in 35% to 60% of PCOS cases,[34] and a 1-unit increase in body mass index (BMI) increases the risk of PCOS by 9%.[35] Moreover, the obesity associated with insulin resistance increases the risk of cardiovascular diseases and type 2 diabetes in PCOS. In many cases, obesity makes the hidden PCOS syndrome clinically apparent in susceptible women. Increases in visceral adipose tissue and central obesity have been observed even in lean women with PCOS compared with healthy women. The combination of obesity and disordered eating habits, which are both commonly associated with PCOS, further exacerbates reproductive, metabolic, and psychological morbidity.[36,37]

The increase in obesity-associated oxidative stress is probably due to the presence of excessive adipose tissue itself. Adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines that are potent stimulators of the production of reactive oxygen and nitrogen species.[38,39]

Abdominal obesity is seen in most patients with PCOS, especially in the viscera,[40] and is strongly associated with the development of IR. Visceral adipose tissue is highly sensitive to lipolysis stimulated by androgens that will contribute to the increased availability of free fatty acids (FFAs). This, in turn, induces accumulation of hepatic fat and decreased hepatic insulin clearance and hepatic IR.[5] There is an interaction between the effects of PCOS and obesity, such that the metabolic abnormalities in women with PCOS are amplified to a greater degree by obesity than in weight-matched controls.[41] Fat cells in women with PCOS appear to be metabolically different, having subcutaneous adipocytes that are larger and less responsive to the lipolytic effects of catecholamines, with lower serum adiponectin production from adipocytes.[42]

Oxidative Stress

The concentration levels of oxidative stress (OS) markers such as lipid peroxidation are higher than normal in PCOS. IR is thought to be a cause of OS, which can lead to the occurrence of hyperglycemia, causing related cells to release ROS. OS can damage cells and activate the expression of proinflammatory cytokines, which in turn promotes the occurrence of IR and hyperandrogenism.[5]

Chronic Low-Grade Inflammation

The role of inflammation in PCOS has been the subject of a number of studies, and direct correlations have been found between increased levels of inflammatory markers and the development of PCOS.[43] PCOS is associated with a chronic low-grade inflammation and predisposition to hemostatic and atherosclerotic complications.[44] The inflammatory

responses are induced by hyperlipidemia and IR, which lead to the development of PCOS and are also involved in the formation of long-term complications. Some studies suggest that hyperandrogenism may induce chronic low-grade inflammation in PCOS, which in turn further promotes the occurrence of IR and hyperandrogenism. Chronic low-grade inflammation in patients with PCOS was found to be closely related to obesity and may be responsible for IR in nonobese patients with PCOS.[5] However, circulating levels of tumor necrosis factor-alpha (TNF-α) are elevated even in the absence of obesity, indicating that PCOS is a proinflammatory state, regardless of obesity. Studies have shown a genetic basis for chronic low-grade inflammation in PCOS.[39]

Patients with PCOS were found to have significantly higher copper concentrations than patients without PCOS.[45–47] Copper contributes to increased oxidative stress, leading to the generation of ROS, and it is possible that elevated copper levels play a role in the low-grade inflammation associated with the pathogenesis of PCOS.[48]

Prenatal Androgen Exposure

The developmental origin of PCOS hypothesis suggests that the capacity to hypersecrete androgens begins in fetal life as a result of genetic and environmental interplay, and that the typical clinical and biochemical features of PCOS are "downstream" effects of exposure to androgen excess at or before puberty.[1,18] Evidence from both clinical and preclinical studies supports the theory that high intrauterine androgen levels increase the susceptibility of female offspring to develop the PCOS phenotype after pubertal onset.[49,50]

Neuroendocrinology Dysfunction of the Hypothalamic–Pituitary Axis

Studies have shown that neuroendocrine dysfunction plays an important role in the pathophysiology of PCOS due to the complex interactions between abnormal ovarian steroidogenesis, hyperinsulinemia, and endocrine dysfunction of the hypothalamic–pituitary–gonadal (HPG) axis.[5] Elevated luteinizing hormone (LH) pulse frequency is suggestive of a hyperactive HPG axis due to impaired negative gonadal hormone feedback. GnRH is increased in response to LH, leading to an increase in the LH/FSH ratio, which causes a decrease in aromatase activity in granulosa cells, resulting in testosterone not aromatizing into estrogen. Androgens in women with PCOS alter central brain circuits, suppressing the negative-feedback effect of progesterone on GnRH production.[5,51]

Adrenal Dysfunction

In women with PCOS, 20% to 30% have excess adrenal androgens such as dehydroepiandrosterone sulfate (DHEAS). The adrenal androgens have feedback effects on increasing the hypothalamic secretion of LH, which, in turn, leads to an increase in the synthesis of ovarian androgens.[5,52]

Ovarian Dysfunction

The World Health Organization classifies PCOS as a group II ovulation disorder, which are dysfunctions of the HPA–ovarian axis.[53] PCOS is the most common cause of anovulatory infertility. There may be genetic morphological changes, but ovulation or anovulation is mainly dependent on the follicular environment. In PCOS, disordered follicular development affects oocyte development. Ovarian hyperandrogenism, mainly due to thecal cells that are overresponsive to LH, can lead to excessive follicular atresia, follicular stasis, and anovulation.

Environmental Toxins

Studies have found significantly higher serum levels of environmental toxins, such as perfluorinated compounds, polychlorinated biphenyls (PCBs), bisphenol A (BPA), pesticides, and polycyclic aromatic hydrocarbons, among women with PCOS compared with control subjects. Endocrine-disrupting chemicals (EDCs) are defined as "substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction."[54] EDCs are a broad class of molecules that include plasticizers such as phthalates and BPA, as well as advanced glycation end products (AGEs).[55] BPA is the most characteristic agent, and it is found in the everyday domestic environment and food chain. These natural or synthetic products mimic natural hormones and/or disrupt their action.

Both experimental and epidemiological evidence support the role of EDC exposure in the physiopathology of PCOS, either through in utero exposure via the mother or through chronic adult exposure interfering with follicular maturation, ovarian steroidogenesis, and/or occurrence of insulin resistance.[56] In addition, EDCs play a role in obesity and type 2 diabetes through an in utero fetal effect. Low concentrations of BPA exposure (as are found in the human population) are able to induce IR. The timing of EDC exposure is crucial for the intensity of adverse health effects, with fetuses, infants, and/or young children being the most susceptible groups, especially in the early development periods. These exposure effects may be expressed across generations.[57] BPA levels are higher in women with PCOS than in those without PCOS, and they correlate positively with total or free testosterone and IR. Hyperandrogenism can slow down the metabolism of BPA in the liver, and BPA can interfere with hyperandrogenism at various levels—circulating sex hormone–binding globulin (SHBG), androgen receptor, synthesis via steroidogenic enzymes, and androgen metabolism—leading to a vicious cycle.[1,32,58]

Genetic Determinants

The origin of hyperandrogenism and hyperinsulinemia has a genetic component, as demonstrated by familial aggregation studies and recent identification of associated genomic variants.[1] To date, more than 100 candidate genes have been implicated in the pathophysiology of PCOS, with particular focus on genes affecting the biosynthesis and function of reproductive hormones, metabolism of insulin, folliculogenesis, cellular metabolism, mitochondrial biogenesis, and chronic inflammation.[12,27,59] Screening for PCOS susceptibility genes has also found variants corresponding to genes involved in the regulation of fat mass.[60] The protective or susceptible genomic variants involved may be influenced by environmental factors through epigenetic modifications (e.g., DNA methylation, histone state, microRNA [miRNA] expression).

Several differences have been found in PCOS in some miRNAs involved in the control of genes necessary for androgen synthesis, inflammation, adipogenesis, and signaling. Two miRNAs appear to be of particular interest as biomarkers in PCOS: miR-222 positively correlates with insulin, and miR-146a negatively correlates with testosterone.[1]

Environmental conditions may mimic hormonal actions and activate preexisting predisposing factors, triggering the characteristic endocrine alterations of PCOS. These can be prenatal (epigenetic fetal programming) or postnatal (diet, obesity, sedentary lifestyle, and environmental toxins).[27]

Attenuated Apoptosis

The majority of human follicles do not reach a stage of final maturation but face elimination by atresia. The process of follicle apoptosis is tightly regulated and influenced by endocrine, paracrine, and autocrine factors.[61] The increased number of viable primary and secondary follicles in the polycystic ovary indicates that the process of apoptosis is attenuated.[62]

DIAGNOSTIC CONSIDERATIONS

No single test is used to diagnose PCOS, and diagnosis criteria vary to some degree according to the consensus group. There are three different diagnostic criteria systems (Table 209.1). Table 209.2 outlines key diagnostics to assess possible underlying causes.

Signs and Symptoms

Women with PCOS demonstrate marked clinical heterogeneity in their symptoms. The wide range of commonly associated features of PCOS are neither uniform nor universal, and they usually worsen with time.[18,63] There are widely ranging signs and symptoms associated with PCOS, which can vary over time in individual women, usually worsening with time. Women might experience many or some of the signs and symptoms; the condition will affect each woman differently.

Classification of Features

- Clinical: including menstrual abnormalities, hirsutism, acne, alopecia, anovulatory infertility, and recurrent miscarriages
- Endocrine: including elevated androgens, LH, estrogen, and prolactin
- Metabolic: including insulin resistance, obesity, lipid abnormalities, and an increased risk of impaired glucose tolerance (IGT) and type 2 diabetes mellitus[12]

Hyperandrogenism

Hyperandrogenism is the most constant and prominent component of PCOS, but reliable detection of this feature is not straightforward, and indices vary considerably depending on age, body weight, and ethnic origin.[6] Hyperandrogenism is assessed by clinical features, biochemical indices, or both. Clinically, hyperandrogenism is diagnosed by the most subjective assessment of cutaneous manifestations of excessive androgen activity, such as hirsutism, acne (especially in young women), and female-pattern baldness (especially in older women).[6]

Anovulation

PCOS is present in around 70% of women who have issues with ovulation, leading to infertility.[64] The major clinical signs of chronic anovulation are oligomenorrhea or amenorrhea. Amenorrhea results from a failure of the HPG axis.[65] The failure of underdeveloped follicles to mature causes menses to be erratic or absent, and the lack of cyclical control in relation to normal mechanisms of feedback between the ovary and pituitary gland can lead to reduced fertility.[66] Women experiencing oligomenorrhea or amenorrhea as a result of PCOS are at increased risk of developing endometrial hyperplasia and endometrial cancer, although there is no increased risk of breast or ovarian cancer.[29]

Hyperinsulinemia

IR is present in 60% to 80% of women with PCOS and in 95% of obese women with PCOS. IR causes LH stimulation of the internal theca LH receptor, which increases hyperandrogenemia and disrupts follicular maturation. Hyperinsulinemia also increases circulating hyperandrogenemia via the decrease of SHBG. Whatever its origin, IR is a mechanism through which environmental factors such as nutrition, energy balance, intestinal microbiota, and chemical pollutants are able to influence the course of PCOS. IR causes an increase in inflammatory markers, which cause endothelial damage, increased vessel wall reactivity to pressure substances, and the development of atherosclerosis, suggesting an increased risk of cardiovascular disease.[1,34]

Hirsutism, Acne, Seborrhea, and Alopecia

Acne, hirsutism, and androgenic alopecia are due to androgenic stimulation of the pilosebaceous unit.[2] Androgen excess contributes to a dysregulation of hair follicle growth, increasing hair follicle size, hair fiber diameter, and the proportion of time terminal hairs remain in the growth phase.[5] Hirsutism can affect approximately 5% to 10% of reproductive-age women, whereas approximately 80% of hirsute patients will have PCOS. Hirsutism is the clinical feature that has the strongest impact on health-related quality-of-life (HRQL) scores in women with PCOS.[67] A Cochrane review found that the treatment of hirsutism needs to incorporate pharmaceutical therapies, cosmetic procedures, and psychological support.[68]

If the sebaceous glands are sensitive to the effects of androgens, they produce excess oil, which can result in the buildup of dead skin cells lining the pores of the skin. When these cells are not adequately shed, they clog the pores and can produce comedones. Alopecia (male-pattern hair loss) is associated with hyperandrogenism.[69]

Obstructive Sleep Apnea

Obstructive sleep apnea appears to be present in a disproportionate number of women with PCOS and may be associated with distinct endocrine and metabolic alterations.[70]

Recognized Phenotypes in PCOS

The phenotypes in PCOS can be broadly classified into four types as per Table 209.3. Metabolically, phenotypes A and B ("classic PCOS") behave similarly, with approximately 75% to 85% demonstrating insulin resistance and some form of metabolic dysfunction.[71] The different phenotypes vary in the degree to which they are associated with an increased risk for metabolic dysfunction and reproductive complications.

THERAPEUTIC CONSIDERATIONS

Treatment often focuses on individual symptoms rather than treatment of the syndrome itself because the underlying pathophysiology of PCOS is not fully understood.[72] There is no medication available that completely reverses the underlying hormonal imbalance and treats all of the associated clinical symptoms of PCOS.[11] Current recommendations focus on controlling weight, hormone balance and insulin resistance via lifestyle modification (e.g., diet, exercise, and weight loss), and insulin-sensitizing agents, which are the more effective management strategies.[5]

Dietary Considerations

It is generally accepted that changes in diet can produce a profound and beneficial effect with regard to symptomatology and coexisting conditions (e.g., subfertility or amenorrhea). The optimal diet for individuals with PCOS is recognized as not just being one that reduces symptomatology and assists with weight management but also one that protects against the associated pathologies of cardiovascular disease, diabetes, impaired fertility, and certain cancers.

TABLE 209.2 Diagnostic Investigations

Investigation	Justification
Biochemical Evaluation	• Testosterone panel including total-testosterone, sex hormone–binding globulin (SHBG), bioavailable testosterone, and free-testosterone (to evaluate hyperandrogenemia).[233] • Anti-Müllerian hormone (AMH) (to assess ovarian follicle/cyst status)—AMH levels correlate with the number of ovarian follicles and cysts and can be used instead of ultrasound for establishing polycystic ovarian morphology (PCOM). AMH concentration is related to the severity of hyperandrogenism and oligo-anovulation in PCOS.[234–236] • Hemoglobin A_{1c} ($HgbA_{1c}$), fasting glucose, fasting GTT, fasting insulin, HOMA-IR, and fasting lipids (to determine insulin resistance, prediabetes, and dyslipidemia; cardiovascular disease risk).[237] • LH:FSH ratio—patients with PCOS usually have a ratio of >3:1, and higher LH values are consistent with more severe hyperandrogenemia. • Estradiol—most women with PCOS are anovulatory and have estradiol levels appropriate for the follicular phase of the menstrual cycle. This measurement has extremely limited value. • Progesterone—needs to be tested 7 days postovulation in the midluteal phase (usually day 21) of the menstrual cycle. Random measurements are uninformative. • Thyroid panel—TSH, T3, T4, reverse T3, thyroid antibodies, urinary iodine or spot iodine • 17-hydroxyprogesterone, prolactin, cortisol (to rule out congenital adrenal hyperplasia, prolactinoma, and Cushing's syndrome) • PCOS cannot be diagnosed accurately while the patient is on contraceptives due to their effects on testosterone and AMH levels.[238]
Radiological Studies	Transabdominal and transvaginal ultrasound
Family and Lifestyle History	• Examination of family history of diabetes mellitus, cardiovascular disease, and hyperlipidemia, possibly with assessment of relevant risk factors in siblings and older family members.[6] • The family history often includes female relatives with irregular menstruation, severe acne, excess hair growth, and difficulty becoming pregnant. • Lifestyle issues, including a history of diet and exercise, should be investigated. • Health-related quality of life is generally worse in women with PCOS than in women without the disorder.[6] A questionnaire of health-related quality of life specific for women has been developed for this purpose.[239]
Developmental and Menstrual History	• Women with PCOS usually report a history of menarche around 12–13 years of age and never develop a consistent pattern of regular menses.[240] • Male hair patterns and skin changes often appear at the same time as secondary sex characteristics.[240] Occasionally, male-pattern alopecia occurs. • Acne can vary from mild to severe. • Symptoms usually begin at menarche and manifest after puberty. • Other causes should be assessed for those women who have a history of regular menstrual cycles and who then develop irregular cycles.
• Polycystic Ovaries on Ultrasound/MRI	• Polycystic ovarian morphology (PCOM) is characterized by more than 12 small follicles (measuring 2–9 mm) in at least one ovary or an ovarian volume of >10.0 cm.[3,16] *Note*: The Androgen Excess and Polycystic Ovary Syndrome Society (AEPCOS) has recommended an updated threshold for PCOM of 25 follicles.[241] • Serum AHM is accepted as a replacement for ultrasound in women under 35.[6] AHM is secreted by granulosa cells of developing follicles, and levels correlate with the number of ovarian follicles and cysts.[238,242–244]

FSH, Follicle-stimulating hormone; *GTT,* glucose tolerance test; *HOMA-IR,* homeostatic model assessment of insulin resistance; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging; *PCOS,* polycystic ovary syndrome; *TSH,* thyroid-stimulating hormone.

TABLE 209.3 Phenotypes for Polycystic Ovary Syndrome (PCOS) Based on the 2012 Modified Rotterdam Criteria[245]

	Hyperandrogenism	Ovulatory Dysfunction	Polycystic Ovarian Morphology
Phenotype A "Classic"	x	x	x
Phenotype B "Classic"	x	x	
Phenotype C "Ovulatory"	x		x
Phenotype D "Nonhyperandrogenic"		x	x

Dieting, alone or in combination with exercise, has been shown to be the most effective way to improve metabolic disturbances in obese women with PCOS. In one study, increased fiber and reduced *trans* fat intake were the primary predictors of weight loss and metabolic improvement.[73] A study on the effects of dietary carbohydrates in PCOS concluded that it is energy restriction and weight loss in PCOS that improve ovulation rates, conception hyperandrogenemia, glucose and insulin levels, insulin resistance, and satiety hormones. The composition of the diet is less important, so long as the weight loss is achieved.[74]

There is limited research in this area in PCOS; however, a systematic review of existing studies including low-glycemic-index (GI) diets concluded no clear benefit of any specific macronutrient composition, and general healthy diets with reduced energy intake were recommended.[75] There is currently a lot of interest in the role of different types of fasting regimes, which can IR and could consequently have beneficial effects on ovarian function, androgen excess, and infertility in women with PCOS.[76]

Glucose and saturated fatty acids are proinflammatory and lead to dyslipidemia independent from IR and obesity in PCOS.[39] Polyunsaturated fatty acids (PUFAs) may facilitate the resolution of inflammation and perhaps confer a protective effect, and alterations in dietary intake of macronutrients may mitigate the low-grade chronic inflammation associated with these disorders and improve fertility. For example, low-GI diets are associated with lower serum concentrations of inflammatory markers such as C-reactive protein (CRP), and dietary consumption of *trans* fat is associated with IR and a proinflammatory state. Replacing animal protein with protein from vegetable sources was associated with a lower risk of ovulatory infertility.[77]

Strong adherence to the healthy dietary pattern (fruits, vegetables, fish, and whole grains) was inversely associated with the hyperandrogenic PCOS phenotype only. The adherence to the unhealthy dietary pattern, however, was not associated with the hyperandrogenic or nonhyperandrogenic PCOS phenotype. Moreover, hyperandrogenic and nonhyperandrogenic patients with PCOS with strong adherence to a healthy dietary pattern also demonstrated a threefold-higher chance of ongoing pregnancy.[9]

An 8-week dietary intervention trial of 24 women with PCOS showed that a low-starch/low-dairy diet improved weight parameters, inulin sensitivity, testosterone levels, and hirsutism. The diet included lean animal protein (meat and poultry), fish and shellfish, eggs, nonstarchy vegetables, low-sugar fruits (berries, apples, oranges, plums, etc.), avocado, olives, nuts and seeds, and oils (olive and coconut) and excluded grains, beans, dairy products (other than 30 g full-fat cheese per day to assist with compliance), and sugar (including fruit juice from concentrate, raw turbinado sugar, evaporated cane juice, high-fructose corn syrup, honey, or agave nectar) because of their insulinemic properties.[78]

Low-GI Diet

High carbohydrate intake is often identified as an exacerbating factor for PCOS, although diet itself is not likely a cause of PCOS.[32] The GI is a classification index of carbohydrate foods based on postprandial glucose response (i.e., it ranks carbohydrate-rich foods in terms of their potential to increase blood glucose levels). Given that insulin resistance and compensatory hyperinsulinemia are key features of PCOS, following a low-GI diet is highly recommended.[79] In PCOS, a high-GI diet is associated with a less favorable anthropometric and metabolic profile.[9] A 12-week high-protein, low-glycemic-load hypocaloric diet (30% of daily energy from protein plus low-glycemic-load foods) resulted in weight loss, reduced androgen levels, increased insulin sensitivity, and decreased inflammatory markers in overweight and obese women with PCOS (n = 60).[80] Another small study showed that a 12-week isocaloric low-GI diet (participants maintained their usual energy and macronutrient intake) improved measures of insulin sensitivity and nonesterified fatty acid in 21 women with PCOS.[81]

Dietary Approaches to Stop Hypertension Diet

The Dietary Approaches to Stop Hypertension (DASH) diet reduces weight and improves the lipid profile in patients with PCOS, and it reduces serum insulin.[82] A study randomly assigned women with PCOS (n = 60) to a control diet or the DASH diet. Both diets consisted of 50% to 55% carbohydrate, 15% to 20% protein, and 25% to 30% total fat, but the DASH diet was high in vegetables, fruits, whole grains, and low-fat dairy products and low in saturated fats, cholesterol, refined grains, and sweets. After 3 months, the DASH diet was associated with significant weight loss and a significant reduction in serum androstenedione, with an increase in antioxidant status and SHBG.[83]

Mediterranean Diet

A Mediterranean-inspired low-GI anti-inflammatory diet based on consumption of legumes, fish, and low-fat dairy products in a Mediterranean context resulted in significant improvements in body composition, hormones and menstrual cycle regulation, blood pressure, glucose homeostasis, dyslipidemia, CRP, serum amyloid A (surrogate measures of cardiovascular risk), and weight loss that is associated with improved fertility outcomes in overweight or obese women with PCOS (n = 100). The 12-week study involved reduced-energy, low-fat, low-saturated-fat, and moderate-to-high-fiber diets (25% proteins, 25% fat, and 50% carbohydrates) and was combined with physical exercise. Of the women in the study, 63% regained menstrual cyclicity, and there was a 12% spontaneous pregnancy rate within the 12 weeks.[84]

Dietary Exclusions

Advanced Glycation End-Products. Advanced glycation end-products (AGEs) are proinflammatory molecules that trigger a state of intracellular oxidative stress and inflammation after binding to their cell membrane receptors (RAGE). Women with PCOS have elevated serum AGEs and an upregulation of RAGE in their ovaries, indicating systemic chronic inflammation at the ovarian level. High AGEs may represent a risk factor for ovulatory dysfunction, metabolic syndrome, type 2 diabetes, and cardiovascular disease[85] and may contribute to the etiology of PCOS and infertility. AGEs accumulate in the ovaries of women with PCOS, potentially contributing to the well-documented abnormal steroidogenesis and folliculogenesis.[86]

AGEs can be formed either endogenously or exogenously. Endogenously, advanced glycation takes place in all cell types via the Maillard reaction between reducing sugars and amino residues present in proteins, lipids, and DNA, resulting in loss of protein structure and function followed in some instances by cellular apoptosis.

Exogenous AGEs can be introduced into the circulation together with nutrients processed by common methods such as dry heat or other food-processing methods, for example, ionization.

About 10% of AGEs contained in a meal could be absorbed into the circulation, of which two thirds remain in the body for 72 hours, long enough to promote oxidative stress and cause tissue injury.[85]

Another exogenous source of AGEs is smoking. Both the aqueous extracts of tobacco and cigarette smoke contain glycotoxins—highly reactive glycation products that can rapidly induce AGE formation on proteins. Smoking has been shown to worsen the already elevated risk for metabolic syndrome in women with PCOS.[85]

Animal-derived foods are the major source of AGEs, and diets containing high protein and fat have higher amounts of AGEs in comparison to a carbohydrate-rich diet. Cooking methods also

affect dietary AGE content—food prepared at low temperatures with high moisture and brief heating times have less AGEs. The use of acidic marinades such as lemon juice and vinegar during cooking significantly reduces the AGE content in the diet. The highest level of AGEs per gram of food is present in dry-heat-processed foods (e.g., chips, crackers, and cookies) due to the presence of oil, butter, cheese, nuts, and eggs as ingredients in these foods. Dry-heat processing also accelerates dietary AGE formation in lean red meats and poultry due to the presence of reactive amino-lipids and reducing sugars (fructose as well as glucose-6-phosphate). Fruits, vegetables, low-fat milk, grains, and legumes have the lowest dietary AGE content.[87–89]

Almost two thirds of women with PCOS are predisposed to developing IR and, ultimately, diabetes, both of which are typically exacerbated by obesity. AGEs have been implicated in the development of IR in PCOS.[85]

Diet-induced elevation of AGE levels and the corresponding increased oxidative stress may directly induce insulin-signaling defects, leading to the development of IR in PCOS. Increased AGEs are also associated with obesity in PCOS.[85–89]

Changes in dietary AGEs parallel changes in insulin sensitivity, oxidative stress, and hormonal status in women with PCOS, and therefore lowering the concentration of AGEs in food may improve these variables.[85]

Caffeine. Women with PCOS need to keep their caffeine intake to a minimum because caffeine affects ovulation and corpus luteum function through changing hormone levels.[90] This is especially relevant to women with PCOS hoping to conceive; most studies have shown that caffeine intake is a risk factor for impaired fertility.[91,92]

Saturated Fats and Deep-Fried Foods. Women with PCOS are at risk of metabolic syndrome and cardiovascular disease and need to keep saturated fat and deep-fried foods to a minimum. These foods have been linked directly to increased risk of cardiovascular disease and metabolic syndrome. Typically, these foods are inflammatory, higher in AGEs, and also deficient in nutrients.

Sugar. Sugar in all its forms needs to be minimized in the patient with PCOS. Sugar contributes to elevated blood sugar and inflammation, contributing to symptomatology. Sugar-containing foods include fruit, which should be kept to a maximum of two pieces daily. The fruit chosen should be low GI (i.e., a cup of berries instead of a cup of pineapple). If sugar is consumed, a protein component should also be included to ensure stable blood sugar levels.

Refined Carbohydrates. Like sugar, refined carbohydrates such as white bread, candies, and pasta (which are ultimately broken down to sugar) aggravate PCOS by causing elevated levels of glucose, insulin, and inflammation. A high-GI diet (i.e., one high in refined carbohydrates and sugars) has been found to worsen postprandial insulin resistance.[93]

Nutritional Supplements

Antioxidants

PCOS is associated with oxidative stress in which increased production of free radicals is followed by decreased serum total antioxidant levels. Free-radical levels have been shown to be elevated in PCOS, resulting in a reduction in nitric oxide (NO) availability, which could play a role in the pathogenesis of PCOS via its effect on insulin resistance and endothelial dysfunction. N-acetylcysteine (NAC) is a thiol-containing antioxidant that has multiple actions, including increasing intracellular glutathione levels, lowering inflammatory markers, and increasing insulin sensitivity in women with PCOS.[94,95]

A small 6-month preliminary study ($n = 8$) showed that treatment with a combination of NAC (1200 mg/day) combined with L-arginine (1600 mg/day) showed a potential to restore gonadal function and improve insulin sensitivity in women PCOS.[96] In women with PCOS undergoing IVF treatment ($n = 60$), 6-week supplementation with NAC (1800 mg/day) has been shown to ameliorate oocyte and embryo quality, representing an alternative to metformin prescription. In this study, the treatment group was compared with a group receiving metformin only (1500 mg/day), a group receiving metformin and NAC (at the same doses), and a placebo group.[97] Studies using NAC as adjuvant therapy to clomiphene citrate showed improved ovulation and pregnancy rates, with no adverse effects or ovarian hyperstimulation.[98,99]

In PCOS, serum total antioxidant status, which combines the concentrations of individual antioxidants, such as vitamins C and E, β-carotene, and thiol groups, is decreased.[94] Sources of oxidative stress in PCOS include the following:

- Adipose tissue—adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines that are potent stimulators of the production of reactive oxygen and nitrogen species.
- Fatty acid oxidation
- Hyperglycemia—induces overproduction of ROS
- NO—endothelial NO release is impaired in insulin-resistant states such as PCOS.
- Mitochondrial dysfunction—mitochondria are the site where the largest amounts of ROS are generated.

It has been shown that even lean women with PCOS exhibit increased oxidative stress.[100,101]

A recent Cochrane review found that in women with PCOS, antioxidant treatment is associated with an increased live birth rate compared with placebo or no treatment.[102]

B Vitamins (Especially Folate and Vitamin B_{12})

Thiamine (B1), via its role in the enzyme system thiamine pyrophosphate, is required for almost every cellular reaction in the body; hence, any deficiency will affect the whole body. Thiamine plays a key role in the metabolism of carbohydrates and fats[103]; thus, it may be useful to improve insulin sensitivity. Patients with a history of oral contraceptive use are at risk of thiamine deficiency, so supplementation may be required to restore levels. Increased glucose metabolism results in increased requirements of thiamine.[104] Thiamine deficiency also leads to impaired glucose tolerance.

Riboflavin (B_2) also plays a key role in metabolism via its role in the flavin adenine dinucleotide/flavin mononucleotide system, which is required for normal carbohydrate metabolism. Vitamin B_3 is required for a multitude of functions within the body and has been used successfully to treat symptoms synonymous with PCOS, including elevated low-density lipoprotein (LDL) cholesterol, triglycerides, and lipoprotein (a). Vitamin B_5 is required for the breakdown of fats and carbohydrates via its function in coenzyme A. Pyridoxine (B_6) has been shown to reduce elevated homocysteine associated with metformin therapy[105] and additionally is recommended to stabilize hormone cascades.

The one-carbon (1-C) pathway plays an essential role in epigenetics, in which methyl groups derived from nutrients such as B vitamins are used for DNA synthesis and phospholipid and protein biosynthesis. Previous studies have shown that dietary patterns rich in these B vitamins are associated with increased chances of pregnancy and decreased chances of adverse pregnancy outcome, confirming the involvement of the 1-C pathway.[9]

All the B vitamins are essential for healthy function because they are required as a cofactor in numerous enzyme pathways, for metabolic functions as well as for energy production. They are especially

important to assist with the breakdown of carbohydrates to energy, to help support the stress response, and to reduce elevated homocysteine levels in women with PCOS taking metformin.[105]

Supplementation with folic acid has been shown to reduce elevated levels of homocysteine in patients with PCOS.[106] Homocysteine levels are significantly elevated in women with PCOS. Levels are particularly elevated in women with PCOS with insulin resistance, and to a lesser degree in women without insulin resistance.[107] Because homocysteine is a marker for cardiovascular disease, homocysteine should be screened for in all patients with PCOS. Supplementation with 5 mg folate/day for 8 weeks in overweight or obese women with PCOS reduced plasma homocysteine, intervention to quantify glucose metabolism and lipid concentrations. Folate supplementation (5 mg), compared with folate (1 mg) and placebo, resulted in reduced plasma homocysteine, homeostatic model assessment of insulin resistance (HOMA-IR) score, and serum CRP levels compared with placebo and treatment with 1 mg folate/day. There was also an increase in total antioxidant capacity and glutathione levels and a beneficial effect on lipid profiles.[108] In contract, a meta-analysis on the effect of folate supplementation on lipid profiles in metabolic disease found no effect on blood pressure or lipid profiles.[109]

Vitamin B_{12} (2000 microgram/day) has been shown to reduce elevated homocysteine associated with metformin therapy.[105]

Chromium

Chromium is an essential element in glucose and insulin homeostasis. Several studies have investigated the effect of chromium supplementation in PCOS, with varying results. An earlier study found that chromium picolinate (1000 μg/day for 2 months) improved glucose disposal in 5 women with PCOS.[110] A more recent 6-month randomized controlled trial of 85 women showed that supplementation with 1000 μg of chromium picolinate daily (in 5 divided doses of 200 μg each) resulted in a significant reduction in BMI and fasting serum insulin. After 5 months, menstrual cycles were more regulated, and there was an increased chance of ovulation in the intervention group.[111] Another 6-month study of chromium picolinate supplementation (1000 μg/day) found that supplementation resulted in a significant reduction in mean ovarian volume, total follicular count, and free testosterone in 35 adolescent girls with PCOS.[112] A randomized, double-blind, placebo-controlled trial of 60 women with PCOS showed that supplementation with 200 μg chromium picolinate per day for 8 weeks had beneficial effects on acne, hirsutism, high sensitivity C-reactive protein (hs-CRP), total antioxidant capacity, and malondialdehyde (MDA) levels compared with placebo. Supplementation did not affect endocrine profiles, nitric oxide, or glutathione levels.[113] A meta-analysis of the literature to date concluded that chromium picolinate supplementation has a significant effect on fasting insulin, free testosterone, and BMI in patients with PCOS.[114] In a study comparing chromium picolinate supplementation (200 μg/day) to metformin (1500 mg/day) over 3 months in women with clomiphene citrate-resistant PCOS ($n = 92$), it was found that the chromium supplementation improved glucose and insulin parameters, with effects comparable to those of metformin. However, metformin treatment decreased hyperandrogenism, unlike chromium picolinate. Chromium picolinate was better tolerated than metformin.[115]

Essential Fatty Acids

Essential fatty acids have shown improved insulin sensitivity and anti-inflammatory properties. Nonalcoholic fatty liver disease has been linked to PCOS, but supplementation with omega-3 fatty acids has been shown to have a beneficial effect on liver fat content and other cardiovascular risk factors in women with PCOS. Supplementation with omega-3 fatty acids (4 g/day of omega-3 fatty acids in the form of 4 × 1000 mg capsules of 56% docosapentaenoic acid [DPA] and 27% eicosapentaenoic acid [EPA]) has been shown to decrease liver fat, triglycerides, and blood pressure in women with PCOS over an 8-week period.[116] Studies have produced mixed results, however, with regard to EFAs and reproductive or metabolic abnormalities in PCOS.[117] A study found that PUFAs modulated hormonal and lipid profiles, and supplementation with long-chain omega-3 PUFAs improved androgenic profiles by reducing plasma bioavailable testosterone concentrations in women with PCOS.[118] However, a 2016 meta-analysis of the antiadrenergic effect of EFAs in PCOS concluded that the results of omega-3 PUFA supplementation studies are inconsistent, and supplementation does not significantly affect the adrenergic profile of women with PCOS. Some studies did show reduced DHEAS levels after supplementation.

There is evidence that omega-2 PUFA supplementation provides beneficial effects on cardio-metabolic risk factors in PCOS, including insulin resistance, dyslipidemia, inflammation, and obesity. In a 6-month study of 88 women with PCOS, omega-3 PUFA supplementation (360 mg EPA and 240 mg DHA per day) was found to improve lipid profile and waist circumference compared with placebo, but there were no changes in hormonal profile.[119] There is some inconsistent evidence that omega-3 PUFA supplementation may elevate fasting blood glucose concentration.[120] A study in 61 women with PCOS found that supplementation with 4 g EFA (equating to 720 mg EPA and 480 mg DHA) per day for 8 weeks resulted in improved insulin sensitivity.[121] However, a 2017 meta-analysis into the effect of omega-3 supplementation on insulin resistance in PCOS concluded that there was no significant effect of omega-3 supplementation compared with placebo.[122] Two recent studies looked at the combined effect of omega-3 and vitamin E supplementation in PCOS. One study found that omega-3 (1000 mg, EPA/DHA ratio not given) and vitamin E (400 IU) per day for 12 weeks improved parameters of mental health (depression, anxiety, and stress) and some parameters of insulin and inflammation (downregulation of PPAR-γ, interleukin [IL]-8, and TNF-α) but had no effect on GLUT-1, IL-6 and TGF-β.[123] Similarly, supplementation with 100 mg flaxseed oil (containing 400 mg α-lipoic acid) combined with 400 IU vitamin E for 12 weeks, resulted in improvements in gene expression of lipoproteins, lipid profiles, and markers of oxidative stress in 68 women with PCOS taking metformin.[124]

Two trials have found a significant effect for total cholesterol in women with PCOS receiving omega-3 fish oils compared with placebo controls. No evidence of an effect was found for free testosterone or SHBG, waist-to-hip ratio, or inflammation.[122,125] Findings were mixed for some secondary outcomes, including triglycerides, high-density lipoprotein (HDL) and LDL, BMI, fasting glucose, and insulin resistance. One trial received treatment of 3.5 g (EPA 258 mg and DHA 242 mg) for 6 weeks,[126] which was less than the other trial, where women received 4 g (EPA 720 mg and DHA 480 mg) over 8 weeks. Significant effects were found for morbidly obese women taking the higher dose of fish oils for fasting glucose, insulin resistance (HOMA-IR), and reduced LDLs.[125]

A small trial of 1500-mg omega-3 supplementation per day for 6 months showed beneficial effects on hirsutism and insulin resistance in nonobese women with PCOS. Serum LH and testosterone levels decreased, and SHBG levels increased significantly in the trial.[127]

Omega-3 fatty acid supplementation from flaxseed oil (2000 mg/day for 12 weeks) resulted in significantly improved gene expression related to insulin and lipid metabolism in women with PCOS ($n = 60$). Beneficial effects were seen in insulin metabolism, hirsutism, serum triglycerides, very low-density lipoprotein (VLDL) cholesterol, and hs-CRP levels.[128–130]

Magnesium

Magnesium, one of the abundant cations in the human body, participates in enzymatic reactions and insulin secretion, and reduced levels have been reported in women with high levels of testosterone or insulin resistance, such as diabetes mellitus type 2 and other metabolic diseases.[45,131] In PCOS, low serum and body magnesium levels have been found, irrespective of steroid hormone concentrations, compared with that of controls.[132] In contrast to this, an observational study of 1137 women found no significant differences in the levels of serum magnesium between PCOS and non-PCOS women.[45] Given the evidence, magnesium replacement seems to be effective in the adjustment and improvement of insulin resistance and should be considered in managing PCOS.

Alpha-Lipoic Acid

Alpha-lipoic acid is a powerful antioxidant that has been shown to improve glycemic control and insulin sensitivity,[133] indicating a beneficial role for it in PCOS. A 4-month study of lean, nondiabetic women with PCOS found that daily supplementation with alpha-lipoic acid (600 mg twice daily) improved insulin sensitivity by 13.5% and lowered triglycerides.[134] An increase in the number of menstrual cycles was observed in patients who were not taking the oral contraceptive pill.

Vitamin E

Oxidative stress and depletion of antioxidants have been suggested to play a role in the pathogenesis of PCOS by contributing to ovarian mesenchymal hyperplasia. Vitamin E via its antioxidant properties has been observed in experimental studies to inhibit these free pro-oxidant constituents, counteracting this growth and highlighting the protective role of vitamin E in PCOS.[135]

Zinc

Zinc contributes to the progress of insulin phosphorylation by increasing antioxidant capacity, which affects insulin synthesis and secretion.[136] In one study, zinc supplementation (30 mg/day for 1 month) in obese individuals with insulin resistance showed improvements in insulin sensitivity,[137] and a higher intake of zinc has been associated with a lower risk of type 2 diabetes.[138] There are conflicting data on serum zinc levels in women with PCOS. Some studies have found that levels are significantly lower in women with PCOS compared with women without PCOS,[136,139] whereas other studies have found no significant difference in levels.[45,47] An observational study of 1137 women found no significant differences in the levels of serum zinc between the two groups, and one study found that zinc levels were significantly higher in those with PCOS.[140] Despite this uncertainty, a clinical trial has shown benefits from zinc supplementation in PCOS. In the randomized, double-blind, placebo-controlled trial of 52 women with PCOS, supplementation with 220 mg zinc sulfate (50 mg elemental zinc) per day over 8 weeks resulted in beneficial effects on metabolic profiles compared with placebo. The women in the treatment group had reduced fasting plasma glucose and serum insulin levels and improved insulin resistance parameters. Secondary outcomes in the treatment group included significant reductions in serum triglyceride levels and VLDL cholesterol concentrations.[141] Given zinc's role in insulin sensitivity, it is likely to be of benefit in managing PCOS. Zinc supplementation may also be useful for managing concurrent PCOS symptoms, such as acne.

Vitamin D

Vitamin D plays important roles in metabolic pathways affected by PCOS, including calcium homeostasis, the insulin pathway, and sex hormone synthesis. The vitamin D receptor (VDR) is expressed throughout the female reproductive tract. VDR polymorphisms (particularly TaqI and Cdx2) are associated with the severity of disease presentation (but not to the same extent as serum vitamin D levels), but not with increased risk of PCOS.[142–144]

There is some evidence to suggest that vitamin D supplementation might be beneficial in PCOS for improving follicular development and regulation of the menstrual cycle; however, the results are inconclusive, and further rigorous trials are needed.[145] In women with PCOS who were vitamin D deficient, 50,000 IU vitamin supplementation once weekly over 8 weeks was found to significantly decrease serum vascular endothelial growth factor (VEGF) compared with placebo, which is another beneficial mechanism for improving PCOS outcomes by reducing the risk of ovarian hyperstimulation syndrome (OHSS).[146]

A systematic review of the data found that vitamin D supplementation significantly reduced total testosterone levels in women with PCOS; however, no effect was found on serum free testosterone or SHBG levels.[147]

One study showed that 8 weeks of vitamin D_3 supplementation increased circulating soluble receptors for AGEs, which bind circulating AGEs and reduce the inflammatory response, exerting a protective effect in PCOS.[148]

Vitamin D deficiency is common in women with PCOS; some estimates indicate that 70% to 85% of women with PCOS may be vitamin D deficient, which may contribute to insulin resistance, ovulatory and menstrual irregularities, lower pregnancy success, hirsutism, hyperandrogenism, obesity, and elevated cardiovascular disease risk factors.[117,149] A meta-analysis found that vitamin D deficiency was more common in obese patients with PCOS than in nonobese women with PCOS, concluding that the risk of vitamin D deficiency is associated with comorbidities of PCOS and not with PCOS per se.[150] Vitamin D deficiency may play a role in exacerbating PCOS, indicating a role for supplementation in the management of the condition. Higher vitamin D levels are associated with an increased likelihood of successful pregnancy and may be of particular benefit to women with PCOS in lowering hyperandrogenism.[149]

Regardless of deficiency, women with PCOS have been found to have markedly lower vitamin D levels than healthy control patients.[151] In a study of 60 women with PCOS, divided into three groups by vitamin D status, insulin resistance was greatest in the patients with the most severe vitamin D deficiency.[152]

Studies have found that 8 weeks of supplementation with vitamin D (50,000 IU/day) and calcium (1000 mg/day) together had beneficial effects on inflammatory markers, total antioxidant capacity, serum insulin levels, insulin resistance markers, insulin sensitivity markers, triglyceride levels, and VLDL-cholesterol levels in overweight vitamin D–deficient women with PCOS. The results were significantly greater than treatment with either vitamin D or calcium alone.[153,154]

In infertile women with PCOS who were vitamin D deficient (n = 100), it was found that treatment with metformin (1500 mg/day) combined with calcium (1000 mg/day) and vitamin D (100,000 IU per month) supplementation over 6 months resulted in improvements in follicle maturation, menstrual regularity, and hyperandrogenism in the treatment group compared with metformin alone.[155]

In another study, the effects of supplementation with vitamin D (200 IU), calcium (500 mg), and vitamin K (90 μg) together, twice a day for 8 weeks, resulted in a significant reduction in serum free testosterone and DHEAS levels in women with PCOS who were vitamin D deficient (n = 60). There was also a significant increase in total antioxidant capacity and plasma malondialdehyde (MDA) concentrations in the treatment group compared with placebo.[156]

A randomized trial in infertile women with PCOS undergoing intracytoplasmic sperm injection (ISCI) treatment (n = 105) found that supplementation with vitamin E (400 mg/day) and vitamin D_3

(50,000 IU fortnightly) over 8 weeks resulted in significant increases in pregnancy, clinical pregnancy, and implantation rates compared with placebo. The study concluded that this result was not due to improved antioxidant status, but the mechanistic pathway was unknown.[157]

Low levels of 25(OH) vitamin D have been associated with increased levels of autoimmune thyroid disease in PCOS.[158] Although the research is not conclusive regarding the role of vitamin D in PCOS, at a minimum, identifying and correcting vitamin D deficiency is recommended.

Inositol

Inositol, particularly the myo-inositol (MI) and D-chiro-inositol (CDI) isomers, is a mediator of insulin action. In the liver, MI promotes glucose uptake, and DCI promotes glycogen synthesis. In reproductive tissue such as the ovary, MI regulates glucose uptake and FSH signaling, whereas DCI is devoted to the insulin-mediated androgen production.[159] MI belongs to the vitamin B complex (it is also known as vitamin B_8) and is found in various foods. The highest concentrations are found in fresh fruits and vegetables, beans, grains (particularly oats and bran), nuts (particularly almonds, walnuts, and brazil nuts), and seeds.[160] MI can also be produced endogenously from glucose. There is some evidence to suggest that MI may help to regulate hormones (LH surge), menstrual cycles, ovulation, androgen levels, and hirsutism in women with PCOS via improved insulin resistance,[117,161] whereas DCI seems to have a positive effect on hyperandrogenism, although studies in DCI have produced mixed results. Inositols are thought to be therapeutic for PCOS because they act as an insulin-sensitizing agent and free-radical scavenger, helping to regulate metabolism and promote ovulation.[159] A 2016 systematic review concluded that MI provided a positive effect on the reproductive axis in PCOS, by improving insulin sensitivity, restoring ovulation, improving oocyte quality, and reducing hyperandrogenism.[162] However, according to a 2017 meta-analysis of the literature, MI supplementation is not able to improve the oocyte or embryo quality and pregnancy rates in women with PCOS who are undergoing ICSI treatment.[163]

A 40:1 ratio of MI:DCI is recommended to improve ovulation and embryo quality.[161] Supplementation of MI alone (2–4 g/d) is optimal to reduce clinical hyperandrogenism and dyslipidemia through the reduction of insulin plasma levels, with few side effects.[164]

A recent 120-week trial conducted on 60 women with PCOS found that supplementation with MI (2 g twice per day with 200 µg folic acid) was more effective in the reduction of testosterone, hirsutism, serum hs-CPR levels, and gene expression of IL-1 compared with metformin (500 mg three times daily). No difference was found in other hormonal profiles between the two treatment groups.[165]

Selenium

Selenium regulates the inflammatory response, proliferation, and differentiation of several immune cells. Moreover, serum selenium levels are related to a decrease in ROS production; therefore, selenium seems to have a protective role against disorders associated with oxidative stress.[48] Selenium levels have been observed to be lower in women with PCOS compared with healthy women, and a negative correlation between testosterone level and serum selenium has been reported.[166] In an 8-week randomized, double-blind, placebo-controlled trial consisting of 64 women with PCOS, women were assigned to either the intervention group ($n = 32$), which received 200 µg selenium daily, or the control group ($n = 32$), which received a placebo. The results showed that the pregnancy rate was higher in the intervention group, and alopecia and acne were decreased compared with the control group. The selenium group also showed decreased DHEA and CRP levels and decreased hirsutism.[167] Recently, two interventional, randomized, double-blind, placebo-controlled studies aimed to investigate the effect of selenium supplementation on glucose homeostasis parameters in patients with PCOS. After 8 weeks of selenium supplementation (200 µg/day), a significant decrease in serum insulin levels was observed in the treatment group compared with placebo.[168] However, a second 12-week trial with the same dose of selenium did not confirm any beneficial effect of selenium supplementation in patients with PCOS. On the contrary, it resulted in worsening of insulin resistance.[169] Further studies are needed to clarify these contradictory results.

Carnitine

Carnitine plays a substantial role in weight loss, glucose tolerance, insulin function, and fatty-acid metabolism. Potential mechanisms of action include increasing mitochondrial efflux of excess acyl groups from insulin-responsive tissues and facilitating transportation of the long-chain free fatty acids into the mitochondrial matrix.[170] Some studies have reported that circulating levels of free and total L-carnitine were significantly lower in women with PCOS.[171,172] In a recent prospective, randomized, double-blind, placebo-controlled trial of 60 overweight women with PCOS, supplementation with carnitine (250 mg/day) for 12 weeks resulted in reductions in weight, BMI, and waist and hip circumference. There were also significant reductions in fasting plasma glucose, serum insulin levels, and HOMA-IR compared with placebo. All participants in the study were taking metformin (500 mg initially, stepped up to 1500 mg/day after 3 weeks). There were no changes to the normal diet and physical activity levels of the participants during the study.[170] This is a very promising result, and further studies are needed to confirm the findings.

Probiotics

Studies have shown that the gut microbiome of individuals with metabolic disorders, such as obesity and diabetes, differs from that of healthy individuals.[173–176] Women with PCOS have a decrease in gut microbiome diversity compared with healthy women,[177–179] and hyperandrogenism, total testosterone, and hirsutism are negatively correlated with microbiome diversity.[180] An analysis of 163 women with PCOS found that higher total testosterone levels, hirsutism, and hyperandrogenism correlate with lower biodiversity in the gut microbiome. The relative abundance of *Porphyromonas* spp., *Bacteroides coprophilus, Blautia* spp., and *Faecalibacterium prausnitzii* was consistently higher in women with PCOS, whereas *Anaerococcus* spp., *Odoribacter* spp., *Roseburia* spp., and *Ruminococcus bromii* were lower. *Porphyromonas* has been reported to increase gut permeability and dysbiosis. The four taxa that had lower abundance in PCOS are all known to synthesize short-chain fatty acids.[180] The findings of the study suggest that androgens may be an important factor in shaping the gut microbiome, and changes in the gut microbiome may influence the development and pathology of PCOS.

Further evidence of the relationship between the gut microbiome and hormonal profiles is that testosterone has been shown to be produced in the gut by *Clostridium scindens.*[181] Another study has shown that the gut microbiota is a principal regulator of circulating estrogens, and disruption in the gut microbiome results in decreased circulating estrogens levels.[182] As an aside, metformin has been shown to alter the composition of the gut microbiota.[182] Obese women with PCOS who have undergone bariatric surgery exhibit reduced symptoms of PCOS, including infertility.[183] The composition of the gut microbiome is altered following bariatric surgery, and that may be the driving factor that increases fertility and resolution of PCOS symptoms.[184] Further large clinical studies are needed to determine whether specific gut bacteria play a causative role in PCOS and will also be important in determining whether probiotics are a treatment option for PCOS.

Botanical Medicines

Actaea racemosa (Black Cohosh)

Actaea racemosa is known to reduce LH and subsequently androgens.[185] A prospective, randomized controlled study investigated the role of *A. racemosa* (Klimadynon, Bionorica) in women with PCOS. The trial involved 100 women. The intervention group ($n = 50$) received *A. racemosa* 20 mg daily for 10 days, starting on the second day of their cycle, whereas the control group received 50 mg clomiphene citrate twice daily, for 5 days starting on the second day of their cycle, for three consecutive cycles. The results showed significant positive changes in LH levels and FSH:LH ratio in the *A. racemosa* group. Progesterone levels were increased from the first treatment cycle (indicating improved ovulation), as was endometrial thickness.[186] A 2014 study of 194 patients found that administering clomiphene citrate in conjunction with *A. racemosa* improved ovulation outcomes and pregnancy rates in PCOS. All patients were administered 150 mg clomiphene per day from days 3 to 7 of their cycle. In addition, the intervention group was administered 120 mg *A. racemosa* (Klimadynon, Bionorica) per day, from day 1 until the day of the pregnancy test or the start of the next cycle. Administration of *A. racemosa* was associated with lower midcycle LH, higher serum estradiol and progesterone in the second half, significantly thicker endometrium, shorter cycles, and increased pregnancy rates.[187]

Cinnamomum spp. (Cinnamon)

Managing IR and, subsequently, the compensatory hyperinsulinemia associated with PCOS is imperative to aid the resolution of the syndrome as well as to prevent complications such as the development of type 2 diabetes. *Cinnamomum* spp. contains polyphenols found to stimulate autophosphorylation of the insulin receptor and inhibit protein tyrosine phosphatase I,[188] thereby improving insulin sensitivity. *Cinnamomum cassia* has been studied in vitro and in humans, with studies showing a positive impact on glycemic control in patients with diabetes.[189,190] A small pilot study involving women with PCOS with either oligomenorrhea or amenorrhea ($n = 15$) showed that taking *Cinnamomum* spp. extract (333 mg of cinnamon three times daily) for 8 weeks resulted in a significant reduction in fasting glucose as well as IR, thought to be mediated through an increase in glucose utilization.[188] This highlights the efficacy of *Cinnamomum* spp. for the management of IR associated with PCOS and provides a rationale for further studies using a larger sample to be conducted. Another small study of 45 women with PCOS found that *Cinnamomum* spp. supplementation (1.5 g/day for 6 months) improved the frequency of menstrual cycles compared with placebo. The study did not find any changes in measures of IR or serum androgen levels in either the intervention or placebo groups.[191] A recent double-blind, randomized, controlled trial ($n = 84$) found that supplementation with cinnamon (*Cinnamomum zeylanicum*) (1500 mg ground cinnamon bark/day) over 8 weeks improved antioxidant status and serum lipid profile in overweight or obese women with PCOS.[192]

Glycyrrhiza glabra (Liquorice)

Hyperandrogenism is characteristic of PCOS, so antiandrogen botanicals such as *Glycyrrhiza glabra* are indicated. *G. glabra* affects androgen metabolism by blocking the effects of 17β-hydroxysteroid dehydrogenase and C17,20 lyase while stimulating the effects of aromatase, reducing serum testosterone, making it useful as an adjuvant therapy of hirsutism associated with PCOS.[193] Glycyrrhizic acid (a triterpenoid saponin glycoside, a bioactive component in *G. glabra*) possesses protective activity against AGE-induced endothelial dysfunction, including antiapoptosis, anti-inflammation, and antioxidant stress, via inhibiting the RAGE/nuclear factor (NF)-kB pathway, and may be potentially useful in the management of PCOS via its anti-inflammatory and antioxidant capacity.[194]

Gymnema sylvestre (Gymnema)

There is abundant evidence of the link between PCOS and insulin resistance. *Gymnema sylvestre* is a botanical that displays blood sugar–regulating properties and is highly indicated for the management of insulin resistance. As yet, no studies have assessed the effects of *Gymnema* in PCOS. *Gymnema* has demonstrated efficacy in a number of human clinical trials in patients with diabetes, where it has been shown to reduce blood sugar; therefore, it is likely to be of help in PCOS.[195,196]

Paeonia lactiflora (Peony)

Paeonia lactiflora has been shown to positively influence low progesterone, reduce elevated androgens (testosterone), and modulate estrogen and prolactin.[185] Administration of a traditional herbal medicine called shakuyaku-kanzo-to, a decoction of *G. glabra* and *P. lactiflora,* has undergone a substantial amount of research, particularly with regard to patients with PCOS and infertility. Shakuyaku-kanzo-to was found to lower plasma testosterone levels in 18 out of 20 patients[197] and increase fertility, resulting in successful conception, as well as reducing LH:FSH ratios.[198]

Schisandra chinensis (Shisandra)

Schisandra chinensis works on both Phase I and Phase II liver metabolism, where it promotes detoxification and healthy liver function. In PCOS, liver support aids in the clearance of sex hormones from the body and prevents them from recirculating. *S. chinensis* functions as an adaptogen, which is also likely to be helpful because stress is involved in the etiology of PCOS.

Tribulus terrestris (Tribulus)

Tribulus assists the patient with PCOS by stimulating the release of FSH. In order to initiate ovulation, the body's production of FSH needs to be stimulated to reduce the raised LH:FSH ratio. Results of human and animal clinical trials support the FSH-stimulating prescription. In one study, 750 mg of active furostanol (TLSE) per day for 5 days was given to women and was shown to increase FSH and estradiol compared with baseline women. The steroidal saponins are thought to bind to and stimulate (weakly) the hypothalamic estrogen-receptor sites.[199] *Tribulus* (1000 mg hydroalcoholic extract per day for 3 months) has also been shown to lower blood glucose levels in women with type 2 diabetes ($n = 98$), which may be relevant in PCOS management because of the high rates of metabolic abnormalities that occur in PCOS.[200]

Note that *Tribulus* leaf standardized extract (TLSE) is a product obtained from the aerial parts of *Tribulus terrestris,* which contain mainly saponins of the furostanol type (not less than 45%, calculated as protodioscin). It was developed in Bulgaria from Mediterranean varieties of *Tribulus. Tribulus* concentrated extract equivalent to fucosterol saponins (protodiosci) is recommended generally in lieu of liquid preparations to obtain the sufficient doses required.

Vitex agnus castus (Chaste Tree Berry)

Vitex agnus castus is a hypothalamic–pituitary–ovarian (HPO) regulator, and because HPO imbalance is associated with PCOS, it is likely to be of benefit in managing the condition. Hyperprolactinemia and an altered response to progesterone are also associated with PCOS[201,202]; therefore, the progestogenic activity of *V. agnus castus* and its ability to regulate prolactin will further assist in the management of PCOS symptoms. Most studies on chaste berry for infertility or PCOS have

used a blend of herbs, making it difficult to determine the role of a solitary component. A 12-week trial of 70 women with PCOS found that a combined low-dose oral contraceptive and *V. agnus castus* were both equally effective in normalizing the menstrual cycle and reducing DHEAS. Neither of the treatments had any effect on testosterone or prolactin levels.[203]

Trigonella foenum-graecum L. (Fenugreek Seed)

Fenugreek is a strongly scented herb that is widely used in cooking and as a traditional medicine for diabetes in Asia, with fenugreek seeds exhibiting potential hypoglycemic and hypolipidemic effects.[190] Fenugreek has also demonstrated beneficial effects in reducing body weight and improving anthropometrical parameters.[204] An open-label nonrandomized trial of 50 women with PCOS demonstrated that administration of a novel fenugreek seed extract enriched in furostanolic saponins resulted in a significant reduction in cyst size (with 36% of participants showing complete dissolution of the cysts) and the normalization of regular menstrual cycles. The novel extract (1 g/day, enriched in 40% furostanolic saponins) was administered daily over a period of 3 months. Results showed significant increases in LH and FSH compared with baseline.[205] These results are supported by findings in a 2-month study (n = 58) in which fenugreek seed restored eumenorrhea in 55% of women with PCOS and significantly decreased the polycystic ovaries.[206] These preliminary studies show very promising results, and further large-scale trials are required.

Camellia sinesus (Green Tea)

Green tea may have an antiandrogen effect due to the actions of epigallocatechins, which inhibit the 5-alpha-reductase conversion of normal testosterone into DHT.[207] A study into the effects of green tea found a small reduction in body weight in the treatment group compared with placebo, but no differences were found in glucose or lipid metabolism.[208] The 3-month study was conducted on 34 obese women with PCOS, and the treatment consisted of boiled Lung Chen tea powder (2% wt/vol), freeze-dried and administered via capsule. Green tea has also been shown to be beneficial in weight loss and reduced waist circumference in women with central adiposity, highlighting another potential benefit in PCOS management and treatment.[209]

Origanum majorana (Marjoram)

A small randomized controlled trial into the effect of marjoram *(Origanum majorana)* tea on the hormonal profile of women with PCOS (n = 25) found that drinking marjoram tea twice daily for 1 month had a beneficial effect on the hormonal profile. Insulin sensitivity was improved, and adrenal androgen levels were reduced in the treatment group compared with placebo. Each tea bag contained 1.4 g of dried organic marjoram.[210]

Combination Botanicals

A 3-month trial (n = 122) found that an individualized lifestyle intervention (healthy food choice and exercise) with a combination of *C. verum* (2250 mg stem bark/day), *G. glabra* (2250 mg root/day), *H. perforatum* (2250 mg flowering herb/day), and *P. lactiflora* (2250 mg root/day) resulted in a 32.9% reduction in oligomenorrhea. Significant improvements were also seen in BMI, insulin, and LH; blood pressure; quality of life; depression, anxiety, and stress; and pregnancy rates in the combination treatment group compared with the control group, which was administered *T. terrestris* (equivalent to 40.5 g aerial parts, standardized to furostanol saponins 330 mg/day), administered during the follicular phase of the menstrual cycle.[211]

THERAPEUTIC APPROACH

Lifestyle Measures

Lifestyle interventions are one of the most effective management strategies for PCOS, with recommendations focusing on controlling weight, hormone balance, and insulin resistance via lifestyle modification (e.g., diet, exercise, and weight loss). Lifestyle interventions are particularly important in patients with prediabetes to delay or prevent the onset of type 2 diabetes.[212]

Weight Management

Anxiety about weight is one of the psychological symptoms of PCOS.[213] Women with PCOS, especially those with concurrent anxiety symptoms but independent of obesity, have a significantly increased risk of eating disorders, including binge-eating behaviors.[214,215] The patient's capacity to adhere to diet and exercise programs and to maintain an appropriate weight over time is paramount.[216]

Weight Loss

Weight loss should be targeted in all overweight women with PCOS through reducing dietary energy intake in the setting of adequate nutritional intake and healthy food choices, irrespective of diet composition.[217] It is important to note that weight loss is difficult to achieve in obese women with PCOS compared with individuals without PCOS.[218] Even 5% to 10% weight loss of body mass is associated with significant improvement in the clinical metabolic and hormonal markers.[219–221] Weight loss also increases the SHBG concentration, reduces the testosterone concentration and androgenic stimulation of the skin, improves menstrual function and conception rates, and reduces miscarriage rates.[222] A 2011 Cochrane review found evidence that a healthy lifestyle (healthy diet, exercise, and maintaining a healthy weight) reduces body weight and abdominal fat, reduces testosterone, and improves hair growth and insulin resistance in women with PCOS.[223] In obese women with PCOS undergoing infertility treatment, delayed treatment preceded by weight loss had improved outcomes for ovulation and live birth compared with immediate treatment without weight loss.[224]

Exercise

Physical activity provides diverse benefits in PCOS, such as improved insulin sensitivity, protection against cardiovascular disease, preservation of lean body mass, positive impact on mood, and reduced morbidity and mortality.[212,225] Studies comparing exercise as an intervention to treat PCOS typically recommend 30 to 45 min of vigorous, but not moderate, exercise three times per week.[226–228] It is necessary to tailor exercise levels to the starting BMI in order to achieve optimum effect.

Stress Management

Due to the interaction between endocrine pathways and subsequent effects of stress on HPA/HPO axes, it is imperative to recommend stress management practices. Exercise may be the best recommendation due to its dual benefit, but meditation, yoga, breathing exercises, and sufficient sleep and rest are also beneficial recommendations. Daily mindfulness breathing and diaphragm breathing exercises reduced stress, anxiety, and depression scores; lowered salivary cortisol levels; and increased Life Satisfaction and quality-of-life scores in women with PCOS.[229]

Women with PCOS who are experiencing psychological stress should be assessed for maladaptive coping, particularly the use of escape–avoidance coping. Studies of coping in women with PCOS in Turkey and Germany indicated that women used maladaptive coping

strategies that were associated with depression, anxiety, and diminished HRQL, including brooding, withdrawal, self-pity, or helplessness. Women with increased psychological stress may need encouragement to use more resourceful problem-solving and positive-reappraisal strategies. Social support appears to be an important coping mechanism. Referral to a mental health specialist may be appropriate.[230]

Diet

- Aim for a diet of 30% complex carbohydrates, 40% protein, and 30% lipids. Low-carbohydrate diets and/or high-protein diets have been found to have significant benefits for weight loss and insulin regulation in PCOS.[218]
- Avoid alcohol, caffeine, smoking, and nutritional stressors.
- Reduce intake of saturated and trans fats.
- Increase fiber intake.
- Improve weight balance and advise to stabilize weight as required.
- Reduce insulin resistance, stabilize blood sugar levels, and avoid fluctuations that compound PCOS presentation by avoiding refined carbohydrates and sugar.
- Support and prevent metabolic syndrome and associated compounding factors by supporting the lipid profile through the health of the cardiovascular system and liver.
- Choose appropriate caloric intake for weight management in lean women with PCOS or weight loss for overweight women with PCOS. Hypocaloric diets have been shown to be effective for weight loss and improvement of metabolic and hormonal abnormalities in PCOS.[218]

Nutritional Supplements

- B complex (all B vitamins) high-dose combination, preferably activated forms
  - Thiamine: 20 to 40 mg/day
  - Riboflavin: 20 to 40 mg/day
  - Niacinamide/nicotinic acid: 50 to 100 mg/day
  - Pantothenic acid: 150 to 300 mg/day
  - Pyridoxine: 20 to 60 mg/day
  - Folate as activated folate (Folinic acid) or methylated folate (L5MTHF) (depending on methylation status of patient): 500 to 1000 microgram/day
  - Cyano-cobalamin or methyl-cobalamin (depending on methylation status of the patient): 500 to 1000 microgram/day
  - Choline: 50 to 100 mg/day
  - Inositol: 50 to 100 mg/day
  - Biotin: 250 to 500 microgram/day
- Chromium picolinate: 600 to 1000 microgram/day[225]
- Essential fatty acids: 4 g/day total omega-3 fatty acids (56% DHA and 27% EPA)[116]
- Magnesium: 600 to 1000 mg/day
- R-Alpha lipoic acid: 1200 mg/day
- Vitamin E: 400 to 800 IU/day
- Zinc: 30 to 60 mg/day
- Vitamin D: 0.5 µg/200IU (RDI); up to 6000 IU/day to correct a deficiency[231]
- Myo-inositol: 2 to 4 g/day[117,164]
- Selenium: 200 micrograms per day[167]
- Carnitine: 250 mg/day[170]

Botanical Medicines

Choose one or more of the following:

A. racemosa (Black Cohosh)
- 20 mg to 120 mg/day for 10 days at start of cycle[186,187]

Cinnamomum spp. (Cinnamon)
- Dietary form is reasonable (0.25–1.0 tsp; 1 tsp = 4.75 g).[225]

G. glabra (Liquorice)
- 10 to 40 mL/week (1:1 liquid)
- 2 to 3 g/day (tablet)

G. sylvestre (Gymnema)
- 25 to 75 mL/week (1:1 liquid)
- 4 to 16 g/day (tablet)

P. lactiflora (Peony)
- 30 to 60 mL/week (1:2 liquid)
- 2.2 to 4.2 g/day (tablet)

S. chinensis (Shisandra)
- 25 to 60 mL/week (1:2 liquid)
- 3 to 5 g/day (tablet)

T. terrestris (Tribulus)
- 50 to 100 mL/week (2:1 liquid)
- Standardized extract delivering 750 mg/day active furastanol[199]

V. agnus-castus (Chaste Tree Berry)
- 20 to 40 mg/day of dried herb[225]
- 5 to 40 mL/week (1:2 liquid)
- 0.25 to 3 g/day (tablet)

T. foenum-graecum L. (Fenugreek Seed)
- 15 to 30 mL/week (1:2 liquid)
- 5 to 100 g seed powder for glycemic control in diabetes[232]

C. sinensis (Green Tea)
- Doses of extract containing 170 to 250 mg of catechins/day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Fenichel P, Rougier C, Hieronimus S, Chevalier N. Which origin for polycystic ovaries syndrome: genetic, environmental or both? *Annal D'endocrinol*. 2017;78(3):176–185.
2. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. *Hum Reproduct Update*. 2016;22(6):687–708.
3. Nayaker BS, Thomas S, Ramachandran S, Loganathan S, Sundari M, Mala K. Polycystic ovarian syndrome-associated cardiovascular complications: an overview of the association between the biochemical markers and potential strategies for their prevention and elimination. *Diabetes Metab Syndr Clin Res Rev*. 2017;11(Suppl 2):S841–S851.
4. Gui J, Wang RH. Cardiovascular autonomic dysfunction in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Reproduct Biomed Online*. 2017;35(1):113–120.
5. Wang F, Wang Z. Diagnosis, pathogenesis and management of polycystic ovary syndrome. In *Testes and Ovaries-Functional and Clinical Differences and Similarities 2017*. InTech.
6. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovarian syndrome. *Lancet*. 2007;370:685–697.
7. Kyritsi EM, Dimitriadis GK, Kyrou I, Kaltsas G, Randeva HS. PCOS remains a diagnosis of exclusion: a concise review of key endocrinopathies to exclude. *Clin Endocrinol*. 2017;86(1):1–6.
8. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reproduct*. 2016;31(12):2841–2455.
9. Huijgen NA, Louwers YV, Willemsen SP, de Vries JH, Steegers-Theunissen RP, Laven JS. Dietary patterns and the phenotype of polycystic ovary syndrome: the chance of ongoing pregnancy. *Reproduct Biomed Online*. 2017;34(6):668–676.
10. Fauser B. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81(1):19–25.
11. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med*. 2010;8(1):41.
12. Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. *Nat Rev Dis Prim*. 2016;2:16057.
13. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome-part 2. *Endocrine Pract*. 2015;21(12):1415–1426.
14. Romanowski MD, Parolin MB, Freitas AC, Piazza MJ, Basso J, Urbanetz AA. Prevalence of non-alcoholic fatty liver disease in women with polycystic ovary syndrome and its correlation with metabolic syndrome. *Arquiv Gastroenterol*. 2015;52(2):117–123.
15. Kim JJ, Kim D, Yim JY, et al. Polycystic ovary syndrome with hyperandrogenism as a risk factor for non–obese non–alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2017;45(11):1403–1412.
16. Chen MJ, Ho HN. Hepatic manifestations of women with polycystic ovary syndrome. *Best Pract Res Clin Obstetr Gynaecol*. 2016;37:119–128.
17. Glintborg D, Rubin KH, Nybo M, Abrahamsen B, Andersen M. Morbidity and medicine prescriptions in a nationwide Danish population of patients diagnosed with polycystic ovary syndrome. *Eur J Endocrinol*. 2015;172(5):627–638.
18. Li S, Zhu D, Duan H, Tan Q. The epigenomics of polycystic ovarian syndrome: from pathogenesis to clinical manifestations. *Gynecol Endocrinol*. 2016;32(12):942–946.
19. Zierau L, Gade EJ, Lindenberg S, Backer V, Thomsen SF. Coexistence of asthma and polycystic ovary syndrome: a concise review. *Respiratory Med*. 2016;119:155–159.
20. Kowalczyk K, Franik G, Kowalczyk D, Pluta D, BLUKACZ P, Madej P. Thyroid disorders in polycystic ovary syndrome. *Eur Rev Med Pharmacol Sci*. 2017;21:346–360.
21. Arduc A, Aycicek Dogan B, Bilmez S, et al. High prevalence of Hashimoto's thyroiditis in patients with polycystic ovary syndrome: does the imbalance between estradiol and progesterone play a role? *Endocrine Res*. 2015;40(4):204–210.
22. Cooney LG, Lee I, Sammel MD, Dokras A. High prevalence of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reproduct*. 2017;32(5):1075–1091.
23. Cesta CE, Månsson M, Palm C, Lichtenstein P, Iliadou AN, Landén M. Polycystic ovary syndrome and psychiatric disorders: co-morbidity and heritability in a nationwide Swedish cohort. *Psychoneuroendocrinology*. 2016;73:196–203.
24. Zangeneh FZ, Jafarabadi M, Naghizadeh MM, Abedinia N, Haghollahi F. Psychological distress in women with polycystic ovary syndrome from Imam Khomeini Hospital, Tehran. *J Reproduct Infertil*. 2012;13(2):111.
25. Deeks AA, Gibson-Helm ME, Teede HJ. Anxiety and depression in polycystic ovary syndrome: a comprehensive investigation. *Fertil Steril*. 2010;93(7):2421–2423.
26. Krishnan A, Muthusami S. Hormonal alterations in PCOS and its influence on bone metabolism. *J Endocrinol*. 2017;232(2):R99–R113.
27. Bellver J, Rodríguez-Tabernero L, Robles A, et al. Polycystic ovary syndrome throughout a woman's life. *J Ass Reproduct Genetic*. 2017:1–5.
28. Kodaman PH, Duleba AJ Statins: do they have potential in the treatment of polycystic ovary syndrome?. *Semin Reproduct Med*. 2008;26(1):127. NIH Public Access.
29. Royal College of Obstetricians and Gynaecologists. *Long-Term Consequences Of Polycystic Ovary Syndrome. Green-top Guideline No 33*. London: RCOG; 2014.
30. Huang Z, Yong EL. Ethnic differences: is there an Asian phenotype for polycystic ovarian syndrome? *Best Pract Res Clin Obstetr Gynaecol*. 2016;37:46–55.
31. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocrine Rev*. 2015;36(5):487–525.
32. Merkin SS, Phy JL, Sites CK, Yang D. Environmental determinants of polycystic ovary syndrome. *Fertil Steril*. 2016;106(1):16–24.
33. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reproduct Update*. 2010;16(4):347–363.
34. Marciniak A, Rutkowska JN, Brodowska A, Wiśniewska B, Starczewski A. Cardiovascular system diseases in patients with polycystic ovary syndrome–the role of inflammation process in this pathology and possibility of early diagnosis and prevention. *Ann Agric Environ Med*. 2016;23(4):537–541.
35. Teede HJ, Joham AE, Paul E, et al. Longitudinal weight gain in women identified with polycystic ovary syndrome: results of an observational study in young women. *Obesity*. 2013;21(8):1526–1532.
36. Moran LJ, Brinkworth GD, Norman RJ. Dietary therapy in polycystic ovary syndrome. *Semin Reproduct Med*. 2008;26(1):85–92.
37. Teede HJ, Misso ML, Deeks AA, et al. Assessment and management of polycystic ovary syndrome: summary of an evidence-based guideline. *Abstract Only Med J Australia*. 2011;195(6):65.
38. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. *Int J Molecul Sci*. 2011;12(5):3117–3132.
39. González F. Nutrient-induced inflammation in polycystic ovary syndrome: role in the development of metabolic aberration and ovarian dysfunction. *Semin Reproduct Med*. 2015;33(4):276–286.
40. Durmus U, Duran C, Ecirli S. Visceral adiposity index levels in overweight and/or obese, and non-obese patients with polycystic ovary syndrome and its relationship with metabolic and inflammatory parameters. *J Endocrinol Investig*. 2017;40(5):487–497.
41. Behboudi-Gandevani S, Ramezani Tehrani F, Rostami Dovom M, et al. Insulin resistance in obesity and polycystic ovary syndrome: systematic

review and meta-analysis of observational studies. *Gynecol Endocrinol*. 2016;32(5):343–353.
42. Mannerås-Holm L, Leonhardt H, Kullberg J, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *J Clin Endocrinol Metabol*. 2011;96(2):E304–E311.
43. Sathyapalan T, Atkin SL. Mediators of inflammation in polycystic ovary syndrome in relation to adiposity. *Mediat Inflamm*. 2010;2010:758656.
44. Carvalho LM, Ferreira CN, Sóter MO, et al. Microparticles: inflammatory and haemostatic biomarkers in polycystic ovary syndrome. *Molecul Cell Endocrinol*. 2017;443:155–162.
45. Li M, Tang Y, Lin C, Huang Q, Lei D, Hu Y. Serum macroelement and microelement concentrations in patients with polycystic ovary syndrome: a cross-sectional study. *Biol Trace Element Res*. 2017;176(1):73–80.
46. Celik C, Bastu E, Abali R, et al. The relationship between copper, homocysteine and early vascular disease in lean women with polycystic ovary syndrome. *Gynecol Endocrinol*. 2013;29(5):488–491.
47. Chakraborty P, Ghosh S, Goswami SK, Kabir SN, Chakravarty B, Jana K. Altered trace mineral milieu might play an aetiological role in the pathogenesis of polycystic ovary syndrome. *Biol Trace Element Res*. 2013;152(1):9–15.
48. Spritzer PM, Lecke SB, Fabris VC, Ziegelmann PK, Amaral L. Blood trace element concentrations in polycystic ovary syndrome: systematic review and meta-analysis. *Biol Trace Element Res*. 2017;175(2):254–262.
49. Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI, Fraser RB. Fetal growth, length of gestation, and polycystic ovaries in adult life. *Lancet*. 1997;350(9085):1131–1135.
50. Xita N, Tsatsoulis A. Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metabol*. 2006;91(5):1660–1666.
51. Moore AM, Campbell RE. Polycystic ovary syndrome: Understanding the role of the brain. *Front Neuroendocrinol*. 2017;46:1–4.
52. Goodarzi MO, Carmina E, Azziz R. DHEA, DHEAS and PCOS. *J Steroid Biochem Molecul Biol*. 2015;145:213–225.
53. ESHRE Capri Workshop Group. Health and fertility in World Health Organization group 2 anovulatory women. *Hum Reproduct Update*. 2012;18(5):586.
54. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine Rev*. 2009;30(4):293–342.
55. Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. *Fertil Steril*. 2016;106(4):948–958.
56. Hewlett M, Chow E, Aschengrau A, Mahalingaiah S. Prenatal exposure to endocrine disruptors: a developmental etiology for polycystic ovary syndrome. *Reproduct Sci*. 2017;24(1):19–27.
57. Rutkowska AZ, Diamanti-Kandarakis E. Polycystic ovary syndrome and environmental toxins. *Fertil Steril*. 2016;106(4):948–958.
58. Du Q, Yang S, Wang YJ, Wu B, Zhao YY, Fan B. Effects of thiazolidinediones on polycystic ovary syndrome: a meta-analysis of randomized placebo-controlled trials. *Adv Ther*. 2012;29(9):763–774.
59. Reddy TV, Govatati S, Deenadayal M, Shivaji S, Bhanoori M. Polymorphisms in the TFAM and PGC1-α genes and their association with polycystic ovary syndrome among South Indian women. *Gene*. 2018;641:129–136.
60. Liu AL, Xie HJ, Xie HY, et al. Association between fat mass and obesity associated (FTO) gene rs9939609 A/T polymorphism and polycystic ovary syndrome: a systematic review and meta-analysis. *BMC Med Genetic*. 2017;18(1):89.
61. van der Spuy ZM, Dyer SJ. The pathogenesis of infertility and early pregnancy loss in polycystic ovarian syndrome. *Best Pract Res Clin Obstetr Gynaecol*. 2004;18(5):755–771.
62. Homberg R, Amsterdam A. Polycystic ovary syndrome- loss of the apoptotic mechanism in the ovarian follicles? *J Clin Endocrinol Metabol*. 1998;21:552–557.
63. Hsu MI. Clinical characteristics in Taiwanese women with polycystic ovary syndrome. *Clin Exp Reproduct Med*. 2015;42(3):86–93.
64. Royal Berkshire NHS Foundation Trust. *Polycystic Ovary Syndrome (PCOS)*. Reading: Royal Berkshire NHS Foundation Trust; 2017. Available from: http://www.royalberkshire.nhs.uk. Accessed 18 Jan 2020.
65. Rebar R. Evaluation of amenorrhea, anovulation, and abnormal bleeding. In: De Groot LJ, Chrousos G, Dungan K, et al., eds. *Endotext*. South Dartmouth: MA; 2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279144/. Accessed 18 Jan 2020.
66. Raisbeck E. Understanding polycystic ovary syndrome. *Nurs Prescribing*. 2009;7(9):390–396.
67. Khomami MB, Tehrani FR, Hashemi S, Farahmand M, Azizi F. Of PCOS symptoms, hirsutism has the most significant impact on the quality of life of Iranian women. *PloS One*. 2015;10(4):e0123608.
68. van Zuuren EJ, Fedorowicz Z, Carter B, Pandis N. *Interventions for Hirsutism (Excluding Laser and Photoepilation Therapy Alone)*. Cochrane Library; 2015.
69. Setji TL, Brown AJ. Polycystic ovary syndrome: update on diagnosis and treatment. *Am J Med*. 2014;127(10):912–919.
70. Nitsche K, Ehrmann DA. Obstructive sleep apnea and metabolic dysfunction in polycystic ovary syndrome. *Best Pract Res Clin Endocrinol Metabol*. 2010;24(5):717–730.
71. Azziz R. Introduction: determinants of polycystic ovary syndrome. *Fertil Steril*. 2016;106(1):4–5.
72. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. *Clin Epidemiol*. 2014;6:1.
73. Nybacka Å, Hellström PM, Hirschberg AL. Increased fibre and reduced trans fatty acid intake are primary predictors of metabolic improvement in overweight polycystic ovary syndrome—substudy of randomized trial between diet, exercise and diet plus exercise for weight control. *Clin Endocrinol*. 2017.
74. Frary JM, Bjerre KP, Glintborg D, Ravn P. The effect of dietary carbohydrates in women with polycystic ovary syndrome. *Min Endocrinol*. 2016;41(1):57–69.
75. Moran LJ, Teede HJ, Noakes M, Clifton PM, Norman RJ, Wittert GA. Sex hormone binding globulin, but not testosterone, is associated with the metabolic syndrome in overweight and obese women with polycystic ovary syndrome. *J Endocrinol Invest*. 2013;36(11):1004–1010.
76. Chiofalo B, Laganà AS, Palmara V, et al. Fasting as possible complementary approach for polycystic ovary syndrome: hope or hype? *Med Hypotheses*. 2017;105:1–3.
77. Riley JK, Jungheim ES. Is there a role for diet in ameliorating the reproductive sequelae associated with chronic low-grade inflammation in polycystic ovary syndrome and obesity? *Fertil Steril*. 2016;106(3):520–527.
78. Phy JL, Pohlmeier AM, Cooper JA, et al. Low starch/low dairy diet results in successful treatment of obesity and co-morbidities linked to polycystic ovary syndrome (PCOS). *J Obes Weight Loss Ther*. 2015;5(2):259.
79. Marsh K, Brand-Miller J. The optimal diet for women with polycystic ovary syndrome? *Br J Nutri*. 2005;94:154–165.
80. Mehrabani HH, Salehpour S, Amiri Z, Farahani SJ, Meyer BJ, Tahbaz F. Beneficial effects of a high-protein, low-glycemic-load hypocaloric diet in overweight and obese women with polycystic ovary syndrome: a randomized controlled intervention study. *J Am Coll Nutri*. 2012;31(2):117–125.
81. Barr S, Reeves S, Sharp K, Jeanes YM. An isocaloric low glycemic index diet improves insulin sensitivity in women with polycystic ovary syndrome. *J Acad Nutri Dietetic*. 2013;113(11):1523–1531.
82. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized clinical trial. *Nutrition*. 2014;30(11):1287–1293.
83. Azadi–Yazdi M, Karimi–Zarchi M, Salehi–Abargouei A, Fallahzadeh H, Nadjarzadeh A. Effects of Dietary Approach to Stop Hypertension diet on androgens, antioxidant status and body composition in overweight and obese women with polycystic ovary syndrome: a randomised controlled trial. *J Hum Nutri Dietetic*. 2017;30(3):275–283.

84. Salama AA, Amine EK, Salem HA, El Fattah NK. Anti-inflammatory dietary combo in overweight and obese women with polycystic ovary syndrome. *North Am J Med Sci*. 2015;7(7):310.
85. Pertynska-Marczewska M, Diamanti-Kandarakis E, Zhang J, Merhi Z. Advanced glycation end products: a link between metabolic and endothelial dysfunction in polycystic ovary syndrome? *Metabol Clin Exp*. 2015;64(11):1564–1573.
86. Garg D, Merhi Z. Relationship between advanced glycation end products and steroidogenesis in PCOS. *Reproduct Biol Endocrinol*. 2016;14(1):71.
87. Bargiota A, Diamanti-Kandarakis E. The effects of old, new and emerging medicines on metabolic aberrations in PCOS. *Therapeutic Adv Endocrinol Metabol*. 2012;3(1):27–47.
88. Garg D, Merhi Z. Advanced glycation end products: link between diet and ovulatory dysfunction in PCOS? *Nutrients*. 2015;7(12):10129–10144.
89. Palimeri S, Palioura E, Diamanti-Kandarakis E. Current perspectives on the health risks associated with the consumption of advanced glycation end products: recommendations for dietary management. *Diabetes Metabol Synd Obes Targets Ther*. 2015;8:415.
90. Klonoff-Cohen H, Bleha J, Lam-Kruglick P. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer. *Hum Reproduct*. 2002;17(7):1746–1754.
91. Stanton CK, Gray RH. Effects of caffeine consumption on delayed conception. *Am J Epidemiol*. 1995;142(12):1322–1329.
92. Wilcox A, Weinberg C, Baird D. Caffeinated beverages and decreased fertility. *Lancet*. 1988;332(8626–8627):1453–1456.
93. Brynes AE, Edwards CM, Ghatei MA, et al. A randomised four-intervention crossover study investigating the effect of carbohydrates on daytime profiles of insulin, glucose, non-esterified fatty acids and triacylglycerols in middle-aged men. *Br J Nutri*. 2003;89(2):207–218.
94. Macut D, Bjekić-Macut J, Savić-Radojević A. Dyslipidemia and oxidative stress in PCOS. *Polycystic Ovary Synd*. 2013;40:51–63.
95. Fulghesu AM, Ciampelli M, Muzj G, et al. N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. *Fertil Steril*. 2002;77(6):1128–1135.
96. Masha A, Manieri C, Dinatale S, Bruno GA, Ghigo E, Martina V. Prolonged treatment with N-acetylcysteine and L-arginine restores gonadal function in patients with polycistic ovary syndrome. *J Endocrinol Invest*. 2009;32(11):870–872.
97. Cheraghi E, Mehranjani MS, Shariatzadeh MA, Esfahani MH, Ebrahimi Z. N-Acetylcysteine improves oocyte and embryo quality in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection: an alternative to metformin. *Reproduct Fertil Develop*. 2016;28(6):723–731.
98. Rizk AY, Bedaiwy MA, Al-Inany HG. N-acetyl-cysteine is a novel adjuvant to clomiphene citrate in clomiphene citrate–resistant patients with polycystic ovary syndrome. *Fertil Steril*. 2005;83(2):367–370.
99. Salehpour S, Akbari Sene A, Saharkhiz N, Sohrabi MR, Moghimian F. N–acetylcysteine as an adjuvant to clomiphene citrate for successful induction of ovulation in infertile patients with polycystic ovary syndrome. *J Obstetr Gynaecol Res*. 2012;38(9):1182–1186.
100. Kuşçu NK, Var A. Oxidative stress but not endothelial dysfunction exists in non–obese, young group of patients with polycystic ovary syndrome. *Acta Obstetr Gynecol Scand*. 2009;88(5):612–617.
101. Macut D, Simic T, Lissounov A, et al. Insulin resistance in non-obese women with polycystic ovary syndrome: relation to byproducts of oxidative stress. *Exp Clin Endocrinol Diabetes*. 2011;119(07):451–455.
102. Showell MG, Mackenzie–Proctor R, Jordan V, Hart RJ. *Antioxidants for Female Subfertility*. Cochrane Library; 2017.
103. Huskisson E, Maggini S, Ruf M. The role of vitamins and minerals in energy metabolism and well-being. *J Int Med Res*. 2007;35(3):277–289.
104. Thornalley PJ. The potential role of thiamine (vitamin B1) in diabetic complications. *Curr Diabetes Rev*. 2005;1(3):287–298.
105. Kilicdag EB, Bagis T, Tarim E, et al. Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. *Human Reproduct*. 2005 Mar 24;20(6):1521–1528.
106. Kazerooni T, Asadi N, Dehbashi S, Zolghadri J. Effect of folic acid in women with and without insulin resistance who have hyperhomocysteinemic polycystic ovary syndrome. *Int J Gynaecol Obstet*. 2008;101(2):156–160.
107. Meng Y, Chen X, Peng Z, Liu X, Sun Y, Dai S. Association between high serum homocysteine levels and biochemical characteristics in women with polycystic ovarian syndrome: a systematic review and meta-analysis. *PloS One*. 2016;11(6):e0157389.
108. Bahmani F, Karamali M, Shakeri H, Asemi Z. The effects of folate supplementation on inflammatory factors and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled clinical trial. *Clin Endocrinol*. 2014;81(4):582–587.
109. Tabrizi R, Lankarani KB, Akbari M, et al. The effects of folate supplementation on lipid profiles among patients with metabolic diseases: a systematic review and meta-analysis of randomized controlled trials. *Diabetes Metabol Synd Clin Res Rev*. 2018;12(3):423–430.
110. Lydic ML, McNurlan M, Bembo S, Mitchell L, Komaroff E, Gelato M. Chromium picolinate improves insulin sensitivity in obese subjects with polycystic ovary syndrome. *Fertil Steril*. 2006;86(1):243–246.
111. Ashoush S, Abou–Gamrah A, Bayoumy H, Othman N. Chromium picolinate reduces insulin resistance in polycystic ovary syndrome: randomized controlled trial. *J Obstetr Gynaecol Res*. 2016;42(3):279–285.
112. Amr N, Abdel-Rahim HE. The effect of chromium supplementation on polycystic ovary syndrome in adolescents. *J Pediatr Adole Gynecol*. 2015;28(2):114–118.
113. Jamilian M, Bahmani F, Siavashani MA, Mazloomi M, Asemi Z, Esmaillzadeh A. The effects of chromium supplementation on endocrine profiles, biomarkers of inflammation, and oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Biol Trace Element Res*. 2016;172(1):72–78.
114. Fazelian S, Rouhani MH, Bank SS, Amani R. Chromium supplementation and polycystic ovary syndrome: a systematic review and meta-analysis. *J Trace Element Med Biol*. 2017;42:92–96.
115. Amooee S, Parsanezhad ME, Shirazi MR, Alborzi S, Samsami A. Metformin versus chromium picolinate in clomiphene citrate-resistant patients with PCOs: a double-blind randomized clinical trial. *Iranian J Reproduct Med*. 2013;11(8):611.
116. Cussons AJ, Watts GF, Mori TA, Stuckey BG. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. *J Clin Endocrinol Metab*. 2009;94(10):3842–3848.
117. Mansour A, Hosseini S, Larijani B, Mohajeri-Tehrani MR. Nutrients as novel therapeutic approaches for metabolic disturbances in polycystic ovary syndrome. *Excli J*. 2016:15.
118. Phelan N, O'Connor A, Kyaw Tun T, et al. Hormonal and metabolic effects of polyunsaturated fatty acids in young women with polycystic ovary syndrome: results from a cross-sectional analysis and a randomized, placebo-controlled, crossover trial–. *Am J Clin Nutri*. 2011;93(3):652–662.
119. Khani B, Mardanian F, Fesharaki SJ. Omega-3 supplementation effects on polycystic ovary syndrome symptoms and metabolic syndrome. *J Res Med Sci Off J Isfahan Univ Med Sci*. 2017;22:64.
120. Hajishafiee M, Askari G, Iranj B, et al. The effect of n-3 polyunsaturated fatty acid supplementation on androgen status in patients with polycystic ovary syndrome: a systematic review and meta-analysis of clinical trials. *Hormone Metabol Res*. 2016;48(05):281–289.
121. Rafraf M, Mohammadi E, Asghari-Jafarabadi M, Farzadi L. Omega-3 fatty acids improve glucose metabolism without effects on obesity values and serum visfatin levels in women with polycystic ovary syndrome. *J Am Coll Nutri*. 2012;31(5):361–368.
122. Sadeghi A, Djafarian K, Mohammadi H, Shab-Bidar S. Effect of omega-3 fatty acids supplementation on insulin resistance in women with polycystic ovary syndrome: meta-analysis of randomized controlled trials. *Diabetes Metabol Synd Clin Res Rev*. 2017;11(2):157–162.
123. Jamilian M, Shojaei A, Samimi M, et al. The effects of omega-3 and vitamin E co-supplementation on parameters of mental health and gene expression related to insulin and inflammation in subjects with polycystic ovary syndrome. *J Affect Disorders*. 2018;229:41–47.

124. Rahmani E, Samimi M, Ebrahimi FA, et al. The effects of omega-3 fatty acids and vitamin E co-supplementation on gene expression of lipoprotein (a) and oxidized low-density lipoprotein, lipid profiles and biomarkers of oxidative stress in patients with polycystic ovary syndrome. *Molecul Cellular Endocrinol*. 2017;439:247–255.
125. Mohammadi E, Rafraf M, Farzadi L, Asghari-Jafarabadi M, Sabour S. Effects of omega-3 fatty acids supplementation on serum adiponectin levels and some metabolic risk factors in women with polycystic ovary syndrome. *Asia Pacific J Clin Nutri*. 2012;21(4):511–518.
126. Vargas ML, Almario RU, Buchan W, Kim K, Karakas SE. Metabolic and endocrine effects of long-chain versus essential omega-3 polyunsaturated fatty acids in polycystic ovary syndrome. *Metabol Clin Exp*. 2011;60(12):1711–1718.
127. Oner G, Muderris II . Efficacy of omega-3 in the treatment of polycystic ovary syndrome. *J Obstetr Gynaecol*. 2013;33(3):289–291.
128. Nasri K, Hantoushzadeh S, Aghadavod E, Taghizadeh M, Asemi Z. The effects of omega-3 fatty acids supplementation on gene expression involved in the insulin and lipid signaling pathway in patients with polycystic ovary syndrome. *Hormone Metabol Res*. 2017;49(06):446–451.
129. Mirmasoumi G, Fazilati M, Foroozanfard F, et al. The effects of flaxseed oil omega-3 fatty acids supplementation on metabolic status of patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Exp Clin Endocrinol Diabetes*. 2018;126(4):222–228.
130. Rahmani E, Jamilian M, Dadpour B, et al. The effects of fish oil on gene expression in patients with polycystic ovary syndrome. *Eur J Clin Invest*. 2018;48(3).
131. Rumawas ME, McKeown NM, Rogers G, Meigs JB, Wilson PW, Jacques PF. Magnesium intake is related to improved insulin homeostasis in the framingham offspring cohort. *J Am Coll Nutri*. 2006;25(6):486–492.
132. Muneyyirci-Delale O, Nacharaju VL, Dalloul M, et al. Divalent cations in women with PCOS: implications for cardiovascular disease. *Gynecol Endocrinol*. 2001;15(3):198–201.
133. Rochette L, Ghibu S, Muresan A, Vergely C. Alpha-lipoic acid: molecular mechanisms and therapeutic potential in diabetes. *Can J Physiol Pharmacol*. 2015;93(12):1021–1027.
134. Masharani U, Gjerde C, Evans JL, Youngren JF, Goldfine ID. Effects of controlled-release alpha lipoic acid in lean, nondiabetic patients with polycystic ovary syndrome. *J Diabetes Sci Technol*. 2010;4(2):359–364.
135. Duleba AJ, Foyouzi N, Karaca M, Pehlivan T, Kwintkiewicz J, Behrman HR. Proliferation of ovarian theca-interstitial cells is modulated by antioxidants and oxidative stress. *Hum Reprod*. 2004;19(7):1519–1524.
136. Guler I, Himmetoglu O, Turp A, et al. Zinc and homocysteine levels in polycystic ovarian syndrome patients with insulin resistance. *Biol Trace Element Res*. 2014;158(3):297–304.
137. Marreiro DN, Geloneze B, Tambascia MA, Lerário AC, Halpern A, Cozzolino SM. Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. *Biol Trace Elem Res*. 2006;112:109–118.
138. Sun Q, van Dam RM, Willett WC, Hu FB. Prospective study of zinc intake and risk of type 2 diabetes in women. *Diabetes Care*. 2009;32(4):629–634.
139. Zheng G, Wang L, Guo Z, et al. Association of serum heavy metals and trace element concentrations with reproductive hormone levels and polycystic ovary syndrome in a Chinese population. *Biol Trace Element Res*. 2015;167(1):1–10.
140. Kurdoglu Z, Kurdoglu M, Demir H, Sahin HG. Serum trace elements and heavy metals in polycystic ovary syndrome. *Hum Exp Toxicol*. 2012;31(5):452–456.
141. Foroozanfard F, Jamilian M, Jafari Z, et al. Effects of zinc supplementation on markers of insulin resistance and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Exp Clin Endocrinol Diabetes*. 2015;123(04):215–220.
142. Zadeh-Vakili A, Tehrani FR, Daneshpour MS, Zarkesh M, Saadat N, Azizi F. Genetic polymorphism of vitamin D receptor gene affects the phenotype of PCOS. *Gene*. 2013;515(1):193–196.
143. Reis GV, Gontijo NA, Rodrigues KF, Alves MT, Ferreira CN, Gomes KB. Vitamin D receptor polymorphisms and the polycystic ovary syndrome: a systematic review. *J Obstetr Gynaecol Res*. 2017;43(3):436–446.
144. Aghadavod E, Mollaei H, Nouri M, Hamishehkar H. Evaluation of relationship between body mass index with vitamin D receptor gene expression and vitamin D levels of follicular Fluid in overweight patients with polycystic ovary syndrome. *Int J Fertil Steril*. 2017;11(2):105.
145. Fang F, Ni K, Cai Y, Shang J, Zhang X, Xiong C. Effect of vitamin D supplementation on polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. *Complement Ther Clin Pract*. 2017;26:53–60.
146. Irani M, Seifer DB, Grazi RV, Irani S, Rosenwaks Z, Tal R. Vitamin D decreases serum VEGF correlating with clinical improvement in vitamin D-deficient women with PCOS: a randomized placebo-controlled trial. *Nutrients*. 2017;9(4):334.
147. Azadi-Yazdi M, Nadjarzadeh A, Khosravi-Boroujeni H, Salehi-Abargouei A. The effect of vitamin D supplementation on the androgenic profile in patients with polycystic ovary syndrome: a systematic review and meta-analysis of clinical trials. *Hormone Metabol Res*. 2017;49(03):174–179.
148. Irani M, Minkoff H, Seifer DB, Merhi Z. Vitamin D increases serum levels of the soluble receptor for advanced glycation end products in women with PCOS. *J Clin Endocrinol Metabol*. 2014;99(5):E886–E890.
149. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clin Endocrinol*. 2012;77(3):343–350.
150. He C, Lin Z, Robb SW, Ezeamama AE. Serum vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. *Nutrients*. 2015;7(6):4555–4577.
151. Jia XZ, Wang YM, Zhang N, et al. Effect of vitamin D on clinical and biochemical parameters in polycystic ovary syndrome women: a meta–analysis. *J Obstetri Gynaecol Res*. 2015;41(11):1791–1802.
152. Patra SK, Nasrat H, Goswami B, Jain A. Vitamin D as a predictor of insulin resistance in polycystic ovarian syndrome. *Diabetes Metabol Synd Clin Res Rev*. 2012;6(3):146–149.
153. Foroozanfard F, Jamilian M, Bahmani F, et al. Calcium plus vitamin D supplementation influences biomarkers of inflammation and oxidative stress in overweight and vitamin D–deficient women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial. *Clin Endocrinol*. 2015;83(6):888–894.
154. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, Jamilian M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. *Clin Nutri*. 2015;34(4):586–592.
155. Firouzabadi RD, Aflatoonian A, Modarresi S, Sekhavat L, MohammadTaheri S. Therapeutic effects of calcium & vitamin D supplementation in women with PCOS. *Complement Ther Clin Pract*. 2012;18(2):85–88.
156. Razavi M, Jamilian M, Karamali M, Bahmani F, Aghadavod E, Asemi Z. The effects of vitamin DK-calcium co-supplementation on endocrine, inflammation, and oxidative stress biomarkers in vitamin D–deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Hormone Metabol Res*. 2016;48(07):446–451.
157. Fatemi F, Mohammadzadeh A, Sadeghi MR, et al. Role of vitamin E and D3 supplementation in Intra-Cytoplasmic Sperm Injection outcomes of women with polycystic ovarian syndrome: a double blinded randomized placebo-controlled trial. *Clin Nutri ESPEN*. 2017;18:23–30.
158. Muscogiuri G, Palomba S, Caggiano M, Tafuri D, Colao A, Orio F. Low 25 (OH) vitamin D levels are associated with autoimmune thyroid disease in polycystic ovary syndrome. *Endocrine*. 2016;53(2):538–542.
159. Nestler JE, Unfer V. Reflections on inositol (s) for PCOS therapy: steps toward success. *Endocrine J*. 2015;62(3):297–298.
160. Porcaro G, Bizzarri M, Monastra G, Filati P, Unfer V. *Strategies For The Treatment Of Polycystic Ovary Syndrome (Pcos) Women: The Role Of Myoinositol (Mi) And D-Chiro-Inositol (Dci) Between Diet And Therapy. Polycystic Ovary Syndrome (PCOS): Clinical Aspects, Potential Complications and Dietary Management*. Hauppauge, NY: Nova Science Publishers; 2016.
161. Facchinetti F, Bizzarri M, Benvenga S, et al. Results from the International consensus conference on myo-inositol and d-chiro-inositol in

Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. *Eur J Obstetric Gynecol Reproduct Biol*. 2015;195:72–76.
162. Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. Effects of inositol (s) in women with PCOS: a systematic review of randomized controlled trials. *Int J Endocrinol*. 2016;2016:1849162.
163. Mendoza N, Pérez L, Simoncini T, Genazzani A. Inositol supplementation in women with polycystic ovary syndrome undergoing intracytoplasmic sperm injection: a systematic review and meta-analysis of randomized controlled trials. *Reproduct Biomed Online*. 2017;35(5):529–535.
164. Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. *J Clin Pharmacol*. 2014;54(10):1079–1092.
165. Jamilian M, Farhat P, Foroozanfard F, et al. Comparison of myo-inositol and metformin on clinical, metabolic and genetic parameters in polycystic ovary syndrome: a randomized controlled clinical trial. *Clin Endocrinol*. 2017;87(2):194–200.
166. Coskun A, Arikan T, Kilinc M, Arikan DC, Ekerbiçer HÇ. Plasma selenium levels in Turkish women with polycystic ovary syndrome. *Eur J Obstetr Gynecol Reproduct Biol*. 2013;168(2):183–186.
167. Razavi M, Jamilian M, Kashan ZF, et al. Selenium supplementation and the effects on reproductive outcomes, biomarkers of inflammation, and oxidative stress in women with polycystic ovary syndrome. *Hormone Metabol Res*. 2016;48(03):185–190.
168. Jamilian M, Razavi M, Fakhrie Kashan Z, Ghandi Y, Bagherian T, Asemi Z. Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Clin Endocrinol*. 2015;82(6):885–891.
169. Hosseinzadeh FM, Hosseinzadeh-Attar MJ, Yekaninejad MS, Rashidi B. Effects of selenium supplementation on glucose homeostasis and free androgen index in women with polycystic ovary syndrome: a randomized, double blinded, placebo controlled clinical trial. *J Trace Element Med Biol*. 2016;34:56–61.
170. Samimi M, Jamilian M, Ebrahimi FA, Rahimi M, Tajbakhsh B, Asemi Z. Oral carnitine supplementation reduces body weight and insulin resistance in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Clin Endocrinol*. 2016;84(6):851–857.
171. Fenkci SM, Fenkci V, Oztekin O, Rota S, Karagenc N. Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. *Hum Reproduct*. 2008;23(7):1602–1606.
172. Vigerust NF, Bohov P, Bjørndal B, et al. Free carnitine and acylcarnitines in obese patients with polycystic ovary syndrome and effects of pioglitazone treatment. *Fertil Steril*. 2012;98(6):1620–1626.
173. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;444(7122):1022.
174. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457(7228):480.
175. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS One*. 2010;5(2):e9085.
176. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490(7418):55.
177. Lindheim L, Bashir M, Münzker J, et al. Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. *PloS One*. 2017;12(1):e0168390.
178. Liu R, Zhang C, Shi Y, et al. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Front Microbiol*. 2017;8:324.
179. Charalampakis V, Tahrani AA, Helmy A, Gupta JK, Singhal R. Polycystic ovary syndrome and endometrial hyperplasia: an overview of the role of bariatric surgery in female fertility. *Eur J Obstetric Gynecol Reproduct Biol*. 2016;207:220–226.
180. Torres PJ, Siakowska M, Banaszewska B, et al. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J Clin Endocrinol Metabol*. 2018;103(4):1502–1511.
181. Ridlon JM, Ikegawa S, Alves JM, et al. *Clostridium scindens:* a human gut microbe with a high potential to convert glucocorticoids into androgens. *J Lipid Res*. 2013;54(9):2437–2449.
182. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen–gut microbiome axis: physiological and clinical implications. *Maturitas*. 2017;103:45–53.
183. Butterworth J, Deguara J, Borg CM. Bariatric surgery, polycystic ovary syndrome, and infertility. *J Obesity*. 2016;2016:1871594.
184. Kaska L, Sledzinski T, Chomiczewska A, Dettlaff-Pokora A, Swierczynski J. Improved glucose metabolism following bariatric surgery is associated with increased circulating bile acid concentrations and remodeling of the gut microbiome. *World J Gastroenterol*. 2016;22(39):8698.
185. Trickey R. *Women, Hormones and the Menstrual Cycle*. 2nd ed. Crows Nest, Australia: Allen & Unwin; 2003.
186. Kamel HH. Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. *Eur J Obstetri Gynecol Reproduct Biol*. 2013;168(1):60–63.
187. Shahin AY, Mohammed SA. Adding the phytoestrogen *Cimicifugae Racemosae* to clomiphene induction cycles with timed intercourse in polycystic ovary syndrome improves cycle outcomes and pregnancy rates–a randomized trial. *Gynecol Endocrinol*. 2014;30(7):505–510.
188. Wang JG, Anderson RA, Graham 3rd GM, et al. The effect of cinnamon extract on insulin resistance parameters in polycystic ovary syndrome: a pilot study. *Fertil Steril*. 2007;88(1):240–243.
189. Akilen R, Tsiami A, Devendra D, Robinson N. Cinnamon in glycaemic control: systematic review and meta analysis. *Clin Nutri*. 2012;31(5):609–615.
190. Bi X, Lim J, Henry CJ. Spices in the management of diabetes mellitus. *Food Chem*. 2017;217:281–293.
191. Kort DH, Lobo RA. Preliminary evidence that cinnamon improves menstrual cyclicity in women with polycystic ovary syndrome: a randomized controlled trial. *Am J Obstetric Gynecol*. 2014;211(5):487.e1.
192. Borzoei A, Rafraf M, Niromanesh S, Farzadi L, Narimani F, Doostan F. Effects of cinnamon supplementation on antioxidant status and serum lipids in women with polycystic ovary syndrome. *J Traditional Complement Med*. 2018;8(1):128–133.
193. Armanini D, Mattarello MJ, Fiore C, et al. Liquorice reduces serum testosterone in healthy women. *Steroids*. 2004;69(11–12):763–766.
194. Feng L, Zhu MM, Zhang MH, et al. Protection of glycyrrhizic acid against AGEs-induced endothelial dysfunction through inhibiting RAGE/NF-κB pathway activation in human umbilical vein endothelial cells. *J Ethnopharmacol*. 2013;148(1):27–36.
195. Pothuraju R, Sharma RK, Chagalamarri J, Jangra S, Kumar Kavadi P. A systematic review of *Gymnema sylvestre* in obesity and diabetes management. *J Sci Food Agricult*. 2014;94(5):834–840.
196. Rathore PK, Arathy V, Attimarad VS, Kumar P, Roy S. In-silico analysis of gymnemagenin from Gymnema sylvestre (Retz.) R. Br. with targets related to diabetes. *J Theoretic Biol*. 2016;391:95–101.
197. Takahashi K, Yoshino K, Shirai T, Nishigaki A, Araki Y, Kitao M. Effect of a traditional herbal medicine (shakuyaku-kanzo-to) on testosterone secretion in patients with polycystic ovary syndrome detected by ultrasound. *(Abstract only) Nihon Sanka Fujinka Gakkai Zasshi*. 40(6):789–792.
198. Takahashi K, Mutiara S, Kita N, Tsuji S, Noda Y, Miyazaki K. Odd variation of 75 g oral glucose tolerance test results in a Japanese patient with polycystic ovary syndrome: a case report. *Archiv Gynecol Obstetr*. 2007;275(5):405–409.
199. Milanov S, Maleeva E, Taskov M, et al. *MBI: Medicobiologic Information*. 1985;4:27.
200. Samani NB, Jokar A, Soveid M, Heydari M, Mosavat SH. Efficacy of the hydroalcoholic extract of Tribulus terrestris on the serum glucose and lipid profile of women with diabetes mellitus: a double-blind randomized placebo-controlled clinical trial. *J Evidence Based Complement Alternat Med*. 2016;21(4):NP91–N97.
201. Bracero N, Zacur H. Polycystic ovary syndrome and hyperprolactinemia. *Obstetr Gynecol Clin North Am*. 2001;28(1):77–84.
202. Shayya R, Chang RJ. Reproductive endocrinology of adolescent polycystic ovary syndrome. *BJOG*. 2010;117(2):150–155.

203. Shahnazi M, Khalili AF, Hamdi K, Ghahremaninasab P. The effects of combined low-dose oral contraceptives and *Vitex agnus* on the improvement of clinical and Paraclinical parameters of polycystic ovarian syndrome: a triple-blind, randomized, controlled clinical trial. *Iranian Red Crescent Med J*. 2016;18(12).
204. Nagulapalli Venkata KC, Swaroop A, Bagchi D, Bishayee A. A small plant with big benefits: fenugreek (*Trigonella foenum-graecum* Linn.) for disease prevention and health promotion. *Molecul Nutri Food Res*. 2017;61(6). https://doi.org/10.1002/mnfr.201600950.
205. Swaroop A, Jaipuriar AS, Gupta SK, et al. Efficacy of a novel fenugreek seed extract (*Trigonella foenum-graecum*, FurocystTM) in polycystic ovary syndrome (PCOS). *Int J Med Sci*. 2015;12(10):825.
206. Bashtian MH, Emami SA, Mousavifar N, Esmaily HA, Mahmoudi M, Poor AH. Evaluation of Fenugreek (*Trigonella foenum-graceum* L.) effects seeds extract on insulin resistance in women with polycystic ovarian syndrome. *Iranian J Pharm Res: IJPR*. 2013;12(2):475.
207. Grant P, Ramasamy S. An update on plant derived anti-androgens. *Int J Endocrinol Metabol*. 2012;10(2):497–502.
208. Chan CC, Koo MW, Ng EH, Tang OS, Yeung WS, Ho PC. Effects of Chinese green tea on weight, and hormonal and biochemical profiles in obese patients with polycystic ovary syndrome—a randomized placebo-controlled trial. *J Soc Gynecol Invest*. 2006;13(1):63–68.
209. Chen IJ, Liu CY, Chiu JP, Hsu CH. Therapeutic effect of high-dose green tea extract on weight reduction: a randomized, double-blind, placebo-controlled clinical trial. *Clin Nutri*. 2016;35(3):592–599.
210. Haj-Husein I, Tukan S, Alkazaleh F. The effect of marjoram (Origanum majorana) tea on the hormonal profile of women with polycystic ovary syndrome: a randomised controlled pilot study. *J Hum Nutri Dietetic*. 2016;29(1):105–111.
211. Arentz S, Smith CA, Abbott J, Fahey P, Cheema BS, Bensoussan A. Combined lifestyle and herbal medicine in overweight women with polycystic ovary syndrome (PCOS): a randomized controlled trial. *Phytother Res*. 2017;31(9):1330–1340.
212. Anwar S, Shikalgar N. Prevention of type 2 diabetes mellitus in polycystic ovary syndrome: a review. *Diabet Metabol Synd Clin Res Rev*. 2017;11(Suppl 2):S913–S917.
213. Larsson I, Hulthén L, Landén M, Pålsson E, Janson P, Stener-Victorin E. Dietary intake, resting energy expenditure, and eating behavior in women with and without polycystic ovary syndrome. *Clin Nutr*. 2016;35(1):213–218.
214. Jeanes YM, Reeves S, Gibson EL, Piggott C, May VA, Hart KH. Binge eating behaviours and food cravings in women with Polycystic Ovary Syndrome. *Appetite*. 2017;109:24–32.
215. Lee I, Cooney LG, Saini S, et al. Increased risk of disordered eating in polycystic ovary syndrome. *Fertil Steril*. 2017;107(3):796–802.
216. Domecq JP, Prutsky G, Mullan RJ, et al. Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. *J Clin Endocrinol Metabol*. 2013;98(12):4655–4663.
217. Moran LJ, Ko H, Misso M, et al. Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutri Dietetic*. 2013;113(4):520–545.
218. Kamran H, Ahmad Z, Aslam M, Jabeen S. Dietary options in polycystic ovary syndrome (PCOS). *Annal King Edward Med Univ*. 2017;23(2).
219. Huber-Buchholz MM, Carey DG, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. *J Clin Endocrinol Metabol*. 1999;84(4):1470–1474.
220. Kiddy DS, Hamilton–Fairley D, Bush A, et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. *Clin Endocrinol*. 1992;36(1):105–111.
221. Pasquali R, Casimirri F, Vicennati V. Weight control and its beneficial effect on fertility in women with obesity and polycystic ovary syndrome. *Hum Reproduct*. 1997;12(suppl 1):82–87.
222. Moran IJ, Noakes M, Clifton PM, Tomlinson L, Galletly C, Norman RJ. Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. *J Clin Endocrinol Metabol*. 2003;88:812–819.
223. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. *Lifestyle Changes in Women with Polycystic Ovary Syndrome*. Cochrane Library; 2011.
224. Legro RS, Dodson WC, Kunselman AR, et al. Benefit of delayed fertility therapy with preconception weight loss over immediate therapy in obese women with PCOS. *J Clin Endocrinol Metabol*. 2016;101(7):2658–2666.
225. Ring M. Women's health: polycystic ovarian syndrome, Menopause, and osteoporosis. *Prim Care Clin Office Pract*. 2017;44(2):377–398.
226. Orio F, Muscogiuri G, Giallauria F, et al. Oral contraceptives versus physical exercise on cardiovascular and metabolic risk factors in women with polycystic ovary syndrome: a randomized controlled trial. *Clin Endocrinol*. 2016;85(5):764–771.
227. Hoeger K, Davidson K, Kochman L, Cherry T, Kopin L, Guzick DS. The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metabol*. 2008;93(11):4299–4306.
228. Greenwood EA, Noel MW, Kao CN, et al. Vigorous exercise is associated with superior metabolic profiles in polycystic ovary syndrome independent of total exercise expenditure. *Fertil Steril*. 2016;105(2):486–493.
229. Stefanaki C, Bacopoulou F, Livadas S, et al. Impact of a mindfulness stress management program on stress, anxiety, depression and quality of life in women with polycystic ovary syndrome: a randomized controlled trial. *Stress*. 2015;18(1):57–66.
230. Carron R, Kooienga S, Boyle DK, Alvero R. Coping in women with polycystic ovary syndrome: implications for practitioners. *J Nurs Pract*. 2017;13(10):700–707.
231. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metabol*. 2011;96(7):1911–1930.
232. Smith JD, Clinard VB. Natural products for the management of type 2 diabetes mellitus and comorbid conditions. *J Am Pharm Ass*. 2014;54(5):e304–e321.
233. Vermeulen A, Verdonck L, Kauffman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metabol*. 1999;84:3666–3672.
234. Łebkowska A, Kowalska I. Anti-Müllerian hormone and polycystic ovary syndrome. *Endokrynologia Polska*. 2017;68(1):74–78.
235. Lie Fong S, Laven JS, Duhamel A, Dewailly D. Polycystic ovarian morphology and the diagnosis of polycystic ovary syndrome: redefining threshold levels for follicle count and serum anti-Müllerian hormone using cluster analysis. *Hum Reproduct*. 2017;32(8):1723–1731.
236. Fraissinet A, Robin G, Pigny P, Lefebvre T, Catteau-Jonard S, Dewailly D. Use of the serum anti-Müllerian hormone assay as a surrogate for polycystic ovarian morphology: impact on diagnosis and phenotypic classification of polycystic ovary syndrome. *Hum Reproduct*. 2017;32(8):1716–1722.
237. Orio F, Muscogiuri G, Nese C, et al. Obesity, type 2 diabetes mellitus and cardiovascular disease risk: an uptodate in the management of polycystic ovary syndrome. *Eur J Obstetr Gynecol Reproduct Biol*. 2016;207:214–219.
238. Karakas SE. New biomarkers for diagnosis and management of polycystic ovary syndrome. *Clin Chim Acta*. 2017;471:248–253.
239. Guyatt G, Weaver B, Cronin L, Dooley JA, Azziz R. Health related quality of life in women with polycystic ovary syndrome, a self-administered questionnaire, was validated. *J Clin Epidemiol*. 2004;57:1279–1287.
240. DuRant E, Leslie NS. Polycystic ovary syndrome: a review of current knowledge. *J Nurse Pract*. 2007;3(3):180–185.
241. Dewailly D, Lujan ME, Carmina E, et al. Definition and significance of polycystic ovarian morphology: a task force report from the androgen excess and polycystic ovary syndrome society. *Hum Reproduct Update*. 2013;20(3):334–352.
242. Qi X, Pang Y, Qiao J. The role of anti-Müllerian hormone in the pathogenesis and pathophysiological characteristics of polycystic ovary syndrome. *Eur J Obstetr Gynecol Reproduct Biol*. 2016;199:82–87.

243. Jeppesen JV, Anderson RA, Kelsey TW, et al. Which follicles make the most anti-Müllerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. *MHR Basic Sci Reproduct Med*. 2013;19(8):519–527.
244. Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Müllerian hormone in women. *Hum Reproduct Update*. 2014;20(3):370–385.
245. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril*. 2016;106(1):6–15.

Porphyrias

Thomas A. Kruzel, MT, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Unexplained abdominal pain, possibly including nausea and vomiting
- Acute peripheral or central nervous system dysfunction
- Mental abnormalities ranging from confusion to acute psychosis
- Urinary porphobilinogen during attack
- Most carriers are usually asymptomatic except when exposed to drugs or chemicals that exacerbate the condition.

GENERAL CONSIDERATIONS

Until recently, porphyrias have been considered rare. It has only been within the past decade or so that an increasing number of patients have been diagnosed with these disorders, leading some clinicians to question their prevalence. Although allopathic medicine offers therapies that are effective in treating them, naturopathic medicine possesses a considerable array of therapeutic modalities that are effective in combating these diseases and preventing their recurrence.

Porphyria as a disease was first described three centuries ago by Sir Theodore Turquet de Mayerne of France, physician to the English court of King James I. He described accounts of paralysis, bilious urine, colic, insanity fits, permanent insanity, deaths at childbirth, and premature death in those afflicted. The disease probably afflicted King George III of England and was thought to have impaired his judgment during the American Revolution.[1]

In 1911, Günther described what is now known as the classic triad of abdominal pain, constipation (or diarrhea), and vomiting. Waldenström later added to the understanding of these diseases by describing acute intermittent porphyria (AIP) as characterized by periods of exacerbation and remission.[2]

Various classifications of porphyria were arrived at as greater recognition of their variations occurred. Genetic and environmentally induced manifestations of the disease were found, with considerable variations in the clinical presentations of the different forms.

The genetic deficits of porphyria affect all tissues but primarily the blood-forming tissues of the bone marrow and the cytochrome P-450 system in the liver because of the greater number of porphyrin precursors required. The liver is the major site where inherited or acquired deficits in heme synthesis occur.[3]

Classifications of Porphyria

Porphyrias are classified as being either hepatic or erythropoietic in origin. In both classes they are characterized by the overproduction of porphyrin or their precursors. Biosynthesis of heme is controlled differently in liver and bone marrow, with the rate-limiting enzyme in the liver being aminolevulinic acid (ALA) synthase, whereas the biosynthesis of heme in the bone marrow is partly regulated by the uptake of iron.

Acute hepatic porphyrias are characterized by a rapid onset of symptoms that are largely neurological; the erythropoietic variety manifests primarily in the skin, leading to cutaneous photosensitivity (Box 210.1).

BIOCHEMISTRY OF PORPHYRIA

The biosynthesis of heme is the main purpose of the porphyrogenic pathway. Heme, a high-molecular-weight metalloprotein, is a member of a group of chemicals that include cytochromes (oxidation/reduction reactions), chlorophyll (photosynthesis), and vitamin B_{12}. Heme is formed from succinyl coenzyme A (CoA) and glycine; after being combined with globin chains, it is ultimately involved in aerobic metabolism. These globin chains are synthesized on the ribosomes in the plasma of the developing erythrocyte. In the liver, heme production is largely used for the production of cytochrome P-450 in response to the body's need to eliminate toxic substances. Heme synthesis begins and ends in the mitochondrion, with part of it occurring in the cytoplasm. All tissues form heme, but the primary sites are the bone marrow and liver.

BOX 210.1 Primary Porphyrias

Hepatic Porphyrias
ALA-dehydrase-deficiency porphyria
Acute intermittent porphyria
Porphyria cutanea tarda
Hereditary coproporphyria
Variegate porphyria

Erythropoietic Porphyrias
X-linked sideroblastic anemia
Congenital erythropoietic porphyria
Erythrocytic protoporphyria

ALA, Aminolevulinic acid.
Modified from Braunwald E, ed. *Harrison's Principles of Internal Medicine.* 15th ed. New York: McGraw-Hill, 2001.

The rate-limiting enzyme for heme synthesis is ALA synthase, which is the initial enzyme in the cascade. ALA synthase requires vitamin cofactors and energy input. All other reactions in the sequence are the result of thermodynamics and are irreversible once initiated.

ALA synthase is regulated by a feedback mechanism that is responsive to the tissues' demand for heme production. With the formation of uroporphyrinogen, branching occurs and results in different porphyrin isomers. Isomer I proceeds only as far as coproporphyrin I and normally is considered inconsequential. Isomer III results in the formation of uroporphyrinogen III, which undergoes modification to render it more lipophilic so that it can be excreted from the body. Thus, most of the pathway favors heme production (Fig. 210.1).

Heme Formation

Final heme formation is regulated by the action of ferrochelatase in the mitochondria. Reducing substances such as ascorbic acid, cysteine, or glutathione are required. Iron must be in the ferrous rather than ferric form. Ferrochelatase activity is inhibited by high concentrations of heme. Thus the feedback system prevents further formation. Heme concentration also feeds back on ALA synthase, the rate-limiting step in the biosynthetic pathway.

Because heme formation is essential for aerobic metabolism, its absence would be lethal. Therefore multiple control systems have evolved to regulate the metabolic pathway, which has made it difficult to elucidate the exact mechanism of the deficit, be it genetic or acquired. Recent advances in identifying specific DNA encoding the heme biosynthetic enzymes, however, have resulted in a more precise diagnosis of the specific genetic deficit.

Red blood cell (RBC) incorporation of heme, iron, and glycine occurs in maturing cells up through and including reticulocytes but is eventually lost as the red cell ages. Hypoxia and erythropoietin will increase ALA synthase activity in RBCs but not the liver, whereas drugs and chemicals will affect the liver but not erythropoietic tissues. Fig. 210.2 illustrates heme synthesis and porphyria.

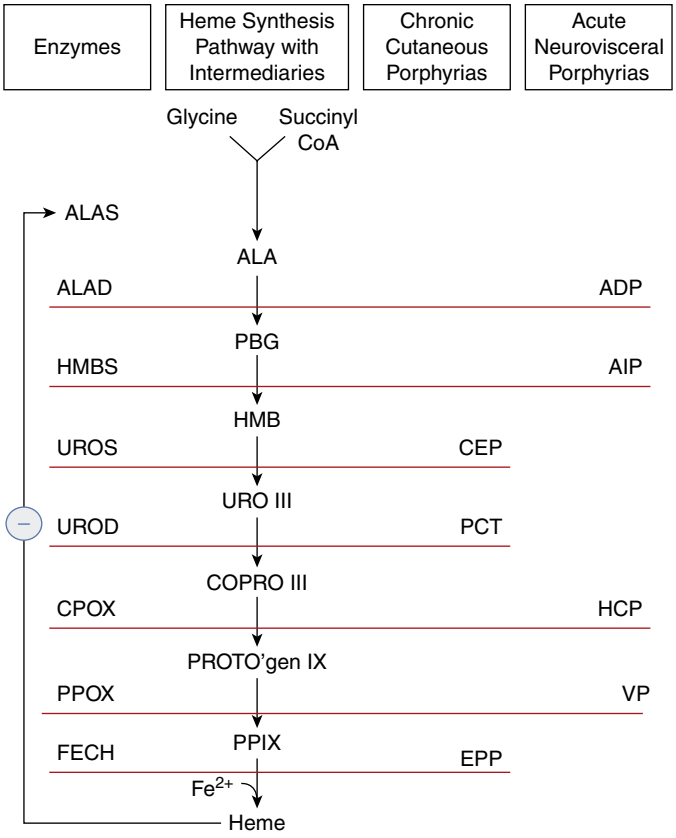


Fig. 210.1 The heme-synthesis pathway: Enzymes involved in the pathway and the associated porphyrias with the disruption of each specific enzyme. **Main (center) core**: Precursors and intermediary products in the heme-synthesis pathway. *ALA,* Aminolevulinic acid; *COPRO III,* coproporphyrinogen III; *Fe2+,* iron.*HMB,* hydroxymethylbilane; *PBG,* porphobilinogen; *PPIX,* protoporphyrin IX; *PROTO'gen IX,* protoporphyrinogen IX; *URO III,* uroporphyrinogen III. **Left of the core:** Enzymes, encoded by genes, catalyze each of the steps. Gene mutations cause deficient enzyme production. Disruptions are indicated by red lines connecting enzymes with the resultant porphyrias. *ALAD,* Aminolevulinic acid dehydratase; *ALAS,* aminolevulinate synthase; *CPOX,* coproporphyrinogen oxidase; *FECH,* ferrochelatase *HMBS,* hydroxymethylbilane synthase; *PPOX,* protoporphyrinogen oxidase; *UROD,* uroporphyrinogen decarboxylase; *UROS,* uroporphyrinogen-III synthase. **Right of the core:** Porphyrias resulting from disruption of enzyme production. *ADP,* Aminolevulinic acid dehydratase porphyria; *AIP,* acute intermittent porphyria; *CEP,* congenital erythropoietic porphyria; *HCP,* hereditary coproporphyria; *EPP,* erythropoietic protoporphyria.*PCT,* porphyria cutanea tarda; *VP,* variegate porphyria. The final product, the heme, exerts control over the whole pathway via a negative-feedback mechanism on the first enzyme—ALAS (indicated by the circle with the minus symbol). (From Edel Y, Mamet R. Porphyria: What is it and who should be evaluated? *Rambam Maimonides Med J.* 2018;9[2]. doi: 10.5041/RMMJ.10333.)

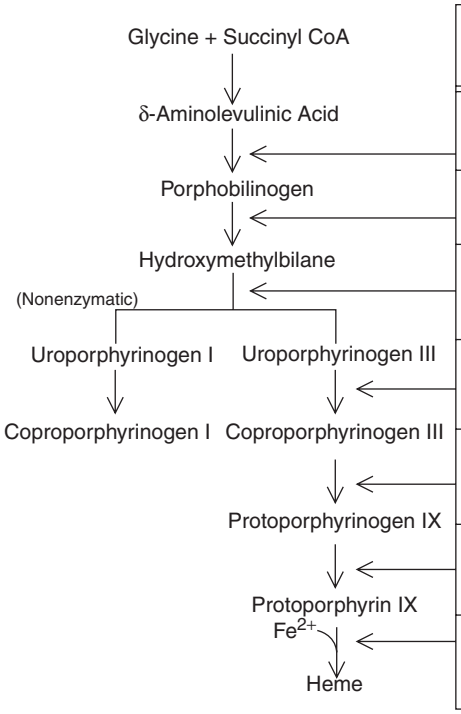
DIAGNOSIS

Manifestation of Porphyria

In patients with porphyria, specific genetic or acquired deficiencies limit the flow of heme precursors through the cascade of steps needed to form hemoglobin. The deficiency can become manifest owing to an increased demand for heme precursors. Certain drugs, chemicals, steroids, estrogens, oral contraceptives, progesterone, testosterone, or any substance that places an excess burden on the cytochrome P-450 system can act to precipitate an acute attack. This results from the partial removal of the feedback mechanism on delta-ALA synthase. Depending on the specific enzyme deficit present, heme precursors follow different pathways, resulting in their accumulation in the tissues. Their presence affects the skin and nervous system and manifests as the signs and symptoms of the disease.

Attacks occur more frequently in women than men, especially premenstrually. As the person ages, the likelihood of exposure of an underlying porphyria deficit increases because of increased exposure to environmental toxins and the aging body's decreasing ability to adapt.

Cutaneous manifestations occur because some porphyritic precursors absorb light at 400 nm, resulting in photosensitivity. The



Enzyme	Disease	Major Symptoms	Accumulation Products
δ-Aminolevulinate Dehydratase	ALA-Dehydrase-Deficiency Porphyria	Neurovisceral	Urinary ALA
Porphobilinigen Deaminase	Acute Intermittent Porphyria	Neurovisceral	Urinary ALA and PBG
Uroporphyrinogen III Cosynthase	Congenital Erythropoietic Porphyria	Photosensitivity	Erythrocyte and Urinary Uroporphyrin I and Coproporphyrin I
Uroporphyrinogen Decarboxylase	Porphyria Cutanea Tarda; Hepatoerythropoietic Porphyria	Photosensitivity	Urinary 7-Carboxylate Porphyrin; Fecal Isocoproporphyrin
Coproporphyrinogen Oxidase	Hereditary Coproporphyria	Neurovisceral and Photosensitivity	Urinary ALA, PBG, and Coproporphyrin; Fecal Coproporphyrin
Protoporphyrinogen Oxidase	Variegate Porphyria	Neurovisceral and Photosensitivity	Urinary ALA and PBG; Fecal Protoporphyrin
Ferrochelatase	Erythropoietic Protoporphyria	Photosensitivity	Erythrocyte, Plasma, and Fecal Protoporphyrin

Fig. 210.2 Heme synthesis and porphyria. (From Sassa S, Kappas A. The porphyrias. *Sci Am.* 2003;9:1–9.)

absorption of light raises the potential energy of the molecule to an "excited" state, resulting in a highly reactive oxygen species. The release of histamine and proteolytic enzymes then results in oxidative damage. Beta-carotene protects against these injuries.[3–5]

Neuropsychiatric changes occur in the hepatic porphyrias (ALA-dehydrase-deficiency porphyria, AIP, porphyria cutanea tarda, hereditary coproporphyria, and variegate porphyria) with excess production of ALA and porphobilinogen (PBG), and there is a somewhat linear relationship between their concentration and the severity and duration of symptoms. A buildup of porphyrin intermediaries has been found in various tissues and may induce the neuropsychiatric symptoms accompanying acute flareups.[6,7] The exact mechanism whereby porphyrins affect the central nervous system is not well understood.

Patients with familial porphyria cutanea tarda (PCT) usually present at a younger age than those who develop PCT spontaneously, despite the fact that there is no difference in the biochemical features of the disease.[8] This may be because of a corresponding genetic predisposition to sequester ferritin in the liver or an increased sensitivity to ethanol ingestion.[8,9] Additionally, even in families with known genetic deficits, manifestations of the disease among family members vary.

Signs and Symptoms of Hepatic Porphyrias

Acute attacks are comprised of a wide variety of symptoms, ranging from skin lesions to abdominal pain to neurological manifestations of varying intensity. Abdominal, back, or extremity pain grows in intensity over a 24- to 48-hour period, which may lead the clinician to consider appendicitis or an acute abdomen. Rebound tenderness is not usually present.

Nausea and vomiting are frequently seen, as are anxiety and restlessness. Often these patients have some degree of constipation that worsens as the condition develops. Tachycardia may also be present, suggesting an infectious process or increased bowel toxicity.

In general, the severity of the symptom pattern seems to the clinician to be out of proportion to the manifesting signs. It is thought that the bowel symptoms are due to a neurological disturbance of normal function.

Mental abnormalities can range from confusion to acute psychosis and may be the first presentation of an attack. With prolonged attacks, sensory and motor functions are impaired and can result in respiratory paralysis and death.[10] Many patients are diagnosed with schizophrenia, and a considerable number of those confined to mental institutions are thought to be afflicted with one of the porphyrias.[11,12]

The majority of carriers are usually asymptomatic, except when they are exposed to drugs or chemicals that exacerbate the condition. Neurological symptoms and the sequelae of an acute or prolonged attack can take months to clear, but eventual recovery occurs in the majority of cases.

LABORATORY DIAGNOSIS OF PORPHYRIA

Small amounts of porphyrins are excreted in normal human urine, the most predominant of which is coproporphyrin. Coproporphyrin is also present in the bile and feces. ALA, PBG, and uroporphyrin are largely excreted in the urine, whereas coproporphyrin is preferentially and protoporphyrin exclusively excreted in the bile and feces.

Fecal excretion is also affected by diet and bowel flora, thus contributing to its wide variation. Geographical distribution, dietary preferences, ethnic diversities, and differences in test methodologies make it difficult to arrive at "normal" values.

PBG excretion in the urine of healthy individuals is usually below 1.5 mg/day and is undetectable by conventional testing. The Watson–Schwartz qualitative test for PBG will be positive in acute attacks of AIP and variegate porphyria and hereditary coproporphyria in latent periods.[13] There is a high false-negative rate owing to its subjective nature. A false increase in porphyrins from a substance present in yeast tablets has been demonstrated to produce a false-positive Watson–Schwartz test.[14]

A cost-effective strategy for the diagnosis of porphyric syndromes in the presence of acute symptoms has been suggested:

If neurovisceral features suggest an acute porphyric syndrome, a rapid screening test for urinary porphobilinogen should be performed. If clinical features suggest a cutaneous porphyria, then for solar urticaria and acute photosensitivity (suggesting protoporphyria) screening tests for increased erythrocytic porphyrins should be done; for vesiculobullous formation (suggesting porphyria cutanea tarda, hereditary coproporphyria, or variegate porphyria) a screening test for urinary porphyrins should be done. Positive screening tests should be confirmed with targeted quantitative testing.[15]

Enzymatic assays and DNA-based testing are not usually necessary for the rapid diagnosis or management of symptomatic subjects with acute disease. Such tests are, however, useful for evaluation and genetic counseling. Isolation of specific DNA encoding the heme biosynthetic pathway enzymes makes it possible to now provide precise heterozygote identification as well as prenatal diagnosis in families with known defects.[4,15,16]

Other cell markers may also help in identifying patients with porphyria in the absence of symptoms. Leukocyte concentrations of manganese, calcium, iron, zinc, and erythrocyte calcium have been shown to be present at different concentrations between groups classified as AIP gene carriers. Of the cell markers, manganese was found to be the most discriminative component of all the variables investigated. An increase in cellular manganese by a factor of four suggests an increase in the likelihood of development of AIP.[17]

Because of the many possible genetic deficits leading to porphyria, it is important that the specific deficit be determined; successful treatment outcomes depend on the correct diagnosis.

See also Chapter 30 for more discussion of the diagnosis of porphyrinopathies.

Etiological Agents Triggering Porphyria

Numerous agents that trigger episodes of porphyria have been identified, and patients afflicted must be careful when taking prescription medications, estrogens, and some herbal medicines; they must also avoid environmental exposures to heavy metals, organophosphates, and any substance that places an excess burden on the cytochrome P-450 system. In particular, exogenous estrogens—whether from oral contraceptives, estrogen patches, or estrogen replacement therapy—are strong contributors to an underlying porphyria deficit.[18,19] This is one reason why acute attacks of porphyria are seen more often in women than men, especially premenstrually. Additionally, some studies have suggested that estrogens enhance the porphyrin-inducing activities of other agents, making women more vulnerable to environmental exposures than their male counterparts.[20,21]

Herbicide-induced porphyria has been shown to decrease the activity of several enzymes involved in the porphyritic pathway as well as to increase the porphyrin content in nerve tissue.[21,22] A considerable number of chemicals have also been linked to porphyria or porphyrinuria in humans; these generally involve chronic industrial exposures or environmental exposures. An example of an epidemic of PCT produced by the accidental ingestion of wheat treated with the fungicide hexachlorobenzene occurred in Turkey in the 1950s.

Some researchers have hypothesized that several otherwise unexplained chemical-associated illnesses, such as multiple chemical sensitivity syndrome (MCSS), may represent mild chronic cases of porphyria or other acquired abnormalities in heme synthesis.[23,24]

According to William Morton, who has written extensively on porphyrias, MCSS as first described by Cullen in 1979 may, in fact, be porphyria. Morton speculates that exposures to porphyrogenic chemicals, medications, or severe infection overpower the already-deficient enzyme system, resulting in an accumulation of the specific porphyrin because of diminished enzyme function. He proposed that the resultant symptoms are due to an increase of the porphyrinogen and not an accumulation of the toxin itself. Many diagnosticians have tried to expedite the distinction between "real" porphyria and secondary porphyrinopathies by requiring that a porphyria diagnosis be based on urinary or fecal porphyrin excretions, or both, of 2 to 20 times the upper limit of normal. This classification would exclude individuals demonstrating lesser degrees of porphyrinogenic activity, possibly some of those whose conditions are in remission or not subject to environmental exposures.[25]

The intensity of porphyria symptoms varies widely. About 10% of the cases present with severe acute attacks, whereas approximately 25% are seen with chronic symptomatology of varying degrees. The remaining percentage (65%) presents with no symptoms but may become susceptible under the right circumstances.[26]

Not all researchers agree with Morton's and others' premise that environmental toxicities induce underlying genetic deficits leading to symptomatic porphyria. According to Hahn and Bonkovsky, "patients with multiple chemical sensitivity syndrome may, at times, have modest increases in urinary coproporphyrin excretion; this is a common finding found in many asymptomatic subjects or patients with diverse other conditions (e.g., diabetes mellitus, heavy alcohol use, liver disease, and many kinds of anemia). Such secondary coproporphyrinuria does not indicate the existence of coproporphyria."[27]

Regardless of which view is correct, an increasing number of cases are being diagnosed by clinicians. Certainly, with the increasing levels of pollutants in the environment, hormonal additives to the food chain, and the unmonitored multiple pharmacy prescriptions encountered by most humans, the potential for unmasking an underlying deficit increases.

THERAPEUTIC CONSIDERATIONS

It is important to identify patients who are predisposed and susceptible to the development of porphyritic episodes. On seeing a patient with a past medical history of periodic psychotic episodes or nervous breakdown, unexplained abdominal pain, or unusual symptoms that have led to numerous diagnoses, the physician should be alerted to the possibility of an underlying porphyria. Prevention is clearly the first option because porphyritic episodes can be difficult to control once initiated.[28]

The treatment of patients afflicted with acute episodes of porphyria can be demanding because of the severity of the condition and the unstable clinical picture. Each patient presentation is different depending on the degree of toxicity, genetic component, and patient response to therapy.

Homeopathic treatment has proved successful, but different homeopathic medicines are required depending on the clinical picture, which, as has been mentioned, can change quickly. Constitutional prescribing does not seem to work as well when the patient is in an acute state, and several prescriptions may be necessary to help the patient to become stable. The prescriptions may have to be changed frequently as the patient progresses through the varying stages of the disease.

During an acute episode, frequent follow-up is necessary in order to assess the clinical state. In some cases, patients must be seen daily, and medications altered depending on the presentation.

Education of the patient as to his or her condition is one of the most important tasks. Often the patient does not want to believe that a genetic affliction exists and will seek another diagnosis. He or she has often received various diagnoses and may still believe that this condition is another disorder. It will be important to delineate which

symptoms go with each condition, but they will all probably be related to the porphyria. Additionally, informing other physicians seen by the patient as to his or her condition will help to keep prescription drugs to a minimum, thus decreasing the likelihood of an exacerbation.

BOX 210.2 Toxins Known to Cause or Exacerbate Porphyria

Intoxications

Alcoholism

Foreign and environmental chemicals (e.g., hexachlorobenzene, polyhalogenated biphenyls, dioxins [2,3,7,8-tetrachlorodibenzo-p-dioxin], vinyl chloride, carbon tetrachloride, benzene, chloroform)

Heavy metals such as lead, arsenic, and mercury

Drugs

Adverse Effect of Drugs

Analgesics

Sedatives

Hypnotics

Anesthetics

Sex hormones

Sulfa-drug antibiotics

The course of therapy required for the patient to reach a state where he or she is not as susceptible to environmental toxins is usually long and involves periods of exacerbation and remission. Helping the patient to understand this will facilitate the recovery.

Environmental and chemical exposures must be eliminated by identifying offending agents and limiting exposures. Lists of prescription medicines and environmental toxins that cause exacerbation of porphyria are available (Boxes 210.2 and 210.3). A complete history as to environmental and work-related exposures is in order. Often, simply by changing the patient's work environment, exposures will be decreased and attacks eliminated.

Diet

During attacks of AIP, an increased intake of complex carbohydrates can help to alleviate symptoms. Often the patient is ingesting high amounts of carbohydrates when first seen, and weight gain often follows. Intravenous glucose can also be given (300–400 g/day). A more complete parenteral nutritional regimen is preferable, however. Fasting or rapid-weight-loss diets can precipitate AIP and should be avoided.

A diet rich in high-fiber fruits and vegetables has been shown to be remarkably effective in improving several measures of porphyria. A 3-week study of 13 male patients with PCT found that 500 kcal/day of fruits and vegetables resulted in a significant decrease in body

BOX 210.3 Drugs Known to Cause or Exacerbate Porphyria[a]

- Antipyrine
- Aminopyrine
- Aminoglutethimide
- Barbiturates
- Captopril
- Carbamazepine
- Carbromal
- Chloral hydrate
- Chloramphenicol
- Chlordiazepoxide
- Chlorpropamide
- Danazol
- Dapsone
- Diazepam
- Diclofenac
- Diltiazem
- Diphenhydramine
- Diphenylhydantoin
- Doxycycline
- Ergot preparations
- Erythromycin
- Estrogen
- Ethanol (acute)
- Ethchlorvynol
- Ethinamate
- Furosemide
- Glutethimide
- Griseofulvin
- Hydralazine
- Hydrochlorothiazide
- Imipramine
- Isopropyl meprobamate
- Lidocaine
- Mephenytoin
- Meprobamate
- Methyldopa
- Methyprylon
- Metoclopramide
- Metronidazole
- N-butylscopolammonium bromide
- Nifedipine
- Nitrous oxide
- Novobiocin
- Oral contraceptives
- Orphenadrine
- Oxycodone
- Pentazocine
- Phenobarbital
- Phenylbutazone
- Phenytoin
- Piroxicam
- Pivampicillin
- Primidone
- Progesterone
- Pyrazinamide
- Pyrazolone preparations
- Sodium valproate
- Succinimides
- Sulfonamide antibiotics
- Sulfonethylmethane
- Sulfomethate
- Synthetic estrogens, progestins
- Terfenadine
- Tetracyclines
- Theophylline
- Tolazamide
- Tolbutamide
- Trimethadione
- Valproic acid
- Verapamil

[a] Although this list includes many of the better-known drugs that can exacerbate porphyria, it should not be considered complete.
Modified from Sassa S, Kappas A. The porphyrias. *Sci Am.* 2003;9:1–9.

mass index (BMI) from 26.8 to 25.8, serum alanine aminotransferase (ALT) activity from 122 to 75.6 U/L, serum aspartate aminotransferase (AST) from 91.8 to 55.2 U/L, serum iron levels from 188.6 to 140.2 mg/dL, and serum ferritin concentrations from 574 to 499 ng/mL. Skin lesions also improved, and urinary coproporphyrin excretion decreased. There was a statistically insignificant reduction in uroporphyrin excretion.[29]

Antioxidants

Antioxidant therapy, along with homeopathy, is the mainstay of case management during acute porphyritic episodes.[30] Once the patient has stabilized, an ongoing regimen of antioxidants such as vitamin C, vitamin E, glutathione, beta-carotene, and *N*-acetylcysteine should be undertaken in order to prevent recurrence.[31] Vitamin C has been found to be low in patients with PCT.[32] Subjective evaluation of the use of high-dose vitamin C (1 g a day) compared with placebo was conducted in 12 patients with erythropoietic protoporphyria photosensitivity for a period of 4 weeks. Although there was no statistical significance to the fact that patients fared better on the high-dose vitamin C, 8 of the 12 participants reported less sensitivity to sunlight.[33]

High-dose vitamin E supplementation (1 g/day of alpha-tocopherol) in patients with PCT decreased urinary excretion of uroporphyrins (50% of the C8 carboxyls), with clinical improvement in all patients.[34] The effect lasted only as long as vitamin E was being supplemented. Treatment with vitamin E was found to reduce residual oxidative stress while not affecting the increased plasma and whole-blood viscosity present in patients with PCT who were receiving only phlebotomy treatment before clinical remission.[35]

Combinations of antioxidants are also being looked at as a mode of treatment for the various porphyrias. A study comparing the protective ability of several antioxidants used concurrently for the phototoxicity of protoporphyrin IX (PP IX) and uroporphyrin I (UP I) was conducted on cell cultures. The researchers found that cell growth and cell-membrane protection showed differences in protection for the two types of porphyrias. Both beta-carotene and lycopene and the combination of beta-carotene, ascorbic acid, and alpha-tocopherol offered cell protection against PP IX phototoxicity. For membrane protection, there was significant protection against UP I by the combination of beta-carotene, ascorbic acid, and alpha-tocopherol compared with any of these antioxidants alone. The authors concluded that a combination of beta-carotene, ascorbic acid, and alpha-tocopherol offers membrane protection against the phototoxicity of both porphyrins, which is believed to occur as a result of synergistic processes. Their results suggest that the treatment of porphyria cutanea tarda and erythropoietic protoporphyria may be improved by the use of a combination of the antioxidants studied.[36]

Additionally, it is thought that the increase in oxidative damage from photodermatitis would contribute to lower vitamin D levels. Lower levels of vitamin D have been implicated more recently as contributing to a number of medical conditions, such as stroke, osteoporosis, hip fracture, and muscle weakness.[37] A cohort of 201 patients with known erythrocytic protoporphyria (EPP) was evaluated over a 7-month period for the deficiency of vitamin D levels due to lowered sun exposure. Thirty-four patients (17%) were deficient in vitamin D, and 126 (63%) were found to have insufficient levels of vitamin D. Both insufficiency and deficiency were significantly associated with the total erythrocyte protoporphyrin concentration and inversely with the time in minutes to the onset of symptoms following sunlight exposure. The authors concluded that in patients with porphyrias associated with photodermatitis, vitamin D therapy should be considered.[38]

In two studies, melatonin as an antioxidant was evaluated for its effects on delta-aminolevulinic acid–induced oxidative toxicity in neural tissue and as a cotreatment in patients suffering from disturbances related to ALA accumulation. In in vivo experiments, it was demonstrated that lipid peroxidation induced by delta-aminolevulinic acid (40 mg/kg) in the cerebellum and hippocampus was reduced by acute melatonin (10 mg/kg) administration.[39] In the other study, it was concluded that melatonin had marked efficacy in protecting against ALA-related oxidative stress and its oncostatic properties. Moreover, it was concluded that its low toxicity constituted a reason to consider the use of melatonin as a cotreatment in patients suffering from disturbances related to ALA accumulation.[40]

B-Complex Vitamins

Because pantothenic acid is required for the formation of succinyl-coenzyme A (CoA) (generated by the tricarboxylic acid cycle) and glycine, which are the precursors to the formation of ALA, supplementation is helpful. Additionally, a step in the transformation of succinyl-CoA requires a vitamin B_{12}–dependent enzyme, and evaluation of vitamin B_{12} and folic acid status may be in order. The initial reaction is oxygen dependent, which may mean that oxygen therapy is contraindicated in delta-ALA porphyria.

ALA formation requires and is dependent on pyridoxal-5-phosphate; hence, any substance that inhibits enzyme systems is countered in part by the addition of pyridoxal-5-phosphate. However, experimental models suggest that this agent does not play an important role.[3]

Carotenoids

All cutaneous porphyrias can be alleviated by the avoidance of sunlight. Treatment of erythropoietic protoporphyria with large doses of beta-carotene has been effective and may improve tolerance to sunlight.[41] Another study showed that mixed carotenoids (alpha-carotene, beta-carotene, and lycopene) were effective in cell cultures, although any one of these used alone was ineffective.

Iron

Several lines of evidence suggest that excessive iron stores and consumption aggravate some porphyrias.[42] Iron should be avoided unless supplementation is clearly indicated by low iron stores.

Detoxification

Detoxification of the liver and colon is indicated as an ongoing process to eliminate toxins that may exacerbate the condition. Regardless of whether the toxin actually precipitates an attack of porphyria, elimination on an ongoing basis reduces the chance of an acute flareup of the disease. Detoxification can be achieved through colon hydrotherapies, constitutional hydrotherapies, saunas, antioxidants, or oxygen therapies such as ozone.

Supplementation with lipotrophic factors that enhance the function of the liver's cytochrome P-450 system is contraindicated during acute attacks but useful when the patient is in a period of normality. Incorporating them, as well as high doses of antioxidants, helps decrease the number of acute attacks.

Additionally, chelation therapies with ethylenediamine tetraacetic acid and ethanol extracts of botanical medicines are contraindicated in patients with a predisposition to developing porphyria.

Constitutional Hydrotherapy

Constitutional hydrotherapy during an acute flareup is effective in normalizing liver function as well as neurological symptoms. Daily sessions may be necessary to accomplish this while allowing the physician to monitor the patient closely.[43]

Ozone Therapy

Ozone therapy is primarily indicated when there is a deficit of coproporphyrinogen oxidase, resulting in a deficit of coporphyrinogen in hereditary coproporphyria. It is contraindicated when there is a deficit of ALA synthase because higher amounts of oxygen enhance the pathway and exacerbate the symptoms.

THERAPEUTIC APPROACH

The primary clinical approach is to identify the condition early and avoid irritating and aggravating factors. Sun exposure should be limited when cutaneous manifestations are present.

Diet

The patient's diet should be rich in fruits and vegetables and low in iron.

Supplements

- Vitamin E (mixed tocopherols): 400 to 1200 IU/day
- Mixed carotenes: 100,000 to 150,000 IU/day
- Vitamin C: 3000 to 5000 mg/day
- Vitamin D: 5000 to 10,000 IU/day (measure blood levels to determine optimal dosage)
- Melatonin: 3 mg/day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lofton E. Porphyrias: demons in disguise? *Diagn Med*. 1984:21–30.
2. Mcalpine I, Hunter R. The "insanity" of King George III: a classic case of porphyria. *Br Med J*. 1966;5479:65–71.
3. Meyer UA, Schmid R. The porphyrias. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *The Metabolic Basis of Inherited Disease*. New York: McGraw-Hill; 1978:1166–1220.
4. Braunwald E, ed. *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill; 2001:2267.
5. Bissell DM. Disorders of porphyrins or metals. In: Wyngaarden JB, Smith Jr LH, eds. *Cecil Textbook of Medicine*. Philadelphia: WB Saunders; 1988.
6. Meyer UA, Schuurmans MM, Lindberg RL. Acute porphyrias: pathogenesis of neurological manifestations. *Semin Liver Dis*. 1998;18:43–52.
7. Krijt J, Stranska P, Maruna P, et al. Herbicide-induced experimental variegate porphyria in mice: tissue porphyrinogen accumulation and response to porphyrogenic drugs. *Can J Physiol Pharmacol*. 1997;75:1181–1187.
8. Siersema PD, Rademakers LH, Cleton MI, et al. The difference in liver pathology between sporadic and familial forms of porphyria cutanea tarda: the role of iron. *J Hepatol*. 1995;23:259–267.
9. Elder GH. Update on enzyme and molecular defects in porphyrias. *Photodermatol Photoimmunol Photomed*. 1998;14:66–69.
10. Ziegler T. Those nonspecific symptoms may mean porphyria. *Diagn Med*. 1983. July/August:21–24.
11. Burgovne K, Swartz R, Ananth J. Porphyria: reexamination of psychiatric implications. *Psychother Psychosom*. 1995;64:121–130.
12. Crimlisk HL. The little imitator—porphyria: a neuropsychiatric disorder. *J Neurol Neurosurg Psychiatry*. 1997;62:319–328.
13. Jacobs D, Demott W, eds. *Laboratory Test Handbook*. Baltimore: Williams & Wilkins; 1990:255.
14. Schoenfeld N, Sztern M, Mamet R. Yeast, creatinine and false diagnosis of porphyria. *Cell Mol Biol*. 1997;43:81–88.
15. Bonkovsky HL, Barnard GF. Diagnosis of porphyric syndromes: a practical approach in the era of molecular biology. *Semin Liver Dis*. 1998;18:57–65.
16. Nissen H, Petersen NE, Mustajoki S, et al. Diagnostic strategy, genetic diagnosis and identification of new mutations in intermittent porphyria by denaturing gradient gel electrophoresis. *Hum Mutat*. 1997;9:122–130.
17. Thunell S, Andersson C, Carlmark B, et al. Markers for vulnerability in acute porphyria: a hypothesis paper. *Eur J Clin Chem Clin Biochem*. 1995;33:179–194.
18. Moore MR, Hift RJ. Drugs in the acute porphyrias: toxicogenetic diseases. *Cell Mol Biol*. 1997;43:89–94.
19. Wetterberg L, Olsson MB, Alm-Agvald I. Estrogen treatment caused acute attacks of porphyria. *Lakartidningen*. 1995;92:2197–2198.
20. Legault N, Sabik H, Cooper SF, et al. Effect of estradiol on the induction of porphyria by hexachlorobenzene in the rat. *Biochem Pharmacol*. 1997;54:19–25.
21. Krijt J, Stranska P, Maruna P, et al. Herbicide-induced experimental variegate porphyria in mice: tissue porphyrinogen accumulation and response to porphyrogenic drugs. *Can J Physiol Pharmacol*. 1997;75:1181–1187.
22. Mylchreest E, Charbonneau M. Studies on the mechanism of uroporphyrinogen decarboxylase inhibition in hexachlorobenzene-induced porphyria in the female rat. *Toxicol Appl Pharmacol*. 1997;145:23–33.
23. Daniell WE, Stockbridge HL, Labbe RF, et al. Environmental chemical exposures and disturbances of heme synthesis. *Environ Health Perspect*. 1997;105(suppl1):37–53.
24. Tenhunen R, Savolainen H, Jappinen P. Changes in haem synthesis associated with occupational exposure to organic and inorganic sulphides. *Clin Sci*. 1983;64:187–191.
25. Morton WE. Chemical-induced porphyrinopathy and its relation to multiple chemical sensitivity (MCS). Newport, RI: Proceedings of the Gordon Research Conference on Biology; 1998.
26. Morton WE. Redefinition of abnormal susceptibility to environmental chemicals. In: *Hazardous Waste: Impacts on Human and Ecological Health*. Princeton, NJ: Princeton Scientific Pub; 1997:320–327.
27. Hahn M, Bonkovsky HL. Multiple chemical sensitivity syndrome and porphyria: a note of caution and concern. *Arch Intern Med*. 1997;157:281–285.
28. Floderus Y, Harper P, Henrichson A, et al. Prevention of acute intermittent porphyria is the best solution: most important are early diagnosis and counseling. *Lakartidningen*. 1998;95:3045–3050.
29. Dabrowska E, Jablonska-Kaszewska I, Falkiewicz B. Effect of high fiber vegetable-fruit diet on the activity of liver damage and serum iron level in porphyria cutanea tarda (PCT). *Med Sci Monit*. 2001;7(suppl 1):282–286.
30. Bohm F, Edge R, Foley S, et al. Antioxidant inhibition of porphyrin-induced cellular phototoxicity. *J Photochem Photobiol B*. 2001;65:177–183.
31. Thunell S, Andersson D, Harper P, et al. Effects of administration of antioxidants in acute intermittent porphyria. *Eur J Clin Chem Clin Biochem*. 1997;35:427–433.
32. Sinclair PR, Gorman N, Shedlofsky SI, et al. Ascorbic acid deficiency in porphyria cutanea tarda. *J Lab Clin Med*. 1997;130:197–201.
33. Boffa MJ, Ead RD, Reed P, et al. A double-blind, placebo-controlled, crossover trial of oral vitamin C in erythropoietic protoporphyria. *Photodermatol Photoimmunol Photomed*. 1996;12(1):27–30.
34. Pinelli A, Trivulzio S, Tomasoni L, et al. High-dose vitamin E lowers urine porphyrin levels in patients affected by porphyria cutanea tarda. *Pharmacol Res*. 2002;45:355–359.
35. Székely E, Vereckei A, Almási A, et al. Effects of vitamin E administration on the hemorheological status and redox homeostasis of patients with porphyria cutanea tarda treated with phlebotomy. *Clin Hemorheol Microcirc*. 2007;36(1):13–23.
36. Böhm F, Edge R, Foley S, et al. Antioxidant inhibition of porphyrin-induced cellular phototoxicity. *J Photochem Photobiol B*. 2001;65(2–3):177–183.
37. Holick M. *Vitamin D deficiency NEJM*. 2007;357:226–281. No 3.
38. Holme SA, Anstey AV, Badminton MN, et al. Serum 25-hydroxyvitamin D in erythropoietic protoporphyria. *Br J Dermatol*. 2008;159(1):211–213. Epub 2008 Jul 1.
39. Carneiro RC, Reiter RJ. Melatonin protects against lipid peroxidation induced by delta-aminolevulinic acid in rat cerebellum, cortex and hippocampus. *Neuroscience*. 1998;82(1):293–299.
40. Karbownik M, Reiter RJ. Melatonin protects against oxidative stress caused by delta-aminolevulinic acid: implications for cancer reduction. *Cancer Invest*. 2002;20(2):276–286.
41. Badminton MN, Elder GH. Management of acute and cutaneous porphyrias. *Int J Clin Pract*. 2002;56:272–278.
42. Rocchi E, Ventura P, Ronzoni A, et al. Pro-oxidant and antioxidant factors in acute intermittent porphyria: family studies. *J Inherit Metab Dis*. 2004;27:251–266.
43. Boyle W, Saine A. *Lectures in Naturopathic Hydrotherapy*. East Palestine, OH: Buckeye Naturopathic Press; 1988.

211

Preconception and Pregnancy Health

Michael Scott, ND, MSA, and *Lindsey Stuart, MS, CNM*

OUTLINE

INTRODUCTION

Pregnancy provides a unique opportunity for the health care provider to support positive pregnancy experiences and birth outcomes by utilizing the preconception and perinatal period to educate and empower parents on best practices to create optimal conditions for the future health of the next generation. The World Health Organization (WHO) defines a positive pregnancy experience as (1) maintaining physical and sociocultural normality, (2) maintaining a healthy pregnancy for mother and baby (including preventing and treating risks, illness, and death), (3) facilitating an effective transition to positive labor and birth, and (4) achieving positive motherhood (including maternal self-esteem, competence, and autonomy).[1]

According to the WHO, a woman in a low- to middle-income developing country is 33 times more likely to die of a maternal-related cause than a woman in a developed country. Data on maternal mortality estimate that worldwide, over 800 women die every day from preventable complications related to pregnancy and childbirth. Preventable complications, including hemorrhage, cardiovascular and coronary conditions, infection, and improper abortion, account for nearly half of all pregnancy-related deaths.[2] The WHO reports that maternal mortality trended downward globally in 157 of 183 countries studied between 1990 and 2013.[3] The United States continues to have the highest maternal mortality rate of all developed countries and is the only high-income country where the maternal mortality rate increased between 1990 and 2015.[4] Among 31 Organization for Economic Cooperation and Development (OECD) countries reporting maternal mortality data, the United States ranks 30th, ahead of only Mexico, and has a rate more than three times higher than rates in Canada and the United Kingdom.[5]

Dramatic discrepancies in maternal-fetal outcomes continue to persist within the United States, where various socioeconomic factors, such as poor maternal health, unintended births, large racial/ethnic disparities, and fewer prenatal visits, are significantly associated with increased maternal mortality.[6] According to most recent Centers for Disease Control and Prevention (CDC) data, black women are more than three times more likely to die of complications related to pregnancy and childbirth compared with white women, and this dire finding has not wavered in decades (40.0 vs. 12.4 maternal deaths per 100,000 live births during 2011–2014).[7] Every year, nearly 4 million women give birth in the United States, with more than 700 of these women dying of complications related to pregnancy and childbirth and more than 50,000 women experiencing a life-threatening complication leading to severe maternal morbidity.[8] The leading causes of pregnancy-related deaths include cardiovascular disease (15.2%), non-cardiovascular diseases (14.7%), infection or sepsis (12.8%), hemorrhage (11.5%), and hypertensive disorders of pregnancy (6.8%).[7] Rates of preventable complications are growing in the United States, despite costs of maternity care exceeding $60 billion in 2012.[9]

When medically necessary, operative deliveries can be a life-saving intervention. However, reducing the frequency of cesarean section (CS) delivery can improve maternal and infant health. Compared with vaginal deliveries, the prevalence of maternal mortality and maternal morbidity is higher after CS, and there is an increased risk of uterine rupture, abnormal placentation, ectopic pregnancy, blood transfusion, stillbirth, and preterm birth (PTB) when CS deliveries are performed for non–medically indicated reasons.[10] A 2018 study found that globally, based on an analysis of data from 169 countries that include 98.4% of the world's births, CS births in 2015 were almost double the number

of births by this method in 2000. CS births were almost five times more frequent in births in the wealthiest versus the poorest quintiles in low-income and middle-income countries.[11] The WHO and UNICEF show that worldwide CS rates increased 3.7% each year between 2000 and 2015. Thus, in 2018, the WHO issued a first-of-its-kind guideline for implementing recommendations on nonclinical interventions to reduce CS births.[12] The WHO has long recommended a rate for CS between 10% to 15% due to medical necessity. However, in the United States, although the rate of CS has declined in recent years, it remains two to three times higher than the WHO guideline.[13]

Due to significant advances in health care, rates of infant mortality in developed countries have declined since 1960, including in the United States. However, the decline in infant mortality rates in the United States has not kept pace with other developed countries, and clear disparities in birth outcomes remain a concern.[14] A 2018 study examined mortality trends for the United States and 19 comparator nations in the OECD for children ages 0 to 19 from 1961 to 2010 using publicly available data. Compared with similar OECD countries, infant mortality rates in the United States were 76% higher, due to extreme immaturity, and U.S. infants were 2.3 times more likely to experience sudden infant death syndrome between 2001 and 2010, the most recent years for which comparable data are available across all the countries. The researchers concluded that if the United States had kept pace with the overall OECD decline in infant mortality since 1960, this would have resulted in approximately 600,000 fewer infant deaths over the course of 50 years. The United States outspends every other OECD nation on health care per capita for children, yet infant and child mortality outcomes remain below targets.[15]

PRECONCEPTION

Maternal health status before conception is strongly linked to healthy pregnancy outcomes.[16] Preconception counseling affords the health care provider an opportunity to support women and parents by discussing a variety of medical, behavioral, and social factors that may be modified before conception, thereby helping individuals to make well-informed and well-considered decisions, with the intention of reducing the risk of poor maternal and birth outcomes.[17]

Preconception counseling should be comprehensive, taking into account the following for optimal prenatal care and attention: (1) review of medical, surgical, and psychiatric histories and immunizations, medications, dietary supplements, family history, and genetic history; (2) infectious disease screening; (3) substance use assessment; (4) exposure to violence, intimate partner violence, and reproductive and sexual coercion; (5) nutritional status assessment; (6) achieving and maintaining healthy body weight; (7) exercise and physical activity assessment; (8) assessment for teratogen and environmental toxicant exposures; and (9) correct pregnancy dating.[18] Culturally appropriate discussion around family planning and prevention of unintended pregnancy should also be included at this visit.

Women's bodies undergo rapid and substantial changes during pregnancy, brought about by both hormonal and mechanical effects. These significant physiological changes occur in order to nurture the developing fetus and prepare the mother for labor and delivery.[19] These physiological adaptations occur in nearly all maternal organ systems. The first sign of pregnancy is usually a missed menstrual period, followed by nonconcerning symptoms such as urinary frequency, fatigue, breast tenderness, and nausea. During the second trimester, the body undergoes postural changes to accommodate the changing center of gravity, which can lead to back pain for many women. In the third trimester, the expanding uterus displaces many internal organs as it grows. Cardiovascular and respiratory changes occur as the body accommodates the growing fetus. For example, circulating blood volume increases by 30% to 50% as the uterus expands, which also increases cardiac output; and there can be reduced movement of the diaphragm, which leads to shortness of breath.[20] Common conditions, including urinary tract infections, anemia, hypertension, and dysglycemia, can present differently during pregnancy, and it is therefore advised to include appropriate screening in prenatal care.

With the average age of U.S. women at their first birth having increased over the past decade, it is important to consider how aging affects fertility and pregnancy. Age has physiological effects on fertility that can reduce conception rates. Once conception does occur, age can increase the risk for complications of pregnancy, including hypertension, diabetes, and birth defects resulting from chromosomal abnormalities.[21]

A growing body of research suggests that increased susceptibility to chronic disease in adulthood originates, in part, during fetal development. During the time of early embryonic development, parental lifestyle can adversely influence the long-term risks of offspring. Fetal organ structures develop at varying points in development, and in utero exposure to toxins can directly affect cardiovascular, metabolic, immune, and neurological development.[16] Inadequate levels of key nutrients during crucial periods of fetal development may lead to reprogramming within fetal tissues, predisposing the infant to neurodevelopmental problems and chronic diseases later in life. These conditions include developmental delays, obesity, cardiovascular disease, and diabetes.[22]

The emerging field of epigenetics involves the study of the process by which stable, heritable alterations to gene expression, and thus the phenotype of cells, are induced without changes to the primary DNA sequence or genotype.[23] These modifications can be altered in response to environmental factors, including periconceptional stress, maternal nutritional status during pregnancy, and fetal exposure to toxicants.

Epigenetics is well exemplified in the case of gestational diabetes mellitus (GDM),[24] which can develop during pregnancy (usually in the late second trimester) when maternal insulin production can no longer cope within increasing adiposity and insulin resistance and affects 5% of all first-time pregnancies worldwide.[25] With GDM already being the most common metabolic disorder occurring in pregnancy, its prevalence continues to grow, given increasing numbers of women of advanced age becoming pregnant and the increasing epidemic of maternal obesity.[26] Adverse potential complications associated with GDM occur during pregnancy, at the time of delivery, and postpartum. Insulin is a major fetal growth hormone, which can lead to macrosomia and increased risk for operative delivery. Intrauterine exposure to GDM causes epigenetic modifications and fetal metabolic programming, which confers increased risk of lifelong consequences for offspring, including the development of type 2 diabetes mellitus and obesity in adulthood.[27] Indeed, mounting evidence suggests that the prenatal period constitutes a critical convergence of short- and long-term modifying factors affecting the lifelong health of both mother and child.[22] Nutritional and lifestyle counseling should thus serve as a fundamental cornerstone of prenatal and pregnancy care in order to optimize a healthy uterine environment for optimal fetal development while supporting maternal health.

Although various complications can occur regardless of preconception counseling and pregnancy planning, antenatal care offers a significant opportunity for promoting positive pregnancy experiences and primary prevention by means of reducing maternal and fetal morbidity and mortality by educating and supporting women throughout pregnancy, preventing unintended pregnancy, managing and even reversing concurrent medical conditions, identifying cases of intimate partner violence, differentiating between expected variations, and

managing potential complications and offering appropriate interventions. Women's positive experiences during antenatal care and childbirth can create the foundations for healthy motherhood.[1] Moreover, health before conception is strongly correlated to the outcome of pregnancy, and life-course research pinpoints the preconception period as crucial for health across generations.[28]

NUTRITION

Healthy food choices and dietary awareness play vital roles before and during pregnancy, with the pregnant woman requiring adequate intake of energy, protein, fats, carbohydrates, vitamins, and minerals to meet both maternal and fetal needs. Maternal metabolism is altered to redirect nutrients to the placenta and fetus. Epigenetic influence, or fetal programming, is transgenerational and long-lasting,[29] with the risk of developing childhood- and adult-onset disease determined, in large part, by maternal nutritional status at conception and during pregnancy and lactation.

With respect to caloric needs and food consumption during pregnancy, the commonly espoused idea that pregnant women should be "eating for two" is a myth. Most pregnant women will need between 2200 and 2900 calories per day, with the estimated energy requirement generally remaining the same as for nonpregnant women in the first trimester. Extra energy needs increase in the second and third trimesters to an estimated 340 kcal per day and 452 kcal per day, respectively.[30] Energy requirements vary based on a woman's age, preconception body mass index (BMI), trimester of pregnancy, gestational weight gain (GWG), and level of physical activity. "Eating for two" is not in any way representational of the nominal 10% to 25% increase in caloric intake actually required to support a healthy pregnancy.[31] For pregnant women who consume unneeded extra calories, rather than focusing caloric intake on nutrient-dense foods, their dietary choices may be 15% to 30% above target calorie levels[32] and lead to excessive and detrimental GWG with respect to National Academy of Medicine 2009 (NAM) guidelines (see "Healthy Weight" section).

Unfortunately, most women in the United States still significantly increase their caloric consumption during pregnancy, with 53% of all women, 70% of overweight women, and 64% of obese women exceeding NAM guidelines for GWG in 2011–2012.[33] Indeed, a recent comprehensive systematic review and meta-analysis evaluated associations between GWG above or below NAM guidelines and pregnancy outcomes. Based on data from 23 studies meeting inclusion criteria involving 1,309,136 pregnant women, GWG was above NAM guidelines in 47% of participants and below guidelines in 23%. Compared with women having GWG within NAM recommended levels, weight gain above and below guidelines was associated with adverse maternal and infant outcomes. GWG greater than recommendations was associated with higher risk of large-for-gestational-age (LGA) infants, macrosomia, and cesarean delivery, whereas GWG below guidelines was associated with higher risk of small-for-gestational-age (SGA) infants and PTB.[34] The study prompts two questions: Are health care providers able to modify the amount of weight women gain in pregnancy, and can altering GWG to within NAM recommendations during pregnancy improve pregnancy outcomes?

These questions were answered in the first large-scale study of its kind, which sought to investigate the impact of dietary and physical activity–based lifestyle interventions on GWG and subsequent pregnancy outcomes. The systematic review and meta-analysis involved more than 50 researchers from 41 institutions in 16 countries and analyzed individual participant data from 36 randomized trials and 12,526 participants. The study compared the effects of healthy dietary recommendations (e.g., restriction of sugar-sweetened beverages, increased servings of fruits and vegetables, choosing low-fat dairy options) combined with physical activity (e.g., moderate intensity, including aerobic classes, stationary cycling, and resistance training as 150 minutes of dedicated activity per week) throughout pregnancy. Participants following the lifestyle modifications demonstrated a reduced GWG by an average of 0.7 kg compared with the control group. The researchers were able to conclude that maintaining a moderated healthy diet and following standard physical activity guidelines during pregnancy had a significant impact on reducing excessive GWG and thus concomitantly decreased the associated risk of GDM, preterm birth, and CS delivery.[35]

Cohort studies have suggested that dietary patterns up to 3 years before pregnancy—characterized by a diet rich in consumption of fruits, vegetables, legumes, whole grains, nuts, and select fish, along with low intake of red and processed meats—are associated with reduced risk of GDM, preeclampsia, and PTB.[36]

Protein is the macronutrient most influential with respect to birth weight.[37] Low birth weight and preterm delivery are considered to be the most relevant determinants of newborn infant morbidity and mortality in both developed and developing countries. Having been studied in many distinct populations, low birth weight has also been associated with placing an infant at greater risk for the development of various adult-onset chronic medical conditions, including type 2 diabetes, insulin resistance, hypertension, coronary artery disease, asthma, osteoporosis, and early menopause, to name a few.[38] The recommended protein intake during pregnancy is a minimum of 60 g per day, which represents an increase from 46 g per day in nonpregnant states, reflecting an increase to 1.1 g of protein/kg/day from 0.8 g of protein/kg/day, respectively.[39] Sixty grams of protein per day will account for approximately 20% to 25% of daily caloric intake.

Pregnant women need the energy provided by complex carbohydrates because glucose is a major fuel used for intrauterine growth. Complex carbohydrates should comprise 45% to 65% of daily calories.[30]

Fats play an essential part of a healthy diet during pregnancy and are used primarily as an energy source. Total dietary fat intake should approximate 20% to 35% of daily calories depending on one's carbohydrate goals.[30] Increased consumption of omega-3 polyunsaturated fatty acids (PUFAs) is emphasized because they play a critical role in brain development. Saturated fats should be limited to less than 10% of total daily caloric intake, and trans fatty acids should be avoided whenever possible.[31]

Maintaining adequate fiber consumption during pregnancy is important, with a goal of 20 g to 35 g per day. More is better, but increase slowly to allow adaptation. Constipation tends to occur more frequently in the first trimester (35% of women) and second trimester (39%), with 20% to 17% of women affected in the third trimester and postpartum, respectively.[40] First-line, nonpharmacological management of constipation includes increasing adequate hydration, getting regular exercise/being physically active, and eating plenty of fiber-rich foods.

An Academy of Nutrition and Dietetics position paper states that well-planned, plant-based diets—whether vegan, lacto-ovo-vegetarian (includes dairy and egg products), lacto-vegetarian, or ovo-vegetarian—are appropriate and can provide the nutrient needs and promote growth at all stages of the life cycle, including pregnancy and lactation. Although limited, research also suggests that where access to healthy food is satisfactory and when compared with nonvegetarian/omnivorous diets, pregnancy outcomes are similar for vegans and vegetarians, including average birth weight and full-term deliveries.[41] One study demonstrated that following a vegetarian diet in the first trimester was inversely associated with excessive GWG.[42] Vegan and vegetarian

women have also been found to have reduced risk of pregnancy complications such as GDM and preeclampsia; lower-than-average rate of CS delivery; lower neonatal and maternal mortality, with no complications or negative outcomes that are higher than average; and less postpartum depression.[43] Consuming a vegan or vegetarian diet composed of organically grown foods also minimizes in utero exposure to toxic chemicals.

Maternal vegan and vegetarian diets do, however, require special attention with respect to obtaining a reliable and adequate intake of critical nutrients, namely, vitamin B_{12}, zinc, iron, iodine, vitamin D, and the essential omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[44] It is therefore advised that pregnant and lactating vegans and vegetarians consider taking a comprehensive daily prenatal multivitamin-mineral dietary supplement as well as an algae-derived omega-3 supplement yielding DHA and EPA. In addition, research is emerging that single-nucleotide polymorphisms (SNPs) can substantially influence the need for specific nutrients in the diet. For example, although it is common to use vitamin A and beta-carotene interchangeably, in fact, a substantial portion of the population makes this conversion very poorly (see Chapter 125, Vitamin A). The more strictly vegetarian a woman's diet, the more important it is for the clinician to monitor for adequate amounts of needed nutrients not typically found in nonanimal sources.

Given that pregnant women and their unborn children are far more susceptible to and at greater risk than the general population of contracting foodborne illnesses, because of hormonal changes that lead to decreased cell-mediated immune function, special caution is recommended with respect to safe food handling and preparation.[45] Of greatest concern are *Listeria monocytogenes, Toxoplasma gondii, Brucella* species, *Salmonella* species, and *Campylobacter jejuni,* and pregnant women should closely adhere to food-safety recommendations.[46]

During pregnancy and lactation, being cognizant of nutritional needs is critical to optimize maternal adaptation, fetal development, and infant health. A majority of women, especially for first-time pregnancies, have heightened concerns about nutritional needs and healthy food choices and confusion over optimal dietary planning. Thus, preconception and pregnancy bring an opportune time for health care providers to offer evidence-based nutritional guidance and reassurance.

A personalized approach to dietary counseling should be the cornerstone of antenatal care and take into account the following factors whenever possible: a woman's access to healthy food, health literacy, physical activity level, socioeconomic status and economic restraints, race/ethnicity, context for cultural attitudes, food practices and preparation methods, as well as a woman's preconception and early pregnancy nutritional status and BMI. A diet rich in organic whole grains, nuts, seeds, fruits, vegetables, and select wild-caught fish is desirable for healthy pregnancy outcomes. Improving and implementing maternal dietary choices and habits before, during, and after pregnancy reduces the risk of medical problems for the mother and her infant.[31]

HYDRATION

Adequate fluid intake is essential for a healthful pregnancy. Water constitutes approximately 55% to 65% of a woman's body weight,[47] and during pregnancy, her fluid requirements increase in order to maintain total body water homeostasis and support cellular metabolism, fetal circulation, amniotic fluid volume, and increased blood volume.

Amniotic fluid is maintained in dynamic equilibrium, and the amniotic fluid volume (AFV) is an important parameter in the assessment of fetal well-being because of the essential role AFV plays in normal fetal growth and development. The AFV progressively increases as gestation progresses. Amniotic fluid is produced once the amniotic sac is formed at 12 days after conception and is initially composed of water provided by the mother. At 10 weeks' gestation, AFV is 10 to 20 mL, rising to 800 mL at 33 weeks, plateauing at 1000 mL at 38 to 39 weeks, and eventually decreasing to 800 mL at 40 weeks. AFV is vital in many ways, such as protecting the fetus from trauma and infection, cushioning the umbilical cord and placenta from compression, allowing fetal movement, providing a supportive medium for growth, and serving as a reservoir of fluid and nutrients.[48] Oligohydramnios is a condition arising from having a too-low AFV than expected for gestational age, affecting approximately 3% to 5% of pregnancies. The concern can occur at any time during pregnancy but more commonly arises in the final trimester and can particularly complicate pregnancies that are postterm. In less than half of cases, the diagnosis is made in the absence of maternal-fetal risk factors and is therefore defined as isolated oligohydramnios (IO).[48] Emerging evidence suggests increasing maternal hydration may be a simple, well-tolerated, and useful strategy to improve AFV in many cases of IO.[49]

Insufficient hydration during pregnancy can lead to constipation, hemorrhoids, edema, muscle cramps, temperature dysregulation (often referred to as "maternal overheating"), cystitis, urinary tract infections, fatigue, low AFV, and preterm labor.

Breastfeeding women are commonly advised to increase their fluid intake in the hope that this may improve breast milk production. Fluid balance continues to be a challenge for nursing mothers, who lose an additional ~700 mL of water per day via breast milk at 8 weeks postpartum.[50] Whether or not additional fluid intake for nursing mothers, beyond what is required to meet physiological needs, increases breast milk supply, however, remains unknown due to a lack of well-conducted trials.[51]

Health authorities such as the National Academy of Medicine (formerly the Institute of Medicine), Office on Women's Health, and European Food Safety Authority state that the requirements for total water intake (TWI, originating from both food moisture and beverage fluid) are increased during pregnancy and breastfeeding.[52] It is recommended that pregnant women approximate 2300 mL per day TWI. This total is equivalent to 10 eight-ounce glasses of water per day. Breastfeeding women are asked to increase this by an extra 700 mL per day, thereby consuming 3000 mL per day TWI.[53]

Women may consider establishing healthy preconception hydration habits. Pregnant women should be encouraged to consume fluids at routine intervals throughout the day and not wait for the feeling of thirst and risk of dehydration. Maternal hydration is, of course, further augmented to account for water loss due to perspiration as a result of exercising or being in a hot/humid environment or having a fever and water loss due to diarrhea or vomiting as a result of "morning sickness" and hyperemesis gravidarum. It is strongly recommended to consider the source of drinking water, given the extensive level of water-supply contamination worldwide, and only consume purified water (e.g., using a carbon block filter or the more expensive reverse-osmosis filtration if arsenic or fluoride is elevated) in order to minimize maternal and fetal exposure to tap-water toxicants that may be endemic to a local area.

LIFESTYLE

Healthy Weight

Over the past several decades, with the global nutrition transition,[54] obesity among women of reproductive age has become a serious and growing public health concern. Although historically associated with affluence and high-resource countries, the increase in obesity is now also being seen in low-income populations. The prevalence of obesity across low- and middle-income countries varies from 3.4% to 73.7%.[55]

TABLE 211.1 Recommendations for Total and Rate of Weight Gain During Prepregnancy, by Prepregnancy or Early Pregnancy (Less Than 10 Weeks) Body Mass Index[a]

Prepregnancy Body Mass Index (BMI)	BMI (kg/m^2) (WHO Criteria)	Total Weight Gain at Term, Gain Range (kg)	Rates of Weight Gain[b] in 2nd and 3rd Trimesters, Mean (Range) (kg/wk)
Underweight	<18.5	12.5–18.0	0.51 (0.44–0.58)
Normal weight	18.5–24.9	11.5–16.0	0.42 (0.35–0.50)
Overweight	25.0–29.9	7.0–11.5	0.28 (0.23–0.33)
Obese	≥30.0	5.0–9.0	0.22 (0.17–0.27)

[a]Calculations are for singleton pregnancies.
[b]Calculations assume a weight gain of 0.5–2.0 kg in the first trimester.
WHO, World Health Organization.
Data from the National Academy of Medicine (2009) http://www.nationalacademies.org/hmd/~/media/Files/Report%20Files/2009/Weight-Gain-During-Pregnancy-Reexamining-the-Guidelines/Report%20Brief%20-%20Weight%20Gain%20During%20Pregnancy.pdf.

Excessive maternal GWG (the amount of weight a pregnant woman gains between the time of conception and the onset of labor) contributes to the global obesity epidemic, given that fetal development and epigenetics are influenced by the metabolic and hormonal milieu of the intrauterine environment, which is shaped by maternal weight status and driven in part by physical activity and dietary factors.[56]

In the United States, based on the 2011–2012 National Health and Nutrition Examination Survey (NHANES), the prevalence of obesity in women of reproductive age is 31.8% and increases to 58% when the overweight and obese categories are combined.[57] Using data from the revised birth certificate for 48 states, the District of Columbia, and New York City, the Centers for Disease Control examined 2011–2015 National Vital Statistics System natality data, which included prepregnancy BMI. The analysis found the overall prevalence of prepregnancy normal weight in 2015 to be 45%. Among 38 jurisdictions, the prevalence of prepregnancy normal weight declined by 5%, whereas the prevalence of overweight increased by 2%, and the prevalence of obesity (all classes) increased by 8%.[58]

A woman's prepregnancy weight is one of the most important indicators and modifiable risk factors affecting a healthy pregnancy. To improve maternal health and neonatal birth outcomes, women should ideally be within a normal BMI range when they conceive and should also gain weight within the ranges established by the NAM. GWG recommendations aim to optimize positive pregnancy outcomes for mothers and infants across whole populations. In today's obesogenic environment, however, a majority of women are anticipated to have difficulty limiting weight gain to the upper limit of the guidelines. Approximately 40% to 70% of women gain in excess of the NAM guidelines during pregnancy, with those most at risk being already overweight or obese at conception.[59]

In the first trimester (initial 12 weeks of gestation), GWG is typically less than 2 kg. Although the rate of GWG may vary during subsequent trimesters, it is normal to gain some weight during pregnancy to meet the needs of a growing baby, with most GWG occurring after 20 weeks' gestation, and GWG should take into account prepregnancy BMI. Overall healthy weight gain represents many components during pregnancy. For example, if a baby's birth weight is 3.5 kg and a mother gained 12.8 kg during pregnancy, the breakdown is as follows: amniotic fluid (0.9 kg), placenta (0.7 kg), growth of womb (0.9 kg), growth of breasts (1.1 kg), increased blood volume (1.5 kg), increased body fluids (1.1 kg), storing of nutrients as fat and protein (3.1 kg).[60]

GWG goals vary according to preconception BMI, and women who meet GWG goals have more positive pregnancy outcomes. According to the most recent revision (May 2009) of the NAM guidelines for GWG, women who are underweight at the beginning of pregnancy (BMI <18.5) should try to gain 12.5 to 18 kg, women who are normal weight (BMI 18.5–24.9) should gain 11.5 to 16 kg, overweight women (BMI 25.0–29.9) should gain 7 to 11.5 kg, and women who are obese (BMI ≥30.0) should gain only 5 to 9 kg during pregnancy (Table 211.1).

Research demonstrates an association between prepregnancy overweight and obesity with excessive GWG, thereby increasing the risk of adverse maternal and neonatal outcomes, including GDM, hypertension, preeclampsia, postpartum weight retention, premature rupture of membranes, fetal growth abnormalities, macrosomia, birth defects, CS delivery, spontaneous miscarriage, and stillbirth.[28,57,61–63]

Similarly, maternal underweight is associated with poor perinatal outcomes, including increased risk of intrauterine growth restriction, SGA infants, low birth weight, and PTB.[61,64]

Given the increasing worldwide prevalence of overweight and obesity in women of reproductive age and the well-established negative effects of poor weight status that lead to adverse maternal and birth outcomes, preconception and early pregnancy offer an ideal opportunity to counsel women so that they can enter pregnancy in as optimal a state of health as possible. Attenuating unhealthy pregnancy weight gain may be accomplished by way of health care provider discussion and education about maternal and fetal risks associated with overweight, obesity, and excessive GWG, as well as receiving personalized treatment with respect to effective behavior and lifestyle modifications, physical activity recommendations, and positive dietary interventions to improve and support healthy maternal weight.

Physical Activity

Regular physical activity in all phases of life promotes optimal health. Despite pregnancy being associated with profound anatomic and physiological changes, it is an ideal time for maintaining or adopting a healthy lifestyle, given physical activity has minimal risks and has been shown to benefit most pregnant women.[65]

The American College of Obstetricians and Gynecologists (ACOG) provides examples of safe physical activities that women with uncomplicated pregnancies may initiate or continue, including walking, swimming, stationary cycling, and low-impact aerobics; running, racquet sports, and strength training (in consultation with an obstetric care provider); and yoga and Pilates (modified). Commonsense activities to avoid while pregnant include hot yoga, contact sports, scuba diving, sky diving, and athletic activities with a high risk of falling (e.g., downhill and Nordic skiing, water skiing, surfing, mountain biking, and horseback riding).[65] Precautions to be aware of when pregnant include avoiding exertion at altitudes greater than 1600 m,[66] avoiding the supine position after the first trimester, and staying well hydrated. Signs and symptoms of when to stop exercising include dizziness,

vaginal bleeding, decreased fetal movement, lower extremity pain or swelling, chest pain, shortness of breath, dyspnea before physical activity, leakage of amniotic fluid, and preterm labor.[67] There are several absolute and relative contraindications to aerobic exercise during pregnancy that women need to be aware of.

The U.S. Department of Health and Human Services recently released a revised edition (November 2018) of the Physical Activity Guidelines (PAG) for Americans. The PAG Advisory Committee concluded that pregnant women benefit from engaging in a physical activity program that leads to an eventual goal of moderate-intensity exercise for 30 minutes per day on most or all days of the week—resulting in at least 150 minutes per week—and adjusted as medically indicated.[68]

A systematic review and meta-analysis of 17 randomized controlled trials, involving 5075 pregnant women, sought to evaluate the effect of exercise on the risk of gestational hypertensive disorders. Compared with control participants, women randomized to aerobic exercise for 30 to 60 minutes, two to seven times per week, had a significantly lower incidence of gestational hypertension (2.5% vs. 4.6%; relative risk [RR] 0.54, 95% confidence interval [CI]: 0.40–0.74; 16 studies, 4641 participants). The occurrence of preeclampsia was similar in both groups. The incidence of CS delivery was decreased by 16% in the exercise group.[69] Other studies have also suggested that regular physical activity may shorten the duration of labor and reduce the risk of CS and operative-assisted vaginal delivery.[67] A meta-analysis of 13 randomized controlled trials (1439 participants) found that physical exercise interventions reduced GWG and the risk of GDM for overweight and obese pregnant women. There were no significant differences in additional outcomes, including gestational hypertension, preeclampsia, birth weight, fetal growth abnormalities, macrosomia, CS delivery, and PTB.[70] No evidence was shown with regard to benefit and/or harm for infants.

Psychological benefits of physical activity during pregnancy include reduced fatigue, stress, anxiety, and depression, as well as improved well-being.[71]

A prospective randomized clinical trial (300 singleton women, >18 years of age, at 10 weeks' gestation and a mean prepregnancy BMI of 26.78 ± 2.75 kg/m^2) assessed whether exercise during pregnancy may prevent GDM and improve pregnancy outcomes in overweight and obese women. Participants assigned to the exercise group followed a cycling program initiated within 3 days of randomization until 37 weeks' gestation, which involved 30-minute sessions, three times per week, with a perceived exertion rating between 12 and 14. Women assigned to the control group continued their usual daily activities. Cycling exercise initiated early in pregnancy was associated with a significant reduction in the incidence of GDM and less GWG before the mid-second trimester. All other secondary outcomes were also lower in the exercise group compared with control but without significant differences.[72]

A systematic review and meta-analysis of nine randomized controlled trials (1502 participants) assessed exercise during pregnancy and the risk of PTB in overweight and obese women with a singleton pregnancy. Compared with controls, women who were randomized in early pregnancy to aerobic exercise for 30 to 60 minutes, three to seven times per week, had a lower percentage of PTB at less than 37 weeks (RR 0.62, 95% CI: 0.41–0.95) and a lower incidence of GDM (RR 0.61, 95% CI: 0.41–0.90). The gestational age at delivery and incidence of CS delivery were similar in both groups, and no differences in birth weight, macrosomia, and stillbirth were found between the intervention and sedentary control groups.[73]

Combining regular exercise and pregnancy benefits both mother and infant. Women with uncomplicated pregnancies should be encouraged to engage in aerobic and strength-conditioning exercises before, during, and after pregnancy.[65]

Sleep

Pregnancy is associated with numerous and dramatic physiological and anatomical changes in a relatively short period of time, predisposing a majority of women to develop sleep disturbances and sleep disorders or unmasking underlying preexisting sleep conditions. Many pregnancy-related factors can result in inadequate sleep, and a growing body of research demonstrates maternal sleep–wake disturbances are predictors of adverse pregnancy outcomes.

In a study seeking to characterize sleep patterns and sleep disorders during pregnancy, 2427 participants completed an Internet-based survey that included multiple screening scales: Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, vitality scale of the Short Form 36 Health Survey, Insomnia Severity Index, Berlin Questionnaire, International Restless Legs Syndrome questions set, and a short version of the Pregnancy Symptoms Inventory. Through all trimesters, women experienced poor sleep quality (76%), insufficient sleep (38%), and significant daytime sleepiness (49%). All women reported frequent awakenings (100%), and others reported insomnia (57%), restless legs syndrome (24%), and sleep-disordered breathing (19%).[74]

Pregnancy-related symptoms tend to arise and predominate in one or another trimester, with disrupted sleep more prevalent and severe in the last 8 weeks of gestation, making it very challenging to achieve consistent and restorative sleep throughout a pregnancy. These symptoms include nausea and vomiting, indigestion and acid reflux, frequent urination, discomfort from fetal movements, Braxton–Hicks contractions, anxiety, difficulty in assuming usual and comfortable sleep positions, low back pain, muscle cramps, restless legs syndrome, and sleep-disordered breathing.

Sleep-Disordered Breathing

Sleep-disordered breathing (SDB) is characterized by abnormalities of respiration during sleep, including obstructive sleep apnea (OSA), and is highly prevalent in pregnant women. Systematic reviews, meta-analyses, and large cohort studies demonstrate that moderate to severe SDB can be significantly associated with the development of many adverse maternal, pregnancy, and neonatal outcomes, including pregnancy-induced hypertension, preeclampsia, gestational diabetes, longer labor induction, increased incidence of cesarean sections, longer hospital stay, increased odds for admission to intensive care unit, intrauterine growth restriction, low birth weight, and preterm delivery.[75–78]

A recent observational, case-control study, involving participants from an original cohort of 3,160,268 women in California between 2007 and 2012, sought to determine the effects of insomnia during pregnancy by separating the effects of sleep disturbance from other factors that also contribute to the risk of preterm birth. Among the women with a diagnosed sleep disorder (2265 participants), more than 30% had insomnia, 56.9% had sleep apnea, 7.5% had a sleep-related movement disorder, and 5.4% had another sleep disorder. The prevalence of preterm birth (before 37 weeks' gestation) was 10.9% in the matched referent group (2172 participants) compared with 14.6% among women with a diagnosed sleep disorder. The odds of early preterm birth (before 34 weeks' gestation) was more than double for women with sleep apnea [odds ratio [OR], 2.2; 95% CI, 1.5–3.1] and almost double for women with insomnia [OR, 1.7; 95% CI, 1.1–2.6]. The odds of preterm birth were not significantly increased for sleep-related movement disorders.[79]

Restless Leg Syndrome

Restless leg syndrome (RLS) is a sensorimotor phenomenon characterized by an urge to move the legs because of an unpleasant sensation, which is worse during the evening or at night.[80] There is a threefold-greater prevalence of RLS among parous women compared with nulliparous, with symptoms tending to occur most frequently

and strongly during the third trimester and usually subsiding after delivery.[81] Present understanding suggests the high prevalence of RLS during pregnancy may be attributed to several factors, including hemodynamic and hormonal changes, childhood and family history; psychomotor behaviors; and maternal iron, folate, and/or vitamin D deficiency.[82]

In a cohort of 1563 women in their third trimester, recruited from prenatal clinics at the University of Michigan between March 2007 and December 2010, 36% had RLS as diagnosed by standard International RLS Study Group criteria. And 50% of these women had moderate to severe symptoms and were more than twice as likely to experience sleep–wake disturbances of poor sleep quality, poor daytime function, and excessive daytime sleepiness in a dose–response relationship compared with pregnant women without RLS. There were no associations between RLS status and adverse delivery outcomes.[83] Other studies have found RLS to be associated with a higher prevalence of preeclampsia, low birth weight, and preterm birth and an increased incidence of CSs.[84–87]

Considering that women experience significant sleep disruption, high prevalence of symptoms, and sleep disorders throughout pregnancy, screening for sleep disturbances and implementing treatment via nonpharmacological interventions can play an important role in limiting adverse pregnancy and neonatal outcomes.

Substance Use

Substance use is a significant, preventable public health issue and contributor to the global burden of disease, and women are the most vulnerable in their reproductive years. Risk factors for problematic substance use in pregnancy include past alcohol, nicotine, or illicit drug use; unintended pregnancy; lower education level; unemployment; younger age; childhood trauma; intimate partner violence; comorbid physical and mental health problems; and lack of knowledge regarding the effect of substances on fetal development.[88]

Alcohol

Alcohol use prenatally and during pregnancy remains a widespread problem, with in utero exposure to this well-established teratogen being correlated to neonatal birth defects, fetal alcohol spectrum disorders (FASDs), and lifelong developmental disabilities. Worldwide, FASDs are the leading known form of preventable birth defects and developmental, behavioral, and learning disabilities.

According to the CDC Behavioral Risk Factor Surveillance System (2011–2013), among pregnant women, 1 in 10 reported alcohol use, and 1 in 33 reported binge drinking. The highest prevalence of any alcohol use was among those who were 35 to 44 years old, not married, and college graduates. Of those who reported binge drinking (defined as ≥4 drinks on the same occasion within the past 30 days), pregnant women reported an average of 4.6 binge-drinking episodes, which was higher than the average 3.1 binge-drinking episodes reported by nonpregnant women.[89] Social factors such as being single or divorced and intimate partner violence victimization before and after recognition of pregnancy are associated with alcohol consumption.[90]

There is no known safe amount of alcohol intake or safe time to drink alcohol during pregnancy. All types of alcohol are equally harmful to the growing fetus and can lead to FASDs. The same holds true for women trying to conceive. A 2014 systematic review and meta-analysis was conducted to ascertain the possible impact of preconception alcohol consumption on maternal and neonatal outcomes, and a nonsignificant 30% increase in spontaneous abortion was found (RR, 1.30; 95% CI, 0.85–1.97).[91] Prenatal alcohol exposure is correlated with growth restriction and birth defects and is the leading preventable cause of neurodevelopmental deficits in children in the United States, as a result of FASDs. Alcohol use in pregnancy is associated with a significantly increased risk of miscarriage, stillbirth, low birth weight, SGA infants, preterm delivery, and infant mortality, as well as other long-term negative childhood outcomes, including behavior problems and cognitive and motor deficits.[88]

Alcohol use, binge drinking, and drinking during pregnancy are increasing among young women in many countries.[92] Moreover, globally, approximately 41% of all pregnancies are unintended and unplanned, which means many women use alcohol before they become aware of pregnancy,[93] leading to an increased risk of involuntary in utero exposure during the earliest weeks of pregnancy, when the fetal central nervous system development is most sensitive to alcohol's effects. Although studies have shown that a positive pregnancy test may lead to abstaining from or a significant decrease in alcohol use in many women, the intervening lapse of time between that and conception is a critical period with respect to fetal susceptibility to alcohol exposure. Evidence supports the importance of including the preconception period in the definition of drinking patterns during pregnancy[94] and promoting early pregnancy awareness[95] in order to influence the risk and rate of alcohol-exposed pregnancies (AEPs).

Clinical trials investigating preconception behavior modification and counseling interventions have demonstrated significant clinical benefit in reducing AEPs.[91,96,97]

Caffeine

Throughout the world, caffeine is the most widely used psychoactive substance, being a central nervous system stimulant. It occurs naturally or is added to medications, foods, and beverages, with coffee, tea, cola soft drinks, and cocoa being the major dietary sources of caffeine commonly consumed. A cup of instant coffee can contain 50 to 80 mg of caffeine per 350-mL (12-oz) serving, whereas commercially brewed coffee typically contains greater than 150 mg of caffeine per serving. Caffeine-containing teas (black tea and green tea) and soft drinks (colas and iced tea) contain less than 50 mg of caffeine per 250-mL (8-oz) serving.[1]

Once ingested, caffeine is metabolized and rapidly absorbed into the bloodstream, passing all biological membranes, including the placental barrier, resulting in an accumulation of caffeine metabolites in the fetus.[98] During pregnancy, caffeine metabolism is prolonged due to the rate of clearance significantly decreasing from the first to third trimester, resulting in the half-life of maternally ingested caffeine doubling, leading to greater exposure risk for the fetus.[99]

Although the precise mechanisms by which caffeine intake affects maternal, newborn, and child health are not fully understood, caffeine exposure may lead to vasoconstriction of the uteroplacental circulation, thereby affecting fetal growth and development, resulting in neonatal adverse outcomes such as low birth weight and SGA infants, which have been well documented in epidemiological studies.[100] Dose–response meta-analyses of prospective observational trials have found that a maternal caffeine intake level of high versus low or no intake is associated with a significantly increased risk of low birth weight, PTB in the first and second trimesters, and pregnancy loss.[99,101–103] Moreover, a comprehensive systematic review and meta-analysis confirmed prior findings (e.g., Tolstrup [2003][104]) that high maternal preconception caffeine intake of greater than 300 mg/day is significantly associated with an increased risk of subsequent spontaneous abortion by upward of 31%.[91]

A maximum level of caffeine intake for pregnant women, and those in the preconception period, has been stipulated by several authorities, including the WHO, European Food Safety Authority, and American College of Obstetricians and Gynecologists; the recommendation is not to exceed 200 mg/day, based on the evidence regarding its adverse fetal and neonatal effects.[105]

Cannabis

Cannabis sativa (marijuana) is the most commonly used recreational drug during pregnancy and among breastfeeding women, with self-reported prevalence ranging from 2% to 5% in most studies and increasing to 15% to 28% depending on the demographics of the population being studied.[106] A cross-sectional study of 3207 respondents from the 2014–2015 Colorado Pregnancy Risk Assessment Monitoring System, which features state-developed questions on cannabis use, evaluated estimates of prenatal and early postnatal cannabis use in a state with legalized medical and recreational cannabis and the association with adverse neonatal outcomes. The study found that the prevalence of cannabis use in Colorado was 5.7% during pregnancy and 5% (95% CI, 4.1%–6.2%) among women who were breastfeeding. Prenatal cannabis use was associated with a 50% increased risk of low birth weight independent of maternal age, race/ethnicity, level of education, and tobacco use during pregnancy, and of the women who used cannabis during pregnancy, 88.6% also breastfed.[107]

Furthermore, studies report that many women perceive cannabis use to be relatively safe during pregnancy.[108] Using data from the 2007–2012 National Surveys on Drug Use and Health—a cross-sectional nationally representative survey including pregnant (4971 participants) and nonpregnant (88,402) women 18 to 44 years of age—researchers found that approximately 70% of both pregnant and nonpregnant women believe there is slight or no risk of harm from using cannabis once or twice a week.[109]

The psychoactive and medicinal properties of cannabis are mediated by cannabinoids, which are highly lipophilic molecules and readily cross the placental and blood–brain barriers. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two major active cannabinoid constituents (see Chapter 61, Cannabis (Marijuana) and Cannabinoids, for a more complete discussion). Cannabinoids and their metabolites have been detected in many human tissues, including the placenta, as well as amniotic fluid and breast milk, with concentrations found to be several times higher in fetal tissue and breast milk than maternal plasma, depending on cannabis potency, duration of use, and method of consumption.[110] Studies have documented the transfer of measurable levels of major metabolite delta-9-tetrahydrocannabinol (Δ9-THC) in the majority of breast milk samples and for up to 6 days after the last-reported maternal cannabis use.[111] A pilot pharmacokinetic study demonstrated that following a standardized maternal dose of inhaled cannabis, Δ9-THC was transferred into the mother's milk such that an exclusively breastfeeding infant ingests an estimated mean 2.5% (range 0.4%–8.7%) of the maternal dose.[112]

A growing body of research is assessing the effects and impact of cannabis use during pregnancy on maternal, neonatal, and childhood health. Recent studies suggest that in utero cannabis exposure affects fetal development and is associated with increased risk for adverse outcomes, including low birth weight, SGA infants, intrauterine growth restriction, reduced head circumference, preterm labor, stillbirth, and increased neonatal intensive care admission.[88,113]

There is limited to no evidence of any statistically significant associations between cannabis use and adverse maternal events (e.g., gestational diabetes, preeclampsia, placental abruption).[113–115]

Nausea and vomiting of pregnancy (NVP) is the most commonly experienced symptom in early pregnancy. NVP typically occurs during the first trimester but can persist to 20 weeks' gestation in up to 20% of pregnancies.[116] A meta-analysis of the reported prevalence of NVP in the United States found a mean rate of 68.6%, which is similar to that found in other countries.[117] One of the primary medicinal uses of cannabis is as an antiemetic.

In a large, diverse cohort study, pregnant women (220,510 participants) in their first trimester underwent universal cannabis screening between 2009 and 2016 as part of standard prenatal care within a single health care system in California. Participants completed a substance use questionnaire and urine toxicology test at approximately 8 weeks' gestation. The prevalence of severe NVP was 2.3%, and the prevalence of mild NVP was 15.3%. The study found that those women with severe NVP had 3.8 times greater odds, and those with mild NVP had 2.37 times greater odds, of prenatal cannabis use than pregnant women without NVP.[118] The only other study to investigate whether women with NVP have an increased prevalence of cannabis use involved 4735 pregnant women in the Hawaii Pregnancy Risk Assessment Monitoring System data representing all live births between 2009 and 2011. This epidemiological study found that 6.0% of participants used cannabis in the month before pregnancy, and 2.6% used cannabis during pregnancy. Severe NVP was experienced by 21.2% of the sample population. Self-reported cannabis use was significantly more prevalent during pregnancy among women with severe NVP (3.7%) compared with those without NVP (2.3%).[119]

With widespread and growing use of cannabis during pregnancy and breastfeeding, accompanied by evolving policies regarding legalization and increased access in many countries, further research and long-term follow-up data are needed in order to (1) understand the safety and potential impact on infants exposed to varying amounts of cannabis via human milk, coupled with concerns over potential negative neurodevelopment outcomes from in utero exposure, and (2) establish guidelines to better counsel women regarding consideration of anticipated risks and birth outcomes. In the meantime, a 2017 American College of Obstetricians and Gynecologists Committee opinion and a 2018 Society of Obstetricians and Gynaecologists of Canada statement recommend that all pregnant women, breastfeeding women, and those contemplating pregnancy should be counseled and encouraged to discontinue use of cannabis for both recreational and medicinal purposes.[106,120]

Tobacco

Tobacco use is a significant global public health concern that is directly correlated to a litany of diseases and total mortality. A recent Global Burden of Disease, Injuries, and Risk Factor Study estimates that tobacco smoking and exposure to passive smoking accounted for more than 6.1 million deaths worldwide in 2013.[121]

In the United States alone, firsthand or mainstream smoke (which is directly inhaled by the user) is the leading cause of preventable disease and risk for death from all causes in men and women, accounting for nearly 1 in 5 deaths every year (480,000 deaths per year).[122] Secondhand or sidestream (passive) smoke (which is released from the burning source of a cigarette, cigar, pipe, hookah, or vaping device and that which is exhaled by the user) contains more than 7000 chemicals, hundreds of which are known toxins and more than 70 of which are identified carcinogens. There is no risk-free level of exposure to secondhand smoke during pregnancy.[122] Thus, in addition to the alkaloid nicotine, the primary addictive agent in firsthand tobacco smoke, a plethora of toxins and carcinogens from maternal smoking and secondhand smoke exposure readily pass to the developing fetus via the placenta, where concentrations can exceed those in the mother.

Recent studies have found that a growing number of women view electronic nicotine delivery systems (ENDSs), commonly referred to as e-cigarettes or vaping devices, to be a safer alternative to cigarettes during pregnancy.[123,124] However, with the current lack of research on the safety and efficacy of ENDS use in pregnancy,[125] the World Health Organization cautions pregnant women against using ENDSs because of the potential risk to fetal development and adverse birth outcomes from nicotine exposure in the same manner as smoking cigarettes.[126]

A recent population-based, longitudinal cohort study conducted in the United States in 2013–2014 among 5105 participants found that ENDS use results in measurable exposure to nicotine and known tobacco-related toxicants, although at lower levels than cigarette smoking.[127]

During preconception and pregnancy, smoking tobacco remains one of the leading modifiable risk factors for preventable causes of multiple adverse neonatal morbidity and mortality outcomes, including significantly increased risk for ectopic pregnancy, intrauterine growth restriction, premature rupture of membranes, placenta previa, placental abruption, preterm delivery, SGA infants, low birth weight, miscarriage, stillbirth, congenital malformations (e.g., orofacial clefts), and sudden infant death syndrome.[128–136] Smoking during pregnancy is estimated to result in more than 1000 infant deaths annually in the United States.[122] Additionally, maternal exposure to secondhand tobacco smoke during pregnancy is significantly associated with detrimental effects on the fetus, including impaired fetal growth, low birth weight, preterm delivery, and stillbirth.[137,138]

A recent meta-analysis found that cigarette smoking also impairs female reproductive potential and clinical outcomes of assisted reproductive technologies. This study reported a significant decrease in the live birth rate per cycle, a lower clinical pregnancy rate per cycle, and an increase in spontaneous miscarriage rate for patients who smoke.[139]

Despite over two decades of incontrovertible evidence that maternal smoking and secondhand smoke exposure are associated with detrimental perinatal outcomes and increased neonatal morbidity and mortality, national health surveys and epidemiological studies continue to find that many women continue to smoke during pregnancy. In February 2018 the CDC presented the first national data with respect to the prevalence of cigarette smoking at any time during pregnancy among women who gave birth in 2016 in the United States. The report found that 1 in 14 women (7.2%) reported smoking during pregnancy, with the highest prevalence occurring among mothers aged 20 to 24 (10.7%) and then declining with increasing maternal age, with 2.0% among those aged 45 and over. Compared with the nation overall, the prevalence of smoking during pregnancy was lower in 19 states and the District of Columbia and higher in 31 states.[140]

Smoking is a modifiable behavior, and clinical trials demonstrate the effectiveness of cessation and psychosocial intervention programs in preconception and all stages of pregnancy, resulting in improved birth outcomes and reduction in intensive care unit admissions.[128,141] Healthcare providers should thus routinely screen all pregnant women about their tobacco use (past and present) and exposure to secondhand smoke. Risks of firsthand and secondhand smoke exposure should be discussed and tobacco cessation intervention options offered to all pregnant women and those planning to conceive and their partners, whether they are current tobacco users, recent quitters, or nonsmokers.[1]

Environmental Toxin Exposure

A rapidly evolving body of rigorous scientific evidence demonstrates disconcerting verification regarding the profound impact that prenatal and gestational environmental toxin exposure has on human reproductive health and development. The associated adverse health outcomes of these voluntary and involuntary exposures are of great concern in women of childbearing age, in whom the exposure has the potential of inflicting harm in utero.[105]

A 2013 American College of Obstetricians and Gynecologists Committee Opinion (reaffirmed in 2018) recognizes that exposure to both persistent organic pollutants and nonpersistent environmental toxicants in the air, water, and soil and in food and in consumer products is ubiquitous during a sensitive period of fetal development and that maternal exposure to these pollutants has a profound adverse and lasting effect on reproductive health across the life course.[142] Indeed, an analysis of the data from the CDC-conducted 2003–2004 NHANES that investigated 163 chemical analytes in 12 chemical classes in subsamples of 268 pregnant women (a nationally representative sample population in the United States) found detectable levels in 8 of 12 classes of chemicals in 100% of pregnant women. These included polychlorinated biphenyls (PCBs), organochlorine pesticides, perfluorocarbons, phenols, polybrominated diphenyl ethers, phthalates, polycyclic aromatic hydrocarbons, and perchlorate. Across chemical classes, the median number detected ranged from 8 of 17 chemical analytes to 50 of 71 chemical analytes.[143]

Although very likely a substantial underestimation, reports suggest approximately 3% of fetal developmental defects are attributable to parental chemical exposures.[144] Prenatal and gestational exposure to environmental chemicals and toxic metals has been associated with a range of adverse pregnancy and birth outcomes and childhood health consequences, such as spontaneous abortion, preterm birth, low birth weight, SGA infants, congenital anomalies, impaired cognitive development, intellectual impairment, impaired neurodevelopment, and childhood cancer[145] (Table 211.2).

Various toxic chemicals and metals in pregnant women can readily cross the placenta, and in some cases, such as with methylmercury, they can accumulate in the fetus, resulting in higher fetal exposure than maternal exposure.[142] Methylmercury and PCBs are also present in the breast milk of lactating mothers. Mercury is ubiquitously found in all human populations that have been tested, with seafood consumption and dental amalgams being the primary sources of exposure, whereas the main source of PCBs is fish that are farmed or taken from bodies of water with accumulated industrial runoff, such as some lakes and the Baltic Ocean. Prenatal exposure to methylmercury has been directly linked to ongoing neurobehavioral and neurocognitive deficits in offspring, and the disease associations for PCBs range from breast cancer to rheumatoid arthritis.[146]

Underlying biological mechanisms associated with environmental chemical exposure during sensitive windows of in utero development include mutagenesis, mitochondrial electron chain decoupling, epigenetic changes, and endocrine disruption. Mutagens affect DNA directly, whereas epigenetic mechanisms modulate gene expression that is integral to orchestrating human development without changing DNA sequences.[142] A heterogeneous group of environmental toxins titled endocrine-disrupting chemicals (EDCs) has been defined by the Endocrine Society as exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural bloodborne hormones present in the body and responsible for homeostasis and reproduction and developmental processes.[147]

Given that hormonal regulation is critical to human reproduction, EDCs pose a significant burden and public health concern, having been implicated in a wide range of adverse health effects. Before the Endocrine Society published its 2014 executive summary regarding the known health impacts of EDCs, the WHO and United Nations Environment Programme launched a joint 2012 assessment report on the global status of scientific knowledge on exposure to and effects of EDCs, including the development of diseases such as obesity, diabetes mellitus, infertility, female and male reproductive disorders, and hormone-sensitive cancers in women.[147,148] EDCs have also been associated with various adverse birth outcomes, such as spontaneous abortion, PTB, SGA infants, and congenital anomalies. EDCs include biologically persistent organic pollutants, including chlorinated pesticides, PCBs, and dioxins, as well as nonpersistent plastic compounds such as bisphenol A and phthalates, organophosphate pesticides, fungicides, and herbicides.[146]

A primary example of a prevalent EDC is bisphenol A (BPA), a high-volume (>10 billion pounds produced per year) industrial chemical for polycarbonate plastic and epoxy resins. It is widely found in household items and consumer products, including plastic food and beverage containers, inner coatings of cans and jar lids, reusable water bottles, plastic toys,

TABLE 211.2 Examples of Reproductive Health Effects of Prenatal Exposure to Environmental Contaminants

Chemicals	Exposure Sources and Pathways	Reproductive or Developmental Health Effects
Pesticides	Pesticides are applied in large quantities in agricultural, community, and household settings. In 2001 more than 1.2 billion pounds of active ingredients were used in the United States. Pesticides can be ingested, inhaled, and absorbed by the skin. The pathways of pesticide exposure include food, water, air, dust, and soil.	Impaired cognitive development Impaired neurodevelopment Impaired fetal growth Increased susceptibility to testicular cancer Childhood cancer
Solvents	Examples include benzene, toluene, xylene, styrene, 1-bromopropane, 2-bromopropane, perchloroethylene, and trichloroethylene. Solvents include some of the highest-production-volume chemicals in the United States. They are used in plastics, resins, nylon, synthetic fibers, rubber, lubricants, dyes, detergents, drugs, pesticides, glues, paints, paint thinners, fingernail polish, lacquers, detergents, printing and leather-tanning processes, insulation, fiberglass, food containers, carpet backing, and cleaning products. Solvents are a component of cigarette smoke. Exposure is primarily through breathing contaminated air.	Fetal loss Miscarriage
Toluene	Exposure occurs from breathing contaminated air at the workplace or in automobile exhaust, some consumer products, paints, paint thinners, fingernail polish, lacquers, and adhesives.	Decreased fetal and birth weight Congenital malformations
Phthalates	Phthalates are synthetically derived. They are used in a variety of consumer goods, such as medical devices, cleaning and building materials, personal care products, cosmetics, pharmaceuticals, food processing, and toys. Exposure occurs through ingestion, inhalation, and dermal absorption.	Reduced masculine play in boys Reduced anogenital distance Shortened gestational age Impaired neurodevelopment in girls
Lead	Occupational exposure occurs in battery manufacturing and recycling, smelting, car repair, welding, soldering, firearm clearing and shooting, and stained-glass ornament and jewelry production. Nonoccupational exposure occurs in older homes where lead-based paints were used, water pipes, imported ceramics and pottery, herbal remedies, traditional cosmetics, hair dyes, contaminated soil, toys, and costume jewelry.	Alterations in genomic methylation Intellectual impairment Increased likelihood of allergies
Mercury	Mercury from coal-fired power plants is the largest man-made source of mercury pollution in the United States. Primary human exposure is by consumption of contaminated seafood.	Reduced cognitive performance Impaired neurodevelopment
Polychlorinated biphenyls	Polychlorinated biphenyls were used as industrial insulators and lubricants. They were banned in the 1970s but are persistent in the aquatic and terrestrial food chains, resulting in exposure by ingestion.	Development of attention deficit hyperactivity disorder–associated behavior Increased body mass index Reduced IQ
Air pollutants	Common air pollutants include carbon monoxide, lead, ground-level ozone, particulate matter, nitrogen dioxide, and sulfur dioxide. Air pollution arises from a variety of sources, including motor vehicles, industrial production, energy (coal) production, wood burning, and small local sources (e.g., dry cleaners).	Low birth weight Birth defects
Cigarette smoke	Cigarette smoke exposure includes active smoking, passive smoking, or both.	Miscarriage Intrauterine growth restriction, low birth weight, and preterm delivery Decreased semen quality
Perchlorate	Perchlorate is used to produce rocket fuel, fireworks, flares, and explosives and also can be present in bleach and some fertilizers. Sources of exposure are contaminated drinking water, food, and other nonwater beverages. Infants also may be exposed through breast milk.	Altered thyroid function
Perfluorochemicals	Perfluorochemicals are widely used man-made organofluoride compounds with many diverse industrial and consumer product applications. Examples are perfluorooctane sulfonate and perfluorooctanoate, which are used in cookware products with nonstick surfaces and in packaging to provide grease, oil, and water resistance to plates, food containers, bags, and wraps that come into contact with food. They persist in the environment. Occupational exposure and general population exposure occur by inhalation, ingestion, and dermal contact.	Reduced birth weight
Polybrominated diphenyl ethers	These include flame-retardant materials that persist and bioaccumulate in the environment. They are found in furniture, textiles, carpeting, electronics, and plastics that are mixed into but not bound to foam or plastic.	Impaired neurodevelopment Premature delivery, low birth weight, and stillbirth
Bisphenol A	Bisphenol A is a chemical intermediate for polycarbonate plastic and resins. It is found in food, consumer products, and packaging. Exposure occurs through inhalation, ingestion, and dermal absorption.	Recurrent miscarriage Aggression and hyperactivity in female children
Formaldehyde	Formaldehyde is used in the production of wood adhesives, abrasive materials, and other industrial products and in clinical laboratories and embalming. It is found in some germicides, fungicides, insecticides, and personal care products. Routes of exposure are oral, dermal, and inhaled.	Spontaneous abortion Low birth weight

TABLE 211.2 Examples of Reproductive Health Effects of Prenatal Exposure to Environmental Contaminants—cont'd

Chemicals	Exposure Sources and Pathways	Reproductive or Developmental Health Effects
Antineoplastic drugs	This class of chemotherapy drugs presents an occupational exposure risk for nurses and other healthcare professionals.	Spontaneous abortion Low birth weight
Anesthetic gases	Anesthetic gases are administered by inhalation in healthcare settings and veterinary care. Occupational exposure is a risk for nurses, physicians, dentists, veterinarians, and other healthcare professionals who work in settings where anesthetic gases are used.	Congenital anomalies Spontaneous abortion
Ethylene oxide	Ethylene oxide is used to sterilize heat-sensitive medical items, surgical instruments, and other objects that come into contact with biological tissues. Occupational exposure is a risk in some healthcare settings, particularly sterilization units. Exposure is through inhalation.	Spontaneous abortion and pregnancy loss Preterm and postterm birth

Modified from Patrice Sutton MPH et al. Toxic environmental chemicals: the role of reproductive health professionals in preventing harmful exposures. *American Journal of Obstetrics and Gynecology*. Volume 207, Issue 3, September 2012, Pages 164-173.

mobile phone cases, eyewear, thermal receipt paper, electronic equipment, dental sealants, and medical devices. The 2003–2004 NHANES conducted by the CDC found detectable levels of BPA in 93% of urine samples (2517 participants) from people 6 years of age and older.[149] The CDC NHANES data are considered representative of exposures in the United States and thus demonstrate the ubiquitous nature of BPA exposure.

In February 2018 the European Commission updated its regulations concerning the use of BPA, and the provisions in the law became effective in September 2018.[150] The new rules are intended to tighten restrictions on the use of BPA in food contact materials and better protect children under the age of 3 years old by extending the ban on the use of BPA to include, in addition to infant feeding bottles, drinking cups or bottles intended for infants and young children. The strengthened regulation lowers the specific migration limit, which is the amount of substance that can migrate from food contact materials (FCMs) into the food, by twelvefold, taking the allowable limit from 0.6 mg BPA per kg of food to 0.05 mg/kg. Denmark and Belgium entirely banned the use of BPA in FCMs for infants and young children. Sweden and France have banned the use of BPA in coatings and varnishes in FCMs intended for infants and young children. In the United States, the Department of Health and Human Services National Toxicity Program convened the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (Clarity-BPA) in February 2012. Despite the results of the Clarity-BPA Core Study Report (released in September 2018) showing that BPA exposure, in the animal model used, can cause adverse health effects at low doses, the U.S. Food and Drug Administration (FDA) asserted that BPA causes "minimal" health effects and is safe for currently authorized uses in food containers and packaging. Elevated BPA levels have been associated with recurrent miscarriages, decreased number of oocytes retrieved in in vitro fertilization, fetuses with an abnormal karyotype, and chromosomal abnormalities.[146]

A retrospective cohort study found that maternal conjugated BPA (cBPA) was associated with a higher risk of first-trimester miscarriage. Of the 115 participants seeking treatment for infertility or recurrent pregnancy loss at the Stanford Fertility Reproductive Medicine Clinic, there were 47 live births and 68 clinical miscarriages (46 aneuploid, 22 euploid). Stored serum samples from 4 to 5 weeks' gestation were analyzed for cBPA concentration. Median serum cBPA levels were higher in women who miscarried (0.101 ng/mL) compared with women who had live birth (0.075 ng/mL; $P = 0.014$). Women with a cBPA level in the highest quartile demonstrated an 83% greater risk of experiencing a first-trimester miscarriage than women with a cBPA level in the lowest quartile.[151]

TABLE 211.3 Prevention and Preconception Care Toxicant Testing

Prevention and Preconception Care Toxicant Testing

Currently available toxicant and toxicant metabolite testing:

- Serum polychlorinated biphenyl
- Serum solvents
- Urinary bisphenol A
- Urinary glyphosate metabolites
- Urinary metals
- Urinary organophosphate pesticide metabolites
- Urinary solvent metabolites

Additional testing:

- Aromatic hydrocarbon exposure
- Perfluorocarbons

Modified from Crinnion WJ, Pizzorno JE. (2018). *Clinical Environmental Medicine: Identification and Natural Treatment of Disease Caused by Common Pollutants*. St. Louis: Elsevier.

Prenatal exposure to environmental toxins is linked to wide-ranging adverse health consequences that can manifest across the life span and potentially be transmitted to the next generation.[152] A significant step toward improving fetal health and pregnancy outcomes should involve preconception counseling that consists of (1) taking a detailed history to identify potential sources of toxicant exposure, (2) raising awareness and informing women of childbearing age about the adverse effects of such exposures, (3) assisting in the identification of chemical exposures, and (4) providing practical lifestyle modifications for toxicant avoidance and removal strategies. Presently, available toxicant and toxicant metabolite testing can assist in identifying exposure to certain compounds that a woman should avoid (Table 211.3).

Mind–Body Medicine

A woman's mental and emotional health during pregnancy and postnatally are of primary importance to the well-being of both the mother and her child, given that it is a time of rapid and sweeping transition in a woman's life, encompassing biological, physical, psychological, and social changes. And although many women welcome the challenges of childbirth, others may feel a significant amount of perceived stress, anxiety, and depression with pregnancy. Prevalence estimates suggest that at least 10% of pregnant women experience perinatal anxiety and

that upward of 20% are affected by depression during pregnancy and are equally likely to suffer from postpartum depression.[153,154]

A growing body of evidence associates both depression and anxiety in pregnancy with an increase in obstetric complications and adverse neonatal outcomes, including PTB, low birth weight, and small fetal head size, which are themselves linked to increased risk of neonatal mortality and morbidity and neurodevelopmental impairment, as well as impaired language development and behavioral and cognitive problems later in life.[155–161]

Reducing maternal stress, anxiety, and depression has been found to result in healthy delivery at term.[162,163] However, up to 10% of women are exposed to selective serotonin reuptake inhibitors (SSRIs) during pregnancy,[164] and clinical studies demonstrate that prescribed antidepressant medication carries a significant risk of adverse neonatal outcomes, including preterm birth, low birth weight, decreased gestational length, low Apgar scores, congenital anomalies, persistent pulmonary hypertension, respiratory distress, convulsions, and hypoglycemia.[165–167] Antidepressant exposure in utero affects birth and neonatal outcomes independently of antenatal maternal depression.[168] A large, international, collaborative meta-analysis found a significantly increased risk for neonatal hospital readmission, major malformations, and infant morbidity due to in utero lithium exposure during the first trimester compared with unexposed babies.[169] Therefore, in addition to preconception discussions with one's healthcare provider regarding the appropriateness and potential risk of medication use in pregnancy, interest in effective nonpharmacological mind–body medicine approaches has been gaining increasing attention for the treatment of anxiety and depression during pregnancy and the postnatal period.

Two recent systematic reviews and meta-analyses assessed the effect of mindfulness-based interventions (MBIs) on maternal perinatal mental health outcomes, including mindfulness-based stress reduction, mindfulness-based cognitive therapy, and integrative mindfulness yoga practice. Maternal MBI participation is associated with reductions in perinatal anxiety, perceived stress, and depression and increased mindfulness.[154] The practice of MBI helps develop and support abilities that are important for pregnant women by encouraging awareness and acceptance of one's thoughts, emotions, and body sensations; reducing reactivity to uncomfortable experiences; and building stress tolerance and resiliency.[170]

DIETARY SUPPLEMENTATION

Prenatal Multivitamin

Adequate nutrition is particularly important during pregnancy because it not only maintains the health of the mother but also determines the course of the pregnancy and its outcome, fetal development, and the child's health after birth and later in life.[171] A growing body of research finds, however, that a majority of women of reproductive age will not be nutritionally prepared for pregnancy because they do not meet even the lower Reference Nutrient Intake (RNI) recommendations.[28] Prenatal multivitamin–mineral supplement recommendations are becoming an attractive option considered by international agencies, such as UNICEF, to improve the nutritional status of pregnant women, particularly in developing countries.[172] Multiple micronutrient deficiencies often coexist among women of reproductive age, particularly in low- to middle-income countries (LMICs) but also in developed countries, and these are exacerbated during pregnancy due to increased physiological demands, whereupon such deficiencies can lead to adverse maternal and neonatal events.[173]

A Cochrane review evaluated the effects of multiple-micronutrient (MMN) supplementation during pregnancy on maternal, fetal, and infant health outcomes. The study included 17 prospective, randomized, controlled trials (138,538 participants), with 15 of the trials conducted in LMICs and 2 carried out in the United Kingdom. Overall, pregnant women who received MMN supplementation (containing iron and folic acid) had reduced rates of low-birth-weight and SGA babies.[173] An additional systematic review found similar findings, in that significant benefit was found with MMN supplementation during pregnancy in reducing low birth weight and SGA.[174] An earlier Cochrane review assessed a total of 40 randomized and quasi-randomized clinical trials comparing MMN supplementation during pregnancy with placebo, no vitamins, or other interventions. Included were studies that began MMN supplementation before conception, periconceptionally, or in early pregnancy (less than 20 weeks' gestation). The use of MMN supplementation (containing iron and folic acid) before pregnancy or in early pregnancy was found to decrease the risk for stillbirth (death of baby before 20 weeks' gestation). No other significant findings were demonstrated with respect to maternal or fetal and neonatal outcomes.[175]

With heightening maternal awareness and scientific evidence demonstrating potential adverse pregnancy and neonatal outcomes with respect to in utero toxicant exposures, increasing widespread attention is being paid to the natural products industry, good manufacturing processes, and minimizing contamination, including toxic metals within prenatal supplements. A 2018 study conducted in Edmonton, Alberta, Canada, sought to evaluate toxic element contamination in 26 "commonly used prenatal vitamin brands including one prescription brand," representing a sample of convenience using readily available over-the-counter products collected from assorted retail outlets, including health food stores, pharmacies, and food retailers. In total, 51 samples were sent to a laboratory for independent analysis. The findings revealed that all samples contained lead, with average amounts being 0.535 mcg per day, which is above the stringent Proposition 65 limits published in California (P65L) of 0.5 mcg/day for prenatal vitamins. Of the 26 products analyzed, 14 had higher-than-acceptable levels of lead, with one supplement yielding 4.0 mcg/day. All samples contained cadmium but did not exceed P65L. Mercury was detected in all samples but did not exceed P65L of <0.3 mcg/day, with the highest level of exposure being 0.095 mcg/day. All samples contained arsenic, with an average exposure of 0.42 mcg/day. Four samples had >1.0 mcg/day exposure, and upon arsenic speciation subanalysis, three out of these four samples contained toxic inorganic arsenic species >0.1 mcg/day, above the acceptable P65L, with one sample having 0.4 mcg/day of exposure. Aluminum, nickel, thallium, and titanium were detected in all samples but did not exceed P65L or *U.S. Pharmacopeia* limits. The authors concluded that cumulative daily intake of prenatal supplementation over many months may constitute a significant source of gestational toxic element exposure for both the mother and the developing fetus.[176]

Although daily prenatal supplementation is widely recommended before conception, through gestation, and during lactation for women in developed countries, such as the United States, Canada, and European countries, according to a 2017 Cochrane systematic review, there is currently insufficient high-quality evidence demonstrating their effect on pregnancy outcomes, and further research is required.[173] In the United States, average daily intake guidelines for individual micronutrients and macronutrients have been established by the NAM for women of childbearing age and for pregnant and lactating women; these are referred to as Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) (Tables 211.4 and 211.5).

Biotin

Biotin, also known as vitamin B_7, is a water-soluble nutrient that plays an essential role as a cofactor in carboxylation reactions and metabolic processes, including fatty-acid synthesis, branched-chain amino acid

TABLE 211.4 Dietary Reference Intakes: Recommended Dietary Allowances and Adequate Intakes—Vitamins

Food and Nutrition Board. National Academies of Sciences, Engineering, and Medicine

Life-Stage Group	Vitamin A (µg/d)[a]	Vitamin C(mg/d)	Vitamin D(µg/d)[b,c]	Vitamin E(mg/d)[d]	Vitamin K(µg/d)	Thiamin (mg/d)	Riboflavin (mg/d)	Niacin (mg/d)[e]	Vitamin B_6(mg/d)	Folate (µg/d)[f]	Vitamin B_{12}(µg/d)	Pantothenic Acid (mg/d)	Biotin(µg/d)	Choline (mg/d)[g]
Infants														
0–6 mo	400*	40*	10[h]	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
7–12 mo	500*	50*	10[h]	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 y	300	15	15	6	30*	0.5	0.5	6	0.5	150	0.9	2*	8*	200*
4–8 y	400	25	15	7	55*	0.6	0.6	8	0.6	200	1.2	3*	12*	250*
Males														
9–13 y	600	45	15	11	60*	0.9	0.9	12	1.0	300	1.8	4*	20*	375*
14–18 y	900	75	15	15	75*	1.2	1.3	16	1.3	400	2.4	5*	25*	550*
19–30 y	900	90	15	15	120*	1.2	1.3	16	1.3	400	2.4	5*	30*	550*
31–50 y	900	90	15	15	120*	1.2	1.3	16	1.3	400	2.4	5*	30*	550*
51–70 y	900	90	15	15	120*	1.2	1.3	16	1.7	400	2.4[i]	5*	30*	550*
>70 y	900	90	20	15	120*	1.2	1.3	16	1.7	400	2.4[i]	5*	30*	550*
Females														
9–13 y	600	45	15	11	60*	0.9	0.9	12	1.0	300	1.8	4*	20*	375*
14–18 y	700	65	15	15	75*	1.0	1.0	14	1.2	400[i]	2.4	5*	25*	400*
19–30 y	700	75	15	15	90*	1.1	1.1	14	1.3	400[i]	2.4	5*	30*	425*
31–50 y	700	75	15	15	90*	1.1	1.1	14	1.3	400[i]	2.4	5*	30*	425*
51–70 y	700	75	15	15	90*	1.1	1.1	14	1.5	400	2.4[i]	5*	30*	425*
> 70 y	700	75	20	15	90*	1.1	1.1	14	1.5	400	2.4[i]	5*	30*	425*

TABLE 211.4 Dietary Reference Intakes: Recommended Dietary Allowances and Adequate Intakes—Vitamins—cont'd

Food and Nutrition Board. National Academies of Sciences, Engineering, and Medicine

Pregnancy														
14–18 y	750	80	15	15	75*	1.4	1.4	18	1.9	600[k]	2.6	6*	30*	450*
19–30 y	770	85	15	15	90*	1.4	1.4	18	1.9	600[k]	2.6	6*	30*	450*
31–50 y	770	85	15	15	90*	1.4	1.4	18	1.9	600[k]	2.6	6*	30*	450*
Lactation														
14–18 y	1,200	115	15	19	75*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*
19–30 y	1,300	120	15	19	90*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*
31–50 y	1,300	120	15	19	90*	1.4	1.6	17	2.0	500	2.8	7*	35*	550*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) m ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR. and thus calculate an RDA. an AI is usually developed For healthy breastfed infants, an AI is the mean intake. The AI for other life-stage and gender groups is believed to cover the needs of all healthy individuals m the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalents (RE), whereas the RAE for preformed vitamin A is the same as RE.

[b]As cholecalciferol. 1 ug cholecalciferol = 40 IU vitamin D.

[c]Under the assumption of minimal sunlight

[d]As α-tocopherol. α-Tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2*R*-stereoisomenc forms of α-tocopherol *(RRR-. RSR-, RRS-.* and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2S-stereoisomeric forms of α-tocopherol (*SRR-, SSR-, SRS-,* and *SSS*-α-tocopherol), also found in fortified foods and supplements

[e]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).

[f]As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.

[g]Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[h]Life-stage groups for infants were 0–5.9 and 6–11.9 months.

[i]Because 10 to 30 percent of older people may malabsorb food-bound B12, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B12 or a supplement containing B12.

[j]In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet

[k]It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period—the critical time for formation of the neural tube.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B*12, *Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and* Sulfate (2005); *and Dietary Reference Intakes for Calcium and Vitamin D* (2011). These reports may be accessed via www.nap.edu.

From http://nationalacademies.org/hmd/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx.

TABLE 211.5 Dietary Reference Intakes: Recommended Dietary Allowances and Adequate Intakes—Elements

Food and Nutrition Board, National Academies of Sciences, Engineering, and Medicine

Life-Stage Group	Calcium (mg/d)	Chromium (μg/d)	Copper (μg/d)	Fluoride (mg/d)	Iodine (μg/d)	Iron (mg/d)	Magnesium (mg/d)	Manganese (mg/d)	Molybdenum (μg/d)	Phosphorus (mg/d)	Selenium (μg/d)	Zinc (mg/d)	Potassium (mg/d)	Sodium (mg/d)	Chloride (g/d)
Infants															
0–6 mo	200*[a]	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	400*	110*	0.18*
7–12 mo	260*[a]	5.5*	220*	0.5*	130*	11	75*	0.6*	3*	275*	20*	3	860*	370*	0.57*
Children															
1–3 y	700	11*	340	0.7*	90	7	80	1.2*	17	460	20	3	2,000*	800*	1.5*
4–8 y	1,000	15*	440	1*	90	10	130	1.5*	22	500	30	5	2,300*	1,000*	1.9*
Males															
9–13 y	1,300	25*	700	2*	120	8	240	1.9*	34	1,250	40	8	2,500*	1,200*	2.3*
14–18 y	1,300	35*	890	3*	150	11	410	2.2*	43	1,250	55	11	3,000*	1,500*	2.3*
19–30 y	1,000	35*	900	4*	150	8	400	2.3*	45	700	55	11	3,400*	1,500*	2.3*
31–50 y	1,000	35*	900	4*	150	8	420	2.3*	45	700	55	11	3,400*	1,500*	2.3*
51–70 y	1,000	30*	900	4*	150	8	420	2.3*	45	700	55	11	3,400*	1,500*	2.0*
>70 y	1,200	30*	900	4*	150	8	420	2.3*	45	700	55	11	3,400*	1,500*	1.8*
Females															
9–13 y	1,300	21*	700	2*	120	8	240	1.6*	34	1,250	40	8	2,300*	1,200*	2.3*
14–18 y	1,300	24*	890	3*	150	15	360	1.6*	43	1,250	55	9	2,300*	1,500*	2.3*
19–30 y	1,000	25*	900	3*	150	18	310	1.8*	45	700	55	8	2,600*	1,500*	2.3*
31–50 y	1,000	25*	900	3*	150	18	320	1.8*	45	700	55	8	2,600*	1,500*	2.3*
51–70 y	1,200	20*	900	3*	150	8	320	1.8*	45	700	55	8	2,600*	1,500*	2.0*
>70 y	1,200	20*	900	3*	150	8	320	1.8*	45	700	55	8	2,600*	1,500*	1.8*

TABLE 211.5 Dietary Reference Intakes: Recommended Dietary Allowances and Adequate Intakes—Elements—cont'd

Food and Nutrition Board, National Academies of Sciences, Engineering, and Medicine

Pregnancy															
14–18 y	**1,300**	29*	**1,000**	3*	**220**	**27**	**400**	2.0*	**50**	**1,250**	**60**	**12**	2,600*	1,500*	2.3*
19–30 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	2,900*	1,500*	2.3*
31–50 y	**1,000**	30*	**1,000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	2,900*	1,500*	2.3*
Lactation															
14–18 y	**1,300**	44*	**1,300**	3*	**290**	**10**	**360**	2.6*	**50**	**1,250**	**70**	**13**	2,500*	1,500*	2.3*
19–30 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	2,800*	1,500*	2.3*
31–50 y	**1,000**	45*	**1,300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	2,800*	1,500*	2.3*

NOTE: This table (taken from the DRI reports, see www.nap.edu) presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk(*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a group. It is calculated from an Estimated Average Requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life-stage and gender groups is believed to cover the needs of all healthy individuals in the groups, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake.

[a]Life-stage groups for infants were 0–5.9 and 6–11.9 months.

SOURCES: *Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride* (1997); *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline* (1998); *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (2000); and *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* (2001); *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and* Sulfate (2005); *Dietary Reference Intakes for Calcium and Vitamin D* (2011); and *Dietary Reference Intakes for Sodium and Potassium* (2019). These reports may be accessed via www.nap.edu.

From http://nationalacademies.org/hmd/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx.

catabolism, and gluconeogenesis. Biotin has been found to have additional roles in the regulation of gene expression, genome stability, and cell proliferation.[177]

Frank, symptomatic biotin deficiency is believed to be rare.[178] However, evidence demonstrates that pregnancy is associated with an increased demand for biotin, and thus a marginal degree of biotin deficiency may occur in upward of 50% of pregnant women during normal gestation.[179,180] The dietary requirement for biotin is currently unknown. The AI for healthy adults and pregnant women is 30 µg biotin per day, although preliminary research suggests an upward adjustment may be necessitated for pregnancy and lactation (AI 35 µg/day). The first human pregnancy trial controlling for dietary biotin intake and quantitating biotin content of the diet sought to assess the impact of reproductive state on biotin-status response. The study demonstrated significant alterations in biomarkers of biotin metabolism and deficiency during pregnancy and lactation and suggested that biotin intakes of greater than 2 to 3 times the current AI of 60 to 90 µg/day are likely needed to meet the demands of these reproductive states.[177]

Marginal biotin deficiency is teratogenic in mouse dam studies.[181] However, further human clinical research is required to ascertain whether suboptimal biotin status spontaneously occurring in a substantial proportion of pregnant women may have an impact on biotin supply to the developing fetus and confer an increased risk of poor neonatal outcomes.

Calcium

Calcium is the most abundant mineral in the body, given its involvement in a multitude of fundamental functions, including muscle contraction, blood clotting, cell signaling, nerve conduction, insulin secretion, and maintenance of bone mineral density. During pregnancy, physiological calcium absorption increases to meet fetal bone mineralization requirements, as well as during lactation to meet the demands of the developing infant. This upregulated calcium requirement is related to transient maternal bone loss associated with childbearing. Randomized clinical trials suggest that calcium supplementation during pregnancy and in the early postpartum period may constitute a practical intervention for reducing bone resorption.[182]

Epidemiological data demonstrate an association between low dietary calcium intake and an increased incidence of preeclampsia, a major cause of maternal morbidity and mortality worldwide.[1] Preeclampsia is a risk factor for preterm birth. A recent Cochrane systematic review and meta-analysis that included 13 randomized controlled trials involving 15,730 participants compared high-dose calcium (≥1000 mg/day) during pregnancy to placebo. The study found a significant reduction in both the average risk of high blood pressure and the risk of preeclampsia, particularly when calcium was taken in the second half of pregnancy. The treatment effect was largest for women having calcium-poor diets and those at higher risk of developing preeclampsia. Both the composite outcome of maternal death or serious morbidity and the average risk of preterm birth were reduced with calcium supplementation.[183] Limited evidence on low-dose calcium supplementation (<1000 mg/day, usually 500 mg/day) suggests a reduction in hypertension, preeclampsia, and admission to neonatal intensive care.[183]

Calcium supplementation prenatally or beginning in early pregnancy and continued at least until mid-pregnancy has also been investigated for its potential in preventing maternal hypertensive disorders. A Cochrane review did not identify any randomized controlled trials where calcium supplementation commenced prepregnancy; the review concluded that there is insufficient evidence on the effectiveness or otherwise of pre- or early-pregnancy calcium supplementation for preventing hypertensive disorders.[184]

The Dietary Reference Intake (DRI) for calcium in pregnancy is the same as that of nonpregnant women of the same age (1000 mg/day) because of the increased efficiency of calcium absorption during pregnancy and maternal bone calcium mobilization.[185] Women with suboptimal dietary calcium intake (<500 mg/day) may need additional supplementation to meet both maternal requirements and fetal development needs.[186]

Carnitine

Carnitine is a hydrophilic amino acid derivative that is critical for energy production, with a primary role in facilitating the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix. Carnitine binds acyl residues and maintains homeostasis of coenzyme-A. It also possesses antioxidant capacity, inhibits free-radical production, and reduces oxidative stress. Carnitine is primarily sourced from dietary intake, with biosynthesis contributing approximately 25% of circulating carnitine levels.[187]

Plasma carnitine concentration is substantially lower during pregnancy compared with nonpregnant women,[188–190] and research demonstrates that normal pregnancy increases urinary loss of carnitine and significantly reduces free and total plasma carnitine concentrations. Total plasma carnitine levels in nonpregnant women of reproductive age average 39 µmol/L[191]; however, carnitine concentrations can decrease precipitously during gestation and at term can be as low as 15 µmol/L, a level also found in carnitine-deficient adults.[192]

Carnitine is the generic term for several compounds, including L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine. A randomized, single-blind, placebo-controlled trial found that plasma carnitine reduction in pregnancy is safely prevented by supplementing with 500 mg of L-carnitine L-tartrate from week 13 of gestation to term versus placebo.[193]

Lower levels of serum L-carnitine may be associated with obesity at term pregnancy. In a study of 118 healthy women with a singleton pregnancy (≥37 weeks), women with a prepregnancy BMI ≥ 29.9 kg/m at term had significantly lower serum L-carnitine levels. Serum total L-carnitine levels correlated significantly and negatively with prepregnancy body weight, prepregnancy BMI, pregnancy bodyweight, pregnancy BMI, and serum triglyceride levels. The authors suggest that nutritional supplementation of L-carnitine can be offered to women who are overweight or obese prenatally or at the beginning of pregnancy.[194]

Elevated levels of plasma free fatty acids (PFFAs) are considered a main cause of insulin resistance and, consequently, type 2 diabetes. L-carnitine supplementation of 2 g per day, beginning in the 20th week of gestation, resulted in a significant decrease in PFFAs. An associated increase (five- to tenfold) was found in the relative mRNA abundances of enzymes involved in regulating fatty acid and glucose oxidation.[195] L-carnitine supplementation may thus help prevent the development of gestational diabetes, especially in overweight women.

Given that suboptimal carnitine levels may lead to complications associated with pregnancy, supplementation with L-carnitine throughout pregnancy may be advisable.[196] Animal-derived foods that provide carnitine-rich dietary sources (the active form in the body and found in food is L-carnitine) include red meat, dairy products, fish, and poultry. Because fruits, vegetables, and grains contain relatively little carnitine, vegans and vegetarians consume considerably less carnitine (10–12 mg per day) versus adults who eat an omnivorous diet (60–180 mg per day)[197] and may therefore consider taking an L-carnitine dietary supplement.

Carotenoids

Carotenoids are a family of naturally occurring fat-soluble pigments synthesized by plants, algae, and photosynthetic microorganisms.

More than 700 carotenoids belonging to groups of carotenes (e.g., β-carotene, lycopene) have been identified, as well as their hydroxylated derivatives—xanthophylls (e.g., lutein, zeaxanthin, astaxanthin).[171] Fruits and vegetables offer the majority of the approximately 50 carotenoids found in the human diet. However, 95% of carotenoids present in plasma and tissues are represented by only six such compounds: α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin.[198] Some carotenoids can be converted to vitamin A, the most common example being β-carotene. However, surprisingly common SNP variants substantially decrease this conversion (see Chapter 125, Vitamin A, for a full discussion). This is of substantial significance for vegetarian and, especially, vegan pregnant women. The findings of the Norwegian Mother and Child Cohort Study confirm that plasma levels of carotenoids in pregnant women are a direct function of their consumption of fruits and vegetables,[199] and carotenoid status in the newborn depends on the nutritional status of the mother.

The beneficial health effects of carotenoids are primarily attributed to their potent antioxidant and anti-inflammatory properties.[200] Oxidative stress has been associated with adverse maternal and neonatal outcomes, including preeclampsia, GDM, SGA infants, intrauterine growth restriction (IUGR), and PTB.[171]

Increases in maternal serum concentration of lycopene have been associated with a decrease in rates of preeclampsia and IUGR. For example, a prospective, randomized controlled trial involving 251 primigravida women assessed the effect of 2 mg lycopene administered twice daily (116 participants) compared with placebo (115 participants), beginning in the second trimester and followed until delivery, on the development of preeclampsia, mode of delivery, and fetal outcomes. Compared with the placebo group, the lycopene group developed significantly less preeclampsia, mean diastolic blood pressure was significantly lower, mean fetal weight was significantly higher, and the mean incidence of IUGR was significantly lower.[201]

A 2018 cohort study of 180 maternal-infant dyads sought to investigate associations between serum lycopene concentrations, including lycopene isomers, and maternal-newborn outcomes. The trial found that maternal serum concentrations of total lycopene and the cis-isoform were positively associated with growth parameters in the newborn, including infant birth weight, length, and head circumference. Maternal concentrations of cis-lycopene were significantly lower in mothers whose infants developed respiratory distress syndrome and in mothers whose babies were admitted to the newborn intensive care unit compared with those who were not.[202]

Approximately 30% to 40% of preterm birth is due to premature rupture of membranes. Increased maternal serum concentrations of carotenoids (α- and β-carotene, β-cryptoxanthin, and lycopene) are associated with decreased risk of premature birth.[203] A higher risk of preterm birth has been observed in women with a lower intake of β-carotene.[204] High maternal plasma concentrations of carotenoids (β-carotenoid, lutein, zeaxanthin, α- and β-carotenoid) during pregnancy decrease the risk of SGA infants and IUGR.[205]

Carotenoids are not considered essential nutrients at this time, so there is currently no dietary intake recommendation for pregnant and breastfeeding women. However, during pregnancy and lactation, diets rich in fruits and vegetables are highly recommended.

Choline

Choline is an essential micronutrient that has far-reaching and diverse functions throughout the life span. Choline is primarily derived exogenously from dietary sources. Small amounts are endogenously synthesized via several metabolic pathways, primarily in the form of phosphatidylcholine; however, the amount is not sufficient to meet human requirements for the nutrient.[206] With respect to one such hepatic pathway, phosphatidylethanolamine N-methyltransferase (PEMT) is estrogen dependent and thus exponentially upregulated and active during pregnancy, when estradiol concentrations can rise to approximately 60 times greater at term than those of nonpregnant women.[207] A study using stable isotope methodology examined the effects of pregnancy on choline partitioning and metabolic activity of the PEMT pathway. Healthy women in their third trimester (26 participants, initially 27th week of gestation) and nonpregnant women (21 participants) consumed 22% of their total choline intake (480–930 mg/day) as a methyl-d9-choline for the final 6 weeks of a 12-week feeding study. Compared with the nonpregnant group, an incremental increase in the selective transfer of PEMT-derived phosphatidylcholine across the placenta and into the fetal compartment was demonstrated in pregnant women.[208] The estrogen-regulated enhanced capacity for endogenous synthesis of choline demonstrates the importance of augmenting choline production in order to meet the heightened requirement for healthy fetal growth and development.

Choline plays a central role in many biochemical processes, being metabolized into four key biological compounds: phosphatidylcholine, acetylcholine, sphingomyelin, and betaine. For example, choline is fundamental to the formation and structural integrity of cell membranes and serves as a source of methyl groups, thereby supporting cellular methylation processes. As a substrate for acetylcholine, choline is critical to the production of this neurotransmitter and nonneuronal cell-signaling molecule necessary for memory, mood, muscle control, and other brain and nervous system functions.[206]

As such, choline and metabolites are in high demand during pregnancy due to the increased rate of sequestration by the growing fetus. The rapid rate of cell division and stem-cell proliferation, expansion of the maternal kidneys and uterus, and growth of placental and fetal tissue, as well as increased lipoprotein requirements for nervous system development, all deplete maternal choline stores. Choline is transferred across the placenta against its concentration gradient, resulting in very high amounts in the fetal compartment to ensure enhanced availability. The choline concentration in amniotic fluid is tenfold greater than that present in maternal blood, and plasma/serum choline concentrations are six- to sevenfold higher in the fetus and newborn than in the adult.[207]

Choline deficiency during pregnancy can alter DNA methylation in the placenta and cord blood and has been associated with neural tube defects, cleft lip, hypospadias, cardiac defects, and suboptimal brain development of the fetus and in infants.[209]

Choline was recognized as an essential nutrient for public health by the NAM in 1998.[210] Similarly, in 2016, the European Food Safety Authority set dietary recommendations for choline.[211] In 2017 delegates at the annual meeting of the American Medical Association voted to pass a resolution in support of including the evidence-based amount of choline (450 mg) in all prenatal multivitamin supplements.[212] However, this has yet to be widely implemented by natural product manufacturers, despite prevailing scientific evidence and clinical recognition of the essential role choline plays during pregnancy and lactation.

There is significant variation in the dietary requirement for choline, due in part to common genetic polymorphisms, and the vast majority of women are not achieving the NAM-established AI)of 450 mg/day for all trimesters and 550 mg/day during lactation.[213] An analysis from the NHANES (2005–2014 data sets for pregnant women) found that only 8.51% ± 2.89% of pregnant women in the United States meet the AI for choline.[206] In other words, approximately 9 out of every 10 pregnant women do not obtain the recommended daily amount of choline.

Moreover, preliminary evidence suggests that the current AI for choline may be suboptimal to meet the significant demand during pregnancy and lactation. A small clinical trial sought to quantify the effects of pregnancy and maternal choline intake on maternal and fetal indicators of choline metabolism. Twenty-six healthy pregnant women

TABLE 211.6 Selected Food Sources of Choline

Food	Milligrams (mg) per Serving	Percent DV[a]
Egg, hard-boiled, 1 large egg	147	27
Beef top round, separable lean only, braised, 3 ounces	117	21
Soybeans, roasted, ½ cup	107	19
Chicken breast, roasted, 3 ounces	72	13
Beef, ground, 93% lean meat, broiled, 3 ounces	72	13
Fish, cod, Atlantic, cooked, dry heat, 3 ounces	71	13
Mushrooms, shiitake, cooked, ½ cup pieces	58	11
Potatoes, red, baked, flesh and skin, 1 large potato	57	10
Wheat germ, toasted, 1 ounce	51	9
Beans, kidney, canned, ½ cup	45	8
Quinoa, cooked, 1 cup	43	8
Milk, 1% fat, 1 cup	43	8
Yogurt, vanilla, nonfat, 1 cup	38	7
Brussels sprouts, boiled, ½ cup	32	6
Broccoli, chopped, boiled, drained, ½ cup	31	6
Cottage cheese, nonfat, 1 cup	26	5
Fish, tuna, white, canned in water, drained in solids, 3 ounces	25	5
Peanuts, dry roasted, ¼ cup	24	4
Cauliflower, 1-inch pieces, boiled, drained, ½ cup	24	4
Peas, green, boiled, ½ cup	24	4
Sunflower seeds, oil roasted, ¼ cup	19	3
Rice, brown, long-grain, cooked, 1 cup	19	3
Bread, pita, whole wheat, 1 large (6½-inch diameter)	17	3
Cabbage, boiled, ½ cup	15	3
Tangerine (mandarin orange), sections, ½ cup	10	2
Beans, snap, raw, ½ cup	8	1
Kiwifruit, raw, ½ cup sliced	7	1
Carrots, raw, chopped, ½ cup	6	1
Apples, raw, with skin, quartered or chopped, ½ cup	2	0

DV = Daily Value.
[a]The U.S. Food and Drug Administration (FDA) developed DVs to help consumers compare the nutrient contents of products within the context of a total diet. The DV for choline is 550 mg for adults.
From National Institutes of Health, Office of Dietary Supplements. (2019). *Choline*. Retrieved from https://ods.od.nih.gov/factsheets/Choline-HealthProfessional/

in their third trimester (27th week of gestation) and 21 nonpregnant control women (aged ≥21 years) were randomly assigned to receive either 480 mg or 930 mg choline per day for 12 weeks in conjunction with a highly controlled choline diet. Fasting blood samples, placental tissue, and umbilical cord venous blood were collected and tested for choline and its metabolites (betaine, dimethylglycine, sarcosine, and methionine).[214] The study found that (1) choline intake modifies both maternal and fetal biomarkers of choline metabolism, and (2) a higher dosage yields higher concentrations of choline and its metabolites in both pregnant and nonpregnant women, as well as neonates (i.e., led to a doubling of dimethylglycine in cord plasma), without changing obligatory maternal free choline urinary excretion. Therefore, more than doubling the choline AI does not exceed physiological needs, and thus 930 mg per day during pregnancy supports maternal and fetal single-carbon metabolism and methylation.

Currently, prenatal multivitamin supplements typically contain little to no choline. A 2016 study found that none of the "top 25 prenatal multivitamins" contain the choline AI for pregnant women. Only two of the products contained 55 mg and 50 mg, respectively, and six others contained merely 30 mg or less.[215] Therefore, to meet the high pre- and postnatal demands for choline, in addition to taking a prenatal supplement, pregnant and lactating women should include a daily single-nutrient choline supplement in order to obtain the minimum AI recommendation of 450 mg per day; dietary recommendations should also emphasize routine consumption of choline-rich dietary sources (Table 211.6).

Folate

Folate is a water-soluble B vitamin occurring naturally in a wide variety of foods, as well as the synthetic form folic acid, which is used in fortified foods and some dietary supplements. Many essential cellular and metabolic pathways are dependent on folate as a single-carbon transfer source, including synthesis and maintenance of nucleic acids (DNA and RNA), methylation of DNA (necessary for cell differentiation and epigenetic modulation), metabolism of amino acids, and the conversion of homocysteine to methionine.[216] Folate status is thus crucial for placental and fetal growth and development, making maternal requirements significantly greater than those of nonpregnant women.

Neural tube defects (NTDs) are congenital malformations of the brain and spinal cord that occur in the first month of gestation. Spina bifida is the most commonly occurring NTD, followed by anencephaly, with encephalocele and iniencephaly being rare types. It has been established that maternal folate deficiency increases the risk of NTD occurrences.

After decades of speculation regarding the role of folic acid in the prevention of NTDs, two randomized, double-blind, controlled trials published in the early 1990s[217,218] concluded that supplementation with folic acid prevents the occurrence and recurrence of these malformations.[219] The results of these and other studies prompted the U.S. Public Health Service to recommend, as a primary care–relevant preventive intervention, that all women capable of becoming pregnant consume 400 mcg per day of folic acid to prevent NTDs. And in 1998 the FDA, Health Canada, and many other governmental public health agencies around the world began implementing policies requiring manufacturers to fortify foods with folic acid, including flours, enriched breads, cereals, rice, and other grain products, to reduce the risk of NTDs. Dramatic results of these established folic acid fortification programs have appeared, with decreases in the rates of NTDs and associated morbidity and mortality by 19% to 32% in the United States and by 19% to 55% in 70 other countries, including Canada, South Africa, Costa Rica, Chile, Argentina, and Brazil, since the initiation of mandatory food-supply fortification.[220] However, more recent population-based studies, systematic reviews, and meta-analyses are beginning to suggest that the reduction in NTD rates by folic acid fortification may be more modest than previously reported.[219,221]

It is now better understood that this shortfall in preventing NTDs via folic acid food fortification is due in part to epigenetic changes and several other factors that can lead to impaired maternal folate status. There are also key genetic polymorphisms that affect the conversion of

dietary and supplemental forms to the activated folates and components of folate-dependent pathways and metabolism, one of which is a very commonly expressed variant and has been directly associated with an increased susceptibility for NTDs. A comprehensive meta-analysis surveyed literature spanning from 1996 to 2011 and investigated the effects of five genetic variants from 47 study populations, for a total of 85 case-control comparisons. The study found a significant association between the methylenetetrahydrofolate reductase *(MTHFR)* C677T variant and increased risk for NTDs.[222]

Recent systematic reviews and meta-analyses suggest that preconceptional folate supplementation is inversely associated with the risk of low birth weight, SGA infants, and PTB.[223–225]

Folic acid, found in fortified foods and many dietary supplements, must be converted into biologically active folate, of which there are primarily two forms: 5-methyltetrahydrofolate (5-MTHF), required in the methylation cycle, and folinic acid, a reduced folate that supports DNA synthesis, for example. SNPs in the genes that regulate folate metabolism can inhibit these folic acid–conversion processes. And as mentioned previously, the *MTHFR* 677CT SNP is strongly associated with NTD risk. Instead of supplying folinic acid, many professionally manufactured prenatal multivitamin–mineral supplements are now utilizing the biologically active form L-5-methyltetrahydrofolate (e.g., Metafolin), which has several potential advantages: (1) bypasses gene mutations affecting folate metabolism, (2) may reduce risk of masking vitamin B_{12} deficiency, and (3) may reduce risk of interaction with drugs that inhibit folate metabolism.[226]

Clinical research demonstrates numerous populations of women who would likely benefit from a significantly higher daily dosage of folate/folic acid (4000–5000 mcg per day) before conception because of factors that individually contribute to insufficient folate status and/or folate deficiency. This includes women who have a history of a prior pregnancy with a NTD, a family history of NTD, a genetic mutation in folate metabolism, a history of taking anti-folate medications, a history of oral contraceptive use, a history of firsthand or secondhand cigarette smoke exposure, an elevated BMI of ≥30 (obesity), poorly controlled diabetes, or malabsorption syndrome (e.g., celiac disease or Crohn's disease).[219]

Worldwide, folate deficiency is most commonly caused by a dietary insufficiency. A daily intake of 400 mcg folate or folinic acid is recommended for most healthy women of reproductive age,[34,213] to be sustained for at least 6 months before conception to maintain minimally adequate folate status. Daily consumption of folate-rich dietary sources is encouraged, including dark-green leafy vegetables, cruciferous vegetables (e.g., Brussels sprouts), citrus fruits, nuts, beans, peas, seafood, eggs, dairy products, meat, beef liver, grains, and fortified foods.[216]

The RDA for folate (as dietary folate equivalents) throughout pregnancy is 600 mcg, and it is 500 mcg during lactation. However, there are several factors that may require an increased recommendation to as high as 4000 to 5000 mcg per day for pregnant women and those planning to become pregnant. High-dose folate supplementation should not extend beyond the first trimester, and it is advisable that women consult with a knowledgeable health care provider during preconception and early pregnancy.

Iodine

Iodine is an essential trace element and is found in every tissue in the body. The primary function of iodine is its role as an intrinsic component for the production of thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Thyroid hormones in turn play a critical role in fetal neurodevelopment and regulating a variety of metabolic processes that are important determinants of health throughout the life span.

Pregnancy has a profound impact on thyroid gland function. The thyroid gland increases in size by 10% in iodine-replete individuals and by 20% to 40% in iodine-deficient populations. Production of T4 and T3 increases by approximately 50%, in conjunction with a separate 50% increase in the daily iodine requirement.[227] The maternal requirement for iodine is heightened during pregnancy due to several factors, including the need for increased T4 synthesis to maintain normal metabolism and the transfer of T4 and iodide ion to the fetus.[228]

Sufficient iodine intake is thus necessary to support increased thyroid functioning and physiological demand during pregnancy and lactation, making the mother and developing fetus vulnerable. The WHO established adequate intake and adequate iodine nutrition for pregnancy as having a median urinary iodine concentration (UIC) of ≥150 mcg/L and defined mild to moderate iodine deficiency during pregnancy as a median UIC of 50 mcg/L to 150 mcg/L.[229] Since 2000, UIC has been measured in the continuous NHANES. Over recent years, the median UIC for pregnant women in the United States sampled in NHANES has been trending below 150 mcg/L. The 2005–2010 survey data revealed a median UIC of 129 mcg/L, which is a decrease from the median UIC of 153 mcg/L per the 2001–2006 NHANES.[228] The NHANES data also show that over 35% of pregnant women have moderately deficient iodine levels.[230]

Although most survey data suggest that the U.S. population is iodine sufficient, a comprehensive 2019 systematic review of the iodine status of women of reproductive age in the United States found emergent iodine deficiency in the population, indicating an alarming public health concern needing immediate attention. The authors also note that public awareness of the importance of iodine consumption, especially in the prenatal period and first trimester of pregnancy, is severely lacking.[231] Moreover, a 2017 study utilizing a web-based survey of 199 midwives (American College of Nurse-Midwives members) and 277 obstetricians (American Medical Association members) found that despite the known consequences of iodine deficiency for pregnant women and the recommendations of various medical organizations in the United States, overall, only 25% of the surveyed participants recommended or would recommend an adequate amount of iodine during preconception, pregnancy, and lactation. Of those respondents who reported prescribing iodine-containing supplements, 85% did so during the first trimester and 75% to 80% during the second and third trimesters; however, only 45% would prescribe the recommended 150 mcg/day during pregnancy.[232]

Iodine-deficiency disorders include clinical hypothyroidism, which is associated with increased risk of poor perinatal outcomes, including preeclampsia, spontaneous abortion, stillbirth, low birth weight, impaired neurocognitive development, and congenital hypothyroidism.[34] Evidence is currently lacking, however, with respect to whether iodine supplementation during pregnancy and lactation is beneficial for the prevention of adverse pregnancy, neonatal, and childhood outcomes that result from iodine deficiency. Well-designed prospective, randomized, controlled trials that examine the neurodevelopmental effects of iodine supplementation in mildly to moderately iodine-deficient pregnant women are urgently needed.[233] A 2017 Cochrane systematic review and meta-analysis evaluated 14 studies meeting inclusion criteria, and 11 randomized and quasi-randomized controlled trials involving 2700 women contributed data for the comparisons in the review. With respect to maternal primary outcomes, no clear differences between groups were found. An evaluation of neonatal and infant primary outcomes found that compared with those who did not receive iodine supplementation, there was a 34% lower likelihood of perinatal mortality, although this difference was not statistically significant; all perinatal deaths occurred in one trial conducted in severely iodine-deficient mothers. There were no clear differences between groups of other neonatal/infant primary outcomes in trials taking place in settings of mild to moderate iodine deficiency. The

authors concluded there were insufficient data to reach any meaningful conclusions on the benefits and harms of routine iodine supplementation in women before, during, or after pregnancy.[234]

The WHO estimates that over 1.8 billion people worldwide have insufficient iodine intake.[229] Initiating iodized salt programs has been the most effective global strategy to mitigate iodine deficiency, with approximately 70% of households worldwide using iodized salt. However, iodine insufficiency is still prevalent in many regions, including Europe, where 52% of the population has insufficient iodine intake, and according to UNICEF, only about 49% of households in Europe (outside of the Western European subregion) have access to iodized salt. Iodine insufficiency is also prevalent in Africa, Southeast Asia, and the eastern Mediterranean WHO regions, where rates of iodized salt use range from approximately 47% to 67%.[235]

The RDA for iodine during pregnancy is 220 mcg, and the RDA is 290 mcg for lactation. Iodine is present in breast milk. As part of the standard American diet, dietary sources of iodine are primarily consumed as dairy products (e.g., milk), eggs, and seafood and seaweed (e.g., kelp, nori, kombu, and wakame) and as potassium iodide in the form of table salt. However, around the world, including the United States and the European Union, there is a high degree of variability in the iodine content of numerous foods that are considered important dietary sources. Authoritative medical organizations, including the European Thyroid Association, American Thyroid Association, Endocrine Society, and American Academy of Pediatrics, have published recommendations stating that women need to supplement their diet with 150 mcg of iodine per day during preconception, pregnancy, and lactation. Fortunately, a large number of prenatal multivitamin–mineral supplements studied provide 150 mcg of potassium iodide per serving.[236]

Iron

Iron is a critical mineral as an essential component of hemoglobin (an erythrocyte protein that carries oxygen to tissues throughout the body) and myoglobin and is important in myelination, neurotransmitter function, various cellular and oxidative processes, energy production, liver cytochrome P450 detoxification enzymes, and thyroid hormone production.[237]

The WHO considers iron deficiency to be the main cause of anemia during pregnancy, and it is a significant worldwide public health issue, especially in low-income developing countries. The overall prevalence of iron deficiency anemia (IDA) in pregnant women in the United States is near 18%, and rates of IDA are increasing across trimesters from 6.9% to 14.3% to 28.4%.[238] In a review considering data from 15 European countries that included national surveys and clinical trials, the evidence suggested that the prevalence of iron deficiency (ID) and IDA in gestational weeks 32 to 39 was 28% to 85% and 21% to 35%, respectively. Women who were taking iron supplements had higher iron status and a lower prevalence of ID and IDA, which were correlated to the iron dosage taken.[239]

The increased risk of developing IDA during pregnancy is due to heightened maternal iron needs and demands from the growing fetus and placenta; increased erythrocyte mass; and expansion of maternal blood volume, especially as the pregnancy progresses into the third trimester.[240] Reduced levels of hemoglobin limit the availability of oxygen to the fetus, and anemia during pregnancy poses risks to both mother and fetus, including altered fetoplacental ratio, IUGR, low birth weight, premature birth, and higher risk of peripartum blood transfusion.[241]

A recent systematic review and meta-analysis evaluating 18 trials (932,090 participants) found that IDA in the first trimester increases the risk of premature birth. This relationship was not significant in the second and third trimesters.[242] Several systematic reviews and meta-analyses have also demonstrated that maternal IDA can be considered a significant risk factor for low birth weight.[243,244]

A Cochrane systematic review and meta-analysis evaluated 44 trials (43,274 participants) comparing the effects of daily supplements containing iron versus no iron or placebo during pregnancy. Preventive iron supplementation lessened maternal anemia at term by 70%, as well as IDA and iron deficiency by 57% at term, respectively, compared with the no-iron or placebo groups. Median hemoglobin concentrations were higher in pregnant women at term and postpartum receiving iron supplementation. There were no significant differences between groups for severe anemia in the second and third trimesters or maternal mortality. Iron supplementation was associated with reduced frequency of low birth weight and preterm deliveries, although these findings did not reach statistical significance. No clear differences were demonstrated between groups for neonatal death or congenital abnormalities.[245]

Given that all pregnant women are at higher risk for IDA, routine screening in preconception and throughout pregnancy is recommended. The RDA for iron during pregnancy is 27 mg per day and 9 to 10 mg per day for lactation. There are various dietary supplemental forms of iron chelate; the most commonly found over-the-counter option is ferrous sulfate. However, a recent randomized, double-blind clinical trial involving 187 women with second-trimester IDA found that after the 8-week study duration, ferrous bis-glycinate (Ferrochel) was more effective in increasing hemoglobin levels, had significantly fewer side effects (e.g., gastrointestinal upset and constipation), and had greater compliance compared with ferrous sulfate.[246] The vast majority of professionally manufactured prenatal multivitamin–mineral formulas include an iron chelate in dosages, allowing for the RDA to be met. Dietary iron occurs in two forms, heme and nonheme. Heme iron has higher bioavailability than nonheme iron. Animal sources such as red meat, poultry, and seafood yield heme iron, whereas plant-based foods like vegetables, fruits, legumes, nuts, seeds, and fortified foods such as cereals contain the nonheme form.

Magnesium

Magnesium is an essential and widely abundant mineral in the body and serves as a cofactor in more than 300 enzyme systems that regulate a plethora of biochemical reactions, including synthesis of nucleic acids and proteins, blood glucose control, blood pressure regulation, maintaining normal nerve conduction, muscle contraction and cardiac rhythm, structural integrity of bone, energy production, oxidative phosphorylation, and glycolysis.[247]

A prospective trial assessing serum magnesium levels during low-risk pregnancies (145 participants) demonstrated that both ionized and total serum magnesium were significantly reduced after the 18th week of gestation compared with measurements before this time.[248] Changes in magnesium homeostasis and deficiency status have been suggested as playing a role in several pregnancy-related conditions and outcomes, including hypertension, preeclampsia, IUGR, PTB, and neonatal hospitalization. One such study showed a correlation between low plasma levels of magnesium in pregnant women and preeclampsia and PTB.[249]

Primigravida women were administered 300 mg magnesium citrate (29 participants) or placebo (30 participants) from the 25th week of gestation until delivery in a randomized, double-blind, controlled trial. Blood pressure was monitored every 2 to 3 weeks throughout pregnancy, in addition to pregnancy outcomes.[250] In the treatment group, both the average diastolic blood pressure (DBP) at week 37 and the number of participants with an increase in DBP of ≥15 mm Hg were significantly lower. Specifically, the proportion of women with

an increase of ≥15 mm Hg was 25% higher in the placebo group as compared with the magnesium intervention group (8%). There were no differences between treatment and placebo groups with respect to gestational length, duration of labor, or birth weight.

A randomized, double-blind, placebo-controlled clinical trial examined the effects of magnesium supplementation on metabolic status and pregnancy outcomes in 70 participants with magnesium deficiency and GDM. Participants were randomly assigned to receive either 250 mg magnesium oxide daily or a placebo for 6 weeks. Fasting blood samples were drawn at baseline and after intervention completion. Compared with placebo, magnesium supplementation was found to have a significant benefit for a variety of metabolic parameters, including fasting plasma glucose, serum insulin concentration, quantitative insulin sensitivity check index, triglycerides, high-sensitivity C-reactive protein, and plasma malondialdehyde concentrations.[251] Magnesium supplementation was also associated with lower incidence of newborn hyperbilirubinemia and hospitalization.

Another trial sought to assess the potential impact of magnesium supplementation on a range of primary outcomes in 180 pregnant women. Serum magnesium levels of women with a pregnancy of gestational age 12 to 14 weeks were measured. Inclusion criteria were single pregnancy and no history of acute renal disease, chronic hypertension, severe anemia, diabetes, pancreatic disease, heart disease, and cigarette or alcohol use. Participants were randomized into treatment or control groups. Those with a serum magnesium level >1.9 mg/dL were considered the control, Group A (60 participants), and received one multimineral tablet daily until the end of pregnancy. Those with serum magnesium levels <1.9 mg/dL (defined as hypomagnesemia) were randomly assigned to Group B and Group C, with 60 participants in each group. Group B participants were administered one multimineral tablet daily until the end of pregnancy, and Group C received one multivitamin tablet daily in addition to a 200-mg effervescent magnesium tablet daily until the end of pregnancy. The multivitamin–mineral tablet contained 100 mg of magnesium. Pregnancy outcomes were compared between groups and included IUGR, preterm labor, maternal BMI, neonatal weight, pregnancy-induced hypertension, preeclampsia, GDM, lower leg muscle cramping, and Apgar scores.[252] The study found that Group C had significant reductions in each of the identified pregnancy outcomes and in the frequency of pregnancy complications when compared with Group A and Group B.

The daily RDA for magnesium in pregnancy ranges from 350 mg to 400 mg and from 310 mg to 360 mg during lactation, depending on maternal age. Prenatal multivitamin–mineral supplements vary widely in the amount of magnesium provided and typically do not meet 100% of the RDA level; it is therefore suggested that women consume magnesium-rich dietary sources as well, including whole grains, dark-green leafy vegetables, legumes, nuts, and seeds.

Omega-3 Fatty Acids

Omega-3 polyunsaturated fatty acids (PUFAs) are considered essential because they must be obtained from the diet, and their nutritional value is critical during fetal growth and development, as well as in early infancy and childhood. The three main omega-3 fatty acids are alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Rich dietary sources of ALA are found primarily in plant-based oils, such as flaxseed, chia seed, walnut, canola, and soybean, whereas DHA- and EPA-rich foods include fish, krill, shrimp, and other seafood, especially cold-water fatty fish such as salmon, mackerel, herring, and anchovies. Foods fortified with DHA can also be readily found and include eggs, dairy products, nondairy beverages, and infant formulas.[253]

Omega-3 fatty acids are particularly critical for fetal brain, nervous system, and retina development. Maternal plasma DHA levels are observed to be significantly increased during early pregnancy, before neural tube closure, suggesting DHA's contribution to embryo development. DHA also rapidly accumulates in the fetal brain in the third trimester, which continues throughout the first 2 years of infancy.[254] Retinal development requires a high concentration of DHA, which optimizes the fluidity of photoreceptor membranes, retinal integrity, and visual function.[255]

The NAM has established AIs for omega-3 fatty acids. During pregnancy and lactation, the AI for omega-3s as ALA is 1.4 g and 1.3 g per day, respectively; the NAM recommends that DHA and EPA contribute 10% of the total omega-3 fatty acid intake. Note that the reason for the NAM using ALA as the required intake is due to the fact that DHA and EPA are not considered essential fatty acids because they can be derived from the desaturation and elongation of their parent fatty acid, ALA. Thus, unlike for ALA, RDAs and DRIs have not been established for DHA and EPA. However, a variety of worldwide groups have made recommendations and issued policy statements on the matter[254]:

- Food and Agriculture Organization of the United Nations: 200 mg DHA and 100 mg EPA
- World Association of Perinatal Medicine: 200 mg DHA per day
- European Food Safety Authority: an additional 100 to 200 mg DHA per day beyond 250 mg EPA + DHA per day
- American College of Obstetricians and Gynecologists: 340 g (two 6-oz servings) seafood per week, providing approximately 200 mg DHA per day
- Dietary Guidelines for Americans (DGA): 8-oz serving per week of a variety of seafood (approximately 250 mg EPA + DHA)
- FDA in concert with the Environmental Protection Agency (EPA): 2 to 3 servings (approximately 8–12 ounces) of fish from the "Best Choices" list they issued

Despite these guidelines, in the United States, a 2018 analysis of data from the 2001–2014 NHANES estimated that the majority of childbearing-age and pregnant women consume significantly lower amounts of seafood than what is recommended. The mean usual intake of seafood was 0.44 ± 0.02 oz equivalent per day, placing 100% of the population studied below the DGA recommendation. This subsequently leads to lower-than-optimal dietary intakes of DHA and EPA. In addition, it was found that dietary supplement use has not eliminated this dietary shortfall of omega-3 fatty acids.[254]

A systematic review and meta-analysis included nine randomized controlled trials, involving 5980 women, and sought to examine the effects of omega-3 fatty acid supplementation on preterm delivery. Six trials evaluated early preterm delivery (<34 weeks' gestation), and three trials evaluated any preterm delivery (<37 weeks' gestation). Interventions were DHA, EPA, or a combination of DHA and EPA, with daily dosages ranging from 133 mg to 3000 mg. Treatment began before 24 weeks' gestation in seven out of nine trials. The study found that omega-3 fatty acid supplementation reduced the risk of early preterm delivery by 58% and the risk of any preterm delivery by 17%. Omega-3 fatty acid supplementation also increased mean gestational age by 1.95 weeks and mean birth weight by 122.1 g, and it decreased perinatal death by 49%.[256]

For decades, epidemiological and observational studies have associated maternal seafood consumption and omega-3 fatty acid intake during pregnancy and lactation with better infant health outcomes, including improved childhood cognitive and visual development. A 2018 systematic review and meta-analysis evaluated 38 randomized controlled trials, with 53 intervention arms, of omega-3 PUFA supplementation in mothers (13 studies) and infants (preterm infants, 7 studies; term infants, 18 studies). The study evaluated standardized

measures of cognitive and visual development up to 18 years of age. Significant differences were not identified by world region, race, maternal education, age at outcome assessment, supplementation duration, DHA or EPA dose, DHA:arachidonic acid ratio, or study quality score. Compared with placebo, omega-3 supplementation was found to improve childhood psychomotor and visual development.[257]

Epidemiological and clinical research findings regarding seafood consumption during pregnancy can often appear contradictory, and this is in part due to the fact that most fish contain competing benefits and risks in the form of omega-3 fatty acids and toxicant levels, particularly methylmercury (MeHg).[258] During pregnancy, MeHg readily transports across the placental barrier and subsequently accumulates in both placental and fetal tissues, thereby directly affecting the developing fetus.[259] Furthermore, data from 34 studies demonstrated that MeHg levels in breast milk were generally higher in populations with high fish consumption.[260] The long half-life of mercury in fish (approximately 2 years) leads to substantial bioaccumulation in larger ocean fish. Given that the half-life of MeHg in humans is 60 days, weekly intake of fish yielding just 0.1 PPM of mercury exposure can result in elevated blood mercury levels.[146]

Given the inherent concerns and risks associated with seafood consumption and in utero toxicant exposure, it is especially well advised that women obtain the majority of their essential DHA and EPA intake via dietary supplementation in the form of either algae-derived oil (i.e., vegetarian source) or fish oil during preconception, pregnancy, and lactation. Methylmercury and other toxins such as dioxins and PCBs found in fish can be effectively removed from omega-3 fatty acid supplements during the molecular distillation and manufacturing process. Such dietary supplementation has been shown to confer a variety of maternal, fetal, and infant health benefits. And ideally, pregnant and lactating women should consume only those fish that are low in mercury levels yet high in omega-3 fatty acids. Plant-based oils contain ALA and should thus be emphasized as part of an overall healthy, pregnancy-supporting diet. However, the endogenous conversion (primarily in the liver) of ALA (the parent omega-3 PUFA) to the more biologically active long-chain omega-3 PUFAs (DHA and EPA) is inefficient in humans and produces only very small amounts of the latter. Therefore, the consumption of preformed omega-3 fatty acids (DHA and EPA) is the only practical way to increase appropriate levels in the body and should be emphasized for optimal health. This is of particular importance for vegetarian and vegan women.

Vitamin A

Vitamin A is a fat-soluble substance derived from both preformed retinoids and provitamin carotenoids (in those who can make the conversion). Vitamin A has three active forms (retinal, retinol, and retinoic acid) and a storage form (retinyl ester). Preformed vitamin A is obtained from animal sources such as liver, kidney, fish, eggs, and dairy products, whereas provitamin A is found in plants, specifically in the form of the pigments beta-carotene, alpha-carotene, and beta-cryptoxanthin. Both provitamin A and preformed vitamin A must be metabolized intracellularly into the active forms of vitamin A, retinoic acid and retinol, to support the vitamin's critical biological functions.[261] In pregnancy, additional vitamin A is required at various distinct stages of embryonic and fetal growth and development, including gene expression and cell differentiation. Target tissues are rather ubiquitous and include the ocular, circulatory, respiratory, urogenital, immune, and central nervous systems.

Vitamin A deficiency remains a prevalent concern in developing low- and middle-income countries. According to the WHO, vitamin A deficiency affects 19 million pregnant women worldwide, and supplementation can reduce infant mortality in regions where this micronutrient deficiency is common, with Africa and Southeast Asian countries affected the most.[1]

Excess vitamin A has potential teratogenic effects and is contraindicated during pregnancy. A single dose of a vitamin A supplement ≥25,000 IU is not recommended because its safety is uncertain. Moreover, this dose may be teratogenic if consumed between days 15 and 60 from conception.[1] Periconception exposures greater than 25,000 IU of preformed vitamin A (retinol and retinyl esters) have not been sufficiently studied to establish specific risk.[262] Preformed vitamin A is correlated with teratogenicity, whereas the provitamin A carotenoid form naturally occurring in food sources and contained in the majority of prenatal multivitamin–mineral supplements is not teratogenic.[263]

A Cochrane systematic review sought to determine the effect of vitamin A supplementation (with or without other supplements) during pregnancy on maternal and newborn clinical outcomes. The study included 19 trials including over 310,000 women, with all trials conducted in lower- and middle-income countries, with the exception of 1 trial in the United Kingdom. Moderate-certainty evidence shows that vitamin A supplementation in deficient populations during pregnancy likely reduces maternal anemia and gestational night blindness but has no effect on maternal mortality. With respect to fetal and neonatal outcomes, high-certainty and moderate-certainty evidence suggest that vitamin A supplementation in the same population makes little or no difference to perinatal mortality, neonatal mortality, stillbirths, low birth weight, and PTB.[264] Another comprehensive systematic review and meta-analysis made similar conclusions, stating that there is little consistent evidence of a benefit of maternal supplementation with vitamin A (or beta-carotene) during pregnancy on maternal mortality and no significant overall effect on birth-weight indicators, PTB, miscarriage, stillbirth, or fetal loss.[265]

In populations where habitual sufficient daily vitamin A intakes exceed 8000 IU (2400 mcg/day), there is no demonstrated benefit from taking vitamin A supplements during pregnancy. And the potential risk of adverse events increases with intakes ≥10,000 IU per day if vitamin A supplements are routinely taken in these populations.[1]

Dosages of vitamin A in excess of 10,000 IU per day are associated with teratogenic effects, including cranial-facial and cardiac birth defects. Therefore, the maximum recommended supplemental dose during pregnancy and lactation is 2400 mcg per day (8000 IU) as retinol. The RDA for vitamin A as retinol equivalents during pregnancy is 750 to 770 mcg (2500 IU) per day, and during lactation, it is 1200 to 1300 mcg (4200 IU).[261] Vitamin A is found as preformed retinol in animal sources, with the highest levels found in beef liver, cod liver oil, dairy products, and fish. And as provitamin A, carotenoids are found in rich supply in leafy green vegetables and red, orange, and yellow vegetables and fruits.

Vitamin C and Vitamin E (Antioxidants)

Oxidative stress has been implicated in various pathophysiological processes during pregnancy. For example, oxidative stress due to increased production of free radicals and lipid peroxidation combined with insufficient antioxidant defenses is a known etiological component of endothelial dysfunction and placental insufficiency and thus may be causally associated with preeclampsia and SGA infants.[266] However, available clinical evidence does not support a preventive or therapeutic use of antioxidant supplementation with vitamin C and/or vitamin E for the prevention of preeclampsia and fetal/neonatal outcomes such as preterm birth, SGA infants, congenital anomalies, and infections.[267]

A systematic review of 29 randomized controlled trials involving 24,300 women from 17 different countries found no significant differences in improved outcomes between women supplemented with

vitamin C alone or in combination with other supplements (mainly vitamin E) compared with the placebo or control groups for risk of stillbirth, neonatal death, perinatal death, SGA infants, PTB, or preeclampsia.[268]

Another systematic review of 17 randomized controlled trials found no clear difference between women supplemented with vitamin E alone or in combination with other supplements (including vitamin C) during pregnancy versus placebo. There were no demonstrated improved clinical outcomes for prevention of stillbirth, neonatal death, preeclampsia, PTB, IUGR, or premature rupture of membranes.[269]

The most commonly used dose in the clinical trials analyzed in the previously referenced Cochrane reviews was vitamin C 1000 mg daily (13 trials) and vitamin E 400 IU daily (15 trials). The majority of the trials began administering supplementation in the second trimester.

Vitamin D

Vitamin D is obtained either from ultraviolet B–induced synthesis in the skin or from dietary intake. Whether derived from sun exposure or food, vitamin D is initially relatively biologically inert and must undergo two sequential hydroxylation steps in the body for it to become active.[270] The first step occurs in the liver, where previtamin D is converted to 25-hydroxyvitamin D [25(OH)D], the main circulating form—which is either bound to vitamin D–binding protein (85%–90%) or albumin (10%–15%) or is in a free form (<1% unbound)—and acts as a reservoir for conversion to the active metabolite.[271] This second step occurs primarily in the kidneys, where 25(OH)D is converted into 1,25-dihydroxyvitamin D [1,25(OH)2D], the physiologically bioactive vitamin D steroid hormone or calcitriol.[272] This conversion also occurs to a lesser extent within the bone and parathyroid glands. Furthermore, during pregnancy, the placenta is also actively converting 25(OH)D to calcitriol on a local-tissue level without significantly contributing to circulating serum 1,25(OH)2D concentrations.[271]

The primary role of vitamin D is in calcium and phosphate homeostasis. It is also required for normal bone mineralization, neuromuscular and immune function, and reduction of inflammation, and it modulates genetic encoding proteins that regulate cell proliferation, differentiation, and apoptosis.[270] Thus, during pregnancy, vitamin D metabolism is under increased demand to support the growth and development of the fetus. Compared with nonpregnant baseline, by 12 weeks' gestation, there is a twofold increase in 1,25(OH)2D serum concentration, and this continues to rise two- to threefold during the course of gestation.[273]

Total serum 25(OH)D concentration is considered the most representative indicator of vitamin D status because it reflects both cutaneous vitamin D synthesis and that obtained from food and dietary supplements. However, there is a lack of universal consensus on defining what constitutes optimal 25(OH)D concentrations during pregnancy and lactation.[274] The NAM has recommended a 25(OH)D concentration threshold of >50 nmol/L (>20 ng/mL) to define sufficiency because this level is associated with the prevention of bone manifestations of vitamin D deficiency (rickets and osteomalacia) in 97.5% of Americans and Canadians.[275] However, the Endocrine Society and Osteoporosis Canada suggest that a higher 25(OH)D serum concentration of >75 nmol/L (>30 ng/mL) is needed for optimal health and bone development. Although no verifiable, agreed-upon serum 25(OH)D concentration has been established for pregnancy, screening for vitamin D sufficiency is advisable during preconception and early pregnancy because it is potentially clinically useful.[276] What is conclusive, however, is that vitamin D deficiency is highly prevalent in pregnant women and newborns worldwide. Research suggests that vitamin D deficiency is associated with an increased risk for preeclampsia, GDM, spontaneous abortion, low birth weight, SGA infants, and PTB.[272,277] Thus a growing body of evidence suggests that vitamin D supplementation during pregnancy may reduce the risk of these adverse gestational outcomes.

Systematic reviews and meta-analyses consistently find that pregnant women supplementing with vitamin D have significantly higher 25(OH)D levels during pregnancy and at term compared with placebo or control grous.[277–279] Clinical trials also support safely and effectively treating vitamin D deficiency in pregnant women via oral supplementation with 4000 IU vitamin D_3 per day from 12 to 16 weeks' gestation to delivery.[280]

The RDA for vitamin D during pregnancy and lactation is 600 IU (15 mcg) per day. However, evidence suggests that this current RDA may not be sufficient for otherwise well-nourished women during pregnancy. A secondary analysis of data from a maternal and infant prospective cohort, the Alberta Pregnancy Outcomes and Nutrition (APrON) study, evaluated total vitamin D intake (diet and supplements) and blood samples of 537 women during their second trimester. Participants were 87% Caucasian, with a mean age of 31.3 years and an average BMI 25.8; 58% were primiparous, 90% completed post–high school education, and 80% had annual family income greater than $70,000. Although a significant relationship was demonstrated between reported maternal vitamin D intake and increased 25(OH)D concentration, the study found that 46% of participants did not meet the Health Canada RDA of 600 IU per day and that 20% did not achieve the recommended sufficiency level of 75 nmol/L serum 25(OH)D concentration. Moreover, approximately 50% of participants assessed as being vitamin D insufficient were found to be consuming the recommended RDA.[275]

High serum 25(OH)D concentrations in early pregnancy (on average, 15 ± 1 weeks' gestation) have been shown to be significantly inversely correlated with and protective against the development of GDM. Women in the lowest quartile for 25(OH)D concentration had a twofold-higher risk of GDM compared with women in the highest quartile.[276,281,282] Pregnant women supplementing with vitamin D and thus sufficient 25(OH)D status are found to have lower risk of preeclampsia.[277,278,283]

A small, randomized, double-blind, controlled trial found that supplementing with vitamin D reduced the risk of unexplained recurrent spontaneous abortion (URSA). Eighty women (aged 18–35 years) with history of experiencing at least two consecutive or three nonconsecutive spontaneous abortions of unknown cause and pregnancy by the same partner in all past and present pregnancies were randomized to either an intervention group (40 participants) and administered 400 IU vitamin D_3 per day or a placebo group (40 participants). Both groups were prescribed 400 mg vaginal progesterone daily, and all participants were administered folic acid and iron supplementation at least 1 month before pregnancy. Primary outcomes included serum 25(OH)D measured at baseline and completion of the study; interleukin 23 (IL-23) measured at baseline and completion of the study, as well as at time of spontaneous abortion; and pregnancy loss, considered as URSA from study onset to 20 weeks' gestation. The number of spontaneous abortions during the study period was 5 in the intervention group and 13 in the control group. The study concluded that compared with placebo, vitamin D_3 supplementation leads to (1) increased serum 25(OH)D levels, (2) decreased serum interleukin (IL)-23 levels, and (3) significantly fewer incidence of spontaneous abortion through the causal pathway with IL-23 among women with URSA.[284]

Recent systematic reviews and meta-analyses have demonstrated an association between vitamin D status and pregnancy outcomes in women undergoing assisted reproductive technology. Compared with women replete in vitamin D at preconception, those with insufficient

or deficient vitamin D status have a significantly decreased probability of achieving clinical pregnancy and live birth after in vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI).[285–287] And in a retrospective cohort study, vitamin D deficiency was associated with a 50% reduction in both clinical pregnancy and live birth rates in recipients of donor eggs compared with participants who had sufficient vitamin D status.[288]

Vitamin D supplementation is frequently required during pregnancy and lactation to achieve sufficient vitamin D status as suggested by nutritional guidelines.[271] A majority of prenatal multivitamin–mineral supplements allow women to achieve the RDA for pregnancy and lactation in addition to dietary sources. Vitamin D is fat soluble and available in fortified foods and dietary supplements in two forms, D_2 (ergocalciferol) and D_3 (cholecalciferol). Vitamin D_2 is derived by ultraviolet irradiation of ergosterol in yeast, whereas D_3 is manufactured from the ultraviolet irradiation of 7-dehydrocholesterol from lanolin and the chemical conversion of cholesterol.[270] Naturally occurring vitamin D_3 is found in a limited number of foods, and these are primarily animal-based sources, including the flesh of fatty fish (e.g., swordfish, tuna, salmon, and mackerel) and fish liver oil, beef liver, dairy products, and egg yolk. The synthetic vitamin D_2 is structurally different from the natural D_3 and has somewhat—and controversial—different physiological and toxicological effects. (See Chapter 203, Osteoporosis, for a comprehensive discussion.)

Zinc

Zinc is one of the most abundant essential trace minerals in the body and is required for the catalytic activity of over 100 enzymes. Zinc is thus involved in a wide array of biological processes, including carbohydrate and protein metabolism, DNA synthesis, cell division, innate and adaptive immune responses, inflammatory processes, and hormone regulation. The significance of zinc to the growth of the fetus is demonstrated by the active transport of the mineral across the placenta into the fetal circulation, resulting in higher cord blood concentrations compared with those in maternal circulation.[289]

Pregnant women are predisposed and vulnerable to low zinc status and deficiency states due to (1) a significantly increased demand associated with accelerated growth and fetal development, (2) the body's lack of a specialized zinc-storage system,[290] and (3) the dependence of dietary intake and bioavailability on the composition of the diet. Globally, zinc deficiency is a rather common clinical finding, and research has shown an association between low maternal circulating zinc concentrations and pregnancy complications.

A systematic review of 64 observational studies assessing maternal circulating zinc concentrations and pregnancy complications found a significant difference in pregnant women with poor zinc status and low-birth-weight infants, as well as an association between low-zinc status and hypertension, particularly in women with severe preeclampsia.[289]

A Cochrane review evaluated 21 randomized controlled trials involving 17,000 participants with respect to zinc supplementation compared with no intervention or placebo and pregnancy outcomes. Low-certainty evidence found that zinc supplementation resulted in a small, relevant reduction (14%) in PTB (7637 participants, 16 trials, primarily representing women of low income) and induction of labor (1 trial). No significant differences were found between the zinc-treatment groups versus the no-zinc or placebo groups for any other primary maternal or neonatal outcomes.[291] Many of the studies were at risk for bias, thus influencing the certainty of the review evidence on the impact of zinc supplementation. Six of the included trials were conducted in the United States, United Kingdom, and Denmark; all others were conducted in low- to middle-income countries. There was wide variation among trials with respect to the following: size (range: 56–4926 participants), zinc dosage (range: 5–90 mg/day), nutritional and zinc status at trial entry, initiation and duration of supplementation (starting before conception in 1 trial, first or second trimester in the majority of trials, or after 26 weeks' gestation in 2 trial, until delivery) and compliance with treatment.[1]

Rich dietary sources of zinc include oysters, shellfish, red meat, chicken, beans, legumes, nuts, seeds, dairy products, and whole grains. However, the bioavailability of zinc can be compromised in vegan and vegetarian diets due to higher amounts of phytate and fiber—found in plants, whole grains, legumes, nuts, and cereals—which bind to zinc and inhibit its absorption. And although plant-based foods are still reasonable dietary sources, plant-derived zinc is inherently less bioavailable compared with animal food sources.[292] A systematic review analyzed six observational studies, and a meta-analysis was conducted to compare the zinc intake/status of vegetarians compared with nonvegetarians during pregnancy. The zinc intake of pregnant vegetarians was found to be lower than that of nonvegetarian pregnant control populations. Neither of the groups met the RDA for zinc, and no differences were found between groups in serum/plasma zinc.[293]

The RDA for zinc during pregnancy is 11 mg/day, and it is 12 mg/day when breastfeeding. Prenatal multivitamin–mineral supplements commonly yield the RDA for zinc. Dietary modifications intended to optimize zinc intake, bioavailability, and status are an important consideration during preconception, pregnancy, and lactation, especially for vegan and vegetarian women. Due to the physiological adaptations associated with gestation and fetal and newborn growth and development, as well as the inherent demands in zinc metabolism, sufficient daily intake should be ensured to meet the increased requirements.

CONCLUSION

Pregnancy offers an unparalleled opportunity to improve maternal health and overall well-being; reduce the risk of adverse maternal, fetal, and childhood outcomes; and address modifiable factors toward encouraging a healthy pregnancy. Implementation of natural medicine approaches serves to support positive pregnancy experiences and birth outcomes by utilizing the preconception and gestational periods to educate and empower parents regarding best practices in order to create optimal conditions for the future health of the next generation.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. World Health Organization. *WHO Recommendations on Antenatal Care for a Positive Pregnancy Experience*. Geneva: World Health Organization; 2016. PubMed PMID: 28079998.
2. World Health Organization. Maternal mortality; 2018. https://www.who.int/en/news-room/fact-sheets/detail/maternal-mortality. Accessed July 24, 2018.
3. World Health Organization. *Trends in Maternal Mortality: 1990 to 2013 Estimates by WHO, UNICEF, UNFPA, the World Bank and the United Nations Population Division*. Geneva, Switzerland: World Health Organization; 2014. ISBN: 978-92-4-150722-6. https://www.who.int/reproductivehealth/publications/monitoring/maternal-mortality-2013/en/. Accessed February 2, 2019.
4. GBD 2015 Maternal Mortality Collaborators. Global, regional, and national levels of maternal mortality, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 388(10053):1775–1812. PubMed PMID: 27733286.
5. MacDorman MF, Declercq E, Cabral H, Morton C. Recent increases in the U.S. maternal mortality rate: disentangling trends from measurement issues. *Obstet Gynecol*. 2016;128(3):447–455. PubMed PMID: 257500333.
6. Moaddab A, Dildy GA, Brown HL, et al. Health care disparity and pregnancy-related mortality in the United States, 2005-2014. *Obstet Gynecol*. 2018;131(4):707–712. PubMed PMID: 29528919.
7. Centers for Disease Control and Prevention. Pregnancy mortality surveillance system. https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pregnancy-mortality-surveillance-system.htm?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Freproductivehealth%2Fmaternalinfanthealth%2Fpmss.html. Accessed February 1, 2019.
8. Lu MC. Reducing maternal morbidity in the United States. *JAMA*. 2018;320(12):1237–1238. PubMed PMID: 30208484.
9. Agrawal P. Maternal mortality and morbidity in the United States of America. *Bull World Health Organ.* 2015;93(3):135. PubMed PMID: 25838608.
10. Sandall J, Tribe RM, Avery L, et al. Short-term and long-term effects of caesarean section on the health of women and children. *Lancet*. 2018;392(10155):1349–1357. PubMed PMID: 30322585.
11. Boerma T, Ronsmans C, Melesse DY, et al. Global epidemiology of use of and disparities in caesarean sections. *Lancet*. 2018;392(10155):1341–1348. PubMed PMID: 30322584.
12. Word Health Organization. WHO recommendations: non-clinical interventions to reduce unnecessary caesarean sections; 2018. https://www.who.int/reproductivehealth/publications/non-clinical-interventions-to-reduce-cs/en/. Accessed February 2, 2019.
13. Betrán AP, Ye J, Moller AB. The increasing trend in caesarean section rates: global, regional and national estimates: 1990-2014. *PLoS One*. 2016;11(2):e0148343. PubMed PMID: 26849801.
14. Tabb KM, Malinqa T, Pineros-Leano M, Andrade FCD. Impact of pre-pregnancy weight and gestational weight gain on birth outcomes by nativity in the United States: a systematic review. *Healthcare (Basel, Switzerland)*. 2017;5(4). PubMed PMID: 28961162.
15. Thakrar AP, Forrest AD, Maltenfort MG, Forrest CB. Child mortality in the US and 19 OECD comparator nations: a 50-year time-trend analysis. *Health Affairs (Project Hope)*. 2018;37(1):140–149. PubMed PMID: 29309221.
16. Fleming TP, Watkins AJ, Velazquez MA, et al. Origins of lifetime health around the time of conception: causes and consequences. *Lancet*. 2018;391(10132):1842–1852. PubMed PMID: 29673874.
17. Mason E, Chandra-Mouli V, Baltag V, et al. Preconception care: advancing from 'important to do and can be done' to 'is being done and is making a difference'. *Reproductive Health*. 2014;11(suppl 3):S38. PubMed PMID: 25415261.
18. American College of Obstetricians and Gynecologists. Committee opinion No. 762: prepregnancy counseling. *Obstet Gynecol*. 2019;133(1):e78–e89. PubMed PMID: 30575679.
19. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. *Cardiovasc J Afr*. 2016;27(2):89–94. PubMed PMID: 27213856.
20. Merck Manual. Physiology of Pregnancy. https://www.merckmanuals.com/professional/gynecology-and-obstetrics/approach-to-the-pregnant-woman-and-prenatal-care/physiology-of-pregnancy Accessed January 31, 2019
21. American College of Obstetricians and Gynecologists. Having a baby after age 35. https://www.acog.org/Patients/FAQs/Having-a-Baby-After-Age-35-How-Aging-Affects-Fertility-and-Pregnancy. Accessed July 20, 2018.
22. Proctor SB, Campbell CG. Position of the academy of nutrition and dietetics: nutrition and lifestyle for a healthy pregnancy outcome. *J Acad Nutr Diet.* 2014;114(7):1099–1103. PubMed PMID: 24956993.
23. Prentice S. They are what you eat: can nutritional factors during gestation and early infancy modulate neonatal immune response? *Front Immunol*. 2017;8:1641. PubMed PMID: 29234319.
24. Dupont C, Armant DR, Brenner CA. Epigenetics: definition, mechanisms and clinical perspective. *Semin Reprod Med*. 2009;27(5):351–357. PubMed PMID: 19711245.
25. Razavi M, Jamilian M, Samimi M, et al. The effects of vitamin D and omega-3 fatty acids co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in patients with gestational diabetes. *Nutrition & Metabolism*. 2017;14:80. PubMed PMID: 29299042.
26. Farrar D. Hyperglycemia in pregnancy: prevalence, impact, and management challenges. *Int J Women's Health*. 2016;8:519–527. PubMed PMID: 27703397.
27. Committee on Practice Bulletins—Obstetrics. Practice bulletin No. 137: gestational diabetes mellitus. *Obstet Gynecol*. 2013;122(2 Pt 1):406–416. PubMed PMID: 2396987.
28. Stephenson J, Heslehurst N, Hall J, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. *Lancet*. 2018;391(10132):1830–1841. PubMed PMID: 29673873.
29. Barker DJ, Thornburg KL. Placental programming of chronic diseases, cancer and lifespan: a review. *Placenta*. 2013;34(10):841–845. PubMed PMID: 23916422.
30. Kaiser LL, Campbell CG, Academy Positions Committee Workgroup. Practice paper of the Academy of Nutrition and Dietetics abstract: nutrition and lifestyle for a healthy pregnancy outcome. *J Acad Nutr Diet.* 2014;114(9):1447. PubMed PMID: 25699300.
31. Lowensohn RI, Stadler DD, Naze C. Current concepts of maternal nutrition. *Obstet Gynecol Surv*. 2016;1(7):413–426. PubMed PMID: 27436176.
32. Britten P, Cleveland LE, Koegel KL, et al. Impact of typical rather than nutrient-dense food choices in the US Department of Agriculture food patterns. *J Acad Nutr Diet.* 2012;112(10):1560–1569. PubMed PMID: 22906562.
33. Caughey AB. Gestational weight gain and outcomes for mothers and infants. *JAMA*. 317(21):2175–2176. PubMed PMID: 28586874.
34. Goldstein RF, Abell SK, Ranasinha S, et al. Association of gestational weight gain with maternal and infant outcomes: a systematic review and meta-analysis. *JAMA*. 2017;317(21):2207–2225. PubMed PMID: 28586887.
35. International Weight Management in Pregnancy (i-WIP) Collaborative Group. Effect of diet and physical activity based interventions in pregnancy on gestational weight gain and pregnancy outcomes: meta-analysis of individual participant data from randomised trials. *BMJ*. 2017;358:j3119. PubMed PMID: 28724518.
36. Stephenson J, Heslehurst N, Hall J, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. *Lancet*. 2018;391:1830–1841. PubMed PMID: 29673873.
37. Imdad A, Bhutta ZA. Effect of balanced protein energy supplementation during pregnancy on birth oucomes. *BMC Public Health*. 2011;11(suppl 3):S17. PubMed PMID: 21501434.
38. Alexander BT, Henry Dasinger J, Intapad S. Effect of low birth weight on women's health. *Clin Ther.* 2014;36(12):1913–1923. PubMed PMID: 25064626.
39. Kominiarek MA, Rajan P. Nutrition recommendations in pregnancy and lactation. *Med Clin North Am*. 2016;100(6):1199–1215. PubMed PMID: 27745590.
40. Gomes CF, Sousa M, Lourenço I, Martins D, Torres J. Gastrointestinal diseases during pregnancy: what does the gastroenterologist need to know? *Ann Gastroenterol*. 2018;31(4):385–394. PubMed PMID: 29991883.

41. Melina V, Craig W, Levin S. Position of the academy of nutrition and dietetics: vegetarian diets. *J Acad Nutr Diet.* 2016;116(12):1970–1980. PubMed PMID: 27886704.
42. Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. *Am J Obstet Gynecol*. 2009;201(1):58. e1-e8. PMID: 19467640.
43. Pistollato F, Sumalla Cano S, Elio I, et al. Plant-based and plant-rich diet patterns during gestation: beneficial effects and possible shortcomings. *Adv Nutr*. 2015;6(5):581–591. PubMed PMID: 26374180.
44. Baroni L, Goggi S, Battaglino R, et al. Vegan nutrition for mothers and children: practical tools for healthcare providers. *Nutrients*. 2018;11(1):5. PubMed PMID: 30577451.
45. U.S. Department of Health & Human Services. Foodsafety.gov. Food safety for pregnant women; 2018. https://www.foodsafety.gov/risk/pregnant/index.html. Accessed October 6, 2018.
46. Procter SB, Campbell CG. Position of the academy of nutrition and dietetics: nutrition and lifestyle for a healthy pregnancy outcome. *J Acad Nutr Diet.* 2014;114(7):1099–1103. PubMed PMID: 24956993.
47. Montgomery KS. Nutrition column: an update on water needs during pregnancy and beyond. *J Perinat Educ*. 2002;11(3):40–42. PubMed PMID: 17273308.
48. Gizzo S, Noventa M, Vitagliano A, et al. An update on maternal hydration strategies for amniotic fluid improvement in isolated oligohydramnios and normohydramnios: evidence from a systematic review of literature and meta-analysis. *PLoS One*. 2015;10(12):e0144334. PubMed PMID: 26658482.
49. McKenzie AL, Perrier ET, Guelinckx I, et al. Relationships between hydration biomarkers and total fluid intake in pregnant and lactating women. *Eur J Nutr*. 2017;56(6):2161–2170. PubMed PMID: 27519184.
50. McKenzie AL, Armstrong LE. Monitoring body water balance in pregnant and nursing women: the validity of urine color. *Ann Nutr Metab*. 2017;70(suppl 1):18–22. PubMed PMID: 28614809.
51. Ndikom CM, Fawole B, Ilesanmi RE. Extra fluids for breastfeeding mothers for increasing milk production. *Cochrane Database Syst Rev*. 2014;6:CD008758. PubMed PMID: 24916640.
52. Bardosono S, Morin C, Guelinckx I, Pohan R. Pregnant and breastfeeding women: drinking for two? *Ann Nutr Metab*. 2017;70(suppl 1):13–17. PubMed PMID: 28614807.
53. EFSA Panel on dietetic products, nutrition, and allergies (NDA). Scientific opinion on dietary reference values for water. *EFSA J*. 2010;8(3):1459. accessed https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1459. Accessed September 28, 2018.
54. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev*. 2012;70(1):3–21. PubMed PMID: 22221213.
55. Kanguru L, McCaw-Binns A, Bell J. The burden of obesity in women of reproductive age and in pregnancy in a middle-income setting: a population based study from Jamaica. *PLoS One.* 12(12):e0188677. PubMed PMID: 29236710.
56. Gilmore LA, Redman LM. Weight gain in pregnancy and application of the 2009 IOM guidelines: toward a uniform approach. *Obesity (Silver Spring, Maryland)*. 2015;23(3):507–511.
57. Committee on Practice Bulletins (Obstetrics), American College of Obstetricians and Gynecologists. Practice bulletin No. 156: obesity in pregnancy. *Obstet Gynecol*. 2016;128(6):1450. PubMed PMID: 28092301.
58. Centers for Disease Control and Prevention. *Prevalence and Trends in Prepregnancy normal Weight—48 States*, New York City, and District of Columbia, 2011-2015; 2018. https://www.cdc.gov/mmwr/volumes/66/wr/mm665152a3.htm Accessed September 25, 2018.
59. Walker R, Bennett C, Blumfield M. Attenuating pregnancy weight gain—what works and why: a systematic review and meta-analysis. *Nutrients*. 2018;10(7):944. PubMed PMID: 30037126.
60. Ministry of Health—Manatū Hauora, New Zealand Government. Healthy weight gain during pregnancy; 2018. https://www.health.govt.nz/your-health/healthy-living/food-activity-and-sleep/healthy-weight/healthy-weight-gain-during-pregnancy. Accessed September 27, 2018.
61. Dean SV, Lassi ZS, Imam AM, Bhutta ZA. Preconception care: nutritional risk and interventions. *Reprod Health*. 2014;11(suppl 3):S3. PubMed PMID: 25415364.
62. Shin D, Lee KW, Song WO. Pre-pregnancy weight status is associated with diet quality and nutritional biomarkers during pregnancy. *Nutrients*. 2016;8(3):162. PubMed PMID: 26978398.
63. Stang J, Huffman LG. Position of the Academy of Nutrition and Dietetics: obesity, reproduction, and pregnancy outcomes. *J Acad Nutr Diet.* 2018;116(4):677–691. PubMed PMID: 27017177.
64. Siega-Riz AM, Deierlein A, Stuebe A. Implementation of the new institute of medicine gestational weight gain guidelines. *J Midwifery Womens Health*. 2010;55(6):512–519. PubMed PMID: 20974413.
65. Committee on Opinion Bulletins (Obstetrics), American College of Obstetricians and Gynecologists. Committee opinion No. 650: physical activity and exercise during pregnancy and the postpartum period. *Obstet Gynecol*. 2015;128(6):e135–e142. PubMed PMID: 26595585.
66. Entin PL, Coffin L. Physiological basis for recommendations regarding exercise during pregnancy at high altitude. *High Alt Med Biol*. 2004;5(3):321–334. PubMed PMID: 15453998.
67. Hinman SK, Smith KB, Quillen DM, Smith MS. Exercise in pregnancy: a clinical review. *Sports Health*. 2015;7(6):527–531. PubMed PMID: 26502446.
68. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for Americans. *JAMA*. 320(19):2020-2028. PubMed PMID: PubMed PMID: 30418471.
69. Magro-Malosso ER, Saccone G, Di Tommaso M, et al. Exercise during pregnancy and risk of gestational hypertensive disorders: a systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 2017;96(8):921–931.
70. Du MC, Ouyang YQ, Nie XF. *Effects of Physical Exercise during Pregnancy on Maternal and Infant Outcomes in Overweight and Obese Pregnant Women: A Meta-Analysis*. Birth; 2018. [Epub ahead of print]. PubMed PMID: 30240042.
71. Evenson KR, Barakat R, Brown WJ, et al. Guidelines for physical activity during pregnancy: comparisons from around the world. *Am J Lifestyle Med*. 2014;8(2):102–141. PubMed PMID: 25346651.
72. Wang C, Wei Y, Zhang X, et al. A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women. *Am J Obstet Gynecol*. 2017;216(4):340–351. PubMed PMID: 28161306.
73. Magro-Malosso ER, Saccone G, Di Mascio D, et al. Exercise during pregnancy and risk of preterm birth in overweight and obese women: a systematic review and meta-analysis of randomized controlled trials. *Acta Obstet Gynecol Scand*. 2017;96(8):921–931. PubMed PMID: 28401531.
74. Mindell JA, Cook RA, Nikolovski J. Sleep patterns and sleep disturbances across pregnancy. *Sleep Medicine*. 2015;16(4):483–488. PubMed PMID: 25666847.
75. Bourjeily G, Danilack VA, Bublitz MH, et al. Obstuctive sleep apnea in pregnancy is associated with adverse maternal outcomes: a national cohort. *Sleep Medicine*. 2017:50–57. PubMed PMID: 29031756.
76. Ding XX, Wu YL, Xu SJ, et al. A systematic review and quantitative assessment of sleep-disordered breathing during pregnancy and perinatal outcomes. *Sleep & Breathing*. 2014;18(4):703–713. PubMed PMID: 24519711.
77. Li L, Zhao K, Hua J, Li S. Association between sleep-disordered breathing during pregnancy and maternal and fetal outcomes: an updated systematic review and meta-analysis. *Front Neurol*. 2018;9:91. PubMed PMID: 29892255.
78. Truong K, Guilleminault C. Sleep disordered breathing in pregnant women: maternal and fetal risk, treatment considerations, and future perspective. *Expert Rev Respir Med*. 2018;12(3):177–189. PubMed PMID: 29363362.
79. Felder JN, Baer RJ, Rand L, et al. Sleep disorder diagnosis during pregnancy and risk of preterm birth. *Obstet Gynecol*. 2017;130(3):573–581. PubMed PMID: 28796676.
80. Oyiengo D, Louis M, Hoot B, Bourjeily G. Sleep disorders in pregnancy. *Clin Chest Med*. 2014;35(3):571–587. PubMed PMID: 25156772.

81. Prosperetti C, Manconi M. Restless legs syndrome/Willis-Ekbom disease and pregnancy. *Sleep Medicine Clinics*. 2015;10(3):323–329. PubMed PMID: 26329442.
82. Gupta R, Dhyani M, Kendzerska T, et al. Restless legs syndrome and pregnancy: prevalence, possible pathophysiological mechanisms and treatment. *Acta Neurol Scand*. 2016;133(5):320–329. PubMed PMID: 26482928.
83. Dunietz GL, Lisabeth LD, Shedden K, et al. Restless legs syndrome and sleep-wake disturbances in pregnancy. *J Clin Sleep Med*. 2017;13(7):863–870. PubMed PMID: 28633715.
84. Howe LD, Signal TL, Paine SJ, et al. Self-reported sleep in late pregnancy in relation to birth size and fetal distress: the E Moe, Māmā prospective cohort study. *BMJ Open*. 2015;5(10):e008910. PubMed PMID: 26438138.
85. Minar M, Habanova H, Rusnak I, et al. Prevalence and impact of restless legs syndrome in pregnancy. *Neuro Endocrinol Lett*. 2013;34(5):366–371. PubMed PMID: 23922045.
86. Oyieng'o DO, Kirwa K, Tong I, et al. Restless legs syndrome and pregnancy and neonatal outcomes. *Clin Ther*. 2016;38(2):256–264. PubMed PMID: 26740290.
87. Ramirez JO, Cabrera SA, Hidalgo H, et al. Is preeclampsia associated with restless legs syndrome? *Sleep Medicine*. 2013;14(9):894–896. PubMed PMID: 23891236.
88. Louw KA. Substance use in pregnancy: the medical challenge. *Obstetric Medicine*. 2018;11(2):54–66. PubMed PMID: 29997687.
89. Centers for Disease Control Behavioral Risk Factor Surveillance System, United States, 2011-2013. https://www.cdc.gov/ncbddd/fasd/data.html. Accessed on July 29, 2018.
90. Devries KM, Child JC, Bacchus LJ, et al. Intimate partner violence victimization and alcohol consumption in women: as systematic review and meta-analysis. *Addiction*. 2014;109(3):379–391. PubMed PMID: 24329907.
91. Lassi ZS, Imam AM, Dean SV, Bhutta ZA. Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. *Reprod Health*. 2014;11(suppl 3):S6. PubMed PMID: 25415846.
92. Sebastiani G, Borrás-Novell C, Casanova MA, et al. The effects of alcohol and drugs of abuse on maternal nutritional profile during pregnancy. *Nutrients*. 2018;10(8). PubMed PMID: 30072661.
93. Singh S, Sedgh G, Hussain R. Unintended pregnancy: worldwide levels, trends, and outcomes. *Stud Fam Plann*. 2010;41(4):241–250. PubMed PMID: 21465725.
94. Fortin M, Muckle G, Anassour-Laouan-Sidi E, et al. Trajectories of alcohol use and binge drinking among pregnant Inuit women. *Alcohol Alcohol*. 2016;51(3):339–346. PubMed PMID: 26409003.
95. Pryor J, Patrick SW, Sundermann AC, et al. Pregnancy intention and maternal alcohol consumption. *Obstet Gynecol*. 2017;129(4):727–733. PubMed PMID: 28277356.
96. Floyd RL, Sobell M, Velasquez MM, et al. Preventing alcohol-exposed pregnancies: a randomized controlled trial. *Am J Prev Med*. 2007;32(1):1–10. PubMed PMID: 17218187.
97. Ingersoll KS, Ceperich SD, Nettleman MD, et al. Reducing alcohol-exposed pregnancy risk in college women: initial outcomes of a clinical trial of a motivational intervention. *J Subst Abuse Treat*. 2005;29(3):173–180. PubMed PMID: 16183466.
98. CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *Br Med J*. 2008;337:a2332. PubMed PMID: 18981029.
99. Chen LW, Wu Y, Neelakantan N, et al. Maternal caffeine intake during pregnancy and risk of pregnancy loss: a categorical and dose-response meta-analysis of prospective studies. *Public Health Nutr*. 2016;19(7):1233–1244. PubMed PMID: 26329421.
100. Sengpiel V, Elind E, Bacelis J, et al. Maternal caffeine intake during pregnancy is associated with birth weight but not with gestational length: results from a large prospective observational cohort study. *BMC Medicine*. 2013;11:42. PubMed PMID: 23421532.
101. Li J, Zhao H, Song JM, et al. A meta-analysis of risk of pregnancy loss and caffeine and coffee consumption during pregnancy. *Int J Gynaecol Obstet*. 2015;130(2):116–122. PubMed PMID: 26026343.
102. Poole R, Kennedy OJ, Roderick P, et al. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. *Br Med J*. 2017;359:j5024. PubMed PMID: 29167102.
103. Rhee J, Kim R, Kim Y, et al. Maternal caffeine consumption during pregnancy and risk of low birth weight: a dose-response meta-analysis of observational studies. *PLoS One*. 2015;10(7):e0132334. PubMed PMID: 26193706.
104. Tolstrup JS, Kjaer SK, Munk C, et al. Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? *Hum Reprod*. 2003;18(12):2704–2710.
105. Papadopoulou E, Botton J, Brantsaeter AL, et al. Maternal caffeine intake during pregnancy and childhood growth and overweight: results from a large Norwegian prospective observational cohort study. *BMJ Open*. 2018;8(3):e018895. PubMed PMID: 29685923.
106. American College of Obstetricians and Gynecologists. Committee opinion No. 722: marijuana use during pregnancy and lactation. *Obstet Gynecol*. 2017;130(4):e205–e209. PubMed PMID: 28937574.
107. Crume TL, Juhl AL, Brooks-Russell A, et al. Cannabis use during the perinatal period in a state with legalized recreational and medical marijuana: the association between maternal characteristics, breastfeeding patterns, and neonatal oucomes. *J Pediatr*. 2018;197:90–96. PubMed PMID: 29605394.
108. Mark K, Gryczynski J, Axenfeld E, et al. Pregnant women's current and intended cannabis use in relation to their views toward legalization and knowledge of potential harm. *J Addict Med*. 2017;11(3):211–216. PubMed PMID: 28252456.
109. Ko JY, Farr SL, Tong VT, et al. Prevalence and patterns of marijuana use among pregnant and nonpregnant women of reproductive age. *Am J Obstet Gynecol*. 2015;213(2):201. PubMed PMID: 25772211.
110. Merritt TA, Wilkinson B, Chervenak C. Maternal use of marijuana during pregnancy and lactation: implications for infant and child development and their well-being. *Acad J PediatrNeonatology*. 2016;2(1):555580.
111. Bertrand KA, Hanan NJ, Bhonerkamp-Smith G, et al. Marijuana use by breastfeeding mothers and cannabinoid concentrations in breast milk. *Pediatrics*. 2018;142(3):e20181076. PubMed PMID: 30150212.
112. Baker T, Datta P, Rewers-Felkins K, et al. Transfer of inhaled cannabis into human breast milk. *Obstet Gynecol*. 2018;131(5):783–788. PubMed PMID: 29630019.
113. Gunn JKL, Rosales CB, Center KE, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. *BMJ Open*. 2016;6(4):e009986. PubMed PMID: 27048634.
114. Leemaqz SY, Dekker GA, McGowen LM, et al. Maternal marijuana use has independent effects on risk for spontaneous preterm birth but not other common late pregnancy complications. *Reproductive Toxicol*. 2016;62:77–86. PubMed PMID: 27142189.
115. Warshak CR, Regan J, Moore B, et al. Association between marijuana use and adverse obstetrical and neonatal outcomes. *J Perinatol*. 2015;35(12):991–995. PubMed PMID: 26401751.
116. Matthews A, Haas DM, O'Mathuna DP, Dowswell T. Interventions for nausea and vomiting in early pregnancy. *Cochrane Database Syst.Rev*. 2015;9:CD007575. PubMed PMID: 26348534.
117. Einarson TR, Piwko C, Koren G. Prevalence of nausea and vomiting of pregnancy in the USA: a meta-analysis. *J Popul Ther Clin Pharmacol*. 2013;20(2):e163–e170. PubMed PMID: 23863545.
118. Young-Wolff KC, Sarovar V, Tucker LY, et al. Association of nausea and vomiting in pregnancy with prenatal marijuana use. *JAMA Intern Med*. 2018;178(10):1423–1424. PubMed PMID: 30128499.
119. Roberson EK, Patrick WK, Hurwitz EL. Marijuana use and maternal experiences of severe nausea during pregnancy in Hawai'i. *Hawaii J Med Public Health*. 2014;73(9):283–287. PubMed PMID: 25285255.
120. Society of Obstetricians and Gynaecologists of Canada. https://sogc.org/files/Cannabis%20campaign_web.pdf. Accessed September 16, 2018.
121. GBD 2013 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 70 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries,

1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015;386(10010):2287–2323. PubMed PMID: 26364544.
122. U.S. Department of Health and Human Services. *The Health Consequences of Smoking—50 Years of Progress. A Report of the Surgeon General*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014. https://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf. Accessed September 1, 2018.
123. Wagner NJ, Camerota M, Propper C. Prevalence and perceptions of electronic cigarette use during pregnancy. *Matern Child Health J*. 2017;21(8):1655–1661. PubMed PMID: 28084577.
124. Wigginton B, Gartner C, Rowlands IJ. Is it safe to vape? Analyzing online forums discussing E-cigarette use during pregnancy. *Womens Health Issues*. 2017;21(1):93–99. PubMed PMID: 27773530.
125. Coleman T, Chamberlain C, Davey MA, et al. Pharmacological interventions for promoting smoking cessation during pregnancy. *Cochrane Database Syst Rev*. 2015;12:CD010078. PubMed PMID: 26690977.
126. WHO Framework Convention on Tobacco Control. *Electronic Nicotine Delivery Systems: Report by WHO*; 2014. http://www.who.int/fctc/cop/cop7/FCTC_COP_7_11_EN.pdf?ua=1&ua=1. Accessed September 6, 2018.
127. Goniewicz ML, Smith DM, Edwards KC, et al. Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes. *JAMA Netw Open*. 2018;1(8):e185937. PubMed PMID: 30646298.
128. Baba S, Wikström AK, Stephansson O, Cnattingius S. Influence of smoking and snuff cessation on risk of preterm birth. *Eur J Epidemiol*. 2012;27(4):297–304. PubMed PMID: 22430122.
129. England MC, Benjamin A, Abenhaim HA. Increased risk of preterm premature rupture of membranes at early gestational ages among maternal cigarette smokers. *Am J Perinatol*. 2013;30(10):821–826. PubMed PMID: 23329562.
130. Källén K. The impact of maternal smoking during pregnancy on delivery outcome. *Eur J Public Health*. 2001;11(3):329–333. PubMed PMID: 11582615.
131. Kaymeba-Kay's S, Ribrault A, Burguet A, et al. Maternal smoking during pregnancy and fetal growth. Effects in preterm infants of gestational age less than 33 weeks. *Swiss Medical Weekly*. 2010;140:w13139. PubMed PMID: 21181569.
132. Murin S, Rafii R, Bilello K. Smoking and smoking cessation in pregnancy. *Clin Chest Med*. 2011;32(1):75–91. PubMed PMID: 21277451.
133. Pineles BL, Park E, Samet JM. Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. *Am J Epidemiol*. 2014;179(7):807–823. PubMed PMID: 24518810.
134. Shobeiri F, Jenabi E. Smoking and placenta previa: a meta-analysis. *J Matern Fetal Neonatal Med*. 2017;30(24):2985–2990. PubMed PMID: 27936997.
135. Vardavas CI, Chatzi L, Patelarou E, et al. Smoking and smoking cessation during early pregnancy and its effect on adverse pregnancy outcomes and fetal growth. *Eur J Pediatr*. 2010;169(6):741–748. PubMed PMID: 19953266.
136. Ward C, Lewis S, Coleman T. Prevalence of maternal smoking and environmental tobacco smoke exposure during pregnancy and impact on birth weight: retrospective study using Millennium Cohort. *BMC Public Health*. 2007;7:81. PubMed PMID: 17506887.
137. Reece S, Morgan C, Parascandola M, Siddiqi K. Secondhand smoke exposure during pregnancy: a cross-sectional analysis of data from Demographic and Health Survey from 30 low-income and middle-income countries. *Tobacco Control*. 2019;28(40):420–426. PMID: 30026189.
138. Wahabi HA, Alzeidan RA, Fayed AA, et al. Effects of secondhand smoke on the birth weight of term infants and the demographic profile of Saudi exposed women. *BMC Public Health*. 2013;13:341. PubMed PMID: 2358716.
139. Budani MC, Fensore S, Di Marzio M, Tiboni GM. Cigarette smoking impairs clinical outcomes of assisted reproductive technologies: a meta-analysis of the literature. *Reproductive Toxicol*. 2018;80:49–59. PMID: 29906539.
140. Centers for Disease Control, National Center for Health Statistics. *(2018). Cigarette Smoking during Pregnancy: United States*; 2016. https://www.cdc.gov/nchs/products/databriefs/db305.htm. Accessed July 31, 2018.
141. Chamberlain C, O'Mara-Eves A, Porter J, et al. Psychosocial interventions for supporting women to stop smoking in pregnancy. *Cochrane Database Syst Rev*. 2017;2:CD001055. PubMed PMID: 28196405.
142. ACOG Committee Opinion No. 575 Exposure to toxic environmental agents. *Obstet Gynecol*. 2013;122(4):931–935. PubMed PMID: 24084567.
143. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environmental Health Perspectives*. 2011;119(6):878–885. PubMed PMID: 21233055.
144. Weck RL, Paulose T, Flaws JA. Impact of environmental factors and poverty on pregnancy outcomes. *Clinical Obstet Gynecol*. 2008;51(2):349–359. PubMed PMID: 18463465.
145. American College of Obstetricians and Gynecologists. *Exposure to Toxic Environmental Agents*; 2013. https://www.acog.org/-/media/Committee-Opinions/Committee-on-Health-care-for-Underserved-Women/ExposuretoToxic.pdf. Accessed February 8, 2019.
146. Crinnion WJ, Pizzorno JE. *Clinical Environmental Medicine: Identification and Natural Treatment of Disease Caused by Common Pollutants*. St. Louis, Missouri: Elsevier, Inc; 2019. ISBN: 978-0-323-480864.
147. Endocrine Society. *Introduction to Endocrine Disrupting Chemicals: A Guide for Public Interest Organizations and Policymakers*; 2014. https://www.endocrine.org/topics/edc/introduction-to-edcs. Accessed February 10, 2019.
148. World Health Organization. *State of the Science of Endocrine Disrupting Chemicals 2012*; 2013. https://www.who.int/ceh/publications/endocrine/en/. Accessed February 10, 2019.
149. National Institute of Environmental Health Sciences. Bisphenol A (BPA). https://www.niehs.nih.gov/health/topics/agents/sya-bpa/index.cfm. Accessed December 23, 2018.
150. European Commission Regulation 2018/213: Official journal of the European Union. On the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials; 2018. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0213&from=EN. Accessed February 10, 2019.
151. Lathi RB, Liebert CA, Brookfield KF, et al. Conjugated bisphenol A in maternal serum in relation to miscarriage risk. *Fertil Steril*. 2014;102(1):123–128. PubMed PMID: 24746738.
152. Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. *Fertil Steril*. 2008;89(suppl 2):e1–e20. PubMed PMID: 18308046.
153. Liberto TL. Screening for depression and help-seeking in postpartum women during well-baby pediatric visits: an integrated review. *J Pediatr Health Care*. 2012;26(2):109–117. PubMed PMID: 22360930.
154. Shi Z, MacBeth A. The effectiveness of mindfulness-based interventions on maternal perinatal mental health outcomes: a systematic review. *Mindfulness (NY)*. 2017;8(4):823–847. PubMed PMID: 28757900.
155. Dole N, Savitz DA, Hertz-Picciotto I, et al. Maternal stress and preterm birth. *Am J Epidemiol*. 2003;157(1):14–24. PubMed PMID: 12505886.
156. Field T, Diego M, Hernandez-Reif M, et al. Comorbid depression and anxiety effects on pregnancy and neonatal outcome. *Infant Behav Dev*. 2010;33(1):23–29. PubMed PMID: 19945170.
157. Maina G, Saracco P, Giolito MR, et al. Impact of maternal psychological distress on fetal weight, prematurity and intrauterine growth retardation. *J Affect Disord*. 2008;111(2–3):214–220. PubMed PMID: 18394713.
158. Quevedo LA, Silva RA, Godoy R, et al. The impact of maternal post-partum depression on the language development of children at 12 months. *Child Care Health Dev*. 2012;38(3):420–424. PubMed PMID: 21651606.
159. Sydsjö G. Long-term consequences of non-optimal birth characteristics. *Am J Reprod Immunol*. 2011;66(suppl 1):81–87. PubMed PMID: 21726342.
160. Talge NM, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry*. 2007;48(3–4):245–261. PubMed PMID: 17355398.

161. Wadhwa PD, Entringer S, Buss C, Lu MC. The contribution of maternal stress to preterm birth: issues and considerations. *Clin Perinatol*. 2011;38(3):351–384. PubMed PMID: 21890014.
162. Glynn L, Schetter CD, Hobel CJ, Sandman CA. Pattern of perceived stress and anxiety in pregnancy predicts preterm birth. *Health Psychology*. 2008;27(1):43–51. PubMed PMID: 18230013.
163. Dunkel Schetter C, Tanner L. Anxiety, depression and stress in pregnancy: implications for mothers, children, research, and practice. *Curr Opin Psychiatry*. 2012;25(2):141–148. PubMed PMID: 22262028.
164. Nezvalová-Henriksen K, Spigset O, Brandlistuen RE, et al. Effect of prenatal selective serotonin reuptake inhibitor (SSRI) exposure on birthweight and gestational age: a sibling-controlled cohort study. *Int J Epidemiol*. 2016;45(6):2018–2029. PubMed PMID: 27188860.
165. Eke AC, Saccone G, Berghella V. Selective serotonin reuptake inhibitor (SSRI) use during pregnancy and risk of preterm birth: a systematic review and meta-analysis. *BJOG*. 2016;123(12):1900–1907. PubMed PMID: 27238775.
166. Grigordiadis S, Vonderporten EH, Mamisashvili L, et al. Prenatal exposure to antidepressants and persistent pulmonary hypertension of the newborn: systematic review and meta-analysis. *BMJ*. 2014;348:f6932. PubMed PMID: 24429387.
167. Huang H, Coleman S, Bridge JA, Yonkers K, Katon W. A meta-analysis of the relationship between antidepressant use in pregnancy and the risk of preterm birth and low birth weight. *Gen Hosp Psychiatry*. 2014;36(1):13–18. PubMed PMID: 24094568.
168. Lewis AJ, Galbally M, Opie G, Buist A. Neonatal growth outcomes at birth and one month postpartum following in utero exposure to antidepressant medication. *Aust N Z J Psychiatry*. 2010;44(5):482–487. PubMed PMID: 20397792.
169. Munk-Olsen T, Liu X, Viktorin A, et al. Maternal and infant outcomes associated with lithium use in pregnancy: an international collaborative meta-analysis of six cohort studies. *Lancet Psychiatry*. 2018;5(8):644–652. PMID: 29929874.
170. Dhillon A, Sparkes E, Duarte RV. Mindfulness-based interventions during pregnancy: a systematic review and meta-analysis. *Mindfulness (NY)*. 2017;8(6):1421–1437. PubMed PMID: 29201244.
171. Zielińska MA, Wesolowska A, Pawlus B, Hamułka J. Health effects of carotenoids during pregnancy and lactation. *Nutrients*. 2017;9(8). PubMed PMID: 28777356.
172. Alwan NA, Greenwood DC, Simpson NA, et al. The relationship between dietary supplement use in late pregnancy and birth outcomes: a cohort study in British women. *BJOG*. 2010;117(7):821–829. PubMed PMID: 20353456.
173. Haider BA, Bhutt ZA. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst Rev*. 2017;4:CD004905. PubMed PMID: 28407219.
174. Zerfu TA, Ayele HT. Micronutrients and pregnancy; effect of supplementation on pregnancy and pregnancy outcomes: a systematic review. *Nutr J*. 2013;12:20. PubMed PMID: 23368953.
175. Balogun OO, da Silva Lopes K, Ota E, et al. Vitamin supplementation for preventing miscarriage. *Cochrane Database Syst Rev*. 2016;5:CD004073. PubMed PMID: 27150280.
176. Schwalfenberg G, Rodushkin I, Genius SJ. Heavy metal contamination of prenatal vitamins. *Toxicol Rep*. 2018;5:390–395. PubMed PMID: 29854609.
177. Perry CA, West AA, Gayle A, et al. Pregnancy and lactation alter biomarkers of biotin metabolism in women consuming a controlled diet. *J Nutr*. 2014;144(12):1977–1984. PubMed PMID: 25122647.
178. Mock DM. Biotin: from nutrition to therapeutics. *J Nutr*. 2017;147(8):1487–1492. PubMed PMID: 28701385.
179. Mock DM. Biotin status assessed longitudinally in pregnant women. *J Nutr*. 1997;127(5):710–716. PubMed PMID: 9164991.
180. Mock DM. Marginal biotin deficiency during normal pregnancy. *Am J Clin Nutr*. 2002;75(2):295–299.
181. Mock DM. Marginal biotin deficiency is teratogenic in mice and perhaps humans: a review of biotin deficiency during human pregnancy and effects of biotin deficiency on gene expression and enzyme activities in mouse dam and fetus. *J Nutr Biochem*. 2005;16(7):435–437. PubMed PMID: 15992686.
182. Ettinger AS, Lamadrid-Figueroa H, Mercado-Garcia A, et al. Effect of calcium supplementation on bone resorption in pregnancy and the early postpartum: a randomized controlled trial in Mexican women. *Nutr J*. 2014;13(1):116. PubMed PMID: 25511814.
183. Hofmeyr GJ, Lawrie TA, Atallah ÁN, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev*. 2018;10:CD001059. PubMed PMID: 30277579.
184. Hofmeyr GJ, Manyame S. Calcium supplementation commencing before or early in pregnancy or food fortification with calcium, for preventing hypertensive disorders of pregnancy. *Cochrane Database Syst Rev*. 2017;9:CD011192. PubMed PMID: 28949421.
185. Procter SB, Campbell CG. Position of the academy of nutrition and dietetics: nutrition and lifestyle for healthy pregnancy. *J Acad Nutr Diet*. 2014;114(7):1099–1103. PubMed PMID: 24956993.
186. Hacker AN, Fung EB, King JC. Role of calcium during pregnancy: maternal and fetal needs. *Nutr Rev*. 2012;70(7):397–409. PubMed PMID: 22747842.
187. Lindsay KL, Hellmuth C, Uhl O, et al. Longitudinal metabolomics profiling of amino acids and lipids across healthy pregnancy. *PLoS One*. 2015;10(12):e0145794. PubMed PMID: 26716698.
188. Cho SW, Cha YS. Pregnancy increases urinary loss of carnitine and reduces plasma carnitine in Korean women. *Br J Nutr*. 2005;93(5):685–691. PubMed PMID: 15975168.
189. Kepka A, Chojnowska S, Okungbowa OE, Zwierz K. The role of carnitine in the perinatal period. *Dev Period Med*. 18(4):417-425. PubMed PMID: 25874778.
190. Schoderbeck M, Auer B, Legenstein E, et al. Pregnancy-related changes of carnitine and acylcarnitine concentrations of plasma and erythrocytes. *J Perinatal Med*. 1995;23(6):477–485. PubMed PMID: 8904477.
191. Cederblad G, Fåhraeus L, Lindgren K. Plasma carnitine and renal-carnitine clearance during pregnancy. *Am J Clin Nutr*. 1986;44:379–383.
192. Duran M, Loof NE, Ketting D, et al. Secondary carnitine deficiency. *J Clin Chem Clin Biochem*. 1990;28:359–363.
193. Keller U, van der Wal C, Seliger G, et al. Carnitine status of pregnant women: effect of carnitine supplementation and correlation between iron status and plasma carnitine concentration. *Eur J Clin Nutr*. 2009;63(9):1098–1105. PubMed PMID: 19491916.
194. Tipi-Akbas P, Arioz DT, Kanat-Pektas M, et al. Lowered serum total L-carnitine levels are associated with obesity at term pregnancy. *J Matern Fetal Neonatal Med*. 2013;26(15):1479–1483. PubMed PMID: 23560471.
195. Lohninger A, Radler U, Jinniate S, et al. Relationship between carnitine, fatty acids and insulin resistance. *Gynakol Geburtshilfliche Rundsch*. 2009;49(4):230–235. PubMed PMID: 20530934.
196. de Bruyn A, Jacquemyn Y, Kinget K, Eyskens F. Carnitine deficiency and pregnancy. *Case Reports in Obstet Gynecol*. 2015:101468. Epub 2015 May 28. PMID: 26113999.
197. Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann N Y Acad Sci*. 2004;1033:30–41. PubMed PMID: 15591001.
198. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med*. 2005;26(6):459–516. PubMed PMID: 16309738.
199. Brantsaeter AL, Haugen M, Rasmussen SE, et al. Urine flavonoids and plasma carotenoids in the validation of fruit, vegetable and tea intake during pregnancy in the Norwegian mother and child cohort study (MoBa). *Public Health Nutr*. 2007;10(8):838–847. PubMed PMID: 17493318.
200. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*. 2014;6(2):466–488.
201. Sharma JB, Kumar A, Kumar A, et al. Effect of lycopene on pre-eclampsia and intra-uterine growth retardation in primigravidas. *Int J Gynaecol Obstet*. 2003;81(3):257–262.
202. Hanson C, Lyden E, Furtado J, et al. Serum lycopene concentrations and associations with clinical outcomes in a cohort of maternal-infant dyads. *Nutrients*. 2018;10(2). PubMed PMID: 29438287.

203. Kramer MS, Kahn SR, Platt RW, et al. Antioxidant vitamins, long-chain fatty acids, and spontaneous preterm birth. *Epidemiology*. 2009;20(5):707–713. PubMed PMID: 19568173.
204. Carmichael SL, Yang W, Shaw GM, et al. Maternal dietary nutrient intake and risk of preterm delivery. *Am J Perinatol*. 2013;30(7):579–588. PubMed PMID: 23208764.
205. Cohen JM, Kahn SR, Platt RW, et al. Small-for-gestational-age birth and maternal plasma antioxidant levels in mid-gestation: a nested case-control study. *BJOG*. 2015;122(10):1313–1321. PubMed PMID: 25677044.
206. Wallace TC, Fulgoni VL. Usual choline intakes are associated with egg and protein food consumption in the United States. *Nutrients*. 2017;9(8). PubMed PMID: 28783055.
207. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. *Annu Rev Nutr*. 2006;26:229–250. PubMed PMID: 16848706.
208. Yan J, Jiang X, West AA, et al. Pregnancy alters choline dynamics: results of a randomized trial using stable isotope methodology in pregnant and nonpregnant women. *Am J Clin Nutr*. 2013;98(6):1459–1467. PubMed PMID: 24132975.
209. Wiedeman AM, Barr SI, Green TJ, et al. Dietary choline intake: current state of knowledge across the life cycle. *Nutrients*. 2018;10:10. PubMed PMID: 30332744.
210. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. *Nutr Rev*. 2009;67(11):615–623. PubMed PMID: 19906248.
211. European Food Safety Authority. *Dietary Reference Values for Choline*; 2016. https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4484. Accessed January 3, 2019.
212. American Medical Association. *AMA Backs Global Health Experts in Calling Infertility a Disease*; 2017. https://www.ama-assn.org/delivering-care/public-health/ama-backs-global-health-experts-calling-infertility-disease. Accessed January 2, 2019.
213. Procter SB, Campbell CG. Position paper of the academy of nutrition and dietetics: nutrition and lifestyle for a health pregnancy. *J Acad Nutr Diet*. 2014;114(7):1099–1103. PubMed PMID: 24956993.
214. Yan J, Jiang X, West AA, et al. Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. *Am J Clin Nutr*. 2012;95(5):1060–1070. PubMed PMID: 22418088.
215. Bell CC, Aujla J. Prenatal vitamins deficient in recommended choline intake for pregnant women. *J Family Med Dis Prev*. 2016;2:048. ISSN: 2469-5793.
216. National Institutes of Health. Office of Dietary Supplements. Folate. https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#en1. Accessed January 19, 2019.
217. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. 1992;327(26):1832–1835. PubMed PMID: 1307234.
218. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the medical research council vitamin study. *Lancet*. 1991;20(8760):338, 131–137. PubMed PMID: 1677062.
219. Chitayat D, Matsui D, Amitai Y, et al. Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. *J Clin Pharmacol*. 2016;56(2):170–175. PubMed PMID.
220. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification—its history, effect, concerns, and future directions. *Nutrients*. 2011;3(3):370–384.
221. Viswanathan M, Treiman KA, Kish-Doto J, et al. Folic Acid Supplementation for the prevention of neural tube defects: an updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA*. 2017;317(2):190–203. PubMed PMID: 28097361.
222. Zhang T, Lou J, Zhong r, et al. Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. *PLoS One*. 2013;8(4):e59570. PubMed PMID: 23593147.
223. Obeid R, Oexle K, Rißmann A, et al. Folate status and health: challenges and opportunities. *J Perinat Med*. 2016;44(3):261–268. PubMed PMID: 25825915.
224. Zhang Q, Wang Y, Xin X, et al. Effect of folic acid supplementation on preterm delivery and small for gestational age births: a systematic review and meta-analysis. *Reproductive Toxicol*. 2017;67:35–41. PubMed PMID: 27856370.
225. Zheng JS, Guan Y, Zhao Y, et al. Pre-conceptional intake of folic acid supplements is inversely associated with risk of preterm birth and small-for-gestational-age birth: a prospective cohort study. *Br J Nutr*. 2016;115(3):509–516. PubMed PMID: 26651997.
226. Pietrzik K, Bailey L, Shane B. Folic acid and L-5-methyltetrahydrofolate: comparison of clinical pharmacokinetics and pharmacodynamics. *Clin Pharmacokinet*. 2010;49(8):535–548. PubMed PMID: 20608755.
227. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid*. 2017;27(3):315–389. PubMed PMID: 28056690.
228. Ershow AG, Goodman G, Coates PM, Swanson CA. Assessing iodine intake, iodine status, and the effects of maternal iodine supplementation: Introduction to articles arising from 3 workshops held by the NIH Office of Dietary Supplements. *Am J Clin Nutr*. 2016;104(suppl 3):859S–863S. PubMed PMID: 27534646.
229. World Health Organization, United Nations Children's Fund. *International Council for the Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination: A Guide for Programme Managers*. 3rd ed. Geneva: World Health Organization; 2007.
230. Caldwell KL, Makhmudov A, Ely E, et al. Iodine status of the U.S. Population, national health and nutrition examination survey, 2005-2006 and 2007-2008. *Thyroid*. 2011;21(4):419–427. PubMed PMID: 21323596.
231. Panth P, Guerin G, DiMarco NM. A review of iodine status of women of reproductive age in the USA. *Biol Trace Elem Res*. 2019. [Epub ahead of print]. PMID: 30617900.
232. De Leo S, Pearce EN, Braverman LE. Iodine supplementation in women during preconception, pregnancy, and lactation: current clinical practice by U.S. obstetricians and midwives. *Thyroid*. 2017;27(3):434–439. PubMed PMID: 27784201.
233. Pearce EN, Lazarus JH, Moreno-Reyes R, Zimmermann MB. Consequences of iodine deficiency and excess in pregnant women: an overview of current knowns and unknowns. *Am J Clin Nutr*. 2016;104(suppl 3):918S–923S. PubMed PMID: 27534632.
234. Harding KB, Peña-Rosas JP, Webster AC, et al. Iodine supplementation for women during the preconception, pregnancy and postpartum period. *Cochrane Database Syst Rev*. 2017;3:CD011761. PubMed PMID: 28260263.
235. National Institutes of Health Office of Dietary Supplements. Iodine. https://ods.od.nih.gov/factsheets/Iodine-HealthProfessional/. Accessed January 8, 2019.
236. Patel A, Lee SY, Stagnaro-Green A, et al. Iodine content of the best-selling United States adult and prenatal multivitamin preparations. *Thyroid*. 2019;29(1):124–127. PubMed PMID: 30266075.
237. Roy A, Evers SE, Campbell MK. Dietary supplement use and iron, zinc and folate intake in pregnant women in London, Ontario. *Chronic Dis Inj Can*. 2012;32(2):76–83. PubMed PMID: 22414304.
238. Cantor AG, Bougatsos C, Dana T, et al. Routine iron supplementation and screening for iron deficiency anemia in pregnancy: a systematic review of the U.S. Preventive Services Task Force. *Ann. Intern Med*. 21:162(8):566-576. PubMed PMID: 25820661.
239. Milman N, Taylor CL, Merkel J, Brannon PM. Iron status in pregnant women and women of reproductive age in Europe. *Am J Clin Nutr*. 2017;106(suppl 6):1655S–1662S. PubMed PMID: 29070543.
240. McDonagh M, Cantor A, Bougatsos C, et al. *Routine Iron Supplementation and Screening for Iron Deficiency Anemia in Pregnant Women: A Systematic Review to Update the U.S. Preventive Services Task Force Recommendation [Internet]*. Rockville (MD): Agency for Healthcare Research and Quality (US), Mar, Report No. 13-05187-EF-2; 2015. PubMed PMID: 25927136.
241. Breymann C. Iron deficiency anemia in pregnancy. *Semin Hematol*. 2015;52(4):339–347. PubMed PMID: 26404445.
242. Rahmati S, MiladAzami PN, Sayehmiri K. The relationship between maternal anemia during pregnancy with preterm birth: a systematic re-

view and meta-analysis. *J Matern Fetal Neonatal Med*. 2018;1:151. [Epub ahead of print]. PMID: 30522368.
243. Figueiredo ACMG, Gomes-Filho IS, Silve RB, et al. Maternal anemia and low birth weight: a systematic review and meta-analysis. *Nutrients*. 2018;10(5). PubMed PMID: 29757207.
244. Rahmati S, Delpishe A, Azami M, et al. Maternal anemia during pregnancy and infant low birth weight: a systematic review and meta-analysis. *Int J Reprod Biomed (Yazd)*. 2017;15(3):125–134. PubMed PMID: 28580444.
245. Peña-Rosas JP, De-Regil LM, Carcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev*. 2015;7:CD004736. PubMed PMID: 26198451.
246. Abbas AM, Abdelbadee SA, Alanwar A, Mostafa S. Efficacy of ferrous bis-glycinate versus ferrous glycine sulfate in the treatment of iron deficiency anemia with pregnancy: a randomized double-blind clinical trial. *J Matern Fetal Neonatal Med*. 2018:1–7. [Epub ahead of print]. PubMed PMID: 29843553.
247. National Institutes of Health Office of Dietary Supplements. Magnesium. https://ods.od.nih.gov/factsheets/Magnesium-HealthProfessional/. Accessed January 6, 2019.
248. Arikan GM, Panzitt T, Gücer F, et al. Course of maternal serum magnesium levels in low-risk gestations and in preterm labor and delivery. *Fetal Diagn Ther*. 1999;14(6):332–336. PubMed PMID: 10640870.
249. Enaruna NO, Ande A, Okpere EE. Clinical significance of low serum magnesium in pregnant women attending the University of Benin Teaching Hospital. *Niger J Clin Pract*. 2013;16(4):448–453. PubMed PMID: 23974737.
250. Bullarbo M, Ödman N, Nestler A, et al. Magnesium supplementation to prevent high blood pressure in pregnancy: a randomised placebo control trial. *Arch Gynecol Obstet*. 2013;288(6):1269–1274. PubMed PMID: 23715924.
251. Asemi Z, Karamali, J.M, et al, Magnesium supplementation affects metabolic status and pregnancy outcomes in gestational diabetes: a randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr*. 102(1):222-229. PubMed PMID: 26016859.
252. Zarean E, Tarjan A. Effect of magnesium supplement on pregnancy outcomes: a randomized control trial. *Advanced Biomedical Research*. 2017;6:109. PubMed PMID: 2890437.
253. National Institutes of Health. Office of Dietary Supplements. Omega-3 Fatty Acids. https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/. Accessed January 27, 2019.
254. Zhang Z, Fulgoni VL, Kris-Etherton PM, Mitmesser SH. Dietary intakes of EPA and DHA omega-3 fatty acids among US childbearing-age and pregnant women: an analysis of NHANES 2001-2014. *Nutrients*. 2018;10(4). PubMed PMID: 29597261.
255. Querques G, Forte R, Souied EH. Retina and omega-3. *J Nutr Metab*. 2011;748361. PubMed PMID: 22175009.
256. Kar S, Wong M, Rogozinska E, Thangaratinam S. Effects of omega-3 fatty acids in prevention of early preterm delivery: a systematic review and meta-analysis of randomized studies. *Eur J Obstet Gynecol Reprod Biol*. 2016;198:40–46. PubMed PMID: 26773247.
257. Shulkin M, Pimpin L, Bellinger D, et al. n-3 fatty acid supplementation in mothers, preterm infants and term infants and childhood psychomotor and visual development: a systematic review and meta-analysis. *J Nutr*. 2018;148(3):409–418. PubMed PMID: 29546296.
258. Kominiarek MA, Rajan P. Nutrition recommendations in pregnancy and lactation. *Med Clin North Am*. 2016;100(6):1199–1215. PubMed PMID: 27745590.
259. Bridges CC, Zalups RK. Mechanisms involved in the transport of mercuric ions in target tissues. *Arch Toxicol*. 2017;91(1):63–81. PubMed PMID: 27422290.
260. Rebelo FM, Caldas ED. Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. *Environ Res*. 2016;151:671–688. PubMed PMID: 27619212.
261. National Institutes of Health Office of Dietary Supplements. Vitamin A. https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/. Accessed January 19, 2019.
262. Miller RK, Hendrickx AG, Mills JL, Hummler H, Wiegand UW. Periconceptional vitamin A use: how much is teratogenic? *Reprod Toxicol*. 1998;12(1):75–88. PubMed PMID: 9431575.
263. Kominiarek MA, Ragan P. Nutrition recommendations in pregnancy and lactation. *Med Clin North Am*. 2016;100(6):1199–1215. PubMed PMID: 27745590.
264. McCauley ME, van den Broek N, Dou L, Othman M. Vitamin A supplementation during pregnancy for maternal and newborn outcomes. *Cochrane Database Syst Rev*. 2015;10:CD008666. PubMed PMID: 26503498.
265. Thorne-Lyman AL, Fawzi WW. Vitamin A and carotenoids during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. *Paediatr Perinat Epidemiol*. 2012;(suppl 1):36–45. PubMed PMID: 22742601.
266. Cohen JM, Beddaoui M, Kramer MS, et al. Maternal antioxidant levels in pregnancy and risk of preeclampsia and small for gestational age birth: a systematic review and meta-analysis. *PLos One*. 2015;10(8):e0125192. PubMed PMID: 26247870.
267. Salles AM, Galvao TF, Silva MT, et al. Antioxidants for preventing preeclampsia: a systematic review. *Sci World J*. 2012;243476. PubMed PMID: 22593668.
268. Rumbold A, Ota E, Nagata C, et al. Vitamin C supplementation in pregnancy. *Cochrane Database Syst Rev*. 2015;9:CD004072. PubMed PMID: 26415762.
269. Rumbold A, Ota E, Hori H, et al. Vitamin E supplementation in pregnancy. *Cochrane Database Syst Rev*. 2015;9:DC004069. PubMed PMID: 26343254.
270. National Institutes of Health. Office of Dietary Supplements. Vitamin D. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/. Accessed January 21, 2019.
271. Pilz S, Zittermann A, Obeid R, et al. The role of vitamin D in fertility and during pregnancy and lactation: a review of clinical data. *Int J Environ Res Public Health*. 2018;15(10). PubMed PMID: 30322097.
272. Curtis EM, Moon RJ, Harvey NC, Cooper C. Maternal vitamin D supplementation during pregnancy. *Br Med Bull*. 2018;126(1):57–77. PubMed PMID: 29684104.
273. Hollis BW, Wagner CL. New insights into the vitamin D requirements during pregnancy. *Bone Research*. 2017;5:17030. PubMed PMID: 28868163.
274. Kominiarek MA, Rajan P. Nutrition recommendations in pregnancy and lactation. *Med Clin North Am*. 2016;100(6):1199–1215. PubMed PMID: 27745590.
275. Aghajafari F, Field CJ, Kaplan BJ, et al. The current recommended vitamin D intake guidelines for diet and supplements during pregnancy is not adequate to achieve vitamin D sufficiency for most pregnant women. *PLoS One*. 2016;11(7):e0157262. PubMed PMID: 27367800.
276. Wilson RL, Leviton AJ, Leemaqz SY, et al. Vitamin D levels in an Australian and New Zealand cohort and the association with pregnancy outcome. *BMC Pregnancy Childbirth*. 2018;18(1):251. PubMed PMID: 29925344.
277. Palacios C, De-Regil LM, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation during pregnancy: updated meta-analysis on maternal outcomes. *J Steroid Biochem Mol Biol*. 2016;164:148–155. PubMed PMID: 26877200.
278. De-Reglis LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. *Sao Paulo Med J*. 2016;134(3):274–275. PubMed PMID: 27355803.
279. Pérez-López ER, Paupuleti V, Mezones-Holguin E, et al. Effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes: a systematic review and meta-analysis of randomized controlled trials. *Fertil Steril*. 2015;103(5):1278–1288. PubMed PMID: 25813278.
280. Hollis BW, Wagner CL. Vitamin D and pregnancy: skeletal effects, nonskeletal effects, and birth outcomes. *Calcif Tissue Int*. 2013;92(2):128–129. PubMed PMID: 22623177.
281. Arnold DL, Enquobahrie DA, Qiu C, et al. Early pregnancy maternal vitamin D concentratioins and risk of gestational diabetes mellitus. *Paediatr Perinat Epidemiol*. 2015;29(3):200–210. PubMed PMID: 25808081.

282. Zhang Y, Gong Y, Xue H, et al. Vitamin D and gestational diabetes mellitus: a systematic review based on data free of Hawthorne effect. *BJOG*. 2018;125(7):784–793. PubMed PMID: 29244241.
283. Serrano-Díaz NC, Gamboa-Delgado EM, Domínguez-Urrego CL. Vitamin D and risk of preeclampsia: a systematic review and meta-analysis. *Biomedica*. 2018;38(suppl 1):43–53. PubMed PMID: 29874709.
284. Samimi M, Foroozanfard F, Amini F, Sehat M. Effect of vitamin D supplementation on unexplained recurrent spontaneous abortion: a double-blind randomized controlled trial. *Glob J Health Sci*. 2017;9(3):95–112. ISSN: 1916-9736.
285. Chu J, Gallos I, Tobias A, et al. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. *Hum Reprod*. 2018;33(1):65–80. PubMed PMID: 29149263.
286. Mumford SL, Garbose RA, Kim K, et al. Association of preconception serum 25-hydroxyvitamin D concentrations with livebirth and pregnancy loss: a prospective study. *Lancet Diabetes Endocrinol*. 2018;6(9):725–732. PubMed PMID: 29859909.
287. Zhao J, Huang X, Xu B, et al. Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. *Reprod Biol Endocrinol*. 2018;16(1):13. PubMed PMID: 29426322.
288. Rudick BJ, Ingles SA, Chung K, et al. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. *Fertil Steril*. 2014;101(2):447–452. PubMed PMID: 24210230.
289. Wilson RL, Grieger JA, Bianco-Miotto T, Roberts CT. Association between maternal zinc status, dietary zinc intake and pregnancy complications: a systematic review. *Nutrients*. 2016;8(10). PubMed PMID: 2775441.
290. Rink L, Gabriel P. Zinc and the immune system. *Proc Nutr Soc*. 2000;59(4):541–552. PubMed PMID: 11115789.
291. Ota E, Mori R, Middleton P, et al. Zinc supplementation for improving pregnancy and infant outcomes. *Cochrane Database Syst Rev*. 2015;2:CD000230. PubMed PMID: 25927101.
292. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *Am J Clin Nutr*. 2003;78(suppl 3):633S–639S. PubMed PMID: 12936958.
293. Foster M, Herulah UN, Prasad A, et al. Zinc status of vegetarians during pregnancy: a systematic review of observational studies and meta-analysis of zinc intake. *Nutrients*. 2015;7(6):4512–4525. PubMed PMID: 26056918.

Premenstrual Syndrome

Joseph Katzinger, ND, and *Tori Hudson*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Recurrent signs and symptoms that develop during the late luteal phase of the menstrual cycle and disappear by the end of the full flow of menses
- Symptoms typically include decreased energy, tension, irritability, depression, headache, altered sex drive, breast pain, backache, abdominal bloating, and edema of the fingers and ankles.

GENERAL CONSIDERATIONS

Premenstrual syndrome (PMS) refers to the cyclic constellation of troublesome symptoms that appears during the luteal phase of the menstrual cycle—more so in the late luteal phase—disappear by the end of the full flow of menses, and do not appear during the follicular phase.[1] Although some 150 symptoms have been listed as premenstrual, the most common symptoms are as follows (Box 212.1):

- Decreased energy
- Tension
- Irritability
- Anger
- Food cravings
- Depression
- Headache
- Altered sex drive
- Breast pain
- Muscle aches
- Abdominal bloating
- Edema of the fingers and ankles

PMS is estimated to affect between 30% and 40% of menstruating women; 80% of women experience premenstrual emotional or physical changes but do not have much difficulty. Peak occurrence is among women in their 30s and 40s. In most cases symptoms are relatively mild; however, in about 3% to 8% of women, symptoms can be severe enough to have a negative impact on their lives, putting home life and work in jeopardy.[2] Severe PMS with depression, irritability, and severe mood swings is referred to as *premenstrual dysphoric disorder* (PMDD)[3] and has a separate diagnostic category in the 5th edition of the American Psychological Association's *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*.

Although PMS has been a well-defined clinical entity for more than 60 years, some physicians still argue that it really does not exist.[4] Therefore, many women suffering from PMS do not receive proper treatment. Until the advent of therapy with selective serotonin reuptake inhibitors (SSRIs), conventional mainstream medicine had not been able to offer women a known cause for PMS, nor has it been able to offer satisfactory interventions. Women were by and large left to self-care or to seek the advice of alternative medicine practitioners.

The etiology of PMS is not yet fully understood, but endocrine studies have clarified that the premenstrual symptoms are not a result of a simple excess or deficiency of any given hormone.[5] Several theories have been proposed to explain the causes of PMS; currently, the strongest is an interaction between the ovarian steroids and the brain's neurotransmitters. Genetic predispositions and sociocultural beliefs about menstruation may also influence what women experience. At present, the dominant thinking is that cyclic changes in the ovarian steroids estrogen and progesterone cause changes in many body systems, including brain neurotransmitters, which then have emotional and physical manifestations.[6] Functional magnetic resonance imaging (MRI) supports this as a plausible etiology and has shown discrete areas of the brain with changes in functional connectivity among women with PMS, including the default mode network, middle frontal gyrus, and the parahippocampal gyrus.[7] Structural MRI has also found evidence for differences in brain morphology, such as changes in gray-matter volume in specific areas of the brain, including the precuneus/posterior cingulate cortex, which correlated with the daily rating of severity of problems index.[8] Therefore, normal ovarian function, and not a true hormonal imbalance, triggers the central nervous system and predisposes a woman to hormone-induced instability.[9] We do not currently know why women differ in their sensitivity

*Previous edition contributor

BOX 212.1 Signs and Symptoms of Premenstrual Syndrome

Behavioral
- Nervousness, anxiety, and irritability
- Mood swings and mild to severe personality change
- Fatigue, lethargy, and depression

Gastrointestinal
- Abdominal bloating
- Diarrhea and/or constipation
- Change in appetite (usually craving of sugar)

Female
- Tender, enlarged breasts
- Uterine cramping
- Altered libido

General
- Headache
- Backache
- Acne
- Edema of fingers and ankles

to the changes in ovarian-steroid–induced neurotransmitters. Of the neurotransmitters studied, serotonin is the principal one implicated in the pathogenesis of PMS and PMDD.[10,11] Whether PMS and PMDD are related to absolute levels or reduced blood levels of serotonin or to serotonin transport remains unclear.[12]

Other neurotransmitter systems may also be involved in PMS and PMDD. They include the adrenergic, opioid, and gamma-aminobutyric acid (GABA) systems. Recent studies have implicated allopregnanolone, a metabolite of progesterone and a potent positive modulator of the $GABA_A$ receptor, as having an important role. For example, an important randomized controlled trial of the 5α-reductase inhibitor dutasteride, which blocks the conversion of progesterone to allopregnanolone, found a significant reduction in core symptoms among women with PMDD, including irritability, sadness, anxiety, food cravings, and bloating, with 75% of women enrolled in the study no longer meeting the criteria for PMDD after 1 month of active treatment.[13] Research over the last 10 to 15 years has shown that SSRIs can alleviate both the psychological and physical symptoms in most women with PMDD. Fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and venlafaxine have all been found to be significantly more effective than placebo for the treatment of PMDD. Interestingly, SSRIs also enhance the conversion of progesterone to allopregnanolone, increasing cerebrospinal fluid (CSF) levels of this neurosteroid, which suggests that fluctuations in allopregnanolone may be more consequential than absolute levels.

DIAGNOSIS AND CLASSIFICATION

The diagnosis of PMS is usually made through the association of the symptoms attributed to PMS with their occurrence during the luteal phase of the menstrual cycle. To aid in the diagnosis, symptom questionnaires are often used. Recalled information is less accurate; therefore, in addition to a symptom questionnaire, the patient may be asked to keep a menstrual symptom diary. This helps to document improvement as well as to further clarify the symptom pattern.

The key defining feature of both PMS and PMDD is the timing of symptoms, which appear only during the luteal phase and disappear before or during menstruation. Fundamental to making the diagnosis is to rule out other psychiatric and medical disorders. The clinician should obtain a medical and psychiatric history and be aware that many mental and physical disorders mimic premenstrual symptoms and may also coexist with them. The results of the medical, psychiatric, and psychosocial evaluations along with the symptom diary offered by the patient can help the clinician to determine whether the symptoms represent PMS or PMDD, a psychiatric or medical illness only, coexisting PMS or PMDD and another illness, or premenstrual exacerbation of an underlying psychiatric or medical illness.

Only one of the following symptoms is required for a diagnosis of PMS: mild psychological discomfort, bloating and weight gain, breast tenderness, swelling of hands and feet, various aches and pains, poor concentration, sleep disturbance, and change in appetite. The symptoms must (1) occur only in the luteal phase, (2) peak close to menstruation, and (3) subside at the onset of menses or during menstrual flow. A diagnosis of PMDD is reserved for women who have at least 5 of 11 specific symptoms at a severe level such that they interfere significantly with daily life and relationships and occur in the premenstrual phase of the cycle.[14]

It must include one (or more) of the following symptoms:
- Marked affective lability (e.g., mood swings, feeling suddenly sad or tearful, or increased sensitivity to rejection)
- Marked irritability or anger or increased interpersonal conflicts
- Marked depressed mood, feelings of hopelessness, or self-deprecating thoughts
- Marked anxiety, tension, and/or feelings of being keyed up or on edge
- It must also include one (or more) of the following symptoms, for a total of five (or more)
- Decreased interest in usual activities (e.g., work, school, friends, hobbies)
- Subjective difficulty concentrating
- Lethargy, easy fatigability, or marked lack of energy
- Marked change in appetite; overeating; or specific food cravings
- Hypersomnia or insomnia
- Feeling overwhelmed or out of control
- Additional physical symptoms (breast tenderness or swelling, joint or muscle pain, a sensation of "bloating," or weight gain)

THERAPEUTIC CONSIDERATIONS

Even though it is now understood that there is no frank increase or decrease in serum estrogen or progesterone levels, a possible relative excess or insufficiency in terms of its effect on the central nervous system should still be considered. Owing to the lack of scientific understanding in this area and the clinical experience in improving estrogen metabolism in women with PMS, some clinical considerations from the past should still be recalled.

Estrogen Metabolism

In the early 1940s, Morton Biskind observed an apparent relationship between B-vitamin deficiency and PMS.[15,16] He postulated that PMS, as well as excessive menstruation and fibrocystic breast disease, was due to an excess in estrogen levels caused by decreased detoxification and elimination in the liver as a result of B-vitamin deficiency. The liver uses various B vitamins to detoxify estrogen and excrete it in the bile.

There appears to be support for Biskind's theory. Estrogen excess is known to produce *cholestasis,* a term that signifies diminished bile flow or stasis of bile. Naturopathic physicians often refer to this condition as a "sluggish liver." It reflects minimal impairment of liver function because normal indicators of liver status (such as concentrations of the liver enzymes alkaline phosphatase,

serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase, and gamma-glutamyl-transpeptidase) are not elevated. These enzyme measurements, the conventional means of assessing liver status, are not very useful because they serve only to indicate liver damage, being elevated only when the liver is compromised. They have little correlation with liver function. Because of the liver's important role in numerous metabolic processes, even a minor impairment of liver function can have profound effects.

Cholestasis can be caused by a large number of factors besides estrogen excess (Box 212.2). The presence of cholestasis may be a predisposing factor in PMS because with cholestasis, there is reduced estrogen detoxification and clearance. Hence, a positive-feedback scenario is produced. The high incidence of gallstones is a clear indication that many American women suffer from cholestasis.

Again, even though elevated serum estrogen values cannot be confirmed in PMS studies, it is interesting to note that estrogen excess during the luteal phase affects endorphin levels negatively. One study found a direct correlation between an increased estrogen:progesterone ratio and endorphin activity in the brain.[17] In essence, when the estrogen:progesterone ratio was increased, there was a decline in endorphin levels. This reduction is significant considering the known ability of endorphins to normalize or improve mood. Other studies have shown that low endorphin levels during the luteal phase are common in women with PMS.[18] Endorphins are lowered by stress and raised by exercise. The role of endorphins is discussed further later in the chapter. Finally, recent data suggest that estrogen-receptor sensitivity may play a role. In a study that enrolled 100 women with PMDD and 96 controls, estrogen levels were not found to be correlated with PMDD. However, among women who carried the G allele of the estrogen receptor (ESR) α-Xbal polymorphism (rs9340799), premenstrual estrogen level was negatively correlated with anxiety and stress; this polymorphism also appeared to affect emotion regulation.[19] Thus the impact of estrogen on premenstrual symptoms may also be influenced by genetic polymorphism in the estrogen receptor.

Estrogen Impairs Vitamin B_6

The way in which estrogen levels during the luteal phase negatively affect neurotransmitter and endorphin levels may be secondary to impairment of the action of vitamin B_6. It is well known that estrogens affect vitamin B_6 function negatively, and this includes more recent lower-dose estrogens.[20] Vitamin B_6 levels are typically quite low in depressed patients, especially women taking estrogens (birth control pills or conjugated estrogens [Premarin]).[21,22] Vitamin B_6 supplementation has been shown to have positive effects on all PMS symptoms, particularly depression, in many women (discussed in greater detail later). This improvement is achieved via a combination of a reduction in midluteal estrogen levels and an increase in midluteal progesterone levels.

Dietary Considerations

Women suffering from PMS typically eat a diet that is even worse than the standard American diet. Abraham[23] reports that, compared with symptom-free women, patients with PMS consume the following substances:

- 62% more refined carbohydrates
- 275% more refined sugar
- 79% more dairy products
- 78% more sodium
- 53% less iron
- 77% less manganese
- 52% less zinc

BOX 212.2 Causes of Cholestasis

- Estrogen excess or birth control pills
- Pregnancy
- Presence of gallstones
- Alcohol
- Endotoxins
- Hereditary disorders such as Gilbert's syndrome
- Anabolic steroids
- Various chemicals and drugs
- Nutritional deficiencies

A diet that is higher in dairy products may also contribute to some PMS symptoms. A survey of 39 women with PMS and 14 women without reported that the women with PMS consumed fivefold more dairy products and threefold more refined sugar than the women without PMS.[24] However, a diet rich in calcium and vitamin D was clearly shown to be associated with a lower risk for PMS in the Nurses' Health Study II cohort.[25] Another study observed that women with PMS also have an increased intake of dietary fat, carbohydrates, and simple sugars and decreased protein intake.[26] This is somewhat consistent with the results from a recent study of over 300 nurses, which found that a Western diet (characterized by a higher intake of red meat, fast foods, vegetable oil and mayonnaise, sweets and desserts, salty snacks, refined grains, sugar and soft drinks, high-fat dairy products, spices, and fried potato) was linked to a higher likelihood of PMS; women in the second and third quintiles of the Western pattern had an adjusted risk of 2.53 and 4.39, respectively, although a dose–response relationship was not observed.[27]

Food cravings are often also higher in women with PMS; this may, in part, be due to a decrease in serotonin during the luteal phase in PMS sufferers. Therefore serotonergic treatments may be helpful in controlling such food cravings.[28]

Another nutritional factor in PMS is the effect of refined sugars on rapid increases in insulin, which then causes the retention of sodium and subsequent water retention, with swelling in the hands and feet, abdominal bloating, and breast engorgement. Sugar has several detrimental actions in PMS. Eating foods high in simple sugars increases insulin secretion and can be harmful to blood sugar control, especially if the patient is hypoglycemic or diabetic. Sugar, especially combined with caffeine, also has a detrimental effect on PMS and mood (discussed later).[29] A high intake of sugar also impairs estrogen metabolism. The evidence is based on the higher frequency of PMS symptoms in women consuming a high-sugar diet and the fact that a high sugar intake is also associated with higher estrogen levels.[30] One study found that a low-fat, high-complex-carbohydrate diet alleviated premenstrual breast tenderness.[31]

C-reactive protein, a marker of inflammation, has been correlated with the severity of both the physical and psychological symptoms of PMS, as have other biomarkers of inflammation.[32–34] A diet that stimulates inflammatory pathways includes sugar, poultry, eggs, cheese, milk, white flour, white rice, and partially hydrogenated oils. Foods that can reduce inflammation include fresh fruits (especially berries), green leafy vegetables, fish, nuts, seeds, turmeric, garlic, and onions.

Vegetarian women have been shown to excrete two to three times more estrogen in their feces and to have 50% lower levels of free estrogen in their blood than omnivores.[35,36] These differences are thought to be due to the lower fat and higher fiber intake of vegetarian women. These dietary differences may also explain the lower incidence of breast cancer, endometriosis, and uterine cancer in vegetarian women. They may also play a role in PMS. For example, a lower intake of plant protein (but not total protein) was recently correlated to symptoms of PMS among athletes.[37]

At the very least, the clinician should recommend a diet lower in saturated fat and cholesterol (achieved by reducing or eliminating the consumption of animal products) and higher in fiber-rich plant foods (fruits, vegetables, grains, and legumes).

Decreasing the percentage of calories as fat, in particular saturated fat, has dramatic effects on the reduction of circulating estrogen.[38,39] In one study, when 17 women switched from the standard American diet (composed of 40% of calories as fat and only 12 g as fiber daily) to a low-fat, high-fiber diet (consisting of 25% of calories as fat and 40 g as fiber daily), there was a 36% reduction in blood estrogen levels, with 16 out of the 17 women demonstrating significant reductions in only 8 to 10 weeks.[40]

It should be noted that not all nutrition research shows a clear-cut association with PMS. In the Study of Women's Health Across the Nation, a cross-sectional analysis was conducted of PMS symptoms in a multiethnic sample of 3302 midlife women.[41] The researchers sought to determine whether the frequency of physical or emotional premenstrual symptoms was associated with dietary intake of phytoestrogens/fiber/fat or calcium, consumption of alcohol or caffeine, cigarette exposure, lack of physical exercise, and race/ethnicity or socioeconomic status. In this study, most dietary factors were not related to PMS. Fat intake was negatively associated with craving and bloating. Fiber intake was positively associated with breast pain. Alcohol intake was negatively associated with anxiety, mood changes, and headaches. Cigarette smoke exposure, whether passive or active, was positively associated with cramps and back pain. Ethnic differences in the reporting of symptoms and comorbidity associations were observed as well.

Thyroid Function

Low thyroid function (hypothyroidism) has been shown to affect a large proportion of women with PMS.[42,43] For example, in one study, 51 of 54 subjects with PMS demonstrated low thyroid status compared with 0 of 12 in the control group.[42] In another study, 7 of 10 subjects in the PMS group had low thyroid status compared with 0 of 9 in the control group.[43] Other studies have also shown hypothyroidism to be only slightly more common in women with PMS than in controls.[44,45] Many women with PMS and confirmed hypothyroidism who are given thyroid hormone experience complete relief of symptoms.[42] For more information, see Chapter 182.

Stress, Coping Style, and Depression

Study of the interaction of stress/serotonin and PMS has shown that serotonin levels in women with PMS fall after ovulation. Those without PMS had much higher levels of serotonin during the last half of the menstrual cycle. In a holistic/natural medicine approach to PMS, the identification of stressors and stress management should not be overlooked.[46] Many women with PMS tend to employ "negative" coping styles,[47] examples of which are listed in Box 212.3. It is important to identify the manner in which the patient deals with stress and to counsel her on more positive ways of coping.

There are some important relationships between PMS and depression. Depression is a common feature in many cases of PMS, and PMS symptoms are typically more severe in depressed women.[1] The reason appears to be a decrease in the brain level of various neurotransmitters, with serotonin and GABA being the most significant.[48,49] The most common class of prescription medications recommended for moderate to severe PMS/PMDD is an SSRI. When they are used for chronic depression, SSRIs must be taken daily. When used for PMS/PMDD, they can be taken just on the days of the month when the symptoms are the most problematic. This would suggest that when used in this way, they are not really increasing neurotransmitter levels but work by altering the neurophysiology and electrical conduction in the brain. Several SSRIs have been used, including fluoxetine, sertraline, and paroxetine. However, various psychotherapy methods have been equally if not more successful in improving the psychological aspects of PMS. In particular, biofeedback and short-term individual counseling (especially cognitive therapy) have documented clinical efficacy.[50,51] One of the advantages of cognitive therapy over antidepressant drug therapy in the treatment of PMS is that learning techniques such as cognitive-behavioral coping skills can produce excellent results that are maintained over time.

BOX 212.3 Negative Coping Styles in Women With Premenstrual Syndrome

- Feelings of helplessness
- Overeating
- Watching too much television
- Emotional outbursts
- Overspending
- Excessive/irrational behavior
- Dependence on chemicals: drugs, legal and illicit; alcohol; cigarettes

Exercise

Several studies have shown that women engaged in a regular exercise program do not suffer from PMS nearly as often as sedentary women.[52–54] In one of the more thorough studies, mood and physical symptoms during the menstrual cycle were assessed in 97 women who exercised regularly and in a second group of 159 women who did not exercise.[52] Mood scores and physical symptoms assessed throughout the menstrual cycle showed significant effects of exercise on negative mood states and physical symptoms. The regular exercisers obtained significantly lower scores for impaired concentration, negative mood, behavior change, and pain.

In another study, 143 women were monitored for 5 days in each of the three phases of their cycles (midcycle, premenstrual, and menstrual).[52] The women were 35 competitive athletes, two groups of exercisers (33 high-frequency exercisers and 36 low-frequency exercisers), and 39 sedentary women. The high-frequency exercisers experienced the greater positive mood scores and sedentary women the least. The high-frequency exercisers also reported the least depression and anxiety. The differences were most apparent during the premenstrual and menstrual phases. These results are consistent with the belief that women who exercise frequently (but not competitive athletes) are protected from PMS symptoms. In particular, regular exercise protects against the deterioration of mood before and during menstruation. A large study published in 2018 also supports the value of regular exercise; among the 7000 women enrolled in this study, those who participated in regular exercise had a reduced frequency of many symptoms, including backaches, abdominal discomfort, depression, and constipation.[55]

These studies provide evidence that women with PMS should partake of the benefits of exercise. Exercise may reduce PMS symptoms by elevating endorphin values, improving glucose tolerance, decreasing catecholamines, and modulating estrogen levels.[56]

Nutritional Supplements

Nutritional supplements have been widely used in the treatment of PMS by alternative practitioners and also by women taking things into their own hands. Despite the inconsistent evidence, the positive results do show that nutritional supplements can offer safe, affordable solutions for many problems.

Vitamin B6

The first use of vitamin B_6 in the management of cyclic conditions in women was in the successful treatment of depression caused by birth control pills, as noted in several studies in the early 1970s. These results led researchers to try to determine the effectiveness of vitamin B_6 in relieving PMS symptoms. Since 1975 at least a dozen double-blind clinical trials have been performed. Some of these studies have shown no effect, but most of the studies have shown a significant effect on the whole range of PMS symptoms at dosage ranges from 50 to 500 mg/day.[57] For example, in one double-blind crossover trial, 84% of the subjects had a lower symptom score during the vitamin B_6 treatment period.[58] In another double-blind crossover trial, 50 mg/day of vitamin B_6 was effective in decreasing premenstrual depression, fatigue, and irritability.[59] Although PMS has multiple causes, vitamin B_6 supplementation alone appears to benefit most patients.

It is important to note, however, that not all double-blind studies of vitamin B_6 have shown a positive effect.[57,60] The negative results in some trials may have been caused by many factors, such as the inability of some women to convert vitamin B_6 to its active form owing to a deficiency in another nutrient (e.g., vitamin B_2 or magnesium) that was not supplemented. These results suggest that supplementing pyridoxine by itself may not produce adequate clinical results for all women suffering from this disorder, and that some women may have difficulty converting vitamin B_6 into its active form, pyridoxal-5-phosphate. To overcome this conversion difficulty, it may be necessary to use a broader-spectrum nutritional supplement or pyridoxal-5-phosphate.

For most indications, the therapeutic dosage of vitamin B_6 is 50 to 100 mg/day. This dose level is generally regarded as safe, even for long-term use. With the use of doses greater than 50 mg, it may be important to divide it into 50-mg doses taken throughout the day. A single dose of 100 mg of pyridoxine did not lead to significantly higher pyridoxal-5-phosphate levels in the blood than a 50-mg dose, possibly indicating that a 50-mg oral dose of pyridoxine is about all the liver can handle at once.[61]

Vitamin B_6 is one of the few water-soluble vitamins associated with some toxicity when taken in large doses or in moderate dosages for long periods. One-time doses larger than 2000 mg/day can produce symptoms of nerve toxicity (tingling sensations in the feet, loss of muscle coordination, and degeneration of nerve tissue) in some individuals. Long-term intake of doses larger than 500 mg a day can be toxic if taken for many months or years.[62] There are also a few rare reports of toxicity occurring at long-term dosages as low as 150 mg/day.[62–64] The toxicity is thought to occur when supplemental pyridoxine overwhelms the liver's ability to add a phosphate group to produce the active form of vitamin B_6 (pyridoxal-5-phosphate). As a result, it is speculated that pyridoxine either is toxic to the nerve cells or actually acts as an antimetabolite by binding to pyridoxal-5-phosphate receptors, thereby creating a relative deficiency of vitamin B_6; this competitive inhibition of the active form of vitamin B_6 was recently shown in cell culture.[65] Another possibility is that minute amounts of contaminants or vitamin B_6 analogs may have been introduced during synthesis. Although not a problem at normal doses of vitamin B_6, contaminants present in larger doses may block vitamin B_6 activation sites. Again, it appears to make sense to limit doses to 50 mg at a time. If more than 50 mg/day is desired, the doses should be spread out throughout the day.

Vitamin B_6 has the ability to increase the synthesis of several neurotransmitters in the brain, including serotonin, dopamine, norepinephrine, epinephrine, taurine, and histamine. There are also extensive interactions between vitamin B_6 and magnesium, which work together in many enzyme systems. In fact, another mechanism by which vitamin B_6 may improve the symptoms of PMS is by increasing the accumulation of magnesium within body cells.[66] Without vitamin B_6, magnesium does not get inside the cell.

Magnesium

Magnesium deficiency has been implicated as a causative factor in PMS.[67] Red blood cell (RBC) magnesium levels in patients with PMS have been shown to be significantly lower than in normal subjects.[23,68] Because magnesium plays such an integral part in normal cell function, magnesium deficiency may account for the wide range of symptoms attributed to PMS. Furthermore, magnesium deficiency and PMS have many common features, and magnesium supplementation has been shown to be an effective treatment of PMS. In one study involving 32 women with PMS, 360 mg of magnesium three times daily was given from midcycle to the onset of menstrual flow.[69] Relief of premenstrual mood fluctuations and depression during magnesium treatment was significant. Women with migraines during their menses also appear to have lower magnesium levels during menstrual attacks, compared with migraines that do not occur during menses or during any part of the cycle without a migraine. The incidence of magnesium deficiency was 45% during menstrual attacks and approximately 15% at all other times.[70]

One study designed to improve understanding of the association between magnesium and the menstrual cycle measured plasma, RBC, and mononuclear blood cell (MBC) magnesium concentrations in 26 women with confirmed PMS and in a control group of 19 women during the follicular, ovulatory, early luteal, and late luteal phases of the menstrual cycle.[71] Although there were no significant differences in plasma magnesium levels between patients with PMS and control subjects and there was no menstrual cycle effect on plasma magnesium, women with PMS had significantly lower RBC magnesium concentrations than those in the control group, which was consistent throughout the menstrual cycle.

The observation of low RBC magnesium concentrations in patients with PMS has now been confirmed by four independent studies. In general, it is thought that women with PMS have a "vulnerability to luteal phase mood state destabilization,"[71] and that chronic and enduring intracellular magnesium depletion serves as a major predisposing factor for destabilization.

In addition to emotional instability, magnesium deficiency in PMS is characterized by excessive nervous sensitivity with generalized aches and pains and a lower premenstrual pain threshold. One clinical trial of magnesium in PMS showed a reduction of nervousness in 89%, breast tenderness in 96%, and weight gain in 95% of subjects.[23] In another double-blind study, high-dose magnesium supplementation (360 mg three times daily) was shown to dramatically relieve PMS-related mood changes.[69]

Although magnesium has been shown to be effective on its own, even better results may be achieved by combining it with vitamin B_6 and other nutrients. Several studies have shown that when patients with PMS are given a multivitamin/multimineral supplement containing high doses of magnesium and pyridoxine, they experience a substantial reduction in PMS symptoms.[72,73] A clinical trial with two intervention groups (magnesium; magnesium combined with vitamin B6) and a placebo group suggests that the combination was more effective for the reduction of premenstrual symptoms than magnesium alone.[74]

The optimal intake of magnesium should be based on body weight, 6 mg/kg. For a 50-kg woman, the recommendation would be 300 mg; for a 90-kg woman, 540 mg. Because these doses are difficult to achieve by diet alone, supplementation is recommended. In the treatment of PMS, a dose of twice this amount, 12 mg/kg, may be needed.

Magnesium bound to aspartate or one of the Krebs cycle intermediates (malate, succinate, fumarate, or citrate) is preferred to magnesium oxide, gluconate, sulfate, or chloride because of better absorption and fewer side effects (laxative effects).[75,76]

Calcium

Calcium has emerged as a common nutrient to supplement for PMS. Because calcium regulation and calcium deficiency can actually mimic some PMS symptoms, supplemental calcium has been tested as a treatment. An important multicenter clinical trial was conducted in 479 women who were given either 1200 mg of calcium carbonate or placebo for three menstrual cycles.[77] A significantly lower symptom score was observed in the calcium group during the luteal phase of the cycle for both the second and third cycles. By the end of the third cycle, calcium resulted in a 48% reduction in total symptom scores from baseline compared with a 30% reduction in the placebo group. Other studies also show improvements in PMS symptomatology with calcium supplementation (1000–1336 mg).[78,79] In one of the later studies, calcium and manganese supplementation (1336 and 5.6 mg, respectively) improved mood, concentration, and behavior. In another study, 1000 mg/day improved mood and water retention.[78]

Zinc

Zinc levels have been shown to be low in women with PMS.[80] In a double-blinded and placebo-controlled trial, 50 mg of elemental zinc given on the 16th day of the cycle through the 2nd day of the next cycle was associated with significant improvements in premenstrual symptoms and, after 3 months, with health-related quality of life.[81] Zinc is required for proper action of many body hormones, including the sex hormones, as well as in the control of the synthesis and secretion of hormones. In particular, zinc serves as one of the control factors for prolactin secretion.[82] When zinc levels are low, prolactin release increases, and high zinc levels inhibit this release. Hence, in high-prolactin states, zinc supplementation is very useful. An effective dose range for zinc supplementation for elevated prolactin levels in women is 30 to 45 mg in the picolinate form.

Vitamin E

Although vitamin E research concerning PMS has focused primarily on breast tenderness, a significant reduction of other PMS symptoms has also been demonstrated in double-blind studies.[23,83] Nervous tension, headache, fatigue, depression, and insomnia were all significantly reduced. In one double-blind study, patients receiving vitamin E (400 IU/day) demonstrated a 33% reduction in physical symptoms (e.g., weight gain and breast tenderness), a 38% reduction in anxiety, and a 27% reduction in depression after 3 months of use. In contrast, the placebo group reported only a 14% reduction in physical symptoms. The group taking vitamin E also noted higher energy levels, fewer headaches, and fewer cravings for sweets.

See Chapter 169 for further research on vitamin E and mastalgia.

Vitamin D

Vitamin D has also emerged as a potential contributor to symptoms of PMS. In 2019 a cross-sectional analysis of nearly 1000 young women found plasma 25-hydroxyvitamin D levels were inversely associated with the risk for cramps, confusion, fatigue, and other emotional symptoms, but unrelated to all symptoms (e.g., acne, bloating, depression, etc.).[84] A nested case-control study from within the Nurses' Health Study II cohort found that dietary intake of vitamin D was also inversely related to risk. Women in the highest quintile of intake (median 706 IU/day) had a 41% lower risk for PMS than women in the lowest quintile (median intake of 112 IU/day).[85] An intervention study that enrolled nearly 900 adolescent girls found that high-dose supplementation improved PMS and related symptoms; unfortunately, blood levels were not determined in this study, so it is difficult to determine how dependent any improvement is on serum levels of vitamin D.[86]

Essential Fatty Acids

Women with PMS have been shown to exhibit abnormalities in essential fatty acids and prostaglandin, the chief abnormality being a decrease in gamma-linolenic acid (GLA).[87] GLA is derived from linoleic acid. This conversion requires adequate levels of vitamin B_6, magnesium, and zinc because these nutrients function in delta-6-desaturase, the key enzyme responsible for this conversion. Given that a deficiency of one or more of these nutrients is common in PMS, decreased GLA levels could almost be expected.

Evening primrose, black currant, and borage oils contain GLA, with typical levels being 9%, 12%, and 22%, respectively. Although these sources of essential fatty acids are quite popular, the research on GLA supplements in the treatment of PMS shows no benefit over placebo. In the four double-blind, crossover, controlled trials of evening primrose oil, this issue may have been complicated by a very high response in the placebo group.[88,89] One of these studies used 3 g/day, and the others used 4 g/day. A meta-analysis of the clinical trials of evening primrose oil concluded that it is of little value in the management of PMS.[88]

Multiple Vitamin and Mineral Supplements

A high-quality multivitamin/multimineral supplement providing all of the known vitamins and minerals can serve as a foundation on which to build. Women with PMS have two very sound reasons for taking a high-potency multiple vitamin:

- Nutritional deficiency is relatively common among women with PMS.
- High-potency multivitamin/multimineral formulations have been shown to have significant benefits in PMS.

The frequency of nutritional supplementation and the calculated intake of select nutrients have been shown to be much lower in patients with PMS than in normal women. Women with PMS have been shown to consume vitamins in their food at levels close to the recommended daily allowance, but compared with women who did not have PMS, their intake levels were only 2.2% as much for thiamin, 2.2% for riboflavin, 16.7% for niacin, 8.7% for pantothenic acid, and 2.7% for pyridoxine.[23]

Several double-blind studies have shown that patients with PMS who were given a multivitamin/multimineral supplement containing high doses of magnesium and pyridoxine experienced reductions (typically at least a 70% reduction) in both premenopausal and postmenstrual symptoms.[72,73]

L-Tryptophan

As discussed earlier in this chapter, decreases in serotonin may be the cause of PMS or at least may exacerbate PMS. Tryptophan is a precursor to serotonin. Studies using L-tryptophan in daily doses of 6 g/day for 17 days from ovulation to day 3 of menses have shown significant reductions in mood swings, insomnia, carbohydrate cravings, tension, irritability, and dysphoria.[90,91] Because 5-hydroxytryptophan (5-HTP) has shown superior results to tryptophan in depression, it may also prove to be a more effective option for raising serotonin levels in PMS. For more information, see Chapter 87.

Botanical Medicines

Many plants have been used throughout traditional botanical medicine for cyclic premenstrual symptoms. These have tended to include what are called reproductive tonics, adaptogens, liver detoxification herbs, and "hormonal balancing" herbs. Unfortunately, these botanical approaches have not been the subject of clinical evaluation. Instead, modern research has focused on the following botanicals for relief of PMS symptoms: chaste berry, St. John's wort, and *Ginkgo biloba.*

Vitex agnus castus (Chaste Tree)

The chaste tree *(Vitex agnus castus)* is native to the Mediterranean, where its berries have long been used for women's health. Chaste berry extract is probably the single most important herb in the treatment of PMS, not only because of its long tradition and known historical uses but also as a result of modern scientific research. In two surveys of gynecological practices in Germany, physicians graded chaste berry extract as good or very good in the treatment of PMS. More than 1500 women participated in the studies.[92,93] One third of the women experienced complete resolution of their symptoms, and another 57% reported significant improvement.

The beneficial effects of chaste tree in PMS and these other conditions appear to be related to alterations in gonadotropin-releasing hormone and follicle-stimulating hormone-releasing hormone. In other words, it appears that chaste berry extract has profound effects on the hypothalamus and pituitary function. As a result, it is able to normalize the secretion of other hormones—for instance, reducing the secretion of prolactin and reducing the estrogen:progesterone ratio.

In a more recent study, a randomized clinical trial compared a chaste tree standardized extract (20-mg tablet standardized for casticin) with placebo in women with PMS.[94] One hundred and seventy women with PMS were assigned to take either the chaste tree or a placebo once daily for three consecutive menstrual cycles. Women were asked to rate changes in PMS symptoms, such as irritability, mood changes, anger, headache, breast tenderness, and bloating. At the end of the trial, women taking the chaste tree reported a 52% overall reduction in PMS symptoms, compared with only 24% for those taking a placebo. Women taking the chaste tree extract reported significantly greater reductions in irritability, mood changes, anger, headache, and breast tenderness than the women taking the placebo. Bloating was the only symptom that did not change significantly with chaste tree. Another study has looked at the effectiveness of chaste tree extract versus fluoxetine in decreasing PMS symptoms and found the two treatments to have comparable results, with the main difference being that fluoxetine was more effective in treating psychological symptoms and chaste berry was more effective with physical symptoms.[95]

In more recent research, Chinese women suffering from moderate to severe PMS were studied in a prospective, double-blind, placebo-controlled, parallel-group multicenter clinical trial.[96] The chaste tree extract contained 4.0 mg of dried ethanolic (70%) extract. The mean total Premenstrual Syndrome Diary (PMSD) score decreased from 29.23 at baseline to 6.41 at the end of the third cycle for the chaste tree group and from 28.14 at baseline to 12.64 at the end of the third cycle for the placebo group. The difference in the PMSD score from baseline to the third cycle was significantly lower in the treatment group than in the placebo group. The Premenstrual Tension Syndrome Self-Rating Scale decreased from 26.17 at baseline to 9.92 for the treatment group and from 27.10 to 14.59 for the placebo group—similar positive results as the PMSD scores.

PMS is also very common in perimenopausal women. A study was undertaken to evaluate the effectiveness of a combination of St. John's wort and chaste berry in the treatment of PMS-like symptoms in perimenopausal women.[97] This clinical trial was conducted over 16 weeks, and information rating PMS scores in perimenopausal women who were experiencing irregular menses was collected at 4-week intervals. The daily dose of herbal products given was 3 tablets containing 5400 mg of St. John's wort standardized to contain 990 mcg hypericin, 9 mg hyperforin, and 18 mg flavonoid glycosides. The daily dose of chaste tree was one tablet of an extract equivalent to 1000 mg of dried fruit. Participants recorded the severity of their PMS symptoms using the Abraham Menstrual Symptom Questionnaire. Results for the active treatment group were statistically superior to placebo for total PMS-like symptoms as well as subgroups of PMS depression and PMS food cravings.

In 2017 a systematic review and meta-analysis analyzed 14 randomized controlled trials of *V. agnus castus* for the treatment of PMS; 13 of the 14 trials reported a positive and large pooled effect, suggesting a high likelihood of clinically meaningful benefit. However, the high risk of bias and publication bias noted in this systematic review call for high-quality trials to clarify its efficacy as well as to determine whether there are optimal doses and/or forms of administration.[115] For example, a double-blind, placebo-controlled trial among 162 women with PMS found that 20 mg per day of a dry 60% ethanol extract was more effective than 8 mg per day but not more effective than 30 mg per day.[98] Two other systematic reviews also published in 2017 found that *Vitex* is likely to have therapeutic benefit for women with PMS.[99,100]

Ginkgo biloba

Several compounds and mechanisms of *Ginkgo* may be involved in physical and psychological benefits for PMS (see Chapter 82). In the first randomized, placebo-controlled clinical trial evaluating *G. biloba* extract (GBE) in PMS, 165 women of reproductive age who had fluid retention, breast tenderness, and vascular congestion were assigned to receive either a GBE of 25% *Ginkgo* flavone glycosides at 80 mg twice daily or a placebo from day 16 to day 5 of their cycles. Symptom diaries kept by patients and physician evaluation of symptoms demonstrated that *G. biloba* extract was effective against the congestive symptoms of PMS, particularly breast pain and breast tenderness.[101]

In a subsequent study, GBE was evaluated using Beck's Depression Inventory, a daily symptom rating questionnaire containing 19 PMS symptoms according to the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*, and items relative to the inclusion criteria included participants experiencing the symptoms for at least two consecutive cycles before the study and experiencing at least 5 of the 19 symptoms for most of the time during the last week before menses. Eighty-five women completed the study. Participants were given 40 mg three times daily of a standardized GBE or a placebo from day 16 of their cycle to day 5 of their next cycle. The overall severity of symptoms in the GBE group was 34.80% before the treatment, which fell to 11.11% after the treatment. In the placebo group, the baseline severity was 34.38%, which dropped to 25.64% after intervention. The severity of the psychological and physical symptoms also declined significantly in both groups, but there was again a significant difference between the two groups, in favor of the GBE.[102]

Hypericum Perforatum

Owing to the influence of St. John's wort on serotonin (see Chapter 88), it should not be surprising that this herb would be an important botanical in the treatment of PMS, and research has affirmed this. A randomized, double-blind, placebo-controlled crossover trial of 36 women with regular menstrual cycles and mild PMS was done in which women were randomly assigned to receive St. John's wort tablets (900 mg/day and standardized to 0.18% hypericin and 3.38% hyperforin) or placebo for two menstrual cycles.[103] After a 1-month no-treatment cycle, women were crossed over to the opposite group for two additional cycles. Symptoms were rated using the Daily Symptom Report, State Anxiety Inventory, Beck Depression Inventory, Aggression Questionnaire, and Barratt Impulsiveness Scale. Numerous hormones and physiological markers were also measured in the follicular and luteal phases: follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, prolactin, testosterone, cytokine, interleukins (ILs; IL-1B, IL-6, and IL-8), interferon, and tumor necrosis factor-alpha.

St. John's wort was statistically more beneficial than a placebo in relieving food cravings, swelling, poor coordination, insomnia, confusion, headaches, crying, and fatigue. There were no significant effects of

St. John's wort compared with placebo in any of the biochemical blood measurements. St. John's wort was not statistically more beneficial in anxiety, irritability, depression, nervous tension, mood swing, feeling out of control, and pain-related symptoms during two cycles of treatment. However, these pain-related symptoms appeared to improve more than with a placebo toward the end of each treatment period.

In a prospective, open, uncontrolled observational study, 19 women who were diagnosed with PMS completed a daily symptom rating questionnaire for one menstrual cycle and underwent a screening interview with physicians. The participants then took St. John's wort tablets (300 mg St John's wort standardized to 900 mcg of hypericin) for two complete menstrual cycles.[104] Symptoms were rated daily by means of the Hospital Anxiety and Depression Scale and modified Social Adjustment Scale, which were administered at baseline and after each of the two cycles. The degree of improvement in overall PMS scores between the beginning of the study and the end was 51%, with more than two thirds of the women having at least a 50% decrease in the severity of symptoms. The mood subscale showed the most improvement (57%); the symptoms with the greatest reductions in scores were crying (92%), depression (85%), confusion (75%), feeling out of control (72%), nervous tension (71%), anxiety (69%), and insomnia (69%). In a more recent prospective, double-blind, randomized, controlled trial, 170 women with PMS were given St. John's wort (two tablets, each standardized to 680 ug hypericin per day) or a placebo. At 8 weeks, a 40% reduction in total symptom scores was observed, with the largest improvements occurring for crying (71%) and depression (52%).[105]

Saffron (*Crocus sativus* L.)

Saffron has been previously shown to have an antidepressant effect in women with mild to moderate depression through a serotonergic mechanism, so it is not surprising that it would be beneficial in PMS. A double-blind, placebo-controlled trial was done to study whether saffron could be used to relieve PMS symptoms. Fifty women of reproductive age with regular menstrual cycles and PMS symptoms for at least the last 6 months were randomly assigned to receive 15 mg of saffron twice daily or placebo twice daily for four full menstrual cycles.[106] The Daily Symptom Report and the Hamilton Depression Rating Scale were used to evaluate the response. According to the Daily Symptom Report, 19 of the 25 women in the saffron group responded with at least a 50% reduction in severity of symptoms versus only 2 of 25 in the placebo group ($P < 0.0001$). A significant difference between the saffron and placebo groups occurred between the third and fourth cycles and was statistically significant by the end of the study ($P < 0.0001$). According to the Hamilton Depression Rating Scale, 15 of 25 women in the saffron group responded to treatment versus only 1 of 25 in the placebo group ($P < 0.0001$). Again, a significant difference was seen between the third and fourth cycles, with a statistically significant difference by the end of the study ($P <0.0001$).

Curcumin

Only recently have clinical trials been conducted to evaluate the effects of this anti-inflammatory component of *Curcuma longa*. A randomized and placebo-controlled trial was conducted in which either placebo or 100 mg b.i.d. curcumin was given 7 days before and until 3 days after menses among 70 young women. Both behavioral and physical symptoms were significantly improved, with total PMS scores dropping to approximately 40% of their pretreatment levels following curcumin therapy. The authors suggest that curcumin's effect on norepinephrine levels may help to explain its benefit.[116] A second publication by the same authors (which appears to be from the same study) found that curcumin also nearly doubled levels of serum brain-derived neurotrophic factor (BDNF) over the 3-month study period, which may also explain its effect, because a decline in BDNF levels has been observed among women with PMS and may precede the onset of symptoms.[107,108]

Bioidentical Progesterone Therapy

Bioidentical progesterone, also called natural progesterone, is a white crystalline powder most often made by extracting diosgenin from Mexican wild yam and then converting this in a manufacturing laboratory into a progesterone molecule that is biochemically identical to the body's own progesterone. It is not wild yam, nor should it be considered an herbal product. What makes natural progesterone "natural" is not so much that it is made from plant material, but rather that it is identical to the progesterone hormone produced in a woman's ovaries. Conventional medical practitioners have historically used a synthetic version, called progestin, which is found in birth control pills as a hormonal treatment for PMS. This has largely been replaced by using select antidepressants for their effect on serotonin levels. Although bioidentical progesterone has been studied in controlled clinical trials for the treatment of PMS, efforts to consistently demonstrate the superiority of progesterone therapy over placebo have failed (possibly because there is a significant placebo response in PMS).[109–112] The studies showing positive effects have used dosages of 200 to 400 mg twice daily as a vaginal or rectal suppository from 14 days before the expected onset of menstruation until the onset of vaginal bleeding.[113,114] Transdermal creams of natural progesterone generally sold in natural foods/nutrition stores have been a popular self-treatment for PMS. These products vary greatly from an herbal wild yam cream only to wild yam cream with bioidentical progesterone added, up to 20 mg per ¼ tsp. Side effects are few and tend to be generally mild, but they may occur in 4% to 5% of individuals using transdermal creams that include 20 mg of USP progesterone per one-quarter teaspoon. In one of the later double-blind studies that did show a positive effect for progesterone therapy (400 mg twice a day by vaginal or rectal administration), adverse events were reported by 51% of patients in the progesterone group compared with 43% in the placebo group.[114] Irregularity of menstruation, vaginal itching, and headache were reported more frequently by the women taking the progesterone.

THERAPEUTIC APPROACH

The approach to the woman with premenstrual symptoms first involves an evaluation of symptoms and the establishment of a diagnosis. In an effort to clarify the treatment approach, the clinician should consider the following seven key steps:

1. Evaluate PMS symptoms by having the patient complete a questionnaire, and ideally 2 consecutive months of symptoms tracking (e.g., Daily Record of Severity of Problems [DRSP]).
2. Rule out psychiatric or other medical conditions.
3. Have the patient adopt the following dietary recommendations for PMS: A vegetarian, low-fat, low-sugar, low-salt, high-fiber diet is advised; if there is mastalgia, caffeine should be avoided.
4. The patient may also benefit from the guidelines for nutritional supplementation given in the following section.
5. Select the appropriate herbal support.
6. Establish a program for stress reduction and recommend regular exercise.
7. If, after at least three complete periods, the patient is not experiencing a significant improvement or complete resolution of symptoms, additional causative factors should be sought, as detailed previously, or change made to the treatment program.

Nutritional Supplements

- High-potency multivitamin/multimineral formula
- Vitamin B6: 50 to 100 mg/day (total day's dose of vitamin B6 in a single ingredient or in the multiple vitamin should not exceed 150 mg). Pyridoxal-5-phosphate is much superior to pyridoxine.
- Magnesium (preferably citrate, malate, aspartate, or glycinate): 250 mg twice a day
- Zinc: 15 to 20 mg/day
- Vitamin E: 200 to 400 IU/day
- Vitamin D: 1000 to 2000 IU/day

Botanical Medicines

V. agnus castus:

- Standardized extract (0.5% agnuside): 175 to 225 mg a day or 20 mg standardized to casticin
- Liquid extract: 2 mL/day

G. biloba:

- 80 to 160 mg standardized extract (24% ginkgoflavonglycosides) twice a day

H. perforatum:

- 900 to 1800 mg standardized extract (0.3% hypericin content) a day

Curcumin:

- 100 mg twice per day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Severino S, Moline M. Premenstrual syndrome: identification and management. *Drugs*. 1995;49:71–82.
2. Halbreich U1, Borenstein J, Pearlstein T, et al. The prevalence, impairment, impact, and burden of premenstrual dysphoric disorder (PMS/PMDD). *Psychoneuroendocrinology*. 2003;28(suppl 3):1–23. PMID:12892987.
3. Steiner M. Premenstrual dysphoric disorder: an update. *Gen Hosp Psychiatry*. 1996;18:244–250.
4. Richardson JT. The premenstrual syndrome: a brief history. *Soc Sci Med*. 1995;41:761–767.
5. Roca C, Schmidt P, Bloch M, et al. Implications of endocrine studies of premenstrual syndrome. *Psychiatr Ann*. 1996;26:576–580.
6. Mortola JF. Premenstrual syndrome. *Trends Endocrinol Metab*. 1996;7:184–189.
7. Liu Q, Li R, Zhou R, et al. Abnormal resting-state connectivity at functional MRI in women with premenstrual syndrome. *PLoS One*. 2015;10(9):e0136029. PMID:26325510.
8. Liu P, Wei Y, Fan Y, et al. Altered brain structure in women with premenstrual syndrome. *J Affect Disord*. 2018;229:239–246. PMID:29329055.
9. Rubinow DR, Schmidt PJ. The treatment of premenstrual syndrome—forward into the past. *N Engl J Med*. 1995;332:1574–1575.
10. Rapkin A. The role of serotonin in premenstrual syndrome. *Clin Obstet Gynecol*. 1992;35:629–636.
11. Rojansky N, Halbreich U, Zander K, et al. Imipramine receptor binding and serotonin uptake in platelets of women with premenstrual changes. *Gynecol Obstet Invest*. 1991;31:146–152.
12. Endicott J, Amsterdam J, Eriksson E, et al. Is premenstrual dysphoric disorder a distinct clinical entity? *J Womens Health Gend Based Med*. 1999;8:663–679.
13. Martinez PE, Rubinow DR, Nieman LK, et al. 5α-Reductase inhibition prevents the luteal phase increase in plasma allopregnanolone levels and mitigates symptoms in women with premenstrual dysphoric disorder. *Neuropsychopharmacology*. 2016;41(4):1093–1102. 26272051.
14. Appleton SM. Premenstrual syndrome: evidence-based evaluation and treatment. *Clin Obstet Gynecol*. 2018;61(1):52–61. 29298169.
15. Biskind MS, Biskind GR. Diminution in ability of the liver to inactivate estrone in vitamin B complex deficiency. *Science*. 1941;94:462.
16. Biskind MS. Nutritional deficiency in the etiology of menorrhagia, metrorrhagia, cystic mastitis and premenstrual tension: treatment with vitamin B complex. *J Clin Endocrinol Metab*. 1943;3:227–234.
17. Facchinetti F, Nappi G, Petraglia F, et al. Oestradiol/progesterone imbalance and the premenstrual syndrome. *Lancet*. 1983;2:1302.
18. Chuong CJ, Hsi BP, Gibbons WE. Periovulatory beta-endorphin levels in premenstrual syndrome. *Obstet Gynecol*. 1994;83:755–760.
19. Yen JY, Wang PW2, Su CH, et al. Estrogen levels, emotion regulation, and emotional symptoms of women with premenstrual dysphoric disorder: the moderating effect of estrogen receptor 1α polymorphism. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;82:216–223. PMID:29146473.
20. Wilson SM, Bivins BN, Russell KA, et al. Oral contraceptive use: impact on folate, vitamin B_6, and vitamin B_{12} status. *Nutr Rev*. 2011;69(10):572–583. PMID:21967158.
21. Wynn V, Adams PW, Folkard J, et al. Tryptophan, depression and steroidal contraception. *J Steroid Biochem*. 1975;6:965–970.
22. Bermond P. Therapy of side effects of oral contraceptive agents with vitamin B6. *Acta Vitaminol-Enzymol*. 1982;4:45–54.
23. Abraham GE. Nutritional factors in the etiology of the premenstrual tension syndromes. *J Reprod Med*. 1983;28:446–464.
24. Goei G, Ralston J, Abraham G. Dietary patterns of patients with premenstrual tension. *J Appl Nutr*. 1982;34:4–11.
25. Bertone-Johnson ER, Hankinson SE, Bendich A, et al. Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med*. 2005;165(11):1246–1252. PMID:15956003.
26. Cross G, Marley J, Miles H, et al. Changes in nutrient intake during the menstrual cycle of overweight women with premenstrual syndrome. *Br J Nutr*. 2001;5(4):475–482.
27. Farasati N, Siassi F, Koohdani F, et al. Western dietary pattern is related to premenstrual syndrome: a case-control study. *Br J Nutr*. 2015;114(12):2016–2021. PMID:26459000.
28. Wurtman J. Carbohydrate craving: the relationship between carbohydrate intake and disorders of mood. *Drugs*. 1990;39(suppl 3):49–52.
29. Rossignol AM, Bonnlander H. Prevalence and severity of the premenstrual syndrome: effects of foods and beverages that are sweet or high in sugar content. *J Reprod Med*. 1991;36:131–136.
30. Yudkin J, Eisa O. Dietary sucrose and oestradiol concentration in young men. *Ann Nutr Metabol*. 1988;32:53–55.
31. Boyd N, McGuire V, Shannon P, et al. Effect of a low-fat high-carbohydrate diet on symptoms of cyclical mastopathy. *Lancet*. 1988;2(8603):128–132.
32. Puder JJ, Blum CA, Mueller B, et al. Menstrual cycle symptoms are associated with low-grade inflammation. *Eur J Clin Invest*. 2006;36:58–64.
33. Gold EB, Wells C, Rasor MO. The association of inflammation with premenstrual symptoms. *J Womens Health (Larchmt)*. 2016;25(9):865–874. PMID: 2713572.
34. Bertone-Johnson ER, Ronnenberg AG, Houghton SC, et al. Association of inflammation markers with menstrual symptom severity and premenstrual syndrome in young women. *Hum Reprod*. 2014;29(9):1987–1994. PMID:25035435.
35. Gorbach SL, Goldin BR. Diet and the excretion and enterohepatic cycling of estrogens. *Prev Med*. 1987;16:525–531.
36. Goldin BR, Adlercreutz H, Gorbach SL, et al. Estrogen patterns and plasma levels in vegetarian and omnivorous women. *New Engl J Med*. 1982;307:1542–1547.
37. Yamada K, Takeda T. Low proportion of dietary plant protein among athletes with premenstrual syndrome-related performance impairment. *Tohoku J Exp Med*. 2018;244(2):119–122. PMID:29434077.
38. Longcope C, Gorbach S, Goldin B, et al. The effect of a low fat diet on estrogen metabolism. *J Clin Endocrinol Metab*. 1987;64:1246–1250.
39. Woods MN, Gorbach SL, Longcope C, et al. Low-fat, high-fiber diet and serum estrone sulfate in premenopausal women. *Am J Clin Nutr*. 1989;49:1179–1183.
40. Jones DY. Influence of dietary fat on self-reported menstrual symptoms. *Physiol Behav*. 1987;40:483–487.
41. Gold E, Bair Y, Block G, et al. Diet and lifestyle factors associated with premenstrual symptoms in a racially diverse community sample: Study of Women's Health Across the Nation (SWAN). *J Womens Health (Larchmt)*. 2007;16(5):641–656.
42. Brayshaw ND, Brayshaw DD. Thyroid hypofunction in premenstrual syndrome. *N Engl J Med*. 1986;315:1486–1487.
43. Roy-Byrne PP, Rubinow DR, Hoban MC, et al. TSH and prolactin responses to TRH in patients with premenstrual syndrome. *Am J Psychiatry*. 1987;144:480–484.
44. Girdler SS, Pedersen CA, Light KC. Thyroid axis function during the menstrual cycle in women with premenstrual syndrome. *Psychoneuroendocrinology*. 1995;20:395–403.
45. Schmidt PJ, Grover GN, Roy-Byrne PP, et al. Thyroid function in women with premenstrual syndrome. *J Clin Endocrinol Metab*. 1993;76:671–674.
46. Rapkin A. The role of serotonin in premenstrual syndrome. *Curr Obster Gynecol*. 1992;35:629–636.
47. Kuczmierczyk AR, Johnson CC, Labrum AH. Coping styles in women with premenstrual syndrome. *Acta Psychiatr Scand*. 1994;89:301–305.
48. Eriksson E, Alling C, Andersch B, et al. Cerebrospinal fluid levels of monoamine metabolites: a preliminary study of their relation to menstrual cycle phase, sex steroids, and pituitary hormones in healthy women and in women with premenstrual syndrome. *Neuropsychopharmacology*. 1994;11:201–213.
49. Halbreich U, Petty F, Yonkers K, et al. Low plasma gamma-aminobutyric acid levels during the late luteal phase of women with premenstrual dysphoric disorder. *Am J Psychiatry*. 1996;153:718–720.
50. Van Zak DB. Biofeedback treatments for premenstrual and premenstrual affective syndromes. *Int J Psychosom*. 1994;41:53–60.
51. Kirkby RJ. Changes in premenstrual symptoms and irrational thinking following cognitive-behavioral coping skills training. *J Consult Clin Psychol*. 1994;62:1026–1032.

52. Aganoff JA, Boyle GJ. Aerobic exercise, mood states and menstrual cycle symptoms. *J Psychosom Res*. 1994;38:183–192.
53. Choi PY, Salmon P. Symptom changes across the menstrual cycle in competitive sportswomen, exercisers and sedentary women. *Br J Clin Psychol*. 1995;34:447–460.
54. Steege JF, Blumenthal JA. The effects of aerobic exercise on premenstrual symptoms in middle-aged women: a preliminary study. *J Psychosom Res*. 1993;37:127–133.
55. Tsai SY, Kuo FC, Kuo HC, et al. The prevalence of self-reported premenstrual symptoms and evaluation of regular exercise with premenstrual symptoms among female employees in Taiwan. *Women Health*. 2018;58(3):247–259. PMID:28318414.
56. Gannon L. The potential role of exercise in the alleviation of menstrual disorders and menopausal symptoms: a theoretical synthesis of recent research. *Women and Health*. 1988;14:105–127.
57. Kliejnen J, Ter Riet G, Knipschild P. Vitamin B_6 in the treatment of premenstrual syndrome: a review. *Br J Obstet Gynaecol*. 1990;97:847–852.
58. Barr W. Pyridoxine supplements in the premenstrual syndrome. *Practitioner*. 1984;228:425–427.
59. Doll H, Brown S, Thurston A, et al. Pyridoxine (vitamin B_6) and the premenstrual syndrome: a randomized crossover trial. *JR Coll Gen Pract*. 1989;39:364–368.
60. Berman MK, Taylor ML, Freeman E. Vitamin B_6 in premenstrual syndrome. *J Am Diet Assoc*. 1990;90:859–861.
61. Zempleni J. Pharmacokinetics of vitamin B_6 supplements in humans. *J Am Coll Nutr*. 1995;14:579–586.
62. Cohen M, Bendich A. Safety of pyridoxine: a review of human and animal studies. *Toxicol Lett*. 1986;34:129–139.
63. Parry GJ, Bredesen DE. Sensory neuropathy with low-dose pyridoxine. *Neurology*. 1985;35:1466–1468.
64. Waterston JA, Gilligan BS. Pyridoxine neuropathy. *Med J Aust*. 1987;146:640–642.
65. Vrolijk MF, Opperhuizen A, Jansen EHJM, et al. The vitamin B_6 paradox: supplementation with high concentrations of pyridoxine leads to decreased vitamin B_6 function. *Toxicol In Vitro*. 2017;44:206–212. PMID: 28716455.
66. Majumdar P, Boylan M. Alteration of tissue magnesium levels in rats by dietary vitamin B_6 supplementation. *Int J Vitamin Nutr Res*. 1989;59:300–303.
67. Posaci C, Erten O, Uren A, et al. Plasma copper, zinc, and magnesium levels in patients with premenstrual tension syndrome. *Acta Obstet Gynecol Scand*. 1994;73:452–455.
68. Piesse JW. Nutritional factors in the premenstrual syndrome. *Int Clin Nutr Rev*. 1984;4:54–81.
69. Facchinetti F, Borella P, Sances G, et al. Oral magnesium successfully relieves premenstrual mood changes. *Obstet Gynecol*. 1991;78:177–181.
70. Mauskop A, Altura BT, Altura BM. Serum ionized magnesium levels and serum ionized calcium/ionized magnesium ratios in women with menstrual migraine. *Headache*. 2002;42(4):242–248. PMID:12010379.
71. Rosenstein DL, Elin RJ, Hosseini JM, et al. Magnesium measures across the menstrual cycle in premenstrual syndrome. *Biol Psychiatr*. 1994;35:557–561.
72. London RS, Bradley R, Chiamori NY. Effect of a nutritional supplement on premenstrual symptomatology in women with premenstrual syndrome: a double-blind longitudinal study. *J Am Coll Nutr*. 1991;10:494–499.
73. Stewart A. Clinical and biochemical effects of nutritional supplementation on the premenstrual syndrome. *J Reprod Med*. 1987;32:435–441.
74. Fathizadeh N, Ebrahimi E, Valiani M, et al. Evaluating the effect of magnesium and magnesium plus vitamin B_6 supplement on the severity of premenstrual syndrome. *Iran J Nurs Midwifery Res*. 2010;15(suppl 1):401–405. PMID:22069417.
75. Lindberg JS, Zobitz MM, Poindexter JR, et al. Magnesium bioavailability from magnesium citrate and magnesium oxide. *J Am Coll Nutr*. 1990;9:48–55.
76. Bohmer T, Roseth A, Holm H, et al. Bioavailability of oral magnesium supplementation in female students evaluated from elimination of magnesium in 24-hour urine. *Magnes Trace Elem*. 1990;9:272–278.
77. Thys-Jacobs S, Starkey P, Bernstein D, et al. Calcium carbonate and the premenstrual syndrome: effects on premenstrual and menstrual symptoms. Premenstrual Syndrome Study Group. *Am J Obstet Gynecol*. 1998;179(2):444–452.
78. Penland JG, Johnson PE. Dietary calcium and manganese effects on menstrual cycle symptoms. *Am J Obstet Gynecol*. 1993;168:1417–1423.
79. Thys-Jacobs S, Ceccarelli S, Bierman A. Calcium supplementation in premenstrual syndrome: a randomized crossover trial. *J Gen Intern Med*. 1989;4:183–189.
80. Chuong CJ, Dawson EB. Zinc and copper levels in premenstrual syndrome. *Fertil Steril*. 1994;62:313–320.
81. Siahbazi S, Behboudi-Gandevani S, Moghaddam-Banaem L, et al. Effect of zinc sulfate supplementation on premenstrual syndrome and health-related quality of life: clinical randomized controlled trial. *J Obstet Gynaecol Res*. 2017;43(5):887–894. PMID:28188965.
82. Judd AM, Macleod RM, Login IS. Zinc acutely, selectively and reversibly inhibits pituitary prolactin secretion. *Brain Res*. 1984;294:190–192.
83. London RS, Sundaram G, Manimekalai S, et al. The effect of alpha-tocopherol on premenstrual symptomatology: a double-blind study. II. Endocrine correlates. *J Am Coll Nutr*. 1984;3:351–356.
84. Jarosz AC, El-Sohemy A. Association between Vitamin D status and premenstrual symptoms. *J Acad Nutr Diet*. 2019;119(1):115–123. PMID:30177298.
85. Bertone-Johnson ER, Hankinson SE, Bendich A, et al. Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med*. 2005;165(11):1246–1252. PMID:15956003.
86. Bahrami A, Avan A, Sadeghnia HR, et al. High dose vitamin D supplementation can improve menstrual problems, dysmenorrhea, and premenstrual syndrome in adolescents. *Gynecol Endocrinol*. 2018;34(8):659–663. PMID:29447494.
87. Horrobin DF, Manku M, Brush M, et al. Abnormalities in plasma essential fatty acid levels in women with premenstrual syndrome and with non-malignant breast disease. *J Nutr Med*. 1991;2:259–264.
88. Budeiri D, Li Wan Po A, Dornan JC. Is evening primrose oil of value in the treatment of premenstrual syndrome? *Control Clin Trials*. 1996;17:60–68.
89. Khoo SK, Munro C, Battistutta D. Evening primrose oil and treatment of premenstrual syndrome. *Med J Austral*. 1990;153:189–192.
90. Steinberg S, Annable L, Young SN, et al. A placebo-controlled study of the effects of L-tryptophan in patients with premenstrual dysphoria. *Adv Exp Med Biol*. 1999;467:85–88.
91. Steinberg S, Annable L, Young SN, et al. Tryptophan in the treatment of late luteal phase dysphoric disorder: a pilot study. *J Psychiatry Neurosci*. 1994;19(2):114–119.
92. Dittmar FW. Premenstrual syndrome. Treatment with a phytopharmaceutical. *Therapiewoche Gynakol*. 1992;5:60–68.
93. Peteres-Welte C, Albrecht M. Menstrual abnormalities and PMS. *Vitex agnus-castus*. *Therapiewoche Gynakol*. 1994;7:49–52.
94. Schellenberg R. Treatment for the premenstrual syndrome with agnus castus fruit extract: prospective, randomized, placebo controlled study. *BMJ*. 2001;322:134–137.
95. Atmaca M, Kumru S, Tezcan C. Fluoxetine versus *Vitex agnus castus* extract in the treatement of premenstrual dysphoric syndrome. *Hum Psychopharmacol*. 2003;3:191–195.
96. He Z, Chen R, Zhou Y, et al. Treatment for premenstrual syndrome with *Vitex agnus castus*: a prospective, randomized, multi-center placebo controlled study in China. *Maturitas*. 2009;63:99–103.
97. Van Die M, Bone K, Burger H, et al. Effects of a combination of *Hypericum perforatum* and *Vitex agnus-castus* on PMS-like symptoms in late-perimenopausal women: findings from a subpopulation analysis. *J Alternative and Complementary Medicine*. 2009;15(9):1045–1048.
98. Schellenberg R, Zimmermann C, Drewe J, et al. Dose-dependent efficacy of the *Vitex agnus castus* extract Ze 440 in patients suffering from premenstrual syndrome. *Phytomedicine*. 2012;19(14):1325–1331. PMID:23022391.
99. Cerqueira RO, Frey BN, Leclerc E, et al. *Vitex agnus castus* for premenstrual syndrome and premenstrual dysphoric disorder: a systematic review. *Arch Womens Ment Health*. 2017;20(6):713–719. PMID:29063202.

100. Rafieian-Kopaei M1. Movahedi M2. Systematic review of premenstrual, postmenstrual and infertility disorders of *Vitex Agnus Castus*. *Electron Physician*. 2017;9(1):3685–3689. PMID:28243425.
101. Tamborini A, Taurelle R. Value of standardized *Ginkgo biloba* extract in the management of congestive symptoms of premenstrual syndrome. *Rev Fr Gynecol Obstet*. 1993;88:447–457.
102. Ozgoli G, Selselei E, Mojab F, et al. A randomized, placebo-controlled trial of *Ginkgo biloba* L. in treatment of premenstrual syndrome. *J Altern Complement Med*. 2009;15(8):845–851.
103. Canning S, Waterman M, Orsi N, et al. The efficacy of *Hypericum perforatum* (St. John's Wort) for the treatment of premenstrual syndrome. *CNS Drugs*. 2010;24(3):207–225.
104. Stevinson C, Ernst E. A pilot study of *Hypericum perforatum* for the treatment of premenstrual syndrome. *BJOG*. 2000;107:870–876.
105. Ghazanfarpour M, Kaviani M, Asadi N, et al. *Hypericum perforatum* for the treatment of premenstrual syndrome. *Int J Gynaecol Obstet*. 2011;113(1):84–85. PMID:21315349.
106. Agha-Hosseini M, Kashani L, Aleyaseen A, et al. *Crocus sativus* L. (saffron) in the treatment of premenstrual syndrome: a double-blind, randomised and placebo-controlled trial. *BJOG*. 2008;115:515–519.
107. Fanaei H, Khayat S, Kasaeian A, et al. Effect of curcumin on serum brain-derived neurotrophic factor levels in women with premenstrual syndrome: a randomized, double-blind, placebo-controlled trial. *Neuropeptides*. 2016;56:25–31. PMID:26608718.
108. Cubeddu A, Bucci F, Giannini A, et al. Brain-derived neurotrophic factor plasma variation during the different phases of the menstrual cycle in women with premenstrual syndrome. *Psychoneuroendocrinology*. 2011;36(4):523–530. PMID:20933336.
109. Freeman E, Rickels K, Sondheimer SJ, et al. Ineffectiveness of progesterone suppository treatment for premenstrual syndrome. *JAMA*. 1990;264:349–353.
110. Maddocks S, Hahn P, Moller F, et al. A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome. *Am J Obstet Gynecol*. 1986;154:573–581.
111. Andersch B, Hahn LJ. Progesterone treatment of premenstrual tension: a double blind study. *Psychosom Res*. 1985;29:489–493.
112. Dennerstein L, Spencer-Gardner C, Gotts G, et al. Progesterone and the premenstrual syndrome: a double blind crossover trial. *Br Med J*. 1985;290:1617–1621.
113. Baker ER, Best RG, Manfredi RL, et al. Efficacy of progesterone vaginal suppositories in alleviation of nervous symptoms in patients with premenstrual syndrome. *J Assist Reprod Genet*. 1995;12:205–209.
114. Magill PJ. Investigation of the efficacy of progesterone pessaries in the relief of symptoms of premenstrual syndrome. Progesterone Study Group. *Br J Gen Pract*. 1995;45:589–593.
115. Verkaik S, Kamperman AM1, van Westrhenen R, et al. The treatment of premenstrual syndrome with preparations of Vitex agnus castus: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2017;217(2):150–166. PMID:28237870.
116. Khayat S, Fanaei H, Kheirkhah M, et al. Curcumin attenuates severity of premenstrual syndrome symptoms: a randomized, double-blind, placebo-controlled trial. *Complement Ther Med*. 2015;23(3):318–324. PMID:26051565.

213

Proctologic Conditions

Thomas A. Kruzel, MT, ND

OUTLINE

ANORECTAL ANATOMY

The anorectal region is divided into the anus, which extends anywhere from 3 to 4 cm above the anal verge to its merge with the rectum, and the rectum, which is 12 to 18 cm long. The anal verge is the demarcation point from perianal skin to anal skin. The anal canal can further be divided into the dentate and anorectal lines. The dentate line is the demarcation point between the ectoderm of the external squamous epithelium and the rectal entodermal mucosa. The anal canal does not contain sebaceous or sweat glands and is innervated by somatic nerves up to and slightly beyond the dentate line, making it sensitive to pain to varying degrees. Above the dentate line, the rectal mucosa is relatively insensitive to pain but registers distention and inflammation as diffuse visceral pain. The anorectal line is the point above which the rectum expands outward into the pelvic bowl.

The area between the dentate and anorectal lines contains the rectal columns, anal valves, anal glands, and anal crypts. The anal glands normally provide mucus for lubrication with the passage of stool and empty into the anal crypts. This area becomes the source of an anal fistula or perirectal abscess if the crypt becomes impacted and cannot discharge its mucus. Anal fissures lie below the dentate line. Midway between the dentate and anorectal lines lies the white line of Hilton, or pectinate line. This is the location of the intersphincteric groove, the region that lies between the internal and external sphincters. The internal sphincter is under the control of the autonomic nervous system, and the external sphincter is under somatic or voluntary control.

The anorectal region is highly vascularized and receives its blood supply from both the inferior mesenteric artery and the internal iliac artery. Arterial flow is then diverted from these sources through the superior, middle, and inferior rectal arteries. There is considerable anastomosis among these branches. Venous return is facilitated by the superior and middle hemorrhoidal veins, which empty into the inferior mesenteric veins and subsequently the portal vein, and the internal iliac vein, which subsequently empties into the vena cava, bypassing the liver.

The levator ani muscle forms the floor of the pelvis and helps form the puborectalis sling. The pull of the puborectalis sling causes the bowel to change direction as it penetrates the pelvic floor, making the passage of stool easier. Along with the internal and external rectal sphincters, these muscles form the sphincter mechanism that controls continence. The anal canal receives its innervation via the pudendal nerves originating from S2, S3, and S4.[1,2]

A wide variety of presenting symptoms are related to disorders of the anorectum. Among them are pain, tenderness, rectal spasm, bleeding, itching, protrusions, eruptions, discharges, constipation, diarrhea, changes in stool pattern, sacral backache, shooting pains down the limbs, crampy and painful menses, urinary disturbances, anemia, prostatitis, restlessness in children, and foreign bodies.[3] The clinician should be aware that seemingly unrelated symptoms might, in fact, have their origin in the anorectal tract.

ANAL DISEASES

Anal Fistula and Anal Abscess

Anal fistula and anal abscess result from an infection of an anal crypt, whose distal end lies near the dentate line. The anal glands, which lie within the intersphincteric groove and empty into the anal crypts, become infected because of impacted feces. The resulting infection makes its way into the intersphincteric space but generally does not penetrate beyond the external sphincter. Most of the anal glands lie posteriorly; therefore most abscesses drain from a line posterior to the ischial tuberosities, whereas

anterior abscesses drain radially from their points of origin. The presence of a rectal fistula or abscess may point to an internal bowel disease such as diverticulitis, trauma to the area, or immune system incompetence, as with the human immunodeficiency virus (HIV).

Under normal conditions, the highly vascularized rectal mucosa is able to provide adequate protection against the spread of infection. The weak points are the anal glands; however, because they may become impacted with fecal matter, setting up an infective process in the anal crypt and intersphincteric space. The surrounding perianal tissue is swollen, red, and painful to palpation. If the infection remains in the intersphincteric space, the patient has rectal pain seemingly without specific findings. Eliciting the pain with pressure upon examination may provide a clue to the source of the lesion. The patient may also present with a fever of unknown origin.

The adjacent ischiorectal fossae are regions filled with fatty tissue that allow for distention of the rectum. Therefore an abscess has many different directions in which to track, setting up areas for pockets of pus. In a true perianal abscess, incision and drainage are usually not recommended because the bulk of the lesion would remain untouched owing to the probable invasion of the underlying tissues and the propensity of such an access to form caverns of pus. Unlike other abscesses formed by the body, perianal abscesses should not be allowed to come to a head and rupture on their own; such an occurrence can lead to excessive inflammatory necrosis of the anal sphincter.

Generally, a surgical consultation is warranted for a rectal abscess. Surgery allows for opening of the tract and healing by secondary intention. However, localized treatment can be undertaken to help the abscess drain if it has ruptured on its own or the patient presents acutely and immediate referral is not possible. Such treatment affords some relief and helps decrease the morbidity associated with this condition. Irrigation with herbal antibacterial agents such as *Hydrastis, Usnea,* and *Calendula succus* in 0.9% saline helps drainage.

Another often-encountered scenario is that of folliculitis with resultant abscess formation. These abscesses can be incised and drained because they do not involve the anal canal or ischiorectal fossa. Homeopathic medicines such as calcarea sulphuricum, silicea, hepar sulphuris, and *Myristica* are some of the medicines indicated most often. Poultices made of onion, garlic, or potatoes exert a drawing action, bringing the lesion to a head faster. Alternating hot and cold sitz baths are also useful. Once the abscess has broken open or has been incised and drained, irrigation with the previously mentioned solution hastens healing.

Anal Cryptitis

Anal crypts are pockets created between the grooves formed by the rectal columns at their distal points ending at the dentate line. The anal glands empty into the crypts and, as previously mentioned, are believed to be the source of anal fistula. That anal cryptitis is a separate entity is open to some debate, and it is rarely seen. The patient complains of pain that is worse with bowel movement. Examination shows localized tenderness and redness at the dentate line, and there may be some rectal spasm. Rectal fissure should be ruled out. Chronic cases suggest bacterial infection, usually gonorrhea.

Treatment is by herbal suppository, sitz baths, increased dietary fiber intake, and, if necessary, localized anesthesia. The crypt may be cleaned out with a crypt hook if needed and irrigated with a solution of *C. succus* and *Hydrastis* in normal saline.

Anal Fissure

An anal fissure is a slit-like separation of the anal mucosa lying below the dentate line. The majority of fissures are found in the posterior midline region (70%–80%), but anterior midline lesions (10%–20%) are more commonly seen in women. Some investigators report that fissures are more common in men than women,[4] but other studies do not bear this out.[5] In some patients, the anal sphincter is more oval than round, coming to a Y or narrow point at the posterior midline, making for a greater predisposition for anal fissure. Anal fissure in children is not all that uncommon, often being associated with chronic diarrhea or hard stool.[6] Fissure may also be a complication of a lesion associated with a congenital anal deformity. Lesions located in the anteroposterior vertical axis suggest an internal bowel disease, such as Crohn's disease, squamous cell carcinoma or adenocarcinoma of the rectum, syphilitic fissure, or ulceration due to tuberculosis.

Anal fissures are very painful because of their somatic innervation, which results from spasm of the anal sphincter in response to stretching during the passage of stool. The presence of an anal fissure triggers a vicious cycle of pain, causing sphincter spasm, which contributes to a tightening of the sphincter and increased pain with the passage of stool. Patients usually have severe pain during and for some time after defecation, and bleeding is not uncommon. As the lesion enlarges, it may ulcerate and become infected. Conventional medical treatment consists of rectal dilation, internal sphincterotomy, electrodessication, or surgical excision.[5]

Anal fissure can be caused by the passage of large, hard stools; childbirth trauma; chronic diarrhea; trauma; food allergy; or prolonged straining to pass stool. Infants and young children who consume large amounts of cow's milk are more likely to have anal fissures and chronic constipation, especially if they were breastfed for a shorter period.[7] Many of the patients in whom anal fissures develop display intense, compulsive personalities that may contribute to the formation of such fissures and the ability to heal. A previous anal or rectal operation, syphilis, or Crohn's disease also predisposes the patient to fissure development.

Examination may be difficult because of the pain and spasm, and anoscopic examination is often out of the question unless anesthesia is used. A localized injection of 1% or 2% lidocaine into the rectal sphincter at the 3-o'clock and 9-o'clock positions can help relax it enough for an examination to occur. One can often see anal fissures without using an anoscope simply by pulling back on the anal skin and examining the tissue. The presence of a sentinel pile or enlarged papilla suggests chronic anal fissure and is the result of inflammation and the body's attempt to protect the inflamed area. Anal spasm may be marked, making it difficult to perform an examination, and anal stenosis and fibrosis may be present if the condition has been chronic.

Clinicians should consider the presence of Crohn's disease, especially if the patient is younger, there is a history of periodic or chronic diarrhea, and the fissure lies in the anteroposterior vertical axis. Squamous cell carcinoma, syphilitic ulcers, and rarely tuberculosis should be considered as part of the differential diagnosis. Spasm of the levator ani muscle may also make one think of anal fissure, but no lesion is present in this case.

Treatment of anal fissure can prove to be one of the more difficult courses of therapy for both the patient and the clinician. Although surgical intervention removes the lesion and alleviates the pain, the lesion invariably returns because the underlying cause has not been addressed. Additionally, surgical intervention often predisposes to fecal incontinence later in life. Therefore medical management both in the short and long terms is necessary for complete resolution. Patients must be cautioned that the healing of a rectal fissure has its periods of exacerbation and remission and that continuing with the treatment protocol is important despite the periodic setbacks that are often encountered. Educating the patient on this point upon initiation of therapy is essential to its success.

Treatment

Initial treatment should be to alleviate the pain and spasm because they have often brought the patient to the office. Homeopathic medicines provide prompt relief if the simillimum is found and aids the healing process. Remedies such as *Chamomilla,* graphites, nitric acid, *Ratanhia, Sepia,* silicea, and *Thuja* are some of the more often indicated medicines, but others may also be needed.[8,9] Frequent dosing is generally the rule. Protease 315 mg, two capsules three times daily between meals and again before bed, will help alleviate pain as well. A preparation of 0.2% glycerol trinitrite can be used topically to relieve rectal spasm,[10] as can 5% lidocaine cream for localized pain. Glycerol trinitrite has been shown in a number of studies to be effective in relieving rectal spasm and increasing blood flow to the anal sphincter.[11–13] Reduced blood flow to the anal sphincter has been postulated as one of the precipitating mechanisms for the development of an anal fissure.

A topical cream consisting of vitamins A and E, panthenol, *Calendula,* and *Comfrey* will considerably enhance the healing process by providing nutrients essential for healing by secondary intention. Some of the commercial preparations also contain boric acid, which acts as a styptic. Initially, the cream should be applied topically after every bowel movement and at bedtime. As healing occurs, twice-daily application usually suffices until the lesion has resolved.

Iontophoresis using a zinc electrode and applying a positive current helps facilitate healing by hardening the underlying fissure, decreasing bleeding, and affording pain relief.[14] The patient lies on the negative dispersing pad, and current is applied for 10 minutes at 10 mA.

The patient should be instructed not to strain during the passage of stool and to use cotton balls that have been moistened with water rather than toilet paper or chemical or alcohol wipes. Some patients are excessive cleaners; they should be instructed that it is unnecessary to wipe deep into the anal canal because doing so makes the condition worse.

Sitz baths also aid in the healing process by providing increased blood flow to the area. If performing a sitz bath is not possible, alternately spraying hot and cold water on the perineal area will achieve the same result.[15]

Increasing dietary fiber is necessary if the condition is due to chronic constipation, whereas chronic diarrhea can be managed through a variety of therapeutic approaches once the cause is found. Dietary changes are a must, especially if the anal fissure is associated with irritable bowel syndrome or Crohn's disease. The higher rates of hemorrhoids and Crohn's disease seen in blood group O individuals are believed to be due to food intolerance.[16]

As previously mentioned, the clinical course varies from patient to patient and seems to depend partially on whether the patient follows the treatment plan religiously. Frequent follow-up is needed to assess the healing process and reassure the patient. Once healing has occurred, proper bowel function and good dietary and bowel habits must be maintained in order to prevent further episodes. Patients who experience an anal fissure are at risk for further episodes.

HEMORRHOIDS

Affliction from hemorrhoids has been noted in the writings of various cultures throughout history—Babylonian, Hindu, Greek, Egyptian, and Hebrew. In the United States and other industrialized countries, hemorrhoidal disease is extremely common. Although most individuals may begin to have hemorrhoids in their 20s, hemorrhoidal symptoms usually do not become evident until the fourth decade of life.[17,18] Estimates have indicated that 50% of persons older than 50 years have symptomatic hemorrhoidal disease at one time or another, and up to one third of the total U.S. population has hemorrhoids to some degree.[19]

The causes of hemorrhoidal disease are similar to those of varicose veins. As with varicose veins, predisposition to the development of hemorrhoids depends on genetic makeup. Excessive venous pressure, pregnancy, long periods of standing or sitting, straining at stool, and heavy lifting are considered the major factors. Most patients have more than one predisposing factor.[20]

Because the portal venous system contains no valves, factors that increase venous congestion in the perianal region can precipitate hemorrhoid formation. They include increasing intraabdominal pressure (e.g., defecation, pregnancy, coughing, sneezing, vomiting, physical exertion, and portal hypertension as a result of cirrhosis), an increase in straining during defecation, and standing or sitting for prolonged periods.

Presenting symptoms are itching, burning, irritation with the passage of stool, swelling of the anus and perianal region, blood on the toilet paper or in the bowl, and seepage of mucus. Most patients attribute all rectal symptoms to hemorrhoids; however, internal hemorrhoids are only rarely painful or cause itching. Usually the hallmark of a hemorrhoidal eruption is bleeding or protrusion noted after the passage of stool. Pain from internal hemorrhoids occurs when they become strangulated from prolapse and with thrombosis. Any other pain associated with hemorrhoids is usually the result of a coexisting lesion, such as a fissure. Itching is rarely associated with internal hemorrhoids except if there is excess mucous discharge.

Classification

Hemorrhoids are typically classified according to location and degree of severity. External hemorrhoids occur below the anorectal line. They may be full of either blood clots (thrombotic hemorrhoids) or connective tissue (cutaneous hemorrhoids). A thrombotic hemorrhoid is produced when a hemorrhoidal vessel has ruptured and formed a thrombus, whereas a cutaneous hemorrhoid consists of fibrous connective tissue covered by anal skin. Cutaneous hemorrhoids can be located at any point on the circumference of the anus. Typically, they are caused by the resolution of a thrombotic hemorrhoid; that is, the thrombus becomes organized and replaced by connective tissue.

Internal hemorrhoids occur above the anorectal line. Occasionally an internal hemorrhoid enlarges to such a degree that it prolapses and descends below the anal sphincter. Internal hemorrhoids are graded by the degree of prolapse.

Grade I: No prolapse
Grade II: Prolapse upon defecation but spontaneous reduction
Grade III: Prolapse upon defecation and manual reduction
Grade IV: Prolapsed and no reduction possible

Internal-external, or mixed, hemorrhoids are a combination of contiguous external and internal hemorrhoids that appear as baggy swellings. The following types of mixed hemorrhoids can occur:

- Without prolapse: bleeding may be present, but there is no pain.
- Prolapsed: characterized by pain and possibly bleeding.
- Strangulated: the hemorrhoid has prolapsed to such a degree and for so long that its blood supply is occluded by the anal sphincter's constricting action; strangulated hemorrhoids are very painful and usually become thrombosed.

Treatment

In contrast to the United States and the United Kingdom, hemorrhoids are rarely seen in parts of the world where high-fiber diets containing unrefined foods are consumed.[19] A low-fiber diet high in refined foods contributes greatly to the development of hemorrhoids. Individuals consuming a low-fiber diet tend to strain more during bowel

movements because their smaller and harder stools are more difficult to pass. This straining raises the pressure in the abdomen, obstructing venous return. The greater pressure will increase pelvic congestion and may significantly weaken the veins, causing hemorrhoids to form.

A high-fiber diet is perhaps the most important component in the prevention of hemorrhoids. A diet rich in vegetables, fruits, legumes, and grains promotes peristalsis because many fiber components attract water and form a gelatinous mass that keeps the feces soft, bulky, and easy to pass. A high-fiber diet leads to significantly less straining during defecation.

Natural bulking compounds can be used to reduce fecal straining. These fibrous substances, particularly psyllium seeds and guar gum, possess a mild laxative action owing to their ability to attract water and form a gelatinous mass. They are generally less irritating than wheat bran and other cellulose fiber products. Several double-blind clinical trials have demonstrated that supplementing the diet with bulk-forming fibers can significantly reduce the symptoms of hemorrhoids (bleeding, pain, pruritus, and prolapse) and improve bowel habits.[21,22] The use of psyllium seed fiber has been shown to afford significant relief in bleeding and pain within 6 weeks in hemorrhoid suffers.[23]

A placebo-controlled randomized study evaluated the efficacy of fiber supplements in 50 patients with bleeding internal hemorrhoids. Patients in the study group were treated with a commercially available preparation of *Plantago ovata,* and those in the control group were treated with a placebo. Endoscopy was performed in every patient before and after treatment to establish the degree of hemorrhoidal prolapse and the numbers of congested hemorrhoidal cushions and contact-bleeding hemorrhoids. During the first 15 days of treatment, the average number of bleeding episodes was 4.8 ± 3.8 for the study group versus 6.4 ± 3 for the control group (NS). During the following 15 days, it decreased to 3.1 ± 2.7 in the study group versus 5.5 ± 3.2 ($P < 0.05$) in the control group, and in the last 10 days of treatment, a further reduction to 1.1 ± 1.4 was found in the study group compared with 5.5 ± 2.9 in the control group ($P < 0.001$). The number of congested hemorrhoidal cushions diminished from 2.6 ± 1 to 1.6 ± 2.2 after fiber treatment ($P < 0.01$), but no differences were found in the control group. In the fiber group, hemorrhoids bled on contact in 5 out of 22 patients before treatment and in none after treatment; no differences were found in the control group. No modification of the extent of prolapse was observed after treatment.[24] Although the effect took a few weeks to develop, the final results were very impressive.

Another important, but only recently recognized, dietary factor is breakfast. An age-, gender-, and pregnancy-matched case-control study carried out in an outpatient clinic found a remarkable 7.5-fold increase in the odds of suffering from hemorrhoids or anal fissures in matched subjects who did not eat breakfast![25]

Flavonoid preparations have been shown to be beneficial in the prevention and treatment of hemorrhoids through their strengthening effect on venous tissues. Although early studies primarily used rutin, later research has used hydroxyethylrutosides (HERs).

Several of the studies have been performed in pregnant women, where HERs were shown to be of great benefit in helping to relieve hemorrhoidal signs and symptoms. In one study, 90% of the women given HERs (1000 mg daily for 4 weeks) experienced improvement of symptoms, compared with only 12% in the placebo group.[26] Similar results in hemorrhoids not associated with pregnancy have been reported.[27,28] Another study provided a micronized flavonoid combination (diosmin 90% and hesperidin 10%) for a median of 8 weeks before delivery and 4 weeks after delivery to 50 pregnant women with acute hemorrhoids. Their therapy was very successful, with 66% of patients reporting relief from symptoms by the fourth day of treatment and fewer suffering relapses during the antenatal period.[29] Treatment was well accepted and did not affect pregnancy, fetal development, birth weight, infant growth, or feeding.

In most circumstances, topical treatments for acute or chronic hemorrhoids involving the use of suppositories, ointments, and anorectal pads provide only temporary relief. Many over-the-counter products for hemorrhoids primarily contain natural ingredients, such as witch hazel *(Hamamelis),* cocoa butter, Peruvian balsam, zinc oxide, allantoin, and homeopathic preparations. Many patients use hydrocortisone cream to help with the itching that they associate with hemorrhoids; prolonged use of this agent can often aggravate the pruritus ani, setting up a cycle of continued use.

Botanical Medicines

Botanical medicines have had a long history of use for hemorrhoids. They can be used topically, administered as suppositories, or taken orally to support redundant tissue and facilitate healing. The use of rectal suppositories after galvanic, infrared, or laser therapy helps facilitate healing and decrease bleeding. Some of the more commonly used botanical medicines are discussed later in this chapter.

Hydrotherapy

The warm sitz bath is an effective noninvasive treatment for uncomplicated hemorrhoids.[15,18] Although a warm sitz bath is a useful treatment for an acute hemorrhoidal flare-up, alternating hot and cold sitz baths are better suited to facilitate the healing of chronic conditions.

Surgical Treatment

Various surgical and nonsurgical methods of treating hemorrhoids exist, the choice of which often depends on the stage of the hemorrhoid and the expertise of the physician. Stage I or II hemorrhoids can often be managed medically, especially if they are acute. Herbal rectal suppositories, along with the indicated homeopathic medicine, often aid in quick resolution of the flare-up. In uncomplicated cases of stage I or II hemorrhoids, unless the patient is exhibiting symptoms, treatment is usually not recommended except for increasing dietary fiber and addressing the patient's dietary choices.

Injection of sclerosing agents is useful in stage I and stage II hemorrhoids, but such treatment is contraindicated in patients with anal fissure, inflammatory bowel disease, Crohn's disease, leukemia, or portal hypertension. Sclerosing therapy is not usually effective against stages III and IV hemorrhoids.

Rubber-band ligation of hemorrhoids is a widely used in-office procedure that removes redundant tissue and causes scarring, which is then replaced by new tissue. Following such treatment, patients often have a sense of fullness or pressure in the rectum, and pain occurs if the band is placed too close to the pectinate line. Postbanding bleeding can occur, and there have been reports of complications resulting in septicemia and death.[20] Rubber-band ligation is used primarily for stage I, stage II, and occasionally stage III hemorrhoids; its success is largely a function of the physician's ability to place the bands.

Cryosurgery involves the application of liquid nitrogen or nitrous oxide to the hemorrhoid, which causes destruction of the venous plexus, scarring, and eventual tissue replacement. Used primarily for stage II and stage III hemorrhoids, the procedure is also useful if condyloma is present. It is contraindicated for stage IV hemorrhoids, chronic ulcers, and acutely thrombosed hemorrhoids. Pain, swelling, and bleeding may occur after cryosurgery.

Hemorrhoidectomy, or the surgical removal of redundant tissue, is by far the most invasive of the hemorrhoidal procedures. It often requires an outpatient surgical setting and causes the patient to lose time from activities of daily living so that healing can take place. Most patients seek alternative treatments in order to avoid surgery and its complications, such as pain and instability of the rectal sphincter.

The monopolar direct current technique for the management of hemorrhoids is becoming a significant treatment of choice in the

United States.[30] According to a report by Machicado et al.,[31] this painless outpatient treatment of all grades of hemorrhoids is effective and safe. This methodology warrants consideration as the treatment of choice for hemorrhoidal disease.

The first published work on monopolar direct current (also called inverse galvanism) was published by Keesey[32] of Chicago. Dr. Keesey dated the first use of this approach to 1897, although the technical problems (producing a smooth, uninterrupted galvanic current source) were not worked out until approximately 1925. The technique then came into use in general practice. Monopolar ablution of hemorrhoidal tissue lost favor in conventional medicine, not because it was ineffective, but because newer techniques and advances in surgery made it less attractive. If the naturopathic medical schools had not been teaching the Keesey treatment over the last 60 years, it is very possible that this irreplaceable technique would have been lost to the healing arts.

A later study showed that direct-current electrotherapy also relieves the chronic anal fissures often associated with internal hemorrhoids. All 10 of the patients in the reported study experienced relief of chronic anal pain within two treatments, and anal fissures healed in 9 within 4 weeks. One patient experienced a perianal abscess and fistula, requiring surgery. There were no recurrences in 20 months (mean) of follow-up.[31]

The monopolar direct current technique is purely an office procedure. No anesthesia is needed except a local anesthetic for the occasional hypersensitive or nervous patient (in such instances, a 2% procaine solution is injected directly into the hemorrhoid). To date, there have been no reported cases of stricture or metastatic abscesses, a fact that speaks well for the procedure's safety.

The chemicophysiological action, which causes the absorption and destruction of a vein, is responsible for the permanence of the cure. The mechanism of action of monopolar direct current is well understood. When introduced into the interior of the hemorrhoid, the negative pole of the galvanic current makes contact with the water of the blood and tissues and generates hydrogen gas and hydroxide ions. The hydroxide destroys the organized structure of the hemorrhoid and its capillary circulation. This produces first a hydrolysis and then a hardening of the hemorrhoid. The final disappearance of the hemorrhoid is brought about in one of two ways. It is either absorbed like any bodily contusion or, if the hemorrhoid is large, it ruptures, causing a discharge of the thrombosed elements into the rectum, followed by a contraction of the residual hemorrhoidal tissues.

What makes the Keesey technique attractive is that the patient may be freely ambulant after completion of the procedure and can return to his or her normal activities. The hemorrhoid disappears 7 to 10 days after the treatment. Each separate hemorrhoid is treated in the same manner, and larger hemorrhoids may have to be treated more than once. Generally it is a good policy to treat only one hemorrhoid or at most two at a time because of the healing that must occur and the higher risk of bleeding with the treatment of several.

Infrared coagulation (IRC) is effective with stage I and stage II hemorrhoids but can be combined with the Keesey treatment for stage III and stage IV lesions. Whereas the Keesey technique uses current, the infrared coagulator uses a burst of intense heat generated internally and shot through a blue anodized sapphire tip to the surface of the hemorrhoid. The IRC "coagulates" the redundant tissue to a depth that is a function of the duration of the light burst, usually 1 to 1.5 seconds. As with the Keesey technique, a 7- to 10-day period of healing should occur between treatments. Many naturopathic physicians who use the Keesey treatment also incorporate IRC because doing so usually reduces the number of treatments needed per hemorrhoid. Compared with rubber-band ligation, laser therapy, and cryotherapy, IRC has been shown to produce better results and less morbidity.[33–35]

Prevention

As with all diseases, the primary treatment of hemorrhoids is prevention. This involves reducing factors that may be responsible for increasing pelvic congestion, such as straining during defecation, sitting or standing for prolonged periods, and underlying liver disease. A high-fiber diet is crucial for the maintenance of proper bowel activity, and nutrients and botanical substances that enhance the integrity of venous structures may also be of benefit.

Warm sitz baths and topical preparations are useful to ameliorate the discomfort but have only transient results. A diet high in complex carbohydrates that are rich in dietary fiber is indicated. The diet should contain liberal amounts of proanthocyanidin- and anthocyanidin-rich foods, such as blackberries, cherries, and blueberries, to strengthen vein structures. Supplements such as vitamins A and B complex, antioxidants such as vitamins C and E, and zinc will help maintain vascular integrity and facilitate healing.

When indicated, the monopolar direct current method, specifically designed for the treatment of hemorrhoids, will give permanent results. This technique is especially useful in advanced hemorrhoidal disease and, when coupled with the necessary diet and lifestyle changes, considerably decreases the likelihood of recurrence.

EXTERNAL HEMORRHOIDS

External hemorrhoids occur when there is dilation of the external rectal plexus or thrombosis after an episode of constipation, diarrhea, or heavy lifting or the Valsalva effect from sneezing, coughing, or childbirth. The patient notices a perianal lump, which is often painful, and some bleeding may be associated with it. External hemorrhoids usually pose mild to little discomfort and largely resolve on their own if homeostasis is restored. External anal skin tags found on examination are the remnants of previous external hemorrhoids.

If, however, the hemorrhoid becomes thrombosed, a cycle of acute edema and pain is set up that may lead to surgical intervention. As the lesion becomes increasingly distended, varying degrees of pain and swelling can arise, which are often exacerbated by the passage of stool or by prolonged sitting. The patient may report bleeding after stool, which is the result of a disruption of the hemorrhoid.[36]

A prolapsed internal hemorrhoid should also be considered as part of the differential diagnosis for external hemorrhoid, as should perianal abscess, rectal fissure with sentinel pile, hypertrophied anal papillae, irritated skin tag, condyloma latum, and condyloma acuminata.

Treatment

Unless the hemorrhoid has thrombosed and the patient is in excessive pain, an external hemorrhoid can usually be managed medically. Initial treatment should be to relieve the pressure and dissolve whatever thrombosis has formed. As healing occurs, long-term management in the form of patient education, dietary changes, and enhancement of vascular integrity should be undertaken to help prevent further episodes.

Initial treatment with the enzyme protease 315 mg, two capsules between meals three times daily and two at bedtime, will help reduce the thrombosis and decrease pain. Alternating hot and cold sitz baths act to relieve pain and increase blood flow. A number of homeopathic medicines, such as *Aesculus,* aloe, *Hamamelis,* muriatic acid, *Ratanhia,* and *Sepia,*[8,37] are effective in relieving pain and speeding the course of healing. A clinical repertory or materia medica should be consulted because there are numerous remedies that will provide relief.

Long-term management should involve identification and removal of the precipitating cause and education of the patient as to the cause and how to prevent further episodes. Dietary changes to eliminate

offending foods while increasing dietary fiber in the form of fruits and vegetables or an over-the-counter fiber supplement are warranted. Herbal medicines such as *Aesculus*, *Collinsonia, Hamamelis,* and *Althea* or *Ulmus*[38] will help restore venous sufficiency and soothe inflamed tissues.

In some cases, regulation of bowel function with the use of herbal laxatives may be necessary while normal intestinal and colon function is restored. Various commercially available herbal medicines and fiber compounds are effective.

In patients who are experiencing an acute episode of a thrombosed external hemorrhoid, prompt surgical excision or incision is in order. Because the external skin is innervated by somatic nerves, administration of anesthesia is needed before evacuation of the clot. Excision leaves a wound that should not be sutured but should be allowed to heal by secondary intention, although this causes greater postoperative pain. Incision and debridement of the clot allow for less pain, but the wound may close too early, leading to re-formation of the thrombus. A text on minor surgery for diseases of the rectum and colon should be consulted as to the proper surgical technique.

Starting the patient on herbal anodynes such as *Piscidia,* belladonna if pain is a result of rectal spasm, and *Hyoscyamus niger* can facilitate postoperative management. Healing and pain relief can be afforded with the topical use of *Arnica, Hypericum,* and in particular *C. succus.*

Alternating hot and cold sitz baths with ½ oz of povidone-iodine added enhances healing and helps reduce the chance of infection. As with medical treatment, protease 2400 milk clotting units (MCUs) can be added for pain relief. Homeopathic agents such as *Arnica, Calendula,* and staphisagria should also be considered for pain relief.

PERIANAL DERMATOLOGICAL DISORDERS

Condylomata Acuminata

A patient presenting with condylomata acuminata may complain of a sticking or foreign-body sensation perianally. If the lesion is large enough, defecation can be interfered with, and hygiene can become a problem. The lesion is usually soft, moist, reddish pink, and pedunculated. Differentiation from condylomata lata of secondary syphilis is necessary because the treatments differ, and the potential for transmission is greater with the latter disorder. A rapid plasma reagin (RPR) test with reflexive fluorescent treponemal antibodies (FTAs) is warranted. If dark-field microscopic examination is available, it should also be performed. Diagnosis of condylomata acuminata is made through biopsy.

A solution of 25% podophyllum that is applied topically for 6 to 8 hours, after which it is washed off by the patient, has been shown to be effective. Several applications may be necessary in order to eradicate the lesion.[39] Cryosurgery is also available, as is IRC after subcutaneous injection of 1% or 2% lidocaine.[40] Although naturopathic and homeopathic philosophy decries the "suppression" of lesions as contributing to a deepening of the disease process, concomitant administration of the proper homeopathic medicine coupled with a good nutritional program rarely causes this to happen.

The administration of homeopathic *Thuja* is often indicated, but a number of other homeopathic remedies cover genital warts. The taking of a constitutional case not only helps with the eradication of the lesion but also acts to decrease the patient's susceptibility to the development of further lesions, which can often happen.

Condyloma Lata

Condyloma lata, or anogenital wart, is caused by human papillomavirus (HPV) types 6, 11, 16, 18, 31, 33, and 35 and is transmitted by sexual contact. Lesions appear as soft, moist, pink to gray, flat to pedunculated excrescences frequently found in clusters. More often located in warm, moist regions of the anogenital region, they are more commonly found clustered around the anus in homosexual men and in women who practice anal intercourse.[41]

Lesions must be differentiated from the flat lesion seen with the condyloma lata of secondary syphilis. A diagnosis of squamous cell carcinoma should be considered if the lesion does not respond to therapy, or biopsy can be performed.[42]

Various naturopathic therapies are available, among them being treatment with 25% podophyllum, *Thuja* ointment, homeopathic prescription, cryotherapy, IRC, and electrosurgical dessication.[43]

Lymphogranuloma Venereum

The infective agent in lymphogranuloma venereum (LGV) is one of the serotypes of *Chlamydia trachomatis,* and the lesion may first appear as a papule that ulcerates and heals rapidly. If the primary lesion is in the rectal mucosa, the patient presents with the signs and symptoms of proctitis. This is then followed by the development of inguinal adenitis or buboes that are extremely painful. The later stages may manifest as fistulization, ulceration, fibrosis, and rectal or anal stenosis and can lead to lymphedema of the legs and genitalia. Non-LGV *Chlamydia* serotypes can also be implicated in proctitis but generally do not have as severe presenting symptoms.

White blood cell counts may reach 20,000/mm^3 if there is lymphatic involvement, and the patient may exhibit anemia. Culture of the chlamydial serotype from an infected lesion is diagnostic, but complement fixation and immunofluorescent antibody tests are available.[44]

If LGV is treated promptly, the prognosis is good; but once the disease has begun to create cellular changes, the prognosis becomes worse because the degree of pathology can be considerable. For the most part, allopathic treatment consists of antibiotics such as doxycycline, erythromycin, and sulfisoxazole, all of which have been found to be effective against LGV.[44] Various homeopathic and herbal medicines can be used. See treatments under "Proctitis."

Herpes

Probably the most commonly encountered perianal lesion, herpes simplex typically manifests as a burning, searing pain that may be confused with that of a rectal fissure. Examination reveals vesicles on an erythematous base that soon erode and form ulcerations. In patients with HIV or other immunocompromising conditions, the lesions can form deep ulcerations and have a prolonged course. Diagnosis is by Tzanck smear or viral culture.

Initially, treatment protocols should address the current eruption because longer-term prevention strategies usually take time to work. Topical preparations consisting of deglycyrrhized licorice (DGL) and *Melissa officinalis* extracts can help alleviate local symptoms of burning and pain and facilitate the healing process. Zinc sulfate 2% has also been used topically with some success. Various homeopathic medicines not only address the pain and eruptions but also help decrease the patient's susceptibility to further outbreaks. Stress reduction is important as well, because herpes eruptions often occur during periods of excessive stress or other illness.

Long-term strategies include decreasing the arginine-containing foods in the diet, such as nuts, legumes (especially peanuts), and chocolate. Supplementing lysine at 500 to 1000 mg/day is useful in eliminating outbreaks. Antioxidants such as vitamins C and E and B complex vitamins, zinc, and copper have all been shown to be of benefit.

Pilonidal Sinus

Pilonidal sinus is a rare congenital tract that runs from the coccyx or sacrum to the perineum with constant drainage. Both congenital and

acquired factors play a role in the development of a pilonidal sinus. The condition is most commonly seen in young white adults, rarely in black persons, and almost never in Asians. Congenital postanal dimples predispose to the formation of a pilonidal tract, as do obesity, a deep intergluteal cleft, and excess body hair.

The condition is usually differentiated from anal fistula and furunculosis because of its midline orientation and through the passing of a probe through the sinus, noting its passage toward the sacrum rather than the anus.

Patients seek treatment because of the often-chronic nature of the discharge. If the sinus becomes infected, incision and drainage may be needed, but irrigation with the herbal formula mentioned for abscess can be performed. Weight reduction and removal of excess hair in the region also helps reduce the risk of infection and symptoms. Homeopathic medicines selected according to the presentation, such as silicea, calcarea sulphuricum, and hepar sulphuris, have proved useful. Additionally, homeopathic *Thuja* as an intercurrent treatment has resolved a number of obstinate cases.

Proctalgia Fugax

Proctalgia fugax, or levator spasm syndrome, is more common in men than women and manifests as pain, often searing, near the coccyx or rectum that lasts for a brief but intense period, usually resolving on its own. The episode often wakens the person from sleep but may occur at any time.

Caused by a spasm of the levator ani or pubococcygeal muscle, proctalgia fugax produces a deep-seated and often heavy, aching, or searing pain that feels as if it were localized in the rectum and/or prostate in men. Examination reveals a tense and tight levator ani muscle that is tender with pressure and is often drawn up above the anal sphincter.

Mechanisms that are thought to trigger an episode are poor posture, chronic anxiety, and mental fixation on the rectal area, as well as misalignment of the coccyx or other bones composing the pelvic bowl.

Differential diagnosis includes lumbar disc disease, coccygodynia, prostatitis, presacral tumor, developing ischiorectal abscess, spinal cord tumor, and rectal lesion.

Treatment often takes the form of reassuring the patient as to the etiology of the condition. Muscle relaxants and hot packs may be of benefit. Spinal and pelvic bone adjustments may also be of use, as is massage to the lower abdomen, lumbosacral spine, and perineum. Chronic cases often respond to deep tissue work, such as Rolfing.

Proctitis

Proctitis usually manifests as rectal pain, tenesmus, rectal discharge, and blood in the stool. White blood cells may be present in large numbers, being evident on wet preparation, Gram stain, or Wright stain. Because there are various causes for the inflammation, an anoscopic examination is warranted to examine the rectum for inflammation, mucopurulent discharge, mucosal friability, bleeding, and ulceration. Severe ulceration and bleeding are associated with infection by the LGV *Chlamydia* serotype.

Among the causative agents are *Chlamydia* (usually of the genital immunotypes but also the LGV immunotypes), yeast, bacteria such as *Neisseria gonorrhoeae,* parasites, trauma, lectins, excessive fiber in the diet, external irradiation, syphilis, *Trichomonas,* and Crohn's disease. One study demonstrated a higher risk for the development of ulcerative proctitis in smokers and persons who had had an appendectomy.[45]

Determination of the etiological agent dictates the course of treatment and expected outcomes. If the cause is external-beam irradiation for colon cancer or radium seed implants for prostate cancer, the therapy should commence before cancer treatment and continue during it and for several weeks after treatment has ceased. In a study examining the effects of radiation and antioxidant therapy,[46] it was demonstrated that a "substantial number of patients with radiation proctitis seem to benefit from antioxidant therapy." Despite admonitions by radiation oncologists that antioxidant therapy should be discontinued, a review by Lamson and Brignall[47,48] demonstrated no ill effects when radiation was combined with antioxidant therapy. As part of the therapeutic regimen for radiation proctitis, concomitant administration of homeopathic radium bromatum 200c before and after treatment helps decrease skin burning. Other homeopathic medicines may also be indicated, such as X-Ray, Sol, and cadmium metallicum.

Additionally, the use of demulcent rectal suppositories helps soothe the inflamed rectal mucosa and decrease pain.

If the proctitis is secondary to an inflammatory bowel condition, treatment of the underlying disease results in healing of the rectum as well. This can be accomplished by addressing the patient's diet, providing adequate fiber, and administering herbal demulcents and enteric-coated peppermint to soothe the inflamed intestinal tract. Short-chain fatty acids administered rectally were shown in one study to alleviate symptoms of proctocolitis, and a deficiency of such acids should be considered as part of the clinical presentation.[49,50]

In cases in which an infective agent such as bacteria, *Chlamydia,* yeast, fungi, trichomonas, or a parasite is identified as the etiological agent, a variety of naturopathic treatments have been found empirically to be effective. Among them are the injection of ozone; herbal suppositories; homeopathic medicines; and a retention enema consisting of *Hydrastis, Usnea, Echinacea,* yarrow for *Trichomonas,* and *Althea* in a base of 0.9% NaCl. This combination can be applied topically during the initial examination to begin treatment. Antioxidants such as vitamins C and E, as previously mentioned, facilitate healing.

As a general rule, treatment of proctitis should continue for 10 to 14 days before reexamination and assessment. In cases of ulcerative disease, treatment may need to be continued longer to allow for complete healing.

Pruritus Ani

Pruritus ani is characterized by chronic itching of the perianal skin with excoriation from scratching and an eventual thickening of chronically affected areas. Patients often scratch at night during sleep or are awakened from sleep by the itching. Patients may complain of intermittent to constant itching, burning, and soreness that often awaken them at night. The use of topical ointments may provide some relief, but symptoms return when they wear off. Examination may show varying degrees of erythema, swelling, excoriation, thickening, and fissuring as a result of scratching.

The stage is set for the development of pruritus ani when the skin's normal protective barriers have broken down. Various agents contribute to this process, such as excessive wiping with harsh toilet papers; use of "wipes" that are impregnated with drying alcohol, dyes, and antibacterial or fungal agents; reactions to synthetic undergarments or detergents used to launder them; and the wearing of too-tight underclothing, which decreases air circulation. Patients with chronic diarrhea or loose stools are at increased risk because of the stools' often acidic nature.[51,52] In some cases, removal of normal hair growth for cosmetic purposes also contributes because it disrupts the normal bacterial flora in this region of the body.

Pinworms and *Candida* are the two most common infecting agents in pruritus ani. In particular, a history of perianal itching largely at night should alert the physician to the possibility of *Enterobius vermicularis* infestation (pinworms). This is a common etiological agent in children and can be spread to other members of the family with considerable ease. A wet preparation will demonstrate *Candida,* and a clear adhesive tape preparation for pinworms helps establish the diagnosis.

Patients with episodes of periodic or chronic diarrhea are also at increased risk of pruritus ani. It can occur because of the acidic nature of the stool but most often is caused by aggressive cleaning of the area afterward. Compulsive cleaners should be instructed on the use of cotton wipes, bath or shower cleansing, or the use of moist towels.

The frequent use of soaps, detergents, bleaches, dyes, or perfumes can also contribute to the development of pruritus ani. A recent onset may be the result of a change in a product, and the physician should ask about it. Certain foods that frequently aggravate the condition are peanuts, coffee, colas, beer, and spicy foods.[5,20] Highly acidic foods and foods that are responsible for an allergic reaction in the patient should be suspected and eliminated for several weeks.

Psoriasis, contact dermatitis, precancerous or cancerous lesions, Bowen's disease (interepithelial epithelioma), keratoacanthoma, melanoma, squamous cell carcinoma, and basal cell carcinoma can also cause pruritus ani.

Laboratory tests to consider are culture and sensitivity testing if bacterial infection is suspected, a potassium hydroxide and/or wet preparation to look for yeast or fungi, Wood's lamp examination for *Tinea cruris,* and a punch biopsy for suspicious lesions.[53]

TREATMENT OPTIONS

Treatment options in anorectal disorders include the elimination of spicy or offending foods or of food allergies and improvement of the patient's digestion. If an infective agent is identified, a course of topical therapy to remove the yeast, fungi, or bacteria should be initiated. Initial washing with 3% hydrogen peroxide followed by application of a topical herbal formula containing *Usnea, Hydrastis, Spilanthes,* oregano, and tea tree oil, which is then allowed to dry, helps eliminate the infective agents. In order to reestablish the skin's defensive barrier, this step should be followed by a healing salve containing *Calendula,* comfrey, and vitamins E and A. Relief should be obtained within 7 to 10 days if the underlying cause has been eliminated.

For pinworms, numerous naturopathic treatments have proved to be effective. Additionally, all clothing and bedding should be washed in warm to hot water to eliminate the eggs that have been laid outside the rectum at night.

Various homeopathic medicines are also effective and may help prevent future episodes in chronic cases. Having the patient use moist cotton wipes after stool and eliminating excess moisture in the perineal area helps break the circle. The topical application of zinc oxide also has proved effective in the treatment of pruritus ani.

Botanicals

Botanical medicines have been used in the treatment of anorectal diseases throughout history by many different cultures. Many of the plants used by the various folk traditions are of the same genus, varying by species only, whereas others are unique to their locations. More recent examinations of the various botanical medicines have elicited their chemical properties and actions, but the eclectic and herbalist traditions have also placed an importance on the symptom specificity of the medicine. Doing so enhances its healing effect.

Aesculus hippocastanum is classified as a tonic, astringent, febrifuge, narcotic, and antiseptic. High in tannic acid and aesculin, this herbal medicine is good for pain relief of internal viscera and is indicated for hemorrhoids and rectal irritation when there is marked congestion and a sense of spasmodic closing of the rectum as if there were a foreign body present. Additionally, it is effective for itching, a sensation of heat, aching, and rectal pains. It has also been found to be useful for rectal neuralgia and proctitis.[38]

Achillea millefolium (yarrow) possesses slightly astringent properties and is an alterative and diuretic. It acts as a tonic on mucous membranes to stem the flow of mild hemorrhage; thus, it is used for bleeding hemorrhoids, especially if they are accompanied by mucoid discharges.[38]

Althea officinalis (marshmallow) is high in mucilage, which helps restore the electrical charge to inflamed rectal mucosa. It is often put into rectal suppositories because of its ability to decrease swelling, irritation, and inflammation.[38]

Cinnamomum (cinnamon) is commonly found in herbal rectal suppositories because of its ability to stem hemorrhaging.[38]

Collinsonia canadensis (stone root) is an alterative, tonic, stimulant, and diuretic. It is used primarily for its effects on the venous system because it tones vascular tissue. It is especially indicated for hemorrhoids caused by chronic constipation and venous insufficiency. Patients so affected have a sense of constriction, weight, and heat in the rectum and pass very dry stools. It is also useful for proctitis, anal fistulas, and rectal ulcer.[38]

Hamamelis virginiana (witch hazel) is primarily a tonic and astringent. It affects the venous system by restoring vascular integrity; therefore it is an excellent medicine for varicose veins, hemorrhages, and hemorrhoids. Indications for its use are venous tissues that are pale and in varying states of flaccidity but may also possess a deep redness because of vascular engorgement and stagnation of blood flow. The hemorrhoidal tissues are usually very painful and may have a tendency to ulceration.[38]

Hydrastis canadensis (goldenseal) contains hydrastine, berberine, canadine, and acrid resins. It exerts its action upon the mucous membranes and affects the healing of ulcerations while controlling mild hemorrhages; it also has antibacterial properties. Additionally, it has an astringent effect upon hemorrhoids. Goldenseal is indicated for hemorrhoids caused by constipation, accompanied by a sinking feeling in the stomach and a dull headache, and for anal fissures that are painful, with severe burning before, during, and after stool.[9,54]

Ruscus aculeatus (butcher's broom) acts upon the venous system, where it reduces swelling and has a vasoconstrictive action, thus reducing bleeding.[55]

Homeopathic Medicines

Various homeopathic medicines have historically been used in the treatment of anorectal diseases. A few key symptoms manifesting during the initial consultation will often guide the practitioner to the correct medicine, thus decreasing the need to take a full constitutional case.

TREATMENT SUMMARY FOR HEMORRHOIDS

As with all diseases, the primary treatment for hemorrhoids is prevention. This goal involves reducing the factors that may be responsible for increasing pelvic congestion, such as straining during defecation, sitting or standing for prolonged periods of time, or underlying liver disease. A high-fiber diet is crucial for the maintenance of proper bowel activity. Fiber supplements, flavonoids, and various botanical medicines such as butcher's broom are appropriate supplementary measures.

Warm sitz baths and topical preparations are useful to ameliorate the discomfort but have only temporary effects.

Diet

A high-complex-carbohydrate diet rich in dietary fiber is recommended. The diet should contain liberal amounts of flavonoid-rich foods, such as blackberries, citrus fruits, cherries, and blueberries, to strengthen vein structures.

Nutritional Supplements

- *Psyllium* seed husks: 5 g at bedtime
- Vitamin C: 500 to 1000 mg three times a day
- Flavonoids (choose one of the following):
  - Citrus bioflavonoids, rutin, and/or hesperidin: 3000 to 6000 mg/ day
  - Micronized diosmin: 500 to 1000 mg/day
  - Grape seed extract: (95% procyanidolic oligomers) 150 to 300 mg/day
  - Pine bark extract: 150 to 300 mg /day

Botanical Medicine

- Butcher's broom (*R. aculeatus*) extract (9%–11% ruscogenin content): 100 mg three times a day

Physical Medicine

- Hydrotherapy: warm sitz baths to relieve uncomplicated hemorrhoids

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Moore K. *Clinically Oriented Anatomy*. Baltimore: Williams & Wilkins; 1980.
2. Clemente C. *Anatomy: A Regional Atlas of the Human Body*. 2nd ed. Baltimore: Urban & Schwarzenberg; 1981.
3. Hirschman L. *Synopsis of Ano-Rectal Diseases*. 2nd ed. St Louis: Mosby; 1942.
4. Janicke DM, Pundt MR. Anorectal disorders. *Emerg Med Clin North Am*. 1996;14:757–788.
5. Goldstein S. Anal fissures and fistulas. *Postgrad Med*. 1987;82:86–92.
6. Kempe H, Silver H, O'Brien D. *Current Pediatric Diagnosis and Treatment*. 8th ed. Los Altos, CA: Lange Medical; 1984.
7. Andiran F, Dayi S, Mete E. Cow's milk consumption in constipation and anal fissure in infants and young children. *J Paediatr Child Health*. 2003;39:329–331.
8. Kruzel T. *The Homeopathic Emergency Guide: A Quick Reference Handbook to Effective Homeopathic Care*. Berkeley, CA: North Atlantic Books; 1992.
9. Vermeulen F. *Concordant Materia Medica.* Haarlem. The Netherlands: Emryss bv Publishers; 1997.
10. Scholefield JH, Lund JN. A nonsurgical approach to chronic anal fissure. *Hosp Pract*. 1997;32:181–188.
11. Kua KB, Kocher HM, Kelkar A, et al. Effect of topical glyceryl trinitrate on anodermal blood flow in patients with chronic anal fissures. *A N Z J Surg*. 2001;71:548–550.
12. Kennedy ML, Sowter S, Nguyen H, et al. Glyceryl trinitrate ointment for the treatment of chronic anal fissure: results of a placebo-controlled trial and long-term follow-up. *Dis Colon Rectum*. 1999;42:1000–1006.
13. Nelson R. A systematic review of medical therapy for anal fissure. *Dis Colon Rectum*. 2004;47:422–431.
14. Mc Dowell B, Kruzel T. *Physiotherapy—Supplemental Notes.* Portland, OR: NCNM Press, compiled; 1997–1998.
15. Boyle W, Saine A. *Lectures in Naturopathic Hydrotherapy*. East Palestine, OH: Buckeye Naturopathic Press; 1988.
16. D'Adamo P. *Eat Right 4 Your Type. Complete Blood Type Encyclopedia: The A-Z Reference Guide for the Blood Type Connection to Symptoms, Disease, Conditions, Vitamins, Supplements, Herbs, and Food*. New York: Riverhead Books; 2002.
17. Robbins SL, Cotran RS, Kumar V. *Pathologic Basis of Disease*. Philadelphia: Saunders; 1984.
18. Berkow R, ed. *The Merck Manual of Diagnosis and Therapy*. Rahway, NJ: Merck Sharp & Dohme Research Laboratories; 1982:560–566.
19. Trowell H, Burkitt D, Heaton K. *Dietary Fibre, Fibre-Depleted Foods and Disease*. London, Orlando: Academic Press; 1985.
20. Leff E. Hemorrhoids. *Postgrad Med*. 1987;82:95–101.
21. Moesgaard F, Nielsen ML, Hansen JB, et al. High-fiber diet reduces bleeding and pain in patients with hemorrhoids. *Dis Colon Rectum*. 1982;25:454–456.
22. Webster DJ, Gough DC, Craven JL. The use of bulk evacuant in patients with haemorrhoids. *Br J Surg*. 1978;65:291–292.
23. Burbrick MP, Benjamin RB. Hemorrhoids and fissures: common problems, current solutions. *Postgrad Med*. 1985;77:165–169, 172–174.
24. Perez-Miranda M, Gomez-Cedenilla A, Leon-Colombo T, et al. Effect of fiber supplements on internal bleeding hemorrhoids. *Hepatogastroenterology*. 1996;43:1504–1507.
25. Ahmed SK, Thomson HJ. The effect of breakfast on minor anal complaints: a matched case-control study. *J R Coll Surg Edinb*. 1997;42:331–333.
26. Annoni F, Boccasanta P, Chiurazzi D, et al. Treatment of acute symptoms of hemorrhoid disease with high-dose oral O-(beta-hydroxyethyl)-rutosides. *Minerva Med*. 1986;77:1663–1668.
27. Wijayanegara H, Mose JC, Achmad L, et al. A clinical trial of hydroxyethylrutosides in the treatment of haemorrhoids of pregnancy. *J Int Med Res*. 1992;20:54–60.
28. Wadworth AN, Faulds D. Hydroxyethylrutosides: a review of its pharmacology, and therapeutic efficacy in venous insufficiency and related disorders. *Drugs*. 1992;44:1013–1032.
29. Buckshee K, Takkar D, Aggarwal N. Micronized flavonoid therapy in internal hemorrhoids of pregnancy. *Int J Gynaecol Obstet*. 1997;57:145–151.
30. Norman DA, Newton R, Nicholas GV. Direct current electrotherapy of internal hemorrhoids: an effective, safe, and painless outpatient approach. *Am J Gastroenterol*. 1989;84:482–487.
31. Machicado GA, Cheng S, Jensen DM. Resolution of chronic anal fissures after treatment of contiguous internal hemorrhoids with direct current probe. *Gastrointest Endosc*. 1997;45:157–162.
32. Keesey WE. Obliteration of hemorrhoids with negative galvanism. *Arch Phys Ther X-ray, Rad*. 1934.
33. Eitan A, Bishara B, Duek DS, et al. Comparison of infrared coagulation and rubber band ligation for treatment of haemorrhoids. *Anus*. 1989:88–91. XII/90.
34. Antonelli A. Infrared coagulation of hemorrhoids. *Endosc Rev*. 1989:75–76.
35. O'Connor JJ. Infrared coagulation of hemorrhoids. *Pract Gastroenterol*. 1986;10:8.
36. Jongen J, Bach S, Stubinger SH, et al. Excision of thrombosed external hemorrhoid under local anesthesia: a retrospective evaluation of 340 patients. *Dis Colon Rectum*. 2003;46:1226–1231.
37. Guernsey W. *Homeopathic Therapeutics of Hemorrhoids*. 2nd ed. Philadelphia: Boericke & Tafel; 1892.
38. Felter HW, Lloyd JU. *King's American Dispensatory*. 18th ed. Cincinnati: Ohio Valley Co; 1983.
39. Beutner KR, von Krogh G. Current status of podophyllum for the treatment of genital warts. *Semin Dermatol*. 1990;9:148–151.
40. Bekassy Z, Westrom L. Infrared coagulation in the treatment of condyloma acuminata in the female genital tract. *Sex Transm Dis*. 1987;14:209–212.
41. Beers MH, Berkow R. *The Merck Manual of Diagnosis and Therapy*. 17th ed. Whitehouse Station, NJ: Merck Research Laboratories; 1999.
42. Lookingbill D, Marks Jr JG. *Principles of Dermatology*. Philadelphia: Saunders; 1986.
43. Traub M. *Dermatological Diagnosis and Natural Therapeutics*. 2nd ed. Portland, OR: NCNM Press; 1985.
44. Tanagho E, McAninch J. *Smith's General Urology*. 14th ed. Norwalk, CT: Appleton & Lange; 1995.
45. Ghirardi M, Nascimbeni R, Mariani PP, et al. Course and natural history of idiopathic ulcerative proctitis in adults. *Ann Ital Chir*. 2002;73:155–158.
46. Kennedy M, Bruninga K, Mutlu EA, et al. Successful and sustained treatment of chronic radiation proctitis with antioxidant vitamins E and C. *Am J Gastroenterol*. 2001;96:1080–1084.
47. Lamson DW, Brignall MS. Antioxidants in cancer therapy: their actions and interactions with oncologic therapies. *Altern Med Rev*. 1999;4:304–329.
48. Lamson D, Brignall M. Antioxidants and cancer therapy II: quick reference guide. *Altern Med Rev*. 2000;5:152–163.
49. Kiely EM, Ajayi NA, Wheeler RA, et al. Diversion procto-colitis: response to treatment with short-chain fatty acids. *J Pediatr Surg*. 2001;36:1514–1517.
50. Head KA, Jurenka JS. Inflammatory bowel disease part I: ulcerative colitis—pathophysiology and conventional and alternative treatment options. *Altern Med Rev*. 2003;8:247–283.
51. Alexander-Williams J. Pruritus ani: what to do, what not to do to control this internal itch. *Postgrad Med*. 1985;77:56–59, 62, 65.
52. Aucoin EJ. Pruritus ani. *Postgrad Med*. 1987;82:76–80.
53. Jacobs DS, ed. *Laboratory Test Handbook with Key Word Index*. Baltimore: Williams & Wilkins; 1990.
54. Boericke W. *Materia Medica with Repertory*. 9th ed. Philadelphia: Boericke & Runyon; 1927.
55. Tilgner S. *Herbal Medicine: From the Heart of the Earth*. Creswell, OR: Wise Acres; 1999.

Psoriasis

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Circumscribed red, thickened plaques overlying silvery-white scale
- Characteristically involves the scalp; the extensor surfaces of the wrists, elbows, knees, buttocks, and ankles; and sites of repeated trauma
- Positive family history in 30% of cases
- Nail involvement results in characteristic pitting. Other nail changes include onycholysis, discoloration, thickening, and dystrophy.
- Possible arthritis

GENERAL CONSIDERATIONS

Psoriasis is a severe, common, multifaceted, chronic, immune-mediated, systemic inflammatory disease resulting from genetic, epigenetic, environmental, and lifestyle factors.[1] It commonly presents as well-demarcated scaly plaques in the skin and scalp (Figs. 214.1 and 214.2). Psoriatic arthritis is a well-known comorbidity of psoriasis. A rapidly increasing body of evidence in diverse populations supports additional associations with gastrointestinal (GI) disease, cardiometabolic disease, kidney disease, malignancy, infections, and mood disorder. Available evidence suggests that their coexistence is not random. The pathogenesis of these comorbidities is emerging and may be related to genetic susceptibility, common risk factors, inflammatory pathways, and cellular mediators.[2] The prevalence of psoriasis in U.S. adults is 3.2% (approximately 7.5 million).[3]

Pathogenesis

The role of environmental triggers such as mechanical trauma, stress, and streptococcal infections is well known in psoriasis. Epidemiological studies demonstrate that there is also a significant genetic basis, and as such, psoriasis is considered a multifactorial disorder caused by the interaction between inherited predisposition and environmental risk factors. In the last decade, genome association studies have identified more than 60 disease susceptibility regions, highlighted by the participation of genes related to the interleukin (IL)-23/Th17 pathway, such as *PSORS1, IL-23R,* and *IL-12 R*.[4,5] The current model of immunopathogenesis derives from evidence for a central role of inflammatory and myeloid dendritic cells (DCs), IL-23 and IL-12, and T-lymphocytes, particularly Th1 and Th17 cells.[6] Targeting DC-derived cytokines such as tumor necrosis factor-alpha (TNF-α), IL-23, and IL-17 produced by Th17 cells is the therapeutic strategy that has resulted in a new generation of biologic drugs that have shown great efficacy in moderate to severe psoriasis. These new drugs have shown superior activity in getting to 90% to 100% clearance. However, they are not curative because when stopped, psoriasis recurs. Moreover, they are very expensive.[7]

Neuropeptides and Skin Microbiome

In addition to immune dysregulation, there are other factors that may be involved in the production of psoriatic inflammation. Notably, there is new evidence of the cutaneous nervous system via neuropeptide release and the skin microbiome interaction with the native and adaptive immune system contributing to the pathogenesis of psoriasis. For example, substance P (SP) functions as a mediator of itch, pain, vasodilation, and inflammation and is involved in propsoriatic activity. Dermal DCs found in close association with cutaneous nerves release SP. The result is that nociceptors via inducing SP modulate the function of DCs and thus the IL-23/IL-17 pathway. Similarly,

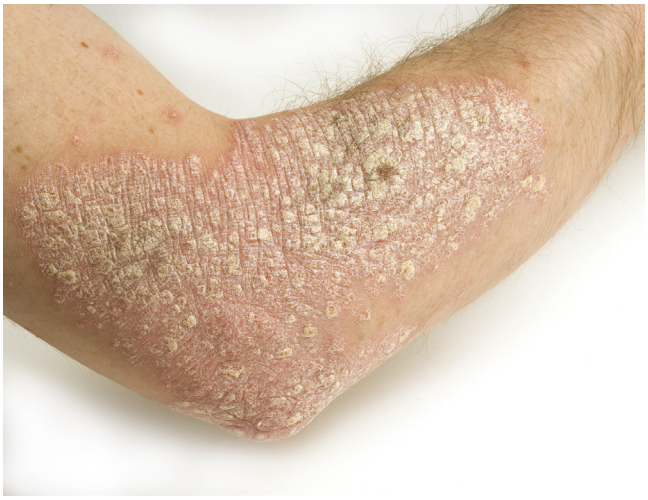


Fig. 214.1 Chronic plague psoriasis on arm. (kenxro/iStock.com.)

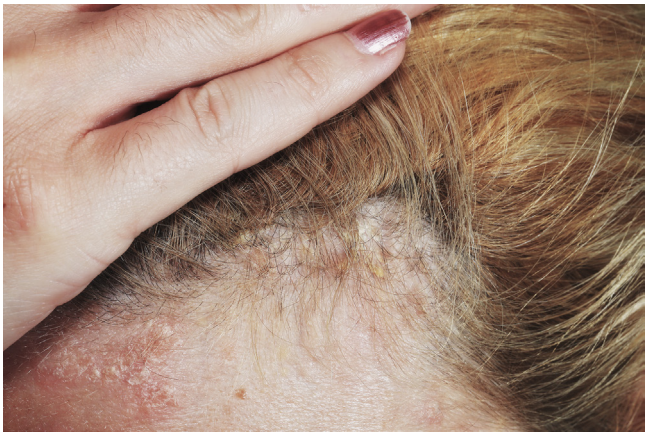


Fig. 214.2 Scalp psoriasis. (petekarici/iStock.com.)

novel antimicrobial peptides produced by the microbiome of the skin may play a role in the pathogenesis of psoriasis. And similarly to the gut microbiome and associated diseases, poor bacterial diversity and strain-level variations could be key determinants of the psoriatic microbiome.[8,9] The commensal microbiota of the skin also play an important role in the maintenance of skin-barrier function, inhibition of the invasion of pathogens, formation of biofilms, production of antibacterial peptides, and prevention of transepidermal water loss.[10]

Beta-hemolytic streptococci and *Malassezia ovalis* have both been found to also play roles in the pathogenesis of psoriasis.[11,12]

Pre- and probiotics exert immunomodulatory effects on skin and can strengthen its barrier function by decreasing the skin bacterial load as well as opposing aggressive commensals.[13] These findings support the idea of a direct link between gut and skin microbiota and imply that a probiotic can modulate the natural microbiota for therapeutic purposes. Research supporting such a claim comes from anecdotal evidence documenting a positive therapeutic response of pustular psoriasis to the administration of the probiotic *Lactobacillus*.[14]

Gastrointestinal Comorbidities

A large proportion of comorbid conditions linked to psoriasis involve the GI tract, including pathologies of the oral cavity, inflammatory bowel disease, celiac disease, increased intestinal permeability, and nonalcoholic fatty liver disease.[15] Common oral psoriatic lesions involve the mucosal surfaces of the tongue, cheeks, and gums. The incidence of geographical tongue in psoriatic patients is estimated at 5.6% to 18.1% and may reflect the severity of psoriasis. Fissured tongue is another nonspecific oral pathology found in 6% to 47.5% of patients psoriasis.

Inflammatory Bowel Disease

The association of inflammatory bowel disease (IBD) with psoriasis can be at least partially explained by a common genetic background. All loci-determining predispositions to both conditions have been found in the 6P212 region encompassing the major histocompatibility complex. Also, the genes encoding IL-23R and IL-12B are implicated in the pathogenesis of both IBD and psoriasis. And both diseases share the same inflammatory pathways in which Th-17 cells and T-regs play a pivotal role.[16] There is an apparent paradoxical iatrogenic link between psoriasis and IBD. It has been reported that some patients with IBD have developed psoriatic lesions during the course of anti-TNF treatment with monoclonal antibodies.

Celiac Disease

Genetic, physiological, epidemiological, and serological links between celiac disease (CD) and psoriasis have been documented.[17] There is a common genetic background between psoriasis and CD. Genome-wide association studies of these two conditions identified genetic susceptibility loci at eight genes regulating innate and adaptive immune responses.[18] Both CD and psoriasis involve an increase in intestinal permeability.[19,20] Malabsorption resulting from CD may predispose to vitamin D deficiency, which is known to contribute to the pathogenesis of psoriasis as well as correlate with psoriasis severity.[21] Th1 and Th17 cells play major roles in the pathogenesis of both psoriasis and CD.[22] Some wheat antigens may trigger an immune response in patients with psoriasis significantly more often than in nonpsoriatic controls, inducing expression of cutaneous lymphocyte antigen (CLA).[23]

Prompted by the discovery of significantly higher mean immunoglobulin (Ig) A antigliadin antibodies (AGAs) in a cohort of 302 patients with psoriasis compared with a reference group, Michaëlsson et al. evaluated the Psoriasis Area Severity Index (PASI) response to a gluten-free diet in patients with positive AGA tests.[24,25] The IgA-AGA-positive group of patients adhering to a gluten-free diet were noted to have a highly significant reduction in PASI compared with the IgA-AGA-negative group. Notably, 60% of IgA-AGA-positive patients experienced a worsening of their psoriasis when they reintroduced their habitual diet. None of the IgA-AGA-negative patients noted any changes in their psoriasis after resuming their habitual diet.[26] The investigators then evaluated histological skin changes in psoriatic and unaffected skin of patients with psoriasis with or without positive IgA and/or IgG AGA on a gluten-free diet. After eating a gluten-free diet for 3 months, patients had a significantly decreased dermal Ki-67+ cell population (an indicator of cell proliferation) in lesional skin. The reduction of the Ki-67+ cell population in unaffected skin was statistically significant in the dermis, whereas in epidermal regions, a gluten-free diet led to a reduction in Ki-67 positivity, although it was not statistically significant. The areas of psoriatic skin expressing Ki-67 showed no regression with the gluten-free diet. Dermal tissue transglutaminase, notably 8× more concentrated in lesional skin compared with uninvolved skin, also decreased by 50% after a gluten-free diet in AGA-positive patients. The gluten-free diet also led to a significant reduction in the lesional CD4+ T-lymphocyte count of AGA-positive patients. No significant changes were noted in the skin of AGA-negative patients after a gluten-free diet. Interestingly, approximately 50% of the AGA-positive patients did not show endoscopic evidence of CD before implementation of the

gluten-free diet, which suggests that such diet may be also beneficial in patients with psoriasis with asymptomatic gluten sensitivity.[27]

Environmental Toxins

Cadmium is a ubiquitous environmental contaminant and is toxic at even low levels. Cadmium also causes the elevation of inflammation markers and influences the immune system. It has been hypothesized that cadmium would be positively associated with the severity of psoriasis. To clarify the relationship between cadmium and psoriasis, 5927 participants, ≥20 years of age, in the National Health and Nutrition Examination Survey (NHANES) 2003–2006 were studied.[28] Psoriasis severity was assessed using self-reported dermatology questionnaires. Cadmium was measured using blood chemistry. Patients with psoriasis had significantly higher blood cadmium (0.67 vs. 0.52 μg/L, $p = 0.006$), and there was a strong linear increase in predicted blood cadmium values with an increase in severity of psoriasis (p for trend = 0.002).

In addition, patients with psoriasis living in a cement factory area had significantly higher mean values of cadmium, chromium, nickel, and lead in scalp hair, blood, and urine samples compared with referents, whereas the concentration of zinc was lower in the scalp hair and blood but higher in the urine samples of patients with psoriasis. It was concluded that the deficiency of zinc in patients with psoriasis may be caused by the toxic element exposures via the cement factory.[29]

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is diagnosed in up to 30% of the population, coinciding with obesity. It is established as a cardiovascular risk factor, is essentially due to insulin resistance, and is thus a common manifestation of metabolic syndrome. Inasmuch as obesity and metabolic syndrome are common in patients with psoriasis, it is not surprising that a high incidence of NAFLD is observed with psoriasis. NAFLD correlates with greater severity of psoriasis, and patients with both conditions are at a higher risk of developing liver fibrosis than nonpsoriatic controls.[30] Some proinflammatory cytokines synthesized by lymphocytes and keratinocytes in psoriatic skin, including IL-6, IL-17, and TNF-α, contribute to systemic insulin resistance.[31] Lifestyle factors, such as cigarette smoking and alcoholism, and iatrogenic factors, such as methotrexate and systemic corticosteroids, should also be considered as fueling this vicious cycle. Preliminary evidence exists that patients with psoriasis are at higher risk of cancers, specifically of the GI tract, but this issue requires further study.[32]

Advanced Glycation End Products

Glycation is a deleterious phenomenon in diabetic complications. One of the underlying features of hyperglycemia is the excessive nonenzymatic glycation of the two major circulating proteins: hemoglobin and albumin. This chemical process consists of a complex cascade of reactions between glucose and its derivatives with proteins, yielding a heterogeneous class of compounds termed *advanced glycation end products* (AGEs).

AGE formation and accumulation in tissue is a physiological process that gradually occurs during aging but accelerates in states of constant hyperglycemia, hyperlipidemia, and oxidative or carbonyl stress, which are common in patients with moderate to severe psoriasis. Exogenous AGEs may come from foods, ultraviolet (UV) irradiation, and cigarette smoking. AGEs activate receptors on epithelial and inflammatory cell surfaces. They amplify inflammation by stimulating the release of cytokines and chemokines and the production of reactive oxygen species and activating metalloproteases. AGE levels are increased in the skin and blood of patients with severe psoriasis independently of associated metabolic disorders. Intensified glycation of proteins in psoriatic skin is thought to play a role in fueling cutaneous inflammation. AGEs released from psoriatic skin may also increase metabolic and cardiovascular risk in patients with severe disease.

The increased keratinocyte proliferation in psoriasis results in increased demand and consumption of glucose, suggesting that AGEs play a role in the pathogenesis of psoriasis. AGEs activate monocytes, macrophages, neutrophils, and endothelial cells that then produce proinflammatory cytokines and additional AGE, stoking the inflammatory response.[33]

Homocysteine and DNA Methylation

Numerous studies have built a foundation for how homocysteine-induced impairment is the common pathway in the pathogenesis of psoriasis and its increased cardiovascular risk and aberrant DNA methylation. However, this area of research remains incomplete, and it is not possible at this time to provide high-quality, evidence-based guidance on how to translate this to clinical practice.[34]

Evidence suggests that the Mediterranean and Paleolithic diets can reduce the risk of cardiometabolic comorbidities in psoriasis.[35,36]

Inflammatory Leukotrienes

Arachidonic acid (AA) metabolites have been of keen interest as possible contributors to the local inflammatory milieu of psoriasis and as promising therapeutic targets. Consumption of fish and omega-3 fatty acids modulates AA metabolism and has been found to moderately improve symptoms of psoriasis.[37]

Depression

Psoriasis does not need to be widespread to be severe. The impact of lesions on the hands, face, genitals, and/or scalp can be very traumatic. Psoriasis can be stigmatizing, and the emotional scar from the condition can still affect quality of life long after the disease is controlled. The main contributors to depression in patients with psoriasis are female gender, beliefs about appearance and its salience to one's self-worth, greater psychological distress, and lower levels of emotional social support. The impact on patients' lives and well-being is often higher than in other skin and chronic diseases. Extensive evidence describes the co-occurrence of psoriasis and depression, suicidal ideation, anxiety, sexual dysfunction, and alcoholism.[38,39]

Considering all of these comorbidities, an interdisciplinary, comprehensive, holistic management plan is necessary.[40]

It is known that psoriasis develops in bone marrow transplant recipients from donors with psoriasis, clears in recipients from donors without psoriasis, and responds positively to various stem cell therapies,[41] and that immunosuppressive drugs are effective in reducing psoriasis.[42,43] Given the genetic predisposition to this disease, what can be done to reduce the phenotypic expression besides resorting to immunosuppressive and biological therapies with their attendant side-effect profiles?

It is interesting that researchers have recognized "unidentified antigens" as ostensibly being the source of the psoriatic cascade. Although a clear relationship of psoriasis with conditions like CD[44] and Crohn's disease[45] has been reported, the intestinal mucosa of patients with psoriasis without GI symptoms has shown microscopic lesions and greater intestinal permeability, even when the mucosa appeared macroscopically normal.[46,47] Given information regarding bowel toxemias, food allergies, and low proteolytic and bile enzymes, as well as suboptimal liver function and food allergies (see the following sections), a plausible naturopathic suggestion here would be to consider that factors leading to poor intestinal function most likely encourage greater intestinal permeability and inflammation, which ultimately allow these antigenic and endotoxic compounds to leave the intestinal confines, travel through the bloodstream, and initiate activated immune cascades in susceptible tissues.

THERAPEUTIC CONSIDERATIONS

Although psoriasis has a significant genetic component, a decrease in the source of blood-borne antigenic immune activators can be substantially achieved through natural medicine intervention. Several controllable factors appear to cause or contribute to etiological bases and pathogenesis of psoriasis, as follows:

- Incomplete protein digestion
- Bowel toxemia
- Impaired liver function
- Bile deficiencies
- Alcohol consumption
- Excessive consumption of animal fats
- Nutritional deficiencies
- Stress

Gastrointestinal Function

Incomplete Protein Digestion

Incomplete protein digestion or poor intestinal absorption of protein breakdown products can result in elevations of amino acids and polypeptides in the bowel. These are metabolized by bowel bacteria into several toxic compounds. The toxic metabolites of the amino acids arginine and ornithine are known as polyamines (e.g., putrescine, spermidine, and cadaverine) and have been shown to be higher in individuals with psoriasis. Polyamines inhibit the formation of cyclic adenosine monophosphate and therefore contribute to the excessive rate of cell proliferation (Fig. 214.3).[48–50] Lowered skin and urinary levels of polyamines are associated with clinical improvement in psoriasis.[48]

A number of natural compounds can inhibit the formation of polyamines and may be of benefit in the treatment of psoriasis. For example, vitamin A and the alkaloids of *Hydrastis canadensis* (goldenseal) such as berberine inhibit bacterial decarboxylase, the enzyme that converts amino acids into polyamines.[51,52] However, the best way to prevent the excessive formation of polyamines is to evaluate digestive function with the aid of Heidelberg Gastric Analysis and/or a comprehensive digestive stool analysis and then take the action necessary (e.g., hydrochloric acid supplementation) to ensure complete protein digestion and absorption (these assessment methods can be found in Chapters 17 and 28).

Bowel Toxemia

A number of gut-derived toxins are implicated in the development of psoriasis, including endotoxins (cell-wall components of gram-negative bacteria), streptococcal products, *Candida albicans,* yeast compounds, and IgE and IgA immune complexes.[53-55] Endotoxins have been found in high levels in the blood of patients with psoriasis,[56] and these compounds lead to increases in cyclic guanosine monophosphate (GMP) levels within skin cells, thereby increasing the rate of keratinocyte proliferation dramatically.

A diet low in dietary fiber is associated with increased levels of gut-derived toxins.[53] Dietary fiber is critical to maintaining a healthy colon. Many fiber components bind bowel toxins and promote their excretion in the feces. It is therefore essential that the diet of an individual with psoriasis be rich in beans, fruits, and vegetables. Natural compounds that bind endotoxins and promote their excretion may also be used. For example, an aqueous extract of the herb *Smilax sarsaparilla* was found in a 1942 study to be effective in psoriasis, particularly the more chronic, large-plaque–forming varient.[57] In this controlled study of 92 patients, *S. sarsaparilla* greatly improved the psoriasis in 62% of the patients and resulted in complete clearance in another 18% (i.e., 80% of the subjects experienced significant benefits). This benefit is apparently due to *S. sarsaparilla*'s components' binding to and promoting the excretion of bacterial endotoxins.

Because clinical severity and therapeutic response have been shown to correlate well with the level of circulating endotoxins, control of gut-derived toxins is important in the treatment of psoriasis. An effort should be made to promote proper binding and elimination of these compounds through support of their excretion in the feces as well as proper handling of absorbed endotoxins by the liver.

Liver Function

The correction of abnormal liver function may be of benefit in the treatment of psoriasis.[58] The connection between the liver and psoriasis relates to one of the liver's basic tasks—filtering and detoxifying the blood returning through the portal circulation from the bowels. Structurally, the hepatic architecture may already be altered in patients with psoriasis.[59] As mentioned previously, psoriasis has been linked to the presence of several microbial by-products in the blood. If hepatic function is compromised by excessive levels of these toxins from the bowel or if there is a decrease in the liver's detoxification ability, the systemic toxin level rises, and the psoriasis worsens.

Alcohol consumption is known to significantly worsen psoriasis.[60] Alcohol has this effect because it both increases the absorption of toxins from the gut (by damaging the gut mucosa) and impairs liver function. Alcohol intake must be restricted in individuals with psoriasis.

Silymarin, the flavonoid component of *Silybum marianum,* has been reported to be of value in the treatment of psoriasis.[58] Presumably this is a result of its ability to improve liver function, inhibit inflammation, and reduce excessive cellular proliferation.[61,62]

Bile Deficiencies

In the patient with psoriasis, endotoxins are able to translocate from the intestine into the bloodstream.[56] Bile acids normally present in

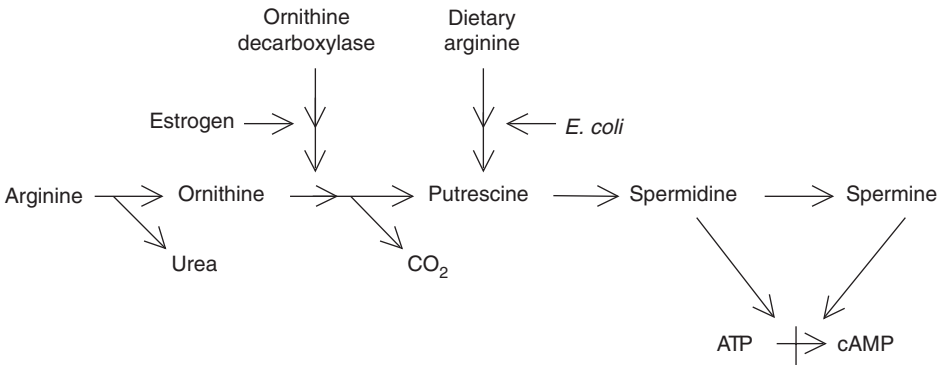


Fig. 214.3 Amino acids, polyamines, and inhibition of adenylate cyclase.

the intestines act to detoxify bacterial endotoxins. In the absence of sufficient amounts of bile acids, endotoxins translocating into the bloodstream can produce pathological conditions that vary in severity depending on their amount, including the release of inflammatory cytokines known to play a role in psoriasis.

A fascinating Hungarian study of 800 patients with psoriasis was conducted in which 551 were treated with oral bile acid (dehydrocholic acid) supplementation for 1 to 6 weeks or 3 to 8 weeks for acute or chronic cases, respectively. Conventional therapies were administered to 249 patients as a comparison group. Both groups were advised to eat a diet high in vegetables and fruits and were instructed to avoid hot spices, alcohol, raw onion, garlic, and carbonated soft drinks. Of the 551 patients receiving bile acid, 434 (78.8%) became asymptomatic, whereas only 62 (24.9%) of the 249 patients receiving conventional therapies demonstrated clinical recovery during this treatment period. Additionally, the curative effect of bile acid supplementation was more pronounced in the acute form of psoriasis; 95.1% of the patients in this group became asymptomatic. In follow-up assessments 2 years later, 319 of the 551 patients with acute and chronic psoriasis who had been treated with bile acid (57.9%) were asymptomatic, compared with only 15 of the 249 patients (6%) who had received the conventional treatment.[56] The bile acid supplements used in the preceding study were either 2 or 3 dehydrocholate sodium (Suprachol) sugar-coated pills once a day or dehydrocholic acid powder (acidum dehydrocholicum pulvis) at two to three doses of 0.25 g/day.

Because there is a theoretical risk of malignant tumors in patients with sluggish intestinal function given long-term bile acid therapy, the investigators in this study recommended that their subjects not continue ingesting bile acids on a regular basis but to supplement with them only after a fatty meal once the initial treatment period had been completed. Two separate evaluations of bile acid effects on the proliferation of colonic mucosa report conflicting results.[63,64] Two other studies actually found a cancer-protective effect of ursodeoxycholic acid. One was an observational study of 114 patients with primary biliary cirrhosis in whom a reduction in the risk for colon adenoma was discovered. The prevalence of colorectal adenomas was 13% in the treated group versus 24% in the untreated group. Additionally, the colon epithelial cell proliferation index was significantly lower in treated patients than in untreated patients.[65] A second randomized controlled clinical trial of 52 subjects found significant declines in the risk for developing colorectal dysplasia or cancer in patients with ulcerative colitis and primary sclerosing cholangitis.[66] More clinical research is needed to examine the different varieties of bile acids and their efficacy as well as their safety profile. Nevertheless, given the preceding information, it seems reasonable to consider the use of bile acids for the short-term treatment of psoriasis and to monitor the colon before and after treatment in patients at high risk for colon cancer.

Nutrition

Omega-3 Fatty Acids

The manipulation of dietary oils is extremely important in the management of psoriasis because serum levels of free fatty acids are typically abnormal in affected patients.[67] Of particular benefit are the omega-3 fatty acids. Most of the clinical research has utilized fish oils rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Several double-blind clinical studies have demonstrated that supplementing the diet with 10 to 12 g/day of fish oils (providing 1.8 g EPA and 1.2 g DHA) results in significant improvement.[68–70] This amount of EPA and DHA would be equivalent to the amount of EPA in about 150 g of salmon, mackerel, or herring.

The improvement in psoriasis from EPA is partly due to the competition of EPA for arachidonic acid binding sites, producing leukotriene B5 (LTB5), which is only one tenth as potent as the inflammatory mediator LTB4. Levels of LTB4 have been shown to be elevated in psoriatic plaques and to demonstrate chemotactic properties necessary for the infiltration of leukocytes and the proliferation of keratinocytes.[71] In the skin of individuals with psoriasis, the production of inflammatory leukotrienes from arachidonic acid is many times greater than normal.[72] Leukotrienes are potent inflammatory agents and promoters of guanylate cyclase activity.

In the psoriatic epidermis, the cellular contents of free arachidonic acid and 12-hydroxyeicosatetraenoic acid (12-HETE; a product of lipoxygenase metabolism of arachidonic acid) are 250 and 810 times greater, respectively, than in uninvolved epidermal tissue.[72]

Immunomodulatory effects of omega-3 fatty acids include the suppression of lymphocyte proliferation, CD4 cells, antigen presentation, adhesion molecule presentation, Th1 and Th2 responses, and proinflammatory cytokine production (such as IL-1, TNF-α, and platelet-derived growth factor [PDGF]), which prevents vascularization within the psoriatic plaque.[73]

Trauma also induces the release of free arachidonic acid and may account for the common clinical observation of plaques at the sites of repeated trauma. These observations are significant because the increase in 12-HETE stimulates the 5-lipoxygenase pathway, promoting leukotriene formation. This pathway is inhibited by EPA and glutathione peroxidase, suggesting that a selenium deficiency may be a contributory factor (see later section "Diet, Fasting, and Food Allergy Control").

As might be expected, cyclooxygenase inhibitors (e.g., aspirin and most other nonsteroidal anti-inflammatory agents) may exacerbate psoriasis, whereas lipoxygenase inhibitors (e.g., benoxaprofen) may bring improvement.[74] Naturally occurring substances such as quercetin (the ubiquitous plant flavonoid), vitamin E, onion, and garlic are known to inhibit lipoxygenase and therefore may be of benefit. However, it is improbable that selective inhibition of one component or enzyme (e.g., a 5-lipoxygenase inhibitor) would do more than create an imbalance in this closely integrated network of mediators, which may not necessarily be beneficial.[75]

Because arachidonic acid is found only in animal tissues, patients with psoriasis may find it helpful to limit their intake of animal products, particularly animal fats and dairy products.

Diet, Fasting, and Food Allergy Control

An evaluation of 316 patients with psoriasis and 366 control subjects, both groups being in the age range of 16 to 65 years, found that psoriasis was positively associated with body mass index and inversely related to the intake of carrots, tomatoes, fresh fruits, and the index of beta-carotene intake.[76] Research at a Swedish hospital studying the effects of fasting and vegetarian regimens on chronic inflammatory disease found that such diets helped patients with psoriasis.[77] The improvement was probably due to decreased levels of gut-derived toxins and polyamines. Patients have also benefited from gluten-free and elimination diets.[78,79] Several herbs used as seasonings—including turmeric, red pepper, cloves, ginger, cumin, anise, fennel, basil, rosemary, garlic, and pomegranate—can block the activation of nuclear factor-kappa B (NF-κB) of inflammatory cytokines.[80]

Individual Nutrients

Decreased levels of vitamin A and zinc are common in patients with psoriasis.[81–83] Given the critical roles of these nutrients in the health of the skin, supplementation might be warranted even without this association. Retinoids are proven effective in treating psoriasis. There is no evidence that zinc is therapeutic, however.

Chromium supplementation may be indicated to increase the sensitivity of insulin receptors because patients with psoriasis typically have increased serum levels of both insulin and glucose and carry an increased risk for type 2 diabetes mellitus and metabolic syndrome.[84]

Substantial evidence indicates that psoriasis is an independent risk factor for cardiovascular disease.[85] It remains difficult to conclude whether risk factors are caused by psoriasis or share a common pathogenesis. Inflammation, characterized by the presence of proinflammatory cytokines and endothelial activation, is a common theme underlying these conditions. Dyslipidemia, coronary calcification, increased highly sensitive C-reactive protein, decreased folate, and hyperhomocysteinemia are found significantly more often in patients with psoriasis.[86]

The inflammatory processes underlying psoriasis suggest the possibility of omega-3 fatty acid, folate, and vitamin B_{12} deficiencies, which are also found in cardiovascular disease.[87] High homocysteine and decreased folate levels correlate with the psoriasis area and severity index (PASI). The rapid keratinocyte turnover rate in psoriasis may result in folate utilization and subsequent deficiency.[88] The authors of one study conclude, "Dietary supplementation of folic acid, B_6, and B_{12} appears reasonable in psoriasis patients, particularly those with elevated homocysteine, low folate and additional cardiovascular risk factors."[89] In supplementing with folic acid or its active form 5-methyltetrahydrofolate, the recommended dose is 1 to 5 mg/day, and testing for *MTHFR* mutations may help guide which form of the vitamin is most advisable.

Glutathione peroxidase (GP) levels are low in psoriatic patients, possibly because of such factors as alcohol abuse, malnutrition, and the excessive loss of skin due to the hyperproliferative disease. The depressed levels of GP normalize with oral selenium and vitamin E therapy.[90] One investigation found that patients with longer-term psoriasis (3 years or more) demonstrated low plasma selenium status.[91] Another study comparing 113 patients with moderate to severe psoriasis and 104 healthy controls found that male patients between 20 and 49 years of age with psoriasis and women with the disease of longer than 20 years' duration had particularly low selenium concentrations. The lowest whole-blood selenium values were found in the subgroup of male patients with widespread disease of long duration, who also required treatment with methotrexate and retinoids.[92]

It has been established that patients with disseminated psoriasis have significantly decreased serum levels of the biologically active form of vitamin D, 1,25-dihydroxycholecalciferol (calcitriol) compared with age- and gender-matched controls and also compared with patients with moderate psoriasis.[93,94]

Whether this is a contributing factor to psoriasis or a result of the disorder has not been elucidated.

Keratinocytes in the epidermis convert 7-dehydrocholesterol to vitamin D_3 in the presence of ultraviolet B. Sunlight, ultraviolet B (UVB) phototherapy, oral calcitriol, and topical vitamin D and its analogs are an effective therapy for psoriasis owing to vitamin D's antiproliferative and prodifferentiating actions on keratinocytes.[95] Vitamin D supplementation enhances the benefit of phototherapy[96] and also encourages a shift toward type 2 helper T-cell cytokine expression, with an increase in IL-10 and a decrease in IL-8, which may be responsible for the improvements seen in psoriasis.[97] Vitamin D analogs like calcipotriol have also been shown to mediate the expression proinflammatory antimicrobial peptides such as β-defensin and to decrease IL-17A, IL-17F, and IL-8 in lesional psoriatic skin.[98] Vitamin D controls plasmacytoid dendritic cell function,[99] and topical calcitriol has also been shown to reduce the number of dendritic cells in the skin.[100]

Calcitriol binding to vitamin D receptors (VDRs) in the skin modulates the expression of a large number of genes, including cell-cycle regulators, growth factors, and their receptors. Polymorphisms of the VDR gene are associated with psoriasis and may predispose to the development of psoriasis and resistance to calcipotriol therapy while also contributing to liver dysfunction in patients with psoriasis.[101]

Given the importance of vitamin D in psoriasis, cancer, inflammatory diseases, and other conditions, it has been suggested by some investigators that recommendations for sun protection and skin cancer prevention may need to be reevaluated to allow for sufficient vitamin D status. However, studies conducted in Honolulu, Miami, and southern Arizona showed that abundant sun exposure did not necessarily ensure vitamin D adequacy, which points to the need for vitamin D supplementation to achieve optimal blood levels while also protecting the skin from sun damage and skin cancer.[102]

It has been demonstrated that oral vitamin D can be safely taken in daily doses of up to 5000 IU/day, with some experts recommending up to 10,000 IU/day to correct a deficiency.[103]

Fumaric Acid

Over the past three decades, fumaric acid therapy has become increasingly popular in Western Europe for psoriasis. Therapy consists of the oral intake of dimethylfumaric acid (240 mg/day) or monoethylfumaric acid (720 mg/day) and the topical application of 1% to 3% of monoethylfumaric acid. Clinical studies have shown that it is useful in many patients with psoriasis,[104] but side effects such as flushing of the skin, nausea, diarrhea, general malaise, gastric pain, and mild liver and kidney disturbances can occur.[105] We recommend using fumaric acid therapy only after other natural therapies have proved ineffective.

Psychological Aspects

Research concerning the role of stress in psoriatic exacerbations is mixed. One investigation revealed that a large proportion (39%) of patients with psoriasis report the occurrence of a specific stressful event within 1 month before their initial episode. Such patients have a better prognosis.[106] Yet other findings show a limited relationship between stress and vulnerability to psoriasis, whereby correlation seemed to occur mostly in patients with repeated stressors occurring four times or more in 1 year.[107] Stress management can benefit individuals with psoriasis.[108] As judged by two independent dermatologists, subjects who listened to a guided meditation tape while undergoing phototherapy cleared four times as quickly as those who received phototherapy only. Psoriasis status was assessed in three ways: direct inspection by unblinded clinic nurses, direct inspection by physicians blinded to the patient's study condition (tape or no tape), and blinded physician evaluation of photographs of psoriatic lesions. Four sequential indicators of skin status were monitored during the study: a First Response Point, a Turning Point, a Halfway Point, and a Clearing Point. Subjects in the tape groups reached the Halfway Point ($P = 0.013$) and the Clearing Point ($P = 0.033$) significantly more rapidly than those in the no-tape condition for both UVB and psoralen and ultraviolet A (PUVA) treatments. Finally, psychotherapy can be an essential adjunct for individuals with persistent unresolved psychological issues such as anxiety, depression, and the psychosocial stress of this chronic skin disease. A few case histories have been reported that document the successful treatment of psoriasis with hypnosis and biofeedback alone.[109]

Physical Therapeutics

Sunlight, Ultraviolet Light, Climatotherapy, and Balneotherapy

Sunlight (which contains UV light) is extremely beneficial for individuals with psoriasis.[110,111] Outdoor 4-week heliotherapy was shown to promote significant clearance of psoriatic symptoms in 84% of 373 subjects.[112] Studies employing nonprescription commercial tanning beds have shown that a majority of patients find them helpful[113]; they

also objectively facilitate improvements in both psoriasis severity and health-related quality of life.[114] In addition, an open-label retrospective trial studied the combined use of the retinoid acitretin (a vitamin A derivative) along with a 4- to 5-day-per-week tanning regimen; 83% of 23 subjects experienced complete or near-complete recovery.[115]

Specific UV exposure may also be of benefit owing to its induction of vitamin D synthesis in the skin. The standard UV medical treatment of psoriasis typically involves the use of the drug psoralen and ultraviolet A (PUVA therapy; 320–340 nm). UVB (280–320 nm) exposure alone also leads to inhibition of cell proliferation; in certain studies, it has been shown to be as effective as PUVA therapy, with fewer side effects.[115] At the Dead Sea, where 80% to 85% of psoriatic conditions clear in 4 weeks, UVB wavelengths are known to be the dominant light.[116,117] In one investigation of 28 patients, UVB had a clear advantage over PUVA therapy.[145] Conversely, another report comparing UVB with PUVA in 100 patients demonstrated clearance of psoriasis in a significantly greater proportion of patients treated with PUVA (84%) than with UVB (63%), and with significantly fewer treatments.[118] Other studies have found both to be effective, with similar untoward side effects.[119,120] It is clear that more study is needed to clarify both the risks and benefits of these therapies and whether certain presentations of psoriasis may respond best to a specific ultraviolet therapy. As noted previously, ultraviolet light deactivates vitamin D topical agents, Therefore, if they are used in conjunction, the topical agent should be applied only after ultraviolet treatment. Furthermore, any light therapy should be monitored carefully, especially in patients at risk for skin cancers.

There is overwhelming evidence that bathing in the Dead Sea is very effective in the treatment of psoriasis.[121]

Traditional Chinese medical bath therapy, in combination with UV irradiation, has also been shown to be safe and effective in the treatment of psoriasis, as documented in a review of 25 randomized controlled trials.[122] A report of a controlled trial of hydrogen-water bathing also showed significant and rapid improvement itching and in PASI score.[123]

The induction of localized elevation of temperature (42°C–45°C) to the affected area by ultrasound and heating pads has been shown to be an effective treatment of psoriasis.[124,125] Italian researchers have conducted small clinical trials using a hypotonic sulfate water (Leopoldine water) in a balneotherapeutic manner. These treatments have yielded favorable immunohistological profiles of the affected tissues, with significant decreases in the numbers of T lymphocytes, Langerhans cells, and markers of keratinocyte inflammatory expression.[126]

Stem Cell Therapy

The effect of stem cells in the epidermis and on T cells is recognized as a primary cause of immune dysregulation in psoriasis. Initial attempts at using stem cells in treatment are being explored.[127] Umbilical cord–Wharton's jelly stem cells yielded complete remission of psoriasis in two cases,[128,129] confirming that mesenchymal stem cells inhibit the activity of the Th17 cell and reduce expression of IL-17.[130]

Thirty patients in Ecuador with severe psoriasis were treated by a single intravenous autologous transplant of hematopoietic cells and compared with 19 patients who received PUVA therapy. PASI 75 reached a statistically significant effect in the stem-cell group, but no significant difference was observed in comparison to the PUVA group.[131]

Topical Treatments

A number of natural proprietary formulas as well as over-the-counter preparations can be used to provide symptomatic relief in mild to moderate psoriasis. The best evidence of effectiveness is found with *Indigo naturalis,* used to treat psoriasis and other inflammatory dermatoses for thousands of years in China. *I. naturalis,* a Chinese herb known as Qing Dai, is a dried pigment prepared from several plant species, including *Baphicacanthus cusia.* The active components include indirubin and tryptanthrin. Indirubin inhibits cyclin-dependent kinase and signal transducer and activator of transcription-3 (STAT3) activities [26] and keratinocyte proliferation in vitro.[132]

Tryptanthrin inhibits vascular endothelial growth factor (VEGF)–induced angiogenesis, resulting in cell-cycle arrest and dose-dependent expressions of cyclin A, cyclin B, and cyclin-dependent kinase (CDK), at both mRNA and protein levels.[133]

A randomized, double-blind, placebo-controlled clinical study was conducted using *I. naturalis* as topical monotherapy to treat moderate plaque psoriasis in 24 Chinese patients; 16 were treated with *I. naturalis* ointment and 8 with matched placebo twice daily for 8 weeks. At week 8, significant improvements in PASI scores from baseline were observed in *I. naturalis*–treated patients (56.3% had 75% improvement [PASI 75] response) compared with placebo (0.0%). A gene expression signature of moderate psoriasis was established from baseline skin biopsies and up-regulation of the IL-17 pathway was seen as a key component. After the treatment, repeat biopsy specimens showed most signature genes returning toward normal, including down-regulation of the IL-17 pathway. Using an in vitro keratinocyte assay, the IL-17-inhibitory effect was observed for tryptanthrin.[134]

In a small trial, 14 patients applied indigo ointment versus vehicle alone on contralateral lesions daily for 8 weeks. Significant reduction was observed in clinical scores of induration, scaling, erythema, and clearing. Analysis of biopsies taken at the end of treatment showed marked improvement of skin histology. Expression of proliferation marker Ki-67 and inflammatory marker CD3 were decreased, but the differentiation marker filaggrin was increased in the epidermis.[135]

Another preliminary clinical trial conducted by Dermatest (Germany) evaluated a proprietary formula containing *Indigofera tinctoria, Rheum palmatum, Portulaca oleracea, Phellodendron amurense, Angelica sinensis, Sapindus mukorossi,* and *Dipotassium glycyrrhizinate.* After 6 weeks of treatment, there was a significant reduction of 58% in PASI, skin thickness (55%), erythema (44%), scaling (73%), and pruritus (82%) (unpublished data). See Figs. 214.4, 214.5, and 214.6.

Scalp Psoriasis

An indigo-containing scalp lotion and an herbal shampoo containing *Sapindus mukorossi* fruit extract with zinc pyrithione and salicylic acid was studied for scalp psoriasis. The scalp lotion also included *Rheum palmatum, Portulaca oleracea, Phellodendron amurense, Angelica polymorpha sinensis,* and *Sapindus mukorossi.* A reduction of 92% in the severity of lesions (Psoriasis Scalp Severity Index [PSSI]) was observed in 67% after 6 weeks of treatment. Improvement of 50% in psoriasis severity (PSSI 50) was observed in 100% of subjects (Prof. A. Lukyanov, Belarus, unpublished data). See Fig. 214.7.

Aloe vera

A double-blind, placebo-controlled study found that topical application of an *Aloe vera* extract in a hydrophilic cream was highly effective in psoriasis vulgaris. Sixty patients with slight to moderate chronic plaque-type psoriasis and PASI scores between 4.8 and 16.7 (mean 9.3) whose mean duration of disease averaged 8.5 years (range 1–21 years) applied either the aloe or a placebo gel three times a day. The treatment was well tolerated by all the patients, with no adverse drug-related symptoms and no dropouts. By the end of the study (4–12 months of treatment), the *A. vera* extract cream had cured 25 of 30 patients

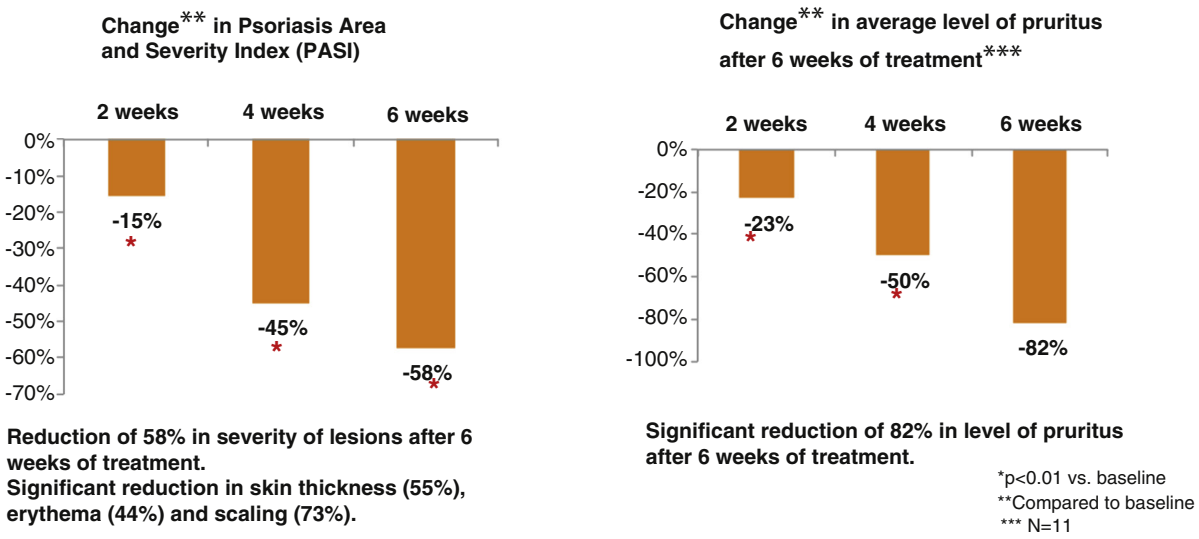


Fig. 214.4 Change in PASI and pruritus from indigo-based proprietary formula.

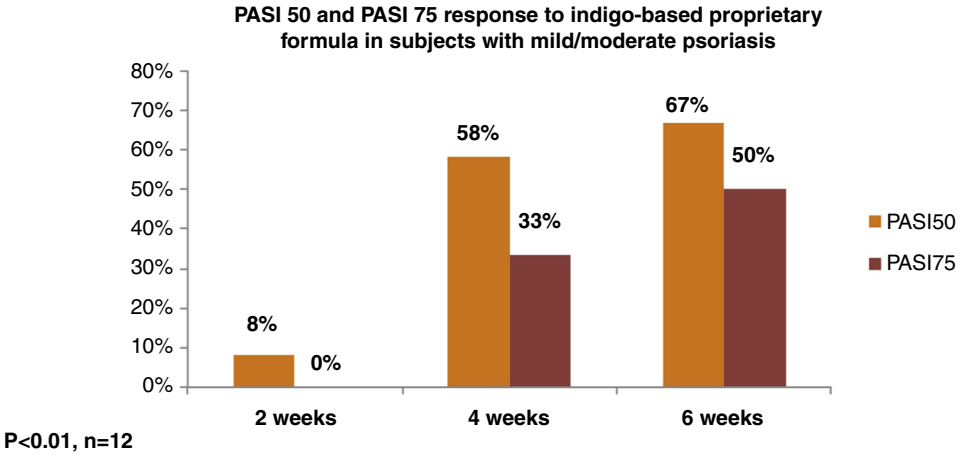


Fig. 214.5 PASI 50 and PASI 75 response to indigo-based proprietary formula in subjects with mild/moderate psoriasis.

(83.3%) compared with the placebo cure rate of only 2 of 30 (6.6%), resulting in significant clearing of the psoriatic plaques (328 of 396 [82.8%] vs. placebo 28 of 366 [7.7%]) and a decrease in PASI score to a mean of 2.2.[136] A systematic review of this trial plus three others revealed methodological weaknesses that made it unable to reach any definitive conclusions on effectiveness.[137]

Curcumin

Curcumin gel yielded 90% resolution of plaques in 50% of patients within 2 to 6 weeks; the remainder of the study subjects showed 50% to 85% improvement.[138] Curcumin was found to be twice as effective as calcipotriene cream (which generally takes 3 months to exert its full effect). Curcumin acts as a selective phosphorylase kinase inhibitor, thereby reducing inflammation through the inhibition of NFκB.

In a randomized, intraindividual, right–left comparative, placebo-controlled, double-blind trial, 34 patients with mild to moderate psoriasis were treated with a topical turmeric microemulgel. Clinical and quality-of-life parameters in treated lesions significantly improved compared with the untreated lesions.[139]

Mahonia aquifolium

Several open and placebo-controlled trials have supported the effectiveness of *Mahonia aquifolium* topical cream in the treatment of psoriasis. On the basis of physician and patient assessments, 71% to 81% of patients improved in these trials.[140] A randomized, double-blind, placebo-controlled trial of 200 patients also demonstrated statistically significant improvement when this agent was applied twice daily for 12 weeks.[141]

Topical Vitamin D

Topical corticosteroids are the most common treatment for psoriasis; however, their long-term use is associated with a potential risk for side effects. Topical vitamin D modulators have been developed as an option for use in place of or in addition to, topical corticosteroids. Topically, vitamin D inhibits keratinocyte proliferation, normalizes differentiation, and modulates immune cell activity with minimal effect on serum calcium.[142] Calcipotriene is the most widely used topical vitamin D. Although evidence suggests that it is approximately as effective as low- to medium-potency corticosteroids in the long term (response is not obtained as quickly as with corticosteroids), it is

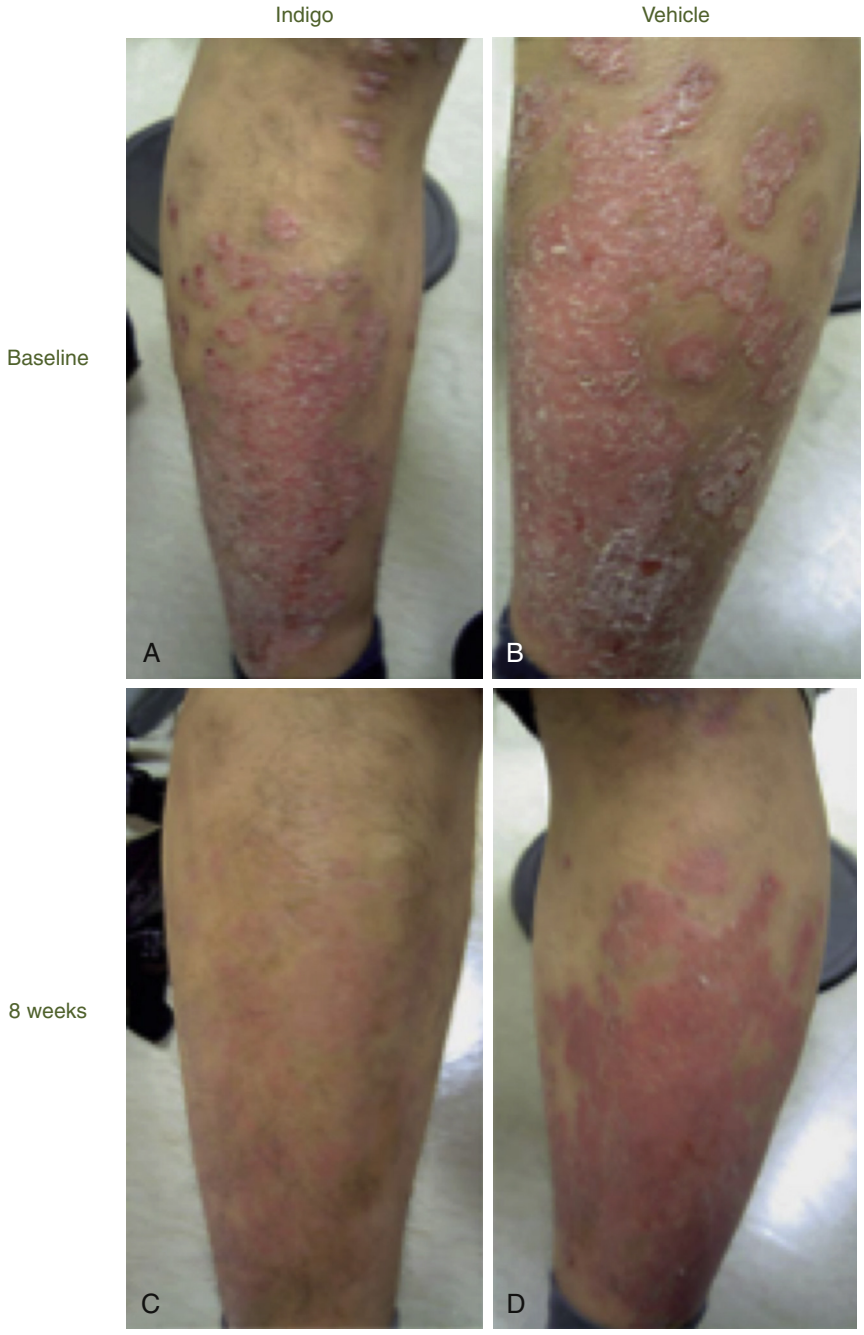


Fig. 214.6 Improvement from baseline after 8 weeks of indigo-based proprietary formula.

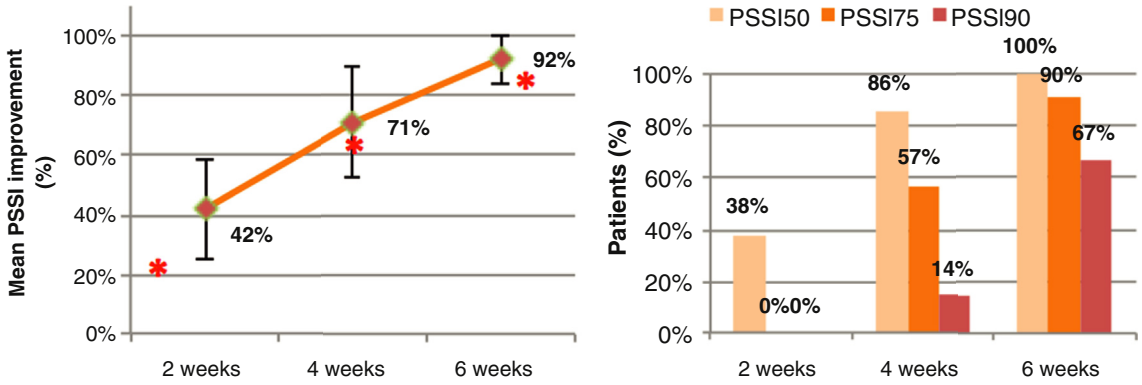


Fig. 214.7 Improvement of Psoriasis Scalp Severity Index and pruritus at 2, 4, and 6 weeks of indigo-based proprietary formula.

associated with cutaneous irritation, especially when used on sensitive skin. Calcitriol ointment contains the naturally occurring active form of vitamin D_3 and is associated with a low rate of cutaneous and systemic adverse effects.

By inhibiting proinflammatory cytokines such as IL-12, IL-23, and TNF-α, topical nicotinamide has been shown to enhance the efficacy of calcipotriene therapy when used in combination.[143]

Emollients

The scaliness of psoriasis benefits from the use of emollients. Intercellular lipids such as ceramides play an important role in regulating the homeostasis of the skin water barrier and the skin's water-holding capacity, and it has been shown that ceramides are decreased in the psoriatic epidermis. Newer ceramide-containing emollients have shown benefit in psoriasis and may improve skin barrier function and decrease water loss.[144]

THERAPEUTIC APPROACH

Despite the complexity of this disease, the therapeutic approach is fairly straightforward: decrease bowel toxemia, rebalance fatty acid levels and inflammatory processes systemically and in the skin, and use the listed therapeutic regimen to further balance the abnormal cell proliferation.

Diet

- Limit sugar, meat, animal fats, and alcohol.
- Increase intake of dietary fiber and cold-water fish, and, if indicated, bring weight to normal levels with a hypocaloric diet.
- Follow Mediterranean or Paleolithic diet if patient has cardiometabolic comorbidities.
- Eliminate sources of gluten if patient is gluten-sensitive.
- Identify and address any food allergies.

Supplements

- High-potency multivitamin/multimineral formula
- Fish oils: 3000 mg EPA+DHA
- Vitamin D: 5000 to 10,000 IU/day based on serum 25(OH)D level
- Consider vitamin E 400 (mixed tocopherols) IU/day, chromium 400 mcg/day, selenium 200 mcg/day, folate 1 mg/day.
- Consider digestive enzymes and/or bile acids with meals.
- Water-soluble fiber (psyllium, pectin, guar gum, etc.): 5 g at bedtime

Botanical Medicines

Consider the following if indicated by impaired digestion or liver function:

H. canadensis (goldenseal)
- The dose should be based on berberine content; because there is a wide range of quality in goldenseal preparations, standardized extracts are preferred three times a day.
- Dried root or as infusion (tea): 2 to 4 g three times a day
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp) three times a day
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content): 250 to 500 mg three times a day

S. sarsaparilla
- Dried root or by decoction: 1 to 4 g three times a day
- Liquid extract (1:1): 8 to 16 mL (2–4 tsp) three times a day
- Solid extract (4:1): 250 to 500 mg three times a day

S. marianum (milk thistle):
- Silymarin: 70 to 210 mg three times a day

Psychological Considerations

- Evaluate stress levels and use stress reduction techniques as appropriate.

Physical Medicines

- Sunbathing—taking precautions not to become sunburned.
- UVB: 295 to 305 nm, 2 mW/cm^2, 3 minutes, three times a week

Topical Treatment

- *Indigo*-based cream, *A. vera,* curcumin, *M. aquifolium,* or vitamin D. Apply to affected areas of the skin two to three times/day.
- Ceramide-containing emollient two to three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Albanesi C, Madonna S, Gisondi P, Girolomoni G. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front Immunol*. 2018;9:1549.
2. Takeshita J, Grewal S, Langan SM, et al. Psoriasis and comorbid diseases part i. Epidemiology. *J Am Acad Dermatol*. 2017;76(3):377–390.
3. Rachakonda TD, Schupp CW, Armstrong AW. Psoriasis prevalence among adults in the United States. *J Am Acad Dermatol*. 2014;70(3):512–516.
4. Capon F. The genetic basis of psoriasis. *Int J Mol Sci.* 2017;18:2526.
5. Alwan W, Nestle FO. Pathogenesis and treatment of psoriasis: exploiting pathophysiological pathways for precision medicine. *Clin Exp Rheumatol.* 2015;33(5 suppl93):S2–6.
6. Alexander H, Nestle FO. Pathogenesis and immunotherapy in cutaneous psoriasis: what can rheumatologists learn? *Curr Opin Rheumatol*. 2017;29(1):71–78.
7. Blauvelt A, Chiricozzi A. The immunologic role of il-17 in psoriasis and psoriatic arthritis pathogenesis. *Clin Rev Allergy Immunol*. 2018. https://doi.org/10.1007/s12016-018-8702-3.
8. Lugar TA, Loser K. Novel insights into the pathogenesis of psoriasis. *Clin Immunol*. 2018;186:43–45.
9. Tett A, Pasolli E, Farina S, et al. Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. *Biofilms Microbiomes*. 2017;3:14.
10. Wang WM, Jin HZ. Skin microbiome: an actor in the pathogenesis of psoriasis. *Chin Med J*. 2018;131:95–98.
11. McFadden J, Valdimarsson H, Fry L. Cross-reactivity between streptococcal M surface antigen and human skin. *Brit J Dermatol*. 1991;125:443–447.
12. Takemoto A, Cho O, Morohoshi Y, et al. Molecular characterization of the skin fungal microbiome in patients with psoriasis. *J Dermatol*. 2015;42:166–170.
13. Nole KLB, Yim E, Keri JE. Probiotics and prebiotics in dermatology. *J Am Acad Dermatol*. 2014;71:814–821.
14. Vijayashankar M, Raghunath N. Pustular psoriasis responding to probiotics—A new insight. *Our Dermatol Online*. 2013;4:326–329.
15. Pietrzak D, Pietrzak A, Krasowska D, et al. Digestive system in psoriasis: an update. *Arch Dermatol Res*. 2017;309:679–693.
16. Skroza N, Skroza N, Proietti I, Pampena R. Correlations between psoriasis and inflammatory bowel diseases. *Biomed Res Int*. 2013:983902.
17. Ojetti V, Aguilar Sanchez J, Guerriero C, et al. High prevalence of celiac disease in psoriasis. *Am J Gastroenterol*. 2003;98(11):2574–2575.
18. Tsoi LC, Spain SL, Knight J, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat Genet*. 2012;44(12):1341–1348.
19. Abenavoli L, Proietti I, Zaccone V, et al. Celiac disease: from gluten to skin. *Expert Rev Clin Immunol*. 2009;5(6):789–800.
20. Humbert P, Bidet A, Treffel P, et al. Intestinal permeability in patients with psoriasis. *J Dermatol Sci*. 1991;2(4):324–326.
21. Lee YH, Song GG. Association between circulating 25-hydroxyvitamin D levels and psoiriasis, and correlation with disease severity: a meta-analysisi. *Clinc exp Dermatol*. 2018.
22. Kupfer SS, Jabri B. Pathophysiology of celiac disease. *Gastrointest Endosc Clin N Am*. 2012;22(4):639–660.
23. Skavland J, Shewry PR, Marsh J, et al. In vitro screening for putative psoriasis-specific antigens among wheat proteins and peptides. *Br J Dermatol*. 2012;166(1):67–73.
24. Michaelsson G, Gerden B, Ottosson M, et al. Patients with psoriasis often have increased serum levels of IgA antibodies to gliadin. *Brit J Dermatol*. 1993;129:667–673.
25. Michaëlsson G, Gerden B, Hagforsen E, et al. Psoriasis patients with antibodies to gliadin can be improved by a gluten-free diet. *Brit J Dermatol*. 2000;142:44–51.
26. Michaëlsson G, Åhs S, Hammarström I, et al. Gluten-free diet in psoriasis patients with antibodies to gliadin results in decreased expression of tissue transglutaminase and fewer Ki67+ cells in the dermis. *Acta Dermato Venereol*. 2003;83:425–429.
27. Chalmers RJ, Kirby B. Gluten and psoriasis. *Br J Dermatol*. 2000;142(1):5–7.
28. Liaw FY, Chen WL, Kao TW, Chang YW, Huang CF. Exploring the link between cadmium and psoriasis in a nationally representative sample. *Scientific Reports*. 2017;7(1):1723. PubMed PMID: 28496169.
29. Afridi HI, Kazi TG, Kazi N, et al. Evaluation of cadmium, chromium, nickel, and zinc in biological samples of psoriasis patients living in Pakistani cement factory area. *Biol Trace Elem Res*. 2011;142(3):284–301. PubMed PMID: 20665125.
30. Miele L, Vallone S, Cefalo C, et al. Prevalence, characteristics and severity of non-alcoholic fatty liver disease in patients with chronic plaque psoriasis. *J Hepatol*. 2009;51(4):778–786.
31. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest*. 2006;116(7):1793–1801.
32. Pouplard C, Brenaut E, Horreau C, et al. Risk of cancer in psoriasis: a systematic review and meta-analysis of epidemiological studies. *J Eur Acad Dermatol Venereol*. 2013;3:36–46.
33. Papagrigoraki A, Maurelli M, del Giglio M, et al. Advanced glycation end products in the pathogenesis of psoriasis. *Int J Mol Sci*. 2017;18.
34. Wang WM, Jin HZ. Homocysteine: a potential common route for cardiovascular risk and DNA methylation in psoriasis. *Chin Med J*. 2017;130:1980–1986.
35. Esposito K, Maiorino MI, Bellastella G, et al. Mediterranean diet for type 2 diabetes: cardiometabolic benefits. *Endocrine*. 2017;56(1):27–32.
36. Frassetto LA, Schloetter M, Mietus-Synder M, et al. Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. *Eur J Clin Nutr*. 2009;63(8):947–955.
37. Yan D, Afifi L, Jeon C, et al. The metabolomics of psoriatic disease. *Psoriasis: targets and therapy*. 2017;7:1–15.
38. Wojtyna E, et al. Gender, body image and social support: biopsychosocial determinants of depression among patients with psoriasis. *Acta Derm Venereol*. 2016.
39. Taketshita J, Grewal BS, Langan SM, et al. Psoriasis and comorbid diseases part i. Epidemiology. *Am Acad Dermatol*. 2017;76(3):377–390.
40. Boehncke WH, Boehncke S. More than skin-deep: the many dimensions of the psoriatic disease. *Swiss Med Wkly*. 2014;144: wt13968.
41. Owczarczyk-Saczonek A, Krajewska-Wlodarczyk M, Kruszewska A, et al. Stem cells as potential candidates for psoriasis cell-replacement therapy. *Int J Mol Sci*. 2017;18:2182.
42. Wahie S, Alexandroff A, Reynolds NJ, et al. Psoriasis occurring after myeloablative therapy and autologous stem cell transplantation. *Br J Dermatol*. 2006;154:194–195.
43. Eedy DJ, Burrows D, Bridges JM, et al. Clearance of severe psoriasis after allogenic bone marrow transplantation. *BMJ*. 1990;300:908.
44. Ojetti V, Aguilar Sanchez J, Guerriero C, et al. High prevalence of celiac disease in psoriasis. *Am J Gastroenterol*. 2003;98:2574–2575.
45. Najarian DJ, Gottlieb AB. Connections between psoriasis and Crohn's disease. *J Am Acad Dermatol*. 2003;48:805–821.
46. Scarpa R, Manguso F, D'Arienzo A, et al. Microscopic inflammatory changes in colon of patients with both active psoriasis and psoriatic arthritis without bowel symptoms. *J Rheumatol*. 2000;27:1241–1246.
47. Humbert P, Bidet A, Treffel P, et al. Intestinal permeability in patients with psoriasis. *J Dermatol Sci*. 1991;2:324–326.
48. Proctor M, Wilkenson D, Orenberg E, et al. Lowered cutaneous and urinary levels of polyamines with clinical improvement in treated psoriasis. *Arch Dermatol*. 1979;115:945–949.
49. Voorhees JJ. Polyamines and psoriasis. *Arch Dermatol*. 1979;115:943–944.
50. McDonald CJ. Polyamines in psoriasis. *J Invest Dermatol*. 1983;81:385–387.
51. Haddox M, Scott KF, Russel D. Retinol inhibition of ornithine decarboxylase induction and G1 progression in Chinese hamster ovary cells. *Cancer Res*. 1979;39:4930–4938.
52. Kuwano S, Yamauchi K. Effect of berberine on tyrosine decarboxylase activity of *Streptococcus faecalis*. *Chem Pharm Bull*. 1960;8:491–496.
53. Rosenberg E, Belew P. Microbial factors in psoriasis. *Arch Dermatol*. 1982;118:1434–1444.

54. Rao M, Field M. Enterotoxins and ion transport. *Biochem Soc Trans*. 1984;12:177–180.
55. Juhlin L, Vahlquist C. The influence of treatment and fibrin microclot generation in psoriasis. *Br J Dermatol*. 1983;108:33–37.
56. Gyurcsovics K, Bertók L. Pathophysiology of psoriasis: coping endotoxins with bile acid therapy. *Pathophysiology*. 2003;10:57–61.
57. Thurmon FM. The treatment of psoriasis with sarsaparilla compound. *N Engl J Med*. 1942;337:128–133.
58. Weber G, Galle K. The liver, a therapeutic target in dermatoses. *Med Welt*. 1983;34:108–111.
59. Pietrzak A, Lecewicz-Torun B, Kadziela-Wypyska G. Changes in the digestive system in patients suffering from psoriasis. *Ann Univ Mariae Curie Sk lodowska [Med]*. 1998;53:187–194.
60. Monk BE, Neill SM. Alcohol consumption and psoriasis. *Dermatologica*. 1986;173:57–60.
61. Hikino H, Kiso Y, Wagner H, et al. Antihepatotoxic actions of flavonolignans from *Silybum marianum* fruits. *Planta Medica*. 1984;50:248–250.
62. Adzet T. Polyphenolic compounds with biological and pharmacological activity. *Herbs Spices Med Plant*. 1986;1:167–184.
63. Ochsenkuhn T, Marsteller I, Hay U, et al. Does ursodeoxycholic acid change the proliferation of the colorectal mucosa? A randomized, placebo-controlled study. *Digestion*. 2003;68:209–216.
64. Ochsenkuhn T, Bayerdorffer E, Meining A, et al. Colonic mucosal proliferation is related to serum deoxycholic acid levels. *Cancer*. 1999;85:1664–1669.
65. Serfaty L, De Leusse A, Rosmorduc O, et al. Ursodeoxycholic acid therapy and the risk of colorectal adenoma in patients with primary biliary cirrhosis: an observational study. *Hepatology*. 2003;38:203–209.
66. Pardi DS, Loftus Jr EV, Kremers WK, et al. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. *Gastroenterology*. 2003;124:889–893.
67. Zlatkov NB, Ticholov JJ, Dourmishev AL. Free fatty acids in the blood serum of psoriatics. *Acta Derm Venereol*. 1984;64:22–25.
68. Bittiner SB, Tucker WF, Cartwright I, et al. A double-blind, randomized, placebo-controlled trial of fish oil in psoriasis. *Lancet*. 1988;1:378–380.
69. Grimmunger F, Mayser P, Papavassilis C. A double-blind, randomized, placebo-controlled n-3 fatty acid based lipid infusion in acute, extended guttate psoriasis. Rapid improvement of clinical manifestations and changes in neutrophil leukotriene profile. *Clin Invest*. 1993;71:634–643.
70. Maurice PD, Allen BR, Barkley AS, et al. The effects of dietary supplementation with fish oil in patients with psoriasis. *Br J Dermatol*. 1987;1117:599–606.
71. Mayser P, Grimm H, Grimminger F. n-3 fatty acids in psoriasis. *Br J Nutr*. 2002;87:S77–S82.
72. Voorhees JJ. Leukotrienes and other lipoxygenase products in the pathogenesis and therapy of psoriasis and other dermatoses. *Arch Dermatol*. 1983;119:541–547.
73. Calder PC. n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr*. 2006;83:S1505–S1519.
74. Kragballe K, Herlin MD. Benoxaphren improves psoriasis: a double-blind study. *Arch Dermatol*. 1983;119:548–552.
75. Greaves MW. Camp RD. Prostaglandins, leukotrienes, phospholipase, platelet activating factor, and cytokines: an integrated approach to inflammation of the skin. *Arch Dermatol Res*. 1988;280(suppl):S33–S41.
76. Naldi L, Parazzini F, Peli L, et al. Dietary factors and the risk of psoriasis: results of an Italian case-controlled study. *Br J Dermatol*. 1996;134:101–106.
77. Lithell H, Bruce A, Gustafsson IB, et al. A fasting and vegetarian diet treatment trial on chronic inflammatory disorders. *Acta Derm Venerool*. 1983;63:397–403.
78. Bazex A. Diet without gluten and psoriasis. *Ann Derm Symp*. 1976;103:648.
79. Douglas JM. Psoriasis and diet. *Calif Med*. 1980;133:450.
80. Aggarwal BB, Shishodia S. Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Ann NY Acad Sci*. 2004;1030:434–441.
81. Majewski S, Janik P, Langer A, et al. Decreased levels of vitamin A in serum of patients with psoriasis. *Arch Dermatol Res*. 1989;280:499–501.
82. Hinks LJ, Young S, Clayton B. Trace element status in eczema and psoriasis. *Clin Exp Dermatol*. 1987;12:93–97.
83. Donadini A, Dazzaglia A, Desirello G. Plasma levels of Zn, Cu and Ni in healthy controls and in psoriatic patients: possible correlations with vitamins. *Acta Vitamin Enzymol*. 1980;1:9–16.
84. Fratino P, Pelfini C, Jucci A, et al. Glucose and insulin in psoriasis: the role of obesity and genetic history. *Panminerva Medica*. 1979;21:167–172.
85. Tobin AM, Veale DJ, Fitzgerald O, et al. Cardiovascular disease and risk factors in patients with psoriasis and psoriatic arthritis. *J Rheumatol*. 2010;37(7):1386–1394.
86. Rocha-Pereira P, Santos-Silva A, Rebelo I, et al. Dyslipidemia and oxidative stress in mild and in severe psoriasis as a risk for cardiovascular disease. *Clin Chim Acta*. 2001;303:33–39.
87. Ludwig RJ, Herzog C, Rostock A, et al. Psoriasis: a possible risk factor for development of coronary artery calcification. *Br J Dermatol*. 2007;156:271–276.
88. Vanizor Kural B, Orem A, Cimsit G, et al. Plasma homocysteine and its relationship with atherothrombotic markers in psoriatic patients. *Clin Chim Acta*. 2003;332:23–30.
89. Malerba M, Gisondi P, Radaeli A, et al. Plasma homocysteine and folate levels in patients with chronic plaque psoriasis. *Br J Dermatol*. 2006;155:1165–1169.
90. Juhlin L, Edqvist LE, Ekman LG, et al. Blood glutathione-peroxidase levels in skin diseases: effect of selenium and vitamin E treatment. *Acta Derm Venereol*. 1982;62:211–214.
91. Serwin AB, Wasowicz W, Gromadzinska J, et al. Selenium status in psoriasis and its relations to the duration and severity of the disease. *Nutrition*. 2003;19:301–304.
92. Michaelsson G, Berne B, Calmark B, et al. Selenium in whole blood and plasma is decreased in patients with moderate and severe psoriasis. *Acta Derm Venereol*. 1989;69:29–34.
93. Staberg B, Oxholm A, Klemp P. Abnormal vitamin D metabolism in patients with psoriasis. *Acta Derm Venereol*. 1987;67:65–68.
94. Lee YH, Song GG. Association between circulating 25-hydroxyvitamin D levels and psoriasis, and correlation with disease severity: a meta-analysis. *Clin Exp Dermatol*. 2018.
95. Reichrath J. Vitamin D and the skin: an ancient friend, revisited. *Exp Dermatol*. 2007;16:618–625.
96. Dua I, Aggarwal K, Jain VK. Comparative evaluation of efficacy and safety of calcipotriol versus tacalcitol ointment, both in combination with NBUVB phototherapy in the treatment of stable plaque psoriasis. *Photodermatol Photoimmunol Photomed*. 2017;33(5):275–281.
97. Mendonça CO, Burden AD. Current concepts in psoriasis and its treatment. *Pharmacol Ther*. 2003;99:133–147.
98. Peric M, Koglin S, Dombrowski Y, et al. Vitamin D analogs differentially control antimicrobial peptide/"alarmin" expression in psoriasis. *PLoS One*. 2009;4(7):e6340.
99. Karthaus N, van Spriel AB, Looman MWG, et al. Vitamin D controls murine and human plasmacytoid dendritic cell function. *J Invest Dermatol*. 2014;134:1255–1264.
100. Gorman S, Judge MA, Hart PH. Immune-modifying properties of topical vitamin D: focus on dendritic cells and T cells. *J Steroid Biochem Mol Biol*. 2010;121(1-2):247–249.
101. Okita H, Ohtsuka T, Yamakage A, et al. Polymorphism of the vitamin D(3) receptor in patients with psoriasis. *Arch Dermatol Res*. 2002;294:159–162.
102. Jacobs ET, Alberts DS, Foote JA, et al. Vitamin D insufficiency in southern Arizona. *Am J Clin Nutr*. 2008;87:608–613.
103. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. *Altern Med Rev*. 2005;10:94–111.
104. Altmeyer PJ, Matthes U, Pawlak F, et al. Antipsoriatic effect of fumaric acid derivatives: results of a multicenter double-blind study in 100 patients. *J Am Acad Dermatol*. 1994;30:977–981.
105. Nieboer C, de Hoop D, van Loenen AC, et al. Systemic therapy with fumaric acid derivates: new possibilities in the treatment of psoriasis. *J Am Acad Dermatol*. 1989;20:601–608.
106. Seville RH. Psoriasis and stress. *Br J Dermatol*. 1977;97:297–302.

107. Picardi A, Pasquini P, Cattaruzza MS, et al. Only limited support for a role of psychosomatic factors in psoriasis: results from a case-control study. *J Psychosom Res*. 2003;55:189–196.
108. Kabat-Zinn J, Wheeler E, Light T, et al. Influence of a mindfulness meditation-based stress reduction intervention on rates of skin clearing in patients with moderate to severe psoriasis undergoing phototherapy (UVB) and photochemotherapy (PUVA). *Psychosom Med*. 1998;60(5):625–632.
109. Winchell SA, Watts RA. Relaxation therapies in the treatment of psoriasis and possible pathophysiologic mechanisms. *J Am Acad Dermatol*. 1988;18:101–104.
110. Kazandjieva J, Grozdev I, Darlenski R, et al. Climatotherapy of psoriasis. *Clin Dermatol*. 2008;269(5):477–485.
111. Ben-Amitai D, David M. Climatotherapy at the Dead Sea for pediatric-onset psoriasis vulgaris. *Pediatr Dermatol*. 2009;26(1):103–104.
112. Snellman E, Lauharanta J, Reunanen A, et al. Effect of heliotherapy on skin and joint symptoms in psoriasis: a 6-month follow-up study. *Br J Dermatol*. 1993;128:172–177.
113. Fleischer Jr AB, Feldman SR, Rapp SR, et al. Alternative therapies commonly used within a population of patients with psoriasis. *Cutis*. 1996;58:216–220.
114. Fleischer Jr AB, Clark AR, Rapp SR, et al. Commercial tanning bed treatment is an effective psoriasis treatment: results from an uncontrolled clinical trial. *J Invest Dermatol*. 1997;109:170–174.
115. Carlin CS, Callis KP, Krueger GG. Efficacy of acitretin and commercial tanning bed therapy for psoriasis. *Arch Dermatol*. 2003;139:436–442.
116. Kudish AI, Abels D, Harari M. Ultraviolet radiation properties as applied to photoclimatherapy at the Dead Sea. *Int J Dermatol*. 2003;42:359–365.
117. Kushelevsky AP, Harari M, Kudish AI, et al. Safety of solar phototherapy at the Dead Sea. *J Am Acad Dermatol*. 1998;38:447–452.
118. Gordon PM, Diffey BL, Matthews JN, et al. A randomized comparison of narrow-band TL-01 phototherapy and PUVA photochemotherapy for psoriasis. *J Am Acad Dermatol*. 1999;41:728–732.
119. Markham T, Rogers S, Collins P, Narrowband UV-B. (TL-01) phototherapy vs oral 8-methoxypsoralen psoralen-UV-A for the treatment of chronic plaque psoriasis. *Arch Dermatol*. 2003;139:325–328.
120. Das S, Lloyd JJ, Walshaw D, et al. Response of psoriasis to sunbed treatment: comparison of conventional ultraviolet A lamps with new higher ultraviolet B-emitting lamps. *Br J Dermatol*. 2002;147:966–972.
121. Talbott W, Duffy N. Complementary and alternative medicine for psoriasis: what the dermatologist needs to know. *Am J Clin Dermatol*. 2015;16(3):147–165.
122. Guan J, Yuan S, Wu H. et al. Effectiveness and safety of traditional Chinese medical bath therapy combined with ultraviolet irradiation in the treatment of psoriasis: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*. 12(3):e0173276. https://doi.org/10.1371/journal.pone.0173276.
123. Zhu Q, Wu Y, Li Y, et al. Positive effects of hydrogen-water bathing in patients of psoriasis and parapsoriasis en plaques. *Nature*. 2018;8:8051.
124. Urabe H, Nishitani K, Kohda H. Hyperthermia in the treatment of psoriasis. *Arch Dermatol*. 1981;117:770–774.
125. Orenberg EK, Deneau DG, Farber EM. Response of chronic psoriatic plaques to localized heating induced by ultrasound. *Arch Dermatol*. 1980;116:893–897.
126. Tsoureli-Nikita E, Menchini G, Ghersetich I, et al. Alternative treatment of psoriasis with balneotherapy using Leopoldine spa water. *J Eur Acad Dermatol Venereol*. 2002;16:260–262.
127. Kaffenberger BH, Wong HK, Jarjour W, et al. Remission of psoriasis after allogeneic, but not autologous, hematopoietic stem-cell transplantation. *J Am Acad Dermatol*. 2013;68:489–492.
128. Held K, Rahmetulla R, Lowe TW, et al. Complete resolution of guttate psoriasis following autolotous SCT for Ewing's sarcoma in a pediatric patient. *Bone Marrow Transplant*. 1997;19:1041–1043.
129. Woods AC, Mant MJ. Amelioration of severe psoriasis with psoriatic arthritis for 20 years after allogeneic hematopoietic stem cell transplantation. *Ann Rheum Dis*. 2006;65:697.
130. Rafei M, Campeau PM, Aguilar-Mahecha A, et al. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. *Immunology*. 2009;182:5994–6002.
131. Zurita G, Geffner L, Maldonado B, et al. autologous stem cells implants in psoriasis. Available online: http://medicosecuador.com/espanolarticulos_medicos/autologous_stem_cells_implants.htm (accessed 10.14.18).
132. Hsieh WL, Lin YK, Tsai CN, et al. Indirubin, an acting component of *Indigo naturalis,* inhibits EGFR activation and EGF-induced CDC25B gene expression in epidermal keratinocytes. *J Dermatol Sci*. 2012;67:140–146.
133. Chang HN, Huang ST, Yeh YC, et al. *Indigo naturalis* and its component tryptanthrin exert anti-angiogenic effect by arresting cell cycle and inhibiting Akt and FAK signaling in human vascular endothelial cells. *J Ethnopharmacol*. 2015;74:474–481.
134. Cheng HM, Wu YC, Wang Q, et al. Clinical efficacy and IL-17 targeting mechanism of *Indigo naturalis* as a topical agent in moderate psoriasis. *BMC Complement Altern Med*. 2017;17(1):439.
135. Lin YK, Wong WR, Chang YC, et al. The efficacy and safety of topically applied *Indigo naturalis* ointment in patients with plaque-type psoriasis. *Dermatology*. 2007;214(2):155–161.
136. Syed TA, Ahmad SA, Holt AH, et al. Management of psoriasis with aloe vera extract in a hydrophilic cream: a placebo-controlled, double-blind study. *Trop Med Int Health*. 1996;1(4):505–509.
137. Miroddi M, Navarra M, Calapai F, et al. Review of clinical pharmacology of *Aloe vera* L. in the treatment of psoriasis. *Phytother Res*. 2015;29(5):648–655.
138. Heng MC, Song MK, Harker J, et al. Drug-induced suppression of phosphorylase kinase activity correlates with resolution of psoriasis as assessed by clinical, histological and immunohistochemical parameters. *Br J Dermatol*. 2000;143(5):937–949.
139. Sarafian G, Afshar M, Mansouri P, et al. Topical turmeric microemulgel in the management of plaque psoriasis; a clinical evaluation. *Iranian J of Pharmaceut Res*. 2015;14(3):865–876.
140. Gulliver WP, Donsky HJ. A report on three recent clinical trials using *Mahonia aquifolium* 10% topical cream and a review of the worldwide clinical experience with *Mahonia aquifolium* for the treatment of plaque psoriasis. *Am J Ther*. 2005;12(5):398–406.
141. Bernstein S, Donsky H, Gulliver W, et al. Treatment of mild to moderate psoriasis with Relieva – a *Mahonia aquifolium* extract: a double blind, placebo-controlled study. *Am J Ther*. 2006;13:121–126.
142. Tanghetti EA. The role of topical vitamin D modulators in psoriasis therapy. *J Drugs Dermatol*. 2009;8(suppl 8):S4–S8.
143. Levine D, Even-Chen Z, Lipets I. Pritulo OA Pilot, multicenter, double-blind, randomized placebo-controlled bilateral comparative study of a combination of calcipotriene and nicotinamide for the treatment of psoriasis. *J Am Acad Dermatol*. 2010;63(5):775–781.
144. Lew BL, Cho Y, Kim J, et al. Ceramides and cell signaling molecules in psoriatic epidermis: reduced levels of ceramides, PKC-alpha, and JNK. *J Korean Med Sci*. 2006;21(1):95–99.
145. Nigam R, El-Nour H, Amatva B, et al. GABA and GABA(A) receptor expression on immune cells in psoriasis: a pathophysiological role. *Arch Dermatol Res*. 2010;302(7):507–515.

Rheumatoid Arthritis

John Nowicki, ND, and *Gregory T. Yasuda*, ND, LMP*

OUTLINE

DIAGNOSTIC SUMMARY

- Insidious onset of chronic, severe joint pain and inflammation
- Polyarticular and symmetrical bilaterally
- Begins in the small joints and progresses to affect other joints in the body
- A systemic disease: fatigue, low-grade fever, weakness, weight loss, joint stiffness, and vague joint pain may precede the appearance of classic symptoms by weeks or months.
- Extraarticular manifestations include vasculitis, subcutaneous and systemic granulomas, pleurisy, pericarditis, pulmonary fibrosis, lymphadenopathy, splenomegaly, and anemia.
- Serum is usually positive for rheumatoid factor (RF) and/or anti–cyclic citrullinated peptide (anti-CCP) antibodies, although no single laboratory test is diagnostic.

GENERAL CONSIDERATIONS

The most common form of inflammatory polyarthritis, rheumatoid arthritis (RA) is a chronic condition with a widely variable course affecting the entire body. Fatigue, low-grade fever, weakness, joint stiffness, and vague joint pain may precede the appearance of joint inflammation by weeks or months, sometimes making early diagnosis difficult. It is estimated that between 1% and 3% of the population is affected, with female patients outnumbering male patients almost 3 to 1.[1] Although RA may begin at any age, the usual age at onset is 40 to 50 years.

The onset of RA is often insidious but occasionally can be quite abrupt. The joints with the greatest ratio of synovium to articular cartilage are most affected (e.g., knees, ankles, feet, wrists, hands). Although any joint may eventually be affected, the distal interphalangeal joints are generally spared. In about one third of persons, joint involvement is confined to one or a few joints. However, the initial presentation is generally polyarticular and bilaterally symmetrical—both hands, both wrists, or both ankles.

Involved joints characteristically present with inflammation and prolonged morning stiffness. As the disease progresses, bony erosion, subluxations, dislocations, and contractures develop and lead to joint deformities, particularly in the hands and feet. Cervical spine involvement is common, and instability of the atlantoaxial joint is a dangerous manifestation owing to the risk of spinal cord compression. Joint destruction and commonly prescribed immunosuppressive drugs predispose patients with RA to septic arthritis, with a mortality rate as high as 11.5%. This must not be confused with a flare of RA.

Focal loss of cartilage characterizes rheumatoid arthritis due to an up-regulation of catabolic pathways induced mainly by proinflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α). These cytokines up-regulate proteolytic enzymes, such as metalloproteinases, that lead to a breakdown of the cartilage macromolecules. Cytokines also blunt the chondrocyte compensatory synthesis pathways required to restore the integrity of the degraded extracellular matrix. The IL-17–producing helper T cells are the dominant cell type in RA and have a critical role in the progression to chronic destructive arthritis. Th17 produces IL-17, IL-17A, IL-17-F, IL-21, and IL-26, which enhance inflammation and cause autoimmune tissue damage. Th17 cells have been linked to rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, psoriasis, and systemic sclerosis, many of which are strongly linked to environmentally toxic compounds.

Pathogenesis

Although the mechanism that produces the characteristic inflammatory joint destruction has not been elucidated completely, the presence

*Previous edition contributor

of circulating immune complexes is one of the major factors thought to contribute to the pathogenesis of RA. The cell-mediated, humoral, and nonspecific immune complexes lead to much of the proliferative inflammation seen in RA. Genetic susceptibility, abnormal bowel permeability, lifestyle and nutritional factors, food allergies/sensitivities (including gluten intolerance), microorganisms, and environmental toxicants have all been investigated as triggers of this autoimmune reaction.

Genetic Susceptibility

Female gender and positive family history are considered risk factors for developing RA, and studies of twins demonstrate other genetic risk factors. There is a 15% concordance rate in monozygotic twins—four times that in dizygotic twins. Genetic studies have isolated a "shared epitope" in HLA-DR1 and HLA-DR4 subtypes that is expressed in 80% of Caucasians with RA. HLA-DRB1 and other factors may be associated with more aggressive disease by affecting antioxidant pathways and detoxification pathways that likely contribute to the pathogenesis of RA. Patients with RA also have changes in fecal flora, and studies have shown that independent of other contributing factors, the flora of monozygotic twins is more similar than that of dizygotic twins.[2]

Environmental Factors

Toxins in cigarette smoke interact with the HLA-DRB1 shared epitope alleles, enhancing the inflammatory process and increasing the risk of seropositive RA. The risk of smokers developing RA is nearly eightfold for carriers of the shared epitope and nearly sixteenfold for homozygous individuals.[3] Smoking also increases RA risk in the general population, threefold for men. Quitting smoking results in a decreased risk of seropositive disease.

Epidemiological studies have reported an increased prevalence of antinuclear antibodies associated with occupational exposures to persistent organic pollutants (POPs). POPs influence the immune system, which may increase the risk of autoimmune conditions such as RA.[4] Based on a job-exposure matrix, substantial handling of organic solvents was associated with an increased relative risk of RA.[5] Exposure to silica particles in dust activates the innate immune system, leading to activation of proinflammatory cytokine production, activation of adaptive immunity, autoantibody production, and tissue damage. Several studies suggest an association between silicosis and RA.[6] However, a large case-controlled study of pottery, sandstone, and refractory workers did not find a statistically significant association between respiratory silica and RA.[7] Data from the Women's Health Initiative Observational Study revealed that participants who self-reported residential or workplace use of pesticides were twice as likely to have been diagnosed with either RA or systemic lupus erythematosus (SLE).

RA rates are 37% higher in persons living <50 m from highways than in those living 150 m away. When individual vehicular exhaust components were assessed for risk, particulate matter of less than 2.5 micrometers ($PM_{2.5}$) showed no association, but ozone levels accounted for a 26% increase in risk. $PM_{2.5}$ levels can increase the risk of juvenile RA by 60%.[8] Women in the 1999 to 2002 National Health and Nutrition Examination Survey (NHANES) trial with blood polychlorinated biphenyl (PCB) levels in the second, third, and fourth quartiles had more than twice the risk of RA as those with PCBs in the first quartile.[9] Hair and blood cadmium, nickel, and lead levels are found to be significantly higher in those with RA than in healthy controls.[10] Low socioeconomic status is associated with increased morbidity and mortality in RA. Factors such as poor diet, drinking more than three cups of coffee per day, and psychological factors may play a role in the development of the disease and affect the levels of pain and physical disability experienced by patients with RA. Oral contraceptives, tea intake, and increased vitamin D consumption have been found to be protective. Additional environmental factors such as low temperature, high atmospheric pressure, and high humidity have been correlated with increased RA pain.

Oxidative Stress

Oxidative stress–induced alterations in cellular signal transduction pathways play a pivotal role in the development of arthritis. Free radicals have both direct and indirect effects in the pathogenesis of arthritis. Indirectly, free radicals act as secondary messengers in inflammatory and immunological cellular responses, activating transcription factors, such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), that control genes involved in inflammation. Directly, free radicals damage hyaluronic acid and degrade the joint cartilage by attacking its proteoglycan and inhibiting its synthesis. Several studies have shown that the risk of RA is highest in people with the lowest levels of nutrient antioxidants. One study examined people who developed RA and SLE 2 to 15 years after they donated blood in 1974.[11] For each case, four controls were selected from the serum bank donors matched for race, gender, and age. Stored serum samples from cases and controls were assayed for α-tocopherol, beta-carotene, and retinol. Patients with both diseases had lower serum concentrations of α-tocopherol, beta-carotene, and retinol in 1974 than their matched controls. Low antioxidant levels may be exacerbated by genetic factors; the shared epitope leads to increased nitrous oxide production and inhibition of antioxidative cellular pathways.[12] The resulting increase in oxidative stress could produce free-radical damage of DNA and mutations that may contribute to the pathogenesis of RA. Once disease is established, synovial concentrations of free radicals are elevated, and concentrations of antioxidants are diminished, accelerating inflammation and joint destruction.

Considering the role of reactive oxygen species in cellular dysfunction and inflammation, biomarkers of oxidative damage, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) and thiobarbituric acid reactive substances (TBARS), may be helpful to assess antioxidant status and toxin load. Studies have shown increased lipid, protein, and DNA oxidation markers and impaired antioxidant status, confirming the role of oxidative stress in the pathogenesis of RA.[13] Another study showed greater baseline 8-OHdG levels among subjects with RA compared with both healthy young ($p < 0.001$) and elderly ($p < 0.05$) subjects, indicating that subjects with RA have higher levels of oxidative stress than healthy individuals.

Autoantibody Production

The serum of most individuals with RA contains rheumatoid factor (RF) and cyclic citrullinated peptide (anti-CCP) autoantibodies. Their presence is thought to contribute to the pathogenesis of RA. The serum titers often correlate well with the severity of arthritis symptoms and prognosis, and the antibodies are not directly responsible for joint destruction. Most RF is formed locally in the affected joints by the inflammatory infiltration of activated B cells and plasma cells. Autoantibodies have been detected up to 10 years before the onset of clinical disease. Inflammatory markers, such as soluble tumor necrosis factor receptor II, have been shown to be elevated for up to 12 years before diagnosis and positively correlated with RA incidence. This suggests a "multiple-hit" model in which pathogenesis progresses in distinct stages, which may explain why autoantibodies are present for years before clinical manifestations appear.

Microbial Influences: Infection and Cross-Reactivity

Microorganisms play important roles in states of health and disease, the most obvious being frank infections that facilitate the pathogenesis of RA. Pathogenic or immunogenic microbes such as yeast,

gram-negative bacteria, protozoa, and amoebas can provoke an immune response that cross-reacts with human body tissues, inducing systemic inflammatory disease as well as tissue-specific inflammation.[14] The onset of RA is preceded by a specific inciting event in 8% to 15% of cases, and antimicrobials such as metronidazole, clotrimazole, acyclovir, roxithromycin, tetracycline, sulfasalazine, and minocycline have been associated with improvement in symptoms and, in some cases, complete remission. Many pathogens, such as Epstein–Barr virus, cytomegalovirus, parvovirus, rubella virus, *Mycoplasma,* amebic organisms, *E. coli,* influenza AH2N2, *Porphyromonas,* and *Proteus,* have been associated with RA. Although no single microbial agent has been consistently isolated in patients with the disease, it may be that a multitude of organisms can directly or indirectly contribute to the disease process by infection, antigen persistence, circulating immune complexes, or immune cross-reactivity. In genetically susceptible individuals, reactive arthritis occurs secondary to an enteric, urogenital, or respiratory infection by over a dozen different microorganisms and persists well beyond, or occurs even in the absence of, clinically evident infection. The condition becomes chronic in 15% to 60% of cases.

Some organisms, such as parvovirus B19, commonly produce an RA-like arthritis and autoantibodies, including RF and antinuclear antibody (ANA), which may be positive in early infection. Although most patients clear the infection, chronic forms are known in up to 20% of cases, where viral DNA is recoverable from a variety of tissues for years postinfection. This raises the possibility that RA development could be driven by chronic infection and/or the persistence of parvovirus B19 antigens. Other pathogens, such as *Proteus mirabilis,* stimulate the production of cross-reactive antibodies, which were found to be elevated in over 90% of patients with active RA.[15] These antibody levels were positively correlated with several markers of disease activity, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and RF. Antibodies to *Campylobacter, Salmonella,* and *Shigella* have also been shown to cross-react with collagen, whereas antibodies to *Klebsiella pneumoniae, Proteus vulgaris,* and *Yersinia enterocolitica* cross-react with other joint tissues. These antibodies are homologous with type XI collagen found in hyaline cartilage, and their immune complexes have been recovered in the synovia of patients with RA.

Dysbiosis and Small Intestinal Bacterial Overgrowth

There is more microflora in the digestive tract than human cells in the body, and the composition of the hundreds of species of microorganisms is known to be affected by genetics, medical treatment, diet, and stress. The flora can contribute to many diseases and have a profound influence on immune function, nutritional status, and stress response. In one study, *Bacteroides thetaiotaomicron,* a member of the normal flora in mice and humans, was inoculated in the gastrointestinal (GI) tracts of germ-free mice.[16] Monitoring the host transcriptional response to this single species, the researchers observed a breadth of influence on intestinal function, including improved nutrient absorption and processing by the host, mucosal barrier fortification, influx of IgA-producing B cells, and prevention of the inflammatory response or complement-mediated mucosal damage. Also affected were xenobiotic metabolism, neurotransmitter production, the enteric nervous system, gut motility, angiogenesis, and postnatal intestinal maturation. Many of these intestinal functions are thought to be involved in the pathogenesis of RA.

Fecal flora is significantly altered in RA, including significantly decreased bifidobacteria and bacteria of the *Bacteroides–Porphyromonas–Prevotella* group. Randomized clinical studies have demonstrated improvement with changes in microbial flora. Up to 51% of patients with RA have small intestinal bacterial overgrowth (SIBO),[17] and the degree of SIBO is associated with the severity of symptoms and disease activity.[18] SIBO has also been linked to the development of bowel-associated dermatosis-arthritis syndrome, which can present as arthralgia and swelling in multiple joints.[19]

The concept of multifocal dysbiosis suggests that the alteration in normal flora is not confined to the gut and cumulatively can produce the immune dysregulation typical of any autoimmune condition. The cumulative load of all microflora, most of which consists of uncultivated and novel organisms, is difficult to predict. This concept of total pathogen burden does, however, appear to correlate with increased CRP and cardiovascular risk and may also contribute to the pathogenesis of RA. Evidence to support this concept can be found in patients with concurrent RA and periodontal disease. One study found identical periodontal bacterial DNA in 100% of synovial fluid samples and 83.5% of serum samples of patients with RA,[20] and another study found a correlation between the severity of periodontitis and the severity of RA symptoms.[21] Antibodies to *Porphyromonas gingivalis* are associated with elevated anti-CCP antibodies and may be involved in breaking immune tolerance to citrullinated antigens and promoting the autoimmune response in RA.[22]

Adverse Food Reactions

It is estimated that up to 10% of the population has food allergies. In patients who have tested positive for allergies on skin-prick testing, consumption of those allergenic foods is associated with increases in inflammatory markers such as TNF-α, IL-1β, CRP, and ESR. Outcomes of food allergy studies in RA have been conflicting. Two possible explanations for negative correlations are that the allergen challenges were too small in quantity, frequency, or both and/or that serum antibody levels are not representative of the mucosal immune response in the intestine. Another confounding factor is that adverse food reactions (e.g., food intolerance, sensitivity, dietary lectins) are not antibody-mediated and therefore not apparent with antibody testing. Still, these reactions may be contributory.

Studies using larger doses of food antigens tend to provide a positive correlation between RA and food allergies in 20% to 40% of patients.[23] Jejunal IgA, IgG, and IgM were significantly elevated against nearly all food antigens in patients with RA compared with controls, and the antibodies were substantially cross-reactive. The investigators concluded that patients with RA may have multiple modest hypersensitivity reactions and that the additive effect could lead to widespread antibody-mediated tissue destruction.

Dietary salt has been shown to interfere with the regulatory mechanisms of both the innate and the adaptive immune systems, enhancing proinflammatory responses by inducing interferon (IFN)-gamma production and reducing the activation of IL-4 and IL-13 macrophages. A cross-sectional study demonstrated a significant dose-dependent association between total sodium intake in the fourth quartile and a diagnosis of RA (odds ratio [OR] 1.5; 95% confidence interval [CI], 1.1–2.1, p for trend = 0.02).[24]

Xenobiotics and Autoantibody Production

Xenobiotics appear to intersect with genetics, pathogenic organisms, and other dietary and environmental factors to stimulate autoantibody production and autoimmunity. Certain xenobiotics may be particularly toxic to both patients with RA and people at risk for RA owing to impaired metabolism in acetylation pathways. A single genetic risk factor, the N-acetyltransferase 2 polymorphism, affects both the acetylation of xenobiotics and individual susceptibility to RA. It also predicts disease severity. The slow-acetylator genotype is associated with a 4.39-times-greater risk of erosive RA than fast types.[25]

Bacterial toxins, such as streptokinase and heat-shock proteins, can bind to mucosal tissue enzymes and stimulate autoantibody

production against peptides and tissue antigens. This same response was also stimulated by gliadin peptides, illustrating that food antigens can elicit responses like those caused by bacterial superantigens. Streptokinase, gliadin, casein (a milk protein), and ethyl mercury (the vaccine adjuvant in thimerosal) bind to different lymphocyte receptors and tissue antigens, producing neoantigens that can induce the production of cross-reactive autoantibodies.[26]

Abnormal Gut Permeability

Individuals with RA have greater intestinal permeability to dietary and bacterial antigens as well as alterations in bacterial flora. Chronic inflammation in the gut can increase intestinal permeability and is associated with joint inflammation. Adverse food reactions and bacterial endotoxins may contribute greatly to chronic gut inflammation. When exposed to gliadin, zonulin receptor-positive IEC6 and Caco2 cells release zonulin, leading to the rearrangement of the cell cytoskeleton, loss of occluding-ZO1 protein–protein interaction, and increased intestinal permeability to macromolecules.[27] Autoimmune diseases, such as RA, have been associated with elevated zonulin. At least half of the individuals with celiac disease have no GI-related symptoms but present with fatigue, subfertility, osteoporosis, joint pain, unexplained iron deficiency, and/or autoimmune disease. Reestablishing the zonulin-dependent intestinal barrier function may provide a mechanism by which processes such as autoimmune disease and inflammatory conditions may be halted in their development and possibly reversed.[28]

Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly prescribed for RA, can also exacerbate gastrointestinal inflammation and intestinal permeability. However, increased gut inflammation and permeability also occur in RA independent of NSAID use. Increased intestinal permeability facilitates the translocation of dietary and gut-derived antigens and bacterial gut flora to blood, mesenteric lymph nodes, the spleen, and the kidneys. In conjunction with dysbiosis and bacterial overgrowth, increased gut permeability to bacterial endotoxins and food antigens can produce immune activation and circulating immune complexes, many of which have been recovered in synovial fluid and could contribute to joint inflammation and degeneration.

Decreased Androgen Levels

Androgens and progesterone are natural immune suppressors and appear to be involved in the apoptosis of activated immune cells. Chronically low levels of testosterone and dehydroepiandrosterone (DHEA), an inhibitor of NF-κB, have been found in early-stage and established RA. One study showed positive effects of testosterone replacement therapy in male patients with RA, including decreasing RF titers, a decrease in the number of affected joints, and decreasing NSAID use.[29] DHEA and testosterone may have to be modulated in concert with estrogens to be optimally effective in RA. Chronic inflammation and elevated TNF-α promote the conversion of testosterone to estrogen by stimulating aromatase activity, and a reduced androgen: estrogen ratio has been identified in both male and female patients with RA.

Abnormal Estrogen Levels

Unlike androgens, estrogens may be involved in sustaining the inflammatory activity of activated T cells and humoral immunity. Estradiol levels have been found to be higher in patients with RA than controls and are strongly and positively associated with indices of inflammation. A 5-year study comprising 689 patients with similar disease duration and severity found that gender is a major predictor of remission in early RA. Women had a lower androgen:estrogen ratio, had more severe disease, and had 16% to 22% fewer remissions than men. This effect could not be explained by any other differences between the groups, including disease duration, age, and treatments used.

Considering that many environmental toxins have estrogenic effects, this may help explain the large number of environmental chemicals associated with RA.

DIAGNOSIS

The most commonly used criteria in the diagnosis of RA have been those issued by the American College of Rheumatology (ACR) in 1987. However, the sensitivity and specificity of these criteria are low, especially in early disease. Earlier diagnosis allows for earlier and more aggressive treatment, which may lead to better outcomes. As a result, the ACR engaged in a joint initiative with the European League Against Rheumatism to create new guidelines that classify a diagnosis of RA based on the presence of synovitis in at least one joint, with no alternative explanation, and a score of 6 or greater in a four-tier rubric (see Table 215.1).[30]

Laboratory Findings

Rheumatoid Factor

RF is made up of a group of antibodies, usually IgM, that bind the Fc fragment of IgG. RF is formed locally in the affected joints by the inflammatory infiltration of activated B cells and plasma cells. Although it is present in 70% to 80% of patients with RA, a variety of conditions can cause elevations of RF, and 4% of apparently healthy individuals also test positive. Despite the relatively low sensitivity and specificity of the RF test, its presence is a confirmatory finding in the diagnosis of RA, and seropositivity is associated with worse outcomes.

TABLE 215.1 Classification Criteria for Rheumatoid Arthritis

Joint Involvement: Select the One With Highest Point Value	
1 medium-large joint	0 points
2–10 medium-large joints	1 point
1–3 small joints	2 points
4–10 small joints	3 points
10+ small joints	5 points
Serology	
Seronegative for RF and anti-CCP	0 points
Low-titer of RF and/or anti-CCP	2 points
High-titer of RF and/or anti-CCP (more than three times the upper limit of normal)	3 points
Duration of Synovitis	
Less than 6 weeks	0 points
6+ weeks	1 point
Acute-Phase Reactants	
Normal ESR and CRP	0 points
Elevated CRP or ESR	1 point

anti-CCP, anti–cyclic citrullinated peptide; *CRP,* C-reactive protein; *ESR,* erythrocyte sedimentation rate; *RF,* rheumatoid factor.
Data from Aletaha D, Neigi T, Silman A, et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis.* 2010 Sept.;62(9):2569–2581.

Anti–Cyclic Citrullinated Peptide Antibodies

Anti-CCP antibodies have emerged as a much more specific indicator of RA than RF as well as a better predictor of clinical course. Whereas the sensitivity of anti-CCP antibodies testing is similar to RF (70%–80%), its specificity is greater than 95%. These autoantibodies, as well as RF, have been identified as much as 10 years before diagnosis, and their presence heralds more aggressive disease and poorer prognosis. No single laboratory test is definitive for the diagnosis of RA, and patients may be seropositive for anti-CCP, RF, both, or neither.

Erythrocyte Sedimentation Rate and C-Reactive Protein

The ESR and CRP are acute-phase reactants that become elevated during active inflammation. Although not specific to RA, they are useful in confirming the diagnosis and monitoring disease activity and response to treatment. Because patients with RA are at increased risk for cardiovascular disease,[31] CRP is a useful marker of cardiovascular risk as well.

Other Laboratory Abnormalities

Anemia, usually normocytic and normochromic or normocytic and hypochromic, is quite common in RA and in chronic inflammatory diseases. Although the serum iron level and total iron-binding capacity are usually low, supplemental iron is of no value and may promote further damage due to free radicals. Because serum ferritin is an acute-phase reactant, iron stores may appear falsely elevated in RA when ESR and/or CRP are elevated. ANAs are found in 20% to 60% of patients with RA. Titers specific for native DNA are typically normal, whereas titers to single-stranded or denatured DNA are usually elevated. Antinuclear antibodies may be present but at lower levels than in SLE. Joint aspiration is not necessary to diagnose RA but may be necessary to rule out crystalline arthropathies or septic arthritis.

Other Diagnostic Testing

It is important to keep in mind that some other conditions can mimic RA and should be ruled out before a diagnosis is made. These include vitamin D deficiency, hemochromatosis, hypothyroidism, and infections such as Lyme disease and hepatitis B and C. Other tests to consider include the following:

- Hormone profiles: DHEA-S, testosterone, and estradiol
- Food allergies (see Chapter 14)
- Lactulose-mannitol test (see Chapter 21)
- Hydrogen-methane breath test (see Chapter 9)
- Stool testing for digestive function and intestinal flora (see Chapter 28)
- Urine testing (see Chapter 29)
- Toxicity assessment

STANDARD MEDICAL THERAPY

Standard medical therapy is limited by its overreliance on pharmacological therapies and focus on suppressing the disease process and its symptoms, most notably controlling the inflammation and free radicals that mediate joint damage, while failing to address the complex underlying causes of this disease.[32] The effects and side effects of these drugs exacerbate many contributing factors to the disease process and possess significant morbidity and mortality risks of their own. Pharmacological therapies generally fall into three categories: NSAIDs, disease-modifying antirheumatic drugs (DMARDs), and biological therapies. Nonpharmacological approaches include reduction of joint stress, physical and occupational therapy, and surgery.

Nonsteroidal Anti-Inflammatory Drugs and Cyclooxygenase Inhibitors

Usually implemented before formal diagnosis, NSAIDs are first-line anti-inflammatory and analgesic medications that suppress symptoms while also accelerating factors that promote the disease process. NSAIDs can inhibit chondrocytes and cartilage formation and promote chondrolysis and joint destruction. Furthermore, they promote several factors thought to contribute to the pathogenesis of RA by increasing hyperpermeability of the GI tract, dysbiosis, free radicals in synovial fluid, enteric inflammation, bacterial overgrowth, and bacterial translocation. NSAIDs also cause serious GI side effects, and long-term therapy has been shown to increase the risk of major GI bleeding,[33] as well as a number of other adverse GI events, including heartburn/acid reflux, excess stomach acid, gastritis, abdominal pain, epigastric burning, stomach ulcer, and duodenal ulcer.[34] Acetaminophen alone is responsible for 50% to 60% of cases of acute liver failure in the United States,[35] and according to one study, NSAIDs led to 107,000 hospitalizations and 16,500 deaths in 1 year among patients with arthritis.

To mitigate this massive GI toxicity, NSAIDs are sometimes coadministered with acid-blocking medications. These drugs help alleviate the upper GI damage but do not protect from the ensuing iatrogenic hypochlorhydria, which is a causative factor in SIBO and another contributing factor to the pathogenesis of RA. Although cyclooxygenase-2 (COX-2) inhibitors have a much-improved GI safety profile compared with nonselective NSAIDs, they may not completely protect the lower GI tract from injury and are associated with adverse cardiovascular events. Because patients with RA are already at increased risk for cardiovascular disease, these drugs may not be appropriate for general use in this population.[36] Finally, all drugs in this class also pose the risk of renal toxicity, and chronic use of high doses in patients with arthritis increases the risk for renal failure.

Corticosteroids

Corticosteroid medications are generally reserved for patients who do not respond to NSAID therapy. By blocking the inflammatory response, including the production and secretion of inflammatory mediators such as histamine, prostaglandins, and leukotrienes, corticosteroids suppress inflammation as well as the normal immune response. Steroid medications may be of great benefit in acute symptom management, but they become problematic with long-term use. Corticosteroids are associated with more frequent serious infections (i.e., those requiring hospitalization) and increased mortality. Even small doses (up to 7.5 mg prednisone or equivalent) carry an increased risk for infection and death when used long term. One study found the risk of mortality increased by 14% after 1 year and 49% after more than 10 years of use compared with the status of patients with RA who had not been treated with low-dose corticosteroids.[37]

Common side effects of long-term corticosteroids at higher doses bring their use further into question because they exacerbate the manifestations of RA itself. Side effects include chondrotoxicity, osteoporosis, increased intestinal permeability, and cardiovascular disease, including an increased risk of myocardial infarction. Furthermore, the risk of peptic ulcers and GI bleeding in patients already using NSAIDs is exacerbated by corticosteroids. Insomnia is also a side effect, and patients with RA are more susceptible to insomnia than the general population. In fact, one study concluded that the fatigue experienced by patients with RA may be more due to sleep fragmentation than a constitutional symptom of the disease itself.[38] Finally, depression is a common side effect of prednisone and is also common among patients experiencing chronic pain from diseases such as RA. Patients with RA

who experience depression have worse outcomes than those who are not depressed; thus, risks and benefits must be considered when recommending corticosteroids.

Synthetic Disease-Modifying Antirheumatic Drugs

Once reserved for severe cases, DMARDs are now used as first-line therapies according to conventional treatment guidelines. Methotrexate is the most common drug used, with the best balance of efficacy and toxicity. Methotrexate was originally used as a chemotherapeutic agent and exerts an immunosuppressive effect by decreasing white blood cell production. The more severe side effects of methotrexate include GI ulceration; severe bone marrow suppression; frequent infections; elevated risk of cancer; and damage to the lungs, liver, and kidneys. Other drugs in this class, including hydroxychloroquine (Plaquenil), azathioprine, cyclophosphamide, and leflunomide, have similar, and sometimes more severe, side effects. Many patients do not continue long-term therapy with any DMARD beyond the first few years because of the adverse effects or lack of efficacy. However, there is evidence that beginning a DMARD within the first 3 months of diagnosis is associated with a decreased risk of joint erosion. Therefore patients presenting with a diagnosis of RA will most often be on DMARDs, individually or increasingly in combination, and perhaps additional drugs to manage the side effects.

Biological Disease-Modifying Antirheumatic Drugs

Biological DMARDs include TNF-α antagonists such as infliximab, etanercept, and adalimumab, as well as tocilizumab, an interleukin inhibitor, and abatacept, a drug acting on T lymphocytes. Some evidence suggests that these medications are no more effective than methotrexate when used as monotherapy.[39] However, in about 10 comparative trials analyzed, the combination of TNF-α antagonists with methotrexate was more effective than methotrexate alone, especially in initially severe RA.[40] The investigators found no firm evidence that other drug combinations were more effective. Unfortunately, drug-free remission is still rarely achieved, and most patients experience higher disease activity upon discontinuation of therapy.

Many patients experience significant improvement within a few weeks. However, blocking TNF-α without addressing the conditions that created the imbalance leads to long-term consequences, such as sepsis and pneumonia and an increased risk of reactivating tuberculosis. A potent inflammatory mediator, TNF-α is also a critical part of the host defense against certain pathogens. For all patients using TNF-α inhibitors, vigilance for hypersensitivities, anemias, infections, malignancy, and accelerated atherosclerotic activity is required. Long-term follow-up studies do not exceed 2 years. However, the risk of severe adverse effects is likely increased with drug combinations.[41]

THERAPEUTIC CONSIDERATIONS

RA is a multifactorial condition that requires a comprehensive therapeutic approach addressing all the factors known to be involved in the inflammatory disease process. Foremost in this approach is the diet. Eliminating alcohol, high-fructose corn syrup (HFCS), and salt; decreasing intake of animal proteins; and avoiding food allergens constitute a good first step.

Diet

Population studies have demonstrated that RA is less common in societies that favor a more "primitive" diet and is found at higher rates in societies consuming a Western diet.[42] A diet of organic whole foods, rich in vegetables and fiber and low in sugar, meat, refined carbohydrates, saturated fat, and additives, appears to offer some protection against the development of RA as well as promise in its treatment. Some clinical evidence suggests the avoidance of foods in the *Solanaceae* (nightshade) family because long-term, low-level consumption of the solanum alkaloids found in tomatoes, potatoes, eggplant, peppers, and tobacco may be proinflammatory and trigger joint inflammation.

Adverse Food Reactions

The elimination of reactive foods has been shown to offer significant benefit to some individuals with RA. The most common triggers in one study included corn (56%); wheat (54%); bacon/pork (39%); oranges (39%); milk and oats (37%); rye (34%); egg, beef, and coffee (32%); malt (27%); cheese and grapefruit (24%); tomato (22%); peanuts and cane sugar (20%); and butter, lamb, soy, and lemon (17%).[43] Nineteen percent of participants who continued to avoid their reactive foods remained well, without medications, for follow-up periods of up to 5 years.

Therapeutic Fasting

Patients with RA have historically benefited from fasting. Short-term fasts of 3 to 5 days' duration are recommended during acute flares, which induce a substantial reduction of joint pain, swelling, morning stiffness, and other symptoms of RA. Fasting increases serum DHEA-S and decreases serum IL-6, CRP, ESR, and disease activity.[44] Fasting also has a favorable effect on intestinal permeability. Juice, broth, and water fasts of longer duration, up to 10 days, have been shown to be effective, but strict water fasting should be done only under direct medical supervision. (For a full discussion, see Chapter 37.)

Vegetarian Diet

One 13-month controlled study began with a 7- to 10-day fast before transitioning to a vegetarian diet.[45] The fast consisted of herbal teas; garlic; vegetable broth; a decoction of potatoes and parsley; and carrot, beet, and celery juices. The diet was followed by a systematic reintroduction of a single food item every 2 days with the elimination of foods that aggravated RA symptoms. The treatment group showed significant improvements compared with controls, and these improvements were maintained at 1-year follow-up in those patients still maintaining the diet. This study supported the positive results of short-term fasting followed by a vegetarian diet, and the pooling of these data showed statistically and clinically significant beneficial long-term effects. Vegetarian diets are also associated with higher fiber intake and improved gut flora, decreased antibodies to *Proteus mirabilis,* reduced SIBO, and an improvement in RA symptomatology. In addition, increased potassium intake from vegetables may lead to improved biosynthesis and release of cortisol as well as a favorable fatty acid profile and increased antioxidant intake.

The Mediterranean Diet

Rich in seasonally fresh plant-derived foods, the Mediterranean diet is similar to the vegetarian diet, with the addition of fish and poultry, low to moderate red meat, dairy and red wine and olive oil as the main source of lipids. Patients with established RA obtained a reduction in inflammatory activity, an increase in physical function, and improved vitality after 12 weeks on a Mediterranean diet.[46] Different types of dietary fats can either alleviate or aggravate RA by influencing eicosanoid metabolism. Both vegetarian and Mediterranean diets are inherently low in saturated fats and arachidonic acid, the precursor to the inflammatory series-2 prostaglandins and leukotrienes, and rich in gamma-linolenic acid (GLA) and α-linolenic acid (ALA), the precursors to anti-inflammatory series-1 and series-3 prostaglandins. The consumption of cold-water fish such as mackerel, herring, sardines, and salmon, which are rich in eicosapentaenoic acid

(EPA) and docosahexaenoic acid (DHA), further promotes anti-inflammatory eicosanoids. A population-based case-control study of women living in the Seattle area compared fish consumption in 324 cases of RA with 1245 control cases.[47] Consumption of broiled or baked fish was associated with a dose-dependent decreased risk of RA. This may explain some of the anti-inflammatory effects of the Mediterranean diet. By excluding meats, not only is preformed, proinflammatory arachidonic acid removed from the diet but also a potential food allergen. Several studies have found that patients with RA are commonly allergic to meats, especially beef and pork.[48] Furthermore, the inclusion of olive oil produces additional benefits in RA, including antioxidant and anti-inflammatory effects, competitive inhibition of omega-6 fatty acids, pain reduction, reduced morning stiffness, and improved patient evaluation of global disease.[49] These effects seem to be synergistic with fish oils.

Dietary Antioxidants

The continuous production of free radicals within arthritic joints promotes joint degeneration, exhausts antioxidant systems, and may cause the low antioxidant levels commonly seen in patients with RA. The importance of consuming a diet rich in fresh fruits and vegetables in the dietary treatment of RA cannot be overstated. These are the best sources of antioxidants such as vitamin C, beta-carotene, vitamin E, selenium, and bioflavonoids, all of which promote healthy joints by neutralizing inflammation and supporting collagen structures. Antioxidants work together synergistically, such as requiring vitamin C to regenerate vitamin E, and may explain why systematic reviews have not found single antioxidant supplements to be effective but have documented benefit for antioxidant-rich diets. The antioxidant content of vegetarian and Mediterranean diets almost certainly contributes to their effectiveness in treating RA. (For more information, see Chapter 97.)

Nutritional Supplements

Gamma-Linolenic Acid

Evening primrose, black currant, and borage oils contain GLA, an omega-6 fatty acid that acts as a precursor to the anti-inflammatory prostaglandins of the 1-series. Although one study showed that the long-term supplementation with GLA produced a paradoxical increase in tissue arachidonic acid levels while decreasing tissue levels of EPA,[50] GLA nevertheless appears to provide anti-inflammatory support in RA. GLA supplementation (1.4 g/day) has been shown to reduce the number of tender joints by 36%, the tender joint score by 45%, the swollen joint count by 28%, and the swollen joint score by 41%.[51] The subjects in the study did, however, continue to take anti-inflammatory pharmaceutical drugs, which may have masked a change in arachidonic acid and EPA levels or acted synergistically with the GLA. Review studies found statistically significant improvements in pain and global function in patients using GLA. Although the investigators emphasized the need for more trials, their conclusions in favor of GLA were stronger than those for fish oil.

Omega-3 Fatty Acids

The richest sources of omega-3 oils are cold-water fish and flaxseed oil. The ALA in flaxseed oil must be enzymatically converted to EPA and DHA to exert an anti-inflammatory effect. Although several studies have shown that flaxseed oil is not as effective as fish oil, these studies failed to address the role of zinc (a cofactor for delta-6-desaturase) and omega-6 fatty acids. Incorporating a low–omega-6 diet and addressing any underlying zinc deficiency while supplementing with 13 g (approximately 1 tbsp) of flaxseed oil a day raises tissue EPA levels to an extent comparable with the effects of fish oil supplementation.[52] Several human and animal studies have demonstrated that flaxseed oil supplementation can inhibit the autoimmune reaction just as well as fish oil.[53]

Supplementing with fish oils bypasses the metabolism of ALA by directly supplying preformed EPA and DHA. Many published studies have documented the efficacy of fish oil. Because it competitively inhibits COX-2, which is overexpressed in the RA synovium, supplementation has been shown to decrease long-term use of NSAIDs.[54] Review studies of fish oil supplementation have concluded that there is convincing evidence of benefit in RA, including reduced morning stiffness, fewer tender or swollen joints, decreased joint pain, less fatigue, and reduced serum markers of inflammation, such as CRP, IL-1β, TNF-α and LTB_4.[55] It may take up to 12 weeks at a minimum daily dose of 3 g of combined EPA and DHA before effects become apparent. It is important to select a product that has been rigorously tested for contaminants such as heavy metals, PCBs, dioxins, and lipid peroxides, or one may rely on cold-water fish and flaxseed oil for the omega-3 oils rather than fish oil capsules to help avoid these toxins. (For more information about fish oils, see Chapter 91.)

Multivitamin/Multimineral Supplementation

A high-potency multiple-vitamin-and-mineral formula is recommended because bone and cartilage are dependent on a constant supply of many nutrients. Blood and/or tissue samples from patients with RA indicate that such patients are deficient in many nutrients, including magnesium, folate, vitamin B_{12}, vitamin B_6, zinc, and selenium. Many factors may contribute to deficiencies, including decreased dietary nutrient intake among patients with RA, decreased absorption due to impaired gut function, increased utilization of antioxidant nutrients resulting from elevated oxidative stress, and increased excretion of nutrients secondary to stress and medications.

Selenium and Vitamin E

An important mineral cofactor for glutathione peroxidase and thioredoxin reductase, selenium is especially important for reducing the production of inflammatory prostaglandins and leukotrienes and controlling tissue damage due to free radicals. Selenium combined with vitamin E has shown a positive effect in patients with RA. Patients already diagnosed with RA who received 600 mg vitamin E twice a day showed significant pain reduction but no changes in clinical and biochemical indices of inflammation.[56]

Zinc

A cofactor for delta-6-desaturase, the rate-limiting enzyme in omega-3 fatty acid metabolism, zinc deficiency is associated with poor conversion of ALA from flaxseed oil to EPA and DHA. Deficiency is common in patients with RA, which may predispose them to increased inflammation due to impaired eicosanoid metabolism. Plasma zinc levels are inversely correlated with ESR, CRP, IL-1β, and TNF-α.[57] Zinc also has antioxidant effects and is a cofactor to the enzyme superoxide dismutase (copper-zinc SOD). Patients using corticosteroids may be at increased risk for zinc deficiency because these medications have been shown to decrease plasma zinc and increase urinary zinc.[58] Several studies have demonstrated a slight therapeutic effect from zinc supplementation in RA patients.

Manganese and Superoxide Dismutase

Manganese-containing SOD is deficient in patients with RA. The injectable form of this enzyme has been shown to be effective in the treatment of RA.[59] Oral manganese supplementation has been shown to increase SOD activity.[60] Although no clinical studies have been

conducted to determine the effectiveness of manganese in the treatment of RA, it appears to be indicated on the basis of the low levels seen in patients with RA as well as its biochemical functions.

Vitamin C

An important antioxidant, vitamin C concentrations in white blood cells and plasma are significantly decreased in patients with RA. Supplementation with vitamin C increases SOD activity, reduces histamine levels, and provides anti-inflammatory action. Studies have demonstrated a negative correlation between plasma vitamin C levels and RA disease activity as well as between vitamin C levels and CRP.[61]

Pantothenic Acid

Whole-blood levels of pantothenic acid have been reported to be lower in patients with RA than in normal controls and inversely correlated with disease activity. Correction of these low levels brings about some alleviation of RA symptoms. In one double-blind study, a subjective improvement of RA symptoms was noted in patients receiving 2 g/day of calcium pantothenate.[62] Patients noted improvements in the duration of morning stiffness, degree of disability, and severity of pain.

Pyridoxine (Vitamin B_6)

There is a clear relationship between low serum levels of pyridoxal 5′-phosphate and inflammatory indicators of RA. Plasma vitamin B_6 levels are lower in patients with more disability, pain, fatigue, and swollen joints, as well as the high ESR and CRP levels associated with ongoing chronic inflammation.[63] Elevated levels of homocysteine were also noted and correlated with low vitamin B_6 status in patients with RA, consistent with the increased risk of cardiovascular disease seen in these patients.

Copper

In patients with RA, copper salicylate yields better results in reducing pain and inflammation than standard aspirin preparations.[64]

Copper is also used in copper-zinc SOD. Deficiency may result in significant susceptibility to free-radical damage as a result of decreased SOD levels. However, an excess intake of copper may be detrimental because of its ability to combine with peroxides and damage joint tissues.

Vitamin D

Vitamin D is a steroid hormone that plays a role in neuromuscular and immune function, modulation of cell growth, and reduction of inflammation. Vitamin D has been shown to increase innate immunity while modulating adaptive immunity, and vitamin D deficiency is associated with an increased prevalence of autoimmune conditions, including RA.[65]

One study identified lower vitamin D levels and a higher incidence of RA in northern European countries compared with southern European countries.[66] In both parts of Europe, however, low 25(OH) D3 levels were significantly correlated with worse RA symptomatology. A high intake of vitamin D is associated with a decreased risk of RA.

Vitamin D also plays an important role in normal calcium metabolism. A study involving 147 women, mean age of 50.9 years, found that 13% had vitamin D levels below the threshold for vitamin D–deficiency osteomalacia.[67] In the winter, 73% had serum concentrations below the normal range. This suggests there is a disturbance in vitamin D metabolism in RA that might play a role in RA-associated osteoporosis.

Pancreatic Enzymes and Hydrochloric Acid

Impaired digestion from inadequate secretion of pancreatic enzymes and/or hydrochloric acid is common in patients with RA and can be a major contributor to the disease process. In addition to poor assimilation of ingested nutrients, poorly digested food molecules can be inappropriately absorbed, stimulating an immune response. In one study, 80% of untreated patients with RA had reduced maximal gastric acid output.[68] Half of patients with RA who had inadequate hydrochloric acid secretion also had SIBO, and restoration of gastric pH helps to resolve the overgrowth.

Pancreatic enzymes may offer additional benefits when taken between meals. Specifically, the proteases in pancreatin have been shown to reduce circulating levels of immune complexes in RA and other autoimmune diseases. Because clinical improvements usually correspond with decreases in levels of immune complexes, proteolytic enzyme supplementation is often warranted. (For more information, see Chapter 100.)

Probiotics

Commensal organisms interact with Toll-like receptors in the intestinal mucosa to promote immune tolerance and regulatory T-cell formation while blocking NF-κB, inflammation, and allergies. These effects in patients with RA may be strain-specific. For example, *Lactobacillus rhamnosus* GG did not show a clinical benefit.[69] However, a study on *Bacillus coagulans* GBI-30, 6080, produced statistically significant improvement in pain scale compared with placebo.[70] Treatment also resulted in greater improvement in patient global assessment, self-assessed disability, CRP, ability to walk 2 miles, and participation in daily activities. Probiotics, prebiotics, and/or antimicrobial compounds may be indicated in cases of dysbiosis and SIBO. (For more information, see Chapters 104 and 105.)

Botanical Medicines

Many botanicals possess significant anti-inflammatory action and are useful in the treatment of RA. The suggestions made here represent some of the more popular and better researched of these botanical medicines.

Curcuma longa

Curcumin, the yellow pigment of *Curcuma longa* (turmeric), exerts excellent anti-inflammatory and antioxidant effects and has been found to be as effective as cortisone or phenylbutazone in models of acute inflammation.[71] Molecular targets include transcription factors (e.g., NF-κB, peroxisome proliferator-activated receptor [PPAR]-gamma), enzymes (e.g., COX-2, 5-lipoxygenase [5-LOX]), and cytokines (e.g., TNF-α, IL-1, IL-6).[72] Animals models also indicate that curcumin may enhance endogenous corticosteroid activity through increased synthesis and/or release, potentiation of receptor sites, or slowing of catabolic pathways.

One double-blind clinical trial compared curcumin (1200 mg/day) with phenylbutazone (300 mg/day) in patients with RA.[73] The improvements in the duration of morning stiffness, walking time, and joint swelling were comparable in both groups. Furthermore, although phenylbutazone is associated with significant adverse effects, curcumin has not been shown to produce any side effects. It is noteworthy that curcumin also lacks any direct analgesic action.

A major concern regarding curcumin is absorption. One method to enhance the absorption is by complexing the curcumin with soy phospholipids to produce the product Meriva. Absorption studies indicate that peak plasma levels of curcumin after administration of Meriva were fivefold higher than those after administration of regular curcumin. Studies with a nano-particle-sized form of curcumin, Theracurmin, show up to 27-times-greater absorption than regular curcumin.[74]

Resveratrol

Resveratrol has been shown to inhibit the interaction of AP-1 and NF-κB with COX-2 promoter in human rheumatoid arthritis synovial

fibroblasts.[75] Resveratrol may also have protective effects against matrix degradation and inflammation in chondrocytes by reversing IL-1β–induced catabolic and inflammatory responses.[76]

Bromelain

Bromelain is a mixture of enzymes found in pineapple. Since 1957, more than 200 scientific papers have appeared in the research literature documenting a wide variety of beneficial effects, including reduced inflammation in RA. Much of the effects of bromelain are due to activation of compounds that break down the inflammation-induced fibrin matrix that leads to inadequate tissue drainage and edema. Bromelain also blocks the production of kinins, compounds produced during inflammation that increase swelling and cause pain. A proteolytic enzyme product containing bromelain, papain (an enzyme from papaya), trypsin, and chymotrypsin (pancreatic enzymes) was shown to decrease excessive levels of transforming growth factor beta in human blood, and polymorphisms of this transforming growth factor have been linked with the progression of joint destruction in rheumatoid arthritis.[77]

Zingiber officinalis

Ginger *(Z. officinalis)* contains antioxidants and exerts anti-inflammatory effects by inhibiting the synthesis of prostaglandins, thromboxanes, and leukotrienes. A preliminary clinical study was conducted with seven patients with RA in whom conventional drugs had provided only temporary or partial relief.[78] All patients were treated with ginger. One patient took 50 g/day of lightly cooked ginger, and the other six took either 5 g/day of fresh ginger or 0.1 to 1 g/day of powdered ginger. Despite the differences in dose, all patients who took ginger reported a substantial improvement, including pain relief, better joint mobility, and a decrease in swelling and morning stiffness.

A follow-up study evaluated 28 patients with RA (18 with osteoarthritis and 10 with muscular discomfort) who had been taking 500 to 4000 mg of powdered ginger for periods ranging from 3 months to 2.5 years.[79] It was reported that 75% of the patients with arthritis and 100% of those with muscular discomfort experienced relief in pain or swelling and that the effects were dose dependent.

Fresh ginger contains higher levels of gingerols as well as a protease that may act on inflammation much like bromelain and therefore may be more effective in the treatment of RA than dried preparations. Most studies have used 1 g of dry powdered ginger root, which is a relatively small dose compared with the daily average of 8 to 10 g consumed in India. A daily dose of 2 to 4 g of dry powdered ginger is safe and may be effective in RA. This is roughly equivalent to 20 g of fresh ginger root, or a 0.5-inch slice, and can easily be incorporated into the diet in fresh fruit and vegetable juices.

Tripterygium wilfordii Hook F

In Chinese medicine, *Tripterygium wilfordii Hook F,* or thunder god vine, has a long history of use in RA and other autoimmune diseases. Although studies have focused almost exclusively on the root, both the folium and the radix of the plant appear to have anti-inflammatory, analgesic, and immunosuppressive effects that may be effective in treating RA. In a double-blind, placebo-controlled study involving patients with long-standing RA in whom conventional therapy had failed, 80% of those who received a high dose (380 mg/day) of ethanol/ethyl acetate root extract had a positive response (i.e., at least 20% improvement according to the ACR criteria).[80] Forty percent of the low-dose (180 mg/day) group also responded positively, compared with none in the placebo group. Another trial demonstrated that 180 mg/day of *Tripterygium* extract was more effective than sulfasalazine 1 g twice a day.[81]

Side effects reported for this herb include GI upset, diarrhea, headache, hair loss, menstrual abnormalities, and hypertension. Randomized controlled trials have demonstrated efficacy and decreased adverse reactions from sustained-release tablet,[82] topical applications,[83] and coadministration with *Glycyrrhiza glabra* (licorice) root.[84] Coadministration of *Tripterygium* with methotrexate may improve efficacy and decrease side effects compared with methotrexate alone.[85]

Valeriana officinalis

Between 54% and 70% of patients with RA report sleep disruptions. *V. officinalis,* or valerian, has been shown to decrease the time between going to bed and the first cycle of deep sleep compared with placebo in patients with RA.[86] Valerian is a sedative and anxiolytic that sensitizes gamma-aminobutyric acid (GABA) receptors and may also affect the actions of serotonin and adenosine. Fatigue, commonly experienced in RA, might be a sign of undiagnosed sleep disturbance rather than a product of the disease process itself. Morning symptoms of RA are correlated with a nonrestorative sleep disorder. Inadequate sleep can lead to inflammation, as measured by increased CRP, and both CRP and sleep problems are inversely related to pain thresholds in patients with RA. Therefore using valerian to improve the quality of sleep may also improve fatigue, pain threshold, and psychological measures of quality of life.

Physical Medicine

Exercises

Exercise can improve strength and performance while maintaining range of motion in patients with RA. Exercise decreases cardiovascular risk; RA disease activity; and systemic inflammation, as evidenced by reduced ESR. Patients with well-developed disease should begin with progressive, passive range-of-motion and isometric exercises, gradually introducing active range-of-motion and isotonic exercises as appropriate. One randomized, controlled, multicenter trial studied the effects of high-intensity exercise in over 300 patients with RA. Participants were given either standard physical therapy or a high-intensity exercise program for 2 years. Whereas radiographic damage of the large joints was not increased, high-intensity exercise improved functionality and mood and provided a sense of well-being.[87]

Hydrotherapy

Heat is typically used to help relieve stiffness and pain, relax muscles, and increase range of motion. Moist heat (e.g., moist packs, hot baths) is more effective than dry heat (e.g., heating pad). Paraffin baths can be used if skin irritation from regular water immersion develops. Cold packs are of value during acute inflammatory flareups or after hot applications.

Constitutional hydrotherapy is a classic naturopathic technique utilizing alternating hot and cold applications to the torso and back in combination with electrical stimulation. As with contrast hydrotherapy in other parts of the body, this creates an alternating vasodilation and vasoconstriction intended to improve the circulation of blood through the viscera, promote detoxification, and tone the nervous system. Rather than treating the symptomatic joints, this therapy is focused on the causative factors underlying the disease: healing impaired gut function and eliminating metabolic wastes and xenobiotic toxins.

Psychological Aspects

Optimistic patients with RA report better psychosocial and physical functioning than pessimistic patients, who adopt passive coping strategies like staying in bed for many hours a day. Patients who believe they can control and decrease pain by using spiritual/religious coping methods tend to

have less joint pain and negative moods and are much more likely to have higher levels of general social support. Positive support from a spouse or other family member is inversely related to depression and can significantly enhance quality of life.[88] Patients who lack support or experience other challenges (e.g., histories of abuse, somatization disorders, obsessive-compulsive disorders) often rely on their healthcare providers for these needs. A supportive and validating doctor–patient relationship is vital in diseases like RA. Counseling is often appropriate to explore common themes experienced by patients with RA, such as coping with severe pain, self-esteem issues, negative feelings, reflections on the past, concentration on recovery from disease, and general support.

THERAPEUTIC APPROACH

RA is often an aggressive disease that requires aggressive treatment. Foremost is the use of diet to reduce the causes and ameliorate the symptoms of RA. Symptom relief can also be attained using physical medicine techniques, botanicals, and nutrients. In severe cases, NSAIDs and other drugs may be necessary, at least in the acute phase. However, the physician should encourage patients not to abandon natural measures because they may enhance the effectiveness of the drugs, allow for lower doses when drugs are necessary, and provide a foundation for healing by addressing the underlying causative factors.

Diet

The first step is a therapeutic fast or elimination diet, followed by careful reintroduction of individual foods to detect those that trigger symptoms. Although any food can cause a reaction, the most common are wheat, corn, dairy products, beef, nightshade family foods (tomato, potato, eggplant, peppers), pork, citrus, oats, rye, egg, coffee, peanuts, cane sugar, lamb, and soy.

After all allergens have been isolated and eliminated, a vegetarian or Mediterranean-style diet rich in organic whole foods, vegetables, cold-water fish (e.g., mackerel, herring, sardines, salmon), olive oil, and berries and low in sugar, meat, refined carbohydrates, and animal fats is indicated.

Supplements

- High-potency multiple-vitamin-and-mineral formula, as described in Chapter 20, Laboratory Tests for the Determination of Vitamin Status
- Fish oil: 3000 mg EPA+DHA a day
- Vitamin C: 500 to 1000 mg/day
- Selenium: 200 to 400 mcg/day
- Vitamin E (mixed tocopherols): 200 to 400 IU/day
- Vitamin D3: 2000 to 4000 IU/day
- A flavonoid-rich extract or other plant-based antioxidant that can provide an oxygen radical absorption capacity (ORAC) of 3000 to 6000 units or higher per day
- Grape seed extract (>95% procyanidolic oligomers): 100 to 300 mg/day
- Pine bark extract (>95% procyanidolic oligomers): 100 to 300 mg/day
- Probiotic (*Lactobacillus* and *Bifidobacter* sp.): minimum of 5 to 10 billion colony forming units daily
- Pancreatin (10× USP): 350 to 750 mg between meals three times daily; or Bromelain: 250 to 750 mg (1800–2000 MCU) between meals three times a day

Botanical Medicines

The following botanicals may be used alone or in combination with others. Severe inflammation and joint destruction require more aggressive therapy.

- Curcumin:
  - Curcumin: 400 mg three times a day between meals

  or
  - Meriva: 1000 to 2000 mg/day

  or
- Theracurmin: 600 to 1200 mg/day
- Ginger: 8 to 10 g of dried ginger or ginger extracts standardized to contain 20% gingerol and shogaol at a dosage of 100 to 200 mg three times a day
- *T. wilfordii Hook F:* 360 to 570 mg/day of ethyl acetate extract
- *V. officinalis:* 1.5 to 3 g/day of dried herb

Physical Medicine

- Heat (moist packs, hot baths, etc.): 20 to 30 minutes, one to three times daily
- Cold packs for acute flareups or following heat: 20 minutes one to three times daily
- Active or passive range-of-motion exercises: 3 to 10 repetitions once or twice a day
- Progressive isometric (and isotonic as the joints improve) exercise: 3 to 10 repetitions several times a day with generous periods of rest

Counseling/Psychological Therapy

A supportive and validating doctor–patient relationship should be established. Priorities include stress management, evaluation for depression, and review of the patient's support network. Attempts should be made to work with spouses and family members if possible.

OTHER CONSIDERATIONS

Positive results from other testing may indicate DHEA, testosterone, vitamin D, and therapeutics for intestinal permeability, SIBO, dysbiosis, and environmental toxicity.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *Am J Manag Care*. 2012;18(suppl 13):S295–S302. 23327517.
2. van de Merwe JP, Stegeman JH, Hazenberg MP. The resident faecal flora is determined by genetic characteristics of the host: implications for Crohn's disease? *Antonie Leeuwenhoek*. 1993;49:119–124.
3. Padyukov L, Silva C, Stolt P, et al. A gene-environment interaction between smoking and shared epitope genes HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum*. 2004;50:3085–3092.
4. Ahmed SA. The immune system as a potential target for environmental estrogens (endocrine disruptors): a new emerging field. *Toxicology*. 2000;150(1–3):191–206. PubMed PMID: 10996675.
5. Lundberg I, Alfredsson L, et al. Occupation, occupational exposure to chemicals and rheumatological disease. A register based cohort study. *Scand J Rheumatol*. 1994;23(6):305–310. PubMed PMID: 7801054.
6. Rosenman KD, Moore-Fuller M, et al. Connective tissue disease and silicosis. *Am J Ind Med*. 1999;35:375–381. PubMed PMID: 10086214.
7. Turner S, Cherry N. Rheumatoid arthritis in workers exposed to silica in the pottery industry. *Occup Environ Med*. 2000;57:443–447. PubMed PMID: 10854495.
8. Zeft AS, Prahalad S, Lefevre S, Clifford B, McNally B, Bohnsack JF, et al. Juvenile idiopathic arthritis and exposure to fne particulate air pollution. *Clin Exp Rheumatol*. 2009;27(5):877–884. PubMed PMID: 19917177.
9. Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. *Environ Health Perspect*. 2007;115(6):883–888. PubMed PMID: 17589595.
10. Afridi HI, Kazi TG, Talpur FN, Naher S, Brabazon D. Relationship between toxic metals exposure via cigarette smoking and rheumatoid arthritis. *Clin Lab*. 2014;60(10):1735–1745. PubMed PMID: 25651721.
11. Comstock GW, Burke AE, Hoffman SC, et al. Serum concentrations of α tocopherol, beta carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis*. 1997;56(5):323–325.
12. Ling S, Li Z, Borschukova O, et al. The rheumatoid arthritis shared epitope increases susceptibility to oxidative stress by antagonizing an adenosine-mediated anti-oxidative pathway. *Arthritis Res Ther*. 2007;9(1):1–5. R5.
13. Seven A, Guzel S, Aslan M, Hamuryudan V. Lipid, protein, DNA oxidation and antioxidant status in rheumatoid arthritis. *Clin Biochem*. 2008;41(7–8):538–543. PubMed PMID: 18313405.
14. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest*. 2001;108(8):1097–1104. PubMed PMID: 11602615.
15. Rashid T, Jayakumar KS, Binder A, et al. Rheumatoid arthritis patients have elevated antibodies to cross-reactive and non cross-reactive proteus microbes. *Clin Exp Rheumatol*. 2007;25(2):259–267.
16. Hooper LV, Wong MH, Thelin A, et al. Olecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001;291(5505):881–884.
17. Henrikksson AE, Blomquist L, Nord CE, et al. Small intestinal bacterial overgrowth in patients with rheumatoid arthritis. *Ann Rheum Dis*. 1993;52(7):503–510.
18. Henriksson AE, Blomquist L, Nord CE, Midtvedt T, Uribe A. Small intestinal bacterial overgrowth in patients with rheumatoid arthritis. *Ann Rheum Dis*. 1993;52(7):503–510. PubMed PMID: 8346978.
19. Patton T, Jukic D, Juhas E. Atypical histopathology in bowel-associated dermatosis-arthritis syndrome: a case report. *Dermatol Online J*. 2009;15(3):3. PubMed PMID: 19379647.
20. Martinez-Martinez RE, Abud-Mendoza C, Patiño-Marin N, et al. Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. *J Clin Periodontol*. 2009;36(12):1004–1010.
21. Mercado FB, Marshall RI, Klestov AC, et al. Relationship between rheumatoid arthritis and periodontitis. *J Periodontol*. 2001;72(6):779–787.
22. Hitchon CA, Chandad F, Ferucci ED, et al. Antibodies to porphyromonas gingivalis are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. *J Rheumatol*. 2010;37(6):1105–1112.
23. Havatum M, Kanerud L, Hällgren R, et al. The gut-joint axis: cross-reactive food antibodies in rheumatoid arthritis. *Gut*. 2006;55:1240–1247.
24. Salgado E, Bes-Rastrollo M, et al. High sodium intake is associated with self-reported rheumatoid arthritis: a cross sectional and case control analysis within the SUN cohort. *Medicine (Baltimore)*. 2015;94(37):e924. PubMed PMID: 26376372.
25. Pawlik A, Ostanek L, Brzosko I, et al. The influence of N-acetyltransferase 2 polymorphism on rheumatoid arthritis activity. *Clin Exp Rheumatol*. 2004;22(1):99–102.
26. Vojdani A, Pangborn JB, Vojdani E, et al. Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism. *Int J Immunopathol Pharmacol*. 2003;16(3):189–199.
27. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, et al. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scand J Gastroenterol*. 2006;41(4):408–419.
28. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev*. 2011;91(1):151–175. PubMed PMID: 21248165.
29. Cutolo M, Balleari E, Giusti M, et al. Androgen replacement therapy in male patients with rheumatoid arthritis. *Arthritis Rheum*. 1991;34(1):1–5.
30. Aletaha D, Neogi T, Silman AJ. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis*. 2010;69(9):1580–1588.
31. Myasoedova E, Gabriel SE. Cardiovascular disease in rheumatoid arthritis: a step forward. *Curr Opin Rheumatol*. 2010;22(3):342–347.
32. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of RA and related disorders. *Br J Nutr*. 2001;85:251–269.
33. De Berardis G, Lucisano G, et al. Association of aspirin use with major bleeding in patients with and without diabetes. *JAMA*. 2012;307(21):2286–2294. PubMed PMID: 22706834.
34. Tournoij E, Peters RJ, et al. The prevalence of intolerance for low-dose acetylsalicylacid in the secondary prevention of atherothrombosis. *Eur J Vasc Endovasc Surg*. 2009;37(5):597–603. PubMed PMID: 19297216.
35. Lee WM. Drug-induced acute liver failure. *Clin Liver Dis*. 2013;17(4):575–586. PubMed PMID: 24099019.
36. Inotai A, Mészáros A. Economic evaluation of nonsteroidal antiinflammatory drug strategies in rheumatoid arthritis. *Int J Technol Assess Health Care*. 2009;25(2):190–195.
37. Sihvonen S, Korpela M, Mustonen J, et al. Mortality in patients with rheumatoid arthritis treated with low-dose oral glucocorticoids: a population-based cohort study. *J Rheum*. 2006;33(9):1740–1746.
38. Mahowald MW, Mahowald ML, Bundlie SR, et al. Sleep fragmentation in rheumatoid arthritis. *Arthritis Rheum*. 1989;32(8):974–983.
39. Anonymous. Rheumatoid arthritis: choice of antirheumatic treatment. Methotrexate first. *Prescrire Int*. 2010;19(105):30–34.
40. Lee SJ, Kavanaugh A. Pharmacological treatment of established rheumatoid arthritis. *Best Pract Res Clin Rheumatol*. 2003;17:811–829.
41. Dahlqvist SR, Engstrand S, Berglin E, et al. Conversion toward an atherogenic lipid profile in rheumatoid arthritis patients during long-term infliximab therapy. *Scand J Rheumatol*. 2006;35:107–111.
42. Trowell H, Burkitt D. *Western Diseases: Their Emergence and Prevention.* Cambridge, MA:Harvard University Press
43. Darlington LG. Dietary therapy for arthritis. *Rheum Dis Clin North Am*. 1991;17:273–285.
44. Fraser DA, Thoen J, Djøseland O, et al. Serum levels of interleukin-6 and dehydroepiandrosterone sulphate in response to either fasting or a ketogenic diet in rheumatoid arthritis patients. *Clin Exp Rheumatol*. 2000;18(3):357–362.
45. Kjeldsen-Kragh J, Hougen M, Borchgrevink CF, et al. Controlled trial of fasting and one-year vegetarian diet in rheumatoid arthritis. *Lancet*. 1991;338(8772):899–902.
46. Bykerk VP, Keystone EC. What are the goals and principles of management in the early treatment of rheumatoid arthritis? *Best Pract Res Clin Rheumatol*. 2005;19(1):147–161.

47. Shapiro JA, Koepsell TD, Voigt LF, et al. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. *Epidemiology*. 1996;7(3):256–263.
48. Grant WB. The role of meat in the expression of rheumatoid arthritis. *Br J Nutr*. 2000;84:589–595.
49. Waterman E, Lockwood B. Active components and clinical applications of olive oil. *Alt Med Rev*. 2007;12(4):331–342.
50. Jantti J, Nikkari T, Solakivi T, et al. Evening primrose oil in rheumatoid arthritis: changes in serum lipids and fatty acids. *Ann Rheum Dis*. 1989;48(2):124–127.
51. Leventhal LJ, Boyce EG, Zurier RB. Treatment of rheumatoid arthritis with gammalinoleic acid. *Ann Intern Med*. 1993;119:867–873.
52. Mantzioris E, James MJ, Gibson RA, et al. Dietary substitution with α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am J Clin Nutr*. 1994;59:1304–1309.
53. Kelley DS. α-linolenic acid and immune response. *Nutrition*. 1992;8:215–217.
54. Ariza-Ariza R, Mestanza-Peralta M, Cardiel MH. Omega-3 fatty acids in rheumatoid arthritis: an overview. *Semin Arthritis Rheum*. 1998;27:366–370.
55. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. *Ann Nutr Metab*. 2009;55:123–139.
56. Edmonds SE, Winyard PG, Guo R, et al. Putative analgesic activity of repeated oral doses of vitamin E in the treatment of rheumatoid arthritis: results of a placebo controlled double blind trial. *Ann Rheum Dis*. 1997;56:649–655.
57. Zoli A, Altomonte L, Caricchio R, et al. Serum zinc and copper in active rheumatoid arthritis: correlation with interleukin 1 beta and tumour necrosis factor α. *Clin Rheumatol*. 1998;17(5):378–382.
58. Peretz A, Neve J, Famaey JP. Effects of chronic and acute corticosteroid therapy on zinc and copper status in rheumatoid arthritis patients. *J Trace Elem Electrolytes Health Dis*. 1989;3(2):103–108.
59. Menander-Huber KB. Orgotein in the treatment of rheumatoid arthritis. *Eur J Rheumatol Inflamm*. 1981;4:201–211.
60. de Rosa GD, Keen CL, Leach RM, et al. Regulation of superoxide dismutase activity by dietary manganese. *J Nutr*. 1980;110(4):795–804.
61. Hagfors L, Leanderson P, Skoldstam L, et al. Antioxidant intake, plasma antioxidants and oxidative stress in a randomized, controlled, parallel, Mediterranean dietary intervention study on patients with rheumatoid arthritis. *Nutr J*. 2003;2:5.
62. Anonymous. General Practitioner Research Group. Calcium pantothenate in arthritic conditions. *Practitioner*. 1980;224:208–211.
63. Chiang EP, Bagley PJ, Selhub J, et al. Abnormal vitamin B_6 status is associated with severity of symptoms in patients with rheumatoid arthritis. *Am J Med*. 2003;114(4):283–287.
64. Sorenson JRJ, Hangarter W. Treatment of rheumatoid and degenerative disease with copper complexes: a review with emphasis on copper salicylate. *Inflammation*. 1977;2:217–238.
65. Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol*. 2008;4(8):404–412. PubMed PMID: 18594491.
66. Cutolo M, Otsa K, Laas K, et al. Circannual vitamin d serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. *Clin Exp Rheumatol*. 2006;24(6):702–704.
67. Kröger H, Penttilä IM, Alhava EM. Low serum vitamin D metabolites in women with rheumatoid arthritis. *Scand J Rheumatol*. 1993;22(4):172–177.
68. Kanerud L, Hafström I, Berg A. Effects of antirheumatic treatment on gastric secretory function and salivary flow in patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 1991;9:595–601.
69. Hatakka K, Martin J, Korpela M, et al. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis: — a pilot study. *Scand J Rheumatol*. 2003;32(4):211–215.
70. Mandel DR, Eichas K, Holmes J. *Bacillus coagulans*: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. *BMC Complement Altern Med*. 2010;10:1.
71. Flynn DL, Rafferty MF, Boctor AM. Inhibition of 5-hydroxy-eicosatetraenoic acid (5-HETE) formation in intact human neutrophils by naturally-occurring diarylheptanoids: inhibitory activities of curcuminoids and yakuchinones. *Prostaglandins Leukot Med*. 1986;22:357–360.
72. Shishodia S, Sethi G, Aggarwal BB. Curcumin: getting back to the roots. *Ann N Y Acad Sci*. 2005;1056:206–217.
73. Deodhar SD, Sethi R, Srimal RC. Preliminary studies on antirheumatic activity of curcumin (diferuloyl methane). *Indian J Med Res*. 1980;71:632–634.
74. Sasaki H, Sunagawa Y, Takahashi K, et al. Innovative preparation of curcumin for improved oral bioavailability. *Biol Pharm Bull*. 2011;34(5):660–665.
75. Yang CM, Chen YW, et al. Resveratrol inhibits BK-induced COX-2 transcription by suppressing acetylation of AP-1 and NF-kβ in human rheumatoid arthritis synovial fibroblasts. *Biochem Pharmacol*. 2017;132:77–91. PubMed PMID: 28288820.
76. Gu H, Jiao Y, et al. Resveratrol inhibits the IL-1β-induced expression of MMP-13 and IL-6 in human articular chondrocytes via TLR4/MyD88-dependent and -independent signaling cascades. *Int J Mol Med*. 2017;39(3):734–740. PubMed PMID: 28204817.
77. Kim SY, Han SW, Kim GW, et al. TGF-beta1 polymorphism determines the progression of joint damage in rheumatoid arthritis. *Scand J Rheumatol*. 2004;33(6):389–394.
78. Srivastava KC, Mustafa T. Ginger *(Zingiber officinale)* and rheumatic disorders. *Med Hypothesis*. 1989;29:25–28.
79. Srivastava KC, Mustafa T. Ginger *(Zingiber officinale)* in rheumatism and musculoskeletal disorders. *Med Hypotheses*. 1992;39:342–348.
80. Tao X, Younger J, Fan FZ, et al. Benefit of an extract of *Trypterygium wilfordii Hook F* in patients with rheumatoid arthritis: a double-blind, placebo-controlled study. *Arthritis Rheum*. 2002;46(7):1735–1743.
81. Goldbach-Mansky R, Wilson M, Fleischmann R, et al. Comparison of *Tripterygium wilfordii Hook F* versus sulfasalazine in the treatment of rheumatoid arthritis: a randomized trial. *Ann Intern Med*. 2009;151(4):229–240. W49-W51.
82. Li RL, Liu PL, Wu Xc. Clinical and experimental study on sustained release tablet of *Tripterygium wilfordii* in treating rheumatoid arthritis. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 1996;16(1):10–13.
83. Cibere J, Deng Z, Lin Y, et al. A ramdomized double blind, placebo controlled trial of topical *Tripterygium wilfordii* in rheumatoid arthritis: reanalysis using logistic regression analysis. *J Rheumatol*. 2003;30(3):465–467.
84. Li YS, Tong PJ, Ma HZ. Toxicity attenuation and efficacy potentiation effect of liquorice on treatment of rheumatoid arthritis with *Tripterygium wilfordii*. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2006;26(12):1117–1119.
85. Wu YJ, Lao ZY, Zhang ZL. Clinical observation on small doses *Trypterygium wilfordii* polyglycoside with methotrexate in treating rheumatoid arthritis. *Zhongguo Xi Yi Jie He Za Zhi*. 2001;21(12):895–896.
86. Taibi DM, Bourguignon C, Taylor AG. Valerian use for sleep disturbances related to rheumatoid arthritis. *Holist Nurs Pract*. 2004;18(3):120–126.
87. de Jong Z, Munneke M, Zwinderman AH, et al. Is a long-term high-intensity exercise program effective and safe in patients with rheumatoid arthritis? Results of a randomized controlled trial. *Arthritis Rheum*. 2003;48(9):2415–2424.
88. Kraaimaat FW, Van Dam-Baggen RM, Bijlsma JW. Association of social support and the spouse's reaction with psychological distress in male and female patients with rheumatoid arthritis. *J Rheumatol*. 1995;22:644–648.

Rosacea

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Chronic acneiform eruption on the face of middle-aged and older adults associated with facial flushing and telangiectasia
- The acneiform component is characterized by papules, pustules, and seborrhea; the vascular component by erythema and telangiectasia; and the glandular component by hyperplasia of the soft tissue of the nose (rhinophyma).
- The primary involvement occurs over the flush areas of the cheeks and nose.
- More common in women (3:1) but more severe in men

GENERAL CONSIDERATIONS

Rosacea is a common, chronic, progressive inflammatory skin disorder in which the nose and cheeks are abnormally red and may be covered with pimples similar to those seen in acne (see Chapter 141). Rosacea was originally called "acne rosacea" because its inflammatory papules and pustules so closely mimic those of acne vulgaris. Unlike acne vulgaris, whose etiology is based on the interaction of abnormal keratinization, increased sebum production, and bacterially induced inflammation, rosacea's inflammation is vascular in nature. Rosacea generally occurs in patients between the ages of 25 and 70 years, and it is much more common in people with fair complexions. Women are three times more likely than men to have rosacea, although the disease is generally more severe in men (Fig. 216.1). At least 13 million Americans are known to be affected.[1,2]

Rosacea is divided into three stages, but because progression does not necessarily occur, rosacea is also often divided into four specific subtypes (erythematous telangiectatic, papulopustular, phymatous, and ocular)[1,3,4]:

- *Stage I:* In this stage (erythematous telangiectatic rosacea), erythema triggered by hot beverages, spicy foods, and alcohol may persist for hours. Telangiectasias are noticeable on the central third of the face, and burning, stinging, and itching after the application of cosmetics, fragrances, and sunscreens become a major complaint.
- *Stage II:* In this stage (papulopustular rosacea), the hallmarks are inflammatory papules and pustules. Flushing, telangiectasias, and seborrhea increase, and minimal enlargement of facial pores becomes obvious.
- *Stage III:* A small number of patients progress to stage III (phymatous rosacea), which exhibits deep inflammatory nodules, large telangiectatic vessels, markedly dilated facial pores, sebaceous gland hyperplasia, and tissue hyperplasia, especially of the nose (rhinophyma).

Ocular rosacea describes the spectrum of eye findings associated with the skin involvement. Ocular rosacea can cause the eyes to have a watery or bloodshot appearance, the sensation of a foreign body, burning or stinging, dryness, itching, light sensitivity, and a host of other signs and symptoms. Styes are a common sign of rosacea-related ocular disease, and some individuals may have decreased visual acuity owing to corneal complications.

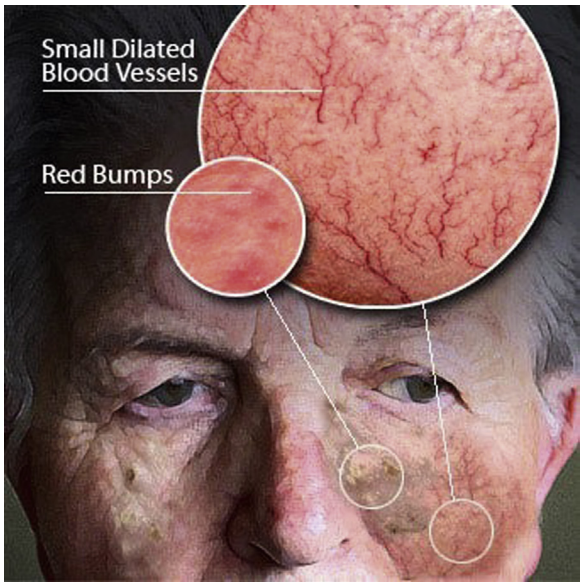


Fig. 216.1 Rosacea.

It is important to point out that the prolonged nature and intensity differentiate the flushing that rosacea patients experience. Many people without rosacea experience evanescent flushing in response to embarrassment, exercise, or hot environments. However, although evanescent flushing episodes last from several seconds to few minutes, the flushing that the typical rosacea patient describes lasts longer than 10 minutes and is more red than pink, with an accompanying burning or stinging sensation. The stimuli that generate flushing in rosacea patients may be acutely felt emotional stress, hot drinks, alcohol, spicy foods, exercise, cold or hot weather, or hot baths or showers. However, the episodes are often without known stimuli.

Etiology

The cause of rosacea is poorly understood, although numerous theories have been offered. Included in the factors that have been suspected of causing acne rosacea are the following:

- The mite *Demodex folliculorum*
- Alcoholism
- Menopausal flushing
- Vasomotor neurosis
- Seborrheic diathesis
- Local infection
- Food allergies
- B-vitamin deficiencies
- Gastrointestinal disorders

Most cases of rosacea are associated with moderate to severe seborrhea, although sebum production is not increased in many. Vasomotor lability is prevalent, and migraine headaches are three times more common in persons with rosacea than in age- and gender-matched controls.

There is also emerging evidence for a role for *Helicobacter pylori* in rosacea. It is known that *H. pylori* infection increases several vasoactive substances, such as histamines, prostaglandins, and leukotrienes, and various cytokines. However, these vascular mediators are found only with *H. pylori* strains that also produce a specific cytotoxin, cytotoxin-associated gene A (CagA). The presence of *H. pylori* capable of producing this cytotoxin may be more important in the etiology of rosacea than other strains. When the presence of CagA was assessed in 60 rosacea patients and compared with age- and gender-matched control subjects with nonulcer dyspepsia, researchers found that when infected with *H. pylori,* 67% of rosacea patients versus only 32% of controls had positive findings for CagA. In addition, these patients had elevated systemic levels of tumor necrosis factor-α and interleukin 8. After the eradication of *H. pylori* infection in the rosacea patients, symptoms disappeared in most (51 of 53), and levels of tumor necrosis factor-α and interleukin 8 normalized.[5,6]

Ultraviolet (UV) radiation is a well-known rosacea trigger. Exposure to UV radiation may cause flushing and worsening of rosacea symptoms. UV-A promotes the expression of matrix metalloproteinase (MMP) and causes collagen denaturation, whereas UV-B increases the production of fibroblast growth factor 2 and vascular endothelial growth factor. Compared with healthy controls, rosacea patients have higher levels of reactive oxygen species, which promote production of inflammatory mediators by keratinocytes and fibroblasts.[7] Correlations have also been made between rosacea and alcohol and smoking.

Regarding the etiology of rosacea, because many of the implicated triggers are experienced by healthy persons who never go on to develop the symptoms or signs of rosacea, it is believed that rosacea-prone individuals have an inherent sensitivity to these triggers.

THERAPEUTIC CONSIDERATIONS

One of the first recommendations to the patient with rosacea is to avoid those stimuli that tend to exacerbate the disease: exposure to extremes of heat and cold, excessive sunlight, and ingestion of hot liquids, alcohol, and spicy foods. The conventional medical treatment of rosacea is usually oral tetracycline, especially for the papular or pustular lesions, although this treatment only controls rather than eradicates the disease. Topical therapy for rosacea with antibiotics or synthetic retinoids is generally less successful than systemic antibiotic treatment. Also, although topical corticosteroids may initially improve signs and symptoms, long-term corticosteroid therapy is not advisable because it may actually lead to rosacea. The treatment of chronic skin changes and severe rhinophyma may require laser treatments and surgical intervention, respectively.

The natural approach in treating rosacea is to identify and eliminate contributing factors if possible. Key factors to address are hypochlorhydria, eradication of *H. pylori,* elimination of food allergies, and optimal intake of B vitamins.

Hypochlorhydria

Gastric analysis of patients with rosacea has led to the postulate that it is the result of hypochlorhydria.[1] Psychological factors, such as worry, depression, and stress, often reduce gastric acidity.[2] Hydrochloric acid supplementation results in a marked improvement in those patients with rosacea who have achlorhydria or hypochlorhydria.[5,8] Patients with rosacea have also been shown to have diminished secretion of lipase (although bicarbonate and chymotrypsin secretion were normal) and to benefit from pancreatic supplementation.[6]

Helicobacter pylori

Given the high incidence of hypochlorhydria, it is not surprising that a high incidence of *H. pylori* overgrowth in the stomach has been found in patients with rosacea.[9] In a pilot study, *H. pylori* was found in 46 of 94 patients with rosacea, 38 of 88 patients with other inflammatory diseases, and 5 of 14 patients without an inflammatory disease. The researchers believed that the flushing reaction in rosacea is caused by gastrin or vasoactive intestinal peptides. They also quoted an Irish study that found that 19 of 20 patients with acne rosacea tested positive for *H. pylori.*

Another study that evaluated histological sections of the stomach mucosa found that 84% of patients ($n = 31$) were *H. pylori*–positive.[10] Interestingly, 20% of the patients who tested histologically positive also tested serologically negative for the organism. The consistency between clinical success in the treatment of rosacea with metronidazole and the abatement of *H. pylori* isolates and serological results after treatment provides additional evidence suggesting an etiological relationship between rosacea and *H. pylori* infection. However, it is also possible that *H. pylori* is simply associated with rosacea and is not a causative factor per se, because patients with rosacea may have rates of *H. pylori* infection similar to those in healthy subjects.[11] Interestingly, patients with rosacea complain significantly more frequently of indigestion and use more antacids than the general population.[11]

Many studies have confirmed a strong association between *H. pylori* and rosacea, with a 2003 study showing a correlation between the severity of rosacea and the level of infection.[12] A single-arm clinical trial demonstrated a 92% (138/150) cure rate among patients with rosacea after receiving eradication therapy for *H. pylori*.[13]

Food Allergy

The statistically significant incidence of indigestion and migraine headaches accompanying rosacea points to food intolerance, as does the reflex flushing caused by vasodilating substances. Some of the later studies seem to support the concept of rosacea as a hypersensitivity disorder. Although these studies have focused on local factors, it is possible that food hypersensitivity plays a significant role given the known association of rosacea with dietary factors exacerbating the skin flushing.

B Vitamins

The administration of large doses of B vitamins has been shown to be quite effective,[14] with riboflavin appearing to be the key factor. For example, researchers examining B vitamins were able to infect the skin of riboflavin-deficient rats with the mite *D. folliculorum*, but not the skin of normal rats.[15] This mite was once considered a causative factor in rosacea and may still be a factor in some patients, especially those with more granulomatous lesions. Evidence suggests that a delayed hypersensitivity reaction in follicles is triggered by *D. folliculorum* antigens, stimulating the progression of the affection to the papulopustular stage.[16]

Although B vitamins are important for patients with rosacea, care must be exercised because some patients' rosacea may be aggravated by large doses of these nutrients. There is a case report of a 53-year-old woman who presented to a dermatology clinic with a 9-month history of a facial eruption resembling acne rosacea. Treatment with oral hydroxychloroquine, ibuprofen, terfenadine, prednisone, erythromycin, and tetracycline had been tried during the 9 months without success. Topical desoximetasone, hydrocortisone, and cosmetic elimination also yielded no benefit. A patch test showed a positive reaction to nickel. The eruption began at the time of personal stress as the patient was going through a marital separation. To help with her stress, the patient began taking 100 mg/day of pyridoxine and 100 mg/day of vitamin B_{12}. Discontinuation of the vitamins resulted in a dramatic improvement; with rechallenge, the condition reappeared. The investigators noted that inflammation and exacerbations of acne related to vitamins B_2, B_6, and B_{12} have been reported in the European literature.[17]

Zinc

Zinc supplementation has been shown to be helpful in acne vulgaris and may also be effective in acne rosacea. To test this hypothesis, 25 patients with rosacea were evaluated and given a clinical score, then randomly allocated to receive either zinc (23 mg from zinc sulfate) or identical placebo capsules three times daily. After 3 months, the patients crossed over. A total of 19 patients completed the study. In the group started on zinc, the scores before therapy ranged from 5 to 11. The mean started to decrease directly after the first month of therapy with zinc sulfate to a significantly lower level. After shifting to placebo treatment, the mean started to rise gradually in the fifth month but remained significantly lower than the levels before therapy. In the group started on placebo, the score before therapy ranged from 5 to 9. The mean remained high in the first 3 months of therapy while the patients were on placebo. After shifting to zinc sulfate, the mean started to decrease after the fourth month to significantly low levels. No significant side effects were reported apart from mild gastric upset in three (12%) patients on zinc sulfate. The authors concluded that zinc is a good option in the treatment of rosacea because it was found to be safe and effective and had no significant side effects.[18]

Topical Azelaic Acid

The topical application of azelaic acid (AzA) appears to be extremely effective in papulopustular rosacea. Initially, AzA was released in a 20% cream formulation and was shown in this vehicle to be effective in the treatment of mild to moderate rosacea. A 15% gel formulation of AzA vastly improved the delivery of AzA and has been shown to be superior in head-to-head studies to the 20% AzA cream. It is equally as effective as metronidazole cream or gel.[19–21] In a meta-analysis of five double-blind trials involving topical azelaic acid (cream or gel) for the treatment of rosacea compared with placebo or other topical treatments, four of five studies demonstrated significant decreases in mean inflammatory lesion count and erythema severity after treatment with AzA compared with placebo, and AzA was found to be equal to metronidazole in papulopustular rosacea. However, no significant decrease in the severity of telangiectasia occurred in any treatment group.[20]

Overexpression of cathelicidin peptide LL-37 has been implicated in the pathophysiology of rosacea, and AzA has been found to inhibit the pathological expression of cathelicidin, as well as the hyperactive protease activity that cleaves cathelicidin into LL-37. A small, prospective, open-label, interventional study was performed to assess the effects of azelaic acid 15% gel on inflammatory lesions of papulopustular rosacea.[22] AzA use was associated with a significant reduction in inflammatory lesions, and these results persisted beyond the active treatment phase.

Kanuka Honey

A pilot study of topical medical-grade kanuka honey as a treatment for rosacea found it to be an acceptable and potentially effective treatment.[23] A parallel-group, randomized, controlled trial with assessor blinding was undertaken at a hospital-based research facility to investigate the efficacy of topical 90% medical-grade kanuka honey and 10% glycerine as a treatment for rosacea.[24] In this study, 138 adults with a diagnosis of rosacea were equally divided into each treatment arm. Participants were randomly allocated to receive control cream (Cetomacrogol) or kanuka honey. After 8 weeks of kanuka honey treatment, about one third of participants had a clinically significant improvement, twofold greater than that observed with the control treatment.

Physical Modalities and Devices

Laser therapy, including vascular lasers or intense pulsed light, may help to reduce refractory background erythema and clinically significant telangiectases, but it will not reduce the frequency of flushing episodes. These should be administered by an experienced and trained laser therapist, and the number of sessions and length of treatment vary for each individual.[25]

Thirty patients with rosacea were enrolled in a randomized, controlled, split-face study to evaluate the efficacy of radiofrequency compared with pulsed dye laser (PDL).[26] Patients were treated with radiofrequency on one side and pulsed dye laser on the other side, with each treatment consisting of three sessions at 4-week intervals and followed up until 4 weeks after the last treatment. Radiofrequency and PDL resulted in significant improvement in severity scores and erythema, and 70% of the patients receiving radiofrequency showed a clinical improvement of >50%. Although no significant difference was noted between radiofrequency and PDL treatment in erythematotelangiectatic rosacea, radiofrequency treatment led to a significantly greater decrease in papulopustular lesion count and routine severity score in papulopustular rosacea.

The effect of fractional microneedling radiofrequency (FMR) on rosacea was studied in a 12-week, prospective, randomized, split-face, clinical trial.[27] Two sessions of FMR were performed on one side of the cheeks with a 4-week interval, and the other side remained untreated. The erythema index decreased by 13.6% at week 12 compared with baseline, and reduced expression of markers related to inflammation, innate immunity, and angiogenesis was observed in immunohistochemical staining of tissue obtained after FMR treatment.

THERAPEUTIC APPROACH

Although the causes of rosacea have not yet been determined, sufficient information is available to treat most affected patients adequately. Eradication of *H. pylori* infection (when present) and control of hypochlorhydria and food intolerance form the basis of therapy.[28] This approach is supported with supplementation of B-complex vitamins and the avoidance of vasodilating foods.

General Recommendations

See Chapter 141 for general recommendations for acne.

Diet

The patient should avoid coffee, alcohol, hot beverages, spicy foods, and any other food or drink that causes a flush. Also to be eliminated from the diet are all refined and/or concentrated sugars; foods containing *trans* fatty acids such as milk, milk products, margarine, shortening, and other synthetically hydrogenated vegetable oils; as well as fried foods and foods high in iodized salt.

Supplements

- Vitamin-B complex: 100 mg/day (avoid niacin)
- Pancreatin (8–10× USP): 350 to 500 mg before meals
- Hydrochloric acid: follow guide provided in Appendix 8
- Zinc: 60 to 75 mg/day for no more than 3 months, followed by 15 to 20 mg/day thereafter
- Topical zinc: application of 15% AzA gel

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Wilkin J, Dahl M, Detmar M, et al. Standard classification of rosacea: report of the National Rosacea Society Expert Committee on the Classification and Staging of Rosacea. *J Am Acad Dermatol*. 2002;46:584–587.
2. Blount BW, Pelletier AL. Rosacea: a common, yet commonly overlooked, condition. *Am Fam Physician*. 2002;66:435–440.
3. Buechner SA. Rosacea: an update. *Dermatology*. 2005;210(2):100–108.
4. Crawford GH, Pelle MT, James WD. Rosacea: I. Etiology, pathogenesis, and subtype classification. *J Am Acad Dermatol*. 2004;51(3):327–341.
5. Poole W. Effect of vitamin B complex and S-factor on acne rosacea. *S Med J*. 1957;50:207–210.
6. Barba A, Rosa B, Angelini G, et al. Pancreatic exocrine function in rosacea. *Dermatologica*. 1982;165:601–606.
7. Ahn CS, Huang WW. Rosacea pathogenesis. *Dermatol Clin*. 2018;36(2):81–86. PubMed PMID: 29499802.
8. Ryle J, Barber H. Gastric analysis in acne rosacea. *Lancet*. 1920;2:1195–1196.
9. Baker B. *Helicobacter pylori* strikes again: this time it's rosacea. *Family Practice News*. 1994:6.
10. Rebora A, Drago F, Parodi A. May *Helicobacter pylori* be important for dermatologists? *Dermatology*. 1995;191:6–8.
11. Sharma VK, Lynn A, Kaminski M, et al. A study of the prevalence of *Helicobacter pylori* infection and other markers of upper gastrointestinal tract disease in patients with rosacea. *Am J Gastroenterol*. 1998;93:220–222.
12. Diaz C, O'Callaghan CJ, Khan A, et al. Rosacea: a cutaneous marker of *Helicobacter pylori* infection? Results of a pilot study. *Acta Derm Venereol*. 2003;83:282–286.
13. Saleh P, Naghavi-Behzad M, Herizchi H, Mokhtari F, Mirza-Aghazadeh-Attari M, Piri R. Effects of *Helicobacter pylori* treatment on rosacea: a single-arm clinical trial study. *J Dermatol*. 2017;44(9):1033–1037. PubMed PMID: 28452093.
14. Tulipan L. Acne rosacea: a vitamin B complex deficiency. *N Y State J Med*. 1929;29:1063–1064.
15. Johnson L, Eckardt R. Rosacea keratitis and conditions with vascularization of the cornea treated with riboflavin. *Arch Ophth*. 1940;23:899.
16. Georgala S, Katoulis AC, Kylafis GD, et al. Increased density of *Demodex folliculorum* and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. *J Eur Acad Dermatol Venereol*. 2001;15:441–444.
17. Sherertz E. Acneiform eruption due to 'megadose' vitamins B_6 and B_{12}. *Cutis*. 1991;48:119–120.
18. Sharquie KE, Najim RA, Al-Salman HN. Oral zinc sulfate in the treatment of rosacea: a double-blind, placebo-controlled study. *Int J Dermatol*. 2006;45(7):857–861.
19. Elewski B, Thiboutot D. A clinical overview of azelaic acid. *Cutis*. 2006;77(suppl 2):12–16.
20. Liu RH, Smith MK, Basta SA, et al. Azelaic acid in the treatment of papulopustular rosacea: a systematic review of randomized controlled trials. *Arch Dermatol*. 2006;142(8):1047–1052.
21. Czernielewski J, Liu Y. Comparison of 15% azelaic acid gel and 0.75% metronidazole gel for the topical treatment of papulopustular rosacea. *Arch Dermatol*. 2004;140(10):1282–1283.
22. Wirth PJ, Henderson Berg MH, Sadick N. Real-world efficacy of azelaic acid 15% gel for the reduction of inflammatory lesions of rosacea. *Skin Therapy Letter*. 2017;22(6):5–7. PubMed PMID: 29091380.
23. Fingleton J, Sheahan D, Cave N, et al. Topical kanuka honey for the treatment of rosacea. *Focus on Alternative and Complementary Therapies*. 2013;18(4):221–222.
24. Braithwaite I, Hunt A, Riley J, et al. Randomized controlled trial of topical kanuka honey for the treatment of rosacea. *BMJ Open*. 2015;5(6):e007651. PubMed PMID: 26109117.
25. Tanghetti E, Del Rosso JQ, Thiboutot D, et al. American Acne and Rosacea Society. Consensus recommendations from the American Acne & Rosacea Society on the management of rosacea, part 4: a status report on physical modalities and devices. *Cutis*. 2014;93(2):71–76. PubMed PMID: 24605343.
26. Kim SJ, Lee Y, Seo YJ, Lee JH, Im M. Comparative efficacy of radiofrequency and pulsed dye laser in the treatment of rosacea. *Dermatol Surg*. 2017;43(2):204–209. PubMed PMID: 27893539.
27. Park SY, Kwon HH, Yoon JY, Min S, Suh DH. Clinical and histologic effects of fractional microneedling radiofrequency treatment on rosacea. *Dermatol Surg*. 2016;42(12):1362–1369. PubMed PMID: 27608206.
28. Szlachcic A, Sliwowski Z, Karczewska E, et al. *Helicobacter pylori* and its eradication in rosacea. *J Physiol Pharmacol*. 1999;50:777–786.

Sarcopenia

David Barry, BS, BAppSci (Hons), DC, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Diagnosis should be suspected in older subjects who are nonambulatory or who cannot rise from a chair unassisted
- Self-reported mobility-related difficulty
- History of recurrent falls
- Recent unintentional weight loss (>5%) in elderly patients
- Recent extended hospital stay or prolonged immobilization
- Presence of both low muscle mass and low muscle function, strength, or performance (European Working Group on Sarcopenia in Older People)
- Gait speed of less than 1 m/sec (International Working Group on Sarcopenia)
- Objectively measured low muscle mass (International Working Group on Sarcopenia)
- Quantified muscle weakness and objectively measured low muscle mass (Foundation for the National Institutes of Health)
- SARC-F scale score ≥4

GENERAL CONSIDERATIONS

Aging is associated with a progressive loss of muscle mass, quality, and strength, resulting in a condition known as sarcopenia. Rosenberg originally proposed the term *sarcopenia* in 1988 to describe the age-related decline in skeletal muscle and physical performance.[1] The term originates from the Greek words *sarx* ("flesh") and *penia* ("loss"). Highly prevalent among older subjects, sarcopenia is associated with a negative prognostic effect on falls, functional independence, disability, and mortality risk. Sarcopenia is a multifactorial phenomenon characterized by changes in muscle morphology, protein and hormonal kinetics, oxidative stress, and inflammation. Lifestyle behaviors such as physical inactivity and poor diet, as well as age-related changes in hormones and cytokine levels, are important risk factors. Sarcopenia can represent both a risk factor for and an adverse event of hospitalization, particularly if the stay is characterized by prolonged immobilization. In a multicenter study of 394 elderly participants, the incidence of sarcopenia during hospitalization was significantly associated with the number of days of bed rest but was not correlated with the total length of stay.[2]

Epidemiology

Sarcopenia is a highly prevalent condition, and wide variations have been reported in the literature, likely due to different criteria for diagnosis[3] and heterogeneity of study populations.[4] Compared with a young reference population, the prevalence of sarcopenia in community-dwelling individuals ranged between 13% and 24% in 60- to 70-year-olds.[5] Based on data from the New Mexico Elder Health Survey, sarcopenia affects about 25% of women between age 70 and 75 years, about 40% of those over age 80 years, and between 20% and 50% of men in the same age ranges.[5] Baumgartner later recognized that these estimates, based on bioelectric impedance measurements, may be biased and published revised prevalence estimates in 2000 based on dual-energy x-ray absorptiometry (DXA) measurements,[6] ranging from 8.8% in women and 13.5% in men aged 60 to 69 years and up to 16% in women and 29% in men older than 80 years. Using similar criteria, only 10% of women had sarcopenia in a cross-sectional study of elderly community-dwelling women in the French EPIDOS study.[7] The age- and sex-adjusted prevalence of sarcopenia varied from 6% to 15% among subjects 65 years of age or older in another population-based survey in the United States.[8] The findings of these studies were based on measurements of muscle mass alone; however, a growing body of research suggests that the prevalence is more accurately predicted when incorporating elements of strength and physical performance in addition to muscle mass.[9–12] A cross-sectional study of 1100 individuals aged 60 years or older living in Brazil determined that the prevalence of sarcopenia was 16% in women and 14% in men, using the European Working Group on Sarcopenia in Older People (EWGSOP) operational definition, which incorporates muscle mass, muscle strength, and physical performance.[13]

Numerous epidemiological and clinical studies report that sarcopenia occurs with advancing age, and the elderly are an increasing proportion of the general population worldwide. Age-related sarcopenia begins in approximately the fifth decade and proceeds, at a population level, at a rate of ~0.8% annually.[14] Sarcopenia occurs to some degree in all individuals as a consequence of aging, but it can be accelerated by a variety of factors, including inactivity, malnutrition, and chronic illness. A high percentage of health care costs for seniors arises from the negative outcomes of the decline in lean muscle mass.[15] The reduction in skeletal muscle mass and strength with advancing age is associated with reduced mobility and disability, hospitalization, and mortality.[16] Mortality was significantly related to age, comorbidity, nutritional status, physical function disability, and frailty in a prospective observational study of elderly hospitalized patients.[17] Metter et al. found that sarcopenia could act as a significant predictor of all-cause mortality.[18]

Sarcopenia represents a significant but modifiable economic burden on health care services worldwide. Sarcopenia has been estimated to result in annual health care costs of approximately $18.5 billion in the United States alone,[19] which represents approximately 1.5% of the total health care expenditures each year. According to statistics from the U.S. Census Bureau, the number of people who are 65 years or older (seniors) equaled 43.1 million in 2012, representing 15% of the U.S. population. By 2050, it is projected that there will be about 83.7 million seniors, representing almost 20% of the population,[20] and healthcare expenditures for seniors in the United States are expected to increase sixfold by 2040.[21] By the year 2050, the global population of older adults (those ≥60 y) is projected to increase from 841 million (2013) to 2 billion, or 21% of the world's population. Moreover, the elderly population is living longer; by 2050, the number of individuals aged ≥80 y is expected to reach 329 million.[22] Improved understanding and treatment of sarcopenia would have a dramatic effect on improving the health and quality of life for the elderly, reducing the associated comorbidity and disability and stabilizing rising health care costs.

PATHOGENESIS

Etiology

Sarcopenia is a universal phenomenon with a complex, multifactorial etiology. Many of the potential causes vary by the age and, to some extent, the gender of the individual. Several processes and mechanisms have been identified that are likely to contribute to the development of sarcopenia, including genetic heritability,[23,24] malnutrition,[25–29] alterations in muscle protein turnover,[30] insulin resistance and altered hormonal status,[31–33] increased production of pro-inflammatory cytokines,[34] loss of α-motor neurons,[35] muscular mitochondrial dysfunction,[36] accelerated myonuclear apoptosis,[37] and impaired satellite cell function.[38,39] The number of muscle fibers begins to decline after the third decade. This development contributes to a decrease in muscle size and an increase in connective-tissue content. The changes that occur in muscle with aging can be categorized as changes in strength, structure, size, fiber type, and contractile characteristics.

Molecular and Biochemical Basis

The aging-related decline in skeletal muscle size and function is well documented; however, it is unclear whether the decline in functional capacity associated with sarcopenia results from the loss of muscle mass and/or the qualitative impairment of muscle tissue. The number of fibers in a muscle and the size of the fibers remain relatively stable from puberty until the fifth decade, at which point a noticeable decline in muscle-fiber size and number begins.[40–42] Lean muscle mass contributes up to 50% of total body weight in young adults but declines with age to 25% at 80 years.[43] Studies have shown that young men have twice as much muscle as fat mass,[44] whereas this ratio is almost reversed in older men. Longitudinal studies have demonstrated a clear decline in skeletal muscle mass, strength, and power beginning at approximately 35 years of age.[45] Muscle mass decreases at a rate of 3% to 5% per decade after the age of 30 years, and its rate of decline accelerates further after the age of 60.[46] Between the ages of 20 and 80 years, there is approximately a 30% reduction in muscle mass[45] and a decline in cross-sectional area of about 20%,[47] which appears to be due to a decline in both muscle-fiber size and number.[48] The reductions in fiber volume may be considerably greater than the percentage declines in cross-sectional area due to the replacement of contractile tissue by connective tissue. In a 7.8-year longitudinal study, Forbes and Reina[49] reported an average loss of 0.25 kg/year of lean muscle mass among participants aged 22 to 53. After 50 years of age, approximately 1% to 2% of muscle mass is expected to be lost per year.[50] Muscle strength also decreases with advancing age and to a greater degree than muscle mass.[51] At the whole-muscle level, there is a reduction in force and a decrease in the number of motor units.[52,53] Muscle strength is reported to decline at an annual rate of approximately 1.5% and accelerates to as much as 3% per year after age 60.[54–57] This decline in muscle function is particularly evident in the lower extremities. The age-associated decline in isometric knee extensor strength has been estimated as being between 55% and 76%.[58] Two longitudinal studies investigating age-related, sex-specific losses of skeletal muscle mass reported losses in both sexes, but the loss of fat-free mass occurred at a faster rate in men even after adjusting for free testosterone, insulin-like growth factor-1 (IGF-1), physical activity, and serum albumin.[59,60]

Muscle atrophy associated with sarcopenia appears to result from a gradual and selective loss of muscle fibers and motor neurons, accompanied by muscle lipid infiltration.[54] Muscle histochemistry in the elderly often shows an unusual pattern of fiber-type distribution. The aging-related decline in fiber size and number affects fast-twitch type II muscle fibers to a greater extent than slow-twitch type I fibers.[47,61] Sarcopenia is characterized by the atrophy of type II muscle fibers and

a reduction in muscle-fiber satellite cells with aging.[62] Early cross-sectional studies demonstrated a shift in muscle-fiber composition, with a higher type I/type II fiber ratio with advancing age.[63] Computed tomography (CT) of individual muscle shows that after age 30 years, there is a decrease in the cross-sectional area of the thigh along with decreased muscle density associated with increased intramuscular fat.[64] Another study has demonstrated that skeletal muscle losses result in a decrease in the total muscle cross-sectional area of about 40% between 20 and 60 years of age, which is even higher in sedentary individuals and twice as high in men compared with women.[57] The combination of a decrease in type II muscle-fiber number, area, and total force production suggests that much of the aging-related decrease in muscle strength is due to a decrease in type II muscle fibers.

Some of the histopathological features of muscle in the elderly therefore include muscle-fiber atrophy (particularly type II fibers), fiber-type grouping, and replacement of muscle fibers with fat and connective tissue. Earlier observations of muscle fibers in the elderly, including visualization under an electron microscope, revealed the following changes[65–70]:

- Hyaline degeneration, lipofuscin pigmentation, and loss of myofibrils
- Necrosis with infiltrations of macrophage cells in degenerating fibers
- Groups of very small fibers with pyknotic nuclei
- Central nucleation of fibers
- Hypertrophy and splitting of muscle fibers
- Increased endomysial connective tissue
- Disorganization of sarcomere spacing, with streaming of Z-discs
- Reductions in mitochondrial size
- Accumulation of reticular material from the sarcoplasmic reticulum and T-tubules

Loss of Neuromuscular Function

Sarcopenia may result from a progressive loss of alpha motor neurons.[35] It has been suggested that the progressive denervation and reinnervation of muscle fibers observed during aging[71–73] may be a mechanism involved in the development of sarcopenia. Yuan et al.[74] found from direct microscopic observation that the number of motor neurons in the spinal cord decreased with aging. Similarly, McNeil and colleagues found differences in the number of motor units in the tibialis anterior of young and older men.[75] Other findings from electrophysiological studies have demonstrated substantive losses of motor units in proximal and distal muscles in both the upper and lower extremities.[76–78] The loss of alpha motor neurons has been estimated on the order of 50%.[79] This decline appears to begin after the seventh decade[80] and affects the lower extremities, with their longer axons, more than the upper limbs.[57] The reduction in the number of alpha motor neurons[54,81] results in a decline in coordinated muscle action and a reduction in muscle strength. These findings, taken together with muscle morphological changes, suggest that age-associated motor-unit loss may be an important contributing factor to sarcopenia.

Protein Metabolism

About 40% of the body's protein is found in muscle, and skeletal muscle mass represents about 43% of the body's mass in healthy adults.[82] Skeletal muscle mass is primarily dictated by the balance between the rates of protein synthesis and degradation, with a net increase in the rate of protein synthesis leading to muscle hypertrophy.[83] The balance between protein synthesis and degradation is crucial in maintaining muscle mass, and this equilibrium may be disrupted in aging. Studies show there is a direct relationship between sarcopenia and changes in muscle metabolism. Balagopal et al.[84] found that the rates of muscle protein synthesis were reduced by 30% with advanced age. Sarcopenia is thought to reflect mainly an age-related decrease in the synthesis of muscle protein rather than an excess catabolic process associated with disease or from reduced caloric intake, although some have hypothesized that low-grade, chronic inflammation with increased protein degradation may contribute.[85–87]

Genetic Influence

The development or progression of sarcopenia may be under significant genetic control. Genetic epidemiological studies suggest that between 36% and 65% of an individual's muscle strength,[88,89] 57% of lower extremity performance,[90] and 34% of the ability to perform the activities of daily living (ADL)[91] are explained by heredity. Results from a number of studies suggest that the heritability of muscle mass and strength may be as high as 60%.[92–96] Polymorphisms of key proteins in the myostatin and other pathways may act as potential mediators of sarcopenia. Cyclin-dependent kinase 2 (CDK2), retinoblastoma (RB1), and IGF-1 were strongly related to muscle strength,[97] and growth/differentiation factor 8 (GDF8), cyclin-dependent kinase inhibitor 1A (CDKN1A), and myogenic differentiation antigen 1 (MYOD1) were identified as positional candidate genes for lower extremity muscle strength.[88] Roth et al. suggest that the ciliary neurotrophic factor gene variant (CNTF A allele) may be related to the loss of muscle power during adulthood, as indicated by findings demonstrating that homozygous individuals had lower quadriceps-strength values.[98] A polymorphism in the angiotensin-converting enzyme *(ACE)* and actinin alpha 3 *(ACTN3)* genes has been shown to influence the response of quadriceps peak power to strength training in older adults.[99] Polymorphisms in the vitamin D receptor (VDR) may be associated with muscle function due to the relationship between vitamin D and its known effects on skeletal muscle.[100,101] Polymorphisms in the VDR were found to be significantly associated with fat-free mass (FFM) and sarcopenia in a cohort of 302 older men.[102] Results from other studies indicate an association of an allelic variant at the VDR locus with muscle strength and body composition in premenopausal women[103] and muscle strength in elderly nonobese women.[104] All of these findings indicate that sarcopenia has a significant heritable component.

There remains considerable unexplained variation in muscle mass and strength among older individuals. This may be partly explained by the observation that muscle mass and strength in later life reflect not only the rate of loss but also the peak attained earlier in life. Most interventional studies to date have focused on factors modifying senescent decline rather than determinants of peak muscle mass and strength attained in early adulthood.

Developmental Influences

Evidence for developmental influences on human muscle has come from a series of observational epidemiological studies linking small size at birth, as a marker of adverse early environmental conditions, with reduced adult muscle mass and strength.[105] Several retrospective cohort studies have investigated whether early life influences may determine the likelihood of developing sarcopenia in later life. In the Hertfordshire Ageing Study, lower birth weight and weight at 1 year were significantly associated with lower grip strength in 700 UK older adults; this association remained significant after allowing for adult size, suggesting that an adverse early life environment may affect the quality of muscle development.[106] A study of older men participating in the Hertfordshire Cohort Study showed that birth weight was significantly positively associated with FFM.[107] Similar findings were observed in studies of women and men using urinary creatinine excretion[108] and dual-energy x-ray absorptiometry[109] to estimate muscle mass. In a study including 2003 participants of the Helsinki Birth

Cohort, low birth weight was found to be associated with lower lean mass and reduced grip strength in adult life.[110] This finding remained consistent after adjusting for adult height, adult body mass index (BMI), and physical activity. These results are in line with the findings of similar studies linking low birth weight with reduced adult lean body mass[111–114] and reduced grip strength,[115,116] which is a significant predictor of survival in elderly men.[117] Lower infant growth was associated with a history of falls in men,[118] whereas standing balance and chair-rise ability were positively predicted by weight gain before age 7 in men[119] independent of later body size, physical activity, or health status. The experience of famine (caloric restriction >1 year) during late childhood was associated with slower walking speed, shorter stride length, and recurrent falls in a study of 4000 community-dwelling older adults in Hong Kong.[120]

Life-course studies suggest that early life programming has an important role in determining functional capacity in aging individuals. Although most muscle growth and fiber differentiation occur postnatally, an initially small reserve of muscle fibers, set at birth, may predispose to premature decline in functional ability via the loss of muscle fiber with aging.[121] The origins of sarcopenia may be found in the prenatal and postnatal environment, and declines in skeletal muscle mass and function during older age may be related to lifestyle factors during early development.[122]

Mitochondrial Dysfunction

Mitochondria are organelles that play an important role in cellular metabolism and regulating nuclear apoptosis. Mitochondrial function may be affected by cumulative damage to muscle mitochondrial DNA (mtDNA) observed with aging. This may result in a reduction of the metabolic rate of the synthesis of muscle-cell protein and ATP,[123] finally leading to the death of muscle fibers and the loss of muscle mass.[87,124] Aging-related declines in the biogenesis of muscle-cell mitochondria[125] as well as the turnover of damaged mitochondria[126] have been observed. It is possible that this decrease in mitochondrial biogenesis and function may contribute to the reductions in aerobic capacity and endurance seen in sarcopenia, although the role of mitochondrial dysfunction in sarcopenia is currently controversial.[36] Some authors report that the decline in mitochondrial function with aging can be attenuated by physical activity,[127] whereas others report that mitochondrial impairment is only partially reversed and that this improvement does not reach the level observed in the young.[87,128,129] Mitochondria appear to play a role in contributing to age-related increases in apoptotic signaling in experimental models.[130]

Accelerated Myonuclear Apoptosis

Accumulating evidence suggests that an age-related acceleration of myonuclear apoptosis may contribute to the onset and progression of sarcopenia. Several reports indicate that enhanced activation of apoptosis occurs in aged skeletal muscle and that myocyte apoptosis is a mechanism underlying sarcopenia.[131] Apoptosis in skeletal muscle displays a unique molecular signature due to the multinucleated nature of myofibrils, and type II fibers appear to be more susceptible than type I.[132] Muscle biopsies of older persons show differences associated with apoptosis compared with those of younger subjects.[133] The mitochondrion is considered the main site for the integration of apoptotic signaling.[134] Mitochondria can induce myocyte apoptosis via caspase-dependent and caspase-independent pathways,[135] although the mediators involved may differ depending on age or muscle-fiber type. Caspases play an important role in mediating cell death. Many of the apoptotic signaling pathways, such as mitochondria-mediated, receptor-mediated, and sarcoplasmic reticulum–mediated pathways, converge in the caspase cascade,[136] and the levels of several caspases are significantly increased with age. Accumulated mutations in muscle-tissue mitochondrial DNA are associated with accelerated apoptosis of myocytes, and apoptosis may provide a link between mitochondrial dysfunction and the loss of muscle mass that occurs with sarcopenia.[135] Aside from mitochondria-mediated apoptosis, other mechanisms may be involved. In animal studies, the death receptor–mediated pathway appears to be upregulated, triggered by tumor necrosis factor-α (TNF-α).[137–140] Increased TNF-α signaling has been shown to induce protein breakdown in myocytes[141] by activation of the ubiquitin-proteasome pathway.[142]

Chronic Low-Grade Inflammation

Physiological aging is associated with chronic low-grade inflammation, a condition that has been termed "inflammaging."[143] Inflammaging is characterized by elevated serum levels of the same proinflammatory cytokines as those produced in metabolic stress, such as interleukin (IL)-1, IL-6, IL-8, interferon-γ (IFN-γ), and TNF-α, as well as acute-phase proteins such as C-reactive protein (CRP).[143,144] The proinflammatory cytokines IL-1, IL-6, and TNF-α are also members of the adipokine family because they are produced by adipose cells as well as by skeletal muscle fibers. Aging is associated with the accumulation of adipose tissue in the subcutaneous, visceral, and intramuscular regions, which may contribute to an inflammatory environment.[145] White adipose tissue (WAT) secretes these adipokines, as well as resistin, visfatin, and adiponectin, which have been shown to promote inflammation.[146] Proinflammatory cytokines and adipokines up-regulate the inflammatory response,[147–149] and increased circulating levels are associated with a decline in muscle mass and strength.[150–153] Cesari et al.[154] reported that CRP and IL-6 were positively associated with total fat mass and negatively associated with lean muscle mass. In the InCHIANTI study of 871 obese community-dwelling men and women, Schrager et al.[155] determined that subjects with low muscle strength had elevated CRP and IL-6 levels compared with those with normal strength. Chronically elevated inflammatory cytokines have been shown to lead to a predisposition to sarcopenia, perhaps through increased activation of the ubiquitin-protease pathway.[151,156] Furthermore, the levels of anti-inflammatory cytokines, such as IL-10, are reduced with age.[144,157] The low-grade, proinflammatory cytokine production that occurs with aging results in a loss of muscle mass and function.[158] There is also growing evidence that chronic inflammation may influence muscle anabolic responses to nutrition and exercise. Muscle protein synthesis in older adults is inversely related to levels of inflammatory cytokines,[159,160] and studies using nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce inflammation have resulted in enhanced gains in muscle mass and strength in response to resistance exercise in older adults.[161] These results suggest that chronic subclinical inflammation may contribute to the dysfunction seen in sarcopenia, particularly among obese individuals.

Impaired Satellite-Cell Function

Satellite cells are the source of the new nuclear material that is required for muscle growth and hypertrophy, and if the muscle fiber is damaged, satellite cells become activated, divide, and fuse to replace the damaged portions. The formation of muscle fibers during embryonic development involves the proliferation, migration, differentiation, and fusion of muscle precursor cells to form postmitotic multinucleated myotubes. Postnatal skeletal muscle growth is accompanied by an increase in the number of myonuclei per muscle fiber[162] that requires the activation of resident muscle stem cells, referred to as satellite cells, which proliferate and fuse with existing muscle fibers.[163] Adult skeletal muscle fibers are multinucleated cells, and their size is dependent, to some extent, on the number of nuclei present within a

fiber.[164] Although nuclei within fibers are capable of undergoing apoptosis during times of disuse or injury, they are unable to replicate.[164] New nuclei added to muscle fibers after periods of injury or inactivity come from satellite cells, which exist between the basal lamina and the fiber plasma membrane. After injury or exercise, satellite cells become activated, migrate to the site, proliferate, and fuse to regenerate the fiber by providing an additional source of nuclei.[165] There is an age-related decline in the density and regenerative capacity of muscle satellite cells,[38,39] which appears to be greater in type II than in type I muscle fibers.[166] These impairments in satellite-cell function can prolong the recovery of muscle from injury and are likely to contribute to the progressive loss of muscle mass that occurs with sarcopenia.

TNF-α interferes with satellite-cell differentiation and therefore muscle growth and regeneration by reducing the expression of myogenic regulatory factors (MRFs).[167] The MRFs are a family of muscle-specific transcription factors (MyoD, myogenin, MRF4, and myf-5) that regulate the transition of satellite cells from proliferation to differentiation.[168,169] TNF-α induces the production of reactive oxygen species (ROS), which activates nuclear factor (NF)-κB, increasing the expression of enzymes of the ubiquitin-proteasome MPD system.[141] TNF-α–induced activation of NF-κB results in a loss of MyoD mRNA[170] and, via activation of the ubiquitin-proteasome pathway (UPP), breakdown of MyoD and myogenin.[171,172] This suggests that part of the attenuated hypertrophic response in elderly versus young muscle[173] could be due to a decrease in MRFs resulting from impaired satellite-cell differentiation.

Myostatin as a Negative Regulator of Muscle Mass

The transition from myoblasts to myotubes is initiated by MRF expression. This process is inhibited by the presence of myostatin, which has been shown to downregulate myoD. Myostatin, otherwise known as growth differentiation factor-8 (GDF-8), is part of the transforming growth factor-β (TGF-β) superfamily. Myostatin is produced in skeletal muscle and acts as a negative regulator of muscle mass. This was demonstrated in mice, where knocking out the *GDF-8* gene led to a two- to threefold increase in skeletal muscle mass,[174] and administration of myostatin resulted in substantial muscle wasting.[175] Animal studies have shown that a lack of myostatin gives rise to muscle hyperplasia and hypertrophy[176] and that pharmacological silencing of myostatin suppresses systemic inflammation and can prevent or reverse muscle atrophy.[177]

Altered MicroRNA Expression

MicroRNAs (miRNAs) are evolutionarily conserved, small (17–22 nucleotides), noncoding posttranscriptional modulators of gene expression that are involved in a variety of biological processes,[178] such as skeletal muscle proliferation and differentiation,[179] apoptosis,[180,181] and aging.[182,183] miRNAs regulate the expression of mRNA targets in a sequence-specific manner by inducing mRNA degradation or translational repression.[184] miRNAs appear to have multiple gene targets, and each target may be regulated by multiple miRNAs.[185] Recent studies have shown that miRNAs have the ability to repress protein synthesis in skeletal muscle,[179] and aging of skeletal muscle has been associated with changes in the levels of miRNA expression.[186] The effect of miRNA expression has been studied in muscle biopsy samples from 17 old and 19 young men.[187] Eighteen miRNAs were found to be differentially expressed in those samples from elderly subjects, with *let-7b* and *let-7e* significantly elevated. *Let-7* family members repress the cell-cycle regulators involved in satellite-cell activity. The authors concluded that aging is characterized by a higher expression of *let-7* family members and that miRNA-mediated regulation of these factors during aging could affect muscle-cell proliferation.[187]

More than 1400 human miRNAs have been identified,[188] and numerous miRNAs have been identified as regulators of the age-related decline in skeletal muscle mass and function.[189] Eisenberg and colleagues[190] described 185 miRNAs that are either up- or downregulated in 10 major muscular disorders in humans. A number of miRNAs, enriched in striated muscle (referred to as myomiRs), have been identified, including miR-1, miR-133a, miR-206, miR-208, miR-486, and miR-499.[191–194] Skeletal muscle biopsies taken from six elderly men revealed elevated levels of primary miR-1-1, miR-1-2, miR-133a-1, and miR-133a-2 compared with biopsies taken from six younger men.[195] TNF-like weak inducer of apoptosis (TWEAK) is a muscle-wasting inflammatory cytokine.[196] Results of an miRNA array demonstrated that TWEAK inhibits the expression of several miRNAs, including miR-1, miR-133, and miR-206.[197] TWEAK induces skeletal muscle atrophy through inhibition of the PI3K/Akt signaling pathway.[198] Still other miRNAs appear to regulate gene-expression patterns that modulate muscle aging, including signaling through the insulin/IGF-1 signaling (IIS) pathway, heat-shock factors (HSFs), AMP-activated protein kinases (AMPKs), mitogen-activated protein kinases (MAPKs), sirtuins, target of rapamycin (TOR), and mitochondria.[199] The IIS pathway is negatively regulated by miR-1, miR-133, miR-206, miR-125b, and miR-486.[178] miR-23a suppresses the translation of both MAFbx/atrogin-1 and MuRF1, which directly inhibits muscle atrophy.[200] Similarly, a decrease in miR-23 is associated with an increase in PGC-1α mRNA and protein in muscle.[201] miR-29 regulates myogenesis by increasing muscle-cell differentiation.[202]

Oxidative Stress

Age-associated oxidative damage in muscle results in atrophy and loss of muscle fibers and muscle function. An imbalance between the generation of ROS and antioxidant defense mechanisms results in oxidative stress, which is considered to be an important contributing factor to the development of degenerative diseases, particularly those mediated by inflammation.[203] With aging, the oxidized proteins generated in muscle may not be completely removed. Oxidative damage to cell components, DNA, proteins, and lipids accumulates with age and contributes to cell degeneration and to the pathogenesis of aging-associated diseases.[204] Antioxidative efficiency decreases with age, and damage from oxidative stress increases with age.[205] Increased production of ROS is implicated in the damage of various macromolecules, immune dysfunction, and muscle damage.[206] Both disuse and reloading, which accelerate the muscle loss seen in sarcopenia, have been shown to greatly increase oxidative stress in the muscles of old animals.[207] Data suggest that reducing high basal levels of oxidative stress in aging could potentially attenuate the loss of skeletal muscle.[208]

Altered Endocrine Function

Normal aging results in decreased circulating levels of several anabolic hormones that may contribute to the changes in muscle mass and strength seen in sarcopenia. Serum levels of testosterone and adrenal androgens decline with age in males.[209] In elderly women, testosterone levels are also decreased, particularly in postmenopausal women and in women who have undergone a hysterectomy.[210] Testosterone has been reported to decrease by 1% per year from age 30,[211–213] and free testosterone levels decrease approximately 3% per year between the ages of 73 and 94.[214] There are epidemiological data suggesting a relationship with reduced testosterone levels and the declines in muscle mass and strength[31] and functional status[215] that occur with aging. Aging decreases the sensitivity of muscle tissue to testosterone,[216] and clinical studies indicate that both the decreased level of testosterone and the insensitivity of muscle tissue to testosterone in the elderly contribute to sarcopenia.[217] Age-related increases in the levels of sex

hormone–binding globulin (SHGB) result in reduced levels of free or bioavailable testosterone.[218] Studies have shown that low testosterone results in lower protein synthesis and a loss of muscle mass, which suggests that low testosterone levels may predict sarcopenia.[219] The steroid hormone dehydroepiandrosterone (DHEA) also declines with age in both females and males.[220] This decline is relatively linear over time and results in a decline of about 10% per decade until age 80, when the decline becomes more rapid.[221] Growth hormone (GH) is important in the growth and maintenance of muscle mass, and multiple studies have found that circulating levels of GH decrease with age. Veldhuis et al. found that GH levels in study participants decreased by approximately 50% between the ages of 20 and 70.[222] Progressive declines in GH results in a decline in plasma IGF-1,[223,224] which is associated with a loss of muscle mass but not necessarily muscle strength.[225] Aging muscle is capable of synthesizing IGF-1; however, it may be less sensitive to IGF-1, which may result in an attenuated ability to promote satellite-cell differentiation. Animal studies have revealed decreased IGF-1/Akt signaling pathways and increased expression of MuRF1 and atrogin-1 in aged rat muscle.[226] Other studies have reported that low levels of anabolic hormones are positively associated with low muscle strength[227] and limited mobility in men[228] and women.[229]

Lack of Physical Activity

Physical activity is known to have a profound effect on muscle mass and function. Inactivity has been shown to lead to a loss of muscle mass and strength at any age, and lifelong physical exercise has been shown to preserve muscle structure and function in elderly men to a degree that is comparable to active men four decades younger.[230] There is a general decline in activity with aging, which causes muscles to be less functional.[231] Increases in midlife leisure activity have been shown to reduce the risk of mobility impairment in old age.[232] However, it may well be that the reduced physical activity seen in the elderly is a response to sarcopenia rather than a cause of it. Reduced physical activity may follow the loss of muscle mass, which then accelerates it by removing the trophic stimulus of performing daily tasks.

Nutritional Status

Several age-related changes influence the nutritional needs and intake of the elderly. There is a decrease in energy requirements, due partially to a decline in physical activity and also to a reduction in muscle mass. The fall in muscle mass reduces the metabolic rate and reduces energy requirements by about 100 kilocalories per decade.[233] In the United States, total energy intake decreases with age, by 1000 to 1200 kcal in men and by 600 to 800 kcal in women in the seventh decade. This is associated with concomitant declines in most nutrient intakes, resulting in an increased risk for macro- and micronutrient deficiencies. Protein-calorie malnutrition is estimated to affect 11% to 22% of community-dwelling elderly outpatients.[234] Fifty percent of older adults have a vitamin and mineral intake less than the RDA, and 10% to 30% have subnormal levels.[235] Inadequate nutrient intake diminishes the immune, antioxidant defense, and acute-phase responses, which may be involved in sarcopenia pathogenesis.

A number of factors may contribute to malnourishment in elderly populations, including inadequate intake (reduced appetite or difficulty preparing food), psychological factors (depression or dementia), social factors (isolation or low income), and physiological factors (reduced sense of smell or taste, drug–nutrient interactions, and reductions in nutrient absorption).[236] Reduced food intake is generally accepted as the main cause of undernutrition in the geriatric population, and hospitalization is a risk factor for inadequate food intake in seniors.[237]

DIAGNOSIS

Classifications

Several consensus definitions of sarcopenia have been proposed, each providing different cut points and methodologies for assessing muscle mass, strength, and physical performance. Early attempts to define sarcopenia were based on measurements of skeletal muscle mass with dual-energy x-ray absorptiometry (DXA) in relation to body size. Baumgartner et al.[5] defined sarcopenia as a reduction of 2 or more standard deviations (SDs) in the muscle mass index (MMI) below the normal mean for a young reference group measured using DXA. MMI was calculated as the appendicular FFM of the combined upper and lower limbs (appendicular skeletal muscle [ASM]) divided by body height squared (ASM/height^2). With this definition, the prevalence of sarcopenia was between 43% and 53% in individuals over 80 years. Janssen et al.[238] proposed a definition of sarcopenia as a skeletal muscle mass index (skeletal muscle mass [kg]/weight [kg] × 100) 1 or 2 SDs below the mean for a younger reference group. The diagnostic criterion was later refined using appendicular lean mass (ALM) adjusted for height and body fat mass,[51] which provided a stronger association with functional performance using the same thresholds.

Since 2010, many expert panels and research groups from the United States and Europe have published definitions of sarcopenia, each recommending diagnostic criteria based on combinations of measurements of muscle mass, muscle function, and physical performance outcomes.[5,9,12,239,240] EWGSOP developed a practical clinical definition using functional measures of performance (gait speed) and strength (grip) preceding a measure of skeletal muscle mass for the diagnosis of sarcopenia.[10] The International Working Group on Sarcopenia (IWGS) proposed that a diagnosis of sarcopenia is consistent with a gait speed of less than 1 m/sec and an objectively measured low muscle mass (ASM mass relative to height^2 that is ≤7.23 kg/m^2 in men and ≤5.67 kg/m^2 in women).[11] The Foundation for the National Institutes of Health (FNIH) recommended a screening algorithm designed for subjects presenting with poor physical function that initially quantifies weakness (grip strength) followed by evaluation of muscle mass adequacy (DXA-determined ALM adjusted for BMI).[3,241–243]

Although a universally adopted definition has not yet been established, there seems to be a consensus among experts that a diagnosis of sarcopenia may be established when at least two of three criteria apply: (1) low physical performance and/or (2) low muscle strength and (3) low muscle mass. Low muscle mass is defined as the presence of a muscle mass ≥2 SD below the sex-specific mean using data from young subjects aged 18 to 39 years from the Third National Health and Nutrition Examination Survey (NHANES III) population.[238] Sarcopenia has recently been recognized as a disease entity, with the awarding of an *International Classification of Diseases,* 10th edition, Clinical Modification (ICD-10-CM) code (M62.84) in September 2016.[244]

The EWGSOP suggests a conceptual staging for sarcopenia, reflecting the severity of the condition, which can help guide clinical management (Table 217.1)[10]:

- Presarcopenia is characterized by a decrease in skeletal muscle mass without a significant decline in muscle strength or physical performance.
- Sarcopenia is characterized by a decrease in skeletal muscle mass accompanied by an impairment of either muscle strength or performance.
- Severe sarcopenia is the stage identified when all three criteria of the definition (decreased muscle mass, strength, and performance) are met.

TABLE 217.1 EWGSOP Conceptual Stages of Sarcopenia

Stage	Muscle Mass	Muscle Strength		Performance
Presarcopenia	↓			
Sarcopenia	↓	↓	or	↓
Severe sarcopenia	↓	↓		↓

EWGSOP, European Working Group on Sarcopenia in Older Persons. Adapted from Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing.* 2010;39(4):412–423.

A recent cross-sectional study investigating the influence of nutritional status in the diagnosis of sarcopenia identified a potential limitation to the sarcopenia staging suggested by the EWGSOP.[245] Data collected from 339 elderly nursing home patients (mean age 84.9 y and 64.3% women) in Spain included measurements of body mass composition, handgrip strength, and physical performance. Nutritional status was assessed using the Mini Nutritional Assessment (MNA), and sarcopenia was defined according to the EWGSOP consensus definition. The authors reported that the presence of malnutrition was statistically higher in individuals with sarcopenia compared with those without it. However, in the diagnostic algorithm proposed by the EWGSOP, muscle mass is not measured in people with normal performance (gait speed) and strength (grip); therefore a subgroup of the population staged as "presarcopenic" (with only low muscle mass) could not be detected.

Differential Diagnosis and Comorbidities

Sarcopenia is a phenomenon that has been reported among healthy, well-nourished, physically active elderly subjects[246] as well as in other syndromes associated with muscle wasting and in those with chronic illnesses such as heart disease or renal failure. A debate exists whether muscle loss should be termed sarcopenia under these conditions as well. Sarcopenia is different from starvation and cachexia, which are also associated with the loss of muscle but for which the causes and therapeutic approaches are different. During starvation, protein-energy deficiency results in a loss of fat and muscle,[247,248] but the losses are reversible with replenishment. Cachexia results in both fat and muscle mass loss; however, it accompanies chronic diseases such as cancer or AIDS.[247,249] In addition, there is increasing evidence that osteoporosis and sarcopenia frequently coexist,[250] and there is a close association between sarcopenia and bone loss and hip fracture, a condition described as *osteosarcopenia*.[250,251]

First described as a loss of muscle mass, sarcopenia has been mostly characterized using mass or strength,[252] although muscle mass is believed to explain only 4% to 60% of muscle strength in older men and women.[253] In 2008 Clark and Manini proposed the term *dynapenia*[254] to distinguish between age-associated deficits of muscle mass (sarcopenia) and muscle strength (dynapenia). Derived from the Greek for "poverty of strength" (*dyna* refers to "power, strength, or force," and *penia* refers to "poverty"), dynapenia describes the age-related loss of muscle strength and power.[255]

Being both obese and sarcopenic is a condition termed *sarcopenic obesity*,[6] originally coined by Heber et al.[256] Accompanying the age-related loss of muscle mass is an increase of fat mass. It is reported that an average adult can expect to gain approximately 0.45 kg (1 lb) of fat and lose about 0.23 kg (0.5 lb) of muscle yearly between 30 and 60 years of age.[257] This shift in body composition is often masked by stable body weight and can result in sarcopenic obesity.[258,259] Clinical measures of BMI and weight are not sensitive to these shifts in body composition; therefore it is more difficult to detect a person who is sarcopenic obese. This condition occurs in about 6% of the community-dwelling elderly,[73] and it is estimated that approximately 30% of men and 10% of women older than 80 years have sarcopenic obesity.[6,55,260] It has been hypothesized that sarcopenic obesity is associated with increased fatty infiltration of skeletal muscle, which is associated with reduced strength[261,262] and functional status.[263] Evidence suggests that fat infiltration decreases motor unit recruitment and contractility,[262] and the excess fatty acids in the muscle fibers appear to interfere with normal cellular signaling.[264] Obesity is linked to inflammation,[265,266] and in elderly subjects with increased visceral adiposity the associated chronic inflammatory state could lead to accelerated muscle loss. Roubenoff has suggested a vicious cycle explaining the role of increased fat mass in the pathogenesis of sarcopenia: loss of muscle mass results in lower physical activity that leads to obesity, which leads to an increase in catabolic over anabolic signals, which accelerates muscle loss.[267] The sarcopenic-obese body composition phenotype has also been associated with poorer physical functioning, falls, and metabolic syndrome, and sarcopenic obesity has been reported to predict the onset of disability more than sarcopenia or obesity alone.[268] Evidence reported by the Framingham and National Health and Nutrition Examination Survey studies confirmed that older adults with large amounts of adipose tissue and low muscle mass had the highest rate of disabilities.[269]

Sarcopenia is assumed to be a major component in the development of frailty.[270–273] Indeed, between 40% and 70% of persons who are frail are also sarcopenic.[273,274] The EWGSOP consensus definition of sarcopenia,[10] which includes criteria for gait speed and muscle strength, is close to and/or overlaps with a physically frail phenotype. Although frailty and sarcopenia overlap, it has been estimated that about a third of persons with sarcopenia do not have frailty, and all frail persons do not have sarcopenia.[275,276] The differentiation between normal aging and frailty appears to be indistinct because some factors, such as the loss of muscle mass (sarcopenia) and strength (dynapenia), occur throughout the process of aging. To distinguish physical frailty from aging, the widely used domains include "shrinking" with weight loss and sarcopenia, weakness with low grip strength, exhaustion or poor endurance, decreased motor performance (e.g., slow walking speed, impaired balance), and decreased physical activity.[277] Although there is not a universally accepted operational definition, the most commonly used definition of a physical phenotype of frailty comes from Fried and colleagues, who proposed the Fried Frailty Index (FFI) to identify frailty.[278] The FFI is used to assess the presence of physical frailty if three or more of the following symptoms are observed: (1) shrinking (unintentional weight loss), (2) weakness, (3) poor endurance or exhaustion, (4) slow walking speed, and (5) low amounts of physical activity.[278] Strong associations have been observed between the physical frailty phenotype and the risk of developing certain health-related outcomes.[279] The difficulty in distinguishing between sarcopenia and frailty has important research and treatment implications.

Categorizing Sarcopenia

Sarcopenia is frequently found in association with comorbidities, such as osteoporosis, obesity, or cancer.[280,281] In such cases, sarcopenia may be considered as a secondary consequence of the coexisting pathological condition. The EWGSOP has proposed to stratify individuals into categories of primary sarcopenia and secondary sarcopenia[10] on the clinical basis that in some individuals, a clear and single cause can be identified, whereas in other cases, no evident cause can be isolated. The EWGSOP suggests defining sarcopenia as "primary" (age-related) when no other cause is evident but aging itself and as "secondary" when

TABLE 217.2 Categories of Sarcopenia

Primary Sarcopenia	Definition
Age-related sarcopenia	No other cause evident except aging
Secondary sarcopenia	**Definition**
Activity-related sarcopenia	From bed rest, sedentary lifestyle, deconditioning
Disease-related sarcopenia	Associated with chronic disease, inflammation, malignancy
Nutrition-related sarcopenia	From inadequate dietary intake of energy and/or protein, as with malabsorption, gastrointestinal disorders

Adapted from Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing.* 2010;39(4):412–423.

one or more causes are evident (Table 217.2). Hospitalization may lead to an acute decline in muscle mass and function due to a combination of acute inflammatory burden, muscle disuse, and/or malnutrition, which may result in hospitalized individuals meeting the criteria for sarcopenia.[282] "Acute sarcopenia" has been proposed to refer to the acute loss of muscle mass and function associated with hospitalization.

Modern approaches to diagnosing sarcopenia are based on physical performance measures and assessment of muscle mass and strength. Muscle mass represents only one of the multiple dimensions of sarcopenia.[283] A decline in mobility (resulting from improper muscle function, coordination, and/or balance) and impairment of physical function are clear manifestations of sarcopenia that significantly affect quality of life.[284,285] Therefore combining assessment of muscle mass and strength with an evaluation of physical performance is an essential requirement for the identification of sarcopenia. A biomarker panel combining muscle-function performance testing, diagnostic imaging, and serum markers has been suggested to establish a clinical diagnosis of sarcopenia.[286]

Assessment of Physical Performance

Standardized physical performance measures complement the objective evaluation of muscle mass for the assessment of sarcopenia.[51] Physical performance measures are correlated with body composition and skeletal muscle parameters[262,287] and predict relevant health-related outcomes such as disability, morbidity, and mortality.[288–291] Walking ability appears to be the best predictor of disability, hospitalization, and healthcare expenditure,[292] and several different measures have been used, including the 400-meter walk test, 6-minute walking test, and usual gait speed test. Gait speed and the Short Physical Performance Battery are brief, quantitative estimates of decline in health and clinical function in older populations.[293]

Gait Speed

Gait speed measurement is the most widely used tool in clinical practice for the assessment of physical performance.[294–297] Gait speed is highly acceptable for subjects and health professionals in clinical settings because it is inexpensive, is easy to perform, and no specialist equipment is required.[298] Short-distance gait speed is a reliable measure associated with falls,[299] hospitalizations,[300] disability,[290] and survival[301,302] in older adults. A recent systematic review determined that gait speed was a consistent risk factor for disability, cognitive impairment, institutionalization, falls, and/or mortality.[303] A large-scale study in more than 2100 elderly subjects found that low walking speed is an independent risk factor of falls.[118] In a population-based sample of 409 adults aged 60 to 96, a nonlinear relationship was determined between leg strength and gait speed that is similar for older men and women,[304] indicating small changes in physiological capacity may have substantial effects on physical performance. Gait speed is frequently measured as part of other assessments, including the 6-minute and 400-meter walking tests, and the Short Physical Performance Battery includes a gait speed subtest.[288,305,306]

Subjects with a gait speed of <0.8 m/s are described as having poor physical performance,[307,308] which is the clinically relevant threshold for gait speed chosen by several international sarcopenia working groups.[10,241] Below 0.8 m/s, there is a strong association with increased risk of disability and reduced survival.[309] Several studies have demonstrated that men walk faster than women even at advanced ages, suggesting that a single cutoff might overestimate the number of women with poor physical performance and underestimate the number of men.[310,311] Recent studies in Asia also suggest that different thresholds for gait speed may be necessary for different ethnic groups.[312,313]

Timed Up and Go

In the timed up and go (TUG) test, subjects are asked to rise from a standard armchair, walk to a marker 3 m away, turn, walk back, and sit down again.[314] The test is a reliable and valid test for quantifying functional mobility that may also be useful in following clinical change over time. Findings from a study of 60 elderly (mean age 79.5 years) patients[315] found that TUG test performance correlated well with log-transformed scores on the Berg Balance Scale ($r = –0.81$), gait speed ($r = –0.61$), and Bartell Index of ADL ($r = –0.78$). The test is quick, requires no special equipment or training, and is easily included as part of the routine medical examination.

Balance Testing

The Berg Balance Scale (BBS) is a valid, efficient measure of postural balance in the geriatric population.[316] A study assessed functional independence, motor performance, and balance regularly over a 9-month period in 113 elderly residents. BBS scores predicted the occurrence of multiple falls among the elderly and were strongly correlated with functional and motor performance in stroke patients.[317]

The BBS consists of 14 items scored on a 5-point ordinal scale, ranging from 0 to 4 (0 indicates lowest level of function; 4 indicates highest level of function), with a maximum total score of 56. Participants presenting a score of 41 to 56 points have been described as "independent"; scores of 21 to 40 are interpreted as "walking with assistance"; and scores of 0 to 20 are generally classified as "wheelchair bound."[317]

Another study used the BBS score as a predictive model to quantify fall risk in community-dwelling older adults.[318] An increased risk of falling was determined as a BBS score of <51 with a personal history of falls or no history of falls and BBS score of <42. An individual BBS score of <40 was associated with almost 100% fall risk. Although the BBS is widely used and can distinguish fallers from nonfallers, it lacks a gait assessment component. A recent small case-control study comparing the BBS against other functional tests of mobility and balance demonstrated that it had better discriminating ability than the Performance-Oriented Mobility Assessment score or the TUG test, with high sensitivity and specificity.[319]

Stair Climb Power Test

The Stair Climb Power Test (SCPT) has been shown to be a clinically relevant measure of lower extremity power and is associated with mobility impairments. SCPT results are consistent with more complex techniques for measuring leg power and physical performance. The SCPT is suitable for clinical use; however, its use is mostly restricted to research settings due to equipment requirements.[320]

Assessment of Muscle Strength

Muscle mass is a well-characterized and easily measured end point; however, a better predictor of muscle function is muscle strength. In elderly men, muscle strength (the maximum capacity of a muscle to generate force) has been positively correlated with muscle fiber cross-sectional area and myonuclear and satellite-cell content.[321] Muscle strength declines much more rapidly than lean muscle mass in both elderly women and men,[322] and low muscle strength was strongly associated with mortality, independently of low muscle mass.[323] Muscle power (the maximum rate of work undertaken by a muscle per unit time) is a strong predictor of functional mobility and risk of falling among older adults[324] and may better predict functional status because it includes dynamic neuromuscular information not captured by measures of muscle mass or strength.[325] Isokinetic dynamometry is the recognized gold standard for measuring muscle strength. As an outcome measure of sarcopenia for use in clinical practice, both muscle strength and power may be limited by the cost and availability of expensive equipment.

Handgrip Dynamometry

Decreased grip strength is a marker for sarcopenia,[326] and handgrip assessment is one of the key tools for clinical assessment.[327–329] Grip strength declines with age and has been reported as a predictor of physical functioning and disability.[330,331] Data from three large population-based surveys of Danes aged 45 to 102 years with a total of 8342 participants reported that grip strength declines almost linearly between 50 and 85 years of age.[332] A meta-analysis of 13 studies involving 44,638 participants reported a reduction in mortality risk for every 1 kg increase in grip strength.[333] Isometric handgrip strength has been found to correlate with leg strength[334] and also with lower extremity power, knee extension torque, and calf cross-sectional muscle area.[307,335] The measurement is easy to perform, is inexpensive, does not require a specialist-trained staff, and is considered a good indicator of overall strength.[336] After multivariable adjustment, a 2.3-fold increase in the risk of falls was reported for patients in the lower third of handgrip strength compared with the upper third.[337] A 25-year prospective cohort study of 6000 men found that handgrip strength was highly predictive of functional limitations and disability.[338] Reference grip strength *t*-scores have been suggested as a promising method for the assessment of age-related losses of muscle strength in older adults.[339] A study was performed as part of the National Institutes of Health (NIH) Toolbox Assessment for Neurological and Behavioral Assessment comparing the greatest magnitudes of grip strength between 558 young adults (aged 20 to 40 years) and 390 older adults (aged 60 to 85 years). In this study, participants with grip strength values of 1.0 or more standard deviations below the means of healthy young men and women were considered dynapenic.[339] Dodds et al. combined data from 12 general population studies to produce normative data for grip strength across the life course, including more than 60,000 observations of grip strength from nearly 50,000 participants.[340] Their findings demonstrated that grip strength increases to a peak in early adult life and is then followed by a period of maintenance before decline with increasing age; this age-related decline begins as early as the fifth decade of life for both men and women.

From a pooled sample including 26,625 participants, the FNIH Sarcopenia Project conference recommended cut points for weakness of grip strength of <16 kg for women and <26 kg for men.[241,341] The EWGSOP defined weakness based on grip strength as <20 kg in women and <30 kg in men.[10,342]

Lower Limb Strength Testing

Muscles in the lower extremities are critical for daily function and allow for close comparison to biopsy data. Further, strength loss in the lower limbs has been seen to confer the greatest risk factors for falls, injury, and disability.[343] A systematic review and meta-analysis of 30 cohort studies[344] determined that for lower extremity weakness, the combined odds ratio (OR) was 3.06 (95% confidence interval [CI] = 1.01 to 2.32) for recurrent falls. In a study of 3075 women and men aged 70 to 79, Visser et al. observed that low quadriceps cross-sectional area (CSA) measured at baseline resulted in a 32% and 22% increased risk of mobility limitations at 2.5 years in women and men, respectively.[345] Lower knee extensor muscle strength was also associated with an increased risk of mobility limitations in the same study. For these reasons, lower body muscle strength and function are important for the clinical assessment of sarcopenia.[346] Knee extension and flexion are often used to measure lower limb power and strength, through isometric, isotonic, or isokinetic actions. Isometric actions measure a muscle's ability to generate force, whereas isotonic and isokinetic actions are measures of muscle power in that they involve translating a weight along an arc of motion within a given time interval. Cross-sectional studies comparing young (age 20 to 40 years) with elderly (70 to 86 years) subjects showed declines in knee extensor torque and power ranging from 20% to 40%.[347–350] A longitudinal study of 23 men (aged 73 to 86 years at baseline) reported that quadriceps muscle strength decreased 10% to 22% over the 7-year period,[351] or roughly 1.4% to 3.1% per year. Studies of age-related changes in lower extremity muscle strength demonstrate similar results as those observed in the upper extremities. Handgrip strength was positively associated with lower extremity performance in a study of 449 community-dwelling elderly men and women.[352] Cross-sectional studies have reported declines of 20% to 40% in measures such as grip strength and elbow extension torque between healthy younger subjects and elderly subjects, and longitudinal studies have demonstrated yearly declines ranging from 1% to 5%.[79]

Repeated Chair Stand

The repeated chair stand test is a timed (30-second) test requiring subjects to rise from a chair without using their arms and return to the seating position five times consecutively, and it has been shown to provide a reasonably reliable and valid indication of lower body strength and performance.[353] Objective measures of lower extremity function among community-dwelling older adults were found to be highly predictive of subsequent disability.[289] Poorer lower extremity function, as measured by repeated chair stand and 6-minute walking tests, was associated with smaller midthigh muscle area and greater intramuscular fat infiltration in older adults.[262] Using baseline data of the Health, Aging and Body Composition study, performance on timed repeated chair stands was found to be independently associated with muscle strength (maximal isokinetic leg extensor torque) but not muscle mass.[354]

Short Physical Performance Battery

The Short Physical Performance Battery (SPPB) is a test scored to a maximum of 12 points comprising three component tasks: gait speed, standing balance, and repeated chair stands.[355] These tasks combine the clinical evaluation of a subject's muscle strength and physical performance and have been shown to correlate with mobility, disability, and patient outcomes, including hospitalization and mortality.[308] Each component task is scored as 0 to 4 (0 = worst to 4 = best), and a composite score is calculated as the sum of scores on each task as 0 to 12.[356] Participants presenting a score ≤8 points have been described as having poor physical performance.[10] Meaningful changes in the SPPB have been defined,[357,358] and studies have demonstrated excellent reliability and sensitivity to clinical change.[359] An international working group[360] has recently recommended the SPPB as a functional outcome measure

in clinical trials in older subjects. The SPPB takes about 10 minutes to complete and can easily be completed in clinical settings.[355]

Assessment of Muscle Mass

Several operational methods have been proposed to quantify the relative muscle mass from objective absolute values,[5,238,243] providing estimates of total muscle mass and appendicular skeletal muscle mass (ASM). These have led to the creation of several different skeletal muscle mass indices.[10,11,312] ASM, a sum of the muscle mass of both arms and legs, is generally used when estimating the skeletal muscle mass index. Subjects with a larger body size may have larger muscle mass, indicating muscle mass is correlated with body size.[361] Therefore prediction equations reflect the absolute value of ASM after adjusting for body size, often using height squared (ASM/ht^2), body weight (ASM/wt), or BMI (ASM/BMI). Among these, the method with the highest predictive value for identifying subjects who are at higher risk for physical performance decline remains uncertain,[362] and the prevalence of sarcopenia varies considerably depending on the calculation used.[51,363,364]

Several techniques can be used to objectively quantify muscle mass, including anthropometry, bioelectrical impedance analysis, ultrasound, dual-photon or dual-energy x-ray absorptiometry, computed tomography, and magnetic resonance (MR) imaging. Imaging methods, specifically magnetic resonance and computed tomography, are considered to be the most accurate approaches for the in vivo quantification of body composition.[365] High cost, limited access to equipment, and radiation concerns limit the use of these imaging methods in routine clinical practice.[366]

Anthropometry

Anthropometry estimates body composition through measurement at various circumference and skin-fold sites. Skin varies in thickness from 0.5 to 2 mm[367]; thus fat beneath this skin typically represents most of the skin-fold measurement. The assumption is that a direct relationship exists between total body fat and subcutaneous fat. Five sites commonly used for measuring skin-fold thickness are the triceps, subscapular, suprailiac, abdomen, and thigh. Measurements from multiple (at least three) sites are deemed better for overall skin-fold assessment than measurements from only one or two sites,[368] and measurements should be repeated at least two or three times and averaged. Several population-specific equations for calculating total body fat from skin-fold sites have been established. Although inexpensive and relatively easy to perform, anthropometric measurement offers limited accuracy due to measurement error and bias associated with the site of the measurement.[369] Circumference or girth measurements may also be used. Typical sites of measurement include the abdomen, buttocks, thigh, and upper arm. As with skin-fold measurements, age- and gender-specific equations using circumferences have been developed. Reproducibility of circumference measurements is good, with only a 2% error in measurement.[370]

Anthropometric measures may predict disability and mortality in elderly populations. The calf circumference measurement predicted self-reported disability in a population of elderly women.[7] Campbell et al.[371] reported that low arm muscle area and triceps skin-fold thickness were associated with significantly increased mortality risk in 758 subjects who were more than 70 years old. Body composition changes throughout the adult life span, which must be considered when evaluating anthropometric indices.[372] Height declines, and there is less FFM in an elderly person than in a younger one of the same gender.[373] Most of the loss in FFM results from a decrease in skeletal muscle.[259] Geriatric-specific anthropometric and bioimpedance body prediction equations have been developed.[374,375]

Bioelectrical Impedance

Bioelectrical impedance analysis (BIA), also called bioelectrical impedance (BEI), is a commonly used technique for estimating body composition. Based on a two-compartment body composition model (fat mass and FFM), BIA determines the resistance (impedance) to small electrical currents as they pass through the body's water pool.[365] BIA measures changes in electrical conductivity via electrodes placed on the extremities, and the lowest resistance values are used to estimate total body water (TBW), from which total body FFM is calculated. Single-frequency BIA (SF-BIA) is the most commonly used, although this technique is limited in its ability to distinguish between intracellular and extracellular water and may be affected by hydration status or electrolyte imbalances. A recent consensus paper by the Society on Sarcopenia, Cachexia and Wasting Disorders[12] has discouraged the use of SF-BIA for the assessment of sarcopenia. Bioimpedance spectroscopy (BIS) or multifrequency BIA (MF-BIA) both allow for the differentiation of TBW into intra- and extracellular compartments[376] and provide information on fat mass and are therefore more useful in evaluating skeletal muscle.[377] BIA is a safe, noninvasive, and rapid means of assessing body composition; however, its validity is influenced by sex, age, and disease state.[378] The associated equipment is fairly portable and easy to operate, although relatively expensive. MF-BIA appears to provide results that are comparable with other methods of body composition analysis.

Ultrasound

Diagnostic ultrasound (US) is accepted as a clinical and research tool for evaluating skeletal muscle mass and quality. US is noninvasive and provides images of tissue configuration or changes in tissue densities,[379] such as increased intramuscular fibrous and adipose tissue.[380] Healthy muscle is echolucent[381]; as subjects age, skeletal muscles increasingly have adipose tissue infiltration and fibrosis that result in new sound reflection planes, which can be quantified.[382] Ultrasound elastography (EUS) can provide information about the mechanical properties of skeletal muscle beyond muscle shape or size.[383,384] Several groups have used US to estimate regional and total skeletal muscle mass, which were found to correlate well with DXA[385] and magnetic resonance[386] criteria. Echo intensity has shown a significant negative correlation with muscle strength in a recent study of elderly subjects.[387] US scanning is a relatively simple and inexpensive technique. Evidence suggests that US is reproducible and accurate,[388] and the technique can provide high test–retest reliability of muscle thickness and cross-sectional area.[389] Its portability makes it useful for mobility-impaired patients; however, results are highly dependent on operator proficiency.

Dual-Energy X-Ray Absorptiometry

DXA is an imaging technique that can distinguish fat, bone mineral, and lean tissues, permitting assessment of whole-body and regional body composition analysis. DXA measures skeletal muscle mass at a molecular level based on differential tissue attenuation of x-ray photons.[390] This whole-body scan exposes the patient to minimal radiation and is therefore a preferable alternative to computed tomography and magnetic resonance for research and clinical use. DXA exposes the subject to a collimated beam of x-rays to determine bone mineral and soft tissue (adipose and muscle) composition.[391] DXA was shown to be superior to anthropometric measurements in evaluating limb muscle mass in elderly subjects.[392] The equipment used is not portable, which is a limitation, particularly for use in large-scale epidemiological studies.[366] Evaluation of muscle mass using DXA (ALM adjusted for BMI) is an accepted component of the algorithms recommended by international working groups to confirm a diagnosis of sarcopenia.[3,10,241] Recent data suggest that the calculation of the percentage of skeletal

muscle mass (total muscle mass/weight × 100) provides a higher estimate of sarcopenia prevalence and is more associated with obesity status in comparison with appendicular lean muscle mass.[393]

Computed Tomography

CT is a gold-standard imaging method for body composition analysis at the tissue-organ level.[394] CT imaging is commonly used to assess the amount of adipose and skeletal muscle tissue and is a valid, precise, and accurate method to evaluate body composition.[395,396] CT exposes a subject to a collimated beam of x-rays that are attenuated as they pass through the body. These attenuations relate to differences in the physical density of the tissues examined, depicted quantitatively as the CT number.[397] There is a linear relationship between tissue density and CT number, reported in Hounsfield units (HU). Water is internationally defined as 0 HU and air as –1000 HU; skeletal muscle has positive HU values, and adipose tissue has negative HU values. The segmented CT scan provides measures of skeletal muscle area, and multiple images at specified intervals can be used to derive regional or total volumes.[398] Total muscle area and fat-free skeletal muscle volume can be calculated from these cross-sectional images.[399] Sarcopenia is often characterized by fat infiltration into muscle, which affects muscle quality and force generation, particularly of the lower extremities.[400] This intermuscular adipose tissue (IMAT) can be quantified by CT, providing a measure of functional changes in skeletal muscle.[401] The use of CT can provide measurements of changes in tissue composition. In a study of institutionalized frail elderly subjects, Fiatarone et al.[402] found a significant increase in midthigh muscle area (9%) in response to 8 weeks of resistance training, without changes in subcutaneous or intramuscular adipose tissue. CT offers high image contrast and clear separation of fat from other soft tissues. Advantages include high-quality image reconstruction that provides a measure of tissue composition and quality. The size of the patient may represent a limitation because morbidly obese subjects may not fit in the CT scan field of view. The radiation exposure of CT and relatively high cost limit current applications mainly to research settings.

Magnetic Resonance

MR imaging is used to measure organ size and structure, body fat and fat distribution (subcutaneous, visceral and intraabdominal), and muscle size as well as body water contents. MR imaging can quantify the distribution of adipose tissue into visceral, subcutaneous, and intermuscular deposits.[400] The technique is noninvasive and safe; however, limitations include high costs, inability to scan claustrophobic persons, and difficulty fitting large subjects (BMI > 40 kg/m^2) within the field of view. Conventional MR imaging cannot determine lipids or water in skeletal muscle; however, chemical shift imaging techniques, including proton magnetic resonance spectroscopy (^{1}H-MRS), have been developed that separate water and fat signals.[365] ^{1}H-MRS is capable of quantifying the lipid content in muscle and has been used to compare intramyocellular lipid (IMCL) changes during exercise therapy in adults[403] and has been used to report on age-related changes in IMCL.[404] Quantitative magnetic resonance (QMR) provides a simple, fast, and noninvasive method for measuring body composition. QMR uses the differences in the nuclear magnetic resonance properties of hydrogen atoms in organic and nonorganic substances to fractionate signals originating from fat, lean tissue, and free water.[405] An initial study[406] in humans reported that QMR underestimated fat mass and overestimated lean mass, suggesting limitations in accuracy and specificity using QMR in the measurement of body composition. Magnetic resonance imaging (MRI) does not rely on ionizing radiation; therefore it is safe across age ranges and groups and allows for serial assessments.[396]

Laboratory Measures of Muscle Mass

Urinary Indicators of Muscle Mass/Degradation

Urinary creatinine excretion is used to assess muscle mass because creatinine is the degradation product of creatine, which constitutes a fairly standard proportion of muscle (approximately 0.3%–0.5% of muscle mass by weight). Urinary creatinine excretion reflects about 1.7% of the total creatine pool per day and is expressed per 24 hours as a coefficient based on height or weight. Urinary creatine is not always an accurate indicator of muscle mass, due to variation in muscle creatine content. An evolving alternative to urinary creatinine is D3-creatine, which provides estimates of total muscle mass that correlate well with MRI measurements.[407] Based on the dilution of an oral dose of creatinine-(methyl-d[3]), urinary D3-creatine has been tested in humans, has a very high bioavailability and steady state after 24 to 48 hours,[408] and has the potential to be superior to DXA.

Urinary excretion of 3-methylhistidine (3-MH) has also been used as an indicator of muscle catabolism.[409] The amino acid histidine is found in high concentrations as 3-MH in the muscle protein actin, and its urinary excretion can be measured as an indicator of muscle breakdown. During muscle catabolism, released 3-MH is neither reused for protein synthesis nor metabolized oxidatively but, instead, is quantitatively excreted in the urine.[409] Studies have shown that endogenous 3-MH was significantly correlated with urinary creatinine excretion[410] and FFM.[411] Urinary 3-MH excretion may not be a reliable marker of muscle protein catabolism, however, because actin is present in other tissues, including intestines and platelets, and may therefore also represent an index of protein breakdown in nonmuscle tissues in the body.[397] A further limitation of both urinary 3-MH and creatinine as markers of skeletal muscle mass is the requirement for dietary (protein) control and 24-hour urine collections.

Serum Biomarkers

Several serum-based biological markers for sarcopenia have been proposed.[412] Many of these are typically related to specific cellular processes[413] and, although providing valuable information on sarcopenia pathophysiology, may provide limited clinical applicability.[414] A range of pathogenic processes is believed to contribute to the development and progression of sarcopenia. This has limited the identification of biological markers, which describe only single aspects of the condition and appear to be only weakly associated with clinically relevant outcomes.[415] Regardless, considerable research efforts have explored the identification and validation of candidate technologies and biomarkers for sarcopenia,[416] which may serve as useful parameters in monitoring pathological skeletal muscle changes. Candidate biomarkers for sarcopenia may be distinguished in four major classes: (1) antecedent biomarkers to estimate risk of developing the condition, (2) diagnostic biomarkers to detect clinically manifest sarcopenia, (3) staging biomarkers to describe categories or severity, and (4) prognostic biomarkers to predict the risk of developing adverse health outcomes.[417] Proposed circulating biomarkers for sarcopenia include hormones, stress proteins and those related to the inflammatory response, antioxidants, and products of oxidative damage.[418,419] In addition, Marzetti et al. recently reported a relationship between telomere length and muscle mass.[420] Telomeres from peripheral blood mononuclear cells were shorter in elderly subjects with sarcopenia relative to nonsarcopenic individuals, suggesting an association between telomere erosion and age-related muscle atrophy.

Interleukin-6

Elevated circulating levels of IL-6 are associated with a reduction in anti-inflammatory factors in the elderly,[421] and elderly subjects with insulin resistance or obesity display chronically elevated serum levels of

proinflammatory cytokines, including IL-6.[422] Evidence exists to suggest that increased IL-6 causes a reduction of physical performance in the elderly through its effect on muscle function. IL-6 has been associated with muscle atrophy[423] and mobility limitations.[424] IL-6, through its interaction with IGF-1, was found to be a significant predictor of handgrip strength, muscle power, and disability in a population-based sample of 526 subjects.[152]

Procollagen Type III N-Terminal Peptide

Plasma concentrations of procollagen type III N-terminal peptide (P3NP) may serve as a biomarker for muscle mass and muscle anabolic therapy.[425] P3NP is a fragment released by the cleavage of procollagen type III to generate collagen III, and its levels have been associated with changes in lean mass during testosterone and GH treatment[426] or resistance training[427] in older adults. Studies measuring serum or plasma P3NP and collagen III gene expression in muscle have demonstrated increased levels of P3NP or procollagen III mRNA after resistance exercise[428,429] and treatment with GH.[430,431] Testosterone administration increases expression of the collagen type III gene that encodes P3NP,[432] which was found to be elevated in a dose-dependent manner in plasma,[425] suggesting P3NP may be a biomarker candidate for skeletal muscle anabolism. As skeletal muscle protein remodels, the collagenous perimysium and endomysium surrounding muscle fibers must also remodel, which would ultimately result in P3NP release and appearance in the circulation, providing further rationale for P3NP as an early biomarker for muscle anabolism.

C-Terminal Agrin Fragment (CAF)

Several studies suggest a role for the circulating CAF as a marker for skeletal muscle mass and function.[427,433] Agrin is a protein of the neuromuscular junction (NMJ), where it is involved in clustering of acetylcholine receptors and stabilization of presynaptic structures.[434] Its activity is regulated by neurotrypsin, which cleaves agrin into CAF that can be detected in human serum.[435,436] Increased circulating CAF concentrations are related to disruption of the NMJ,[437] which has been associated with muscle fiber denervation, atrophy, and dysfunction.[438] Age-related degeneration of the NMJ may be associated with the development of sarcopenia.[35,439] In a study of 22 healthy older subjects, serum CAF concentration was inversely related to the onset of neuromuscular fatigue in men, independent of age and BMI.[440] Another study found that vitamin D supplementation and strength training significantly reduced the CAF concentrations of 69 community-dwelling older adults.[437] A multicenter, nonrandomized clinical study demonstrated that CAF was significantly elevated in 73 subjects diagnosed with sarcopenia compared with matched controls, suggesting agrin-dependent sarcopenia shows a clear neurogenic component and may be distinguishable from natural muscle aging.[433]

Indirect Measures of Muscle Mass/Damage

Stress Proteins

A number of studies suggest that heat-shock proteins (HSPs) may be potential biomarkers for sarcopenia. HSPs are a family of cytoprotective proteins that maintain normal cellular function by interacting with denatured or misfolded proteins, preventing their aggregation into larger complexes.[441] HSP synthesis is triggered by diverse stresses, including elevated temperatures, toxin exposure, ischemia, and hypoxia.[442] Age-induced alterations in filament structure and contractile elements are associated with increased levels of denatured muscle proteins.[443] HSPs function to maintain cellular integrity in skeletal muscle by repairing damaged proteins or degrading irreversibly damaged ones.[444] The production of extracellular HSPs has been linked with inflammation[445] and apoptosis of motor neurons.[446] An age-related increase in HSP expression in human skeletal muscle has been reported.[447] It has been proposed that this upregulation may represent an auto-protective mechanism of damaged muscle fibers[448] related to the structural and metabolic alterations associated with sarcopenia.[449] A review by Liu et al.[450] determined that HSP70 plays a pivotal role in maintaining cellular homeostasis by preventing apoptosis, facilitating muscle adaptation, and helping stabilize metabolic pathways. A diminished HSP70 response in aged muscle is associated with age-related functional deficits.[451] Thalacker-Mercer and colleagues reported increased expression of HSP70 in aged versus young human muscle after unaccustomed high-intensity resistance loading.[452] In another study of 665 elderly subjects, higher plasma HSP-72 was found to be associated with lower muscle mass, weaker grip strength, and slower walking speed.[453]

Oxidative Stress

Sarcopenia has been associated with oxidative damage. Biomarkers of oxidative stress may reflect an increased production of ROS or a decrease in antioxidant capacity. ROS accumulation during aging results in oxidative stress that can damage cellular components such as lipids, proteins, and DNA.[454] Increased production of ROS results in increased lipid peroxidation or increased protein oxidation.[455] Thiobarbituric acid reactive substances (TBARS) are indicators of lipid peroxidation and oxidative stress, and they increase with age.[456] The increase in TBARS during exercise is accompanied by reduced HSP production, suggesting that oxidative stress during exercise may result from insufficient production of HSPs.[457] Oxidized low-density lipoprotein (oxLDL) is an independent predictor of incident mobility limitation.[458] Accumulation of oxidized proteins in muscle is thought to result in cellular damage or tissue dysfunction,[459] decreased satellite-cell activation/proliferation, and decreased excitation–contraction coupling in skeletal muscle.[460] Accumulation of carbonyl groups on protein side chains occurs as a result of oxidation, and this is considered a biochemical marker of oxidative stress.[461] Protein carbonyls have been associated with lower grip strength[462] and decreased walking speed[463] in older adults. Similarly, elevated serum advanced glycation end products have been associated with poor grip strength in community-dwelling older women.[464] Total antioxidant capacity (TAC) comprises the entire pool of antioxidants within a cell and includes antioxidants (glutathione [GSH]; ascorbic acid; coenzyme-Q; tocopherols; carotenoids; and the amino acids cysteine, methionine, and tyrosine), enzymes (glutathione peroxidase [GPX], catalase, and superoxide dismutase [SOD]), and metal chelates.[465] TAC is age and sex dependent.[466] Intake of carotenoids and vitamin C and plasma levels of α- and γ-tocopherols are inversely associated with sarcopenia among older community-dwelling women.[467]

Myostatin

Myostatin, also known as growth/differentiation factor-8 (GDF-8), is a member of the transforming growth factor-beta (TGF-β) superfamily and is secreted mainly by skeletal muscle fibers.[468] Myostatin has been shown to be an inhibitor of muscle growth.[469] Through GDF-8 gene targeting, myostatin knockout mice were shown to have an increase in skeletal muscle mass.[174] Adult myostatin-deficient mice had greater maximum isometric force production compared with their wild-type counterparts,[470,471] and antagonism of myostatin significantly enhanced muscle tissue regeneration in aged mice by increasing satellite-cell proliferation.[472] Although a mutation in the myostatin gene has been shown to result in muscle hypertrophy in humans,[473] systemic administration of myostatin in adult mice was found to induce profound muscle loss.[175] High muscular myostatin mRNA has been

associated with impaired metabolism, systemic inflammation, obesity, and poor physical fitness in healthy subjects.[474] Myostatin is a candidate biomarker for skeletal muscle atrophy because it directly mediates catabolic signaling and is found in plasma.[475] Activin A, which binds to the same surface receptor complex as myostatin, also stimulates muscle wasting.[476] Both myostatin and activin A share follistatin as their antagonist.[477] Because myostatin, activin A, and follistatin are sensitive to age- and disease-associated muscle changes, they may be involved in sarcopenia pathogenesis.[478]

Vitamin D

Vitamin D plays an important role in skeletal muscle metabolism, and serum 25-hydroxyvitamin D (25[OH]D) levels have been found to decrease longitudinally with aging.[479] Vitamin D deficiency induces proximal muscle atrophy, loss of type II muscle fibers, and downregulation of myogenic markers and transcription factors related to muscle hypertrophy.[480,481] Several cross-sectional studies have demonstrated that vitamin D levels are inversely correlated with various parameters of muscle mass and function. Persons with low serum 25(OH)D level were shown to have decreased muscle mass measured with DXA and diminished grip strength.[29,482] Serum 25(OH)D was found to be positively correlated with SPPB, gait speed, 6-minute walking distance, and grip strength[483] and inversely associated with poor balance and an increased risk of falls.[484] Low serum 1,25(OH)D and 25(OH)D concentrations at baseline were independently associated with the incidence of sarcopenia over a 5-year follow up in 1705 elderly (>70 years) men participating in the Concord Health and Ageing in Men Project,[485] and a strong inverse association between 25(OD)D level and sarcopenia was found in a cohort of 3169 adults in the Korea National Health and Nutrition Examination Survey (KNHANES IV).[486] A prospective cohort study with 1231 participants demonstrated that 25(OH) vitamin D_3 levels lower than 10 ng/mL were independently associated with an increased risk of falling in older adults.[487] Studies show that in obese individuals, serum levels of cholecalciferol, ergocalciferol, and 25(OH)D are lower than in nonobese individuals.[488,489] A cross-sectional study of 302 nursing home residents revealed that 32% of participants had serum 25(OH)D levels below 30 ng/mL.[490] Similarly, 49.4% of long-term care facility residents were found to have vitamin D levels <30 ng/mL, despite receiving vitamin D supplementation (400 to 800 IU/day).[491] Vitamin D insufficiency was found in 72.2% of subjects in a recent study,[492] which also demonstrated that the serum 25(OH)D level was inversely correlated with body weight and BMI, and waist circumference was positively correlated with leptin and negatively correlated with adiponectin. Of United States and European community-dwelling older adults, 40% to 100% show vitamin D insufficiency (serum 25[OH]D levels <30 ng/mL or <75 nmol/L), and it is estimated that more than 1 billion people worldwide have vitamin D deficiency.[493]

Rapid Screening SARC-F

A rapid screening test for sarcopenia has been developed,[494,495] based on cardinal features or consequences of sarcopenia. SARC-F is a five-question scale designed to detect muscle dysfunction in older people (Table 217.3) and includes five components: strength, need for assistance in walking, ability to rise from a chair and climb stairs, and frequency of falls. Scores range from 0 to 10 (0 = best to 10 = worst) and are dichotomized to represent healthy (0 to 3) versus symptomatic (4+) status. SARC-F has been proven internally consistent and valid for detecting persons at risk for adverse outcomes from sarcopenia,[496] is able to predict physical limitation,[497] and can be used successfully to screen for sarcopenia.[273] Cao et al. showed that a low SARC-F score was associated with poor physical performance, poor grip strength, and hospitalization.[498] SARC-F appears to be a brief, inexpensive, reliable clinical tool that may be useful in screening for sarcopenia, similar to the FRAX questionnaire for osteoporotic fracture risk assessment.[499,500]

TABLE 217.3 SARC-F Screen for Sarcopenia

Component	Question	Scoring
Strength	How much difficulty do you have in lifting and carrying 10 pounds?	None = 0 Some = 1 A lot or unable = 2
Assistance in walking	How much difficulty do you have walking across a room?	None = 0 Some = 1 A lot, use aids, unable = 2
Rise from a chair	How much difficulty do you have transferring from a chair or bed?	None = 0 Some = 1 A lot or unable without help = 2
Climb stairs	How much difficulty do you have climbing a flight of ten stairs?	None = 0 Some = 1 A lot or unable = 2
Falls	How many times have you fallen in the past year?	None = 0 1–3 falls = 1 4 or more falls = 2

Adapted from Malmstrom TK, Morley JE. SARC-F: a simple questionnaire to rapidly diagnose sarcopenia. *J Am Med Dir Assoc.* 2013;14(8):531–532.

Assessment of Nutritional Status

Malnutrition is believed to be involved in the pathogenesis of sarcopenia. An assessment of nutritional status in elderly patients presenting with strength or performance deficits may be helpful. The Mini Nutritional Assessment (MNA) is widely used to screen elderly patients for malnutrition[501] and incorporates BMI and anthropometric measurements.[502] Initially developed for the frail elderly, the MNA has been validated for use in other elderly populations.[503] The MNA consists of 18 questions relating to anthropometrics (weight loss, BMI, circumference measurements), dietary intake (change in appetite, number meals/day, autonomy of feeding), global assessment (mobility, lifestyle, medication use), and subjective assessment (self-perception of health and nutritional status), with a maximum score of 30 points.[504,505] Patients who score >24 points are considered well nourished, those scoring 17 to 23.5 points are classified as being at risk of malnutrition, and those scoring <17 points may have existing malnutrition. The MNA is a validated assessment instrument for nutritional problems[506]; however, its length limits its usefulness for screening. The Mini Nutrition Assessment–Short Form (MNA-SF) was developed to identify persons with undernutrition as part of a two-step screening process in which persons identified as "at risk" on the MNA-SF would receive additional assessment. The MNA-SF score (Table 217.4) was strongly correlated with the total MNA score, and for predicting undernutrition, the sensitivity was found to be 97.9%, the specificity was 100%, and the diagnostic accuracy was 98.7%.[507]

Currently, several well-validated tools exist to assess physical performance, muscle mass, and strength.[306,327] Despite the relatively large number of tools available, their use may not always be feasible in daily clinical practice, and some of them may be of greater utility for the assessment of sarcopenia in research settings than in clinical practice. These tools are summarized in Table 217.5.

TABLE 217.4 Mini Nutritional Assessment-Short Form (MNA-SF)

A.	Has food intake declined over the past 3 months as a result of loss of appetite, digestive problems, or chewing or swallowing difficulties?	0 = severe loss of appetite 1 = moderate loss of appetite 2 = no loss of appetite
B.	Weight loss during past 3 months?	0 = weight loss >3 kg (6.6 lb) 1 = does not know 2 = weight loss between 1 and 3 kg (2.2 and 6.6 lb) 3 = no weight loss
C.	Mobility	0 = bed or chair bound 1 = able to get out of bed/chair but does not go out 2 = goes out
D.	Has suffered psychological stress or acute disease in the past 3 months	0 = yes 1 = no
E.	Neuropsychological problems	0 = severe dementia or depression 1 = mild dementia 2 = no psychological problems
F.	Body mass index (BMI) (weight in kg/height in m^2)	0 = BMI < 19 1 = BMI 19–21 2 = BMI 21–23 3 = BMI > 23

Screening score (subtotal max. 14 points):
12 points or greater: normal, no further need for further assessment.
11 points or lower: possible malnutrition, continue assessment.
Adapted from Rubenstein LZ, Harker JO, Salva A, et al. Screening for undernutrition in geriatric practice: developing the Short-Form Mini-Nutritional Assessment (MNA-SF). *J Gerontol A Biol Sci Med Sci.* 2001;56(6):M366–372.

TABLE 217.5 Applicability of Existing Tools for the Assessment of Physical Performance, Muscle Strength, and Muscle Mass in Clinical and Research Settings

	APPLICABILITY		
	Primary Care Settings	Specialist Clinical Settings	Research Settings
Assessment of Physical Performance			
Gait speed	+++	+++	+++
6-minute walk test	+	+	++
400-meter walk test	+	+	++
Timed up and go test	+	+	++
Balance Test	+	+	+
Stair Climb Power Test	+	+	++
Short Physical Performance Battery	+	++	+++
Assessment of muscle strength			
Handgrip strength	+++	+++	+++
Lower limb muscle strength	+	++	+++
Repeated chair stand test	++	+	+
Assessment of muscle mass			
Anthropometric measurements	++	++	++
Bioelectrical impedance analysis	+	++	+++
Ultrasound	++	++	+++
DXA	+	+++	+++
Computed tomography	+	++	+++
Magnetic resonance	+	++	+++

DXA, Dual-energy x-ray absorptiometry.
+++, best recommended tool; ++, best alternative tool; +, less recommended tool—based on availability, cost, utility, and availability of robust cutoff points.
Adapted from Beaudart C, McCloskey E, Bruyere O, et al. Sarcopenia in daily practice: assessment and management. *BMC Geriatr.* 2016;16(1):170.

STANDARD MEDICAL THERAPY

At present, no pharmacological treatment exists that is able to definitively halt the progression of sarcopenia or prevent the onset of age-associated muscle wasting; however, several are currently at various stages of development (Table 217.6).[508]

Myostatin Inhibition

The negative role of myostatin in muscle growth has led to the development of specific inhibitors of myostatin signaling shown to stimulate muscle growth or prevent muscle loss.[468] Antibodies to myostatin have been developed.[509] Myostatin antibodies have been shown to increase muscle mass and muscle fiber diameter in mice,[510] and there is some evidence they may have similar effects in humans with sarcopenia.[475]

Soluble activin type IIB receptor (ACVR2B) has been shown to be a potent myostatin inhibitor. Administration of soluble ACVR2B led to muscle hypertrophy in normal and myostatin-knockout mice.[511] Bimagrumab, a monoclonal antibody that binds to type II activin receptors (ActRII), was shown to dramatically increase skeletal muscle mass in mice. Bimagrumab (BYM338) enhances differentiation of primary human skeletal myoblasts and prevents myostatin- or activin A-induced atrophy through inhibition of Smad2/3 phosphorylation.[512] A recent pilot study investigated the effects of bimagrumab in treatment of sporadic inclusion body myositis (sIBM), a muscle-wasting disease characterized by enhanced muscle Smad2/3 phosphorylation. The bimagrumab-treated subjects had increased thigh muscle volume and lean body mass compared with placebo, as well as an improved 6-minute walking distance.[513] In a study of older adults with sarcopenia, a single dose of BYM338 increased thigh muscle volume, whereas there was no change with placebo. Participants receiving bimagrumab with a slower walking speed at baseline had a statistically significant greater improvements in gait speed and 6-minute walk distance than those receiving a placebo.[514]

Angiotensin-Converting Enzyme Inhibitors

Experimental and clinical studies have given insight into the role of the renin–angiotensin system (RAS) in skeletal muscle. Evidence suggests that angiotensin-converting enzyme (ACE) inhibitors may be beneficial in the prevention[515,516] and treatment[517] of sarcopenia. RAS blockade may decrease the decline of muscle performance and reduce the occurrence of frailty.[518] Treatment with ACE inhibitors may improve exercise capacity by inducing favorable changes in skeletal muscle myosin heavy-chain composition.[519] A randomized controlled trial (RCT) of 130 elderly participants (mean age 78.7) evaluated the effects of an ACE inhibitor on physical performance.[520] At 20 weeks, 6-minute walking distance was significantly improved in the treatment group relative to placebo. Reduced expression of ACE resulting from a genetic polymorphism is associated with greater muscle anabolic response

TABLE 217.6 Comparison of Operational Definitions for Sarcopenia

	OPERATIONAL DEFINITION		
Group	**Physical Performance**	**Muscle Strength**	**Muscle Mass**
FNIH-SP[3]	Gait speed ≤0.8 m/s	HGS Women: <16 kg Men: <26 kg	ALM_{BMI} Women: <0.512 Men: <0.789
IWG[11]	Gait speed <1.0 m/s	—	ALM/ht^2 Women: ≤5.67 kg/m^2 Men: ≤7.23 kg/m^2
EWGSOP[10]	Gait speed ≤0.8 m/s	HGS Women: <20 kg Men: <30 kg	ALM/ht^2 Women: ≤5.67 kg/m^2 Men: ≤7.23 kg/m^2
AWGS[312]	Gait speed ≤0.8 m/s	HGS Women: <16 kg Men: <26 kg	ALM/ht^2 Women: ≤5.67 kg/m^2 Men: ≤7.23 kg/m^2
ESPEN-SIG[9]	Gait speed ≤0.8 m/s OR reduced performance in any functional test used in geriatric assessment	—	Low muscle mass (% muscle mass >2 SD below mean in individuals aged 18–39 y in NHANES III cohort)
SCWD[12]	Gait speed <1.0 m/s OR 6-min walk <400 m	—	ALM/ht^2 >2 SD below mean of healthy individuals aged between 20–30 y of the same ethnic group

ALM_{BMI}, ratio of appendicular lean mass over body mass index; *ALM/ht^2,* ratio of appendicular lean mass over height squared; *AWGS,* Asian Working Group on Sarcopenia; *ESPEN-SIG,* European Society for Clinical Nutrition and Metabolism—Special Interest Group (Nutrition in Geriatrics); *EWGSOP,* European Working Group on Sarcopenia in Older Persons; *FNIH-SP,* Foundation for the National Institutes of Health—Sarcopenia Project; *IWG,* International Working Group Sarcopenia Task Force; *HGS,* handgrip strength; *NHANES III,* Third National Health and Nutrition Examination Survey; *SCWD,* sarcopenia, cachexia and wasting disorders; *SD,* standard deviation.

and improved skeletal muscle performance after training.[521,522] The mechanisms of ACE inhibitors are unclear but have been suggested to involve positive modulation of the IGF-1 system, improved perfusion of skeletal muscle, and reductions in inflammatory cytokines.[523]

Anabolic Hormone Repletion

Testosterone

Testosterone increases muscle mass and power, but it has a number of potentially limiting side effects.[225,524,525] The therapeutic benefit of testosterone therapy for sarcopenia is thought to suppress skeletal muscle myostatin expression while simultaneously stimulating the Akt pathway,[526] leading to increased muscle protein synthesis (MPS) and decreased muscle protein breakdown (MPD).[527] Testosterone can increase muscle mass in persons with low testosterone levels[528,529] and has been shown to improve function in older persons with frailty.[530–532] Intramuscular (IM) injection of testosterone enanthate in healthy individuals induced a twofold increase in protein synthesis compared with control subjects,[533] and a supraphysiological dose for 10 weeks in trained men produced a significant increase in strength and CSA of quadriceps.[534] A 6-month, randomized, placebo-controlled trial reported increased lean body mass and strength in older men.[535] These changes were associated with an increase in IGF-1 expression. Testosterone replacement also has been shown to increase muscle strength in women.[536] Testosterone supplementation in elderly men for 20 weeks found dose-dependent increases in both type I and type II muscle fibers.[537] At higher doses, testosterone enhances muscle strength through satellite-cell recruitment.[538] Expression of androgen receptors has been demonstrated in satellite cells,[539] which appear to be associated with androgen-induced muscle hypertrophy.[540] Testosterone appears to be better than growth hormone at reversing the decline in strength that occurs with sarcopenia[541]; however, testosterone replacement is not currently recommended in the treatment of sarcopenia due to high rates of side effects and low physical performance benefits.[542]

Growth Hormone and Insulin-Like Growth Factor-1

GH replacement therapy lowers fat mass and increases lean body mass. Rudman et al.[543] reported that older (>61 years) men who received GH injections for 18 months increased their FFM by 6% while decreasing fat mass by 15%. GH administration resulted in a significant increase in mixed muscle protein synthesis in elderly women[544]; however, one study reported that GH increased muscle strength only when coupled with a weight-training program.[545] Insulin-like growth factor-1 (IGF-1) has been shown to activate satellite-cell proliferation and differentiation in muscle and to increase protein synthesis in existing fibers.[546] In animal models, aging muscle has been shown capable of synthesizing IGF-1; however, it may be less sensitive to its stimulatory effects[226]; this anabolic resistance may be reversed through resistance exercise. Although there is little clinical research support for the use of GH in the treatment of sarcopenia, GH supplementation is limited by safety issues.[547] Potential serious side effects occur with GH supplementation in healthy adults, such as arthralgia, edema, and glucose intolerance.[548] GH has been shown to increase mortality in hospitalized persons,[549] and IGF-1 has been shown to correlate with the risk of prostate cancer in men, premenopausal breast cancer in women, and lung and colorectal cancer in both men and women.[550]

Dehydroepiandrosterone

Cross-sectional studies have shown a progressive decline in blood DHEA levels after the second decade, resulting in about a 10% decline per decade.[220,221] At 70 to 80 years of age, peak DHEAS concentrations are only 10% to 20% of those in young adults.[551] Administration of DHEA in elderly subjects has been shown to increase DHEA blood levels and increase biologically active IGF-1,[552] which has been shown

to stimulate MPS in humans.[553] DHEA administered for 6 months resulted in reduced body fat mass and increased muscle strength in men but not in women.[554] Oral administration of 50 mg DHEA for 1 year in healthy, ambulatory, elderly (60 to 80 years) individuals restored DHEA concentrations to the normal range for young adults (aged 20 to 50 years); however, no positive effect on muscle strength or CSA was observed.[555] Other studies using DHEA doses between 50 to 100 mg/day have shown no beneficial effects on improving muscle mass.[556,557]

Ghrelin Agonists

Ghrelin, a 28–amino acid peptide primarily produced in the gastric mucosa, has been identified as the endogenous ligand for the growth hormone secretagogue receptor (GHSR).[558] Apart from its well-established effects in appetite stimulation,[559] ghrelin possesses anti-inflammatory effects[249] and has been shown to enhance growth hormone release.[560,561] Studies have demonstrated that ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells[562] and NF-κB activation in human endothelial cells.[563] Plasma ghrelin levels have been found to be lower in elderly compared with younger subjects,[564–566] and elderly subjects with sarcopenia have lower ghrelin levels than those without sarcopenia.[567] Intravenous infusion of ghrelin in patients with congestive heart failure (CHF) for 3 weeks showed improvements in exercise capacity, muscle strength, and lean body mass,[568] which were unchanged in matched controls. Anamorelin, a ghrelin agonist, has been shown to enhance appetite and lean body mass, but not muscle function, in persons with cancer cachexia.[569,570] A randomized, double-blind, placebo-controlled crossover trial found that oral administration of the ghrelin mimetic ibutamoren (MK-677) for 12 months in healthy elderly subjects significantly increased GH and IGF-1 levels to that of younger adults and prevented lean mass loss without severe side effects.[571]

Selective Androgen-Receptor Modulators

Selective androgen-receptor modulators (SARMs) are a class of androgen-receptor ligands that display tissue-selective activation of androgenic signaling and have shown promise in increasing muscle mass and physical performance measures. A number of SARMs have undergone Phase I, II, and III trials. A 12-week Phase II clinical trial demonstrated that Enobosarm (GTx-024) treatment resulted in significant dose-dependent increases in total lean body mass ($P < 0.001$) and improvements in the Stair Climb Test ($P = 0.013$) compared with a placebo in 120 healthy elderly subjects.[572] Results from a Phase III clinical trial evaluating Enobosarm for the prevention and treatment of muscle wasting in patients with non–small-cell lung cancer (NSCLC) showed a statistically significant increase in lean body mass (LBM) in subjects receiving 1 mg ($P = 0.0012$) and 3 mg ($P = 0.046$) of the drug but not in placebo controls.[573] A 21-day ascending-dose study of the nonsteroidal SARM LGD-4033 in 76 healthy young men showed that the drug increased LBM and leg-press strength and was well tolerated.[574] In a study of 170 elderly women (age >65) with sarcopenia, treatment with another SARM, MK-773, produced statistically significant increases in LBM compared with placebo at 6 months; however, no significant improvement was seen in muscle strength or function.[575] Both treatment and placebo groups in this study also received vitamin D and protein supplementation. SARMs have the potential to increase muscle mass and improve physical function across several populations without the unwanted effects on the prostate, skin, or hair that are commonly associated with testosterone or other nonselective anabolic steroids. The effect of SARMs on muscle mass and function, however, has been modest in comparison to the effects from treatment with supraphysiological doses of testosterone.[530]

THERAPEUTIC CONSIDERATIONS

Exercise and Physical Activity

Exercise and physical activity are important considerations for both sarcopenia prophylaxis[576,577] and management.[578,579] Exercise training is a simple and inexpensive strategy to delay the onset of sarcopenia and reduce the rate of functional decline. Regular exercise has been shown to help maintain muscle strength, flexibility, and balance. Results from interventional studies have demonstrated that resistance training (RT) is the most effective intervention. A Cochrane review of 121 trials provided evidence that progressive resistance training, performed two to three times weekly by older people, improved gait speed, TUG, stair climbing, and overall muscle strength.[580] Trials of resistance exercise training in a frail older population have shown significant increases in 1-repetition maximal (1-RM) leg strength and improvements in SPPB scores.[11] Jubrias et al.[128] studied 40 elderly subjects (mean age 69.2 years) who were randomly assigned to one of three groups: control, resistance training, or endurance training. Training for 24 weeks resulted in substantial improvement. Endurance training resulted in a substantial increase in the intensity of exercise that could be sustained for 40 minutes, and endurance-trained subjects had less phosphocreatine breakdown and less acidosis during a standard stimulation exercise. Resistance training resulted in increased muscle cross-sectional area and increased mitochondrial volume density. Subjects in the resistance group increased their leg press by 64%. Similar findings were reported in other strength-training studies in elderly subjects,[581–584] and RT has been shown to increase muscle size and strength in old,[585] very old,[586] and frail individuals.[587] These results indicate that skeletal muscle adaptability is well maintained in older populations and provides evidence that some of the functional muscular deficits seen with aging can be reversed with regular physical activity, resistance exercise in particular. Maintaining the benefits from RT has been shown possible with as little as one training session per week.[588]

Resistance and isometric training techniques have demonstrated substantial improvements in muscle strength and metabolic capabilities. Resistance exercise has been shown to increase muscle protein synthesis,[589,590] reduce inflammation,[159] increase mitochondrial function,[129] and improve myogenic signaling[591] and satellite-cell activity.[592] Resistance training performed over many weeks is a potent stimulator for skeletal muscle growth and strength gains.[593] The dramatic increase in muscle size after resistance exercise is primarily the result of an increase in muscle fiber size (hypertrophy), with a minor contribution from an increase in fiber number (hyperplasia).[594] According to Grimby,[595] type II fibers show the most prominent increases in cross-sectional area after strength training in the elderly, and the increased mitochondrial volume that accompanies resistance training is consistent with improved endurance.[596] Physical activity has been shown to attenuate mitochondrial dysfunction in the elderly.[127] Based on the correlation between muscle cross-sectional area and maximum force-generating capacity,[597–599] it is not surprising that an increase in muscle size after resistance exercise is accompanied by an increase in maximal muscle strength.[600,601] This increase can enhance the functional performance of an individual and also reduce the elevated risk of falling in older people. A structured, moderate-intensity physical activity program over 2.6 years reduced major mobility disability compared with a health education program in 1635 sedentary men and women aged 70 to 89.[602] The American College of Sports Medicine (ACSM) and the American Heart Association have recommended the promotion of physical activity in older adults, emphasizing moderate-intensity aerobic activity and muscle-strengthening activity and reducing sedentary behavior.[603]

(See Chapter 36, The Exercise Prescription, for a comprehensive presentation on strength-training protocols.)

Dietary Modification

Nutritional modification or supplementation has been widely evaluated as a potential intervention for the prevention or treatment of age-related physical impairments.[604] Suboptimal micronutrient intake is very common in older adults,[605] and a proper diet is critically important to slowing sarcopenia progression.[606,607] Insufficient total caloric intake may lead to the development of sarcopenia[608] or accelerate its progression.[609] Research is focused on specialized nutritional products rich in immunomodulating nutrients, such as omega-3 fatty acids, phytochemicals (polyphenols, resveratrol, and catechins), and vitamin D, to attenuate inflammaging.[610] Significant increases in circulating proinflammatory cytokines and cortisol due to caloric insufficiency (CI) are among the triggers thought to contribute to age-related muscle decline.[611–613] Further data indicate that lean muscle mass in older adults is significantly associated with dietary protein intake. In one study, 2066 women and men aged 70 to 79 years in the highest quintile of protein intake (~1.1 g/kg/day) lost approximately 40% less LBM and appendicular LBM than did those in the lowest quintile (~0.7 g/kg/day).[606]

It is well known that resistance exercise increases aging muscle mass and strength, and these physiological adaptations from exercise may be further enhanced with certain nutritional interventions. Uptake of amino acids by skeletal muscle readily occurs after the ingestion of a protein-containing meal, during which time skeletal muscles typically experience a net protein synthesis. Both resistance exercise[590,614] and amino acid/protein ingestion[615,616] stimulate muscle protein synthesis independently, and a combination of the two further augments muscle protein synthesis.[617,618] Combined RT and nutritional intervention (whey protein and vitamin D) in 34 elderly patients for 3 months was found to significantly increase muscle strength, more than RT alone.[619]

Studies have shown that the timing and amount of amino acid ingestion are important for optimizing muscle protein synthesis after resistance exercise. Ingesting amino acids immediately before an exercise bout promotes a greater increase in muscle protein synthesis compared with ingestion immediately after,[620] which is attributed to increased blood flow during exercise leading to increased amino acid delivery. The amount of protein ingested is also crucial to optimize the anabolic response from RT. The MPS dose response to ingested protein after an exercise bout appears to be altered with aging. It has been demonstrated that older individuals need to ingest twice as much protein (40 g) to maximally stimulate MPS after exercise,[621] compared with the 20-g dose in healthy young subjects.[622] This change in the dose–response relationship suggests older muscle may be less sensitive to amino acids, which has been termed *anabolic resistance*. Older persons may obtain inadequate protein in their diet, which may impair MPS,[623] and older individuals suffering from protein-energy malnutrition (PEM) are unlikely to gain muscle mass and strength while engaging in RT.[624]

The Recommended Daily Allowance (RDA) for protein is 0.8 g/kg of body weight.[625] Although many older adults consume adequate protein on the basis of current standards, a subset of older individuals have been shown to ingest protein intakes below the RDA.[626] Kersetter et al. reported that 32% to 41% of women and 22% to 38% of men older than 50 years ingested less than the RDA for protein.[627] Protein can therefore be considered a key shortfall nutrient for aging populations, and it has been suggested that the current RDA for protein is inadequate for older adults because it fails to prevent muscle loss with aging.[28,607,628–630] Increasing protein intake above the RDA, especially in frail elderly individuals, could minimize the muscle loss seen in sarcopenia.[631] Based on a nitrogen balance study, Campbell and colleagues recommend that protein intake should be increased to 0.91 g/kg/day in healthy older men and women.[27] An increase in muscle was significantly enhanced in those elderly subjects who were supplemented with protein during strength training, compared with their nonsupplemented counterparts.[632] However, protein supplementation without exercise has been shown to have little effect on improving muscle mass.[633] Another study found that 1.0 g of protein/kg/day was the minimal amount required to maintain muscle mass.[634] The PROT-AGE study group, appointed by the European Union Geriatric Medicine Society (EUGMS), recommends average daily protein intake in the range of 1.0 to 1.2 g/kg body weight to maintain and regain lean body mass and function in older (>65 years) people,[635] particularly in those who are actively participating in endurance- or resistance-type exercise. Levels of protein intake as high as 1.6 g/kg/day have been demonstrated to increase exercise-induced muscle hypertrophy in older persons.[636] For individuals already diagnosed with sarcopenia, protein intake of at least 1.5 g/kg/day has been recommended.[637] Cross-sectional analysis of dietary intake data has shown a relationship between total[638] and per-meal[639] protein intake on both muscle mass and function. There is a saturable dose–response relationship between the amount of protein consumed in a single meal/bolus and MPS, which has led some researchers to promote a balanced pattern of dietary protein intake—rather than generic per-diem guidelines—to provide a more optimal per-meal stimulation of MPS, especially in the prevention or treatment of sarcopenia.[640] Results from muscle protein anabolism, appetite regulation, and satiety research support recommendations for a per-meal protein threshold (25 to 30 g protein per meal, containing approximately 2.5 to 2.8 g of leucine)[641] for older individuals in lieu of the less-specific RDA.[637,642]

Muscle tissue preferentially catabolizes some amino acids more than others, which can influence the anabolic response of muscle to protein. Six amino acids (aspartate, asparagine, glutamate, leucine, isoleucine, and valine) appear to be catabolized to a greater extent in skeletal muscle than in other tissues. Supplementation of essential amino acids (EAAs) has been shown to improve grip strength and 6-minute walking distance in elderly subjects after 3 months.[643] Treatment with 8 g of EAAs over 18 months in a randomized trial of 41 elderly persons (aged 66 to 84 years) with sarcopenia resulted in increased muscle mass, a significant increase in IGF-1 and a significantly reduced TNF-α.[644] The branched-chain amino acids (BCAAs) valine, isoleucine, and leucine appear to be the primary stimulus of protein synthesis[645] and have been estimated to represent 14% to 18% of the total amino acid content of skeletal muscles.[646] Leucine appears to be the most potent of these BCAAs,[647] producing its anabolic effects in muscle by stimulating the mTOR pathway[648] via the leucine-binding protein Sestrin2.[649,650] Leucine supplementation stimulates muscle protein accretion in cultured cells,[651] and infusion with L-leucine has been shown to reduce muscle protein degradation in healthy men.[652] Leucine is one of the few amino acids completely oxidized in the muscle for energy. When combined with exercise training, BCAA supplementation increases testosterone and decreases cortisol.[653] The ingestion of whey protein has been shown to stimulate postprandial muscle protein accretion more efficiently than casein,[654] which was attributed to whey protein's higher leucine content and absorption kinetics.

Manipulation of Gut Microbiota

The composition and function of the human gut microbiota have been linked to the age-related decline of physical performance, and microbiome-targeted interventions may hold therapeutic potential in preventing or treating sarcopenia. The human gastrointestinal tract is known to house more than 1000 distinct bacterial species, for a total of about 10^{14} microorganisms.[655] Although the human microbiome is dominated by four bacterial phyla (*Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria*),[656] each host has a unique biological relationship

with its gut microbiota.[657] Many host properties, including diet, age, gender, genome, and relative pharmaceutical load, can exert selective pressure on the microbiota.[655] The gut microbiota influence nutrient bioavailability, glucose and lipid metabolism, immune system conditioning, and drug metabolism,[658–660] and dysregulated host–microbe interactions have been implicated in the pathogenesis of a number of conditions, including obesity, insulin resistance, inflammatory bowel disease, and multiple sclerosis.[661–663] Aging has been associated with specific changes in gut microbiota,[664] and resilience of the enteric microflora is generally reduced with advancing age.[665] The structure and function of the gut microbiota can be affected by age-related physiological changes in gastrointestinal function or altered diet and lifestyle factors, which may eventually lead to low-grade chronic inflammation, malnutrition, adverse drug reactions, and greater infection susceptibility as well as possibly accelerated progression of chronic diseases, frailty, and sarcopenia.[666,667] Claesson et al. demonstrated that species richness (the number of taxa that metagenomic analyses are able to detect) of the fecal microbiota of 178 older subjects is inversely related to physical performance.[668] Analysis of data from 371 ELDERMET cohort subjects revealed that the presence of frailty in community-dwelling elderly individuals was associated with a gut microbiome profile similar to that of nursing home residents,[669] and gut dysbiosis was associated with reduced survival in a study of 76 elderly hospitalized patients with frailty or disability.[670] Altered gut microbiota may be involved in the onset of physical frailty: reduced representation of *Faecalibacterium prausnitzii,* which is known to produce fecal short-chain fatty acids (SCFAs), has been observed in frail subjects.[667]

Evidence suggests that therapeutic manipulation of the enteric microflora might be harnessed to obtain therapeutic gains in old age,[671] although studies investigating prebiotic or probiotic administration on skeletal muscle function have mainly focused on animal models.[672] In a recent 13-week trial of 60 elderly subjects investigating the effects of prebiotics on muscle function, the treatment group (fructooligosaccharides and inulin) demonstrated improved handgrip strength compared with placebo.[673] Evidence from animal studies suggests that physical exercise can modulate gut microbiota composition,[674] leading to increased gut biodiversity and mucosal integrity, reduced inflammation, and improved metabolic function.[675–677]

Nutritional Supplements

β-Hydroxy-β-Methyl Butyrate

β-Hydroxy-β-methyl butyrate (HMB), a metabolite of leucine, is believed to improve protein anabolism in muscle, and its supplementation has been used as a potential treatment for sarcopenia.[678] HMB has been shown to attenuate body weight and muscle loss in experimental cancer cachexia by inhibiting the ubiquitin-proteasome pathway[679] and to stimulate skeletal muscle hypertrophy in rats.[680] HMB supplementation may promote hepatic IGF-1 production, resulting in skeletal muscle protein synthesis and attenuation of muscle mass loss via increased mTOR expression[681] and increases in satellite-cell content, myonuclei number, and total DNA content.[682] Treatment with HMB resulted in reduced apoptotic signaling and increased satellite-cell content after disuse in aged rat muscles compared with controls.[683,684] Based on these observations, HMB is claimed to increase strength and FFM and to maximize muscle mass gains during resistance training.[685,686] Results from a meta-analysis of nine studies[687] indicate that HMB supplementation promotes gains in muscle mass and strength with RT. Interestingly, these positive effects appear to be evident only in conditions in which muscle proteolysis is more pronounced, such as in unconditioned individuals exposed to acute exercise training; these responses are not demonstrated in athletes and well-trained individuals, which is believed to result from training-induced suppression of muscle protein degradation.[688–690] A more recent meta-analysis of seven trials concluded that HMB supplementation contributes to the preservation of muscle mass in older adults and may help prevent bed-rest–induced muscle atrophy.[691] HMB is present in some foods, such as citrus fruits, fish, and breast milk, and a small amount is endogenously produced (a 70-kg individual produces about 0.2 g to 0.4 g daily) in consequence of leucine oxidation.[692] Most studies with human subjects have employed 3 g/day, and supplementation would be required to achieve therapeutic levels that demonstrate muscle gains and inhibition of proteolysis. Doses higher than 3.0 g/day do not appear to further increase strength or FFM. Gallagher et al. demonstrated that 3.0 g/day produced better results for FFM gain than did 1.5 and 6.0 g/day.[693] In an earlier study, untrained individuals were given one of three doses of HMB (0, 1.5, or 3.0 g/day) and were subjected to a resistance-training regimen for 3 weeks. Despite no alteration in body composition, total strength increased in a dose-dependent manner: 8%, 13%, and 13.4% for 0, 1.5, and 3.0 g/day, respectively.[692] Vukovich et al.[694] randomized 31 70-year old men and women into two groups, placebo and HMB supplementation (3 g/day), in conjunction with a 5-day/week exercise program. HMB supplementation promoted an increased percentage of body fat loss assessed by skin-fold estimation and CT. The effects of HMB supplementation on strength does not appear to differ by gender.[695] Up to 76 mg/kg/day (equivalent to ~ 5.3 g/day in a 70-kg individual) for 8 weeks appears to be safe and does not adversely affect hepatic and renal function in young adult males.[696] HMB as a free acid gel, compared with HMB calcium salt, resulted in faster and greater plasma concentrations (+185%) and improved clearance (+25%) of HMB from plasma, suggesting HMB as a free acid gel may be the optimal delivery form due to increased bioavailability.[697]

Creatine

Creatine has been shown to exert anabolic effects on muscle mass. Creatine supplementation may increase muscle accretion during resistance training through up-regulation of myogenic transcription factors.[698] Oral creatine supplementation (20 g/day) was found to attenuate the loss of muscle mass and strength during 7 days of unilateral upper arm immobilization,[699] and 0.5 g/day of creatine monohydrate for 14 weeks resulted in significantly greater increases in FFM and total body mass compared with a placebo[700] in elderly adults participating in a resistance exercise program 3 days per week. In a similar study of elderly (67 years to 80 years) subjects, 20 g creatine monohydrate for 8 weeks did not result in additional improvements in body composition, strength, or endurance.[701] Creatine supplementation, when combined with resistance training, was found to increase lean tissue mass and improve muscle strength, endurance, and power in elderly subjects,[702,703] whereas other studies found no benefit.[704,705] Volek et al.[706] demonstrated a significant increase in muscle fiber CSA after 12 weeks of resistance training in conjunction with creatine supplementation, which was greater than the increase with resistance training alone. In a study of women and men 65 to 86 years, creatine supplementation for 14 days increased maximal isometric grip strength and physical working capacity at fatigue threshold.[707] A recent meta-analysis found that creatine supplementation during RT programs (>6 weeks) enhanced gains in LBM, strength, and functional performance over RT alone.[708] Creatine alone or with conjugated linolenic acid (CLA) increased lean body mass and improved strength.[709,710] Low-dose creatine (0.1g/kg body weight) together with a protein supplement (0.3 g/kg) increased lean mass and upper limb strength.[711] Exogenous sources of creatine are predominantly animal foods, especially meat and fish. Hepatic synthesis contributes an estimated 1 to 2 g per day, involving arginine, glycine, and methionine.[712] Approximately 95% of creatine stores are found in skeletal muscle,[713] and a decrease in intramuscular creatine levels in advanced age has been documented.[714]

Omega-3 Polyunsaturated Fatty Acids

Omega-3 polyunsaturated fatty acids (n3-PUFAs) may be useful for the prevention and treatment of sarcopenia. Dietary PUFAs reduce adipose tissue inflammation in humans[715] and have been shown to repress TNF-α signaling pathways in mice.[716] Eicosapentaenoic acid (EPA) was found to restore skeletal muscle mitochondrial oxidative capacity[717] and was associated with increased transcriptional regulators of mitochondrial biogenesis in old mice.[718] A recent open-label intervention of 3.9 g n3-PUFA per day (2.7 and 1.2 g EPA and docosahexaenoic acid [DHA], respectively) for 16 weeks was shown to induce favorable adaptations within skeletal muscle in older adults (65 to 85 years), including decreased mitochondrial ROS production, increased muscle protein synthesis rates, and enhanced anabolic response to a single bout of exercise.[719] When incorporated with strength training in elderly women, n3-PUFA supplementation was shown to enhance skeletal muscle activation and force more than with strength training alone.[720] n3-PUFA supplementation, in association with an anabolic stimulus, has been shown to counteract anabolic resistance and sarcopenia. Long-chain n3-PUFAs have been shown to have anabolic properties in healthy young and middle-aged adults,[721] and studies show that the anabolic stimuli from amino acids, hormones, and/or physical activity in skeletal muscle can be enhanced by long-term fish oil supplementation.[722] Omega-3 fatty acids have been shown to attenuate muscle wasting in cancer treatment[723] and improve the MPS response to amino acid administration.[724] Individuals receiving 4 g/day of omega-3 fatty acids for 8 weeks demonstrated a significant increase in MPS with coadministration of amino acids, which the authors suggest may be due to amplification of the stimulatory effects of amino acids on the mTOR signaling pathway. Recent studies suggest that intake of omega-3 fatty acids is inversely associated with biomarkers of inflammation such as CRP and IL-6.[725] EPA has been shown to inhibit NF-κB[726,727] and reduce TNF-α production.[728]

Epidemiological data also suggest that consumption of fatty fish rich in anti-inflammatory omega-3 fatty acids may prevent sarcopenia. In a cross-sectional retrospective study of 2983 older adults in the UK, an increase in grip strength (of 0.48 kg in women and 0.43 kg in men) was observed for each additional portion of fatty fish consumed per week.[729] These relationships were independent of adult height, age, or birth weight. There is no established Dietary Reference Intake for n3-PUFAs; however, the Adequate Intake (AI) for α-linolenic acid (ALA) is set at 1.1 and 1.6 g/day for adult women and men, respectively, and the AI for linoleic acid (LA) for adult women and men is 11 and 14 g/day, respectively.[730]

Vitamin D

Research indicates that vitamin D may have beneficial effects on aging muscle biology, and there is evidence to suggest a protective role for vitamin D supplementation in older adults to preserve lean body mass and muscle strength and prevent frailty. The active hormone 1α, 25-dihydroxyvitamin D_3 promotes muscle cell growth, which may lead to improved muscle function.[731] Evidence from randomized studies in institutionalized frail elderly subjects demonstrated that vitamin D supplementation significantly improves body sway and lower extremity strength, reducing the risk of falls.[732] A meta-analysis of five RCTs including 1237 elderly individuals evaluated the effect of treatment with vitamin D for 2 months up to 3 years on falls risk (doses ranged from 400 to 800 IU/day with or without 1200 mg calcium). Treatment with vitamin D reduced the risk of falling by 22% (OR, 0.78; 95% CI, 0.64 to 0.92) compared with patients receiving calcium or placebo.[733] A more recent meta-analysis of eight RCTs involving 2426 individuals >65 years determined that supplemental vitamin D (700 IU to 1000 IU daily), which resulted in 25(OH)D concentrations ≥60 nmol/L, reduced fall risk by 23%.[734] A Cochrane review including 60 trials and 60,345 participants concluded that vitamin D supplementation is effective in reducing the rate of falls in care facilities.[735] Prolonged (2 months to 20 months) vitamin D supplementation with 800 IU/day has been demonstrated to improve muscle strength and/or physical performance in frail older adults[736] and older individuals with vitamin D deficiency.[737] Older ambulatory subjects (n = 139, ≥65 years) with a history of falls and 25(OH)D levels ≤12 μg/L were randomized to receive a single intramuscular injection of 600,000 IU of ergocalciferol or a placebo.[738] Aggregate Function Performance Time (AFPT), Choice Reaction Time (CRT), and postural sway measures improved in the intervention group and deteriorated in the control group; no significant difference in change in muscle strength was observed between the groups. In community-dwelling older adults who had low vitamin D status (<16 ng/mL), 400 IU daily over a period of 9 months along with exercise training resulted in a significant improvement in physical performance (TUG test) over training alone.[739,740] Recent meta-analyses concluded that supplemental vitamin D in daily doses of 800 to 1000 IU consistently demonstrated beneficial effects on strength[741] and balance,[742] and repletion in vitamin D-deficient persons has been shown to increase muscle strength and prevent falls.[742,743] Supplementation with vitamin D and leucine-enriched whey protein for 13 weeks improved appendicular muscle mass and lower extremity function among 380 older adults with sarcopenia.[744] Another study of 160 postmenopausal women demonstrated that vitamin D administration (1000 IU/day) for 9 months significantly increased muscle strength and prevented loss of muscle mass compared with placebo.[745] Vitamin D supplementation in older persons has been shown to reduce the incidence of falls, positively affect muscle-fiber composition,[746,747] and enhance RT-induced increases in muscle mass, strength, and function.[748]

For many elderly individuals, it may be difficult to reach the daily recommendation for vitamin D consumption without supplementation. Certain age-related changes contribute to an increased requirement for vitamin D in older adults, including decreased endogenous synthesis, decreased intestinal absorption, and impaired conversion to active D_3 (calcitriol). Evidence indicates a reduced ability of the aging kidney to dihydroxylate 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D,[749] which may be due to reduced expression of 25-hydroxyvitamin D-1α-hydroxylase.[750] Intestinal absorption of vitamin D is adversely affected with aging via increased resistance of the duodenal Ca^{2+} transport mechanism to the stimulatory action of 1,25(OH)$_2$ D.[751,752] An age-related decrease occurs in the rate of skin conversion of 7-dehydrocholesterol to vitamin D_3. The ability to synthesize vitamin D decreases with aging, and vitamin D synthesis has been shown to decrease by approximately 75% by age 70.[493] Many chronic diseases can cause vitamin D deficiency, including malabsorption syndromes, kidney disease, and hyperthyroidism, and several medications can alter vitamin D absorption, synthesis, or metabolism.[493,753] The AI for vitamin D among men and women between the ages of 51 and 70 years is 400 IU (10 μg) per day; for men and women above the age of 70 years, the AI increases to 600 IU (15 μg) per day.[753] A serum 25(OH) D level below 25 nmol/L has been associated with decreased muscle strength[29] and a level below 50 nmol/L with increased body sway.[100] It has been recommended that 25(OH)D levels be measured in long-term care residents[754] and all older patients with muscle loss.[624] The 2005 Dietary Guidelines for Americans recommended 1000 IU (15 μg) of vitamin D (which has since been reduced, as noted previously) in high-risk populations such as the elderly; this amount aims to increase the circulating concentration of 25 (OH) D to 80 nmol/L.[755] Evidence from research trials and dosing studies has shown that 40 IU (1 μg) of cholecalciferol daily raises serum 25(OH)D by 2 nmol/L in persons with serum levels <50 nmol/L.[756] Concentrations of 25(OH)D in older

persons have been increased 50 nmol/L to 65 nmol/L to means of 100 nmol/L with 800 IU/day.[757,758]

A supplemental dose of vitamin D_3 of 4000 IU/day is the "official" safe upper limit.[759] Evidence suggests that absorption of vitamin D from supplements ranges from 55% to 99% and may be greater than absorption from food sources.[753] Elderly persons have the same absorption rate of pharmacological doses as younger subjects,[760] and doses of 50,000 IU a week are safe.[761] Although vitamin D_3 appears to have greater bioavailability that D_2,[762] vitamin D_2 has been shown to be equally as effective as vitamin D_3 in maintaining 25(OH)D levels.[763] However, this is a different molecule than that formed from natural D3 and is less physiologically active. In vitro studies indicate that D_3 supplementation can affect reproductive hormone levels in a tissue-specific manner.[764–768]

Vitamin E

Evidence suggests that vitamin E may enhance muscle regeneration and mitigate the age-associated skeletal muscle dysfunction seen in sarcopenia.[769] Ble et al. found that age- and gender-adjusted plasma levels of vitamin E were significantly lower in frail individuals compared with nonfrail counterparts, suggesting an association between low vitamin E and the presence of frailty.[770] Vitamin E is protective against oxidative stress and inflammatory age-related diseases.[771] Preclinical and human experimental studies show that vitamin E positively affects muscle mass, muscle contractile properties,[772] exercise capacity,[773] mitochondrial function,[774] and myoblast proliferation and differentiation.[775] Vitamin E supplementation (800 IU/day α-tocopherol) for 28 days resulted in decreased oxidative stress markers after unaccustomed exercise compared with a placebo.[776] Tocotrienols reduced DNA damage in a trial of 64 subjects (aged 37 to 78 years) who received 160 mg tocotrienols or a placebo for 6 months.[777] Interestingly, in the same study, tocotrienol supplementation was found to decrease protein damage and reduce serum advanced glycosylation end products (AGEs) in adults aged >50 years.[778] Increased levels of AGEs in skeletal muscle have been observed with advancing age,[779] and AGE accumulation appears to contribute to decreased muscle function with aging.[780] Several studies have shown a significant association between increased AGEs and reduced muscle strength and physical performance.[464,781,782] Studies have demonstrated positive associations between serum tocopherol level and muscle strength. Vitamin E daily intake has been shown to positively correlate with knee extension and physical performance in a data subset of the InCHIANTI study.[783] Vitamin E deficiency can lead to nutritional myopathy,[784] and animal models of α-tocopherol deficiency have been shown to affect skeletal muscle strength and function.[785,786] Vitamin E deficiency is associated with sarcopenia, and both high intake and serum concentrations of vitamin E have been associated with reduced risk of incident frailty and decreased physical function.

Inadequate vitamin E intake is highly prevalent in community-dwelling elderly populations in the United States, with only 2% to 8% of women and 8% to 11% of men meeting the estimated average requirement (EAR) for vitamin E from foods alone in the Continuing Survey of Food Intakes by Individuals (CSFII) conducted from 1994 to 1996[787] and the National Health and Nutrition Examination Survey (NHANES) conducted from 2001 to 2002.[788] Although suboptimal micronutrient intake is common, particularly for older adults, hospitalization influences nutritional status because both pathological conditions and their treatment can cause or worsen malnutrition.[789] An observational study of 166 participants (mean age 76.3 years, 65% female) observed a high prevalence of inadequate nutritional status and a clear decreasing trend with age for α-tocopherol (mean 1.38 ng/dL in 65 to 75 age group; 1.26 ng/dL between 76 to 85; and 1.14 mg/dL >85 years).[790] The prevalence of inadequacy was especially high in hospitalized patients compared with outpatients (1.19 mg/dL and 1.35 mg/dL, respectively, $p < 0.011$). The normal serum range of vitamin E (α-tocopherol) for adults is 5 to 18 mg/L (14 to 40 μmol/L).[791] The EAR for vitamin E in adults ≥51 years is 12 mg/day (27.9 μmol/L), the RDA for the same age group is 15 mg/dL (35 μmol/L), and the TUIL has been set at 1000 mg/day.[792] Vitamin E is found in many plants, especially seeds, oil derivatives, grains, fruits, and vegetables.[793] Evidence exists to suggest tocotrienol has superior antioxidant properties[794] and possesses unique biological functions not shared by tocopherols.[795] It is recommended that vitamin E supplementation should include the full spectrum of vitamin E isomers (4 tocopherols and 4 tocotrienols) insofar as possible.[784,796,797] Researchers are investigating vitamin E gene interactions and specific polymorphisms affecting vitamin E bioactivity to help guide the development of personalized, nutrigenomic vitamin E supplementation and better elucidate therapeutic implications in the treatment of sarcopenia.[771]

Vitamin B_{12}

Sarcopenia may be related to vitamin B_{12} deficiency, which may occur independently of nutritional intake. Vitamin B_{12} deficiency is common in the elderly, and the prevalence increases with age.[798] In one study, 4-meter walking speed, handgrip strength, skeletal muscle mass, and serum B_{12} levels were assessed in 403 outpatient older adults.[799] The prevalence of sarcopenia in this study cohort was 24.8%, whereas the frequency of sarcopenia was 31.6% in patients with B_{12} levels <400 pg/mL. In addition, lean body mass and skeletal muscle mass index were lower in subjects with low vitamin B_{12} levels (<400 pg/mL) compared with those with B_{12} levels >400 pg/mL ($p < 0.05$). A cross-sectional study of 680 elderly participants (mean age 79, 65% female) found no association between serum B_{12} levels and sarco-osteoporotic status.[800] Analysis of data from a subset of 238 participants from the Singapore Longitudinal Aging Study[801] demonstrated that aging and frailty are associated with a higher prevalence of functional B_{12} deficiency as detected by increased methyl-malonic acid (MMA) in plasma. MMA values tend to rise in the elderly,[802] which is believed to reflect inadequate B_{12} intake or absorption. The range for serum MMA that represents expected variability is 73 to 271 nmol/L.[803] Amnionless, a vitamin B_{12} coreceptor that anchors the B_{12} transport complex to epithelial membranes, was also measured and was found to positively correlate with MMA ($p = 0.00068$). These findings suggest that amnionless may serve as a candidate biomarker for perturbed B_{12} bioavailability during aging and/or physical frailty. The EAR for vitamin B_{12} in adults ≥51 years is 2 μg/day, the RDA for the same age group is 2.4 μg/day, and there is insufficient data for deriving a TUIL for B_{12}.[804] It is estimated between 10% and 15% of people over the age of 60 have vitamin B_{12} deficiency.

Resveratrol

Research suggests that resveratrol may attenuate the decline in aging skeletal muscle through anti-inflammatory and antioxidant actions and enhanced satellite-cell regulation. In aged rats, resveratrol was shown to result in a modest improvement in satellite-cell proliferation after hindlimb suspension compared with control,[806] resulting in improved muscle mass during reloading. Resveratrol was shown to have a protective effect against oxidative stress in skeletal muscle through the expression of antioxidant enzymes in young and old rats.[208] Results from other animal studies suggest that resveratrol may have profound protective effects against oxidative stress in aged muscle.[807–809] Resveratrol appears to enhance the activity of sirtuins 1 and

2[810] and NAD+-dependent histone deacetylases, which have been identified as important regulators of skeletal muscle gene expression.[811,812] Resveratrol may therefore possess anti-inflammatory actions in skeletal muscle, through activation of PGC1α, which in turn activates farsenoid x receptor (FXR), PPARβ, and PPARδ, which have anti-inflammatory effects.[813,814] Although these results are promising, the beneficial effects of resveratrol on aspects of sarcopenia have yet to be determined in human clinical trials.

Epigallocatechin-3-Gallate and Green Tea Catechins

Epigallocatechin-3-gallate (EGCg), one of the most abundant catechins in green tea *(Camellia sinensis),* has been shown to have strong antioxidant and anti-inflammatory properties, which may be beneficial in the treatment of sarcopenia. A green tea extract containing approximately 50% EGCg was found to increase satellite-cell proliferation and differentiation in the muscles of aged rats after hindlimb suspension.[815] Markers for satellite-cell activation (Myf5, MyoD) were improved after EGCg administration for 7 days, and treatment with EGCg was shown to reduce myostatin.[816] Green tea catechin supplementation has been shown to reduce oxidative stress in both cultured cells[817] and after eccentric exercise.[818] Compared with controls, treatment with green tea catechins appears to reduce apoptotic signaling in activated satellite cells,[815] which may be due to up-regulation of antioxidants and a reduction in oxidative stress and/or inflammation.[819,820]

(See Chapter 60, *Camellia sinensis,* for a comprehensive presentation on this useful botanical medicine.)

Antioxidants

Natural defense mechanisms against oxidative damage include specific and nonspecific antioxidants. Antioxidants, including vitamin C and alpha- and gamma-tocopherol, are inversely associated with measures of sarcopenia.[467,783] An animal aging study demonstrated that supplementation with rutin, vitamin E, vitamin A, zinc, and selenium restores the ability to stimulate protein synthesis subsequent to leucine administration.[821] Epidemiological studies in community-dwelling older adults have shown that low serum carotenoids are independently associated with low skeletal muscle strength and development of functional limitation and walking disability.[822] Results from the InCHIANTI study revealed that low plasma levels of selenium[823] and carotenoids[824] may be independent predictors of all-cause mortality among community-dwelling older adults. Plasma carotenoids are considered a valid biological marker for fruit and vegetable intake.[825] Higher total serum carotenoid concentrations were associated with faster walking speeds in 687 elderly women,[826] suggesting that higher fruit and vegetable intakes may be protective against declines in lower extremity performance. Other studies have shown that decreased muscle strength is associated with low levels of selenium,[827,828] carotenoids,[829,830] and vitamin E.[770,831]

Protective effects from fruit and vegetable consumption have been attributed to the presence in these foods of bioactive compounds such as flavonoids, carotenoids, and other plant polyphenols. These naturally occurring phytochemicals have the ability to act as antioxidants and inhibit oxidative stress.[832] Mechanisms by which phytochemicals contribute to natural antioxidant defense systems include the inhibition of ROS formation and the interruption of radical chain reactions. Phytochemical antioxidants prevent the formation of ROS by inhibiting enzymes or chelating trace elements involved in free-radical production.[833] In addition to their antioxidant properties, many phytochemicals have been shown to be biologically active and protect against disease-related biological pathways, such as cell signaling, cell-cycle regulation, and inflammation.[834] Recent studies suggest that high fruit and vegetable intake reduces biomarkers of inflammation, such as IL-6, IL-18, and CRP.[835] Carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene) act as free-radical scavengers, modulate immune responses, and reduce inflammation.[836] Flavonoids account for about two thirds of polyphenols, the most abundant group of dietary antioxidants. Polyphenols have been shown to reduce inflammation and oxidative stress.[837] Manach et al. reviewed 97 bioavailability studies of various classes of polyphenols, which revealed wide variability.[838] They found that the polyphenols that are most well absorbed in humans are isoflavones and gallic acid, followed by catechins, flavanones, and quercetin glucosides; the least well-absorbed polyphenols are the proanthocyanidins, the galloylated tea catechins, and the anthocyanins. Bioavailability does not necessarily predict biological activity in vivo, however, as demonstrated by Williamson and Manach in a review of 93 intervention studies.[839] Polyphenols shown to have effects on plasma antioxidant biomarkers include monomeric catechins (found in high concentrations in tea), procyanidins (oligomeric catechins found in red wine, grapes, cocoa, cranberries, apples, and some supplements such as Pycnogenol and grape seed extract), and quercetin (flavonol found in onions, apples, red wine, broccoli, tea, and *Ginkgo biloba*). Whole-food sources of antioxidants include pinto, red, and black beans; russet potatoes; plums; apples; cherries; prunes; pecans; and berries such as cranberry, blueberry, raspberry, and blackberry.[840]

Specific antioxidant micronutrients appear to suppress oxidative pathways believed to contribute to the inflammatory characteristics observed in sarcopenia. Evidence suggests that antioxidants decrease the levels of inflammatory markers such as IL-6 and TNF-α,[841,842] elevated levels of which are associated with physical decline in older persons.[843] Longitudinal analyses of data from the Women's Health and Aging Study I (WHAS I) demonstrated that participants with the lowest levels of α- and β-carotene, lutein/zeaxanthin, and total carotenoids were significantly more likely to have increasing IL-6 levels over a 2-year period.[841] Those with the lowest selenium levels had a significantly higher risk of all-cause 5-year mortality. Vitamin C and E supplementation attenuated the increase in markers of oxidative stress in response to chronic repetitive muscle loading in aged rats,[844] and vitamin C and E coadministration with a blend of polyphenols and carotenoids for 10 months resulted in significantly increased GSH activity in the skeletal muscle of senescence-accelerated mice.[845] Low serum selenium was independently associated with low muscle mass in a cross-sectional observational study of 327 community-dwelling elderly (mean age 71.5 years) subjects.[846]

(See Chapter 81, Flavonoids—Quercetin, Citrus Flavonoids, and Hydroxyethylrutosides, for a comprehensive discussion of the use of nutritional supplements to increase muscle mass.)

Botanical Medicines

Muscle Atrophy Prevention

Evidence from animal studies has shown that *Curcuma longa* can prevent muscle atrophy by stimulating the expression of glucose-regulated protein 94 kDa (Grp94) in myogenic cells.[847] Grp94 is a sarcoendoplasmic reticulum chaperone involved in the attenuation of myofiber atrophy, the levels of which decrease significantly in unloaded muscle. Oral supplementation of 400 mg/day of curcumin was associated with blunting of serum inflammatory markers (CK, IL-8, and TNF-α), but had no positive effect muscle soreness, in an experimental model of exercise-induced muscle damage.[848]

Consumption of *Chlorella* appears to prevent age-related muscle atrophy in mice, possibly due to its antioxidant properties, its BCAA content, or through enhanced mitochondrial function.[849]

Epigallocatechin-3-gallate, the most abundant catechin in *Camellia sinensis,* attenuated TNF-α–induced depression of protein synthesis in murine myotubes,[850] suggesting EGCg may be effective in halting the skeletal muscle catabolism seen in sarcopenia.

Isoflavones (genistein and daidzein) from *Glycine max* were shown to induce expression of SIRT1 mRNA and phosphorylation of AMP kinase in vitro as well as suppress MuRF1 promoter activity and TNF-α–induced atrophy in C2C12 myotubes,[851] suggesting isoflavones may be effective in reducing inflammation-associated muscle atrophy. However, a 6-month clinical study of 70 sedentary postmenopausal women found that irrespective of physical exercise, isoflavones (70 mg/day) did not improve muscle strength or reduce risks of mobility impairment.[852]

Muscle Regeneration and Differentiation

There is evidence to suggest that several botanical medicines can positively influence skeletal muscle anabolism and improve muscle mass. In a proof-of-concept trial in humans, 7-day treatment with epicatechin (flavanol in *C. sinensis*) at 1 mg/kg/day was found to increase grip strength and the ratio of plasma follistatin:myostatin.[816] Green tea extracts were found to significantly decrease NF-κB activity of regenerating muscle fibers in mdx mice compared with controls.[853] In a 3-month trial of 128 elderly women with sarcopenia, Kim et al. demonstrated that green tea catechin supplementation (350-mL beverage fortified with 540 mg catechins) combined with physical exercise had a beneficial effect on physical function and muscle mass compared with control.[854]

Proanthocyanidins of *Vitis vinifera* (125 mg/kg/d) enhanced the growth of skeletal muscle fibers in aged rats, which led to significantly increased muscle weight.[806] In a pilot study of 91 middle-aged (40 to 60 years) women, grape seed proanthocyanidins (100 or 200 mg/d) for 8 weeks resulted in increased muscle mass.[855] Resveratrol (125 mg/kg/day) from *Vitis vinifera* resulted in favorable changes to type IIA and type IIB muscle fiber CSA and reduction of apoptotic signaling in muscles of old male rats.[806] A prospective, open-label study evaluating the safety and tolerability of escalating doses of *Withania somnifera* (750 to 1250 mg/day over 30 days) in young (aged 18 to 30 years) healthy volunteers demonstrated a significant increase in muscle strength,[856] and 500 to 750 mg twice daily for 3 months of *W. somnifera* improved muscle strength in 35 elderly individuals.[857]

Physical Medicine

Whole-body vibration (WBV) appears to positively affect balance, muscle function, and physical performance, which would be of benefit in the treatment of the skeletal muscle decline seen in sarcopenia.[858] Several human trials have demonstrated improvements in muscle strength[859] and reduced risk of falls.[860] A randomized controlled trial investigated the effects of 6 months of WBV training and/or vitamin D supplementation on balance, physical performance, and estimated fall risk in 113 elderly (mean age 79.6 years) institutionalized women.[861] All participants received either 880 or 1600 IU of vitamin D_3 per day and were assigned to either the no-training group or WBV (performing exercises on a vibration platform 3×/week). Sway velocity and maximal isometric knee extension improved only in the WBV group, and the improvements in the WBV group in endurance capacity, walking at referred speed, and TUG performance were significantly larger than the changes with vitamin D supplementation alone.

A recent meta-analysis of seven studies involving 933 participants found that side-alternating sinusoidal WBV seems to have a beneficial effect on dynamic balance in the elderly.[862] Training sessions typically consisted of 3 to 10 series of 30 to 60 seconds of WBV with 60 seconds of rest between at frequencies ranging between 5 and 26 Hz. Commonly, participants performed dynamic physical exercises during WBV. Another systematic review of 16 trials found that WBV training significantly improved knee muscle isometric strength, muscle power, and balance control in comparison with a control group.[863] These results suggest that WBV may be a safe, low-demand, and effective candidate therapy for musculoskeletal strengthening, postural control, and performance improvement that can easily be carried out in clinical settings.

Whole-body electromyostimulation (WB-EMS) has similarly been shown to have beneficial effects on muscle mass, strength, and power parameters in the elderly, with equal effects compared with conventional RT.[864] The Training and ElectroStimulation Trial (TEST-III) enrolled 76 elderly women (mean age 75 years), who were randomly assigned to either WB-EMS (performed 18 minutes, 1.5 sessions per week) or control groups.[865] After 54 weeks, significant intergroup differences were determined for ALM ($p = 0.009$) and maximal isometric strength ($p = 0.003$). WBV or WB-EBM may prove to be safe, effective alternatives for maintaining lean body mass and strength in elderly subjects unable or unwilling to perform dynamic strength exercises.

THERAPEUTIC APPROACH

Age-related sarcopenia can compromise physical function, increase the risk of disability, and lower the quality of life in older adults. In May 2012, a Sarcopenia Consensus Summit was convened by the Foundation of the National Institutes of Health, National Institute of Aging, and U.S. Food and Drug Administration. A task force of worldwide sarcopenia experts from the International Conference on Frailty and Sarcopenia Research (ICFSR) proposed that progress in treating sarcopenia will require strengthening of global partnerships among academia, industry, and government agencies to reach consensus on diagnostic criteria, optimize clinical trial design, and identify improved treatment and preventive strategies.[866]

There are no available pharmaceutical treatments for sarcopenia, but evidence shows that resistance training is a viable and relatively low-cost treatment. Maintaining an active lifestyle with regular periods of aerobic and resistance training, in combination with adequate protein, is believed to reduce the prevalence of sarcopenia in elderly populations. Nutritional supplementation is effective in the treatment of sarcopenia, and its positive effects increase when associated with physical exercise.[867] A recent interventional trial in older persons with limited mobility showed that twice-daily intake of a supplement providing 21 g protein, 10 g EAAs, 3 g leucine, and 800 IU vitamin D for a period of 12 weeks significantly improved muscle mass and the results on a chair stand test.[868] It is currently recommended to measure serum 25(OH)D levels in all patients with sarcopenia and to prescribe supplementation (at least 800 IU/day) to those with values lower than 40 ng/mL (100 nmol/L).[869]

Physical Activity

Endurance and resistance exercises are recommended at individualized levels that are safe and tolerated. Progressive and dynamic resistance training that uses both concentric and eccentric movements should be encouraged, and a program targeting lower extremity muscles should be prioritized due to their importance in mobility, gait, and balance.[870] The ACSM recommends that elderly subjects participate in RT of 65% to 75% maximum 1 to 3 days per week, for no more than 1 hour per session and with at least 48 hours between sessions.[547,603]

Diet

Encourage a well-balanced diet, rich in fruits and vegetables, along with adequate amounts (≥1.5 g/kg/day) of quality protein and fatty acids. Aim to provide 0.3 to 0.5 g/kg protein at each meal to optimize MPS. Supplement with whey protein as a source of leucine, if tolerated.

Supplements

- Amino acids: 5 to 15 g/day to include 100 mg/kg/d BCAAs (leucine, isoleucine, valine)
- HMB: 3 g/day
- Creatine: 5 to 20 g/day
- Omega-3 fatty acids: 4 g/day (2.7 g EPA and 1.2 g DHA, or 1.6 g ALA)
- Vitamin D_3: 800 IU/day (repletion to maintain blood levels ≥40 ng/mL)
- Vitamin E: 800 IU/day of mixed tocopherols and tocotrienols (to maintain serum levels between 5 and 18 mg/L)
- Vitamin B_{12}: 2 to 4 μg/d from all intakes (to maintain blood levels ≥400 pg/mL)

Botanical Medicines

- *Vitis vinifera:* 100 to 200 mg/day proanthocyanidins
- *Curcuma longa:* 400 mg/day curcumin
- *Withania somnifera:* 500 to 750 mg/day alkaloids and steroidal lactones
- *Glycine max:* 70 mg/day isoflavones
- *Camellia sinensis:* 540 mg catechins or 1 mg/kg/day epicatechin

Physical Medicine

WBV (side-alternating sinusoidal, 5 to 26 Hz) for 10 to 15 minutes, one to three times weekly

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr*. 1997;127(suppl 5):990S–991S.
2. Martone AM, Bianchi L, Abete P, et al. The incidence of sarcopenia among hospitalized older patients: results from the Glisten study. *J Cachexia Sarcopenia Muscle*. 2017;8(6):907–914.
3. Dam TT, Peters KW, Fragala M, et al. An evidence-based comparison of operational criteria for the presence of sarcopenia. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):584–590.
4. von Haehling S, Morley JE, Anker SD. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle*. 2010;1(2):129–133.
5. Baumgartner RN, Koehler KM, Gallagher D, et al. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol*. 1998;147(8):755–763.
6. Baumgartner RN. Body composition in healthy aging. *Ann N Y Acad Sci*. 2000;904:437–448.
7. Rolland Y, Lauwers-Cances V, Cournot M, et al. Sarcopenia, calf circumference, and physical function of elderly women: a cross-sectional study. *J Am Geriatr Soc*. 2003;51(8):1120–1124.
8. Melton 3rd LJ, Khosla S, Crowson CS, et al. Epidemiology of sarcopenia. *J Am Geriatr Soc*. 2000;48(6):625–630.
9. Muscaritoli M, Anker SD, Argiles J, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics. *Clin Nutr*. 2010;29(2):154–159.
10. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. 2010;39(4):412–423.
11. Fielding RA, Vellas B, Evans WJ, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International Working Group on Sarcopenia. *J Am Med Dir Assoc*. 2011;12(4):249–256.
12. Morley JE, Abbatecola AM, Argiles JM, et al. Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc*. 2011;12(6):403–409.
13. Alexandre Tda S, Duarte YA, Santos JL, et al. Prevalence and associated factors of sarcopenia among elderly in Brazil: findings from the SABE study. *J Nutr Health Aging*. 2014;18(3):284–290.
14. Janssen I. Evolution of sarcopenia research. *Appl Physiol Nutr Metab*. 2010;35(5):707–712.
15. Deschenes MR. Effects of aging on muscle fibre type and size. *Sports Med*. 2004;34(12):809–824.
16. Srikanthan P, Karlamangla AS. Muscle mass index as a predictor of longevity in older adults. *Am J Med*. 2014;127(6):547–553.
17. Hernandez-Luis R, Martin-Ponce E, Monereo-Munoz M, et al. Prognostic value of physical function tests and muscle mass in elderly hospitalized patients. A prospective observational study. *Geriatr Gerontol Int*. 2018;18(1):57–64.
18. Metter EJ, Talbot LA, Schrager M, et al. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J Gerontol A Biol Sci Med Sci*. 2002;57(10):B359–B365.
19. Janssen I, Shepard DS, Katzmarzyk PT, et al. The healthcare costs of sarcopenia in the United States. *J Am Geriatr Soc*. 2004;52(1):80–85.
20. Ortman JVV, Hogan H. *An Aging Nation: The Older Population in the United States. Population Estimates and Projections*. Washington, DC: US Census Bureau; 2014.
21. Schneider EL, Guralnik JM. The aging of America. Impact on health care costs. *JAMA*. 1990;263(17):2335–2340.
22. United Nations. *World Population Ageing*. New York: United Nations; 2013.
23. Welle S, Brooks AI, Delehanty JM, et al. Gene expression profile of aging in human muscle. *Physiol Genomics*. 2003;14(2):149–159.
24. Carey KA, Farnfield MM, Tarquinio SD, et al. Impaired expression of Notch signaling genes in aged human skeletal muscle. *J Gerontol A Biol Sci Med Sci*. 2007;62(1):9–17.
25. Chapman IM, MacIntosh CG, Morley JE, et al. The anorexia of ageing. *Biogerontology*. 2002;3(1-2):67–71.
26. Volpi E, Sheffield-Moore M, Rasmussen BB, et al. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA*. 2001;286(10):1206–1212.
27. Campbell WW, Crim MC, Dallal GE, et al. Increased protein requirements in elderly people: new data and retrospective reassessments. *Am J Clin Nutr*. 1994;60(4):501–509.
28. Campbell WW, Trappe TA, Wolfe RR, et al. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci*. 2001;56(6):M373–M380.
29. Visser M, Deeg DJ, Lips P, et al. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab*. 2003;88(12):5766–5772.
30. Combaret L, Dardevet D, Bechet D, et al. Skeletal muscle proteolysis in aging. *Curr Opin Clin Nutr Metab Care*. 2009;12(1):37–41.
31. Baumgartner RN, Waters DL, Gallagher D, et al. Predictors of skeletal muscle mass in elderly men and women. *Mech Ageing Dev*. 1999;107(2):123–136.
32. Morley JE. Hormones and the aging process. *J Am Geriatr Soc*. 2003;51(suppl 7):S333–S337.
33. Guillet C, Boirie Y. Insulin resistance: a contributing factor to age-related muscle mass loss?. *Diabetes Metab*. 2005;31. Spec No 2:5S20–25S26.
34. Ferrucci L, Penninx BW, Volpato S, et al. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. *J Am Geriatr Soc*. 2002;50(12):1947–1954.
35. Edstrom E, Altun M, Bergman E, et al. Factors contributing to neuromuscular impairment and sarcopenia during aging. *Physiol Behav*. 2007;92(1–2):129–135.
36. Dirks AJ, Hofer T, Marzetti E, et al. Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle. *Ageing Res Rev*. 2006;5(2):179–195.
37. Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. *Exp Gerontol*. 2006;41(12):1234–1238.
38. Conboy IM, Rando TA. Aging, stem cells and tissue regeneration: lessons from muscle. *Cell Cycle*. 2005;4(3):407–410.
39. Kadi F, Charifi N, Denis C, et al. Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve*. 2004;29(1):120–127.
40. Faulkner JA, Davis CS, Mendias CL, et al. The aging of elite male athletes: age-related changes in performance and skeletal muscle structure and function. *Clin J Sport Med*. 2008;18(6):501–507.
41. Janssen I, Heymsfield SB, Wang ZM, et al. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol (1985)*. 2000;89(1):81–88.
42. Lexell J. Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci*. 1995;50:11–16.
43. Short KR, Vittone JL, Bigelow ML, et al. Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab*. 2004;286(1):E92–E101.
44. Cohn SH, Vartsky D, Yasumura S, et al. Compartmental body composition based on total-body nitrogen, potassium, and calcium. *Am J Physiol*. 1980;239(6):E524–530.
45. Frontera WR, Hughes VA, Fielding RA, et al. Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol (1985)*. 2000;88(4):1321–1326.
46. Patel HP, Syddall HE, Jameson K, et al. Prevalence of sarcopenia in community-dwelling older people in the UK using the European Working Group on Sarcopenia in Older People (EWGSOP) definition: findings from the Hertfordshire Cohort Study (HCS). *Age Ageing*. 2013;42(3):378–384.
47. Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J Neurol Sci*. 1988;84(2–3):275–294.
48. Lexell J, Henriksson-Larsen K, Winblad B, et al. Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve*. 1983;6(8):588–595.

49. Forbes GB, Reina JC. Adult lean body mass declines with age: some longitudinal observations. *Metabolism*. 1970;19(9):653–663.
50. Ferrucci L, Guralnik JM, Buchner D, et al. Departures from linearity in the relationship between measures of muscular strength and physical performance of the lower extremities: the Women's Health and Aging Study. *J Gerontol A Biol Sci Med Sci*. 1997;52(5):M275–M285.
51. Newman AB, Kupelian V, Visser M, et al. Sarcopenia: alternative definitions and associations with lower extremity function. *J Am Geriatr Soc*. 2003;51(11):1602–1609.
52. Power GA, Dalton BH, Behm DG, et al. Motor unit number estimates in masters runners: use it or lose it? *Med Sci Sports Exerc*. 2010;42(9):1644–1650.
53. Trappe S, Gallagher P, Harber M, et al. Single muscle fibre contractile properties in young and old men and women. *J Physiol*. 2003;552(Pt 1):47–58.
54. Evans W. Functional and metabolic consequences of sarcopenia. *J Nutr*. 1997;127(suppl 5):998S–1003S.
55. Morley JE, Baumgartner RN, Roubenoff R, et al. Sarcopenia. *J Lab Clin Med*. 2001;137(4):231–243.
56. Roubenoff R, Hughes VA. Sarcopenia: current concepts. *J Gerontol A Biol Sci Med Sci*. 2000;55(12):M716–M724.
57. Vandervoort AA. Aging of the human neuromuscular system. *Muscle Nerve*. 2002;25(1):17–25.
58. Rolland YM, Perry 3rd HM, Patrick P, et al. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. *J Gerontol A Biol Sci Med Sci*. 2007;62(3):330–335.
59. Dey DK, Bosaeus I, Lissner L, et al. Changes in body composition and its relation to muscle strength in 75-year-old men and women: a 5-year prospective follow-up study of the NORA cohort in Goteborg, Sweden. *Nutrition*. 2009;25(6):613–619.
60. Zamboni M, Zoico E, Scartezzini T, et al. Body composition changes in stable-weight elderly subjects: the effect of sex. *Aging Clin Exp Res*. 2003;15(4):321–327.
61. Claflin DR, Larkin LM, Cederna PS, et al. Effects of high- and low-velocity resistance training on the contractile properties of skeletal muscle fibers from young and older humans. *J Appl Physiol (1985)*. 2011;111(4):1021–1030.
62. Verdijk LB, Koopman R, Schaart G, et al. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab*. 2007;292(1):E151–E157.
63. Larsson L. Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study. *Acta Physiol Scand Suppl*. 1978;457:1–36.
64. Imamura K, Ashida H, Ishikawa T, et al. Human major psoas muscle and sacrospinalis muscle in relation to age: a study by computed tomography. *J Gerontol*. 1983;38(6):678–681.
65. Shafiq SG, Dimino LC, Schutta HS. Electron microscopic study of skeletal muscle in elderly subjects. In: Kaldor GB, Dibattista WJ, eds. *Aging in Muscle*. New York: Raven; 1978:68–85.
66. Serratrice G, Roux H, Aquaron R. Proximal muscular weakness in elderly subjects. Report of 12 cases. *J Neurol Sci*. 1968;7(2):275–299.
67. Tomlinson BE, Walton JN, Rebeiz JJ. The effects of ageing and of cachexia upon skeletal muscle. A histopathological study. *J Neurol Sci*. 1969;9(2):321–346.
68. Tomonaga M. Histochemical and ultrastructural changes in senile human skeletal muscle. *J Am Geriatr Soc*. 1977;25(3):125–131.
69. Orlander J, Kiessling KH, Larsson L, et al. Skeletal muscle metabolism and ultrastructure in relation to age in sedentary men. *Acta Physiol Scand*. 1978;104(3):249–261.
70. Rubinstein LJ. Ageing changes in muscles. In: Bourne GH, ed. *The Structure and Function of Muscle*. New York: Academic Press; 1960:209–226.
71. Andersen JL, Terzis G, Kryger A. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. *Muscle Nerve*. 1999;22(4):449–454.
72. Essen-Gustavsson B, Borges O. Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiol Scand*. 1986;126(1):107–114.
73. Oertel G. Changes in human skeletal muscles due to ageing. Histological and histochemical observations on autopsy material. *Acta Neuropathol*. 1986;69(3–4):309–313.
74. Yuan H, Goto N, Akita H, et al. Morphometric analysis of the human cervical motoneurons in the aging process. *Okajimas Folia Anat Jpn*. 2000;77(1):1–4.
75. McNeil CJ, Doherty TJ, Stashuk DW, et al. Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. *Muscle Nerve*. 2005;31(4):461–467.
76. de Koning P, Wieneke GH, van der Most van Spijk D, et al. Estimation of the number of motor units based on macro-EMG. *J Neurol Neurosurg Psychiatry*. 1988;51(3):403–411.
77. Stalberg E, Borges O, Ericsson M, et al. The quadriceps femoris muscle in 20-70-year-old subjects: relationship between knee extension torque, electrophysiological parameters, and muscle fiber characteristics. *Muscle Nerve*. 1989;12(5):382–389.
78. Doherty TJ, Brown WF. Motor unit number estimation: methods and applications. In: Brown WF, Bolton CF, Aminoff MJ, eds. *Neuromuscular Function and Disease: Basic, Clinical and Electrodiagnostic Aspects*. Philadelphia, PA: Saunders; 2002:274–290.
79. Doherty TJ. Invited review: Aging and sarcopenia. *J Appl Physiol (1985)*. 2003;95(4):1717–1727.
80. McComas AJ. 1998 ISEK Congress Keynote Lecture: motor units: how many, how large, what kind? International Society of Electrophysiology and Kinesiology. *J Electromyogr Kinesiol*. 1998;8(6):391–402.
81. Doherty TJ, Vandervoort AA, Taylor AW, et al. Effects of motor unit losses on strength in older men and women. *J Appl Physiol (1985)*. 1993;74(2):868–874.
82. Poortmans JR, Carpentier A, Pereira-Lancha LO, et al. Protein turnover, amino acid requirements and recommendations for athletes and active populations. *Braz J Med Biol Res*. 2012;45(10):875–890.
83. Goldberg AL. Protein synthesis during work-induced growth of skeletal muscle. *J Cell Biol*. 1968;36(3):653–658.
84. Balagopal P, Rooyackers OE, Adey DB, et al. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol*. 1997;273(4 Pt 1):E790–E800.
85. Friedman PJ, Campbell AJ, Caradoc-Davies TH. Prospective trial of a new diagnostic criterion for severe wasting malnutrition in the elderly. *Age Ageing*. 1985;14(3):149–154.
86. Girasole G, Giuliani N, Modena AB, et al. Oestrogens prevent the increase of human serum soluble interleukin-6 receptor induced by ovariectomy in vivo and decrease its release in human osteoblastic cells in vitro. *Clin Endocrinol (Oxf)*. 1999;51(6):801–807.
87. Waters DL, Brooks WM, Qualls CR, et al. Skeletal muscle mitochondrial function and lean body mass in healthy exercising elderly. *Mech Ageing Dev*. 2003;124(3):301–309.
88. Huygens W, Thomis MA, Peeters MW, et al. Linkage of myostatin pathway genes with knee strength in humans. *Physiol Genomics*. 2004;17(3):264–270.
89. Frederiksen H, Gaist D, Petersen HC, et al. Hand grip strength: a phenotype suitable for identifying genetic variants affecting mid- and late-life physical functioning. *Genet Epidemiol*. 2002;23(2):110–122.
90. Carmelli D, Reed T. Stability and change in genetic and environmental influences on hand-grip strength in older male twins. *J Appl Physiol (1985)*. 2000;89(5):1879–1883.
91. Christensen K, McGue M, Yashin A, et al. Genetic and environmental influences on functional abilities in Danish twins aged 75 years and older. *J Gerontol A Biol Sci Med Sci*. 2000;55(8):M446–452.
92. Zhai G, Ding C, Stankovich J, et al. The genetic contribution to longitudinal changes in knee structure and muscle strength: a sibpair study. *Arthritis Rheum*. 2005;52(9):2830–2834.
93. Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *J Bone Miner Res*. 1997;12(12):2076–2081.
94. Tiainen K, Sipila S, Alen M, et al. Heritability of maximal isometric muscle strength in older female twins. *J Appl Physiol (1985)*. 2004;96(1):173–180.

95. Carmelli D, Kelly-Hayes M, Wolf PA, et al. The contribution of genetic influences to measures of lower-extremity function in older male twins. *J Gerontol A Biol Sci Med Sci*. 2000;55(1):B49–B53.
96. Reed T, Fabsitz RR, Selby JV, et al. Genetic influences and grip strength norms in the NHLBI twin study males aged 59–69. *Ann Hum Biol*. 1991;18(5):425–432.
97. Huygens W, Thomis MA, Peeters MW, et al. Quantitative trait loci for human muscle strength: linkage analysis of myostatin pathway genes. *Physiol Genomics*. 2005;22(3):390–397.
98. Roth SM, Schrager MA, Ferrell RE, et al. CNTF genotype is associated with muscular strength and quality in humans across the adult age span. *J Appl Physiol (1985)*. 2001;90(4):1205–1210.
99. Delmonico MJ, Kostek MC, Doldo NA, et al. Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women. *J Gerontol A Biol Sci Med Sci*. 2007;62(2):206–212.
100. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. *Osteoporos Int*. 2002;13(3):187–194.
101. Girgis CM, Baldock PA, Downes M. Vitamin D, muscle and bone: Integrating effects in development, aging and injury. *Mol Cell Endocrinol*. 2015;410:3–10.
102. Roth SM, Zmuda JM, Cauley JA, et al. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. *J Gerontol A Biol Sci Med Sci*. 2004;59(1):10–15.
103. Grundberg E, Brandstrom H, Ribom EL, et al. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. *Eur J Endocrinol*. 2004;150(3):323–328.
104. Geusens P, Vandevyver C, Vanhoof J, et al. Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. *J Bone Miner Res*. 1997;12(12):2082–2088.
105. Sayer AA, Syddall H, Martin H, et al. The developmental origins of sarcopenia. *J Nutr Health Aging*. 2008;12(7):427–432.
106. Sayer AA, Cooper C, Evans JR, et al. Are rates of ageing determined in utero? *Age Ageing*. 1998;27(5):579–583.
107. Sayer AA, Syddall HE, Dennison EM, et al. Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. *Am J Clin Nutr*. 2004;80(1):199–203.
108. Phillips DI. Relation of fetal growth to adult muscle mass and glucose tolerance. *Diabet Med*. 1995;12(8):686–690.
109. Gale CR, Martyn CN, Kellingray S, et al. Intrauterine programming of adult body composition. *J Clin Endocrinol Metab*. 2001;86(1):267–272.
110. Yliharsila H, Kajantie E, Osmond C, et al. Birth size, adult body composition and muscle strength in later life. *Int J Obes (Lond)*. 2007;31(9):1392–1399.
111. Kensara OA, Wootton SA, Phillips DI, et al. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. *Am J Clin Nutr*. 2005;82(5):980–987.
112. Lapillonne A, Braillon P, Claris O, et al. Body composition in appropriate and in small for gestational age infants. *Acta Paediatr*. 1997;86(2):196–200.
113. Hediger ML, Overpeck MD, Kuczmarski RJ, et al. Muscularity and fatness of infants and young children born small- or large-for-gestational-age. *Pediatrics*. 1998;102(5):E60.
114. Singhal A, Wells J, Cole TJ, et al. Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? *Am J Clin Nutr*. 2003;77(3):726–730.
115. Kuh D, Bassey J, Hardy R, et al. Birth weight, childhood size, and muscle strength in adult life: evidence from a birth cohort study. *Am J Epidemiol*. 2002;156(7):627–633.
116. Sayer AA, Syddall HE, Gilbody HJ, et al. Does sarcopenia originate in early life? Findings from the Hertfordshire cohort study. *J Gerontol A Biol Sci Med Sci*. 2004;59(9):M930–934.
117. Rantanen T, Harris T, Leveille SG, et al. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. *J Gerontol A Biol Sci Med Sci*. 2000;55(3):M168–M173.
118. Sayer AA, Syddall HE, Martin HJ, et al. Falls, sarcopenia, and growth in early life: findings from the Hertfordshire cohort study. *Am J Epidemiol*. 2006;164(7):665–671.
119. Kuh D, Hardy R, Butterworth S, et al. Developmental origins of midlife physical performance: evidence from a British birth cohort. *Am J Epidemiol*. 2006;164(2):110–121.
120. Woo J, Leung JC, Wong SY. Impact of childhood experience of famine on late life health. *J Nutr Health Aging*. 2010;14(2):91–95.
121. Maltin CA, Delday MI, Sinclair KD, et al. Impact of manipulations of myogenesis in utero on the performance of adult skeletal muscle. *Reproduction*. 2001;122(3):359–374.
122. Hanson MA, Cooper C, Aihie Sayer A, et al. Developmental aspects of a life course approach to healthy ageing. *J Physiol*. 2016;594(8):2147–2160.
123. Stump CS, Short KR, Bigelow ML, et al. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci U S A*. 2003;100(13):7996–8001.
124. Bua EA, McKiernan SH, Wanagat J, et al. Mitochondrial abnormalities are more frequent in muscles undergoing sarcopenia. *J Appl Physiol (1985)*. 2002;92(6):2617–2624.
125. Reznick RM, Zong H, Li J, et al. Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis. *Cell Metab*. 2007;5(2):151–156.
126. Brunk UT, Terman A. The mitochondrial-lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. *Eur J Biochem*. 2002;269(8):1996–2002.
127. Kent-Braun JA, Ng AV, Young K. Skeletal muscle contractile and noncontractile components in young and older women and men. *J Appl Physiol (1985)*. 2000;88(2):662–668.
128. Jubrias SA, Esselman PC, Price LB, et al. Large energetic adaptations of elderly muscle to resistance and endurance training. *J Appl Physiol (1985)*. 2001;90(5):1663–1670.
129. Melov S, Tarnopolsky MA, Beckman K, et al. Resistance exercise reverses aging in human skeletal muscle. *PLoS One*. 2007;2(5):e465.
130. Marzetti E, Lawler JM, Hiona A, et al. Modulation of age-induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle. *Free Radic Biol Med*. 2008;44(2):160–168.
131. Dupont-Versteegden EE. Apoptosis in muscle atrophy: relevance to sarcopenia. *Exp Gerontol*. 2005;40(6):473–481.
132. Solomon A, Bouloux P. Endocrine therapies for sarcopenia in older men. *Br J Hosp Med (Lond)*. 2006;67(9):477–481.
133. Giresi PG, Stevenson EJ, Theilhaber J, et al. Identification of a molecular signature of sarcopenia. *Physiol Genomics*. 2005;21(2):253–263.
134. Wenz T, Rossi SG, Rotundo RL, et al. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. *Proc Natl Acad Sci USA*. 2009;106(48):20405–20410.
135. Marzetti E, Hwang JC, Lees HA, et al. Mitochondrial death effectors: relevance to sarcopenia and disuse muscle atrophy. *Biochim Biophys Acta*. 2010;1800(3):235–244.
136. Leeuwenburgh C. Role of apoptosis in sarcopenia. *J Gerontol A Biol Sci Med Sci*. 2003;58(11):999–1001.
137. Marzetti E, Groban L, Wohlgemuth SE, et al. Effects of short-term GH supplementation and treadmill exercise training on physical performance and skeletal muscle apoptosis in old rats. *Am J Physiol Regul Integr Comp Physiol*. 2008;294(2):R558–567.
138. Marzetti E, Carter CS, Wohlgemuth SE, et al. Changes in IL-15 expression and death-receptor apoptotic signaling in rat gastrocnemius muscle with aging and life-long calorie restriction. *Mech Ageing Dev*. 2009;130(4):272–280.
139. Phillips T, Leeuwenburgh C. Muscle fiber specific apoptosis and TNF-alpha signaling in sarcopenia are attenuated by life-long calorie restriction. *FASEB J*. 2005;19(6):668–670.
140. Pistilli EE, Jackson JR, Alway SE. Death receptor-associated pro-apoptotic signaling in aged skeletal muscle. *Apoptosis*. 2006;11(12):2115–2126.
141. Li YP, Schwartz RJ, Waddell ID, et al. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. *FASEB J*. 1998;12(10):871–880.

142. Llovera M, Garcia-Martinez C, Agell N, et al. TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. *Biochem Biophys Res Commun*. 1997;230(2):238–241.
143. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000;908:244–254.
144. Bartlett DB, Firth CM, Phillips AC, et al. The age-related increase in low-grade systemic inflammation (inflammaging) is not driven by cytomegalovirus infection. *Aging Cell*. 2012;11(5):912–915.
145. Pedersen BK. The diseasome of physical inactivity—and the role of myokines in muscle—fat cross talk. *J Physiol*. 2009;587(Pt 23):5559–5568.
146. Cave MC, Hurt RT, Frazier TH, et al. Obesity, inflammation, and the potential application of pharmaconutrition. *Nutr Clin Pract*. 2008;23(1):16–34.
147. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol*. 2005;115(5):911–919. quiz 920.
148. Fontana L, Eagon JC, Trujillo ME, et al. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007;56(4):1010–1013.
149. Hung J, McQuillan BM, Thompson PL, et al. Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. *Int J Obes (Lond)*. 2008;32(5):772–779.
150. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 2002;57(5):M326–M332.
151. Schaap LA, Pluijm SM, Deeg DJ, et al. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 2006;119(6). 526 e529–517.
152. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab*. 2003;284(3):E481–E487.
153. Roth SM, Metter EJ, Ling S, et al. Inflammatory factors in age-related muscle wasting. *Curr Opin Rheumatol*. 2006;18(6):625–630.
154. Cesari M, Kritchevsky SB, Baumgartner RN, et al. Sarcopenia, obesity, and inflammation—results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. *Am J Clin Nutr*. 2005;82(2):428–434.
155. Schrager MA, Metter EJ, Simonsick E, et al. Sarcopenic obesity and inflammation in the InCHIANTI study. *J Appl Physiol (1985)*. 2007;102(3):919–925.
156. De Martinis M, Franceschi C, Monti D, et al. Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol*. 2006;80(3):219–227.
157. Lio D, Scola L, Crivello A, et al. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. *Genes Immun*. 2002;3(1):30–33.
158. Argiles JM, Anker SD, Evans WJ, et al. Consensus on cachexia definitions. *J Am Med Dir Assoc*. 2010;11(4):229–230.
159. Greiwe JS, Cheng B, Rubin DC, et al. Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. *FASEB J*. 2001;15(2):475–482.
160. Toth MJ, Matthews DE, Tracy RP, et al. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. *Am J Physiol Endocrinol Metab*. 2005;288(5):E883–891.
161. Trappe TA, Carroll CC, Dickinson JM, et al. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(3):R655–662.
162. Delhaas T, Van der Meer SF, Schaart G, et al. Steep increase in myonuclear domain size during infancy. *Anat Rec (Hoboken)*. 2013;296(2):192–197.
163. Jacquemin V, Furling D, Bigot A, et al. IGF-1 induces human myotube hypertrophy by increasing cell recruitment. *Exp Cell Res*. 2004;299(1):148–158.
164. Favier FB, Benoit H, Freyssenet D. Cellular and molecular events controlling skeletal muscle mass in response to altered use. *Pflugers Arch*. 2008;456(3):587–600.
165. Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol (1985)*. 2001;91(2):534–551.
166. Jozsi AC, Campbell WW, Joseph L, et al. Changes in power with resistance training in older and younger men and women. *J Gerontol A Biol Sci Med Sci*. 1999;54(11):M591–596.
167. Degens H. The role of systemic inflammation in age-related muscle weakness and wasting. *Scand J Med Sci Sports*. 2010;20(1):28–38.
168. Szalay K, Razga Z, Duda E. TNF inhibits myogenesis and downregulates the expression of myogenic regulatory factors myoD and myogenin. *Eur J Cell Biol*. 1997;74(4):391–398.
169. Langen RC, Van Der Velden JL, Schols AM, et al. Tumor necrosis factor-alpha inhibits myogenic differentiation through MyoD protein destabilization. *FASEB J*. 2004;18(2):227–237.
170. Guttridge DC, Mayo MW, Madrid LV, et al. NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science*. 2000;289(5488):2363–2366.
171. Reid MB, Li YP. Tumor necrosis factor-alpha and muscle wasting: a cellular perspective. *Respir Res*. 2001;2(5):269–272.
172. Saini A, Al-Shanti N, Faulkner SH, et al. Pro- and anti-apoptotic roles for IGF-I in TNF-alpha-induced apoptosis: a MAP kinase mediated mechanism. *Growth Factors*. 2008;26(5):239–253.
173. Welle S, Totterman S, Thornton C. Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci*. 1996;51(6):M270–275.
174. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*. 1997;387(6628):83–90.
175. Zimmers TA, Davies MV, Koniaris LG, et al. Induction of cachexia in mice by systemically administered myostatin. *Science*. 2002;296(5572):1486–1488.
176. Langley B, Thomas M, Bishop A, et al. Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *J Biol Chem*. 2002;277(51):49831–49840.
177. Zhang L, Rajan V, Lin E, et al. Pharmacological inhibition of myostatin suppresses systemic inflammation and muscle atrophy in mice with chronic kidney disease. *FASEB J*. 2011;25(5):1653–1663.
178. Wang XH. MicroRNA in myogenesis and muscle atrophy. *Curr Opin Clin Nutr Metab Care*. 2013;16(3):258–266.
179. Chen JF, Mandel EM, Thomson JM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet*. 2006;38(2):228–233.
180. Brennecke J, Hipfner DR, Stark A, et al. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell*. 2003;113(1):25–36.
181. Xu C, Lu Y, Pan Z, et al. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. *J Cell Sci*. 2007;120(Pt 17):3045–3052.
182. Boehm M, Slack F. A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science*. 2005;310(5756):1954–1957.
183. Wang E. MicroRNA, the putative molecular control for mid-life decline. *Ageing Res Rev*. 2007;6(1):1–11.
184. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281–297.
185. Lewis BP, Shih IH, Jones-Rhoades MW, et al. Prediction of mammalian microRNA targets. *Cell*. 2003;115(7):787–798.
186. Hamrick MW, Herberg S, Arounleut P, et al. The adipokine leptin increases skeletal muscle mass and significantly alters skeletal muscle miRNA expression profile in aged mice. *Biochem Biophys Res Commun*. 2010;400(3):379–383.
187. Drummond MJ, McCarthy JJ, Sinha M, et al. Aging and microRNA expression in human skeletal muscle: a microarray and bioinformatics analysis. *Physiol Genomics*. 2011;43(10):595–603.

188. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res*. 2011;39:D152–157 (Database issue).
189. Callis TE, Deng Z, Chen JF, et al. Muscling through the microRNA world. *Exp Biol Med (Maywood)*. 2008;233(2):131–138.
190. Eisenberg I, Eran A, Nishino I, et al. Distinctive patterns of microRNA expression in primary muscular disorders. *Proc Natl Acad Sci U S A*. 2007;104(43):17016–17021.
191. Small EM, O'Rourke JR, Moresi V, et al. Regulation of PI3-kinase/Akt signaling by muscle-enriched microRNA-486. *Proc Natl Acad Sci U S A*. 2010;107(9):4218–4223.
192. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol (1985)*. 2007;102(1):306–313.
193. Kim VN, Nam JW. Genomics of microRNA. *Trends Genet*. 2006;22(3):165–173.
194. van Rooij E, Quiat D, Johnson BA, et al. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Dev Cell*. 2009;17(5):662–673.
195. Drummond MJ, McCarthy JJ, Fry CS, et al. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. *Am J Physiol Endocrinol Metab*. 2008;295(6):E1333–1340.
196. Guller I, Russell AP. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. *J Physiol*. 2010;588(Pt 21):4075–4087.
197. Panguluri SK, Bhatnagar S, Kumar A, et al. Genomic profiling of messenger RNAs and microRNAs reveals potential mechanisms of TWEAK-induced skeletal muscle wasting in mice. *PLoS One*. 2010;5(1):e8760.
198. Dogra C, Changotra H, Wedhas N, et al. TNF-related weak inducer of apoptosis (TWEAK) is a potent skeletal muscle-wasting cytokine. *FASEB J*. 2007;21(8):1857–1869.
199. Smith-Vikos T, Slack FJ. MicroRNAs and their roles in aging. *J Cell Sci*. 2012;125(Pt 1):7–17.
200. Wada S, Kato Y, Okutsu M, et al. Translational suppression of atrophic regulators by microRNA-23a integrates resistance to skeletal muscle atrophy. *J Biol Chem*. 2011;286(44):38456–38465.
201. Safdar A, Abadi A, Akhtar M, et al. miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. *PLoS One*. 2009;4(5):e5610.
202. Zhou L, Wang L, Lu L, et al. A novel target of microRNA-29, Ring1 and YY1-binding protein (Rybp), negatively regulates skeletal myogenesis. *J Biol Chem*. 2012;287(30):25255–25265.
203. Peake JM, Suzuki K, Coombes JS. The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. *J Nutr Biochem*. 2007;18(6):357–371.
204. Scalbert A, Manach C, Morand C, et al. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr*. 2005;45(4):287–306.
205. Bouzid MA, Hammouda O, Matran R, et al. Changes in oxidative stress markers and biological markers of muscle injury with aging at rest and in response to an exhaustive exercise. *PLoS One*. 2014;9(3):e90420.
206. Samaras A, Tsarouhas K, Paschalidis E, et al. Effect of a special carbohydrate-protein bar and tomato juice supplementation on oxidative stress markers and vascular endothelial dynamics in ultra-marathon runners. *Food Chem Toxicol*. 2014;69:231–236.
207. Pellegrino MA, Desaphy JF, Brocca L, et al. Redox homeostasis, oxidative stress and disuse muscle atrophy. *J Physiol*. 2011;589(Pt 9):2147–2160.
208. Jackson JR, Ryan MJ, Hao Y, et al. Mediation of endogenous antioxidant enzymes and apoptotic signaling by resveratrol following muscle disuse in the gastrocnemius muscles of young and old rats. *Am J Physiol Regul Integr Comp Physiol*. 2010;299(6):R1572–R1581.
209. Tenover JL. Testosterone and the aging male. *J Androl*. 1997;18(2):103–106.
210. Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, et al. Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study. *J Clin Endocrinol Metab*. 2000;85(2):645–651.
211. Morley JE, Kaiser FE, Perry 3rd HM, et al. Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *Metabolism*. 1997;46(4):410–413.
212. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab*. 2002;87(2):589–598.
213. Sattler FR, Castaneda-Sceppa C, Binder EF, et al. Testosterone and growth hormone improve body composition and muscle performance in older men. *J Clin Endocrinol Metab*. 2009;94(6):1991–2001.
214. van den Beld AW, de Jong FH, Grobbee DE, et al. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. *J Clin Endocrinol Metab*. 2000;85(9):3276–3282.
215. Perry 3rd HM, Miller DK, Patrick P, et al. Testosterone and leptin in older African-American men: relationship to age, strength, function, and season. *Metabolism*. 2000;49(8):1085–1091.
216. Bhasin S, Tenover JS. Age-associated sarcopenia—issues in the use of testosterone as an anabolic agent in older men. *J Clin Endocrinol Metab*. 1997;82(6):1659–1660.
217. Gooren LJ. Endocrine aspects of ageing in the male. *Mol Cell Endocrinol*. 1998;145(1–2):153–159.
218. Korenman SG, Morley JE, Mooradian AD, et al. Secondary hypogonadism in older men: its relation to impotence. *J Clin Endocrinol Metab*. 1990;71(4):963–969.
219. Morley JE, Kaiser F, Raum WJ, et al. Potentially predictive and manipulable blood serum correlates of aging in the healthy human male: progressive decreases in bioavailable testosterone, dehydroepiandrosterone sulfate, and the ratio of insulin-like growth factor 1 to growth hormone. *Proc Natl Acad Sci U S A*. 1997;94(14):7537–7542.
220. Gray A, Feldman HA, McKinlay JB, et al. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *J Clin Endocrinol Metab*. 1991;73(5):1016–1025.
221. Birkenhager-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulphate (DHEAS) in the oldest old, aged 85 and over. *Ann N Y Acad Sci*. 1994;719:543–552.
222. Veldhuis JD, Iranmanesh A, Weltman A. Elements in the pathophysiology of diminished growth hormone (GH) secretion in aging humans. *Endocrine*. 1997;7(1):41–48.
223. Kim MJ, Morley JE. The hormonal fountains of youth: myth or reality?. *J Endocrinol Invest*. 2005;28:5–14 (11 Suppl Proceedings).
224. Poehlman ET, Copeland KC. Influence of physical activity on insulin-like growth factor-I in healthy younger and older men. *J Clin Endocrinol Metab*. 1990;71(6):1468–1473.
225. Morley JE. Scientific overview of hormone treatment used for rejuvenation. *Fertil Steril*. 2013;99(7):1807–1813.
226. Clavel S, Coldefy AS, Kurkdjian E, et al. Atrophy-related ubiquitin ligases, atrogin-1 and MuRF1 are up-regulated in aged rat tibialis anterior muscle. *Mech Ageing Dev*. 2006;127(10):794–801.
227. Ceda GP, Dall'Aglio E, Maggio M, et al. Clinical implications of the reduced activity of the GH-IGF-I axis in older men. *J Endocrinol Invest*. 2005;28:96–100 (11 suppl Proceedings).
228. Schaap LA, Pluijm SM, Smit JH, et al. The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. *Clin Endocrinol (Oxf)*. 2005;63(2):152–160.
229. Cappola AR, Bandeen-Roche K, Wand GS, et al. Association of IGF-I levels with muscle strength and mobility in older women. *J Clin Endocrinol Metab*. 2001;86(9):4139–4146.
230. Zampieri S, Pietrangelo L, Loefler S, et al. Lifelong physical exercise delays age-associated skeletal muscle decline. *J Gerontol A Biol Sci Med Sci*. 2015;70(2):163–173.
231. Manini TM, Everhart JE, Patel KV, et al. Daily activity energy expenditure and mortality among older adults. *JAMA*. 2006;296(2):171–179.
232. Hinrichs T, von Bonsdorff MB, Tormakangas T, et al. Inverse effects of midlife occupational and leisure time physical activity on mobility limitation in old age—a 28-year prospective follow-up study. *J Am Geriatr Soc*. 2014;62(5):812–820.

233. Gariballa SE, Sinclair AJ. Nutrition, ageing and ill health. *Br J Nutr*. 1998;80(1):7–23.
234. Miller AS, Furness JB, Costa M. The relationship between gastrin cells and bombesin-like immunoreactive nerve fibres in the gastric antral mucosa of guinea-pig, rat, dog and man. *Cell Tissue Res*. 1989;257(1):171–178.
235. Wakimoto P, Block G. Dietary intake, dietary patterns, and changes with age: an epidemiological perspective. *J Gerontol A Biol Sci Med Sci*. 2001;56:65–80. Spec No 2.
236. Payette H, Boutier V, Coulombe C, et al. Benefits of nutritional supplementation in free-living, frail, undernourished elderly people: a prospective randomized community trial. *J Am Diet Assoc*. 2002;102(8):1088–1095.
237. Sullivan DH, Sun S, Walls RC. Protein-energy undernutrition among elderly hospitalized patients: a prospective study. *JAMA*. 1999;281(21):2013–2019.
238. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc*. 2002;50(5):889–896.
239. Manini TM, Clark BC. Dynapenia and aging: an update. *J Gerontol A Biol Sci Med Sci*. 2012;67(1):28–40.
240. Studenski S. Evidence-based criteria for sarcopenia with clinically important weakness. *Semin Arthritis Rheum*. 2013;42(4):447–449.
241. Studenski SA, Peters KW, Alley DE, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):547–558.
242. McLean RR, Shardell MD, Alley DE, et al. Criteria for clinically relevant weakness and low lean mass and their longitudinal association with incident mobility impairment and mortality: the foundation for the National Institutes of Health (FNIH) sarcopenia project. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):576–583.
243. Cawthon PM, Peters KW, Shardell MD, et al. Cutpoints for low appendicular lean mass that identify older adults with clinically significant weakness. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):567–575.
244. Cao L, Morley JE. Sarcopenia is recognized as an independent condition by an International Classification of Disease, Tenth Revision, Clinical Modification (ICD-10-CM) Code. *J Am Med Dir Assoc*. 2016;17(8):675–677.
245. Lardies-Sanchez B, Sanz-Paris A, Perez-Nogueras J, et al. Influence of nutritional status in the diagnosis of sarcopenia in nursing home residents. *Nutrition*. 2017;41:51–57.
246. Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. *J Gerontol A Biol Sci Med Sci*. 2001;56(5):B209–B217.
247. Thomas DR. Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia. *Clin Nutr*. 2007;26(4):389–399.
248. Morley JE. Weight loss in the nursing home. *J Am Med Dir Assoc*. 2007;8(4):201–204.
249. Morley JE, Thomas DR, Wilson MM. Cachexia: pathophysiology and clinical relevance. *Am J Clin Nutr*. 2006;83(4):735–743.
250. Huo YR, Suriyaarachchi P, Gomez F, et al. Phenotype of osteosarcopenia in older individuals with a history of falling. *J Am Med Dir Assoc*. 2015;16(4):290–295.
251. Morley JE. Frailty and sarcopenia: the new geriatric giants. *Rev Invest Clin*. 2016;68(2):59–67.
252. Chumlea WC, Guo SS, Vellas B, et al. Techniques of assessing muscle mass and function (sarcopenia) for epidemiological studies of the elderly. *J Gerontol A Biol Sci Med Sci*. 1995;50:45–51 (Spec No).
253. Beliaeff S, Bouchard DR, Hautier C, et al. Association between muscle mass and isometric muscle strength in well-functioning older men and women. *J Aging Phys Act*. 2008;16(4):484–493.
254. Clark BC, Manini TM. Sarcopenia ≠ dynapenia. *J Gerontol A Biol Sci Med Sci*. 2008;63(8):829–834.
255. Clark BC, Manini TM. What is dynapenia? *Nutrition*. 2012;28(5):495–503.
256. Heber D, Ingles S, Ashley JM, et al. Clinical detection of sarcopenic obesity by bioelectrical impedance analysis. *Am J Clin Nutr*. 1996;64(suppl 3):472S–477S.
257. Forbes GB. Longitudinal changes in adult fat-free mass: influence of body weight. *Am J Clin Nutr*. 1999;70(6):1025–1031.
258. Hughes VA, Frontera WR, Roubenoff R, et al. Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. *Am J Clin Nutr*. 2002;76(2):473–481.
259. Gallagher D, Ruts E, Visser M, et al. Weight stability masks sarcopenia in elderly men and women. *Am J Physiol Endocrinol Metab*. 2000;279(2):E366–E375.
260. Roubenoff R. Sarcopenia and its implications for the elderly. *Eur J Clin Nutr*. 2000;54(suppl 3):S40–S47.
261. Goodpaster BH, Carlson CL, Visser M, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol (1985)*. 2001;90(6):2157–2165.
262. Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *J Am Geriatr Soc*. 2002;50(5):897–904.
263. Sipila S, Suominen H. Knee extension strength and walking speed in relation to quadriceps muscle composition and training in elderly women. *Clin Physiol*. 1994;14(4):433–442.
264. Corcoran MP, Lamon-Fava S, Fielding RA. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *Am J Clin Nutr*. 2007;85(3):662–677.
265. Yudkin JS, Kumari M, Humphries SE, et al. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148(2):209–214.
266. Ryan AS, Nicklas BJ. Reductions in plasma cytokine levels with weight loss improve insulin sensitivity in overweight and obese postmenopausal women. *Diabetes Care*. 2004;27(7):1699–1705.
267. Roubenoff R. Sarcopenic obesity: does muscle loss cause fat gain? Lessons from rheumatoid arthritis and osteoarthritis. *Ann N Y Acad Sci*. 2000;904:553–557.
268. Baumgartner RN, Wayne SJ, Waters DL, et al. Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly. *Obes Res*. 2004;12(12):1995–2004.
269. Davison KK, Ford ES, Cogswell ME, et al. Percentage of body fat and body mass index are associated with mobility limitations in people aged 70 and older from NHANES III. *J Am Geriatr Soc*. 2002;50(11):1802–1809.
270. Abellan van Kan G, Rolland YM, Morley JE, et al. Frailty: toward a clinical definition. *J Am Med Dir Assoc*. 2008;9(2):71–72.
271. Abellan van Kan G, Rolland Y, Bergman H, et al. The I.A.N.A Task Force on frailty assessment of older people in clinical practice. *J Nutr Health Aging*. 2008;12(1):29–37.
272. Morley JE. Frailty screening comes of age. *J Nutr Health Aging*. 2014;18(5):453–454.
273. Woo J, Yu R, Wong M, et al. Frailty screening in the community using the FRAIL scale. *J Am Med Dir Assoc*. 2015;16(5):412–419.
274. Mijnarends DM, Schols JM, Meijers JM, et al. Instruments to assess sarcopenia and physical frailty in older people living in a community (care) setting: similarities and discrepancies. *J Am Med Dir Assoc*. 2015;16(4):301–308.
275. Morley JE, Anker SD, von Haehling S. Prevalence, incidence, and clinical impact of sarcopenia: facts, numbers, and epidemiology-update 2014. *J Cachexia Sarcopenia Muscle*. 2014;5(4):253–259.
276. Morley JE, von Haehling S, Anker SD, et al. From sarcopenia to frailty: a road less traveled. *J Cachexia Sarcopenia Muscle*. 2014;5(1):5–8.
277. Walston J, Hadley EC, Ferrucci L, et al. Research agenda for frailty in older adults: toward a better understanding of physiology and etiology: summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *J Am Geriatr Soc*. 2006;54(6):991–1001.
278. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56(3):M146–156.

279. Aubertin-Leheudre M, Woods AJ, Anton S, et al. Frailty clinical phenotype: a physical and cognitive point of view. *Nestle Nutr Inst Workshop Ser*. 2015;83:55–63.
280. Sayer AA, Dennison EM, Syddall HE, et al. Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? *Diabetes Care*. 2005;28(10):2541–2542.
281. Reginster JY, Beaudart C, Buckinx F, et al. Osteoporosis and sarcopenia: two diseases or one? *Curr Opin Clin Nutr Metab Care*. 2016;19(1):31–36.
282. Welch C, Hassan-Smith KZ, Greig AC, et al. Acute sarcopenia secondary to hospitalisation—an emerging condition affecting older adults. *Aging Dis*. 2018;9(1):151–164.
283. Marzetti E. Editorial: imaging, functional and biological markers for sarcopenia: the pursuit of the golden ratio. *J Frailty Aging*. 2012;1(3):97–98.
284. Cummings SR, Studenski S, Ferrucci L. A diagnosis of dismobility—giving mobility clinical visibility: a Mobility Working Group recommendation. *JAMA*. 2014;311(20):2061–2062.
285. Studenski S. What are the outcomes of treatment among patients with sarcopenia? *J Nutr Health Aging*. 2009;13(8):733–736.
286. Scharf G, Heineke J. Finding good biomarkers for sarcopenia. *J Cachexia Sarcopenia Muscle*. 2012;3(3):145–148.
287. Delmonico MJ, Harris TB, Lee JS, et al. Alternative definitions of sarcopenia, lower extremity performance, and functional impairment with aging in older men and women. *J Am Geriatr Soc*. 2007;55(5):769–774.
288. Newman AB, Simonsick EM, Naydeck BL, et al. Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability. *JAMA*. 2006;295(17):2018–2026.
289. Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med*. 1995;332(9):556–561.
290. Cesari M, Kritchevsky SB, Penninx BW, et al. Prognostic value of usual gait speed in well-functioning older people—results from the Health, Aging and Body Composition Study. *J Am Geriatr Soc*. 2005;53(10):1675–1680.
291. Rolland Y, Lauwers-Cances V, Cesari M, et al. Physical performance measures as predictors of mortality in a cohort of community-dwelling older French women. *Eur J Epidemiol*. 2006;21(2):113–122.
292. Cesari M, Rolland Y, Abellan Van Kan G, et al. Sarcopenia-related parameters and incident disability in older persons: results from the "Invecchiare in Chianti" study. *J Gerontol A Biol Sci Med Sci*. 2015;70(4):457–463.
293. Studenski S, Perera S, Wallace D, et al. Physical performance measures in the clinical setting. *J Am Geriatr Soc*. 2003;51(3):314–322.
294. Dodge HH, Mattek NC, Austin D, et al. In-home walking speeds and variability trajectories associated with mild cognitive impairment. *Neurology*. 2012;78(24):1946–1952.
295. Ries JD, Echternach JL, Nof L, et al. Test-retest reliability and minimal detectable change scores for the timed "up & go" test, the six-minute walk test, and gait speed in people with Alzheimer disease. *Phys Ther*. 2009;89(6):569–579.
296. Kaufman KR, Hughes C, Morrey BF, et al. Gait characteristics of patients with knee osteoarthritis. *J Biomech*. 2001;34(7):907–915.
297. Afilalo J, Eisenberg MJ, Morin JF, et al. Gait speed as an incremental predictor of mortality and major morbidity in elderly patients undergoing cardiac surgery. *J Am Coll Cardiol*. 2010;56(20):1668–1676.
298. Karpman C, Lebrasseur NK, Depew ZS, et al. Measuring gait speed in the out-patient clinic: methodology and feasibility. *Respir Care*. 2014;59(4):531–537.
299. Biderman A, Cwikel J, Fried AV, et al. Depression and falls among community dwelling elderly people: a search for common risk factors. *J Epidemiol Community Health*. 2002;56(8):631–636.
300. Montero-Odasso M, Schapira M, Soriano ER, et al. Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older. *J Gerontol A Biol Sci Med Sci*. 2005;60(10):1304–1309.
301. Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. *JAMA*. 2011;305(1):50–58.
302. Hardy SE, Perera S, Roumani YF, et al. Improvement in usual gait speed predicts better survival in older adults. *J Am Geriatr Soc*. 2007;55(11):1727–1734.
303. Abellan van Kan G, Rolland Y, Andrieu S, et al. Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force. *J Nutr Health Aging*. 2009;13(10):881–889.
304. Buchner DM, Larson EB, Wagner EH, et al. Evidence for a non-linear relationship between leg strength and gait speed. *Age Ageing*. 1996;25(5):386–391.
305. Steffen TM, Hacker TA, Mollinger L. Age- and gender-related test performance in community-dwelling elderly people: six-minute walk test, Berg Balance Scale, timed up & go test, and gait speeds. *Phys Ther*. 2002;82(2):128–137.
306. Mijnarends DM, Meijers JM, Halfens RJ, et al. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. *J Am Med Dir Assoc*. 2013;14(3):170–178.
307. Lauretani F, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol (1985)*. 2003;95(5):1851–M1860.
308. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *J Gerontol A Biol Sci Med Sci*. 2000;55(4):M221–M231.
309. Landi F, Cruz-Jentoft AJ, Liperoti R, et al. Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study. *Age Ageing*. 2013;42(2):203–209.
310. Asher L, Aresu M, Falaschetti E, et al. Most older pedestrians are unable to cross the road in time: a cross-sectional study. *Age Ageing*. 2012;41(5):690–694.
311. Beavers KM, Beavers DP, Houston DK, et al. Associations between body composition and gait-speed decline: results from the Health, Aging, and Body Composition study. *Am J Clin Nutr*. 2013;97(3):552–560.
312. Chen LK, Liu LK, Woo J, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc*. 2014;15(2):95–101.
313. Zeng P, Wu S, Han Y, et al. Differences in body composition and physical functions associated with sarcopenia in Chinese elderly: reference values and prevalence. *Arch Gerontol Geriatr*. 2015;60(1):118–123.
314. Mathias S, Nayak US, Isaacs B. Balance in elderly patients: the "get-up and go" test. *Arch Phys Med Rehabil*. 1986;67(6):387–389.
315. Podsiadlo D, Richardson S. The timed "up & go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc*. 1991;39(2):142–148.
316. Berg KO, Maki BE, Williams JI, et al. Clinical and laboratory measures of postural balance in an elderly population. *Arch Phys Med Rehabil*. 1992;73(11):1073–1080.
317. Berg KO, Wood-Dauphinee SL, Williams JI, et al. Measuring balance in the elderly: validation of an instrument. *Can J Public Health*. 1992;83(suppl 2):S7–S11.
318. Shumway-Cook A, Baldwin M, Polissar NL, et al. Predicting the probability for falls in community-dwelling older adults. *Phys Ther*. 1997;77(8):812–819.
319. Chiu AY, Au-Yeung SS, Lo SK. A comparison of four functional tests in discriminating fallers from non-fallers in older people. *Disabil Rehabil*. 2003;25(1):45–50.
320. Bean JF, Kiely DK, LaRose S, et al. Is stair climb power a clinically relevant measure of leg power impairments in at-risk older adults? *Arch Phys Med Rehabil*. 2007;88(5):604–609.
321. Verdijk LB, Snijders T, Beelen M, et al. Characteristics of muscle fiber type are predictive of skeletal muscle mass and strength in elderly men. *J Am Geriatr Soc*. 2010;58(11):2069–2075.
322. Goodpaster BH, Park SW, Harris TB, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci*. 2006;61(10):1059–1064.
323. Newman AB, Kupelian V, Visser M, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci*. 2006;61(1):72–77.

324. Reid KF, Callahan DM, Carabello RJ, et al. Lower extremity power training in elderly subjects with mobility limitations: a randomized controlled trial. *Aging Clin Exp Res*. 2008;20(4):337–343.
325. Reid KF, Fielding RA. Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exerc Sport Sci Rev*. 2012;40(1):4–12.
326. Sayer AA, Robinson SM, Patel HP, et al. New horizons in the pathogenesis, diagnosis and management of sarcopenia. *Age Ageing*. 2013;42(2):145–150.
327. Cooper C, Fielding R, Visser M, et al. Tools in the assessment of sarcopenia. *Calcif Tissue Int*. 2013;93(3):201–210.
328. Garcia-Pena C, Garcia-Fabela LC, Gutierrez-Robledo LM, et al. Handgrip strength predicts functional decline at discharge in hospitalized male elderly: a hospital cohort study. *PLoS One*. 2013;8(7):e69849.
329. Sallinen J, Stenholm S, Rantanen T, et al. Hand-grip strength cut points to screen older persons at risk for mobility limitation. *J Am Geriatr Soc*. 2010;58(9):1721–1726.
330. Nybo H, Gaist D, Jeune B, et al. Functional status and self-rated health in 2,262 nonagenarians: the Danish 1905 Cohort Survey. *J Am Geriatr Soc*. 2001;49(5):601–609.
331. Al Snih S, Markides KS, Ottenbacher KJ, et al. Hand grip strength and incident ADL disability in elderly Mexican Americans over a seven-year period. *Aging Clin Exp Res*. 2004;16(6):481–486.
332. Frederiksen H, Hjelmborg J, Mortensen J, et al. Age trajectories of grip strength: cross-sectional and longitudinal data among 8,342 Danes aged 46 to 102. *Ann Epidemiol*. 2006;16(7):554–562.
333. Cooper R, Kuh D, Hardy R, Mortality Review G, Falcon, Teams HAS. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. *BMJ*. 2010;341:c4467.
334. Stevens PJ, Syddall HE, Patel HP, et al. Is grip strength a good marker of physical performance among community-dwelling older people? *J Nutr Health Aging*. 2012;16(9):769–774.
335. Bohannon RW, Magasi SR, Bubela DJ, et al. Grip and knee extension muscle strength reflect a common construct among adults. *Muscle Nerve*. 2012;46(4):555–558.
336. Rantanen T, Era P, Heikkinen E. Maximal isometric strength and mobility among 75-year-old men and women. *Age Ageing*. 1994;23(2):132–137.
337. Wickham C, Cooper C, Margetts BM, et al. Muscle strength, activity, housing and the risk of falls in elderly people. *Age Ageing*. 1989;18(1):47–51.
338. Rantanen T, Guralnik JM, Foley D, et al. Midlife hand grip strength as a predictor of old age disability. *JAMA*. 1999;281(6):558–560.
339. Bohannon RW, Magasi S. Identification of dynapenia in older adults through the use of grip strength t-scores. *Muscle Nerve*. 2015;51(1):102–105.
340. Dodds RM, Syddall HE, Cooper R, et al. Grip strength across the life course: normative data from twelve British studies. *PLoS One*. 2014;9(12):e113637.
341. Alley DE, Shardell MD, Peters KW, et al. Grip strength cutpoints for the identification of clinically relevant weakness. *J Gerontol A Biol Sci Med Sci*. 2014;69(5):559–566.
342. Fox B, Henwood T, Schaap L, et al. Adherence to a standardized protocol for measuring grip strength and appropriate cut-off values in adults over 65 years with sarcopenia: a systematic review protocol. *JBI Database System Rev Implement Rep*. 2015;13(10):50–59.
343. Nevitt MC, Cummings SR, Kidd S, et al. Risk factors for recurrent nonsyncopal falls. A prospective study. *JAMA*. 1989;261(18):2663–2668.
344. Moreland JD, Richardson JA, Goldsmith CH, et al. Muscle weakness and falls in older adults: a systematic review and meta-analysis. *J Am Geriatr Soc*. 2004;52(7):1121–1129.
345. Visser M, Goodpaster BH, Kritchevsky SB, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *J Gerontol A Biol Sci Med Sci*. 2005;60(3):324–333.
346. Lang T, Streeper T, Cawthon P, et al. Sarcopenia: etiology, clinical consequences, intervention, and assessment. *Osteoporos Int*. 2010;21(4):543–559.
347. Larsson L, Grimby G, Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. *J Appl Physiol Respir Environ Exerc Physiol*. 1979;46(3):451–456.
348. Murray MP, Gardner GM, Mollinger LA, et al. Strength of isometric and isokinetic contractions: knee muscles of men aged 20 to 86. *Phys Ther*. 1980;60(4):412–419.
349. Young A, Stokes M, Crowe M. Size and strength of the quadriceps muscles of old and young women. *Eur J Clin Invest*. 1984;14(4):282–287.
350. Young A, Stokes M, Crowe M. The size and strength of the quadriceps muscles of old and young men. *Clin Physiol*. 1985;5(2):145–154.
351. Aniansson A, Hedberg M, Henning GB, et al. Muscle morphology, enzymatic activity, and muscle strength in elderly men: a follow-up study. *Muscle Nerve*. 1986;9(7):585–591.
352. Visser M, Deeg DJ, Lips P, et al. Skeletal muscle mass and muscle strength in relation to lower-extremity performance in older men and women. *J Am Geriatr Soc*. 2000;48(4):381–386.
353. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. *Res Q Exerc Sport*. 1999;70(2):113–119.
354. Visser M, Newman AB, Nevitt MC, et al. Reexamining the sarcopenia hypothesis. Muscle mass versus muscle strength. Health, Aging, and Body Composition Study Research Group. *Ann NY Acad Sci*. 2000;904:456–461.
355. Guralnik JM, Simonsick EM, Ferrucci L, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol*. 1994;49(2):M85–M94.
356. Miller DK, Wolinsky FD, Andresen EM, et al. Adverse outcomes and correlates of change in the Short Physical Performance Battery over 36 months in the African American health project. *J Gerontol A Biol Sci Med Sci*. 2008;63(5):487–494.
357. Perera S, Mody SH, Woodman RC, et al. Meaningful change and responsiveness in common physical performance measures in older adults. *J Am Geriatr Soc*. 2006;54(5):743–749.
358. Kwon S, Perera S, Pahor M, et al. What is a meaningful change in physical performance? Findings from a clinical trial in older adults (the LIFE-P study). *J Nutr Health Aging*. 2009;13(6):538–544.
359. Ostir GV, Volpato S, Fried LP, et al. Reliability and sensitivity to change assessed for a summary measure of lower body function: results from the Women's Health and Aging Study. *J Clin Epidemiol*. 2002;55(9):916–921.
360. Working Group on Functional Outcome Measures for Clinical Trials. Functional outcomes for clinical trials in frail older persons: time to be moving. *J Gerontol A Biol Sci Med Sci*. 2008;63(2):160–164.
361. Gallagher D, Visser M, De Meersman RE, et al. Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *J Appl Physiol (1985)*. 1997;83(1):229–239.
362. Kim KM, Jang HC, Lim S. Differences among skeletal muscle mass indices derived from height-, weight-, and body mass index-adjusted models in assessing sarcopenia. *Korean J Intern Med*. 2016;31(4):643–650.
363. Scott D, Hayes A, Sanders KM, et al. Operational definitions of sarcopenia and their associations with 5-year changes in falls risk in community-dwelling middle-aged and older adults. *Osteoporos Int*. 2014;25(1):187–193.
364. Batsis JA, Barre LK, Mackenzie TA, et al. Variation in the prevalence of sarcopenia and sarcopenic obesity in older adults associated with different research definitions: dual-energy X-ray absorptiometry data from the National Health and Nutrition Examination Survey 1999-2004. *J Am Geriatr Soc*. 2013;61(6):974–980.
365. Lee SY, Gallagher D. Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care*. 2008;11(5):566–572.
366. Chien MY, Huang TY, Wu YT. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. *J Am Geriatr Soc*. 2008;56(9):1710–1715.
367. Clarys JP, Martin AD, Drinkwater DT, et al. The skinfold: myth and reality. *J Sports Sci*. 1987;5(1):3–33.
368. McArdle WDKF, Katch VL. *Exercise Physiology: Energy Nutrition & Human Performance*. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.
369. Martorell RMF, Mueller W, Pawson I. *Which Side to Measure: Right or Left*. Champaign, IL: Human Kinetics Publishers; 1988.

370. van der Kooy K, Seidell JC. Techniques for the measurement of visceral fat: a practical guide. *Int J Obes Relat Metab Disord*. 1993;17(4):187–196.
371. Campbell AJ, Spears GF, Brown JS, et al. Anthropometric measurements as predictors of mortality in a community population aged 70 years and over. *Age Ageing*. 1990;19(2):131–135.
372. Baumgartner RN. Body composition in elderly persons: a critical review of needs and methods. *Prog Food Nutr Sci*. 1993;17(3):223–260.
373. Baumgartner RN, Stauber PM, McHugh D, et al. Cross-sectional age differences in body composition in persons 60+ years of age. *J Gerontol A Biol Sci Med Sci*. 1995;50(6):M307–316.
374. Baumgartner RN, Heymsfield SB, Lichtman S, et al. Body composition in elderly people: effect of criterion estimates on predictive equations. *Am J Clin Nutr*. 1991;53(6):1345–1353.
375. Goran MI, Toth MJ, Poehlman ET. Cross-validation of anthropometric and bioelectrical resistance prediction equations for body composition in older people using the 4-compartment model as a criterion method. *J Am Geriatr Soc*. 1997;45(7):837–843.
376. Heymsfield SB, Wang Z, Visser M, et al. Techniques used in the measurement of body composition: an overview with emphasis on bioelectrical impedance analysis. *Am J Clin Nutr*. 1996;64(suppl 3):478S–484S.
377. Pietrobelli A, Morini P, Battistini N, et al. Appendicular skeletal muscle mass: prediction from multiple frequency segmental bioimpedance analysis. *Eur J Clin Nutr*. 1998;52(7):507–511.
378. Rush EC, Chandu V, Plank LD. Prediction of fat-free mass by bioimpedance analysis in migrant Asian Indian men and women: a cross validation study. *Int J Obes (Lond)*. 2006;30(7):1125–1131.
379. Lukaski HC. Methods for the assessment of human body composition: traditional and new. *Am J Clin Nutr*. 1987;46(4):537–556.
380. Wagner DR. Ultrasound as a tool to assess body fat. *J Obes*. 2013;2013:280713.
381. Harris-Love MO, Monfaredi R, Ismail C, et al. Quantitative ultrasound: measurement considerations for the assessment of muscular dystrophy and sarcopenia. *Front Aging Neurosci*. 2014;6:172.
382. Pedersen M, Fredberg U, Langberg H. Sonoelastography as a diagnostic tool in the assessment of musculoskeletal alterations: a systematic review. *Ultraschall Med*. 2012;33(5):441–446.
383. Sarvazyan AP, Urban MW, Greenleaf JF. Acoustic waves in medical imaging and diagnostics. *Ultrasound Med Biol*. 2013;39(7):1133–1146.
384. Drakonaki EE, Allen GM, Wilson DJ. Ultrasound elastography for musculoskeletal applications. *Br J Radiol*. 2012;85(1019):1435–1445.
385. Abe T, Loenneke JP, Young KC, et al. Validity of ultrasound prediction equations for total and regional muscularity in middle-aged and older men and women. *Ultrasound Med Biol*. 2015;41(2):557–564.
386. Sanada K, Kearns CF, Midorikawa T, et al. Prediction and validation of total and regional skeletal muscle mass by ultrasound in Japanese adults. *Eur J Appl Physiol*. 2006;96(1):24–31.
387. Watanabe Y, Yamada Y, Fukumoto Y, et al. Echo intensity obtained from ultrasonography images reflecting muscle strength in elderly men. *Clin Interv Aging*. 2013;8:993–998.
388. Bazzocchi A, Filonzi G, Ponti F, et al. Accuracy, reproducibility and repeatability of ultrasonography in the assessment of abdominal adiposity. *Acad Radiol*. 2011;18(9):1133–1143.
389. Mayans D, Cartwright MS, Walker FO. Neuromuscular ultrasonography: quantifying muscle and nerve measurements. *Phys Med Rehabil Clin N Am*. 2012;23(1):133–148. xii.
390. Heymsfield SB, Smith R, Aulet M, et al. Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. *Am J Clin Nutr*. 1990;52(2):214–218.
391. Laskey MA. Dual-energy X-ray absorptiometry and body composition. *Nutrition*. 1996;12(1):45–51.
392. Fuller NJ, Sawyer MB, Laskey MA, et al. Prediction of body composition in elderly men over 75 years of age. *Ann Hum Biol*. 1996;23(2):127–147.
393. Lee WJ, Liu LK, Peng LN, et al. Comparisons of sarcopenia defined by IWGS and EWGSOP criteria among older people: results from the I-Lan longitudinal aging study. *J Am Med Dir Assoc*. 2013;14(7). 528 e521–527.
394. Heymsfield SB, Wang Z, Baumgartner RN, et al. Human body composition: advances in models and methods. *Annu Rev Nutr*. 1997;17:527–558.
395. Ross R, Janssen I. Computed tomography and magnetic resonance imaging. In: Heymsfield SB, Lohman T, Wang Z, Going S, eds. *Human Body Composition*. 2nd ed. Champaign, IL: Human Kinetics; 2005:89–108.
396. Prado CM, Heymsfield SB. Lean tissue imaging: a new era for nutritional assessment and intervention. *JPEN J Parenter Enteral Nutr*. 2014;38(8):940–953.
397. Lukaski H. Sarcopenia: assessment of muscle mass. *J Nutr*. 1997;127(suppl 5):994S–997S.
398. Heymsfield SB, Gonzalez MC, Lu J, et al. Skeletal muscle mass and quality: evolution of modern measurement concepts in the context of sarcopenia. *Proc Nutr Soc*. 2015;74(4):355–366.
399. Rubbieri G, Mossello E, Di Bari M. Techniques for the diagnosis of sarcopenia. *Clin Cases Miner Bone Metab*. 2014;11(3):181–184.
400. Gallagher D, Kuznia P, Heshka S, et al. Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. *Am J Clin Nutr*. 2005;81(4):903–910.
401. Addison O, Marcus RL, Lastayo PC, et al. Intermuscular fat: a review of the consequences and causes. *Int J Endocrinol*. 2014;2014:309570.
402. Fiatarone MA, Marks EC, Ryan ND, et al. High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA*. 1990;263(22):3029–3034.
403. White LJ, Ferguson MA, McCoy SC, et al. Intramyocellular lipid changes in men and women during aerobic exercise: a (1)H-magnetic resonance spectroscopy study. *J Clin Endocrinol Metab*. 2003;88(12):5638–5643.
404. Nakagawa Y, Hattori M, Harada K, et al. Age-related changes in intramyocellular lipid in humans by in vivo H-MR spectroscopy. *Gerontology*. 2007;53(4):218–223.
405. Taicher GZ, Tinsley FC, Reiderman A, et al. Quantitative magnetic resonance (QMR) method for bone and whole-body-composition analysis. *Anal Bioanal Chem*. 2003;377(6):990–1002.
406. Napolitano A, Miller SR, Murgatroyd PR, et al. Validation of a quantitative magnetic resonance method for measuring human body composition. *Obesity (Silver Spring)*. 2008;16(1):191–198.
407. Clark RV, Walker AC, O'Connor-Semmes RL, et al. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. *J Appl Physiol (1985)*. 2014;116(12):1605–1613.
408. Stimpson SA, Turner SM, Clifton LG, et al. Total-body creatine pool size and skeletal muscle mass determination by creatine-(methyl-D3) dilution in rats. *J Appl Physiol (1985)*. 2012;112(11):1940–1948.
409. Young VR, Munro HN. Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Fed Proc*. 1978;37(9):2291–2300.
410. Lukaski HC, Mendez J, Buskirk ER, et al. Relationship between endogenous 3-methylhistidine excretion and body composition. *Am J Physiol*. 1981;240(3):E302–E307.
411. Lukaski H, Mendez J. Relationship between fat-free weight and urinary 3-methythistidine excretion in man. *Metabolism*. 1980;29(8):758–761.
412. Calvani R, Marini F, Cesari M, et al. Biomarkers for physical frailty and sarcopenia. *Aging Clin Exp Res*. 2017;29(1):29–34.
413. Marzetti E, Lees HA, Wohlgemuth SE, et al. Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction. *Biofactors*. 2009;35(1):28–35.
414. Marzetti E, Calvani R, Cesari M, et al. Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials. *Int J Biochem Cell Biol*. 2013;45(10):2288–2301.
415. Calvani R, Marini F, Cesari M, et al. Biomarkers for physical frailty and sarcopenia: state of the science and future developments. *J Cachexia Sarcopenia Muscle*. 2015;6(4):278–286.
416. Nedergaard A, Karsdal MA, Sun S, et al. Serological muscle loss biomarkers: an overview of current concepts and future possibilities. *J Cachexia Sarcopenia Muscle*. 2013;4(1):1–17.
417. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89–95.
418. Cesari M, Fielding RA, Pahor M, et al. Biomarkers of sarcopenia in clinical trials-recommendations from the International Working Group on Sarcopenia. *J Cachexia Sarcopenia Muscle*. 2012;3(3):181–190.

419. Gueugneau M, Coudy-Gandilhon C, Gourbeyre O, et al. Proteomics of muscle chronological ageing in post-menopausal women. *BMC Genomics*. 2014;15:1165.
420. Marzetti E, Lorenzi M, Antocicco M, et al. Shorter telomeres in peripheral blood mononuclear cells from older persons with sarcopenia: results from an exploratory study. *Front Aging Neurosci*. 2014;6:233.
421. Michaud M, Balardy L, Moulis G, et al. Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir Assoc*. 2013;14(12):877–882.
422. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol*. 2012;8(12):709–716.
423. Catoire M, Kersten S. The search for exercise factors in humans. *FASEB J*. 2015;29(5):1615–1628.
424. Lippi G, Sanchis-Gomar F, Montagnana M. Biological markers in older people at risk of mobility limitations. *Curr Pharm Des*. 2014;20(19):3222–3244.
425. Chen F, Lam R, Shaywitz D, et al. Evaluation of early biomarkers of muscle anabolic response to testosterone. *J Cachexia Sarcopenia Muscle*. 2011;2(1):45–56.
426. Bhasin S, He EJ, Kawakubo M, et al. N-terminal propeptide of type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone. *J Clin Endocrinol Metab*. 2009;94(11):4224–4233.
427. Fragala MS, Jajtner AR, Beyer KS, et al. Biomarkers of muscle quality: N-terminal propeptide of type III procollagen and C-terminal agrin fragment responses to resistance exercise training in older adults. *J Cachexia Sarcopenia Muscle*. 2014;5(2):139–148.
428. Garma T, Kobayashi C, Haddad F, et al. Similar acute molecular responses to equivalent volumes of isometric, lengthening, or shortening mode resistance exercise. *J Appl Physiol (1985)*. 2007;102(1):135–143.
429. Crameri RM, Langberg H, Teisner B, et al. Enhanced procollagen processing in skeletal muscle after a single bout of eccentric loading in humans. *Matrix Biol*. 2004;23(4):259–264.
430. Wallace JD, Cuneo RC, Lundberg PA, et al. Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. *J Clin Endocrinol Metab*. 2000;85(1):124–133.
431. Longobardi S, Keay N, Ehrnborg C, et al. Growth hormone (GH) effects on bone and collagen turnover in healthy adults and its potential as a marker of GH abuse in sports: a double blind, placebo-controlled study. The GH-2000 Study Group. *J Clin Endocrinol Metab*. 2000;85(4):1505–1512.
432. Nelson AE, Meinhardt U, Hansen JL, et al. Pharmacodynamics of growth hormone abuse biomarkers and the influence of gender and testosterone: a randomized double-blind placebo-controlled study in young recreational athletes. *J Clin Endocrinol Metab*. 2008;93(6):2213–2222.
433. Hettwer S, Dahinden P, Kucsera S, et al. Elevated levels of a C-terminal agrin fragment identifies a new subset of sarcopenia patients. *Exp Gerontol*. 2013;48(1):69–75.
434. Stephan A, Mateos JM, Kozlov SV, et al. Neurotrypsin cleaves agrin locally at the synapse. *FASEB J*. 2008;22(6):1861–1873.
435. Bolliger MF, Zurlinden A, Luscher D, et al. Specific proteolytic cleavage of agrin regulates maturation of the neuromuscular junction. *J Cell Sci*. 2010;123(Pt 22):3944–3955.
436. Frischknecht R, Fejtova A, Viesti M, et al. Activity-induced synaptic capture and exocytosis of the neuronal serine protease neurotrypsin. *J Neurosci*. 2008;28(7):1568–1579.
437. Drey M, Sieber CC, Bauer JM, et al. C-terminal Agrin Fragment as a potential marker for sarcopenia caused by degeneration of the neuromuscular junction. *Exp Gerontol*. 2013;48(1):76–80.
438. Butikofer L, Zurlinden A, Bolliger MF, et al. Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. *FASEB J*. 2011;25(12):4378–4393.
439. Jang YC, Van Remmen H. Age-associated alterations of the neuromuscular junction. *Exp Gerontol*. 2011;46(2–3):193–198.
440. Stout JR, Fragala MS, Hoffman JR, et al. C-terminal agrin fragment is inversely related to neuromuscular fatigue in older men. *Muscle Nerve*. 2015;51(1):132–133.
441. Voellmy R, Boellmann F. Chaperone regulation of the heat shock protein response. *Adv Exp Med Biol*. 2007;594:89–99.
442. Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet*. 1988;22:631–677.
443. Navarro A, Lopez-Cepero JM, Sanchez del Pino MJ. Skeletal muscle and aging. *Front Biosci*. 2001;6:D26–D44.
444. Nishimura RN, Sharp FR. Heat shock proteins and neuromuscular disease. *Muscle Nerve*. 2005;32(6):693–709.
445. Asea A, Kraeft SK, Kurt-Jones EA, et al. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med*. 2000;6(4):435–442.
446. Robinson MB, Tidwell JL, Gould T, et al. Extracellular heat shock protein 70: a critical component for motoneuron survival. *J Neurosci*. 2005;25(42):9735–9745.
447. Yamaguchi T, Arai H, Katayama N, et al. Age-related increase of insoluble, phosphorylated small heat shock proteins in human skeletal muscle. *J Gerontol A Biol Sci Med Sci*. 2007;62(5):481–489.
448. Koh TJ. Do small heat shock proteins protect skeletal muscle from injury? *Exerc Sport Sci Rev*. 2002;30(3):117–121.
449. McArdle A, Jackson MJ. Exercise, oxidative stress and ageing. *J Anat*. 2000;197(Pt 4):539–541.
450. Liu Y, Gampert L, Nething K, et al. Response and function of skeletal muscle heat shock protein 70. *Front Biosci*. 2006;11:2802–2827.
451. McArdle A, Vasilaki A, Jackson M. Exercise and skeletal muscle ageing: cellular and molecular mechanisms. *Ageing Res Rev*. 2002;1(1):79–93.
452. Thalacker-Mercer AE, Dell'Italia LJ, Cui X, et al. Differential genomic responses in old vs. young humans despite similar levels of modest muscle damage after resistance loading. *Physiol Genomics*. 2010;40(3):141–149.
453. Ogawa K, Kim HK, Shimizu T, et al. Plasma heat shock protein 72 as a biomarker of sarcopenia in elderly people. *Cell Stress Chaperones*. 2012;17(3):349–359.
454. Mecocci P, Fano G, Fulle S, et al. Age-dependent increases in oxidative damage to DNA, lipids, and proteins in human skeletal muscle. *Free Radic Biol Med*. 1999;26(3–4):303–308.
455. Finsterer J, Drory VE. Wet, volatile, and dry biomarkers of exercise-induced muscle fatigue. *BMC Musculoskelet Disord*. 2016;17:40.
456. Finsterer J. Biomarkers of peripheral muscle fatigue during exercise. *BMC Musculoskelet Disord*. 2012;13:218.
457. Jammes Y, Steinberg JG, Delliaux S, et al. Chronic fatigue syndrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses. *J Intern Med*. 2009;266(2):196–206.
458. Cesari M, Kritchevsky SB, Nicklas BJ, et al. Lipoprotein peroxidation and mobility limitation: results from the Health, Aging, and Body Composition Study. *Arch Intern Med*. 2005;165(18):2148–2154.
459. Fulle S, Protasi F, Di Tano G, et al. The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Exp Gerontol*. 2004;39(1):17–24.
460. Buonocore D, Rucci S, Vandoni M, et al. Oxidative system in aged skeletal muscle. *Muscles Ligaments Tendons J*. 2011;1(3):85–90.
461. Dalle-Donne I, Rossi R, Giustarini D, et al. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta*. 2003;329(1–2):23–38.
462. Howard C, Ferrucci L, Sun K, et al. Oxidative protein damage is associated with poor grip strength among older women living in the community. *J Appl Physiol (1985)*. 2007;103(1):17–20.
463. Semba RD, Ferrucci L, Sun K, et al. Oxidative stress and severe walking disability among older women. *Am J Med*. 2007;120(12):1084–1089.
464. Dalal M, Ferrucci L, Sun K, et al. Elevated serum advanced glycation end products and poor grip strength in older community-dwelling women. *J Gerontol A Biol Sci Med Sci*. 2009;64(1):132–137.
465. Kerasioti E, Kiskini A, Veskoukis A, et al. Effect of a special carbohydrate-protein cake on oxidative stress markers after exhaustive cycling in humans. *Food Chem Toxicol*. 2012;50(8):2805–2810.
466. Hammouda O, Chtourou H, Chahed H, et al. Diurnal variations of plasma homocysteine, total antioxidant status, and biological markers of muscle injury during repeated sprint: effect on performance and muscle fatigue—a pilot study. *Chronobiol Int*. 2011;28(10):958–967.
467. Semba RD, Blaum C, Guralnik JM, et al. Carotenoid and vitamin E status are associated with indicators of sarcopenia among older women living in the community. *Aging Clin Exp Res*. 2003;15(6):482–487.
468. Kalinkovich A, Livshits G. Sarcopenia—The search for emerging biomarkers. *Ageing Res Rev*. 2015;22:58–71.

469. Artaza JN, Bhasin S, Magee TR, et al. Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. *Endocrinology*. 2005;146(8):3547–3557.
470. Mendias CL, Marcin JE, Calerdon DR, et al. Contractile properties of EDL and soleus muscles of myostatin-deficient mice. *J Appl Physiol (1985)*. 2006;101(3):898–905.
471. Gentry BA, Ferreira JA, Phillips CL, et al. Hindlimb skeletal muscle function in myostatin-deficient mice. *Muscle Nerve*. 2011;43(1):49–57.
472. Siriett V, Salerno MS, Berry C, et al. Antagonism of myostatin enhances muscle regeneration during sarcopenia. *Mol Ther*. 2007;15(8):1463–1470.
473. Schuelke M, Wagner KR, Stolz LE, et al. Myostatin mutation associated with gross muscle hypertrophy in a child. *N Engl J Med*. 2004;350(26):2682–2688.
474. Brandt C, Nielsen AR, Fischer CP, et al. Plasma and muscle myostatin in relation to type 2 diabetes. *PLoS One*. 2012;7(5):e37236.
475. Elkina Y, von Haehling S, Anker SD, et al. The role of myostatin in muscle wasting: an overview. *J Cachexia Sarcopenia Muscle*. 2011;2(3):143–151.
476. Han HQ, Zhou X, Mitch WE, et al. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45(10):2333–2347.
477. Vamvini MT, Aronis KN, Chamberland JP, et al. Energy deprivation alters in a leptin- and cortisol-independent manner circulating levels of activin A and follistatin but not myostatin in healthy males. *J Clin Endocrinol Metab*. 2011;96(11):3416–3423.
478. Bowser M, Herberg S, Arounleut P, et al. Effects of the activin A-myostatin-follistatin system on aging bone and muscle progenitor cells. *Exp Gerontol*. 2013;48(2):290–297.
479. Perry 3rd HM, Horowitz M, Morley JE, et al. Longitudinal changes in serum 25-hydroxyvitamin D in older people. *Metabolism*. 1999;48(8):1028–1032.
480. Cipriani C, Pepe J, Piemonte S, et al. Vitamin D and its relationship with obesity and muscle. *Int J Endocrinol*. 2014;2014:841248.
481. Dirks-Naylor AJ, Lennon-Edwards S. The effects of vitamin D on skeletal muscle function and cellular signaling. *J Steroid Biochem Mol Biol*. 2011;125(3-5):159–168.
482. Houston DK, Cesari M, Ferrucci L, et al. Association between vitamin D status and physical performance: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2007;62(4):440–446.
483. Dhaliwal R, Mikhail M, Usera G, et al. The relationship of physical performance and osteoporosis prevention with vitamin D in older African Americans (PODA). *Contemp Clin Trials*. 2018;65:39–45.
484. Stein MS, Wark JD, Scherer SC, et al. Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel. *J Am Geriatr Soc*. 1999;47(10):1195–1201.
485. Hirani V, Cumming RG, Naganathan V, et al. Longitudinal associations between vitamin D metabolites and sarcopenia in older Australian men: the Concord Health and Aging in Men Project. *J Gerontol A Biol Sci Med Sci*. 2017;73(1):131–138.
486. Kim MK, Baek KH, Song KH, et al. Vitamin D deficiency is associated with sarcopenia in older Koreans, regardless of obesity: the Fourth Korea National Health and Nutrition Examination Surveys (KNHANES IV) 2009. *J Clin Endocrinol Metab*. 2011;96(10):3250–3256.
487. Snijder MB, van Schoor NM, Pluijm SM, et al. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. *J Clin Endocrinol Metab*. 2006;91(8):2980–2985.
488. Arunabh S, Pollack S, Yeh J, et al. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab*. 2003;88(1):157–161.
489. Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72(3):690–693.
490. Drinka PJ, Krause PF, Nest LJ, et al. Determinants of vitamin D levels in nursing home residents. *J Am Med Dir Assoc*. 2007;8(2):76–79.
491. Hamid Z, Riggs A, Spencer T, et al. Vitamin D deficiency in residents of academic long-term care facilities despite having been prescribed vitamin D. *J Am Med Dir Assoc*. 2007;8(2):71–75.
492. Karonova T, Belyaeva O, Jude EB, et al. Serum 25(OH)D and adipokines levels in people with abdominal obesity. *J Steroid Biochem Mol Biol*. 2018;175:170–176.
493. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–281.
494. Malmstrom TK, Morley JE. SARC-F: a simple questionnaire to rapidly diagnose sarcopenia. *J Am Med Dir Assoc*. 2013;14(8):531–532.
495. Morley JE, Cao L. Rapid screening for sarcopenia. *J Cachexia Sarcopenia Muscle*. 2015;6(4):312–314.
496. Malmstrom TK, Miller DK, Simonsick EM, et al. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J Cachexia Sarcopenia Muscle*. 2016;7(1):28–36.
497. Woo J, Leung J, Morley JE. Defining sarcopenia in terms of incident adverse outcomes. *J Am Med Dir Assoc*. 2015;16(3):247–252.
498. Cao L, Chen S, Zou C, et al. A pilot study of the SARC-F scale on screening sarcopenia and physical disability in the Chinese older people. *J Nutr Health Aging*. 2014;18(3):277–283.
499. Hoff M, Meyer HE, Skurtveit S, et al. Validation of FRAX and the impact of self-reported falls among elderly in a general population: the HUNT study, Norway. *Osteoporos Int*. 2017;28(10):2935–2944.
500. Kanis JA, Harvey NC, Johansson H, et al. FRAX update. *J Clin Densitom*. 2017;20(3):360–367.
501. Vellas B, Guigoz Y, Baumgartner M, et al. Relationships between nutritional markers and the Mini-Nutritional Assessment in 155 older persons. *J Am Geriatr Soc*. 2000;48(10):1300–1309.
502. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: The Mini Nutritional Assessment as part of the geriatric evaluation. *Nutr Rev*. 1996;54(1 Pt 2):S59–S65.
503. Omran ML, Morley JE. Assessment of protein energy malnutrition in older persons, part I: history, examination, body composition, and screening tools. *Nutrition*. 2000;16(1):50–63.
504. Guigoz Y, Lauque S, Vellas BJ. Identifying the elderly at risk for malnutrition. The Mini Nutritional Assessment. *Clin Geriatr Med*. 2002;18(4):737–757.
505. Vellas B, Guigoz Y, Garry PJ, et al. The Mini Nutritional Assessment (MNA) and its use in grading the nutritional state of elderly patients. *Nutrition*. 1999;15(2):116–122.
506. Kaiser MJ, Bauer JM, Ramsch C, et al. Validation of the Mini Nutritional Assessment Short-Form (MNA-SF): a practical tool for identification of nutritional status. *J Nutr Health Aging*. 2009;13(9):782–788.
507. Rubenstein LZ, Harker JO, Salva A, et al. Screening for undernutrition in geriatric practice: developing the Short-Form Mini-Nutritional Assessment (MNA-SF). *J Gerontol A Biol Sci Med Sci*. 2001;56(6):M366–372.
508. Beaudart C, McCloskey E, Bruyere O, et al. Sarcopenia in daily practice: assessment and management. *BMC Geriatr*. 2016;16(1):170.
509. Whittemore LA, Song K, Li X, et al. Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. *Biochem Biophys Res Commun*. 2003;300(4):965–971.
510. Morley JE. Pharmacologic options for the treatment of sarcopenia. *Calcif Tissue Int*. 2016;98(4):319–333.
511. Lee SJ, Reed LA, Davies MV, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proc Natl Acad Sci U S A*. 2005;102(50):18117–18122.
512. Lach-Trifilieff E, Minetti GC, Sheppard K, et al. An antibody blocking activin type II receptors induces strong skeletal muscle hypertrophy and protects from atrophy. *Mol Cell Biol*. 2014;34(4):606–618.
513. Amato AA, Sivakumar K, Goyal N, et al. Treatment of sporadic inclusion body myositis with bimagrumab. *Neurology*. 2014;83(24):2239–2246.
514. Rooks D, Praestgaard J, Hariry S, et al. Treatment of sarcopenia with bimagrumab: results from a phase II, randomized, controlled, proof-of-concept study. *J Am Geriatr Soc*. 2017;65(9):1988–1995.
515. Carter CS, Onder G, Kritchevsky SB, et al. Angiotensin-converting enzyme inhibition intervention in elderly persons: effects on body composition and physical performance. *J Gerontol A Biol Sci Med Sci*. 2005;60(11):1437–1446.
516. Onder G, Penninx BW, Balkrishnan R, et al. Relation between use of angiotensin-converting enzyme inhibitors and muscle strength and physical function in older women: an observational study. *Lancet*. 2002;359(9310):926–930.

517. Sartiani L, Spinelli V, Laurino A, et al. Pharmacological perspectives in sarcopenia: a potential role for renin-angiotensin system blockers? *Clin Cases Miner Bone Metab*. 2015;12(2):135–138.
518. Anker SD, Negassa A, Coats AJ, et al. Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study. *Lancet*. 2003;361(9363):1077–1083.
519. Vescovo G, Dalla Libera L, Serafini F, et al. Improved exercise tolerance after losartan and enalapril in heart failure: correlation with changes in skeletal muscle myosin heavy chain composition. *Circulation*. 1998;98(17):1742–1749.
520. Sumukadas D, Witham MD, Struthers AD, et al. Effect of perindopril on physical function in elderly people with functional impairment: a randomized controlled trial. *CMAJ*. 2007;177(8):867–874.
521. Puthucheary Z, Skipworth JR, Rawal J, et al. The ACE gene and human performance: 12 years on. *Sports Med*. 2011;41(6):433–448.
522. Onder G, Vedova CD, Pahor M. Effects of ACE inhibitors on skeletal muscle. *Curr Pharm Des*. 2006;12(16):2057–2064.
523. Witham MD, Sumukadas D, McMurdo ME. ACE inhibitors for sarcopenia—as good as exercise training? *Age Ageing*. 2008;37(4):363–365.
524. Bassil N, Morley JE. Late-life onset hypogonadism: a review. *Clin Geriatr Med*. 2010;26(2):197–222.
525. Cappola AR. Testosterone therapy and risk of cardiovascular disease in men. *JAMA*. 2013;310(17):1805–1806.
526. Kovacheva EL, Hikim AP, Shen R, et al. Testosterone supplementation reverses sarcopenia in aging through regulation of myostatin, c-Jun NH2-terminal kinase, Notch, and Akt signaling pathways. *Endocrinology*. 2010;151(2):628–638.
527. Vingren JL, Kraemer WJ, Ratamess NA, et al. Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. *Sports Med*. 2010;40(12):1037–1053.
528. Wittert GA, Chapman IM, Haren MT, et al. Oral testosterone supplementation increases muscle and decreases fat mass in healthy elderly males with low-normal gonadal status. *J Gerontol A Biol Sci Med Sci*. 2003;58(7):618–625.
529. Morley JE. Hypogonadism, testosterone, and nursing home residents. *J Am Med Dir Assoc*. 2013;14(6):381–383.
530. Travison TG, Basaria S, Storer TW, et al. Clinical meaningfulness of the changes in muscle performance and physical function associated with testosterone administration in older men with mobility limitation. *J Gerontol A Biol Sci Med Sci*. 2011;66(10):1090–1099.
531. Kenny AM, Kleppinger A, Annis K, et al. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels, low bone mass, and physical frailty. *J Am Geriatr Soc*. 2010;58(6):1134–1143.
532. Srinivas-Shankar U, Roberts SA, Connolly MJ, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. *J Clin Endocrinol Metab*. 2010;95(2):639–650.
533. Ferrando AA, Tipton KD, Doyle D, et al. Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. *Am J Physiol*. 1998;275(5 Pt 1):E864–E871.
534. Bhasin S, Woodhouse L, Casaburi R, et al. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab*. 2001;281(6):E1172–E1181.
535. Ferrando AA, Sheffield-Moore M, Yeckel CW, et al. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am J Physiol Endocrinol Metab*. 2002;282(3):E601–607.
536. Davis SR, McCloud P, Strauss BJ, et al. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas*. 1995;21(3):227–236.
537. Sinha-Hikim I, Cornford M, Gaytan H, et al. Effects of testosterone supplementation on skeletal muscle fiber hypertrophy and satellite cells in community-dwelling older men. *J Clin Endocrinol Metab*. 2006;91(8):3024–3033.
538. Haren MT, Siddiqui AM, Armbrecht HJ, et al. Testosterone modulates gene expression pathways regulating nutrient accumulation, glucose metabolism and protein turnover in mouse skeletal muscle. *Int J Androl*. 2011;34(1):55–68.
539. Sinha-Hikim I, Roth SM, Lee MI, et al. Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *Am J Physiol Endocrinol Metab*. 2003;285(1):E197–E205.
540. Chen Y, Zajac JD, MacLean HE. Androgen regulation of satellite cell function. *J Endocrinol*. 2005;186(1):21–31.
541. Bhasin S. Testosterone supplementation for aging-associated sarcopenia. *J Gerontol A Biol Sci Med Sci*. 2003;58(11):1002–1008.
542. Bhasin S, Buckwalter JG. Testosterone supplementation in older men: a rational idea whose time has not yet come. *J Androl*. 2001;22(5):718–731.
543. Rudman D, Feller AG, Cohn L, et al. Effects of human growth hormone on body composition in elderly men. *Horm Res*. 1991;36(suppl 1):73–81.
544. Butterfield GE, Thompson J, Rennie MJ, et al. Effect of rhGH and rhIGF-I treatment on protein utilization in elderly women. *Am J Physiol*. 1997;272(1 Pt 1):E94–E99.
545. Yarasheski KE, Zachwieja JJ, Campbell JA, et al. Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am J Physiol*. 1995;268(2 Pt 1):E268–E276.
546. Musaro A, McCullagh KJ, Naya FJ, et al. IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATc1. *Nature*. 1999;400(6744):581–585.
547. Borst SE. Interventions for sarcopenia and muscle weakness in older people. *Age Ageing*. 2004;33(6):548–555.
548. Blackman MR, Sorkin JD, Munzer T, et al. Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. *JAMA*. 2002;288(18):2282–2292.
549. Takala J, Ruokonen E, Webster NR, et al. Increased mortality associated with growth hormone treatment in critically ill adults. *N Engl J Med*. 1999;341(11):785–792.
550. Pollak M. Insulin-like growth factor physiology and cancer risk. *Eur J Cancer*. 2000;36(10):1224–1228.
551. Genazzani AD, Lanzoni C, Genazzani AR. Might DHEA be considered a beneficial replacement therapy in the elderly? *Drugs Aging*. 2007;24(3):173–185.
552. Yen SS, Morales AJ, Khorram O. Replacement of DHEA in aging men and women. Potential remedial effects. *Ann N Y Acad Sci*. 1995;774:128–142.
553. Fryburg DA, Jahn LA, Hill SA, et al. Insulin and insulin-like growth factor-I enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *J Clin Invest*. 1995;96(4):1722–1729.
554. Morales AJ, Haubrich RH, Hwang JY, et al. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. *Clin Endocrinol (Oxf)*. 1998;49(4):421–432.
555. Percheron G, Hogrel JY, Denot-Ledunois S, et al. Effect of 1-year oral administration of dehydroepiandrosterone to 60- to 80-year-old individuals on muscle function and cross-sectional area: a double-blind placebo-controlled trial. *Arch Intern Med*. 2003;163(6):720–727.
556. Flynn MA, Weaver-Osterholtz D, Sharpe-Timms KL, et al. Dehydroepiandrosterone replacement in aging humans. *J Clin Endocrinol Metab*. 1999;84(5):1527–1533.
557. Welle S, Jozefowicz R, Statt M. Failure of dehydroepiandrosterone to influence energy and protein metabolism in humans. *J Clin Endocrinol Metab*. 1990;71(5):1259–1264.
558. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656–660.
559. Muller TD, Nogueiras R, Andermann ML, et al. Ghrelin. *Mol Metab*. 2015;4(6):437–460.
560. Gaskin FS, Farr SA, Banks WA, et al. Ghrelin-induced feeding is dependent on nitric oxide. *Peptides*. 2003;24(6):913–918.
561. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. *Nature*. 2001;409(6817):194–198.

562. Dixit VD, Schaffer EM, Pyle RS, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest*. 2004;114(1):57–66.
563. Li WG, Gavrila D, Liu X, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. *Circulation*. 2004;109(18):2221–2226.
564. Rigamonti AE, Pincelli AI, Corra B, et al. Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. *J Endocrinol*. 2002;175(1):R1–5.
565. Akamizu T, Murayama T, Teramukai S, et al. Plasma ghrelin levels in healthy elderly volunteers: the levels of acylated ghrelin in elderly females correlate positively with serum IGF-I levels and bowel movement frequency and negatively with systolic blood pressure. *J Endocrinol*. 2006;188(2):333–344.
566. Nass R, Farhy LS, Liu J, et al. Age-dependent decline in acyl-ghrelin concentrations and reduced association of acyl-ghrelin and growth hormone in healthy older adults. *J Clin Endocrinol Metab*. 2014;99(2):602–608.
567. Serra-Prat M, Papiol M, Monteis R, et al. Relationship between plasma ghrelin levels and sarcopenia in elderly subjects: a cross-sectional study. *J Nutr Health Aging*. 2015;19(6):669–672.
568. Nagaya N, Moriya J, Yasumura Y, et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. *Circulation*. 2004;110(24):3674–3679.
569. Garcia JM, Boccia RV, Graham CD, et al. Anamorelin for patients with cancer cachexia: an integrated analysis of two phase 2, randomised, placebo-controlled, double-blind trials. *Lancet Oncol*. 2015;16(1):108–116.
570. Anker SD, Coats AJ, Morley JE. Evidence for partial pharmaceutical reversal of the cancer anorexia-cachexia syndrome: the case of anamorelin. *J Cachexia Sarcopenia Muscle*. 2015;6(4):275–277.
571. Nass R, Pezzoli SS, Oliveri MC, et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. *Ann Intern Med*. 2008;149(9):601–611.
572. Dalton JT, Barnette KG, Bohl CE, et al. The selective androgen receptor modulator GTx-024 (enobosarm) improves lean body mass and physical function in healthy elderly men and postmenopausal women: results of a double-blind, placebo-controlled phase II trial. *J Cachexia Sarcopenia Muscle*. 2011;2(3):153–161.
573. Dobs AS, Boccia RV, Croot CC, et al. Effects of enobosarm on muscle wasting and physical function in patients with cancer: a double-blind, randomised controlled phase 2 trial. *Lancet Oncol*. 2013;14(4):335–345.
574. Basaria S, Collins L, Dillon EL, et al. The safety, pharmacokinetics, and effects of LGD-4033, a novel nonsteroidal oral, selective androgen receptor modulator, in healthy young men. *J Gerontol A Biol Sci Med Sci*. 2013;68(1):87–95.
575. Papanicolaou DA, Ather SN, Zhu H, et al. A phase IIA randomized, placebo-controlled clinical trial to study the efficacy and safety of the selective androgen receptor modulator (SARM), MK-0773 in female participants with sarcopenia. *J Nutr Health Aging*. 2013;17(6):533–543.
576. Sipila S, Suominen H. Effects of strength and endurance training on thigh and leg muscle mass and composition in elderly women. *J Appl Physiol (1985)*. 1995;78(1):334–340.
577. Burton LA, Sumukadas D. Optimal management of sarcopenia. *Clin Interv Aging*. 2010;5:217–228.
578. Lenk K, Schuler G, Adams V. Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. *J Cachexia Sarcopenia Muscle*. 2010;1(1):9–21.
579. Beaudart C, Dawson A, Shaw SC, et al. Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review. *Osteoporos Int*. 2017;28(6):1817–1833.
580. Liu CJ, Latham NK. Progressive resistance strength training for improving physical function in older adults. *Cochrane Database Syst Rev*. 2009;(3):CD002759.
581. Aniansson A, Grimby G, Rundgren A, et al. Physical training in old men. *Age Ageing*. 1980;9(3):186–187.
582. Orlander J, Aniansson A. Effect of physical training on skeletal muscle metabolism and ultrastructure in 70 to 75-year-old men. *Acta Physiol Scand*. 1980;109(2):149–154.
583. Aniansson A, Ljungberg P, Rundgren A, et al. Effect of a training programme for pensioners on condition and muscular strength. *Arch Gerontol Geriatr*. 1984;3(3):229–241.
584. Kauffman TL. Strength training effect in young and aged women. *Arch Phys Med Rehabil*. 1985;66(4):223–226.
585. Reeves ND, Narici MV, Maganaris CN. Effect of resistance training on skeletal muscle-specific force in elderly humans. *J Appl Physiol (1985)*. 2004;96(3):885–892.
586. Harridge SD, Kryger A, Stensgaard A. Knee extensor strength, activation, and size in very elderly people following strength training. *Muscle Nerve*. 1999;22(7):831–839.
587. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med*. 1994;330(25):1769–1775.
588. Taaffe DR, Duret C, Wheeler S, et al. Once-weekly resistance exercise improves muscle strength and neuromuscular performance in older adults. *J Am Geriatr Soc*. 1999;47(10):1208–1214.
589. Chesley A, MacDougall JD, Tarnopolsky MA, et al. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol (1985)*. 1992;73(4):1383–1388.
590. Phillips SM, Tipton KD, Aarsland A, et al. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol*. 1997;273(1 Pt 1):E99–107.
591. Kosek DJ, Kim JS, Petrella JK, et al. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J Appl Physiol (1985)*. 2006;101(2):531–544.
592. Mackey AL, Esmarck B, Kadi F, et al. Enhanced satellite cell proliferation with resistance training in elderly men and women. *Scand J Med Sci Sports*. 2007;17(1):34–42.
593. Narici MV, Hoppeler H, Kayser B, et al. Human quadriceps cross-sectional area, torque and neural activation during 6 months strength training. *Acta Physiol Scand*. 1996;157(2):175–186.
594. Kelley G. Mechanical overload and skeletal muscle fiber hyperplasia: a meta-analysis. *J Appl Physiol (1985)*. 1996;81(4):1584–1588.
595. Grimby G. Physical activity and effects of muscle training in the elderly. *Ann Clin Res*. 1988;20(1–2):62–66.
596. Todd IC, Wosornu D, Stewart I, et al. Cardiac rehabilitation following myocardial infarction. A practical approach. *Sports Med*. 1992;14(4):243–259.
597. Kanehisa H, Ikegawa S, Fukunaga T. Comparison of muscle cross-sectional area and strength between untrained women and men. *Eur J Appl Physiol Occup Physiol*. 1994;68(2):148–154.
598. Bamman MM, Newcomer BR, Larson-Meyer DE, et al. Evaluation of the strength-size relationship in vivo using various muscle size indices. *Med Sci Sports Exerc*. 2000;32(7):1307–1313.
599. Fukunaga T, Miyatani M, Tachi M, et al. Muscle volume is a major determinant of joint torque in humans. *Acta Physiol Scand*. 2001;172(4):249–255.
600. Erskine RM, Jones DA, Williams AG, et al. Resistance training increases in vivo quadriceps femoris muscle specific tension in young men. *Acta Physiol (Oxf)*. 2010;199(1):83–89.
601. Jones DA, Rutherford OM. Human muscle strength training: the effects of three different regimens and the nature of the resultant changes. *J Physiol*. 1987;391:1–11.
602. Pahor M, Guralnik JM, Ambrosius WT, et al. Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. *JAMA*. 2014;311(23):2387–2396.
603. Nelson ME, Rejeski WJ, Blair SN, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc*. 2007;39(8):1435–1445.
604. Wall BT, van Loon LJ. Nutritional strategies to attenuate muscle disuse atrophy. *Nutr Rev*. 2013;71(4):195–208.
605. Marian M, Sacks G. Micronutrients and older adults. *Nutr Clin Pract*. 2009;24(2):179–195.
606. Houston DK, Nicklas BJ, Ding J, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health,

Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr*. 2008;87(1):150–155.
607. Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care*. 2009;12(1):86–90.
608. Morley JE, Kim MJ, Haren MT, et al. Frailty and the aging male. *Aging Male*. 2005;8(3–4):135–140.
609. Visvanathan R, Chapman IM. Undernutrition and anorexia in the older person. *Gastroenterol Clin North Am*. 2009;38(3):393–409.
610. Berger MM, Chiolero RL. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit Care Med*. 2007;35(suppl 9):S584–590.
611. Bales CW, Ritchie CS. Sarcopenia, weight loss, and nutritional frailty in the elderly. *Annu Rev Nutr*. 2002;22:309–323.
612. Douyon L, Schteingart DE. Effect of obesity and starvation on thyroid hormone, growth hormone, and cortisol secretion. *Endocrinol Metab Clin North Am*. 2002;31(1):173–189.
613. Bouchard DR, Dionne IJ, Brochu M. Sarcopenic/obesity and physical capacity in older men and women: data from the Nutrition as a Determinant of Successful Aging (NuAge)—the Quebec longitudinal Study. *Obesity (Silver Spring)*. 2009;17(11):2082–2088.
614. Biolo G, Maggi SP, Williams BD, et al. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol*. 1995;268(3 Pt 1):E514–E520.
615. Tang JE, Moore DR, Kujbida GW, et al. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol (1985)*. 2009;107(3):987–992.
616. Yang Y, Churchward-Venne TA, Burd NA, et al. Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutr Metab (Lond)*. 2012;9(1):57.
617. Biolo G, Tipton KD, Klein S, et al. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol*. 1997;273(1 Pt 1):E122–E129.
618. Tipton KD, Ferrando AA, Phillips SM, et al. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol*. 1999;276(4 Pt 1):E628–E634.
619. Molnar A, Jonasne Sztruhar I, Csontos AA, et al. Special nutrition intervention is required for muscle protective efficacy of physical exercise in elderly people at highest risk of sarcopenia. *Physiol Int*. 2016;103(3):368–376.
620. Tipton KD, Rasmussen BB, Miller SL, et al. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab*. 2001;281(2):E197–206.
621. Yang Y, Breen L, Burd NA, et al. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr*. 2012;108(10):1780–1788.
622. Moore DR, Robinson MJ, Fry JL, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr*. 2009;89(1):161–168.
623. Morley JE. Anorexia of aging: physiologic and pathologic. *Am J Clin Nutr*. 1997;66(4):760–773.
624. Rolland Y, Czerwinski S, Abellan Van Kan G, et al. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. *J Nutr Health Aging*. 2008;12(7):433–450.
625. National Research Council. *Recommended Dietary Allowances*. 10th ed. Washington DC: National Academies Press; 1989.
626. Vesnaver E, Keller HH, Payette H, et al. Dietary resilience as described by older community-dwelling adults from the NuAge study "if there is a will—there is a way!". *Appetite*. 2012;58(2):730–738.
627. Kerstetter JE, O'Brien KO, Insogna KL. Low protein intake: the impact on calcium and bone homeostasis in humans. *J Nutr*. 2003;133(3):855S–861S.
628. Volpi E, Campbell WW, Dwyer JT, et al. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? *J Gerontol A Biol Sci Med Sci*. 2013;68(6):677–681.
629. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. *Clin Nutr*. 2008;27(5):675–684.
630. Evans WJ. Protein nutrition, exercise and aging. *J Am Coll Nutr*. 2004;23(suppl 6):601S–609S.
631. Morais JA, Chevalier S, Gougeon R. Protein turnover and requirements in the healthy and frail elderly. *J Nutr Health Aging*. 2006;10(4):272–283.
632. Meredith CN, Frontera WR, O'Reilly KP, et al. Body composition in elderly men: effect of dietary modification during strength training. *J Am Geriatr Soc*. 1992;40(2):155–162.
633. Blumberg J. Nutritional needs of seniors. *J Am Coll Nutr*. 1997;16(6):517–523.
634. Arnal MA, Mosoni L, Boirie Y, et al. Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr*. 1999;69(6):1202–1208.
635. Bauer J, Biolo G, Cederholm T, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc*. 2013;14(8):542–559.
636. Campbell WW. Synergistic use of higher-protein diets or nutritional supplements with resistance training to counter sarcopenia. *Nutr Rev*. 2007;65(9):416–422.
637. Paddon-Jones D, Leidy H. Dietary protein and muscle in older persons. *Curr Opin Clin Nutr Metab Care*. 2014;17(1):5–11.
638. Gregorio L, Brindisi J, Kleppinger A, et al. Adequate dietary protein is associated with better physical performance among post-menopausal women 60–90 years. *J Nutr Health Aging*. 2014;18(2):155–160.
639. Loenneke JP, Loprinzi PD, Murphy CH, et al. Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance. *Clin Nutr*. 2016;35(6):1506–1511.
640. Murphy CH, Oikawa SY, Phillips SM. Dietary protein to maintain muscle mass in aging: a case for per-meal protein recommendations. *J Frailty Aging*. 2016;5(1):49–58.
641. Pennings B, Groen B, de Lange A, et al. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol Endocrinol Metab*. 2012;302(8):E992–999.
642. Bauer JM, Diekmann R. Protein supplementation with aging. *Curr Opin Clin Nutr Metab Care*. 2015;18(1):24–31.
643. Scognamiglio R, Piccolotto R, Negut C, et al. Oral amino acids in elderly subjects: effect on myocardial function and walking capacity. *Gerontology*. 2005;51(5):302–308.
644. Solerte SB, Gazzaruso C, Bonacasa R, et al. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. *Am J Cardiol*. 2008;101(11A):69E–77E.
645. Anthony JC, Yoshizawa F, Anthony TG, et al. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr*. 2000;130(10):2413–2419.
646. Riazi R, Wykes LJ, Ball RO, et al. The total branched-chain amino acid requirement in young healthy adult men determined by indicator amino acid oxidation by use of L-[1-13C]phenylalanine. *J Nutr*. 2003;133(5):1383–1389.
647. Rieu I, Balage M, Sornet C, et al. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol*. 2006;575(Pt 1):305–315.
648. Drummond MJ, Rasmussen BB. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Curr Opin Clin Nutr Metab Care*. 2008;11(3):222–226.
649. Chantranupong L, Wolfson RL, Orozco JM, et al. The sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. *Cell Rep*. 2014;9(1):1–8.
650. Wolfson RL, Chantranupong L, Saxton RA, et al. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science*. 2016;351(6268):43–48.
651. Haegens A, Schols AM, van Essen AL, et al. Leucine induces myofibrillar protein accretion in cultured skeletal muscle through mTOR dependent and -independent control of myosin heavy chain mRNA levels. *Mol Nutr Food Res*. 2012;56(5):741–752.
652. Nair KS, Schwartz RG, Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol*. 1992;263(5 Pt 1):E928–934.

653. Sharp CP, Pearson DR. Amino acid supplements and recovery from high-intensity resistance training. *J Strength Cond Res*. 2010;24(4):1125–1130.
654. Pennings B, Boirie Y, Senden JM, et al. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am J Clin Nutr*. 2011;93(5):997–1005.
655. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–214.
656. Ley RE, Knight R, Gordon JI. The human microbiome: eliminating the biomedical/environmental dichotomy in microbial ecology. *Environ Microbiol*. 2007;9(1):3–4.
657. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. 2006;124(4):837–848.
658. Clayton TA, Lindon JC, Cloarec O, et al. Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature*. 2006;440(7087):1073–1077.
659. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. *Science*. 2001;292(5519):1115–1118.
660. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science*. 2000;288(5474):2222–2226.
661. Calvani R, Miccheli A, Capuani G, et al. Gut microbiome-derived metabolites characterize a peculiar obese urinary metabotype. *Int J Obes (Lond)*. 2010;34(6):1095–1098.
662. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. *Genome Med*. 2011;3(3):14.
663. Manichanh C, Varela E, Martinez C, et al. The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. *Am J Gastroenterol*. 2008;103(7):1754–1761.
664. Salazar N, Valdes-Varela L, Gonzalez S, et al. Nutrition and the gut microbiome in the elderly. *Gut Microbes*. 2017;8(2):82–97.
665. Claesson MJ, Cusack S, O'Sullivan O, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci USA*. 2011;108(suppl 1):4586–4591.
666. Biagi E, Candela M, Fairweather-Tait S, et al. Aging of the human metaorganism: the microbial counterpart. *Age (Dordr)*. 2012;34(1):247–267.
667. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, et al. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. *ISME J*. 2017;11(4):841–852.
668. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature*. 2012;488(7410):178–184.
669. Jeffery IB, Lynch DB, O'Toole PW. Composition and temporal stability of the gut microbiota in older persons. *ISME J*. 2016;10(1):170–182.
670. Ticinesi A, Milani C, Lauretani F, et al. Gut microbiota composition is associated with polypharmacy in elderly hospitalized patients. *Sci Rep*. 2017;7(1):11102.
671. Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: a player in aging and a target for anti-aging intervention. *Ageing Res Rev*. 2017;35:36–45.
672. Ticinesi A, Lauretani F, Milani C, et al. Aging gut microbiota at the cross-road between nutrition, physical frailty, and sarcopenia: is there a gut-muscle axis? *Nutrients*. 2017;9(12).
673. Buigues C, Fernandez-Garrido J, Pruimboom L, et al. Effect of a prebiotic formulation on frailty syndrome: a randomized, double-blind clinical trial. *Int J Mol Sci*. 2016;17(6).
674. Cerda B, Perez M, Perez-Santiago JD, et al. Gut microbiota modification: another piece in the puzzle of the benefits of physical exercise in health? *Front Physiol*. 2016;7:51.
675. Denou E, Marcinko K, Surette MG, et al. High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. *Am J Physiol Endocrinol Metab*. 2016;310(11):E982–993.
676. Campbell SC, Wisniewski PJ, Noji M, et al. The effect of diet and exercise on intestinal integrity and microbial diversity in mice. *PLoS One*. 2016;11(3):e0150502.
677. Welly RJ, Liu TW, Zidon TM, et al. Comparison of diet versus exercise on metabolic function and gut microbiota in obese rats. *Med Sci Sports Exerc*. 2016;48(9):1688–1698.
678. Fuller Jr JC, Baier S, Flakoll P, et al. Vitamin D status affects strength gains in older adults supplemented with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and lysine: a cohort study. *JPEN J Parenter Enteral Nutr*. 2011;35(6):757–762.
679. Aversa Z, Bonetto A, Costelli P, et al. beta-hydroxy-beta-methylbutyrate (HMB) attenuates muscle and body weight loss in experimental cancer cachexia. *Int J Oncol*. 2011;38(3):713–720.
680. Pimentel GD, Rosa JC, Lira FS, et al. beta-Hydroxy-beta-methylbutyrate (HMbeta) supplementation stimulates skeletal muscle hypertrophy in rats via the mTOR pathway. *Nutr Metab (Lond)*. 2011;8(1):11.
681. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol*. 2005;37(10):1974–1984.
682. Fiorotto ML, Schwartz RJ, Delaughter MC. Persistent IGF-I overexpression in skeletal muscle transiently enhances DNA accretion and growth. *FASEB J*. 2003;17(1):59–60.
683. Alway SE, Pereira SL, Edens NK, et al. beta-Hydroxy-beta-methylbutyrate (HMB) enhances the proliferation of satellite cells in fast muscles of aged rats during recovery from disuse atrophy. *Exp Gerontol*. 2013;48(9):973–984.
684. Hao Y, Jackson JR, Wang Y, et al. Beta-hydroxy-beta-methylbutyrate reduces myonuclear apoptosis during recovery from hind limb suspension-induced muscle fiber atrophy in aged rats. *Am J Physiol Regul Integr Comp Physiol*. 2011;301(3):R701–R715.
685. Kraemer WJ, Hatfield DL, Volek JS, et al. Effects of amino acids supplement on physiological adaptations to resistance training. *Med Sci Sports Exerc*. 2009;41(5):1111–1121.
686. Wilson JM, Kim JS, Lee SR, et al. Acute and timing effects of beta-hydroxy-beta-methylbutyrate (HMB) on indirect markers of skeletal muscle damage. *Nutr Metab (Lond)*. 2009;6:6.
687. Nissen SL, Sharp RL. Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. *J Appl Physiol (1985)*. 2003;94(2):651–659.
688. Kreider RB, Ferreira M, Wilson M, et al. Effects of calcium beta-hydroxy-beta-methylbutyrate (HMB) supplementation during resistance-training on markers of catabolism, body composition and strength. *Int J Sports Med*. 1999;20(8):503–509.
689. Ransone J, Neighbors K, Lefavi R, et al. The effect of beta-hydroxy beta-methylbutyrate on muscular strength and body composition in collegiate football players. *J Strength Cond Res*. 2003;17(1):34–39.
690. Slater G, Jenkins D, Logan P, et al. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. *Int J Sport Nutr Exerc Metab*. 2001;11(3):384–396.
691. Wu H, Xia Y, Jiang J, et al. Effect of beta-hydroxy-beta-methylbutyrate supplementation on muscle loss in older adults: a systematic review and meta-analysis. *Arch Gerontol Geriatr*. 2015;61(2):168–175.
692. Nissen S, Sharp R, Ray M, et al. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. *J Appl Physiol (1985)*. 1996;81(5):2095–2104.
693. Gallagher PM, Carrithers JA, Godard MP, et al. Beta-hydroxy-beta-methylbutyrate ingestion, Part I: effects on strength and fat free mass. *Med Sci Sports Exerc*. 2000;32(12):2109–2115.
694. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. *J Nutr*. 2001;131(7):2049–2052.
695. Panton LB, Rathmacher JA, Baier S, et al. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) during resistance training. *Nutrition*. 2000;16(9):734–739.
696. Gallagher PM, Carrithers JA, Godard MP, et al. Beta-hydroxy-beta-methylbutyrate ingestion, part II: effects on hematology, hepatic and renal function. *Med Sci Sports Exerc*. 2000;32(12):2116–2119.
697. Fuller Jr JC, Sharp RL, Angus HF, et al. Free acid gel form of beta-hydroxy-beta-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt. *Br J Nutr*. 2011;105(3):367–372.

698. Candow DG, Chilibeck PD. Effect of creatine supplementation during resistance training on muscle accretion in the elderly. *J Nutr Health Aging*. 2007;11(2):185–188.
699. Johnston AP, Burke DG, MacNeil LG, et al. Effect of creatine supplementation during cast-induced immobilization on the preservation of muscle mass, strength, and endurance. *J Strength Cond Res*. 2009;23(1):116–120.
700. Brose A, Parise G, Tarnopolsky MA. Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *J Gerontol A Biol Sci Med Sci*. 2003;58(1):11–19.
701. Bermon S, Venembre P, Sachet C, et al. Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults. *Acta Physiol Scand*. 1998;164(2):147–155.
702. Chrusch MJ, Chilibeck PD, Chad KE, et al. Creatine supplementation combined with resistance training in older men. *Med Sci Sports Exerc*. 2001;33(12):2111–2117.
703. Gotshalk LA, Volek JS, Staron RS, et al. Creatine supplementation improves muscular performance in older men. *Med Sci Sports Exerc*. 2002;34(3):537–543.
704. Eijnde BO, Van Leemputte M, Goris M, et al. Effects of creatine supplementation and exercise training on fitness in men 55–75 yr old. *J Appl Physiol (1985)*. 2003;95(2):818–828.
705. Jakobi JM, Rice CL, Curtin SV, et al. Neuromuscular properties and fatigue in older men following acute creatine supplementation. *Eur J Appl Physiol*. 2001;84(4):321–328.
706. Volek JS, Duncan ND, Mazzetti SA, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc*. 1999;31(8):1147–1156.
707. Stout JR, Sue Graves B, Cramer JT, et al. Effects of creatine supplementation on the onset of neuromuscular fatigue threshold and muscle strength in elderly men and women (64–86 years). *J Nutr Health Aging*. 2007;11(6):459–464.
708. Devries MC, Phillips SM. Creatine supplementation during resistance training in older adults-a meta-analysis. *Med Sci Sports Exerc*. 2014;46(6):1194–1203.
709. Tarnopolsky M, Zimmer A, Paikin J, et al. Creatine monohydrate and conjugated linoleic acid improve strength and body composition following resistance exercise in older adults. *PLoS One*. 2007;2(10):e991.
710. Burke DG, Chilibeck PD, Parise G, et al. Effect of alpha-lipoic acid combined with creatine monohydrate on human skeletal muscle creatine and phosphagen concentration. *Int J Sport Nutr Exerc Metab*. 2003;13(3):294–302.
711. Candow DG, Little JP, Chilibeck PD, et al. Low-dose creatine combined with protein during resistance training in older men. *Med Sci Sports Exerc*. 2008;40(9):1645–1652.
712. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev*. 2000;80(3):1107–1213.
713. Gualano B, Artioli GG, Poortmans JR, et al. Exploring the therapeutic role of creatine supplementation. *Amino Acids*. 2010;38(1):31–44.
714. Moller P, Bergstrom J, Furst P, et al. Effect of aging on energy-rich phosphagens in human skeletal muscles. *Clin Sci (Lond)*. 1980;58(6):553–555.
715. Spencer M, Finlin BS, Unal R, et al. Omega-3 fatty acids reduce adipose tissue macrophages in human subjects with insulin resistance. *Diabetes*. 2013;62(5):1709–1717.
716. Oh DY, Talukdar S, Bae EJ, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell*. 2010;142(5):687–698.
717. Johnson ML, Lalia AZ, Dasari S, et al. Eicosapentaenoic acid but not docosahexaenoic acid restores skeletal muscle mitochondrial oxidative capacity in old mice. *Aging Cell*. 2015;14(5):734–743.
718. Lanza IR, Blachnio-Zabielska A, Johnson ML, et al. Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. *Am J Physiol Endocrinol Metab*. 2013;304(12):E1391–1403.
719. Lalia AZ, Dasari S, Robinson MM, et al. Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. *Aging (Albany NY)*. 2017;9(4):1096–1129.
720. Rodacki CL, Rodacki AL, Pereira G, et al. Fish-oil supplementation enhances the effects of strength training in elderly women. *Am J Clin Nutr*. 2012;95(2):428–436.
721. Smith GI, Atherton P, Reeds DN, et al. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci (Lond)*. 2011;121(6):267–278.
722. Di Girolamo FG, Situlin R, Mazzucco S, et al. Omega-3 fatty acids and protein metabolism: enhancement of anabolic interventions for sarcopenia. *Curr Opin Clin Nutr Metab Care*. 2014;17(2):145–150.
723. Fearon KC, Von Meyenfeldt MF, Moses AG, et al. Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. *Gut*. 2003;52(10):1479–1486.
724. Smith GI, Atherton P, Reeds DN, et al. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clin Nutr*. 2011;93(2):402–412.
725. Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr*. 2004;134(7):1806–1811.
726. Babcock T, Helton WS, Espat NJ. Eicosapentaenoic acid (EPA): an anti-inflammatory omega-3 fat with potential clinical applications. *Nutrition*. 2000;16(11–12):1116–1118.
727. Singer P, Shapiro H, Theilla M, et al. Anti-inflammatory properties of omega-3 fatty acids in critical illness: novel mechanisms and an integrative perspective. *Intensive Care Med*. 2008;34(9):1580–1592.
728. Babcock TA, Helton WS, Hong D, et al. Omega-3 fatty acid lipid emulsion reduces LPS-stimulated macrophage TNF-alpha production. *Surg Infect (Larchmt)*. 2002;3(2):145–149.
729. Robinson SM, Jameson KA, Batelaan SF, et al. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. *J Am Geriatr Soc*. 2008;56(1):84–90.
730. Aranceta J, Perez-Rodrigo C. Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *Br J Nutr*. 2012;107(suppl 2):S8–S22.
731. Ceglia L. Vitamin D and skeletal muscle tissue and function. *Mol Aspects Med*. 2008;29(6):407–414.
732. Boonen S, Bischoff-Ferrari HA, Cooper C, et al. Addressing the musculoskeletal components of fracture risk with calcium and vitamin D: a review of the evidence. *Calcif Tissue Int*. 2006;78(5):257–270.
733. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, et al. Effect of vitamin D on falls: a meta-analysis. *JAMA*. 2004;291(16):1999–2006.
734. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ*. 2009;339:b3692.
735. Cameron ID, Gillespie LD, Robertson MC, et al. Interventions for preventing falls in older people in care facilities and hospitals. *Cochrane Database Syst Rev*. 2012;12:CD005465.
736. Bischoff HA, Stahelin HB, Dick W, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res*. 2003;18(2):343–351.
737. Pfeifer M, Begerow B, Minne HW, et al. Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos Int*. 2009;20(2):315–322.
738. Dhesi JK, Jackson SH, Bearne LM, et al. Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing*. 2004;33(6):589–595.
739. Bunout D, Barrera G, Leiva L, et al. Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol*. 2006;41(8):746–752.
740. Hirsch S, Leiva L, de la Maza P, et al. Effects of a nutritional supplement fortified with vitamin B-12 on well nourished, free-living elderly subjects. *Int J Vitam Nutr Res*. 2006;76(2):95–99.
741. Beaudart C, Buckinx F, Rabenda V, et al. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic

review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab*. 2014;99(11):4336–4345.
742. Muir SW, Montero-Odasso M. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. *J Am Geriatr Soc*. 2011;59(12):2291–2300.
743. Tomlinson PB, Joseph C, Angioi M. Effects of vitamin D supplementation on upper and lower body muscle strength levels in healthy individuals. A systematic review with meta-analysis. *J Sci Med Sport*. 2015;18(5):575–580.
744. Bauer JM, Verlaan S, Bautmans I, et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc*. 2015;16(9):740–747.
745. Cangussu LM, Nahas-Neto J, Orsatti CL, et al. Effect of vitamin D supplementation alone on muscle function in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. *Osteoporos Int*. 2015;26(10):2413–2421.
746. Sato Y, Iwamoto J, Kanoko T, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis*. 2005;20(3):187–192.
747. Montero-Odasso M, Duque G. Vitamin D in the aging musculoskeletal system: an authentic strength preserving hormone. *Mol Aspects Med*. 2005;26(3):203–219.
748. Yamada M, Arai H, Yoshimura K, et al. Nutritional supplementation during resistance training improved skeletal muscle mass in community-dwelling frail older adults. *J Frailty Aging*. 2012;1(2):64–70.
749. Tsai KS, Heath 3rd H, Kumar R, et al. Impaired vitamin D metabolism with aging in women. Possible role in pathogenesis of senile osteoporosis. *J Clin Invest*. 1984;73(6):1668–1672.
750. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest*. 2005;35(5):290–304.
751. Wood RJ, Fleet JC, Cashman K, et al. Intestinal calcium absorption in the aged rat: evidence of intestinal resistance to 1,25(OH)2 vitamin D. *Endocrinology*. 1998;139(9):3843–3848.
752. Pattanaungkul S, Riggs BL, Yergey AL, et al. Relationship of intestinal calcium absorption to 1,25-dihydroxyvitamin D [1,25(OH)2D] levels in young versus elderly women: evidence for age-related intestinal resistance to 1,25(OH)2D action. *J Clin Endocrinol Metab*. 2000;85(11):4023–4027.
753. Institue of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Flouride*. Washington DC: National Academies Press; 1997.
754. Morley JE. Should all long-term care residents receive vitamin D? *J Am Med Dir Assoc*. 2007;8(2):69–70.
755. Johnson MA, Kimlin MG. Vitamin D, aging, and the 2005 Dietary Guidelines for Americans. *Nutr Rev*. 2006;64(9):410–421.
756. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006;84(1):18–28.
757. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. *N Engl J Med*. 1992;327(23):1637–1642.
758. Dawson-Hughes B, Harris SS, Krall EA, et al. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med*. 1997;337(10):670–676.
759. National Research Council. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington DC: National Academic Press; 2011.
760. Clemens TL, Zhou XY, Myles M, et al. Serum vitamin D_2 and vitamin D_3 metabolite concentrations and absorption of vitamin D_2 in elderly subjects. *J Clin Endocrinol Metab*. 1986;63(3):656–660.
761. Hathcock JN, Shao A, Vieth R, et al. Risk assessment for vitamin D. *Am J Clin Nutr*. 2007;85(1):6–18.
762. van den Berg H. Bioavailability of vitamin D. *Eur J Clin Nutr*. 1997;51(suppl 1):S76–S79.
763. Holick MF, Biancuzzo RM, Chen TC, et al. Vitamin D_2 is as effective as vitamin D_3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab*. 2008;93(3):677–681.
764. Enjuanes A, Garcia-Giralt N, Supervia A, et al. Functional analysis of the I.3, I.6, pII and I.4 promoters of CYP19 (aromatase) gene in human osteoblasts and their role in vitamin D and dexamethasone stimulation. *Eur J Endocrinol*. 2005;153(6):981–988.
765. Enjuanes A, Garcia-Giralt N, Supervia A, et al. Regulation of CYP19 gene expression in primary human osteoblasts: effects of vitamin D and other treatments. *Eur J Endocrinol*. 2003;148(5):519–526.
766. Lou YR, Murtola T, Tuohimaa P. Regulation of aromatase and 5alpha-reductase by 25-hydroxyvitamin D(3), 1alpha,25-dihydroxyvitamin D(3), dexamethasone and progesterone in prostate cancer cells. *J Steroid Biochem Mol Biol*. 2005;94(1-3):151–157.
767. Pino AM, Rodriguez JM, Rios S, et al. Aromatase activity of human mesenchymal stem cells is stimulated by early differentiation, vitamin D and leptin. *J Endocrinol*. 2006;191(3):715–725.
768. Yague JG, Garcia-Segura LM, Azcoitia I. Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone, and mifepristone in human glioma cells. *Endocrine*. 2009;35(2):252–261.
769. Chung E, Mo H, Wang S, et al. Potential roles of vitamin E in age-related changes in skeletal muscle health. *Nutr Res*. 2018;49:23–36.
770. Ble A, Cherubini A, Volpato S, et al. Lower plasma vitamin E levels are associated with the frailty syndrome: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2006;61(3):278–283.
771. Mocchegiani E, Costarelli L, Giacconi R, et al. Vitamin E-gene interactions in aging and inflammatory age-related diseases: implications for treatment. A systematic review. *Ageing Res Rev*. 2014;14:81–101.
772. Dahlin KJ, Chan AC, Benson ES, et al. Rehabilitating effect of vitamin E therapy on the ultrastructural changes in skeletal muscles of vitamin E-deficient rabbits. *Am J Clin Nutr*. 1978;31(1):94–99.
773. Wray DW, Nishiyama SK, Monnet A, et al. Antioxidants and aging: NMR-based evidence of improved skeletal muscle perfusion and energetics. *Am J Physiol Heart Circ Physiol*. 2009;297(5):H1870–1875.
774. Magalhaes J, Ferreira R, Neuparth MJ, et al. Vitamin E prevents hypobaric hypoxia-induced mitochondrial dysfunction in skeletal muscle. *Clin Sci (Lond)*. 2007;113(12):459–466.
775. Lim JJ, Ngah WZ, Mouly V, et al. Reversal of myoblast aging by tocotrienol rich fraction posttreatment. *Oxid Med Cell Longev*. 2013;2013:978101.
776. Meydani M, Evans WJ, Handelman G, et al. Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am J Physiol*. 1993;264(5 Pt 2):R992–R998.
777. Chin SF, Hamid NA, Latiff AA, et al. Reduction of DNA damage in older healthy adults by Tri E Tocotrienol supplementation. *Nutrition*. 2008;24(1):1–10.
778. Chin SF, Ibahim J, Makpol S, et al. Tocotrienol rich fraction supplementation improved lipid profile and oxidative status in healthy older adults: a randomized controlled study. *Nutr Metab (Lond)*. 2011;8(1):42.
779. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest*. 1997;99(3):457–468.
780. Haus JM, Carrithers JA, Trappe SW, et al. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *J Appl Physiol (1985)*. 2007;103(6):2068–2076.
781. Momma H, Niu K, Kobayashi Y, et al. Skin advanced glycation end product accumulation and muscle strength among adult men. *Eur J Appl Physiol*. 2011;111(7):1545–1552.
782. Semba RD, Bandinelli S, Sun K, et al. Relationship of an advanced glycation end product, plasma carboxymethyl-lysine, with slow walking speed in older adults: the InCHIANTI study. *Eur J Appl Physiol*. 2010;108(1):191–195.
783. Cesari M, Pahor M, Bartali B, et al. Antioxidants and physical performance in elderly persons: the Invecchiare in Chianti (InCHIANTI) study. *Am J Clin Nutr*. 2004;79(2):289–294.
784. Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. *FASEB J*. 1999;13(10):1145–1155.
785. Rafique R, Schapira AH, Coper JM. Mitochondrial respiratory chain dysfunction in ageing; influence of vitamin E deficiency. *Free Radic Res*. 2004;38(2):157–165.

786. Nier B, Weinberg PD, Rimbach G, et al. Differential gene expression in skeletal muscle of rats with vitamin E deficiency. *IUBMB Life*. 2006;58(9):540–548.
787. Maras JE, Bermudez OI, Qiao N, et al. Intake of alpha-tocopherol is limited among US adults. *J Am Diet Assoc*. 2004;104(4):567–575.
788. Gao X, Wilde PE, Lichtenstein AH, et al. The maximal amount of dietary alpha-tocopherol intake in U.S. adults (NHANES 2001-2002). *J Nutr*. 2006;136(4):1021–1026.
789. Alvarez-Hernandez J, Planas Vila M, Leon-Sanz M, et al. Prevalence and costs of malnutrition in hospitalized patients; the PREDyCES Study. *Nutr Hosp*. 2012;27(4):1049–1059.
790. Granado-Lorencio F, Blanco-Navarro I, Perez-Sacristan B, et al. Determinants of fat-soluble vitamin status in patients aged 65 years and over. *Eur J Clin Nutr*. 2013;67(12):1325–1327.
791. Painter PC, Cope JY, Smith JL. Reference information for the clinical laboratory. In: Burtis CA, Ashwood ER, eds. *Tietz Textbook of Clinical Chemistry*. 3rd ed. Philadelphia: Saunders; 1999.
792. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington DC: National Academies Press; 2000.
793. Karmowski J, Hintze V, Kschonsek J, et al. Antioxidant activities of tocopherols/tocotrienols and lipophilic antioxidant capacity of wheat, vegetable oils, milk and milk cream by using photochemiluminescence. *Food Chem*. 2015;175:593–600.
794. Yoshida Y, Niki E, Noguchi N. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. *Chem Phys Lipids*. 2003;123(1):63–75.
795. Aggarwal BB, Sundaram C, Prasad S, et al. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. *Biochem Pharmacol*. 2010;80(11):1613–1631.
796. Wu D, Meydani SN. Age-associated changes in immune and inflammatory responses: impact of vitamin E intervention. *J Leukoc Biol*. 2008;84(4):900–914.
797. Sen CK, Khanna S, Roy S. Tocotrienols: vitamin E beyond tocopherols. *Life Sci*. 2006;78(18):2088–2098.
798. Herrmann W. The importance of hyperhomocysteinemia as a risk factor for diseases: an overview. *Clin Chem Lab Med*. 2001;39(8):666–674.
799. Bulut EA, Soysal P, Aydin AE, et al. Vitamin B_{12} deficiency might be related to sarcopenia in older adults. *Exp Gerontol*. 2017;95:136–140.
800. Huo YR, Suriyaarachchi P, Gomez F, et al. Comprehensive nutritional status in sarco-osteoporotic older fallers. *J Nutr Health Aging*. 2015;19(4):474–480.
801. Pannerec A, Migliavacca E, De Castro A, et al. Vitamin B_{12} deficiency and impaired expression of amnionless during aging. *J Cachexia Sarcopenia Muscle*. 2018;9(1):41–52.
802. Joosten E, Lesaffre E, Riezler R. Are different reference intervals for methylmalonic acid and total homocysteine necessary in elderly people? *Eur J Haematol*. 1996;57(3):222–226.
803. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc*. 1992;40(12):1197–1204.
804. Institute of Medicine. *Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Biotin and Choline*. Washington DC: National Academies Press; 1998.
805. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. Annu Rev Nutr. 1999;19:357-77.
806. Bennett BT, Mohamed JS, Alway SE. Effects of resveratrol on the recovery of muscle mass following disuse in the plantaris muscle of aged rats. *PLoS One*. 2013;8(12):e83518.
807. Durbin SM, Jackson JR, Ryan MJ, et al. Resveratrol supplementation preserves long bone mass, microstructure, and strength in hindlimb-suspended old male rats. *J Bone Miner Metab*. 2014;32(1):38–47.
808. Joseph AM, Malamo AG, Silvestre J, et al. Short-term caloric restriction, resveratrol, or combined treatment regimens initiated in late-life alter mitochondrial protein expression profiles in a fiber-type specific manner in aged animals. *Exp Gerontol*. 2013;48(9):858–868.
809. Jackson JR, Ryan MJ, Alway SE. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. *J Gerontol A Biol Sci Med Sci*. 2011;66(7):751–764.
810. Liao ZY, Chen JL, Xiao MH, et al. The effect of exercise, resveratrol or their combination on sarcopenia in aged rats via regulation of AMPK/Sirt1 pathway. *Exp Gerontol*. 2017;98:177–183.
811. Fulco M, Schiltz RL, Iezzi S, et al. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol Cell*. 2003;12(1):51–62.
812. Rathbone CR, Booth FW, Lees SJ. Sirt1 increases skeletal muscle precursor cell proliferation. *Eur J Cell Biol*. 2009;88(1):35–44.
813. Galuppo M, Di Paola R, Mazzon E, et al. Role of PPAR-delta in the development of zymosan-induced multiple organ failure: an experiment mice study. *J Inflamm (Lond)*. 2010;7:12.
814. Xu Z, Huang G, Gong W, et al. FXR ligands protect against hepatocellular inflammation via SOCS3 induction. *Cell Signal*. 2012;24(8):1658–1664.
815. Alway SE, Bennett BT, Wilson JC, et al. Green tea extract attenuates muscle loss and improves muscle function during disuse, but fails to improve muscle recovery following unloading in aged rats. *J Appl Physiol (1985)*. 2015;118(3):319–330.
816. Gutierrez-Salmean G, Ciaraldi TP, Nogueira L, et al. Effects of (-)-epicatechin on molecular modulators of skeletal muscle growth and differentiation. *J Nutr Biochem*. 2014;25(1):91–94.
817. Casanova E, Baselga-Escudero L, Ribas-Latre A, et al. Epigallocatechin gallate counteracts oxidative stress in docosahexaenoic acid-treated myocytes. *Biochim Biophys Acta*. 2014;1837(6):783–791.
818. Haramizu S, Ota N, Hase T, et al. Catechins attenuate eccentric exercise-induced inflammation and loss of force production in muscle in senescence-accelerated mice. *J Appl Physiol (1985)*. 2011;111(6):1654–1663.
819. Haramizu S, Ota N, Hase T, et al. Catechins suppress muscle inflammation and hasten performance recovery after exercise. *Med Sci Sports Exerc*. 2013;45(9):1694–1702.
820. Andrade JP, Assuncao M. Protective effects of chronic green tea consumption on age-related neurodegeneration. *Curr Pharm Des*. 2012;18(1):4–14.
821. Marzani B, Balage M, Venien A, et al. Antioxidant supplementation restores defective leucine stimulation of protein synthesis in skeletal muscle from old rats. *J Nutr*. 2008;138(11):2205–2211.
822. Semba RD, Lauretani F, Ferrucci L. Carotenoids as protection against sarcopenia in older adults. *Arch Biochem Biophys*. 2007;458(2):141–145.
823. Lauretani F, Semba RD, Bandinelli S, et al. Low plasma selenium concentrations and mortality among older community-dwelling adults: the InCHIANTI Study. *Aging Clin Exp Res*. 2008;20(2):153–158.
824. Lauretani F, Semba RD, Dayhoff-Brannigan M, et al. Low total plasma carotenoids are independent predictors of mortality among older persons: the InCHIANTI study. *Eur J Nutr*. 2008;47(6):335–340.
825. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academies Press; 2000.
826. Alipanah N, Varadhan R, Sun K, et al. Low serum carotenoids are associated with a decline in walking speed in older women. *J Nutr Health Aging*. 2009;13(3):170–175.
827. Lauretani F, Semba RD, Bandinelli S, et al. Association of low plasma selenium concentrations with poor muscle strength in older community-dwelling adults: the InCHIANTI Study. *Am J Clin Nutr*. 2007;86(2):347–352.
828. Beck J, Ferrucci L, Sun K, et al. Low serum selenium concentrations are associated with poor grip strength among older women living in the community. *Biofactors*. 2007;29(1):37–44.
829. Lauretani F, Semba RD, Bandinelli S, et al. Low plasma carotenoids and skeletal muscle strength decline over 6 years. *J Gerontol A Biol Sci Med Sci*. 2008;63(4):376–383.
830. Semba RD, Bartali B, Zhou J, et al. Low serum micronutrient concentrations predict frailty among older women living in the community. *J Gerontol A Biol Sci Med Sci*. 2006;61(6):594–599.
831. Bartali B, Frongillo EA, Guralnik JM, et al. Serum micronutrient concentrations and decline in physical function among older persons. *JAMA*. 2008;299(3):308–315.

832. Kim J, Lee Y, Kye S, et al. Association of vegetables and fruits consumption with sarcopenia in older adults: the Fourth Korea National Health and Nutrition Examination Survey. *Age Ageing*. 2015;44(1):96–102.
833. Pietta PG. Flavonoids as antioxidants. *J Nat Prod*. 2000;63(7):1035–1042.
834. Le Marchand L. Cancer preventive effects of flavonoids—a review. *Biomed Pharmacother*. 2002;56(6):296–301.
835. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr*. 2004;80(4):1029–1035.
836. McEligot AJ, Yang S, Meyskens Jr FL, et al. Redox regulation by intrinsic species and extrinsic nutrients in normal and cancer cells. *Annu Rev Nutr*. 2005;25:261–295.
837. Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol*. 2005;16(1):77–84.
838. Manach C, Williamson G, Morand C, et al. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(suppl 1):230S–242S.
839. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr*. 2005;81(suppl 1):243S–255S.
840. Halvorsen BL, Holte K, Myhrstad MC, et al. A systematic screening of total antioxidants in dietary plants. *J Nutr*. 2002;132(3):461–471.
841. Walston J, Xue Q, Semba RD, et al. Serum antioxidants, inflammation, and total mortality in older women. *Am J Epidemiol*. 2006;163(1):18–26.
842. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition*. 2002;18(10):872–879.
843. Schaap LA, Pluijm SM, Deeg DJ, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. *J Gerontol A Biol Sci Med Sci*. 2009;64(11):1183–1189.
844. Ryan MJ, Dudash HJ, Docherty M, et al. Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats. *Exp Gerontol*. 2010;45(11):882–895.
845. Rebrin I, Zicker S, Wedekind KJ, et al. Effect of antioxidant-enriched diets on glutathione redox status in tissue homogenates and mitochondria of the senescence-accelerated mouse. *Free Radic Biol Med*. 2005;39(4):549–557.
846. Chen YL, Yang KC, Chang HH, et al. Low serum selenium level is associated with low muscle mass in the community-dwelling elderly. *J Am Med Dir Assoc*. 2014;15(11):807–811.
847. Vitadello M, Germinario E, Ravara B, et al. Curcumin counteracts loss of force and atrophy of hindlimb unloaded rat soleus by hampering neuronal nitric oxide synthase untethering from sarcolemma. *J Physiol*. 2014;592(12):2637–2652.
848. McFarlin BK, Venable AS, Henning AL, et al. Reduced inflammatory and muscle damage biomarkers following oral supplementation with bioavailable curcumin. *BBA Clin*. 2016;5:72–78.
849. Nakashima Y, Ohsawa I, Nishimaki K, et al. Preventive effects of Chlorella on skeletal muscle atrophy in muscle-specific mitochondrial aldehyde dehydrogenase 2 activity-deficient mice. *BMC Complement Altern Med*. 2014;14:390.
850. Mirza KA, Pereira SL, Edens NK, et al. Attenuation of muscle wasting in murine C2C 12 myotubes by epigallocatechin-3-gallate. *J Cachexia Sarcopenia Muscle*. 2014;5(4):339–345.
851. Hirasaka K, Maeda T, Ikeda C, et al. Isoflavones derived from soy beans prevent MuRF1-mediated muscle atrophy in C2C12 myotubes through SIRT1 activation. *J Nutr Sci Vitaminol (Tokyo)*. 2013;59(4):317–324.
852. Choquette S, Dion T, Brochu M, et al. Soy isoflavones and exercise to improve physical capacity in postmenopausal women. *Climacteric*. 2013;16(1):70–77.
853. Evans NP, Call JA, Bassaganya-Riera J, et al. Green tea extract decreases muscle pathology and NF-kappaB immunostaining in regenerating muscle fibers of mdx mice. *Clin Nutr*. 2010;29(3):391–398.
854. Kim H, Suzuki T, Saito K, et al. Effects of exercise and tea catechins on muscle mass, strength and walking ability in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. *Geriatr Gerontol Int*. 2013;13(2):458–465.
855. Terauchi M, Horiguchi N, Kajiyama A, et al. Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: a randomized, double-blind, placebo-controlled pilot study. *Menopause*. 2014;21(9):990–996.
856. Raut AA, Rege NN, Tadvi FM, et al. Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha *(Withania somnifera)* in healthy volunteers. *J Ayurveda Integr Med*. 2012;3(3):111–114.
857. Mishra SKaT B. A clinical trial with *Withania somnifera (Solanaceae)* extract in the management of sarcopenia. *Signpost J Organic Biomolec Chem*. 2013;1:187–194.
858. Wysocki A, Butler M, Shamliyan T, et al. Whole-body vibration therapy for osteoporosis: state of the science. *Ann Intern Med*. 2011;155(10):680–686. W206–W613.
859. Bautmans I, Van Hees E, Lemper JC, et al. The feasibility of whole body vibration in institutionalised elderly persons and its influence on muscle performance, balance and mobility: a randomised controlled trial [ISRCTN62535013]. *BMC Geriatr*. 2005;5:17.
860. von Stengel S, Kemmler W, Engelke K, et al. Effects of whole body vibration on bone mineral density and falls: results of the randomized controlled ELVIS study with postmenopausal women. *Osteoporos Int*. 2011;22(1):317–325.
861. Bogaerts A, Delecluse C, Boonen S, et al. Changes in balance, functional performance and fall risk following whole body vibration training and vitamin D supplementation in institutionalized elderly women. A 6 month randomized controlled trial. *Gait Posture*. 2011;33(3):466–472.
862. Rogan S, Hilfiker R, Herren K, et al. Effects of whole-body vibration on postural control in elderly: a systematic review and meta-analysis. *BMC Geriatr*. 2011;11:72.
863. Sitja-Rabert M, Rigau D, Fort Vanmeerghaeghe A, et al. Efficacy of whole body vibration exercise in older people: a systematic review. *Disabil Rehabil*. 2012;34(11):883–893.
864. Kemmler W, von Stengel S. Alternative exercise technologies to fight against sarcopenia at old age: a series of studies and review. *J Aging Res*. 2012;2012:109013.
865. Kemmler W, Bebenek M, Engelke K, et al. Impact of whole-body electromyostimulation on body composition in elderly women at risk for sarcopenia: the Training and ElectroStimulation Trial (TEST-III). *Age (Dordr)*. 2014;36(1):395–406.
866. Vellas B, Fielding R, Miller R, et al. Designing drug trials for sarcopenia in older adults with hip fracture—a task force from the International Conference on Frailty and Sarcopenia Research (ICFSR). *J Frailty Aging*. 2014;3(4):199–204.
867. Malafarina V, Uriz-Otano F, Iniesta R, et al. Effectiveness of nutritional supplementation on muscle mass in treatment of sarcopenia in old age: a systematic review. *J Am Med Dir Assoc*. 2013;14(1):10–17.
868. Verreijen AM, Verlaan S, Engberink MF, et al. A high whey protein-, leucine-, and vitamin D-enriched supplement preserves muscle mass during intentional weight loss in obese older adults: a double-blind randomized controlled trial. *Am J Clin Nutr*. 2015;101(2):279–286.
869. Morley JE, Argiles JM, Evans WJ, et al. Nutritional recommendations for the management of sarcopenia. *J Am Med Dir Assoc*. 2010;11(6):391–396.
870. Taaffe DR. Sarcopenia—exercise as a treatment strategy. *Aust Fam Physician*. 2006;35(3):130–134.

218

Seborrheic Dermatitis

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Branny or greasy scaling over erythematous skin patterned on the sebum-rich areas of the scalp, face, and trunk. Facial areas include the forehead, eyebrows, eyelashes, nasolabial folds, and beard. Truncal involvement includes the presternal region, umbilicus, axillae, inframammary and inguinal folds, and perineum.
- The scalp appearance varies from mild, patchy dandruff to widespread, thick, adherent crusts; it may involve the anterior and posterior hairline and the periauricular skin. In infants, it occurs as "cradle cap."
- Usually nonpruritic, although active phases can manifest with burning and itching
- Seasonal; worse in winter

GENERAL CONSIDERATIONS

Seborrheic dermatitis (SD) is a common papulosquamous condition similar in appearance to psoriasis. Clinically, it may be associated with excessive oiliness (seborrhea) and dandruff. The scale may be yellowish and either dry or greasy. The erythematous, follicular, scaly papules may coalesce to form large plaques or circinate patches. The condition occurs either in infancy (usually between 2 and 12 weeks of age) or in the middle-aged or elderly and has a prognosis of lifelong recurrence.

Various environmental and intrinsic factors have been identified as predisposing factors for SD, but its etiology remains poorly understood. Genes contributing to SD etiology play a role either in the immune response or epidermal differentiation.[1]

Malassezia yeast organisms are probably not the cause but a cofactor linked to depressed helper T cells (SD is very common in AIDS). Other contributing factors include increased natural killer cells, which increase inflammatory cytokines; increased sebum levels; activation of the alternate complement pathway; and genetic susceptibility to a skin-barrier dysfunction.[2] *Malassezia* species have lipase activity, which releases inflammatory arachidonic acid. SD is aggravated by changes in humidity, scratching, emotional stress, diet, various medications, and androgen excess.

DIAGNOSIS

As can be seen in Figs. 218.1 through 218.6, diagnosis is typically made by the patient's skin presentation.

THERAPEUTIC CONSIDERATIONS

Food Allergy

SD usually begins as cradle cap, and although it is not primarily an allergic disease, it has been associated with food allergy (67% of patients exhibit some form of allergy by 10 years of age).[3]

Nutritional Supplements

Vitamin A

Off-label use of low-dose isotretinoin (10 mg every other day) is an effective therapy for SD because it reduces sebum production and sebaceous gland size and is anti-inflammatory.[4] However, due to potential adverse effects, it is safer to employ vitamin A, which, even in high doses, is better tolerated. However, vitamin A, like isotretinoin, does carry a high risk for teratogenicity and therefore is not to be prescribed for women of childbearing potential.

Biotin

The underlying factor in infants may be a biotin deficiency.[5] A syndrome clinically similar to SD has been produced by feeding rats a diet high in raw egg white (high in avidin, a glycoprotein that binds biotin, making it unavailable for absorption). Because a large portion of the human biotin supply is provided by intestinal bacteria, it has been postulated that the absence of normal intestinal flora may be responsible for biotin deficiency in infants.[3] A number of articles have demonstrated successful treatment of SD with biotin in both the nursing mother and the infant.[5,6]

In adults, treatment with biotin alone is usually of no value.

Pyridoxine

Both the administration of desopyridoxine, which induces pyridoxine deficiency in humans, and the placing of rats on a pyridoxine-deficient diet cause dermatological lesions indistinguishable from SD.[7] Despite

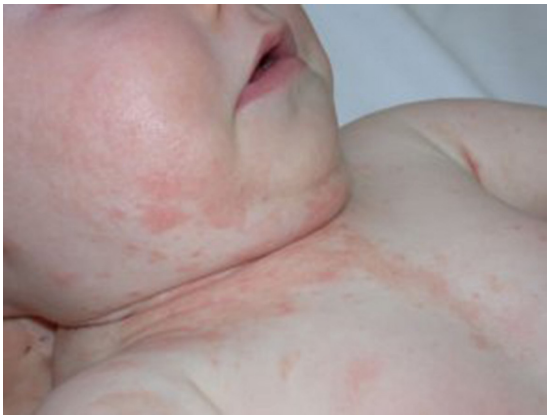


Fig. 218.1 Presentation in a child.

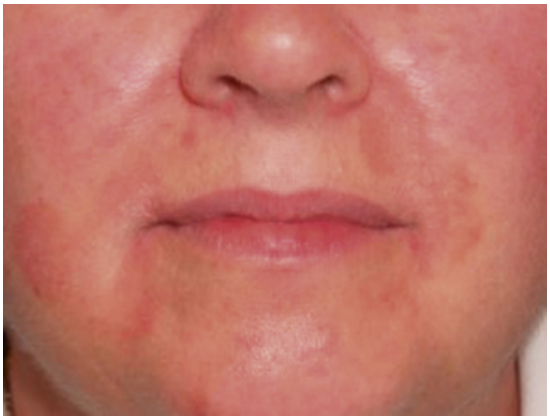


Fig. 218.2 Mouth.

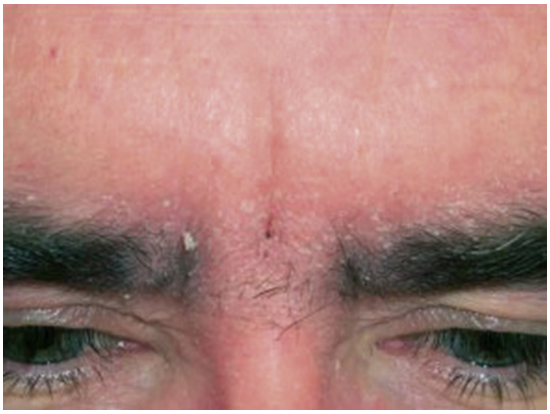


Fig. 218.3 Eyebrows.

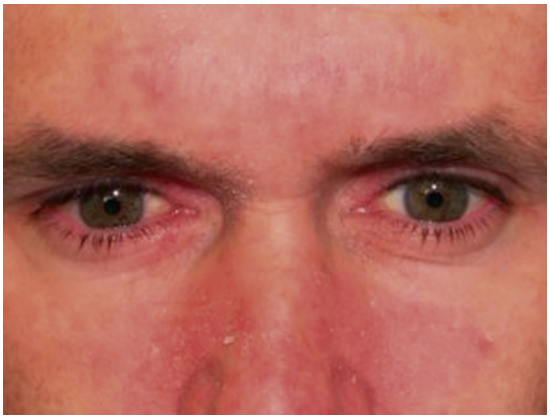


Fig. 218.4 Eyes and nose.

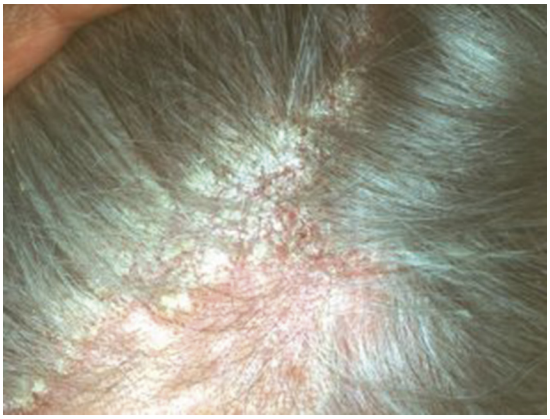


Fig. 218.5 Scalp.

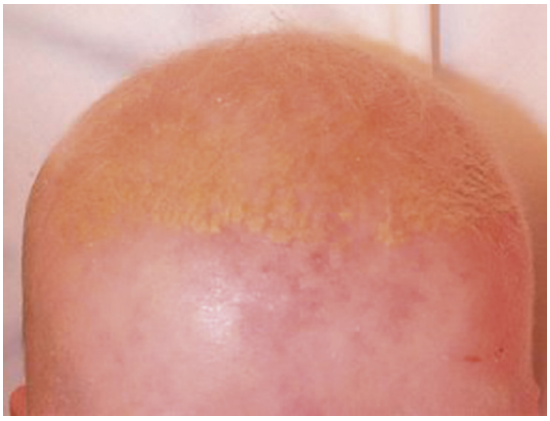


Fig. 218.6 Cradle cap.

these results, oral and parenteral applications of pyridoxine have shown little success. However, in the sicca form of the disorder (involvement of the scalp [dandruff], brow, nasolabial folds, and bearded area with varying degrees of greasy adherent scales on an erythematous base), all cases cleared completely within 10 days of local application of a water-soluble ointment containing 50 mg/g of pyridoxine. Other types of SD, particularly flexural and infected, did not respond to this mode of therapy.

In one study of patients with elevated levels of urinary xanthurenic acid, oral, parenteral, and local applications of pyridoxine all returned excretion levels to normal, implying transcutaneous absorption of pyridoxine.[7,8] These results are clouded, however, by those of another study indicating that the improvement from topical application may be due more to a reduction in the sebaceous secretion rate by the ointment itself, with the added pyridoxine having no effect.[9]

The patient should be asked about exposure to pyridoxine antimetabolites. Examples are the hydrazine dyes (U.S. Food Drug and Cosmetic Act [FD&C] yellow no. 5) and drugs (isoniazid and hydralazine), dopamine, penicillamine, oral contraceptives, and excessive protein intake.[10]

Botanical Medicines

Proprietary Herbal Shampoo

An open-label study of a shampoo and scalp lotion that includes botanical antifungal and anti-inflammatory agents in addition to zinc pyrithione in 50 patients with moderate to severe scalp SD showed a statistically significant reduction from baseline in erythema and loose flaking after 2 weeks. The average total severity score improved by 49% after 6 weeks of treatment. Patient satisfaction with the product was very good. The botanical formula included *Phellodendron amurense* bark extract, *Portulaca oleracea* extract, *Sapindus mukorossi* fruit extract, *Indigofera tinctoria* extract, and *Rheum palmatum* root extract.[11]

Aloe vera

Aloe vera gel can be quite helpful when applied topically. In one double-blind trial in people with SD, the application of a 30% crude aloe emulsion cream twice a day for 4 to 6 weeks produced improvements in scaling and itching in 62% of subjects compared with improvements in only 25% of the placebo group.[12]

Melaleuca alternifolia

Melaleuca alternifolia (tea tree) oil has demonstrated activity against *Malassezia* species, which may be of benefit in the treatment of SD. Honey and cinnamic acid have similar activity.[13] A study of 126 patients using 5% tea tree oil shampoo showed 41% improvement in severity versus 11% in the placebo group.[14] Tea tree oil may be added to the patient's favorite shampoo as a way of increasing compliance.

Homeopathic Therapy

A 2002 study evaluated the efficacy of a homeopathic combination remedy in the control of SD and chronic dandruff. The homeopathic therapy of Kali brom 1x, Natrum brom 2x, Niccolum sulf 3x, and Natrum mur 6x (potassium bromide, sodium bromide, nickel sulfate, and sodium chloride) was studied in a placebo crossover trial in 41 patients with SD and/or chronic dandruff. At the end of 10 weeks, all patients crossed over to the active medication under a different label for an additional 10 weeks in an open-study format. Twenty-nine patients completed the 10-week blinded portion of the study. After 10 weeks of treatment, the disease state of the patients receiving active medication showed significant improvement compared with that of the patients receiving placebo ($P < 0.04$). Ten weeks after crossover, the placebo patients experienced improvement as well ($P < 0.01$).[15]

THERAPEUTIC APPROACH

Although the optimal approach to treating all patients with SD is not clear at this time, effective therapy is available for most patients. In infants, alleviation of the biotin deficiency and control of the food allergies are key. For adults, using the aforementioned herbal and zinc pyrithione-based shampoo and scalp lotion, *Aloe vera,* and *M. alternifolia* oil are the primary therapies.

Diet

The physician should detect and treat food allergens. In nursing infants, the food allergies of the mother should be considered.

Supplements

- Biotin: 1 mg daily for infants[16]
- Vitamin A 50,000 IU daily (contraindicated in women of childbearing potential)
- Homeopathic combination remedy once a day

Topical Treatments

- Proprietary herbal shampoo and scalp lotion: shampoo 2 to 3 days/week; scalp lotion applied to affected areas daily at bedtime
- Pyridoxine ointment: 50 mg/g (in water-soluble base)
- *Aloe vera* gel: Apply twice a day to affected areas.
- Tea tree: 5% solution (may be added to patient's shampoo of choice)

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Karakadze MA, Hirt PA, Wikramanayake TC. The genetic basis of seborrhoeic dermatitis: a review. *J Eur Acad Dermatol Venereol*. 2018;32(4):529–536.
2. Faergemann J, Bergbrant IM, Dohse M. Seborrheic dermatitis and *Pityrosporum folliculitis:* characterization of inflammatory cells and mediators in the skin by immunohistochemistry. *Br J Dermatol*. 2001;144:549–556.
3. Eppig JJ. Seborrhea capitis in infants: a clinical experience in allergy therapy. *Ann Allergy*. 1971;29:323–324.
4. Kamamoto CSL, Sanudo A, Hassun KM, et al. Low-dose oral isotretinoin for moderate to severe seborrhea/seborrheic dermatitis: a randomized and comparative trial. *Int J Dermatol*. 2017;56:80–85.
5. Nisenson A. Seborrheic dermatitis of infants and Leiner's disease: a biotin deficiency. *J Pediatr*. 1957;51:537–548.
6. Nisenson A, Barness LA. Treatment of seborrheic dermatitis with biotin and vitamin B complex. *J Pediatr*. 1972;81:630–631.
7. Schreiner A, Slinger W, Hawkins V, et al. Seborrheic dermatitis: a local metabolic defect involving pyridoxine. *J Lab Clin Med*. 1952;40:121–130.
8. Callaghan T. The effect of folic acid on seborrheic dermatitis. *Cutis*. 1967;3:584–588.
9. Andrews GC, Post CF, Domonkos AN. Seborrheic dermatitis: supplemental treatment with vitamin B_{12}. *N Y State J Med*. 1950;50:1921–1925.
10. Schreiner A, Rockwell E, Vilter R. A local defect in the metabolism of pyridoxine in the skin of persons with seborrheic dermatitis of the "sicca" type. *J Invest Dermatol*. 1952;19:95–96.
11. Barak-Shinar D, Green L. Scalp seborrheic dermatitis and dandruff therapy using a herbal and zinc pyrithione-based therapy of shampoo and scalp lotion. *J Clin Aesthet Dermato*. 2018;11(1):26–31.
12. Effersoe H. The effect of topical application of pyridoxine ointment on the rate of sebaceous secretion in patients with seborrheic dermatitis. *Acta Derm Venereol*. 1954;3:272–278.
13. Gupta AK, Nicol K, Batra R. Role of antifungal agents in the treatment of seborrheic dermatitis. *Am J Clin Dermatol*. 2004;5(6):417–422.
14. Satchell AC, Sauragen A, Bell C. Treatment of dandruff with 5% tea tree oil shampoo. *J Am Acad Dermatol*. 2002;47(6):852–855.
15. Smith SA, Baker AE, Williams JH. Effective treatment of seborrheic dermatitis using a low dose, oral homeopathic medication consisting of potassium bromide, sodium bromide, nickel sulfate, and sodium chloride in a double-blind, placebo-controlled study. *Altern Med Rev*. 2002;7:59–67.
16. Messaritakis J, Kattamis C, Karabula C. Generalized seborrhoeic dermatitis: clinical and therapeutic data of 25 patients. *Arch Dis Child*. 1975;50(11):871–874.

Senile Cataracts

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Clouding or opacity in the crystalline lens of the eye
- Absence or alteration of red reflex (small cataracts stand out as dark defects)
- Gradual loss of vision

GENERAL CONSIDERATIONS

Cataracts are the leading cause of impaired vision and blindness in the United States. Approximately 4 million people have some degree of vision-impairing cataract, and at least 40,000 people in the United States are blind because of cataracts. Cataracts are a source of a tremendous financial burden on our society, and cataract surgery is the most common major surgical procedure performed in the United States each year (600,000 per year) for persons receiving Medicare benefits.

Cataracts may be classified by location and appearance of the lens opacities, by cause or significant contributing factor, and by age at onset. Many factors may cause or contribute to the progression of lens opacity, including ocular disease, injury, surgery, systemic diseases (e.g., diabetes mellitus, galactosemia), toxins, ultraviolet and near-ultraviolet light, radiation exposure, and hereditary disease.

Aging-related (or senile) cataracts are discussed in this chapter; diabetes- and galactose-induced cataracts (sugar cataracts) are discussed in Chapter 165.

The crystalline lens is a vital component of the optical system owing to its ability to focus light (via changes in shape) while maintaining optical transparency. Unfortunately, this transparency diminishes with age. Most of the geriatric population displays some degree of cataract formation (Fig. 219.1). In the normal aging eye, there is a progressive increase in the size, weight, and density of the lens throughout life.

Cataract formation is characterized histopathologically by the following features:

- Fibrous metaplasia of the epithelium
- Liquefaction of fibers, resulting in morgagnian globule formation (drops of fluid beneath the capsule and between the lens fibers)
- Sclerosis (melding of fibers)
- Posterior migration and swelling of the epithelium

According to a topoanatomic classification, these basic alterations result in the following five types of cataracts:

- *Anterior subcapsular cataract:* Fibrous metaplasia of lens epithelium (usually follows iritis and adherence of the iris to the lens–posterior synechiae)
- *Anterior cortical cataract:* Liquefaction of lens fibers occurs, and morgagnian globules form in the cortex anteriorly.
- *Nuclear cataract:* An exaggeration of the normal aging-related melding of fibers in the nucleus.
- *Posterior cortical cataract:* Liquefaction and globular degeneration of the posterior lens cortex.
- *Posterior subcapsular cataract:* Epithelial cells migrate posteriorly under the capsule and form large irregular nucleated cells.

About 75% of senile cataracts are cortical, and the rest are nuclear. Clinically cortical cataracts take three forms:

- Spoke wheel, beginning in the periphery and coursing anteriorly and posteriorly to the nucleus
- Perinuclear punctate opacities
- Granular opacities under the posterior capsule (subcapsular cataracts)

THERAPEUTIC CONSIDERATIONS

Age-related cataracts are closely associated with lens chronological aging, oxidation, calcium imbalance, hydration, and crystallin modifications. Age-related changes in the lens cause a reduction in transparency, presbyopia, an increase in the scattering and aberration of light waves, and a degradation of the optical quality of the eye. Accumulating evidence indicates that misfolded proteins are generated in the endoplasmic reticulum (ER) by most cataractogenic stresses.[1] The cells activate a clean-up machinery called the ER stress/unfolded protein response (UPR) to eliminate misfolded proteins from cells before they can induce senescence. The UPR activates the nuclear factor-erythroid-2-related factor 2 (Nrf2), a central transcriptional factor for cytoprotection against stress; activates mobilization of ER-Ca^{2+} to the cytoplasm, which leads to the activation of Ca^{2+}-dependent proteases to cleave various enzymes and proteins, causing the loss of normal lens function; and enhances the overproduction of reactive oxygen species (ROS), which damage lens constituents. The etiology

of cataract formation is ultimately related to an inability to maintain normal homeostatic concentrations of Na^+, K^+, and Ca^{2+} within the lens. These abnormalities are apparently the result of decreased Na^+, K^+-ATPase activity,[2–7] a defect usually due to free-radical damage to some of the sulfhydryl proteins in the lens, including Na^+, K^+-ATPase, which contains a sulfhydryl component.

In cataract formation, the normal protective mechanisms are unable to prevent free-radical damage. The lens, like many other tissues of the body, depends on adequate levels and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), as well as adequate levels of accessory antioxidants such as lutein, vitamins E and C, and selenium, to help prevent damage by free radicals (Fig. 219.2). The SOD, CAT, and GSH-peroxidase content in the aqueous fluid and lenses decreases significantly with increasing lenticular nucleus hardness grading, with lenses at hardness level V having the lowest content of antioxidants.[8]

Fig. 219.1 Cataract. (sdigital/iStock.com.)

Individuals with higher dietary intakes of vitamin C and E, selenium, and carotenes (especially lutein) have a much lower risk of developing cataracts.[9] Several studies have shown that various nutritional supplements—multiple-vitamin formulas, vitamins C and E, B vitamins (especially vitamin B_{12} and folic acid), and vitamin A—also offer significant protection against both nuclear and cortical cataracts (Fig. 219.3).[10–13] Studies conducted by the Age-Related Eye Disease Study Research Group and others indicate that a combination of these nutrients will likely produce better results than any single nutrient alone or even limited combinations of three or fewer nutrients in the prevention of both age-related macular degeneration and cataracts (see Chapter 195 for more information).

Antioxidants

Lutein

Lutein, the yellow-orange carotene that offers significant protection against macular degeneration, also exerts protection against cataract formation.[14] Like the macula, the human lens concentrates lutein. In 1992 a prospective cohort study showed that consumption of spinach (high in lutein) was inversely related to the risk of cataracts severe enough to require extraction.[15] This initial investigation was followed by three prospective studies showing that the intake of lutein was inversely associated with cataract extraction (20% to 50% risk reduction).[16–18] In a double-blind intervention trial, 17 patients clinically diagnosed with age-related cataracts were randomly assigned to receive dietary supplementation with lutein (15 mg), α-tocopherol (100 mg), or placebo three times a week for up to 2 years.[19] Visual performance (visual acuity and glare sensitivity) improved in the lutein group, whereas there was a trend toward the maintenance of visual acuity with α-tocopherol and a decrease with placebo supplementation. Lutein-containing supplements were found to be effective in reducing oxidation in the aqueous humor of patients with senile cataracts by increasing superoxide scavenging activity.[20]

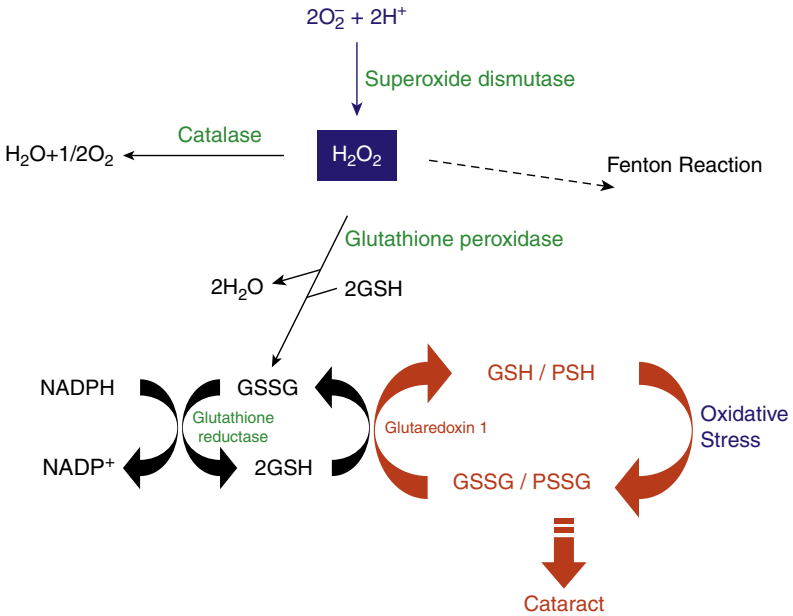


Fig. 219.2 Antioxidant defense systems in the lens. H_2O_2 generated by the dismutation of superoxide anion is degraded by several pathways, including catalase, glutathione peroxidase, and the Fenton reaction. A decreased SH/S–S ratio by oxidation can be reversed by glutathione reductase or glutaredoxin 1; the latter specifically reduces proteinthiol mixed disulfides. (From Weikel KA, Garber C, Baburins A, Taylor A. Nutritional modulation of cataract. *Nutr. Rev.* 2014;72[1]:30–47. PubMed PMID: 24279748.)

Vitamin C

A high dietary intake of vitamin C from either dietary sources or supplements has been shown to protect against cataract formation.[10–13,21] In addition to preventing cataracts, antioxidant nutrients like vitamin C may offer some therapeutic benefits. Several clinical studies have demonstrated that vitamin C supplementation can halt cataract progression and in some cases significantly improve vision. For example, in a study conducted in 1939, 450 patients with cataracts were started on a nutritional program that included 1 g/day of vitamin C, resulting in a significant reduction in cataract development.[2] Similar patients had previously required surgery within 4 years, but in the vitamin C–treated patients, only a handful needed surgery, and in most, there was no evidence that the cataracts had progressed over the 11-year study period.

It appears that the daily dose of vitamin C necessary to increase the vitamin C content of the lens is 1000 mg.[3] The lens of the eye and active tissue of the body require higher concentrations of vitamin C. The level of vitamin C in the blood is about 0.5 mg/dL, whereas that in the adrenal and pituitary glands is 100 times that. In the liver, spleen, and lens of the eye, the vitamin C level is increased by at least a factor of 20. In order for these concentrations to be maintained, the body must generate enormous amounts of energy to pull vitamin C out of blood against this tremendous gradient. Keeping blood vitamin C concentrations elevated helps the body concentrate vitamin C into active tissue by reducing the gradient. That is probably why such a high dose is required to raise the vitamin C content of the lens.

In another study, 450 patients with incipient cataracts were started on a nutritional program including 1 g/day of vitamin C, which led to a significant reduction in cataract development.[3]

In a large double-blind trial, 11,545 apparently healthy U.S. male physicians 50 years or older without a diagnosis of cataracts at baseline were randomly assigned to receive 400 IU of vitamin E or placebo on alternate days and 500 mg of vitamin C or placebo daily.[22] After 8 years of treatment and follow-up, there was no significant difference in cataract formation in the groups. This study may have failed to show benefit because it was below the threshold of 1 g/day of vitamin C.

A meta-analysis summarizing the evidence from epidemiological studies of vitamin C and the risk of age-related cataracts found that higher vitamin C intake and serum ascorbate may be inversely associated.[23] The relative risk (RR) and 95% confidence interval (CI) of cataract for the highest versus the lowest category of vitamin C intake was 0.814 (0.707–0.938), and the associations were significant in America and Asia. A significant association of cataract risk with the highest versus the lowest category of serum ascorbate was found in general (0.704 [0.564–0.879]). Inverse associations were also found between serum ascorbate and nuclear cataract and posterior subcapsular cataract.

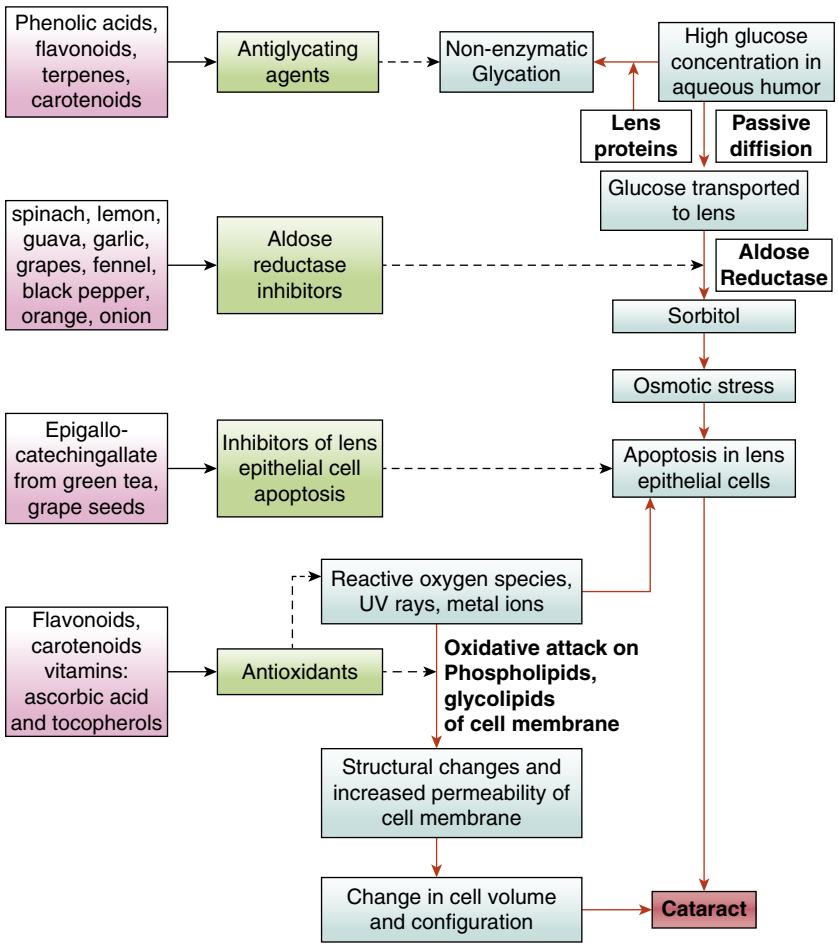


Fig. 219.3 Diagrammatic representation of the mechanisms of action of various nutraceuticals in the prevention of cataracts. (Kaur, A., Gupta, V., Christopher, A.F., Ahmad Malik, M., Bansal, P., (2017). Nutraceuticals in prevention of cataract - An evidence based approach, *Saudi Journal of Ophthalmology,* 31(1), 30-37.)

Glutathione

A tripeptide composed of glycine, glutamic acid, and cysteine, GSH is found at very high concentrations in the lens. GSH plays a vital role in maintaining a healthy lens and has been postulated as a key protective factor against toxins of both intralenticular and extralenticular origin. It functions as an antioxidant, maintains reduced sulfhydryl bonds within the lens proteins, acts as a coenzyme of various enzyme systems, participates in amino acid transport with gamma-glutamyl transpeptidase, and is involved in cation transport.[5] GSH levels are diminished in virtually all forms of cataracts.

Ascorbic acid and glutathione interactions. The antioxidants ascorbic acid (AA) and GSH work in close conjunction and are the most important of all the host-protective factors against the induction of cataracts. The reactions between them are as follows:

$$\text{AA} + \text{superoxide} \rightarrow \text{dehydroascorbate} + H_2O_2$$

Light may also cause oxidation of AA, leading to hydrogen peroxide formation, as follows:

$$\text{AA} + \text{light} \rightarrow \text{dehydroascorbate} + H_2O_2$$

An interesting cycle is then set into motion. The dehydroascorbate and hydrogen peroxide produced are reduced by selenium-containing glutathione peroxidase, as follows:

$$2\text{GSH} + \text{dehydroascorbate} \rightarrow \text{GSSG} + \text{AA}$$

$$2\text{GSH} + H_2O_2 \rightarrow \text{GSSG} + 2H_2O$$

The oxidized glutathione (GSSG) serves as an inducer of the hexose monophosphate shunt, which provides the nicotinamide adenine dinucleotide phosphate (NADPH; reduced form) necessary for reducing GSSG via riboflavin-dependent glutathione reductase, as follows:

$$\text{GSSG} + 2\text{NADPH} \rightarrow 2\text{GSH} + 2\text{NADP}$$

The NADP is reduced by hexose monophosphate dehydrogenase, as follows:

$$\text{NADP} + \text{glucose} - 6 - (\text{P}) \rightarrow \text{NADPH} + \text{ribulose5} - (\text{P}) + CO_2$$

This combination of enzymatic and nonenzymatic scavenging of free radicals is a key mechanism for the protection of the lens from photochemical and other forms of oxidative damage.

Selenium and Vitamin E

Selenium and vitamin E, both antioxidants, are known to function synergistically. The maintenance of proper selenium levels appears to be especially important because human lens glutathione peroxidase is selenium-dependent. Low selenium levels strongly promote cataract formation. Previous studies have shown that the selenium content in the cataractous human lens is only 15% of normal.[6]

A later study was conducted to better examine the role of selenium in cataract formation.[7] Selenium levels in the serum, lens, and aqueous humor were determined in 48 patients with cataracts and compared with levels in matched controls. Selenium levels in the serum and aqueous humor were found to be significantly lower in the patients with cataracts (serum, 0.28 mg/mL; aqueous humor, 0.19 mg/mL) than in normal controls (serum, 0.32 mg/mL; aqueous humor, 0.31 mg/mL). However, the selenium level in the lens itself did not significantly differ between the patients with cataracts and the controls.

The most important finding of the study was the decreased level of selenium in the aqueous humor in patients with cataracts. Excess hydrogen peroxide levels, up to 25 times normal, are found in the aqueous humor in patients with cataracts. An excess of hydrogen peroxide is associated with higher lipid peroxidation and altered lens permeability as a result of damage to the sodium–potassium pump. These changes ultimately leave the lens unprotected against free-radical and sun damage. As a result, a cataract is formed. Because selenium-dependent glutathione peroxidase is responsible for the breakdown of hydrogen peroxide, it is obvious that low levels of selenium are a major factor in the development of a cataract.

As previously described, vitamin E supplementation alone does not slow the progression of cataract formation.[19] A double-blind study in which vitamin E was given at a dose of 500 IU daily also found that supplementation did not slow cataract formation.[24] In a 7-year trial, supplementation with vitamin E (400 IU) combined with vitamin C (500 mg) and beta-carotene (15 mg) had no effect on the development or progression of cataracts.[25] However, a meta-analysis indicated that dietary vitamin E intake, dietary and supplemental vitamin E intake, and high levels of serum tocopherol were significantly associated with reduced age-related cataract (ARC) risk.[26] The pooled relative risk was 0.73 (95% CI 0.58, 0.92), 0.86 (95% CI 0.75, 0.99), and 0.77 (95% CI 0.66, 0.91), respectively. The findings from dose–response analysis showed evidence of a nonlinear association between dietary vitamin E intake and ARC. The risk of ARC decreased with dietary vitamin E intake from 7 mg/d (relative risk = 0.94; 95% CI 0.90, 0.97).

Superoxide Dismutase

The activity of SOD is lower in the human lens than in other tissues owing to the higher levels of ascorbate and glutathione in the lens, and a progressive decrease in SOD is encountered in cataract progression. Oral supplementation is probably of little value because it does not affect tissue SOD activity.[27] Of greater value is supplementation with the trace mineral cofactors of SOD, the levels of which are greatly reduced in the cataractous lens (copper by >90%, manganese by 50%, and zinc by >90%).[28]

Catalase

Catalase is concentrated in the epithelial portion of the lens (anterior surface), with very low levels found in the rest of the lens. Its primary function is to reduce (to water and oxygen) the hydrogen peroxide formed from the oxidation of ascorbate.

Tetrahydrobiopterin

Pteridine compounds are believed to play a protective role against cataract formation via the prevention of oxidation and damage by ultraviolet light. This action prevents the formation of high-molecular-weight proteins in the lens. Tetrahydrobiopterin functions as an essential coenzyme in the hydroxylation of monoamines such as phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase. Studies of human senile cataracts have demonstrated decreased levels of pteridine-synthesizing enzymes and tetrahydrobiopterin.[29] Supplemental folic acid may help to compensate for this deficiency.

Other Nutritional Factors

B Vitamins

Lenticular GSH requires flavin adenine dinucleotide (FAD) as a coenzyme for GSH.[30,31] Deficiency of riboflavin, the precursor of FAD, is believed to enhance cataract formation by depressing GSH activity. Although riboflavin deficiency is fairly common in the geriatric population (33%), original studies demonstrating an association between riboflavin deficiency and cataract formation were followed by studies demonstrating no such association. The patient's riboflavin status can

be determined by measuring GSH activity in red blood cells before and after stimulation with FAD.[31]

Although correction of the deficiency is warranted, no more than 10 mg/day of riboflavin should be prescribed for patients with cataracts because it is a photosensitizing substance—superoxide radicals are generated by the interaction of light, ambient oxygen, and riboflavin/FAD. Riboflavin and light (at physiological levels) have been used experimentally to induce cataracts. The evidence appears to suggest that excess riboflavin does more harm than good in patients with cataracts.

The Age-Related Eye Disease Study (AREDS) evaluated whether the dietary intake of B vitamins is associated with cataract prevalence and incidence.[32] At baseline, increased dietary riboflavin and B_{12} were inversely associated with nuclear and cortical lens opacities. In comparisons of individuals with and without cataract, those with the highest riboflavin intake versus those with the lowest intake had the following associations: mild nuclear cataract—odds ratio (OR), 0.78; 95% confidence interval (CI), 0.63 to 0.97; moderate nuclear cataract—OR, 0.62; 95% CI, 0.43 to 0.90; and mild cortical cataract—OR, 0.80; 95% CI, 0.65 to 0.99. For B_{12}, the results were as follows: mild nuclear cataract—OR, 0.78; 95% CI, 0.63 to 0.96; moderate nuclear cataract—OR, 0.62; 95% CI, 0.43 to 0.88; and mild cortical cataract—OR, 0.77; 95% CI, 0.63 to 0.95. The highest dietary B_6 intake was associated with a decreased risk of the development of moderate nuclear lens opacity compared with the lowest quintile (OR, 0.67; 95% CI, 0.45 to 0.99). The highest dietary intake levels of niacin and B_{12} were associated with a decreased risk of the development of mild nuclear or mild cortical cataracts in participants not taking multivitamins.

Amino Acids

Methionine is a component of the lenticular antioxidant enzyme methionine sulfoxide reductase and a precursor of cysteine, a component of GSH. Cysteine, along with the other amino acid precursors of GSH, has been shown to be of some aid in cataract treatment.[33]

Zinc, Vitamin A, and Beta-Carotene

The antioxidants zinc, vitamin A, and beta-carotene are known to be essential to normal epithelial integrity. Adequate amounts of these nutrients are vitally important to the health of the epithelial portion of the lens. In particular, beta-carotene may act as a filter, protecting against light-induced damage to the fibrous portion of the lens. Beta-carotene is the most significant of the singlet oxygen free-radical scavengers and is used in treating photosensitive disorders.[34] However, in long-term studies, beta-carotene supplementation (50 mg on alternate days) on its own has been found to have no impact on cataract prevention in either women or men.[35,36]

Melatonin

Melatonin is a very efficient free-radical scavenger and antioxidant that can neutralize hydroxyl and peroxyl radicals as well as enhance endogenous and exogenous antioxidant efficiency. In animal models, melatonin has been an effective inhibitor of DNA damage, lipid peroxidation, and cataract formation. Melatonin is present at significant levels in the cell nucleus, aqueous cytosol, and lipid-rich cellular membranes.[37]

Multivitamin/Mineral

A systematic review and meta-analysis were conducted to evaluate the effectiveness of multivitamin/mineral supplements for decreasing the risk of age-related cataracts.[38] Twelve prospective cohort studies and two randomized controlled trials (RCTs) were included. Pooled results from the cohort studies indicated that multivitamin/mineral supplements have a significant beneficial effect in decreasing the risk of nuclear cataracts (RR: 0.73; 95% CI: 0.64–0.82), cortical cataracts (RR: 0.81; 95% CI: 0.68–0.94), and any cataracts (RR: 0.66; 95% CI: 0.39–0.93). In addition, there were no decreases in the risk of posterior capsular cataracts (RR: 0.96; 95% CI: 0.72–1.20) or cataract surgery (RR: 1.00; 95% CI: 0.92–1.08). The two RCTs demonstrated that multivitamin/mineral supplements could decrease the risk of nuclear cataracts.

Curcumin

Curcumin has been widely exploited due to its anti-inflammatory and antioxidant properties. Research indicates curcumin has several anti-cataract mechanisms. Curcumin inhibits lens membrane lipid peroxidation, supplements as well as induces the expression of antioxidant enzymes, maintains lens calcium homeostasis, aids lens chaperone proteins, regulates transcription factors, and modulates the levels of enzyme systems in the lens that are associated with disease conditions leading to cataract (Fig. 219.4).[39]

Lanosterol

Lanosterol is an amphipathic molecule enriched in the lens synthesized by lanosterol synthase. The genetic roots of severe cataracts have been traced to a mutation of the gene that encodes lanosterol synthase. Treating cells that express disordered crystallin proteins with lanosterol significantly decreased preformed protein aggregates in vitro and in cell-transfection experiments, resulting in solubilization of the cataracts.[40] A nanoparticle preparation of lanosterol when applied as eye drops to one eye of a dog with bilateral cataracts for 6 weeks led to a marked dissolution of that cataract, suggesting that dietary-derived small molecules may not only reduce the risk of lens cataracts but may even be used in the therapeutic reversal of cataracts. Human studies are needed to verify this encouraging potential treatment.

Dairy Products

Cataracts often develop in infants with a homozygous deficiency of either galactokinase or galactose-1-phosphate uridyl transferase and in laboratory animals fed a high-galactose diet. Abnormalities of galactose metabolism can be identified by measurements of the activity of these enzymes in red blood cells. It has been suggested that such abnormalities are an important mechanism in approximately 30% of cataracts.[31] However, this mechanism of cataract formation appears to be significant only in diabetic cataract formation and is probably not relevant to senile cataract formation (for further discussion, see Chapter 165).

Toxic Metals

Several toxic metals have been shown to have higher concentrations in both the aging lens and the cataractous lens. Although the levels are higher in the latter, the significance of this finding is unknown.[28]

The cadmium concentration is two to three times higher in cataractous lenses than in lenses of age-matched controls. Because cadmium displaces zinc from binding in enzymatic proteins by binding to the sulfhydryl groups, it may contribute to deactivation of free-radical quenching and other protective/repair mechanisms.

Cadmium is a common component of cigarette smoke, which, along with free-radical formation, may be the reason cigarette smoking is associated with lens opacities. Moreover, levels of nitrite, a stable metabolite of nitric oxide, are markedly increased by cigarette smoking and have been found in higher concentration in human cataractous lenses.[41]

Other elevated elements of unknown significance are bromine, cobalt, iridium, and nickel.[28]

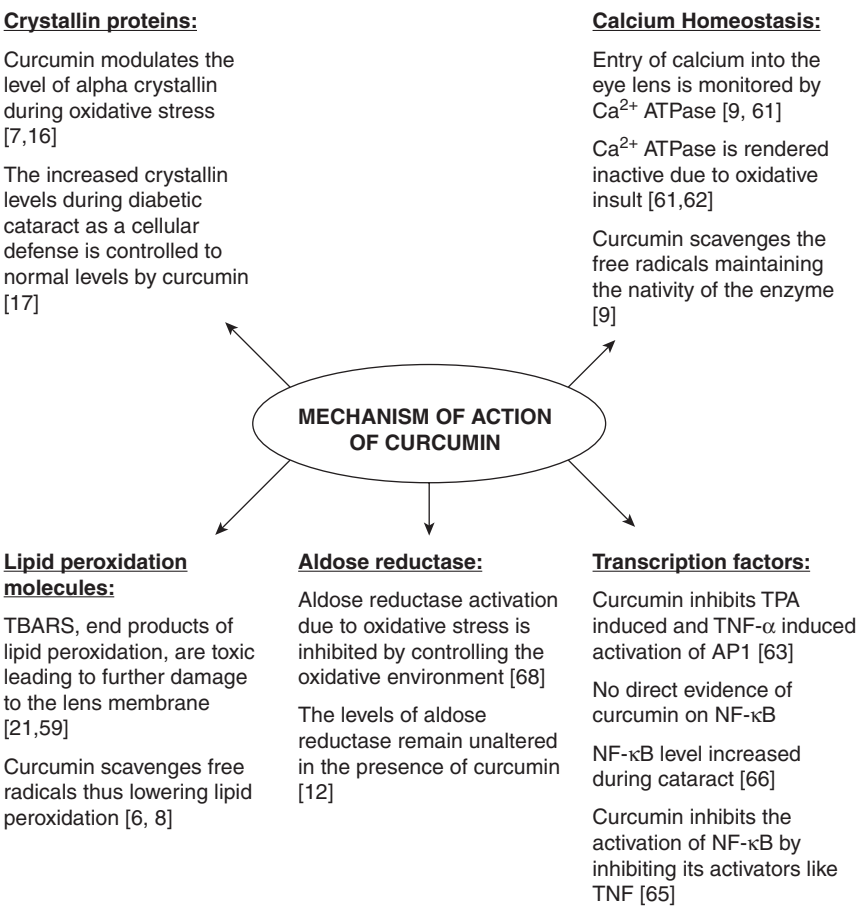


Fig. 219.4 Mechanisms by which curcumin protects the eye from cataract formation. (From Raman T, Ramar M, Arumugam M, Nabavi SM, Varsha MK. Cytoprotective mechanism of action of curcumin against cataract. *Pharmacol. Rep.* 2016;68[3]:561–569.)

Botanical Medicines

Flavonoid-Rich Extracts

Several excellent choices from the botanical world are available to help with antioxidant mechanisms. Among the best may be flavonoid-rich extracts from *Vaccinium myrtillus* (bilberry), *Vitis vinifera* (grape seed), and *Pinus maritima* (pine bark). The occurrence of cataracts in rats can be retarded by changing their diet from a commercial laboratory chow to a "well-defined diet."[42] Preliminary research suggests that flavonoid components in the well-defined diets may be responsible for the protective effects.[43]

Of the flavonoid-rich extracts, bilberry anthocyanosides may offer the greatest protection. In one human study, bilberry extract plus vitamin E stopped the progression of cataract formation in 97% of 50 patients with senile cortical cataracts.[44]

Hachimijiogan

An ancient Chinese herbal formula, Hachimijiogan, has been shown to raise the antioxidant level of the lens of the eye.[45] This activity may explain its use in the treatment of cataracts for hundreds of years. According to clinical research, its therapeutic effect is quite impressive in the early stages of cataract formation. In one study, 60% of the subjects receiving Hachimijiogan noted significant improvement, 20% of the group showed no progression, and only the remaining 20% displayed progression. Hachimijiogan contains the following eight herbs (per 24 g):

- *Rehmania glutinosa:* 6000 mg
- *Poria cocos sclerotium:* 3000 mg
- *Dioscorea opposita:* 3000 mg
- *Cormus officinalis:* 3000 mg
- *Epimedium grandiflorum:* 3000 mg
- *Alisma plantago:* 3000 mg
- *Astragalus membranaceus:* 2000 mg
- *Cinnamonum cassia:* 1000 mg

THERAPEUTIC APPROACH

In cases of marked visual impairment, cataract removal and lens implantation may be the only alternative. As with most diseases, prevention or treatment at an early stage is most effective. Free-radical damage appears to be the primary factor in the induction of senile cataracts, so avoidance of oxidizing agents and the promotion of free-radical scavenging are critical to successful treatment. The patient should avoid direct ultraviolet light, bright light, and photosensitizing substances; wear protective lenses when outdoors; and greatly increase intake of antioxidant nutrients. Progression of the pathological process can be stopped, and early lesions can be reversed. However, significant reversal of well-developed cataracts does not appear to be possible at this time. Because the geriatric population is especially susceptible to nutrient deficiencies, every effort should be made to ensure that the patient is ingesting and assimilating adequate macronutrients and micronutrients.

Diet

Patients should avoid rancid foods and other sources of free radicals and increase consumption of legumes (high in sulfur-containing amino acids), yellow vegetables (carotenes), and foods rich in vitamins E and C.

Supplements

- High-potency multivitamin/multimineral formula
- Lutein: 5 to 15 mg/day
- Vitamin C: 1 g one to three times a day
- Vitamin E: 600 to 800 IU/day
- Selenium: 400 mcg/day
- L-Cysteine or *N*-acetylcysteine: 400 mg/day
- L-Glutamine: 200 mg/day
- L-Glycine: 200 mg/day

Botanical Medicines

- Bilberry extract (25% anthocyanidin content): 80 mg three times a day
- Hachimijiogan formula: 1000 mg three times a day

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Periyasamy P, Shinohara T. Age-related cataracts: role of unfolded protein response, Ca 2+ mobilization, epigenetic DNA modifications, and loss of Nrf2/Kaep1 dependent cytoprotection. *Prog Retin Eye Res*. 2017;60:1–19. PubMed PMID:28864287.
2. Bouton S. Vitamin C and the aging eye. *Arch Intern Med*. 1939;63:930–945.
3. Ringvold A, Johnsen H, Blika S. Senile cataract and ascorbic acid loading. *Acta Ophthalmol (Copenh)*. 1985;63:277–280.
4. Atkinson DT. Malnutrition as an etiological factor in senile cataract. *Eye Ear Nose Throat Mon*. 1952;31:79–83.
5. Rathbun W, Hanson S. Glutathione metabolic pathway as a scavenging system in the lens. *Ophthalmic Res*. 1979;11:172–176.
6. Swanson AA, Truesdale AW. Elemental analysis in normal and cataractous human lens tissue. *Biochem Biophys Res Comm*. 1971;45:1488–1496.
7. Karakucuk S, Ertugrul Mirza G, Faruk Ekinciler O. Selenium concentrations in serum, lens, and aqueous humour of patients with senile cataract. *Arch Ophthalmol Scand*. 1995;73:329–332.
8. Wang X, Sun J, Dang GF, Gao Y, Duan L, Wu XY. Antioxidant content and cytological examination of aqueous fluid from patients with age-related cataracts at different stages. *Genet Mol Res*. 2015;14(2):6251–6255. PubMed PMID:26125826.
9. Taylor A. Cataract: relationships between nutrition and oxidation. *J Am Coll Nutr*. 1993;12:138–146.
10. Taylor A, Jacques PF, Chylack Jr LT, et al. Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. *Am J Clin Nutr*. 2002;75:540–549.
11. Jacques PF, Chylack Jr LT, Hankinson SE, et al. Long-term nutrient intake and early age-related nuclear lens opacities. *Arch Ophthalmol*. 2001;119:1009–1019.
12. Kuzniarz M, Mitchell P, Cumming RG, et al. Use of vitamin supplements and cataract: the Blue Mountains Eye Study. *Am J Ophthalmol*. 2001;132:19–26.
13. Mares-Perlman JA, Lyle BJ, Klein R, et al. Vitamin supplement use and incident cataracts in a population-based study. *Arch Ophthalmol*. 2000;118:1556–1563.
14. Granado F, Olmedilla B, Blanco I. Nutritional and clinical relevance of lutein in human health. *Br J Nutr*. 2003;90:487–502.
15. Hankinson SE, Stampfer MJ, Seddon JM, et al. Nutrient intake and cataract extraction in women: a prospective study. *BMJ*. 1992;305:335–339.
16. Brown L, Rimm EB, Seddon JM, et al. A prospective study of carotenoid intake and risk of cataract extraction in U.S. men. *Am J Clin Nutr*. 1999;70:517–524.
17. Chasan-Taber L, Willett WC, Seddon JM, et al. A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in U.S. women. *Am J Clin Nutr*. 1999;70:509–516.
18. Lyle BJ, Mares-Perlman JA, Klein BE, et al. Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. *Am J Epidemiol*. 1999;149:801–809.
19. Olmedilla B, Granado F, Blanco I, et al. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. *Nutrition*. 2003;19:21–24.
20. Hayashi R, Hayashi S, Arai K, Sakai M, Okamoto H, Chikuda M. The gender-differentiated antioxidant effects of a lutein-containing supplement in the aqueous humor of patients with senile cataracts. *Exp Eye Res*. 2014;129:5–12. PubMed PMID:25305576.
21. Valero MP, Fletcher AE, De Stavola BL, et al. Vitamin C is associated with reduced risk of cataract in a Mediterranean population. *J Nutr*. 2002;132:1299–1306.
22. Christen WG, Glynn RJ, Sesso HD, et al. Age-related cataract in a randomized trial of vitamins E and C in men. *Arch Ophthalmol*. 2010;128(11):1397–1405.
23. Wei L, Liang G, Cai C, Lv J. Association of vitamin C with the risk of age-related cataract: a meta-analysis. *Acta Ophthalmologica*. 2016;94(3):e170–e176. PubMed PMID:25735187.
24. McNeil JJ, Robman L, Tikellis G, et al. Vitamin E supplementation and cataract: randomized controlled trial. *Ophthalmology*. 2004;111:75–84.
25. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. *Arch Ophthalmol*. 2001;119:1439–1452. Age-Related Eye Disease Study Research Group.
26. Zhang Y, Jiang W, Xie Z, Wu W, Zhang D. Vitamin E and risk of age-related cataract: a meta-analysis. *Public Health Nutr*. 2015;18(15):2804–2814. PubMed PMID:25591715.
27. Whanger P, Weswig P. Effects of selenium, chromium and antioxidants on growth, eye cataracts, plasma cholesterol and blood glucose in selenium deficient, vitamin E supplemented rats. *Nutr Rep Int*. 1975;12:345–358.
28. Swanson AA, Truesdale AW. Elemental analysis in normal and cataractous human lens tissue. *Biochem Biophys Res Comm*. 1971;45:1488–1496.
29. Rao GN, Cotlier E. The enzymatic activities of GTP cyclohydrolase, sepiapterin reductase, dihydropteridine reductase and dihydrofolate reductase; and tetrahydrobiopterin content in mammalian ocular tissues and in human senile cataracts. *Comp Biochem Physiol B*. 1985;80B:61–66.
30. Skalka H, Prchal J. Cataracts and riboflavin deficiency. *Am J Clin Nutr*. 1981;34:861–863.
31. Prchal JT, Conrad ME, Skalka HW. Association of pre-senile cataracts with heterozygosity for galactosemic states and riboflavin deficiency. *Lancet*. 1978;1:12–13.
32. Glaser TS, Doss LE, Shih G, et al. The association of dietary lutein plus zeaxanthin and B vitamins with cataracts in the Age-Related Eye Disease Study: AREDS report No. 37. *Ophthalmology*. 2015;122(7):1471–1479. PubMed PMID:25972257.
33. Rathbun WB. Influence on lenticular glutathione research. *Ophthalmic Res*. 1995;27(suppl 1):13–17.
34. Burton GW, Ingold KU. Beta-carotene: an unusual type of lipid antioxidant. *Science*. 1984;224:569–573.
35. Christen W, Glynn R, Sperduto R, et al. Age-related cataract in a randomized trial of beta-carotene in women. *Ophthalmic Epidemiol*. 2004;11(5):401–412.
36. Christen WG, Manson JE, Glynn RJ, et al. A randomized trial of beta carotene and age-related cataract in U.S. physicians. *Arch Ophthalmol*. 2003;121(3):372–378.
37. Reiter RJ. Oxygen radical detoxification processes during aging: the functional importance of melatonin. *Aging (Milano)*. 1995;7:340–351.
38. Zhao LQ, Li LM, Zhu H. The Epidemiological Evidence-Based Eye Disease Study Research Group EY. The effect of multivitamin/mineral supplements on age-related cataracts: a systematic review and meta-analysis. *Nutrients*. 2014;6(3):931–949. PubMed PMID:24590236.
39. Raman T, Ramar M, Arumugam M, Nabavi SM, Varsha MK. Cytoprotective mechanism of action of curcumin against cataract. *Pharmacol Rep*. 2016;68(3):561–569. PubMed PMID:26894964.
40. Zhao L, Chen XJ, Zhu J, et al. Lanosterol reverses protein aggregation in cataracts. *Nature*. 2015;523(7562):607–611. PubMed PMID:26200341.
41. Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Folic acid, vitamin B6, and vitamin B12 in combination and age-related cataract in a randomized trial of women. *Ophthalmic Epidemiol*. 2016;23(1):32–39. PubMed PMID:26786311.
42. Hess HH, Knapka JJ, Newsome DA, et al. Dietary prevention of cataracts in the pink-eyed RCS rat. *Lab Anim Sci*. 1985;35:47–53.
43. Pautler EL, Maga JA, TKengerdy C. A pharmacologically potent natural product in the bovine retina. *Exp Eye Res*. 1986;42:85–88.
44. Bravetti G. Preventive medical treatment of senile cataract with vitamin E and anthocyanosides: clinical evaluation. *Ann Ottalmol Clin Ocul*. 1989;115:109.
45. Yoshida H, Kusukawa R, Watanabe N, et al. The effects of Ba-wei-wan (Hachimijiogan) on plasma levels of high density lipoprotein-cholesterol and lipoperoxide in aged individuals. *Am J Clin Med*. 1985;13:71–76.

220

Streptococcal Pharyngitis

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Abrupt onset of sore throat, fever, malaise, nausea, and headache
- Throat red and edematous, with or without exudate
- Tender cervical lymph nodes
- Positive rapid detection of streptococcal antigen
- Group A streptococci on throat culture

GENERAL CONSIDERATIONS

The signs and symptoms of streptococcal pharyngitis ("strep throat") resemble those of viral pharyngitis. In clinical settings, approximately 10% of children swabbed with a sore throat have serologically confirmed group A beta-hemolytic streptococci (GABHS). However, this increases to 50% to 60% when the child is GABHS culture–positive. Throat cultures yield GABHS in less than 20% of adult patients presenting clinically with a sore throat. However, it must be kept in mind that 10% to 25% of the general, asymptomatic population is a carrier for group A streptococci. Therefore, the actual number of cases of pharyngitis due to GABHS is probably lower than reported. It should be noted that if the prevalence of GABHS detection approaches the asymptomatic carriage rate, there may be little benefit from antibiotic treatment because the majority of culture-positive patients are likely carriers.

Rapid "strep" screens that detect the presence of group A streptococcal antigens are a major clinical advancement. Because definitive diagnosis with a positive culture usually takes 2 days, antibiotic therapy during this period for presumed group A strep throat leads to unnecessary exposure to antibiotics and a greater likelihood of the development of antibiotic-resistant organisms. Rapid strep screens, such as the Strep A OIA test, have now shown excellent sensitivity and specificity and will soon replace throat culture as the diagnostic gold standard.[1] In addition, the use of rapid strep screens may eliminate the unnecessary use of antibiotics (Fig. 220.1). That said, these tests remain underutilized. One analysis found that rapid strep screens were performed in only 53% of cases of acute pharyngitis where an antibiotic was prescribed.[2] Many physicians continue to rely on antibiotics as a precaution against the sequelae of streptococcal pharyngitis even in the absence of a positive diagnosis, resulting in unnecessary prescriptions for antibiotics. Even in positive cases, antibiotics may not be necessary, because strep throat is usually a self-limited disease, and most research has shown that clinical recovery is similar in cases where antibiotics are prescribed and those where they are not.[3–5]

The primary concern surrounding the nonuse of antibiotics is the development of "nonsuppurative poststreptococcal syndromes" (rheumatic fever, poststreptococcal glomerulonephritis, etc.). However, antibiotic administration does not significantly reduce the incidence of these sequelae. In developed countries, most cases of rheumatic fever and glomerulonephritis due to group A beta-hemolytic strep throat occur because the affected persons do not consult a physician.[6] Although the dogma holds that acute rheumatic fever can be caused only by group A streptococcal infection of the upper respiratory tract, epidemiology indicates that streptococcal pyoderma is a major cause in the Aboriginal people of Northern Australia and perhaps other high-incidence communities. In contrast, in settings where rheumatic fever has become rare, the group A streptococcal strains causing pharyngitis are of relatively low virulence regarding the development of rheumatic fever.[6]

The use of antibiotics should be reserved for those patients who are suffering from severe infection or whose sore throats are unresponsive to therapy (i.e., no response after 1 week of immune-supportive therapy) and in those with a prior history of rheumatic fever or glomerulonephritis. Penicillin, amoxicillin, erythromycin, and first-generation cephalosporins are the recommended antibiotics for the treatment of sore throat due to GABHS. Amoxicillin tends to be the most common prescription given to children, primarily for compliance reasons. Macrolides, such as erythromycin and cephalosporins, have been recommended as the best first-line antibiotics because penicillin fails to eradicate the streptococci in more than 20% of patients.[7] The presence of beta-lactamase–positive organisms (*Staphylococcus aureus* and *Bacteroides* species) shield streptococci by deactivating penicillin. In these instances, stronger antibiotics, such as a cephalosporin, may be required.[8] Nonetheless, because of the low cost and absence of resistance, penicillin is still regarded as an acceptable first-choice treatment for both adults and children.[9]

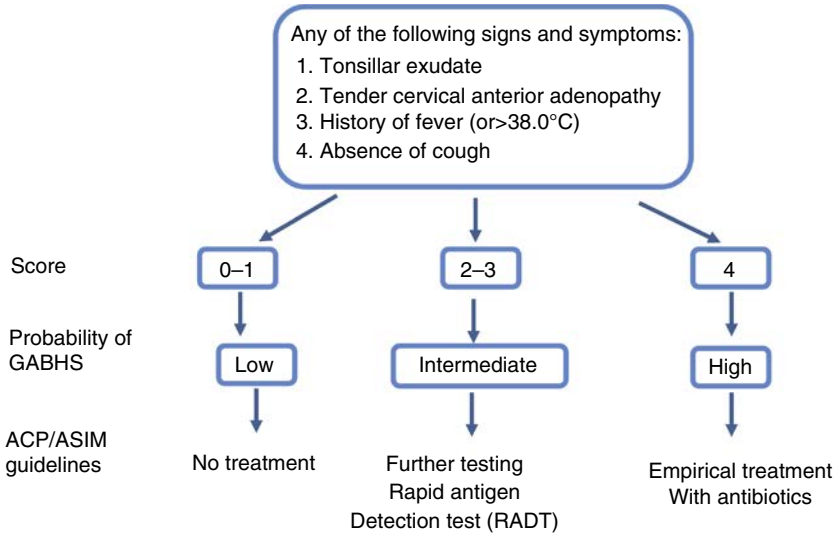


Fig. 220.1 Flow chart for management of pharyngitis. (Wallace E, Smith SM, Perera-Salazar R, et al. Framework for the impact analysis and implementation of Clinical Prediction Rules (CPRs). BMC Med Inform Decis Mak. 2011;11:62. Published 2011 Oct 14. PubMed PMID: 21999201.)

Antibiotics are often praised for their role in effectively eliminating rheumatic fever as a serious concern. However, the dramatic decrease in the incidence of rheumatic fever began before the advent of effective antibiotics.[10] Similar to the eradication of most infectious diseases, improvements in socioeconomic, hygienic, and nutritional conditions have contributed significantly more than the liberal use of antibiotics. The present attack rates after a streptococcal infection are 0.4% to 2.8% for rheumatic fever and 0.2% to 20% for glomerulonephritis. Such a wide range of reported sequelae makes accurate evaluation of the risk difficult.

THERAPEUTIC CONSIDERATIONS

The primary therapeutic consideration is the status of the patient's immune system. If the patient's immune system is functioning well, the illness will be short-lived. Enhancing general immune function, as described in Chapter 136, may shorten the course. In cases of poor immune function, every effort should be made to strengthen the immune system by following the recommendations given in that chapter.

Vitamin C

During the 1930s there was considerable interest in the relationship between malnutrition and the development of the sequelae of streptococcal pharyngitis. Both experimental animal work and epidemiological surveys demonstrated a correlation between vitamin C deficiency and the development of sequelae. Rheumatic fever is virtually nonexistent in the tropics, where vitamin C intake is higher. Elsewhere, as many as 18% of children in high-risk groups have subnormal serum levels of vitamin C.

When streptococcus-infected, vitamin C–deficient, rheumatic fever–susceptible guinea pigs are supplemented with vitamin C, the development of rheumatic fever is totally prevented.[11,12] Uncontrolled clinical studies have demonstrated very positive results when children were given orange juice supplementation. Unfortunately, this promising line of research appears to have been dropped, probably owing to the advent of antibiotics.

Hydrastis canadensis and *Echinacea* Species

Hydrastis canadensis and *Echinacea* species are well respected as supports for the immune system during GABHS infections. The berberine alkaloid of *Hydrastis* exerts antibiotic activity against streptococci and, perhaps more importantly, has been shown to inhibit the attachment of group A streptococci to pharyngeal epithelial cells. *Echinacea* also exerts action against streptococcal infection. To promote the spread of colonies, streptococci secrete large amounts of hyaluronidase. This enzyme is inhibited by *Echinacea* as well as by many bioflavonoids. *Echinacea* also inactivates group A streptococci and reduces the proinflammatory response to strep infection as well as promoting greater phagocytosis, natural killer cell activity, and properdin levels.[13] See Chapters 75 and 86 for further discussion.

Pelargonium sidoides (South African Geranium)

Extracts from the rhizomes and tubers of *Pelargonium sidoides* have been shown to exert several effects that are beneficial in upper respiratory tract infections, and such extracts are an approved drug in Germany for the treatment of acute bronchitis (see Chapter 155). *P. sidoides* has demonstrated immune-enhancing effects as well as antibacterial effects and the ability to prevent adhesion of bacteria to epithelial cells.[14] In a double-blind study, an extract of *P. sidoides* (EPs 7630) was shown to be superior to placebo for the treatment of non-GABHS tonsillopharyngitis in children.[15] A total of 143 children, aged 6 to 10 years, with non-GABHS tonsillopharyngitis were given either EPs 7630 or placebo (20 drops three times daily) for 6 days. The decrease in the Tonsillopharyngitis Severity Score from baseline (day 0) to day 4 was 7.1 points with EPs 7630 and 2.5 points in the placebo group. Treatment with EPs 7630 reduced the severity of symptoms and shortened the duration of illness by at least 2 days, consistent with the results seen in acute bronchitis. Although this study was in non-GABHS, it does raise the possibility that *P. sidoides* may have benefit in GABHS.

Bacteriotherapy

Colonizing the throat with group A non–beta-hemolytic streptococci may prove to be an effective treatment for recurrent group A beta-hemolytic streptococcal pharyngitis. In a double-blind study, 130 patients with recurrence of group A beta-hemolytic streptococcal pharyngotonsillitis received antibiotic treatment for 10 days, followed by 10 days of spray treatment with either placebo or group A non–beta-hemolytic streptococcal alpha-streptococci.[16] The clinical recurrences (bacteriologically verified) in the alpha-streptococci- and placebo-treated patient groups were 2% and 23%, respectively, in patients given spray for at least 5 days. No side effects were reported.

In another double-blind study, a total of 342 patients with verified GABHS pharyngitis by the rapid strep test and culture received antibiotic treatment for 10 days, followed by 10 days of alpha-streptococcal or placebo spray treatment.[17] Pharyngeal status, throat culture, and adverse events were investigated up to 75 days after treatment. The frequencies of bacteriologically verified clinical recurrence at the last valid visit after 45 to 75 days were 19% and 30%, respectively—a statistically significant difference. Recolonization with alpha-streptococci seemed to hinder late recurrences of GABHS pharyngotonsillitis.

Probiotics

Streptococcus salivarius K12 (BLIS K12) is a probiotic strain producing the bacteriocins salivaricin A2 and salivaricin B, both of which strongly antagonize the growth of *Streptococcus pyogenes*, the most important bacterial cause of pharyngeal infections in humans. It successfully colonizes and exhibits persistence in the oral cavity, is endowed with an excellent safety profile, and reduces the occurrence of streptococcal and viral pharyngitis. A potential basis for this effect has been shown, whereby the administration of BLIS K12 in adults can increase salivary γ-interferon levels without modifying the levels of either interleukin (IL)-1β or tumor necrosis factor-α but considerably reducing IL-8 release.[18]

Several studies have confirmed the benefits of *S. salivarius*. A retrospective analysis of pediatric subjects with nonrecurrent streptococcal infection demonstrated that K12 use decreased the incidence of pharyngo-tonsillitis by about 90%.[19] The role of BLIS K12 was evaluated in the control of streptococcal disease and acute otitis media in children attending the first year of kindergarten.[20] By randomization, 222 enrolled children attending the first year of kindergarten were divided into a treated group ($N = 111$) receiving a daily treatment with BLIS K12 (Bactoblis) for 6 months and a control group ($N = 111$) that was monitored as untreated controls. During the 6-month trial, the incidence of streptococcal pharyngo-tonsillitis, scarlet fever, and acute otitis media was approximately 16%, 9%, and 44%, respectively, in the treated group and 48%, 4%, and 80% in the control group. During the 3-month follow-up, the corresponding rates of infection were 15%, 0%, and 12% in the treated group and 26%, 6%, and 36% in the controls. Prophylactic administration of *S. salivarius* K12 to children with a history of recurrent oral streptococcal disease resulted in a considerable reduction of episodes of both streptococcal and viral infections, reduced the number of days under antibiotic and/or antipyretic therapy, and reduced days of absence from school or work.[21] The benefits to children may also extend to a reduction of nonstreptococcal diseases, including tracheitis, viral pharyngitis, rhinitis, flu, laryngitis, acute otitis media, and enteritis.[22] In adults with a history of recurrent oral streptococcal pathology, prophylactic administration of *S. salivarius* K12 reduced the number of episodes of streptococcal pharyngeal infections and/or tonsillitis by 80%, with an approximately 60% reduction in the incidence of reported pharyngitis in the 6-month period following use of the product.[23]

Lactoferrin

Low concentrations of bovine lactoferrin have significantly hindered the in vitro invasion of cultured epithelial cells by group A streptococci isolated from patients with pharyngitis. The ability of lactoferrin to decrease streptococcal invasion was confirmed in a trial carried out in 12 children with pharyngitis and already scheduled for tonsillectomy. A lower number of intracellular group A streptococci were found in tonsil specimens from children treated for 15 days before tonsillectomy with both oral erythromycin (500 mg three times daily) and lactoferrin gargles (100 mg three times daily) than in those from children treated with erythromycin alone (500 mg three times daily).[24]

THERAPEUTIC APPROACH

For further information, consult Chapter 136. If antibiotics are used, follow the recommendations for supplementation with *Lactobacillus acidophilus* given in Chapter 105.

Supplements

- Vitamin C: 500 mg every 2 hours
- Bioflavonoids: 1000 mg/day
- Zinc: 30 mg/day

Botanical Medicines

Echinacea species

- Dried root (or as tea): 0.5 to 1 g/day
- Freeze-dried plant: 325 to 650 mg/day
- Juice of aerial portion of *Echinacea purpurea* stabilized in 22% ethanol: 2 to 3 mL/day
- Tincture (1:5): 2 to 4 mL (0.5 to 1 tsp)/day
- Fluid extract (1:1): 2 to 4 mL (0.5 to 1 tsp)/day
- Solid (dry powdered) extract (6.5:1 or 3.5% echinacoside): 150 to 300 mg/day

Hydrastis canadensis

The dosage of *H. canadensis* should be based on berberine content. Because there is a wide range of quality in goldenseal preparations, standardized extracts are recommended.

- Dried root or as infusion (tea): 2 to 4 g three times a day
- Tincture (1:5): 6 to 12 mL (1.5 to 3 tsp) three times a day
- Fluid extract (1:1): 2 to 4 mL (0.5 to 1 tsp) three times a day
- Solid (powdered dry) extract (4:1 or 8% to 12% alkaloid content): 250 to 500 mg three times a day

Pelargonium sidoides

Dosage recommendations for EPs 7630 or equivalent preparation:

Adults: 1.5 mL three times a day or a 20-mg tablet three times a day for up to 14 days

Children: age 7 to 12 years, 20 drops (1 mL) three times a day; age 6 years or less, 10 drops (0.5 mL) three times a day

Local Treatment

The patient should gargle with lactoferrin (100 mg) dissolved in water three times a day. An alternative recommendation is a saltwater gargle consisting of 1 tablespoon of salt per 240 mL of warm water.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Corneli HM. Rapid detection and diagnosis of group A streptococcal pharyngitis. *Curr Infect Dis Rep*. 2004;6:181–186.
2. Linder JA, Bates DW, Lee GM, et al. Antibiotic treatment of children with sore throat. *JAMA*. 2005;294(18):2315–2322.
3. Zwart S, Rovers MM, de Melker RA, et al. Penicillin for acute sore throat in children: randomised, double blind trial. *BMJ*. 2003;327:1324. https://doi.org/10.1136/bmj.327.7427.1324.
4. Dagnelie CF, van der Graaf Y, De Melker RA. Do patients with sore throat benefit from penicillin? A randomized double-blind placebo-controlled clinical trial with penicillin V in general practice. *Br J Gen Pract*. 1996;46:589–593.
5. McIsaac WJ, Goel V, Slaughter PM, et al. Reconsidering sore throats. Part I: problems with current clinical practice. *Can Fam Physician*. 1997;43:485–493.
6. McDonald M, Currie BJ, Carapetis JR. Acute rheumatic fever: a chink in the chain that links the heart to the throat? *Lancet Infect Dis*. 2004;4:240–245.
7. Passàli D, Lauriello M, Passàli GC, et al. Group A streptococcus and its antibiotic resistance. *Acta Otorhinolaryngol Ital*. 2007;27(1):27–32.
8. Casey JR, Pichichero ME. Meta-analysis of cephalosporin versus penicillin treatment of group A streptococcal tonsillopharyngitis in children. *Pediatrics*. 2004;113:866–882.
9. van Driel ML, De Sutter AI, Habraken H, Thorning S, Christiaens T. Different antibiotic treatments for group A streptococcal pharyngitis. *Cochrane Database Syst Rev*. 2016;9:CD004406.
10. Quinn RW. Comprehensive review of morbidity and mortality trends for rheumatic fever, streptococcal disease, and scarlet fever: the decline of rheumatic fever. *Rev Infect Dis*. 1989;11:928–953.
11. Rinehart JF. Studies relating vitamin C deficiency to rheumatic fever and rheumatoid arthritis: experimental, clinical, and general considerations. I. Rheumatic fever. *Ann Intern Med*. 1935;9:586–599.
12. Rinehart JF. Studies relating vitamin C deficiency to rheumatic fever and rheumatoid arthritis: experimental, clinical, and general considerations. II. Rheumatoid (atrophic) arthritis. *Ann Intern Med*. 1935;9:671–689.
13. Sharma SM, Anderson M, Schoop SR, et al. Bactericidal and antiinflammatory properties of a standardized Echinacea extract (Echinaforce): dual actions against respiratory bacteria. *Phytomedicine*. 2010;17(8–9):563–568.
14. Brendler T, van Wyk BE. A historical, scientific and commercial perspective on the medicinal use of *Pelargonium sidoides* (Geraniaceae). *J Ethnopharmacol*. 2008;119(3):420–433.
15. Bereznoy VV, Riley DS, Wassmer G, et al. Efficacy of extract of *Pelargonium sidoides* in children with acute non-group A beta-hemolytic streptococcus tonsillopharyngitis: a randomized, double-blind, placebo-controlled trial. *Altern Ther Health Med*. 2003;9(5):68–79.
16. Roos K, Holm SE, Grahn-Hakansson E, et al. Recolonization with selected alpha-streptococci for prophylaxis of recurrent streptococcal pharyngotonsillitis: a randomized placebo-controlled multicenter study. *Scand J Infect Dis*. 1996;28:459–462.
17. Falck G, Grahn-Håkansson E, Holm SE, et al. Tolerance and efficacy of interfering alpha-streptococci in recurrence of streptococcal pharyngotonsillitis: a placebo-controlled study. *Acta Otolaryngol*. 1999;119(8):944–948.
18. Wescombe PA, Hale JD, Heng NC, Tagg JR. Developing oral probiotics from *Streptococcus salivarius*. *Future Microbiol*. 2012;7(12):1355–1371. PubMed PMID: 23231486.
19. Di Pierro F, Risso P, Poggi E, et al. Use of *Streptococcus salivarius* K12 to reduce the incidence of pharyngo-tonsillitis and acute otitis media in children: a retrospective analysis in not-recurrent pediatric subjects. *Minerva Pediatrica*. 2018;70(3):240–245. PubMed PMID: 29322750.
20. Di Pierro F, Colombo M, Giuliani MG, et al. Effect of administration of *Streptococcus salivarius* K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children. *Eur Rev Med Pharmacol*. 2016;20(21):4601–4606. PubMed PMID: 27874935.
21. Di Pierro f, Colombo M, Zanvit A, Risso P, Rottoli AS. Use of *Streptococcus salivarius* K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. *Drug Healthc Patient*. 2014;6:15–20. PubMed PMID: 24600248.
22. Di Pierro F, Colombo M, Zanvit A, Rottoli AS. Positive clinical outcomes derived from using *Streptococcus salivarious* K12 to prevent streptococcal pharyngotonsillitis in children: a pilot investigation. *Drug Healthc Patient*. 2016;8:77–81. PubMed PMID: 27920580.
23. Di Pierro F, Adami T, Rapacioli G, Giardini N, Streitberger C. Clinical evaluation of the oral probiotic *Streptococcus salivarius* K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* in adults. *Expert Opin Biol Ther*. 2013;13(3):339–343. PubMed PMID: 23286823.
24. Ajello M, Greco R, Giansanti F, et al. Anti-invasive activity of bovine lactoferrin towards group A streptococci. *Biochem Cell Biol*. 2002;80:119–124.

Trichomoniasis

Michael T. Murray, ND

OUTLINE

DIAGNOSTIC SUMMARY

- Profuse malodorous white to green discharge from the vagina
- Discharge usually has a pH greater than 4.5, a weak amine odor, and large numbers of white blood cells and trichomonads on wet mount.
- Vulvovaginal pruritus, burning, and/or irritation
- Vulva and introitus usually show erythema.
- Cervix may or may not have a mottled erythema—"strawberry cervix" (less than 5%).
- Dysuria and/or dyspareunia may be present.
- Rule out trichomoniasis in males exhibiting signs of prostatitis, urethritis, or epididymitis.

GENERAL CONSIDERATIONS

Trichomonas vaginalis infection is a common cause of vaginal irritation in women and is the most common nonviral sexually transmitted disease in the world. It is estimated to affect 278 million people each year globally, including over 5 million women in the United States. One in five women in the United States will have trichomoniasis at some time in her life. Trichomonal infections are significant for several reasons, including the following[1,2]:

- Gonorrhea and trichomoniasis are common coexisting infections, with up to 40% of women with trichomoniasis having gonorrhea, and vice versa.
- Trichomoniasis is a common cause (90%) of cervical erosion and therefore may be a factor in malignant transformation.
- Trichomoniasis may complicate interpretation of Papanicolaou smears, increasing the number of false-positive results.
- Trichomoniasis raises the rate of sterility among males and females, in the latter as a result of salpingitis and in the former because of toxic products that decrease the motility of spermatozoa.
- The rate of postpartum fever and discharge is higher in women in whom *T. vaginalis* infection occurs at delivery.
- Neonates infected via the birth canal may manifest serious illness (rare).
- Prostatitis and epididymitis are common in infected males.
- Trichomoniasis increases the transmission and infectivity of human immunodeficiency virus (HIV), such that HIV-seropositive men with concomitant trichomoniasis may have a sixfold higher concentration of HIV RNA in their seminal plasma.
- Infection may confuse and/or complicate other urinary or genital tract problems.

DIAGNOSIS

T. vaginalis is a flagellate 15 to 18 micrometers in length. It is shaped like a turnip, with three to four anterior flagella and one posterior flagellum mounted in an undulating membrane. It is transmitted via sexual intercourse. Although it has been thought that women have been the primary reservoir for *Trichomonas* and men merely the vector, it is generally accepted that men are also reservoirs.[3]

Diagnosis is made from clinical signs and symptoms (see the diagnostic summary), saline wet mount, and culture. Trichomonal cultures (using the Feinberg *Trichomonas* medium) have been advocated to improve diagnostic sensitivity (Fig. 221.1). Although the wet mount is one of the most commonly used and quickest methods to achieve a diagnosis, multiple studies have demonstrated that, compared with culture, the sensitivity of a wet mount ranges from only 45% to 60%. In men, a reliable culture site has not been established, and cultures from urine and seminal samples have consistently afforded a low yield. Among patients with trichomonal vaginitis, the organism can be cultured from the vagina and paraurethral glands in 98%, from the urethra in 82%, and from the endocervix in 13%. *T. vaginalis* is seen in only 56% to 65% of patients on a Papanicolaou smear, thus making the smear an unreliable form of diagnosis. However, data suggest that the positive predictive value of this test is acceptable for a diagnosis of trichomoniasis when it is found incidentally on Papanicolaou smear. A meta-analysis found a sensitivity of 57% and a specificity of 97%.[4]

Rapid point-of-care tests for trichomonal vaginitis are now available; they include the OSOM Trichomonas Rapid Test (Genzyme Diagnostics, Cambridge, MA), an immunochromatographic capillary-flow dipstick technology, and the Affirm VP III

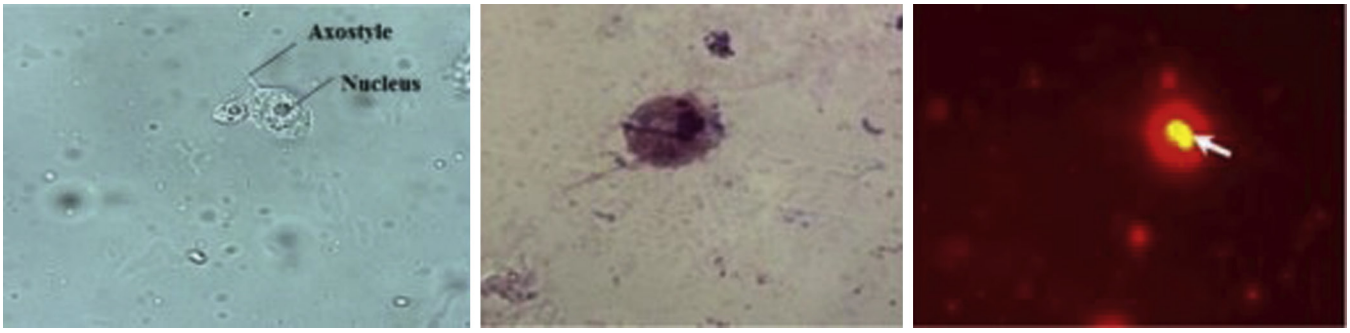


Fig. 221.1 *Trichomonas vaginalis* trophozoite, as shown by wet-mount smear (*left,* X630), Giemsa stain (*middle,* X1000), and Acridine orange (*right,* trophozoite stained brick red with a yellowish nucleus, X400). (From Hussein AH, Saleh MH, Nagaty IM, Ghieth K, El-Azab NA. Prevalence, clinical criteria and sociodemographic predictors of *Trichomonas vaginalis* infection in suspected Egyptian women, using direct diagnostic techniques. *Iran J Parisitol.* 2015:10[3]:432–440. PubMed PMID: 26622298.)

(Becton Dickinson, Franklin Lakes, NJ), a nucleic acid probe test that evaluates for trichomonal vaginitis, *Gardnerella vaginalis,* and *Candida albicans.*[5] Both of these tests are performed on vaginal secretions and have a sensitivity of greater than 83% and a specificity of greater than 97%. The results of the OSOM Trichomonas Rapid Test are available in about 10 minutes, and the results of the Affirm VP III are available within 45 minutes. These tests tend to greatly assist physicians in the accurate and timely diagnosis of trichomoniasis.

Trichomonal Vaginitis

Sexual transmission is the clear route of *Trichomonas* infection. Prevalence is highest among women with multiple sex partners and in those with other sexually transmitted infections. Transmission rates from men to women seem to be high—an 80% to 100% prevalence rate is found in the female partners of infected men.[3] In the female, *T. vaginalis* usually infests the vagina and urethra. However, infection may involve the endocervix, Bartholin glands, Skene glands, or bladder. The vagina appears to be a good reservoir for the organism. Under the stimulation of estrogen, the vaginal walls are well glycogenated—essential for *T. vaginalis* to thrive. Prepubescent and postmenopausal women seldom have symptomatic trichomonal infections.

In addition to estrogen, an elevated pH increases susceptibility to *Trichomonas.* The normal adult vagina maintains a pH of 3.5 to 4.5 owing to the metabolism of free glucose into lactic acid by vaginal *Lactobacillus acidophilus.* A decrease in the number of lactobacilli raises the pH. *Trichomonas* organisms grow optimally at a vaginal pH of 5.5 to 5.8. Other conditions that increase vaginal pH include the following:

- An increase in progesterone, which rises in the latter half of the menstrual cycle and during pregnancy
- Excess intravaginal secretions (i.e., cervical mucus)
- Overgrowth of certain bacteria, such as *Streptococcus* and *Proteus*

Trichomonas in the Male

Although the incidence is lower in men, 5% to 15% of cases of nongonococcal urethritis are estimated to be caused by trichomonal infections.[1,2] The estimated transmission rate is 70% for men who have had sexual contact with infected women in the previous 48 hours.[2] Men with *T. vaginalis* are most often asymptomatic, yet mild cases of urethritis, prostatitis, and epididymitis have been reported. As might be expected, trichomoniasis is a factor in male infertility.[4] Trichomonads have been identified in semen, urethral discharge, urine, and prostatic fluid and have been found in the prostatic secretions and semen in up to 23% of men with chronic nongonococcal prostatitis.[6]

In several independent studies, a history of trichomoniasis, as measured by serum antibodies against *T. vaginalis* α-actinin protein, was found to be associated with increased prostate cancer risk. Preliminary reports indicate that *T. vaginalis* adherence or binding of specific trichomonad adhesin proteins to normal prostate epithelial cells triggers a cell-signaling cascade through known proto-oncogenes, PIM1, c-MYC, and HMGA1, which may ultimately lead to prostate carcinogenesis (Fig. 221.2).[7]

Although men were thought to be only vectors for *Trichomonas,* the parasite is now known to persist in the male reproductive tract. The reinfection of treated females who are sexually active is well documented.[4] Therefore treatment of both sex partners is necessary. Furthermore, among both women and men, the association of *T. vaginalis* with enhanced HIV acquisition and transmission has been well documented.[2]

THERAPEUTIC CONSIDERATIONS

Conventional Treatment

Conventional therapy of trichomoniasis involves metronidazole and tinidazole, a second-generation nitroimidazole used to treat metronidazole-resistant infection.[6] Randomized controlled trials comparing tinidazole (2-g single oral dose) and either metronidazole (2-g single oral dose) or short-course metronidazole have demonstrated parasitological cure rates of 86% to 100% for all treatments, although tinidazole is slightly more effective but also more expensive. In a Cochrane Database meta-analysis of randomized trials comparing short-course therapy with tinidazole and short-course therapy with metronidazole for trichomoniasis, metronidazole had significantly higher rates of parasitological failure, clinical failure, and adverse effects.[8] Again, in order to reduce recurrence rates, sex partners should be treated at the same time.

Common side effects of metronidazole and tinidazole are nausea, vomiting, metallic taste, and gastrointestinal upset. With long-term high-dose use, rare occurrences of peripheral neuropathy have been reported.

If metronidazole or tinidazole treatment is chosen, probiotic supplementation should also be included. In the treatment of bacterial vaginosis, the administration of vaginal insertion with *L. acidophilus* has led to cure rates superior to those with metronidazole.[9,10] This suggests some benefit in vaginal trichomoniasis, given the frequent disruption of proper vaginal flora in these women.

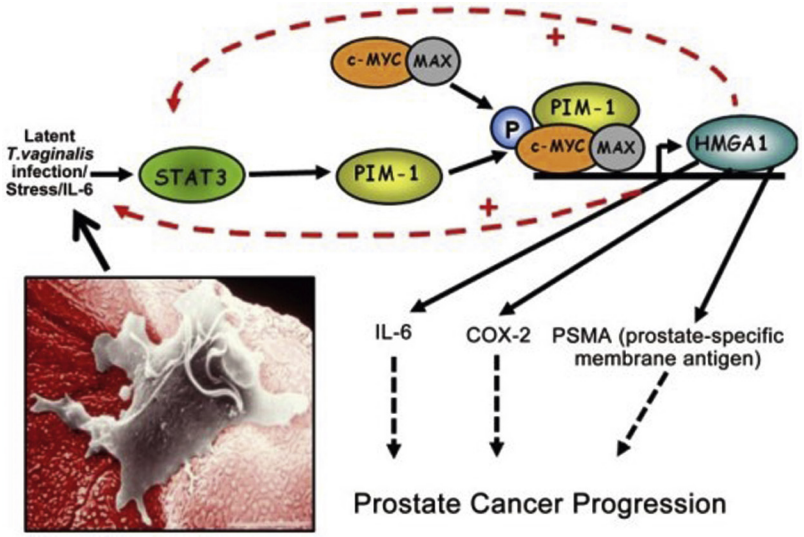


Fig. 221.2 A working model of how chronic, latent *Trichomonas vaginalis* infection of prostate tissue up-regulates the signaling cascade leading to prostate carcinogenesis. (From Sutcliffe S, Neace C. Magnuson NS, Reeves R, Alderete JF. Trichomoniasis, a common curable STI, and prostate carcinogenesis—a proposed molecular mechanism. *PLoS Pathogens.* 2012;8[8]:e1002801. PubMed PMID: 22912571.)

Diet

Dietary factors affect the body's ability to defend itself against foreign invaders and substances both directly and indirectly. As with any infection, it is not the pathogenicity of the organism but rather the "fertility of the soil" that allows the organism to grow and flourish. A well-balanced diet high in natural fiber (vegetable, fruits) and low in fat, sugar, and refined carbohydrates aids immune function (see Chapter 136 for more discussion) and may discourage any concomitant overgrowth of *Candida.*

Lifestyle

Depression and anxiety have been associated with exacerbations of trichomonal infections. Therefore efforts to reduce stress are indicated. Reduction may be achieved by a variety of means, including exercise and meditation.

The practice of safe sex or abstinence (during infection) also lowers the incidence of infection and reinfection.

Nutritional Supplements

In addition to the basic supplements for immune support described in Chapter 136, zinc supplementation appears to be an important consideration in the treatment of trichomonal infections in both men and women. The antimicrobial spectrum of zinc is broad and comprises many potential genitourinary pathogens, including *T. vaginalis* as well as *C. albicans* and *Chlamydia trachomatis* and many viruses.[11] Trichomonads are readily killed by zinc at a concentration of 0.042% (6.4 mmol/L), a concentration that can occur in the prostatic fluid of men. The zinc concentration of prostatic fluid ranges from 0.015% to 0.10% (2.3–15.3 mmol/L).[11] This finding suggests that persistent trichomonal infections in men may be due to a low-level zinc deficiency. Zinc sulfate (220 mg twice daily for 3 weeks) has been recommended as a possible treatment for trichomonal infections that are refractory to metronidazole.[12]

For women with drug-resistant trichomoniasis, zinc douches in combination with metronidazole may provide welcome relief. In a small study, the women with recalcitrant trichomoniasis (4 months to 4 years culture-positive despite conventional treatment) all became culture-negative using a combination of 1% zinc sulfate douching (for 3 days after each menstrual period) and 1.6 to 2.2 g/day of metronidazole (suppositories plus oral administration).[13]

Topical Trichomonacides

Povidone-Iodine

Iodine has long been recognized as a highly potent trichomonacide. Povidone-iodine (PVP) has a broad therapeutic effect in killing many different microorganisms that cause vaginitis, including *T. vaginalis.*[14,15] PVP (iodine, which is absorbed into polyvinyl pyrrolidine) has several advantages over iodine in that it has little sensitizing potential, does not sting, is water-soluble, and washes out of clothing.

A success rate of 98.1% has been reported in patients with intractable trichomonal, monilial, nonspecific, and mixed vaginitis for a 2-week treatment regimen using PVP (Betadine) preparations.[16,17] Other studies suggest a 28-day course of povidone-iodine pessaries, particularly if the patient is using oral contraceptives.[18]

Propolis

An ethanol extract of propolis (150 mg/mL) has been shown to have a 100% lethal effect in vitro on the protozoans *T. vaginalis* and *Toxoplasma gondii* after 24 hours of contact.[19] This extract has also been shown to diminish the inflammation associated with trichomonal vaginitis.

Essential Oils

The diverse antimicrobial action of essential oils has been well demonstrated. Many possess strong antitrichomonal properties. In a study of 40 essential oils tested for their ability to kill *Trichomonas, Mentha piperita* (peppermint) and *Lavandula angustifolia* (lavender) had the fastest killing effects (20 and 15 minutes, respectively).[20]

Melaleuca alternifolia

M. alternifolia (tea tree) oil is a powerful antimicrobial agent (see Chapter 91). Commonly used as a germicidal agent in Great Britain and Australia, a 40% solution of tea tree oil has been found to be a highly effective treatment.[21] The 40% solution of the oil produced no

irritation, burning, or other side effects. Daily vaginal douches with a 0.4% solution of *Melaleuca* oil in 1 L of water was also found to be effective.[22]

Berberine-Containing Botanicals

The plant alkaloid berberine sulfate has been shown in vitro to inhibit the growth of several protozoa, including *Entamoeba histolytica, Giardia lamblia,* and *T. vaginalis.*[23,24] No clinical trials of this agent have been reported in trichomonas vaginalis (see Chapter 86 for further discussion).

THERAPEUTIC APPROACH

Given the risk of serious sequelae of trichomoniasis and the high success rate of conventional pharmaceutical intervention, systemic metronidazole should be carefully considered as a possible first-line treatment, along with simultaneous and subsequent naturopathic therapies to decrease the risk of future recurrence and to treat some of the contributing underlying susceptibilities. Naturopathic therapies may be used as first-line therapy in patients who are allergic to metronidazole or are pregnant. The use of metronidazole in pregnancy remains somewhat controversial in spite of its apparent lack of teratogenic action. Despite the role of *T. vaginalis* in perinatal morbidity, metronidazole treatment may increase the risk of preterm birth.[25,26] Discussion with the patient of such factors as diet, sexual habits, and lifestyle is recommended. The clinician should inform the patient that *Trichomonas* infection is a sexually transmitted disease and that treatment of the sex partners is necessary to prevent reinfection. During the treatment, sexual intercourse should be avoided. If intercourse does occur, a condom must be used.

Diet

The patient should decrease the consumption of refined carbohydrates, alcohol, and fats and increase the intake of fiber.

Nutritional Supplements

- Zinc (picolinate): 10 to 15 mg/day
- Vitamin E (mixed tocopherols: 400 IU/day
- *L. acidophilus:* 5 to 10 billion live bacteria twice a day orally as well as the intravaginal application in vaginal trichomoniasis. See Chapter 105 for further discussion.

Topical Treatments

- Betadine douche, pessary, or saturated tampon: twice a day for 14 days
- *M. alternifolia* oil (40% solution): swabbed on affected area two times/day *or* used as a douche, 1 L of a 0.4% solution twice a day or a suppository, one at night (always check for sensitivity first!)
- Zinc sulfate douche: 1% solution twice a day
- *Lactobacillus* insertions or douches once a day, preferably in the morning

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Menezes CB, Frasson AP, Tasca T. Trichomoniasis—are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide? *Microb Cell*. 2016;3(9):404–419. https://doi.org/10.15698/mic2016.09.526. Review.
2. van de Wijgert JHHM, Jespers V. The global health impact of vaginal dysbiosis. *Res Microbiol*. 2017;168(9-10):859–864.
3. Saperstein AK, Firnhaber GC. Clinical inquiries: should you test or treat partners of patients with gonorrhea, chlamydia, or trichomoniasis? *J Fam Pract*. 2010;59(1):46–468.
4. Wiese W, Patel SR, Patel SC, et al. A meta-analysis of the Papanicolaou smear and wet mount for the diagnosis of vaginal trichomoniasis. *Am J Med*. 2000;108:301–308.
5. Gaydos CA, Klausner JD, Pai NP, et al. Rapid and point-of-care tests for the diagnosis of Trichomonas vaginalis in women and men. *Sex Transm Infect*. 2017;93(S4):S31–S35.
6. Meites E, Gaydos CA, Hobbs MM. A Review of evidence-based care of symptomatic trichomoniasis and asymptomatic trichomonas vaginalis infections. *Clin Infect Dis*. 2015;61(suppl 8):S837–S848. https://doi.org/10.1093/cid/civ738.
7. Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF. Trichomoniasis, a common curable STI, and prostate carcinogenesis—a proposed molecular mechanism. *PLoS Pathogens*. 2012;8(8):e1002801. PubMed PMID:22912571.
8. Forna F, Gulmezoglu AM. Interventions for treating trichomoniasis in women. *Cochrane Database Syst Rev*. 2003;2:218.
9. Anukam KC, Osazuwa E, Osemene GI, et al. Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes Infect*. 2006;8(12-13):2772–2776.
10. Marcone V, Calzolari E, Bertini M. Effectiveness of vaginal administration of Lactobacillus rhamnosus following conventional metronidazole therapy: how to lower the rate of bacterial vaginosis recurrences. *New Microbiol*. 2008;31(3):429–433.
11. Krieger JN, Rein MF. Zinc sensitivity of *Trichomonas vaginalis:* in vitro studies and clinical implications. *J Inf Disease*. 1982;146:341–345.
12. Willmott F, Say J, Downey D, et al. Zinc and recalcitrant trichomoniasis. *Lancet*. 1983;1:1053.
13. Houang ET, Ahmet Z, Lawrence AG. Successful treatment of four patients with recalcitrant vaginal trichomoniasis with combination of zinc sulfate douche with metronidazole therapy. *Sex Transm Dis*. 1997;24:116–119.
14. Gershenfeld L. Povidone-iodine (PVP-I) as a trichomonacide. *Am J Pharm Sci Support Public Health*. 1962;134:324–331.
15. Gershenfeld L. Povidone-iodine as a topical antiseptic. *Am J Surg*. 1957;94:938–939.
16. Shook DM. Clinical study of povidone-iodine regimen for resistant vaginitis. *Curr Ther Res Clin Exp*. 1963;5:256–263.
17. Mayhew SR. Vaginitis: a study of the efficacy of povidone-iodine in unselected cases. *J Int Med Res*. 1981;9:157–159.
18. Henderson JN, Tait IB. The use of povidone-iodine ("Betadine") pessaries in treatment of candidal and trichomonal vaginitis. *Curr Med Res Opinion*. 1975;3:157–162.
19. Starzyk J, Scheller S, Szaflarski J, et al. Biological properties and clinical application of propolis. II. studies on the antiprotozoan activity of ethanol extract of propolis. *Arzneimittelforschung*. 1977;27:1198–1199.
20. Jankov N, Baltova E, Topalov V, et al. Action of some essential oils on *Trichomonas vaginalis*. *Folia Med (Plodiv).* 1968;10:308.
21. Humphrey EM. New Australian germicide. *Med J Aus*. 1930;1:417.
22. Pena EF. Melaleuca alternifolia oil: its use for trichomonal vaginalis and other vaginal infections. *Obstet Gynecol*. 1962;19:793–795.
23. Kaneda Y, Torii M, Tanaka T, et al. In vitro effects of berberine sulphate on the growth and structure of *Entamoeba histolytica, Giardia lamblia* and *Trichomonas vaginalis*. *Ann Trop Med Parasitol*. 1991;85:417–425.
24. Kaneda Y, Tanaka T, Saw T. Effects of berberine, a plant alkaloid, on the growth of anaerobic protozoa in axenic culture. *Tokai J Exp Clin Med*. 1990;15:417–423.
25. Klebanoff MA, Carey JC, Hauth JC, et al. Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. *N Engl J Med*. 2001;345:487–493.
26. Kigozi GG, Brahmbhatt H, Wabwire-Mangen F, et al. Treatment of trichomonas in pregnancy and adverse outcomes of pregnancy: a subanalysis of a randomized trial in Rakai, Uganda. *Am J Obstet Gynecol*. 2003;189:1398–1400.

Urticaria

Michael Traub, ND, DHANP, FABNO

OUTLINE

DIAGNOSTIC SUMMARY

- Urticaria (hives): Well-circumscribed pruritic erythematous wheals with raised serpiginous borders and blanched centers that may coalesce to become giant wheals; limited to the superficial portion of the dermis.
- Angioedema: Eruptions similar to those of urticaria but with larger, well-demarcated edematous areas that involve subcutaneous structures as well as the dermis and may provoke a burning sensation.
- Chronic versus acute: Recurrent episodes of urticaria and/or angioedema of less than 6 weeks' duration are considered acute, whereas attacks persisting beyond this period are designated chronic and may persist for several months or years.[1]
- Special forms: Special forms have characteristic features—dermographism (also known as dermatographism), cholinergic urticaria, solar urticaria, cold urticaria.

INTRODUCTION

Urticaria is a skin disorder manifesting in wheals in 30% to 40%, angioedema in 10% to 20%, or both in one third of cases. Urticaria is defined as "inducible" when wheals are induced by a known trigger and as "chronic spontaneous urticaria" (CSU) when the trigger is unidentified.[2] Up to 45% of patients with CSU, besides developing spontaneous wheals, may also show typical symptoms induced by a specific trigger (Fig. 222.1).[3]

Urticaria and angioedema are relatively common conditions and have a lifetime prevalence of about 9%.[4,5]

Although persons in any age group may experience acute or chronic urticaria and/or angioedema, young adults (postadolescence through the third decade of life) are most often affected.[6,7] Females are affected approximately twice as often as males.[8] Urticaria has a complex pathogenesis, along with a high disease burden, a significant impact on quality of life, and high health care costs.

PATHOPHYSIOLOGY

The signs and symptoms of acute and chronic urticaria are consistent despite the many diverse etiological and initiating factors (see

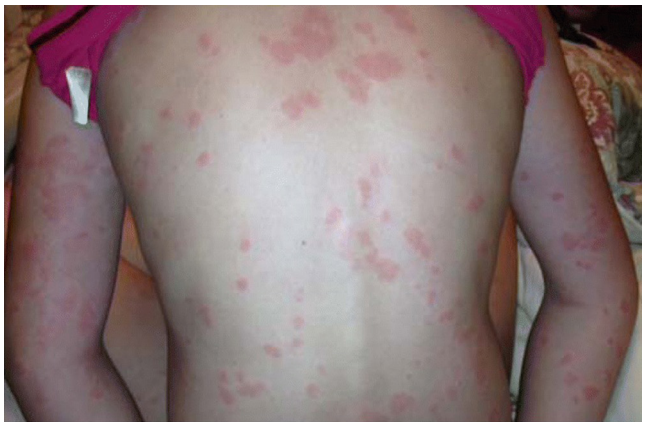


Fig. 222.1 Chronic spontaneous urticaria. (From Sussman G, Hebert J, Gulliver W, et al. Insights and advances in chronic urticaria: a Canadian perspective. *Allergy Asthma Clin Immunol.* 2015;11[1]:7. PubMed PMID: 25705232. (Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC4336710_13223_2015_72_Fig1_HTML&query=urticaria&req=4&npos=46 [accessed January 6, 2019])

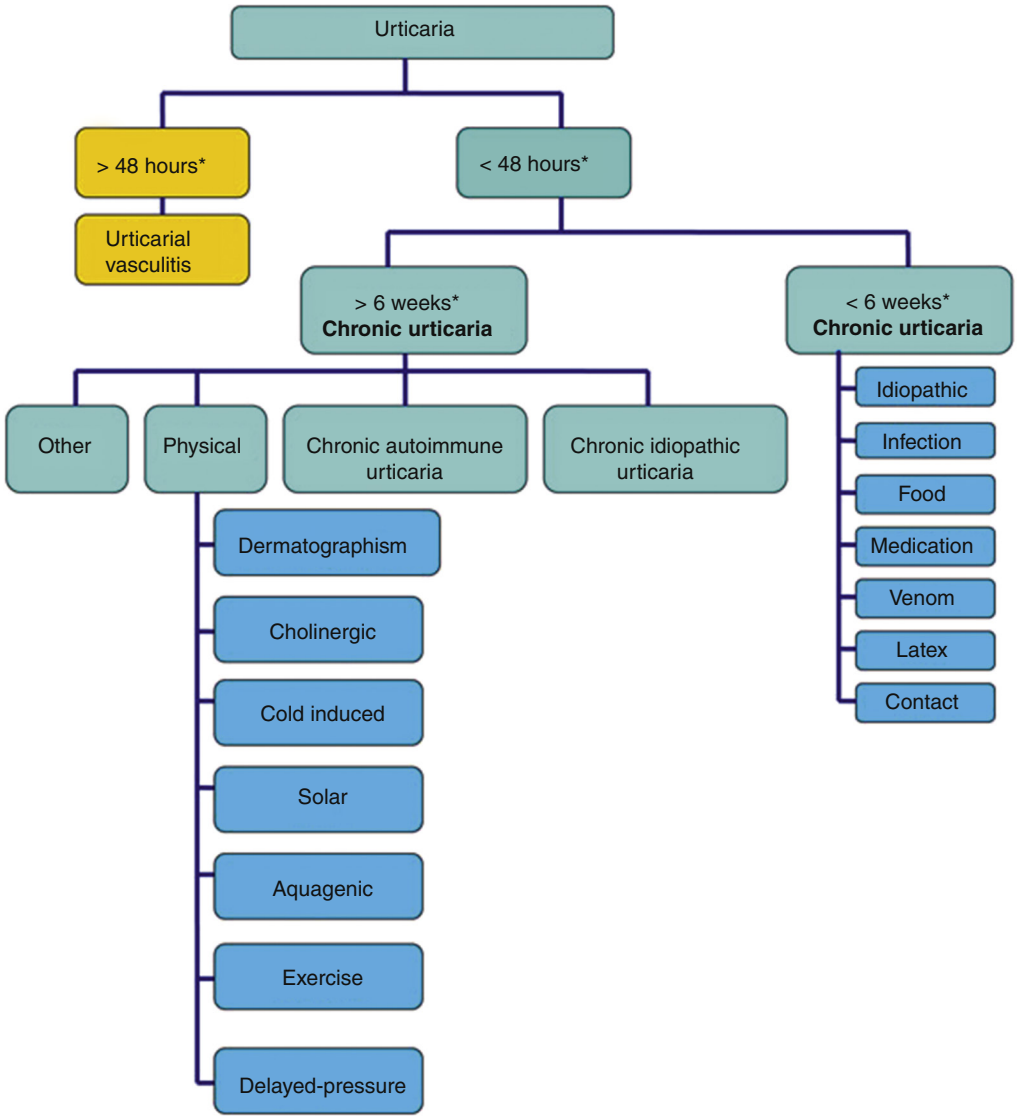


Fig. 222.2 Classification of urticaria: overview. *The 48-hour cut-off refers to individual lesions, whereas the 6-week cut-off refers to the condition as a whole. (From Kanani A, Schellenberg R, Warrington R. Urticaria and angioedema. *Allergy Asthma Clin Immunol.* 2011;7[Supp 1]:S9. PubMed PMID: 22165855. (Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC3245442_1710-1492-7-S1-S9-2&query=urticaria&req=4&npos=73 [accessed January 7, 2019])

later) that have been found, yet the pathogenesis cannot be entirely ascribed to any one mechanism. The spinothalamic tract is thought to play an important role in the pathway of pruritus.[9] Primary afferent neurons, also known as pruriceptors, detect itch-inducing substances like histamine and chloroquine. The most well-known pruritogen is histamine; however, nonhistaminergic mediators also exist. Initially it was thought that the nerve fibers only responded to histamine/non-histamine stimulus, but it is now accepted that these fibers can also be stimulated by noxious stimuli.

Acute urticaria has identified precipitating factors in <50% of cases. When present, the most common triggers are upper respiratory infections (40%), drug reactions (9.2%), and food intolerance (0.9%).[10] Chronic urticaria may be subclassified into chronic spontaneous urticaria or chronic inducible urticaria (CIndU). Up to 30% of cases are associated with functional immunoglobulin (Ig)-G antibodies to the high-affinity IgE receptor FcεRIα or to IgA. Among patients in which an etiology is suspected, infections, drugs, food, and psychological factors are the most commonly associated (Fig. 222.2). CIndU is recognized by its ability to be triggered consistently and reproducibly in response to a specific stimulus.

Mast cells, mast cell–dependent mediators, and basophils play the most prominent role in the pathogenesis of urticaria.[11,12] Mast cells are widely distributed throughout the body and are found primarily near small blood vessels, particularly in the skin. The granule-containing mast cell is a secretory cell capable of releasing both preformed and newly synthesized mediators (listed in Box 222.1) that play key roles in the pathogenesis of both immunological and nonimmunological inflammatory reactions. The three distinct sources of mediators are as follows:

- Preformed mediators, which are contained in the granules and are released immediately
- Secondarily formed mediators, which are generated immediately or within minutes by the interaction of the primary mediators and nearby cells and tissues
- Granule matrix–derived mediators, which are preformed but slowly dissociate from the granule after discharge and remain in the tissues for hours

BOX 222.1 Mast Cell–Derived Mediators

Preformed, rapidly released:
- Histamine
- Eosinophil chemotactic factors of anaphylaxis (ECF-A)
- Eosinophil chemotactic oligopeptides
- Neutrophil chemotactic factor
- Superoxide anions
- Exoglycosidases (β-hexosaminidase, β-δ-galactosidase,[a] β-glucuronidase)
- Serotonin
- Arylsulfatase A

Secondary or newly generated:
- Slow-reacting substances (SRS-A): leukotrienes (LT) C, LTD, LTE
- Prostaglandins
- Monohydroxyeicosatetraenoic acids (HETEs)
- Hydroperoxyeicosatetraenoic acids (HPETEs)
- Thromboxanes
- Platelet-activating factor (PAF)
- Prostaglandin-generating factor of anaphylaxis (PGF-A)

Preformed, granule-associated:
- Heparin
- Proteases (chymotrypsin/trypsin)
- Peroxidase
- Superoxide dismutase
- Arylsulfatase B
- Inflammatory factors of anaphylaxis (IFA)

[a]Found in mast cells of species other than human.
Data from Keahey TM. The pathogenesis of urticaria. *Dermatol Clin* 1985;3:13–28.

The most common immunological mechanism is mediated by IgE. The early vascular changes appear to be the result of mast cell–dependent vasoactive mediators, particularly histamine and some secondarily generated end products of arachidonic acid metabolism (prostaglandins). The wheal-and-flare reactions occur within minutes of initiation and last 30 to 60 minutes.

Histamine and other mediators can be released by other nonallergic mechanisms as well. Neuropeptides are known to cause mast cell degranulation and may well be involved in dermographism and emotional exacerbation of urticaria. The more prolonged and delayed reactions reflect leukocytic infiltration in response to the release of mast cell granule–derived chemotactic factors. These late-phase reactions develop over time and are characterized by erythema, edema, and induration beginning within 2 hours and lasting 12 to 24 hours. Leukocyte infiltration may contribute to the urticarial tissue response by inducing a second wave of mast cell activation or by releasing toxic lysosomal enzymes and mediators characteristic of the type of infiltrating cell.

The actual events initiated by the mediators also depend on the tissues into which they are released. For example, the release of histamine into the skin primarily produces pruritus and vascular permeability, whereas histamine release into the lungs may induce bronchospasm. Prostaglandin release, rather than histamine mediation, seems to be involved in contact urticaria.

Helicobacter pylori has been implicated as a causative agent in chronic urticaria, and a protein component has been shown to induce mast cell degranulation.[13,14]

However, two reviews of the trials showing the benefit of *H. pylori* eradication in patients with chronic urticaria have arrived at conflicting conclusions, with one saying the benefit is weak and another that it is statistically significant.[15,16] Including testing for *H. pylori* seems reasonable; if positive, a decision to proceed with this management should be considered carefully in the context of relative harms/burdens and benefits as well as patient values and preferences.[17]

CAUSES OF URTICARIA

Fundamental to the treatment of urticaria are the recognition and control of causative factors.

Physical Urticarias

Urticaria can be produced as a result of reactions to various physical stimuli. The most common forms of physical urticarias are dermographic, cholinergic, and cold urticarias (Table 222.1). These are briefly described here. Less common types of physical urticarias or angioedema are as follows[6]:
- Contact
- Solar
- Pressure
- Heat contact
- Aquagenic
- Vibratory
- Exercise-induced (a subtype of cholinergic)

Dermographism

Dermographism or dermatographic urticaria is a readily elicited wheal of the skin that evolves rapidly when moderate amounts of pressure are applied. It may occur as a result of simple contact with furniture, garters, bracelets, watch bands, towels, or bedding. The incidence of dermographic urticaria has been estimated at 2% to 5% in the general population.[18]

The most common type of physical urticaria, it is found twice as frequently in women as in men, with the average age at onset being in the third decade. The incidence is much greater among the obese, especially those who wear tight clothing. There are several inexpensive instruments for determining provocation thresholds in patients with symptomatic dermographism.[19,20]

Dermographic lesions usually start within 1 to 2 minutes of contact as an erythema, which is replaced within 3 to 5 minutes by edema and surrounding reflex urticaria. Maximal edema is usually produced within 10 to 15 minutes. Although the erythema generally regresses within an hour, the edema can persist for up to 3 hours.

Dermographism may be associated with other diseases, including the following[6]:
- Parasitosis
- Insect bites
- Neuropsychiatric disorders
- Hormonal changes
- Thyroid disorders
- Pregnancy
- Menopause
- Diabetes
- Immunological alterations
- Other urticarias
- During or following drug therapy
- Infection with *Candida albicans*
- Angioedema
- Hypereosinophilia

Cholinergic Urticaria (Heat Urticaria)

Cholinergic, or heat-reflex, urticaria is the second most common physical urticaria. These lesions, which depend on the stimulation of the

TABLE 222.1 Clinical Aspects of Physical Urticarias

Type	Eliciting Stimulus	Time of Onset	Duration of Lesion	Diagnostic Test	Associated Symptoms
Dermographic urticaria (tarda)	Stroking, scratching (rubbing for red dermographism)	2–5 min (0.5–5 hr)	1–5 hr (48 hr)	Firm stroking of skin	Headache, malaise
Cholinergic urticaria	Physical exercise + overheating; mental stress	2–20 min	30–60 min	Bicycling, running, sauna	Headache, gastrointestinal upset, wheezing, salivation, lacrimation, syncope
Cold urticaria	Cold contact	2–5 min	1–2 hr	Ice cube, cold arm bath, cold air	Wheezing, syncope
Solar urticaria	Light of varying wavelengths	2–15 min	0.25–3 hr	Phototest	Wheezing, dizziness, syncope
Pressure urticaria	Pressure	3–8 hr	8–24 hr	Locally applied weights	Flulike syndrome, fever, leukocytosis, arthralgias
Heat contact urticaria	Contact with heat	2–15 min	30–60 min	Hot arm bath	Gastrointestinal upset, dizziness, fatigue, wheezing, dyspnea
Aquagenic urticaria	Contact with water	2–30 min	30–60 min	Bath, compresses	None
Vibratory angioedema	Vibration	0.5–4 min	1 hr	Vibrating motor	Faintness, headache
Exercise-induced anaphylaxis	Exercise after a heavy meal	2–5 min	10–30 min	Exercise	Flushing, headache, disorientation, glottis edema, dyspnea, collapse
Familial cold urticaria	Cold wind, change from cold to warm air	0.5–3 hr	48 hr	Cold wind and subsequent rewarming	Tremor, headache, arthralgias, fever

Data from Czarnetzki BM. *Urticaria*. New York: Springer-Verlag; 1986.

sweat gland via cholinergic afferent fibers, consist of pinpoint wheals surrounded by reflex erythema. The wheals arise at or between follicles and develop preferentially on the upper trunk and arms.

The three basic types of stimuli that may produce cholinergic urticaria are passive overheating, physical exercise, and emotional stress. Typical eliciting activities, in addition to physical exercise, may include taking a warm bath or sauna, eating hot spices, and drinking alcoholic beverages. The lesions usually arise within 2 to 10 minutes after provocation and last for 30 to 50 minutes.

A variety of systemic symptoms may also occur, suggesting a more generalized mast cell release of the mediators than in the skin. Headache, periorbital edema, lacrimation, and burning of the eyes are common symptoms. Less common symptoms are nausea, vomiting, abdominal cramps, diarrhea, dizziness, hypotension, and asthma attacks.[6]

Cold Urticaria

Cold urticaria is an urticarial and/or angio-edematous reaction of the skin that develops after contact with cold objects, water, or air. Lesions are usually restricted to the area of exposure and develop within a few seconds to minutes after the removal of the cold object and rewarming of the skin. The lower the object's or element's temperature, the faster the reaction. Children with cold urticaria have a higher risk of anaphylaxis, especially triggered by swimming.[21]

Widespread local exposure and generalized urticaria can be accompanied by the following symptoms:

- Flushing
- Headaches
- Chills
- Dizziness
- Tachycardia
- Abdominal pain
- Nausea
- Vomiting
- Muscle pain
- Shortness of breath
- Wheezing
- Unconsciousness

Cold urticaria has been observed to accompany a variety of clinical conditions, including the following[22]:

- Viral infections
- Parasitical infestations
- Syphilis
- Multiple insect bites
- Penicillin injections
- Dietary changes
- Stress

The association of cold urticaria with infectious mononucleosis is well established. Other conditions associated with cold urticaria are cryoglobulinemia and myeloma, in which cold urticaria may precede the diagnosis by several years.[6] Cold urticaria is often found in patients with other chronic inducible urticarias (e.g., physical urticaria) as well as other allergic diseases.[23]

Autoimmune Urticaria

Although much remains unknown regarding the specific pathophysiological mechanisms underlying most cases of chronic urticaria, some seem to have an autoimmune basis. Research has discovered that a significant percentage of patients previously categorized as having chronic idiopathic urticaria (CIU) have autoantibodies to IgE or the FcεRIα subunit of the high-affinity IgE mast cell receptor (Fig. 222.3).[24] Studies have identified these autoantibodies in 24% to 76% of patients with chronic urticaria. From a clinical perspective, these patients tend to have a somewhat more severe, prolonged course of disease. Middle-aged women are disproportionately represented in the population of patients with urticaria, and higher rates of autoimmunity in this group, particularly for thyroid disease, may account for this preponderance. Thyroid autoantibodies are found more frequently in patients with these IgE-receptor autoantibodies (see later discussion of thyroid). However, investigations have revealed that epitopic

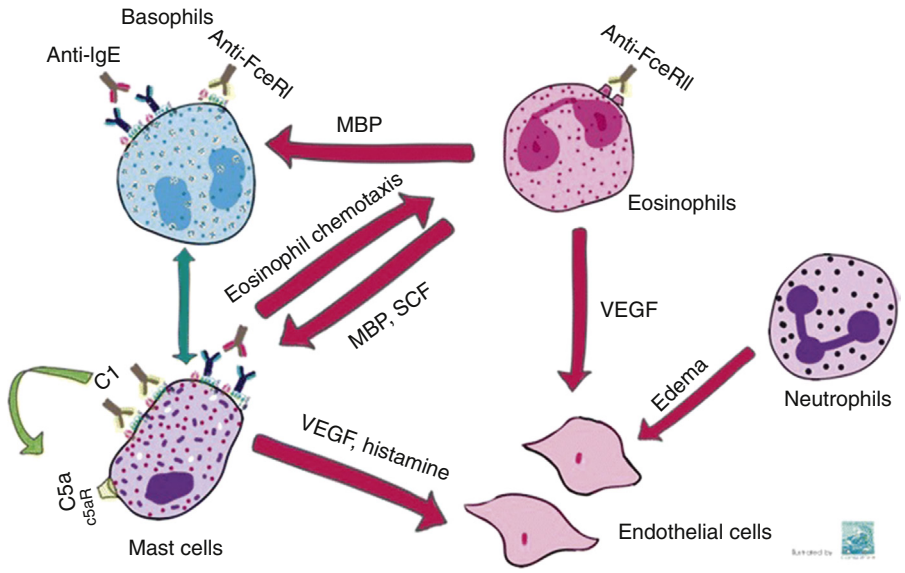


Fig. 222.3 Possible mechanisms of activation among cells implicated in chronic idiopathic urticaria. Cross-talk between mast cells and basophils remains to be defined. (From Ferrer M. Immunological events in chronic spontaneous urticaria. *Clin Trans Allergy*. 2015;5:30. PubMed PMID: 26309723. (Retrieved from: https://openi.nlm.nih.gov/detailedresult.php?img=PMC4549074_13601_2015_74_Fig1_HTML&query=urticaria+autoimmune&req=4&npos=91 [accessed January 7, 2019])

cross-reactivity does not explain the higher prevalence of Hashimoto's thyroiditis (HT) in patients with CIU. The frequent concurrence of HT and CIU most likely is due to a genetic predisposition to autoimmune disease.[25]

One third to half of patients with CSU show a positive response against their own serum (positive autologous serum skin test [ASST]). The ASST is the most generally available test to screen for autoantibodies against either IgE or FcεRIα. These patients may need higher doses of antihistamines or additional immunomodulators. Thus the ASST is a useful tool in patients who are not responding to traditional therapy.[26]

Autoimmune diseases are well known to be associated with bowel permeabilities.[27–30] Because subclinical impairments of small bowel enterocyte function have been postulated to induce a higher sensitivity to histamine in the digestive tract,[31] it makes sense from a naturopathic standpoint to treat overt and subclinical digestive system permeabilities to decrease systemic inflammatory responses to endotoxins in these autoimmune conditions.

Drugs

Drugs are the leading cause of urticarial reactions in adults. In children, the reactions are usually due to foods, food additives, or infections.[6]

Most drugs are composed of small molecules incapable of inducing antigenic/allergenic activity on their own. Typically, they act as haptens that bind to endogenous macromolecules, ultimately causing the production of hapten-specific antibodies. Alternatively, drugs can interact directly with mast cells to induce degranulation. Many drugs have been shown to produce urticaria. Box 222.2 provides a condensed list.[32] The three most common urticaria-inducing drugs, penicillin, aspirin, and angiotensin-converting enzyme (ACE) inhibitors, are briefly discussed here.

Penicillin

Antibiotics, including penicillin and related compounds, are the most common cause of drug-induced urticaria. The allergenicity of penicillin in the general population is thought to be at least 10%. Nearly 25% of these individuals display urticaria, angioedema, or anaphylaxis on ingestion of penicillin.[6,33]

BOX 222.2 Drugs That Can Cause Urticaria

- Acetylsalicylic acid
- Allopurinol
- Angiotensin-converting enzyme (ACE) inhibitors
- Antimony
- Antipyrines
- Barbiturates
- Bismuth
- Chlorhydrate
- Chlorpromazine
- Corticotropin (adrenocorticotropic hormone)
- Eucalyptus
- Fluorides
- Gold
- Griseofulvin (cold urticaria)
- Insulin
- Iodines
- Liver extract
- Menthol
- Meprobamate
- Mercury
- Morphine, opium
- Para-aminosalicylic acid
- Penicillin
- Phenacetin
- Phenobarbital
- Pilocarpine
- Poliomyelitis vaccine
- Potassium sulfocyanate
- Procaine
- Promethazine
- Quinine
- Reserpine
- Saccharin
- Thiamine chloride
- Thiouracil

Data from Andrews GC. *Andrews' diseases of the skin*. 7th ed. Philadelphia: WB Saunders; 1982: 131.

An important characteristic of penicillin is that it cannot be destroyed by boiling or steam distillation. This is a problem because penicillin and related contaminants can exist undetected in foods. To what degree penicillin in the food supply contributes to urticarial reactions is not known. However, urticaria and anaphylactic symptoms have been traced to penicillin in milk,[34] soft drinks,[35] and frozen dinners.[36] In one study of 245 patients with chronic urticaria, 24% had positive skin test results, and 12% had positive results on the radioallergosorbent test for penicillin sensitivity.[37] Of those 42 patients sensitive to penicillin, 22 experienced clinical improvement with a dairy-free diet, whereas only 2 out of 40 patients with negative skin test results experienced improvement with the same diet. This study

TABLE 222.2 Provocation Tests With Food Additives in Chronic Urticaria

			PERCENT POSITIVE TESTS					
Study	Year	No. of Patients	Tartrazine	Other Azo Dyes	Annatto	Benzoates	Bha/Bht	Aspirin
Samter and Beers[44]	1969	40	8	—	—	—	—	—
Michaelsson and Juhlin[45]	1973	52	36	20	—	44	—	67
Thune and Granholt[46]	1975	100	21	14	—	10	14	33
Doeglas[47]	1975	23	30	—	—	23	—	24
Warin and Smith[48]	1976	108	12	—	—	16	—	—
Settipane et al.[49]	1976	38	8	—	—	—	—	—
Kaaber[50]	1978	65	5	5	8	3	—	—
Fujita et al.[51]	1978	57	—	—	—	23	—	—
Neuman et al.[52]	1978	30	23	—	—	—	—	—
August[53]	1979	86	23	—	—	22	—	—
Meynadier et al.[54]	1979	24	24	46	—	25	—	—
Lindemayr and Schmidt[55]	1979	90	19	16	—	29	—	—
Mikkelsen et al.[54]	1979	24	24	46	26	—	—	—
Wutrich and Hacki-Herrmann[57]	1980	81	21	—	—	18	11	—
Gibson and Clancy[58]	1980	76	28	—	—	34	—	54
Juhlin[59]	1981	330	—	18	11	11	15	10
Wutrich and Fabro[60]	1981	306	6	—	—	6	—	—
Kirchoff et al.[61]	1981	100	15	10	—	8	—	—
Egyedi and Torok[62]	1982	40	37	7	—	17	—	37
Merk and Gorez[63]	1983	25	24	—	—	—	—	—
Hannuksela[64]	1983	137	1	—	—	4	—	18
Ortolani et al.[65]	1984	75	13	—	—	21	10	43
Simon[66]	1984	25	0	—	—	—	—	—
Genton et al.[67]	1985	17	59	—	—	30	—	—
Pigatto et al.[68]	1985	61	10	—	—	—	15	—
Kemp and Schembri[69]	1985	23	7	—	—	7	—	36
Zieger and Hamstein[70]	1986	100	10	—	—	—	—	—
Supramaniam and Warnor[71]	1986	43	26	—	—	15	—	2

provides indirect evidence of the importance of penicillin in the food supply in urticaria.

To provide direct evidence, penicillin-contaminated pork was given to penicillin-allergic volunteers. No significant reactions were noted other than transient pruritus in two volunteers.[38] Penicillin in milk appears to be more allergenic than penicillin in meat.[34] Presumably, the reason is that penicillin can be degraded into more allergenic compounds in the presence of carbohydrate and metals, suggesting that penicillin in milk may be more allergenic than artificially contaminated meat.[34]

Aspirin and Nonsteroidal Anti-Inflammatory Drugs

Urticaria is a more common indicator of aspirin sensitivity than is asthma (see Chapter 148). The incidence of aspirin sensitivity in patients with chronic urticaria is at least 20 times greater than that in normal controls.[39–43] Studies (summarized in Table 222.2)[44–73] have demonstrated that 2% to 67% of patients with chronic urticaria are sensitive to aspirin.

Aspirin inhibition of cyclooxygenase apparently shunts eicosanoid metabolism toward leukotriene synthesis, thereby increasing smooth muscle contraction and vascular permeability. In addition, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to increase gut permeability dramatically and may alter the normal handling of antigens.[69,74] NSAIDs are also known to be associated with prolonged and more pronounced autoreactivity in urticaria.[73] The daily administration of 650 mg of aspirin for 3 weeks has been shown to desensitize patients with urticaria and aspirin sensitivity. While taking the aspirin, patients also became nonresponsive to foods to which they usually reacted, such as pineapple, milk, egg, cheese, fish, chocolate, pork, strawberries, and plums.[75] Others have noted this effect in patients with asthma, but they have also found that the effect disappears within 9 days after the treatment is stopped, suggesting the loss of effect or a possible placebo response.[76]

Angiotensin-Converting Enzyme Inhibitors

ACE inhibitors are the other most commonly implicated drugs in chronic urticaria, due to the nonimmunological accumulation of bradykinin and other neurokinins. Chronic urticaria can occur from weeks to years after treatment is initiated.[77]

Environmental Toxicants

Exogenous toxins are comprised of toxic agents that arise from external factors. Sources are primarily environmental and include metals, chemicals (inorganic, fluoride, organic, persistent organic pollutants [POPs], drugs, etc.), molds, and particulate matter. The skin can eliminate toxic metals and chemical xenobiotics through perspiration, which may lead to urticarial eruptions. More commonly, reactions to exogenous toxins occur from topical exposure and can range from contact dermatitis to urticaria.

Nickel is the most common allergen found by patch testing, and contact urticaria (CU) to nickel (immediate reaction) has been reported. Patients with nickel allergy often report reactions to such objects as jewelry (e.g., earrings, necklaces, bracelets, watches), buttons on clothing, and belt buckles within minutes to hours of exposure.

Ambient air pollution exposure has been associated with several health conditions, limited not only to respiratory and cardiovascular systems but also to cutaneous tissues. The Air Quality Health Index (AQHI) was developed in Canada and is based on the combined effects of three ambient air pollutants, ozone (O_3), nitrogen dioxide (NO_2),

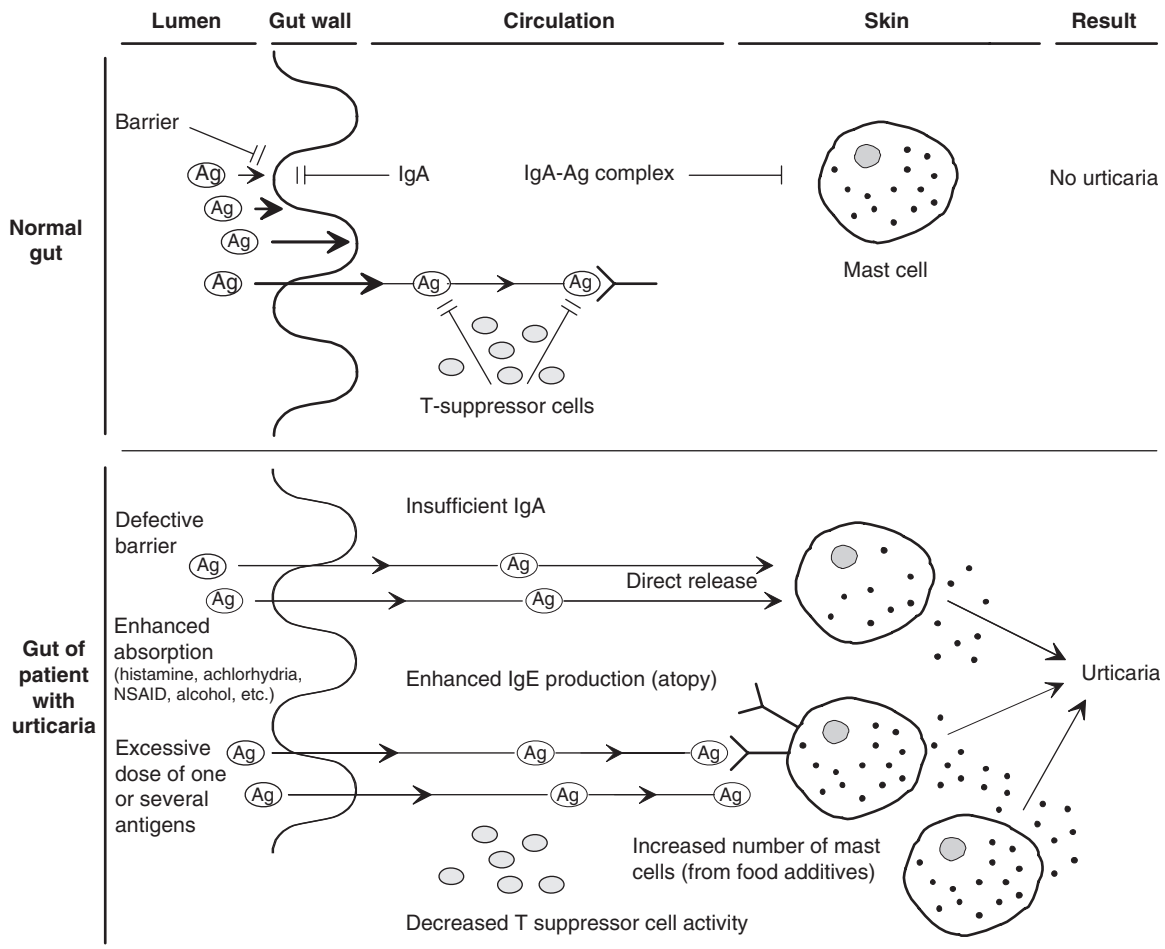


Fig. 222.4 Hypothetical model of immune defense in the normal and urticarial gut.

and fine particulate matter ($PM_{2.5}$), on mortality. One Canadian study examined the associations of short-term changes in the AQHI with emergency department (ED) visits for urticaria in Windsor-area hospitals and found positive and significant results between AQHI levels and odds ratio for ED visits for urticaria for lags of 2 and 3 days.[78]

Topical insect repellants, such as N,N-diethyl-meta-toluamide (DEET), can produce contact urticaria from direct application as well as from contact with individuals who have used DEET-containing repellents.[79]

Food-Related Causes

Food Allergy

IgE-mediated urticaria can occur with the ingestion of a specific reactive antigens. Although any food can be the agent, the most common offenders are milk, fish, meat, eggs, beans, and nuts.[6,33,80–86] Other common allergens are citrus, kiwis, peanuts, and apples.[80] Individuals with atopy are most likely to experience urticaria as a result of IgE-related mechanisms, although reports confirm the presence of pseudoallergic reactions, whereby direct mast cell histamine release is involved in this reactivity. Aromatic volatile ingredients in tomatoes, wine, and culinary herbs (basil, fenugreek, cumin, dill, ginger, coriander, caraway, turmeric, parsley, pepper, rosemary, and thyme) have been considered the initiating factors in some of these non-IgE events.[87,88]

Food allergies may contribute greatly to the increased permeability of the gut in urticaria. A basic requirement for the development of a food allergy is the absorption of the allergen through the intestinal barrier. Several factors are known to significantly increase gut permeability, including alcohol, NSAIDs, vasoactive amines ingested in foods or produced by bacterial action on essential amino acids, and possibly many food additives (see Chapter 19 for a full discussion). Increased intestinal permeability is also associated with autoimmune conditions.[27–30] Because chronic urticaria is now known to be at least partially related to an autoimmune process, it is possible that, depending on the genetic susceptibility of individual, bowel hyperpermeability may play a key role in the instigation of immune activity and its prolongation, leading to chronic urticaria.

In addition, several investigators have reported alterations in gastric acidity, intestinal motility, and the function of the small intestine and biliary tract in up to 85% of patients with chronic urticaria.[89–93] Selective IgA deficiency, gastroenteritis, hypochlorhydria or achlorhydria, and other disruptive factors reported in patients with chronic urticaria may temporarily or permanently alter the barrier and immune function of the gut wall, predisposing an individual to allergic sensitization.

In one study of 77 patients with chronic urticaria, 24 (31%) were diagnosed as achlorhydric, and 41 (53%) were shown to be hypochlorhydric.[91] Treatment with hydrochloric acid and a vitamin B complex gave impressive clinical results, highlighting the importance of correcting any underlying factor in the treatment of chronic urticaria (see Chapter 23).

Although IgE-mediated reactions are thought to predominate in immunological urticaria, it has been suggested that IgG-mediated reactions are probably responsible for the majority of adverse reactions to foods, as seen in general practice (see Chapter 134). IgG antigen–antibody complexes are capable of promoting complement activation and the subsequent generation of anaphylatoxins that promote mast cell degranulation. This could be a significant factor in some cases of urticaria.

Fig. 222.4 summarizes the basic aspects of a hypothetical model of immune defense in the normal gut and the urticarial gut.

Food Colorants

Food additives are a major factor in many cases of chronic urticaria in children. Colorants (azo dyes), flavorings (salicylates, aspartame), preservatives (benzoates, nitrites, sorbic acid), antioxidants (hydroxytoluene, sulfite, gallate), and emulsifiers/stabilizers (polysorbates, vegetable gums) have all been shown to produce urticarial reactions in sensitive individuals.[2,17,28–57,66,69,81,115–118]

The importance of the control of food additives was well demonstrated in a study of 64 patients with common urticaria. After 2 weeks on an additive-free diet, 73% of the patients had a significant reduction in symptoms. The diet strictly forbade preservatives, dyes, and antioxidants. No fruits or refined sugars were allowed except honey. Rice, potatoes, and unprocessed cereals were allowed, as well as fresh milk.[94]

Tartrazine. In 1959 the azo dye tartrazine (Food, Drug and Cosmetic Act [FD&C] Yellow no. 5) was the first food dye reported to induce urticaria.[95] Tartrazine is one of the most widely used colorants. It is added to almost every packaged food as well as many drugs, including some antihistamines, antibiotics, steroids, and sedatives.[96] Reactions to this food additive are so common that its use has been banned in Sweden.[97]

In the United States the average daily per capita consumption of certified dyes is 15 mg, of which 85% is tartrazine. Among children, consumption is usually much higher. Tartrazine sensitivity has been calculated as occurring in 0.1% of the population.[98]

Tartrazine sensitivity is extremely common (20%–50%) in individuals sensitive to aspirin.[33,96] Like aspirin, tartrazine is a cyclooxygenase inhibitor and a known inducer of asthma, urticaria, and other allergic conditions, particularly in children. Inhibition of cyclooxygenase by tartrazine or aspirin apparently shunts eicosanoid metabolism toward leukotriene synthesis, thereby increasing smooth muscle contraction and vascular permeability.

In addition, tartrazine, as well as benzoate and aspirin, increases the production of the lymphokine leukocyte inhibitory factor.[98] This results in an increase in perivascular mast cells and mononuclear cells. Histological examination of patients with urticaria shows that more than 95% have an increase in perivascular mast cells and mononuclear cells.[99]

Table 222.2 summarizes the findings in studies using provocation tests to determine sensitivity to tartrazine and other food additives in patients with urticaria. Rates have varied from 5% to 46%. Diets eliminating tartrazine as well as other azo dyes and food additives in sensitive individuals have in many cases been shown to be of great benefit. These studies are summarized in Table 222.3. Table 222.4 shows a recommended test battery.[100]

Food Flavorings

Salicylates. A broad range of salicylic acid esters are used to flavor foods such as cake mixes, puddings, ice cream, chewing gum, and soft drinks. The mechanism of action of these agents is thought to be like that of aspirin.[33]

Salicylates are also found naturally in many foodstuffs. It is estimated that daily salicylate intake from foods is in the range of 10 to 200 mg/day.[101] Because this is very close to the level of salicylates used in clinical testing (usually 300 mg), dietary salicylate may be a significant factor in aspirin-sensitive individuals.

Most fruits, especially berries and dried fruits, contain salicylates. Raisins and prunes have the highest amounts. Salicylates are also found in appreciable amounts in candies made of licorice and peppermint. Moderate levels of salicylate are found in nuts and seeds. Vegetables, legumes, grains, meat, poultry, fish, eggs, and dairy products typically contain insignificant levels of salicylates. Salicylate levels are especially high in some herbs and condiments, including curry powder (turmeric), paprika, thyme, dill, and oregano. Although the intake of these herbs and spices is relatively small, they can contribute to total dietary salicylate.[101]

TABLE 222.3 Response to a Diet Free of Added Dyes and Benzoates

Study	Year	No. of Patients	PERCENTAGE Free of Urticaria	Better	Same
Michaelsson and Juhlin[45]	1973	16	81	6	13
Thune and Granholt[48]	1975	100	12	50	38
Ros et al.[147]	1976	75	24	57	19
Warin and Smith[48]	1976	58	75	75	25
Douglas[93]	1977	18	67	67	33
Kaaber[50]	1978	23	44	30	26
August[53]	1979	22	45	23	32
Meynadier et al.[54]	1979	98	80	12	8
Lindemayr and Schmidt[55]	1979	90	20	55	25
Gibson and Clancy[58]	1980	65	75	15	10
Valverde et al.[149]	1980	258	62	22	16
Wutrich and Fabro[60]	1981	51	31	57	12
Kirchoff et al.[61]	1981	41	44	29	27
Verschave et al.[150]	1983	67	73	73	37
Kemp and Schembri[69]	1985	18	39	39	22

Other Flavoring Agents. Other flavoring agents, such as cinnamon, vanilla, menthol, and other volatile compounds, may produce urticaria in some individuals.[33] For example, the artificial sweetener aspartame has been shown to induce urticaria.[102]

Food Preservatives

Benzoates. Benzoic acid and benzoates are the most commonly used food preservatives. Although the incidence of adverse reactions to these compounds is thought to be less than 1% for the general population, the frequency of positive challenges in patients with chronic urticaria varies from 4% to 44%, as illustrated in Table 222.2.

Fish and shrimp frequently contain extremely high quantities of benzoates. This may be one reason why adverse reactions to these foods are so common in patients with urticaria.

Butylated Hydroxytoluene and Butylated Hydroxyanisole. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole are the primary antioxidants used in prepared and packaged foods. Typically, 15% of patients with chronic urticaria test positive to oral challenge with BHT.[46,59,68,72] The use of chewing gum containing BHT was enough to induce urticaria in one patient.[103]

Sulfites. Sulfites, like tartrazine, have been shown to induce asthma, urticaria, and angioedema in sensitive individuals.[104,105] The source may be varied because these compounds are ubiquitous in foods and drugs. They are typically added to processed foods to prevent microbial spoilage and keep them from browning or changing color. The earliest known use of sulfites was in the treatment of wines with sulfur dioxide by the Romans. Sulfites are sprayed on fresh foods such as shrimp, fruits, and vegetables.

TABLE 222.4 Test Battery for Patients With Recurrent Urticaria

Day	Substance(s) Tested	Amount (mg)
1	Control (lactose)	100,000
2	Azo dyes:	
	Tartrazine	0.1, 1, 10
	New coccine	0.1, 1, 10
	Sunset yellow	0.1, 1, 10
3	Control (lactose)	100, 100
4	Benzoates:	
	Sodium benzoate	50, 500
	4-Hydroxybenzoic acid	50, 200
5	Carotene	50, 100, 100
	Canthazanthine	10, 200, 200
6	Annatto	5, 10
7	BHT-BHA	1, 10, 50, 50
8	Yeast extract	600
9	Control (lactose)	100, 100
10	Aspirin	0.1, 1, 10, 100, 250, 500
11	Sorbic acid	50, 200, 200
12	Control	
13	Sodium nitrite	100
	Sodium nitrate	100
14	Sodium glutamate	100, 200
15	Quinoline yellow	1, 5, 10
16	Potassium metabisulfate	1, 5, 10, 50

Data from Juhlin L. Recurrent urticaria. Clinical investigation of 330 patients. *Br J Dermatol.* 1981;104:369–381; and Valverde E, Vich JM, Garcia-Calderon JV, et al. In vitro stimulation of lymphocytes in patients with chronic urticaria induced by additives and food. *Clin Allergy.* 1980;10:691–698.

They are also used as antioxidants and preservatives in many pharmaceuticals. Sulfites have caused such a wide range of health problems, such as asthma and urticaria, that their use on fresh fruits and vegetables (except potatoes) was banned in the United States in 1986.[97]

The average person consumes an average of 2 to 3 mg/day of sulfites. Wine and beer drinkers typically consume up to 10 mg/day, and individuals who frequent restaurants for meals may ingest up to 150 mg/day.[104]

Normally, the enzyme sulfite oxidase metabolizes sulfites to safer sulfates, which are excreted in the urine. People with a poorly functioning sulfoxidation system, however, have an increased ratio of sulfite to sulfate in their urine. Sulfite oxidase depends on the trace mineral molybdenum. Although most nutrition textbooks list molybdenum deficiency as uncommon, an Austrian study of 1750 patients found that 41.5% were molybdenum deficient.[102]

Food Emulsifiers and Stabilizers

A variety of compounds are used to emulsify and stabilize many commercial foods to ensure that the solids, oils, and liquids do not separate out. Most of the foods containing these compounds are heterogeneous because they usually contain antioxidants, preservatives, and dyes. Polysorbate in ice cream has been reported to induce urticaria, and vegetable gums such as acacia, gum arabic, tragacanth, quince, and carrageenan may also induce urticaria in susceptible individuals.[33]

Infections

Infections are a major cause of urticaria in children.[6] Apparently, in adults, immunological tolerance occurs to many microorganisms owing to repeated massive antigen exposure. The role of bacteria, viruses, and yeast *(C. albicans)* in urticaria is briefly reviewed here. Chronic *Trichomonas* infections have also been found to cause urticaria.

Bacteria

As noted previously, *H. pylori* has been implicated in chronic urticaria. Bacterial infections contribute to urticaria in two other major settings—acute streptococcal tonsillitis in children and chronic dental infections in adults. In the first setting, acute urticaria predominates, whereas in the second, chronic urticaria predominates.[6]

Viruses

Hepatitis B is the most common cause of virally induced urticaria. One study found that 15.3% of patients with chronic urticaria had anti–hepatitis B surface antibodies.[106] Urticaria has also been strongly linked to infectious mononucleosis and may develop several weeks before clinical manifestations. The incidence of urticaria during infectious mononucleosis is 5%.[6]

Candida albicans

The association between *C. albicans* and chronic urticaria has been suggested in several clinical studies. The number of patients with chronic urticaria who react positively to an immediate skin test with *Candida* antigens is 19% to 81%, compared with 10% to 15% of normal subjects.[48,107–113] It appears that sensitivity to *C. albicans* is an important factor in at least 25% of patients with chronic urticaria.[110] Approximately 70% of patients with a positive skin reaction also react to oral provocation tests using foods prepared with yeasts.

Treatment with nystatin has demonstrated that elimination of the organism can achieve a cure in several individuals with positive skin test results, although a placebo response cannot be ruled out. However, more patients have responded to a "yeast-free" diet than to the simple elimination of the organism in clinical trials. The yeast-free diet employed excluded breads, buns, sausage, wine, beer, cider, grapes, sultanas, Marmite, Bovril, vinegar, tomato, ketchup, pickles, and prepared foods containing food yeasts. For example, in a study of 49 patients with proven sensitivity to *Candida,* 9 showed responses to a 3-week course of nystatin, whereas 18 became symptom-free only after adopting the yeast-free diet.[110] This finding would seem to support the importance of diet along with elimination of the yeast.

Further support for the importance of diet can be found in a study of 36 patients with a positive skin-prick test response to *Candida.* Only 3 patients became asymptomatic with nystatin alone, compared with 23 with diet therapy after the nystatin therapy.[111]

Desensitizing patients to *C. albicans* with the use of a *Candida* cell-wall extract has also demonstrated encouraging results in some patients, although the treatment of these individuals also included increasing gastrointestinal fermentation and acidity as well as the elimination of yeast.[112,113]

Vitamin D Deficiency

A systematic review found that patients with CSU have significantly lower serum vitamin D levels than controls in most studies. These results, however, do not prove causation, and the mechanisms have

not been elucidated. The review showed that a high dose (60,000 IU/week) of vitamin D supplementation for 4 to 12 weeks might help to decrease the disease activity in some patients with CSU.[114] Note that although the total weekly dose is reported, bone health research shows that daily administration is more effective than a weekly bolus.

THERAPEUTIC CONSIDERATIONS

Psychological Aspects

In one retrospective study involving 236 cases of chronic urticaria, psychological factors, such as stress, were reported to be the most common primary cause.[115] Stress appears to play an important role through a reduction of intestinal secretory IgA levels.

In one study of 15 patients with chronic urticaria, relaxation therapy and hypnosis were shown to provide significant benefit.[116] Patients were given an audiotape and asked to use the relaxation techniques described on the tape at home. At a follow-up examination 5 to 14 months after the initial session, six patients were free of hives, and seven others reported improvement.

Ultraviolet Light Therapy

Ultraviolet light has been shown to be of some benefit to patients with chronic urticaria.[117,118] Both ultraviolet A (UVA) and ultraviolet B (UVB) light therapies have been used. Patients with cold, cholinergic, and dermographic urticarias display the greatest therapeutic response.

Thyroid

The association of thyroid disease and autoimmunity with urticaria has been established for decades.[119–124] The prevalence of antithyroid antibodies in the normal population has been estimated at 3% to 6%, and they are commonly found in association with other autoimmune conditions, such as pernicious anemia and vitiligo.[122] A subset of patients with chronic urticaria may be best helped by suppressing thyroiditis, by thyroid hormone therapy, by surgery, or by antithyroid medication.

One study evaluated a group of 624 patients with presumed idiopathic chronic urticaria and angioedema. From these, 90 patients were found to have thyroid autoimmunity.[123] Forty-six of these patients were treated with L-thyroxine therapy, eight of whom had remission within 4 weeks of therapy. Four patients with high thyroid antibody titers had repeated exacerbations when therapy was discontinued and had repeated remissions when therapy with L-thyroxine was resumed. Although in most cases treatment with L-thyroxine did not improve the patient's urticaria or angioedema, a few did demonstrate a dramatic response.

Another study reported that thyroid hormone replacement therapy dramatically improved chronic urticaria in patients who had normal thyroid function but had evidence of thyroid autoimmunity. Of seven patients with chronic urticaria, five were started on thyroid hormone; doses were increased in two. Their urticaria resolved within 2 to 4 weeks, at which time the thyroid therapy was discontinued. In five patients, the symptoms returned within 4 weeks, then resolved within 4 weeks of restarting the hormone therapy. Antithyroid antibodies did not correlate with clinical response.[123]

There have been cases in which resolution of chronic urticaria associated with thyrotoxicosis has also been seen in patients treated with antithyroid medication and in those treated with radioactive iodine.[122] Subclinical hypothyroidism is an increasingly recognized entity and may underlie the thyroid–urticaria association in some cases.

Although the usefulness of thyroid hormone therapy in patients who are euthyroid and have chronic urticaria has not been demonstrated in a controlled trial, testing for thyroid autoimmunity is certainly warranted in cases refractory to other forms of therapy.

Conventional Drug Treatment

CSU is a disease that is particularly difficult to treat. Although nonsedating antihistamines are recommended as first-line agents, a substantial proportion of patients remain poorly responsive to these agents even if H_2-receptor antagonists and/or leukotriene pathway inhibitors are added. Such patients are often treated with corticosteroids, cyclosporine, dapsone or omalizumab, and alternatives to these agents would be a welcome addition if efficacy could be shown with an acceptable tolerability profile and cost.[125,126]

Diphenhydramine, an anticholinergic drug available without prescription, is often recommended for both acute and chronic urticaria. Higher cumulative anticholinergic use is associated with an increased risk for dementia ($p < 0.001$).[127] Efforts to increase awareness among health care professionals and older adults about this potential medication-related risk are important to minimize anticholinergic use over time.

Phototherapy

A randomized, prospective, observer-blinded comparative study of 50 patients with steroid-dependent CRU (6 months of spontaneous urticaria with no response after 3 consecutive months of antihistamines and steroid dependence) were administered either psoralen and UVA (PUVA; group A) or narrowband UVB (NB-UVB; group B) for 90 days, with a posttreatment follow-up of 90 days. The Average Urticaria Activity Score 7 (aUAS7) decreased at 90 days in both groups and continued to decrease at 180 days. The Outcome Scoring Scale (OSS) favorably increased at 90 days and persisted at 180 days. NB-UVB was statistically better than PUVA at different time points. The authors concluded that phototherapy, especially NB-UVB, is an effective, safe, and affordable therapeutic modality for steroid-dependent CRU and should be tried before third-line treatment options such as omalizumab, ciclosporin, and other immunosuppressants.[128]

Traditional Chinese Medicine/Acupuncture Therapy

Acupuncture has long been used in traditional Chinese medicine (TCM) to effectively treat urticaria[129,130] and type I allergic conditions.[131] Known as "wind wheal," urticaria is also referred to by Chinese practitioners as the "hidden rash," owing to its ephemeral nature.

Three standard TCM etiologies are considered for urticaria. Urticarial disease due to wind heat typically manifests as red rashes with pruritus. Wind damp etiology correlates with lighter-colored rashes and a heavy sensation in the body. Alternatively, red rashes that appear with concomitant epigastric or abdominal pain and diarrhea or constipation are considered a derivation of stomach and small intestinal heat. Acute urticaria can be easily and effectively treated with acupuncture, in which LI11 (Quchi), Sp10 (Xuehai), Sp6 (Sanyinjiao), and S36 (Zusanli) are the acupuncture points most commonly prescribed.[131] Auricular acupuncture may also accompany body acupuncture points for enhanced effect. The traditional Chinese herbal formula Jade Screen has been found to enhance the effectiveness of the antihistamine desloratadine in the treatment of chronic urticaria ($P < 0.00001$).[132] Jade Screen formula consists of three traditional Chinese medicines, including *Astragalus membranaceus*, Rhizoma Atractylodis Macrocephalae, and Radix Saposhnikoviae. External herbal washes with *Fructus arctii* (burdock) are also used in TCM.

A systematic review of six randomized controlled trials concluded that acupuncture might be effective and safe for chronic urticaria in relieving symptoms, although based on a low level of evidence.[133]

Supplements

Vitamin B_{12}

Vitamin B_{12} has been anecdotally reported to be of value in the treatment of acute and chronic urticaria.[134,135] A study published in 2004 investigated the prevalence of B_{12} deficiency in patients with CIU and also its relationship to gastric *H. pylori* infection and serological markers of autoimmunity (antithyroglobulin, thyroid microsomal, gastric parietal cell [GPC], and antinuclear autoantibodies). Thirty-three patients with CIU and 27 healthy controls were included in the study. Serum B_{12} levels were below the normal reference range in 11/33 patients with CIU. The mean serum B_{12} levels among patients with CIU and the controls were significantly lower in those with CIU ($p = 0.0001$). Antithyroid antibodies were positive in 6 of 11 patients with low B_{12} levels but only in 4 of 27 healthy controls ($p = 0.019$). Anti-GPC antibodies were positive in 4 of 11 patients with CIU and low B_{12} levels, but only in 2 of 27 healthy controls ($p = 0.047$). In patients with CIU, there was no difference in the frequency of IgG *H. pylori* antibodies between those with low B_{12} levels and normal B_{12} levels. Among the 19 patients who underwent gastric endoscopy, 15 patients had chronic antral gastritis, 2 patients had atrophic gastritis, and there were normal findings in 2 patients. The higher frequency of antithyroid and anti-GPC antibodies in patients with low B_{12} levels suggest that low B_{12} levels in CIU may be of an autoimmune nature.[136] Although serum vitamin B_{12} levels are normal in most patients, additional vitamin B_{12} appears to be of value.

Vitamin D_3

As mentioned previously, high-dose vitamin D_3 therapy may be beneficial for some patients with chronic urticaria.

Quercetin

Considering the importance of mast cell degranulation in the pathogenesis of urticaria, quercetin's significant in vitro activity as a mast cell stabilizer (and inhibitor of many of the pathways of inflammation; see Chapter 84) suggests that it may be very useful in treating urticaria. This possibility is strengthened by the observation that sodium cromoglycate (at 200–400 mg four times daily), a compound similar to quercetin, confers excellent protection against the development of urticaria and angioedema in response to ingested food allergens.[137]

Fish Oil

Three patients with salicylate-induced urticaria experienced alleviation of symptoms following dietary supplementation with omega-3 fatty acids. All three patients experienced severe urticaria, asthma requiring systemic steroid therapy, and anaphylactic reactions. After dietary supplementation with 10 g daily of fish oil providing 3000 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 6 to 8 weeks, all three experienced complete or virtually complete resolution of symptoms, allowing discontinuation of systemic corticosteroid therapy. Symptoms relapsed after dose reduction.[138]

Probiotics

A pilot study of *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 administered twice daily for 8 weeks modestly reduced the symptoms scores and improved quality-of-life scores in a minority of patients with refractory CSU. No statistical significance was reported.[139]

DIAGNOSIS

Physical Evaluation and History

A thorough history and physical evaluation are imperative for the proper diagnosis of urticaria and should include a careful history listing all medications, supplements, and botanicals. Evaluation should also detail travel, recent infection, occupational exposure, timing and onset of lesions, morphology, associated symptoms, family medical history, and preexisting allergies. Contact sensitivities such as latex allergy[140] and exposure to physical stimuli should be documented. History of tattoos should be explored for possible correlations. A diet and lifestyle diary that chronicles date and time, foods eaten, major activities, bowel and urine habits, and stress should be gathered in order to fully characterize the potential temporal relationships of urticaria with specific ingested foods and activities. A comprehensive physical examination can also uncover important diagnostic clues that may help to diagnose comorbidities. A list of pertinent questions to ask during the history-taking for urticaria suggested by the European Academy of Allergy and Clinical Immunology/Global Allergy and Asthma European Network/European Dermatology Forum/World Allergy Organization guidelines can be found in Table 222.5.[141]

TABLE 222.5 Recommended History Intake for Urticaria

Pertinent questions
Time of onset of disease
Frequency/duration of and provoking factors for wheals
Diurnal variation
Occurrence in relation to weekends, holidays, and foreign travel
Shape, size, and distribution of wheals
Associated angioedema
Associated subjective symptoms of lesions, for example, pruritus and pain
Family and personal history regarding urticaria or atopy
Previous or current allergies, infections, internal diseases, or other possible causes
Psychosomatic and psychiatric diseases
Surgical implantations and events during surgery, for example after local anesthesia
Gastric/intestinal problems
Induction by physical agents or exercise
Use of drugs (i.e., nonsteroidal anti-inflammatory drugs, immunizations, hormones, laxatives, ear and eye drops, and supplements)
Observed correlation to food
Relationship to the menstrual cycle
Smoking habits (especially use of perfumed tobacco products or cannabis)
Type of work
Hobbies
Stress
Quality of life related to urticaria and emotional impact
Previous therapy and response to therapy

Data from European Academy of Allergy and Clinical Immunology/Global Allergy and Asthma European Network/European Dermatology Forum/World Allergy Organization 2013 urticaria guidelines.

Laboratory Testing

In cases of CSU, neither US nor European guidelines recommend extensive laboratory testing because it rarely identifies the cause or affects long-term management. Provocation testing can be performed based on the patient's history (application of heat, cold, pressure, etc.). In cases where an etiology is suspected, infections are the most common identified cause.[142]

One review of 29 large studies of laboratory testing in chronic urticaria, involving 6462 patients, demonstrated no relationship between numbers of identified diagnoses and numbers of performed tests.[143] This review noted that systemic diseases (which excluded physical urticarias, allergens, infections, and psychological causes) were identified

and believed to be related causally to the urticaria in only 1.6% of patients; these disorders included vasculitis, thyroid disease, systemic lupus erythematosus, other rheumatic disease, hereditary angioedema, and hematological or oncological conditions.

If symptoms do not remit after proper history and physical examination have been performed and the therapies listed here have been tried, it may be useful to perform a complete blood count with differential, urinalysis, erythrocyte sedimentation rate measurement, liver function tests, antinuclear antibody titer measurements, and thyroid panel with antithyroid autoantibody measurements. These tests may help ferret out most of the uncommon cases with a detectable but unsuspected underlying cause (e.g., infectious, parasitical, rheumatic, thyroid, and hematological conditions).

Although biopsy is not recommended for most cases of chronic urticaria, biopsy can provide useful information to rule out urticarial vasculitis when this condition is suspected clinically.[24]

THERAPEUTIC APPROACH

Regarding the conventional management of chronic urticaria, patients often show incomplete responses to antihistamines and are often uncomfortable and frustrated by chronic pruritus. Some cases do respond to systemic corticosteroids, but the potentially serious side effects associated with these medications preclude their safe chronic use.[24] See Box 222.3 for a complete list of conventional drugs used to treat urticaria.

The basic natural therapeutic approach involves the identification and control of all factors that promote the patient's urticarial response. Obtaining a careful and thorough history is paramount to this process. Acute urticaria is usually a self-limiting disease, especially once the eliciting agent has been removed or reduced. Chronic urticaria also responds to the removal of the eliciting agent or agents if they can be identified. In severe anaphylaxis, appropriate emergency care procedures should be followed.

Diet

Although rarely used in conventional medical therapy, evidence is mounting to support the wisdom of the elimination diet.[24,144]

An elimination or oligoantigenic diet is of utmost importance in the treatment of chronic urticaria (see Chapters 18 and 134). The diet should eliminate not only suspected allergens but also all food additives.

The strictest elimination diets allow only water, lamb, rice, pears, and vegetables. Those foods most commonly associated with inducing urticaria (i.e., milk, eggs, chicken, fruits, nuts, and additives) should be avoided.[85] Foods containing vasoactive amines should be eliminated even if no direct allergy to them is noted; the primary foods to eliminate are cured meat, alcoholic beverages, cheese, chocolate, citrus fruits, and shellfish.

The importance of eliminating food additives cannot be overstated. If food additives do in fact increase the number of perivascular mast cells in the skin, they may also do the same in the small intestine, thereby greatly raising the risk of developing a "leaky gut."

Supplements

- Vitamin C: 1 g three times/day
- Vitamin B12: 1000 mcg intramuscularly per week
- Vitamin D_3: 2000 to 6000 IU per day
- Fish oil: 3000 mg EPA+DHA a day
- Probiotics: *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 twice daily

Psychological

The patient should perform relaxation techniques daily. Listening to audio relaxation programs may be an appropriate way to induce the desired state. Appropriate mind–body management programs leading to good clinical outcomes for chronic urticaria are dependent on the clinician's ability to solicit and discern unique patient "stories." Cognitive therapy and psychospiritual approaches can assist patients to release habitual thought patterns and emotional patterns that can play a role in the initiation and persistence of chronic urticaria.[145,146]

Physical Medicine

Phototherapy is recommended for chronic urticaria. Tepid oatmeal baths may also help calm urticarial symptoms, but caution should be exercised with physical modalities when physical triggers such as heat, cold, and water are salient for the patient.

BOX 222.3 Drugs Used to Treat Urticaria

Second-generation histamine$_1$ (H_1) receptor antagonists:
- Cetirizine (Zyrtec)
- Fexofenadine (Allegra D)
- Desloratadine (Clarinex)
- Loratadine (Claritin)

First-generation H_1 receptor antagonists:
- Hydroxyzine (Atarax)
- Diphenhydramine (Benadryl)
- Ranitidine (Zantac)

H_1 and histamine$_2$ (H_2) receptor antagonists:
- Doxepin (Sinequan)—sedation, increased appetite, resultant weight gain
- Cyproheptadine (Periactin)
- Cimetidine (Tagamet)—interference with hepatic microsomal enzymes and androgen receptors

Leukotriene modifiers:
- Montelukast (Singulair)
- Zafirlukast (Accolate)
- 5-Lipoxygenase inhibitors (e.g., Zileuton [Zyflo])
- Nonsteroidal anti-inflammatory drugs

Corticosteroid:
- Prednisone

Immunosuppressive treatments:
- Cyclosporin A
- Tacrolimus
- Azathioprine
- Cyclophosphamide
- Mycophenolate mofetil
- Plasmapheresis
- Intravenous immunoglobulin

Others:
- Omalizumab
- Calcium channel blocker antihypertensives
- Hydroxychloroquine
- Dapsone
- Colchicine
- Methotrexate
- Sulfasalazine
- Intramuscular gold
- Capsaicin
- Impeded androgens
- Warfarin

Data from references Wedi B, Raap U, Wieczorek D, Kapp A. Urticaria and infections. *Allergy Asthma Clin Immunol*. 2009 Dec 1;5(1):10; and Muller BA. Urticaria and angioedema: a practical approach. *Am Fam Physician*. 2004;69:1123–1128.

Traditional Chinese Medicine/Acupuncture

Acupuncture and Chinese herbal medicine work quite well together with conventional and naturopathic therapies to treat the causes and symptoms of urticaria.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Hiragun M, Hiragun T, Mihara S, et al. Prognosis of chronic spontaneous urticaria in 117 patients not controlled by a standard dose of antihistamine. *Allergy*. 2013;68(2):229–235.
2. Zuberbier T, Aberer W, Asero R, et al. The EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. *Allergy*. 2018;73(7):1393–1414.
3. Magerl M, Altrichter S, Borzova E, et al. The definition, diagnostic testing, and management of chronic inducible urticarias - the EAACI/GA² LEN/EDF/UNEV consensus recommendations 2016 update and revision. *Allergy*. 2016;71(6):780–802.
4. Zuberbier T, Balke M, Worm M, et al. Epidemiology of urticaria: a representative cross-sectional population survey. *Clin Exp Dermatol*. 2010;35:869–873.
5. Sanchez J, Amaya E, Acevedo A, et al. Prevalence of inducible urticaria in patients with chronic spontaneous urticaria: associated risk factors. *J Allergy Clin Immunol Pract*. 2017;5:464–470.
6. Czarnetzki BM. *Urticaria.* Springer-Verlag: New York.
7. Mathews KP. A current view of urticaria. *Med Clin North Am*. 1974;58:185–205.
8. Curto-Barredo L, Riba Archilla L, Roura Vives G, et al. Clinical features of chronic spontaneous urticaria that predict disease prognosis and refractoriness to standard treatment. *Acta Derm Venereol*. 2018;98(7):641–647.
9. Davidson S, Zhang X, Yoon CH, et al. The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. *J Neurosci*. 2007;27:10007–10014.
10. Zuberbier T, Ifflander J, Semmler C, et al. Acute urticaria: clinical aspects and therapeutic responsiveness. *Acta Derm Venereol*. 1996;76:295–297.
11. Keahey TM. The pathogenesis of urticaria. *Dermatol Clin*. 1985;3:13–28.
12. Antia C, Bquerizo K, Korman A, et al. Urticaria: a comprehensive review: epidemiology, diagnosis and work-up. *JAAD*. 2018;79:599–614.
13. Rasooly MM, Moye NA, Kirshenbaum AS. Helicobacter pylori: a significant and treatable cause of chronic urticaria and angioedema. *Nurse Pract*. 2015;40(10):1–6.
14. Mogica-Mogica JD, Gomez-Lopez VM. Association between Helicobacter pylori and allergic and non-allergic chronic urticaria [in Spanish]. *Rev Alerg Mex*. 2013;60:161–163.
15. Shakouri A, Compalati E, Lang DM, Khan DA. Effectiveness of Helicobacter pylori eradication in chronic urticaria: evidence-based analysis using the grading of recommendations assessment, development, and evaluation system. *Curr Opin Allergy Clin Immunol*. 2010 Aug;10(4):362–369.
16. Wedi B, Raap U, Wieczorek D, Kapp A. Urticaria and infections. *Allergy Asthma Clin Immunol*. 2009;5(1):10.
17. Ludvigsson JF, Lindelöf B, Rashtak S, et al. Does urticaria risk increase in patients with celiac disease? A large population-based cohort study. *Eur J Dermatol*. 2013;23(5):681–687.
18. Schoepke N, Młynek A, Weller K, et al. Symptomatic dermographism: an inadequately described disease. *J Eur Acad Dermatol Venereol*. 2015;29(4):708–712.
19. Schoepke N, Abajian M, Church MK, et al. Validation of a simplified provocation instrument for diagnosis and threshold testing of symptomatic dermographism. *Clin Exp Dermatol*. 2015;40(4):399–403.
20. Lembo C, Patruno C, Balato N, et al. Rational ideation and empiric validation of an innovative digital dermographic tester. *Clin Exp Dermatol*. 2018;43(3):268–273.
21. Alangari AA, Twarog FJ, Shih MC, et al. Clinical features and anaphylaxis in children with cold urticaria. *Pediatrics*. 2004;113:e313–e317.
22. Muller BA. Urticaria and angioedema: a practical approach. *Am Fam Physician*. 2004;69:1123–1128.
23. Stepaniuk P, Vostretsova K, Kanani A. Review of cold-induced urticaria characteristics, diagnosis and management in a Western Canadian allergy practice. *Allergy Asthma Clin Immunol*. 2018 Dec 18;14:85.
24. Dibbern Jr DA, Dreskin S. Urticaria and angioedema: an overview. *Immunol Allergy Clin North Am*. 2004;24:141.
25. Mozena JD, Tiñana A, Negri J, et al. Lack of a role for cross-reacting anti-thyroid antibodies in chronic idiopathic urticaria. *J Invest Dermatol*. 2010;130(7):1860–1865.
26. Antia C, Bquerizo K, Korman A, et al. Urticaria: a comprehensive review: Epidemiology, diagnosis and work-up. *JAAD*. 2018;79:599–614.
27. Katz JP, Lichtenstein GR. Rheumatologic manifestations of gastrointestinal diseases. *Gastroenterol Clin North Am*. 1998;27:533–562.
28. Parke AL. Gastrointestinal disorders and rheumatic diseases. *Curr Opin Rheumatol*. 1991;3:160–165.
29. Zanjanian MH. The intestine in allergic diseases. *Ann Allergy*. 1976;37:208–218.
30. Yacyshyn B, Meddings J, Sadowski D, et al. Multiple sclerosis patients have peripheral blood CD45RO? B cells and increased intestinal permeability. *Dig Dis Sci*. 1996;41:2493–2498.
31. Guida B, De Martino CD, De Martino SD, et al. Histamine plasma levels and elimination diet in chronic idiopathic urticaria. *Eur J Clin Nutr*. 2000;54:155–158.
32. Andrews GC. *Andrews' Diseases of the Skin.* 7th ed. WB Saunders: Philadelphia.
33. Winkelmann RK. Food sensitivity and urticaria or vasculitis. In: eds: Brostoff J, Challacombe SJ. *Food Allergy and Intolerance.* WB Saunders: Philadelphia; 602–617.
34. Ormerod AD, Reid TM, Main RA. Penicillin in milk: its importance in urticaria. *Clin Allergy*. 1987;17:229–234.
35. Wicher K, Reisman RE. Anaphylactic reaction to penicillin in a soft drink. *J Allergy Clin Immunol*. 1980;66:155–157.
36. Schwartz HJ, Sher TH. Anaphylaxis to penicillin in a frozen dinner. *Ann Allergy*. 1984;52:342–343.
37. Boonk WJ, Van Ketel WG. The role of penicillin in the pathogenesis of chronic urticaria. *Br J Dermatol*. 1982;106:183–190.
38. Lindemayr H, Knobler R, Kraft D, et al. Challenge of penicillin allergic volunteers with penicillin contaminated meat. *Allergy*. 1981;36:471–478.
39. Settipane RA, Constatine HP, Settipane GA. Aspirin intolerance and recurrent urticaria in normal adults and children. *Allergy*. 1980;35:149–154.
40. Warin RP. The effect of aspirin in chronic urticaria. *Br J Dermatol*. 1960;72:350–351.
41. Moore-Robinson M, Warin RP. Effects of salicylates in urticaria. *Br Med J*. 1967;4:262–264.
42. Champion RH, Roberts SO, Carpenter RG, et al. Urticaria and angio-oedema: a review of 554 patients. *Br J Dermatol*. 1969;81:588–597.
43. James J, Warin RP. Chronic urticaria: the effect of aspirin. *Br J Dermatol*. 1970;82:204–205.
44. Samter M, Beers Jr RF. Concerning the nature of intolerance to aspirin. *J Allergy*. 1967;40:281–291.
45. Michaelsson G, Juhlin L. Urticaria induced by preservatives and dye additives to food and drugs. *Br J Derm*. 1973;88:525–534.
46. Thune P, Granholt A. Provocation tests with antiphlogistica and food additives in recurrent urticaria. *Dermatologica*. 1975;151:360–372.
47. Doeglas HM. Reactions to aspirin and food additives in patients with chronic urticaria, including the physical urticaria. *Br J Dermatol*. 1975;93:135–144.
48. Warin RP, Smith RJ. Challenge test battery in chronic urticaria. *Br J Dermatol*. 1976;94:401–410.
49. Settipane GA, Chafee FH, Postman IM, et al. Significance of tartrazine sensitivity in chronic urticaria of unknown etiology. *J Allergy Clin Immunol*. 1976;57:541–549.
50. Kaaber K. Colouring and preservative agents and chronic urticaria. Value of a provocative trial and elimination diet. *Ugeskr Laeger*. 1978;140:1473–1476.
51. Fujita M, Yakimoto T, Aoki T, et al. Provocation tests with aspirin and sodium benzoate in urticaria. *Japan J Derm*. 1978;88:709–713.
52. Neuman I, Elian R, Nahum H, et al. The danger of "yellow dyes" (tartrazine) to allergic subjects. *Clin Allergy*. 1978;8:65–68.
53. August PJ. Successful treatment of urticaria due to food additive with sodium cromoglycate and an exclusion diet. In: Pepys J, Edward AM eds. *The Mast Cell: Its Role in Health and Disease.* Tunbridge Wells, UK: Pitman Medical

54. Meynadier J, Guilhou J, Meynadier J, et al. Chronic urticaria. Etiologic and therapeutic evaluation of 150 cases. *Ann Dermatol Venereol*. 1979;106:153–158.
55. Lindemayr H, Schmidt J. Intolerance to acetylsalicylic acid and food additives in patients suffering from chronic urticaria. *Wien Klin Wochenschr*. 1979;91:817–822.
56. Mikkelsen H, Larsen JC, Tarding F. Hypersensitivity reactions to food colours with special reference to the natural colour annatto extract (butter colour). *Arch Toxicol Suppl*. 1978;1:141–143.
57. Wutrich B, Hacki-Herrmann D. Zur atiologie der urticaria. Eine retropektive studie anhand von 316 konsekutiven fallen. *Z Hautkr*. 1980;55:102–111.
58. Gibson A, Clancy R. Management of chronic idiopathic urticaria by the identification and exclusion of dietary factors. *Clin Allergy*. 1980;10:699–704.
59. Juhlin L. Recurrent urticaria. Clinical investigation of 330 patients. *Br J Dermatol*. 1981;104:369–381.
60. Wutrich B, Fabro L. Acetylsalicylsaure unc lebensmittel-additiva-intleranz bei urticaria, asthma bronchiale and chronischer rhinopathie. *Schweiz Med Wochenschr*. 1981;111:1445–1450.
61. Kirchhof B, Haustein UF, Rytter M. Acetylsalicylic acid: additive intolerance phenomenon in chronic recurring urticaria. *Dermatol Monatsschr*. 1982;168:513–519.
62. Egyedi K, Torok L. Nachweis einer intoleranz von lebensmittel-additvstoffen durch proben mit salizylaten bei chronischer urticaria. *Allergologie*. 1982;5:234–235.
63. Merk Goerz G. Analgetika-intoleranz. *Z Hautkr*. 1983;58:535–554.
64. Hannuksela M. Food allergy and skin diseases. *Ann Allergy*. 1983;51:269–271.
65. Ortolani C, Pastorello E, Luraghi MT, et al. Diagnosis of intolerance to food additives. *Ann Allergy*. 1984;53:587–591.
66. Simon RA. Adverse reactions to drug additives. *J Allergy Clin Immunol*. 1984;74:623–630.
67. Genton C, Frei PC, Pecoud A. Value of oral provocation tests to aspirin and food additives in the routine investigation of asthma and chronic urticaria. *J Allergy Clin Immunol*. 1985;76:40–45.
68. Pigatto PD, Riva F, Cattaneo A, et al. Chronic urticaria: clinico-therapeutic study on 300 cases. *G Ital Dermatol Venereol*. 1985;120:113–117.
69. Kemp AS, Schembri G. An elimination diet for chronic urticaria of childhood. *Med J Aust*. 1985;143:234–235.
70. Ziegler B, Haustein UF. Intolerance reactions to non-steroidal antiphlogistics and analgesics in chronic recurrent urticaria. *Dermatol Monatsschr*. 1986;172:313–317.
71. Supramaniam G, Warner JO. Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet*. 1986;2:907–909.
72. Perry CA, Dwyer J, Gelfand JA, et al. Health effects of salicylates in foods and drugs. *Nutr Rev*. 1996;54:225–240.
73. Grattan CE. Aspirin sensitivity and urticaria. *Clin Exp Dermatol*. 2003;28:123–127.
74. Bjarnason I, Williams P, Smethurst P, et al. Effect of non-steroidal antiinflammatory drugs and prostaglandins on the permeability of the human small intestine. *Gut*. 1986;27:1292–1297.
75. Asad SI, Youlten LJ, Lessof MH. Specific desensitization in aspirin sensitive urticaria; plasma prostaglandin levels and clinical manifestations. *Clin Allergy*. 1983;13:459–466.
76. Kowalski ML, Grzelewski-Ryzmowski I, Roznieki J, et al. Aspirin tolerance induced in aspirin-sensitive asthmatics. *Allergy*. 1984;39:171–178.
77. Scalese MJ, Reinaker TS. Pharmacologic management of angioedema induced by angiotensin-converting enzyme inhibitors. *Am J Health Syst Pharm*. 2016;73:873–879.
78. Kousha T, Valacchi G. The air quality health index and emergency department visits for urticaria in Windsor, Canada. *J Toxicol Environ Health A*. 2015;78(8):524–533. PubMed PMID: 25849769.
79. Shutty B, Swender D, Chernin L, Tcheurekdjian H, Hostoffer R. Insect repellants and contact urticaria: differential response to DEET and picaridin. *Cutis*. 2013;91(6):280–282. PubMed PMID: 23837149.
80. Mattila L, Kilpeläinen M, Terho EO, et al. Food hypersensitivity among Finnish university students: association with atopic diseases. *Clin Exp Allergy*. 2003;33:600–606.
81. Atkins FM. The basis of immediate hypersensitivity reactions to foods. *Nutrition Rev*. 1983;41:229–234.
82. Wraith DG, Merrett J, Roth A, et al. Recognition of food-allergic patients and their allergens by the RAST technique and clinical investigation. *Clin Allergy*. 1975;9:25–36.
83. Golbert TM, Patterson R, Pruzansky JJ. Systemic allergic reactions to ingested antigens. *J Allergy*. 1969;44:96–107.
84. Galant SP, Bullock J, Frick OL. An immunological approach to the diagnosis of food sensitivity. *Clin Allergy*. 1973;3:363–372.
85. Pachor ML, Andri L, Nicolis F, et al. Elimination diet and challenge test in diagnosis of food intolerance. *Italian J Med*. 1986;2:1–6.
86. Treudler R, Tebbe B, Steinhoff M, et al. Familial aquagenic urticaria associated with familial lactose intolerance. *J Am Acad Dermatol*. 2002;47:611–613.
87. Zuberbier T, Pfrommer C, Specht K, et al. Aromatic components of food as novel eliciting factors of pseudoallergic reactions in chronic urticaria. *J Allergy Clin Immunol*. 2002;109:343–348.
88. Henz BM, Zuberbier T. Most chronic urticaria is food-dependent, and not idiopathic. *Exp Dermatol*. 1998;7:139–142.
89. Rawls WB, Ancona VC. Chronic urticaria associated with hypochlorhydria or achlorhydria. *Rev Gastroenterol*. 1951;18:267–271.
90. Baird PC. Etiology and treatment of urticaria: diagnosis, prevention and treatment of poison-ivy dermatitis. *N Engl J Med*. 1941;224:649–658.
91. Allison JR. The relation of hydrochloric acid and vitamin B complex deficiency in certain skin diseases. *South Med J*. 1945;38:235–241.
92. Gloor M, Heinkel K, Schulz U. Pathogenetic significance of gastric functional disorders in allergic, chronic urticaria. *Dermatol Monatsschr*. 1972;158:96–102.
93. Husz S, Berko G, Szabo, et al. Immunoelectrophoresis in the dermatologic practice. III. Dysproteinemias (chronic urticaria, drug allergy). *Dermatol Monatsschr*. 1974;160:93–100.
94. Zuberbier T, Chantraine-Hess S, Hartmann K, et al. Pseudoallergen-free diet in the treatment of chronic urticaria: a prospective study. *Acta Derm Venereol (Stockh)*. 1995;75:484–487.
95. Lockey SD. Allergic reactions to F D and C Yellow No. 5, tartrazine, an aniline dye used as a coloring and identifying agent in various steroids. *Ann Allergy*. 1959;17:719–721.
96. Collins-Williams C. Clinical spectrum of adverse reactions to tartrazine. *J Asthma*. 1985;22:139–143.
97. Lessof MH. Reactions to food additives. *Clin Exp Allergy*. 1995;25(suppl 1):27–28.
98. Warrington RJ, Sauder PJ, McPhillips S. Cell-mediated immune responses to artificial food additives in chronic urticaria. *Clin Allergy*. 1986;16:527–533.
99. Natbony SF, Phillips ME, Elias JM, et al. Histologic studies of chronic idiopathic urticaria. *J Allergy Clin Immunol*. 1983;71:177–183.
100. Juhlin L. Additives and chronic urticaria. *Ann Allergy*. 1987;59:119–123.
101. Swain AR, Dutton SP, Truswell AS. Salicylates in foods. *J Am Diet Assoc*. 1985;85:950–960.
102. Birkmayer JGD, Beyer W. Biological and clinical relevance of trace elements. *Artzl Lab*. 1990;36:284–287.
103. Moneret-Vautrin DA, Faure G, Bene MC. Chewing-gum preservative induced toxidermic vasculitis. *Allergy*. 1986;41:546–548.
104. Yang WH, Purchase EC. Adverse reactions to sulfites. *CMAJ*. 1985;133:865–880.
105. Vally H, Misso NL, Madan V. Clinical effects of sulphite additives. *Clin Exp Allergy*. 2009;39(11):1643–1651.
106. Vaida GA, Goldman MA, Bloch KJ. Testing for hepatitis B virus in patients with chronic urticaria and angioedema. *J Allergy Clin Immunol*. 1983;72:193–198.
107. Schade C, Kaben U, Westphal HJ. Occurrence of yeasts and therapeutic results in chronic urticaria. *Dermatol Monatsschr*. 1975;161:187–195.
108. Holti G. Management of pruritus and urticaria. *Br Med J*. 1967:155–158.
109. Serrano H. Hypersensitivity to "candida albicans" and other fungi in patients with chronic urticaria. *Allergol Immunopathol*. 1975;3:289–298.
110. James J, Warin RP. An assessment of the role of *Candida albicans* and food yeast in chronic urticaria. *Br J Dermatol*. 1971;84:227–237.

111. Rives H, Pellerat J, Thivolet J. Chronic urticaria and Quincke's oedema. 100 case reports: allergology and therapeutic results. *Dermatologica*. 1972;144:193–204.
112. Vivarelli I, Mancosu A. Data on 2 therapeutic trends in the treatment of urticaria: desensitization to *Candida*. Use of vitamin K. *Minerva Dermatol*. 1967;42:441–442.
113. Westphal HJ, Schade C, Kaben U. Specific desensitization in patients with chronic urticaria due to yeasts and intestinal colonization of yeasts. *Dermatol Monatsschr*. 1976;162:912–915.
114. Tuchinda P, Kulthanan K, Chularojanamontri L, et al. Relationship between vitamin D and chronic spontaneous urticaria: a systematic review. *Clin Transl Allergy*. 2018;8:51.
115. Green G, Koelsche G, Kierland R. Etiology and pathogenesis of chronic urticaria. *Ann Allergy*. 1965;23:30–36.
116. Shertzer CL, Lookingbill DP. Effects of relaxation therapy and hypnotizability in chronic urticaria. *Arch Dermatol*. 1987;123:913–916.
117. Hannuksela M, Kokkonen EL. Ultraviolet light therapy in chronic urticaria. *Acta Derm Venereol*. 1985;65:449–450.
118. Olafsson JH, Larko O, Roupe G, et al. Treatment of chronic urticaria with PUVA or UVA plus placebo: a double-blind study. *Arch Derm Res*. 1986;278:228–231.
119. Chapman EW, Maloof F. Bizarre clinical manifestations of hyperthyroidism. *N Engl J Med*. 1956;254:1–5.
120. Caravati Jr CM, Richardson DR, Wood BT, et al. Cutaneous manifestations of hyperthyroidism. *South Med J*. 1969;62:1127–1130.
121. Isaacs NJ, Ertel NH. Urticaria and pruritus: uncommon manifestations of hyperthyroidism. *J Allergy Clin Immunol*. 1971;48:73–81.
122. Rumbyrt JS, Schocket AL. Chronic urticaria and thyroid disease. *Immunol Allergy Clin North Am*. 2004;24:215.
123. Leznoff A, Sussman GL. Syndrome of idiopathic chronic urticaria and angioedema with thyroid autoimmunity: a study of 90 patients. *J Allergy Clin Immunol*. 1989;84:66–71.
124. Cusack C, Gorman DJ. Role of thyroxine in chronic urticaria and angio-oedema. *J R Soc Med*. 2004;97:257.
125. Liang SE, Hoffmann R, Peterson E, et al. Use of dapsone in the treatment of chronic idiopathic and autoimmune urticaria. *JAMA Dermatol*. 2019;155(1):90–95.
126. Incorvaia C, Mauro M, Makri E, et al. Two decades with omalizumab: what we still have to learn. *Biologics*. 2018;12:135–142.
127. Gray SL, Anderson ML, Dublin S, et al. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Intern Med*. 2015;175(3):401–407.
128. Bishnoi A, Parsad D, Vinay K, et al. Phototherapy using narrowband ultraviolet B and psoralen plus ultraviolet A is beneficial in steroid-dependent antihistamine-refractory chronic urticaria: a randomized, prospective observer-blinded comparative study. *Br J Dermatol*. 2017;176(1):62–70.
129. Chen CJ, Yu HS. Acupuncture treatment of urticaria. *Arch Dermatol*. 1998;134:1397–1399.
130. Chen CJ, Yu HS. Acupuncture, electrostimulation, and reflex therapy in dermatology. *Dermatol Ther*. 2003;16:87–92.
131. Lai X. Observation on the curative effect of acupuncture on type I allergic diseases. *J Tradit Chin Med*. 1993;13:243–248.
132. Yuan SF, Guan JZ, Hao Y, et al. Effectiveness and safety of the Combination of the traditional Chinese medicine prescription jade screen and desloratadine in the treatment of chronic urticaria: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Complement Alternat Med*. 2017;2017:1390301.
133. Yao Q, Li S, Liu X, et al. The effectiveness and safety of acupuncture for patients with chronic urticaria: a systematic review. *Biomed Res Int*. 2016;2016:5191729.
134. Simon SW. Vitamin B_{12} therapy in allergy and chronic dermatoses. *J Allergy*. 1951;22:183–185.
135. Simon SW, Edmonds P. Cyanocobalamin (B12): Comparison of aqueous and repository preparations in urticaria: possible mode of action. *J Am Geriatr Soc*. 1964;12:79–85.
136. Mete N, Gulbahar O, Aydin A, et al. Low B12 levels in chronic idiopathic urticaria. *J Investig Allergol Clin Immunol*. 2004;14(4):292–299.
137. Canonica GW, Ciprandi G, Bagnasco M, et al. Oral cromolyn in food allergy: in vivo and in vitro effects. *Clin Immunol Immunopathol*. 1986;41:154–158.
138. Healy E, Newell L, Howarth P, et al. Control of salicylate intolerance with fish oils. *Br J Dermatol*. 2008;159(6):1368–1369.
139. Nettis E, Di Leo E, Pastore A, et al. Probiotics and refractory chronic spontaneous urticaria. *Eru Ann Allergy Clin Immunol*. 2016;48(5):182–187.
140. Valks R, Conde-Salazar L, Cuevas M. Allergic contact urticaria from natural rubber latex in healthcare and non-healthcare workers. *Contact Dermatitis*. 2004;50:222–224.
141. Zuberbier T, Aberer W, Asero R, et al. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*. 2014;69:868–887.
142. Antia C, Bquerizo K, Korman A, et al. Urticaria: a comprehensive review: Epidemiology, diagnosis and work-up. *JAAD*. 2018;79:599–614.
143. Kozel MM, Bossuyt PM, Mekkes JR, et al. Laboratory tests and identified diagnoses in patients with physical and chronic urticaria and angioedema: a systematic review. *J Am Acad Dermatol*. 2003;48:409–416.
144. Guida B, De Martino CD, De Martino SD, et al. Histamine plasma levels and elimination diet in chronic idiopathic urticaria. *Eur J Clin Nutr*. 2000;54(2):155–158.
145. Bahmer JA, Kuhl J, Bahmer FA. How do personality systems interact in patients with psoriasis, atopic dermatitis and urticaria? *Am J Clin Hypn*. 2018;61(1):34–44.
146. Shenefelt PD. Mindfulness-based Cognitive Hypnotherapy and skin disorders. *Acta Derm Venereol*. 2007;87(4):317–324.
147. Ros AM, Juhlin L, Michaelsson G. A follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes. *Br J Dermatol*. 1976;95:19–24.
148. Doeglas HM. Dietary treatment of patients with chronic urticaria and intolerance to aspirin and food additives. *Dermatologica*. 1977;154:308–310.
149. Valverde E, Vich JM, Garcia-Calderon JV, et al. In vitro stimulation of lymphocytes in patients with chronic urticaria induced by additives and food. *Clin Allergy*. 1980;10:691–698.
150. Verschave A, Stevens E, Degreef H. Pseudo-allergen free diet in chronic urticaria. *Dermatologica*. 1983;167:256–259.

Uterine Fibroids

Joseph Katzinger, ND, and *Tori Hudson*, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Most are asymptomatic.
- Symptoms include a vague feeling of discomfort, pressure, congestion, bloating, heaviness; can include pain with vaginal sexual activity, urinary symptoms (urgency, frequency, and/or incontinence), backache, abdominal enlargement, dysmenorrhea, pelvic pain, and abnormal bleeding.
- Abnormal bleeding occurs in 30% of women with fibroids; heavy bleeding may lead to severe anemia, which can be life-threatening.[1]
- Fibroids may also impair fertility and increase the risk for miscarriage, as well as the risk for preterm delivery (odds ratio [OR], 1.5) and cesarean section (OR, 3.7).[2]
- Fibroids can undergo degenerative changes with necrosis, resulting in cystic degeneration.
- Calcification can occur.
- Examination is by pelvic palpation and/or pelvic ultrasonography.
- The main diagnostic consideration is differentiating a possible fibroid from a malignant ovarian tumor, abscess in the fallopian tube/ovarian region, diverticulum from the colon, pelvic kidney, endometriosis, adenomyosis, congenital anomalies, and uterine sarcoma.

GENERAL CONSIDERATIONS

Uterine fibroids are not actually fibrous but consist of muscle, both smooth muscle cells and large amounts of extracellular matrix, which includes collagen, fibronectin, and proteoglycans.[3] The growth of fibroids is thought to be stimulated by estrogen. The tendency of fibroids to arise during the reproductive years, grow during pregnancy, and regress postmenopausally implicates estrogen as one factor in their cause and growth. Fibroids often demonstrate a growth spurt in the perimenopausal years, likely because of anovulatory cycles with a relative estrogen excess that commonly occurs irregularly during this time. Somewhat surprisingly, testosterone appears to influence the risk for fibroids also; higher levels have been associated with both an increase in the risk for incident fibroids among midlife women with no prior history and a decrease in the risk for recurrent fibroids (when combined with high estradiol).[4]

Uterine fibroids occur in 50% to 60% of women by age 40 and as many as 70% to 80% of women by age 50, with African American women experiencing a higher incidence.[5] This is twice the prevalence as in the first edition of the *Textbook of Natural Medicine*. Fibroids are the most common indication for major surgery in women and the most common solid tumor in women.

The cause of uterine fibroids remains poorly understood. Increases in local estradiol concentration within the fibroid itself may play a role in its cause and growth. Concentrations of estrogen receptors in fibroid tissue are higher than in the surrounding myometrium but lower than in the endometrium. Risk factors include early menarche, obesity, a higher intake of red meat, and more (see Fig. 223.1).

Fifty percent to 80% of fibroids do not cause symptoms. Abnormal bleeding, including menorrhagia and metrorrhagia, occurs in 30% of women with fibroids, is worse among African American women, and in some cases the resulting anemia may be life-threatening.[6] Other symptoms are pelvic pressure, bloating, congestion, urinary frequency and urgency, backache, and pain with vaginal sexual activity. Sometimes the urinary complications may be a cause for concern because they may be due to compression of the ureter, which can then cause hydronephrosis. Fibroids are thought to be the cause of 2% to 10% of cases of infertility, and myomectomy has been associated with a 68% increase in pregnancy rates among previously infertile women.[7] Large fibroids can also interfere with a normal pregnancy by disrupting fetal growth or causing premature rupture of membranes, retained placenta, postpartum hemorrhage, abnormal labor, or an abnormal fetal lie. The risk for caesarian section is nearly fourfold higher among women with fibroids, and the risk for placental abruption is over threefold higher.[8] The incidence of miscarriage due to fibroids (most commonly submucosal fibroids) is estimated to be two to three times greater than that in women without fibroids.

*Previous edition contributor

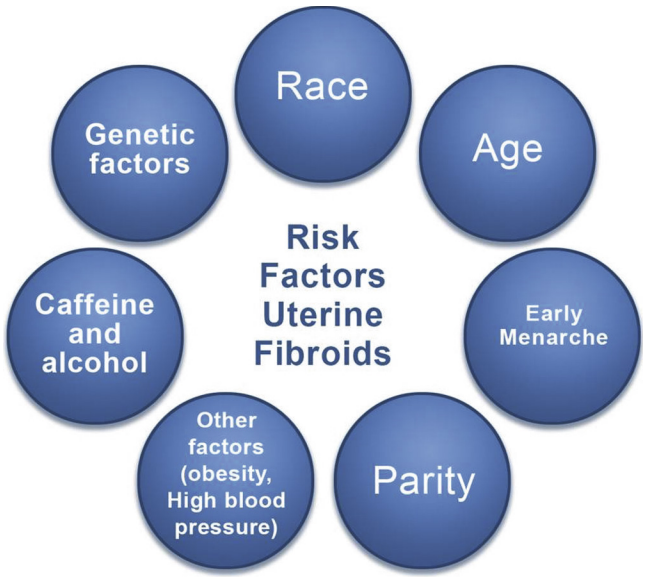


Fig. 223.1 Risk factors for uterine fibroids. These include race, age, delayed pregnancy, early menarche, parity (protective effect), caffeine, genetic alterations, and others, such as obesity and a diet rich in red meat. (From Donnez J, Dolmans MM. Uterine fibroid management: from the present to the future. *Hum Reprod Update*. 2016 Nov;22[6]:665–686. PMID: 27466209. Reproduced from an Open Access article distributed under the terms of the Creative Commons Attribution License [http://creativecommons.org/licenses/by/4.0/].)

Fibroids can undergo degenerative changes. One type of degenerative change occurs when the continued growth of the fibroid outpaces the blood supply. This occurs in approximately 10% of pregnant women with fibroids, and the resultant ischemia and necrosis can be painful. A more common type of degenerative change involves a loss of cellular detail as a result of a decrease in the tumor's vascularity. Necrosis leads to cystic degeneration. Calcification can occur over time and is usually seen in postmenopausal women.

Environmental Endocrine Disrupters

Consistent with a hormonal influence on fibroma development, environmental exposures to endocrine-disrupting pollutants also influence risk. Analysis of the 1999–2004 National Health and Nutrition Examination Survey (NHANES) data found that higher exposure to monobutyl phthalate (MBP) was associated with a 56% higher risk for self-reported fibroids, whereas another phthalate metabolite (mono(2-ethylhexyl) phthalate [MEHP]) was associated with a 37% reduction in risk.[9] Subsequent studies have also found phthalate exposure to be positively associated with uterine volume among women undergoing surgery for fibroid treatment, and phthalates may have a combined effect with genetic factors, including a nearly sixfold increase in risk among women with high exposure and the GSTM1 null genotype.[10,11] Other persistent organic pollutants, including polychlorinated biphenyl (PCBs), have been associated with an elevated risk for fibroid incidence.[12]

DIAGNOSIS

On the basis of mitotic count, nuclear atypia, and other morphological features, uterine smooth muscle tumors can be classified as leiomyomas, smooth muscle tumors of uncertain malignant potential, and leiomyosarcomas.

A presumptive diagnosis of fibroids can often be made simply with a thorough history and adequate pelvic examination. An enlarged, firm uterus or a uterus with irregular edges is the main palpation finding. The diagnostician must distinguish a possible fibroid from a malignant ovarian tumor, fallopian tube or ovarian-region abscess, diverticulum, pelvic kidney, endometriosis, congenital anomalies, pelvic adhesions, and a retroperitoneal tumor (rare). Not all of these distinctions can be made with a medical history, physical examination, and pelvic ultrasonography. Submucosal pedunculated fibroids can be visualized with laparoscopy, as can intramural and subserous fibroids.

Numerous classifications for uterine fibroids exist, typically according to their location, as follows:

- Submucosal (just under the endometrium)
- Intramural (within the uterine muscle wall)
- Subserosal (from the outer wall of the uterus)
- Interligamentous (in the cervix between the two layers of the broad ligament)
- Pedunculated (on a stalk, either submucosal or subserous)

More recent classification systems, such as that approved by the International Federation of Gynecology and Obstetrics (FIGO) Executive Board, also include hybrid types and specify the degree of intramural extension and/or uterine cavity distortion.[13]

THERAPEUTIC CONSIDERATIONS

Natural therapies for uterine fibroids largely involve managing any troublesome symptoms. Women who are seeking an alternative to surgical intervention for uterine fibroids will not find a reliable alternative to shrink their tumors. The following therapies may be able to help keep a fibroid from growing larger and are usually able to successfully resolve or improve most symptoms related to the tumor. Expectations to reduce the size of fibroids must be low, although there are individual case reports of such results. Reduction in fibroid size due to treatment is difficult to assess, especially in perimenopausal women in their 40s, who at some point will have lower endogenous estrogen production, with resultant diminution of the fibroid.

Diet

No dietary approach is known to prevent or shrink uterine fibroids. However, there are very strong rationales for several dietary interventions. For example, a diet high in saturated fats is associated with higher blood levels of estrogen and therefore could have the potential to exacerbate fibroids. Also, low-fiber diets are associated with elevated estrogen levels and poor excretion of estrogen. Dietary change by itself is unlikely to reduce the size of fibroids, but good dietary habits are still important for general health; moreover, they may reduce some of the gastrointestinal symptoms that can be associated with large fibroids or pedunculated fibroids and an enlarged uterus. Furthermore, specific foods and dietary patterns have been associated with a reduced risk for fibroid development and may play a role in preventing a recurrence. Specifically, greater consumption of fruits and vegetables appears protective, and a higher intake of broccoli, cabbage (cruciferous vegetables), tomato, and apple has been associated with lower risk.[14,15] Surprisingly, dietary omega-3 intake has been associated with a slightly greater risk for fibroid development, at least among African American women.[16]

Clinical observation has demonstrated that natural therapies work best in the context of a healthy lifestyle, including dietary changes. Improving one's diet can help to decrease heavy bleeding or the pain and discomfort caused by the fibroids. In addition to these potential benefits, dietary improvements enhance general well-being.

The tradition of naturopathic medicine holds firmly to the influence of the health of the liver on the health and vitality of an individual. The liver metabolizes estrogen, which can be eliminated from the body by

converting it to estrone and finally to estriol, a weaker form of estrogen that has very little ability to stimulate the uterus. Failure of the liver to effectively metabolize estradiol may be one mechanism by which the uterus becomes overestrogenized and responds with fibroid growths. Support for the role of estrogen also stems from genetic associations with fibroma risk; polymorphisms in the *COMT, CYP1A1,* and *CYP1B1* genes all have been linked to a two- to threefold increase in risk for fibromas and are involved in estrogen metabolism. Similarly, in one small study, women with homozygous polymorphisms in both *CYP17A1* (estrogen synthesis) and estrogen receptor 1 *(ESR1)* had nearly a twentyfold increase in the risk for fibroids compared with controls.[17,18]

Saturated fats, sugar, caffeine, alcohol, and "junk foods" are presumably problematic in two main ways. First, they interfere with the body's ability to metabolize estradiol to estrone to estriol. Second, some of them are deficient in B vitamins or interfere with B-vitamin metabolism. If B vitamins are lacking in the diet, the liver will lack some of the raw materials it needs to carry out its metabolic processes and regulate estrogen levels.

Whole grains—such as brown rice, oats, buckwheat, millet, and rye—are excellent sources of B vitamins. Whole grains also help the body excrete estrogens through the bowel. The role of whole-grain fiber in lowering estrogen levels was first reported in 1982.[19] This study found that vegetarian women who eat a high-fiber, low-fat diet have lower blood estrogen levels than omnivorous women with low-fiber diets. Once again, the logic of a high-fiber diet would have implications in preventing and perhaps reducing uterine fibroids because of the stimulating effect of estrogen on fibroid growth.

A high-fiber diet may also help to relieve some of the bloating and congestion that are sometimes associated with fibroids. Fiber, by bulking up the stool and regulating bowel movements, may relieve some of these symptoms. Some women have a hard time tolerating increased fiber in their diets because their digestive function is otherwise compromised.

Because there is an association between having uterine fibroids and a fourfold increase in the risk of endometrial cancer,[19] three dietary considerations stand out above the others: to increase dietary fiber, lower dietary fat, and increase the intake of soy products and other legumes, although this last component has come into question because it may contribute to fibroid incidence. A case-control study in a multiethnic population (Japanese, white, Native Hawaiian, Filipino, and Chinese) examined the roles of dietary soy, fiber, and related foods and nutrients in relation to the risk of endometrial cancer.[20] In total 332 women with endometrial cancer were compared with women in the general multiethnic population, and all were interviewed by means of a dietary questionnaire. The researchers found positive associations between a higher level of fat intake and endometrial cancer, between a higher level of fiber intake and a reduction in the risk for endometrial cancer, and between a higher consumption of soy products and other legumes and a decreased risk of endometrial cancer. Similar reductions in risk were found for greater consumption of other sources of phytoestrogens, such as whole grains, vegetables, fruits, and seaweeds. The researchers concluded that plant-based diets low in calories from fat, high in fiber, and rich in legumes (especially soybeans), whole-grain foods, vegetables, and fruits reduce the risk of endometrial cancer. These dietary associations may explain, at least in part, the lower rates of uterine cancer in Asian countries compared with the United States. Recent studies have supported this protective benefit; a study among women in northern Italy found that a higher consumption of vegetables is associated with roughly only one third the risk for endometrial cancer, and adherence to the Mediterranean diet is associated with approximately one-half the risk.[21] In 2019, data from the Women's Health Initiative showed that the "healthy lifestyle index," a composite of diet, alcohol consumption and tobacco use, and physical activity and body mass index (BMI), was associated with a reduced risk for endometrial cancer overall as well as cancer subtypes.[22]

Some have suggested that because soy foods are high in phytoestrogens (specifically isoflavones), which have a weak estrogenic effect, women with uterine fibroids or endometrial cancer should avoid phytoestrogens. This issue deserves some clarification. Soy phytoestrogens do not appear to have an estrogenic effect on the human uterus except in doses higher than 150 mg isoflavones daily in postmenopausal women. They appear to be selective in terms of the tissues on which they have an estrogenic effect and the tissues or organs on which they have an antiestrogenic effect. Soy foods may be analogous to a class of drugs called selective estrogen-receptor modulators: they act one way in one part of the body and another way in a different part of the body. It seems that in the uterus, soy isoflavones have an antiestrogenic effect, although recent clinical data show their relation to fibroids as more complicated.

Nutritional Supplements

Many of the symptoms related to uterine fibroids can be effectively treated with natural therapies. This section presents nutritional supplements that may control or diminish the growth of fibroids. Unfortunately, these recommendations are based more on tradition, theory, logic, and clinical experiences than on scientific evidence.

Lipotropic Factors

Supplements such as inositol and choline exert a lipotropic effect—that is, they promote the removal of fat from the liver. Lipotropic supplements usually are a combination vitamin-and-herbal formulation and sometimes an animal liver extract designed to support the liver's function in removing fat, detoxifying the body's wastes, detoxifying external harmful substances (pesticides, fossil fuels, etc.), and metabolizing and excreting estrogens. These lipotropic products vary in their formulations, depending on the manufacturer, but they are all similar and are intended for the same uses. Many now contain anticancer phytonutrients found in cabbage-family vegetables, such as indole-3-carbinol (I3C), diindolylmethane (DIM), and sulforaphane. Research has shown that these compounds help to break down cancer-causing forms of estrogens to nontoxic forms, making them especially important in women with uterine fibroids.[23,24]

Pancreatic Enzymes

Supplementation with pancreatic enzymes is usually done to treat pancreatic insufficiency with symptoms of abdominal bloating, gas, indigestion, undigested food in the stool, and malabsorption. The logic for their use in the treatment of uterine fibroids is that the pancreatic enzymes help digest the fibrous/smooth muscle tissue and thus dissolve the fibroids. When used for this purpose, the pancreatic enzyme supplement must be taken between rather than with meals.

Vitamin D

In recent years, much research has been done on the role of vitamin D deficiency as a risk factor for fibroids, suggesting that vitamin D physiology may affect fibroma development through a variety of pathways (Fig. 223.2). Furthermore, unlike most other natural therapies for fibroma treatment, there is some clinical data to suggest that vitamin D supplementation may have efficacy as treatment, not just prevention. Vitamin D supplementation was shown to reduce the progression to extensive disease among women with "small burden" fibroids; in this group, only 13.2% receiving vitamin D needed surgical or medical treatment after 1 year, versus 30.9% in the control group.[25] It has been suggested that a target concentration of 40 to 60 ng/mL 25-OH vitamin D may be optimal.[26]

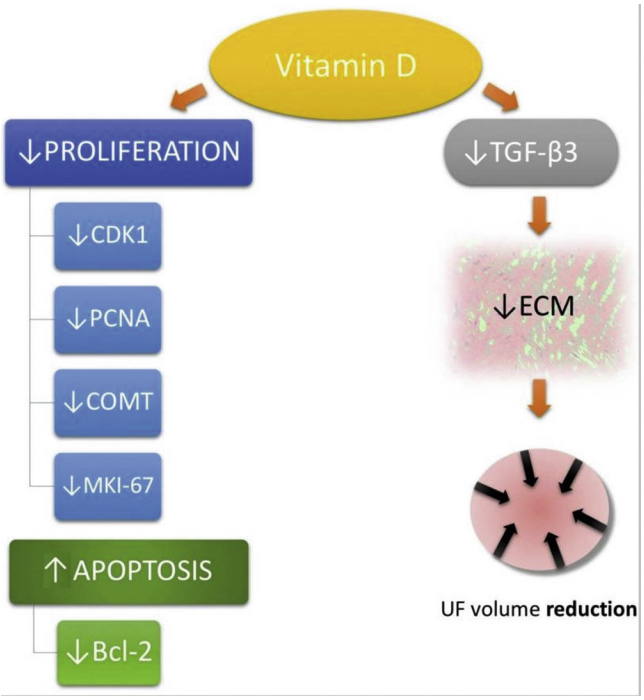


Fig. 223.2 Vitamin D influence on uterine fibroid pathophysiological pathways. *CDK1,* Cyclin-dependent kinase 1; *COMT,* catechol-O-methyltransferase; *ECM,* extracellular matrix; *MKI-67,* Bcl-2 protein, proliferation marker protein Ki-67; *PCNA,* proliferating cell nuclear antigen; *TGF-β3,* transforming growth factor-beta; *UF,* uterine fibroid. (From Ciebiera M, Włodarczyk M, Ciebiera M, et al. Vitamin D and uterine fibroids—review of the literature and novel concepts. *Int J Mol Sci.* 2018 Jul 14;19[7]: E2051. PMID: 30011902. Reproduced from an open-access article distributed under the terms and conditions of the Creative Commons Attribution [CC BY] license [http://creativecommons.org/licenses/by/4.0/].)

Traditional Botanical Medicines

Many plants have been used in traditional herbal medicines in an attempt to treat women with uterine fibroids. The plants and herbal formulations described here are used in attempting to reduce uterine fibroids or slow their growth. They have been anecdotally reported to achieve modest success in reducing the size and number of uterine fibroids. There is no scientific research to document the outcomes, but the protocol is presented here as an option.

Scudder's Alterative

Scudder's alterative should be used at 30 to 40 drops in a small amount of warm water three times daily. Its components are as follows:

- Corydalis tubers *(Dicentra canadensis)*
- Black alder bark *(Alnus serrulata)*
- Mayapple root *(Podophyllum peltatum)*
- Figwort flowering herb *(Scrophularia nodosa)*
- Yellow dock root *(Rumex crispus)*

Compounded Echinacea/Red Root

- Echinacea (*Echinacea* spp.)
- Red root *(Ceanothus americanus)*
- Baptisia root *(Baptisia tinctoria)*
- Thuja leaf *(Thuja occidentalis)*
- Stillingia root *(Stillingia sylvatica)*
- Blue flag root *(Iris versicolor)*
- Prickly ash bark *(Xanthoxylum clava-herculus)*

Compounded Fraxinus/Ceonothus

The constituents of compounded *Fraxinus/Ceonothus* are as follows:

- Mountain ash bark *(Fraxinus americanus)*
- Red root *(C. americanus)*
- Life root *(Senecio aureus)*
- Mayapple root *(P. peltatum)*
- Helonias root *(Chamaelirium luteum)*
- Goldenseal root *(Hydrastis canadensis)*
- Lobelia *(Lobelia inflata)*
- Ginger root *(Zingiber officinalis)*

Compounded *Gelsemium/Phytolacca*

Compounded *Gelsemium/Phytolacca* is also known as the Turska formula. It has the following constituents:

- Gelsemium root *(Gelsemium sempervirens)*
- Poke root *(Phytolacca americana)*
- Aconite *(Aconitum napellus)*
- Bryonia root *(Bryonia dioica)*

Other Herbal Extracts to Consider

- Chaste tree *(Vitex agnus castus)*
- Nettles *(Urtica dioica)*
- Burdock root *(Arctium lappa)*
- Dandelion root *(Taraxacum officinalis)*
- Oregon grape *(Berberis aquifolium)*
- Crila *(Crinum latifolium)*
  - Crila has been a part of Vietnamese history and folklore for generations. Numerous benefits of *Crinum* have been reported over the years, but none had been clinically studied until recently. Of the 12 varieties of Crila, one specific variety in particular, the *C. latifolium* "Tram," named after the leading researcher, has been studied in women with uterine fibroids. A 3-month study of 195 women with uterine fibroid tumors was conducted in three hospitals in Vietnam in 2007. Crila decreased the size or stopped the growth of the fibroid tumors in 79.5% of the women. In 20.5%, tumor growth continued at a very slow rate. Whereas a heavy menstrual flow was reported by 36% of the women before taking Crila, this had decreased to only 1% after treatment. Side effects reported were slight, including nausea, headache, vaginal dryness, and hot flashes, but these decreased over time.[27]

Topical Herbal Applications

- Poke root oil: Rub onto the belly over the uterus nightly before bed for 1 month, then two to three times weekly.
- Castor oil packs: Apply over pelvis three to five times weekly.

Herbal Phytoestrogens

Three types of phytoestrogens are found in medicinal plants: resorcylic acid lactones, steroids and sterols, and phenolics. Phytoestrogens are present in virtually every plant in at least modest levels, with some plants having particularly high levels. Resorcylic acid lactones are not true phytoestrogens because they come from plant mold contamination rather than being synthesized by the plant.

Steroids are the classic steroidal estrogens, estradiol and estrone, which are found in very minute amounts in a few plants such as apple seeds, date palms, and pomegranate seeds in the range of 1 to 10 parts per billion.[28–30] Diosgenin is a steroid derivative found in at least 20 plants, including wild yam species.

Beta-sitosterol is the most common phytosterol and hence is distributed widely throughout the plant kingdom. It is found in plant oils such as wheat germ oil, cottonseed oil, and soybean oil and is the dominant phytosterol found in garlic and onions. Herbal sources include

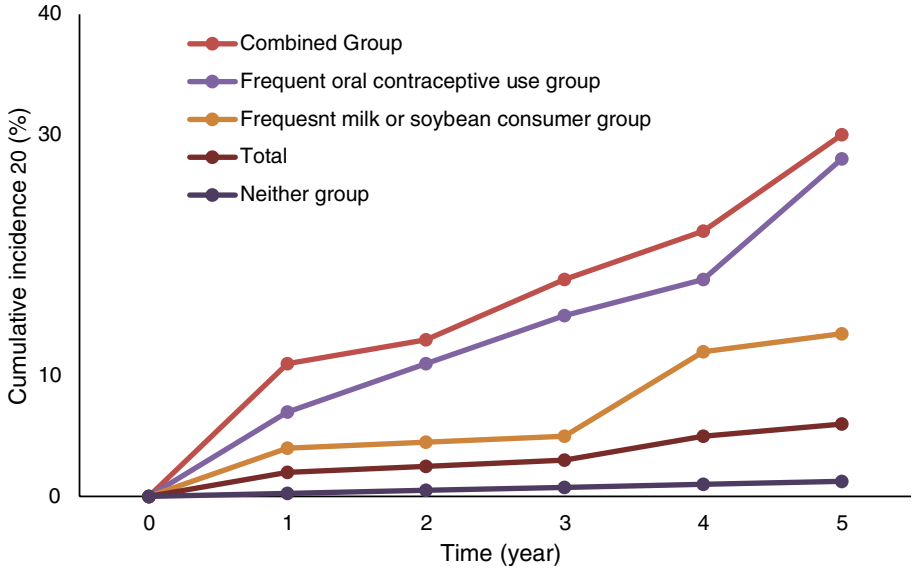


Fig. 223.3 Cumulative incidence of uterine leiomyoma. (From Gao M, Wang H. Frequent milk and soybean consumption are high risks for uterine leiomyoma: A prospective cohort study. *Medicine (Baltimore).* 2018 Oct;97[41]:e12009. PMID: 30313022. Reproduced from an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 [CCBY-NC] [http://creativecommons.org/licenses/by-nc/4.0.])

licorice root, saw palmetto, and red clover. Stigmasterol is closely related to beta-sitosterol. Soybean oil is an important source of stigmasterol; it is a better source for laboratory synthesis of progesterone than beta-sitosterol. Some herbal sources are burdock, fennel, licorice, alfalfa, anise, and sage. The phenolic phytoestrogens are members of the largest single family of plant substances, the flavonoids, which has over 4000 individual members. The term *flavonoid* derives from the Latin *flavus,* meaning "yellow," because the flavonoids are responsible for the yellow, red, white, and blue pigments in plants.

Phenolics include the following substances:

- Isoflavones, which are present in higher concentrations in legumes, especially soybeans, than in any other plants
- Coumestans, of which coumestrol is the one known estrogenic member. Coumestrol is approximately six times more estrogenic than the isoflavones.[31] Red clover is a medicinal plant with considerable coumestans.
- Lignans, found in high concentrations in grains and cereals and highest in flaxseeds

There has been some concern and controversy about how phytoestrogens affect the uterus as well as whether, because they have an estrogenic effect, they should be avoided in women with uterine fibroids or endometrial cancer. Research has shown that phytoestrogens actually reduce the incidence of uterine cancer.

Most of the research on the effects of phytoestrogens on the uterus is found in relation to the agriculture industry and the health of grazing animals. In the 1940s it was reported that the grazing of sheep on red clover in Australia was responsible for their infertility.[32] A Finnish study of pasture legumes identified red clover as containing the highest concentrations of phytoestrogens[33] and showed that abundant intake of red clover resulted in fertility problems in cattle.[34]

In one study on the effects of phytoestrogens in sheep, it was noted that both coumestans and isoflavones produce changes similar to stimulation with steroidal hormones such as estradiol in all of the target organs.[35] Among these changes was an increase in uterine weight. Other investigators have examined the binding of phytoestrogens to the uterus and vagina. Coumestrol has temporarily enhanced the uptake of estradiol by the uterus and vagina only 1 hour after injection into mice.[36] The researchers of this study also noted that coumestrol actually inhibited the uptake of estradiol by the uterus in a prolonged manner; they postulated that there was actually an inhibitory effect at the estradiol receptor sites. Other researchers have observed that coumestans and isoflavones compete with estradiol for uterine receptor sites but have less affinity for them than estradiol does.[37]

Coumestrol has been found to increase uterine weight at a 100-mcg dose when given to rats at a certain time in the development of glands.[38] The estrogenic effect of phytoestrogens appears to be dose dependent. When given in high enough doses, phytoestrogens have estrogenic effects on all the tissues that are targets of estradiol. Two questions must be answered: What is an excessively high dose, and are the observations in animals translatable to humans? One way of answering the first question is to note that countries with a high intake of phytoestrogens (Japan, Thailand, China) do not have increased rates of uterine fibroids.

As in the case of breast cancer, data on women of different cultures support the conclusion that soy phytoestrogens are not an estrogen stimulus for the endometrium. Soy is probably an estrogen antagonist in the uterus, at least at doses of less than 150 mg/day, and it is associated with low rates of endometrial cancer in countries where the intake of soy phytoestrogens is high.[39]

One recent study did raise some concern about high doses of soy phytoestrogens and endometrial effects.[40] A total of 298 women completed a 5-year study of either 150 mg/day of soy isoflavones or a placebo tablet. Of the women using 150 mg of soy isoflavones, 70% had an endometrium classified as atrophic, versus 81% of those receiving the placebo. The occurrence of endometrial hyperplasia was higher in the soy group versus the placebo group as well (3.37% vs. 0%). There were no endometrial cancers in either group. The estrogenic effect was not detectable at 30 months but only at the end of the 5-year study. A second recent study also suggests that soy may be problematic. In an analysis of nearly 1300 women (213 uterine leiomyomas), in addition to oral contraceptive use, soybean and soymilk intake was associated with an increase in risk for fibroma development (OR ,7.3), suggesting that soy intake should be limited (Fig. 223.3).[41]

Tripterygium wilfordii Hook F

An uncontrolled but well-designed study looked at the efficacy of a traditional Chinese herbal therapy, *Tripterygium wilfordii* Hook F, on uterine fibroids.[42] The researchers used several objective measures for clinical response, including ultrasonographic measurement of fibroid size and determination of blood estradiol, progesterone, testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin levels by radioimmunoassay. Measurements were taken at baseline and 3 and 6 months. Treatment was effective and time dependent; 28% of fibroids responded in 3 to 4 months and 52% in 5 to 6 months. A total of 25 of the 65 patients were amenorrheic during the course of treatment. Compared with pretreatment values, treatment with *T. wilfordii* induced a rise in mean LH and FSH levels and a decrease in mean estradiol and progesterone levels. The researchers speculated that this result was likely due to a reversible inhibitory effect on the ovaries.

Bioidentical Progesterone

Several older studies have suggested that progesterone may inhibit the growth of uterine fibroids. Lipschutz[43] demonstrated that progesterone administered to guinea pigs prevented the formation of tumors that would otherwise be induced by estrogen. In 1946 Goodman[44] reported six cases of clinically diagnosed uterine fibroids that regressed after the use of progesterone therapy. In relation to uterine fibroids, the expression and responsiveness of estrogen and progesterone receptor sites to these hormones is a complex issue.[45]

Lee[46] posited that because uterine fibroids are a result of estrogen stimulation and what he calls "estrogen dominance," the problem is a relative progesterone deficiency, and the corrective is to use natural progesterone. He asserted that estrogen dominance is a much greater problem than is recognized by conventional medicine:

> *Because many women in their mid-thirties begin to have nonovulating cycles, they are producing much less progesterone than expected but still producing normal (or more) estrogen. They retain water and salt, their breasts swell and become fibrocystic, gain weight (especially around the hips and torso), become depressed and lose sex drive; in addition, their bones suffer mineral loss and they develop fibroids. All are signs of estrogen dominance relative to a progesterone deficiency. When sufficient natural progesterone is replaced, fibroid tumors no longer grow in size (they generally decrease in size) and can be kept from growing until menopause, after which they will atrophy. This is the effect of reversing estrogen dominance.*

The preferred form of natural progesterone for treating fibroids (unless the patient has heavy bleeding) is a topical cream with about 400 mg of progesterone per one ounce of cream. One-quarter teaspoon of the cream is to be applied, one to two times daily for 1 week after the menses, and then one-quarter to one-half teaspoon twice daily for the next 2 weeks (the second half of the menstrual cycle). The progesterone cream is not used for 1 week during the menstrual flow. The cream is applied to the inner arms, chest, inner thighs, and/or palms.

Another theory and counter-opinion about the relationship of progesterone to uterine fibroids raises some questions about the use of the hormone in women with fibroids. Rein et al.[47] at Brigham and Women's Hospital published a report in 1995 stating not only that is there no evidence of estrogen directly stimulating myoma growth but also that progesterone and progestins promote the growth of fibroids.[47] The report cites the biochemical, histological, and clinical evidence supporting an important role for progesterone and progestins in the growth of uterine myomas. This comprehensive hypothesis is based on the analysis of many different technical studies and, according to the reviewers, suggests that the development and growth of myomas involve a multistep chain of events.

THERAPEUTIC APPROACH

Diet

The patient should consume a diet low in fat and high in fiber, vegetables, whole grains, and flaxseeds, consistent with a Mediterranean diet, while avoiding saturated fats, sugar, caffeine, and alcohol.

Nutritional Supplements

- Vitamin D_3: Oral supplementation sufficient to achieve a 25-OH vitamin D level of 40 to 60 ng/mL. This is likely to require at least 2000 IU/day or more for most women.
- Lipotropic factors: 1000 mg of choline and 1000 mg of methionine and/or cysteine a day. Alternatively, S-adenosylmethionine (SAMe) can be used at a dosage of 200 to 400 mg a day.
- Choose one or a combination of the following:
  - Indole-3-carbinol: 300 to 600 mg a day
  - Diindolylmethane (DIM): 100 to 200 mg a day taken with food
- Proteolytic enzymes such as mixed enzyme preparations or pancreatin (8–10× USP): 350 to 750 mg between meals three times/day; or Bromelain: 250 to 750 mg (1800–2000 MCU) between meals three times/day

Herbal Medicines

- Echinacea/red root compound: 30 drops in a small amount of warm water three times a day
- *Fraxinus*/*Ceonothus* compound: 30 drops in a small amount of warm water three times a day
- Scudder's alternative: 30 to 40 drops in a small amount of warm water three times a day
- Turska formula: 5 drops in a small amount of warm water three times a day

Topical Treatment

- Bioidentical progesterone cream: one-quarter tsp twice a day from day 15 to day 26
- Topical poke root oil over uterus every night for 1 month, then once to three times a week

Conventional Medicine Therapies

Where fibroids cause heavy bleeding, medical management with progestogens or oral contraceptives may be used to manage the bleeding. Some fibroids and uteri are large enough that presurgical treatment with leuprolide acetate (Lupron) may facilitate a less radical surgical option. High-intensity focused ultrasound is a new technique being used to treat fibroids. In addition, selective progesterone-receptor modulators are being investigated as a treatment for fibroid pain and bleeding and for reducing the size of fibroids.

Surgery

Some women will have to consider their surgical options, which are based on the size, number, and location of fibroids, as follows, although today only myomectomy and hysterectomy are commonly used:

- Myomectomy
- Laparoscopic surgery for subserous and pedunculated fibroids
- Uterine embolization
- Hysteroscopic resection of submucous fibroids
- Supracervical hysterectomy (some fibroids may lend themselves to a supracervical laparoscopic hysterectomy)
- Laparoscopy-assisted vaginal hysterectomy

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Nelson AL, Ritchie JJ. Severe anemia from heavy menstrual bleeding requires heightened attention. *Am J Obstet Gynecol*. 2015;213:97. PMID: 25935784.
2. Ezzedine D1, Norwitz ER. Are women with uterine fibroids at increased risk for adverse pregnancy outcome? *Clin Obstet Gynecol*. 2016;59(1):119–127. PMID: 26670833.
3. Donnez J1, Dolmans MM2. Uterine fibroid management: from the present to the future. *Hum Reprod Update*. 2016;22(6):665–686. PMID: 27466209.
4. Wong JY1, Gold EB1, Johnson W, et al. Circulating sex hormones and risk of uterine fibroids: Study of Women's Health Across the Nation (SWAN). *J Clin Endocrinol Metab*. 2016;101(1):123–130. PMID: 26670127.
5. Wise LA1, Laughlin-Tommaso SK. Epidemiology of uterine fibroids: from menarche to menopause. *Clin Obstet Gynecol*. 2016;59(1):2–24. PMID: 26744813.
6. Stewart EA1, Nicholson WK, Bradley L, et al. The burden of uterine fibroids for African-American women: results of a national survey. *J Womens Health (Larchmt)*. 2013;22(10):807–816. PMID: 24033092.
7. Whynott RM1, Vaught KCC2, Segars JH. The effect of uterine fibroids on infertility: a systematic review. *Semin Reprod Med*. 2017;35(6):523–532. 29100240.
8. Ezzedine D1, Norwitz ER. Are women with uterine fibroids at increased risk for adverse pregnancy outcome? *Clin Obstet Gynecol*. 2016;59(1):119–127. PMID: 26670833.
9. Weuve J1, Hauser R, Calafat AM, et al. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004. *Environ Health Perspect*. 2010;118(6):825–832. PMID: 20185384.
10. Huang PC1, Tsai EM, Li WF. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. *Hum Reprod*. 2010;25(4):986–994. PMID: 20147336.
11. Zota AR1, Geller RJ2, Calafat AM, et al. Phthalates exposure and uterine fibroid burden among women undergoing surgical treatment for fibroids: a preliminary study. *Fertil Steril*. 2019;111(1):112–121. PMID: 30447935.
12. Trabert B1, Chen Z2, Kannan K3, et al. Persistent organic pollutants (POPs) and fibroids: results from the ENDO study. *J Expo Sci Environ Epidemiol*. 2015;25(3):278–285. PMID: 24802554.
13. Munro MG, Critchley HO, Broder MS, et al. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. *Int J Gynaecol Obstet*. 2011;113(1):3–13. PMID: 21345435.
14. Parazzini F1, Di Martino M, Candiani M, et al. Dietary components and uterine leiomyomas: a review of published data. *Nutr Cancer*. 2015;67(4):569–579. PMID: 25826470.
15. Shen Y1, Wu Y1, Lu Q1, Ren M1. Vegetarian diet and reduced uterine fibroids risk: a case-control study in Nanjing, China. *J Obstet Gynaecol Res*. 2016;42(1):87–94. PMID: 26458740.
16. Wise LA1, Radin RG, Kumanyika SK, et al. Prospective study of dietary fat and risk of uterine leiomyomata. *Am J Clin Nutr*. 2014;99(5):1105–1116. PMID: 24598152.
17. Shen Y1, Xu Q, Ren M, et al. Role of single nucleotide polymorphisms in estrogen-metabolizing enzymes and susceptibility to uterine leiomyoma in Han Chinese: a case-control study. *J Obstet Gynaecol Res*. 2014;40(4):1077–1084. PMID: 24320736.
18. Huang PC1, Li WF, Liao PC, et al. Risk for estrogen-dependent diseases in relation to phthalate exposure and polymorphisms of CYP17A1 and estrogen receptor genes. *Environ Sci Pollut Res Int*. 2014;21(24):13964–13973. PMID: 25030786.
19. Goldin B, Allercreutz H, Gorbach SL, et al. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. *N Engl J Med*. 1982;307:1542–1547.
20. Goodman MT, Wilkens LR, Hankin JH, et al. Association of soy and fiber consumption with the risk of endometrial cancer. *Am J Epidemiol*. 1997;146:294–306.
21. Ricceri F1,2, Giraudo MT3, Fasanelli F, et al. Diet and endometrial cancer: a focus on the role of fruit and vegetable intake, Mediterranean diet and dietary inflammatory index in the endometrial cancer risk. *BMC Cancer*. 2017;17(1):757. PMID: 29132343.
22. Arthur R1, Brasky TM2, Crane TE, et al. Associations of a healthy lifestyle index with the risks of endometrial and ovarian cancer among women in the women's health initiative study. *Am J Epidemiol*. 2019;188(2):261–273. PMID: 30407487.
23. Michnovicz JJ, Bradlow HL. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr Cancer*. 1991;16(1):59–66.
24. Rajoria S, Suriano R, Parmar PS, et al. 3,3'-diindolylmethane modulates estrogen metabolism in patients with thyroid proliferative disease: a pilot study. *Thyroid*. 2011;21(3):299–304.
25. Ciavattini A1, Delli Carpini G, Serri M, et al. Hypovitaminosis D and "small burden" uterine fibroids: opportunity for a vitamin D supplementation. *Medicine (Baltimore)*. 2016;95(52):e5698. PMID: 28033263.
26. Ciebiera M1, Włodarczyk 3 M2, Ciebiera M, et al. Vitamin D and uterine fibroids-review of the literature and novel concepts. *Int J Mol Sci*. 2018;19(7). pii: E2051. PMID: 30011902.
27. Nguyen Thi Ngoc Tram. To evaluate the effect and possibility of accepting of CRILA in uterus fibroid tumor treatment. Vietnam National Institute of Gerontology Hospital. http://www.crilahealth.com/wp-content/uploads/2011/03/Uterus-Fibroid-Tumor-research-10-09.pdf. Accessed 9 Dec 2019.
28. Dean D, Exley D, Goodwin T. Steroid oestrogens in plants: re-estimation of oestrone in pomegranate seeds. *Phytochemistry*. 1971;10:2215–2216.
29. Verdeal R, Ryan D. Naturally-occurring oestrogens in plant foodstuffs: a review. *J Food Protection*. 1979;42:577–583.
30. Price K, Fenwick G. Naturally occurring oestrogens in foods: a review. *Food Addit Contam*. 1985;2:73–106.
31. Miksicek R. Estrogenic flavonoids: structural requirements for biological activity. *Proc Soc Exp Biol Med*. 1995;208:44–50.
32. Bennetts H, Underwood E, Shier F. A breeding problem of sheep in the south-west division of Western Australia. *J Dept Agric West Aust*. 1946;23:1–12.
33. Saloniemi H, Wahala K, Nykanen-Kurki P, et al. Phytoestrogen content and effect of legume fodder. *Proc Soc Exp Biol Med*. 1995;208:13–17.
34. Kallela K, Heinonen K, Saloniemi H. Plant oestrogens: the cause of decreased fertility in cows. A case report. *Nord Vet Med*. 1984;36:124–128.
35. Adams N. Cervical mucus and reproductive efficiency in ewes after exposure to oestrogenic pastures. *Aust J Agric Res*. 1977;28:481–489.
36. Folman Y, Pope G. Effect of norethisterone acetate, dimethylstilbestrol, genistein, and coumestrol on uptake of oestradiol by uterus, vagina, and skeletal muscle of immature mice. *J Endocrinol*. 1969;44:213–218.
37. Shutt D, Cox R. Steroid and phyto-oestrogen binding to sheep uterine receptors in vitro. *J Endocrinol*. 1972;52:299–310.
38. Medlock K, Branham W, Sheehan D. Effects of coumestrol and equol on the developing reproductive tract of the rat. *Proc Soc Exp Biol Med*. 1995;208:67–71.
39. Parkin D, Muir C, Whelan S, et al., eds. Cancer incidence in five continents. *International Agency for Research on Cancer Scientific Publications* No. 120: Lyon; 486–509.
40. Unfer V, Casini M, Costabile L, et al. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. *Fertil Steril*. 2004;82(1):145–148.
41. Gao M, Wang H. Frequent milk and soybean consumption are high risks for uterine leiomyoma: a prospective cohort study. *Medicine (Baltimore)*. 2018;97(41):e12009. PMID: 30313022.
42. Gao Y, Chen D. Clinical study on effect of Tripterygium wilfordii Hook. f. on uterine leiomyoma. *Zhonghua Fu Chan Ke Za Zhi*. 2000;35:430–432.
43. Lipschutz A. Experimental fibroids and the antifibromatogenic action of steroid hormones. *JAMA*. 1942;120:171–175.
44. Goodman A. Progesterone therapy in uterine fibromyoma. *J Clin Endocrinol Metab*. 1946;6:402–408.
45. Bodner K, Bodner-Adler B, Kimberger O, et al. Estrogen and progesterone receptor expression in patients with uterine smooth muscle tumors. *Fertil Steril*. 2004;81:1062–1066.
46. Lee J. *What Your Doctor May not Tell you About Menopause*. New York: Warner Books.
47. Rein M, Barbieri R, Friedman A. Progesterone: a critical role in the pathogenesis of uterine myomas. *Am J Obstet Gynecol*. 1995;172:14–18.

Vaginitis, Vulvovaginitis, and Vulvodynia

Mollie Parker Szybala, ND, MPH, and *Cory Szybala, ND*

OUTLINE

DIAGNOSTIC SUMMARY[1,2]

- Change in volume, color, or odor of vaginal discharge
- Localized erythema, irritation, itching, or burning
- Spotting
- Dysuria or dyspareunia may be present.
- Introitus may show patchy erythema, and vaginal mucosa may exhibit congestion or petechiae.

GENERAL CONSIDERATIONS

Vaginitis is the most common gynecological diagnosis in primary care. Most women will have at least one episode of vaginitis during their lifetime, making it the most common reason for women to seek medical attention.[1] It affects all age groups, although it is most common among women of reproductive age, and has a variety of etiologies.[3,4] To accurately diagnose vaginitis, a practitioner should include a complete history, physical examination, and laboratory evaluation as a part of the initial clinical workup.[5] The treatment for acute vaginitis is relatively simple, making it easily treatable; recurrent vaginitis, however, can be among the most troublesome and challenging conditions for both practitioners and patients alike. Early misdiagnosis or undetected coinfections (in approximately 30% of symptomatic women, no etiological agent is identified) can lead to repeat office visits, inappropriate or delayed treatment, and sometimes serious consequences, especially in pregnant women.[1]

In addition to causing physical discomfort and relationship and self-esteem issues, and negatively affecting a woman's overall quality of life, vaginitis can also lead to several known obstetrical and gynecological complications, including an increased risk of acquiring sexually transmitted infections, late miscarriage, premature rupture of membranes, premature delivery, low-birth-weight delivery, development of pelvic inflammatory disease (PID), and postoperation infections after gynecological procedures.[6–12] New research has shown that bacterial vaginosis (BV), the most common cause of vaginitis, may lead to a twofold increased risk of acquiring sexually transmitted infections, including chlamydia, gonorrhea, herpes simplex virus type 2, and HIV.[13–17] Some of the causal organisms may also be able to ascend and lead to PID. PID may in turn lead to chronic pelvic pain, tubal scarring, infertility, or ectopic pregnancy.[18] Chronic asymptomatic vaginal infections have been implicated in recurrent urinary tract infections through their action as reservoirs of the infectious agent.[19,20] During pregnancy, vaginal infections, such as BV, are associated with preterm birth, premature rupture of membranes, amnionitis, and postpartum endometritis; if present at delivery, they may be associated with an increased incidence of neonatal infection, with potentially serious or fatal consequences.[5]

Vaginitis is often the result of disruption to the local microbiome. A healthy vaginal ecosystem is maintained by vaginal microflora, estrogen, vaginal secretions, metabolic products, the vaginal immune system, and pH level. Vaginal flora is thought to be the first line of defense against infection by competitively excluding exogenous organisms, mediating the host immune response, and direct release of metabolites, including lactic acid.[21–23] There is a large variation in vaginal microflora between ethnic groups. *Lactobacillus* species are more likely to be dominant in Asian and white females, whereas Black and Hispanic females are more likely to have an abundance of anaerobes.[21] The most common species of *Lactobacillus* in reproductive-age individuals include *L. crispatus, L. iners, L. gasseri,* and *L. jensenii*.[21] Microbes important for the diversity and function of the vaginal ecosystem include anaerobes (*Garnerella vaginalis, Prevotella, Atopobium, Megasphaere*); gram-negative aerobic bacteria; and mycological species, including *Candida albicans*.[21] The composition of the vaginal microbiota shifts frequently. Females may transition between a *Lactobacillus*- to an anaerobe-dominant state with triggers, including menstruation, changes in sexual behavior or partners, antibiotic use, hormonal shifts, and host immune status.[21,23] Although vaginal ecosystems that are not predominant in *Lactobacillus* species are considered normal in some women, they create an increased risk for sexually transmitted infections.[23]

A microflora dominated by lactobacilli is capable of inhibiting the adhesion and growth of pathogens. It depletes the nutrients available to pathogens and modulates the host immune response and the

vaginal environment.[23–25] Lactobacilli act via at least four mechanisms: (1) They help produce lactic acid and provide a normal vaginal acidic environment of 3.5 to 4.5. Many pathological microbes cannot survive or flourish in this pH. (2) Certain species of *Lactobacillus* produce hydrogen peroxide, which may inhibit microbial growth. (3) Lactobacilli produce bacteriocins, which inhibit the growth of species such as *Klebsiella* and *Escherichia coli.* (4) Lactobacilli are competitive with the pathogenic microorganisms for adherence to the vaginal epithelial cells.[5,23–25] The vaginal epithelium contains immune-related cells and receptors that work symbiotically with the microflora to initiate the host immune defense. Vaginal flora that is not *Lactobacillus* predominant is more likely to elicit a proinflammatory response when experiencing changes in bacterial community states.[23]

The microbiome derives its resources from the host, and overall host physiology determines which bacterial species can successfully colonize the vagina.[21] Most nutrients are obtained from vaginal secretions and sloughed vaginal epithelial cells. Estrogen increases the volume of vaginal secretions and thickens the vaginal epithelium, providing adequate nutrition, namely, glycogen, to bacterial colonies. Glycogen is broken down by host amylase into carbohydrates, which are then fermented by lactobacilli to produce lactic acid. There is a strong correlation between estrogen levels and the relative abundance of *Lactobacillus* species. Estrogen levels are lower in women during menstruation, after ovulation, and in menopause.[21]

TYPES OF VAGINITIS

Vaginitis is most commonly associated with BV, vulvovaginal candidiasis, and trichomoniasis, but it may also present as atrophic or inflammatory vaginitis, irritant vaginitis, or allergic vaginitis.[1,5] Historically, the diagnostic criteria for vaginitis was limited to BV, vulvovaginal candidiasis, trichomoniasis, and atrophic vaginitis, leaving gaps in the proper diagnosis and treatment of several atypical scenarios.[1,5,26] Experts in the field have since added inflammatory vaginitis, irritant vaginitis, and allergic vaginitis as diagnosable causes of atypical vaginitis.

Infectious Vaginitis

In 75% to 95% of vaginitis cases, where a cause is identified, the cause is linked to an infectious agent, including BV (40%–50%), vulvovaginal candidiasis (20%–25%), trichomoniasis (15%–20%), and many less common infections, such as herpes simplex virus, gonorrhea, and chlamydia.[1] There are other infections associated with the surrounding tissues that may cause symptoms similar to those seen in vaginitis, including local itching and/or discharge. These other infections include folliculitis, hidradenitis, scabies, condyloma, herpes, syphilis, human papillomavirus, and candida and, in rarer instances, chancroid, lymphogranuloma venereum inguinale, and molluscum contagiosum.

Infectious vaginitis may be sexually transmitted (e.g., trichomoniasis) or may arise from a disturbance to the delicate balance of a healthy vaginal ecosystem (e.g., candida and BV). Vaginal "infections" frequently involve common organisms found in the cervix and vagina of healthy, asymptomatic women.[27]

The unifying factor in the pathogenesis of vaginal infections is not so much a matter of the organisms present in the patient's genital tract but rather of what risk factors are present that make the patient more susceptible to the infection.

Factors influencing the vaginal ecosystem include antibiotic use; changes in estrogen levels (e.g., pregnancy, breastfeeding, or menopause); glycogen content, which directly influences pH; glucose levels; presence of pathogenic (e.g., BV, candida, and trichomoniasis) and nonpathogenic (e.g., lactobacilli) organisms; presence of a foreign body (e.g., retained tampon, condom, or intrauterine devices); irritants and allergens (e.g., semen, spermicide, douching); menstrual blood; sexual history, including gender of sex partners and practices; and contraceptive choice.[2,5,28] Many of these factors are, in turn, affected by the individual's daily habits, internal milieu, and general health.

Immune status also plays a major role in the susceptibility, expression, and severity of disease, including vaginal infections. Immune system dysfunction, or depression, may occur as a result of nutritional deficiencies, medications (e.g., antibiotics, immunosuppressants, or steroids), pregnancy, a serious illness such as HIV, or genetics. Other medical conditions may predispose a woman to infectious agents, such as diabetes mellitus, hypothyroidism, leukemia, Addison's disease, and Cushing's syndrome.[2,5]

Predisposing factors for sexually transmitted infections (STIs) should also be considered when vaginitis is suspected or diagnosed. These factors include a baseline presence of STIs, unprotected sex, multiple sexual partners, advanced age, drug use, low socioeconomic status, douching, race, smoking, and incarceration.[1,5,29] Table 224.1 summarizes the diagnostic differentiation of the most common causes of infectious vaginitis.

Bacterial Vaginosis

BV, the most common cause of vaginitis among reproductive-age women, is any shift from a normal vaginal flora, which is lactobacilli dominant, to a predominance of anaerobes and facultative bacteria.[5] This shift results in the degradation of the mucins that form a natural barrier on the vaginal epithelium, leading to a proinflammatory response and clinical symptoms (e.g., vaginal discharge).[5] In addition, destruction of these mucins exposes the cervical epithelium, allowing BV-associated organisms to affect the cervix, resulting in the appearance of clue cells. These changes on the epithelial surface also cause an immunological shift leading to upregulation of interleukin-1-beta, a proinflammatory cytokine, and a decrease in protectant molecules such as secretory leukocyte protease inhibitor.[5]

Three main factors have been identified to explain how the shift from a lactobacilli-dominant environment to one in which the anaerobes and facultative bacteria dominate and therefore why some women experience BV: sexual activity, douching, and the absence of peroxide-producing lactobacilli in the vagina. The incidence of BV increases with multiple sexual partners, frequency of sexual activity, younger age at onset of sexual activity, practicing anal and oral sex, and using vaginal sex toys.[5] Overall, the sexual transmission of BV is not well understood. It may not be truly sexually transmitted but rather may occur by a variety of other mechanisms. Increased frequency of douching, for example, is associated with a loss of vaginal lactobacilli species and a twofold increase of BV.[30] It is hypothesized that those women with an absence of peroxide-producing lactobacilli in the vagina never had the normal inhabitation of lactobacilli species at menarche, or perhaps the lactobacilli were present but eliminated through the use of certain medications, including broad-spectrum antibiotics.

Other factors that have been associated with an increased prevalence of BV include cigarette smoking, ethnicity, and lower educational attainment. The exact correlation between cigarette smoking and BV is unknown, but it may be related to the downregulation of the immune system. Women of a nonwhite ethnicity (e.g., Hispanic and African American) are 50% more likely to develop BV.[31] Although the reasons for these differences are not clear, we do know that African American women practice douching twice as often as white women and that African American women are less likely to have lactobacilli in the vagina.[32]

Diagnosing BV involves taking a thorough medical history, visualization of any discharge, and determination of vaginal pH. Of women who develop BV, 84% do not report any symptoms, BV has a characteristic discharge that is thin, dark, or dull gray and has a strong fish-like odor.[5] Itching is not common with BV but may be present if there

TABLE 224.1 Diagnostic Differentiation of the Common Causes of Infectious Vaginitis

	Bacterial Vaginosis	*Candida*	*Trichomonas*	*Neisseria Gonorrheae*	Herpes	*Chlamydia*
Keynote symptoms	Discharge	Itching	Odor and itching	Asymptomatic or cervicitis	Vesicles or ulcers	Asymptomatic
Discharge	Malodorous (fishy odor), homogenous, clear, white or gray	White, thick, lacking any odor	Greenish yellow and frothy			
pH	>4.5	<4.5	>5.0	<4.5	<4.5	<4.5
Odor	Malodorous (fishy)	None	May be fishy	None	None	None
Appearance	Homogenous, clear, white or gray	Curdy, adherent, scant to thick	Greenish, yellow, frothy	Mucopurulent cervicitis	None	None
Pelvic examination	Unremarkable	Adherent white patches with an erythematous border	May show petechial lesions on cervix or vagina mucosa; a "strawberry cervix"	Cervical discharge; may have adnexal tenderness	Small multiple vesicles or ulcers on cervix or vulva	Unremarkable or may show signs of pelvic inflammatory disease
Microscopic examination	Presence of at least 20% "clue cells" on wet mount or Gram stain	Mycelia (10% KOH)	Motile flagellated organisms; few WBCs	Many WBCs with gram-negative intracellular diplococci	Unremarkable	Unremarkable
Culture media		Sauerbaud	Diamond	Thayer-Martin	Live cell	Live cell or antibody test

WBC, White blood cell.

is profuse discharge. A whiff test should be performed, as well as a wet mount to look for clue cells and observe the vaginal flora. An abundance of various bacteria, from 100-fold to 1000-fold, and the absence of or a decrease in lactobacilli is consistent with a diagnosis of BV. The pH in most cases is elevated to 5 or above, and there appears to be a correlation between elevated pH and the presence of odor.[33] To clinically diagnose BV using the Amsel criteria, three of the following four criteria must be met:

- Vaginal pH greater than 4.5
- The presence of a homogenous, thin, dark or dull-gray, malodorous vaginal discharge
- Positive KOH (whiff/amine) test
- Presence of clue cells on wet-mount microscopic examination

There is no definition of recurrent BV, but approximately 30% of women experience a recurrence within 3 months after treatment, and up to 50% after 6 to 12 months.[5, 34] It is not always clear whether the recurrence represents a relapse or reinfection. In women who experience four or more episodes in a year, it is thought that many have an underlying issue that interrupts the reestablishment of their normal vaginal flora. Another possibility is the development of a chronic abnormal vaginal ecosystem causing symptomatic and asymptomatic episodes.

Depending on concomitant risk factors, severity of infection, and treatment success, a diagnosis of BV can lead to serious health risks, including increased susceptibility to other STIs (e.g., HIV, chlamydia, and gonorrhea), increased chances of passing HIV to a sex partner, preterm delivery, and PID. Those women who have BV and insufficient lactobacilli species are more susceptible to HIV and gonorrhea.[35,36] During pregnancy, the organisms present during BV can ascend the genital tract, leading to preterm delivery and an increased risk of postpartum endometritis. Women with BV are also at a greater risk of infection after gynecological surgery.

Vulvovaginal Candidiasis

Vulvovaginal candidiasis (VVC), commonly referred to as a "yeast" infection, is caused when *Candida* species, a normal part of our microflora, transition from a commensal to pathogenic role due to overgrowth.[5] VVC is currently the second most common type of vaginal infection after BV, and several key factors are thought to play a role in the increased incidence, including repeated courses of antibiotics, use of hormonal contraceptives, corticosteroids, genetic predisposition, intrauterine devices, and the continuing rise in the incidence of diabetes mellitus.[5,37]

The idea that host-environment disruption plays a role in the prevalence of candidiasis was first validated by Miles et al.[38] in the 1970s when his team found a link between genital and gastrointestinal candidiasis. This systemic relationship is now thought to play an important role in recurrent VVC and the increased prevalence of VVC during pregnancy.[38–40] During pregnancy, when the prevalence of candidiasis is between 10% to 75%, it is believed that elevated estrogen and glycogen levels in vaginal secretions play an important role in the higher incidence.[40–42] Certain fabrics can also increase the risk of developing candidiasis, VVC is three times more prevalent in women wearing pantyhose than those wearing cotton underwear due to lack of breathability.[43]

The diagnosis of VVC may be classified as either uncomplicated, a mild-to-moderate infection in an immunocompetent, nonpregnant individual that recurs less than four times per year, or complicated, a moderate to severe infection associated with pregnancy or another concomitant condition (e.g. diabetes mellitus, immunocompromise) or an infection that recurs more than four times per year in an immunocompetent, nonpregnant individual.[5] Those who have four or more episodes of symptomatic VVC within 1 year are classified as having recurrent vulvovaginal candidiasis (RVVC).[44] Most cases of RVVC are caused by *C. albicans*, but non-*albicans Candida* species, such as *C. glabrata*, have a higher prevalence (10%–20%) in RVVC cases. Many of these non-*albicans Candida* species are resistant to conventional treatment, making them more problematic in recent years as women have been using more and more antifungal agents (Box 224.1). There are three main theories to explain why women have RVVC: (1) the intestinal reservoir theory, which hypothesizes that a patient's reinoculation is due to *Candida*

BOX 224.1 Factors Predisposing to Candidal Vaginitis[37]

- Recent or current antibiotic use
- Diabetes mellitus
- Gastrointestinal candidiasis
- Hormonal contraceptive use
- Pregnancy
- Weakened immunity (e.g., HIV infection, steroid use, or chemotherapy)
- Wearing pantyhose

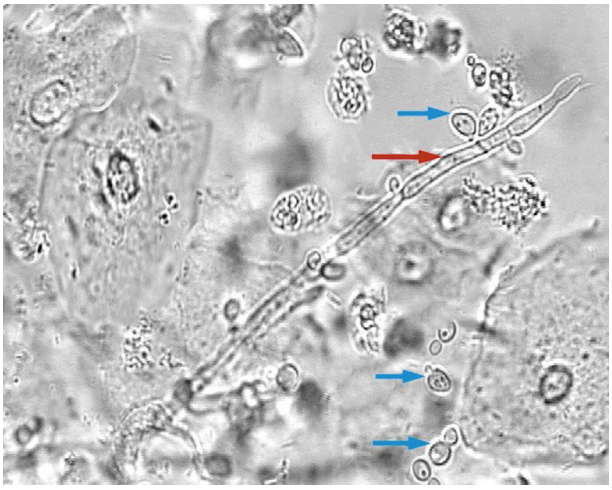


Fig. 224.1 Vaginal saline wet mount with multiple yeast forms.[5] Budding yeast or conidia *(blue arrows)* and pseudohyphae or mycelia *(red arrow)*. Magnification 40×.

populating the gastrointestinal tract and migrating into the vagina; (2) the sexual transmission theory, which points to the possibility that the sex partner is the source of the recurrence; and (3) the vaginal relapse theory, which maintains that some women retain small numbers of yeasts, even after treatment, that later cause a resurgence of symptoms. A considerable body of research supports this last theory.[45,46] These studies include immunological research suggesting that women with recurrent infections have an abnormal immune response to infection, which leaves them susceptible to further episodes.[47]

The primary symptom of VVC is pruritus followed by soreness; dyspareunia; burning exacerbated by urination or vaginal sexual activity; dysuria; and a thick, clumpy, "cottage-cheese-like" discharge.[5] The presence of such a discharge is strong evidence of VVC, but its absence does not rule out candidiasis. Fewer than 20% of symptomatic patients with candidal infection actually display classic thrush patches. Other signs of a candidal etiology include the presence of vulvar erythema and excoriations, due to scratching. The vaginal pH is not usually altered. Neither the character of the discharge nor the symptomatology is sufficient alone to make a diagnosis of VVC. Visualization of pseudohyphae (mycelia) and/or budding yeast (conidia; Fig. 224.1) under microscopy using a saline wet mount or 10% KOH wet prep will confirm the diagnosis.[5] It should be noted that budding forms of yeast may be found in both normal and symptomatic vaginas, but the mycelial stage is found only in symptomatic women.

Trichomoniasis

Trichomoniasis is caused by the single-celled, flagellated, anaerobic, protozoan parasite *Trichomonas vaginalis* and is one of the most common curable STIs worldwide.[37] *T. vaginalis* appears to affect only humans, and sexual transmission appears to be its primary mode of dissemination. It is the only known protozoan parasite that infects the lower urogenital tract of both men and women, and it can remain dormant for months to years, making it difficult to distinguish between a persistent subclinical infection and acute sexual acquisition.[37] This ability to lay dormant is thought to be the reason why many (~70%) who are infected with *T. vaginalis* in the United States are asymptomatic. For those who do develop symptoms, most will start to show signs within 5 to 28 days, and those symptoms, depending on the individual, can range from mild to severe.[37] The most common, or characteristic, symptoms include a malodorous, frothy gray or yellow-green discharge and pruritis.[37] The "strawberry cervix" with punctate hemorrhages is found in only a small percentage of patients with trichomoniasis. Trichomonads grow optimally at a pH of 5.5 to 5.8.[48] Thus, conditions that elevate the pH, such as increased progesterone, will favor the overgrowth of *T. vaginalis*. Conversely, a vaginal pH of 4.5 in a woman with vaginitis is suggestive of an agent other than *T. vaginalis*. Microscopic examination of a saline wet mount of fresh vaginal fluid reveals the presence of small motile organisms to confirm the diagnosis in 80% to 90% of symptomatic carriers.[38,49]

BOX 224.2 Predisposing Factors That Increase the Risk of Developing Atrophic Vaginitis[50,154]

- Antiestrogen medications (e.g., tamoxifen, danazol, medroxprogesterone, leuprolide, nafarelin)
- Cessation of sexual activity
- Childbrith by cesarean section
- Decreased ovarian function (e.g., radiation and chemotherapy)
- Lactation (elevated prolactin level)
- Menopause
- Natural estrogen deficiency (premenopausal)
- Never having been pregnant
- Nonfluctuating estrogen levels
- Postpartum loss of placental estrogen
- Smoking (all types)
- Surgial intervention (e.g., oophorectomy)
- Vaginal nulliparity

Atrophic Vaginitis

Atrophic vaginitis is primarily a problem of estrogen deficiency, most commonly affecting peri- and postmenopausal women, although it can affect women of all ages. Those not of peri- or postmenopausal age may develop an estrogen-like deficiency from a multitude of risk factors (Box 224.2). The vagina undergoes several changes with declining estrogenic stimulation, including a decrease in *Lactobacillus* species, a shift to a more alkaline pH, thinning of the vaginal wall, less lubrication, more easily irritated and inflamed tissue, and greater susceptibility to infection. The most commonly reported genital symptoms include dryness; burning; dyspareunia; loss of vaginal secretions; leukorrhea; itching; and a thin, watery discharge, which may occasionally be tinged with blood.[50] (Note that any vaginal bleeding in a postmenopausal woman requires a complete evaluation to rule out endometrial hyperplasia and endometrial carcinoma.) The vaginal pH is typically equal to or greater than 5.5, but there is no infection. Other common complaints include urethral discomfort, frequency, hematuria, urinary tract infection, dysuria, and stress incontinence.

THERAPEUTIC CONSIDERATIONS

Dietary Considerations

The internal milieu of the vagina is a reflection of the condition of the entire body. Vaginal secretions are continuously released that affect

and are affected by the microbial flora. These secretions contain water, nutrients, electrolytes, and proteins, such as secretory immunoglobulin (Ig) A. The quantity and character of these components are altered by hormonal and dietary factors. A general healthful diet is recommended in all cases to ensure the availability of all nutrients in sufficient quantity to optimize the body's ability to respond to changing conditions.

A longitudinal study evaluated the association between diet and the presence of bacterial vaginosis in 1521 participants.[51] It was found that a diet with excess energy intake primarily derived from saturated fats incurred the highest risk of having BV. It is hypothesized that high fat intake alters the vaginal microflora and the immune response initiated by gut-associated lymphoid tissue, increasing the risk of BV. The majority of participants also ate a low-fiber diet.[51]

A well-balanced diet low in sugars, saturated fats, and refined foods is particularly important in vaginitis due to infectious organisms. Maintaining balanced blood sugar levels is particularly important for *Candida* infections. A diet high in fiber, from a variety of fiber sources, is important to support the diversity and abundance of the microbiome. Patients with depressed immunity are susceptible to higher rates of infection, particularly those due to *Candida, Trichomonas,* and herpes (see Chapter 18 and 136 for further discussion of the effects of diet on immune function). Nutrients particularly important for proper immune function are zinc; vitamins A, C, D, and E; manganese; and vitamin B complex.

A diet high in lysine-containing foods and low in arginine-containing foods reduces the number and severity of herpetic outbreaks. Many animal products are high in lysine, and it is important to choose organic, hormone- and antibiotic-free products. Patients can choose a low-arginine diet combined with lysine supplements to limit animal product intake.

Live active culture yogurt may reduce the incidence of VVC and BV infections. A 1992 study investigated the effects of the daily ingestion of yogurt containing *L. acidophilus* on 33 women with five or more episodes per year of candidal vaginitis. Thirteen women completed the study. The women were randomized into two groups, with the first group receiving 8 oz of *L. acidophilus* yogurt daily for 6 months and then no yogurt for 6 months. The other group consumed the yogurt-free diet for the first 6 months and then the diet including yogurt for the second 6 months. There was a threefold reduction in infections and in candidal colonization during the yogurt diet compared with the nonyogurt diet.[52] Another study did not support a role for yogurt in the prevention of recurrent candidal vaginitis but also showed that daily intake of probiotic yogurt may reduce episodes of recurrent BV.[53] Other fermented foods, particularly lacto-fermented foods, including kefir, sauerkraut, miso, pickled vegetables, and kimchi, may provide similar benefits.

Nutritional Supplements

For general maintenance of health and immune competence, a high-quality multivitamin/multimineral supplement provides low-cost compensation for most dietary inadequacies. In addition, the nutrients discussed here may be useful in one or more types of vaginitis.

Vitamin A and Beta-Carotene

Both vitamin A and beta-carotene are necessary for the normal growth and integrity of epithelial tissues, such as the vaginal mucosa. Vitamin A is essential for adequate immune response and resistance to infection. Secretory IgA, a major factor in resistance to infection, is lower in vitamin A–deficient subjects.[54,55] In addition, it has been shown to enhance T-cell numbers and to favorably alter their ratios.[56] Excessive vitamin A can be toxic and teratogenic. This is of particular concern in women of reproductive age. Total vitamin A intake should be limited to 5000 IU/day. If larger doses of vitamin A are used, patients should be cautioned to be particularly careful about contraception. There is a hypothesis that the teratogenic effects of excessive intake of vitamin A may actually be due to inadequate intake of vitamin D because the two fat-soluble vitamins have opposite effects on epigenetic expression. Balanced intake is critical.

B Vitamins

The body requires one or more of the B vitamins for virtually every metabolic activity. B vitamins are needed for carbohydrate metabolism, protein catabolism and synthesis, cell replication, and immune function. Vitamins B_2 and B_6 have been shown to have estrogen-like effects and to act synergistically with estradiol. Vitamin B_1 and pantothenic acid enhance the action of estradiol, although they have no estrogenic activity themselves.[29] This finding suggests that B vitamins may be of use in estrogen-deficiency conditions, such as atrophic vaginitis, especially if combined with phytoestrogens.

Vitamin C and Bioflavonoids

Vitamin C and bioflavonoids are essential in any process related to immune function. A deficiency of vitamin C reduces the phagocytic activity of leukocytes. Both vitamin C and bioflavonoids improve connective tissue integrity, thus reducing the spread of infection. Both nutrients are also useful in diminishing the frequency and severity of herpetic outbreaks.[57–62]

Intravaginal vitamin C has been used to treat BV. Vitamin C is thought to increase the acidity of the vagina and reduce the growth of pathogenic bacteria, allowing for the growth of normal vaginal flora.[63] To date, several randomized controlled trials have established that a 250-mg tablet of intravaginal vitamin C, for 6 consecutive days, is often an effective treatment for BV.[63] These studies have shown that vitamin C 250-mg tablets are more effective than placebo and as effective as metronidazole gel.[64–66] Vitamin C application can also improve vaginal microflora composition and reestablish an acidic pH, and it may prevent the recurrence of BV.[67–69] These studies have also demonstrated that intravaginal vitamin C tablets are safe and well tolerated.

One of the randomized, double-blind, placebo-controlled studies used a 250-mg tablet of vitamin C inserted vaginally once a day for 6 days.[65] Of the 100 participants, 50 were given the active treatment and 50 were given a placebo. At the end of the study, significantly more patients still had BV in the placebo group (35.7%) compared with the vitamin C tablet group (14%). There were no clue cells in 79% of patients receiving the vitamin C, versus 53% in the placebo group. Bacteria disappeared in 77% of the vitamin C group versus 54% in the placebo group, and lactobacilli reappeared in 79.1% of the vitamin C group versus 53.3% in the placebo group.

Vitamin D

Vitamin D deficiency has been associated with concurrent BV. One randomized trial of 208 women with asymptomatic BV used 2000 IU vitamin D versus placebo for 15 weeks as the sole treatment intervention. The placebo group was 10.8 times more likely to have BV at the end of intervention compared with those who received vitamin D.[70] This study had several weaknesses, however. Vitamin D status should be assessed during treatment because it influences the local immunity of the vagina and has been shown to play an important role in the proliferation of vaginal epithelium (which provides nutrients to lactobacilli).[70,71] One study found that postmenopausal women taking vitamin D for at least 1 year experienced improved growth in cells of the superficial vaginal wall.[72] Reduction of vaginal atrophy may reduce the incidence of vaginitis.

Vitamin E

Lack of vitamin E depresses the immune response and host resistance. This situation may be corrected by high doses of supplemental vitamin

E. Several experiments have shown increased resistance to chlamydial infection when subjects were supplemented with vitamin E. Vitamin E also regulates retinol in humans, and an inadequacy of vitamin E hinders the utilization of vitamin A despite adequate retinol intake.[73] The use of vitamin E for the treatment of atrophic vaginitis has been reported since the 1930s.

Excessive vitamin E intake (>1200 IU/day), however, may be immunosuppressive and may transiently elevate blood pressure. This vitamin is reported to improve glycogen storage and the tone of the heart muscle. Thus extra caution should be exercised in patients with diabetes, hypoglycemia, hypertension, or heart disease. For such high-risk patients, supplementation should begin with a daily dose of 100 IU (mixed tocopherols), with monitoring of blood glucose or blood pressure values, and the dose should be increased slowly over time (e.g., by 50 IU/day).

Zinc

All DNA and RNA polymerases and repair and replication enzymes require zinc. Zinc enhances prostaglandin (PG) E_1 synthesis, normalizes lymphocyte activity, and enhances epithelial growth. Low levels of zinc are associated with depressed immunity and thymic atrophy, both of which are correctable when zinc is replenished. Zinc is also essential for the proper utilization of vitamin A. Many otherwise well-nourished adults receive less than 50% of the recommended dietary allowance of zinc from their diets and have one or more measurable signs of zinc deficiency.[74–76]

Treatment with topical and oral zinc has been shown to reduce the duration and severity of herpes outbreaks. This may be due either to the effect of zinc on the production of prostaglandins or to the direct antiviral activity of the zinc ion. High levels of zinc are also toxic to *Chlamydia* and *Trichomonas* and have been used successfully in vaginitis that did not respond to antibiotic therapy.[77–81] A 2015 case study reported the successful treatment of patients with metronidazole-resistant *T. vaginalis* with a 1% zinc sulfate douche twice daily with or without 2 g oral tinidazole for 14 to 28 days.[82] Seven patients were followed, with two patients receiving only the 1% zinc sulfate douche; five patients were treated with a combination of 1% zinc sulfate douche and 2 g oral tinidazole. All patients experienced clinical improvement, and six of the seven patients were successfully treated at follow-up 2 months to 5 years after treatment.[82]

Botanical Medicines

Glycyrrhiza glabra

Licorice, a traditional herbal medicine used for its antiviral, analgesic, and carminative effects, has been shown to be effective against *Candida* as an isolate (18-beta glycyrrhetinic acid), an ethanolic and methanolic extract, a suppository, and a topical cream.[83–86] The effects of this herb, as a topical vaginal cream, also play a role in mitigating the symptoms of atrophic vaginitis in postmenopausal women.[87] *G. glabra* is well known for its antiviral activity, specifically against RNA and DNA viruses, and has been used successfully in the treatment of herpes. The number and severity of recurrences may be reduced by the repeated application of licorice gel to active lesions.[88] (For further discussion, see Chapter 85.)

Chlorophyll

Chlorophyll has both bacteriostatic and soothing actions. Water-soluble chlorophyll may be added to douching solutions for symptomatic relief of vaginitis.[89–91]

Allium sativum

Garlic *(A. sativum)*—which is antibacterial, antiviral, and antifungal—has been shown to be effective even against some antibiotic-resistant organisms.[92–96] The major growth-inhibitory component in garlic extract is allicin; therefore garlic products with the highest amount of allicin are preferable. Studies comparing daily oral intake of garlic, in tablet form, to fluconazole (for treatment of candidiasis) and metronidazole (for treatment of BV) found the garlic tablets to be a suitable alternative in both instances.[97, 98]

Hydrastis canadensis and *Berberis vulgaris*

Goldenseal *(Hydrastis canadensis)* and Oregon grape *(Berberis vulgaris)* contain berberine, a constituent known to demonstrate significant antimicrobial activity (topically and internally) and enhance immune function (internally only).[99] Berberine has been shown to be effective against a number of different pathogenic species, including *C. albicans, C. tropicalis,* and *T. mentagrophytesas,* among others.[100, 101] When applied locally, via a cream, suppository, or douche, it offers localized symptomatic relief and soothes inflamed mucous membranes.[102–105] It has also been shown to increase the efficacy of a metronidazole-based gel in the treatment of bacterial vaginosis.[106] (For further discussion, see Chapter 86.)

Melaleuca alternifolia

The volatile essential oil derived from the Australian native tea tree plant, *Melaleuca alternifolia*, is known most commonly for its strong antibacterial and antifungal properties.[107] It was shown in one study to be effective in treating trichomoniasis, candidiasis, and cervicitis. Treatment consists of daily douching combined with saturated tampons used weekly. No adverse reactions were reported, and patients commented favorably on its soothing effect[108] (for further discussion, see Chapter 91). Various tea tree oil preparations have demonstrated antimicrobial activity against *Staphylococcus aureus* and *C. albicans.*[109]

Humulus lupulus

A study was conducted using a combination gel containing phytoestrogens from an extract of the hop *(Humulus lupulus)* plant, hyaluronic acid, liposomes, and vitamin E.[110] This open, noncontrolled trial was performed on 150 postmenopausal women presenting with vaginal dryness and related symptoms. One vaginal suppository per day was inserted for the first 14 days and then one suppository every other day for 14 days. The primary endpoint was the evaluation of vaginal dryness by both the patient and the investigator. The secondary endpoints were the evaluation of other symptoms, including vaginal itching, burning, dyspareunia, inflammation, swelling, or irritation and vulvovaginal abrasions. Among the 130 women who completed the study, the average score on the vaginal dryness scale decreased from 7.92 to 0 by the end of the treatment. Itching disappeared progressively throughout the treatment period, and only four women still had itching at the end of the treatment. Burning was severe in 92 women at baseline, moderate in 26, and mild in 11. By the end of the treatment, only four women complained of mild burning. Dyspareunia also improved progressively; by the end, only 5 women of the 130 who reported mild to moderate or severe dyspareunia had mild dyspareunia. Inflammation and irritation of vulvar and vaginal mucosa also improved significantly from the beginning to the end of the treatment period.

Botanical Mixture

Women with abnormal vaginal discharge who presented to a gynecological clinic in India were randomly assigned to receive either a cream containing *Azadirachta indica* (Neem) seed oil, *Sapindus mukerossi* (Reetha) saponin extract, and quinine or placebo. They applied the cream intravaginally at bedtime for 14 days. The symptomatic and microbial assessment showed that 10 of 12 women with *C. trachomatis* vaginitis recovered within 1 week, and 10 of 17 with BV recovered

within 2 weeks. No benefit was found in women with candidal or trichomonal infections, and none of the women using the placebo recovered from any of the infections.[111]

Other Agents

Lactobacillus Probiotics

Lactobacilli are frequently found to be the most dominant organisms in the vagina of a healthy, reproductive-aged woman. Properties of these strains—including their adhesiveness and their ability to produce lactic acid, hydrogen peroxide, bacteriocidins, and biosurfactants—confer protection on the host. Selection of *Lactobacillus* species and strains for therapeutic purposes with these properties should be a guiding principle for their use in treatment. Substantial data have been published on a number of strains, their properties, and their ability to fight pathogens. Many species and specific strains have demonstrated antipathogenic activity; these include *L. rhamnosus* GG, *L. acidophilus* NCFM, *L. casei Shirota, L. reuteri* MM-53, *L. casei* CRL-431, *L. rhamnosus* GR-1, *L. rheuteri* (formerly *fermentum* RC-14), *L. plantarum* 299V, and *L. salivarius.*[112] *L. rhamnosus* GR-1 and *L. reuteri* RC-14 have been shown to have antifungal effects against *C. glabrata.*[113] *L. rhamnosus* GG has been shown to protect oral epithelia against *C. albicans* infection by reducing fungal adhesion, invasion, and damage by depleting fungal nutrition and reducing ergosterol synthesis.[114] Although communication among bacteria is multifactorial and complex, it is thought that orally consumed probiotics ascend the vaginal tract after rectal excretion to colonize the vagina.[114]

Probiotic therapies, oral or vaginal, may be useful in the prevention or treatment of BV.[115,116] Supplementation with vaginal *Lactobacillus* probiotics after antibiotic therapy promotes the growth of healthy vaginal flora.[115,116] One review suggested that studies using higher doses of lactobacilli (around 10^9 colony-forming units [CFUs]) incurs greater efficacy.[117] Another study found that both vaginal administration and oral-plus-vaginal administration of lactobacilli was effective in lowering the vaginal pH, treating the current infection, and preventing recurrence over the subsequent 3 months.[118] After any conventional treatment with antibiotics, vaginal lactobacilli can be restored by the coadministration of *Lactobacillus* and low-dose vaginal estriol.[119] The use of oral probiotics containing *L. rhamnosus* GR-1 and *L. reuteri* daily for 6 weeks has been shown to restore normal vaginal microflora after infection.[120]

A randomized, double-blind, placebo-controlled trial of BV was conducted with 100 women who were given a 2% vaginal clindamycin cream for 7 days and then randomized to receive vaginal capsules for 10 days containing either a placebo or a combination of *L. gasseri* and *L. rhamnosus* (10 billion CFUs/capsule); this was then repeated for three cycles. The probiotics did not improve the efficacy of BV treatment during the first month of treatment. However, women initially "cured" were followed for six menstrual cycles or until relapse within that time. At the end of 6 months, 64.9% of the probiotic-treated group were still BV-free compared with 46.2% in the placebo group.[121]

Another study enrolled 125 premenopausal women diagnosed with BV by the presence of vaginal irritation, discharge, and "fishy" odor; Nugent criteria; and the detection of sialidase enzyme. The subjects were treated with oral metronidazole (500 mg) twice daily from days 1 to 7, and randomized to receive oral *L. rhamnosus* GR-1 (1×10^9) and *L. reuteri* RC-14 (1×10^9) or placebo twice daily from days 1 to 30. The primary outcome was the cure of BV as determined by normal Nugent score, negative sialidase test, and no symptoms or signs of BV at day 30. A total of 106 subjects returned for 30-day follow-up, of which 88% were cured in the antibiotic/probiotic group compared with 40% in the antibiotic/placebo group ($P < 0.001$). Of the remaining subjects, 30% subjects in the placebo group and none in the probiotic group had BV, whereas 30% in the placebo and 12% in the probiotic group fell into the intermediate category based on Nugent score, sialidase result, and clinical findings. High counts of *Lactobacillus* spp. ($>10^5$ CFU/mL) were recovered from the vaginas of 96% probiotic-treated subjects compared with 53% of controls at day 30. In summary, this study showed efficacious use of lactobacilli and antibiotics in the eradication of BV in black African women.[122]

There are more studies showing these specific species in the treatment of BV. In another clinical trial, 64 premenopausal women with diagnosed BV received a single dose of tinidazole (2 g) and either one capsule of *L. rhamnosus* GR-1 (1×10^9) and *L. reuteri* RC-14 (1×10^9) or placebo orally, twice a day, from days 1 to 28. At day 28, the probiotic group had a higher cure rate (Nugent score and Amsel test) of BV compared with placebo (87.5% vs. 50%; $P = 0.001$). According to the Gram-stain Nugent score, more women in the probiotic group were assessed with "normal" vaginal microbiota compared with placebo (75% vs. 34.4%; $p = 0.011$).[123]

Another way to use these probiotic species is after conventional treatment. In one clinical trial, 95 women (39 with BV, 45 with VVC, and 11 with both infections) were randomized to receive a vaginal capsule containing *L. gasseri* LN40, *L. fermentum* LN99, *L. casei rhamnosus* LN113, *P. Pediococcus acidilactici* LN23 (10^8–10^{10} CFU), or placebo for 5 days after conventional treatment. Probiotic strains were present in vaginal cultures 2 to 3 days after administration (53% colonized after one menstruation). Ninety-three percent of women in the probiotic group were cured after 2 to 3 days compared with 83% in the placebo group (78% vs. 71% after first menstruation). The probiotic group also had significantly less malodorous discharge.[124]

A 2017 Cochrane review observed the effect of oral or vaginally administered probiotics in 1656 participants in 10 clinical trials with vulvovaginal candidiasis. This review determined that probiotics as adjuvant therapy could increase the rate of cure within 5 to 10 days, can normalize laboratory results, and may prevent relapse at 1 month. In these studies, probiotics did not influence the relapse rate at 1 to 3 months. The use of probiotics is considered safe. The researchers concluded that there is low confidence for the use of probiotics adjunctively or as a standalone treatment until studies of larger size and duration are available.[125]

Although additional studies would improve confidence, several smaller studies have shown that probiotic use may benefit outcomes. A randomized, double-blind, clinical trial showed that the use of oral probiotics and fluconazole treatment for VVC significantly reduced 6-month recurrence of candidiasis.[126] Another clinical trial for VVC employed *Lactobacillus* GG suppositories, which were given twice a day for 7 days to women with more than five infections per year.[127] Four of the five women with positive yeast cultures had negative cultures after receiving this treatment. All the women reported improvement of their vaginal symptoms of erythema and discharge.[127]

A 2015 clinical trial showed intravaginal honey and yogurt to be an effective treatment for vaginal candidiasis. This randomized, triple-blind trial of 70 women with vaginal candidiasis compared the effectiveness of a 5-g vaginal cream made from yogurt and honey to 5 g of 1% clotrimazole vaginal cream for 7 days.[128] The results showed that the yogurt and honey cream had similar efficacy to clotrimazole cream but also alleviated the symptoms of VVC. It is thought that the dual effect of *Lactobacillus* with honey's ability to inhibit the growth of various fungi due to its high acidity, osmolarity, and the presence of hydrogen peroxide and other metabolites is more effective than mono treatment. Flavonoids from the honey may also stimulate the host immune system to modulate bacterial populations.[128]

Another study examined the effectiveness of weekly intravaginal *L. acidophilus* versus clotrimazole (antifungal) tablets in HIV-positive

women, a group highly susceptible to recurrent yeast vaginitis, and found the two treatments to be similarly effective at preventing candidiasis.[129] These women were randomized into three groups, with one group receiving *L. acidophilus* intravaginally once a week. The second group received vaginal clotrimazole weekly, and the third group received a placebo. During the 21-month study, the relative risk of developing VVC was 0.5 for the lactobacilli group and 0.4 for the prescription antifungal group compared with placebo. In addition, women who used the lactobacilli went for a longer period of time until they became infected compared with the women who received a placebo.

Other studies have demonstrated that when lactobacilli are given orally, they do colonize the vagina and/or reduce vaginal candidal infections. Three studies, all using *L. rhamnosus* GR-1 and *L. fermentum* RC-14, have been positive and showed a significant reduction in yeast[130] or reduction in the recurrences of yeast vaginitis[131] or restoration of normal vaginal flora in women with a history of VVC.[132]

A review of *Lactobacillus* treatments for VVC in 2003[133] found that vaginally administered or orally ingested *Lactobacillus* is able to colonize the vaginal ecosystem and that supplementation generally had to continue for 2 to 6 months to sustain continued colonization. The author also concluded that controlled trials are encouraging but few and that these trials had small numbers of participants, inadequate controls or lack of blinding, and high attrition rates and were not consistent in the form of *Lactobacillus* used. In addition, they produced conflicting results.

There are a few studies that do not support a role for probiotics in the prevention of recurrent VVC or BV.[53,134]

Alone or in combination with other vaginal or oral therapies, selected combination species and strains of *Lactobacillus* can provide the key to establishing normal vaginal microflora, preventing recurring infections, and treating acute candidal and bacterial infections of the vagina.

Iodine

Iodine used topically is effective against a wide range of organisms, including *Trichomonas, Candida, Chlamydia,* and nonspecific vaginitis. Povidone-iodine (Betadine) has all the advantages of iodine without the disadvantages of stinging and staining. A study published in 1969 found povidone-iodine to be effective in treating 100% of cases of candidal vaginitis, 80% of cases due to *Trichomonas,* and 93% of combination infections. Although douching has not been as strongly recommended, this study found a douching solution diluted to 1 part iodine in 100 parts water (e.g., 1.5–3 teaspoons povidone-iodine to 1 quart of water) used twice daily for 14 days to be effective against most organisms.[133,135–142]

Boric Acid

Boric acid suppositories have been around for a very long time and are both antibacterial and antifungal, although the exact mechanism of action is not clear. One hypothesis involves the vaginal acidification of boric acid, which then leads to penetration of fungal cell walls and disruption of the fungal cell membrane. However, because boric acid's minimum inhibitory concentration works best at a pH similar to that of a normal, uninfected vagina, the hypothesis of increased acidity may not explain its therapeutic effects.

Vaginal capsules of boric acid have been shown to treat candidiasis with success rates equal to or better than those for nystatin. In the most impressive study, 100 women with chronic resistant yeast vaginitis for whom extensive and prolonged conventional therapy had failed were treated with vaginal suppositories containing 600 mg of boric acid twice a day for 2 or 4 weeks. This regimen was effective in curing 98% of the women with failure of response to the most commonly used antifungal agents,[142] thus offering an inexpensive, easily accessible therapy for vaginal yeast infections.[143,144]

A recent review of boric acid for recurrent *Candida* vaginitis sheds some light on its effectiveness.[145] Fourteen studies, including two randomized clinical trials, nine case series, and four case reports, were included in this review of the clinical evidence using intravaginal boric acid for vulvovaginal candidiasis. Boric acid was compared with nystatin, terconazole, flucytosine, itraconazole, clotrimazole, ketoconazole, fluconazole, butoconazole, and miconazole. The mycological and clinical cure rates were as follows:

- Butoconazole: 100%
- Miconazole: 100%
- Boric acid: varied from 40% to 100%
- Fluconazole: 28.6% to 92.3%
- Itraconazole: 90.9%
- Flucytosine: 90%
- Terconazole: 70%
- Nystatin: 50%
- Ketoconazole: 50%
- Clotrimazole: 36%

The recurrence rates ranged from 0% to 45.5% in those using boric acid. None of the studies found statistically significant recurrence rates. A vaginal burning sensation was reported in less than 10% of the cases, and watery discharge during treatment and vaginal erythema were reported in seven studies.

Kudzu *(Pueraria mirifica)*

Pueraria mirifica was examined for its effect on vaginal symptoms, vaginal health index, vaginal pH, and vaginal cytology in postmenopausal women.[146] In this randomized, double-blind, placebo-controlled study, the participants were given either 20, 30, or 50 mg of *P. mirifica* in capsule or placebo daily for 24 weeks. The average vaginal dryness symptoms in the treatment group decreased after 12 weeks, and the maturation index increased after 24 weeks. This effect is evidence of an estrogenic effect on vaginal tissue due to this plant and points to its clinical use for vaginal dryness and dyspareunia due to vaginal atrophy.

Estrogens

Estrogen therapy is the most effective treatment for moderate to severe atrophic vulvovaginitis. Systemic estrogen therapies and estrogen-progestogen therapies are effective for treating these symptoms. However, when urogenital atrophy is the main or only indication for estrogen, topical vaginal estrogen therapies (creams, pessaries, intravaginal tablets, and estradiol rings) are preferred due to low systemic absorption and reduced risk of adverse effects.[1]

Vaginal Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is used for the treatment of atrophic vaginitis and associated symptoms, including dyspareunia. Ovules or suppositories dosed at 0.25% to 1% daily for 12 weeks can help improve vaginal atrophy and restore normal vaginal pH.[147, 148] DHEA is produced in the adrenal glands and serves as a precursor to estrogens and androgens. Elevation of vaginal estrogen levels has been shown to increase the proliferation of superficial vaginal epithelium, reducing the symptoms of atrophic vaginitis. Additionally, the elevation of local androgens by DHEA has been shown to improve collagen formation in the lamina propria, strengthening the vaginal wall.[147] Application of vaginal DHEA has not been shown to significantly increase serum estrogen levels, limiting systemic effects.[147,148] Prasterone, or Intrarosa, is a form of 0.5% DHEA

approved by the U.S. Food and Drug Administration (FDA) for dyspareunia due to vaginal atrophy.[149] Over-the-counter DHEA has not been FDA approved for use.

A prospective, randomized, double-blind, Phase III, placebo-controlled trial evaluated the effect of daily local intravaginal DHEA ovules for 12 weeks in postmenopausal women. The main assessment criteria were sexual function parameters of libido, arousal, orgasm, and dyspareunia in postmenopausal women with vaginal atrophy.[150] A total of 218 postmenopausal women were randomized to receive a daily ovule of either no DHEA, 0.25% (3.25 mg) DHEA, 0.5% (6.5 mg) DHEA, or 1.0% (13 mg) DHEA. The ovules contained Prasterone, in a lipophilic agent manufactured by Recipharm of Sweden. At 12 weeks, compared with placebo, the 1.0% DHEA ovule improved symptoms by 68% in the abbreviated sex function/arousal/sensation domain, in the arousal/lubrication domain by 39%, orgasm by 75%, and dryness during intercourse by 57%. DHEA also fared better than placebo in the desired domain of a menopause-specific quality-of-life inventory by 49% to 23%. Similar results using 0.5% (6.5 mg) DHEA have been reproduced in a Phase III, randomized, double-blind, placebo-controlled clinical trial published in 2018.[151] One review of 14 randomized controlled trials confirmed that intravaginal DHEA appears to be a safe and effective treatment for most women with vulvovaginal atrophy and dyspareunia.[152]

In a similar trial, serum levels of vaginal DHEA showed no or minimal changes during the study period, which lasted up to 12 weeks. All values remained within the normal range of postmenopausal women.[153] Additional studies need to be performed to determine whether intravaginal DHEA is safe for women with a history of cardiovascular disease, thrombosis, and hormone-sensitive neoplasms.[152]

DIAGNOSTIC APPROACH

Some women are hesitant to mention symptoms of vaginitis to their doctors out of embarrassment, associating these symptoms with poor personal hygiene or promiscuity. Because of the relative frequency of occurrence of vaginitis and the potential for serious consequences if it is untreated, all female patients should be questioned directly regarding the presence of pruritus, vaginal discharge, dysuria, or other symptoms of vaginitis. The following protocol outlines the basic approach to diagnosis:

1. Obtain a complete gynecological and sexual history, including details of the sexual activity and practices of the patient and her partner or partners. Determine method of contraception, personal hygiene habits, and any self-medication. Rule out the presence of associated symptoms suggestive of PID or systemic infection. Inquire about previous occurrences and their diagnosis, treatment, and resolution.
2. Identification of the causative agent is essential for successful treatment and evaluation. To facilitate diagnosis, the patient should be instructed to avoid douching, intercourse, and vaginal medications for 1 to 2 days before the office visit.
3. Determine by speculum examination whether the discharge emanates from the vagina or the cervix. Note the condition of the vaginal mucosa and the character of any discharge. Specimens should be collected and then placed on slides for saline and KOH examination. Use pH paper, amines testing strips, and microscopy of wet mounts.
4. Measure pH.
5. Use microscopy when needed for diagnosis (e.g., KOH wet prep).
6. Appropriate culture specimens should be taken if the diagnosis remains in question or if screening for gonococcus or *Chlamydia* is desired (highly recommended). An abdominal/bimanual examination should be done. A genital culture can be used to diagnose beta strep, yeast, BV (not well), *S. aureus,* and *E. coli.* A yeast culture can be ordered, and a request to identify different strains of *Candida* can be made. A group B strep DNA probe or group B strep culture should be ordered for pregnant patients.
7. The Affirm VP III test is used to diagnose *Candida* species, BV, and trichomoniasis.
8. The Thin prep can actually test for many different pathogens, including the detection and identification of human papillomavirus and its genotype (HPV 16/18), *Chlamydia,* gonococcus, *Trichomonas,* yeast, *Actinomyces,* herpesviruses I and II, Group B strep, BV, syphilis, *Ureaplasma,* and *Mycoplasma.*

THERAPEUTIC APPROACH

Because approximately 90% of all cases of vaginitis are due to *Candida, Trichomonas,* or *Gardnerella* infections, the following recommendations are primarily directed to the treatment of these organisms. Owing to the infectious nature of these organisms, immune support (through proper diet, nutritional supplementation, and botanical medicines) is an important aspect of the therapy. For further recommendations on treating atrophic vaginitis, see Chapter 196; for herpes simplex, see Chapter 177. All women should be advised to not wear synthetic materials that cover the vaginal opening.

Diet

For all causes of vaginitis, a nutrient-dense diet is recommended. All refined foods and simple carbohydrates should be eliminated, and trans fats and saturated fats should be kept to a minimum. If food allergies are suspected, they should be determined and eliminated.

Supplements

- Vitamin A: 5000 IU/day
- Beta-carotene: 50,000 IU/day
- Vitamin C: 500 to 1000 mg every 4 hours
- B complex: a well-balanced B complex averaging 20 to 50 mg/day of each of the major components
- Zinc: 10 to 15 mg/day
- Vitamin E (mixed tocopherols): 200 IU/day
- *Lactobacillus* species orally and vaginally; dose depends on duration and kind of infection

Botanical Medicines

Hydrastis canadensis (Goldenseal)

Dosages three times a day are as follows:

- Dried root or infusion (tea): 2 to 4 g
- Tincture (1:5): 6 to 12 mL (1.5–3 tsp)
- Fluid extract (1:1): 2 to 4 mL (0.5–1 tsp)
- Solid (powdered dry) extract (4:1 or 8%–12% alkaloid content): 250 to 500 mg

Allium sativum (garlic)

- 4000 mcg to 400 mg dose equivalent of allicin.

General Recommendations

1. In treating infectious vaginitis, the following natural medicine concepts should be kept in mind:
   - Remove/limit obstacles to cure.
   - Improve vaginal immunity.
   - Support systemic immunity.
   - Restore vaginal pH.
   - Restore vaginal microenvironment.

- Restore gut ecology.
- Decrease inflammation/irritation.
- Provide symptom relief.
- Correct coexisting medical conditions.

2. In all cases of chronic vaginitis, *Lactobacillus* capsules or *Lactobacillus* yogurt should be used daily, at least orally if not vaginally, to reinoculate the vagina with these desirable organisms. Oral use should continue for 2 to 6 months to ensure colonization.
3. Treatment failures may be due to an incorrect diagnosis, reinfection, failure to treat predisposing factors, or patient resistance to the treatment used.
4. *Trichomonas* infections in women require concurrent treatment of the male partner.
5. In cases of recurrent or chronic BV or yeast vaginitis, the clinician should consider treating both male and female partners, infection in whom is a possible explanation for recurrent disease.

Specific Recommendations/Sample Treatments

Acute Candidal Vaginitis

- 8 oz of *Acidophilus* yogurt every day
- *Lactobacillus* spp. 2 to 10 billion CFUs twice a day for 14 days
- Boric acid: 600 mg placed in capsules twice a day for 3 to 7 days

Chronic Candida Vaginitis

- 8 oz of *Acidophilus* yogurt every day
- *Lactobacillus* spp. (e.g., *L. rhamnosus, L. rheuteri*) 1 to 5 billion CFUs; twice daily for 1 to 6 months
- Boric acid: 600 mg vaginal suppositories twice a day for 2 weeks (4 weeks if patient is not free of symptoms and wet-mount examination still shows organisms at 2 weeks)
- To prevent vulvar irritation from the boric acid dispensed from the dissolved capsule, vitamin E oil or petroleum jelly can be applied to the external genitalia.

Bacterial Vaginosis

- Vitamin C vaginal tablet for 6 days and then boric acid suppositories once a day for 1 week
- Multiple *Lactobacillus* species/strains 2 billion CFUs or more a day for 2 weeks
- Systemic immune support

Trichomonas

- Povidone-iodine: applied twice daily for 14 days
- Zinc sulfate: 1% zinc sulfate douche twice daily
- *M. alternifolia* oil : 1%-20% emulsified solution for vaginal douching or a saturated tampon, applied weekly for 4 weeks; or vaginal pessaries (0.2 g essential oil) applied nightly for 30 to 90 days

Atrophic Vaginitis

- Intravaginal Estriol cream, 1 mg/g. Insert 1 g a day for 2 weeks, then 1 g twice a week as maintenance. Other vaginal estrogen options include vaginal estradiol 0.1% cream, Premarin cream, Estring, and Vagifem.
- Vaginal DHEA ovules 0.5% (6.5 mg)/day for 12 weeks, then twice a week

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Paladine HL, Desai UA. Vaginitis: diagnosis and treatment. *Am Fam Physician*. 2018;97(5):321–329.
2. Sobel J. *Approach to women with symptoms of vaginitis*. In: Eckler K, Barbieri R, eds. UpToDate. https://www.uptodate.com/contents/approach-to-women-with-symptoms-of-vaginitis. Accessed November 6, 2018.
3. Koumans EH, Sternberg M, Bruce C, et al. *The prevalence of bacterial vaginosis in the United States*, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis*. 2007; 34(11):864–869.
4. Bautista CT, Wurapa E, Sateren WB, et al. Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. *Mil Med Res*. 2016;3:4.
5. Kinney RG, Spach DH. "Vaginitis". In: Marrazzo J, ed. *National STD Curriculum*. 2017. www.std.uw.edu/go/syndrome-based/vaginal-discharge/core-concept/all. Accessed November 5, 2018.
6. Berger A, Kane KY. Clindamycin for vaginosis reduces prematurity and late miscarriage. *J Fam Pract*. 2003;52(8):603–604.
7. Cherpes TL, Wiesenfeld HC, Melan MA, et al. The associations between pelvic inflammatory disease, *Trichomonas vaginalis* infection, and positive herpes simplex virus type 2 serology. *Sex Transm Dis*. 2006;33(12):747–752.
8. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. *N Engl J Med*. 1995;333(26):1737–1742.
9. Laxmi U, Agrawal S, Raghunandan C, Randhawa VS, Saili A. Association of bacterial vaginosis with adverse fetomaternal outcome in women with spontaneous preterm labor: a prospective cohort study. *J Matern Fetal Neonatal Med*. 2012;25(1):64–67.
10. Nelson DB, Hanlon A, Hassan S, et al. Preterm labor and bacterial vaginosis-associated bacteria among urban women. *J Perinat Med*. 2009;37(2):130–134.
11. Oakeshott P, Kerry S, Hay S, Hay P. Bacterial vaginosis and preterm birth: a prospective community-based cohort study. *Br J Gen Pract*. 2004;54(499):119–122.
12. Soper DE. Bacterial vaginosis and postoperative infections. *Am J Obstet Gynecol*. 1993;169(Pt 2):467–469.
13. Brotman RM, Klebanoff MA, Nansel TR, et al. Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *J Infect Dis*. 2010;202(12):1907–1915.
14. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis*. 2003;37(3):319–325.
15. Cohen CR, Lingappa JR, Baeten JM, et al. Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. *PLoS Med*. 2012;9(6):e1001251.
16. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis*. 1999;180(6):1863–1868.
17. Myer L, Kuhn L, Stein ZA, Wright TC, Denny L. Intravaginal practices, bacterial vaginosis, and women's susceptibility to HIV infection: epidemiological evidence and biological mechanisms. *Lancet Infect Dis*. 2005;5(12):786–794.
18. *"Pelvic Inflammatory Disease (PID) - CDC Fact Sheet." Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention. 27; 2017. www.cdc.gov/std/pid/stdfact-pid-detailed.htm. Accessed August 20, 2019.
19. Stamey T. The role of introital enterobacteria in recurrent urinary infections. *J Urol*. 1973;109:467–472.
20. Netto NR, Rangel P, DaSilva R, et al. The importance of vaginal infection on recurrent cystitis in women. *Int Surg*. 1979;64:79–82.
21. Nunn KL, Forney LJ. Unraveling the dynamics of the human vaginal microbiome. *Yale J Biol Med*. 2016;89(3):331–337.
22. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. *Annu Rev Microbiol*. 2012;66:371–389.
23. Smith SB, Ravel J. The vaginal microbiota, host defence and reproductive physiology. *J Physiol*. 2017;595(2):451–463.
24. Erickson K, Hubbard N. Probiotic immunomodulation in health and disease. *J Nutr*. 2000;130(suppl 25):S403–S409.
25. Reid G, Cook R, Bruce A. Examination of strains of lactobacilli for properties that may influence bacterial interference in the urinary tract. *J Urol*. 1987;138:330–335.
26. Fowler S. Expansion of altered vaginal flora states in vaginitis to include a spectrum of microflora. *J Reproductive Med*. 2007;52(2):93–99.
27. Larsen B, Galask R. Vaginal microbial flora: practical and theoretic relevance. *Ob Gyn*. 1980;55:S100–S113.
28. "Vaginitis - Frequently Asked Questions." American College of Obstetricians and Gynecologist. Am Col Obstetric Gynecol; 2017. https://www.acog.org/-/media/For-Patients/faq028.pdf?dmc=1&ts=20181106T1845081326. Accessed August 20, 2019.
29. Centers for Disease Control and Prevention. *Trichomoniasis [CDC Fact Sheet]*; 2017. Retrieved from https://www.cdc.gov/std/trichomonas/stdfact-trichomoniasis.htm. Accessed November 6, 2018.
30. Hawes S, Hillier S, Benedetti J, et al. Hydrogen-peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis*. 1996;174:1058–1063.
31. Goldenberg R, Klebanoff M, Nugent R, et al. Bacterial colonization of the vagina during pregnancy in four ethnic groups. *Am J Obstet Gynecol*. 1996;174:1618–1624.
32. Fettweis, JM, Brooks JP, Serrano MG, et al. "Differences in vaginal microbiome in African American women versus women of European ancestry" *Microbiol (Reading, England)*. 2014;160(10):2272–2282.
33. Fleury FJ. Is there a "non-specific" vaginitis? *Med Times*. 1978;106:37–43.
34. Blackwell A, Fox A, Phillips I, et al. Anaerobic vaginosis (nonspecific vaginitis): clinical, microbiological, and therapeutic findings. *Lancet*. 1983;2:1379–1382.
35. Martin J, Richardson B, Nyange P, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis*. 1999;180:1863–1868.
36. Taha T, Hoover D, Dallabeta G, et al. Bacterial vaginosis and disturbances of vaginal flora: association with an increased acquisition of HIV. *AIDS*. 1998;12:1699–1706.
37. Centers for Disease Control and Prevention. *Vaginal Candidiasis*; 2017. Retrieved from https://www.cdc.gov/fungal/diseases/candidiasis/genital/index.html. Accessed November 7, 2018.
38. Miles MR, Olsen L, Rogers A, et al. Recurrent vaginal candidiasis: importance of an intestinal reservoir. *JAMA*. 1977;238:1836–1837.
39. Lin XL, Li Z, Zuo XL. [Study on the relationship between vaginal and intestinal candida in patients with vulvovaginal candidiasis]. *Zhonghua Fu Chan Ke Za Zhi*. 2011;46(7):496–500.
40. Soong D, Einarson A. Vaginal yeast infections during pregnancy. *Can Fam Physician*. 2009;55(3):255–256.
41. Malgaard-Nielsen D, Svanstrom H, Melbye M, et al. Association between use of oral fluconazole during pregnancy and risk of spontaneous abortion and stillbirth. *JAMA*. 2016;315:58–67.
42. Aslam M, Hafeez R, Ijaz S, Thair M. Vulvovaginal candidiasis in pregnancy. *Biomedica*. 2008;24:54–56.
43. Heidrich F, Berg A, Gergman F, et al. Clothing factors and vaginitis. *J Fam Pract*. 1984;19:491–494.
44. Centers for Disease Control and Prevention. *Vulvovaginal Candidiasis*. 2015. Retrieved from https://www.cdc.gov/std/tg2015/candidiasis.htm. Accessed November 7, 2018.
45. O'Connor M, Sobel J. Epidemiology of recurrent vulvovaginal candidiasis: identification and strain differentiation of *Candida albicans*. *J Infect Dis*. 1986;154:358–363.
46. Mercure S, Poirier S, Lemay G, et al. Application of biotyping and DNA typing of *Candida albicans* to the epidemiology of recurrent vulvovaginal candidiasis. *J Infect Dis*. 1993;168:502–507.
47. Fidel P, Sobel J. Immunopathogenesis of recurrent vulvovaginal candidiasis. *Clin Microbiol Rev*. 1996;9:335–348.

48. Hildebrandt RJ. Trichomoniasis: always with us—but controllable. *Med Times*. 1978;106:44–48.
49. Sparling PF. Introduction to sexually transmitted diseases and common syndromes. In: Bennett JC, Plum F, eds. *Cecil Textbook of Medicine*. Philadelphia: Saunders; 1669–1700.
50. Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. *Am Fam Physician*. 2000;16(10):3090–3096.
51. Neggers YH, Nansel TR, Andrews WW, et al. Dietary intake of selected nutrients affects bacterial vaginosis in women. *J Nutr*. 2007;137(9):2128–2133.
52. Hilton E, Isenberg H, Alperstein P, et al. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. *Ann Intern Med*. 1992;116:353–357.
53. Shaley E, Battino S, Weiner E, et al. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. *Arch Fam Med*. 1996;5:593–596.
54. Sirisinha S. The pleiotropic role of vitamin A in regulating mucosal immunity. *Asian Pac J Allergy Immunol*. 2015;33(2):71–89. Review. PubMed PMID: 26141028.
55. Sirisnha S, Daziy M, Moongkarndi P, et al. Impaired local immune response in vitamin A deficient rats. *Clin Exp Immunol*. 1980;40:127–135.
56. Alexander MM, Newmark H, Miller R. Oral betacarotene can increase the number of $OKT4^+$ cells in human blood. *Immunol Letters*. 1985;9:221–224.
57. Sharaf A, Gomaa N. Interrelationship between vitamins of the B-complex group and oestradiol. *J Endo*. 1974;62:241–244.
58. Beisel WR, Edelman R, Nauss K, et al. Single-nutrient effects on immunologic functions. *JAMA*. 1981;245:53–58.
59. Stankova L, Gerhardt N, Nagel L, et al. Ascorbate and phagocyte function. *Infect Immun*. 1975;12:252–256.
60. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharm*. 1983;32:1141–1148.
61. Holden M, Resnick R. The in-vitro action of synthetic crystalline vitamin C (ascorbic acid) on Herpes virus. *J Immunol*. 1936;31:455–462.
62. Terezhalmy G, Bottomley W, Pelley G. The use of water soluble bioflavonoid-ascorbic acid complex in the treatment of recurrent herpes labialis. *Oral Surg*. 1978;45:60–62.
63. Welch C, Baker K. The effectiveness of intravaginal vitamin C versus placebo for the treatment of bacterial vaginosis: a systematic review protocol. *JBI Database System Rev Implement Rep*. 2015;13(6):96–113.
64. Petersen EE, Genet M, Caserini M, Palmieri R. Efficacy of vitamin C vaginal tablets in the treatment of bacterial vaginosis: a randomised, double blind, placebo controlled clinical trial. *Arzneimittelforschung*. 2011;61(4):260–265.
65. Petersen E, Magnani P. Efficacy and safety of vitamin C vaginal tablets in the treatment of non-specific vaginitis. *Eur J Obstet Gynecol Reprod Biol*. 2004;117(1):70–75.
66. Abbaspoor Z, Goodarzy F, Abbaspoor MR. Comparison of the effectiveness of vitamin C vaginal tablet with metronidazole vaginal gel in the treatment of bacterial vaginosis. *Afr J Pharm Pharmacol*. 2010;4(7):484–489.
67. Polatti F, Rampino M, Magnani P, Mascarucci P. Vaginal pH-lowering effect of locally applied 62. vitamin C in subjects with high vaginal pH. *Gynecol Endocrinol*. 2006;22(4):230–234.
68. Zodzika J, Rezeberga D, Donders G, et al. Impact of vaginal ascorbic acid on abnormal vaginal microflora. *Arch GynecolObstet*. 2013;288(5):1039–1044.
69. Krasnopolsky VN, Prilepskaya VN, Polatti F, et al. Efficacy of vitamin C vaginal tablets as prophylaxis for recurrent bacterial vaginosis: a randomised, double-blind, placebo-controlled clinical trial. *J Clin Med Res*. 2013;5(4):309–315.
70. Taheri M, Baheiraei A, Foroushani AR, et al. Treatment of vitamin D deficiency is an effective method in the elimination of asymptomatic bacterial vaginosis: a placebo-controlled randomized clinical trial. *Indian J Med Res*. 2015;141(6):799–806.
71. Lee A, Lee MR, Lee HH, et al. Vitamin D proliferates vaginal epithelium through RhoA expression in postmenopausal atrophic vagina tissue. *Mol Cells*. 2017;40(9):677–684.
72. Yildirim B, Kaleli B, Düzcan E, Topuz O. The effects of postmenopausal Vitamin D treatment on vaginal atrophy. *Maturitas*. 2004;49:334–337.
73. Stephens L, McChesney A, Nockels C. Improved recovery of vitamin E treated lambs that have been experimentally infected with intertracheal Chlamydia. *Br Vet J*. 1979;135:291–293.
74. Pories W, Henzel J, Rob C, et al. Acceleration of wound healing in man with zinc sulphate given by mouth. *Lancet*. 1967;1:121–124.
75. Sandstead H, Lanier Jr V, Shepard G, et al. Zinc and wound healing. *Am J Clin Nutr*. 1970;23:514–519.
76. Liszewski R. The effect of zinc on wound healing: a collective review. *J Am Osteopath Assoc*. 1981;81:104–106.
77. Greenberg S, Harris D, Giles P, et al. Inhibition of *Chlamydia trachomatis* growth by zinc. *Antimicrob Agents Chemother*. 1985;27:953–957.
78. Krieger J, Rein M. Zinc sensitivity of *Trichomonas vaginalis*: in vitro studies and clinical implications. *J Infect Dis*. 1982;146:341–345.
79. Willmott F, Say J, Downey D, et al. Zinc and recalcitrant trichomoniasis [letter]. *Lancet*. 1983;1:1053.
80. Tennican P, Carl G, Frey J, et al. Topical zinc in the treatment of mice infected intravaginally with *Herpes genitalis* virus. *Proc Soc Exp Biol Med*. 1980;164:593–597.
81. Gordon Y, Asher Y, Becker Y. Irreversible inhibition of herpes simplex virus replication in BSC-cells by zinc ions. *Antimicrob Agents Chemother*. 1975;8:377–380.
82. Byun JM, Jeong DH, Kim YN, et al. Experience of successful treatment of patients with metronidazole-resistant *Trichomonas vaginalis* with zinc sulfate: a case series. *Taiwan J Obstet Gynecol*. 2015;54(5):617–620.
83. Pellati D, Fiore C, Armanini D, et al. In vitro effects of glycyrrhetinic acid on the growth of clinical isolates of Candida albicans. *Phytother Res*. 2009;23(4):572–574.
84. Mahmoudabadi AZ, Iravani M, Khazeri A. Anti-fungal activity of *Glycyrrhiza glabra* (Licorice) against vaginal isolates of candida. *BioTechnol*. 2009;3(2):75–77.
85. Sirilun S, Sivamaruthi BS, Kesika P, et al. Development and stability evaluation of vaginal suppository containing *Glycyrrhiza glabra* L. For the treatment of *Candida albicans* infection. *Asian J Pharm Clin Res*. 2018;11(7):205–209.
86. Azemi ME, Morshedi Z, Moghimipour E. Preparation and evaluation of glycine max, trigonella foenum, glycyrrhiza glabra and aloe barbadensis extracts vaginal cream. *Int J Curr Res Chem Pharm Sci*. 2015;2(2):44–50.
87. Sadeghi M, Abbaspoor Z, Namjouyan F, Cheraghian B. Effect of *Glycyrrhiza glabra* vaginal cream on the mental status symptoms of vaginal atrophy in postmenopausal women. *J Gynecol Infertil*. 2018;7(21):53–61.
88. Mitchell W. *Naturopathic Applications of the Botanical Remedies*. Seattle, WA: JBC Publications.
89. Mowbray S. The antibacterial activity of chlorophyll. *Br Med J*. 1957;1:268–270.
90. Goldberg S. The use of water soluble chlorophyll in oral sepsis. *Am J Surg*. 1943;62:117–122.
91. Smith L, Livingston A. Chlorophyll: an experimental study of its water soluble derivatives in wound healing. *Am J Surg*. 1943;62:358–369.
92. Sharma V, Sethi MS, Kumar V, et al. Antibacterial property of *Allium sativum* Linn. in vivo and in vitro studies. *Ind J Exp Bio*. 1977;15:466–468.
93. Moore G, Atkins R. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast-like fungi. *Mycologia*. 1977;69:341–348.
94. Cavallito C, Bailey J. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J Am Chem Soc*. 1944;66:1950–1951.
95. Barone F, Tansey M. Isolation, purification, identification, synthesis, and kinetics of activity of the anticandidal component of *Allium sativum*, and a hypothesis for its mode of action. *Mycologia*. 1977;69:793–825.
96. Prat M. Algunas consideraciones sobre la accion antibiotica del Allium sativum y sus preparados [trans]. *Biol Abstr*. 1950;24:264.
97. Ebrahimy F, Dolatian M, Moatar F, Alavi Majd H. Comparison of the therapeutic effects of Garcin(R) and fluconazole on *Candida vaginitis*. *Singapore Med J*. 2015;56(10):567–572.
98. Mohammadzadeh F, Dolatian M, Jorjani M, Alavi Majd H, Borumandnia N. Comparing the therapeutic effects of garlic tablet and oral met-

ronidazole on bacterial vaginosis: a randomized controlled clinical trial. *Iran Red Crescent Med J*. 2014;16(7):e19118.
99. Chu M, Xiao R, Yin Y, et al. Berberine: a medicinal compound for the treatment of bacterial infections. *Clin Microbial*. 2014;3:150. https://doi.org/10.4172/2327-5073.1000150.
100. Romm A, Hardy ML, Mills S. *Botanical Medicine for Women's Health: Vaginal Infections and Sexually Transmitted Diseases*. London: Elsevier; 2010.
101. Baery N, Amin G, Amin M, et al. Herbal remedies used in treatment of bacterial vaginitis: a mini-review. *Indo Am J Pharm Sci*. 2017;4(11):3830–3839.
102. Hahn F, Ciak J. *Berberine. Antibiotics*. 1976;3:577–588.
103. Sabir M, Bhide N. Study of some pharmacological actions of berberine. *Ind J Phys Pharm*. 1971;15:111–132.
104. Sabir M, Mahajan V, Mohaptra L, et al. Experimental study of the antitrachoma action of berberine. *Ind J Med Res*. 1976;64:1160–1167.
105. Dutta N, Panse M. Usefulness of berberine (an alkaloid from *Berberis aristata*) in the treatment of cholera (experimental). *Ind J Med Res*. 1962;50:732–735.
106. Masoudi M, Rafieian Kopaei M, Miraj S. Comparison between the efficacy of metronidazole vaginal gel and *Berberis vulgaris (Berberis vulgaris)* combined with metronidazole gel alone in the treatment of bacterial vaginosis. *Electronic Physician*. 2016;8(8):2818–2827.
107. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 20016;19(1):50–62.
108. Pena EF. *Melaleuca alternifolia* oil. Its use for trichomonal vaginitis and other vaginal infections. *Ob Gyn*. 1962;19:793–795.
109. Williams L, Home V. A comparative study of some essential oils for potential use in topical applications for the treatment of the yeast *Candida albicans*. *Aust J Med Herbal*. 1995;7:57–62.
110. Costantino D, Guaraldi C. Effectiveness and safety of vaginal suppositories for the treatment of the vaginal atrophy in postmenopausal women: an open, non-controlled clinical trial. *Eur Rev Med Pharm Sci*. 2008;12:411–416.
111. Mittal A, Kapur S, Garg S, et al. Clinical trial with Praneem polyherbal cream in patients with abnormal vaginal discharge due to microbial infections. *Aust N Z J Obstet Gyn*. 1995;35:190–191.
112. Reid G. The scientific basis for probiotic strains of *Lactobacillus*. *Appl Environ Microbiol*. 1999;65:3763–3766.
113. Chew SY, Cheah YK, Seow HF, Sandai D, Than LT. Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exhibit strong antifungal effects against vulvovaginal candidiasis-causing *Candida glabrata* isolates. *J Appl Microbiol*. 2015;118(5):1180–1190. https://doi.org/10.1111/jam.12772. Epub 2015 Mar 16.
114. Mailänder-Sánchez D, Braunsdorf C, Grumaz C, et al. Antifungal defense of probiotic *Lactobacillus rhamnosus* GG is mediated by blocking adhesion and nutrient depletion. *PLoS One*. 2017;12(10):e0184438.
115. Homayouni A, Bastani P, Ziyadi S, et al. Effects of probiotics on the recurrence of bacterial vaginosis: a review. *J Low Genit Tract Dis*. 2014;18(1):79–86.
116. Parma M, Stella Vanni V, Bertini M, Candiani M. Probiotics in the prevention of recurrences of bacterial vaginosis. *Altern Ther Health Med*. 2014;20(suppl 1):52–57.
117. Mastromarino P, Vitali B, Mosca L. Bacterial vaginosis: a review on clinical trials with probiotics. *New Microbiol*. 2013;36(3):229–238.
118. Delia A, Morgante G, Rago G, et al. Effectiveness of oral administration of *Lactobacillus paracasei* subsp. paracasei F19 in association with vaginal suppositories of *Lactobacillus acidofilus* in the treatment of vaginosis and in the prevention of recurrent vaginitis. *Minerva Ginecol*. 2006;58(3):227–231.
119. Ozkinay E, Terek MC, Yayci M, et al. The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections. *BJOG*. 2005;112(2):234–240.
120. Vujic G, Jajac Knez A, Despot Stefanovic V, et al. Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: a double-blind, randomized, placebo-controlled study. *Eur J Obstet Gynecol Reprod Biol*. 2013.
121. Larrsson P, Stray-Pedersen B, Ryttig K, et al. Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate: a 6-month, double-blind, randomized, placebo-controlled study. *BMC Women's Health*. 2008;8:3.
122. Anukam Km, Osazuwa E, Ahonkhai I, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: a randomized, double-blind, placebo controlled trial. *Microbes Infect*. 2006;8:1450–1454.
123. Martinez R, Franceschini S, Patta M, et al. Improved cure of bacterial vaginosis with single dose of tinidazole (2 g), *Lactobacillus rhamnosus* GR-1, and *Lactobacillus reuteri* RC-14: a randomized, double-blind, placebo-controlled trial. *Can J Microbiol*. 2009;55:133–138.
124. Ehrström S, Daroczy K, Rylander E, et al. Lactic acid bacteria colonization and clinical outcome after probiotic supplementation in conventionally treated bacterial vaginosis and vulvovaginal candidiasis. *Microbes Infect*. 2010;12(10):691–699.
125. Xie HY, Feng D, Wei DM, et al. Probiotics for vulvovaginal candidiasis in non-pregnant women. *Cochrane Database Syst Rev*. 2017;11:Art. No.: CD010496. https://doi.org/10.1002/14651858.CD010496.pub2.
126. Davar R, Nokhostin F, Eftekhar M, et al. Comparing the recurrence of vulvovaginal candidiasis in patients undergoing prophylactic treatment with probiotic and placebo during the 6 months. *Probiotics Antimicrob Proteins*. 2016;8(3):130–133.
127. Hilton E, Rindos P, Isenberg H. Lactobacillus GG vaginal suppositories and vaginitis. *J Clin Microbiol*. 1995;33:1433.
128. Darvishi M, Jahdi F, Hamzegardeshi Z, et al. The comparison of vaginal cream of mixing yogurt, honey and clotrimazole on symptoms of vaginal candidiasis. *Glob J Health Sci*. 2015;7(6):108–116.
129. Williams A, Yu C, Tashima K, et al. Evaluation of two self care treatments for prevention of vaginal candidiasis in women with HIV. *J Assoc Nurses AIDS Care*. 2001;12:51–57.
130. Reid G, Charbonneau D, Erb J, et al. Oral use of *Lactobacillus rhamnosus* GR01 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immun Med Microbiol*. 2003;35:131–134.
131. Reid G, Bruce A, Fraser N, et al. Oral probiotics can resolve urogenital infections. *FEMS Immun Med Microbiol*. 2001;30:49–52.
132. Reid G, Beueman D, Heinemann C, et al. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. *FEMS Immu Med Microbiol*. 2001;32:37–41.
133. Gershenfeld L. Povidone iodine as a vaginal microbicide. *Am J Pharm*. 1962;134:278–291.
134. Pirotta M, Gunn J, Chondros P, et al. Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomized controlled trial. *BMJ*. 2004;329:548–552.
135. Shook D. A clinical study of a povidone-iodine regimen for resistant vaginitis. *Curr Ther Res*. 1963;5:256–263.
136. Maneksha S. Comparison of povidone-iodine (Betadine) vaginal pessaries and lactic acid pessaries in the treatment of vaginitis. *J Int Med Res*. 1974;2:236–239.
137. Reeve P. The inactivation of *Chlamydia trachomatis* by povidone iodine. *J Antimicrob Chemo*. 1976;2:77–80.
138. Ratzen J. Monilial and trichomonal vaginitis: topical treatment with povidone iodine treatments. *Cal Med*. 1969;110:24–27.
139. Mayhew S. Vaginitis. A study of the efficacy of povidone iodine in unselected cases. *J Int Med Res*. 1981;9:157–159.
140. Gershenfeld L. Povidone iodine as a trichomoniacide. *Am J Pharm*. 1962;134:324–331.
141. Singha H. The use of a vaginal cleansing kit in non-specific vaginitis. *Practitioner*. 1979;223:403–404.
142. Jovanovic R, Congema E, Nguyen H. Antifungal agents vs boric acid for treating chronic mycotic vulvovaginitis. *J Reprod Med*. 1991;36:593–597.
143. Swate T, Weed J. Boric acid treatment of vulvovaginal candidiasis. *Ob Gyn*. 1974;43:894–895.
144. Keller Van Slyke K. Treatment of vulvovaginal candidiasis with boric acid powder. *Am J Obstet Gynecol*. 1981;141:145–148.
145. Iavazzo C, Gkegkes I, Zarkada I, et al. Boric acid for recurrent vulvovaginal candidiasis: the clinical evidence. *J Women's Health*. 2011;20(8):1245–1255.

146. Manonai J, Chittacharoen A, Theppisai U, et al. Effect of *Pueraria mirifica* on vaginal health. *Menopause: The Journal of the North American Menopause Society*. 2007;14(5):919–924.
147. Labrie F, Archer D, Bouchard C, et al. High internal consistency and efficacy of intravaginal DHEA for vaginal atrophy. *Gynecol Endocrinol*. 2010;26(7):524–532.
148. Labrie F, Archer D, Bouchard C, et al. Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy. *Menopause*. 2009;16(5):907–922.
149. FDA News Release: *FDA approves Intrarosa for postmenopausal women experiencing pain during sex. FDA Press Announcements*. November 17, 2016. Available at: https://www-fda-gov.nunm.idm.oclc.org/NewsEvents/Newsroom/PressAnnouncements/ucm529641.htm. Accessed November 17, 2016.
150. Labrie F, Archer D, Bouchard C, et al. Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women. *Menopause*. 2009;16:923–931.
151. Labrie F, Archer DF, Koltun W, et al. Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause*. 2018;25(11):1339–1353.
152. Sauer U, Talaulikar V, Davies MC. Efficacy of intravaginal dehydroepiandrosterone (DHEA) for symptomatic women in the peri- or postmenopausal phase. *Maturitas*. 2018;116:79–82.
153. Labrie F, Archer D, Bouchard C, et al. Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration. *Menopause*. 2009;16(5):897–906.
154. Atrophic Vaginitis | UVA Health. Uvahealth.com. https://uvahealth.com/services/womens-health/atrophic-vaginitis. Published 2020. Accessed February 9, 2020.

Varicose Veins

Michael T. Murray, ND, and *John Nowicki, ND*

OUTLINE

DIAGNOSTIC SUMMARY

- Dilated, tortuous superficial veins in the lower extremities
- May be asymptomatic or associated with fatigue, aching discomfort, feeling of heaviness, or pain
- Edema, pigmentation, and ulceration of the skin of the distal leg may develop.
- Women are affected four times as frequently as men.

GENERAL DISCUSSION

Veins are frail structures. Defects in the wall of a vein lead to dilation of the vein and damage to the valves. When the valves become damaged, the higher static pressure results in the bulging veins known as *varicose veins.*

Varicose veins affect nearly 50% of middle-aged adults. The subcutaneous veins of the legs are the veins most commonly affected, owing to gravitational pressure. When an individual stands for long periods, the pressure buildup in the vein can increase up to 10 times. Hence, individuals with occupations that require long periods of standing are at greatest risk for the development of varicose veins.

Women are affected about four times as frequently as men. Obese individuals have a much greater risk, and the risk rises with age owing to loss of tissue tone, loss of muscle mass, and weakening of the walls of the veins. Pregnancy, which increases venous pressure in the legs, may also lead to the development of varicose veins.

In general, varicose veins pose little risk if the involved vein is near the surface. These types of varicose veins are, however, cosmetically unappealing (Fig. 225.1). Although significant symptoms are not common, the legs may feel heavy, tight, and tired. If the varicose veins are associated with significant chronic venous insufficiency (CVI), leg ulcers may form that are often difficult to resolve.

A more serious form of varicose vein involves obstruction and valve defects of the deeper veins of the leg. This type of varicose vein can lead to problems such as thrombophlebitis, pulmonary embolism, myocardial infarction, and stroke. Phlebography and Doppler ultrasonography are the most accurate methods of diagnosing deep venous involvement.

ETIOLOGY

The following theories have been advanced to explain the cause of varicose veins:

- Genetic or functional weakness of the veins or venous valves
- Excessive venous pressure due to increased straining during defecation, often caused by a low-fiber diet
- Long periods of standing and/or heavy lifting
- Damage to the veins or venous valves secondary to thrombophlebitis

The major cause of varicose veins is weakness of the vascular walls due to either abnormalities in the proteoglycans of the interendothelial cement substance or excessive expression/activity/release of matrix metalloproteinases (MMPs) such as β-*N*-acetylglucosaminidase, β-glucuronidase, and arylsulfatase, which degrade extracellular matrix proteins and affect the structural integrity of the vein wall, leading to increased capillary permeability and loss of venous structural integrity. The MMPs also affect the endothelium and smooth muscle components of the vein wall, thereby causing changes in the properties of venous constriction/relaxation. Endothelial cell injury also triggers leukocyte infiltration, activation, and inflammation, which leads to further damage to the vein wall and thus chronic and progressive venous insufficiency and varicose vein formation.[1,2]

THERAPEUTIC CONSIDERATIONS

The treatment of varicose veins ranges from conservative measures to surgical interventions. Conservative therapy involves the following:

- Elevating the legs periodically
- Wearing graduated compression stockings with variable pressure gradients, especially if standing for long periods of time is unavoidable
- Exercise, especially walking, riding a bike, or jogging, which is thought to be helpful because contraction of the leg muscles pushes pooled blood back into circulation
- Achieving or maintaining one's ideal body weight
- Maintaining adequate intake of dietary fiber to avoid increasing venous pressure consequent to straining during defecation
- Using nutritional and botanical agents to assist in improving the structural integrity of the veins

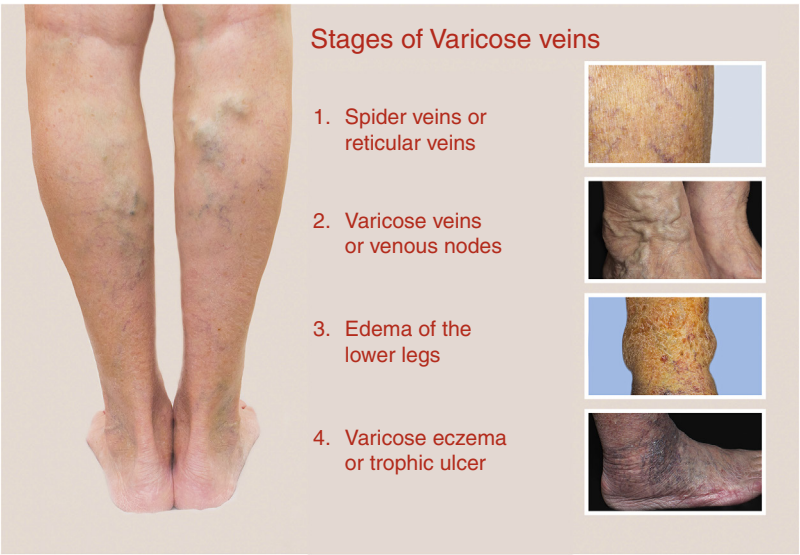


Fig. 225.1 Stages of varicosities.

For severely affected veins, more aggressive treatment may be necessary. The traditional surgical treatment has been vein stripping to remove the affected veins. Newer, less invasive treatments that seal the main leaking vein at the highest point of valvular dysfunction on the thigh include ultrasound-guided foam sclerotherapy, radiofrequency ablation, and endovenous laser treatment. Because most of the blood in the legs is returned by the deep veins, the superficial veins, which return only about 10% of the total blood of the legs, can usually be removed or ablated without serious harm. Sclerotherapy with segmental phlebectomy (stripping of the vein) is also popular. In sclerotherapy with segmental phlebectomy, a sclerosing agent is injected at the highest portion of the affected vein. Only the most severely diseased segments of vein are stripped away with segmental phlebectomy.

Dietary Factors

Fiber

A low-fiber diet that is high in refined foods contributes to the development of varicose veins.[3,4] Individuals consuming a low-fiber diet tend to strain more during bowel movements because smaller and harder stools are more difficult to pass. Straining raises the pressure in the abdomen, obstructing the flow of blood up the legs. Over time, this increased pressure may significantly weaken the vein walls, leading to the formation of varicose veins or hemorrhoids, or it may weaken the wall of the large intestine and produce diverticuli.[5]

A diet rich in vegetables, fruits, legumes, and grains promotes peristalsis, and many fiber components attract water and form a gelatinous mass, which keeps the feces soft, bulky, and easy to pass. The net effect of a high-fiber diet is significantly less straining during defecation.

Natural bulking compounds can also be used. These substances, particularly psyllium seed, pectin, and guar gum, possess a mild laxative action owing to their ability to attract water and form a gelatinous mass. This process, as previously mentioned, keeps the feces soft and promotes peristalsis, significantly reducing straining during defecation. These types of fibers are generally less irritating than wheat bran and other cellulose fiber products.

Flavonoid-Rich Berries

Berries, such as hawthorn berries, cherries, blueberries, and blackberries, appear to be beneficial in the prevention and treatment of varicose veins. These berries are very rich sources of proanthocyanidins and anthocyanidins.[6–8] Flavonoids are noted for their ability to improve the integrity of ground substance and the vascular system. Extracts of several of these berries are used widely in Europe for various circulatory conditions.[6–8]

Another rich source of flavonoids is buckwheat, which is high in rutin. In one double-blind, placebo-controlled study, 77 patients with CVI were given placebo tea or *Fagopyrum esculentum* (buckwheat) tea for 12 weeks.[9] The tea was standardized to contain 5% total flavonoids, yielding a daily dosage of 270 mg of rutin. A statistically significant reduction in total leg volume was seen in the treated group, along with statistically insignificant improvements in capillary permeability and symptoms. No adverse effects were noted.

The efficacy of these extracts is related to their ability to accomplish the following[6–8]:

- Reduce capillary fragility
- Increase the integrity of the venous wall
- Inhibit the breakdown of the compounds composing the ground substance
- Improve the muscular tone of the vein

Consumption of these berries or of their extracts or other flavonoid-rich extracts like grape seed or pine bark (see Chapter 106) is indicated for individuals with varicose veins as well as those who wish to prevent them.

Botanical Medicines

Aesculus hippocastanum

Horse chestnut *(Aesculus hippocastanum)* was once native to western Asia but has been widely distributed all over the world because of its beauty. In addition to enhancing the appearance of city streets and parks, this hearty tree possesses significant medicinal effects. In particular, the seeds of the horse chestnut tree have been valued for centuries for their ability to improve hemorrhoids and varicose veins.[10] This historical use, seemingly successful, ultimately has led to the development of topical and oral preparations with confirmed clinical benefits for these same conditions.[11,12]

The chief component of horse chestnut seed extract (HCSE) is escin, a triterpenic saponin, although other molecules such as proanthocyanidin A_2 and esculin also exert significant effects.[11] These compounds have shown several pharmacological effects beneficial in

the treatment of CVI. First, all three active components have been shown to exert significant vasoprotective and venotonic effects, such as antioxidant effects, combined with an ability to inhibit enzymes that destroy venous structures, such as collagenase, hyaluronidase, β-glucuronidase, and elastase, thus shifting the equilibrium between degradation and synthesis of proteoglycans and other critical venous structures toward a net synthesis. In addition, HCSE prevents the accumulation of leukocytes in varicose vein–affected limbs and their subsequent activation, an important pathophysiological mechanism in varicose veins. It appears that the ultimate effect of HCSE treatment is the prevention of vascular leakage along with an increase in the tone of the vein itself.[13]

The therapeutic benefits of HCSE have been confirmed in more than 16 double-blind clinical trials demonstrating a positive effect in the treatment of varicose veins and thrombophlebitis,[11] and a Cochrane review concluded that evidence suggests HCSE is an efficacious and safe treatment for CVI.[14] Extracts of HCSE standardized for escin appear to be as effective as compression stockings but without the nuisance. For example, in a well-designed study, the effectiveness of HCSE versus compression stockings was examined in 240 patients with varicose veins.[15] Patients received either HCSE (50 mg/day of escin), compression stockings, or a placebo for 12 weeks. Effectiveness was evaluated by a plethysmograph—a machine that measures the volume of fluid in the leg. After the 12-week trial, the lower-leg volume of the more severely affected leg decreased an average of 56.5 mL with compression therapy and 53.6 mL with HCSE, whereas it rose by 9.8 mL with the placebo.

In the treatment of varicose veins, escin can be given orally as well as topically. The topical formula is also of benefit in the treatment of bruises, owing to escin's ability to reduce capillary fragility and swelling. In a meta-analysis of five clinical studies on the treatment of CVI with HCSE, preparations that contain HCSE were demonstrated to be effective through the objective measure of the reduction in lower-leg edema and the subjective alleviation of leg pain, heaviness, and itching.[16] In fact, the authors reported on one trial of 39 patients with varicose veins who took 1 to 2 tablets (20 mg escin each) three times daily and also applied a 2% escin gel topically twice daily (average = 4.4 mL daily) for 8 weeks. Of the subjects, 58% reported good overall efficacy for the combination therapy, and at the end of the treatment period, patients had improvement in blue discoloration, pain, edema, and leg heaviness compared with baseline.

Centella asiatica

When given orally, an extract of *Centella asiatica* containing 70% triterpenic acids (asiatic acid, madecassic acid, and asiatoside) has demonstrated impressive clinical results in the treatment of cellulite, venous insufficiency of the lower limbs, and varicose veins (see Chapter 64 for more information).[17–21]

Several experimental studies have discovered that *Centella* exerts a normalizing effect on the metabolism of connective tissue. Specifically, it possesses an ability to enhance connective tissue integrity by stimulating glycosaminoglycan synthesis without promoting excessive collagen synthesis or cell growth.[18] Glycosaminoglycans are the major components of the amorphous intercellular matrix (ground substance), in which collagen fibers are embedded. The net outcome of *Centella*'s effect on connective tissue is the development of normal tissue.

The effect of *Centella* in venous insufficiency and varicose veins appears to be related to its ability to enhance connective tissue structure, reduce sclerosis, and improve blood flow through the affected limbs.[17–21] (For further information, see Chapter 64.)

Ruscus aculeatus (Butcher's Broom)

The shrub butcher's broom *(Ruscus aculeatus)* is a member of the lily family that grows in the Mediterranean region. The rhizome from butcher's broom has a long history of use in treating venous disorders such as hemorrhoids and varicose veins. This historical effect has been confirmed in experimental and double-blind studies in patients with varicose veins.[22–24] The active ingredients in butcher's broom are ruscogenins. These compounds have demonstrated a wide range of pharmacological actions, including anti-inflammatory and vasoconstrictor effects. In Europe, butcher's broom extracts are used extensively, both internally and externally, in the treatment of varicose veins and hemorrhoids. Double-blind clinical studies have shown that these preparations offer benefits in terms of both symptomatic relief and improved venous blood flow.[22–24] In addition, a multicenter, double-blind, randomized, placebo-controlled trial of extracts from Butcher's broom rhizome concluded that *Ruscus* extract is a safe and effective treatment for patients suffering from CVI.[25] After 12 weeks, the treatment group had significant positive changes in leg volume; ankle and leg circumferences; and subjective symptoms of heavy and tired legs, tingling, and sensation of tension compared with the placebo group.

Systematic reviews have confirmed that *Ruscus,* especially when combined with hesperidin methyl chalcone and vitamin C, is highly effective in reducing leg symptoms (pain, heaviness, fatigue, feeling of swelling, cramps, itching, and paresthesia) and edema in patients with chronic venous disorders.[26] In addition to its vasoconstrictive effect on veins, its pharmacological action is on the microcirculation impairment caused by venous hypertension that is at the heart of the pathophysiological mechanism underlying venous disease.[27]

Nutritional Supplements

Flavonoids and Flavonoid-Rich Extracts

As mentioned previously, flavonoids and flavonoid-rich extracts are useful in the prevention and treatment of varicose veins. The most useful single flavonoid for varicose veins may be micronized diosmin. Micronization involves a high-technology grinding process with a jet of air at supersonic velocities, reducing the size of standard particles from more than 20 to less than 2 micrometers. As a result, there is more efficient and increased absorption and thus an increased bioavailability, which lends greater clinical efficacy. In addition to showing considerable improvements in signs and symptoms, purified micronized diosmin decreases the levels of some plasma markers of endothelial activation, including soluble endothelial adhesion molecules.[28] Micronized diosmin has also shown considerable benefits in promoting the healing of venous ulcers and hemorrhoids.[29] (For more information, see Chapter 81.)

Bromelain and Other Fibrinolytic Compounds

Individuals with varicose veins have a decreased ability to break down fibrin.[30] This is important because fibrin is deposited in the tissue near the varicose veins. The skin then becomes hard and "lumpy" owing to the presence of the fibrin and fat (lipodermatosclerosis). In addition, decreased fibrinolytic activity raises the risk of thrombus formation, which may result in thrombophlebitis, myocardial infarction, pulmonary embolism, or stroke.

Herbs that increase the fibrinolytic activity of the blood are therefore indicated. Capsicum (cayenne),[31] garlic,[32] onion,[33] and ginger[34] all promote fibrin breakdown. Liberal consumption of these spices in foods is recommended for individuals with varicose veins and other disorders of the cardiovascular system.

The proteolytic enzyme from pineapple, bromelain, also appears to be indicated in the treatment of varicose veins. Vein walls are an important source of plasminogen activator, which promotes the breakdown of fibrin. Varicosities have decreased levels of plasminogen

activator. Bromelain acts in a similar manner to plasminogen activator to cause fibrin breakdown.[35] Bromelain may help prevent the development of the hard and lumpy skin found around varicose veins.

THERAPEUTIC APPROACH

Conservative therapy, as described previously, should be employed as early as possible in patients with varicose veins. It may halt the progression and prevent further need for more aggressive therapy.

Diet

A diet rich in fiber is indicated. The diet should contain liberal amounts of proanthocyanidin- and anthocyanidin-rich foods, such as blackberries, cherries, and blueberries. Garlic, onions, ginger, and cayenne should also be consumed liberally.

Botanical Medicines

Choose one or more of the following:

- *A. hippocastanum:* bark of root, 500 mg three times a day
- Escin: 50 mg two or three times a day; alternatively, escin preparations may be applied topically in a 1% concentration.
- *C. asiatica* extract (70% triterpenic acid content): 30 mg three times a day
- *R. aculeatus* extract (9%–11% ruscogenin content): 100 mg three times a day
- Grape seed *(Vitis vinifera)* extract (95% procyanidolic oligomers): 150 to 300 mg/day
- Pine bark extract *(Pinus pinaster)*: 150 to 300 mg/day
- Micronized diosmin: 500 to 1000 mg/day
- Hydroxyethyrutosides: 1000 to 3000 mg/day

The following may be useful as an adjunct:

- Bromelain (minimum 1500 MCU): 500 to 750 mg three times a day between meals

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Lim CS, Davies AH. Pathogenesis of primary varicose veins. *Br J Surg*. 2009;96(11):1231–1242.
2. Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and wall dilation. *Phlebology*. 2008;23(2):85–98.
3. Trowell H, Burkitt D, Heaton K. *Dietary Fibre, Fibre-Depleted Foods and Disease*. London: Academic Press; 1985.
4. Vahouny G, Kritchevsky D. *Dietary Fiber in Health and Disease*. New York: Plenum Press; 1982.
5. Latto C, Wilkinson RW, Gilmore OJ. Diverticular disease and varicose veins. *Lancet*. 1973;1:1089–1090.
6. Gabor M. Pharmacologic effects of flavonoids on blood vessels. *Angiologica*. 1972;9:355–374.
7. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet*. 1976;24:117–191.
8. Pourrat H. Anthocyanidin drugs in vascular disease. *Plant Med Phytother*. 1977;11:143–151.
9. Ihme N, Kieswetter H, Jung F, et al. Leg edema protection from buckwheat herb tea in patients with chronic venous insufficiency: a single-center, randomized, double-blind, placebo-controlled clinical trial. *Eur J Clin Pharmacol*. 1996;50:443–447.
10. Felter HW, Lloyd JU. *King's American Dispensatory*. 18th ed. Portland, OR: Eclectic Medical Publications; 1983.
11. Pittler MH, Ernst E. Horse-chestnut seed extract for chronic venous insufficiency: a criteria-based systematic review. *Arch Dermatol*. 1998;134:1356–1360.
12. Incandela L, De Sanctis MT, Cesarone MR, et al. Treatment of superficial vein thrombosis: clinical evaluation of Essaven gel: a placebo-controlled, 8-week, randomized study. *Angiology*. 2001;52(suppl 3):S69–S72.
13. Annoni F, Mauri A, Marincola F, et al. Venotonic activity of escin on the human saphenous vein. *Arzneimittelforschung*. 1979;29:672–675.
14. Pittler MH, Ernst E. Horse chestnut seed extract for chronic venous insufficiency. *Cochrane Database Syst Rev*. 2012;11:CD003230. PubMed PMID: 23152216.
15. Diehm C, Trampisch HJ, Lange S, et al. Comparison of leg compression stocking and oral horse-chestnut seed extract therapy in patients with chronic venous insufficiency. *Lancet*. 1996;347:292–294.
16. Suter A, Bommer S, Rechner J. Treatment of patients with venous insufficiency with fresh plant horse chestnut seed extract: a review of 5 clinical studies. *Adv Ther*. 2006;23(1):179–190. PubMed PMID: 16644618.
17. Allegra C, Pollari G, Criscuolo A, et al. Centella asiatica extract in venous disorders of the lower limbs: comparative clinico-instrumental studies with a placebo. *Clin Ter*. 1981;99:507–513.
18. Brinkhaus B, Lindner M, Schuppan D, et al. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*. *Phytomedicine*. 2000;7:427–448.
19. Allegra C. Comparative capillaroscopic study of certain bioflavonoids and total triterpenic fractions of *Centella asiatica* in venous insufficiency. *Clin Ter*. 1984;110:555–559.
20. Pointel JP, Boccalon H, Cloarec M, et al. Titrated extract of *Centella asiatica* (TECA) in the treatment of venous insufficiency of the lower limbs. *Angiology*. 1987;38:46–50.
21. Marastoni F, Baldo A, Redaelli G, et al. *Centella asiatica* extract in venous pathology of the lower limbs and its evaluation as compared with tribenoside. *Minerva Cardioangiol*. 1982;30:201–207.
22. Boccalon H, Causse C, Yubero L. Comparative efficacy of a single daily dose of two capsules of Cyclo 3 Fort in the morning versus a repeated dose of one capsule morning and noon: a one month study. *Int Angiol*. 1998;17:155–160.
23. Cappelli R, Nicora M, Di Perri T. Use of extract of *Ruscus aculeatus* in venous disease in the lower limbs. *Drugs Exp Clin Res*. 1988;14:277–283.
24. Beltramino R, Penenory A, Buceta AM. An open-label, randomized multicenter study comparing the efficacy and safety of Cyclo 3 Fort® versus hydroxyethyl rutoside in chronic venous lymphatic insufficiency. *Angiology*. 2000;51:535–544.
25. Vanscheidt W, Jost V, Wolna P, et al. Efficacy and safety of a butcher's broom preparation (*Ruscus aculeatus* L. extract) compared to placebo in patients suffering from chronic venous insufficiency. *Arzneimittelforschung*. 2002;52(4):243–250. PubMed PMID: 12040966.
26. Kakkos SK, Allaert FA. Efficacy of *Ruscus* extract, HMC and vitamin C, constituents of Cyclo 3 fort®, on improving individual venous symptoms and edema: a systematic review and meta-analysis of randomized double-blind placebo-controlled trials. *Int Angiol*. 2017;36(2):93–106. PubMed PMID: 28225220.
27. Allaert FA. Combination of Ruscus aculeatus extract, hesperidin methyl chalcone and ascorbic acid: a comprehensive review of their pharmacological and clinical effects and of the pathophysiology of chronic venous disease. *Int Angiol*. 35(2):111-116. PubMed PMID: 26928296.
28. Nicolaides AN. From symptoms to leg edema: efficacy of Daflon 500 mg. *Angiology*. 2003;54(suppl 1):S33–S44.
29. Lyseng-Williamson KA, Perry CM. Micronised purified flavonoid fraction: a review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. *Drugs*. 2003;63:71–100.
30. Kreysel HW, Nissen HP, Enghofer E. A possible role of lysosomal enzymes in the pathogenesis of varicosis and the reduction in their serum activity by Venostasin. *Vasa*. 1983;12:377–382.
31. Visudhiphan S, Poolsuppasit S, Piboonnukarintr O, et al. The relationship between high fibrinolytic activity and daily capsicum ingestion in Thais. *Am J Clin Nutr*. 1982;35:1452–1458.
32. Bordia A, Sharma KD, Parmar YK, et al. Protective effect of garlic oil on the changes produced by 3 weeks of fatty diet on serum cholesterol, serum triglycerides, fibrinolytic activity and platelet adhesiveness in man. *Indian Heart J*. 1982;34:86–88.
33. Baghurst KI, Raj MJ, Truswell AS. Onions and platelet aggregation. *Lancet*. 1977;1:101.
34. Srivas KC. Effects of aqueous extracts of onion, garlic and ginger on the platelet aggregation and metabolism of arachidonic acid in the blood vascular system. In vitro study. *Prostaglandins Leukot Med*. 1984;13:227–235.
35. Ako H, Cheung AH, Matsuura PK. Isolation of a fibrinolysis enzyme activator from commercial bromelain. *Arch Int Pharmacodyn Ther*. 1981;254:157–167.

Candida Questionnaire[1]

Michael T. Murray, ND

PURPOSE

The purpose of this questionnaire is to estimate the likelihood of having an overgrowth of intestinal yeast.

QUESTIONNAIRE

HISTORY

	Point Score
1. Have you taken antibiotics for acne for 1 month or longer?	25
2. Have you, at any time in your life, taken other broad-spectrum antibiotics for respiratory, urinary, or other infections for 2 months or longer, or in short courses four or more times in a 1-year period?	20
3. Have you ever taken a broad-spectrum antibiotic (even a single course)?	6
4. Have you, at any time in your life, been bothered by persistent prostatitis, vaginitis, or other problems affecting your reproductive organs?	25
5. Have you been pregnant?	
One time	3
Two or more times	5
6. Have you taken birth control pills?	
For 6 months to 2 years	8
For more than 2 years	15
7. Have you taken prednisone or other cortisone-type drugs?	
For 2 weeks or less	6
For more than 2 weeks	15
8. Does exposure to perfumes, insecticides, fabric shop odors, and other chemicals provoke:	
Mild symptoms	5
Moderate to severe symptoms	20
9. Are your symptoms worse on damp, muggy days or in places with mold?	20
10. Have you had athlete's foot, ringworm, "jock itch," or other chronic infections of the skin or nails?	
Mild to moderate	10
Severe or persistent	20
11. Do you crave sugar?	10
12. Do you crave breads?	10
13. Do you crave alcoholic beverages?	10
14. Does tobacco smoke really bother you?	10
Total score for this section	___

Major Symptoms

For each of your symptoms, enter the appropriate number in the "Point Score" column:

- If a symptom is occasional or mild, score 3 points.
- If a symptom is frequent or moderately severe, or both, score 6 points.
- If a symptom is severe or disabling, or both, score 9 points.

	Point Score
1. Fatigue or lethargy	___
2. Feeling of being "drained"	___
3. Poor memory	___
4. Feeling "spacey" or "unreal"	___
5. Depression	___
6. Numbness, burning, or tingling	___
7. Muscle aches	___
8. Muscle weakness or paralysis	___
9. Pain and/or swelling in joints	___
10. Abdominal pain	___
11. Constipation	___
12. Diarrhea	___
13. Bloating	___
14. Persistent vaginal itch	___
15. Persistent vaginal burning	___
16. Prostatitis	___
17. Impotence	___
18. Loss of sexual desire	___
19. Endometriosis	___
20. Cramps or other menstrual irregularities, or both	___
21. Premenstrual tension	___
22. Spots in front of eyes	___
23. Erratic vision	___
Total score for this section	___

Other Symptoms

For each of your symptoms, enter the appropriate figure in the "Point Score" column:

- If a symptom is occasional or mild, score 1 point.
- If a symptom is frequent or moderately severe, or both, score 2 points.
- If a symptom is severe or disabling, or both, score 3 points.

	Point Score
1. Drowsiness	___
2. Irritability	___
3. Incoordination	___
4. Inability to concentrate	___
5. Frequent mood swings	___
6. Headache	___
7. Dizziness/loss of balance	___
8. Pressure above ears, feeling of head swelling and tingling	___
9. Itching	___
10. Other rashes	___
11. Heartburn	___
12. Indigestion	___
13. Belching and intestinal gas	___
14. Mucus in stools	___
15. Hemorrhoids	___
16. Dry mouth	___
17. Rash or blisters in mouth	___
18. Bad breath	___
19. Joint swelling or arthritis	___
20. Nasal congestion or discharge	___

TABLE A1.1 Interpretation

Yeast-Connected Health Problems	Women	Men
Almost certainly present	>180	>140
Probably present	120–180	90–140
Possibly present	60–119	40–89
Less likely present	<60	<40

	Point Score
21. Postnasal drip	___
22. Nasal itching	___
23. Sore or dry throat	___
24. Cough	___
25. Pain or tightness in chest	___
26. Wheezing or shortness of breath	___
27. Urinary urgency or frequency	___
28. Burning on urination	___
29. Failing vision	___
30. Burning or tearing of eyes	___
31. Recurrent infections or fluid in ears	___
32. Ear pain or deafness	___
Total score for this section	___
TOTAL SCORE FOR ALL THREE SECTIONS	___

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCE

1. Modified from Crook WG. *The yeast connection*, ed 2. Jackson, TN: Professional Books, 1984.

Cervical Escharotic Treatment

Joseph E. Pizzorno, ND

1. Before beginning the treatment, prepare the following items:
   a. Zinc chloride ($ZnCl_2$)/*Sanguinaria* solution (90 g $ZnCl_2$/60 mL sterilized water; made at a compounding pharmacy). Take ¼ tsp $ZnCl_2$ solution and place in an empty cup. Add ¾ tsp *Sanguinaria* tincture to this same cup. This will now be the mixture used for one treatment.
   b. 1 cup distilled water
   c. ⅓ cup *Calendula succus*
   d. A cup containing two powdered bromelain capsules or tablets
2. Insert speculum and visualize the cervix.
3. Blot the cervix dry with large cotton swab or cotton ball on the end of a ring forceps.
4. Dip a large cotton swab into the distilled water, and then squeeze out the water with your fingers. Place the damp swab into the bromelain and attempt to thickly cover the face of the cervix with the powder. This will have to be repeated two to four times for proper coverage. The same step must be done in the endocervical canal with a small cotton-tip applicator (i.e., dampen it, place in the bromelain, and apply to endocervix one to three times). Use a new cotton-tip applicator each time.
5. Leave the bromelain on the cervix and in the endocervical canal for 15 minutes. A gynecologic lamp should be placed facing the vagina so that gentle heat is provided during this portion of the treatment.
6. Now remove the bromelain by placing a large cotton swab in the *C. succus* and then applying it to the cervix, thus washing off the bromelain. This must also be done to the endocervical canal with a small cotton-tip applicator. Be liberal; repeat two to four times. Take a large dry swab and absorb the washings that have pooled in the vagina.
7. Now soak a large swab in the $ZnCl_2$/*Sanguinaria* mixture prepared earlier. Apply this to the cervix once. Repeat this procedure with a small cotton-tip applicator and insert in the endocervical canal. Leave on for 1 minute. If this causes pain, wash the cervix with a small amount of distilled water. Avoid contact of the $ZnCl_2$/*Sanguinaria* mixture with the vaginal wall.
8. Wash off the $ZnCl_2$/*Sanguinaria* mixture with swabs of *C. succus.* Wash the endocervical canal as well with a cotton-tip applicator. Absorb the liquid that has pooled in the vagina with a dry cotton swab.
9. Insert two "vag pack" suppositories. (See Appendix 14, "Vaginal Depletion Pack—Traditional Method.") Using forceps or other appropriate instruments, attempt to have the suppositories lie lengthwise across the cervix. Instruct the patient to leave the suppositories in place for 24 hours (using a small sanitary napkin due to leakage).
10. After the last escharotic treatment:
    Week 1: Vitamin A suppository every night for 6 nights
    Week 2: Herbal vaginal suppository every night (herbal suppository: myrrh, echinacea, slippery elm, goldenseal root, marshmallow root, geranium, and yarrow) for 6 nights or alternate suppository (vitamin A, thuja, lomatium isolate, green tea)
    Week 3: Vitamin A suppository every night for 6 nights
    Week 4: Herbal vaginal or alternate suppository every night for 6 nights

Note: The escharotic treatment is best done twice a week with 2 full days between treatments.

Crohn's Disease Activity Index

Gerard E. Mullin, MD

Patient name: _____ Chart no.: _____
Date: _____

		Day 1 2 3 4 5 6 7	Sum	×	Factor	=	Subtotal
X_1	Number of liquid or very soft stools	| | | | | | |	___	×	2	=	____
X_2	Abdominal pain (0 = none, 1 = mild, 2 = moderate, 3 = severe)	| | | | | | |	___	×	5	=	____
X_3	General well-being (0 = well, 1 = slightly under par, 2 = poor, 3 = very poor, 4 = terrible)	| | | | | | |	___	×	7	=	____
X_4	Number of six listed categories the patient now has: 1. Arthritis/arthralgia 2. Iritis/uveitis 3. Erythema nodosum/pyoderma gangrenosum/ aphthous stomatitis 4. Anal fissure, fistula, or abscess 5. Other fistula 6. Fever higher than 100°F during past week		___	×	20	=	____
X_5	Taking Lomotil/opiates for diarrhea (0 = no, 1 = yes)		___	×	30	=	____
X_6	Abdominal mass (0 = none, 2 = questionable, 5 = definite)		___	×	10	=	____
X_7	Hematocrit (HCT): ___ (standard: males = 47, females = 42)	Standard – HCT (add or subtract according to sign)		×	6	=	____
X_8	Body weight (BW): ___ Standard weight (SW): ___	(SW – BW)/SW (add or subtract according to sign)		×	100	=	____
Crohn's disease activity index (CDAI; sum of X_1 through X_8)					CDAI	=	___

The Role of Essential Oils in the Treatment of Chronic Inflammatory Processes and Infectious Diseases

Jade Dandy, ND, MSiMR, and edited by *Nicole Pierce, ND*

INTRODUCTION

A universal challenge for both physicians and patients is the often-frustrating limitations of the current approach to the treatment of inflammatory diseases and the sequelae that follow chronic infections. Although conventional medicine's standard of care, antibiotics, still play a crucial role in the initial treatment of many infections, there remains a desperate need for alternative and complementary therapeutics for the treatment of micropathogens that are becoming more resistant to the current standard of care. Integrative therapies such as botanicals, nutraceuticals, homeopathics, pharmaceuticals, and diet and lifestyle counseling must all be implemented to successfully combat this growing epidemic. Essential oil protocols are an exciting addition to this holistic treatment plan.

There are over 200,000 published articles in the medical literature on the therapeutic benefits of aromatherapy and essential oils in the treatment of a vast array of diseases, including depression, anxiety, and microbial infections. Essential oils (EOs) are aromatic and volatile liquid extracts derived from plant materials such as flowers, roots, bark, leaves, seeds, peels, fruits, wood, and whole plants. They are considered to be secondary metabolites, which play an important role in a plant's defense because they often possess antimicrobial and antioxidant properties.

In vitro studies have demonstrated considerable antibacterial activity of EOs and their chemical constituents against *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O157:H7, *Shigella dysenteria, Bacillus cereus,* and *Staphylococcus aureus.*[1] Thus far, gram-negative organisms have been shown to be less susceptible than gram-positive bacteria to these extracts.[2,3]

Although EO research is vast, it often lacks a focused approach for support of disease treatment. The mechanism of action regarding EOs and their antibacterial activity is not presently fully understood, and more research is needed to determine which constituents, combinations, and concentrations are most effective. For the same reasons, no particular bacterial resistance or adaptation to essential oils has been described in the literature, and secondary effects have not yet been confirmed.[4]

Encouragingly, the naturally occurring compounds found in essential oils have been found to disrupt bacterial membranes.[5] Likely this is due to the presence of lipophilic compounds such as cyclic hydrocarbons, terpenes, and aromatics.[6] Essential oils target bacteria by changing their membrane structure and function. Specifically, the changes in membrane function often involve the transformation of energy as well as the activity of membrane-bound enzymes.[7] In many cases the activity of essential oils results from the complex interaction between the different classes of compounds, such as phenols, aldehydes, ketones, alcohols, esters, ethers, and hydrocarbons, all found in EOs, suggesting that a single oil is less effective than a combination of oils used together (Fig. A4.1).

One main challenge to treating infections is the potential of microbial mutation of the infecting species. Essential oils may play an important role in preventing mutations. For example, the multicomponent nature of tea tree oil *(Melaleuca alternifolia)* could reduce a bacteria's ability to undergo a sufficient number of simultaneous mutations to avoid the membrane-targeting constituents.[8] This means that the bacteria's numerous cell proteins would be forced to adapt to overcome the effects of the oils while also overcoming the pharmaceutical antibiotics.

Essential oils have many therapeutic effects, but in this chapter the focus will remain on their ability to improve the immune system and alter the function and virulence of bacteria. The following essential oil protocol has been developed by physicians as a complementary therapy to the standard-of-care treatment for chronic infection and also to improve immune function. Essential oils have shown to be effective in treating inflammatory disease and chronic infections by the following mechanisms:

- Direct cidal activity on the microbes
- Decreasing the inflammatory pathways
- Interfering with communication between infecting organisms

KEY ESSENTIAL OILS

Oregano Essential Oil

Botanical Name: *Origanium vulgare* (Fig. A4.2)
Alternative Names: Wild Marjoram
Botanical Family: *Lamiaceae*
Primary Constituent: Carvacrol
Other Main Constituents: Thymol
Distillation Method: Steam
Plant Part: Leaf

The ability of hydrocarbons to interact with cell membranes facilitates the penetration of carvacrol into the cell.[9] In one study, two fractions of an oregano *(O. vulgare)* extract obtained by supercritical fluid extraction (liquid CO_2) were used to test anti-inflammatory effects on activated human THP-1 cells. The main compounds present were transsabinene hydrate, thymol, and carvacrol. Concentrations higher than 30 μg/mL of both supercritical S1 and S2 oregano fractions caused a reduction in cell viability in a dose-dependent manner. Oxidized low-density lipoprotein (oxLDL)–activated THP-1 macrophages were used as cellular models of atherogenesis, and the release/secretion of cytokines (tumor necrosis factor-alpha [TNF-α], interleukin [IL]-1β, IL-6, and IL-10) and their respective mRNA expressions were quantified both in the presence and absence of supercritical oregano extracts. The results showed a decrease in proinflammatory TNF-α, IL-1β, and IL-6 cytokines synthesis, as well

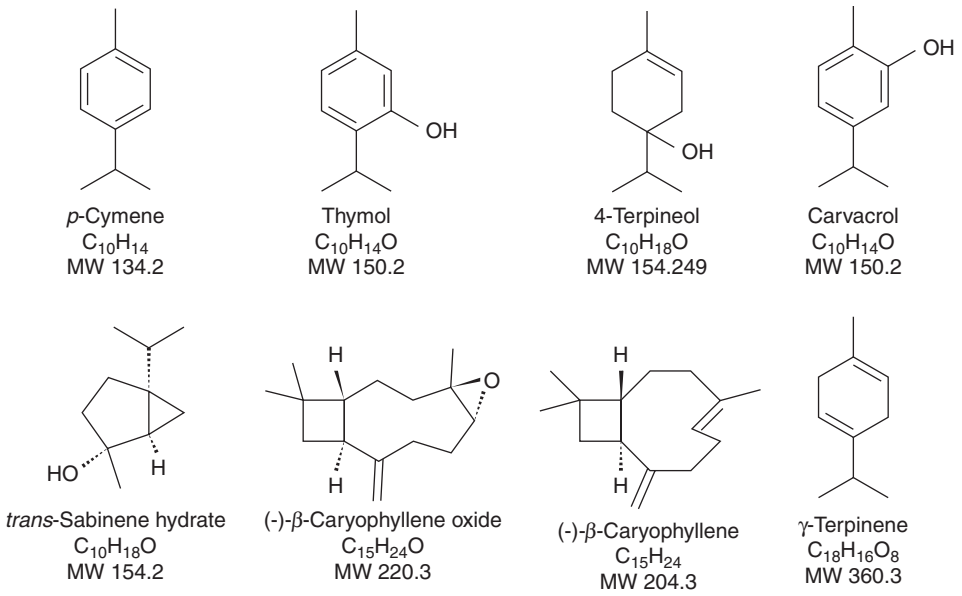


Fig. A4.1 Common active constituents of essential oils.

Fig. A4.2 *Origanium vulgare* (oregano). (cunfek/iStock.com.)

Fig. A4.3 *Thymus vulgaris* (thyme). (From backyardpatch.com.)

as an increase in the production of anti-inflammatory cytokine IL-10, when oregano fractions were present.[10]

This finding is significant because the physiopathology of many bacterial and viral organisms is to increase TNF-α in order to invade the immune system.[11,12] If carvacrol can inhibit this cytokine, it may shorten the duration of treatment and decrease the likelihood of coinfections. Another study in a mouse model used carvacrol in doses of 20, 40, and 80 mg/kg by intragastric gavage 6 days before induced inflammation. Proinflammatory cytokines TNF-α and IL-6, as well as arginase activity, were decreased by carvacrol. These results are extremely promising and indicate that carvacrol may have a potent anti-inflammatory effect.[13]

Thyme Essential Oil

Botanical Name: *Thymus vulgaris* (Fig. A4.3)
Alternative Names: Common Thyme
Botanical Family: *Lamiaceae*
Primary Constituent: Thymol
Other Main Constituents: Para-Cymene, Gamma-Terpinene, Carvacrol
Distillation Method: Steam
Plant Part: Leaf

Thyme is also an anti-inflammatory agent, containing carvacrol among its other constituents. As a cellular model of inflammation/atherogenesis, one study used human macrophages derived from THP-1 monocytes and activated by oxLDLs. These cells were incubated with the thyme fraction oils, and the productions and gene expressions of the inflammatory mediators TNF-α, IL-1B, IL-6, and IL-10 were determined. Thyme extracts significantly reduced production and gene expression of the proinflammatory mediators TNF-α, IL-1β, and IL-6 and highly increased these parameters on the anti-inflammatory IL-10 cytokine.[14] After just 24 hours of incubation, activated cells treated with any of the thyme fractions showed a significant decrease in TNF-α release compared with nontreated cells.

Cinnamon Essential Oil

Botanical Name: *Cinnamomum zeylanicum* (Fig. A4.4)
Alternative Names: Seychelles cinnamon, Ceylon cinnamon
Botanical Family: *Lauraceae*
Primary Constituent: Cinnamaldehyde

Fig. A4.4 *Cinnamomum zeylanicum* (cinnamon). (From photogram.com.)

Fig. A4.5 *Eugenia caryophyllata* (clove). (quietcorner.com)

Other Main Constituents: Eugenol, Linalool
Distillation Method: Steam
Plant Part: Bark, Leaf

The major constituents of cinnamon oil are the monoterpenes 1,8-cineole (17.0%) and santolina triene (14.2%) and the sesquiterpenes spathulenol (15.7%) and caryophyllene oxide (11.2%).[15] In an anti-inflammatory activity assay, it was demonstrated that cinnamon essential oil has a significant capacity to inhibit IL-1 protein expression at dosages of 60 μg/mL. Furthermore, a dose of 60 μg/mL of cinnamon essential oil was effectively inhibitory for IL-1 and IL-6 production but not for TNF-α, suggesting that cinnamon essential oil may be bioactive in anti-inflammatory processes specifically.[16] This is a significant finding because the amount of IL-6 in human serum and cerebrospinal fluid (CSF) has been shown to correlate with disease activity in neurological diseases.[17]

Clove Essential Oil

Botanical Name: *Eugenia caryophyllata* (Fig. A4.5)
Alternative Names: Clove Bud
Botanical Family: *Myrtaceae*
Primary Constituent: Eugenol
Other Main Constituents: Caryophyllene, Eugenol Acetate
Distillation Method: Steam
Plant Part: Bud, Leaf

Eugonal, clove oil's primary constituent, has been shown to disrupt oral biofilms formed by *S. pneumonia*. Eugonal extracted from *E. caryophyllata* has been shown to produce a synergistic effect against *S. pneumonia* in combination with penicillin.[18] When some organisms mutate into a cyst form, they, too, create biofilms, making it more difficult to eradicate the pathogen from the body. If an agent disrupts these biofilms, bacterial exposure to the agent is facilitated, leading to faster eradication.[19]

Fig. A4.6 *Melissa officianalis* (lemon balm). (Leonsbox/iStock.com.)

Melissa Essential Oil

Botanical Name: *Melissa officianalis* (Fig. A4.6)
Alternative Names: Lemon Balm
Botanical Family: *Lamiaceae*
Primary Constituent: Geranial
Other Main Constituents: Neral, Germacrene, Caryophyllene
Distillation Method: Steam
Plant Part: Leaf Top, Leaf

The effect of the essential oil derived from the leaves of *Melissa* was investigated for anti-inflammatory properties by using carrageenan to induce hind paw edema in rats. The essential oil extracted from the lemon balm leaves by hydrodistillation was characterized by gas chromatography–mass spectrometry (GC-MS). *M. officinalis* contains Nerol (30.44%), Citral (27.03%), Isopulegol (22.02%), Caryophyllene (2.29%), Caryophyllene oxide (1.24%), and Citronella (1.06%).[20] Anti-inflammatory properties of oral administration of this essential oil at the doses of 200 and 400 mg/kg p.o., respectively, showed significant inhibition of edema with 61.76% and 70.58%, respectively ($P < 0.001$), compared with standard drug control of Indomethacin.[21]

Patchouli Essential Oil

Botanical Name: *Pogostemon cablin* (Fig. A4.7)
Alternative Names: Patchouly
Botanical Family: *Lamiaceae*
Primary Constituent: Patchouli Alcohol
Other Main Constituents: Alpha-Bulnasene, Alpha-Guaiene
Distillation Method: Steam
Plant Part: Leaf

One notable study using an in vivo model of inflammation suggested that *P. cablin* extract plays a role in the anti-inflammatory activities in the model of Carr-induced paw edema of mice through the inhibition of TNF-α and cyclooxygenase-2 (COX-2) level.[22] Research has found that Patchouli oil has a specific molecule α-bulnesene, which is a sesquiterpenoid isolated from the water extract of *P. cablin*. It demonstrates a potent and concentration-dependent inhibitory effect on rabbit platelet aggregation induced by platelet-activating factor (PAF) and arachidonic acid (AA). In a radioligand binding assay for the PAF receptor, α-bulnesene competitively inhibited [3H]PAF, binding to the PAF receptor with an IC50 value of 17.62 ± 5.68 μM. These results indicate that the inhibitory effect of α-bulnesene on platelet aggregation was

Fig. A4.7 *Pogostemon cablin* (patchouli). (From everythingsoulful.com.)

due to a dual activity; specifically, the chemical blocked PAF-induced intracellular signal transduction and interfered with cyclooxygenase activity, which resulted in a decrease in thromboxane formation. This study demonstrates that α-bulnesene is a PAF receptor antagonist as well as an antiplatelet aggregation agent.[23]

CONCLUSION

As discussed previously, antibiotics are not always effective for bacterial and viral pathogens and have a short window of opportunity. It is important to recognize that there is not a "silver bullet" protocol for complementary treatment of disease with essential oils; integration is key in eradicating the pathogen, alleviating the associated symptoms, and improving the patient's quality of life. A treatment plan that combines a carefully selected essential oil protocol with conventional medicine's standard of care is an innovative and promising strategy in the ongoing battle against these increasingly prevalent diseases.

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCES

1. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol*. 2004;94(3):223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022. Accessed Dec 11, 2018.
2. Benach J, Bosler E, Habicht G, et al. Spirochetes isolated from the blood of two patients with Lyme disease. *N Engl J Med*. 1983;308:740–742. https://doi.org/10.1056/NEJM198303313081302. Accessed Dec 11, 2018.
3. Djenane D, Yangüela J, Montañés L. Antimicrobial activity of *Pistacia lentiscus* and *Satureja montana* essential oils against *Listeria monocytogenes* CECT 935 using laboratory media: efficacy and synergistic potential in minced beef. *Food Control*. 2011;22(7):1046–1053. http://www.sciencedirect.com/science/article/pii/S0956713510004433. Accessed Dec 11, 2018.
4. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine*. 2009;16(2-3):97–110. https://doi.org/10.1016/j.phymed.2008.12.018. Accessed Dec 11, 2018.
5. Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components and antibiotics: a review. *Crit Rev Microbiol*. 2014;40(1):76–94. https://doi.org/10.3109/1040841X.2013.763219. Accessed Dec 11, 2018.
6. Sikkema J, de Bont JA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev*. 1995;59(2):201–222. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=239360&tool=pmcentrez&rendertype=abstract. Accessed Dec 11, 2018.
7. Wilson WW, Wade MM, Holman SC, Champlin FR. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. *J Microbiol Methods*. 2001;43(3):153–164. http://www.ncbi.nlm.nih.gov/pubmed/11118650, Accessed date: 11 December 2018.
8. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006;19(1):50–62. https://doi.org/10.1128/CMR.19.1.50-62. Accessed Dec 11, 2018.
9. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules*. 2012;17(4):3989–4006. https://doi.org/10.3390/molecules17043989. Accessed Dec 11, 2018.
10. Ocaña-Fuentes A, Arranz-Gutiérrez E, Señorans FJ, Reglero G. Supercritical fluid extraction of oregano *(Origanum vulgare)* essentials oils: anti-inflammatory properties based on cytokine response on THP-1 macrophages. *Food Chem Toxicol*. 2010;48(6):1568–1575. https://doi.org/10.1016/j.fct.2010.03.026. Accessed Dec 11, 2018.
11. Radolf JD, Norgard MV, Brandt ME, Isaacs RD, Thompson PA, Beutler B. Lipoproteins of *Borrelia burgdorferi* and *Treponema pallidum* activate cachectin/tumor necrosis factor synthesis. Analysis using a CAT reporter construct. *J Immunol*. 1991;147(6):1968–1974. http://www.jimmunol.org/content/147/6/1968.abstract. Accessed Dec 11, 2018.
12. Mason LMK, Veerman CC, Geijtenbeek TBH, Hovius JWR. Ménage à trois: Borrelia, dendritic cells, and tick saliva interactions. *Trends Parasitol*. 2014;30(2):95–103. https://doi.org/10.1016/j.pt.2013.12.003. Accessed Dec 11, 2018.
13. Kara M, Uslu S, Demirci F, Temel HE, Baydemir C. Supplemental carvacrol can reduce the severity of inflammation by influencing the production of mediators of inflammation. *Inflammation*. 2015;38(3):1020–1027. https://doi.org/10.1007/s10753-014-0066-0. Accessed date: 11 December 2018.
14. Braga PC, Dal Sasso M, Culici M, Bianchi T, Bordoni L, Marabini L. Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil elastase. *Pharmacology*. 2006;77(3):130–136. https://doi.org/10.1159/000093790. Accessed Dec 11, 2018.
15. Smith-Palmer A. Influence of subinhibitory concentrations of plant essential oils on the production of enterotoxins A and B and α-toxin by Staphylococcus aureus. *J Med Microbiol*. 2004;53(10):1023–1027. https://doi.org/10.1099/jmm.0.45567-0. Accessed Dec 11, 2018.
16. AromaticScience.com. Study on the antiinflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. *J Agr Food Chem*. 2005;53(18):7274–7278. http://www.aromaticscience.com/study-on-the-antiinflammatory-activity-of-essential-oil-from-leaves-of-cinnamomum-osmophloeum/. Accessed Dec 11, 2018.
17. Weller M, Stevens A, Sommer N, Wietholter H, Dichgans J. Cerebrospinal fluid interleukins, immunoglobulins, and fibronectin in neuroborreliosis. *Arch Neurol*. 1991;48(8):837–841. https://doi.org/10.1001/archneur.1991.00530200079022. Accessed Dec 11, 2018.
18. Yadav MK, Park SW, Chae SW, Song JJ, Kim HC. Antimicrobial activities of *Eugenia caryophyllata* extract and its major chemical constituent eugenol against *Streptococcus pneumoniae*. *APMIS*. 2013;121(12):1198–1206. https://doi.org/10.1111/apm.12067. Accessed Dec 11, 2018.
19. Xu JS, Li Y, Cao X, Cui Y. The effect of eugenol on the cariogenic properties of *Streptococcus mutans* and dental caries development in rats. *Exp Ther Med*. 2013;5(6):1667–1670. https://doi.org/10.3892/etm.2013.1066. Accessed Dec 11, 2018.
20. Jalal Z, El Atki Y, Lyoussi B, Abdellaoui A. Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. *Asian Pac J Trop Biomed*. 2015;5(6):458–461. https://doi.org/10.1016/j.apjtb.2015.03.003. Accessed Dec 11, 2018.
21. In Vivo Potential Anti-Inflammatory Activity of *Melissa officinalis* L. Essential Oil. *Adv Pharmacol Sci*. 2013;2013:101759. http://www.aromaticscience.com/in-vivo-potential-anti-inflammatory-activity-of-melissa-officinalis-l-essential-oil/. Accessed Dec 11, 2018.
22. Beutler B, Cerami A. The biology of cachectin/TNF—a primary mediator of the host response. *Annu Rev Immunol*. 1989;7:625–655. https://doi.org/10.1146/annurev.iy.07.040189.003205. Accessed Dec 11, 2018.
23. Hsu HC1, Yang WC, Tsai WJ, Chen CC, Huang HY, Tsai YC. Alpha-bulnesene, a novel PAF receptor antagonist isolated from *Pogostemon cablin*. *Biochem Biophys Res Commun*. 2006;345(3):1033–1038. https://doi.org/10.1016/j.bbrc.2006.05.006. Accessed Dec 11, 2018.

Fasting—Patient Guidelines

Alan Goldhamer, DC, and *Toshia R. Myers, BS, MA, MPhil, PhD*

Preliminary research suggests that fasting results in positive outcomes for various illnesses, such as hypertension, diabetes, and arthritis. Although longer fasts are required in chronic disease cases, a short fast (3–5 days) is a good chance for the body to acquire optimal rest, both mental and physical, and is an opportunity to build health, break old habits, and increase self-awareness. It can also be conducted at home rather than in an inpatient facility. A short fast can be implemented at any time, but it is better to instruct the patient to begin on a weekend or during a time when the patient can be inactive. More rest generally garners better results because energy can be directed toward healing instead of other body functions.

Before instructing a patient to undertake a short fast, it is important to determine that there are no contraindications by conducting a thorough medical history, physical examination (including comprehensive metabolic panel and complete blood count), and review of medications (including herbs and nutritional supplements). The patient must be able to discontinue all medications and supplements before fasting. The patient's vital signs, amount of rest, and water intake should be monitored daily. These steps will ensure that the fast can be completed safely and effectively.

FASTING INSTRUCTIONS

Starting the Fast

The day before the fast begins, instruct the patient to eat only fresh fruits and vegetables for at least the last meal of the day (some authorities recommend a full day of raw food to start a fast). In addition, the patient should finish all last-minute errands and tasks to establish an environment as free of stress and responsibility as possible.

During the Fast

The patient should only consume purified water—distilled is best—while fasting. (Some authorities recommend fruit or vegetable juice, but this is actually an elimination diet rather than a fast.) The quantity of water should be dictated by thirst, but at least 40 ounces every day should be consumed. Coffee, tea, juice, soft drinks, cigarettes, or anything else should not be consumed while fasting. If possible, the patient should avoid the smell and sight of food and keep the mind occupied by reading a book (especially a book on fasting), listening to music, writing a letter, or watching a movie.

Exercise should be avoided while fasting to conserve energy and allow maximal healing. Increased activity will increase the need for glucose, which will in turn result in increased gluconeogenesis and the loss of protein. Light stretching may be useful, but heavy workouts tax the system and inhibit repair and elimination. Cleansing the skin with lukewarm water is encouraged, but extremes of temperature can be tiring. The patient should try to avoid deodorants, soaps, sprays, detergents, synthetic shampoos, and exposure to other chemicals. These only hinder elimination and add to the body's detoxification and elimination burden. Patients are often more sensitive to chemicals and irritants during fasting.

Sunlight is essential for healthy cells, but excessive exposure will strain the body's protective systems. At least 10 to 20 minutes of direct sun exposure per day appears to be beneficial without causing adverse effects. Rest is one of the most important aspects of the fast, and one or two naps a day is recommended. Patients often require less sleep at night because daily activity has decreased. Enemas are not usually necessary. If constipation is a concern, a longer prefast period of fresh fruits and vegetables will assist elimination. The body temperature usually drops during a fast, as do blood pressure and pulse and respiratory rates—all measures of the slowing metabolic rate of the body. Therefore it is important for the patient to stay warm. If needed, a hot water bottle can be used at night.

Breaking the Fast

Breaking the fast is a good time for the patient to carefully consider what he or she is eating and why. How to break the fast is outlined in Table A5.1. While breaking the fast and over the days that follow, instruct the patient to carefully record what he or she eats and how he or she feels after eating, and then you can review the record with the patient. This is the perfect time to educate the patient so that he or she can reestablish healthy eating habits, such as eating slowly, chewing thoroughly, limiting quantities, and eating foods at room temperature.

PROBLEMS WHILE FASTING

Most discomfort during fasting is usually brief as the body works quickly to restore homeostasis. The patient may experience headaches, dizziness, nausea, coated tongue, bad odor, mild palpitations, and mucus discharge. Instruct the patient to contact you or break the fast (with fresh fruit or vegetable juices for 2 days) if the symptoms are intolerable and persist. The patient can resume fasting when symptoms disappear.

SUMMARY

Although fasting is an effective way to improve health, it is only one part of a total health-building program, not a panacea. Fresh air, rest, exercise, sunshine, water, emotional health, and wholesome foods eaten in appropriate quantities are equally important in building and maintaining health.

TABLE A5.1 TrueNorth Standard Refeeding Guidelines for Breaking Your Fast

Phase I: Juice	Phase II: Raw	Phase III: Steamed	Phase IV: Grains, etc.	Phase V: Unrestricted
1 day juicing for every 7–10 days fasting	1 day raw for every 7–10 days of fasting Chew! Chew!	1 day of raw and steamed plants for every 7–10 days of fasting Chew! Chew!	1–2 days of raw and steamed plants and grains for every 7–10 days of fasting	Vegan SOS-Free Diet (to be followed for 50 years)
Juices will be delivered to your suite refrigerator at the following approximate times: 9 a.m. 12 p.m. 3 p.m. 5 p.m. If the first juices taste too strong, please dilute them with water, and do drink them all down.	Raw fruits (except citrus) Raw, juicy vegetables (including lettuce, cucumber tomato, celery, jicama, sprout, etc.)	Raw fruits Steamed vegetables Light soups (without grains) Potatoes, yams, squash Premade, grain-free salads Dressings (small amounts)	Raw fruits Raw vegetables Steamed vegetables Premade salads and all cold foods Grains (oatmeal, rice, quinoa, corn) NO LEGUMES Avocado—up to ½ per day Nuts or seeds—½ oz	Unrestricted Raw fruits Raw vegetables Steamed vegetables Premade salads and all cold foods Grains (oatmeal, rice, quinoa, corn) Thick soups Avocado (up to ½ per day) Nuts and seeds (up to 1 oz per day) Prepared desserts

Things to remember:

- Chew thoroughly!
- Eat small, simple meals.
- Remember to drink 1–2 glasses of water 20 minutes before meals.
- Bowel movements will gradually restart within 6 days of refeeding. The first bowel movement can be hard and/or difficult. Do not strain. If a problem arises, ask the rounding team for further instructions.

Phase A:	Phase B:	Phase C:	Phase D:	Phase E:
Liquids 1 day juicing for every 7–10 days fasting	Steamed 1 day for every 7–10 days of fasting	Steamed 1 day for every 7–10 days of fasting	Raw 1–2 days of raw and steamed veggies for every 7–10 days of fasting	Raw/Unrestricted Vegan SOS-Free Diet (to be followed for 50 years)
Broth or green juices will be delivered to your suite refrigerator at the following approximate times: 9 a.m. 12 p.m. 3 p.m. 5 p.m. If the first juices taste too strong, please dilute them with water, and do drink them all down.	Steamed or blended zucchini and/or steamed squash	Steamed vegetables (no potatoes) Steamed squash or zucchini Steamed fruit	Steamed vegetables (no potatoes) Raw vegetables Raw fruits (very small amounts) Premade salads and all cold foods	Steamed vegetables (including potatoes) Raw vegetables Raw fruits (small amounts) Premade salads and all cold foods Grains (oatmeal, rice, quinoa, corn) Thick soups w/grains and beans Avocado (up to ½ per day) Nuts or seeds (up to 1 oz per day) No prepared desserts

Things to remember:

- Chew thoroughly!
- Eat small, simple meals.
- Remember to drink 1–2 glasses of water 20 minutes before meals.
- Bowel movements will gradually restart within 6 days of refeeding. The first bowel movement can be hard and/or difficult. Do not strain. If a problem arises, ask the rounding team for further instructions.

Gluten and Gliadin Content of Select Foods

Joseph E. Pizzorno, ND

Food	Total Protein	Prolamines[a] (% of Total Protein)	Gliadin (mg/100 g)	Glutelins[b] (% of Total Protein)	Cross-Reactivity With Gliadin
Wheat	10–15	40–50	6900	30–40	++++
Rye	9–14	30–50	580	30–50	+++
Oats	8–14	10–15	100	≈5	++
Corn (maize)	7–13	50–55	0	30–45	+
Rice	8–10	1–5	0	85–90	—
Sorghum	9–13	>60	NA	NA	NA
Millet	7–16	57	0	30	NA
Buckwheat	13–15	0–11	4	High	—

NA, No data available; +, degree of reactivity; —, no reactivity.
[a]Primarily gliadin.
[b]Primarily gluten.
Data from Baker PG. Facts about gluten. *Lancet.* 1975;ii:1307; Friis SU. Enzyme-linked immunosorbent assay for quantitation of cereal proteins toxic in coeliac disease. *Clin Chim Acta.* 1988;178:261–270; and Chartrand LJ, Russo PA, Duhaime AG, et al. Wheat starch intolerance in patients with celiac disease. *J Am Diet Assoc.* 1997;97:612–618. https://onlinelibrary.wiley.com/doi/full/10.1111/1541-4337.12240 (accessed 12/30/19).

Grain Species	Components	Molecular Weight (% Total)	Polymers or Monomers
HMW Protamins			
Wheat	HMW subunits of glutenin	65–90 kDa (6%–10%)	Polymers
Barley	D-hordeins	>100 kDa (2%–4%)	Polymers
Rye	HMW secalins	>100 kDa (2%)	Polymers
S-rich Prolamins			
Wheat	γ-gliadins	30–45 kDa (70%–80%)	Monomers
	α-gliadins		Monomers
	B- and C-type LMW subunits of glutenin		Polymers
Barley	B-hordeins and γ-hordeins	32–45 kDa (80%)	Aggregated type, monomers or single-chain polypeptide
Rye	γ-secalins	40–75 kDa (80%)	Polymers
S-poor Prolamins			
Wheat	ω-gliadins	30–75 kDa (10%–20%)	Monomers
	D-type LMW subunits of glutenin		Aggregated type, polymers
Barley	C-hordeins	40–72 kDa (10%–15%)	Monomers
Rye	ω-secalins	48–55 kDa (10%–15%)	Monomers
Other Gluten Prolamins			
Oat	avenins	18.5–23.5 kDa (10%)	Monomers

HMW, high molecular weight; *LMW,* low molecular weight.
From Balakireva AV, Zamyatnin AA. Properties of gluten intolerance: gluten structure, evolution, pathogenicity and detoxification capabilities. *Nutrients.* 2016;8(10):E644.

Glycemic Index, Carbohydrate Content, and Glycemic Load of Select Foods

Michael T. Murray, ND

A complete list of the glycemic index (GI) and glycemic load (GL) of all tested foods is beyond the scope of this book—it would be a book in itself. Therefore we have selected the most common foods. Table A7.1 provides a general sense of what are considered high- and low-GL foods. We have listed the items by food groups, from low to high GLs. Some food groups are not listed. For example, nuts, seeds, fish, poultry, and meats are not listed—they have little impact on blood sugar levels because they are low in carbohydrates.

For a more complete listing, visit http://www.mendosa.com, a free website operated by medical writer Rick Mendosa.

TABLE A7.1 Low- to High-Glycemic Foods by Food Groups

Food	Glycemic Index	Carbohydrates (g)	Fiber (g)	Glycemic Load
Beans (Legumes)				
Soybeans, cooked, ½ cup, 100 g	14	12	7	1.6
Peas, green, fresh, frozen, boiled, ½ cup, 80 g	48	5	2	2
White navy beans, boiled, ½ cup, 90 g	38	11	6	4.2
Kidney beans, boiled, ½ cup, 90 g	27	18	7.3	4.8
Peas, split, yellow, boiled, ½ cup, 90 g	32	16	4.7	5.1
Lentils, ½ cup, 100 g	28	19	3.7	5.3
Lima beans, baby, ½ cup cooked, 85 g	32	17	4.5	5.4
Black beans, canned, ½ cup, 95 g	45	15	7	5.7
Pinto beans, canned, ½ cup, 95 g	45	13	6.7	5.8
Chickpeas, canned, drained, ½ cup, 95 g	42	15	5	63
Kidney beans, canned and drained, ½ cup, 95 g	52	13	7.3	6.7
Broad beans, frozen, boiled, ½ cup, 80 g	79	9	6	7.1
Peas, dried, boiled, ½ cup, 70 g	22	4	4.7	8
Baked beans, canned in tomato sauce, ½ cup, 120 g	48	21	8.8	10
Black-eyed peas, soaked, boiled, ½ cup, 120 g	42	24	5	10
Bread				
Multigrain, unsweetened, 1 slice, 30 g	43	9	1.4	4
Oat bran and honey loaf, 1 slice, 40 g	31	14	1.5	4.5
Sourdough, rye, 1 slice, 30 g	48	12	0.4	6
Stoneground whole wheat, 1 slice, 30 g	53	11	1.4	6
Wonder, enriched white bread, 1 slice, 20 g	73	10	0.4	7
Sourdough, wheat, 1 slice, 30 g	54	14	0.4	7.5
Pumpernickel, 1 slice, 60 g	41	21	0.5	8.6
Whole wheat, 1 slice, 35 g	69	14	1.4	9.6
Healthy Choice, hearty 7-grain, 1 slice, 38 g	56	18	1.4	10
White (wheat flour), 1 slice, 30 g	70	15	0.4	10.5
Healthy Choice, 100% whole grain, 1 slice, 38 g	62	18	1.4	11
Gluten-free multigrain, 1 slice, 35 g	79	15	1.8	12
French baguette, 30 g	95	15	0.4	14
Hamburger bun, 1 prepacked bun, 50 g	61	24	0.5	15
Rye, 1 slice, 50 g	65	23	0.4	15
Light rye, 1 slice, 50 g	68	23	0.4	16
Dark rye, black, 1 slice, 50 g	76	21	0.4	16
Croissant, 1, 50 g	67	27	0.2	18
Kaiser roll, 1 roll, 50 g	73	25	0.4	18

Continued

TABLE A7.1 Low- to High-Glycemic Foods by Food Groups—cont'd

Food	Glycemic Index	Carbohydrates (g)	Fiber (g)	Glycemic Load
Pita, 1 piece, 65 g	57	38	0.4	22
Bagel, 1, 70 g	72	35	0.4	25
Breakfast Cereals				
Oat bran, raw, 1 tbsp, 10 g	55	7	1	4
Bran with psyllium, ⅓ cup, 30 g	47	12	12.5	5.6
Bran, ⅓ cup, 30 g	58	14	14	8
All-Bran Soy 'n Fiber, ½ cup, 45 g	33	26	7	8.5
All-Bran, ½ cup, 40 g	42	22	6.5	9.2
Oatmeal (cooked with water), 1 cup, 245 g	42	24	1.6	10
Shredded wheat, ⅓ cup, 25 g	67	18	1.2	12
Mini Wheats (whole wheat), 1 cup, 30 g	58	21	4.4	12
All-Bran Fruit 'n Oats, ½ cup, 45 g	39	33	6	13
Weet-Bix, 2 biscuits, 30 g	69	19	2	13
Cheerios, ½ cup, 30 g	74	20	2	15
Frosties, ½ cup, 30 g	55	27	1	15
Corn Bran, ½ cup, 30 g	75	20	1	15
Honey Smacks, ¾ cup, 30 g	56	27	1	15
Wheatbites, 30 g	72	22	2	16
Total, 30 g	76	22	2	16.7
Healthwise for Heart Health, 45 g	48	35	2	16.8
Mini Wheats (blackcurrant), 1 cup, 30 g	71	24	2	17
Puffed wheat, 1 cup, 30 g	80	22	2	17.6
Bran Flakes, ¾ cup, 30 g	74	24	2	18
Crunchy Nut Cornflakes (Kellogg's), 30 g	72	25	2	18
Froot Loops, 1 cup, 30 g	69	27	1	18
Cocoa Puffs, ¾ cup, 30 g	77	26	1	20
Team, 30 g	82	25	1	20.5
Corn Chex, 30 g	83	25	1	20.75
Just Right, ¾ cup, 30 g	60	36	2	21.6
Corn Flakes, 1 cup, 30 g	84	26	0.3	21.8
Rice Krispies, 1 cup, 30 g	82	27	0.3	22
Rice Chex, 1 cup, 30 g	89	25	1	22
Crispix, 30 g	87	26	1	22.6
Just Right Just Grains, 1 cup, 45 g	62	38	2	23.5
Oat 'n Honey Bake, 45 g	77	31	2	24
Raisin Bran, 1 cup, 45 g	73	35	4	25.5
Grape Nuts, ½ cup, 58 g	71	47	2	33.3
Cake				
Angel food, 1 slice, 30 g	67	17	<1	11.5
Sponge cake, 1 slice, 60 g	46	32	<1	14.7
Cupcake, with icing and cream filling, 1 cake, 38 g	73	26	<1	19
Chocolate fudge, mix (Betty Crocker), 73 g cake + 33 g frosting	38	54	<1	20.5
Banana cake, 1 slice, 80 g	47	46	<1	21.6
Pound cake, 1 slice, 80 g	54	42	<1	22.6
French vanilla (Betty Crocker), 73 g cake + 33 g frosting	42	58	<1	24.4
Lamingtons, 1, 50 g	87	29	<1	25
Flan, 1 slice, 80 g	65	55	<1	35.75
Scones, made from packet mix, 1 scone, 40 g	92	90	<1	83
Crackers				
Corn Thins, puffed corn cake, 2, 12 g	87	9	<1	7.8
Kavli, 4, 20 g	71	13	3	9.2
Breton wheat crackers, 6, 25 g	67	14	2	9.4
Ryvita or Wasa, 2, 20 g	69	16	3	11
Stoned Wheat Thins, 5, 25 g	67	17	1	11.4
Premium soda crackers, 3, 25 g	74	17	0	12.5
Water cracker, 5, 25 g	78	18	0	14

TABLE A7.1 Low- to High-Glycemic Foods by Food Groups—cont'd

Food	Glycemic Index	Carbohydrates (g)	Fiber (g)	Glycemic Load
Graham, 1, 30 g	74	22	1.4	16
Rice cake, 2, 25 g	82	21	0.4	17
Milk, Soy Milk, and Juices				
Milk, full fat, 1 cup, 250 mL	27	12	0	3
Soy, 1 cup, 250 mL	31	12	0	3.7
Milk, skim, 1 cup, 250 mL	32	13	0	4
Grapefruit juice, unsweetened, 1 cup, 250 mL	48	16	1	7.7
Nesquik chocolate powder, 3 tsp in 250 mL milk	55	14	0	7.7
Milk, chocolate flavored, low fat, 1 cup, 250 mL	34	23	0	7.8
Orange juice, 1 cup, 250 mL	46	21	1	9.7
Gatorade, 1 cup, 250 mL	78	15	0	11.7
Pineapple juice, unsweetened, canned, 250 mL	46	27	1	12.4
Apple juice, unsweetened, 1 cup, 250 mL	40	33	1	13.2
Cranberry juice cocktail (Ocean Spray USA), 240 mL	68	34	0	23
Coca-Cola, 375 mL	63	40	0	25.2
Other soft drinks, 375 mL	68	51	0	34.7
Milk, sweetened condensed, 1/2 cup, 160 g	61	90	0	55
Fruit				
Cherries, 20 cherries, 80 g	22	10	2.4	2.2
Plums, 3–4 small, 100 g	39	7	2.2	2.7
Peach, fresh, 1 large, 110 g	42	7	1.9	3
Apricots, fresh, 3 medium, 100 g	57	7	1.9	4
Apricots, dried, 5–6 pieces, 30 g	31	13	2.2	4
Kiwi, 1, raw, peeled, 80 g	52	8	2.4	4
Orange, 1 medium, 130 g	44	10	2.6	4.4
Peaches, canned, natural juice, ½ cup, 125 g	38	12	1.5	4.5
Pears, canned in pear juice, ½ cup, 125 g	43	13	1.5	5.5
Watermelon, 1 cup, 150 g	72	8	1	5.7
Pineapple, fresh, 2 slices, 125 g	66	10	2.8	6.6
Apple, 1 medium, 150 g	38	18	3.5	6.8
Grapes, green, 1 cup, 100 g	46	15	2.4	6.9
Apple, dried, 30 g	29	24	3.0	6.9
Prunes, pitted (Sunsweet), 6 prunes, 40 g	29	25	3.0	7.25
Pear, fresh, 1 medium, 150 g	38	21	3.1	8
Fruit cocktail, canned in natural juice, ½ cup, 125 g	55	15	1.5	8.25
Apricots, canned, light syrup, ½ cup, 125 g	64	13	1.5	8.3
Peaches, canned, light syrup, ½ cup, 125 g	52	18	1.5	9.4
Mango, 1 small, 150 g	55	19	2.0	10.4
Figs, dried, tenderized (water added), 50 g	61	22	3.0	13.4
Sultanas, ¼ cup, 40 g	56	30	3.1	16.8
Banana, raw, 1 medium, 150 g	55	32	2.4	17.6
Raisins, ¼ cup, 40 g	64	28	3.1	18
Dates, dried, 5, 40 g	103	27	3.0	27.8
Grains				
Rice bran, extruded, 1 tbsp, 10 g	19	3	1	0.57
Barley, pearled, boiled, ½ cup, 80 g	25	17	6	4.25
Millet, cooked, ½ cup, 120 g	71	12	1	8.52
Bulgur, cooked, 2/3 cup, 120 g	48	22	3.5	10.6
Brown rice, steamed, 1 cup, 150 g	50	32	1	16
Couscous, cooked, ⅔ cup, 120 g	65	28	1	18
White rice, boiled, 1 cup, 150 g	72	36	0.2	26
Arborio risotto rice, white, boiled, 100 g	69	35	0.2	29
Basmati rice, white, boiled, 1 cup, 180 g	58	50	0.2	29
Buckwheat, cooked, ½ cup, 80 g	54	57	3.5	30
Instant rice, cooked, 1 cup, 180 g	87	38	0.2	33

Continued

TABLE A7.1 Low- to High-Glycemic Foods by Food Groups—cont'd

Food	Glycemic Index	Carbohydrates (g)	Fiber (g)	Glycemic Load
Tapioca (steamed 1 h), 100 g	70	54	<1	38
Tapioca (boiled with milk), 1 cup, 265 g	81	51	<1	41
Jasmine rice, white, long grain, steamed, 1 cup, 180 g	109	39	0.2	42.5
Ice Cream				
Low-fat French vanilla, 100 mL	38	15	0	5.7
Full fat, 2 scoops, 50 g	61	10	0	6.1
Jam				
No sugar, 1 tbsp, 25 g	55	11	<1	6
Sweetened, 1 tbsp	48	17	<1	8
Muffins and Pancakes				
Chocolate butterscotch muffins, from mix, 50 g	53	28	1	15
Apple muffins, oat and sultana, from mix, 50 g	54	28	1	15
Apricot muffins, coconut and honey, from mix, 50 g	60	27	1.5	16
Banana muffins, oat and honey, from mix, 50 g	65	28	1.5	18
Apple muffins, 1 muffin, 80 g	44	44	1.5	19
Bran muffins, 1 muffin, 80 g	60	34	2.5	20
Blueberry muffins, 1 muffin, 80 g	59	41	1.5	24
Buckwheat pancakes, from dry mix, 40 g	102	30	2	30
Pancake, from dry mix, 1 large, 80 g	67	58	1	39
Pasta				
Tortellini, cheese, cooked, 180 g	50	21	2	10.5
Ravioli, meat-filled, cooked, 1 cup, 220 g	39	30	2	11.7
Vermicelli, cooked, 1 cup, 180 g	35	45	2	15.7
Rice noodles, fresh, boiled, 1 cup, 176 g	40	44	0.4	17.6
Spaghetti, whole meal, cooked, 1 cup, 180 g	37	48	3.5	17.75
Fettuccini, cooked, 1 cup, 180 g	32	57	2	18.2
Spaghetti, gluten-free in tomato sauce, 1 small tin, 220 g	68	27	2	18.5
Macaroni and cheese, packaged, cooked, 220 g	64	30	2	19.2
Star pastina, cooked, 1 cup, 180 g	38	56	2	21
Spaghetti, white, cooked, 1 cup, 180 g	41	56	2	23
Rice pasta, brown, cooked, 1 cup, 180 g	92	57	2	52
Sugars				
Fructose, 10 g	23	10	0	2.3
Honey, ½ tbsp, 10 g	58	16	0	4.6
Lactose, 10 g	46	10	0	4.6
Sucrose, 10 g	65	10	0	6.5
Glucose, 10 g	102	10	0	10.2
Maltose, 10 g	105	10	0	10.5
Snacks				
Corn chips, Doritos original, 50 g	42	33	<1	13.9
Snickers, 59 g	41	35	0	14.3
Tofu frozen dessert (nondairy), 100 g	115	13	<1	15
Real fruit bars, strawberry, 20 g	90	17	<1	15.3
Twix cookie bar (caramel), 59 g	44	37	<1	16.2
Pretzels, 50 g	83	22	<1	18.3
Mars candy bar, 60 g	65	41	0	26.6
Skittles, 62 g	70	55	0	38.5
Soups				
Tomato, canned, 220 mL	38	15	1.5	6
Black bean, 220 mL	64	9	3.4	6
Lentil, canned, 220 mL	44	14	3	6
Split pea, canned, 220 mL	60	13	3	8

TABLE A7.1 Low- to High-Glycemic Foods by Food Groups—cont'd

Food	Glycemic Index	Carbohydrates (g)	Fiber (g)	Glycemic Load
Vegetables				
Carrots, raw, ½ cup, 80 g	16	6	1.5	1
Low-glycemic vegetables:	≈20	≈7	≈1.5	≈1.4
Asparagus, 1 cup cooked or raw				
Broccoli, 1 cup cooked or raw				
Brussels sprouts, 1 cup cooked or raw				
Cabbage, 1 cup cooked or raw				
Cauliflower, 1 cup cooked or raw				
Cucumber, 1 cup				
Celery, 1 cup cooked or raw				
Eggplant, 1 cup				
Green beans, 1 cup cooked or raw				
Kale, 1 cup cooked, 2 cups raw				
Lettuce, 2 cups raw				
Mushrooms, 1 cup				
Spinach, 1 cup cooked, 2 cups raw				
Tomatoes, 1 cup				
Zucchini, 1 cup cooked or raw				
Carrots, peeled, boiled, 1/2 cup, 70 g	49	3	1.5	1.5
Beets, canned, drained, 2–3 slices, 60 g	64	5	1	3
Pumpkin, peeled, boiled, ½ cup, 85 g	75	6	3.4	4.5
Parsnips, boiled, ½ cup, 75 g	97	8	3	8
Sweet corn on the cob, boiled 20 min, 80 g	48	14	2.9	8
Corn, canned and drained, ½ cup, 80 g	55	15	3	8.5
Sweet potato, peeled, boiled, 80 g	54	16	3.4	8.6
Sweet corn, ½ cup boiled, 80 g	55	18	3	10
Potatoes, peeled, boiled, 1 medium, 120 g	87	13	1.4	10
Potatoes, with skin, boiled, 1 medium, 120 g	79	15	2.4	11
Yam, boiled, 80 g	51	26	3.4	13
Potatoes, baked in oven (no fat), 1 medium, 120 g	93	15	2.4	14
Potatoes, mashed, ½ cup, 120 g	91	16	1	14
Potatoes, instant, prepared, ½ cup	83	18	1	15
Potatoes, new, unpeeled, boiled, 5 small (cocktail), 175 g	78	25	2	20
Cornmeal (polenta), ⅓ cup, 40 g	68	30	2	20
French fries, fine cut, small serve, 120 g	75	49	1	36
Gnocchi, cooked, 1 cup, 145 g	68	71	1	48
Yogurt				
Yogurt, low fat, artificial sweetener, 200 g	14	12	0	2
Yogurt, with fruit, 200 g	26	30	0	8
Yogurt, low fat, 200 g	33	26	0	8.5

Hydrochloric Acid Supplementation: Patient Instructions

Michael T. Murray, ND

PURPOSE

The purpose of this self-test is to estimate the amount of supplemental hydrochloric acid (HCl) you need to reestablish adequate stomach acid. Adequate stomach acid is critical for initiating digestion and protecting the intestinal tract from microbial pathogens.

DIRECTIONS

1. Begin by taking one HCl capsule (500–600 mg) at your next large meal. At every meal after that of the same size, take one more capsule (one capsule at the next meal, two at the meal after that, then three at the next meal, and so on).
2. Continue to increase the dose until you reach seven capsules or you feel a warmth in your stomach, whichever occurs first. A feeling of warmth in the stomach means that you have taken too many capsules for a meal of that size. Take one fewer capsule the next time. However, it is a good idea to try the larger dose again at another meal to make sure that it was the HCl that caused the warmth and not something else.
3. After you have determined the largest dose that you can take at your large meals without feeling any warmth, maintain that dose at all meals of similar size. Take fewer capsules with smaller meals.
4. When taking several capsules, it is best to take them throughout the meal rather than all at once.
5. As your stomach begins to regain the ability to produce the amount of HCl needed to properly digest your food, you will notice the warm feeling again. This is the time to start decreasing the dose level.
6. Every 3 days, decrease the dose by one capsule per meal. If the warmth continues, decrease more rapidly. If maldigestion symptoms return, add capsules back until digestion improves again.

Optimal Health Food Pyramid

Michael T. Murray, ND

The Optimal Health Food Pyramid, shown in Fig. A9.1, incorporates the best from two of the most healthful diets ever studied: the traditional Mediterranean diet and the traditional Asian diet. In addition, the Optimal Health Food Pyramid more clearly defines what the healthy components within the categories are and stresses the importance of vegetable oils and regular fish consumption as part of a healthful diet (Table A9.1).

FOODS TO AVOID ENTIRELY

- Refined white flour products: pastas, cakes, muffins, pretzels, and so forth
- Refined sugar–loaded cereals, candies, baked goods, and so forth
- Processed foods packed full of empty calories (sugar and fat) or salt (e.g., soups, theater-style popcorn, chips, etc.)
- Margarine, butter, and shortening
- Smoked or cured meats: bacon, hot dogs, smoked luncheon meats, sausages, ham, Spam, and so forth
- Meats cooked at extremely high temperatures or cooked to well done
- Heavily sweetened or artificially sweetened soft drinks, juice-flavored drinks, and so forth
- Fried foods, including French fries, potato chips, corn chips, and doughnuts

VEGETABLES: FIVE TO SEVEN SERVINGS DAILY

In Latin, the word *vegetable* means "to enliven or animate." Vegetables give us life and should be the main focus of any health-promoting diet. Vegetables provide the broadest range of nutrients of any food class. They are rich sources of vitamins, minerals, carbohydrates, and protein. Vegetables also provide high quantities of anticancer phytochemicals.

It is very important not to overcook vegetables. Overcooking not only results in loss of important nutrients, but it also alters the flavor of the vegetable. Light steaming, baking, and quick stir-frying are the best ways to cook vegetables. Do not boil vegetables, unless you are making soup, because much of the nutrients will leach into the water. If fresh vegetables are not available, frozen vegetables are preferred over their canned counterparts. The only exception is tomato products (e.g., soup, paste, sauce, etc.), especially when they also contain oil; canned tomato products actually provide more absorbable lycopene than raw tomatoes.

We have divided vegetable intake into three categories: green leafy and cruciferous vegetables, low-glycemic vegetables, and starchy vegetables. This approach encourages eating a variety of these life-giving foods, helps achieve a "rainbow assortment" in the diet, and enables a focus on low-glycemic choices.

One serving of vegetables equals any of the following:

- 1 cup raw leafy vegetables (such as lettuce or spinach)
- ½ cup raw nonleafy vegetables
- ½ cup cooked vegetables or fresh vegetable juice

Box A9.1 offers easy tips to increase the daily intake of fruits and vegetables.

GREEN LEAFY AND CRUCIFEROUS VEGETABLES: TWO TO FOUR SERVINGS DAILY

- Alfalfa sprouts
- Beet greens
- Bok choy
- Broccoli
- Brussels sprouts
- Cabbage
- Cauliflower
- Chard
- Chinese cabbage
- Collard greens
- Dandelion
- Endive
- Escarole

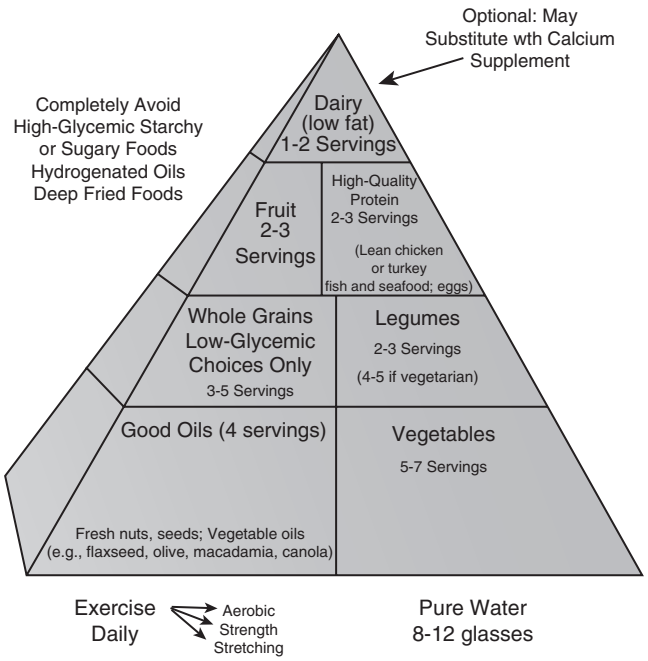


FIG. A9.1 The Optimal Health Food Pyramid.

TABLE A9.1 Daily Food Group Recommendations for a 2000-Calorie Diet

Food	Number of Servings
Vegetables:	
Total servings	5–7
Green leafy and cruciferous vegetables	2–4
Low-glycemic vegetables	2–3
Other vegetables	1–2
Good Oils:	
Total servings	4
Nuts and seeds	1
Olive, macadamia, flaxseed, or canola oil	2–3
Whole grains	3–5
Legumes	2–3 (4–5 if vegetarian)
High-quality protein	2–3
Fruit	2–3
Dairy	1–2 (optional)

- Kale
- Lettuce (the darker, the better)
- Mustard greens
- Parsley
- Spinach
- Turnip greens
- Watercress

LOW-GLYCEMIC VEGETABLES: TWO TO THREE SERVINGS DAILY

- Artichoke (one medium)
- Asparagus
- Bean sprouts
- Bell peppers
- Carrots
- Celery
- Cucumber
- Fennel
- Mushrooms
- Okra
- Onions
- Peas (fresh or frozen)
- Radishes
- Rhubarb
- String beans, green or yellow
- Summer squash
- Tomatoes, tomato paste, tomato sauce, tomato juice, vegetable juice cocktail
- Zucchini

STARCHY VEGETABLES: ONE TO TWO SERVINGS DAILY

- Beets
- Parsnip
- Potato
- Pumpkin
- Rutabaga
- Winter, acorn, or butternut squash
- Yam or sweet potato

BOX A9.1 Easy Tips to Increase the Daily Intake of Fruits and Vegetables

- Buy many kinds of fruits and vegetables when grocery shopping so that plenty of choices are available.
- Stock up on frozen vegetables for easy cooking so that a vegetable dish can easily be served with every dinner.
- Use the fruits and vegetables that go bad quickly (peaches, asparagus) first. Save hardier varieties (apples, acorn squash) or frozen goods for later in the week.
- Keep fruits and vegetables where they are easily and frequently seen. The more often they are seen, the more likely they are to be eaten.
- Keep a bowl of cut-up vegetables on the top shelf of the refrigerator.
- Make a big tossed salad with several kinds of greens, cherry tomatoes, cut-up carrots, red pepper, broccoli, scallions, and sprouts. Refrigerate in a large glass bowl with an airtight lid so that a delicious mixed salad will be ready to enjoy for several days.
- Keep a fruit bowl on your kitchen counter, table, or desk at work.
- Treat yourself to a fruit sundae: top a bowl full of your favorite cut-up fruits with vanilla yogurt, shredded coconut, and a handful of nuts.
- Pack a piece of fruit or some cut-up vegetables in your briefcase or backpack; carry moist towelettes for easy cleanup.
- Add fruits and vegetables to lunch by having them in soup or salad or cutting them up to eat raw.
- Use thinly sliced pears or apples in omelets.
- At dinner, serve steamed or microwaved vegetables.
- Increase portions when serving vegetables. One easy way of doing so is by adding fresh greens such as Swiss chard, collard greens, or beet greens to stir-fry dishes.
- Serve fresh fruit for dessert. Add visual and taste appeal by serving with nuts, cheese, or yogurt. For example, serve grapes or sliced apple with cheese, or serve a fruit parfait made with low-fat yogurt or topped with berries.
- Add extra varieties of vegetables to soups, sauces, and casseroles (e.g., add grated carrots and zucchinis to spaghetti sauce).
- Take advantage of salad bars, which offer ready-to-eat raw vegetables and fruits and prepared salads made with fruits and vegetables.
- Use vegetable-based sauces, such as marinara sauce, and juices, such as low-sodium vegetable juice (V-8) or tomato juice.
- Freeze lots of blueberries and grapes to use as summer replacements for ice cream, popsicles, and other sugary foods.

NUTS, SEEDS, AND GOOD OILS: FOUR SERVINGS DAILY

Nuts and seeds provide beneficial oils, especially monounsaturated and medium-chain fatty acids. Regular consumption of nuts has been shown to improve blood sugar regulation and lower the risk for diabetes, heart disease, obesity, and cancer. Focus on raw nuts and seeds. Definitely avoid nuts and seeds roasted in oils or coated with sugar. Nuts and seeds make excellent additions to salads and sautéed greens. Try to eat a variety of nuts and seeds, such as almonds, Brazil nuts, walnuts, pecans, flaxseeds, sunflower seeds, and pumpkin seeds.

Use olive, macadamia, flaxseed, or coconut oil both in salad dressings and to replace butter, margarine, and the shortening used for cooking. Never cook with flaxseed oil; it is too rich in polyunsaturated fats, which are easily damaged by heat. Coconut and macadamia nut oils are the best cooking oils because of their ability to remain stable during high temperatures, but olive oil is a good choice for sautéed vegetables, and canola oil is usually best for baked goods because it has the mildest flavor. Coconut oil is also very stable in cooking and is fine to use in small quantities; it contains saturated fat but is metabolized differently from

TABLE A9.2 Single Servings of Whole Grains

Bread: whole wheat, rye, or other whole grain	1 slice
Cereals: whole grain	½ cup
Corn:	
Cooked whole kernel corn	½ cup
Corn on the cob	1 small
Flour and flour products:	
Whole-wheat flour (uncooked)	2–½ tbsp
Whole-grain pasta (cooked)	½ cup
Whole grains (cooked): rice, oats, wheat, barley, quinoa, spelt, etc.	½ cup

animal-derived saturated fats and can be used safely in moderation. Avoid using safflower, sunflower, soy, and corn oils because they contain too much omega-6 fatty acid (as discussed in Chapter 44).

We recommend at least one serving of nuts or seeds (one serving equals ¼ cup) and 3 tbsp of healthy oils daily. In addition, we recommend taking a high-quality fish oil supplement.

WHOLE GRAINS: THREE TO FIVE SERVINGS DAILY

It is very important to choose whole-grain products (e.g., whole-grain breads, whole-grain flour products, brown rice) over their processed counterparts (white bread, white flour products, white rice, etc.). Whole grains provide substantially more nutrients and health-promoting properties. Whole grains are a major source of complex carbohydrates, dietary fiber, magnesium and other minerals, and B vitamins. The content and quality of the protein in whole grains are also greater than in refined grains. Diets rich in whole grains have been shown to be helpful in both the prevention and treatment of diabetes, heart disease, and cancer.

Table A9.2 defines servings of various whole grains.

BEANS (LEGUMES): TWO TO THREE SERVINGS DAILY

Beans are a mainstay in most diets of the world and are second only to grains in supplying calories and protein to the world's population. Compared with grains, they supply about the same number of total calories, but they usually provide two to four times as much protein and are a richer source of the soluble fiber that lowers cholesterol and stabilizes blood glucose levels. Although we do not recommend using canned vegetables or fruit, canned beans retain their fiber content and anticancer flavonoids. Plus, given the long preparation time for cooking beans, canned beans are extremely quick and convenient.

A serving size for beans is ½ cup.

FRUITS: THREE TO FOUR SERVINGS DAILY

Fruits are a rich source of many beneficial nutrients, and regular fruit consumption has been shown to offer significant protection against chronic degenerative diseases, including cancer, heart disease, cataracts, diabetes, and stroke. Fruits make nutrient-dense, easy between-meal snacks and desserts (e.g., nothing could be simpler than phytonutrient-rich fresh berries alone). It is easy to get into the habit of eating only a few varieties of fruit. We encourage eating a "rainbow assortment" of fruits over the course of a week.

BOX A9.2 Fish Consumption Caution

Fish consumption offers significant protection against heart disease and many forms of cancer, especially the major cancers, like those of the lung, colon, breast, and prostate. Although we encourage the eating of more fish, some guidelines are necessary. Nearly all fish contain trace amounts of methyl mercury. In most cases, this is of little concern because the level is so low. The fish most likely to have the lowest level of methyl mercury are salmon (usually nondetectable levels), sardines, Atlantic mackerel, cold-water tuna, farm-raised catfish, and herring. However, certain seafood, particularly swordfish, shark, and some other large predatory fish, may contain high levels of methyl mercury. Fish absorb methyl mercury from water and aquatic plants. Larger predatory fish also absorb mercury from their prey. Methyl mercury binds tightly to the proteins in fish tissue, including muscle; cooking does not reduce the mercury content significantly.

We suggest limiting intake to no more than about 2 lb (1 kg) per week. That translates to six 7-oz servings per week maximum. Swordfish, shark, marlin, tilefish, and king mackerel should be limited to no more than once per month and should be avoided completely in women of childbearing age who might get pregnant.

A general rule of thumb is that one serving of fruit equals one of the following:

- One medium fruit
- ½ cup of small or cut-up fruit
- 4 oz of 100% juice
- ¼ cup dried fruit

See Box A9.1 for easy tips to increase the daily intake of fruits and vegetables.

HIGH-QUALITY PROTEIN: TWO TO THREE SERVINGS DAILY

The detriment of diets high in saturated fat and cholesterol has been stressed for decades. Likewise, the importance of omega-3 fatty acids in the battle against the development of chronic disease is now also well known. Fish consumption, in particular, has shown tremendous protection against heart disease and cancer. Choose smaller species of fatty fish, such as wild salmon, mackerel, herring, and sardines. Their smaller size and shorter life span translate into a lower accumulation of mercury (Box A9.2).

We therefore recommend eating small fish at least three, but no more than six, times per week. Avoid fish such as swordfish, Atlantic cod, farmed salmon, and tilapia. Keep intake of red meat (beef, veal, or lamb) to no more than two servings per month, and choose the leanest cuts possible; keep the portion size limited to about the size of a deck of cards, and do not charbroil or cook the meat until well done because this practice increases the formation of cancer-causing compounds. Also, consider some of the alternatives to beef, such as venison, buffalo, elk, rabbit, and ostrich. These emerging beef alternatives are lower in saturated fat and provide higher levels of omega-3 fatty acids.

Chicken and turkey can also provide excellent protein with very little fat, especially if only the white meat (breast) without the skin is eaten. Eggs are also a good source of high-quality protein, and if produced by free-range hens fed flaxseed meal, they will be rich in beneficial omega-3 fatty acids.

One serving equals about the size of a deck of cards; that translates to roughly 4 oz.

DAIRY: ONE OR TWO SERVINGS DAILY (OPTIONAL)

We have found that many people are allergic to milk or lack the enzymes necessary to digest dairy products. Even for people who do tolerate dairy foods, milk consumption should be limited to no more than one or two servings per day. Use nonfat or reduced-fat dairy products rather than whole-milk varieties. Also, fermented dairy products like yogurt, kefir, and acidophilus-fortified milk are preferred over milk. Some of the soy milk alternatives to cow's milk are delicious, especially when flavored with vanilla or chocolate. If dairy products are not consumed, we recommend a calcium supplement.

One serving of dairy products equals 1 cup of milk, yogurt, or cottage cheese or 1 oz of cheese.

Patient Instructions for Measuring Basal Body Temperature

Michael T. Murray, ND

PURPOSE

Your body temperature reflects your metabolic rate, which is largely determined by hormones secreted by the thyroid gland and by the ability of your cells to convert these hormones to their more active form. Your level of thyroid activity can be determined simply by measuring your basal body temperature. All you need is a high-quality thermometer.

PROCEDURE

1. Place the thermometer by your bed before going to sleep at night. If using a mercury thermometer, shake it down to below 95°F.
2. On waking, place the thermometer in your armpit for a full 10 minutes. It is important to make as little movement as possible. Lying and resting with your eyes closed is best. Do not get up until the 10-minute measurement is completed.
3. After 10 minutes, read and record the temperature and date (a convenient form is printed as Table A10.1).
4. Record the temperature for at least three mornings (preferably at the same time of day) and give the information to your physician. Menstruating women must perform the test on the second, third, and fourth days of menstruation. Men and postmenopausal women can perform the test at any time.

INTERPRETATION

Your basal body temperature should be between 97.6°F and 98.2°F. Low basal body temperatures are quite common and may reflect hypothyroidism. Common signs and symptoms of hypothyroidism are as follows:

- Depression
- Difficulty in losing weight
- Dry skin
- Headaches
- Lethargy or fatigue
- Menstrual problems
- Recurrent infections
- Sensitivity to cold

High basal body temperatures (higher than 98.6°F) are less common but may be evidence of hyperthyroidism. Common signs and symptoms of hyperthyroidism are as follows:

- Bulging eyeballs
- Fast pulse
- Hyperactivity
- Inability to gain weight
- Insomnia
- Irritability
- Menstrual problems
- Nervousness

TABLE A10.1 Form for Recording Temperature and Date When Measuring Basal Body Temperature

NAME_____

Month	Day	Temp.	Month	Day	Temp.	Month	Day	Temp.
____	1	____	____	1	____	____	1	____
	2	____		2	____		2	____
	3	____		3	____		3	____
	4	____		4	____		4	____
	5	____		5	____		5	____
	6	____		6	____		6	____
	7	____		7	____		7	____
	8	____		8	____		8	____
	9	____		9	____		9	____
	10	____		10	____		10	____
	11	____		11	____		11	____
	12	____		12	____		12	____
	13	____		13	____		13	____
	14	____		14	____		14	____
	15	____		15	____		15	____
	16	____		16	____		16	____
	17	____		17	____		17	____
	18	____		18	____		18	____
	19	____		19	____		19	____
	20	____		20	____		20	____
	21	____		21	____		21	____
	22	____		22	____		22	____
	23	____		23	____		23	____
	24	____		24	____		24	____
	25	____		25	____		25	____
	26	____		26	____		26	____
	27	____		27	____		27	____
	28	____		28	____		28	____
	29	____		29	____		29	____
	30	____		30	____		30	____
	31	____		31	____		31	____

Rotation Diet Master Chart and Plan

Sally J. Rockwell, PhD, CCN

TABLE A11.1 Rotation Diet Master Chart and Plan

Food Family	Day 1 (Green)	Day 2 (Yellow)	Day 3 (Blue)	Day 4 (Red)
Proteins				
Fin fish	Fish may be eaten daily as long as a different fish is selected each day			
Shellfish	Mussel, escargot, clam	Shrimp	Oysters, scallops, abalone	Crab, squid, octopus
Fowl flesh and eggs	Chicken, goose	Turkey ↔	Cornish game hen, quail, pheasant	Duck
Red meat	Beef (veal, liver, etc.)		Pork (ham, bacon, etc.), lamb	Rabbit
Dairy	Cow (milk, yogurt, cheeses)		Goat (milk, yogurt, cheeses)	
Legumes	Kidney, soy, and lima beans		Pinto, garbanzo, and black beans; black-eyed and split peas	
Sprouts	Nuts, seeds, and legumes	Nuts and seeds	Nuts, seeds, and legumes	Nuts and seeds
Nuts, seeds, and oils	Soy, walnuts	Almonds	Peanuts, pecans, pumpkin seeds	Cashews, pistachios
	Filberts ↔	Pine nuts ↔	Chestnuts ↔	Safflower, corn
	Sesame ↔	Brazil nuts ↔	Macadamias ↔	Avocado ↔
		Sunflower, apricots		Olive ↔
Grains and flours	Buckwheat, soy, quinoa, tofu, arrowroot, guar gum	Rice, millet, rye, potato, flaxseed, xanthan gum	Amaranth, garbanzo, kudzu root, chestnut	Barley oats, wheat, corn, tapioca, agar ↔
Sweeteners	Honey, raisins	Dates, molasses, rice syrup	Figs, currants	Coconut, malt, sago palm, maple syrup
Vegetables				
Beet	Beets, Swiss chard	Carrots, parsley	Spinach	Avocado
Mustard	Cabbage, broccoli, white radish, kale, napa cabbage		Bok choy, red radish, Brussels sprouts, cauliflower	Parsnip, celery
Composite		Leaf and bib lettuce, common artichoke		Iceberg lettuce, romaine
Gourd	Cucumber, winter squash		Summer squash, pumpkin, zucchini	
Lily	Onion, garlic		Chives, leeks, shallots, asparagus	
Nightshade		White potato, peppers	Chestnuts	Tomato, eggplant, peppers
Miscellaneous	String beans	Yams ↔	Peas	Bamboo shoots
		Jicama ↔	Okra ↔	Jerusalem artichoke ↔
		Mushrooms ↔	Olives ↔	Sweet potato ↔
Fruits				
Plum		Plum, prune, apricot		Peach, cherry, nectarine
Apple	Apple		Pear, quince	
Citrus		Lemon, orange, kumquat		Lime, grapefruit, tangerine
Berries	Boysenberry, blackberry, strawberry	Huckleberry, cranberry	Loganberry, raspberry, gooseberry	Blueberry
Gourd	Melons		Cantaloupe, melons	Pomegranate ↔
Miscellaneous	Rhubarb, grapes	Kiwi ↔	Pineapple ↔	Mango, banana ↔
	Papaya ↔	Guava ↔		
	Persimmon ↔			
Bulk	Pectin	Flaxseed ↔	Chia seed ↔	Psyllium seed

↔, A food that is essentially the only food of a family and that will not cross-react with other foods.

Seligman's Attributional Style Questionnaire

Michael T. Murray, ND

What distinguishes an optimist from a pessimist is the way in which they explain both good and bad events. Dr. Martin Seligman developed a simple test to determine your level of optimism (from *Learned Optimism*, Knopf, 1981).

INSTRUCTIONS

Take as much time as you need. There are no right or wrong answers. It is important that you take the test before you read the interpretation.

Read the description of each situation and vividly imagine it happening to you. Choose the response that most applies to you by circling either A or B. Ignore the letter and number codes for now; they will be explained after you take the test.

1.	The project you are in charge of is a great success.	PsG
	A. I kept a close watch over everyone's work.	1
	B. Everyone devoted a lot of time and energy to it.	0
2.	You and your spouse (boyfriend/girlfriend) make up after a fight.	PmG
	A. I forgave him or her.	0
	B. I'm usually forgiving.	1
3.	You get lost driving to a friend's house.	PsB
	A. I missed a turn.	1
	B. My friend gave me bad directions.	0
4.	Your spouse (boyfriend/girlfriend) surprises you with a gift.	PsG
	A. He or she just got a raise at work.	0
	B. I took him or her out to a special dinner the night before.	1
5.	You forget your spouse's (boyfriend's/girlfriend's) birthday.	PmB
	A. I'm not good at remembering birthdays.	1
	B. I was preoccupied with other things.	0
6.	You get a flower from a secret admirer.	PvG
	A. I am attractive to him or her.	0
	B. I am a popular person.	1
7.	You run for a community office position, and you win.	PvG
	A. I devote a lot of time and energy to campaigning.	0
	B. I work very hard at everything I do.	1
8.	You miss an important engagement.	PvB
	A. Sometimes my memory fails me.	1
	B. I sometimes forget to check my appointment book.	0
9.	You run for a community office position, and you lose.	PsB
	A. I didn't campaign hard enough.	1
	B. The person who won knew more people.	0
10.	You host a successful dinner.	PmG
	A. I was particularly charming that night.	0
	B. I am a good host.	1
11.	You stop a crime by calling the police.	PsG
	A. A strange noise caught my attention.	0
	B. I was alert that day.	1
12.	You were extremely healthy all year.	PsG
	A. Few people around me were sick, so I wasn't exposed.	0
	B. I made sure I ate well and got enough rest.	1
13.	You owe the library $10 for an overdue book.	PmB
	A. When I am really involved in what I am reading, I often forget when it's due.	1
	B. I was so involved in writing the report that I forgot to return the book.	0
14.	Your stocks make you a lot of money.	PmG
	A. My broker decided to take on something new.	0
	B. My broker is a top-notch investor.	1
15.	You win an athletic contest.	PmG
	A. I was feeling unbeatable.	0
	B. I train hard.	1
16.	You fail an important examination.	PsB
	A. I wasn't as smart as the other people taking the examination.	1
	B. I didn't prepare for it well.	0

17. You prepared a special meal for a friend, and he or she barely touched the food. PvB
    A. I wasn't a good cook. 1
    B. I made the meal in a rush. 0
18. You lose a sporting event for which you have been training for a long time. PvB
    A. I'm not very athletic. 1
    B. I'm not good at that sport. 0
19. Your car runs out of gas on a dark street late at night. PsB
    A. I didn't check to see how much gas was in the tank. 1
    B. The gas gauge was broken. 0
20. You lose your temper with a friend. PmB
    A. He or she is always nagging me. 1
    B. He or she was in a hostile mood. 0
21. You are penalized for not returning your income tax forms on time. PmB
    A. I always put off doing my taxes. 1
    B. I was lazy about getting my taxes done this year. 0
22. You ask a person out on a date, and he or she says no. PvB
    A. I was a wreck that day. 1
    B. I got tongue-tied when I asked him or her on the date. 0
23. A game show host picks you out of the audience to participate in the show. PsG
    A. I was sitting in the right seat. 0
    B. I looked the most enthusiastic. 1
24. You are frequently asked to dance at a party. PmG
    A. I am outgoing at parties. 1
    B. I was in perfect form that night. 0
25. You buy your spouse (boyfriend/girlfriend) a gift he or she doesn't like. PsB
    A. I don't put enough thought into things like that. 1
    B. He or she has very picky tastes. 0
26. You do exceptionally well in a job interview. PmG
    A. I felt extremely confident during the interview. 0
    B. I interview well. 1
27. You tell a joke and everyone laughs. PsG
    A. The joke was funny. 0
    B. My timing was perfect. 1
28. Your boss gives you too little time to finish a project, but you get it finished anyway. PvG
    A. I am good at my job. 0
    B. I am an efficient person. 1
29. You've been feeling run down lately. PmB
    A. I never get a chance to relax. 1
    B. I was exceptionally busy this week. 0
30. You ask someone to dance, and he or she says no. PsB
    A. I am not a good enough dancer. 1
    B. He or she doesn't like to dance. 0
31. You save a person from choking to death. PvG
    A. I know a technique to stop someone from choking. 0
    B. I know what to do in crisis situations. 1
32. Your romantic partner wants to cool things off for a while. PvB
    A. I'm too self-centered. 1
    B. I don't spend enough time with him or her. 0
33. A friend says something that hurts your feelings. PmB
    A. My friend always blurts things out without thinking of others. 1
    B. My friend was in a bad mood and took it out on me. 0
34. Your employer comes to you for advice. PvG
    A. I am an expert in the area about which I was asked. 0
    B. I'm good at giving useful advice. 1
35. A friend thanks you for helping him or her get through a bad time. PvG
    A. I enjoy helping him or her through tough times. 0
    B. I care about people. 1
36. You have a wonderful time at a party. PsG
    A. Everyone was friendly. 0
    B. I was friendly. 1
37. Your doctor tells you that you are in good physical shape. PvG
    A. I make sure I exercise frequently. 0
    B. I am very health-conscious. 1
38. Your spouse (boyfriend/girlfriend) takes you away for a romantic weekend. PmG
    A. He or she needed to get away for a few days. 0
    B. He or she likes to explore new areas. 1
39. Your doctor tells you that you eat too much sugar. PsB
    A. I don't pay much attention to my diet. 1
    B. You can't avoid sugar; it's in everything. 0
40. You are asked to head an important project. PmG
    A. I just successfully completed a similar project. 0
    B. I am a good supervisor. 1
41. You and your spouse (boyfriend/girlfriend) have been fighting a great deal. PsB
    A. I have been feeling cranky and pressured lately. 1
    B. He or she has been hostile lately. 0

42.	You fall down a great deal while skiing.	PmB
	A. Skiing is difficult.	1
	B. The trails were icy.	0
43.	You win a prestigious award.	PvG
	A. I solved an important problem.	0
	B. I was the best employee.	1
44.	Your stocks are at an all-time low.	PvB
	A. I didn't know much about the business climate at the time.	1
	B. I made a poor choice of stocks.	0
45.	You win the lottery.	PsG
	A. It was pure chance.	0
	B. I picked the right numbers.	1
46.	You gain weight over the holidays, and you can't lose it.	PmB
	A. Diets don't work in the long run.	1
	B. The diet I tried didn't work.	0
47.	You are in the hospital, and few people come to visit.	PsB
	A. I'm irritable when I am sick.	1
	B. My friends are negligent about things like that.	0
48.	They won't honor your credit card at a store.	PvB
	A. I sometimes overestimate how much money I have.	1
	B. I sometimes forget to pay my credit card bill.	0

SCORING KEY

PmB: ___ PmG: ___
PvB: ___ PvG: ___
PsB: ___ PsG: ___
Total B: ___ Total G: ___
G – B: ___

INTERPRETING YOUR TEST RESULTS

The test results will give you a clue as to your explanatory style. In other words, the results will tell you about how you explain things to yourself, or your habit of thought. Again, remember that there are no right or wrong answers.

There are three crucial dimensions to your explanatory style: permanence, pervasiveness, and personalization. Each dimension, plus a couple of others, will be evaluated on the basis of your answers to the questionnaire.

Permanence

When pessimists are faced with challenges or bad events, they view them as being permanent. In contrast, people who are optimists tend to view the challenges or bad events as temporary. Here are some statements that reflect the subtle differences:

Permanent (Pessimistic)	Temporary (Optimistic)
"My boss is always a jerk."	"My boss is in a bad mood today."
"You never listen."	"You are not listening."
"This bad luck will never stop."	"My luck has got to turn."

To determine how you view bad events, look at the eight items coded PmB (for Permanent Bad): 5, 13, 20, 21, 29, 33, 42, and 46. Each answer with a "0" after it is optimistic; each one followed by a "1" is pessimistic. Total the numbers at the right-hand margin of the questions coded PmB, and write the total on the PmB line on the scoring key.

If you totaled 0 or 1, you are very optimistic on this dimension; 2 or 3 is a moderately optimistic score; 4 is average; 5 or 6 is quite pessimistic; and 7 or 8 is extremely pessimistic.

Now let us take a look at the difference in explanatory style between pessimists and optimists when there is a positive event in their lives. It is just the opposite of what happens with a bad event. Pessimists view positive events as temporary, whereas optimists view them as permanent. Here again are examples of some subtle differences in how pessimists and optimists might communicate their good fortune:

Temporary (Pessimistic)	Permanent (Optimistic)
"It's my lucky day."	"I am always lucky."
"My opponent was off today."	"I am getting better every day."
"I tried hard today."	"I always give my best."

Now total all the questions coded PmG (for Permanent Good): 2, 10, 14, 15, 24, 26, 38, and 40. Write the total on the line in the scoring key marked PmG.

If you totaled 7 or 8, you are very optimistic on this dimension; 6 is a moderately optimistic score; 4 or 5 is average; 3 is pessimistic; and 0, 1, or 2 is extremely pessimistic.

Are you starting to see a pattern? If you are scoring as a pessimist, you may want to learn how to be more optimistic. Your anxiety may be due to your belief that bad things are always going to happen and that good things are only "flukes."

Pervasiveness

Pervasiveness refers to the tendency to describe things either in universals (everyone, always, never, etc.) versus specifics (a specific individual, a specific time, etc.). Pessimists tend to describe things in universals, whereas optimists describe things in specifics, as shown in the following examples:

Universal (Pessimistic)	Specific (Optimistic)
"All lawyers are jerks."	"My attorney was a jerk."
"Instruction manuals are worthless."	"This instruction manual is worthless."
"He is repulsive."	"He is repulsive to me."

Total your score for the questions coded PvB (for Pervasive Bad): 8, 17, 18, 22, 32, 44, and 48. Write the total on the PvB line.

If you totaled 0 or 1, you are very optimistic on this dimension; 2 or 3 is a moderately optimistic score; 4 is average; 5 or 6 is quite pessimistic; and 7 or 8 is extremely pessimistic.

Now let us look at the level of pervasiveness of good events. Optimists tend to view good events as universal, and pessimists view them as specific. Again, it is just the opposite of how each views a bad event.

Total your score for the questions coded PvG (for Pervasive Good): 6, 7, 28, 31, 34, 35, 37, and 43. Write the total on the line labeled PvG.

If you totaled 7 or 8, you are very optimistic on this dimension; 6 is a moderately optimistic score; 4 or 5 is average; 3 is pessimistic; and 0, 1, or 2 is extremely pessimistic.

Hope

Our level of hope or hopelessness is determined by our combined level of permanence and pervasiveness. Your level of hope may be the most significant score for this test. Take your PvB and add it to your PmB score. This is your hope score.

If it is 0, 1, or 2, you are extraordinarily hopeful; 3, 4, 5, or 6 is a moderately hopeful score; 7 or 8 is average; 9, 10, or 11 is moderately hopeless; and 12, 13, 14, 15, or 16 is severely hopeless.

People who make permanent and universal explanations for their troubles tend to have stress, anxiety, and depression; they tend to collapse when things go wrong. According to Dr. Seligman, no other score is as important as your hope score.

Personalization

The final aspect of explanatory style is personalization. When bad things happen, we can either blame ourselves (internalize) and lower our self-esteem as a consequence, or we can blame things beyond our control (externalize). Although it may not be right to deny personal responsibility, people who tend to externalize blame in relation to bad events have higher self-esteem and are more optimistic.

Total your score for those questions coded PsB (for Personalization Bad): 3, 9, 16, 19, 25, 30, 39, 41, and 47.

A score of 0 or 1 indicates very high self-esteem and optimism; 2 or 3 indicates moderate self-esteem; 4 is average; 5 or 6 indicates moderately low self-esteem; and 7 or 8 indicates very low self-esteem.

Now let us take a look at personalization and good events; the pattern is the exact opposite of that for bad events. When good things happen, the person with high self-esteem internalizes, whereas the person with low self-esteem externalizes.

Total your score for those questions coded PsG (for Personalization Good): 1, 4, 11, 12, 23, 27, 36, and 45. Write your score on the line marked PsG on the scoring key.

If you totaled 7 or 8, you are very optimistic on this dimension; 6 is a moderately optimistic score; 4 or 5 is average; 3 is pessimistic; and 0, 1, or 2 is extremely pessimistic.

Your Overall Scores

To compute your overall scores, first add the three B scores (PmB + PvB + PsB); the total is your B (bad event) score. Do the same for all of the G scores (PmG + PvG + PsG); the total is your G score. Subtract B from G to obtain your overall score.

If your B score is 3 to 6, you are marvelously optimistic when bad events occur; a score of 7 to 9 shows you are optimistic; 10 or 11 is average; 12 to 14 is pessimistic; anything above 14 is extremely pessimistic.

If your G score is 19 or above, you think about good events extremely optimistically; a score of 17 to 18 shows you are optimistic; 14 to 16 is average; 11 to 13 indicates pessimism; and a score of 10 or less indicates great pessimism.

If your overall score (G – B) is above 8, you are very optimistic across the board; if it is 6 to 8, you are moderately optimistic; 3 to 5 is average; 1 or 2 is pessimistic; and a score of 0 or less is very pessimistic.

Supplier Certification: Compliance Guide and Questionnaire[1]

Joseph E. Pizzorno, ND

INTRODUCTION

This guidance was prepared to assist practitioners in their review of suppliers. To effectively use the supplier certification and quality assurance questionnaires, some background information is needed. The questionnaire asks a series of questions regarding the quality practices and Good Manufacturing Practice (GMP) compliance of the supplier. These questions should be reviewed using a graded approach to determine the level of quality practices the supplier has in place.

In this version of the questionnaire (total of 22 questions), the following questions and their related answers are considered critical, major, or minor points of an appropriate quality system.

Critical Questions: 8, 12, 14A, 15, 18, 19, 20, and 22

Missing answers to the critical questions show potential adulteration and/or contamination problems with the materials and/or products supplied. These questions deal with information about the supplier's quality control personnel, specifications, and related testing of raw materials and/or finished products; possible contamination of materials and/or products due to inadequate sanitation practices; inability to substantiate label information; and noncompliance with current food labeling regulations for potential allergens. If the supplier's answers indicate possible contamination and/or adulteration issues with materials and/or products, the use of the supplier should be rejected.

Major Questions: 6, 7, 11, 14B, 16, 17, and 21

Missing answers to the major questions show serious gaps in the supplier's quality program. These gaps include a lack of the following: designated quality control personnel, necessary quality procedures, suitable employee training program, adequate internal GMP audit program, suitable review of raw material suppliers, appropriate monitoring of contract laboratories, required testing of raw materials, adequate retesting of released raw materials, suitable use of expiration dates, appropriate retained samples, and appropriate monitoring and/or verification of subcontracted production. If the supplier's answers indicate possible gaps in the quality program, the number of gaps must be reviewed to determine how much of the quality system is missing or compromised. If the majority of these questions are not answered appropriately, it is recommended these be treated in the same manner as critical issues.

Minor Questions: 1, 2, 3, 4, 5, 9, 10, and 13

Information required by the minor questions should be readily available because it deals with the supplier's name and address, manufacturer's name and address, and contact personnel. The remaining minor questions deal with items that are not mandatory for food producers, such as the following: GMP audit of the supplier by a third party, written employee training program, frequency of internal GMP audits and how reports are handled, auditing of raw material suppliers, and basis for retesting of released raw materials.

Any issues surrounding the testing of raw materials and/or finished products should be carefully scrutinized. If it appears the supplier is not performing adequate testing and/or examination to provide proof of a material's identity, purity, strength, or composition, the material should be considered suspect and not eligible for use. If suppliers are relying on outside sources for analytic data, it is necessary to ask how they validate the source of that information.

Typically, it only takes one critical issue question to derail the use of a supplier because the questions concern material and/or product contamination and/or adulteration issues. In each situation, after the initial review is completed, the supplier should be notified of the results of the review. Suppliers should be given the opportunity to correct deficiencies and provide proof of the corrections.

Practice Name _____

Address _____

_____

_____

FOREWORD

Our suppliers are the essence of our success. We cannot succeed without quality materials and services. We have therefore embarked on a program designed to develop working partnerships with our valued suppliers. We feel that development of an open, trusting, cooperative relationship with our suppliers is a prerequisite to a meaningful certification process.

Vendor certification is an important component of a total quality management system that ensures that a supplier's product is produced, packaged, and shipped under a controlled process that results in consistent conformance to our requirements. It supports the concept of quality at the source by doing it right the first time, thereby substantially reducing or eliminating the need for final quality inspections by the supplier or the customer. The primary objective of the certification process is to ensure consistently high quality, as demonstrated by predictable conformance to our requirements. The basic premise is that we want to identify suppliers that have adequate process controls in place and that *provide legitimate proof* that their products are consistently fit for use, are authentic, meet label claims, and are free from contamination or have maximum freedom from contamination.

It is our goal as a medical practice to offer products that meet or exceed all the applicable regulatory requirements; are clinically effective; and are safe for our patients, customers, and friends. We feel very strongly about the importance of and need for certification and have included it as a major cornerstone of our business philosophy. We seek to identify and do business with natural product suppliers that attain and maintain full compliance with the proposed U.S. Food and Drug Administration Good Manufacturing Practice guidelines for the manufacture of nutritional supplements currently published in the *Federal Register*.

OBJECTIVES

We are seeking suppliers that:

a. Are interested in making certification a standard part of doing business, and we invite you to join us in our pursuit of excellence. Our suppliers have in place or are willing to put in place a documented quality system.
b. Are committed to partnering with us and developing internal programs to ensure consistent quality, good communication, timely delivery, and best overall cost.
c. Have or are willing to put in place a comprehensive quality assurance testing program that ensures the authenticity of raw materials and finished products, label-claim potency, freedom from contamination, and stability over the expiration dating period.

Please answer the questions and return the completed audit to the address at the top of the form. In an effort to protect your intellectual property (IP) and confidentiality, you may provide the first and last sheet only for standard operating procedures, multipage forms, audit reports, and so forth as proof of proper documentation.

Please answer each question with Yes, No, or N/A or provide further data.

Corporate and Personnel Information

1. Supplier's name:
2. Supplier's address, telephone/fax number, and web address:
3. Manufacturer's name, address, and telephone number (including all manufacturing sites):
4. Name, address, telephone number, and contact person for any/all of your QA/QC Contract Laboratories:
5. Contact personnel: (provide name and e-mail address)
   Plant Manager:
   QA/QC Manager:
   Purchasing Agent:

GMP and Quality of Procedures Information

Check one column or circle one letter for each.	Y	N	N/A
6. Does the company have a Quality Control Unit?			
7. Does the quality unit have the authority to approve/reject the following:			
a. Procedures	a	a	a
b. Specifications	b	b	b
c. Test methods and results	c	c	c
d. Raw ingredients/components	d	d	d
e. Finished ingredients	e	e	e
f. Packaging materials	f	f	f
g. Labels	g	g	g
h. Processing records	h	h	h
i. Forms	i	i	i
j. Instrument/control calibrations	j	j	j
k. Reprocessing operations	k	k	k
8. Do you have a GMP system in place? If so, which do you follow:			
a. Food GMPs	a	a	
b. FDA GMPs for dietary supplements	b	b	
9. Have you ever been independently certified for GMP compliance? If so, by whom?			
a. NPA Date of Last Audit: _____	a	a	
b. NSF Date of Last Audit: _____	b	b	
c. USP Date of Last Audit: _____	c	c	
d. TGA Date of Last Audit: _____	d	d	
e. Other: Date of Last Audit: _____	e	e	
10. If independently certified for GMPs, please provide proof that you successfully passed the GMP audit. (Please attach audit report as proof)			
11. Please provide a copy of: The table of contents for your SOP forms (Please attach)			
12. Do written records exist of employee training and education? If yes, please attach an example.			
13. Does a written GMP training program exist for new and veteran employees?			

Raw Materials

Check one column or circle one letter for each.	Y	N	N/A
14A. If you have an in-house laboratory:			
a. Name/e-mail of supervisor:			
b. How many analysts by level of education are in the laboratory? GED____BS____MS____PhD____			

14B. If you use contract laboratory personnel are they audited by:

a. Company personnel a
b. A third party b
c. Not audited c
d. If audited, how often? Yearly. Every 2 yrs. Every 5 yrs. Other d

15A. When doing in-house or independent testing of "BOTANICAL" raw materials, are they checked for:
(Please provide two examples of test data for each item, a-g)

a. Identity (To authenticate material or botanical genus and species) a a
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

b. Microbiology Profile (Bacteria, Yeast, and Mold) b b
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

c. Potency (if potency claim exists) c c
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

d. Heavy Metals (Lead, Mercury, Cadmium, Arsenic) d d
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

e. Chemical Solvent Residue e e
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

f. Aflatoxins f f
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

g. Herbicides and Pesticides Residue g g
If yes, are SOME or ALL materials tested? (circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

If your company does not test one or more of these items on every batch of material please provide a detailed rationale for how you prove that omitting the analysis is not missing a quality parameter.

15B. When doing in-house or independent testing of "NON-BOTANICAL" raw materials, are they checked for: (Provide two examples of test data for each item, a-g)

a. Identity (To authenticate material) a a
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

b. Microbiology Profile (Bacteria, Yeast, and Mold) b b
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)

1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

c. Potency (if potency claim exists) c c
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

d. Heavy Metals (Lead, Mercury, Cadmium, Arsenic) d d
If yes, are SOME or ALL materials tested? (Circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

e. Chemical Solvent Residue e e
If yes, are SOME or ALL materials tested? (circle answer)
If yes, how often? (Choose one)
1. Each batch received _____
2. Skip lot testing (If so, how often?) _____
3. Other _____

If your company does not test one or more of these items on every batch of material, please provide a detailed rationale for how you prove that omitting the analysis is not overlooking or missing a quality parameter.

16. Do you accept a Certificate of Analysis in lieu of independent testing of raw materials?
17. If you answered yes to the preceding question, please provide a written, detailed rationale for how you quality assure your raw materials at the time of receipt.
18. Metal Detection:
Are all in-process materials metal detected?
If so, by what method? _____
Are finished products metal detected?
If so, by what method? _____
19. How is the effectiveness of metal detection measures evaluated?
(Please attach rationale)

Finished Products

Check one column or circle one letter for each.	Y	N	N/A
20. Are your finished products tested for label claim potency before release for sale? If yes, please provide full test data for three different products If not, please provide a rationale for how you prove you meet the label claim.			
21. Do you put expiration dates or a use by date on your products?			
22. Do you do label claim potency testing (Stability Testing) to verify that the product meets the label claim throughout the expiration dating period? If yes, please provide stability potency assays on three different finished product batches that were tested to verify the expiration date claim. If not, please provide a detailed rationale for how you prove that you have met the label claim through the dated period.			

REFERENCES

See www.expertconsult.com for a complete list of references.

REFERENCE

1. This questionnaire is based on the work of Rick Liva, ND, RPh.

Vaginal Depletion Pack

Joseph E. Pizzorno, ND

INTRODUCTION

The vaginal depletion pack ("vag pack") was originally developed by the eclectic physicians of the 19th century and later modified to its present form by John Bastyr, ND. Over the past 75 years, it has been regularly used by many, if not most, naturopathic physicians in the treatment of various vaginal disorders.

Although no controlled studies of the clinical efficacy of the vag pack have been published, it has a long, and apparently successful, history of use. Its primary application has been in the treatment of cervical erosions, cervical dysplasia, pelvic inflammatory disease, and pelvic congestion. The mode of action is apparently through its local antibacterial, anhydrous, alterative, and sclerotic activities. These are thought to inhibit local microbial growth, stimulate the body to slough off abnormal cervical cells, and promote lymphatic drainage. However, no research has been done to determine the actual mechanism.

Nonetheless, this author has seen many women with precancerous abnormalities of the cervix return to normal. However, they were also treated nutritionally, so the benefit cannot be isolated.

PROCEDURE

Supplies

- Open-mouth 1-lb container with tight-sealing lid
- Anhydrous magnesium sulfate ($MgSO_4$; keep tightly closed)
- Glycerin
- *Hydrastis canadensis* tincture: 0.5 oz
- *Thuja occidentalis* oil (not thuja in oil): 0.5 oz
- *Melaleuca alternifolia* oil: 0.25 oz
- Bitter orange oil: 0.25 oz
- Vita Minerals 120 (iron sulfate solution): 1 oz
- Cotton: 0.5 in. thick, 2 in. wide, and 3 in. long
- Cotton string (surgical grade): 4 in. long (dental floss also works well)

Mixing the Solution

The following steps must be followed carefully. Deviation results in either a runny, inconvenient liquid or a rock-hard, unusable precipitate. Each step requires vigorous stirring before proceeding to the next!

1. Fill the container half full with anhydrous $MgSO_4$.
2. Add glycerin until soupy. The solution will get warm to hot. Mix until the solution begins to cool.
3. Add oils.
4. Add *H. canadensis.*
5. Add Vita Minerals 120.
6. Stir occasionally over the next 2 to 3 hours until the solution cools. It should have a thick, tar-like consistency.
7. Store in a tightly sealed container in a cool, dry place. The solution will require stirring if not used regularly.

The Tampon

The cotton is folded lengthwise and tied at one end to make a tampon 1 in. thick and 3 in. long, with approximately 3 in. of string hanging from the tied end.

Application

The best time for application is during times of normal uterine drainage—right before or after the menses and during ovulation. The procedure is as follows:

1. Fold 1 tbsp of the formula into the untied end of the cotton tampon (some find it easier to put the solution into the cotton tampon before folding and tying it).
2. Expose the cervix with a vaginal speculum and open it wide enough to insert the tampon.
3. Slide the vag pack, formula-end first, through the speculum until it fits tightly against the cervix. (A uterine forceps can be used to hold the pack and aid its insertion.)
4. Carefully remove the speculum while leaving the pack in place. Avoid touching the sides of the vagina because the fresh pack can cause some irritation. The uterine forceps are used to hold the pack in contact with the cervix as the speculum is removed. Ensure that the string is left exposed in the introitus.
5. Leave the pack in for 24 hours, at which time the patient can remove it by simply pulling on the string.
6. Have the patient douche after an additional 24 hours. A mild vinegar solution is recommended.
7. The procedure can be performed weekly until the desired results are obtained.

CLINICAL APPLICATIONS

Typically, the patient notices an increase in drainage, both while the vag pack is in place and during the next 24 to 48 hours. If she experiences any burning or discomfort, the pack can be removed early, followed by douching. Suggest that she wear a sanitary pad to absorb the typical secretions. Be careful to not touch the sides of the vagina with the solution because it will cause irritation.

Cervical Dysplasia

When the vag pack is used in the treatment of abnormal cervical cells, inspection of the cervix within a few days of the application reveals a red, eroded appearance where the cells have sloughed off. This typically heals within 7 to 10 days.

The number of applications needed varies with the patient and the condition. This is described in the cervical dysplasia chapter (see Chapter 158).

INDEX

Page numbers followed by *f*, *t*, or *b* indicate figures, tables, or boxes, respectively.

D

H

S

W